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Solution and fluorous synthesis of bioactive carbohydrates: phosphorylated sugars, hyaluronic acid, and isobutyl-C-galactoside

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Solution and fluorous synthesis of bioactive carbohydrates:

Phosphorylated sugars, hyaluronic acid, and isobutyl-*C*-galactoside

by

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ac	Acetyl
AcOH	Acetic acid
Allyl	allyl
BAIB	Bisacetoxyiodobenzene
Bn	Benzyl
Bz	Benzoyl
CAN	Ceric ammonium nitrate
CSA	Camphorsulfonic acid
DBU	1,8-Diazabicycloundec-7-ene
DDQ	2,2-Dichloro-5,6-dicyano-p-benzoquinone
DMAP	N, N-Dimethylaminopyridine
DMF	N, N,-Dimethylformamide
ESI	Electrospray ionization
FSPE	Fluorous solid-phase extraction
F-tag	Fluorous tag
Gal	Galactose
Glc	Glucose
GlcNAc	N-Acetylglucosamine
HRMS	High resolution mass spectrometry
IBCG	Isobutyl-beta-C-galactoside
Man	Mannose
mCPBA	meta-Chloroperoxybenzoic acid
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
MeOTf	Methyl trifluoromethanesulfonate
NBS	N-Bromosuccinimide
NIS	N-Iodosuccinimide
NMR	Nuclear magnetic resonance
Ph	Phenyl
PMB	para-methylbenzyl
PPTS	pyridinium *p*-toluenesulfonate
PTSA	para-Toluenesulfonic acid
SGC	Silica gel chromatography
SPE	Solid-phase extraction
TBAF	Tetrabutylammonium fluoride
TBS	Tert-Butyldimethylsilyl
TCA	Trichloroacetyl

TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidinyloxy
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
TfOH	Triflic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSE	Trimethylsilylethyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate

CHAPTER 1

General Introduction

Dissertation organization

This dissertation is divided into six chapters. Chapter 1 is a general introduction to the thesis. Carbohydrates play important roles in biology; however, studies of the bioactivities of carbohydrates clearly fall behind studies regarding nucleic acids and peptides. This lag clearly in large part is due to the difficulties of obtaining homogenous carbohydrates from natural sources, and the challenges of synthesizing carbohydrates chemically. Whereas nucleic acids and peptides can be synthesized using automation as a routine practice, the synthesis of carbohydrates is still a difficult task. In this thesis, through the development of new methods, including multi-gram scale approaches to building blocks, amenable to automation for the solution and fluorous-assisted synthesis of complex carbohydrates, we discuss the progress toward solving the obstacles in the synthesis of carbohydrates.

Chapter 2 was published in *Organic Letters* in 2011.[1] This chapter discusses the synthesis of the first fluorous protecting group for phosphate and its application in carbohydrate synthesis. Fluorous-assisted synthesis provides a convenient way of purification, and has attracted significant attention in the past decade. The Pohl group has developed an automated solution-phase synthesis platform based in part on fluorous solid-phase extractions. A fluorous phosphate protecting group

based on halo ethyl protecting groups was designed and synthesized. The stability of this group towards acidic conditions and basic conditions has been determined. This protecting group can be used as a facile tag for purification and be removed under mild reducing conditions using zinc and ammonium formate. Synthesis of a disaccharide from *Leishmania* using this fluorous protecting group demonstrated the group's stability to the acidic conditions necessary for glycosylation as well as its orthogonality to several other common protecting groups.

Chapter 3 discusses the synthesis of a series of maltotriose phosphate as probes to be used in the study of glycogen storage diseases. There have been extensive studies regarding the synthesis of maltose structures, but the synthesis of maltose with a phosphate at either the 2- or 3-position of the glucose residue has received very little attention in the past. In this chapter, different synthetic strategies are presented depending on the position of the phosphate to find a general and efficient synthetic route of the maltose phosphates. Partially as an expansion of the work presented in chapter 2, the fluorous phosphate protecting group is evaluated in the synthesis of maltotriose phosphates to probe the feasibility and limitations of using this particular new fluorous protecting group.

Chapter 4 was published in *The Journal of Organic Chemistry* in 2012.[2] This chapter reports a new synthetic route for isobutyl-*β*-*C*-galactoside (IBCG). The synthesis of IBCG was reported by our group in 2003. Since then, the compound has attracted attention for its potential use in animal studies. However, the previous synthesis is not easily suitable for larger scale preparation. In this chapter, the

synthesis of IBCG was achieved in 5 steps from galactose in 81% overall yield without any chromatographic separation steps. This new route allows obtaining the multigram quantities of material required for animal studies more feasible. An optimized microwave-assisted reaction at high concentration was crucial to making the *C*-glycosidic linkage. In the synthesis, it was demonstrated that the protecting groups have a profound influence of the reactivity of the substrate. The key Wittig reaction to install the extra carbon on a per-*O*-silylated gives drastically improved yield compared with the reactions on the per-*O*-acetylated or -benzylated substrate. The co-authors, Basma Abdel Motaal and Marc Schmidt-Supprian in the Max Planck Institute of Biochemistry in Germany, showed in their cell-based assays that IBCG is also a promising inducer of gene expression in mammalian cells.

Chapter 5 discusses the development of synthetic methods amenable to automation for the production of hyaluronic acid fragments, members of the glycosaminoglycan family. Obtaining homogeneous oligosaccharides for biological studies is a major goal of carbohydrate chemistry. However, the chemical synthesis of carbohydrates, especially glycosaminoglycans, is still a difficult task. Several different synthetic strategies to hyaluronic acid fragments were studied and compared. To overcome the severely reduced reactivity because of the strong electron withdrawing properties of the uronic acid, which is one of the major difficulties in glycosaminoglycan synthesis, a new method to install benzyl groups at the 4- and 6-positions on the trichloroacetyl protected glucosamine and at the 2- and 3-positions on glucuronic acid allowed the synthesis of the more electron rich hyaluronic disaccharide

building blocks. Using fluorous assisted synthesis, a tetrasaccharide of hyaluronic acid was synthesized. The conditions developed should be readily applicable for future automated syntheses of this class of glycosaminoglycans.

Chapter 6 discusses the conclusions and future directions for the entire thesis.

References

(1) Liu, L.; Pohl, N. L. B.: A Fluorous Phosphate Protecting Group with Applications to Carbohydrate Synthesis. *Org. Lett.* **2011**, *13*, 1824-1827.

(2) Liu, L.; Abdel Motaal, B.; Schmidt-Supprian, M.; Pohl, N. L. B.: Multigram Synthesis of Isobutyl-β-C-galactoside as a Substitute of Isopropylthiogalactoside for Exogenous Gene Induction in Mammalian Cells. *J. Org. Chem.* **2012**, *77*, 1539-1546.

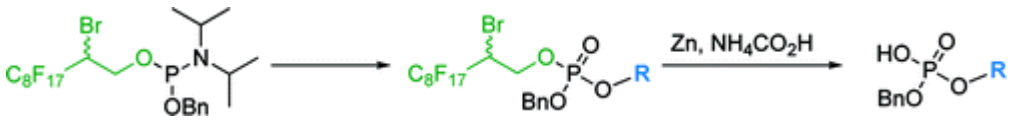
CHAPTER 2

A Fluorous Phosphate Protecting Group with Applications to Carbohydrate Synthesis

A paper published in *Organic Letters*[1]

Lin Liu and Nicola L. B. Pohl

Abstract



The first fluorous protecting group for phosphate is reported. This group can be used as a facile tag for purification and be removed under mild reducing conditions using zinc and ammonium formate. Synthesis of a disaccharide from *Leishmania* using this fluorous protecting group demonstrated the group's stability to the acidic conditions necessary for glycosylation as well as its orthogonality to several other common protecting groups.

[1] Reprinted with permission from *Organic Letters*, **2011**, 13(7), 1824 – 1827. Copyright 2011 American Chemical Society.

Introduction

Phosphate groups are a common motif in a range of bioactive molecules. Phosphodiester bonds make up the backbone of nucleic acids, and phosphate groups are an important part of phospholipids.[1,2] Phosphorylation is a key modification for numerous proteins, and many complex carbohydrates found on the cell surface are phosphorylated.[3,4] Consequently, the chemistry of phosphate has received a lot of attention. One of the central problems in phosphate chemistry is the protecting group. The phosphate itself is acidic and charged at neutral pH and therefore difficult to carry through and purify by standard organic synthetic methods. Numerous protecting group strategies have been developed for the protection of the phosphate group, most of which have been used in the synthesis of nucleotides, especially on a solid phase.[5] Given the growing interest in fluorous-assisted synthesis[6,7] and our own interest in the synthesis of phosphate-containing complex carbohydrates, we were intrigued by the possibility of combining a protecting group for phosphate with a fluorous tag for easy purification using fluorous solid phase extraction (FSPE)[8-13] of the protected compound.

Many fluorous versions of protecting groups have been developed for a variety of functional groups. Our group has used fluorous tags as a handle for purification[14-16] and also has shown that these fluorous tags/protecting groups could be used to directly array compounds for screening.[17-21] However, surprisingly no fluorous protecting group for phosphate has yet been reported. Fluorous groups for the

temporary tagging of the hydroxyls or permanent tagging of the phosphates of nucleotides have been reported in the context of nucleic acid synthesis to assist separation, and some of these fluorous tags have been commercialized.[22-25] However, since the tags for phosphates cannot be removed, they cannot serve a dual purpose also as a protecting group. We envisioned fluorous protecting groups for phosphates that could function as tags, but also could be removed under mild conditions when necessary, would be useful in the synthesis of phosphate-containing molecules (Figure 1), allowing both easy purification and a handle for microarray formation. Herein we report the design and synthesis of the first fluorous protecting group for phosphate and demonstrate its use in carbohydrate synthesis.

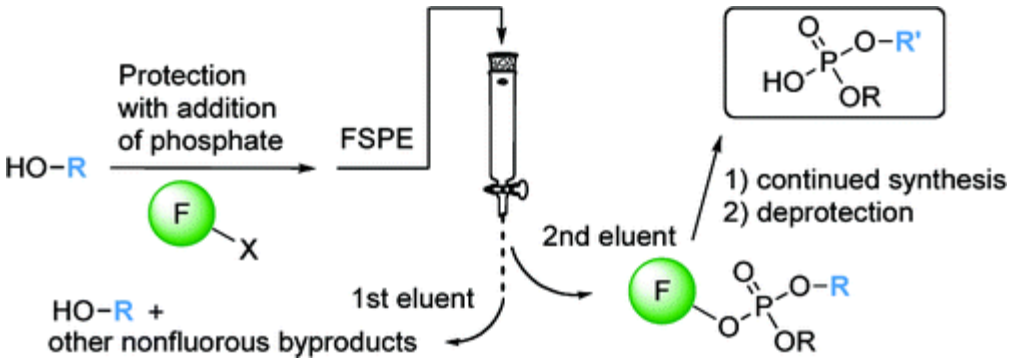


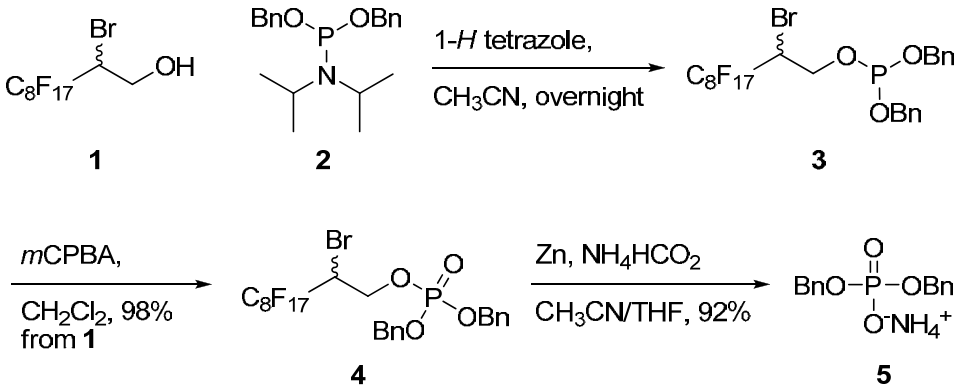
Figure 1. Concept of the fluorous protecting and tagging group for phosphate.

Results and Discussion

In the search for a fluorous protecting group for phosphate, 3-(perfluorooctyl)propanol—which contains a C_8F_{17} moiety with a simple three carbon alkyl linker and is commercially available—was a natural starting point. If this fluorous alkyl alcohol could be readily added and removed from a

phosphate, it could serve as a protecting group. To test the reactivity of this fluorous alcohol, a model study using dibenzyl 3-(perfluorooctyl)propyl phosphate was initiated. The benzyl groups on the phosphate ideally would serve as the same sort of "permanent" protecting group often used on the hydroxyls of carbohydrates that is removed by hydrogenolysis only at the very end of a synthesis. Generally alkyl protecting groups of phosphates are removed using small nucleophiles.[26] Unfortunately, various nucleophiles such as azide or iodide served only to remove one of the benzyl groups in quantitative yields; the fluorous alcohol largely remained in position.

In the continued search for a fluorous protecting group for phosphate that could be easily removed under conditions in which the benzyl phosphate was stable, a haloethyl ester of phosphate caught our attention. These haloethyl groups can generally be removed under mild reducing conditions. Fluorous bromo-alcohol **1**, first reported in 1984,[27] had shown use as a carbamate-type protecting group for amines associated with carbohydrate and peptide structures and could be deprotected using $Zn/Ac_2O/Et_3N$ to provide an *N*-acetyl.[28] We reasoned that this fluorous alcohol **1** with a bromide at the β-position could potentially be suitable for phosphate protection if conditions for its easy removal could be found. However, a concern was the extra stereogenic center of the haloalkyl group, coupled with the stereogenicity of the phosphate ester with a benzyl and a carbohydrate substituent and the chirality inherent in sugars; the resulting diastereomers could make separations and structure elucidation challenging enough to render the fluorous phosphate protecting group more trouble than it was worth.



Scheme 1. Synthesis of Fluorous Protected Dibenzyl Phosphate

To first test the relative stability of the fluorous haloalkyl group and the benzyl group on phosphate, a simple dibenzyl phosphate was made using standard phosphoramidite chemistry (Scheme 1). The fluorous bromo-alcohol **1** was coupled with dibenzyl phosphoramidite **2** in the presence of tetrazole to yield phosphite **3**, which was then oxidized to phosphate **4** using *m*-chloroperoxybenzoic acid (*m*-CPBA) in 98% yield in two steps after FSPE purification. Various conditions were tested to remove the fluorous protecting group, including $Zn/NH_4HCO_2/CH_3OH$, Zn/HOAc/THF, and $Pd/C/CH_3OH/NH_4HCO_2$.[29,30] The reaction was monitored by TLC and ^{31}P NMR. All of these conditions successfully removed the fluorous group on **4**, yielding the desired phosphate **5**, without removal of either benzyl group. The Zn/NH_4HCO_2 conditions in methanol provided the fastest and cleanest reaction. Further optimization of the deprotection conditions showed that by using Zn/NH_4HCO_2 in CH_3CN/THF (4:1) the reaction could go to completion in 1−2 h. The resulting ammonium salt of phosphoric acid was purified by a short silica gel column using CH_2Cl_2/CH_3OH as

the eluent with 1% NH_4OH to remove the small amount of $ZnBr_2$. The fluorous byproduct of the deprotection, the fluorous alkene, has a boiling point of 146−147 °C and could be removed easily by evaporation. The fraction was concentrated, followed by the addition of water, and subjected to lyophilization to give the pure product.

To study the stability of this protecting group under typical acidic and basic conditions used to remove other protecting groups, compound **4** (0.006 M) was treated with 10% trifluoroacetic acid (TFA, 217 equiv) or 10% piperdine (168 equiv) in deuterated chloroform and monitored by ^{1}H NMR. Haloethyl compounds are known to be unstable to piperdine; our results showed only a 24-h half-life for the group. In contrast, the fluorous haloalkyl protecting group showed a half-life of over 120 h in the presence of 10% TFA, and no decomposition was found in the presence of 5 equiv of TsOH after 48 h (Table 1). Compound **4** itself is stable at room temperature for several months.

Table 1. Assessment of Protecting Group Stability

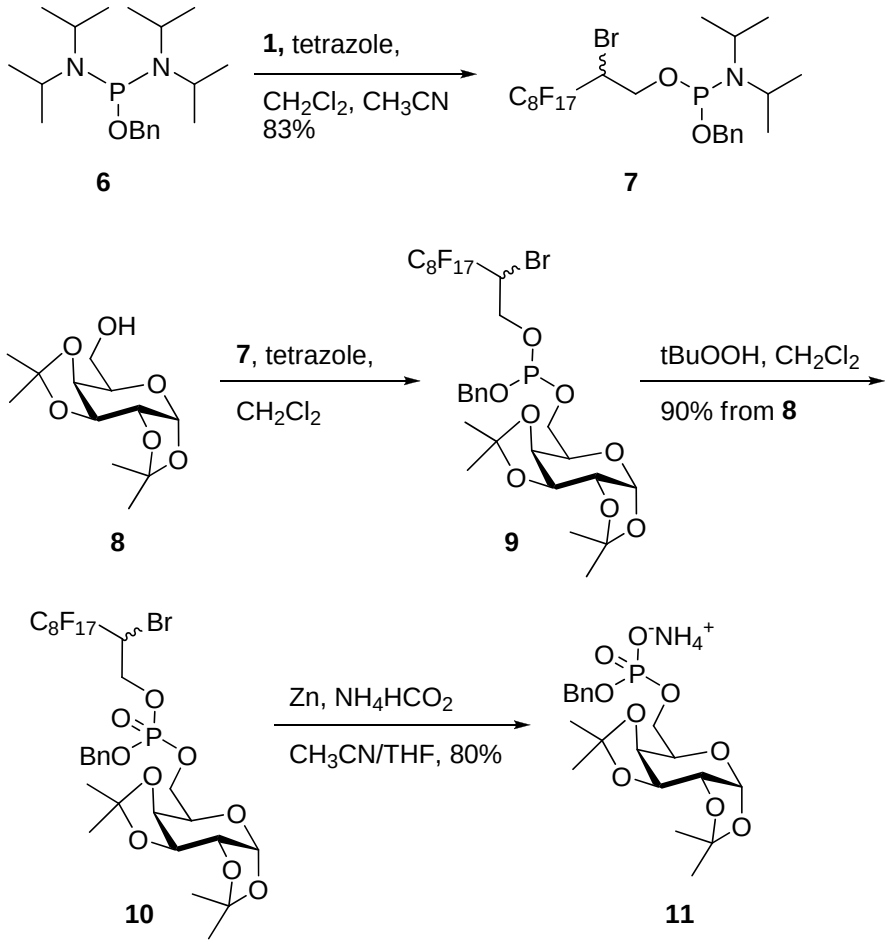
	Percentage (%) Decomposition of **4**						
Time (h)	0.5	12	24	48	72	96	120
10% TFA[a]	0	9	17	31	33	42	45
10% piperdine[a]	0	21	50	91	100	--	--
5 equiv TsOH[b]	0	0	0	0	0	--	--

a) in $CDCl_3$; b) in 9:1 $CDCl_3/CD_3OD$

Encouraged by these results, we decided to use this protecting group as a protecting group for a phosphate monoester on a carbohydrate to ascertain its affect on the separation and characterization of the resulting diastereomeric compounds. As before, we used a benzyl group as the second group on the phosphate ester, since the widely used benzyl group would not make a final deprotection scheme more complicated by the addition of another step. This strategy was first tested on a galactose monosaccharide (Scheme 2).

Benzyl phosphoramidite **6** [31] was coupled with fluorous bromo-alcohol **1** in the presence of tetrazole to yield the desired fluorous phosphoramidite **7**. The ^{31}P NMR of **7** reveals two peaks at 149.57 and 149.44; these peaks reflect the diastereomeric nature of this compound based on the presence of two stereogenic centers. The product was then reacted with diisopropylidene galactose **8** [32] and oxidized with *t*-BuOOH to afford the desired protected phosphate **10** in 90% yield after FSPE purification without the need of a silica gel column. Two sets of closely spaced peaks in the ^{31}P NMR spectrum showed the influence of the newly added stereogenic center from the carbohydrate, theoretically resulting in four diastereomers. Even so, the ^{1}H NMR spectrum of the product was still clean and did not show signs of a mixture. The chiral center on the fluorous bromo-alkylalcohol does not complicate the proton NMR analysis likely because of the remoteness of this center from the influence of the carbohydrate. However, ^{31}P NMR can be used to ensure that the group has actually been successfully added. Removal of the protecting group using $Zn/NH_4HCO_2/CH_3OH$ required 6−8

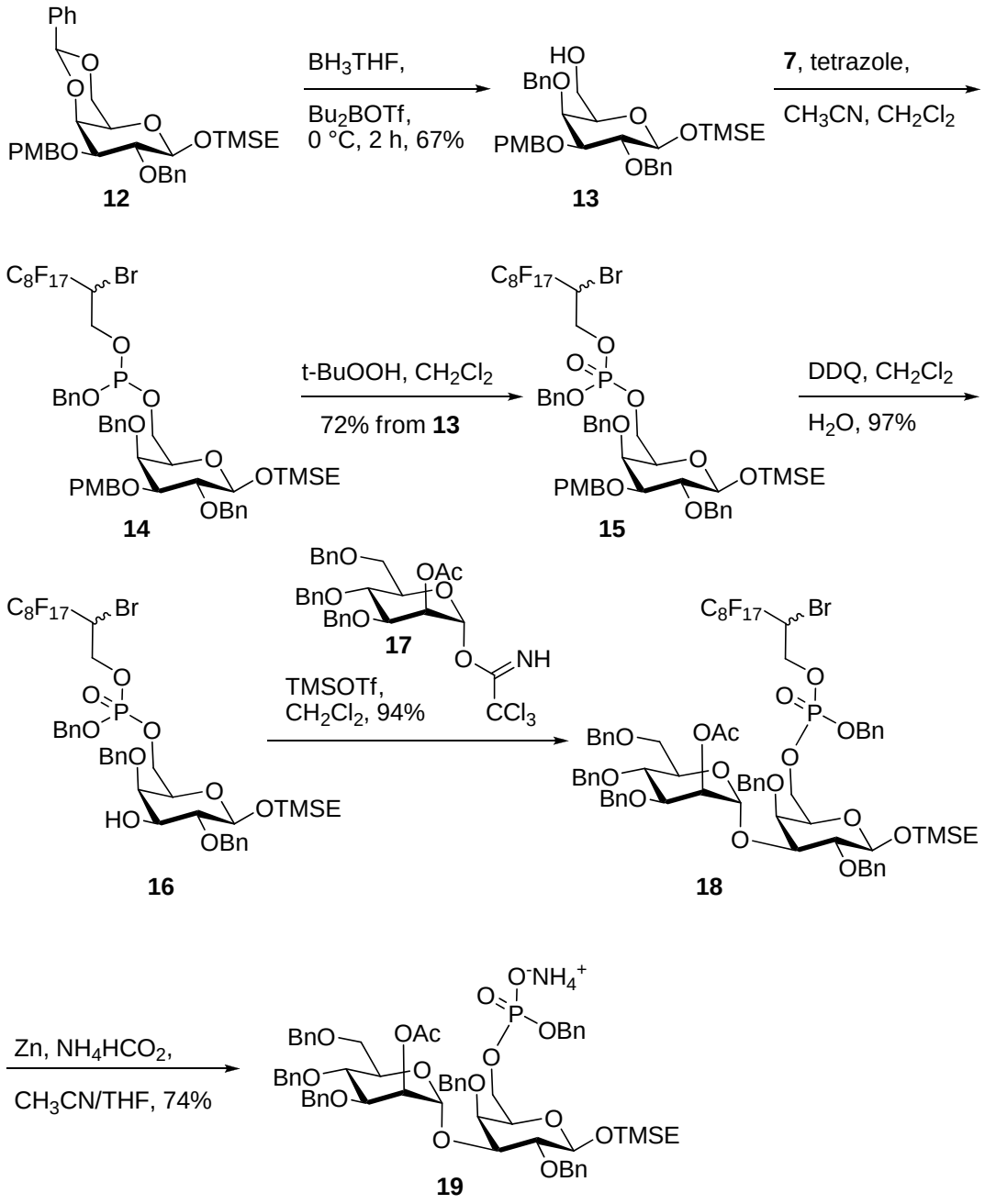
h for completion whereas using CH_3CN/THF (4:1) as the solvent reduced the reaction time to 1−2 h. A short silica gel column, followed by concentration and lyophilization, gave the product **11** as the ammonium salt in 80% yield.



Scheme 2. Fluorous Phosphite Synthesis, Addition to a Monosaccharide with Formation of the Phosphate Ester, and Deprotection of the Fluorous Tag

We next wanted to probe the robustness of this new fluorous phosphate protecting group with a set of

glycosylation/deprotection conditions used in oligosaccharide synthesis by making a disaccharide from *Leishmania* [33](Scheme 3). To this end, galactose building block **12** was obtained from d-galactose.[34] The benzylidene was opened selectively using Bu_2BOTf and BH_3THF[35] to yield **13** with a free C-6 hydroxyl. Compound **13** was then coupled with fluorous phosphoramidite **7** to yield **14**. At this stage, four closely spaced peaks in the ^{31}P NMR spectrum (δ 139.52, 139.44, 139.33, 139.26) revealed the product as four diastereomers as expected. After oxidation, phosphate **15** was purified by FSPE and obtained as the only product in 79% yield. The ^{31}P NMR spectrum of **15** showed only two close peaks separated by just 0.01 ppm at −1.90 and −1.91. This small difference demonstrates that the chemical environments of the phosphorus in the diastereomeric products are close enough to make little difference in the ^{31}P NMR response. The *p*-methoxybenzyl (PMB) group at C-3 of compound **15** was removed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ),[36] followed by FSPE purification, to afford**16** with a free hydroxyl acceptor in 97% yield. The acceptor was coupled with trichloroacetimidate donor **17**[37] using trimethylsilyltriflate as a promoter followed by another FSPE to yield the desired disaccharide **18** in 94% yield. The fluorous protecting group was removed using Zn/NH_4HCOO in CH_3CN/THF within 2 h to yield the desired disaccharide **19** as the ammonium salt in 74% yield.



Scheme 3. Synthesis of a *Leishmania* Disaccharide

Conclusion

In conclusion, a fluorous protecting group for the phosphate group was synthesized. Although this new protecting group contains a stereogenic center, this center does not complicate structure elucidation by ^{1}H NMR and, in fact, adds diagnostic signals in the ^{13}C and ^{31}P NMR spectra. The fluorous phosphate protecting group greatly simplified the purification through the use of FSPE in the synthesis of phosphate-containing compounds. The fluorous bromo-ethanol could be removed easily under mild reducing conditions using zinc and ammonium formate in CH_3CN/THF. The fluorous byproduct has a relatively low boiling point that allows its easy removal under reduced pressure. We are currently probing the utility of this new protecting group for the synthesis of a series of maltose-related phosphates and for solution-phase automated oligosaccharide synthesis.

Experiment section

General Methods: Reactions were performed using flame-dried glassware under argon using anhydrous solvents unless otherwise noted. Ambient temperature in the laboratory was usually 20 °C. CH_2Cl_2 and CH_3CN were distilled freshly from CaH_2. Zinc dust was obtained from Fisher and activated according to *Purification of Laboratory Chemicals* (5th Edition), 2003, Elsevier. Other commercially available reagents were obtained from Aldrich, Fisher or TCI and used as received.

Thin layer chromatography (TLC) was performed using glass backed Silica Gel HL TLC plates w/UV254 from Sorbent Technologies. Visualization of TLC plates was performed by UV, 5% sulfuric acid/ethanol, or *p*-anisaldehyde/ethanol. Silica gel flash chromatography was performed using silica gel (60 Å, 40-63 μm) from ZEOChem AG.

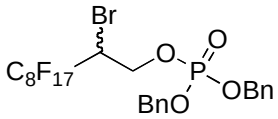
The lyophilization was performed on a Labcono Freezone 4.5. Distilled water was used for the freezing of the sample for lyophilization.

NMR spectra were recorded on a Agilent-Varian 400MR (400 MHz for ^{1}H, 101 MHz for ^{13}C, 376MHz for ^{19}F, 162 MHz for ^{31}P), Varian VXR400 (400 MHz for ^{1}H, 101 MHz for ^{13}C) or Bruker DRX400 (400 MHz for ^{1}H, 101 MHz for ^{13}C, 162 MHz for ^{31}P). Chemical shifts are reported in parts per million (ppm) on the δ scale. ^{13}C, ^{31}P spectra were obtained with ^{1}H decoupling only unless otherwise noted. ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ was referenced the solvent peak at 7.260 ppm (^{1}H) and 77.0 ppm (^{13}C). ^{1}H NMR and ^{13}C NMR taken in d_6-DMSO was referenced the solvent peak at 2.50 ppm (^{1}H) and 39.5 ppm (^{13}C). Note that due to the extensive ^{19}F-^{13}C and ^{31}P-^{13}C coupling and overlap in some of the ^{13}C spectra, the coupling constants of those couplings are not reported. The peak values are listed instead. The assignments of ^{1}H NMR peaks were made primarily from 2D ^{1}H-^{1}H COSY and edited ^{1}H-^{13}C HSQC spectra. ^{1}H-^{13}C HMBC and ^{1}H-^{1}H TOCSY spectra were obtained to aid the assignments when necessary.

High resolution mass spectra (HRMS, ESI mode) were obtained using an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS at Iowa State University. For the fluorous phosphate containing compounds, acetonitrile was used instead of methanol in the ESI/MS to obtain reliable results.

General Procedure for Fluorous Solid Phase Extraction (FSPE): A FSPE cartridge (2 g. Fluorous Technologies, Inc., Pittsburgh, PA) was preconditioned by passing 80:20 MeOH:H_2O (6 mL) through it under a vacuum. The crude mixture was loaded onto the cartridge by using no more than 2 mL of a 9:1 DMF:H_2O solution. The non-fluorous containing compounds were eluted by passing 6-8 mL of 80:20 MeOH:H_2O through the cartridge. The fluorous containing compounds were eluted by passing 6-8 mL of MeOH through the cartridge. The MeOH wash was concentrated under reduced pressure and the residue was coevaporated with toluene to provide the fluorous compounds. The cartridge was regenerated by washing using acetone.

Synthesis protocols and data of new compounds:



Dibenzyl 2-bromo-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl phosphate (4). Fluorous bromo-alcohol **1**[38] (0.5 mmol, 272 mg) was dissolved in CH_3CN (3 mL) and tetrazole (0.72 mmol, 50.4 mg) was added. The reaction was cooled to 0 °C and dibenzyl *N*,*N*-di isopropylphosphoramidite **2** (0.6 mmol, 207 mg) was added. The reaction was brought to ambient

temperature (20 ° C), then stirred overnight. The reaction was cooled to –40 °C; a solution of *m*CPBA (1.0 mmol, 223 mg, 77% purity) in CH_2Cl_2 (4 mL) was added dropwise. The mixture was allowed to warm to ambient temperature and stirred for 2.5 h, then diluted with CH_2Cl_2, washed with 10% $Na_2S_2O_3$ (aq), sat $NaHCO_3$ (aq) and dried over anhydrous Na_2SO_4. Solvents were removed under reduced pressure and the mixture was purified using FSPE to afford **4** as a white solid (396 mg, 0.49 mmol, 98%).

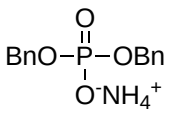
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.31 (m, 10H, ArH), 5.16 – 5.01 (m, 4H, OC*H*$_2$Ph), 4.47 – 4.32 (m, 2H, OC*H*HCBr, C*H*Br), 4.24 (dt, *J* = 11.2, 6.7 Hz, 1H, OC*H*HCBr);

^{13}C NMR (101 MHz, $CDCl_3$) δ 135.36, 135.29, 128.77, 128.64, 128.09, 69.90, 69.85, 64.81, 43.89, 43.80, 43.64, 43.56, 43.40, 43.31;

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.78;

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.73 (t, *J* = 10.0 Hz, 3F), -109.87 (ABq, *J* = 280 Hz, 1F), -113.11 (ABq, *J* = 280 Hz, 1F), -118.55 (ABq, *J* = 295 Hz, 1F), -120.29 (ABq, *J* = 295 Hz, 1F), -121.40 – -121.99 (m, 6F), -122.69 (s, 2F), -126.08 (d, *J* = 7.6 Hz, 2F);

HRMS calcd for $C_{24}H_{18}BrF_{17}O_4P$ $[M+H]^+$: 802.9849, found 802.9856.



Ammonium dibenzyl phosphate (5). Compound **4** (110 mg, 0.014 mmol) was dissolved in CH_3CN (2 mL) and 0.5 mL THF. NH_4HCOO (56 mg, 0.89 mm0l) was added to the reaction followed by Zn

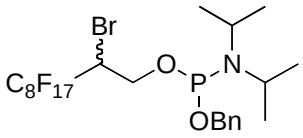
powder (40 mg, 0.61 mmol). The mixture was stirred for 2 h at ambient temperature, then filtered via Celite. The solid was washed with CH_3OH. The filtrates were combined, concentrated, and purified via silica gel column using CH_2Cl_2/CH_3OH (5:1) with 1% NH_4OH as eluent to afford a white solid. Water was added to the solid and the mixture was lyophilized twice to afford the product **5** as a white solid (37 mg, 0.125 mol, 92%).

^{1}H NMR (400 MHz, d_6-DMSO) δ 7.29 (s, 10H, ArH), 6.67 (bs, 4H, NH_4), 4.81 (bs, 4H, OCH_2Ph);

^{13}C NMR (101 MHz, d_6-DMSO) δ 139.01, 128.56, 127.73, 127.61, 67.20;

^{31}P NMR (162 MHz, d_6-DMSO) δ -2.79;

HRMS calcd for $C_{14}H_{14}O_4P$ $[M-NH_4]^-$: 277.0635, found 277.0632



Benzyl 2-bromo-3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 9, 9, 10, 10, 10-heptadecafluorodecyl diisopropylphosphoramidite (7). Fluorous bromo-alcohol **1** (227 mg, 0.42 mmol) and benzyl di(*N*,*N*-diisopropyl)phosphoramidite **6**[39] (150 mg, 0.44 mmol) were dissolved in CH_2Cl_2 (3 mL). A solution of tetrazole (33 mg, 0.47 mmol) in THF (1.5 mL) was added to the reaction at ambient temperature. The mixture was stirred overnight. Et_3N was added to the reaction and the mixture was concentrated. Silica gel column purification using hexanes/ethyl acetate (5:1) with 3% Et_3N as eluent yielded product **7** as a colorless syrup (270 mg, 0.35 mmol, 83%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.20 (m, 5H, ArH), 4.77 – 4.54 (m, 2H, OCH_2Ph), 4.48 – 4.32

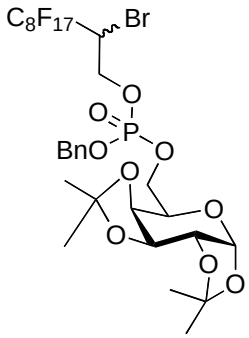
(m, 1H, CHBr), 4.20 – 4.02 (m, 1H, C*H*HCBr), 4.02 – 3.82 (m, 1H C*H*HCBr), 3.65-3.56 (m, 2H, C*H*$(CH_3)_2$), 1.19-1.15 (m, 3H, CH_3), 1.14-1.12 (m, 6H, 2xCH_3), 1.12 -1.10 (m,3H, CH_3);

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.12, 139.05, 138.98, 128.31, 127.46, 127.46, 127.42, 127.07, 127.07, 127.00, 65.71, 65.59, 65.53, 65.41, 62.40, 62.24, 62.05, 46.92, 46.89, 46.69, 46.62, 46.47, 46.40, 46.24, 46.17, 46.00, 45.93, 43.33, 43.30, 43.21, 43.18, 24.55, 24.49, 24.42;

^{31}P NMR (162 MHz, $CDCl_3$) δ 149.57, 149.44;

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.89 (t, *J* = 10.0 Hz, 3F), -109.22 (ABq, *J* = 280 Hz, 1F), -113.44 (ABq, *J* = 280 Hz, 1F), -118.75 (ABq, *J* = 295 Hz, 1F), -120.42 (ABq, *J* = 295 Hz, 1F), -121.39 – -122.24 (m, 6F), -122.80 (s, 2F), -126.22 (dd, *J* = 14.2, 8.2 Hz, 2F);

HRMS calcd for $C_{23}H_{25}BrF_{17}NO_2P$ $[M+H]^+$: 780.0529, found 780.0532.



6-(Benzyl 2-bromo-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl phosphate)-1,2: 3,4-di-*O*-isopropylidene-α-D-galactopyranose (10). 7 (170 mg, 0.22 mmol) and di-acetone galactose **8**[40] (170 mg, 0.66 mmol) was dissolved in CH_2Cl_2 (10 mL) at ambient temperature. 1 mL tetrazole in CH_3CN (0.45 M) solution (0.45 mmol) was added dropwise and the mixture was stirred overnight. A

solution (5-6 M) of tBuOOH (200 µL) in nonane was added, and the reaction was stirred for another 4 h. The reaction was diluted with CH_2Cl_2, washed with sat $NaHCO_3$ (aq) and dried with anhydrous Na_2SO_4. Solvents were removed under reduced pressure and the mixture was purified using FSPE to afford **10** as a white solid (188 mg, 0.196 mmol) in 90% yield.

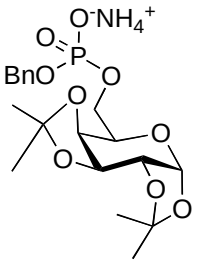
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.29 (m, 5H), 5.52 (d, *J* = 4.9 Hz, 1H, H-1), 5.13 (d, *J* = 8.1 Hz, 2H, OCH_2Ph), 4.61 (dd, *J* = 7.3, 2.9 Hz, 1H, H-3), 4.59 – 4.47 (m, 2H, C*H*Br, C*H*HCBr), 4.37-4.28(m, 1H, C*H*HCBr), 4.33 (dd, *J* = 5.0, 2.5 Hz, 1H, H-2), 4.26 – 4.15 (m, 3H, H-4, H-6a, H-6b), 4.10 – 4.00 (m, 1H, H-5), 1.56-1.51 (m, 3H, CH_3), 1.51-1.41 (m, 3H,), 1.31 (s, 6H, 2xCH_3);

^{13}C NMR (101 MHz, $CDCl_3$) δ 135.44, 135.37, 128.76, 128.68, 128.62, 128.58, 128.28, 128.07, 128.05, 109.67, 109.64, 108.83, 108.81, 96.21, 70.63, 70.57, 70.33, 69.86, 69.83, 69.81, 69.77, 67.01, 66.96, 66.92, 66.90, 66.85, 66.83, 66.77, 66.71, 66.69, 64.96, 64.89, 64.83, 43.71, 29.66, 25.82, 24.79, 24.32;

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.98, -2.04, -2.12;

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.95 (dt, *J* = 18.1, 9.7 Hz, 3F), -109.99 (ABq, *J* = 285 Hz, 1F), -113.10 (ABq, *J* = 285 Hz, 1F), -118.56 (ABq, *J* = 295Hz, 1F), -120.32 (ABq, *J* = 295 Hz, 1F), -121.36 – -122.36 (m, 6F), -122.82 (s, 2F), -125.61 – -126.65 (m, 2F);

HRMS calcd for $C_{29}H_{27}BrF_{17}O_9PNa$ $[M+Na]^+$: 977.0353, found 977.0358.



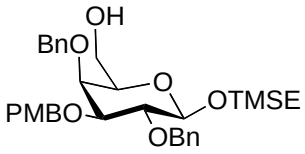
6-(Benzyl ammonium phosphate)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (11). Compound **10** (35 mg, 0.036 mmol) was dissolved in CH_3CN (2 mL) and THF (0.5 mL). NH_4HCOO (28 mg, 0.44 mmol) was added to the reaction followed by Zn powder (20 mg, 0.30 mmol). The mixture was stirred for 2 h at ambient temperature, then filtered via Celite. The solid was washed with CH_3OH. The filtrates were combined, concentrated, and purified via silica gel column using $CH2Cl_2/CH_3OH$ 5:1 with 1% NH_4OH as eluent to afford a white solid. Water was added to the solid and the mixture was lyophilized twice to afford the product **11** as a white solid (13 mg, 0.029 mol, 80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.01 (m, 9H, ArH, NH_4), 5.44 (bs, 1H, H-1), 5.01 (bs, 2H, OCH_2Ph), 4.48 (bs, 1H, H-3), 4.24 (bs, 1H, H-2), 4.17 – 3.78 (m, 4H, H-4, H-5, H-6a, H-6b), 1.43 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.30 – 1.16 (m, 6H, 2x CH_3);

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.18, 128.04, 127.79, 127.11, 109.17, 108.81, 96.03, 70.43, 67.64, 64.47, 25.82, 24.76, 24.18;

^{31}P NMR (162 MHz, $CDCl_3$) δ -3.50;

HRMS calcd for $C_{19}H_{26}O_9P$ $[M\text{-}NH_4]^-$: 429.1320, found 429.1322.

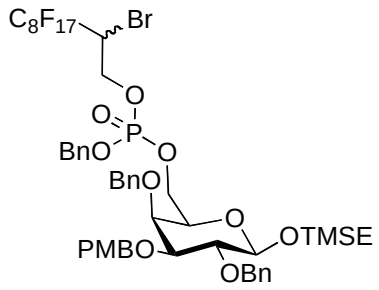


2-(Trimethylsilyl)ethyl 2,4-di-O-Benzyl-3-*O*-*p*-methoxybenzyl-β-D-galactopyranoside(13). 2-(Trimethylsilyl)ethyl 2,4-*O*-dibenzyl 3-*O*-*p*-methoxybenzyl-ß-D-galactopyranoside **12**[4141] (210 mg, 0.36 mmol) was dissolved in 1 M BH_3THF (3.62 mL) at 0 °C. Bu_2BOTf (1 M in CH_2Cl_2, 376 µL) was added and the reaction was stirred for 1h. Additional 110 µL Bu_2BOTf (1 M in CH_2Cl_2) was added and the reaction was stirred for other 40 minutes. The reaction was quenched by adding 600 µL Et_3N. 20 mL CH_3OH was added, and the solvents were removed at reduced pressures. The residue was purified by silica gel chromatography (hexanes/ethyl acetate 9:1 to 2:1) to afford the product **13** as a syrup (140 mg, 0.24 mmol, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 12H, ArH), 6.86 (d, *J* = 8.6 Hz, 2H, ArH), 4.95 (d, *J* = 11.7 Hz, 2H, OCH_2Ar), 4.77 (d, *J* = 10.9 Hz, 1H, OCH_2Ar), 4.73 (d, *J* = 11.5 Hz, 1H, OCH_2Ar), 4.67 (d, *J* = 10.2 Hz, 1H, OCH_2Ar), 4.64 (d, *J* = 11.8 Hz, 1H, OCH_2Ar), 4.36 (d, *J* = 7.7 Hz, 1H, H-1), 3.99 (dd, *J* = 17.5, 9.5 Hz, 1H, OC*H*HCH_2TMS), 3.81 (s, 3H, OCH_3), 3.79 (t, *J* = 8.8 Hz, 1H, H-2), 3.78 – 3.74 (m, 1H, H-6a), 3.73 (d, *J* = 3.1 Hz, 1H, H-4), 3.56 (dd, *J* = 17.5, 9.5 Hz, 1H, OC*H*HCH_2TMS), 3.52 – 3.42 (m, 2H, H-3, H-6b), 3.35 (t, *J* = 6.1 Hz, 1H, H-5), 1.45 (dd, *J* = 8.8, 4.1 Hz, 1H, OH), 1.02 (dd, *J* = 9.5, 8.0 Hz, 2H, C*H*$_2$TMS), -0.00 (s, 9H, TMS);

^{13}C NMR (101 MHz, $CDCl_3$) δ 147.71, 138.78, 133.09, 133.07, 130.42, 129.17, 128.51, 128.29, 128.15, 127.97, 127.41, 113.70, 103.48, 81.86, 79.67, 75.10, 74.34, 73.99, 72.82, 67.40, 61.70, 55.17, 30.35, 7.77, -1.49;

HRMS calcd for $C_{33}H_{44}O_7SiNa$ $[M+Na]^+$:603.2749, found 603.2746.



2-(Trimethylsilyl)ethyl 2,4-di-O-Benzyl-6-(Benzyl 2-bromo-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10 -heptadecafluorodecyl phosphate)-3-*O*-*p*-methoxybenzyl-β-D-galactopyranoside (15). Fluorous phosphoramidite **7** (111 mg, 0.142 mmol) and **13** (82 mg, 0.142 mmol) was dissolved in 10 mL CH_2Cl_2 at ambient temperature. A 0.45M solution of tetrazole (470 µL , 0.21 mmol) in CH_3CN was added dropwise and the mixture was stirred for 12 h. A 5-6 M solution of tBuOOH (250 µL) in nonane was then added; the reaction was stirred overnight. The reaction was diluted with CH_2Cl_2, washed with sat $NaHCO_3$ (aq) and dried with anhydrous Na_2SO_4. Solvents were removed under reduced pressure and the mixture was purified using FSPE to afford colorless syrup **15** (130 mg, 0.102 mmol, 72%).

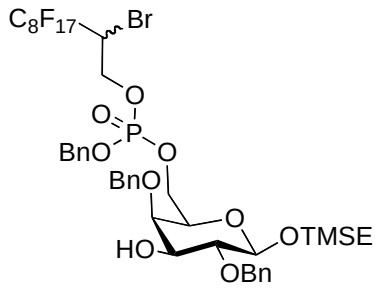
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.11 (m, 17H, ArH), 6.86 (d, J = 8.7 Hz, 2H, ArH), 5.07 (ddd, J = 9.5, 5.2, 3.3 Hz, 2H, OCH_2Ar), 5.00 – 4.89 (m, 2H, OCH_2Ar), 4.77 (d, J = 11.0 Hz, 1H, OCH_2Ar), 4.68 (dd, J = 11.2, 3.7 Hz, 2H, OCH_2 Ar), 4.62 – 4.55 (m, 1H, OCH_2 Ar), 4.51 – 4.36 (m, 2H, CHBr, C*H*HCBr), 4.34 (d, J = 7.7 Hz, 1H, H-1), 4.30 – 4.22 (m, 1H, C*H*HCBr), 4.21 – 4.14 (m, 1H, H-6a), 4.11 – 4.03 (m, 1H, H-6b), 3.99 (dd, J = 18.2, 9.5 Hz, 1H, C*H*HCH_2TMS), 3.80 (s, 3H, OCH3), 3.79

– 3.77 (m, 1H, H-2), 3.77-3.72 (m, 1H, H-4), 3.56 (d, J = 9.8 Hz, 1H,C*H*HCH$_2$TMS), 3.49 (dd, J = 6.8, 2.5 Hz, 1H, H-5), 3.48 – 3.45 (m, 1H, H-3), 1.07 – 0.98 (m, 2H, CH$_2$TMS), 0.11 – -0.15 (m, 9H, TMS);

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.20, 138.80, 138.32, 135.27, 130.44, 129.20, 128.94, 128.91, 128.72, 128.70, 128.25, 128.23, 128.20, 128.16, 128.08, 127.58, 127.52, 113.77, 103.47, 81.57, 79.42, 75.17, 74.39, 73.04, 72.89, 72.64, 72.57, 70.09, 70.03, 67.57, 66.44, 64.90, 55.20, 18.46, -1.52, -1.53, -1.54;

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.90, -1.91; ^{19}F NMR (376 MHz, $CDCl_3$) δ -80.73 (t, *J* = 9.9 Hz, 3F), -109.94 (ABq, *J* = 280 Hz, 1F), -112.89 (ABq, *J* = 280 Hz, 1F), -118.50 (ABq, *J* = 295 Hz, 1F), -120.24 (ABq, *J* = 295 Hz, 1F), -121.13 – -122.23 (m, 6F), -122.68 (s, 2F), -126.09 (s, 2F);

HRMS calcd for $C_{50}H_{53}BrF_{17}O_{10}PSiNa$ $[M+Na]^+$: 1297.1950, found 1297.1956.



2-(Trimethylsilyl)ethyl 2,4-di-O-benzyl-6-(benzyl 2-bromo-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 10-heptadecafluorodecyl phosphate)-β-D-galactopyranoside (16). To a solution of **15** (65 mg, 0.051 mmol) in CH_2Cl_2 (5 mL) / H_2O (277 µL) at 0 °C was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (23 mg, 0.1 mmol). The reaction was allowed to warm to ambient temperature. After

stirring for 2.5 h, the reaction was diluted with CH_2Cl_2, washed with sat $NaHCO_3$ (aq) and dried over anhydrous Na_2SO_4. Solvents were removed under reduced pressure and the mixture was purified using FSPE to afford **16** as a syrup (57 mg, 0.049 mmol, 97%).

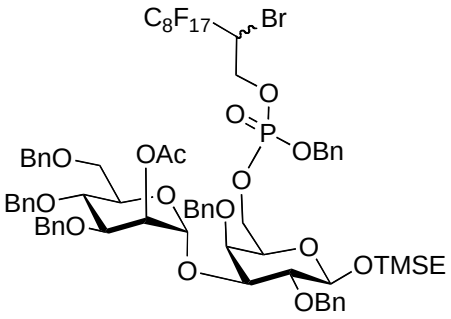
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.17 (m, 15H, ArH), 5.08 (ddd, J = 11.1, 5.9, 2.6 Hz, 2H, OCH_2Ar), 4.98 (d, J = 11.4 Hz, 1H, OCH_2Ar), 4.81 (dd, J = 11.6, 4.4 Hz, 1H, OCH_2Ar), 4.66 (d, J = 11.5 Hz, 1H, OCH_2Ar), 4.60 (ddd, J = 11.5, 6.1, 1.5 Hz, 1H, OCH_2Ar), 4.47 – 4.35 (m, 2H, C*H*HCBr, CHBr), 4.32 (d, J = 7.2Hz, 1H, H-1), 4.30 – 4.24 (m, 1H, C*H*HCBr), 4.22 – 4.15 (m, 1H, H-6a), 4.12 – 4.03 (m, 1H, H-6b), 3.97 (dd, J = 17.1, 9.2 Hz, 1H, C*H*HCH_2TMS), 3.77-3.70 (m, 1H, H-4), 3.65 – 3.48 (m, 4H, C*H*HCH_2TMS, H-3, H-2, H-5), 2.26 (s, 1H, OH), 1.07 – 0.93 (m, 2H, CH_2TMS), 0.06 – -0.08 (m, 9H, TMS);

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.42, 138.17, 135.23, 135.17, 128.94, 128.79, 128.71, 128.65, 128.50, 128.42, 128.29, 128.21, 128.21, 128.17, 128.11, 128.06, 128.02, 127.95, 127.85, 127.67, 127.56, 126.74, 103.20, 79.15, 74.89, 74.88, 74.58, 74.54, 74.00, 73.98, 72.75, 72.67, 70.11, 70.05, 67.47, 66.15, 66.10, 64.90, 43.55, 43.47, 29.68, 26.47, 26.09, 18.45, -1.51, -1.52, -1.53;

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.81, -1.84, -1.86;

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.73 (t, *J* = 9.9 Hz, 3F), -109.92 (ABq, *J* = 285 Hz, 1F), -112.90 (ABq, *J* = 285 Hz, 1F), -118.50 (ABq, *J* = 295 Hz, 1F), -120.24 (ABq, *J* = 295 Hz, 1F), -121.41 – -121.99 (m, 6F), -122.68 (s, 2F), -125.96 – -126.41 (m, 2F);

HRMS calcd for $C_{42}H_{45}BrF_{17}O_9PSiNa$ $[M+Na]^+$:1177.1375, found 1177.1387.



2-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-(trimethylsilyl)ethyl 2,4-di-*O*-benzyl-6- (benzyl 2-bromo-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10- heptadecafluorodecyl phosphate)-β-D-galactopyranoside (18). To a solution of acceptor **16** (25 mg, 0.022 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TMSOTf (0.42 µL , 0.0024 mmol). Donor **17** (42 mg, 0.066 mmol) in CH_2Cl_2 (5 mL) was added to the reaction dropwise. The reaction was stirred for 1 h and quenched by Et_3N. The mixture was concentrated, purified using FSPE to afford product **18** as a syrup (33 mg, 0.020 mmol, 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 6.86 (m, 30H, ArH), 5.22 – 5.17 (m, 1H, H-2'), 5.15 – 5.01 (m, 3H, H-1', OCH_2Ph), 4.97 – 4.76 (m, 4H, OCH_2Ph), 4.68 – 4.45 (m, 6H, OCH_2Ph), 4.46-4.39 (m, 2H, C*H*CBr, C*H*Br), 4.28 (d, *J* = 9.4 Hz, 1H, H-1), 4.25 – 4.21 (m, 1H, C*H*CBr), 4.21 – 4.17 (m, 1H, H-6a), 4.14 (d, *J* = 12.9 Hz, 1H, H-4'), 4.11 – 4.05 (m, 1H, H-6b), 4.03 – 3.93 (m, 3H, H-3', H-5', C*H*HCH$_2$TMS), 3.84-3.78 (m, 1H, H-4), 3.77 – 3.73 (m, 1H, H-3), 3.68 (d, *J* = 7.7 Hz, 1H, H-2), 3.61-3.52(m,2H, H-6a', C*H*HCH$_2$TMS), 3.53 – 3.44 (m, 2H, H-5, H-6b'), δ 2.18 – 2.13 (m, 3H, OAc), 1.07 – 0.96 (m, 2H, CH_2TMS), -0.00 (s, 9H, TMS);

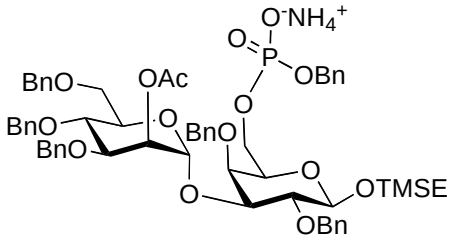
^{13}C NMR (101 MHz, $CDCl_3$) δ 172.27, 140.29, 139.83, 139.76, 139.35, 139.31, 136.76, 136.70, 130.52, 130.48, 130.25, 130.19, 129.91, 129.82, 129.79, 129.78, 129.69, 129.65, 129.55, 129.33,

129.31, 129.23, 129.14, 129.05, 129.01, 128.95, 105.05, 95.36, 79.11, 78.72, 77.63, 76.85, 76.64, 76.47, 75.74, 74.94, 74.46, 73.80, 73.72, 73.31, 72.99, 72.43, 71.66, 71.60, 70.61, 69.96, 69.27, 67.36, 66.43, 45.11, 31.22, 22.63, 22.61, 20.11, -0.01;

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.75, -1.80, -1.84;

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.72 (t, *J* = 10.0 Hz, 3F), -109.95 (ABq, *J* = 280Hz, 1F), -112.87 (ABq, *J* = 280Hz, 1F), -118.50 (ABq, *J* = 290Hz, 1F), -120.19 (ABq, *J* = 287Hz, 1F), -121.36 – -122.03 (m, 6F), -122.68 (t, *J* = 17.2 Hz, 2F), -126.08 (t, *J* = 15.2 Hz, 2F);

HRMS calcd for $C_{71}H_{76}BrF_{17}O_{15}PSi$ $[M+H]^+$: 1629.3598, found 1629.3594.



2-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-(trimethylsilyl)ethyl 2,4-di-*O*-benzyl-6-(benzyl ammonium phosphate)-β-D-galactopyranoside (19). 18 (33 mg, 0.021 mmol) was dissolved in CH_3CN (2 mL) and THF (0.5 mL). NH_4HCOO (28 mg, 0.44 mmol) was added to the reaction followed by Zn powder (20 mg, 0.30 mmol). The mixture was stirred for 2 h at ambient temperature, and then filtered via celite. The solid was washed with CH_3OH. The filtrates were combined, concentrated, and purified via silica gel column using CH_2Cl_2/CH_3OH (5:1) with 1% NH_4OH as eluent to afford a white solid. Water was added to the solid and the mixture was lyophilized three times to afford the product **19** as a white solid (17 mg, 0.015 mol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 6.69 (m, 34H, ArH, NH_4), 5.19 (s, 1H, H-2'), 5.10 (s, 1H, H-1'), 4.94 (d, *J* = 7.2 Hz, 2H, OCH_2Ph), 4.89 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 4.79 (d, *J* = 10.7 Hz, 2H, OCH_2Ph), 4.73 – 4.65 (m, 1H, OCH_2Ph), 4.57 (d, *J* = 11.8 Hz, 2H, OCH_2Ph), 4.49 (dd, *J* = 19.3, 10.3 Hz, 2H, OCH_2Ph), 4.28 (d, *J* = 11.9 Hz, 2H, OCH_2Ph), 4.17 – 4.05 (m, 2H, H-4', H-6a), 4.04 – 3.86 (m, 5H, H-6b, H-3', H-5', H-4, C*H*HCH_2TMS), 3.77 – 3.62 (m, 2H, H-3, H-2), 3.61 – 3.42 (m, 3H, H-6a', H-6', H-5), 2.12 (s, 3H, OAc), 1.06 – 0.91 (m, 2H, CH_2TMS), 0.13 – -0.07 (m, 9H, TMS);

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.18, 140.23, 140.06, 139.66, 139.40, 139.25, 130.35, 129.85, 129.80, 129.75, 129.64, 129.58, 129.47, 129.30, 129.21, 128.96, 128.90, 128.85, 128.77, 104.93, 95.27, 79.10, 78.65, 77.77, 76.67, 76.48, 76.41, 75.67, 74.31, 73.32, 73.10, 72.23, 70.41, 69.83, 69.23, 31.14, 22.55, 20.04, -0.01;

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.01.

HRMS calcd for $C_{61}H_{72}O_{15}P$ $[M\text{-}NH_4]^-$: 1103.4384, found 1103.4392.

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CHAPTER 3

Synthesis of a Series of Maltotriose Phosphates and Evaluation of the Utility of a Fluorous Phosphate Protecting Group

Abstract

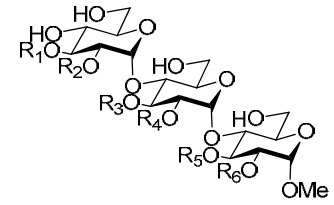
A series of methyl maltotrioside phosphates were synthesized for application in the determination of the actual molecular substrate of the Lafora enzyme involved in Lafora disease. Several different synthetic routes were applied for the successful synthesis of six methyl maltotrioside phosphate regioisomers. The utility of a new fluorous phosphate protecting group was evaluated, but found not to be practical in this particular late stage introduction.

Introduction

α-Glucans, including starch and glycogen, are important molecules in biological systems functioning as energy storage.[1] A series of specific enzymes are involved in the synthesis of starch and glycogens and determine their structures and properties.[2] Lafora disease,[3] or Lafora progressive myoclonic epilepsy, is a fatal disease with no cure or treatment today.[4] Hyperphosphorylation of glycogen is the cause of Lafora disease and it is related to mutations in the genes that code for the protein laforin.[5] In the synthesis of glycogen, phosphates are added to the 2- or 3-position hydroxyls of glucose at a rate of one phosphate in *ca.* 10000 glucose residues.[6,7] Laforin would remove those phosphates groups subsequently under normal circumstances.[8,9] With the mutated laforin, this dephosphorylation cannot be performed and eventually leads to Lafora disease.[10,11] However, the actual molecular mechanism of the laforin enzyme is still unknown. A detailed understanding of enzyme function requires the synthesis of molecules with well-defined chemical structures as substrates. To study the dephosphorylation of laforin, a series of maltotriose analogs with phosphate groups at one of either the 2- or 3-position hydroxyl was designed and synthesized as substrates for the dephosphorylation enzyme. (Figure 1)

Although the synthesis of α-glucans has been extensively studied and reviewed,[12] the synthesis of maltose analogs with phosphate at the 2-or 3-hydroxyl of a sugar ring remains relatively understudied. The desired 1,4-α-glycosidic linkage generally requires non-participating protecting groups at the

2-position of the donor, which would be need to be cleaved selectively later to install the phosphate at the 2-position. The temporary protecting group at the 3-position should also be chosen carefully, since the protecting group could also effect the glycosylation reaction itself.



$R_1 = PO_3^{2-}$; $R_2 = R_3 = R_4 = R_5 = R_6 = H$
$R_2 = PO_3^{2-}$; $R_1 = R_3 = R_4 = R_5 = R_6 = H$
$R_3 = PO_3^{2-}$; $R_1 = R_2 = R_4 = R_5 = R_6 = H$
$R_4 = PO_3^{2-}$; $R_1 = R_2 = R_3 = R_5 = R_6 = H$
$R_5 = PO_3^{2-}$; $R_1 = R_2 = R_3 = R_4 = R_6 = H$
$R_6 = PO_3^{2-}$; $R_1 = R_2 = R_3 = R_4 = R_5 = H$

Figure 1 Six methyl maltotrioside phosphates as substrates for the laforin protein.

In thinking about an efficient strategy to form all six desired regioisomers, we considered the possible use of our recently developed fluorous protecting group for phosphate groups.[13] This group can easily be added to a phosphate and then be used as a tag to separate the sugar chain from other reagents using fluorous solid-phase extraction (FSPE).[14,15] In our previous work, this approach was used for the synthesis of a disaccharide form *Leishmania* and had to advantage of easy purification and could be deprotected under mild conditions. However, in the current case, it was unclear whether introduction of the group so close to the anomeric center might negatively affect the glycosylation and whether a relatively late stage introduction of the fluorous group would actually facilitate the synthesis of the various methyl maltotrioside phosphate regioisomers. In many cases of the synthesis of a phosphate bearing compound, the phosphate group is installed in the last few steps.[16] We

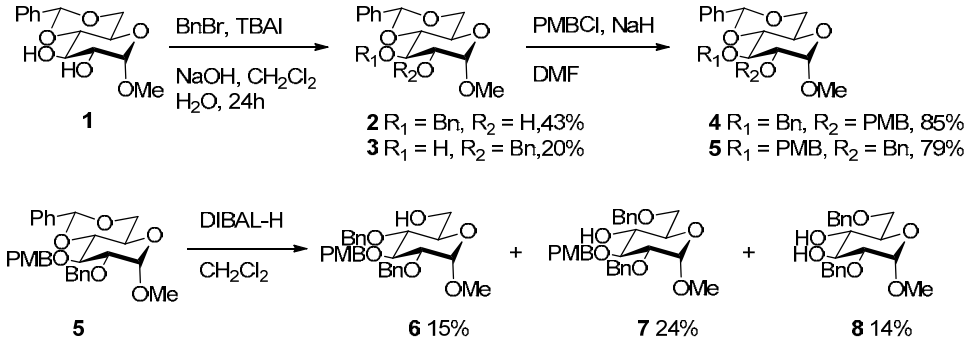
reasoned that if the purification of the non-fluorous phosphate group-bearing compound created a problem, it might be useful to perform a late stage fluorous phosphate installation; otherwise it might not be very practical to carry out a late stage installation. Opposed to late stage installation, the other possibilty is to perform an early stage installation of the fluorous phosphate group and carry the fluorous group throughout the synthesis. In this way, the fluorous group functions as a tag. In the previous cases of fluorous tag assisted synthesis, most tags were attached at the anomeric position of the reducing end of the oligosaccharide, so the tag is away from the reaction center. In the synthesis of maltose phosphates, the fluorous phosphate group would be closer to the reaction site inevitably. In the massive amount of fluorous literatures, few reports discuss the effect of fluorous tags on the reactions. Our concern is although fluorous groups could be used as a convenient way of purification, if the stereoselectivity is not ideal in the synthesis of the α-glycosidic linkage, it would be difficult to separate the α/β mixture, thus diminishing the usefulness of the fluorous assisted synthesis. Van der Marel reported another fluorous phosphate protecting group earlier this year to be used in the synthesis of carbohydrates.[17] The reaction utilized in their study did not involve the glycosidic bond formation, so the fluorous tag proved to be very efficient in their synthesis. Herein we probe how the fluorous tag would perform in a situation when the selectivity of the reaction might not be ideal.

Results and discussion

The desired six methyl maltotrioside phosphates could be broken into three categories depending on

the position of the phosphate group: phosphates at the reducing end, phosphates at the non-reducing end, and phosphates in the middle saccharides.

We started the synthesis of the analogs with phosphates at the reducing end. In this case, the temporary protecting group for future installation of phosphates only exists in the acceptor, simplifying the glycosylation reaction. Commercially available α-methyl glucoside **1** was used as starting material for the acceptor. We proposed by using a p-methoxybenzyl (PMB) group as the temporary protecting group, the synthesis could be simplified. After benzylidene formation, phase-transfer-catalyzed benzylation using sodium hydroxide as a base yielded an easily separable mixture of alcohols **2** and **3**.[18] PMB was installed to give the two proper protected monosaccharides **4** and **5**, (Scheme 1) which were synthesized by known routes.[19,20]



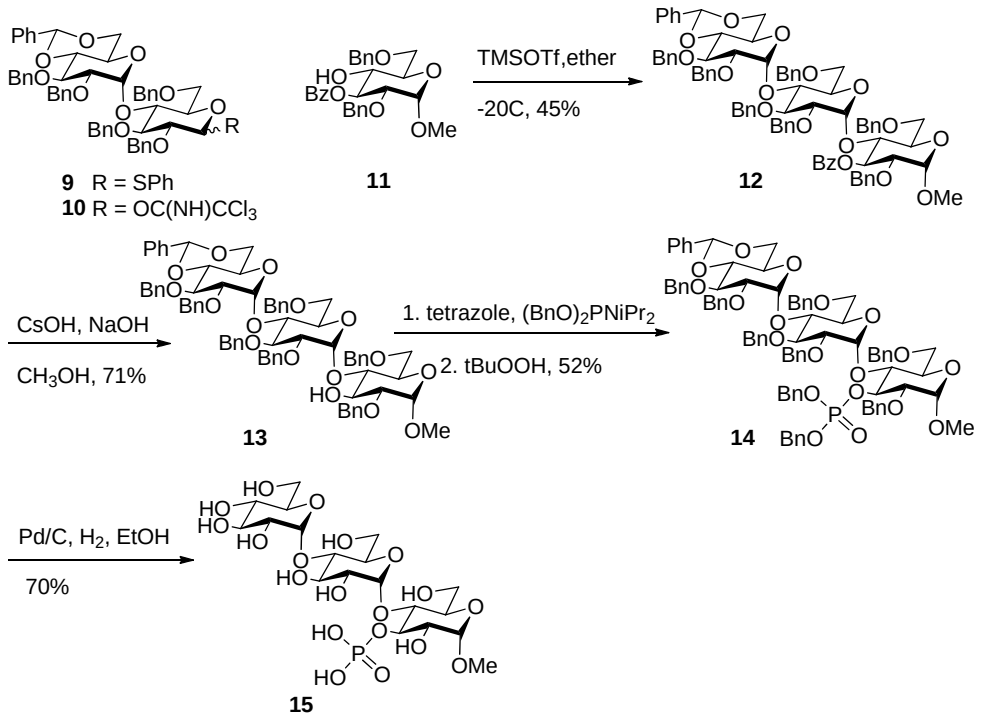
Scheme 1 The synthesis of PMB protected reducing end of methyl maltotriose and the benzylidene opening reaction.

Unfortunately, problems were encountered in selective opening of the benzylidene acetal in the

presence of the PMB group. Various conditions were studied using **5** as a model compound (Scheme 1). TfOH/Et_3SiH[21] and $NaBH_3CN$/HCl[22] gave a low yield of the desired 4-hydroxyl compound **7** and lead to extensive decomposition, which was presumably related to the acid lability of PMB groups.[23] A milder condition using DIBALH in CH_2Cl_2[24] gave the desired product in modest yield. However, it was found that if the PMB is at the 3-postion, extended reaction times would lead to PMB cleavage and afford diol **8**.

To circumvent this problem, we attempted to use benzoyl groups instead of acid-labile PMB groups as the temporary protecting group at the reducing end of maltotriose. Alocohol **3** was reacted with benzoyl chloride and pyridine followed by benzylidene opening using $NaBH_3CN$/HCl[22] to provide an excellent yield of the desired alcohol **11** which was synthesized via other routes before [25] (Scheme 2).

The maltose donor **9** and **10** were synthesized via a modified known procedure starting from maltose. One pot acetylation and bromination of maltose afforded the maltose bromide, which was converted to a thioglycoside under phase-transfer conditions.[26] Deacetylation, followed by benzylidene formation and benzylation yielded the previously reported fully protected maltose thioglycoside donor.[27]



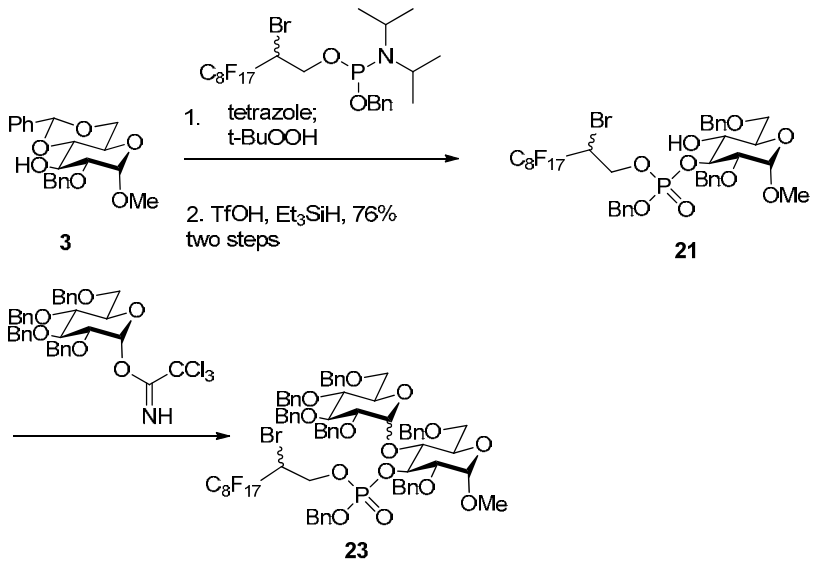
Scheme 2. Synthesis of the 3-phosphate methyl maltotrioside via a benzoyl-protected acceptor

The glycosylation reaction was performed using the thioglycoside donor **9** first (Scheme 2). In the presence of NIS and TMSOTf at 0 °C in ether/dichloromethane (1:1), the reaction proceeded well to give a mixture of α/β glycosides in the ratio of 2:3, and the alpha glycoside was separated in a 22% yield. To try to improve the stereoselectivity of this glycosylation reaction, we carried out the glycosylation of benzoylated acceptors with maltose imidate donors **10** in light of Motawia's work on the 'blockwise three-stage glycosylation strategy'[28] by converting the thiophenyl group to a trichloroacetimidate using standard chemistry.[29] In a mixture of ether/dichloromethane, the glycosylation between the imidate donor and the acceptor gave the desired α product **12** in 53% yield with no β anomer detected.

To remove the benzoyl group in **12** at the 3-position, various conditions were tested. Routine conditions including using $NaOCH_3$ in methanol or using NaOH or in water/THF could only remove the benzoyl group slowly, probably due to steric hindrance. It was found that using cesium hydroxide and sodium hydroxide together could speed up the reaction greatly and give a better result for the deprotection of the benzoyl ester, possibly due to the cesium effect.[26] Proton NMR was used to determine when the deprotection reaction had finished. The doublet peak from the benzoyl group moves slightly down field, indicating the formation of benzoic acid. We envisioned that if there would be any problem in the purification of trisaccharide **14**, the fluorous phosphate protecting group would be beneficial. After the deprotection, a phosphate group was installed using phosphoramidite chemistry[30] to yield **14**, followed by hydrogenolysis to provide the desired phosphate **15**. No problems were encountered in the purification of **14**—a finding that eliminates the need for installing a fluorous phosphate group at this late stage.

If late stage installation of the fluorous phosphate group is not worthwhile, how about performing an early stage installation? Van der Marel reported the synthesis of teichoic acids using a fluorous phosphate group.[17] In their synthesis, the fluorous protecting group is far away from the reaction center, and no glycosylation was involved in the synthesis. As a fluorous protecting group instead of a tag, it would be closer to the reaction site inevitably. The size of the fluorous protecting group and the change of the molecule polarity due to the attachment of the fluorous portion might influence the reaction and the following separation in a reaction that might give a mixture of products like

glycosylation reactions. Not many reports focus on the situations when the reaction would lead to a mixture. If the fluorous protecting group-modified product was inseparable, a fluorous assisted synthesis would not be efficient or practical.

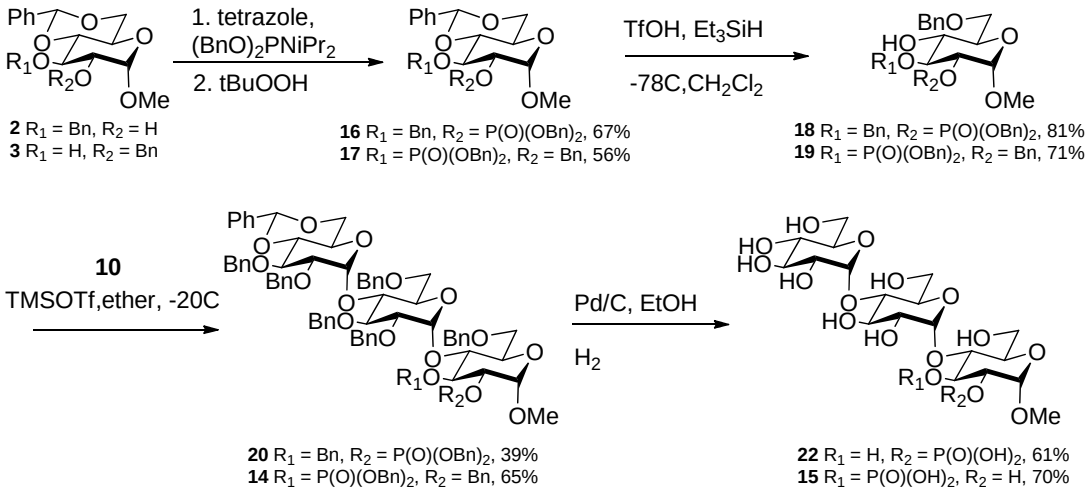


Scheme 3. Early stage introduction of the fluorous phosphate protecting group.

We decided to use alcohol **3** as a model compound to test the early stage installation of the fluorous phosphate protecting group. Compound **3** was coupled with fluorous phosphoramidate followed by selective benzylidene opening to provide glycosyl acceptor **21**. However, the glycosylation between **21** and the perbenzylated donor gave an inseparable α/β mixture in low yield. In our synthesis of *Leishmania* saccharide, we also witnessed a phenomenon with change of stereochemical outcomes. These results showed that even though fluorous protecting groups and tags could provide a convenient way of purification, it could lead to subtle changes in the reaction and might render its

usage not practical (Scheme 3).

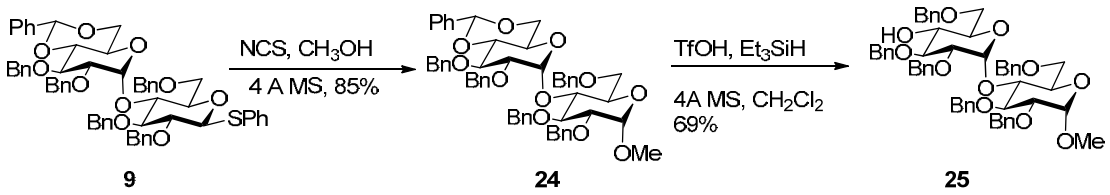
Since using benzoyl as a temporary protecting group worked well, and the early stage installation of the fluorous phosphate group leads to a mixture, we wanted to further examine the possibility of using phosphorylated acceptors directly. In this way, the protection/deprotection could be omitted by using phosphate directly as the protecting group. The free hydroxyl in compounds **2** or **3** was directly protected as dibenzyl phosphates to give **16** or **17**. The benzylidene was opened smoothly to give the phosphorylated acceptor **18** or **19**. The glycosylation reaction between donor **10** and **18** or **19** in ether at -20 °C gave a separable α/β mixture of the methyl maltotriose phosphate in the ratio of 3:1. Hydrogenolysis of **18** or **19** gave **22** or **23** respectively (Scheme 4).



Scheme 4. Synthesis of methyl maltotrioside phosphates using phosphorylated acceptors.

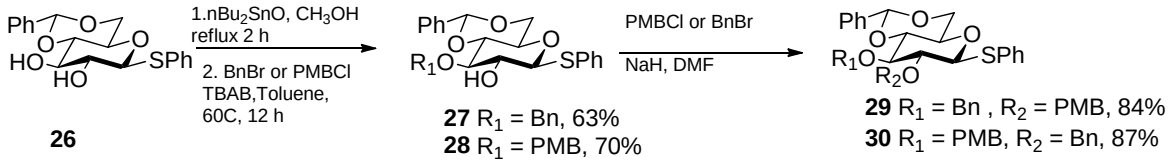
Encouraged by these results, we moved to synthesize the maltotriose with a phosphate at the

non-reducing end. To prepare the maltose acceptor, the thiophenyl group on the maltose donor **9** was converted to an α-methoxy group by reacting with methanol in the presence of *N*-chlorosuccinimide.[27,31] It was found that the formation of methyl glycoside **24** required the presence of molecular sieves, otherwise large amounts of side product, presumably NHS-substituted maltoside, would be formed. We also found out that using freshly recrystallized NCS would slow the reaction down; the un-recrystallized NCS gave much better results. Selective opening of the benzylidene group using TfOH/Et_3SiH afforded the acceptor **25** with a free hydroxyl group at the 4-position (Scheme 5).



Scheme 5. Preparation of the methyl maltoside acceptor.

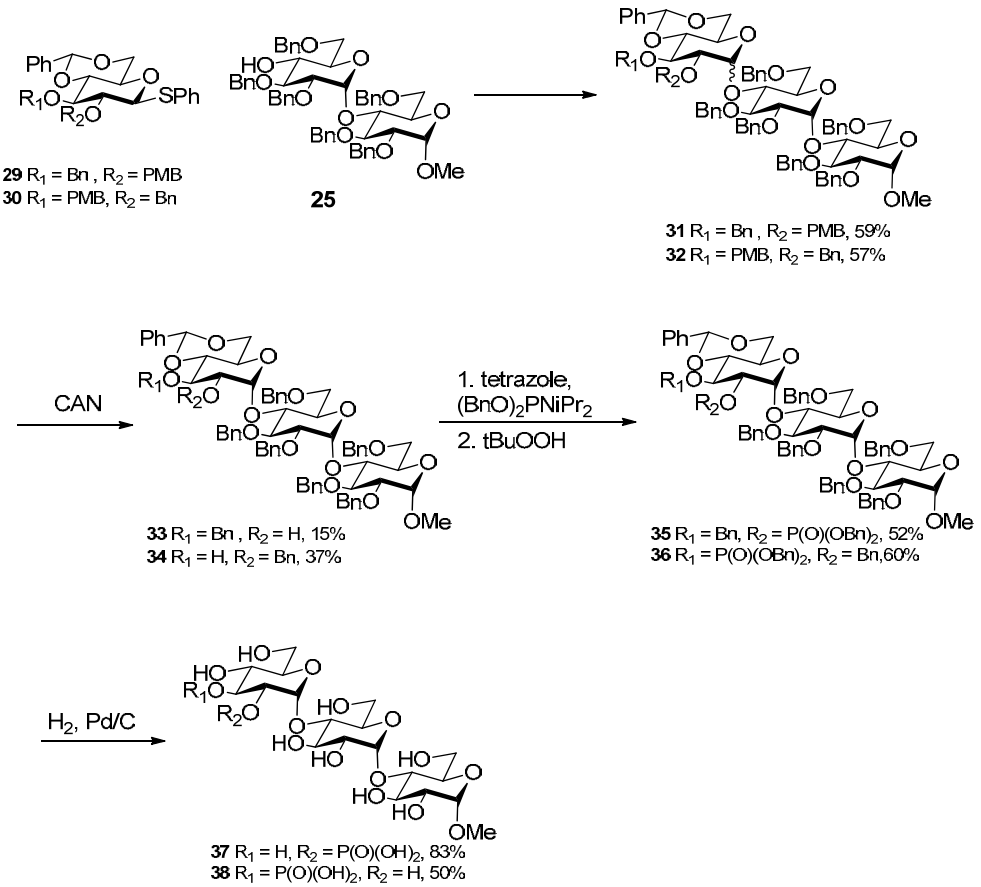
To obtain the desired α-linkage, a PMB-substituted donor were used. The PMB-protected glucose donor was synthesized from the 4,6-*O*-benzylidene-protected thioglycoside **26**. Tin-mediated selective benzylation afforded 3-OBn substituted product **27** or the 3-PMB substituted product **28**.[32] Then the 2-position was protected using PMB or Bn groups respectively to give the donors **29** or **30** in the desired protecting group pattern (Scheme 6).



Scheme 6. Synthesis of the glucose donor.

Glycosylation using the thioglycoside donor **29** and maltose acceptor **25** was studied under different conditions. $Tf_2O/PhS_2O/TTBP$ at -78 °C failed to give the desired glycosylation product **31**, and using NIS/TfOH as the promoter at -45 °C led to an inseparable mixture of α/β products in the ratio of 2:1. Changing the donor from thioglycoside to imidate did not improve the stereoselectivity of the glycosylation reaction at all. Fortunately the mixture became separable after the cleavage of the PMB group. It was found that the deprotection of PMB on **31** using CAN gave a much cleaner reaction than using DDQ; however, if the reaction using CAN took too long, the acidic conditions could cleave the benzylidene. The desired α product **33** was phosphorylated to give **35**, and hydrogenolysis gave the final product **37**. Compound **38** was synthesized via the same route (Scheme 7).

For the maltotriose with phosphates on the middle saccharide, we surveyed the methods that could potentially lead to selective protection and differentiation of hydroxyls on maltose, in the hope of finding an easier way of installing the phosphates. However, the selective protection of hydroxyls on disaccharides is far from developed, so we decided to synthesize the desired maltotriose via two glycosylation reactions.

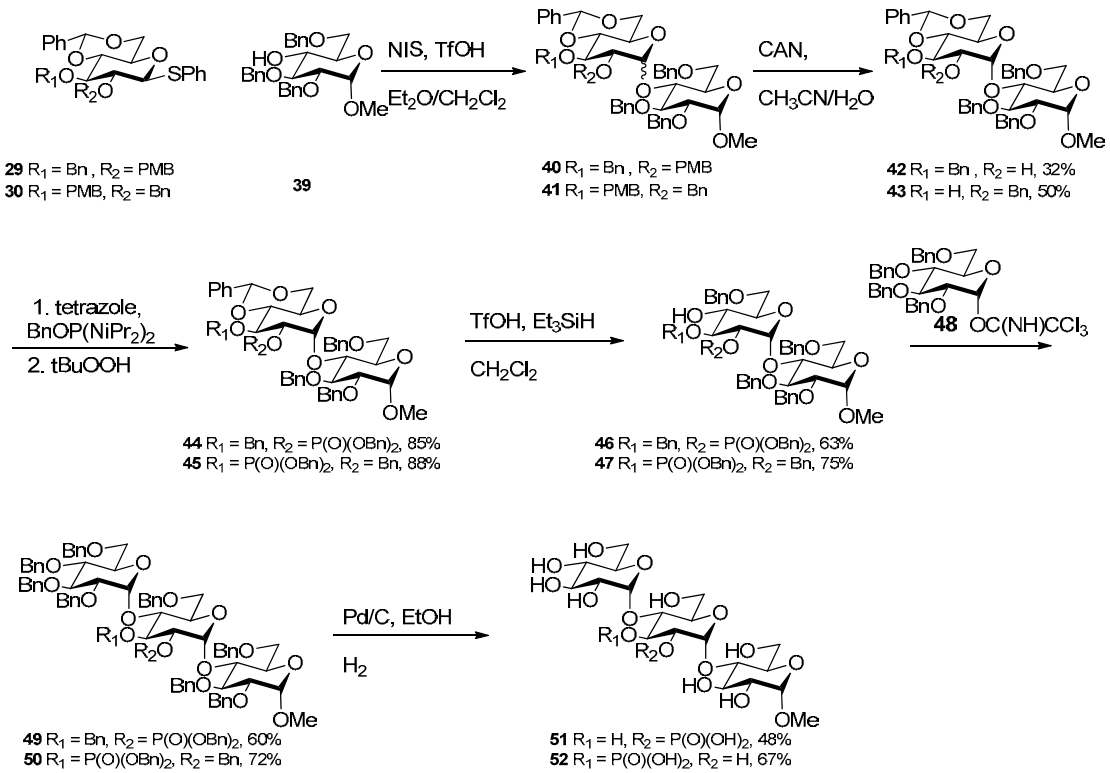


Scheme 7. Synthesis of methyl maltotrioside 2'' and 3''-phosphates.

The glycosylation between the donor **29** with a PMB group at the 2-position and acceptor **39** gave an inseparable mixture of α/β stereoisomers in the ratio of 3:1. As in the case of the previous trisaccharide, the mixture became separable after PMB cleavage. The free hydroxyl in **42** was phosphorylated to give **44**, followed by selective benzylidene acetal opening to give the disaccharide acceptor **46**. The glycosylation between **46** and donor **48** in ether gave the desired methyl maltotrioside 2'-phosphate **49**, followed by hydrogenolysis to give **51**. The methyl maltotrioside 3'-phosphate **52** was synthesized in a similar straightforward fashion to successfully provide the last

of the desired phosphate regioisomers (Scheme 8.)

We have reported the first fluorous phosphate protecting group previously[13] and would like to evaluate if this fluorous protecting group could be used in the synthesis of maltose phosphate isomers to facilitate the synthesis. In the studies of *Leishmina* carbohydrates, we have reported the synthesis of a phosphorylated disaccharide utilizing our fluorous phosphate protecting group. When we were trying to expand the study, an interesting result caught our attention. In the glycosylation coupling between the donor and a per-acetylated galactose donor, a mixture of α/β isomers was formed instead of the desired β product, which was not readily separable from the mixture. Interestingly, most of the previously reported fluorous assisted synthesis focused on fairly straightforward reactions, so the formation of a mixture with a fluorous tag is minimized. For example. in our automated solution-phase synthesis of oligosaccharides,[33,34] the fluorous tag is attached at the anomeric position of the reducing end, so the tag remains far away from the reaction site.



Scheme 8 Synthesis of methyl maltotrioside 2' and 3' phosphate

Conclusion

A series of methyl maltotrioside phosphate regioisomers were synthesized via a modular synthesis. The formation of an α-glucosidic bond is still a significant challenge, as shown in the synthesis of maltotriose. Different protecting groups and glycosylations could all alter the outcome of the glycosylations. Although fluorous tags and protecting groups can provide a convenient method of purification, this work demonstrates that the tag can introduce some subtle changes in reactions at nearby sites and provide little advantage when introduced at a late stage of a synthesis. Future studies

will use these new methyl maltotrioside phosphate isomers to probe the activity of the Laforin enzyme and thereby determine the most likely in vivo substrate for this enzyme.

Experiment section

General Experimental Methods: Reactions were performed using flame-dried glassware under argon using anhydrous solvents unless otherwise noted. Thin layer chromatography (TLC) was performed using glass-backed silica gel plates w/UV254. Visualization of TLC plates was performed by UV light and 5% sulfuric acid/ethanol. NMR spectra were recorded on a 400 MHz for ^{1}H (101 MHz for ^{13}C, 162 MHz for ^{31}P) spectrometer or on a 600 MHz for ^{1}H (150 MHz for ^{13}C, 243 MHz for ^{31}P). ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ spectra were referenced to the solvent peak at 7.260 ppm (^{1}H) and 77.0 ppm (^{13}C). ^{31}P NMR was not referenced. High resolution mass spectra (HRMS, ESI mode) were obtained using a Q-TOF LC/MS.

General Procedure for Selective Benzylidene Opening Reaction: The substrate (1 eq) was dissolved in CH_2Cl_2 (0.1 M), and ASW-3000 (powder, 100mg/mol) was added. The reaction was cooled to -78 °C, and Et_3SiH (3 eq) was added followed by TfOH (1.5 eq). The reaction was stirred at -78 °C until TLC indicated the conversion had finished. Methanol and Et_3N were added, and the reaction was filtered, concentrated, and subject to SGC for purification.

General Procedure for Thioglycoside Removal: The substrate (1 eq) was dissolved in Acetone/water (9:1, 0.1 M). NBS (2 eq) was added and the reaction was stirred until TLC indicated the conversion had finished. The reaction was quenched with adding solid $NaHCO_3$, concentrated, diluted with EtOAc, washed with $Na_2S_2O_3$, $NaHCO_3$, brine, dried and concentrated. The crude mixture was purified via SGC.

General Procedure for PMB Removal: The substrate (1 eq) was dissolved in CH_3CN/H_2O (9:1 0.05 M), and CAN (4 eq) was added. The reaction was stirred at r.t. until TLC indicated the conversion had finished. The reaction was diluted with EtOAc, washed with water, $NaHCO_3$ (aq), dried, concentrated and purified via SGC.

General Procedure for Glycosylation Using Thioglycoside donor: The acceptor (1 eq) and donor (1.5 eq) was co-evaporated with toluene for 3 times and dissolved in Et_2O/CH_2Cl_2 (3:1, 0.05 M). ASW-3000 (powder, 100 mg/mmol) was added, and the reaction was cooled to -78 °C. NIS (1.8 eq) was added, and the reaction was stirred at -78 °C for 20 min then warmed up to -45 C. TfOH(1.8 eq) was added and the reaction was stirred at -45 °C until TLC indicated the conversion had finished. The reaction was quenched by adding Et_3N, concentrated, and purified via SGC.

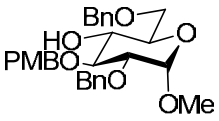
General Procedure for Trichloroacetimidate Formation: The substrate (1 eq) was dissolved in CH_2Cl_2 (0.1 M), Cs_2CO_3 (0.5 eq) was added, followed by CCl_3CN (3 eq). The reaction was stirred at

r.t. until TLC indicated the conversion had finished. The reaction was filtered through Celite, and concentrated to give the crude imidate.

General Procedure for Glycosylation Using Trichloroacetimidate Donor: The acceptor (1 eq) and donor (1.5 eq) was co-evaporated with toluene for 3 times and dissolved in Et_2O (0.05 M). The reaction was cooled to -20 °C, and TMSOTf (0.1 eq, 0.0268 M in CH_2Cl_2) was added and the reaction was stirred at -20 °C until TLC indicated the conversion had finished. The reaction was quenched by adding Et_3N, concentrated, and purified via SGC.

General Procedure for Phosphorylation: The substrate (1 eq) was dissolved in CH_2Cl_2 (0.1 M). Dibenzyl *N*,*N*-di isopropylphosphoramidite (2 eq) was added followed by 1-H tetrazole (3 eq, 0.45M in CH_3CN). The reaction was stirred at r.t. until TLC indicated the conversion had finished. *t*-BuOOH (5 eq, 5 – 6 M in decane) was added, and the reaction was stirred for 1 h. The mixture was concentrated, and purified via SGC.

General Procedure for Global Deprotection: The substrate was dissolved in EtOH, and Pd/C was added. The reaction was stirred under 800 psi of H_2 for 8 hour, filtered, and concentrated to give the product.

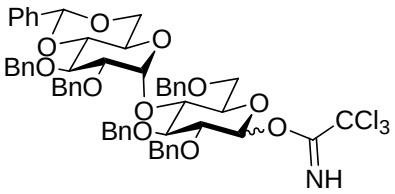


Methyl 2, 6 di- *O*-benzyl-3- *O*-(4-methoxybenzyl)- D-glucopyranoside (7) 5 (101 mg, 0.203 mmol) was dissolved in CH_2Cl_2 (2 mL) at 0 C. DIBAL-H in CH_2Cl_2 (0.608 mmol) was added, and the reaction was stirred for 8 hours. The reaction was purified via SGC (hexanes/EtOAc 2:1) to give **7** (26 mg, 0.053 mmol, 20%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 – 7.91 (m, 14H), 7.72 – 7.51 (m, 2H), 5.69 (d, *J* = 11.11 Hz, 1H), 5.54 (d, *J* = 12.11 Hz, 1H), 5.46 – 5.36 (m, 3H), 5.36 – 5.24 (m, 2H), 4.56 (s, 3H), 4.53 – 4.45 (m, 1H), 4.43 (q, *J* = 1.31, 2.06 Hz, 2H), 4.38 – 4.21 (m, 2H), 4.14 (d, *J* = 2.67 Hz, 3H), 3.07 (d, *J* = 2.36 Hz, 1H).

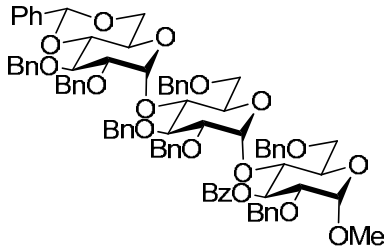
^{13}C NMR (101 MHz, $CDCl_3$) δ 159.33, 138.08, 138.01, 130.91, 129.67, 128.58, 128.46, 128.33, 128.30, 128.15, 128.10, 127.93, 127.59, 126.32, 114.00, 113.93, 98.19, 81.09, 79.59, 79.57, 75.04, 73.55, 73.15, 70.60, 69.92, 69.44, 55.26, 55.23.

HRMS(ESI): calcd for $C_{29}H_{34}O_7Na$ $[M+Na]^+$:517.2197, found 517.2194



Trichloroacetimido 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl -(1→4)- 2,3,6-tri-*O*-benzyl-D- glucopyranoside (10) Compound **9** (500mg, 0.513mmol) was subjected to the conditions in the general method for thiolphenol removal to give the hemiacetal (320mg, 0.367mmol). The

hemiacetal was treated with CCl_3CN and Cs_2CO_3 as described in the general procedure for trichloroacetimidate formation to give a yellow foam (350 mg, 0.34 mmol, 67% for two steps) and used without further purification.



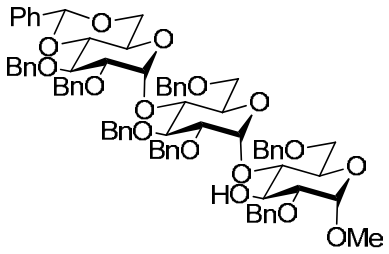
Methyl 2,3-di-*O*-benzyl--4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)- 2,3,6-tri-*O*- benzyl-α-D -glucopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-benzoyl-α- D-glucopyranoside (12) Donor **9** (98 mg, 0.98 mmol) and acceptor **11** (28 mg, 0.058 mmol) was glycosylated using the general glycosylation method for thioglycoside and purified via SGC (hexanes/EtOAc 3:1) to give **12** (35 mg, 0.026 mmol, 45%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 7.94 (m, 2H), 7.54 – 7.00 (m, 43H), 5.88 (t, *J* = 9.58 Hz, 1H), 5.73 (d, *J* = 3.87 Hz, 1H), 5.53 (s, 1H), 4.92 (d, *J* = 3.24 Hz, 1H), 4.87 (d, *J* = 11.20 Hz, 1H), 4.81 – 4.75 (m, 2H), 4.75 – 4.63 (m, 2H), 4.63 – 4.53 (m, 6H), 4.49 (d, *J* = 11.79 Hz, 1H), 4.42 (dd, *J* = 5.65, 12.06 Hz, 2H), 4.19 (d, *J* = 12.25 Hz, 1H), 4.16 – 4.05 (m, 3H), 4.05 – 3.90 (m, 5H), 3.91 – 3.76 (m, 3H), 3.71 (dd, *J* = 3.54, 9.98 Hz, 2H), 3.67 – 3.55 (m, 3H), 3.55 – 3.46 (m, 2H), 3.43 (s, 3H), 3.28 (h, *J* = 5.07 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.50, 138.91, 138.70, 138.14, 137.94, 137.87, 137.86, 137.71, 137.58, 132.69, 130.69, 129.83, 128.81, 128.38, 128.35, 128.31, 128.27, 128.24, 128.23, 128.20,

128.16, 128.15, 128.14, 127.98, 127.96, 127.85, 127.80, 127.79, 127.67, 127.61, 127.59, 127.52, 127.42, 127.38, 126.97, 126.53, 126.05, 101.12, 97.81, 97.69, 96.92, 82.18, 81.26, 79.71, 78.90, 78.67, 76.89, 75.51, 75.23, 73.79, 73.53, 73.44, 73.29, 73.23, 72.77, 72.41, 71.25, 70.55, 69.86, 68.71, 63.20, 55.29.

HRMS (ESI) calcd for $C_{82}H_{84}O_{17}Na$ $[M+Na]^+$:1363.5601, found 1363.5561



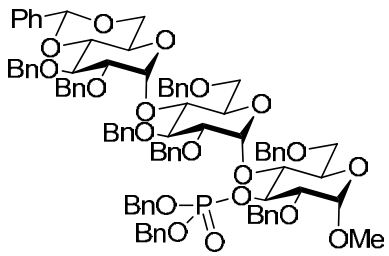
Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)- 2,3,6-tri-*O*- benzyl-α-D-glucopyranosyl-(1→4) -2,6-tri-*O*-benzyl-α-D-glucopyranoside (13) Compound **12** (23 mg, 0.017 mmol) was dissolved in CH_3OH (3 mL). $CsOH\text{-}H_2O$ (50 mg) was added, followed by Na (23 mg). The reaction was stirred for 16 h, concentrated, extracted with EtOAc, and purified with SGC (hexanes/EtOAc 3:1) to give **13** (15 mg, 0.012 mmol, 71%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.04 (m, 40H), 5.69 (d, *J* = 3.83 Hz, 1H), 5.55 (s, 1H), 5.00 – 4.79 (m, 5H), 4.79 – 4.66 (m, 4H), 4.66 – 4.52 (m, 5H), 4.51 – 4.41 (m, 2H), 4.35 (d, *J* = 11.79 Hz, 1H), 4.15 (dq, *J* = 5.23, 5.88, 10.96 Hz, 2H), 4.12 – 4.03 (m, 2H), 4.00 (t, *J* = 9.28 Hz, 2H), 3.83 (td, *J* = 4.50, 9.79 Hz, 1H), 3.78 – 3.42 (m, 10H), 3.37 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.62, 138.58, 138.15, 137.88, 137.74, 137.56, 136.80, 128.88, 128.83, 128.56, 128.43, 128.38, 128.36, 128.32, 128.30, 128.21, 128.18, 128.00, 127.93, 127.81,

127.77, 127.74, 127.70, 127.62, 127.58, 127.54, 127.23, 126.50, 126.05, 101.19, 100.39, 98.36, 97.62, 82.34, 82.14, 82.07, 80.28, 78.74, 78.70, 78.02, 77.26, 75.28, 74.31, 74.26, 74.02, 73.40, 73.35, 73.15, 71.90, 70.77, 68.96, 68.77, 68.58, 63.40, 55.23, 29.73, 29.69, 22.73, 14.17.

HRMS (ESI) calcd for $C_{75}H_{80}O_{16}Na$ $[M+Na]^+$:1259.5339, found 1559.5326



Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl- (1→4)- 2,3,6-tri-*O*- benzyl-α-D-glucopyranosyl-(1→4) -2,6-di-*O*-benzyl-3-dibenzylphosphate- α-D- glucopyranoside (14)

Compound **13**(15 mg, 0.012 mmol) was subjected to the general procedure for phosphorylation described above and purified via SGC (hexanes/EtOAc 3:2) to give **14** (9.3 mg, 0.006 mmol, 52%).

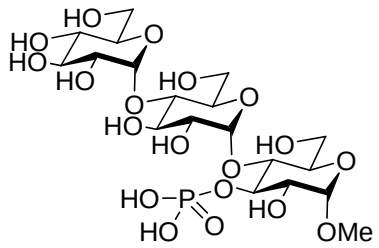
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 6.97 (m, 50H), 5.86 (d, *J* = 3.45 Hz, 1H), 5.76 (d, *J* = 3.89 Hz, 1H), 5.53 (s, 1H), 5.05 – 4.76 (m, 9H), 4.70 (s, 2H), 4.63 (td, *J* = 7.03, 11.35 Hz, 4H), 4.58 – 4.47 (m, 5H), 4.43 (d, *J* = 12.19 Hz, 2H), 4.24 (t, *J* = 9.54 Hz, 2H), 4.17 (t, *J* = 9.07 Hz, 1H), 4.10 (dd, *J* = 4.68, 10.17 Hz, 1H), 4.01 (t, *J* = 8.84 Hz, 1H), 3.93 (t, *J* = 9.27 Hz, 1H), 3.90 – 3.74 (m, 4H), 3.72 – 3.56 (m, 5H), 3.50 (ddt, *J* = 3.28, 6.35, 10.07 Hz, 3H), 3.28 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.83, 138.66, 138.33, 138.21, 137.76, 137.64, 137.52, 135.98, 128.79, 128.55, 128.51, 128.48, 128.44, 128.38, 128.34, 128.30, 128.26, 128.23, 128.20, 128.18,

128.16, 128.13, 128.09, 128.03, 127.90, 127.76, 127.73, 127.64, 127.56, 127.49, 127.47, 127.45, 127.40, 127.36, 127.29, 127.09, 126.97, 126.73, 126.06, 101.11, 97.44, 96.80, 93.79, 82.25, 81.88, 81.80, 81.31, 79.81, 79.12, 78.69, 78.65, 75.02, 73.82, 73.52, 73.29, 73.02, 72.34, 71.14, 70.40, 69.85, 69.82, 69.76, 69.28, 69.23, 68.93, 68.77, 68.71, 68.10, 65.37, 63.14, 55.11, 29.70.

^{31}P NMR (162 MHz, $CDCl_3$) δ -2.96.

HRMS (ESI) calcd for $C_{89}H_{93}O_{19}PNa$ $[M+Na]^+$:1519.5914, found 1519.5949



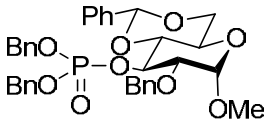
Methyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)- 3-phosphate-α-D- glucopyra noside (15) Hydrogenolysis of **14** (12 mg, 0.007 mmol) according to the general method for global deprotection gave **15** (3 mg, 0.005 mmol, 71%)

^{1}H NMR (400 MHz, CD_3OD) δ 5.44 (d, *J* = 3.4 Hz, 1H), 5.15 (d, *J* = 3.7 Hz,1H), 4.69 (d, *J* = 4.4 Hz,1H), 3.82 (d, *J* = 11.41 Hz, 5H), 3.65 (dq, *J* = 8.78, 11.26, 20.37 Hz, 5H), 3.52 (d, *J* = 8.68 Hz, 1H), 3.42 (d, *J* = 18.32 Hz, 4H), 3.30 (d, *J* = 2.99 Hz, 12H).

^{13}C NMR (101 MHz, CD_3OD) δ 101.52, 99.43, 99.14, 79.92, 78.24, 75.29, 73.65, 73.35, 72.87, 71.82, 70.65, 70.07, 61.30, 60.76, 60.67, 54.11, 46.42, 29.33.

^{31}P NMR (162 MHz, CD_3OD) δ 1.57.

HRMS (ESI) calcd for $C_{19}H_{34}O_{19}P$ $[M-H]^-$:597.1437, found 597.1447



Methyl 2-*O*-benzyl-3-dibenzylphosphate-4,6-*O*-benzylidene-α-D-glucopyranoside (17)

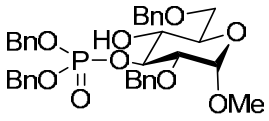
Compound **3** (220 mg, 0.59 mmol) was subjected to the general procedure for phosphorylation described above and purified via SGC (hexanes/EtOAc 3:2) to give **17** (210 mg, 0.33 mmol, 56%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 – 6.95 (m, 20H), 5.52 (s, 1H), 4.98 (dddd, *J* = 7.30, 12.17, 26.00, 44.62 Hz, 5H), 4.80 (d, *J* = 12.19 Hz, 1H), 4.73 – 4.55 (m, 2H), 4.30 (dd, *J* = 4.86, 10.30 Hz, 1H), 3.90 (td, *J* = 4.79, 9.89 Hz, 1H), 3.83 – 3.62 (m, 3H), 3.40 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 137.82, 136.90, 136.25, 136.21, 136.20, 129.18, 128.48, 128.35, 128.31, 128.28, 128.26, 128.13, 128.08, 128.00, 127.97, 127.55, 127.40, 126.42, 101.98, 98.99, 80.21, 80.19, 78.38, 78.35, 76.92, 76.88, 73.36, 69.08, 69.05, 69.04, 69.00, 68.96, 62.22, 55.44.

^{31}P NMR (243 MHz, $CDCl_3$) δ 15.82.

HRMS (ESI) calcd for $C_{35}H_{38}O_9PNa$ $[M+H]^+$:633.2248, found 633.2257



Methyl 2-,6-di-*O*-benzyl-3-dibenzylphosphate-α-D-glucopyranoside (19) Compound **17** (91 mg, 0.143 mmol) was subjected to the conditions described in the general procedure for selective benzylidene opening and purified via SGC (hexanes/EtOAc 3:2) to give **19** (64 mg, 0.101 mmol, 71%).

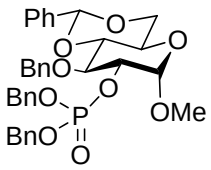
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35 (s, 20H), 5.25 – 5.00 (m, 2H), 4.81 – 4.69 (m, 3H), 4.69 – 4.57

(m, 3H), 3.88 – 3.72 (m, 4H), 3.59 (ddd, *J* = 4.03, 10.10, 13.15 Hz, 1H), 3.40 (d, *J* = 12.26 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.20, 137.73, 135.61, 135.57, 128.73, 128.71, 128.63, 128.61, 128.59, 128.54, 128.49, 128.47, 128.36, 128.32, 128.11, 128.05, 128.02, 127.97, 127.94, 127.65, 127.62, 127.59, 97.78, 81.95, 81.91, 73.63, 73.22, 70.26, 70.03, 69.99, 69.92, 69.79, 69.75, 68.91, 55.23.

^{31}P NMR (243 MHz, $CDCl_3$) δ 18.83.

HRMS (ESI) calcd for $C_{35}H_{40}O_9P$ $[M+H]^+$:635.2404, found 635.2411



Methyl 2-dibenzylphosphate-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (16)

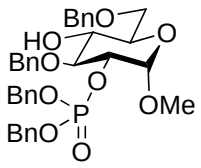
Compound **2** (76 mg, 0.20 mmol) was subjected to the general procedure for phosphorylation described above to and purified via SGC (hexanes/EtOAc 2:1) give **16** (85 mg, 0.134 mmol, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.14 (m, 20H), 5.57 (s, 1H), 5.04 (d, *J* = 7.51 Hz, 2H), 5.02 – 4.94 (m, 3H), 4.91 (d, *J* = 11.35 Hz, 1H), 4.73 (d, *J* = 11.32 Hz, 1H), 4.41 (ddd, *J* = 3.81, 7.70, 9.40 Hz, 1H), 4.30 (dd, *J* = 4.66, 10.12 Hz, 1H), 4.06 (t, *J* = 9.28 Hz, 1H), 3.87 (td, *J* = 4.66, 9.90 Hz, 1H), 3.76 (t, *J* = 10.25 Hz, 1H), 3.67 (t, *J* = 9.34 Hz, 1H), 3.37 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.25, 137.19, 135.66, 128.97, 128.52, 128.47, 128.45, 128.33, 128.23, 128.21, 127.89, 127.84, 127.67, 127.55, 125.99, 101.33, 98.61, 82.03, 82.01, 74.98, 69.37, 69.32, 69.27, 69.22, 68.94, 62.24, 55.44.

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.77.

HRMS (ESI) calcd for $C_{35}H_{38}O_9P$ $[M+H]^+$:633.2248, found 633.2244



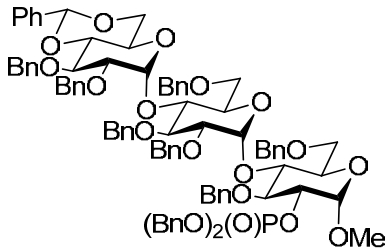
Methyl 2-dibenzylphosphate-3,6-di-*O*-benzyl- -α-D-glucopyranoside (18) Compound **16** (104 mg, 0.165 mmol) was subjected to the conditions described in the general procedure for selective benzylidene opening and purified via SGC (hexanes/EtOAc 2:1) to give **18** (84 mg, 0.133 mmol, 81%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.16 (m, 20H), 5.05 (d, *J* = 7.64 Hz, 2H), 5.03 – 4.96 (m, 3H), 4.87 (d, *J* = 11.45 Hz, 1H), 4.71 (d, *J* = 11.37 Hz, 1H), 4.64 – 4.52 (m, 2H), 4.36 (ddd, *J* = 3.67, 7.22, 9.60 Hz, 1H), 3.84 (t, *J* = 9.14 Hz, 1H), 3.80 – 3.61 (m, 4H), 3.35 (s, 3H), 2.56 (d, *J* = 2.75 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.40, 137.88, 135.77, 135.70, 135.63, 128.56, 128.52, 128.51, 128.49, 128.45, 128.43, 128.40, 127.91, 127.88, 127.80, 127.78, 127.71, 127.64, 97.94, 80.15, 80.08, 76.99, 75.19, 73.63, 71.25, 71.23, 69.79, 69.60, 69.41, 69.36, 69.28, 69.22, 55.32.

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.59.

HRMS (ESI) calcd for $C_{35}H_{39}O_9PNa$ $[M+Na]^+$:657.2224, found 657.2209



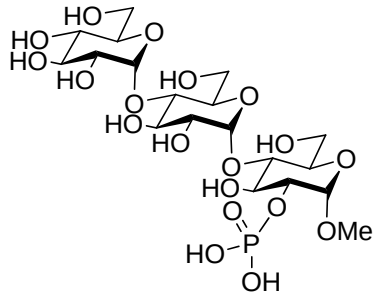
Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl- (1→4)- 2,3,6-tri-*O*- benzyl-α-D-glucopyranosyl-(1→4)-3-dibenzylphosphate- ,6-di-*O*-benzyl-α-D-glucopyranoside (20) Donor **10** (72 mg, 0.071 mmol) and acceptor **18** (30 mg, 0.047 mmol) were subjected to the conditions for general method of glycosylation and purified via SGC (hexanes/EtOAc 2:1) to give **20** (27 mg, 0.018 mmol, 39%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 6.85 (m, 50H), 5.72 – 5.61 (m, 1H), 5.45 – 5.35 (m, 1H), 4.95 (td, *J* = 4.00, 9.37, 9.82 Hz, 4H), 4.83 (tq, *J* = 4.36, 5.86, 11.30 Hz, 5H), 4.73 – 4.57 (m, 3H), 4.57 – 4.33 (m, 7H), 5.56 – 5.45 (m, 1H), 4.16 – 3.84 (m, 7H), 3.78 (dd, *J* = 8.78, 14.01 Hz, 2H), 3.72 – 3.51 (m, 5H), 3.53 – 3.39 (m, 3H), 3.31 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.25, 138.13, 137.97, 137.93, 137.65, 137.56, 137.54, 137.16, 137.07, 135.27, 135.20, 135.13, 135.05, 128.38, 128.05, 128.03, 127.98, 127.96, 127.86, 127.83, 127.79, 127.76, 127.70, 127.64, 127.55, 127.42, 127.39, 127.35, 127.33, 127.26, 127.23, 127.21, 127.18, 127.15, 127.11, 127.09, 127.07, 127.02, 126.98, 126.95, 126.71, 126.68, 126.62, 126.35, 126.23, 126.11, 125.56, 100.66, 97.26, 97.05, 96.82, 96.28, 91.37, 81.78, 81.23, 80.16, 80.08, 79.87, 79.79, 79.11, 78.42, 78.17, 74.65, 73.55, 73.51, 73.29, 73.01, 72.95, 72.86, 72.71, 72.55, 71.53, 70.87, 70.49, 70.09, 69.41, 68.88, 68.83, 68.79, 68.73, 68.68, 68.46, 68.18, 68.01, 62.74, 54.80, 54.67.

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.74.

HRMS (ESI) calcd for $C_{89}H_{93}O_{19}PNa$ $[M+Na]^+$:1519.5914, found 1519.5934



Methyl α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-2-phosphate-α-D-gluco pyranoside (22)

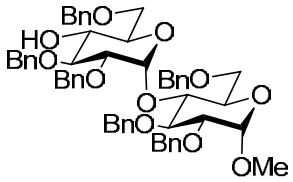
Hydrogenation of **20** (5 mg, 0.003 mmol) according to the general method for global deprotection gave **15** (1 mg, 0.002 mmol, 61%).

^{1}H NMR (400 MHz, CD_3OD) δ 5.15 (s, 1H), 5.11 (d, *J* = 3.14 Hz, 1H), 4.63 – 4.55 (m, 1H), 4.00 (s, 2H), 3.77 (h, *J* = 9.54, 10.19 Hz, 7H), 3.70 – 3.51 (m, 5H), 3.45 (q, *J* = 10.22, 10.70 Hz, 2H), 3.37 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 101.45, 101.12, 97.89, 79.84, 79.48, 73.63, 73.49, 73.34, 72.81, 72.41, 71.99, 71.93, 70.58, 70.21, 70.07, 61.29, 60.68, 60.50, 54.34, 54.21.

^{31}P NMR (162 MHz, CD_3OD) δ -0.43.

HRMS (ESI) calcd for $C_{19}H_{34}O_{19}P$ $[M-H]^-$: 597.1437, found 597.1433

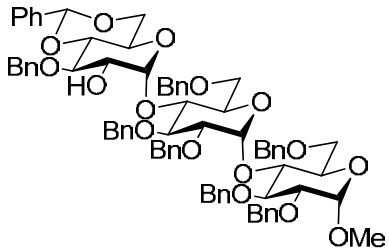


Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl -(1→4)- 2,3,6-tri-O-benzyl-D- glucopyranoside (25) Compound **24** (59 mg, 0.066mmol) was subjected to the conditions according to the general procedure for selective benzylidene opening to and purified via SGC (hexanes/EtOAc 3:1) afford **25** (41 mg, 0.046 mmol, 69%) as white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.06 (m, 30H), 5.72 (d, *J* = 3.59 Hz, 1H), 5.07 (d, *J* = 11.62 Hz, 1H), 4.91 (d, *J* = 11.29 Hz, 1H), 4.82 (d, *J* = 11.70 Hz, 1H), 4.77 – 4.68 (m, 2H), 4.66 – 4.41 (m, 7H), 4.35 (d, *J* = 12.09 Hz, 1H), 4.10 (d, *J* = 8.80 Hz, 2H), 3.96 – 3.81 (m, 2H), 3.81 – 3.42 (m, 8H), 3.40 (s, 3H), 2.52 (d, *J* = 2.43 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.93, 138.78, 138.22, 137.95, 137.91, 137.87, 128.47, 128.44, 128.34, 128.32, 128.25, 128.21, 127.94, 127.87, 127.73, 127.71, 127.66, 127.64, 127.39, 127.33, 127.12, 126.72, 97.74, 96.55, 82.05, 81.27, 80.19, 78.99, 75.32, 74.39, 73.54, 73.35, 73.17, 73.08, 72.27, 71.46, 70.53, 69.76, 69.53, 69.02, 55.18.

HRMS(ESI): calcd for $C_{55}H_{61}O_{11}$ $[M+H]^+$: 897.4206, found 897.4208



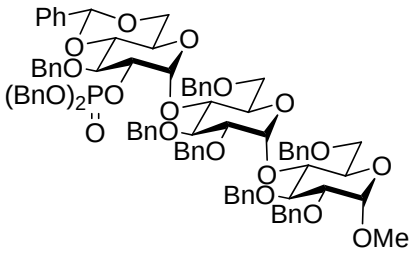
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)- 2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4) -2 ,3,6-tri-*O*-benzyl-D-glucopyranoside (33)

Donor **29** (19 mg, 0.033 mmol) and acceptor **25** (22 mg, 0.023 mmol) was glycosylated using the general glycosylation method for thiolglycoside to give a 3:1 alpha/beta mixture of **31**(17.5 mg, 0.013 mmol)**. 31** was treated with DDQ according to the conditions in the general methods for PMB removal, and purified by SGC (hexanes/ethyl acetate 3:1) to give **33** (6 mg, 0.005 mmol, 15% for two steps)**.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 6.95 (m, 40H), 5.69 (dd, *J* = 3.59, 9.73 Hz, 1H), 5.54 (s, 1H), 5.19 (d, *J* = 3.53 Hz, 1H), 5.06 (dd, *J* = 11.33, 22.77 Hz, 2H), 4.84 – 4.66 (m, 5H), 4.66 – 4.31 (m, 11H), 4.08 (dq, *J* = 3.79, 6.48, 11.88 Hz, 4H), 4.00 – 3.81 (m, 6H), 3.80 – 3.42 (m, 11H), 3.39 (d, *J* = 4.84 Hz, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 137.84, 137.64, 136.99, 136.89, 136.87, 136.64, 136.39, 136.35, 127.86, 127.43, 127.40, 127.34, 127.31, 127.28, 127.26, 127.24, 127.21, 127.16, 127.01, 126.93, 126.83, 126.78, 126.75, 126.73, 126.69, 126.62, 126.53, 126.49, 126.42, 126.38, 126.29, 126.12, 125.68, 125.60, 124.97, 100.11, 100.01, 96.75, 95.02, 80.91, 80.41, 79.27, 79.17, 78.76, 78.27, 74.06, 73.64, 73.38, 72.58, 72.43, 72.31, 72.20, 71.76, 71.31, 69.99, 68.56, 67.87, 67.30, 62.76, 54.23.

HRMS (ESI) calcd for $C_{75}H_{80}O_{16}Na$ $[M+Na]^+$:1259.5339, found 1559.5337



Methyl 2-dibenzylphosphate-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)- 2,3,6-tri-*O*-benzyl-α- D-glucopyranosyl-(1→4) -2,3,6-tri-*O*-benzyl-α-D- glucopyranoside (35)

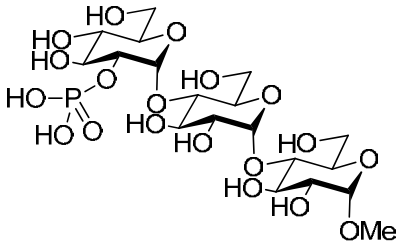
Compound **33** (13 mg, 0.011 mmol) was subjected to the general procedure for phosphorylation described above and purified by SGC (hexanes/ethyl acetate 4:1) to give **35** (8.6 mg, 0.0055 mmol, 52%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 6.98 (m, 50H), 5.82 (d, *J* = 3.69 Hz, 1H), 5.62 (d, *J* = 3.49 Hz, 1H), 5.55 (s, 1H), 5.06 – 4.97 (m, 2H), 4.88 (dqd, *J* = 5.15, 8.96, 10.19, 18.94 Hz, 6H), 4.81 – 4.63 (m, 6H), 4.63 – 4.37 (m, 11H), 4.15 (q, *J* = 7.88, 8.73 Hz, 2H), 4.09 – 3.94 (m, 5H), 3.95 – 3.75 (m, 5H), 3.74 – 3.55 (m, 4H), 3.54 – 3.39 (m, 2H), 3.37 (d, *J* = 7.73 Hz, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.89, 138.51, 138.30, 138.08, 137.97, 137.88, 137.35, 128.88, 128.55, 128.54, 128.49, 128.43, 128.42, 128.38, 128.35, 128.32, 128.25, 128.20, 128.16, 127.92, 127.84, 127.79, 127.75, 127.71, 127.68, 127.64, 127.60, 127.57, 127.53, 127.50, 127.42, 127.37, 127.31, 127.25, 127.10, 126.97, 126.73, 126.70, 126.02, 101.22, 97.77, 95.99, 82.21, 81.99, 81.04, 80.11, 79.64, 77.21, 76.83, 74.79, 74.29, 73.99, 73.41, 73.32, 73.07, 73.00, 72.13, 71.54, 70.47, 69.49, 69.28, 68.82, 68.65, 68.56, 62.98, 55.19.

^{31}P NMR (162 MHz, $CDCl_3$) δ -1.19.

HRMS (ESI) calcd for $C_{89}H_{93}O_{19}PNa$ $[M+Na]^+$:1519.5914, found 1519.5942



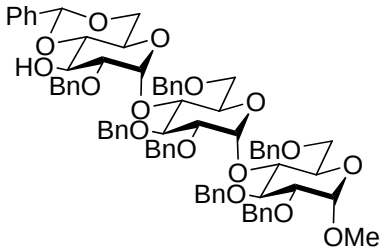
Methyl 2-phosphate-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranoside (37) Compound **35** (8.6 mg, 0.0055 mmol) was subjected to the general procedure for global deprotection described above to give **37** (2.7 mg, 0.0045 mmol, 83%).

^{1}H NMR (400 MHz, CD_3OD) δ 5.52 (s, 1H), 5.15 (s, 1H), 4.66 (s, 1H), 4.07 – 3.92 (m, 2H), 3.92 – 3.72 (m, 11H), 3.72 – 3.56 (m, 2H), 3.56 – 3.34 (m, 6H), 3.12 – 2.83 (m, 2H).

^{13}C NMR (101 MHz, CD_3OD) δ 101.11, 99.70, 98.75, 80.25, 74.79, 73.52, 73.24, 72.67, 72.26, 71.69, 70.76, 70.54, 61.39, 60.67, 54.20, 45.89, 29.35, 29.04.

^{31}P NMR (162 MHz, CD_3OD) δ 3.43.

HRMS (ESI) calcd for $C_{19}H_{34}O_{19}P$ $[M-H]^-$:597.1437, found 597.1455



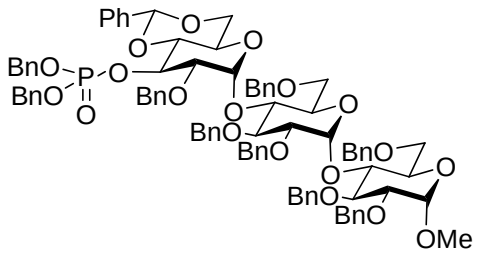
Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4) -2 ,3,6-tri-*O*-benzyl-α- D-glucopyranoside (34) Donor **30** (72 mg, 0.126 mmol) and acceptor **25** (63 mg, 0.078 mmol) was glycosylated using the general glycosylation

method for thioglycoside to give a 3:1 alpha/beta mixture of **32** (56 mg, 0.040 mmol, 52%)**. 32** (22 mg, 0.016 mmol) was treated with DDQ according to the conditions in the general methods for PMB removal, and purified by SGC (hexanes/EtOAc 3:1) to give **34** (8 mg, 0.006 mmol, 37%)**.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 6.82 (m, 40H), 5.68 (d, *J* = 3.76 Hz, 1H), 5.55 (d, *J* = 3.52 Hz, 1H), 5.41 (s, 1H), 4.98 (d, *J* = 11.65 Hz, 1H), 4.91 (d, *J* = 11.78 Hz, 1H), 4.73 (q, *J* = 9.98, 10.69 Hz, 1H), 4.68 – 4.57 (m, 2H), 4.57 – 4.45 (m, 4H), 4.45 – 4.26 (m, 5H), 4.15 – 3.89 (m, 6H), 3.90 – 3.64 (m, 5H), 3.54 (dd, *J* = 7.82, 12.39 Hz, 3H), 3.51 – 3.36 (m, 3H), 3.36 – 3.26 (m, 4H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.83, 138.28, 138.12, 138.03, 137.80, 137.64, 137.30, 129.13, 128.46, 128.44, 128.36, 128.32, 128.30, 128.28, 128.25, 128.21, 127.95, 127.89, 127.84, 127.73, 127.69, 127.48, 127.43, 127.40, 127.30, 127.15, 126.76, 126.56, 126.37, 101.85, 97.83, 96.40, 96.02, 81.95, 81.85, 81.28, 80.09, 79.74, 79.20, 74.38, 73.86, 73.38, 73.16, 72.89, 72.63, 71.39, 70.50, 70.24, 69.61, 68.92, 68.75, 68.62, 62.89, 55.23, 29.72.

HRMS (ESI) calcd for $C_{75}H_{80}O_{16}Na$ $[M+Na]^+$:1259.5339, found 1559.5332



Methyl 2-*O*-benzyl- 3-dibenzylphosphate -4,6-*O*-benzylidene-α-D-glucopyranosyl- (1→4)-2,3,6-tri-*O*-benzyl-α- D-glucopyranosyl-(1→4) -2 ,3,6-tri-*O*-benzyl- D-glucopyranoside (36) Compound **34** (12 mg, 0.01 mmol) was subjected to the general procedure for phosphorylation

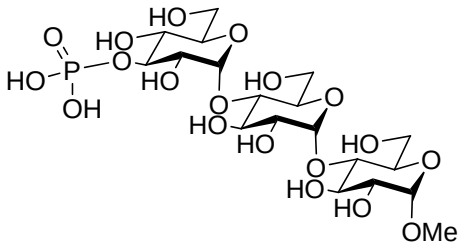
described above and purified by SGC (hexanes/EtOAc 2:1) to give **36** (8 mg, 0.006 mmol, 60 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 – 7.06 (m, 50H), 5.08 – 4.87 (m, 4H), 4.86 – 4.69 (m, 3H), 4.71 – 4.60 (m, 3H), 4.60 – 4.38 (m, 8H), 5.53 – 5.43 (m, 1H), 4.14 (td, *J* = 7.75, 12.95, 15.48 Hz, 4H), 4.07 (q, *J* = 7.79, 9.07 Hz, 1H), 3.97 – 3.77 (m, 5H), 3.66 (dt, *J* = 10.94, 16.65 Hz, 4H), 5.73 – 5.63 (m, 1H), 3.63 – 3.48 (m, 4H), 5.83 – 5.76 (m, 1H), 3.44 (d, *J* = 10.38 Hz, 8H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 139.02, 138.89, 138.30, 138.21, 138.05, 137.62, 137.13, 135.84, 135.79, 129.14, 128.72, 128.61, 128.57, 128.53, 128.49, 128.46, 128.36, 128.31, 128.28, 128.25, 128.23, 128.20, 128.08, 128.04, 127.96, 127.93, 127.88, 127.83, 127.67, 127.57, 127.49, 127.47, 127.39, 127.36, 127.32, 127.30, 127.27, 127.25, 127.13, 127.05, 126.75, 126.50, 126.46, 126.45, 101.95, 97.82, 96.82, 96.03, 82.02, 81.73, 80.13, 79.71, 78.04, 74.99, 74.36, 73.81, 73.35, 73.33, 73.07, 72.89, 72.48, 71.73, 70.41, 69.59, 69.33, 69.29, 68.98, 68.95, 68.73, 68.53, 63.03, 55.22.

^{31}P NMR (243 MHz, $CDCl_3$) δ 16.09.

HRMS (ESI) calcd for $C_{89}H_{93}O_{19}PNa$ $[M+Na]^+$:1519.5914, found 1519.5944



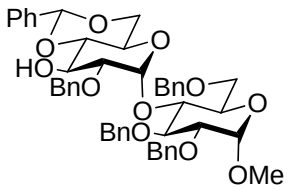
Methyl 3-phosphate-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-gluco pyranoside (38) Hydrogenation of **36** (10 mg, 0.006 mmol) according to the general method for global deprotection gave **38** (2.5 mg, 0.003 mmol, 50%).

^{1}H NMR (600 MHz, MeOD) δ5.23 (d, *J* = 3.40 Hz, 1H), 5.18 (d, *J* = 3.71 Hz, 1H), 4.70 (d, *J* = 3.69 Hz, 1H), 3.36 – 3.30 (m, 8H), 3.91 – 3.78 (m, 9H), 5.12 – 5.10 (m, 1H), 3.63 (d, *J* = 11.74 Hz, 5H), 3.53 (m, 4H)

^{13}C NMR (101 MHz, MeOD) δ 101.25, 101.18, 99.70, 89.67, 80.23, 80.14, 73.49, 73.01, 72.33, 71.82, 71.72, 70.71, 61.01, 60.67, 54.20, 52.35.

^{31}P NMR (162 MHz, MeOD) δ 1.25.

HRMS (ESI) calcd for $C_{19}H_{34}O_{19}P$ $[M-H]^-$: 597.1437, found 597.1425



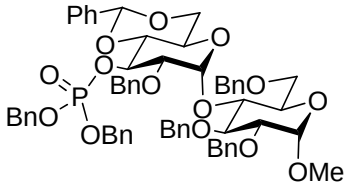
Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4) -2 ,3 ,6-tri-*O*–benzyl -α-D-glucopyranoside (43) Donor **30** (82 mg, 0.144 mmol) and acceptor **39** (38 mg, 0.082 mmol) were subjected to the conditions for general method of glycosylation to give **41** as a mixture, followed by PMB removal and purified by SGC (hexanes/EtOAc 4:1) to give **43** (33 mg, 0.041 mmol, 50%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.16 (m, 20H), 5.80 (d, *J* = 3.69 Hz, 1H), 5.50 (s, 1H), 5.12 (d, *J* = 11.73 Hz, 1H), 4.78 (d, *J* = 11.80 Hz, 1H), 4.71 (d, *J* = 12.11 Hz, 2H), 4.68 – 4.45 (m, 5H), 4.21 – 4.05 (m, 4H), 3.93 – 3.78 (m, 3H), 3.74 – 3.57 (m, 3H), 3.48 (t, *J* = 9.46 Hz, 1H), 3.44 – 3.28 (m, 4H), 2.48 (bs, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.91, 138.09, 137.87, 137.60, 137.23, 129.12, 128.50, 128.47,

128.35, 128.29, 128.26, 128.19, 127.98, 127.94, 127.92, 127.47, 127.43, 127.21, 126.67, 126.32, 101.80, 97.70, 96.51, 82.14, 81.30, 80.30, 79.06, 74.24, 73.42, 73.26, 72.97, 71.45, 70.20, 69.42, 68.89, 68.72, 62.96, 55.23.

HRMS (ESI) calcd for $C_{56}H_{55}O_4P$ $[M+H]^+$:821.3832, found 821.3834



Methyl 2-*O*-benzyl-3-dibenzylphosphate- 4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4) -2 ,3,6 -tri-*O*-benzyl-α-D-glucopyranoside (45) Compound **43** (33 mg,0.041 mmol) was subjected to the general procedure for phosphorylation described above and purified by SGC (hexanes/EtOAc 4:1) to give **45** (38 mg, 0.036 mmol, 88%).

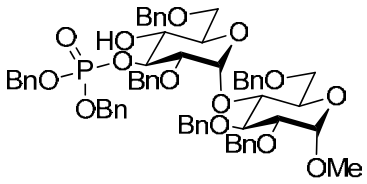
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 – 6.99 (m, 35H), 5.84 (d, *J* = 3.67 Hz, 1H), 5.50 (s, 1H), 5.18 – 5.04 (m, 8H), 5.04 – 4.91 (m, 3H), 4.86 (ddd, *J* = 7.69, 12.12, 14.64 Hz, 2H), 4.75 – 4.67 (m, 4H), 4.66 – 4.57 (m, 3H), 4.54 (d, *J* = 11.69 Hz, 2H), 4.23 – 4.11 (m, 3H), 3.94 (ddd, *J* = 9.85, 16.41, 20.21 Hz, 3H), 3.68 (dt, *J* = 10.19, 20.97 Hz, 5H), 3.61 (dd, *J* = 3.76, 9.57 Hz, 1H), 3.45 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 139.00, 138.16, 137.92, 137.46, 137.09, 136.25, 136.20, 136.12, 135.62, 135.58, 129.11, 128.74, 128.72, 128.59, 128.49, 128.32, 128.30, 128.28, 128.27, 128.23, 128.20, 128.17, 128.08, 128.06, 127.99, 127.95, 127.64, 127.55, 127.45, 127.43, 127.31, 127.14, 126.62, 126.49, 101.93, 97.73, 96.85, 82.03, 80.36, 80.23, 78.09, 74.14, 73.44, 73.27, 72.92, 71.69,

69.39, 69.06, 69.02, 68.99, 68.96, 68.86, 68.75, 67.34, 67.30, 63.20, 55.30.

^{31}P NMR (243 MHz, $CDCl_3$) δ 16.02.

HRMS (ESI) calcd for $C_{62}H_{65}O_{14}PK$ $[M+K]^+$:1103.3744, found 1103.3756



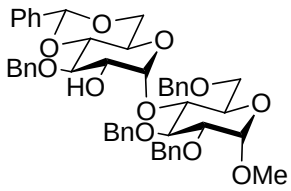
Methyl 2,6-di-*O*-benzyl-3-dibenzylphosphate-α-D-glucopyranosyl-(1→4) -2 ,3 ,6-tri-*O*-benzyl-α-D-glucopyranoside (47) Compound **45** (38 mg, 0.036 mmol) was subjected to the conditions according to the general procedure for selective benzylidene opening and purified by SGC (hexanes/EtOAc 3:2) to afford **47** (29 mg, 0.027 mmol,75 %) as syrup.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 – 7.09 (m, 35H), 5.77 (d, *J* = 3.68 Hz, 1H), 5.18 – 4.95 (m, 7H), 4.73 (dd, *J* = 11.86, 21.93 Hz, 2H), 4.68 – 4.57 (m, 4H), 4.58 – 4.44 (m, 5H), 4.12 – 4.03 (m, 2H), 3.84 (qd, *J* = 2.91, 10.86, 11.77 Hz, 3H), 3.75 (dq, *J* = 3.62, 4.30, 10.72 Hz, 1H), 3.70 – 3.58 (m, 3H), 3.51 (ddd, *J* = 3.08, 10.16, 23.29 Hz, 2H), 3.44 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 139.00, 138.22, 138.18, 137.96, 137.60, 135.60, 135.57, 135.55, 135.53, 128.73, 128.71, 128.56, 128.49, 128.45, 128.31, 128.28, 128.26, 128.21, 128.19, 128.05, 127.97, 127.95, 127.91, 127.85, 127.66, 127.62, 127.59, 127.53, 127.43, 127.40, 127.06, 126.65, 97.73, 96.48, 82.02, 81.96, 81.92, 80.22, 74.26, 73.59, 73.31, 73.22, 73.00, 72.78, 71.11, 69.89, 69.85, 69.73, 69.69, 69.51, 69.02, 68.63, 67.33, 67.30, 55.27.

^{31}P NMR (243 MHz, $CDCl_3$) δ 18.69.

HRMS (APCI) calcd for $C_{62}H_{68}O_{14}P$ $[M+H]^+$:1067.4341, found 1067.4342

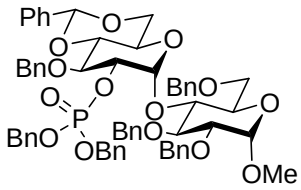


Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4) -2,3 ,6-tri-*O*-benzyl-α-D-glucopyranoside (42) Donor **29** (50 mg, 0.088 mmol) and acceptor **39** (22 mg, 0.047 mmol) were subjected to the conditions for general method of glycosylation to give **40** as a mixture, followed by PMB removal and purified by SGC (hexanes/EtOAc 4:1) to give **42** (12 mg, 0.015 mmol, 32 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.21 (m, 25H), 5.52 (s, 1H), 5.26 (d, *J* = 3.47 Hz, 1H), 5.09 (d, *J* = 10.91 Hz, 1H), 4.81 – 4.73 (m, 2H), 4.73 – 4.51 (m, 7H), 4.13 (dd, *J* = 4.84, 10.27 Hz, 1H), 3.99 (t, *J* = 9.29 Hz, 1H), 3.95 – 3.80 (m, 3H), 3.77 – 3.51 (m, 7H), 3.46 – 3.30 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 138.60, 137.83, 137.78, 137.66, 137.37, 128.88, 128.51, 128.42, 128.32, 128.20, 128.06, 127.88, 127.71, 127.62, 127.48, 126.00, 101.17, 100.92, 97.82, 81.47, 80.71, 80.27, 79.22, 77.19, 76.83, 75.33, 74.74, 73.50, 73.43, 73.19, 69.94, 68.90, 68.46, 63.73, 55.32, 29.69.

HRMS (ESI) calcd for $C_{48}H_{52}O_{11}PNa$ $[M+Na]^+$:827.3402, found 827.3402



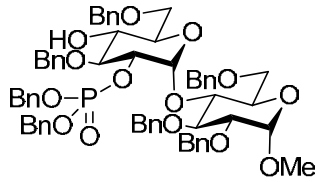
Methyl 2-dibenzylphosphate-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl-(1→4) -2 ,3 ,6-tri-*O*-benzyl-α-D-glucopyranoside (44) Compound **42** (11 mg, 0.015 mmol) was subjected to the general procedure for phosphorylation described above and purified by SGC (hexanes/EtOAc 3:1) to give **44** (13 mg, 0.013 mmol, 85%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.20 (m, 35H), 4.51 – 4.44 (m, 1H), 5.91 (d, *J* = 3.81 Hz, 1H), 5.59 (s, 1H), 5.16 – 4.84 (m, 8H), 4.80 – 4.67 (m, 3H), 4.64 (d, *J* = 3.56 Hz, 1H), 4.59 (dd, *J* = 6.56, 12.17 Hz, 2H), 4.21 (dd, *J* = 4.84, 10.31 Hz, 1H), 4.16 (t, *J* = 9.15 Hz, 1H), 4.10 (t, *J* = 9.05 Hz, 1H), 4.05 (t, *J* = 9.34 Hz, 1H), 3.96 (ddt, *J* = 5.43, 9.92, 24.67 Hz, 1H), 3.90 – 3.81 (m, 2H), 3.77 – 3.67 (m, 3H), 3.61 – 3.47 (m, 3H), 3.40 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.74, 138.36, 138.11, 138.03, 137.41, 137.14, 137.09, 128.73, 128.71, 128.58, 128.53, 128.47, 128.44, 128.40, 128.34, 128.29, 128.24, 128.20, 128.10, 128.05, 127.99, 127.97, 127.93, 127.74, 127.60, 127.49, 127.47, 101.27, 97.90, 96.06, 82.20, 81.41, 80.40, 76.63, 76.59, 74.88, 74.47, 73.50, 73.38, 71.70, 69.54, 69.42, 68.87, 68.77, 67.34, 67.30, 67.24, 67.21, 63.12, 55.31.

^{31}P NMR (243 MHz, $CDCl_3$) δ 12.20.

HRMS (ESI) calcd for $C_{62}H_{65}O_{14}PNa$ $[M+Na]^+$: 1087.4004, found 1087.3988



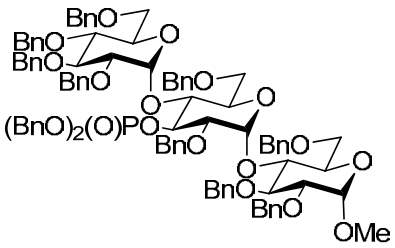
Methyl 3-dibenzylphosphate-2,6-di-*O*-benzyl- α-D-glucopyranosyl-(1→4) -2 ,3 ,6-tri-*O*-benzyl-α-D-glucopyranoside (46) Compound **44** (8 mg, 0.008 mmol) was subjected to the conditions according to the general procedure for selective benzylidene opening and purified by SGC (hexanes/EtOAc 3:1) to afford **46** (5 mg, 0.005 mmol, 63%) as syrup.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 6.99 (m, 35H), 5.69 (d, *J* = 3.57 Hz, 1H), 4.98 – 4.70 (m, 8H), 4.70 – 4.55 (m, 2H), 4.54 – 4.42 (m, 4H), 4.36 (d, *J* = 12.00 Hz, 1H), 4.32 – 4.23 (m, 2H), 4.02 – 3.91 (m, 2H), 3.84 – 3.68 (m, 4H), 3.68 – 3.54 (m, 2H), 3.43 (dtd, *J* = 4.21, 10.24, 17.47 Hz, 3H), 3.28 (s, 3H), 2.53 (d, *J* = 2.51 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.85, 138.51, 138.12, 137.85, 135.81, 135.76, 128.72, 128.57, 128.46, 128.43, 128.41, 128.39, 128.34, 128.28, 128.24, 128.10, 128.04, 127.96, 127.92, 127.88, 127.81, 127.70, 127.65, 127.45, 127.27, 97.88, 95.69, 81.38, 80.24, 79.63, 75.03, 74.50, 73.62, 73.37, 73.27, 72.45, 72.09, 70.32, 70.06, 69.63, 69.38, 69.34, 68.94, 67.33, 55.27.

^{31}P NMR (162 MHz, $cdcl_3$) δ -0.99.

HRMS (ESI) calcd for $C_{62}H_{68}O_{14}P$ $[M+H]^+$: 1067.4341, found 1067.4319



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3-dibenzylphosphate-2,6-di-*O*-benzyl- α-D-glucopyranosyl-(1→4) -2 ,3 ,6-tri-*O*-benzyl-α-D-glucopyranoside (50)

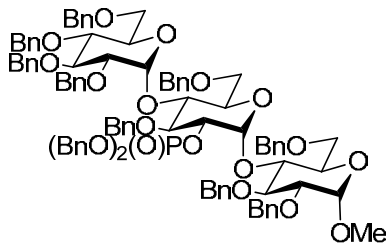
Donor **48** (29 mg, 0.042 mmol) and acceptor **47** (15 mg, 0.014 mmol) was subjected to the conditions according to the glycosylation procedure and purified by SGC (hexanes/EtOAc 4:1) to give **50** (16 mg, 0.010 mmol, 72%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 – 6.98 (m, 55H), 5.83 (d, *J* = 3.58 Hz, 1H), 5.66 (d, *J* = 3.54 Hz, 1H), 5.05 – 4.95 (m, 3H), 4.95 – 4.89 (m, 1H), 4.89 – 4.83 (m, 5H), 4.83 – 4.79 (m, 2H), 4.77 (d, *J* = 11.12 Hz, 1H), 4.75 – 4.68 (m, 1H), 4.66 – 4.58 (m, 6H), 4.56 – 4.38 (m, 8H), 4.32 (dd, *J* = 12.11, 16.95 Hz, 2H), 4.27 (dd, *J* = 6.52, 9.76 Hz, 1H), 4.06 – 3.99 (m, 2H), 3.99 – 3.94 (m, 1H), 3.83 (dd, *J* = 7.14, 11.48 Hz, 2H), 3.79 – 3.74 (m, 2H), 3.74 – 3.66 (m, 3H), 3.66 – 3.51 (m, 7H), 3.51 – 3.42 (m, 5H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.93, 138.84, 138.74, 138.39, 138.24, 138.22, 138.14, 137.97, 137.93, 128.53, 128.45, 128.42, 128.38, 128.30, 128.27, 128.24, 128.21, 128.18, 128.13, 128.06, 128.00, 127.97, 127.94, 127.87, 127.77, 127.73, 127.69, 127.64, 127.50, 127.44, 127.43, 127.41, 127.36, 127.28, 127.18, 126.90, 97.78, 95.20, 94.55, 82.02, 81.66, 80.18, 80.07, 75.39, 74.87, 74.30, 73.36, 73.33, 73.25, 72.89, 72.59, 72.33, 71.84, 71.03, 70.41, 69.50, 69.45, 69.41, 69.24, 69.21, 68.82, 68.71, 68.50, 55.28.

^{31}P NMR (243 MHz, $CDCl_3$) δ 15.13.

HRMS (ESI) calcd for $C_{96}H_{101}O_{19}PNa$ $[M+Na]^+$: 1611.6567, found 1611.6524



Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2-dibenzylphosphate-3,6-di-*O*-benzyl- α-D-glucopyranosyl-(1→4) -2 ,3 ,6-tri-*O*-benzyl-α-D-glucopyranoside (49) Donor **48** (10 mg, 0.012 mmol) and acceptor **46** (5 mg, 0.005 mmol) was subjected to the conditions according to the glycosylation procedure and purified by SGC ($PhCH_3$/EtOAc 8:1) to give **50** (5 mg, 0.003 mmol, 60 %).

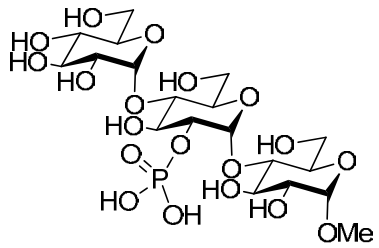
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 – 7.00 (m, 50H), 5.73 (d, *J* = 3.51 Hz, 1H), 5.59 (d, *J* = 3.63 Hz, 1H), 5.09 – 4.98 (m, 2H), 4.98 – 4.88 (m, 2H), 4.88 – 4.77 (m, 6H), 4.77 – 4.68 (m, 3H), 4.66 – 4.41 (m, 11H), 4.32 (d, *J* = 12.19 Hz, 1H), 4.19 – 4.08 (m, 2H), 4.08 – 4.02 (m, 2H), 4.02 – 3.94 (m, 1H), 3.94 – 3.87 (m, 2H), 3.80 (d, *J* = 7.13 Hz, 1H), 3.79 – 3.73 (m, 2H), 3.72 – 3.66 (m, 2H), 3.57 (dd, *J* = 2.60, 10.17 Hz, 2H), 3.51 (tt, *J* = 3.50, 8.64, 9.82 Hz, 2H), 3.41 (dd, *J* = 2.00, 10.75 Hz, 1H), 3.38 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 138.96, 138.72, 138.42, 138.21, 138.00, 128.98, 128.63, 128.40, 128.32, 128.28, 128.24, 128.19, 128.16, 128.07, 127.98, 127.83, 127.78, 127.73, 127.69, 127.64, 127.62, 127.53, 127.49, 127.43, 127.38, 127.21, 127.10, 126.74, 97.93, 97.05, 95.65, 82.09, 81.19,

80.03, 79.52, 77.60, 75.43, 74.93, 74.66, 73.65, 73.48, 73.34, 73.07, 71.14, 71.08, 69.75, 69.24, 68.81, 68.20, 55.28.

^{31}P NMR (243 MHz, $CDCl_3$) δ 16.68.

HRMS (ESI) calcd for $C_{96}H_{101}O_{19}PNa$ $[M+Na]^+$: 1611.6567, found 1611.6565



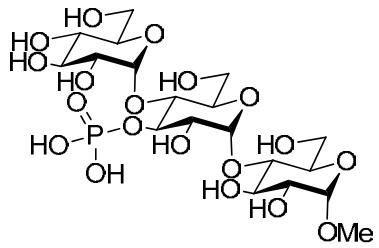
Methyl α-D-glucopyranosyl-(1→4)-2-phosphate-α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (51) Hydrogenation of **50** (3 mg, 0.0018 mmol) according to the general method for global deprotection gave **15** (0.5 mg, 0.001 mmol, 48%).

^{1}H NMR (700 MHz, MeOD) δ 5.75 (s, 1H), 5.24 (s, 1H), 4.73 (s, 1H), 4.20 – 4.02 (m, 3H), 3.99 – 3.81 (m, 5H), 3.70 (dt, *J* = 9.96, 44.31 Hz, 6H), 3.54 – 3.14 (m, 6H).

^{13}C NMR (176 MHz, MeOD) δ 104.08, 102.25, 99.04, 82.14, 80.76, 80.39, 78.37, 76.48, 76.27, 76.11, 75.46, 74.41, 74.09, 73.01, 72.93, 72.80, 63.98, 63.60, 63.25, 56.85.

^{31}P NMR (243 MHz, MeOD) δ 3.20.

HRMS (ESI) calcd for $C_{19}H_{34}O_{19}P$ $[M-H]^-$: 597.1437, found 597.1435



Methyl α-D-glucopyranosyl-(1→4)-3-phosphate-α-D-glucopyranosyl-(1→4)-α-D-gluco pyranoside (52) Hydrogenation of **50** (10 mg, 0.006 mmol) according to the general method for global deprotection gave **15** (3 mg, 0.004 mmol, 67%).

^{1}H NMR (600 MHz, MeOD) δ 5.49 (bs, 1H), 5.19 (bs, 1H), 5.14 (bs, 1H), 4.07 – 3.76 (m, 4H), 3.76 – 3.58 (m, 5H), 3.58 – 3.36 (m, 6H), 3.24 – 3.14 (m, 6H).

^{13}C NMR (151 MHz, MeOD) δ 99.70, 96.82, 92.57, 76.71, 76.64, 74.91, 73.50, 73.04, 72.46, 71.58, 71.37, 70.49, 70.36, 61.48, 61.37, 60.86, 54.21.

^{31}P NMR (162 MHz, MeOD) δ 2.20.

HRMS (ESI) calcd for $C_{19}H_{34}O_{19}P$ $[M-H]^-$: 597.1437, found 597.1436

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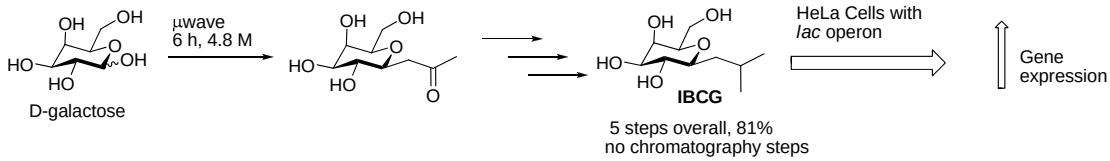
CHAPTER 4

Multigram Synthesis of Isobutyl-*β*-*C*-galactoside as a Substitute of Isopropylthiogalactoside for Exogenous Gene Induction in Mammalian Cells

A paper published in *Journal of Organic Chemistry*[1]

Lin Liu, Basma Abdel Motaal, Marc Schmidt-Supprian, and Nicola L. B. Pohl

Abstract



Herein we report that isobutyl-*β*-*C*-galactoside (IBCG) is also a promising inducer of gene expression in mammalian cells and report a new synthetic route to the compound that should make obtaining the multigram quantities of material required for animal studies more feasible. A convenient synthesis of IBCG—an inducer of genes controlled by the *lac* operon system in bacterial cells—was achieved in 5 steps from galactose in 81% overall yield without any chromatographic separation steps. An optimized microwave-assisted reaction at high concentration was key to making the *C*-glycosidic linkage. A Wittig reaction on a per-*O*-silylated rather than per-*O*-acetylated or -benzylated substrate proved most effective in installing the final carbon atom.

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Introduction

Genetic engineering demands a tight regulation system to control the expression of the introduced exogenous genes, and this requirement is generally achieved via inducible gene expression systems.[1-3] Among the current available inducible systems, *lac* operon-based systems are the most widely studied and used.[4,5] Isopropyl-*β*-D-thiogalactopyranoside (IPTG), a lactose analog, is routinely used as an inducer of the *lac* operon in bacterial systems for *in vitro* studies to induce the expression of exogenously introduced genes.[4,6] This sugar analog binds to *lac* repressor to activate the gene transcription machinery (Figure 1). Studies have also shown that this inducible *lac* operon/repressor system can be implemented in mammalian cells and animal systems.[7-10] Such an inducible system in mammalian cells ensures the exogenous genes are expressed only when the inducer is added and tightly regulates the gene transcription. When IPTG is used as the *lac* operon inducer in the animal models, the inducer is usually dissolved in drinking water and fed to the animals. Since decomposition products resulting from IPTG have an unpleasant thiol smell, IPTG solutions have to be put into light-protected bottles and carefully monitored.[11,12] Even so, IPTG's rapid-clearance and short half-life limit its usage and present a major drawback of using this popular gene induction system in animal systems.[13-15]

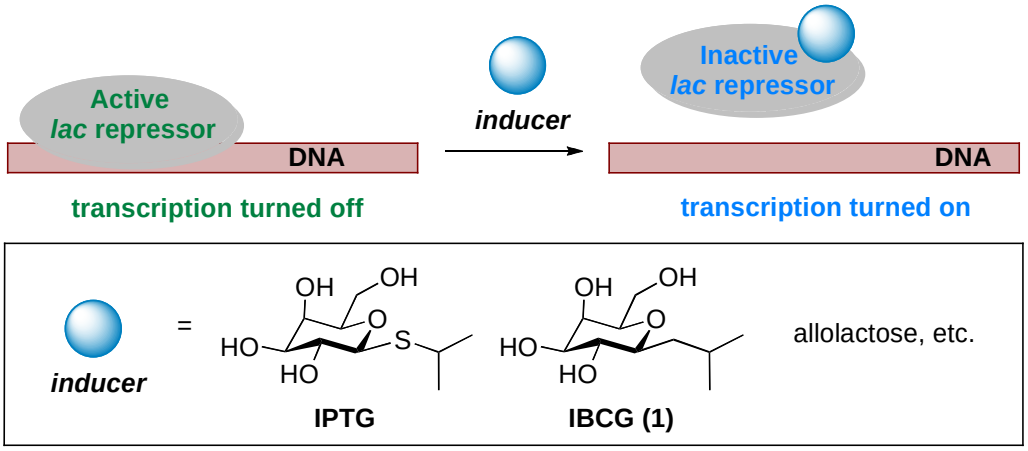


Figure 1. Lactose analogs such as allolactose, IPTG and IBCG can serve as inducers to bind the *lac* repressor and activate gene transcription in *lac* operon regulated gene transcription.

To circumvent this stability problem, our group has reported the design and synthesis of a new *lac* inducer, isobutyl-*β*-*C*-galactoside (IBCG, **1**),[16] as a *C*-glycoside analog of IPTG. Not only does this C-glycoside show at least equal gene induction ability in bacterial systems as the *S*-glycoside, but replacement of the *S*-glycosidic bond by a *C*-glycosidic bond renders the resulting molecule much more stable. Herein we report that IBCG is also a promising inducer of gene expression in mammalian cells and report a new synthetic route to the compound that should make obtaining the multigram quantities of material required for animal studies more feasible.

Previously, the synthesis of IBCG was achieved via either a Lewis acid-promoted reaction between galactose pentaacetate and methallyltrimethylsilane or a Grignard reaction between bromoacetogalactose and excess isobutylmagnesium bromide.[16] The former method employs a large

excess of a relatively expensive material (methallyltrimethylsilane, > $20/g) and shows no α/β selectivity in the *C*-glycosidic bond formation reaction. The latter method utilizes a Grignard approach, which is highly exothermic and cumbersome to scale up. Both of the methods require silica gel chromatography to purify the product. To satisfy the need for larger amounts of IBCG required for animal studies, we hoped to find a new route that could offer a high overall yield on a multigram scale without any chromatography steps.

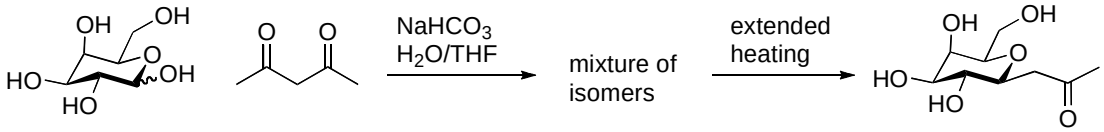
Results and discussion

Obviously key to any successful synthesis of IBCG is the method for installation of the *C*-glycoside. Stereoselective formation of *C*-glycosidic bonds have attracted significant attentions in the recent years.[17,18] Among methods for the synthesis of *β*-*C*-glycosides, the one step condensation between free sugars in aqueous solutions and 2,4-pentanedione was particularly attractive as a way to quickly make *β*-*C*-glycosidic ketones without prior protection of the carbohydrate hydroxyl groups.[19] A mixture of *C*-glycoside stereoisomers are formed in the initial Knoevenagel condensation; however, extended heating under basic conditions allow the equilibrium to shift to the *β*-*C*-glycosidic pyranose ketone (Scheme 1).[20] Many recent studies utilized this strategy as a starting point to synthesize *β*-*C*-glycoside analogs.[21-27] Ideally, IBCG could be obtained via a simple methylenation on the ketone by using a Wittg-type reaction. However, even though the carbonyl group in the *β*-*C*-glycosidic ketones obtained from natural reducing sugars should provide an excellent opportunity to perform

C-C bond formation via a Wittig-type reaction to obtain other *β*-*C*-glycoside analogs, this possibility remains relatively unexplored. Most of the existing studies are based on Aldol reactions between the *β*-*C*-glycosidic ketone and an aldehyde to form an *α*, *β* unsaturated ketone. We found this apparent absence of Wittig-type reaction on *β*-*C*-glycosidic ketones quite intriguing, and speculated that the base sensitivity of the ketone substrates might be a contributing factor. We decided to pursue our IBCG synthesis through this route with the hope of developing an efficient method for Wittig reactions on the *β*-*C*-glycosidic ketones.

First, an optimized synthesis of our desired intermediate ketone 4 had to be developed. The synthesis of **4** has been reported on a 500-mg scale at 0.15 M concentrations of galactose after heating at 90 °C for 24 h.[28] Unfortunately, the long reaction time and dilute reaction conditions made the reaction less desirable for multigram syntheses. We therefore set out to probe the limits of this reaction. By careful monitoring of the reaction by ^{1}H NMR (Figure 2), we found that formation of the *C*-glycosidic bond itself was a relatively fast process; the mixture of isomers (mainly *α*-pyranose) was then converted to the thermodynamically more stable *β*-pyranose product **4** slowly upon extended heating. The characteristic peaks of *β*-pyranose product **4** are two doublets of doublets at δ 3.02 and 2.74 corresponding to the protons from H-1'. The disappearance of peaks corresponding to H-1' from *α*-pyranose product around δ 2.92 indicates the shift of the equilibrium has finished (Figure 2). To possibly accelerate the reaction, microwave irradiation was attempted instead of conventional heating.[29-31] We found that the *C*-glycosidic bond formation was finished under microwave irradiation

in only 30 minutes under reflux at 70 – 75 °C. The top layer of the mixture containing the excess 2, 4-pentanedione was then discarded and the reaction was heated in the microwave reactor during which time the temperature of the reaction was slowly raised to 100 °C as the excess THF evaporated. After 5.5 h, ^{1}H NMR showed most of the mixture had been converted to the desired *β-C-gly*coside **4** (Figure 2). We also found out that the reaction could be run at much higher concentrations than reported. The reaction was run at a concentration from 1.2 M to as high as 4.8 M instead of the reported 0.15 M with similar results. The water was removed from the reaction mixture under reduced pressure, and then methanol/ethyl acetate was added to separate the product from extra sodium bicarbonate. The crude products still contained some sodium acetate, but could be either used directly in the following reaction or purified by passing through a short silica gel plug followed by recrystallization to give the desired *β-C-gly*cosidic ketone **4** in 92% yield for a 5-gram scale reaction.



2 **3** **4**

	Heating method	Reaction time	Concentration	Yield
Previous[28]	Conventional, 90 ℃	24 h	0.15 M	90%
Modified method	Microwave, 70 – 100 ℃	6 h	As high as 4.8 M	92%

Scheme 1. Comparison of heating methods and reaction concentrations/times for the synthesis of *β-C*-glycosidic ketone **4**

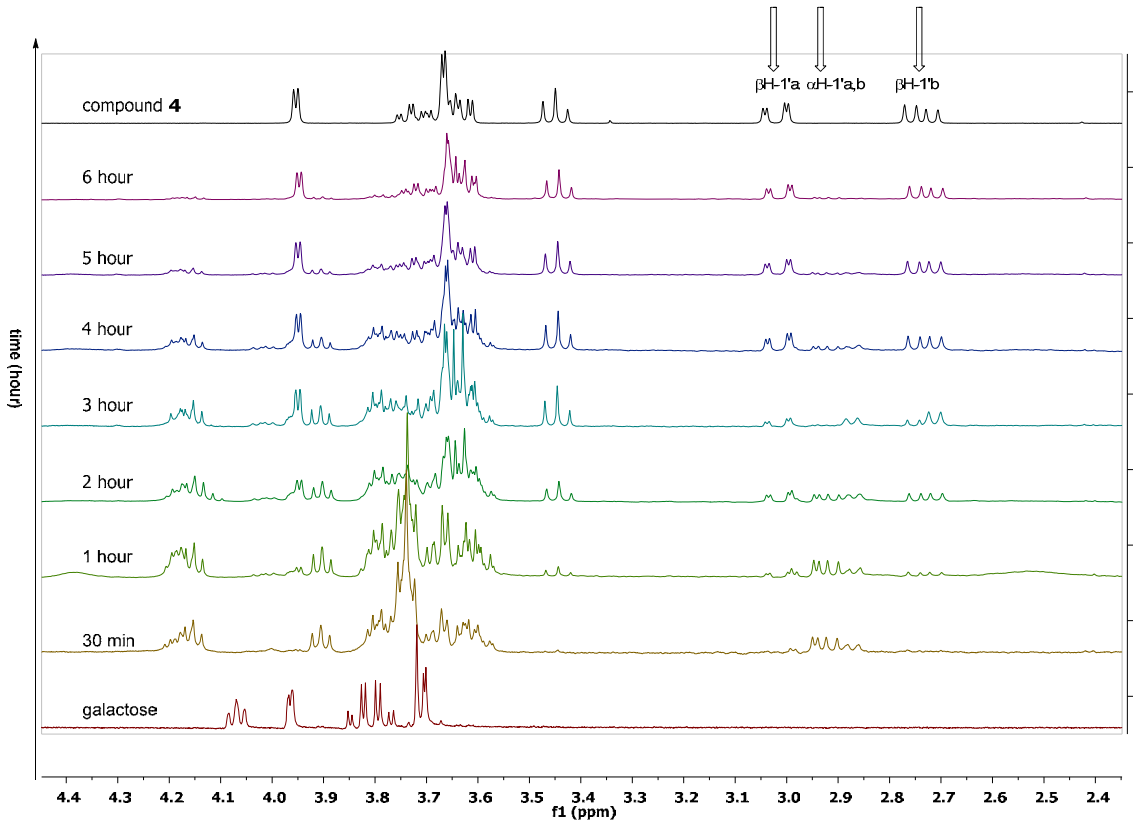
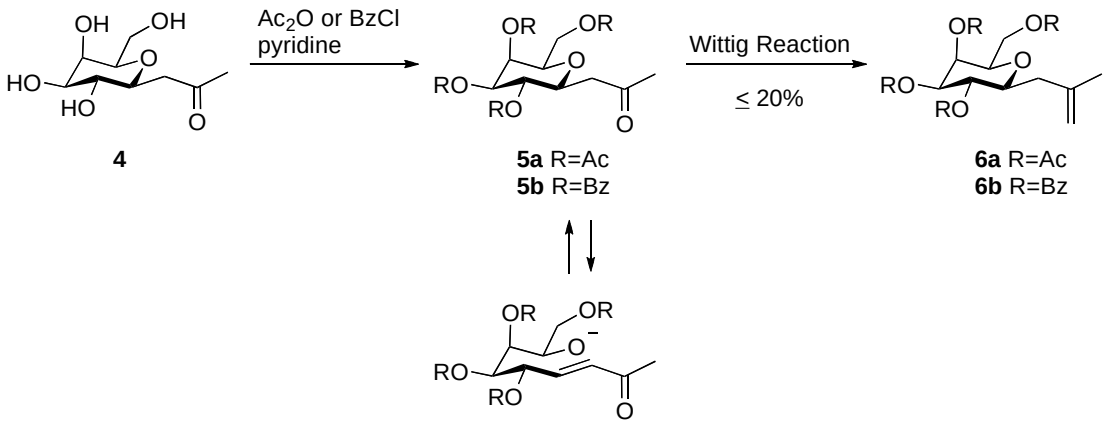


Figure 2. The ^{1}H NMR spectra in D_2O of a 10.00 g reaction (2.4 M) under microwave heating conditions. Samples aliquots were removed at 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h.

With a method for the larger scale synthesis of the key intermediate ketone **4** in hand, only a few steps should allow elaboration of this ketone to the final compound **1**. The synthesis of *β-C*-glycoside **4** utilizes a well-studied property that 2-carbonylalkyl-*C*-glycopyranosides tend to do a retro-Michael type addition initiated by the enolate formation and followed by ring-opening under mild basic conditions.[32-35] However, this property that makes for a convenient synthesis of *β-C*-glycoside ketones also makes the following transformations under basic conditions troublesome. A straightforward way to do the homologation is to use the Wittig reaction.[36] No precedents for such a

Wittig reaction on substrates like compound 4 have been reported. Even though the Wittig reagent is relatively basic,[37] we anticipated that there was a reasonable chance to do the desired methylenation on the base sensitive ketone substrate if we could fine tune the electronic properties of the pyranose ring to make the homologation, and not the enolate formation and ring opening reaction, more favorable. The Tebbe reagent provides a less basic alternative to Wittig regents that could possibly give superior results on a base-sensitive substrate,[38] but is less desirable in large scale reactions due to its high cost. Therefore, we decided to try the Wittig reaction on the per-*O*-acetylated ketone **5a**, since the per-*O*-acetylation of ketone **4** was reported to give a high yield easily[28] and provides a readily available starting point for the methylenation. The ketone **4** was per-acetylated using acetic anhydride and pyridine to give per-*O*-acetylated compound **5a**,[28] which was then subjected to a Wittig reaction (Scheme 2). However, no combination of varying base, temperature or order of addition was found in which the desired alkene **6a** was had in over 20% yield. The possible side products in this reaction involved a retro-Michael addition under basic conditions, followed by possible polymerization and decomposition. The less base-sensitive benzoyl group was then installed in place of acetyl groups, but the results were worse. The protected sugar derivative **5b** was obtained in only 30% yield, and the Wittig reactions on **5b** gave the desired product in less than 5% yield. Apparently the Wittig reagent was too basic for use with these peracetylated substrates. Non-basic methylenation conditions using $TiCl_4/Mg/CH_2Cl_2$[39] on substrate **5a** were attempted, but the yield was also low (~20%). A Grignard reaction using methylmagnesium bromide on **5a** to install a methyl group also did not give satisfactory results.

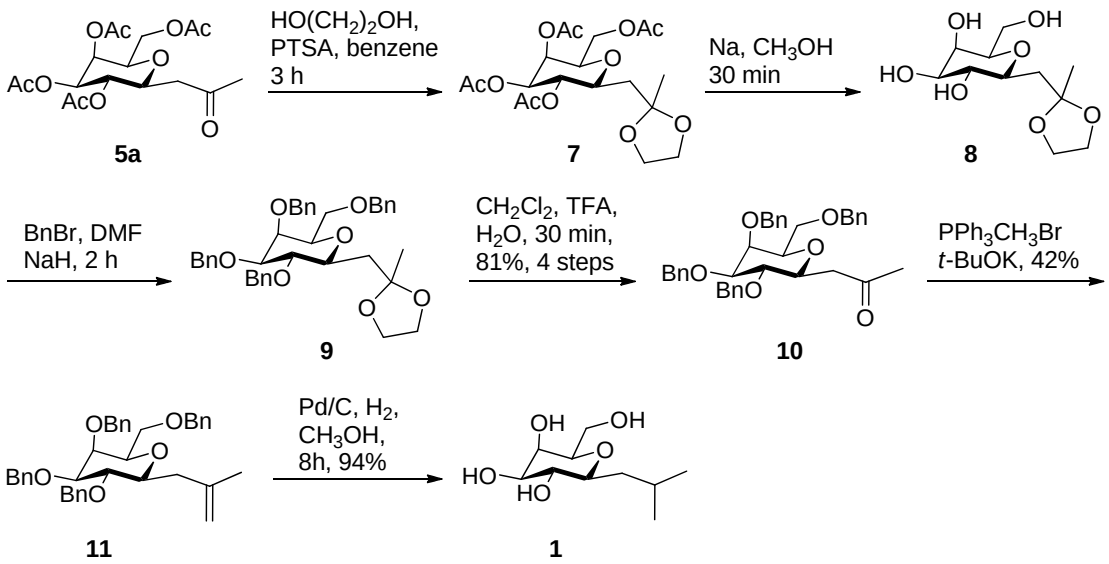


Scheme 2. Wittig reaction on acyl-protected substrates.

The electron withdrawing acyl groups appeared to make the retro-Michael addition more favorable, hence the side reactions and low yields. Using more electron donating groups like benzyl as the hydroxyl protecting groups might help. Per-*O*-benzylated compound **10** (Scheme 3) has been made previously from epimerization under basic conditions of the corresponding α-ketone,[33] which was obtained via oxymercuration followed by oxidation of α-allyl-*C*-galactoside. Benzylation of **4** seems to be a much more straightforward route to obtain **10**. However, this perbenzylation reaction turned out to be more problematic than expected. Direct benzylation of the ketone **5a** using NaH/BnBr gave complex mixtures, presumably due to ring-opening under basic conditions. Similar issues were reported on the glucose ketone substrate.[40] Using NaOH/BnBr in THF with a phase transfer reagent did not give much improvement. We therefore decided to protect the ketone first, although this strategy would add undesirable additional steps to the sequence. Using ethylene glycol, PTSA and compound **4** in a mixture of acetonitrile/benzene did not yield the ketal, possibly due to the poor

solubility of the initial ketone. Interestingly, the glucose analog could react under similar conditions.[40] The attempt at protecting ketone **4** in methanol as a dimethyl ketal also did not proceed well.

Finally the per-acetylated ketone **5a** was protected to form ketal **7** using ethylene glycol and pyridinium *p*-toluenesulfonate (PPTS) as a catalyst[40] (using TsOH as catalyst led to the decomposition of the substrate) in benzene in 3 h (Scheme 3). Deacetylation, followed by benzylation and ketal removal gave the per-benzyl substituted ketone **10** in 81% overall yield over 4 steps (Scheme 3).

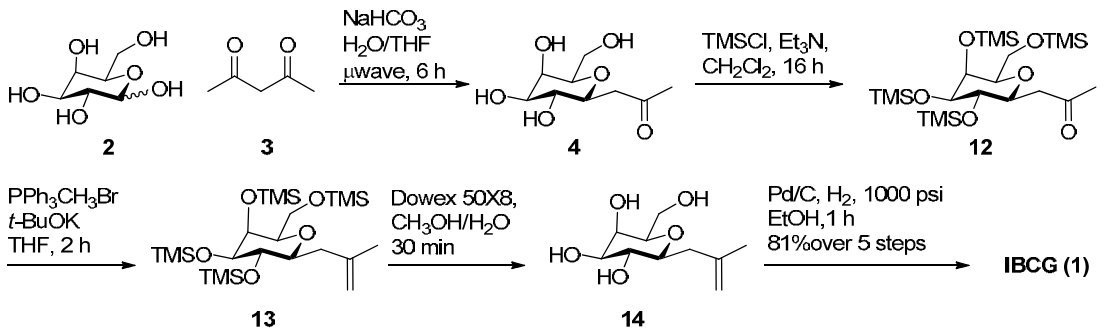


Scheme 3. Synthesis of IBCG via a per-benzylated ketone.

The Wittig reaction on the per-benzyl substituted ketone **10** proceeded better than on the per-acetyl substituted ketone. Using two equivalents each of Wittig salt and *t*-BuOK, the alkene **11** was formed in 42% yield. When slightly excess base was used, the yield of **11** dropped significantly. Hydrogenation of **11** gave IBCG **1** in 94% yield. This route yields IBCG from galactose in 8 steps in

30% overall yield. However, this route is still not very satisfactory, given the low yields and long step count.

To further improve the synthesis route, further fine-tuning of the electronic effects was needed. Ideally, the Wittig substrate would be even more electron rich than the perbenzylated substrate employed. Several examples of protected *O*-glycosides using silyl groups as temporary protecting groups have been reported.[41-45] The per-*O*-TMS protected substrates are readily prepared on large scale, and the ease of deprotection makes them very convenient to use. However, the current studies mainly focused on utilizing the improved solubility of the per-*O*-silylated substrate in organic solvents and on the Lewis acid-catalyzed reactions of silyl protected hydroxyl groups. In the latter case, the silyl groups were used as a proton surrogate. Only a few studies used per-*O*-silylation as a method to change the electronic properties of the parent compound. For example, Gervay-Hague's studies of glycosyl iodides,[42,46,47] showed that the per-*O*-trimethlysilyl glycosyl iodides were more reactive than per-*O*-benzyl donors in glycosylation reactions. We proposed that by per-silylation, we might be able to circumvent the troublesome side reaction encountered in the Wittig reaction by making the ketone substrate more electron rich.



Scheme 4. Revised synthesis of IBCG using TMS as a protecting group.

The per-TMS-silylation of **4** was tested under different conditions, including using pyridine, Et_3N/DMF, and Et_3N/CH_2Cl_2. Fortunately, it was found out that using crude **4** containing large amounts of NaOAc from the condensation did not interfere with the per-silylation reaction. Since ketone **12** has good solubility in hexanes, it could be extracted from the crude mixture directly to give pure product, leaving Et_3N•HCl and NaOAc as insoluble salts. The reaction in Et_3N/CH_2Cl_2 is slower than in DMF or pyridine, but the work-up process is easier. The Wittig reaction on **12** proceeded surprisingly well and gave the methylenation product **13** in high yield. After solvent removal, the crude mixture of the Wittig reaction containing **13** and triphenylphosphine oxide was redissolved in methanol/water and subjected to acidic silyl group cleavage using Dowex 50X8 (H^+ form). After filtration and solvent removal, the crude product was redissolved in water and extracted with EtOAc to remove triphenylphosphine oxide and provide an aqueous solution of alkene **14**. However, we experienced problems in the hydrogenation of this alkene. Unlike alkenes **6a** or **11**, the hydrogenation of **14** proceeded very slowly; the reaction required more than 48 h to go to completion under 1000 psi of H_2. We reasoned that even though the ^{1}H NMR of crude **14** seemed pure, trace amounts of

phosphorus-containing compounds left due to the large amount of Wittig salt used in the methylenation reaction might be interfering with the hydrogenation reaction. Therefore, the work-up procedure for the Wittig reaction was revisited. Most of the solvent was removed, and then hexane was added to remove most of the triphenylphosphine oxide by filtration. The resulting filtrate was concentrated, and the residue was dissolved in methanol/water and subjected to Dowex 50X8 to provide **14**. After EtOAc/water extraction, the hydrogenation of **14** in ethanol took less than 1 h under high pressure and could be done under atmospheric pressures of H_2 in 8 h to give **1** as a white solid. The ^{1}H NMR of crude **1** showed that it was sufficiently pure and no further purification was needed. By using this modified route, we were able to perform a larger scale synthesis of IBCG from 10.00 g of galactose **2** (55.5 mmol), and obtained 9.90 g of **1** (45.4 mmol) in 81% yield over 5 steps (95% average yield per step) without any chromatography purification (Scheme 4). The large difference in the Wittig reaction using a per-*O*-benzylated substrate versus a per-*O*-silylated substrate shows the significant affect a change in electronic properties of the substrate can make in reaction yields.

Now that a route was available to readily provide the multigram quantities of IBCG necessary to carry out animal studies, the utility of the compound in inducing gene expression in mammalian rather than bacterial cells needed to be ascertained. Mammalian cells such as HeLa cells have less diverse metabolic capabilities compared to bacterial systems and therefore decomposition of IPTG is less of an issue on the time scale of the experiments. Such issues become problematic when whole animal experiments are envisioned. However, the permeability of IBCG compared to IPTG was

unclear. In other words, would IBCG reach the site of action to induce gene expression? To test if IBCG will work as a *lac* operon inducer in mammalian cells in the induction of protein expression, assays of repressor activity utilizing the expression of fluorescent proteins in HeLa cells were preformed (Figure 3). To this end we cloned a construct placing the far-red fluorescent protein Neptune behind the LacI-repressible Chicken –actin CMV enhancer promoter sequences (CAGop)[48] in between recognition sites for the *Sleeping Beauty* transposase.[49] As control, we used a *Sleeping Beauty* transposon containing green fluorescent protein (GFP) expressed from the original CAG promoter. HeLa cells were made transgenic for these constructs by co-transfection with a plasmid encoding SB100x,[50] an enhanced version of the *Sleeping Beauty* transposase. HeLa cells expressing a fixed ration between Neptune and GFP were purified by cell sorting. We then used SB100x-mediated transposition to make the CAGop-Neptune/CAG-GFP HeLa cells transgenic for LacI (Figure 3A). Expression of lacI resulted in an over 40-fold repression of Neptune expression, while leaving the GFP levels unaltered (data not shown). The addition of the inducers IPTG or IBCG resulted in an increase of Neptune expression over time. Our results showed that IBCG was very well tolerated by HeLa cells at 1 mM concentration and was comparable to IPTG in inducing gene expression (Figure 3B).

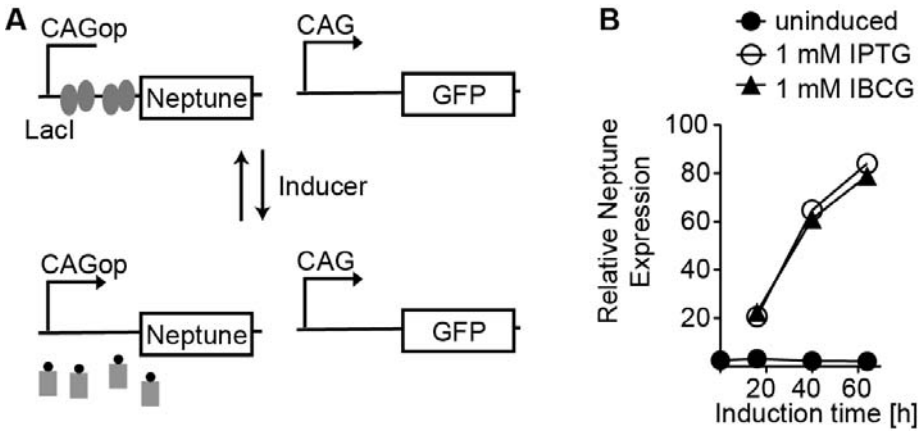


Figure 3. A) Scheme of promoters used to evaluate the induction capabilities of IPTG and IBCG in HeLa cells. B) Neptune expression from the CAGop promoter was normalized to GFP expression and to maximal Neptune expression in absence of LacI. The relative Neptune expression after induction with IPTG or IBCG is shown. Fluorescence intensities were determined by flow cytometry. Data shown are the average of two independent experiments.

Conclusion

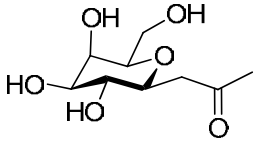
In summary, a convenient route for the synthesis IBCG (**1**) from galactose was developed that includes as key steps a microwave-assisted synthesis of an intermediate *β*-*C*-glycoside and a Wittig reaction on a per-*O*-silylated base-sensitive substrate. Microwave-assisted conditions could significantly reduce the reaction time for the *β*-*C*-glycoside formation and also easily allows the reaction to be run at much higher concentrations. The large yield differences of the Wittig reaction on per-acetyl, per-benzyl, and per-silyl-protected substrates further demonstrate the profound impact of

protecting groups. TMS groups cannot only be used to improve solubility of poly-hydroxyl bearing compounds and react as a proton surrogate under Lewis acid-promoted conditions, they can also change the electronic properties of the substrate greatly. The Wittig reaction on the per-silyl substituted *β-C*-glycosidic ketones provides a new and efficient way of synthesizing novel *β-C*-glycoside analogs. In addition, the developed route can produce material sufficiently pure for biological studies without a single chromatographic separation. Finally, IBCG has been shown capable of inducing *lac* operon promoters for induction of gene expression in not only bacterial systems, but also in mammalian cells. This improved route amenable to larger scale production of IBCG coupled with the promising cell-based studies now sets the stage for testing of this system in animal studies.

Experiment section

General Experimental Methods: Reactions were performed using flame-dried glassware under argon using anhydrous solvents unless otherwise noted. Microwave-assisted reactions were performed using a CEM Discover® Microwave system. Thin layer chromatography (TLC) was performed using glass-backed silica gel plates w/UV254. Visualization of TLC plates was performed by UV light and 5% sulfuric acid/ethanol. NMR spectra were recorded on a 400 MHz for ^{1}H (100 MHz for ^{13}C) spectrometer. ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ spectra were referenced to the solvent peak at 7.260 ppm (^{1}H) and 77.0 ppm (^{13}C). Due to the severe overlap of ^{13}C signals from aromatic carbons in the range of 129 – 127 ppm

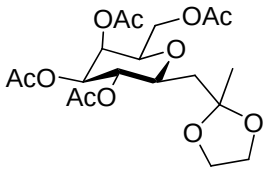
in the ^{13}C NMR of the tetra-benzyl protected compounds **9** and **11**, only clearly discernable peaks from aromatic carbons on the benzyl groups are reported. The assignments of ^{1}H NMR peaks were made primarily from 2D ^{1}H-^{1}H COSY and edited ^{1}H-^{13}C HSQC spectra. High resolution mass spectra (HRMS, ESI mode) were obtained using a Q-TOF LC/MS.



1-C-(*β*-D-Galactopyranosyl)-propan-2-one (4). To a round bottom flask was added D-galactopyranose **2** (4.00 g, 22.2 mmol), $NaHCO_3$ (7.40 g, 88.8 mmol), water (20 mL) and THF (10 mL). 2, 4-Pentanedione **3** (4.6 mL, 44 mmol, freshly distilled) was added; the reaction started to turn light yellow. The flask was attached to a condenser and put into a microwave reactor. The temperature of the reaction was measured using the external sensor equipped in the microwave reactor. The reaction was heated to reflux at 70-75 °C at 80 W with stirring. After 30 min, ^{1}H NMR spectrum showed that all the galactose had been consumed, and the top layer was separated and discarded. The remaining solution was extracted with EtOAc (20 mL); then the undissolved solid was returned to the flask, the aqueous solution was heated at 75 °C at 80 W for another 30 min, and then gradually heated to 90 °C for 3 h, then to 100 °C for another 2 h. The ^{1}H NMR spectrum indicated that the reaction was finished, and the yellow/orange reaction mixture was cooled and concentrated under reduced pressure at room temperature. Methanol/ethyl acetate (1:1) (100 mL) was added to the mixture to dissolve the product, and then the remaining salt was removed by filtration. The solution was concentrated to give a crude product of **4** containing NaOAc, which could be used directly in the

following experiments. The crude product was passed through a short column using methanol/ethyl acetate 1:4 as eluting solvent, and recrystallization using methanol/ethyl acetate gave **4** as white crystals (3.21 g, 14.5 mmol). The mother liquor was concentrated and purified by silica gel column chromatography to give additional **4** (1.02 g, 4.6 mmol). ^{1}H NMR data matches data reported in the literature.[28]

The reaction was also performed on a 10.00 g scale and on a 20.00 g scale of galactose at concentrations of 2.4 M and 4.8 M respectively.



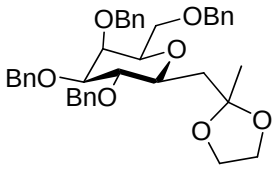
1-*C*-(2, 3, 4, 6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-propan-2-one ethylene ketal (7). To a solution of **5a** (500 mg, 1.30 mmol) in benzene (15 mL) was added ethylene glycol (0.145 mL, 2.60 mmol), and pyridinium *p*-toluenesulfonate (50 mg, 0.2 mmol). The reaction was heated to reflux with a Dean-Stark apparatus. After 3 h, ^{1}H NMR indicated completion of the reaction. The solvent was removed under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with $NaHCO_3$ (aq), and then dried over Na_2SO_4. Solvents were removed under reduced pressure to provide **7** (550 mg, 1.27 mmol) as a white foam that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (dd, *J* = 1.2, 3.3 Hz, 1H, H-4), 5.05 (t, *J* = 9.7 Hz, 1H, H-2), 5.02 (dt, *J* = 3.3, 9.7 Hz, 1H, H-3), 4.13 (dd, *J* = 7.2, 11.3 Hz, 1H, H-6a), 4.05 (dd, *J* = 6.1, 11.3 Hz, 1H, H-6b), 3.98-3.82 (m, 5H, -OCH_2CH_2O-, H-5), 3.64 (dt, *J* = 1.6, 9,3 Hz, 1H, H-1), 2.14 (s, 3H, OAc),

2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.91 (dd, *J* = 8.5, 14.7 Hz, 1H, H-1'a), 1.75 (dd, *J* = 1.6, 14.7 Hz, 1H, H-1'b), 1.37 (s, 3H, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 170.1, 169.9, 169.7, 108.4, 75.1, 74.0, 72.1, 68.9, 67.7, 64.4, 64.3, 61.8, 39.9, 24.5, 20.6, 20.53, 20.49, 20.42.

HRMS(ESI): calcd for $C_{19}H_{28}NaO_{11}$ $[M+Na]^+$: 455.1524, found 455.1517.



1-*C*-(2, 3, 4, 6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl)-propan-2-one ethylene ketal (9).

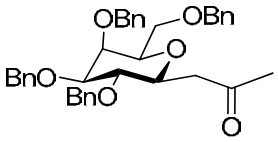
Compound **7** (550 mg, 1.27 mmol) was dissolved in CH_3OH (15 mL), cooled to 0 °C, and treated with Na (23 mg, 1.0 mmol). After 2 h, the solvent was removed under reduced pressure to yield crude **8**. The crude product was dissolved in DMF (10 mL) and cooled to 0 °C. NaH (262 mg, 60%, 7.8 mmol) was added and the reaction was stirred for 30 min. Benzyl bromide (0.8 mL) was added, and the reaction was stirred at room temperature for 6 h before methanol (1 mL) was added. The mixture was extracted with EtOAc, washed with water, and dried over Na_2SO_4. The solvents were removed under reduced pressure to yield **9** as a syrup that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.29 (m, 20H, PhH), 5.05 (d, *J* = 11.2 Hz, 1H, -OC*H*Ph), 5.01 (d, *J* = 11.8 Hz, 1H, -OC*H*Ph), 4.82 (d, *J* = 11.8 Hz, 1H, -OC*H*Ph), 4.74 (d, *J* = 11.8 Hz, 1H, -OC*H*Ph), 4.73 – 4.67 (m, 2H, -CH_2Ph), 4.58 (d, *J* = 11.9 Hz, 1H, -OC*H*Ph), 4.53 (d, *J* = 11.9 Hz, 1H, -OC*H*Ph), 4.06 (d, *J* = 2.5 Hz,, H-4), 3.99 – 3.83 (m, 4H, -OCH_2CH_2O-), 3.75 – 3.65 (m, 2H, H-2,

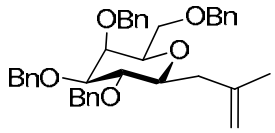
H-3), 3.64 – 3.59 (m, 3H, H-6a, H-6b, H-5), 3.52 (t, *J* = 9.0 Hz, 1H, H-1), 2.19 (d, *J* = 15.5 Hz, 1H,H-1'a), 1.87 (dd, *J* = 9.0, 15.5 Hz, 1H,H-1'b), 1.48 (s, 3H, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.6, 138.4, 138.1, 128.6, 128.49, 128.45, 128.40, 128.37, 128.28, 127.98, 127.91, 127.78, 127.70, 127.67, 127.65, 127.60, 126.99, 109.4, 85.1, 78.6, 77.0, 76.87, 76.83, 73.8, 73.5, 72.4, 69.2, 65.3, 64.39, 64.35, 39.6, 24.7.

HRMS(ESI): calcd for $C_{39}H_{44}NaO_7$ $[M+Na]^+$: 647.2979, found 647.2987.



1-*C*-(2, 3, 4, 6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-propan-2-one (10). Crude benzyl ketal **9** was dissolved in CH_2Cl_2/TFA/H_2O (10:1:0.1, 10 mL total) and the reaction was stirred for 30 min. The reaction was diluted with CH_2Cl_2, washed with water and saturated $NaHCO_3$ solution, and dried over Na_2SO_4. Silica gel chromatography purification using hexanes/ethyl acetate 5:1 to 3:1 afforded the product as a white solid (675 mg, 1.16 mmol, 89.2% for 4 steps). The ^{1}H and ^{13}CNMR of **10** spectra matches previously reported data.[33]



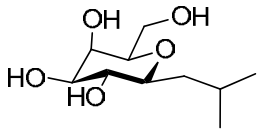
2-(1-*C*-(2, 3, 4, 6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl) methyl)-propene (11). To a flask containing PPh_3CH_3Br (830 mg, 2.32 mmol) and THF (5 mL) was added *t*BuOK (1 M in THF, 2.9 mL, 2.9 mmol) at 0 °C and the reaction was stirred for 30 min. A solution of **10** (675 mg, 1.16 mmol) in THF (7 mL) was added dropwise into the reaction, and the mixture was stirred for 8 h. A saturated

NH_4Cl solution (10 mL) was added to quench the reaction, and the reaction was extracted with EtOAc, washed with water, and dried over Na_2SO_4. Solvents were removed under reduced pressure, and silica gel column purification using hexanes/ethyl acetate 6:1 to 4:1 offered the product **11** (281 mg, 0.49 mmol, 42%).

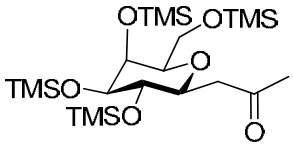
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.23 (m, 20H, PhH), 4.96 (d, *J* = 11.2 Hz, 1H, -OC*H*Ph), 4.95 (d, *J* = 11.6 Hz, 1H, -OC*H*Ph), 4.77 (m, 2H, C=CH_2), 4.76 (d, *J* = 12.9 Hz, 1H, -OC*H*Ph), 4.68 (d, *J* = 12.9 Hz, 1H, -OC*H*Ph), 4.66 (d, *J* = 11.2 Hz, 1H, -OC*H*Ph), 4.64 (d, *J* = 11.6 Hz, 1H, -OC*H*Ph), 4.45(ABq, *J* = 11.8 Hz, 2H, -OC*H*2Ph), 3.99 (m, 1H, H-4), 3.67 (t, 1H, *J* = 8.8 Hz, H-2), 3.63(dd, 1H, *J* = 2.5, 8.8 Hz, H-3), 3.59-3.48 (m, 3H, H-6a, H-6b, H-5), 3.41 (dt, *J* = 1.6, 9.4 Hz, H-1), 2.57 (d, 1H, *J* = 14.6 Hz, H-1'a), 2.25 (dd, 1H, *J* = 9.4, 14.6 Hz, H-1'b), 1.76 (s, 3H, CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 143.1, 138.8, 138.5, 138.4, 138.1, 128.42, 128.36, 128.19, 128.14, 128.02, 127.85, 127.68, 127.65, 127.62, 127.55, 127.53, 112.1, 85.0, 78.9, 78.7, 77.2, 75.4, 74.4, 73.8, 73.5, 72.3, 69.2, 39.8, 23.0.

HRMS(ESI): calcd for $C_{38}H_{42}NaO_5$ $[M+Na]^+$: 601.2924, found 601.2939.



Isobutyl-*C*-galactoside (1) by the benzyl ketone route: To a solution of **11** (281 mg, 0.49 mmol) in methanol was added 10% Pd/C (50 mg), and the reaction was stirred under H_2 for 8 h. The reaction was filtered and concentrated to give **1** as a hygroscopic white foam (101 mg, 0.46 mmol, 94%). The ^{1}H and ^{13}C NMR data of **1** matches the reported.[16]



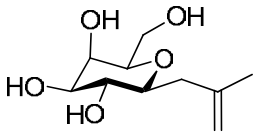
1-*C*-(2, 3, 4, 6-Tetra-*O*-trimethylsilyl-*β*-D-galactopyranosyl)-propan-2-one (12). Crude **4** (22.60 g) was synthesized from galactose (10.00 g, 55.5 mmol), $NaHCO_3$ (18.65 g, 222 mmol), and 2,4-pen-tanedione **3** (11.45 mL, 111 mmol, freshly distilled) in water (50 mL) and THF (25 mL) according to the above procedure. The crude product obtained from methanol/ethyl acetate extraction contained *ca.* 10.40 g NaOAc as indicated by ^{1}H NMR and used directly.

To a round bottom flask containing crude **4** was added CH_2Cl_2 (100 mL), and Et_3N (263 mL, 1.89 mol). The reaction was cooled to 0 °C, and freshly distilled chlorotrimethylsilane (48.3 mL, 380 mmol) was added dropwise. The reaction was stirred at ambient temperature for 16 h before the solvents were removed at reduced pressure. Hexanes were added to the mixture, and the solution was filtered through Celite, washed with hexanes, and concentrated to yield crude **12** as a yellow liquid that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88(d, *J* = 2.3 Hz, 1H, H-4), 3.65 – 3.60 (m, 2H, H-1, H-5), 3.57 -3.47 (m, 2H, H-6a, H-6b), 3.45-3.38 (m, 2H, H-2, H-3), 2.68 (dd, *J* = 1.8, 15.6 Hz, 1H, H-1'a), 2.58 (dd, *J* = 7.0, 15.6 Hz, 1H, H-1'b), 2.18 (s, 3H, CH_3), 0.17 (s, 9H, TMS), 0.13 (s, 9H, TMS),0.128 (s, 9H, TMS), 0.08 (s, 9H, TMS)

^{13}C NMR (101 MHz, $CDCl_3$) δ 208.07, 79.43, 78.16, 77.23, 72.68, 71.84, 61.49, 46.88, 31.27, 1.50, 1.26, 0.97, 0.03.

HRMS(ESI) calcd for $C_{21}H_{48}NaO_6Si_4$ $[M+Na]^+$ 531.2420 found 531.2408



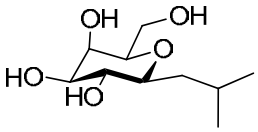
2-(1-*C*-*β*-D-Galactopyranosyl methyl)-propene (14). To a round bottom flask was added anhydrous PPh_3CH_3Br (29.80 g, 83.4 mmol) and *t*BuOK (9.36 g, 83.4 mmol). The reaction was cooled to 0 °C and THF (200 mL) was added via cannula. The resulting yellow solution was stirred at 0 °C for 5 min and warmed up to room temperature in 25 min. The solution was stirred at room temperature for another 30 min and re-cooled to 0 °C. A solution of crude **12** in THF (30 mL) was added dropwise to the reaction over 10 min. After stirring at 0 °C for another 10 min, the ice bath was removed and the reaction was stirred for 90 min. Acetone (5 mL) was added, the reaction was partially concentrated to slurry, diluted with hexanes and filtered. The filtrate was concentrated to yield crude **13** as a yellow liquid that was used without purification.

The crude product **13** was dissolved in methanol/water (120 mL/10 mL). Dowex 50X8 (H^+ form, 10.00 g) was added, and the reaction was stirred for 25 min, filtered, and concentrated under reduced pressure. The resulting residue was suspended in water (250 mL) and extracted with ethyl acetate (3 x 100 mL). The aqueous layer was concentrated to give crude **14** as a pale yellow liquid that was used without further purification.

^{1}H NMR (400 MHz, D_2O) δ 4.70 (d, 2H, *J* = 9.8 Hz, C=CH_2), 3.79 (d, 1H, *J* = 3.3 Hz, H-4), 3.58 – 3.48 (m, 2H, H-6ab), 3.48 – 3.41 (m, 2H, H-3, H-5), 3.31 – 3.26 (m, 2H, H-1, H-2), 2.44 (d, *J* = 15.7 Hz, 1H, H-1'a), 2.03 (dd, *J* = 9.0, 15.7 Hz, H-1'b), 1.61 (s, 3H, CH_3).

^{13}C NMR (100 MHz, D_2O) δ 143.9, 111.9, 78.5, 77.6, 73.9, 71.0, 70.0, 61.2, 39.2, 21.6.

HRMS(ESI): calcd for $C_{20}H_{36}NaO_{10}$ $[2M+Na]^+$:459.2201, found 459.2201.



Isobutyl-*C*-galactoside (1) from the per-*O*-silylated ketone. Crude **14** was dissolved in ethanol (100 mL) and a slurry of 10% Pd/C (950 mg) in water (5 mL) was added. The reaction was stirred in a Parr apparatus under H_2 at 1000 psi for 1 h. The pressure was dropped to 800 psi and then stayed stable, indicating the hydrogenation had finished. The mixture was filtered and concentrated to give **1** as a white solid (9.90 g, 45.4 mmol, 81% for 5 steps). The ^{1}H and ^{13}CNMR of **1** spectra matches previously reported data.[16]

Induction of *lac* operon using IBCG in Hela cells.

HeLa cells were cultured in DMEM (Dubelcco's Modified Eagle Medium) supplemented with 10% FCS (fetal calf serum) and penicillin/streptomycin at 37 °C with 5% CO_2. A detailed description of the construct cloning and generation of transgenic cells will be reported elsewhere. For the induction 1 mM IPTG (Fermentas GmbH) or 1 mM IBCG (both dissolved in water) were added. At the indicated time points HeLa cells were trypsinized and acquired on a FACSCanto II (BD Biosciences). Dead cells were excluded from the analysis by staining with 7-aminoactinomyxin (7-AAD). Data analysis was conducted with FlowJo software (Tree Star). For calculating the normalized Neptune values, mean fluorescent Neptune intensities were divided by the mean fluorescent intensities of GFP in the same cells. This value was then divided by the Neptune to GFP ratio in CAGop-Neptune/CAG-GFP HeLa cells lacking lacI and multiplied by 100.

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CHPATER 5

Studies Towards the Automated Synthesis of Hyaluronic Acid Fragments

Abstract

Hyaluronic acid is repeating polymer of two sugar building blocks that serves a variety of biological functions and is structurally the simplest of the larger class of glycosaminoglycans. To synthesize hyaluronic acid oligosaccharide fragments for applications such as mass spectrometry and for biological assays, six different synthetic strategies were studied and compared. To overcome the severely reduced reactivity due to the strong electron withdrawing properties of the uronic acid building block—one of the major difficulties in glycosaminoglycan synthesis—a new method was developed to install benzyl groups at the 4-,6- positions on the trichloroacetyl-protected glucosamine building block and at the 2-,3- positions on glucuronic acid. This strategy allowed the synthesis of the more electron rich hyaluronic disaccharide building blocks that provided the best results in subsequent glycosylation reactions of the disaccharide unit. Using fluorous-assisted synthesis and conditions amenable to solution-phase automated synthesis, a tetrasaccharide fragment of hyaluronic acid was successfully synthesized as the first step towards the automated synthesis of fragments of this size and longer.

Introduction

Hyaluronic acid (HA) is a linear, unbranched repeating polymer of *N*-acetyl glucosamine and D-glucuronic acid. It is the only member of the glycosaminoglycan family that is not sulfated and therefore is a relatively simple structure. Hyaluronic acid is distributed widely throughout connective, epithelial, and neural tissues and is involved with myriad functions including cellular proliferation, cell–cell recognition, and cell migration.[1,2] As the main function of this glycosaminoglycan family member was believed to be as a polymer for lubrication of joints, HA has received relatively little attention for years. However, more recently biological functions of shorter hyaluronic acid fragments are being discovered.[3] The degradation products of hyaluronic acid, small oligosaccharides with varying lengths, exhibit pro-angiogenic properties.[4] Short fragments of hyaluronic acid can induce inflammatory responses in macrophages and dendritic cells in tissue injury and in skin transplant rejection.[5] Hyaluronic acid is also the main component of the capsular polysaccharide of Group A streptococci (*Streptococcus pyogenes* or GAS).[6] Abundant production of hyaluronic acid in GAS is a key virulence determinant and is related with severe GAS infections.[7,8] Low molecular weight hyaluronic acid is now being studied as a treatment and prevention of infection and disease caused by group A and group C Streptococci.[9]

Given the increasing biological interest in HA, there have been many reports on the synthesis of hyaluronic acid fragment;[10] those syntheses mainly differ in the choice of protecting groups

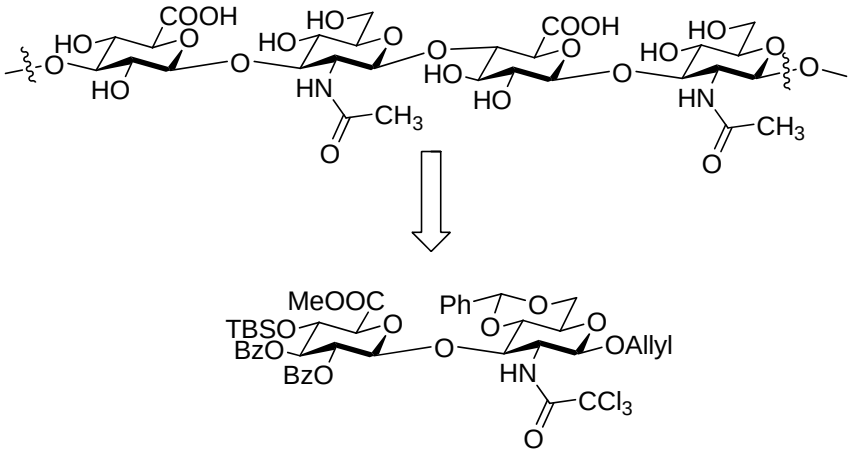
(especially on the amino group), the choice of anomeric activating group and the strategy of the oxidation.[11-21] In recent years, great advancements were made in this area. For example, van der Marel and coworkers have reported the synthesis of the tri-, penta-, and heptamer of hyaluronic acid.[22,23] Huang and coworkers have reported the synthesis of a dodecamer of hyaluronic acid.[10,24,25] Nonetheless, it is still a daunting task to perform the synthesis to obtain different lengths of hyaluronic acid fragments; ideally a strategy amenable to automation could be developed to eliminate some of the tedium in producing this class of glycosaminoglycans to accelerate biological studies.

The major difficulty in the synthesis of hyaluronic acid is also the biggest issue in the synthesis of glycosaminoglycans.[26-28] The uronic acid residues in the glycosaminoglycan structure have very low chemical relativities. The strong electron withdrawing nature of the uronic acid makes the building block relatively electron deficient, making it both a weak glycosyl donor and also a weak glycosyl acceptor, especially when functioning as a donor at the 4-position with a uronic acid at the 6-position. At the monosaccharide stage, the low reactivity issue is not so severe; however, the reactivity becomes a serious issue when the oligosaccharides get longer. As the chain lengthens, the reactivity of the donor and acceptor are reduced even more. The synthesis of HA fragments also requires a sophisticated protecting group strategy to achieve the desired coupling patterns.

In addition to the challenges of finding a suitable protecting group pattern for each building block, a second challenge is finding methods to automate the process of stringing together the building blocks.

Recently the Pohl group has developed an automated solution-phase synthesis platform based in part on fluorous solid-phase extractions.[29,30] This new automation platform provides a promising new way of synthesizing oligosaccharides without the use of large excesses of building blocks at each coupling stage as is required for biphasic processes such as solid-phase synthesis. The fluorous tag used to aid in the automated purification scheme can also function as the immobilization tail for fluorous microarrays,[31-34] providing a convenient way of detecting the binding of sugars with biomolecules such as lectins. However, the existing methods for the synthesis of hyaluronic acid are not suitable for the automated solution-phase synthesis. Herein we report studies toward developing a simple and efficient strategy which is amenable to automation for the solution and fluorous-assisted synthesis of HA fragments.

Results and discussion

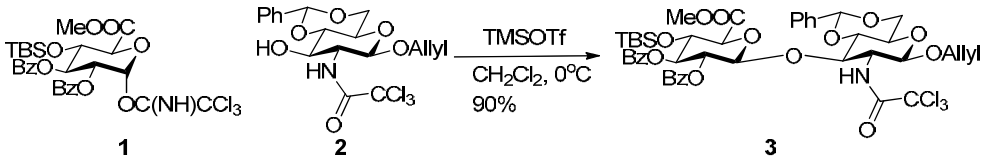


Scheme 1 The retrosynthesis of hyaluronic acid

Due to the polymeric nature of hyaluronic acid, we decided to use a disaccharide building block—the basic repeating unit of the polymer—approach in the synthesis (Scheme 1). Both pre-oxidation and post-oxidation have been used in the synthesis of hyaluronic acids. It is reported that post-oxidation sometimes causes problems on larger oligosaccharide,[24] so we first chose to use pre-oxidation at the monosaccharide stage. To alleviate the low reactivity problems caused by the strong electron withdrawing group by the uronic acid, glucosamine was put at the reducing end of the disaccharide building block. This way, the disaccharide donor could have better reactivity in glycosylation reactions. Both literature and our previous studies indicated that phthalamide group as N-protecting group had problems in removal,[10] so trichloroacetyl group was used as the protecting group for the amino group. We have use thiol phenol group as the temporary protecting group at the anomeric position of the disaccharide, but it suffered from low yield during removal when forming the disaccharide hemiacetal. After a survey of different protecting groups for anomeric positions, allyl group was chosen as the temporary protecting group for glucosamine at the anomeric position for its stability under various conditions and also for it could be removed under mild conditions in high yield. A transition metal catalyzed isomerization followed by mild hydrolysis should provide the hemiacetal.

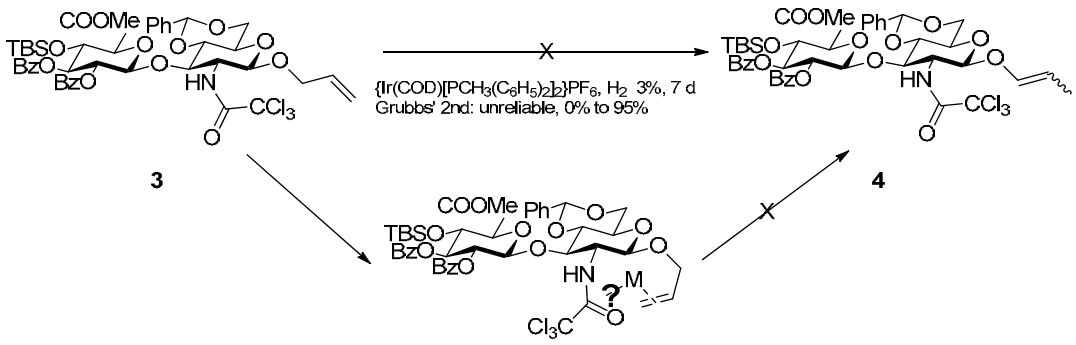
The glucuronic trichloroacetimidate donor **1** was synthesized from glucose in 10 steps via a modified route from literature procedure.[35,36] TBS group was chosen as the temporary protecting group for 4 position on the glucuronic acid building block, and the 2, 3 position were protected as benzoyl esters.

The allyl NHTCA-glucosamine donor **2** was synthesized from glucosamine in 6 steps.[37] 4,6 position of the glucosamine building block was protected as benzylidene acetal. The glycosylation between the glucuronic acid donor **1** and the glucosamine acceptor **2** in the presence of TMSOTf as a promoter in CH_2Cl_2 worked smoothly to give the disaccharide **3** with allyl group at the anomeric position, through an orthoester intermediate, in 90% yield. (Scheme 2)



Scheme 2 Synthesis of disaccharide building block **3**.

However, we encountered problems when trying to remove the anomeric allyl protecting group on **3** to synthesis the disaccharide donor. Route catalyst for the isomerization of the alkene didn't work. The widely used $[Ir(COD)(PMePh_2)_2]PF_6$ catalyst[38] gave a very slow reaction, which only gave ~3% conversion after 7 days. Wilkinson's catalyst and $Pd(PPh_3)_4$ gave no isomerization reaction at all. (Scheme 3)



Scheme 3 Isomerization problem with the benzylidene-protected disaccharide **3**

This was quite an unexpected situation. In Hsieh-Wilson's synthesis of chondroitin sulfates,[35] they had encountered a similar situation when trying to remove the anomeric allyl on a trichloroacetyl protected galactosamine monosaccharide building block. They proposed that the problem encountered in the isomerization was from the trichloroacetyl group, because the allyl group on a NHAc substrate instead of on the NHTCA substrate isomerized without any problem. In their study, they finally used the Grubbs' second-generation catalyst to perform the isomerization. We found out that using Grubbs' catalyst gave very unreliable results. The conversion we achieved ranged from 0% to 95%, depending on different batches of catalyst used. Grubbs had reported the actual active catalyst for the alkene isomerization reaction was a decomposition product of Grubbs' catalyst,[39,40] and we thought the irreproducibility of this reaction might had been caused by the batch and age differences of the Grubbs' catalyst.

Another choice to remove the allyl group is to use stoichiometric metal reagents. In Sleeman and Brãse's synthesis of hyaluronic acid,[37] they used 2 eq of $PdCl_2$ in neat acetic acid to cleave the allyl group. Given that most of the catalytic version of the alkene isomerization reaction only requires less than 1mol% catalyst, apparently the deallylation on trichloroacetyl protected amino sugars was still a problem.

Before switching to other anomeric protecting group like silyl groups, we tried to find out why the catalytic alkene isomerization wouldn't work on the NHTCA substrate. Finally, the 4, 6 benzylidene

acetal on the glucosamine building block caught our attention. Benzylidene acetal is one of the most common protecting groups used in carbohydrate chemistry. 4, 6-benzylidene would form a six-membered ring. Fused with glucose, the whole molecule would form a trans-decalin structure. This decalin structure provided a very rigid ring structure, a scaffold suitable to serve as metal ligand if proper groups were presented correctly. Indeed, in several cases, benzylidene protected carbohydrates-derived diarylphosphinites were used to as ligands for metal.[4] In Hsieh-Wilson's studies, they proved that trichloroacetyl group was involved with the isomerization problem. In our study, we found out that even though Iridium catalyzed reaction couldn't give a useable conversion, there was still some conversion. Apparently the deactivation of the catalyst occurred in the reaction.

Based on these examples, we reasoned that due to the presence of benzylidene on the glucosamine building block, the trichloroacetyl group and double bond were close with each other in space due to the rigid conformation.(Scheme 3) Both the rigid conformation led by the benzylidene and the presence of trichloroacetyl group could contribute to the binding. What exactly was the deactivating mechanism for the catalyst was unknown. If benzylidene could be removed, the hexose ring could flip freely, so the alkene and the trichloroacetyl group might not be in a pre-arranged configuration, the isomerization reaction might be able to work. And since 4, 6- dibenzyl group would increase the electron density of the ring, the isomerization could be benefited from the increased electron density.

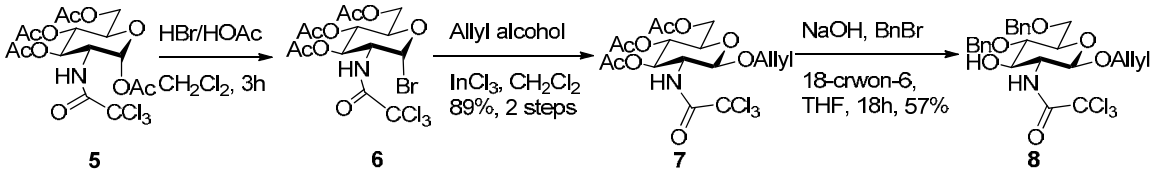
To test this hypothesis, we decide to remove the benzylidene on the glucosamine building block and

use benzyl groups on the 4 and 6 positions instead. However, looking into the literature of carbohydrate chemistry, there were very few examples with 4, 6 dibenzyl protecting groups on a trichloroacetyl protected 2-amion sugar. Even though the 4, 6-dibenzyl protection was widely used on the 2-azido glucosamine building blocks, it was rarely used on trichloroacetyl protected glucosamine. Apparently synthesizing 4, 6-dibenzyl trichloroacetyl protected glucosamine was non trivial at all because of the base sensitivity of trichloroacetyl group. Benzylation of the 3-OH on the trichloroacetyl protected glucosamine had been achieved using NaH/BnBr at low temperatures; however, using the routine conditions to benzylate the 4, 6 position would cleave the trichloroacetyl group, and then the amine would be benzylated. A few existing examples either started with the 2-azide protected glucosamine,[41] or involved the selective opening of the benzylidene acetal to form a primary alcohol at 6 position,[42] and then the 6-OH was benzylated under non-basic conditions like BnBr/Ag_2O or 2-Benzyloxy-1-methylpyridinium triflate.[43] However, none of these methods gave satisfactory results due to low yield of the benzylation step and the long step count.

The ongoing chemistry being developed in our lab allowed the installation of benzyl groups selectively on the trichloroacetyl protected glycosamines by using benzyl bromide with sodium hydroxide powder in the presence of catalytic amount of 18-crown-6 in THF. Due the inherent steric hindrance, if the reaction was monitored carefully, on a 3,4, 6 triol, the 4, 6 position would be benzylated selectively, leaving 3-OH unprotected. No N-benzylation or trichloroacetyl group cleavage was detected in the reaction. The method was further modified in this study by using a large

excess of sodium hydroxide, and adding equal weight of 18-crown-6 as sodium hydroxide. These modifications greatly speed up the reaction. This method provide a way of synthesizing the desired 4, 6-dibenzyl protected NHTCA protected glucosamine building block.

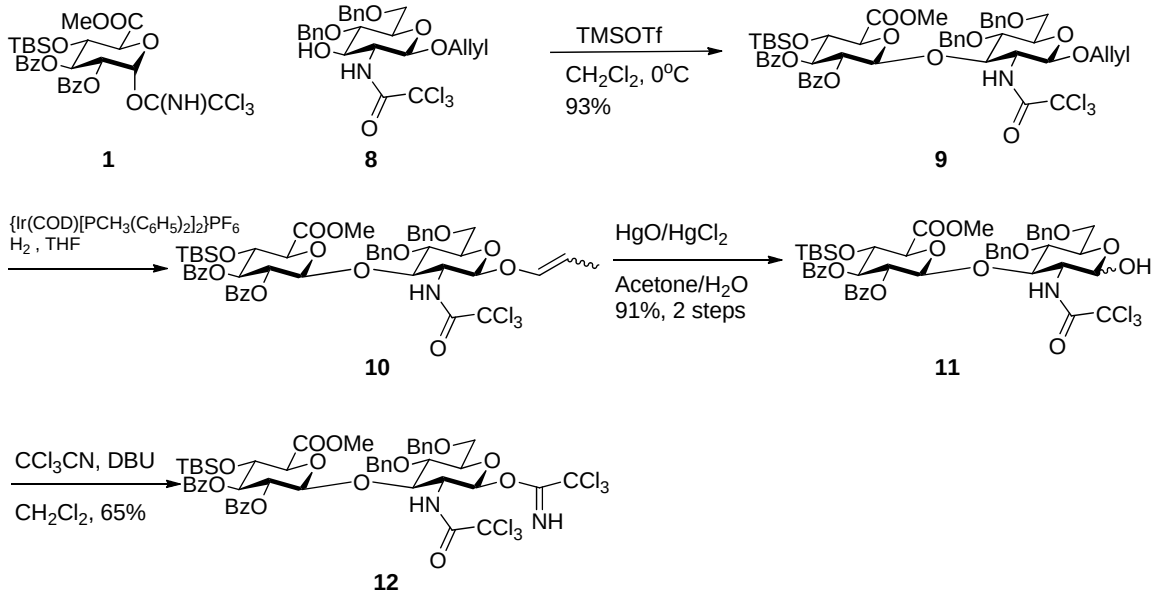
Utilizing an $InCl_3$ promoted glycosylation between easily prepared glycosyl bromide **6** and allyl alcohol,[37] the allyl 3,4,6-tri acetyl 2-trichloroacetyl amino glucosamine **7** was obtained in high yield and could be used without purification. Under the BnBr/NaOH/18-crown-6 conditions, the desired 4,6-dibenyl product was obtained in 57% yield along with small amount of tri-benzyl protected product in grams scale in a single step.(Scheme 4) If the routine benzylidene opening-non-basic benzylation route was used, it would have to use 6 steps with very low yield.



Scheme 4 Synthesis of 4,6 dibenzyl NHTCA building block **8** using phase transfer conditions.

The glycosylation between the new glucosamine acceptor **8** and the glucuronic donor **1** worked without problem to give the disaccharide in 93% yield. If our hypothesis is correct, the catalytic allyl isomerization reaction should work much better than the benzylidene protected version. As expected, the Ir catalyzed isomerization worked very nicely with full conversion in only 2 hours with 1% of

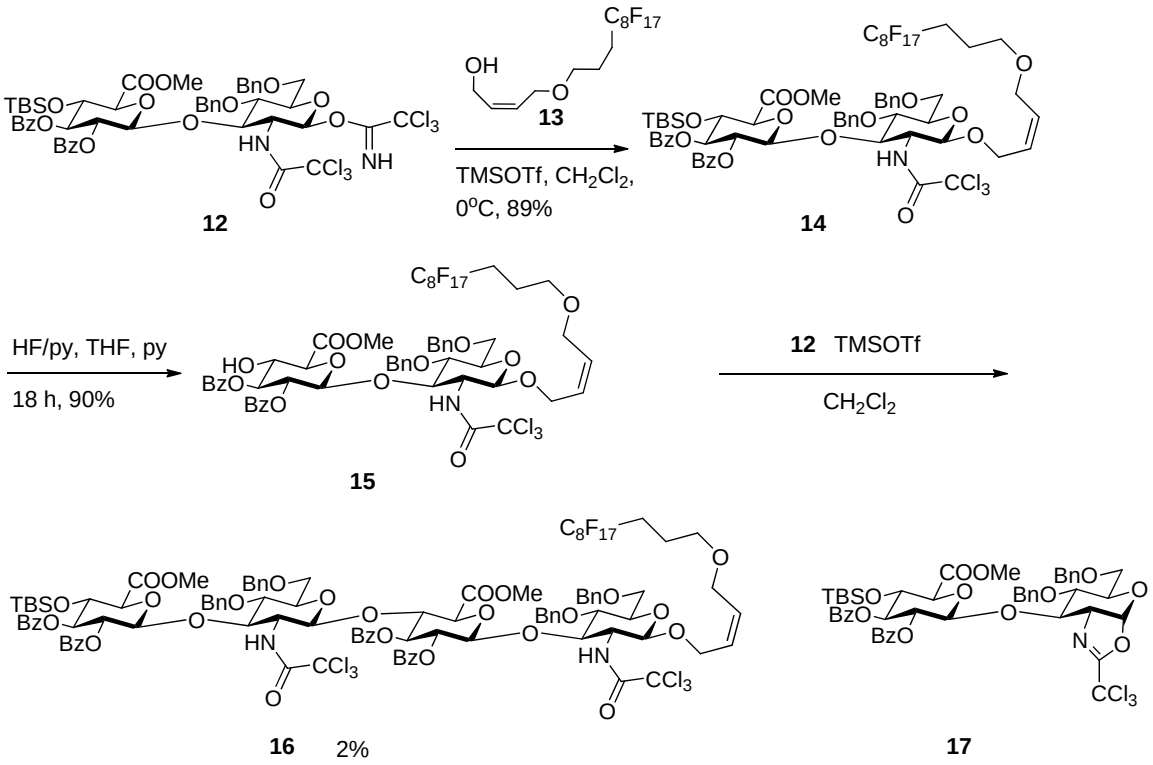
catalyst loading to give **10**. The isomerized alkene **10** was hydrolyzed, and converted to the trichloroacetimidate donor **12**. (Scheme 5) More detailed studies would be needed to elucidate the exact deactivating mechanism of the Iridium catalyst. This again proved that the protecting groups used in carbohydrate chemistry cannot be treated as inert groups severing solely protecting functions; they can also have a profound influence of the reactivity of the substrate.



Scheme 5 Ally deprotection and synthesis of the disaccharide donor.

Fluorous tag **13** [44] was used as the acceptor in the glycosylation reaction with donor **12** to install the fluorous handle for FSPE purification. The TBS group was removed by using HF/pyridine to afford the disaccharide acceptor **14** with the free 4-OH on the guluronic acid residue as the chain elongation point. However, we encountered problems in the [2+2] coupling reaction. The disaccharide acceptor **15** and donor **12** were reacted in CH_2Cl_2 in the presence of catalytic amount of TMSOTf as promoter.

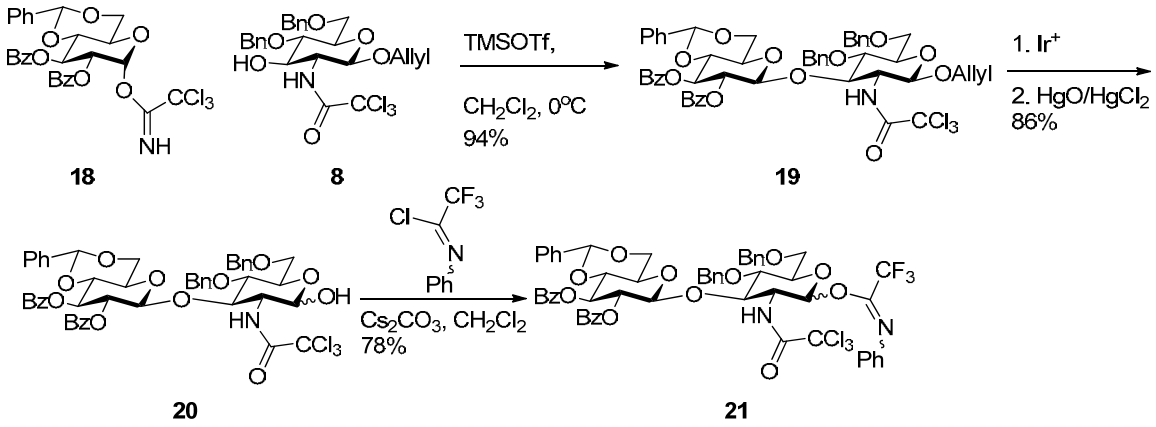
The majority of the imidate donor formed oxazoline byproduct **17**; only trace amount of the desired tetrasaccharide **16** was formed. Using various conditions to improve the reaction failed. Failure of the glycosylation was attributed to the low reactivity of the acceptor, possibly due to the strong electron withdrawing ester group and disarming benzoyl groups.



Scheme 6 Attempt 2+2 glycosylation to synthesis the tetrasaccharide

Since the electron withdrawing uronic acid lowered the reactivity of the acceptor, post oxidation strategy was probed in the synthesis of hyaluronic oligomers and analogs. Acceptor **8** was glycosylated with 4,6 benzylidene protected glucose donor **18**, which was synthesized from glucose in 7 steps.[45] The glycosylation gave the disaccharide **19**, which was suitable for post-oxidation. The

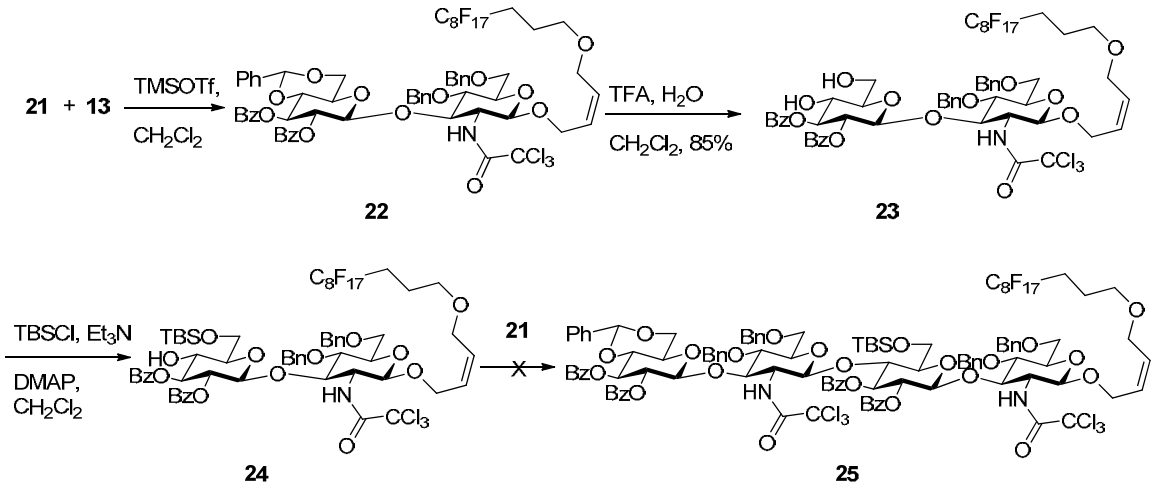
allyl group on **19** was removed, and the anomeric hydroxyl was activated using N-phenyltrifluoroacetimidates.[46] Trifluoroacetimidates could give the donor a little more stability.(Scheme 7)



Scheme 7 Synthesis of disaccharide donor **21** used in post oxidation

The coupling of the trifluo imidate **21** with fluorous tag as the acceptor proceeded smoothly at 0 °C in CH_2Cl_2 in 94% yield. After benzylidene removal, the primary hydroxyl at 6' -position was protected with TBS group using TBSCl in CH_2Cl_2 in the presence of DMAP using Et_3N as base.[47] The reaction was very slow at room temperature, but we found out that the solution could be heated to 38 – 40 °C to speed up the reaction greatly. The [2 + 2] glycosylation was attempted under different conditions, however, only tract amount of the product was detected. In CH_2Cl_2 at 0 °C using 0.1 eq TMSOTf, the donor hydrolyzed very quickly. In toluene as the solvent, the donor was considerably more stable; however, the coupling was still not going. (Scheme 8) The reason of the failed glycosylation was attributed to the steric hindrance from the TBS group at the 6' position and the dibenzoyl disarming

protecting groups.



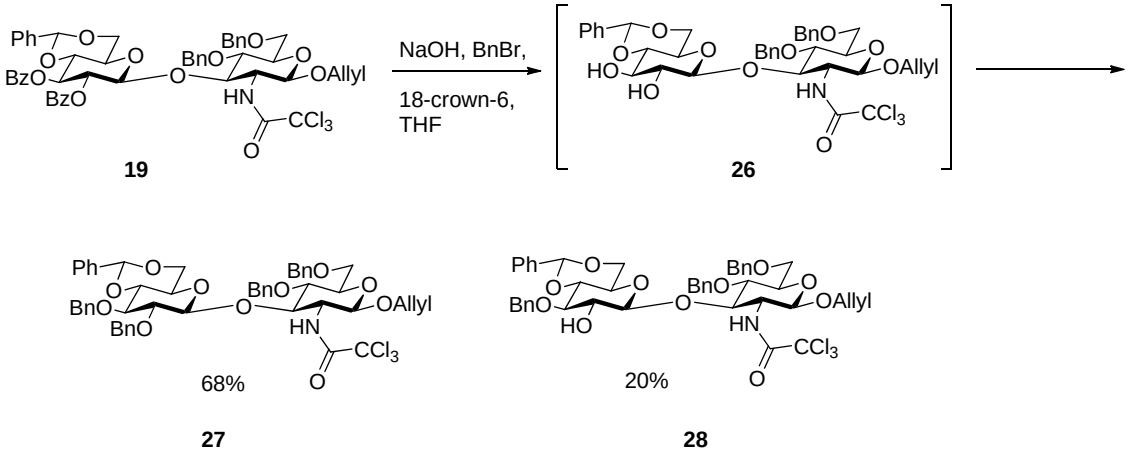
Scheme 8 Attempt synthesis of tetrasaccharide applying post-oxidation strategy

When the donor and acceptor became larger, their reactivity decreased due to steric and electronic reasons. So increasing the reactivity, especially for uronic acid disaccharide acceptor had become more important. To increase the reactivity of the acceptor, more electron donating groups, instead of electron withdrawing groups should be installed on the glucuronic building block. In the former synthesis of hyaluronic acids, 2-Bz 3-Bn protected uronic acid building blocks had been used several times to improve the activity issue.[25,48] We reasoned that if we could synthesize a disaccharide building block with a uronic acid residue bearing 2,3-dibenzyl protecting groups, the reactivity of the building block might be further improved. 2-Bz 3-Bn protected uronic acid building blocks could be synthesized fairly easily; however, synthesizing a disaccharide building block with a 2, 3-dibenzyl uronic acid residue was much more difficult and hadn't been reported. The benzyl group cannot be

installed on the 2 position glucuronic building block prior to glycosylation since the neighboring group participation from acyl group was needed to form beta-glycosidic bond. Therefore, a method to do benzylation on the disaccharide had to be developed to synthesize the disaccharide building block with a 2,3-dibenzyl glucuronic acid residue.

We decide to use the benzylation method which was applied earlier using BnBr/NaOH/18-crown-6 in THF in the synthesis of the 4,6 dibenzyl NHTCA building block to perform the 2, 3 dibenzylation on the disaccharide. In this case, oxidation after the formation of the benzyl ether at the disaccharide stage to the glucuronic acid had to be used.

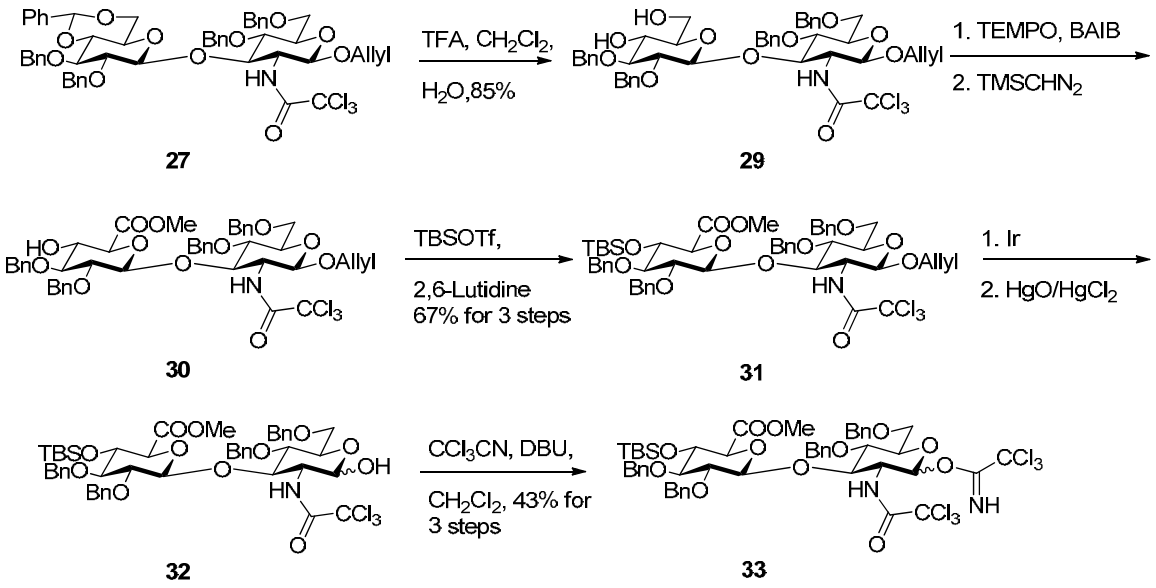
The 2, 3-dibenzoyl disaccharide was subject to the BnBr/NaOH/18-crown-6 benzylation reaction, and the 2, 3 –dibenzyl disaccharide was isolated in 68% yield, along with the 3-Bn product in 20% yield. (Scheme 9)



19 **26** **27** **28**

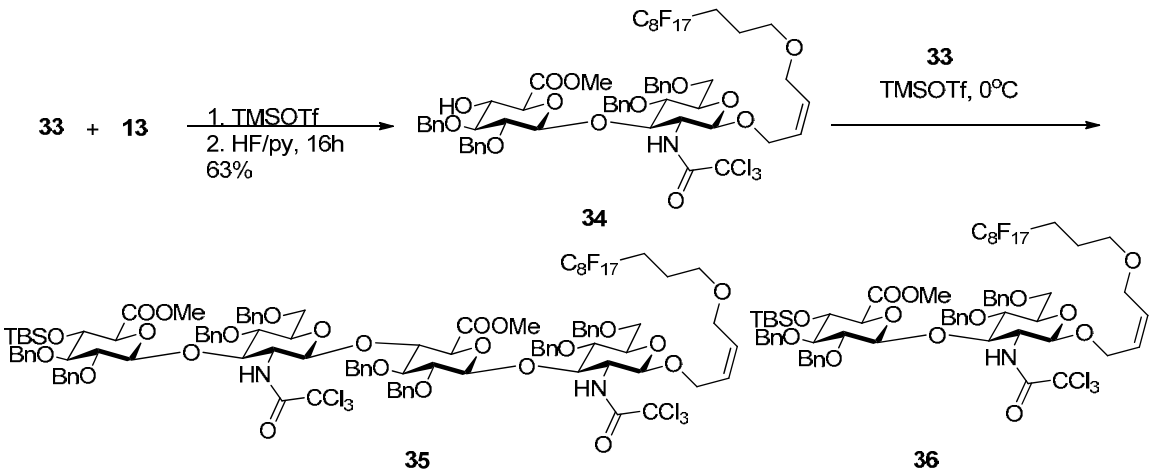
Scheme 9 Synthesis of the tetra benzyl substituted disaccharide.

With the desired tetra- benzyl substituted disaccharide in hand, the benzylidene was cleaved, primary alcohol oxidized into carboxylic acid using BAIB/TEMPO and protected as methyl ester using $TMSCHN_2$. The 4-OH was then protected using TBS group with TBSOTf/Lutidine. Ally group was removed, and the disaccharide was transformed into the imidate donor in 3 steps. (Scheme 10)



Scheme 10 Synthesis the disaccharide uronic acid donor **33**.

The imidate **33** was coupled with the fluorous tag. TBS group was removed with HF/py to furnish the disaccharide acceptor. (Scheme 11) In the initial experiment, the desired tetrasaccharide **35** was obtained in about 10% yield in CH_2Cl_2. The glycosylation on this tetra-benzyl disaccharide worked better than the previous di benzyl building blocks. Further optimization is still under way.



Scheme 11 Synthesis of the tetrasaccharide **35** using the tetra benzyl donor and acceptor

Conclusion

In the studies toward the synthesis of hyaluronic acid oligomers, several different protecting group patterns were tested. We encountered an unexpected problem when performing the transitional metal catalyzed terminal alkene isomerization on the benzylidene acetal protected NHTCA glucosamine. This problem was solved by changing the protection pattern to 4,6-dibenzyl. An optimized method was developed for the benzylation of NHTCA substrates. A tetra-benzyl substituted disaccharide building block was synthesized, and in the initial glycosylation studies, it showed better reactivity than the previously used di-benzoyl building blocks. The optimization of the glycosylation is the next work in this study.

Experiment section

General Methods: Reactions were performed using flame-dried glassware under argon using

anhydrous solvents unless otherwise noted. Ambient temperature in the laboratory was usually 20 °C. CH_2Cl_2 were distilled freshly from CaH_2. Commercially available reagents were obtained from Aldrich, Fisher or TCI and used as received.

Thin layer chromatography (TLC) was performed using glass backed Silica Gel HL TLC plates w/UV254 from Sorbent Technologies. Visualization of TLC plates was performed by UV, 5% sulfuric acid/ethanol, or *p*-anisaldehyde/ethanol. Silica gel flash chromatography was performed using silica gel (60 Å, 40-63 μm) from ZEOChem AG.

NMR spectra were recorded on a Agilent-Varian 400MR (400 MHz for ^{1}H, 101 MHz for ^{13}C, 376MHz for ^{19}F, 162 MHz for ^{31}P), Varian VXR400 (400 MHz for ^{1}H, 101 MHz for ^{13}C) , Bruker DRX400 (400 MHz for ^{1}H, 101 MHz for ^{13}C, 162 MHz for ^{31}P), or Bruker AVIII600 (600 MHz for ^{1}H, 150 MHz for ^{13}C, 564MHz for ^{19}F). Chemical shifts are reported in parts per million (ppm) on the δ scale. ^{1}H NMR and ^{13}C NMR taken in $CDCl_3$ was referenced the solvent peak at 7.260 ppm (^{1}H) and 77.0 ppm (^{13}C). The assignments of ^{1}H NMR peaks were made primarily from 2D ^{1}H-^{1}H COSY and edited ^{1}H-^{13}C HSQC spectra. ^{1}H-^{13}C HMBC and ^{1}H-^{1}H TOCSY spectra were obtained to aid the assignments when necessary.

High resolution mass spectra (HRMS, ESI mode) were obtained using an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS at Iowa State University.

General Procedure for Fluorous Solid Phase Extraction (FSPE): A FSPE cartridge (2 g. Fluorous Technologies, Inc., Pittsburgh, PA) was preconditioned by passing 80:20 MeOH:H_2O (6 mL) through it under a vacuum. The crude mixture was loaded onto the cartridge by using no more than 2 mL of a 9:1 DMF:H_2O solution. The non-fluorous containing compounds were eluted by passing 6-8 mL of 80:20 MeOH:H_2O through the cartridge. The fluorous containing compounds were eluted by passing 6-8 mL of MeOH through the cartridge. The MeOH wash was concentrated under reduced pressure and the residue was coevaporated with toluene to provide the fluorous compounds. The cartridge was regenerated by washing using acetone.

General Procedure for Deallylation Reaction: Allyl protected compound (1 eq) and $[Ir(COD)(PMePh_2)_2]PF_6$ catalyst (0.01 eq) was dissolved in THF (0.05 M) to give a pink solution. H_2 was applied to the reaction for 5 – 10 seconds until the reaction turned yellow. The H_2 gas was purged out with Ar, then the reaction was stirred at room temperature until the NMR indicated the reaction was finished.

The solvent was removed under reduced pressure, and the crude mixture was dissolved in acetone/water 5/1. $HgCl_2$ (0.6 eq) and HgO (0.6 eq) was added, and the reaction was stirred until TLC indicated the reaction had finished. The reaction was filtered through Celite, concentrated, re-dissolved with CH_2Cl_2, washed with KI (aq), and dired with Na_2SO_4. The crude mixture was

purified using SGC.

General Procedure for Benzylidene Removal: The benzylidene bearing compound was dissolved in a mixture of CH_2Cl_2:TFA:H_2O (0.06 M, 10/1/0.1) and stirred at room temperature for 1 h. The reaction was diluted with CH_2Cl_2, washed with water, $NaHCO_3$ (aq), dried with Na_2SO_4, and concentrated. The crude mixture was purified using SGC.

General Procedure for Oxidation and Methylation of Primary Alcohol to Methyl Ester: The diol substrate (1 eq) was dissolved in CH_2Cl_2/H_2O 2:1 (0.3 M). TEMPO (0.2 eq) and BAIB (2.5 eq) was added, and the reaction was stirred until TLC indicated the oxidation had finished. The reaction mixture was extracted with EtOAc, washed with $Na_2S_2O_3$ (aq), dried and concentrated. The crude acid was dissolved in toluene/methanol 4:1 (0.1 M), and $TMSCHN_2$ (2 M in ether, 2 eq) was added. The yellow mixture was stirred for 30 min and quenched with HOAc. The reaction was concentrated and purified via SGC.

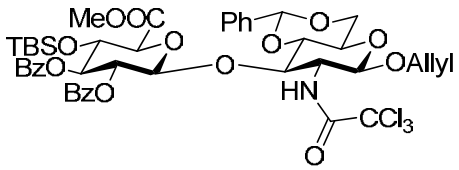
General Procedure for Trichloroacetimidate Formation: The substrate (1 eq) was dissolved in CH_2Cl_2 (0.1 M), Cs_2CO_3 (0.5 eq) was added, followed by CCl_3CN (3 eq). The reaction was stirred at r.t. until TLC indicated the conversion had finished. The reaction was filtered through Celite, and concentrated to give the crude imidate.

General Procedure for Glycosylation Reaction: The acceptor (1 eq) and donor (1.5 eq) was

co-evaporated with toluene for 3 times and dissolved in CH_2Cl_2 (0.05 M). The reaction was cooled to 0 °C, and TMSOTf (0.1 eq, 0.0268 M in CH_2Cl_2) was added and the reaction was stirred at 0 °C until TLC indicated the conversion had finished. The reaction was quenched by adding Et_3N, concentrated, and purified via SGC.

General Procedure for Selective TBS Protection: The diol substrate (1 eq) was dissolved in CH_2Cl_2, and Et_3N (10 eq), TBSCl (8 eq), DMAP (1 eq) was added. The reaction was stirred at 35 – 40 °C for 8 h. The reaction was quenched by adding methanol, concentrated, and purified by SGC.

General Procedure for TBS Cleavage: The substrate (1 eq) was dissolved in THF (0.1 M), and HF-pyridine (150 eq) was added. The reaction was stirred at r.t. until TLC indicated the conversion had finished. EtOAc was added to the reaction, and the mixture was washed with water, $NaHCO_3$(aq), brine, dried and concentrated. The crude mixture was purified via SGC.



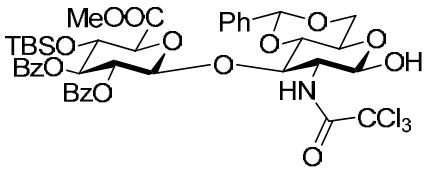
Allyl *O*-(methyl 2,3–*O*–di–benzoyl–4–*O*-tert-butyldimethylsilyl-β-D- glucopyrafnosyl uronate)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D- glucopyranoside (3) To a

round bottom flask was added acceptor **2** (100 mg, 0.221 mmol), donor **1** (224 mg, 0.331 mmol). The reactants were co-evaporated with toluene.5 mL CH_2Cl_2 was added, and the reaction was cooled to 0 °C. 4 μL TMSOTf was added, and the reaction was stirred at 0 °C for 50 min, then warmed up to room temperature. The reaction was quenched by adding 400 μL Et_3N, and concentrated. Purification using silica gel chromatography using hexanes/ethyl acetate 4:1 as eluting solution give product **3** (175 mg, 0.181 mmol, 82%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) 7.84 (d, *J* = 7.4 Hz, 4H, OBz), 7.54 – 7.24 (m, 11H, PhH), 6.81 (d, *J* = 7.0 Hz, 1H, NH), 5.85 – 5.72 (m, 1H, -*H*C=CH_2), 5.53 (s, 1H, PhCH), 5.44 (t, *J* = 9.1 Hz, 1H, H-3'), 5.34(t, *J* = 7.5 Hz, 1H, H-2'), 5.22 (dd, *J* = 1.6, 17.7 Hz, 1H, C=CH*H*), 5.16 (dd, *J* = 1.6, 10.0 Hz, 1H, C=CH*H*), 5.07 (d, *J* = 8.7 Hz, 1H, H-1), 4.99 (t, *J* = 7.4 Hz, 1H, H-1'), 4.66 (t, *J* = 9.5 Hz, 1H, H-3), 4.38 – 4.24 (m, 3H, H-6a, H-4', OC*H*HC=CH_2), 4.03 (dd, *J* = 6.6, 12.8 Hz, 1H, OC*H*HC=CH_2), 3.91 (d, *J* = 8.7 Hz, 1H, H-5'), 3.84 – 3.71 (m, 2H, H-4, H-6b), 3.67(s, 3H, $COOCH_3$), 3.55 – 3.49 (m, 1H, H-5), 3.29 (dd, *J* = 7.0, 16.5 Hz, 1H, H-2), 0.70 (s, 9H, 3*CH_3), -0.07 (s, 3H, $SiCH_3$), -0.23 (s, 3H, $SiCH_3$);

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3, 165.6, 165.1, 161.9, 137.1, 133.2, 133.1, 133.0, 129.9, 129.65, 129.38, 128.99, 128.94, 128.33, 128.28, 126.11, 118.54, 101.21, 99.96, 97.9, 91.9, 79.72, 77.20, 76.41, 75.85, 75.14, 72.31, 70.78, 70.63, 68.58, 66.14, 59.52, 52.41, 25.64, 25.39, 17.71, -4.46, -5.19.

HRMS(ESI): calcd for $C_{45}H_{53}Cl_3NO_{14}Si$ $[M+H]^+$: 964.2295, found 964.2285



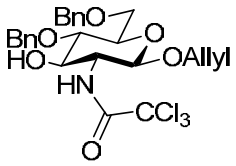
Methyl 2,3–*O*–di–benzoyl–4–*O*-tert-butyldimethylsilyl-β-D-glucopyrafnosyluronate- (1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside

3 (66 mg, 0.068 mmol) and Grubbs' 2nd catalyst (14.3 mg, 20 mol %) was dissolved in CH_2Cl_2 (2 mL). The reaction was stirred for 8 h and NMR indicated the reaction has finished. The reaction was concentrated, dissolved in Acetone/water (5 mL/1 mL), and added HgO(13 mg), $HgCl_2$ (13 mg). The reaction was stirred for 16 h, concentrated and purified via SGC (hexanes/ethyl acetate 2:1) to give the product (53 mg, 0.058 mmol, 85%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.77 (m, 4H), 7.56 – 7.27 (m, 11H), 6.75 (d, *J* = 8.3 Hz, 1H), 5.58 (s, 1H), 5.44 (t, *J* = 8.7 Hz, 1H), 5.32 (t, *J* = 3.8 Hz, 1H), 5.23 (t, *J* = 8.1 Hz, 1H), 5.17 (d, *J* = 7.5 Hz, 1H), 4.34 – 4.22 (m, 3H), 4.21 – 4.13 (m, 1H), 4.10 (dd, *J* = 9.9, 4.8 Hz, 1H), 3.90 – 3.83 (m, 2H), 3.83 – 3.76 (m, 1H), 3.71 (s, 3H), 3.00 (dd, *J* = 3.8, 1.6 Hz, 1H), 0.69 (s, 9H), -0.07 (s, 3H), -0.23 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.29, 165.61, 165.15, 161.71, 137.02, 133.11, 129.89, 129.83, 129.74, 129.70, 129.44, 129.19, 129.13, 128.35, 128.32, 128.27, 128.23, 126.16, 126.10, 101.59, 99.74, 91.51, 80.48, 77.20, 75.15, 74.91, 73.22, 70.35, 68.80, 62.74, 54.87, 53.75, 52.45, 29.70, 29.25, 25.40, 17.71, -4.45, -5.23.

HRMS(ESI): calcd for $C_{42}H_{48}Cl_3NO_{14}Si$ $[M+Na]^+$: 946.1802, found 946.1803



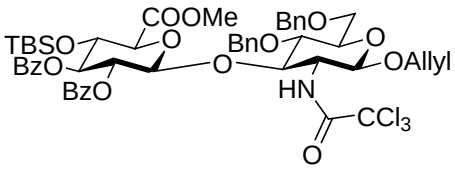
Allyl 4,6-O–di-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (8)

To a round bottom flask was added **7** (4.2 g, 8.5 mmol) and powdered NaOH (1.71 g, 42.8 mmol). 50 mL THF was added and the reaction was stirred for 5 h. Another portion of NaOH (1.71 g, 42. 8 mmol) and 18-crown-6 (3.6 g) was added. After 25 min, BnBr (2.14 mL, 18 mmol) was added dropwisely. The reaction was stirred for 12 h, and then HOAc (5.1g, 85.6 mmol) was added to the reaction slowly. The solvent was removed at reduced pressure, and EtOAc was added to the mixture to extract the product. The organic phase was washed with sat. NH_4Cl (aq), water, brine, and dried by Na_2SO_4. Solvents were removed under reduced pressure, and the product was purified using silica gel chromatography with hexanes/ethyl acetate 2:1 as eluting solution to give product **8** as a white solid (2.68 g, 4.9 mmol, 57%).

^{1}H NMR (400 MHz, $CDCl_3$) 7.41 – 7.22 (m, 10 H, PhH), 7.20 (d, *J* = 6.4 Hz, 1H, NH), 5.93 – 5.81 (m, 1H, -*H*C=CH_2), 5.28 (d, *J* = 17.0 Hz, 1H, C=CH*H*), 5.19 (dd, *J* = 9.9 Hz, 1H, C=CH*H*), 4.79 – 4.71 (m, 2H, H-1, PhC*H*H), 4.67 – 4.51 (m, 3H, PhC*H*H, PhCH_2), 4.36 (dd, *J* = 5.7, 13.2 Hz, 1H, OC*H*HC=CH_2), 4.14 – 4.03 (m, 2H, OC*H*HC=CH_2, H-3), 3.79 – 3.70 (m, 2H, H-6a, H-6b), 3.69 – 3.56 (m, 3H, H-2, H-4, H-5), 3.21 (br, 1H, OH).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 162.4, 137.9, 133.4, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 118.0, 98.6, 92.5, 78.2, 74.8, 74.5, 73.5, 72.6, 70.0, 68.9, 58.2

HRMS(ESI): calcd for $C_{25}H_{28}Cl_3NO_6Na$ $[M+Na]^+$: 566.0874, found 566.0874



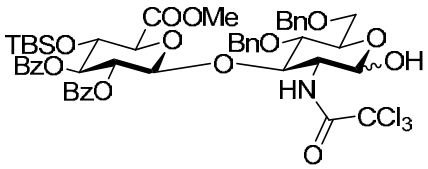
Allyl *O*-(methyl 2,3–*O*–di–benzoyl–4–*O*-tert-butyldimethylsilyl-β-D-glucopyrafnosyl -uronate)-(1→3)- 4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido-β-D- glucopyranoside (9) To a round bottom flask was added acceptor **8** (44 mg, 0.081 mmol), donor **1** (110 mg, 0.162 mmol). The reactants were co-evaporated with toluene.5 mL CH_2Cl_2 was added, and the reaction was cooled to 0 °C. 1.4 μL TMSOTf was added, and the reaction was stirred at 0 °C for 4 h, then quenched by adding 300 μL Et_3N, and concentrated. Purification using silica gel chromatography using hexanes/ethyl acetate 4:1 as eluting solution give product **9** (79 mg, 0.075 mmol, 93%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) 7.83 (t, *J* = 8.4 Hz, 4H, OBz), 7.47 – 7.16 (m, 16H, PhH), 6.93 (d, *J* = 7.8 Hz, 1H, NH), 5.62 – 5.52 (m, 1H, -*H*C=CH_2), 5.44 (t, *J* = 9.8 Hz, 1H, H-3'), 5.34(t, *J* = 8.4 Hz, 1H, H-2'), 5.06 (d, *J* = 17.3 Hz, 1H, C=CH*H*), 5.01 (d, *J* =10.0 Hz, 1H, C=CH*H*), 4.95 (d, *J* = 7.3 Hz, 1H, H-1'), 4.90 (d, *J* = 11.4 Hz, 1H, PhC*H*H), 4.73 (d, *J* = 7.3 Hz, 1H, H-1), 4.51 – 4.39 (m, 4H, H-6a, PhC*H*H, PhCH_2, H-3), 4.27 (t, *J* = 8.4 Hz, 1H, H-4'), 4.08 – 3.95 (m, 2H, OC*H*HC=CH_2, H-5'), 3.85(dd, *J* = 6.7, 12.6Hz, 1H, OC*H*HC=CH_2), 3.66 – 3.57 (m, 7H, $COOCH_3$, H-4, H-5, H-6a, H-6b), 3.30 (dd, *J* = 8.4, 16.5 Hz, 1H, H-2), 0.65 (s, 9H, 3*CH_3), -0.09 (s, 3H, $SiCH_3$), -0.26 (s, 3H, $SiCH_3$)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 165.7, 165.3, 163.6, 161.8, 138.0, 137.9, 133.3, 133.2, 133.1, 129.8, 129.7, 129.66, 129.62, 129.4, 129.0, 128.4, 128.4, 128.34, 128.30, 127.8, 127.70, 127.6, 117.9,

100.3, 97.1, 92.3, 77.4, 76.7, 76.6, 75.8, 74.9, 74.5, 74.1, 73.3, 72.0, 71.0, 69.8, 69.0, 57.5, 52.4, 25.41, 25.39, 17.7, -3.6, -4.4, -5.2.

HRMS(ESI): calcd for $C_{52}H_{60}Cl_3NO_{14}SiNa$ $[M+Na]^+$: 1078.2741, found 1078.2765



Methyl 2,3–*O*–di–benzoyl–4–*O*-tert-butyldimethylsilyl-β- D- glucopyrafnosyluronate) - (1→3) - 4, 6- *O* – di - benzyl - 2 - deoxy - 2- trichloroacetamido - β - D - glucopyranoside

(11) Compound **9** (79 mg, 0.075 mmol) and Ir (3.4 mg, 0.004 mmol) was added to a round bottom flask. THF (5 mL) was added. The reaction was subject to H_2 balloon for a few seconds until the red color faded, and then stirred for 2 h. NMR indicated the isomerization had finished, and solvents were removed under reduced pressure.

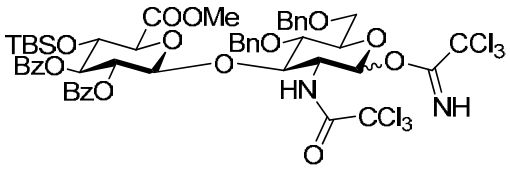
The crude mixture was dissolved in acetone (5 mL) and water (1 mL), and then HgO (40 mg, 0.18 mmol), $HgCl_2$ (40 mg, 0.15 mmol) was added. The reaction was stirred overnight, filtered through Celite, concentrated, diluted with EtOAc, washed with KI(aq), brine, and dried with Na_2SO_4. The solution was concentrated and purified by SGC to give product **11** as white foam (65 mg, 0.064 mmol, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) 7.91 – 7.83 (m, 4H, OBz), 7.53 – 7.27 (m, 16H, PhH), 6.91 (d, *J* = 10.0 Hz, 1H, NH), 5.47 (t, *J* = 9.3 Hz, 1H, H-3'), 5.34 (t, *J* = 8.4 Hz, 1H, H-2'), 5.12 – 5.00 (m, 3H,

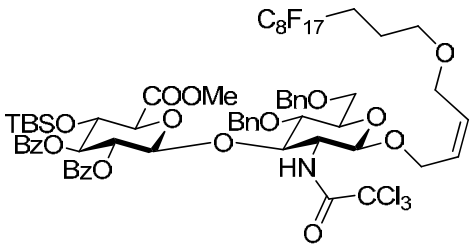
H-1', H-1, PhC*H*H), 4.51 – 4.43 (m, 3H, PhC*H*H, PhCH_2), 4.36 – 4.24 (m, 2H, H-4', H-3), 4.15 – 4.01 (m, 3H, H-2, H-5', H-5), 3.74 (s, 3H, $COOCH_3$), 3.67 – 3.61 (m, OH, H-6a), 3.59 – 3.53 (m, H-6b), 3.50 (t, *J* = 8.4 Hz, 1H, H-4), 0.73 (s, 9H, 3*CH_3), -0.02 (s, 3H, $SiCH_3$), -0.20 (s, 3H, $SiCH_3$)

^{13}C NMR (101 MHz, $CdCl_3$) δ 168.0, 165.7, 165.5, 163.4, 161.2, 137.9, 137.6, 133.1, 133.0, 130.0, 129.6, 129.4, 128.9, 128.7, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 100.3, 92.6, 91.0, 77.2, 76.9, 76.8, 75.8, 75.1, 74.8, 73.5, 72.2, 72.1, 71.0, 70.5, 69.1, 54.7, 52.5, 29.7, 25.7, 25.4, 17.7, 1.0, -4.3, -5.1, -5.2

HRMS(ESI): calcd for $C_{49}H_{56}Cl_3NO_{14}SiNa$ $[M+Na]^+$: 1038.2428, found 1038.2433



Trichloroacetimido Methyl 2,3–*O*–di–benzoyl–4–*O*-tert-butyldimethylsilyl-β- D-glucopyrafnosyluronate) - (1→3) - 4, 6- *O* – di - benzyl - 2 - deoxy - 2- trichloroacetamido - β - D – glucopyranoside (12) 11 (65 mg, 0.064 mmol) was dissolved in CH_2Cl_2 (5 mL), and CCl_3CN (139 mg, 0.9 mmol), DBU (4 mg, 0.026 mmol) was added. The reaction was stirred at 0 °C for 30 min. The reaction was concentrated and passed through a silica gel plug using hexanes/acetate 2:1 as eluting solvent to give the crude imidate (64 mg, 0.055 mmol) and used directly without further purification.



cis-4-(1H, 1H, 2H, 2H, 3H, 3H -Perfluoroundecyloxy) -2-butenyl *O*-(methyl 2,3 –*O*– di –benzoyl–4 –*O*-tert-butyldimethylsilyl - β- D - glucopyrafnosyluronate) - (1→3) - 4,6- *O* –di-benzyl-2-deoxy-2- trichloroacetamido-β-D-glucopyranoside (14) To a round bottom flask was added acceptor **13** (10 mg, 0.019 mmol), donor **12** (23 mg, 0.020 mmol). The reactants were co-evaporated with toluene. CH_2Cl_2 (1.5 mL) was added, and the reaction was cooled to 0 °C. TMSOTf (0.7 μL, 0.002 mmol) was added, and the reaction was stirred at 0 °C for 90 min, then quenched by adding 100 μL Et_3N, and concentrated. Purification using FSPE give product **14** (22.5 mg, 0.014 mmol, 76 %) as a white solid.

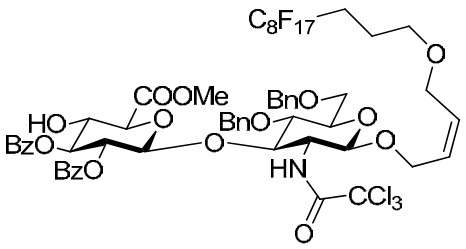
^{1}H NMR (400 MHz, $CDCl_3$) 7.84 (dd, *J* = 6.9, 9.7Hz, 4H, OBz), 7.46 – 7.19 (m, 16H, PhH), 6.85 (d, *J* = 6.9 Hz, 1H, NH), 5.58 – 5.50 (m, 1H, -*H*C=CH-), 5.47 (t, *J* = 9.8 Hz, 1H, H-3'), 5.35 (m, 2H, -*H*C=CH-, H-2'), 4.91 (d, *J* =7.9 Hz, 1H, H-1'), 4.90 (d, *J* = 9.8 Hz, 1H, PhC*H*H), 4.74 (d, *J* = 6.3 Hz, 1H, H-1), 4.50 – 4.39 (m, 4H, H-3, PhC*H*H, PhCH_2), 4.27 (t, *J* = 9.7 Hz, 1H, H-4'), 4.07 – 3.96 (m, 3H, C=C-CH_2-O, H-5'), 3.84 (dd, *J* = 2, 6Hz, 2H, C=C-CH_2-O), 3.65 – 3.59 (m, 7H, $COOCH_3$, H-4, H-5, H-6a, H-6b), 3.29 (t, *J* = 6.4 Hz, 2H, CH_2-CF_2), 3.23 (dd, *J* = 8.4, 14.6 Hz, 1H, H-2), 2.16 – 1.97 (m, 2H, C$H_2CH_2CF_2$), 1.81 – 1.69 (m, 2H, CH_2CH_2CF_2), 0.66 (s, 9H, 3*CH_3), -0.09 (s, 3H, $SiCH_3$), -0.25 (s, 3H, $SiCH_3$)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0, 165.5, 165.1, 161.7, 138.0, 137.8, 133.3, 133.1, 130.2, 129.9,

129.7, 129.6, 129.4, 129.0, 128.6, 128.5, 128.4, 128.33, 127.8, 127.76, 127.73, 127.67, 127.6, 100.4, 96.7, 92.2, 77.2, 76.6, 75.8, 74.8, 74.4, 74.2, 74.1, 73.3, 72.0, 70.9, 69.1, 68.6, 66.3, 64.1, 57.4, 52.4, 29.6, 27.9, 25.3, 20.7, 17.7, -4.4, -5.1.

^{19}F NMR (376 MHz, $CDCl_3$) -80.73 (t, *J* = 9.9 Hz, 3F), -114.4 (t, *J* = 20 Hz, 2F), -121.60 - -122.0 (m, 6F), -122.73 (m, *2*F), -123.44 (m, 2F), -126.10 (m, 2F);

HRMS(ESI): calcd for $C_{64}H_{67}Cl_3F_{17}NO_{15}SiNa$ $[M+Na]^+$: 1568.2966, found 1568.2974



cis-4-(1H, 1H, 2H, 2H, 3H, 3H -Perfluoroundecyloxy) -2-butenyl *O*-(methyl 2,3–*O*–di–benzoyl-β-D-glucopyrafnosyluronate)-(1→3)-4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido - β - D – glucopyranoside (15) **14** (18.5 mg, 0.012 mmol) was dissolved in pyridine (1.5 mL) and THF (4 mL). HF/py (450 μL) was added at 0 °C, and the reaction was stirred for 18 h, diluted with EtOAc, washed with 10% $CuSO_4$, $NaHCO_3$(aq), and purified with FSPE to give the product **15** (17 mg, 0.012 mmol, 98%).

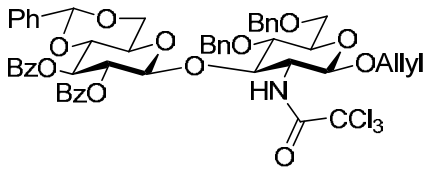
^{1}H NMR (400 MHz, $CDCl_3$) 7.98 – 7.91 (m, 4H, OBz), 7.56 – 7.17 (m, 16H, PhH), 7.02 (d, *J* = 6.9 Hz, 1H, NH), 5.51 – 5.37 (m, 3H, -*H*C=CH-, H-3', H-2'), 4.99 (d, *J* =7.5 Hz, 1H, H-1'), 4.93 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.80 (d, *J* = 6.3 Hz, 1H, H-1), 4.60 – 4.45 (m, 4H, H-3, PhC*H*H, PhCH_2), 4.22 – 4.15 (m, 1H, H-4'), 4.13 – 4.01 (m, 3H, C=C-CH_2-O, H-5'), 3.90 (d, *J* = 6.2 Hz, 2H, C=C-CH_2-O), 3.65 – 3.59 (m, 7H, $COOCH_3$, H-4, H-5, H-6a, H-6b), 3.41 – 3.32 (m, 3H, H-2, CH_2-CF_2), 3.29 (br,

1H, OH), 2.21 – 2.04 (m, 2H, C*H*$_2$CH$_2$CF$_2$), 1.87 – 1.77 (m, 2H, CH$_2$C*H*$_2$CF$_2$);

^{13}C NMR (151 MHz, $CDCl_3$) δ 168.97, 166.47, 165.16, 161.79, 138.05, 137.99, 133.48, 130.88, 130.28, 129.90, 129.83, 129.01, 128.92, 128.81, 128.55, 128.41, 128.37, 128.30, 128.09, 127.82, 127.78, 127.70, 127.67, 100.23, 96.89, 92.28, 75.74, 74.86, 74.60, 74.29, 74.25, 73.39, 71.37, 70.67, 69.20, 68.69, 68.18, 66.83, 66.35, 64.16, 52.84, 38.76, 31.94, 30.43, 30.38, 29.71, 29.61, 29.37, 28.94, 28.00, 24.50, 23.81, 23.77, 23.00, 22.98, 22.70, 14.12, 14.05, 10.99, 10.97, 1.03.

^{19}F NMR (376 MHz, $CDCl_3$) -80.70 (t, *J* = 10.0 Hz, 3F), -114.5 (t, *J* = 20 Hz, 2F), -121.60 - -122.0 (m, 6F), -122.73 (m, *2*F), -123.44 (m, 2F), -126.10 (m, 2F);

HRMS(ESI): calcd for $C_{58}H_{53}Cl_3F_{17}NO_{15}Na$ $[M+Na]^+$: 1454.2102, found 1454.2090

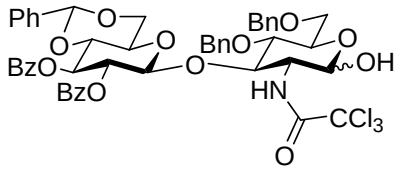


Allyl *O* - (2, 3 –*O*–di – benzoyl – 4,6 –*O*-benzylidene-β-D-glucopyrafnosyl)-(1→3)- 4,6- *O* –di-benzyl-2-deoxy-2- trichloroacetamido-β-D-glucopyranoside (19) To a round bottom flask was added acceptor **8** (530 mg, 0.97 mmol), donor **18** (724 mg, 1.17 mmol). The reactants were co-evaporated with toluene. 15 mL CH_2Cl_2 was added, and the reaction was cooled to 0 °C. TMSOTf (8.7 μL, 0.048 mmol) was added, and the reaction was stirred at 0 °C for 2 h, then quenched by adding 500 μL Et_3N, and concentrated. The crude product was purified with SGC to afford **19** as white foam (910 mg, 0.91 mmol, 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.87 (m, 4H), 7.59 – 7.16 (m, 21H), 6.93 (d, *J* = 7.4 Hz, 1H), 5.75 – 5.60 (m, 2H), 5.54 – 5.42 (m, 2H), 5.19 – 5.05 (m, 2H), 4.99 (d, *J* = 7.9 Hz, 1H), 4.94 (d, *J* = 10.7 Hz, 1H), 4.85 (d, *J* = 7.0 Hz, 1H), 4.69 – 4.59 (m, 1H), 4.59 – 4.47 (m, 2H), 4.35 (d, *J* = 5.7 Hz, 1H), 4.14 (dd, *J* = 13.3, 5.9 Hz, 1H), 3.94 (dd, *J* = 13.0, 6.4 Hz, 1H), 3.87 (t, *J* = 8.7 Hz, 1H), 3.77 – 3.55 (m, 5H), 3.29 (q, *J* = 7.6 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ165.5, 165.2, 161.8, 138.1, 138.0, 136.6, 133.5, 133.3, 133.1, 129.8, 129.7, 129.3, 129.05, 129.01, 128.57, 128.43, 128.38, 128.29, 128.18, 127.95, 127.83, 127.78, 127.67, 126.1, 118.1, 101.5, 100.8, 96.9, 92.2, 78.8, 75.8, 74.3, 74.2, 73.4, 72.6, 71.9, 70.0, 68.9, 68.5, 66.7, 58.1.

HRMS(ESI): calcd for $C_{52}H_{50}Cl_3NO_{13}Na$ $[M+Na]^+$: 1024.2240, found 1024.2251



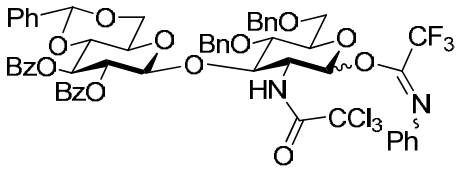
2,3–*O*–di–benzoyl–4,6–*O*-benzylidene-β-D-glucopyrafnosyl-(1→3)-4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (20) Compound **19** (200 mg, 0.2 mmol) was subjected to the conditions in the general deallylation method. The mixture was purified via SGC to afford compound **20** (142 mg, 0.15 mmol, 75%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 7.77, 12.45 Hz, 4H), 7.52 – 7.10 (m, 21H), 6.78 (d, *J* = 9.58 Hz, 1H), 5.60 (t, *J* = 9.56 Hz, 1H), 5.47 – 5.33 (m, 2H), 5.05 (d, *J* = 7.78 Hz, 1H), 4.99 – 4.88 (m,

2H), 4.52 – 4.38 (m, 3H), 4.38 – 4.30 (m, 1H), 4.26 (t, *J* = 9.05 Hz, 1H), 4.02 (ddt, *J* = 5.36, 11.04, 18.39 Hz, 4H), 3.83 – 3.70 (m, 1H), 3.69 – 3.51 (m, 4H), 3.43 (t, *J* = 8.90 Hz, 1H), 3.37 – 3.21 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.74, 165.57, 161.11, 138.09, 137.46, 136.62, 133.17, 130.15, 129.78, 129.20, 129.16, 128.97, 128.59, 128.41, 128.36, 128.29, 128.26, 128.20, 128.11, 127.87, 126.17, 101.51, 100.37, 92.68, 91.05, 79.02, 76.56, 75.79, 74.89, 73.48, 72.62, 71.96, 70.57, 69.12, 68.56, 66.67, 57.03, 56.84, 54.72, 11.89, 11.86.

HRMS(ESI): calcd for $C_{49}H_{46}Cl_3NO_{13}Na$ $[M+Na]^+$: 984.1927, found 984.1926



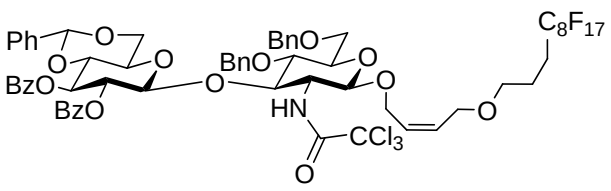
2,3–*O*–di–benzoyl–4,6–*O*-benzylidene-β-D-glucopyrafnosyl-(1→3)-4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido-β-D–glucopyranosideN-Phenyl-2,2,2-trifluoroacetimidate (21) Compound **20** (35 mg, 0.036 mmol) was dissolved in CH_2Cl_2, and Cs_2CO_3 (20.3 mg, 0.054 mmol) was added, followed by N -phenyltrifluoroacetimidoyl chloride (13 mg, 0.054 mmol) and stirred for 8 h. The reaction was filtered and purfied via SGC (hexanes/EtOAc 4:1) to give **21** (22 mg, 0.022 mmol, 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 3.76, 7.86 Hz, 4H), 7.54 – 7.13 (m, 21H), 7.00 (t, *J* = 7.44 Hz, 1H), 6.63 (t, *J* = 7.96 Hz, 3H), 6.16 (bs, 1H), 5.63 (t, *J* = 9.57 Hz, 1H), 5.48 – 5.32 (m, 2H),

5.07 (d, *J* = 7.67 Hz, 1H), 4.89 (d, *J* = 10.72 Hz, 1H), 4.52 (d, *J* = 10.02 Hz, 2H), 4.44 (d, *J* = 12.01 Hz, 1H), 4.38 – 4.16 (m, 3H), 4.10 – 3.96 (m, 1H), 3.90 (d, *J* = 8.97 Hz, 1H), 3.86 – 3.68 (m, 3H), 3.68 – 3.49 (m, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.62, 165.50, 161.19, 142.82, 137.78, 137.72, 136.58, 133.31, 133.17, 130.93, 129.99, 129.77, 129.22, 129.12, 128.82, 128.77, 128.46, 128.43, 128.33, 128.31, 128.22, 128.07, 127.99, 127.94, 127.82, 126.12, 124.60, 119.17, 101.55, 100.43, 93.20, 92.34, 78.73, 74.92, 74.73, 73.57, 73.30, 72.81, 71.86, 68.48, 67.80, 66.86, 53.65, 27.73, 19.17.

HRMS(ESI): calcd for $C_{63}H_{66}Cl_3F_3NO_{13}Na$ $[M+Et_3N+H]^+$: 1234.3608, found 1034.3584



cis-4-(1H,1H,2H,2H,3H,3H-Perfluoroundecyloxy)-2-butenyl (2,3–*O*–di–benzoyl–4,6–*O*-benzylidene-β-D–glucopyrafnosyl)-(1→3)-4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (22) Donor **21** (22 mg, 0.020 mmol) and acceptor **13** (11 mg, 0.020 mmol) were subjected to the conditions in the general glycosylation method. The reaction was purified via FSPE to give **22** (16 mg, 0.011 mmol, 55%).

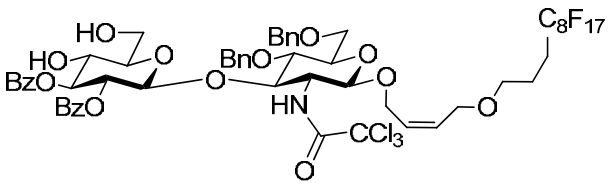
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 – 7.99 (m, 2H), 7.99 – 7.94 (m, 2H), 7.59 – 7.30 (m, 21H), 6.91 (d, *J* = 7.33 Hz, 1H), 5.73 (t, *J* = 9.64 Hz, 1H), 5.70 – 5.62 (m, 1H), 5.57 – 5.44 (m, 3H), 5.01 (d, *J* = 7.89 Hz, 1H), 4.97 (d, *J* = 10.75 Hz, 1H), 4.88 (d, *J* = 6.97 Hz, 1H), 4.67 – 4.63 (m, 1H), 4.63 – 4.52

(m, 3H), 4.38 (d, *J* = 5.62 Hz, 1H), 4.22 – 4.16 (m, 1H), 4.16 – 4.07 (m, 1H), 3.95 (dd, *J* = 1.52, 6.47 Hz, 2H), 3.90 (ddd, *J* = 1.52, 7.17, 9.12 Hz, 1H), 3.77 (d, *J* = 2.25 Hz, 2H), 3.73 – 3.67 (m, 2H), 3.65 (d, *J* = 7.09 Hz, 2H), 3.41 (td, *J* = 2.52, 6.10 Hz, 2H), 3.29 (d, *J* = 7.67 Hz, 1H), 2.29 – 2.10 (m, 2H), 1.89 – 1.80 (m, 2H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 165.49, 165.23, 161.84, 138.10, 138.05, 136.66, 133.50, 133.14, 130.41, 129.86, 129.77, 129.32, 129.07, 128.59, 128.40, 128.32, 128.20, 127.96, 127.83, 127.81, 127.77, 127.71, 126.13, 101.50, 100.90, 96.80, 92.24, 78.86, 76.99, 75.82, 74.36, 74.32, 73.44, 72.68, 71.93, 69.01, 68.90, 68.69, 68.47, 66.77, 66.53, 66.34, 64.30, 58.11, 29.72, 28.00, 20.82.

^{19}F NMR (564 MHz, $CDCl_3$) -80.74 (t, *J* = 8.9 Hz, 3F), -114.4 (t, *J* = 20 Hz, 2F), -121.60 - -122.0 (m, 6F), -122.70 (m, *2*F), -123.41 (m, 2F), -126.10 (m, 2F);

HRMS(ESI): calcd for $C_{64}H_{57}Cl_3F_{17}NO_{14}SiNa$ $[M+Na]^+$: 1514.2465, found 1514.2439



cis-4-(1H,1H,2H,2H,3H,3H-Perfluoroundecyloxy)-2-butenyl (2,3–*O*–di–benzoyl-β-D–glucopyrafnosyl)-(1→3)-4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside

(23) Compound **22** (16 mg, 0.011 mmol) was subjected to the condtion in the general benzylidene cleavage method and purified by FSPE to give **23** (14 mg, 0.010 mmol, 91%).

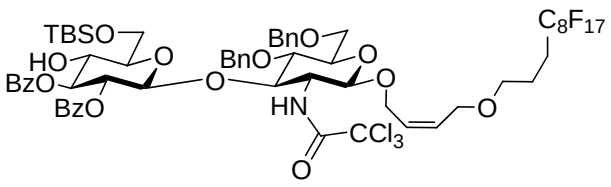
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 – 7.87 (m, 4H), 7.68 – 7.25 (m, 16H), 7.10 (d, *J* = 7.75 Hz, 1H),

5.69 (ddt, *J* = 6.43, 12.05, 23.92 Hz, 1H), 5.53 (dt, *J* = 6.72, 12.01 Hz, 1H), 5.44 (dd, *J* = 7.88, 9.85 Hz, 1H), 5.35 (t, *J* = 9.44 Hz, 1H), 4.94 (t, *J* = 8.89 Hz, 2H), 4.80 (d, *J* = 7.08 Hz, 1H), 4.68 – 4.45 (m, 4H), 4.32 – 4.04 (m, 3H), 3.96 (d, *J* = 6.39 Hz, 2H), 3.93 – 3.81 (m, 2H), 3.81 – 3.57 (m, 5H), 3.57 – 3.43 (m, 2H), 3.41 (td, *J* = 2.10, 6.08 Hz, 2H), 2.18 (dtt, *J* = 9.09, 18.17, 27.04 Hz, 2H), 2.01 – 1.67 (m, 2H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 167.30, 165.37, 162.59, 138.00, 137.95, 133.59, 133.45, 130.35, 129.92, 129.82, 129.12, 128.98, 128.83, 128.59, 128.53, 128.46, 128.38, 127.93, 127.82, 127.79, 127.71, 127.54, 99.92, 97.37, 92.39, 77.25, 77.09, 77.03, 76.96, 76.82, 76.70, 76.22, 75.69, 74.45, 74.18, 73.41, 71.66, 69.90, 69.02, 68.71, 66.37, 64.30, 61.90, 57.59, 29.71, 27.98, 20.81, 1.06.

^{19}F NMR (564 MHz, $CDCl_3$) -80.74 (t, *J* = 8.9 Hz, 3F), -114.4 (t, *J* = 20 Hz, 2F), -121.60 - -122.0 (m, 6F), -122.70 (m, *2*F), -123.41 (m, 2F), -126.10 (m, 2F);

HRMS(ESI): calcd for $C_{57}H_{53}Cl_3F_{17}NO_{14}Na$ $[M+Na]^+$:1426.2152, found 1426.2131



cis-4-(1H,1H,2H,2H,3H,3H-Perfluoroundecyloxy)-2-butenyl (2,3–*O*–di–benzoyl-6–*O*-tert-butyldimethylsilyl-β-D–glucopyrafnosyl)-(1→3)-4,6-*O*–di-benzyl-2-deoxy-2-trichloroacetamido -β-D-glucopyranoside (24)

Compound **23** (14 mg, 0.010 mmol) was subjected to the conditions for selective TBS protection and

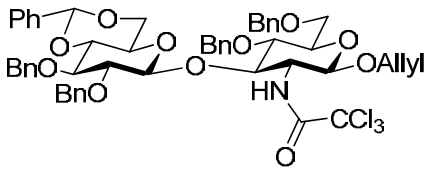
purified by FSPE to give compound **24** (16 mg, 0.010 mmol, 96%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 – 7.94 (m, 4H), 7.59 – 7.20 (m, 17H), 7.04 (d, *J* = 7.47 Hz, 1H), 5.63 (dq, *J* = 8.05, 9.71, 16.16 Hz, 1H), 5.50 (q, *J* = 9.36 Hz, 1H), 5.44 (td, *J* = 7.24, 10.20 Hz, 2H), 4.98 – 4.89 (m, 2H), 4.86 (d, *J* = 6.59 Hz, 1H), 4.61 – 4.53 (m, 3H), 4.51 (d, *J* = 12.04 Hz, 1H), 4.10 (dd, *J* = 6.66, 23.55 Hz, 2H), 4.01 – 3.88 (m, 4H), 3.79 – 3.69 (m, 5H), 3.67 (dq, *J* = 3.69, 7.39 Hz, 1H), 3.60 (ddd, *J* = 5.22, 7.08, 9.33 Hz, 1H), 3.46 – 3.30 (m, 3H), 2.17 (ddq, *J* = 7.93, 8.60, 18.39, 27.18 Hz, 2H), 1.92 – 1.76 (m, 2H), 0.91 (s, 9H), 0.17 – 0.06 (m, 6H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 166.53, 165.32, 161.80, 138.09, 138.02, 133.36, 133.23, 130.29, 129.90, 129.80, 129.24, 128.52, 128.36, 128.31, 128.26, 127.91, 127.85, 127.76, 127.74, 127.65, 99.86, 96.79, 92.26, 76.28, 75.71, 74.23, 74.06, 73.96, 73.37, 73.00, 71.76, 69.25, 68.67, 66.34, 65.08, 64.13, 57.19, 29.71, 28.00, 25.89, 25.85, 20.81, 18.18, 1.16.

^{19}F NMR (564 MHz, $CDCl_3$) -80.74 (t, *J* = 8.9 Hz, 3F), -114.4 (t, *J* = 20 Hz, 2F), -121.60 - -122.0 (m, 6F), -122.70 (m, *2*F), -123.41 (m, 2F), -126.10 (m, 2F);

HRMS(ESI): calcd for $C_{63}H_{67}Cl_3F_{17}NO_{14}SiNa$ $[M+Na]^+$:1540.3017, found 1540.2978



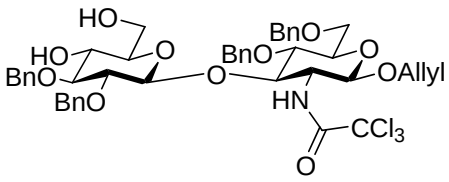
Allyl *O* - (2, 3 –*O*–di – benzyl – 4,6 –*O*-benzylidene-β-D-glucopyrafnosyl)-(1→3)- 4,6- *O*

–di-benzyl-2-deoxy-2- trichloroacetamido-β-D-glucopyranoside (27) Compound **19** (990 mg, 0.99 mmol) was dissolved in THF (15 mL). NaOH (450 mg, 11.25 mmol) added, and the reaction was stirred for 8 h. To the milky mixture was added 18-crown-6 (410 mg), and the reaction was stirred for 40 min. BnBr (0.31 mL, 22 mmol) was added. After 8 hours, NaOH (450mg, 11.25 mmol) and BnBr (0.31 mL, 22 mmol) was added. The reaction was stirred for another 8 h and diluted with EtOAc, washed with water, dried with Na_2SO_4. Silica gel chromatography using Hexanes/EtOAc 4:1 as eluting solution gave product **27** (660 mg, 0.67 mmol, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.16 (m, 25H), 7.02 (d, *J* = 7.0 Hz, 1H), 5.86 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.55 (s, 1H), 5.26 (d, *J* = 17.2 Hz, 1H), 5.16 (dd, *J* = 15.4, 8.9 Hz, 2H), 4.98 (dd, *J* = 11.1, 3.9 Hz, 2H), 4.94 (d, *J* = 10.5 Hz, 1H), 4.86 (d, *J* = 11.5 Hz, 1H), 4.80 (d, *J* = 10.4 Hz, 1H), 4.71 – 4.56 (m, 3H), 4.56 – 4.46 (m, 2H), 4.36 (dd, *J* = 13.0, 5.3 Hz, 1H), 4.28 (dd, *J* = 10.5, 4.9 Hz, 1H), 4.08 (dd, *J* = 13.0, 6.0 Hz, 1H), 3.82 (d, *J* = 3.3 Hz, 2H), 3.70 (dtd, *J* = 17.6, 9.0, 4.0 Hz, 4H), 3.59 (t, *J* = 10.2 Hz, 1H), 3.49 (t, *J* = 8.1 Hz, 1H), 3.36 (dq, *J* = 8.8, 5.4, 3.5 Hz, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz) δ 161.5, 138.3, 138.23, 138.17, 138.13, 137.2, 133.7, 129.0, 128.7, 128.5, 128.44, 128.43, 128.28, 128.26, 128.24, 128.15, 128.08, 128.05, 127.89, 127.83, 127.79, 127.75, 126.0, 117.5, 103.8, 101.1, 97.3, 92.1, 82.5, 81.8, 81.0, 79.2, 76.1, 76.0, 75.0, 74.4, 74.3, 73.6, 70.2, 69.0, 68.7, 66.2, 59.7.

HRMS(ESI): calcd for $C_{52}H_{54}Cl_3NO_4Na$ $[M+Na]^+$: 996.2655, found 996.2644

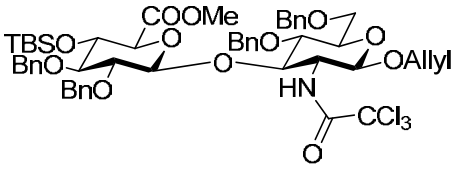


Allyl *O* - (2, 3 – *O* – di – benzyl - β- D - glucopyrafnosyl) - (1→3) - 4, 6- *O* – di - benzyl - 2 - deoxy - 2- trichloroacetamido - β - D – glucopyranoside (29) 28 (163 mg, 0.17 mmol) was dissolved in CH_2Cl_2/TFA/H_2O (10:1:0.1, 5 mL) and stirred at r.t. for 1 h. The reaction was diluted with EtOAc, washed with water, sat. $NaHCO_3$ (aq), dried with Na_2SO_4, and concentrated. The crude mixture was passed through a short silica gel plug to give **29** (127 mg, 0.14 mmol, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) 7.40 – 7.21 (m, 20H, PhH), 7.05 (d, *J* = 7.3 Hz, 1H, NH), 5.88 – 5.77 (m, 1H, -*H*C=CH_2), 5.24 (dd, *J* = 1.6, 17.2 Hz, 1H, C=CH*H*), 5.15 (dd, *J* = 1.4, 10.3 Hz, 1H, C=CH*H*), 5.09 – 4.97 (m, 3H, H-1, PhC*H*H , PhC*H*H), 4.88 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.86 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.80 (d, *J* = 11.0 Hz, 1H, PhC*H*H), 4.69 (d, *J* = 11.7 Hz, 1H, PhC*H*H), 4.64 (d, *J* = 12.2 Hz, 1H, PhC*H*H) 4.58 – 4.51 (m, 3H, $PhCH_2$, H-1'), 4.39 – 4.28 (m, 2H, H-3, OC*H*HC=CH), 4.05 (dd, *J* = 6.6, 12.8 Hz, 1H, OC*H*HC=CH), 3.82 (dd, *J* = 3.0, 10.6 Hz, 1H, H-6a), 3.78 – 3.69 (m, 2H, H-4, H-6b), 3.69 – 3.60 (m, 2H, H-5a, H-6'a), 3.54 – 3.42 (m, 3H, H-2, H-4', H-6'b), 3.39 – 3.30 (m, 2H, H-2', H-3'), 3.21 – 3.15 (m, 1H, H-5'), 2.16 (d, *J* = 2.7 Hz, 4'-OH), 1.83 (t, *J* = 6.9 Hz, 6'-OH)

^{13}C NMR (100 MHz, $CDCl_3$): δ 161.5, 138.4, 138.3, 138.0, 137.9, 133.7, 128.7, 128.52, 128.47, 128.45, 128.40, 128.38, 128.04, 128.00, 127.98, 127.93, 127.90, 127.8, 117.5, 103.2, 98.3, 92.5, 83.8, 82.2, 78.2, 76.0, 75.7, 75.2, 75.1, 74.5, 74.2, 73.6, 70.5, 70.1, 68.8, 62.4, 58.4

HRMS(ESI): calcd for $C_{45}H_{50}Cl_3NO_{11}Na$ $[M+Na]^+$: 908.2343, found 908.2353



Allyl *O*-(methyl 2,3–*O*–di–benzyl–4–*O*-tert-butyldimethylsilyl-β-D-glucopyrafnosyl -uronate)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D- glucopyranoside (31)

The diol **29** (127 mg, 0.14 mmol) was dissolved in $CH_2Cl_2H_2O$ (2 mL/1 mL). BAIB (113 mg, 0.35 mmol) and TMEPO (4 mg, 0.03 mmol) was added, and the reaction was stirred for 1 h. $Na_2S_2O_3$(aq, 10%, 10 mL) was added, and the reaction was extracted with EtOAc for 3 times, dried with Na_2SO_4, and concentrated to give the crude acid.

The crude acid was dissolved in toluene/methanol (12 mL/3 mL), and $TMSCHN_2$ (2 M in ether, 0.14 mL, 0.28 mmol) was added. After stirring for 50 min, HOAc (0.15 mL) was added, and the reaction was concentrated to give the crude ester.

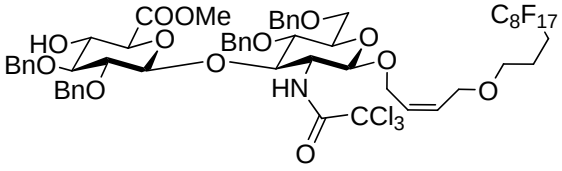
The crude ester was dissolved in CH_2Cl_2 (5 mL). 2,6 – Lutidine (0.1 mL, 0.84 mmol) and TBSOTf (0.1 mL, 0.56 mmol) was added, and the reaction was stirred for 12 h, quenched by adding CH_3OH (0.1 mL), washed with 10% $CuSO_4$, $NaHCO_3$ (aq), dried with Na_2SO_4, and concentrated. Silica gel chromatography using hexanes/EtOAc 4:1 to 3:1 as eluting solution gave the product **33** (96 mg, 0.093 mmol, 67% for 3 steps).

^{1}H NMR (400 MHz, $CDCl_3$) 7.43 – 7.20 (m, 20H, PhH), 7.05 (d, *J* = 8.0 Hz, 1H, NH), 5.88 – 5.76

(m, 1H, -*H*C=CH_2), 5.23 (d, *J* = 16.6Hz, 1H, C=CH*H*), 5.14 (d, *J* =10.3 Hz, 1H, C=CH*H*), 5.09 – 4.97 (m, 3H, H-1, PhC*H*H , PhC*H*H), 4.86 – 4.69 (m, 2H, PhC*H*H , PhC*H*H), 4.70 – 4.54 (m, 3H, H-1', PhCH_2), 4.50 (d, *J* = 10.0 Hz, 1H, PhC*H*H), 4.42 (t, *J* = 6.9 Hz, 1H, H-3), 4.32 (dd, *J* = 5.2, 12.7 Hz,1H, OC*H*HC=CH), 4.04 (dd, *J* = 5.6, 12.7 Hz,1H, OC*H*HC=CH), 3.97 (t, *J* = 8.9, 1H, H-4'), 3.86 – 3.67 (m, 5H, H-5', H-4, H-5, H-6a, H-6b), 3.64 (s, 3H, $COOCH_3$), 3.52 (t, *J* = 8.9 Hz, 1H, H-2'), 3.47 – 3.38 (m, 2H, H-2, H-3;), 0.85 (s, 9H, 3*CH_3), 0.00 (s, 6H, 2*$SiCH_3$)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5, 161.2, 138.3, 137.9, 137.8, 137.6, 133.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.4, 126.9, 126.5, 117.1, 103.2, 97.1, 91.9, 83.9, 82.2, 78.9, 76.3, 75.9, 75.1, 74.7, 74.2, 74.1, 73.2, 72.0, 69.8, 68.9, 58.5, 51.9, 25.5, 17.7, -4.1, -5.4, -5.7.

HRMS(ESI): calcd for $C_{52}H_{64}Cl_3NO_{12}SiNa$ $[M+Na]^+$: 1050.3156, found 1050.3145



cis-4-(1H,1H,2H,2H,3H,3H-Perfluoroundecyloxy)-2-butenyl *O*-(methyl 2,3–*O*–di– benzyl –β-D-glucopyrafnosyluronate)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido -β-D-glucopyranoside (34) 31 (75 mg, 0.073 mmol) and Ir (3.4 mg, 0.004 mmol) was added to a round bottom flask. THF (5 mL) was added. The reaction was subject to H_2 balloon for a few seconds until the red color faded, and then stirred for 2 h. NMR indicated the isomerization had finished, and solvents were removed under reduced pressure.

The crude mixture was dissolved in acetone (5 mL) and water (1 mL), and then HgO (70 mg, 0.32 mmol), $HgCl_2$ (70 mg, 0.26 mmol) was added. The reaction was stirred overnight, filtered through Celite, concentrated, diluted with EtOAc, washed with KI(aq), brine, and dried with Na_2SO_4. The solution was concentrated and passed through a short silica gel column to give crude product **32** and used directly without purification.

Imidate **33** was formed using the method depicted in the general methods and used without further purification (35 mg, 0.032 mmol, 43% overall for 3 steps).

Glycosylation of **33** and **13** followed by silyl removal using the methods depicted in the general method gave **34** (63% overall, 2 steps).

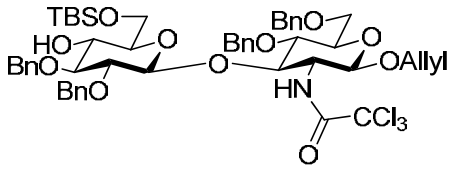
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 20H), 7.08 (d, *J* = 7.1 Hz, 1H), 5.63 (tp, *J* = 11.5, 6.0 Hz, 2H), 5.06 (d, *J* = 7.1 Hz, 1H), 4.99 (d, *J* = 10.6 Hz, 1H), 4.89 (q, *J* = 11.4 Hz, 2H), 4.84 – 4.75 (m, 2H), 4.59 (dd, *J* = 9.5, 2.6 Hz, 2H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.49 (d, *J* = 10.8 Hz, 1H), 4.42 (t, *J* = 7.7 Hz, 1H), 4.33 (dd, *J* = 12.7, 5.5 Hz, 1H), 4.16 (dd, *J* = 12.5, 6.5 Hz, 1H), 3.96 (d, *J* = 5.7 Hz, 2H), 3.86 (t, *J* = 8.9 Hz, 1H), 3.80 – 3.63 (m, 5H), 3.58 (s, 3H), 3.51 – 3.32 (m, 5H), 2.88 (bs, 1H), 2.15 (dp, *J* = 22.5, 7.9 Hz, 3H), 1.83 (dq, *J* = 12.0, 6.1 Hz, 2H).

^{13}C NMR (101 MHz, $cdcl_3$) δ 169.61, 161.42, 138.24, 138.20, 137.99, 137.92, 130.06, 128.63, 128.54, 128.37, 128.33, 128.23, 128.18, 128.08, 128.03, 127.98, 127.91, 127.79, 127.70, 127.58, 103.40, 97.12, 92.09, 83.09, 81.39, 79.25, 76.13, 75.58, 75.31, 74.37, 74.25, 74.13, 73.47, 72.08, 69.10, 68.64, 66.41, 64.60, 58.88, 52.53, 29.69, 27.98, 20.81, 20.76.

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.56 (m, 3F), -114.30 (m, 2F), -121.60 (m, 6F), -122.62 (m, 2F),

-123.32 (m, 2F), -126.10 (m, 2F).

HRMS(ESI): calcd for $C_{58}H_{57}Cl_3F_{17}NO_{13}SiNa$ $[M+Na]^+$: 1426.2516, found 1426.2498



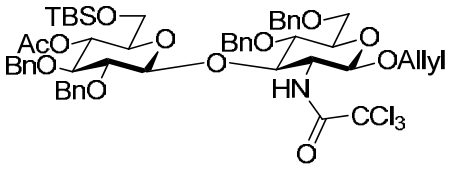
Allyl *O* - (2, 3 – *O* – di – benzoyl – 6 – *O* - tert-butyldimethylsilyl - β- D - glucopyrafnosyl) -(1→3) - 4, 6- *O* – di - benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (37)

Compound **29** (12 mg, 0.014 mmol) was treated with the conditons in the selective TBS protection followed by FSPE to give **37** (9 mg, 0.009 mmol, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.17 (m, 20H), 7.01 (d, *J* = 7.1 Hz, 1H), 5.77 (ddt, *J* = 16.3, 10.8, 5.6 Hz, 1H), 5.17 (dd, *J* = 17.1, 1.7 Hz, 1H), 5.09 (dd, *J* = 10.3, 1.7 Hz, 1H), 5.02 (d, *J* = 7.2 Hz, 1H), 4.87 (d, *J* = 11.9 Hz, 3H), 4.81 – 4.69 (m, 2H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.53 – 4.43 (m, 3H), 4.26 (dd, *J* = 12.9, 5.2 Hz, 1H), 3.99 (dd, *J* = 12.9, 6.0 Hz, 1H), 3.46 – 3.27 (m, 3H), 3.23 (ddd, *J* = 9.3, 7.3, 4.7 Hz, 1H), 0.06 – -0.06 (m, 6H), 4.38 (dt, *J* = 8.0, 3.4 Hz, 1H), 3.81 – 3.68 (m, 3H), 3.69 – 3.59 (m, 3H), 3.56 (dd, *J* = 10.0, 7.2 Hz, 1H), 0.83 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$) δ161.5, 138.7, 138.4, 138.3, 138.1, 133.7, 128.6, 128.44, 128.40, 128.3, 128.2, 128.1, 127.99, 127.91, 127.8, 127.68, 127.64, 117.5, 103.1, 97.3, 92.2, 84.2, 81.9, 78.6, 76.1, 75.5, 75.3, 74.9, 74.4, 74.1, 73.5, 73.3, 70.2, 69.2, 65.4, 59.1, 29.7, 25.9, 18.2, -5.5, -5.6.

HRMS(ESI): calcd for $C_{51}H_{64}Cl_3NO_{11}Na$ $[M+Na]^+$: 1022.3206, found 1022.3216

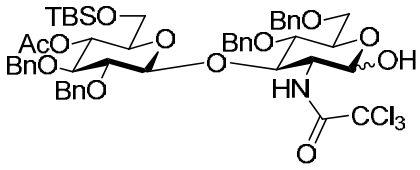


Allyl *O* - (2, 3 – *O* – di – benzoyl – 4 - *O* – acetyl – 6 – *O* - tert-butyldimethylsilyl - β- D - glucopyrafnosyl) - (1→3) - 4, 6- *O* – di - benzyl - 2 - deoxy - 2- trichloroacetamido - β - D – glucopyranoside (38) Compound **37** (8 mg, 0.008 mmol) was dissolved in pyridine (3 mL). Ac_2O (0.5 mL) was added followed by DMAP (4 mg). The reaction was stirred for 4 h, and concentrated. SGC (hexances/EtOAc 3:1) afford the product (8 mg, 0.076 mmol, 95%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.17 (m, 20H), 7.14 (d, *J* = 7.6 Hz, 1H), 5.75 (ddt, *J* = 16.0, 10.5, 5.3 Hz, 1H), 5.16 (d, *J* = 17.2 Hz, 1H), 5.07 (d, *J* = 10.5 Hz, 1H), 4.96 – 4.88 (m, 2H), 4.85 – 4.73 (m, 3H), 4.67 (d, *J* = 10.8 Hz, 1H), 4.62 – 4.54 (m, 2H), 4.54 – 4.44 (m, 3H), 4.34 (t, *J* = 7.0 Hz, 1H), 4.24 (dd, *J* = 12.8, 5.2 Hz, 1H), 3.99 (dd, *J* = 12.9, 6.2 Hz, 2H), 3.78 – 3.63 (m, 4H), 3.63 – 3.45 (m, 5H), 3.38 (t, *J* = 8.5 Hz, 2H), 3.30 (dt, *J* = 9.1, 4.3 Hz, 1H), 1.86 (s, 3H), 0.79 (s, 9H), -0.08 (d, *J* = 5.9 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ169.6, 161.3, 138.2, 138.1, 138.1, 138.0, 133.6, 128.5, 128.39, 128.37, 128.28, 128.23, 128.13, 127.96, 127.93, 127.77, 127.69, 127.67, 117.5, 102.5, 97.5, 92.2, 82.4, 81.9, 77.6, 77.2, 75.7, 75.3, 75.1, 74.8, 74.3, 73.7, 73.4, 70.8, 69.9, 69.5, 63.0, 57.6, 29.7, 25.9, 20.9, 18.3, -5.45, -5.47.

HRMS(ESI): calcd for $C_{53}H_{66}Cl_3NO_{12}SiNa$ $[M+Na]^+$: 1064.3312, found 1064.3321

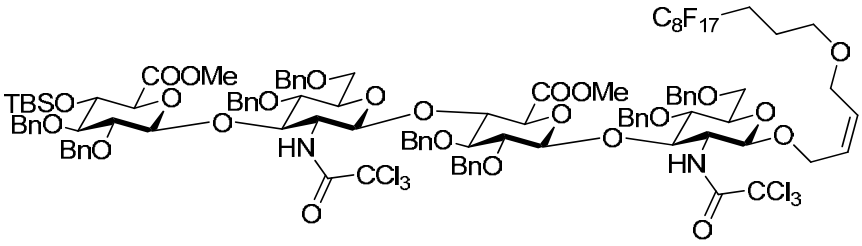


2, 3–*O*–di–benzoyl–4-*O*–acetyl–6–*O*-tert-butyldimethylsilyl-β-D-glucopyrafnosyl-(1→3)- 4,6-*O*–di-benzyl-2-deoxy-2- trichloroacetamido - β - D – glucopyranoside (39) Compound **38** (46 mg, 0.04 mmol,was treated with the general deallylation proceudre to yield compound **39** (25 mg, 0.025 mmol, 63%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.09 (m, 20H), 7.06 – 6.96 (m, 1H), 5.23 (d, *J* = 3.06 Hz, 1H), 4.96 – 4.89 (m, 1H), 4.85 (dd, *J* = 6.26, 10.34 Hz, 2H), 4.77 – 4.68 (m, 2H), 4.69 – 4.61 (m, 1H), 4.56 (dd, *J* = 8.13, 11.56 Hz, 3H), 4.52 – 4.45 (m, 1H), 0.06 – -0.04 (m, 6H), 4.45 – 4.36 (m, 1H), 4.33 (td, *J* = 3.23, 9.40 Hz, 1H), 4.12 (dt, *J* = 3.99, 8.66 Hz, 1H), 3.67 (d, *J* = 4.00 Hz, 2H), 3.64 – 3.57 (m, 2H), 3.51 (dt, *J* = 8.72, 14.17 Hz, 2H), 3.41 (ddt, *J* = 4.21, 9.56, 14.62 Hz, 2H), 3.31 (d, *J* = 3.59 Hz, 1H), 1.94 (s, 3H), 0.86 (d, *J* = 3.13 Hz, 9H), 0.02 (s, *3*H), 0.01 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.29, 166.95, 143.88, 143.71, 143.57, 143.17, 134.21, 134.07, 134.01, 133.95, 133.85, 133.81, 133.73, 133.71, 133.66, 133.62, 133.47, 133.45, 133.22, 133.12, 133.09, 107.73, 98.12, 96.63, 87.85, 86.99, 82.74, 81.45, 81.00, 80.76, 80.58, 80.36, 80.11, 78.98, 76.49, 76.34, 74.40, 68.77, 60.60, 35.21, 31.41, 26.47, 23.79, 6.55, 0.19, 0.00.

HRMS(ESI): calcd for $C_{50}H_{62}Cl_3NO_{12}SiNa\,[M+Na]^+$: 1024.2999, found 1024.3008



cis-4-(1H,1H,2H,2H,3H,3H-Perfluoroundecyloxy)-2-butenyl *O*-(methyl 2,3–*O*–di– benzyl -4– *O* -tert-butyldimethylsilyl –β-D-glucopyrafnosyluronate)-(1→3)-(4,6-*O*-benzylidene -2-deoxy-2-trichloroacetamido - β-D-glucopyranosyl)-(1→4)- (methyl 2,3–*O*–di– benzyl –β-D-glucopyrafnosyluronate)- (1→3)-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido - β-D-glucopyranoside (35)

Donor **33** (36mg, 0.031 mmol) and acceptor **34** (16 mg, 0.011 mmol) were reacted under the conditions of general glycosylations. After FSPE purification, and the mixture was further purified by SGC (hexanes/EtOAc 2:1) to give **35** (2.3 mg, 0.001 mmol, 10%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.16 (m, 40H), 0.06 – -0.06 (m, 7H), 7.08 (d, *J* = 8.90 Hz, 1H), 6.93 (d, *J* = 8.69 Hz, 1H), 5.75 – 5.61 (m, 2H), 5.09 (d, *J* = 7.36 Hz, 1H), 5.06 – 4.92 (m, 4H), 4.85 (dd, *J* = 5.64, 10.91 Hz, 1H), 4.81 – 4.68 (m, 3H), 4.68 – 4.53 (m, 4H), 4.53 – 4.43 (m, 3H), 4.44 – 4.32 (m, 2H), 4.27 – 4.18 (m, 2H), 4.06 – 3.91 (m, 3H), 3.91 – 3.76 (m, 3H), 3.76 – 3.57 (m, 9H), 3.58 – 3.30 (m, 6H), 2.25 – 2.10 (m, 2H), 1.95 – 1.85 (m, 2H), 0.86 (s, 9H), 0.00 (s, 3H), -0.02 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.48, 174.02, 166.71, 166.16, 143.29, 133.82, 133.67, 133.59, 133.51, 133.38, 133.26, 133.16, 133.11, 132.93, 132.81, 132.38, 132.16, 108.32, 103.06, 102.44, 97.40, 89.16, 81.47, 80.89, 80.84, 80.23, 79.69, 79.69, 79.56, 78.79, 78.61, 77.54, 74.45, 74.36, 74.28,

74.10, 73.93, 73.93, 71.72, 71.72, 64.32, 57.46, 35.00, 31.00, 23.20, 19.42, 1.32, 0.00.

^{19}F NMR (376 MHz, $CDCl_3$) δ -80.74 (m, 3F), -114.41 (m, 2F), -121.81 (m, 6F), -122.70 (m, 2F), -123.43 (m, 2F), -126.10 (m, 2F).

HRMS(ESI): calcd for $C_{107}H_{115}Cl_6F_{17}N_2O_{24}SiNa$ $[M+Na]^+$: 2395.5361, found 2395.5396

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CHAPTER 6

Conclusions and future directions

In this dissertation, methods for the solution and fluorous-assisted synthesis of complex carbohydrates including maltotriose phosphate analogs and an oligomer of hyaluronic acid have been developed along with the development of process scale synthesis of a *C*-glycoside as a substitute for IPTG.

The first fluorous protecting group for phosphate was developed. In future work, this tag and additional novel fluorous tags could be tested for their utility in the automated synthesis of oligosaccharides. The fluorous phosphate protecting group was then evaluated in the synthesis of maltotriose phosphate analogs. A series of maltotriose phosphates was synthesized for the study of Lafora disease. The α/β mixtures resulting from the glycosylation reactions proved difficult to separate, but in the end six maltotriose phosphate analogs were synthesized for the first time. The usage of the fluorous phosphate protecting group was evaluated. In case of reactions that would give a mixture of fluorous tagged product, it might not be very convenient to use a fluorous assisted synthesis. In the future, this fluorous protecting group could be used together with other fluorous tags, combined with F-HPLC to perform mix-tag synthesis.

We developed a much more efficient and practical synthesis for the larger scale synthesis of IBCG

that should enable future experiments in mice. This work demonstrated the problems which can be encountered during optimizing and scaling up of a synthetic route. In synthetic carbohydrate chemistry, one of the major practical difficulties is to synthesize building blocks in large amounts. The automated solution-phase synthesis of carbohydrates also calls for multi-gram-scale building block synthesis. In the future, optimization of the syntheses for the existing building blocks is clearly an important direction.

Finally, an approach amenable to automation for the synthesis of hyaluronic acid fragments was developed. The synthesis of glycosaminoglycans is still a daunting challenge, but a protecting group pattern that shows promise has been found for each of the constituent building blocks. This initial work sets the stage for future studies in the automated synthesis of a range of glycosaminoglycans.

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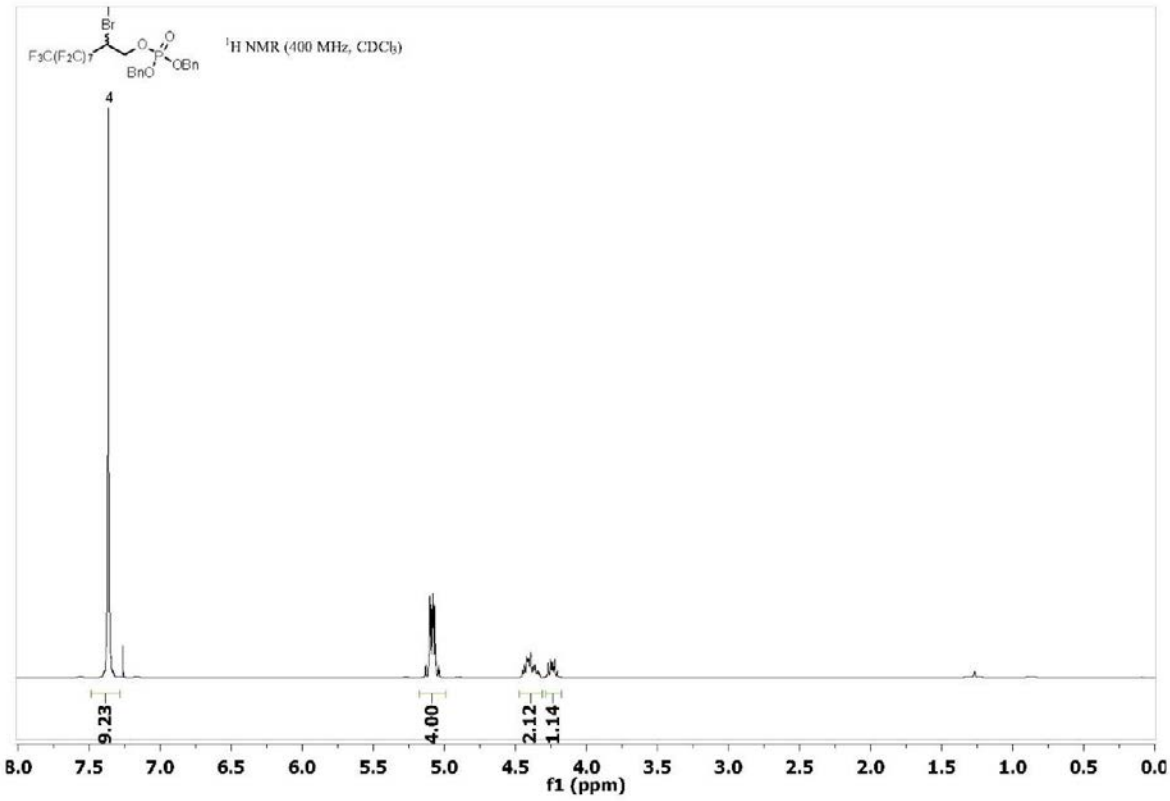
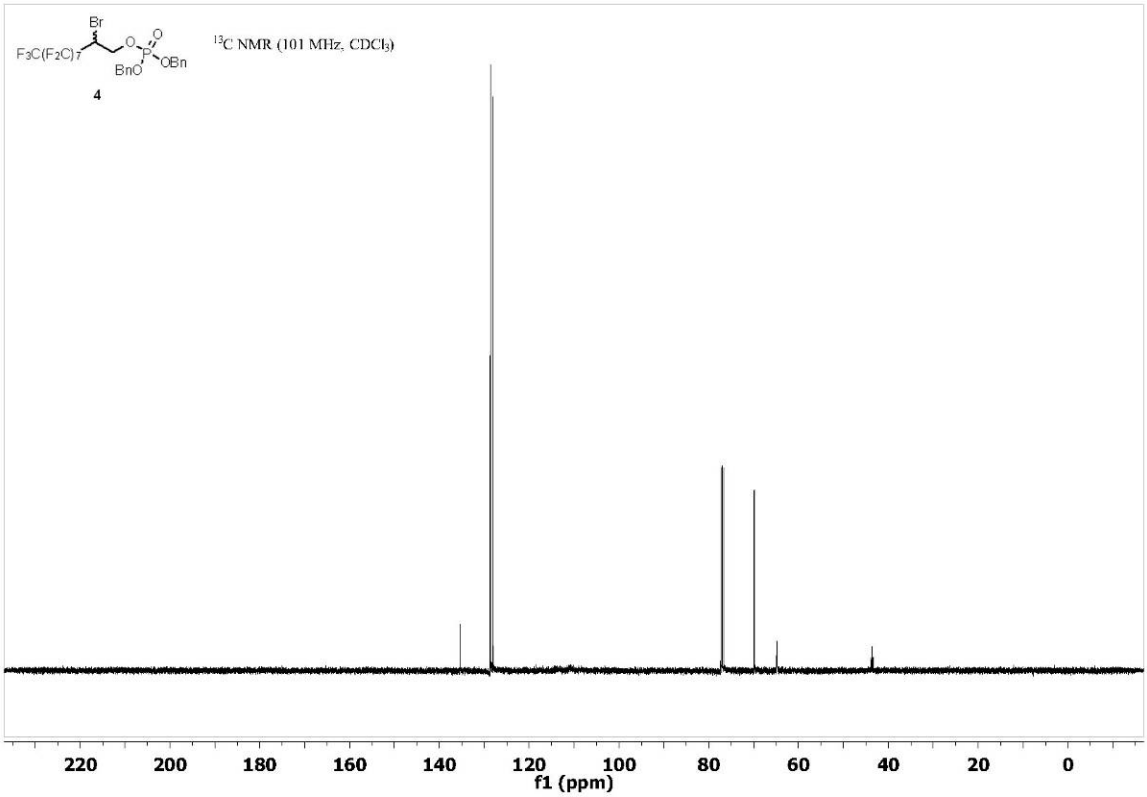
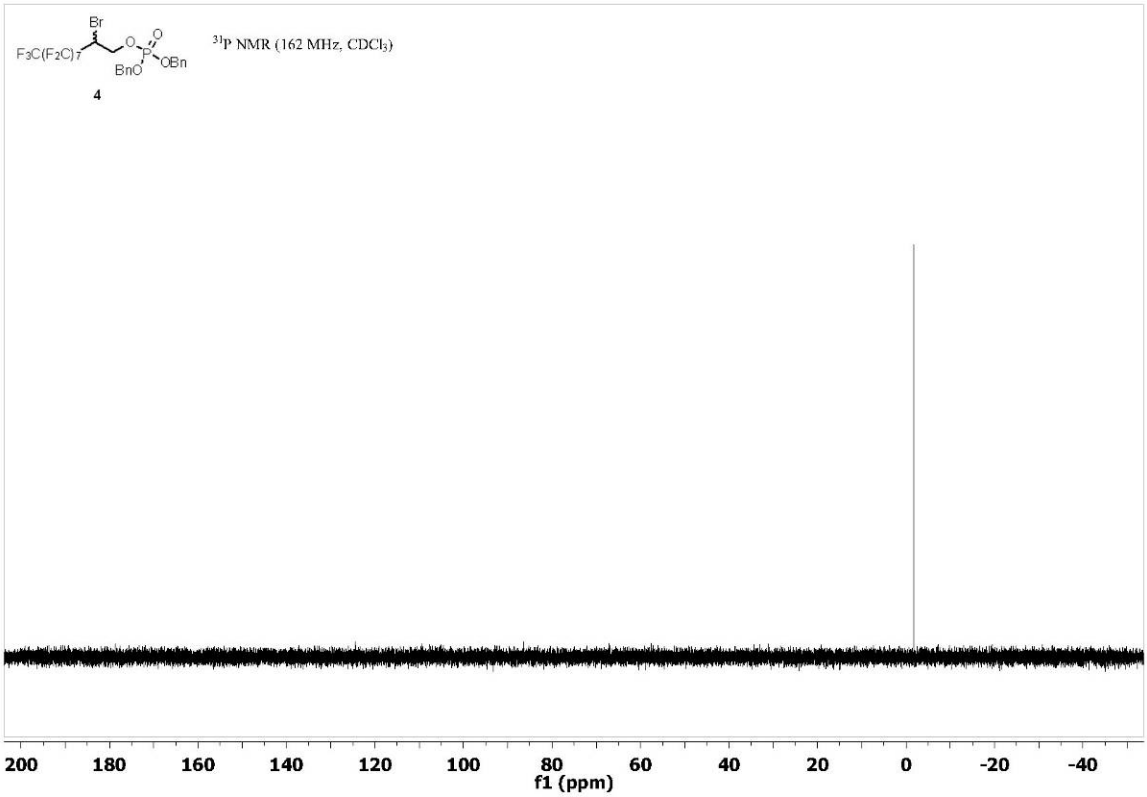
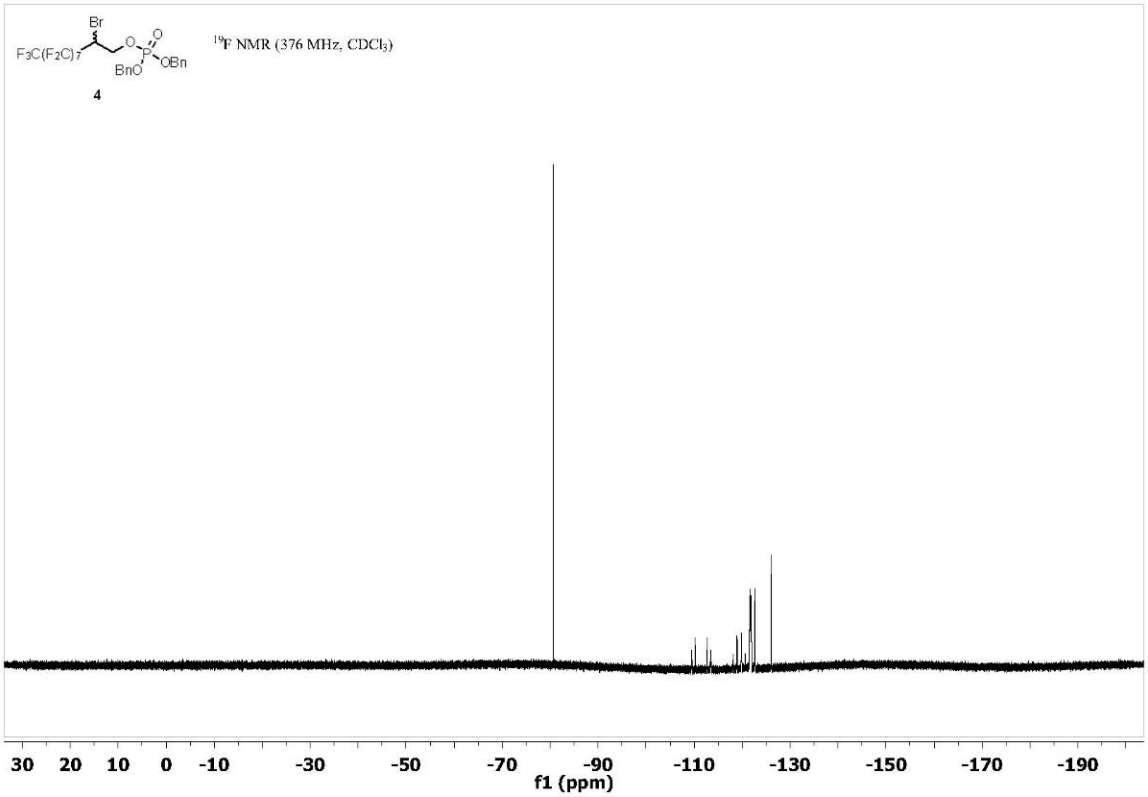
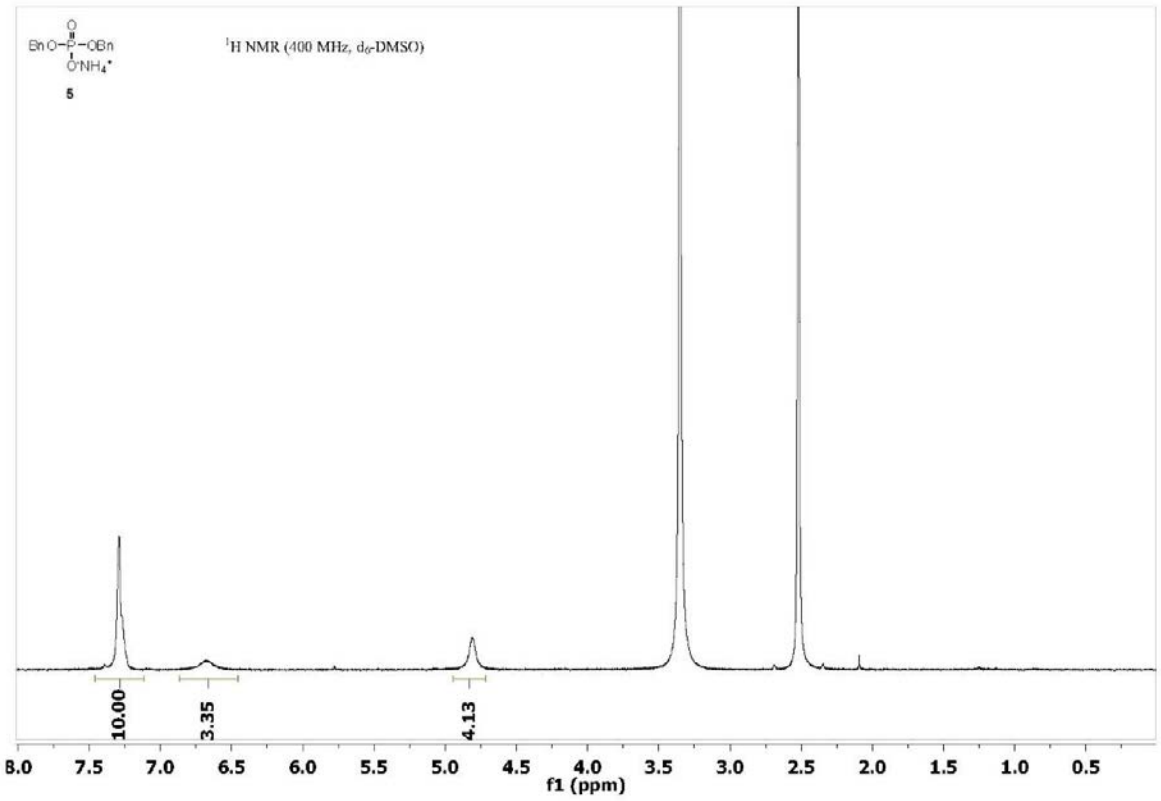
APPENDIX A. CHAPTER 2 ^{1}H AND ^{13}C NMR SPECTRA

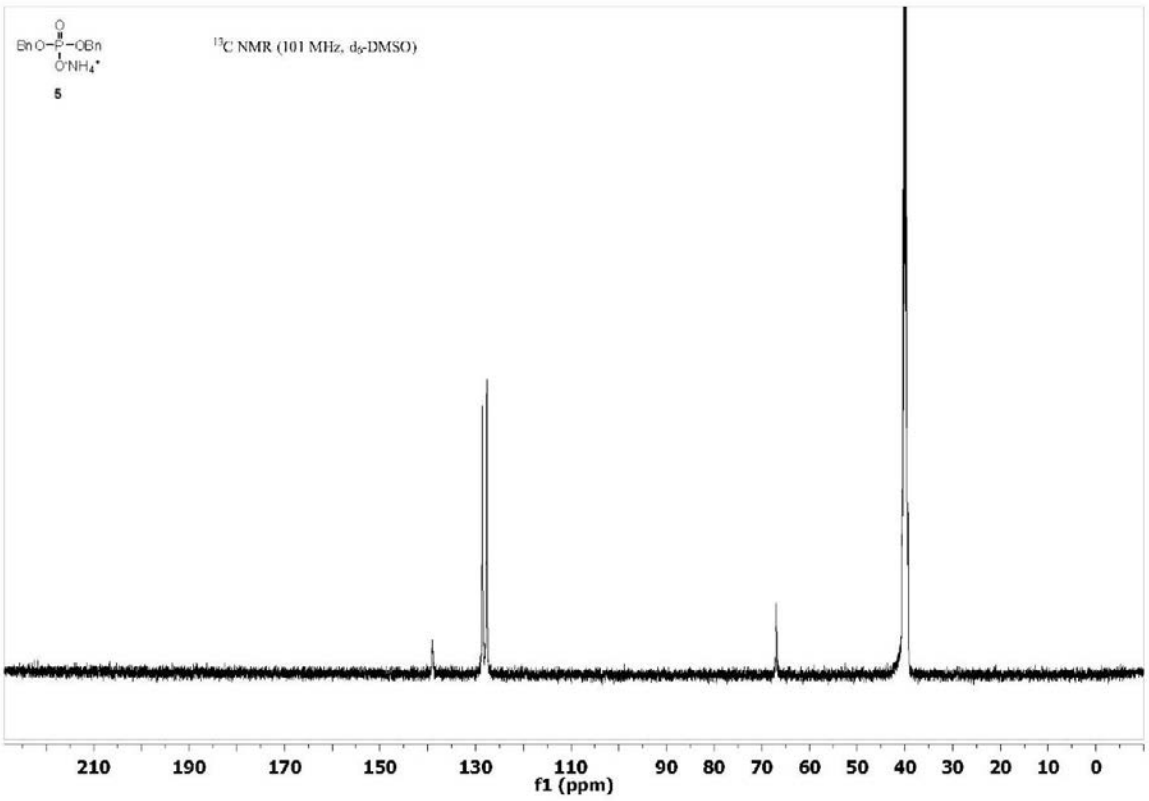


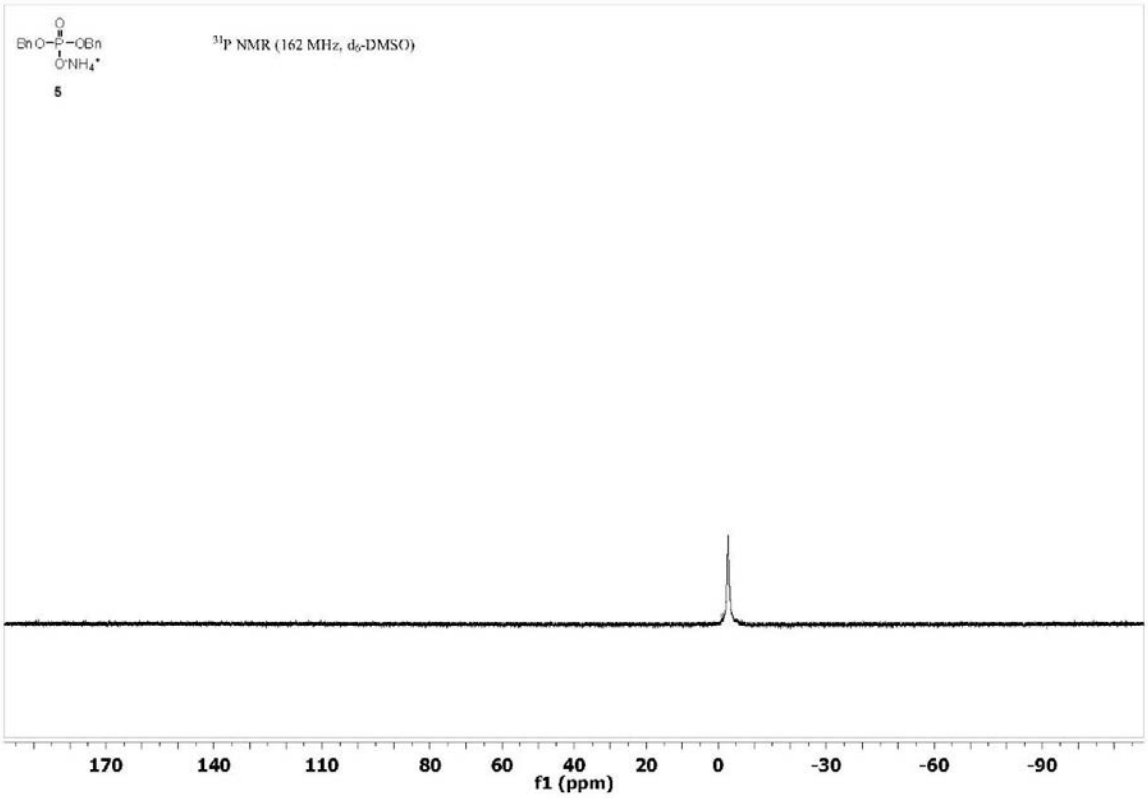


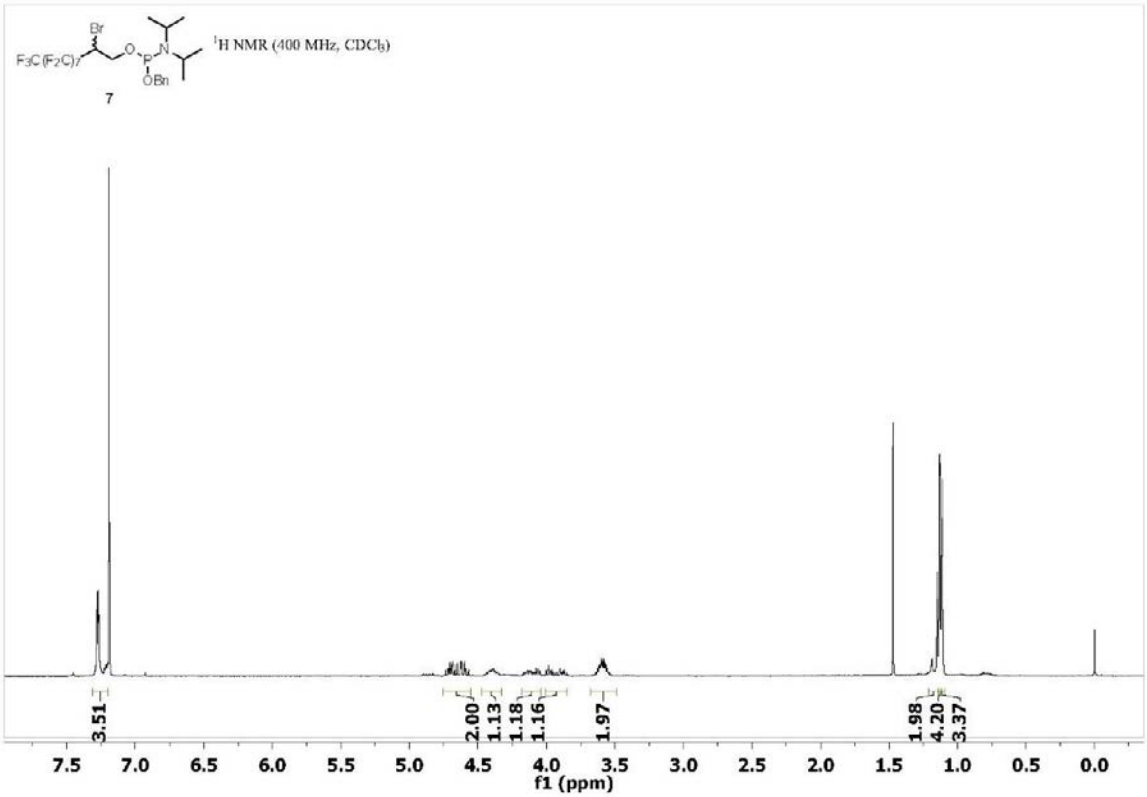


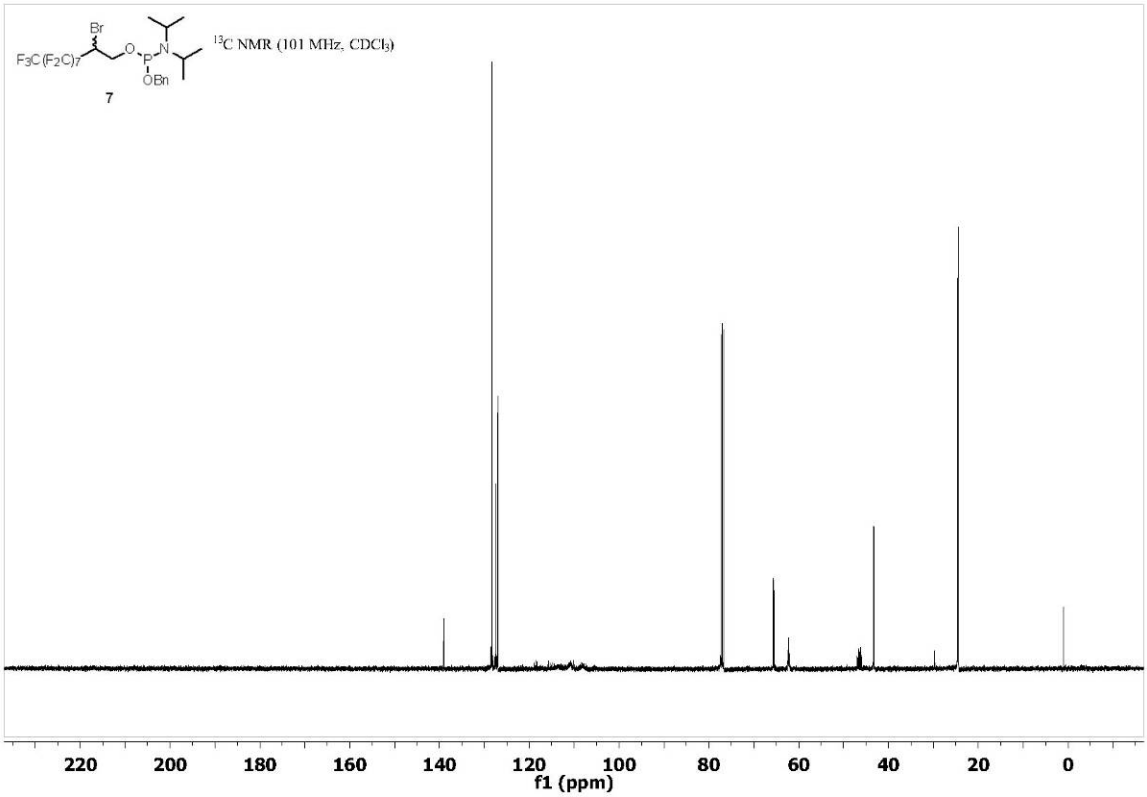


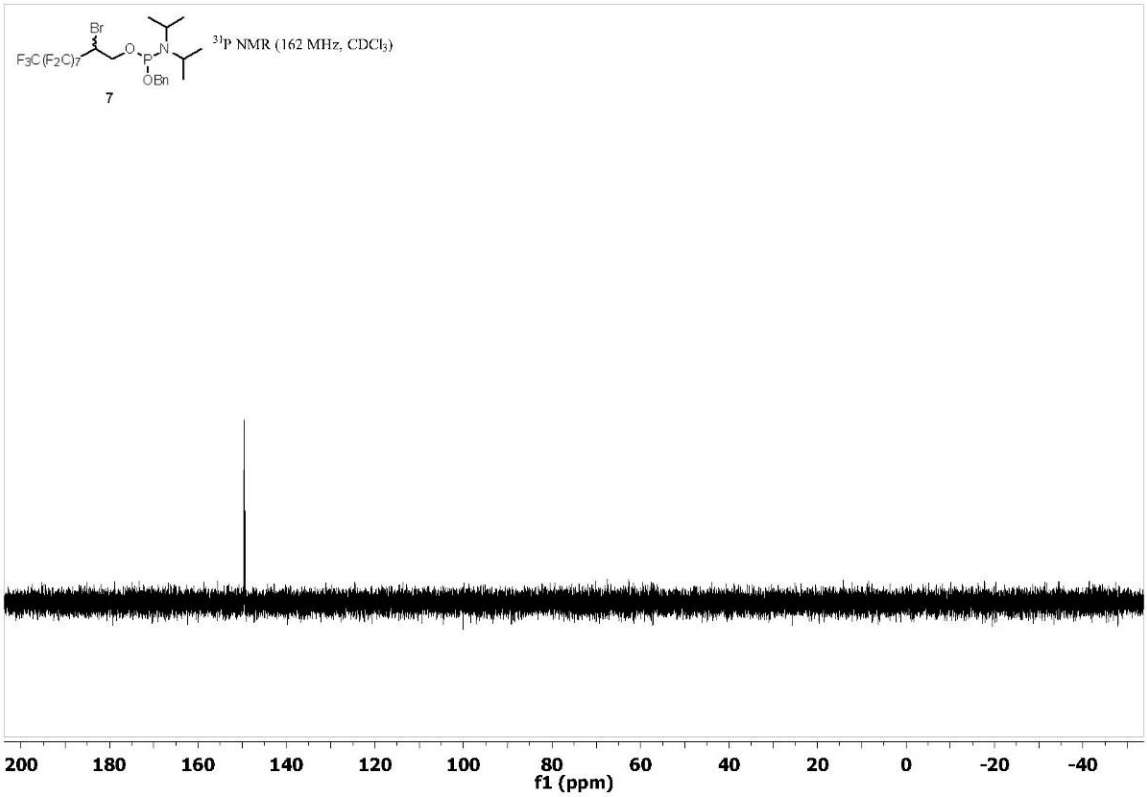


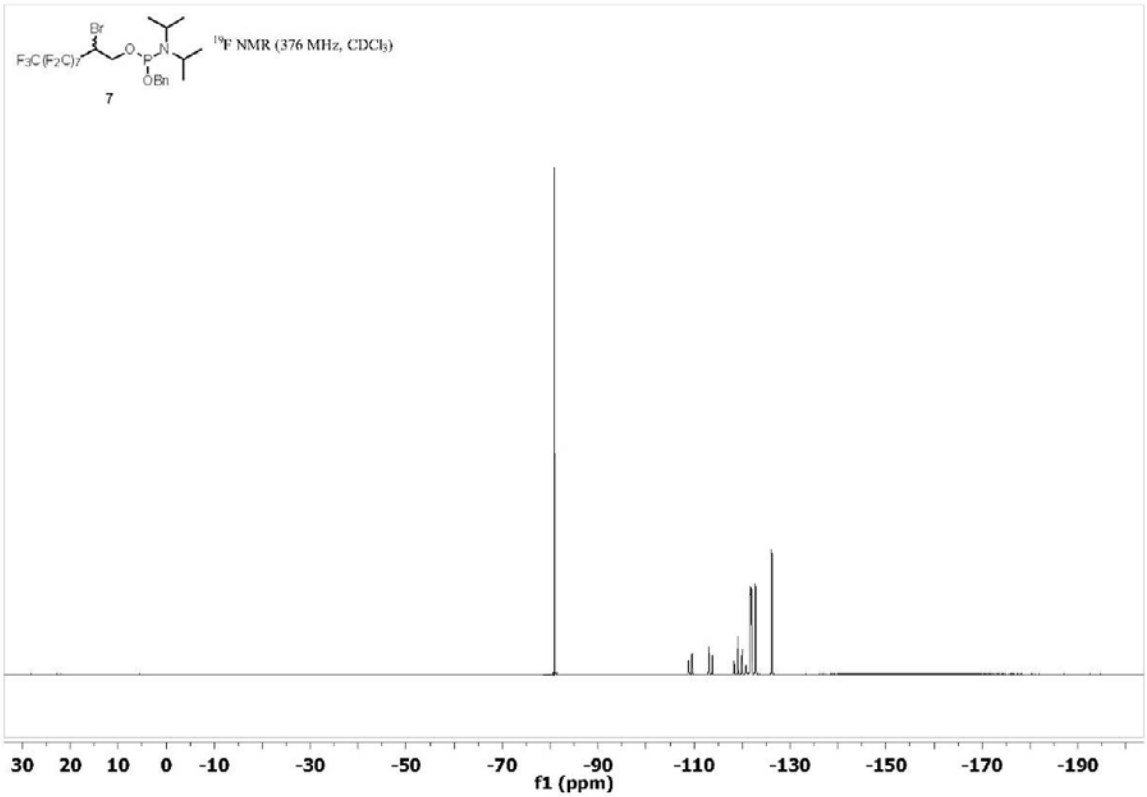


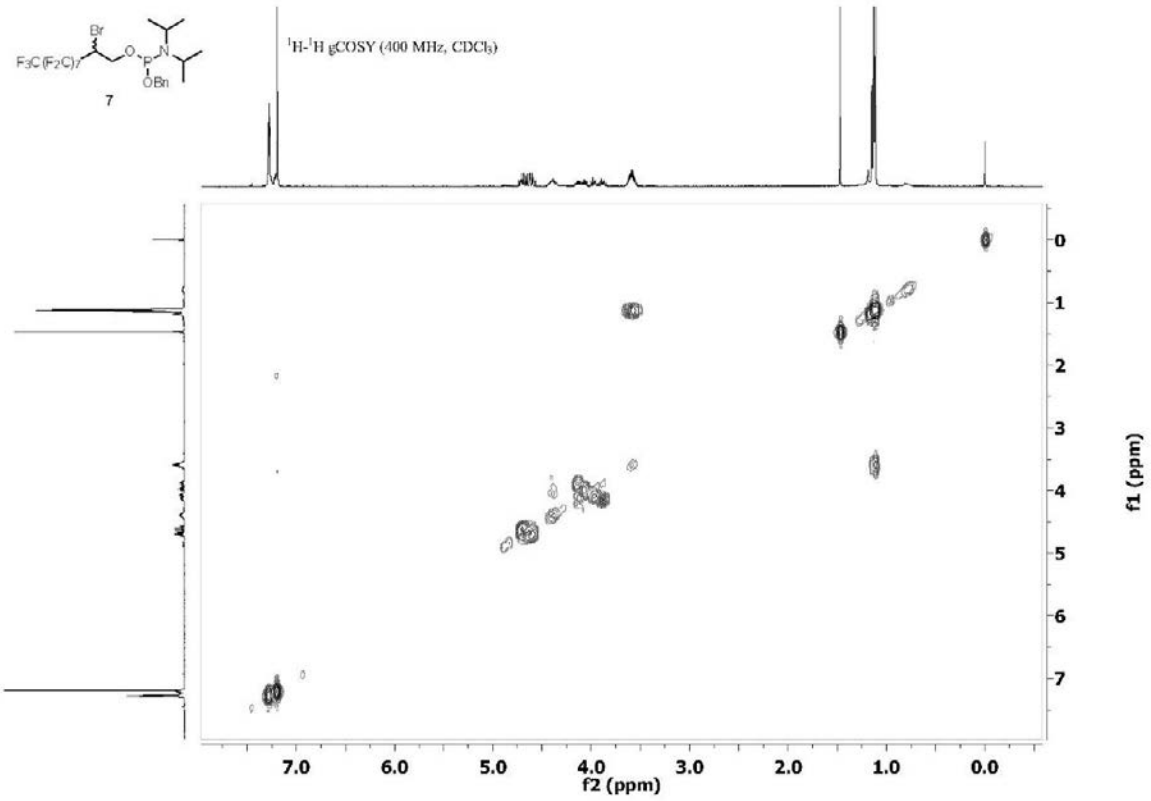


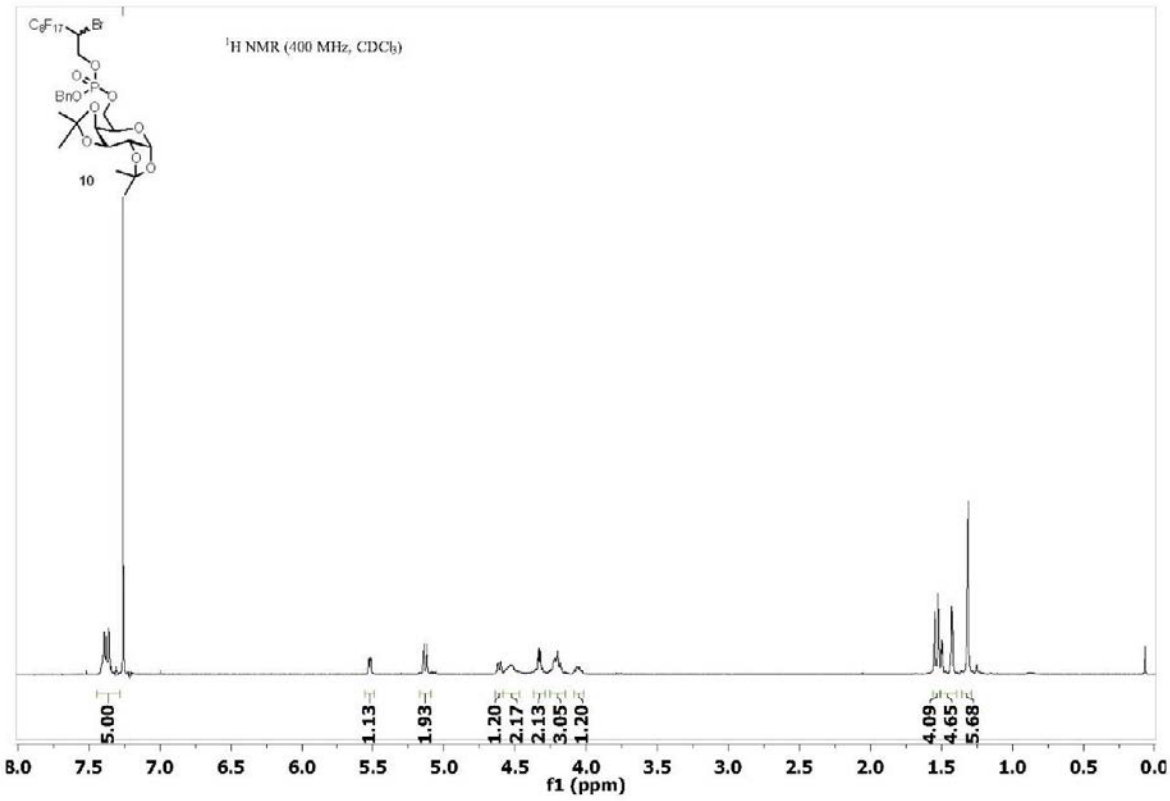


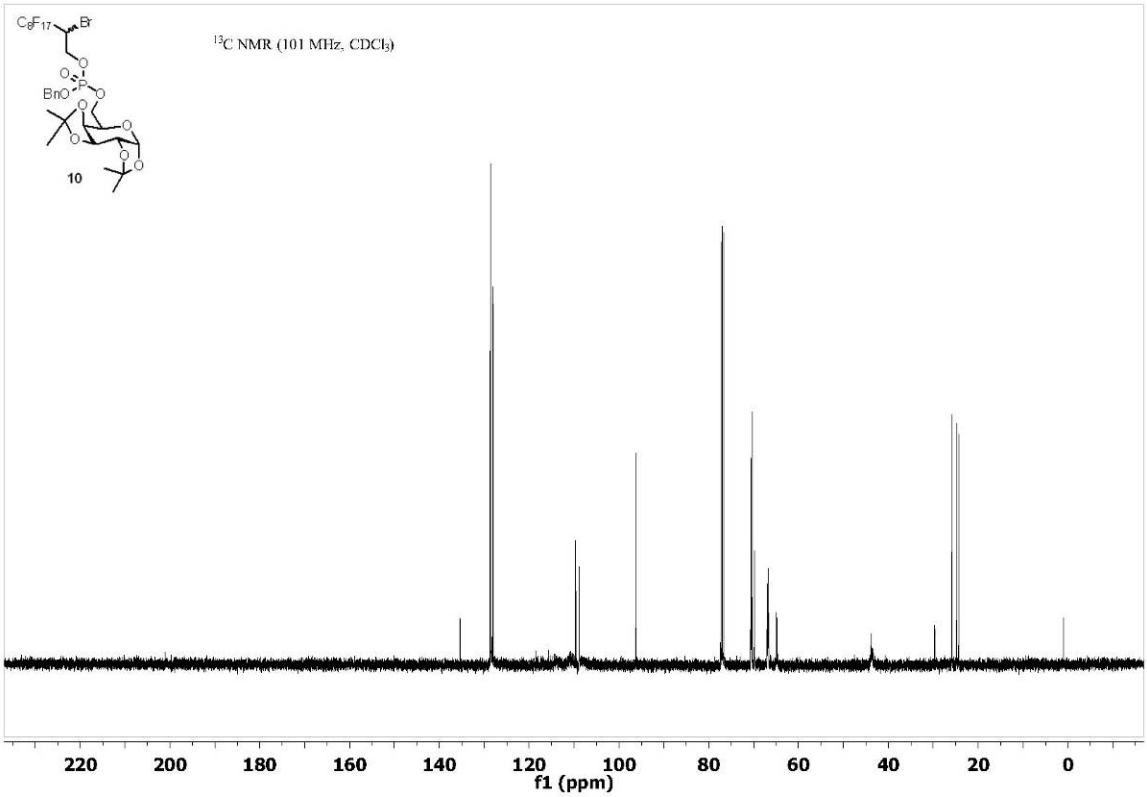


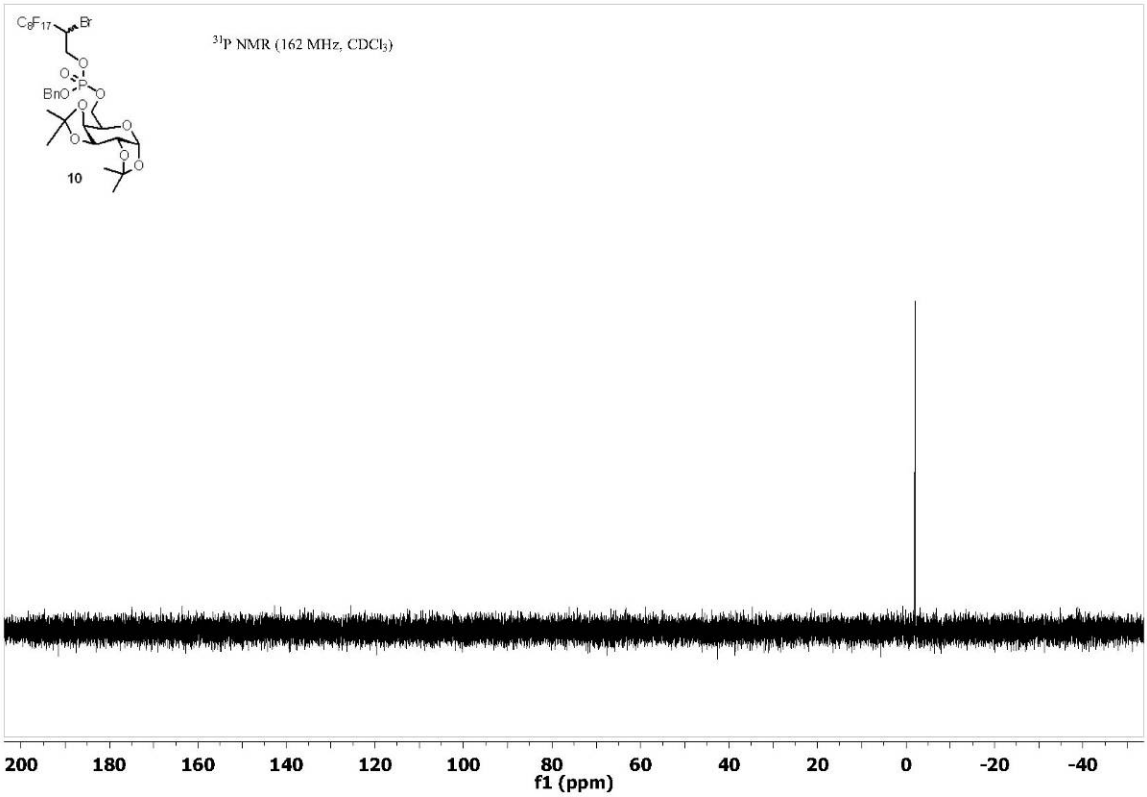


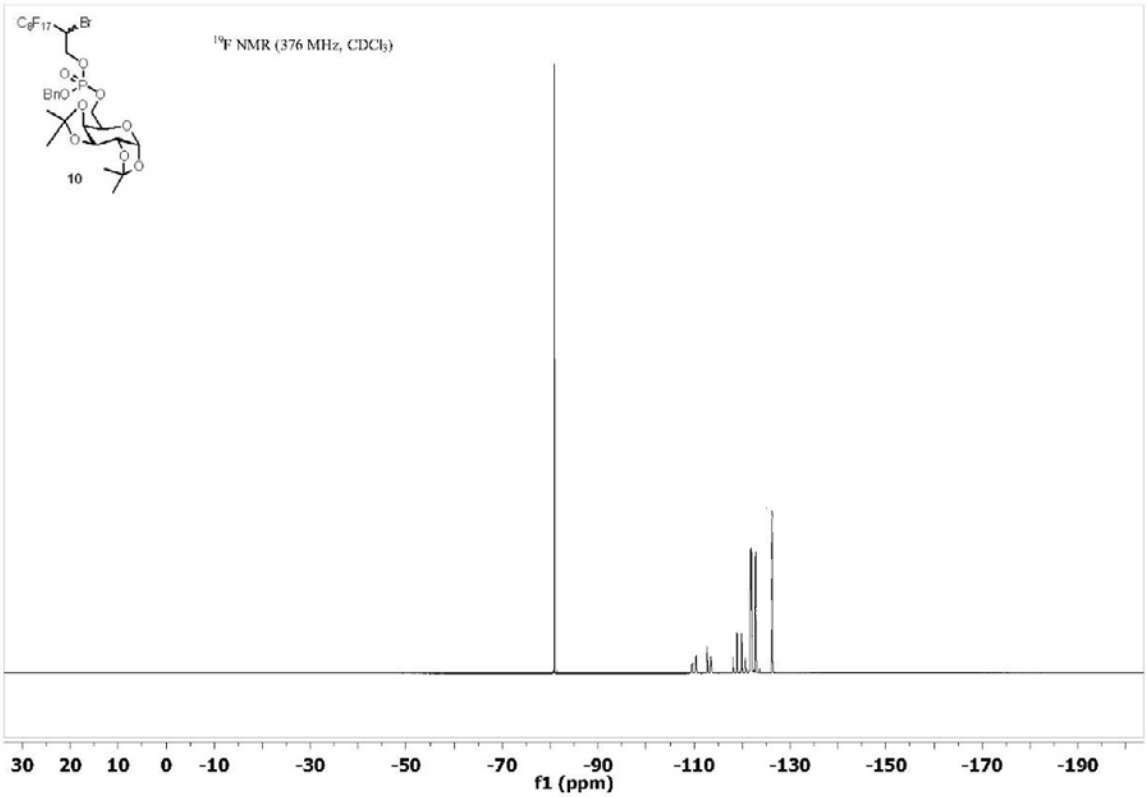


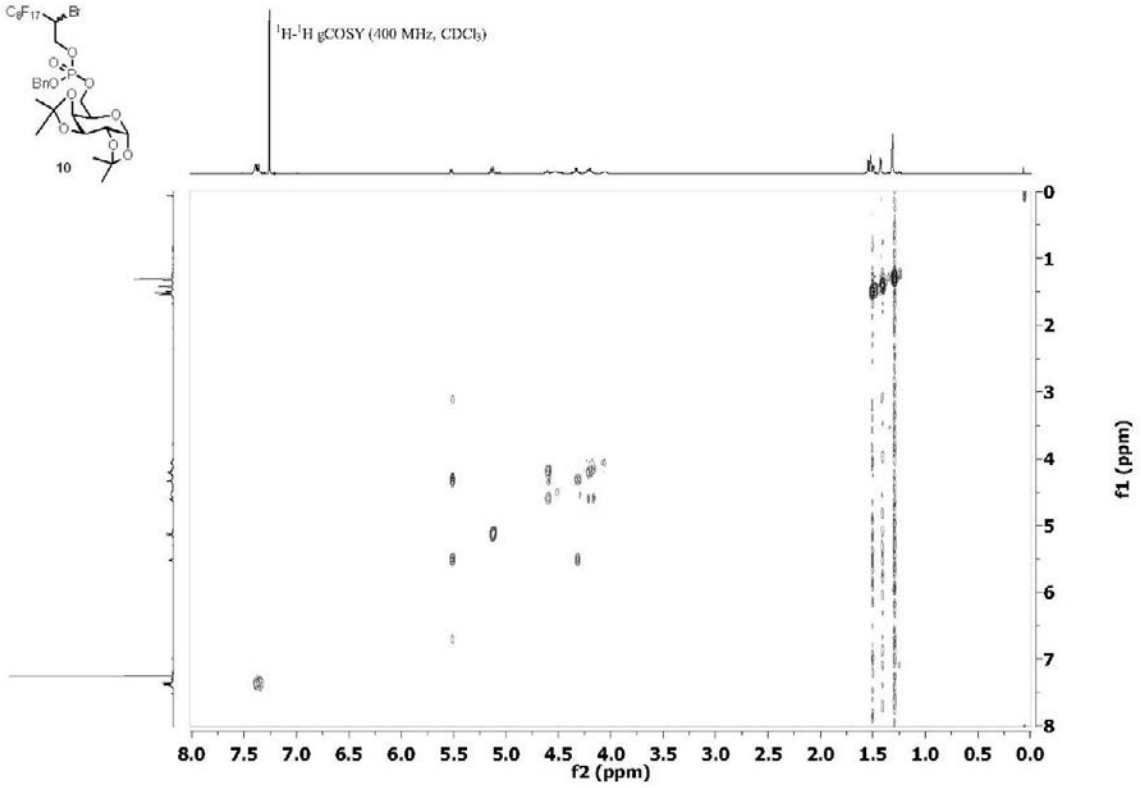


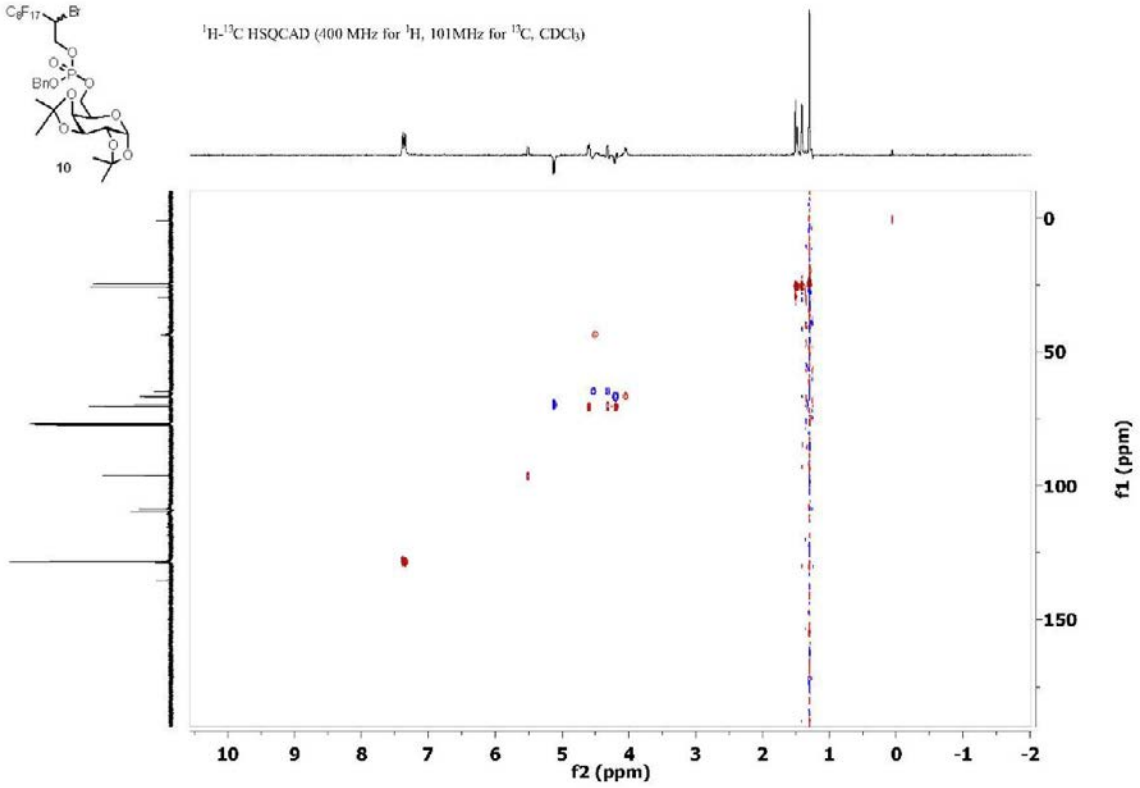


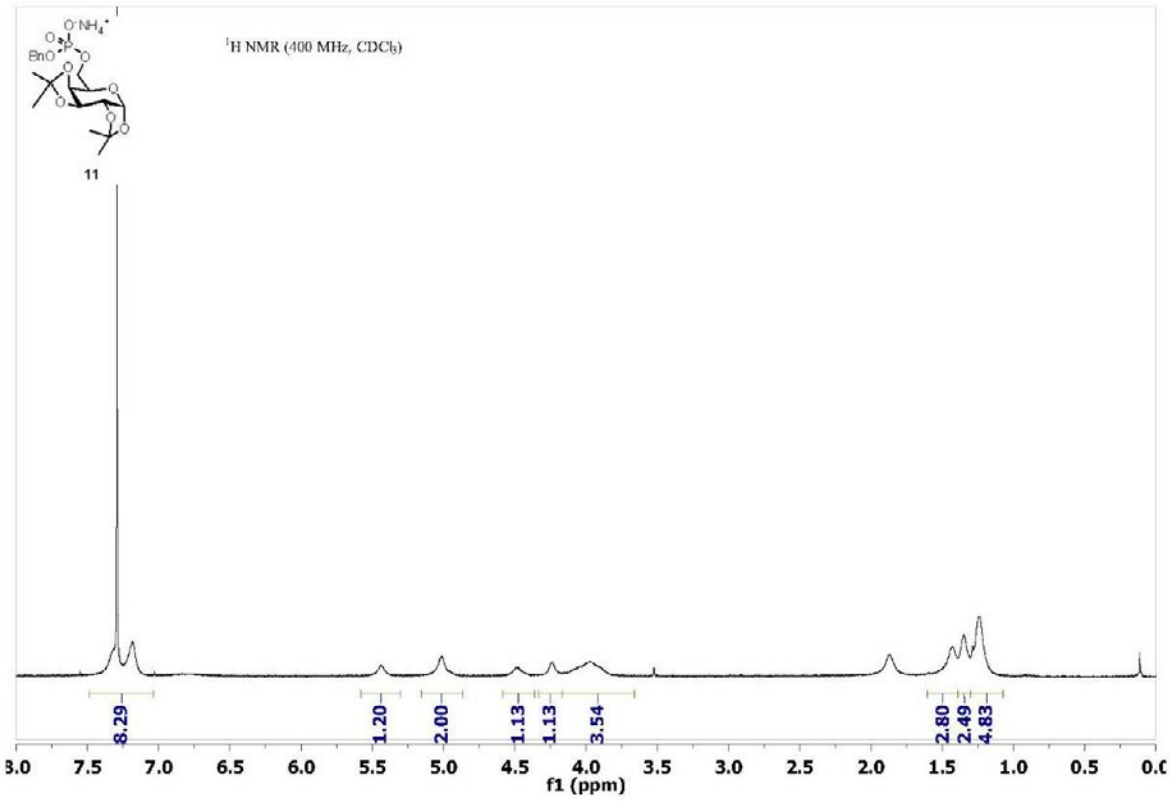


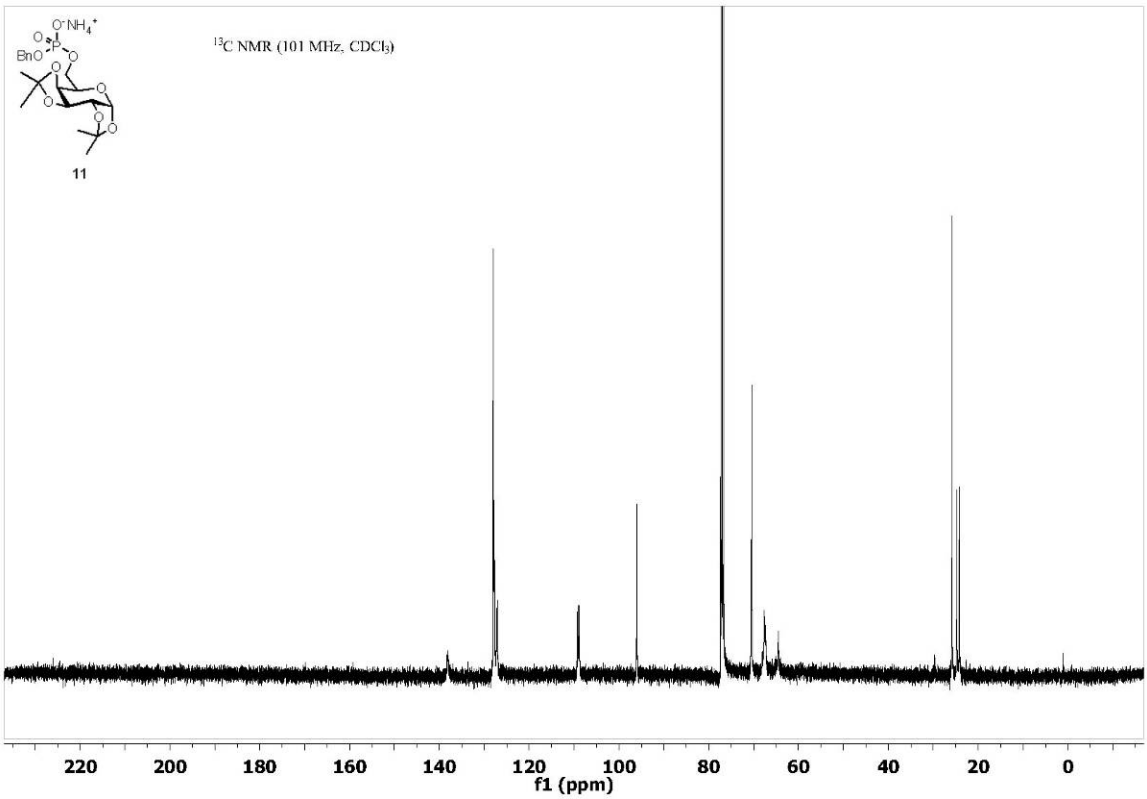


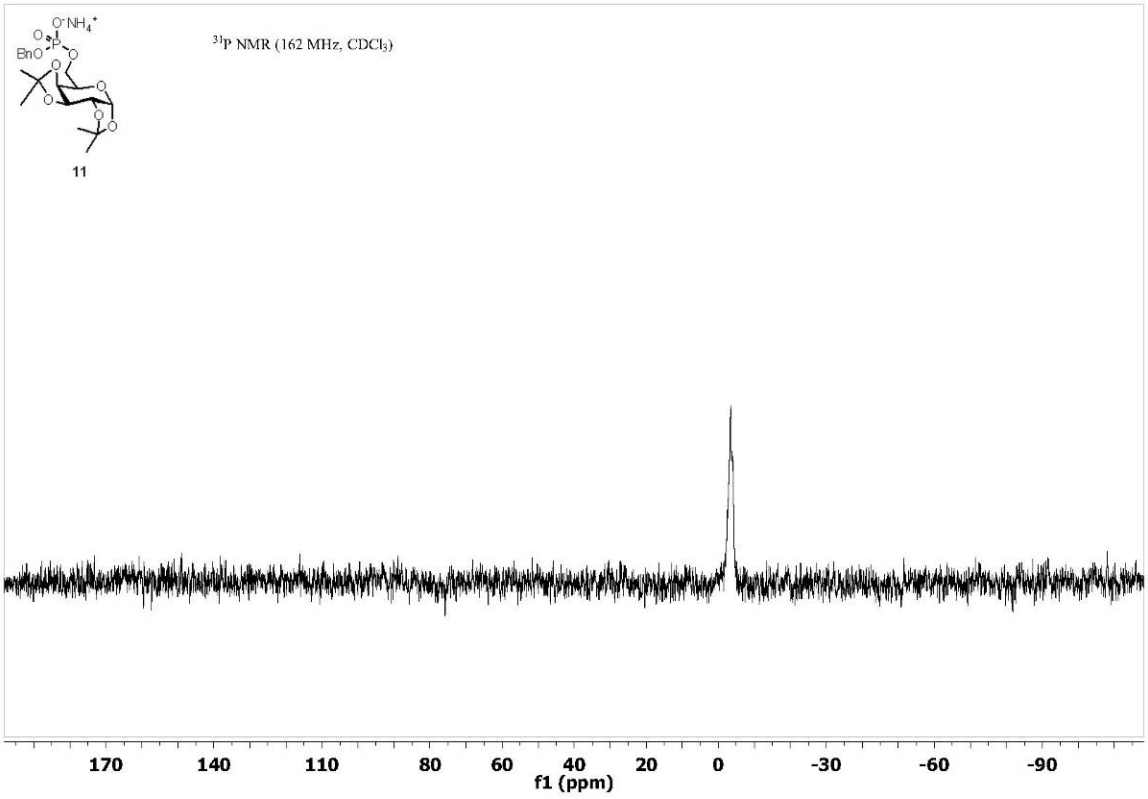


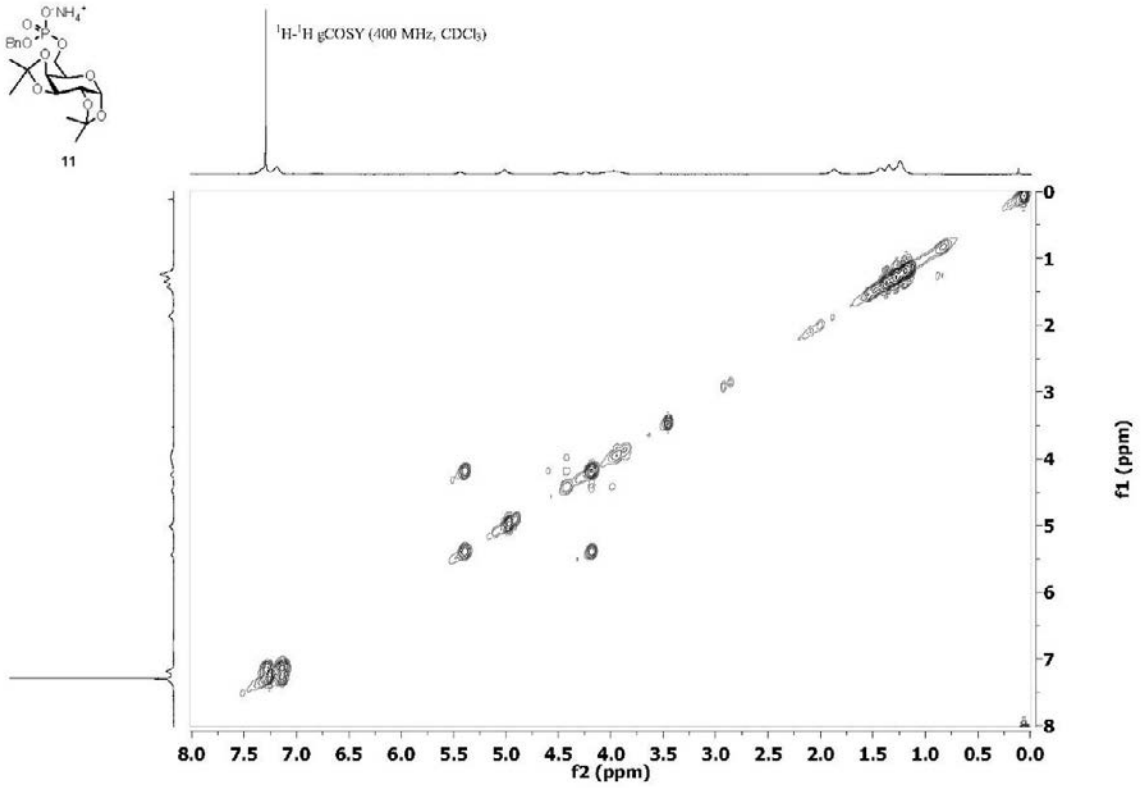


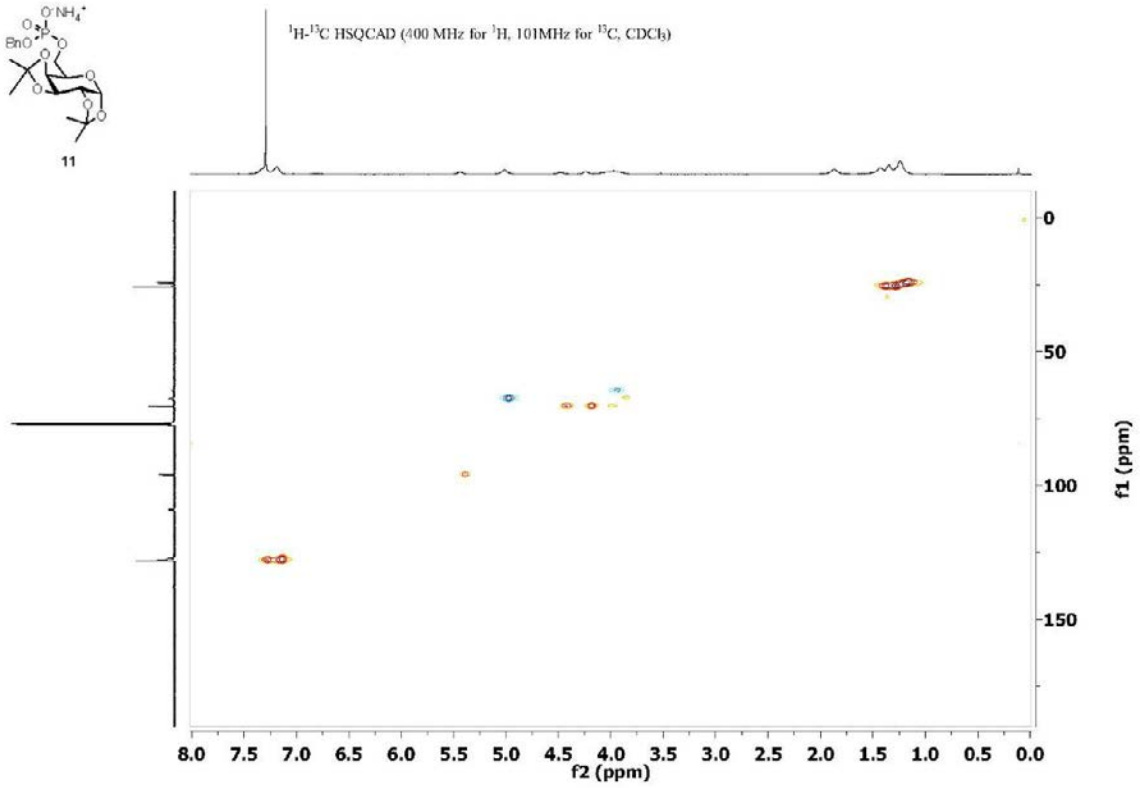


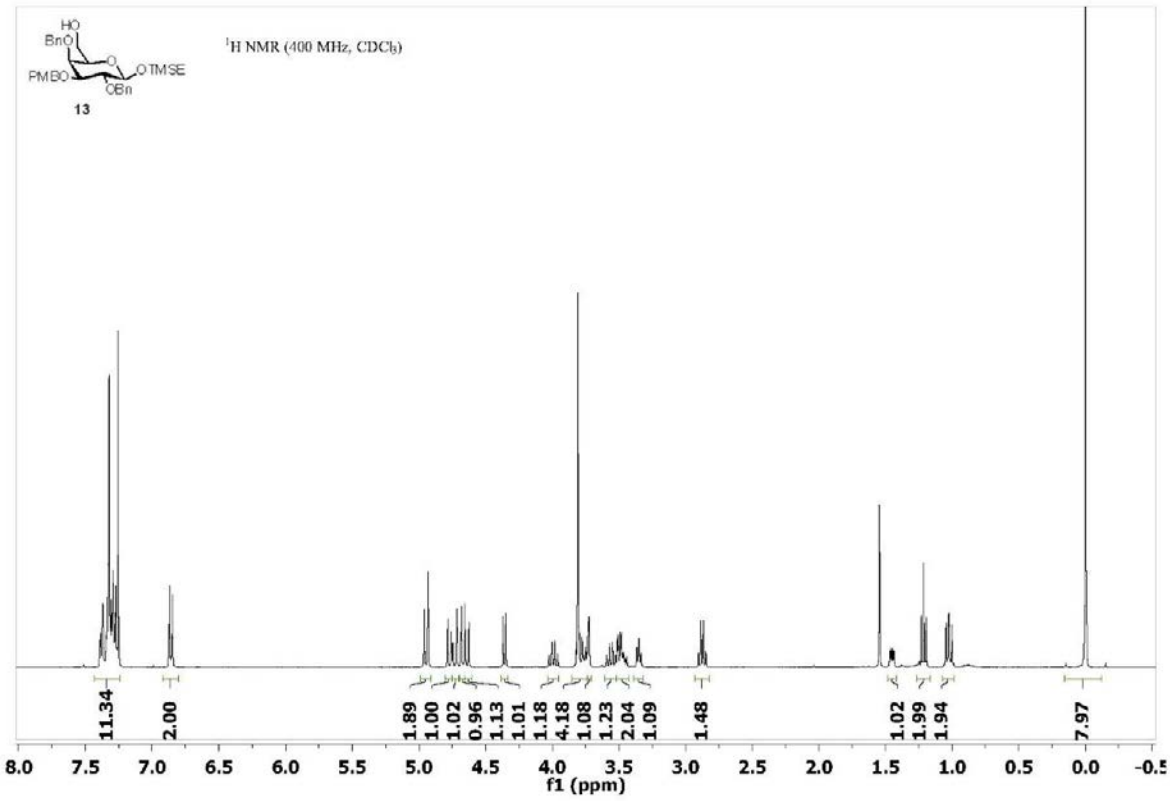


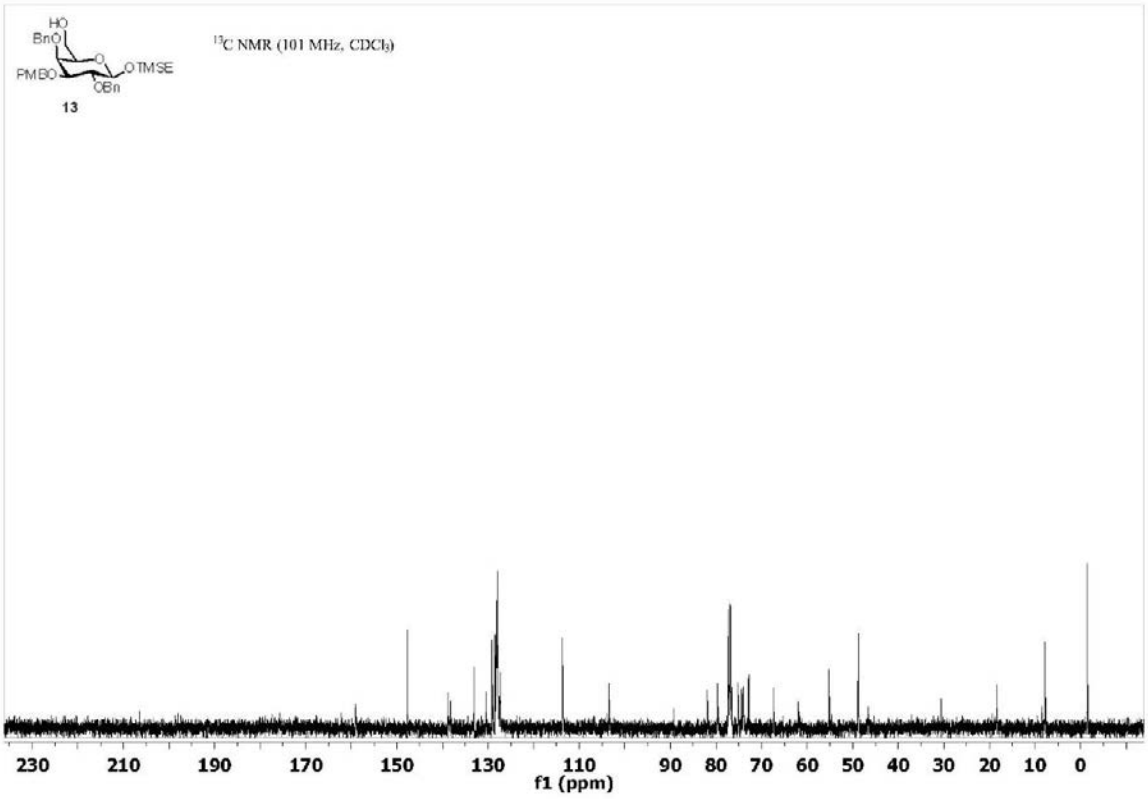


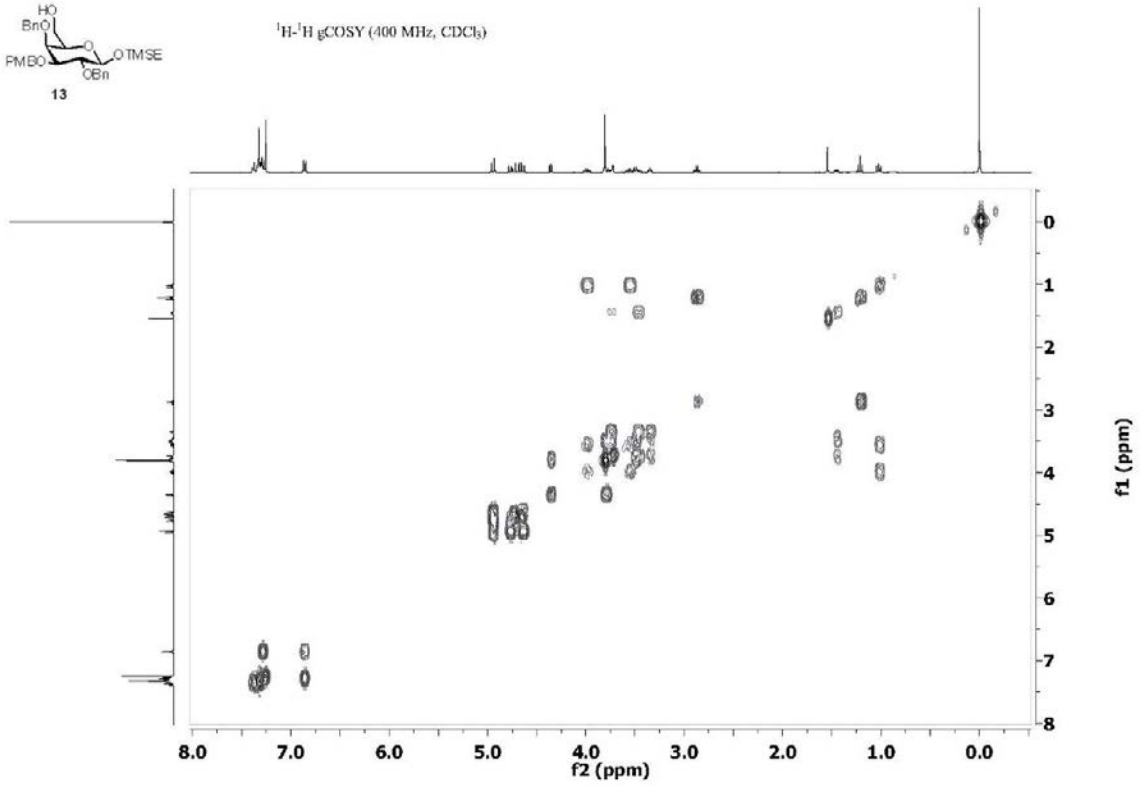


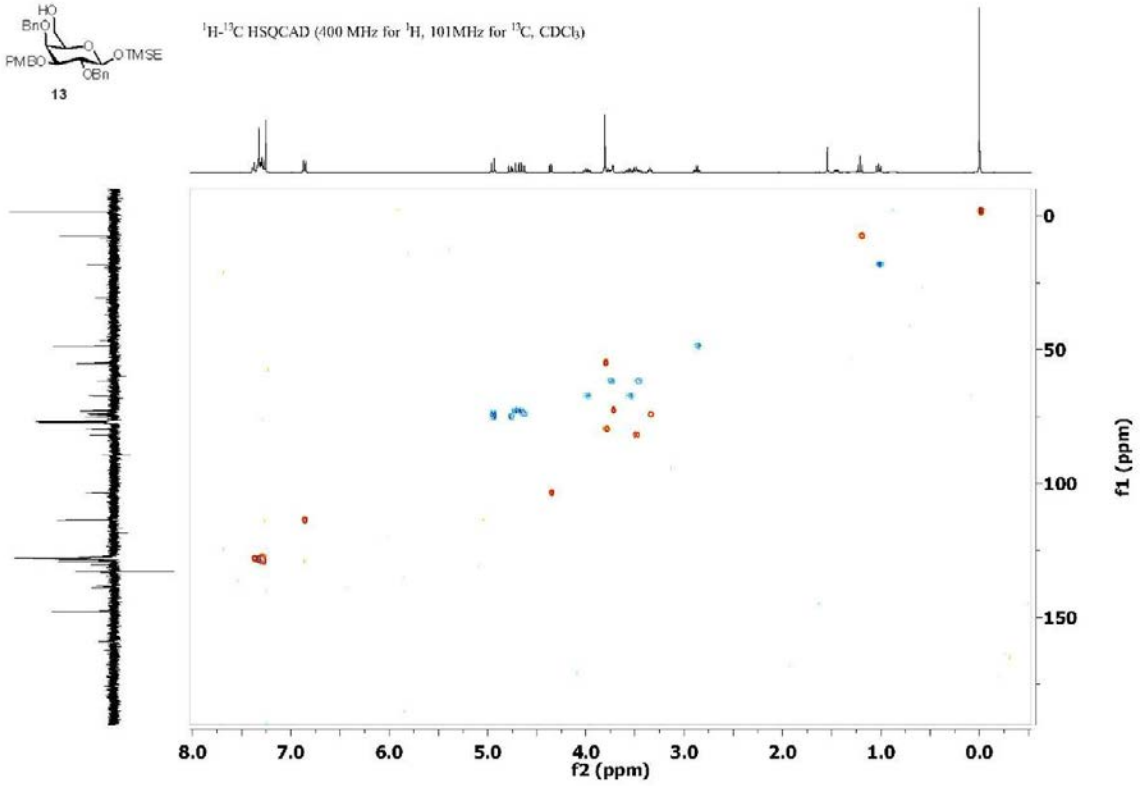


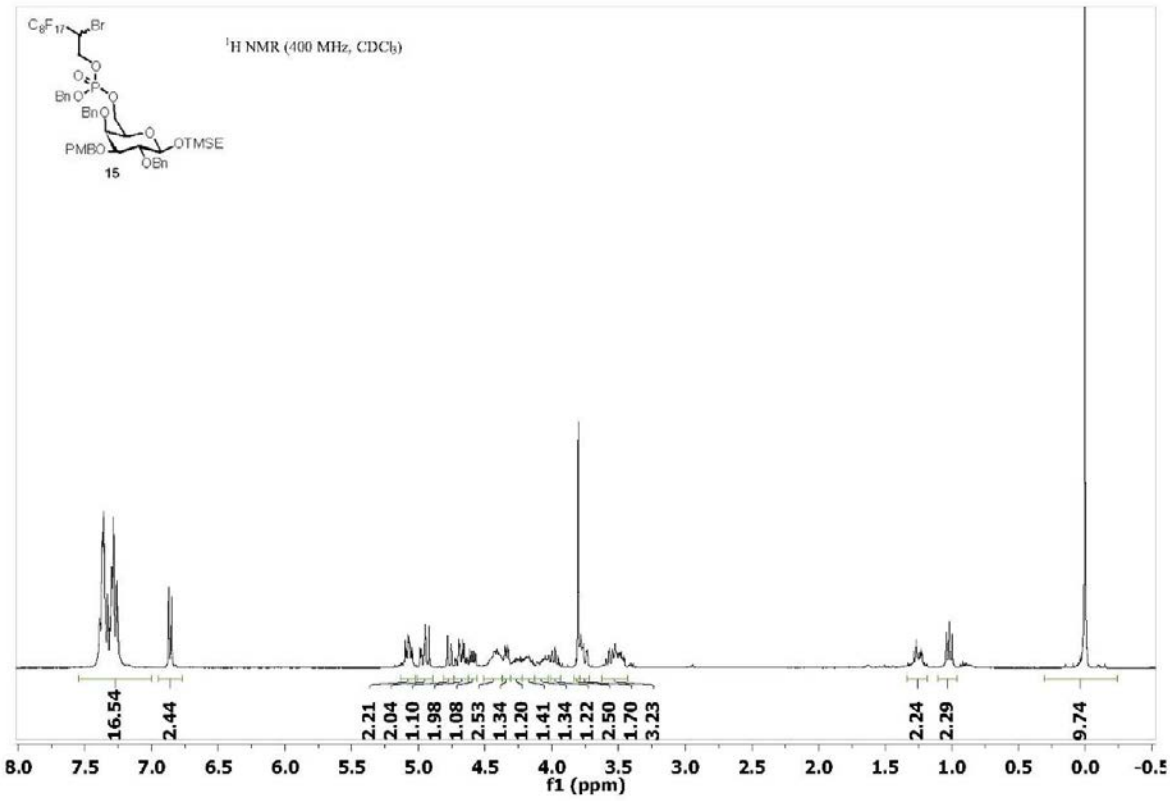


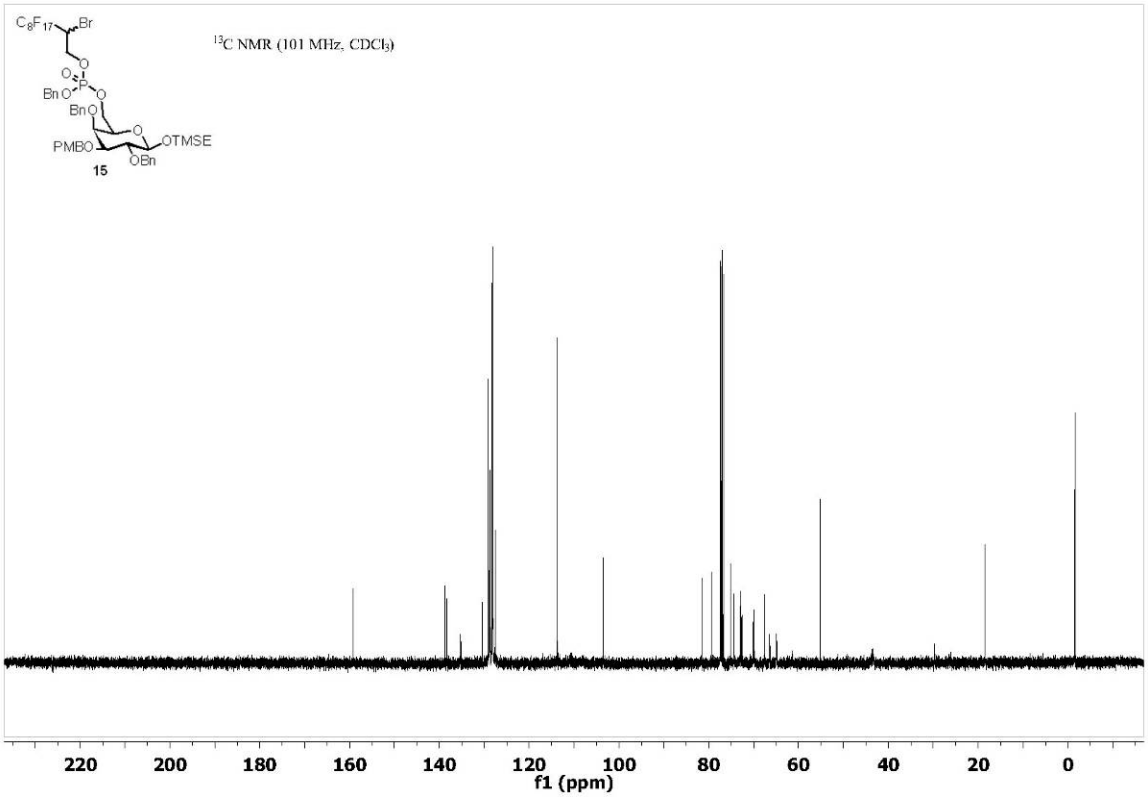


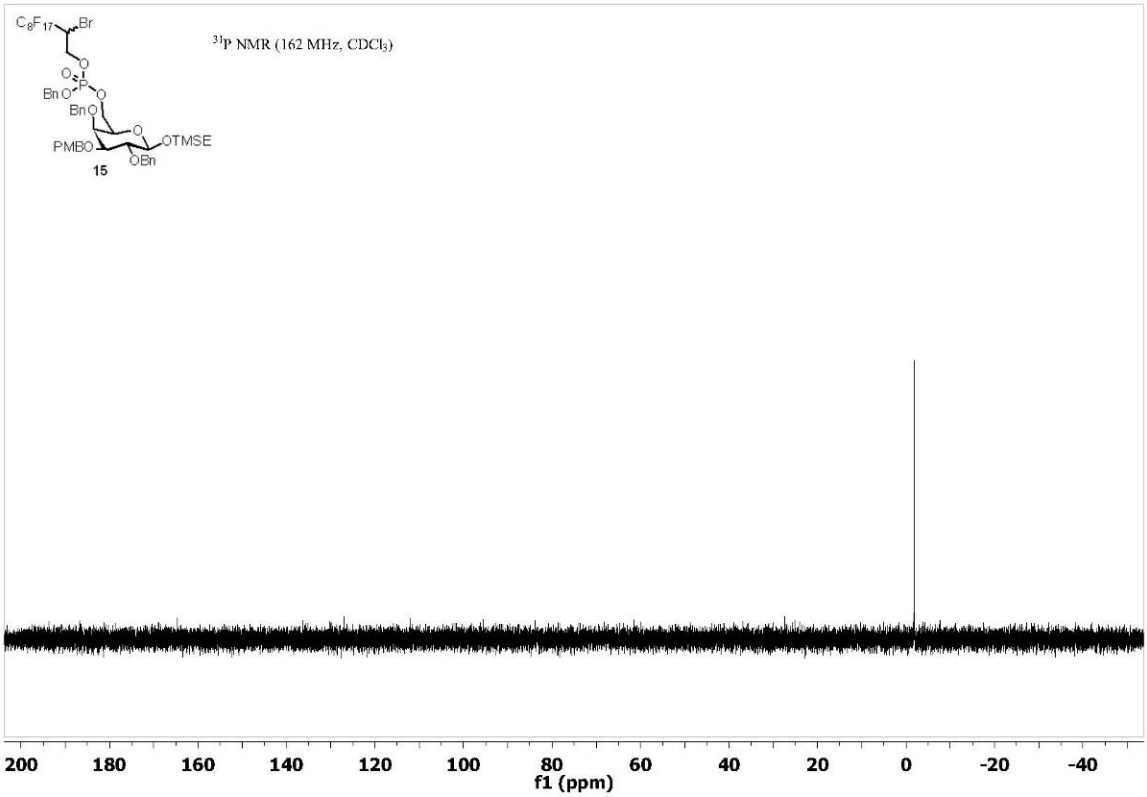


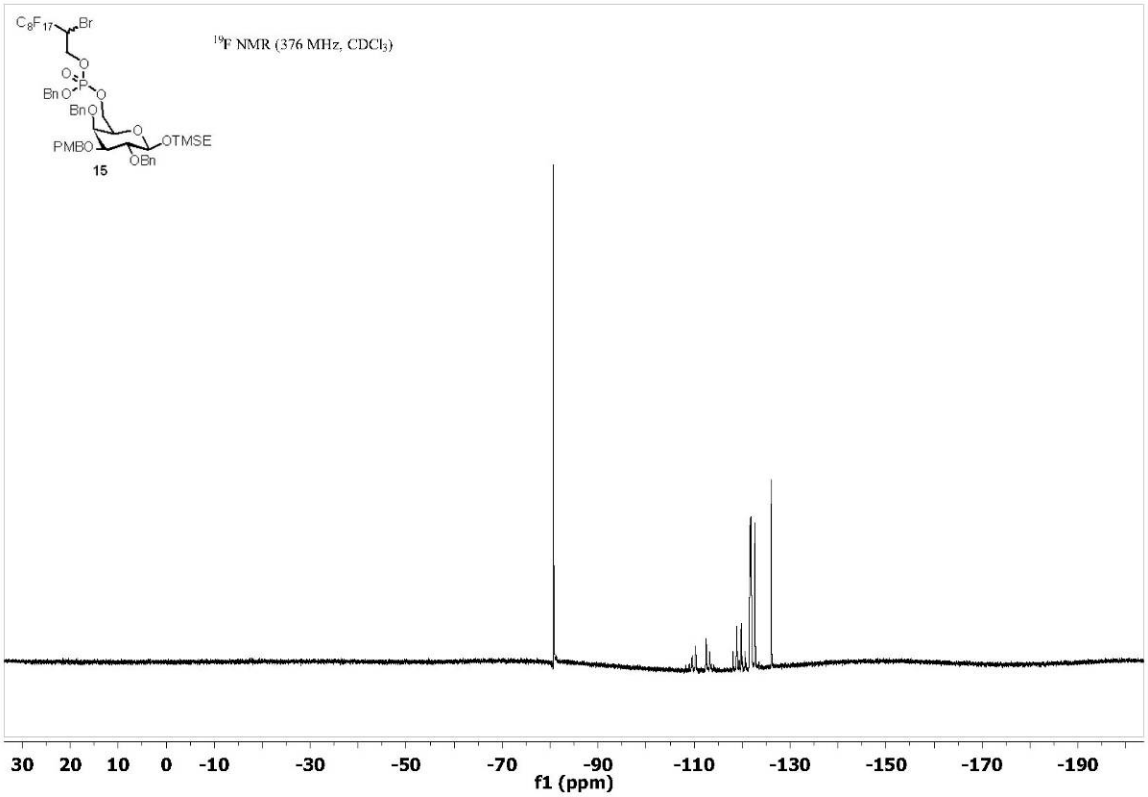


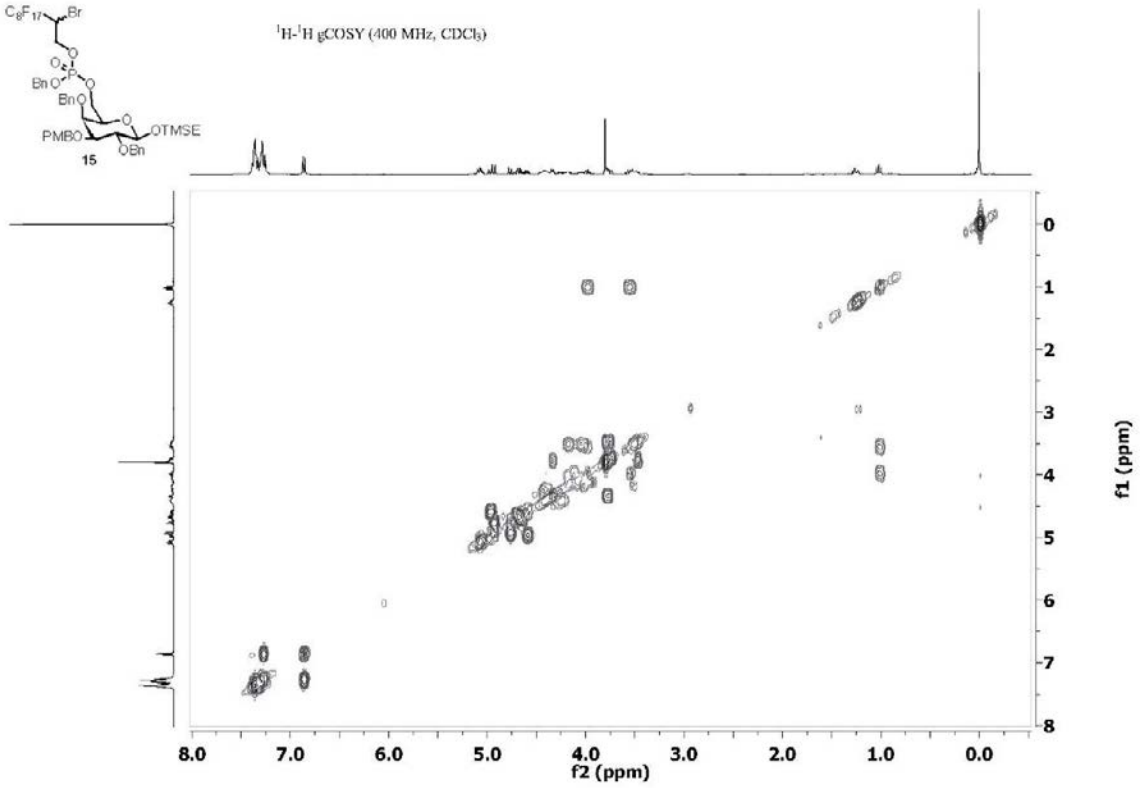


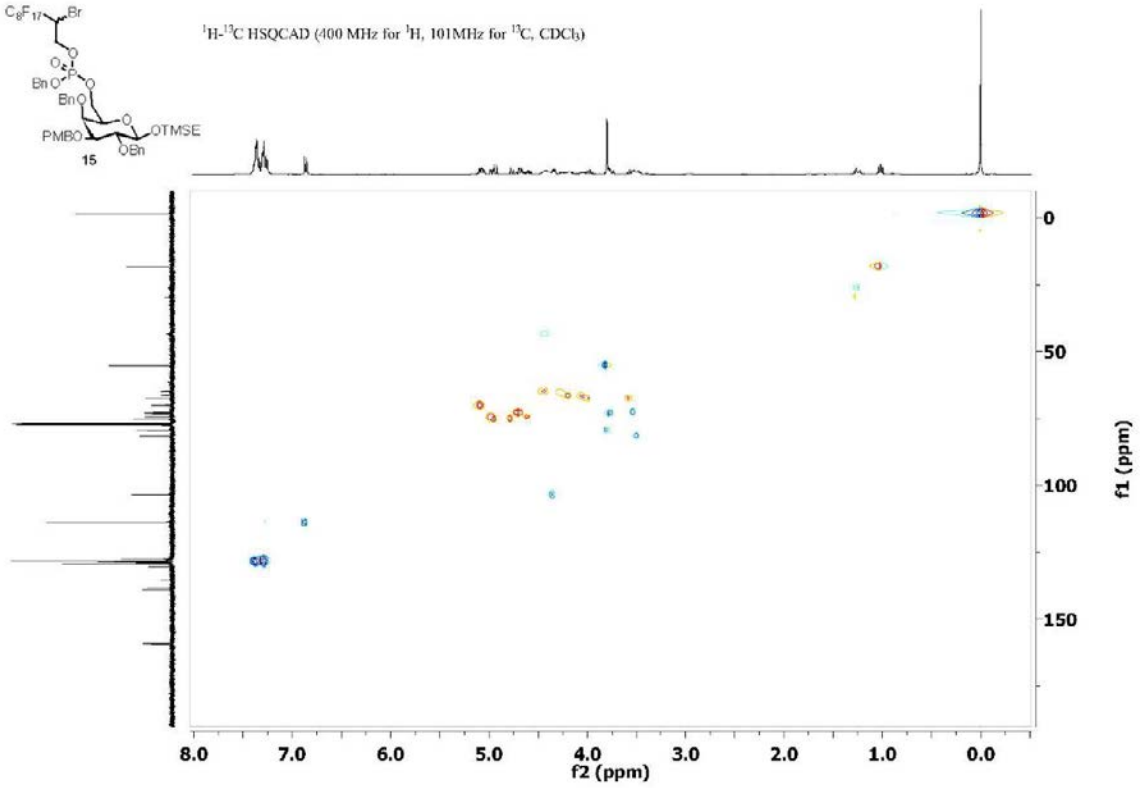


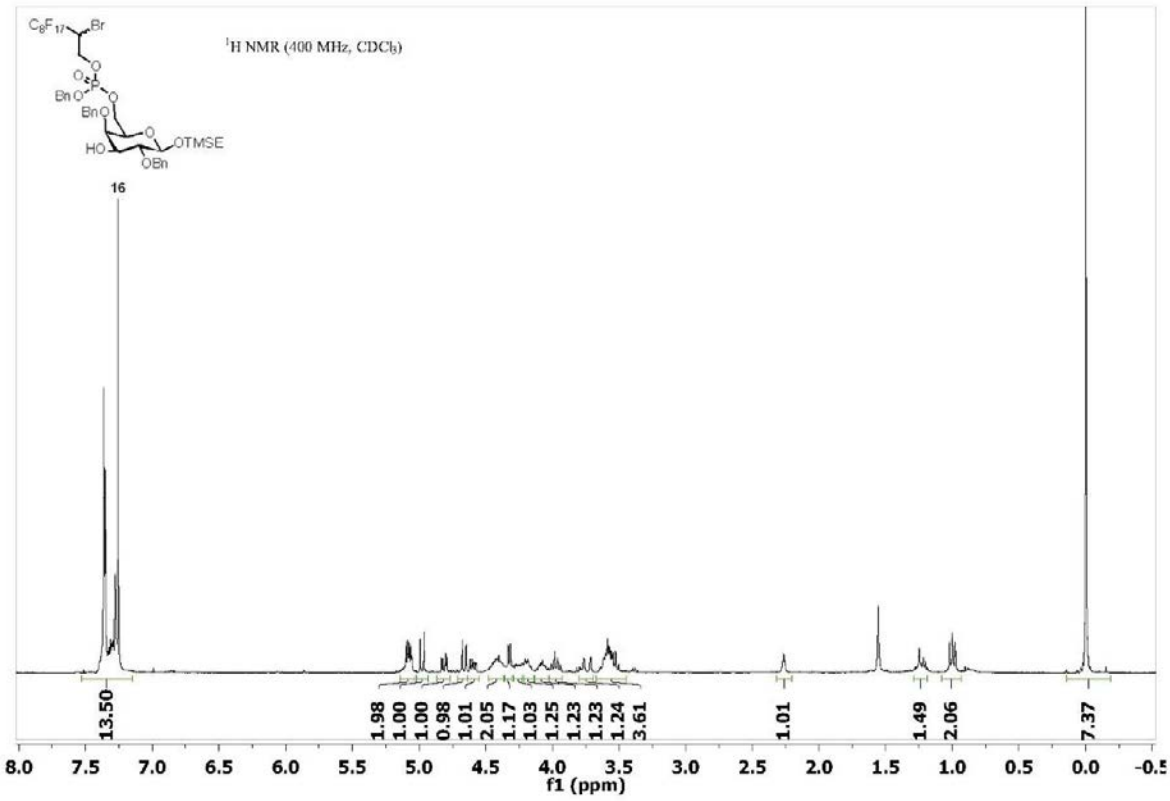


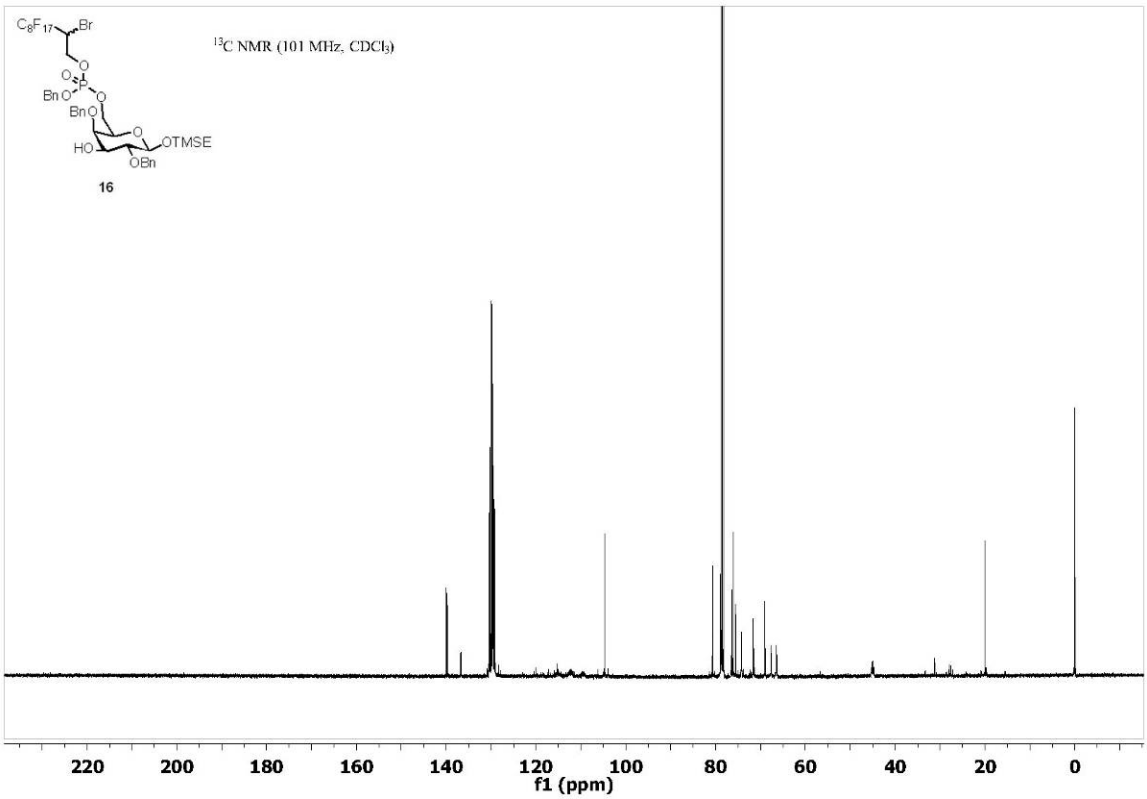


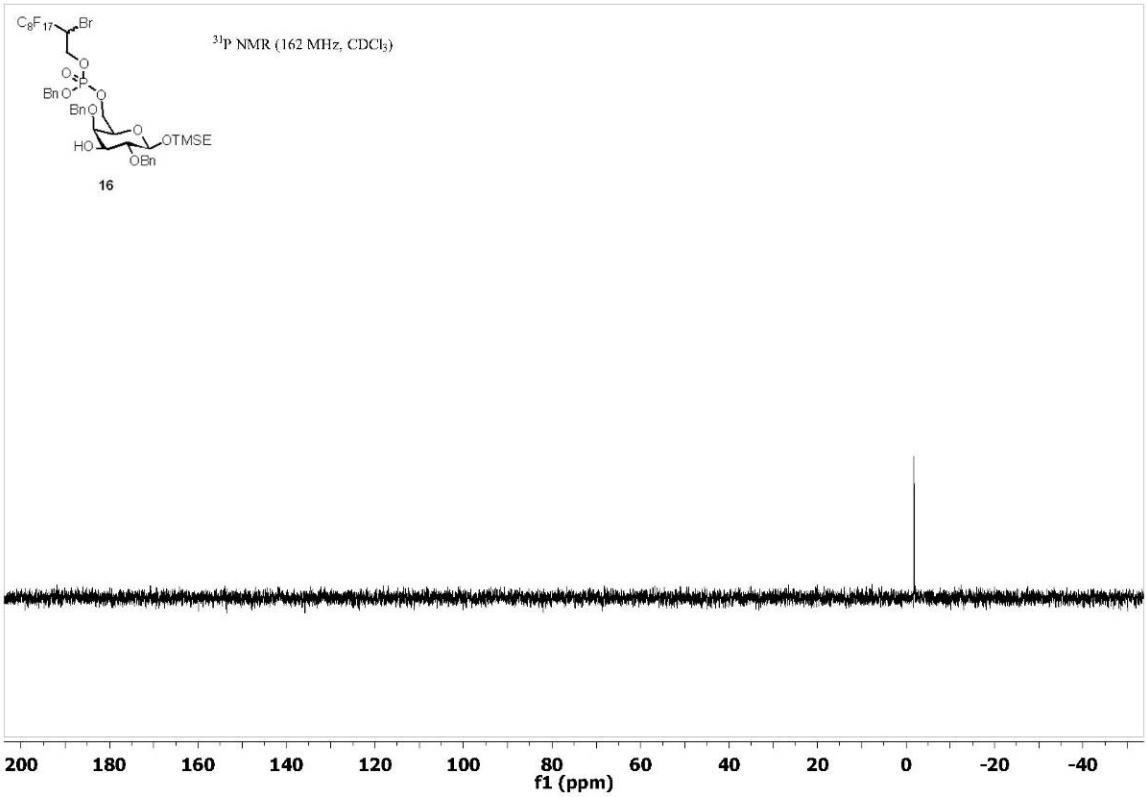


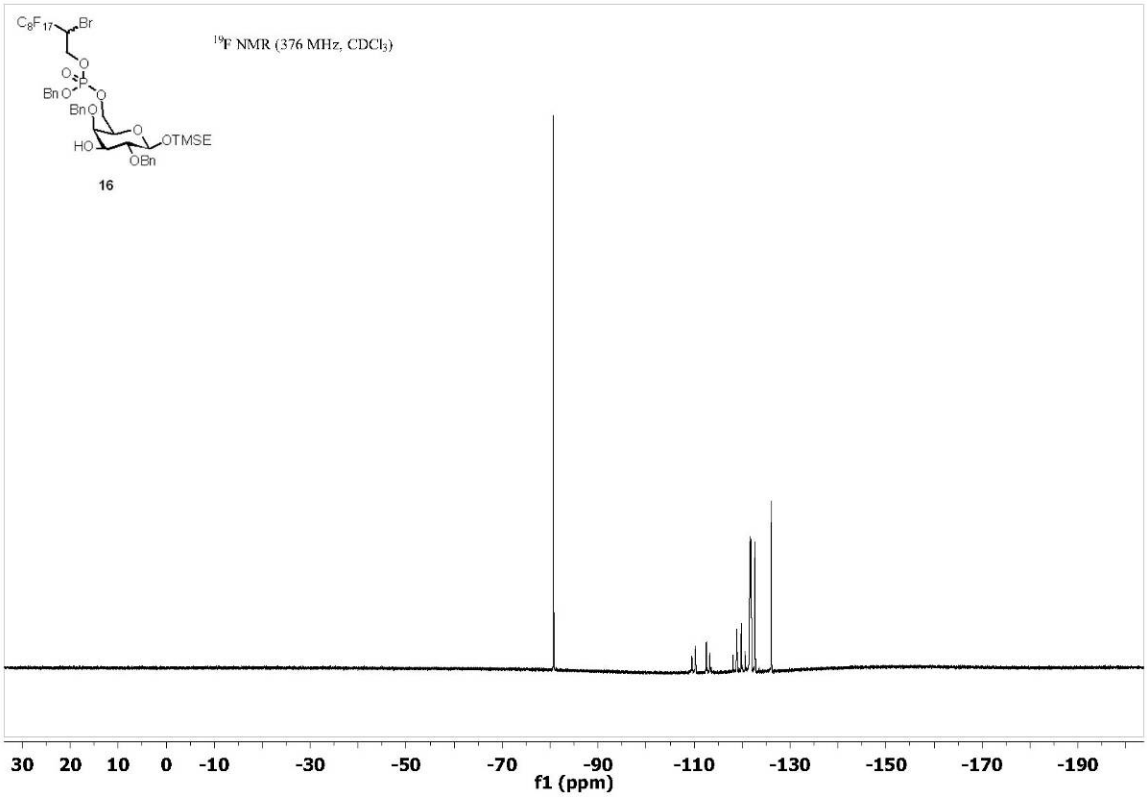


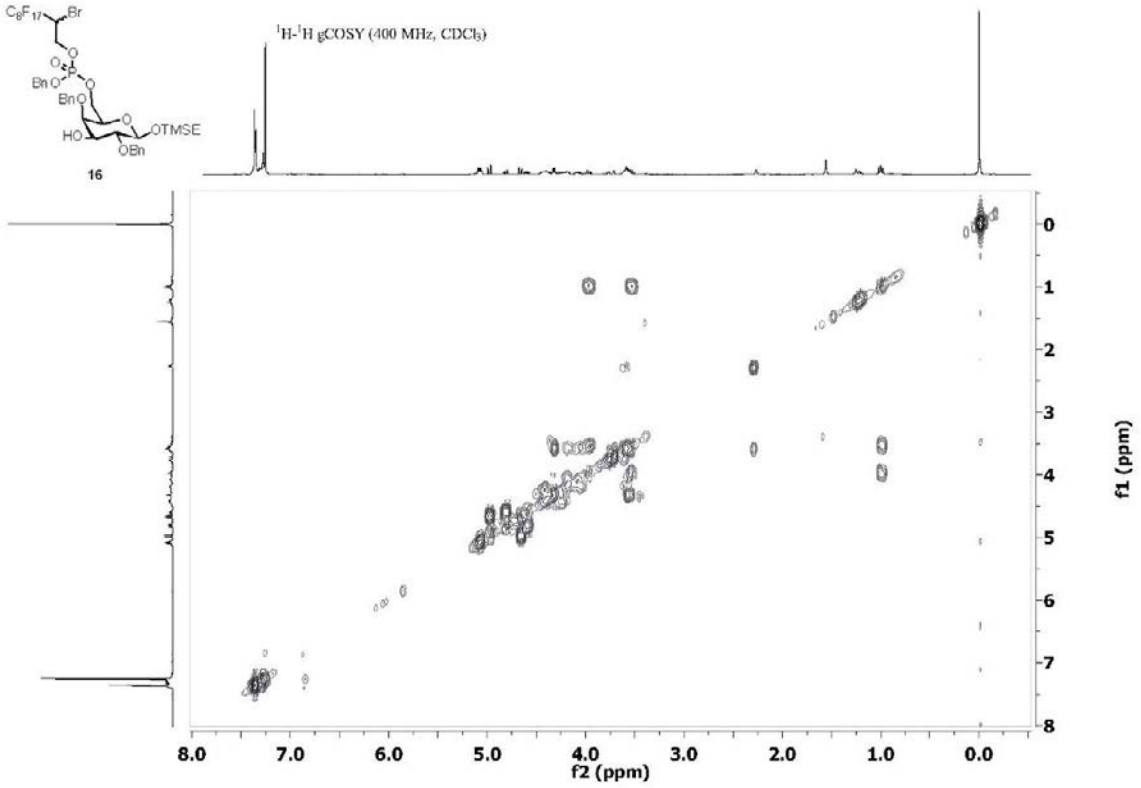


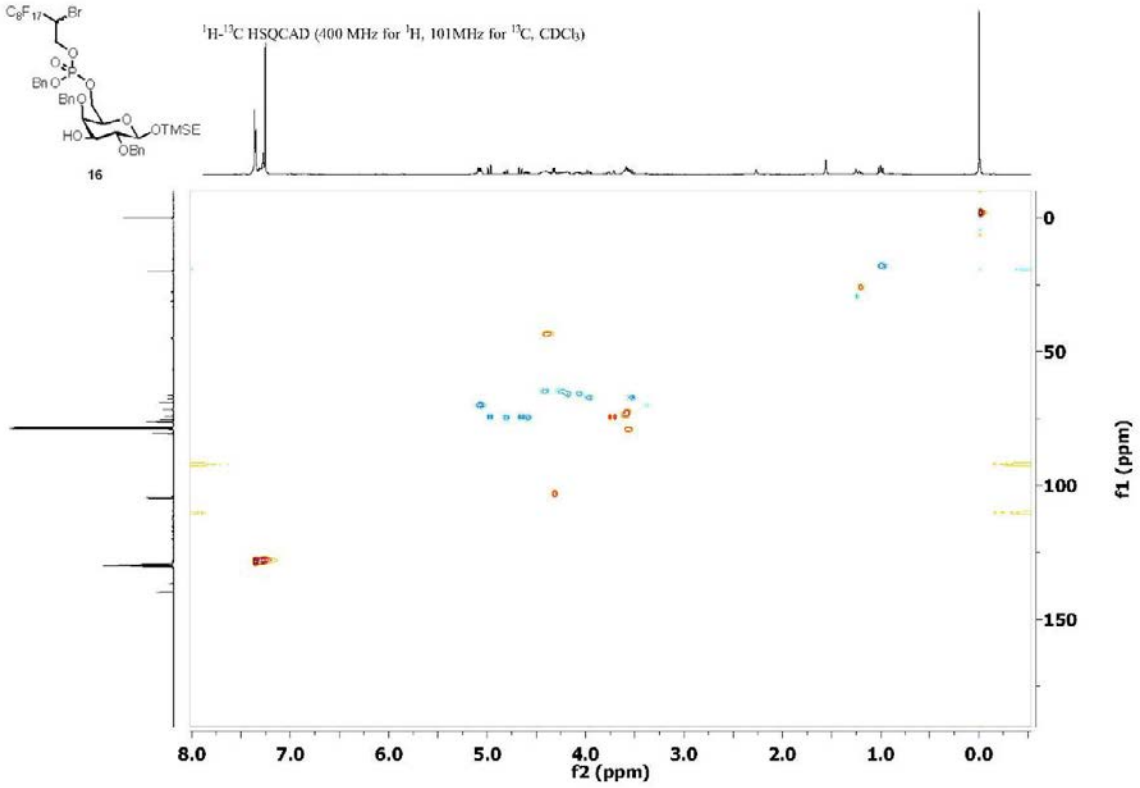


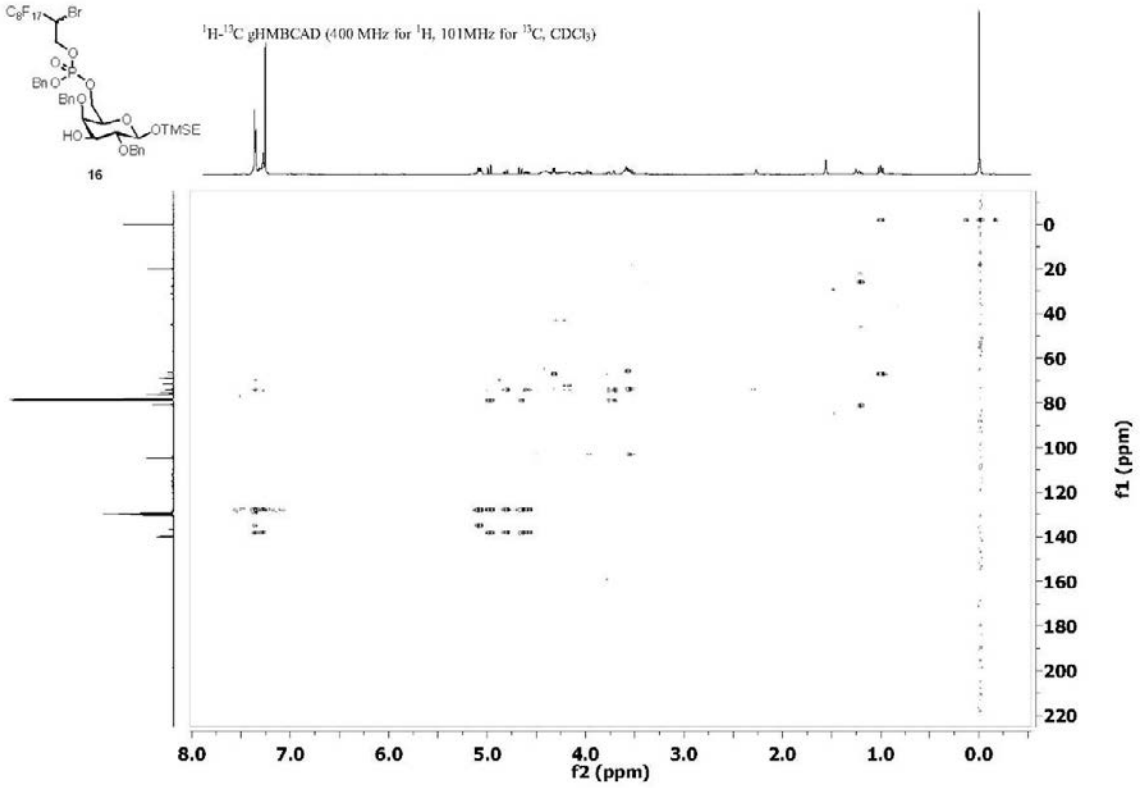


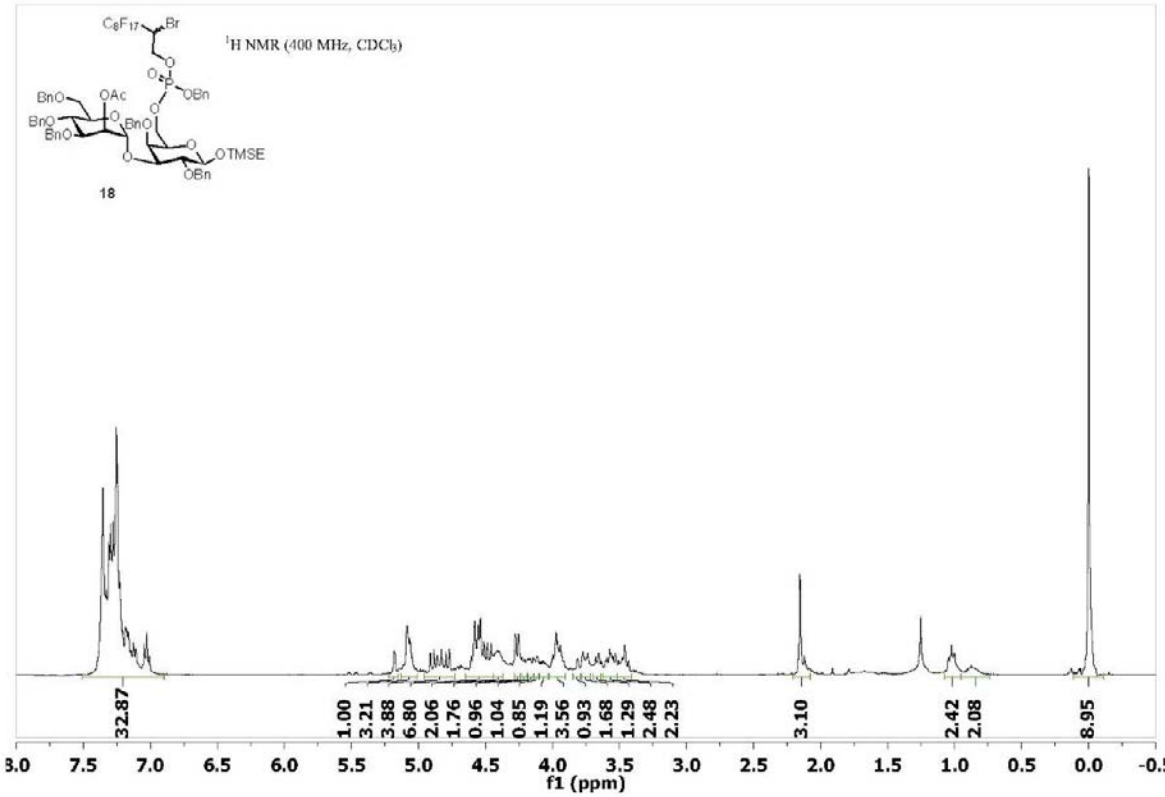


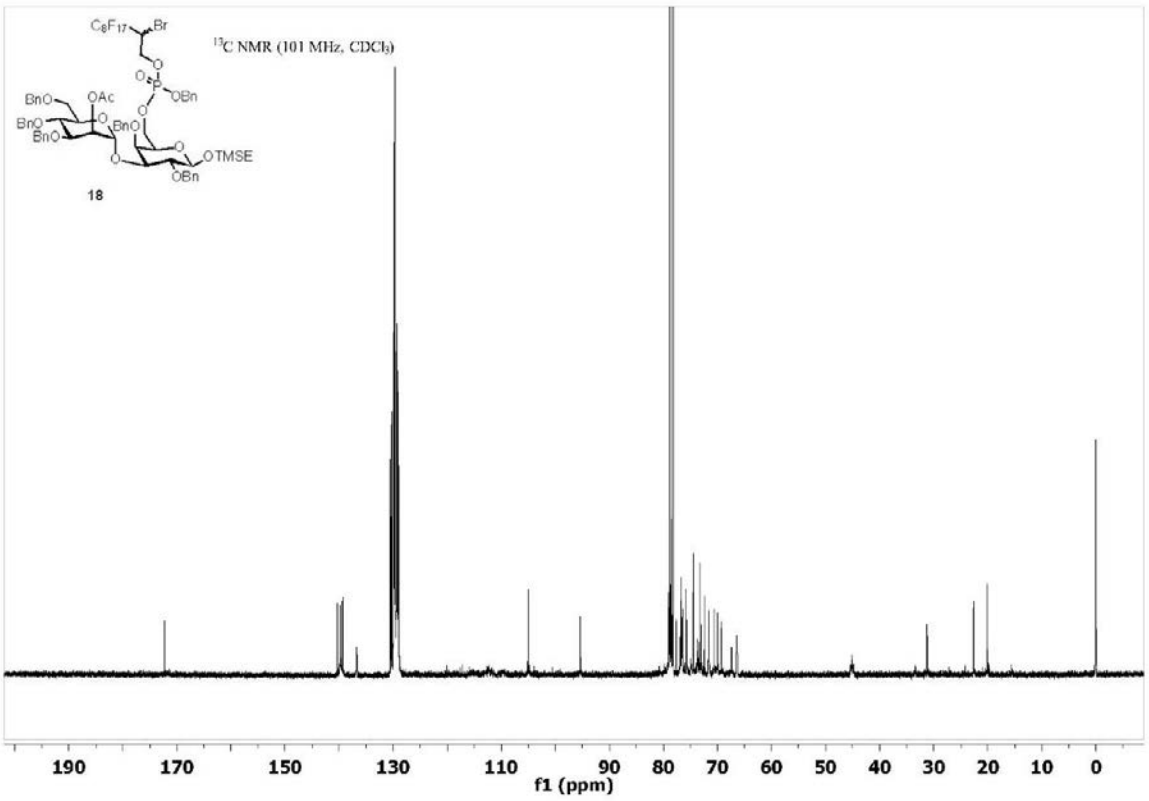


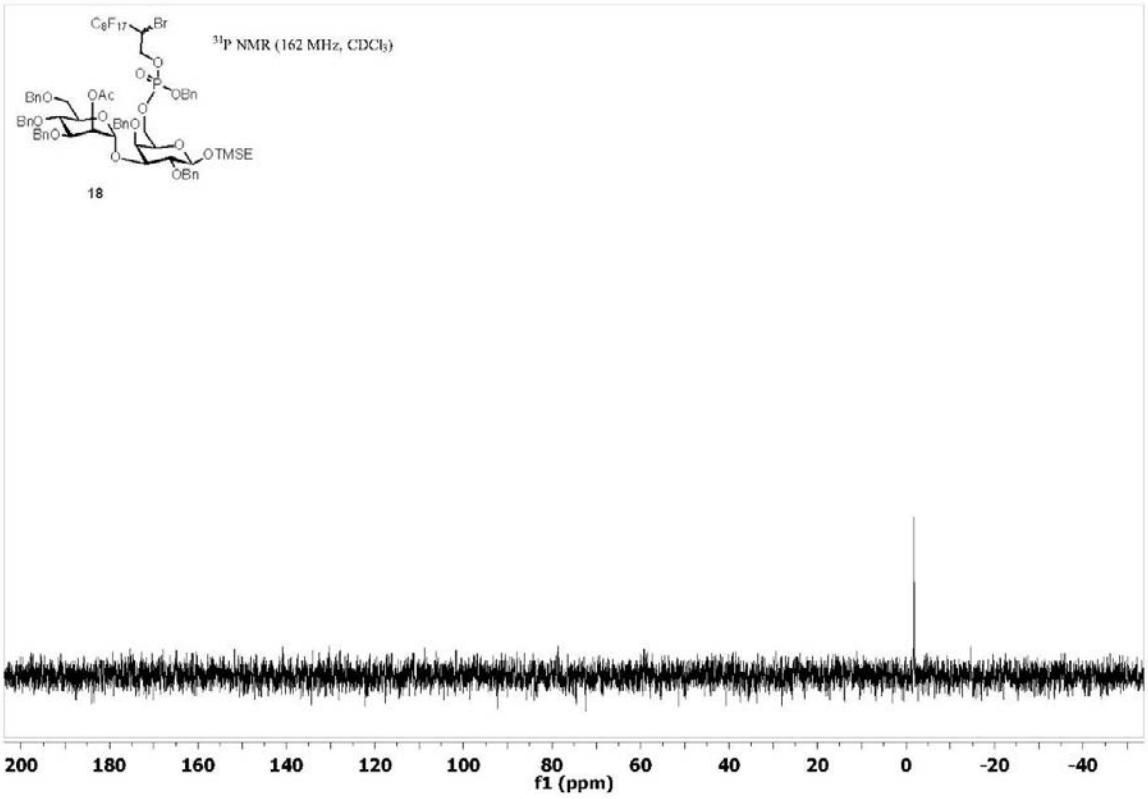


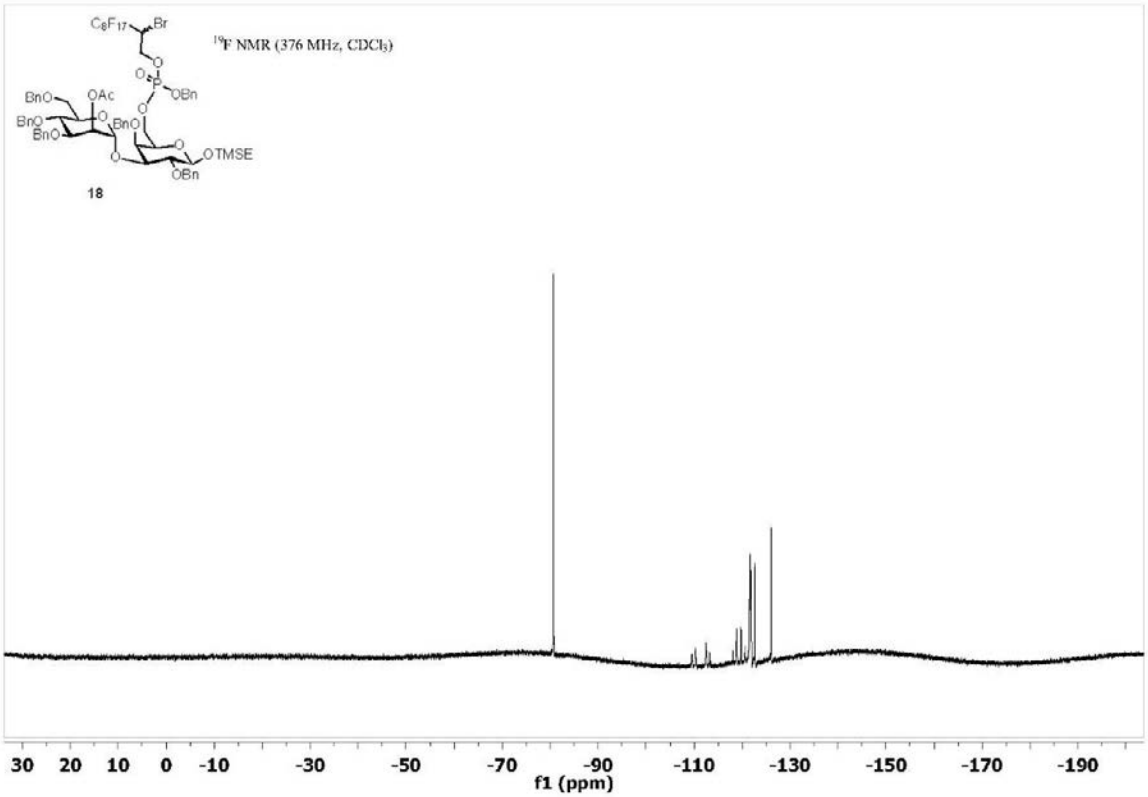


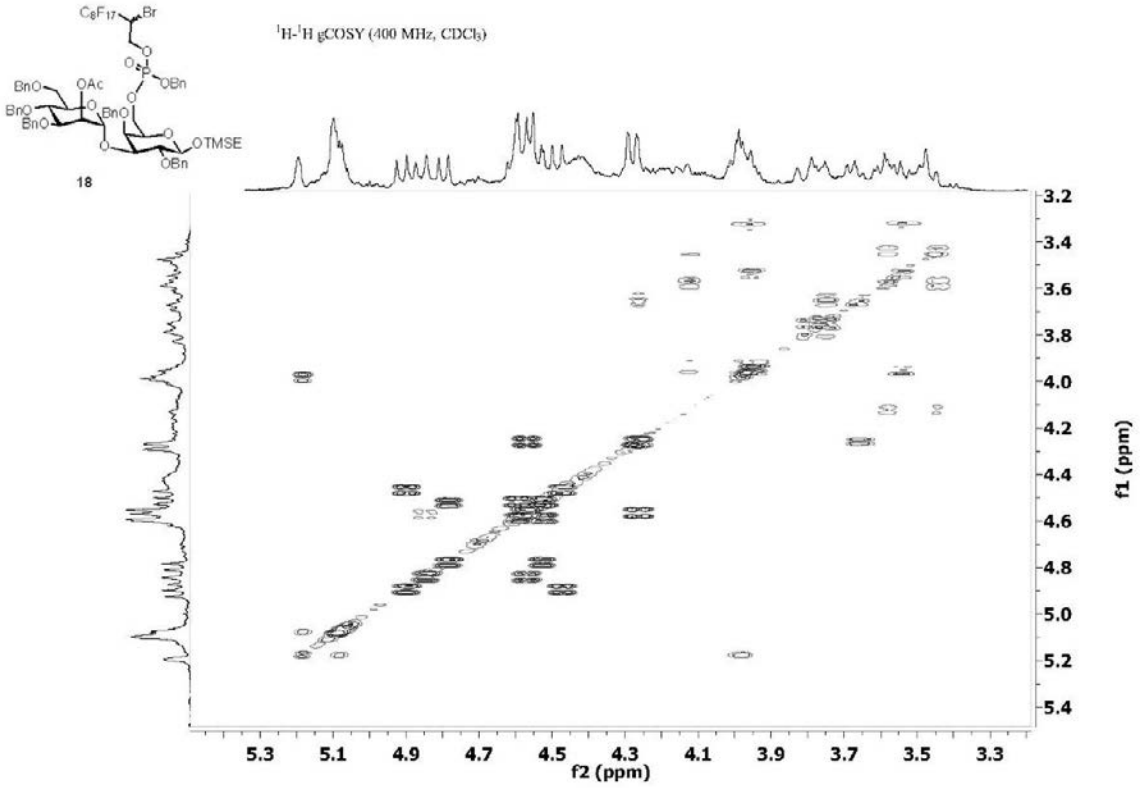


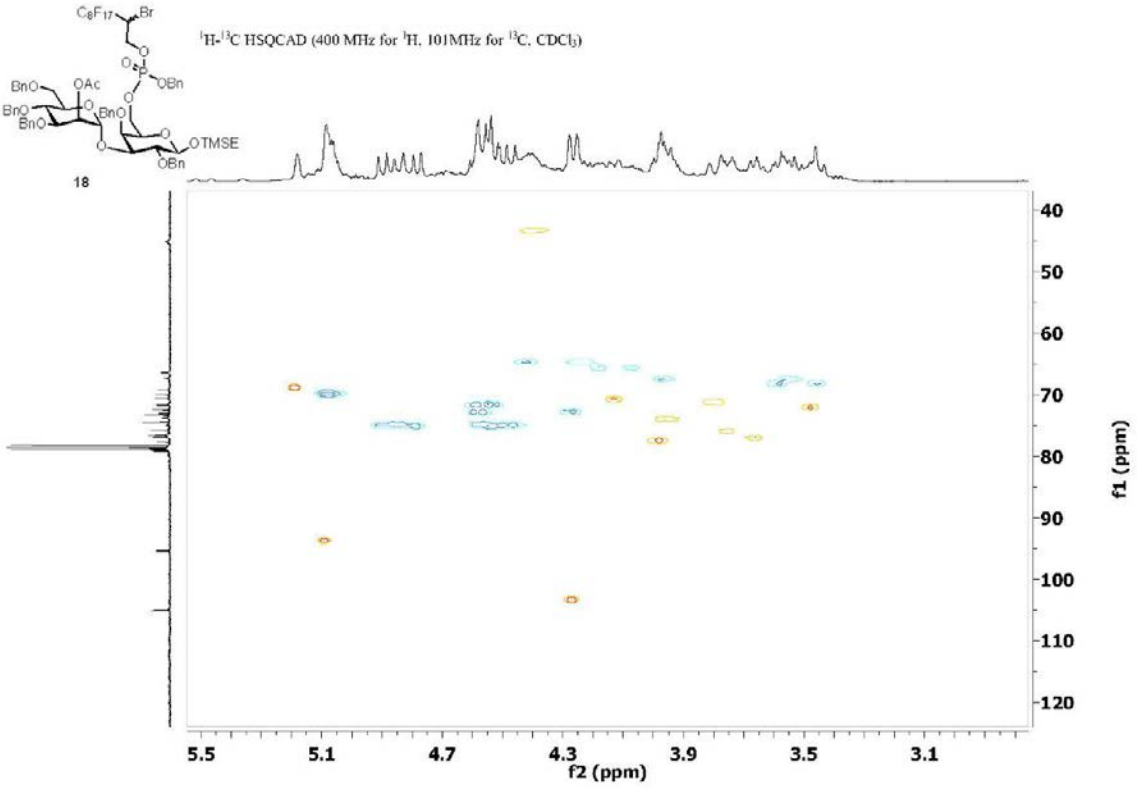


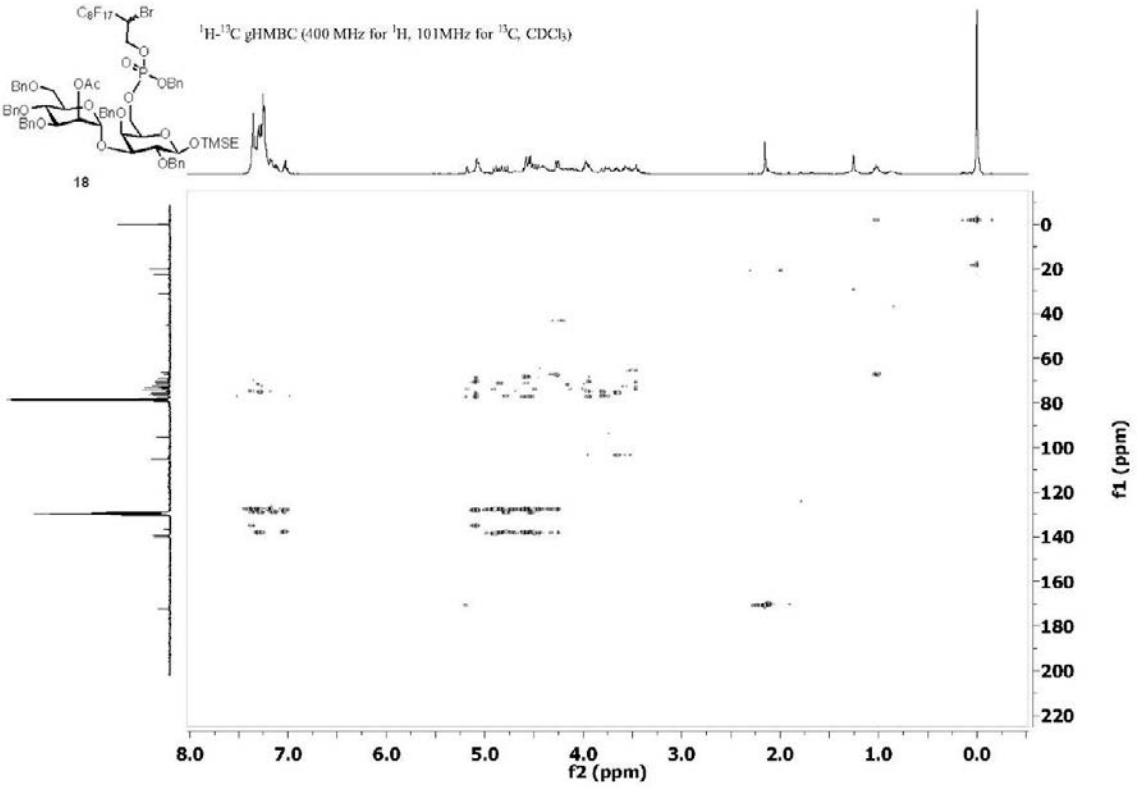


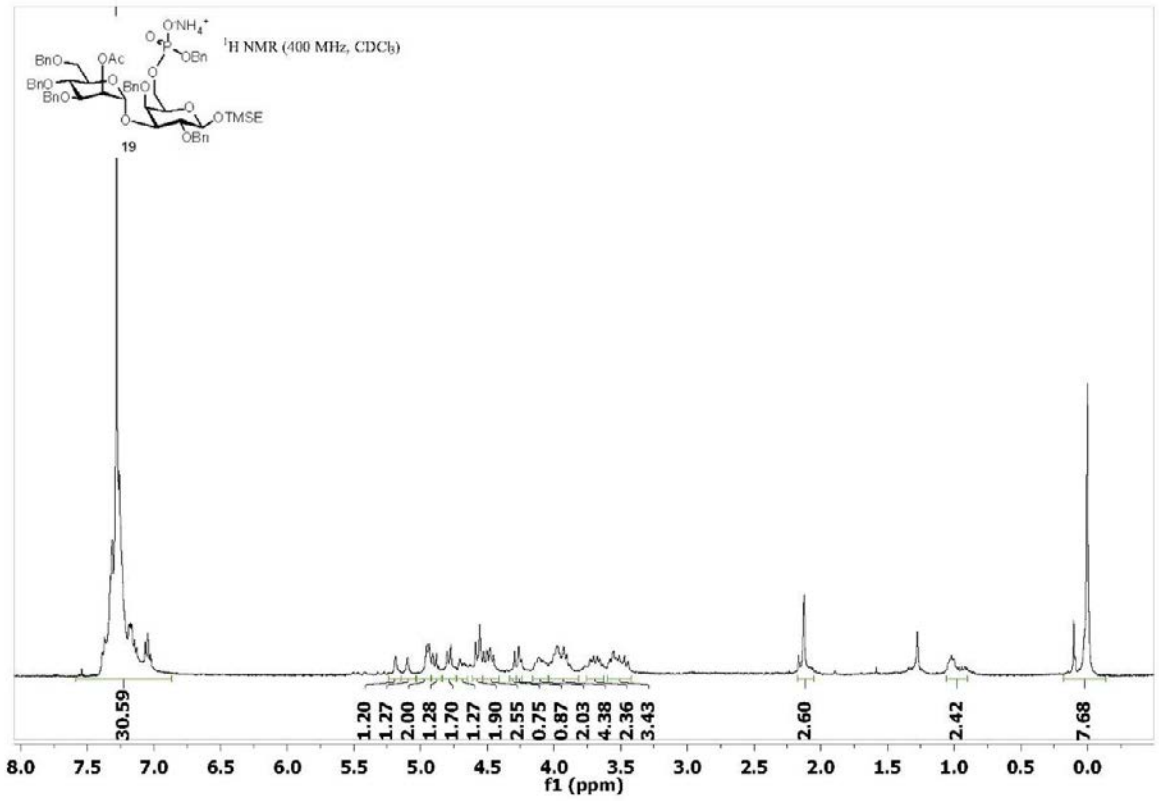


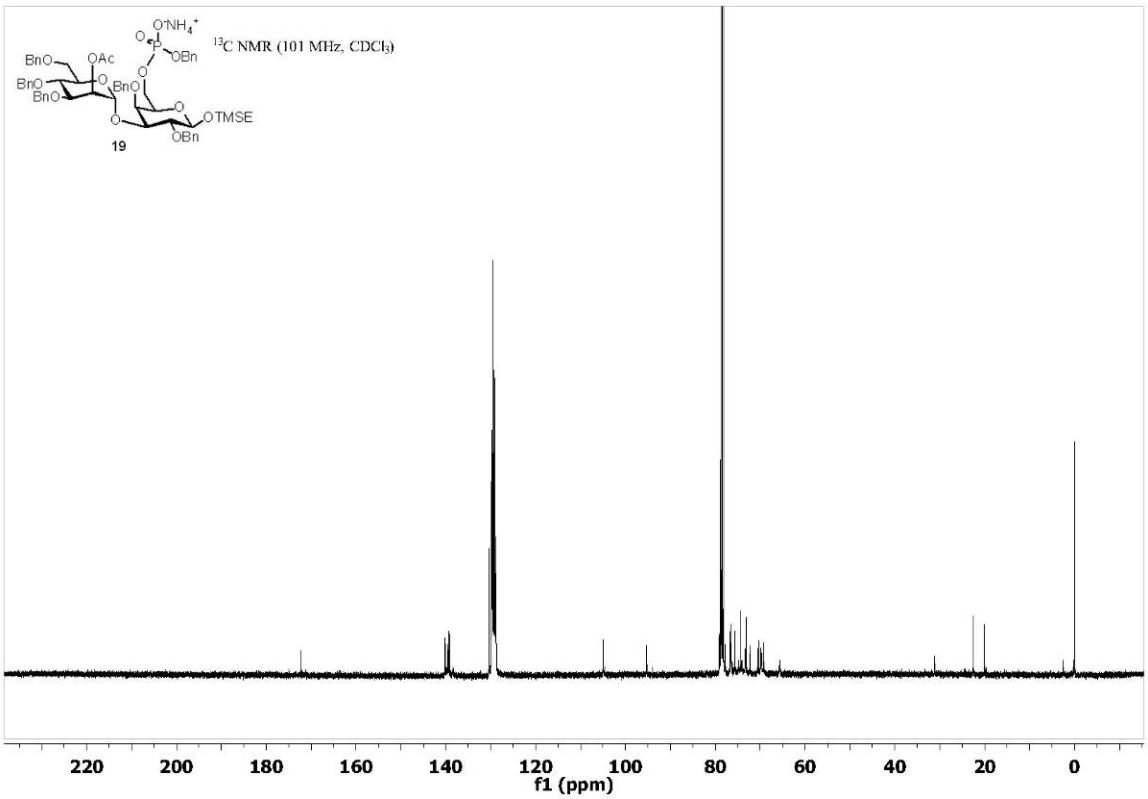


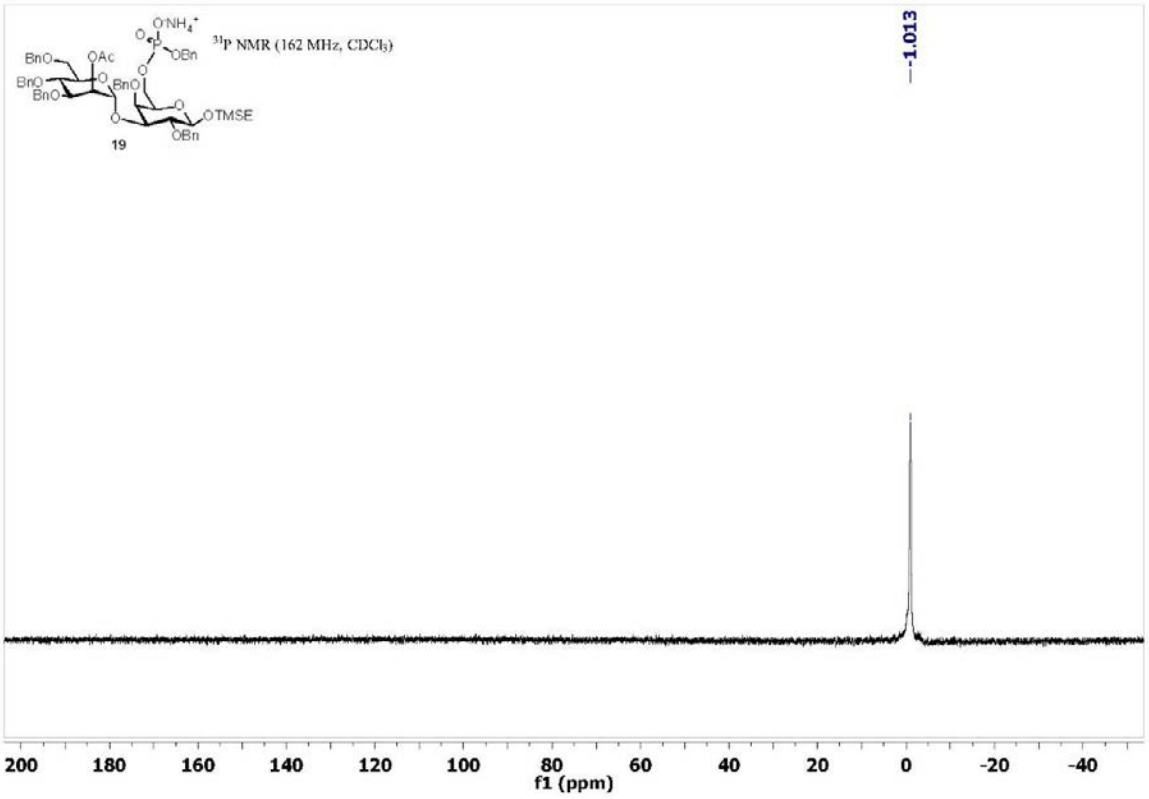


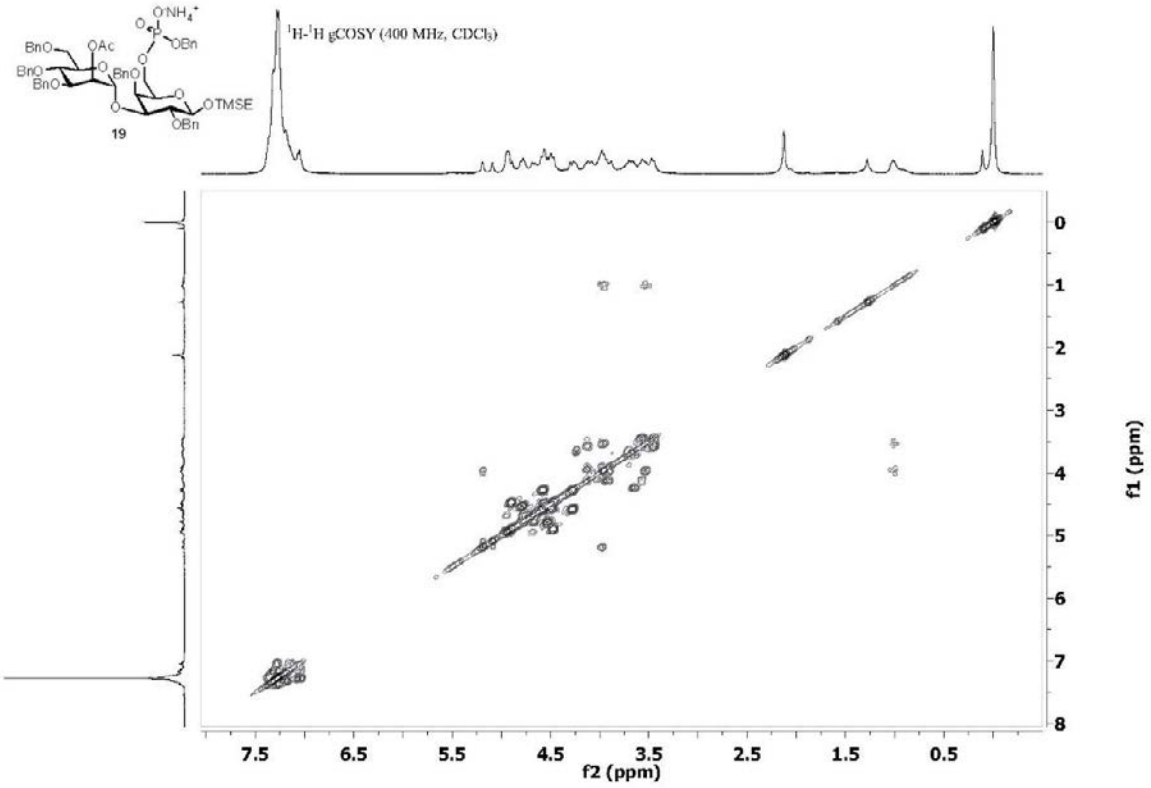


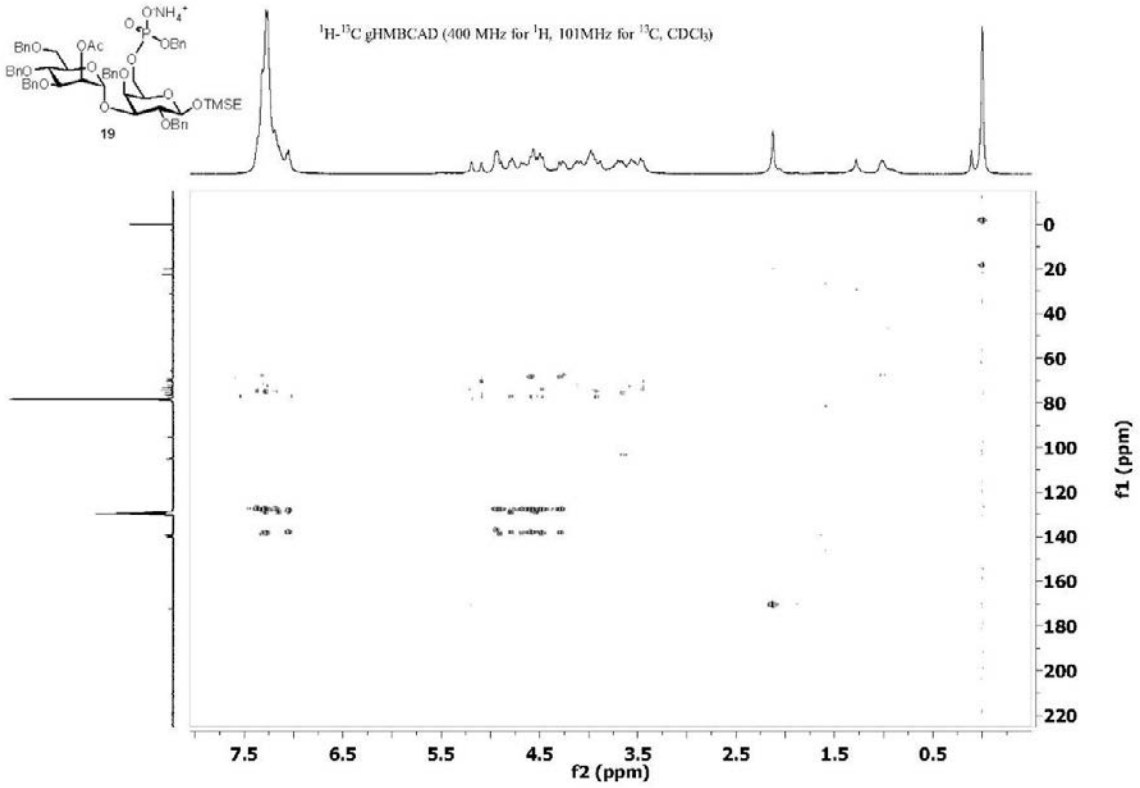


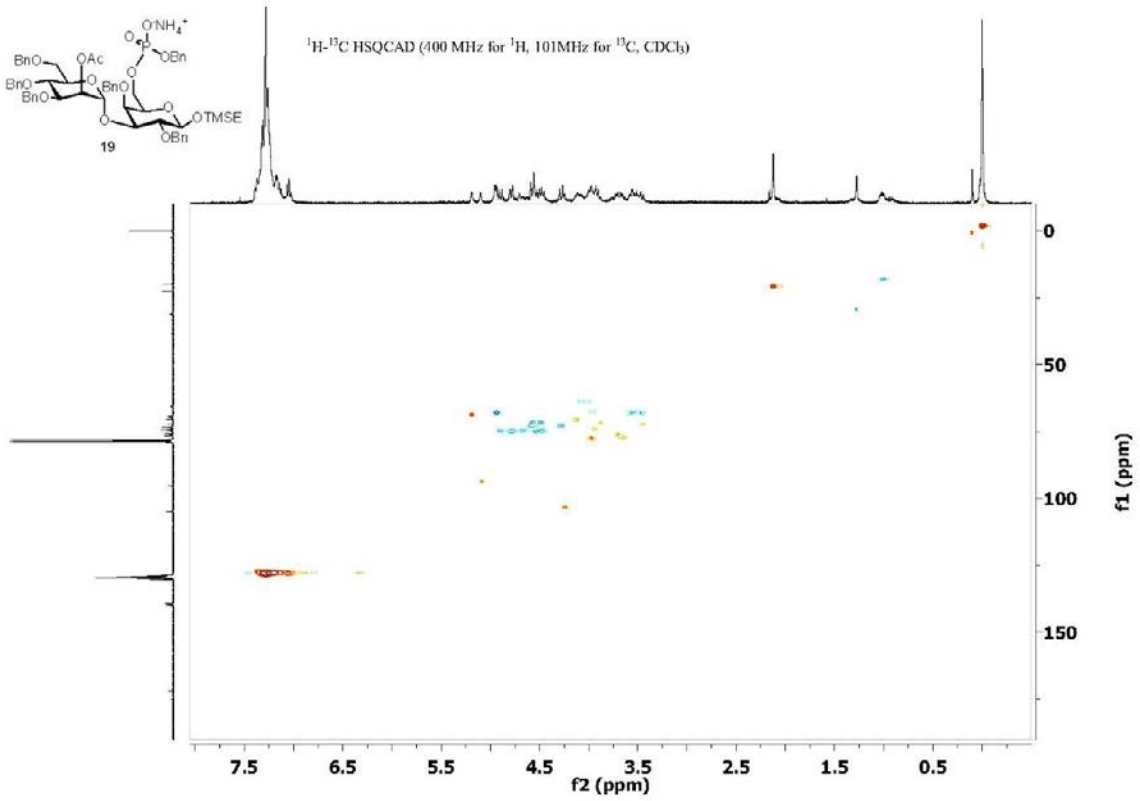




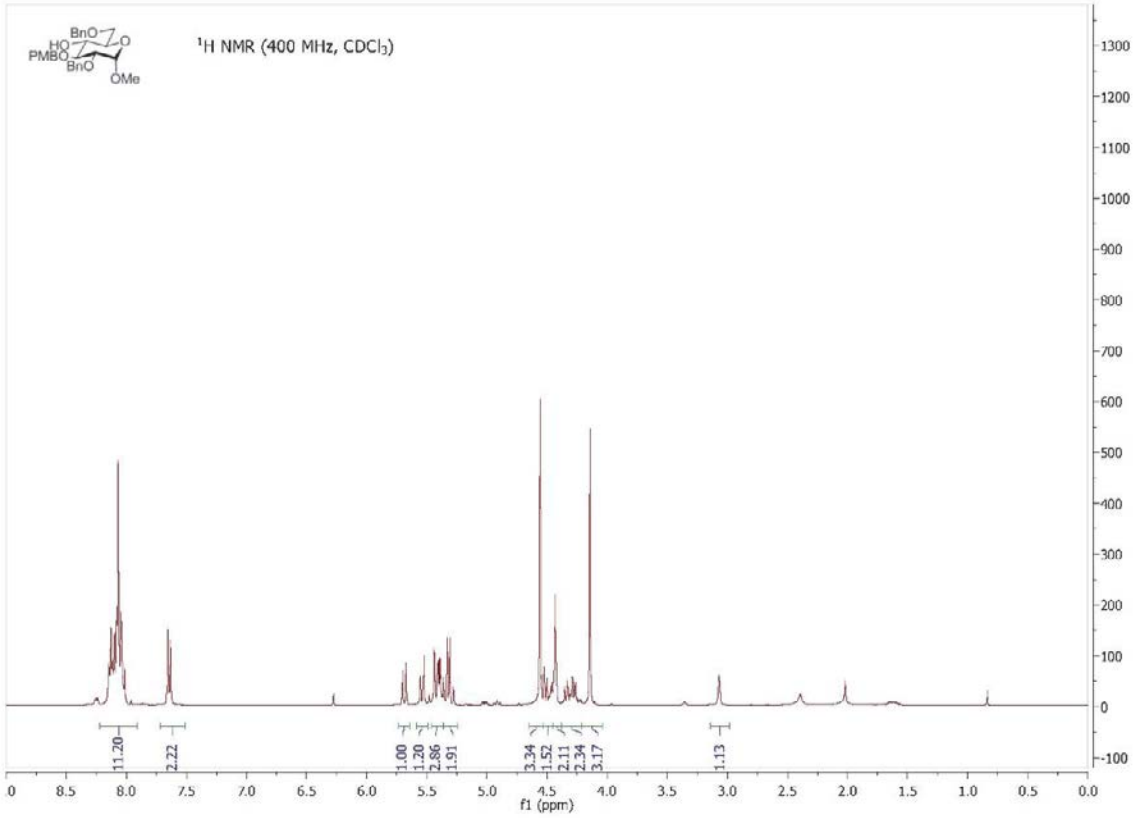


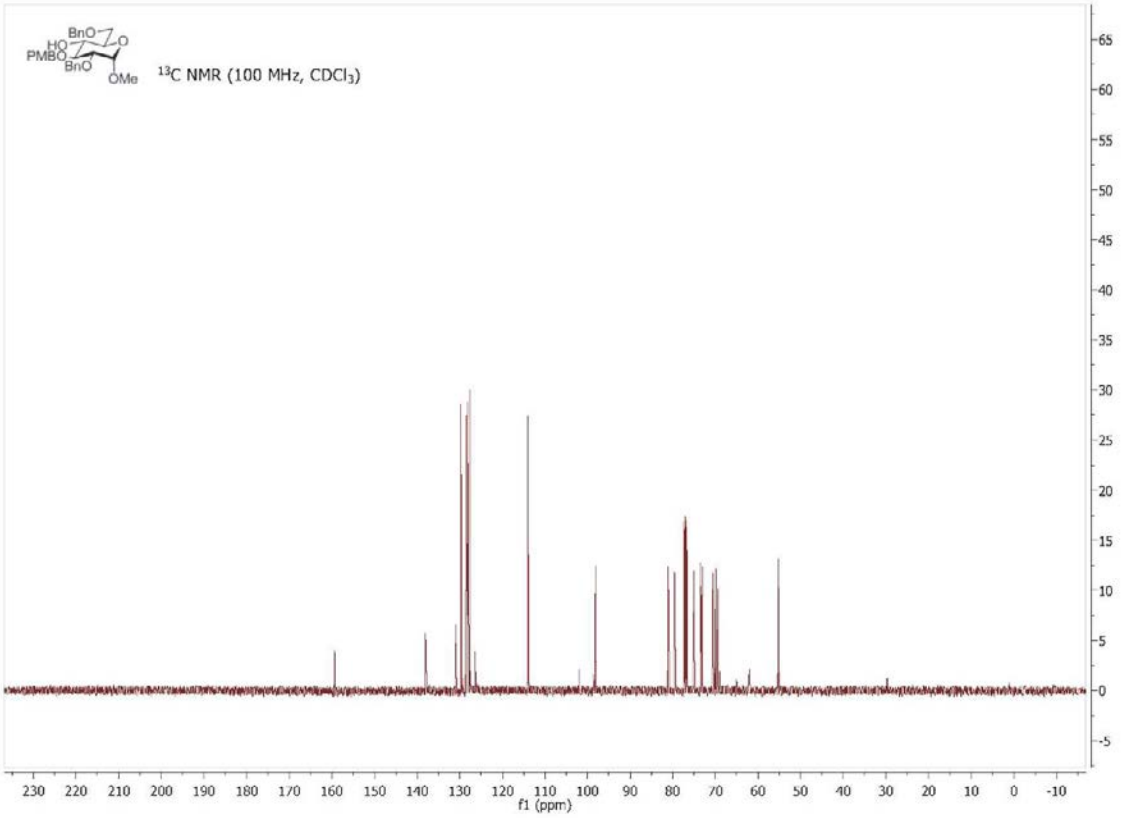


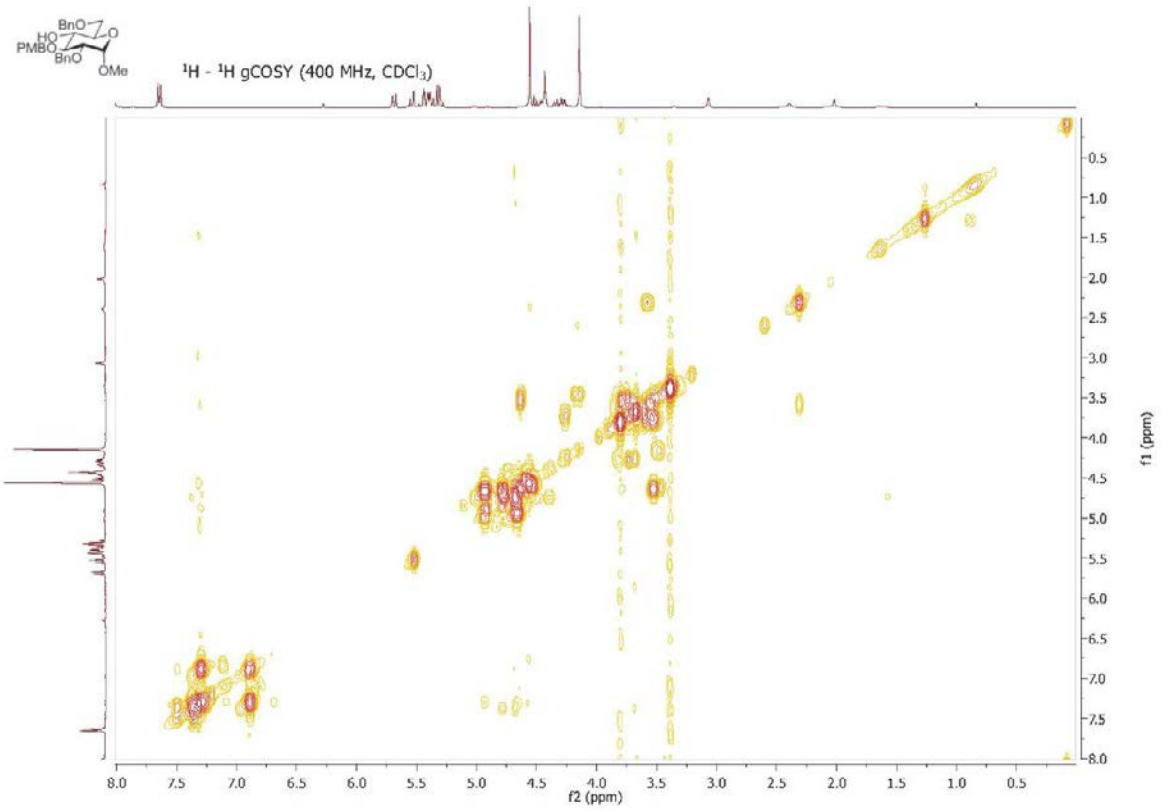


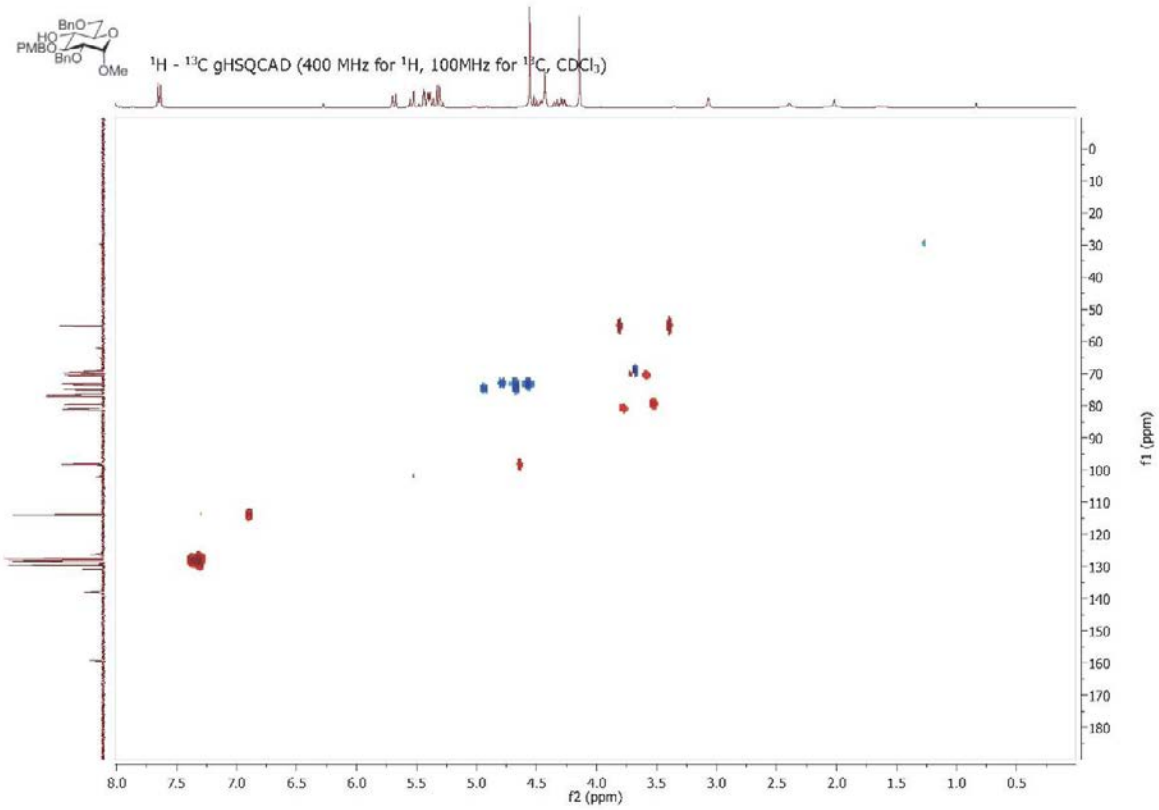


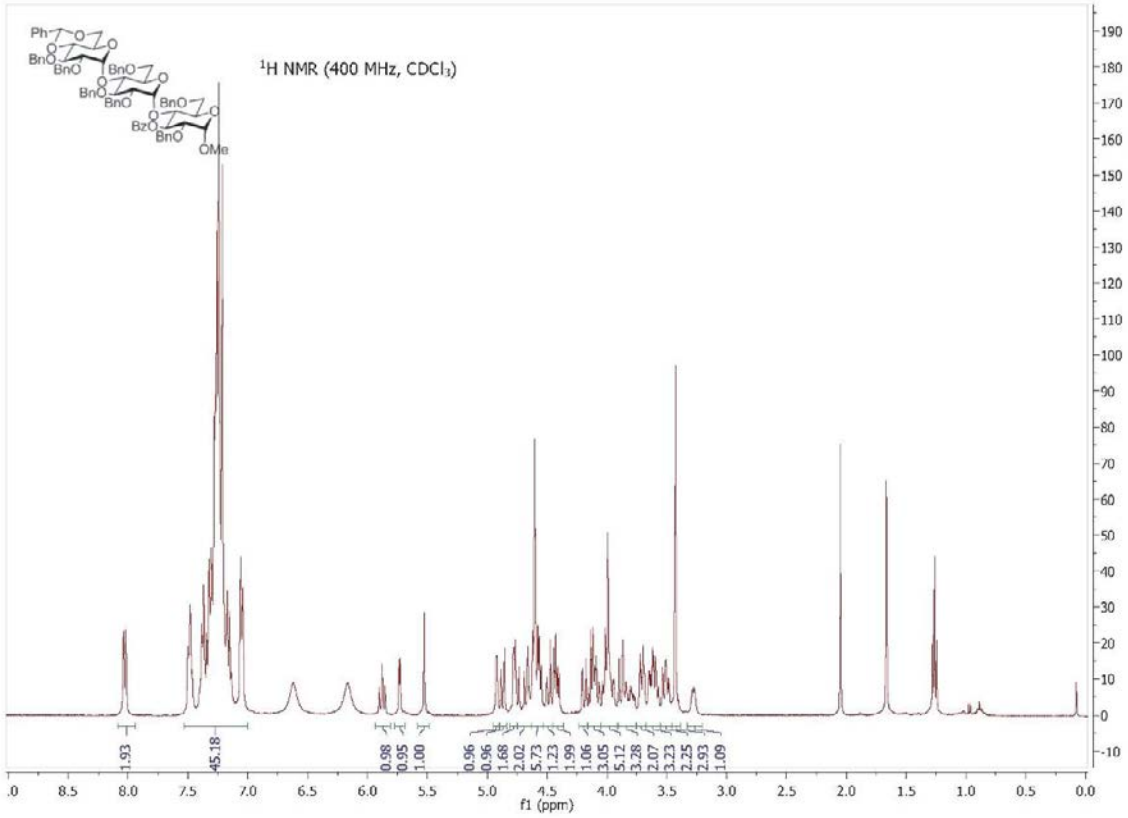
APPENDIX B. CHAPTER 3 ^{1}H AND ^{13}C NMR SPECTRA

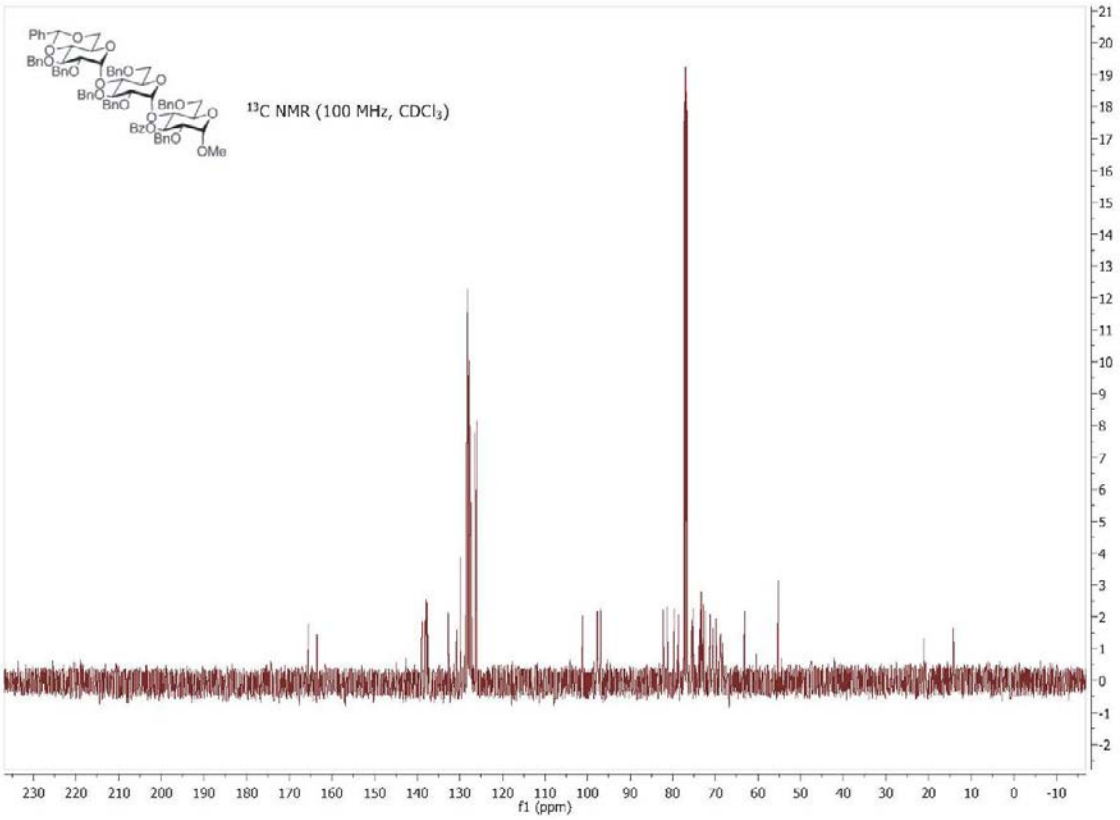


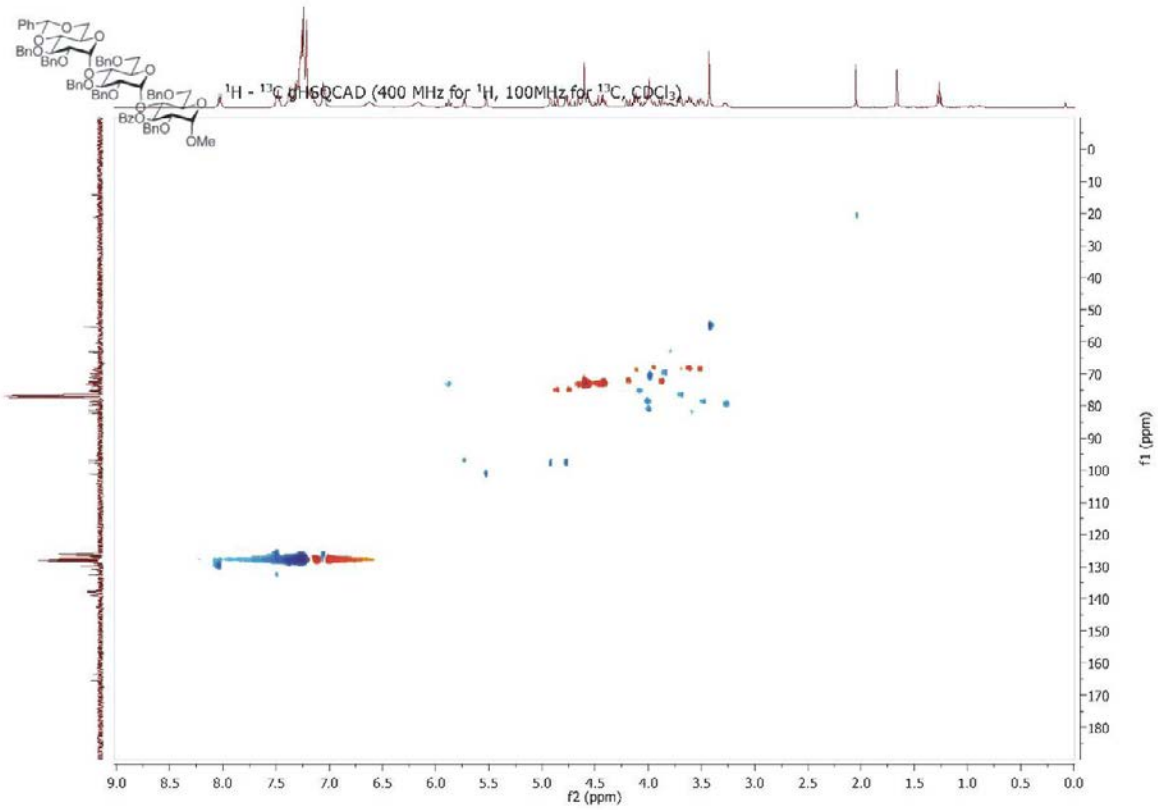


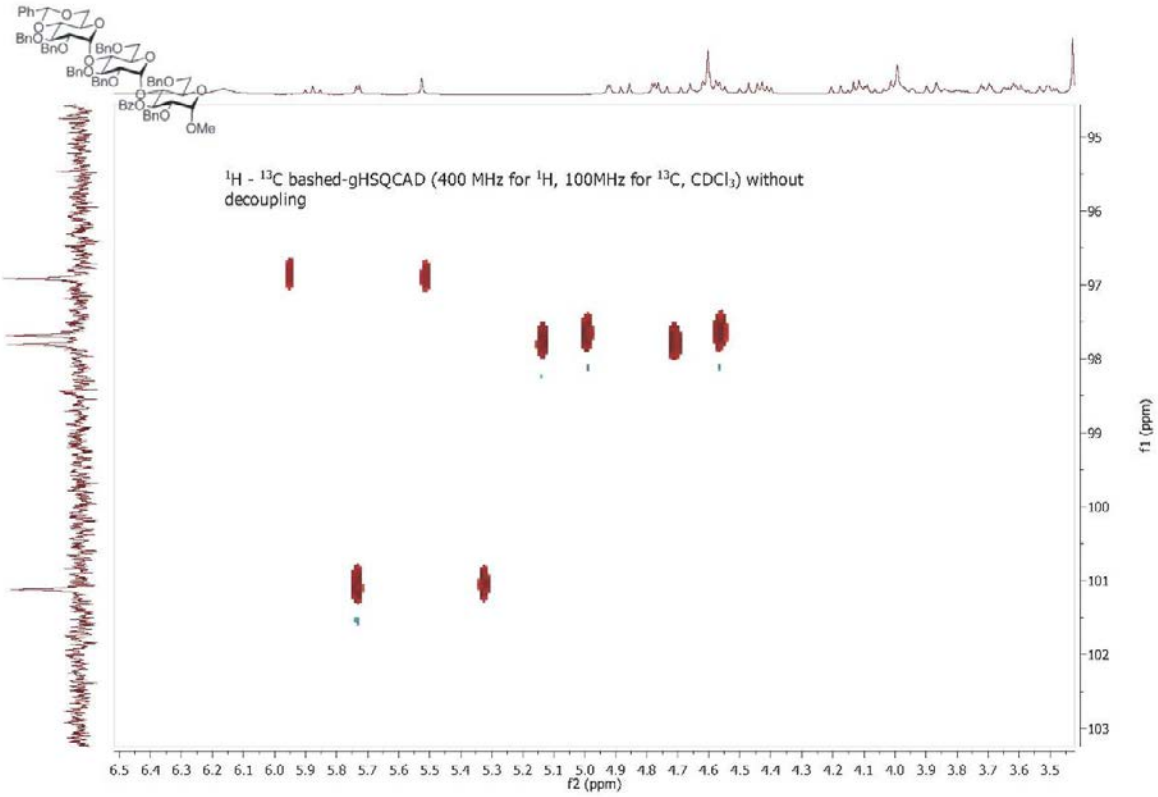


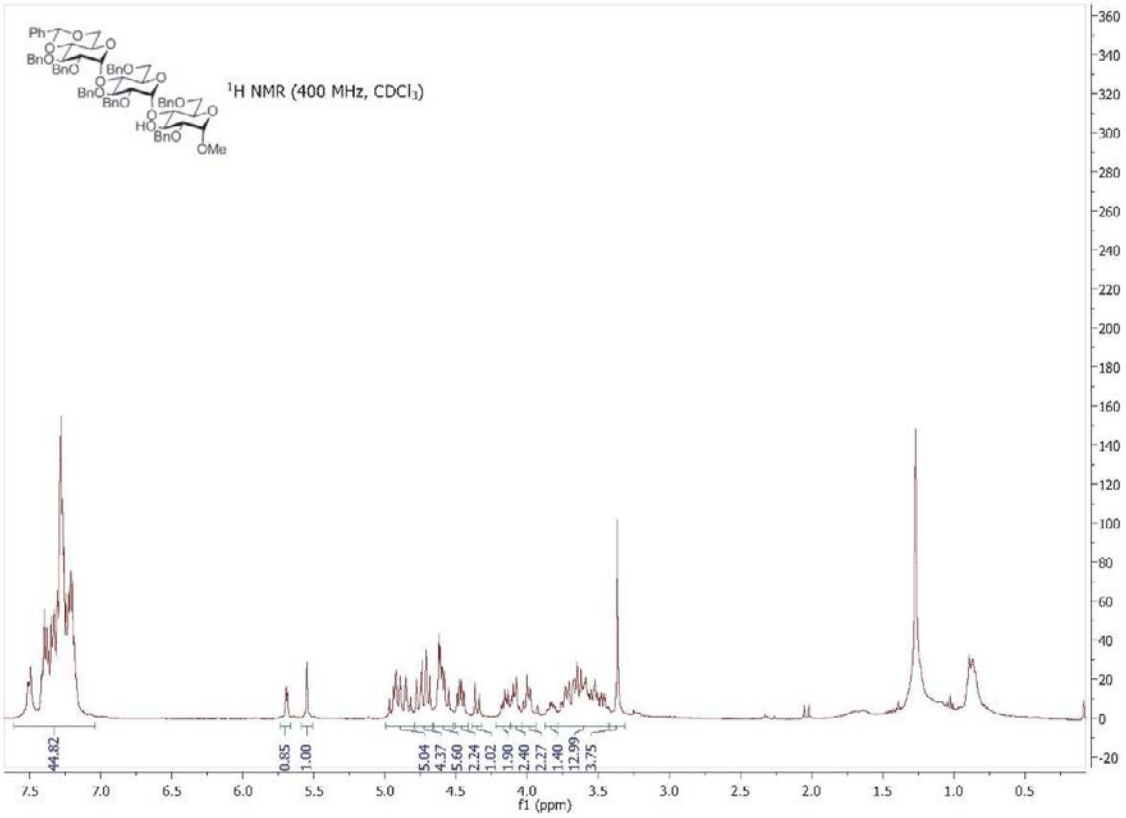


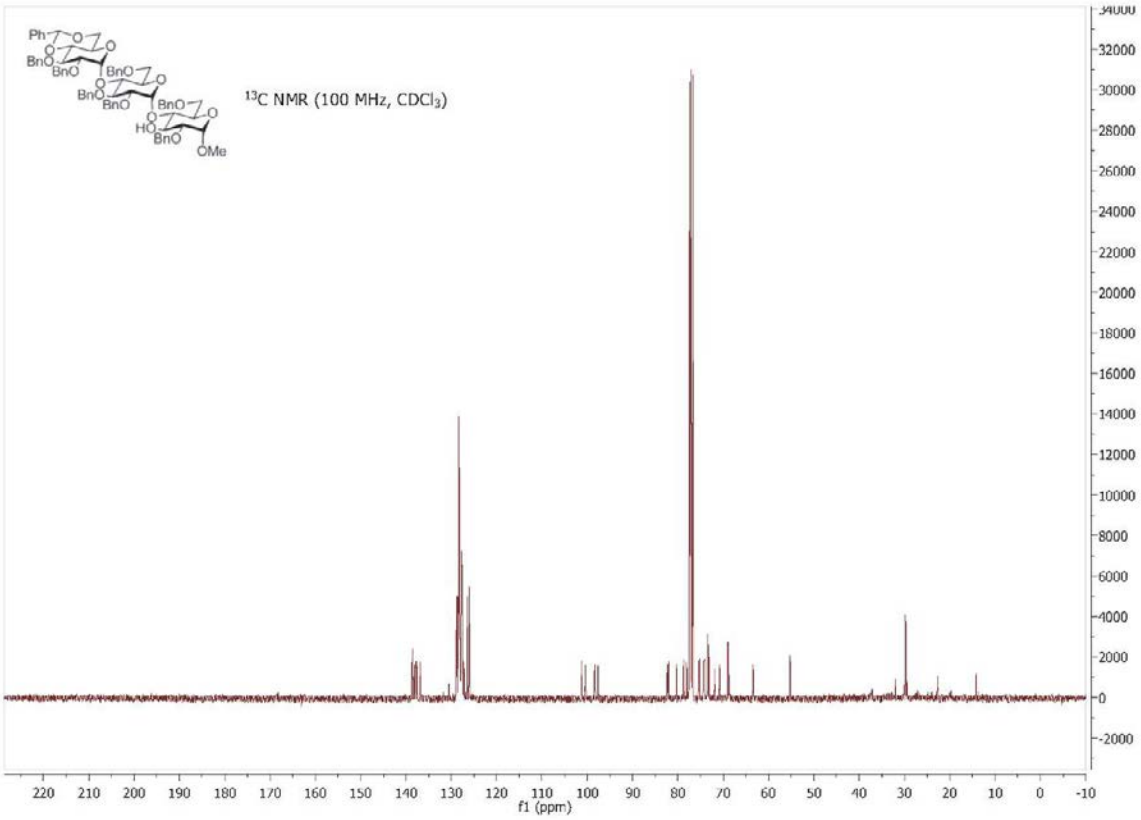


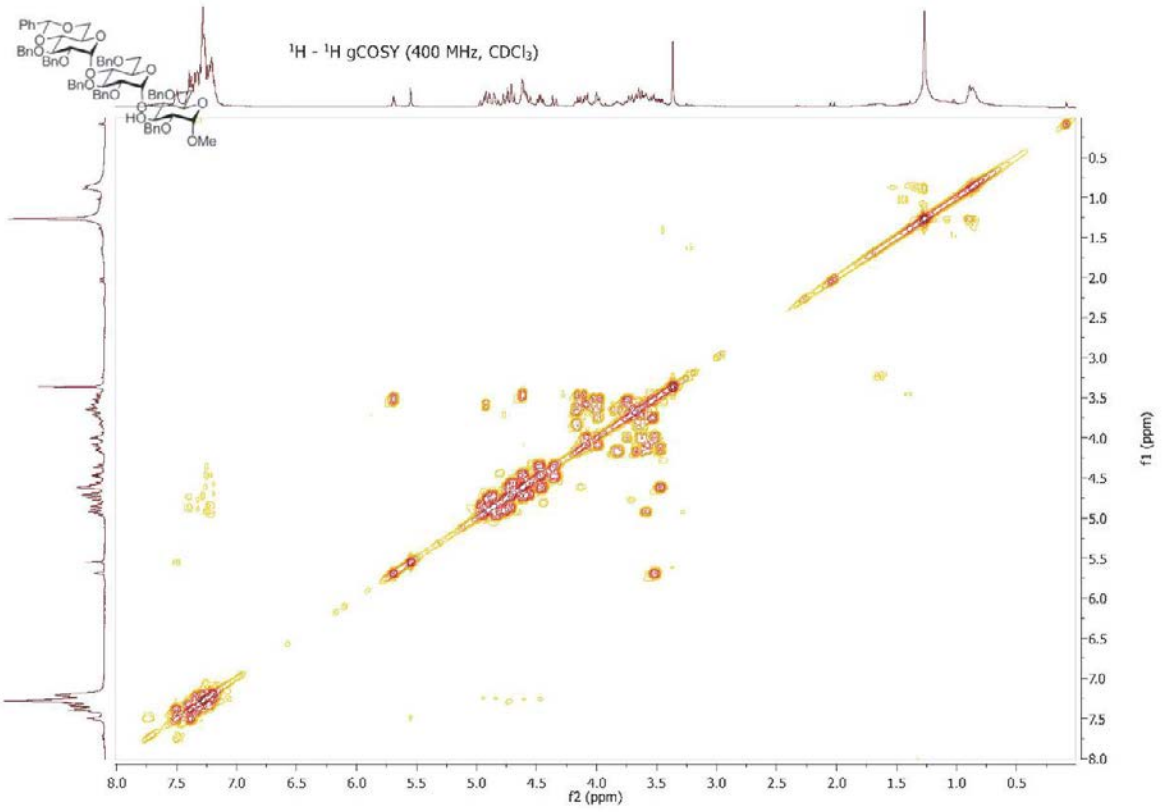


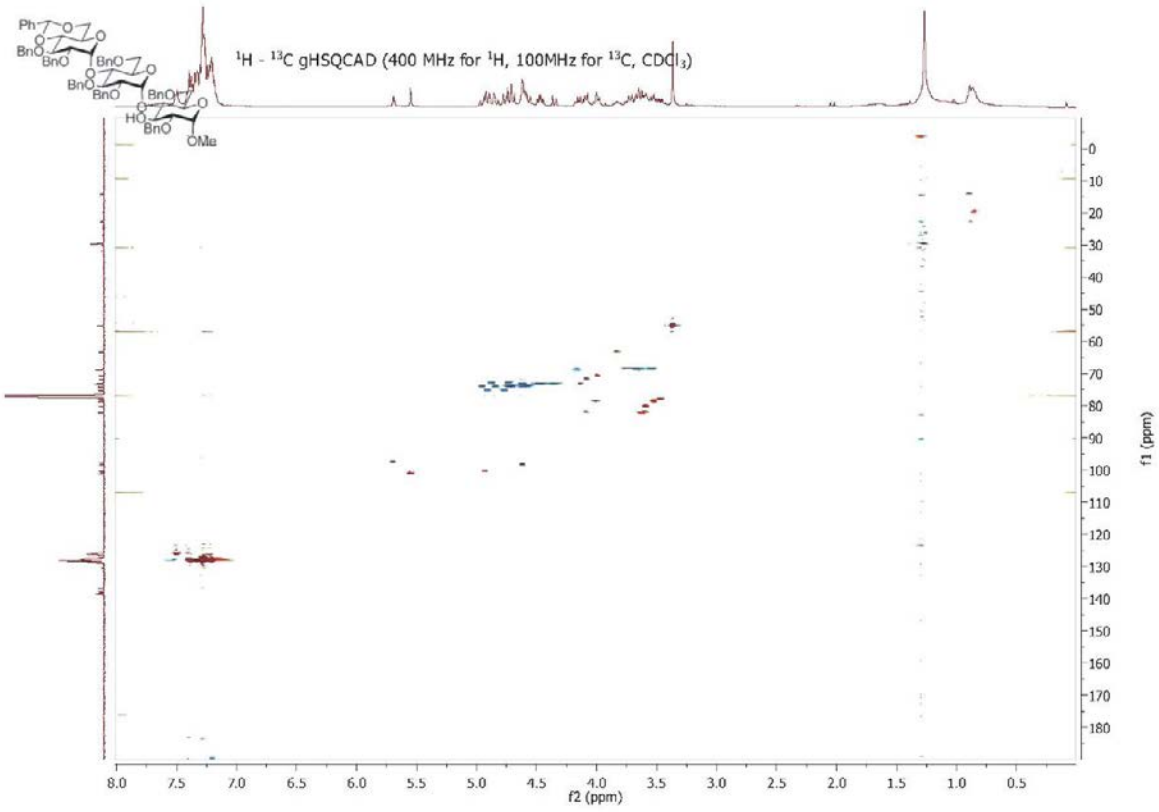


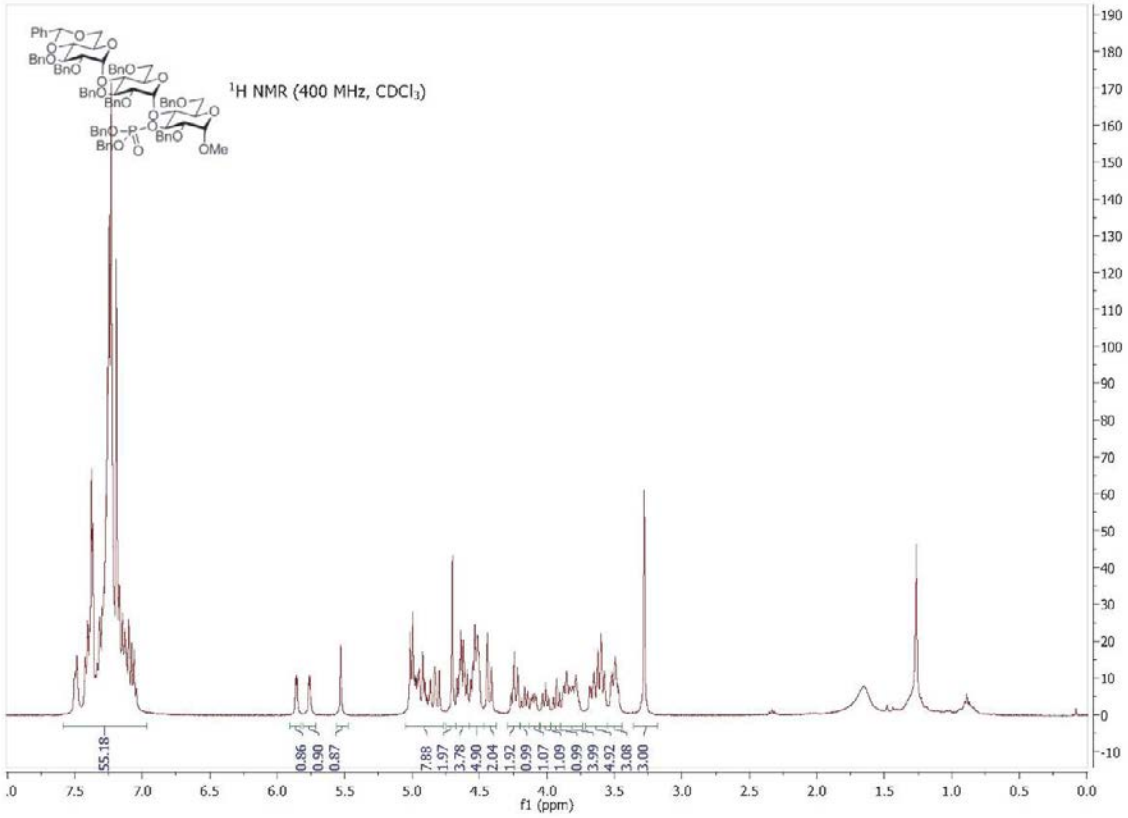


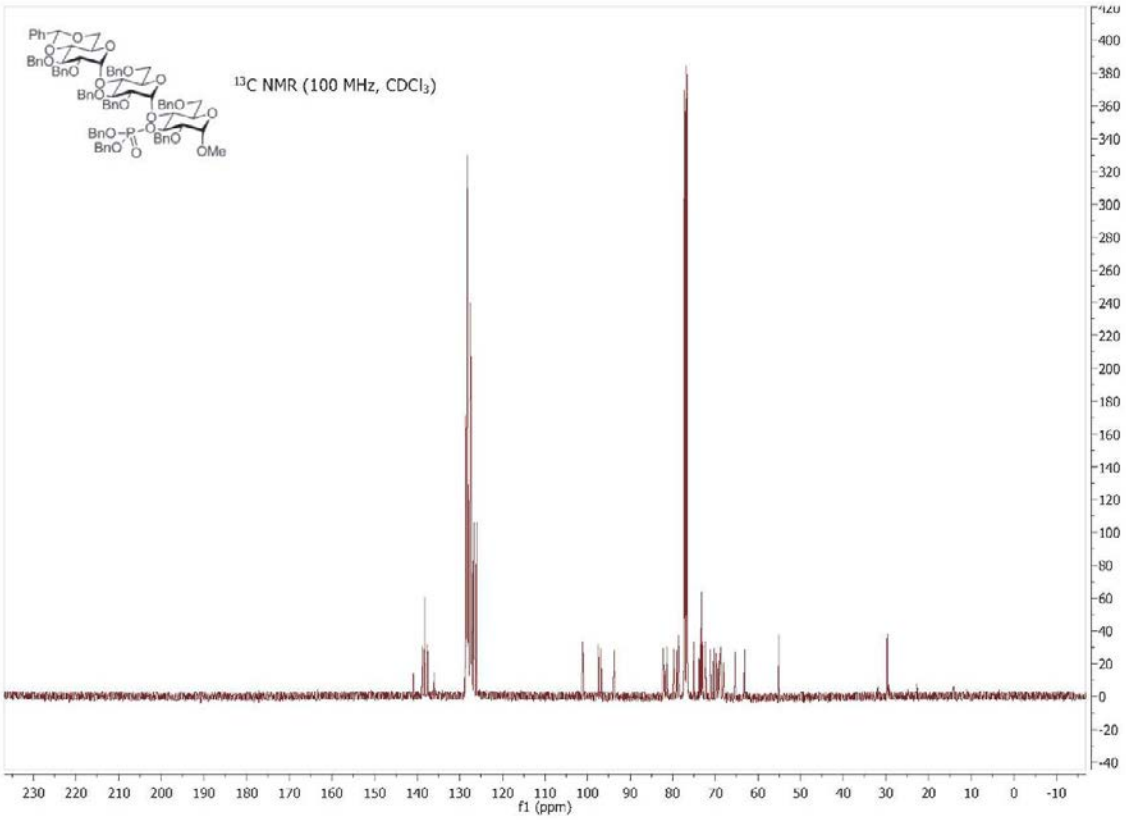


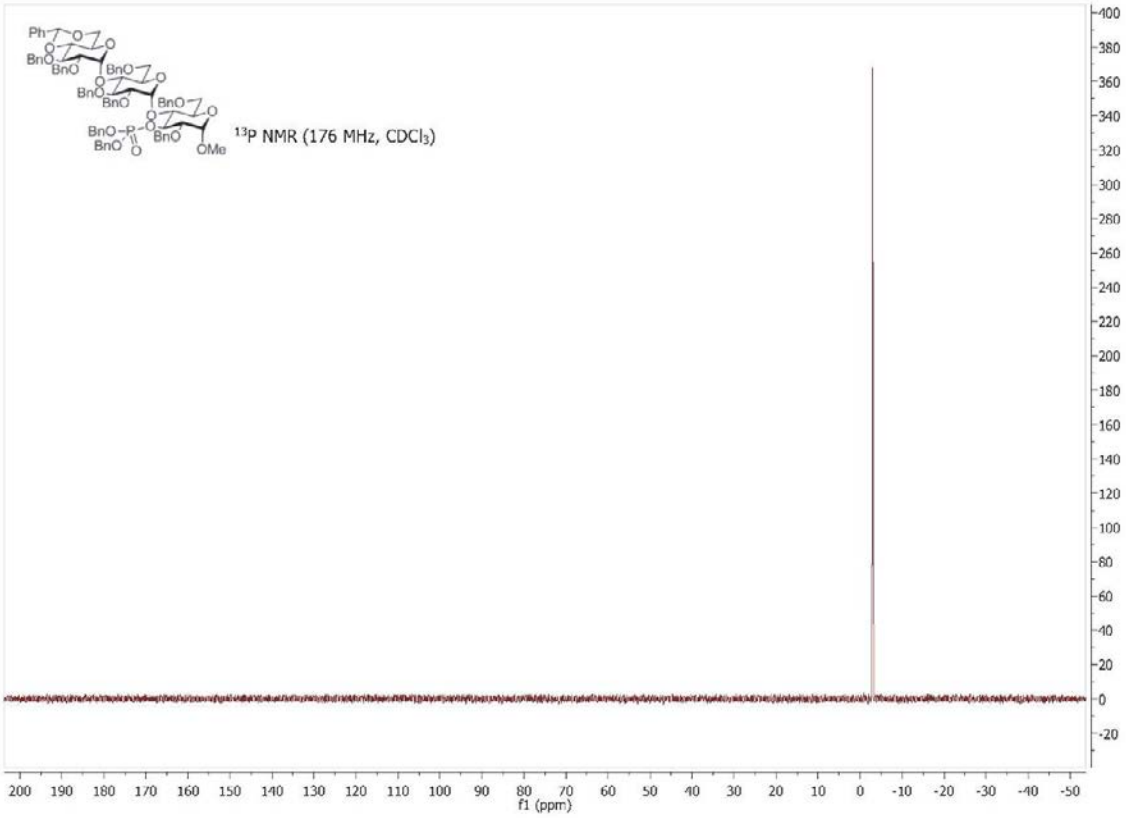


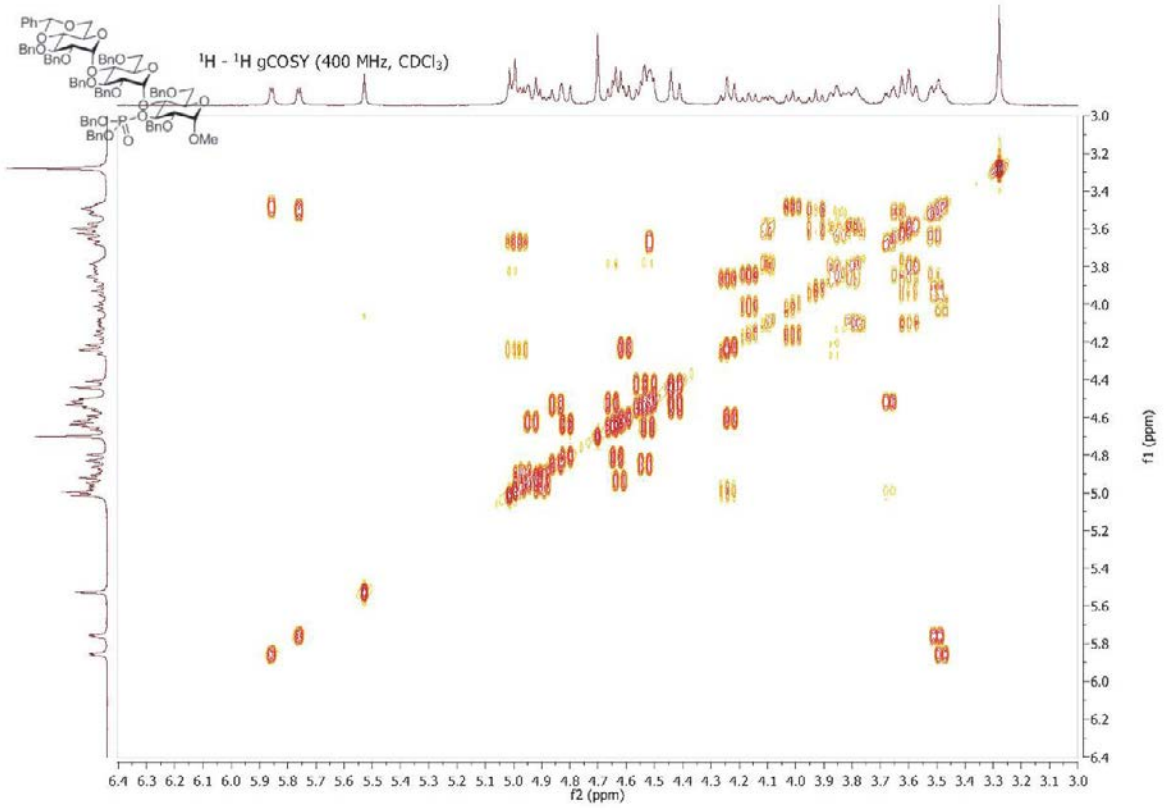


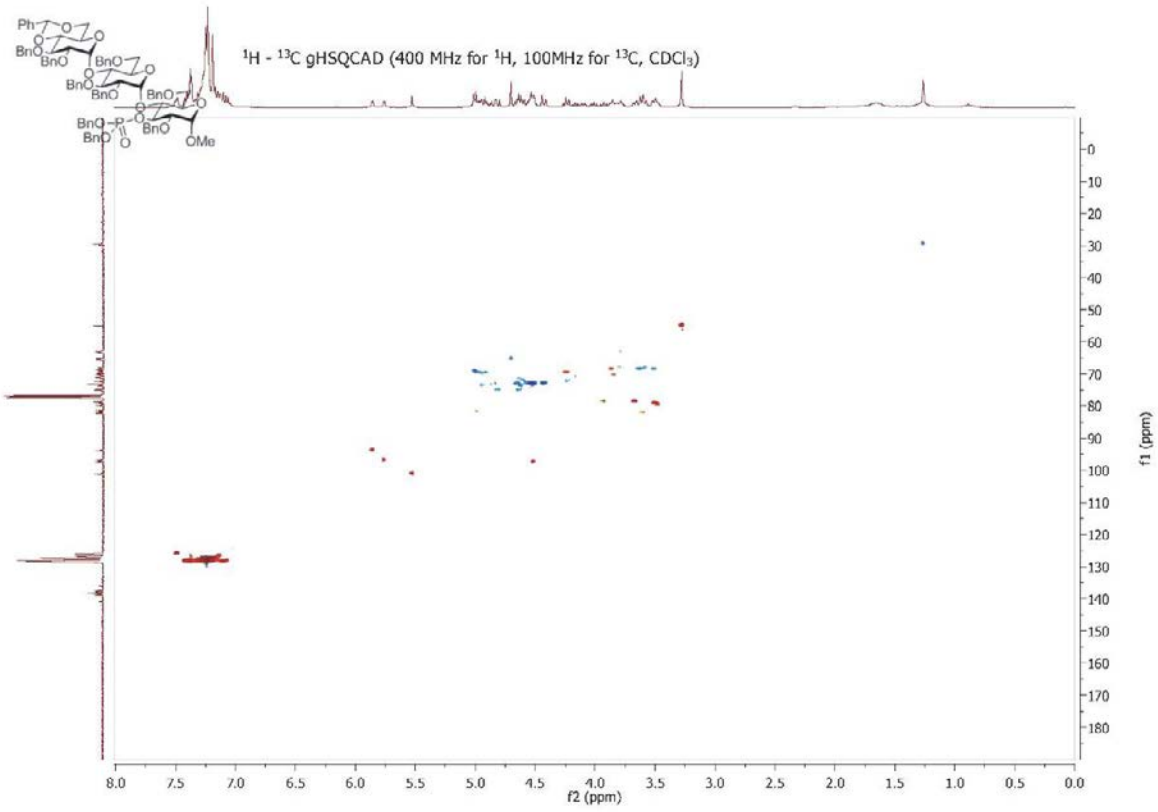


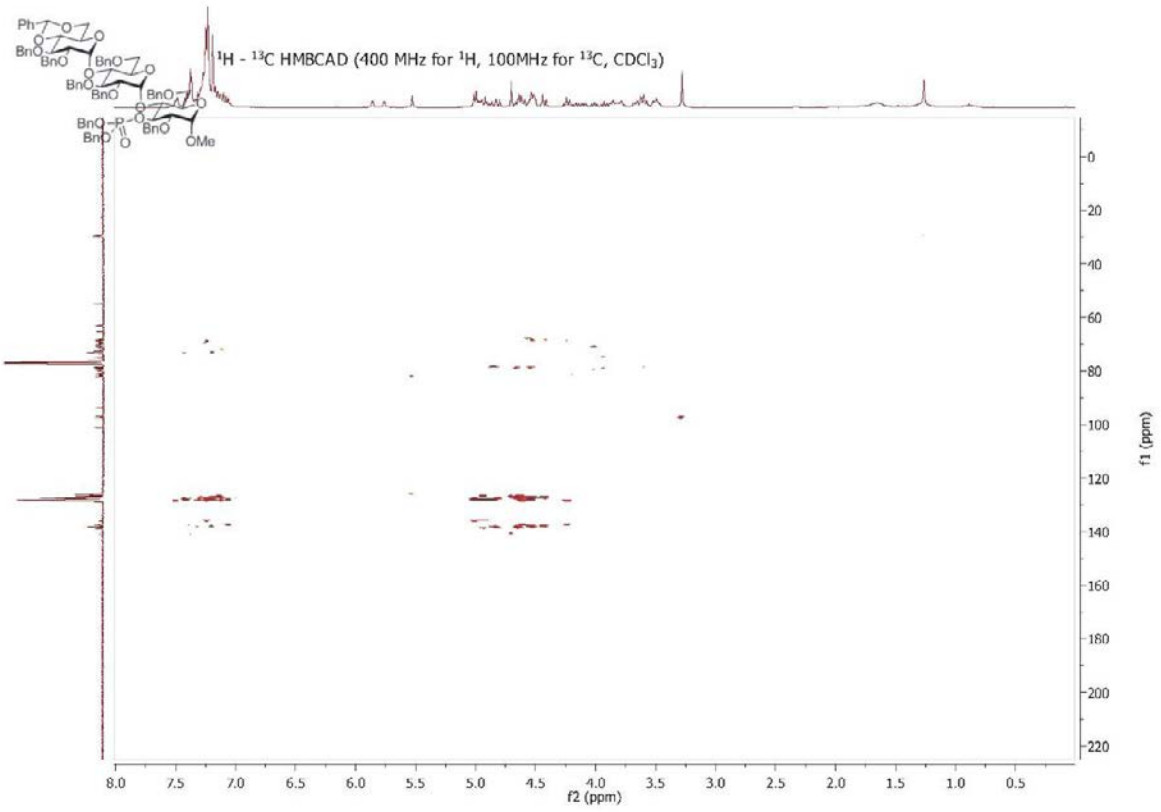


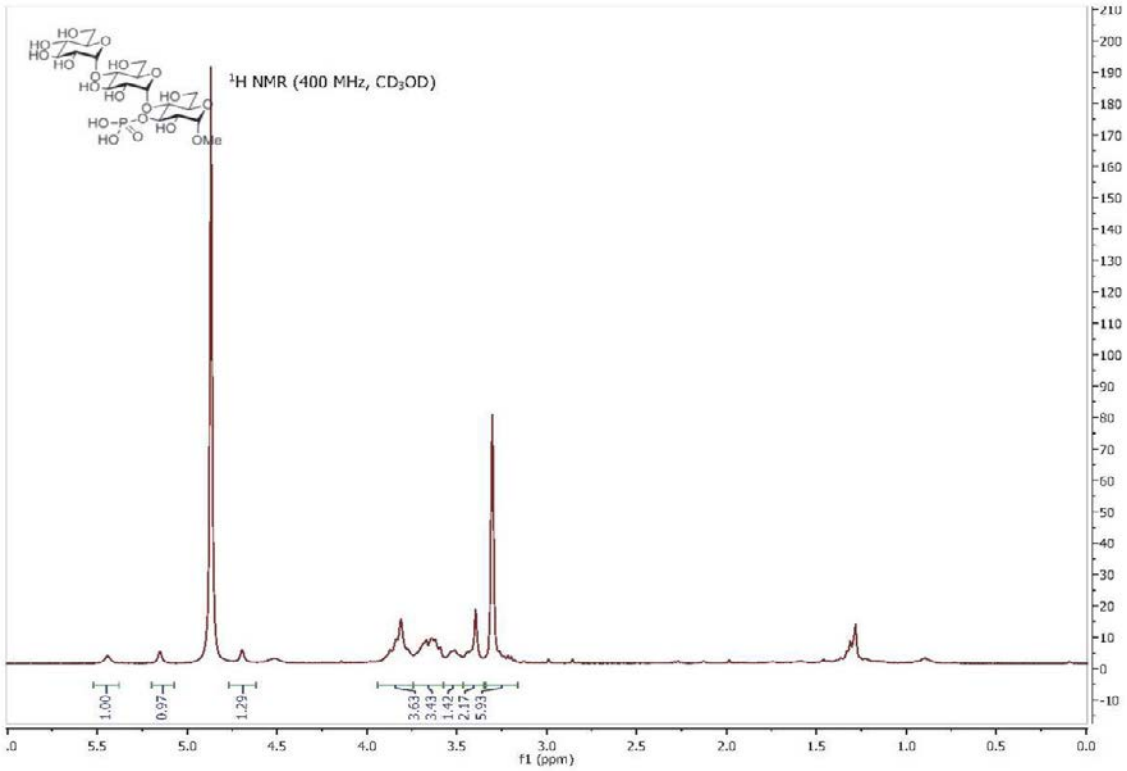


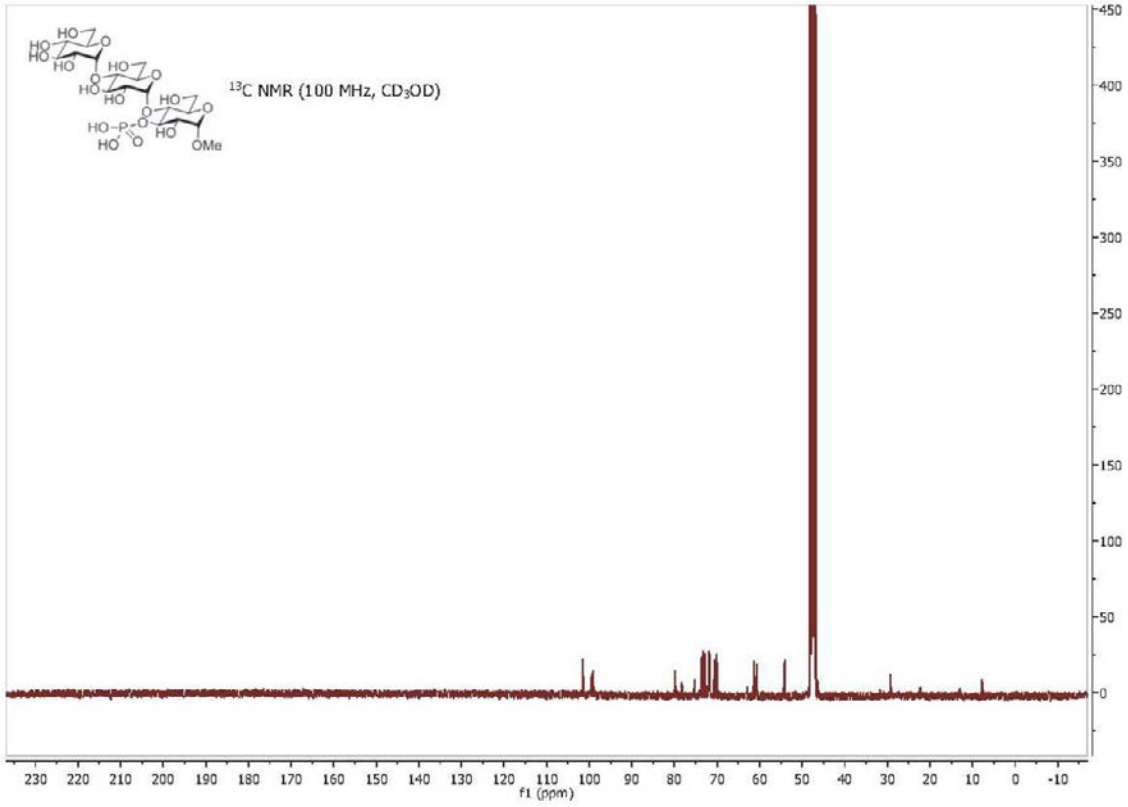


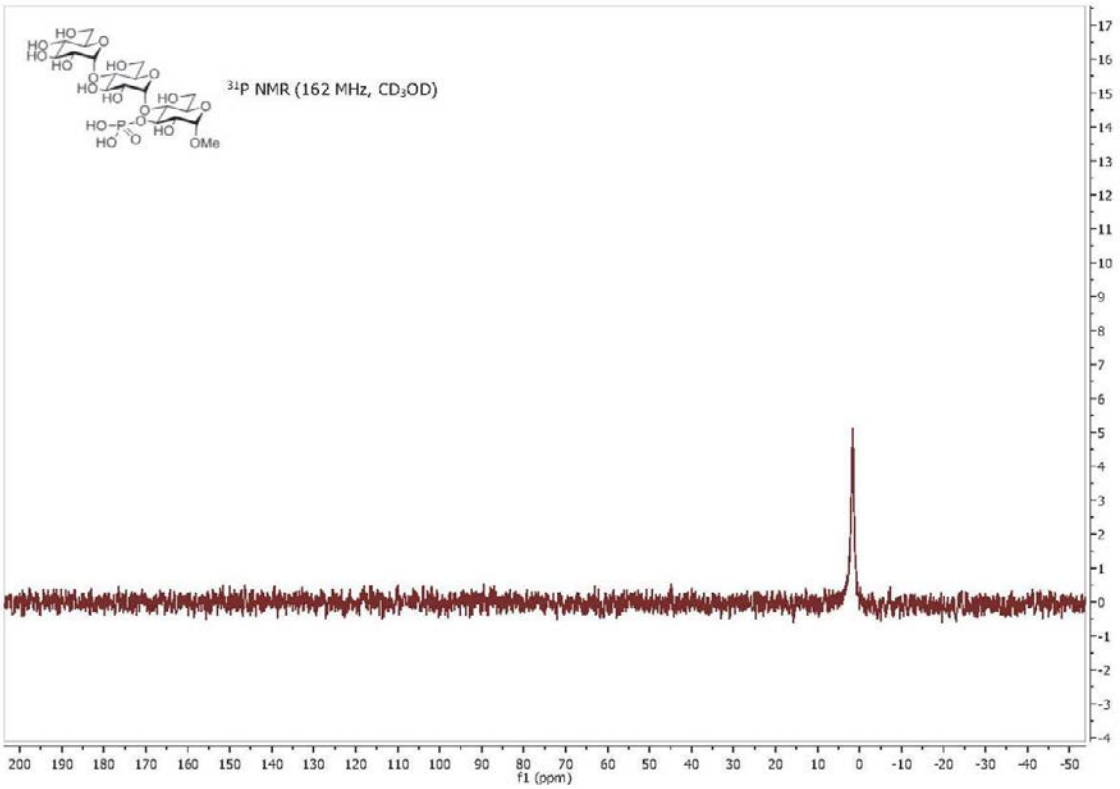


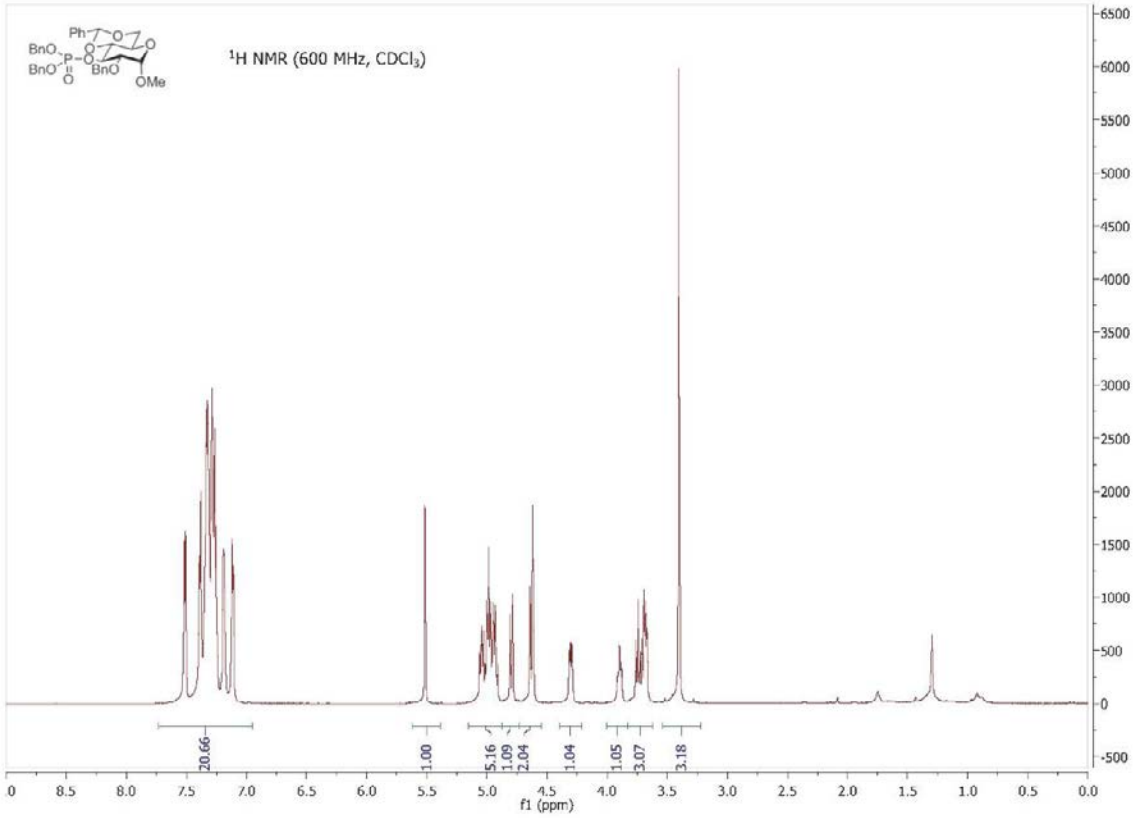


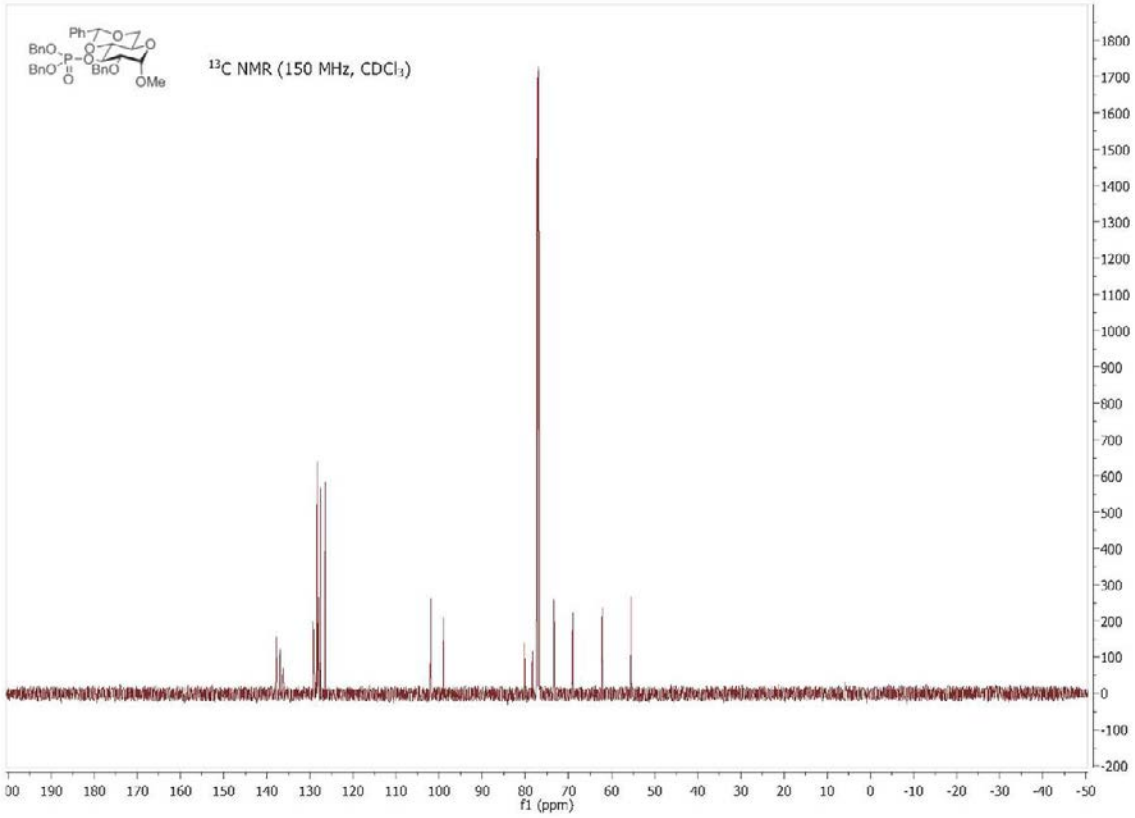


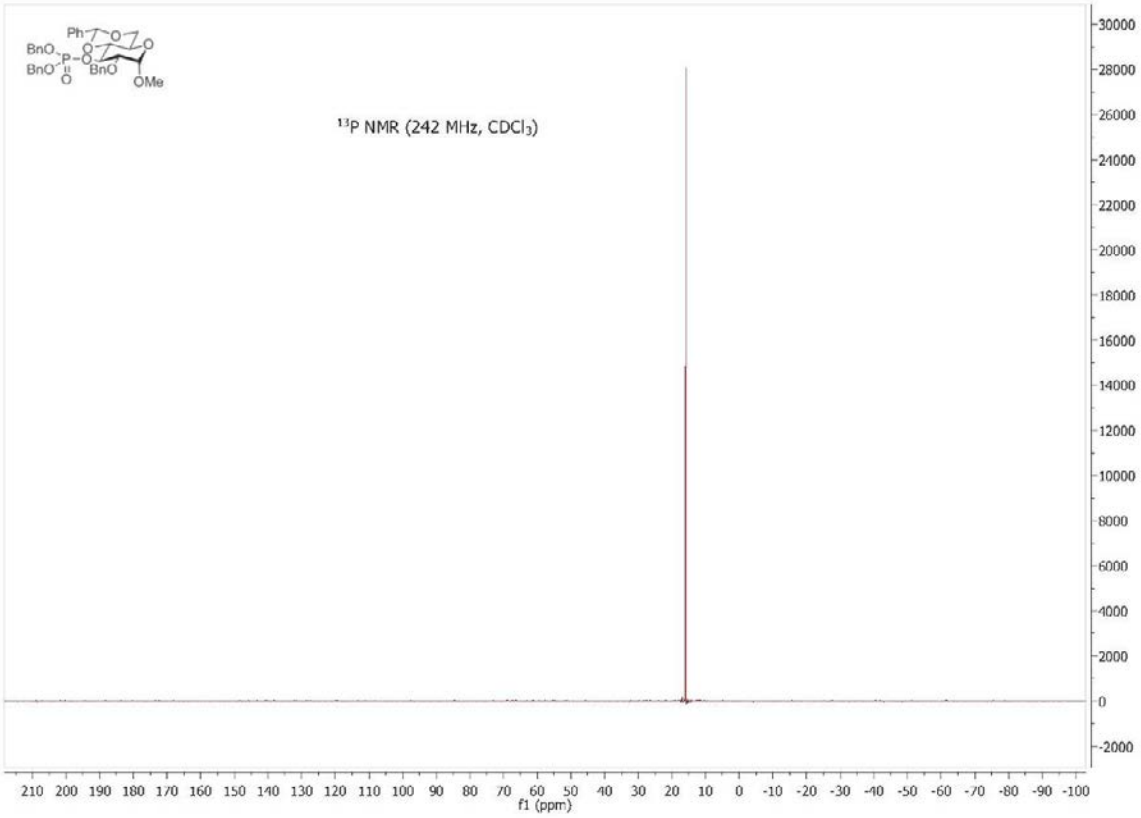


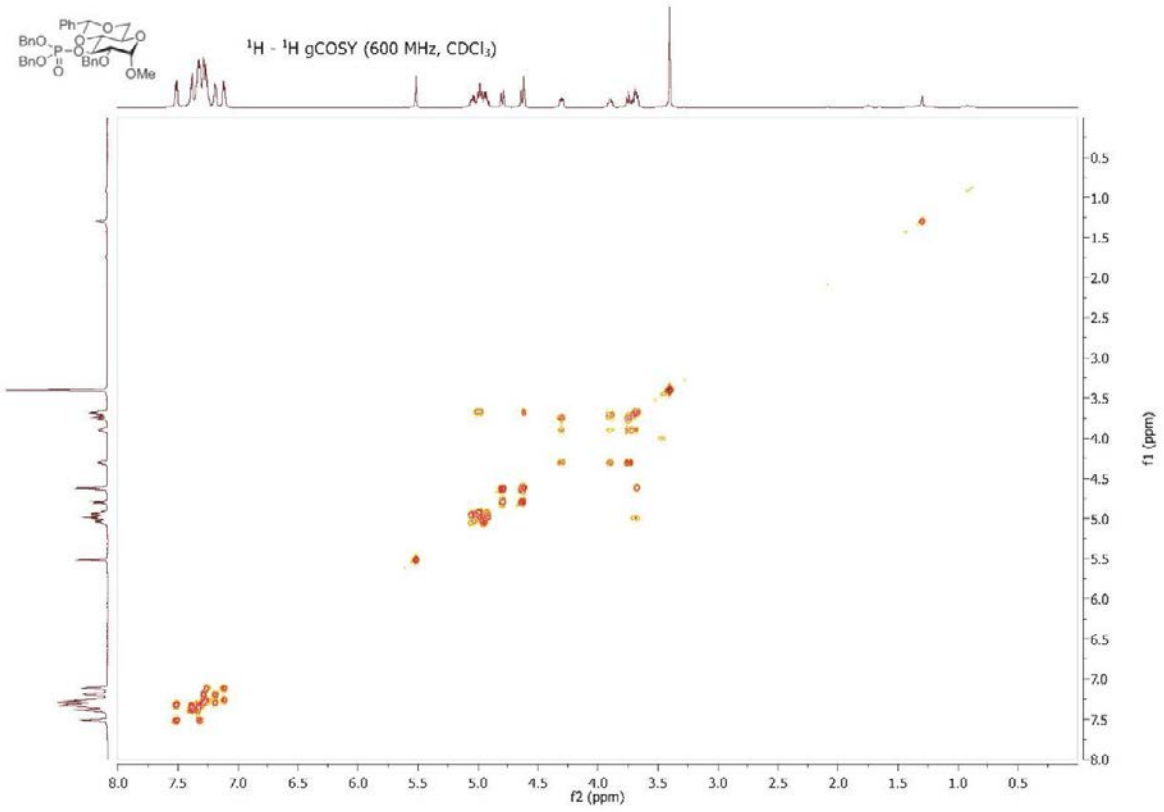


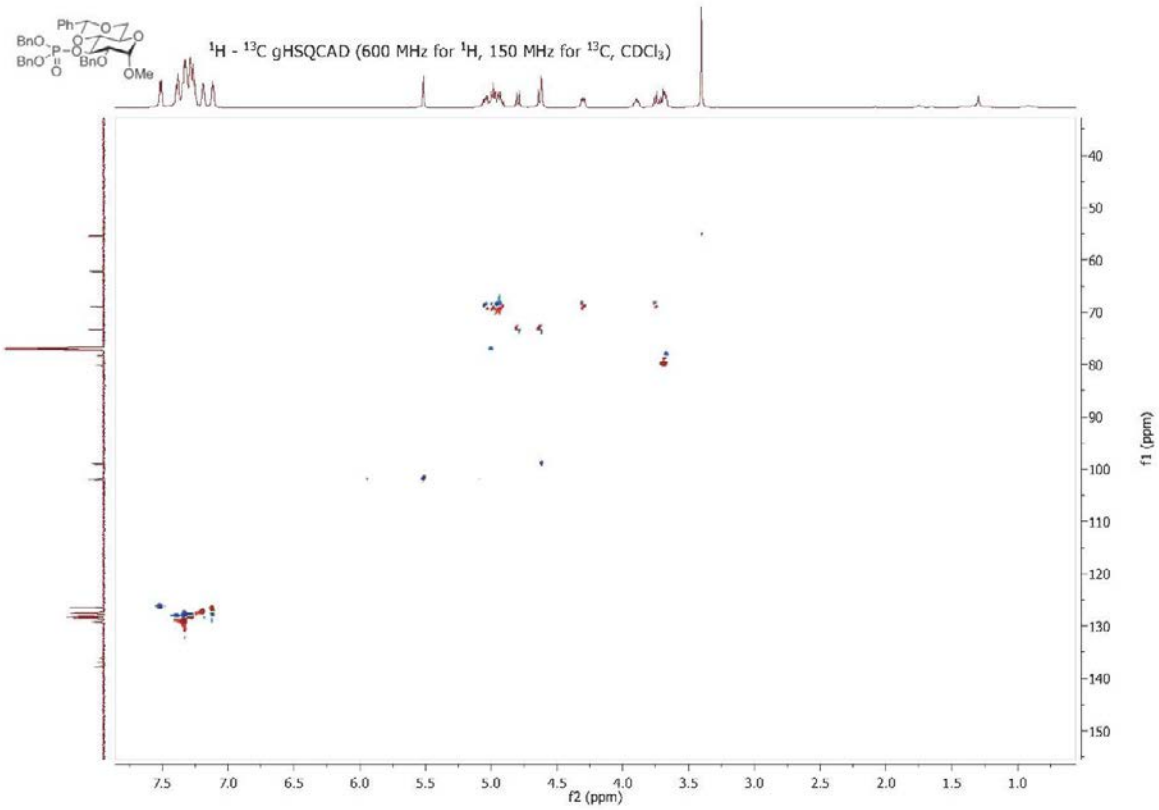


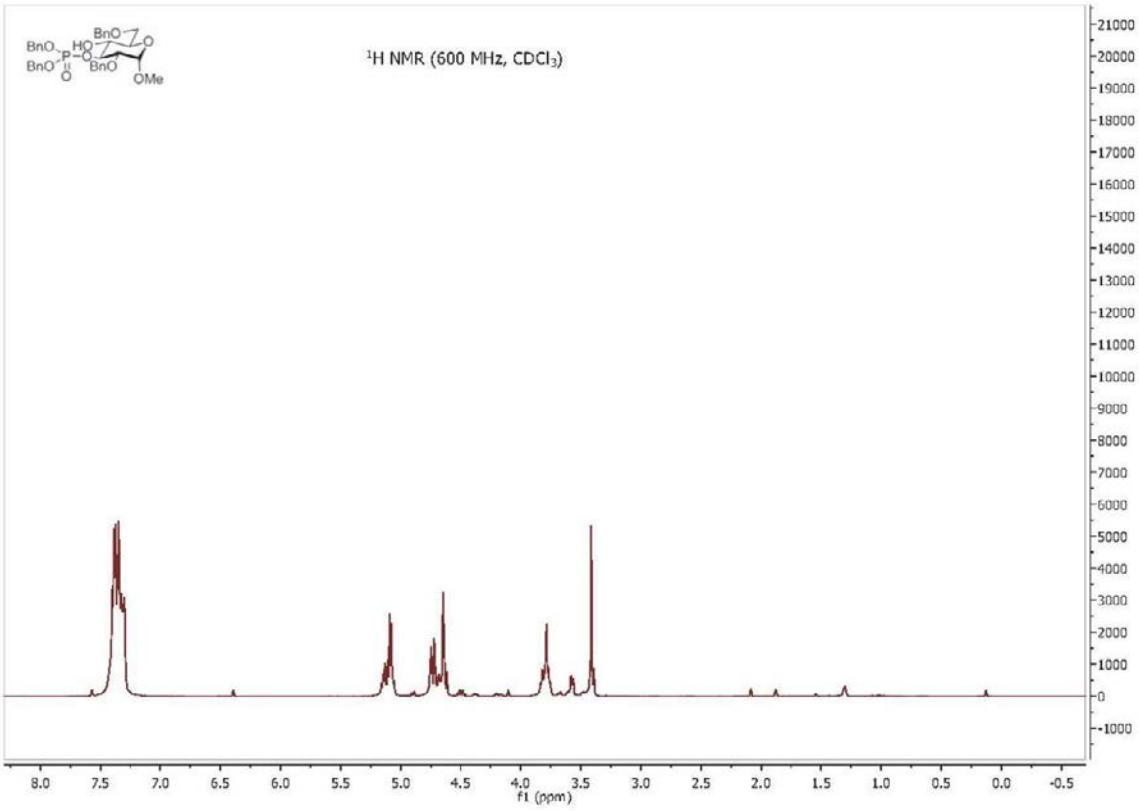


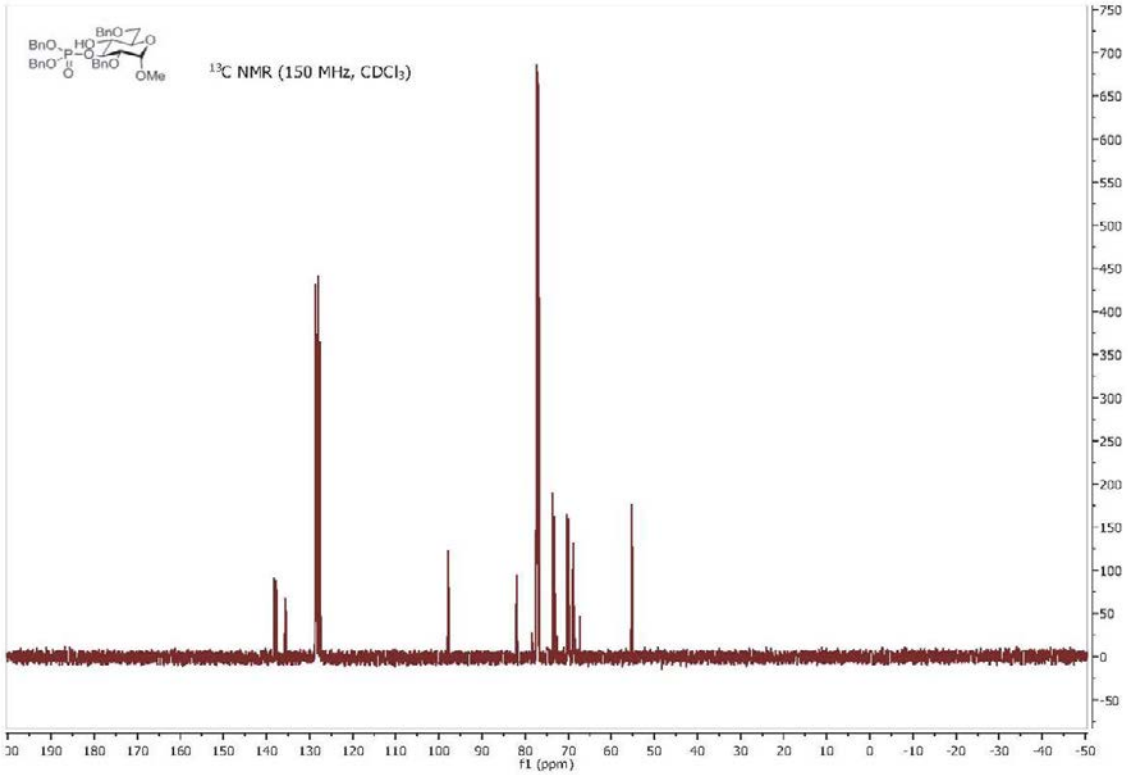


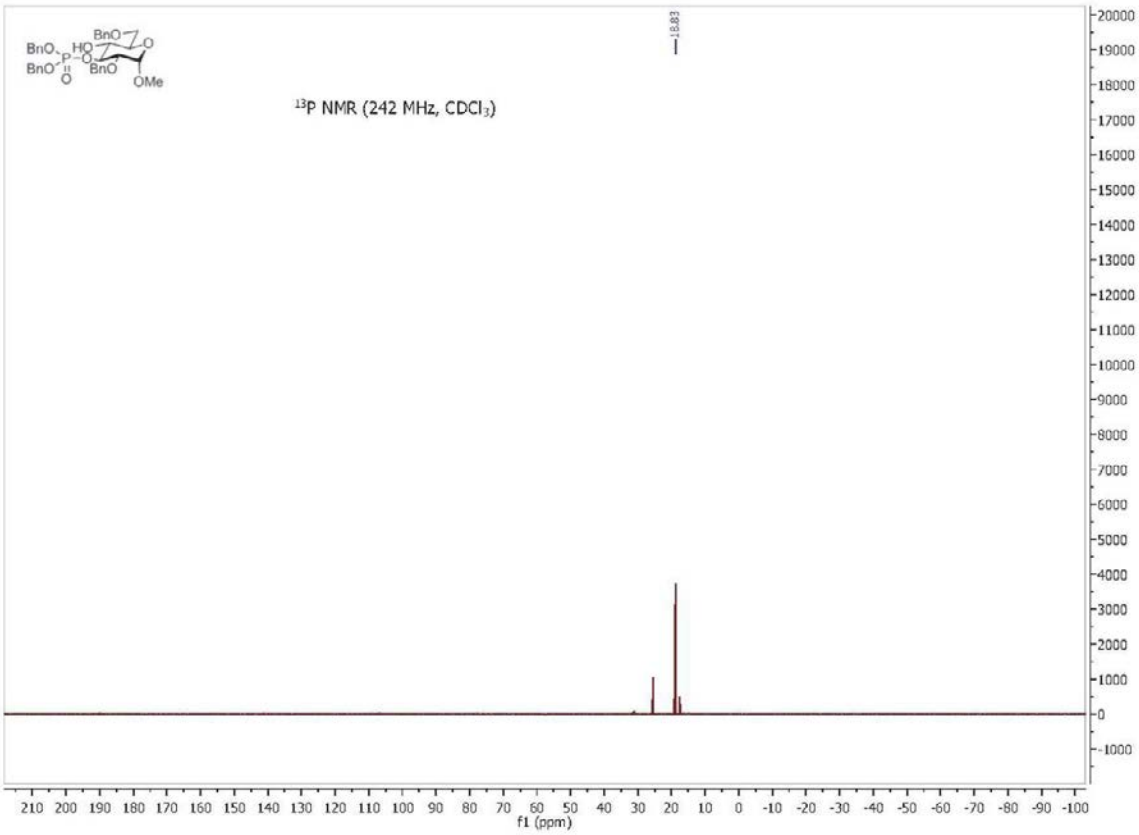


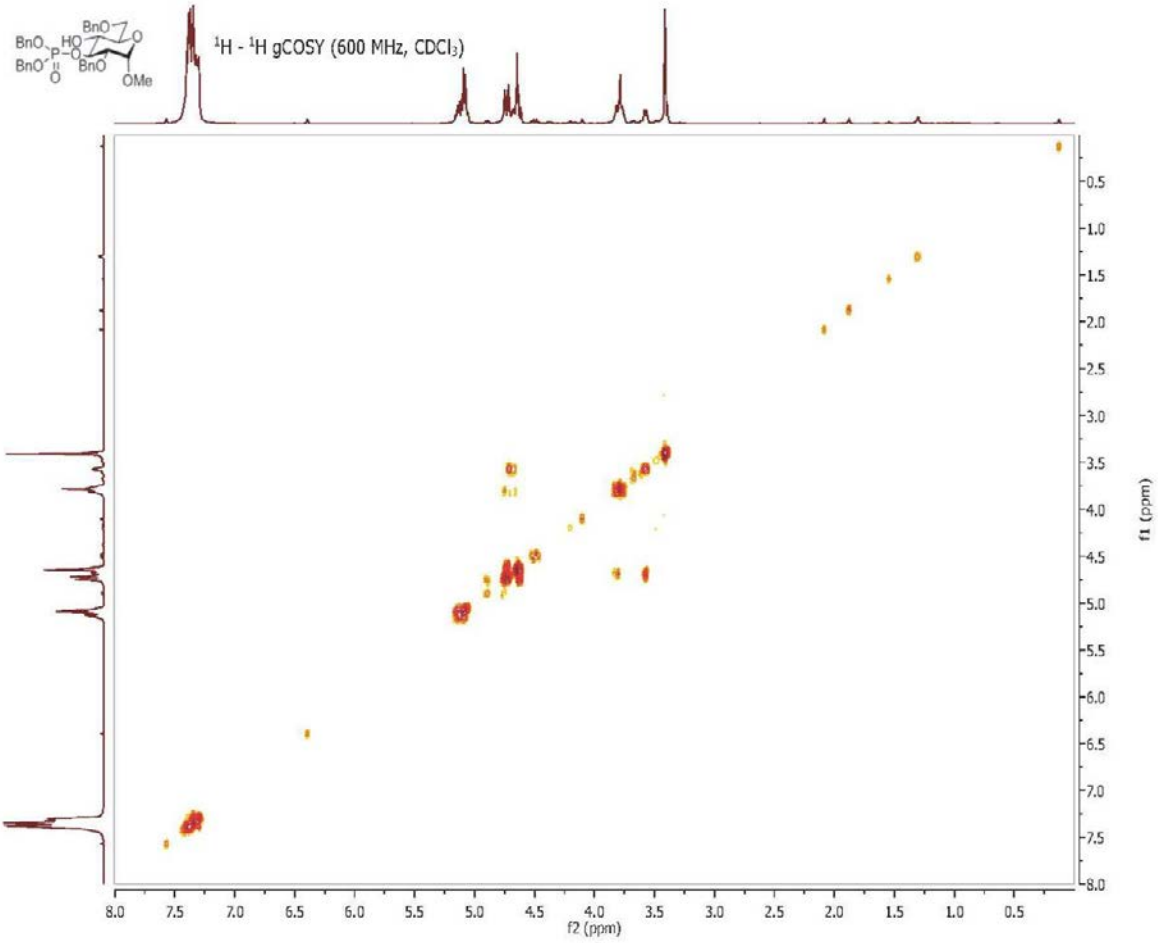


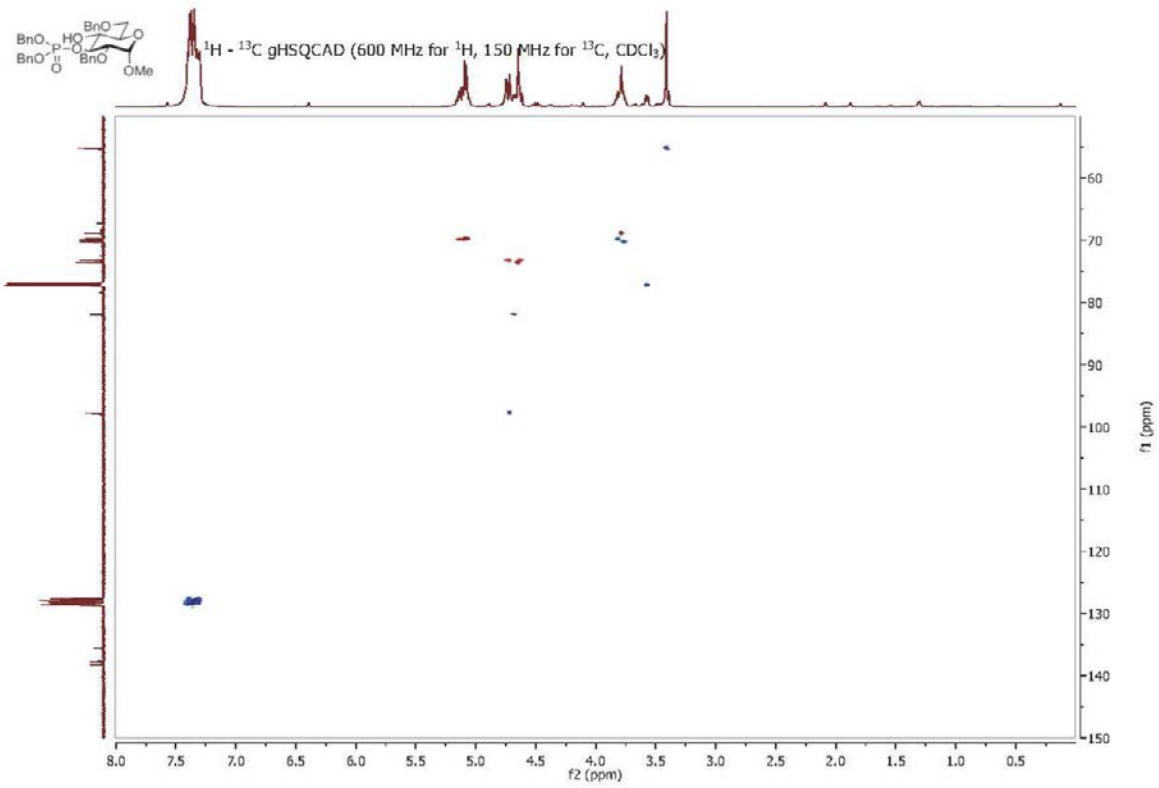


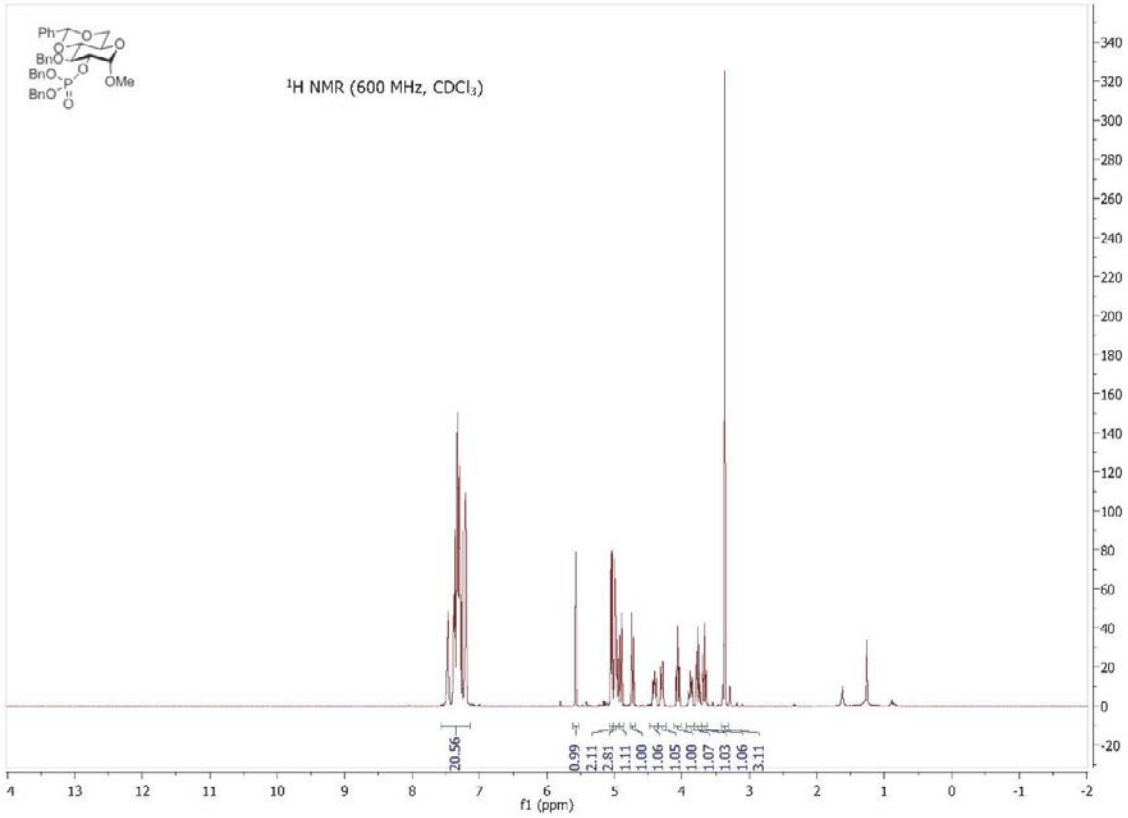


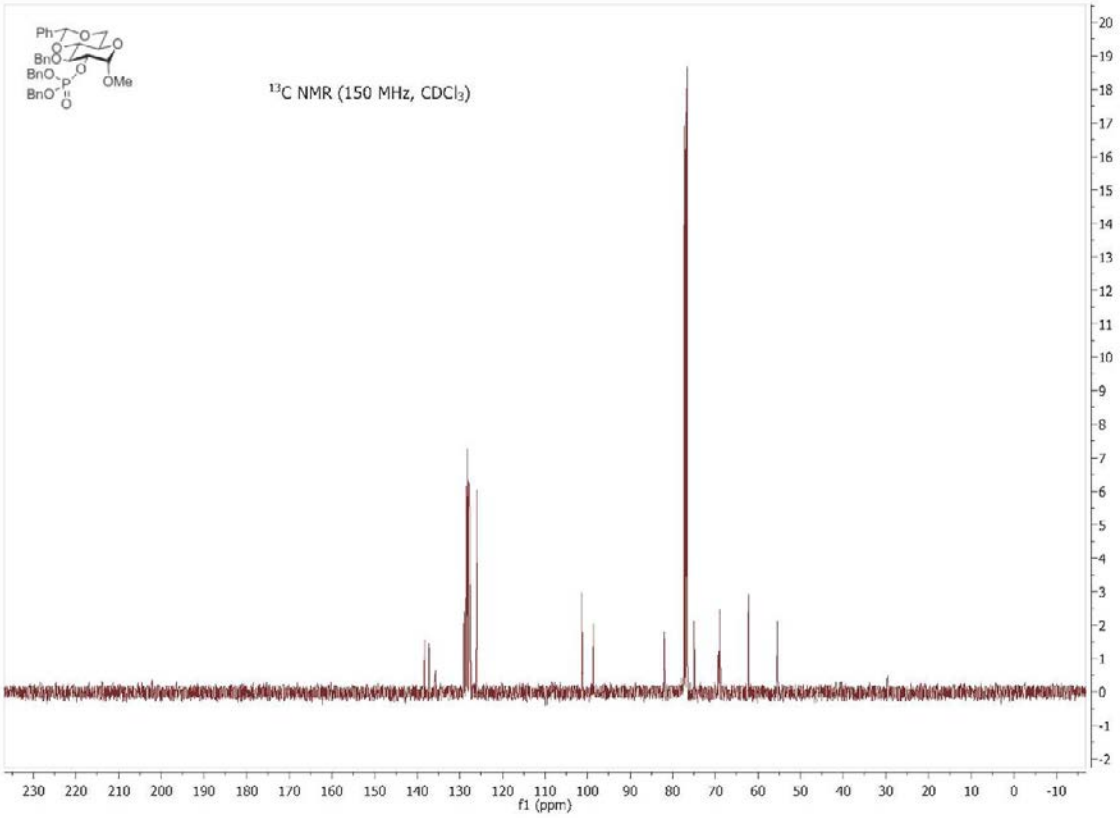


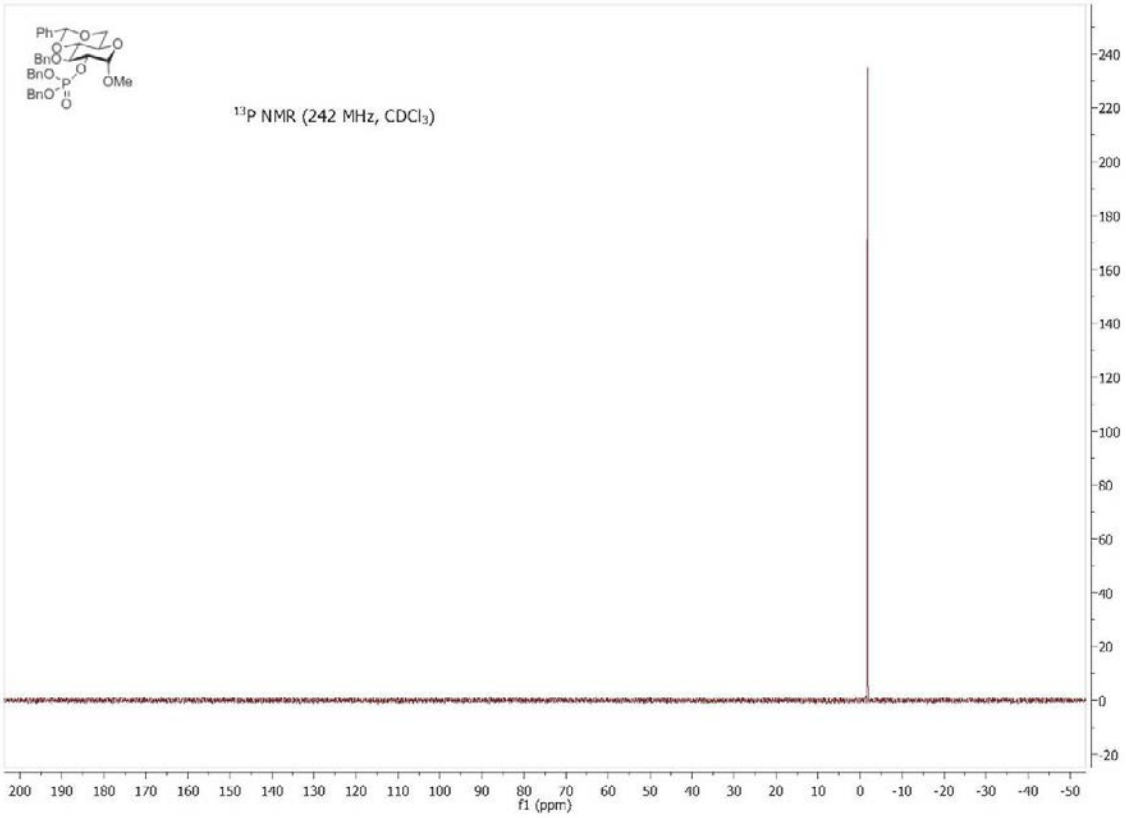


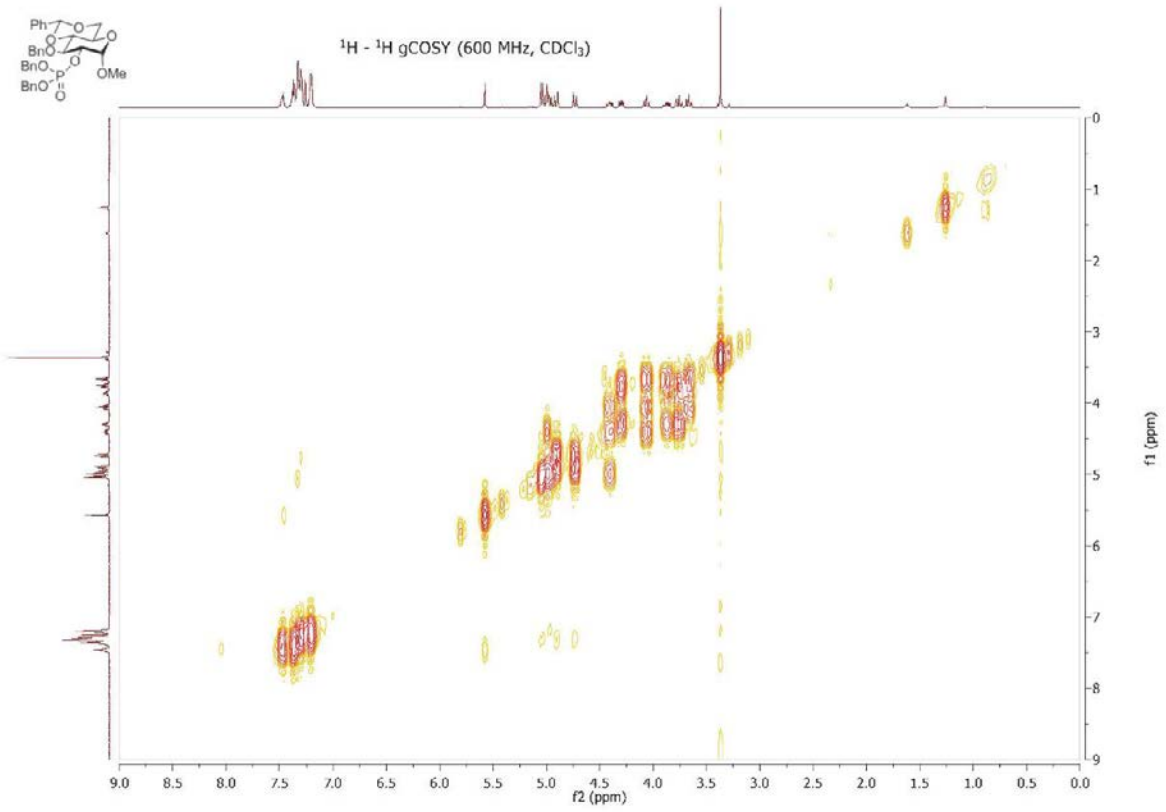


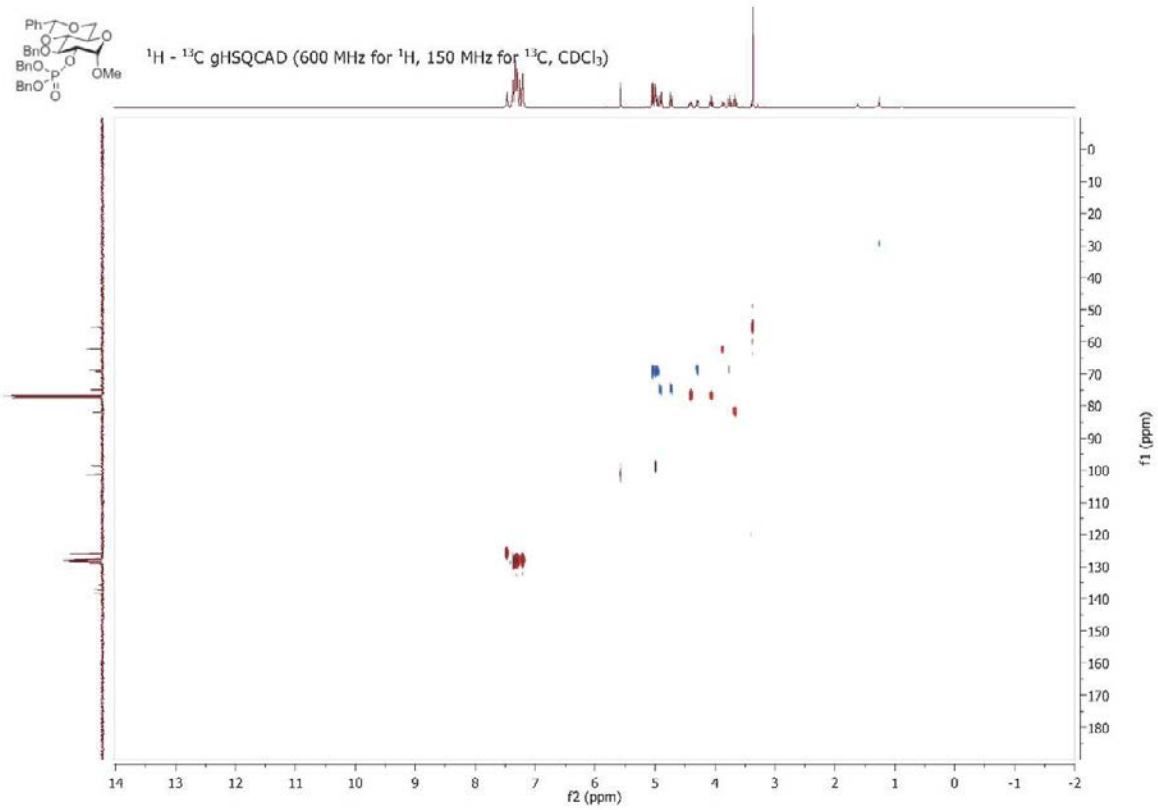


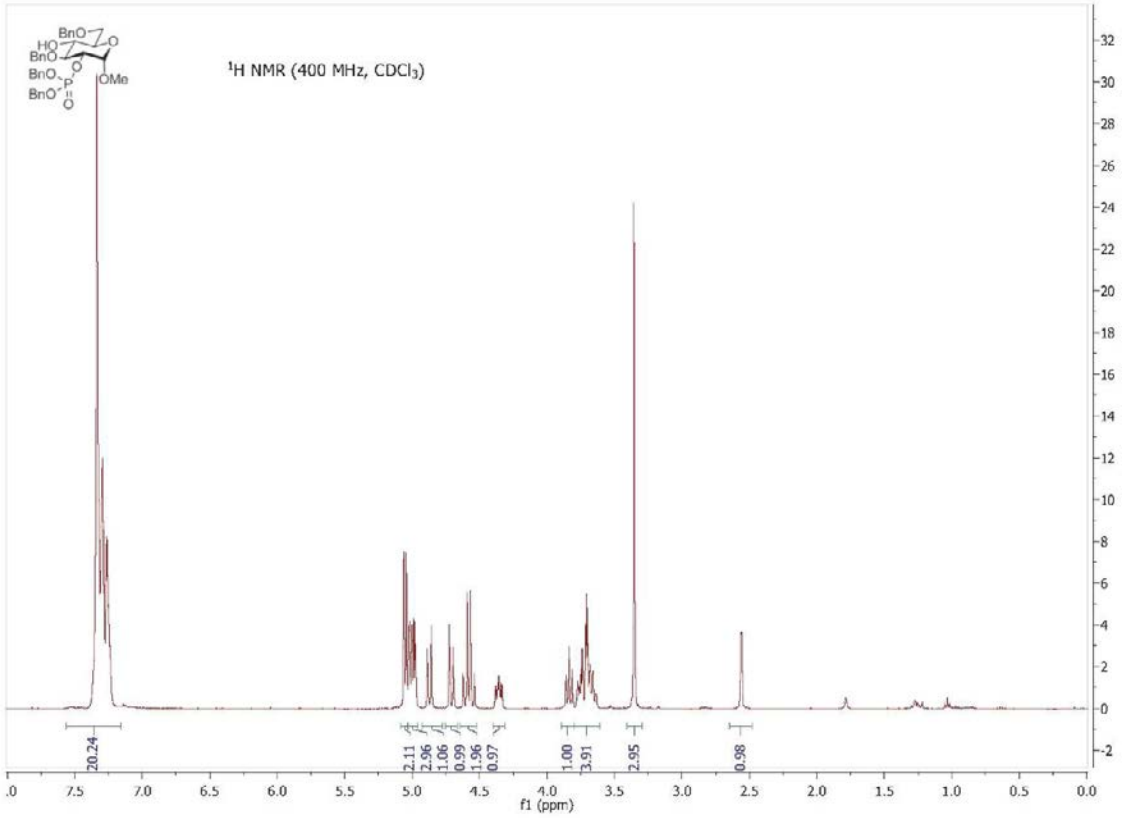


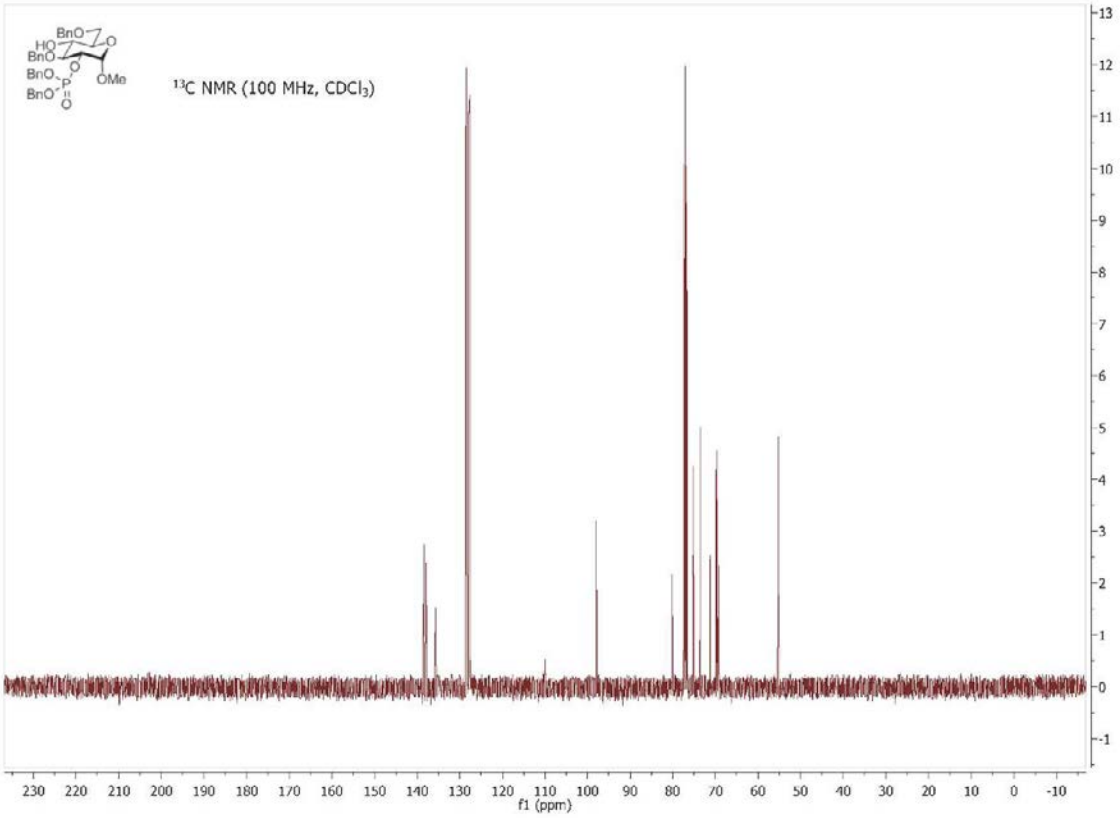


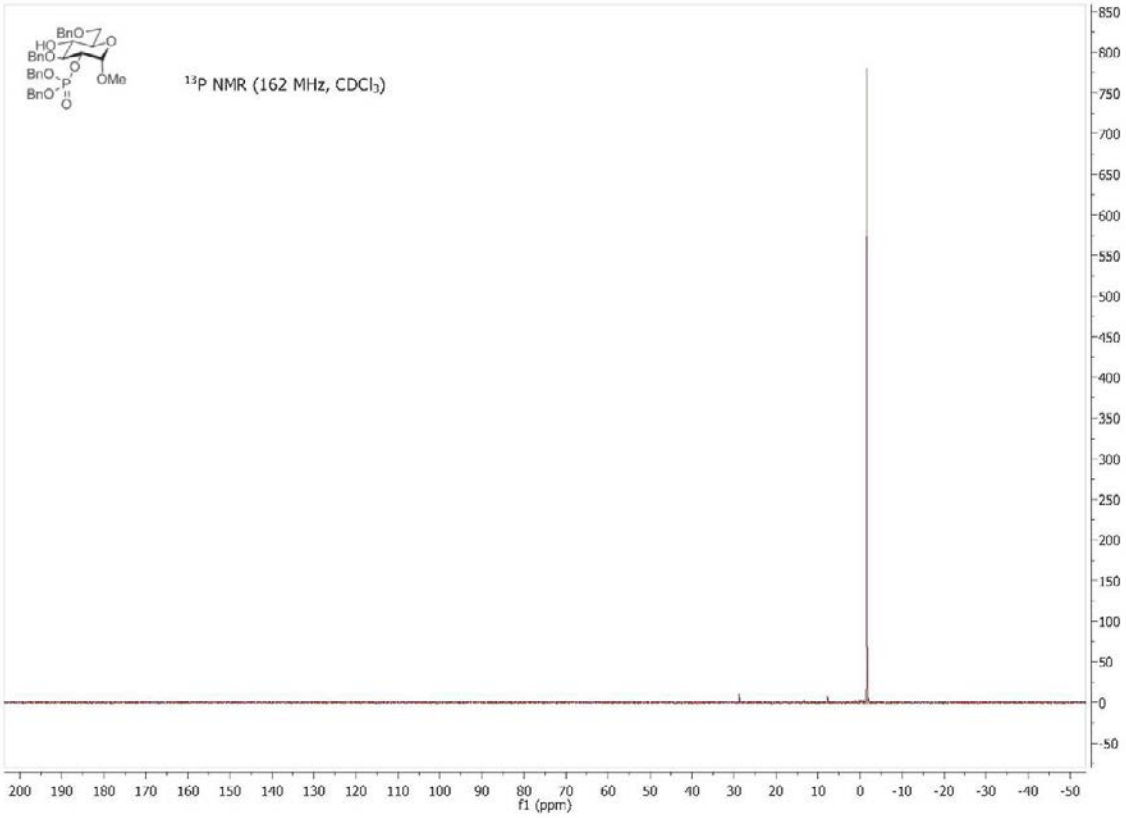


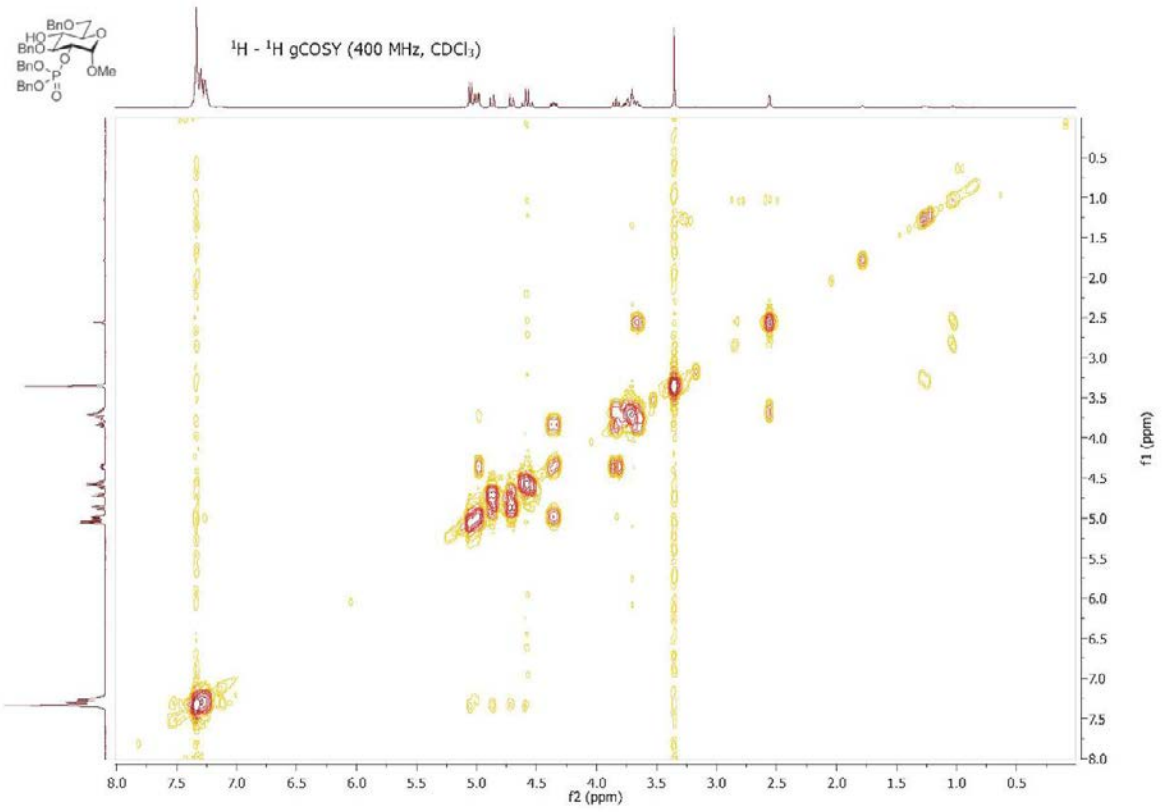


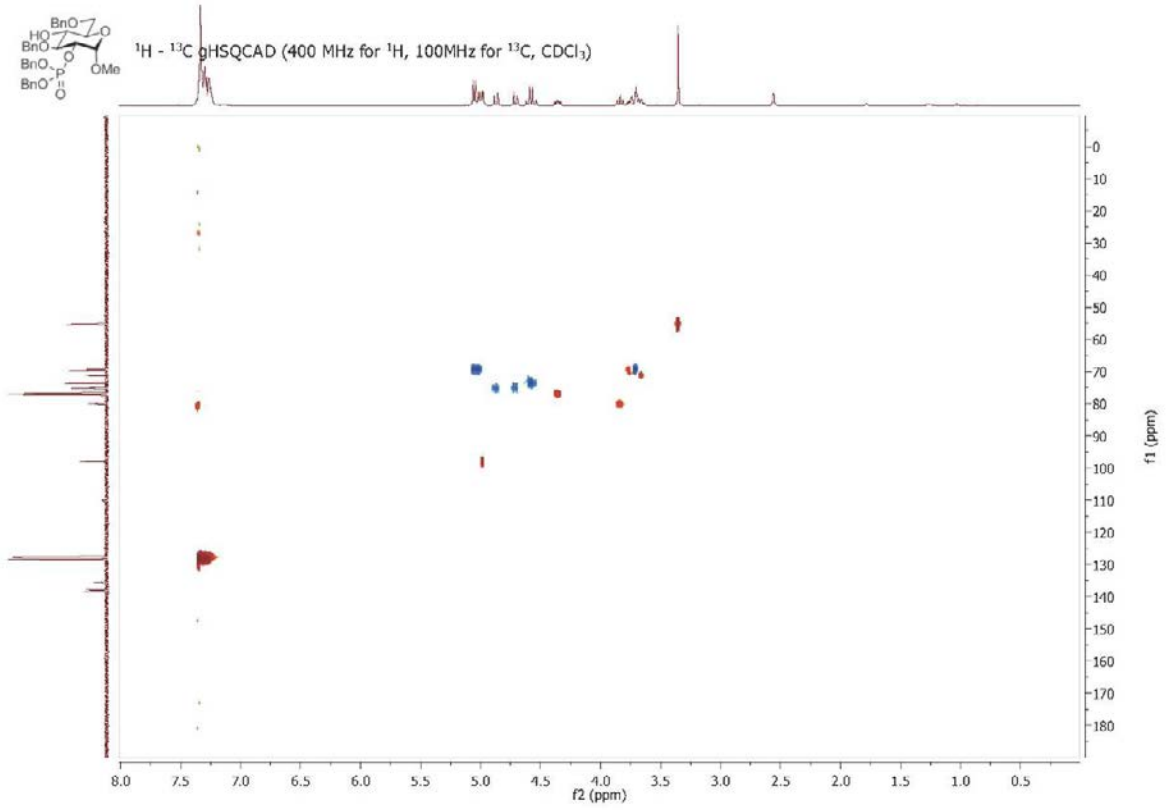


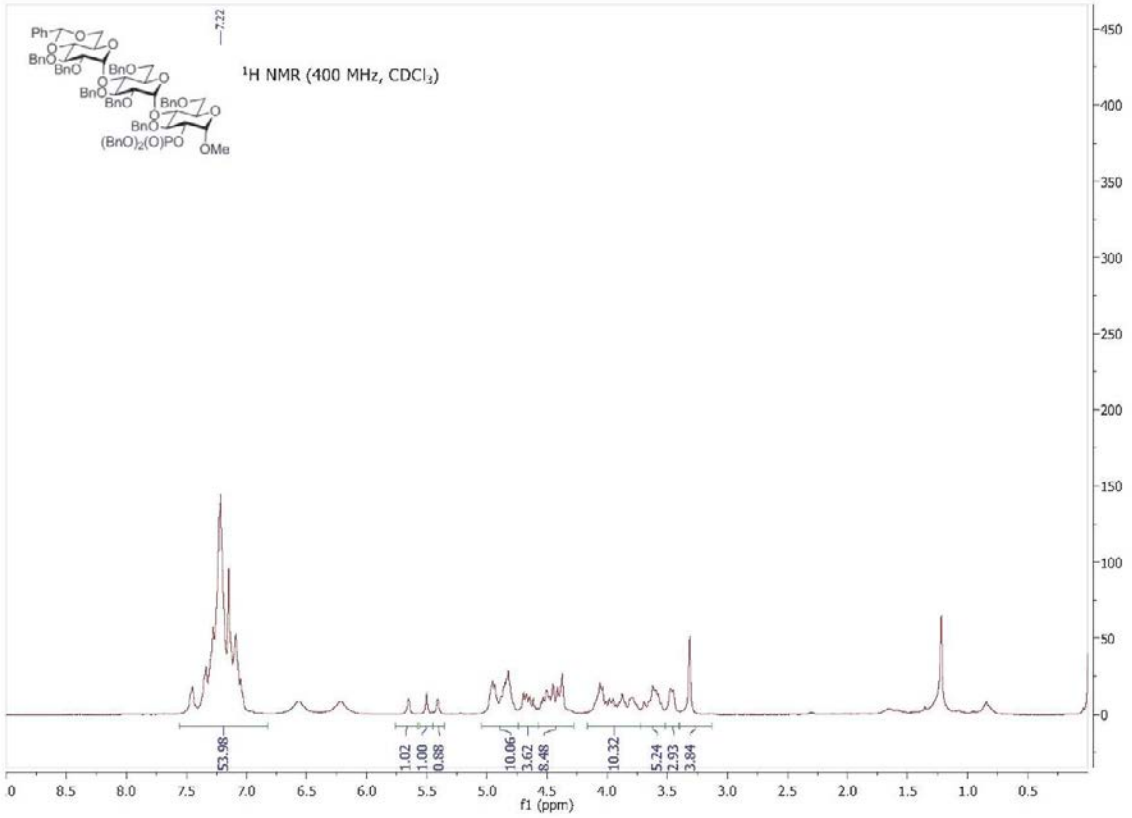


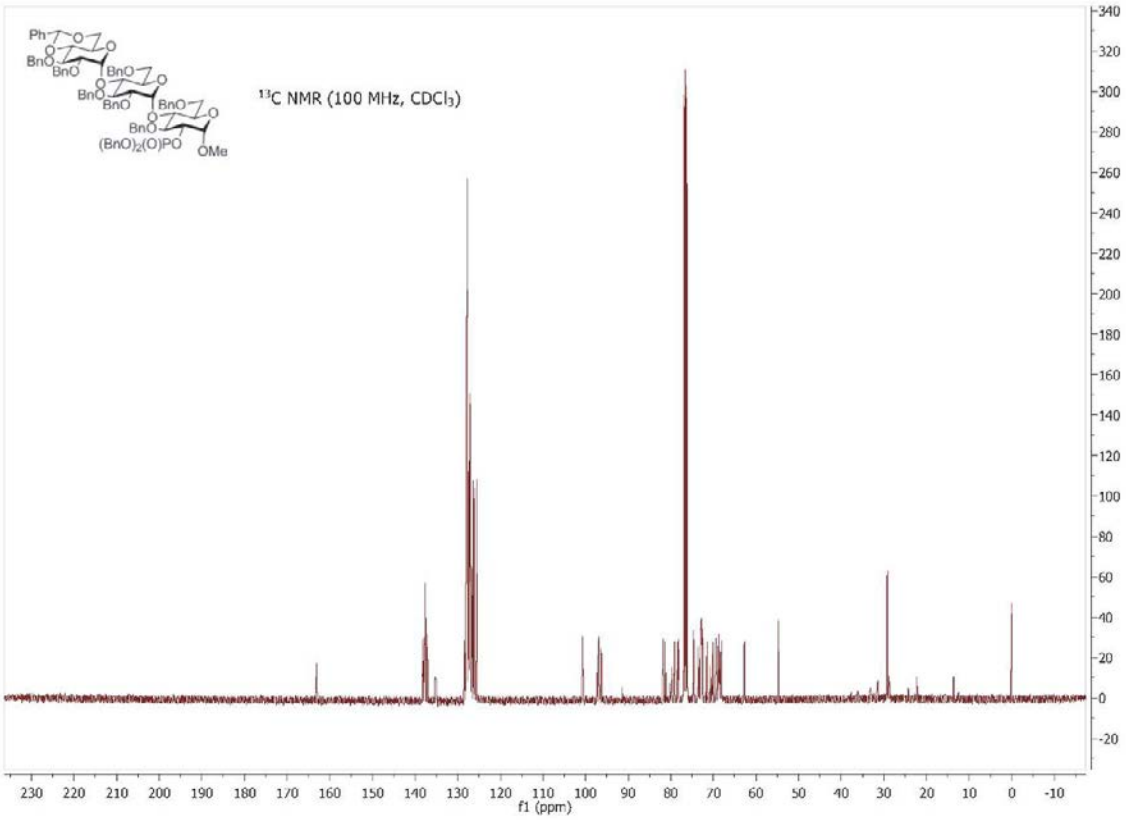


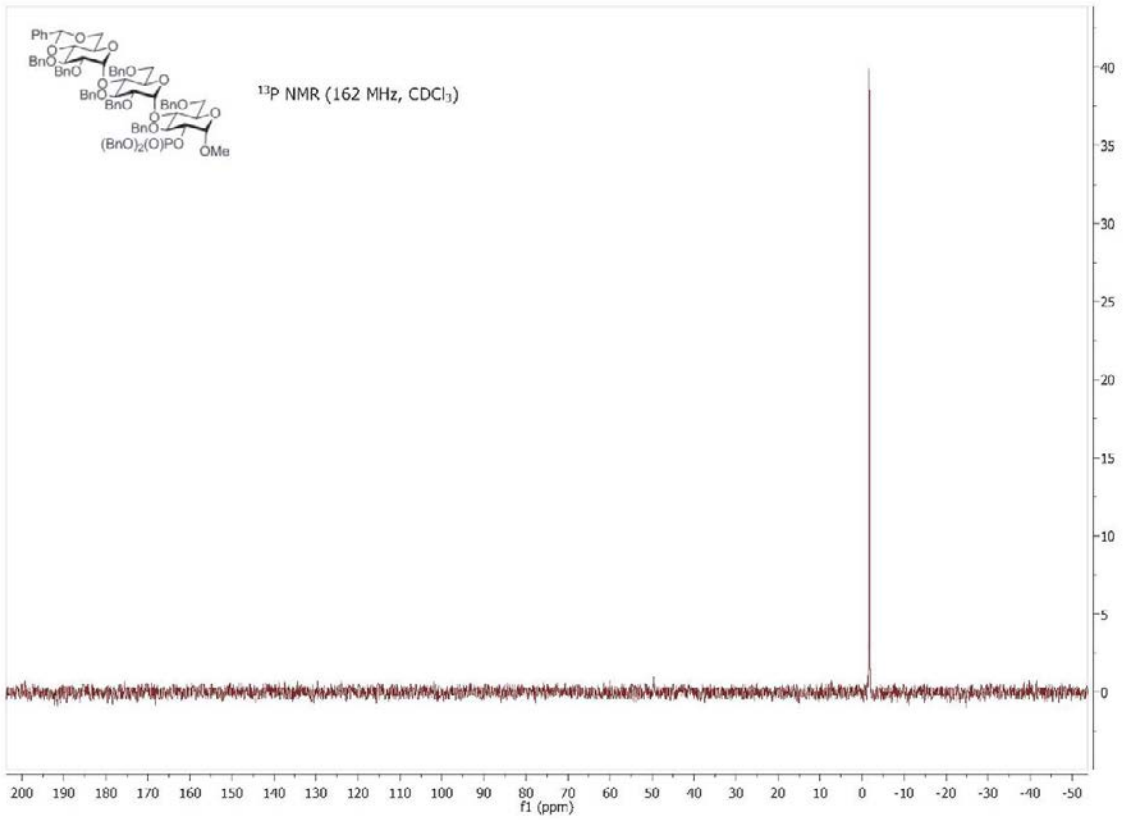


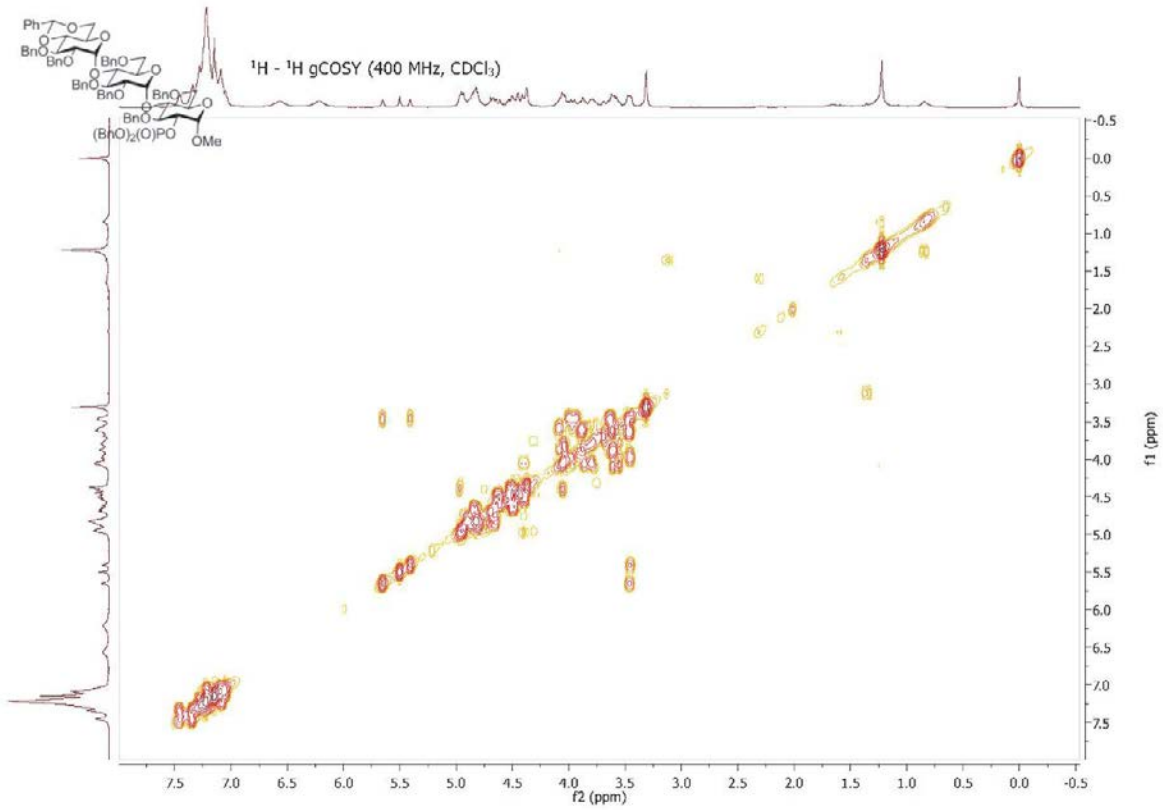


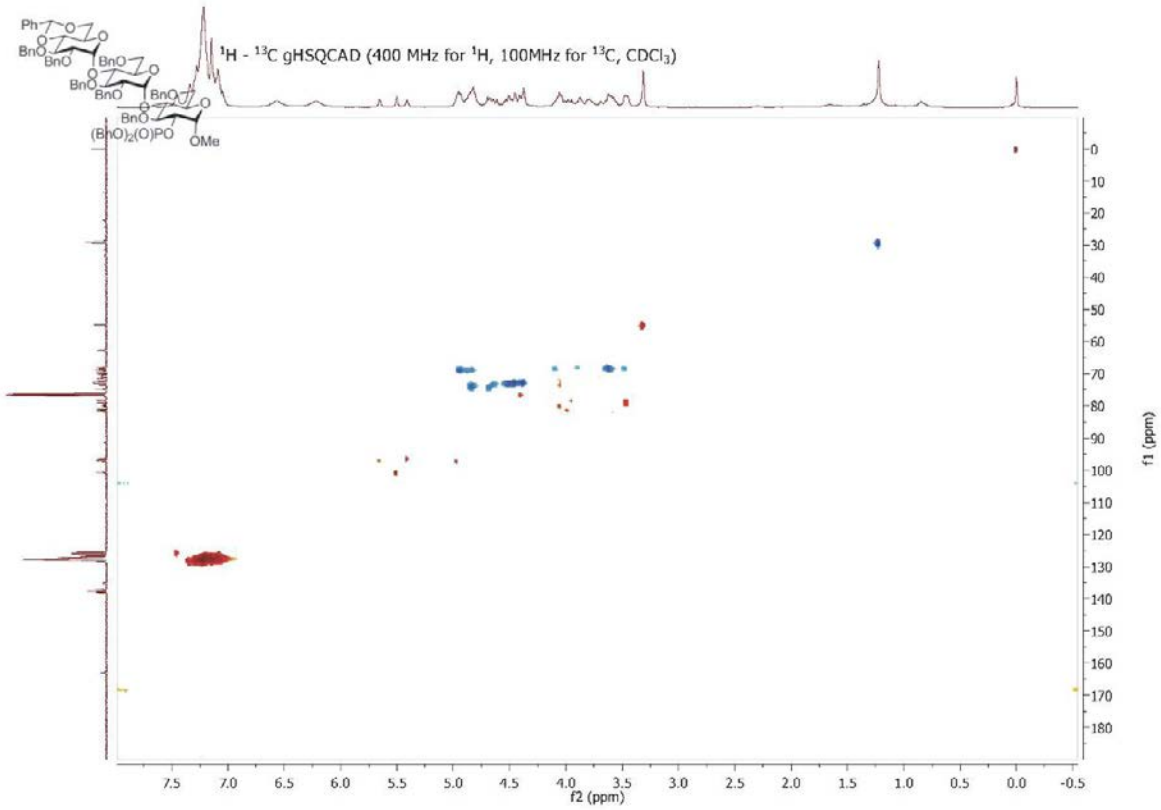


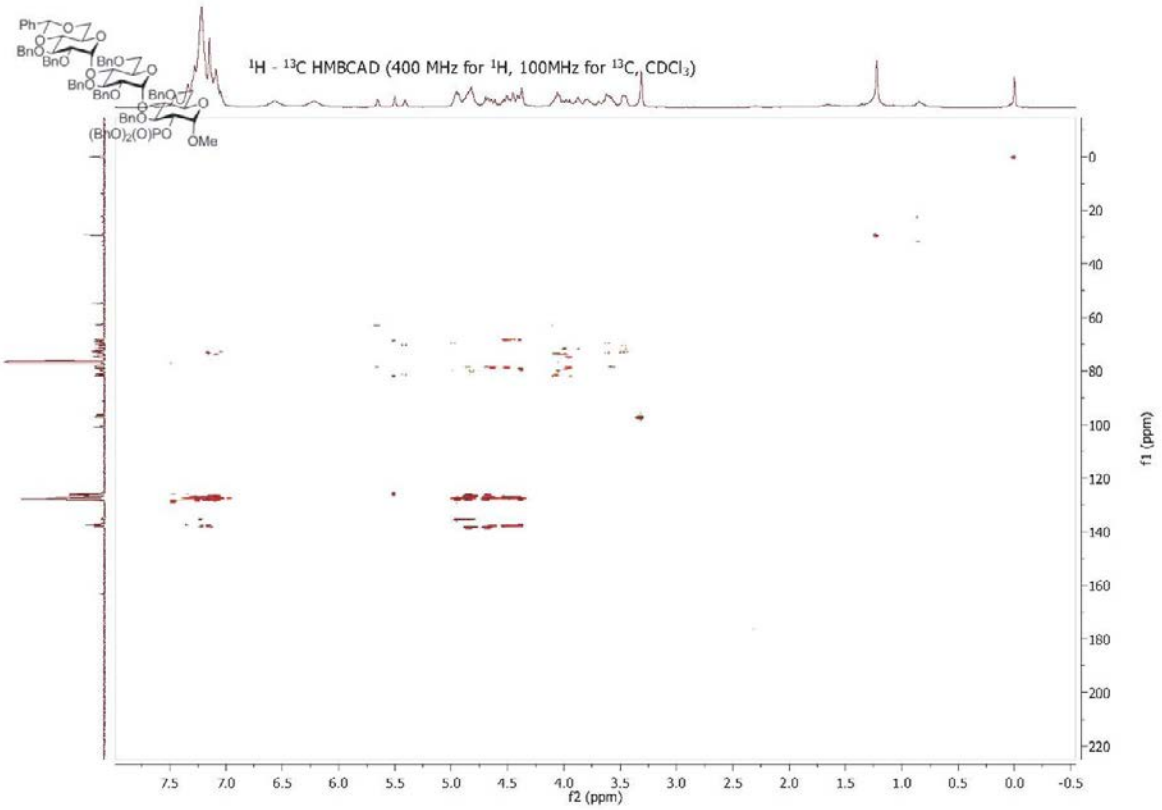


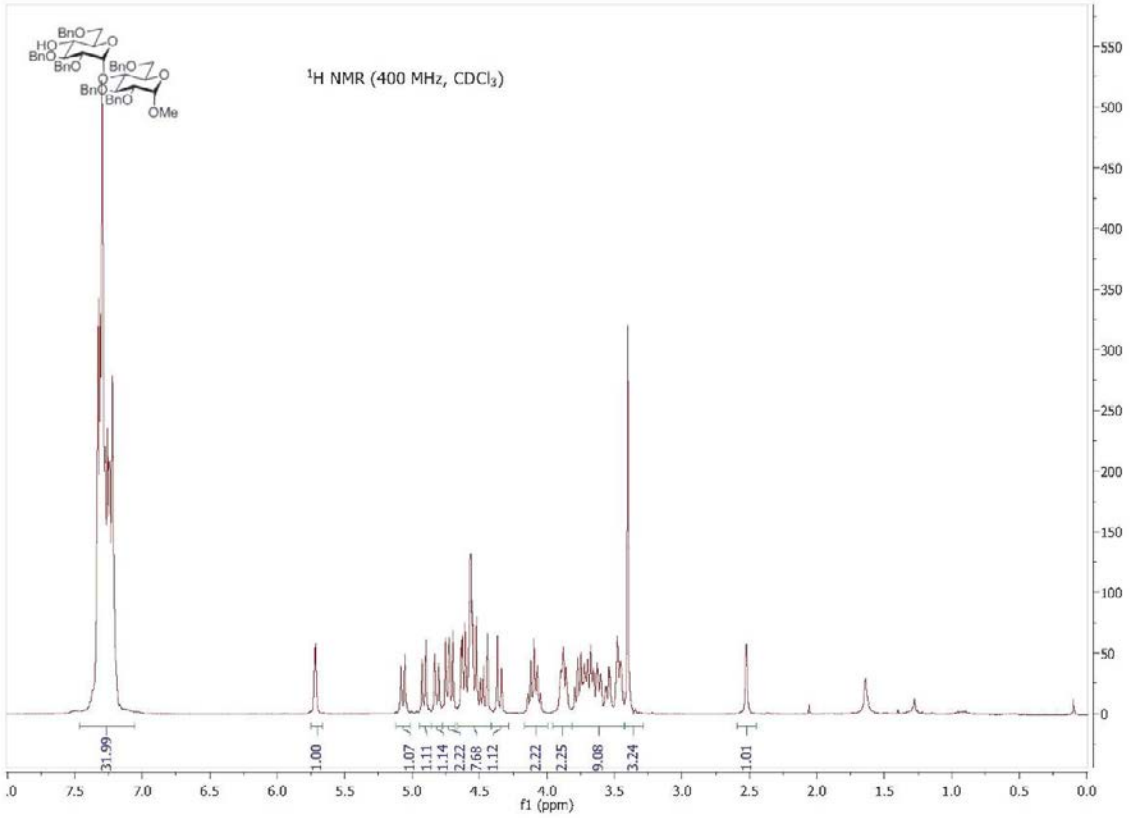


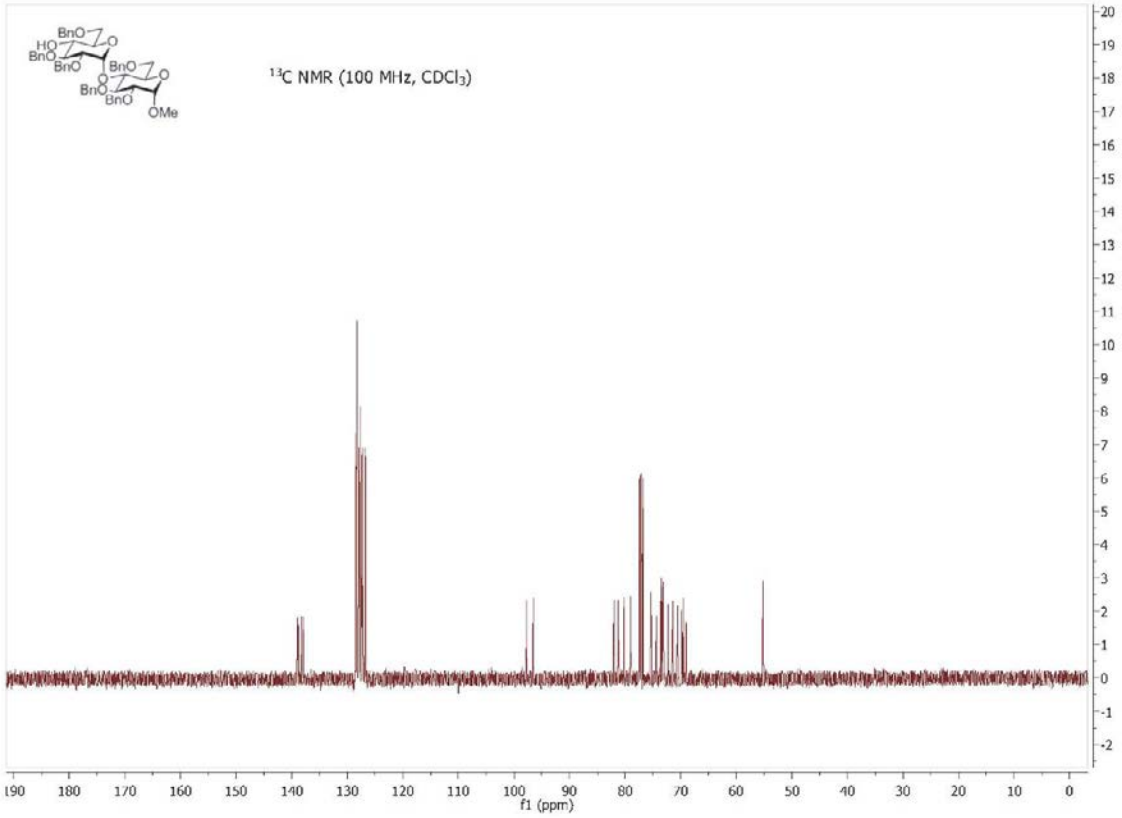


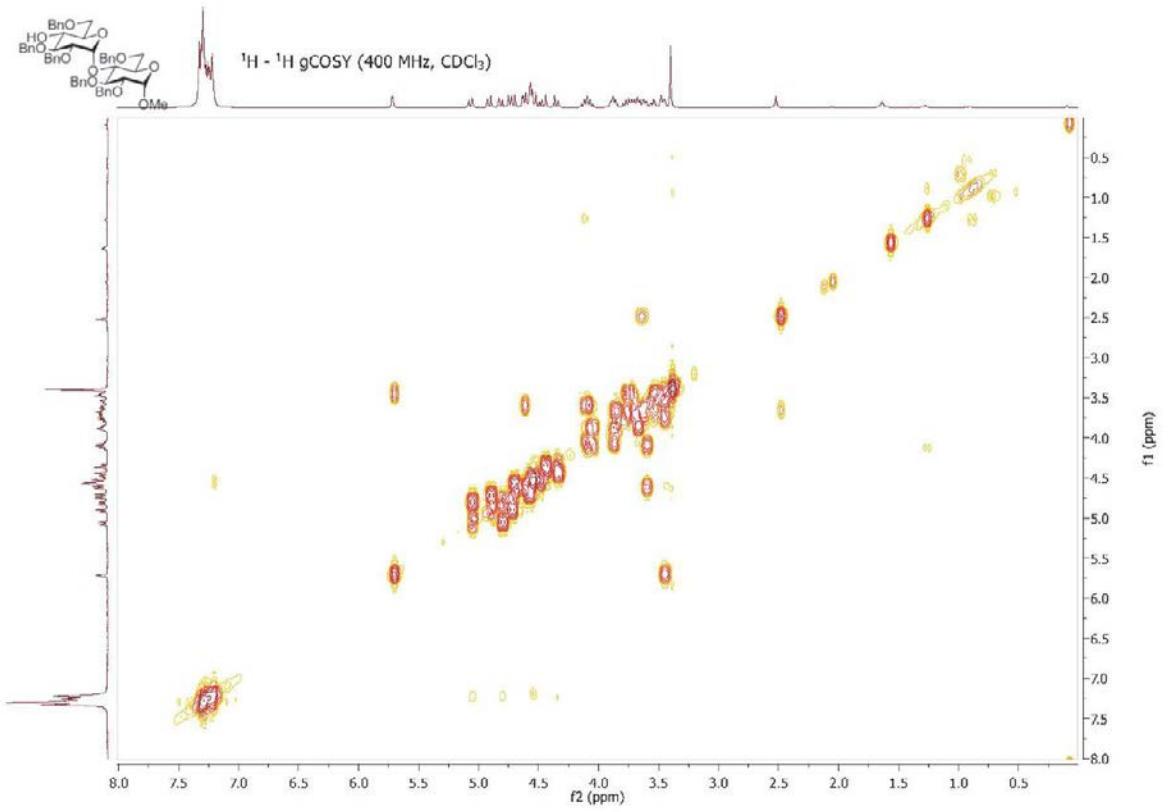


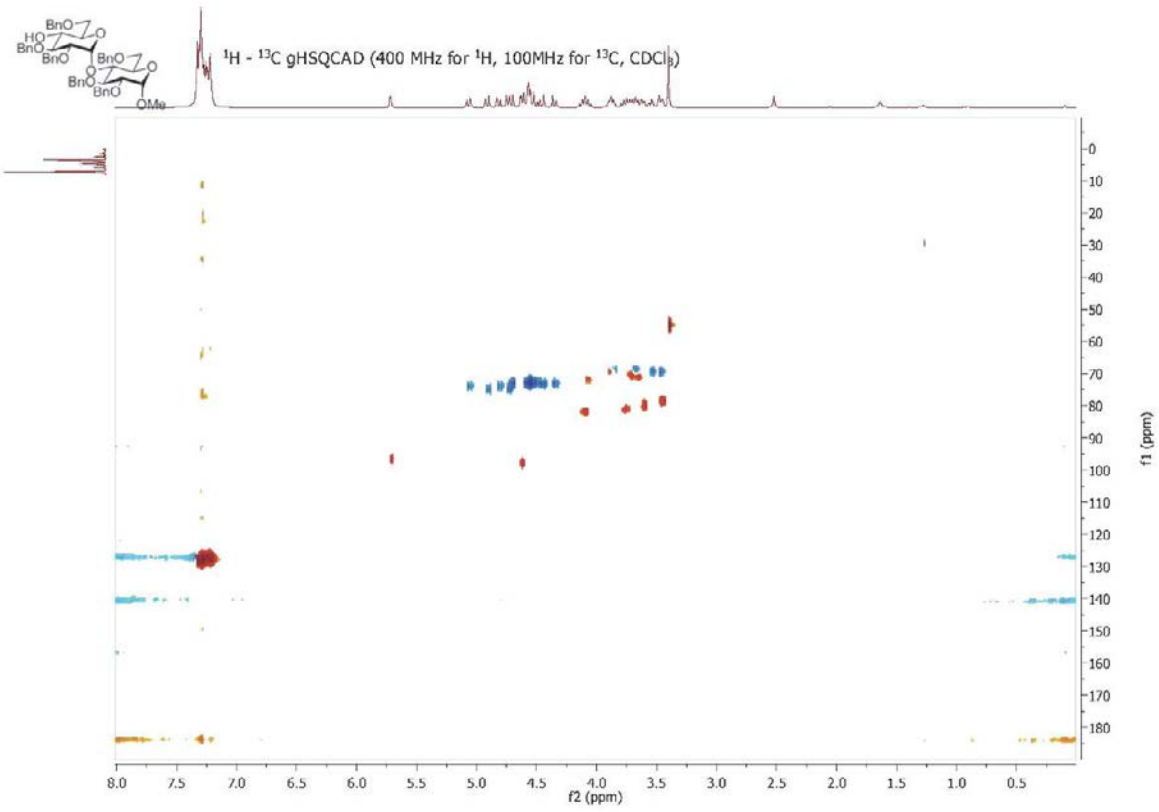


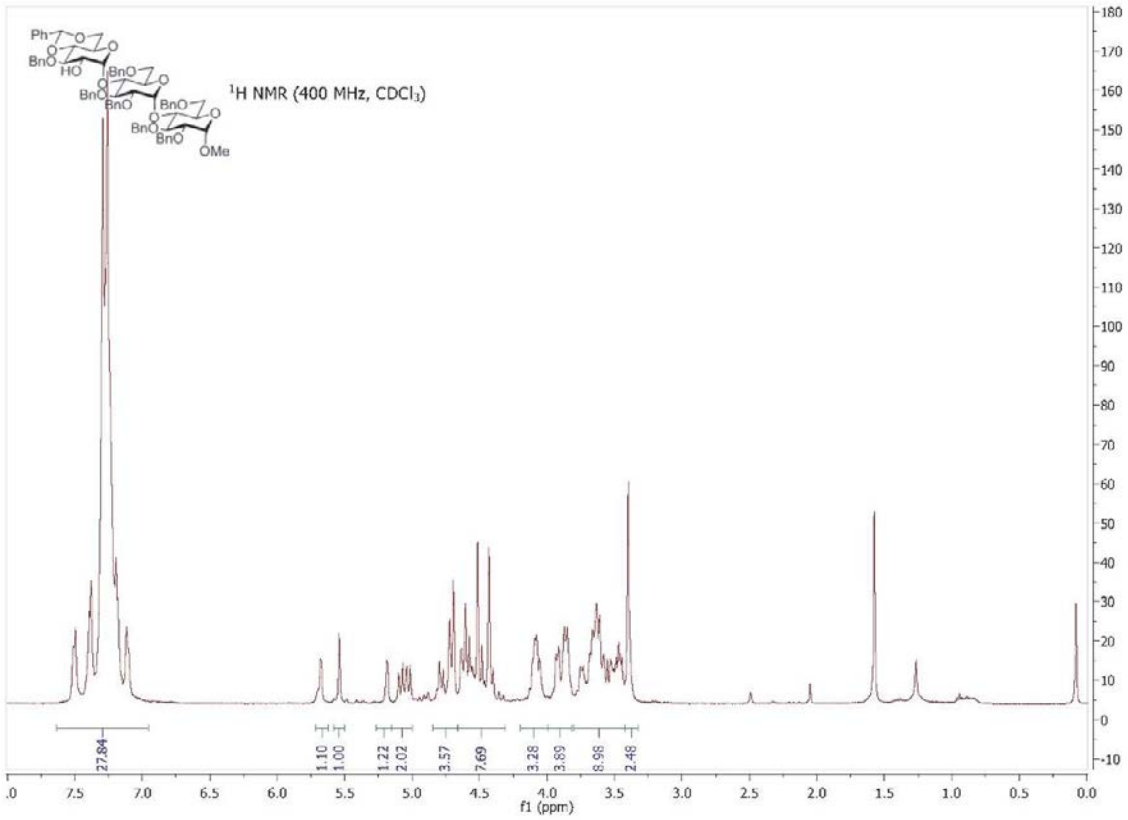


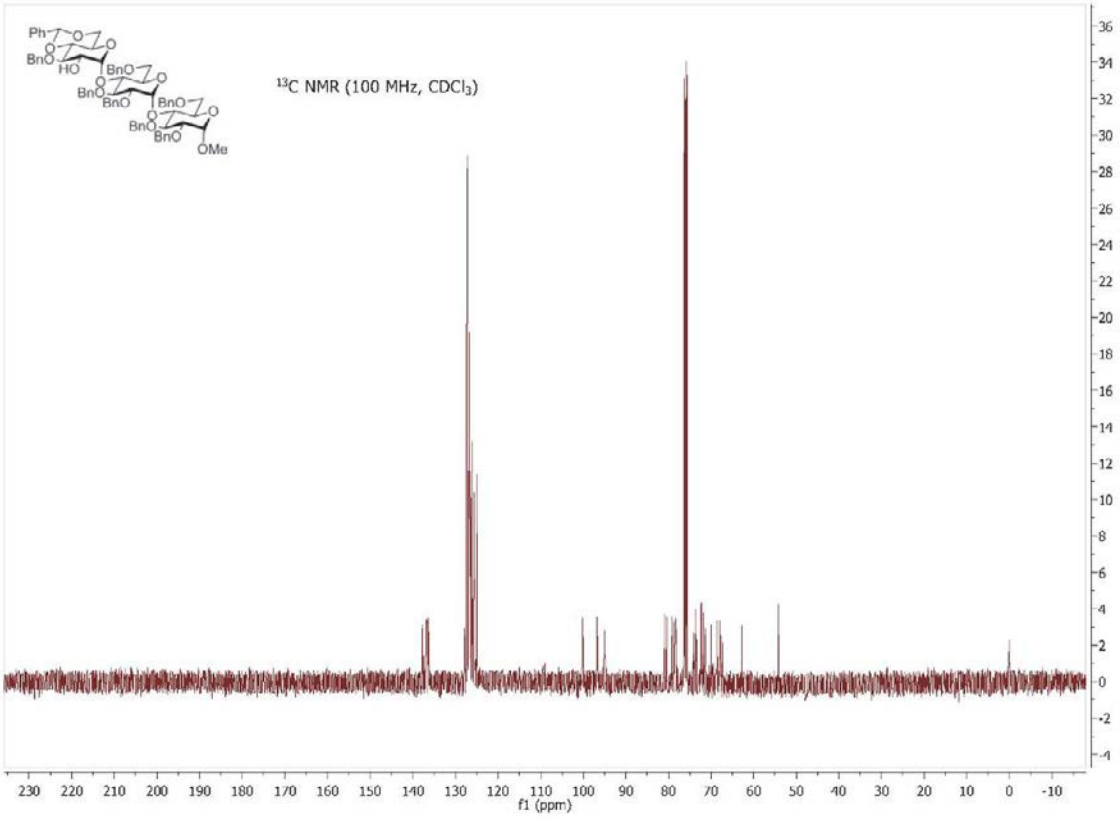


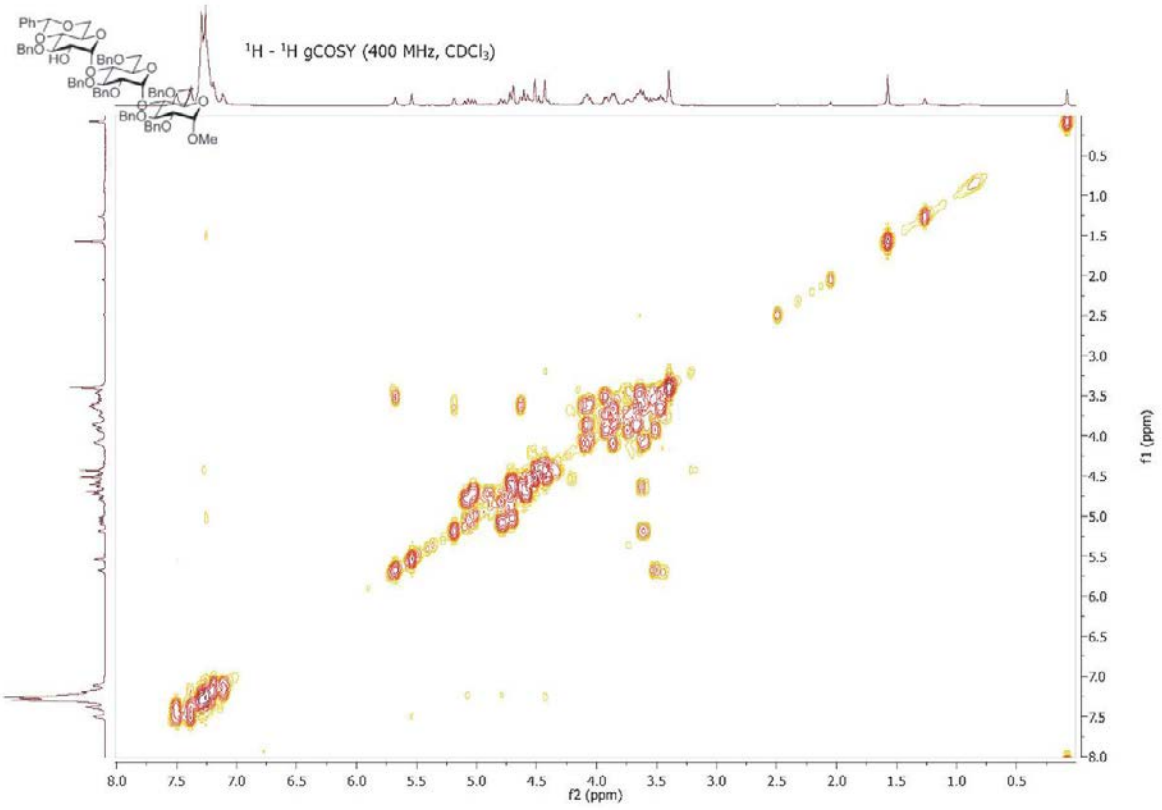


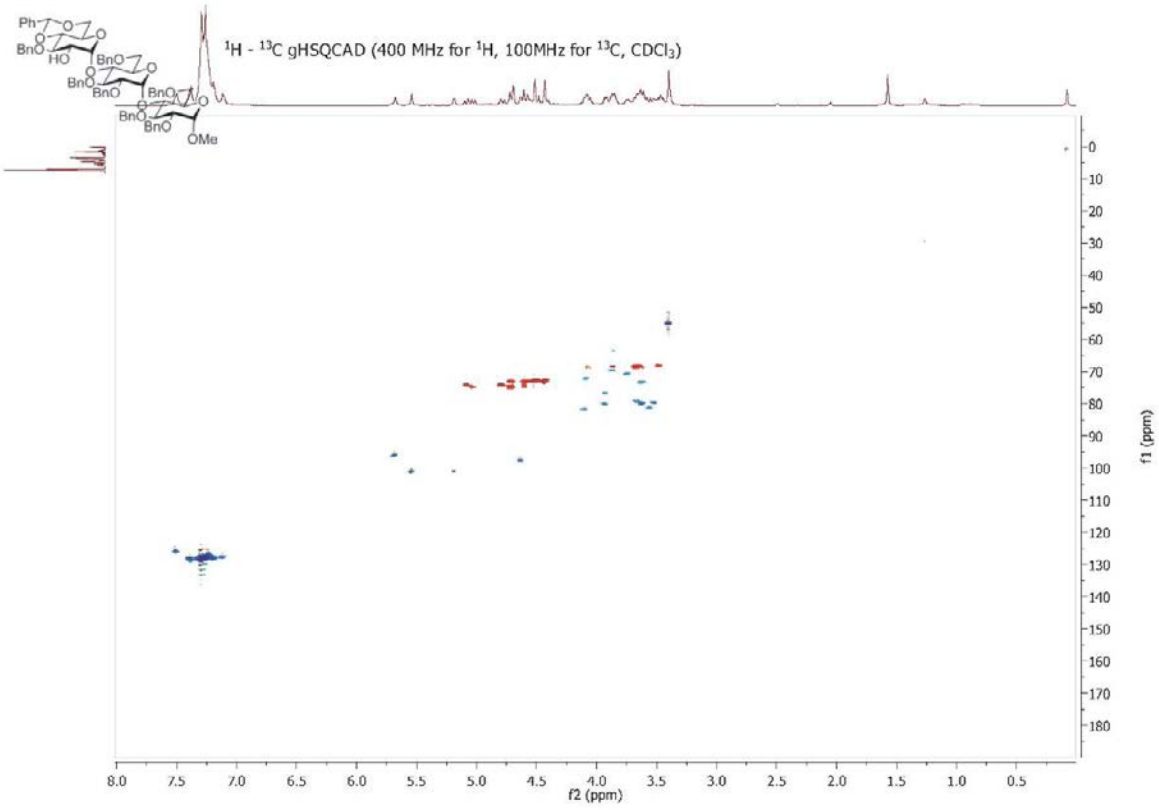


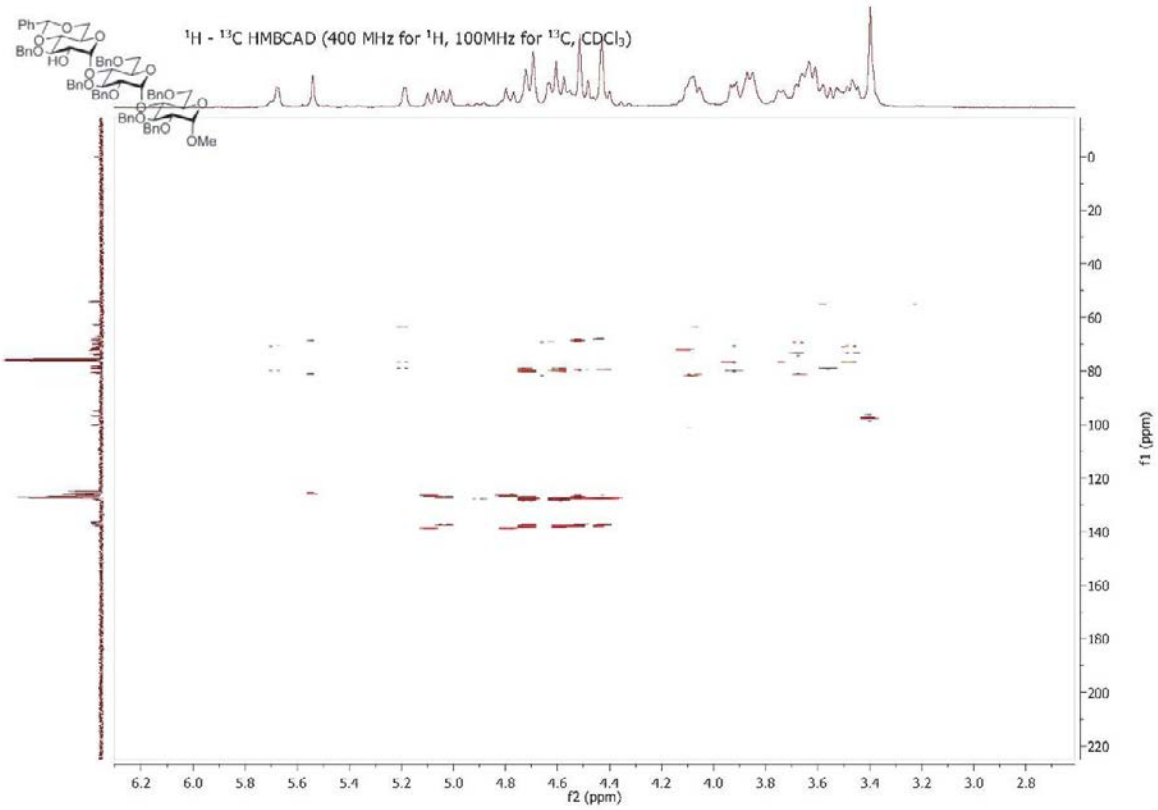


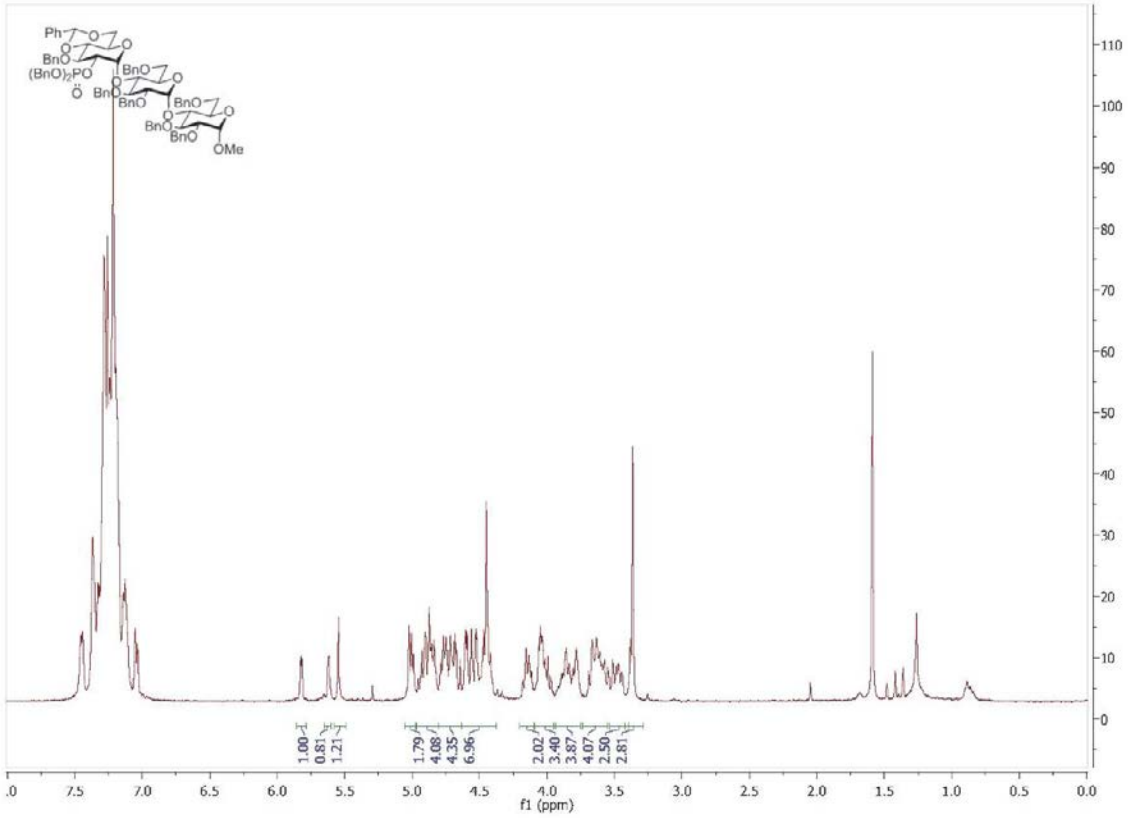


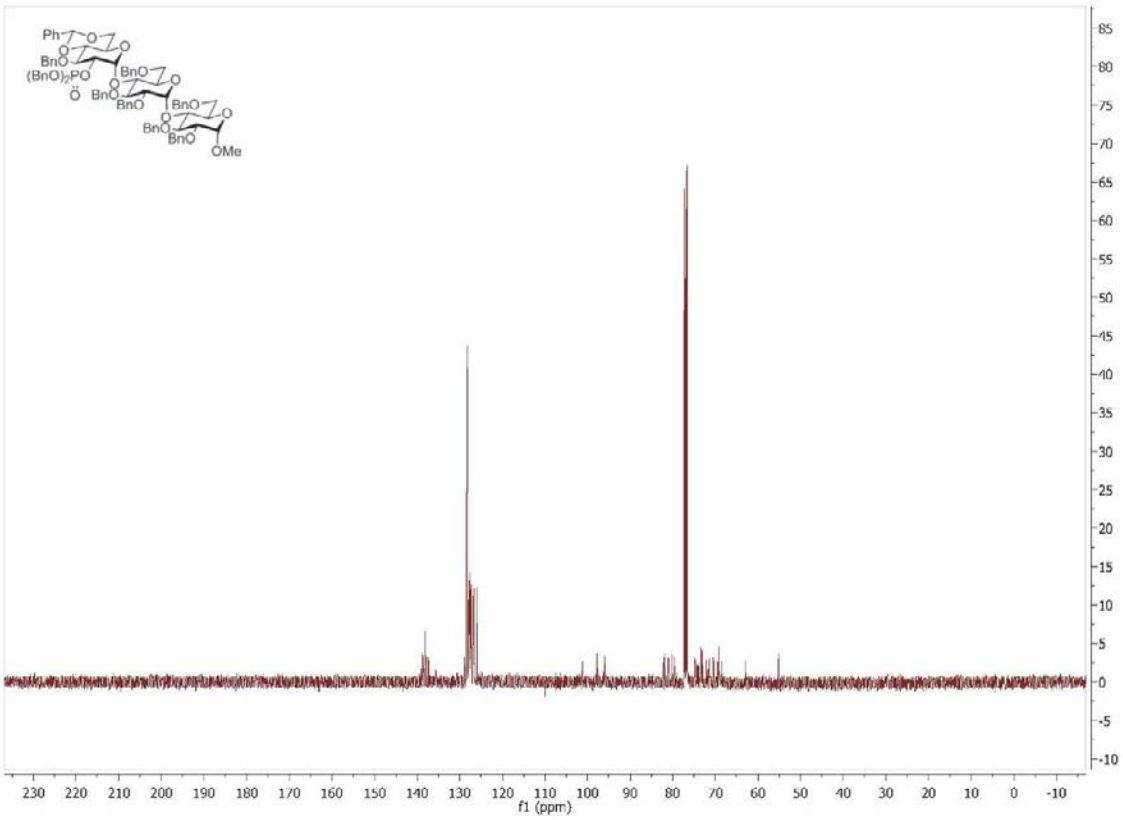


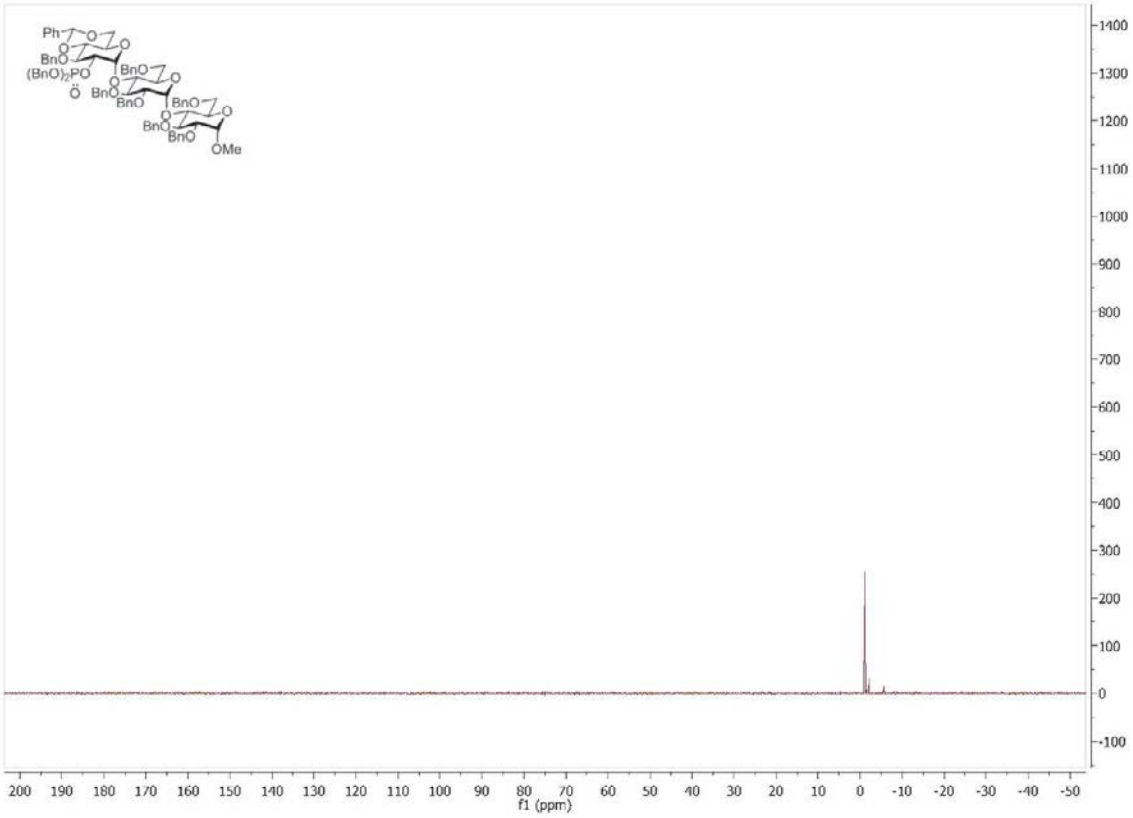


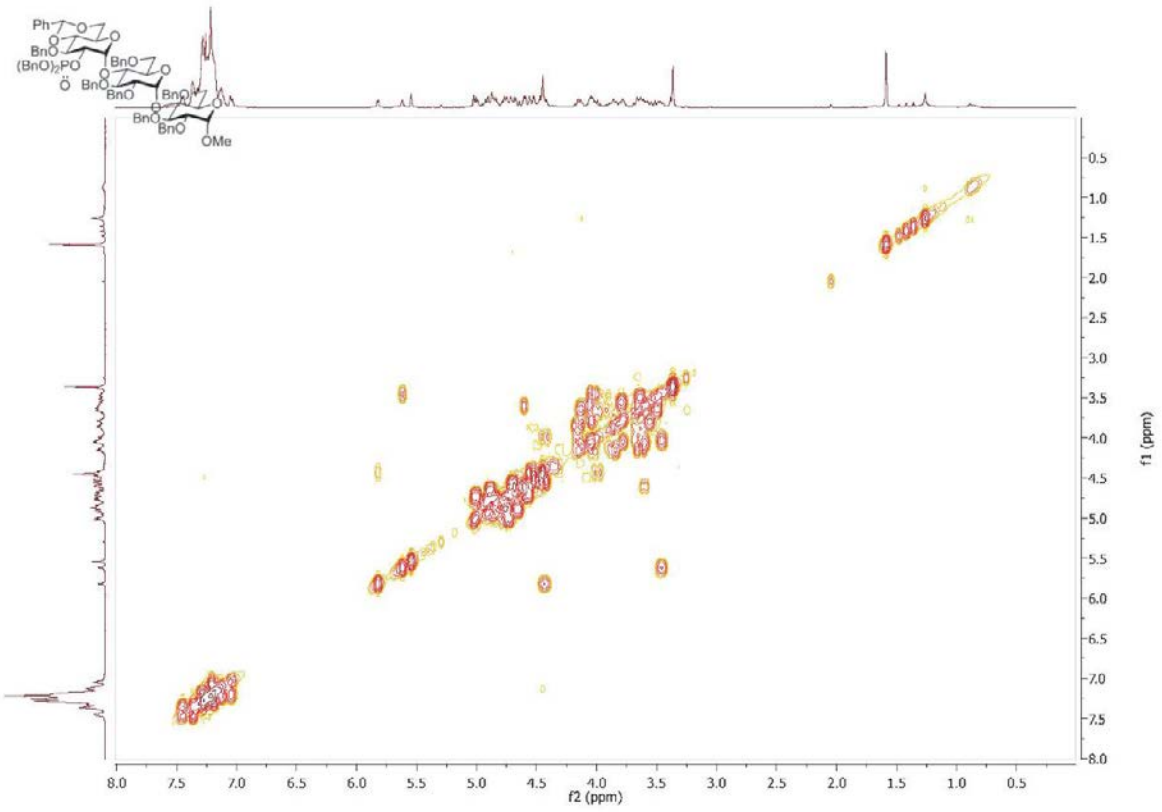


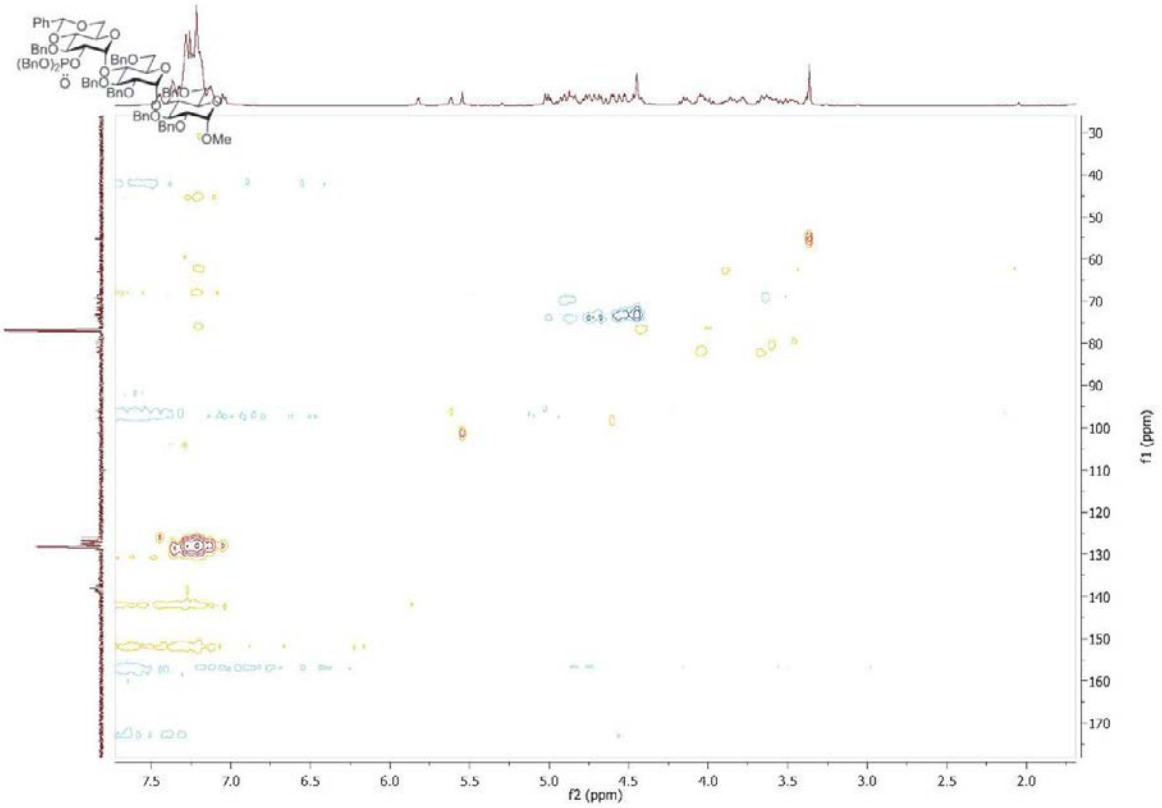


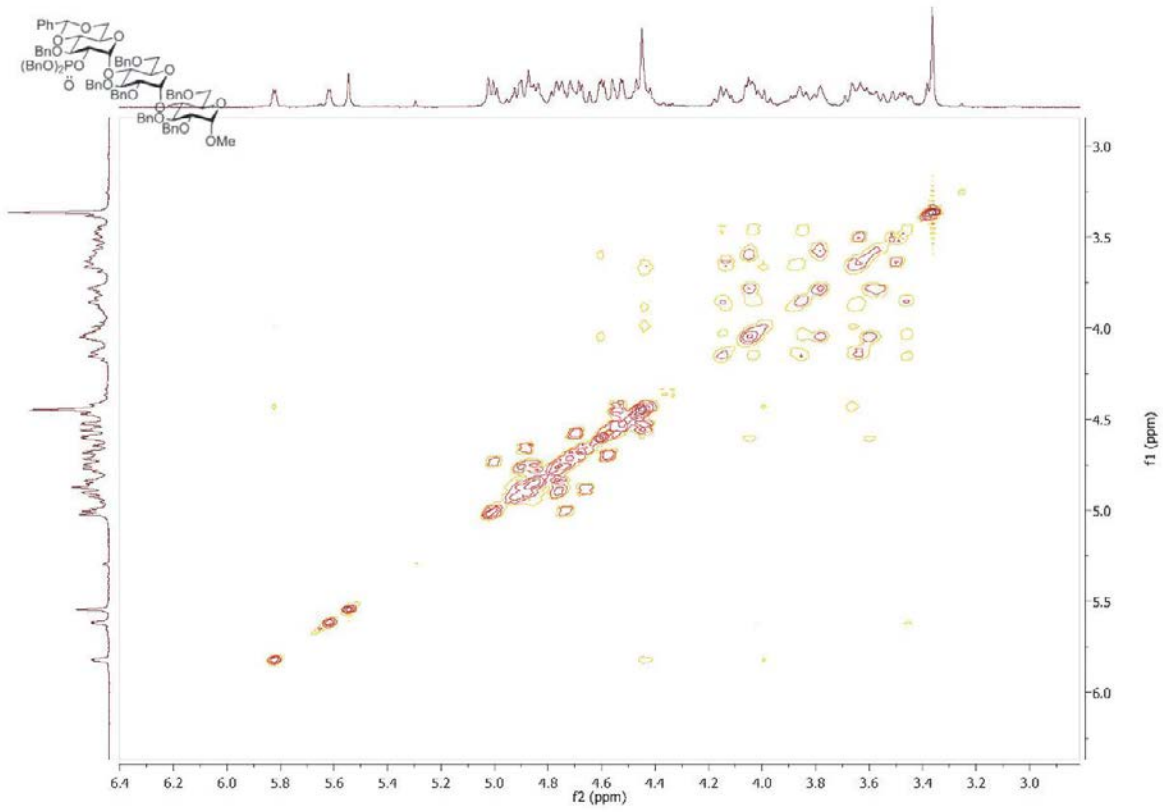


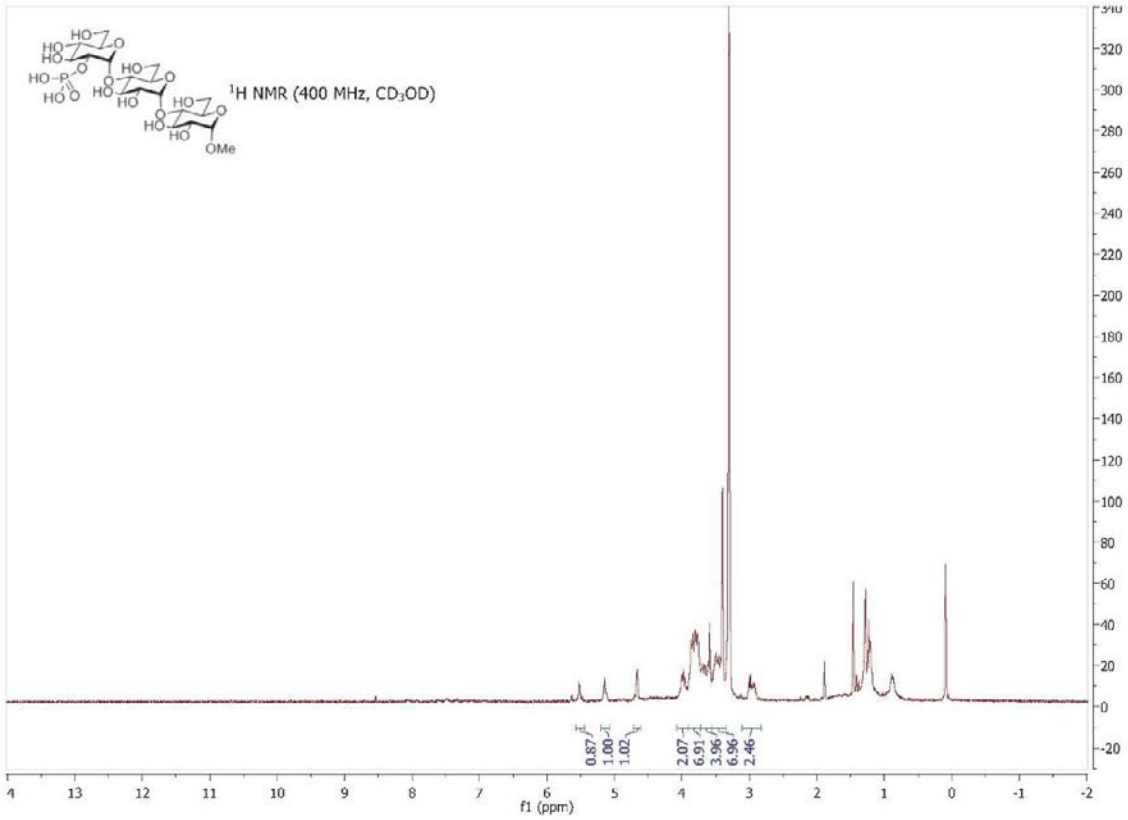


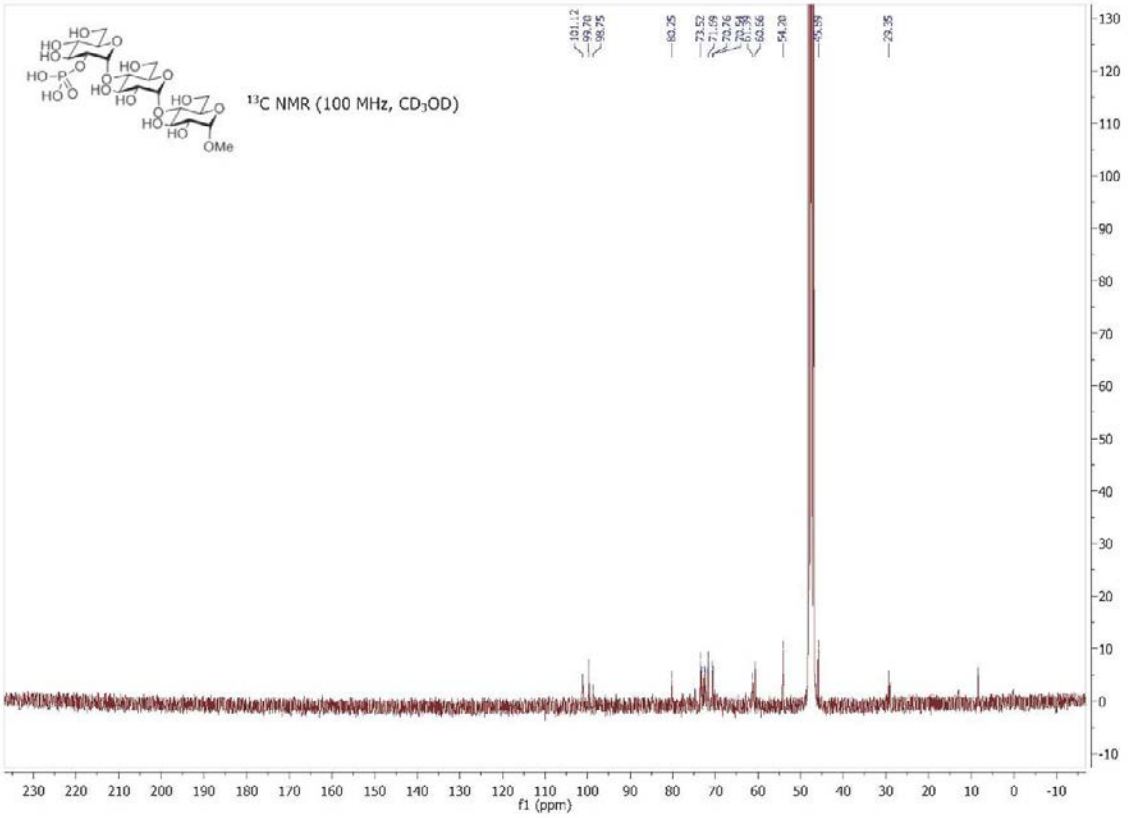


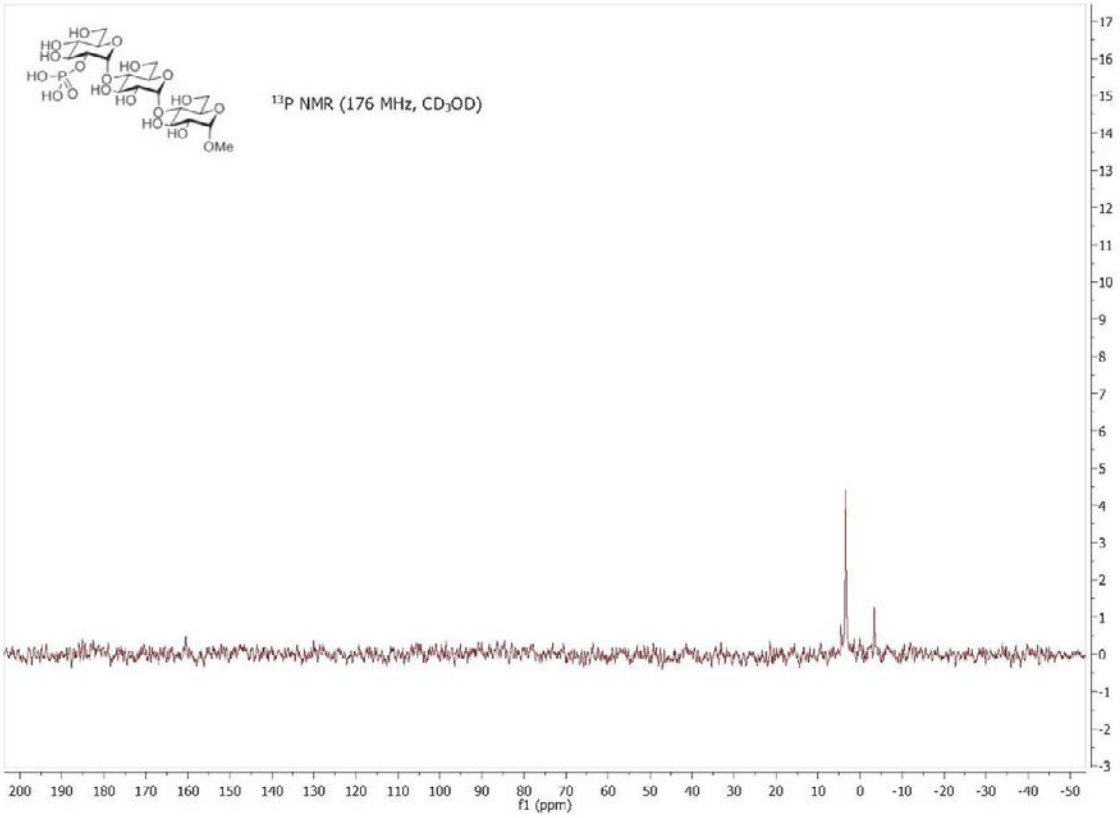


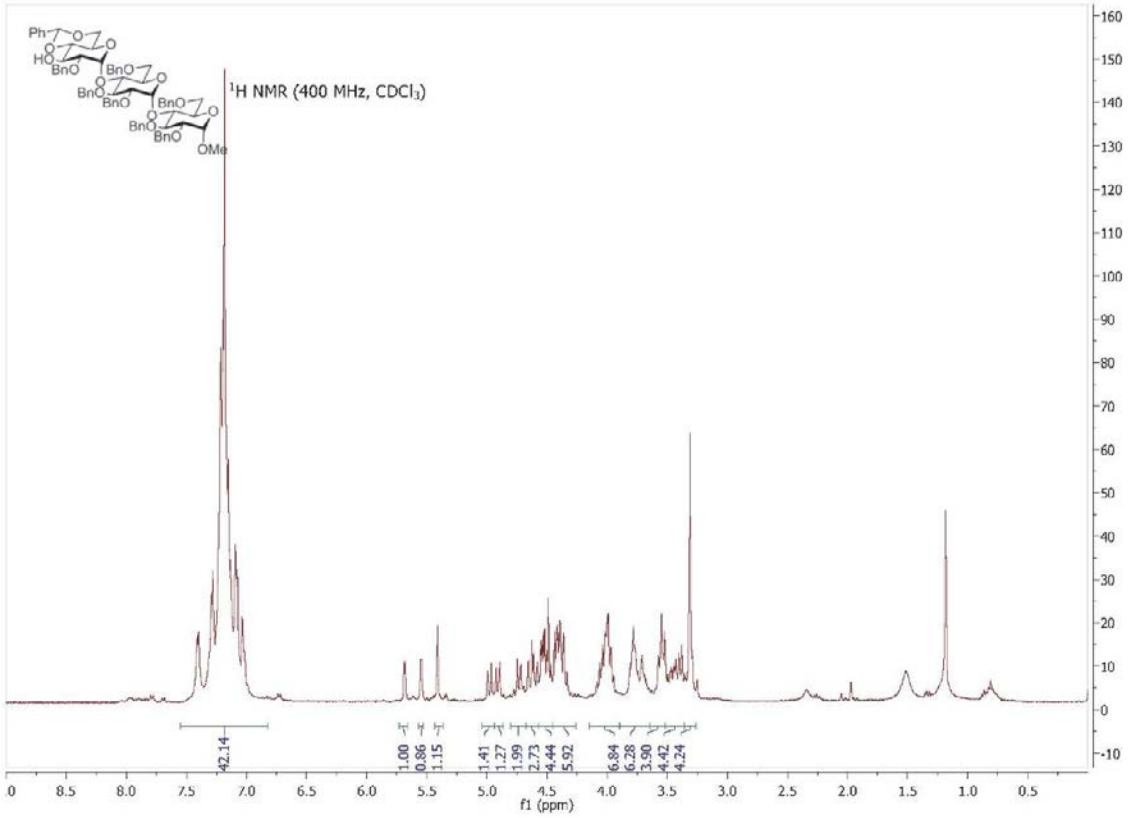


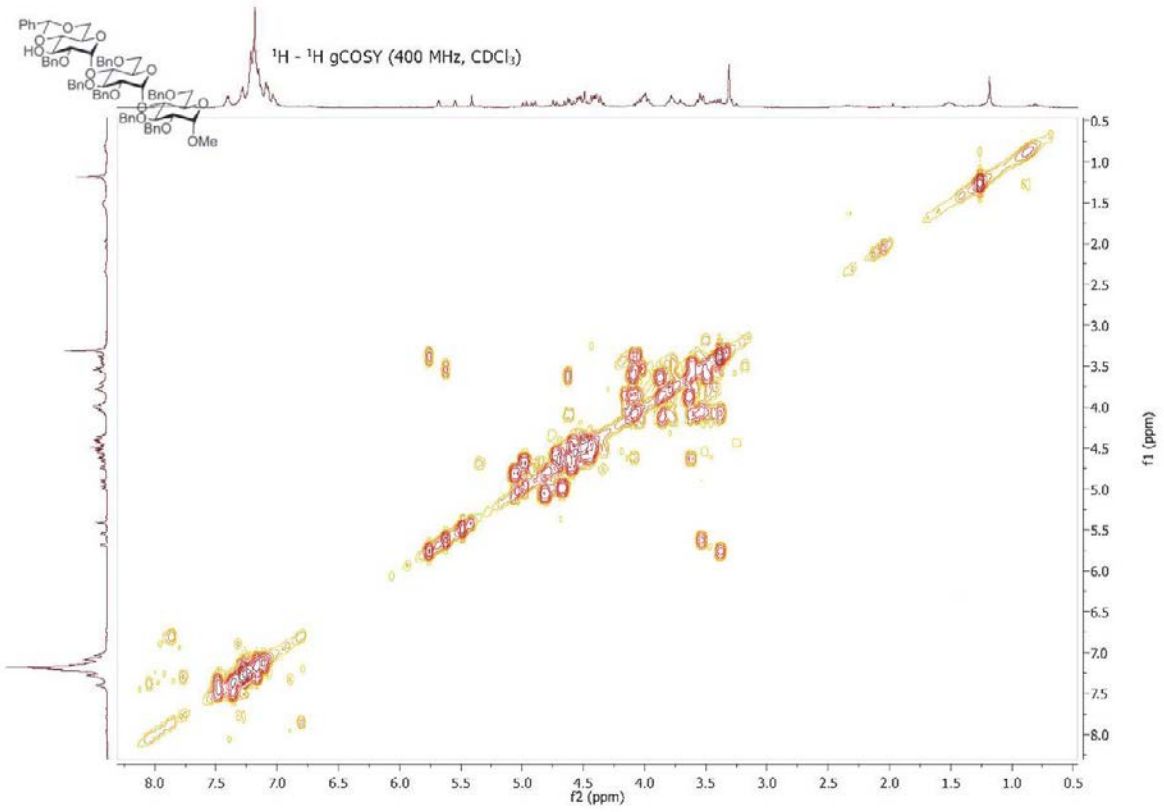


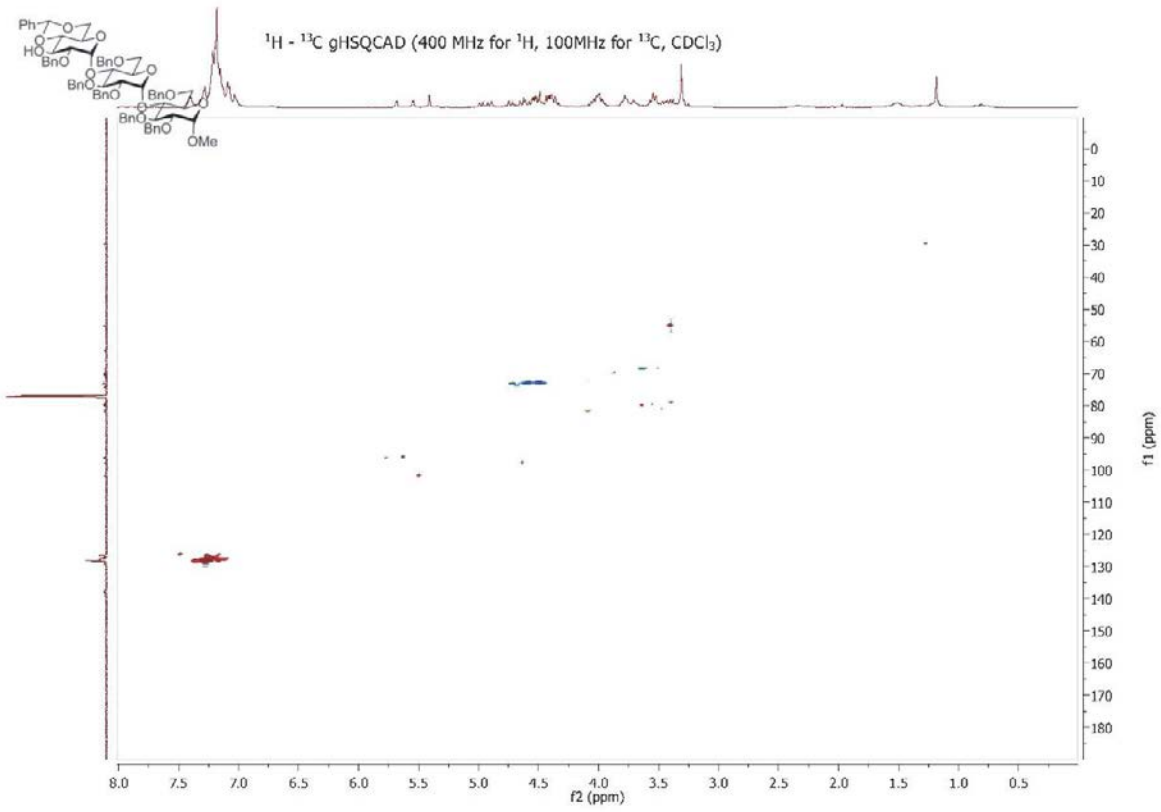


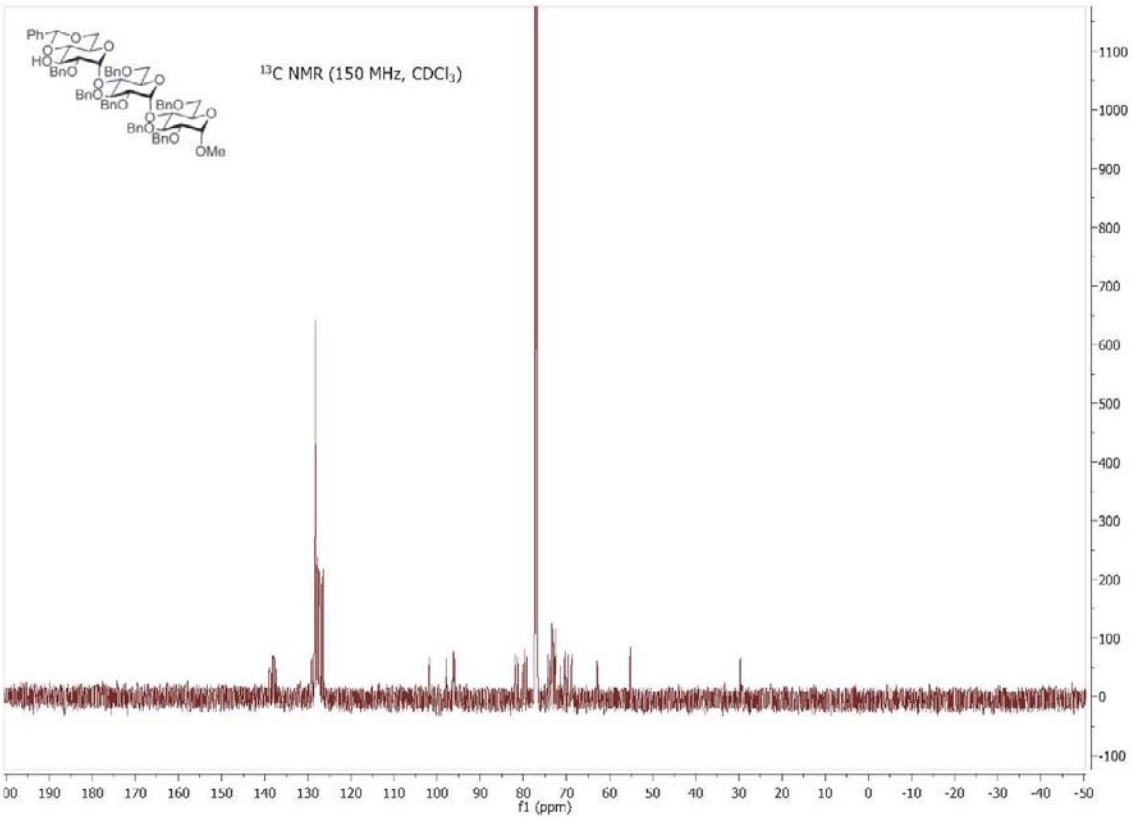


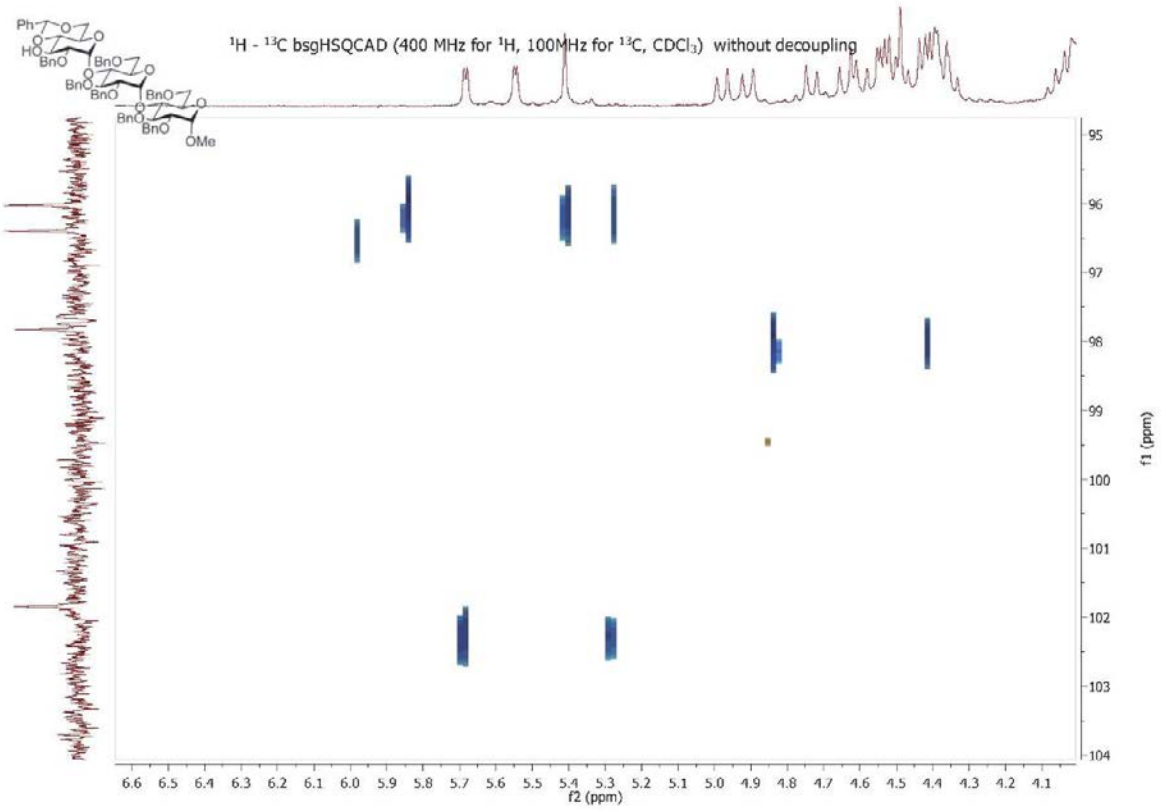


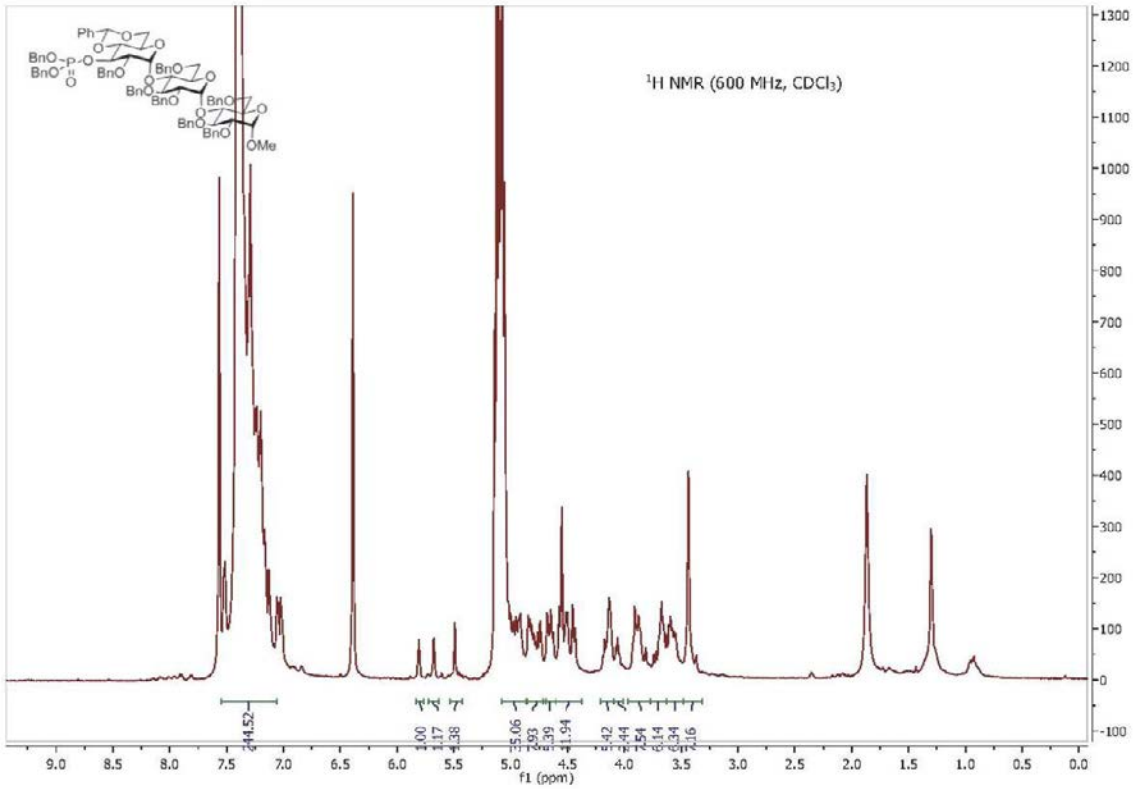


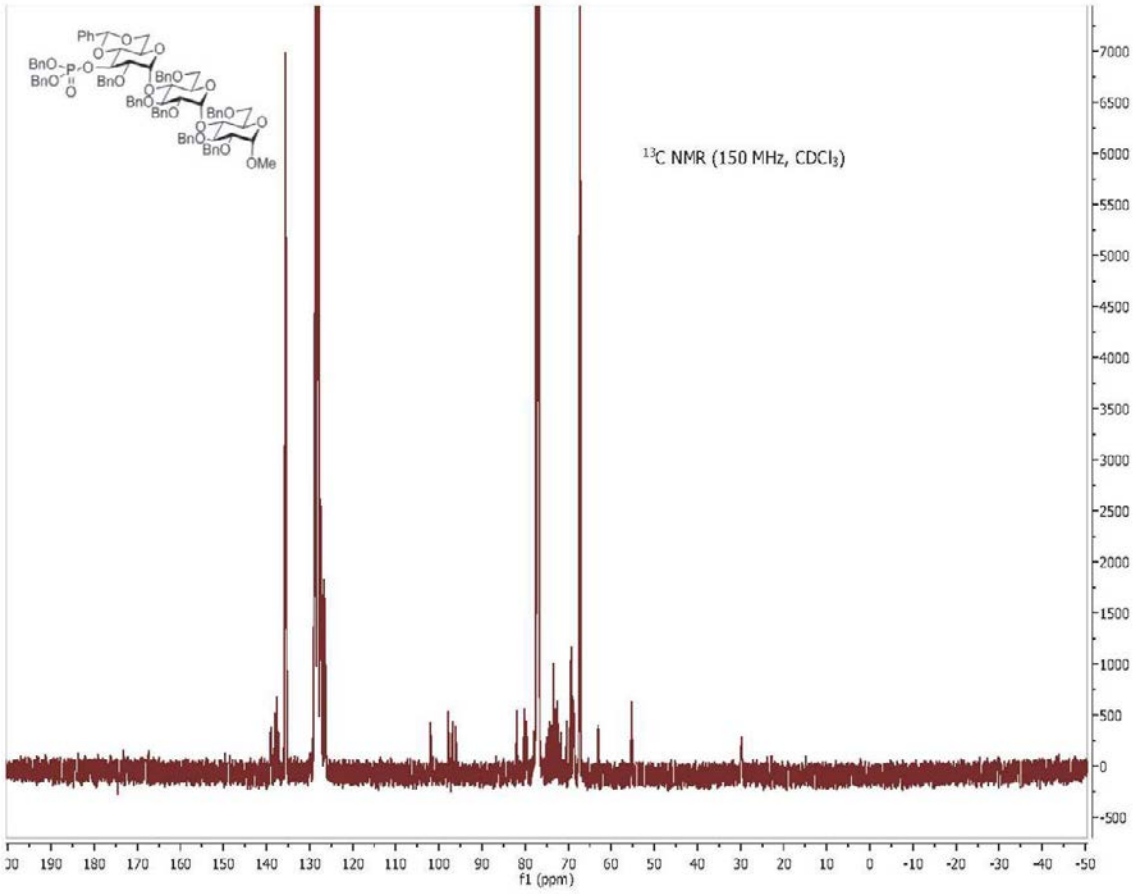


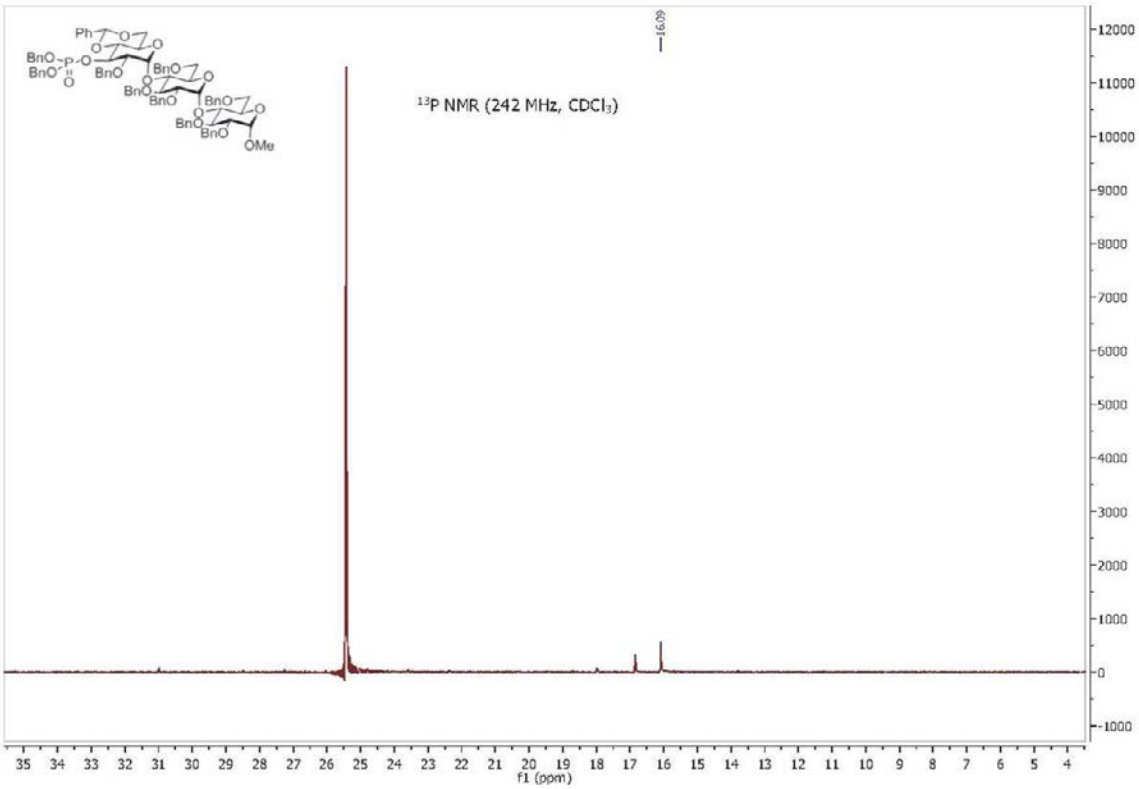


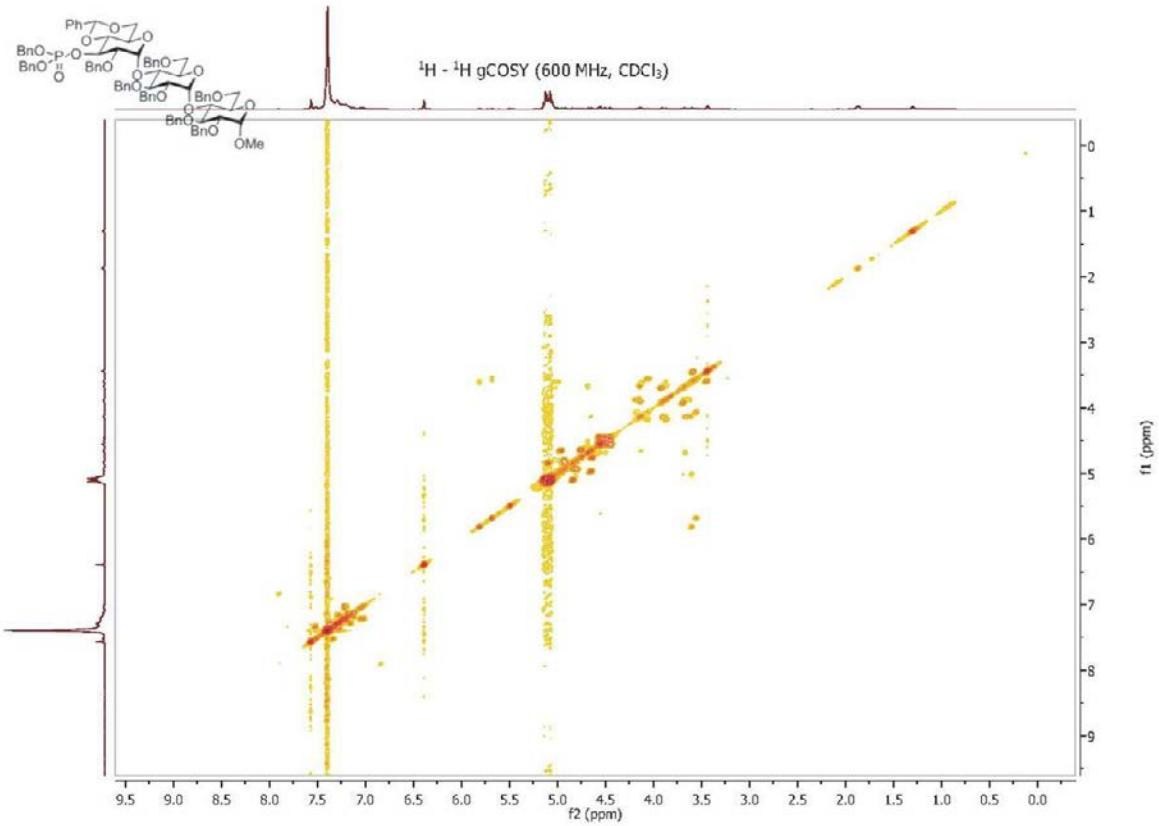


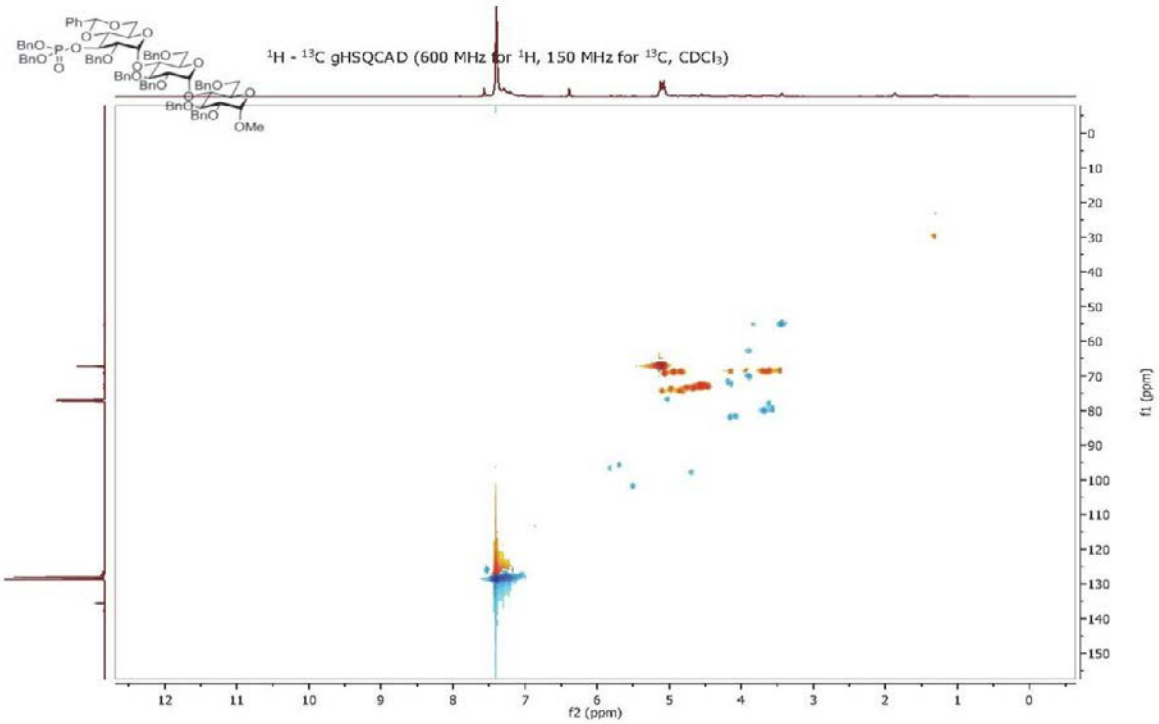


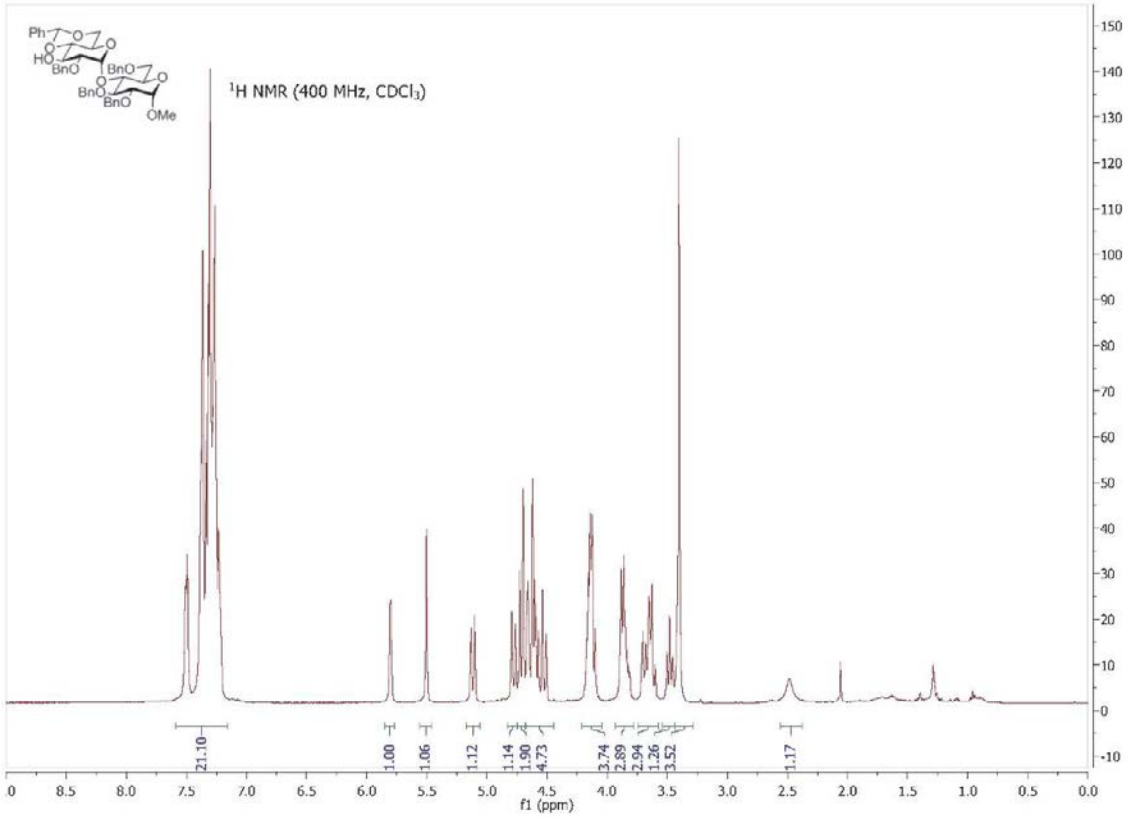


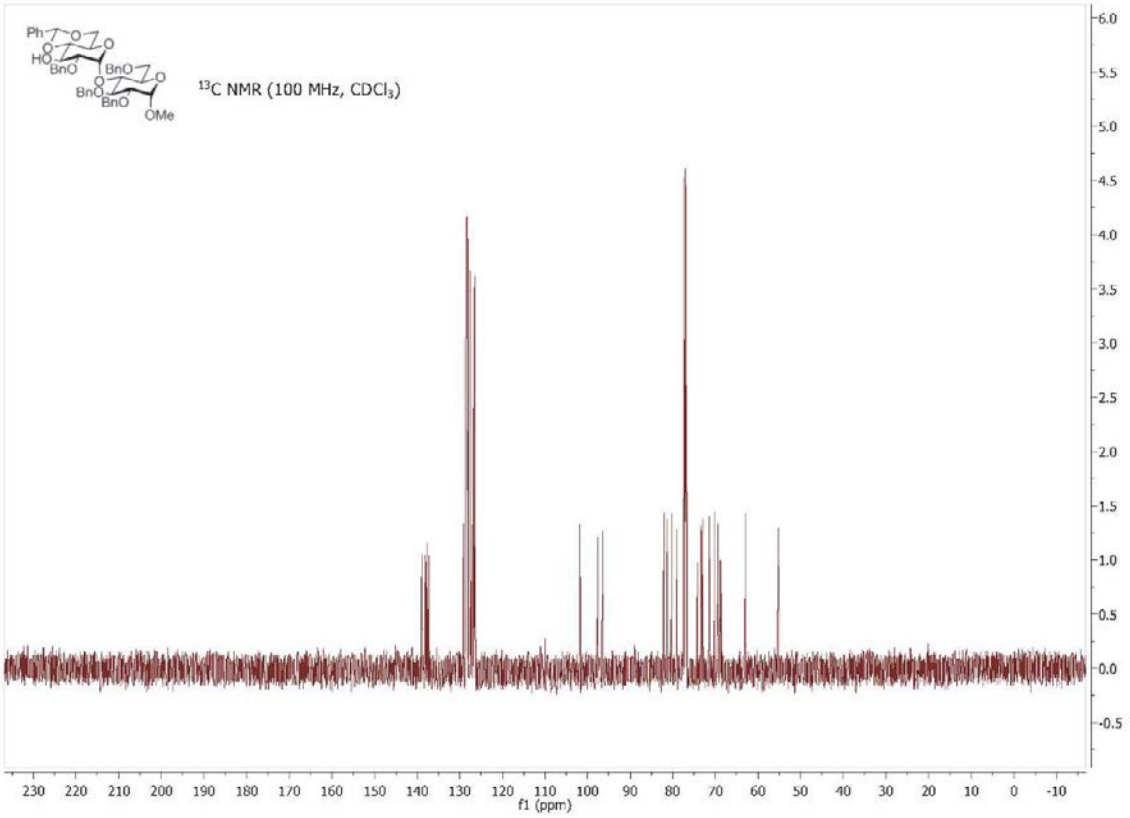


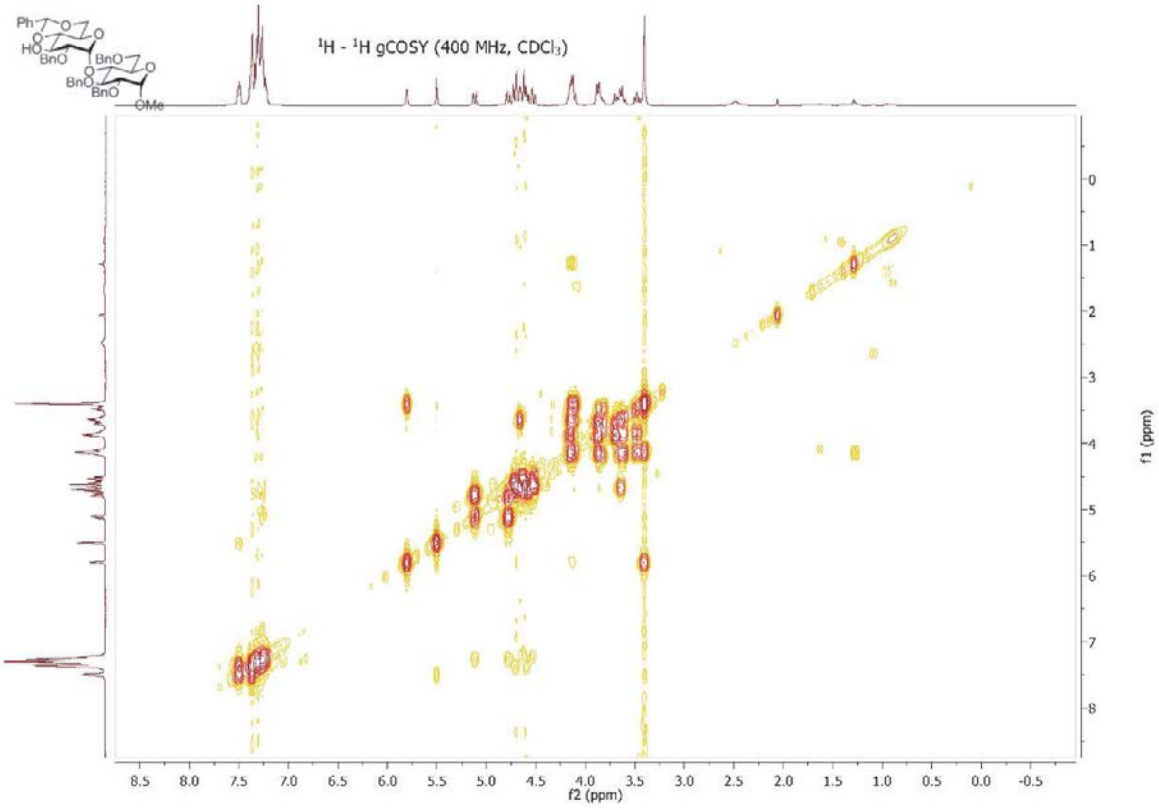


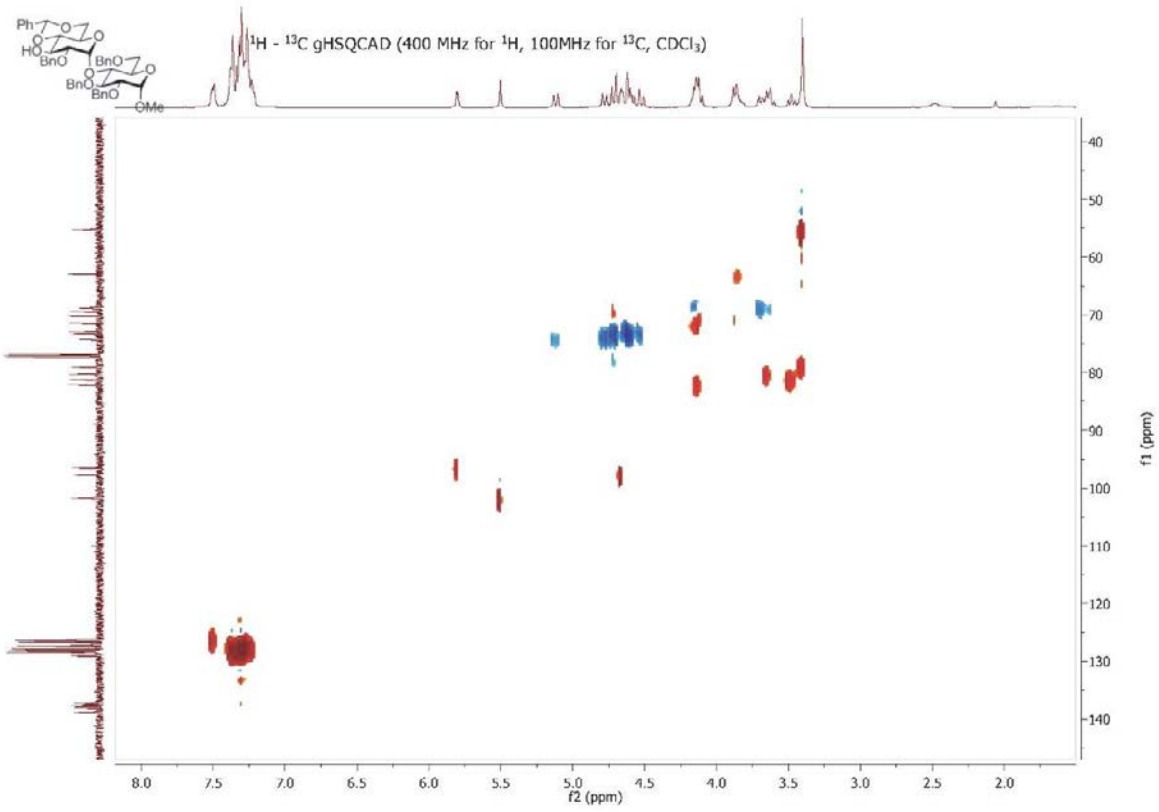


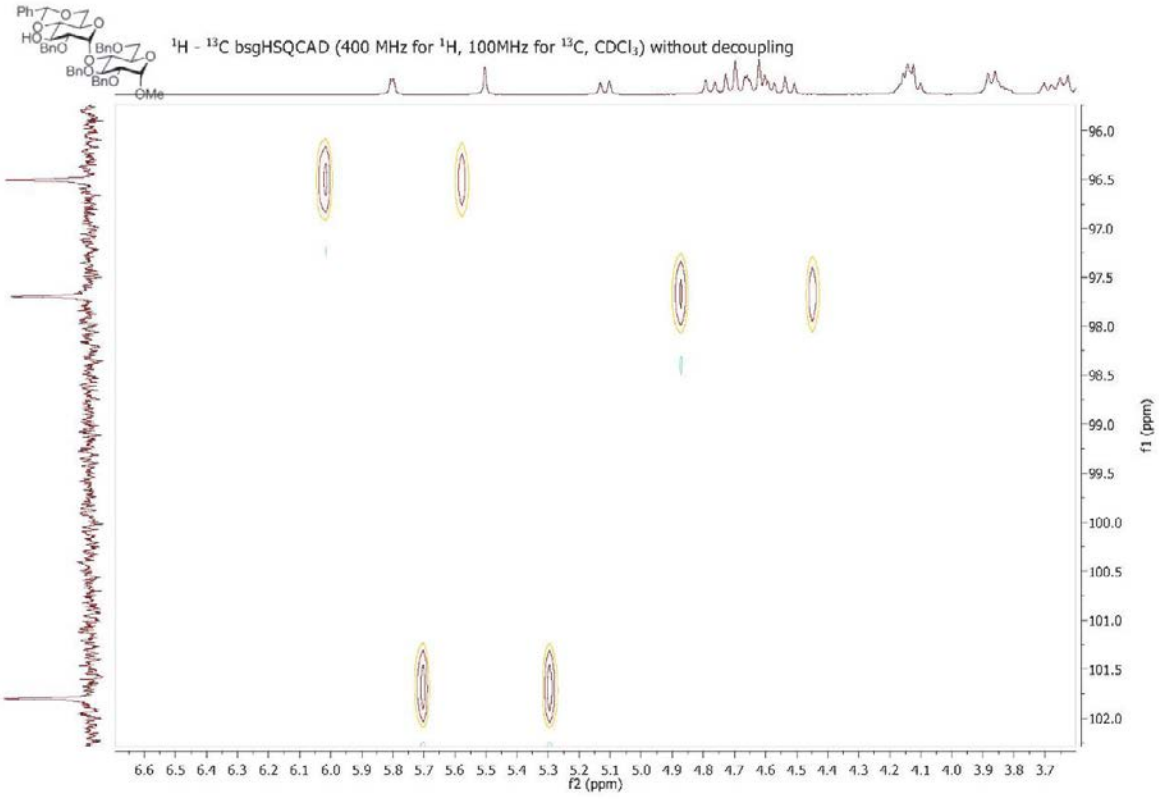


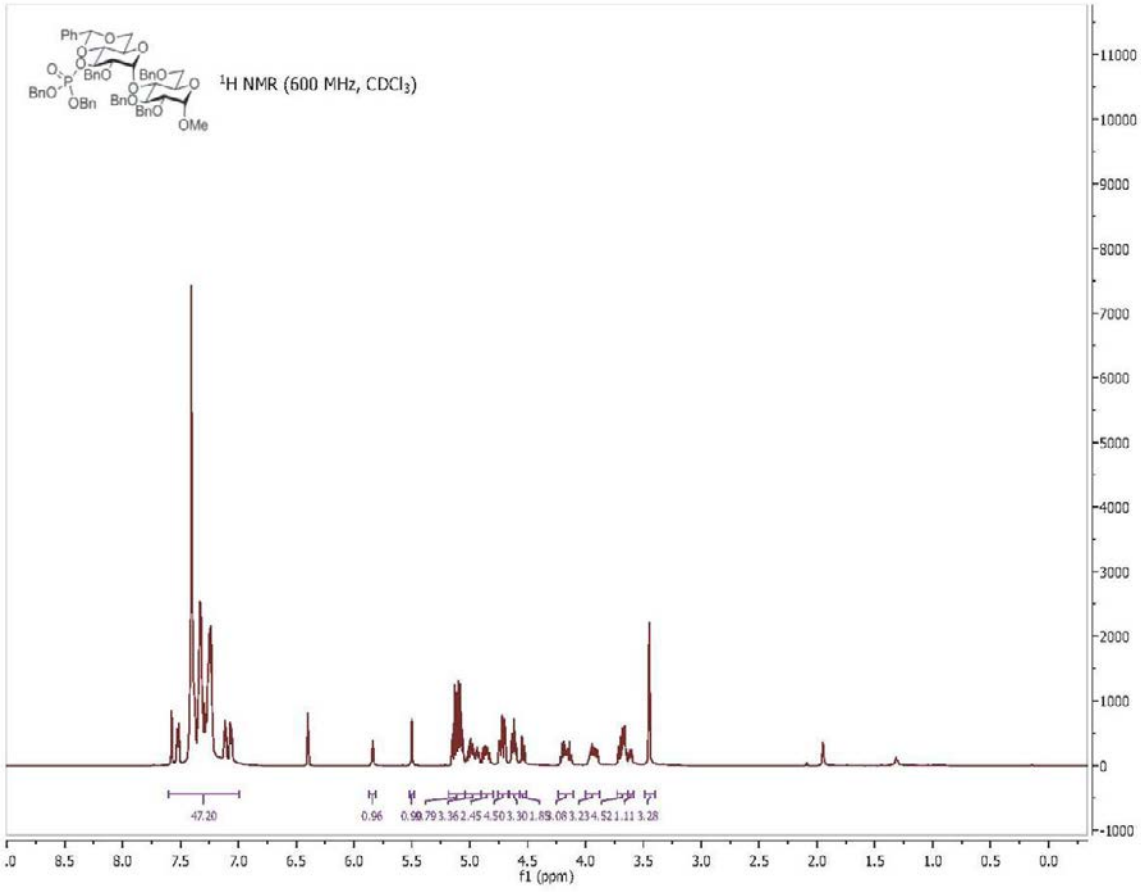


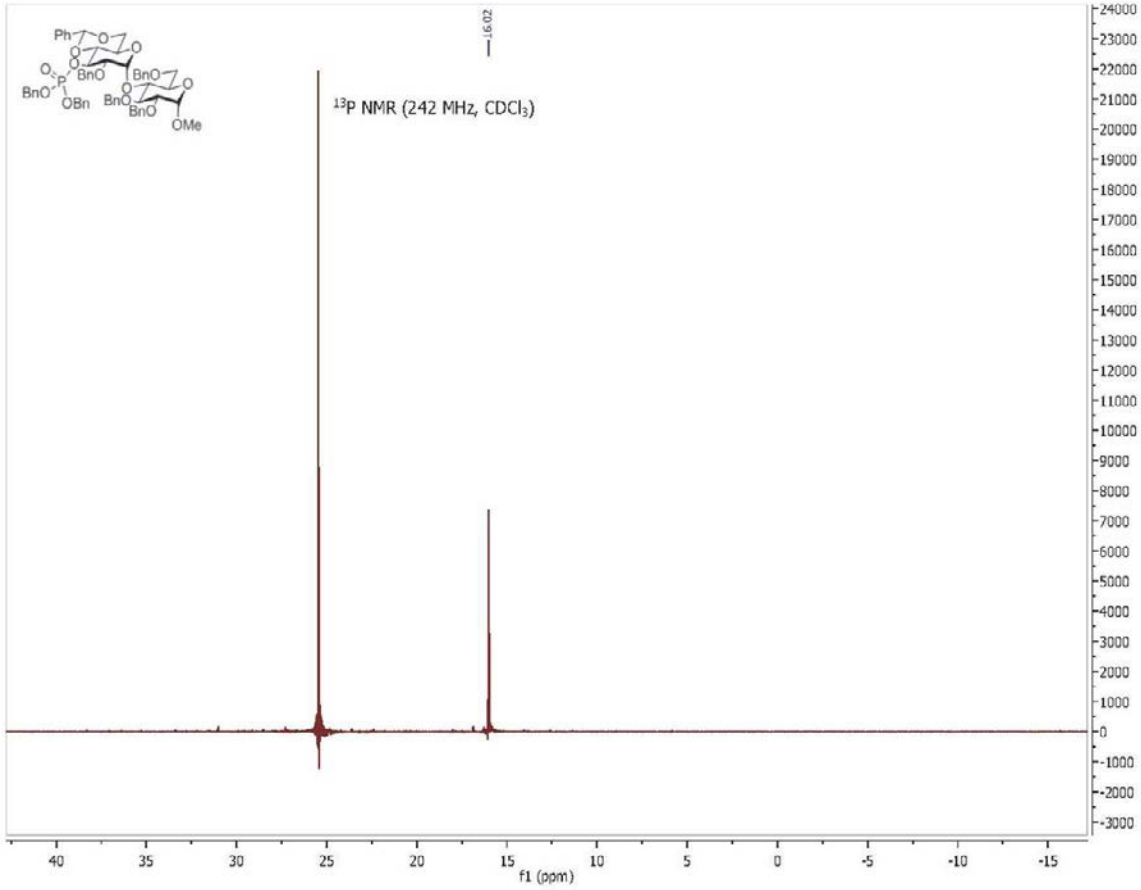


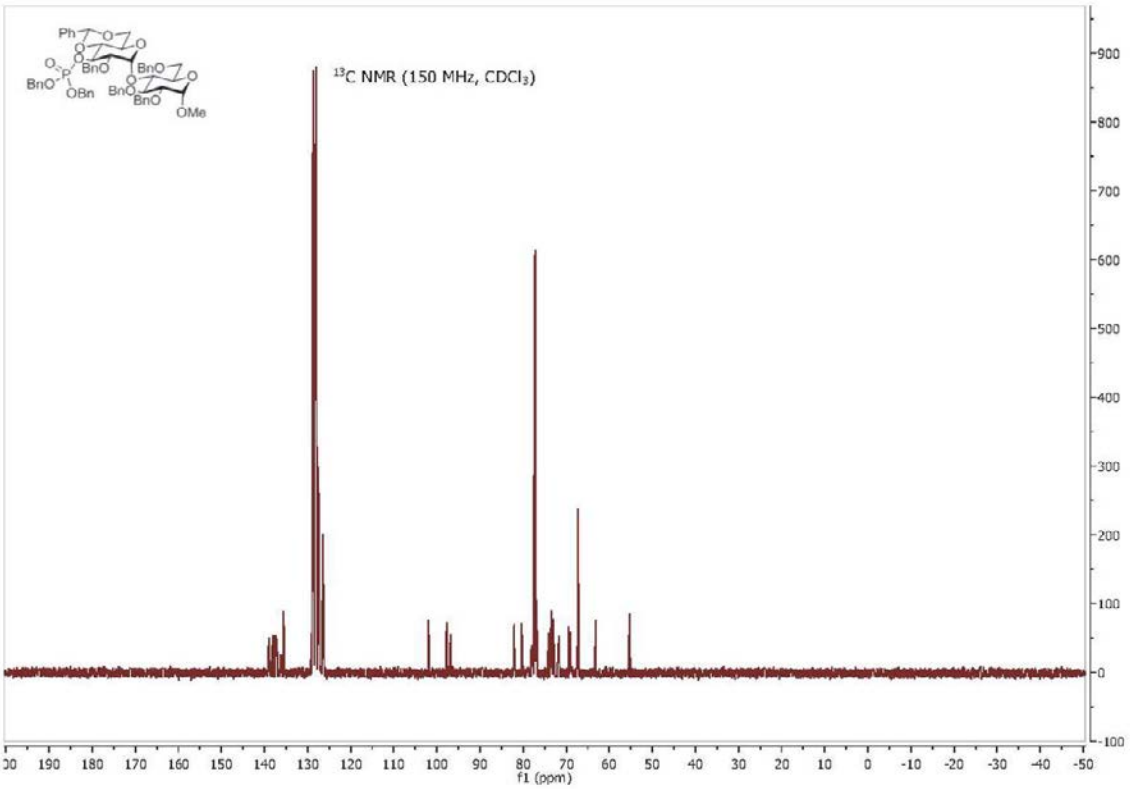


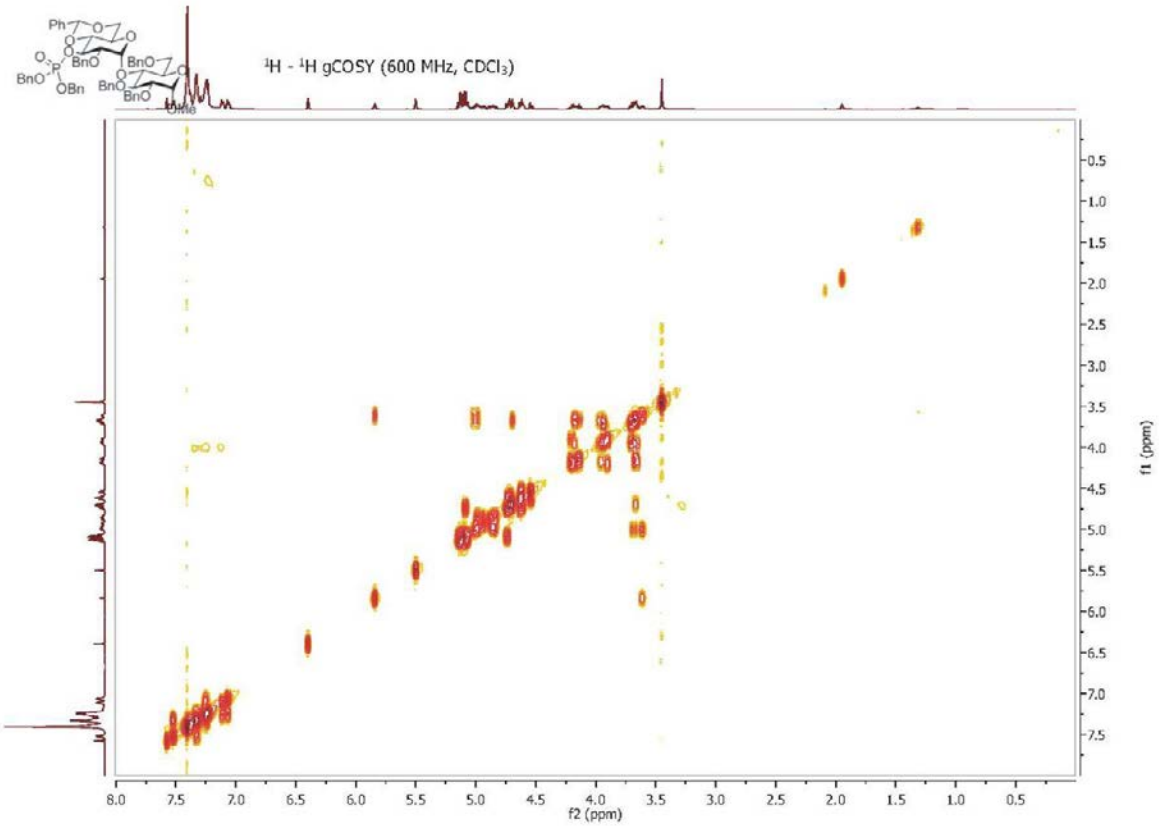


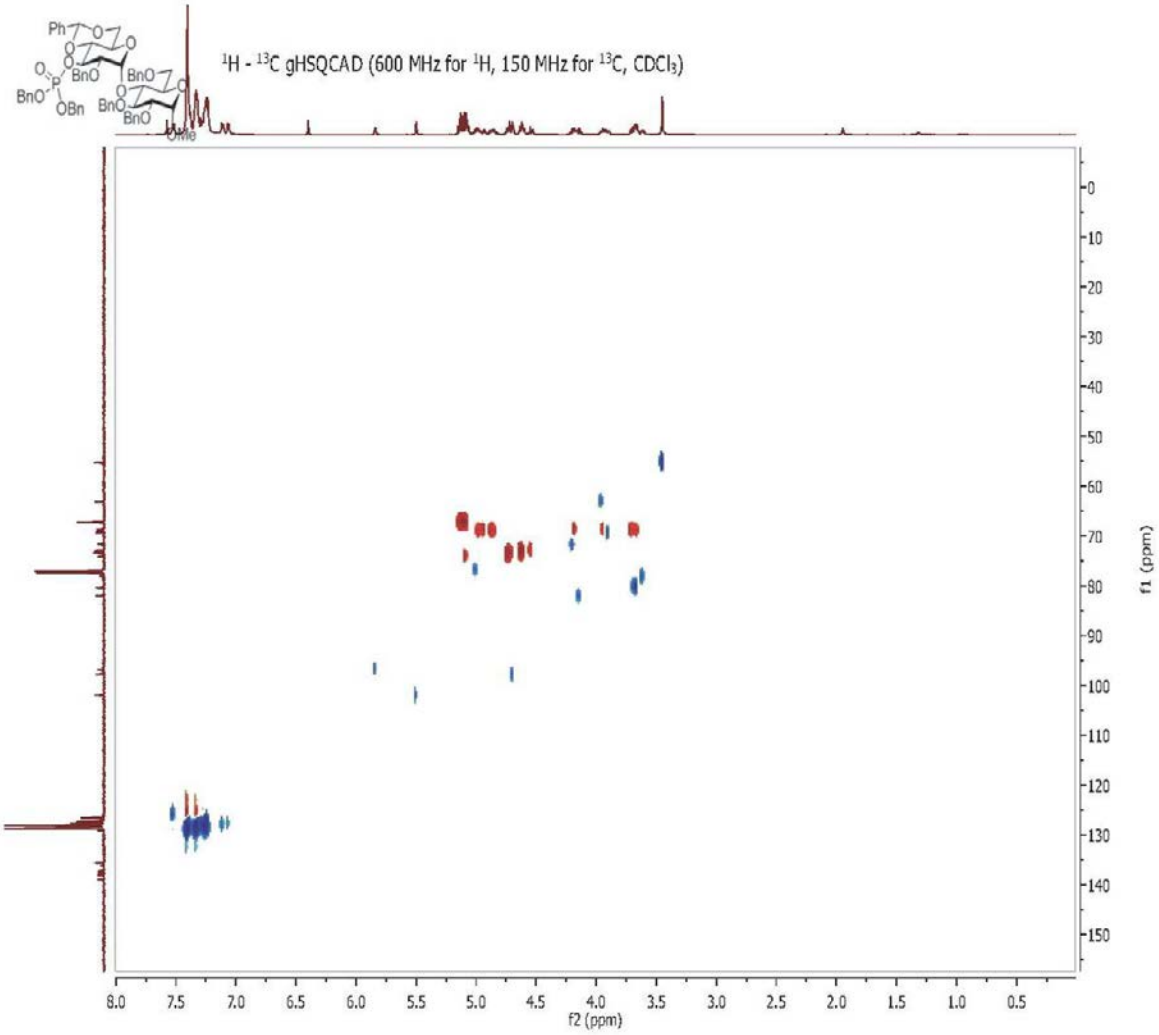


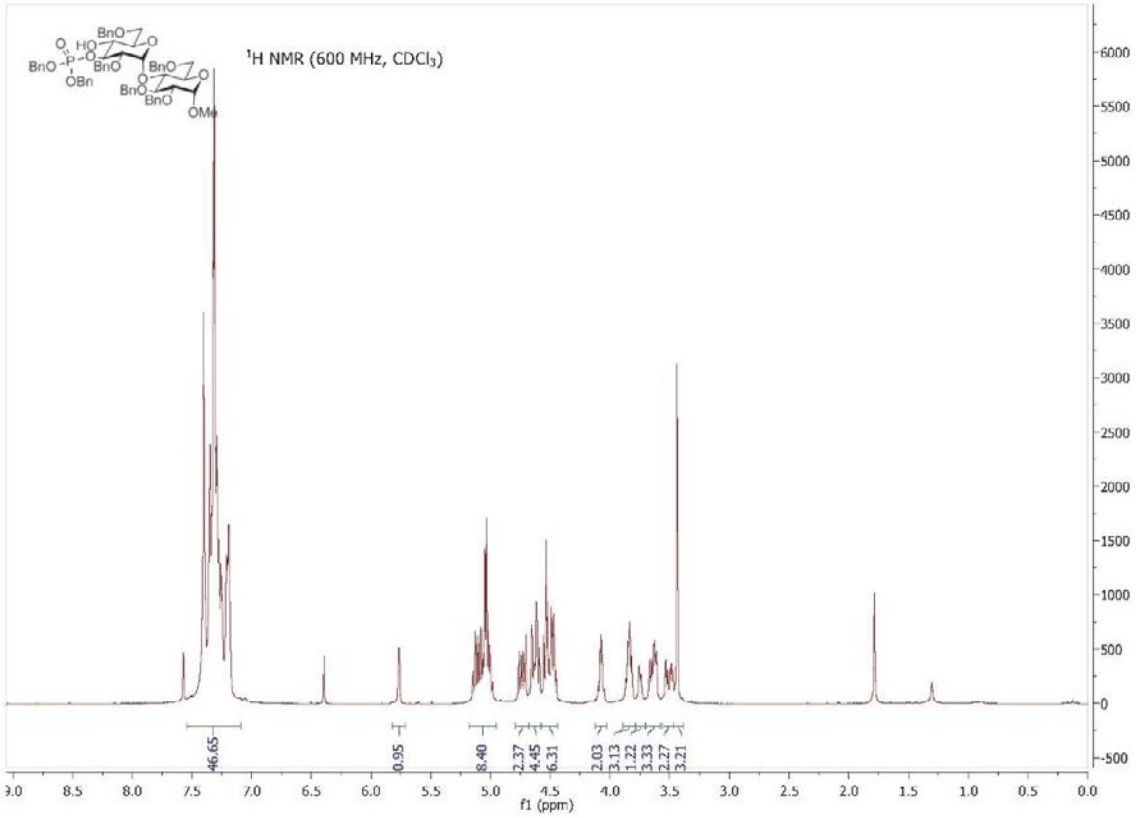


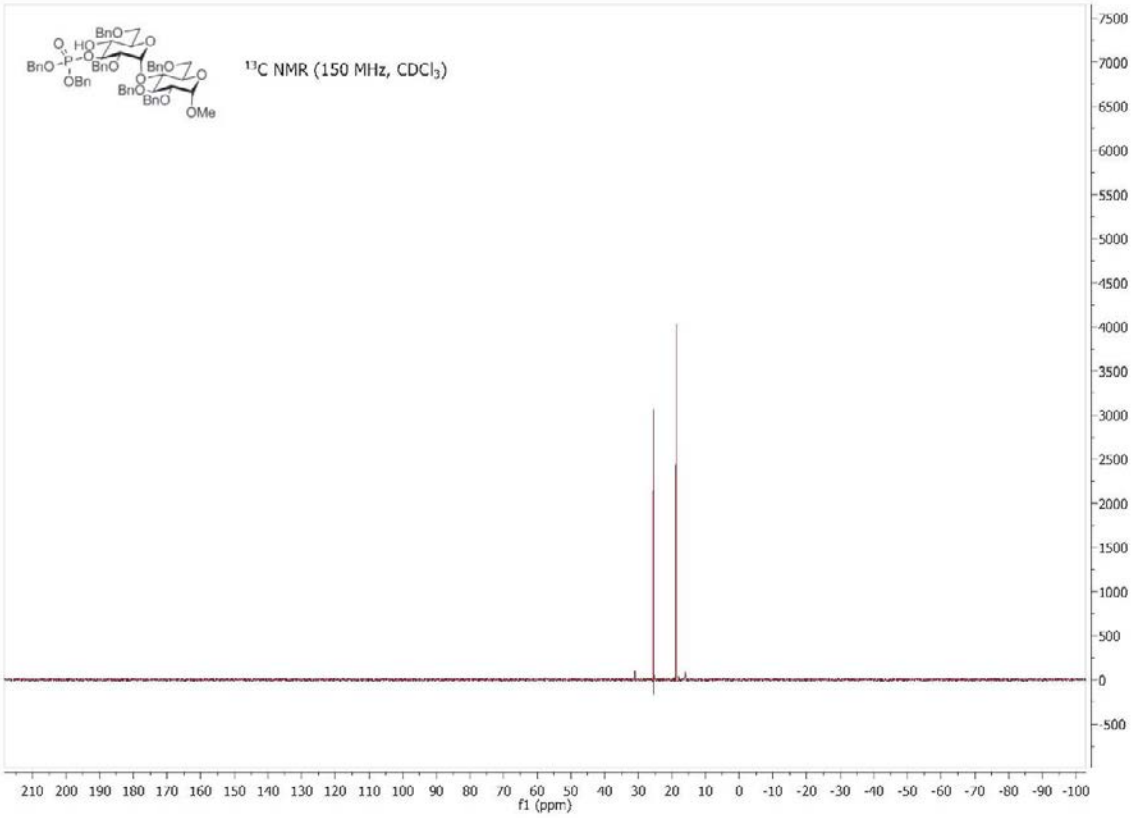


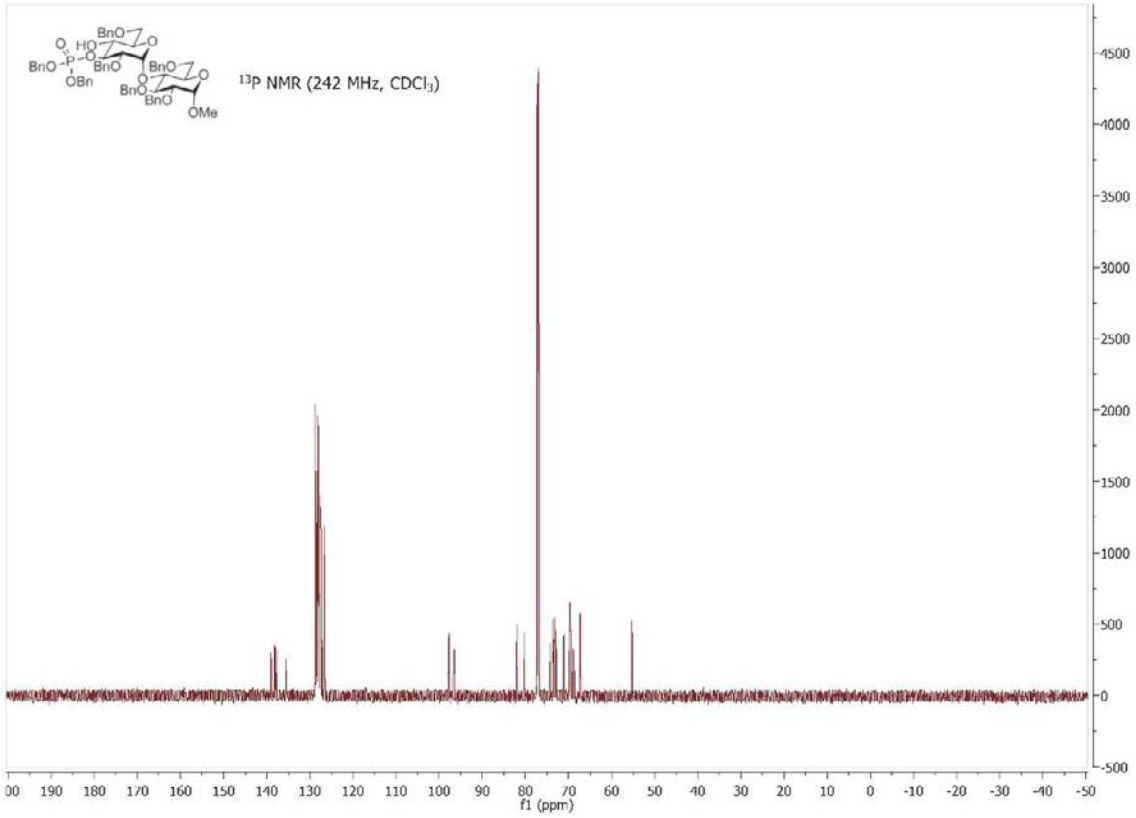


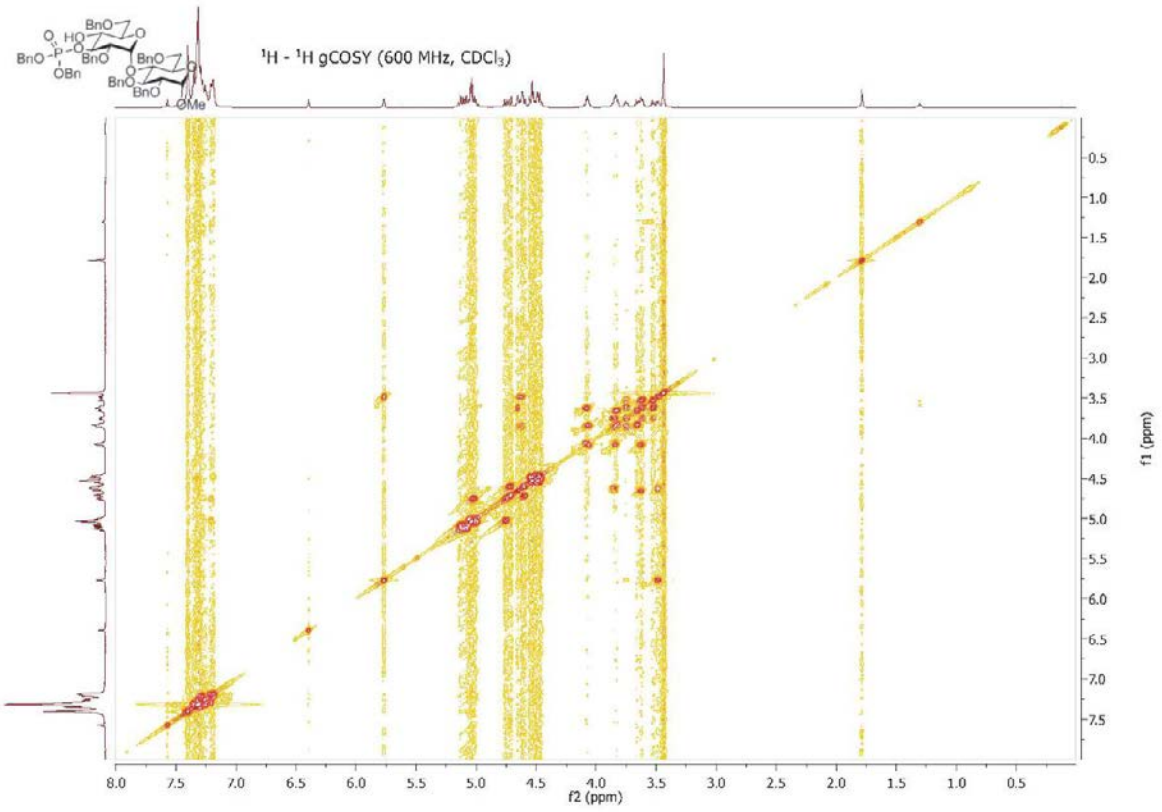


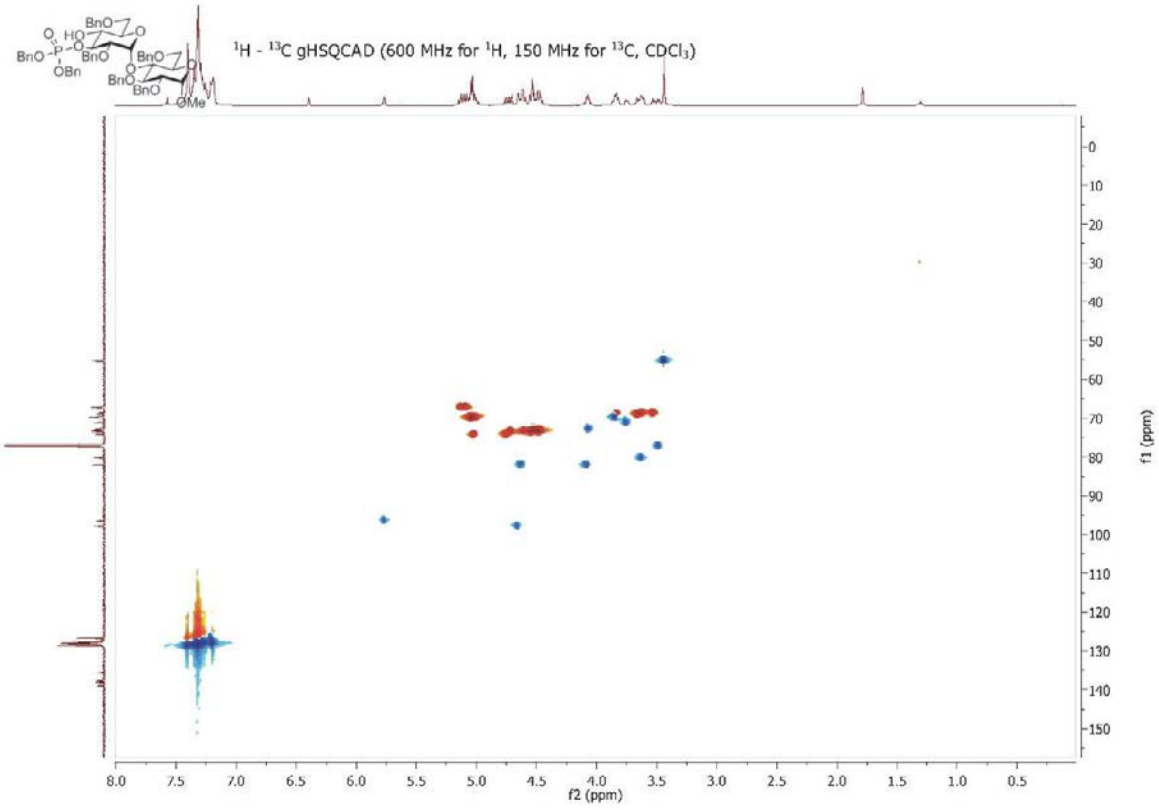


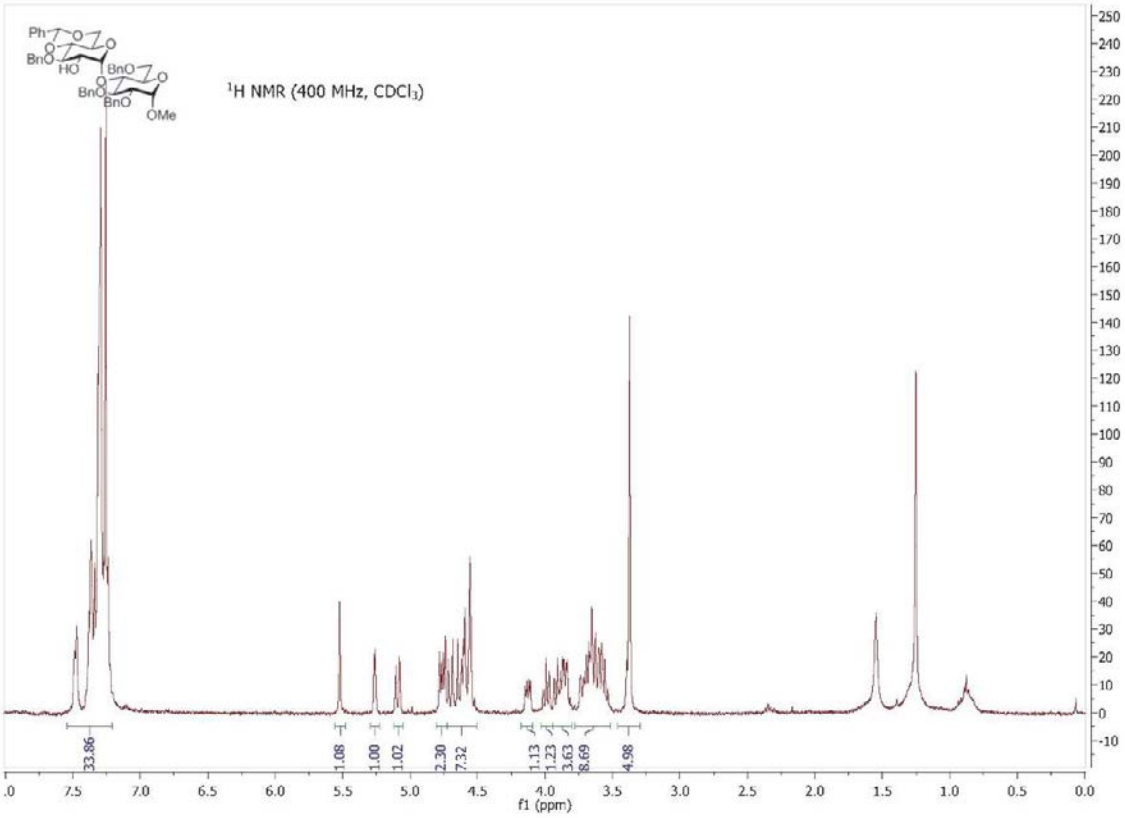


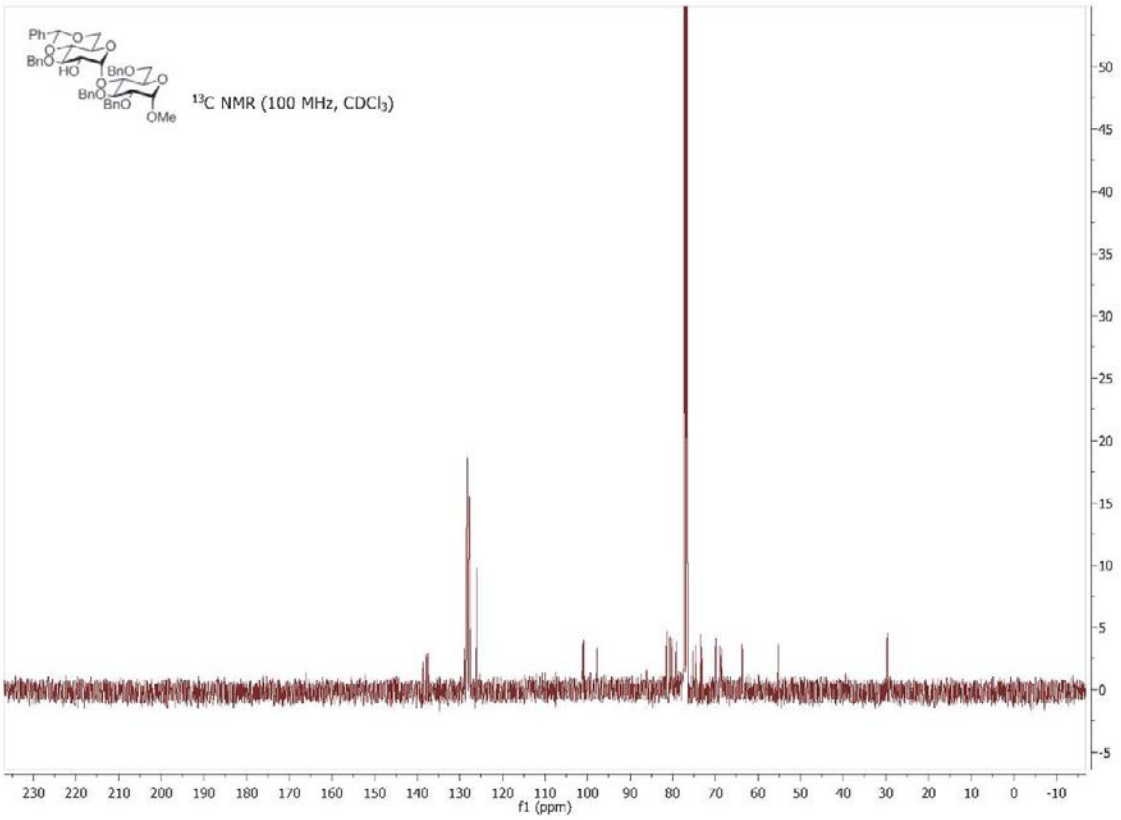


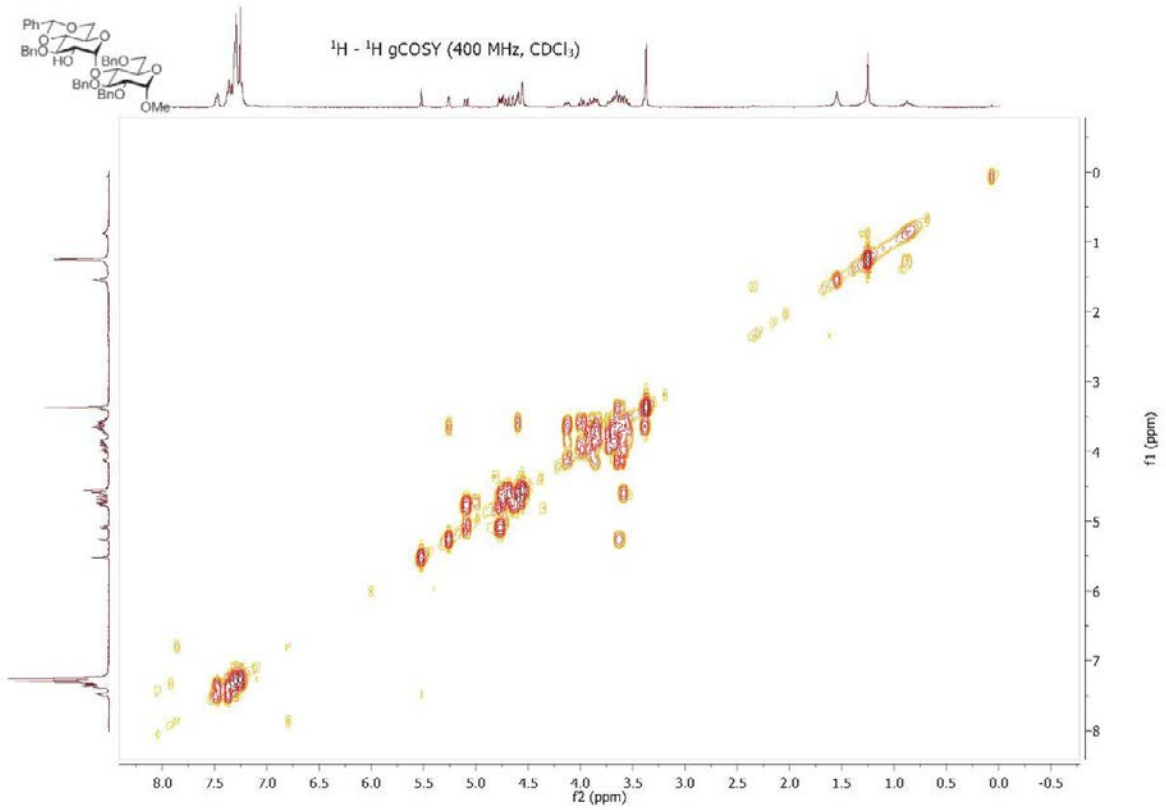


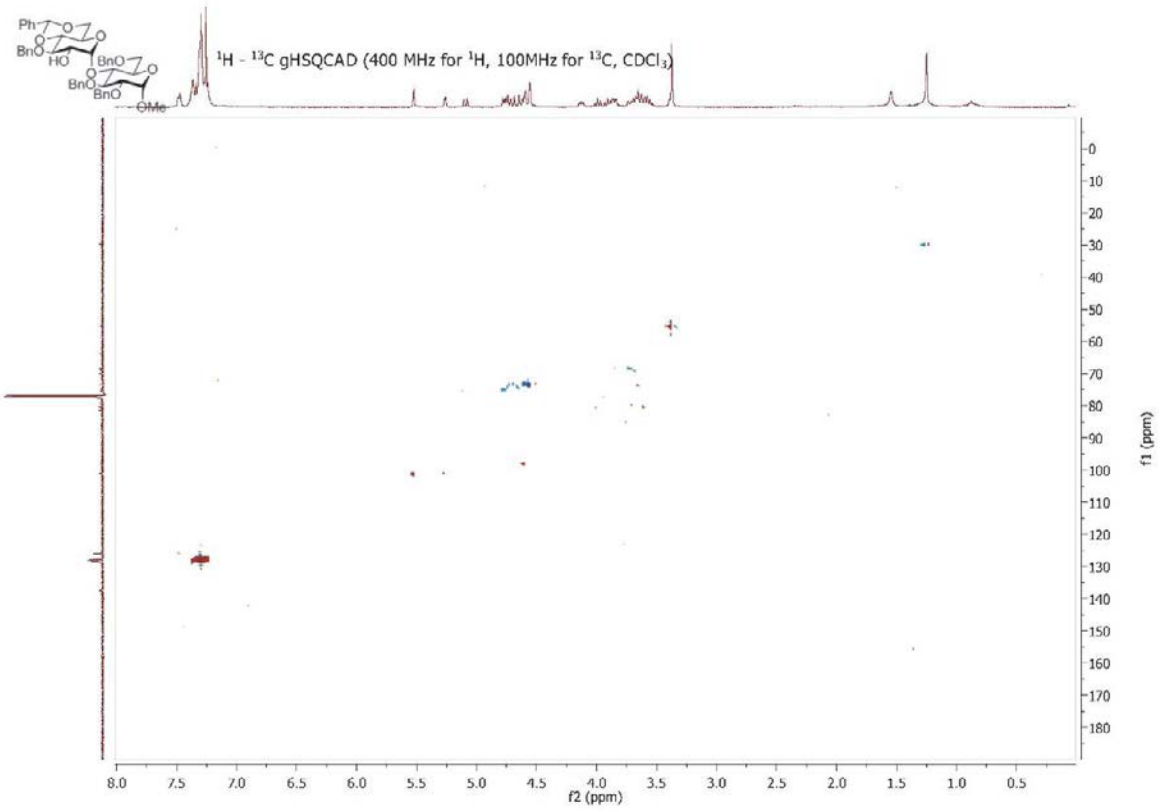


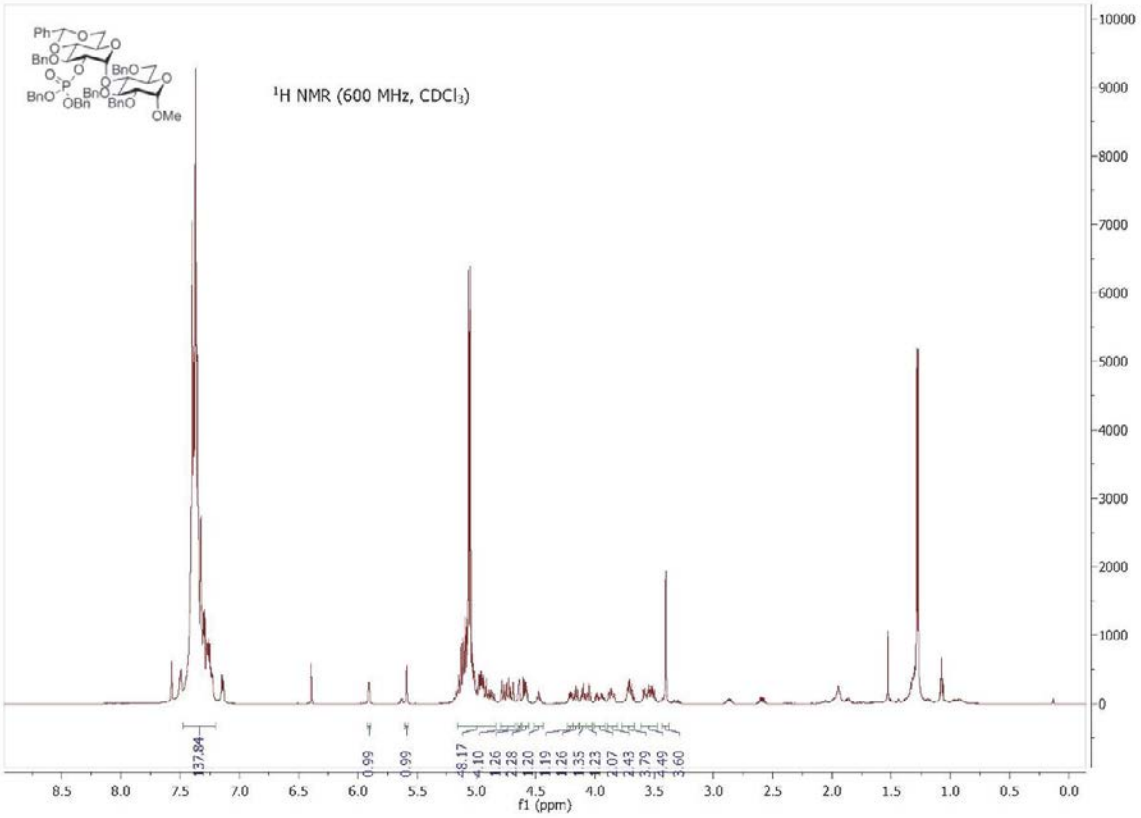


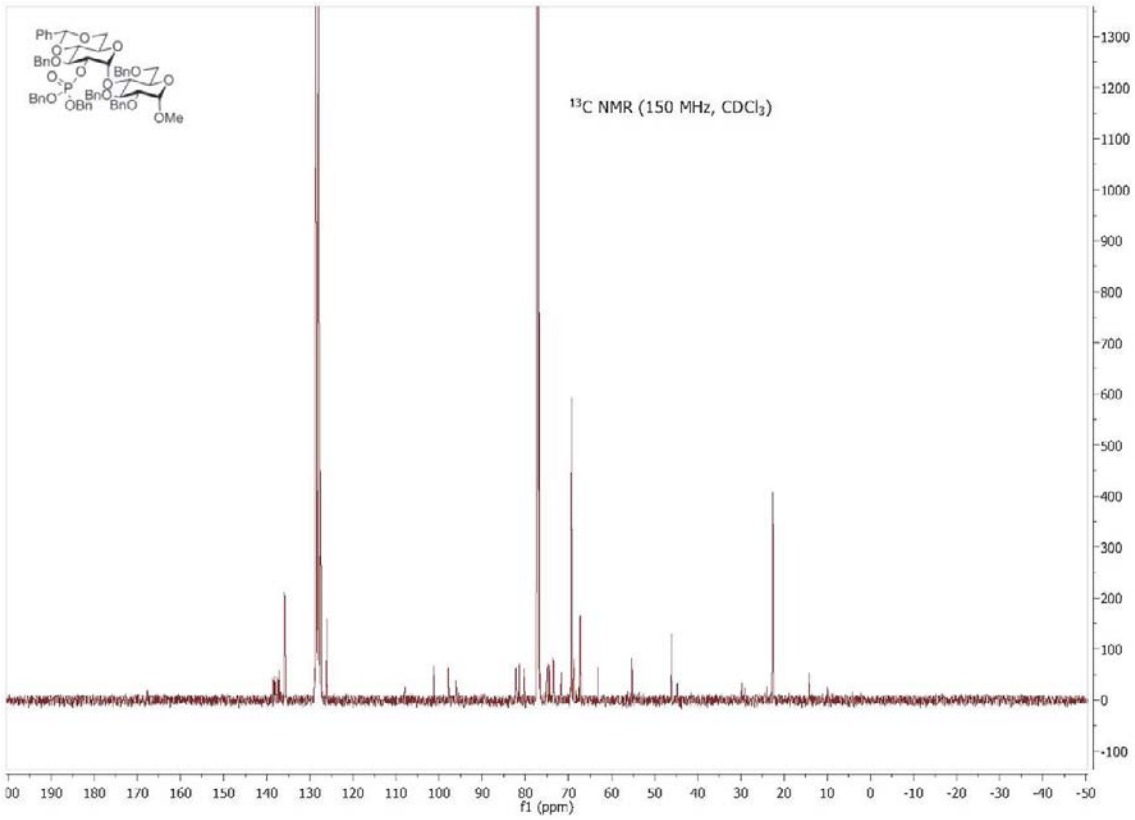


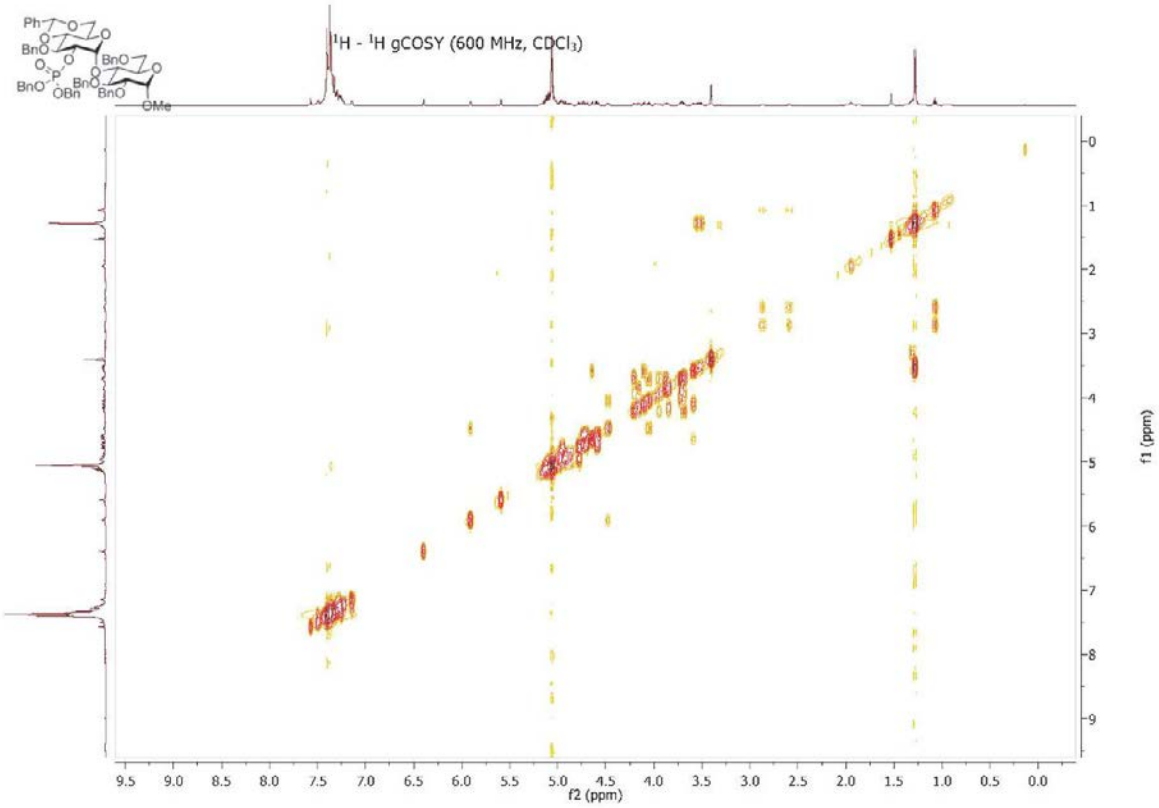


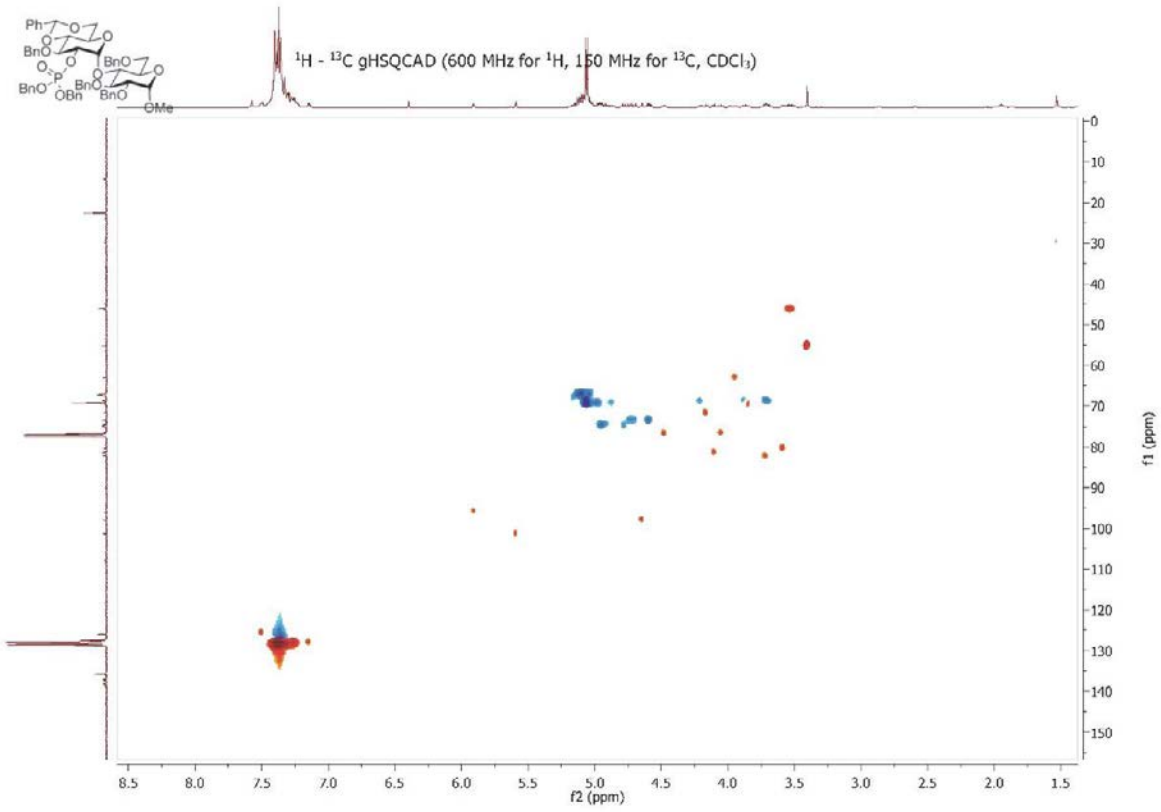


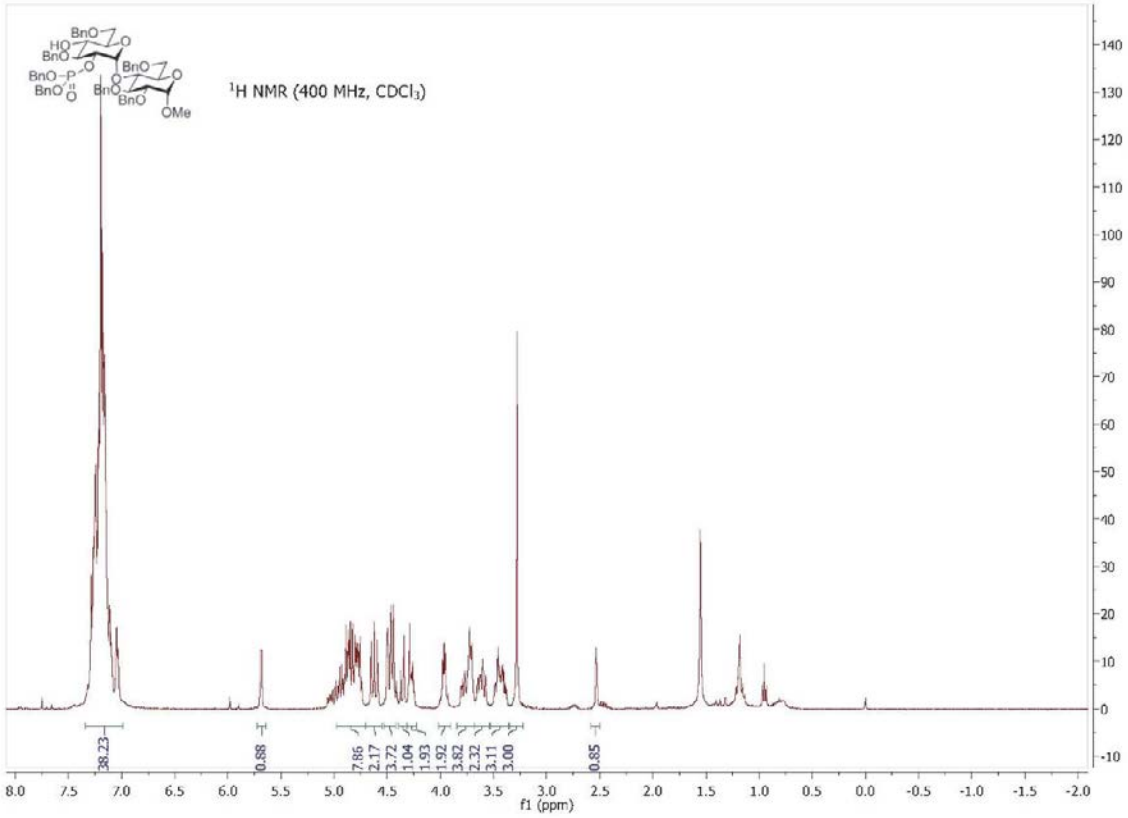


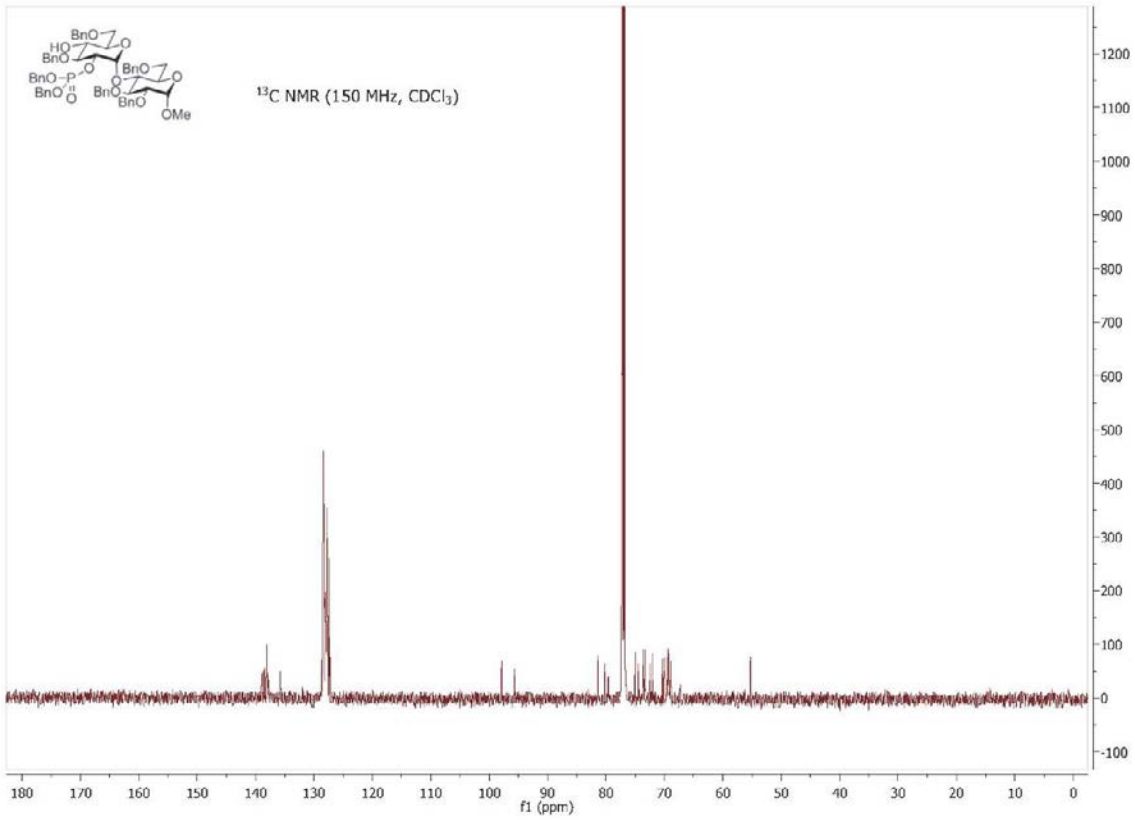


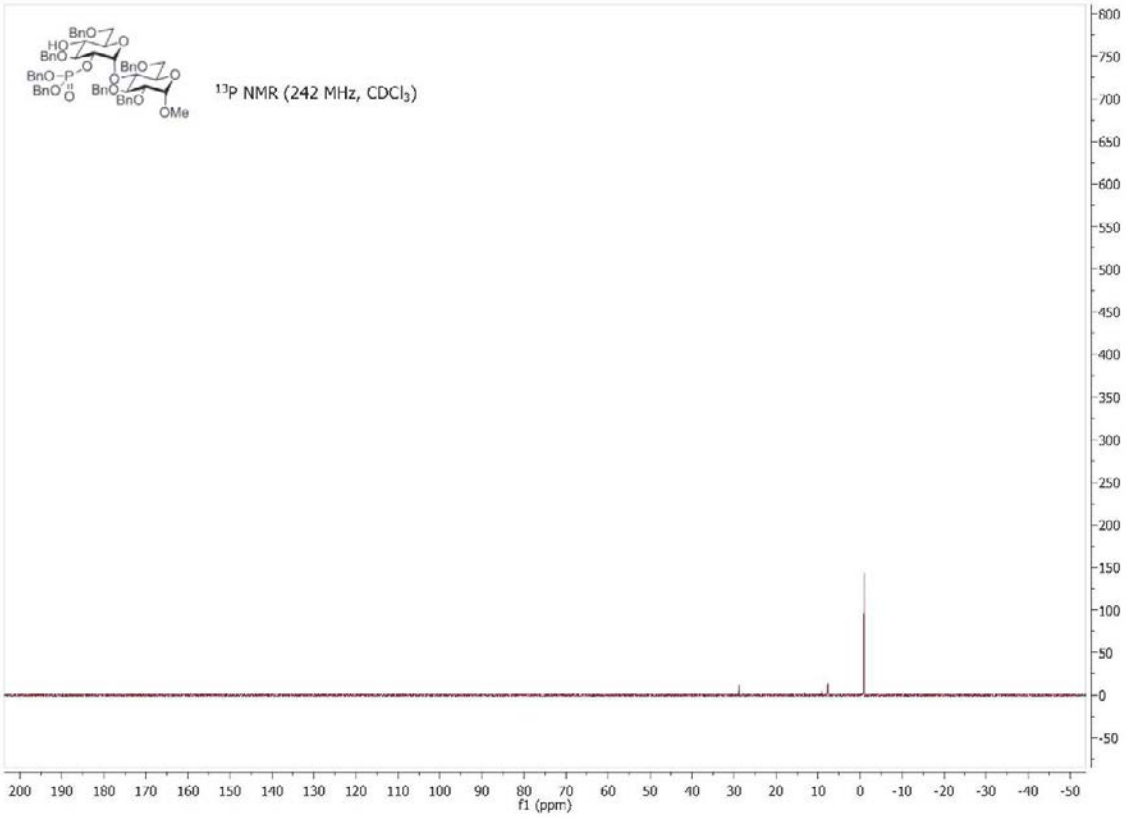


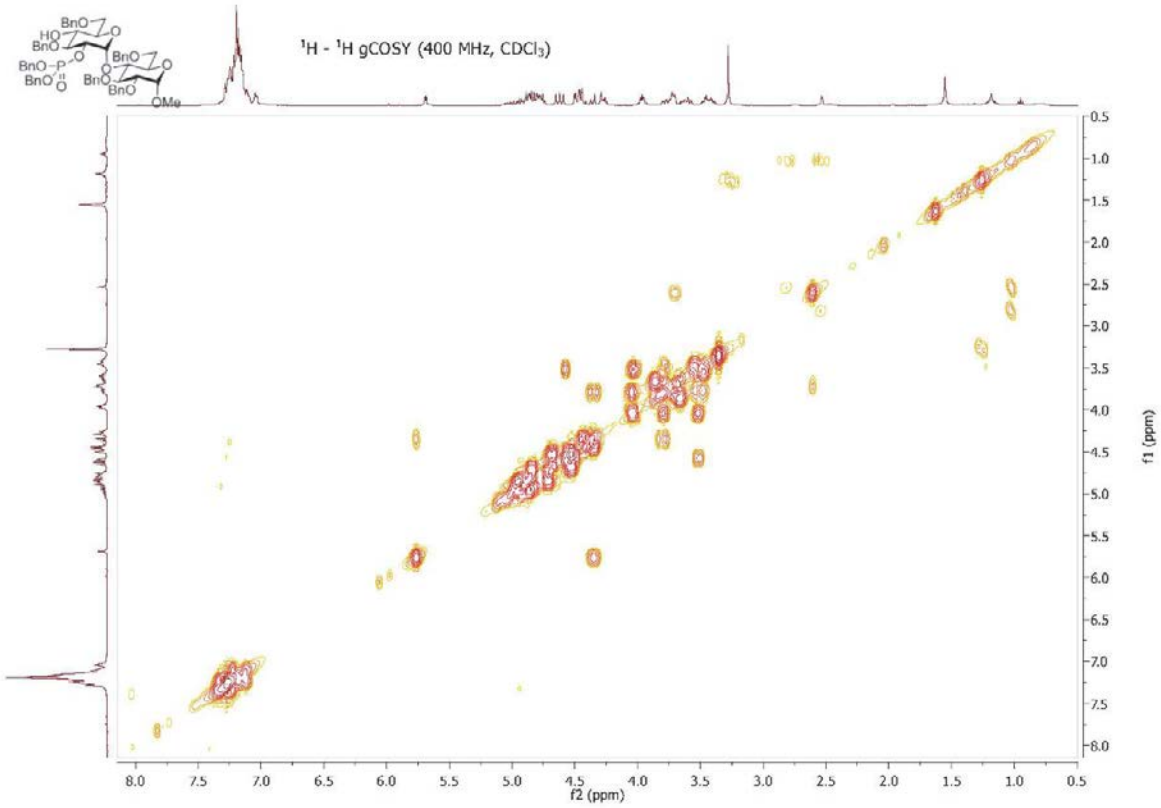


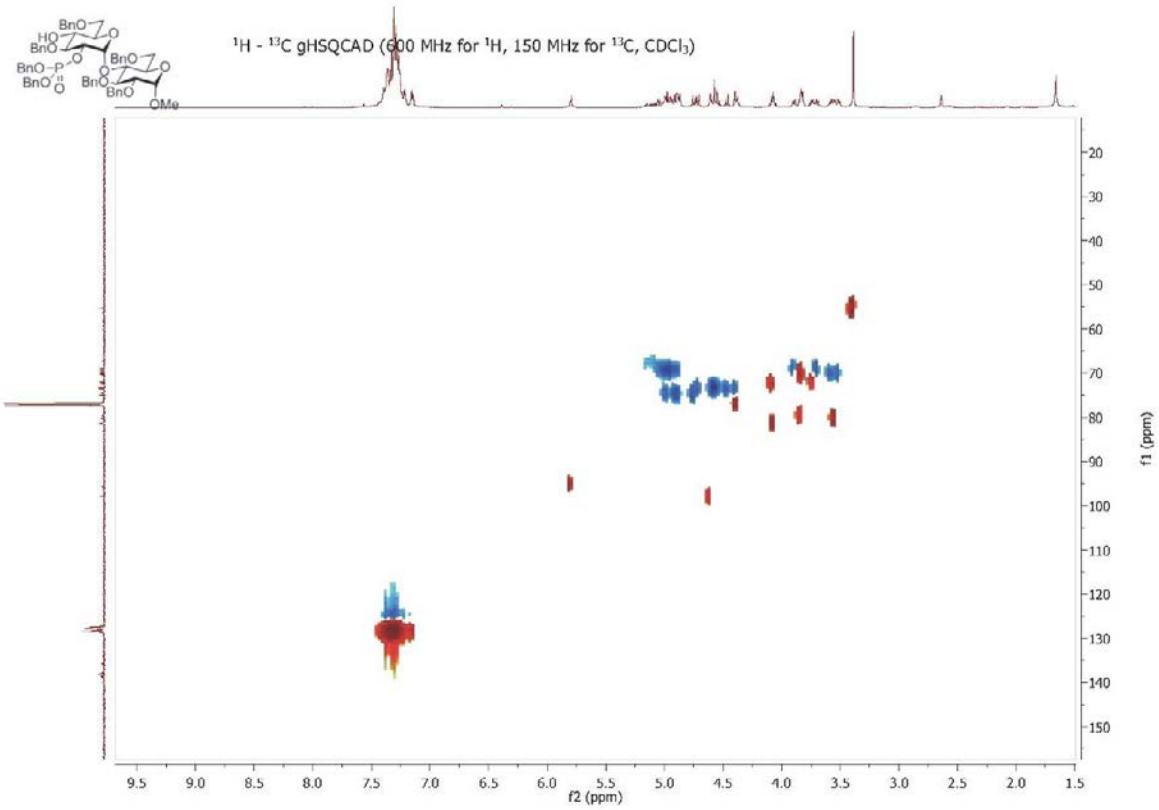


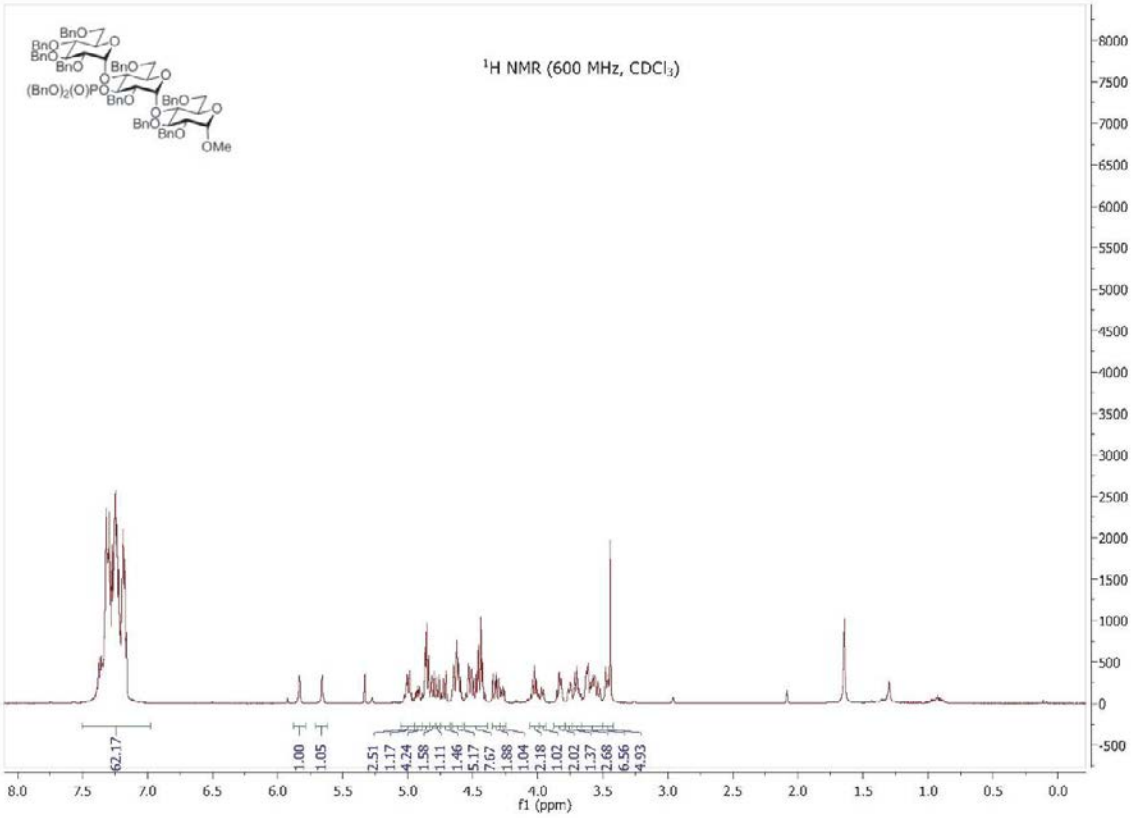


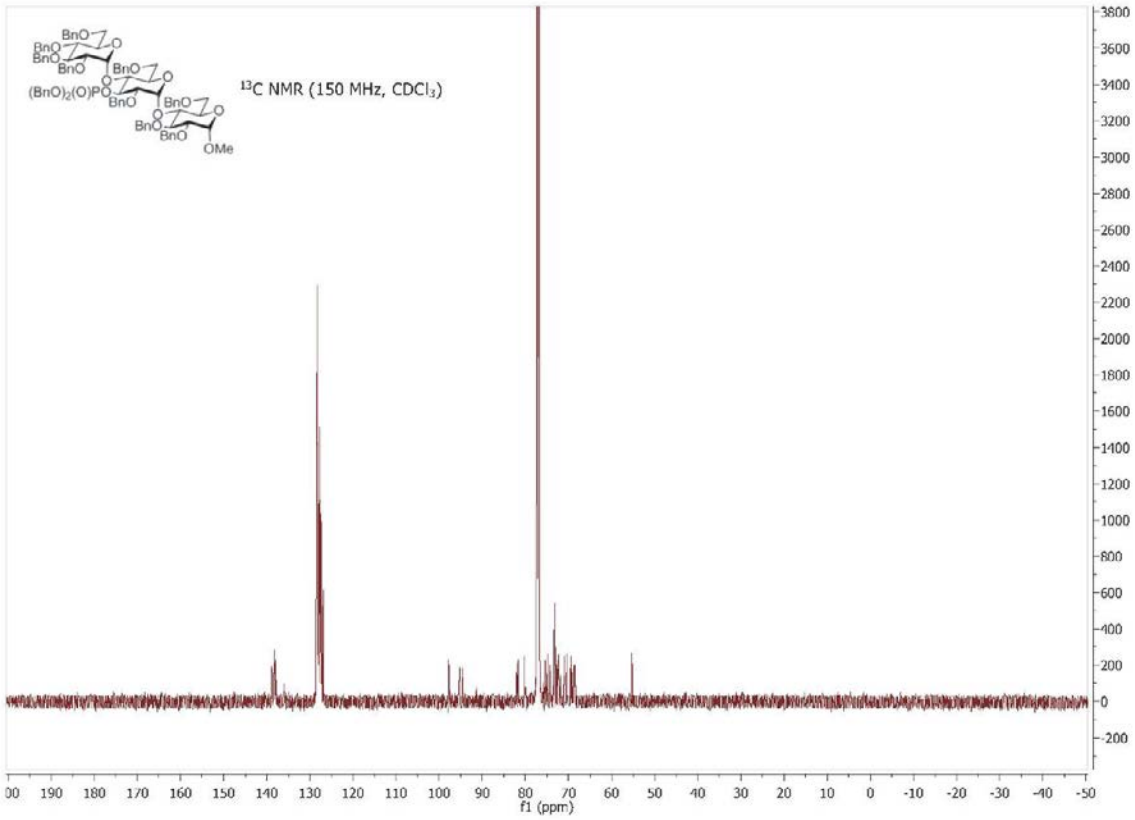


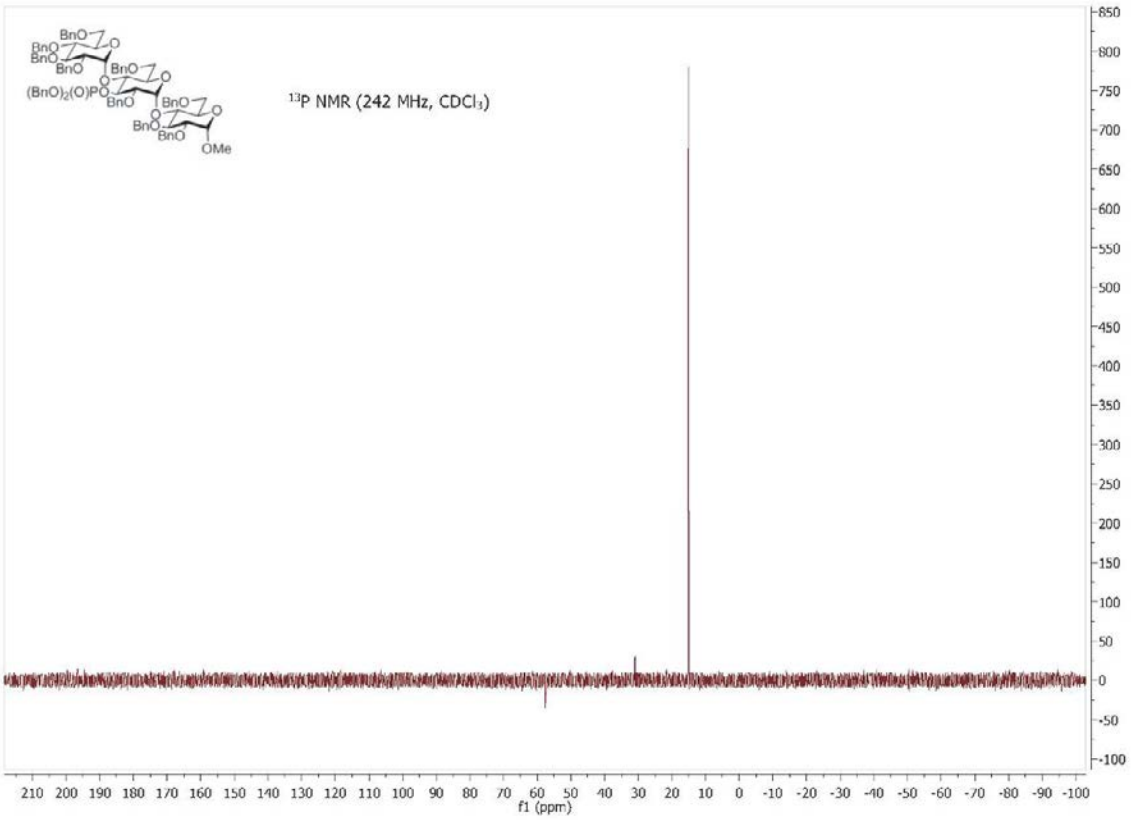


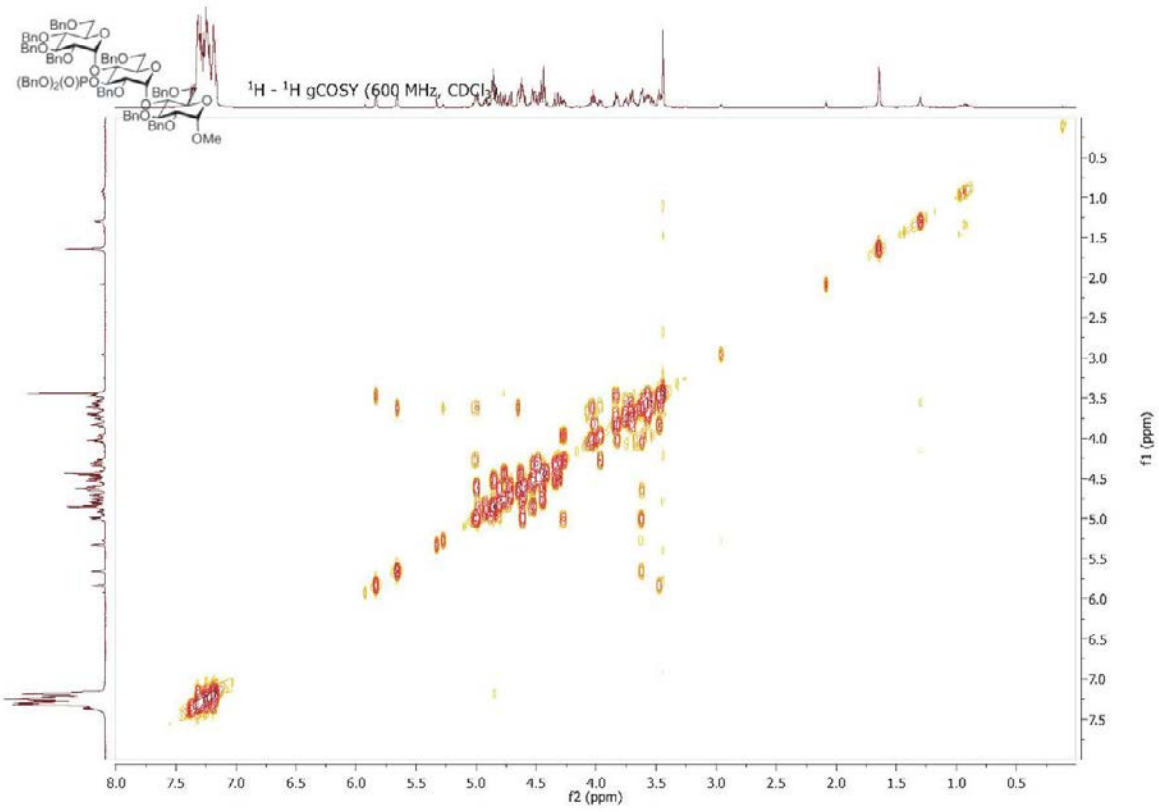


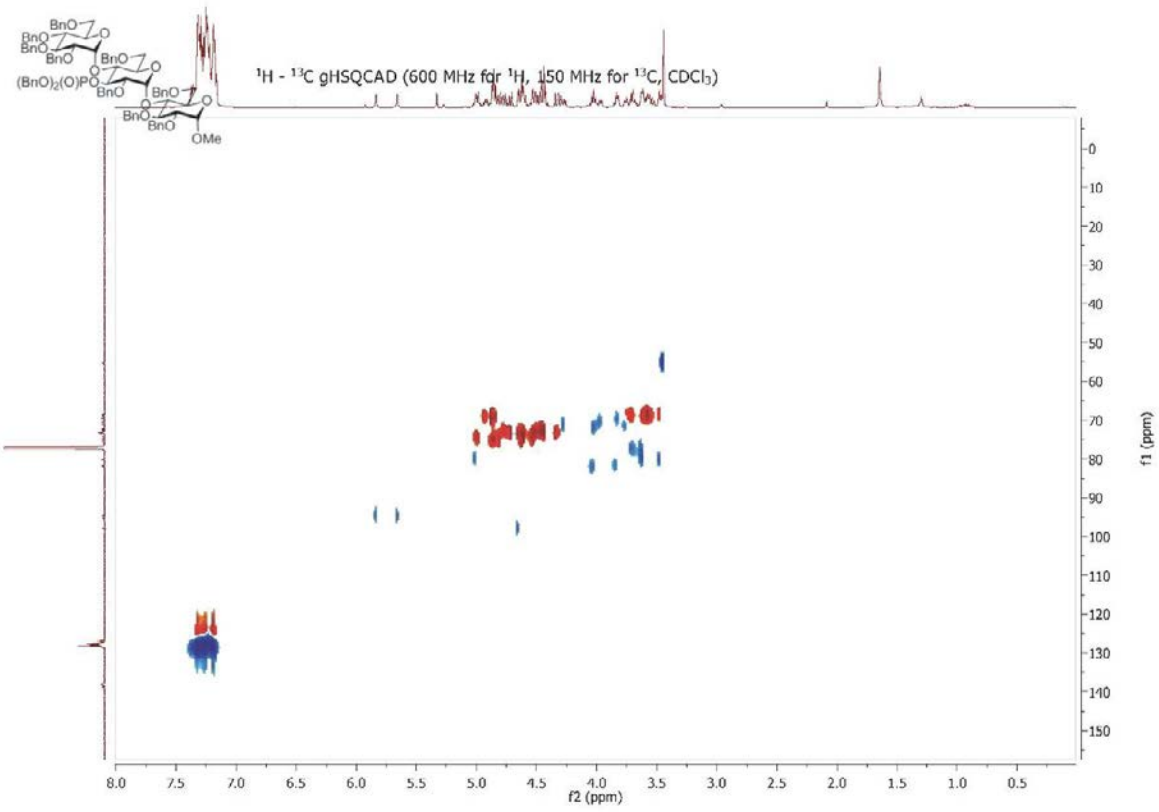


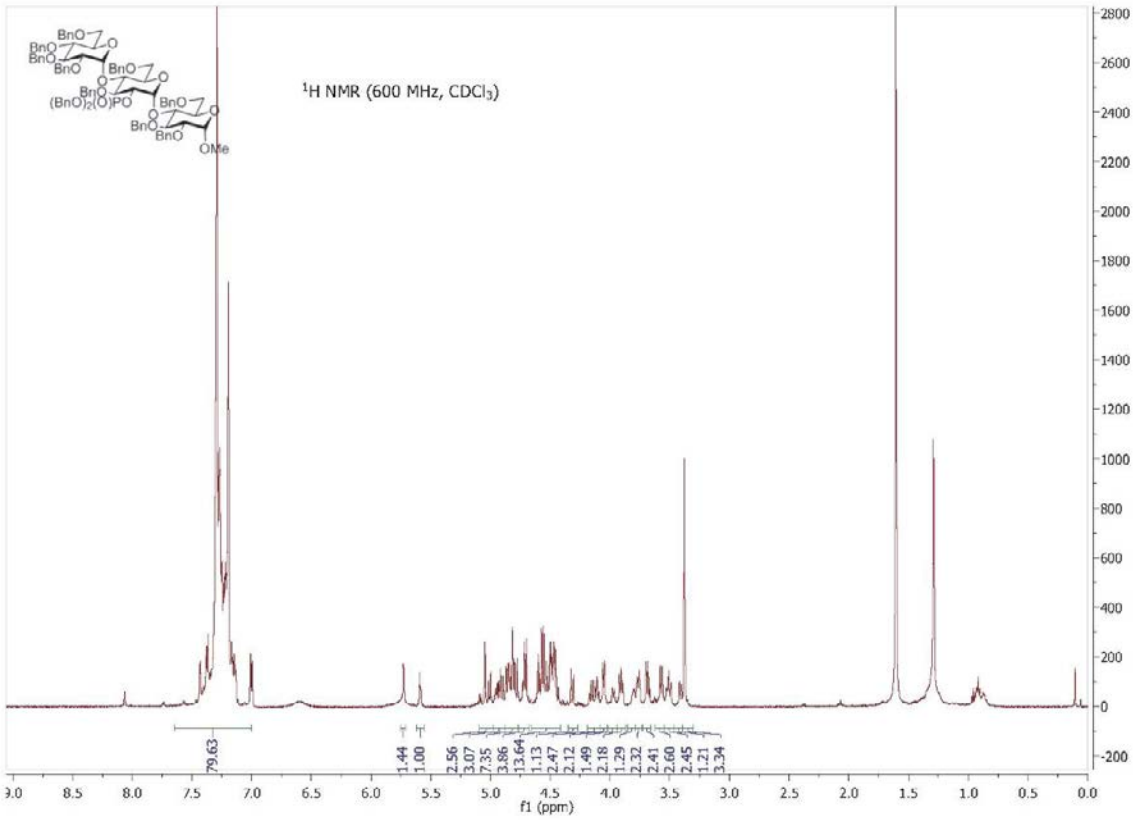


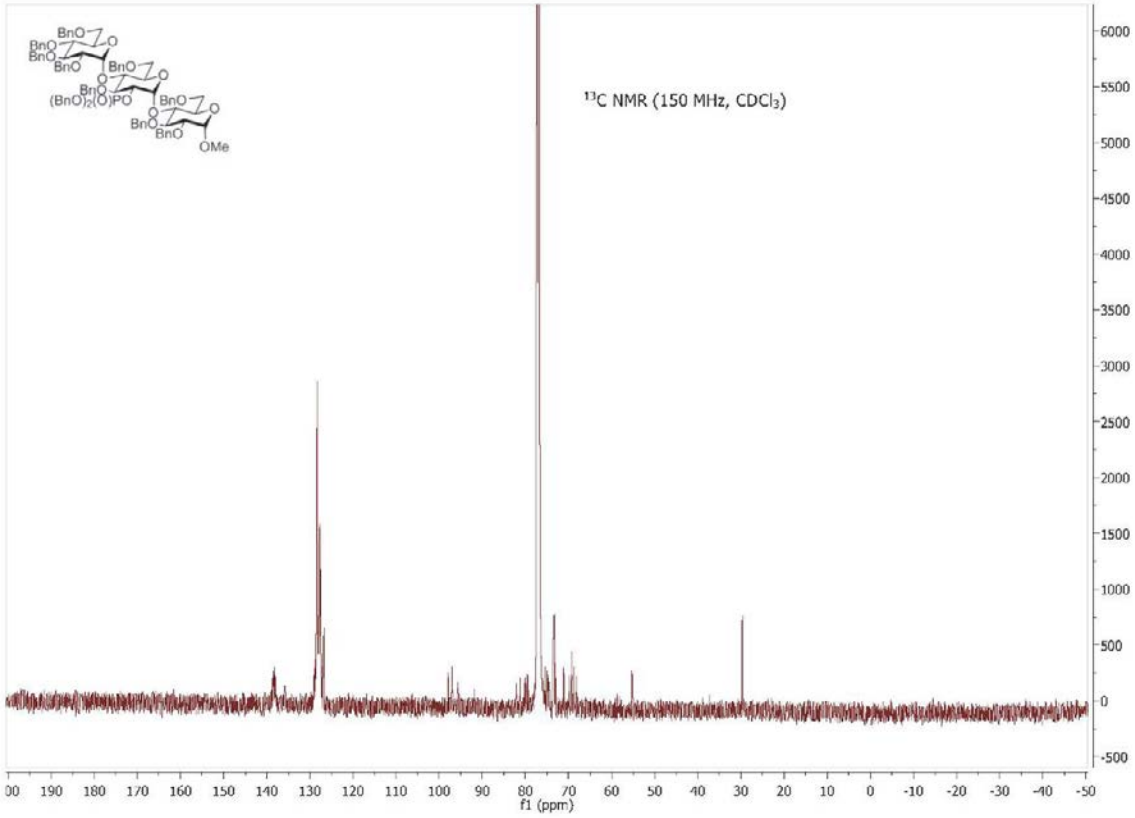


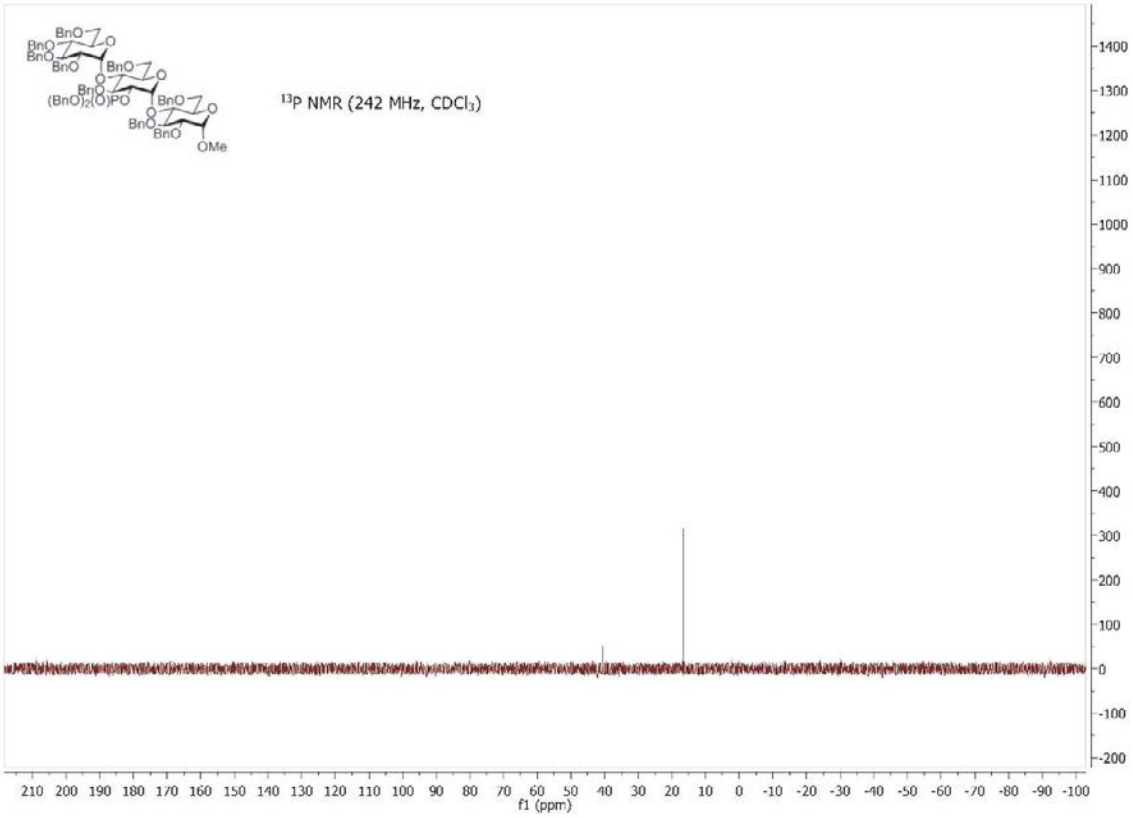


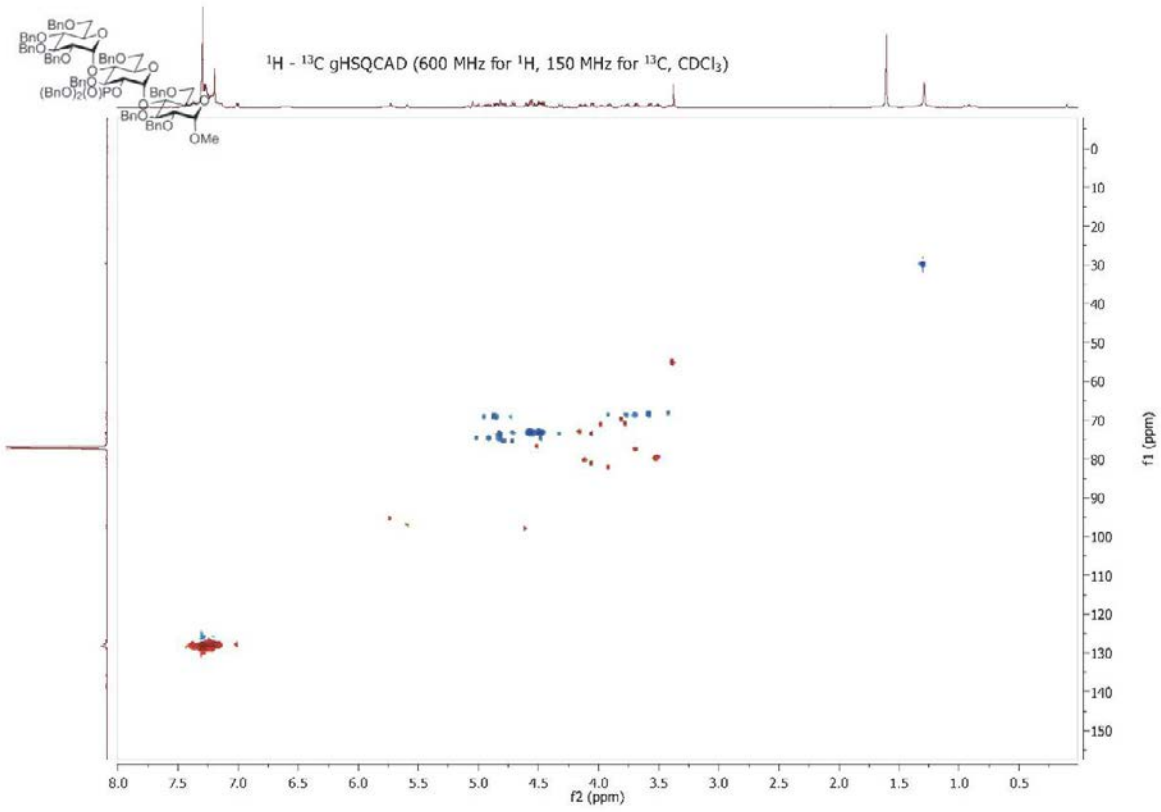


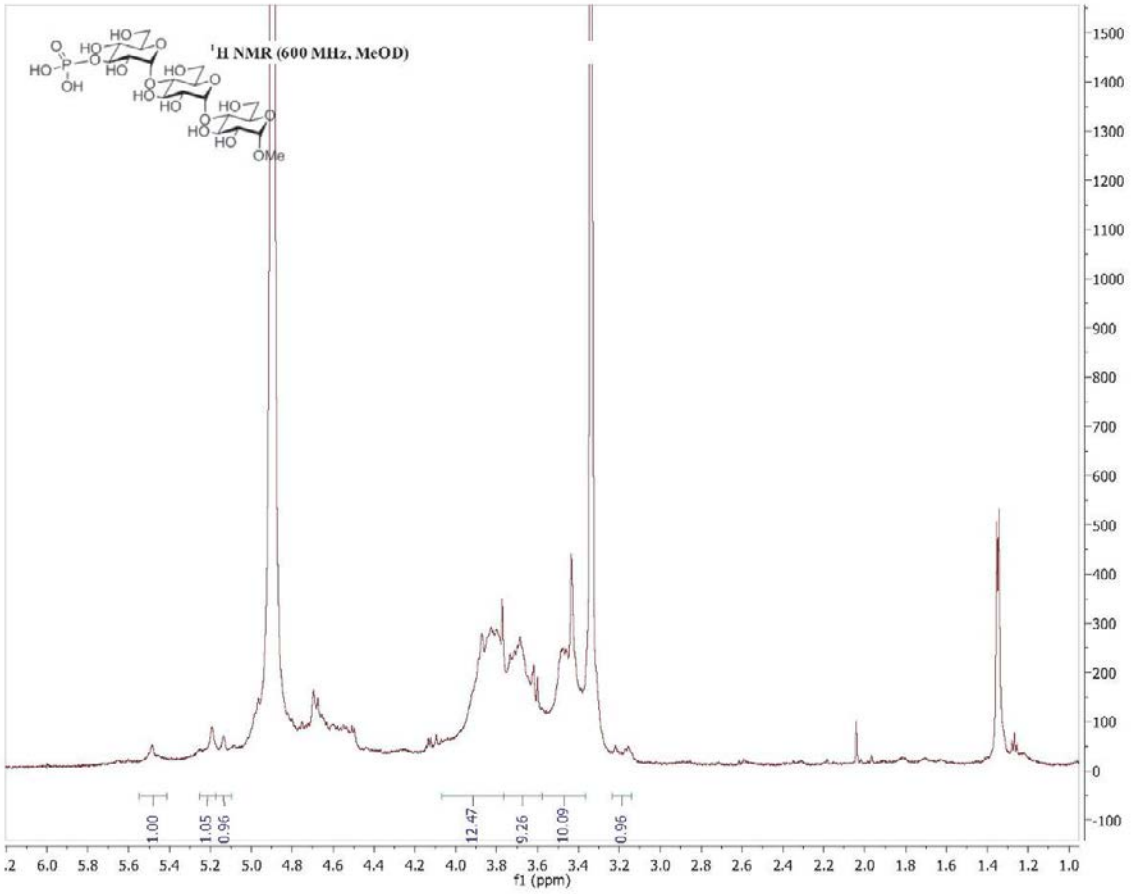


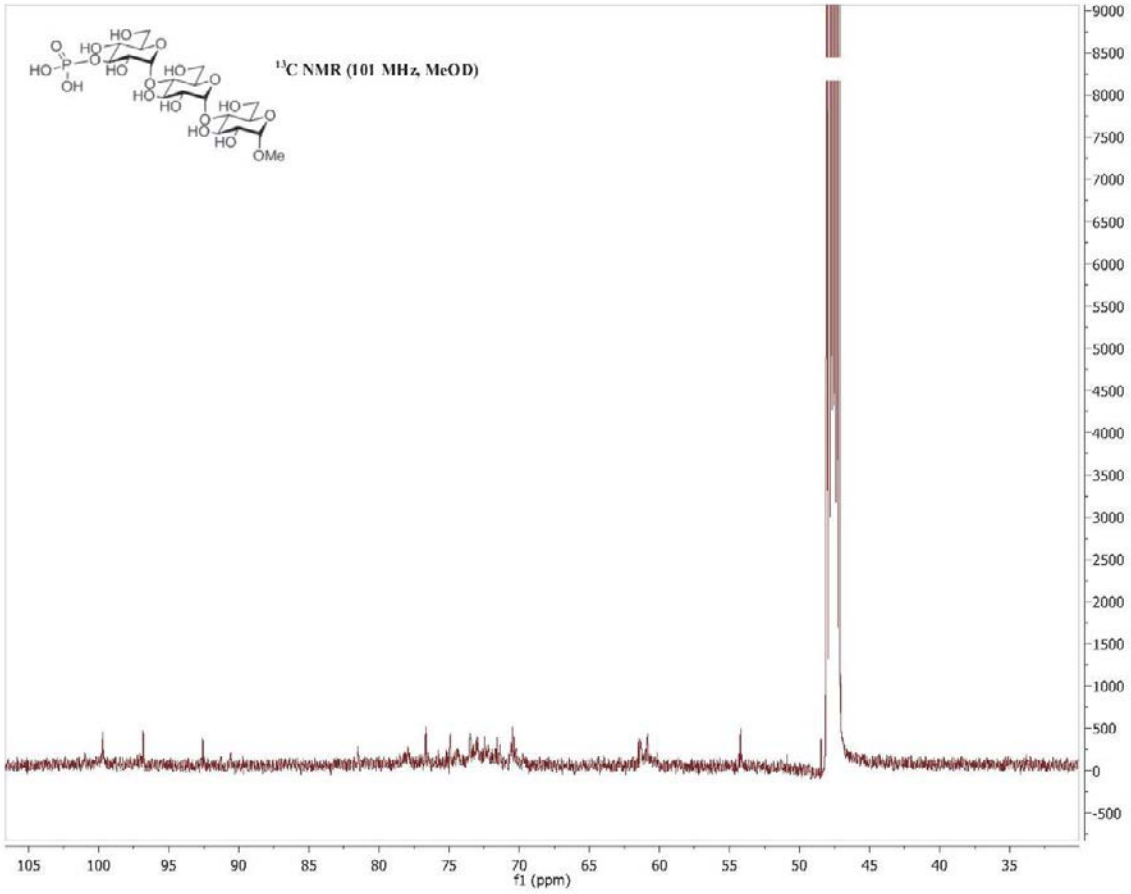


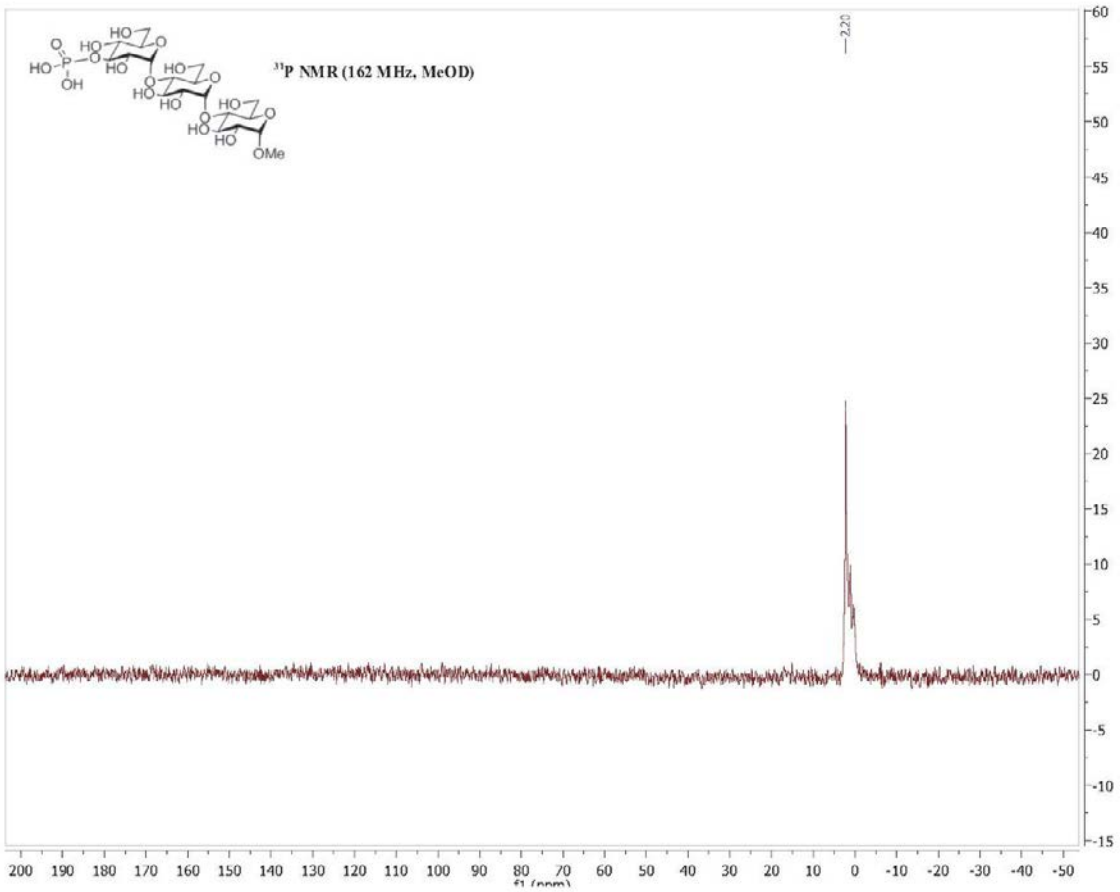


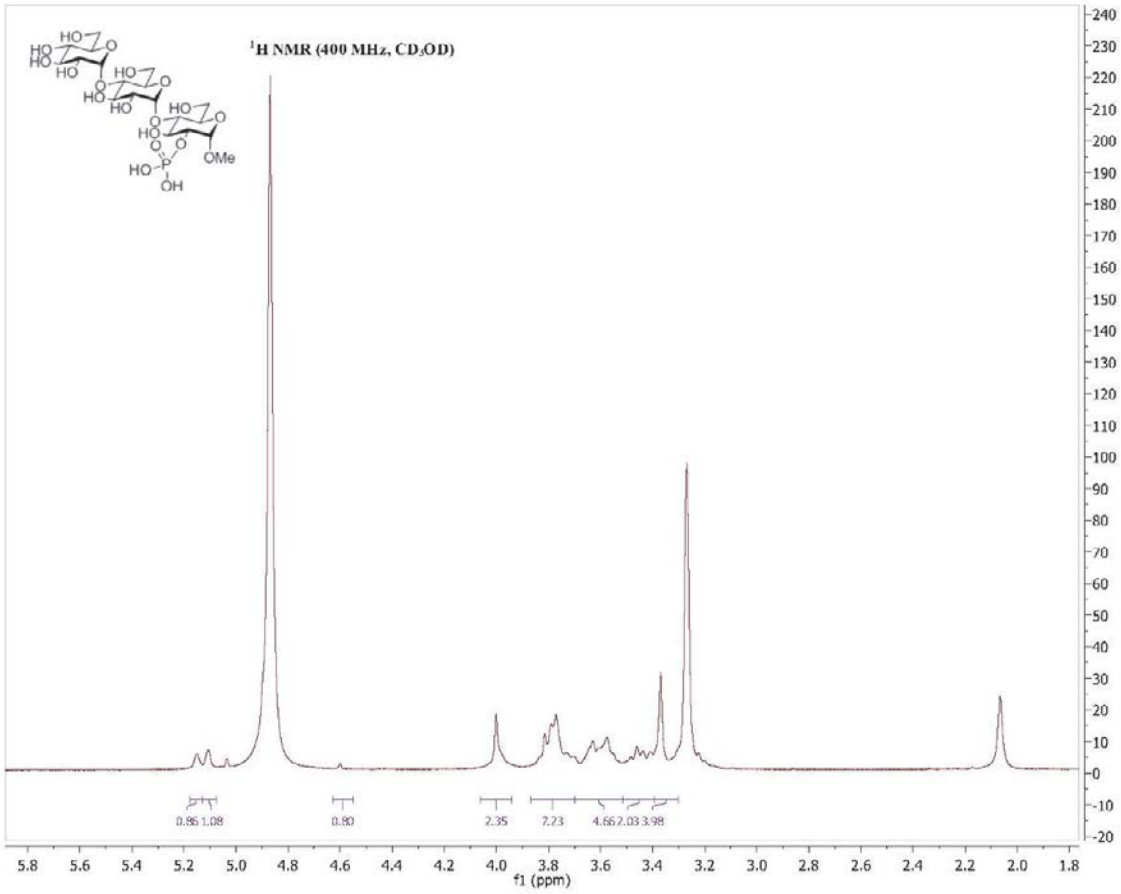


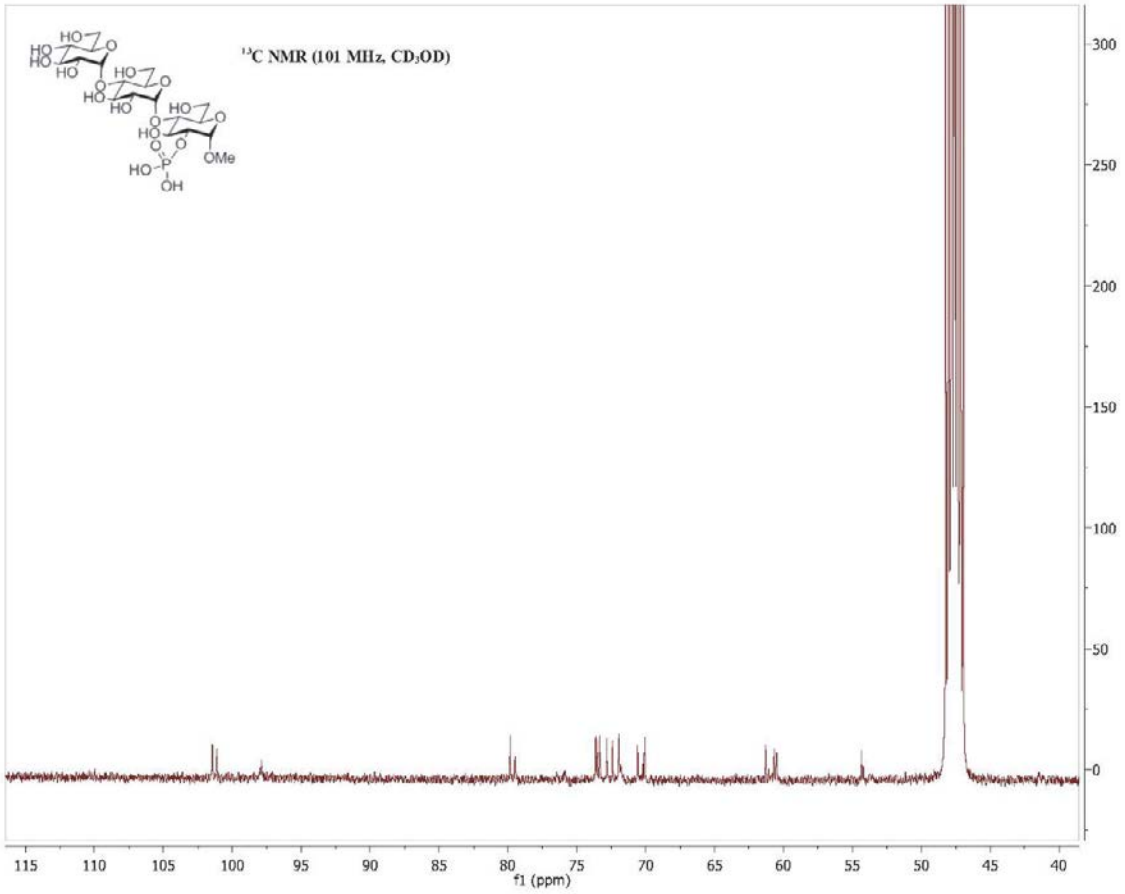


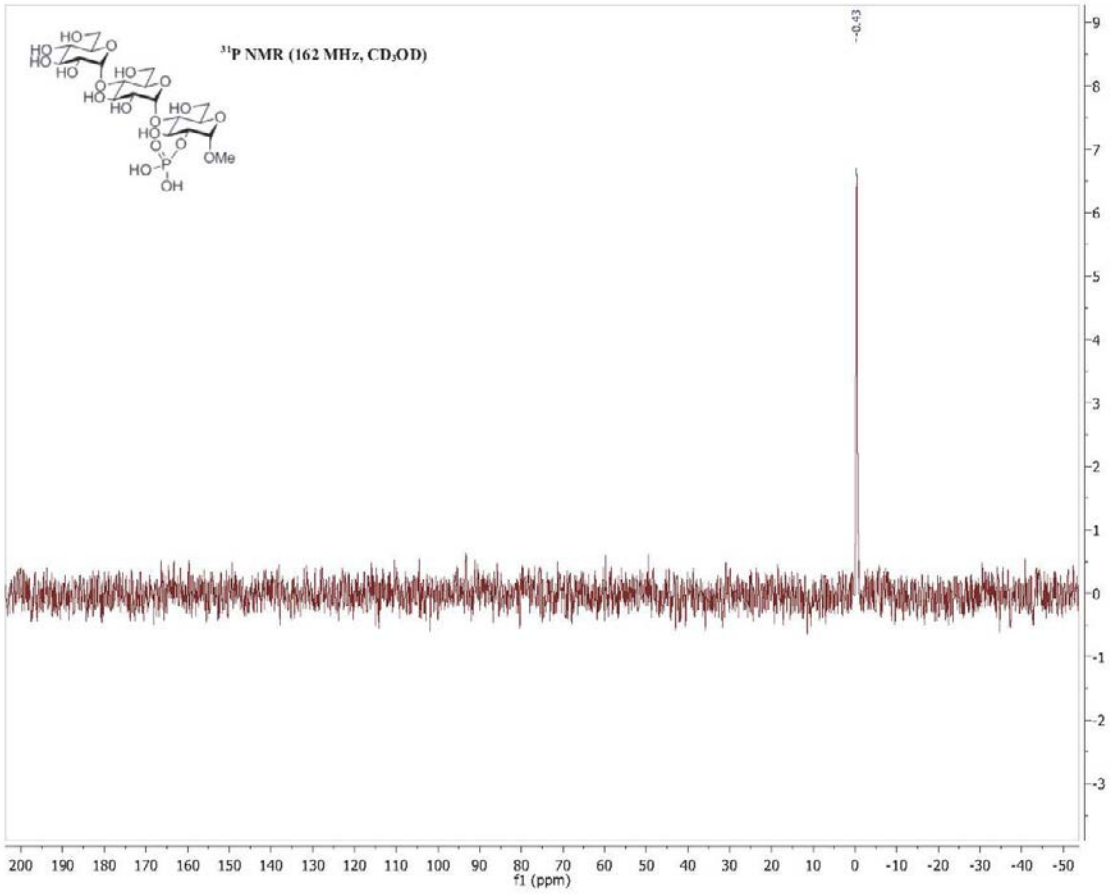


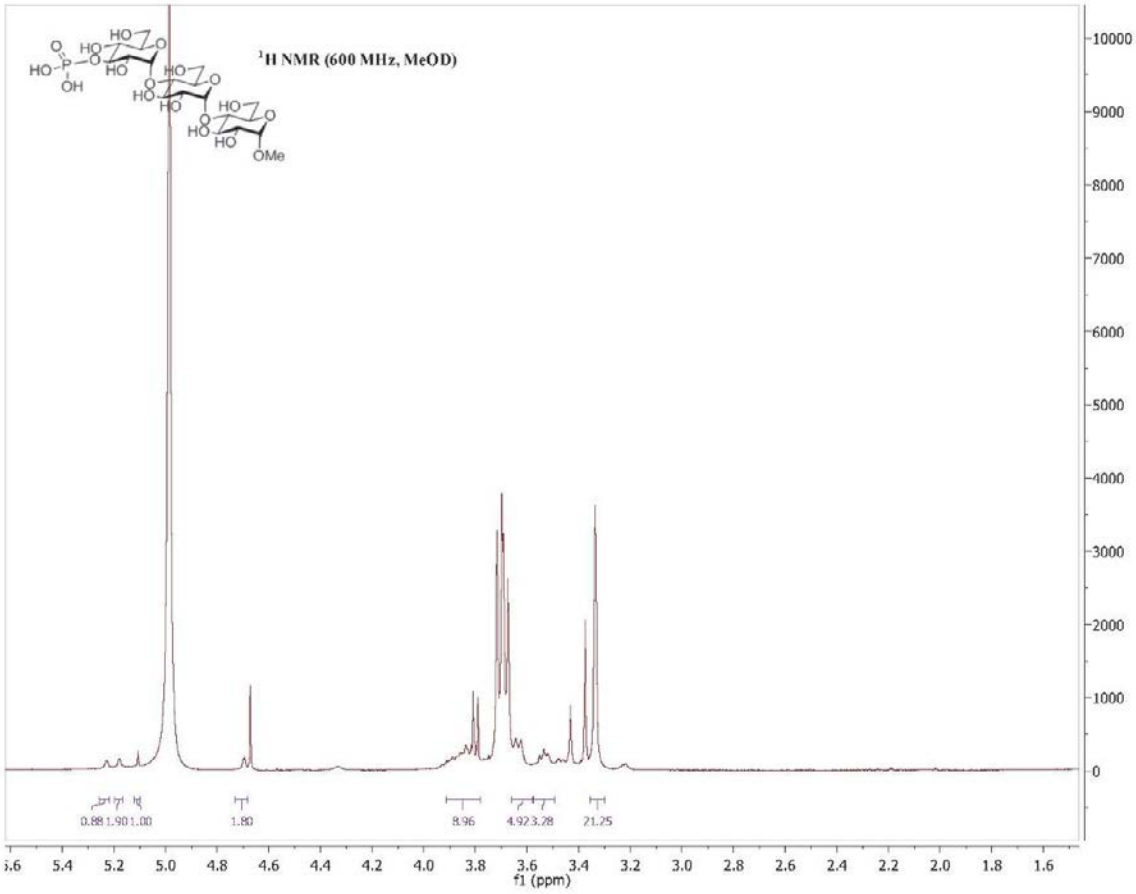


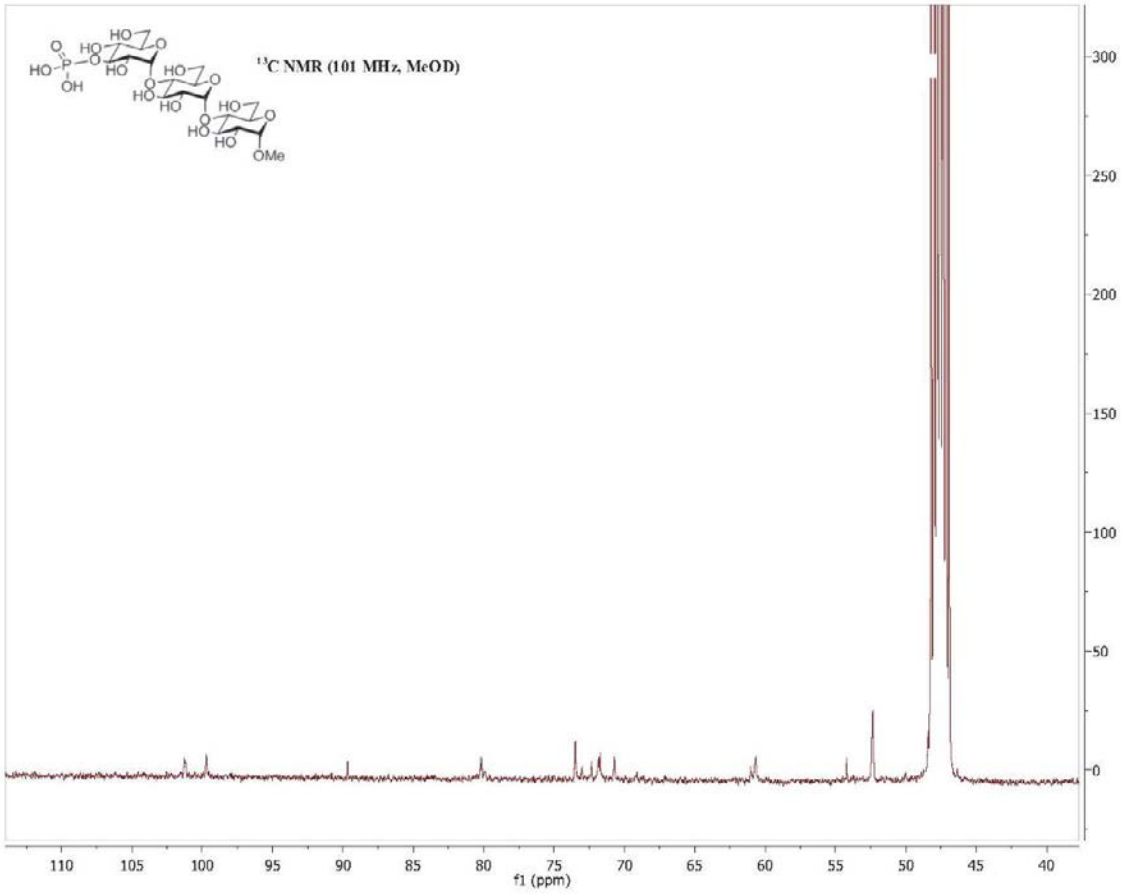


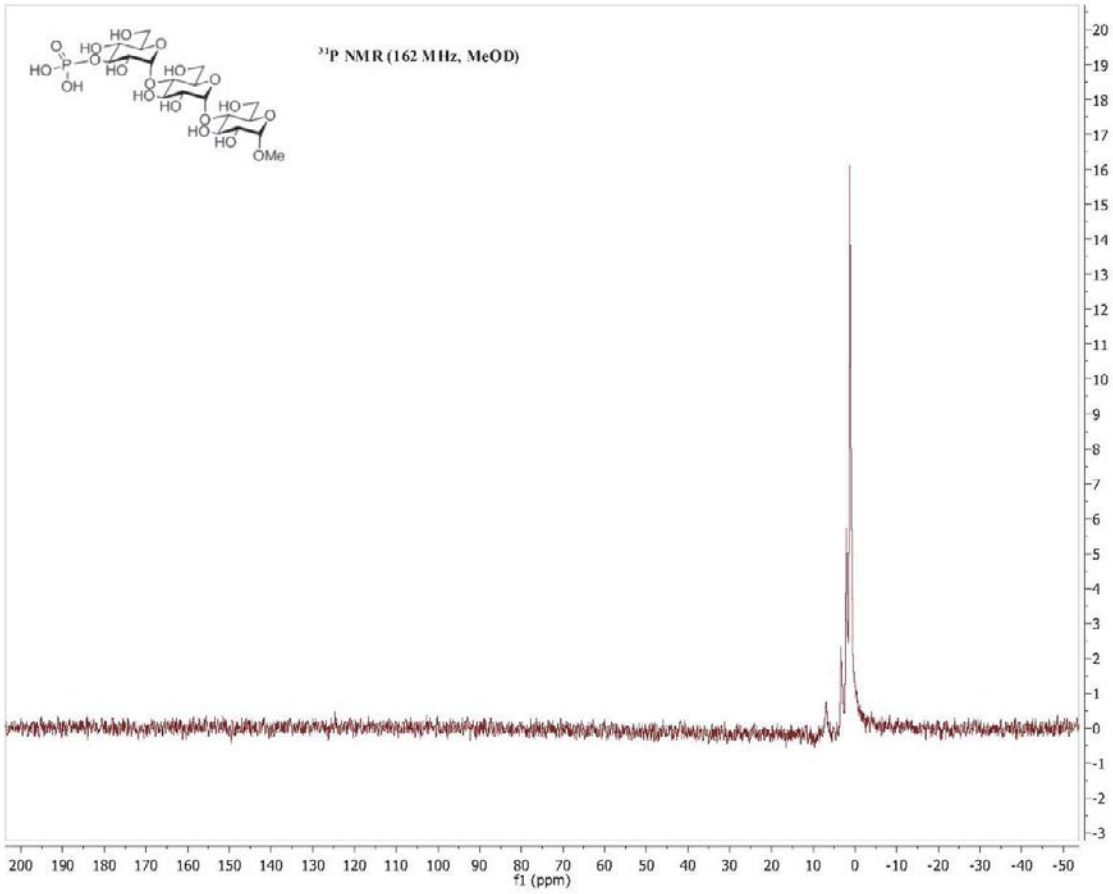


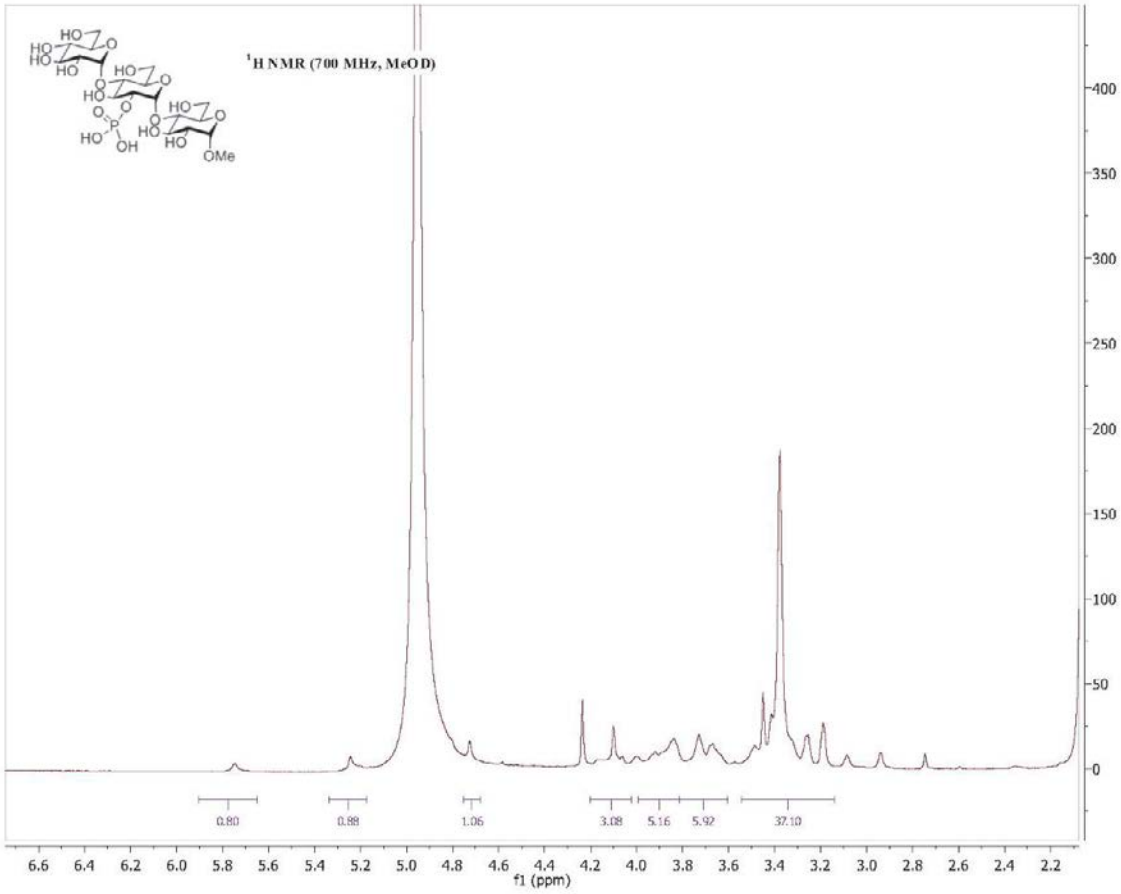


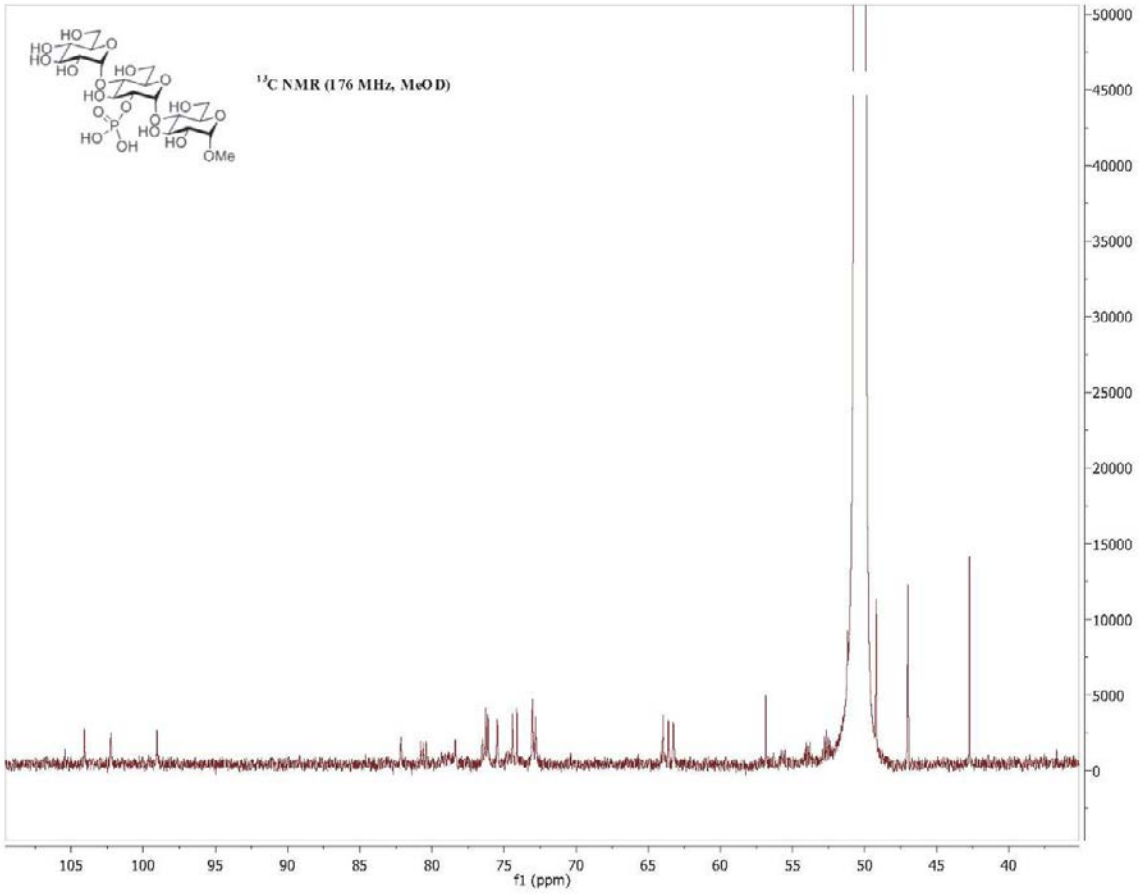


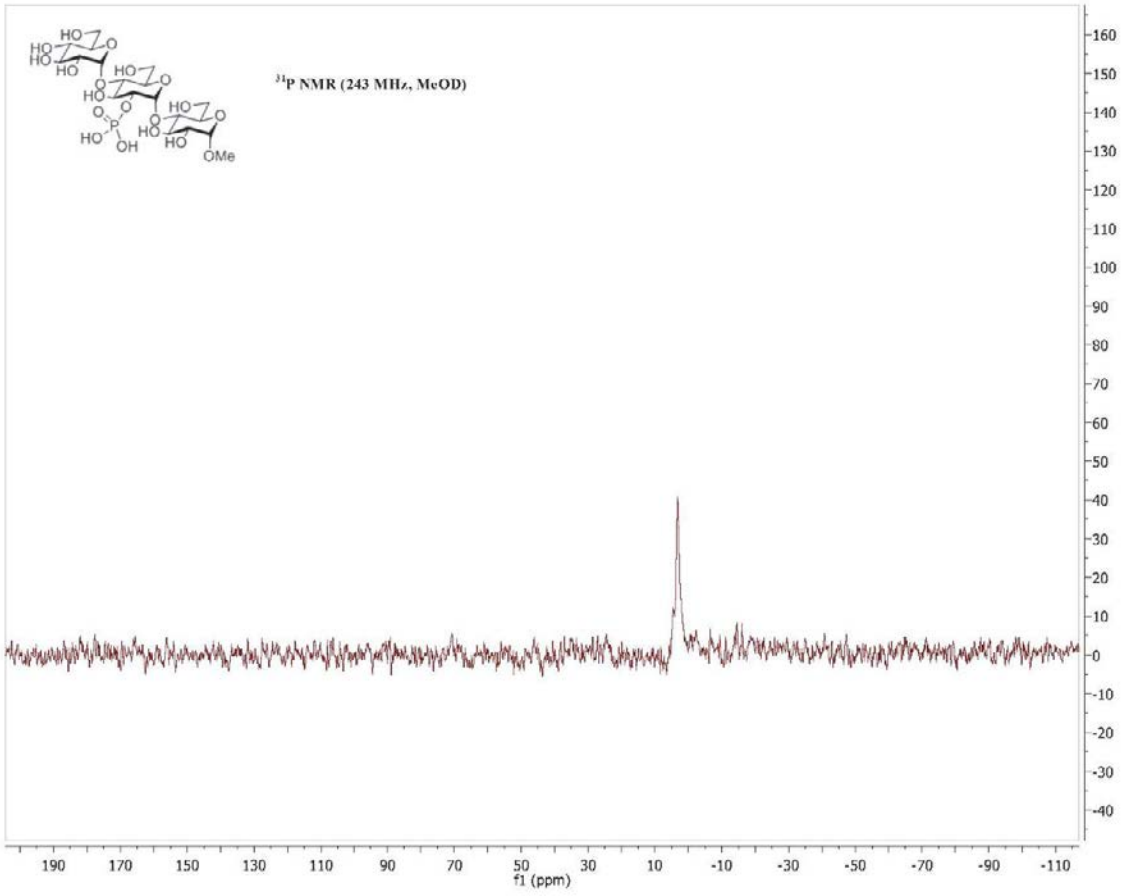




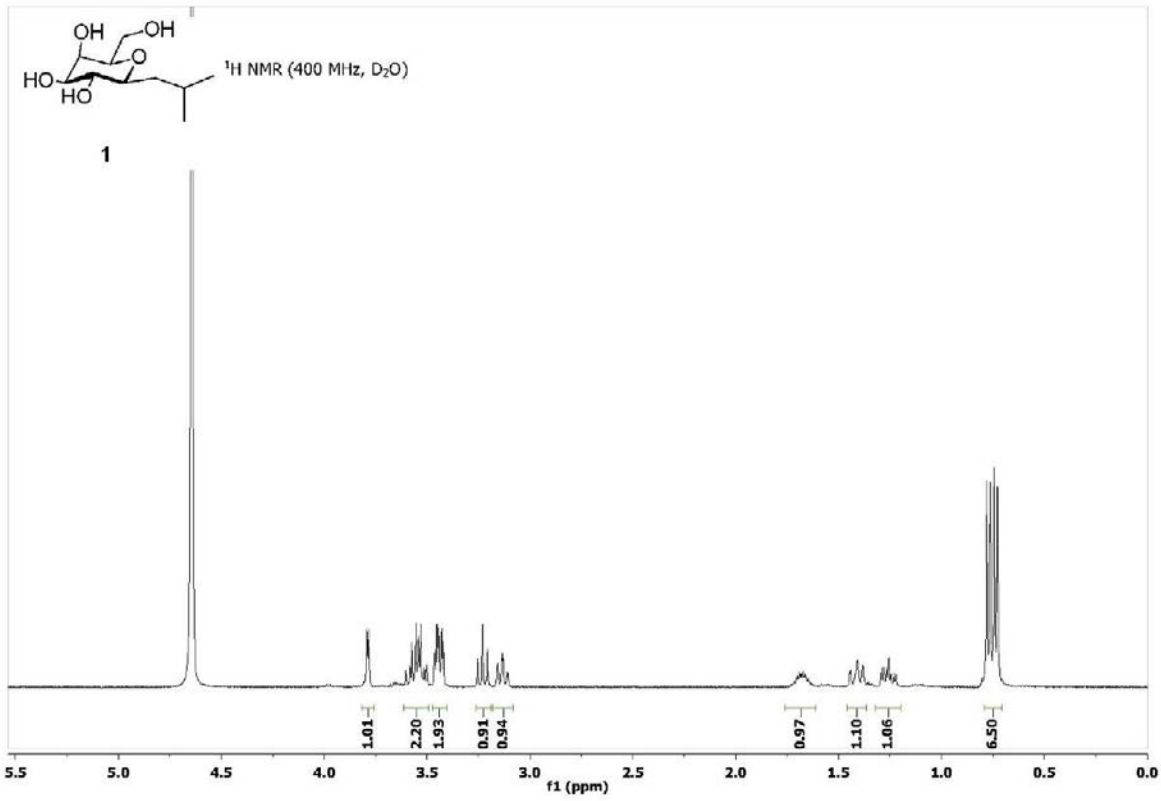


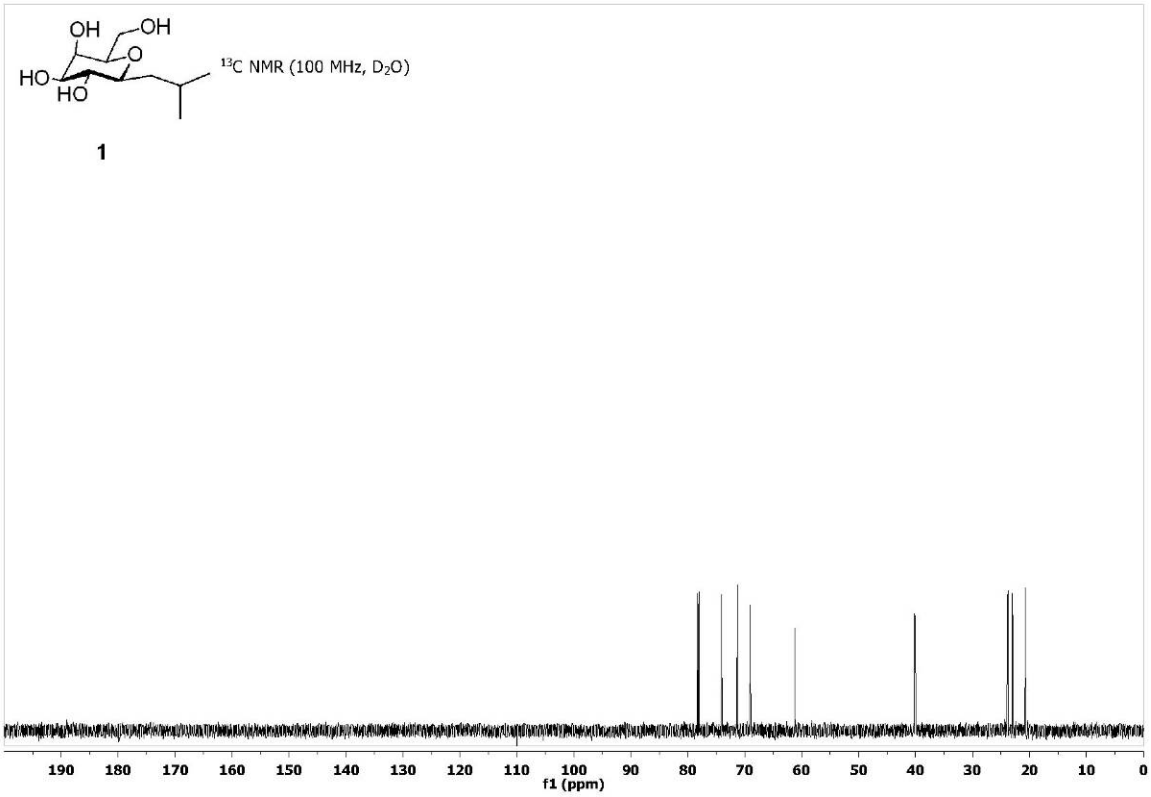


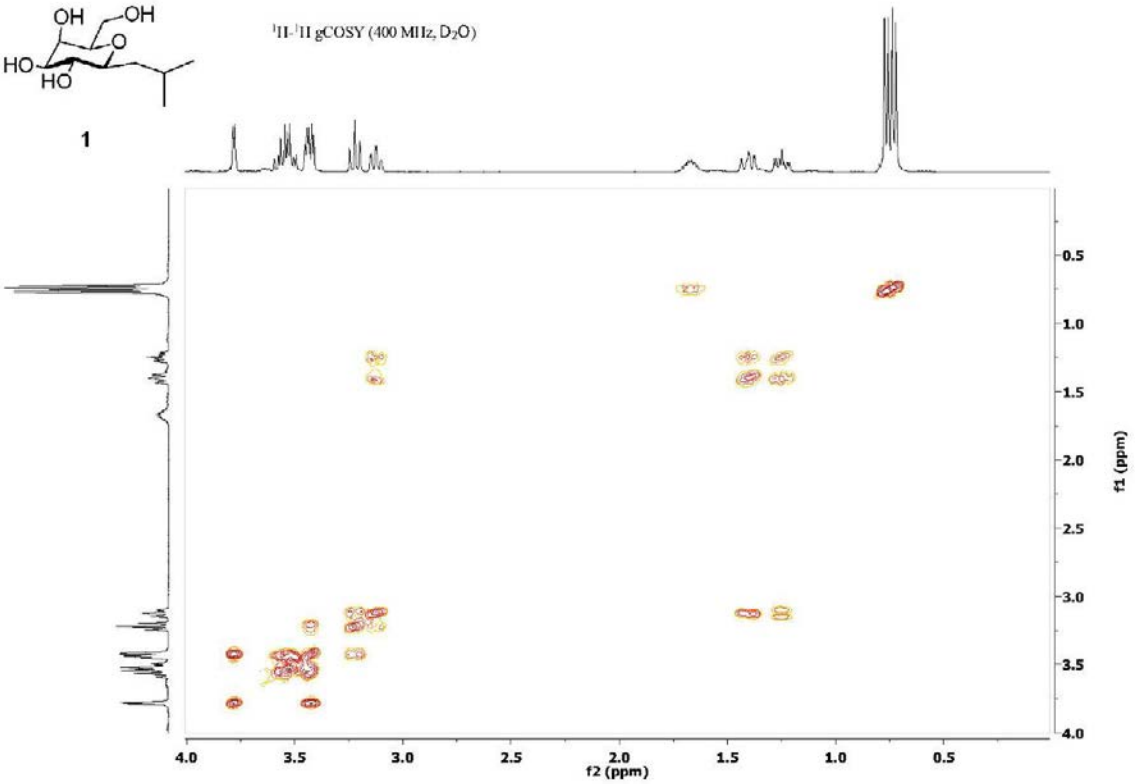


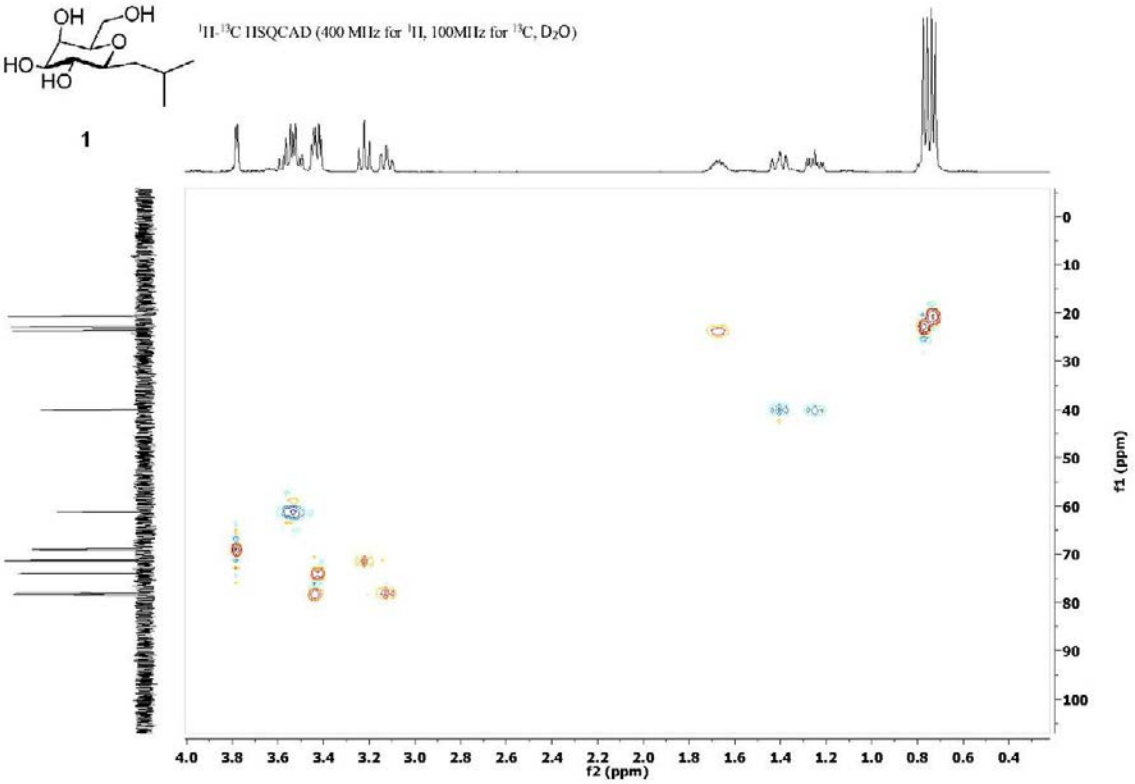


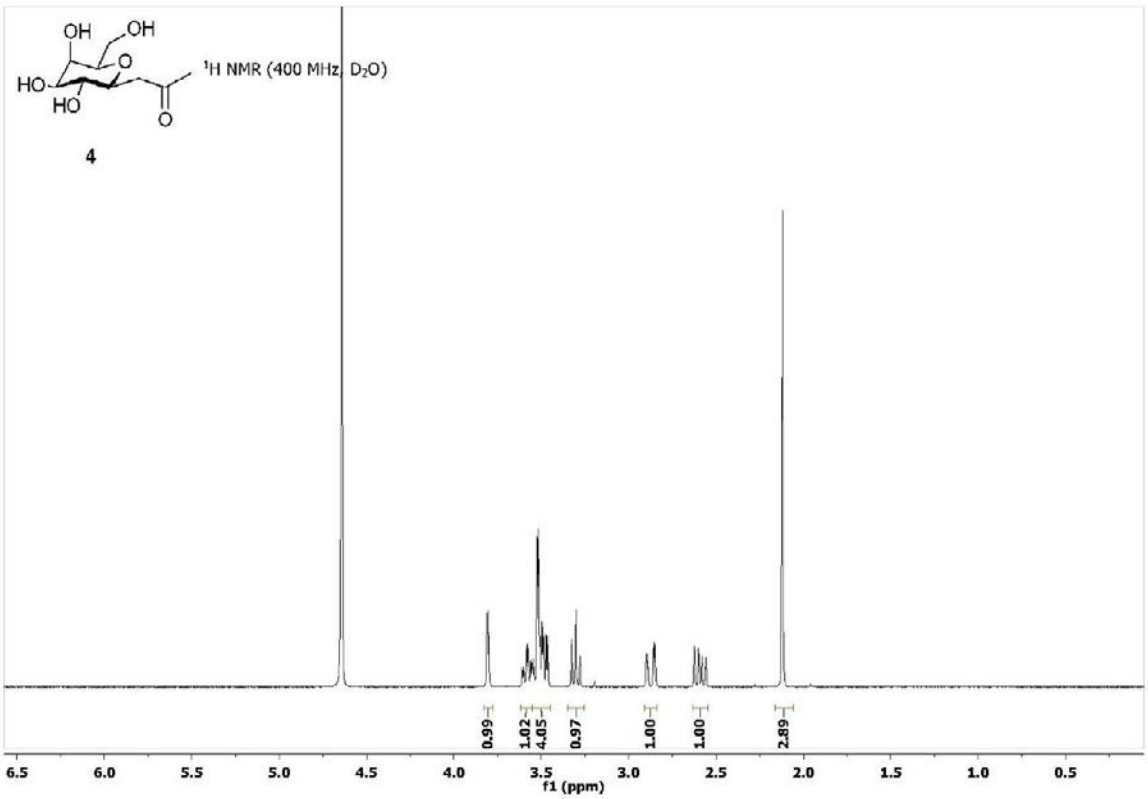
APPENDIX C. CHAPTER 4 ^{1}H AND ^{13}C NMR SPECTRA

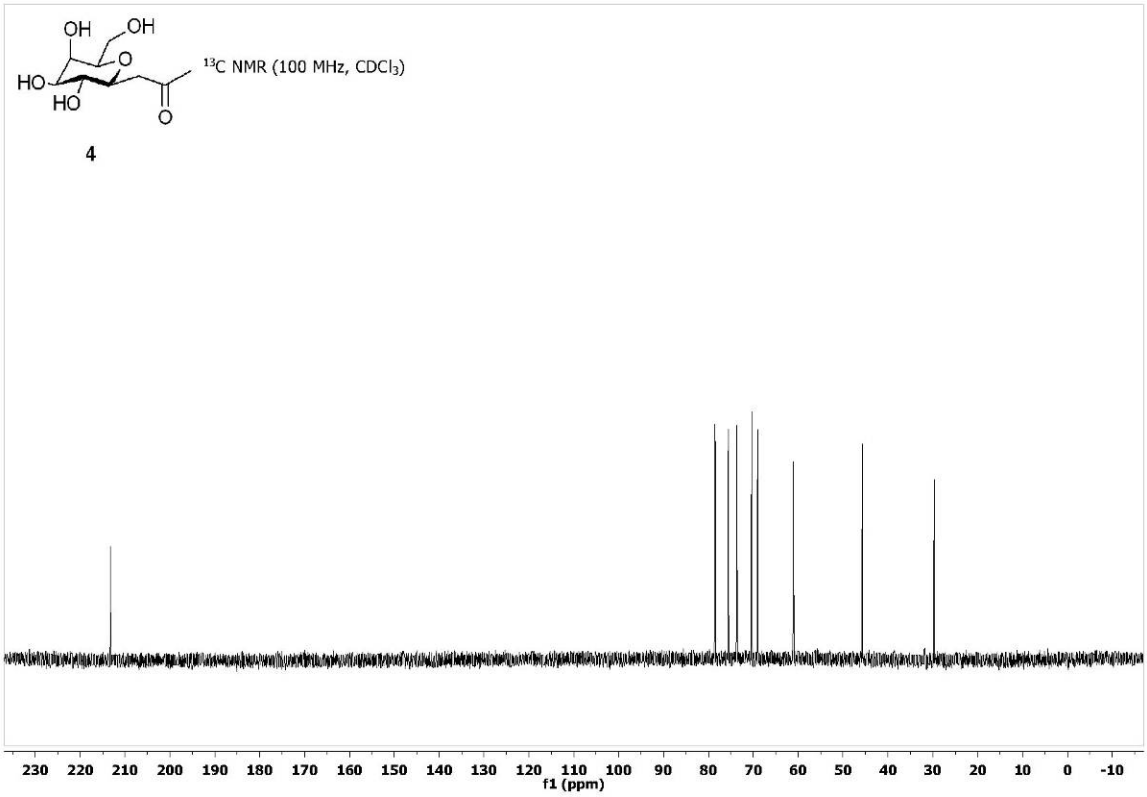


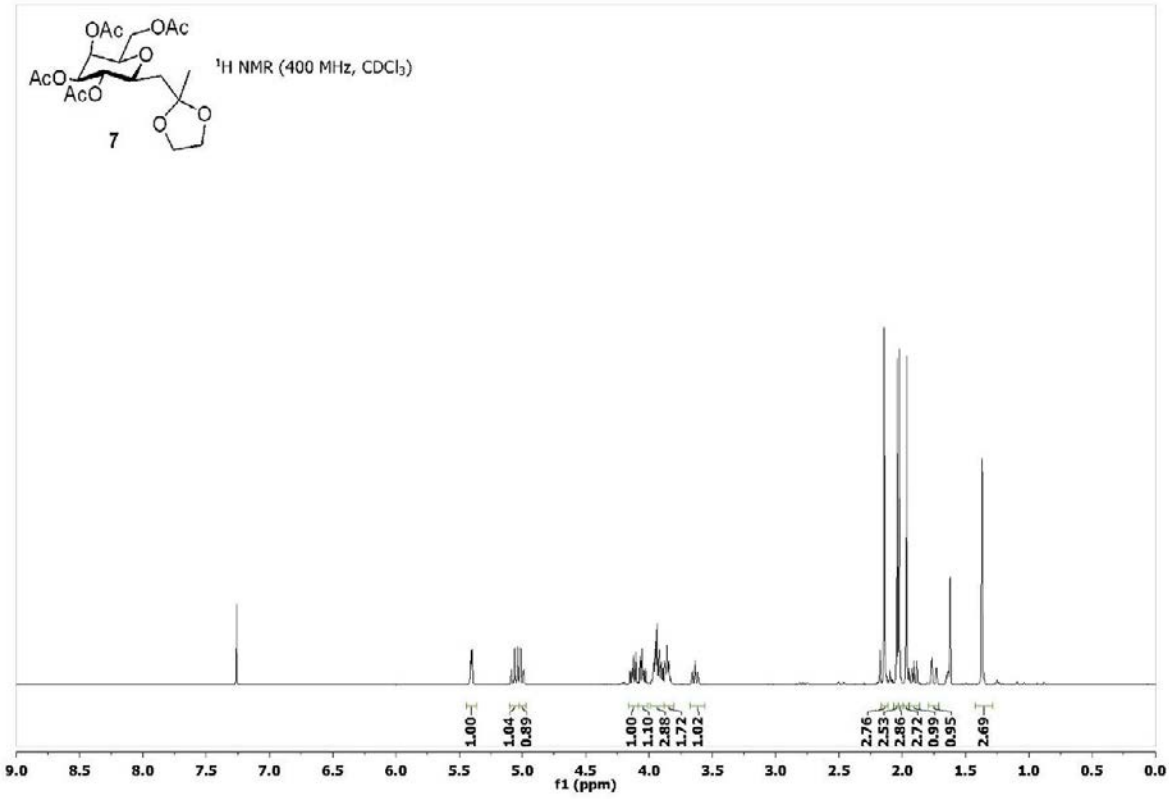


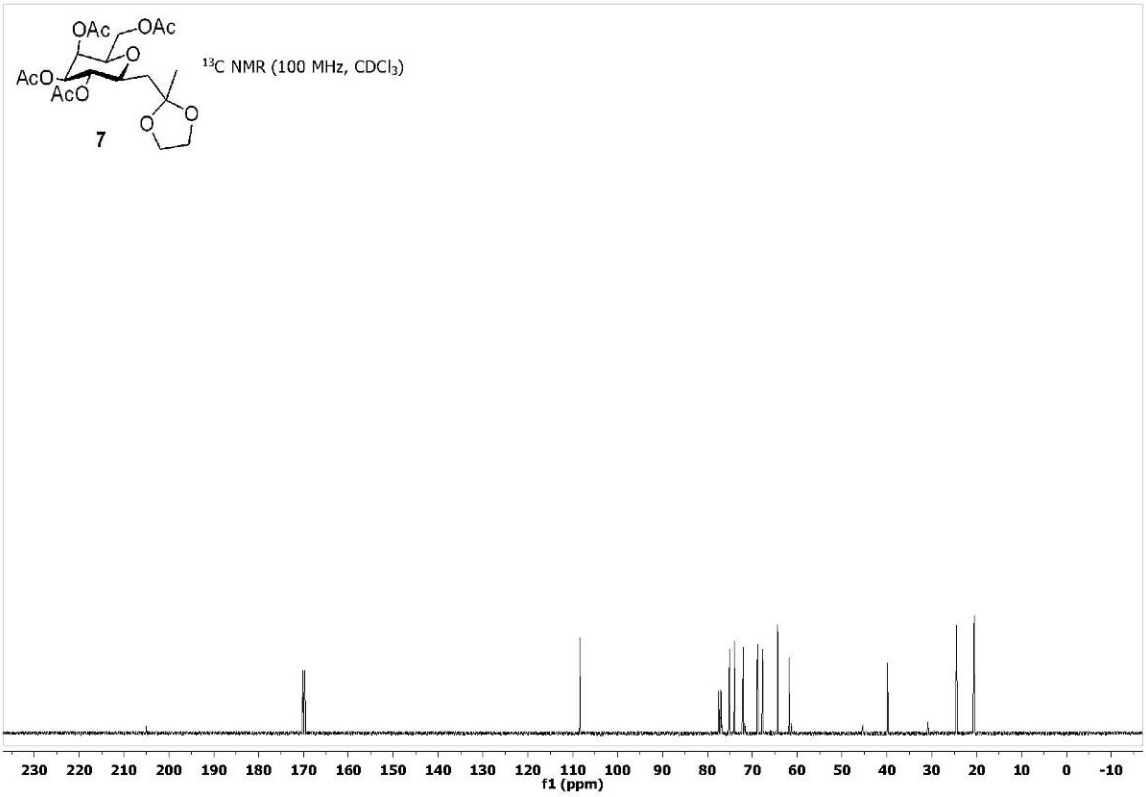


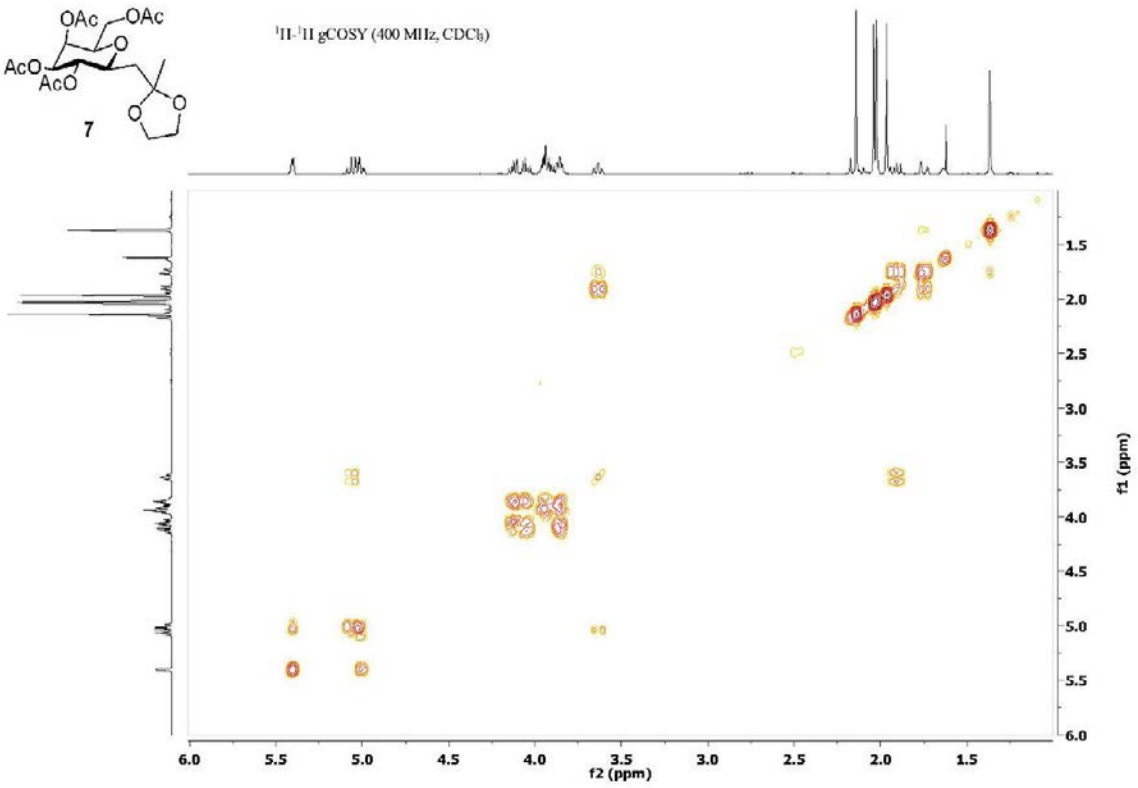


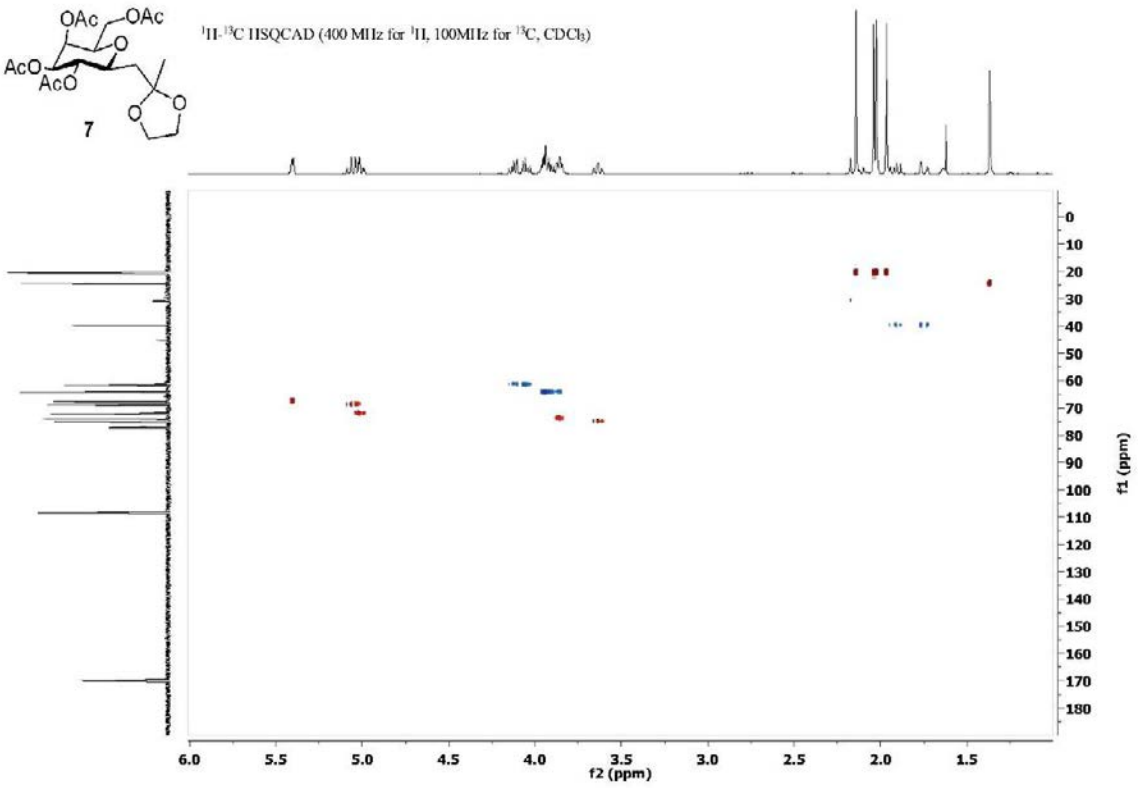


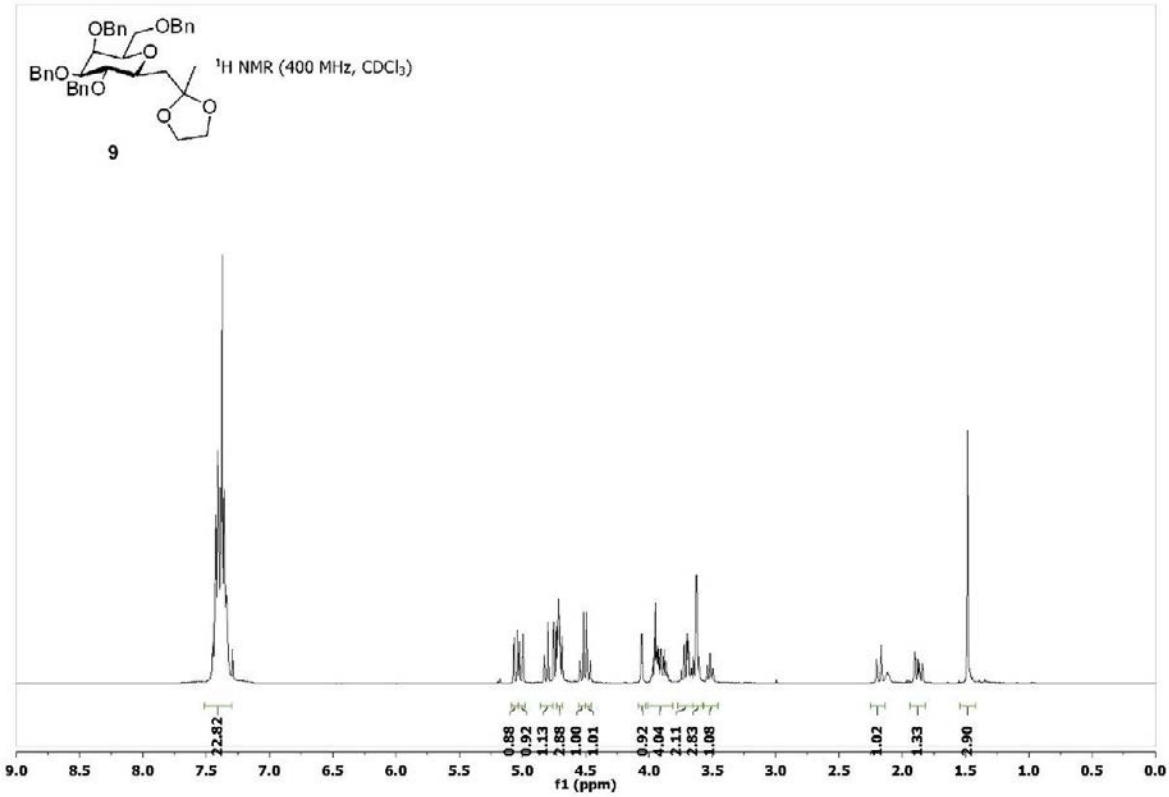


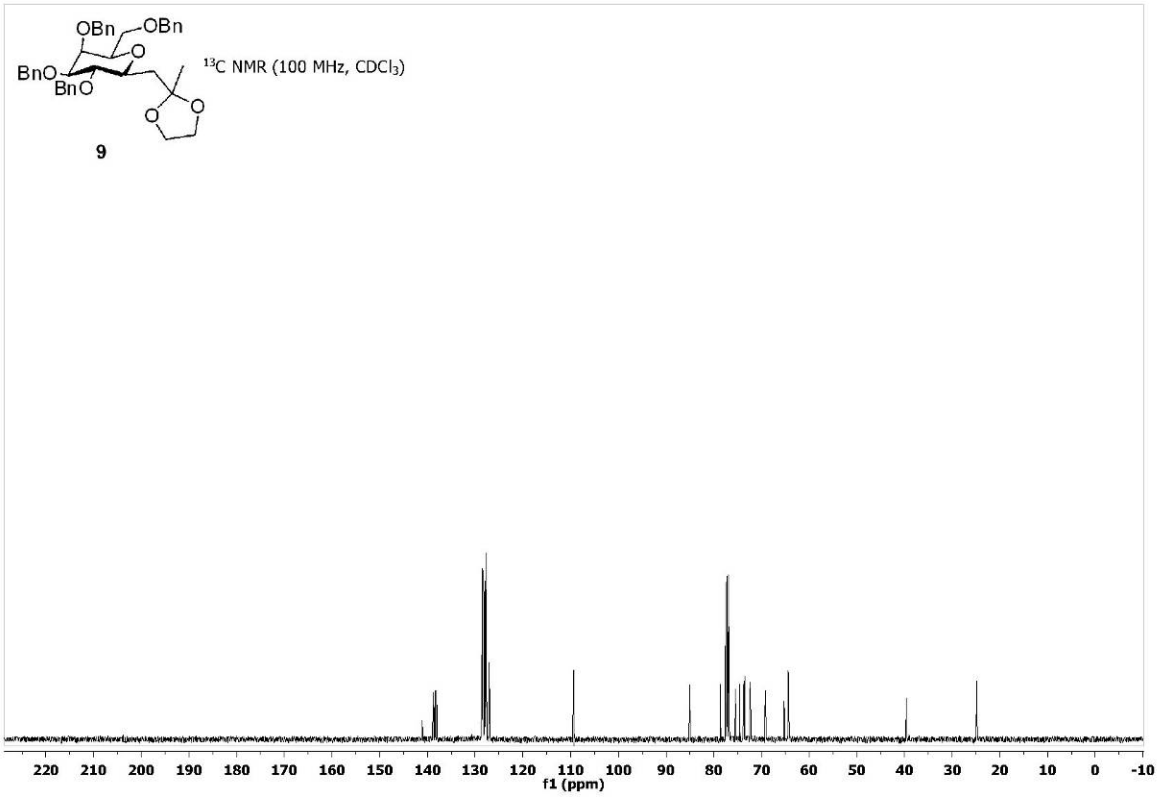


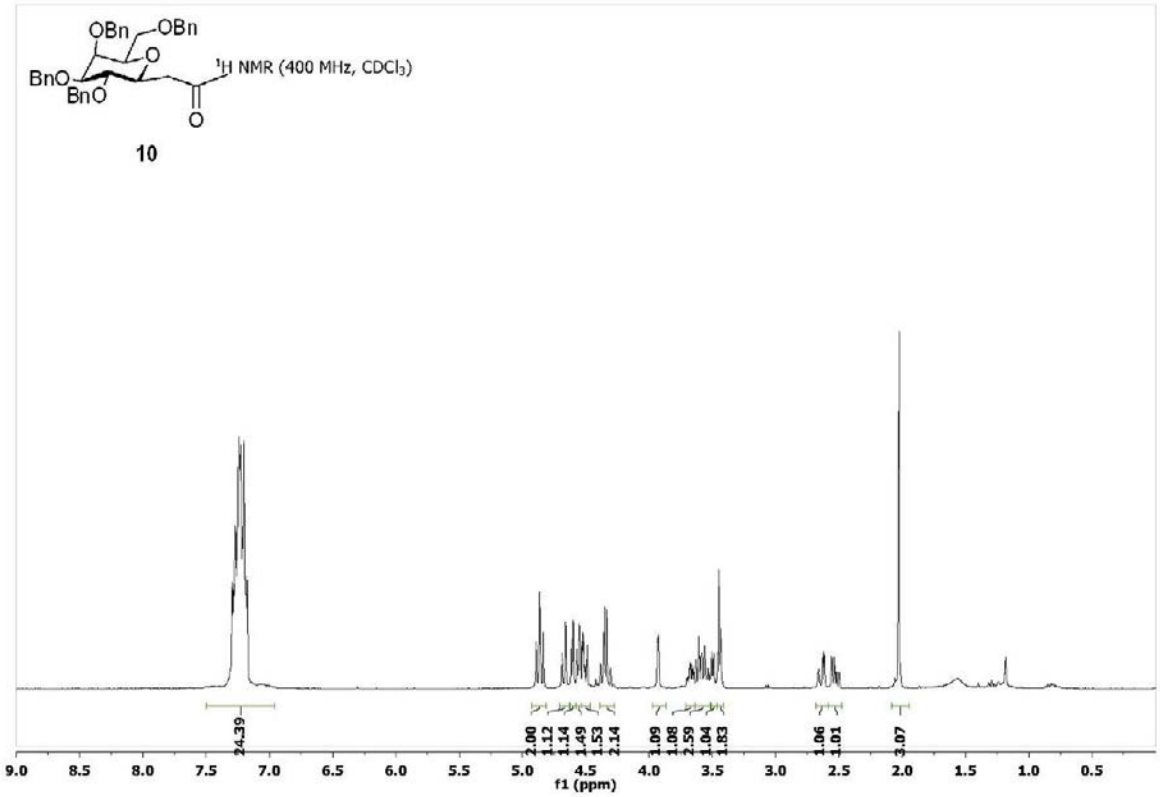


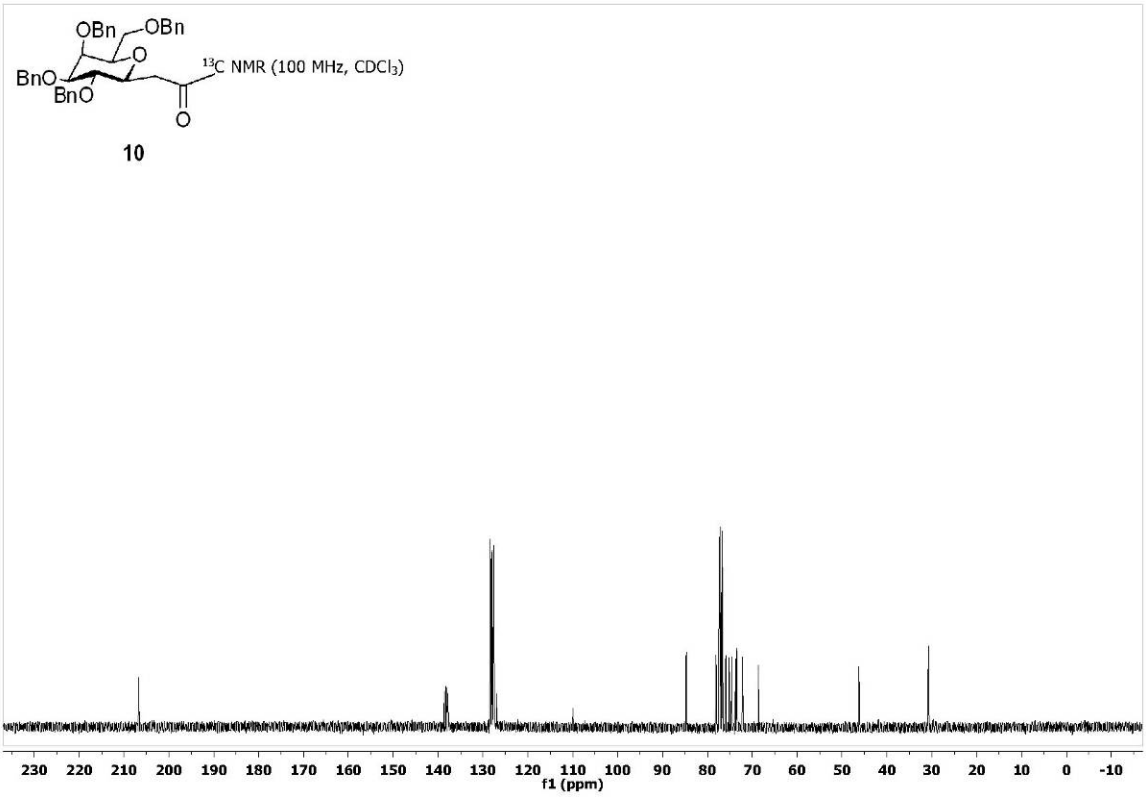


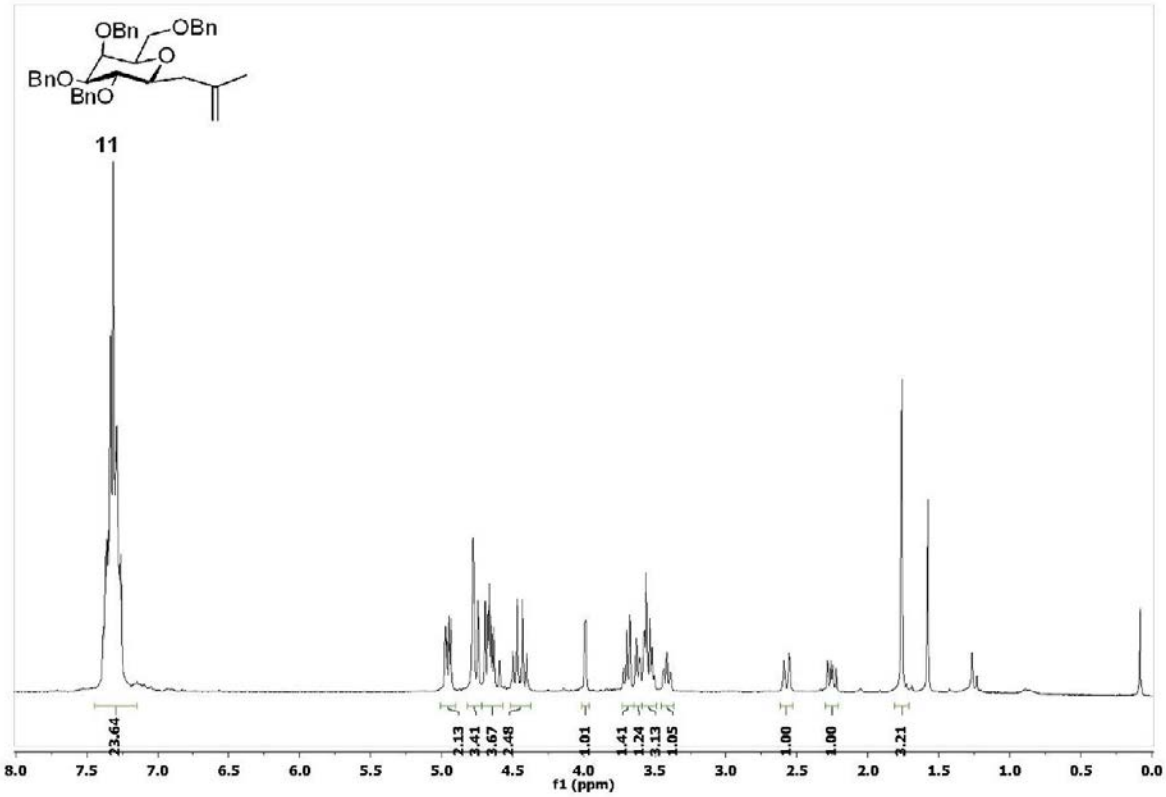


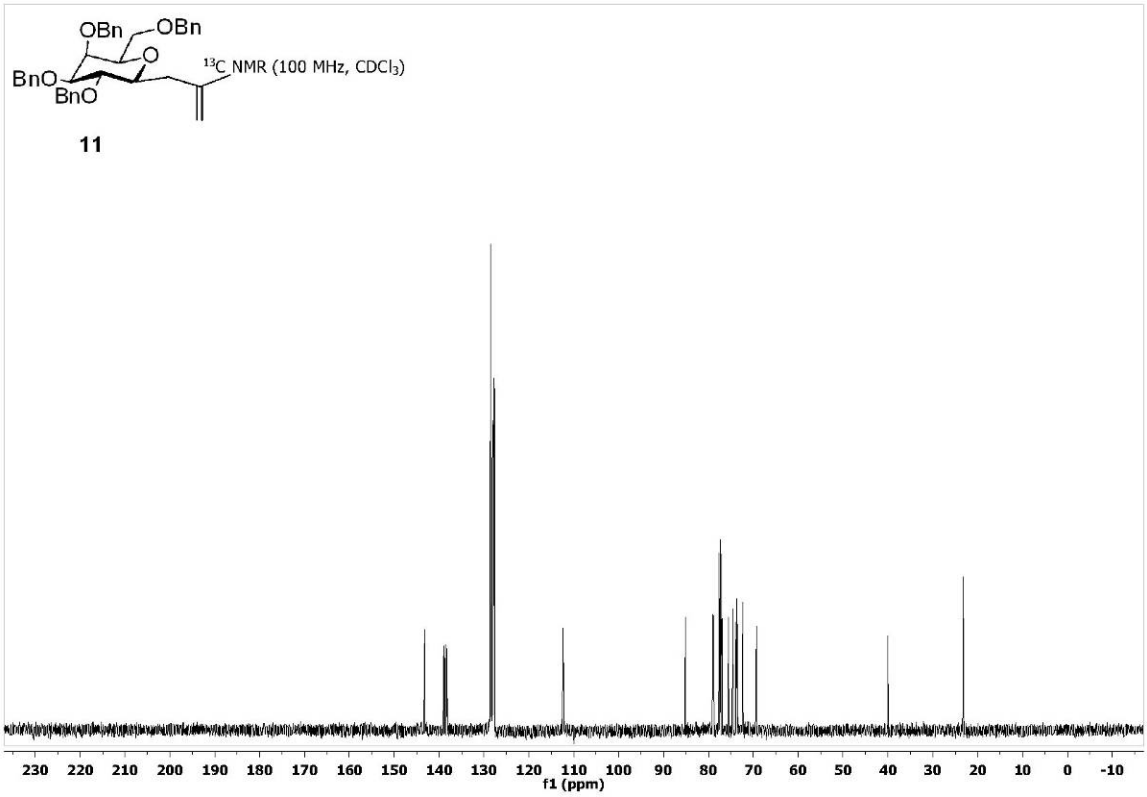


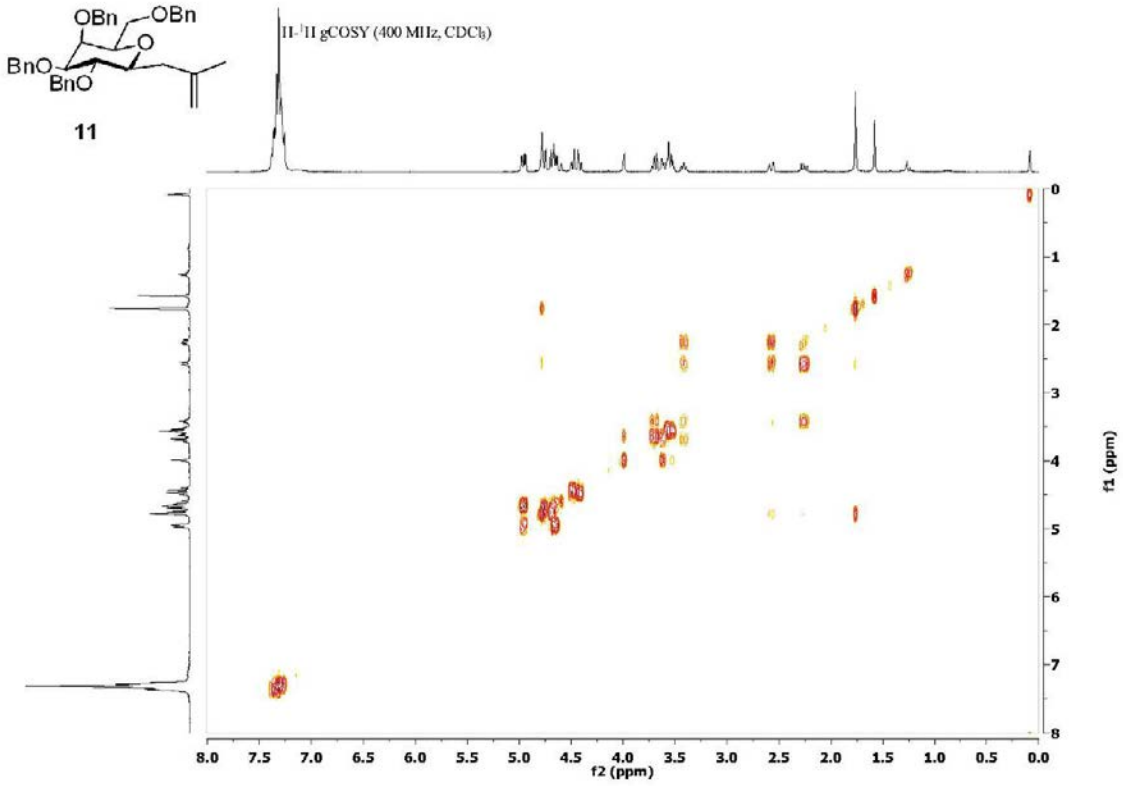


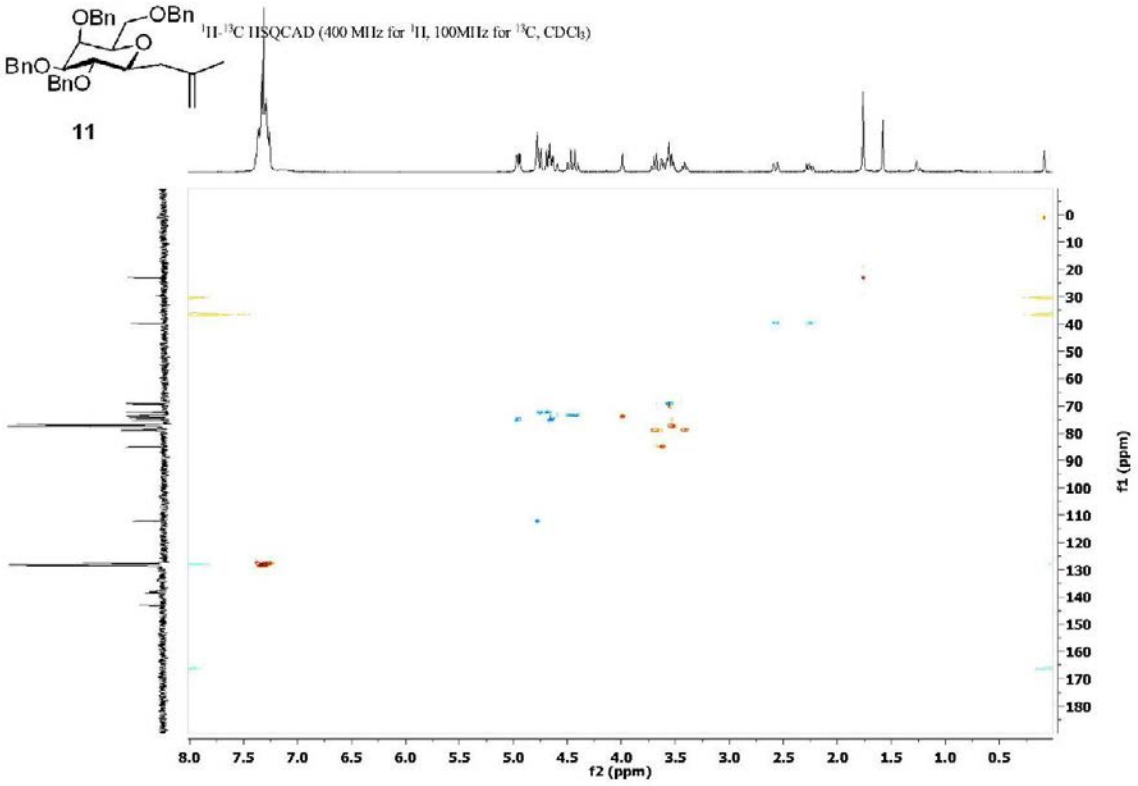


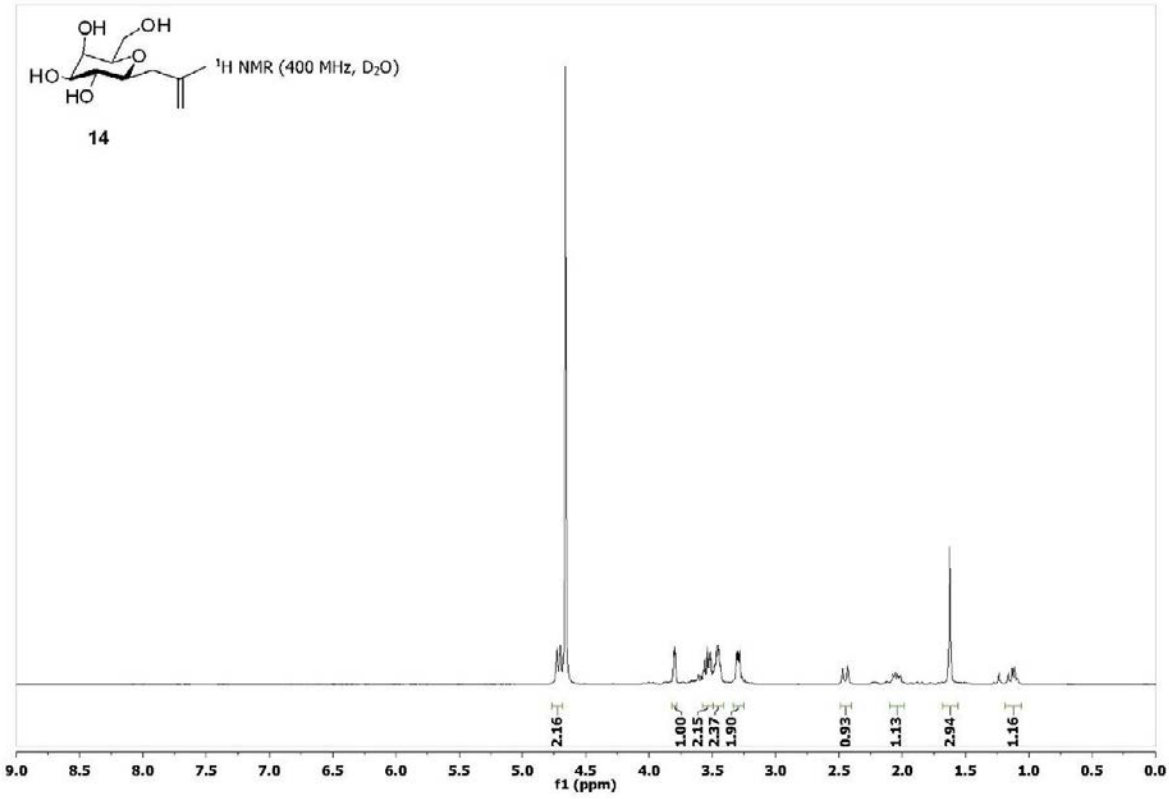


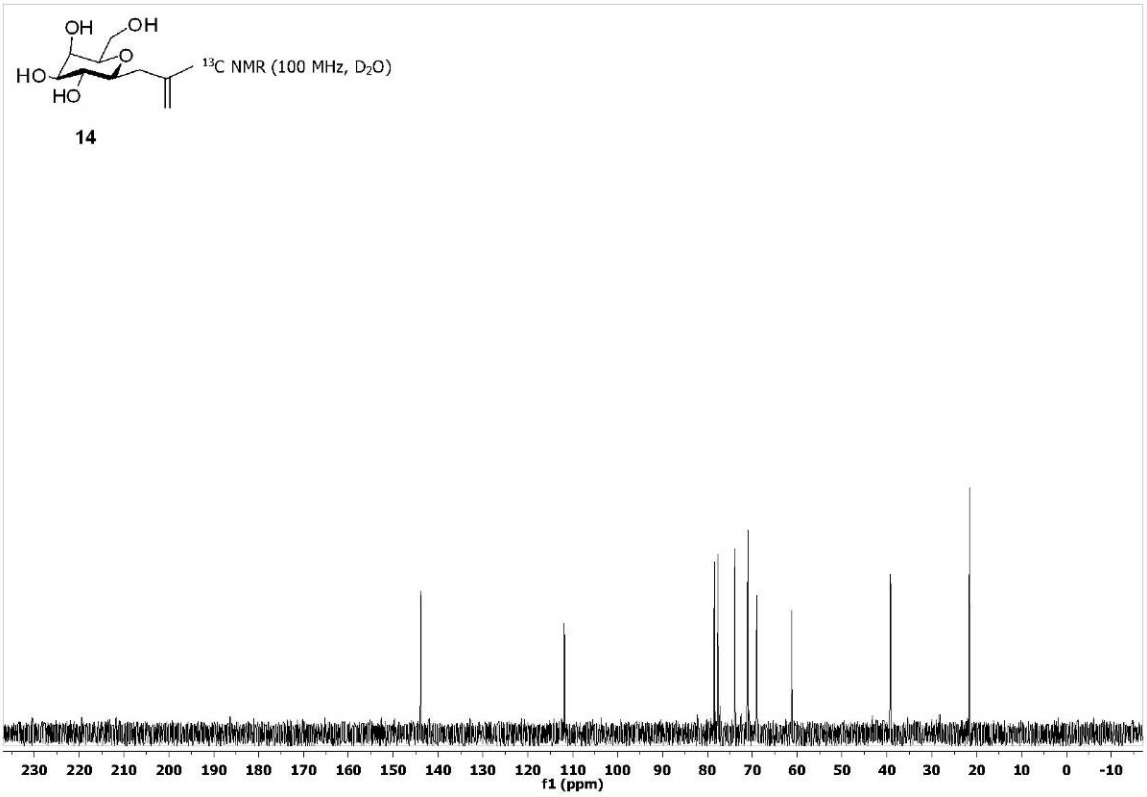


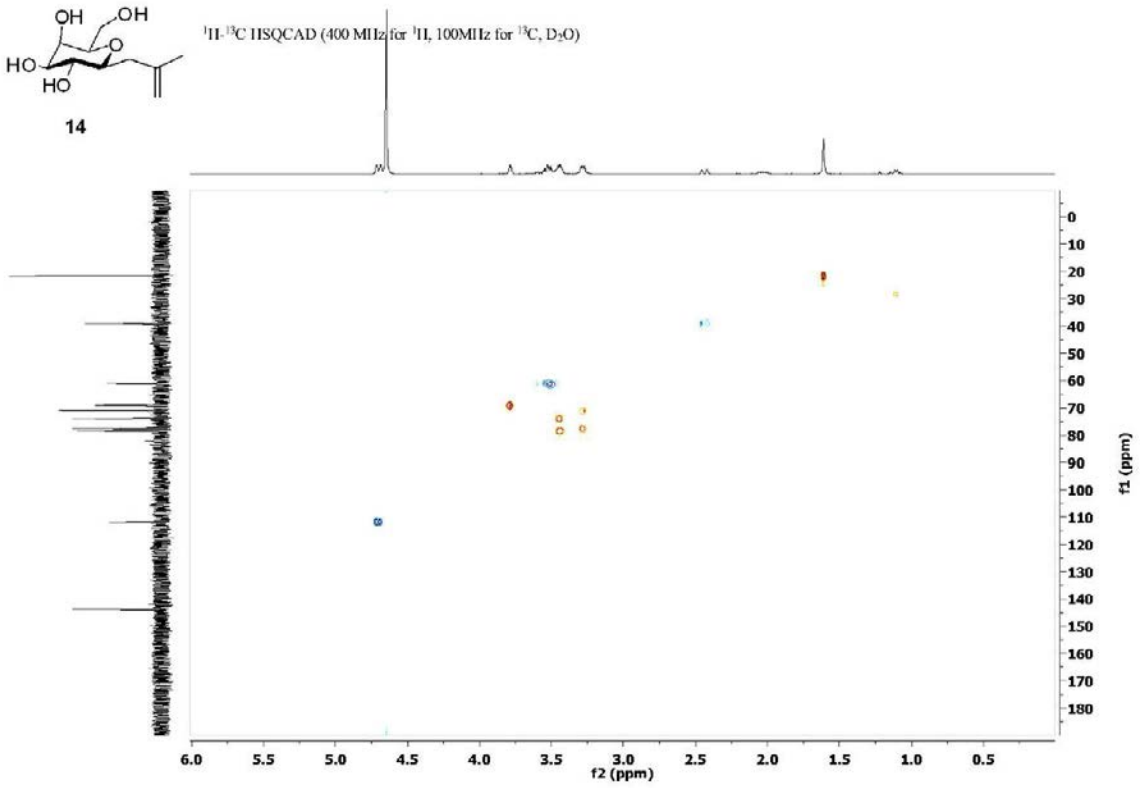












APPENDIX D. CHAPTER 5 ^{1}H AND ^{13}C NMR SPECTRA