Preparation of Oligosaccharides and Related Structures

By

Cleo Sabrina Evans

Thesis

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

MASTER IN SCIENCE

in

Chemistry

August 12, 2022

Nashville, Tennessee

Approved:

Steven D. Townsend, Ph.D.

Jennifer A. Gaddy, Ph.D.

ACKNOWLEDGEMENTS

This work was supported financially by the Department of Chemistry at Vanderbilt University. This work was supported emotionally by my fiancé, Joshua Elder, my cats, my family, and my friends. I would also like to acknowledge Javier Ortiz Alvarado for his help screening glycosylation conditions last summer.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

Significance and Preparation of Oligosaccharide Repeating Units

1.1 Introduction and Background

Bacterial cells contain sugar residues on their outer membranes referred to as capsular polysaccharides (CPSs) or lipopolysaccharides (LPSs).[1, 2] CPSs are comprised of oligosaccharide repeating units that connect to form polysaccharides. (**Figure 1A**) Repeating units are unique to bacterial species and they can vary across strains. Because of this, repeating units are used to differentiate strains of bacteria, even within the same species.[2, 3] LPSs are CPSs that contain long-chain carbon residues on some of the sugars' hydroxyl groups. As such, this thesis refers to the sugar coatings of bacteria as CPSs since LPSs serve similar functions.[2]

Bacteria primarily thwart host immune cells through molecular mimicry.[1, 3] Bacteria secrete molecules that mimic host proteins. This camouflages the bacteria from the host immune system.[4, 5] In some cases, CPSs contain similar sugar residues to the caps on host glycoproteins; so, they also contribute to this defense mechanism.[6] Since CPSs are considered the strongest virulence factor for bacterial species, and since they are used to differentiate strains, antibiotic therapies are designed with CPSs in mind. CPSs enable the infiltration and colonization of host cells by bacterial species by increasing adhesion to host cells, increasing biofilm formation by stabilizing extracellular matrix formation between planktonic cells, and encapsulating the bacteria to shield them from host antibodies and antibiotics[1, 3] (**Figure 1A**). Additionally, bacterial species can modify their CPSs or LPSs to thwart these targeted antibiotic therapies.[5, 7, 8]

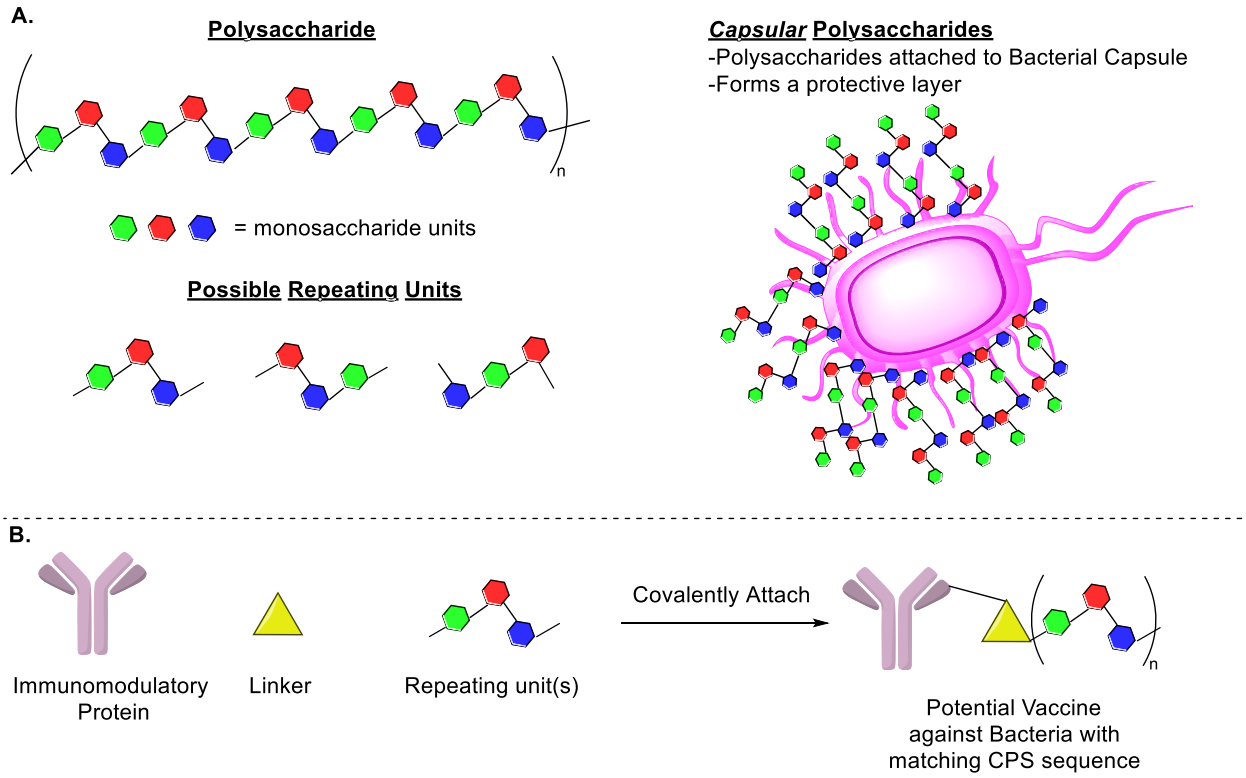


Figure 7. A. Capsular Polysaccharides B. Vaccine Development

Antibodies identify virulent cells by targeting their unique CPSs or LPSs. As such, repeating units and their respective polysaccharides have garnered significant interest as vaccine components.[9] Oligosaccharide repeating units can be appended to immunogenic carrier proteins for direct use in vaccines (**Figure 1 B)**.[10] If a single repeating unit adduct fails to elicit an immune response, the polysaccharide can be accessed by polymerizing the repeating unit using methods such as Ring Opening Metathesis Polymerization (ROMP) or newer 3D-printing photopolymerization. [11, 12] These polysaccharides, then, are then bound to immunogenic carrier proteins for use in immunological studies and vaccines.

1.2 Previous Syntheses of Oligosaccharide Repeating Units

Isolation procedures may produce low yields of these polysaccharides, and the conditions used can compromise the structural integrity of the CPSs. Synthetic routes toward polymerizable CPS repeating units provide a facile, scalable alternative to messy, low-yielding isolation procedures.[11]

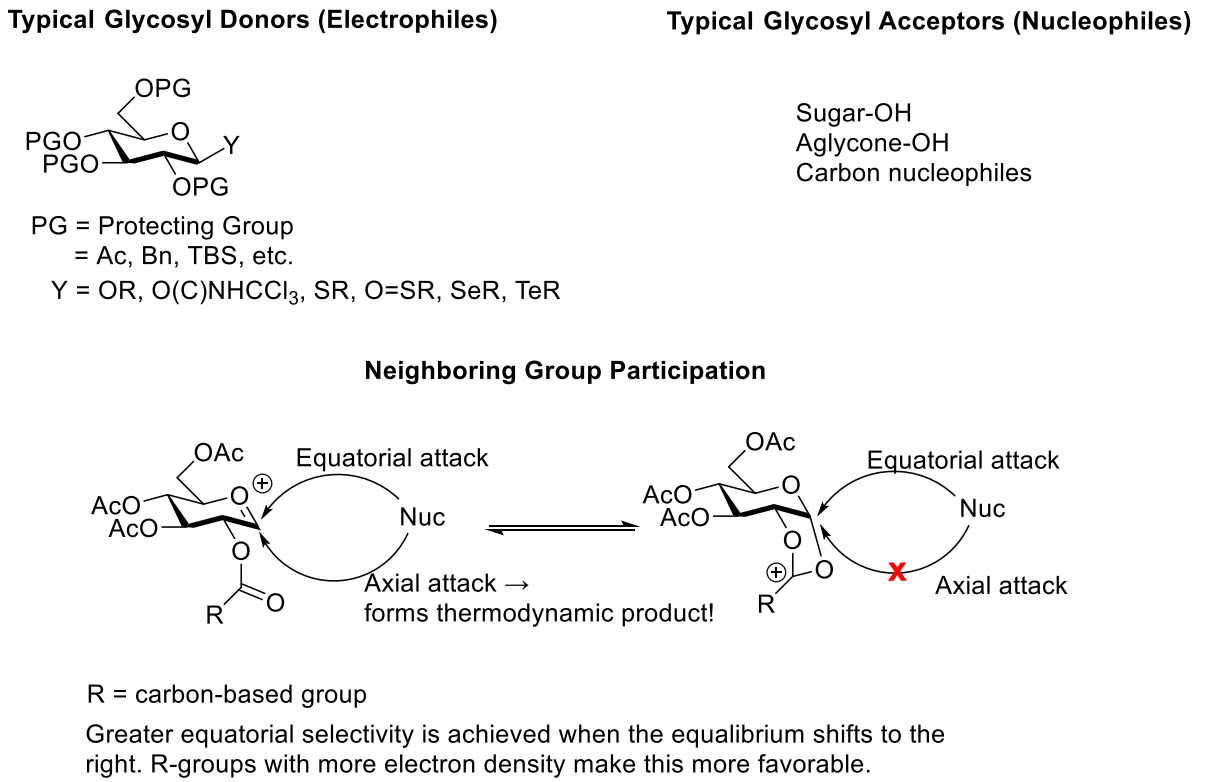


Figure 8. Glycosyl Donors, Acceptors, and Neighboring Group Participation

Oligosaccharide repeating units typically are accessed through sequential glycosylations of mono- or oligosaccharide acceptors (nucleophiles) with protected mono- or oligosaccharide donors (electrophiles) (**Figure 2)**.[13-15] Regioselectivity usually is not an issue under typical glycosylation conditions because there is one electrophilic site on the donor and one nucleophilic site on the acceptor. However, glycosylations between sugar residues require stringent anhydrous

conditions because even minute quantities of water represent stoichiometric amounts of a competing acceptor.

Attaining the desired stereoselectivity at the newly-forming anomeric center poses the greatest challenge, particularly when trying to selectively synthesize the kinetic product of the glycosylation. The kinetic product typically is the product with the glycosidic oxygen in the equatorial position.[16] Low temperatures (-78 °C to -20 °C) may contribute to this selectivity; but, the kinetic product typically is synthesized through the employ of neighboring group effects. Installing sterically hindered protecting groups at an equatorial C2 position may block the bottom face of the sugar and force the incoming nucleophile to attack equatorially (**Figure 2)**. Neighboring group participation is achieved through the installation of acyl groups at the C2 position. The pi system can form a cyclic intermediate with the oxonium ion glycosylation intermediate during the S_N1-type reaction (**Figure 2**). When this occurs, the nucleophile can only attack from the equatorial side in an S_N2-type fashion to attain the desired product. Using a sterically hindered and/or less electron-poor acyl group increases selectivity by blocking the bottom face or stabilizing the cyclic intermediate, respectively.

In more recent studies, acylated O-glycosides are seldom employed as donors due to their inherently low reactivity. Donors typically are thioglycosides (SH, SR, S-Aryl, O=SR, O=S-Aryl)[17] or acetimidates ($O(C)NHCCl_3$ or $O(C)NPhCF_3$).[13] Selenoglycosides (SeH, SeR, Se-Aryl) and telluroglycosides (TlR, Tl-Aryl) also have been employed.[18, 19] Selenoxides (Se(O)Aryl or Se(O)Alkyl) were successfully synthesized in one study from the early 1990s;[20] but, they were only used in one test glycosylation with peracetylated glucose, and no scope was ever generated for this donor type. No total or partial syntheses have been conducted using selenoxide donors.

The following literature examples employed these aforementioned strategies to access repeating units in high selectivity and yield. The first synthesis comes from the Townsend group in 2018. Dr. Jamin Keith synthesized a zwitterionic trisaccharide repeating unit of *Morganella morganii* to pave the way for investigations into the immunomodulatory properties of the zwitterionic polysaccharide (ZPS).[11] This zwitterionic CPS is able to activate T-cells without appendage to an immunogenic carrier protein, making it an attractive target.[11] The trisaccharide contains one β-linkage and one α-linkage. In Keith's 1+1 glycosylation, he used trimethylsilyltriflate as an activator and employed a trichloroacetimidate donor with a C2 acetyl protecting group to get β-selectivity (equatorial attack). For his 2+1 glycosylation, he employed a similar activator and donor-type *without* a participating group at C2 to get α-selectivity (axial attack).

Zhang and Seeberger recently synthesized two trisaccharide repeating units from *Acinetobacter baumanii* AB5075 ([→3)-β-d-ManpNAcA-(1→4)-β-d-ManpNAcA-(1→3)-α-d-QuipNAc4NR-(1→] where R indicates (*S*)-3-hydroxy butanoyl or acetyl in a ratio of approximately 2.5:1). [21] They ran their glycosylations at -20 °C in dichloromethane using triflic acid as the activator. They employed thioglycoside donors with N-iodosuccinimide (NIS) co-reagent in their 1+1 and 1+2 glycosylations. Levulinyl ester protecting groups at the C2 positions of the donors imparted the desired β-selectivity (equatorial attack) through neighboring group participation. Using these conditions, they achieved complete β-selectivity at each new anomeric center. They also included a linker on the trisaccharide to make conjugation to an immunogenic carrier protein easier.

The following synthesis of the disaccharide repeating unit from *Aeromonas veronii* strain Bs8 employs similar strategies to access the desired linkage.[22]

CHAPTER 2

Preparation of Disaccharide Repeating Unit from *Aeromonas veronii* strain Bs8

This chapter is adapted from "Synthesis of the *Aeromonas veronii* strain Bs8 disaccharide repeating unit" published in *Carbohydrate Research* and has been reproduced with the permission of the publisher and my co-authors (Johny M Nguyen, Nicole M Wright, and Steven D Townsend).[22]

2.1 Background

Virulent bacterial species such as *Aeromonas veronii* deplete fish populations in fisheries and aquaculture farms by causing lesions, infections, and death.[23, 24] This contributes to significant loss of revenue for the industry.[25] Antibiotics have been weaponized to fight pathogenic species such as these since the early 1900s.[26, 27] Unfortunately, overuse of antibiotics led to the development of antibiotic resistant strains that now pose a major threat to global health.[28] The use of antibiotics in aquaculture farms exacerbates the resistance crisis by giving rise to new mutants.[29, 30]

Aeromonas species are transmissible to humans through food and water sources. They have been shown to be toxic to the human gut and cause gastroenteritis.[31] *A. veronii,* specifically, is one known cause of sepsis in Thailand and ulcerative syndrome in Saudi Arabia.[32, 33] Because of antibiotic resistance amongst *Aeromonas* species, it is increasingly important to develop vaccines.[34, 35] As mentioned, this can be achieved by appending CPSs or CPS repeating units to an immunogenic carrier protein. Several representative *Aeromonas* polysaccharide structures are shown in **Figure 3**.

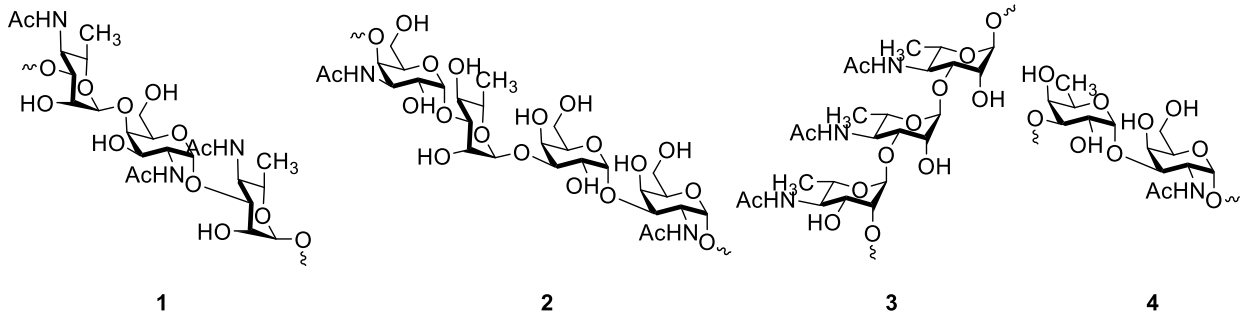


Figure 9. Sample *Aeromonas* polysaccharides

We chose to investigate the synthesis of the disaccharide repeating unit **4** because of its presence in a new strain of *Aeromonas veronii* called Bs8 (**Figure 3**).[24] The disaccharide repeating unit consists of repeating α-D-Fucp and β-D-GalpNAc units connected by 1,3 glycosidic bonds. We hope that the facile, scalable route toward this disaccharide presented in this dissertation will allow for more immunological studies and vaccine development against this and future strains. Herein I report our 11-step, gram-scale total synthesis of this O-specific polysaccharide repeating unit.

2.2 Preparation of Disaccharide Repeating Unit from *Aeromonas veronii* strain Bs8

The disaccharide repeating unit target has the following structure: α-D-Fucp-(1→3)-β-D-GalpNAc with a PMP group capping the reducing end of the sugar. We postulated that we could access PMP-capped target **5** from commercially available D-Fucose and D-Galactosamine (**Figure 4**).

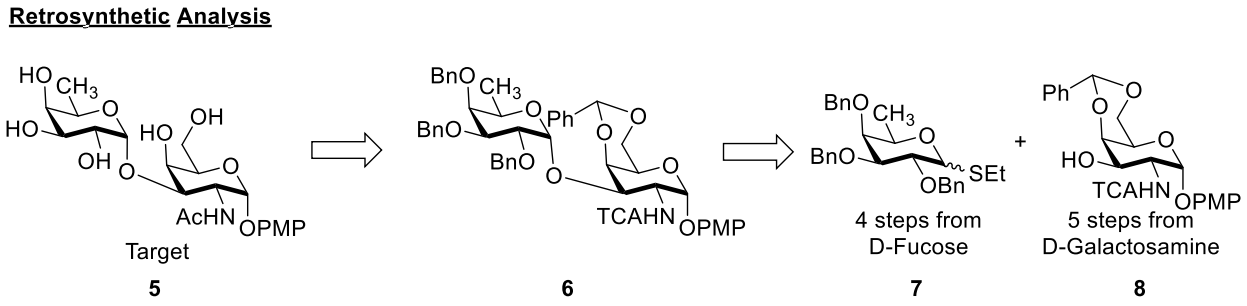
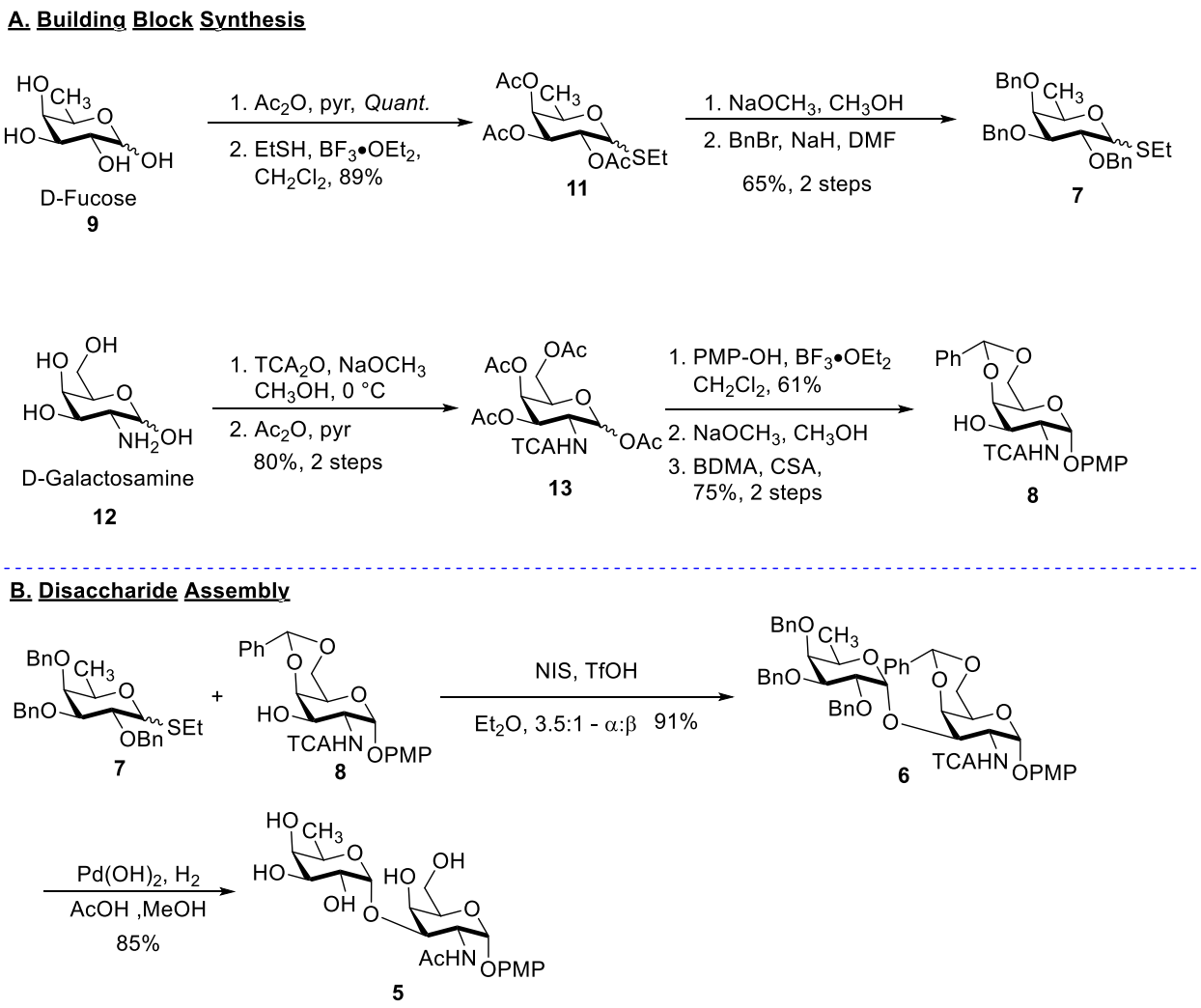


Figure 10. Retrosynthetic Analysis

Peracetylation of D-Fucose proceeded in quantitative yield (**Scheme 1A**). Thioglycosylation with boron trifluoride diethyl etherate and ethane thiol provided donor **11** in 89% yield. From there, we swapped the acetyl groups for benzyl groups. This served two purposes: activating the donor with electron-rich aryl rings *and* eliminating the potential for β-directing effects from the C2 acyl protecting group. With fucosyl residue **7** in-hand, we turned our attention to constructing the second building block.

We treated D-Galactosamine with trichloroacetic anhydride and sodium methoxide at low temperature to selectively protect the C2 amine. Then, we acetylated the hydroxyl groups with acetic anhydride and pyridine to produce intermediate **13** in 80% yield over two steps. Glycosylation with para-methoxyphenol and boron trifluoride diethyl etherate capped and

protected the reducing end of the sugar. This was followed by deacetylation of the hydroxyl moieties with sodium methoxide and subsequent benzylidene acetal formation between the C4 and C6 hydroxyls to yield acceptor **8**.



Scheme 9. A. Building Block Synthesis B. Disaccharide Assembly

After synthesizing the two necessary building blocks, selective alpha glycosylation (3.5:1, α:β) was achieved in high yield by reacting the donor and acceptor together in diethyl ether at low temperature with NIS and catalytic triflic acid as activators (**Scheme 1B**). The final hydrogenolysis

with Pearlman's catalyst in acetic acid and methanol served to remove the three benzyl ethers, remove the benzylidene acetal, and convert the trichloroacetamide to the acetamide to produce target disaccharide **5** in 11 steps. NMR analysis between target **5** and repeating unit isolates were comparable.

2.3 Experimental Methods & Instrumentation

Commercial reagents were used as received. Anhydrous solvents were taken from an MBRAUN solvent purification system (MB SPS) and stored over 4 Å or 3 Å molecular sieves. All moisture-sensitive reactions were performed in flame- or oven-dried round bottom flasks under an argon atmosphere. All air- or moisture-sensitive liquids were transferred via oven-dried stainless-steel syringes or cannula. Reaction temperatures were monitored and controlled via thermocouple thermometer and corresponding hot plate stirrer. Flash column chromatography was performed as described by Still et al. using silica gel 230–400 mesh. Analytical thin-layer chromatography (TLC) was performed on glass-backed Silica gel 60 F254 plates (EMD/Merck KGaA) and visualized using UV, cerium ammonium molybdate stain, and anisaldehyde stain. ^{1}H NMR spectra were obtained on a Bruker 400 or 600 MHz spectrometer with reporting relative to residual solvent signals ($CDCl_3$, 7.26 ppm; CD_3OD, 3.31 ppm; D_2O, 4.79 ppm). ^{1}H NMR spectral data are presented as follows: chemical shifts (δ, ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz), integration, proton assignment. ^{13}C NMR spectra were obtained on a Bruker 100 MHz spectrometer with reporting relative to residual solvent signals ($CDCl_3$, 77.16 ppm; CD_3OD, 49.0 ppm). ^{13}C NMR spectral data are presented as follows: chemical shifts (δ, ppm), carbon assignment. Proton and carbon assignments were made with the aid of 2D NMR techniques (COSY, HSQC, and HMBC). High resolution mass spectra were

recorded on a high resolution Thermo Electron Corporation MAT 95XP-Trap by use of electro-spray ionization (ESI) by the Indiana University Mass Spectrometry facility and a SYNAPT G2 or SYNAPT G2-S spectrometer (Waters, for TOFMS) by the McLean lab of Vanderbilt University. Optical rotations were obtained using a Perkin Elmer 341 polarimeter.

2.3.1 Compound Preparation and Characterization

2,2,2-trichloro-N-((2R,4aR,6S,7R,8R,8aR)-6-(4-methoxyphenoxy)-2-phenyl-8-(((2S,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-methyltetrahydro-2H-pyran-2-yl)oxy)hexahydropyrano [3,2-d] [1,3]dioxin-7-yl)acetamide (6**)**

Donor **7** (1.0 eq, 0.500 g, 1.04 mmol) and acceptor **8** (1.5 eq, 0.813 g, 1.6 mmol) were co-evaporated with benzene (2 × 8 mL) and placed in a vacuum desiccator containing P_2O_5 overnight. The donor/acceptor mixture was dissolved in diethyl ether (14 mL) and the resulting solution was cannulated into a reaction flask containing 4 Å powdered molecular sieves. The mixture was stirred under argon atmosphere for 1 h; then it was cooled to −78 °C and TfOH (0.1 eq, 0.038 mL in 0.2 mL CH_2Cl_2) was added. The reaction was stirred 2 h at -78 °C then quenched with Et_3N. The reaction was diluted with CH_2Cl_2, filtered through celite, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude residue was purified via flash column chromatography (2:1 hexanes/EtOAc) to yield disaccharide **6** (0.890 g, 0.95 mmol, 91%) as a yellow foam. R_F: 0.35 (1:1 hexanes/EtOAc); $[\alpha]D^{25}$ = +38.7° (c = 0.012, $CHCl_3$) (α major product),^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.25 (m, 20H), 7.02 (d, *J* = 9.1 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 1H,-NH), 6.85 (d, *J* = 9.0 Hz, 2H), 5.71 (d, *J* = 3.5 Hz, 1H), 5.26 (s, 1H), 5.16 (d, *J* = 3.5 Hz, 1H), 5.02 (d, *J* = 11.5 Hz, 1H), 4.85 (d, *J* = 11.9 Hz, 1H), 4.79–4.77 (m, 1H), 4.75 (d, *J* = 9.2 Hz, 1H), 4.72 (d, *J* = 7.2 Hz, 1H), 4.65 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 11.4 Hz, 1H), 4.40 (d, *J* = 3.3 Hz, 1H), 4.24 (d, *J* = 13.3 Hz, 1H), 4.20 (dd, *J* = 11.0, 3.2 Hz, 1H), 4.13 (dd, *J* = 10.1, 3.5 Hz, 1H), 4.07–

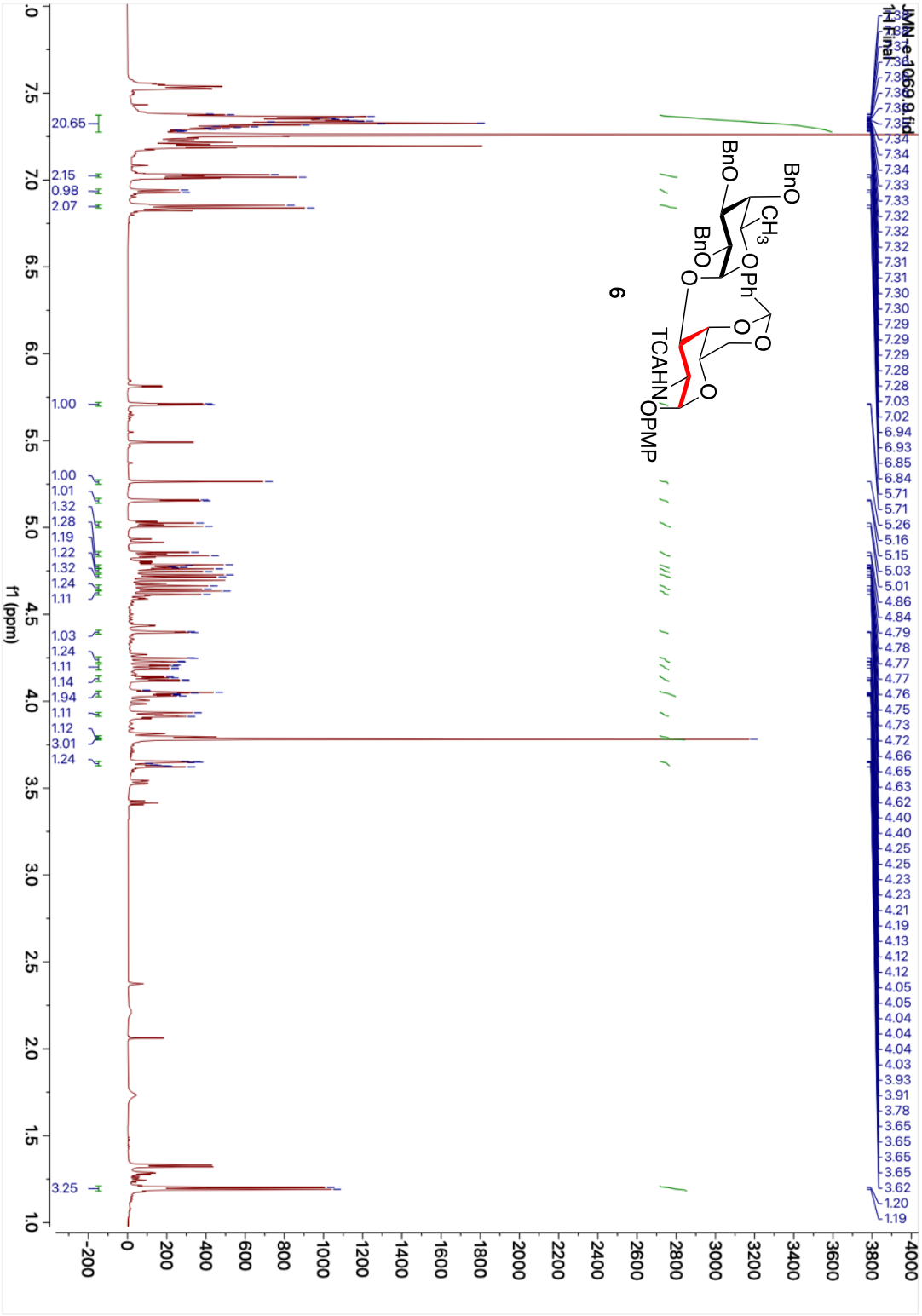
4.01 (m, 2H), 3.92 (d, J = 13.3 Hz, 1H), 3.80 (m, 1H), 3.78 (s, 3H), 3.64 (m, 1H), 1.20 (d, J = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.6, 155.5, 150.4, 138.8, 138.5, 137.5, 128.4, 128.2, 128.2, 128.1, 128.0, 127.4, 117.9, 114.8, 100.6, 97.5, 96.8, 92.6, 78.9, 78.0, 75.9, 75.0, 73.3, 73.1, 72.8, 72.0, 69.2, 67.7, 63.7, 55.6, 50.5, 16.8. HRMS (ESI) calcd for $C_{49}H_{50}Cl_3NO_{11}[M+Na]^+$ 956.2347, found 956.2348.

N-((2R,3R,4R,5R,6R)-5-hydroxy-6-(hydroxymethyl)-2-(4-methoxyphenoxy)-4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (5**)**

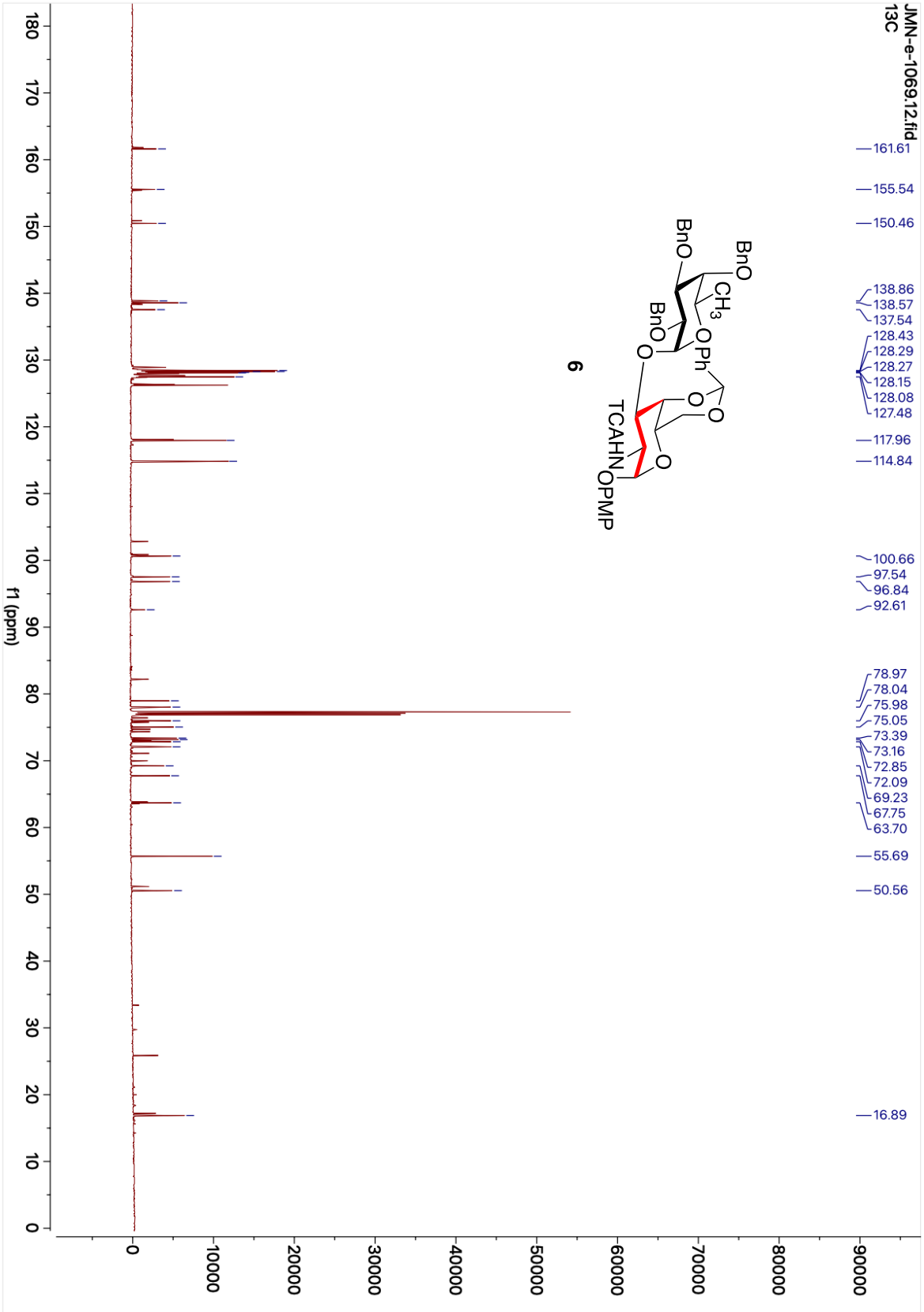
To a solution of **6** (1.0 eq, 0.500 g, 0.535 mmol) in CH_3OH (10 mL) and AcOH (1 mL), $Pd(OH)_2$ was added (2.0 eq, 0.75 g g, 1.07 mmol). The reaction was stirred under H_2 for 3 days then was diluted with CH_3OH, filtered through celite, concentrated in vacuo. The crude material was purified by size exclusion chromatography (Bio-Gel P2 gel) using a 1:1 mixture of deionized H_2O:CH_3OH as an eluant. Fractions containing the desired product (determined from MS) were combined and lyophilized to yield **5** (0.214 g, 0.452 mmol, 85%) as a white solid. $[\alpha]D^{25}$ = +60.7° (c = 0.012, CH_3OH), ^{1}H NMR (600 MHz, CD_3OD) δ 7.18 (d, J = 9.2 Hz, 2H), 7.01 (d, J = 9.3 Hz, 2H), 5.50 (s, 1H), 5.12 (s, 1H), 4.62–4.51 (m, 1H), 4.35–4.28 (m, 1H), 4.18–4.12 (m, 2H), 4.06–4.05 (m, 1H), 3.85 (s, 3H), 3.84–3.83–3.75 (m, 5H), 2.11 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.4, 156.2, 151.8, 120.2, 120.0, 116.3, 98.8, 97.9, 75.8, 73.2, 72.6, 71.0, 69.3, 68.7, 66.7, 62.3, 57.0, 50.2, 23.3, 16.9. HRMS (ESI) calcd for $C_{21}H_{31}NO_{11}$ $[M+Na]^+$ 496.1794, found 496.1101. $[\alpha]D^{25}$ = +60.7° (c = 0.015, $CDCl_3$)

2.3.2. ^{1}H, ^{13}C, HSQC, and HMBC Spectra, and Data Table

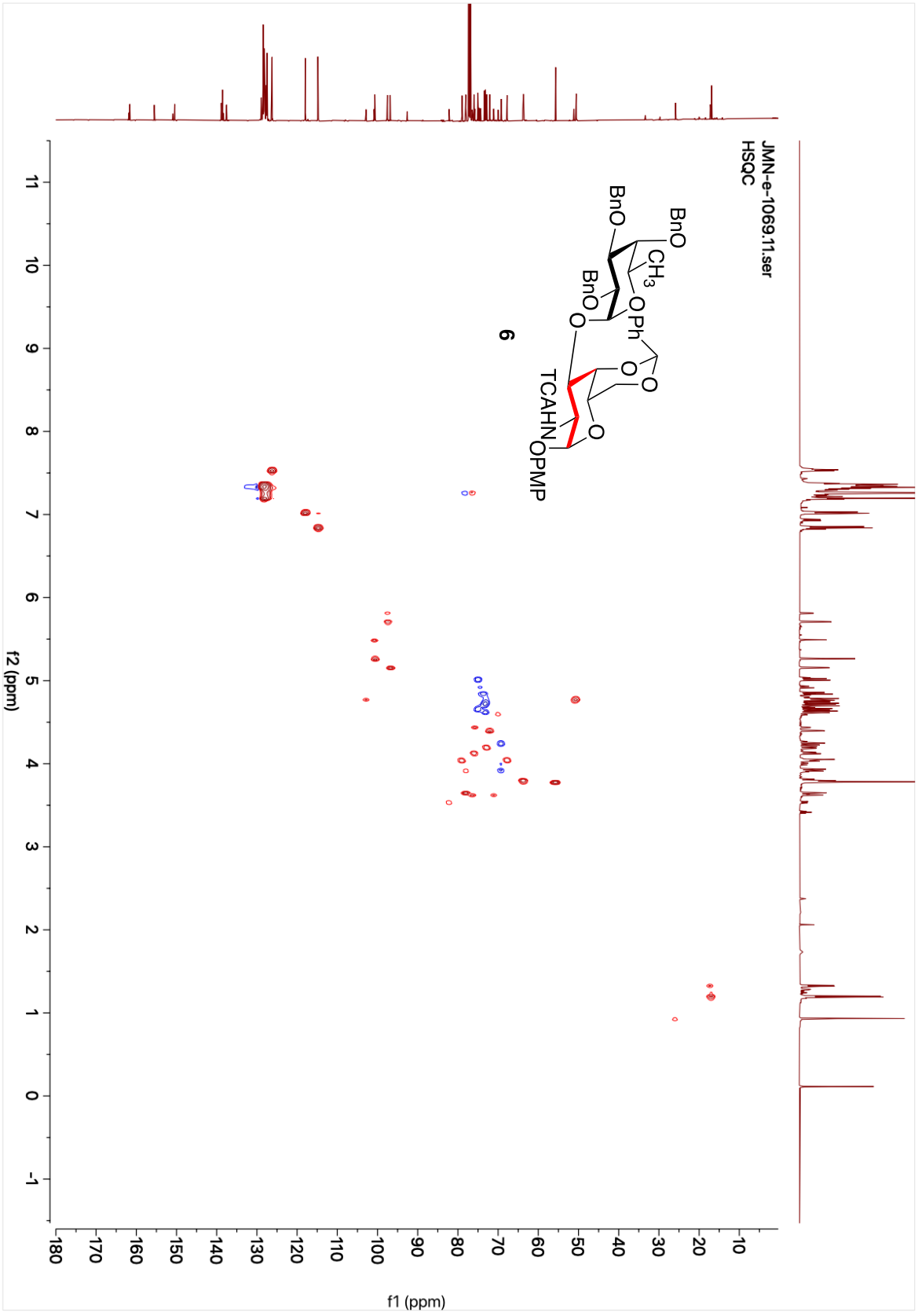
^{1}H NMR (600 MHz, $CDCl_3$) of Compound 6



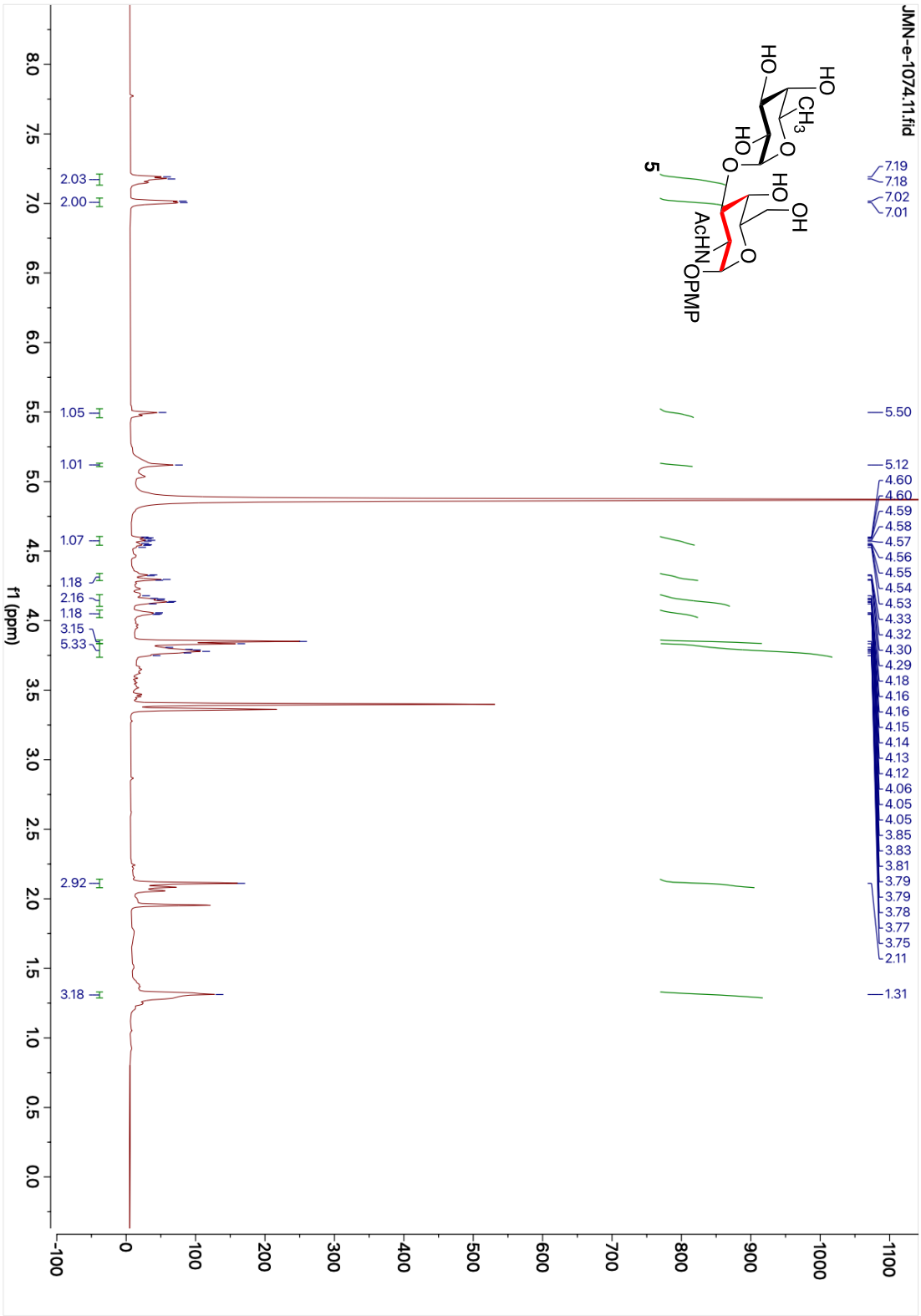
^{13}C NMR (151 MHz, $CDCl_3$) of Compound 6



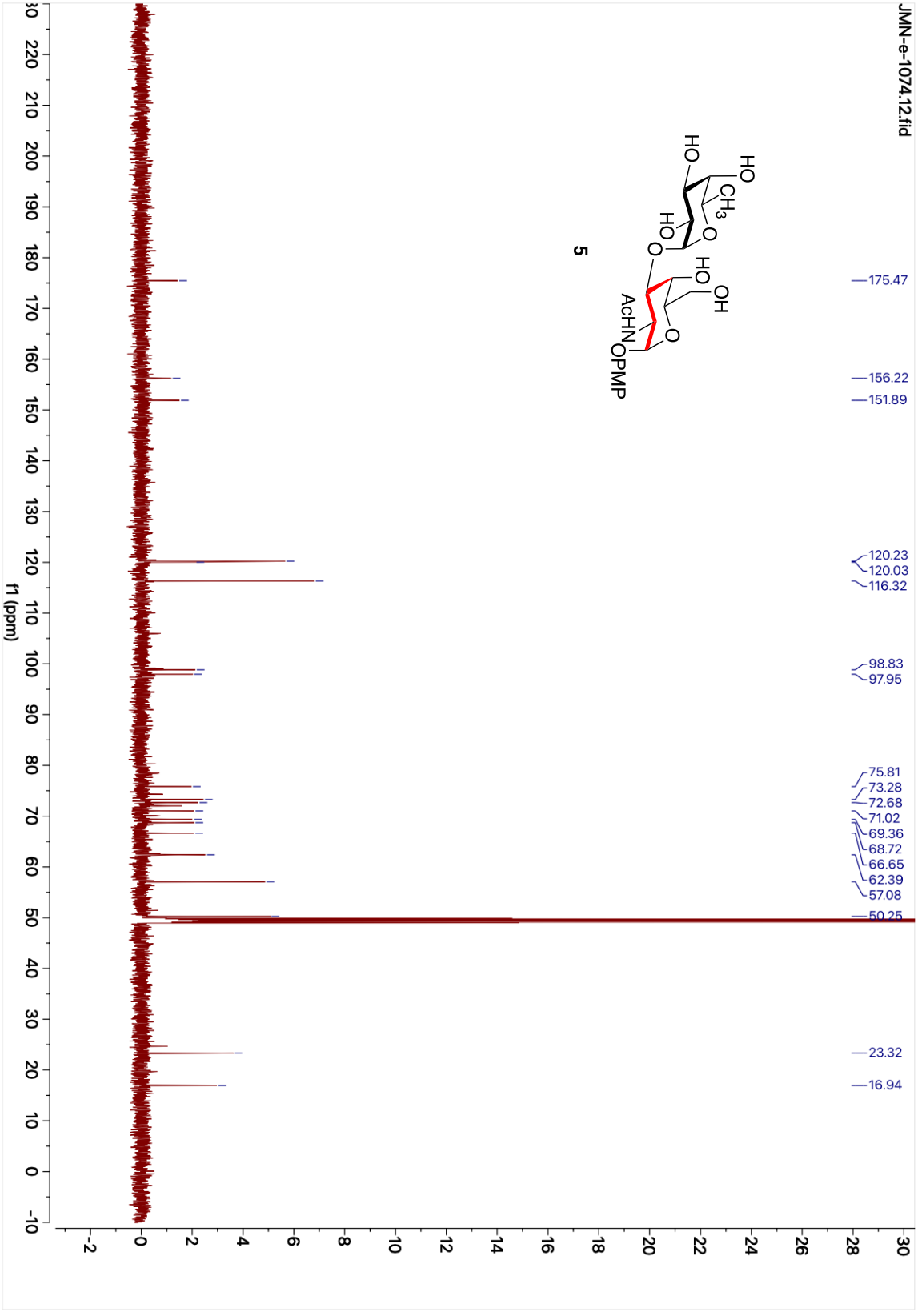
^{1}H-^{13}C HSQC ($CDCl_3$) of Compound 6



^{1}H NMR (600 MHz, CD_3OD) of Compound 5



^{13}C NMR (151 MHz, CD_3OD) of Compound 5



^{1}H-^{13}C HSQC (CD_3OD) of Compound

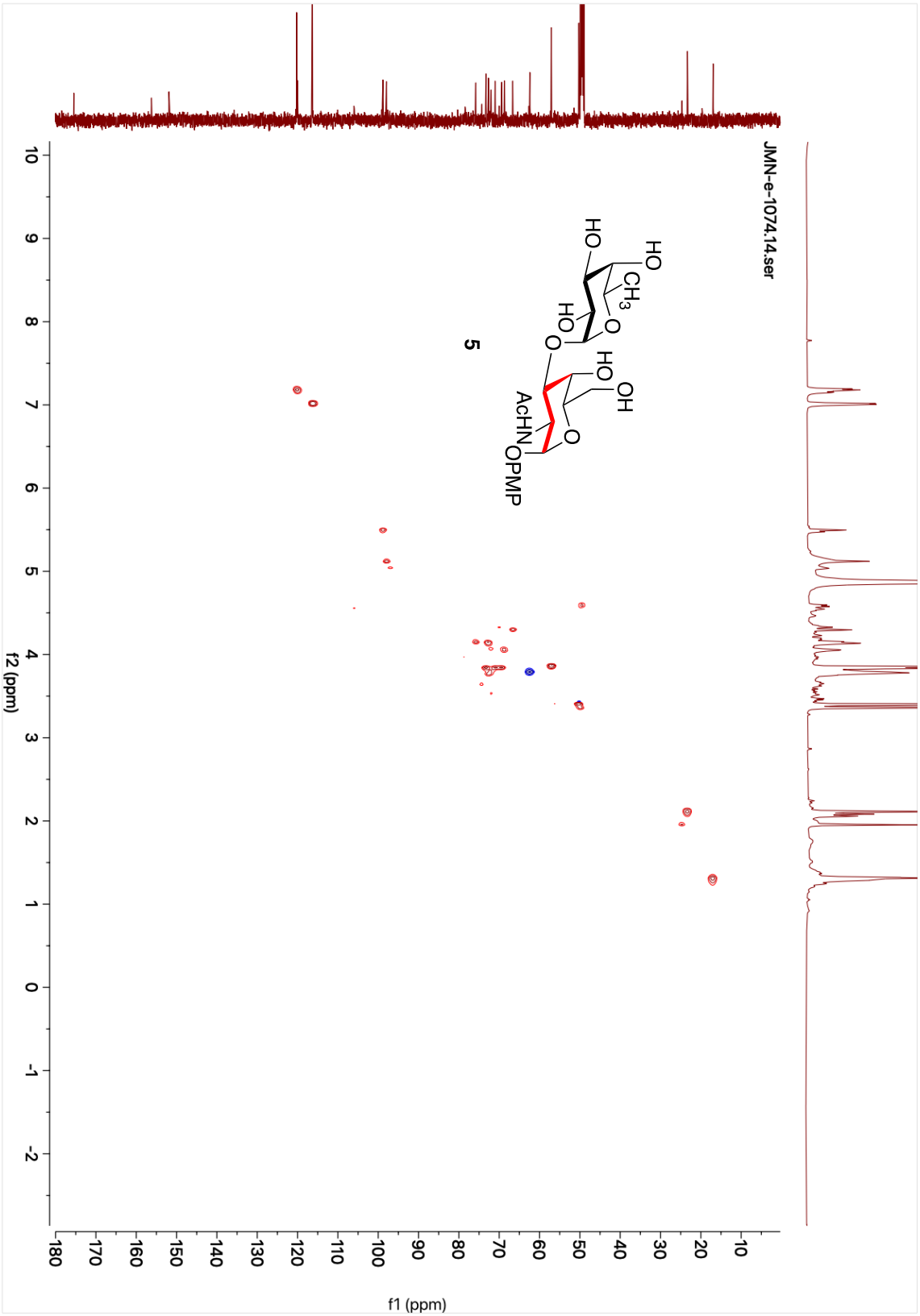


Table S1. ^{1}H and ^{13}C NMR chemical shifts (ppm) between isolated polysaccharide

Residue		Chemical shifts (ppm)					
		H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-6
→3-α-D-Fuc*p*-(1→	Isolated	5.04	3.88	3.88	4.06	4.00	1.24
		97.2	68.0	80.7	72.7	68.0	16.6
→3-β-D-Gal*p*NAc-(1→	Isolated	4.60	4.10	3.81	4.16	3.69	3.80
		104.2	52.3	77.3	65.6	76.0	62.1
→3-α-D-Fuc*p*-(1→	Synthetic	5.10	4.05	3.83	3.82	3.84	1.31
		97.9	68.7	73.2	71.0	69.3	16.9
→3-β-D-Gal*p*NAc-(1→	Synthetic	5.50	4.62	4.30	4.14	3.75	3.79
		98.8	50.2	66.7	75.8	72.6	62.3
Chemical shifts for NAc are ^{1}H 2.11 and ^{13}C 23.3/175.4							

CHAPTER 3

Significance of Saponins

3.1 Introduction and Background

Saponins are isolated from plants and are found in the fruit, bark, stems, leaves, and roots of various species, including several common foods.[36] Olives contain tormentic acid saponins, and monk fruit, an increasingly popular sugar substitute, contains Mogroside V, a glucose-substituted saponin that is 300 times sweeter than table sugar.[37, 38] Saponins are ubiquitous in treatment of diseases and disease-causing species. As treatments, saponins have been used as vaccine adjuvants,[39] antibacterial agents,[40] antiparasitic agents,[41] antifungal agents,[42, 43] antiviral agents,[44] biofilm inhibitors,[45] chemotherapeutics,[46] and supplements for autoimmune disorders like diabetes.[47]

Structurally, saponins are quite simple, but they have hundreds of derivatives. Saponins are composed of terpene units assembled into an aglycone core. This aglycone core takes on several forms, such as terpenoid, steroid, or alkaloid.[48] Within these forms, the aglycone carbon skeletons vary. Examples of some 30-carbon terpenoid (triterpenoid) skeletons are dammaranes, oleananes, ursanes, hopanes. Oleananes are derivatives of oleanolic acid. The biosynthesis of oleanane saponins has been extensively studied.[36, 49] Hederagenin, a triterpenoid discussed later, is an oxidized derivative of oleanolic acid, the namesake of oleanane aglycones.

The structure-activity-relationships (SARs) of the aglycone core also have been studied extensively. One facile method of derivatization involves the oxidation and/or substitution of different positions on the aglycone core.[50] In these instances, oxidations are performed both chemically and enzymatically. Biosynthetically, all triterpenoid saponins assemble from

sesquiterpene, a 30-carbon terpene unit. Two rearrangements occur during the cyclization of sesquiterpene when forming the final two six-membered rings of oleanane and ursane aglycones. After cyclization, the aglycone may be oxidized by P450 enzymes to produce an array of aglycones at different oxidation states.[51]

The second component of a saponin is sugars. The attachment of one or more monosaccharides or oligosaccharides occurs after the formation of the aglycone core. If the aglycone is attached to one sugar moiety, the saponin is monodesmosidic.[48] If the aglycone is attached to two sugar moieties, it is bidesmosidic. Chains of sugars may be linear or branched, and each sugar can be attached to the aglycone or other sugars by an α- or β-linkage. The incorporation of sugars exponentially increases the amount of possible saponin structures.

An accepted and common mechanism of action of saponins involves localization to certain cell types, differentiated by the various sugar moieties.[52, 53] Then, the aglycone core may incorporate into the hydrophobic cell membrane, dragging along the more highly oxidized sugar chain(s). The sugars cannot incorporate into the membrane, so they end up forming pores across the cell membrane. This causes increased cell permeability, making saponins excellent adjuvants in vaccines.[52] Many saponins exhibit cytotoxicity, themselves. When pore-formation increases to the point that the cell membrane ruptures, the cell immediately dies.[54] There are covalent mechanisms in which the aglycone core of a saponin can directly bind to an extracellular receptor. This is the case with QS-21, a common vaccine adjuvant. The aglycone of QS-21 has an aldehyde which directly bonds to and activates T-cells. [53] QS-21 also activates T-cells *indirectly*, as it exhibits the typical increase of cell permeability in dendritic cells, thereby allowing the infiltration of dendritic cells by vaccine components.

3.2 Established Methods for Preparing Saponins

Saponins can be accessed in three ways. As mentioned previously, they may be extracted directly from plants. The second way to access them is to isolate the respective saponin's aglycone from plants, usually in larger amounts, because aglycones are not subject to deglycosylation in acidic extraction conditions.[55] In this second method, saponins are accessed by coupling a protected aglycone acceptor to a protected sugar donor. The final way to access saponins is via complete total synthesis of the aglycone core and its sugars. The sugars can be isolated in large quantities and protected; or, they can be accessed *de novo* from linear, chiral starting materials. The remainder of this chapter is devoted to further details regarding these methods with a focus on oleanane-type aglycones.

Extraction of Saponins:

Bioactive components of medicinal organisms are discovered through extraction and stringent purification. New saponins were isolated from the fruit of *Sapindus saponaria* using a typical workflow.[41] After extraction in ethanol, silica gel column, extraction with n-butanol, and two more silica gel columns, two previously undiscovered saponins were isolated (<8% total mass). This isolation process took over one week. Though plant materials are cheap, the time, materials, and equipment used to isolate saponin components are costly. Furthermore, once isolated, they are not easily derivatized. As such, the tests and experiments that can be run are limited. Once discovered, new compounds can be accessed faster synthetically.

Extraction and Synthesis:

Access to multiple saponins can be achieved by isolating a desired aglycone core from a plant, subjecting that core to chemical derivatizations, and appending sugars. Greatrex *et al* accessed six QS-21 saponin mimics from hederagenin using this method.[55] First, they isolated the aglycone, hederagenin, from *Helix hedera* in the amount of 15 g/kg, using acidic extraction procedures to crystalize the aglycone. Then, they esterified the acid of hederagenin and protected the primary alcohol. Alternatively, after esterifying the acid, they oxidized the primary alcohol to access gypsogenin. From these aglycones, they generated six unique saponins via glycosylations with three different disaccharides and one trisaccharide. For more methods of derivatizing saponins and forming glycosidic linkages, see the publication by Yang *et al*.[56]

Total Synthesis:

This final method avoids extraction procedures altogether. Amyrins are key biosynthetic intermediates for the synthesis of oleanane- and dammarane-type aglycones. Because amyrins are elusive biosynthetic intermediates, they are attractive targets for chemical synthesis. Tamelen *et al* synthesized α- and β-amyrins in 1972 using a convergent synthesis with a longest-linear sequence of 8 steps.[57] Over twenty years later, in 1993, E.J. Corey and Jaemoon Lee synthesized three oleanane aglycones (oleanolic acid, β-amyrin, and erythrodiol) enantioselectively for the first time in 26-28 step. These were accessed starting from commercially-available 7-methoxy-1-tetralone, and the syntheses only diverged for the last three steps.[58] Since these syntheses, a more recent article focused on accessing α- and β-amyrin from the more-available oleanolic acid.[59]

CHAPTER 4

Progress Toward Synthesis of Saponin S1

4.1 Background

Soapberry trees grow well in tropical and sub-tropical areas, and their parts have been used as medicines for thousands of years. In fact, the use of soapberry in India can be traced back to 300 B.C.[60] Unfortunately, after these plants garnered the attention of Western scientists in the mid-1900s, the researchers seldom credit the ancient and indigenous knowledge used to prompt their investigations.[61] In 1964, Dr. Lemma, an African researcher, discovered a new bioactivity of African Soapberry and reported this to the Tropical Products Institute in Britain. Without informing Lemma, they patented his discovery and left him off of the patent.[61] Such appropriation is so commonplace and historically ingrained, it is easily overlooked. That is why, in this thesis, I would like to take at least a couple of sentences to thank the people who originally cultivated and used the Western Soapberry Tree as medicine. Without their indigenous knowledge, Western researchers never would have investigated the compounds in Western Soapberry, and I never would have worked on Saponin S1.

A Brazilian group isolated Saponin S1 from the fruit of native *Sapindus saponaria*, more commonly known as the Western Soapberry Tree, back in 2007.[43] The new saponin was tested against spermatozoa, a protozoan parasite called *Trichomonas vaginalis*, and two yeast infection-causing *Candida* species (*albicans* and non-*albicans*).[41-43] Its activity as a spermicide is comparable to nonoxynol-9 (N-9), the only commercially available spermicide in the United States of America. However, unlike N-9, Saponin S1 protects against a sexually transmitted infection (STI), prevents yeast infections, *and* does not harm a primary species of commensal bacteria in

the human vagina, *Lactobacillus vaginalis*. N-9 is usually safe, but extended and prolonged use can *increase* risks of STI transmission and yeast infections because of its disruption of commensal biofilm.[62, 63] Soapberry saponin extracts would provide a safer birth control method that concomitantly *reduces* risk of STI transmission and yeast infections.

Saponin S1 contains a hederagenin (Hed) aglycone core and a trisaccharide composed of arabinose (Ara), rhamnose (Rha), and partially acylated xylose (Xyl). Because there is only one sugar chain attached, Saponin S1 is categorized as a monodesmosidic saponin. As mentioned previously, hederagenin is an oxidized biosynthetic derivative of oleanolic acid, an oleanane triterpenoid. The trisaccharide is a linear chain connected in a *cis* fashion to the secondary alcohol on Hed (**Figure 5**).

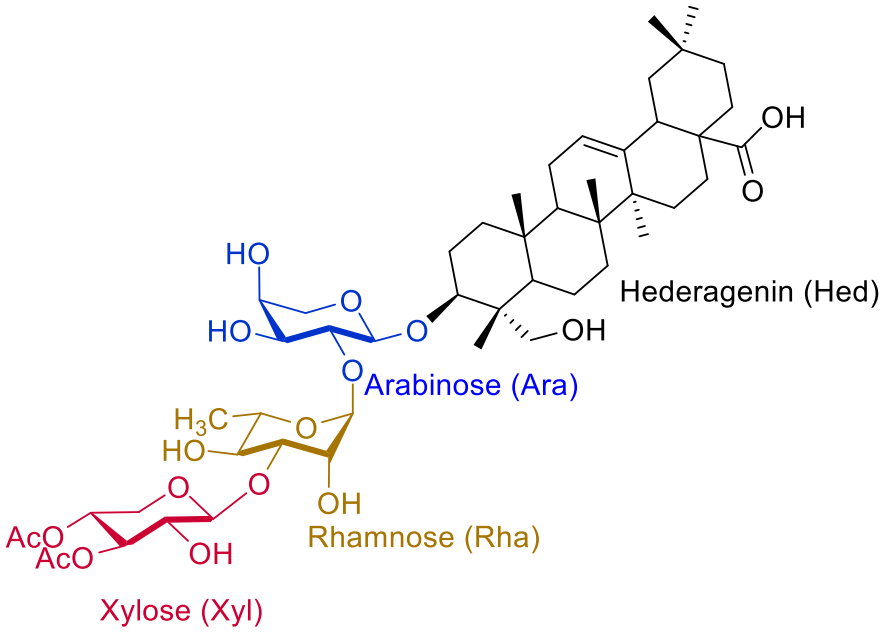


Figure 11. Saponin S1

A disaccharide saponin of Hed, Ara, and Rha has been made.[64, 65] However, the partially-acylated nature of the terminal Xyl significantly complicates the synthesis of Saponin S1. To attain the desired linkages in Saponin S1, acyl protecting groups would be installed at the C2 positions of the glycosyl donors to facilitate attack from the top face through neighboring group participation. Then, the acyl groups would be removed after all glycosylations were accomplished.[13, 16] The presence of C3 and C4 acetyl groups on Xyl complicate this because only *some* of the acyl groups must be removed in the final synthetic steps. To avoid removal of the desired acyl groups, acyl groups that can be removed orthogonally are required at the C2 position of Ara and Xyl. Chloroacetyl groups are an excellent solution because they can be removed with thiourea in the presence of other acyl groups.[66] Unfortunately, the neighboring group participation of these groups is reduced by the presence of an electron-withdrawing group at the would-be site of a partial positive charge. Employment of the 2,2-dimethyl-2-(*o*-nitrophenyl)acetyl group (DMNPA), a bulky acyl group with *two* neighboring group effects, provides an incredibly strong directing effect.[67] However, the strong acidic conditions necessary to remove this group could break the glycosidic linkages. Also, DMNPA-anhydride, the needed reagent, is not commercially available and must be made over 5 steps.[67] As I discuss later, the C2 protection and glycosylation yields employing DMNPA were lower, so this in-combination with the aforementioned factors meant that the DMNPA protecting group was not viable for this synthesis.

4.2 Current Progress

Orthogonality issues made it difficult to plan a synthetic route toward Saponin S1. The presence of acyl groups on the terminal Xyl on the saponin invalidated the commonplace use of acetyl or benzoyl protecting groups at C2 to facilitate equatorial addition of Xyl to Rha. Furthermore, the linkage of Rha to the C2 position of Ara meant that the trisaccharide could not be formed before performing a glycosylation with Hed as the acceptor. This led me to employ a 1+1 (Hed + Ara), 1+1 (Rha + Xyl), and 2+2 (Hed-Ara + Rha-Xyl) approach to the glycosylations (**Figure 6**).

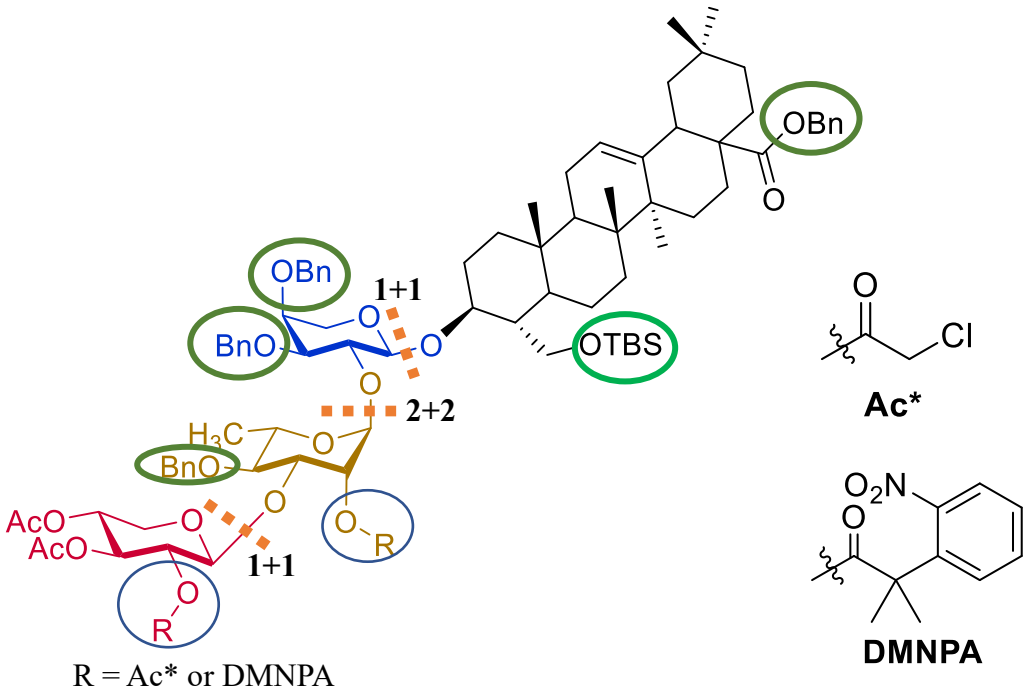
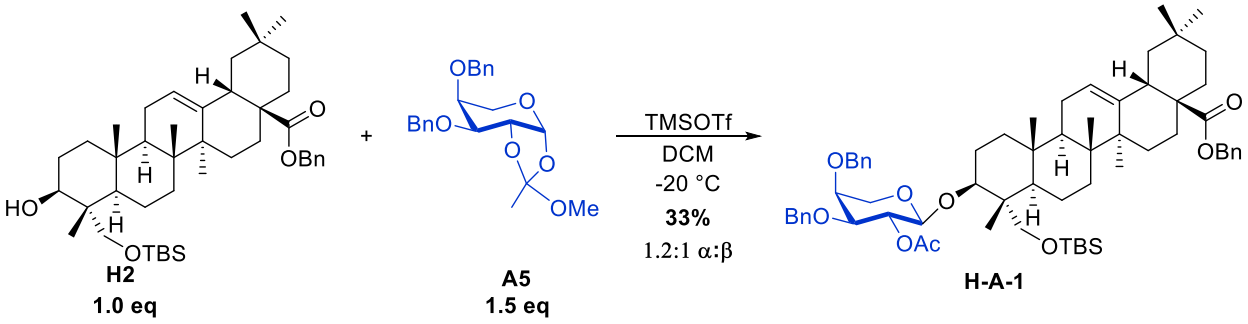


Figure 12. Glycosylation and Protecting Group Strategy

Peracetylation of Ara and conversion to the thiogylcoside provided a simple test-subject donor to make sure the glycosylation with Hed occurred. Previous syntheses involving the

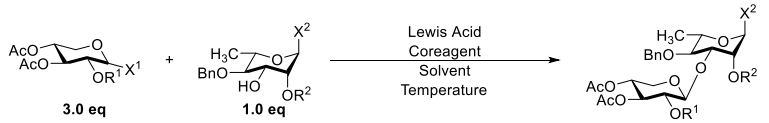
glycosylation of Hed used trichloroacetimidate donors.[64, 65] I quickly realized that this was because, when using co-reagent NIS with thioglycosides, the reagent reacts with the olefin on Hed. Thus, an acetimidate donor, a glycal donor, or an orthoester donor is required. Glycals, especially of pentose sugars, are inherently unstable and provide low diastereoselectivity,[68] whereas the main problem with using acetimidates is the risk of rearrangement to the amide.[69] The rearrangement can be avoided by using the acetimidate within a day *or* employing an N-phenyltrifluoroacetimidate rather than the more common trichloroacetimidate.[70] However, my current strategy has been to employ an orthoester Ara donor and do a simple S_N2-type attack of the anomeric center with Hed to achieve the desired selectivity (**Scheme 2**).



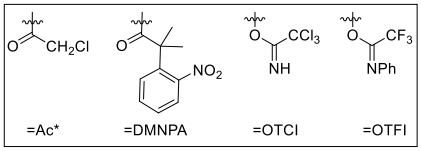
Scheme 10. Hederagenin-Arabinose Glycosylation

Facilitating the other 1+1 glycosylation between Rha and Xyl took more time and effort. There were two solutions that I investigated to solve the problem of partial acylation on the terminal Xyl. The use of either chloroacetyl or DMNPA protecting groups at C2 would allow for their orthogonal removal at the end of the synthesis. Chloroacetyl protecting groups have less neighboring group participation than their acetyl counterparts, but they may be removed orthogonally using thiourea. DMNPA provides a stronger directing effect, but DMNPAA, the reagent necessary to add the protecting group, is not commercially available. Furthermore, the

removal requires the use of acid which could prove detrimental to the glycosidic linkages in Saponin S1.



	R^1	X^1	R^2	X^2
Combo 1	Ac*	OTCI	Ac*	SPh
Combo 2	DMNPA	OTCI	Ac*	SPh
Combo 3	DMNPA	OTFI	Ac*	SPh
Combo 4	Ac*	STol	H	OPMP



Combo 1	Solvent	Lewis Acid	Temp.	Co-reagent	Yield	β:α
Condition 1	DCM (0.1 M)	TMSOTf	-78 °C → 0°C	none	<3%	0: 100
Condition 2	DCM (0.1 M)	TfOH	0 °C → 23 °C	none	0% (decomp.)	——
Condition 3	MeCN (1.0 M)	TMSOTf	0 °C → 23 °C	none	0% (low solubility)	——
Condition 4	EtCN (1.0 M)	TMSOTf	23 °C	none	< 3%	——

Combo 2	Solvent	Lewis Acid	Temp.	Co-reagent	Product?
Condition 1	DCM (1.0 M)	TMSOTf	23 °C	none	Yes
Condition 2	DCM (1.0 M)	TfOH	23 °C	none	decomp.
Condition 3	DCM (1.0 M)	BF_3•OEt_2	23 °C	none	Yes
Condition 4	DCM (1.0 M)	Tf_2NH	23 °C	none	Yes
Condition 5	EtCN (0.1 M)	TMSOTf	23 °C	none	Yes
Condition 6	EtCN (0.1 M)	TfOH	23 °C	none	No
Condition 7	EtCN (0.1 M)	BF_3•OEt_2	23 °C	none	Yes
Condition 8	EtCN (0.1 M)	Tf_2NH	23 °C	none	Yes
Condition 9	DCM: EtCN, 1:5 (0.1 M)	TMSOTf	23 °C	none	decomp.
Condition 10	DCM: EtCN, 1:5 (0.1 M)	TfOH	23 °C	none	decomp.
Condition 11	DCM: EtCN, 1:5 (0.1 M)	BF_3•OEt_2	23 °C	none	No
Condition 12	DCM: EtCN, 1:5 (0.1 M)	Tf_2NH	23 °C	none	No
Condition 13	DCM: EtCN, 5:1 (0.1 M)	TMSOTf	23 °C	none	decomp.
Condition 14	DCM: EtCN, 5:1 (0.1 M)	TfOH	23 °C	none	decomp.
Condition 15	DCM: EtCN, 5:1 (0.1 M)	BF_3•OEt_2	23 °C	none	Yes
Condition 16	DCM: EtCN, 5:1 (0.1 M)	Tf_2NH	23 °C	none	Yes

Combo 3	Solvent	Lewis Acid	Temp.	Co-reagent	Yield	β:α
Condition 1	DCM (0.3 M)	TMSOTf	23 °C	none	0%	——

Combo 4*	Solvent	Lewis Acid	Temp.	Co-reagent	Yield	β:α
Condition 1	DCM (0.1 M)	TMSOTf	-78 °C	NIS (1.7 eq)	64%	**1.1:1**
Condition 2	EtCN (1.0 M)	TMSOTf	-78 °C	NIS (1.7 eq)	46%	**1.2:1**
Condition 3	DCM: EtCN, 1:5 (0.1 M)	TMSOTf	-78 °C	NIS (1.7 eq)	49%	**1.4:1**
Condition 4	DCM: EtCN, 5:1 (0.1 M)	TMSOTf	-78 °C	NIS (1.7 eq)	55%	**1.3:1**

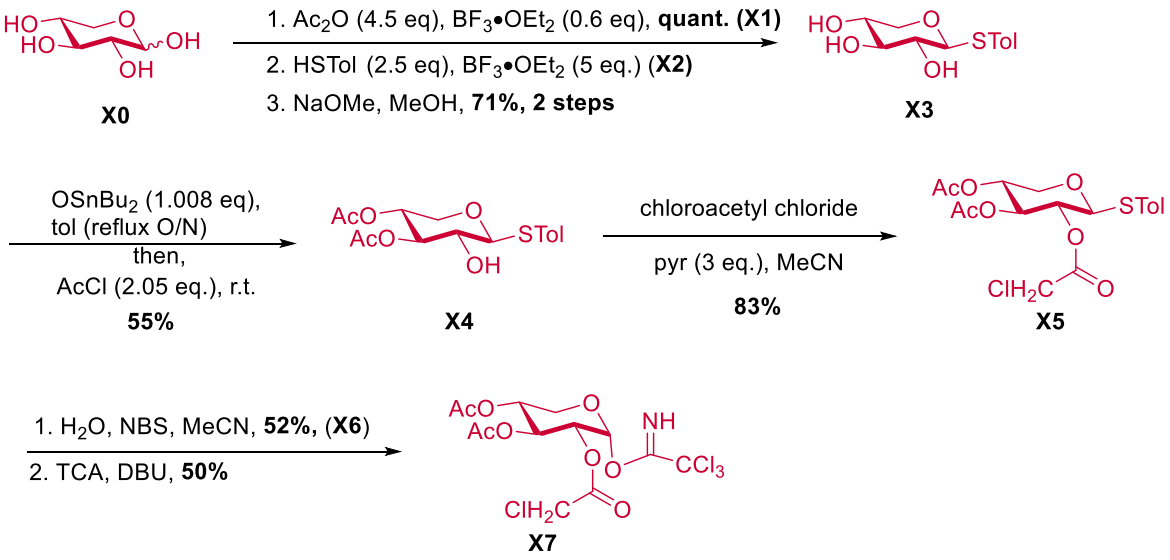
*Javier Ortiz Alvarado ran these glycosylations; he also made >1 g of Xylose donor.

Scheme 11. Rhamnose-Xylose Glycosylations

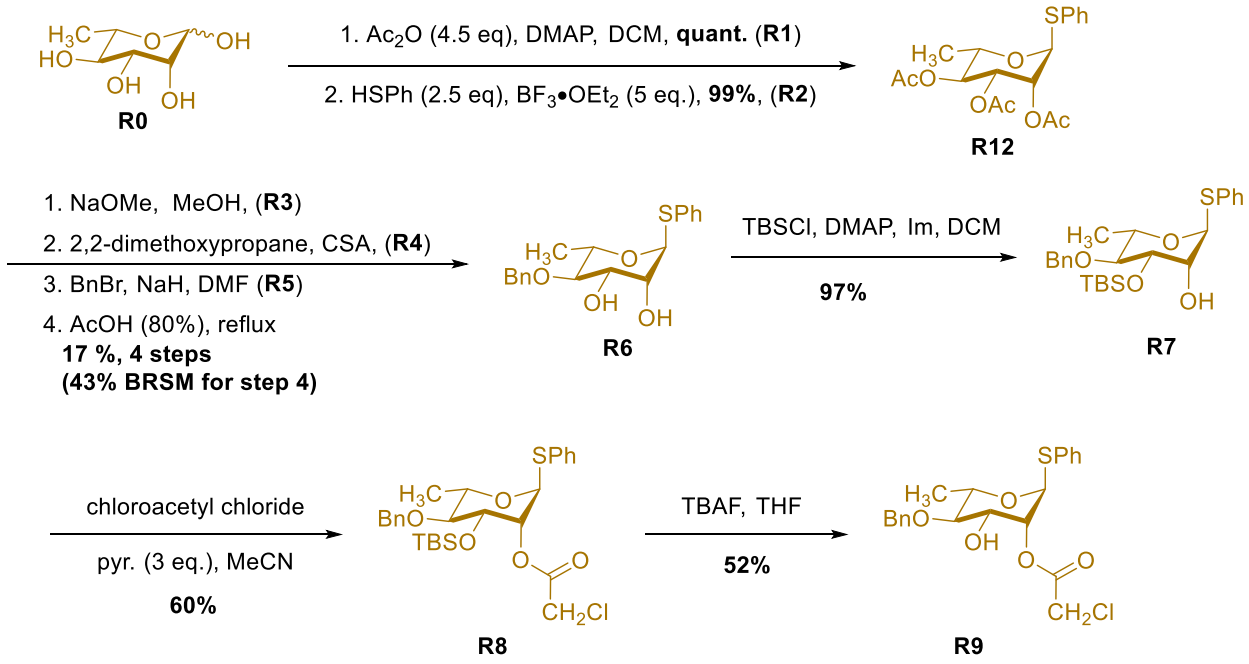
After optimizing routes, the chosen Xyl building block could be attained within a week, and the Rha building block could be attained within two weeks, both on multi-gram scale. However, I ended up testing two generations of rhamnose acceptors and three generations of xylose donors before landing on the mostly-optimal combination. I will go through these combinations in the following paragraphs. **Scheme 3** provides an overview of all four combinations and results from attempted glycosylations. For combination four, I synthesized the Rha acceptor and Javier Ortiz Alvarado synthesized the Xyl donor and conducted the glycosylations.

Combo 1

The Xyl acetimidate donor for Combo 1 was accessed in 7 steps from commercially-available D-Xyl (**Scheme 4**). The rhamnose acceptor was accessed in 9 steps from commercially-available L-Rha (**Scheme 5**). At low temperatures, acetimidates can be selectively activated over thioglycosides, especially in the absence of a co-reagent such as NIS. In theory, this reaction should have produced a disaccharide that was a thioglycoside donor. In reality, the Rha acceptor was not nucleophilic enough to react with activated donor until at least 0 °C, at which point selectivity from the acyl participating group on Xyl was negligible. Even with this, there was minimal to no conversion of starting materials to product. Furthermore, at higher temperatures, I observed activation of the thioglycoside, even without the presence of NIS.



Scheme 12. First Xylose Donor Synthesis

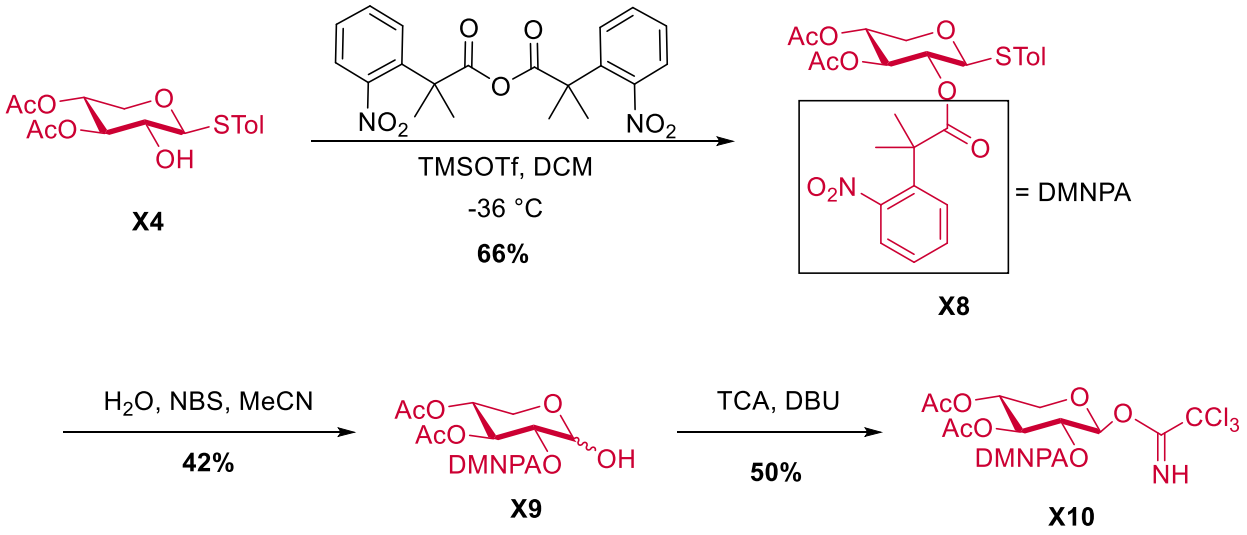


Scheme 13. First Rhamnose Acceptor Synthesis

Combo 2

By switching the chloroacetyl protecting group on the C2 position of the Xyl donor with the bulkier, stronger participating group DMNPA, I aimed to increase the selectivity, even at

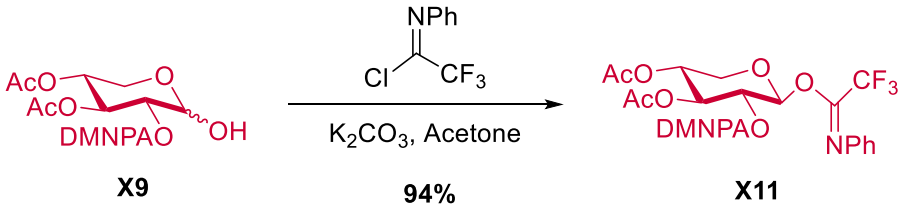
higher temperatures. I generated this donor alongside the chloroacetyl Xyl so I could compare them. Unfortunately, I saw the same co-activation of the Rha thioglycoside; this occurred faster than the nucleophilic attack of Rha. The glycosylation reaction gave excellent selectivity (only the desired linkage) but *very* poor conversion (trace); I mostly recovered starting material or activated Rha thioglycoside.



Scheme 14. Second Xylose Donor Synthesis

Use of DMNPA increased selectivity, but it presented significant scalability issues. Trying to increase the reaction scale for the hydrolysis or the subsequent acetimidate formation resulted in a two-fold decrease in yield. Also, the reagent needed to protect the C2 position of Xyl with DMNPA is *not* commercially available, and it requires 4 steps to make. This process takes multiple days, and the C2 protection yield is lower in-comparison with typical acyl protections.

Combo 3:



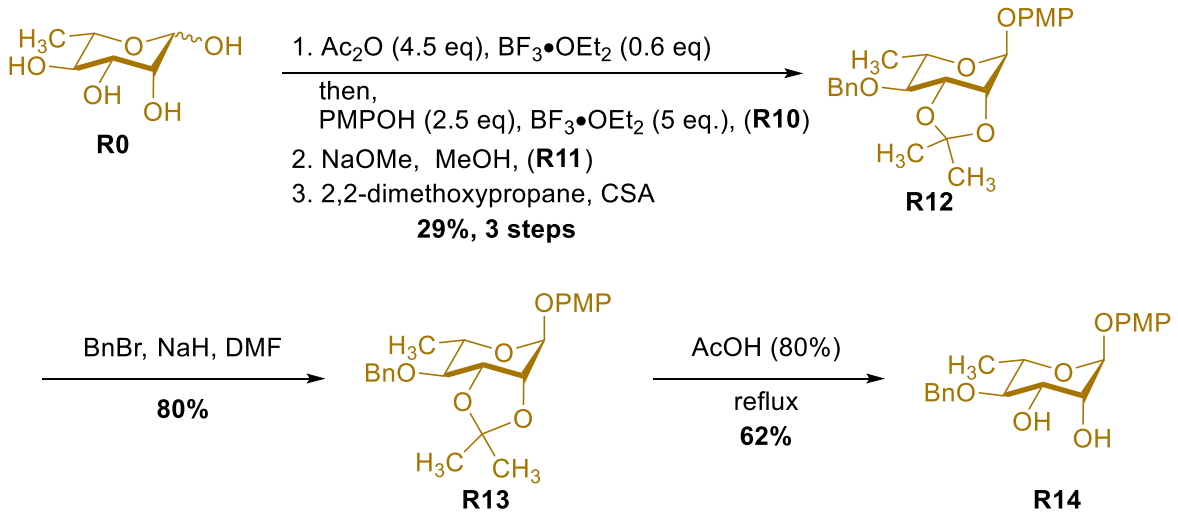
Scheme 15. Third Xylose Donor Synthesis

To fix the bottleneck effect of bringing up the Xyl donor with DMNPA, I used N-phenyl trifluoroacetimidoyl chloride as a reagent to form an acetimidate donor in higher yield (**Scheme 7**). The yield was significantly higher (94% versus <50%) and the reaction proceeded in less than one hour (versus >5 hours). The increased stability of the N-phenyl trifluoroacetmidate donor also was desirable; the presence of the phenyl ring on nitrogen prevents rearrangement to the amide. Despite this benefit, the preceding hydrolysis reaction remained unscalable, and the glycosylation results were just as poor for this new donor. This led me to Combo 4.

Combo 4:

At this point, I realized that the Rha acceptor was the biggest issue. The presence of the sulfide at the anomeric position meant unwanted side-products could form during the glycosylation. To fix this issue, I employed para-methoxyphenol as an anomeric protecting group. The second issue to solve was the inherent lack of nucleophilicity. I hypothesized that the electron-withdrawing C2 acyl group might contribute to a decrease in nucleophilicity. Furthermore, I thought there might be steric hindrance from the C2 acyl group.

When forming the C2-protected Rha, I first had to selectively protect C3 with a bulky silyl group. Equatorial hydroxyl groups tend to have a greater electron density than axial hydroxyl groups. The C2 position of Rha is an axial hydroxyl, and the C3 is equatorial. I hypothesized that if the C3 position would selectively attack a bulky silyl group, it would selectively attack a much larger monosaccharide. This approach takes advantage of the inherent nucleophilicity difference between equatorial and axial hydroxyl groups to selectively attack the xylose donor with the C3 hydroxyl. Therefore, I decided to employ Rha building block **R14** (**Scheme 8**) as the acceptor. As for Xyl, I decided to use thioglycoside intermediate **X5** and perform the Rha-Xyl glycosylation using the co-reagent NIS. This shortened the building block synthesis by two steps and eliminated the concern over the acetimidate rearranging. Also, chloroacetyl chloride is commercially available and produces higher yields in the C2 protection of Xyl intermediate **X4**.



Scheme 16. Second Rhamnose Acceptor Synthesis

The glycosylation reaction finally proceeded at lower temperatures (slowly at -78 °C, reasonably paced at -40 °C, and instantaneous at 0 °C, with selectivity decreasing with increasing

temperature). After a column and several co-evaporations, I attained disaccharide (**R-X-1**) as a brown powder. Heteronuclear Multiple Bond Correlation Nuclear Magnetic Resonance (HMBC NMR) seemed to suggest the correct attachment, but a crystal structure would provide more definitive results. Though the disaccharide was a brown solid, it was in a powder form rather than a crystalline form. Derivatizing this disaccharide to produce crystals and taking x-ray crystal structures would give the most conclusive results on the regioselectivity and stereoselectivity exhibited by this disaccharide.

Future Directions

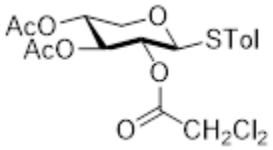
Two out of the three desired glycosidic linkages have been accessed, but there is room for improvement. The Hed-Ara glycosidic linkage has been formed (**H-A-1**), but the use of the orthoester Ara donor results in low yield and selectivity of product. The employment of a traditional acetimidate donor with C2 acyl group should produce better results.

The selectivity of the Rha-Xyl glycosylation could be increased by employing DMNPA at C2 again (**X8**) in-combination with the current Rha building block (**R14**). Using DMNPA might be beneficial, as the resultant disaccharide might produce nice crystals without any derivatization. However, DMNPA removal should be tested with the disaccharide prior to performing the last 2+2 glycosylation to avoid the potential of losing more expensive material.

4.3 Experimental Methods and Instrumentation

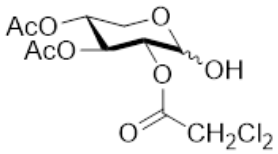
Commercial reagents were used as received. Anhydrous solvents were taken from an MBRAUN solvent purification system (MB SPS) and stored over 4 Å or 3 Å molecular sieves. All moisture-sensitive reactions were performed in flame- or oven-dried round bottom flasks under an argon atmosphere. All air- or moisture-sensitive liquids were transferred via oven-dried stainless-steel syringes or cannula. Reaction temperatures were monitored and controlled via thermocouple thermometer and corresponding hot plate stirrer. Flash column chromatography was performed as described by Still et al. using silica gel 230–400 mesh. Analytical thin-layer chromatography (TLC) was performed on glass-backed Silica gel 60 F254 plates (EMD/Merck KGaA) and visualized using UV, cerium ammonium molybdate stain, and anisaldehyde stain. ^{1}H NMR spectra were obtained on a Bruker 400 or 600 MHz spectrometer with reporting relative to residual solvent signals ($CDCl_3$, 7.26 ppm; CD_3OD, 3.31 ppm; D_2O, 4.79 ppm). ^{1}H NMR spectral data are presented as follows: chemical shifts (δ, ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz), integration, proton assignment. ^{13}C NMR spectra were obtained on a Bruker 100 MHz spectrometer with reporting relative to residual solvent signals ($CDCl_3$, 77.16 ppm; CD_3OD, 49.0 ppm). ^{13}C NMR spectral data are presented as follows: chemical shifts (δ, ppm), carbon assignment. Proton and carbon assignments were made with the aid of 2D NMR techniques (COSY, HSQC, and HMBC). High resolution mass spectra were recorded on a high resolution Thermo Electron Corporation MAT 95XP-Trap by use of electro-spray ionization (ESI) by the Indiana University Mass Spectrometry facility and a SYNAPT G2 or SYNAPT G2-S spectrometer (Waters, for TOFMS) by the McLean lab of Vanderbilt University. Optical rotations were obtained using a Perkin Elmer 341 polarimeter.

4.3.1 Compound Preparation and Characterization



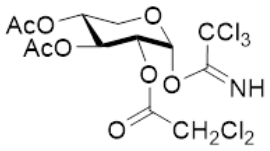
(3R,4S,5R,6S)-5-(2-chloroacetoxy)-6-(p-tolylthio)tetrahydro-2H-pyran-3,4-diyl diacetate (X5)

(3R,4R,5R,6S)-5-hydroxy-6-(p-tolylthio)tetrahydro-2H-pyran-3,4-diyl diacetate (210.4 mg, 0.618 mmol, 1.0 eq) was added to a flame-dried RBF with magnetic stirring bar and dissolved in acetonitrile (7.7 mL). Pyridine (0.15 mL, 1.85 mmol, 3 eq) was added and the reaction mixture was cooled to 0 °C. 2-chloroacetyl chloride (0.06 mL, 0.8 mmol, 1.25 eq) was diluted with acetonitrile (0.05 mL) and added dropwise to the reaction mixture. The reaction was stirred at 0 °C for 1 hour. Then, the mixture was diluted with dichloromethane (10 mL), washed sequentially with 1 N HCl (10 mL), saturated aqueous sodium bicarbonate (10 mL), and water (10 mL). Then, the organic layer was dried (sodium sulfate), filtered, concentrated (rotary evaporator), and purified via FCC (25% EtOAc/Hexanes) to produce a viscous yellow oil (213.8 mg) in 83% yield (9.1:1 β:α by ^{1}H NMR). $\mathbf{R_F}$ = 0.65 (50% in EtOAc/Hexanes) **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 7.39 – 7.34 (m, 1H), 7.14 (d, *J* = 7.9 Hz, 1H), 5.20 (t, *J* = 8.5 Hz, 0H), 4.96 – 4.88 (m, 1H), 4.71 (d, *J* = 8.7 Hz, 0H), 4.25 (dd, *J* = 11.7, 5.1 Hz, 1H), 4.13 – 4.03 (m, 1H), 3.39 (dd, *J* = 11.7, 9.2 Hz, 1H), 2.35 (s, 2H), 2.03 (d, *J* = 1.1 Hz, 3H).**^{13}C NMR:** (151 MHz, $CDCl_3$) δ 170.15, 169.89, 166.10, 139.09, 133.98, 130.01, 127.51, 86.03, 72.33, 71.60, 68.52, 65.78, 40.69, 21.34, 20.84, 20.83.



(3R,4S,5R,6R)-5-(2-chloroacetoxy)-6-hydroxytetrahydro-2H-pyran-3,4-diyl diacetate (X6)

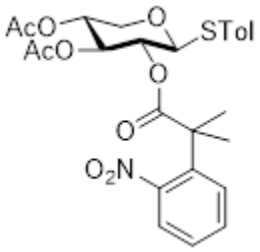
X5 (300.0 mg, 0.720 mmol, 1.00 eq) was added to a flame-dried RBF with magnetic stirring bar and dissolved in acetonitrile (7 mL, 0.1 M). To this mixture, N-bromosuccinimide (192.1 mg, 1.08 mmol, 1.5 eq) and water (19 μL, 19 μg, 1.08 mmol, 1.5 eq) were added sequentially. This was allowed to stir at room temperature for 5 hours. Then, the reaction was quenched with aqueous sodium thiosulfate (5 mL). The contents were transferred to a separatory funnel. The organic layer was washed with sodium thiosulfate (3X, 5 mL), dried (sodium sulfate), filtered, and concentrated (rotary evaporator). The product was purified via FCC (50% EtOAc/Hexanes) to isolate a viscous yellow oil (116.1 mg) in 52% yield. **R_F**: 0.21 in 50% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 8.66 (s, 1H), 6.48 (d, *J* = 3.6 Hz, 1H), 5.66 – 5.53 (m, 1H), 5.30 (s, 0H), 5.16 – 5.03 (m, 3H), 4.12 (q, *J* = 7.1 Hz, 1H), 4.05 – 3.95 (m, 2H), 3.88 – 3.78 (m, 1H), 2.06 (s, 6H), 2.05 (s, 3H), 2.02 (s, 3H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 169.95, 161.04, 93.17, 69.94, 69.36, 68.61, 60.79, 20.69, 20.48.



(3R,4S,5R,6R)-5-(2-chloroacetoxy)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,4-diyl diacetate (X7)

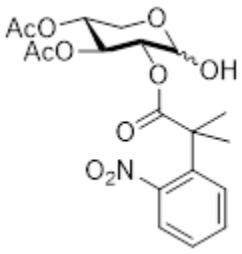
X6 (185.5 mg, 0.60 mmol, 1.0 eq) was added to a flame-dried RBF equipped with magnetic stirring bar and dissolved in dry dichloromethane (6 mL, 0.1 M). The flask was purged with argon, and

then 2,2,2-trichloroacetonitrile (0.30 mL, 3.0 mmol, 5.0 eq) and DBU (2 drops, cat.) were added sequentially. After 1 hour and 45 minutes, the reaction mixture was quenched with 1 N HCl (5 mL) and transferred to a separatory funnel. The aqueous layer was washed with DCM (3X, 10 mL); combined organics were washed sequentially with saturated aqueous sodium bicarbonate (15 mL) and brine (15 mL), dried (sodium sulfate), filtered, and concentrated (rotary evaporator). The resultant crude product was purified via FCC (10% EtOAc/Hexanes) to yield a viscous oil (137 mg) in 50% yield. $\mathbf{R_F}$: 0.56 in 50% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.68 (d, *J* = 3.6 Hz, 1H), 7.70 (dq, *J* = 6.9, 3.9, 3.4 Hz, 1H), 7.52 (dt, *J* = 6.0, 3.0 Hz, 1H), 7.26 (s, 3H), 6.49 (dt, *J* = 6.9, 3.1 Hz, 1H), 6.02 (dt, *J* = 22.1, 4.0 Hz, 1H), 5.60 (td, *J* = 12.7, 11.3, 8.8 Hz, 1H), 5.25 (dt, *J* = 7.0, 3.4 Hz, 1H), 5.19 (p, *J* = 5.7, 4.9 Hz, 2H), 5.16 – 5.06 (m, 3H), 4.98 (ddd, *J* = 24.8, 6.6, 3.7 Hz, 2H), 4.32 – 4.26 (m, 2H), 4.21 (dddd, *J* = 17.6, 11.0, 8.4, 2.2 Hz, 3H), 4.15 – 3.94 (m, 8H), 3.82 (tt, *J* = 11.1, 3.3 Hz, 1H), 3.70 (td, *J* = 12.4, 11.6, 6.1 Hz, 2H), 2.17 – 1.98 (m, 19H), 1.71 – 1.63 (m, 2H), 1.48 – 1.32 (m, 5H), 1.32 – 1.28 (m, 5H), 1.25 (h, *J* = 2.9 Hz, 3H), 0.90 (dtd, *J* = 14.8, 7.3, 2.6 Hz, 8H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 169.97, 169.94, 169.91, 169.67, 169.18, 167.89, 166.78, 166.63, 166.47, 165.93, 161.02, 160.72, 160.62, 132.57, 131.01, 128.93, 95.19, 94.71, 93.18, 92.96, 90.66, 71.55, 71.44, 71.13, 69.83, 69.54, 69.24, 68.58, 68.53, 68.39, 68.28, 68.15, 67.56, 67.44, 62.14, 61.97, 60.95, 60.83, 60.52, 40.60, 40.57, 40.45, 40.36, 38.84, 30.48, 29.82, 29.04, 23.86, 23.11, 21.18, 20.93, 20.88, 20.83, 20.78, 20.72, 20.57, 14.32, 14.18, 11.08.



(3R,4S,5R,6S)-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)-6-(p-tolylthio)tetrahydro-2H-pyran-3,4-diyl diacetate (X8)

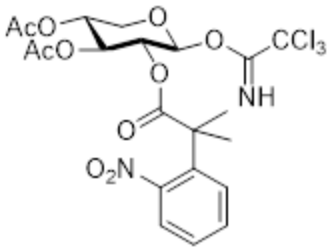
(3R,4R,5R,6S)-5-hydroxy-6-(p-tolylthio)tetrahydro-2H-pyran-3,4-diyl diacetate (589 mg,1.73 mmol, 1.0 eq) and 2-methyl-2-(2-nitrophenyl)propanoic anhydride (DMNPAA; 831 mg, 2.08 mmol, 1.2 eq) were added to a flame-dried round-bottom flask charged with magnetic stirring bar and dissolved in dichloromethane (13 mL, 0.13 M). The flask was purged with argon gas. Then, mixture was cooled to -36 °C. Trimethylsilyltriflate (1.2 mL, 1.4 g, 6.4 mmol, 3.7 eq) was added dropwise via single-use syringe. The reaction was stirred for 1 hour before being quenched with triethylamine (3 mL). The reaction mixture was transferred to a separatory funnel and washed sequentially with saturated aqueous sodium bicarbonate (2X, 10 mL) and brine (1X, 10 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated (rotary evaporator). The crude material was purified via FCC (25% EtOAc/Hexanes) and isolated as a white solid (610 mg) in 66% yield. $\mathbf{R_F}$: 0.3 in 25% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 7.74 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.54 (ddd, *J* = 8.7, 7.4, 1.4 Hz, 1H), 7.46 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.39 – 7.36 (d, *J* = 8.0 Hz, 2H), 7.34 (ddd, *J* = 8.5, 7.4, 1.4 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 5.17 (t, *J* = 8.4 Hz, 1H), 4.95 – 4.87 (m, 2H), 4.71 (d, *J* = 8.5 Hz, 1H), 4.25 (dd, *J* = 11.7, 5.0 Hz, 1H), 3.39 (dd, *J* = 11.7, 9.0 Hz, 1H), 2.34 (s, 3H), 2.10 (s, 3H), 2.04 (d, *J* = 1.9 Hz, 3H), 1.59 (s, 3H), 1.54 (s, 3H). **^{13}C NMR:**



(3R,4S,5R)-6-hydroxy-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)tetrahydro-2H-pyran-3,4-diyl diacetate (X9)

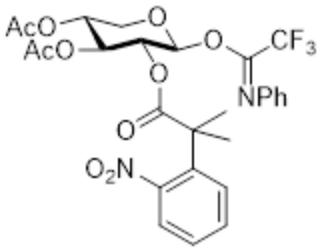
(3R,4S,5R,6S)-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)-6-(p-tolylthio)tetrahydro-2H-pyran-3,4-diyl diacetate (2.43 g, 4.57 mmol, 1.0 eq) was added to a RBF with magnetic stirring bar and dissolved in acetonitrile (51 mL, 0.09 M). To this mixture, N-bromosuccinimide (895 mg, 5.03 mmol, 1.1 eq) and water (90 μL, 90 μg, 5.0 mmol, 1.1 eq) were added sequentially. This was allowed to stir at room temperature for 2 hours. Then, the reaction was quenched with aqueous sodium thiosulfate (20 mL). The contents were transferred to a separatory funnel. The organic layer was washed with sodium thiosulfate (3X, 25 mL), dried (sodium sulfate), filtered, and concentrated (rotary evaporator). The product was purified via FCC (50% EtOAc/Hexanes) to isolate a white solid (819 mg) as a 2.18:1.00 β:α anomeric mixture in 42% yield. **R_F**: 0.24 in 40% EtOAc/Hexanes. **^{1}H NMR (β anomer): (**600 MHz, $CDCl_3$) δ 7.99 – 7.95 (m, 1H), 7.66 – 7.60 (m, 1H), 7.46 – 7.41 (m, 1H), 6.15 (d, *J* = 3.8 Hz, 1H), 5.52 (t, *J* = 9.5 Hz, 1H), 5.39 (d, *J* = 3.5 Hz, 1H), 5.25 (td, *J* = 9.4, 7.3 Hz, 1H), 5.03 – 4.93 (m, 1H), 4.86 (dd, *J* = 9.8, 3.5 Hz, 1H), 3.94 – 3.85 (m, 1H), 3.86 – 3.79 (m, 1H), 2.77 (s, 1H), 2.09 (s, *3H*), 2.04 (d, *J* = 1.7 Hz, 6H),1.72 (s, 3H) **^{1}H NMR (α anomer): (**600 MHz, $CDCl_3$) δ 7.99 – 7.95 (m, 1H), 7.66 – 7.60 (m, 2H), 7.46 – 7.41 (m, 1H), 6.15 (d, *J* = 3.8 Hz, 1H), 5.25 (td, *J* = 9.4, 7.3 Hz, 1H), 5.03 – 4.93 (m, 1H), 4.82 (dd, *J* = 9.4, 7.5 Hz, 1H), 4.68 (d, *J* = 7.6 Hz, 1H), 4.17 – 4.10 (m, 1H), 3.94 – 3.85 (m, 1H), 3.86 – 3.79 (m, 1H), 3.63 (t, *J* = 10.8 Hz, 1H), 3.38 (dd, *J* = 11.8, 9.9 Hz, 1H), 2.20 (s, 3H), 2.13 (s, 3H), 2.05 (d, *J* = 3.2 Hz, 6H),

^{13}C NMR: (151 MHz, $CDCl_3$) δ 171.73, 170.34, 170.20, 133.52, 128.22, 128.08, 125.91, 96.09, 91.77, 90.49, 73.24, 72.81, 71.53, 71.27, 70.16, 69.34, 69.27, 69.17, 68.26, 62.85, 60.82, 58.72, 46.33, 29.69, 27.41, 21.08, 20.89, 20.84.



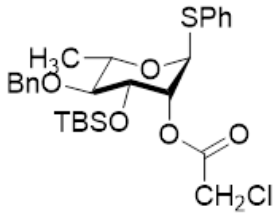
(3R,4S,5R)-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,4-diyl diacetate (X10)

(3R,4S,5R)-6-hydroxy-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)tetrahydro-2H-pyran-3,4-diyl diacetate (106 mg, 0.25 mmol, 1.0 eq) was added to a flame-dried RBF equipped with magnetic stirring bar and dissolved in dry dichloromethane (0.4 mL, 0.6 M). The flask was purged with argon, and then 2,2,2-trichloroacetonitrile (1.37 mL, 1.98 g, 13.7 mmol, 55 eq) and DBU (0.05 mL, 0.05 g, 0.33 mmol, 1.3 eq) were added sequentially. After 1 hour, the reaction mixture was diluted with dichloromethane (15 mL) and transferred to a separatory funnel. The organic layer was sequentially washed with water (3X, 10 mL) and brine (3X, 10 mL), dried (sodium sulfate), filtered, and then concentrated (rotary evaporator). The resultant crude product was purified via FCC (20% EtOAc/Hexanes) to yield a white solid (70 mg) in 49% yield. $\mathbf{R_F}$: 0.58 in 50% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 9.85 (s, 1H), 7.71 (dd, *J* = 5.6, 3.5 Hz, 1H), 7.53 (dd, *J* = 5.6, 3.5 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.23 (dd, *J* = 17.1, 8.0 Hz, 2H), 7.14 (d, *J* = 7.8 Hz, 1H), 4.22 (qd, *J* = 10.9, 5.9 Hz, 2H), 2.42 (s, 1H), 2.38 (s, 1H), 2.03 (dd, *J* = 27.0, 8.2 Hz, 1H), 1.74 – 1.61 (m, 3H), 1.43 (dq, *J* = 15.6, 7.3 Hz, 3H), 1.39 – 1.32 (m, 3H), 1.30 (td, *J* = 7.7, 3.9 Hz, 5H), 1.23 (s, 1H), 1.21 (t, *J* = 6.9 Hz, 2H), 1.07 (s, 1H), 0.93 (d, *J* = 7.4 Hz, 2H), 0.92 – 0.86 (m, 5H), 0.86 – 0.78 (m, 3H), 0.72 (q, *J* = 11.0, 7.5 Hz, 1H), 0.07 (s, 1H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 130.91, 128.81, 68.19, 38.74, 30.37, 29.72, 28.94, 23.76, 10.98.



(3R,4S,5R)-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)-6-((Z)-2,2,2-trifluoro-1-(phenylimino)ethoxy)tetrahydro-2H-pyran-3,4-diyl diacetate (X11)

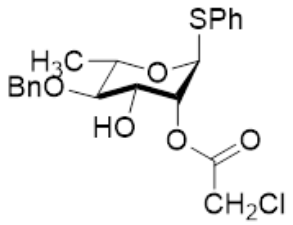
(3R,4S,5R)-6-hydroxy-5-((2-methyl-2-(2-nitrophenyl)propanoyl)oxy)tetrahydro-2H-pyran-3,4-diyl diacetate (77.4 mg, 0.18 mmol, 1.0 eq) was added to a flame-dried RBF with magnetic stirring bar and dissolved in dry acetone (4.6 mL, 40 mM); the reaction flask was purged with argon. Then, (Z)-2,2,2-trifluoro-N-phenylacetimidoyl chloride (71.8 mg, 0.35 mmol, 1.9 eq) was added. Oven-dried potassium carbonate (37.7 mg, 0.27 mmol, 1.5 eq) was added and the reaction mixture was allowed to stir overnight. The reaction mixture was concentrated *in vacuo* and charged onto a silica gel column. The crude mixture was purified via FCC (20% EtOAc/Hexanes) to produce a white solid (101.9 mg) in 94% yield. $\mathbf{R_F}$: 0.52 in 40% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 7.27 (s, 1H), 7.31 – 7.19 (m, 4H), 7.07 (t, *J* = 7.5 Hz, 2H), 6.80 (d, *J* = 8.0 Hz, 3H), 6.75 (d, *J* = 7.8 Hz, 2H), 5.49 (t, *J* = 9.8 Hz, 1H), 5.08 (d, *J* = 20.3 Hz, 2H), 5.04 (dd, *J* = 11.5, 5.6 Hz, 2H), 4.89 (q, *J* = 5.0 Hz, 1H), 4.19 (d, *J* = 11.7 Hz, 1H), 3.93 (td, *J* = 13.3, 12.4, 5.6 Hz, 1H), 3.73 (d, *J* = 10.9 Hz, 1H), 2.06 (s, 5H), 2.04 (s, 3H), 2.03 (s, 6H), 2.01 (s, 7H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 169.88, 169.83, 169.80, 169.76, 169.51, 169.04, 143.08, 143.02, 128.83, 128.81, 124.58, 119.33, 69.62, 69.27, 68.50, 68.43, 67.63, 67.33, 61.61, 60.72, 20.77, 20.69, 20.64, 20.57, 20.54, 20.48.



(2S,3R,4R,5S,6S)-5-(benzyloxy)-4-((tert-butyldimethylsilyl)oxy)-6-methyl-2-(phenylthio)tetrahydro-2H-pyran-3-yl 2-chloroacetate (R8)

(2S,3R,4S,5S,6S)-5-(benzyloxy)-4-((tert-butyldimethylsilyl)oxy)-6-methyl-2-(phenylthio)tetrahydro-2H-pyran-3-ol (2.82 g, 6.12 mmol, 1.0 eq) was added to a flame-dried RBF with magnetic stirring bar and dissolved in dry acetonitrile (77 mL, 0.08 M). Pyridine (1.49 mL, 18.4 mmol, 3.0 eq) was added, the reaction flask was purged with argon gas, and the mixture was brought to 0 °C. 2-chloroacetyl chloride (0.61 mL, 1.25 eq) was diluted with acetonitrile (0.5 mL) and added dropwise, and then the reaction was allowed to warm to room temperature. After 30 minutes, only starting material was observed on TLC, so more 2-chloroacetyl chloride (2 mL) was added (total of 2.61 mL, 33 mmol, and 5.33 eq of 2-chloroacetyl chloride). After an additional hour of stirring, the reaction went to completion. The mixture was diluted with dichloromethane (50 mL) and transferred to a separatory funnel. The organic layer was sequentially washed with 1 N HCl (60 mL), saturated aqueous sodium bicarbonate (60 mL), dried (sodium sulfate), decanted, and concentrated (rotary evaporator). The resultant crude product was purified via FCC (10% EtOAc/Hexanes) to give a viscous oil (2.82 g) in 70% yield. $\mathbf{R_F}$: 0.54 in 20% EtOAc/Hexanes **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 7.55 – 7.28 (m, 9H), 4.89 (dd, *J* = 11.2, 4.0 Hz, 1H), 4.68 (s, 1H), 4.62 (d, *J* = 11.0 Hz, 1H), 4.15 (s, 1H), 4.11 – 4.05 (m, 1H), 3.95 – 3.90 (m, 1H), 3.86 (d, *J* = 15.0 Hz, 1H), 3.46 – 3.39 (m, 1H), 1.42 – 1.18 (m, 6H), 1.05 – 0.79 (m, 9H), 0.22 – -0.02 (m, 8H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 166.76, 166.74, 166.68, 138.24, 138.09, 133.82, 133.72, 132.03,

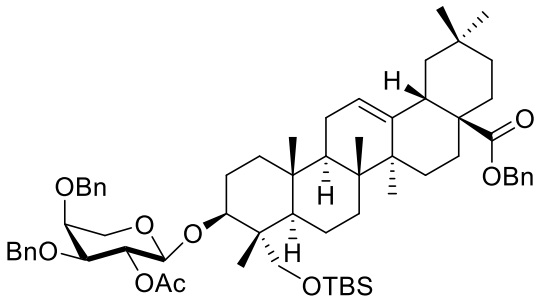
131.97, 131.90, 131.69, 131.43, 131.25, 129.30, 129.25, 129.23, 129.21, 129.16, 129.13, 128.60, 128.49, 128.48, 128.36, 128.12, 128.06, 128.00, 127.93, 127.91, 127.87, 127.84, 127.64, 127.60, 127.54, 88.66, 86.35, 85.99, 84.79, 81.32, 80.55, 80.38, 78.72, 76.47, 76.41, 76.35, 76.26, 76.08, 75.66, 75.18, 74.60, 73.27, 71.57, 71.48, 71.06, 69.52, 69.45, 67.98, 40.98, 40.93, 40.91, 40.79, 40.27, 31.70, 26.34, 26.20, 25.94, 25.89, 25.85, 25.82, 25.75, 25.73, 18.28, 18.23, 18.09, 18.07, 18.03, 17.92, 17.86, 17.58, -2.82, -4.14, -4.50, -4.59, -4.63, -4.66, -4.69, -4.81, -4.84, -4.94.



(2S,3R,4R,5R,6S)-5-(benzyloxy)-4-hydroxy-6-methyl-2-(phenylthio)tetrahydro-2H-pyran-3-yl 2-chloroacetate (R9)

(2S,3R,4R,5S,6S)-5-(benzyloxy)-4-((tert-butyldimethylsilyl)oxy)-6-methyl-2-(phenylthio)tetrahydro-2H-pyran-3-yl 2-chloroacetate (308 mg, 0.572 mmol, 1.00 eq) was added to a flame-dried round bottom flask under argon atmosphere equipped with magnetic stirring bar. This was dissolved in THF (1.9 mL, 0.3 M). The mixture was cooled to 0 °C, and 1 M TBAF, (0.80 mL, 0.80 mmol, 1.4 eq) was added dropwise. The ice bath was removed and the reaction was allowed to warm to and stir at room temperature for 3 hours. The reaction mixture was concentrated *in vacuo* and diluted with ethyl acetate (5 mL). The mixture was transferred to a separatory funnel and washed with aqueous ammonium chloride (3 mL). Then, the aqueous layer was extracted with ethyl acetate (3X, 5 mL). Combined organics were dried (sodium sulfate), filtered, and concentrated (rotary evaporator). The resultant crude product was purified via FCC

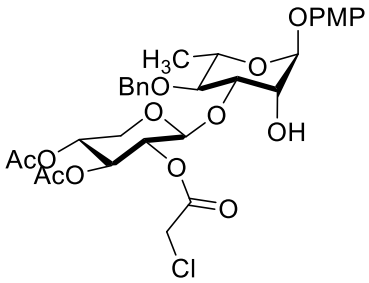
(20% EtOAc/Hexanes) to produce a viscous yellow oil (127 mg) in 52% yield. **R_F**: 0.48 in 20% EtOAc/Hexanes **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 7.52 – 7.27 (m, 10H), 5.48 (s, 1H), 4.90 – 4.67 (m, 2H), 4.28 – 4.17 (m, 1H), 3.93 (dt, *J* = 8.5, 4.4 Hz, 1H), 3.49 – 3.34 (m, 1H), 2.53 – 2.37 (m, 1H), 2.32 (d, *J* = 5.0 Hz, 1H), 1.46 – 1.28 (m, 3H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 138.23, 138.18, 134.37, 134.35, 134.25, 131.69, 131.53, 131.39, 129.26, 129.22, 129.17, 129.14, 128.80, 128.78, 128.74, 128.34, 128.21, 128.13, 128.11, 127.65, 127.62, 127.52, 87.54, 86.89, 85.06, 81.92, 81.21, 79.67, 76.04, 75.33, 75.21, 75.00, 74.33, 72.81, 72.69, 72.45, 72.10, 72.01, 69.19, 68.75, 25.80, 18.42, 18.04, 17.60.



Benzyl (6aS,6bR,9R,10S,12aR)-10-(((2S,3R,4S,5S)-3-acetoxy-4,5-bis(benzyloxy)tetrahydro-2H-pyran-2-yl)oxy)-9-(((tert-butyldimethylsilyl)oxy)methyl)-2,2,6a,6b,9,12a-hexamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (H-A-1)

Compounds **H2** (52.6 mg, 0.08 mmol, 1 eq) and **A5** (45.0 mg, 0.18 mmol, 1.5 eq) were co-evaporated with benzene (0.5 mL, 3X) and dried inside of a vacuum desiccator over P_2O_5 for two nights. Then, they were dissolved in dichloromethane (0.8 mL) and transferred to a flame-dried vial with magnetic stirring bar and activated 4Å molecular sieves under argon atmosphere. This mixture was allowed to stir for one hour. Then, the flask was placed in a -20 °C ice bath; TMSOTf (2 drops) was added, and the mixture was allowed to stir for one hour. After one hour, more

TMSOTf was added (40 μL) and the mixture was stirred for an additional hour. The mixture was quenched with triethylamine, filtered to remove sieves, and concentrated. Then, the crude was purified via FCC on silica gel (20% EtOAc/Hexanes) and isolated as a white solid in 33% yield as a 1.2:1 mixture of α:β anomers. $\mathbf{R_F}$: 0.59 (α) and 0.63 (β) in 50% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 7.34 (d, *J* = 7.4 Hz, 1H), 7.33 – 7.27 (m, 3H), 4.11 – 4.04 (m, 1H), 3.92 – 3.83 (m, 2H), 3.35 (s, 1H), 3.30 – 3.21 (m, 1H), 2.24 (dt, *J* = 12.7, 5.4 Hz, 1H), 2.02 (s, 1H), 1.90 – 1.77 (m, 4H), 1.64 – 1.55 (m, 8H), 1.47 (dd, *J* = 9.4, 4.4 Hz, 3H), 1.46 – 1.43 (m, 2H), 1.43 – 1.32 (m, 5H), 1.31 (s, 2H), 1.29 (s, 2H), 1.25 (s, 15H), 1.21 (s, 5H), 1.18 – 1.09 (m, 9H), 1.07 (s, 2H), 0.99 – 0.80 (m, 18H), 0.79 – 0.68 (m, 3H), 0.18 – 0.04 (m, 4H). **^{13}C NMR*:** (151 MHz, $CDCl_3$) δ 179.2, 172.5, 170.7, 166.9, 138.9, 129.0, 128.0, 126.2, 100.2, 85.4, 81.2, 78.0, 77.7, 74.5, 73.8, 72.1, 51.3, 45.0, 39.4, 36.2, 33.0, 31.6, 29.9, 27.8, 26.7, 23.5, 23.2, 20.7, 20.0, 18.8, 17.9, 17.2, 14.0, 13.0, 12.6, 10.9, 7.7. *Values from HSQC and HMBC



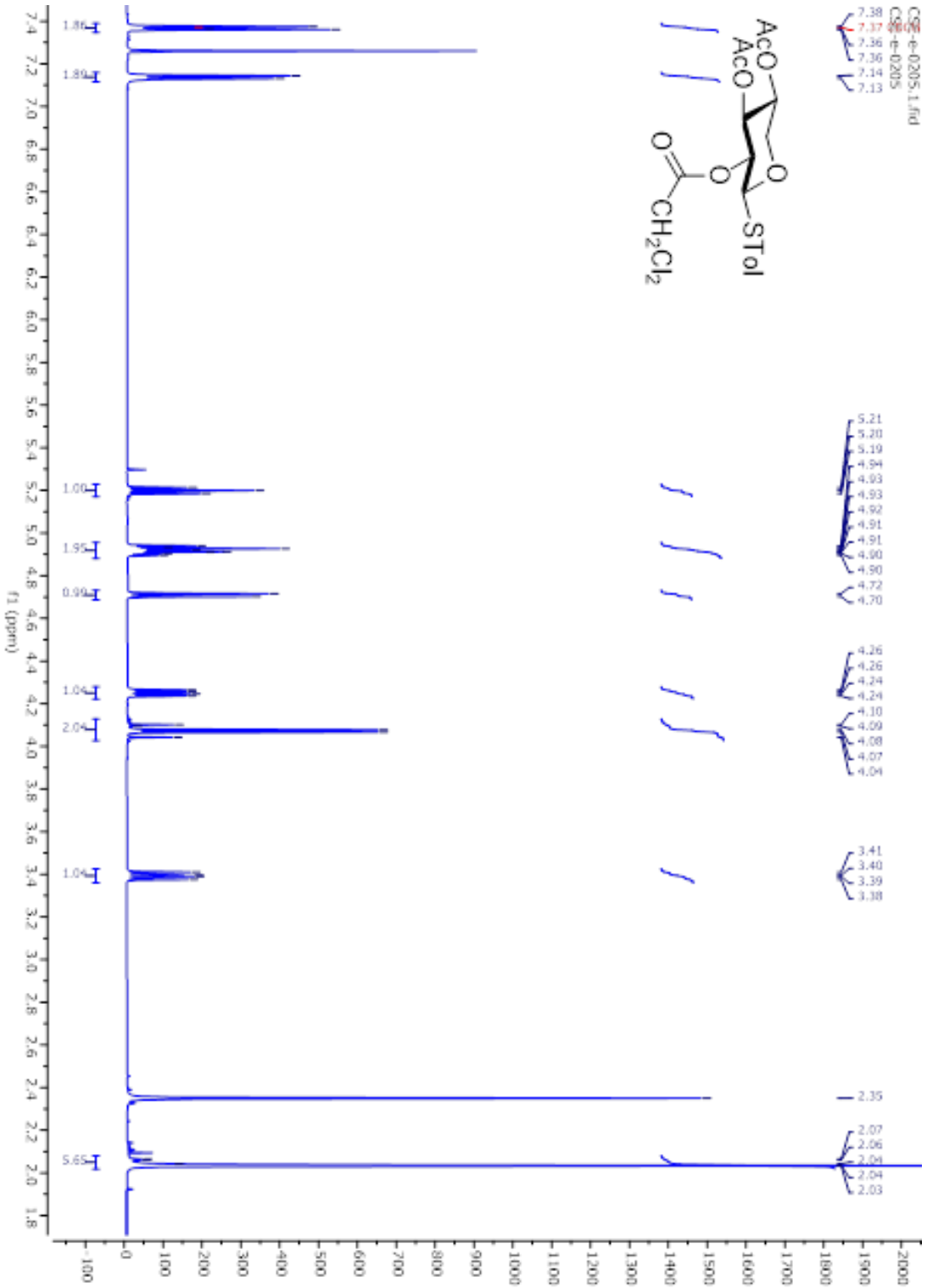
(3R,4S,5R,6S)-6-(((2S,3S,4S,5R,6S)-3-(benzyloxy)-5-hydroxy-6-(4-methoxyphenoxy)-2-methyltetrahydro-2H-pyran-4-yl)oxy)-5-(2-chloroacetoxy)tetrahydro-H-pyran-3,4-diyl diacetate (R-X-1)

Compounds **X5** (347 mg, 0.832 mmol, 2 eq) and **R14** (150 mg, 0.416 mmol, 1 eq) were co-evaporated in benzene (0.5 mL, 3X). They were dried in a vacuum desiccator over P_2O_5 overnight. Then, they were dissolved in propionitrile (4.2 mL) and transferred to a flame-dried vial with

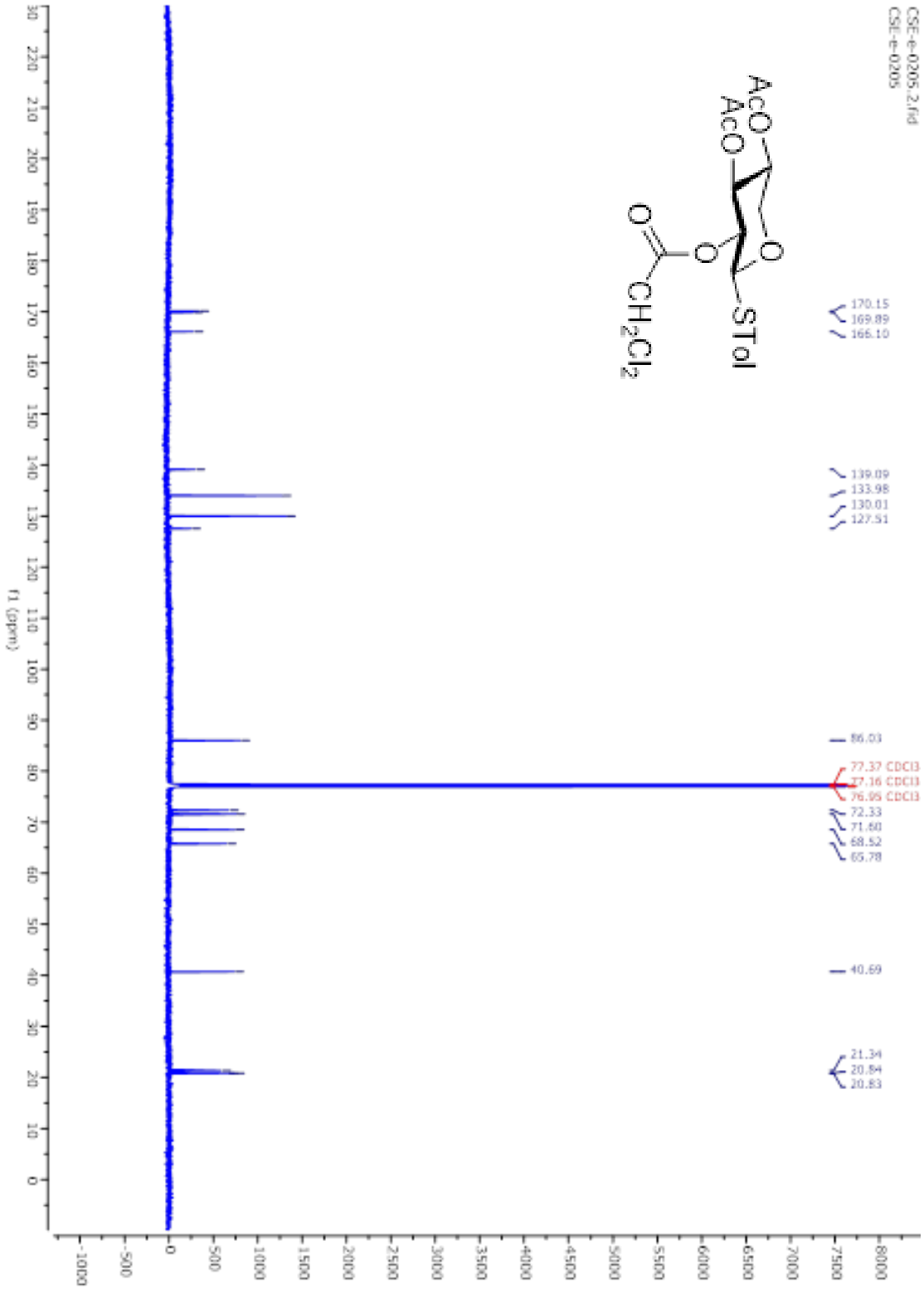
magnetic stirring bar. Activated 4Å molecular sieves were added to the vial until it became cloudy. Then, the vial was placed under argon atmosphere and the mixture was allowed to stir for 1 hour. After an hour, the flask was cooled to -78 °C. NIS (187 mg, 0.832 mmol, 2 eq) was added in one portion and TMSOTf (22.6 μL, 27.7 mg, 0.125 mmol, 0.3 eq) was added dropwise. The mixture was stirred for 1 hour before quenching with triethylamine (0.1 mL). The mixture was filtered through celite and concentrated, and the crude compound was purified via FCC (20% EtOAc/Hexanes) to yield a brown solid in 55% yield. **R_F:** 0.16 in 20% EtOAc/Hexanes. **^{1}H NMR:** (600 MHz, $CDCl_3$) δ 7.46 – 7.38 (m, 1H), 7.38 – 7.27 (m, 2H), 7.26 (s, 1H), 7.02 – 6.87 (m, 1H), 6.85 – 6.76 (m, 1H), 6.17 (d, *J* = 3.8 Hz, 0H), 5.49 – 5.30 (m, 1H), 5.17 – 4.91 (m, 1H), 4.91 – 4.82 (m, 1H), 4.82 – 4.62 (m, 1H), 4.33 – 3.93 (m, 3H), 3.88 – 3.80 (m, 1H), 3.80 – 3.68 (m, 2H), 3.68 – 3.49 (m, 1H), 3.45 – 3.34 (m, 1H), 2.76 (s, 0H), 2.45 – 2.28 (m, 1H), 2.25 – 1.99 (m, 5H), 1.37 – 1.17 (m, 2H), 0.23 – 0.11 (m, 1H). **^{13}C NMR:** (151 MHz, $CDCl_3$) δ 177.1, 175.6, 170.1, 170.0, 169.4, 156.1, 153.8, 128.8, 128.5, 128.1, 117.7, 114.7, 99.4, 98.0, 91.7, 89.9, 74.6, 71.7, 69.9, 69.1, 68.1, 60.7, 58.5, 55.8, 40.8, 29.8, 29.7, 21.1, 20.9, 14.9.

4.3.2 ^{1}H, ^{13}C, and 2D NMR Spectra

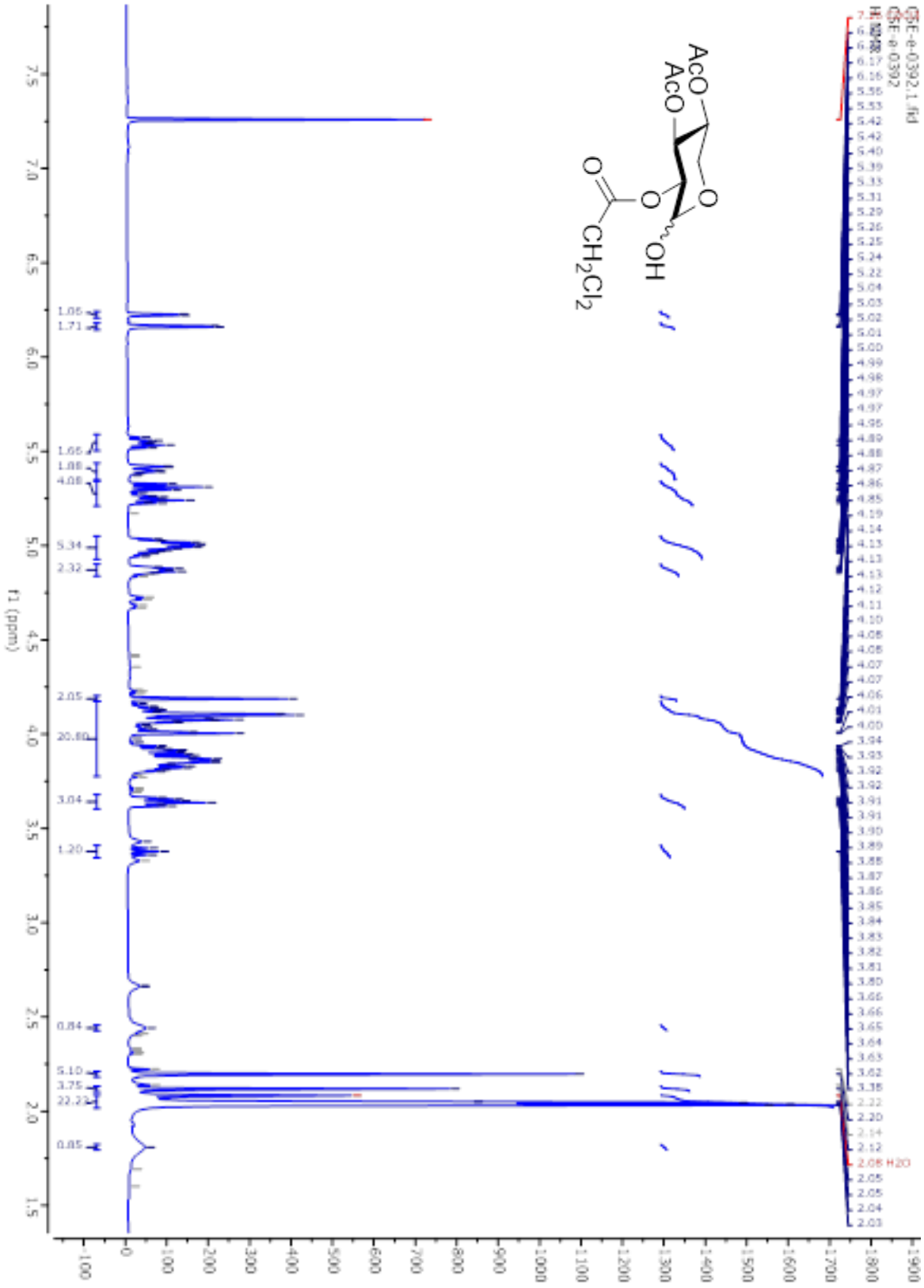
^{1}H NMR of Compound X5



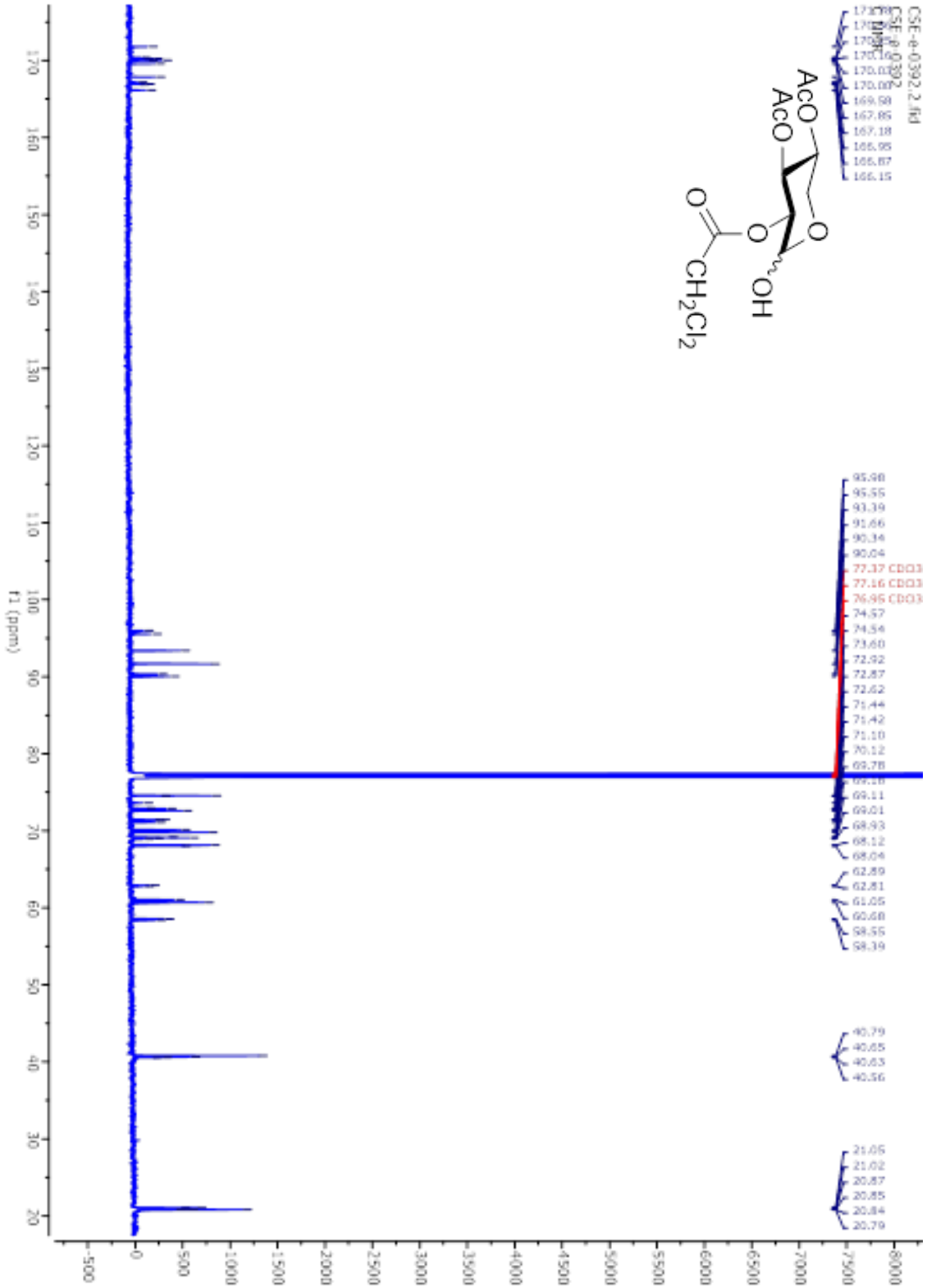
^{13}C NMR of Compound X5



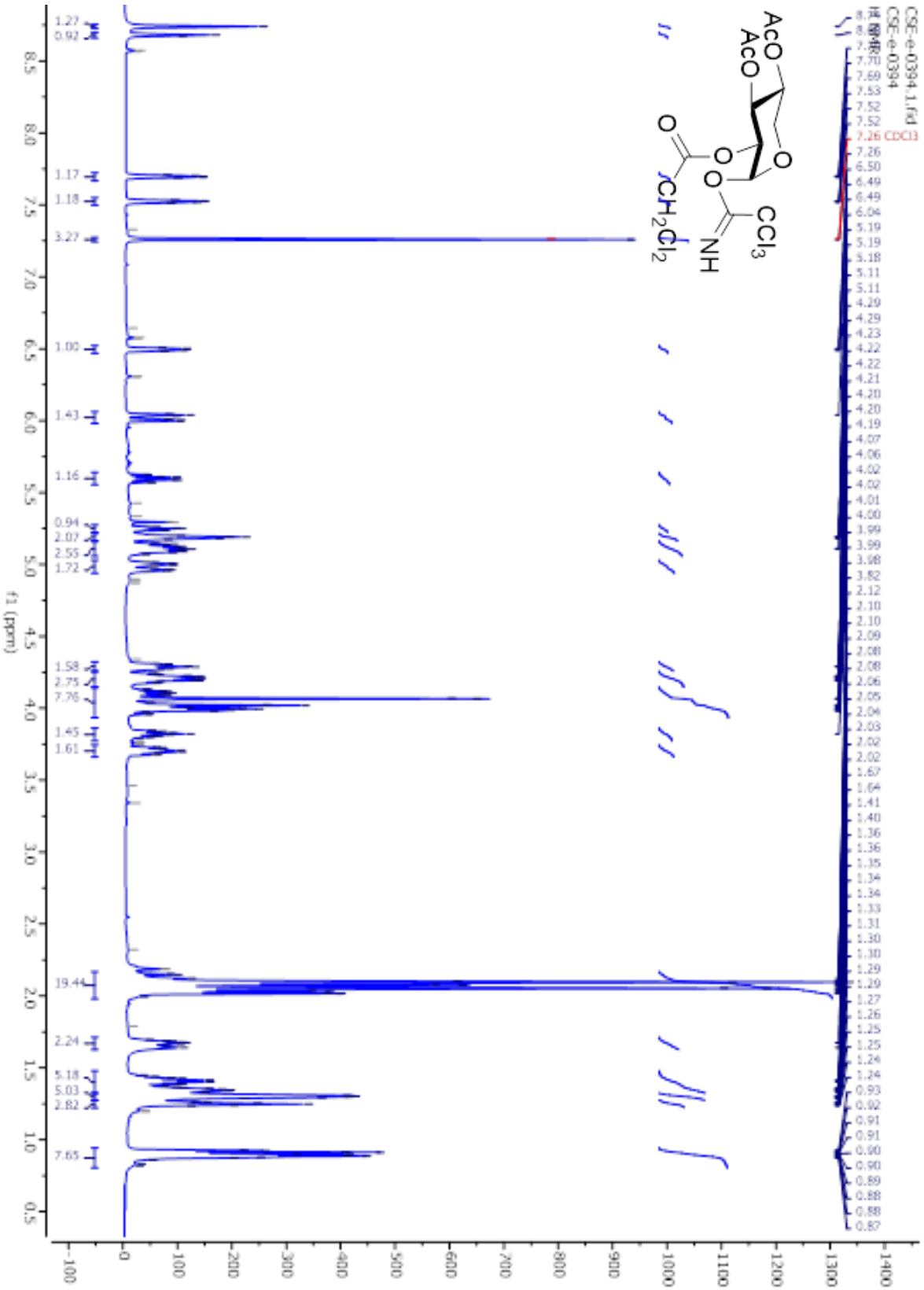
^{1}H NMR of Compound X6



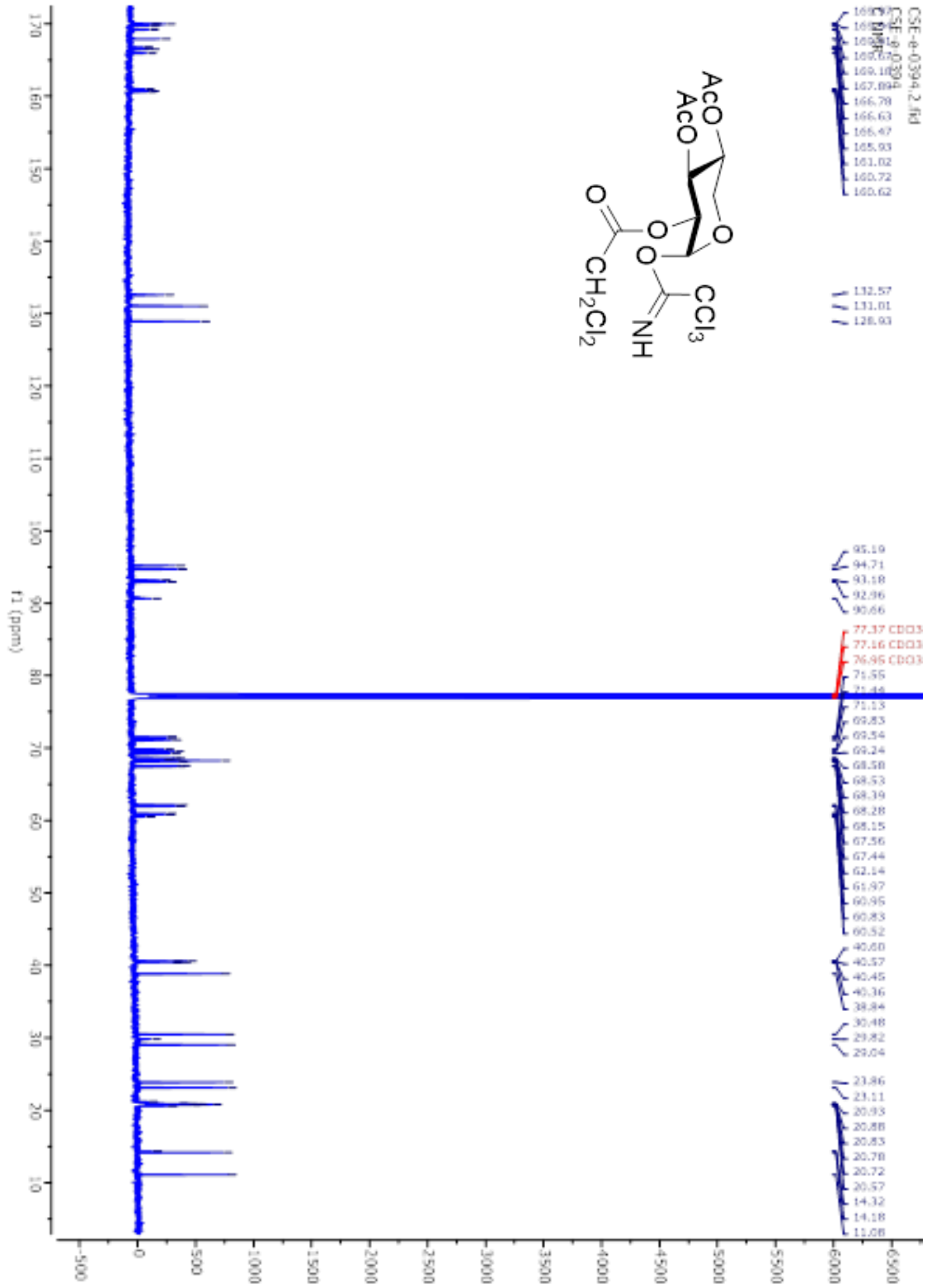
^{13}C NMR of Compound X6



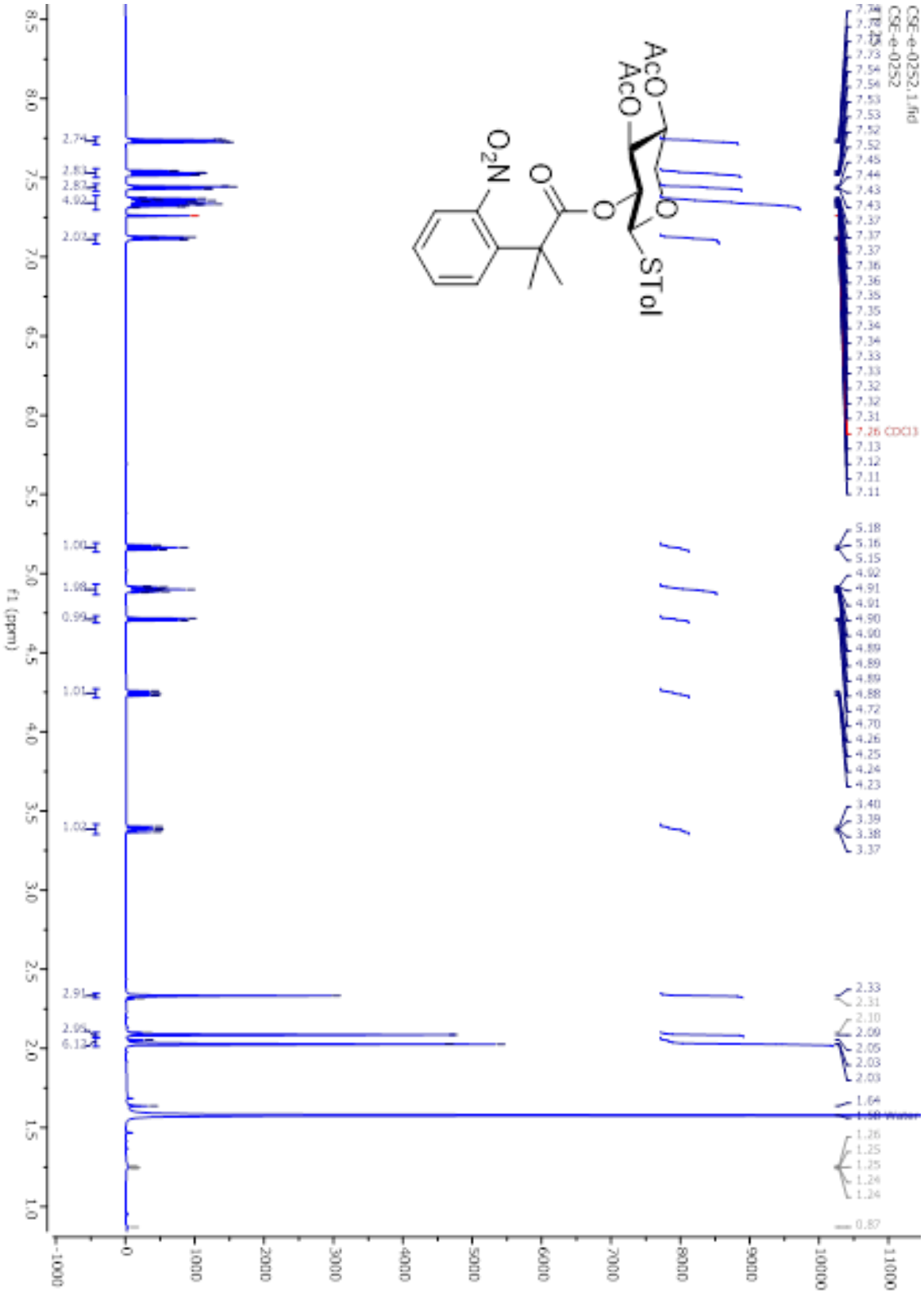
[1]H NMR of Compound X7



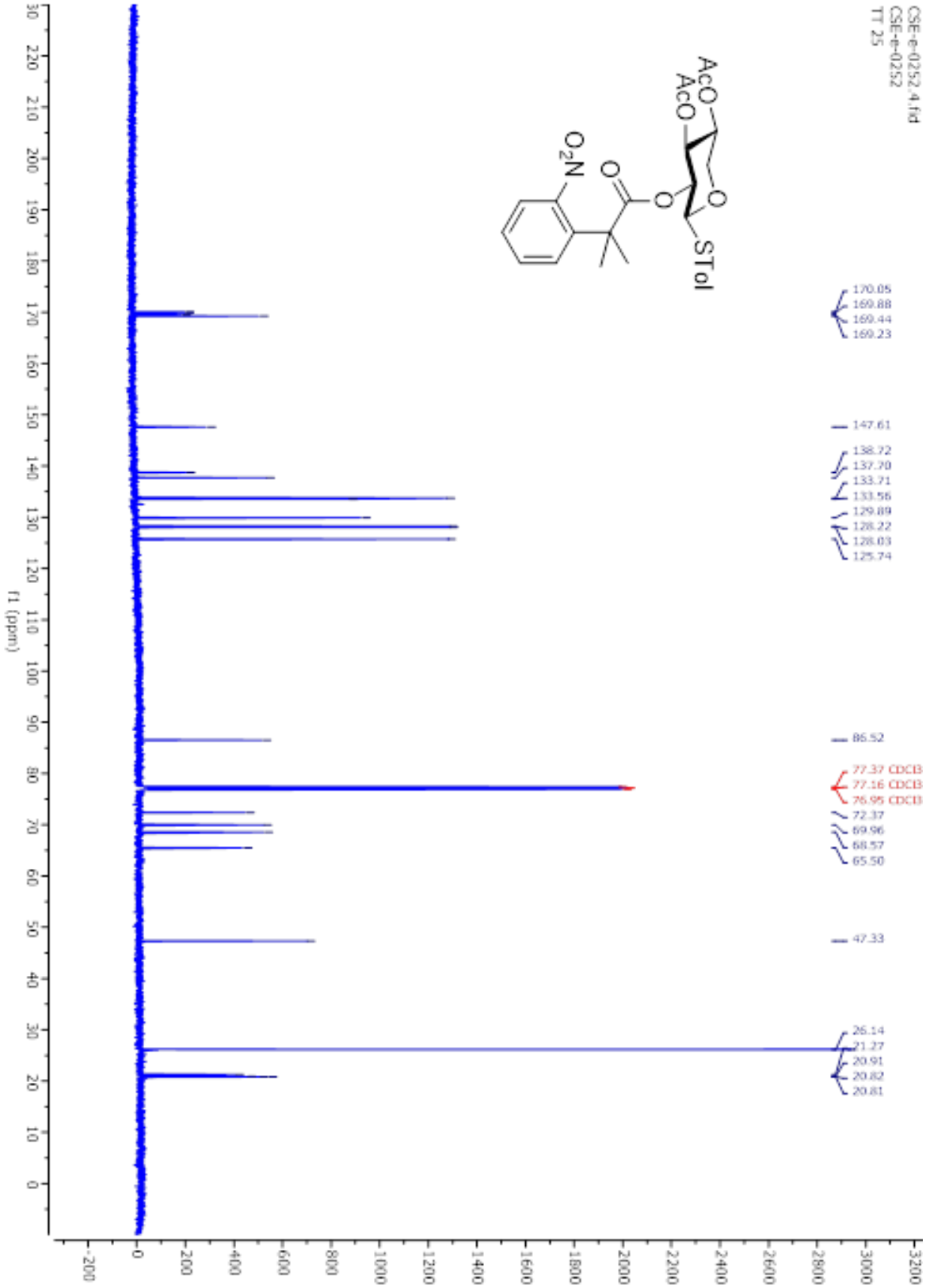
^{13}C NMR of Compound X7



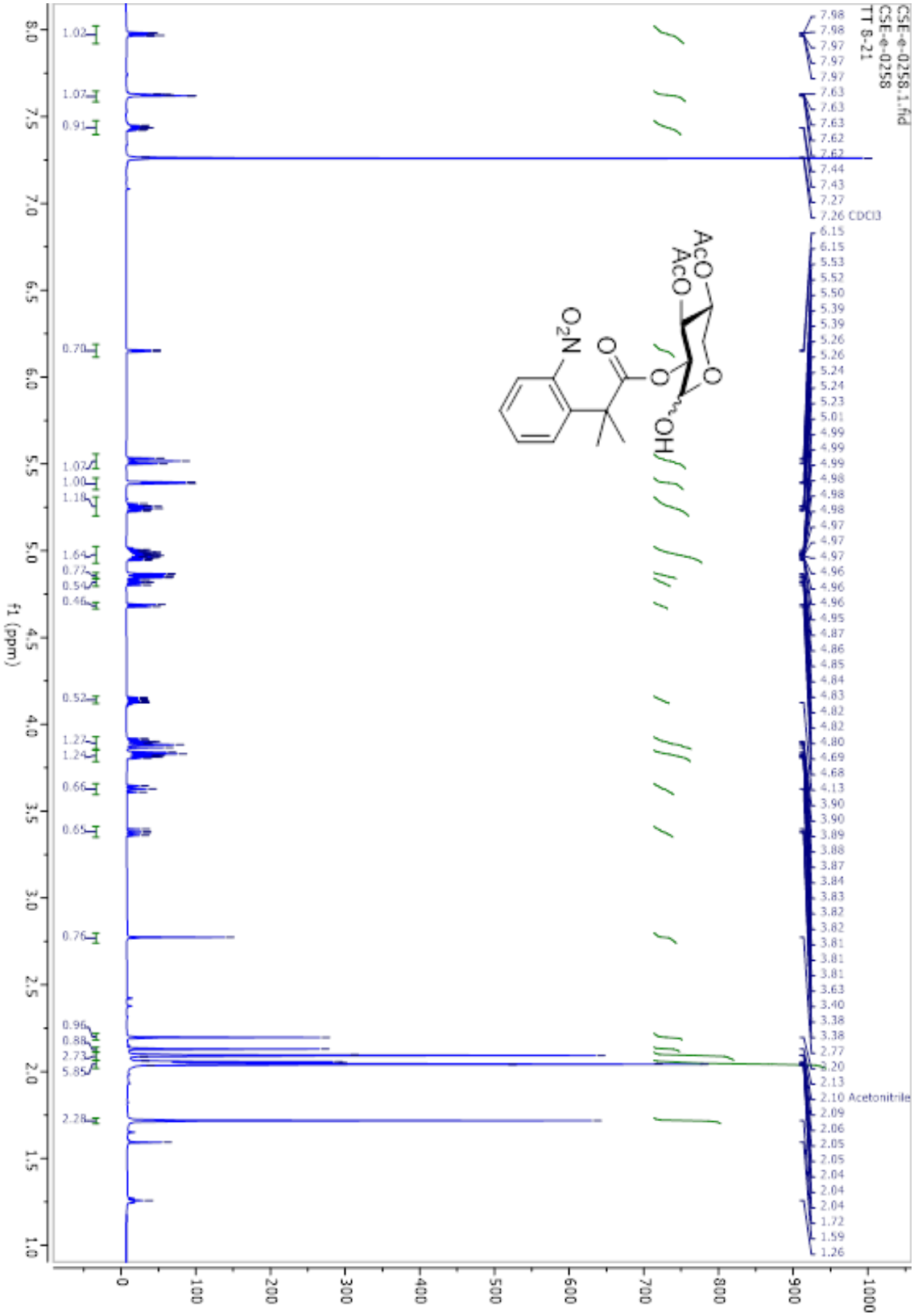
^{1}H NMR of Compound X8



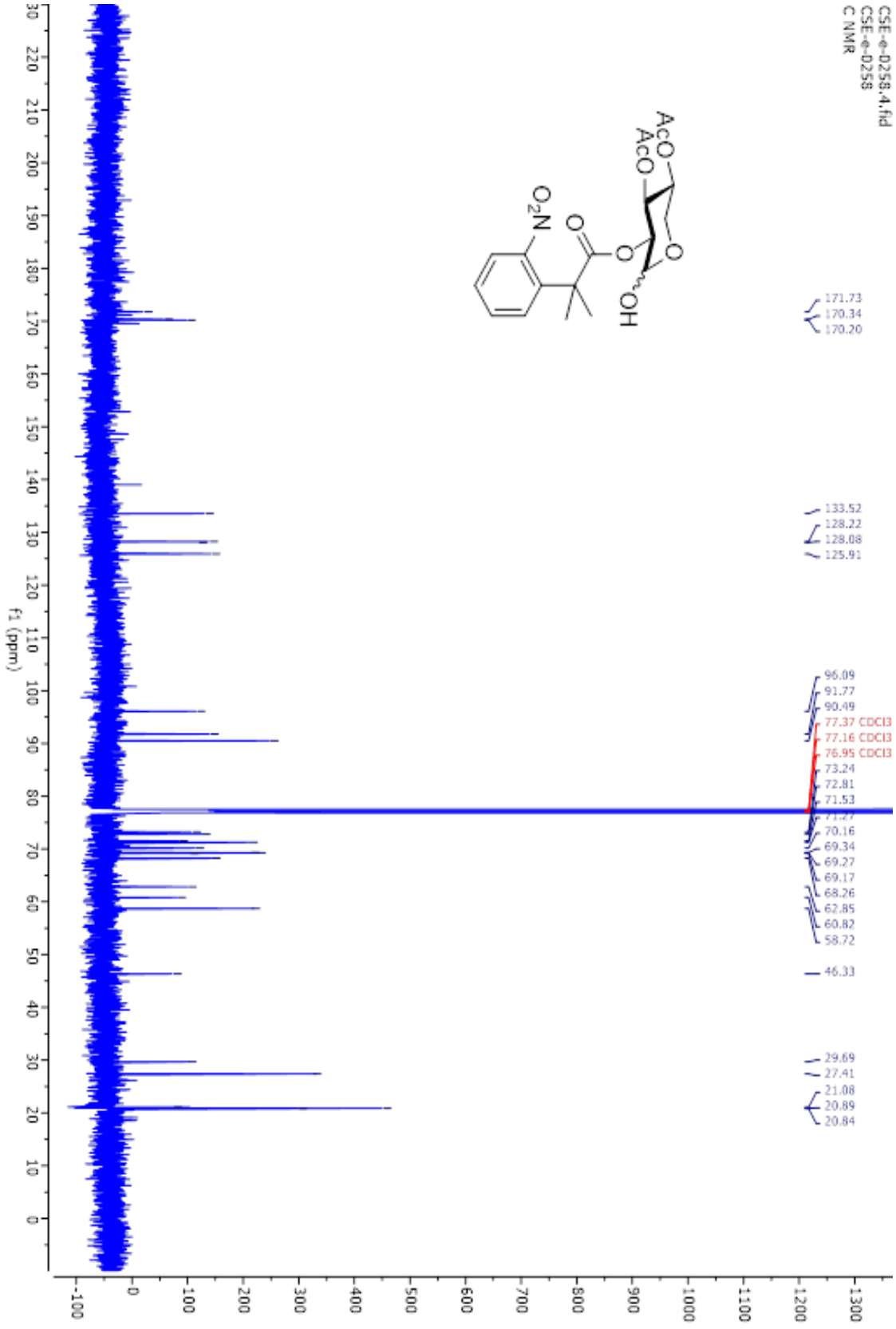
^{13}C NMR of Compound X8



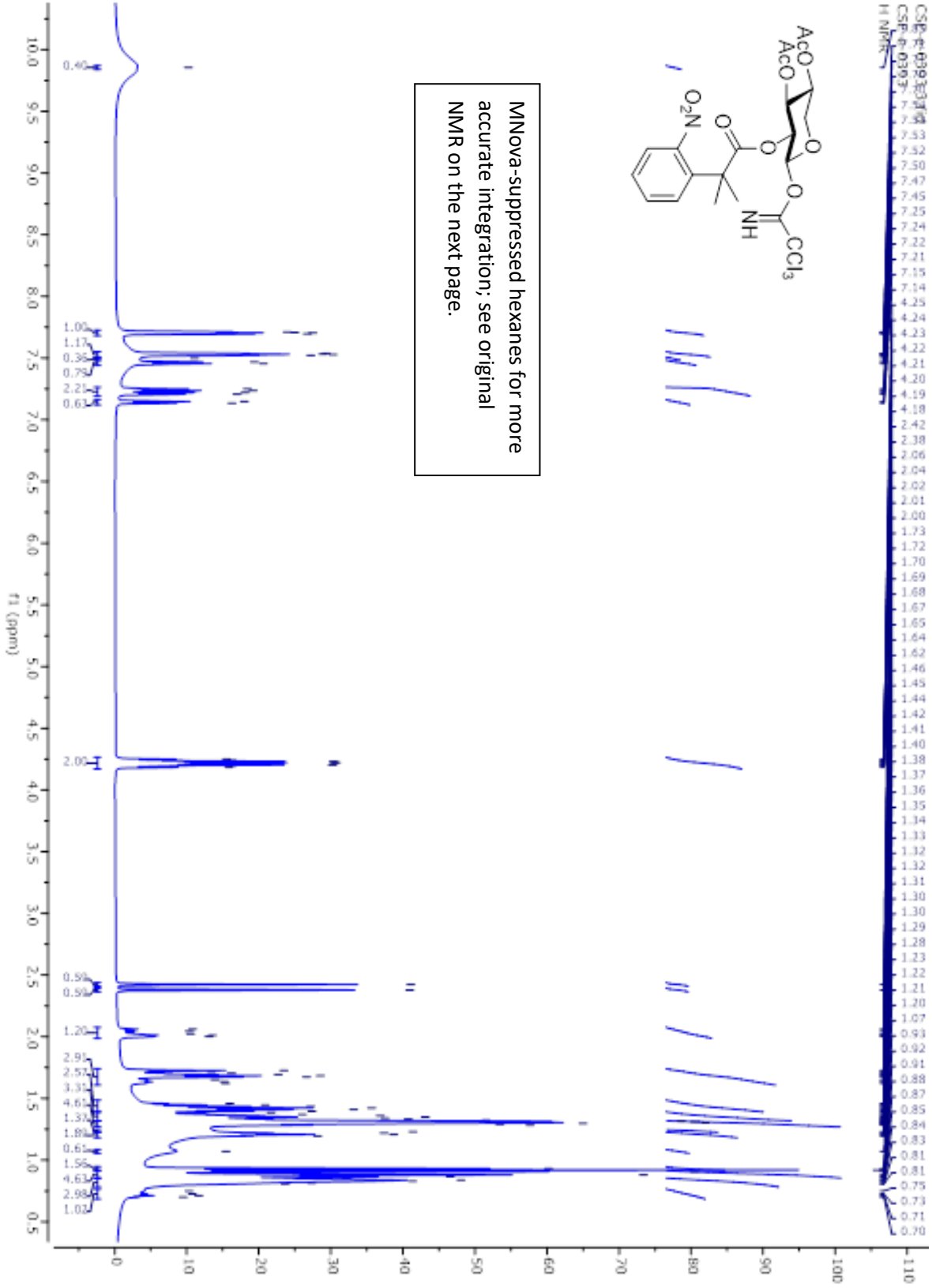
[1]H NMR of Compound X9



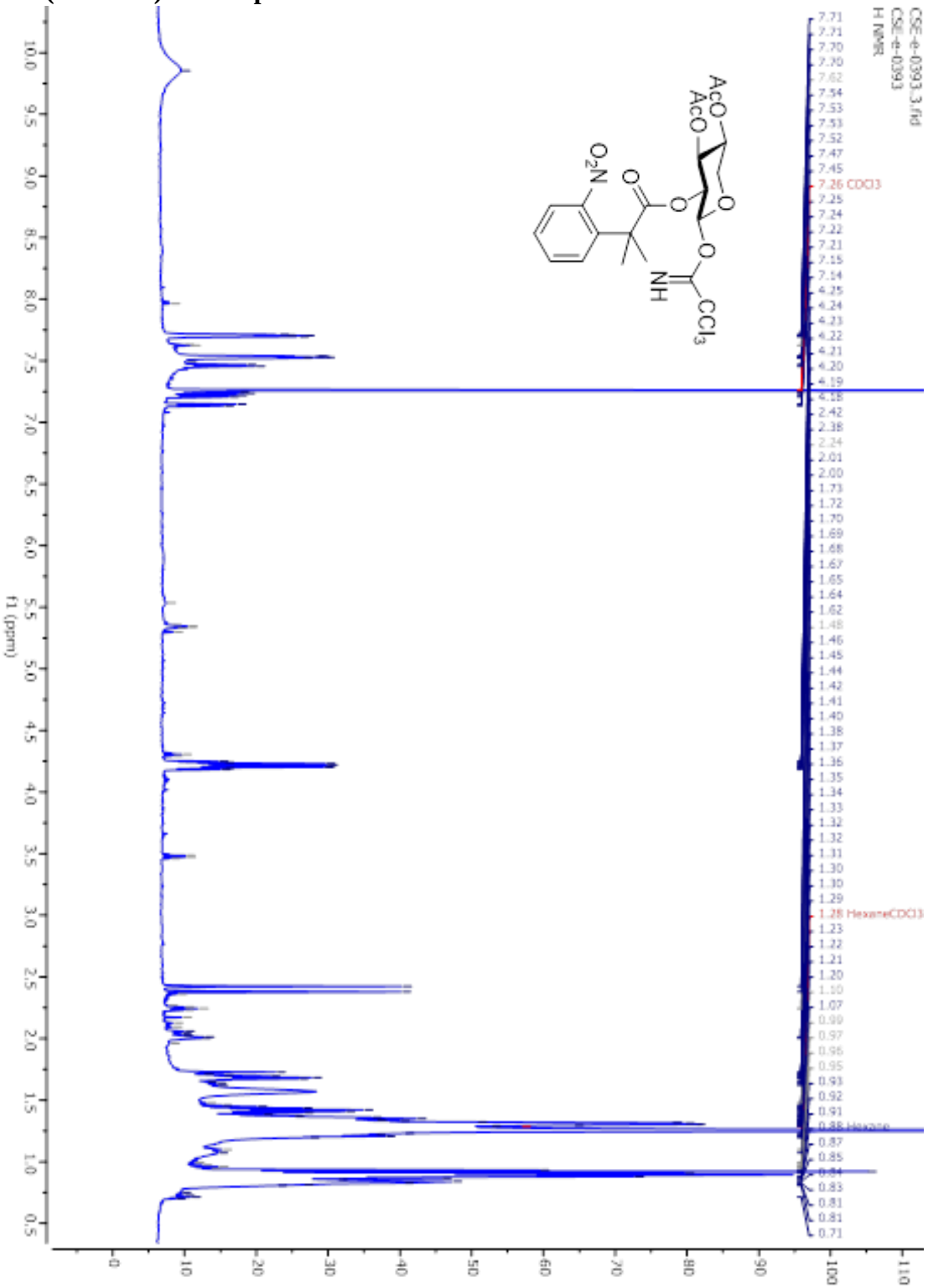
^{13}C NMR of Compound X9



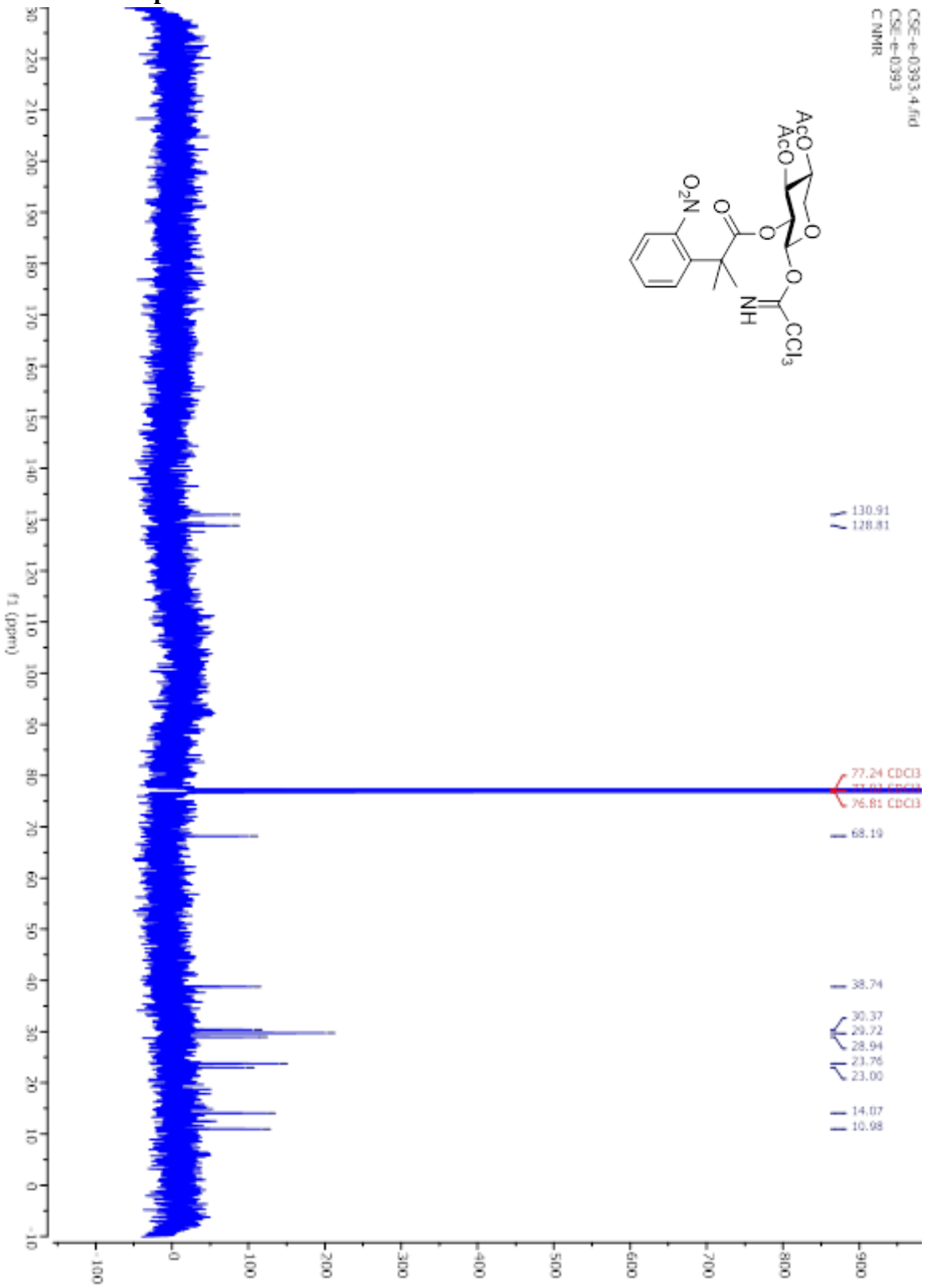
^{1}H NMR of Compound X10



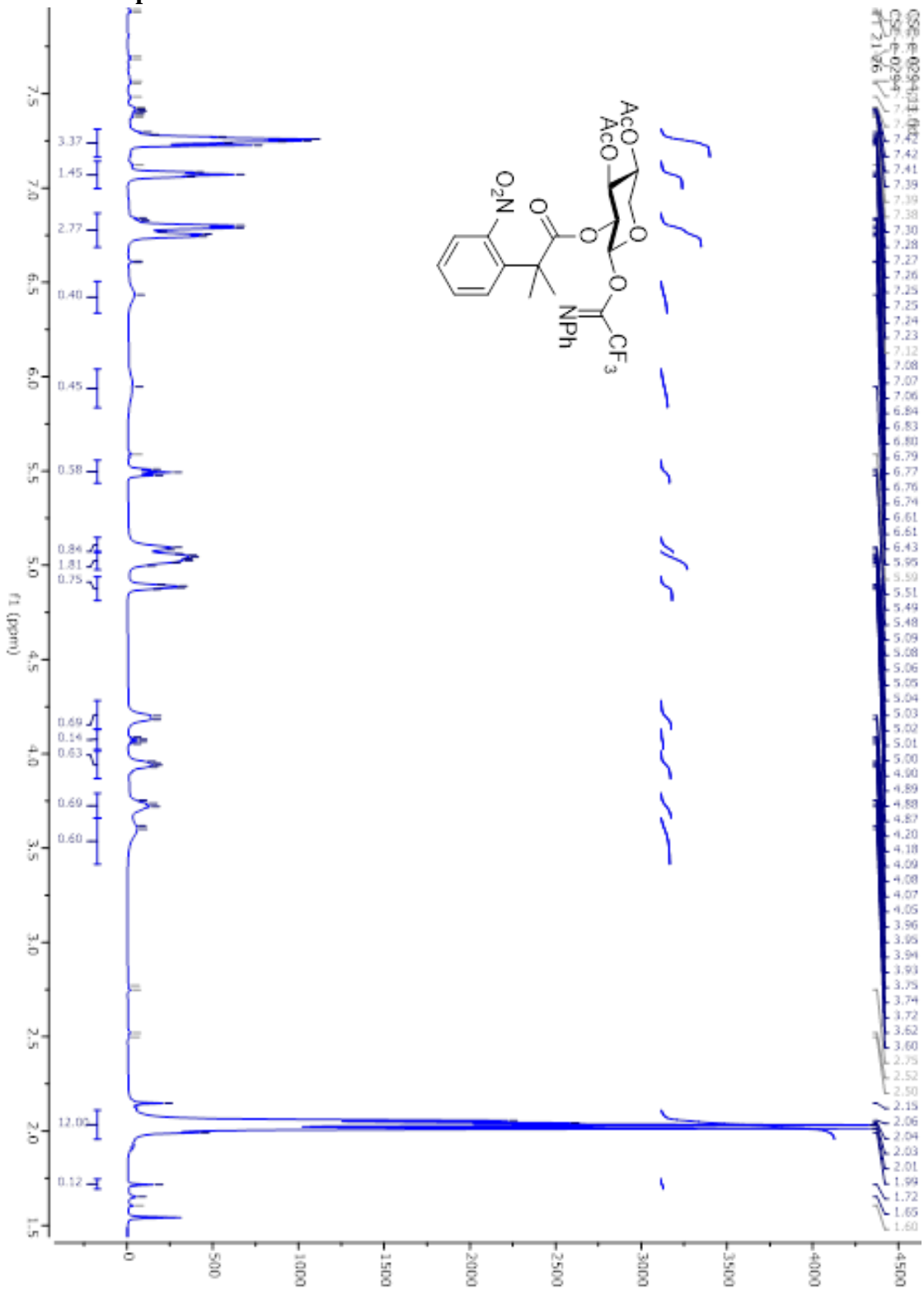
^{1}H NMR (unedited) of Compound X10



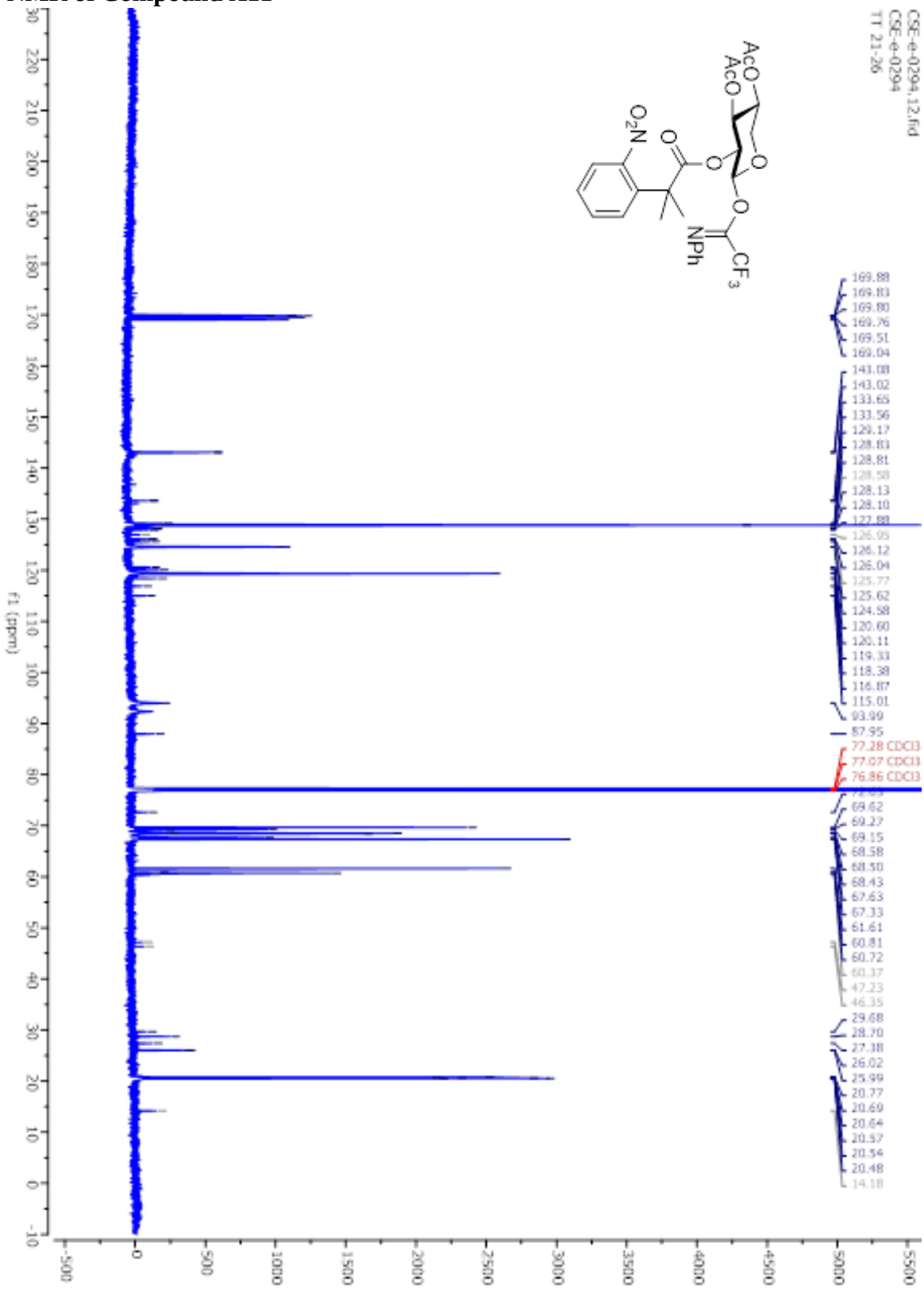
^{13}C NMR of Compound X10



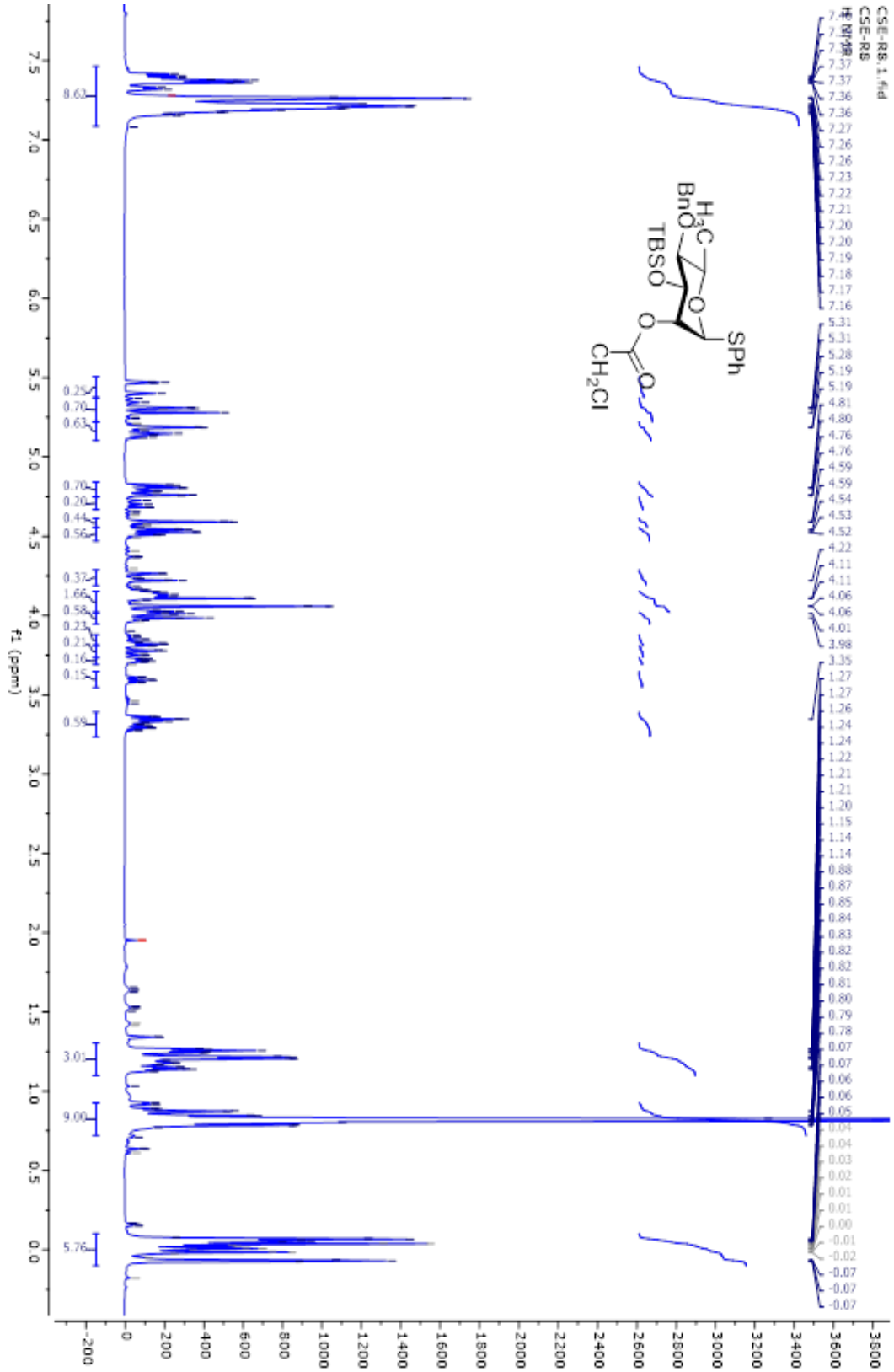
[1]H NMR of Compound X11



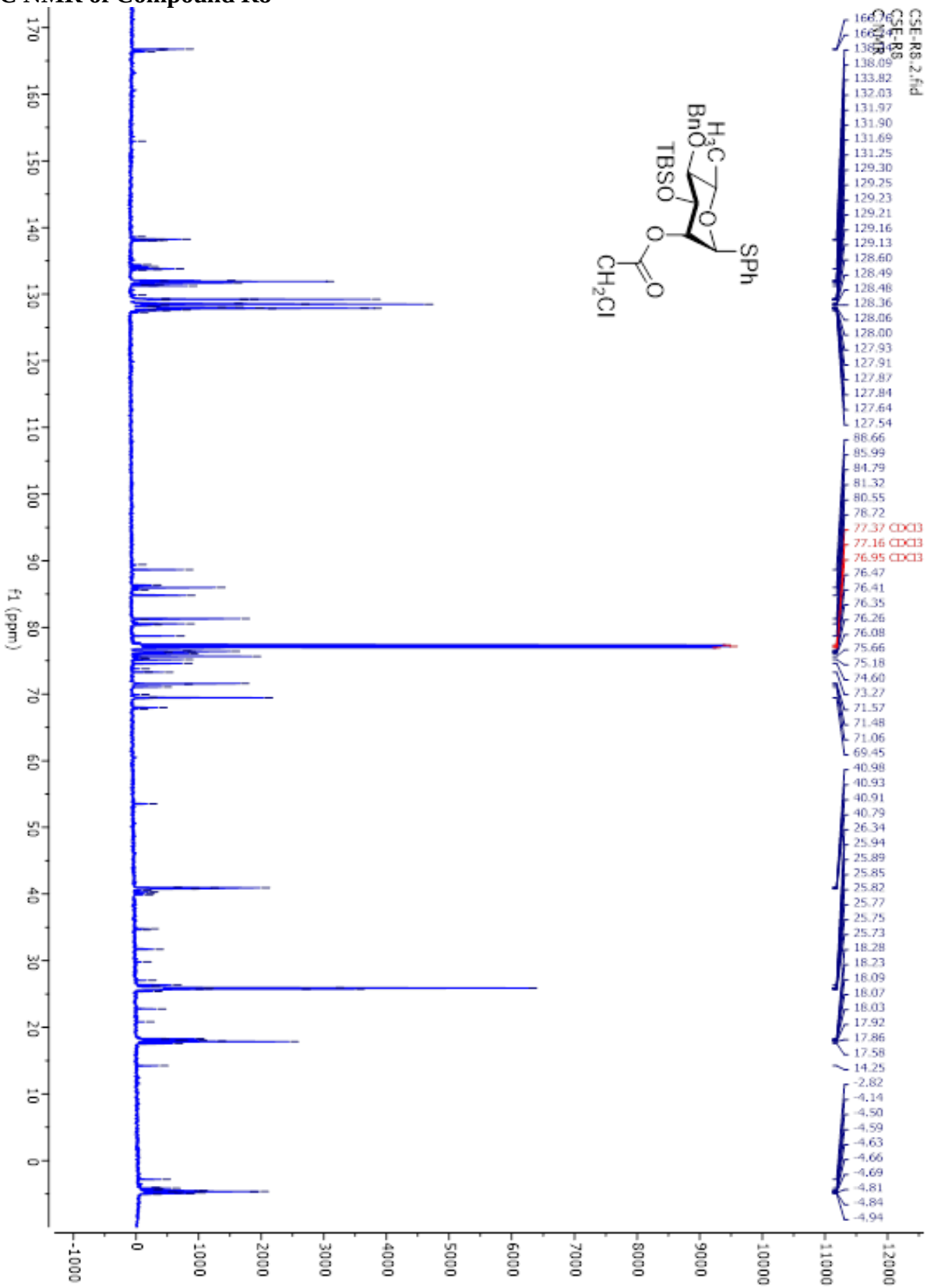
^{13}C NMR of Compound X11



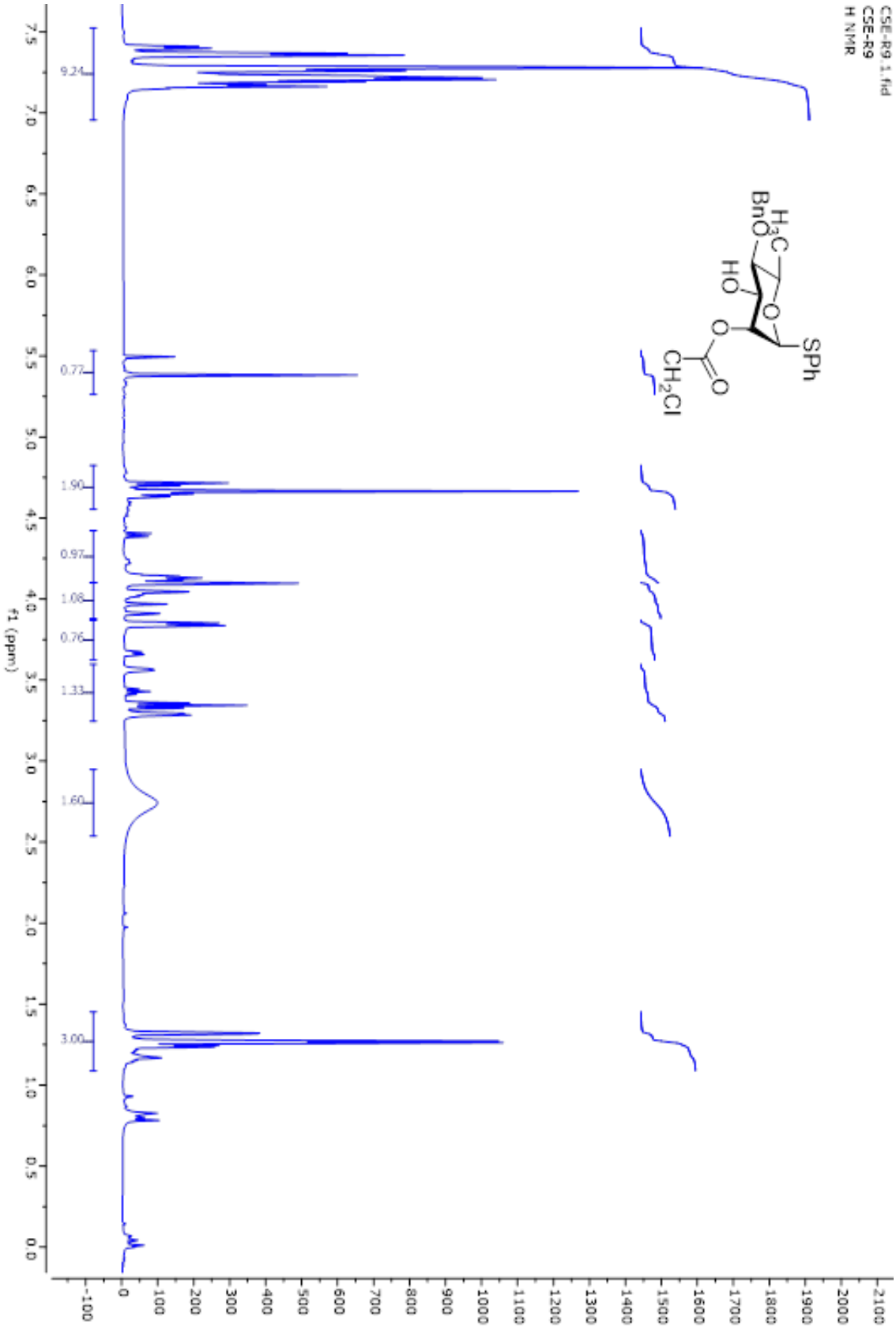
^{1}H NMR of Compound R8



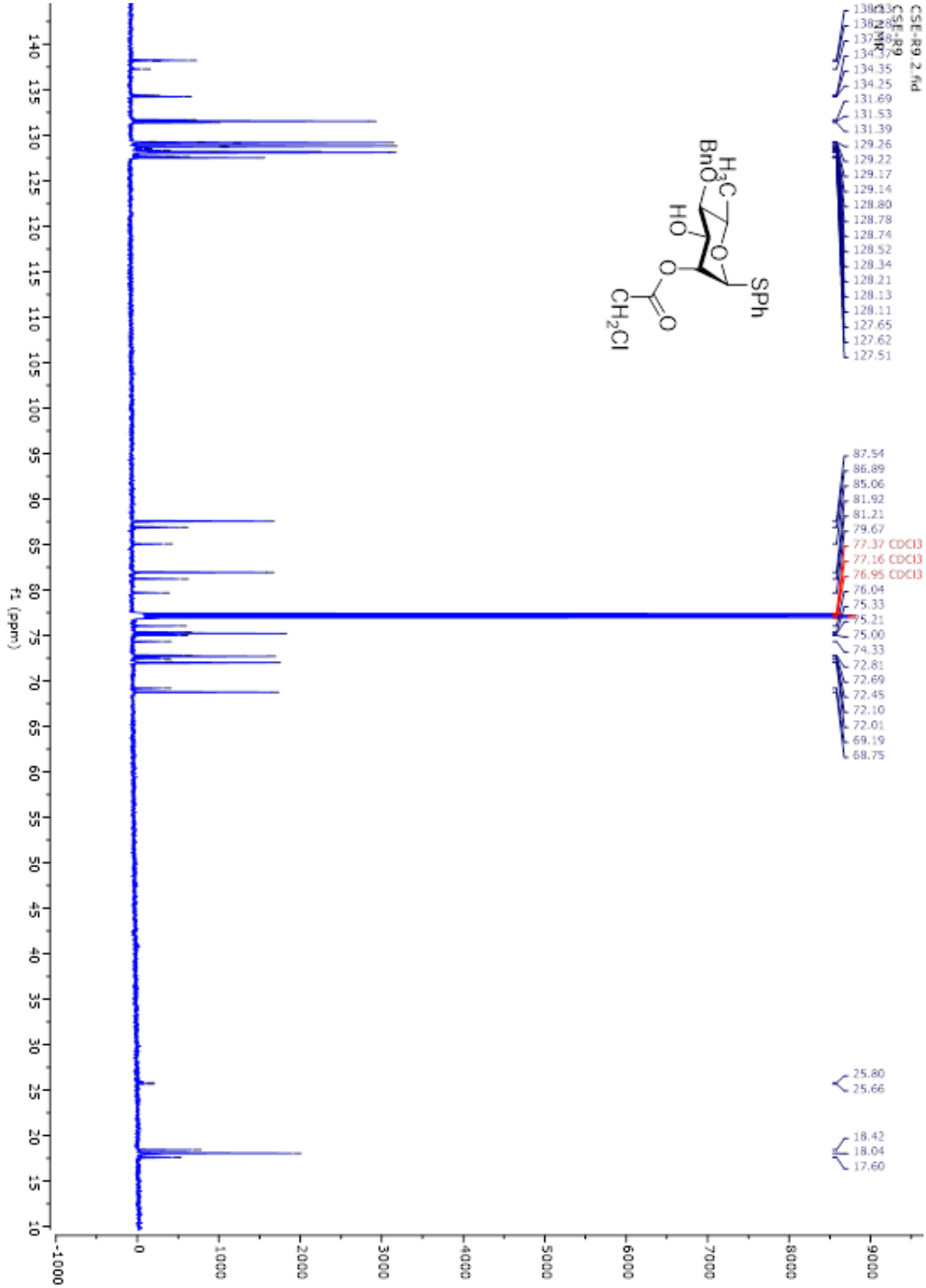
^{13}C NMR of Compound R8



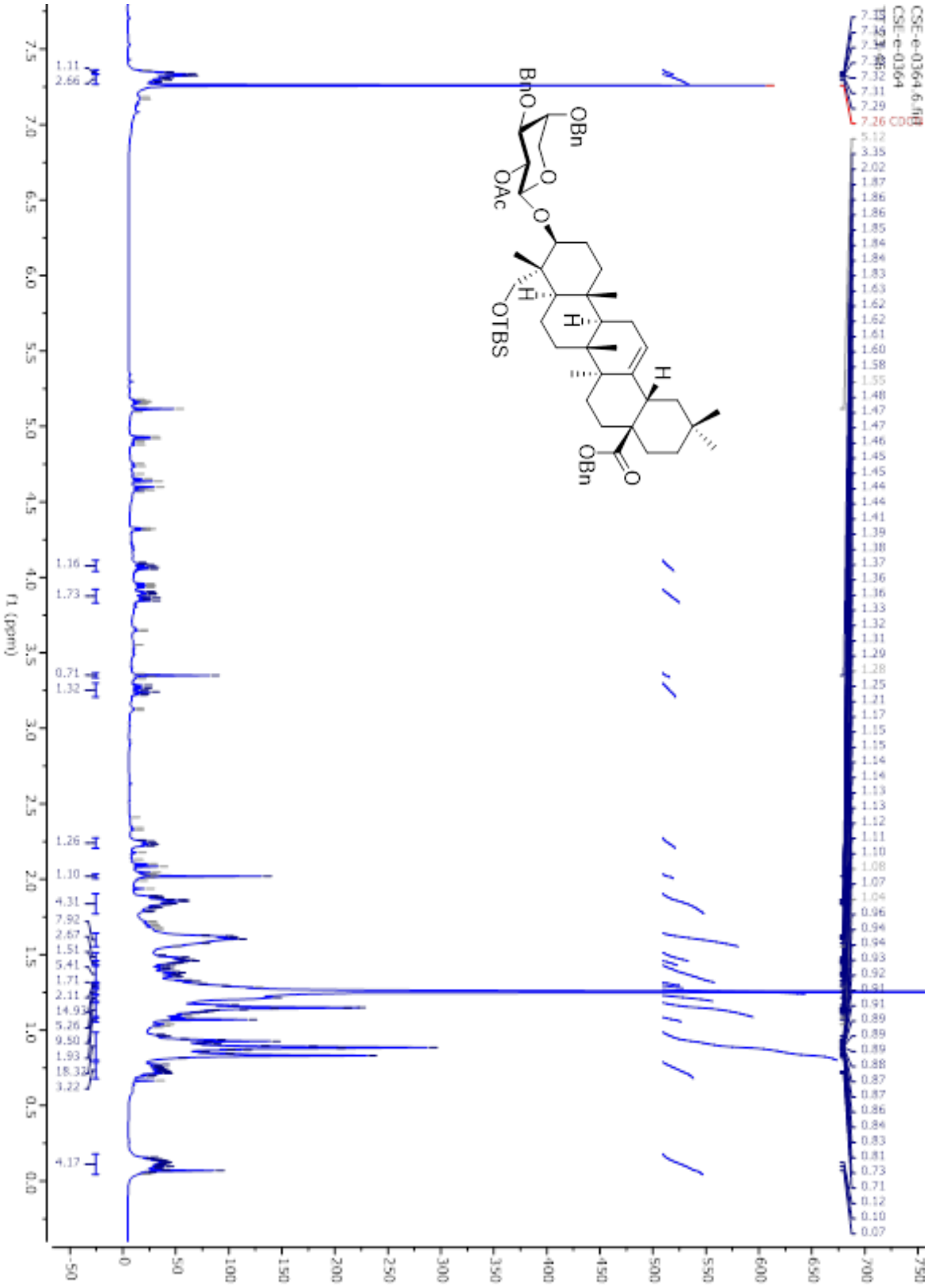
^{1}H NMR of Compound R9



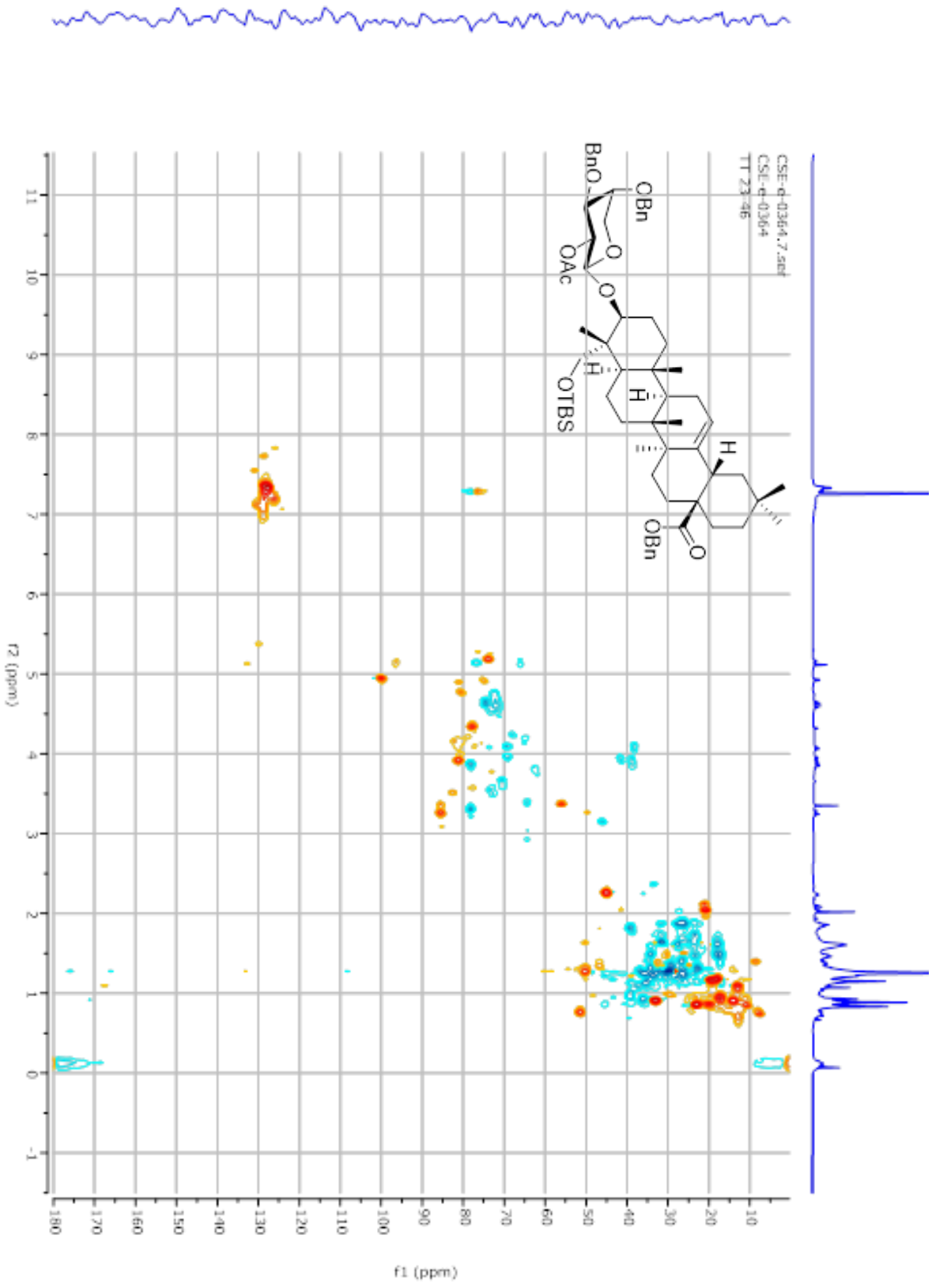
^{13}C NMR of Compound R9



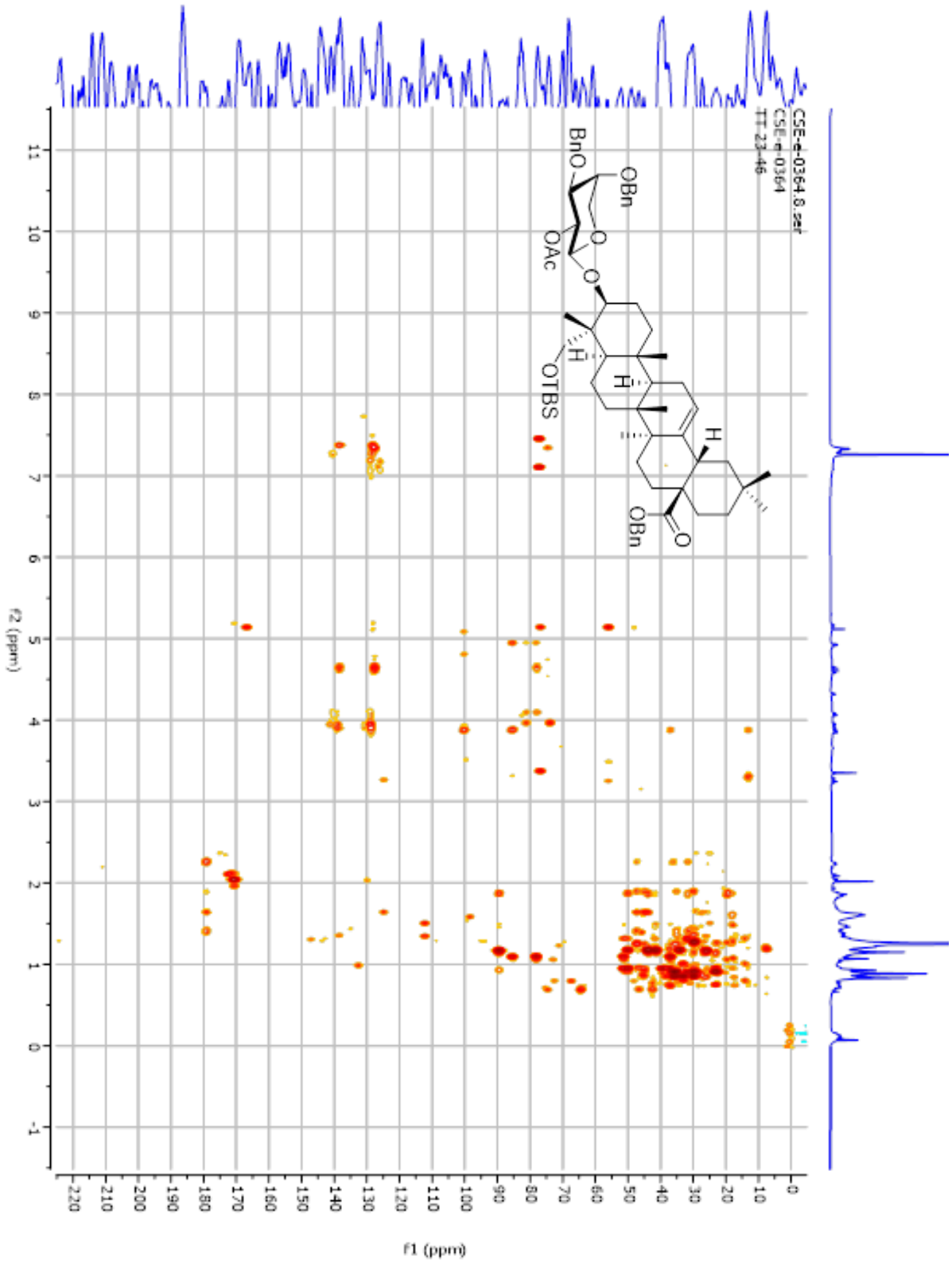
^{1}H NMR of Compound H-A-1



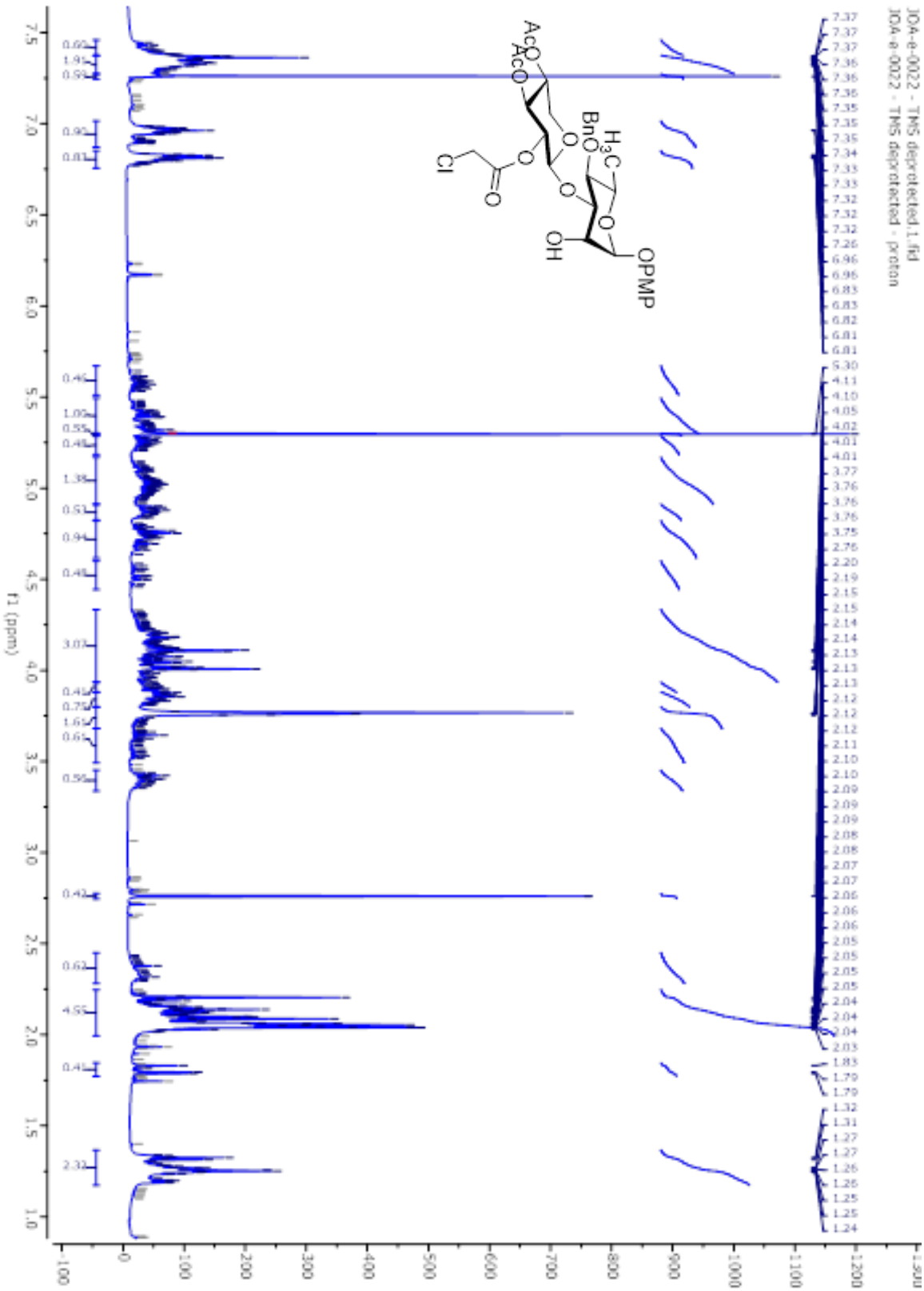
HSQC NMR of Compound H-A-1



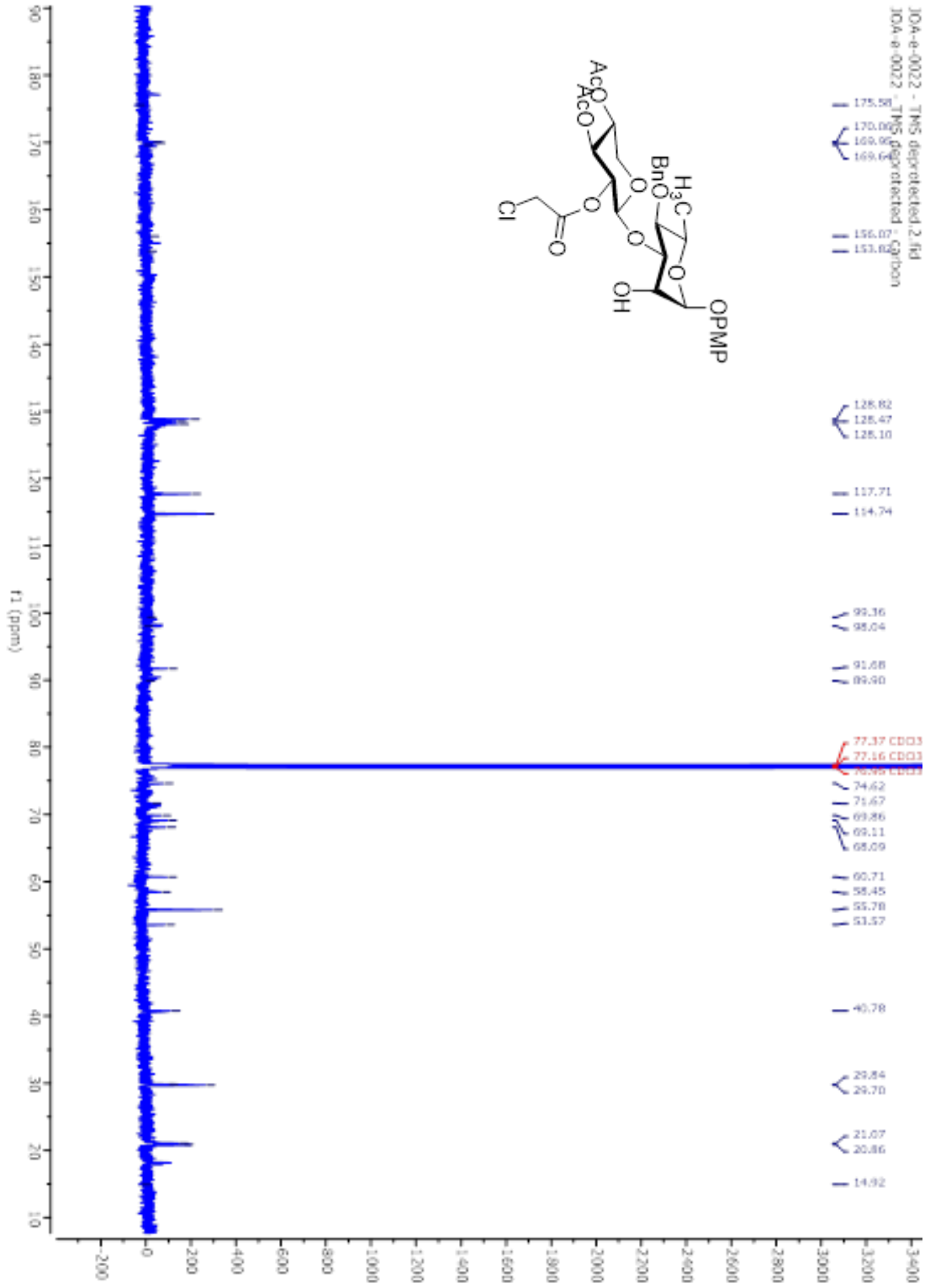
HMBC NMR of Compound H-A-1



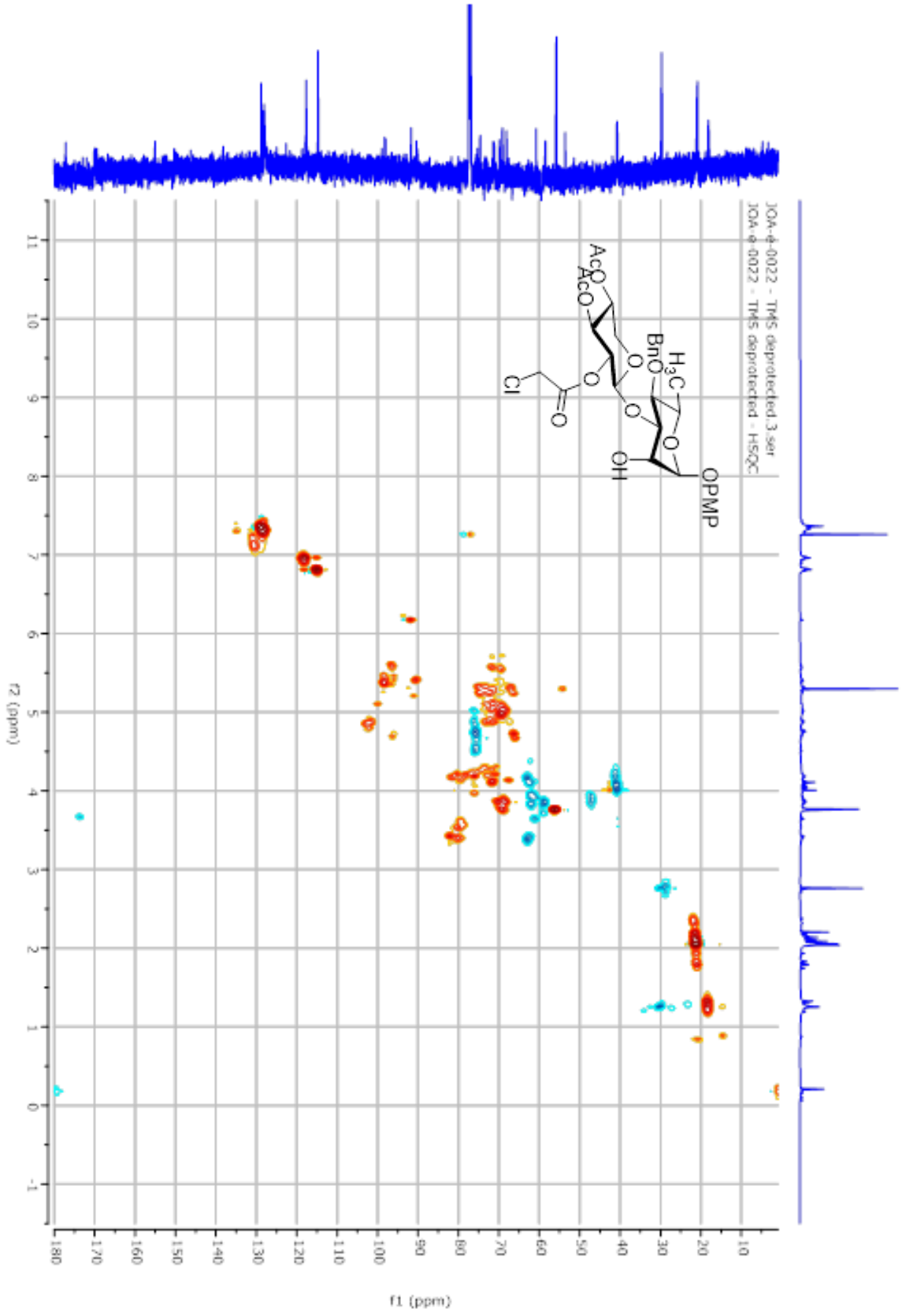
^{1}H NMR of Compound R-X-1



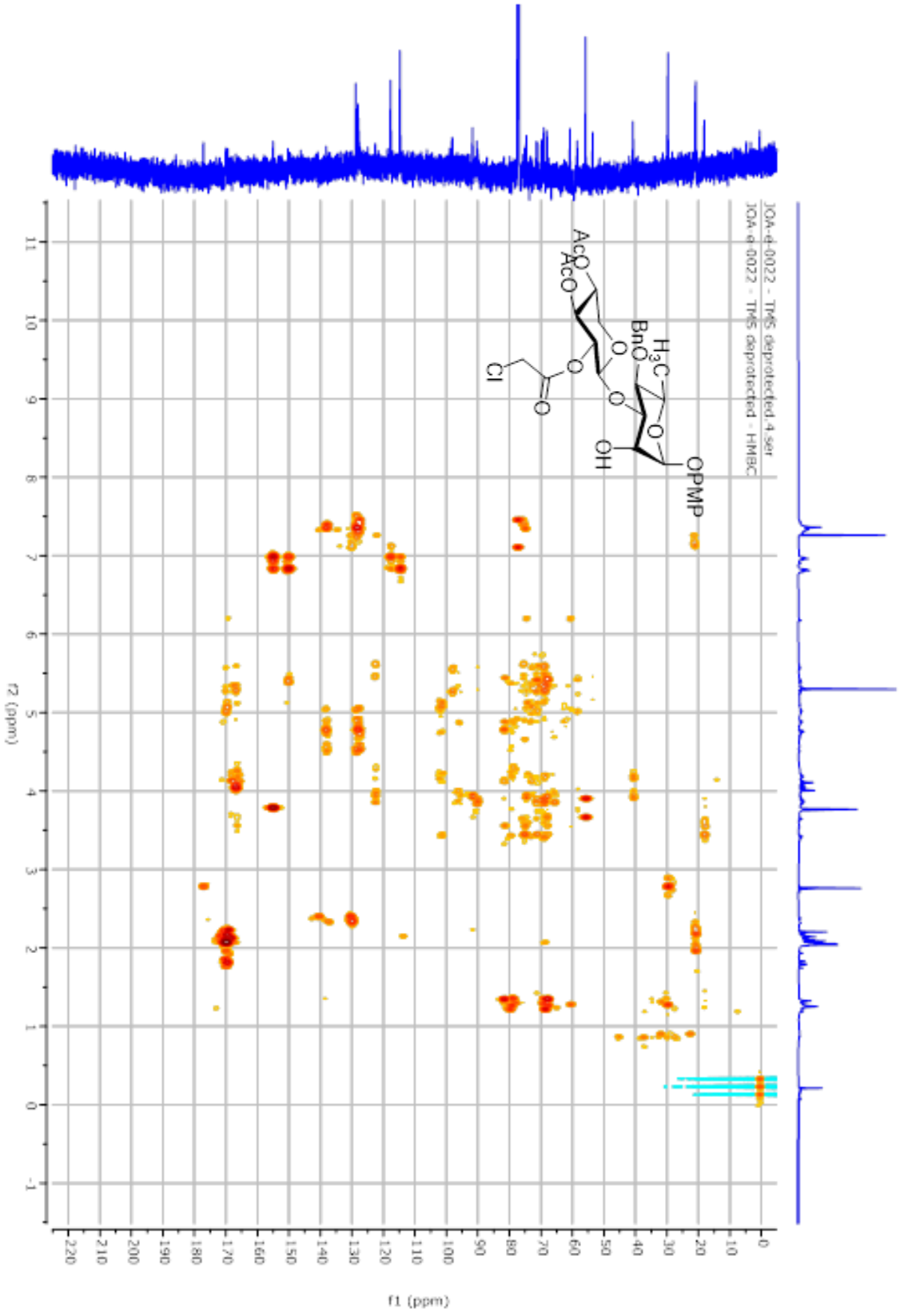
^{13}C NMR of R-X-1



HSQC NMR of R-X-1



HMBC NMR of Compound R-X-1



References

[1] Inge Lerouge, J. V. (2002) O-antigen structural variation: mechanisms and possible roles in animal/plant–microbe interactions, *FEMS Microbiology Reviews 26*, 17-47.

[2] Ewa Katzenellenbogen, N. A. K., George V. Zatonsky, Alexander S. Shashkov, Maria Bogulska, Yuriy A. Knirel. (2005) Structures of the biological repeating units in the O-chain polysaccharides of Hafnia alvei strains having a typical lipopolysaccharide outer core region, *FEMS Immunology & Medical Microbiology 45*, 269-278.

[3] Rossella Grande, V. P., & Raffaella Muraro. (2020) Antibiotic resistance and bacterial biofilm, *Expert Opinion on Therapeutic Patents 30*, 897-900.

[4] Mondino, S., Schmidt, S., Buchrieser, C., and Garsin, D. A. (2020) Molecular Mimicry: a Paradigm of Host-Microbe Coevolution Illustrated by Legionella, *mBio 11*, e01201-01220.

[5] Singh, J. K., Adams, F. G., and Brown, M. H. (2019) Diversity and Function of Capsular Polysaccharide in Acinetobacter baumannii, *Frontiers in Microbiology 9*.

[6] Carlin, A. F., Uchiyama, S., Chang, Y.-C., Lewis, A. L., Nizet, V., and Varki, A. (2009) Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response, *Blood 113*, 3333-3336.

[7] Boyce, J. H. M. H. D. (2019) Mechanisms of Polymyxin Resistance, In *Polymyxin Antibiotics: From Laboratory Bench to Bedside: Advances in Experimental Medicine and Biolog* (Li J., N. R., Kaye K., Ed.), pp 55-71, Springer, Cham.

[8] Milad Abdi, S. M., Nour Amirmozafari. (2019) Bacterial resistance to antimicrobial peptides, *Journal of Peptide Science 25*.

[9] Peter H Seeberger, C. L. P., and Subramanian Govindan. (2017) Total synthesis of a Streptococcus pneumoniae serotype 12F CPS repeating unit hexasaccharide, *Beilstein Journal of Organic Chemistry 13*, 164-173.

[10] Guochao Liao , J. G., Deying Yang, Zhifang Zhou, Zhongqiu Liu and Zhongwu Guo (2019) Synthesis of a dimer of the repeating unit of type Ia group B Streptococcus extracellular capsular polysaccharide and immunological evaluations of related protein conjugates, *Org. Chem. Front. 6*, 2833-2838.

[11] D. Jamin Keith, S. D. T. (2019) Total Synthesis of the Congested, Bisphosphorylated Morganella morganii Zwitterionic Trisaccharide Repeating Unit, *JACS 141*, 12939-12945.

[12] Zamboulis, A., Michailidou, G., Koumentakou, I., and Bikiaris, D. N. (2022) Polysaccharide 3D Printing for Drug Delivery Applications, *Pharmaceutics 14*, 145.

[13] Nielsen, M. M., and Pedersen, C. M. (2018) Catalytic Glycosylations in Oligosaccharide Synthesis, *Chemical Reviews 118*, 8285-8358.

[14] Krasnova, L., and Wong, C.-H. (2019) Oligosaccharide Synthesis and Translational Innovation, *Journal of the American Chemical Society 141*, 3735-3754.

[15] Van Der Vorm, S., Hansen, T., Van Hengst, J. M. A., Overkleeft, H. S., Van Der Marel, G. A., and Codée, J. D. C. (2019) Acceptor reactivity in glycosylation reactions, *Chemical Society Reviews 48*, 4688-4706.

[16] Li, B.-H., and Ye, X.-S. (2020) Recent advances in glycan synthesis, *Current Opinion in Chemical Biology 58*, 20-27.

[17] Zeng, J., Liu, Y., Chen, W., Zhao, X., Meng, L., and Wan, Q. (2019) Glycosyl Sulfoxides in Glycosylation Reactions, In *Sulfur Chemistry* (Jiang, X., Ed.), pp 367-398, Springer International Publishing, Cham.

[18] Guan, Y., and Townsend, S. D. (2017) Metal-Free Synthesis of Unsymmetrical Organoselenides and Selenoglycosides, *Organic Letters 19*, 5252-5255.

[19] Das, A., Ray, D., and Banik, B. K. (2022) Tellurium in carbohydrate synthesis, *Physical Sciences Reviews*.

[20] Marco Tingoli, A. T., Lorenzo Testaferri, Marcello TIecco, and Giuseppe Resnati. (1998) Glycosylation Reaction Using Anomeric Selenoixdes, *Carbohydrate Letters 3*, 39-46.

[21] Zhang, S., and Seeberger, P. H. (2021) Total Syntheses of Conjugation-Ready Repeating Units of Acinetobacter baumannii AB5075 for Glycoconjugate Vaccine Development, *Chemistry – A European Journal*.

[22] Nguyen, J. M., Evans, C. S., Wright, N. M., and Townsend, S. D. (2022) Synthesis of the Aeromonas veronii strain Bs8 disaccharide repeating unit, *Carbohydrate Research 514*, 108530.

[23] Marta Zdanowicz, Z. J. M. P. P. (2020) Abundance and antibiotic resistance of Aeromonas isolated from the water of three carp ponds, *Veterinary Research Communications 44*, 9-18.

[24] Dworaczek, K., , M. K., , M. L., , A. P.-S., , Marczak, M., and , A. T.-S. (2021) Structure of the disaccharide repeating unit of O-specific polysaccharide

isolated from Aeromonas veronii strain Bs8 pathogenic to common carp

(Cyprinus carpio) *Carbohydrate Research 500*.

[25] Joy E. M. Watts, H. J. S., Lauma Lanska, and Michelle S. Hale. (2017) The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions, *Mar Drugs. 15*.

[26] WALSH, M. A. F. A. C. T. (2009) Antibiotics for Emerging Pathogens, *Science 325*, 1089-1093.

[27] Siang Yong Tan, Y. T. (2015) Alexander Fleming (1881–1955): Discoverer of penicillin, *Singapore Med J 56*, 366-367.

[28] Organization, W. H. (2015) Global action plan on antimicrobial resistance, p 28, World Health Organization, https://ahpsr.who.int/publications/i/item/global-action-plan-on-antimicrobial-resistance.

[29] Daniel Schar, E. Y. K., Ramanan Laxminarayan, Marius Gilbert & Thomas P. Van Boeckel (2020) Global trends in antimicrobial use in aquaculture, *Scientific Reports 10*.

[30] AzmaiAmalag, M.-S. Y.-S.-S. T. Y. R. L. S. N. (2021) The prevalence, putative virulence genes and antibiotic resistance profiles of Aeromonas spp. isolated from cultured freshwater fishes in peninsular Malaysia, *Aquaculture 540*.

[31] Handfield, M., Simard, P., Couillard, M., and Letarte, R. (1996) Aeromonas hydrophila isolated from food and drinking water: hemagglutination, hemolysis, and cytotoxicity for a human intestinal cell line (HT-29), *Applied and Environmental Microbiology 62*, 3459-3461.

[32] Hatrongjit, R., Kerdsin, A., Takeuchi, D., Wongsurawat, T., Jenjaroenpun, P., Chopjitt, P., Boueroy, P., Akeda, Y., and Hamada, S. (2020) Genomic Analysis of Aeromonas veronii C198, a Novel Mcr-3.41-Harboring Isolate from a Patient with Septicemia in Thailand, *Pathogens 9*, 1031.

[33] Khalifa, A. Y., and Bekhet, G. (2018) First isolation and characterization of the pathogenic Aeromonas veronii bv. veronii associated with ulcerative syndrome in the indigenous Pelophylax ridibundus of Al-Ahsaa, Saudi Arabia, *Microbial pathogenesis 117*, 361-368.

[34] Fauzi, N. N. F. N. M., Hamdan, R. H., Mohamed, M., Ismail, A., Zin, A. A. M., and Mohamad, N. F. A. (2021) Prevalence, antibiotic susceptibility, and presence of drug resistance genes in Aeromonas spp. isolated from freshwater fish in Kelantan and Terengganu states, Malaysia, *Veterinary World 14*, 2064.

[35] Lim, J., and Hong, S. (2020) Characterization of Aeromonas salmonicida and A. sobria isolated from cultured salmonid fish in Korea and development of a vaccine against furunculosis, *Journal of Fish Diseases 43*, 609-620.

[36] Sawai, S., and Saito, K. (2011) Triterpenoid Biosynthesis and Engineering in Plants, *Frontiers in Plant Science 2*.

[37] Chipenzi, T., Baloyi, G., Mudondo, T., Sithole, S., Fru Chi, G., and Mukanganyama, S. (2020) An Evaluation of the Antibacterial Properties of Tormentic Acid Congener and Extracts From Callistemon viminalis on Selected ESKAPE Pathogens and Effects on Biofilm Formation, *Advances in Pharmacological and Pharmaceutical Sciences 2020*, 8848606.

[38] Zhou, G., Zhang, Y., Li, Y., Wang, M., and Li, X. (2018) The metabolism of a natural product mogroside V, in healthy and type 2 diabetic rats, *Journal of Chromatography B 1079*, 25-33.

[39] Zhu, D., and Tuo, W. (2016) QS-21: A Potent Vaccine Adjuvant, *Nat Prod Chem Res 3*, e113.

[40] Tagousop, C. N., Tamokou, J.-D.-D., Kengne, I. C., Ngnokam, D., and Voutquenne-Nazabadioko, L. (2018) Antimicrobial activities of saponins from Melanthera elliptica and their synergistic effects with antibiotics against pathogenic phenotypes, *Chemistry Central Journal 12*.

[41] Damke, E., Tsuzuki, J. K., Chassot, F., Cortez, D. A. G., Ferreira, I. C. P., Mesquita, C. S. S., da-Silva, V. R. S., Svidzinski, T. I. E., and Consolaro, M. E. L. (2013) Spermicidal and anti-Trichomonas vaginalis activity of Brazilian Sapindus saponaria, *BMC Complement Altern Med 13*, 196-196.

[42] Damke, E., Tsuzuki, J. K., Cortez, D. A., Ferreira, I. C., Bertoni, T. A., Batista, M. R., Donati, L., Svidzinski, T. I., and Consolaro, M. E. (2011) In vivo activity of Sapindus saponaria against azole-susceptible and-resistant human vaginal Candida species, *BMC Complement Altern Med 11*, 1-9.

[43] Tsuzuki, J. K., Svidzinski, T. I., Shinobu, C. S., Silva, L. F., Rodrigues-Filho, E., Cortez, D. A., and Ferreira, I. C. (2007) Antifungal activity of the extracts and saponins from Sapindus saponaria L, *Anais da Academia Brasileira de Ciências 79*, 577-583.

[44] Mair, C. E., Grienke, U., Wilhelm, A., Urban, E., Zehl, M., Schmidtke, M., and Rollinger, J. M. (2018) Anti-Influenza Triterpene Saponins from the Bark of Burkea africana, *Journal of Natural Products 81*, 515-523.

[45] Tatli Cankaya, I. I., and Somuncuoglu, E. I. (2021) Potential and Prophylactic Use of Plants Containing Saponin-Type Compounds as Antibiofilm Agents against Respiratory Tract Infections, *Evid Based Complement Alternat Med 2021*, 6814215-6814215.

[46] Elekofehinti, O. O., Iwaloye, O., Olawale, F., and Ariyo, E. O. (2021) Saponins in Cancer Treatment: Current Progress and Future Prospects, *Pathophysiology 28*.

[47] Choudhary, N., Khatik, L. G., and Suttee, A. (2021) The Possible Role of Saponin in Type-II Diabetes- A Review, *Current Diabetes Reviews 17*, 107-121.

[48] Soica, C., Antal, D., Andrica, F., Racoviceanu Babuta, R., Tăculescu, E., Ardelean, F., Ghiulai, R., Avram Feflea, S., Danciu, C., Coricovac, D., and Dehelean, C. (2017) Lupan-Skeleton Pentacyclic Triterpenes with Activity against Skin Cancer: Preclinical Trials Evolution.

[49] Han, J. Y., Chun, J. H., Oh, S. A., Park, S. B., Hwang, H. S., Lee, H., and Choi, Y. E. (2018) Transcriptomic Analysis of Kalopanax septemlobus and Characterization of KsBAS, CYP716A94 and CYP72A397 Genes Involved in Hederagenin Saponin Biosynthesis, *Plant Cell Physiol 59*, 319-330.

[50] Sun, H., Fang, W., Wang, W. Z., and Hu, C. (2006) Structure-activity relationships of oleanane- and ursane-type triterpenoids, *Botanical Studies 47*, 339-368.

[51] Liu, Q., Khakimov, B., Cárdenas, P. D., Cozzi, F., Olsen, C. E., Jensen, K. R., Hauser, T. P., and Bak, S. (2019) The cytochrome P450 CYP72A552 is key to production of hederagenin-based saponins that mediate plant defense against herbivores, *New Phytol 222*, 1599-1609.

[52] Aboutalebi, R., and Monfared, A. H. (2016) Saponin Terpenoids; A Brief Review of Mechanisms of Actions and Anti-cancerous Effects, *American Chemical Science Journal 12*, 1-8.

[53] Marciani, D. J. (2018) Elucidating the Mechanisms of Action of Saponin-Derived Adjuvants, *Trends in Pharmacological Sciences 39*, 573-585.

[54] Francis, G., Kerem, Z., Makkar, H. P. S., and Becker, K. (2002) The biological action of saponins in animal systems: a review, *British Journal of Nutrition 88*, 587-605.

[55] Greatrex, B. W., Daines, A. M., Hook, S., Lenz, D. H., McBurney, W., Rades, T., and Rendle, P. M. (2015) Synthesis, Formulation, and Adjuvanticity of Monodesmosidic Saponins with Olenanolic Acid, Hederagenin and Gypsogenin Aglycones, and some C-28 Ester Derivatives, *ChemistryOpen 4*, 740-755.

[56] Yang, Y., Laval, S., and Yu, B. (2014) Chapter 2 - Chemical Synthesis of Saponins, In *Advances in Carbohydrate Chemistry and Biochemistry* (Horton, D., Ed.), pp 137-226, Academic Press.

[57] Van Tamelen, E. E., Seiler, M., and Wierenga, W. (1972) Biogenetic-type total synthesis.. delta.-Amyrin,. beta.-amyrin, and germanicol, *Journal of the American Chemical Society 94*, 8229-8231.

[58] Corey, E. J., and Lee, J. (1993) Enantioselective total synthesis of oleanolic acid, erythrodiol, .beta.-amyrin, and other pentacyclic triterpenes from a common intermediate, *Journal of the American Chemical Society 115*, 8873-8874.

[59] Serbian, I., and Csuk, R. (2018) An Improved Scalable Synthesis of α- and β-Amyrin, *Molecules 23*, 1552.

[60] Singh, S., and Ali, M. (2019) Sapindus mukorossi: a review article, *J. Pharm. Innov 8*, 88-96.

[61] Singh, O., Khanam, Z., and Ahmad, J. (2011) Neem (Azadirachta indica) in Context of Intellectual Property Rights (IPR), *Recent Research in Science and Technology 3*.

[62] Schreiber, C. A., Meyn, L. A., Creinin, M. D., Barnhart, K. T., and Hillier, S. L. (2006) Effects of long-term use of nonoxynol-9 on vaginal flora, *Obstet Gynecol 107*, 136-143.

[63] Parenthood, P. (2022) Spermicide and Gel, In *Birth Control*, Planned Parenthood, https://www.plannedparenthood.org/learn/birth-control/spermicide.

[64] Plé, K., Chwalek, M., and Voutquenne-Nazabadioko, L. (2005) Synthesis of l-arabinopyranose containing hederagenin saponins, *Tetrahedron 61*, 4347-4362.

[65] Gauthier, C., Legault, J., Lavoie, S., Rondeau-Gagné, S., Tremblay, S., and Pichette, A. (2008) Synthesis of Two Natural Betulinic Acid Saponins Containing a-L-Rhamnopyranosyl-(1->2)-a-L-Arabinopyranose and their Analogues, *Tetrahedron 64*, 7386-7399.

[66] Masaki, M., Kitahara, T., Kurita, H., and Ohta, M. (1968) A new method for the removal of chloroacetyl groups, *Journal of the American Chemical Society 90*, 4508-4509.

[67] Liu, H., Zhou, S.-Y., Wen, G.-E., Liu, X.-X., Liu, D.-Y., Zhang, Q.-J., Schmidt, R. R., and Sun, J.-S. (2019) The 2,2-Dimethyl-2-(ortho-nitrophenyl)acetyl (DMNPA) Group: A Novel Protecting Group in Carbohydrate Chemistry, *Organic Letters 21*, 8049-8052.

[68] Randolph, J. T., and Danishefsky, S. J. (1993) Application of the glycal assembly strategy to the synthesis of a branched oligosaccharide: the first synthesis of a complex saponin, *Journal of the American Chemical Society 115*, 8473-8474.

[69] Larsen, K., Olsen, C. E., and Motawia, M. S. (2008) Acid-catalysed rearrangement of glycosyl trichloroacetimidates: a novel route to glycosylamines, *Carbohydrate Research 343*, 383-387.

[70] Tsabedze, S. B., Kabotso, D. E. K., and Pohl, N. L. B. (2013) The development of N-aryl trifluoroacetimidate-based benzyl and allyl protecting group reagents, *Tetrahedron Letters 54*, 6983-6985.