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Progress towards the Synthesis of Carbohydrate-Based Biomedical and Material-Science Relevant Molecules

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By

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Abstract

Cancer cells express unique carbohydrate signatures on their surfaces known as tumour-associated carbohydrate antigens (TACAs). These antigens are not typically found on healthy cells and therefore are promising targets for immunotherapy using adaptive immune response tools. If the immune system could theoretically be "trained" to target this molecule, then the immune system could be used to help eradicate any cancer cells from the host. A problem with carbohydrate-based targets is they are not stable in the body because of their inherent acetal functionality: the glycosidic bond. This research aims to remove the unstable acetal functionality in carbohydrates by replacing the exocyclic anomeric oxygen with a methylene group (C-glycoside) to make new *N*-Fmoc protected acetal-free C-glycoside analogues of the Tn antigen, a TACA found on many tumor cells. Removing the labile functionality should result in a greatly enhanced lifetime and bioavailability relative to the native system with no likely loss in recognition specificity as the exocyclic oxygen is not generally involved in molecular recognition events.

The Thomsen-Friedenreich antigen is another tumour-associated carbohydrate, which is found on specific types of cancer cells, such as CD34+ T-cells in leukemia and sarcoma, as well as CD 44+-cells in colon and breast cancers. The application of this antigen confronts the same limitation as to the Tn antigen. In order to be more effective, it has to be modified to increase its stability and have a higher chance of getting to the target and inducing the required immune response. The synthesis of a fully acetal-free TF is challenging because of the unique 1,3-β one carbon linkage between the galactose and *N*-acetyl galactosamine moieties. Through the removal of both the exocyclic oxygens in the anomeric position and the linkage between the two sugar moieties, and replacing them with

methylene groups, the stability of this antigen under biological conditions in the body greatly increases, and as a result, can better activate the immune system. This type of double C-glycoside-amino acid conjugate has never been reported in the literature for any carbohydrate. This thesis will discuss significant progress towards the synthesis of this promising, but challenging, fully acetal-free TF antigen.

The next portion of the thesis expresses the synthesis of two glycolipids isolated from *S. pneumoniae*, the leading cause of neonatal sepsis and meningitis. Two main fractions of antigenic *S. pneumoniae* glycolipids were identified. One contains a monosaccharide, and the second fraction contains a disaccharide. Both the monosaccharide and the disaccharide appear capable of activating invariant natural killer T cells (iNKT), a subclass of white blood cells that produce non-specific cytokines whose activation can lead to a systemic uncontrolled immune response. The total synthesis of both the mono- and the disaccharide will be discussed in this thesis and the biological activity of these synthesized saccharides will be measured in order to confirm their structures, which have never been examined.

The next part of the thesis reports the efforts toward the study of the limitations of the selective removal of acetates in the presence of long-chain esters. This area of research remains under development as selective deprotection of the acetyl group, especially in the pyranose sugar moiety is largely unexplored.

The final section of the thesis outlines the synthesis of an acetylated lactose bearing free hydroxyl groups at the C-6 and C-6′ for attachment to conjugated polymers, with applications in electronic devices.

Dedication

Dedicated to my family, my father, mother, brother, and sister, but most of all, Nazanin as my wife, partner, and best friend.

I would like to dedicate this thesis to my son, Arteen, who makes my life so beautiful and gives me passion and motivation.

In the memory of my father: Mohammad and my sister: Parisa

Acknowledgement

In this part, I would like to write about individuals who helped and supported me during this journey. First of all, my special thanks go to my supervisor, Prof. John Trant for his unlimited help, support and encouragement, his assistance and suggestions during my Ph.D. and being a great supervisor and friend makes him unique that I don't think many students could have this chance to take advantage of, many thanks to John because of that. I would like to thank him for his willingness to set me free to explore and pursue my ideas and learn from my mistakes. Moreover, I appreciate all his effort to build a fun and friendly environment in the group.

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Here, I would like to thank my undergraduate students and visiting students from abroad and also visiting high school students who I spend most of my time in the lab with them, and all of whom make our lab fun and enjoyable place, Emmanuel Igbokwe, Adan Sadiq Ahmed, Asma Ghafoor, Greg Usef, Advait Desai, Joy Kobti, Peter Bahnam, Thomson Ly, Khushali Parikh, Abdul Aljoudi, Emma Flynn, Maya Sutherland, Vrunjal Gandhi, Emmanuel Perez Escalante, Chitra Bidlon. I would like to thank especially those who spent

the first two years of my Ph.D. period with me when we had to do chemistry in the tiny lab at the same time, we had an enjoyable time, and I will never forget that.

I would like to thank the entire Trant team for their support and encourage including postdoctoral fellowships and graduate and undergraduate students, particularly graduate students who are trained by me. Here I would also thank Michael Reynolds (graduate student in Trant team) I can definitely call him my close friend, who assisted and helped me in editing my document and being my extra set of eyes for the preparation of this thesis and thanks for all the laughs and all the good moments that we had. I would also thank Dr. Fraser Pick, who read my thesis and gave me instructive comments and the corrections. Moreover, I should thank Dr. Bukola Rhoda Aremu, who helped me with Endnote problem solving.

In the most important part, I would like to thank my wife for being for me unconditionally, being supportive, helpful, and the best friend, not only in my academic career but also in my life, thanks for that and I love you.

Here I would like to thank my son, Arteen, who is almost three years old. In the first thought it doesn't seem to he did anything for me as anyone else involved in this thesis, but it is not true, he gave me more passion and motivation to go deeper in my path and pursue my dream for my family and myself, and he made my days beautiful and bright with his laugh and energy, I love you.

Last but not least, many thanks go to my family, who have supported and encouraged me to pursue my dreams. Thanks to my mother, who always has supported me, she has always been full of positive energy, which transfers it to me, my little sister, who gives

me her kindness and full support and my brother for his support and friendship. I owe a debt of great thanks to my father and sister, who passed away. They were very supportive and encouraging me to chase my dreams. I know they are very happy and watching me now. I would like to thank my in-laws, thanks to being supportive, kind, and treating me as your own son and brother.

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List of Abbreviations

Ac_2O	Acetic anhydride
AIBN	Azobisisobutyronitrile
allyl-TMS	Allyltrimethylsilane
APC	Antigen-presenting cell
BALB	Binaural Alternate Loudness Balance
BbGL	Burgdorferi glycolipid
9-BBN	9-Borabicyclo(3.3.1)nonane
BF_3•OEt_2	Boron trifluoride diethyl etherate
BnBr	Benzyl bromide
Boc	*tert*-butyloxycarbonyl
CAN	Ceric ammonium nitrate
CBz	Carboxybenzyl
CDR	Complementarity-determining region
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino- morpho-lino-carbenium hexafluorophosphate
DAG	Diacylglycerol
DBU	Diazabicycloundecene
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DMTr	4,4′-Dimethoxytrityl chloride
DIBAL-H	Diisobutylaluminum hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMPM	3,4-dimethoxybenzyl
DPP	Diketopyrrolopyrole
DTBS	Di-tert-butylsilyl bis(trifluoromethanesulfonate)

EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ELISA	Enzyme-linked immunosorbent assay
FMPT	2-fluoromethylphosphotyrosine
Fmoc	9-fluorenylmethoxycarbonyl
GSL	glycosphingolipid
HLA	Human leukocyte antigen
HPLC	High-performance liquid chromatography
HWE	Horner-Wadsworth-Emmons
iNKT	Invariant natural killer T
KHMDS	Potassium bis(trimethylsilyl)amide
KLH	Keyhole limpet haemocyanin
LDA	Lithium diisopropylamide
LHMDS	Lithium bis(trimethylsilyl)amide
lysoPC	Lysophosphatidylcholine
MBS	*m*-Maleimidobenzoyl *N*-hydroxysuccinimide
*m*CPBA	*meta*-chloroperoxybenzoic acid
MHC	Major histocompatibility complex
MsCl	Methanesulfonyl chloride
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance spectroscopy
Nap	Naphthyl methyl
NPM	*p*-nitrophenylmethyl
OFET	Organic field-effect transistors
PC	Phosphatidylcholine
PCC	Pyridinium chlorochromate
PivCl	Pivaloyl chloride
PLA2	Phospholipase A2

PMB	*p*-Methoxybenzyl ether
PRE	Paramagnetic relaxation enhancement
PUFA	Polyunsaturated fatty acid
RP-HPLC	Reversed phase- High-performance liquid chromatography
TACAs	Tumour-associated carbohydrate antigens
TBAB	Tetra-*n*-butylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBS-	*tert*-butyldiphenylsilyl-
TFE	2,2,2-trifluoroethanol
THF	Tetrahydrofuran
TIPS-	Triisopropylsilyl
TMSI	Trimethylsilyl iodide
TBS	*tert*-Butyldimethylsilyl
TBSOTF	*tert*-Butyldimethylsilyl trifluoromethanesulfonate
t-Bu	tert-butyl
TCR	T-cell receptor
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TF	Thomsen−Friendreich
TFA	Trifluoroacetic anhydride
TF_2O	Trifluoromethanesulfonic anhydride
TfOH	Triflic acid
TH	T helper type
TLC	Thin Layer Chromatography
TMSCN	Trimethylsilyl cyanide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
p-TsOH	*para*-Toluenesulfonic acid
TTBP	2,4,6-Tri-tert-butylpyrimidine

Chapter 1: Introduction

Carbohydrates are complex biomolecules which fill essential roles in cellular communications such as signaling to cell surface receptors and diverse cellular targets.[1] A wide variety of glycans composed of oligosaccharide and monosaccharide residues are linked *via* glycosidic linkages to access to very important glycoconjugates, such as glycolipids, glycoproteins, and glycosylated biologically active molecules. The biosynthesis of those glycans is conducted by several enzymes, so any modification in the structure of cell-surface glycans empowers them to assist in the release of the information critical for disease development.[2] Therefore, sugar molecules which participate in carbohydrate-mediated communications, are targets for medicinal therapeutics and remedies for various pathological illnesses. A large portion of materials, which are used for the immunotherapeutic studies are composed of carbohydrates targeting cancers.

Cancer is one of the most common deadly diseases, which takes a lot of lives annually all around the world.[3] Scientists have made promising progress in traditional forms of cancer therapy, including radiation, surgery, and chemotherapy over the past decades. However, a cure or preventative treatment continues to elude researchers. Consequently, it is critical to establish other approaches to fight cancer. Immunotherapy using a vaccine approach is an attractive target as an alternative method for cancer prevention and treatment as the application of vaccines has proven to be effective against other infectious diseases. The first efforts towards a vaccine against cancer were reported by William Coley in 1893, when live or inactivated Streptococcus pyogenes and Serratia marcescens were injected into tumor tissues. The result indicated the activation of immune system against established sarcomas.[4] In recent years, antitumor monoclonal antibodies (mAbs) has been successfully applied for the passive immune therapy in clinics around the world.[5, 6]

At first, many glycans employed in the cancer diagnostic field were isolated from natural sources. Nowadays, scientists try to synthesis these compounds rather than isolate them because of the lengthy isolation procedure, low quantities isolated and low purity of isolated glycans process from natural sources. These include glycolipids and tumour-associated carbohydrate antigens (TACAs).[7] The standard application of vaccines provides prevention and protection against infectious diseases. However, most experimental cancer vaccines aim to induce an immune response to eliminate the threats, including cancers and infections.[4–7] Vaccines are also applied for metastatic cancers that often occurs after removing the tumour by chemotherapy or surgery. The current cancer vaccines can be classified as dendritic cell vaccines, whole-cell vaccines, DNA vaccines, viral vectors, and antigen-specific vaccines, the last of which has recently attracted the most attention from researchers.[8]

After the discovery of tumour-associated carbohydrate antigens (TACA) development of antigen-specific vaccines has become possible. In recent years, progress has been made towards vaccines that use these antigens for cancer immunotherapy through controlled activation of the immune system to attack to the cancer cells. However, many challenges are left to be addressed in order to get effective treatments. These challenges include low stability and immunogenicity of these antigens, both of which are under study in this thesis, and overcoming these challenges could be the key to eradicating cancer cells.

TACAs are characterized in two categories: Glycoprotein antigens including Tn, Thomsen−Friendreich (TF), and sialyl-Tn (sTn), which are O-linked to the amino acid serine or threonine and glycolipid antigens, which are carbohydrates O-linked to ceramide

lipids with fatty chains. The glycolipids can be subclassified into blood group determinants such as Lewis^{x}, Lewis^{y}, the gangliosides, GD2, GM2, Globo class Globo-H, Gb3. Although, glycolipid vaccines appeared to not have a significant effect on tumour growth,[9] recently, new methodology associated with a better in-depth understanding of glycolipids properties and interactions paved the path for new immunotherapy development.[10] Glycolipids are also widely applied as an adjuvant in cancer immunotherapy.[11-14] More recently, α-Linked glycolipids attracted scientists' interest after the therapeutic potential of α-galactosylceramide KRN7000 showed promising result in treatments of tumours, autoimmune diseases and microbial infections.[15]

Despite promising preliminary results to activate the immune system against the threats, more information is necessary to get this massive response under control as immune response generated by these types of glycolipid antigens contributes to dangerous effects.[16] As a result, research is required in order to synthesize glycolipids analogues with the ability to activate the immune system in a controlled way against a specific target. Consequently, carbohydrates in the form of glycoconjugate are essential in the battle against cancer and serve as the backbone of an immunotherapy. Recent studies have shown progress towards finding a way to prevent or cure cancer, however, synthesis of more relevant carbohydrates such as modified tumour associated carbohydrates, glycolipids and glycoproteins, and their application against cancer heavily is still required.[17, 18]

1.1 Biostable acetal-free Tn and TF antigen-based vaccines as an immunotherapy for cancer

1.1.1 Cancer and immunotherapy

Cancer has been a major cause of death among humans for a long time and is still taking lives. Despite an enormous global effort to try and mitigate the effects of this disease, there has not been a major breakthrough in terms of an absolute cure for this category of disease.[19, 20] Traditionally, most of the initiatives were focused on finding innovative treatments for all kind of cancers. Surgery, radiotherapy, chemotherapy, and newer targeted drug therapies are all major targets for researchers. A major issue, however, lies in the endless obstacles that must be overcome. For example: Surgery, followed by chemotherapy, and/or radiation therapy has the potential to effectively terminate tumours. However, the mutations that are responsible for developing certain cancers may remain hidden in nearby pre-cancerous tissue, and because of this, the disease can often return. These treatments can work if the disease is diagnosed in an early stage and the tumour is located in tissue that can be easily sacrificed. Even targeted therapies, which initially show promising results in the early stages of research, have often led to drug resistance as the long-term outcome.[21]

These results have led researchers to try to find a way to activate the immune system against cancer cells to terminate them when they are introduced into the body.[22] Immunologists suggest using a vaccine to activate the immune system. Usually the immune system responds to foreign proteins, however, proteins that exist in cancer cells can also be found in healthy cells as well. This means that they are not an appropriate target for a vaccine therapy. Fortunately, some antigens are unique and are not found in healthy cells. This class of antigens are normally called tumour-associated carbohydrate antigens (TACAs) and can be found in different cancers such as ovarian, breast, prostate and lung.[23, 24]

1.1.2 TACAs in Cancer

The TACAs are a subclass of mucin-glycopeptides in carcinomas. These mucin-glycopeptides usually exist in fetal tissue in the early stages, and are expressed on the surface of carcinomas; however, they are not present in adult healthy cells.[25, 26] Carcinomas include lung, prostate, colon, breast, oral, pancreas, ovarian, and cervical cancers that make up the surface of an organ. Epithelial cells secrete mucins which are glycosylated proteins that form a backbone of mucous and other biological lubricants. The mucins often consist of large, complicated oligosaccharides.[27] In the carcinomas, glycosylation and/or glycosidase procedures can undergo misregulation, which leads to truncated carbohydrates, but scientists are uncertain whether or not this is a cause of carcinoma progression or merely a common side effect. Regardless, the presence of these carbohydrates has correlated with the invasion of healthy tissue, metastasis, and more aggressive tumours. Furthermore, this interaction has been associated with their role in mediating tumour-cell adhesion.[28]

The TACAs can be classified into three classes: Firstly, the mucin-based glycopeptides, Tn **101**, TF **102** and sialyl-Tn **103**; the lactosamine-series depicted as both glycoproteins and glycolipids, the sialyl-LewisX **104**, LewisX **105**, sialyl LewisA **106**, LewisY **107** **(Figure 1.1)**. Furthermore, we have the truncated gangliosides which are represented by not only the Globo-H **108** antigen but the stage-specific embryonic antigens 3, **109**, and ganglioside monosialic 2 (GM2) **110 (Figure 1.1)**. The first category is the primary focus of this dissertation which focuses heavily on both the Tn and TF antigens. These antigens have received the majority of both synthetic and biological attention because of comparable simplicity and their exclusivity to carcinomas. The second class includes blood-group an-

tigens. Their structural complexity has limited their use as potential vaccine targets, especially the unnatural multimeric forms. The final ganglioside class shows good potential, and these TACA glycolipids continue to be reported and they may open up new source of immunological targets in the future. The remainder of part of this dissertation will focus exclusively on the Tn and TF antigens.[7, 29-34]

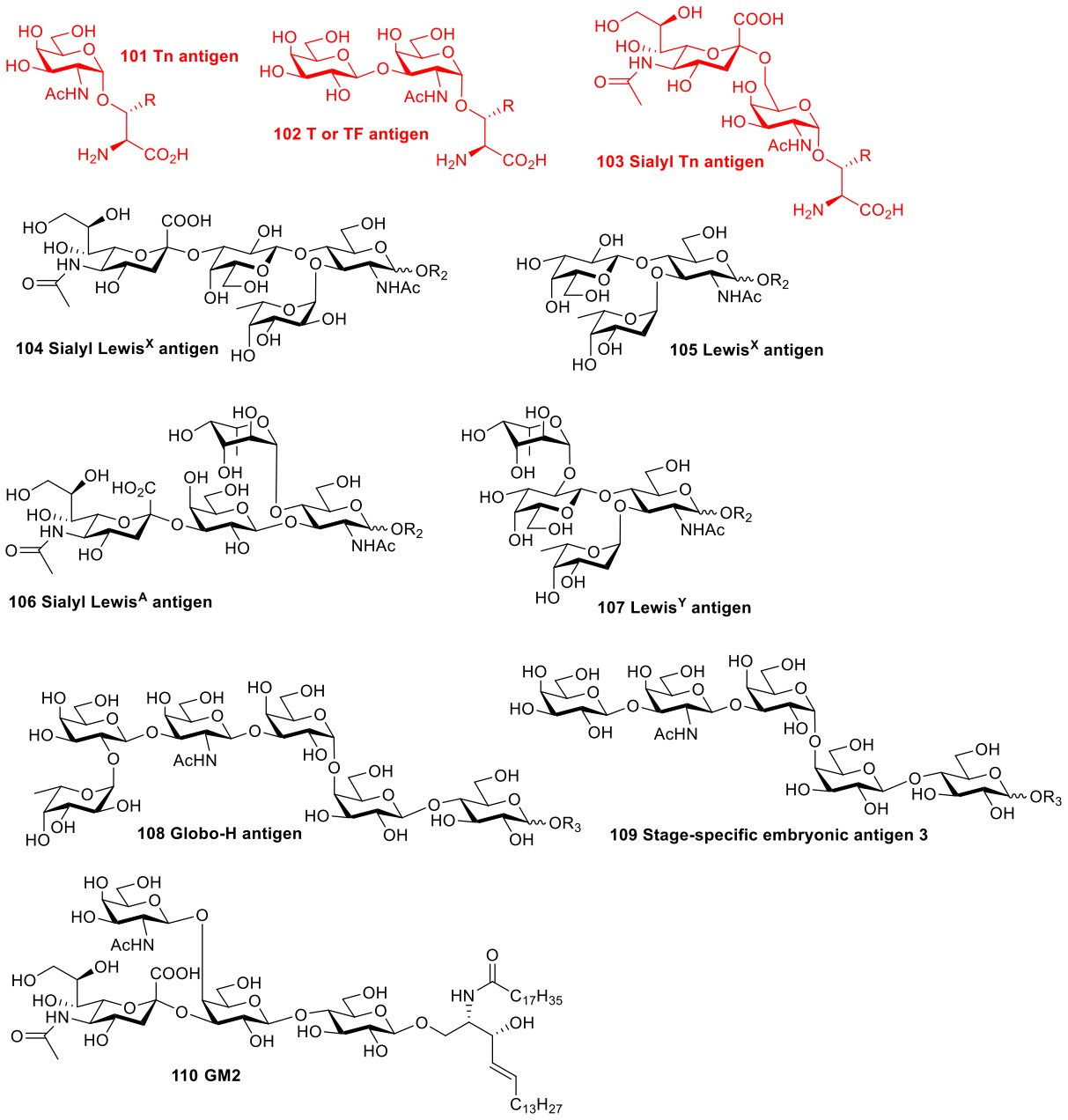


Figure 1. 1 Structures of the most common tumour-associated carbohydrate antigens. R represents either H or CH_3 in the case of the mucin antigens, and R2 represents either a peptide or a lipid

1.1.3 Synthesis of the natural TACAs, historical overview

1.1.3.1 Synthesis of Tn Antigen

The Tn antigen is comprised of two parts, (2-deoxy-2-acetamido-galactose (GalNHAc))α-O-linked to either serine **101a**, or threonine **101b** through their side chains as depicted in **Figure 1.2**.[35] It is the simplest possible antigen that exists currently.

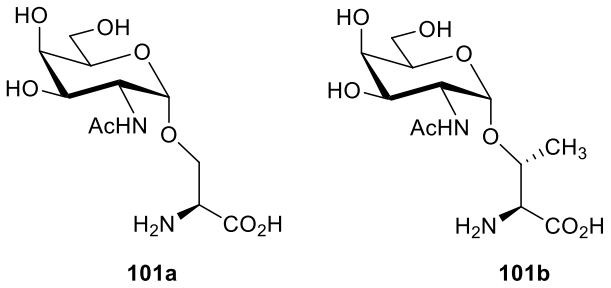
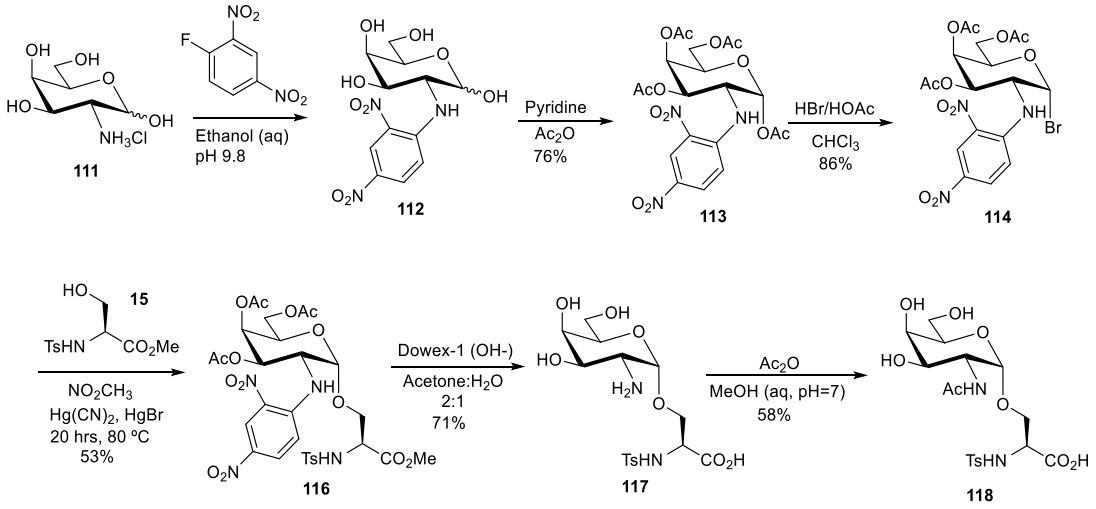


Figure 1. 2 Serine-linked **101a** and threonine linked **101b** forms of the Tn antigen; both are often co-expressed on the same glycopeptide

The Tn antigen was discovered in 1957 by Moreau as the "T antigene nouvelle" to differentiate it from the Thomsen-Friedenreich antigen (TF antigen).[36] In 1975 Dahr determined the exact structure of the carbohydrate portion of this antigen as GalNHAc using co-elution in gas-liquid chromatography. Moreover, they also identified the major glycopeptides affected.[37] The Tn antigen attracted additional attention when it was correlated with breast carcinomas by Springer in 1974, where it was shown to be present in over 90% of cases.[38] It has also been identified in ovarian,[9] bladder,[39] cervical,[40, 41] colon,[42, 43] lung,[44] stomach,[45] pancreatic[46] and prostate tumours,[47] and it usually has been associated with tumour expansion and metastatic behaviour. Its presence in normal healthy fetal tissue may be consistent with its role in facilitating rapid cell division.[48] Tn antigen backbone structure is generated in the Golgi body (or apparatus) but is always then rapidly glycosylated with additional residues before ever being introduced on the surface of healthy cells. These further steps often do not happen in many carcinomas.

The cancer-specificity and simple structure of the Tn antigen has attracted the attention of chemists to synthesis this target. In 1977 Osawa reported the first synthesis of this antigen.[33] A Koenigs-Knorr glycosylation using mercuric cyanide as an activator of the glycosyl donor was performed (**Scheme 1.1)**. In this synthesis, the C-2 amine **111** was protected as a 2,4-dinitroanilino group in **112** to prevent any anchimeric assistance.[34] The glycosyl donor **114** was formed through an acetylation to the α-acetate **113**, followed by an acid catalyzed anomeric bromination. The glycosylation was performed between the *N*-tosyl-L-serine methyl ester **115** and the glycosyl donor **114**, to provide the first reported synthetic TACA glycoconjugate **116**. A subsequent global deprotection under basic conditions provided tosylamine **117**, followed by a selective protection of the C-2 amine as the *N*-acetamide performed to generate Tn antigen **118**.

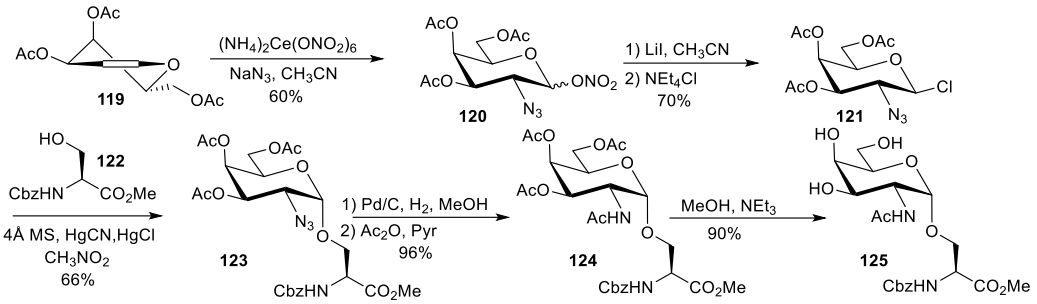


Scheme 1. 1 First synthesis of the Tn antigen reported by Osawa using a 2,4-dinitroanilino protecting group approach

The formation of a syn-1,2-relationship between the α-orientation of the respective amino acid and the 2-acetylamino group in **118** has always been challenging due to neighbouring group participation.

Oxocarbenium cations are not useful for generating α-glycosides if the acetamido group is present and intact. Different approaches have been examined to increase the probability of making this syn relationship using an appropriate choice of protecting groups for the amine, including imines, electron-withdrawing amines **(Scheme 1.1)**, azides, nitro and dinitroanilino groups.

Paulsen pioneered using the azide group as a masked NHAc group towards the synthesis of the TF-glycon.[49, 50] Since then, it has used in the vast majority of synthetic procedures to date. Expanding from this research, Pavia used this approach to generate the Tn antigen **(Scheme 1.2)**.[51] Starting with the introduction of the azide and an anomeric nitrate to galactal **119** using the oxidative addition, β-glycosyl chloride **121** was formed through a selective α-anomeric iodide formation, this was subsequently followed by an S_N2 inversion, which provided the β-chloride **121** in a 70% overall yield. Compound **121** underwent a mercuric-mediated glycosylation with the serine acceptor derivative **122**. The glycoconjugate **125** was formed *via* conversion of the azide to the acetamide group followed by a mild deprotection of the acetates. Although this approach has become popular among chemists, different methods have been applied to modern glycoside donors, including the use of anomeric fluorides and trichloroacetimidates which save a few synthetic steps. These compounds have been extensively examined as bioconjugates however, this discussion remains beyond the scope of this dissertation.



Scheme 1. 2 Pavia synthesis of the Tn antigen using a C-2 azide as an amine masking group as developed by Paulsen

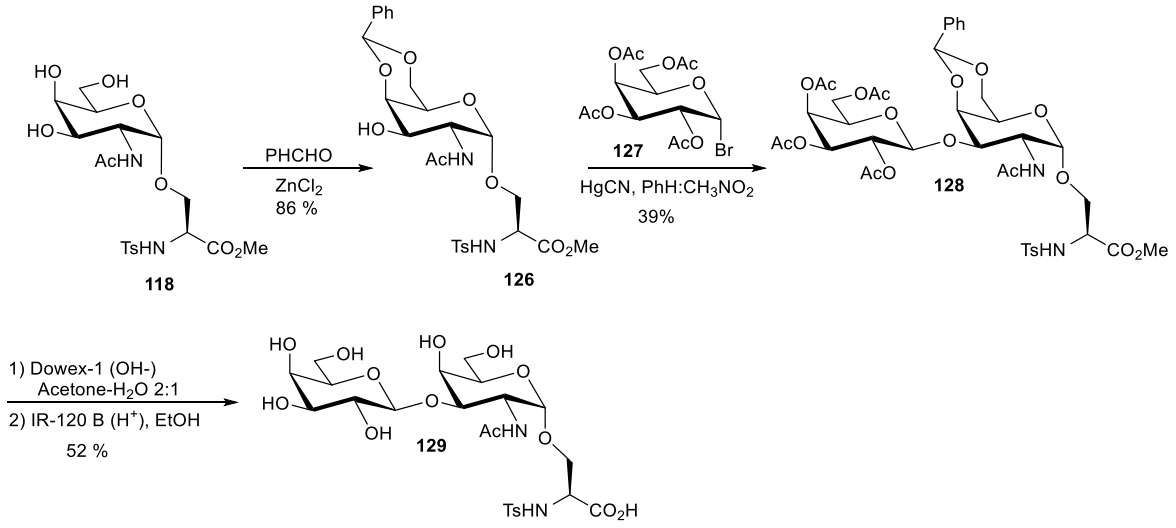
1.1.3.2 Synthesis of the TF antigen

The first series of TACAs that were identified were both the T or TF antigens, established by Huebener in 1925, Thomsen in 1926, and further defined by Friedenreich, and as a result, should be called the HTF antigen on that basis, though Huebener has been "deleted" by the scientific community.[52, 53] The structure was first reported by Kim and Uhlenbruck in 1966.[54] It was represented as the disaccharide, β-D-galactosyl-1,3-linked to 2-deoxy-2-acetamido-galactose (GalNHAc) α-O-linked to either serine or threonine. The TF antigen, like the Tn antigen, is a cancer biomarker that does not exist in normal healthy cells. The TF antigen, as with other non-oncogenic markers, interacts selectively with a protein which is specific for β-galactose residues called galectin-3.[55] Galectin-3 is responsible for antimicrobial activity, mediating cell adhesion, apoptosis, and regulating cell cycle checkpoints. However, galectin-3's precise role in healthy or cancerous cells remains unclear.[55] The TF antigen and galectin-3 co-over-expression is accompanied with the ability of cancer cells to bond better to the extracellular matrix and can result in metastasis.[55]

The TF antigen is present in several cancers, including ovarian (>99% of cases), breast (85%), colon cancer (60%) and acute lymphoblastic leukemia (50%).[56] Although the TF antigen has only been presented on adult cancer cells, the disaccharide unit itself,

(which is referred to as the Core 1 carbohydrate), is used to build simple oligosaccharides in healthy mucins. As a result, any antibodies produced against the TF antigen should recognize the carbohydrate-protein linkage in addition to the carbohydrates alone.

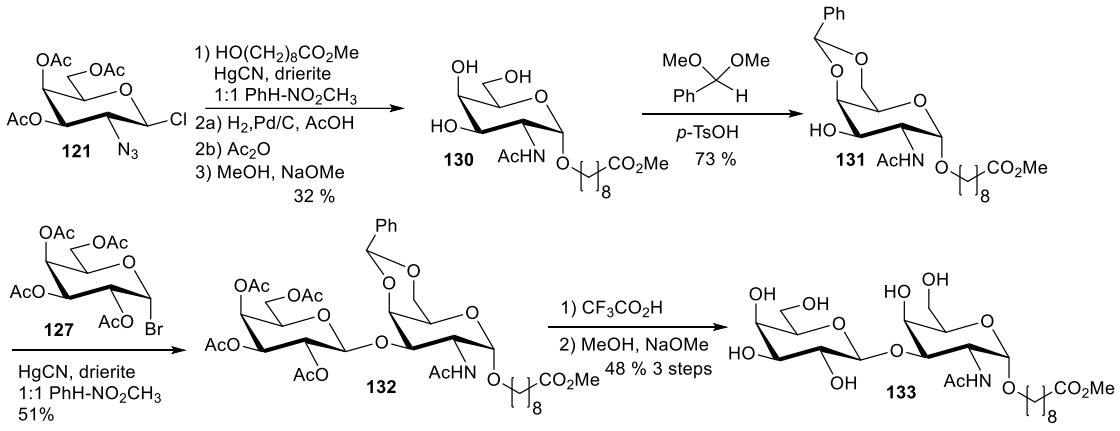
To save time and reduce more challenges toward the synthesis of the TF antigen, the majority of the synthetic pathway is quite similar to synthesis of the Tn antigen. The TF antigen is composed of the C-3 oxygen of the Tn antigen unit which is β-linked to the anomeric position of galactose. The first synthesis of the TF antigen reported by Kaifu and Osawa follows directly from the synthesis of the Tn antigen **(Scheme 1.3)**.[57] The glycosylation was performed between **126** and **127** to generate a protected disaccharide **128**. Global deprotection in both basic and acidic conditions respectively provided compound **129**. This is a relatively efficient synthesis of **129** from galactosamine **111** in 10 linear steps.



Scheme 1. 3 Osawa and Kaifu's synthesis of the TF antigen from their previously prepared Tn antigen

Lemieux reported the first synthesis of the glycon alone in 1981 which is depicted in **Scheme 1.4**.[58]Azido-chloride **121** was prepared by Paulsen's approach and then glyco-

sylated with a long chain hydroxy-ester. The acetamide was generated by an azide reduction, and subsequent deacetylation afforded triol **130**. Hydroxyl groups in C-4 and C-6 were protected with a benzylidene protecting group. The corresponding compound **131** underwent a glycosylation with a 2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide donor **127** to provide compound **132**. A global deprotection afforded glycoside **133**, which then applied for inhibition of a T-antigen-specific agglutination. A similar approach was used by Paulsen to prepare the glycoconjugate with both the serine and threonine analogues attached.[59]



Scheme 1. 4 Lemieux's synthesis of the TF antigen glycon, and its conjugation to a hydrophobic linker

1.1.4 Synthetic TACA antigens as potential immunotherapeutic

TACAs are only present on cancer cells and cannot be found on healthy cells, this inspires researchers to target these compounds when designing a vaccine to induce the immune system to target cancer cells. The general mechanism of a vaccine is as follows: Initially, after being introduced to the serum where the most common enzymes are present, endocytosis occurs by antigen-presenting cells in a lysozyme. This cellular compartment is responsible for breaking down the components into fragments. These fragments, using

the Golgi apparatus, are delivered and bind to the human leukocyte antigen receptor and placed on the cell surface. Recognition of the antigen-HLA complex by a T-cell receptor plays an essential role in receiving a robust immune response. T-cell dependent and independent pathways are the two common ways to activate the immune system. The independent T-cell activation is when antigens cannot bind to the T-cell receptor (HLA), and consequently, T-cells can not recognize them. In this case, the immune system will be activated only through the activation of B-cell *via* the B-cell receptor (BCR); however, this activation would be inferior due to the low affinity of Immunoglobulin M (IgM), which is the first antibody that exists mainly in blood and lymph fluid, produced by the body to fight against a new infection. The B-cell response is usually more potent when they are activated *via* a dependent pathway through activation of T-cell.

T-cell dependent pathway occurs when antigens survive the biological conditions. This includes the glycosidases that are present in their path and bind to the T-cell receptor (HLA) and activate T-cells. T-cells release cytokines to activate a cytotoxic T-cell, which can eliminate damaged cells, cells infected by viruses and can also kill cancer cells. In addition, these T-cells can cause activation of B-cells. B-cells can release a high affinity of Immunoglobulin G (IgG), which is the most common antibody present in blood and other body fluids capable of forming after immunization or infection and fighting against bacterial and viral infections. *via* plasma cells. Moreover, inducing memory B-cells plays an essential role in the immunotherapy. These cells remain last longer in the body compared to the rest of the immune system cells in order to recognize and fight infections or cancer cells when they return.

1.1.4.1 Challenges in TACA vaccine preparation

The first TACA-targeting vaccines were isolated from natural sources. For example, Hakomori's Tn-dense sialo-ovine submaxillary mucin, which was able to assist inoculated mice in resisting a highly invasive Tn-antigen-overexpressing tumour line.[28] However, these are still challenges left to be addressed. The first question is, are antigens capable of activating the immune system? In other words, can they survive through the harsh biological conditions and activate T-cells? As many of the tumour-associated carbohydrate antigens are expressed exclusively on cancer cells, the initial postulate was that these systems might be able to act as monomeric vaccines because TACAs are T-cell independent antigens. For example: the human leukocyte antigen (HLA) receptors on the surface of antigen-presenting cells cannot recognize sugars instead they interact with approximately 8-12 amino acids of antigenic peptides through their binding grooves.[60, 61] As above mentioned, the human leukocyte antigen (HLA) receptors are responsible for binding prospective antigens and displaying them to T-cells. The second question is due to the presence of the acetal group, which is susceptible to cleavage by biological conditions, mainly through enzymes cleavage how can we improve the low biostability of TACAs? The third question is how do we improve the typically low immunogenicity of TACAs?

The new antigen design with the ability to activate T-cells was the first question that researchers tried to address. Knowing that T-cells are good at binding to peptides, antigen synthesis was imperative and therefore relied on conjugation to a carrier protein KLH to activate a T-cell. Dansihefsky developed monomeric TACA antigens and a multivalent vaccine that incorporated all three of the mucin carbohydrates as well as Globo-H

and GM2 in a genuinely great synthetic campaign (**Figure 1.3a)**.[62, 63] The first case indicated limited success in early-phase clinical trials, and for the second case, the clinical trials are yet to be reported. As an alternative, a monovalent Tn antigen vaccine was provided in one molecule as a complete package by the Boons group **(Figure 1.3b)**.[64]

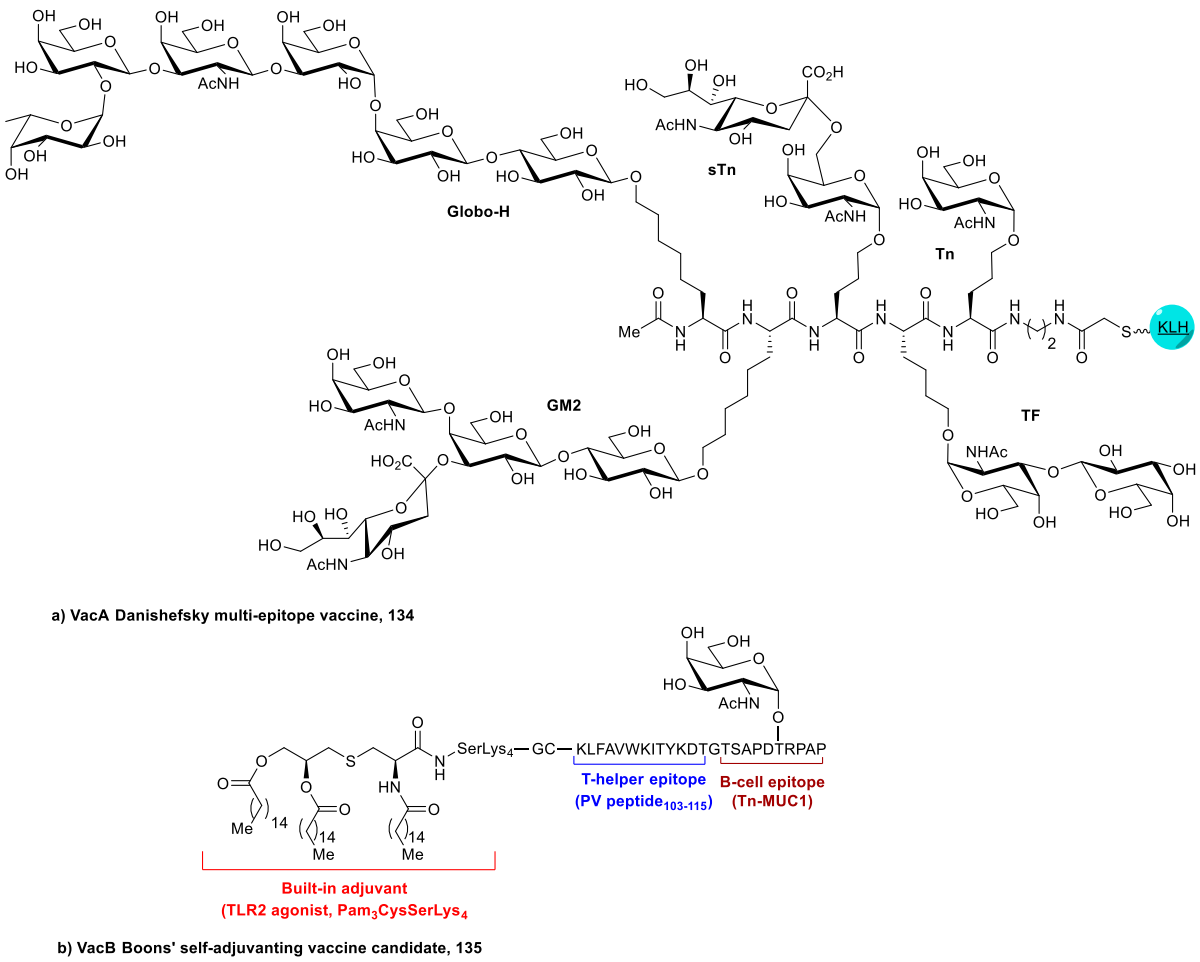


Figure 1. 3a,b Structure of Danishefsky's multiantigen vaccine candidate and Boons' self- adjuvating Tn-vaccine system as examples of existing TACA immunotherapeutic constructs

These approaches express a promising future for the development of vaccines. Unfortunately, despite the significant production of Immunoglobulin-G responses in animal models, these carbohydrate vaccines remain a work in progress and have yet to be tested in or delivered to the human population. The reasons for this are partly because of low

immunogenicity of carbohydrates and partly the potential lack of specificity of the immune response for the carbohydrate counterpart of the vaccine rather than the T-helper epitopes and the development of immunotolerance.[25]

The low biological half-life of TACAs, which degrade *via* enzymatic cleavage of the acetal group, poses another question. The biological half-life of TACAs is usually only a few hours, and before they can get to the T-cell and activate the immune system, they are catabolized by the body. The difference between human and mouse immune systems might explain this failure in translation. Rodents have many α-galactose residues on their cells, but these are far rarer in humans. This difference is extreme enough that α-galactosidase is the most common enzyme circulating in human serum, which is precisely the enzyme necessary to cleave these sugars and deactivate the vaccine. This enzyme is also greatly overexpressed and present in the lysozymes of antigen-presenting cells. But they are not common in rodents. This means that vaccines that work well in rodents are much more readily cleaved in humans; this highlights again the caution needed when translating animal models to human systems.[65] Chemists have proposed two ways to remove the acetal group; The endocyclic oxygen can be removed and replaced with a methylene group, namely a carbasugar or the exocyclic oxygen can be replaced with a methylene group which is called a C-glycoside.

The synthesis of a C-glycoside attracted more attention among chemists compared to the synthesis of a carbasugar simply because of a simpler potential synthetic pathway. Although the instability of TACAs can be addressed through C-glycosides and carbasugars, these compounds are synthesized predominantly to showcase the novel chemistry and the biological behaviour of these compounds remains mostly neglected. There are only a

few reported cases to date using these compounds to explore improvements to the stability of TACAs.[66-68]

For the multivalency to overcome low immunogenicity, preliminary preclinical data suggests that the immunogenicity could be improved by increasing the number of carbohydrates and, as a result, exponentially increasing the number of carbohydrates should elicit significantly higher immunogenicity.[69] Dendrons are monodisperse, highly symmetric branched macromolecule synthesized in a stepwise process, which each step exponentially increases the number of branches.[70] This highly symmetric multiple branches in a single molecule versus polymers, where the presence of many different lengths is unavoidable, facilitates the precise characterization of the expected co-operativity effects and simplifies any clinical characterization.

Trant, Gillies, Haeryfar and Lecommandoux reported the first application of multivalent antigen-loading, which resulted in higher immunogenicity using acetal-free antigens on dendron support, showing the number of antigens doubled, the immunogenicity appeared to improve exponentially.[71] They synthesized the first acetal-free glycodendron–lipid hybrids based on glycerides and applied it to activate iNKT cells. The result showed enhanced activation of iNKT cells using higher generation systems (a single molecule with 16 carbohydrate antigens). This result indicates a direct relationship between antigen multivalency and immunogenic response, however, the designed molecule only produced moderate immunogenic result as it does not closely resemble the natural glycolipid antigen.[71] The interleukin-2 was measured by ELISA with introducing 20 ng/mL of samples to mouse DN32.D3 cells **(Figure 1.4)**.

Although dendrons can be functionalized with TACAs[27] and rarely with AFCs,[71] only a few of them biologically evaluated, [71, 45] however, there appears to be no investigation on effect on multiple antigen-loading in a single molecule (antigens on dendron support) with immunogenicity. This multivalency effect could be applied to functionalize a carrier protein, which would be novel and further increase immunogenicity, and solve the low immunogenicity of the TACA vaccine candidates.

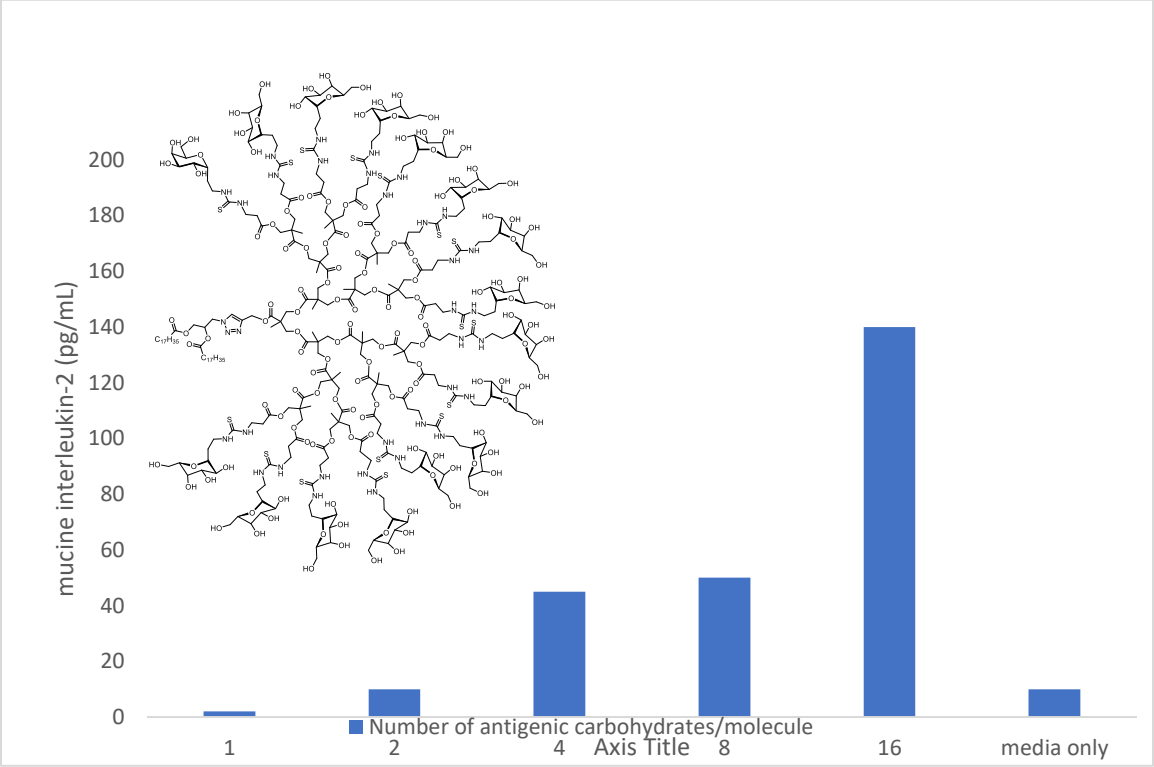


Figure 1. 4 Relationship between antigen multivalency and immunogenic response from Trant et al 2016.[71]

Hence, the significant challenges of vaccine therapies remain the same regardless of promising results that have been achieved. Both immunotolerance and immunogenicity remain problematically low, and the immune response is often specific for the peptide or carrier protein and produces a minimal response against the carbohydrate component. These challenges can be attributed to a variety of different reasons. Many of the individual

carbohydrates exist in the body in diverse forms, which means only a few antibodies exist in the body against these particular forms. T-cell receptors are not trained nor optimized for identifying glycopeptides. Lastly, the antigen processing of carbohydrate-bearing materials, (including the enzymatic profile of the liposomes and survival of glycopeptides through this process) is still poorly understood and currently under exploration.

1.1.5 Acetal-free carbohydrate antigens

The immune system is not designed to target carbohydrates, and this could be an important reason for the lack of success in this field, despite significant efforts and considerable promise, to generate anti-cancer vaccine candidates. It should be noted, however that these vaccine candidates have been capable of producing antibodies although the response is not sufficient to target the cancer cells. Three factors could explain the inadequate immune response generated by vaccines. First, the fact that cancer cells are often resistant toward carbohydrate antibodies causes problems for carbohydrate based vaccines and this immunotolerance is a reason for the weak immune response provided by these vaccines. Second, it is still not clear whether or not the specificity of the antibodies generated from the antigen vaccine candidate can be congruent towards actual glycopeptides (cancer cells). The immune system cannot recognize glycopeptides in the presence of peptide support alone. This is mostly due to the higher immunogenicity of peptides. Third, the lack of stability of the antigen vaccine (mainly in the antigen processing step), could be another factor contributing to the inadequate immune response. Carbohydrates have a short physiological half-life in the body, mostly because of the acetal functionality which is susceptible to cleavage in biological conditions, including acidic, basic, and enzymatic processes. If the physiological half-life of the antigen is sufficiently short, the carbohydrate antigens may

not survive antigen processing in the immune system, and before they can fulfill their duty, they can be decomposed and excreted by the body. As mentioned above, chemists can theoretically solve this problem by making the acetal-free carbohydrate molecules. This includes: removing the acetal group by replacing the *exo*- or endocyclic oxygen with different elements including sulfur (exocyclic oxygen replacement), nitrogen (endocyclic oxygen replacement), and more importantly carbon (both exocyclic C-glycoside and endocyclic oxygen replacement carbasugar). The first two cases, azasugars, and thioglycosides as well as carbasugars are beyond the scope of this dissertation and have been well-reviewed elsewhere.[72, 73] In this subsection, three general routes toward the synthesis of a C-glycoside is presented: cross-coupling, ring-closing metathesis and allylation reactions. For further detail refer to the review by Sadraei et al.[74]

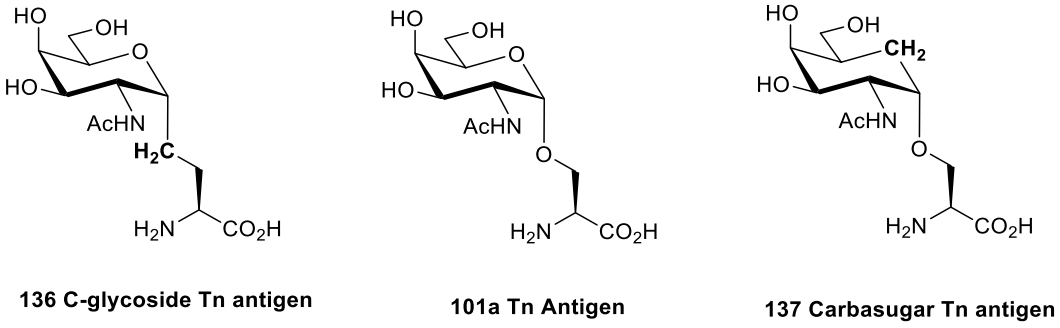


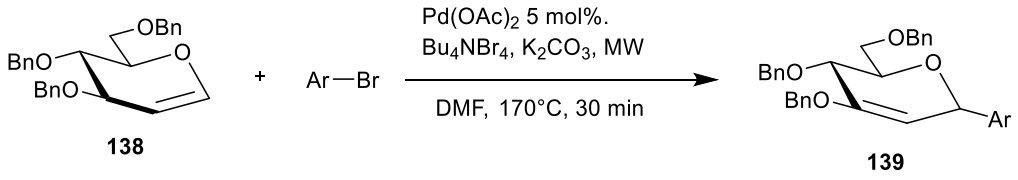
Figure 1. 5 Structural comparison between C-glycoside 1**36**, natural acetal-system, **101a**, and carbasugar **137**, analogues of the Tn antigen

1.1.5.1 Cross-Coupling

Cross-coupling reactions are potent synthetic methods and have attracted much interest, particularly relevant is the development of sp^3-sp^2 couplings in carbohydrate chemistry. Carbon-carbon bond formation, especially in the anomeric position, has always been a challenge in carbohydrate chemistry.[75] Cross-coupling approaches can be used to provide novel solutions to this challenge, as an example, both C-glycosides[76, 77] and C-

nucleoside analogues[78] have been generated from this approach. The cross-coupling reactions are usually catalyzed by transition metal complexes, most commonly based on palladium. Heck, Suzuki and Negishi cross couplings are the most well-known reactions belong to this group.

The Heck reaction is a palladium-catalyzed cross-coupling under basic conditions to generate a sp^2-sp^2 bond between an alkene containing at least one proton and an aryl or alkenyl halide or triflate. A limitation of this approach is that it has often been used to make a sp^2-sp^2 carbon bonds in carbohydrate chemistry, rather than sp^2-sp^3 or sp^3-sp^3, the latter of which is needed for carbohydrate chemistry where we have few modifications. There are only a few examples of using Heck reactions to make bioconjugated glycosides. In one of those examples, Yang and co-workers reported benzyl-protected glucal **138** could be coupled with a variety of different aryl bromides under microwave irradiation to form the corresponding α-2-deoxy-dihydro-C-aryl glycosides.

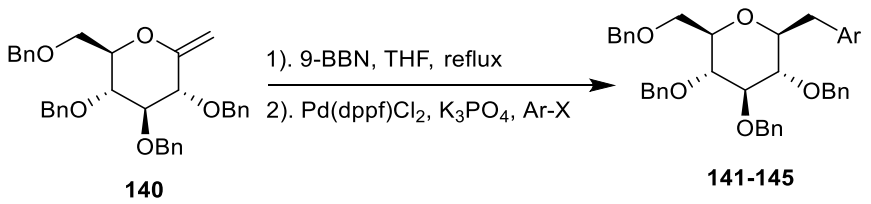


Scheme 1. 5 Aryl-Bromide Coupling Under Microwave Conditions by Yang

The Suzuki-Miyarua coupling is another well-known cross-coupling reaction, usually it is catalyzed with either Palladium or Nickel complexes. These catalysts mediate carbon-carbon bond formations between boronic acids (or boronic esters) and alkyl halides (or triflates) in what has become a powerful synthetic tool.[79, 80]

The Suzuki coupling approach has been used in carbohydrate chemistry in two slightly different ways. In a report by Johnson, the authors were able to formally alkylate

gluconolactone producing exo-glycals *via* introducing a 9-BBN functionality through a hydroboration, followed by palladium catalyst mediated Suzuki coupling reaction, which could be an elegant way to achieve further functionalization including the synthesis of C-glycosides **(Scheme 1.6)**.[81]

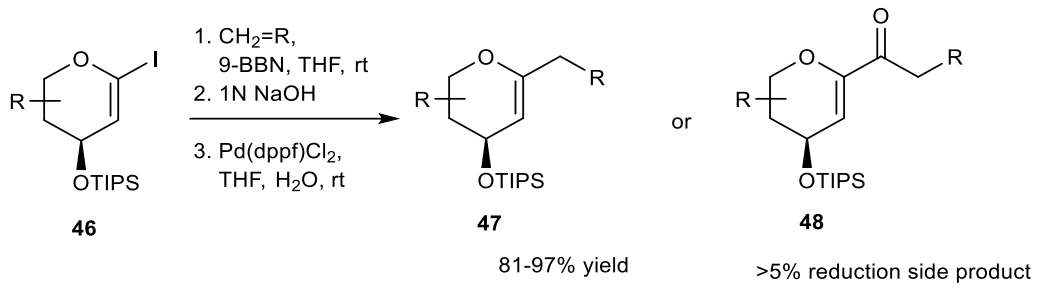


140 **141-145**

Ar	X	Yield (%)
Ph	Br	67
4-*t*-BuC_6H_4	Br	82
4-$HOCH_2C_6H_4$	I	74
4-BrC_6H_4	Br	60
5-pyridyl	Br	21

Scheme 1. 6 9-BBN hydroboration and Suzuki coupling approach for the synthesis of benzyl C-glycosides

In a second report, C-glycals have been generated by the Tan group using a Suzuki coupling between olefinic species, which can be *in situ* borylated using 9-BBN and halogenated glycals **(Scheme 1.7)**. This chemistry is tolerant of Fmoc amino acids and other differentially-protected carbohydrates producing products in very good isolated yields.[82]

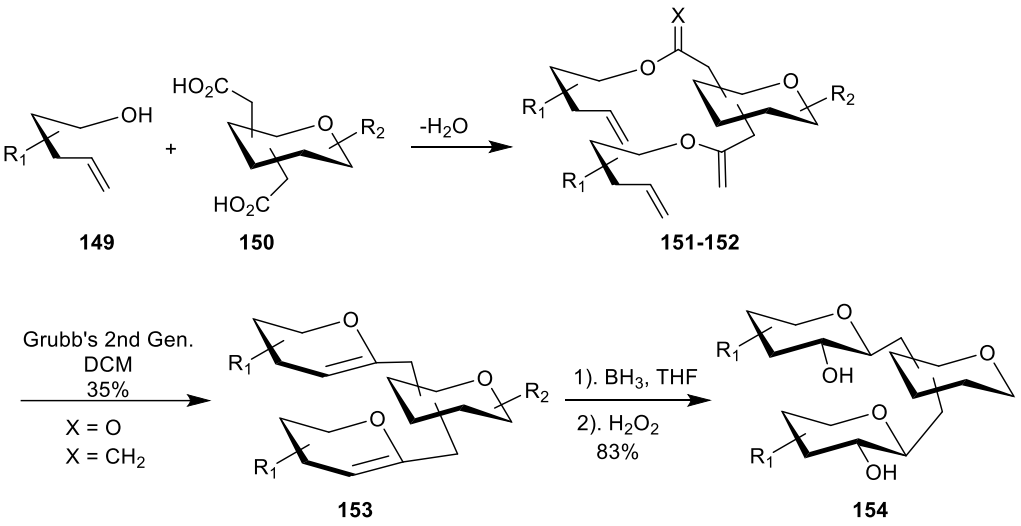


Scheme 1. 7 Synthesis of C-glycals by Tan using Suzuki coupling reaction

The Negishi Coupling is a compelling methodology amongst the cross-coupling reactions because it provides selective and rapid access to α-C-glycosides. In the Negishi coupling, an organohalide is coupled with an organozinc nucleophile through a catalytic process using either palladium or nickel complexes. The highly selective and rapid synthesis of α-C-glycosides were reported *via* the same chemistry using α-glycosyl halides and alkyl-zinc reagents by Gagné.[83]

1.1.5.2 Ring-Closing Metathesis

The ring-closing metathesis reaction involves the formation of a cyclic olefin between two sp^2-sp^2 carbons of an alkene mediated by a ruthenium catalyst. This approach has widely been used in synthesis and an example of its utility in carbohydrate chemistry can be seen in the reported synthesis of a complicated acetal-free trisaccharide shown in **Scheme 1.8**.[84, 85]

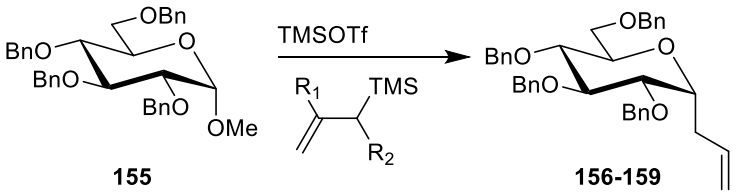


Scheme 1. 8 Overview of Postema's approach to a trisaccharide using a double RCM

1.1.5.3 Allylation and Related Reactions

The anomeric allylation method is an effective and efficient approach to generate a C-glycoside in the anomeric position. It has been so effective that it dominates the literature, bring used far more than any other methodology. High selectivity and rapid access to α-allylation, in addition to straight forward manipulation for the attachment of amino acids, have made this method an outstanding choice for the synthesis of TACA antigens. This methodology is extremely important in the synthesis of acetal-free analogues of TACAs when an allyl group is used as a tool to connect the amino acid to the sugar moiety in order to generate C-glycoside derivatives of the TACA antigens.

Hosomi and co-workers reported the addition of allyl silyl nucleophiles to glycopyranosides using TMSOTf as a Lewis acid obtaining moderate to high yields;[86, 87]

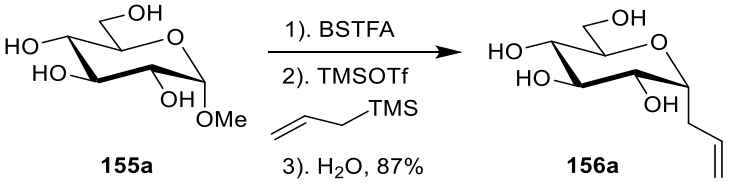


155 156-159

Product	Allylsilane	R_1	R_2	Yield	α:β Ratio
156	$Me_3SiCH_2CH=CH_2$	H	H	86	10:1
157	$Me_3SiCH_2CH=CHMe$	H	Me	87	6:1
158	Z-$Me_3SiCH_2CH=CHMe$	Me	H	68they	undetermined
159	$Me_3SiCH_2C(Br)=CH_2$	H	Br	71	1:0

Table 1. 1 Allylation of glycopyranosides using allyl silyl nucleophiles and TMSOTf

In 1987, Bennek and Gray reported rapid access to the fully deprotected C-allyl glycosides.[88] For example, (trimethylsilyl)trifluoroacetamide (BSTFA) *in situ* silylates the hydroxyl group before allylation which, after aqueous workup, cleavage of the silyl protecting groups afforded the desired C-allyl glycosides **(Scheme 1.9)**.



155a 156a

Scheme 1. 9 Bennek and Gray's one-pot allylation of unprotected glycosides

Allyltributyltin can be used to allylate acetylated galactose as an alternative for allyl silyl nucleophiles. Guindon reported allylation with retention of configuration when they used alkyl halides adjacent to electron-withdrawing functionalities with AIBN or Et_3B as a radical initiator.[89]

A large number of C-linked glycoconjugates of antifreeze studies have been carried out in good selectivity by Ben, Leclere, Trant, and co-workers using an allyltributyltin toward the formation of anomeric allylation.[90-93]

Using yet another method, Danishefsky and co-workers[94] reported the first Ferrier-Type 1 rearrangement of glycal acetates *via* a carbon nucleophile, using allyl-TMS, or $TiCl_4$ as a Lewis acid, to form 2,3-unsaturated glycosides (**Table 1.1)**. Despite an appealing outcome of high yields and excellent regioselectivity, the reformation of the desired carbohydrate requires further manipulation.

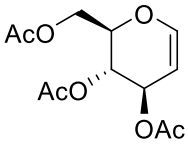
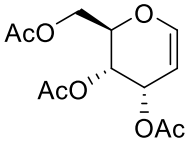
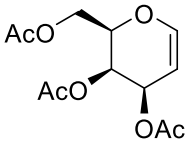
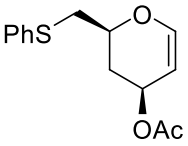
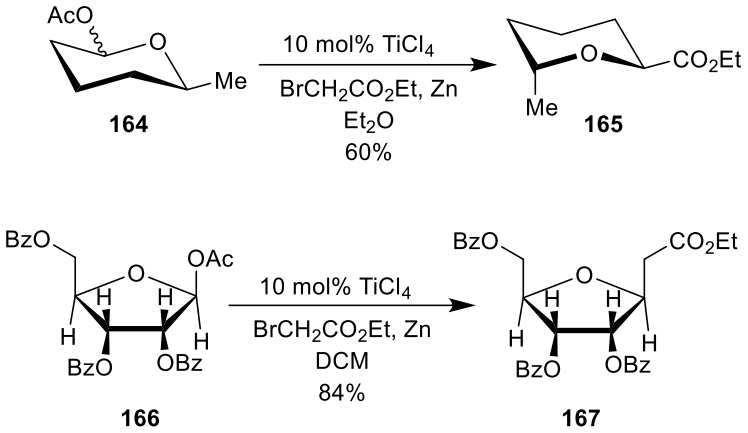
Glycals	Yield	α:β
 **160**	85%	16:1
 **161**	95%	6:1
 **162**	93%	30:1
 **163**	95%	1:0

Table 1. 2 Danishefsky's allylation of disparate glycals with 1 equiv. of $TiCl_4$ and 1.5 equiv. of allyl TMS at –78 ºC

All of the previously mentioned allylation approaches predominantly afford the *α*-anomer. The *β*-C-glycoside formation was accomplished by Hanessian using the SmI_2-mediated Reformatsky type reaction on both aldonolactones and oxocarbeniums generated

from an anomeric acetate using $TiCl_4$ as a Lewis acid.[95] The carbon in oxocarbenium ion can act as an electrophile for the nucleophilic attack of the organozinc compound produced by ethyl bromoester and zinc reported by Oguni **(Scheme 110)**.[96]



Scheme 1. 10 Titanium-mediated Reformatsky-like, *β*-selective alkylations

The allylation chemistry is the most powerful tool for the synthesis of acetal-free carbohydrate (C-glycoside), in particular TACAs, however, the synthesis of these compounds has been done only to represent a novel methodology toward the synthesis of these antigens, and the biological evaluation of these antigens mostly remained unexplored. It is worth mentioning that, despite only a few other approaches reported as mentioned above, still more reliable procedures required in order to develop new and elegant synthetic routes, especially when applying the allylation, would be challenging and involves multiple manipulations.

1.1.5.4 Synthesis of an Acetal-Free Tn antigen

The Tn antigen is a sugar moiety known as *N*-acetyl galactosamine, where an *α*-linked amino acid is attached to the sugar. The amino acids are usually either serine or threonine. The Tn antigen has become the centre of attention in the field of tumour-associated carbohydrate antigens, because this antigen belongs to the simplest branch of

amino acid-monosaccharide conjugates. As a result, the synthesis of this antigen should be less challenging compared to the rest of the TACAs. Although there are four possibile acetal-free Tn antigen analogues, the few attempts carried out to date have focused on the synthesis of only **136 (Figure 1.6)**. Despite remarkable progress for the synthesis of the C-glycoside analogue of the Tn antigen (See Section 1.1.5.3), the true acetal-free analogue of the natural Tn antigen has never been synthesized. In addition, the other three analogues have yet to be prepared. We will now show the various approaches used towards the synthesis of **136**.

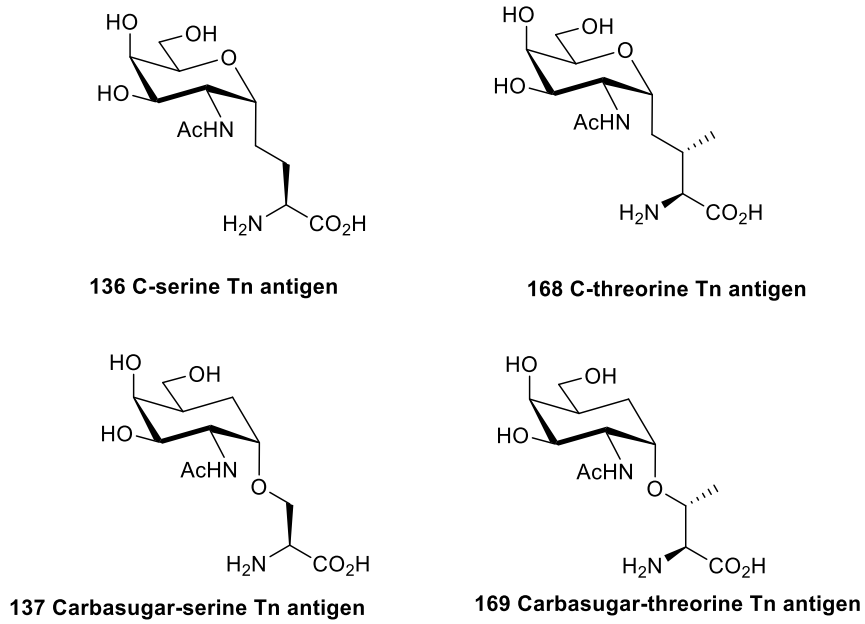
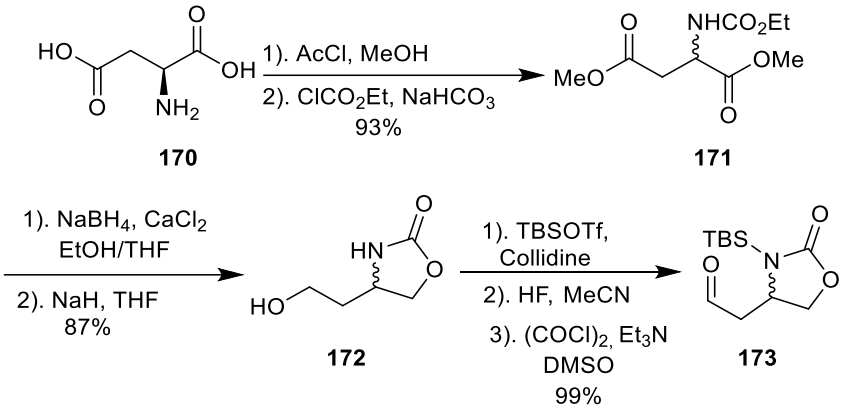


Figure 1. 6 The four possible direct acetal-free Tn analogues; the C-glycoside and carbasugar derivatives of both the threonine and serine conjugates

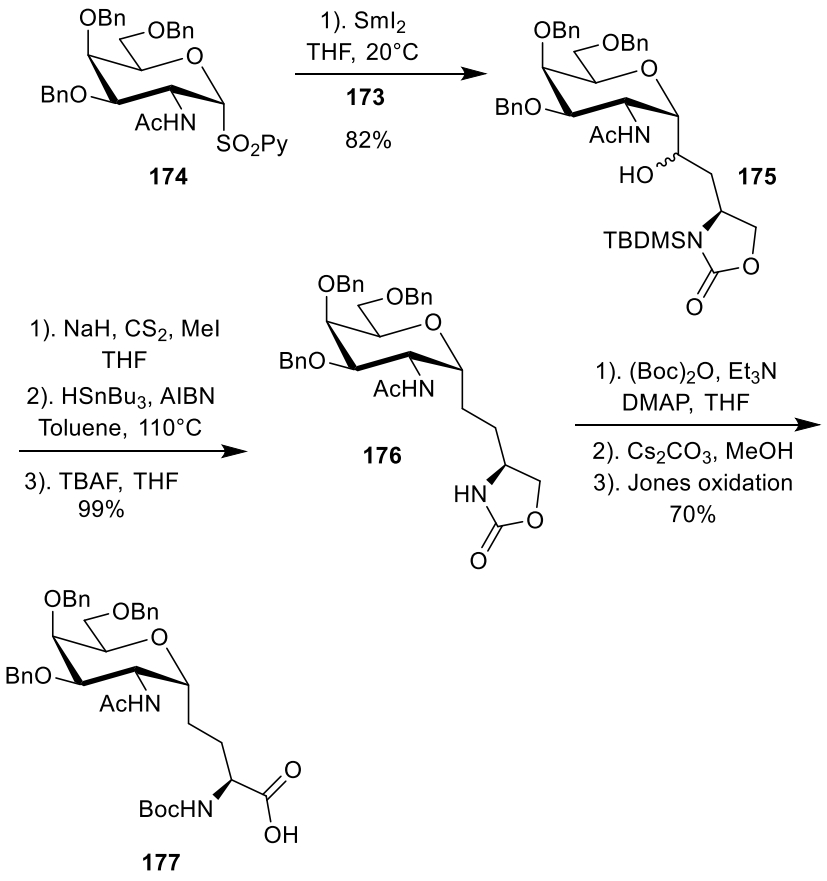
Beau and co-workers,[97] reported the first synthesis of the C-Glycoside derivative of the Tn antigen, which is only the second example of the preparation of C-2 aminohexose C-glycoside in the literature.[98] Synthesis of this compound initiated with the formation of serine- analogue amino acid components, starting from L-aspartic acid **170 (Scheme 1.10)**. This compound was selectively protected with a carbamate, cyclized in two steps, and then

deprotected and replaced with a TBS group. Finally, the terminal alcohol was oxidized to form the desired compound **173**. This report indicates that having a TBS protecting group is necessary to afford the aldehyde coupling partner **173** with an optimal yield.



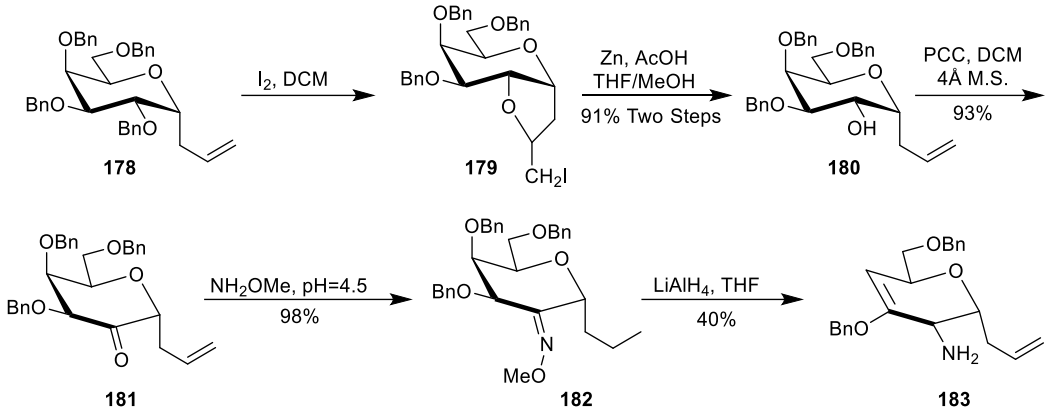
Scheme 1. 11 Beau's synthesis of the amino acid required for samarium-mediated C- glycosylation

After a coupling reaction between the aldehyde **173** and the glycosyl pyridyl sulfone **174**, which was prepared using an azido nitration sequence from tri-*O*-benzyl galactal, a mixture of diastereomeric alcohols **175** was obtained.[99] The hydroxyl group was removed *via* a tin hydride reduction after conversion to their respective xanthates. Consequently, the first reported hydrolytically stable C-glycoside Tn antigen mimics were obtained in the four additional steps. Lastly, TBS was replaced with a carbamate Boc protecting group, followed by a ring-opening reaction *via* cesium carbonate and a subsequent Jones oxidation, generated the desired product **177 (Scheme 1.12)**.



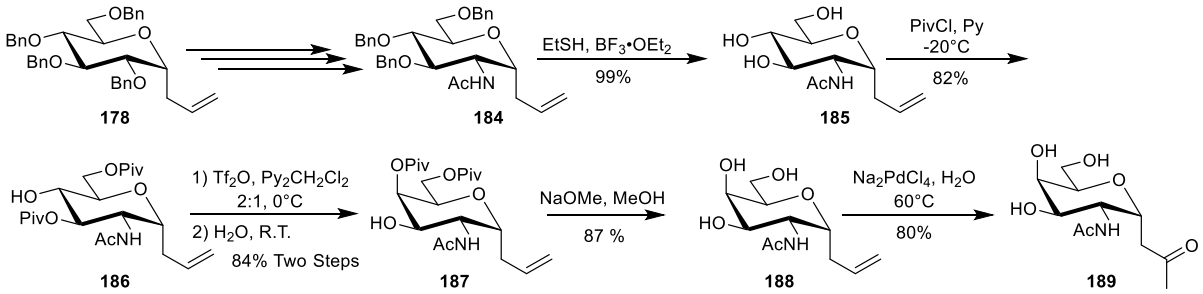
Scheme 1. 12 Beau's synthesis of the Tn antigen

In 2000, Cipolla and co-workers reported[100] the synthesis of C-glycoside analogues of *N*-acetylgalactosamine through a synthetic strategy based on their previous work with the mannosamine and glucosamine derivatives.[101] The reaction of previously reported allylated C-glycoside, compound **178**,[102] with iodine affords cyclic iodoether **179 (Scheme 1.13)**. Compound **179** undergoes a ring opening with Zn in acetic acid followed by selective deprotection of the C-2 hydroxyl to prepare **180**. Compound 1**83** was afforded in a moderate yield *via* oxidation followed by oxime formation (compound **182**) using NH_2OMe in acidic media, and finally, reduction using $LiAlH_4$. However, the oxime reduction in these systems always proceeded with an associated elimination of the C-4 benzyl alcohol which would require multiple challenging manipulations to re-install.



Scheme 1. 13 Cipolla's Initial approach to an *α*-C-glycosidic analogue of *N*- acetylgalactosamine

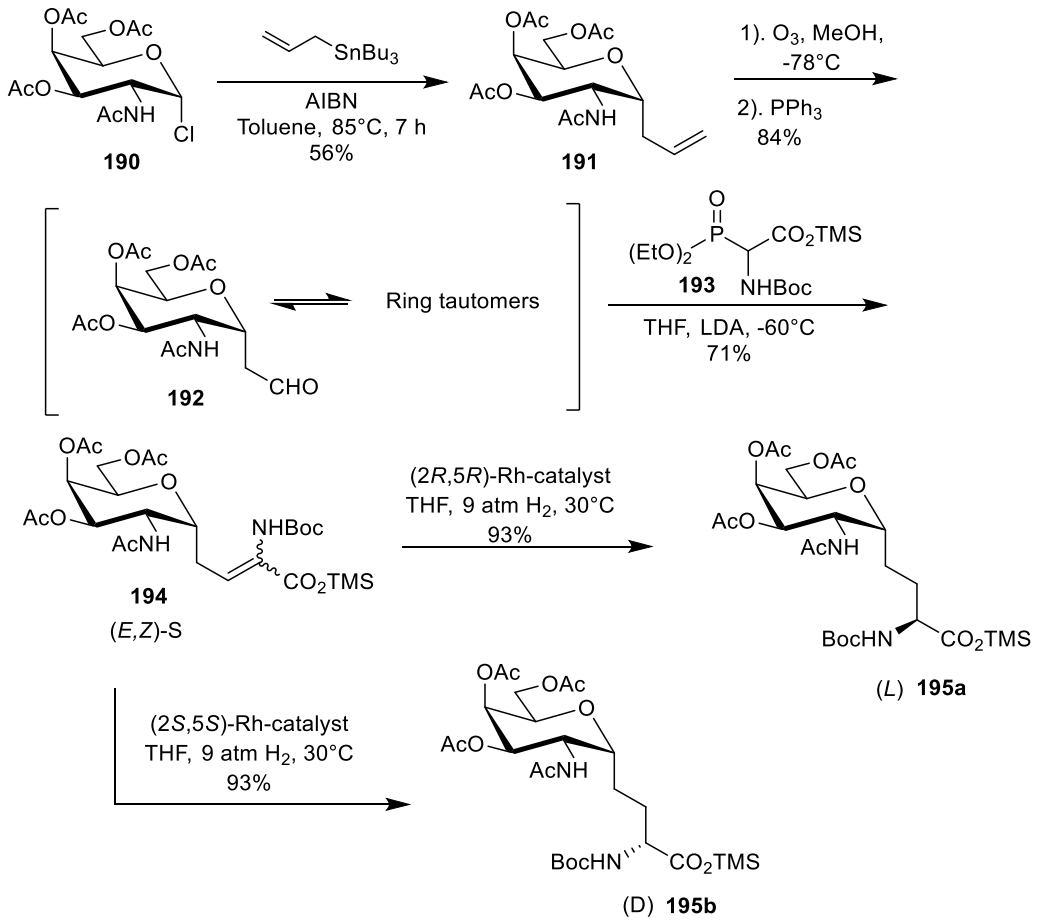
In a revised approach, Cipolla and co-workers synthesized a Tn building block starting from simple D-glucose. Although, the starting material is cost-effective, this approach is step-intensive which drives up the overall cost of the synthesis. In the revised approach the starting material glucosamine hydrochloride was readily converted to C-allyl-glucoside **178**,[87] then compound **184** was formed according to the previous report,[101] followed by the benzyl deprotection to generate **185 (Scheme 1.14)**. Hydroxyl groups at the C-4 and C-6 positions of **186** were protected using trimethyl acetyl chloride (PivCl) in order to convert glucosamine to galactosamine with the assistance of triflic anhydride, pyridine, and water in two subsequent steps. Finally, the methyl ketone **189** afforded *via* the Zemplén deprotection protocol followed by the oxidation of the allyl group using sodium tetrachloropalladate in water.



Scheme 1. 14 Cipolla's revised approach to an α-C-glycosidic analogue of *N*-acetylgalactosamine

A different approach was applied by Schmidt and co-workers for the synthesis of the Tn mimic using a Horner-Wadsworth-Emmons olefination instead of using a samarium coupling as the key conjugation step.[103] Although this approach was efficient and effective with a high degree of stereoselectivity, the ultimate synthesis of the exact analogue of the natural Tn antigen motif (which involves adding further synthetic steps and applications of this compound as an anti-cancer vaccine), remains to be explored.

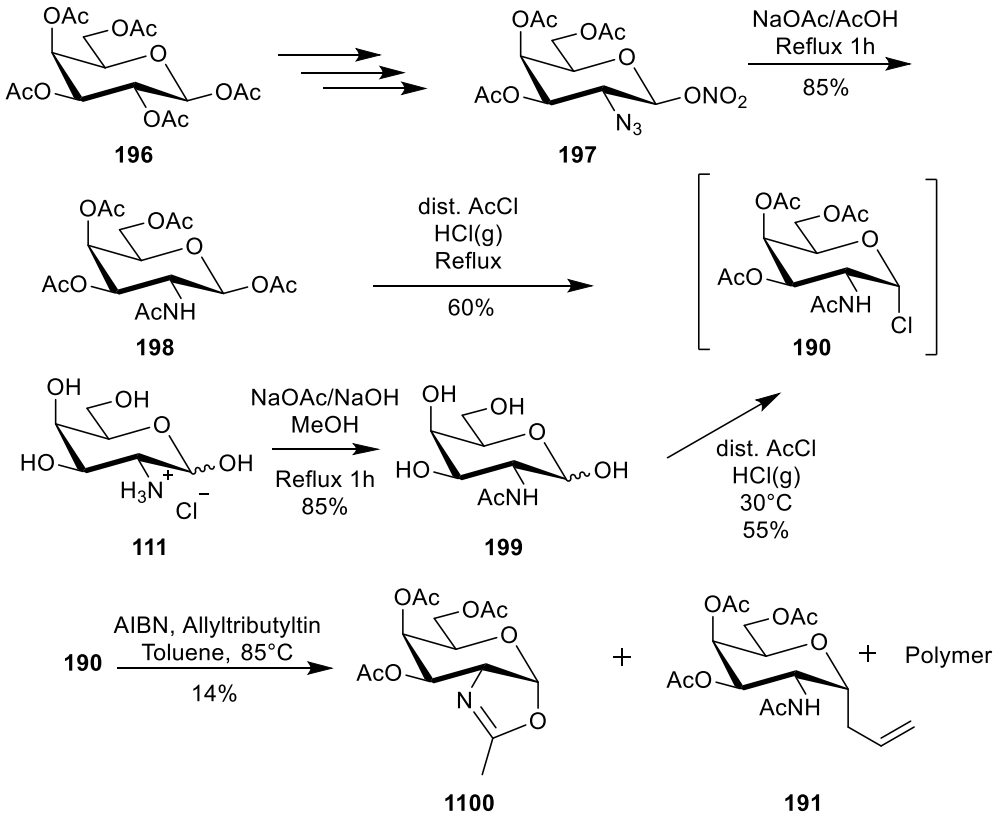
Galactosamine hydrochloride was used as a starting material, which in the first step was converted to 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl chloride **190** according to the known procedure reported by Jeanloz.[104] Aldehyde **92** was formed after an allylation using AIBN and allyl tributylstannane was then followed by the ozonolysis process, generating **192**. A Horner-Wadsworth-Emmons olefination applied between aldehyde **192** and glycine-derived phosphonate **193** resulted in a mixture of two diastereomers with good yield **(Scheme 1.15)**. An asymmetric halogenation performed using a chiral Rhodium complex catalyst to afford either the L-**195a** or D-**195b** amino acid in excellent 98:2 ratios depending on the choice of catalyst. It is worth mentioning here that using an alternative starting material seems be preferable as galactosamine hydrochloride is not readily available and is very costly. Ben and his group tried to address this problem using two different starting materials in two separate experiments; with both galactose and glucosamine hydrochloride investigated as potential galactosamine replacements.[105]



Scheme 1. 15 Schmidt's HWE-mediated approach towards the C-glycosyl analogue of the Tn antigen

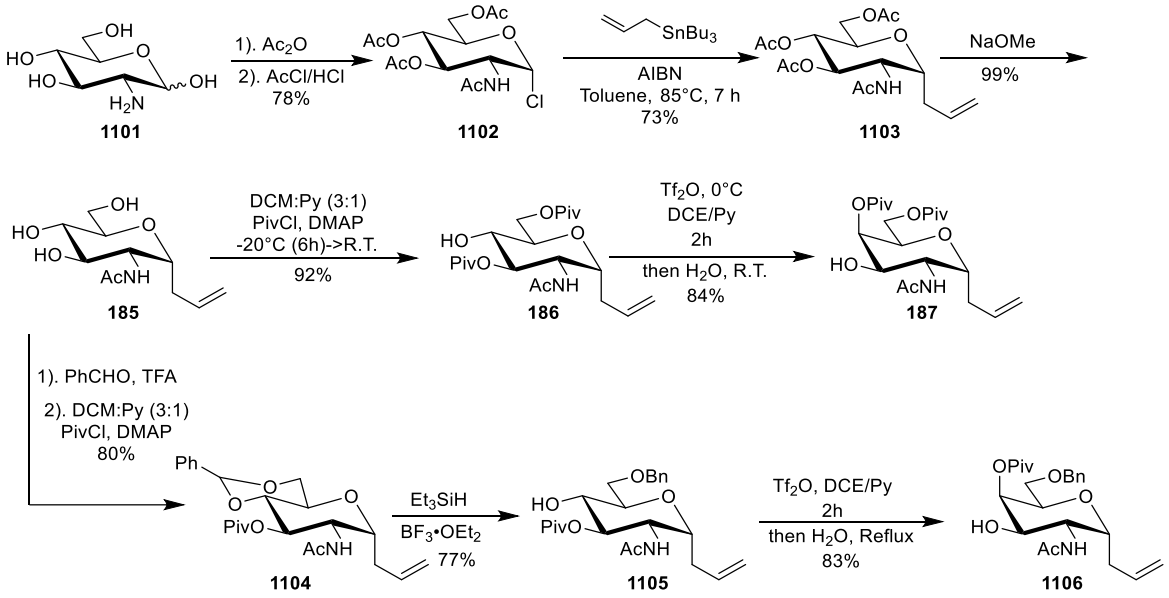
The first route, starting from galactose, did not afford an acceptable yield and turned out to be an inefficient alternative for previous approaches. As shown in **Scheme 1.16**, the nitroso azide **197** was obtained *via* three sequence steps: acetylation, galactal formation, followed by azidonitration. Consequently, peracetylated 2-Deoxy-2-acetamido-α-D-galactopyranose was afforded using sodium methoxide and acetic acid. Although the chlorination step was done successfully using HCl gas and acetyl chloride, allylation only provides a 14% yield regardless of the previous success of Schmidt with this transformation. This low yield was explained by the presence of minor impurities. A more direct route was then

applied to examine the viability of this approach. The 2-Acetamido-2-deoxy-D-galactopyranose obtained from selective acetylation of galactosamine hydrochloride and then converted to per-acetylated α-chloro galactoside **190** using the same chlorination protocol in their previous method. Finally, they tried allylation, which again failed to provide more than a 14% yield. The result indicated that oxazoline **1100** and a polymer is always favoured over the desired allylated compound. The Bromo-substituted pyranose was examined to improve the yield of this reaction, despite obtaining a slightly better result, the purification step seemed to be extremely challenging.



Scheme 1. 16 Ben's initial approach to *N*-acetyl C-allyl galactosamine **191**

After those unsuccessful attempts, Ben and Bouvet began with inexpensive D-glucosamine **(Scheme 1.17)**.[105] The same acetylation and chlorination protocol were implemented, followed by an allylation resulting in good yields using AIBN and allyl tributyltin in toluene. The rest of the procedure followed as Cipolla outlined above.[100] After deacetylation, the glucosamine was converted to galactosamine **187** using a Pivoyl protecting group, TF_2O, and water, respectively. In the parallel procedure, compound **1106** was obtained in five steps from **185**. Subsequent protection of both alcohols at C-4 and C-6 respectively with benzylidene protecting groups were subsequently followed by a protection of the C-3 hydroxyl group with PivCl, and therefore, afforded compound **1104**. Continuing on, compound **1106** was obtained *via* a selective benzylidene deprotection, followed by the conversion of the glucosamine analogue to the respective galactosamine analogue through the previously reported procedure using TF_2O and water.



Scheme 1. 17 Ben's alternate approach to differentially protected *N*-acetyl C-allyl galactosamine derivatives 1**87** and 1**106**

Consequently, both intermediates **187** and **1106** may therefore, be synthesized in 8 and 6 steps, respectively: with both high yields and selectivity, using either Beau or Cipolla chemistry. This represents rapid, efficient, and cost-effective routes for the syntheses of these useful building blocks.

Finally, we will conclude this section by discussing the synthesis of conformationally-restricted acetal-free Tn antigen mimics by Peregrina and co-workers.[106] These C-glycoside bicyclic systems are designed to investigate the effect of the carbohydrate component of antigens on the cancer cells; particularly, the interactions between lectins and the Tn antigen. The lectin-antigen interaction may have a destructive impact on cancer metastasis and therefore, interfering with this process may show some therapeutic effect.[106]

The bicyclic compounds prepared by Peregrina and co-workers are in a locked conformation which force the carbohydrate into a similar conformation as the energy minima for the natural Tn antigen. In these compounds, the methyl group is eliminated from the acetamido group of GalNAc and as a result, the amide is locked into a *cis*-geometry. Peregrina prepared three structurally relevant bicyclic acetal-free Tn antigen analogues to provide additional information. In those three compounds: **1107** and **1108** in **Figure 1.7**, the C-2 amido group and the anomeric positions are attached to the same α-carbon of the amino acid. The only difference between these compounds pertains to the carboxylic acid carbon of the amino acid in compound **1108**. This is present in the form of an alcohol functionality. However, in the other two members of the series, a carboxylic acid carbon is removed.

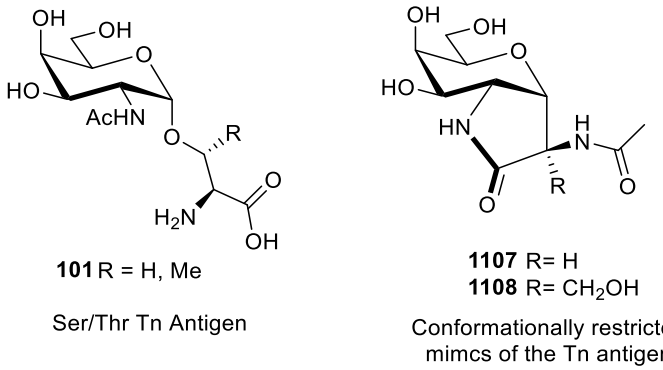
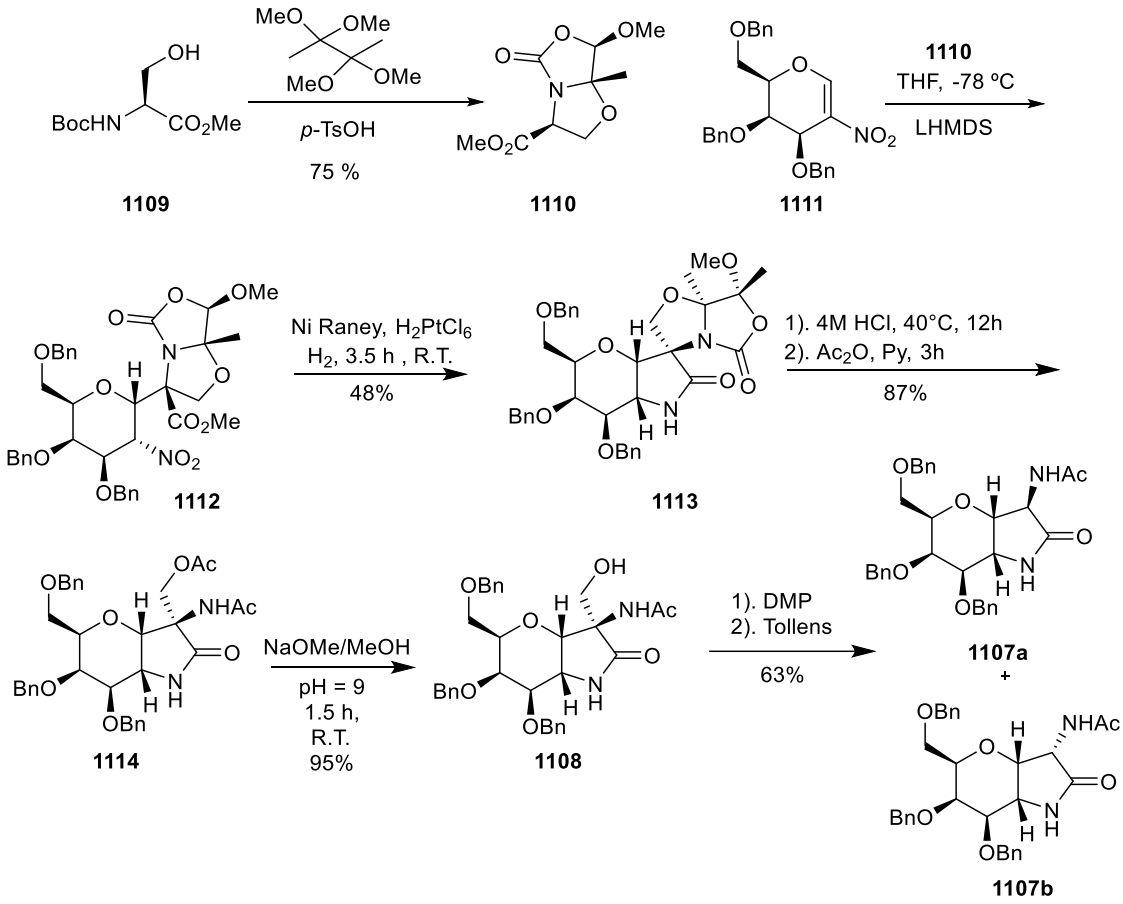


Figure 1. 7 Peregrina's targeted conformationally-restricted Tn antigens **1107** and **1108**, compared to the Tn antigen **101**

To begin the synthesis, the serine-equivalent C-nucleophile **1110** was prepared from (S)-*N*-Boc-serine benzyl ester in mostly a single diastereomeric form then underwent conjugate addition with a nitrogalactal derivative **1111** as a carbohydrate moiety in the presence of LHMDS (lithium bis(trimethylsilyl)amide). The functionalized tetracyclic **1113** was obtained *via* two subsequent steps. Initially, a reduction of the nitro group using a hydrogenation with platinized Raney nickel and an intramolecular cyclization resulted. Secondly, the acetal group was hydrolyzed, followed by the acetylation of the alcohols and finally, the amine-to- amide conversion produces compound **1108 (Scheme 1.18)**. Consequently, an isomeric mixture of **1107a** and **1107b** was prepared *via* oxidation and Tollens decarbonylation. These conformationally restricted Tn antigen mimics resulted in moderate bioactivity for the lectin-carbohydrate interactions.[106]



Scheme 1. 18 Peregrina's synthesis of conformationally-restricted Tn antigens **1107a** and **b,** and **1108**

In this section, many useful syntheses of acetal-free (C-glycoside) Tn antigen analogues were shown with promising leads for future synthesis. In the next section, we will focus on previous attempts towards the synthesis of the acetal-free TF antigen, which is much more challenging than the synthesis of the Tn antigen.

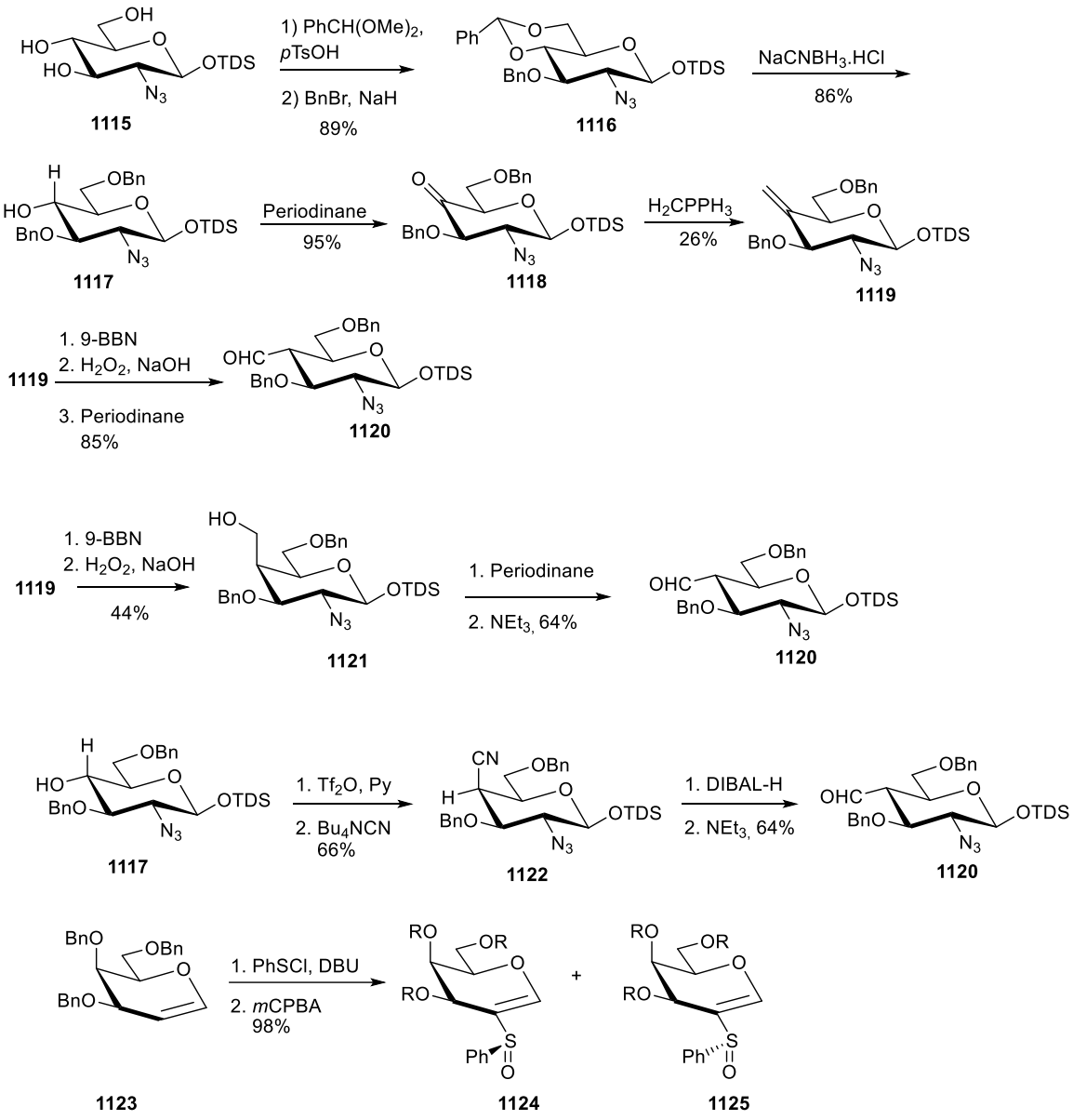
1.1.5.5 Synthesis of Acetal-Free TF Antigens

Another prominent and complex target, the TF antigen, which consists of the Tn antigen unit 1,3-β-linked to a galactose moiety, is a difficult synthetic target. The construction of a one carbon 1,3-β-linkage between two sugars, removing both the acetal group in sugar units of TF antigen and the attachment of the amino acid to the sugar moiety in the

α orientation are the three most critical challenges that chemists currently are trying to address. The first challenge facing chemists in the synthesis of the acetal-free TF antigen is to form a one-carbon 1,3-β link between the galactose and the galactosamine moieties. The previous research suggests that the best biological result would be achieved using acetal-free antigens that are structurally quite similar or exactly resemble the natural antigen with peptide chain linked to an amino acid moiety.[67] Consequently, the construction of a one carbon linkage with a β orientation between the two sugar moieties is essential. At the same time, this requirement could present significant challenges to the synthesis of the TF antigen. The stereoselective formation of the second C-glycoside in the galactosamine moiety is the second obstacle to obtaining a fully acetal-free TF antigen. Due to this challenge there is no example of a fully acetal-free TF antigen in the literature. Although the third challenge seems to have been resolved by Beau and Schmidt, which are the only two reported ways to install an α-oriented amino acid onto the sugar moiety in the syntheses of Tn antigen derivatives, these approaches use samarium iodide chemistry or Horner–Wadsworth–Emmons chemistry, which require specific synthetically challenging precursors.[97, 103] These three challenges are most likely the main reasons why there is no example of the synthesis of a fully acetal-free TF antigen analogue in the literature to date. The formation of an aldehyde functionality seems to have attracted the majority of attention amongst the chemists as both an electrophile, and an essential precursor in the one-carbon coupling strategy between two sugars. Schmidt reported the first attempt to solve the first challenge, installing a one-carbon linkage between two sugar moieties, however, this linkage did not have the same connectivity as native TF antigen.[107]

Aldehyde **1120** was synthesized from two separate precursors to find optimized conditions, see Scheme 1.18. The first precursor, **1119,** was synthesized through the Wittig reaction from **1118**, which itself was obtained from **1115** in three steps:[108] protection, selective deprotection, and oxidation. The compound **1119** was then subjected to a hydroboration to afford a mixture of epimers in a 2:1 ratio of the glucose (not shown)-galactose **1121** diastereomers. These two diastereomers were separated by column chromatography and oxidized separately. The undesired α-aldehyde (not shown) was epimerized with triethylamine to afford a 2:1 ratio of glucosyl-galactosyl aldehydes. The process could be subsequently repeated to drive the material towards the desired product. This process was not an efficient route however, because of a poor yield in the olefination step **(Scheme 1.19)**.

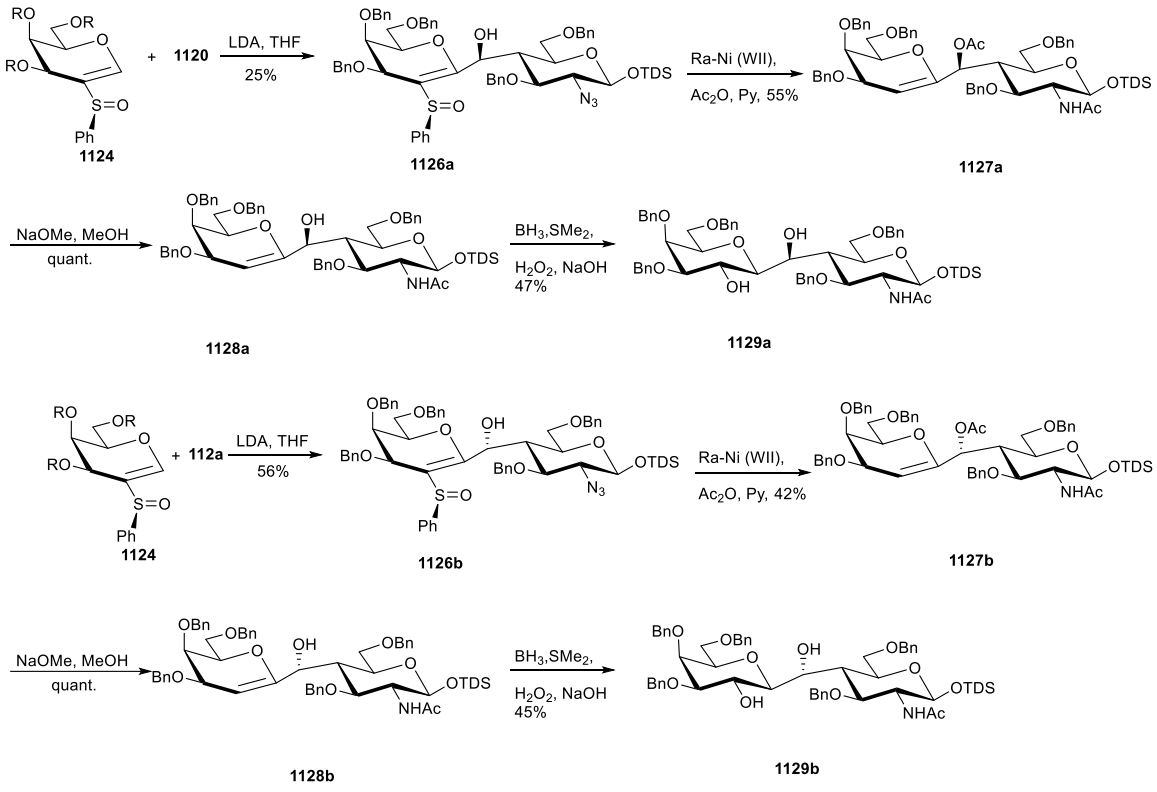
An alternative method was examined using compound **1117**, which was converted to the aldehyde through a cyanation followed by an aldehyde formation. Alcohol **1117** was treated with triflic anhydride in pyridine, followed by tetrabutylammonium cyanide to replace alcohol with nitrile functionality in an S_N2 fashion. A reduction of the nitrile group with DIBAL-H, followed by epimerization with triethylamine was performed to generate the desired aldehyde **1120** in good yield. The coupling partner, nucleophile **1124** was formed from D-galactal **1123 (Scheme 1.19)** and was subsequently coupled with aldehyde **1120.**



Scheme 1. 19 First steps for Schmidt's synthesis of a C-linked *N*-acetyllactosamine

After synthesis of the vinyl sulfoxides, two epimeric hydroxyl methylene-bridged C-disaccharides (**1126a** and **1127b**) were obtained through an LDA deprotonation of the vinyl sulfoxides, followed by nucleophilic attack to the aldehyde. These compounds were successfully separated in parallel syntheses and subjected to an azide reduction using Raney nickel to generate a free amine, which, upon *in situ* acetylation, obtained **1127a,b**.

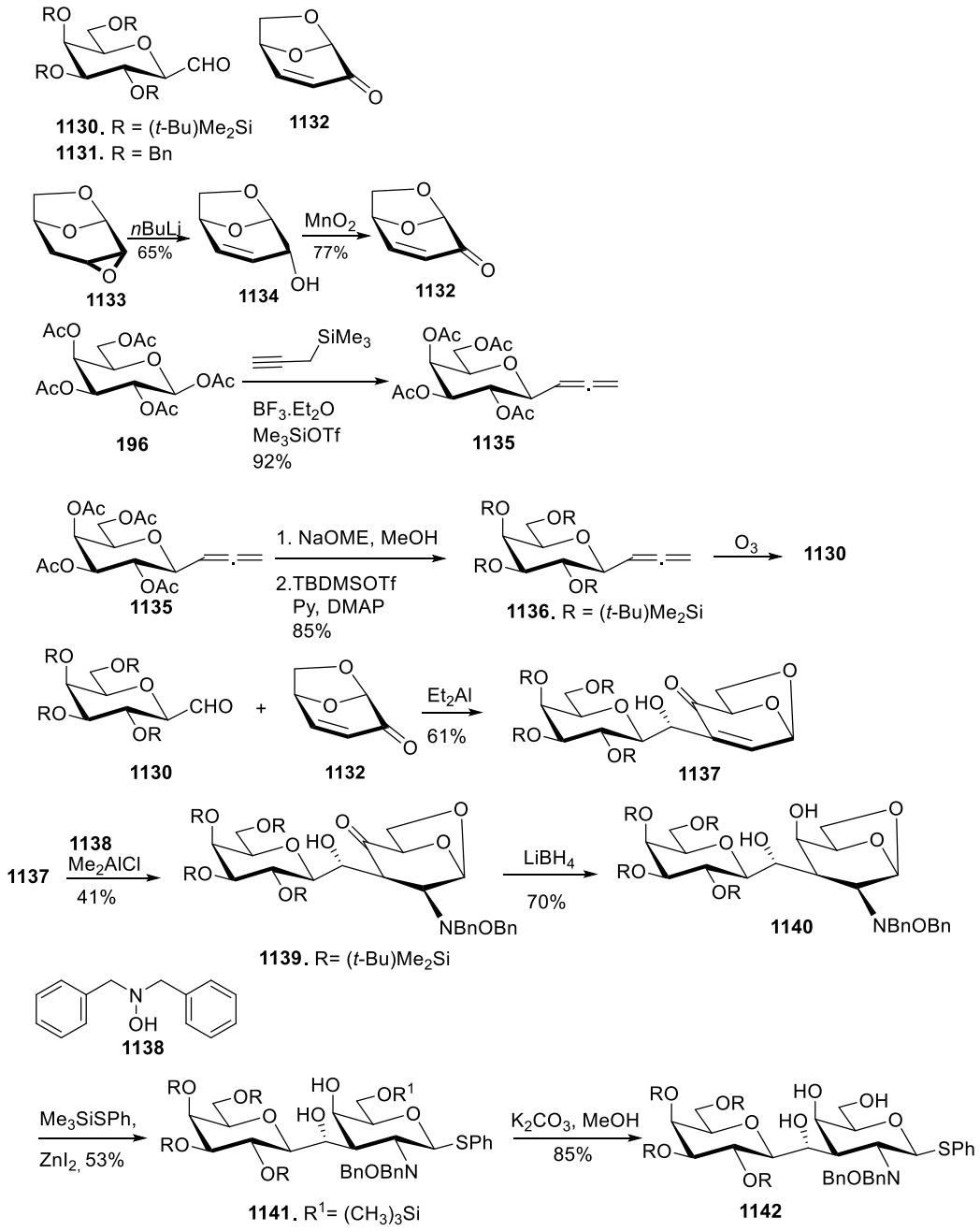
The alcohol was deprotected *via* Zemplén deacetylation, then hydroboration was performed to produce compounds **1129a,b**. It is worth mentioning that, although the hydroxyl group in the bridge was not removed in this synthetic step, the corresponding chirality and differentiation between the two epimers were furnished using *O-iso*-propylidenation of **1129a,b** with 2,2-dimethoxypropane in the presence of acid. The protection of the *R* isomer with 2,2-DMP led to the generation of a 6-membered ring with the glucosamide residue, lying in the equatorial position of a typical chair conformation. On the other hand, the *S* isomer could not adapt a chair conformation and therefore rearranged towards a boat conformation to minimize the steric demands (not shown here) **(Scheme 1.20)**.



Scheme 1. 20 Completion of Schmidt's synthesis of a C-linked *N*-acetyllactosamine

Vogel was the first chemist to accomplish the synthesis of a *β*- D-galactosyl-1,3 carbon-linked to 2-deoxy-2-acetamido-galactose using Baylis-Hillman chemistry, which is

the earliest reported formation of a 1,3-β carbon linkage between galactose and galactosamine components (**Scheme 1.21)**.[109, 110] The coupling partner of isolevoglucosenone was synthesized according to a known procedure by Horton.[111] The Compound **1132** was furnished in three subsequent steps, epoxidation, hemiacetal formation, and lactone formation using manganese dioxide from the anhydro sugar glycal **(Scheme 1.21)**. The second Baylis-Hillman coupling partner was prepared from peracetylated galactose by replacing the acetyl group at the anomeric position with an allene group using propargyl-TMS and a superacid consisting of and TMSOTf and BF_3•OEt_2, followed by replacement of the acetyl protecting group with a TBS group. Finally, the allene functionality underwent oxidative cleavage with ozone to afford the β-anomeric aldehyde **1130**. The Baylis-Hillman reaction assisted by diethyl aluminum iodide was exploited to provide the C-glycoside **1137**, primarily as one diastereomer, from the coupling components. Compound **1137** was subjected to Michael addition to introducing the nitrogen source in the C-2 position using *N*,*O*-dibenzyl hydroxylamine, and dimethyl aluminum chloride as a Lewis acid catalyst. An undesired imine functionality accompanied by 1,4 conjugate addition was observed when they used *O*-benzylhydroxylamine as a nucleophile. The glycosyl donor **1142** was then successfully prepared in three steps. First, selective reduction of the ketone was performed with $LiBH_4$ as the rest of the common reducing agents failed to produce the desired absolute galactose configuration product. Second, a cyclic acetal cleavage with an SPh group and subsequent C-6 hydroxyl protection with a TMS protecting group was carried out using ZnI_2 and Me_3SiSPh. Finally, desialylation of the C-6 position afforded desired product **1142**.



Scheme 1. 21 Synthesis of the disaccharide component in Vogel's synthesis of a C-linked *N*-acetyllactosamine (R= TBS)

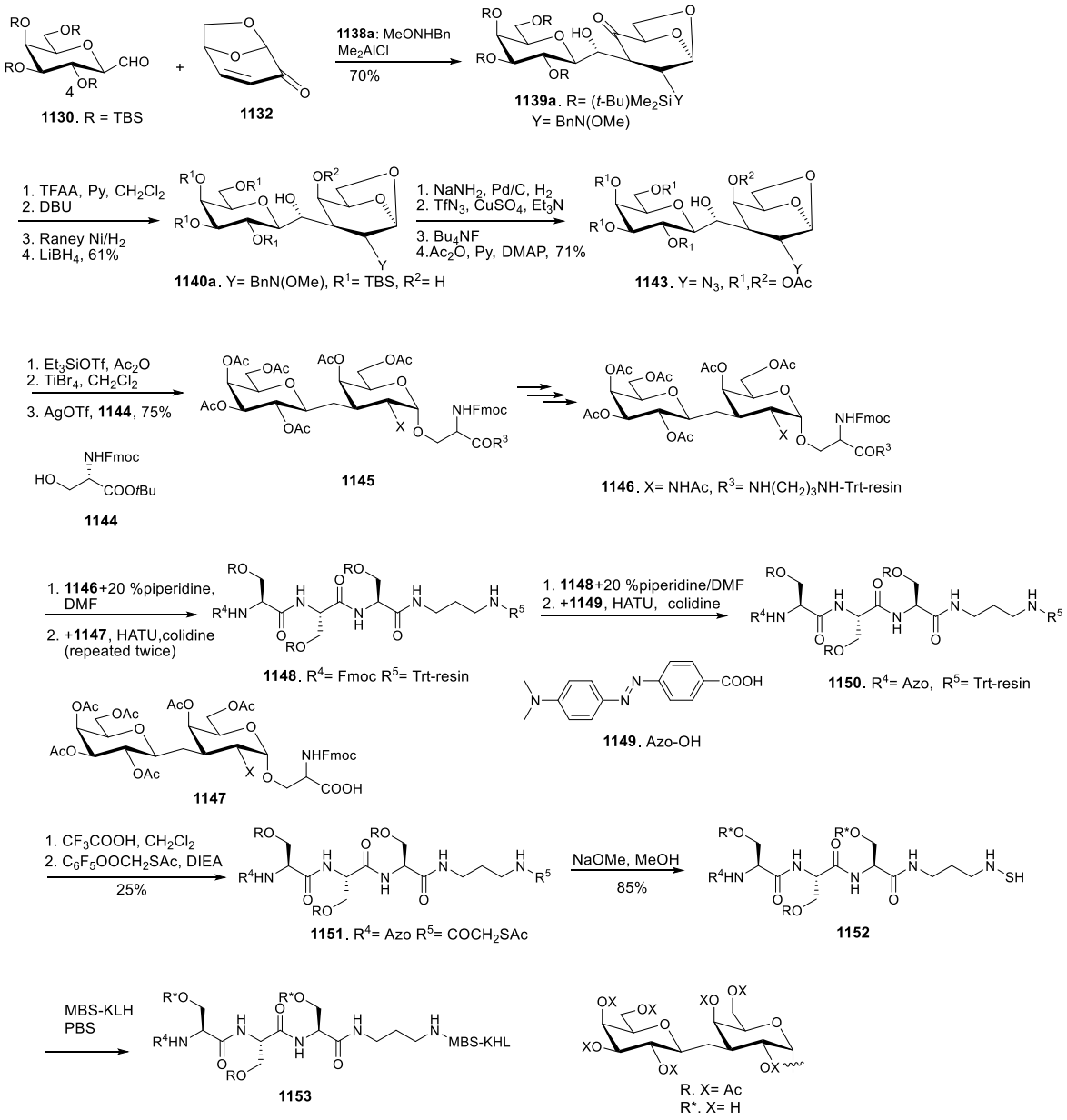
A few years later, Vogel reported the second-generation synthesis of a C-linked disaccharide using the Baylis-Hillman approach. In this research the authors investigated C-linked disaccharides of the TF antigen as potential anti-cancer vaccine candidates.[66] To

determine the effect of the C-anomeric linkage between the two carbohydrates in the immunogenicity of the antigen, both anomeric α and β disaccharides **1137** and **1137a** were synthesized using the same Baylis-Hillman approach between isolevoglucosenone and both anomers of TBS-protected C-1 aldehyde **1130** and **1154**.

A Michael addition was performed to introduce an amine source in the C-2 position using *O*-methyl hydroxybenzylamine. The hydroxyl group in the bridge was then removed using TFAA and DBU to generate the acyclic enone, which was then reduced by Raney Nickel, followed by a selective reduction of the ketone to form an axial hydroxyl group at the C-4 position in a galactose configuration. As a next step, BnNOMe was reduced to an amine and then converted to the respective azide functionality using a diazo transfer, and compound **1143** was then formed by acetylation of the hydroxyl group at the C-4 position. Consequently, the glycoconjugate **1146** was successfully obtained in five steps. A cyclic acetal cleavage using triethyl silyl triflate/acetic anhydride, followed by bromination of the anomeric alcohol using titanium (IV) bromide generated the desired glycosyl donor. Koenigs-Knorr glycosylation was performed using Fmoc-Ser-*t*-Bu, followed by acetamide formation and *t*-butyl carboxyl deprotection, produced compound **1145.**

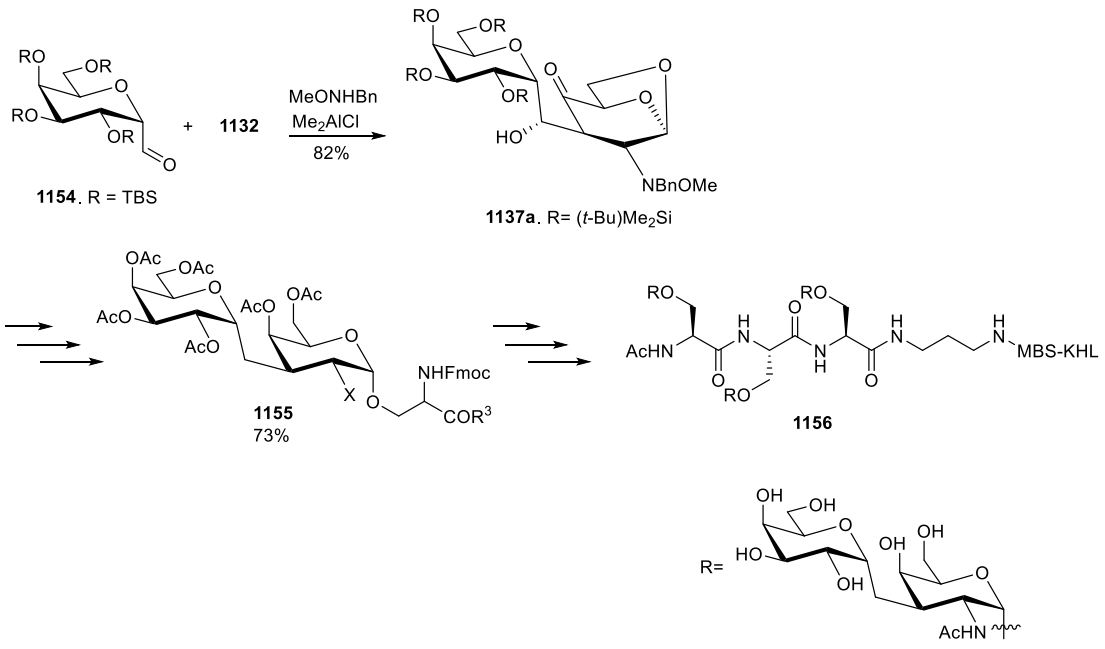
The solid-supported core, compound **1148**, was then prepared by loading the glycoconjugate onto a solid support and an amino-functionalized trityl resin. The short tripeptides of repeating glycoconjugate monomers load three antigens per peptide resulting in an antigen-dense system, **1149**, which is prepared *via* solid-phase peptide synthesis. The diazo protecting group at the C-terminus of the peptide was installed to simplify the purification of **1150**, and the free glycopeptide was obtained after its cleavage of the resin under standard acidic conditions. The amino terminus was then attached to an acetylthioacetate, **1152,**

using pentafluorophenyl acetylthioacetate. A Zemplén deacetylation was then performed to generate a conjugate that could be linked to the carrier protein KLH mediated through an m-maleimidobenzoic acid *N*-hydroxysuccinimide ester (MBS), providing Antigen **1153 (Scheme 1.22)**.



Scheme 1. 22 Completion of the synthesis of **1153**, an acetal-free TF analogue

The same synthetic procedure was applied for the α-anomeric aldehyde, with only a few exceptions; The Itoh-Nozaki coupling procedure yielded the epimeric alcohols rather than the single major product. A flexible diaminopropane spacer is used to conjugate the tripeptide to the resin in the synthesis of the TF β-mimic. In this second case, a more rigid *p*-xylylenediamine linker was exploited to provide a suitable chromophore to help in HPLC detection during the purification. The antigen **1156** was then prepared using peptide synthesis, followed by hydrolysis of the resin, and direct protection of the *N*-terminus as the acetate and ligation to the KLH-MBS provides the glycopeptide **1156 (Scheme 1.23)**.



Scheme 1. 23 Completion of the synthesis of **1156**, an α-anomer version of the acetal-free TF analogue

Although the first synthesis of a mono C-glycoside disaccharide toward the synthesis of an acetal-free TF antigen was accomplished, many obstacles still remain. For example, the *O*-glycosylic linkage between the glycan and the peptide remained in the molecule, which makes the molecule vulnerable towards enzymatic or acidic-mediated hydrolysis *in*

vivo. Second, an extended multi-step synthesis is required for the generation of these compounds, and an effort to reduce the step count is necessary. Third, despite the positive biological results of this research, regarding producing a robust immune response, as determined by serum immunoglobulin G concentrations, the specificity of these antigens towards cancer cells has yet to be conducted and biological testing has to be performed on the corresponding C-glycoside TF antigen analogue and actual natural TF antigen for a useful comparison.

1.1.6 Biological Conjugation and Biological Evaluation of Acetal-Free Mimics

The immunogenicity of natural TACAs is usually low because the immune system cannot recognize the sugar, and there are sugars all over the surface of these tumours. As a result, these antigens cannot activate T-cells, and therefore generate a T-cell-independent immune response. Consequently, antibodies generated from the immune system will be weak and will not stay in the body for a long time. The low stability of TACAs is another major challenge that should be solved to get an adequate response from the immune system to eliminate these cancer cells. Glycosidases are mostly responsible for the cleavage of the glycosyl functionalities from peptides as part of the antigen-processing process, which are present in human serum and the liposomes of antigen-presenting cells.[65,112] In this section, conjugation and biological evaluation of acetal-free mimics will be discussed.

1.1.6.1 Tn Antigen

The C-glycoside derivative of the Tn antigen was prepared by Cipolla for the immunological evaluation, which is described above. Based on a general supposition, an ideal carbohydrate-based vaccine candidate should express three main features: they should have the ability to activate B-cells, they should have the ability to activate T-cells to strongly

activate the immune system, and should include an adjuvant to boost the immune sensitivity to the candidate vaccine.[69]

In order to meet these features the OVA peptide, a T-cell epitope which is readily recognized by the immune system of DO11.10 mice, was chosen for this research.[113] The protected hydroxylamine-containing peptide was obtained *via* solid-phase peptide synthesis using a Fmoc/*t*-butyl protection approach.[68] The linkage was cleaved using standard cation-scavenging conditions to afford the deprotected peptide that was incubated with the glycosyl ketone in a pH 4.5 acetate buffer for 90 minutes and monitored by RP-HPLC to produce a mixture of the syn and anti-oxime isomers. Although the Major histocompatibility complex (MHC) found on the surfaces of cells, which are the class of genes that code for proteins that assist the immune system in recognizing foreign treats recognizes the isolated neo-glycopeptide to the same degree as the peptide aglycon, the oxime linkage does not resemble a natural glycopeptide linkage. They mentioned that the vaccine did not require internalization to bind with the MHC receptor, they postulated that it maybe because of the competitive displacement of the antigen interacting with surface-localized receptors. The result showed that T-cell activation of both the hydroxylamine aglycon and neo-glycopeptide were reduced compared to the native unmodified peptide. Thus, the unnatural linkage interferes with either MHC-binding or T-cell receptor (TCR) activation.

A few years later, Cipolla reported the synthesis of mono and divalent neo-glycopeptides with modified peptide fragments,[114] as the previous sequence, does not precisely match the antigenic sequence proposed by the NCBI and is shifted by an additional residue.[115, 116] Both neo-glycopeptides are shown in **Figure 1.8**. The results indicate that the new peptide could interact with the MHC, and the oxime successfully survives antigen

processing. The successful survival of antigen processing occurs when the peptide is introduced into an unfixed compared to fixed dendritic cells similar to the aglycons and in turn. As a result of this interaction the TCR activation was improved.

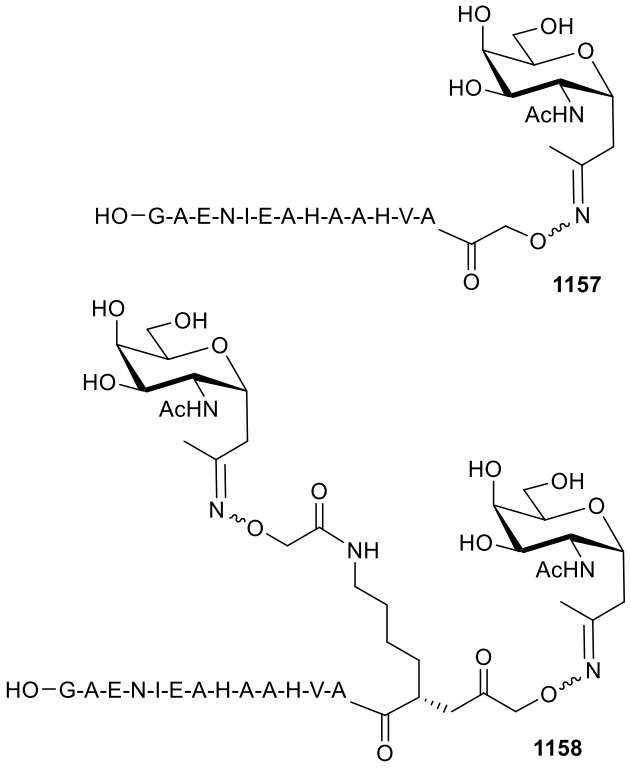
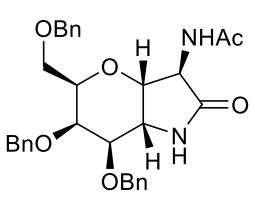


Figure 1. 8 The structure of the revised neoglycopeptide **1157** based on the OVA peptide, and the divalent analogue **1158**

It is worth mentioning that the divalent system showed a more robust TCR activation which may be due to crosslinking of dendritic cell receptors and suggests a multivalency effect may be operative.[91, 117-119] However, inter-trial reliability is not particularly strong, and the effect is only moderate with these types of cellular assays which precludes confirming the multivalency affect. The result indicated that the antigen with a new peptide sequence interacts with the MHC and therefore, the oxime successfully survived antigen processing. This occurs because, when the peptide was introduced into unfixed compare to fixed dendritic cells similar to the aglycons, the TCR activation was improved. Despite

exciting preliminary work, some key questions are left unanswered. First, the specificity of the native antigen towards cancer cells should have been evaluated. Second, as an immunological response is similar in magnitude for both the glycon and peptide alone, it is essential to determine if the glycon is actually responsible for an immune response or if it was only courtesy of the peptide alone. As a neo-glycopeptide linkage is different from the natural Tn antigen linkage, it is beneficial to compare these two antigens to understand the importance of introducing an oxime linkage rather than a traditional glycosidic linkage. Lastly, the examination of the effect of this mono-and divalent neo-glycopeptide on tumours *in vivo* remains an unanswered question.

The bicyclic Tn antigen mimics synthesized by Peregrina, which locks the conformation of the ring have been screened, which as mentioned above, this was examined in a competitive lectin-binding assay with a Tn-specific lectin and soybean agglutinin lectin.[120] Although, the bicyclic systems can interact with the lectin with approximately 80% of the efficiency of the Tn antigen, the lectin preferred the natural antigen rather than the bicyclic forms and their enantiomers in competition experiments. Computational simulations rationalized this lower recognition due to the possible hydrogen-bonding interactions between the ligand and the carbohydrate-binding site of the lectin **(Figure 1.9)**. Finally, it should be mentioned that the Tn antigens synthesized by Ogawa, Beau, Ben, and Schmidt, as mentioned above, were not examined for their biological activity as Tn antigens.



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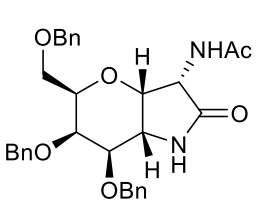

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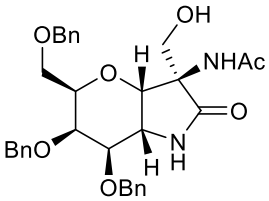

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Figure 1. 9 The structures of the conformationally restricted Tn antigens examined for lectin-binding affinity by Peregrina

1.1.6.2 TF Antigen

Not many syntheses of analogues of the natural C-glycoside TF antigen exist to date. It is noteworthy that there is no reported synthesis of a fully acetal-free analogue of the TF antigen yet. Partial analogues, the mono C-glycoside TF antigen α-*O*-linkage to the serine building block, as mentioned above, was first reported by Vogel and Awad.[110] However, even this compound was never examined for biological applications. Seven years later, they reported the synthesis of the clustered glycopeptide as both linkages between two sugars, the α (unnatural) and β (natural) anomers with galactose amine α-*O*-linked to the serine building block.[66]

These antigens were constructed with the belief that the TACA would activate the B-cells, and the KLH would activate the T-cells. This compound was accompanied by the carbohydrate adjuvant QS21,[121, 122] and applied against BALB/c adult mice. This particular strain of albino mice is frequently chosen for immunological studies because they of their generally potent immune response.[23] The structures of the two vaccines is shown in **Figure 1.10**. As expected, the α-linkage did not generate significant an immune response, as it is not the same analogue of natural TF antigen. However, the β-isomer provided a robust immune response, as determined by serum immunoglobulin G concentrations. The result indicated that the serum antibodies induced by the β-vaccine were also specific for the β-glycopeptide, and it could not recognize the α-glycopeptide.

Despite the response of the immune system towards the KLH carrier protein as expected, a few antibodies induced by α vaccine, could not recognize either the α or the β-glycopeptides. For this reason, the immune response of the α-vaccine was not examined

against the natural antigen. Finally, this C-glycoside analogue of the TF antigen is not an entirely acetal-free antigen. In order to reach the maximum potential of this antigen (by increasing biostability), a synthesis of fully acetal-free TF antigen seems to be necessary.

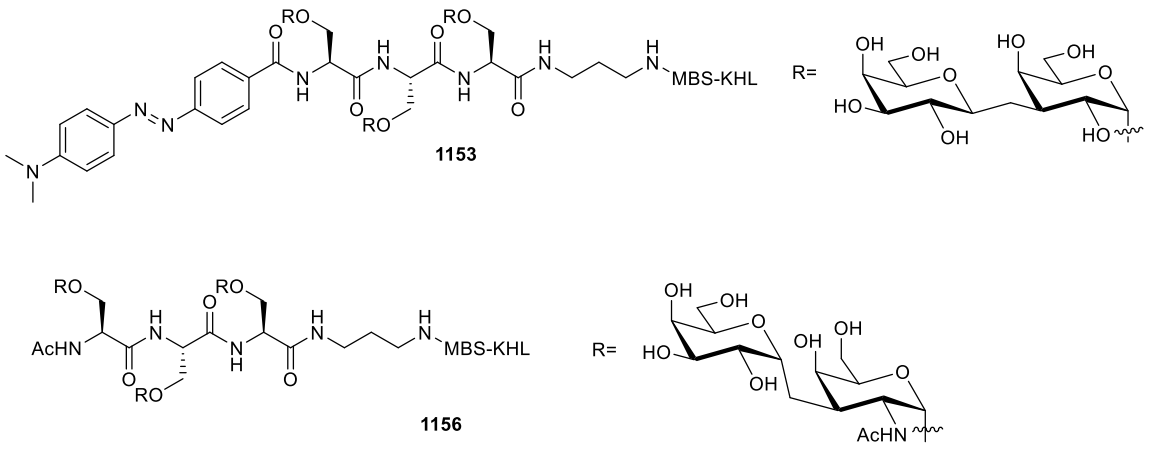


Figure 1. 10 The structures of the trivalent vaccine TF vaccine candidates investigated by Vogel

1.1.6.3 sTn Antigen

Synthesis of an acetal-free sTn antigen have attracted the attention of chemists and biologists because they belong to the TACAs antigen class, which are expressed in many cancers, including breast, esophageal, gastric, prostate, lung, and colon cancer. In this section, only the biological activity of the C-glycoside analogues of these compounds are discussed because they are the only ones showing a 1:1 comparison to the TACA immunogenicity of a *C*- and an *O*-linked carbohydrate antigen. The synthesis of these compounds is not discussed in this thesis.

Linhardt synthesized the double C-glycoside and mono C-glycoside sTn antigens and applied them to the same protocol in the one described above by Vogel, for the measurement of the IgM and IgG titres.[67] The carbohydrate in the sTn antigen is not part of a

glycopeptide or neo-glycopeptide as above and makes it an unusual mucin antigen. Although the *O,C*-disaccharide **1159b** (**Figure 1.11)** showed the highest immune response, the sugar-amino acid *O*-linkage remained susceptible to enzymatic cleavage. This is not surprising as one acetal group is still present in the molecule. The *C,C*-disaccharide **1160**, which was expected to show better immune activity than *O,C*-disaccharide, proved to be less potent immunologically. To explain the lower immune activity of the fully acetal-free disaccharide compared to the *O,C*-disaccharide we note that the structure of the *C,C*-disaccharide was not a direct analogue of the native *O,O*-antigen. The linkage between the two sugars is one carbon short. Also, the hydroxyl functionality introduced to the bridge is a mismatch, which changes the hydrogen bonding and solvation properties of the antigen. Despite the remarkable results showing a direct comparison of *O,C*-disaccharide, the sTn antigen analogue versus the native sTn antigen, and its better immunogenic response, **(Table 1.2)** the specificity of this antigen towards cancer cells and the antibodies produced by the immune system remains unclear. Consequently, these issues remain to be addressed before the application of TACA antigens can be fully realized.

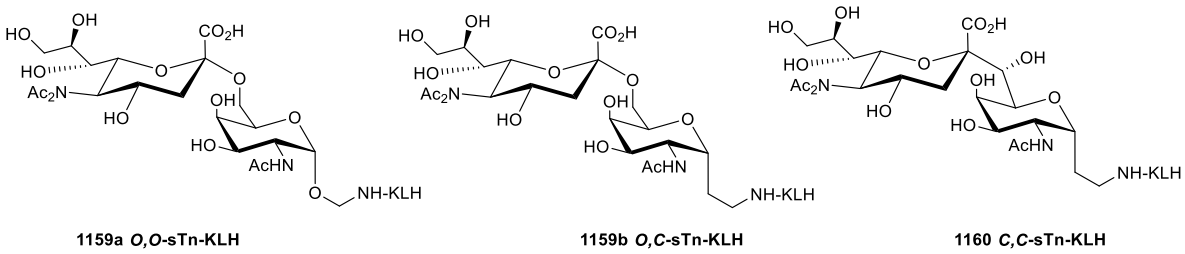


Figure 1. 11 The structures of Linhardt's differentially linked sTn antigen analogues

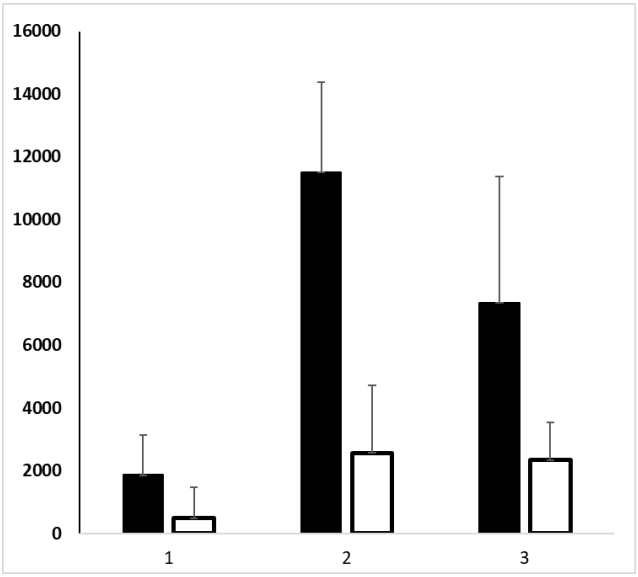


Table 1. 3 Antibody titres from BALB/c mice immunized thrice with either 1 μg/mouse of (O,C) or 10 μg/mouse of (C,C or O,O) of the KLH conjugate. After 1 week, mice were sacrificed and serum IgG and IgM titres were measured through ELISA; error represents the standard deviation from 5 mice

1.1.7 Conclusions and perspectives

At first, the native antigens were targeted for anti-cancer vaccine applications. Despite remarkable preclinical data results reported by Danishefsky,[123] the following reports for the synthesis and application of clustered Tn and TF mucin candidates showed that the antibodies generated were specific for the natural antigens. Therefore, these candidates have not been introduced as commercial vaccines. A few years later the acetal-free carbohydrate, introduced to compensate for the low biostability of the O-linked carbohydrate in the synthesis of TACAs, showed better immunogenicity by a factor of 6. Although there are many ways to synthesize the C-glycoside and carbasugar, there are few reports of the biological application of these compounds. Furthermore, there is not a single example of immunological studies on carbasugar derivatives of TACAs, despite their potent immune activity when applied as glycomimetics in other systems.[124, 125] This is most likely because the structural complexity of these antigens makes their synthesis very tempting targets for chemists to showcase novel synthetic chemistry, rather than for their potential as vaccine candidates.

Consequently, there are three main questions in this field that remain to be addressed:

1) What is the reason for enhancing the immunogenicity of acetal-free candidates compared to natural antigens?

2) Are acetal-free analogues capable of inducing an antibody response that is specific for the glycopeptide conjugate?

3) Does the increased immune response observed for these acetal-free systems have an impact on tumour growth, and do these materials have potential as anti-cancer vaccines?

All the research done previously in this field has paved the path for the bright future, however, until research addresses these questions, we cannot realize that future of a real anti-cancer vaccine.

1.2 Total synthesis of glycolipids as antigens and the biological evaluation of their immunological activity

1.2.1 Invariant natural T killer cells

Invariant natural T killer cells (iNKT) are a subclass of white blood cells that use their T cell receptors (TCRs) interact with self and foreign lipids and as a result, capable of inducing immune system *via* a robust and rapid cytokine release.[126] These lipids are presented by CD1d, a non-polymorphic major histocompatibility complex class I-like antigen-presenting protein.[126] It is a member of the lipids antigen family that includes the CD1a, CD1b, and CD1c molecules.[126] Invariant natural killer T cells are conserved between humans and mice. These cells have been shown to be very useful and, at the same time, destructive in many pathological states, including autoimmune disease,[127] allergic

disease,[128] cancer,[129] and microbial infection.[130, 131] Activation of iNKT cell leads to producing a mixture of cytokines that often work against each other. Many significant efforts have been expended trying to uncover a better understanding of lipid antigen recognition by iNKT cell and TCR and interactions between iNKT cells and other leukocytes.

After activation of iNKT cells, cytokines are released, which are the small proteins responsible for cell signaling in an innate-like manner. Consequently, they engage in the effector functions, such as NK transactivation, T cell activation and differentiation, B cell activation. When iNKT cells are activated, cytokines are involved in direct and indirect B-cell activation, cognate, and non-cognate interactions. They are also responsible for dendritic cells activation and cross-presentation activity and macrophage activation. Furthermore, invariant natural killer T cells can regulate and recruit dendritic cells, neutrophils, and lymphocytes through cytokine release. The multi-functional responses of iNKT cells have been shown to enhance microbial and tumour immunity as well as promoting tolerance and suppressing autoimmune disease.[132] The α-galactosylceramide (α-GalCer, KRN7000) was reported as the first CD1d presented lipid antigen that can activate iNKT cells by Natori and coworkers in 1994 **(Figure 1.12)**.[133] This antigen was isolated from a sea sponge off the coast of Okinawa Japan, however, there is still some debate as to whether this lipid may be isolated from a bacterium present in the sample rather than from the sea sponge itself.

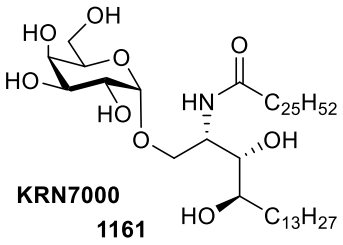
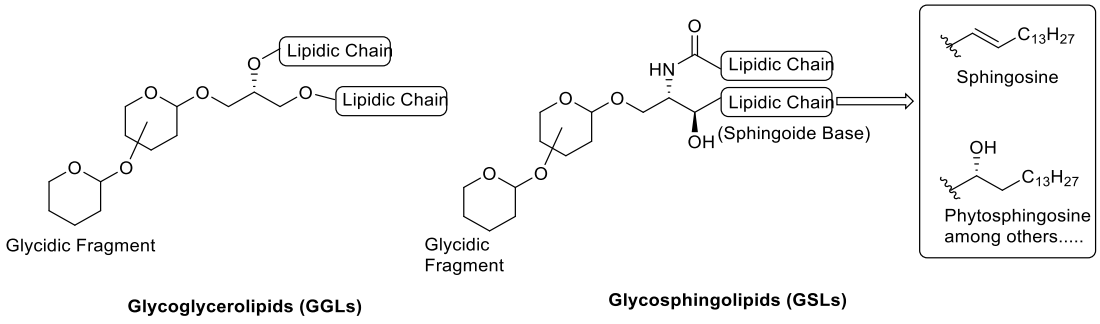


Figure 1. 12 Structure of KRN 7000

The lipid antigens capable of activating iNKT cells are classified into two categories: glycerol-based lipids and glycosphingolipids (ceramide-based glycolipids) depicted in **Scheme 1.24**. The glycerol-based lipids contain two parts: the sugar moiety in which the anomeric oxygen is linked to the glycerol moiety bearing two long acyl chains. Conversely the glycosphingolipids are composed of a sugar and a ceramide. The sphingosine moiety consists of 18-carbon amino alcohol bearing an unsaturated hydrocarbon chain and fatty acid.

This α orientation does not exist in the mammalian lipids, and it is unique for the foreign lipidic antigens. *Sphingomonas spp* are antigenic glycosphingolipids bearing α-linked glucuronic or galacturonic acid.[134-136] *Borrelia burgdorferi*[137] and *Streptococcus pneumoniae*[138] are examples of α-linked glucosyl and galactosyl diacylglycerols.



Scheme 1. 24 General structural feature of glycolipids

A cholesterol ester produced by *Helicobacter pylori* and lipopeptidophosphoglycans found in *Leishmania donovani*[139] have been proposed to bind to the iNKT cells TCR. The antigenicity of these lipids is less well understood than the other foreign lipid antigens. This α orientation may be a structural feature which explains how iNKT cells can be activated during infection; however, to date the direct and actual mechanism of action of these lipids with this linkage orientation is uncertain.

A study on lipid self-antigens attracted much attention among biologists, when iNKT cells were activated during viral infections, cancer, and autoimmune diseases, without the presence of a foreign lipid antigen.[140-142] Generally, in the absence of an infection, and as a result any pathogen associated with it, iNKT cells still can be activated by antigen-presenting cells (APCs). This activation happens through Toll-like receptor (TLR) agonists, and therefore, the presence of lipid–CD1d complex signal still seems to be necessary for the activation of iNKT cells. Consequently, this has led to the isolation and characterization of lipid self-antigens from CD1d with which the glycerol-based phospholipids, glycosphingolipids, and lysophospholipids were identified.[143-145] The results showed that among human-CD1d self-antigen lipids, only single-branched fatty acid lysophospholipids can activate iNKT cells. Lysophosphatidylcholine (lysoPC) was also found to be antigenic towards the activation of iNKT cells. This lysoPC results from cleavage of phosphatidylcholine (PC), *via* a phospholipase A2 (PLA2).[146, 147] These self-antigen lipids are capable of inducing the immune system by releasing granulocyte-macrophage colony-stimulating factors from iNKT cells. However, these antigens cannot use another cytokine type to strongly activate iNKT cells. Indeed, there are not many foreign lipid antigens presented

by CD1d that can strongly activate iNKT cells. Before 2011, the glycolipid α-galactosylceramide, known as KRN7000, was the only glycolipid identified as being capable of strongly activating this cell.

1.2.2 KRN7000

KRN7000 belongs to ceramide-based glycolipids, which are isolated from a marine sponge, *Agelas Mauritius* in Japan.[133] This glycolipid, composed of a sugar moiety (Galactose) α-linked to ceramide, is specific for only iNKT cells to activate the immune system. The ligand should be presented by CD1d protein, then the iNKT cell is activated through the T-cell receptor (TCR). Consequently, iNKT cells release cytokines to provoke immune system components, and as mentioned above, many defensive cells will be activated to eliminate the foreign antigens. In this process, two types of defence mechanisms are applied: TH1, for the fight against tumours and antimicrobial functions and TH2, against autoimmune diseases **(Figure 1.13)**.[16] KRN 7000 showed a strong ability to prevent asthma development[148, 149] by suppressing the growth of a variety of tumour cells, such as liver,[150] melanoma,[151] hepatitis and lung,[152] as well as fighting against inflammation,[153] pathogens,[154] and autoimmune diseases. Hence, this lipid antigen has become an interesting therapeutic target for researchers.[155]

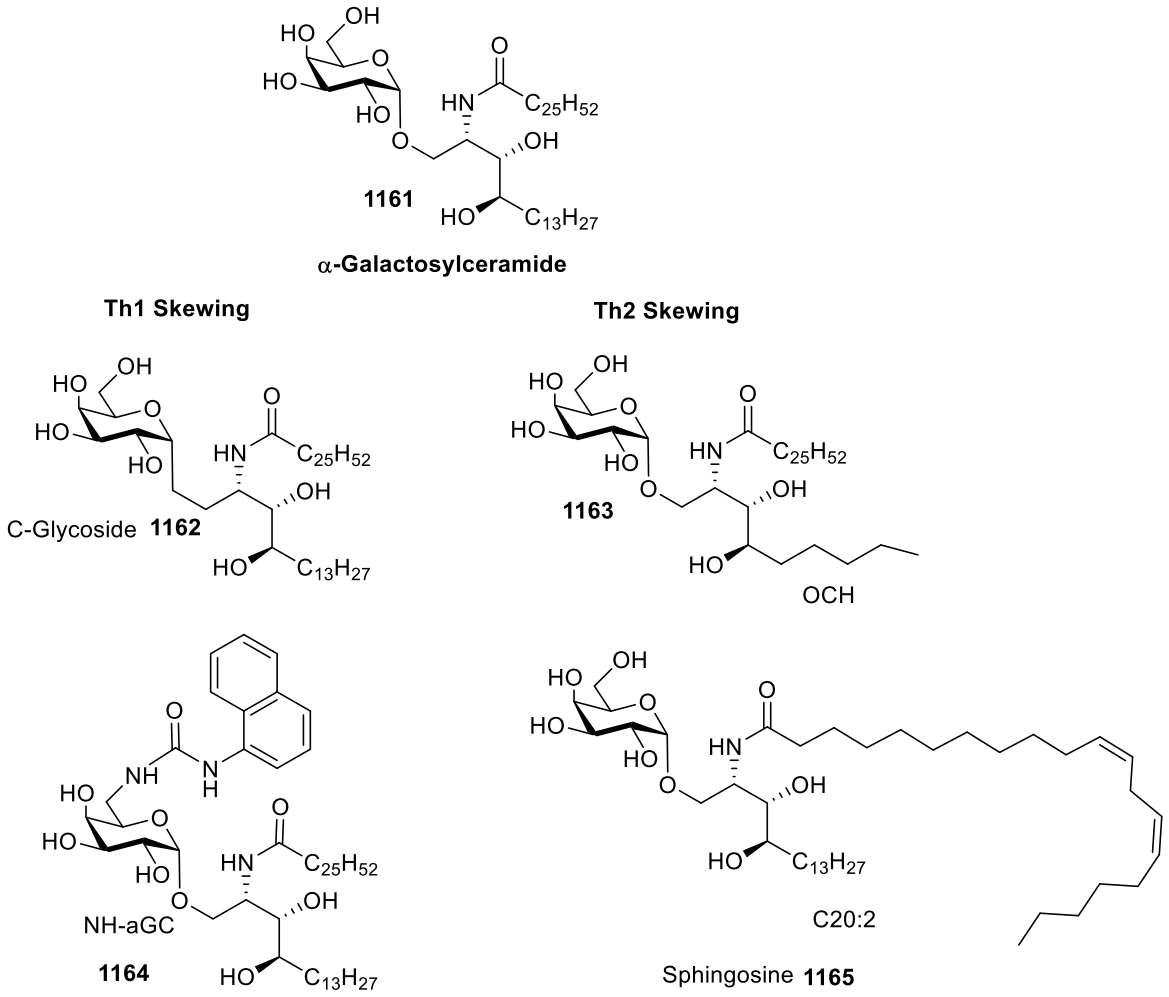


Figure 1. 13 KRN 7000 derivatives and their defense mechanisms

A wide range of modifications have been performed on KRN 7000 to increase the activity of this molecule. These include modifications of the polar sugar portion, the orientation of the linkage, the polar part of ceramide, and the fatty acid chains. Using galactose showed better iNKT cells activation results than glucose and mannose derivatives.[156] Attempts to replace hydroxyl groups with different functionalities resulted in interesting outcomes. Replacement of C-2-hydroxyl groups with methoxide, fluoride- and sulfate showed no activity at all. However, replacement of C-3 and C-4 with fluoro- and methoxide promoted the production of Interleukin-2 (IL-2), which was is a type of cytokine signaling

molecule responsible for in protein that blood cell activity adjustment. The amount of production of this cytokine was not compatible with the amount of this protein produced by KRN7000. 3-*O*-sulfate substitution resulted in similar iNKT cell activity as KRN7000, this is probably due to the presence of a strong hydrogen bonding network. The formation of a disaccharide using different sugars in different positions, as expected, showed the same activity as KRN7000 *in vivo*. This is, of course, due to cleavage of the acetal linkage by glycosidases. However, the C-6 linked disaccharide(s) showed iNKT cell activity. This result is explained by the unique structure and orientation of this molecule, which can fit itself in TCR space between the active areas CDR1a and CDR3a. Consequently, researchers have turned their attention to optimize the activity of iNKT cells by changing the C-6 substitution in KRN7000. Various functionalities were introduced, including acid,[157] amide, acyl amides,[158] biotin,[159] methoxyethers[160] and aryl ring with electron-withdrawing group substituted.[161] Unfortunately, none of these resulted in stronger activation of the T cells.

Subsequently, the polar ceramide portion of the KRN 7000 molecule was subjected to modification looking for enhanced anti-tumour activity. Based on previous research by Trappeniers, the presence of an amide functionality and 3′-OH are necessary for TCR recognition.[162] The removal of both hydroxyl groups resulted in complete loss of activity, and any changes at the 4′-OH, including any alteration of the conformation or even complete removal of the 4’-OH afforded no notable difference in iNKT cell activity. Although the replacement of the hydroxyl group with an amide group showed good TCR affinity, the production of cytokines was reduced.

Next, alterations to the fatty chains were investigated and analogues with different chain lengths were synthesized. Unfortunately, these compounds did not produce a consistent trend and showed a wide range of lower and higher activities, none of which were significantly higher than KRN7000. Finally, the linkage orientation was evaluated and α-KRN7000 indicated excellent TCR binding compare to the β linkage. This result showed better iNKT cell activation, however, using the β-anomer resulted in better CD1d interaction.

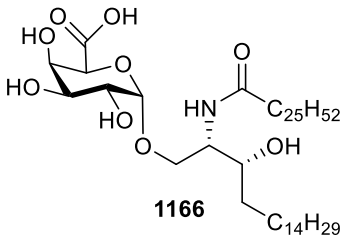
Finally, the oxygen at the anomeric position was replaced by sulfur[157] and also CH_2.[159] The replacement with sulfur showed no iNKT cell activity, possibly, due to lack of efficiency of hydrogen bonding, and when the *exo*cyclic oxygen was replaced with a methylene group, a robust TH1 cytokine response was observed in mice. However, this effect did not translate to humans with the same strength. Consequently, the presence of oxygen at the anomeric position seems to be necessary for efficient activation of iNKT cells, particularly in humans. So far, all the modifications on KRN 7000 have not resulted in significant improvement of iNKT cell activity in humans, and the majority of them showed no or low activity in comparison to KRN7000 itself.

1.2.3 Bacterial antigens for invariant natural killer T-cells

Five major bacterial antigens, which can activate invariant natural killer T-cells, have been isolated. The previous studies showed that phospholipids from *Mycobacterium tuberculosis*, which was the first reported glycolipid antigen presented by group 1 CD1 protein, are capable of activating any T-lymphocytes.[163-165] Careful screening of the bonding between *Mycobacterium* lipids and CD1d in mammals showed a strong binding between CD1d and phosphatidylinositol tetramannoside. This lipid is isolated from a related

bacterium, *Mycobacterium Bovis bacillus*, contains different fatty chains and is responsible for *bovine tuberculosis*.[166]

S. paucimobilis, *Sphingomonas capsulate* and *Sphingomonas yanoikuyae* belong to the *Sphingomonas spp* family. Among them, *Sphingomonas paucimobilis* is the most pathogenic bacteria, and in both synthetic and natural forms the glycosphingolipids (GSLs) from *S. paucimoblis* showed the ability to activate iNKT cells.[136] However, the activity was weaker than that observed for KRN7000. Glycosphingolipids (GSLs) GSL-1, isolated from *S. yanoikuyae* and GSL-1', isolated from *S. paucimobilis*, are highly structurally similar to KRN7000 **(Figure 1.14)** and are capable of the highest activation of iNKT cells compared to the rest of the *Sphingomonas spp* family, however, as mentioned above, none are as high as αGalCer.

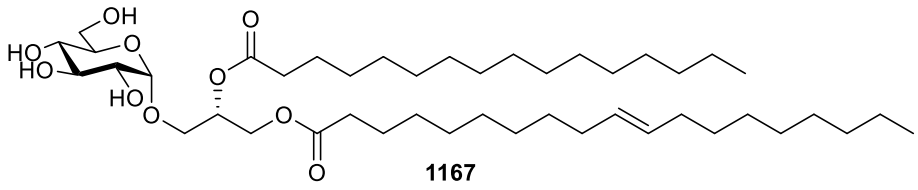


Gal-GSL (*Sphingomonas spp.*)

Figure 1. 14 Structure of Gal-GSL **1166** (isolated from *Sphingomonas spp*.)

Spirochete Borrelia burgdorferi is the only pathogenic member of the *Borrelia burgdorferi* family, which can be introduced to humans by *Ixodes scapularis* (deer tick) bites. These lipids are composed of a variety of galactosyl diacylglycerols (DAGs), including BbGL-2, which is capable of activating iNKT cells in both natural and synthetic forms.[137] These antigens include a galactose sugar moiety, an *α-O*-glycosidic bond, and lipid chains, which are linked with ester bonds to a glycerol moiety bearing saturated or

unsaturated fatty chains. In the mice study BbGL-2c **(Figure 1.15)**, which contains oleic acid in the sn-1 and palmitic acid in the sn-2, was identified as the most effective member of this family in activating iNKT cells.[137] The length and degree of saturation of the fatty acid plays an essential role in the activation of the iNKT cells through TCR recognition of the sugar component. In this case, sn-1 fatty acid picks A′ or F′ pockets of mouse CD1d to bind, whereas, sn-2, which in this case is oleic acid, picks the A′ pocket. Simply binding is insufficient however as the effectiveness of these antigens is related to how strong they can bind in the pocket. This is very important as the lipid chain binding to the pocket is directly related to the orientation of the sugar moiety for the TCR recognition. Changing this orientation could improve the interaction between TCR and the sugar moiety or may have adverse effects.



BbGL-2c (*B. burgdorfei*)

Figure 1. 15 Structure of *B. burgdorfei*, BbGL-2c

Streptococcus pneumonia is a gram-positive bacterium and is the leading cause of neonatal sepsis and bacterial meningitis, both of which were leading causes of death among humans before the discovery of antibiotics. The two main fractions of antigenic *S. pneumoniae* glycolipids were identified, one contains a monosaccharide glucose sugar α-linked to diacylglycerol (DAG), the second fraction was identical except it had a disaccharide moiety attached to the DAG.[167] The lipid chains were composed of palmitic acid in the sn-1 and *cis*-vaccenic acid in the sn-2, which is (C18:1) fatty acid with a C11-

C12 unsaturated bond present in low concentrations in mammalian cells. The glycolipid response was dependent on *cis*-vaccenic acid due to improved TCR recognition caused by changing the orientation of the sugar moiety, particularly the rearrangement of an axial hydroxyl group in the C-4 position of glucose.[131] These antigens can activate iNKT cells by releasing IFN-γ, Interferon gamma, a cytokine responsible for activation of macrophages, neutrophils and natural killer cells.

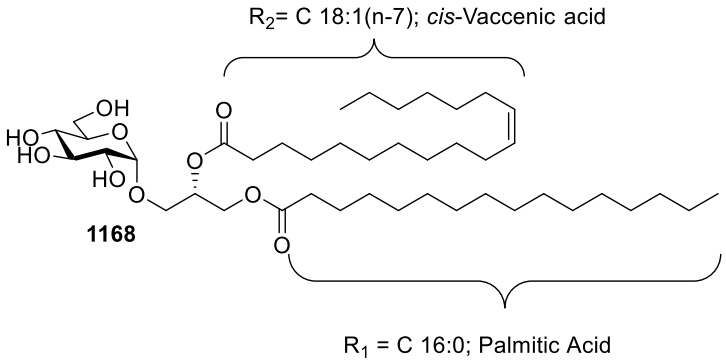


Figure 1. 16 Structure of *S. pneumoniae* (Glc-DAG) **1168**

Helicobacter pylori is a gram-negative helical bacterium and are the leading cause of duodenal and peptic ulcers, gastritis, gastric cancer, and lymphoma.[168] This bacterium is composed of cholesterol *α*-linked to a glucoside sugar moiety, and they are capable of activation of iNKT cells. PI57 **(Figure 1.17)** is a glycolipid, which belongs to the *Helicobacter pylori* family and has been used for asthma symptom protection studies.[169] This glycolipid has also been shown to activate iNKT cells in both mice and humans. However, studies towards biochemical analysis and structural analysis of binding a cholesterol-containing antigen to the CD1d, remain to be explored.

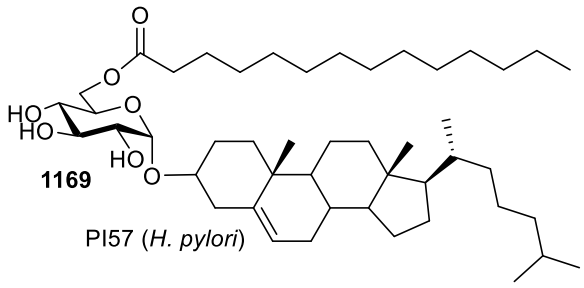


Figure 1. 17 Structure of *H. pylori* (PI57) **1169**

Aspergillus fumigatus is a fungus, which leads to infections in the lungs and sinuses.[170] Among the *Aspergillus fumigatus* family, a microbial *Asperamide B* **(Figure 1.18)** lipid can activate iNKT cells in both synthetic and natural forms. This lipid is composed of the glucose sugar moiety, linked to the ceramide bearing lipid chains *via* β-*O*-glycosidic bond. The sn-1 chain contains the 9-methyl-4,8-sphingadienine, which is commonly found in fungi[171] and the sn-2 chain is a β-γ unsaturated acyl chain with a hydroxyl group.[172]

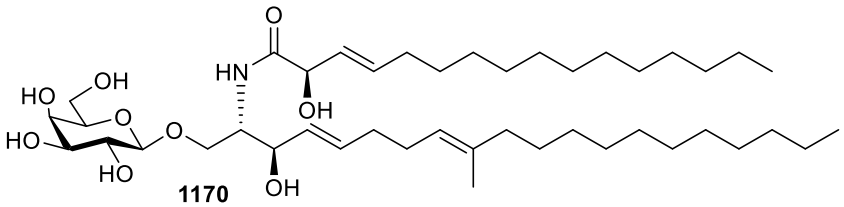


Figure 1. 18 Structure of *Asperamide B* (*A. fumigatus*) **1170**

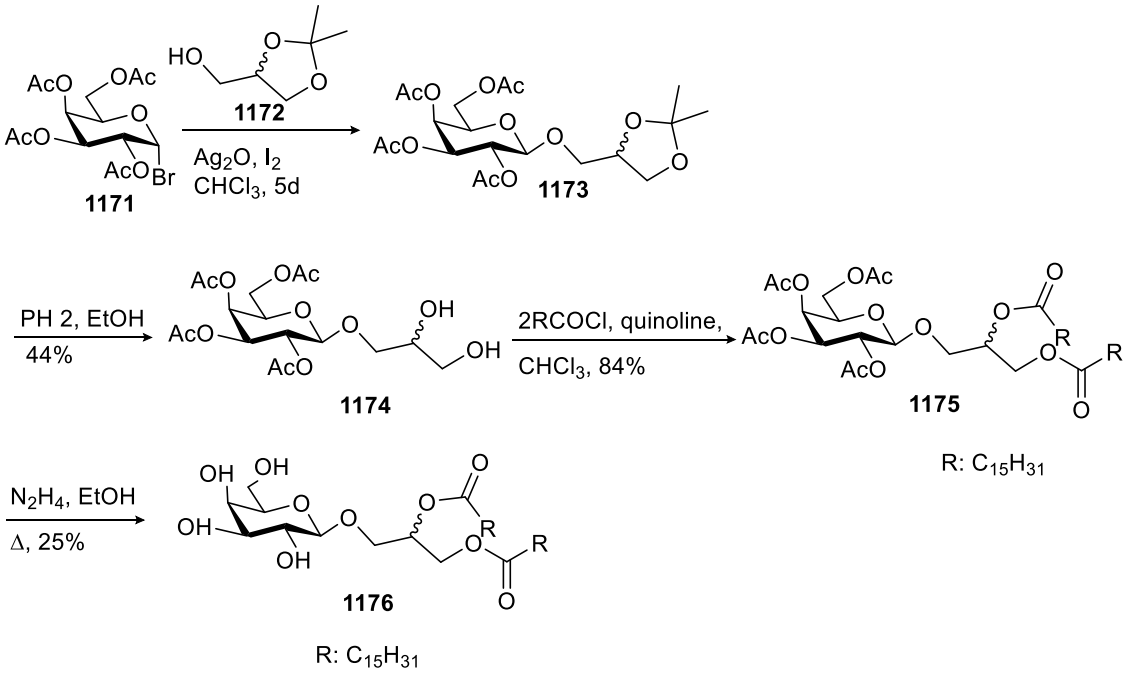
In conclusion, iNKT cell, which are part of the immune system, could be activated internally (self-antigen) or externally by bacterial and fungal lipids. The lipid antigens presented by the CD1d molecule induce a response from iNKT cells, which leads to the release of cytokines, both *gamma* (IFNg) and interleukin-4 responses, which work against each other. This is the most challenging problem in the application of these antigens for acquiring constructive iNKT cell responses. The study, design, and synthesis of antigens that are capable of inducing an iNKT cell response by releasing only the strong interleukin-4 or

robust interferon-*gamma* (IFNg) drew considerable attention.[173] Despite very optimistic preliminary results, both the control and specificity mechanisms of the iNKT cell response have yet to be explored.

1.2.4 Background of the synthesis of glycerol-based glycolipids

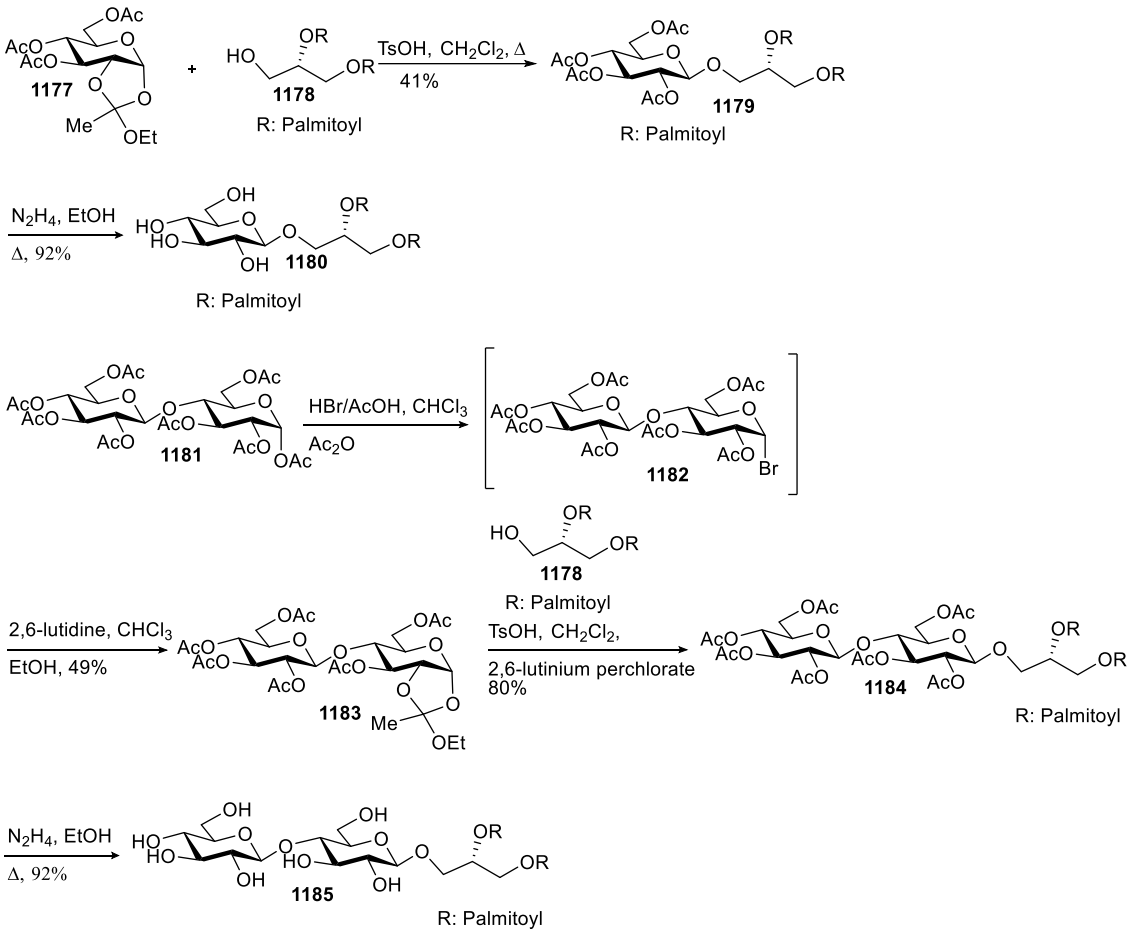
1.2.4.1 Synthesis of monosaccharide glycerol-based glycolipids

This section only discusses the background of the synthesis of glycerol-based glycolipids, as the synthesis of the rest of the glycolipid family is beyond the scope of this thesis. The first full synthesis of glycerol-based glycolipids was reported by Wehrli and Pomeranz.[174] They used racemic isopropylidene glycerol with an acetobromogalactose donor in the glycosylation step. This glycosylation afforded a β-oriented product. The hydroxyl groups in the glycerol moiety were deprotected and then esterified with palmitoyl chloride. Finally, a selective deprotection of the sugar moiety provided the galactosyl diglyceride bearing a racemic long-chain ester **1176** in 25% yield. Although the synthesis of this compound did not provide a practical biologically active glycolipid, their approach could be applied for the synthesis of optically active glycolipids with a brief modification and is shown below in **Scheme 1.25**.



Scheme 1. 25 The first full synthesis of glycerol-based glycolipids was reported by Wehrli and Pomeranz

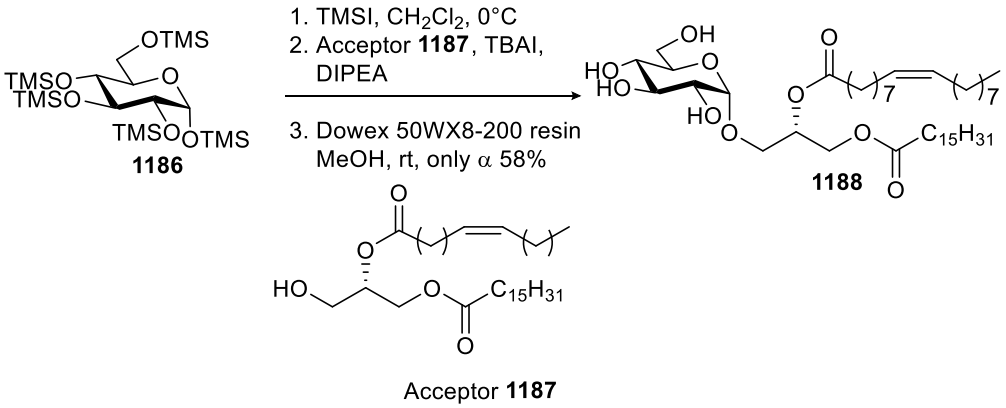
Four years letter, Evstigneeva reported the first synthesis of optically active versions of both mono- and disaccharide glycolipids bearing a β glycosidic bond using the orthoester method of 1,2-transglycosylation **(Scheme 1.26**).[175] A 1,2-di-*O*-palmitoyl-sn-glycerol **1178** was directly used instead of isopropylidene glycerol as an acceptor in the glycosylation. Consequently, the acetylated glycolipids were selectively deacetylated using hydrazine hydrate. In the subsequent attempt, cellobiosyl diglyceride **1185** was prepared from the orthoester, which itself was synthesized through glycosylation, from the corresponding glycosyl donor and the 1,2-di-*O*-hexadecanoyl-sn-glycerol acceptor. The desired glycolipid was then formed using selective deacetylation using hydrazine hydrate.



Scheme 1. 26 The first synthesis of optically active versions of both mono- and disaccharide glycolipids

Gervay-Hague and coworkers[176] reported the first synthesis of the glucosyl analogue of the bacterial antigen BbGL-II. The TMS group was used as a protecting group in the glycosyl iodide donor to perform a glycosylation with functionalized unprotected acceptors **(Scheme 1.27)**. The fully protected TMS glucose **1186** was employed as a starting material. α-glycosyl iodide was prepared using TMSI, subsequently, the α-glycosyl iodide was converted to the β-glycosyl donor using TBAI and DIPEA, which is a more reactive anomer of the initial glycosyl iodide. Then, the glycosyl donor was subjected to an *in situ* glycosylation with the corresponding glycosyl acceptor **1187**. Finally, the desired glycoli-

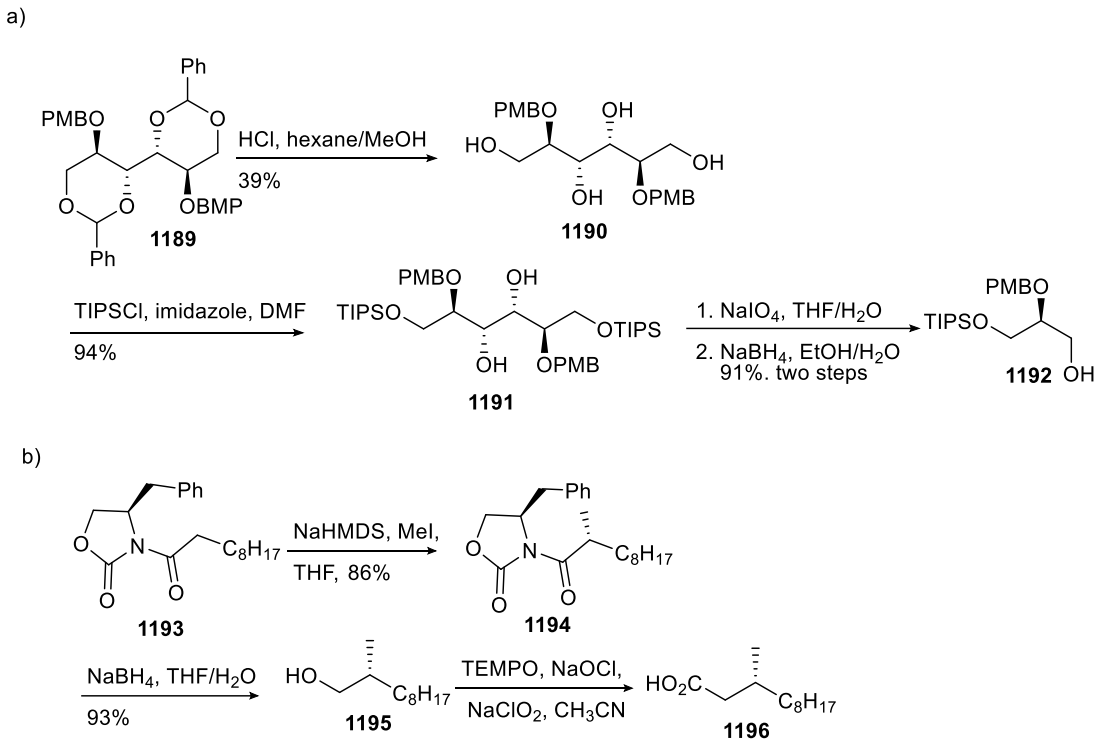
pid was obtained after removal of the protecting group using Dowex 50WX8-200 ion-exchange resin and methanol in 58% yield. Although this approach provided efficient access to biologically relevant glycolipids with α-orientation, this approach has not become a popular method. Reasons include the use of a TMS-protected glycoside donor does not afford acceptable results in terms of both selectivity and yield with specific acceptors. In addition, conditions could be problematic in some cases due to the sensitivity of the TMS protecting group towards reaction conditions.



Scheme 1. 27 The first synthesis of the glucosyl analogue of the bacterial antigen BbGL-II

In 2013, McConville and Williams reported the biochemical analysis and synthesis of acyl-isoforms of GlcAGroAc2, which were isolated from *C. glutamicum* and *M. smegmatis*, bearing two acylation patterns.[177] The first containing (*R*)-tuberculostearic acid (C19:0) and palmitic acid (C16:0) and the second containing oleic acid (C18:1) and palmitic acid (C16:0). It is worth mentioning that these glucuronosyl diacylglycerides include carboxylic acid functionality at the C-6 position of the sugar moiety. The glycosyl donor **1197** was synthesized based on Thiem report.[178] Simultaneously, the homochiral glycerol moiety was bearing two different hydroxyl protecting groups in TIPS and PMB, which

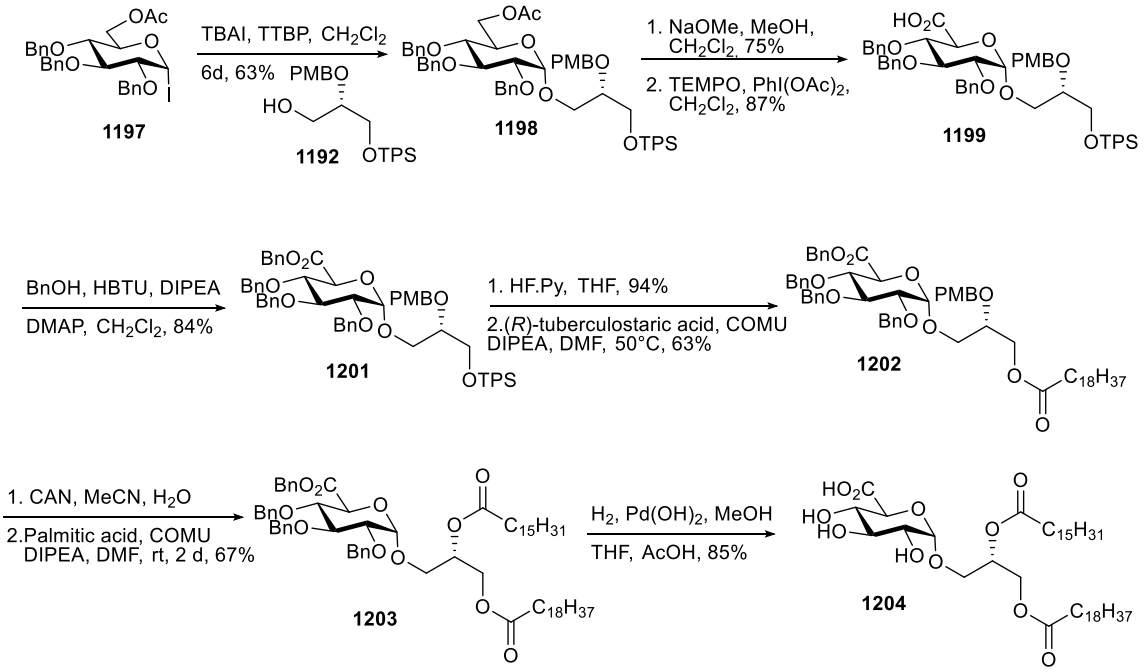
were synthesized from compound **1189** in 5 steps **(Scheme 1.28a)**. As the third part of the glycolipids construction, the enantiopure (*R*)- tuberculostearic acid **1196** was targeted using an Evans' chiral auxiliary to provide the 10-(*R*)-methyl stereocenter.[179] They applied *N*-decanoyloxazolidinone **1193**,[180] as a starting material for the diastereoselective methylation using NaHMDS and iodomethane. The desired product was obtained by reductive cleavage of chiral auxiliary using sodium borohydride, followed by oxidation of primary alcohol to the carboxylic acid *via* TEMPO, NaOCl and $NaClO_2$ in CH_3CN **(Scheme 1.28b)**.



Scheme 1. 28a,b Synthesis of the glycosyl acceptor **1192**, a, and (*R*)-Tuberculostearic Acid **1196**, b

After the preparation of the main components of glycolipids, they tried to link them together **(Scheme 1.29)**. First, a glycosylation was performed over four days between the acceptor **1192** and the donor **1197**,[178] using TBAI and TTBP in dichloromethane. Then,

the acetyl group at the C-6 position was deprotected and oxidized to the resulting carboxylic acid using TEMPO and $PhI(OAc)_2$ in a biphasic mixture of CH_2Cl_2/H_2O. In the next step, the TIPS protecting group in sn-1 was first removed, and then subjected to an esterification with the corresponding fatty acid. This was followed by a PMB deprotection in sn-2, and finally an esterification with the other fatty acid. The same procedure was repeated with the rearrangement of fatty acids in the glycerol moiety to provide glycolipids with different fatty chains. Finally, a global deprotection of **1203** afforded the glycolipids **1204** in 85% yield *via* hydrogenation using $Pd(OH)_2$.

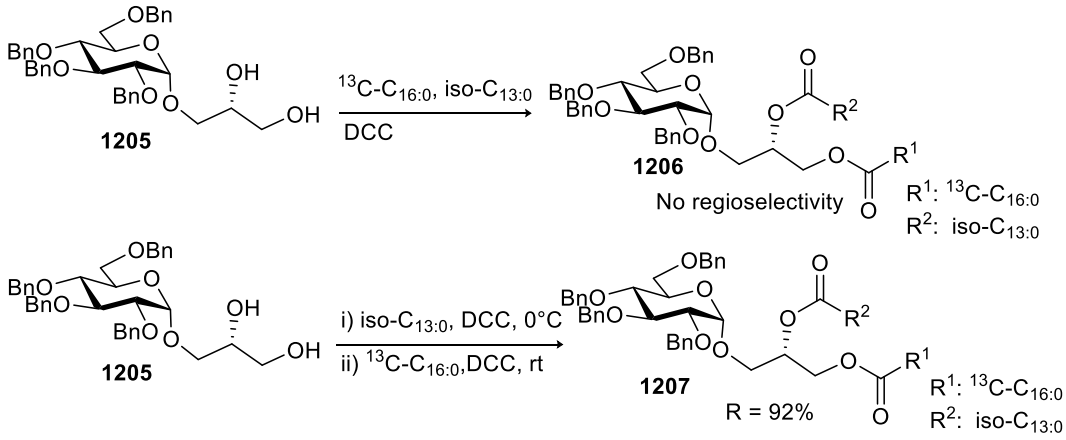


Scheme 1. 29 Synthesis of acyl-isoforms of GlcAGroAc2

Richardson and Williams reported the total synthesis of an *α*-glucosyl diglyceride isolated from *Streptococcus pneumonia*.[181] Different methods for acylation of the glyceride moiety was examined to investigate the fidelity of acylation, particularly acyl migration. To this end, a quantitative ^{13}C NMR spectroscopy study was done *via* carbon labelling at

the carbonyl carbon of one of the fatty acyl chains and then measuring the abundance of the ^{13}C label at each position using a paramagnetic relaxation enhancement (PRE) agent.

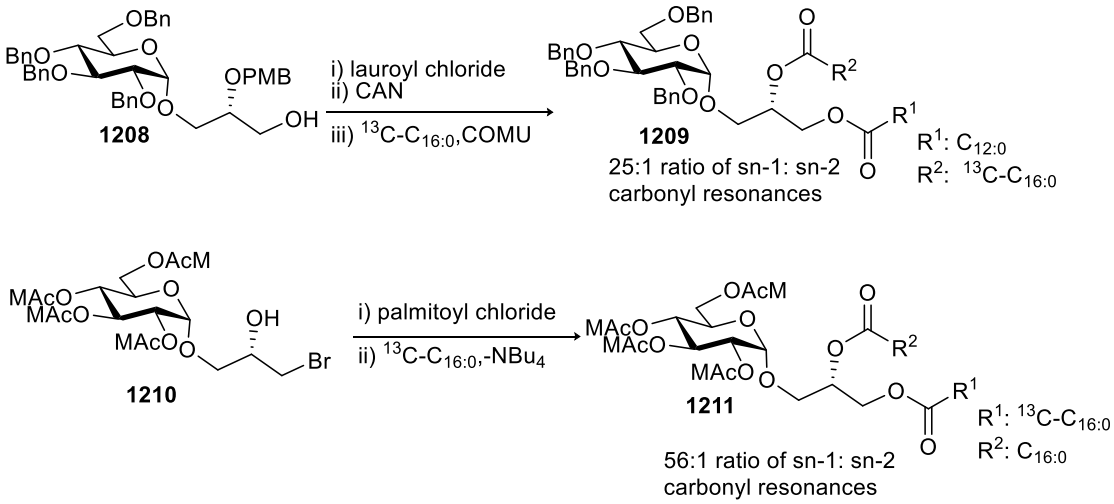
First, a nonselective acylation of the diol **1205**, was performed in the presence of a 1:1 mixture of commercial 1-^{13}C-palmitic acid and 10-methyl decanoic acid (*iso*-C13:0). As expected, no regioselectivity was observed. Next, they tried a stepwise esterification of glycerol using 10-methyl decanoic acid, DMAP and DCC at 0°C. After careful monitoring of the reaction, the crude was subjected to rapid purification at cold temperature to minimize the acyl migration, then the secondary alcohol was esterified with ^{13}C-labelled palmitic acid at the same reaction conditions as the primary alcohol acylation. After purification and a carbon NMR study, the result showed the desired regioisomer in R = 92%.



Scheme 1. 30 Synthesis of α-glucosyl diglyceride isolated from *Streptococcus pneumonia*

In the next experiments the acylation was conducted in a stepwise acylation manner and was controlled by the appropriate choice of different protecting groups as shown in **Scheme 1.31**. First, compound **1208** was acylated using lauroyl chloride, DMAP, and pyridine. The PMB protecting group was then removed *via* ceric ammonium nitrate in acetonitrile and water and (without purification) was subjected to the second acetylation to

minimize acyl migration even further. The resulting secondary alcohol was subsequently treated with ^{13}C-labelled palmitic acid in the presence of COMU and *N, N*-Diisopropylethylamine. The result showed R = 97.2% desired regioisomer in the product **1209**. In the next experiment, the primary alcohol was replaced by bromide to eliminate the possibility of having a free primary hydroxyl group as this should decrease the possibility of any acyl migration. First, the secondary alcohol was esterified using palmitic acid, followed by the replacement of bromide with 1-^{13}C-palmitic acid through nucleophilic substitution. The result afforded the product **1211** with a 56:1 ratio of sn-1 and sn-2 carbonyl resonances, corresponding to an R > 99%.

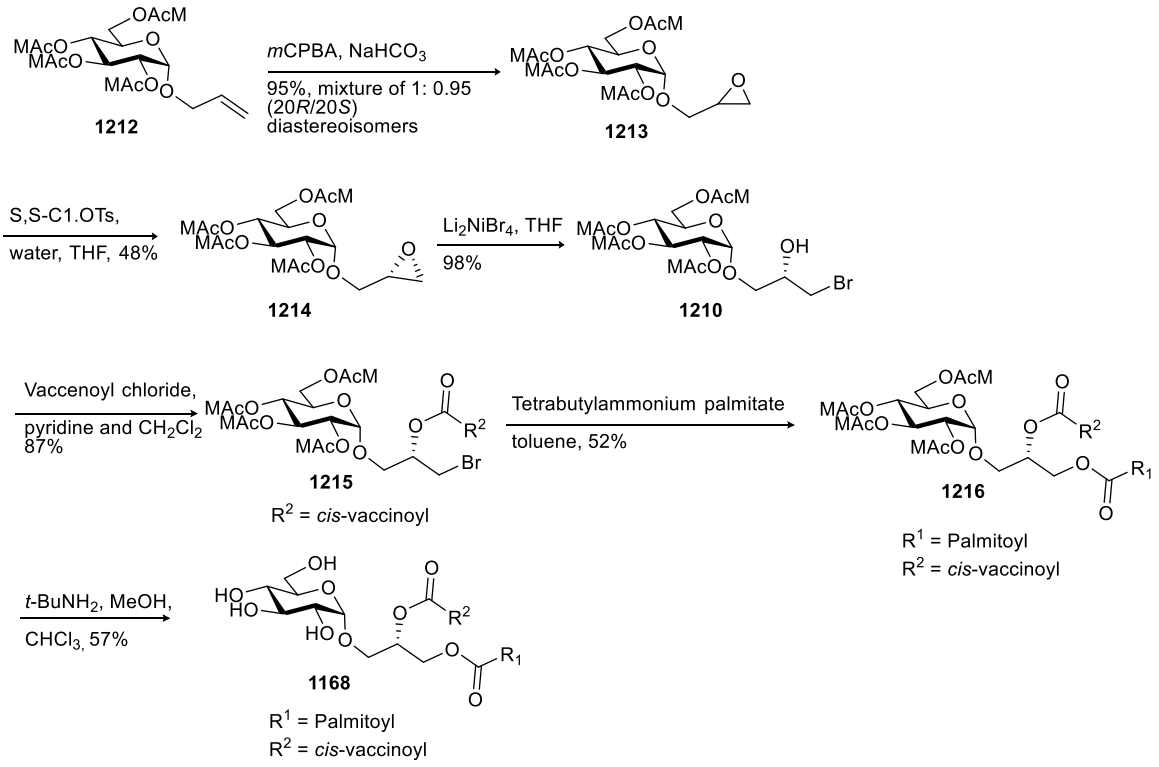


Scheme 1. 31 Quantitation of acylation fidelity by ^{13}C NMR spectroscopy

The same bromohydrin strategy was applied for the synthesis of the *S. pneumoniae* isolate Glc-DAG-s2 and is shown in **Scheme 1.32**. A glycidol-derived bromohydrin **1210** was prepared based on their previous report using commercial provided allyl α- D-glucopyranoside as a starting material.[182] Compound **1212** was treated with *m*CPBA to generate an epoxide in 1: 0.95 (20*R*/20*S*) mixture of diastereoisomers. A single stereoisomer was obtained in 48% yield using *S,S*-C1.OTs in water and THF.[182] Finally, compound **1168**

was prepared after regioselective ring-opening using Li_2NiBr_4. The first esterification was performed using vaccinoyl chloride, pyridine, and CH_2Cl_2. Then a palmitic acid salt, which was prepared from the reaction of tetrabutylammonium salt, corresponding carboxylic acid and base, was applied for the nucleophilic substitution of the alkylbromide.

Although this hydrobromin approach provides glycolipids with high regioselectivity in the acylation part, the total synthesis of these compounds starting from common starting materials such as D-glucose and D-galactose requires an extended multi-step synthesis compare to other approaches. In addition, the selectivity and yields of some of the reactions (especially with regards to the epoxide formation) needs to be improved.

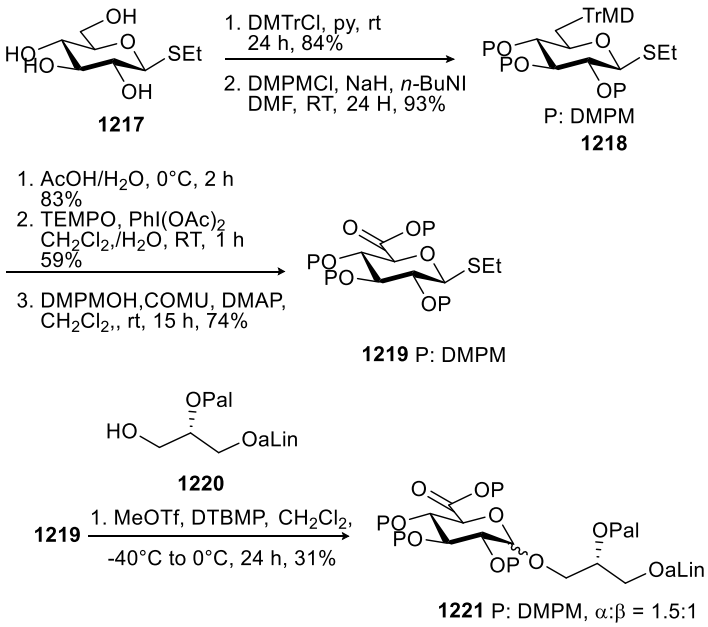


Scheme 1. 32 Synthesis of *Streptococcus pneumonia* Glc-DAG-s

In 2017, Sodeoka and Hirai reported the synthesis of GlcADG containing polyunsaturated fatty acids (PUFAs), which includes α-linolenic acid (α-Lin, 18:3(n-3)) in sn-1,

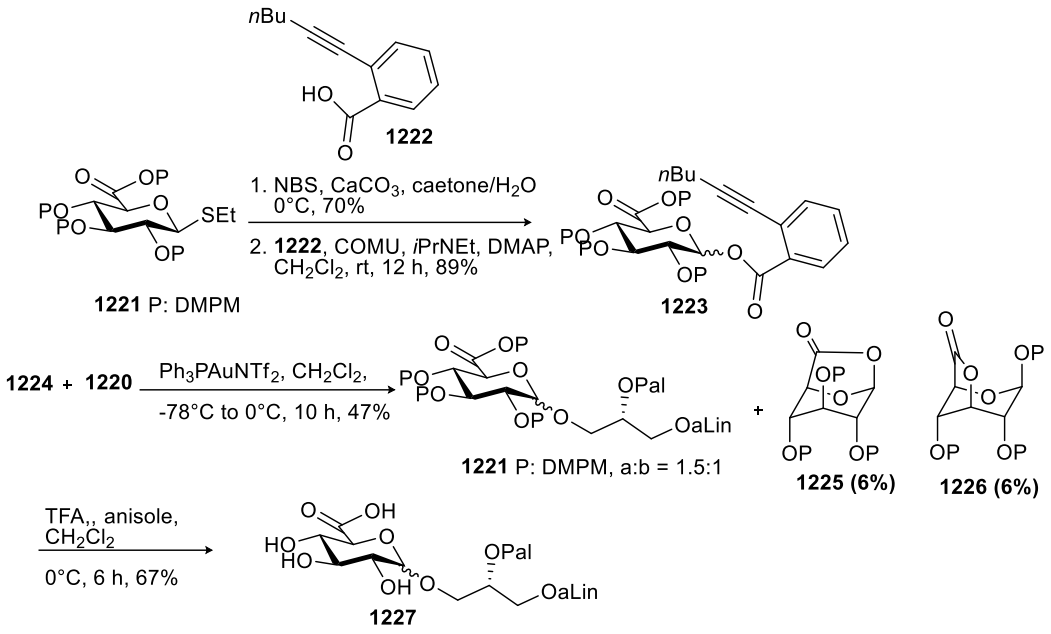
palmitic acid in sn-2 and a carboxylic acid functionality in the sugar moiety.[183] This synthesis, shown in **Scheme 1.33**, represents the first synthesis of GlcADG bearing a PUFA. They explained that due to the poor stability of the oxocarbenium ion intermediate bearing an electron-withdrawing ester functionality at the C-6 position, and also weak reactivity of glycosyl acceptor because of the presence of two acyl electron-withdrawing functionalities, 3,4-dimethoxybenzyl (DMPM) protecting groups were applied to increase the stability of oxocarbenium ion intermediate and enhance the α-selectivity of the glycosylation.

The commercially available(*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol was used for the synthesis of the glycosyl acceptor **1220**. The two hydroxyl groups were regioselectively acylated by α-linolenic and palmitic acids. In parallel, the glucuronide donor bearing the DMPM protecting group was synthesized, starting from compound **1217**, which then was selectively protected by DMTr group using DMTrCl in pyridine, then the rest of hydroxyl groups were protected by DMPMCl in the presence of NaH and TBAI in DMF. The DMTr were removed by 80% acetic acid in the water and oxidized using TEMPO and bis(acetoxy)iodobenzene and finally, esterification of carboxylic acid with DMPMOH in the presence of COMU and DMAP afforded thioglucuronide donor **1219**. The glycosyl acceptor and donor were then subjected to glycosylation reactions in the presence of methyl triflate as an activator. The result showed poor yield and weak selectivity with a 1.5:1 α:β ratio, this result expected in glycosylation of a thioglucuronide donor with a less reactive acceptor bearing two acyl withdrawing groups. As a result, it seems necessary to have a highly reactive glycosyl acceptor to get high selectivity during the formation of the α-glucuronide product.



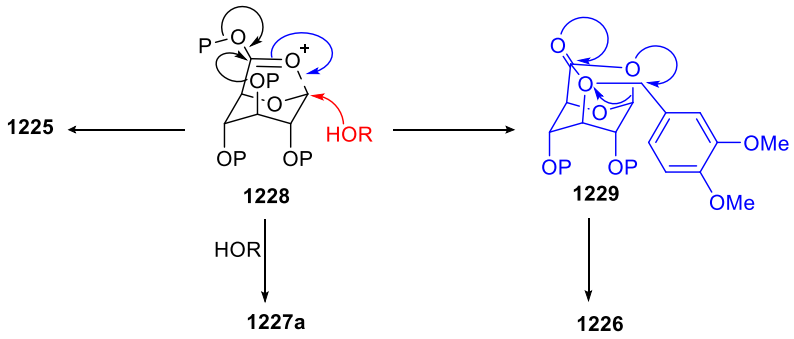
Scheme 1. 33 Synthesis of compound **1221**

The authors tried to optimize the reaction by changing the functionality at the anomeric position. They replaced the thioethyl functionality with ortho-alkynylbenzoates in two subsequent steps: hydrolysis of thioethyl, followed by the installation of ortho-alkynylbenzoates at the anomeric position using ortho-alkynylbenzoic acid, COMU, DMAP, *i*-Pr_2NEt in CH_2Cl_2 as shown in **Scheme 1.34**. In this case the glycosylation between glycosyl acceptor **1220** and glycosyl donor **1224** was performed using $Ph_3PAuNTf_2$ as an activator. The result indicated a slightly better yield, and α selectivity improved to a 4:1 α:β ratio. It is worth mentioning that these two anomers were separated by HPLC. They reported the presence of 1,6-lactone **1225** and 3,6-lactone **1226** as byproducts**.**



Scheme 1. 34 Synthesis of compound **1227**

The authors postulated that the formation of byproduct **1225** occurs through the formation of intermediate **1228** in **Scheme 1.35**. First, the sugar moiety inverts, then, the carbonyl oxygen in the C-6 position attacks to the cationic carbon at the anomeric position. Consequently, byproduct **1225** is formed by the release of the DMPM group. In the case of byproduct **1226**, intermediate **1228** was subjected to the nucleophilic attack of the oxygen at the C-3 position to afford intermediate **1229**. Then, the DMPM protecting group at the C-6 position was removed and the nucleophilic attack of O1 to the benzylic carbon of the DMPM group on the C-3 position provides byproduct **1226**.

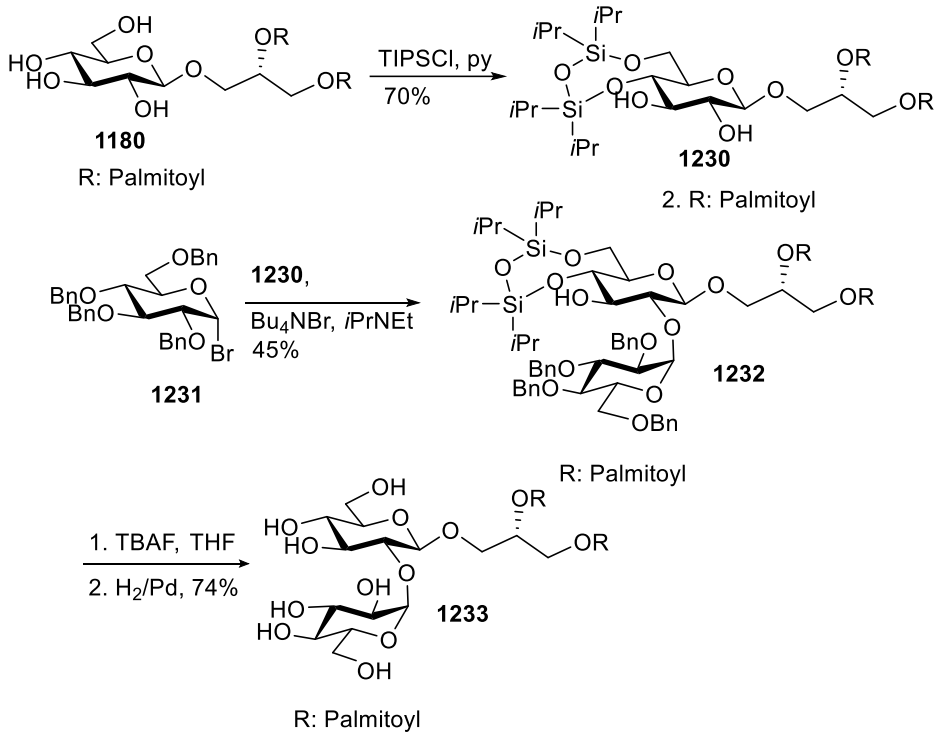


Scheme 1. 35 Formation of compounds **1225**, **1226** and **1227a** through intermediates **1228** and **1229**

With the glycosylation complete, the final product **1227** was obtained by removal of the DMPM groups using TFA and anisole in 67% yield. The glycosyl donor (ortho-alkynylbenzoates), protecting group (DMPM), and a gold catalyst for the activation of glycosyl donor (which are not very common in carbohydrate glycosylation chemistry) were used in this modified synthesis. However, it did not afford efficient results in either productivity or selectivity. This report shows that exploring a more convenient route for the synthesis of these glucuronosyl-diacylglycerol lipids seems to be necessary.

1.2.4.2 Synthesis of disaccharide glycerol-based glycolipids

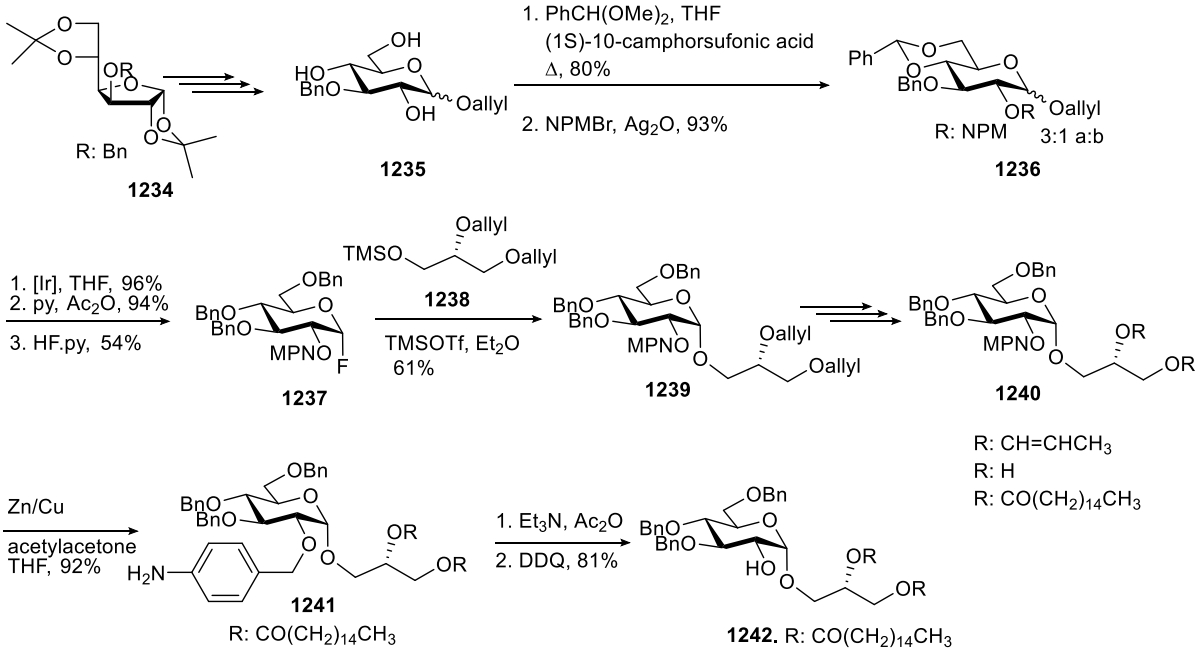
There are not many syntheses of the diglycosyl diglyceride glycolipids previously reported in the literature. In 1985 Boom and coworkers reported the first synthesis of 2,1-α linked glucose-glucose disaccharide and the second synthesis of diglycosyl diglyceride glycolipids.[184] They prepared phosphatidyl-α-diglucosyl using compound **1180** as glycosyl acceptor in **Scheme 1.36** as a starting material, which was previously synthesized by the same group.[185] The oxygens at the C-4 and C-6 positions were protected *via* a cyclic silicon protecting group using TIPSCl, after which selective glycosylation was performed using bromoglycosyl donor **1231**, TBAB and DIPEA. Consequently, disaccharide **1233** was revealed by the removal of the TIPS and benzyl protecting group *via* tetrabutylammonium fluoride and hydrogenation, respectively.



Scheme 1. 36 The first synthesis of 2,1-α linked disaccharide **1233**

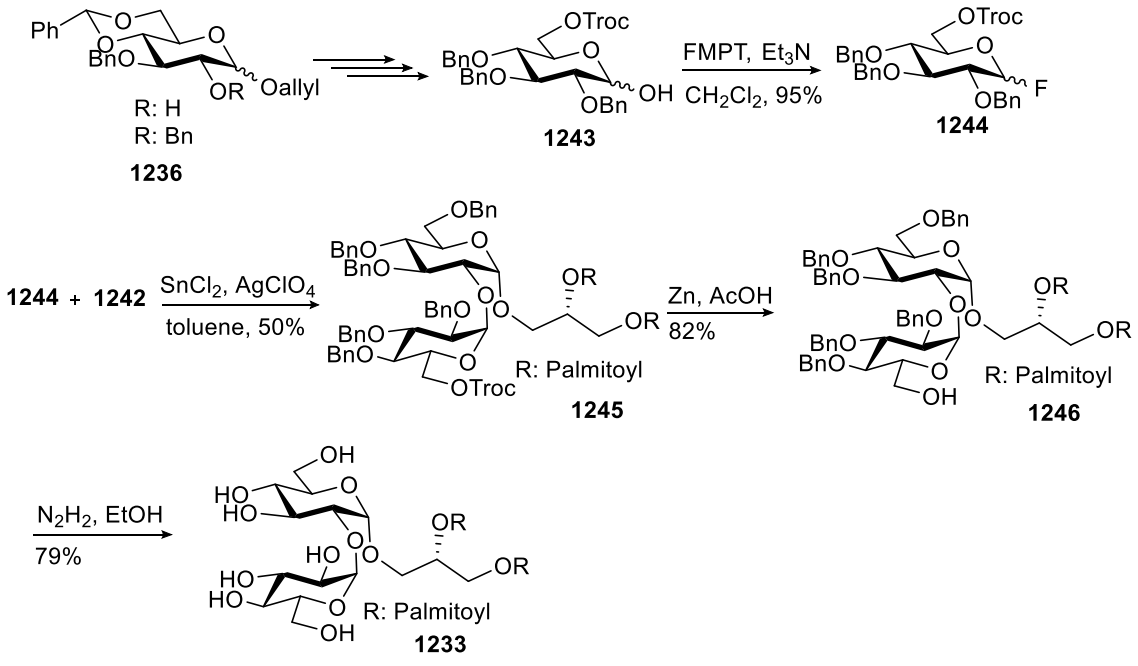
In 1992, Kusumoto reported the synthesis of the same molecule, **1233**, in the synthesis of *Streptococcus Pyogenes LTA*.[186] Their approach is quite lengthy and required further manipulation. They synthesized glycosyl donor **1237** using *O*-diisopropylidine glucose as the starting material. This compound was benzylated, followed by an allylation at the anomeric position with allyl alcohol and HCl. This reaction gave the desired product **1235** in a 3:1 α:β ratio. The oxygens at C-6 and C-4 carbons were protected with a benzylidene protecting group and then a hydroxyl group at C-2 position was blocked with an NPM group using NPMBr and Ag_2O. The anomeric allyl group was first removed *via* an iridium complex then acetylated, finally, fluorination using HF.Py afforded compound **1233**.

The glucosyl acceptor **1228** was used, bearing allyl groups to prevent acyl migration during the acidic glycosylation. The alcohol was then converted to an OTMS and subjected to the first glycosylation with glycosyl donor **1237** using TMSOTf as an activator to afford the desired product **1239** in 3 to 1 α:β ratio. The allyl groups in the glycerol moiety were converted to acyl groups in three subsequent steps. First the allyl groups were isomerized, then hydrolyzed to free alcohols, and finally acylated afforded compound **1240**. To generate glycosyl acceptor **1242**, the NPM protecting group was removed *via* a three-step procedure. The first nitro group was reduced to an amine using a mixture of zinc and copper and acylated in the presence of acetylacetone. With the amine functionality acetylated, the acetamido benzyl protecting group was then removed *via* oxidative cleavage using DDQ as shown in **Scheme 1.37**.



Scheme 1. 37 Synthesis of glycosyl acceptor **1242**

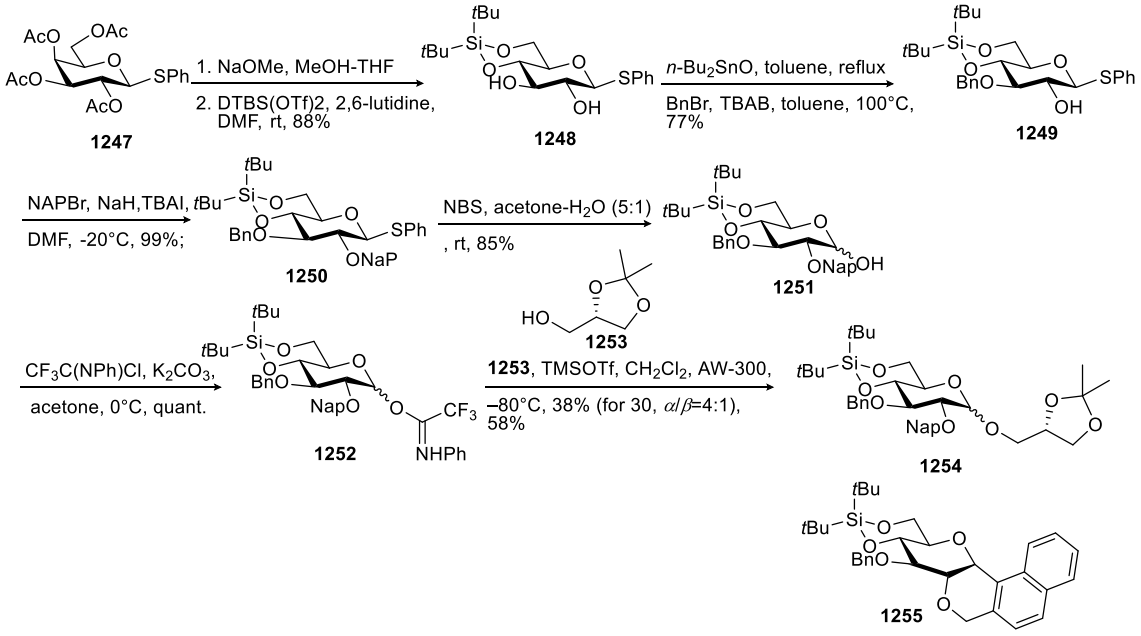
The glycosyl donor **1244** was prepared using compound **1236** as a starting material as shown in **Scheme 1.38**. The 2-hydroxyl group was benzylated, then a benzylidine protecting group was selectively removed and the C-6 hydroxyl group was blocked with a Troc protecting group. The allyl group at the anomeric position was replaced with fluorine in two subsequent steps. First, hydrolysis of the allyl functionality, then fluorination using FMPT and triethylamine. The last glycosylation was performed between glycosyl donor **1244** and glycosyl acceptor **1242** to afford desired compound **1245** in a 3 to 1 α:β ratio. Compound **1245** was then deprotected using zinc in acetic acid to remove the Troc group, followed by a benzyl deprotection to provide glycolipid diglycosyl diglyceride **1246**. However, the result did not show any antitumor activity or any production of cytokines such as TNF or IL-6.



Scheme 1. 38 Synthesis of *Streptococcus Pyogenes LTA*

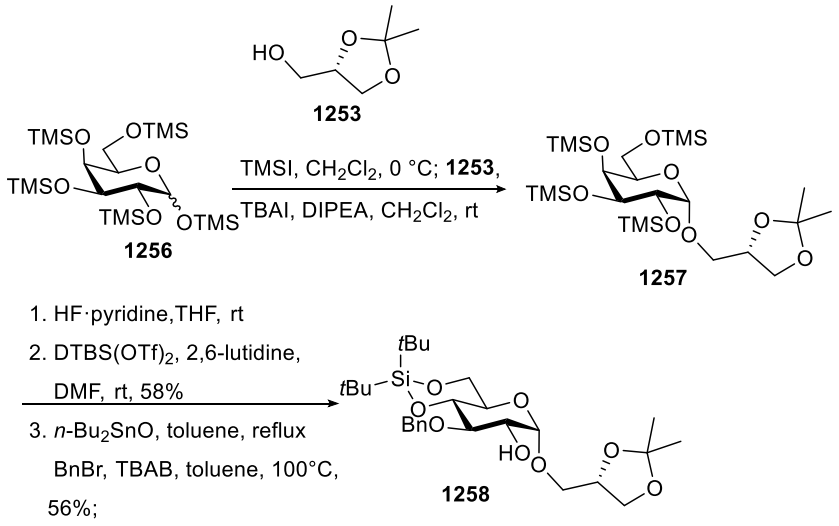
Recently, Imamura and Ishida reported the synthesis of α-diglycosyl diglyceride **1262** bearing an unsaturated fatty chain in a glyceride moiety using the cyclic silyl protecting group strategy that we discussed previously to achieve the desired selectivity for the glycosylation procedure.[187] This approach is distinct from the previous reported α-diglycosyl diglyceride synthesis. Their synthetic design involves using the isopropylidene protected glyceride moiety as a glycosyl acceptor, and the fatty acid chains are then selectively added by acylation at the end of the synthesis. Cyclic silyl protecting groups were exploited to improve α-selectivity of glycosylation.

The first attempt to produce glycosyl acceptor gave a low yield due to the generation of byproduct **1255 (Scheme 1.39)**. They start by manipulating protecting groups and finally applying the naphthyl methyl (Nap) group to protect the 2-hydroxyl functionality. At this point the glycosylation was performed but the result showed the oxocarbenium ion promotes a Friedel-Crafts reaction with 2-naphthyl methyl functionality resulting in the formation of byproduct **1255**.



Scheme 1. 39 Synthesis of compound **1254** and byproduct **1255**

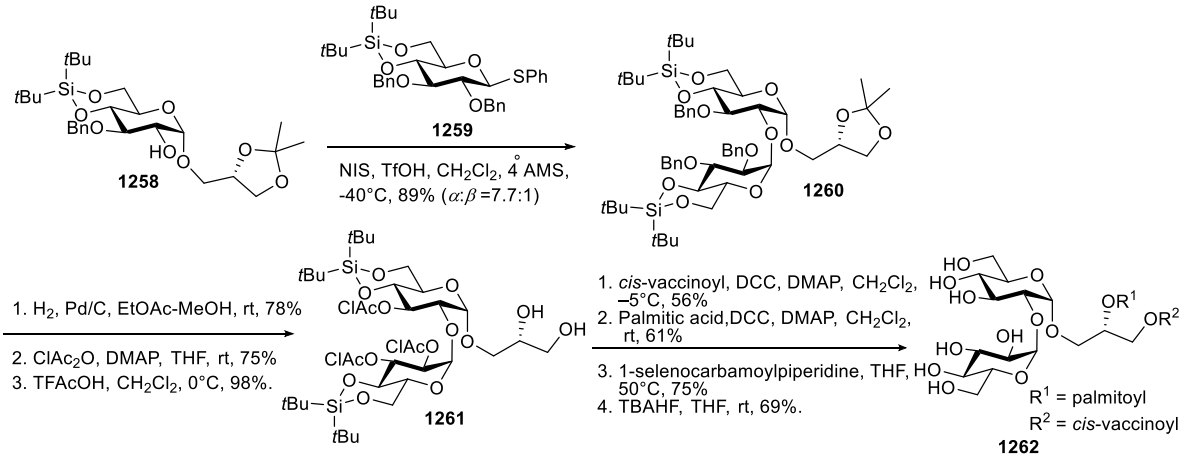
After the first synthesis was found unacceptable, compound **1258** was prepared based on known procedure. In this protocol the C-4 and C-6 hydroxyl groups were first protected by the DTBS protecting group using DTBS(OTf)$_2$ and 2,6-lutidine in DMF. The 3-hydroxyl group was then selectively protected *via* the benzyl group followed by acetal formation using *n*-Bu$_2$SnO in toluene and finally, a benzylation using benzyl bromide and TBAB in toluene afforded glycosyl donor **1258** in two steps.



Scheme 1. 40 Synthesis of glycosyl acceptor **1258**

The second glycosylation was performed using the glycosyl acceptor **1258** and the known 4,6-*O*-DTBS–protected glucosyl donor **1259**,[188] using NIS and TfOH as activators to obtain the desired disaccharyl glycerol **38** in an 8 to 1 α:β ratio **(Scheme 1.41**). Due to complications of removing the benzyl protecting group in the presence of an unsaturated fatty chain in the last step, they had to replace the benzyl groups with monochloroacetyl protecting groups. This procedure was done in two stages. First, hydrogenation of the benzyl groups afforded free hydroxyl groups, then monochloroacetylation was performed using monochloroacetic anhydride and DMAP.

The target compound **1262** was revealed in three steps, initially removal of the isopropylidene protecting group was done using trifluoroacetic acid. Then, selective acylation of sn-1 with *cis*-13-octadecenoic acid and the second acylation of sn-2 was performed using palmitic acid, DCC and DMAP. The final α-diglycosyl diglyceride **1262** was obtained *via* deprotection using 1-selenocarbamoylpiperidine and tributylamine hydrofluoride to remove the monochloroacetyl and DTBS groups, respectively.



Scheme 1. 41 Synthesis of α-diglycosyl diglyceride **1262**

Although, the approach previously discussed above could be a convenient route for the synthesis of disaccharyl glycerol glycolipids bearing 1-2-*O*-α-linkage between two sugar moieties, this is an extended multi-step synthesis, and a reduction in the reduced number of synthetic steps seemed to be required for this synthetic route to be viable. The 1-2-α-linkage between the two sugar moieties and the presence of an unsaturated acyl chain makes the synthesis of disaccharyl glycerol glycolipids difficult. This is mainly due to the excessive manipulation of protecting groups needed in both sugar moieties and two stereoselective glycosylation strategies required, which is one of the most challenging procedures in carbohydrate chemistry.[184] Despite the increasing number of isolated disaccharyl

glycerol glycolipids from natural sources,[189, 190] only a few synthetically prepared derivatives of these glycolipids have been synthesized to date. As a result, a general and efficient methodology for the preparation of synthetic disaccharyl glycerol glycolipids seems to be required to allow for the biological examination of their properties in activation of immune system.

1.3 Tertbutyl amine as a mild and effective for the selective deacetylation

There are many approaches for protecting hydroxyl group in organic synthesis. However, esters have emerged as the preferred option because of the diversity of ester choices, for example: acetate, benzoate, pivaloate, and also the convenience of installation and removal under mild conditions.[191] Acetate esters in particular are commonly used to protect hydroxyl groups in organic chemistry due to the availability of simple reactions for attachment and removal of this protecting group. Many reagents and reaction conditions can facilitate the removal of this protecting group, allowing chemists to optimize the deprotection of a wide range of organic compounds. During the past decades, more attention was paid to the selective removal of the acetate functionality than any other protecting group. This functionality often needs to be deprotected in different steps of a synthesis to prevent having any conflict in protecting and deprotecting strategies.[192] Many attempts have been made to develop reactions that selectively remove acetate esters in the presence of other ester functionalities. For example, DBU in benzene has been reported to remove the acetate group selectively in the presence of a benzoate group. Furthermore, Guanidine[193] or magnesium methoxide[194] have both been used to deprotect acetate groups in the presence of benzoates, pivaloates, and acetamides. In addition, ammonia,[195] hydrazine,[196] and Mg

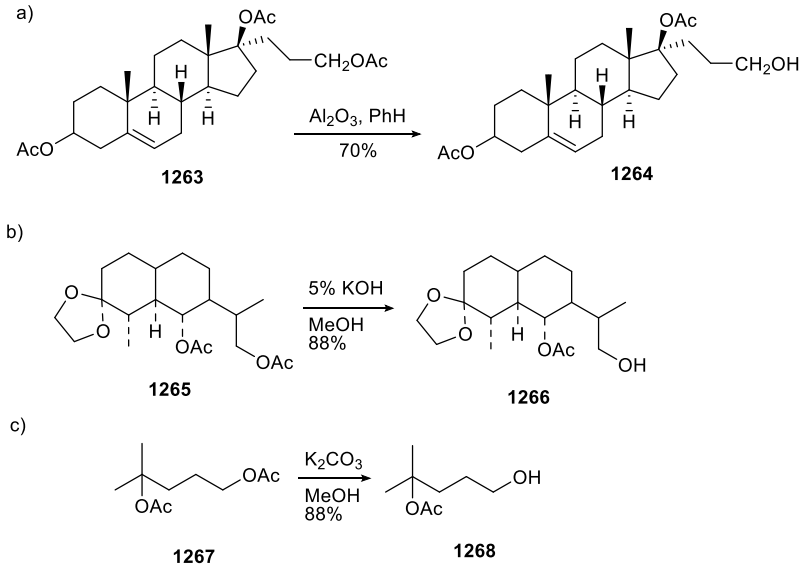
metal in methanol are also the reagents found capable of removing acetate groups in the presence of other esters.[197]

The selective deprotection of the ester group in the presence of the same type of esters is the other important subject in carbohydrate chemistry. It has great potential to be beneficial in complex syntheses by reducing the step counts for the protection processes. Primary, secondary, and tertiary acetates can be differentiated based on steric and electronic properties and thus can be selectively removed by proper choice of reagent. In carbohydrate chemistry, this process is more complicated because of the unique structure of carbohydrates, which can contain primary alcohols and a number of distinct secondary alcohols. Other than enzymes,[198] which are well known to be selective towards the cleavage of ester groups, BF_3•OEt_2[199] and Bu_3SnOMe[200] are chemical reagents that have been used to selectively remove acetates at the anomeric position in the presence of other acetates. Aluminum oxide,[201]potassium carbonate,[202] potassium cyanide[203]and potassium hydroxide[204] have all shown the ability to selectively deacetylate primary alcohols.[191]

Johns and Jerina reported the selective hydrolysis of steroidal acetates in 1963.[201] The primary acetate in **1263** was selectively deacetylated in the presence of a secondary acetate, using alumina in benzene, in moderate yield **(Scheme 1.42a)**. In a second example, 8 years later, Hirata and Yamamura reported the synthesis of Shyobunone and Sesquiterpene derivatives.[204] In their report 5% KOH in methanol was applied for the selective deprotection of a primary acetate in the presence of a secondary acetate and gave a successful result with 88% yield **(Scheme 1.42b)**.

In the synthesis of Eldanolide, Schmidt reported the removal of primary acetates in the presence of a tertiary acetate.[202] Schmidt exploited a methanolic solution of K_2CO_3 for the

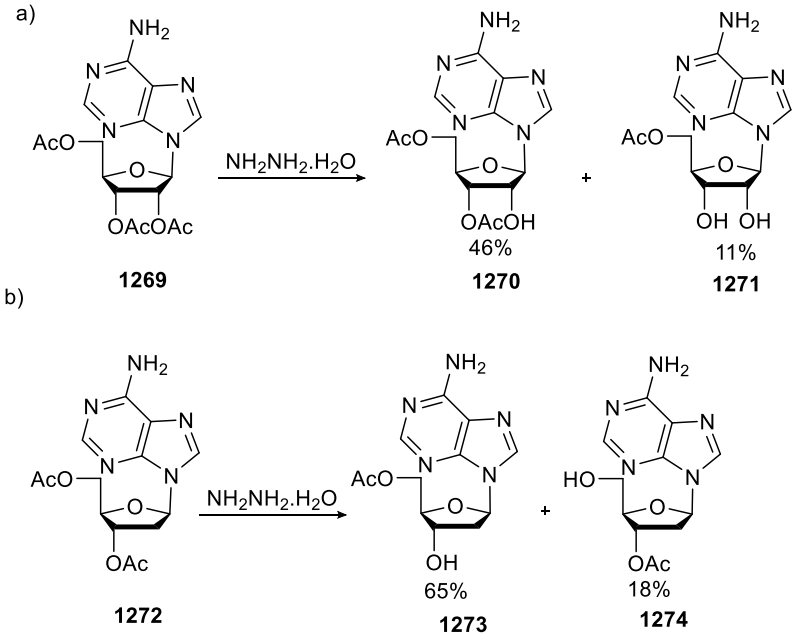
selective deacetylation of the primary hydroxyl group in excellent yield **(Scheme 1.42c)**. The selective deprotection of the primary acetyl group in the previous two examples seemed to garner their selectivity from the steric and electronic effects which make the primary acetate more reactive than the secondary acetate. In contrast, the relative stability of the acetate protecting group in carbohydrates are mostly based on the geometry and acidity of the masked hydroxyl groups.



Scheme 1. 42a,b,c Selective hydrolysis of steroidal acetates, a, selective deprotection of a primary acetate in the synthesis of Shyobunone, b, and selective removal of primary acetates in the presence of a tertiary acetate, c

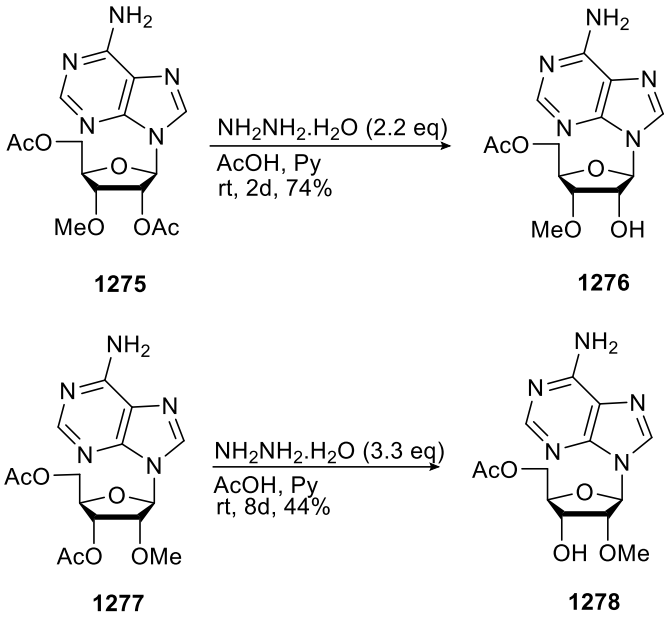
In 1979 Ishido and coworkers reported regioselective deacetylation of acetylated Purine and pyrimidine ribonucleosides using hydrazine hydrate.[196] This study showed that hydrazine hydrate reacts first with the 2′-O-acetyl group, then the acetyl group at the C-3 position and lastly, the acetyl group at C-5 **(Scheme 1. 43a)**. They rationalized that the hydroxyl group at the C-2 position, after acetyl deprotection, could interact with the C-3 acetyl group which may explain the rate enhancement of the C-3 compared to the C-5 acetyl deprotection. In order to eliminate this effect, they investigated compound **1272**

without a C-2 substitution **(Scheme 1. 43b)**. The deprotection procedure showed that the C-3 acetate group is deprotected faster than C-5 with or without C-2 participation. This result showed presence of C-2 does not affect in rate of deprotection of C-3 versus C-5.



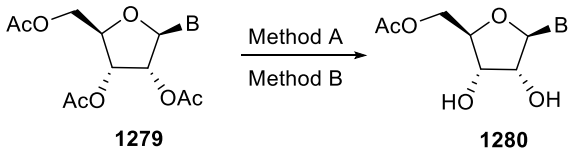
Scheme 1. 43a,b Regioselective deacetylation rate of acetylated compounds **1269** and **1272**

In the next experiment, they applied *N*- 6,2′,5′-triacetyl-3′-*O*-methyl- **1275** and *N*-6,3′,5′-triacetyl-2′-*O*-mehtyladenosine **1277** to evaluate the reactivity of 2′-*O*-acetyl group compared to 3′-*O*-acetyl group deacetylation **(Scheme 1.44)**. These experiments were performed in the presence of hydrazine hydrate in a mixture of pyridine and acetic acid. The results indicated that 2′-*O*-acetyl group is far more reactive than the 3′-*O*-acetyl functionality, as the 2′-*O*-acetyl was deprotected with 2.2eq. in 2 days and 74% yield, whereas the 3′-*O*-acetyl group was removed with 3.3 eq. of hydrazine in 8 days and 44% yield.



Scheme 1. 44 Selective deacetylation of *N*- 6,2′,5′-triacetyl-3′-O-methyl- **1275** and *N*-6,3′,5′-triacetyl-2′-*O*-mehtyladenosine **1277**

Robins tried the selective deacetylation of the 2′- and 3′-*O*-Acyl groups from 2′,3′,5′-Tri-*O*-acylribonucleoside derivatives using two separate procedures, both Dowex (CF_3CH_2O-) in 2,2,2-trifluoroethanol (TFE) and lithium trifluoroethoxide in TFE **(Scheme 1.45)**.[205] The experiments afforded the 5′-O-acyl derivatives in high yields and purity, especially using the latter set of conditions. This research illustrated a mild and rapid procedure for selective deacetylation that can tolerate a fused furan ring in furo[2,3-d]pyrimidin-2(3H)-one derivative, an amino group and a double bond in pyrimidines as well as the 6-(imidazol-1-yl) group in purines, because of the low nucleophilicity of $CF_3CH_2O^-$ ion.



1279 **1280**

	Mothod 1: Dowex 1x2 [$CF_3CH_2O^-$] Resin in TFE	Method 2:$LiOCH_2CF_3$/TFE
B: TrAde	66	88
TrCyt	–	93
BnUra	69	91

TrAde: 6-N-trityladenin-9- yl
TrCyt: 4-N-tritylcytosin-1-yl
BnUra: 3-benzyluracil-1-yl

Scheme 1. 45 Selective deacetylation of the 2′- and 3′-*O*-Acyl groups from compound **1279** using two separate procedures

It is important to mention a couple gaps in the literature at this point. First, the selective deprotection of the acetyl group in pyranosyl carbohydrate moieties are rarely investigated. Second, a reliable method does not exist for controlling the selective deprotection of acetate protecting group in the presence of alkyl acetates group of different lengths.

1.4 Non-biomedical application of carbohydrates, as facilitating agent for green solubility and degradability

This section highlights the potential application of carbohydrates outside of the biomedical applications discussed. It is related to the research reported in Chapter 7. This technology and the devices created from them have become critical in many aspects of our lives including healthcare, communications, entertainment, and manufacturing.[206] Faster than ever, electronic products are being updated, upgraded, and replaced as the lifetime of these devices become shorter and shorter. This planned obsolescence has led to a growing surplus of electronic waste around the world which has had a substantial environmental impact.[207]

Recycling is a difficult and expensive process, and as a result, most of these toxic materials are being deposited directly into landfills. The United Nations estimates that 50 million metric tons of electronic waste is discarded worldwide each year.[208] Further, the Environmental Protection Agency estimates that only 15%-20% of electronic waste is recycled.[208] The remainder of that waste ends up in traditional landfilling which may result in leaching of toxic components of electronic waste, such as lead, mercury, and cadmium, into the ground water.[208] It is clear, that there is an imperative to develop more environmentally friendly materials to be used in these products.

Green chemistry offers us the tools needed to build a sustainable industrial process. In 1997, Paul Anastas and John Warner defined green chemistry as the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances.[209] Green chemistry has 12 principles which include the following:

prevention;

atom economy;

less hazardous chemical syntheses;

designing safer chemicals;

safer solvents and auxiliaries;

design for energy efficiency;

use of renewable feedstocks;

reduce derivatives;

catalysis;

design for degradation;

real-time analysis for pollution prevention;

and inherently safer chemistry for accident prevention. [2]

The development of economically feasible and ecologically benign alternatives to traditional synthetic reactions is highly desirable.[210] From an industrial perspective, the interest in a green chemistry approach include the public relations benefits of being labeled as a "green" company, lower production costs by using fewer materials, lower waste costs due to less waste being processed and decreased toxicity in the waste streams produced. In this regard, the application of green chemistry for the development of low-waste and energy efficient synthetic approaches to organic electronic synthesis is advantageous.

To address the environmental issues associated with the ever-growing problem of electronic waste, degradable, organic electronic materials have been gaining interest for many low-cost applications.[211] Biodegradable electronics, which are electronic devices manufactured with biocompatible and biodegradable materials, are in high demand due to their positive financial and environmental aspects.[208] They have several advantageous qualities including their environmental safety, nontoxicity, low cost, which can obtained in large scale, and ease of disposal compared to traditional silicon-based electronics.[208] The degradability is crucial for eliminating hazardous waste products as well as the associated health risks and costs associated with recycling operations.[208] Additionally, biodegradable electronics can be used in implantable biomedical applications.[208] Their use enables the electronic devices to be completely or partially absorbed into and excreted from the body after functioning under physiological conditions.[208]

Conjugated polymers, which are based on alternating electron donor-acceptor units, have gained interest in the field of organic electronics.[212] Specifically, diketopyrrolopyrole

(DPP)-based polymers are good candidates for use in degradable technologies. This polymer is environmentally benign and is known for its favourable charge carrier properties. Diketopyrrolopyrole (DPP)-based polymers show high charge carrier mobility in organic field-effect transistors (OFETs).[212] This is due to the coplanar nature of the conjugated backbone, short-contact oxygen-sulfur interactions, and cross-axis dipoles which result in strong intermolecular pi bonding interactions and therefore long-range order.[208] The rigid polymer backbones, high molecular weights, and strong aggregation properties of conjugated polymers is essential for good charge transport. However, these properties result in challenges in regard to the dissolution of high-mobility polymers for organic field-effect transistors (OFETs).[213] The conjugated polymers exhibit strong intermolecular interactions, which cause large free volume dissimilarity between polymer and solvent molecules and the free energy change of solvation is less negative.[213] It is therefore clear that improving the solubility properties of (DPP)-based polymers, while maintaining the properties which make them good charge transporters, is a worthwhile goal that will lead to the creation of polymers which are ideal candidates for use in degradable electronics.

When evaluating the environmental performance of a chemical processes, solvents have a major impact on cost, safety, and health issues. Furthermore, solvents are a major input in the synthetic processes and typically comprise the largest fraction of waste generated.[210] Traditional solvents account for most of the mass wasted in synthetic and industrial processes and many common solvents are toxic, flammable, or corrosive.[214] Often, the large volumes of solvents used are not directly responsible for the composition of a reaction product, nor are they an active component of a formulation.[214] Therefore, the use of environmentally damaging solvents, which have no impact on the function or progress of the

system, seems unnecessary. When possible, even the recovery and reuse of these solvents is associated with energy-intensive distillation which creates unwanted air emissions and the safety risks associated with worker exposure.[209] The concept of green solvents is intrinsically linked to the principles of green chemistry. Introducing a green solvent to a chemical process provides a large reduction of the environmental impact of said processes.[214] These solvents, which are bio-sourced and non-toxic, are derived from renewable feedstock and the processing of agricultural crops. To be useful these solvents must be capable of replacing traditional solvents for use in extractions, separations, formulations, and reaction chemistry.[214] Environmentally preferable solvents can be determined by the GSK Solvent Selection Guide.[215] The greenest option for solvents is, of course, water. The saccharide-containing backbone with hydroxyl functionality inserted into the modified conjugated polymers makes them soluble in water.

The ever-increasing need for non-toxic and environmentally friendly electronic devices is evident in our world. The field of green chemistry, including organic electronics and green solvents, allows us the opportunity to utilize sustainable processes and bio-sourced materials to minimize the environmental impact at each stage of the product lifecycle.

1.5 Summary

This thesis focuses on the application of novel synthesis for accessing carbohydrates for diverse applications. The focus is entirely on the use of the end-goal carbohydrates, target-oriented synthesis; however, this provides an extremely useful platform for conducting discovery research as we shall see in the coming chapters. Carbohydrates have been underexplored in immunoregulation and materials science, and this work aims to help

the global refocus on these extremely diverse, useful, yet synthetically challenging biomolecules as they offer new solutions to longstanding challenges to society.

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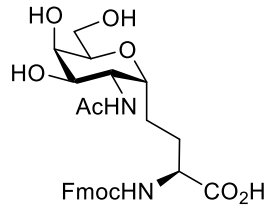
Chapter 2: Goals and Objectives

2.1 Synthesis of biostable *N*-Fmoc protected acetal-free exact analogue of Tn antigen

The first objective of this research, discussed in Chapter 3, is to accomplish the synthesis of the exact C-glycoside analogue of the Tn antigen. This molecule depicted in **Figure 2.1** has not been synthesized to date. In particular, a *N*-Fmoc protected acetal-free analogue of the Tn antigen is targeted in order to enhance the stability of this antigen. The Fmoc protecting group is already attached to the amino acid moiety so that solid phase peptide synthesis can be conducted resolving the T-cell recognition problem discussed in Chapter 1. Once synthesized a secondary goal will be to attach compound **201** to a short peptide chain and send it for biological examination. Both acetal-free and native Tn antigens will be evaluated together to see if the difference in immune response between the two antigens. We want to determine whether an immune response is generated from our acetal-free Tn antigen, specifically to see if these antigens can trigger the immune system to attack cancer cells.



201

Figure 2. 1 Structure of acetal-free Tn antigen **201**

2.2 Toward the synthesis of biostable acetal-free TF antigen

To date, only one reported TF antigen analogue has been synthesized and evaluated in immunological studies. This analogue was prepared and examined by Vogel in 2012 and it shown in **Scheme 2.1**.[1] This was the first synthesis of the single C-glycoside version of the TF antigen, which was synthesized in more than 40 steps, however, some questions remain unanswered. Is the

vaccine candidate prepared by Vogel specific for cancer cells? Or, is this activity only observed because of the peptide chain, as it is independently capable of activating T-cells. Moreover, this C-glycoside antigen was never examined against the natural TF antigen to show whether the generated immune response is also specific for the native antigen.

The second objective of this research, discussed in Chapter 4, is to remove the unstable acetal functionality in the TF antigen by replacing the exocyclic anomeric oxygen with a methylene group (C-glycoside), thus creating the first fully-acetal-free C-glycoside analogues of the TF antigen. We will attempt to design this synthesis with a reasonable overall step count, and ultimately try to attach a peptide chain to this antigen for biological evaluation. Removing the labile functionality should result in enhanced biostability relative to the native system, likely, with no loss in recognition specificity as the exocyclic oxygen is not generally involved in molecular recognition events.

2.3 Total Synthesis of glycolipids from *S. pneumoniae* and the biological re-evaluation

The third objective for this thesis is the synthesis of mono- and disaccharide glycolipids **(Figure 2.2)**, which are isolated from *pneumonia*, for the purpose of biological evaluation. Despite a few reported syntheses of the monosaccharide glycolipid, to date, no biological examination has been performed on these synthetic analogues. Kinjo, in 2011, reported for the first-time isolation and identification of these glycolipids and showed their ability to activate iNKT cells.[2] However, these tests were carried out using minimal amounts of the isolated glycolipids in questionable purity.[2] As a result, it is impossible to ascertain whether the activity observed was due to these glycolipids or due to some minor impurity. Also, there remains some questions about the unusual structures of the glycolipids due to insufficient structural evidence in the 2011 report to confirm

the proposed structures conclusively. Synthesis of this type of glycoside diglycerides, which includes either the same or different fatty acid residues, requires significant effort to manipulate the protecting groups in both sugar and glyceride parts in order to get the desired selectivity and avoid any conflict in protecting and deprotecting strategies. These approaches commonly suffer from lack of high efficiency, flexibility and stereopurity.[3-5] On the other hand, synthetic procedures using the starting material with well-established stereopure glycoside associated with an appropriate protecting group is costly.[5, 6] Here, an efficient, total synthesis of both *S. pneumonia* glycolipids is reported, and our surprising re-evaluation of their immunological activity will be discussed.

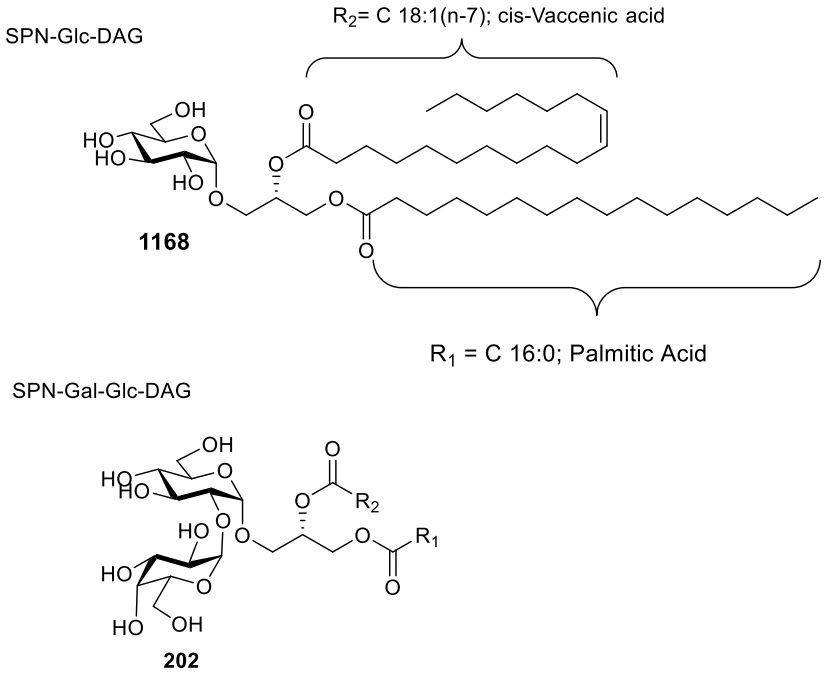


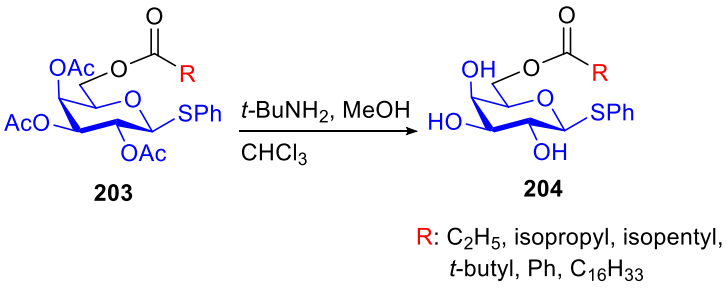
Figure 2. 2 Structure of mono- and disaccharide glycolipids **1168** and **202.** Synthetic targets for Chapter 5

2.4 *t*-Butylamine as a mild and selective deacetylation agent

The selective hydrolysis of esters has always been an exciting subject among carbohydrate chemists, especially when the result is saving a few synthetic steps which can become essential for an efficient synthesis. In particular, the selective removal of acetates in the presence of longer

chain esters remains a significant challenge in carbohydrate chemistry. In the synthesis of the disaccharide glycolipids project discussed in Chapter 5, the acetyl protecting group had to remove selectively in the presence of glycerol ester chains. The only reagent reported in the literature suitable for this transformation was not able to selectively deprotect acetyl groups without also degrading the disaccharide glycolipid. All listed chemical methods in Green and Wutz either failed to provide selectivity or degraded the complex carbohydrate. To address this challenge, we had turned to methoxyacetates as an alternative protecting group. Standard conditions for their cleavage involve the use of *tert*-butylamine, although the precise mechanism of action remains unclear. Furthermore, this reagent has never been used for the deacetylation of carbohydrates.

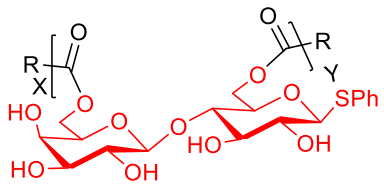
A fourth objective of this thesis, discussed in Chapter 6, is to study the mechanism and evaluate the steric and electronic effects of *t*-butyl amine as a mild, selective, and inexpensive reagent for the selective deprotection of the acetyl group in the presence of different ester functionalities. The potential application for the selective hydrolysis of acetates in protected carbohydrates, as well as our explorations into the chemoselectivity, kinetic profile, and scope of this reaction will be discussed.



Scheme 2. 1 Selective acetyl deprotection in the presence of different esters at C-6 position using *t*-$BuNH_2$

2.5 Conjugated polymer backbone modifications for greener solubility and degradability

The final goal of this research is to utilize the hydrogen bonding inherent in saccharides to improve the solubility of conjugated polymers in greener solvents and introduce a degradable component to the polymer backbone. The objectives of the project include developing a series of saccharide-containing backbone modified conjugated polymers for electronic devices (shown in red in **Figure 2.3**); achieve solubility in greener solvents for eco-friendly device processing; and introduce the possibility of enzyme-catalyzed degradability to the polymer backbone through ester linkages for waste reduction. Saccharides have several characteristics that make them an advantageous choice for organic electronics: low cost, bio-sourced, inherent degradability, and are already found to improve aqueous solubility in pharmaceuticals. In this thesis the acetylated lactose bearing free hydroxyl groups at the C-6 and C-6′ is targeted. This target molecule will then be sent to our collaborator for polymerization and evaluation of the resulting properties.



205

Figure 2. 3 Proposed oligosaccharides conjugation-breaking polymer units **205**

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Chapter 3. Synthesis of an *N*-Fmoc protected, biostable acetal-free analogue of the Tn antigen

3.1 Synthesis of the C-glycoside acetylated glucosamine

The synthesis of the *N*-Fmoc protected acetal-free Tn antigen, **201**, has attracted chemists' attention as a way to increase the biostability compared to the native antigen. Even with this attention, there is no synthetic analogue of this antigen linked to the peptide reported, compound **301 in Figure 3.1**, and no biological examination has been performed to date.[1] Only two attempts have been made towards the synthesis of the exact analogue of the native Tn antigen, **101a (Figure 3.1)**. Although these two reports were valuable contributions, both required modifications to the synthetic route in order to be completed, and as a result, the ultimate target was never synthesized, and the biological activity never examined.

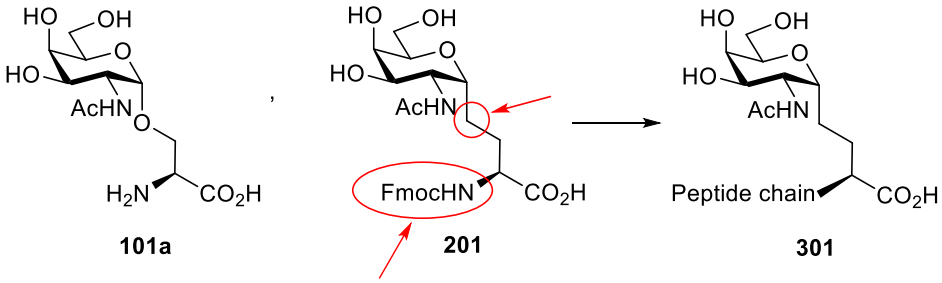
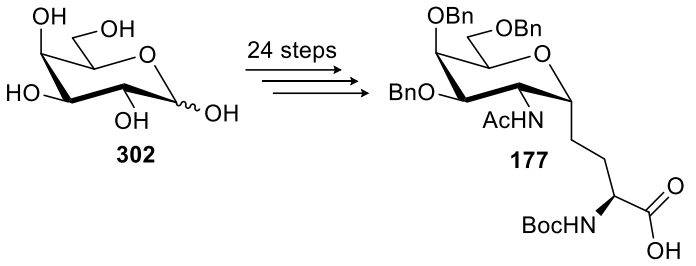


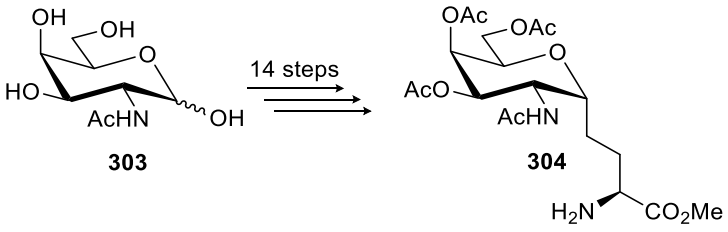
Figure 3. 1 Structure of the Acetal-free Tn antigen target **301** and native Tn antigen **101a**

As mentioned in the introduction, the first attempted synthesis of a C-Glycoside analogue of the Tn antigen was reported by Beau and coworkers in 1998 using a samarium iodide strategy.[2] This synthesis was performed in 24 steps to generate a C-glycoside analogue of the Tn antigen; however, the sugar moiety was left protected with the benzyl group, and the Boc amino protecting group remained in the amino acid moiety **(Scheme 3.1)**. Deprotection of the benzyl group and replacement of the Boc group with an Fmoc protecting group, necessary for most modern solid phase peptide synthesis, are still required in order to achieve the desired acetal-free Tn antigen; this represents a minimum of three additional steps.



Scheme 3. 1 The first synthesis of the C-Glycoside Analogue of Tn antigen reported by Beau

Three years later, Schmidt reported a relatively short synthesis of the acetal-free analogue of the Tn antigen.[3] This compound, however, was never biologically evaluated, as it was never integrated into a peptide. This synthesis was performed in 14 steps, however, the synthetic approach saved multiple steps by using galactosamine hydrochloride as a starting material **(Scheme 3.2)**. This starting material is very costly (~$328/g, Sigma Aldrich). Moreover, both the acetyl protecting group in the sugar moiety and the methyl ester need to be removed, and more importantly, the free amine needs to be protected with an Fmoc functionality for the peptide synthesis. This synthesis was clearly never intended to provide a building block. Consequently, a more streamlined synthetic strategy which uses a more readily available starting material and allows for complete deprotection of the sugar and attachment of an Fmoc groups to the amino acid is required.

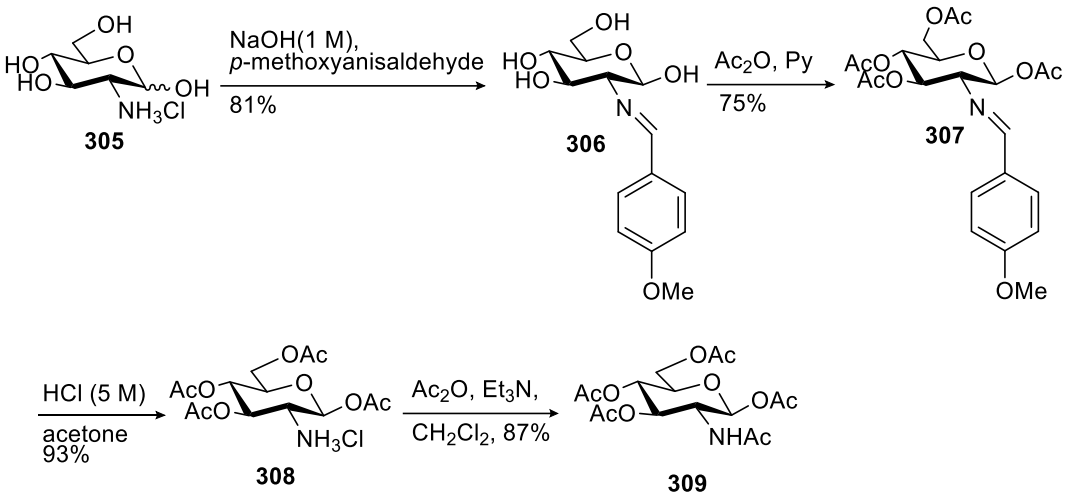


Scheme 3. 2 Short synthesis of the acetal-free analogue of the Tn antigen by Schmidt

To begin the synthesis of **301**, the glucosamine hydrochloride was used as a starting material, as commercial galactosamine hydrochloride is not economically available, and we planned to

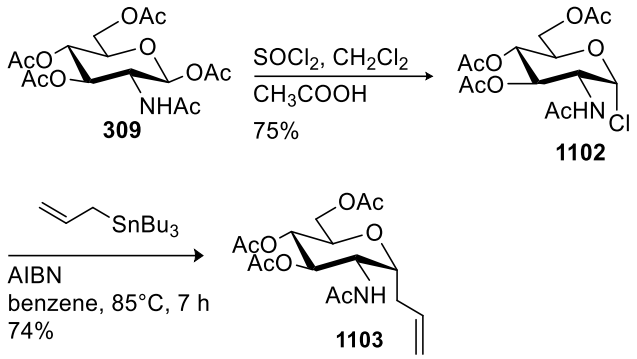
convert to galactosamine moiety in further steps, allylation of the galactosamine is also more challenging than of glucosamine from experience.[4] The very first step, acetylation of glucosamine hydrochloride, which was supposed to be an easy step, failed to give the right β-anomer as α-anomer is usually inactive towards the glycosylation reactions, because α-anomer is more stable due to anomeric effect. Despite trying multiple published protocols, which reportedly would provide the β anomer from acetic anhydride, including catalysis with zinc chloride[5] and pyridine,[6] all exclusively afforded the α-acetyl anomer. Finally, fully acetylated glucosamine was synthesized based on the Zhang's 4 step procedure, which seems to be the only way to synthesize the β-anomer of acetylated glucosamine.[7] This is yet another example of the lack of reproducibility in the carbohydrate literature.

In this procedure, shown in **Scheme 3.3**, the amine group of **305** was protected using anisaldehyde and a 1M solution of NaOH, then pyridine and acetic anhydride were applied for acetylation of the hydroxyl groups. In this reaction, the large steric bulk of the imine functionality pushes an anomeric acetyl group to re-orient to the β-anomer. The imine functionality was then hydrolyzed *via* 5M HCl in acetone. Finally, the acetamide functionality was generated using Et_3N and acetic anhydride. It is worth mentioning that, although this procedure seems to be lengthy, this procedure can be performed on a large-scale without chromatographic purification at any step of the preparation of **309**, and the *p*-methoxyanisaldehyde can be easily recovered to make it quite economical.



Scheme 3. 3 Synthesis of acetylated glucosamine **309**

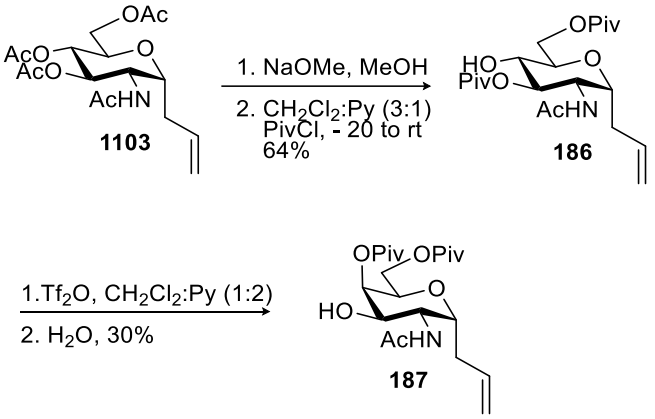
The next steps involved the synthesis of C-glycoside glucosamine starting from acetylated glucosamine based on a report by Cipolla **(Scheme 3.4)**.[8] The allyl group was chosen because it can be exploited in the construction of the amino acid linkage later in the synthesis. First, the anomeric acetyl group was replaced with chlorine using thionyl chloride and acetic acid with a 75% yield. The allylic C-glycoside compound **1103** must be prepared carefully as this reaction is sensitive to any moisture or air. A solution of compound **1102** in distilled benzene, AIBN and allyl tributylstannane was degassed for 45 minutes by bubbling N_2 through the solution. The reaction mixture was then heated to reflux to afford the desired allylic compound **1103** in moderate yield. It should be mentioned that there is a limitation for using this protocol on larger scales; AIBN must be added portionwise to maintain acceptable yields.



Scheme 3. 4 Synthesis of compound **1103**

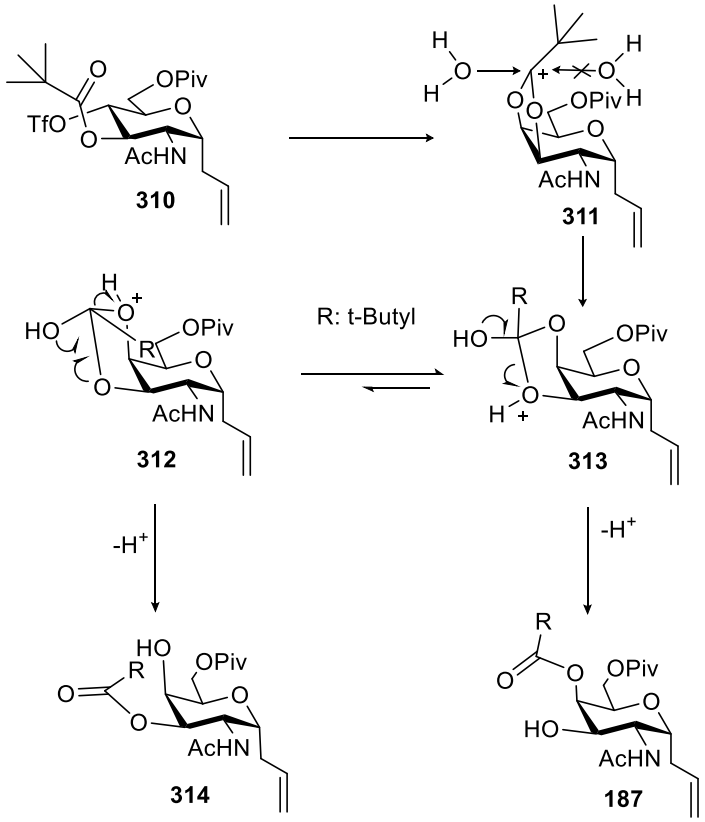
3.2 Synthesis of galactosamine aldehyde 317

The glucosamine moiety was converted to galactosamine using four subsequent steps shown in **Scheme 3.5**. First, the initial acetyl groups were removed *via* a solution of sodium methoxide in methanol. Hydroxyl groups in C-3 and C-6 were then selectively protected with the Piv group using pivaloyl chloride in pyridine and dichloromethane. The galactose derivative was subsequently achieved by successful inversion of the C-4 hydroxyl group *via* S_N2 substitution using Tf_2O in pyridine and dichloromethane and subsequent hydrolyzed with the addition of water.



Scheme 3. 5 Conversion of glucosamine to galactosamine **187**

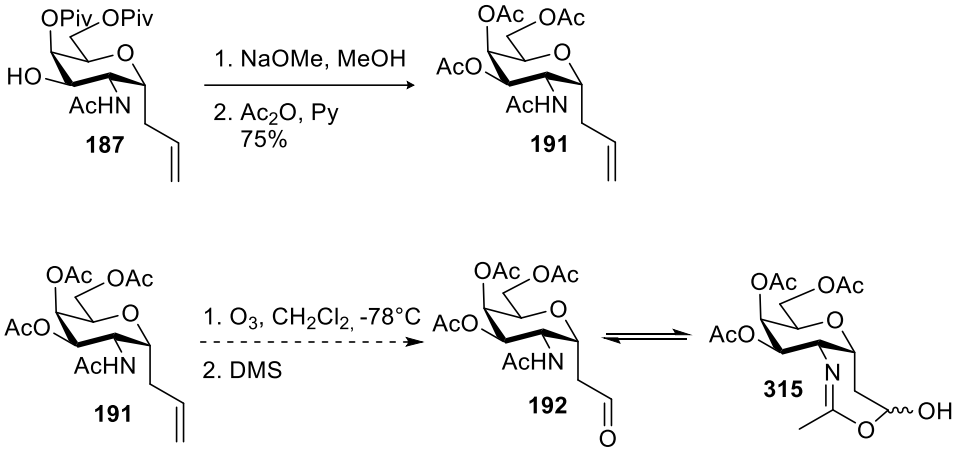
The mechanism of this procedure was well explained by Ben and is shown below.[9] First the carbonyl oxygen of C-2 removes the triflate, followed by carbocation hydrolysis from the opposite side of the *tert*-butyl orientated face. The intermediate **313 (Scheme 3.6)** is generated after a proton transfer to the oxygen at the C-3 position. The galactose moiety with having a pivaloyl ester protecting group at C-4 and C-6 positions **187** forms with the oxygen atom's assistance and deprotonation. However, compound **314** is not observed for two reasons, first, intermediate **312** is less favourable due to steric complication between the *tert*-butyl and sugar moieties. Second, better stability of protonated oxygen at the equatorial position in compound **313** compares to protonated oxygen at the axial position in compound **312**.



Scheme 3. 6 The proposed mechanism for the formation of galactosamine **187**

The pivaloyl-protected galactosamine was subjected to a Zemplén reaction to remove the protecting group, then hydroxyl groups were protected with an acetyl group using pyridine and acetic anhydride, which is necessary for asymmetric hydrogenation in later steps.[10] To attach the amino acid to the sugar moiety through an allyl functionality, the allyl group had to be converted to an aldehyde, which can be linked to the amino acid fragment using the Horner-Wadsworth-Emmons olefination reaction.

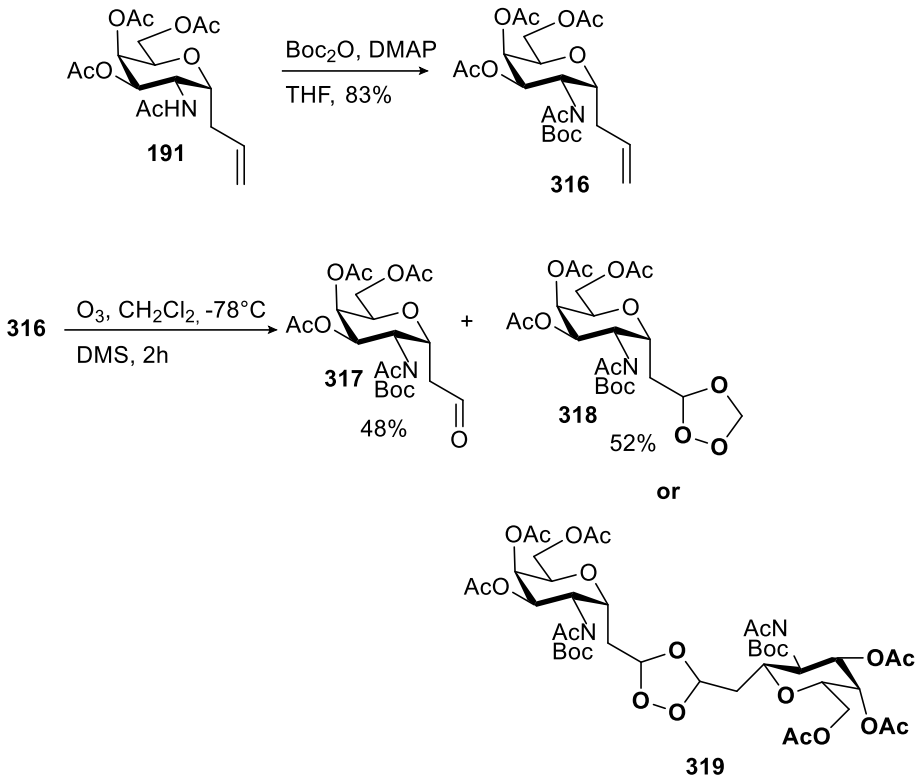
The production of Aldehyde **192** was expected without difficulties, as reported by Schmidt.[3] However, this reaction did not afford acceptable product usable for the next step. The presence of an equilibrium of a five- and/or seven-membered cyclic forms of aldehyde were expected;[11, 12] however, an attempt to purify and apply the mixture for the next step did not result in the desired product.



Scheme 3. 7 Attempt to the formation of aldehyde **192**

In order to eliminate the equilibrium forms of the aldehyde, the nitrogen in the acetamide functionality was inactivated by protection with the Boc group. A mixture of Boc_2O, DMAP and pyridine was treated with compound **191** to afford **316 (Scheme 3.8)**. Next, the ozonolysis was performed at –78°C and dimethyl sulfide was used to generate the aldehyde. Although this reaction

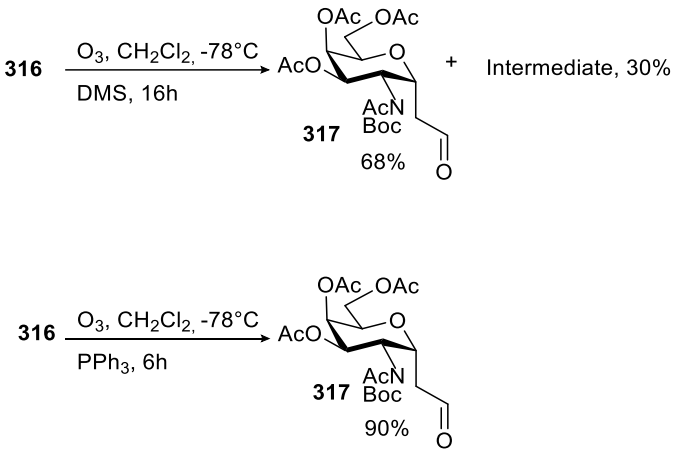
gave pure desired aldehyde after purification, the yield was low, and surprisingly the trioxolane intermediate was stable enough to be isolated. The exact structure of a mono-or disaccharide trioxolane intermediates could not be determined using standard NMR techniques and mass spectrometry. X- ray diffraction studies will have to be performed to determine the exact structure of this intermediate. Regardless, the result showed clearly that the generation of the aldehyde from the trioxolane is a slow process, presumably due to the low reactivity of dimethyl sulfide.



Scheme 3. 8 Synthesis of aldehyde **317** accompanied by intermediates **318** and **319**

Two sets of reaction conditions, **(Scheme 3.9)**, were set up to optimize the yield of this reaction. First, the ozonolysis was performed, and dimethyl sulfide was added, and the mixture stirred for 16 h at room temperature. The second approach used the same initial ozonolysis, but this time triphenylphosphine was used to open the trioxolane intermediate. The result of the first reaction indicated the presence of an intermediate by TLC. Increasing the reaction time did lead

to a slightly improved yield but was not ideal when compared to the PPh_3 reaction. The second reaction was completed in 6 hours with a 90% yield indicating that PPh_3 is far more reactive compared to dimethyl sulfide in this specific reaction. As the reader is aware, these are the two standard agents for resolving ozonides, and the relative merits of each are beyond the scope of this thesis.

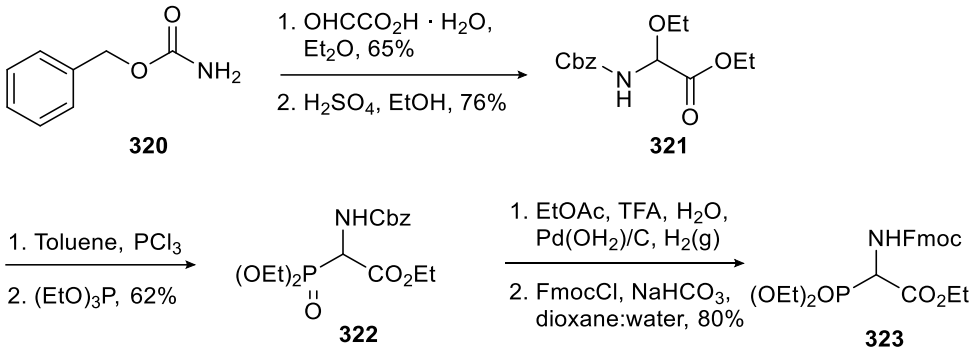


Scheme 3. 9 Optimization of aldehyde formation through ozonolysis procedure

3.3 Synthesis of biostable acetal-free Tn antigen bearing *N*- Fmoc protected amino acid

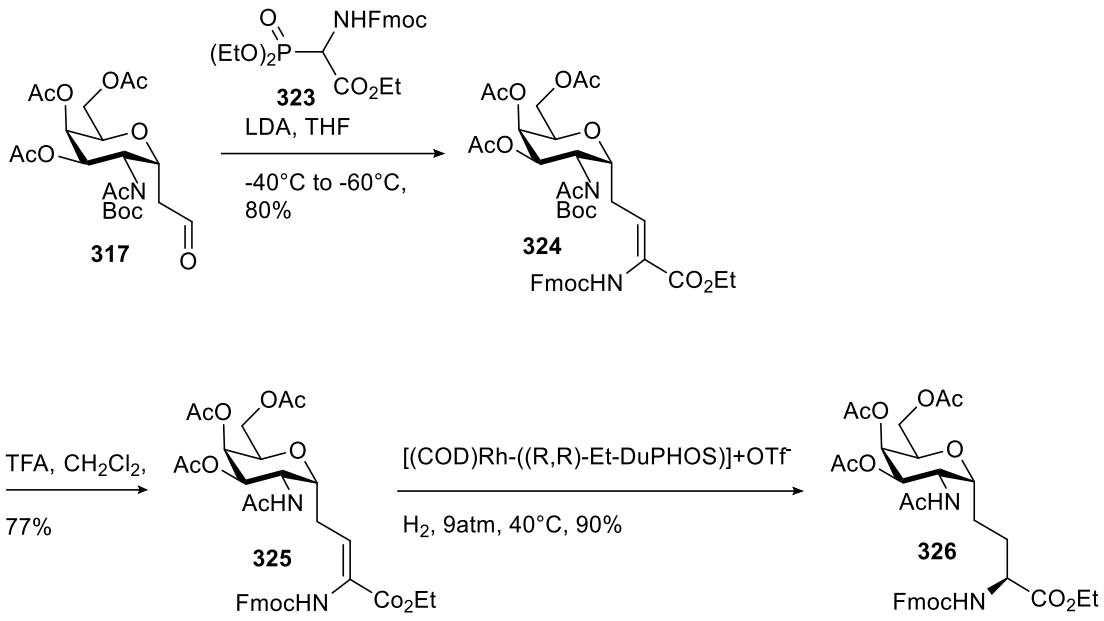
With aldehyde **317** in hand, attachment of the amino acid appeared to be straight forward. First, the synthesis of phosphonate **323** can be performed as shown in **Scheme 3.10** and subsequently linked to the sugar fragment by a Horner-Wadsworth-Emmons olefination reaction that will result in the serine amino acid addition.[13] Synthesis of phosphonate **323** started from glyoxylic acid monohydrate and benzyl carbamate, which reacted in diethyl ether to produce α-Hydroxy-*N*-benzyloxycarbonylglycine in moderate yield.[14] The concentrated H_2SO_4 and EtOH were added to **320a** to generate the ethyl ester and protect the alcohol functionality.[13] Compound **321** then underwent a reaction with phosphorus trichloride and triethyl phosphite to produce the phosphonate. The CBz protecting group was converted to Fmoc in two sequential steps. The CBz group was first removed using Pearlman's catalyst and TFA in ethyl acetate, then Fmoc was applied for the

protection of the amine using fluorenylmethyloxycarbonyl chloride and $NaHCO_3$ in dioxane and water. This reaction afforded the desired Fmoc protected Serine amino acid derivative ready for olefination in the next step**.**



Scheme 3. 10 Synthesis of *N*-Fmoc protected amino acid **323**

Gratifyingly, aldehyde **317** and amino acid **323** underwent a Horner-Wadsworth-Emmons reaction in the presence of LDA to give the desired product in good yield. The sugar moiety was successfully linked to the amino acid counterpart through the alkene functionality to generate the first Tn antigen backbone containing the *N*-Fmoc protecting group **324 (Scheme 3.11)**. The Boc protecting group in the sugar moiety was then successfully removed using TFA in 77% yield. The olefin group was asymmetrically reduced using a chiral rhodium complex in a hydrogen atmosphere at 200 Psi.[10, 15-19] The result gave a 90% yield of compound **326**, ready to use for the last step, final deprotection.



Scheme 3. 11 Synthesis of compound **326**

The [1]HNMR spectrum of **326** showed broad peaks, which was most likely caused by the restricted rotation between Fmoc rotamers depicted in **Figure 3.2**. This phenomenon is well known as the Fmoc protection group is widely utilized in solid-phase peptide synthesis.[20] The high barrier for rotation around the C–N bond usually leads to a broadening or in some cases even increasing and decreasing the intensities of some of the peaks due to the durability of the two major rotamers. In order to prove that restricted rotation was the cause of the atypical NMR spectrum VT-NMR spectroscopy was performed. The sample was heated at three temperature points 22°C (room temperature), 35°C and 50°C and spectrum were recorded to observe any changes to the molecule`s NMR signals. As was expected, changing the temperature changed the intensities of some of the peaks associated with the Fmoc protecting group, which supports the idea of restricted Fmoc rotation around the C–N bond (see supplementary data page 344).

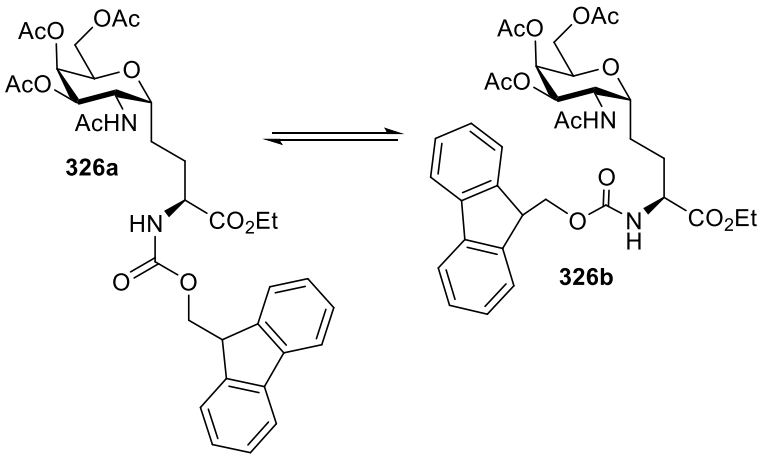
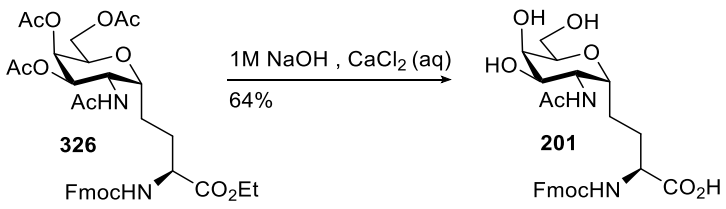


Figure 3. 2 Fmoc rotation in an equilibrium

The final step, selective hydrolyzation of the acetate group and the ethyl ester functionality appeared to be challenging because under basic conditions all the carbamate and ester functionalities are usually removed and selective hydrolyzation of ester groups in the presence of carbamates is extremely challenging. Three reaction procedures were examined to selectively remove two different ester groups in the presence of an Fmoc group. In the beginning, two sets of acidic conditions were applied for the selective deprotection of both acetyl and ethyl ester groups. First, concentrated HCl with acetic acid and second, anhydrous HCl in dioxane were applied. Both reactions started at room temperature and were then heated to reflux. Both reactions did not afford the desired product, but instead gave a mixture of partially deprotected compounds containing some remaining acetyl group. Finally, the acetal-free Tn antigen analogue **201** was obtained in moderate yield using a 0.8 molar solution of $CaCl_2$ and NaOH in the solution of isopropyl alcohol and water.[21]



Scheme 3. 12 Synthesis of C-glycoside Tn antigen **201**

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Chapter 4: Synthesis of biostable acetal-free TF antigen

As mentioned in Chapter 1, carbohydrate-based antigens have great potential as cancer vaccines. In particular, the TF antigen, which is responsible for many carcinomas, attracted scientist's attention for the cancer vaccine application, however, these attempts ultimately did not lead to the desired vaccine, mainly due to the low stability of this antigen. On the other hand, these results led to the idea of attempting to synthesize the acetal-free TF antigen in order to address the low stability of this antigen. Despite several promising attempts, still, synthesis of fully acetal-free TF antigen remained under exploration. In order to improve the stability of the TF antigen an acetal-free TF-antigen was selected as a synthetic target. The Tn antigen serves as the core of both the TF antigen (**Figure 4.1)**. One underlying problem of how to link an amino acid to a carbohydrate conjugate needs to be solved to synthesize the target molecule. Conveniently, solutions to this problem were explored in the synthesis of the Tn antigen (Chapter 3) and can be applied here. The two carbon linker between the amino acid and the carbohydrate brings a certain degree of flexibility to the potential synthetic solutions. A more challenging obstacle is the synthesis of an anomeric carbon-carbon bond using the one-carbon linkage required for the preparation of the inter-glycosidic bond. This is an exceptionally challenging problem, and there are only a few existing solutions for this challenge. In many aspects, this is more challenging for the TF antigen than the sTn antigen. In particular, the glycosidic linkage in the sTn antigen is formed through the primary C-6 OH of the sugar, which provides an extra carbon allowing for a bit more synthetic flexibility.

The first challenge involves the attachment of the *N*-Fmoc protected amino acid to the galactosamine moiety was already solved in the synthesis of the Tn antigen. The most challenging part is the formation of a carbon-carbon bond between an anomeric carbon of galactose and the C-

3 position of the galactosamine moieties using a one-carbon linkage. Although Vogel solved this problem using Baylis Hillman reaction, his synthetic route is quite lengthy. It contains over 40 steps and did not end in the fully acetal-free TF antigen. Thus, completing his synthesis towards an actual fully acetal-free analogue of TF antigen would not be practical.

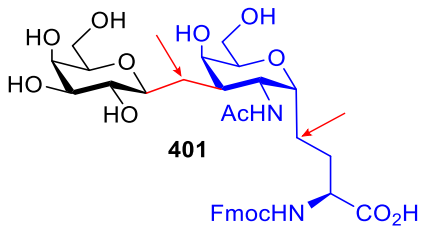
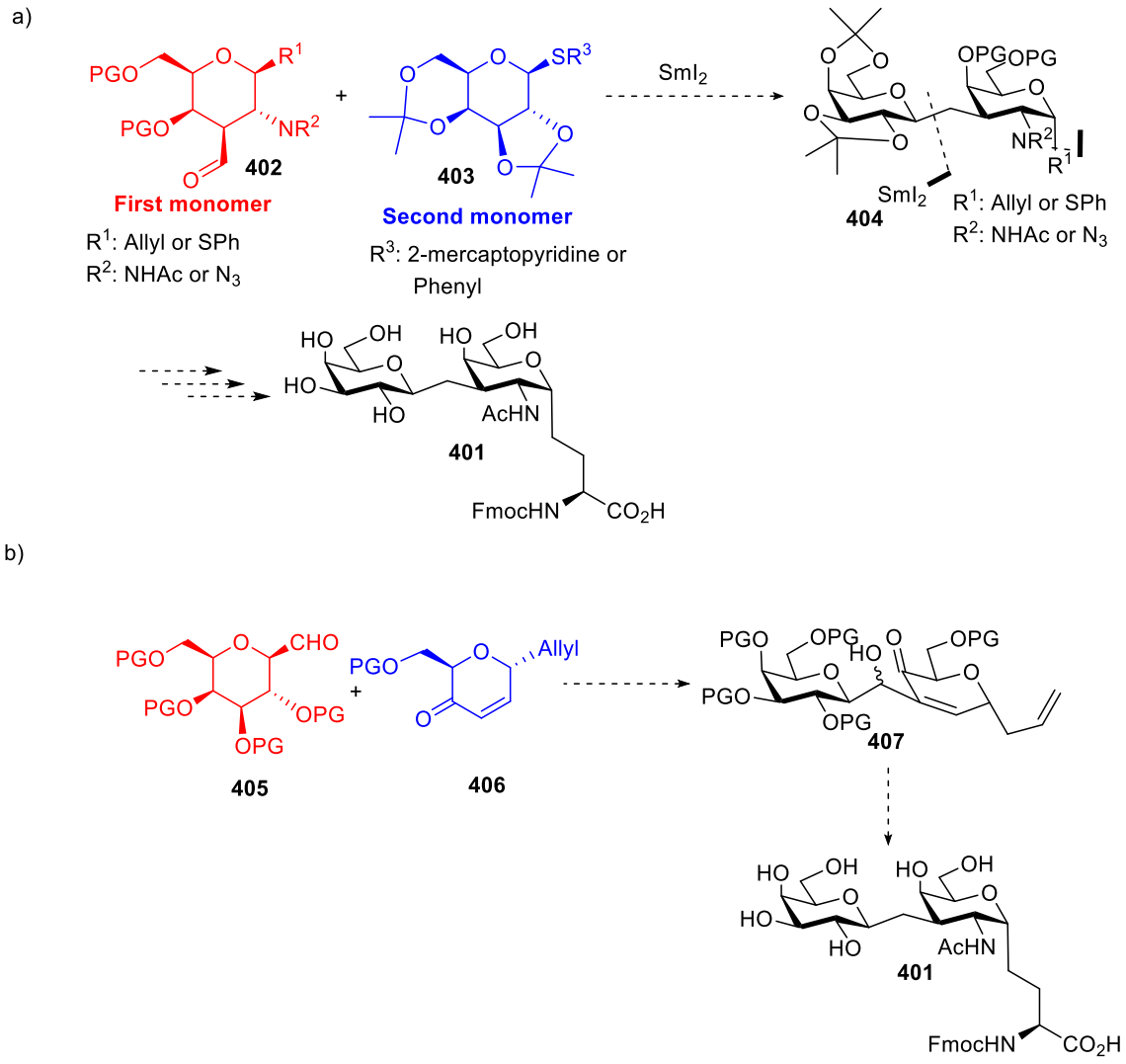


Figure 4. 1 Fully acetal-free TF antigen target **401**

The aldehyde functional group, either in the galactose or the galactosamine component, seems to be the key in the synthesis of acetal-free TF antigen. This aldehyde moiety can be converted into other useful functionalities or participate directly in nucleophilic or radical substitution approaches. In this research the two main synthetic approaches, were attempted. The first involves the synthesis of aldehyde **402** in the galactosamine moiety and using samarium iodide chemistry as the key step to attach the aldehyde to the second monomer bearing the sulfone functionality **(Scheme 4.1a)**. The second is the synthesis of aldehyde **405** in the galactose moiety, which can be linked to the enone monomer, **406**, which already has an allyl group attached to the anomeric position to form double C-glycoside, through a Baylis-Hillman reaction as the key step.[1] Both routes would then require further transformation to achieve the desired acetal-free TF antigen **(Scheme 4.2b)**. In addition to those main approaches, a few side approaches were attempted as well, which will be discussed in this chapter.



Scheme 4. 1a,b Two different proposed synthesis of acetyl free TF antigen, a and b

Although the installation of the aldehyde functionality initially seems easier in a galactose sugar due to the lack of a nitrogen source at the C-2 position, the proposed synthesis of this molecule would require a longer synthesis than the galactosamine derivatives, because after forming the linkage between two sugars, more manipulation must be done in order to achieve the final product, including; introducing nitrogen at the C-2 position, dihydroxylation, selective reduction of ketone and lastly, attachment of amino acid to the galactosamine sugar part **(Figure 4.2**).

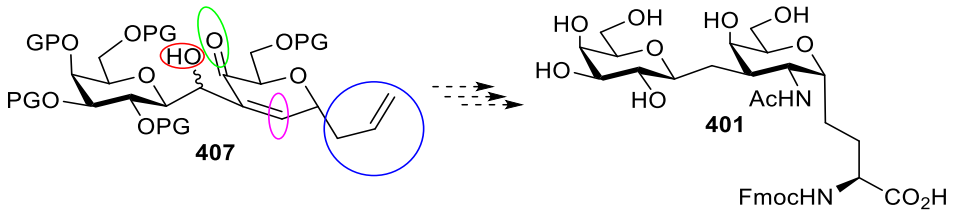


Figure 4. 2 Highlighted challenges towards the synthesis of **401** from **407**

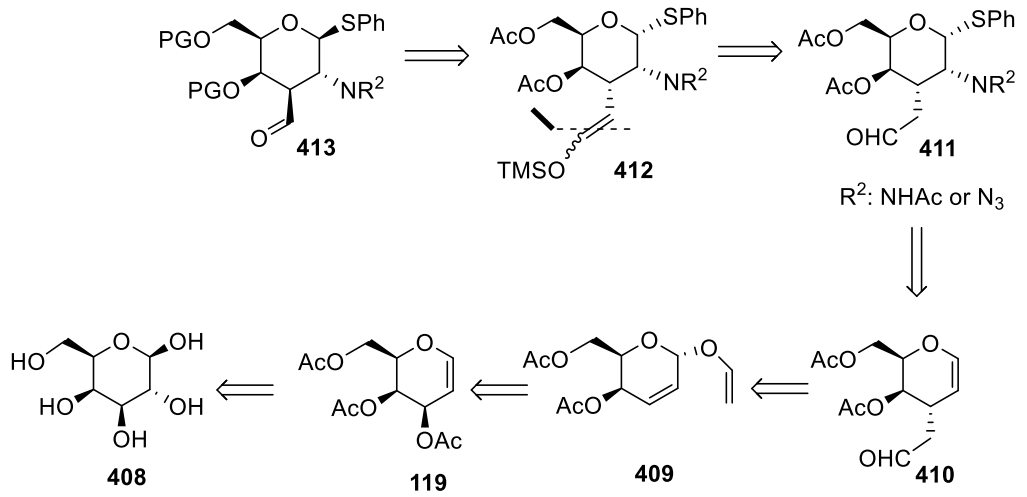
On the other hand, the formation of the aldehyde at the C-3 position in the galactosamine moiety is extremely challenging because of the presence of nitrogen source at the C-2 position close to the aldehyde. There are no reports of such an aldehyde in the literature. It is evident that both routes will require significant synthetic innovation to generate the required aldehydes. In this chapter, the synthesis of acetal-free TF antigen *via* samarium iodide chemistry through synthesis of aldehyde **402**, followed by investigation of some related side reactions, and lastly, the synthesis of acetal-free TF antigen *via* Baylis Hillman reaction are discussed.

4.1 Attempt to synthesis of acetal-free TF antigen *via* samarium iodide chemistry

4.1.1 Synthesis of the first monomer aldehyde 413 through the synthesis of aldehyde 410 precursor

The aldehyde in the galactosamine is the key component of the first approach. It is required to react with the sulfone galactose derivative as a coupling partner. Coupling using the samarium iodide method is proposed to take advantage of a single electron transfer to connect aldehyde to the anomeric position of galactose moiety. The proposed synthesis of aldehyde **413** is shown in **Scheme 4.2** and has been designed so as to go through compound **410**, reported by Seshadri.[2] As shown in the retrosynthesis of the aldehyde, there are two key steps in this proposed approach. The first aldehyde, **410,** generated from the Claisen rearrangement is one carbon longer than the target aldehyde, **413,** and attaches to the sugar moiety with the wrong stereoselectivity. However, we proposed to convert compound **410** to aldehyde **413** through trimethylsilyl vinyl ether formation,

followed by oxidative cleavage *via* ozonolysis. The epimerization of the α aldehyde can correct the stereoselectivity to the more stable β oriented aldehyde. The second key feature in this method is the formation of a galactal moiety bearing a nitrogen source at the C-2 position and installing an R group at the anomeric position in order to transform it to the C-Glycoside linked to amino acid (Serine). The aziridine formation approach was chosen to simultaneously introduce the nitrogen source and R group at the C-2 and anomeric positions, respectively.

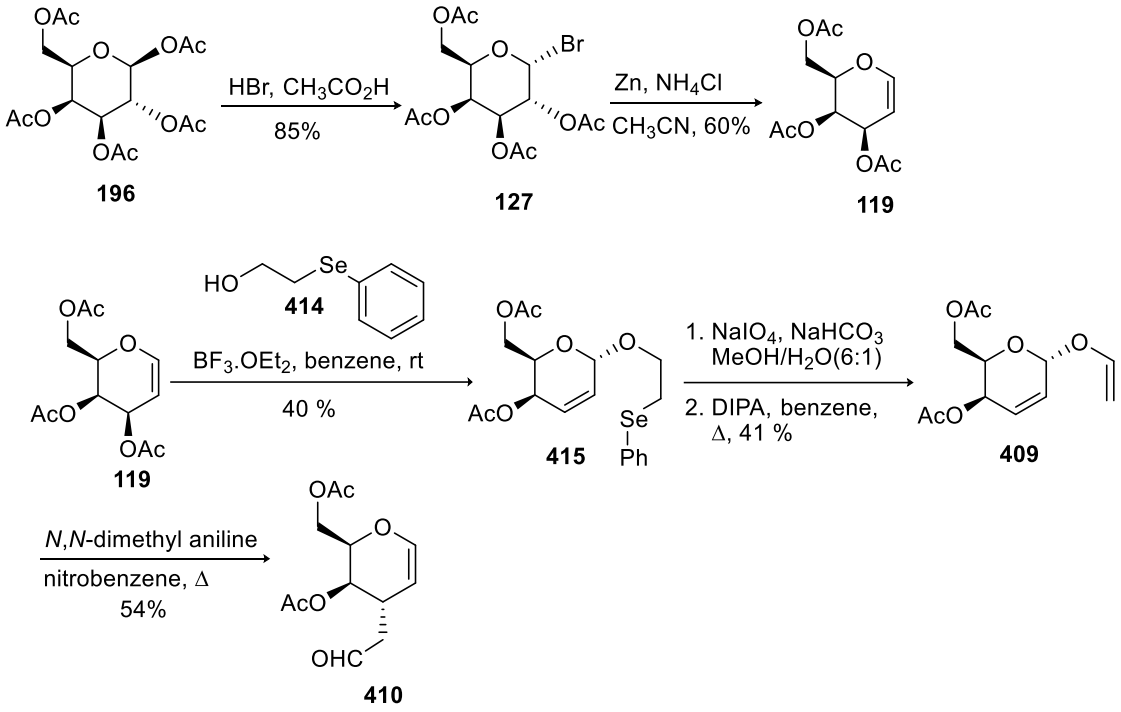


Scheme 4. 2 Retrosynthesis of the first monomer aldehyde **413** *via* aldehyde **410** formation

The aldehyde **410** was prepared starting from galactose which was acetylated *via* sodium acetate and acetic anhydride. Acetylated galactal was prepared in two steps **(Scheme 4.3)**. First, a bromination of the anomeric position of acetylated galactose *via* HBr in acetic acid, then galactal formed using zinc and ammonium chloride.

A Ferrier rearrangement[3] was performed between acetylated galactal **119** and 2-(phenylselenyl)ethanol using $BF_3 \bullet OEt_2$ to generate compound **415**. The oxidation was done *via* sodium periodate and sodium bicarbonate to establish selenone glycoside. An allyl vinyl ether was then generated through a base-mediated thermal fragmentation with the assistance of DIPA in benzene.

The desired product was finally achieved by a Claisen [3,3]-sigmatropic rearrangement[4] at high temperature using *N*,*N*-dimethylaniline and nitrobenzene **(Scheme 4.3)**.

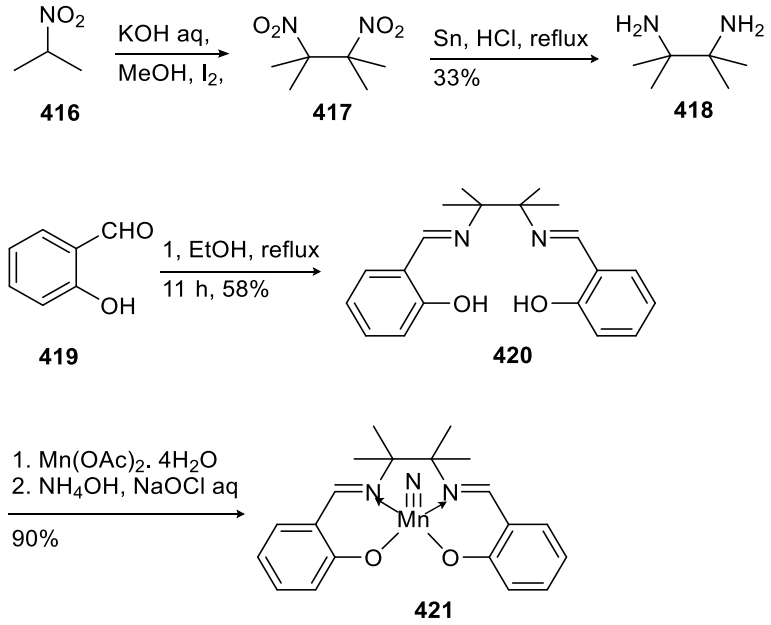


Scheme 4. 3 Synthesis of aldehyde **410**

Next, we attempted to introduce a nitrogen source into the molecule. First, the aldehyde was treated with ethylene glycol in the presence of *p*-TsOH to protect aldehyde **410**; however, the presence of an olefin was problematic. Consequently, the protection of the aldehyde failed and produced a complex mixture.

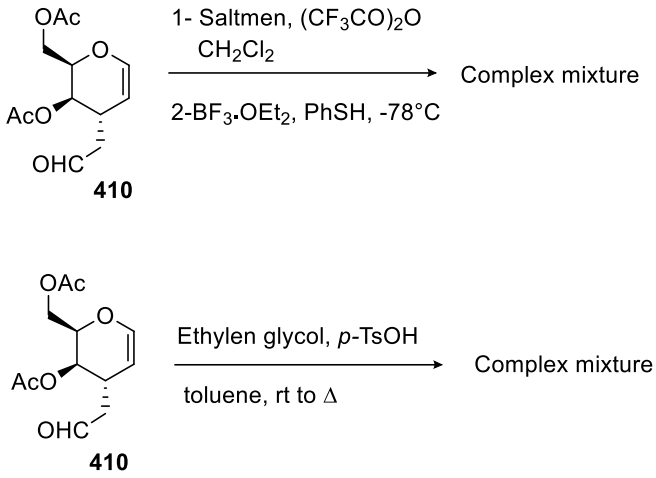
Not being able to protect the aldehyde we hypothesized that aziridine formation on compound **410** could be accomplished using (saltmen)Mn(N) as a reagent to deliver nitrogen to the alkene forming the aziridine ring. (saltmen)Mn(N)[5] was freshly prepared based on a published procedure reported by Carrieira,[6] *via* reaction of salicylaldehyde with diamine **418**. This diamine was synthesized in two steps **(Scheme 4.4)**.[7] 2-nitropropane was converted to the dinitro compound **417** *via* iodine and KOH, and then the nitro group was reduced to the diamine functionality

using tin and concentrated HCl. The sialic aldehyde was treated with diamine compound **418** to form imine **420**, which then reacted with $Mn(OAc)_2.4H_2O$ to afford the desired (saltmen)Mn(N) complex.



Scheme 4. 4 Synthesis of (saltmen)Mn(N) **421** as nitrogen donor

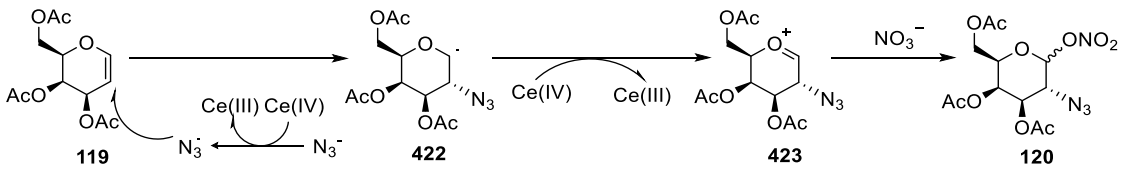
Unfortunately, the aziridine formation using (saltmen)Mn(N) and TFAA failed. This reaction afforded a complex mixture in which no useful information could be obtained from the crude produc(s) using NMR techniques. This result may be explained by the high reactivity of the aldehyde and/or acetyl protecting group, which could destructively interfere with Saltmen's reagent. It is worth mentioning again that the attempt to protect the aldehyde functionality using ethylene glycol and *p*-TsOH failed to generate an acetal group and afforded a complex mixture, the conditions for both reactions are shown in **Scheme 4.5**.



Scheme 4. 5 Attempts to aziridination and protect aldehyde **410**

To investigate the applicability of this procedure, a peracetylated galactal was used for aziridine formation; however, this reaction gave no conversion whatsoever, which eliminated the possibility of acetyl protecting group interference. The galactal **119** seems to have inherently low reactivity towards the Saltmen complex for aziridine formation.

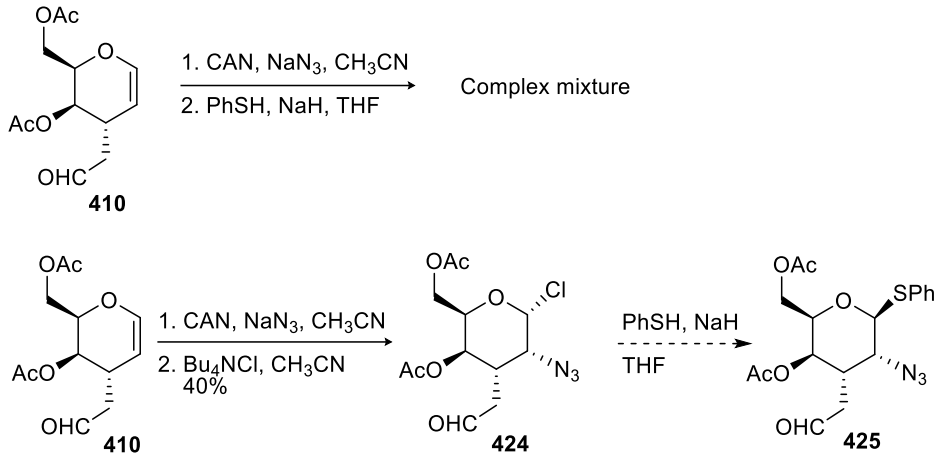
The second method chosen to introduce a nitrogen source to **410** was the azidonitration strategy.[8] The plan was to attach the azide functionality *via* azidonitration using ceric ammonium nitrate (CAN) and NaN_3, followed by adding the SPh group to the anomeric position. The mechanism of azidonitration has been studied by Lemieux and Ratcliffe and is depicted in **Scheme 4.6**.[8] First, Ceric ammonium nitrate is reduced to generate an azide radical, then the radical azide attacks to the C-2 position of galactal. The anomeric radical then changes to an oxocarbenium ion through a one-electron transfer to the ceric ammonium nitrate ion, and finally the nitrate ion attacks the anomeric position to form a mixture of nitro galactosamine derivatives.



Scheme 4. 6 Azido nitration mechanism of galactal **119**

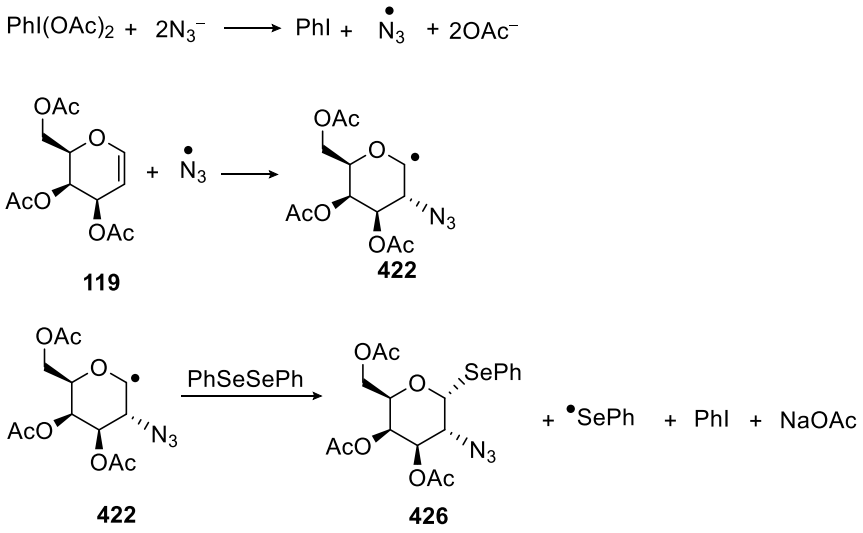
Although this reaction works fine based on TLC and HNMR technique as previous report mentioned,[8] the next step was extremely challenging. In our first attempt, the thiophenyl applied directly to replace with the nitro group using thiophenol and sodium hydride **(Scheme 4.7)**. This reaction afforded a complex mixture, which could not be used for the next step.

To solve this problem, we tried to install the SPh functional group at the anomeric position through chlorination. Based on TLC and crude NMR the reaction proceeded to completion; however, this compound was not stable at room temperature for more than half an hour and also attempt for the purification was impossible due to lack of stability. In a final attempt, azidonitration and chlorination were performed, but this time the chlorinated compound was used *in situ* after work up. After successful chlorination, based on a crude NMR spectrum, this compound was treated with thiophenol and sodium hydride. This compound again decomposed in about half an hour indicating an overall stability issue with compound **124 (Scheme 4.7)**. Synthesis of aldehyde **413** seems to be more challenging than expected. Protection of aldehyde **410** through a simple protection and deprotection strategy such as using TBS group to mask aldehyde could be a solution in order to convert aldehyde **410** to **413**, however, taking this approach would be lengthy.



Scheme 4. 7 Attempt to synthesis of aldehyde **425** *via* azidochlorination procedure

As an alternative, an azido phenylselenylation was attempted with the hope that the phenylselenide could be an appropriate replacement for the SPh group.[9] Moreover, the one pot synthesis eliminates the need for chlorination and thus the possibility of the formation of an unstable intermediate. A proposed mechanism for this transformation is shown in **Scheme 4.8** based on the mechanism proposed by Tingoli.[9] The azide radical is first generated *via* iodosobenzene diacetate, which reacts with the alkene of the radical to produce radical intermediate **422**. The radical **422** is trapped with diphenyl diselenide to form the desired compound **426** accompanied by iodobenzene and sodium acetate. Unfortunately, this procedure produced a complex mixture again. Clearly, these methods, which readily work with acetylated galactal, do not work in the presence of an aldehyde and our attempts to regioselectively introduce the nitrogen group at the C-2 position failed to produce the galactosamine moiety.



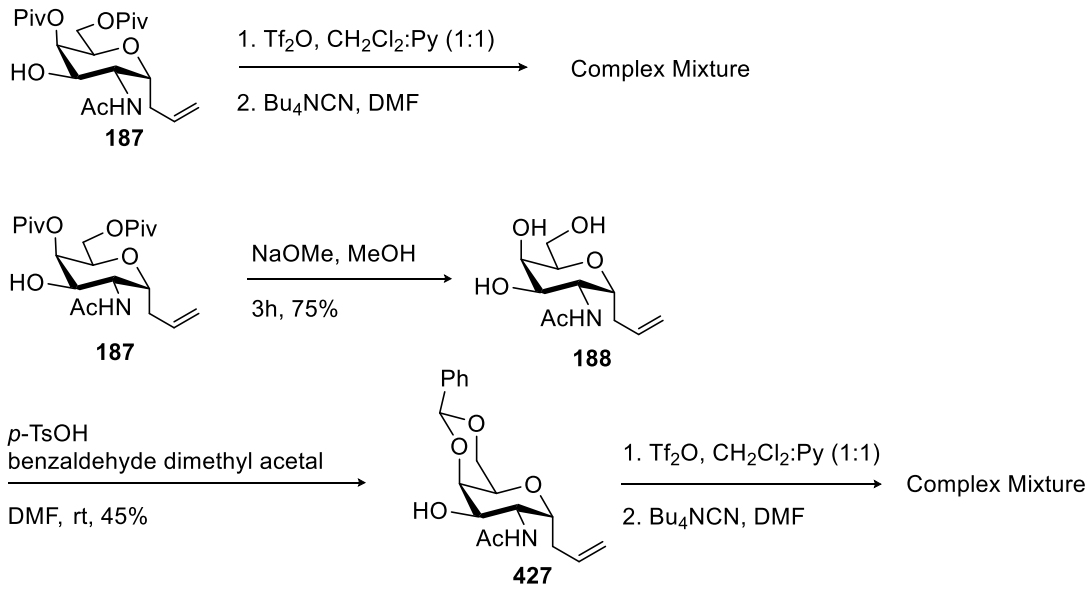
Scheme 4. 8 Mechanism of compound **426** formation

4.1.2 Attempt toward the synthesis of aldehyde at C-3 position through a cyanation procedure

Introducing a nitrogen source to compound **410** failed and as a result the desired aldehyde **413** could not be produced. As an alternative strategy, glucosamine **187** was chosen for the synthesis of the aldehyde at C-3 position, which already has an amine functionality attached to the C-2 position. Although this molecule saves some synthetic steps, the presence of an amine group made the synthesis of the aldehyde far more challenging. We initially attempted to install the aldehyde on the glucosamine residue *via* cyanation, and those attempts are discussed below.

Initially we attempted direct cyanation of **187 (Scheme 4.9)**, this reaction was performed in two steps. First, the alcohol **187** was turned into a triflate leaving group which was then substituted through an S_N2 substitution using tetrabutylammonium cyanide. This procedure afforded a complex mixture from which product could not be isolated. It was assumed that the Piv protecting group is labile in this reaction. To solve this problem, the Piv groups were replaced with a benzylidene group in two sequential steps. A Zemplén rection to remove the Piv groups, followed by the

protection of alcohol groups at the C-4 and C-6 positions with a benzylidene group using benzaldehyde dimethyl acetal and *p*-TsOH, to produce compound **187 (Scheme 4.9)**. Hoping that we had reduced the reactivity of the protecting group towards nucleophilic substitution, the cyanation was repeated, this time using compound **427**. Unfortunately, a complex intractable mixture was again obtained. The result suggests that the Piv protecting the group were not an issue in the original cyanation of **187**. Therefore, the main problem with the cyanation is most likely the presence of the acetamido group. Consequently, the only option left was to try and remove the acetamide group from the molecule and replace it with another source of nitrogen.

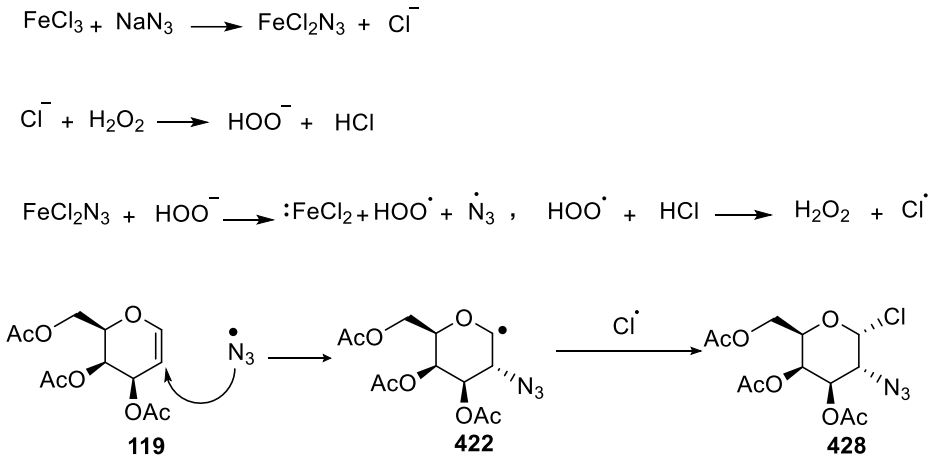


Scheme 4. 9 Attempt to cyanation of alcohol

An azide functionality was targeted to replace the acetamido group, which was thought to be slightly less reactive and can be easily converted to the acetamido group in later steps. The galactal **119** was subjected to azidochlorination using $FeCl_3.6H_2O$, NaN_3 and H_2O_2, followed by an S_N2 substitution of chlorine with SPh functionality. We are hoping to be able to convert the SPh group to an allyl group in later steps for the eventual attachment of amino acid to the sugar moiety.

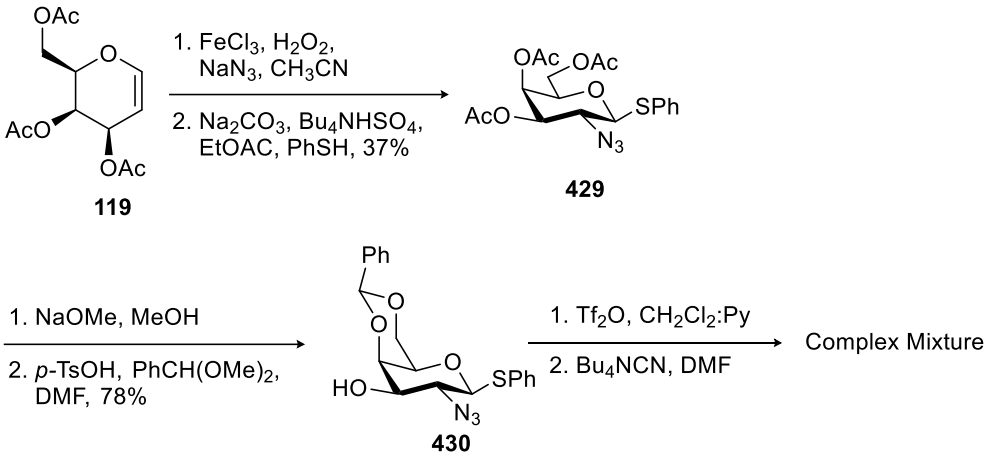
There is no exact known mechanism using this approach, however, we proposed a mixture of an ionic pathway accompanied by a radical addition/atom transfer sequence **(Scheme 4.10)**.

We proposed that the nucleophilic attack of NaN_3 to $FeCl_3$ happens first, followed by HOO- formation in order to convert to HOO and azide radicals using $FeCl_2N_3$. The azide radical attacks the C-2 position of galactal to produce intermediate **422**. This radical intermediate reacts with Cl radical, prepared from the reaction between HOO radical and HCl to form compound **428**.



Scheme 4. 10 Proposed azidochlorination mechanism using $FeCl_3.6H_2O$ and H_2O_2

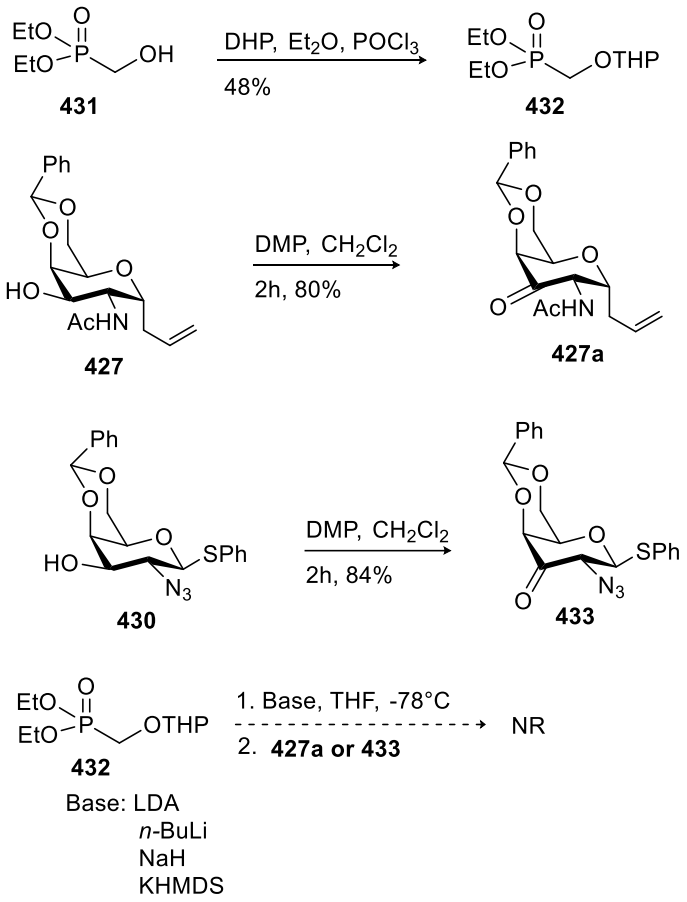
The one pot azidochlorination and nucleophilic substitution successfully afforded the desired product **429**. The acetyl protecting group were then replaced with more inert benzylidene group in good yield. Finally, to convert the alcohol to a cyano group, compound **430** was treated with TF_2O, followed by the addition of *tert*-butyl ammonium cyanide based on the same procedure as above, however, the result of the reaction is again a highly complex mixture. This appears to be a nearly intractable problem.



Scheme 4. 11 Attempt to cyanation of alcohol **430**

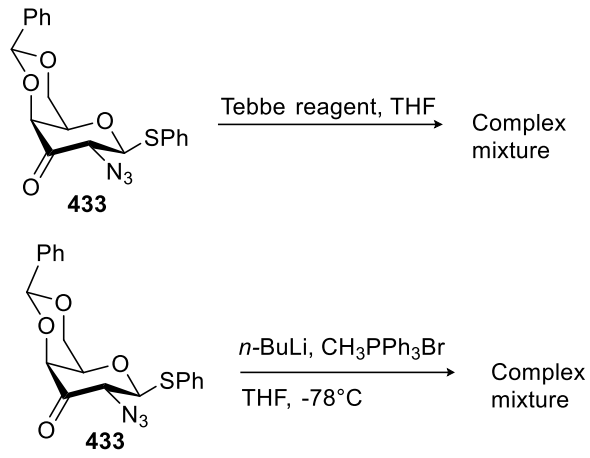
4.1.3 Attempt to the synthesis of aldehyde at C-3 position *via* an olefination procedure

As the cyanation strategy had failed to provide adequate results, we turned our attention to the Wittig reaction. Compound **432** was prepared as a Wittig reagent so that it once the Wittig reaction was complete the resulting compound could be converted directly to the aldehyde through a hydrolyzation process. This reagent was synthesized using diethyl hydroxymethyl phosphonate, dihydropyran and phosphorus oxychloride in moderate yield **(Scheme 4.12)**. The other Wittig counterpart was formed upon oxidation of alcohols **427** and **430** to the ketone using Dess-Martin periodinane[10] in good yields. Compound **432,** treated with base and then exposed to ketones **427a** and **433** separately**,** underwent a Wittig reaction in THF. No matter what type of base was applied, compounds **427a** and **433** did not show any conversion to the desired compound.



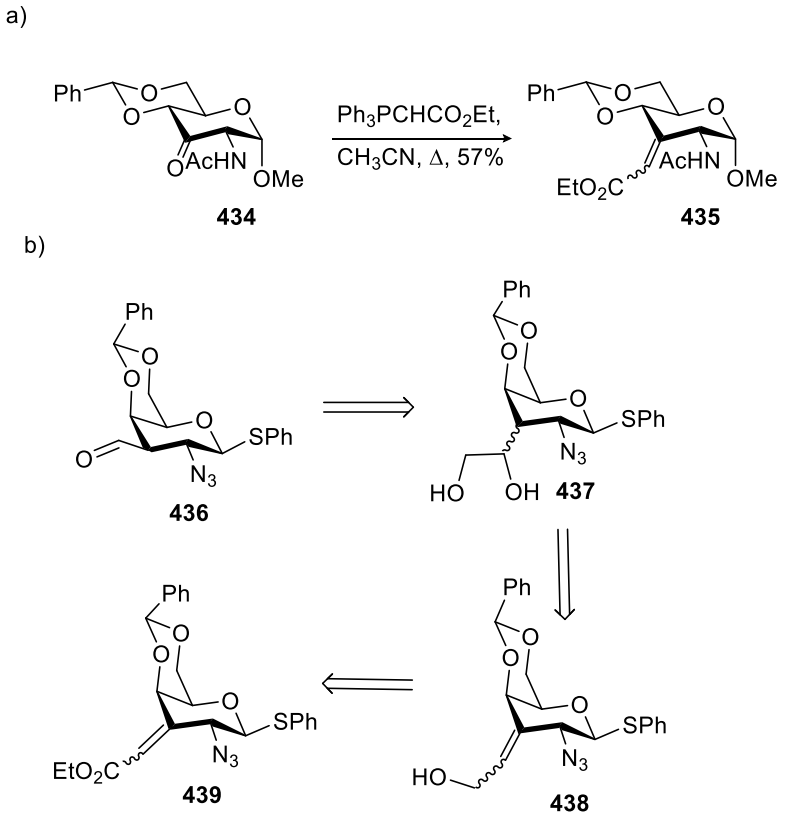
Scheme 4. 12 Attempt to the synthesis of aldehyde through a Wittig reaction

With ketone **433** in hand, we attempted to form the olefin using other synthetic techniques. The olefin was targeted as the key compound for eventual aldehyde formation through hydroboration, followed by oxidation. The olefination was attempted using two separate methods **(Scheme 4.13)**. First, the Tebbe reagent was used to convert the ketone to the alkene functionality;[11] however, this reaction did not afford the desired product and produced a complex mixture. Again, a Wittig reaction was performed, this time in the presence of methyl triphenylphosphonium bromide to achieve the alkene functionality. The phosphonium ylides were attempted to be generated using *n*-BuLi, but a complex mixture was obtained, and no desired product was observed.



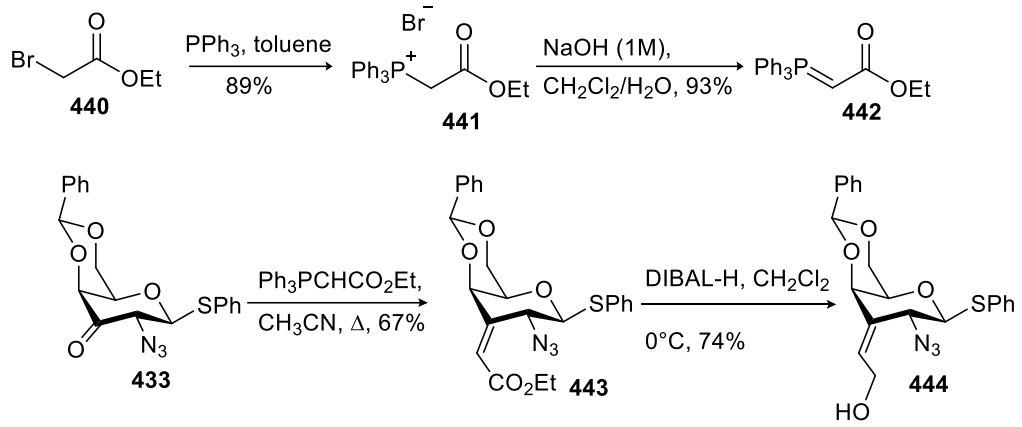
Scheme 4. 13 Attempt to olefination of compound **433**

Based on the previous research done by Heras using a Wittig strategy to form a C-C bond between a stabilized ylide reagent and C-3 of the glucosamine derivative **334** (**Scheme 4.14a)**, it is clear that having a less hindered stabilized ylide is necessary for the C-C bond formation.[12] We proposed that aldehyde **436** could be accessible through the following procedures: Wittig reaction, reduction of ester, hydroboration of alkene and an oxidative cleavage. The retrosynthetic analysis is shown in **Scheme 4.14b**. The first step in this procedure is a Wittig reaction using ethoxycarbonylmethyl and triphenylphosphine as a stabilized ylide reagent.



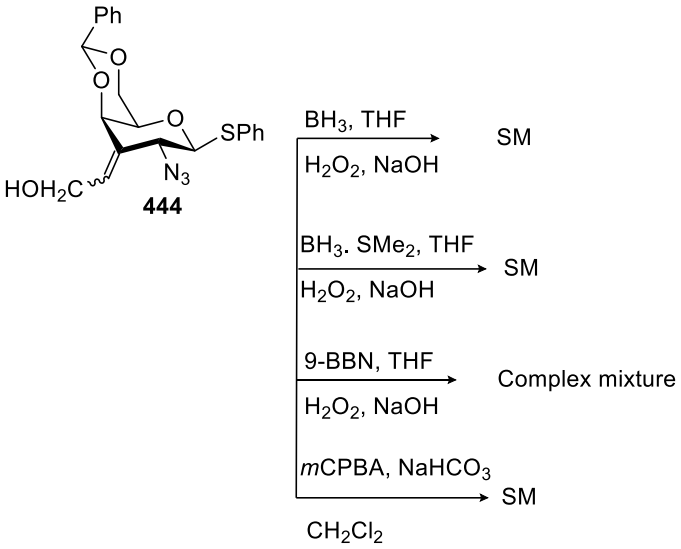
Scheme 4. 14a,b An example of Wittig reaction on C-3 ketone **434**, a, retrosynthesis of aldehyde **436** *via* a Wittig reaction, b

To this end, the ylide regent was synthesized in two steps. First, ethyl bromoacetate was treated with triphenylphosphine, followed by ylide formation using NaOH. This reagent was then used for a Wittig reaction with ketone **433**. The reaction afforded the desired α,β-unsaturated system with a 3:1 ratio of E to in 67% yield. The E and Z isomers then successfully were separated, and E isomer subjected to the next step. This result was promising as we thought C-C bond formation would be the most challenging part of the synthesis. The ester group was subsequently reduced to the alcohol *via* DIBAL-H to generate the allylic alcohol **444** in 74% yield.



Scheme 4. 15 Synthesis of allyl alcohol **444**

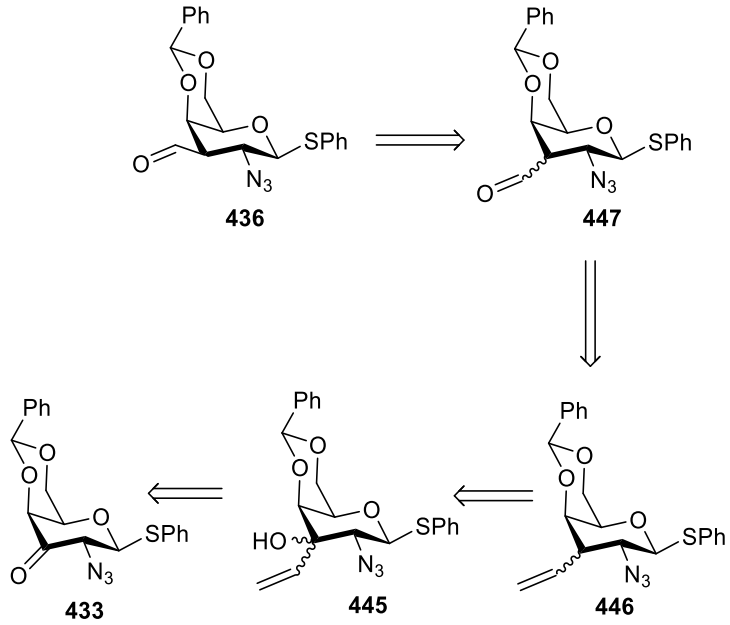
With alcohol **444** in hand, hydroboration was then attempted to form the dihydroxyl groups and remove the alkene functionality; however, this reaction did not work, despite several trials with various borane sources such as BH_3, $BH_3.SMe_2$ and 9-BBN. Regardless of the conditions employed the result did not afford the desired product **(Scheme 4.16)**. To avoid the problematic hydroboration, epoxidation was attempted in order to generate the alcohol functionality through a selective epoxide ring-opening using *m*CPBA and $NaHCO_3$. Unfortunately, the result showed no conversion of starting material. Although this approach failed to produce aldehyde **436**, Ketone **433** seemed to be reactive for small and stabilized Wittig reagents which inspired us to apply this strategy for other similar nucleophiles to generate an sp^3-sp^3 carbon bond, which will be discussed later in the following section.



Scheme 4. 16 Attempt to synthesis of aldehyde **436** through hydroboration and epoxide formation

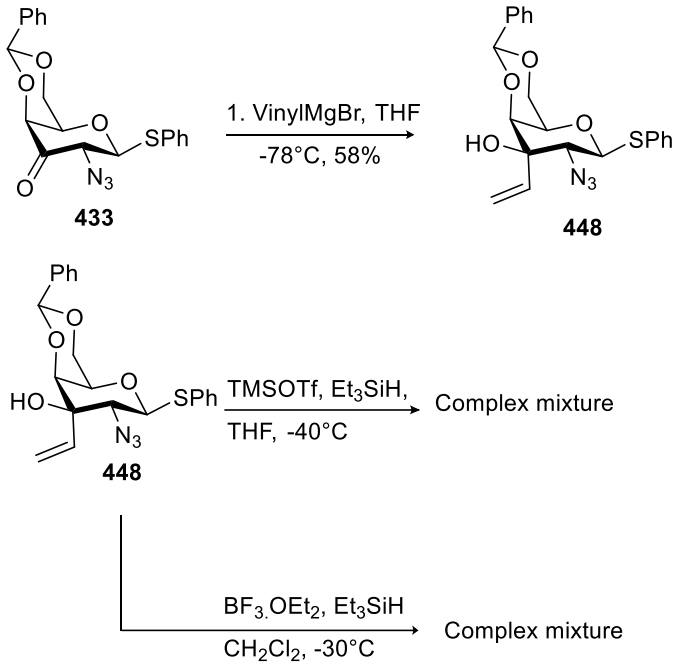
4.1.4 Attempt to synthesize aldehyde 436 using Grignard chemistry

As mentioned, the reactivity of ketone **433** towards small nucleophiles inspired us to attempt a final approach to the aldehyde using Grignard chemistry. In particular, the use of Grignard chemistry to introduce the vinyl group at the C-3 position as the key intermediate towards the synthesis of aldehyde **436**. This approach is proposed to require 4 steps and the retrosynthetic analysis is presented in **Scheme 4.17**.



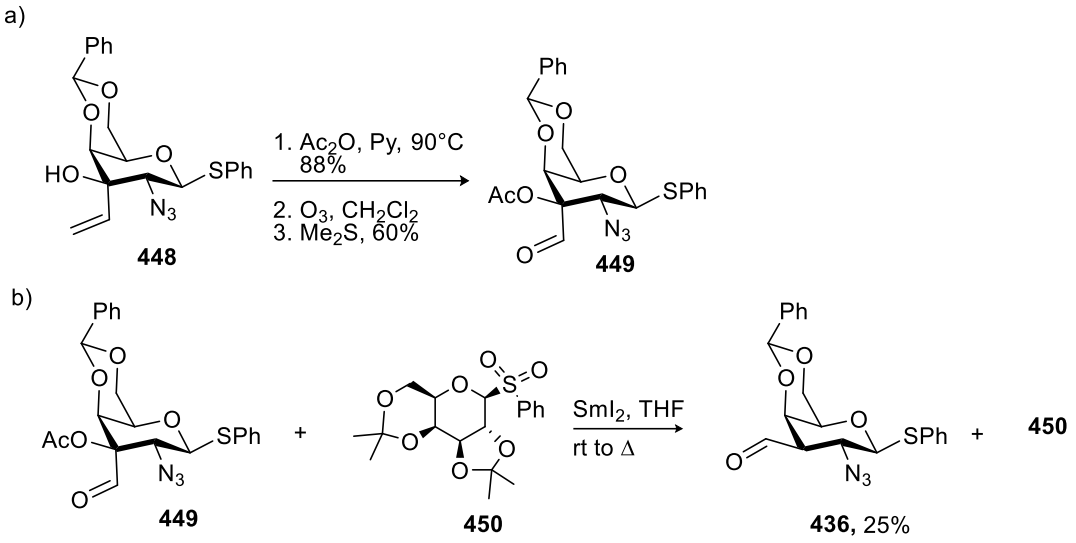
Scheme 4. 17 Retro synthesis of aldehyde **436** *via* Grignard reaction

Compound **433** was treated with vinyl magnesium bromide in THF at –78°C. The results indicated the vinyl group was attached to the C-3 position, which was a promising result for further transformations; however, this reaction afforded exclusively an axially oriented vinyl group with a hydroxyl group in the equatorial position, which is not the desired orientation for aldehyde **436 (Scheme 4.18)**. This orientation of the substituents may be explained by the structure of sugar **433** which makes the bottom face less sterically encumbered. A hydroxyl group elimination was attempted using TMSOTF and BF_3•OEt_2 in the presence of Et_3SiH in two separate experiments. However, we failed to dehydroxylate compound **436** and instead obtained a complex mixture of benzylidene deprotected product accompanied with an isomerized alkene group **(Scheme 4.18)** .[13, 14]



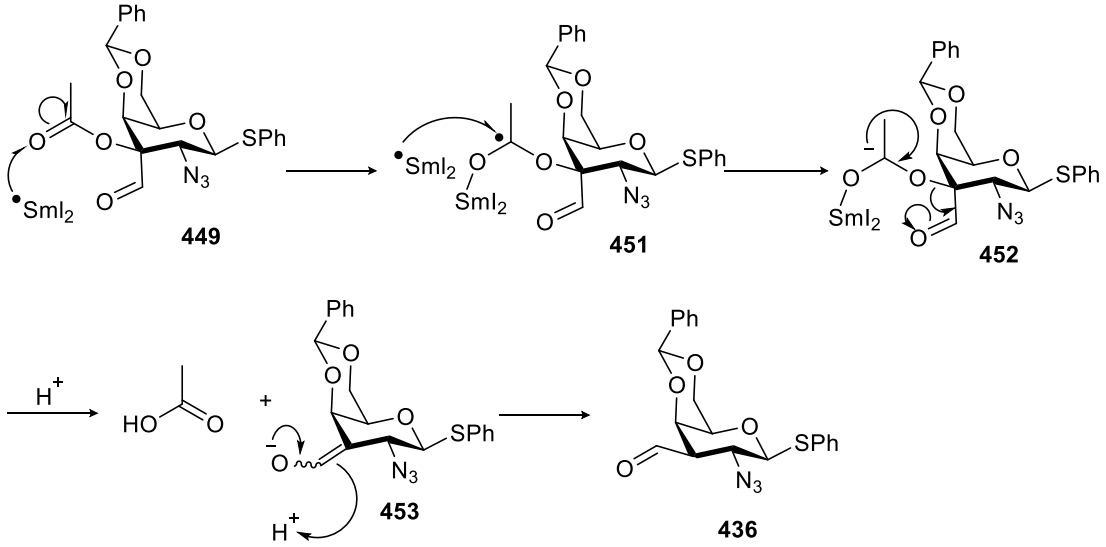
Scheme 4. 18 Synthesis of allylic alcohol **448** *via* Grignard reaction and Attempt to dehydroxylate compound **448**

With the carbon-carbon bond formed, albeit in the wrong orientation, we decided to continue the synthesis, hoping to fix this problem after the attachment of the two sugar moieties. The hydroxyl group in compound **448** was protected using acetic anhydride and pyridine under reflux, as expected protection of a tertiary alcohol, especially in carbohydrates, require harsh reaction conditions. After acetylation of the alcohol, the ozonolysis process was performed and aldehyde **449 (Scheme 4.19a)** was formed in 60% yield, bearing an acetyl group at the β position. An initial attempt to couple the sugar moieties, mediated by samarium iodide, was performed between aldehyde **449** and sulfone **450** (The synthesis of sulfone 450 will be discussed in section 4.1.5). The coupling was unsuccessful, and the sulfone **450** was recovered, surprisingly, the aldehyde **449** was converted to our desired aldehyde **436** in 25% yield **(Scheme 4.19b)**. It seems SmI_2 first reacted with aldehyde **449** and, through radical reaction, eliminated the acetyl group and epimerized the aldehyde to the most favoured equatorial orientated position.



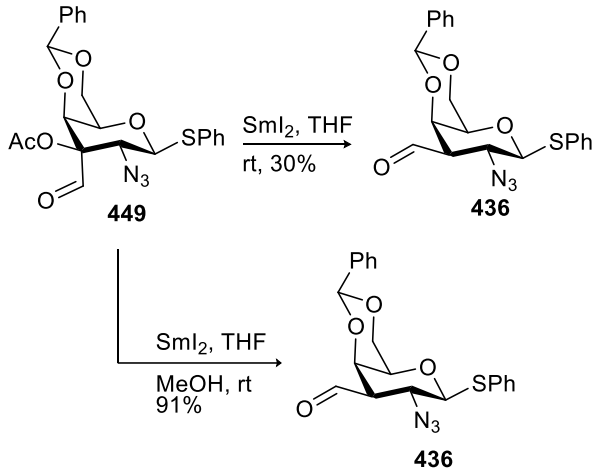
Scheme 4. 19a,b Synthesis of compound **449**, a, and aldehyde **436** using SmI_2, b

A mechanism for this transformation is proposed in **Scheme 4.20**. First the samarium transfers one electron to the carbonyl oxygen of the ester to generate a radical at the C-3 position, then, samarium transfers another single electron to generate minus charge, which then shares electron with oxygen to release an acetate group and form enolate. The enolate convert to the more stable, *β*-oriented aldehyde **436**; however, this reaction appeared not to be scalable as we were not able to repeat the reaction when we scaled up (it is perfectly reproducible on small scale).



Scheme 4. 20 Proposed mechanism of SmI_2 mediated aldehyde formation

In order to optimize this reaction, compound **449** alone was subjected to the same reduction procedure using SmI_2. The result showed a slight improvement in the yield, by 5% (**Scheme 4.21)**. In the following reaction, methanol was applied to the mixture of aldehyde **449** and samarium iodide and compound **436** was afforded in excellent yield. However, scaling up this reaction failed to provide the desired product and afforded a complex mixture, consequently, this result, lead us to set up multiple small scale synthesis in order to achieve enough material for our next step synthesis. It is worth mentioning that, despite a few reported syntheses of aldehyde at the C-4 and C-6 positions of a sugar bearing a nitrogen source at the C-2 position, compound **436** is the first reported synthesis of an aldehyde at the C-3 position of a sugar moiety in the presence of any nitrogen source at the C-2 position.[15, 16]

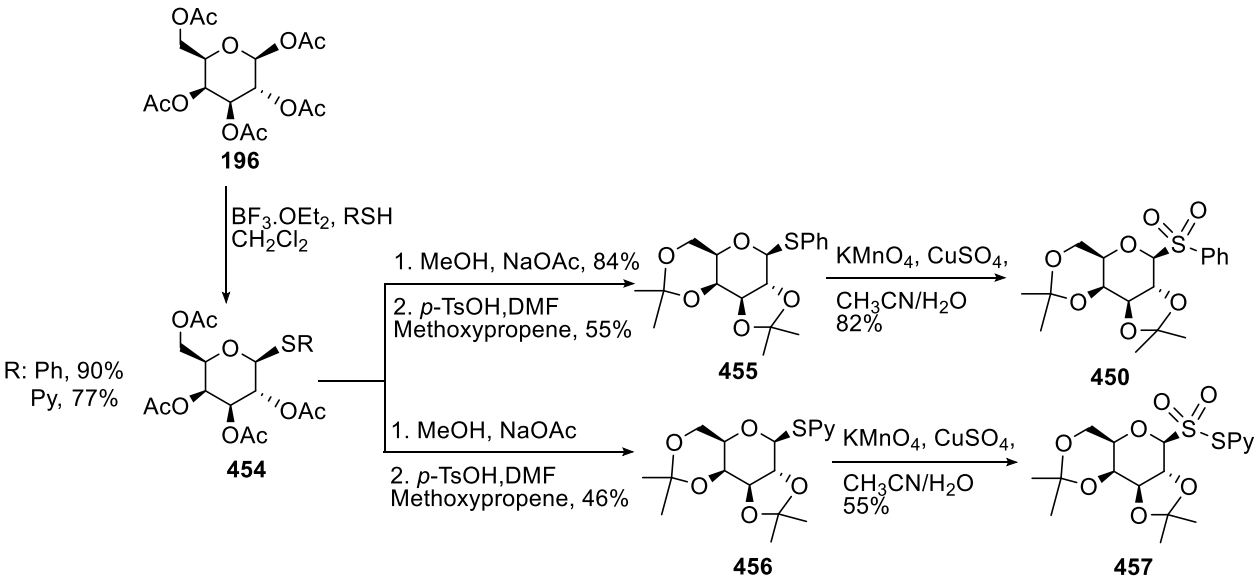


Scheme 4. 21 Attempt to optimize aldehyde synthesis **436**

4.1.5 Attempt toward the sp3-sp3 one carbon coupling formation *via* samarium iodide chemistry

After a successfully synthesis of the more challenging aldehyde **436**, we turned our attention to the synthesis of the second sugar partner and the coupling reaction between them. When we began synthesis of the second monomer, we decided to target both the 2-mercaptopyridine and

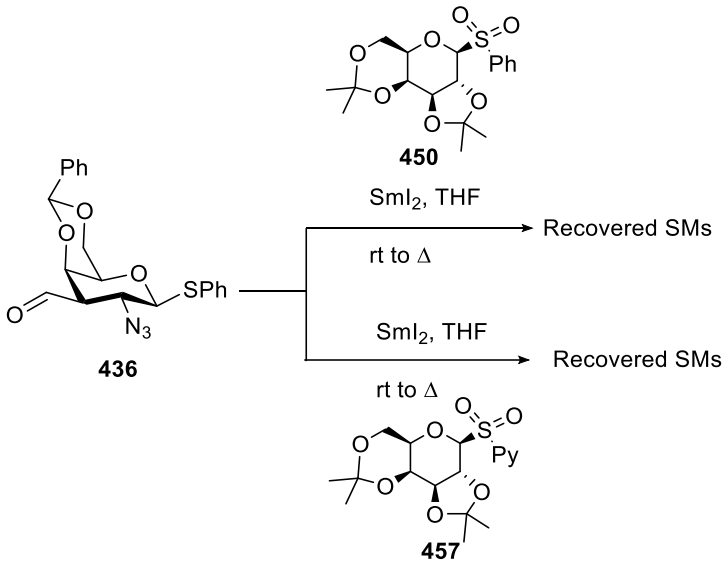
phenyl sulfone galactose derivatives which were prepared in the same reaction conditions **(Scheme 4.22)**. Initially, the galactose was acetylated and then the acetyl group at the anomeric position was replaced with SPh or SPy using BF_3•OEt_2. The acetyl groups were removed *via* the Zemplén procedure and replaced with a more stable isopropylidene protecting group using 2-Methoxypropene and *p*-TsOH in DMF. Then, potassium permanganate and copper sulfate were applied for the oxidation of sulfur to form the desired sulfone compounds **450** and **457** in good yields.



Scheme 4. 22 Synthesis of the second monomer **450** and **457**

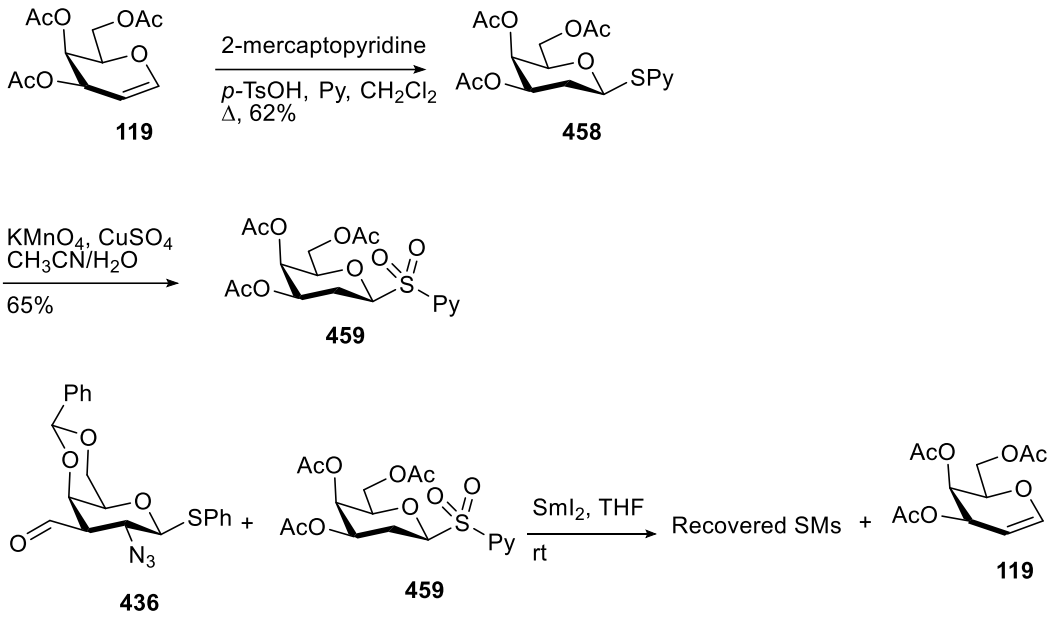
Having both coupling partners in hand, the first coupling reaction was performed between aldehyde **436** and sulfone **450** with the assistance of SmI_2. The SmI_2 was prepared freshly based on the known procedure by Procter,[17] and the SmI_2 was titrated prior to use in the reaction.[18] Using the reaction conditions shown in **Scheme 4.23** did not show any consumption of either aldehyde **436** or sulfone **450** even when heated to reflux. SmI_2 usually reacts readily with common sulfones derivatives which may suggest that isopropylidene groups of sulfones **450** were sterically or electronically preventing the samarium from fulfilling its role in this reaction. The more reactive

pyridyl sulfone **457** was then used for the radical reaction with aldehyde **436** mediated by SmI_2; however, the result was the same, and no conversion was observed.



Scheme 4. 23 Attempt to form 1,3-*β* one carbon link between first and second monomers

The isopropylidene had to be replaced by other protecting groups, but we knew that using common protecting groups such as acetyl and benzyl would increase the possibility of producing a 1,2-elimination byproduct. Therefore, we attempted to remove substitution at the C-2 position in order to reduce the chance of producing the 1,2-elimination byproduct mediated by SmI_2, 2-pyridyl 2-deoxy-sulfone moiety was then prepared using peracetylated galactal **119** as a starting material. The acetylated galactal **119** underwent a 1,2-addition reaction using anhydrous *p*-TsOH and 2-mercaptopyridine in the reflux condition. The compound **458** then oxidized to form sulfone *via* $KMnO_4$ and $CuSO_4$ (**Scheme 4.24)**. Surprisingly, even after removing the substituent on the C-2 position the coupling reaction between deoxy sulfone **459** and aldehyde **436** afforded acetylated galactal, the 1,2-elimination product **(Scheme 4.24)**.



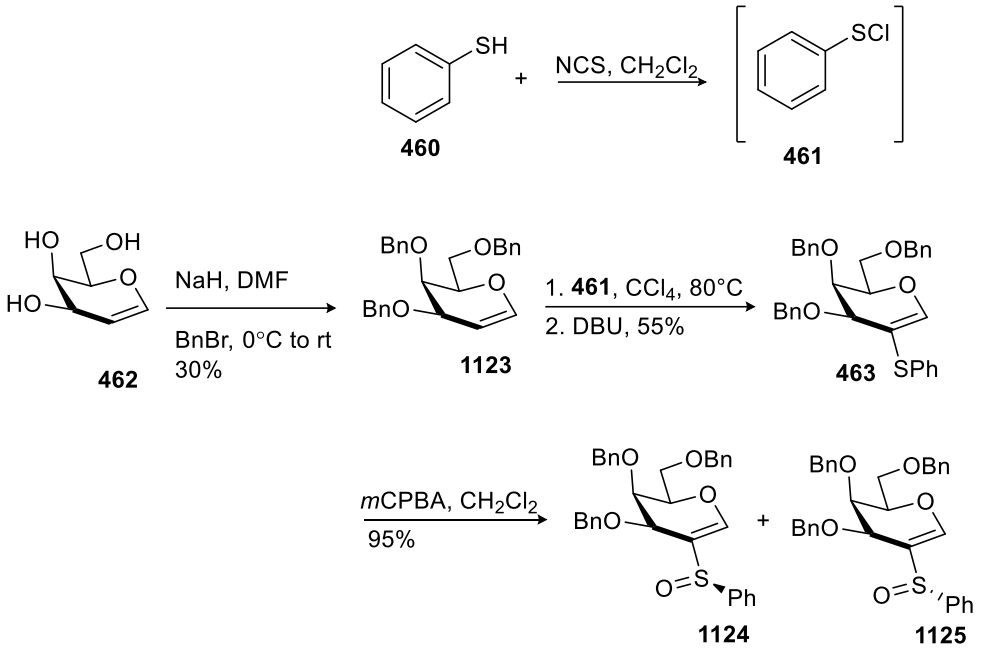
Scheme 4. 24 Synthesis of 2-pyridyl 2-deoxy-sulfone **459** and its reaction with aldehyde **436**

This result shows that aldehyde **436** reacts slowly, which gives time for the 1,2-elimination to proceed. This is most likely due to the structural conformation of aldehyde and its sugar backbone. In the case of isopropylidine protected sulfones, the recovery of starting material proved the low reactivity of aldehyde **436** and sulfones towards the samarium activated sugar, which may be cause by steric hinderance and/or low reactivity due to electronic effects of the sugar moiety.[16] Usually samarium mediated coupling reactions fails for two reasons: either because of steric hindrance from the sugar ketone or aldehyde coupling partner, or due to the highly reactive intermediate generated from reaction of the sulfone with samarium iodide undergoing a premature intramolecular transformation, often, an elimination reaction. It seems having an electron-withdrawing group in the same carbon as sulfone to stabilized carbon radical and removing any C-2 substitution to reduce the chance of the side reaction are necessary, and the generation of an intermediate samarium enolate, which assumes to facilitate the coupling reaction between samarium enolate and the aldehyde and suppress the elimination reaction is required.[16] The synthesis of acetal-free TF

antigen failed *via* samarium iodide chemistry; however, we were able to synthesize an aldehyde, **436**, that could be used as an electrophile for the nucleophilic reactions, which led us to try one more additional attempt.

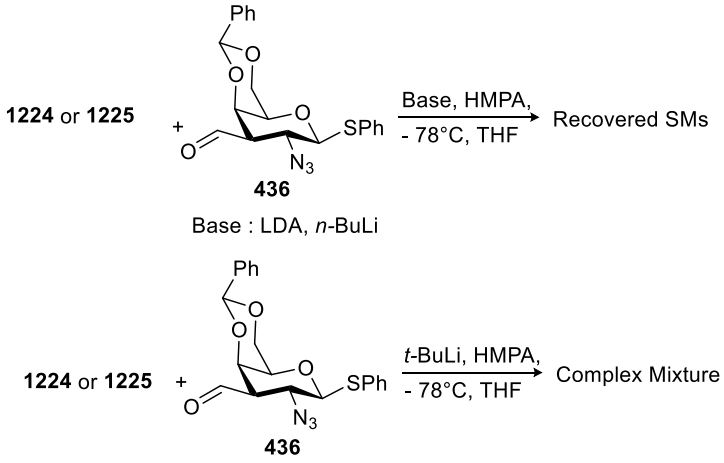
4.2 Attempt toward the sp2-sp3 one carbon coupling formation between aldehyde 436 and sulfoxides 1124 and 1125

In order to further investigate the reactivity of aldehyde **436**, the sulfoxides **1124** and **1125** were prepared, which was previously shown to act as a nucleophile capable of attacking an aldehyde at the axial position C-4 position of a galactose moiety bearing an azide group at the C-2 position.[15] The sulfoxides **1124** and **1125** were prepared by replacement of the acetyl protecting group of galactal by benzyl group in two steps, followed by the addition of phenyl sulfenyl chloride and subsequent hydrogen chloride elimination using DBU **(Scheme 4.25)**. The acetyl group was then removed *via* Zemplén deacetylation and the hydroxyl groups were benzylated using NaH and benzyl bromide. The phenyl sulfenyl chloride, freshly prepared from *N*-Chlorosuccinimide, was used directly for addition to the benzylated galactal. After the addition reaction, hydrogen chloride was eliminated with DBU,[19, 20] and upon oxidation using *m*CPBA, a diastereomeric mixture of sulfoxide **1124** and **1125** was obtained.[21]



Scheme 4. 25 Synthesis of sulfoxide **1124** and **1125**

The nucleophilic addition of both sulfoxide **1124** and **1125** with the aldehyde were performed in the presence of strong bases, however, the result did not show the desired product. Using different bases including LDA, *n*-BuLi and *t*-BuLi accompanied by HMPA to enhance the nucleophilicity of the nucleophile did not improve matters. Using LDA and *n*-BuLi gave no conversion of aldehyde **436** and using *t*-BuLi indicated the decomposition of both the nucleophile and electrophile.



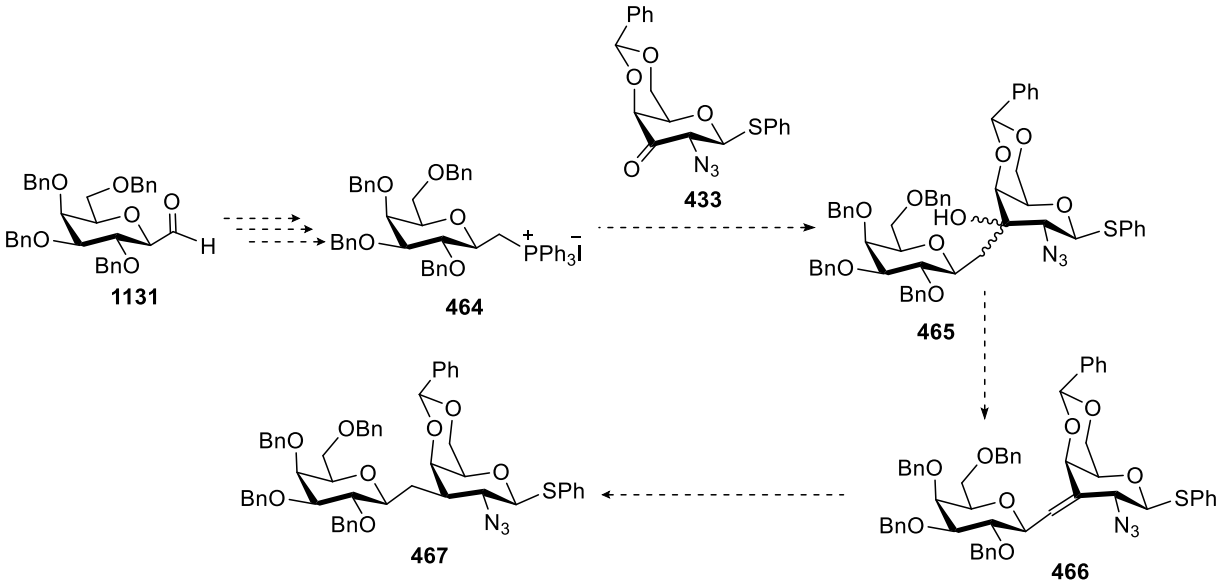
Scheme 4. 26 Nucleophilic addition reaction between sulfoxide **1224**,**1225** and aldehyde **436**

Consequently, the synthesis of aldehyde at the C-3 position through Claisen rearrangement, olefination, and Wittig reaction as the key step, did not afford the desired result, however using Grignard reaction, followed by samarium iodide chemistry resulted in desired aldehyde **436**.

Although our attempts did not lead us to the final product, after trying multiple approaches, we were able to synthesis novel aldehyde **436**, which seems necessary for the construction of a single sp3-sp3 carbon linkage between two sugar moieties in reasonable steps counts.

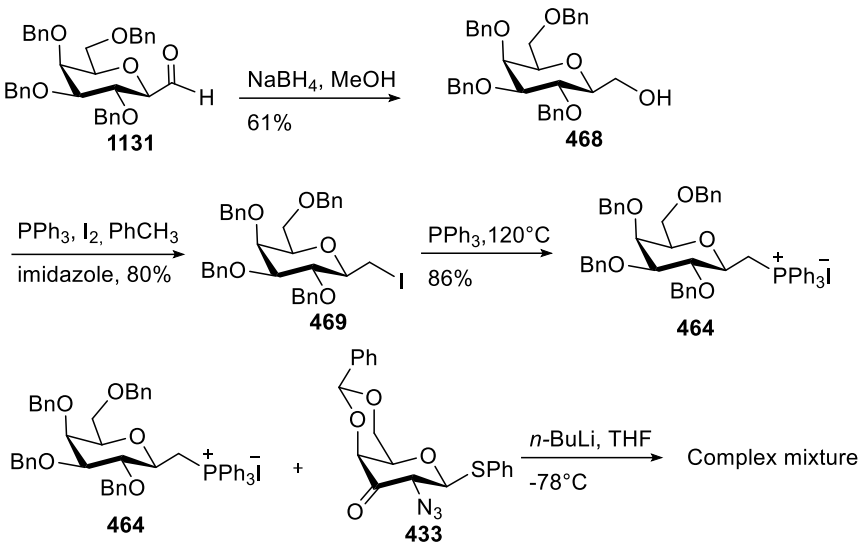
4.3 Alternative meanderings: Attempt to synthesize acetal-free TF antigen

As our previous research showed, (Section 4.1.3) using Wittig reactions for the synthesis of aldehyde **436**, ketone **433** could behave as an electrophile if small stabilized ylide reagents were used. However, we did not know how small the ylide should be to be effective. We then targeted Wittig and Horner–Wadsworth–Emmons reactions to build one carbon linkages between two sugar moieties **(Scheme 4.27)**. First, we introduced *β*-aldehyde functionality to the anomeric position of a galactose moiety to be converted to and unstabilized ylide. Compound **464** could then react with ketone **433** *via* ylide formation to form one carbon linkage between sugars. Elimination of water would afford compound **466** bearing an alkene group in the bridge, which upon selective reduction would provide the desired compound **467**.



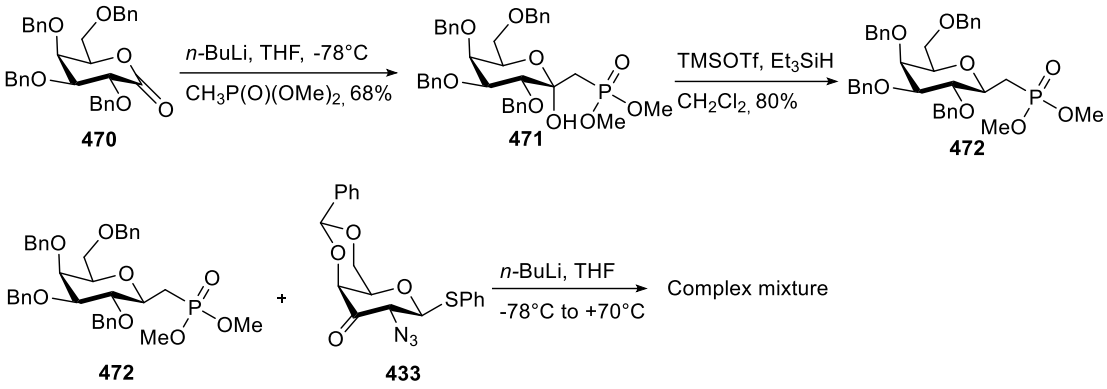
Scheme 4. 27 Proposed synthesis of fully C-glycoside disaccharide **468** *via* a Wittig reaction

The triphenylphosphonium iodide **464** was prepared in three steps as shown in **Scheme 4.28**. Aldehyde **1131 (**Synthesis of aldehyde **1131** will be discussed in section 4.4**)** was readily reduced to alcohol **468** using $NaBH_4$, then the alcohol was replaced with an Iodide *via* PPh_3, I_2 and imidazole. The desired salt **464** was prepared using neat PPh_3 and heated at 120°C for 2 hours. Then *n*-BuLi was used to convert compound **464** to the triphenyl phosphonium ylide for the Wittig reaction with ketone **433**. Unfortunately, the Wittig reaction gave a complex mixture from which no product could be observed.



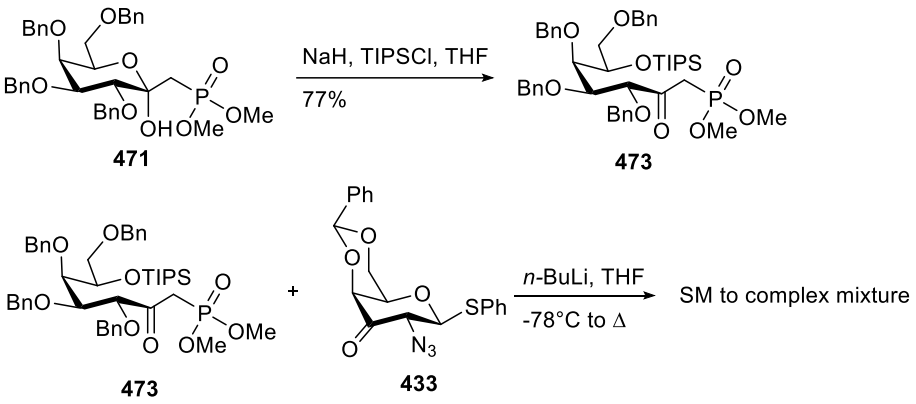
Scheme 4. 28 Synthesis of compound **464** and a Wittig reaction

Next, we tried to use a slightly more activated ylide for the Wittig reaction, specifically compound **472**, which was synthesized starting from lactone **470** (Synthesis of aldehyde **470** will be discussed in section 4.4). In the first step, a nucleophilic attack of dimethyl methyl phosphonate ion, generated by *n*-BuLi to the lactone **470**, afforded compound **471**, which after dihydroxylation with trimethylsilyl triflate and triethylsilane converted to the desired compound **472**. A Horner–Wadsworth–Emmons reaction between compound **472** and ketone **433** was attempted through an *in situ* ylide formation using *n*-BuLi, however, this reaction did not proceed and trying to push the reaction forward with heating to the reflux resulted in a complex and intractable mixture **(Scheme 4.29)**.



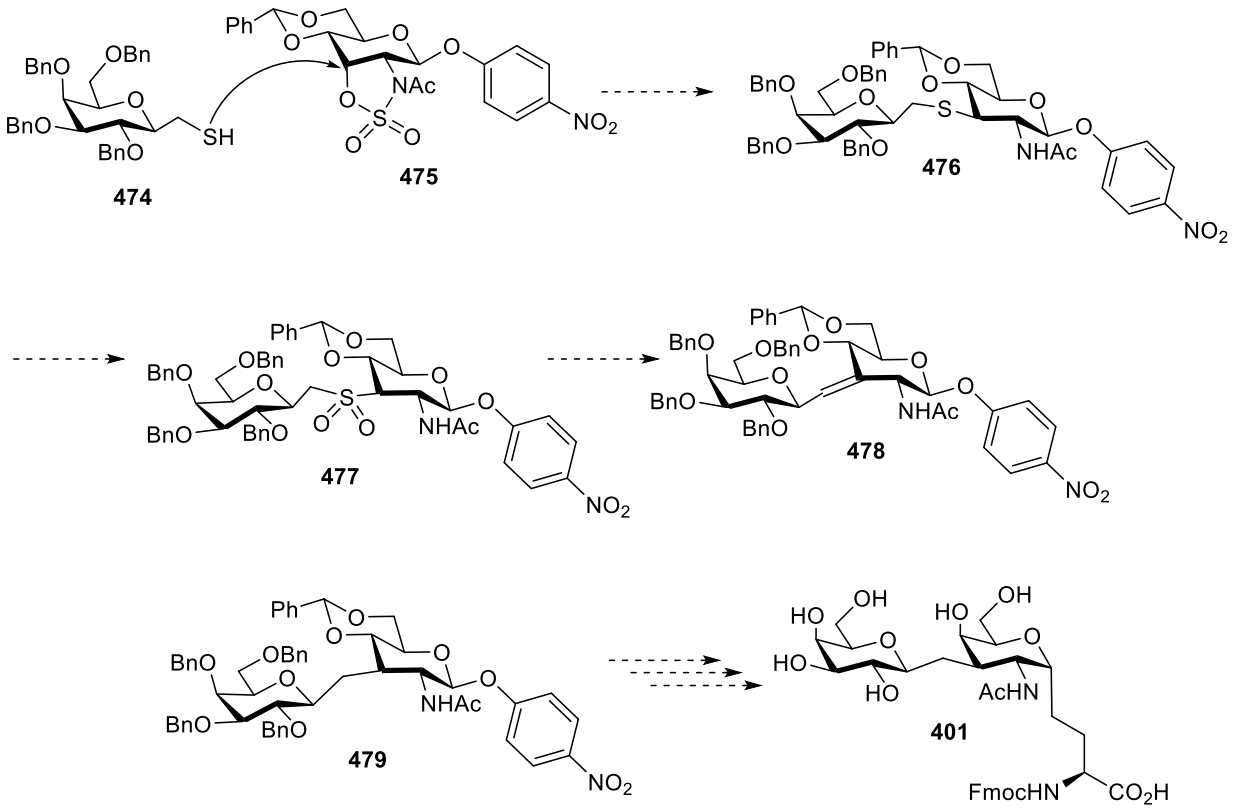
Scheme 4. 29 A Wittig reagent attempt between ketone **433** and compound **472**

After two failed attempts at using Wittig chemistry to link the two sugar moieties together, the next step was to design a stabilized ylide for use in the Horner–Wadsworth–Emmons reaction. The synthesis of compound **473** was initiated using compound **471** as a starting material **(Scheme 4.30)**. This compound was synthesized using NaH to form an open chain and trapped the hydroxyl group with TIPSCl. This compound was used for a Horner–Wadsworth–Emmons reaction with ketone **433**. We attempted to generate the corresponding ylide *in situ* using *n*-BuLi, but the desired product never formed, and the reaction gave a complex mixture. This result may be explained by too much combined steric hinderance on both the ylide and the electrophile, ketone **433**.



Scheme 4. 30 Synthesis of compound **473** and a Horner–Wadsworth–Emmons attempt between compound **473** and **433**

Our next proposed approach was built based on the recent application of cyclic sulfamidates, derived from serine, to activate the β-position to nucleophilic attack.[22, 23] A Julia-Lythgoe olefination would then allow us to make our target, and this inspired us to combine these methods for the synthesis of TF antigen. This approach, **(Scheme 4.31)**, involves a thiol functionality in the galactose moiety that would β attack a cyclic sulfamidate **475** in glucosamine sugar to connect two sugar moieties together. Sulfur was oxidized to form the sulfone, which upon Ramberg-Bäcklund Reaction would generate the bridge-bearing alkene group. This olefin then could be selectively reduced to build a one-carbon bridge with a β orientation.



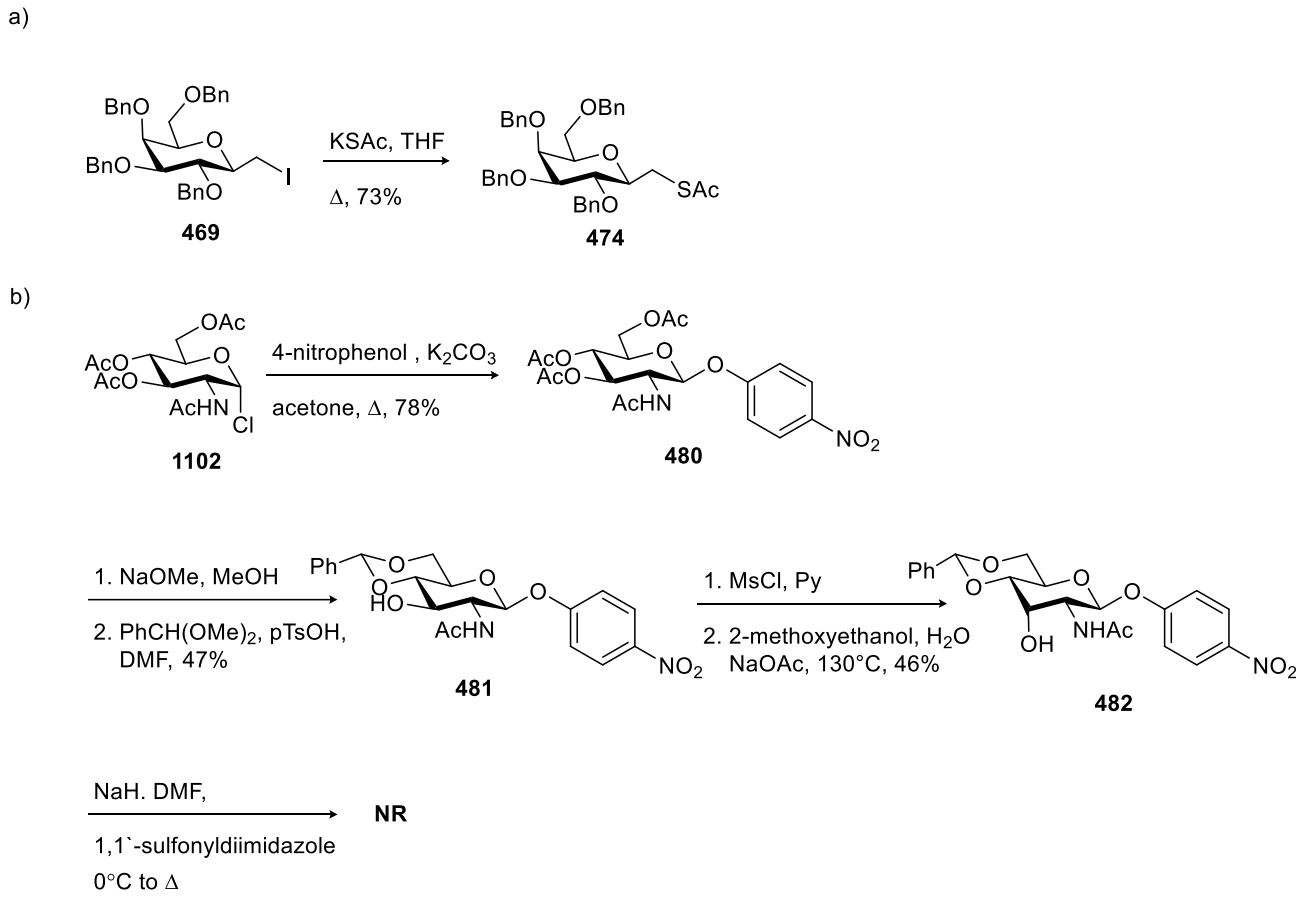
Scheme 4. 31 Proposed synthesis of acetal TF antigen through a cyclic sulfamidate **475** formation as the key step

To begin our synthesis, compound **474** was synthesized from compound **469**, which is to be used as a nucleophile in an S_N2 reaction to attack a cyclic sulfamidate **475** and open the cyclic

ring. The nucleophile synthesis was straight forward with a simple nucleophilic substitution between thioacetate and iodide afforded the compound **474** in 73% yield, **(Scheme 4.32a**) which then could easily convert to the resulting thiol using sodium methoxide**.**

The synthesis of the galactosamine moiety was more challenging. The nucleophilic substitution between glucose bearing thiol directly attaching to the anomeric position and compound **475** was already reported by Fernández-Mayoralas,[24] however, in our case, -CH_2SH is attached to the anomeric position of galactose and electrophile has to be galactosamine moiety. First, we tried to investigate whether we could successfully perform an S_N2 reaction between compound **474** and compound **475** bearing glucosamine moiety, hoping to convert to galactosamine in later steps.

Synthesis of compound **475** began based on known procedure reported by Withers[25] using compound **1102** as the starting material **(Scheme 4.32b**). 4-nitrophenol was applied as a glycosyl acceptor to react with the glycosyl chloride donor in the presence of K_2CO_3 to generate compound **480**. The acetyl group was then deprotected, and the hydroxyl group at C-6 and C-4 were blocked by a benzylidene protecting group using benzaldehyde dimethyl acetal and *p*-TsOH. The equatorial hydroxyl group was then converted to the axial orientation through another S_N2 reaction. A mesylation of the hydroxyl group using MsCl and pyridine followed by nucleophilic substitution with water yielded the axially oriented hydroxyl group on compound **482**. Despite the known procedure for sulfonation using 1,1′-sulfonyldiimidazole, we were not able to obtain the same result.



Scheme 4. 32a,b Synthesis of the first monomer **474**, a, and Attempt to synthesis of the second monomer, b

Unfortunately, none of these routes toward the synthesis of acetal-free TF antigen resulted in the desired product, and we had to move on the next approach, the synthesis of acetal-free TF antigen *via* Baylis-Hillman reaction, which will be discussed in the next section.

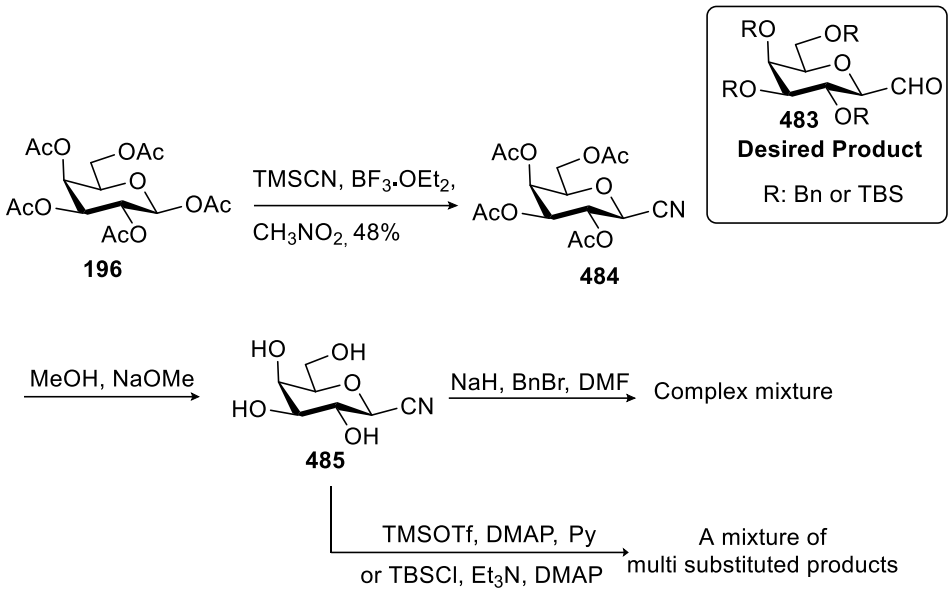
4.4 Attempt to synthesis of acetal-free TF antigen *via* Baylis-Hillman reaction

This synthetic route was inspired by Vogel's synthesis of the mono C-glycoside analogue of the TF antigen.[26] We tried to modify the synthesis with two goals in mind. First, we are targeting the fully acetal-free analogue of the TF antigen. Second, we are trying to build a more efficient route for the synthesis antigen. In particular, we concluded that starting from the synthesis of the mono C-glycoside, reported by Vogel which itself was performed in more than 40 steps, to access

a fully acetal-free version would not be practical if it were to be actually used in a glycopeptide as we intend.[26]

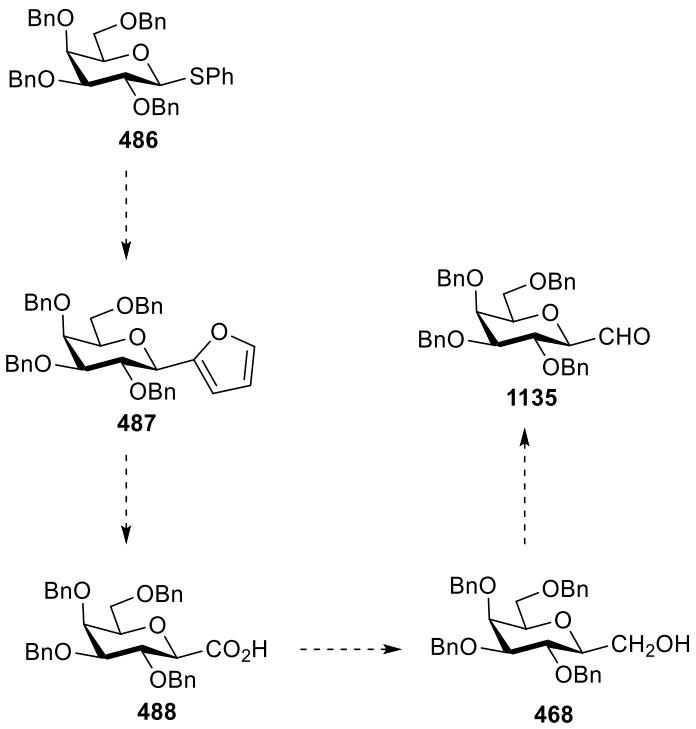
Our first proposed synthesis of a β-oriented anomeric aldehyde of a galactose moiety goes through a cyanation procedure and the results are shown in **Scheme 4.33**. TMSCN was used as the cyanide source that replaced the acetyl group at the anomeric position *via* a Lewis acid assisted nucleophilic substitution using BF_3•OEt_2. Next, the rest of the acetyl groups were removed *via* a Zemplén deacetylation. Although the cyanation and acetyl deprotection procedures worked smoothly the next step, protection of the hydroxyl group, was problematic. First, compound **485** was subjected to the standard benzylation procedure using NaH, BnBr in DMF, which did not result in the desired product. It may be explained by the electron-withdrawing nature of the cyanide group, which makes the proton at the anomeric position more acidic and using a strong base such as NaH drives the reaction towards an elimination product.

As an alternative, the TBS protecting group was then applied to protect the hydroxyl group in two separate reactions. First TBSCl was used with Et_3N, DMAP and imidazole. This reaction afforded a mixture of multi-substituted products resulting from incomplete protection. Forcing the reaction toward completion by heating and increasing the amount of each reagent still did not provide the desired outcome. TBSOTf was then applied as a TBS source accompanied by DMAP and pyridine; however, no desired product was observed. Despite using a milder base and a stronger electrophilic TBS source, the steric effect and decreased reactivity of the alcohols toward the electrophile results in the same incomplete protection.



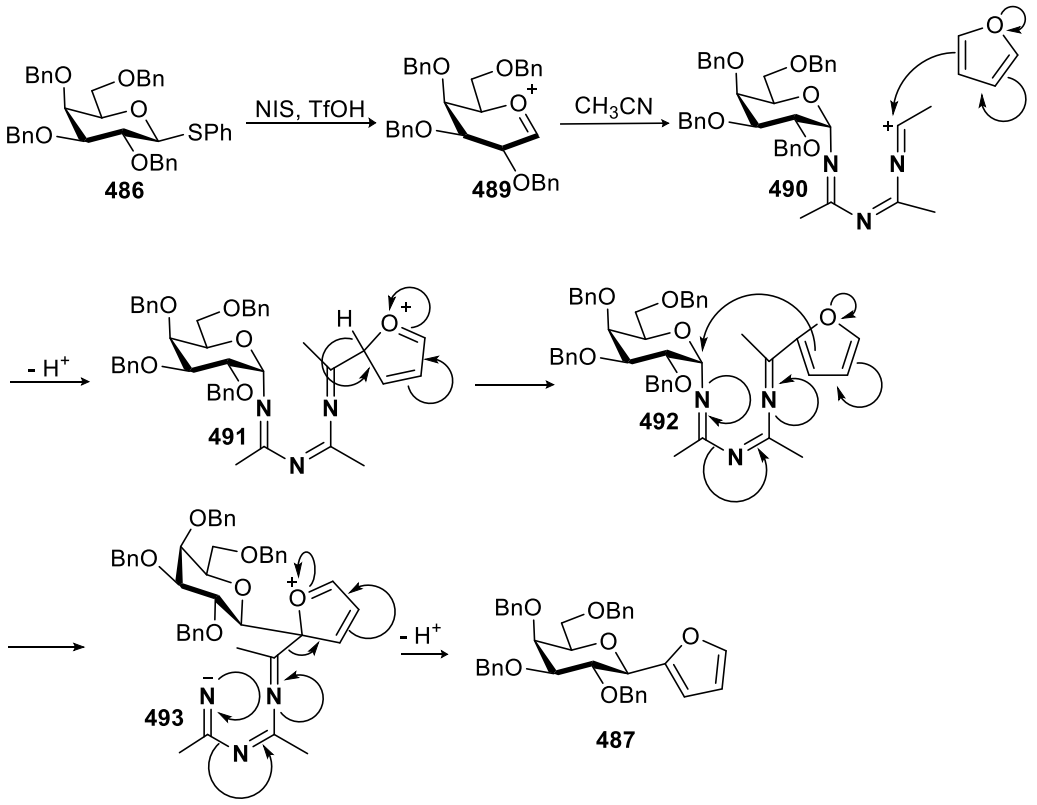
Scheme 4. 33 Synthesis of aldehyde **483** *via* cyanation procedure

Next, we turned our attention to the use of a furan substituent at the anomeric position which we could convert to the aldehyde in three steps. The proposed synthetic analysis is presented in **Scheme 4.34**. First, oxidative cleavage of furan, reduction of the carboxylic acid and then subsequent oxidation of the alcohol would form the aldehyde. A solvent assisted procedure was proposed to push the furan to attack from the equatorial site of the anomeric position.



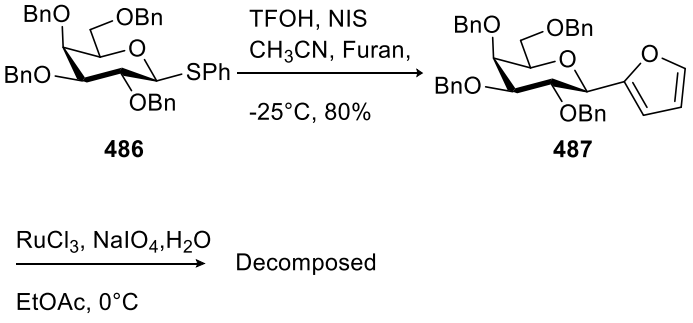
Scheme 4. 34 Proposed synthesis of aldehyde **1135** through furan oxidative cleavage

The first glycosyl donor was synthesized from fully acetylated galactose. First, SPh was installed at the anomeric position, then the acetyl protecting groups were replaced with non-participating benzyl groups to facilitate glycosylation. Glycosyl donor **486** was then activated by NIS and TfOH to form the oxocarbenium ion. In order to get a β-oriented furan at the anomeric position, acetonitrile is employed as a solvent for glycosylation to take advantage of a solvent assisted procedure reported by Schmidt.[27] This was successful, producing the furyl glycoside in 80% yield. The mechanism originally proposed by Schmidt is shown in **Scheme 4.35**. After formation of the oxocarbenium ion, acetonitrile then could attack to the C-1 position to build a network. This network encumbered the axial face and nucleophile, furan, in this case, can occupy β position. However, the presence of this network and solvent assistance in general is under debate and are currently under investigation in the Trant group.



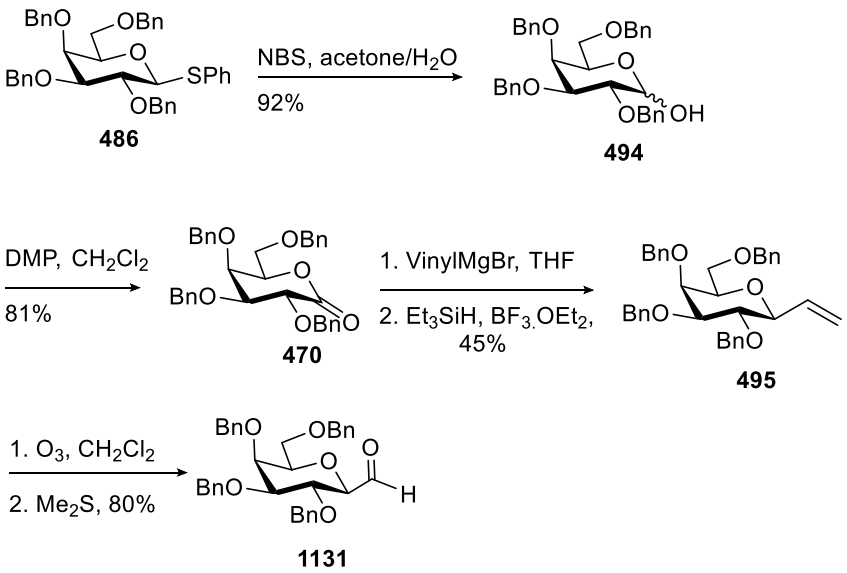
Scheme 4. 35 Proposed solvent effect of acetonitrile for *β*-glycosylation through an axial network by Schmidt

Oxidative cleavage of the furan substituted galactose **487** based on the standard Sharpless procedure was performed successfully using $RuCl_3$, $NaIO_4$;[28] however, the carboxylic acid produced was unstable and decomposed in one hour. This is not a very useful reaction.



Scheme 4. 36 Attempt to synthesis of aldehyde **1131** *via* oxidative cleavage of furan

The final approach, which successfully produced the desired aldehyde **1131** was designed with a Grignard reaction as the key step **(Scheme 4.37)**. First, lactone **470** was prepared from compound **486**, which was previously synthesized for the previous furan glycosylation approach. This molecule underwent hydrolyzation of the SPh group using NBS and water, and then the hydroxyl group was oxidized using Dess-Martin Periodinane to generate lactone **470** in 81% yield. The lactone **470** was subjected to a Grignard reaction using a vinyl magnesium bromide solution in THF. Subsequent dihydroxylation of the anomeric alcohol using triethylsilane and BF_3•OEt_2 afforded the desired β-vinylic compound **495** in moderate yield in two steps from **470**. Having a vinyl group in the β orientation at the anomeric position in hand, we tried the ozonolysis process to obtain aldehyde **1131**. The ozonolysis was performed, and subsequently dimethyl sulfide was used to cleave an O-O bond of the trioxolane intermediate produced from ozonolysis. This reaction gave the desired aldehyde in good yield. With aldehyde **1131** in hand, we turned our attention to the second component necessary for the Baylis-Hillman reaction, the enone **499**, and finally to linking the two sugar moieties and forming the first double C-glycoside disaccharide.

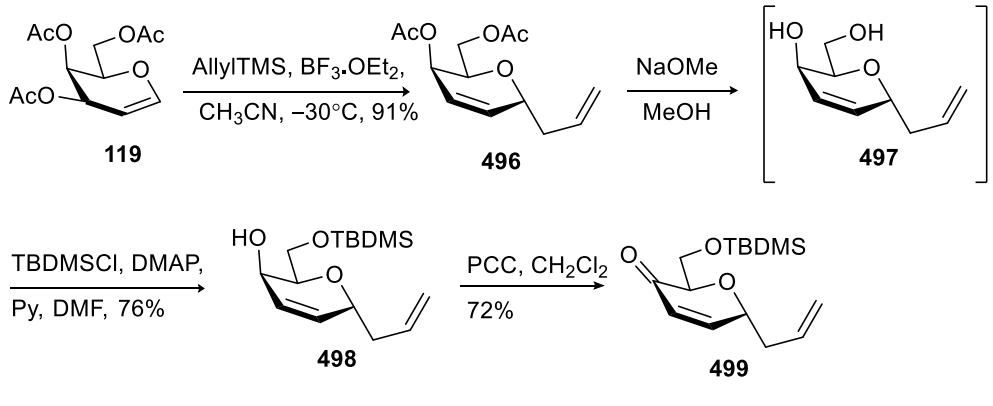


Scheme 4. 37 Synthesis of aldehyde **1131** through a Grignard reaction as the key step

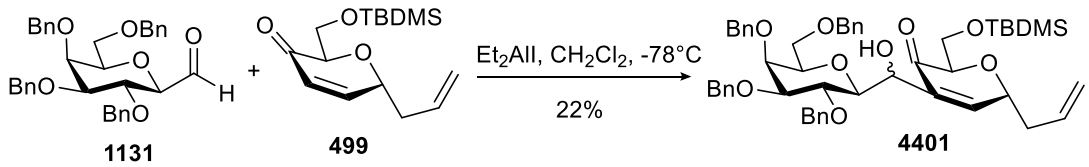
In order to save some steps, an allyl group was introduced to the second monomer at the anomeric position, which was intended to be used to convert to the galactosamine moiety in later steps. The installation of an allyl group before a Baylis-Hillman reaction allows the formation of the first double C-glycoside of a disaccharide in fewer steps and provides the tools for the attachment of an amino acid to the allylic functionality.

The Ferrier rearrangement[29, 30] was performed to install the allyl group at the anomeric position in the presence of BF_3•OEt_2 in acetonitrile. As expected, an absolute *α* oriented allylated product was obtained, then acetyl groups were removed *via* NaOMe, MeOH. The hydroxyl group at the C-6 position was then selectively protected by the TBS group using Et_3N and DMAP,[31] which upon oxidation using PCC, formed the desired enone **499** in good yield **(Scheme 4.38a)**. Finally, the Baylis-Hillman reaction was carried out using Et_2AlI to couple enone **499** and aldehyde **1131**. This reaction afforded the first disaccharide bearing double C-glycoside **4401** in low yield **(Scheme 4.38b)**.

a)

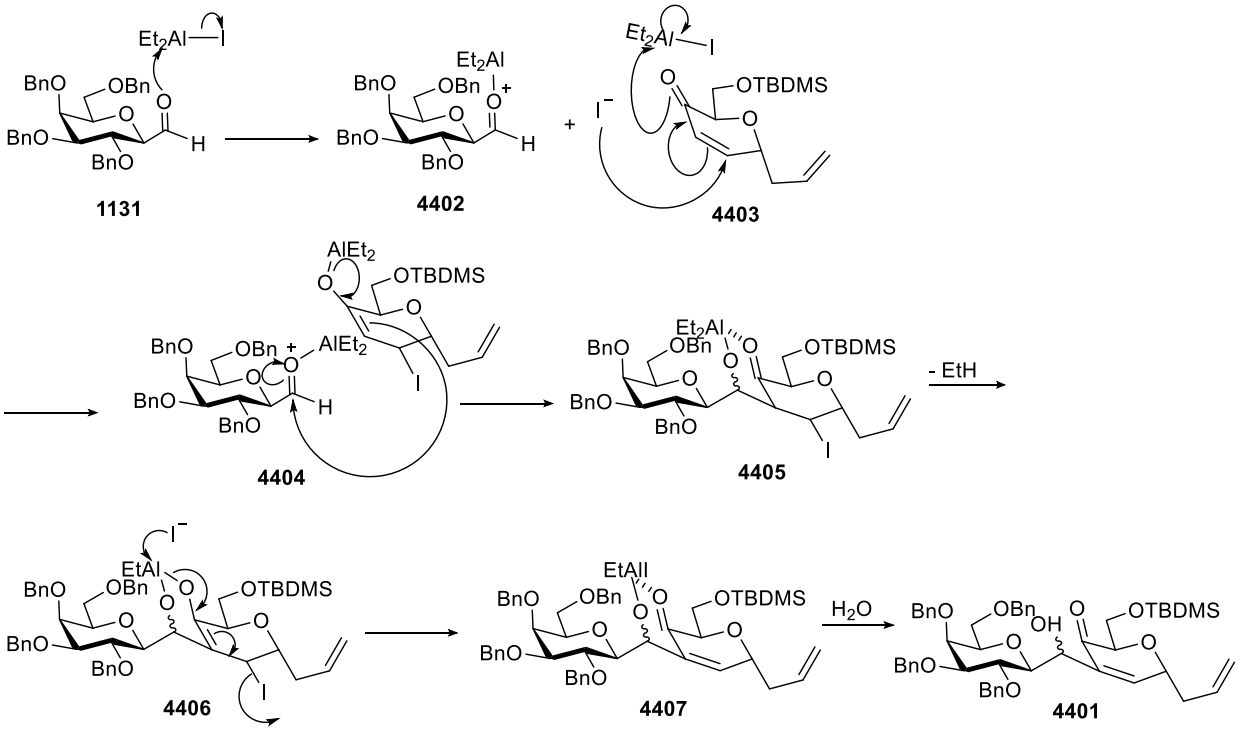


b)



Scheme 4. 38a,b Synthesis of the second monomer **499**, a, and Synthesis the first double C-glycoside of disaccharide *via* a Baylis-Hillman reaction, b

A mechanism for this reaction is proposed in **Scheme 4.39**. Initially, the aldehyde **1131** is activated by the Lewis acidic aluminum species. Iodide ion then attacks in Michael 1,4-addition fashion to the enone, which upon collapse of the enolate establishes the C-C bond formation. The enolate again forms by removing an ethanol molecule, which upon the nucleophilic attack of iodide ion to the aluminum, regenerates the Et_2AlI and the enone. The desired product **4401** obtained after work up *via* hydrolysis.



Scheme 4. 39 Mechanism of a Baylis-Hillman reaction between aldehyde **1131** and enone **499**

With the linkage of the sugars complete and the double C-glycoside in hand, we set up a plan to get to the target TF antigen analogue. Our proposed strategy includes, first, introducing nitrogen source to the C-2 position of the enone, dihydroxylation of the bridge, a selective reduction of ketone followed by linking the amino acid to the galactosamine moiety, the sites of these

manipulation are highlighted in **Figure 4.3**. At first glance this approach seems lengthy, however, if the plan works it would still be less than 40 steps and would be a more efficient route for the synthesis of a very important biological relevant and completely acetal-free TF antigen analogue.

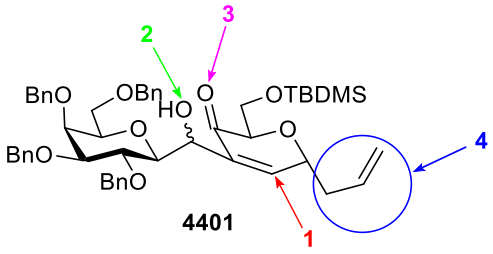
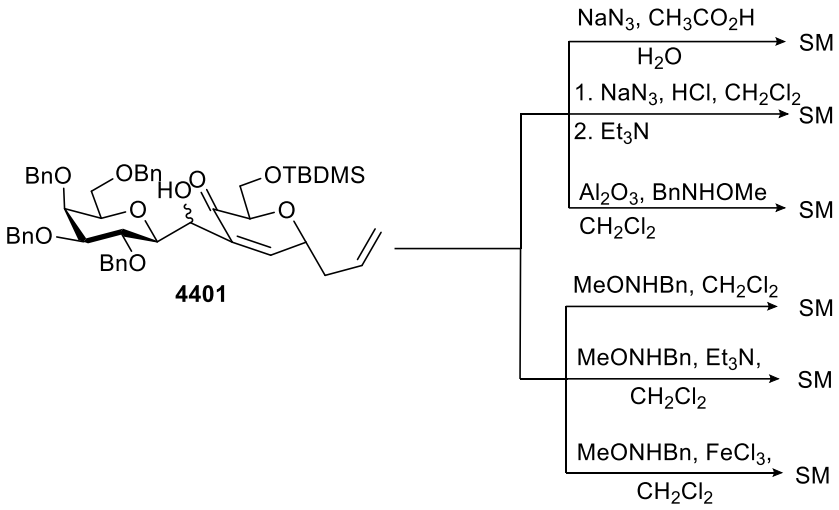


Figure 4. 3 Manipulation sites of C-glycoside disaccharide towards synthesis of acetal-free TF antigen **401**

We first tried to introduce a nitrogen source at the C-2 position. First, we tried using neat MeONHBn, which is a known reagent to do conjugate addition with isolevoglucosenone reported by Vogel.[32] Unfortunately, this reagent did not work with our α,β-unsaturated system, and the starting material was recovered. NaN_3 and MeONHBn were then used with a wide range of acidic and basic conditions to push the reaction forward which are summarized in **Scheme 4.40**. Acetic acid, HCl and alumina were used for the azidation reactions; however, none of them showed any conversion. Introducing a nitrogen source using MeONHBn was then again examined, this time *via* basic, using Et_3N and acidic, using $FeCl_3$, conditions. Again, no conversion was observed. The results suggest low reactivity of the nitrogen sources or, more likely, the α,β-unsaturated system **500**.



Scheme 4. 40 Michael addition procedures of enone **500**

Two main challenges have to be addressed in order to move forward using this approach. First, the key step in the yield and scalability of the Baylis-Hillman reaction must be optimized as a lengthy synthesis remains in order to get to the target TF anitgen.[26] Second, Identification of a more reactive nitrogen source with higher nucleophilicity is required. Depending on the identity of this reagent, further manipulation may be required in order to convert it to the acetamide group with the correct stereochemistry. Once these two issues are solved, the selective reduction of the ketone could be accomplished using $LiBH_4$, as previously published by Vogel.[26] Finally introducing an amino acid to the sugar moiety has already been solved in the synthesis of the Tn antigen in Chapter 3 and similar methodology could be applied here.

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Chapter 5: Total synthesis of glycolipids from *S. pneumoniae* and the biological re-evaluation of their immunological activity

5.1 Synthesis of mono- and disaccharide glycolipids

Glycosphingolipids are very important in biological compounds. Invariant natural T killer cells are a subclass of white blood cells, which activate the immune system through releasing cytokines. These cells are activated with glycolipid antigens. Once activated, they engage in many effector functions. They can activate T cell, B cell, dendritic cells, and macrophages. Also, they can be involved in the cross-presentation activity and NK transactivation. This means we can expect a robust immune response from the activation of iNKT cells. Unfortunately, there is no known mechanism up to date to control this massive immune response. However, this type of robust immune response could be applied as the last line of defense against cancers, or pathogenic infections.

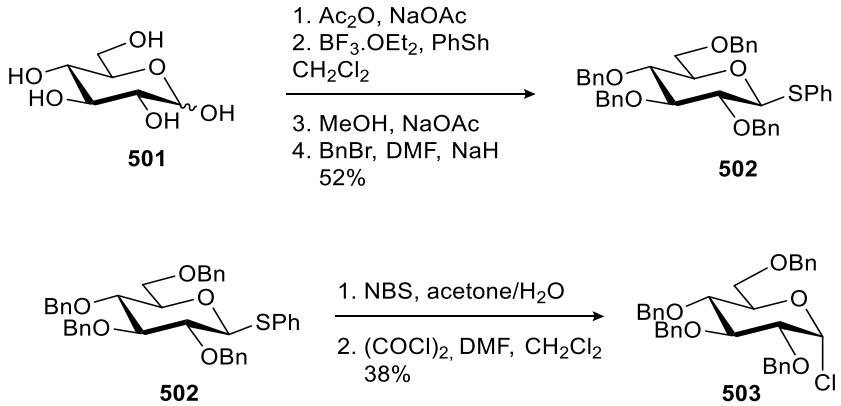
iNKT cells recognize lipid antigens presented by CD1d, a non-polymorphic major histocompatibility complex class I-like antigen-presenting molecule. These cells are conserved between humans and mice, specifically the glycolipid antigens. KRN 7000 was the first glycolipid antigen known to have the ability to activate iNKT cells, this antigen was isolated from sea sponge in japan. KRN 7000 was isolated as a result of structure-activity studies on glycolipid cell extracts of sea sponge. KRN 7000 acts as an antigen causing a systemic immune response in mammalian cells, by the release of signaling molecules called cytokines, and has potential as immunotherapy of last resort. However, it is still unclear how the mammalian immune system responds to the glycolipid antigen isolated from a sea sponge.

Two main fractions of antigenic *S. pneumoniae* glycolipids, which were isolated from *S. pneumoniae*, have been identified; one contains a monosaccharide glucose sugar α-linked to diacylglycerol (DAG), the second was identical except it had a galactose moiety attached to the DAG shown in **Figure 2.2.** Both the monosaccharide and the disaccharide appear capable of activating

iNKT cells, however, the exact biological properties of these two glycolipids remained uncertain. Despite a few reported synthesis of the monosaccharide, biological evaluation of this antigen remained incomplete, and synthesis of disaccharide glycolipids has yet to be reported. Here an efficient and well manipulated total synthesis of both *S. pneumoniae* glycolipids and our re-evaluation of their immunological activity are discussed.

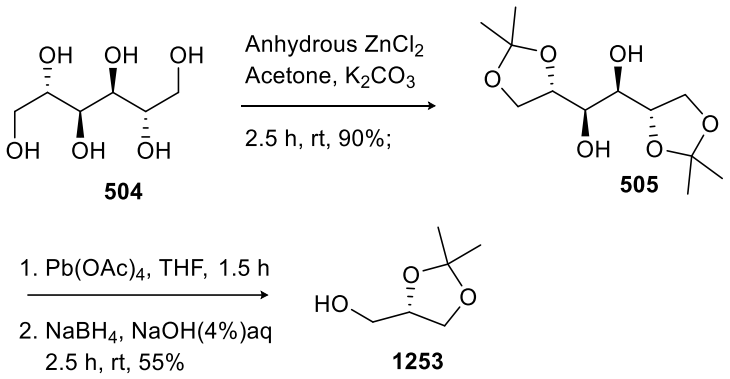
5.1.1 Synthesis of monosaccharide glycolipid 1168

The synthesis of **1168** was initiated using glucose as a starting material **(Scheme 5.1)**. Glucose was acetylated using the standard acetylation protocol, then the acetyl group at the anomeric position was replaced with an SPh functionality. The acetyl protecting groups were replaced with non-participating benzyl protecting groups to prevent the anchimeric assistance of the acetyl group for the glycosylation step. Zemplén deacetylation, followed by benzylation using BnBr and NaH in DMF afforded compound **502**.[1] Finally, the glycosyl chloride donor was prepared for use in the glycosylation reaction. This compound was synthesized by hydrolyzing SPh in compound **502** using NBS and H_2O,[2] then chlorination of the anomeric position *via* oxalyl chloride afforded glycosyl donor **503** in 38% yield (**Scheme 5.1)**.[3]



Scheme 5. 1 Synthesis of compound **502** and chlorination of the anomeric position

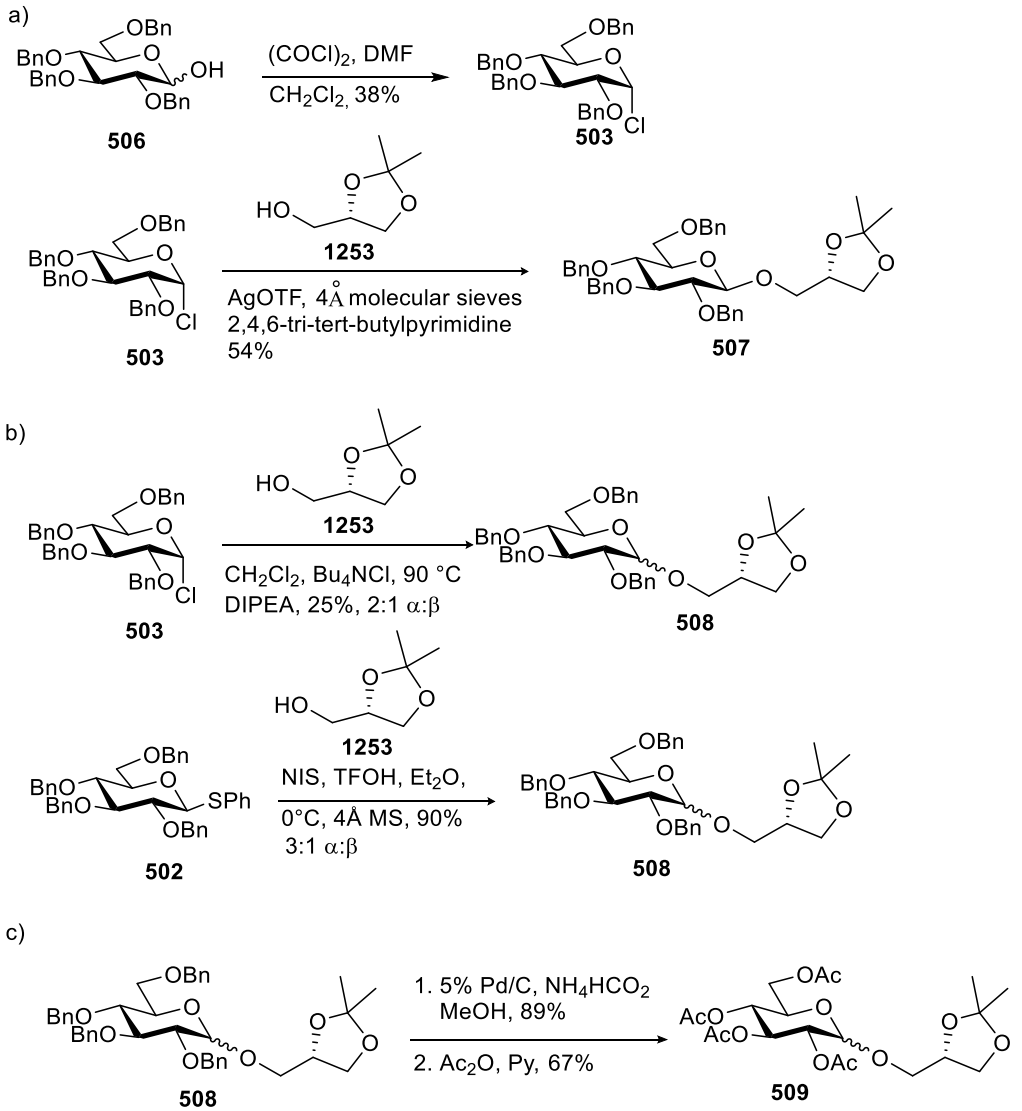
The diglycerides acceptor was synthesized based on Trivedi's reported procedure as shown in **Scheme 5.2**.[4] D-mannitol was protected with zinc chloride and acetone to form mannitol di-acetonide. An oxidative cleavage followed by reduction afforded the glycosyl acceptor **1253**. it is worth mentioning that the oxidative cleavage step of mannitol di-acetonide, was done using lead IV acetate as sodium periodate did not work according to Trivedi's report.[4]



Scheme 5. 2 Synthesis of glycosyl acceptor **1253**

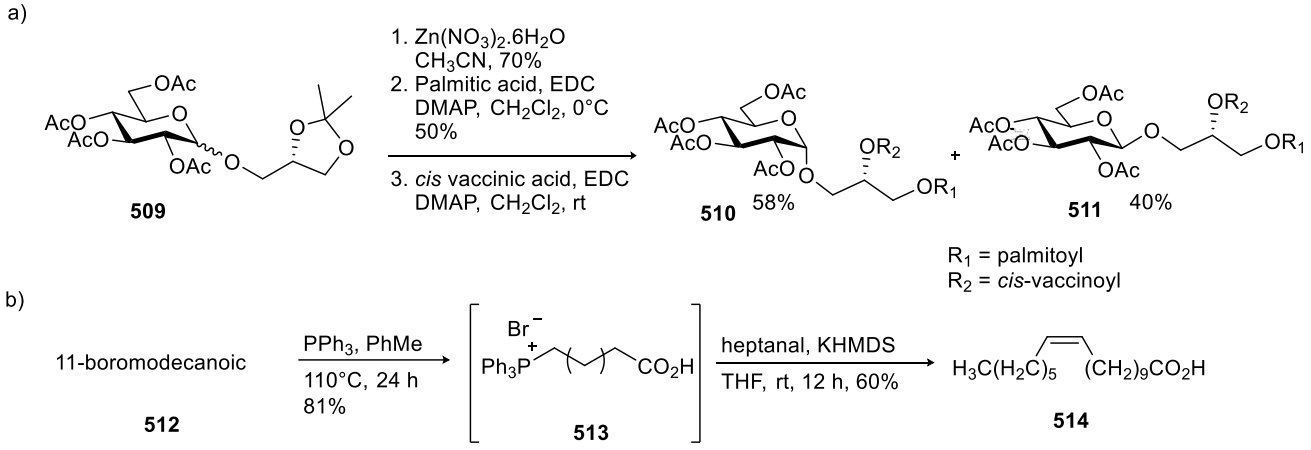
The first glycosylation reaction between glycosyl donor **503**[3] and glycosyl acceptor **1253** was performed based on the Pozsgay approach using AgOTf and 2,6-di-*tert*-butylpyrimidine.[5] The result showed exclusively β oriented glycosylic bond against our expectation based on Pozsgay's outcome **(Scheme 5.3a)**.[5] The next glycosylation procedure was done using the glycosylation procedure reported by Sugawara with the exact glycosyl donor and acceptor using Bu_4NCl and DIPEA.[6] However, despite numerous attempts, we were not able to reproduce the same yield or selectively reported by Sugawara. In our hands, it instead afforded a mixture of products in a 2:1 α:β ratio with low yield. We then pivoted to a solvent assistant strategy using glycosyl donor **502** and glycosyl acceptor **1253 (Scheme 5.3b)**.[7] This resulted in a 90% yield with slightly better selectivity toward the α anomeric oriented product in a 3:1 α:β ratio. However, the standard purification procedure was not successful, and a mixture of both anomers was used for the next step planning to

separate them in later synthetic steps. Next, the benzyl protecting group was replaced with an acetyl group **(Scheme 5.3c)** to prevent complications at the last deprotection step as the *cis*-vaccenic acid fatty chain contains an alkene functionality, which is prone to reduction in the hydrogenation step. Pd/C was used under a H_2 atmosphere to remove the benzyl groups, then the resulting hydroxyl groups were acetylated *via* Ac_2O and pyridine in a 67% overall yield.



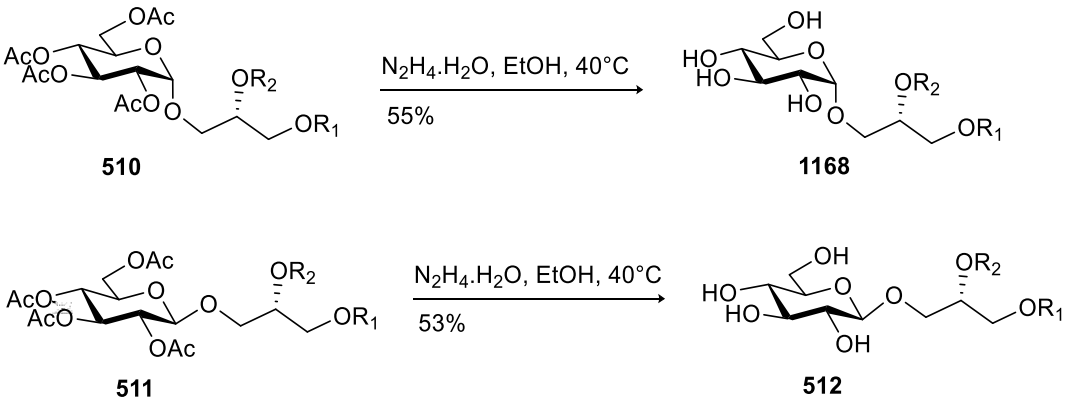
Scheme 5. 3 Glycosylation procedures a,b and c

The isopropylidene group was removed using zinc nitrate to form free hydroxyl groups ready for acylation.[8] The first selective esterification of the primary alcohol was performed by controlling the temperature at 0°C in the presence of Palmitic acid, EDC and DMAP **(Scheme 5.4a)**. The *cis*-vaccenic acid was freshly prepared as shown in **Scheme 5.4b** and applied for the second esterification. The synthesis of *cis*-vaccenic acid followed the protocol published by Duffy in two steps.[9] First, a triphenylphosphonium fatty acid was formed *via* reaction of 11-bromodecanoic acid with PPh_3. Then an ylide, generated using KHMDS, was able to attack heptanal through a Wittig reaction resulting in *cis*-vaccenic acid. Then *cis*-vaccenic acid was successfully used in the esterification of the secondary alcohol in the presence of EDC and DMAP. Fortunately, at this stage the mixture of α and β anomers were easily separated using column chromatography.



Scheme 5. 4 Synthesis of compound **510** and **511,** a, and *cis*-vaccenic acid **514,** b

The final deprotection was carried out using N_2H_4 at 40°C, which is the only reported reagent capable of deprotecting acetyl group in the presence of long acyl chains.[10] This reaction afforded both α and β anomers of glycolipids in 55% and 53%, respectively.



Scheme 5. 5 Synthesis of both α and β glycosylic bond of monosaccharide glycolipids**, 1168,** and **512.**

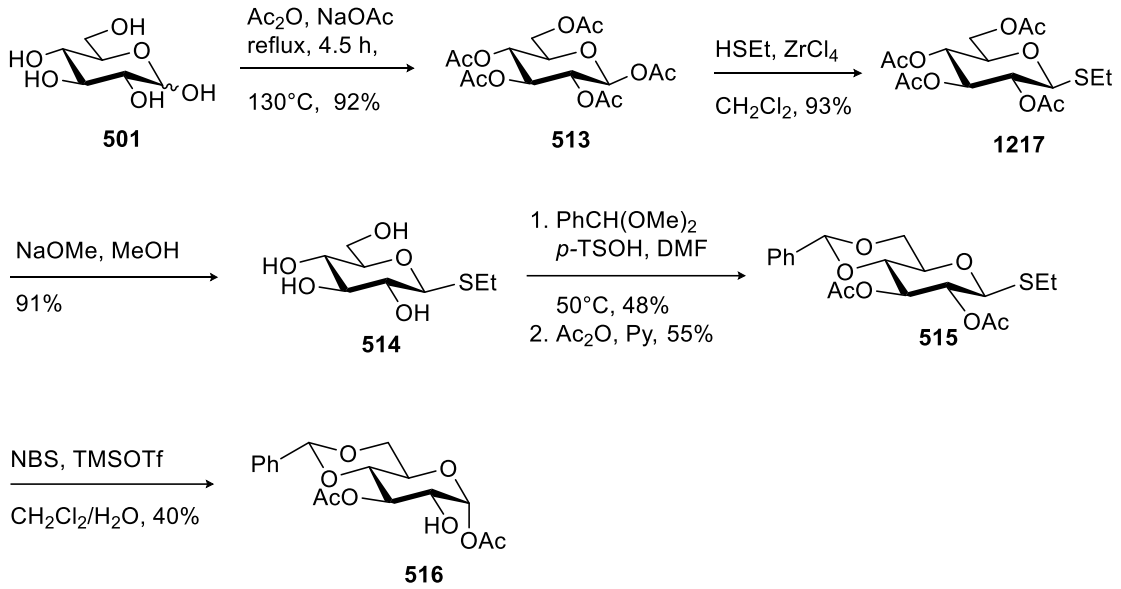
The monosaccharide glycolipid **1168** was synthesized successfully starting from glucose in eleven steps, and ready to be sent for the further biological evaluation. In the next section, the synthesis of disaccharide glycolipid **202** will be discussed.

5.1.2 Synthesis of disaccharide glycolipid

Our proposed approach for the synthesis of disaccharide glycolipid **202** was designed to link glucose to the galactose moieties through a glycosylation procedure, then the second glycosylation between disaccharide and (*S*)-(+)-Solketal would generate the backbone of the disaccharide glycolipid. The fatty acid chains could then be introduced to the glycerol moiety in the same manner as described in Section 5.1.1 to afford disaccharide glycolipid antigen **202**.

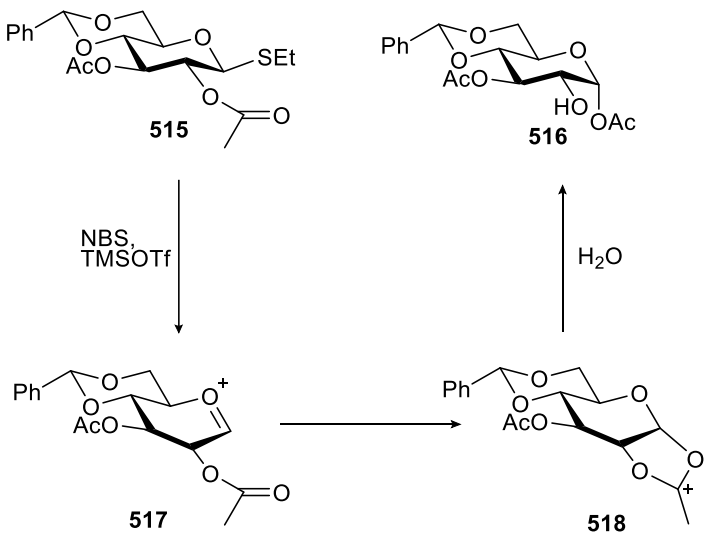
First, the glycosyl acceptor **516** was targeted and synthesized, based on a known procedure reported by Ensley, in 5 steps starting from glucose **(Scheme 5.6)**.[11] The acetylation of glucose was performed based on the standard protocol and then the acetyl group at the anomeric position was replaced with a SEt functionality using $ZrCl_4$ in 93% yield. The acetyl protecting groups were then removed *via* Zemplén deacetylation procedure. The hydroxyl groups at C-4 and C-6 positions were blocked with the benzylidene functionality *via* benzaldehyde dimethyl acetal and *p*-TsOH at

50°C, and the rest of the hydroxyl group protected by acetyl group using Ac_2O and pyridine. In order to get a free hydroxyl group at the C-2 position, (which is necessary for the glycosylation), an acetal migration assisted by NBS and TMSOTf has been done to afford glycosyl acceptor **516** in 40% yield.



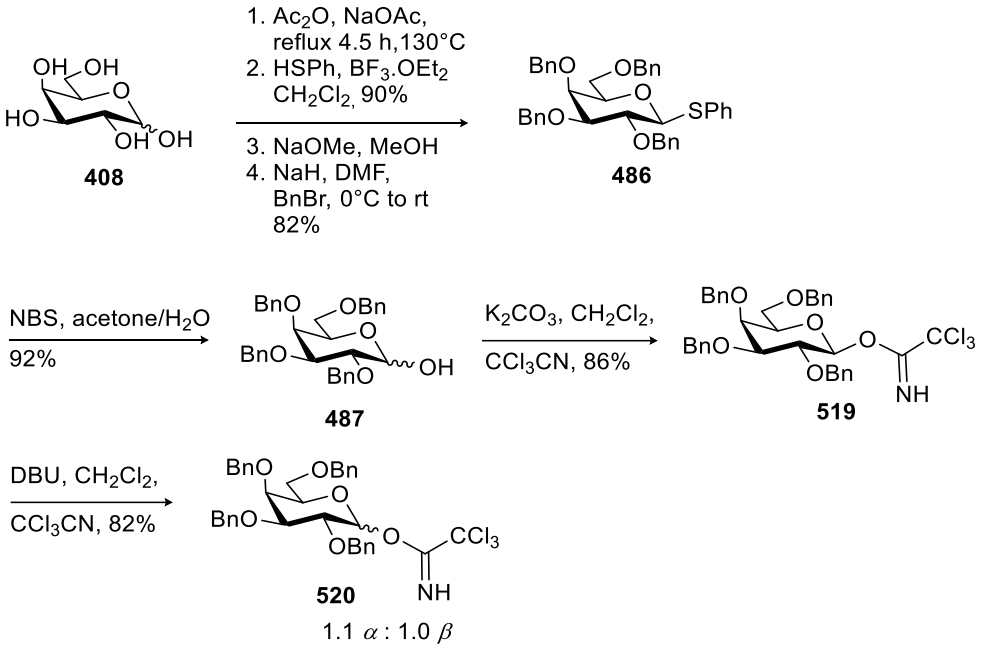
Scheme 5. 6 Synthesis of glycosyl acceptor **516**

This acetal migration is rationalized by the mechanism depicted in **Scheme 5.7.** First, the activation of the glycosyl donor bearing the SEt group at the anomeric position *via* NBS and TMSOTf to make an oxocarbenium **517**. The oxocarbenium then undergoes a nucleophilic attack by the carbonyl oxygen of the acetyl group at the C-2 position, which upon addition of water, the acetyl group migrated to the C-1 and the free hydroxyl group is formed to make acceptor **516**.



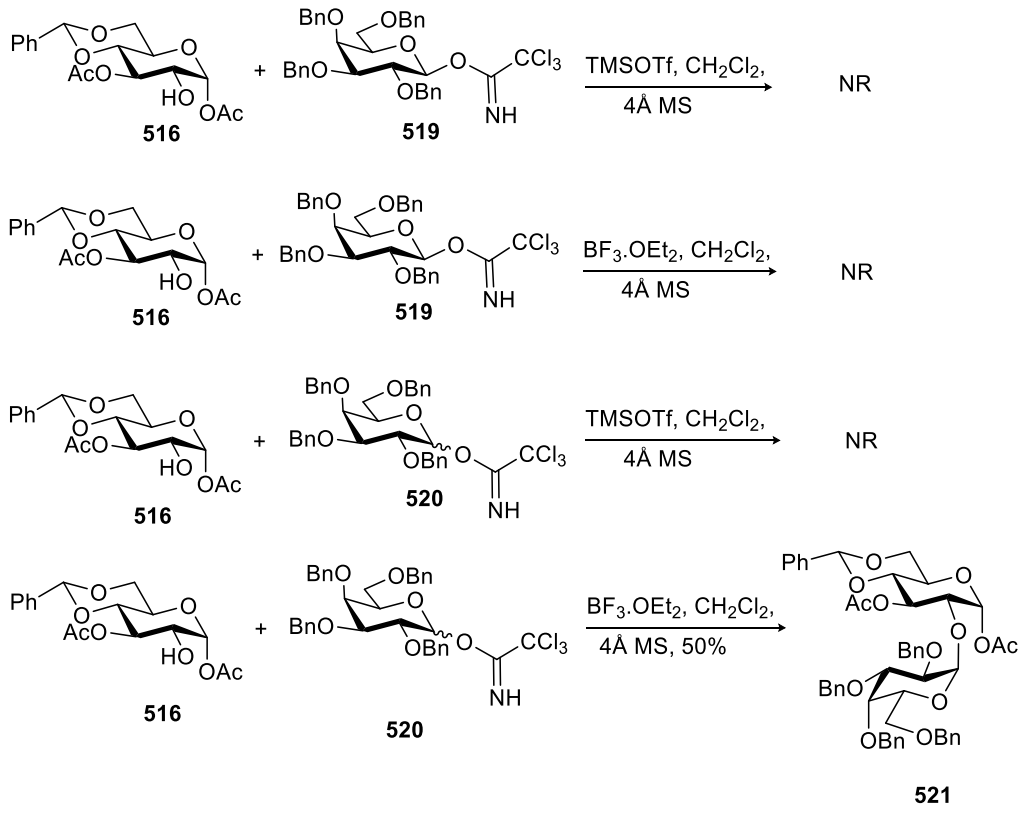
Scheme 5. 7 Mechanism of acetyl migration to the anomeric position

With the glycosyl acceptors in hand, the glycosyl donors **519** and **520** were prepared for the glycosylation in two separate procedures starting from compound **408 (Scheme 5.8)**.[12] After hydrolyzation of SPh using NBS and H_2O compound **487** treated with trichloroacetonitrile and K_2CO_3 to produce β oriented glycosyl donor **519**. The attempts to provide α oriented glycosyl donor did not afford exclusively α anomer. The treatment of compound **487** with trichloroacetonitrile in the presence of DBU gave a mixture of anomeric glycosyl donor **519** and **520** in 82% yield. It is worth mentioning that these glycoside donors needed to be used for glycosylation directly after synthesis due to their instability.



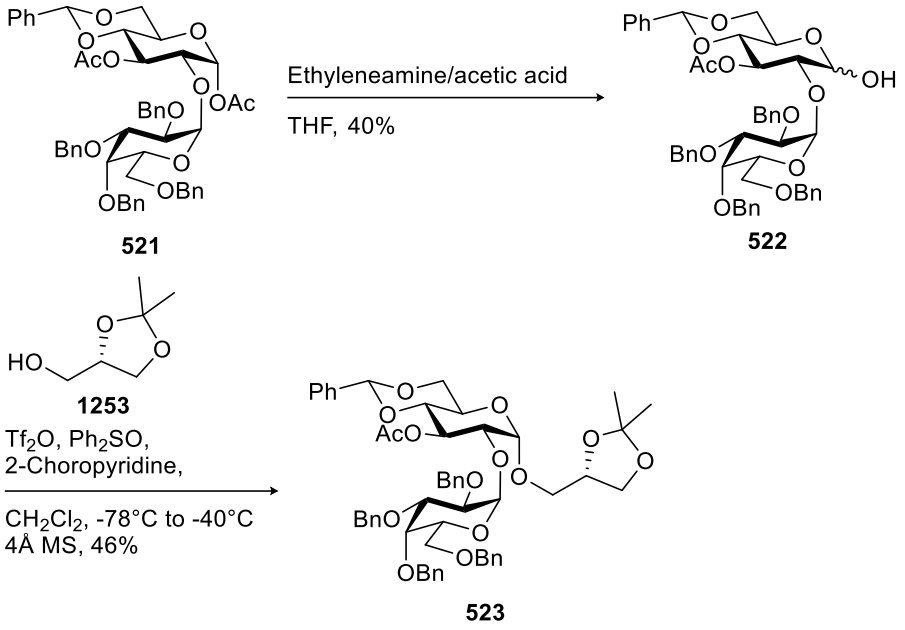
Scheme 5. 8 Synthesis of glycosyl donors **519** and **520**

In order to optimize the glycosylation reaction a set of experiments were examined under anhydrous conditions using various reaction conditions in order to achieve the highest yield and selectivity. BF_3•OEt_2 and TMSOTf were used as activators accompanied by α or β glycosyl donors in the glycosylation reaction **(Scheme 5.9)**. The results showed that TMSOTf was not an appropriate activator, and importantly, the β glycosyl donor was utterly inactive in glycosylation. The mixture of glycosyl donors was exploited for glycosylation in order to use the α glycosyl donor, as this donor could not be separated through standard purification procedures. This glycosyl donor was activated *via* BF_3•OEt_2 for the glycosyl acceptors attack and the product was achieved exclusively in the desired α orientation. The overall yield of this reaction indicates full conversion of the α glycosyl donor.



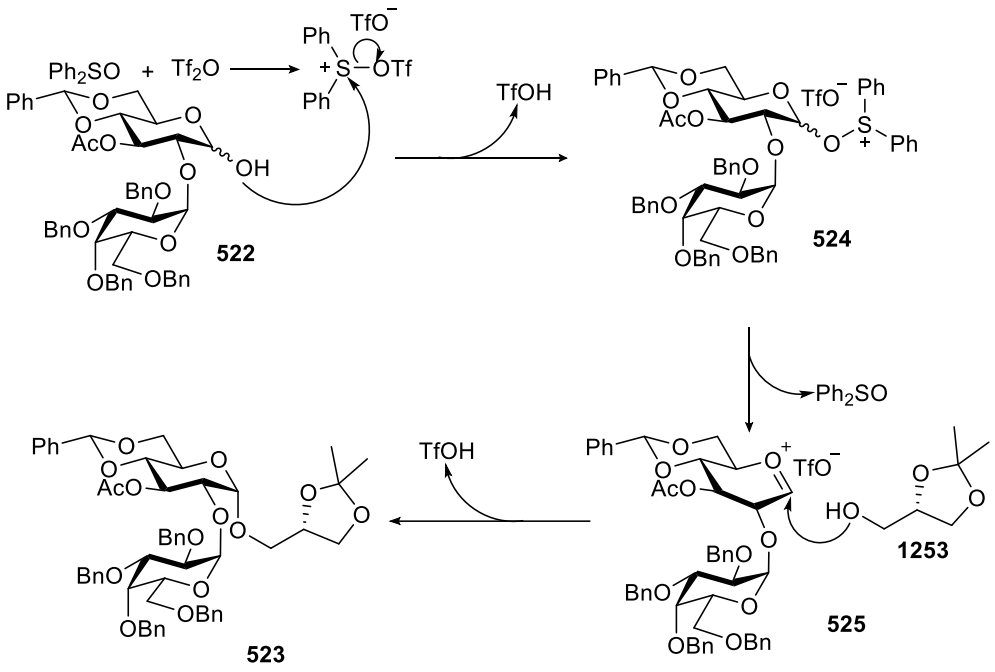
Scheme 5. 9 Glycosylation procedures between glycosyl acceptor **516** and glycosyl donors **519** and **52**0

Based on Savage's procedure, a regioselective deacetylation at the anomeric position was performed using a mixture of ethylenediamine and acetic acid **(Scheme 5.10)**.[13] After successful deacetylation, the second glycosylation was examined. First, diphenyl sulfoxide, TF_2O and 2,4,6-tri-*tert*-butylpyrimidine were applied to acceptor **1253**;[13] However, this reaction did not afford the desired product. Finally, 2,4,6- tri-*tert*-butylpyrimidine replaced with 2-chlropyridine to afford compound **523** in 46% yield with absolute α configuration.[14]



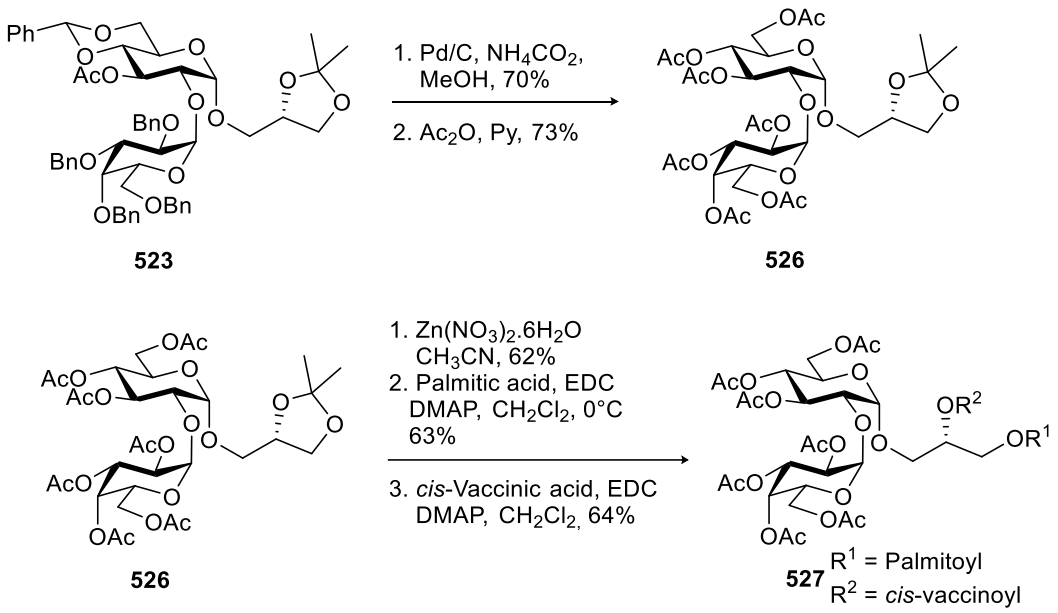
Scheme 5. 10 Synthesis of compound **150** through the second glycosylation

A proposed mechanism for this glycosylation is shown in **Scheme 5.11**. First, attack of sulfoxide oxygen to TF_2O forms the active diphenyl sulfide bis(trifluoromethanesulfonate) specious, which upon nucleophilic substitution release triflic acid and form the oxosulfonium trifluoromethanesulfonate **524**. The oxocarbenium trifluoromethanesulfonate **525** then forms by regeneration of diphenyl sulfoxide and finally the oxocarbenium ion reacts with the glycosyl acceptor **1253** to obtain compound **523**.



Scheme 5. 11 Mechanism of the second glycosylation

After this step, the same protocol was applied to add the long chain ester functionality as was used in the synthesis of the monosaccharide **(Scheme 5.12)**. First, the benzylidine and benzyl protecting groups were removed at the same time *via* hydrogenation, and then the hydroxyls were reprotected with acetyl groups. The isopropylidene group was then removed using zinc nitrate and primary and secondary alcohols in diglycerides part esterified with palmitic acid and *cis*-vaccenic acid, respectively.

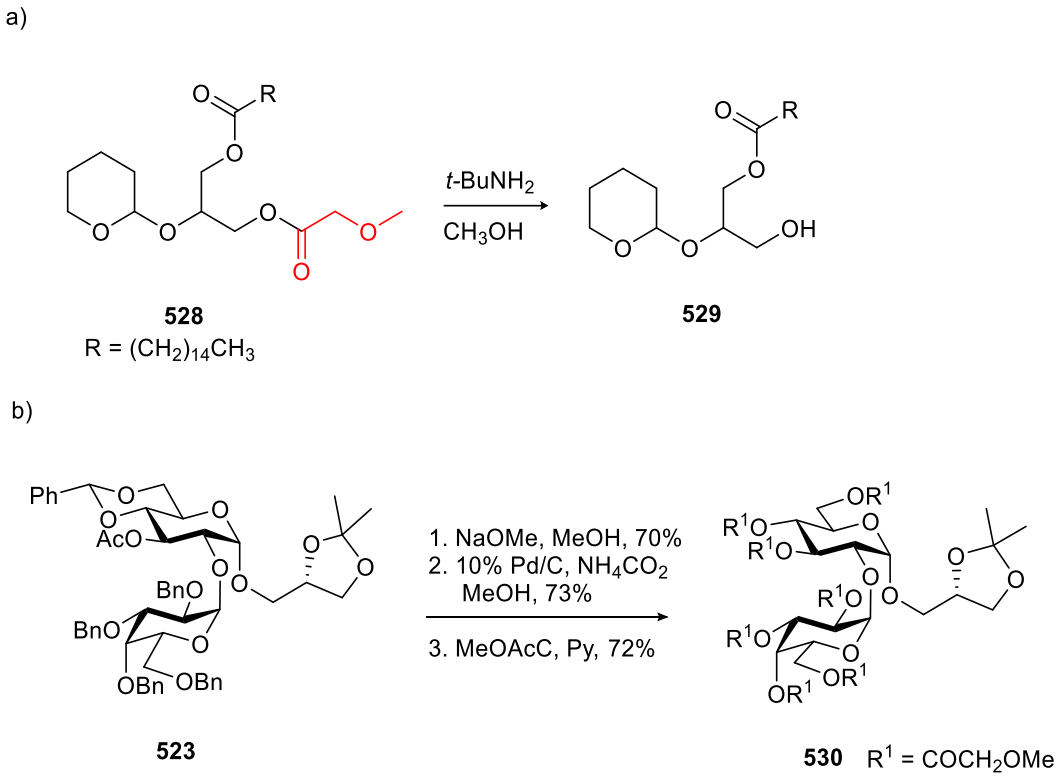


Scheme 5. 12 Synthesis of compound **527**

The final step, the chemoselective removal of the acetyl group, which was well accomplished in the previous examples, did not work out for the disaccharide **527**. The disaccharide **527** fragmented upon using hydrazine hydrate at standard conditions, however cooling down the temperature did not show any product formation. Evidently, hydrazine hydrate was too reactive to deprotect disaccharide glycolipid and disaccharide **527** decomposed to its monosaccharides and the glyceride in both room temperature and at 0°C.

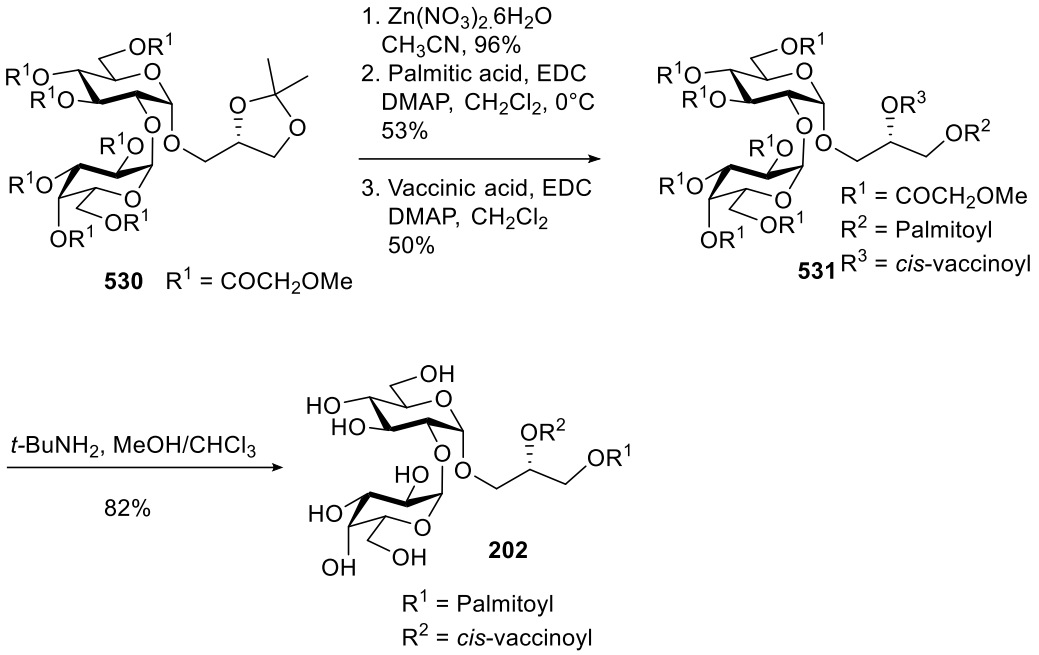
In order to get the final product, we had to try another protecting group, which requires a milder condition for the deprotection. We found methoxyacetyl (MeOAc) can be used as an alternative for the acetyl protecting group, which can be selectively removed *via t*-$BuNH_2$, a milder nucleophile than hydrazine hydrate **(Scheme 5.13a)**.[15, 16] Switching acetyl protecting groups to the MeOAc involved adding one extra deprotection step in our proposed approach. The full deprotection of compound **523** was performed in two separate steps. First, The acetyl group was removed with sodium methoxide, and then both benzylidine and benzyl protecting groups were

deprotected with palladium on carbon in methanol in 73% yield. This time, methoxyacetyl groups were applied as a protecting group.[15, 16] The full deprotected of compound **523** was treated with methoxyacetyl chloride and pyridine to generate fully methoxyacetylated disaccharide **530** **(Scheme 5.13b)**.



Scheme 5. 13a,b. A *t*-BuNH$_2$ as deprotecting agent for methoxyacetyl protecting group,a, and synthesis of compound **530**, b

The same protocol was successfully applied *via* isopropylidene removal, followed by stepwise esterification using palmitic and *cis*-vaccenic acids. The final product was obtained by selective deprotection of methoxyacetyl groups using *tert*-butyl amine in 82% yield **(Scheme 5.14)**.



Scheme 5. 14 Synthesis of disaccharide glycolipid target **202**

Although *t*-$BuNH_2$ is a milder and cheaper reagent than hydrazine hydrate and capable of deprotecting of methoxyacetyl groups, there is no reported application of *t*-$BuNH_2$ for the selective removal of acetyl groups in the synthesis of glycolipids. This discovery led us to consider another project, a selective deacetylation procedure using *t*-Butylamine, which will be discussed in the next chapter.

5.2 The First preliminary biological result

With the glycolipid antigens in hand, we proceeded to evaluate their biological activity. We employed mouse hydridoma models using two different cell lines: DN32.D3, and N38.3C3 with bone marrow-derived dendritic cells from a DR4 transgenic mouse, expressing human HLA-DRA-IEα and DRB1*0401-IEβ, on a B6 background, lacking any endogenous type II MHC receptors. The DN32.D3 hydridoma line is especially well designed for this experiment as it possesses both the CD1d receptor for these antigenic glycolipids and the unique conserved, invariant Natural Killer T-cell receptor. The N38.3C3 does not express the Cd1d receptor. Consequently, it

does require more additional antigen presenting cells to display the glycolipid antigen. These two cell lines have been applied in the past to measure iNKT response expressed by interleukin-2 (IL-2, other cytokines produce similar results).[17-19] IL-2 is secreted when KRN7000 binds to the CD1d receptor; making KRN7000 a great positive control. Several other glycolipid formulations have also been demonstrated to induce an immune response from these cell types, including bacterial superantigens (SAgs),[11] a series of glycolipids from pathogenic bacteria,[13] including the antigens synthesized here,[5] and also a multivalent acetal-free,[13] dendron-supported carbohydrate previously presented by this team.[12c] Kinjo and co-workers examined the immunogenicity of their isolated glycolipids using a similar system to the one applied by our team: mouse Vα14iNKT cell hybridomas were applied. Kinjo isolated glycolipids showed good elicitation of IL-2 from Vα14iNKT+ cells, but no activation in the absence of Vα14iNKT+, then the monosaccharide synthesized and evaluated the immunogenicity of that system (while also confirming the structure and regiochemistry of the ester groups) and demonstrated the similar activity to the isolated monosaccharide, however, our result was different. We observed very poor activity in Vα14iNKT cells using our synthesized glycolipids at any concentration from 1 ng/mL to 1 mg/mL. α-GalCer (KRN7000) shows its typical potent activity, but the glycolipids show a very low affinity to interact with the CD1d receptor. The difference between the cell lines used by our research group and that of the Kronenberg group is that our cells, and mice, have had the murine MHC receptors eliminated, leaving on CD1d. The Kronenberg group's cells still have other receptors present. Glycolipids do not generally bind with other MHC receptors, but the possibility remains that this could explain this discrepancy. The authors comment on this themselves showing that some of the CD1d-dependent *in vivo* activation of the iNKT cells could be related to self-antigens presented by CD1d

and finish their description by stating that the isolated antigens likely "make a contribution to the production of cytokines by Vα14iNKT cells"[5]

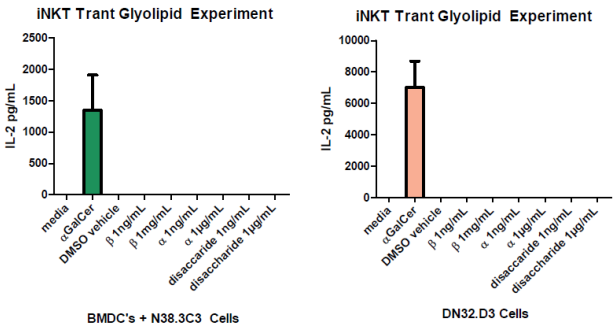


Figure 5. 1 Elicited IL-2 levels obtained from exposing cells to the synthesized glycosphingolipids

Although this preliminary result disagrees with the Kinjo result, it is worth mentioning that the Kinjo results regarding iNKT activation was obtained with *in vivo* biological examination, however our biological evaluation performed using two different cell lines *in vitro*. Therefore, it is not a completely fair comparison nor can any valid conclusions regarding low iNKT cell activity be drawn. The biological evaluation *in vivo* is currently under study by our collaborator at the University of Windsor and will be reported soon. With this *in vivo* study and our preliminary biological data, the extremely low activity of these glycolipids antigen *in vitro* obtained by our collaborators, it will result in two possibilities. First, if our *in vivo* study confirms the Kinjo result, it implies that *in vitro* results are not an appropriate tool for measuring the biological activity of iNKT activators without an accompanying *in vivo* study. Second, if our results disagree with the Kinjo result, and no activity is observed *in vivo*, it may mean that the biological results noted by Kinjo may have resulted from the presence of impurities in their isolated samples, and possibly in the synthetic batches as full synthetic characterization was not provided for their monosaccharide. There are currently more than 300 publications that have cited the Kinjo paper, and this figure is

expected to grow in the future. It would be problematic if these studies have been based on an inaccurate result or a poorly characterized compound. This study highlights the need for resynthesize and the importance of careful isolation and characterization of natural products from complex biological matrices.

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Chapter 6. *t*-Butylamine as a mild and selective deacetylation agent

6.1 Synthesis of starting materials for the deprotection procedure using *t*-Butylamine

Based on our previous challenge, the deacetylation of a disaccharide glycolipid in the presence of longer ester chains in the glyceride moiety, we were inspired to study the use of *t*-$BuNH_2$ as a deacetylation reagent. If successful, this compound could be an excellent alternative to traditional deprotection conditions because it is a milder nucleophile and more inexpensive than the reagents typically employed. However, an essential requirement is the deprotection of acetyl protecting group must be selective for acetyl groups in the presence of other esters.

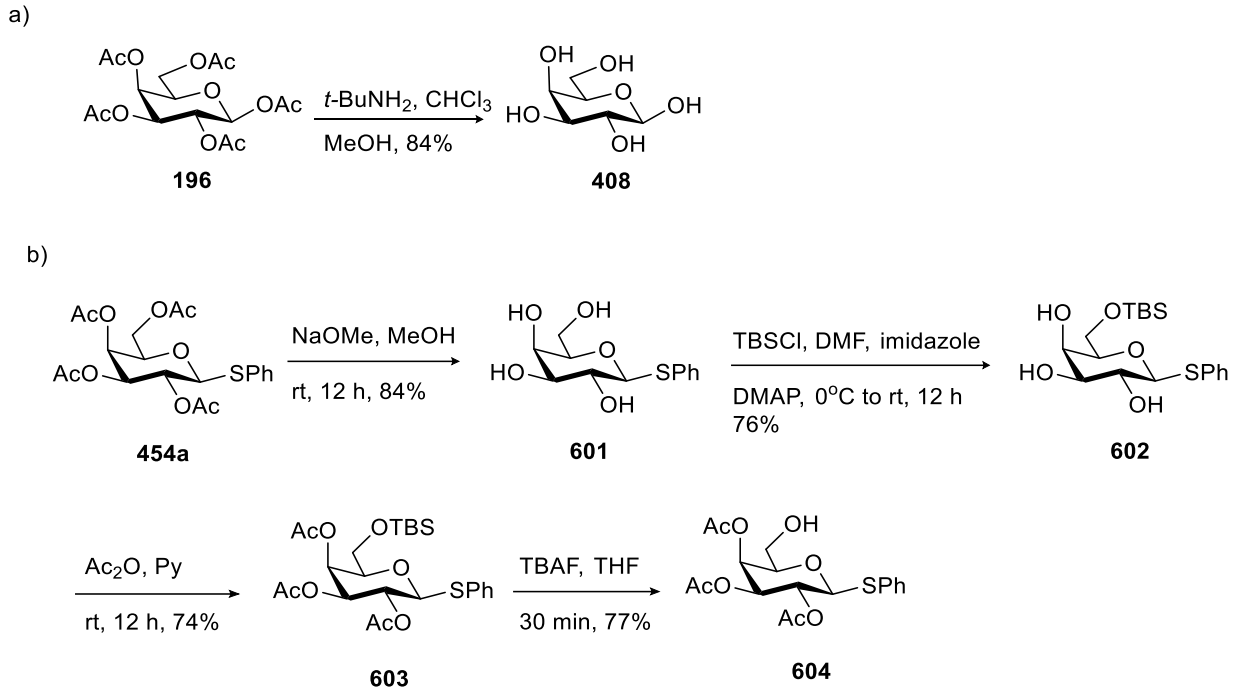
Although primary esters are typically more prone to hydrolysis than secondary or tertiary ones, mainly due to steric effects, in carbohydrates this is not always the case. The primary alcohol in pyranose carbohydrates has lower acidity[1] and it seems that the steric effects do not play as important a role, especially when using small bases and/or nucleophiles for the ester hydrolyzation. However, due to the lack of adequate research in the carbohydrate field, more detailed studies evaluating the reactivity of acetate protecting groups at different positions of the pyranosyl form are required: is it possible to selectively remove some acetates/esters in the presence of others?

The regioselectivity of the deacetylation of nucleosides are well studied;[2, 3] however, it is difficult to extend these results to pyranoses due to a lack of sufficient data. Selective deacetylations have been accomplished when a small base/nucleophile is used for the deprotection; Furthermore, the reactivity order of nucleophilic sites remains to be explored. The steric effects of the deprotecting reagent are important as, in some cases, these effects work against the electronic preferences in carbohydrates. The higher acidity of secondary acetyl protecting groups makes it more susceptible to deprotection than the primary acetate group, however, steric effects in the

more sterically hindered reagent suggests primary alcohol should deprotect faster than secondary acetates.

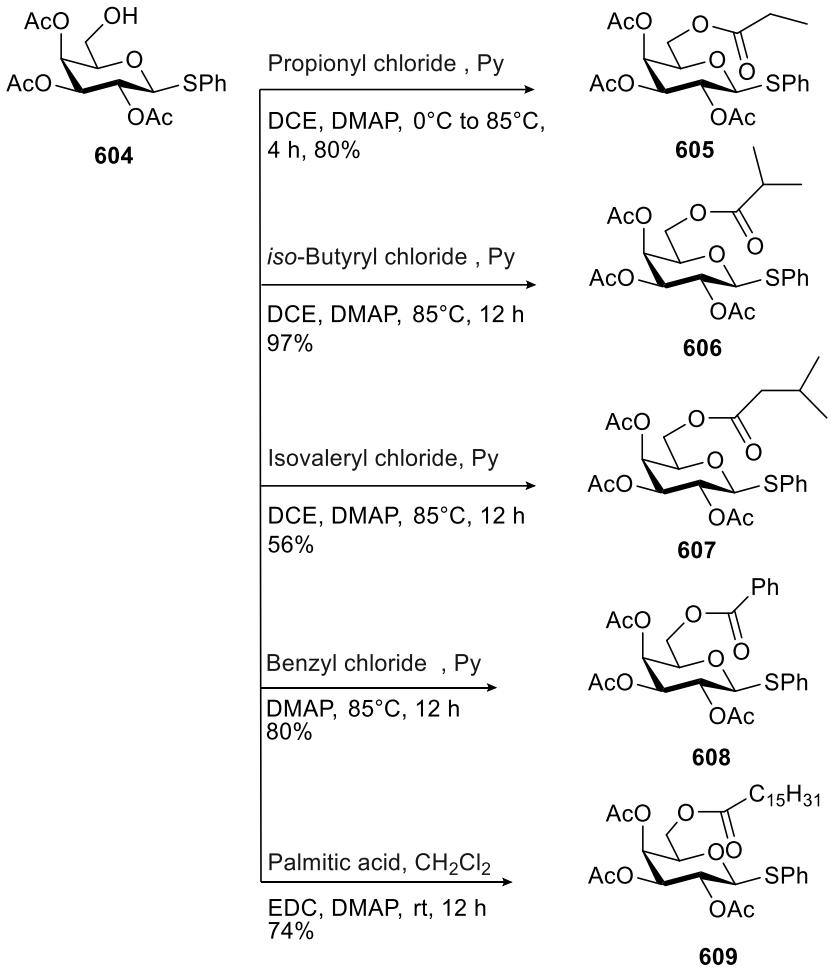
The focus of this study will be examining the potential selectivity of the *t*-$BuNH_2$ deprotecting agent and its limitations. In particular, t*ert*-butyl amine is studied as a sterically hindered reagent for the selective deprotection of acetyl groups in the presence of different ester chains synthesized from propionyl chloride, *iso*-butyryl chloride, *iso*-pentanoyl chloride, benzoyl chloride, pivaloyl chloride and palmitoyl chloride. Before examining the selectivity, we needed to confirm that *t*-$BuNH_2$ can deprotect acetyl groups. This was successful as galactose was acetylated, then deprotected *via t*-$BuNH_2$ in the presence of MeOH and $CHCl_3$ in an 84% yield **(Scheme 6.1a)**.

Next, the acetyl group at the anomeric position was replaced with the SPh group, and the rest of the acetyl groups were removed using NaOMe and MeOH.[4] The alcohol at C-6 was selectively protected with a TBS group using TBSCl, imidazole, and DMAP in DMF, then the rest of hydroxyl groups protected with acetic anhydride and pyridine in 74% yield. The TBS protecting group was removed **(Scheme 6.1b)** using TBAF in THF to generate a free C-6 hydroxyl group ready to be esterified with different ester chains.



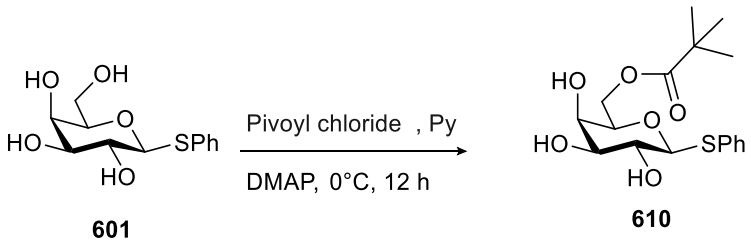
Scheme 6. 1 Deprotection of acetyl group using *t*-$BuNH_2$, a, and synthesis of compound **604**, b

Esterification of the hydroxyl group at the C-6 position was then performed using different acyl chlorides, the esterification of propionyl chloride, *iso*-butyl chloride and *iso*-pentyl chloride were done using DMAP and pyridine in 80%, 97% and 56% yield, respectively. Those reactions, shown in **Scheme 6.2** were done in DCE at 85°C as performing the reaction in dichloromethane at ambient temperature did not afford full conversion. The benzoyl chloride reacted with primary alcohol in the presence of DMAP and pyridine to form the desired product in 80% yield. The alcohol **604** was subjected to esterification using palmitic acid with EDC, DMAP in CH_2Cl_2, to afford compound **609** in 74% yield.



Scheme 6. 2 Esterification of alcohol **604** with different acyl chlorides

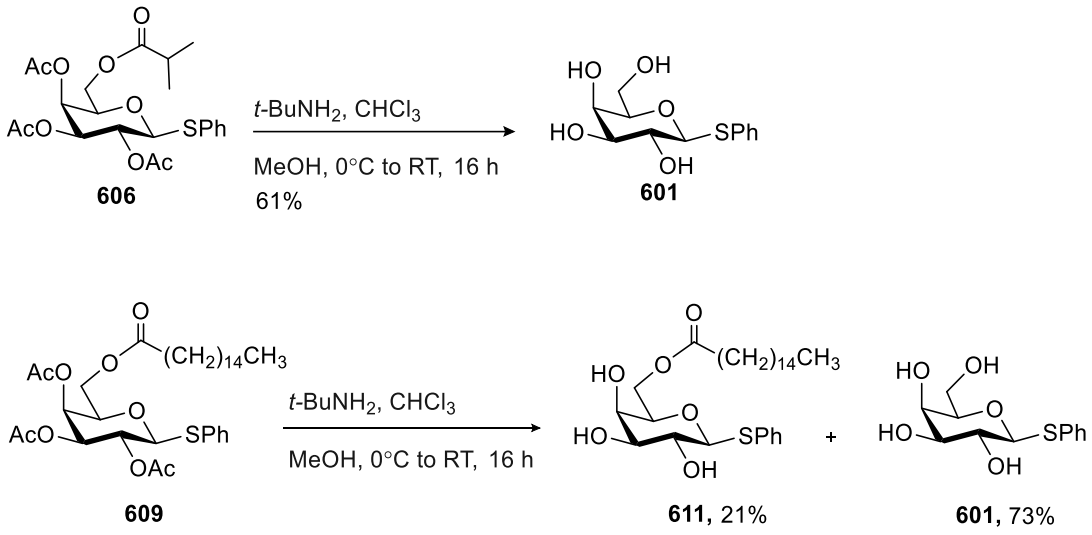
The PivCl was introduced selectively at the C-6 alcohol of fully deprotected compound **601** directly through the acylation. The synthesis provided the product as per the crude NMR, but due to the COVID-19 situation, we were not able to purify this compound **(Scheme 6.3)**.



Scheme 6. 3 Synthesis of compounds **610**

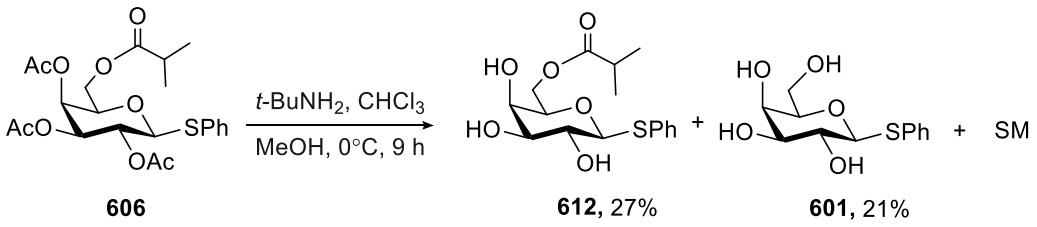
6.2 Selective acetyl deprotection of compound 605-609 using *t*-BuNH$_2$

First, deprotection was carried out in parallel on compound **606** and **609** and was allowed to proceed until the full consumption of starting material was observed by TLC **(Scheme 6.4)**. The *t*-butylamine was used in a 1:1 ratio with the starting materials. The solvent and reagent were added at 0°C, and the reaction was stirred for 16 hours and allowed to warm to ambient temperature (20-23°C). The *iso*-butyl substituted compound **606** was treated with *t*-BuNH$_2$, MeOH and $CHCl_3$ starting at 0°C then left it to reach ambient temperature. The result showed the starting material was fully deprotected. However, when the same reaction conditions were applied to the palmitoyl substituted compound **609** the result indicated the desired selective deprotected product was produced but contaminated with the fully deprotected ester. This result demonstrated that the steric demand of ester chains at the C-6 position may play an important role in the selective acetyl deprotection in the presence of *t*-BuNH$_2$ providing a form of substrate control. The outcome of this reaction could surely be improved by controlling the rate of deprotection *via* temperature adjustment.



Scheme 6.4 Deprotecting reactions of compounds **606** and **609** at 0°C to room temperature

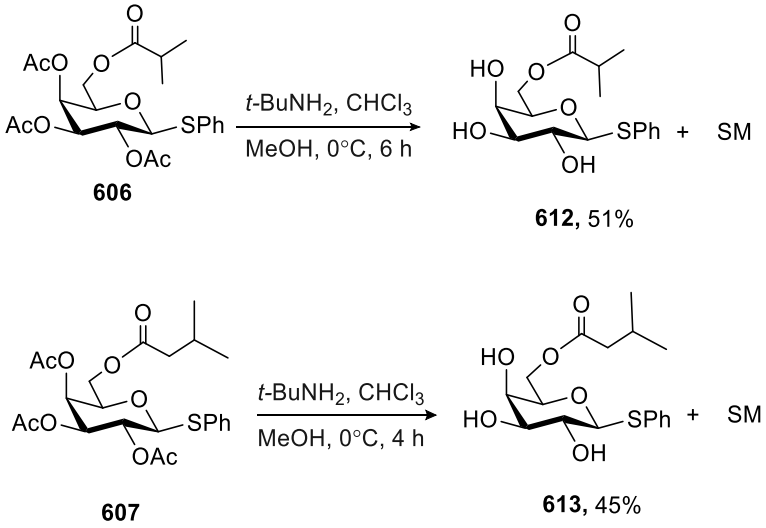
In the next step, we tried to control the progress of this reaction by optimizing the reaction conditions. A, 1:1.5 ratio of *iso*-butyl substituted compound **606** to *t*-butylamine was examined starting at 0°C, however in order to slow down the rate, the reaction mixture was kept at 0°C throughout the reaction **(Scheme 6.5)**. In 30 to 45-minute intervals, TLCs of the reaction showed the formation of the desired product; however, after 1.5 hours, the fully deprotected acetyl group compound began to form. After 9 hours, the reaction was stopped, and the compounds purified. The result showed a 27% yield of the desired product accompanied by fully deprotected compound **601** in 21% yield along with starting material.



Scheme 6.5. Deprotecting reactions of compound **606** at 0°C

For our next attempt, we decided to keep the temperature at 0°C and stop it when the un-desired fully deprotected compound begins to form, these reactions are shown in **Scheme 6.6**. This time the same reaction set up at 0°C with 3:1 reagent to starting material ratio was monitored closely *via* TLC techniques. After 6 hours, the intensity of the product grew stronger, judged by TLC, and then the second spot began to produce a shadow. At this point, the reaction was stopped and purified. The result showed a 51% yield of the desired product with the mass balance being recovered starting material. In a second reaction, starting material **607** was added to 3 equivalents of *t*-butylamine at 0°C. The TLC observations after 4 hours indicated the desired product spot had become stronger and minimal amount of fully deprotected compound start to form. The reaction

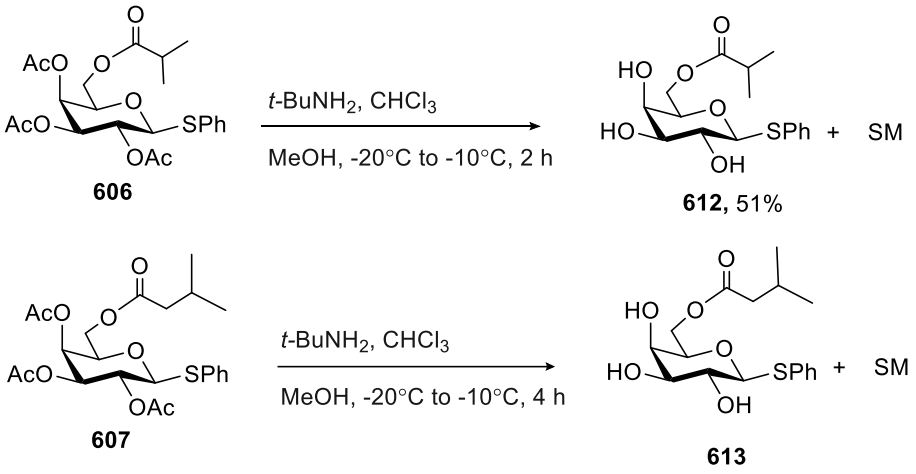
was stopped and purified to generate compound **613** in a 45% yield, again with the recovery of starting material and trace fully deprotected material.



Scheme 6. 6 Deprotecting reactions of compounds **606** and **607** at 0°C

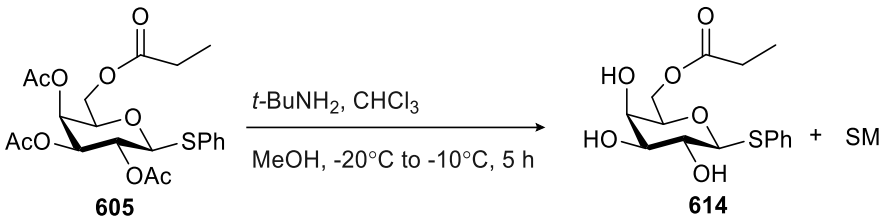
In the next stage, we decided to determine the minimum temperature that the reaction can progress at and stop it when the undesired fully deprotected compound begins to form. A series of experiments were set up in parallel with a lower starting temperature, –20°C. This temperature was raised or maintained depending on the progress of the reaction for specific substrates. Compound **606** was treated with *tert*-butyl amine in a 1:3 ratio, at –20°C and monitored closely *via* TLC. After 3 hours, when no conversion was observed, the temperature was increased to –10°C. The desired product began to form after 1 hour. The product spot became stronger after one more hour, and a very faint, fully deprotected compound appeared. The reaction was stopped, and the mixture was stored at –78°C in a freezer. Due to the sudden lab shutdown, purification of this compound was not completed. However, it is predicted that this yield would be the highest of the reactions attempted based on the TLC and crude HNMR.

In a similar protocol, the isopentyl group **607** was subjected to the deprotection at –20°C. Similarly, no reaction was observed after 3.5 hours. The temperature increased to –10°C, and after 1 hour, the product started to appear. The reaction continued for one more hour and stopped when the byproduct began to form. The mixture was stored at –78°C freezer. Due to the sudden lab shutdown, purification of this compound was not completed. However, the crude ^{1}H NMR indicates the product **613** is present in the reaction mixture.



Scheme 6. 7 Deprotecting reactions of compounds **606** and **607** at –20°C to –10°C

The last deprotection was done on propionyl substituted compound **605**, as a challenging substituted because of size similarity with the acetyl group. The starting material **605** was treated with *tert*-butyl amine in a 1:3 ratio at –20°C, the temperature was increased after 3.5 hours when no reaction was observed. Like the other esters, the product spot reached maximum intensity after one hour. The reaction stopped, and like the rest of the reactions that have been done in parallel, the mixture was stored at –78°C freezer. Due to the sudden lab shutdown, purification of this compound was also not completed.



Scheme 6. 8 Deprotecting reactions of compound **605** at –20°C to –10°C

This research forced to be stopped due to the COVID-19 pandemic situation. Once allowed back in the lab, in future work we will complete the purification of the esters investigated during the first phase. Then we will investigate selective deprotection of acetyl group in the presence of longer chains in different position of galactose as well as glucose and glucosamine.

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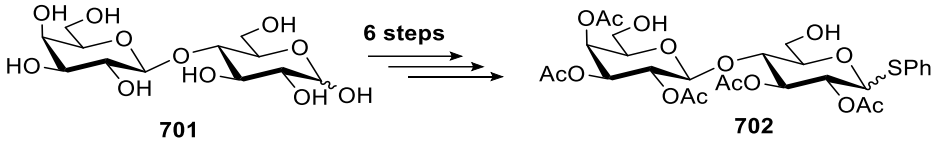
Chapter 7: Synthesis of oligosaccharides conjugation-breaking units in semiconducting polymers

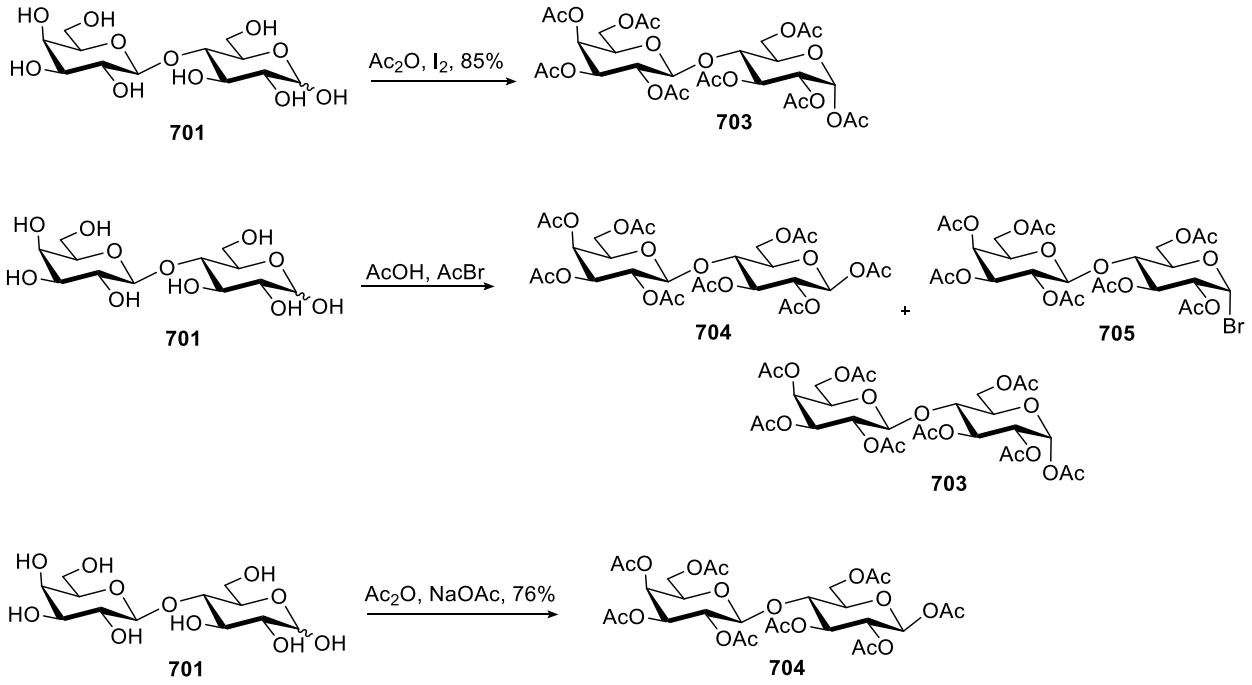
Organic electronics are often processed with halogenated solvents.[1, 2] In order to replace these harmful halogenated solvents with greener ones, we need polymers that are soluble in greener, generally protic solvents. Carbohydrates seem to be a good candidate to be added to the polymer to increase the solubility due to their high hydrogen-bonding ability. In this section we discuss the synthesis of a carbohydrate insulating spacer that can be integrated into conductive polymers. An additional advantage of using carbohydrates in the backbone is the introduction of a biodegradable component into the polymer framework. This project will be done in two phases; the first one, which is discussed in this section, is the synthesis of protected disaccharide bearing the free hydroxyl group at the C-6 positions, the second phase involves further manipulation and polymerization. The second phase of this research is currently underway and is being performed in the research group of our collaborator, Professor Rondeau-Gagné at the University of Windsor.



Scheme 7. 1 Synthesis of disaccharide **702** from lactose

We needed a divalent scaffold to allow for polymerization from both "ends" of the molecule, while also ensuring the presence of an enzymatically/chemically labile linker. A disaccharide, like lactose, satisfies these conditions. The interglycosidic bond is highly sensitive to omnipresent lactosidases while also being sensitive to less specific endoglycosidases and mild aqueous acid. At the same time, it clearly provides two similar sites for polymerization, the sterically unencumbered C-6 hydroxyls. This target **702** also has a clear and simple strategy available for its synthesis.

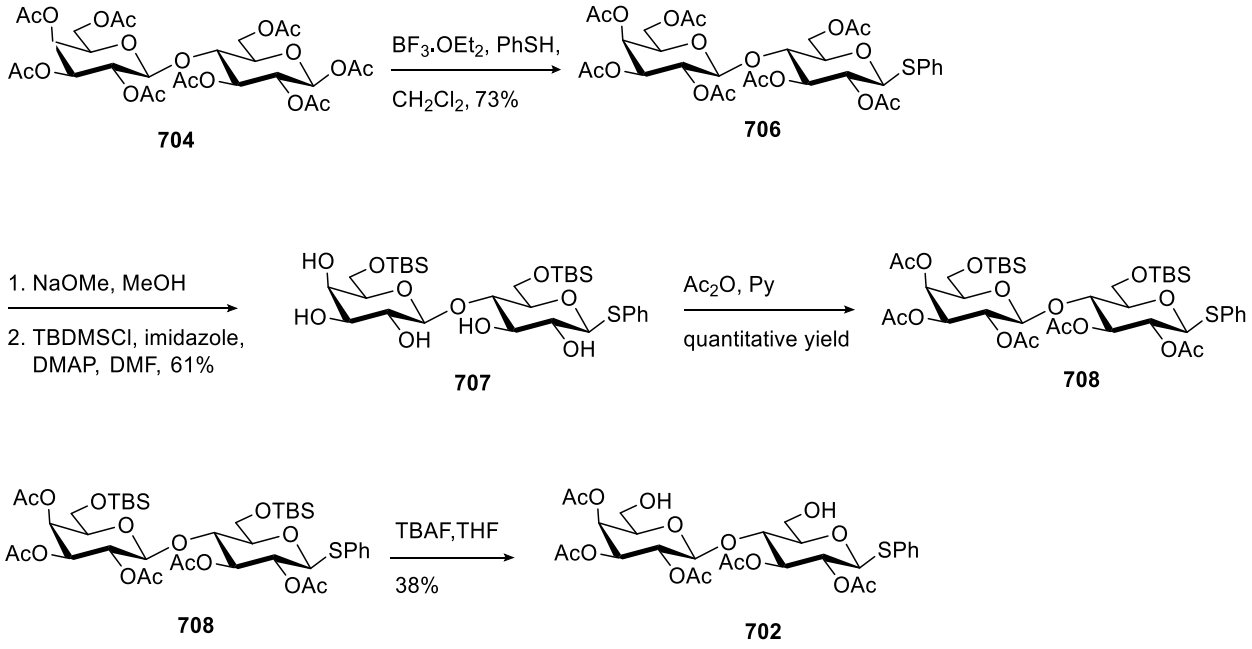
Surprisingly, it is a new compound **(Scheme 7.1)**. The project was initiated by acetylation of lactose; however, the reported acetylation of lactose using I_2 and acetic anhydride[3] failed to produce β oriented acetyl at the anomeric position and results showed the production of the α acetylated product exclusively **(Scheme 7.2)**. As the readers will know, this is not a new problem encountered in this thesis: many supposedly selective glycosylations have proven to be irreproducible. Acetobromination employing AcBr and AcOH also did not give the desired outcome and afforded a mixture of undesired products with only a small amount of the desired product.[4, 5] Finally, the β anomeric conformer of peracetylated lactose **704** was obtained using our normal β-selective acetylation system: sodium acetate and acetic anhydride at elevated temperatures with a yield of 76% after recrystallization.



Scheme 7. 2 Lactose acetylation procedures

Next, the acetate at the anomeric position was replaced with the SPh group using thiophenol and BF_3•OEt_2. This reaction afforded the desired product in a 73% yield. The acetyl protecting

groups were then deprotected *via* the Zemplén deacetylation procedure and the C-6 primary hydroxyl groups selectively protected by the TBS group using imidazole, DMAP and TBDSCl. The result showed 61% of the desired product, which upon acetyl protection based on standard acetylation procedure using pyridine and acetic anhydride converted to the compound **708** with a quantitative yield **(Scheme 7.3)**. The final step, TBS deprotection, was successfully done *via* one molar TBAF solution in THF at 0°C for 4 h. Although this reaction worked smoothly, the result was not great, due to acetyl migration, and a 38% yield was obtained **(Scheme 7.3)**. This compound was sent out to our collaborator, Rondeau-Gagné group, at the University of Windsor for further transformation toward polymerization.



Scheme 7. 3 Synthesis of compound **702**

If the disaccharide polymer shows a promising result, then we can work on yield optimization for future work. The final step, especially, might benefit from buffering with acetic acid to avoid undesirable side reactions involving general base/nucleophile catalysis that can occur in the presence of F^-.

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Chapter 8. Conclusions, synthesis, and future work

8.1 The synthesis of the Tn antigen

The primary objective for Chapter 3 was completed, which was the synthesis of an acetal-free, *N*-Fmoc protected Tn antigen. The acetal-free Tn antigen bearing *N*-Fmoc protecting group was targeted in this research to facilitate solid-phase synthesis for peptide attachment to the antigen. This antigen was synthesized in order to investigate whether it can activate T-cells and to investigate the immune response produced by this vaccine. Specifically, there are questions regarding whether the immune response is specific for the whole antigen unit or the response comes from only peptide chain.

For future work, the acetal-free Tn antigen **301** should be integrated into longer peptides using solid-phase peptide synthesis to link the peptide chain to the Tn antigen unit for the T-cell activation study **(Figure 8.1)**. After preparation of antigen **801**, this compound will be sent to our collaborator at Laval University for biological examinations, these tests will be performed against the natural antigen, which is currently under preparation in our group. Our ultimate goal is to use T-cell activation approach developed by Boons and Danishefsky[1-3] by conjugating highly immunogenic T-cell dependent peptides to short B-cell peptide sequences incorporating the acetal-free Tn antigen, or using a rod-shaped viral nanoparticle comprised of the coat protein (CP) of papaya mosaic virus (PapMV), which is self-assembled around a single-stranded RNA, developed by our collaborator Dr. Denis Leclerc at Laval University.[4-6] The single-stranded RNA determines the length of the nanorod (PMVN).

Papaya mosaic virus nanoparticles (PMVN), act as a toll-like receptor 7/8 agonist. These have been applied to improve the immunogenicity of a peptide antigen fused to the nanoparticle framework as a vaccine platform technology.[7-10] PMVN nanoparticles are used to trigger innate immunity and increase the immune response against cancer.

The way that the PMVN nanoparticle vaccine platform works is, after self-assembly around a ssRNA, Sortase, which is a bacterial enzyme responsible for attachment of specific proteins to the cell wall of Gram-positive bacteria, ligates our glycopeptide (acetal-free Tn antigen attached to a peptide chain with the sortase-specific GGG amino acid sequence at the C terminus) to the surface. Consequently, an immunogenic cylinder can be readily prepared to act as a promising vaccine candidate. This cylinder could be converted to the multivalent antigen cylinder for further immunogenicity enhancement.

This biological evaluation includes: the measurement of quantity and quality of the antibodies production and specificity of these antibodies toward the cancer cells. In addition, a second set of experiments will be done to determine if the T-cells are activated by antigen **801** or only by the peptide chain alone thus determining the specificity of the immune response activated by T-cells towards antigen **801**.

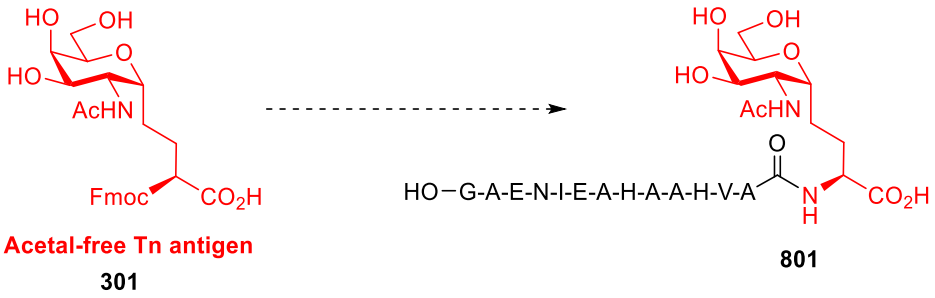


Figure 8. 1 Future work, peptide attachment to the antigen **301**

Based on previous results expressing multivalency increases immunogenicity,[11-13] which was also discussed in both sections 1.1.4.2,[14] and 1.1.6.1,[15] this effect could be applied in the next step of this research to solve low immunogenicity of TACAS vaccine candidates. If the biological evaluation of **801** shows promising results then, as an ultimate goal, the acetal-free Tn antigen could be converted to a dendron, **(Figure 8.2)** in order to increase the immunogenicity of the antigen taking advantage of the multivalent effect.

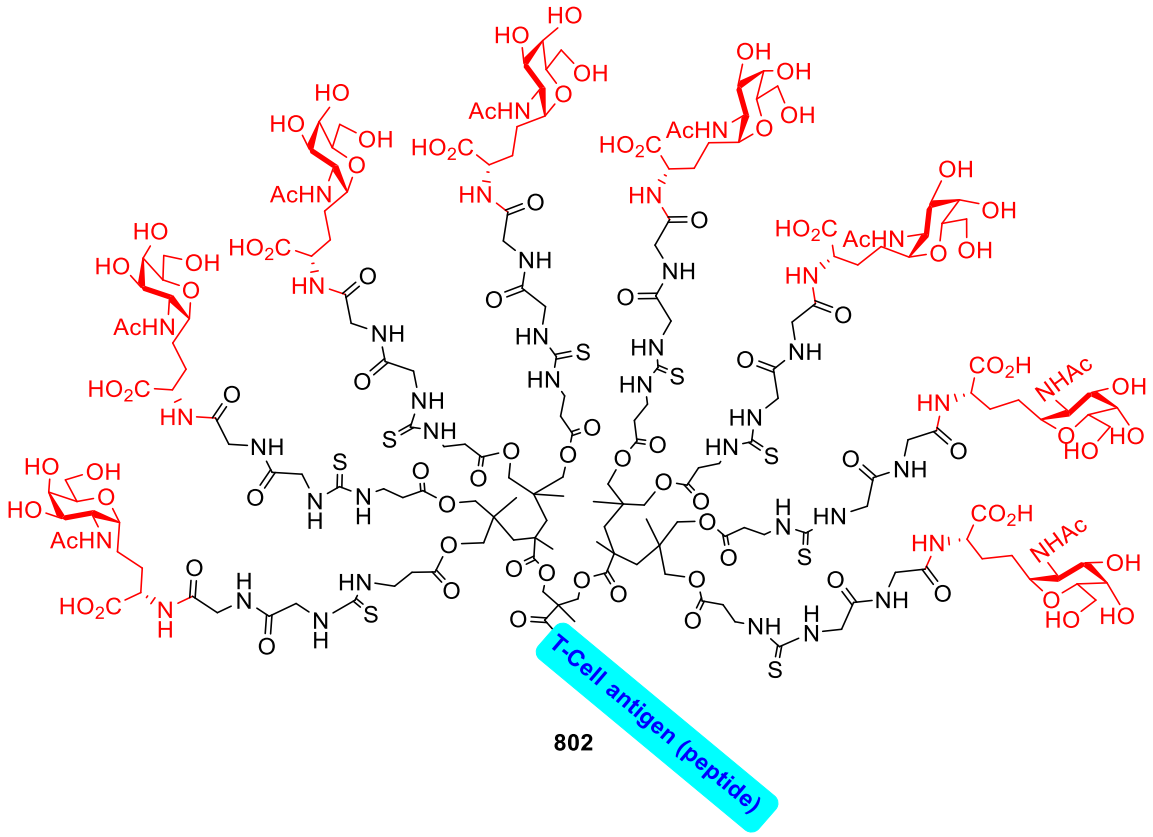
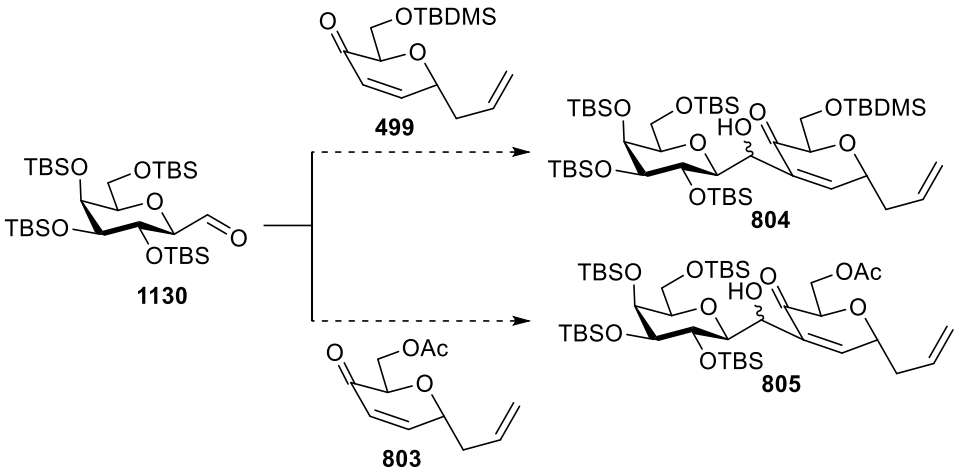


Figure 8. 2 Proposed dendron **802**

8.2 The synthesis of the TF antigen

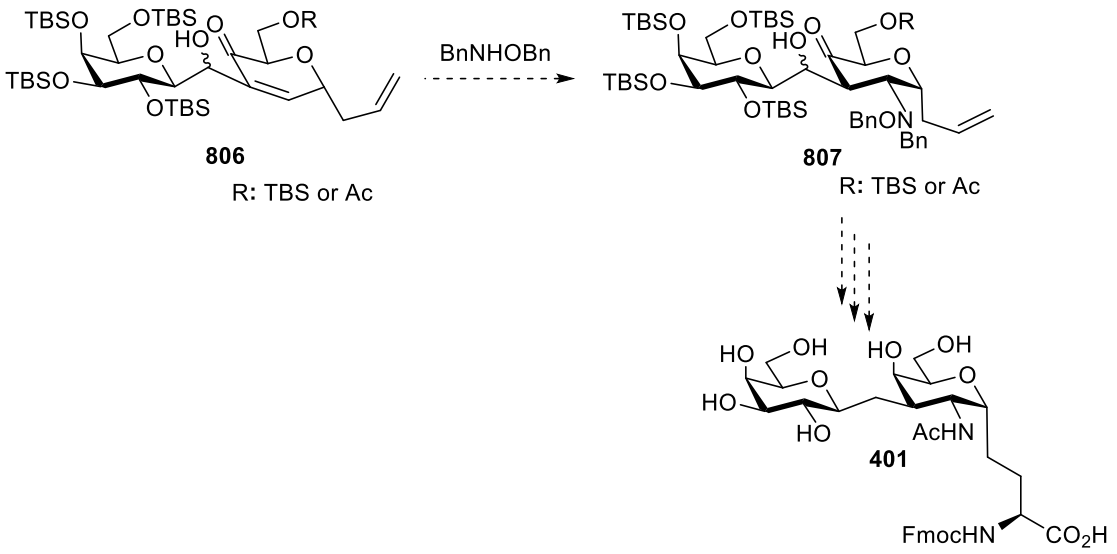
In the synthesis of the acetal-free TF antigen discussed in Chapter 4, the main objective was the synthesis of a fully C-glycoside version of the TF antigen in a reasonable number of synthetic steps such that we can integrate it into vaccine candidates. This overarching objective was not met, although progress was made towards it. However, we have been able to solve several key synthetic challenges including the first synthesis of a sugar bearing an aldehyde at the C-3 position and a nitrogen source at the C-2 position. In addition, the synthesis resulted in the first ever example of a double C-glycoside disaccharide. With these two lead compounds in hand, the synthesis will be completed in future work by a different lab member. We have learnt a lot in this endeavour, it was the majority of the thesis work, and several steps will need to be addressed to move forward: first, optimization of the double C-glycoside disaccharide formation must be performed. This will

likely involve modifying the protecting groups and functionalities on the two partners used in the Baylis-Hillman Reaction in addition to standard reaction optimization screens **(Scheme 8.1)**. For example, the TBS protecting group could be used instead of a Bn protecting group in the galactose moiety. Vogel reported a better yield using the TBS group rather than using a Bn protecting group, which may be valid for our research as well.[16] Second, a different, smaller, protecting group in the enone moiety such as an electron withdrawing C-6 acetate could be tried to achieve a better result.

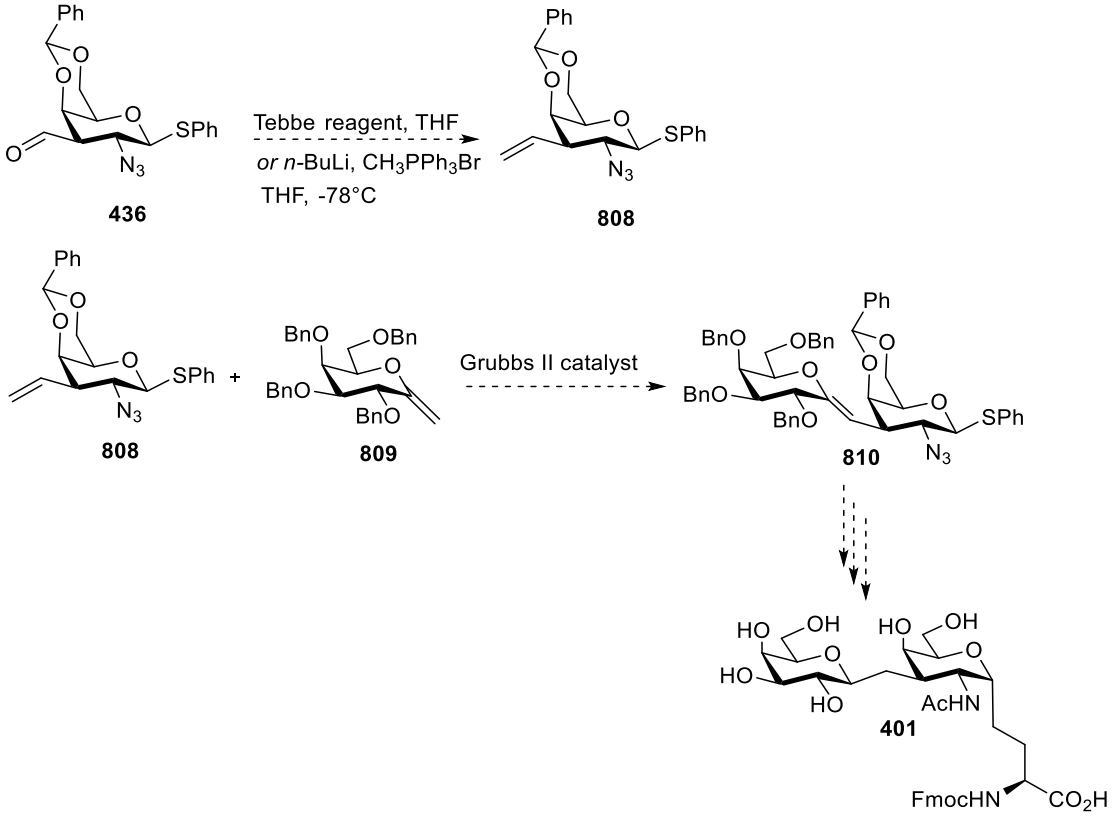


Scheme 8. 1 Proposed Baylis-Hillman Reaction between aldehyde **1130** and enones **499** and **803**

Once the disaccharide formation has been optimized, a more reactive amine, such as BnNHOBn, could be used for the conjugate addition to enone **806** as shown in **Scheme 8.2**. As an alternative plan, aldehyde **436** could be converted to the olefin using the Tebbe reagent or CH_3PPh_3Br through a Wittig reaction, followed by olefin metathesis with olefin **809** mediated by a Grubbs 2nd generation catalyst. An asymmetric olefin reduction should provide access to disaccharide **810**, which after a few manipulations, should be easily converted to the target **401** as shown in **Scheme 8.3**. This is a challenging target, but it should be attainable.



Scheme 8. 2 Proposed synthesis of compound **401** through a conjugate addition reaction



Scheme 8. 3 Proposed synthesis of compound **401** *via* a Grubbs 2nd generation catalyst

8.3 Synthesis of an immunogenic glycolipids target 1168 and 202

The main objectives of Chapter 5, the synthesis of a glycolipidic antigen, were fully met. However, the biological evaluation has yet to be completed. These evaluations are required in order to form conclusions regarding the activity of these antigens towards iNKT cell activation. Our result was totally different from the Kinjo report, but as mentioned before, a similar system, mouse Vα14iNKT cell hybridomas were applied. Glycolipids do not generally interact with other MHC receptors; however, this could be the reason for discrepancy between the Kinjo result and ours. Moreover, as they mentioned,[17] some of the CD1d-dependent *in vivo* activation of the iNKT cells could be obtained from self-antigens presented by CD1d. Consequently, further biological examination remained for the future using the exact same conditions *in vivo* as the Kronenberg group applied to have valid conclusions. Either; these antigens are capable of activating the iNKT cell, which means the biological result produced *in vitro* cannot be extended to the *in vivo* result meaning further biological evaluation *in vivo* to validate *in vitro* results are always necessary, or there is no significant activation meaning this type of so-called antigen are not actual antigens and the study and synthesis towards these molecules based on the previous reports are not useful and should be stopped.

8.4 *tert*-butylamine as a mild deacetylation agent

The application of *t*-$BuNH_2$ as a selective deacetylation agent mostly remains to be explored in the future because of the pandemic situation. We had identified this reaction in early 2019 and were pursuing it as a side project in the current academic year; under normal circumstances we would be further ahead, but the pandemic intervened. Before the shutdown, the selective deacetylation of the C-2, C-3, and C-4 in the presence of other esters chain at the C-6 position was accomplished by controlling the temperature and closely monitoring the progress of the reactions. Still a few reactions remained to be performed to complete this part of the research. As an ultimate goal, in the future, the location of the esters chain and acetyl group should be changed to

further investigate the utility of using *t*-BuNH$_2$ in the selective deacetylation of acyl groups in different locations, in the presence of varying ester chains. This research also could be extended by using different sugar moieties, such as glucose, glucosamine and galactosamine. Some proposed target molecules are shown below in **Figure 8.3**. We want to ensure that this is a general reaction and it is robust. As noted throughout the thesis, when we applied published protocols to our systems, they often failed to provide the reported product which does lead to doubts as to the validity of the original research. We want to ensure that, when we publish these results and share them with the community, that we can highlight the advantages and limitations. Acetates are likely the most flexible protecting group in carbohydrate chemistry. It would be a very great benefit if they could be used freely in the presence of other esters as the designer would have confidence that they could be selectively removed in reasonable yield from most any system. We are confident that the data suggests this is likely, but we want to be very confident. One of the methods we will be employing is to repeat the reaction with different chemists conducting the study. We want to ensure that these reactions are robust and usable.

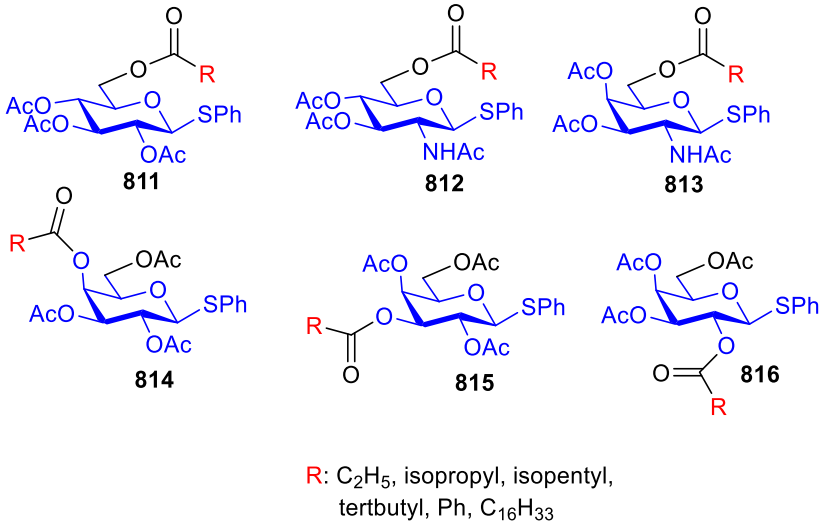


Figure 8. 3 The proposed structure of future targets

8.5 Lactose as an insulator in conductive polymers

The objective of the final chapter of this thesis, Chapter 7, was met in full: the synthesis of acetylated lactose bearing free hydroxyl group at the C-6 and C-6´positions. This compound was synthesized successfully and will be sent in the future to our collaborator for polymerization through an esterification reaction. This will result in a polymer which hopefully is more soluble in greener, protic solvents and, which will introduce enzyme-catalyzed degradability to the polymer backbone through the ester linkages. A depiction of a targeted block co-polymer is shown in **Figure 8.4.**

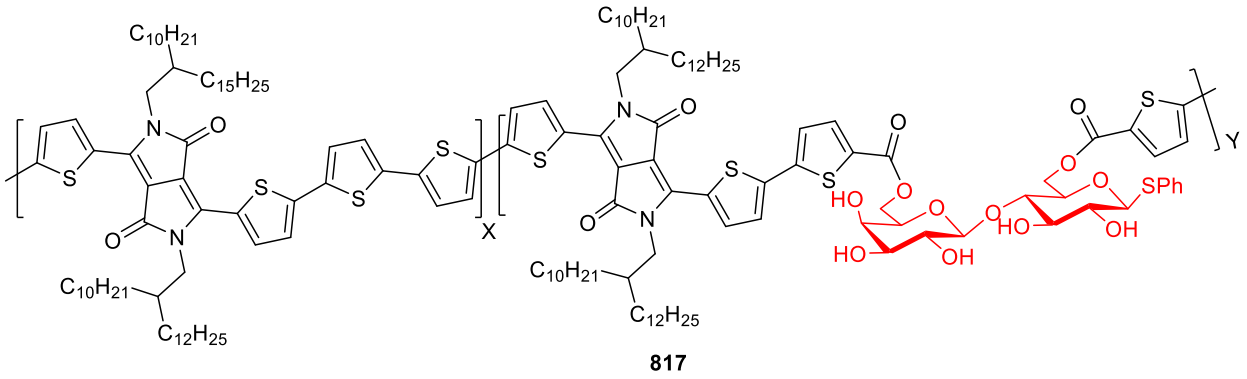


Figure 8. 4 Proposed conjugated polymers for greener solubility and degradability

8.6 Synthesis of the thesis

This thesis, and my PhD work more broadly, focused on the interface of carbohydrate and organic chemistry. In many ways, these have historically been treated as two separate sub-disciplines, with the carbohydrate community using its own conventions and approaches to solve problems that have solutions in the synthetic organic chemistry space. My interest was in applying the tools of synthetic organic chemistry to make unusual carbohydrates, and to integrate them into biomedically or material-science relevant molecules.

The synthesis of these molecules is challenging. This thesis shows our effort to solve these challenges using organic chemistry approaches and trying to explore fast, efficient and cost-effec-

tive ways to overcome these obstacles. Applying the tools of synthetic organic chemistry in carbohydrate research could be used for the deprotection of acetyl group in the presence of other ester functionality, which may practically save a significant number of steps in carbohydrate synthesis or can be used for providing carbohydrate-based vaccines that may save lives. Both could be aligned in the same direction for the bigger picture. This thesis and my PhD work focused on using this approach especially for applications in immunology. Carbohydrates play important roles in the pathogenicity of many diseases and could also help provide the solution. However, the immune system has a complicated relationship with recognizing carbohydrates, and this is further complicated by the inherent physiological instability of glycosides; consequently, my main goal was to induce specific immune responses by synthesizing specific carbohydrates, including acetal-free Tn and TF antigens and also glycolipids. This has required new chemistry.

Synthesizing these carbohydrates is challenging, and scientists spend a lot of time and resources making these biomedically relevant molecules, however, despite much effort, including my own, much more research is required to improve our understanding of the role of carbohydrates in immunology; our tools could prove very helpful.

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Experimental protocols and characterization data

9.1 General methods and materials for chemical reactions

Solvents were purchased from Caledon Labs (Caledon, Ontario), Sigma-Aldrich (Oakville, Ontario) or VWR Canada (Mississauga, Ontario). Other chemicals were purchased from Sigma-Aldrich, AK Scientific, Oakwood Chemicals, Alfa Aesar or Acros Chemicals and were used without further purification unless otherwise noted. Anhydrous toluene, tetrahydrofuran (THF), diethyl ether and *N*,*N*-dimethylformamide (DMF) were obtained from an Innovative Technology (Newburyport, USA) solvent purification system based on aluminium oxide columns. CH_2Cl_2, pyridine, acetonitrile, *N*,*N*-diisopropylethylamine (DIPEA) and NEt_3 were freshly distilled from CaH_2 prior to use. Purified water was obtained from a Millipore deionization system. All heated reactions were conducted using oil baths on IKA RET Basic stir plates equipped with a P1000 temperature probe. Thin layer chromatography was performed using EMD aluminum-backed silica 60 F254-coated plates and were visualized using either UV-light (254 nm), $KMnO_4$, vanillin, Hanessian's stain, Dragendorff or phosphomolybdic Acid (PMA)'s stain. Preparative TLC was done using glass-backed silica plates (Silicycle) of either 250, 500, 1000 or 2000 μm thickness depending on application. Column chromatography was carried out using standard flash technique with silica (Siliaflash-P60, 230-400 mesh Silicycle) under compressed air pressure. Standard work-up procedure for all reactions undergoing an aqueous wash involved back extraction of every aqueous phase, a drying of the combined organic phases with anhydrous magnesium sulphate, filtration either using vacuum and a sintered-glass frit or through a glass-wool plug using gravity, and concentration under reduced pressure on a rotary evaporator (Buchi or Synthware). ^{1}H NMR spectra were obtained at 300 MHz or 500 MHz, and ^{13}C NMR spectra were obtained at 75 or 125 MHz on Bruker instruments. NMR chemical shifts (δ) are reported in ppm and are calibrated against residual solvent signals of $CHCl_3$ (δ 7.26), DMSO-d5 (δ 2.50), acetone-d5 (δ 2.05), or methanol-

d3 (δ 3.31). HRMS were conducted on a Waters XEVO G2-XS TOF instrument with an ASAP probe in CI mode.

9.2 Procedure for the preparation of SmI_2 from "Inactive" Samarium metal (Chapter 4)

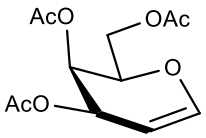
A 100 mL three neck Schlenk flask equipped with a Teflon-coated magnetic stir bar and a septum was flame-dried under vacuum. The flask to cool to room temperature and evacuated/back-filled with argon three times, then (3.30 g, 22.0 mmol) of "inactive" samarium metal was added, the flask was sealed with a septum and subjected to three evacuation/backfilling cycles, then Sm metal was stirred in the high speed under nitrogen atmosphere for 24 h. The samarium metal was dissolved in THF (90 mL) and then a solution of iodine (2.80 g, 11 mmol) in THF (20 mL) was added. The reaction flask was sealed with Parafilm and heated at 60°C for 18 h, then stirring was turned off and the solution of SmI_2 settled for 2 h before cannulating to the Serum Bottle.[1]

9.3 General deprotection protocol using *t*-$BuNH_2$ (Chapter 6)

The deprotection of the acetyl protecting groups in the presence of the ester groups followed a general protocol adapted by literature.[2] The adaptation was based on using *t*-$BuNH_2$ as the deprotecting agent for *O*-methoxy acetates in the presence of esters. The galactose molecule with thiophenol on the anomeric carbon, the ester groups on C-6, and the acetyl protecting groups on C-2, C-3, C-4 was subjected to deprotection with $CHCl_3$ and MeOH as the solvents in every reaction. The temperature and ratio of *t*-$BuNH_2$ were both varied. All reactions were done in a sealed tube. After the reaction is done, there is no work-up, and the reaction mixtures concentrated under reduced pressure.

9.4 Experimental protocols for Chapter 1

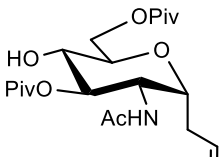
(2R,3R)-2-(acetoxymethyl)-3,4-dihydro-2H-pyran-3,4-diyl diacetate 119



119

The glycopyranosyl bromide **127** (354 mg, 0.86 mmol) was dissolved in CH_3CN (10 mL), and then zinc dust (422 mg, 6.46 mmol) and ammonium chloride (354 mg, 6.46 mmol) were added, and stirred for 4 h at 60°C. Inorganic salts and excessive zinc dust were removed by filtration. The filtrate was concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **119**, the title compound, as a colourless liquid (141 mg, 60% yield). On replication, yields varied between 53% and 60% for scales between 100 mg and 50.0 g. Rf = 0.25. 9:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 6.47 (dd, *J* = 6.28, 1.70 Hz, 1H), 5.62-5.50 (m, 1H), 5.49-5.36 (m, 1H), 4.73 (ddd, *J* = 6.29, 2.62, 1.46 Hz, 1H), 4.34-4.19 (m, 3H), 2.14 (s, 3H), 2.09 (s, 1H), 2.03 (s, 3H). Consistent with the literature.[3]

((2R,3S,4R,5S,6R)-6-allyl-3-hydroxy-5-methyl-4-(pivaloyloxy)tetrahydro-2H-pyran-2-yl)methyl pivalate 186

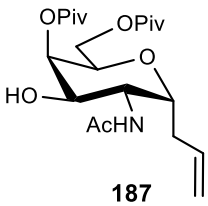


186

Compound **1103** (5.50 g, 14.8 mmol) was dissolved in methanol (100 mL) then NaOMe solution was added until a pH of 12 was reached. Upon stirring for 1 h, Dowex 50WX8 was added to neutralize the solution. The solution was filtered and concentrated under reduced pressure. The residue (3.45 g, 14.1 mmol) was dissolved in a 2:1 mixture of dry pyridine/CH_2Cl_2 (72 mL), and the solution was cooled to –20°C. Pivaloyl chloride (6.90 mL, 56.3 mmol) was added in portions and the reaction mixture was allowed to warm to 0°C. After 7 h, CH_2Cl_2 (50 mL) was added and the organic phase was washed sequentially with 5% aqueous HCl (2 x 50 mL) and water (2 x 50 mL). The organic phase was dried, filtered,

and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **186**, the title compound, as a white solid (3.70 g, 64% yield). On replication, yields varied between 52% and 64% for scales between 100 mg and 5.00 g. Rf = 0.45. 1:1 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 6.12 (d, J = 8.2 Hz, 1H), 5.85–5.71 (m, 1H), 5.00-5.16 (m, 3H), 4.49 (dd, J=12.0, 5.8 Hz, 1H), 4.25–4.13 (m, 3H), 3.77 (ddd, J=8.1, 6.0, 2.8 Hz), 3.53 (t, J = 7.7 Hz, 1H), 3.14 (bs, 1H), 2.51–2.40 (m, 1H), 2.25-2.32 (m, 1H), 1.93 (s, 3H), 1.23–1.19 (m, 18H). Consistent with the literature.[4]

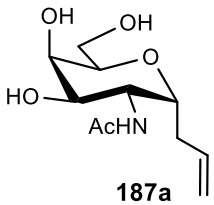
(2R,3R,4R,5R,6R)-6-allyl-4-hydroxy-5-methyl-2-((pivaloyloxy)methyl)tetrahydro-2H-pyran-3-yl pivalate 187



187

Compound **186** (3.70 g, 8.95 mmol) was dissolved in a 2:1 pyridine/CH_2Cl_2 mixture (63 mL) and the solution was cooled to 0°C. Triflic anhydride (3.9 mL, 23.3 mmol) was added in portions until complete consumption of the starting material. Water (9.3 mL) was then added, and the reaction mixture was allowed to stir overnight at room temperature. The reaction was diluted with CH_2Cl_2 (200 mL) and the organic phase was washed sequentially with 5% aqueous HCl (2 x 100 mL) and water (2 x 100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1, EtOAc/hexanes +0.2% EtOH) and passed through a silica gel column to produce **187**, the title compound, as a yellowish oil (1.10 g, 30% yield). On replication, yields varied between 24% and 30% for scales between 150 mg and 5.00 g. Rf = 0.25. 1:1 EtOAc/hexanes +0.2% EtOH. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 5.83–5.74 (m, 1H), 5.17-5.07 (m, 3H), 4.62 (bt, J = 9.7 Hz, 1H), 4.37 (ddd, J=8.9, 5.0, 3.3 Hz, 1H), 4.20 (dt, J=7.7, 3.3 Hz, 1H), 4.16-4.01 (m, 3H), 2.34–2.18 (m, 2H), 2.03 (s, 3H), 1.29–1.19 (m, 18H). Consistent with the literature.[4]

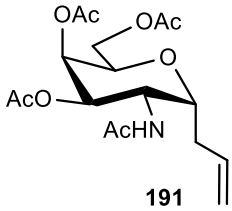
(2R,3R,4R,5R,6R)-6-allyl-2-(hydroxymethyl)-5-methyltetrahydro-2H-pyran-3,4-diol 187a



187a

Compound **187** (1.10 g, 2.66 mmol) was dissolved in methanol (100 mL) then NaOMe solution was added until a pH of 12 was reached. Upon stirring for 1 h, Dowex 50WX8 was added to neutralize the solution. The solution was filtered and concentrated under reduced pressure to produce **187a**, title compound as a colourless jelly liquid (600 mg, 92%). On replication, yields varied between 82% and 92% for scales between 500 mg and 5.00 g. Rf = 0.3. 9:1 EtOAc/MeOH. ^{1}H NMR (300 MHz, D_2O): δ_{ppm} 5.88–5.66 (m, 1H), 5.00 (dd, J=17.1, 2.3 Hz, 1H), 4.95 (dd, J=9.1, 2.3 Hz, 1H), 4.17–4.03 (m, 2H), 3.83 (bs, 1H), 3.72–3.57 (m, 4H), 2.43–2.32 (m, 1H), 2.05–2.14 (m, 1H), 1.89 (s, 3H). Consistent with the literature.[4]

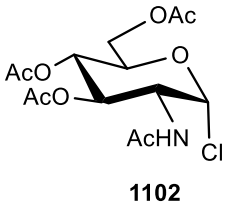
(2R,3R,4R,5S,6R)-2-(acetoxymethyl)-6-allyl-5-methyltetrahydro-2H-pyran-3,4-diyl diacetate 191



191

To a solution of compound **187a** (600 mg, 2.45 mmol) in pyridine (50 mL), Ac_2O (25 mL) was added. The reaction mixture was then stirred for 3 h, and then CH_2Cl_2 (100 mL) was added and the mixture was washed with 10% HCl (2 x 50 mL), water (2 x 50 mL) and brine (1 x 50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to produce **191**, title compound as a colourless liquid (680 mg, 75% yield). On replication, yields varied between 58% and 75% for scales between 100 mg and 5.00 g. Rf = 0.33. 2:1 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.89-5.66 (m, 2H), 5.32 (t, J = 3.02, 3.02 Hz, 1H), 5.21-5.04 (m, 3H), 4.48 (dt, J = 8.98, 8.87, 4.88 Hz, 1H), 4.32 (td, J = 9.87, 4.88, 4.88 Hz, 1H), 4.22 (d, J = 8.03 Hz, 1H), 4.15-4.01 (m, 2H), 2.50-2.31 (m, 1H), 2.32-2.24 (m, 1H), 2.11 (s, 3), 2.06 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H). Consistent with the literature.[5]

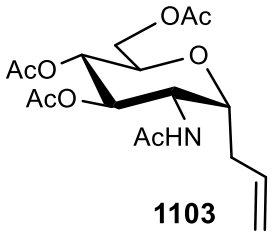
(2R,3S,4R,5R,6R)-5-acetamido-2-(acetoxymethyl)-6-chlorotetrahydro-2H-pyran-3,4-diyl diacetate 1102



1102

To a solution of **309** (57.0 g, 0.15 mol) in CH_2Cl_2 (500 mL) at 0°C, thionyl chloride (170 mL) and acetic acid (28.5 mL) were added. The mixture was stirred at ambient temperature for 4 h then concentrated in vacuo. The residue was recrystallized from Et_2O to produce **1102**, title compound as a brown solid (40.2 g, 75%). On replication, yields varied between 70% and 75% for scales between 100 mg and 100 g. Rf = 0.3. 2:1 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 6.20 (d, J = 3.7 Hz, 1H), 5.83 (d, J = 8.6 Hz, 1H), 5.35–5.28 (m, 1H), 5.22 (t, J = 9.7 Hz, 1H), 4.54 (ddd, J = 12.4, 8.7, 3.8 Hz, 1H), 4.32–4.25 (m, 2H), 4.16–4.12 (m, 1H), 2.11 (s, 3H), 2.06 (s, 6H), 1.99 (s, 3H). Consistent with the literature.[6]

(2R,3S,4R,5S,6R)-2-(acetoxymethyl)-6-allyl-5-methyltetrahydro-2H-pyran-3,4-diyl diacetate 1103

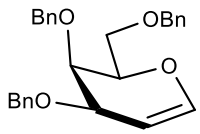


1103

In a flame dried round bottom flask, compound **1102** (10.0 g, 27.4 mmol) was dissolved in anhydrous benzene under nitrogen. Allyltributyltin (8.50 mL, 27.4 mmol) and AIBN (680 mg, 4.10 mmol) then were added to the mixture and the reaction mixture was degassed for 30 minutes by bubbeling nitrogen through the mixture. The reaction mixture then was heated to reflux at 85ºC and strred for 8 h. The solution was cooled to room temperature and concentrated under reduced pressure. The remaining residue was partitioned between CH_3CN (200 mL) and hexanes (400 mL). The CH_3CN phase was extracted with additional hexanes (3 x 400 mL) to remove the remaining organotin compounds and then concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and passed through a silica gel column to produce **1103**, the

title compound, as a waxy solid (7.50 g, 74% yield). On replication, yields varied between 52% and 74% for scales between 100 mg and 18.0 g. Rf = 0.33. 2:1 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 5.90-5.66 (m, 2H), 5.21-5.00 (m, 3H), 4.95 (t, J = 6.83, 6.83 Hz, 1H), 4.36-4.15 (m, 3H), 4.10 (dd, J = 11.99, 3.64 Hz, 1H), 3.88 (dt, J = 6.57, 6.53, 3.75 Hz, 1H), 2.52-2.33 (m, 1H), 2.33-2.19 (m, 1H), 2.09 (s, 3H), 2.07 (s, 6H), 1.97 (s, 3H). Consistent with the literature.[5]

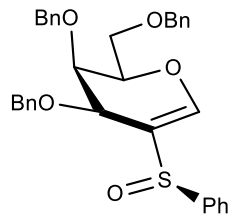
(2R,3R,4R)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-3,4-dihydro-2H-pyran 1123



1123

Compound **119** (4.02 g, 14.8 mmol) was dissolved in methanol (100 mL) then NaOMe solution was added until pH of 12 was reached. The reaction was stirred at room temperature for 1 h to produce a faint yellow coloured solution. DOWEX 50W X8 ion exchange resin was added to the reaction mixture until a pH of 7 was reached. The solution was filtered and concentrated under reduced pressure. The crude was dissolved in DMF (9 mL) and was cooled to 0°C. It was then subsequently treated with sodium hydride (2.20 g, 55 mmol, 60% suspension in mineral oil) and benzyl bromide (6.4 mL, 53.7 mmol) and stirred for 12 h. Methanol (10 mL) was then added slowly, diluted with ethyl acetate (250 mL), and washed with water (150 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:9) and passed through a silica gel column to produce **1123**, the title compound, as a colourless liquid (1.80 g, 30% yield). On replication, yields varied between 22% and 30% for scales between 100 mg and 5.00 g. Rf = 0.32. 9:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.61-7.26 (m, 15H), 6.36 (dd, *J* = 6.21, 1.41 Hz, 1H), 4.91-4.83 (m, 2H), 4.63 (dd, *J* = 8.09, 3.80 Hz, 2H), 4.46 (q, *J* = 11.91, 11.91, 11.86 Hz, 2H), 4.24-4.13 (m, 2H), 4.00-3.89 (m, 1H), 3.78 (dd, *J* = 10.08, 7.26 Hz, 1H), 3.64 (dd, *J* = 10.10, 5.10 Hz, 1H). Consistent with the literature.[7]

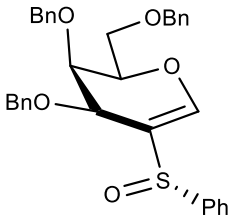
(2R,3S)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-((S)-phenylsulfinyl)-3,4-dihydro-2H-pyran 1124



1124

Compound **463** (200 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) was treated with *m*CPBA (126 mg, 0.40 mmol) and stirred for 2 h at –20°C. The reaction mixture was filtere and diluted with a saturated aqueous $NaHCO_3$ solution (5 mL) and extracted with CH_2C1_2 (2 x 5 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce ratio l : l **1124** and **1125**, the title compounds, as colourless liquid (195 mg, 95% total yield). **1125**: Rf = 0.52 and **1125**: Rf = 30. 1:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.57-7.37 (m, 2H), 7.36-7.21 (m, 11H), 7.21-7.08 (m, 6H), 6.77-6.66 (m, 2H), 4.63 (d, *J* = 11.73 Hz, 1H), 4.53-4.40 (m, 6H), 3.89 (t, *J* = 3.80, 3.80 Hz, 1H), 3.82 (dd, *J* = 11.40, 8.28 Hz, 2H), 3.68 (dd, *J* = 11.32, 3.18 Hz, 1H).[8]

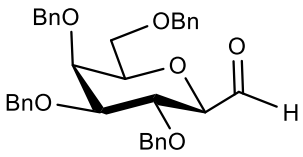
(2R,3S)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-((R)-phenylsulfinyl)-3,4-dihydro-2H-pyran 1125



1125

1125: 7.57-7.48 (m, 2H), 7.42 (dd, J = 5.23, 1.79 Hz, 3H), 7.38-7.14 (m, 13H), 7.05 (dd, J = 4.50, 1.29 Hz, 2H), 7.02 (s, 1H), 4.69 (d, *J* = 11.58 Hz, 1H), 4.52 (d, *J* = 11.30 Hz, 2H), 4.45 (d, *J* = 4.05 Hz, 2H), 4.42-4.37 (m, 1H), 4.26 (d, *J* = 11.95 Hz, 1H), 4.18-4.03 (m, 1H), 4.05-3.74 (m, 3H), 3.63 (t, *J* = 3.91, 3.91 Hz, 1H).[8]

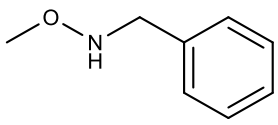
(2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-carbaldehyde 1131



1131

A solution of **495** (3.50 g, 6.36 mmol) in CH_2Cl_2 (50 mL) was cooled to –78°C. Ozone was then bubbled through the solution under vigorous stirring. Upon a notable colour change to a stable deep blue colour, dimethylsulfide (1.2 mL, 16.0 mmol) was added and the solution was allowed to warm up to room temperature while stirring was continued overnight. The clear solution was concentrated under reduced pressure, then water (50 mL) was added and extracted with EtOAc (3 x 40 mL), and the combined organics were washed with water (20 mL) and brine (20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to produce **1131**, the title compound as a colourless liquid (2.80 g, 80% yield). On replication, yields varied between 72% and 80% for scales between 110 mg and 3.50 g. Rf = 0.3. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 9.65 (d, J = 1.33 Hz, 1H), 7.43-7.23 (m, 20H), 4.91 (dd, J = 19.16, 11.02 Hz, 2H), 4.80-4.57 (m, 4H), 4.46 (q, J = 11.97, 11.84, 11.84 Hz, 2H), 4.15-3.92 (m, 2H), 3.83-3.47 (m, 5H). Consistent with the literature.[9]

***N*-benzyl-O-methylhydroxylamine 1138a**

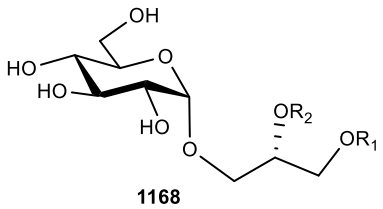


1138a

To the *O*-methylhydroxylamine hydrochloride (10.0 g, 0.20 mol) was added CH_2Cl_2 (120 mL) and pyridine (16 mL) then benzaldehyde (8.7 mL, 0.09 mol) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the mixture was washed with 1M HCl (100 mL), and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in acetic acid (60 mL), then $NaBH_3CN$ (12.6 g, 0.20 mol) was added and stirred for 1 h at room temperature. Acetic acid was removed, then 0.05M HCl (100 mL) was added and the mixture was washed with CH_2Cl_2 (3 x 50 mL). The aqueous phase basified with saturated aqueous Na_2CO_3, then extracted with CH_2Cl_2 (4

x 50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to produce **1138a**, the title compound, as a colourless liquid (3.50 g, 20% yield, note: yield is sensitive regarding large scale synthesis, and it will be lower). Rf = 0.27. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.40-7.26 (m, 5H), 5.80-5.63 (m, 1H), 4.05 (s, 2H), 3.51 (d, *J* = 0.43 Hz, 3H). Consistent with the literature.[10]

1-*O*-hexadecanoyl-2-*O*-*cis*-Octadec-12-enoyl-3-*O*-(*α*-D-glucopyranosyl)-sn-glycerol 1168

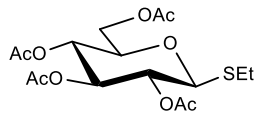


1168

R_1 = palmitoyl
R_2 = *cis*-vaccinoyl

Compound **510** (0.11 g, 0.12 mmol) was dissolved in EtOH (2.5 mL) and $N_2H_4{\cdot}H_2O$ 35% (0.3 mL) was added and refluxed at 40°C for 4.5 h. EtOH was then evaporated under reduced pressure and (50 mL) of H_2O (20 mL) was added. The aqueouse phase was extracted with CH_2Cl_2 (4 x 20 mL) and organic phase was washed with brine (40 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in ethyl acetate and passed through a silica gel column to produce **1168**, title compound as a colourless liquid (0.05 g, 55% yield). Rf = 0.28. 98:2 EtOAc/MeOH. ^{1}H NMR (500 MHz, MeOD) δ_{ppm} 5.37 (t, J = 5.10, 2H), 5.28 (m, 1H), 4.8 (t, J = 3.15, 1H), 4.45 (m, 1H), 4.2 (m, 1H), 3.87 (dd, J = 10.78, 5.79, 1H), 3.79 (ddd, J = 2.46, 5.25, 11.83, 1H), 3.51-3.72 (m, 4H), 3.39 (m, 1H), 3.31, (m, 1H), 2.33 (m, 4H), 2.04 (m, 4H), 1.62 (m, 4H), 1.32 (s, 44H), 0.91 (td, J = 1.95, 6.82, 6H). 1H NMR (500 MHz d_6-Me_2SO-$CDCl_3$ (5:1, v/v) δ_{ppm} 5.37-5.20 (m, 2H), 5.18-5.07 (m, 1H), 4.90-4.81 (m, 1H), 4.81-4.73 (m, 1H), 4.70-4.58 (m, 2H), 4.44-4.35 (m, 1H), 4.35-4.26 (m, 1H), 4.18-4.08 (m, 1H), 3.77-3.65 (m, 1H), 3.63-3.55 (m, 1H), 3.53-3.43 (m, 2H), 3.43-3.33 (m, 2H), 3.22-3.16 (m, 1H), 3.13-3.06 (m, 1H), 2.28-2.22 (m, 4H), 2.00-1.93 (m, 4H), 1.55-1.45 (m, 4H), 1.32-1.15 (m, 44H), 0.85 (dd, *J* = 7.38, 6.65 Hz, 1H). Consistent with the literature.[11, 12]

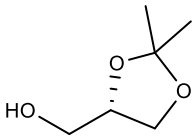
(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(ethylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 1217



1217

To a solution of compound **513** (30.0 g, 76.9 mmol) in CH_2Cl_2 (150 mL) at 0°C and ethanethiol (7.2 mL, 99.9 mmol) and BF_3**.**OEt_2 (27 mL, 220 mmol) were added, and stirred for 12 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with 2M NaOH (3 x 100 mL), water (100 mL) and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **1217**, the title compound, as a colourless liquid (28.0 g, 93% yield). On replication, yields varied between 85% and 93% for scales between 100 mg and 30.0 g. Rf = 0.32. 3:7 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.69 (d, J = 5.79 Hz, 1H), 5.37 (t, J = 9.78, 9.78 Hz, 1H), 5.13-4.98 (m, 2H), 4.57-4.39 (m, 1H), 5.47-5.30 (m, 1H), 4.31 (dd, J = 12.32, 4.59 Hz, 1H), 4.08 (dd, J = 12.33, 2.15 Hz, 1H), 2.66-2.48 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.28 (t, J = 7.42, 7.42 Hz, 3H). Consistent with the literature.[13]

(S)-(+)-1,2-isopropylideneglycerol or (S)-(+)-Solketal 1253



1253

Compound **505** (10.0 g, 38.1 mmol) was dissolved in THF (150 ml) and lead (IV) acetate (16.8 g, 38.1 mmol) was added. The reaction was stirred for 30 min at 0°C and 1 h at room temperature. The reaction mixture was filtered and $NaBH_4$ (2.88 g, 76.2 mmol) was added in 4% NaOH (aq) to the filtrate. The reaction mixture was stirred at 0°C for 30 minutes and then 2 h at room temperature. The reaction mixture was quenched with NH_4Cl, concentrated under reduced pressure, and diluted with CH_2Cl_2 (250 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 150 mL) and the organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate

(3:1) and passed through a silica gel column to produce **1253**, title compound as a colourless liquid (2.80 g 55% yield). On replication, yields varied between 48% and 55% for scales between 50 mg and 10.0 g. Rf = 0.4. 3:7 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 4.20-4.27 (m, 1H), 4.05 (t, 1H J = 8.28), 3.81 (dd, 1H J = 6.53, 8.17), 3.71 (d, 1H J = 3.81), 3.60 (dd, 1H J = 4.79, 11.70), 1.43 (s, 3H), 1.37 (s, 3H). Consistent with the literature.[14]

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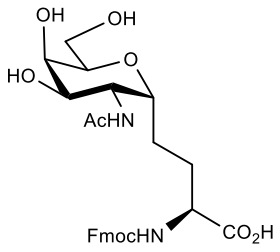
9.5 Experimental protocols for Chapter 2

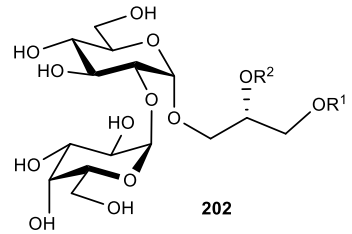
(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)butanoic acid 201



201

To solution of **326** (20.0 mg, 0.03 mmol) in isopropyl alcohol (3 mL) and water (1.5 mL), solution of 0.8M calcium chloride (0.3 mL) was added and stirred for 2 h at room temperature, then ethyl acetate (10 mL) was added and organic phase was washed with 1M HCl (3 mL). The crude product was dissolved in hexanes/ethyl acetate (9.5:0.5) and passed through a silica gel column to produce (*L*)-**201**, the title compound as a colourless liquid (10.2 mg, 44% yield). On replication, yields varied between 30% and 44% for scales between 20.0 mg and 965 mg. Rf = 0.29. 9.5:0.5 hexanes/EtOAc. ^{1}H NMR (500 MHz, MeOD) δ_{ppm} 7.86-7.75 (m, 2H), 7.74-7.62 (m, 2H), 7.46-7.25 (m, 4H), 4.47-4.31 (m, 2H), 4.31-4.08 (m, 5H), 3.94-3.83 (m, 1H), 3.83-3.74 (m, 1H), 3.74-3.60 (m, 3H), 1.93 (s, 3H), 1.32-1.20 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 174.1, 173.6, 158.7, 145.2, 142.6, 128.8, 128.1, 126.1, 120.9, 74.6, 69.5, 62.3, 55.6, 51.8, 40.1, 31.6, 30.1, 24.9, 24.0, 22.6, 14.5. **EI-MS *m/z*** was not collected.

(Z)-(S)-1-(((2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-3-(palmitoyloxy)propan-2-yl octadec-6-enoate 202



202

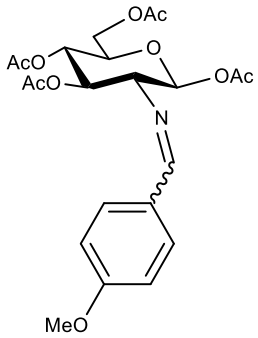
R^1 = Palmitoyl
R^2 = *cis*-vaccinoyl

Compound **531** (6.60 mg, 0.005 mmol) was dissolved in *t*-butylamine (0.05 mL, 0.43 mmol), $CHCl_3$ (0.09 mL) and MeOH (0.30 mL), and stirred at 0°C for 10 min. The reaction mixture then warmed to room temperature and stirred for 2 h. The solvents were evaporated under high vacuum. The crude product was dissolved in

methanol/ethyl acetate (1:9) and passed through a silica gel column to produce **202**, title compound as a colourless liquid (3.50 mg, 82% yield). Rf = 0.2. 1:9 EtOAc/MeOH.^{1}H NMR (500 MHz, MeOD) δ_{ppm} 5.39-5.31 (m, 2H), 5.29-5.20 (m, 1H), 5.04 (dd, *J* = 16.54, 3.66 Hz, 2H), 4.58 (s, 1H), 4.49 (dd, *J* = 12.20, 2.89 Hz, 1H), 4.26-4.20 (m, 1H), 4.13-4.07 (m, 1H), 3.94-3.85 (m, 2H), 3.83-3.61 (m, 8H), 3.61-3.56 (m, 2H) , 3.40-3.33 (m, 1H), 2.34 (td, *J* = 14.23, 7.24, 7.24 Hz, 4H), 2.09-2.00 (m, 4H), 1.67-1.55 (m, 4H), 1.36-1.26 (m, 44H), 0.93-0.87 (m, 6H) ^{13}C NMR (125 MHz, MeOD) δ_{ppm} 174.7, 174.0, 101.2, 100.6, 98.2, 97.7, 77.7, 73.8, 73.5, 73.3, 72.6, 71.7, 71.7, 71.5 x 2, 71.4, 71.2, 71.1, 71.0, 70.3, 70.3, 70.2, 67.0, 63.9, 62.8, 62.5, 35.1, 35.0, 34.9, 33.1, 31.6, 30.8 x 4, 30.6 x 2, 30.5, 30.3, 30.2, 30.0, 28.1, 26.0, 23.7, 14.4. (Three peaks are under solvent peak) **EI-MS *m/z*** calcd for $C_{49}H_{90}O_{15}$ $[M+Na]^+$: 941.61. Found: 941.62.

9.6 Experimental protocols for Chapter 3

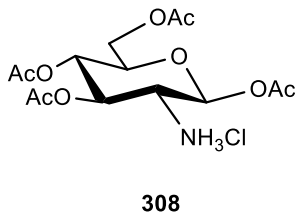
(2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-((4-methoxybenzylidene)amino)tetrahydro-2H-pyran-2,4,5-triyl triacetate 307



307

Ac_2O (250 mL) was added slowly to pyridine at 0°C (550 mL) and **306** (80.0 g, 271 mmol) was added. The reaction mixture then warmed to room temperature whie stirring overnight. The reaction was poured into a beaker containing crushed ice (2.15 L) under stirring conditions and precipitate was formed after 2 h. The precipitate was filtered and washed with ice cold water to produce **307**, title compound as a white solid (93.2 g, 75% yield). On replication, yields varied between 62% and 75% for scales between 5.00 g and 80.0 g. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 8.14 (s, 1H), 7.64 (d, *J* = 8.77 Hz, 2H), 6.90 (d, *J* = 8.76 Hz, 2H), 5.93 (d, *J* = 8.29 Hz, 1H), 5.42 (t, *J* = 9.60, 9.60 Hz, 1H), 5.13 (t, *J* = 9.75, 9.75 Hz, 1H), 4.37 (dd, *J* = 12.47, 4.50 Hz, 1H), 4.12 (dd, *J* = 12.44, 2.00 Hz, 1H), 3.96 (ddd, *J* = 10.11, 4.45, 2.07 Hz, 1H), 3.83 (s, 3H), 3.44 (dd, *J* = 9.62, 8.39 Hz, 1H), 2.09 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.87 (s, 3H). Consistent with the literature[1]

(2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-aminotetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride 308

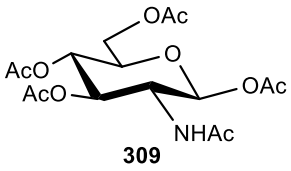


308

To a solution of compound **307** (93.0 g, 0.20 mol) in acetone (600 mL), hydrochloric acid (51 mL, 5 N aqueous solution) was added dropwise. The reaction mixture was stirred for 1 h at 0°C until precipitate formed. The precipitate was removed by filtration and washed with Et_2O (200 mL). It was then dried to produce **308**, title compound as a white solid (73.0 g, 93% yield). On replication, yields varied between 85% and 93% for scales between 820 mg and 93.0 g. Rf = 0.3.

EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 5.70 (d, J = 8.78 Hz, 1H), 5.50 (d, J = 9.48 Hz, 1H), 5.29-4.94 (m, 2H), 4.39-4.21 (m, 2H), 4.13 (dd, J = 12.49, 2.21 Hz, 1H), 3.88-3.72 (m, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H). Consistent with the literature[1]

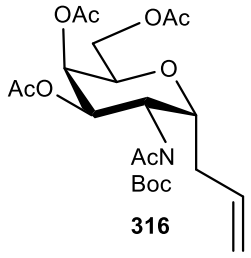
(2S,3R,4R,5S,6R)-3-acetamido-6-(acetoxymethyl)tetrahydro-2H-pyran-2,4,5-triyl triacetate 309



309

Et_3N (82.2 mL, 0.575 mol) was added dropwise to a solution of **308** (73.5 g, 0.19 mol) in CH_2Cl_2 (500 mL) under rapid stirring conditions. Ac_2O (233 mL, 2.46 mol) was then added and the mixture was stirred for an additional 4 h. The reaction mixture quenched with methanol (28 mL) and organic phase was washed with 1M HCl (2 x 250 mL), saturated aqueous $NaHCO_3$ (2 x 250 mL), water (250 mL) and brine (250 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to obtain the desired product **309** (65.0 g, 87% yield). On replication, yields varied between 77% and 87% for scales between 12.0 g and 73.5 g. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm} 5.70 (d, J = 8.8 Hz, 1H), 5.65 (d, J = 9.6 Hz, 1H), 5.19–5.10 (m, 2H), 4.35–4.24 (m, 2H), 4.13 (dd, J = 12.5, 2.1 Hz, 1H), 3.84–3.75 (m, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.94 (s, 3H). Consistent with the literature.[2]

(2R,3R,4R,5S,6R)-2-(acetoxymethyl)-6-allyl-5-methyltetrahydro-2H-pyran-3,4-diyl diacetate 316

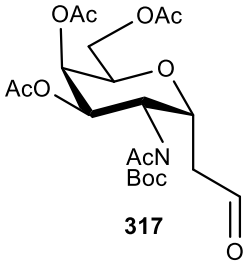


316

To a solution of compound **191** (200 mg, 0.54 mL) in THF (5 mL), Boc_2O (1.20 g, 5.43 mmol) and DMAP (27.0 mg, 0.22 mmol) were added and stirred at 80°C for 2 h. The reaction mixture was then concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate

(8:2) and passed through a silica gel column to produce **316** the title compound, as a white solid (210 mg, 83% yield). Rf = 0.31. 8:2 hexanes/EtOAc. On replication, yields varied between 76% and 83% for scales between 200 mg and 3.50 g. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.97-5.57 (m, 2H), 5.51 (d, *J* = 2.05 Hz, 1H), 5.15-5.01 (m, 2H), 4.28-4.15 (m, 1H), 4.13-3.98 (m, 4H), 2.70-2.52 (m, 1H), 2.37 (s, 3H), 2.28-2.14 (m, 1H), 2.12 (s, 3H), 2.01 (s, 3H), 1.93 (s, 1H), 1.51 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 173.4, 170.8, 170.6, 170.0, 153.0, 134.4, 117.5, 84.9, 77.5, 68.4, 68.1, 67.7, 62.4, 32.2, 32.0, 30.0, 30.0, 29.7, 28.1, 23.0, 21.0, 14.4. **EI-MS *m/z*** calcd for $C_{22}H_{33}NO_{10}$: 471.21. Found: $[M+Na]^+$ 494.20.

(2R,3R,4R,5S,6R)-2-(acetoxymethyl)-5-(*N*-(tert-butoxycarbonyl)acetamido)-6-(2-oxoethyl)tetrahydro-2H-pyran-3,4-diyl diacetate 317

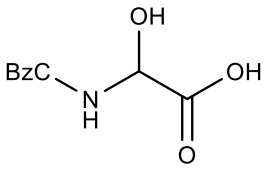


317

A solution of **316** (200 mg, 0.42 mmol) in CH_2Cl_2 (50 mL) was cooled to –78°C and ozone was bubbled through the solution under vigorous stirring. Upon a notable colour change to a stable deep blue colour, triphenylphosphine (330 mg, 1.26 mmol) was added and the solution was allowed to warm up to room temperature while stirring was continued overnight. The clear solution was concentrated under reduced pressure, then water (50 mL) was added and extracted with EtOAc (3 x 40 mL), and then organic phase washed with water and brine, dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **317,** the title compound, as a white solid (180 mg, 90% yield). On replication, yields varied between 84% and 90% for scales between 200 mg and 2.50 g Rf = 0.28. Rf = 0.28. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 9.69 (d, *J* = 1.24 Hz, 1H), 5.85-5.65 (m, 1H), 5.57-5.48 (m, 1H), 4.84 (dd, *J* = 12.94, 6.56 Hz, 1H), 4.33-3.80 (m, 4H), 3.11-2.93 (m, 1H), 2.93-2.72 (m, 1H), 2.37 (s, 3H), 2.15 (s, 3H), 2.04 (s, 3H), 1.96 (s, 3H), 1.51

(s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 198.9, 172.9, 170.4, 170.2, 169.9, 169.5, 152.5, 84.8, 77.6, 77.1, 76.7, 68.9, 68.8, 67.7, 66.7, 61.8, 42.9, 27.7, 27.5, 26.8, 20.8, 20.6. **EI-MS *m/z*** calcd for $C_{21}H_{31}NO_{11}$: 473.19. Found: $[M+Na]^+$ 496.18.

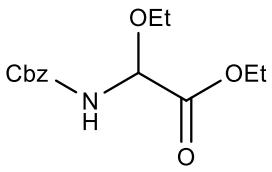
α-Hydroxy-*N*-benzyloxycarbonylglycine 320a



320a

A mixture of benzyl carbamate (15.0 g, 99.2 mmol) and glyoxylic acid monohydrate (10.0 g, 109 mmol) in anhydrous ether (100 mL) was stirred overnight. The crystalline product was filtered and washed with ether to produce **320a**, title compound as a white solid (13.7 g, 65%). ^1H NMR (300 MHz, DMSO-d_6): δ_{ppm} ^1H NMR (500 MHz, $CDCl_3$) δ_{ppm} 8.14 (d, J = 8.50 Hz, 1H), 7.39-7.23 (m, 5H), 5.21 (d, J = 8.91 Hz, 1H), 5.05 (s, 2H). Consistent with the literature.[3]

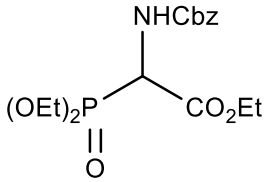
***N*-Carbobenzoxy-α-ethoxyglycine ethyl ester 321**



321

Compound **320a** (7.50 g, 33.3 mmol) was dissolved in EtOH (90 mL), to which conc. H_2SO_4 (1.65 mL) was added at 0°C. After stirring for 3 days at room temperature, the reaction mixture was cooled to 0°C and 8.5 M NaOH was added dropwise until pH 7. The mixture was extracted with EtOAc and the organic phase was washed with 0.5 M $NaHCO_3$ (2 x 50 mL), 0.5 M HC1(2 x 50 mL), and brine (2 x 50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to produce **321**, title compound as a white solid (7.10 g, 76% yield). ^1H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.42 (t, J = 12.42 Hz, 1H), 5.26-5.02 (m, 2H), 4.36-4.19 (m, 1H), 4.13 (q, J = 7.15 Hz, 1H), 3.92-3.51 (m, 2H), 1.38-1.27 (m, 3H), 1.27-1.20 (m, 3H). Consistent with the literature.[4]

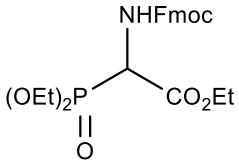
Ethyl2-(Benzyloxycarbonylamino)-2-(diethoxyphosphoryl)acetate 322



322

Compound **321** (7.10 g, 25.2 mmol) was dissolved in toluene (45 mL) at 50°C under nitrogen gas. The solution was cooled to room temperature and PCl_3 (3.27 mL, 37.8 mmol) was added. The solution was stirred for 18 h at 70°C, and then cooled to room temperature, $(EtO)_3P$ (5.34 mL, 31.1 mmol) was added and heated to 70°C for 3 h. The mixture was concentrated under reduced pressure and the residue was partitioned between Et_2O (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:2) and passed through a silica gel column to produce **322** the title compound, as a white solid (5.82 g, 62% yield) as a white waxy solid. Rf = 0.35. 2:1, EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.41-7.30 (m, 5H), 5.61 (d, J = 7.40 Hz, 1H), 5.27-5.02 (m, 2H), 4.87 (dd, J = 22.19, 9.22 Hz, 1H), 4.41-3.95 (m, 6H), 1.42-1.11 (m, 9H). Consistent with the literature.[5]

Ethyl 2-(*tert*-butoxycarbonylamino)-2-(diethoxyphosphoryl) acetate 323

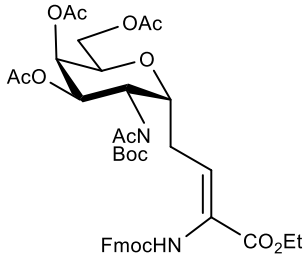


323

Compound **322** (200 mg, 0.54 mmol) was dissolved in EtOAc (25 mL). To this solution was subsequently added TFA (54 µL, 0.67 mmol), water (0.2 mL) and Pearlman's catalyst $Pd(OH)_2/C$ (60 mg). The mixture was treated with hydrogen gas for 24 h at ambient pressure and room temperature. Thereafter, the black suspension was filtered through a pad of Celite and concentrated under reduced pressure. The residue was diluted with water (10 mL) and dioxane (10 mL), then $NaHCO_3$ (135 mg, 1.61 mmol) and FmocCl (288 mg, 1.11 mmol) were added and stirred for 16 h at room temperature. The reaction mixture diluted with EtOAc (50 mL) and aqeouse phase extracted with EtOAc (10 mL). The combined organic phase washed with water (50 mL). The organic phase was

dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and passed through a silica gel column to produce **323**, the title compounds as white solid (200 mg, 80% yield). On replication, yields varied between 75% and 80% for scales between 200 mg and 3.50 g. Rf = 0.27. 1:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.78 (d, J = 7.40 Hz, 2H), 7.61 (dd, J = 6.92, 3.27 Hz, 2H), 7.42 (t, J = 7.47, 7.47 Hz, 2H), 7.36-7.29 (m, 2H), 5.78-5.56 (m, 1H), 4.90 (dd, J = 22.32, 9.32 Hz, 1H), 4.42 (d, J = 7.28 Hz, 2H), 4.39-3.92 (m, 6H), 1.40-1.30 (m, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 166.6, 155.6, 155.4, 143.4, 141.0, 127.5, 126.9, 124.9, 124.8, 119.8, 67.5, 63.6, 63.5, 62.3, 53.4, 51.5, 46.8, 16.2, 16.1, 13.8. **EI-MS *m/z*** was not collected.

(2R,3R,4R,5S,6R)-6-((Z)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-ethoxy-4-oxo-but-2-en-1-yl)-2-(acetoxymethyl)-5-(*N*-(tert-butoxycarbonyl)acetamido)tetrahydro-2H-pyran-3,4-diyl diacetate 324

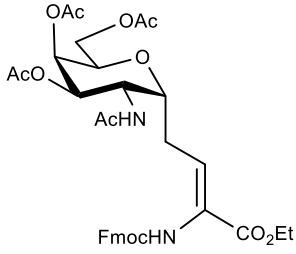


324

To 0.85 M LDA (0.13 mL, 0.11 mmol) was added anhydrous THF (2 mL), and cooled to –40°C and stirring for 20 min. amino acid **323** (50.0 mg, 0.11 mmol) was dissolved in anhydrous THF (1 mL) and added to the mixture. Within 20 min, the solution was allowed to warm to −10°C, then the solution was cooled to −60 °C and **317** (36.0 mg, 0.08 mmol) was dissolved in anhydrous THF (1 mL), and was added to the solution. The reaction mixture warmed up to room temperature, and then was quenched with saturated aqueous NH_4Cl (30 mL), and then Et_2O (30 mL) was added. The aqueous phase was then extracted with Et_2O (3 x 30 mL), the combined organic phase was washed with water (50 mL) and brine (50 mL). The organic phase dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce (E,Z)- **324**, the title

compound, as a colourless liquid (46.0 mg, 80% yield). On replication, yields varied between 72% and 80% for scales between 36.0 mg and 1.30 g. Rf = 0.29. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.76 (d, J = 7.25 Hz, 2H), 7.57 (d, J = 7.14 Hz, 2H), 7.40 (t, J = 7.79, 7.79 Hz, 2H), 7.35-7.26 (m, 2H), 7.00-6.70 (m, 1H), 6.68-6.48 (m, 1H), 6.02-5.67 (m, 1H), 5.52 (s, 1H), 5.38-5.00 (m, 1H), 4.56-3.94 (m, 9H), 3.27 (ddd, J = 16.29, 10.98, 8.49 Hz, 1H), 2.88-2.57 (m, 1H), 2.40 (s, 3H), 2.13 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.52 (s, 9H), 0.87-0.82 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 173.1, 170.5, 170.3, 170.3, 170.2, 169.6, 164.1, 163.5, 154.1, 153.5, 152.6, 152.6, 143.6, 141.2, 127.7, 127.0, 124.9, 119.9, 84.8, 84.6, 68.0, 67.9, 67.2, 67.0, 62.4, 62.1, 61.9, 61.5, 47.0, 31.5, 29.0, 28.2, 27.7, 27.6, 26.8, 26.5, 26.3, 22.6, 20.6, 20.5, 20.3, 14.1, 14.0. **EI-MS *m/z*** calcd for $C_{40}H_{48}N_2O_{14}$: 780.31. Found: $[M+Na]^+$ 803.33.

(2R,3R,4R,5S,6R)-6-((Z)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-ethoxy-4-oxo-but-2-en-1-yl)-5-acetamido-2-(acetoxymethyl)tetrahydro-2H-pyran-3,4-diyl diacetate 325

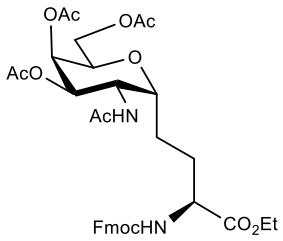


325

To a solution of **324** (120 mg, 0.16 mmol) in CH_2Cl_2 (3 mL) at room temperature, TFA (2 mL) was added and stirred for 2 h. The solution concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:2) and passed through a silica gel column to produce (E,Z)-**325**, the title compound, as a colourless liquid (88.0 mg, 77% yield). On replication, yields varied between 70% and 77% for scales between 120 mg and 1.50 g. Rf = 0.29. 1:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.76 (d, J = 7.36 Hz, 2H), 7.72-7.52 (m, 2H), 7.47-7.36 (m, 2H), 7.37-7.28 (m, 2H), 6.97-6.76 (m, 1H), 6.76-6.59 (m, 1H), 6.16 (d, J = 8.50 Hz, 1H), 5.41-5.26 (m, 1H), 5.27-5.10 (m, 1H), 4.65-3.94 (m, 10H), 3.07-2.70 (m, 1H), 2.58-2.28 (m, 1H), 2.11 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.41-1.24 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.6, 170.1, 164.1, 163.6, 155.0, 153.6, 143.6, 141.2,

134.4, 127.7, 127.0, 126.2, 124.9, 124.9, 120.0, 68.2, 67.7, 67.6, 67.1, 66.9, 62.0, 61.4, 48.7, 46.9, 23.0, 22.9, 20.6, 20.6, 14.1. **EI-MS *m/z*** calcd for $C_{35}H_{40}N_2O_{12}$: 680.26. Found: $[M]^+$ 681.26.

(2R,3R,4R,5S,6R)-6-((S)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-ethoxy-4-oxobutyl)-5-acetamido-2-(acetoxymethyl)tetrahydro-2H-pyran-3,4-diyl diacetate 326



326

[(COD)Rh-((R,R)-Et-DuPHOS)]+OTf $^-$ catalyst precursor (10.0 mg, 0.01 mmol) and (E,Z)-**325** (75.0 mg, 0.11 mmol) were dissolved in deoxygenated anhydrous THF (10 mL) in a 100 mL reactor. The reactor was vacuumed and refilled with H_2 three times, and finally was pressurized with 9 atm of H_2 and stirred at 40°C for 48 h. The reactor was then depressurized, and the solvent was removed in vacuo. The crude product was dissolved in hexanes/ethyl acetate (1:1) and passed through a silica gel column to produce (*L*)-**326**, the title compound as a colourless liquid (68.0 mg, 90% yield). On replication, yields varied between 84% and 90% for scales between 75.0 mg and 1.20 g. Rf = 0.31. 1:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.77 (d, J = 7.38 Hz, 2H), 7.69-7.52 (m, 2H), 7.41 (t, J = 7.29, 7.29 Hz, 2H), 7.32 (t, J = 7.27, 7.27 Hz, 2H), 6.91-6.37 (m, 1H), 5.77 (dd, J = 27.00, 8.45 Hz, 1H), 5.50 (dd, J = 16.42, 8.08 Hz, 1H), 5.44-5.25 (m, 1H), 4.79-3.47 (m, 13H), 2.96-2.19 (m, 1H), 2.11 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.32-1.25 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 172.1, 170.8, 170.7, 170.6, 170.5, 170.3, 170.2, 170.1, 169.8, 169.8, 169.7, 169.6, 169.5, 169.3, 164.1, 156.0, 155.0, 155.0, 143.8, 143.7, 143.6, 143.5, 141.3, 134.6, 127.8, 127.1, 125.0, 120.0, 68.9, 68.2, 68.1, 67.7, 67.1, 67.0, 66.9, 66.8, 61.9, 61.7, 61.3, 53.8, 48.9, 47.1, 29.7, 28.8, 27.9, 27.9, 23.2, 20.9, 20.7, 14.2. **EI-MS *m/z*** calcd for $C_{35}H_{42}N_2O_{12}$: 682.27. Found: $[M]^+$ 683.28.

Reference

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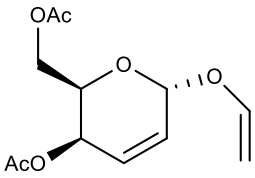
9.7 Experimental protocols for Chapter 4

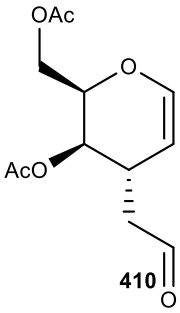
((2R,3R,6S)-3-acetoxy-6-(vinyloxy)-3,6-dihydro-2H-pyran-2-yl)methyl acetate 409



409

The crude selenoxide **415a** (190 mg, 0.48 mmol) was dissolved in benzene (5 mL), diisopropylamine (0.4 mL, 2.41 mmol), was added, and the mixture was heated at reflux for 1 h. The reaction mixture was concentrated under reduced pressure, and the crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **409**, the title compounds, as colourless liquid (50.0 mg, 41% yield). On replication, yields varied between 35% and 41% for scales between 50.0 mg and 10.0 g. Rf = 0.28. 8:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 6.45 (dd, *J* = 14.00, 6.49 Hz, 1H), 6.18 (ddd, J = 10.00, 5.47, 0.82 Hz, 1H), 6.05 (dd, *J* = 10.01, 3.03 Hz, 1H), 5.36 (d, *J* = 2.87 Hz, 1H), 5.04 (dd, *J* = 5.47, 2.44 Hz, 1H), 4.55 (dd, *J* = 14.00, 1.65 Hz, 1H), 4.37-4.26 (m, 1H), 4.23-4.18 (m, 3H), 2.06 (s, 3H), 2.03 (s, 3H).[1]

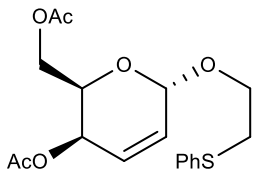
((2R,3R,4S)-3-acetoxy-4-(2-oxoethyl)-3,4-dihydro-2H-pyran-2-yl)methyl acetate 410



410

Compound **409** (50.0 mg, 0.19 mmol) in nitrobenzene (3 mL) was treated with *N*,*N*-dimethylaniline (2 mL), and the mixture was heated at 160°C for 3 h, then crude was subjected to column chromatography using hexanes/ethyl acetate (9:1) to remove nitro benzene then (1:1) to obtain **410**, the title compounds as colourless liquid (27.0 mg, 54% yield). On replication, yields varied between 50% and 54% for scales between 50.0 mg and 10.0 g. Rf = 0.25. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 9.88 (t, J = 1.42, 1.42 Hz, 1H), 6.51 (dd, J = 6.18, 1.52 Hz, 1H), 4.99-4.89 (m, 1H), 4.89-4.82 (m, 1H), 4.31 (d, J = 5.84 Hz, 2H), 4.21-4.14 (m, 1H), 2.91-2.76 (m, 1H), 2.76-2.52 (m, 2H), 2.19 (s, 3H), 2.19-2.18 (m, 3H).[1]

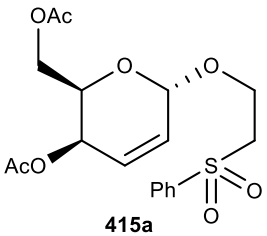
((2R,3R,6S)-3-acetoxy-6-(2-(phenylthio)ethoxy)-3,6-dihydro-2H-pyran-2-yl)methyl acetate 415



415

The solution of acetylated galactal **119** (375 mg, 1.38 mmol) and compound **414** (277 mg, 1.38 mmol) in benzene (5 mL) at 0°C was treated with BF_3**.**OEt_2 (0.05 mL, 0.4 mmol), then stirred for 30 min and quenched with Et_3N (1 mL). The reaction mixture concentrated under reduced pressure, then dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **415**, the title compound, as a colourless liquid (200 mg, 40% yield). On replication, yields varied between 36% and 40% for scales between 30.0 mg and 15.0 g. Rf = 0.28. 9:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.61-7.38 (m, 2H), 7.27-7.23 (m, 3H), 6.19-6.08 (m, 1H), 6.01 (dd, *J* = 9.81, 3.05 Hz, 1H), 5.10 (d, *J* = 2.95 Hz, 1H), 5.02 (dd, *J* = 5.47, 2.60 Hz, 1H), 4.42-4.30 (m, 1H), 4.21 (dd, *J* = 6.33, 3.00 Hz, 2H), 4.07-3.93 (m, 1H), 3.89-3.77 (m, 1H), 3.26-3.01 (m, 2H), 2.09 (s, 3H), 2.03 (s, 3H).[1]

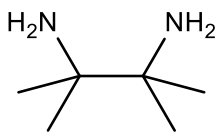
((2R,3R,6S)-3-acetoxy-6-(2-(phenylsulfonyl)ethoxy)-3,6-dihydro-2H-pyran-2-yl)methyl acetate 415a



415a

Compound **415** (200 mg, 0.48 mmol) in methanol (6 mL) and water (1 mL) was treated with sodium periodate (155 mg, 0.78 mmol) and $NaHCO_3$ (44.0 mg, 0.53 mmol). The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The crude was dissolved in ethyl acetate (20 mL) and the mixture was washed with water (10 mL), and the organic phase was concentrated under reduced pressure and used without purification for next step.[1]

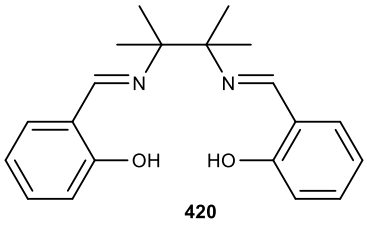
2,3-dimethylbutane-2,3-diamine 418



418

2-nitropropane (2.00 g, 22.5 mmol) and KOH (1.25 g, 22.5 mmol) in methanol (7 mL) was treated with a solution of iodine (2.82 g, 22.5 mmol) in methanol (20 mL) and heated to reflux. The solution was cooled and concentrated under reduced pressure and used without purification. The crude compound (2.20 g, 12.5 mmol) was dissolved in concentrated HCl (19 mL) and Sn (2.37 g, 79.0 mmol) was added and heated at 80°C for 1h. The reaction mixture was then cooled to room temperature and washed with diethyl ether (100 mL). Sodium hydroxide (7.90 g) was added to the aqueous phase and passed through a pad of Celite, then extracted with CH_2Cl_2 (2 x 100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to produce **418**, the title compound as a colourless liquid (470 mg, 33% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 1.31 (s, 4H), 1.12 (s, 12H). Consistent with the literature.[2]

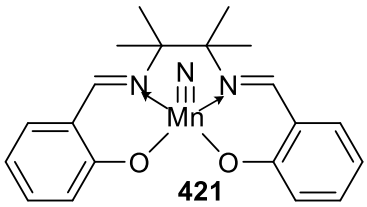
2,2'-((1E,1'E)-((2,3-dimethylbutane-2,3-diyl)bis(azanylylidene))bis(methanylylidene))di-phenol 420



420

To a solution of salicylaldehyde **419** (700 mg, 5.73 mmol) in ethanol (10 mL), diamine **418** (320 mg, 2.78 mmol) was added and heated to reflux for 15 h, then water (2.5 mL)was added and heated to reflux for 10 min. The reaction mixture was cooled to –20°C and kept for 16 h to which the solid was filtered and dried to produce **420**, the title compound as a yellow solid (525 mg, 58% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 14.09 (s, 2H), 8.38 (s, 2H), 7.34-7.22 (m, 4H), 6.95 (dd, J = 8.25, 0.45 Hz, 2H), 6.87 (dt, J = 7.67, 7.65, 1.07 Hz, 2H), 1.40 (s, 12H). Consistent with the literature.[3]

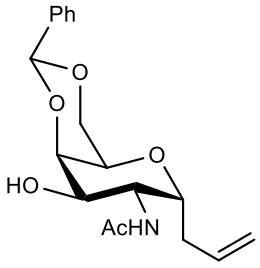
(saltmen)Mn(N) 421



421

Compound **420** (420 mg, 1.30 mmol) in methanol (5 mL), heated to 60°C for 10 min then the $Mn(OAc)_2 .4H_2O$ (335 mg, 1.37 mmol) was added and refluxed for 1 h. The reaction mixture was cooled to room temperature and NH_4OH (1.5 mL, 19.5 mmol) was added followed by addition of Clorox bleach (NaOC1, 12 mL, 7.8 mmol) over 40 min. The reaction mixture was cooled to room temperature and diluted with CH_2Cl_2 (100 mL). The combined organics were washed with water (4 x 50 mL) and the organic phase was dried, filtered, and concentrated under reduced pressure. The crude was dissolved in CH_2Cl_2 (200 mL) passed through a pad of Celite. The organic phase was dried, filtered, and concentrated under reduced pressure to produce **421**, the title compound as a green solid (460 mg, 90% total yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.08 (s, 2H), 7.38 (ddd, *J* = 8.64, 6.93, 1.81 Hz, 2H), 7.22 (dd, *J* = 7.86, 1.70 Hz, 2H), 7.16 (d, *J* = 8.39 Hz, 2H), 6.74-6.65 (m, 2H), 5.31 (s, 2H), 1.49 (s, 12H). Consistent with the literature.[4]

***N*-((2S,4aR,6R,7R,8R,8aR)-6-allyl-8-hydroxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)acetamide 427**

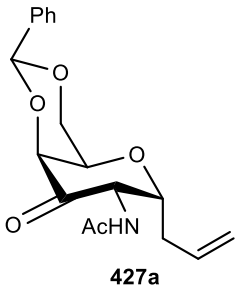


427

Compound **188** (60.0 mg, 0.24 mmol) was dissolved in acetonitrile (4 mL), then benzaldehyde dimethyl acetal (0.05 mL, 0.30 mmol) and *p*-TsOH (9.00 mg, 0.05 mmol) were added and stirred for 3 h at 50°C temperature. The saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixture and extracted with Et_2O (4 x 10 mL). The organic phase was washed with water (20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:3) and passed through a silica gel column to produce **427**, the title compound, as a white solid (36.0 mg, 45% yield). On replication, yields varied between 40%

and 45% for scales between 40.0 mg and 500 mg. Rf = 0.29. 1:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.52 (dd, J = 6.55, 3.07 Hz, 2H), 7.44-7.34 (m, 3H), 5.96-5.72 (m, 1H), 5.60-5.57 (m, 1H), 5.56 (s, 1H), 5.20-5.04 (m, 2H), 4.61 (td, J = 10.47, 5.37, 5.37 Hz, 1H), 4.51-4.37 (m, 1H), 4.24 (dd, J = 12.01, 1.79 Hz, 2H), 4.04 (dd, J = 12.53, 1.66 Hz, 1H), 3.80 (dt, J = 11.15, 11.11, 3.59 Hz, 1H), 3.55 (s, 1H), 2.55 (d, J = 11.25 Hz, 1H), 2.47-2.26 (m, 2H), 2.01 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 171.23, 134.58, 129.10, 128.56, 127.09, 126.71, 117.45, 101.73, 96.83, 75.57, 73.66, 72.92, 70.12, 68.96, 68.00, 64.55, 64.02, 63.36, 51.57, 23.69. **EI-MS *m/z*** calcd for $C_{18}H_{23}NO_5$: 333.16. Found: $[M]^+$ 334.16.

***N*-((2S,4aR,6R,7S,8aS)-6-allyl-8-oxo-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)acetamide 427a**

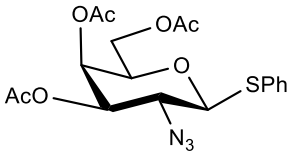


427a

Compound **427** (50.0 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (3 mL) and Dess–Martin periodinane (95.3 mg, 0.22 mmol) was added and stirred at room temperature for 4 h. The mixture was diluted with CH_2Cl_2 (20 mL) and the mixture was washed with sat. $Na_2S_2O_3$ (10 mL), sat. $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:2) and passed through a silica gel column to produce **427a**, the title compound, as a white solid 40.0 mg, 80% yield). On replication, yields varied between 72% and 80% for scales between 50.0 mg and 200 mg. Rf = 0.31. 1:1 hexanes/EtOAc. ^{1}H NMR (75 MHz, $CDCl_3$) δ_{ppm} 7.58-7.43 (m, 2H), 7.43-7.32 (m, 3H), 6.39-6.20 (m, 1H), 5.87-5.62 (m, 1H), 5.59 (s, 1H), 5.15-4.93 (m, 3H), 4.51 (d, J = 1.28 Hz, 1H), 4.34 (dd, J = 12.74, 1.41 Hz, 1H), 4.10 (dd, J = 12.76, 1.74 Hz, 1H), 3.72 (d, J = 1.41 Hz, 1H), 2.29-2.12 (m, 1H), 2.12-2.02 (m, 1H), 2.01 (s, 3H).^{13}C NMR (75 MHz,

$CDCl_3$) δ_{ppm} 200.19, 169.88, 137.00, 133.14, 129.57, 128.47, 126.41, 117.70, 100.99, 81.08, 69.34, 65.80, 58.14, 30.22, 29.83, 23.18. **EI-MS *m/z*** calcd for $C_{18}H_{21}NO_5$: 331.14. Found: $[M]^+$ 332.15.

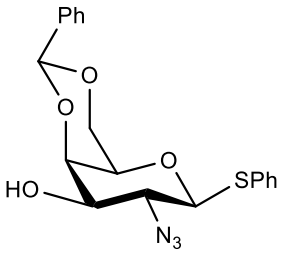
(2R,3R,4R,5R,6S)-2-(acetoxymethyl)-5-azido-6-(phenylthio)tetrahydro-2H-pyran-3,4-diyl diacetate 429



429

To a stirring solution of 3,4,6-Tri-O-acetyl- D-galactal **119** (30.0 g, 110 mmol) in acetonitrile (430 mL), ferric chloride hexahydrate (24.7 g, 91.5 mmol), sodium azide (8.10 g, 121 mmol) (portionwise) and hydrogen peroxide (20 mL, 121 mmol 33% aq. solution) were added subsequently and the solution was stirred at −20 °C for 5 h until the starting material was consumed. The mixture was diluted with diethyl ether (500 mL) and washed with water (4 x 250 mL), saturated aqueous $NaHCO_3$ (250 mL), and brine (250 mL) until the organic phase was colorless. The crude product was used without further purification. The crude mixture was dissolved in ethyl acetate (830 mL) to which 1M Na_2CO_3 (830 mL) was added, followed by addition of both Bu_4NHSO_4 (46.0 g, 138 mmol) and thiophenol (40 mL, 392 mmol) and stirred for 16 h. The two phases were separated, and the organic phase was washed with water (4 x 400 mL), sat. $NaHCO_3$ (400 mL), and brine (400 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and taken up through a silica gel column to produce **429**, the title compound, as a colourless liquid (17.0 g, 37% yield in two steps). On replication, yields varied between 32% and 37% for scales between 100 mg and 30.0 g. Rf = 0.28. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.75-7.54 (m, 2H), 7.42-7.31 (m, 3H), 5.39-5.34 (m, 1H), 4.87 (dd, *J* = 10.25, 3.18 Hz, 1H), 4.53 (d, *J* = 10.09 Hz, 1H), 4.13 (dd, *J* = 6.71, 2.10 Hz, 2H), 3.93-3.86 (m, 1H), 3.66 (t, *J* = 10.19, 10.19 Hz, 1H), 2.10 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H).[5]

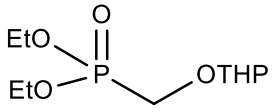
(4aR,6S,7R,8R,8aR)-7-azido-2-phenyl-6-(phenylthio)hexahydropyrano[3,2-d][1,3]dioxin-8-ol 430



430

Compound **429** (280 mg, 0.66 mmol) was dissolved in methanol (45 mL) and NaOMe (10 mL) was added until a pH of 11 was reached. Upon stirring for 16 h, Dowex 50WX8 was added to neutralize the solution. The mixture was filtered and concentrated under reduced pressure to afford the crude product which was used in next step without purification. The crude product (150 mg, 0.50 mmol) was then dissolved in acetonitrile (8 mL), to which benzaldehyde dimethyl acetal (0.13 mL, 0.85 mmol) and *p*-TsOH (14.0 mg, 0.08 mmol) were added and stirred for 3 h at room temperature. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **430**, the colorless liquid compound (150 mg, 78% yield). On replication, yields varied between 70% and 78% for scales between 100 mg and 10.0 g. Rf = 0.34. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.96-7.58 (m, 2H), 7.55-7.30 (m, 8H), 5.55 (s, 1H), 4.49-4.36 (m, 2H), 4.26-4.17 (m, 1H), 4.05 (dd, *J* = 12.52, 1.68 Hz, 1H), 3.73-3.61 (m, 1H), 3.61-3.49 (m, 2H), 2.74-2.19 (m, 1H). Consistent with the literature.[6]

diethyl (((tetrahydro-2H-pyran-2-yl)oxy)methyl)phosphonate 432

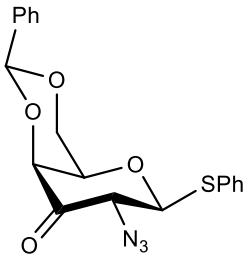


432

Diethyl hydroxymethylphosphonate **431** (5.00 g, 29.7 mmol) was dissolved in diethyl ether (20 mL) and treated with dihydropyran (4.1 mL, 44.6 mmol) and cooled to 0°C. Phosphorus oxychloride (4.1 mL, 44.6 mmol) was then added and stirred for 3 h at room temperature. After the reaction mixture was diluted with diethyl ether (50 mL), the combined organics were washed with saturated aqueous $NaHCO_3$ (30 mL) water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate

(7:3) and taken up through a silica gel column to produce **432**, the title compound as a light-yellow liquid (3.60 g, 48% yield). Rf = 0.30. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 4.70 (t, J = 3.04, 3.04 Hz, 1H), 4.29-4.10 (m, 4H), 4.02 (dd, J = 13.90, 9.17 Hz, 1H), 3.89-3.67 (m, 2H), 3.61-3.48 (m, 1H), 1.94-1.43 (m, 6H), 1.35 (dt, J = 7.07, 7.06, 1.59 Hz, 6H)s. Consistent with the literature.[7]

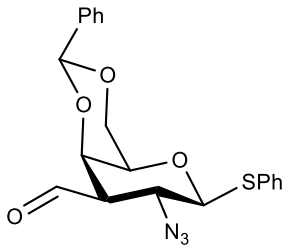
(4aR,6S,7R,8aS)-7-azido-2-phenyl-6-(phenylthio)tetrahydropyrano[3,2-d][1,3]dioxin-8(8aH)-one 433



433

To a stirring solution of compound **430** (50.0 mg, 0.13 mmol) in CH_2Cl_2 (3 mL) was added Dess–Martin periodinane (100 mg, 0.26 mmol) and the solution was stirred at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with sat. $Na_2S_2O_3$ (10 mL), sat. $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and taken up through a silica gel column to produce **433**, the title compound, as a colourless liquid (41.0 mg, 84% yield in two steps). On replication, yields varied between 78% and 84% for scales between 50.0 mg and 11.0 g. Rf = 0.29. 1:1 hexanes/EtOAc.^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.89-7.80 (m, 2H), 7.48-7.41 (m, 8H), 5.63 (s, 1H), 4.74 (d, *J* = 10.17 Hz, 1H), 4.59-4.54 (m, 2H), 4.49 (d, J = 10.17 Hz, 1H), 4.17 (dd, *J* = 12.66, 1.50 Hz, 1H), 3.74 (d, *J* = 1.24 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 196.9, 136.9, 139.2, 129.8, 129.3, 129.3, 129.1, 128.5, 126.5, 101.0, 87.0, 80.7, 71.2, 68.8, 65.2. **EI-MS *m/z*** calcd for $C_{19}H_{17}N_3O_4S$: 383.09. Found: $[M+K]^+$ 424.09 note: One of the carbon oxygen bond in the benzylidene protecting group is cleaved.

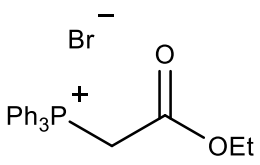
(2S,4aR,6S,7R,8R,8aR)-7-azido-2-phenyl-6-(phenylthio)hexahydropyrano[3,2-d][1,3]dioxine-8-carbaldehyde 436



436

To the compound **449** (83.0 mg, 0.18 mmol) was added methanol (64 μL) then excess amount of freshly prepared 0.08 M SmL_2 (24 mL, 1.92 mmol) was added and stirred for 6 h at room temperature. The reaction mixture was diluted with ethyl acetate (100 mL) and the mixture was washed with saturated aqueous $NaHCO_3$ (70 mL), water (70 mL) and brine (70 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **436**, the title compound, as a colourless liquid (65.0 mg, 91% yield). Note: yield is sensitive regarding large scale synthesis, and it will be lower). On replication, yields varied between 85% and 91% for scales between 15.0 mg and 85.0 mg. Rf = 0.27. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 9.75 (d, *J* = 1.76 Hz, 1H), 7.82-7.67 (m, 2H), 7.52-7.29 (m, 8H), 5.55 (s, 1H), 4.86 (d, *J* = 10.32 Hz, 1H), 4.42 (dd, *J* = 12.58, 1.57 Hz, 1H), 4.08-3.98 (m, 2H), 3.92 (d, *J* = 1.04 Hz, 1H), 3.61 (dd, *J* = 10.31, 1.71 Hz, 1H), 3.40 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 202.4, 137.0, 134.7, 129.8, 129.7, 129.1, 128.7, 128.4, 126.5, 101.9, 82.8, 78.0, 73.9, 69.1, 68.5, 65.0. **EI-MS *m/z*** calcd for $C_{20}H_{19}N_3O_4S$: 397.11. Found: $[M+NH_4]^+$ 309.07-benzylidine protecting group.

(2-ethoxy-2-oxoethyl)triphenylphosphonium bromide 441

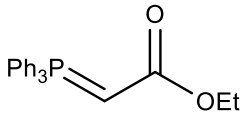


441

Triphenylphosphine (39.0 g, 149 mmol) was dissolved in toluene and cooled to 0°C. Ethyl bromoacetate (16.6 mL, 65.8 mmol) was added and the mixture was stirred for 18 h. Upon which it the mixture was allowed to stand and settle for 2 h to which the product was filtered and dried to produce compound **441**, the

title compound as a white solid (25.1 mg, 89% yield). On replication, yields varied between 75% and 89% for scales between 1.00 g and 39.0 g. Rf = 0.32. 1:9 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.01-7.83 (m, 6H), 7.84-7.74 (m, 3H), 7.73-7.63 (m, 6H), 5.55 (d, *J* = 13.75 Hz, 2H), 4.04 (q, *J* = 7.13, 7.13, 7.12 Hz, 2H), 1.07 (t, *J* = 7.14, 7.14 Hz, 3H). Consistent with the literature.[8]

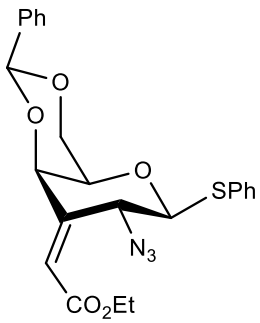
ethyl 2-(triphenylphosphoranylidene)acetate 442



442

Sodium hydroxide (868 mg) was dissolved in H_2O (36 mL) and added to a solution of salt **441** (62 g, 145 mmol) in H_2O (435 mL) and CH_2Cl_2 (435 mL) and stirred for 1 h. The organic phase was separated from aqueous phase and washed with water (300 mL) and brine (300 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to produce **442**, the title compound as a white solid (46.5 g, 93% yield). On replication, yields varied between 87% and 93% for scales between 800 mg and 8.00 g. Rf = 0.32. 4:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.10-7.50 (m, 15H), 5.61 (bs, 1H), 4.00 (q, *J* = 7.12, 7.12, 7.10 Hz, 2H), 1.04 (t, *J* = 7.10, 7.10 Hz, 3H). Consistent with the literature.[8]

Ethyl 2-((4aR,6S,7R,8aR)-7-azido-2-phenyl-6-(phenylthio)tetrahydropyrano[3,2-d][1,3]dioxin-8(8aH)-ylidene)acetate 443

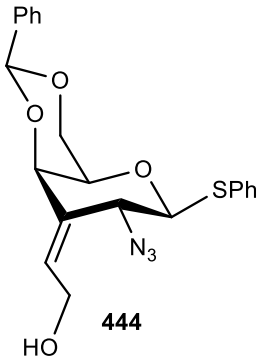


443

The ylide **442** (16.8 g, 48.2 mmol) and ketone **433** (7.70 g, 20.1 mmol) were dissolved in toluene (700 mL) and heated for 1 h, then the reaction mixture was concentrated under reduced pressure and diluted with CH_2Cl_2 (400 mL). The organic phase was washed with water (200 mL) and brine (200 mL) and the combined organics were dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in

hexanes/ethyl acetate (7:3) and taken up through a silica gel column to produce **443**, the title compound as a light-yellow liquid (6.10 g, 67% yield). On replication, yields varied between 51% and 67% for scales between 100 mg and 8.00 g. Rf = 0.28. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.84-7.77 (m, 2H), 7.43 (dd, *J* = 6.80, 3.00 Hz, 2H), 7.40-7.34 (m, 3H), 7.34-7.28 (m, 1H), 7.28-7.21 (m, 2H), 6.27 (d, *J* = 1.43 Hz, 1H), 6.11 (d, *J* = 1.09 Hz, 1H), 5.66 (s, 1H), 4.53-4.46 (m, 2H), 4.41 (dd, *J* = 12.44, 1.59 Hz, 1H), 4.19 (dq, *J* = 7.12, 7.09, 7.09, 0.92 Hz, 2H), 4.14 (dd, *J* = 12.46, 1.60 Hz, 1H), 3.53 (d, *J* = 1.34 Hz, 1H), 1.30 (t, *J* = 7.13, 7.13 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 165.4, 149.1, 137.7, 134.3, 129.2, 128.4, 126.5, 118.7, 101.3, 88.7, 71.8, 70.7, 69.4, 60.9, 60.4, 14.2. **EI-MS *m/z*** calcd for $C_{23}H_{23}N_3O_5S$: 453.14. Found: $[M+ Na]^+$ 476.12.

2-((4aR,6S,7R,8aR)-7-azido-2-phenyl-6-(phenylthio)tetrahydropyrano[3,2-d][1,3]dioxin-8(8aH)-ylidene)ethanol 444

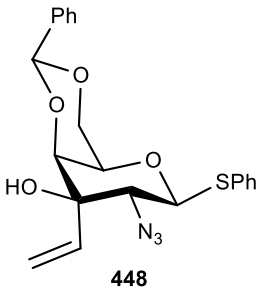


444

To a solution of compound **443** (2.70 g, 5.96 mmol) in CH_2Cl_2 (55 mL) at 0°C was added (1M) DIBAL-H (12.0 mL, 12.0 mmol) and the mixture was stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and 3M HCl (40 mL) was added. The organic phase was washed with water (40 mL) and brine (40 mL) and the combined organics were dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and taken up through a silica gel column to produce **444**, the title compound as a light-yellow liquid (1.82 g, 74% yield). On replication, yields varied between 68% and 74% for scales between 100 mg and 3.00 g. Rf = 0.28. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.62 (dd, *J* = 8.17, 1.10 Hz, 2H), 7.49-7.44 (m, 2H), 7.44-7.39 (m, 3H), 7.19-7.06 (m, 1H), 6.95 (t, *J* = 7.76, 7.76 Hz, 2H), 5.99 (d, *J* = 1.29 Hz, 1H), 5.68-5.64 (m, 1H), 5.63 (s, 1H), 4.50 (dd, *J* = 12.74, 1.11 Hz, 1H), 4.35 (t, *J* = 1.43, 1.43 Hz, 1H), 4.20 (dd, *J* = 12.75, 2.09 Hz,

1H), 3.98 (t, *J* = 6.88, 6.88 Hz, 1H), 3.58-3.52 (m, 1H), 3.38 (t, *J* = 6.47, 6.47 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 137.6, 134.6, 134.5, 133.1, 130.5, 129.3, 128.5, 128.4, 128.4, 126.0, 100.7, 80.7, 70.0, 69.1, 67.3, 66.7, 63.0. **EI-MS *m/z*** calcd for $C_{21}H_{21}N_3O_4S$: 411.13. Found: $[M+ NH_4]^+$ 429.16.

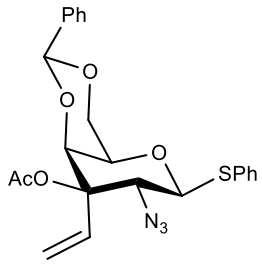
(2S,4aR,6S,7R,8R,8aS)-7-azido-2-phenyl-6-(phenylthio)-8-vinylhexahydropyrano[3,2-d][1,3]dioxin-8-ol 448



448

To a solution of ketone **433** (6.70 g, 17.5 mmol) in THF (160 mL) at −78 °C was added a vinylmagnesium bromide solution 1.0 M in THF (21 mL, 21.0 mmol). The reaction mixture was stirred for 2 h and then allowed to warm to 0°C. The reaction was then quenched with sat. NH_4Cl (100 mL) and extracted four times with ethyl acetate (70 mL), and then organic phase was washed once with brine (150 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **448**, the title compound, as a colourless liquid (4.20 g, 58% yield). On replication, yields varied between 52% and 58% for scales between 40.0 mg and 7.00 g. Rf = 0.27. 9:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.88-7.75 (m, 3H), 7.58-7.48 (m, 2H), 7.45-7.25 (m, 5H), 6.10 (dd, *J* = 16.95, 10.90 Hz, 1H), 5.85 (dd, *J* = 16.95, 1.29 Hz, 1H), 5.63-5.51 (m, 2H), 4.70 (d, *J* = 10.35 Hz, 1H), 4.45 (dd, *J* = 12.47, 1.61 Hz, 1H), 4.03 (dd, *J* = 12.48, 1.61 Hz, 1H), 3.88 (d, *J* = 0.54 Hz, 1H), 3.75 (d, *J* = 1.01 Hz, 1H), 3.70 (d, *J* = 10.35 Hz, 1H), 3.15 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 137.3, 134.7, 132.4, 130.2, 129.7, 129.1, 128.6, 128.4, 126.6, 120.6, 101.6, 83.9, 79.1, 75.5, 69.4, 68.5, 64.6. **EI-MS *m/z*** calcd for $C_{21}H_{21}N_3O_4S$: 411.12. Found: $[M+Na]^+$ 434.11.

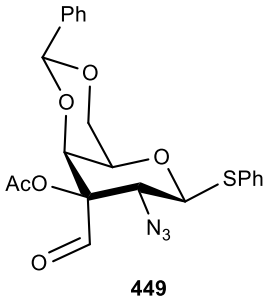
(2S,4aR,6S,7R,8R,8aS)-7-azido-2-phenyl-6-(phenylthio)-8-vinylhexahydropyrano[3,2-d][1,3]dioxin-8-yl acetate 448a



448a

To a solution of compound **448** (3.50 g, 8.51 mmol) in pyridine (36 mL), acetic anhydride (18 mL, 190 mmol) was added and stirred at 115°C for 16 h. The reaction mixture was cooled to room temperature and diluted with CH_2Cl_2 (150 mL), washed with 10% HCl (2 x 70 mL), water (70 mL) and brine (150 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **448a**, the title compound, as a colourless liquid (3.40 g, 88% yield). On replication, yields varied between 78% and 88% for scales between 240 mg and 3.50 g. Rf = 0.27. 9:1 hexanes/EtOAc. 1H NMR ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.64-7.52 (m, 2H), 7.31-7.22 (m, 5H), 7.21-7.08 (m, 1H), 5.88 (dd, *J* = 17.42, 11.55 Hz, 1H), 5.53-5.50 (m, 1H), 5.49 (d, *J* = 7.61 Hz, 1H), 5.39 (s, 1H), 4.96-4.95 (m, 1H), 4.41 (d, *J* = 10.52 Hz, 1H), 4.26 (dd, *J* = 12.43, 1.65 Hz, 1H), 3.91 (dd, *J* = 12.45, 1.69 Hz, 1H), 3.82 (d, *J* = 10.51 Hz, 1H), 3.63-3.58 (m, 1H), 1.85 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 169.0, 137.7, 134.2, 130.2, 129.0, 128.9, 128.4, 128.1, 126.1, 122.5, 100.8, 83.4, 82.4, 73.9, 69.4, 68.7, 62.6, 21.7. **EI-MS *m/z*** calcd for $C_{23}H_{23}N_3O_5S$: 453.14. Found: $[M+NH_4]^+$ 471.14.

(2S,4aR,6S,7R,8R,8aS)-7-azido-8-formyl-2-phenyl-6-(phenylthio)hexahydropyrano[3,2-d][1,3]dioxin-8-yl acetate 449

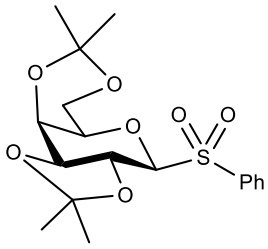


449

To a solution of compound **448a** (50.0 mg, 0.11 mmol) in CH_2Cl_2 (10 mL), was bubbled ozone through the solution. Upon a notable colour change to a stable deep blue colour, dimethylsulfide (0.02 mL, 0.29 mmol) was added to which the solution was left to warm up to room temperature. After 2 h, CH_2Cl_2 was removed and water (10 mL) was added and extracted with ethyl acetate (3 x 10 mL). The organic phase was then washed with brine (20 mL). The organic

phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **449**, the title compound, as a colourless liquid (30.0 mg, 60% yield). On replication, yields varied between 55% and 60% for scales between 50.0 mg and 1.20 g. Rf = 0.31. 9:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 9.62 (d, *J* = 1.17 Hz, 1H), 7.82-7.59 (m, 2H), 7.39 (s, 5H), 7.36-7.24 (m, 3H), 5.50 (s, 1H), 4.71 (dd, *J* = 5.74, 4.69 Hz, 2H), 4.39 (dd, *J* = 12.60, 1.58 Hz, 1H), 4.26-4.19 (m, 1H), 4.06 (dd, *J* = 12.61, 1.67 Hz, 1H), 4.01 (dd, *J* = 10.43, 0.97 Hz, 1H), 2.15 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 198.5, 170.8, 137.6, 134.1, 130.0, 129.2, 129.1, 128.6, 128.3, 126.1, 100.9, 83.1, 72.0, 69.8, 61.7, 20.7. **EI-MS *m/z*** calcd for $C_{22}H_{21}N_3O_6S$: 454.11. Found: $[M+NH_4]^+$ 473.15.

(3aR,4S,9bS)-2,2,8,8-tetramethyl-4-(phenylsulfonyl)hexahydro-[1,3]dioxolo[4',5':4,5]pyrano[3,2-d][1,3]dioxine 450

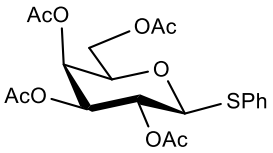


450

To the compound **455** (200 mg, 0.57 mmol) in acetonitrile (4.7 mL) and water (0.9 mL), $KMnO_4$ (360 mg, 2.30 mmol) and $CuSO_4.5H_2O$ (122 mg, 1.50 mmol) were added and stirred for 2 h at room temperature. Then solvent was removed and CH_2Cl_2 (50 mL) was added. The organic phase was washed water (30 mL) and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (6:4) and passed through a silica gel column to produce **450**, the title compound, as a colourless liquid (180 mg, 82% yield). Rf = 0.29. 6:4 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 8.02 (d, *J* = 7.35 Hz, 2H), 7.68 (t, *J* = 7.46, 7.46 Hz, 1H), 7.57 (t, *J* = 7.77, 7.77 Hz, 2H), 4.55 (d, *J* = 8.98 Hz, 1H), 4.36 (s, 1H), 3.98 (dq, *J* = 13.14, 13.14, 13.12, 1.61 Hz, 2H), 3.66-

3.56 (m, 2H), 3.43 (s, 1H), 1.47 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H), 1.09 (s, 3H). Consistent with the literature.[9]

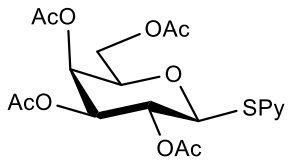
(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl tri-acetate 454a



454a

To a solution of compound **196** (30.0 g, 77.0 mmol) in CH_2Cl_2 (600 mL) at 0°C, thiophenol (20 mL, 0.20 mol) and BF_3**.**OEt_2 (25 mL, 0.20 mol) were added, and stirred for 12 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the mixture was washed with 2M NaOH (3 x 200 mL), water (400 mL) and brine (400 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **454a**, the title compound, as a colourless liquid (30.0 g, 90% yield). On replication, yields varied between 76% and 90% for scales between 100 mg and 30.0 g. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.71-7.38 (m, 2H), 7.39-7.27 (m, 3H), 5.42 (dd, *J* = 3.29, 0.85 Hz, 1H), 5.25 (t, *J* = 9.96, 9.96 Hz, 1H), 5.05 (dd, *J* = 9.95, 3.34 Hz, 1H), 4.72 (d, *J* = 9.96 Hz, 1H), 4.16 (ddd, *J* = 17.49, 11.34, 6.69 Hz, 2H), 4.02-3.87 (m, 1H), 3.49 (s, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H). Consistent with the literature.[10]

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(pyridin-2-ylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 454b

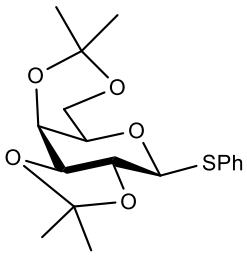


454b

Peracetylated galactose compound **196** (2.00 g, 5.13 mmol) was dissolved in CH_2Cl_2 (40 mL) and treated with 2-mercaptopyridine (1.14 g, 10.2 mmol) and $ZrCl_4$ (2.40 mg, 10.2 mmol) and heated to 60°C for 16 h. The reaction mixture was cooled to room temperature and diluted with CH_2Cl_2 (150 mL). The reaction mixture was washed with sat $NaHCO_3$ (100 mL). and the combined organics were dried,

filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **454b**, the title compound, as a colourless liquid (1.75 g, 77% yield). Rf = 0.32. 8:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.55-8.35 (m, 1H), 7.53 (dt, J = 7.92, 7.89, 1.85 Hz, 1H), 7.26-7.16 (m, 1H), 7.07 (ddd, J = 7.34, 4.90, 0.94 Hz, 1H), 5.79 (d, J = 10.39 Hz, 1H), 5.52-5.43 (m, 1H), 5.41 (t, J = 10.16, 10.16 Hz, 1H), 5.18 (dd, J = 9.92, 3.41 Hz, 1H), 4.23-4.00 (m, 4H), 2.16 (s, 3H), 2.03 (3s, 9H).[11]

(3aR,4S,9bS)-2,2,8,8-tetramethyl-4-(phenylthio)hexahydro-[1,3]dioxolo[4',5':4,5]pyrano[3,2-d][1,3]dioxine 455

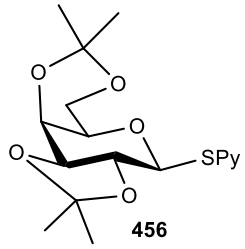


455

Compound **454a** (165 mg, 0.37 mmol) was dissolved in methanol (50 mL) then NaOMe solution was added till pH 12. The reaction was stirred at room temperature for 1 h to produce a faint yellow colour solution. DOWEX 50W X8 ion exchange resin was added to the reaction mixture until pH 7 was reached. The solution was filtered and concentrated under reduced pressure, then the crude was dissolved in DMF (5 mL), then 2-methoxy propane (0.16 mL, 1.92 mmol), and *p*-TsOH (6.00 mg, 0.03 mmol) were added and the reaction mixture was stirred for 1 h at room temperature. Saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixture and extracted with Et_2O (4 x 10 mL). The organic phase was washed with water (20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **455**, the title compound, as a colourless liquid (58.5 mg, 48% yield). On replication, yields varied between 35% and 48% for scales between 200 mg and 10.0 g. Rf = 0.31. 7:3 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.65 (dd, J = 6.62, 2.96 Hz, 2H), 7.47-7.14 (m, 3H), 4.80 (d, J = 9.56 Hz, 1H), 4.44 (d, J =

1.38 Hz, 1H), 4.08 (dq, J = 12.97, 12.97, 12.95, 1.76 Hz, 2H), 3.86 (t, J = 9.39, 9.39 Hz, 1H), 3.57 (dd, J = 9.21, 2.74 Hz, 1H), 3.38 (d, J = 1.14 Hz, 1H), 1.46 (s, 6H), 1.39 (s, 3H), 1.38 (s, 3H). Consistent with the literature.[12]

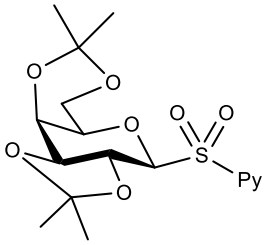
2-(((3aR,4S,9bS)-2,2,8,8-tetramethylhexahydro-[1,3]dioxolo[4',5':4,5]pyrano[3,2-d][1,3]dioxin-4-yl)thio)pyridine 456



456

Compound **454b** (165 mg, 0.37 mmol) was dissolved in methanol (50 mL) and a NaOMe solution was added until a pH of 12 was reached. The reaction was stirred at room temperature for 1 h to produce a faint yellow coloured solution. DOWEX 50W X8 ion exchange resin was added to the reaction mixture until a pH of 7 was reached. The solution was filtered and concentrated under reduced pressure. The crude was then dissolved in DMF (5 mL) and 2-methoxy propane (0.16 mL, 1.92 mmol), to which *p*-TsOH (6.00 mg, 0.03 mmol) was added and stirred for 1 h at room temperature. The saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixture and extracted with Et_2O (4 x 10 mL). The organic phase was washed with water (20 mL) and the combined organics were dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **456**, the title compound, as a colourless liquid (65.0 mg, 46% yield). Rf = 0.29. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.40 (d, J = 4.26 Hz, 1H), 7.57-7.41 (m, 1H), 7.29-7.22 (m, 1H), 7.04-6.96 (m, 1H), 5.80 (d, J = 10.06 Hz, 1H), 4.50 (s, 1H), 4.28-4.01 (m, 2H), 3.94 (d, J = 13.02 Hz, 1H), 3.82-3.60 (m, 2H), 3.49 (s, 1H), 1.47 (s, 3H), 1.46 (s, 3H), 1.44 (s, 3H), 1.43 (s, 3H). ^{13}C NMR and **EI-MS *m/z*** were not collected.

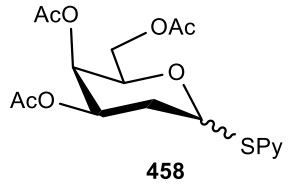
2-(((3aR,4S,9bS)-2,2,8,8-tetramethylhexahydro-[1,3]dioxolo[4',5':4,5]pyrano[3,2-d][1,3]dioxin-4-yl)sulfonyl)pyridine 457



457

Compound **456** (50.0 mg, 0.14 mmol) in acetonitrile (1.2 mL) and water (0.25 mL) was treated with $KMnO_4$ (90.0 mg, 0.56 mmol) and $CuSO_4.5H_2O$ (62.0 mg, 0.39 mmol) and stirred for 2 h. The reaction mixture was then mixture concentrated under reduced pressure and diluted with CH_2Cl_2 (20 mL). The organic phase was washed with water (10 mL) and brine (10 mL) and the combined organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (4:1) and passed through a silica gel column to produce **457**, the title compounds, as colourless liquid (30.0 mg, 55% yield). Rf = 0.32. 4:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.76 (ddd, *J* = 4.68, 1.60, 0.82 Hz, 1H), 8.17-8.12 (m, 1H), 7.95 (dt, *J* = 7.78, 7.77, 1.70 Hz, 1H), 7.55 (ddd, *J* = 7.67, 4.69, 1.10 Hz, 1H), 4.97 (d, *J* = 9.61 Hz, 1H), 4.42-4.40 (m, 1H), 4.28-4.18 (m, 2H), 3.95 (dd, *J* = 13.13, 2.23 Hz, 1H), 3.73 (dd, *J* = 13.17, 1.50 Hz, 1H), 3.63 (dd, *J* = 9.24, 2.62 Hz, 1H), 3.37 (d, *J* = 1.31 Hz, 1H), 1.48 (s, 3H), 1.24 (s, 3H), 1.45 (s, 3H), 1.38 (s, 3H) ^{13}C NMR and **EI-MS *m/z*** were not collected.

(2R,3R,4R)-2-(acetoxymethyl)-6-(pyridin-2-ylthio)tetrahydro-2H-pyran-3,4-diyl diacetate 458

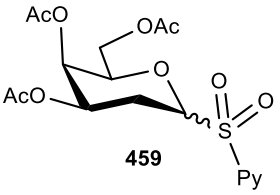


458

Compound **119** (2.00 g, 7.36 mmol) in CH_2Cl_2 (40 mL) was treated with 2-mercaptopyridine (1.06 mL, 9.56 mmol) and anhydrous *p*-TsOH (800 mg, 4.80 mmol) and the mixture was heated under reflux for 30 min. The rection mixture was cooled and poured into water (20 mL), and the organic phase was washed with aqueous 2% KOH (20 mL) and water (20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (2:1) and passed through a silica gel column to produce **458**, the title compound, as colourless liquid (1.75 g, 62% yield). Rf = 0.31. 2:1 hexanes/EtOAc. Mixtures of α and

β anomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.48 (dd, J = 10.49, 4.86 Hz, 1H), 7.56 (dt, J = 7.85, 7.71, 1.78 Hz, 1H), 7.40-7.28 (m, 1H), 7.08 (ddd, J = 7.36, 4.91, 1.01 Hz, 1H), 6.52 (d, J = 5.67 Hz, 1H), 5.36 (dd, J = 12.77, 2.89 Hz, 1H), 5.26 (ddd, J = 12.58, 4.81, 3.06 Hz, 1H), 4.54 (t, J = 6.74, 6.74 Hz, 1H), 4.18-3.95 (m, 3H), 2.60 (dt, J = 13.01, 12.97, 5.87 Hz, 1H), 2.14 (s, 3H), 2.12-2.05 (m, 1H), 2.01 (s, 3H), 1.91 (s, 3H). Consistent with the literature.[13]

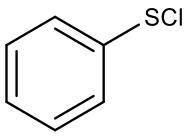
(2R,3R,4R)-2-(acetoxymethyl)-6-(pyridin-2-ylsulfonyl)tetrahydro-2H-pyran-3,4-diyl diacetate 459



459

Compound **458** (170 mg, 0.44 mmol) in acetonitrile (3.7 mL) and water (0.75 mL) was treated with $KMnO_4$ (282 mg, 1.78 mmol) and $CuSO_4.5H_2O$ (190 mg, 1.20 mmol) and stirred for 2 h then the reaction mixture was concentrated under reduced pressure and diluted with CH_2Cl_2 (20 mL). The combined organic phase was washed with water (10 mL) and brine (10 mL), dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (3:1) and passed through a silica gel column to produce **459**, the title compounds, as colourless liquid (120 mg, 65% yield). Rf = 0.29. 3:1 hexanes/EtOAc. Mixtures of α and β anomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.84-8.63 (m, 1H), 8.18-8.02 (m, 1H), 8.03-7.89 (m, 1H), 7.61-7.50 (m, 1H), 5.57 (ddd, J = 12.43, 5.38, 2.88 Hz, 1H), 5.51 (d, J = 7.36 Hz, 1H), 5.37 (s, 1H), 5.23 (d, J = 2.36 Hz, 0.5H), 5.11-5.01 (m, 1H), 4.80 (t, J = 6.30, 6.30 Hz, 1H), 3.95-3.81 (m, 3H), 3.82-3.68 (m, 0.5H), 2.66 (dd, J = 14.53, 5.48 Hz, 1H), 2.48-2.28 (m, 2H), 2.05 (s, 3H), 1.96 (s, 3H), 1.84 (s, 3H) . ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 169.9, 169.7, 169.6, 169.4, 155.1, 154.4, 150.4, 150.1, 137.8, 127.5, 124.6, 123.9, 85.6, 85.4, 75.2, 71.1, 68.2, 65.5, 65.2, 65.1, 62.0, 61.1, 22.9, 21.2, 20.5, 20.4, 20.2. **EI-MS *m/z*** was not collected.

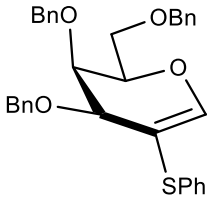
Benzenesulfenyl Chloride (Phenyl hypochlorothioite) 461



461

NCS (2.67 g, 0.02 mmol) in CH_2Cl_2 (30 mL), thiophenol (1.85 g, 0.02 mmol) was added slowly and stirred for 30 min and used directly without any purification. Consistent with the literature.[14]

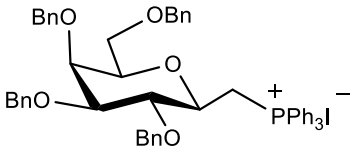
(2R,3S)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-(phenylthio)-3,4-dihydro-2H-pyran 463



463

To a stirring solution of compound **1123** (470 mg, 1.13 mmol) in CCl_4 (3 mL), phenylsulfenyl chloride (0.5 mL, 1.80 mmol) was added and heated for 5 h at reflux. 70% of the CCl_4 is then evaporated, and DBU (0.25 mL, 1.68 mmol) was added. This mixture was stirred for 16 h at 50°C for 14 h. The mixture was cooled to room temperature and diluted with a saturated aqueous NH_4CI solution (10 mL) and extracted with CH_2Cl_2 (2 x 10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:1) and taken up through a silica gel column to produce **463**, the title compound, as a colourless liquid (335 mg, 55% yield). Rf = 0.29. 7:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.39-7.27 (m, 10H), 7.24-7.03 (m, 10H), 4.77 (d, J = 11.68 Hz, 1H), 4.70-4.35 (m, 7H), 4.13 (d, J = 3.62 Hz, 1H), 3.98-3.63 (m, 3H). Consistent with the literature.[15]

Triphenyl(((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)methyl)phosphonium iodide 464

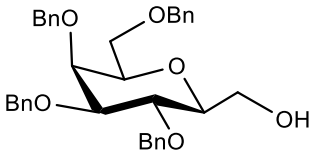


464

Compound **469** (265 mg, 0.40 mmol) in toluene (5 mL) was treated with triphenylphosphine (215 mg, 0.80 mmol), and heated at 120°C for 2 h. The reaction mixture was cooled and concentrated under reduced pressure. The crude was triturated with Et_2O (10 mL) three times to produce crude salt **464**, the title compound, as white solid (320 mg, 86% yield). Rf = 0.32

10:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.37-7.27 (m, 35H), 5.08-4.87 (m, 6H), 4.79-4.68 (m, 3H), 4.56 (d, J = 12.36 Hz, 2H), 4.23-4.06 (m, 2H), 3.96-3.75 (m, 1H), 3.49 (d, J = 6.92 Hz, 1H), 3.18-3.09 (m, 1H), 3.07-3.00 (m, 1H). Consistent with the literature.[16]

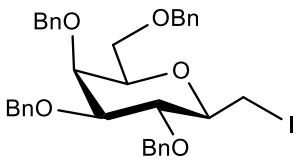
((2S,3S,4R,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)methanol 468



468

Compound **1131** (460 mg, 0.83 mmol) in methanol (4 mL) and diethylether (2 mL) was treated with sodium borohydride (35.0 mg, 0.93 mmol) and stirred for 30 min at 0°C. Water (10 mL) was added and then the reaction mixture was concentrated under reduced pressure to remove methanol. The aqueous phase was extracted with ethyl acetate (2 x 10 mL) and then washed with brine (10 mL). The organic phase phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **468**, the title compounds, as colourless liquid (280 mg, 61% total yield). Rf = 0.26. 9:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.43-7.23 (m, 20H), 4.95 (dd, J = 11.20, 6.24 Hz, 1H), 4.83-4.56 (m, 3H), 4.46 (q, J = 11.85, 11.85, 11.82 Hz, 2H), 4.02-3.78 (m, 3H), 3.77-3.50 (m, 5H), 3.42-3.30 (m, 1H). Consistent with the literature.[16]

(2R,3S,4S,5R,6R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(iodomethyl)tetrahydro-2H-pyran 469

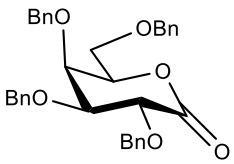


469

Compound **468** (280 mg, 0.5 mmol) in toluene (5 mL) was treated with triphenylphosphine (398 mg, 1.50 mmol), imidazole (104 mg, 1.50 mmol) and iodine (256 mg, 1 mmol). The reaction mixture then was heated at 110°C and stirred for 2 h. The reaction mixture was cooled and diluted with Et_2O (10 mL), washed with 5% aqueous $Na_2S_2O_3$ (2 x 20 mL), and concentrated under

reduced pressure. The crude was triturated with Et_2O (10 mL) and filtered through a pad of Celite to remove most of the triphenylphosphine oxide. The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **469**, the title compounds, as colourless liquid (265 mg, 80% yield). Rf = 0.32. 10:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.46-7.25 (m, 20H), 4.98 (dd, J = 11.24, 5.74 Hz, 2H), 4.84-4.39 (m, 6H), 4.01 (d, J = 2.58 Hz, 1H), 3.80 (t, J = 9.20, 9.20 Hz, 1H), 3.68-3.60 (m, 3H), 3.54 (dd, J = 10.43, 2.25 Hz, 1H), 3.37-3.24 (m, 1H), 3.24-3.15 (m, 1H), 2.37-2.30 (m, 1H). Consistent with the literature.[16]

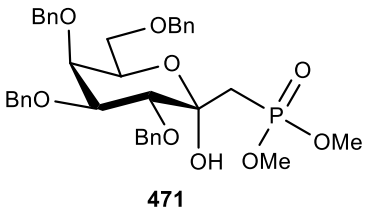
(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-one 470



470

Compound **494** (11.4 g, 20.6 mmol) was dissolved in CH_2Cl_2 (175 mL) then Dess-Martin Periodinane (9.30 g, 21.6 mmol) was added at room temperature. The reaction was stirred at room temperature for 2 h and then filtered through a pad of Celite. The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **470**, the title compound, as a colourless liquid (8.92 g, 81% yield). On replication, yields varied between 77% and 81% for scales between 100 mg and 12.0 g. Rf = 0.30. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.49-7.40 (m, 2H), 7.39-7.34 (m, 6H), 7.32-7.22 (m, 12H), 5.20 (d, J = 11.00 Hz, 1H), 4.95 (d, J = 11.29 Hz, 1H), 4.72 (ddd, J = 35.63, 17.38, 8.75 Hz, 4H), 4.56-4.41 (m, 3H), 4.39-4.31 (m, 1H), 4.18 (t, J = 1.81, 1.81 Hz, 1H), 3.90 (dd, J = 9.57, 2.15 Hz, 1H), 3.80-3.61 (m, 2H). Consistent with the literature.[17]

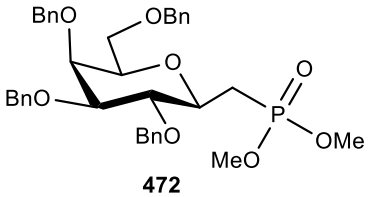
dimethyl (((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-2-hydroxytetrahydro-2H-pyran-2-yl)methyl)phosphonate 471



471

Lactone **470** (1.35 g, 2.50 mmol) in THF (7.5 mL) was cooled to –78°C and 1M *n*-BuLi (3.75 mL, 3.75 mmol) was added and the mixture was stirred for 20 min. It was then treated slowly with solution of $CH_3P(O)(OMe)_2$ (0.46 mL, 3.75 mmol) in THF (5 mL) and left to reach –40°C. The reaction mixture was diluted with saturated aqueous NH_4Cl (20 mL) and extracted with CH_2Cl_2 (2 x 20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and passed through a silica gel column to produce **471**, the title compound, as a colourless liquid (1.10 g, 68% yield). Rf = 0.33. 1:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.48-7.06 (m, 20H), 4.95 (dd, *J* = 11.08, 10.36 Hz, 2H), 4.84-4.51 (m, 4H), 4.42 (q, *J* = 11.65, 11.65, 11.65 Hz, 2H), 3.99 (d, *J* = 1.78 Hz, 1H), 3.72-3.48 (m, 12H), 2.32 (ddd, *J* = 20.06, 15.50, 1.46 Hz, 1H), 1.92 (ddd, *J* = 16.34, 12.96, 9.39 Hz, 1H). Consistent with the literature.[18]

dimethyl (((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)methyl)phosphonate 472

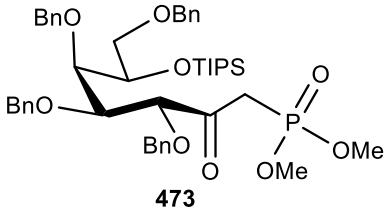


472

Compound **471** (1.10 g, 1.66 mmol) in CH_2Cl_2 (2 mL) was treated with triethylsilane (2.7 mL, 16.6 mmol) and trimethylsilyl triflate (0.3 mL, 1.66 mmol). The mixture was stirred at room temperature for 45 min, then diluted with Et_3N (1 mL). The crude concentrated under reduced pressure and product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **472**, the title compounds, as colourless liquid (860 mg, 80% yield). Rf = 0.29. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.45-7.05 (m, 20H), 4.95 (dd, J = 11.08, 10.36 Hz, 2H), 4.74 (d, J = 11.64 Hz, 1H), 4.64 (dd, J = 18.62, 7.82 Hz, 3H), 4.42 (q, J = 11.65, 11.65, 11.65

Hz, 2H), 4.03-3.95 (m, 1H), 3.87-3.24 (m, 12H), 2.32 (ddd, J = 20.06, 15.50, 1.35 Hz, 1H), 1.92 (dt, J = 15.87, 15.54, 9.67 Hz, 1H). Consistent with the literature.[18]

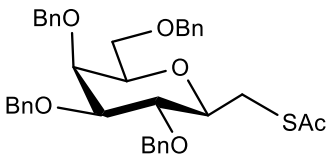
dimethyl((3S,4S,5R)-3,4,5,7-tetrakis(benzyloxy)-2-oxo-6-((triisopropylsilyl)oxy)heptyl)phosphonate 473



473

To a solution of compound **471** (100 mg, 0.15 mmol) in THF (5 mL) at 0°C was added NaH (11.0 mg, 1.18 mmol) portionwise and stirred for 30 min. TIPSCl (0.06 mL, 0.30 mmol) was then added and stirred for 18 h at room temperature. The reaction mixture was quenched slowly with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The organic phase washed with water (20 mL) and brine (20 mL) and the organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and passed through a silica gel column to produce **473**, the title compound, as a colourless liquid (142 mg, 77% yield). Rf = 0.31. 1:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.56-7.00 (m, 20H), 4.73 (dd, *J* = 11.52, 4.69 Hz, 2H), 4.60 (d, *J* = 14.73 Hz, 3H), 4.51 (d, *J* = 11.98 Hz, 1H), 4.46-4.40 (m, 2H), 4.39-4.28 (m, 2H), 4.17-4.08 (m, 1H), 4.06 (t, *J* = 5.22, 5.22 Hz, 1H), 3.72-3.48 (m, 8H), 3.36 (dd, *J* = 22.02, 14.54 Hz, 1H), 2.98 (dd, *J* = 21.12, 14.52 Hz, 1H), 1.05 (s, 21H).^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 200.6, 200.5, 138.8, 138.2, 138.1, 137.9, 128.4, 128.2, 128.0, 127.9, 127.7, 127.2, 84.2, 80.1, 79.6, 77.5, 77.1, 76.7, 74.8, 73.3, 72.7, 72.2, 72.0, 71.9, 52.8, 52.8, 38.9, 37.2, 18.3, 18.2, 13.1. **EI-MS *m/z*** calcd for $C_{46}H_{63}O_9PSi$: 818.40. Found: $[M]^+$ 819.41.

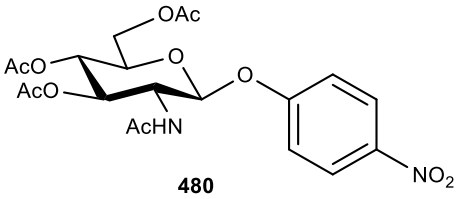
S-(((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)methyl) ethanethioate 474



474

Compound **469** (200 mg, 0.32 mmol) was dissolved in THF (13 mL) and treated with KSAc (75.0 mg, 0.66 mmol). The reaction mixture then was heated at 60°C and stirred for 16 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. EtOAc (50 mL) was added and washed with saturated $NaHCO_3$ (30 mL), water (30 mL) and brine (30 mL). The combined organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and taken up through a silica gel column to produce **474**, the title compound, as a colourless liquid (95.0 mg, 73% yield). Rf = 0.32. 8:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.43-7.27 (m, 20H), 4.94 (dd, J = 11.25, 2.53 Hz, 2H), 4.68 (td, J = 17.73, 11.77, 11.77 Hz, 4H), 4.45 (q, J = 11.83, 11.78, 11.78 Hz, 2H), 3.97 (d, J = 2.70 Hz, 1H), 3.76 (t, J = 9.30, 9.30 Hz, 1H), 3.63-3.50 (m, 5H), 3.44-3.32 (m, 1H), 2.99 (dd, J = 13.56, 7.97 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 195.2, 138.6, 138.1, 138.0, 137.8, 128.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.6, 127.4, 84.4, 78.5, 77.7, 77.1, 76.5, 75.3, 74.3, 73.6, 73.4, 72.2, 68.7, 31.3, 30.4. **EI-MS *m/z*** was not collected.

(2R,3S,4R,5R,6S)-5-acetamido-2-(acetoxymethyl)-6-(4-nitrophenoxy)tetrahydro-2H-pyran-3,4-diyl diacetate 480

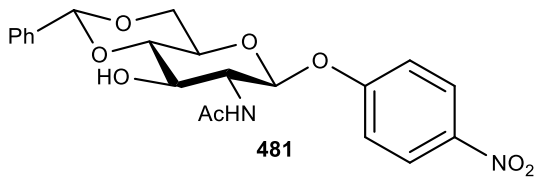


480

Compound **1102** (2.08 g, 5.47 mmol) in acetone (150 mL, HPLC-grade) was treated with *p*-nitrophenol (1.8 g, 12.5 mmol) and K_2CO_3 (2.07 g, 15 mmol) and the suspension was stirred for 1 h at 50 °C. The reaction mixture was concentrated under reduced pressure and diluted with CH_2Cl_2 (150 mL) and the mixture was washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was recrystallized from MeOH to produce **480**, the title compounds,

as brown solid (2.00 g, 78% yield). On replication, yields varied between 74% and 78% for scales between 2.00 g and 5.00 g. Rf = 0.26. 1:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.24 (d, J = 9.26 Hz, 2H), 7.11 (d, J = 9.27 Hz, 2H), 5.65 (d, J = 8.24 Hz, 1H), 5.55-5.44 (m, 2H), 5.18 (t, J = 9.52, 9.52 Hz, 1H), 4.32 (dd, J = 12.33, 5.54 Hz, 1H), 4.26-4.07 (m, 2H), 3.97 (ddd, J = 9.69, 5.51, 2.48 Hz, 1H), 2.12 (s, 6H), 2.10 (s, 3H), 2.00 (s, 3H). Consistent with the literature.[19]

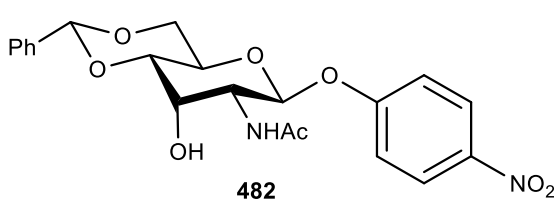
_N_-((4aR,6S,7R,8R,8aS)-8-hydroxy-6-(4-nitrophenoxy)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)acetamide 481



481

Compound **480** (2.33 g, 4.97 mmol) was dissolved in methanol (100 mL) and then NaOMe solution was added until a pH of 12 was reached. The reaction was stirred at room temperature for 5 h. DOWEX 50W X8 ion exchange resin was added to the reaction mixture until a pH of 7 was reached. The solution was filtered and concentrated under reduced pressure. The crude was dissolved in anhydrous DMF (50 mL), and treated with benzaldehyde dimethyl acetal (0.9 mL, 5.4 mmol) and *p*-TsOH (136 mg, 0.78 mmol) and heated at 50°C. The reaction mixture was cooled and concentrated under reduced pressure. The saturated aqueous Na-HCO_3 (100 mL) was added and stirred for 0.5 h at room temperature. The dark brown precipitate was filtered and washed with water (50 mL) and diethyl ether (50 mL) to produce **481**, the title compounds, as brown solid (1.00 g, 47% yield). On replication, yields varied between 35% and 47% for scales between 1.70 g and 4.70 g. Rf = 0.42. 1:9 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.20 (d, J = 9.26 Hz, 2H), 7.98 (d, J = 8.40 Hz, 1H), 7.50-7.42 (m, 2H), 7.43-7.35 (m, 4H), 7.24 (d, J = 9.28 Hz, 2H), 5.64 (s, 1H), 5.50 (s, 1H), 5.40 (d, J = 8.08 Hz, 1H), 4.24 (d, J = 5.20 Hz, 1H), 3.93-3.65 (m, 3H), 3.56 (t, J = 8.75, 8.75 Hz, 1H), 1.82 (s, 3H). Consistent with the literature.[19]

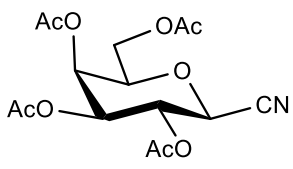
***N*-((4aR,6S,7R,8S,8aS)-8-hydroxy-6-(4-nitrophenoxy)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)acetamide 482**



482

Compound **481** (1.00 g, 2.50 mmol) in CH_2Cl_2 (10 mL) and pyridine (10 mL) was treated with MsCl (0.56 mL, 7.20 mmol). The reaction mixture was stirred for 16 h at room temperature, quenched with MeOH (1 mL) and concentrated under reduced pressure. The crude was dissolved in MeOH (20 mL) and stirred for 30 min at room temperature. The precipitate was filtered and washed with MeOH to give the mesylate as a solid, which was dissolved in 2-methoxyethanol (25 mL) and water (2.5 mL), and then reacted with sodium acetate (1.15 g, 14.0 mmol) at 130°C for 8 h. The mixture was cooled to room temperature, and the solvents were concentrated under reduced pressure and distilled water (150 mL) was added, and the mixture was stirred for an additional 30 min at room temperature. The dark brown precipitate was filtered and washed with water and diethyl ether to produce **482**, the title compound, as a brown solid (0.58 g, 46% yield). On replication, yields varied between 35% and 46% for scales between 1.00 g and 4.80 g. Rf = 0.4, EtOAc. ^{1}H NMR (300 MHz, acetone-d6) δ_{ppm} 8.27-8.17 (m, 1H), 8.16-8.10 (m, 1H), 7.56-7.44 (m, 2H), 7.43-7.31 (m, 3H), 7.32-7.22 (m, 1H), 7.06-6.92 (m, 1H), 5.70 (s, 1H), 5.55 (dd, J = 8.53, 1.00 Hz, 1H), 4.45-4.14 (m, 3H), 3.81 (dd, J = 19.68, 9.47 Hz, 2H), 3.66-3.51 (m, 1H), 3.43-3.34 (m, 1H), 3.30-3.25 (m, 1H), 1.93 (s, 3H). Consistent with the literature.[19]

(2R,3S,4R,5S,6S)-2-(acetoxymethyl)-6-cyanotetrahydro-2H-pyran-3,4,5-triyl triacetate 484

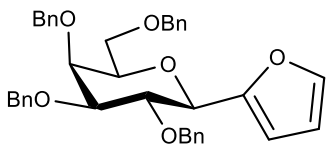


484

Compound **196** (5.00 g, 29.70 mmol) was dissolved in nitro methane (20 mL) and trimethylsilyl cyanide was added and the mixture was cooled to 0°C. The solution was then treated with BF_3**.**OEt_2 (4.1 mL,

44.6 mmol) and stirred at room temperature for 2 h. The reaction mixture was quenched with Et_3N and concentrated under reduced pressure. The crude was dissolved in acetyl acetate and washed with a saturated aqueous $NaHCO_3$ solution (50 mL), H_2O (50 mL), and brine (50 mL) and stirred for 3 h at room temperature. The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was recrystallized with ethyl acetate to produce **484**, the title compound as an orange solid (3.60 g, 48% yield). Rf = 0.31. 9:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.52 (t, *J* = 10.18, 10.18 Hz, 1H), 5.42 (dd, *J* = 3.25, 0.92 Hz, 1H), 4.99 (dd, *J* = 10.15, 3.30 Hz, 1H), 4.28 (d, *J* = 10.21 Hz, 1H), 4.10 (d, *J* = 6.34 Hz, 2H), 4.00-3.88 (m, 1H), 2.17 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H). Consistent with the literature.[20]

(2R,3S,4S,5R,6R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(furan-2-yl)tetrahydro-2H-pyran 487

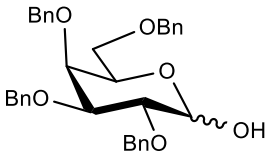


487

Compound **486** (2.60 g, 4.11 mmol) was dissolved in distilled acetonitrile (35 mL) over activated power 4Å molecular sieves and stirred for 30 min. The flask was cooled to –25°C and distilled furan was added and stirred for 30 min, then NIS (1.85 g, 8.22 mmol) and TfOH (0.73 mL, 8.22 mmol) were added and stirred for 2 h. The reaction mixture warmed to room temperature and diluted with CH_2Cl_2 (50 mL) then passed through a pad of Celite. The solution was washed with saturated $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **486**, the title compounds, as colourless liquid (1.95 g, 80% yield). On replication, yields varied between 52% and 80% for scales between 50.0 mg and 2.60 g. Rf = 0.32. 9:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.48-7.28 (m, 15H), 7.26-7.18 (m, 5H), 7.06-7.00 (m, 2H), 6.44 (dd, J = 3.22, 0.67 Hz, 1H), 6.39 (dd, J =

3.22, 1.84 Hz, 1H), 5.00 (d, J = 11.72 Hz, 1H), 4.76 (s, 2H), 4.65 (dd, J = 19.42, 11.02 Hz, 2H), 4.42 (q, J = 11.79, 11.79, 11.79 Hz, 2H), 4.35-4.26 (m, 2H), 4.18-4.07 (m, 2H), 4.04 (d, J = 2.45 Hz, 1H), 3.72-3.62 (m, 2H), 3.63-3.55 (m, 2H). Consistent with the literature.[21]

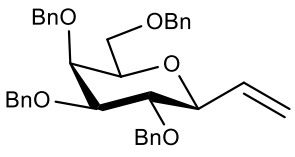
(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol 494



494

N-Bromosuccinimide (700 mg, 4.72 mmol) was added to a stirred solution of **486** (700 mg, 1.11 mmol) in 9:1 acetone/water (16 mL) and the reaction mixture was stirred for 5 min at room temperature, then $NaHCO_3$ (5.00 g) was added and the solvent concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL) and water (50 mL) and the organic phase was separated and the mixture was washed with saturated aqueous $NaHCO_3$ (2 x 50 mL), water (50 mL), and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **494**, the title compound, as a colourless liquid (550 mg, 92% yield). On replication, yields varied between 85% and 92% for scales between 100 mg and 11.0 g. Rf = 0.28. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.47-7.18 (m, 20H), 5.34-5.26 (m, 1H), 5.00-4.88 (m, 1H), 4.88-4.80 (m, 1H), 4.79-4.72 (m, 3H), 4.71-4.55 (m, 1H), 4.45 (q, *J* = 11.92, 11.90, 11.90 Hz, 2H), 4.17 (t, *J* = 6.64, 6.64 Hz, 1H), 4.09-4.01 (m, 1H), 4.01-3.95 (m, 1H), 3.95-3.87 (m, 1H), 3.84-3.70 (m, 1H), 3.66-3.45 (m, 3H). Consistent with the literature.[22]

(2R,3S,4R,5S,6S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-vinyltetrahydro-2H-pyran 495

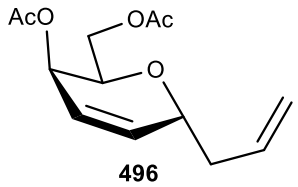


495

To a solution of lactone **470** (8.90 g, 0.57 mmol) in THF (160 mL) at −78°C was added allylmagnesium bromide solution 1.0 M in THF (20 mL, 20.0 mmol). The reaction mixture was stirred for 2 h and then allowed to warm to 0°C. The reaction was then quenched with saturated aqueous NH_4Cl (50 mL) water and

extracted with ethyl acetate (4 x 50 mL). The combined organic phases were washed with brine (150 mL), dried, filtered, and concentrated under reduced pressure. The crude was immediately dissolved in CH_2Cl_2 (100 mL) and cooled to –40 °C. Triethylsilane (16 mL, 99.4 mmol) and then BF_3**.**Et_2O (8.2 mL, 66.4 mmol) were added and the reaction mixture stirred for 1 h. The reaction was then allowed to warm to room temperature. The reaction was quenched by Et_3N (8 mL) and was washed with water (30 mL) and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (10:1) and passed through a silica gel column to produce **495**, the title compound, as a colourless liquid (4.00 g, 45% yield). On replication, yields varied between 40% and 45% for scales between 150 mg and 9.00 g. Rf = 0.29. 10:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.40-7.14 (m, 20H), 6.06-5.82 (m, 1H), 5.38 (dd, *J* = 17.30, 1.47 Hz, 1H), 5.23 (dd, *J* = 10.50, 1.67 Hz, 1H), 4.91 (d, *J* = 11.68 Hz, 1H), 4.78 (d, *J* = 10.53 Hz, 1H), 4.69 (d, *J* = 2.44 Hz, 2H), 4.61 (d, *J* = 2.05 Hz, 1H), 4.58 (s, 1H), 4.40 (d, *J* = 4.96 Hz, 2H), 3.95 (d, *J* = 2.72 Hz, 1H), 3.79-3.61 (m, 2H), 3.62-3.46 (m, 4H). Consistent with the literature.[23]

((2R,3R,6R)-3-acetoxy-6-allyl-3,6-dihydro-2H-pyran-2-yl)methyl acetate 496

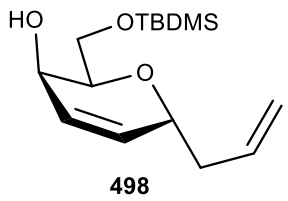


496

A solution of **119** (4.10 g, 15.0 mmol) and allyltrimethylsilane (1.5 mL, 18.0 mmol) in CH_2Cl_2 (50 mL) was cooled to –30°C and BF_3**.**OEt_2 (1.9 mL, 15 mmol) was added. The reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ (50 mL) and EtOAc (100 mL) was added. The phases were separated, and the organic phase was washed with water (50 mL) and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **496**, the title compound, as a colourless liquid (3.45 g, 91% yield). On replication, yields varied between 85% and 91% for scales between 50 mg and 4.00 g. Rf = 0.32. 8:2

hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 6.06 (dd, J = 10.33, 2.83 Hz, 1H), 5.99 (ddd, J = 10.26, 4.90, 1.83 Hz, 1H), 5.94-5.75 (m, 1H), 5.20-5.04 (m, 3H), 4.44-4.31 (m, 1H), 4.27-4.17 (m, 2H), 4.17-4.10 (m, 1H), 2.53-2.37 (m, 1H), 2.36-2.23 (m, 1H), 2.09 (s, 3H), 2.07 (s, 3H). Consistent with the literature.[24]

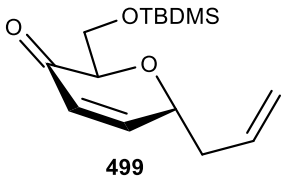
(2R,3R,6R)-6-allyl-2-(((tert-butyldimethylsilyl)oxy)methyl)-3,6-dihydro-2H-pyran-3-ol 498



498

Compound **496** (525 mg, 2.20 mmol) was dissolved in methanol (100 mL) then NaOMe solution was added till pH 12. The reaction was stirred at room temperature for 1 h to produce a faint yellow colour solution. DOWEX 50W X8 ion exchange resin was added to the reaction mixture until pH 7 was reached. The solution was filtered and concentrated under reduced pressure, then the crude was dissolved in CH_2Cl_2 (25 mL) and cooled to 0°C and Et_3N (0.42 mL, 3.00 mmol), DMAP (30.0 mg, 0.24 mmol) and TBSCl (430 mg, 2.86 mmol) were added and stirred 4 h. The reaction mixture was washed with water (50 mL) and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (10:1) and passed through a silica gel column to produce **498**, the title compound, as a colourless liquid (480 mg, 76% yield). On replication, yields varied between 69% and 76% for scales between 50.0 mg and 2.00 g. Rf = 0.33. 10:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.99-5.77 (m, 2H), 5.20-5.03 (m, 2H), 4.27 (ddd, J = 10.83, 5.39, 2.53 Hz, 1H), 4.00-3.90 (m, 1H), 3.89-3.73 (m, 3H), 2.53-2.34 (m, 1H), 2.35-2.19 (m, 1H), 1.98 (d, J = 8.71 Hz, 1H), 0.91 (s, 9H), 0.09 (s, 6H). Consistent with the literature.[24]

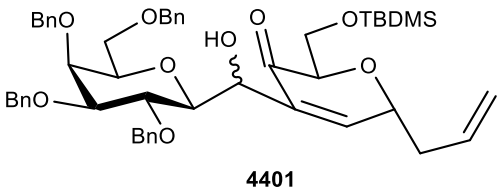
(2R,6R)-6-allyl-2-(((tert-butyldimethylsilyl)oxy)methyl)-2H-pyran-3(6H)-one 499



499

Compound **498** (190 mg, 0.54 mmol) was dissolved in CH_2Cl_2 (3 mL) and treated with PCC (240 mg, 1.09 mmol) and silica (240 mg), The reaction mixture was stirred for 4 h, diluted with CH_2Cl_2 (30 mL) and then pass through Celite. The solution was washed with saturated aqueous $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (90:1) and passed through a silica gel column to produce **499**, the title compound, as a colourless liquid (110 mg, 72% yield). On replication, yields varied between 65% and 72% for scales between 50.0 mg and 1.00 g. Rf = 0.28. 90:1 hexanes/EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 6.97 (dd, J = 10.52, 2.02 Hz, 1H), 6.13 (dd, J = 10.52, 2.20 Hz, 1H), 5.96-5.79 (m, 1H), 5.27-5.09 (m, 2H), 4.93-4.72 (m, 1H), 4.23 (dd, J = 4.27, 2.71 Hz, 1H), 4.02 (dq, J = 11.14, 11.13, 11.13, 3.49 Hz, 2H), 2.60-2.47 (m, 1H), 2.47-2.39 (m, 1H), 0.85 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 195.4, 151.0, 133.3, 126.8, 118.1, 79.6, 71.1, 64.9, 38.7, 25.7, 18.1, -5.5, -5.6. **EI-MS *m/z*** calcd for $C_{15}H_{26}O_3Si$: 282.16. Found: $[M]^+$ 283.18.

(2R,6R)-6-allyl-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(hydroxy((2S,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)methyl)-2H-pyran-3(6H)-one 4401



4401

A mixture of aldehyde **1131** (70.0 mg, 0.13 mmol) and compound **499** (65.0 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was cooled to –78°C and treated with solution of 1 M Et_2AlI (0.13 mL, 0.13 mmol) in toluene for 2 h. The reaction mixture was diluted with Et_2O (20 mL) and then 2M HCl (10 mL) was added. The aqueous phase was extracted with Et_2O (2 x 10

mL), and the combined organic phases was washed with water (20 mL), and brine (20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **500**, the title compounds, as colourless liquid (25.0 mg, 22% yield). On replication, yields varied between 6% and 22% for scales between 20.0 mg and 1.60 g. Rf = 0.33. 9:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.52-7.17 (m, 20H), 7.12-6.86 (m, 1H), 6.01-5.72 (m, 1H), 5.26-5.08 (m, 1H), 5.02 (dd, J = 11.24, 2.54 Hz, 2H), 4.97-4.88 (m, 1H), 4.84 (d, J = 11.30 Hz, 2H), 4.81-4.70 (m, 3H), 4.62 (d, J = 11.56 Hz, 1H), 4.56-4.38 (m, 3H), 4.29-4.12 (m, 2H), 4.09-3.94 (m, 3H), 3.74-3.63 (m, 1H), 3.63-3.52 (m, 2H), 3.52-3.41 (m, 2H), 2.76-2.22 (m, 2H), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 195.0, 146.5, 138.9, 138.8, 138.7, 138.5, 138.5, 138.4, 138.3, 138.2, 138.1, 138.0, 136.5, 133.7, 128.6, 128.5, 128.5, 128.3, 128.0, 127.9, 127.7, 127.6, 127.3, 127.2, 127.1, 118.0, 84.7, 79.7, 78.8, 77.6, 77.3, 76.9, 75.3, 74.9, 74.6, 73.9, 73.6, 72.5, 71.6, 68.7, 66.0, 64.2, 38.3, 31.0, 26.0, 18.3, -5.2, -5.3. **EI-MS *m/z*** calcd for $C_{50}H_{62}O_9Si$: 834.42. Found: $[M+ NH_4]^+$ 852.45.

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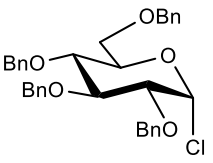
9.8 Experimental protocols for Chapter 5

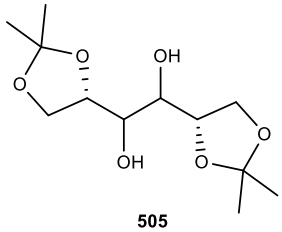
2,3,4,6-tetra-*O*-benzyl-*α*- D-glucopyranosyl Chloride 503



503

2,3,4,6-tetra-*O*-benzyl- D-glucopyranose **502** (820 mg, 1.52 mmol) and DMF (128 *u*L) were dissolved in CH_2Cl_2 (12 mL). Oxalyl chloride (0.13 mL) was added dropwise at 0°C and the reaction was stirred for 30 min at 0°C, then for 1 h at room temperature. The crude product was concentrated under reduced pressure, dissolved in hexanes, and passed through a silica gel column to to produce **503**, title compound as a colourless liquid (320 mg, 38% yield). On replication, yields varied between 32% and 38% for scales between 50.0 mg and 820 mg. Rf = 0.31. 3:7 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.53-7.30 (m, 18H), 7.29-7.20 (m, 2H), 6.16 (d, *J* = 3.69 Hz, 1H), 5.07 (d, *J* = 10.81 Hz, 1H), 4.93 (dd, *J* = 10.79, 5.15 Hz, 2H), 4.81 (d, *J* = 1.66 Hz, 2H), 4.73-4.50 (m, 3H), 4.28-4.04 (m, 2H), 3.92-3.78 (m, 3H), 3.74 (dd, *J* = 10.91, 1.86 Hz, 1H). Consistent with the literature.[1]

1,2,5,6-di-*O*-isopropyliidene-D-mannitol 505

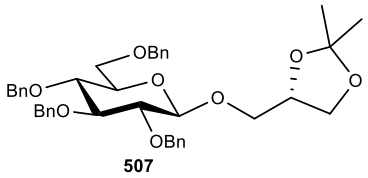


505

Acetone (50 ml) was saturated with anhydrous $ZnCl_2$ (10.0 g, 76.8 mmol) and the milky solution was allowed to settle down. Solution was cannulated under nitrogen to a round bottom flask containing D-mannitol (5.00 g, 27.4 mmol). The reaction mixture was stirred for 2.5 h at room temperature and saturated K_2CO_3 (40 mL) was then added. The reaction mixture was stirred at room temperature for 0.5 h before being filtered. The filtrate and the residue extracted with CH_2Cl_2 (3 x 50 mL) and the organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was recrystallized from 9:1 hexanes/CH_2Cl_2 to produce **505**, title compound as a white solid (6.00 g, 90% yield). On replication, yields varied between 84% and 90% for scales between 2.50 g and 5.00 g. Rf = 0.37. 1:7 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 4.19

(q, 2H J = 6.03), 4.13 (t, 2H, J = 8.33), 4.0 (d, 1H J = 5.58), 3.97 (d, 1H J = 5.6), 3.76 (t, 2H J = 6.17), 2.72 (d, 2H J = 6.66), (s, 6H), 1.35 (s, 6H). Consistent with the literature.[2]

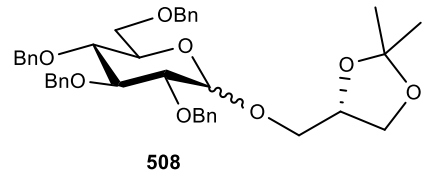
(*2R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-*β*-D-glucopyranosyl)-2,3-*O*-isopropylideneglycerol 507



507

Compound **503** (160 mg, 0.28 mmol), 2-6-di-tert-butylpyrimidine (180 mg, 0.95 mmol), molecular sieves and **(***S*)-(+)-Solketal **1253** (50 mg, 0.378 mmol) were stirred in anhydrous CH_2Cl_2 (2 mL) under nitrogen. AgOTf (160 mg, 0.60 mmol) was added to the reaction mixture. After the disappearance of starting material, Bu_4NBr (120 mg) and saturated aqueous $NaHCO_3$ (10 mL) were added, and the mixture was filtered through a pad of Celite, diluted with CH_2Cl_2 (30 mL) and the mixture was washed with water (30 x 2 mL), and the organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (3:1) and passed through a silica gel column to produce **507**, title compound as a colourless liquid (100 mg, 54% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.29 (m, 18H), 7.18 (m, 2H), 5.02-4.25 (m, 10H), 4.09 (m, 2H), 3.84 (dd, J = 5.99, 8.28, 1H), 3.71 (m, 2H), 3.62 (m, 3H), 3.46 (t, J = 7.93, 2H), 1.42 (s, 3H), 1.37 (s, 3H). Consistent with the literature.[3]

(2R)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-*α*-D-glucopyranosyl)-2,3-*O*-isopropylideneglycerol 508

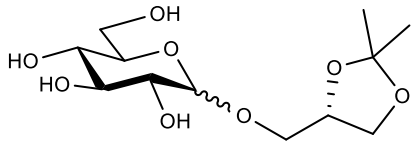


508

Compound **502** (4.80 g, 7.60 mmol), (*S*)-(+)-Solketal **1253** (1.00 g, 7.60 mmol) and activated 5Å MS stirred in Et_2O (100 ml) for 0.5 h and cooled to –40°C then TfOH (0.14 mL, 16.2 mmol), and NIS (1.71 g, 7.60 mmol) were added. The reaction mixture was then stirred for 2 h and then passed through Celite upon completion. The reaction was diluted with CH_2Cl_2 (100 mL) and the organic phase was washed with $Na_2S_2O_3$ (100 mL), saturated aqueous $NaHCO_3$ (100 mL)

and brine (100 mL), then concentrated. The crude product was dissolved in toluene/ethyl acetate (9:1) and passed through a silica gel column to produce **508**, the title compound as a colorless liquid (4.50 g, 90% yield). On replication, yields varied between 84% and 90% for scales between 50.0 mg and 4.80 g. Rf = 0.29. 3:7 EtOAc/hexanes. Mixtures of α and β anomer : ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.41-7.27 (m, 14H), 7.25-7.08 (m, 6H), 5.05-4.90 (m, 1H), 4.90-4.71 (m, 3H), 4.71-4.62 (m, 1H), 4.62-4.55 (m, 1H), 4.48 (d, J = 11.64 Hz, 2H), 4.40-4.28 (m, 1H), 4.12-4.03 (m, 1H), 4.03-3.86 (m, 1H), 3.84-3.35 (m, 8H), 1.43 (s, 3H), 1.37 (s, 3H). Consistent with the literature.[3, 4]

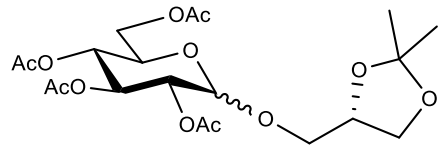
(2R)-1-*O*-(α-D-glucopyranosyl)-2,3-*O*-isopropylideneglycerol 508a



508a

To a solution of compound **508** (3.50 g, 5.32 mmol) MeOH (200 mL), Pd/C (21.0 g, 99.0 mmol) and ammonium formate (6.40 g, 102 mmol) were added and refluxed at 70°C for 5 h. After cooling to room temperature, the reaction mixture was passed through a pad of Celite and washed with MeOH (100 mL). The solvent was evaporated and then co-evaporated with chloroform to produce **508a**, the title compound as a colourless liquid (1.40 g, 89% yield). On replication, yields varied between 80% and 89% for scales between 50.0 mg and 3.50 g. Rf = 0.25. 95:5 EtOAc/MeOH. Mixtures of alpha and beta: ^{1}H NMR (300 MHz, MeOD) δ_{ppm} 4.80-478 (m, 1H), 4.32-4.28 (m, 1H), 4.05-3.98 (m, 1H), 3.91-3.48 (m, 6H), 3.47-3.04 (m, 4H), 1.36 (s, 3H), 1.29 (s, 4H). Consistent with the literature.[4, 5]

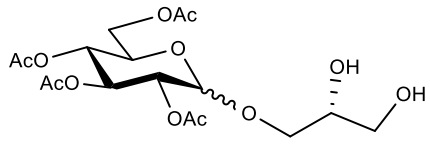
(2R)-1-*O*-(α-D-2,3,4,6-tetra-*O*-acetyl-glucopyranosyl)-2,3-*O*-isopropylideneglycerol 509



509

Compound **508a** (1.80 g, 6.12 mmol) was added to pyridine (13 mL) and Acetic Anhydride (7 mL) at 0°C, under nitrogen. The reaction was stirred overnight for 12 h and then poured into a beaker containing ice water (100 mL), and then CH_2Cl_2 (100 mL) was added and the aqueous and organic phase were separated. The organic phase was washed once with $CuSO_4$ aq (2 x 50 mL), saturated aqueous $NaHCO_3$ (50 mL) and water CH_2Cl_2 (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **509**, the colourless liquid compound (1.90 g, 67% yield). On replication, yields varied between 58% and 67% for scales between 50.0 mg and 1.80 g. Rf = 0.45. 3:7 EtOAc/hexanes. Mixtures of α and β: ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.49 (t, J = 9.83, 1H), 5.26-4.95 (m, 2H), 4.89-4.81 (m, 1H), 4.27 -4.22 (m, 2H), 4.19-3.98 (m, 3H), 3.82- 3.61 (m, 2H), 3.52-3.44 (m, 1H), 2.06 (acetate singlets, 12H), 1.42 (s, 3H), 1.37 (s, 3H). Consistent with the literature.[6]

3-O-(α-D-2,3,4,6-tetra-*O*-acetyl-glucopyranosyl)-sn-glycerol 509a

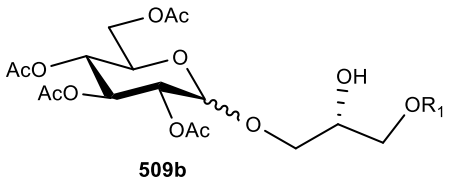


509a

To a solution of compound **509** (1.80 g, 3.90 mmol) in acetonitrile (18 mL), $Zn(NO_3)_2.6H_2O$ (5.80 g, 19.5 mmol) was added. The mixture was stirred for 6 h at 50°C, and the solvent was then evaporated under reduced pressure. The reaction mixture diluted with CH_2Cl_2 (150 mL) and water (100 mL) added, and the organic phase was washed with water (2 x 100 mL) and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (5:1) and passed through a silica gel column to produce **509a**, the colourless liquid compound (1.20 g, 70% yield). On replication, yields varied between 58% and 70% for scales between 50.0 mg and 1.80 g. Rf = 0.3. EtOAc.

Mixtures of α and β: ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.55-5.40 (m, 1H), 5.15-5.00 (m, 2H), 5.00-4.87 (m, 1H), 4.32-4.17 (m, 1H), 4.17-4.04 (m, 2H), 4.01-3.59 (m, 6H), 2.63-2.54 (m, 1H), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H). Consistent with the literature.[7]

1-*O*-hexadecanoyl-3-*O*-(α-D-2,3,4,6-tetra-*O*-acetyl-glucopyranosyl)-sn-glycerol 509b

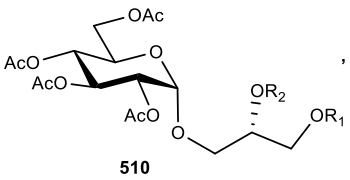


509b

R_1 = palmitoyl

To a solution of compound **509a** (1.20 g, 2.76 mmol) in anhydrous CH_2Cl_2 (50 mL) under nitrogen, palmitic acid (0.72 g, 2.76 mmol), EDC (0.53 g, 2.76 mmol), and DMAP (0.33 g, 0.28 mmol) were added under nitrogen at 0°C and the reaction was stirred 0°C for 6 h. The reaction mixture diluted with CH_2Cl_2 (150 mL) and water (100 mL) added, and the organic phase was washed with water (2 x 100 mL) and with brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (2:1) and passed through a silica gel column to produce **509b**, the title compound as a colourless liquid (0.90 mg, 50% yield). Rf = 0.22. 3:7 EtOAc/hexanes. Mixtures of α and β: ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.47 (t, J = 9.94, 1H), 5.32 (t, J = 5.65, 1H), 5.12-4.98 (m, 2H), 4.9 (d, J = 10.05, 1H), 4.05-4.3 (m, 5H), 3.89 -3.63 (m, 2H), 3.54-3.50 (m, 1H), 2.52 (dd, J = 20.46, 4.95 Hz, 1H), 2.35-2.29 (m, 2H), 2.11 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.03(s, 3H), 1.61 (m, 2H), 1.31 (s, 24H), 0.91 (t, J = 6.96, 3H). ^{13}C NMR was not collected.

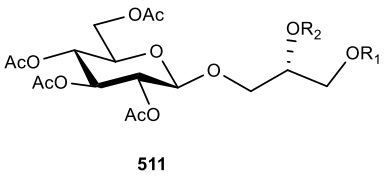
1-*O*-hexadecanoyl-2-*O*-*cis*-Octadec-12-enoyl-3-*O*-(α-D-2,3,4,6-tetra-*O*-acetyl-glucopyranosyl)-sn-glycerol 510 and 1-*O*-hexadecanoyl-2-*O*-*cis*-Octadec-12-enoyl-3-*O*-(β-D-2,3,4,6-tetra-*O*-acetyl-glucopyranosyl)-sn-glycerol 511



510

R_1 = palmitoyl
R_2 = *cis*-vaccinoyl

,



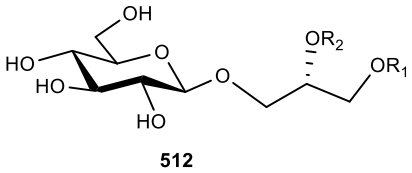
511

R_1 = palmitoyl
R_2 = *cis*-vaccinoyl

To a solution of compound **509b** (0.85 g, 1.29 mmol) in anhydrous CH_2Cl_2 (30 mL), *cis*-vaccenic acid (0.43 g, 1.52 mmol), EDC (0.30 g, 1.52 mmol), and DMAP (6 pinches) were added under nitrogen at 0°C and the reaction was stirred for 16 h at room temperature. CH_2Cl_2 (50 mL) and water (50 mL) were added to the reaction mixture and the organic phase was washed with water (2 x 40 mL) and brine (40 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (2:1) and passed through a silica gel column to produce α-compound **510,** the title compound as a colourless liquid (0.70 g, 58% yield). Rf = 0.33 (30% EtOAc, 70% Hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.44 (t, J = 9.87, 1H), 5.34-5.25 (m, 2H), 5.20-5.11 (m, 1H), 5.09-5.06 (m, 1H), 5.05 (d, J = 9.80, 1H) 4.85 (dd, J = 3.79, 10.27, 1H), 4.32 (td, J = 4.27, 11.70, 1H), 4.24 (dt, J = 3.69, 12.37, 1H), 4.14-4.10 (m, 1H), 4.09-4.03 (m, 1H), 4.01 (t, J = 9.82, 1H), 3.81 (dd, J = 4.76, 11.17, 1H), 3.62-3.51 (m, 1H), 2.32-2.28 (m, 4H), 2.09-2.01(acetate singlets plus $H_2CCH{=}CHCH_2$, m, 16H), 1.62 (t, J = 6.87, 4H), 1.29 (s, 44H), 0.88 (td, J = 1.65, 6.83, 6H). ^{13}C NMR (500 MHz, $CDCl_3$) δ_{ppm} 173.3, 172.9, 170.6, 170.2, 170.1, 169.6, 130.1, 130.0, 129.9, 129.8, 96.3, 77.6, 77.2, 76.8, 70.7, 70.1, 70.0, 69.8, 69.8, 68.4, 67.6, 67.6, 66.7, 66.3, 62.2, 62.1, 61.8, 34.3, 34.2, 34.1, 32.0, 31.9, 29.8, 29.6, 29.6, 29.4, 29.4, 29.2, 29.1, 27.3, 24.9, 22.7, 20.7, 20.6, 14.2 and β-compound **511** (0.48 g, 40% yield). Rf = 0.31 (30% EtOAc, 70% Hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.32 (t, J = 4.73, 4.73 Hz, 2H), 5.24-5.12 (m, 2H), 5.11-5.02 (m, 1H), 5.01-4.92 (m, 1H), 4.51 (dd, J = 7.86, 4.03 Hz, 1H), 4.39-4.17 (m, 2H), 4.18-3.99 (m, 2H), 3.93 (dd, J = 10.95, 4.87 Hz, 1H), 3.75-3.55 (m, 2H), 2.39-2.16 (m, 4H), 2.10-1.89

(m, 16H), 1.68-1.50 (m, 4H), 1.36-1.18 (m, 44H), 0.86 (t, J = 6.10, 6.10 Hz, 6H). ^{13}C NMR was not collected.

1-*O*-hexadecanoyl-2-*O*-*cis*-Octadec-12-enoyl-3-*O*-(*β*-D-glucopyranosyl)-sn-glycerol 512

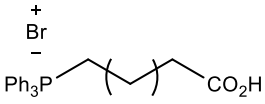


512

R_1 = palmitoyl
R_2 = *cis*-vaccinoyl

Compound **511** (85 mg, 0.09 mmol) was dissolved in EtOH (2.5 mL) and $N_2H_4 \cdot H_2O$ 35% (0.3 mL) was added and refluxed at 40°C for 2.5 h. EtOH was then evaporated under reduced pressure and H_2O (20 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (4 x 20 mL) and organic phase was washed with brine (40 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in ethyl acetate and passed through a silica gel column to produce **512**, title compound as a colourless liquid (48 mg, 53% yield). Rf = 0.29 (98% EtOAc, 2% MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.49-5.30 (m, 1H), 5.30-5.21 (m, 1H), 4.94 (ddd, J = 26.58, 19.21, 1.34 Hz, 1H), 4.74-4.47 (m, 1H), 4.44-4.34 (m, 2H), 4.31 (d, J = 7.43 Hz, 2H), 4.16 (ddd, J = 18.57, 12.05, 6.57 Hz, 1H), 3.97-3.86 (m, 1H), 3.83 (s, 2H), 3.75-3.65 (m, 1H), 3.63-3.47 (m, 3H), 3.37 (t, J = 7.97, 7.97 Hz, 1H), 3.30 (d, J = 5.75 Hz, 1H), 2.38-2.20 (m, 4H), 2.04-1.96 (m, 4H), 1.66-1.51 (m, 4H), 1.37-1.13 (m, 44H), 0.87 (t, J = 6.91, 6.91 Hz, 6H). Consistent with the literature.[8, 9]

(10-Carboxydecyl)triphenylphosphonium bromide 513

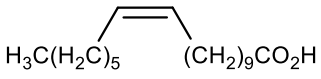


513

11-bromodecanoic acid **512** (10.0 g, 37.5 mmol) and PPh_3 (10.9 g, 41.2 mmol) in toluene (60 mL) were stirred at reflux temperature under nitrogen for 24 h. After cooling to room temperature two phases were apparent and the upper phase was decanted. CH_2Cl_2 was added to the viscous residue before the solvent was rotovaped off, and the resultant solid was washed with boiling ethyl acetate 3 times. A white

solid was collected *via* filtration and dried under reduced pressure to produce the title compound **513** (16 g, 81% yield).[10]

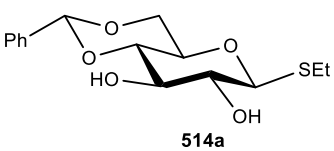
cis-Octadec-12-enoic acid (_cis_-vaccenic acid) 514



514

(10-Carboxydecyl)triphenylphosphonium bromide **513** (2.00 g, 3.80 mmol) in THF (25 mL) was cooled to –78°C under nitrogen. KHMDS 0.5M solution in toluene (20 mL) was added and the reaction was warmed to room temperature over 2 h. The solution was recooled to –78°C before heptanal (0.54 mL, 3.80 mmol) was added dropwise. The reaction was then warmed to room temperature and stirred for 12 h. EtOAc (50 ml) and 1M HCl (50 ml) were added to the reaction mixture and the aqueous phase was extracted with ethyl acetate (2 x 50 ml). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (6:1) and taken up through a silica gel column to produce the title compound (640 mg, 60%) ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.48-5.27 (m, 2H), 2.45-2.31 (m, 2H), 2.10-1.95 (m, 4H), 1.74-1.56 (m, 2H), 1.40-1.25 (m, 20H), 0.90 (t, J = 5.70, 5.70 Hz, 3H). Consistent with the literature.[10]

(2R,4aR,6S,7R,8R,8aS)-6-(ethylthio)-2-phenylhexahydropyrano[3,2-d][1,3]dioxine-7,8-diol 514a

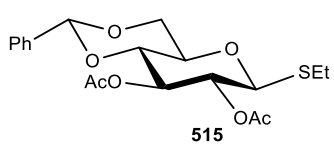


514a

Compound **1217** (3.67 g, 9.03 mmol) was dissolved in methanol (100 mL) then NaOMe solution was added until a pH of 12 was reached. The reaction was stirred at room temperature for 5 h. DOWEX 50W X8 ion exchange resin was added to the reaction mixture to neutralize the solution. The solution was filtered and concentrated under reduced pressure. The crude was dissolved in anhydrous DMF (18 mL), and treated with benzaldehyde dimethyl acetal (1.6 mL, 10.8 mmol) and *p*-TsOH (70 mg,

0.36 mmol) and heated at 50°C for 18 h. The reaction mixture diluted with ethyl acetate (200 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL) water (100 mL), and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:1) and passed through a silica gel column to produce **514a**, the title compounds, as colourless liquid (1.35 g, 48% yield). On replication, yields varied between 42% and 48% for scales between 100 mg and 3.70 g. Rf = 0.30. 1:1 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.57-7.44 (m, 2H), 7.46-7.30 (m, 3H), 5.55 (s, 1H), 4.48 (d, J = 9.78 Hz, 1H), 4.37 (dd, J = 10.46, 4.66 Hz, 1H), 3.97-3.69 (m, 2H), 3.67-3.46 (m, 3H), 2.86-2.71 (m, 3H), 2.59 (d, J = 2.07 Hz, 1H), 1.34 (t, J = 7.44, 7.44 Hz, 3H). Consistent with the literature.[11]

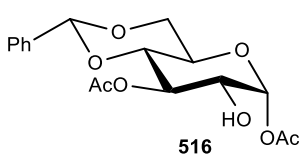
(2R,4aR,6S,7R,8S,8aR)-6-(ethylthio)-2-phenylhexahydropyrano[3,2-d][1,3]dioxine-7,8-diyl diacetate 515



515

Compound **514a** (1.50 g, 3.03 mmol) was dissolved in pyridine (85 mL) then acetic anhydride (12 mL) was added and stirred at room temperature for 16 h, then diluted with CH_2Cl_2 (200 mL) and washed with 5% HCl aq (2 x 100 mL), water (100 mL) and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **514**, the title compound, as a colourless liquid (1.16 g, 55% yield). On replication, yields varied between 45% and 55% for scales between 50.0 mg and 5.00 g. Rf = 0.32. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.49-7.40 (m, 2H), 7.40-7.31 (m, 3H), 5.50 (s, 1H), 5.34 (t, J = 9.33, 9.33 Hz, 1H), 5.04 (dd, J = 9.98, 9.09 Hz, 1H), 4.58 (d, J = 10.05 Hz, 1H), 4.37 (dd, J = 10.41, 4.77 Hz, 1H), 3.73 (td, J = 19.01, 9.76, 9.76 Hz, 2H), 3.64-3.49 (m, 1H), 2.83-2.60 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.27 (t, J = 7.43, 7.43 Hz, 3H). Consistent with the literature.[12]

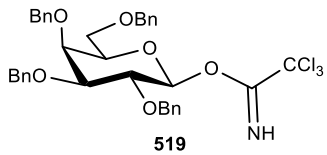
(2R,4aR,6R,7R,8R,8aR)-7-hydroxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxine-6,8-diyl diacetate 516



516

Compound **515** (1.20 g, 3.03 mmol) was dissolved in CH_2Cl_2/H_2O (100:1) and cooled to 0°C and *N*-bromosuccinimide (540 mg, 3.03 mmol) and TMSOTf (0.06 mL, 0.30 mmol) were added and stirred at 0°C for 5 min. The reaction mixture poured into saturated aqueous $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **516**, the title compound, as a light-yellow solid (426 mg, 40% yield). On replication, yields varied between 35% and 40% for scales between 50.0 mg and 5.00 g. Rf = 0.29. 7:3 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.54-7.39 (m, 2H), 7.42-7.33 (m, 3H), 6.20 (d, *J* = 3.82 Hz, 1H), 5.52 (s, 1H), 5.35 (t, *J* = 9.78, 9.78 Hz, 1H), 4.32 (dd, *J* = 10.42, 4.69 Hz, 1H), 3.99-3.79 (m, 2H), 3.81-3.57 (m, 2H), 2.85 (s, 1H), 2.21 (s, 3H), 2.16 (s, 3H). Consistent with the literature.[13]

(2S,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl 2,2,2-trichloroacetimidate 519

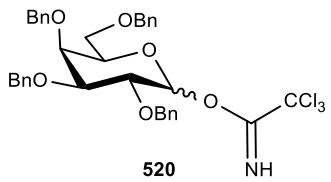


519

Compound **487** (5.20 g, 9.62 mmol) was dissolved in CH_2Cl_2 (50 mL) and Cl_3CCN (5 mL, 49.9 mmol) and K_2CO_3 (5.00 g, 36.2 mmol) were added. The reaction mixture was stirred for 5 h at room temperature. The reaction mixture passed through Celite and concentrated under reduced pressure to produce **519**, the title compound without purification (5.70 g, 86% yield). On replication, yields varied between 81% and 86% for scales between 100 mg and 5.20 g. Rf = 0.12 only *β*-anomer 1:7

EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.63 (s, 1H), 7.37-7.27 (m, 20H), 5.75 (d, J = 8.03 Hz, 1H), 5.01-4.87 (m, 2H), 4.82 (d, J = 10.70 Hz, 1H), 4.74 (s, 2H), 4.64 (d, J = 11.53 Hz, 1H), 4.46 (d, J = 4.80 Hz, 2H), 4.10 (dd, J = 9.61, 8.05 Hz, 1H), 3.99 (d, J = 2.02 Hz, 1H), 3.76 (dd, J = 6.56, 6.04 Hz, 1H), 3.69-3.57 (m, 3H). Consistent with the literature.[14]

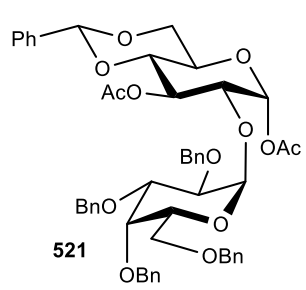
(3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl 2,2,2-trichloroacetimidate 520



520

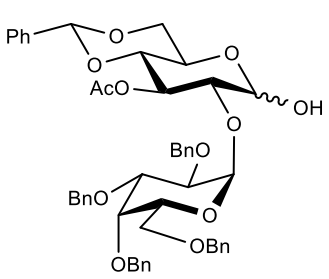
Compound **487** (325 mg, 0.60 mmol) was dissolved in CH_2Cl_2 (6 mL) and cooled to 0°C then Cl_3CCN (0.6 mL, 6.00 mmol) and DBU (0.01 mL, 0.06 mmol) were added. The reaction mixture was stirred for 3 h before being concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:1) and passed through a silica gel column to produce **520** the title compound as a colorless liquid (335 mg 82% yield α:β 1:0.5). On replication, yields varied between 78% and 82% for scales between 50.0 mg and 5.00 g. Rf = 0.31 α-anomer, 0.12 β-anomer 1:7 EtOAc/hexanes. α-Glycosyl donor: ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 8.51 (s, 1H), 7.39-7.21 (m, 20H), 6.51 (d, J = 3.44 Hz, 1H), 4.97-4.88 (m, 1H), 4.85-4.77 (m, 2H), 4.61 (t, J = 11.56, 11.56 Hz, 2H), 4.50-4.36 (m, 2H), 4.23 (dd, J = 9.87, 3.48 Hz, 1H), 4.07-3.98 (m, 3H), 3.69-3.48 (m, 3H). ^{13}C NMR was not collected.

(2R,4aR,6R,7R,8S,8aR)-2-phenyl-7-(((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydropyrano[3,2-d][1,3]dioxine-6,8-diyl diacetate 521



Acceptor **516** (3.00 g, 8.52 mmol) and donor **17** (9.70 g, 14.2 mmol) were dissolved in CH_2Cl_2 (500 mL) and (10 g) activated 5Å MS was added and stirred at room temperature for 30 min, and then cooled to 0°C. BF_3**.**OEt_2 (1.1 mL, 8.52 mmol) was then added dropwise and stirred for 4 h at room temperature. The mixture passed through a pad of Celite and solvent concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **521**, title compound as a colourless liquid (3.80 g, 50% yield). On replication, yields varied between 42% and 50% for scales between 50.0 mg and 10.0 g. Rf = 0.34. 3:7 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.47-7.39 (m, 2H), 7.40-7.27 (m, 21H), 7.26-7.24 (m, 2H), 6.35 (d, J = 3.73 Hz, 1H), 5.55 (t, J = 9.77, 9.77 Hz, 1H), 5.46 (s, 1H), 4.90 (dd, J = 17.93, 7.44 Hz, 1H), 4.80 (d, J = 11.78 Hz, 1H), 4.73 (dd, J = 11.65, 2.51 Hz, 2H), 4.62 (d, J = 11.83 Hz, 1H), 4.52 (d, J = 11.36 Hz, 1H), 4.47 (d, J = 11.77 Hz, 1H), 4.40 (d, J = 11.69 Hz, 1H), 4.32-4.27 (m, 1H), 4.03-3.89 (m, 5H), 3.85 (dd, J = 10.13, 2.69 Hz, 1H), 3.68 (t, J = 10.32, 10.32 Hz, 1H), 3.59-3.51 (m, 2H), 3.50 (dd, J = 8.89, 5.55 Hz, 1H), 1.99 (s, 3H), 1.94 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 169.9, 169.6, 138.9, 138.8, 138.7, 138.5, 138.0, 136.9, 129.6, 129.1, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.4, 127.4, 127.2, 126.1, 103.8, 101.5, 99.9, 97.5, 89.3, 79.2, 78.7, 75.4, 75.1, 74.9, 74.7, 74.1, 73.6, 73.5, 73.4, 73.3, 73.3, 73.2, 73.0, 72.7, 70.1, 70.0, 69.8, 68.8, 68.6, 68.5, 64.7, 21.0, 20.8. **EI-MS *m/z*** calcd for $C_{51}H_{54}O_{13}$ 874.36. Found: $[M+H_2O]^+$ 892.39

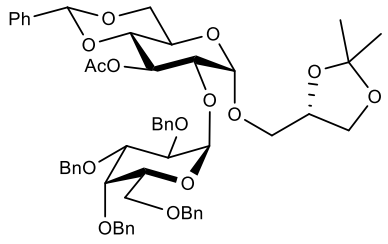
(2R,4aR,7R,8S,8aR)-6-hydroxy-2-phenyl-7-(((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-8-yl acetate 522



522

Compound **521** (8.00 g, 9.15 mmol) was dissolved in THF (550 mL) and prepared salt of ethyleneamine and acetic acid (18.0 g) was added, which was formed by mixing acetic acid (36 mL) with ethylene diamine (29 mL) at room temperature to 0°C for 15 minutes. The reaction mixture was stirred at room temperature for 24 h. The mixture was concentrated, then CH_2Cl_2 (300 mL) was added and the organic phase was washed with water (2 x 200 mL) and with brine (200 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (9:1) and passed through a silica gel column to produce **522**, the title compound as a white solid, mixtures of α and β anomers (ratio: 1/1 determined by ^{1}H NMR) (3.00 g, 40% yield). On replication, yields varied between 32% and 40% for scales between 100 mg and 8.00 g. Rf = 0.31. 1:9 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.44-7.20 (m, 25H), 5.45 (d, J = 4.52 Hz, 2H), 5.31 (t, J = 9.32, 9.32 Hz, 1H), 5.17 (d, J = 3.54 Hz, 1H), 4.97-4.82 (m, 3H), 4.78-4.65 (m, 4H), 4.53 (dd, J = 11.31, 6.53 Hz, 1H), 4.44 (dd, J = 24.09, 11.80 Hz, 2H), 4.38-4.30 (m, 2H), 4.08-3.87 (m, 5H), 3.82-3.71 (m, 1H), 3.63-3.47 (m, 3H), 3.46 (dd, J = 6.32, 5.10 Hz, 1H), 3.43-3.37 (m, 1H), 1.97 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.1, 138.6, 138.5, 138.4, 138.3, 137.9, 137.8, 137.6, 137.2, 137.2, 137.0, 128.7, 128.5, 128.3, 128.3, 128.0, 127.8, 127.6, 127.5, 126.3, 126.2, 101.5, 101.5, 97.5, 97.2, 91.2, 84.3, 79.5, 79.3, 79.1, 78.8, 75.2, 75.0, 74.4, 74.3, 73.6, 73.5, 72.9, 72.5, 71.8, 70.7, 70.3, 70.1, 69.0, 68.8, 68.7, 68.0, 66.9, 62.9, 60.5, 14.3. **EI-MS *m/z*** calcd for $C_{49}H_{52}O_{12}$ 832.34. Found: $[M+H_2O]^+$ 850.38.

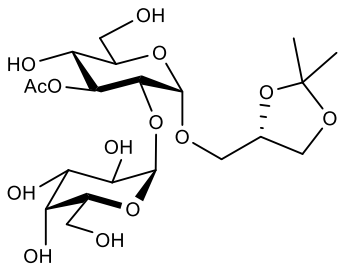
(2R,4aR,6S,7R,8S,8aR)-6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-phenyl-7-(((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-8-yl acetate 523



523

Compound **522** (50.0 mg, 0.06 mmol) and diphenylsulfoxide (36.0 mg, 0.17 mmol) were stirred over (130 mg) of activated 4Å MS for 45 min in CH_2Cl_2 (4 mL), then the mixture was cooled to –78°C, and triflic anhydride (14 μL, 0.08 mmol) and 2-chloro-pyridine (30 μL, 0.32 mmol) were added and warmed to –40°C and stirred for 45 min, then compound **523** (25.0 mg, 0.19 mmol) in CH_2Cl_2 (1 mL) was added and allowed to stir for 16 h. The reaction mixture was quenched with Et_3N (0.5 mL). The mixture passed through a pad of Celite and washed with CH_2Cl_2 (50 mL). The organic phase was washed with water (2 x 30 mL), saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (10:1) and passed through a silica gel column to produce **523**, the title compound as a colorless liquid compound (25.0 mg, 46% yield). On replication, yields varied between 40% and 46% for scales between 50.0 mg and 1.00 g. Rf = 0.36. 1:9 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.71-7.60 (m, 1H), 7.48-7.42 (m, 3H), 7.37-7.28 (m, 19H), 7.41-7.38 (m, 2H), 5.52 (t, *J* = 9.72, 9.72 Hz, 1H), 5.45 (s, 1H), 5.00 (d, *J* = 3.58 Hz, 1H), 4.94 (dd, *J* = 7.38, 3.88 Hz, 2H), 4.79 (dd, *J* = 21.55, 11.34 Hz, 3H), 4.68 (d, *J* = 11.53 Hz, 1H), 4.56 (d, *J* = 11.32 Hz, 1H), 4.45 (dd, *J* = 30.60, 11.78 Hz, 2H), 4.27 (dd, *J* = 10.27, 4.93 Hz, 1H), 4.17-4.11 (m, 1H), 4.06-3.97 (m, 3H), 3.96-3.88 (m, 3H), 3.79-3.63 (m, 3H), 3.61-3.55 (m, 2H), 3.52-3.45 (m, 2H), 3.41 (dd, *J* = 10.63, 5.01 Hz, 1H), 1.96 (s, 3H), 1.41 (s, 3H), 1.31 (s, 3H)) ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 169.8, 145.7, 138.7, 138.7, 138.6, 138.0, 137.1, 131.0, 129.3, 129.0, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 126. 2, 124.8, 109.4, 101.5, 98.7, 97.8, 79.7, 78.8, 77.1, 76.1, 74.9, 74.8, 74.4, 73.5, 73.4, 72.8, 70.1, 70.0, 69.0, 68.9, 68.3, 66.7, 62.6, 26.7, 25.6, 21.0. **EI-MS *m/z*** calcd for $C_{55}H_{62}O_{14}$ 946.41. Found: $[M+Na]^+$ 969.40.

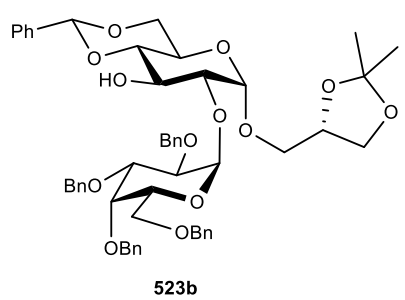
(2R,3R,4S,5R,6R)-2-(((2S,3R,4S,5S,6R)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol 523a



523a

To a solution of compound **523** (200 mg, 0.21 mmol) in MeOH (10 mL) Pd/C (1.27 g, 5.90 mmol) and ammonium formate (0.73 g, 11.6 mmol) were added and refluxed at 70°C for 7 h. After cooling to room temperature, the reaction mixture passed through a pad of Celite and washed with MeOH (50 mL). The solvent was evaporated and then co-evaporated with toluene. The crude product was dissolved in ethyl acetate/methanol (9:1) and passed through a silica gel column to produce **523a**, the title compound as a colorless liquid (72.0 mg, 70% yield). On replication, yields varied between 62% and 70% for scales between 50.0 mg and 500 mg. Rf = 0.29. EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.07 (dd, *J* = 32.12, 3.61 Hz, 2H), 4.47-4.29 (m, 1H), 4.20-4.00 (m, 2H), 4.00-3.86 (m, 1H), 3.86-3.65 (m, 9H), 3.64-3.45 (m, 4H), 1.92 (s, 3H), 1.41 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 177.9, 109.3, 96.7, 96.3, 76.1, 74.7, 72.3, 72.0, 71.0, 70.2, 70.0, 69.7, 68.9, 68.5, 66.1, 61.4, 25.6, 24.2, 22.1, 19.4. **EI-MS *m/z*** calcd for $C_{20}H_{34}O_{14}$: 498.19. Found: $[M+ Na]^+$ 479.17, note: Ac removed in the process.

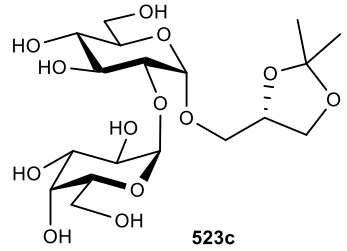
(2R,4aR,6S,7R,8S,8aS)-6-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-phenyl-7-(((2R,3R,4S,5S,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-8-ol 523b



523b

To a solution of compound **523** (90.0 mg, 0.09 mmol) in methanol (50 mL), solution of sodium methoxide was added until a pH of 12 was reached, then stirred for 16 h. Dowex 50WX8 was added to neutralize solution. Mixture filtered and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **523b**, title compound as a colourless liquid (60.0 mg, 70% yield). On replication, yields varied between 65% and 70% for scales between 50.0 mg and 500 mg. Rf = 0.36. 2:8 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.56-7.49 (m, 2H), 7.43-7.34 (m, 19H), 7.34-7.25 (m, 4H), 5.53 (s, 1H), 5.05 (d, *J* = 3.75 Hz, 1H), 4.95 (dd, *J* = 7.59, 3.90 Hz, 2H), 4.84 (d, *J* = 11.55 Hz, 2H), 4.77 (d, *J* = 11.88 Hz, 1H), 4.69 (d, *J* = 11.74 Hz, 1H), 4.58 (d, *J* = 11.51 Hz, 1H), 4.51 (d, *J* = 11.75 Hz, 1H), 4.41 (d, *J* = 11.75 Hz, 1H), 4.29 (dd, *J* = 10.22, 4.83 Hz, 1H), 4.26-4.19 (m, 2H), 4.13-4.07 (m, 2H), 4.02-3.87 (m, 4H), 3.83 (dd, *J* = 8.28, 6.29 Hz, 1H), 3.77-3.65 (m, 3H), 3.60-3.49 (m, 3H), 3.49-3.39 (m, 2H), 1.43 (s, 3H), 1.35 (s, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 138.6, 138.5, 138.3, 137.8, 137.7, 137.2, 129.1, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 126.4, 109.4, 102.0, 98.2, 98.1, 81.1, 81.1, 78.7, 76.3, 75.6, 75.3, 75.0, 74.7, 74.7, 74.5, 74.5, 74.3, 73.7, 73.6, 73.5, 73.4, 73.4, 73.0, 72.8, 70.3, 69.2, 69.2, 69.0, 68.9, 66.6, 62.4, 26.7, 25.5. **EI-MS *m/z*** calcd for $C_{53}H_{60}O_{13}$ 904.40. Found: $[M+Na]^+$ 927.39.

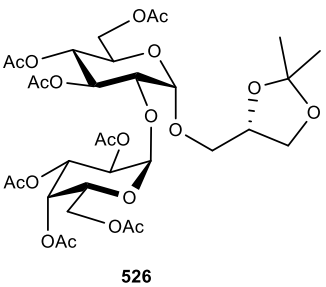
(2R,3R,4S,5R,6R)-2-(((2S,3R,4S,5S,6R)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol 523c



523c

To a solution of acetyl deprotected of **523b** (60.0 mg, 0.06 mmol) in MeOH (3 mL), Pd/C (400 mg, 1.86 mmol) and ammonium formate (230 mg, 3.65 mmol) were added and refluxed at 70°C for 7 h. After cooling to room temperature, the reaction mixture was then passed through a pad of Celite and washed with MeOH (20 mL). The solvent was concentrated under reduced pressure and then co-evaporated with toluene. The crude product was dissolved in ethyl acetate/methanol (9.5:0.5) and passed through a silica gel column to produce **523c**, title compound as a colourless liquid (23.0 mg, 73% yield). On replication, yields varied between 68% and 73% for scales between 60.0 mg and 200 mg. Rf = 0.29. 95:5 EtOAc/MeOH. ^{1}H NMR (500 MHz, MeOD) δ_{ppm} 5.12 (d, *J* = 3.55 Hz, 1H), 5.06 (d, *J* = 3.73 Hz, 1H), 4.69-4.51 (m, 2H), 4.44-4.30 (m, 1H), 4.19-4.02 (m, 2H), 3.92 (dd, *J* = 3.12, 1.08 Hz, 1H), 3.87-3.66 (m, 8H), 3.66-3.55 (m, 3H), 1.43 (s, 3H), 1.37 (s, 3H) ^{13}C NMR (125 MHz, MeOD) δ_{ppm} 109.3, 96.7, 76.2, 74.7, 73.0, 72.3, 72.0, 71.0, 70.2, 70.0, 69.7, 68.9, 68.5, 66.1, 61.4, 61.2, 25.6, 24.2. **EI-MS *m/z*** calcd for $C_{18}H_{32}O_{13}$ 456.18. Found: $[M+Na]^{+}$ 479.17.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 526

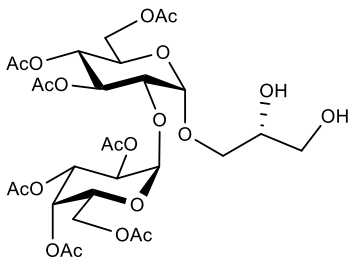


526

Compound **523a** (220 mg, 0.44 mmol) was dissolved in pyridine (6.4 mL), and cooled to 0°C, then acetic anhydride (3.2 mL) was added dropwise and stirred for 16 h. The reaction mixture was diluted EtOAc (100 mL), and the mixture was washed with 10% HCl aq (50 mL), with water (50 mL) and with brine (50 mL). The organic phase was

dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (2:8) and passed through a silica gel column to produce **526**, the yellow liquid compound (322 mg, 73% yield). On replication, yields varied between 70% and 73% for scales between 50.0 mg and 220 mg. Rf = 0.27. 9:1 hexanes/ethyl acetate. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.51-5.39 (m, 1H), 5.31-5.22 (m, 2H), 5.18-5.11 (m, 1H), 5.11-5.04 (m, 1H), 5.03-4.93 (m, 1H), 4.36-4.22 (m, 3H), 4.23-4.15 (m, 1H), 4.16-4.05 (m, 4H), 3.98 (dd, J = 10.96, 6.19 Hz, 1H), 3.84-3.68 (m, 3H), 3.59 (dd, J = 10.72, 5.32 Hz, 1H), 2.09 (7s, 21H), 1.44 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.7, 170.5, 170.4, 170.2, 170.1, 170.0, 169.9, 109.8, 96.2, 95.2, 74.8, 74.5, 71.3, 69.0, 68.6, 68.3, 67.9, 67.7, 67.4, 67.3, 66.6, 62.0, 61.3, 26.9, 25.5, 20.9, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6. **EI-MS *m/z*** was not collected.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-2-((R)-2,3-dihydroxypropoxy)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 526a

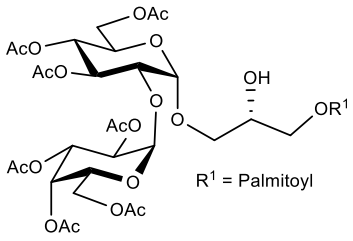


526a

Compound **526** (130 mg, 0.17 mmol) was dissolved in acetonitrile (6 mL) and $Zn(NO_3)_2.6H_2O$ (260 mg, 1.37 mmol) was added. The mixture was stirred for 10 h at 50°C, and the solvent was then evaporated under reduced pressure, then CH_2Cl_2 (50 mL) was added and the organic phase was washed with water (2 x 30 mL) and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in methanol/ethyl acetate (1:9) and passed through a silica gel column to produce **526a**, title compound as a liquid (75.0 mg, 62% yield). On replication, yields varied between 57% and 62% for scales between 30.0 mg and 500 mg. Rf = 0.31. 7:3 hexanes/ethyl acetate. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.48-5.41 (m, 2H), 5.39-5.27 (m, 2H), 5.19-5.03 (m, 2H), 4.99

(t, J = 9.80, 9.80 Hz, 1H), 4.34-4.21 (m, 2H), 4.18 (dd, J = 11.02, 7.04 Hz, 1H), 4.13-4.02 (m, 2H), 4.03-3.90 (m, 2H), 3.85 (dd, J = 10.57, 3.50 Hz, 1H), 3.82-3.68 (m, 2H), 3.66-3.53 (m, 2H), 2.99 (d, J = 4.48 Hz, 1H), 2.29 (t, J = 6.01, 6.01 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.7, 170.6, 170.3, 170.2, 170.1, 170.0, 169.7, 128.4, 127.7, 96.9, 96.0, 71.3, 70.6, 70.4, 68.6, 68.4, 67.7, 67.6, 67.5, 67.2, 63.5, 61.9, 61.0, 20.8, 20.7, 20.7, 20.7, 20.6, 20.6. **EI-MS *m/z*** calcd for $C_{29}H_{42}O_{20}$: 710.23. Found: $[M+ Na]^+$ 733.22.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-2-((S)-2-hydroxy-3-(palmitoyloxy)propoxy)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 526b

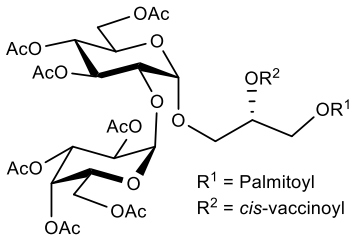


526b

Compound **526a** (250 mg, 0.35 mmol), was dissolved in anhydrous CH_2Cl_2 (4 mL) under nitrogen and palmitic acid (90.0 mg, 0.35 mmol), EDC (67.0 mg, 0.35 mmol), and DMAP (2 pinches) were added under nitrogen at 0°C and the reaction was stirred on ice for 6 h. CH_2Cl_2 (30 mL) and water (10 mL) were added to the reaction mixture and the organic phase was washed water (2 x 10 mL) and once with brine (10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in ethyl acetate and passed through a silica gel column to produce **526b**, title compound as a colourless liquid (210 mg, 63% yield). Rf = 0.55. EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.53-5.42 (m, 2H), 5.41-5.34 (m, 1H), 5.34-5.22 (m, 1H), 5.13 (d, *J* = 3.53 Hz, 1H), 5.08 (dd, *J* = 10.77, 3.59 Hz, 1H), 5.01 (t, *J* = 9.71, 9.71 Hz, 1H), 4.35-4.24 (m, 2H), 4.25-4.04 (m, 6H), 4.01 (dd, *J* = 11.03, 6.17 Hz, 1H), 3.85-3.77 (m, 2H), 3.53 (dd, *J* = 10.83, 7.58 Hz, 1H), 3.05 (d, *J* = 4.09 Hz, 1H), 2.38 (t, *J* = 7.61, 7.61 Hz, 2H), 2.13 (7s, 21H), 1.69-1.61 (m, 2H),1.33-1.25 (m,

24H), 0.91 (t, *J* = 6.94, 6.94 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 173.8, 173.7, 170.8, 170.5, 170.3, 170.0, 170.0, 169.7, 96.8, 95.3, 74.9, 71.1, 70.1, 69.2, 68.5, 68.5, 67.8, 67.6, 67.4, 67.2, 64.9, 61.9, 61.1, 34.1, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.2, 29.1, 27.2, 27.2, 24.9, 22.7, 20.8, 20.7, 20.6, 20.6, 20.6, 14.1. **EI-MS *m/z*** calcd for $C_{45}H_{72}O_{21}$: 948.46. Found: $[M]^+$ 949.46.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)-2-((S)-2-((Z)-octadec-6-enoyloxy)-3-(palmitoyloxy)propoxy)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 527

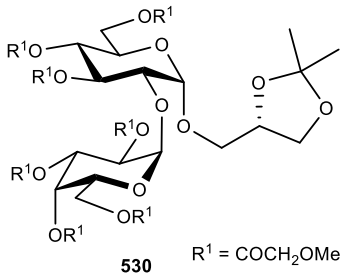


527

Compound **526b** (50.0 mg, 0.05 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) under nitrogen and *cis*-vaccenic acid (23.0 mg, 0.08 mmol), EDC (17.0 mg, 0.08 mmol), and DMAP (2 pinches) were added under nitrogen at 0°C and the reaction was stirred on ice for 16 h. CH_2Cl_2 (30 mL) and water (10 mL) were added to the reaction mixture and the organic phase was washed with water (2 x 10 mL) and once with brine (10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:2) and passed through a silica gel column to produce **527**, title compound as a colourless liquid (62.0 mg, 64% yield). Rf = 0.29. 8:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 5.48-5.43 (m, 1H), 5.41 (t, J = 9.65, 9.65 Hz, 1H), 5.37-5.33 (m, 2H), 5.29-5.18 (m, 3H), 5.07 (dd, J = 10.86, 3.58 Hz, 1H), 5.02-4.92 (m, 2H), 4.42 (dd, J = 12.00, 3.48 Hz, 1H), 4.32-4.14 (m, 4H), 4.08-3.94 (m, 3H), 3.83 (dd, J = 10.68, 5.53 Hz, 1H), 3.78 (dd, J = 9.98, 3.62 Hz, 1H), 3.67 (dd, J = 10.67, 4.82 Hz, 1H), 2.38-2.27 (m, 4H), 2.16-1.93 (m, 25H), 1.67-1.54 (m, 4H), 1.40-1.17 (m, 48H), 0.88 (dd, J = 7.81, 6.07 Hz, 6H)). ^{13}C NMR (125 MHz, $CDCl_3$) 168.7, 168.4,

166.0, 165.9, 165.8, 165.5, 165.4, 165.2, 165.0, 125.4, 125.3, 92.0, 91.1, 70.6, 66.7, 65.0, 63.9, 63.8, 63.3, 63.3, 63.2, 62.9, 62.9, 62.7, 62.4, 57.7, 57.6, 57.3, 57.2, 56.7, 56.6, 29.7, 29.6, 29.5, 27.4, 27.3, 25.3, 25.2, 25.2, 25.2, 25.0, 25.0, 25.0, 24.9, 24.9, 24.8, 24.7, 24.6, 24.5, 22.7, 20.4, 20.4, 20.4, 20.3, 20.2, 18.2, 18.1, 18.1, 16.2, 16.2, 16.2, 16.1, 9.6. **EI-MS *m/z*** calcd for $C_{63}H_{104}O_{22}$: 1213.70. Found: $[M]^+$ 1214.70.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-2-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 530

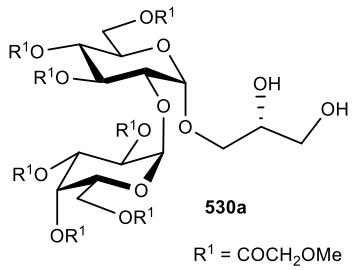


530 $R^1 = COCH_2OMe$

Compound **523c** (40.0 mg, 0.09 mmol) was dissolved in pyridine (2 mL), and cooled to 0°C, then methoxyacetyl chloride (3 mL, 1.10 mmol) was added dropwise and stirred for 16 h. The reaction mixture diluted with EtOAc (50 mL) and the organic phase was washed with 10% HCl aq (30 mL), water (30 mL) and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (2:8) and passed through a silica gel column to produce **530**, title compound as a yellow liquid (60.0 mg, 72% yield). On replication, yields varied between 68% and 72% for scales between 20.0 mg and 100 mg. Rf = 0.3. 2:8 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.55-5.48 (m, 2H), 5.38-5.31 (m, 2H), 5.13 (dd, *J* = 10.77, 3.50 Hz, 1H), 5.10-5.04 (m, 2H), 4.40-4.34 (m, 2H), 4.33-4.26 (m, 2H), 4.25-3.93 (m, 17H), 3.86 (d, *J* = 16.51 Hz, 1H), 3.82 (dd, *J* = 9.95, 3.54 Hz, 1H), 3.73 (ddd, *J* = 15.34, 9.62, 5.30 Hz, 2H), 3.59 (dd, *J* = 10.96, 5.67 Hz, 1H), 3.46-3.39 (m, 21H), 1.43 (s, 3H), 1.36 (s, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.0, 169.8, 169.8, 169.7, 169.6, 169.4, 169.3, 109.8, 95.8, 94.8, 74.5, 74.5, 71.8, 69.7, 69.5, 69.3, 69.0, 68.6, 68.3, 68.2,

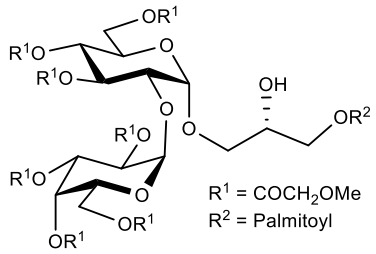
68.1, 67.5, 67.3, 67.1, 66.2, 61.9, 61.0, 61.0, 59.5, 59.4, 38.8, 30.4, 29.0. 26.7, 25.4, 23.8, 23.0, 14.1, 11.0. **EI-MS *m/z*** calcd for $C_{39}H_{60}O_{27}$ 960.33. Found: $[M+Na]^+$ 983.33.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-2-((R)-2,3-dihydroxypropoxy)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 530a



Compound **530** (17.0 mg, 0.02 mmol) was dissolved in acetonitrile (2 mL) and $Zn(NO_3)_2.6H_2O$ (32.0 mg, 0.11 mmol) was added. The mixture was stirred for 6 h at 50°C, and the solvent was then evaporated under reduced pressure, then CH_2Cl_2 (30 mL) was added and the organic phase was washed with water (20 mL) and brine (20 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in methanol/ethyl acetate (1:9) and passed through a silica gel column to produce **530a**, title compound as a colourless liquid (15.0 mg, 96% yield). On replication, yields varied between 89% and 96% for scales between 17.0 mg and 100 mg. Rf = 0.38. 9:1 EtOAc/MeOH. ^{1}H NMR (500 MHz, MeOD) δ_{ppm} 5.59 (dd, *J* = 3.27, 1.21 Hz, 1H), 5.55 (t, *J* = 9.69, 9.69 Hz, 1H), 5.44 (d, *J* = 3.60 Hz, 1H), 5.38 (dd, *J* = 10.80, 3.42 Hz, 1H), 5.24 (dd, *J* = 10.76, 3.57 Hz, 1H), 5.20 (d, *J* = 3.52 Hz, 1H), 5.11 (dd, *J* = 10.20, 9.40 Hz, 1H), 4.47-4.39 (m, 2H), 4.36 (dd, *J* = 11.11, 6.25 Hz, 1H), 4.27-4.17 (m, 6H), 4.16-3.98 (m, 12H), 3.92 (d, *J* = 17.02 Hz, 1H), 3.89-3.81 (m, 2H), 3.67-3.59 (m, 2H), 3.57 (dd, *J* = 10.03, 6.05 Hz, 1H), 3.50-3.38 (m, 22H) ^{13}C NMR (125 MHz, MeOD) δ_{ppm} 171.94, 171.92, 171.82, 171.68, 171.47, 171.22, 171.14, 97.25, 95.97, 75.21, 73.18, 72.06, 70.97, 70.64, 70.57, 70.43, 70.36, 70.25, 70.20, 70.16, 70.14, 69.77, 69.28, 69.22, 68.56, 68.47, 64.25, 63.17, 62.77, 61.55, 59.81, 59.72, 59.68, 59.65, 59.61, 59.48. **EI-MS *m/z*** calcd for $C_{36}H_{56}O_{27}$ 920.30. Found: $[M+H]^+$ 921.31.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-2-((S)-2-hydroxy-3-(palmitoyloxy)propoxy)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 530b

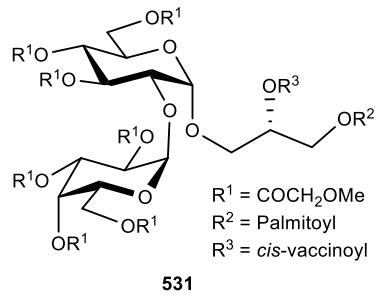


530b

Compound **530a** (12.0 mg, 0.01 mmol), was dissolved in anhydrous CH_2Cl_2 (2 mL) under nitrogen and palmitic acid (4.00 mg) , 0.01 mmol), EDC (2.50 mg, 0.01 mmol), and DMAP (2 pinches) were added under nitrogen at 0°C and the reaction was stirred on ice for 6 h. then CH_2Cl_2 (20 mL) was added and the organic phase was washed with water (10 mL) and brine (10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in ethyl acetate and passed through a silica gel column to produce **530b**, title compound as a colourless liquid (8.00 mg, 53% yield). On replication, yields varied between 48% and 53% for scales between 12.0 mg and 50.0 mg. Rf = 0.55 EtOAc. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.62-5.45 (m, 2H), 5.41-5.29 (m, 2H) 5.18-5.02 (m, 3H), 4.43-4.34 (m, 2H), 4.34-4.25 (m, 1H), 4.21-3.95 (m, 19H), 3.90 (s, 1H), 3.88-3.82 (m, 1H), 3.77 (dd, *J* = 11.03, 3.09 Hz, 1H), 3.53 (dd, *J* = 10.97, 7.30 Hz, 1H), 3.49-3.33 (m, 21H), 3.06 (d, *J* = 4.27 Hz, 1H), 2.36-2.32 (m, 2H), 1.66-1.60 (m, 2H), 1.37-1.17 (m, 24H), 0.89 (t, *J* = 6.96, 6.96 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 171.94, 171.92, 171.82, 171.68, 171.47, 171.22, 171.14, 97.25, 95.97, 75.21, 73.18, 72.06, 70.97, 70.64, 70.57, 70.43, 70.36, 70.25, 70.20, 70.16, 70.14, 69.77, 69.28, 69.22, 68.56, 68.47, 64.25, 63.17, 62.77, 61.55, 59.81, 59.72, 59.68, 59.65, 59.61, 59.48. **EI-MS *m/z*** calcd for $C_{70}H_{118}O_{29}$ 1158.53. Found: $[M]^+$ 1159.54.

(2R,3R,4S,5S,6R)-2-(((2S,3R,4S,5R,6R)-4,5-bis(2-methoxyacetoxy)-6-((2-methoxyacetoxy)methyl)-2-((S)-2-((Z)-octadec-6-enoyloxy)-3-(palmitoyloxy)propoxy)tetrahydro-2H-

pyran-3-yl)oxy)-6-((2-methoxyacetoxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methoxyacetate) 531



531

Compound **530b** (11.0 mg, 0.01 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) under nitrogen and *cis*-vaccenic acid (4.00 mg, 0.01 mmol), EDC (3.00 mg, 0.01 mmol), and DMAP (2 pinches) were added under nitrogen at 0°C and the reaction was stirred on ice for 16 h. then CH_2Cl_2 (20 mL) was added and the organic phase was washed with water (10 mL) and brine (10 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:3) and passed through a silica gel column to produce **531**, title compound as a colourless liquid (7.00 mg, 50% yield). Rf = 0.4. 2.5:7.5 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.57-5.50 (m, 1H), 5.47 (t, J = 9.56, 9.56 Hz, 1H), 5.39 (dd, J = 10.70, 3.27 Hz, 1H), 5.36-5.33 (m, 2H), 5.27 (d, J = 3.51 Hz, 1H), 5.25-5.18 (m, 1H), 5.15 (dd, J = 10.76, 3.54 Hz, 1H), 5.10-5.02 (m, 1H), 5.00 (d, J = 3.52 Hz, 1H), 4.46-4.33 (m, 3H), 4.29 (dd, J = 11.08, 7.13 Hz, 1H), 4.23 (dd, J = 12.01, 6.49 Hz, 1H), 4.19-3.94 (m, 16H), 3.90-3.77 (m, 3H), 3.71 (dd, J = 10.93, 4.75 Hz, 1H), 3.52-3.31 (m, 21H), 2.40-2.25 (m, 4H), 2.06-1.96 (m, 4H), 1.70-1.61 (m, 4H), 1.39-1.09 (m, 44H), 0.92-0.83 (m, 6H) ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 173.4, 173.1, 170.0, 169.9, 169.9, 169.8, 169.7, 169.4, 169.3, 130.1, 129.9, 96.8, 96.7, 96.1, 76.3, 76.2, 76.2, 76.1, 76.1, 76.0, 75.8, 75.8, 71.9, 71.9, 69.7, 69.7, 69.5, 69.5, 69.4, 69.4, 68.9, 68.6, 68.5, 68.3, 67.7, 67.6, 67.6, 67.5, 67.4, 62.3, 61.8, 61.0, 59.5, 59.5, 59.5, 34.3, 34.2, 34.1, 32.0, 31.9, 29.9, 29.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.5, 29.4, 29.3, 29.3, 29.3, 29.1, 27.3, 25.0, 25.0, 22.8, 22.8, 14.2, 14.2. **EI-MS *m/z*** calcd for $C_{70}H_{118}O_{29}$ 1422.77. Found: $[M+H]^+$ 1423.78.

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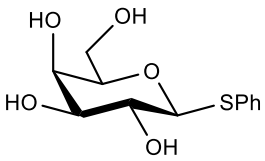
9.9 Experimental protocols for Chapter 6

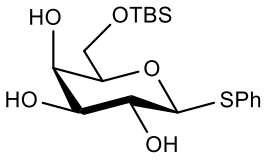
(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triol 601



601

Compound **454a** (20.7 g, 47 mmol) was dissolved in methanol (300 mL) then NaOMe solution was added until a pH of 12 was reached. Upon stirring for 16 h, Dowex 50WX8 was added to neutralize the solution. The solution was filtered and concentrated in reduced pressure, and co-evaporated with toluene. The crude product was dissolved in ethyl acetate /methanol (8:2) and passed through a silica gel column to produce **601**, the title compound, as a colourless liquid (10.7 g, 84% yield). On replication, yields varied between 80% and 84% for scales between 100 mg and 40.0 g. Rf = 0.30 (80% ethyl acetate, 20% methanol). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.55 (dd, *J* = 8.1, 1.6 Hz, 2H), 7.35 – 7.18 (m, 3H), 4.59 (d, *J* = 9.7 Hz, 1H), 3.86 (dd, J = 12.07, 1.75 Hz, 1H), 3.74-3.47 (m, 2H), 3.44-3.33 (m, 1H), 3.26-3.15 (m, 2H). Consistent with the literature.[1]

(2R,3R,4S,5R,6S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triol 602

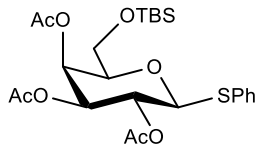


602

To a solution of compound **601** (4.95 g, 18 mmol) in anhydrous DMF (20 mL), *tert*-butyldimethylsilyl chloride (3.06 g, 20 mmol), imidazole (2.74 g, 40 mmol), and DMAP (0.45 g, 4 mmol) were added at 0°C. The ice bath was then removed, and the reaction mixture stirred at ambient temperature for 12 h. The reaction was diluted with saturated aqueous $NaHCO_3$ (60 mL) and ethyl acetate (100 mL) was added, and the organic phase was separated and organic phase was washed with saturated aqueous $NaHCO_3$ (2 x 50 mL), water (50 mL), and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **602**, the title compound, as a colourless liquid

(2.34 g, 76% yield). On replication, yields varied between 64% and 76% for scales between 50 mg and 5.00 g. Rf = 0.30. 8:2 EtOAc/hexanes. ^{1}H NMR (500 MHz, MeOD) δ_{ppm} 7.51 (d, *J* = 7.2 Hz, 2H), 7.27 (t, *J* = 5.2 Hz, 3H), 5.23 (dd, *J* = 16.3, 5.3 Hz, 1H), 4.20 – 4.00 (m, 2H), 4.00 – 3.89 (m, 1H), 3.82 (s, 1H), 3.76 – 3.51 (m, 3H), 3.43 (s, 1H), 1.27 (d, *J* = 10.2 Hz, 1H), 0.97 – 0.76 (m, 9H), 0.21 – -0.08 (m, 6H). Consistent with the literature.[2]

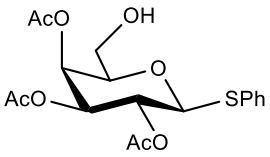
(2R,3S,4S,5R,6S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 603



603

To a solution of compound **602** (1.53 g, 4.20 mmol) in pyridine (145 mL), acetic anhydride (20 mL) was added. The reaction mixture was stirred at room temperature for 12 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 5% HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **603**, the title compound, as a colourless liquid (1.61 g, 74%yield). On replication, yields varied between 68% and 74% for scales between 50.0 mg and 2.00 g. Rf = 0.40. 3:7 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.50 (dd, *J* = 6.7, 2.9 Hz, 2H), 7.31 (dd, *J* = 5.1, 2.1 Hz, 3H), 5.50 (d, *J* = 3.3 Hz, 1H), 5.23 (t, J = 9.97, 9.97 Hz, 1H), 5.06 (dd, J = 9.91, 3.34 Hz, 1H), 4.72 (d, J = 10.06 Hz, 1H), 3.81-3.67 (m, 2H), 3.60 (dd, J = 9.18, 6.63 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 1.96 (s, 3H), 0.84 (s, 9H), 0.00 (d, J = 4.94 Hz, 6H). Consistent with the literature.[3]

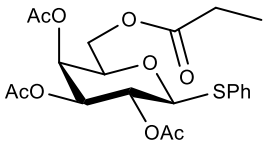
(2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyltriacetate 604



604

Compound **603** (1.64 g, 3.20 mmol) was dissolved in THF (20 mL) and TBAF (4 mL) was added and stirred at room temperature for 30 min. The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (6:4) and passed through a silica gel column to produce **604**, the title compound, as a colourless liquid (0.98 g, 77% yield). On replication, yields varied between 68% and 77% for scales between 50.0 mg and 2.00 g. Rf = 0.30. 4:6 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.57-7.37 (m, 2H), 7.34-7.26 (m, 3H), 5.09-4.97 (m, 1H), 4.96-4.85 (m, 1H), 4.76-4.63 (m, 1H), 4.51-4.40 (m, 1H), 4.34 (d, J = 12.05 Hz, 1H), 3.58-3.48 (m, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H). Consistent with the literature.[3]

(2S,3R,4S,5S,6R)-2-(phenylthio)-6-((propionyloxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate 605

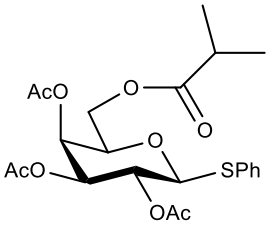


605

To a solution of compound **604** (250 mg, 0.63 mmol) in $C_2H_4Cl_2$ (5 mL), propionyl chloride (0.16 mL, 1.83 mmol), pyridine (0.15 mL) and DMAP (30.0 mg, 0.25 mmol) were added at 0°C and slowly warmed up to the refluxed at 85°C for 4 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 2M HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **605**, the title compound, as a colourless liquid (230 mg, 80% yield). Rf = 0.26 3:7 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.47 – 7.37 (m, 2H), 7.31 – 7.18 (m, 3H), 5.17 (t, *J* = 9.3 Hz, 1H), 4.95 (dt, *J* = 22.7, 9.5 Hz, 2H), 4.65 (d, *J* = 10.1 Hz, 1H), 4.21 – 4.05 (m, 2H), 3.67 (ddd, *J* = 10.0, 4.8, 2.8 Hz, 1H), 2.39 – 2.13 (m, 2H), 2.09 – 1.86 (m, 9H), 1.12 – 0.94 (m, 3H). ^{13}C NMR

(76 MHz, MeOD) δ 170.48, 170.09, 169.30, 165.13, 133.70, 133.12, 129.85, 128.98, 128.63, 128.44, 85.86, 75.96, 73.72, 70.10, 69.10, 62.64, 20.79, 20.68, 20.54. **EI-MS *m/z*** was not collected.

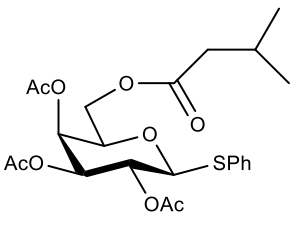
(2R,3S,4S,5R,6S)-2-((isobutyryloxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 606



606

To a solution of compound **604** (225 mg, 0.56 mmol) in $C_2H_4Cl_2$ (5 mL), *iso*-butyryl chloride (0.18 mL, 1.72 mmol), pyridine (0.14 mL), and DMAP (30.0 mg, 0.25 mmol) were added to the reaction in 0°C and slowly warmed up to the refluxed at 85°C for 4 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 2M HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **606**, the title compound, as a colourless liquid (260 mg, 97% yield). Rf = 0.27. 3:7 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.55 – 7.42 (m, 2H), 7.37 – 7.28 (m, 3H), 5.25 (t, *J* = 9.4 Hz, 1H), 5.15 – 4.88 (m, 2H), 4.71 (d, *J* = 10.1 Hz, 1H), 4.28 – 4.06 (m, 2H), 3.73 (ddd, *J* = 10.0, 4.7, 2.9 Hz, 1H), 2.50 (p, *J* = 7.0 Hz, 1H), 2.14 – 1.91 (m, 9H), 1.10 (dd, *J* = 7.0, 5.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 170.5, 170.0, 169.3, 133.0, 131.8, 128.9, 128.4, 85.8, 76.0, 73.8, 70.0, 67.8, 62.2, 33.9, 20.7, 20.5, 18.8, 18.7. **EI-MS *m/z*** was not collected.

(2R,3S,4S,5R,6S)-2-(((3-methylbutanoyl)oxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 607

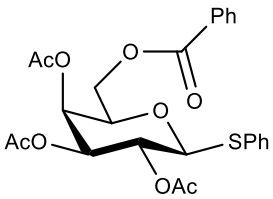


607

To a solution of compound **604** (212 mg, 0.53 mmol) in $C_2H_4Cl_2$ (5 mL), *iso*-pentanoyl chloride (0.19 mL, 1.56 mmol), pyridine (0.13 mL), and DMAP (30.0 mg, 0.25 mmol) were added to the reaction in 0°C, and

slowly warmed up to the refluxed at 85°C for 4 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 2M HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **607**, the title compound, as a colourless liquid (144 mg, 56% yield). Rf = 0.28. 3:7 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.51 – 7.35 (m, 2H), 7.32 – 7.23 (m, 3H), 5.20 (t, *J* = 9.4 Hz, 1H), 5.09 – 4.85 (m, 2H), 4.67 (d, *J* = 10.1 Hz, 1H), 4.14 (d, *J* = 3.9 Hz, 2H), 3.67 (dt, *J* = 10.0, 3.9 Hz, 1H), 2.31 – 1.76 (m, 12H), 0.86 (dd, *J* = 6.5, 0.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_{ppm} 171.4, 170.5, 170.0, 169.2, 133.1, 131.7, 128.9, 128.4, 85.7, 76.0, 73.8, 70.1, 67.8, 62.2, 42.9, 25.4, 22.2, 20.7, 20.7, 20.6. **EI-MS *m/z*** was not collected.

(2R,3S,4S,5R,6S)-2-((benzoyloxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 608

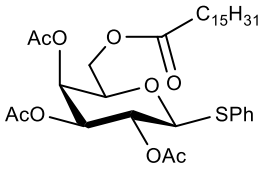


608

To a solution of compound **604** (130 mg, 0.33 mmol) in pyridine (5 mL), benzoyl chloride (0.12 mL, 1.03 mmol), and DMAP (20.0 mg, 0.16 mmol) were added and stirred at 0°C and slowly warmed up to the re-fluxed at 85°C for 4 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 2M HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure and co-evaporated with toluene. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **608**, the title compound, as a colourless liquid (125 mg, 80% yield). Rf = 0.30. 2:8 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.89 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.58 – 7.42 (m, 3H), 7.37 (dd, *J* = 8.4, 7.0 Hz, 2H), 7.32 – 7.17 (m, 3H), 5.38 (t, *J* = 9.4 Hz, 1H), 5.24 (t, *J* =

9.7 Hz, 1H), 4.99 (dd, *J* = 10.0, 9.2 Hz, 1H), 4.73 (d, *J* = 10.1 Hz, 1H), 4.17 (d, *J* = 4.2 Hz, 2H), 3.82 (dt, *J* = 9.8, 4.2 Hz, 1H), 2.16 – 1.74 (m, 9H). ^{13}C NMR and **EI-MS *m/z*** were not collected.

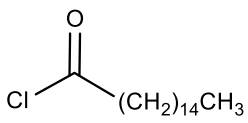
(2R,3S,4S,5R,6S)-2-((palmitoyloxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate 609



609

To a solution of compound **604** (125 mg, 0.31 mol) in CH_2Cl_2 (10 mL), palmitic acid (121 mg, 0.47 mmol), EDC (94.0 mg, 0.49 mmol), and DMAP (20.0 mg, 0.16 mmol) were added at 0°C and stirred at room temperature for 12 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 2M HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **609**, the title compound, as a colourless liquid (160 mg, 74% yield). Rf = 0.41. 3:7 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.53 – 7.46 (m, 2H), 7.31 (qd, *J* = 4.3, 1.6 Hz, 3H), 5.23 (t, *J* = 9.4 Hz, 1H), 5.06 (t, *J* = 9.8 Hz, 1H), 5.01 – 4.92 (m, 1H), 4.71 (d, *J* = 10.1 Hz, 1H), 4.24 – 4.11 (m, 2H), 3.72 (ddd, *J* = 10.1, 4.7, 3.1 Hz, 1H), 2.34 – 2.18 (m, 2H), 2.08 (d, *J* = 4.1 Hz, 6H), 1.97 (s, 3H), 1.66 – 1.43 (m, 2H), 1.38 – 1.09 (m, 24H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 172.24, 170.57, 170.12, 169.32, 133.11, 131.75, 128.98, 128.44, 85.79, 75.94, 73.95, 70.06, 67.92, 62.24, 34.05, 31.97, 30.53, 29.81, 29.74, 29.64, 29.57, 29.54, 29.47, 29.41, 29.37, 29.24, 29.16, 29.10, 29.07, 24.84, 22.74, 20.81, 20.66, 14.18. **EI-MS *m/z*** was not collected.

Palmitoyl chloride 609a

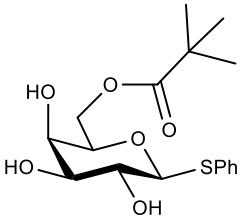


609a

Palmitic acid (5.00 g, 19.5 mmol) was dissolved in thionyl chloride (30 mL). The reaction was stirred under reflex for 12 h. The product was dried, fil-

tered, and concentrated under reduced pressure to produce palmitoyl chloride **609a**, crude: (5.73 g, 106% yield)). Rf = 0.31. 1:1 EtOAc/hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 2.88 (t, *J* = 7.3 Hz, 2H), 1.76 – 1.65 (m, 2H), 1.36 – 1.21 (m, 22H), 0.93 – 0.83 (m, 3H).[4]

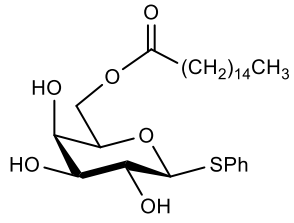
(2S,3R,4S,5S,6R)-2-(phenylthio)-6-((pivaloyloxy)methyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate 610



610

To a solution of compound **601** (105 mg, 0.39 mmol) in pyridine (1 mL), pivaloyl chloride (0.06 mL, 0.49 mmol) was added at 0°C and stirred for 12 h. The reaction was diluted with CH_2Cl_2 (50 mL) and the mixture was washed with 2M HCl (2 x 30 mL), water (30 mL), and brine (30 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. Due to the sudden lab shut-down, purification was not completed. (crude: 381 mg). Rf = 0.29. 9:1 EtOAc/MeOH. ^{1}H NMR (301 MHz, $CDCl_3$) δ_{ppm} 7.94 – 7.78 (m, 2H), 7.63 – 7.52 (m, 3H), 4.88 (dd, *J* = 9.7, 3.3 Hz, 1H), 4.62 (d, *J* = 9.8 Hz, 1H), 4.40 – 4.20 (m, 2H), 4.01 (s, 1H), 3.94 – 3.73 (m, 2H), 1.34 – 1.11 (m, 18H).[5]

((2R,3R,4S,5R,6S)-3,4,5-trihydroxy-6-(phenylthio)tetrahydro-2H-pyran-2-yl)methyl palmitate 611

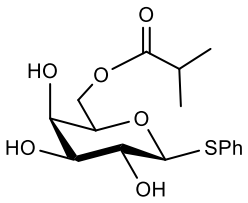


611

To solution of **609** (20.0 mg, 0.03 mmol) in $CHCl_3$/MeOH (1 mL), *t*-$BuNH_2$ (5 μL, 0.04 mmol) was added at 0°C and stirred for 12 h, then the same amount of reagent was added, as a sealed tube was not used in this reaction and the reagent evaporated and stirred for 12 h. The solvent concentrated under reduced pressure. The crude product was dissolved in ethyl acetate/methanol (9.5:0.5) and passed through a silica gel column to produce **611** (3.20 mg, 21% yield). Rf = 0.32. 9.5:0.5 EtOAc/MeOH. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.56 (dd, J = 6.56, 3.08 Hz, 2H), 7.35-

7.29 (m, 3H), 4.53 (q, J = 3.31, 3.31, 3.10 Hz, 1H), 4.29 (ddd, J = 9.06, 8.00, 2.26 Hz, 2H), 3.70-3.55 (m, 1H), 3.56-3.46 (m, 1H), 3.33 (t, J = 8.95, 8.95 Hz, 1H), 3.09-2.97 (m, 1H), 2.89-2.73 (m, 1H), 2.53 (s, 1H), 2.38 (t, J = 7.62, 7.62 Hz, 1H), 2.14-1.86 (m, 2H), 1.52-1.39 (m, 2H), 1.37-1.19 (m, 24H), 0.95 (t, J = 7.45, 7.45 Hz, 3H). Consistent with the literature.[6]

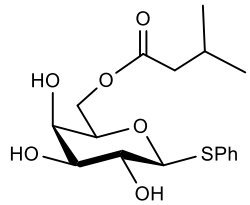
((2R,3R,4S,5R,6S)-3,4,5-trihydroxy-6-(phenylthio)tetrahydro-2H-pyran-2-yl)methyl isobutyrate 612



612

To a solution of Compound **606** (27.0 mg, 0.06 mmol) in $CHCl_3$/MeOH (1 mL), *t*-$BuNH_2$ (20 μL, 0.18 mmol) was added at 0°C and stirred for 6 h. The solvent concentrated under reduced pressure. The crude product was dissolved in ethyl acetate/methanol (9:1) and passed through a silica gel column to produce **612** (10.0 mg, 51% yield). Rf = 0.32. 9:1 EtOAc/MeOH. ^{1}H NMR (301 MHz, CD_3OD) δ_{ppm} 7.53 (dd, *J* = 7.5, 2.2 Hz, 2H), 7.34 – 7.20 (m, 3H), 4.58 (d, *J* = 9.8 Hz, 1H), 4.42 (dd, *J* = 11.9, 2.1 Hz, 1H), 4.15 (dd, *J* = 11.9, 6.5 Hz, 1H), 3.50 (ddd, *J* = 9.0, 6.5, 2.1 Hz, 1H), 3.38 (t, *J* = 8.8 Hz, 1H), 3.30 (p, *J* = 1.7 Hz, 2H), 3.27 – 3.12 (m, 2H), 2.57 (p, *J* = 7.0 Hz, 1H), 1.15 (dd, *J* = 7.0, 2.4 Hz, 6H). ^{13}C NMR and **EI-MS *m/z*** were not collected.

((2R,3R,4S,5R,6S)-3,4,5-trihydroxy-6-(phenylthio)tetrahydro-2H-pyran-2-yl)methyl-3-methylbutanoate 613



613

To a solution of compound **607** (18.0 mg, 0.04 mmol) in $CHCl_3$/MeOH(1 mL), *t*-$BuNH_2$ (15 μL, 0.13 mmol) was added at 0°C and stirred for 4 h. The solvent concentrated under reduced pressure. The crude product was dissolved in ethyl acetate/methanol (9:1) and passed through a silica gel column to produce **613** (6.00 mg, 45% yield). Rf = 0.31. 9:1 EtOAc/MeOH. ^{1}H NMR (301 MHz, $CDCl_3$) δ_{ppm} 7.53 (dd, J = 7.24, 2.03 Hz, 2H), 7.39-7.19 (m, 3H), 4.57 (d, J = 9.74 Hz, 1H), 4.43

(dd, J = 11.85, 1.87 Hz, 1H), 4.15 (dd, J = 11.88, 6.54 Hz, 1H), 3.56-3.41 (m, 1H), 3.37 (t, J = 8.76, 8.76 Hz, 1H), 3.21 (dd, J = 19.19, 9.72 Hz, 2H), 2.21 (d, J = 7.10 Hz, 2H), 2.16-1.97 (m, 1H), 0.95 (d, J = 6.57 Hz, 6H). ^{13}C NMR and **EI-MS *m/z*** were not collected.

Reference

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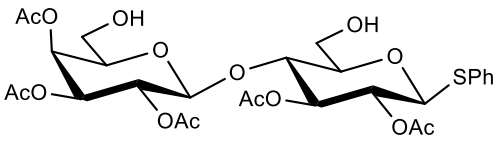
9.10 Experimental protocols for Chapter 7

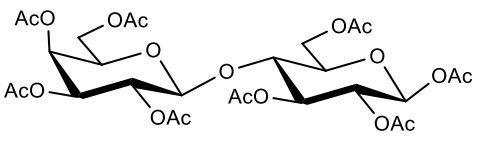
(2S,3R,4S,5S,6R)-2-(((2R,3R,4S,5R,6S)-4,5-diacetoxy-2-(hydroxymethyl)-6-(phenylthio)tetrahydro-2H-pyran-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate 702



702

Compound **708** (270 mg, 0.31 mmol) was dissolved in THF (10 mL) and cooled to 0°C and 1M solution of TBAF in THF (3 mL) was added and stirred for 4 h. The reaction mixture concentrated in reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:2) and passed through a silica gel column to produce **702**, the title compound, as a colourless liquid (75.0 mg, 38% yield). Rf = 0.26. 1:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.47 (dd, *J* = 6.38, 3.06 Hz, 2H), 7.40-7.28 (m, 3H), 5.34 (d, *J* = 3.29 Hz, 1H), 5.28 (t, *J* = 9.39, 9.39 Hz, 1H), 5.00-4.85 (m, 2H), 4.75 (d, *J* = 10.04 Hz, 1H), 4.52 (d, *J* = 7.62 Hz, 1H), 4.18-4.07 (m, 2H), 4.05-3.94 (m, 3H), 3.89 (t, *J* = 6.52, 6.52 Hz, 1H), 3.74 (dd, *J* = 10.27, 7.67 Hz, 1H), 3.53 (d, *J* = 9.89 Hz, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.06 (s, 6H), 2.04 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.43, 170.05, 170.01, 169.85, 169.53, 132.81, 131.98, 129.12, 128.41, 103.57, 86.13, 79.04, 77.25, 75.18, 74.56, 72.65, 70.88, 70.48, 69.76, 61.18, 31.97, 29.74, 29.70, 29.40, 22.73, 20.83, 20.61, 14.15. **EI-MS *m/z*** calcd for $C_{28}H_{36}O_{15}S$: 644.18. Found: $[M+NH_4]^+$ 662.21.

(2S,3R,4S,5R,6R)-6-(acetoxymethyl)-5-(((2S,3R,4S,5S,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2,3,4-triyl triacetate 704

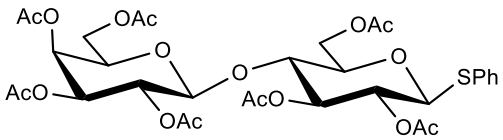


704

Lactose (10.0 g, 0.03 mol) was dissolved in acetic anhydride (32 mL) and sodium acetate (2.50 g ,0.03 mol) and stirred at 100°C for 16 h. The reaction mixture was diluted

with (200 mL) CH_2Cl_2 (200 mL) and washed with saturated aqueous $NaHCO_3$ (150 mL), water (150 mL) and brine (150 mL) and the crude compound was recrystallized with ethanol to produce **704**, the title compound, as a white solid (15.0 g, 76%). On replication, yields varied between 70% and 76% for scale in only 10.0 g each time. Rf = 0.37. 1:1 EtOAc/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 5.67 (d, *J* = 8.21 Hz, 1H), 5.34 (d, *J* = 2.59 Hz, 1H), 5.30 (s, 1H), 5.25 (d, *J* = 9.24 Hz, 1H), 5.17-4.86 (m, 3H), 4.57-4.36 (m, 2H), 4.21-4.02 (m, 3H), 3.85 (dd, *J* = 16.55, 7.60 Hz, 2H), 2.06 (8 of s, 24H). Consistent with the literature.[1]

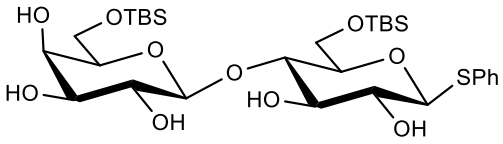
(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6S)-4,5-diacetoxy-2-(acetoxymethyl)-6-(phenylthio)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate 706



706

To a solution of fully acetylated lactose **704** (7.00 g, 0.10 mol) in CH_2Cl_2 (80 mL) was added thiophenol (2.73 mL, 0.03 mol) and BF_3**.**OEt_2 (3.3 mL ,0.03 mol) at 0°C and the mixture was stirred for 16 h. Then trimethylamine was added and CH_2Cl_2 were added (200 mL) and the organic phase was washed with (2M) NaOH (3 x 200 mL), water (3 x 200 mL) and brine (200 mL). The crude product was dissolved in hexanes/ethyl acetate (7:3) and passed through a silica gel column to produce **706**, the title compound, as a colourless gel (5.50 g, 73% yield). On replication, yields varied between 70% and 73% for scales between 70.0 mg and 15.0 g. Rf = 0.27. 8:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.47 (dd, J = 6.63, 2.99 Hz, 2H), 7.35-7.27 (m, 3H), 5.37-5.30 (m, 1H), 5.21 (t, J = 9.05, 9.05 Hz, 1H), 5.10 (dd, J = 10.44, 7.85 Hz, 1H), 5.00-4.84 (m, 2H), 4.67 (d, J = 10.07 Hz, 1H), 4.53 (dd, J = 11.91, 1.85 Hz, 1H), 4.46 (d, J = 7.86 Hz, 1H), 4.12-4.02 (m, 3H), 3.86 (t, J = 6.83, 6.83 Hz, 1H), 3.80-3.69 (m, 1H), 3.69-3.58 (m, 1H), 2.14, 2.10, 2.09, 2.04, 1.96 (7of s, 21H). Consistent with the literature.[2]

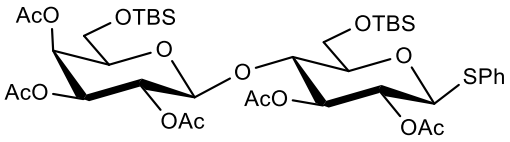
(2R,3R,4S,5R,6S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-6-(((2R,3S,4R,5R,6S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4,5-dihydroxy-6-(phenylthio)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triol 707



707

Compound **706** (5.00 g, 6.87 mmol) was dissolved in methanol (50 mL) then NaOMe solution was added until a pH of 12 was reached. The reaction was stirred at room temperature for 5 h to produce a faint yellow coloured solution. DOWEX 50W X8 ion exchange resin was added to the reaction mixture until a pH of 7 was reached. The solution was filtered and concentrated under reduced pressure to obtain the desired product (2.25 g, 75%). Rf = 0.3 in 9:1 EtOAc/MeOH, then crude (1.23 g, 2.84 mmol) in DMF (7 mL) cooled to 0°C and DMAP (145 mg, 1.19 mmol), imidazole (870 mg, 12.8 mmol) and TBSCl (970 mg, 6.44 mmol) were added and stirred for 16 h. The reaction mixture poured into $NaHCO_3$ aq (50 mL) and stirred for 10 min. The aqueous phase was extracted with ethyl acetate (3 x 50 mL) and the combined organic phase was washed with water (2 x 100 mL) and brine (100 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (1:9) and taken up through a silica gel column to produce **707**, the title compound, as a colourless liquid (1.15 g, 61% yield in two steps). On replication, yields varied between 57% and 61% for scales between 5.00 g and 8.00 g. Rf = 0.27. 1:9 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.65-7.41 (m, 2H), 7.33-7.26 (m, 3H), 4.53 (d, *J* = 9.71 Hz, 1H), 4.33 (d, *J* = 7.65 Hz, 1H), 4.06-3.99 (m, 1H), 3.97-3.91 (m, 2H), 3.87 (d, *J* = 5.79 Hz, 2H), 3.73-3.46 (m, 5H), 3.46-3.29 (m, 2H), 2.95 (s, 1H), 2.90-2.86 (m, 1H), 0.89 (s, 9H), 0.87 (s, 9H), 0.10 (d, *J* = 1.94 Hz, 6H), 0.07 (s, 6H). Consistent with the literature.[3]

(2R,3S,4S,5R,6S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-6-(((2R,3R,4S,5R,6S)-4,5-diacetoxy-2-(((tert-butyldimethylsilyl)oxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate 708



708

Compound **707** (3.65 g, 5.50 mmol) was dissolved in pyridine (16 mL) then acetic anhydride (8 mL) was added and stirred at room temperature for 16 h then diluted with CH_2Cl_2 (100 mL) and the mixture was washed with 5% HCl aq (2 x 50 mL), water (50 mL) and brine (50 mL). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was dissolved in hexanes/ethyl acetate (8:2) and passed through a silica gel column to produce **708**, the title compound, as a colourless liquid (4.80 g, quantitative yield). On replication, yields varied between 95% and 100% for scales between 250 mg and 3.65 g. Rf = 0.28. 8:2 hexanes/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ_{ppm} 7.49 (dd, *J* = 6.65, 2.96 Hz, 2H), 7.33-7.28 (m, 3H), 5.44 (d, *J* = 2.77 Hz, 1H), 5.16 (t, *J* = 9.37, 9.37 Hz, 1H), 5.05 (dd, *J* = 10.30, 7.80 Hz, 1H), 4.95 (d, *J* = 3.41 Hz, 1H), 4.86 (t, *J* = 9.64, 9.64 Hz, 1H), 4.65 (d, *J* = 2.12 Hz, 1H), 4.62 (s, 1H), 4.02-3.78 (m, 3H), 3.72 (dd, *J* = 8.30, 4.67 Hz, 1H), 3.65-3.47 (m, 2H), 3.41-3.29 (m, 1H), 2.12 (s, 3H), 2.08 (s, 3H), 2.01 (s, 6H), 1.96 (s, 3H), 0.94 (s, 9H), 0.88-0.84 (m, 9H), 0.13 (d, *J* = 5.00 Hz, 6H), 0.02 (d, *J* = 3.97 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.22, 170.01, 169.65, 169.39, 168.97, 133.34, 132.51, 128.98, 128.28, 101.55, 100.40, 86.05, 85.61, 80.16, 79.77, 78.86, 74.67, 74.27, 74.06, 73.83, 73.41, 71.67, 71.63, 70.38, 69.83, 69.27, 66.88, 61.06, 60.26, 26.03, 25.89, 25.81, 20.88, 20.80, 20.73, 18.39, -4.90, -5.15, -5.47. **EI-MS *m/z*** calcd for $C_{40}H_{64}O_{15}SSi$: 872.35. Found: $[M+ NH_4]^+$ 890.38.

Reference

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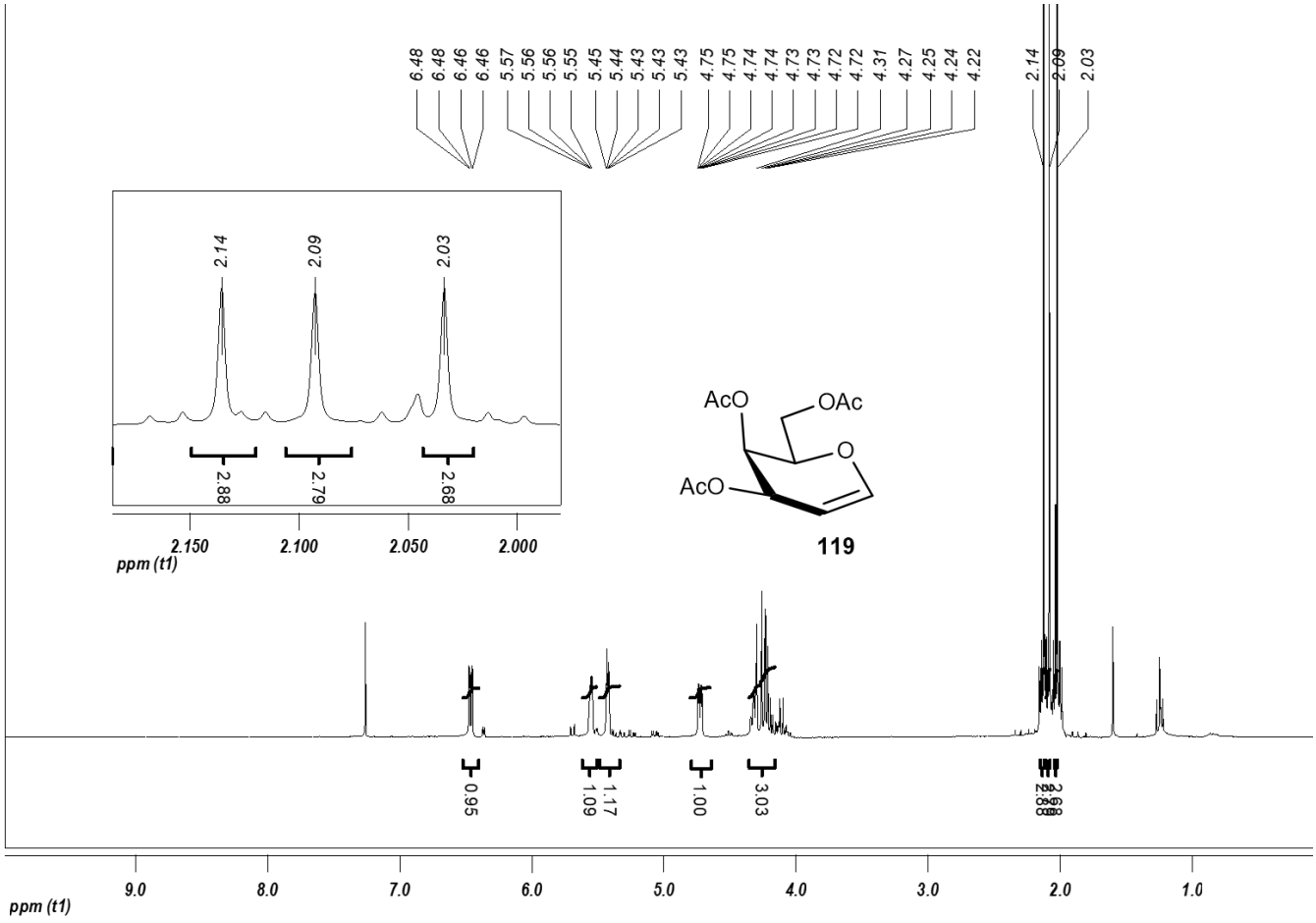
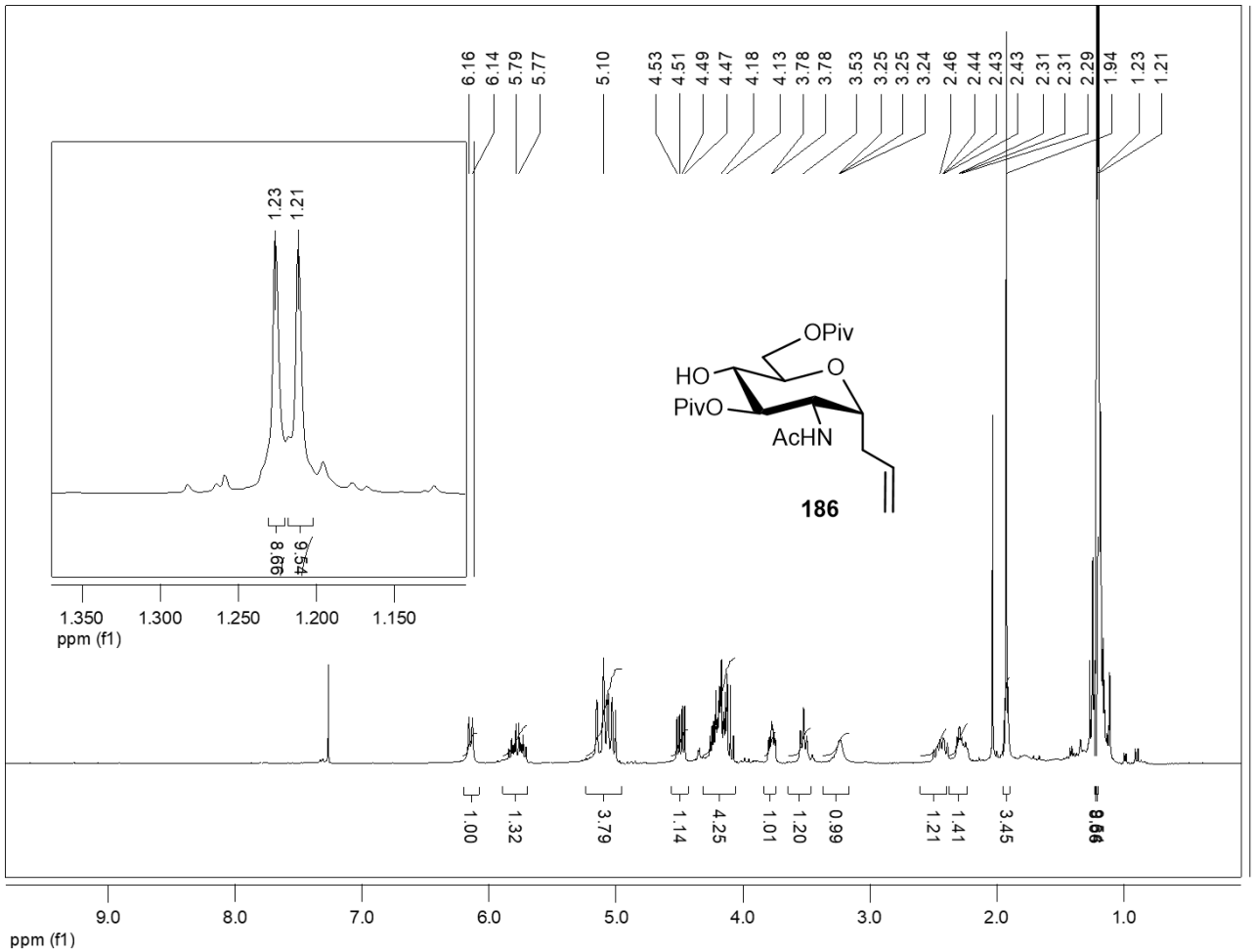
Supplementary Spectra Data

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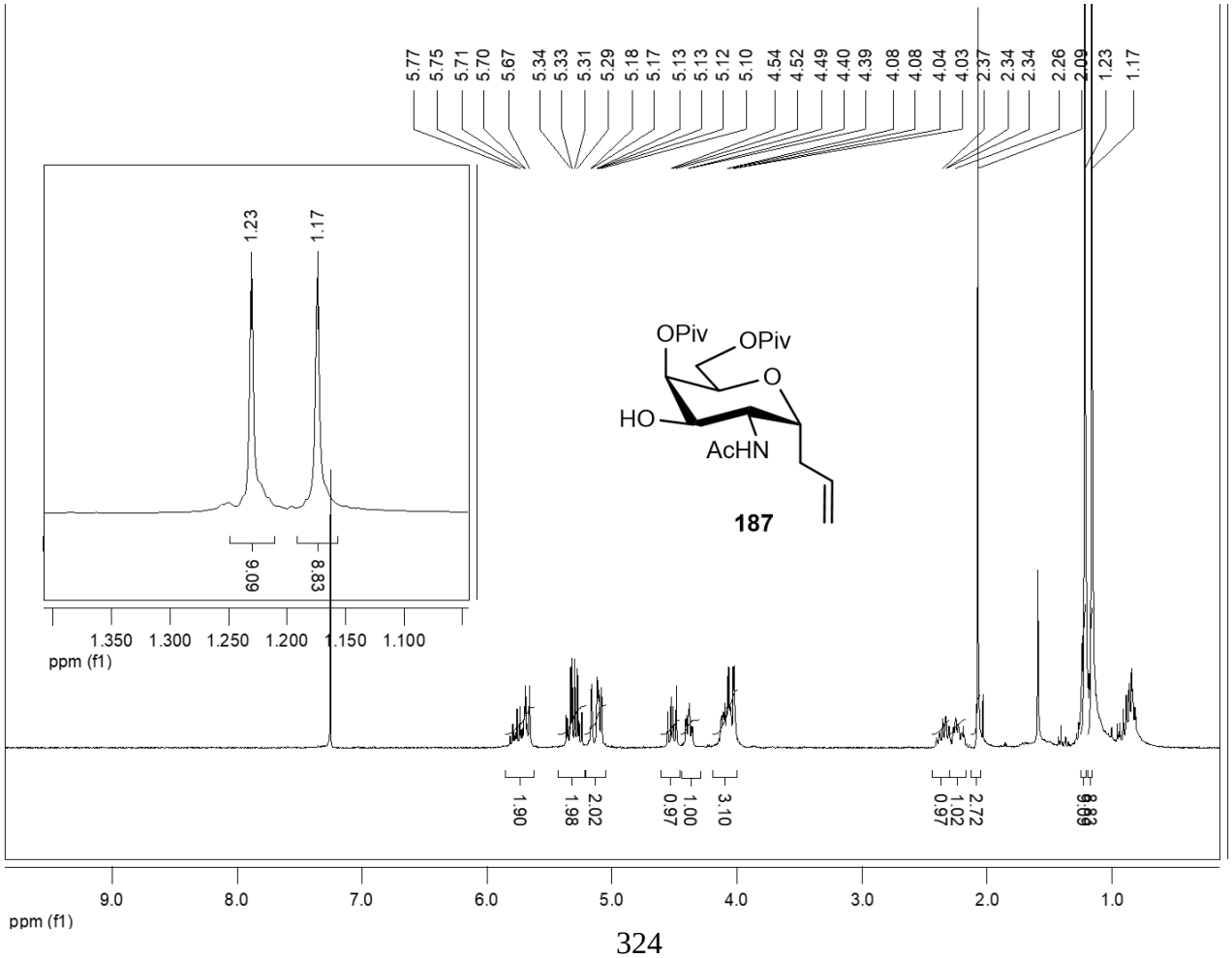
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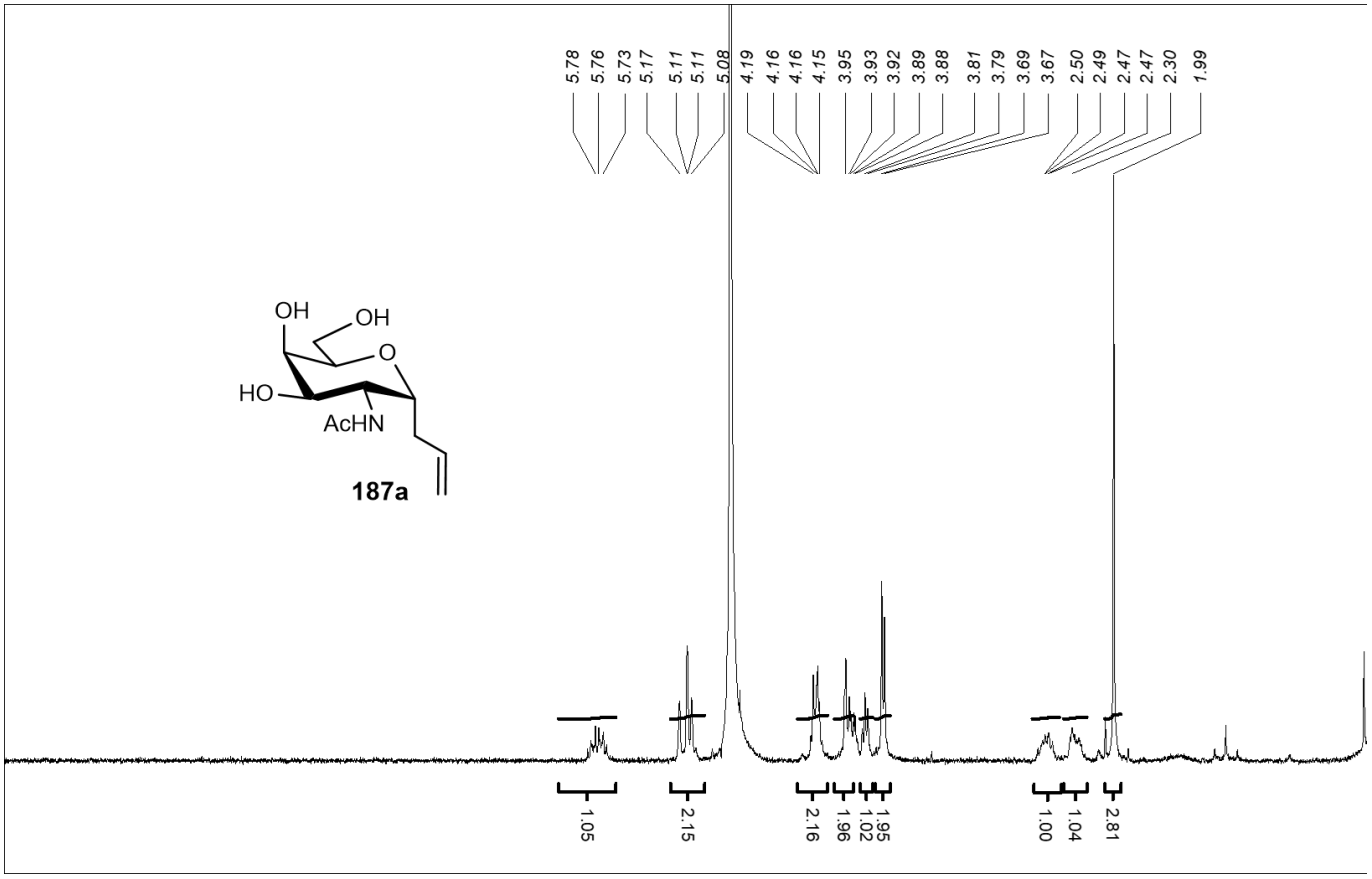
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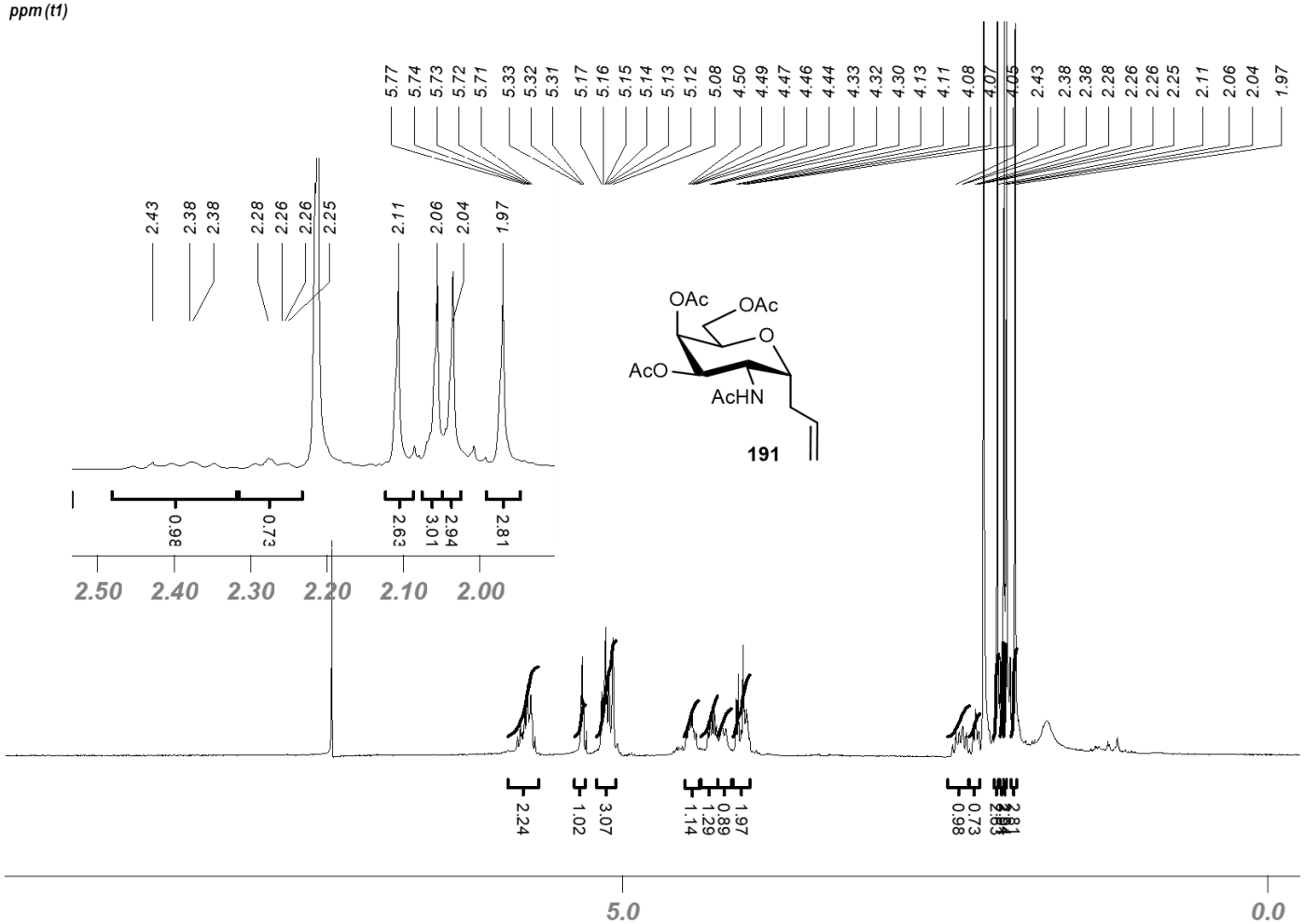
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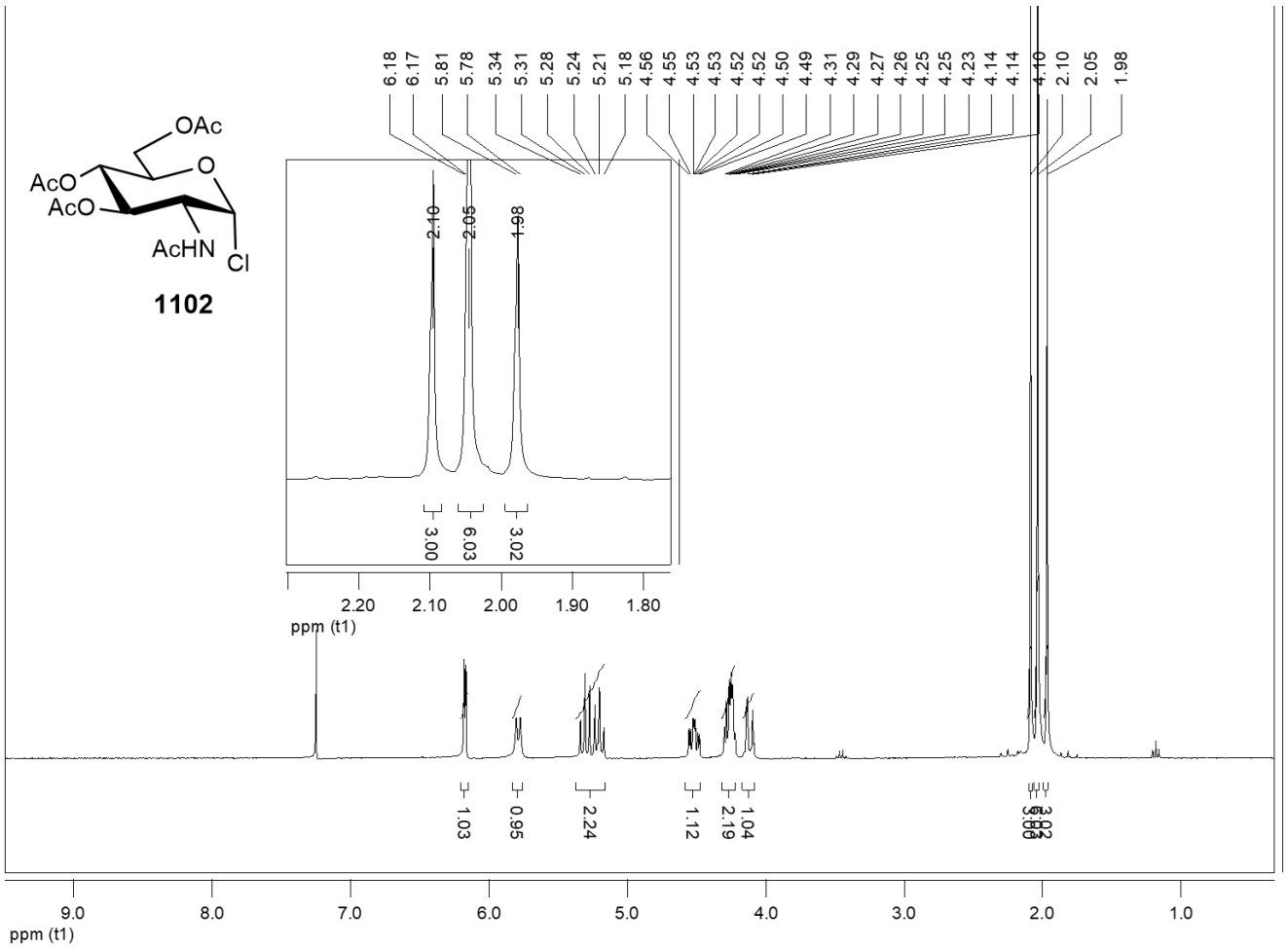
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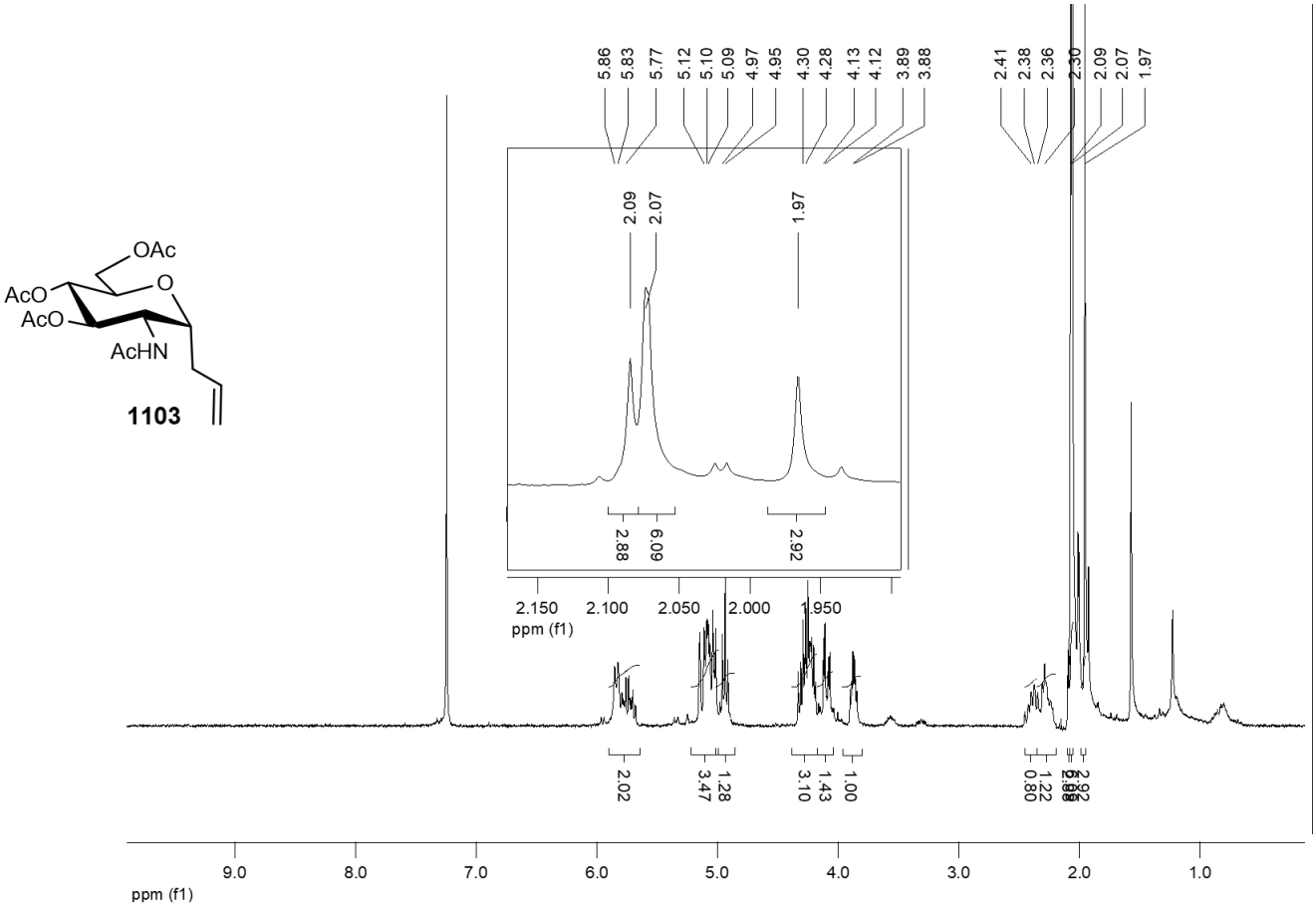
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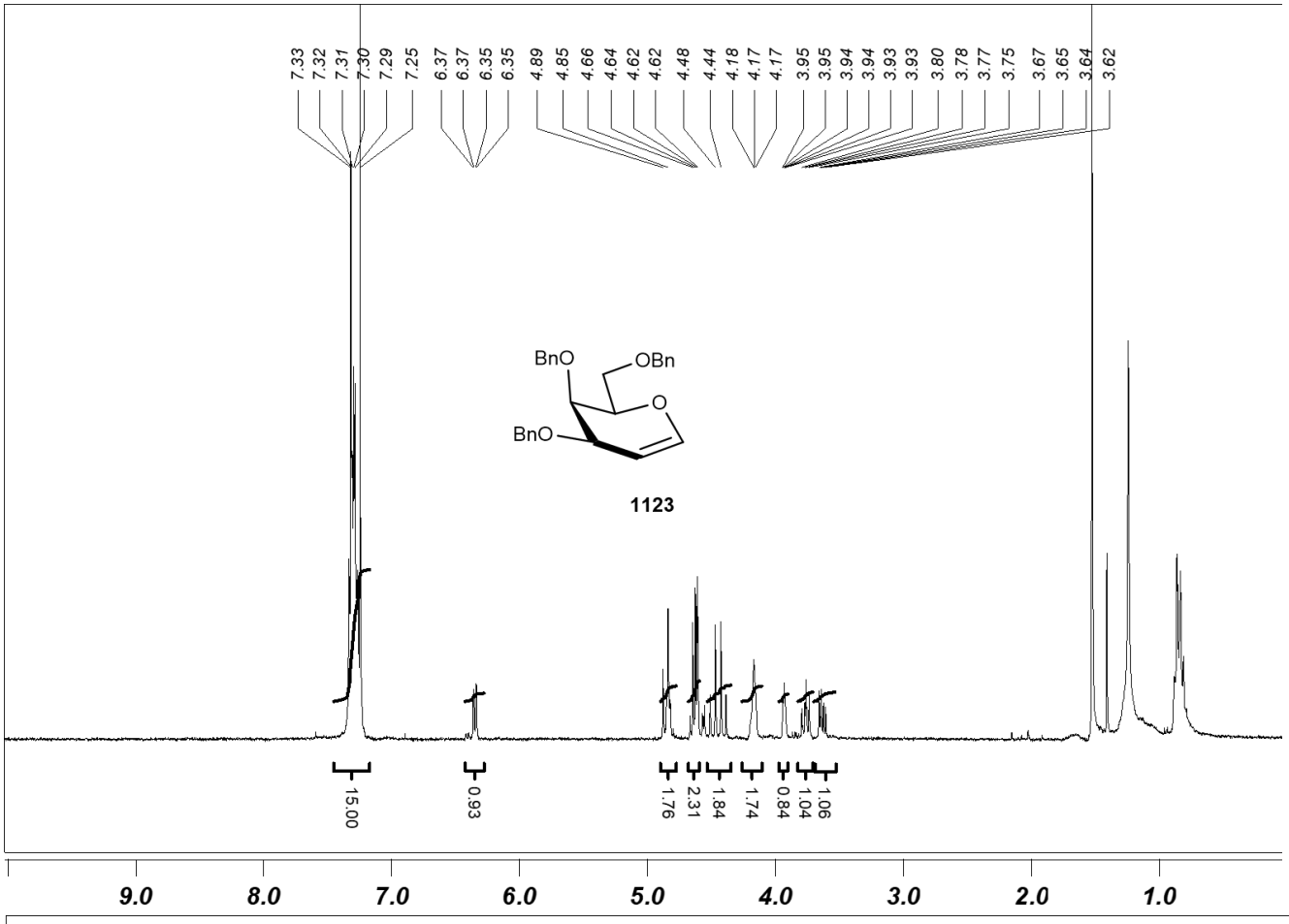
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O
BnO
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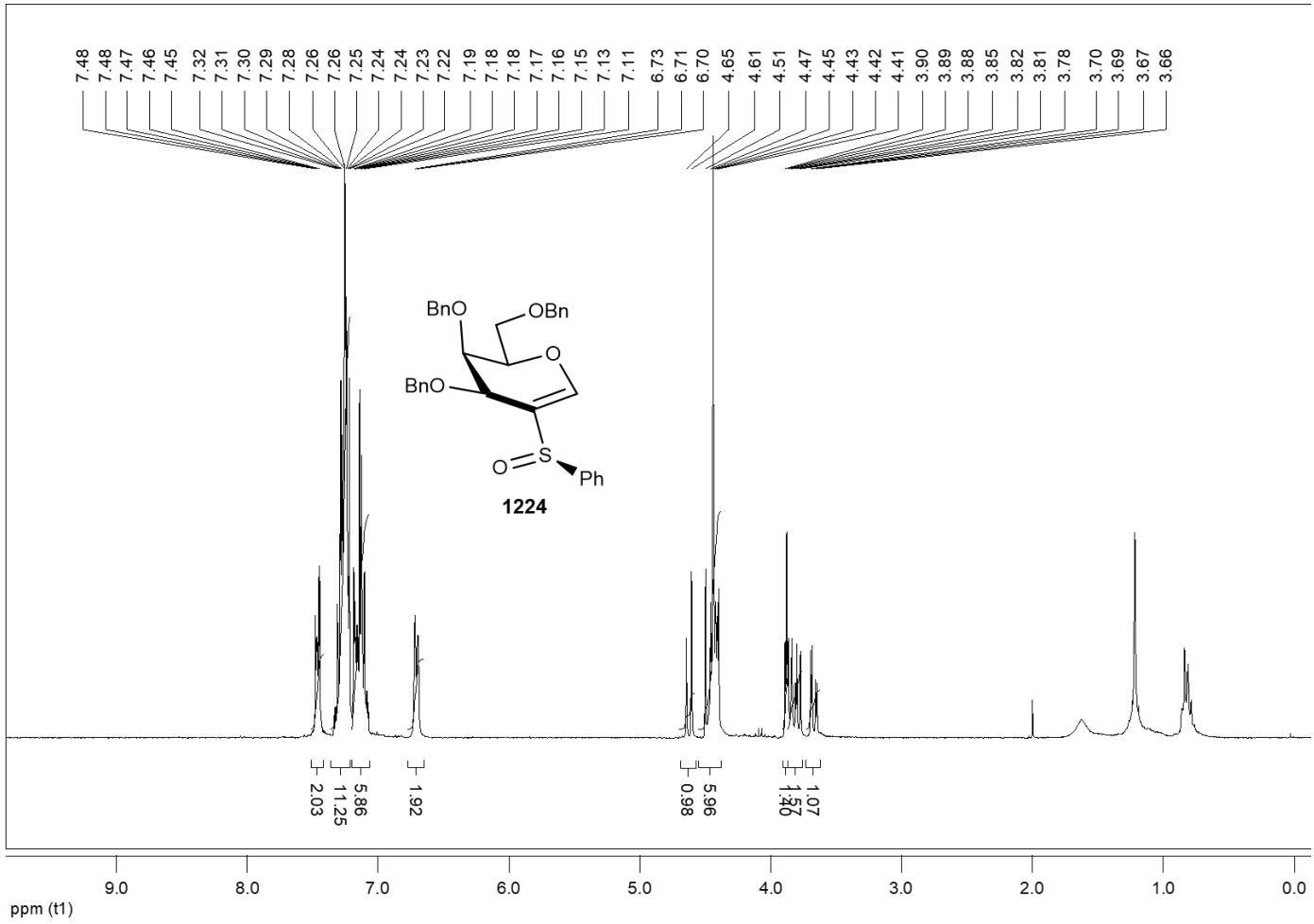
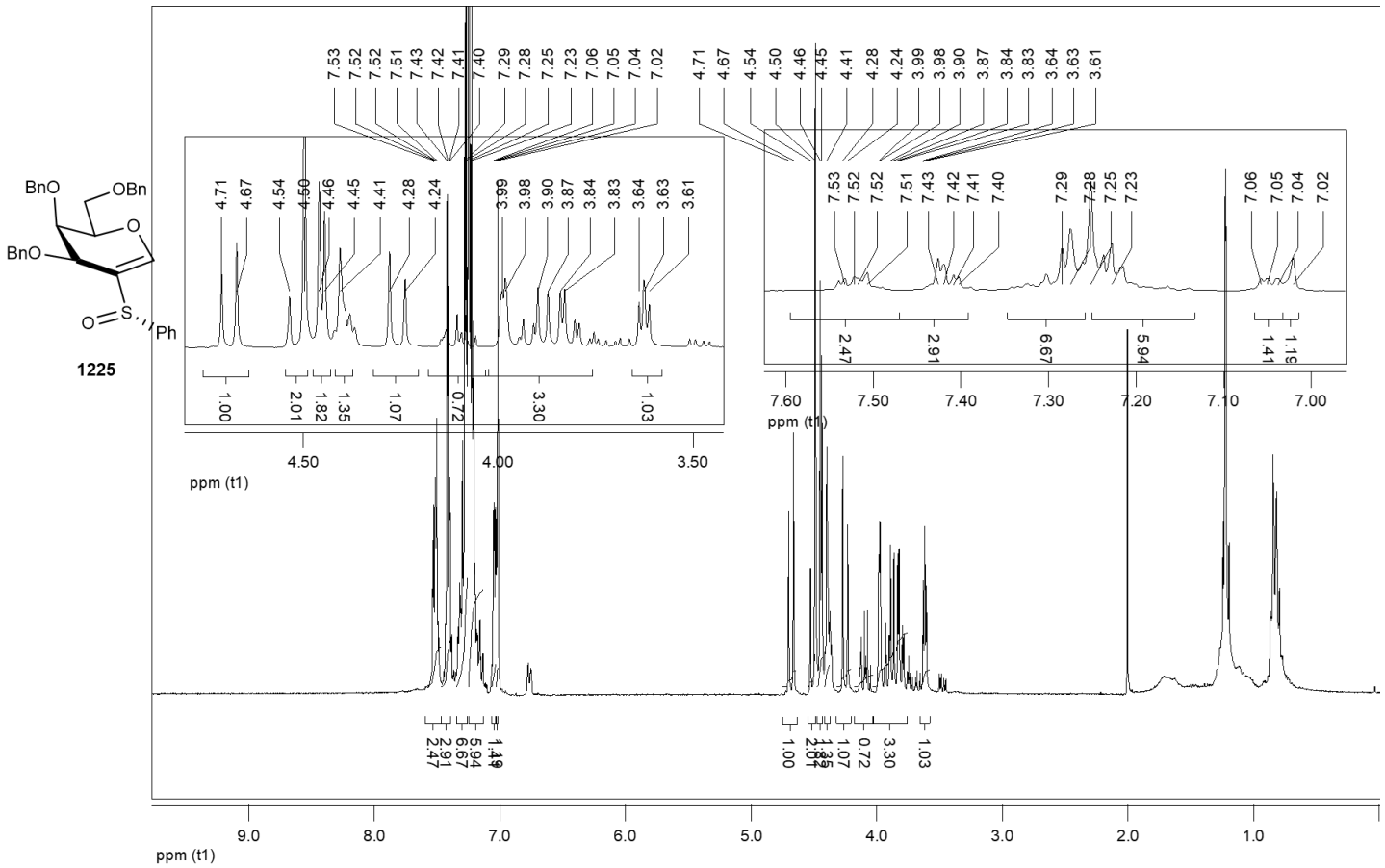
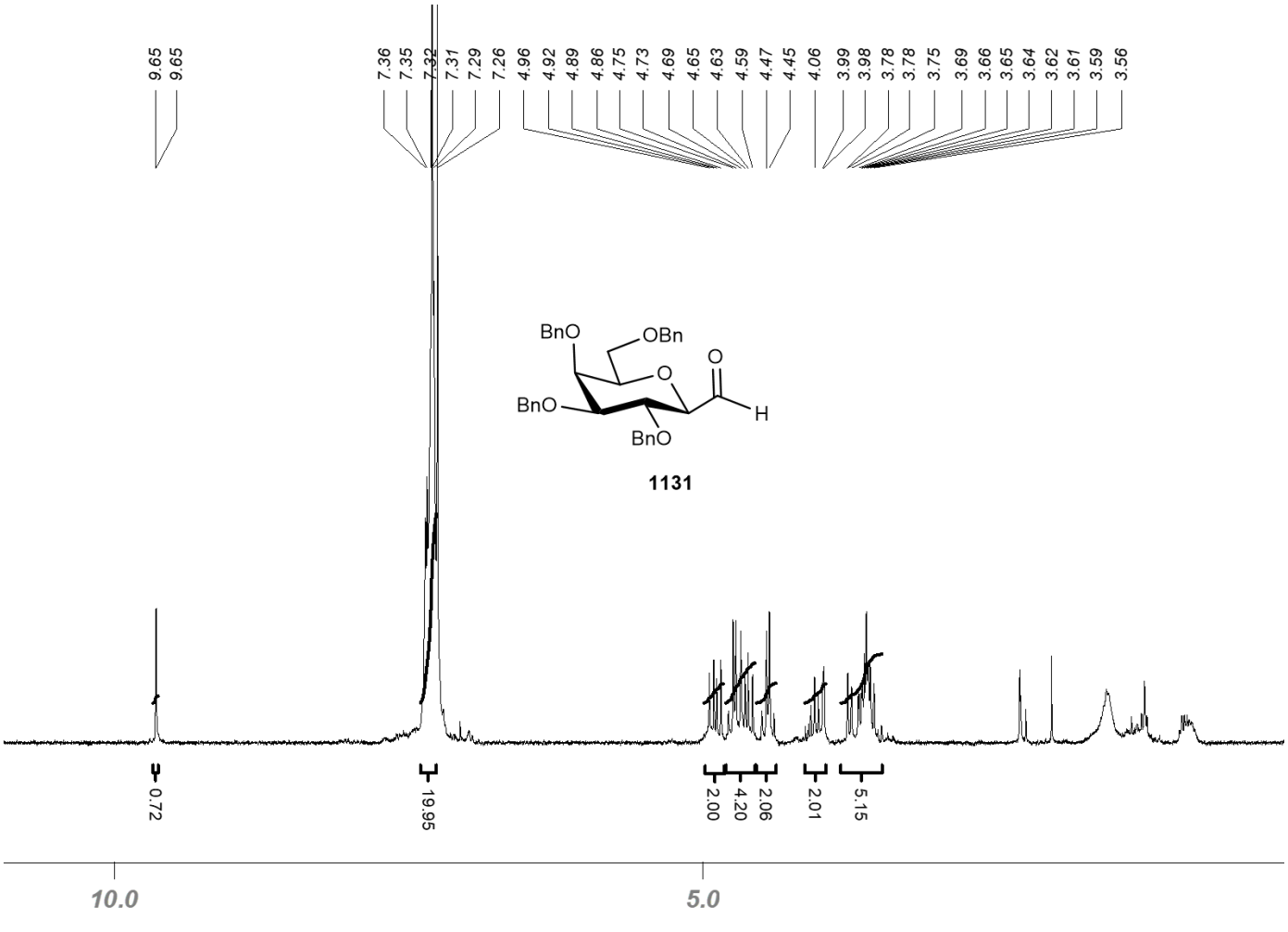
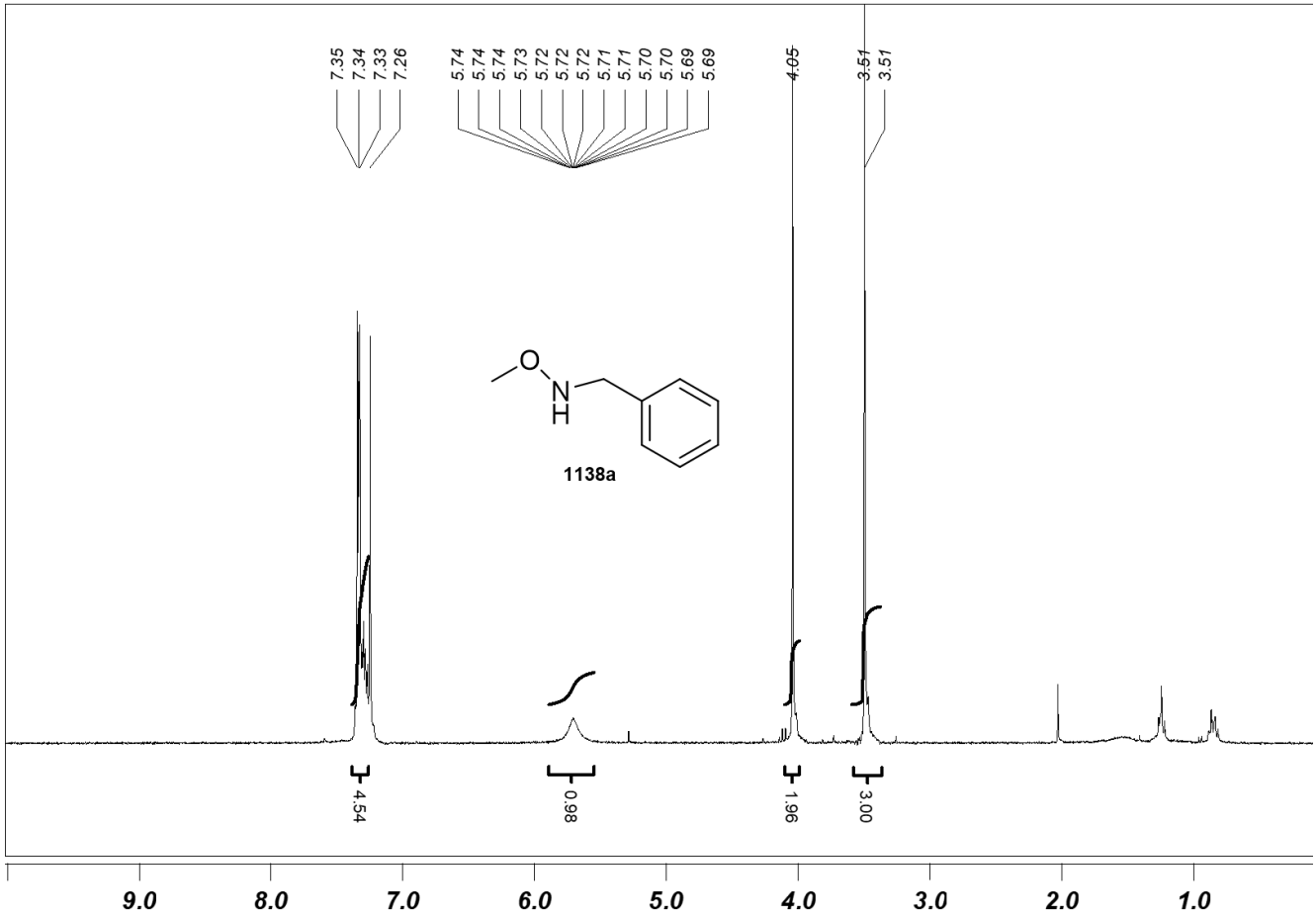
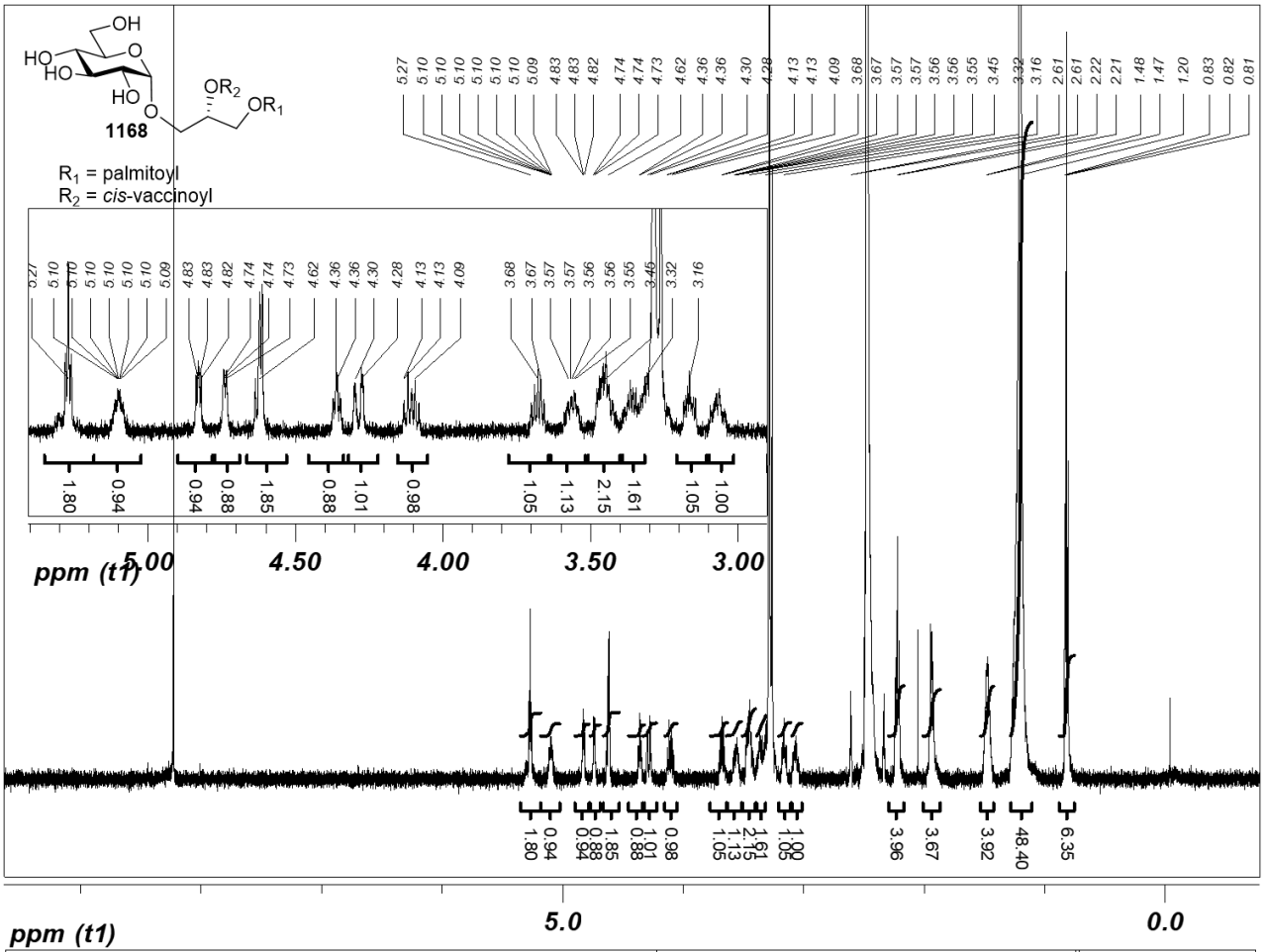
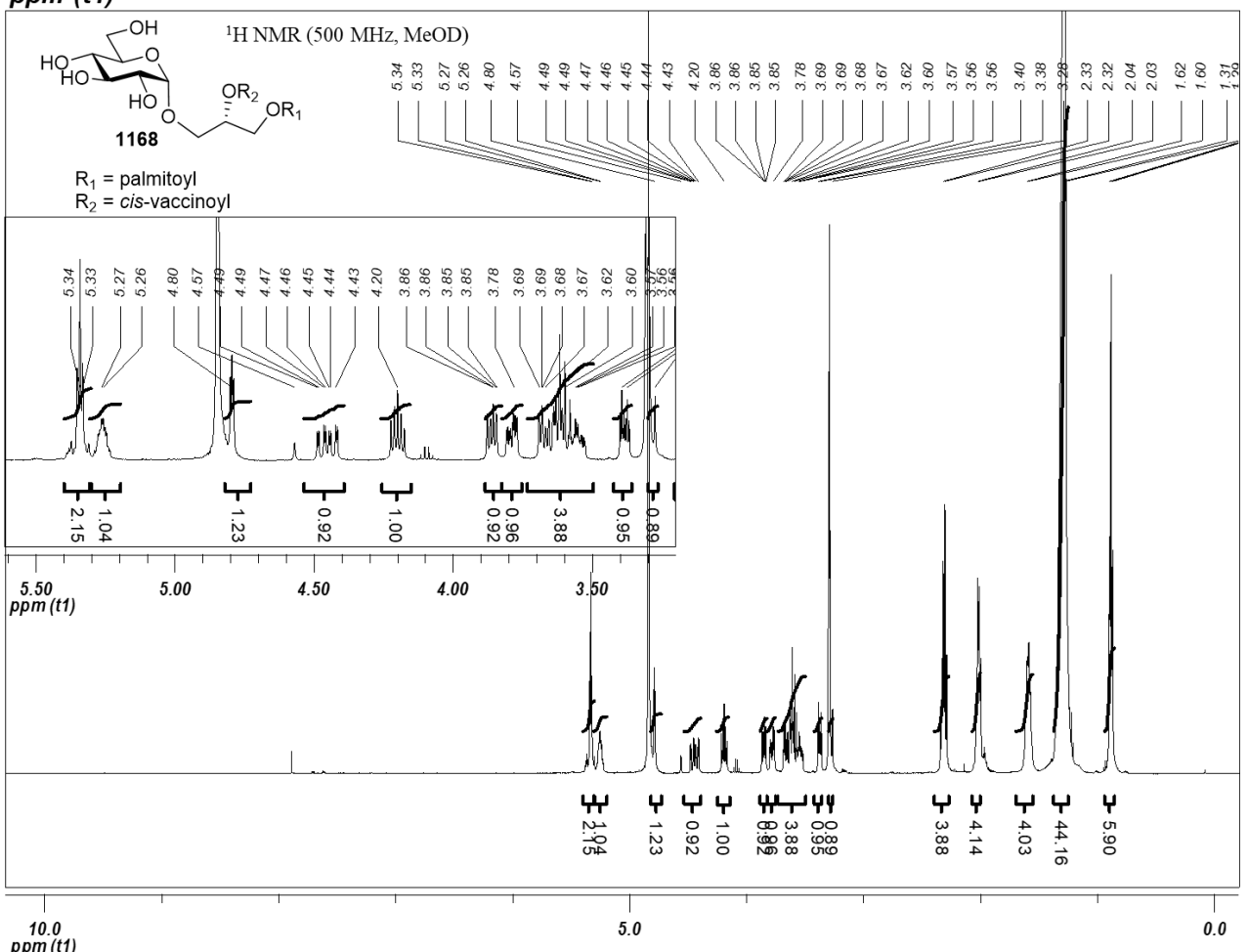

7.48 7.48 7.47 7.46 7.45 7.32 7.31 7.30 7.29 7.28 7.26 7.26 7.25 7.24 7.24 7.23 7.22 7.19 7.18 7.18 7.17 7.16 7.15 7.13 7.11 6.73 6.71 6.70 4.65 4.61 4.51 4.47 4.45 4.43 4.42 4.41 3.90 3.89 3.88 3.85 3.82 3.81 3.78 3.70 3.69 3.67 3.66
BnO
OBn
O
BnO
O
S
Ph
1224
2.03 11.25 5.89 1.92 0.98 5.96 1.07 1.57 1.40
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
ppm (t1)

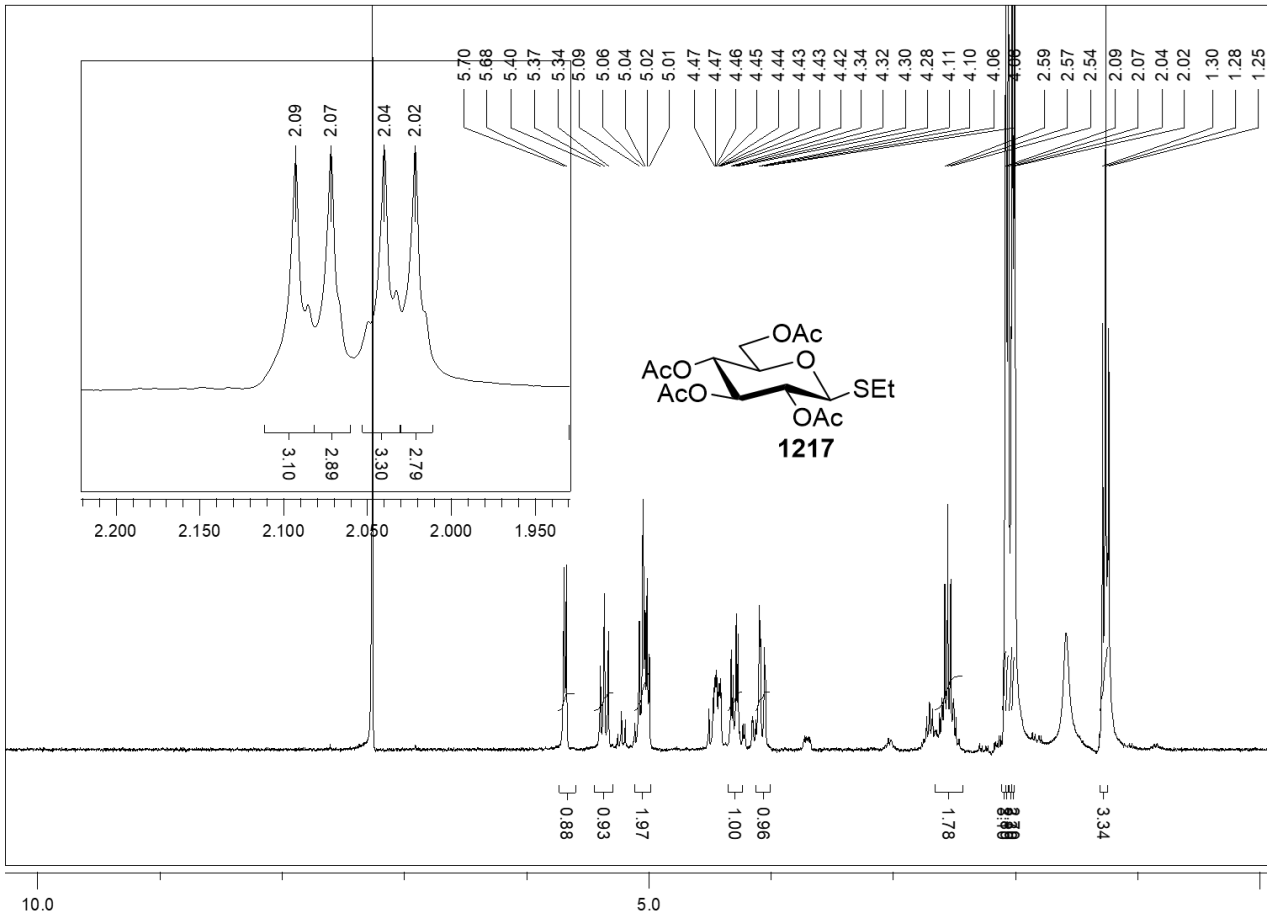

BnO
OBn
O
BnO
O
S
Ph
1225
ppm (t1)

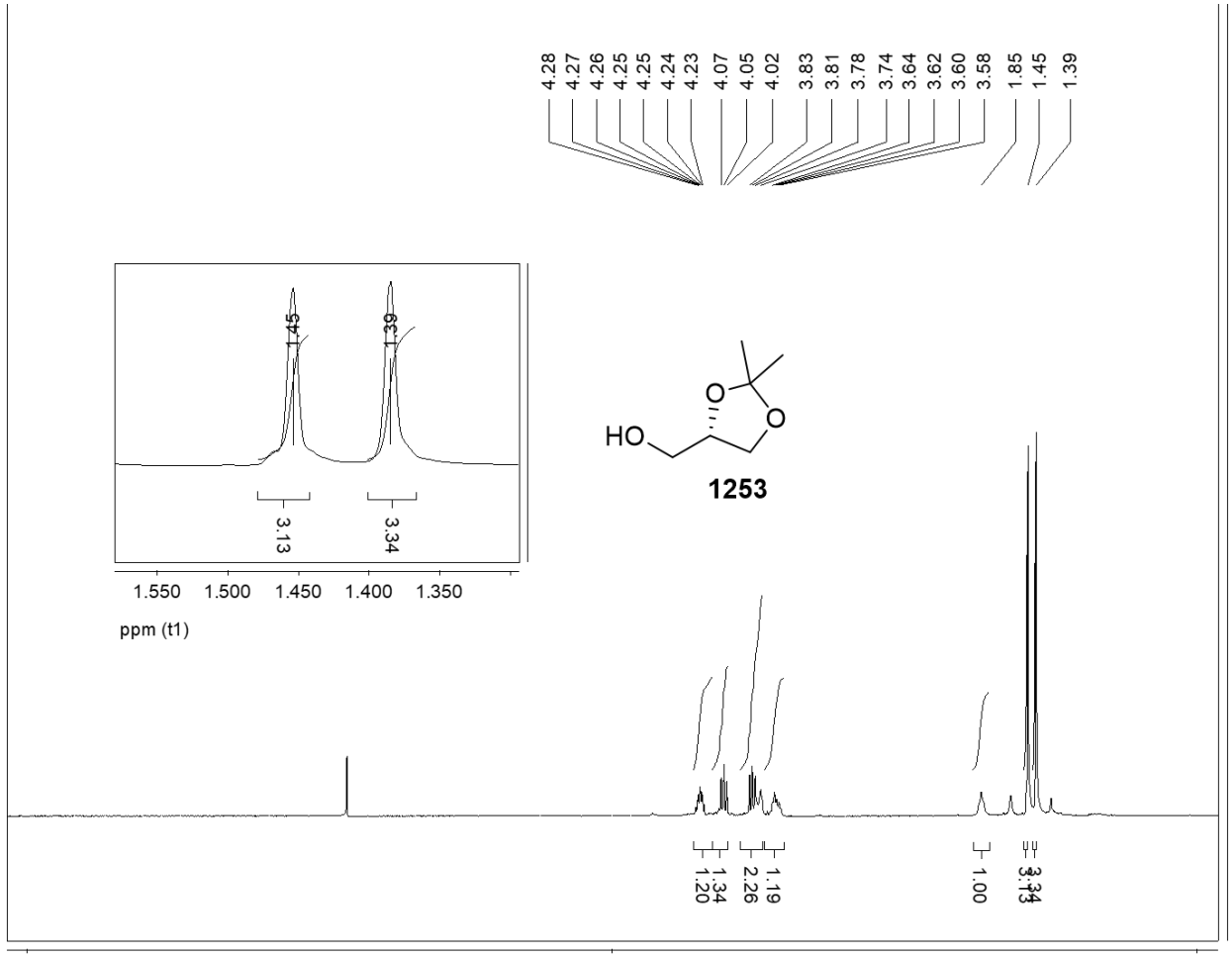

BnO
OBn
O
BnO
O
H
BnO
1131
ppm (t1)

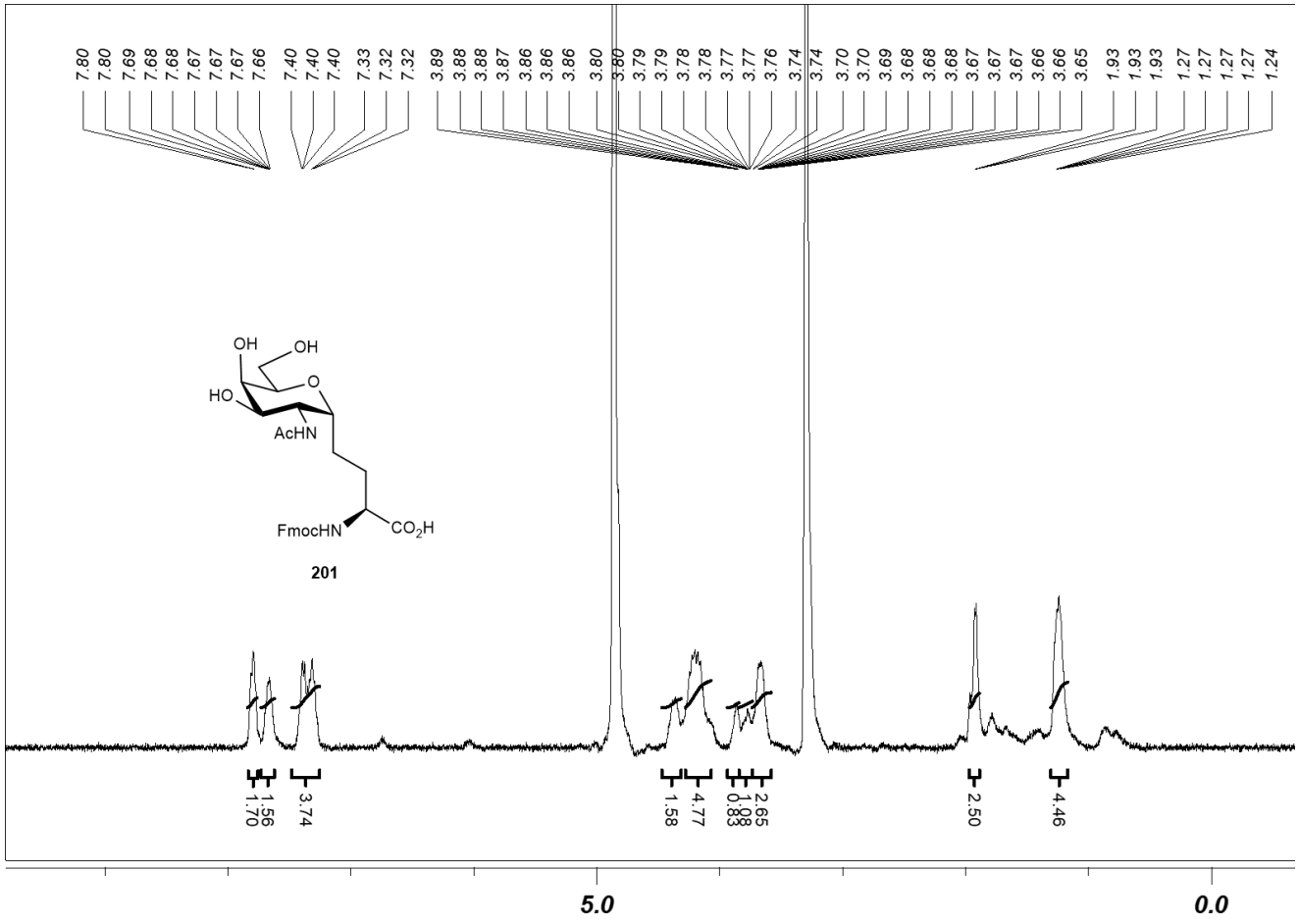

7.35
7.34
7.33
7.26
5.74
5.74
5.74
5.73
5.72
5.72
5.72
5.71
5.71
5.70
5.70
5.69
5.69
4.05
3.51
3.51
O
N
H
1138a
4.54
0.98
1.96
3.00
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

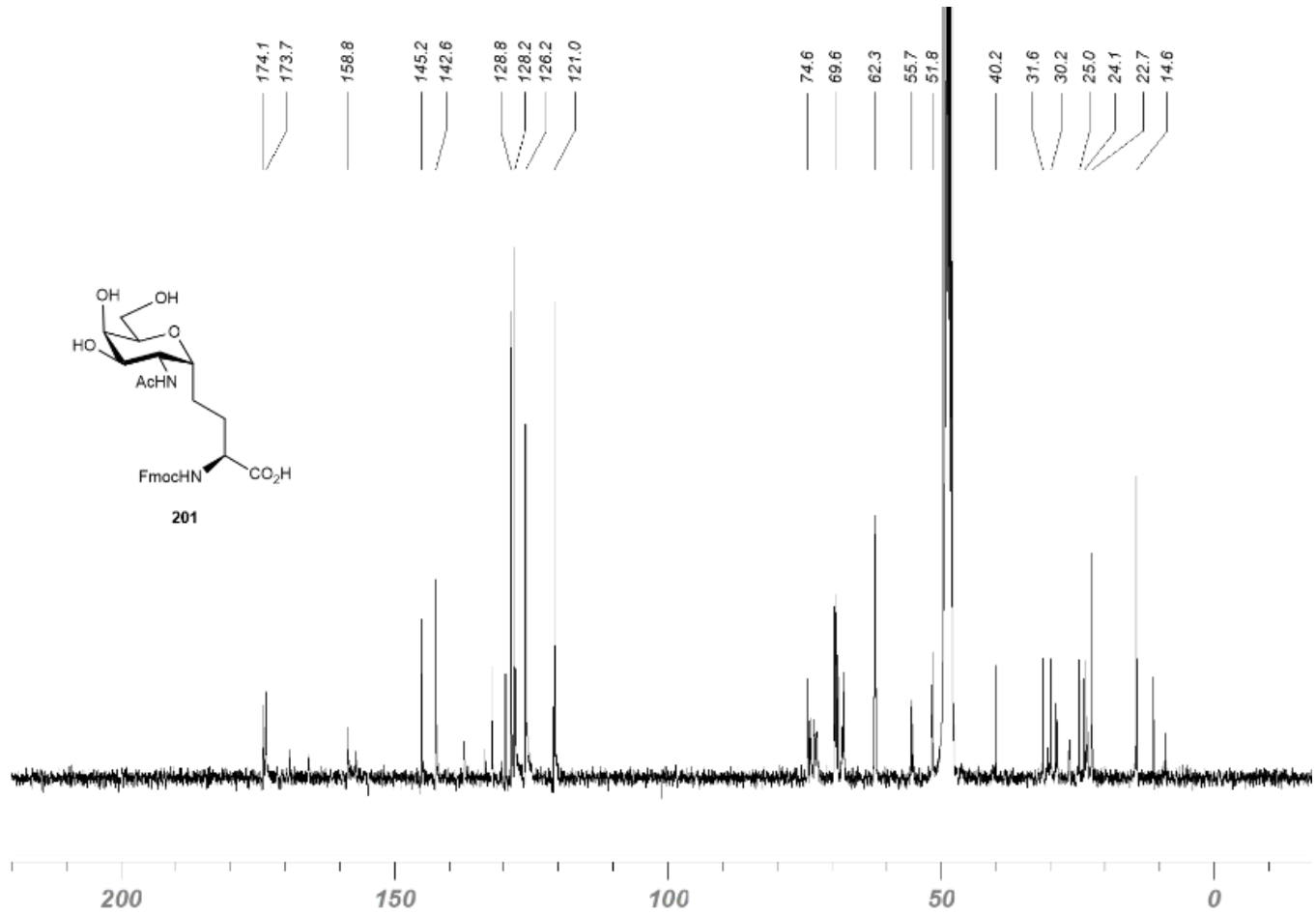

1168
R1 = palmitoyl
R2 = cis-vaccinoyl
ppm (t1)
5.00
4.50
4.00
3.50
3.00
5.0
0.0

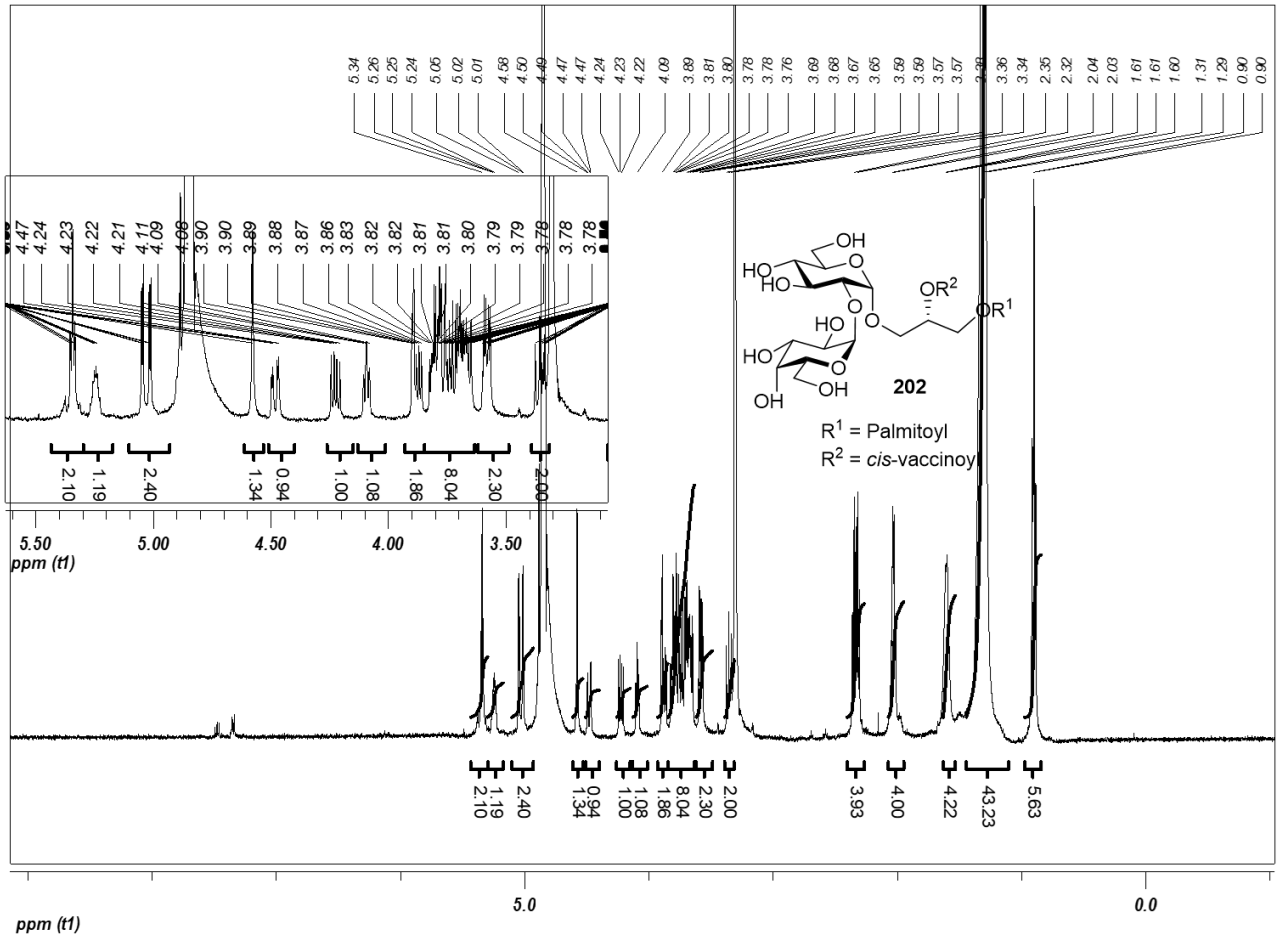

1H NMR (500 MHz, MeOD)
1168
R1 = palmitoyl
R2 = cis-vaccinoyl
5.50
ppm (t1)
5.00
4.50
4.00
3.50
10.0
ppm (t1)
5.0
0.0

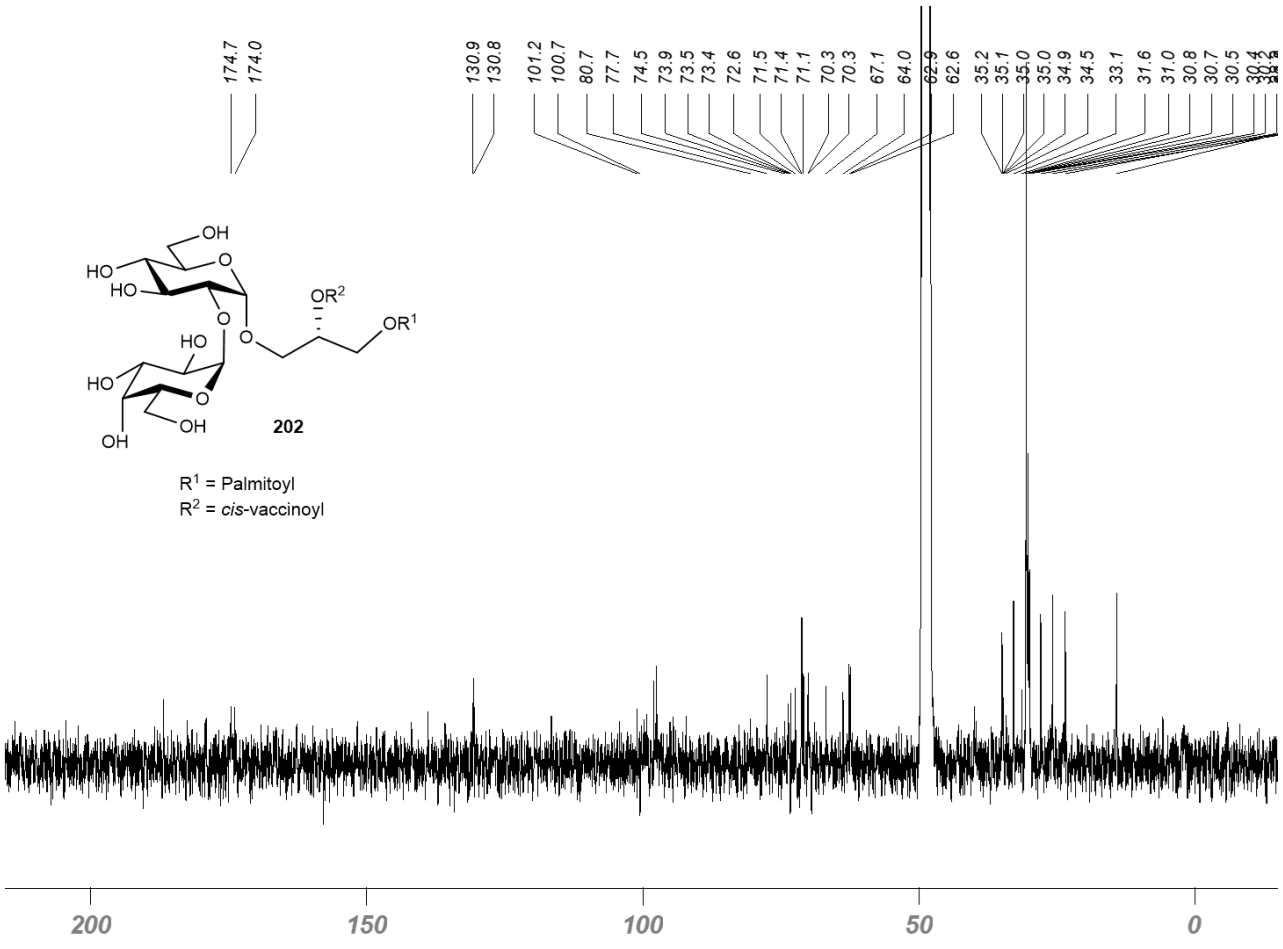

5.70 5.68 5.40 5.37 5.34 5.09 5.06 5.04 5.02 5.01 4.47 4.47 4.46 4.45 4.44 4.43 4.43 4.42 4.34 4.32 4.30 4.28 4.11 4.10 4.06 2.59 2.57 2.54 2.09 2.07 2.04 2.02 1.30 1.28 1.25
2.09 2.07 2.04 2.02
3.10 2.89 3.30 2.79
2.200 2.150 2.100 2.050 2.000 1.950
OAc
AcO
AcO
O
SEt
OAc
1217
0.88 0.93 1.97 1.00 0.96 1.78 3.34
10.0 5.0

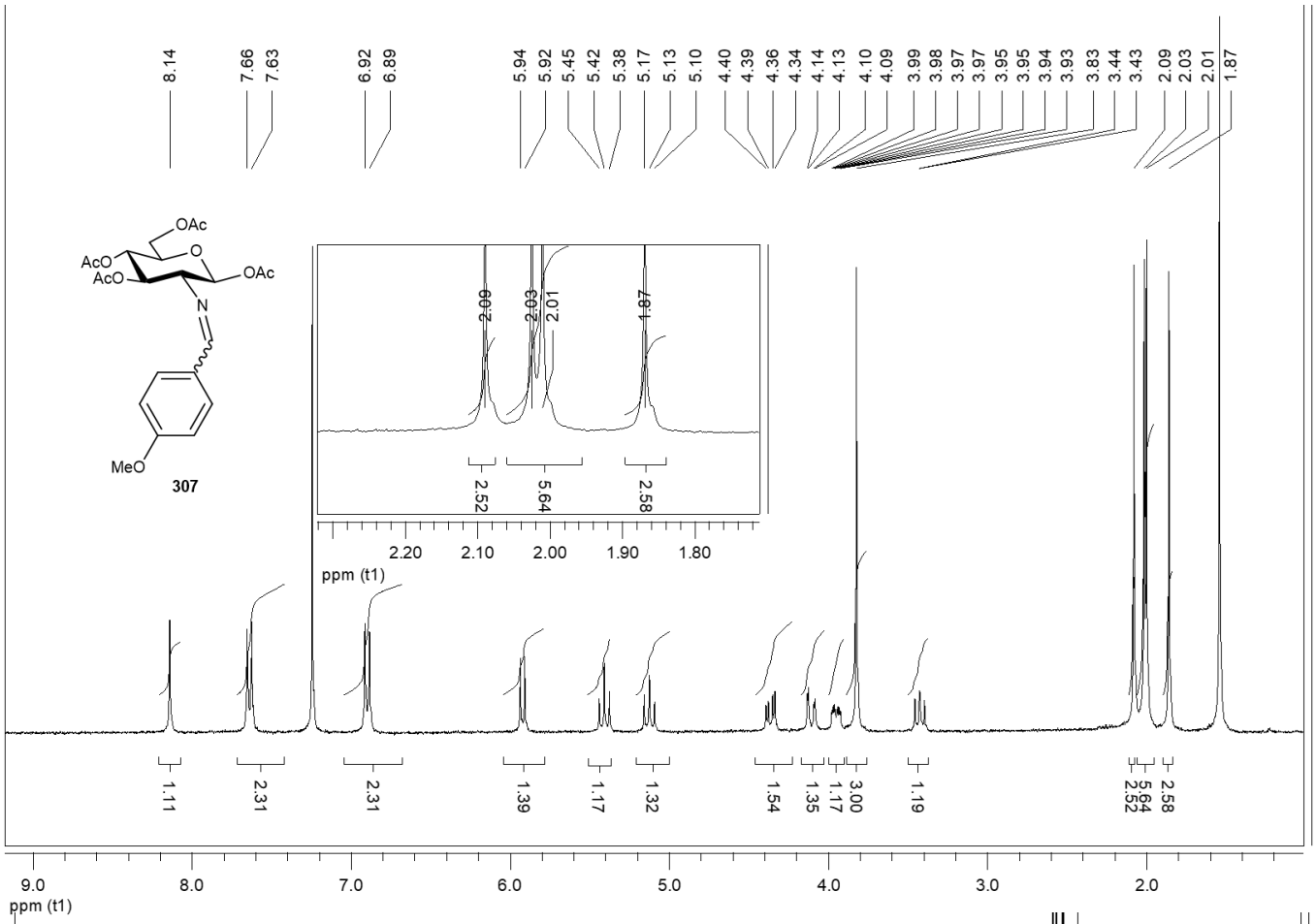

4.28 4.27 4.26 4.25 4.25 4.24 4.23 4.07 4.05 4.02 3.83 3.81 3.78 3.74 3.64 3.62 3.60 3.58 1.85 1.45 1.39
1.45 1.39
3.13 3.34
1.550 1.500 1.450 1.400 1.350
ppm (t1)
HO
O
O
1253
1.20 1.34 2.26 1.19 1.00 3.34 3.13
10.0 5.0 0.0
ppm (t1)

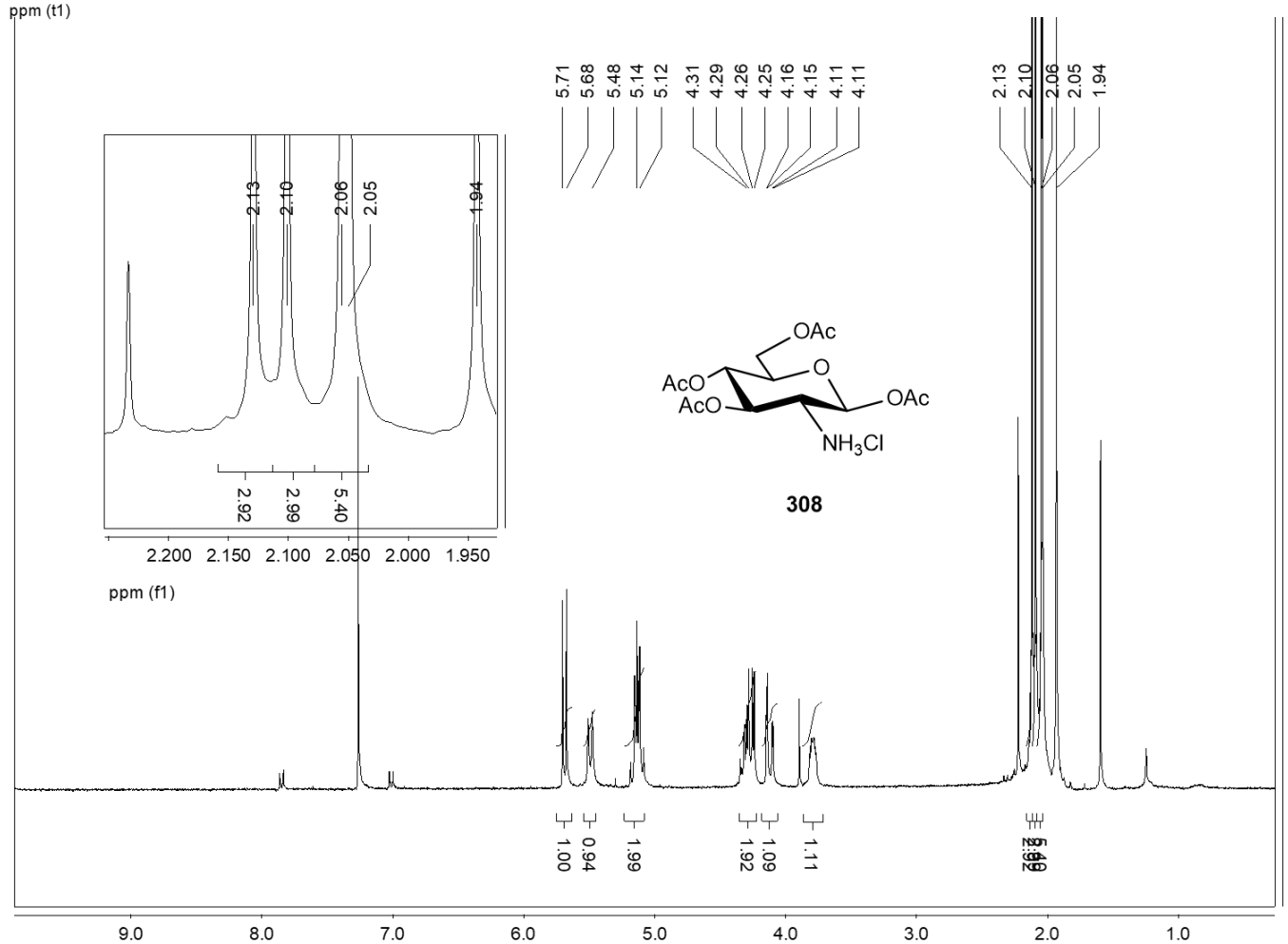

7.80 7.80 7.69 7.68 7.68 7.67 7.67 7.67 7.66 7.40 7.40 7.40 7.33 7.32 7.32
3.89 3.88 3.88 3.87 3.86 3.86 3.86 3.80 3.80 3.79 3.79 3.78 3.78 3.77 3.77 3.76 3.74 3.74 3.70 3.70 3.69 3.68 3.68 3.68 3.67 3.67 3.67 3.66 3.66 3.65
1.93 1.93 1.93 1.27 1.27 1.27 1.27 1.24
OH
OH
HO
O
AcHN
FmocHN
CO2H
201
1.70
1.56
3.74
1.58
4.77
0.83
1.08
2.65
2.50
4.46
5.0
0.0

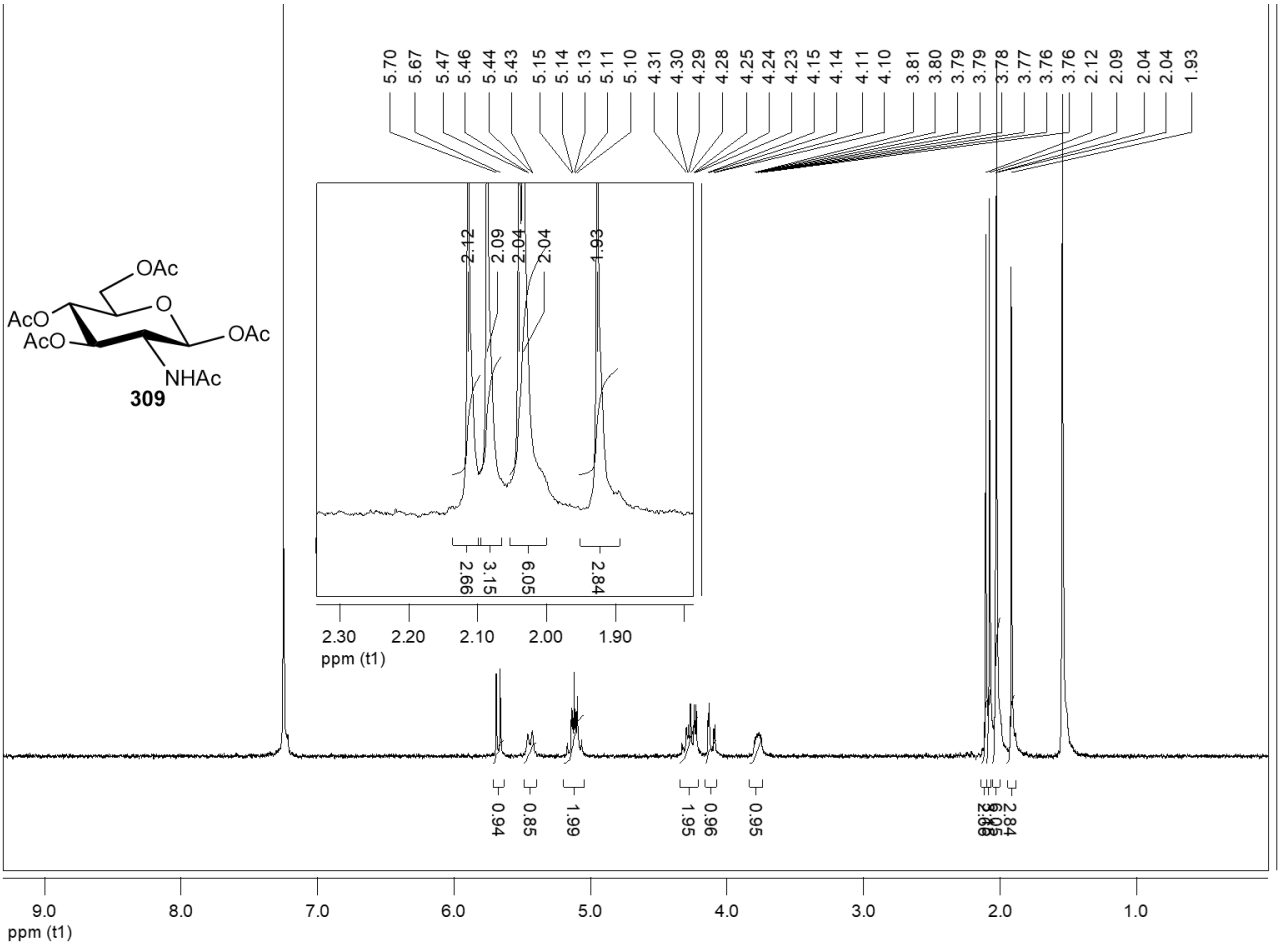

174.1 173.7 158.8 145.2 142.6 128.8 128.2 126.2 121.0
74.6 69.6 62.3 55.7 51.8 40.2 31.6 30.2 25.0 24.1 22.7 14.6
OH
OH
HO
O
AcHN
FmocHN
CO2H
201
200
150
100
50
0

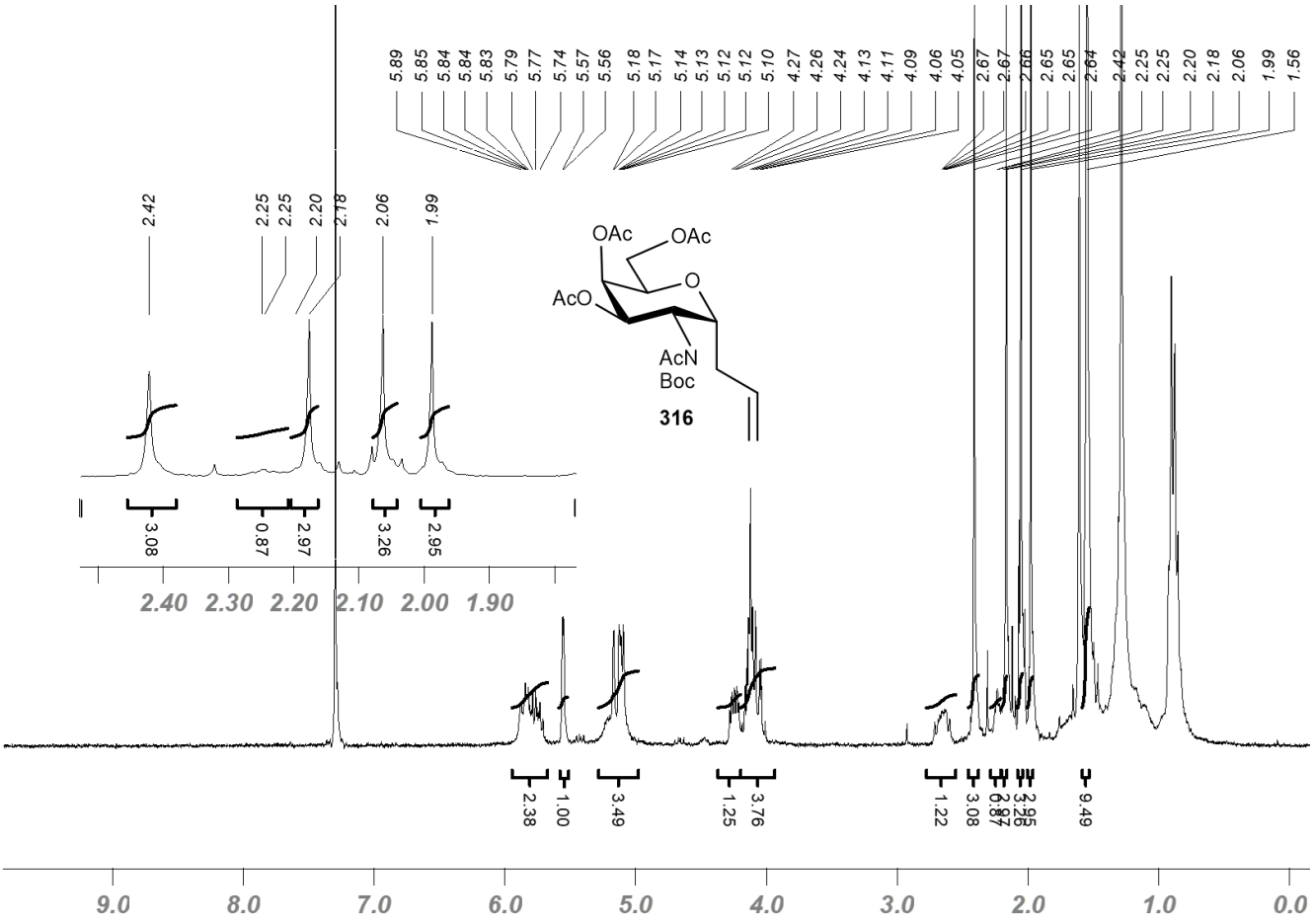

202
R1 = Palmitoyl
R2 = cis-vaccinoyl
ppm (t1)

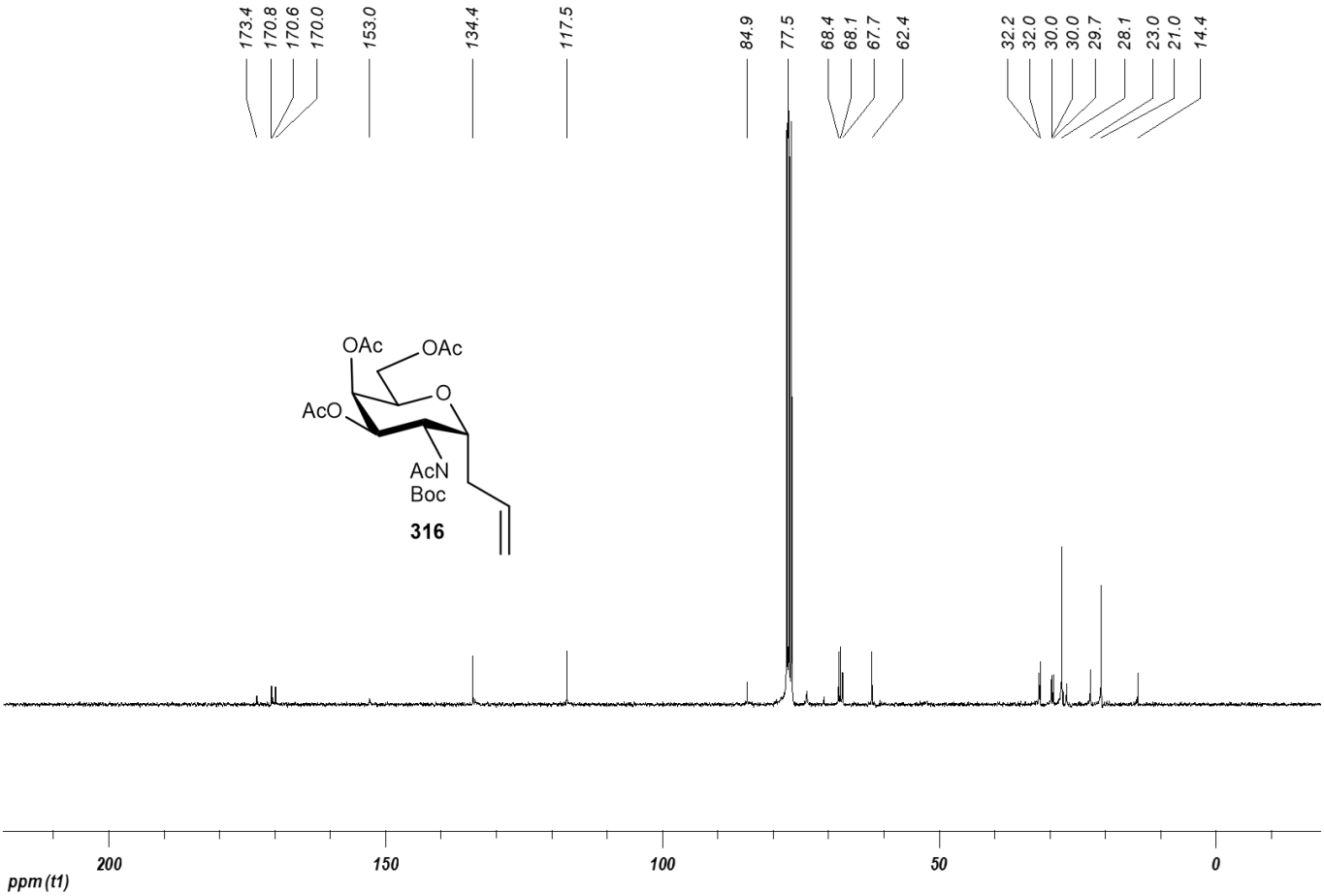

202
R1 = Palmitoyl
R2 = cis-vaccinoyl
ppm (t1)

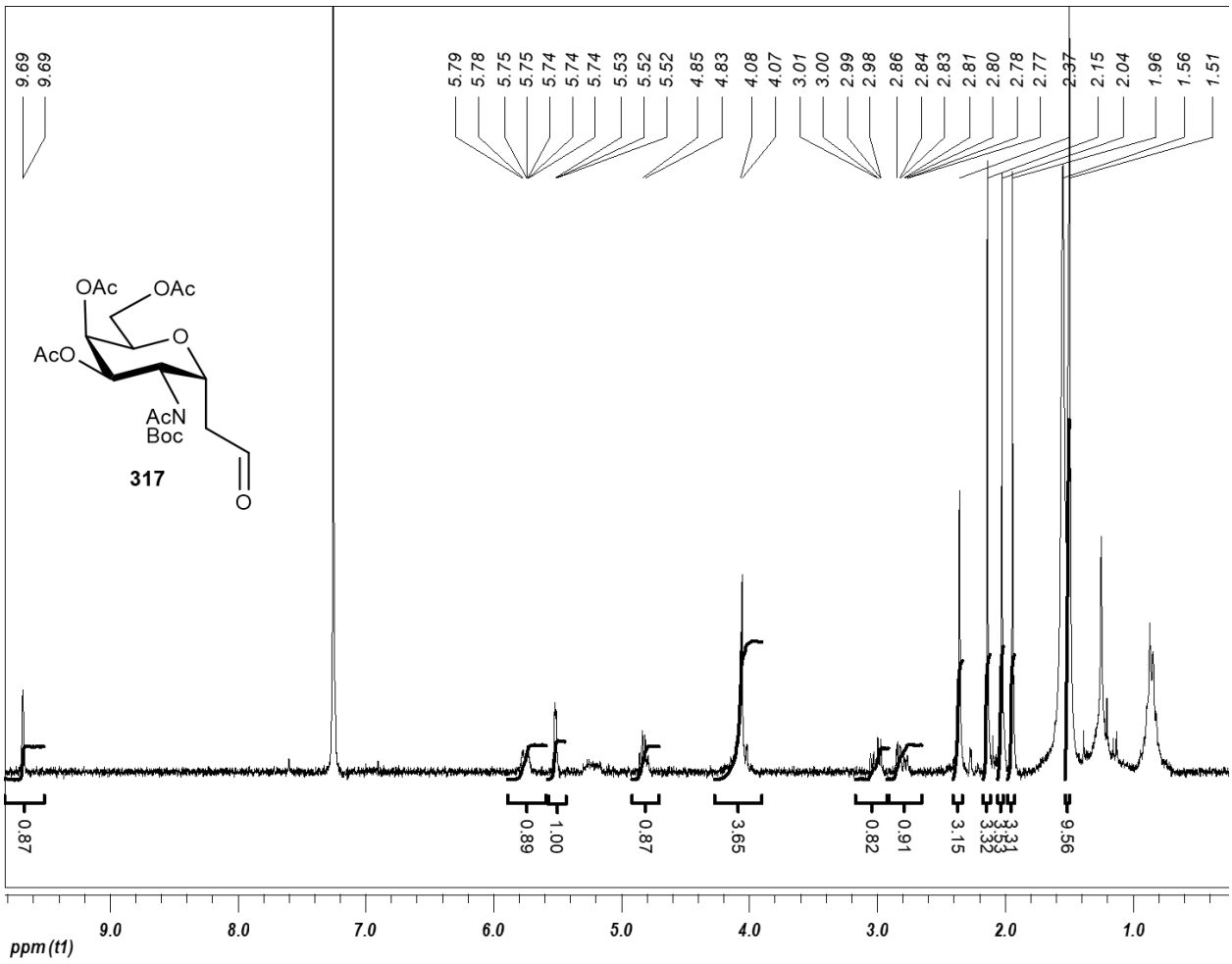

8.14
7.66
7.63
6.92
6.89
5.94
5.92
5.45
5.42
5.38
5.17
5.13
5.10
4.40
4.39
4.36
4.34
4.14
4.13
4.10
4.09
3.99
3.98
3.97
3.97
3.95
3.95
3.94
3.93
3.83
3.44
3.43
2.09
2.03
2.01
1.87
OAc
AcO
AcO
O
OAc
N
MeO
307
2.09
2.09
2.01
1.87
2.52
5.64
2.58
2.20
2.10
2.00
1.90
1.80
ppm (t1)
1.11
2.31
2.31
1.39
1.17
1.32
1.54
1.35
1.17
3.00
1.19
2.52
5.64
2.58
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)


5.71
5.68
5.48
5.14
5.12
4.31
4.29
4.26
4.25
4.16
4.15
4.11
4.11
2.13
2.10
2.06
2.05
1.94
2.13
2.10
2.06
2.05
1.94
2.92
2.99
5.40
2.200
2.150
2.100
2.050
2.000
1.950
ppm (f1)
OAc
AcO
AcO
O
OAc
NH3Cl
308
1.00
0.94
1.99
1.92
1.09
1.11
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


OAc
AcO
AcO
O
OAc
NHAc
309
5.70 5.67 5.47 5.46 5.44 5.43 5.15 5.14 5.13 5.11 5.10 4.31 4.30 4.29 4.28 4.25 4.24 4.23 4.15 4.14 4.11 4.10 3.81 3.80 3.79 3.79 3.78 3.77 3.76 3.76 2.12 2.09 2.04 2.04 1.93
2.12 2.09 2.04 2.04 1.93
2.66 3.15 6.05 2.84
2.30 2.20 2.10 2.00 1.90
ppm (t1)
0.94 0.85 1.96 1.95 0.96 0.95 2.84
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
ppm (t1)


5.89 5.85 5.84 5.84 5.83 5.79 5.77 5.74 5.57 5.56 5.18 5.17 5.14 5.13 5.12 5.12 5.10 4.27 4.26 4.24 4.13 4.11 4.09 4.06 4.05 2.67 2.67 2.66 2.65 2.65 2.64 2.42 2.25 2.25 2.20 2.18 2.06 1.99 1.56
2.42 2.25 2.25 2.20 2.18 2.06 1.99
3.08 0.87 2.97 3.26 2.95
2.40 2.30 2.20 2.10 2.00 1.90
OAc OAc
O
AcO
AcN
Boc
316
2.38 1.00 3.49 1.25 3.76 1.22 3.08 9.49
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0


173.4 170.8 170.6 170.0 153.0 134.4 117.5 84.9 77.5 68.4 68.1 67.7 62.4 32.2 32.0 30.0 30.0 29.7 28.1 23.0 21.0 14.4
OAc OAc
O
AcO
AcN
Boc
316
200 150 100 50 0
ppm (t1)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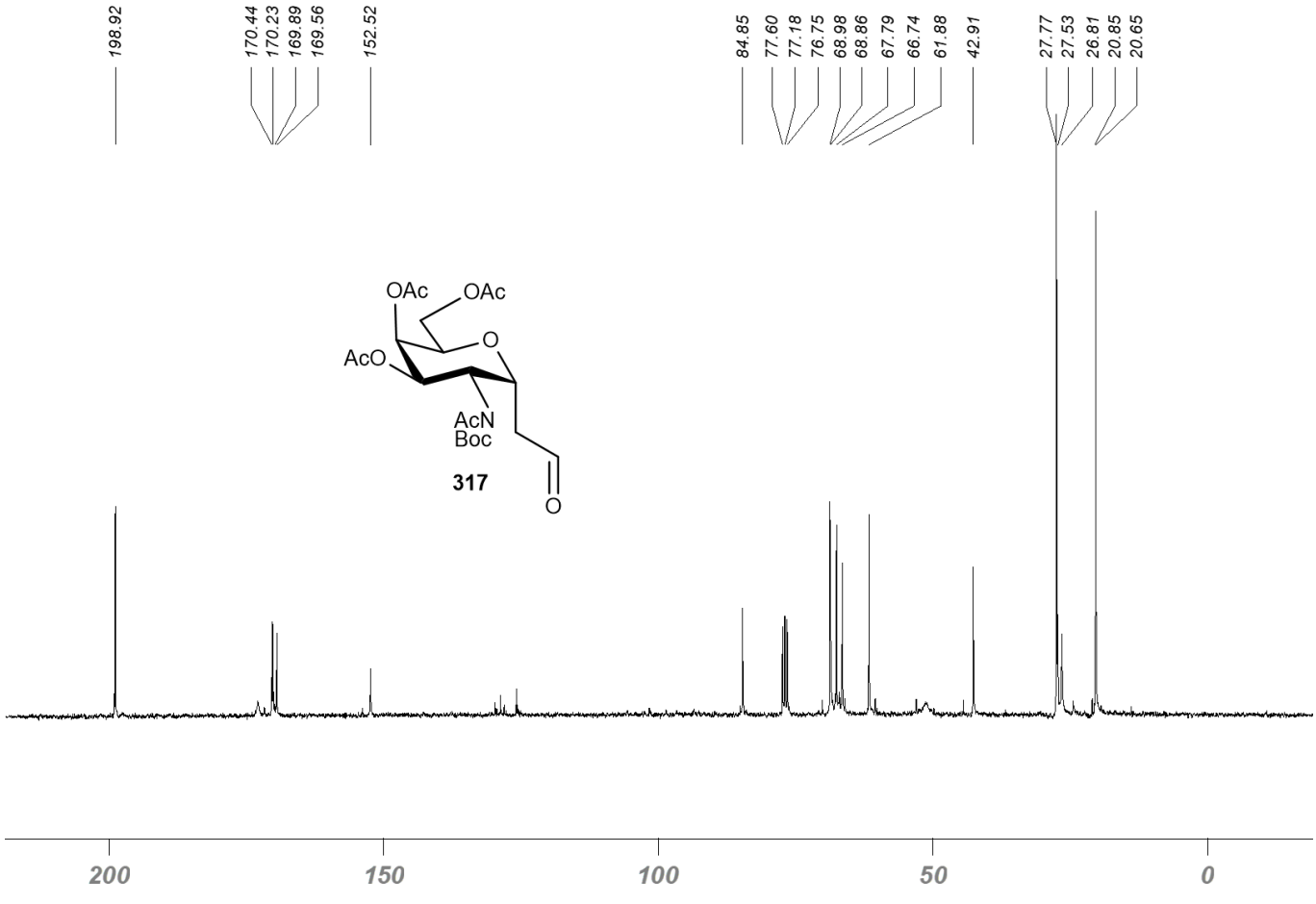
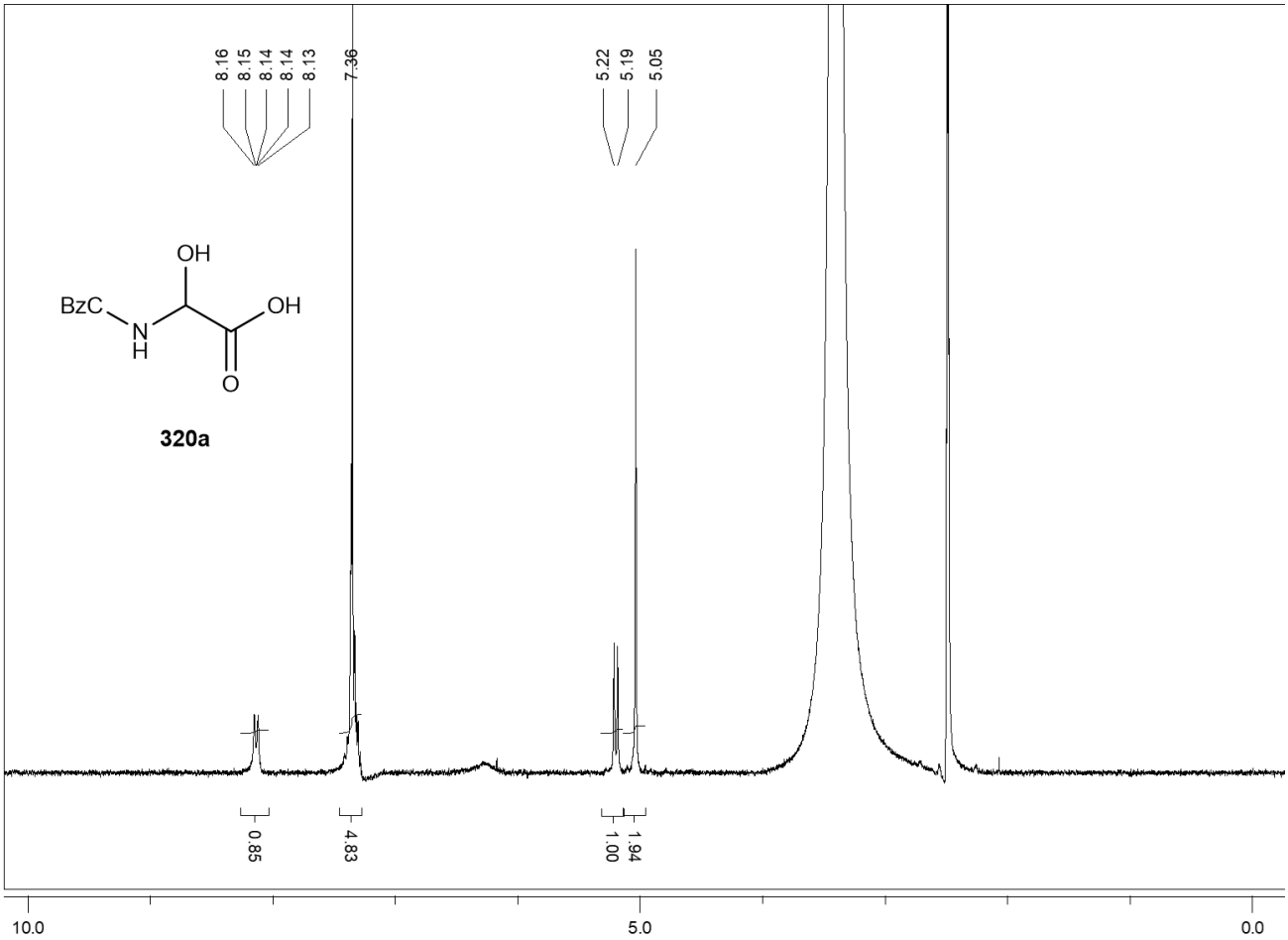
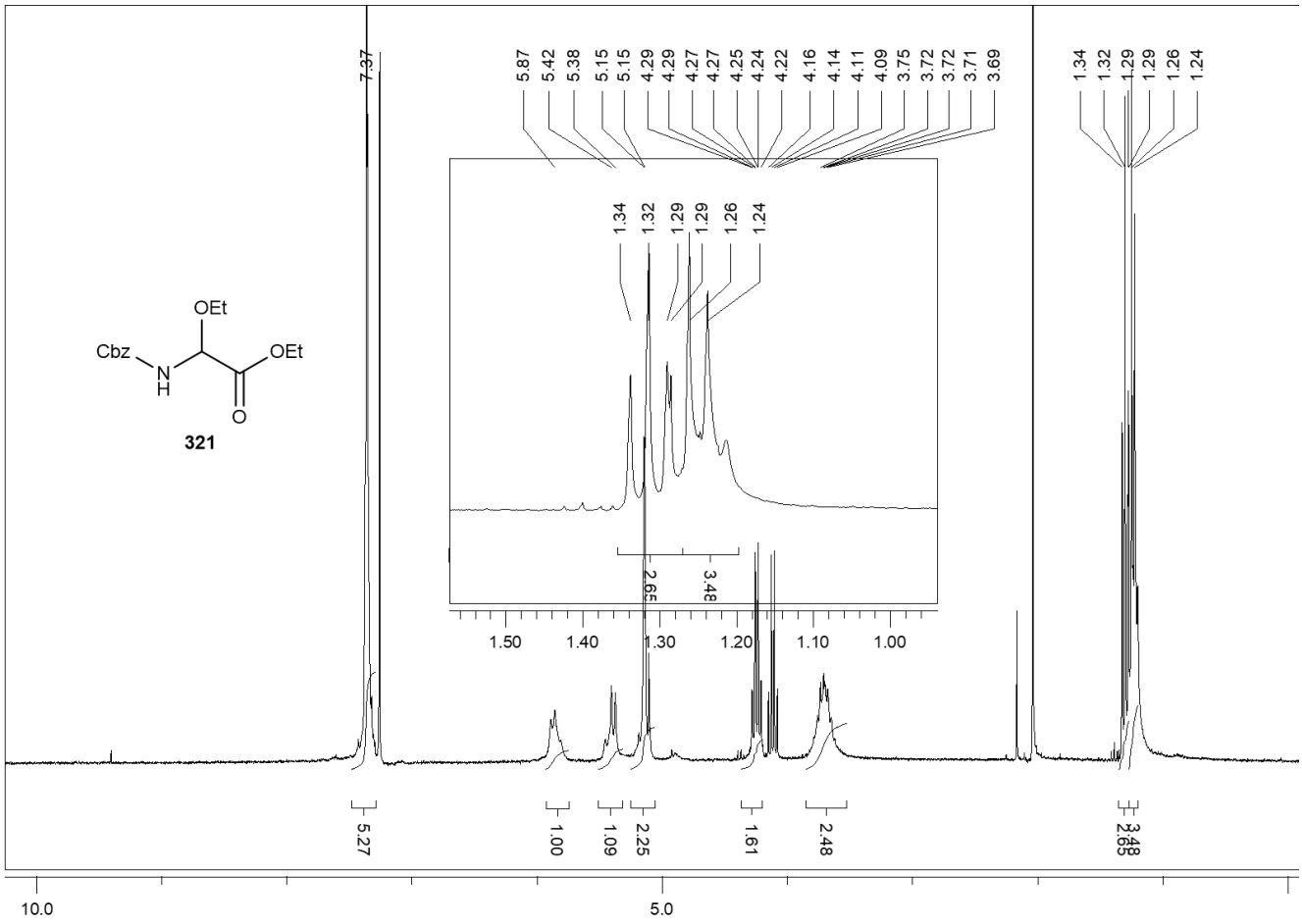
9.69
5.79
5.78
5.75
5.75
5.74
5.74
5.74
5.53
5.52
5.52
4.85
4.83
4.08
4.07
3.01
3.00
2.99
2.98
2.86
2.84
2.83
2.81
2.80
2.78
2.77
2.37
2.15
2.04
1.96
1.56
1.51
OAc
OAc
O
AcO
AcN
Boc
317
O
0.87
0.89
1.00
0.87
3.65
0.82
0.91
3.15
3.32
3.33
3.53
6.96
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


198.92
170.44
170.23
169.89
169.56
152.52
84.85
77.60
77.18
76.75
68.98
68.86
67.79
66.74
61.88
42.91
27.77
27.53
26.81
20.85
20.65
OAc
OAc
O
AcO
AcN
Boc
317
O
200
150
100
50
0


8.16
8.15
8.14
8.14
8.13
7.36
5.22
5.19
5.05
OH
BzC
N
H
OH
O
320a
0.85
4.83
1.00
1.94
10.0
5.0
0.0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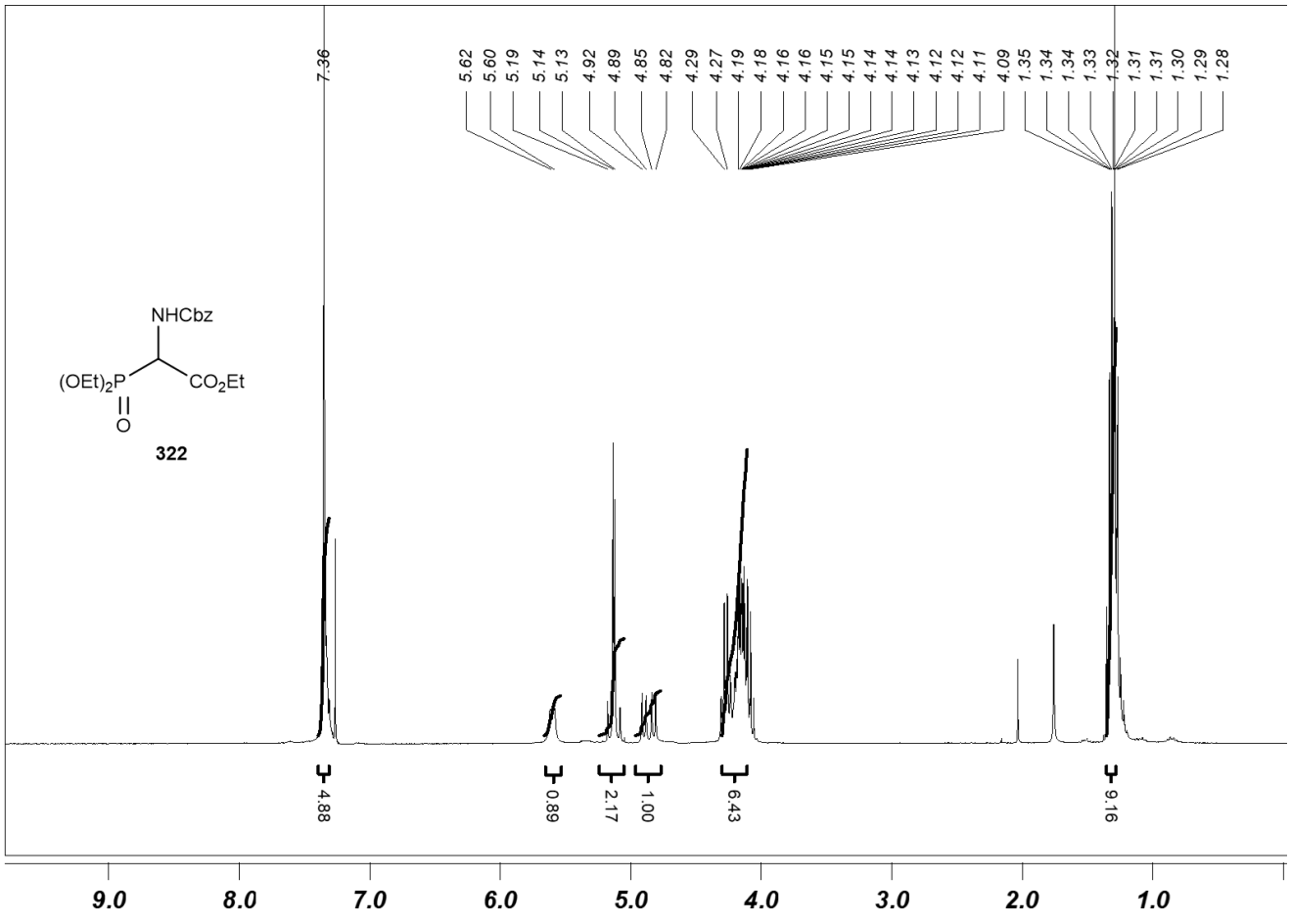
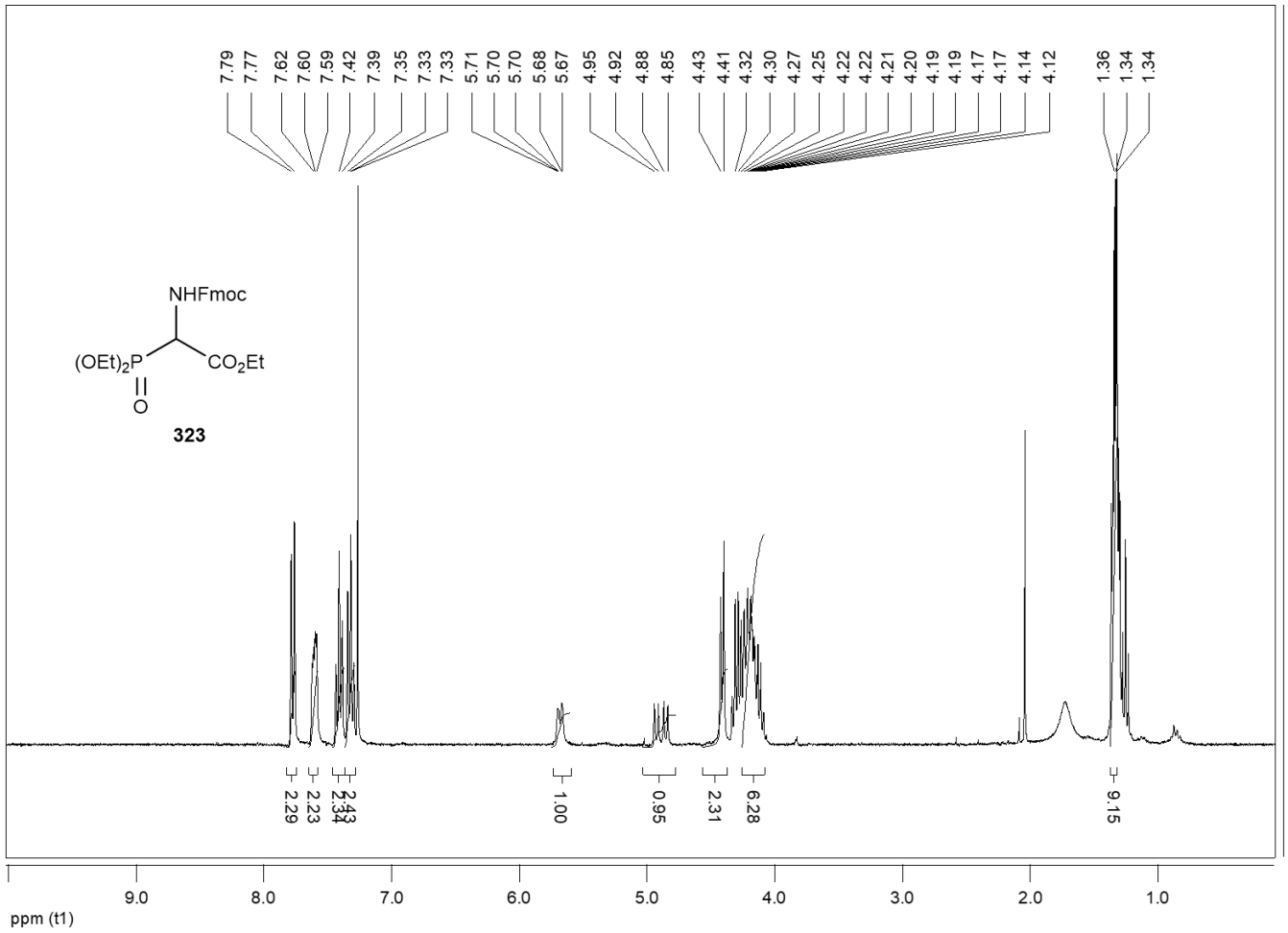
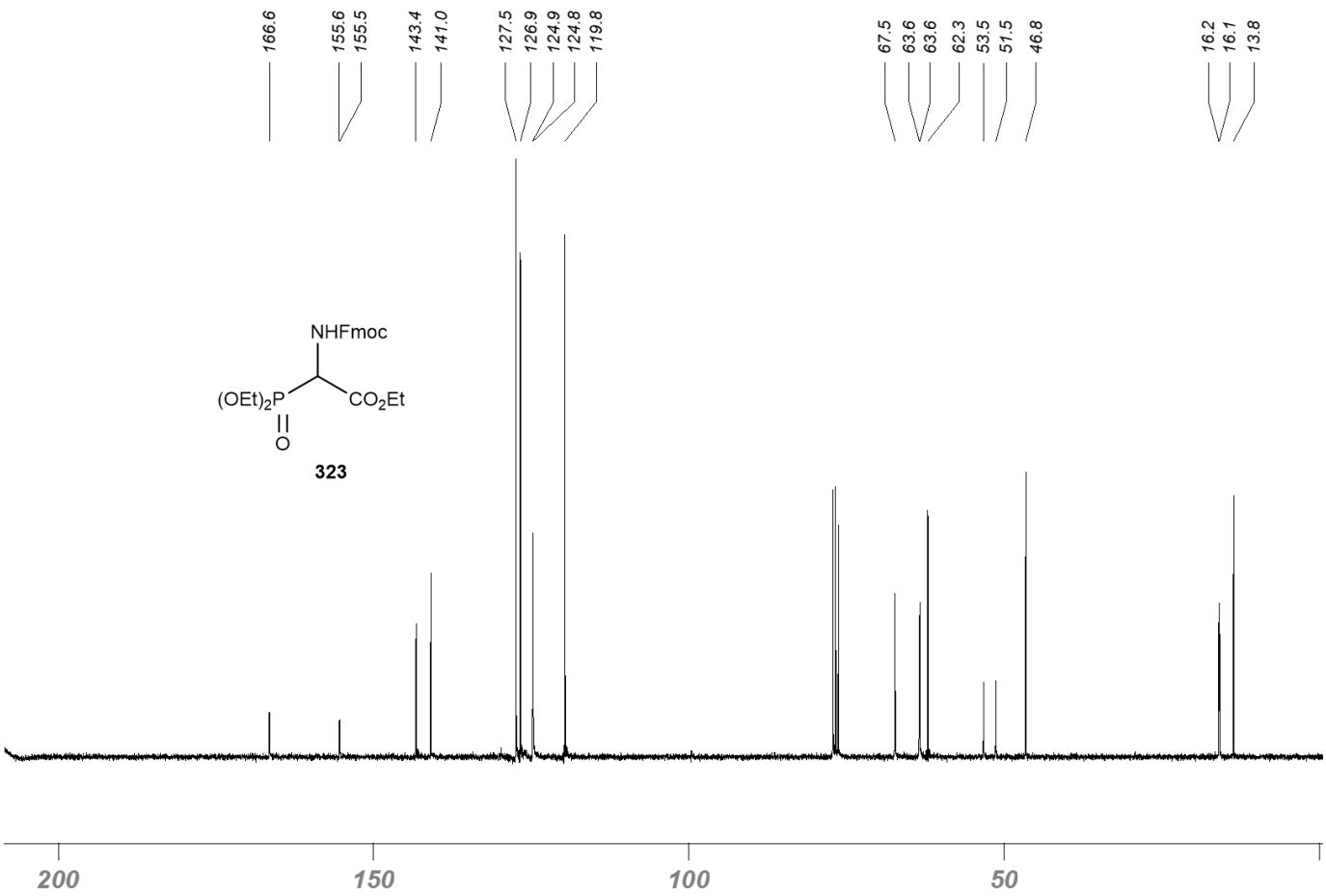
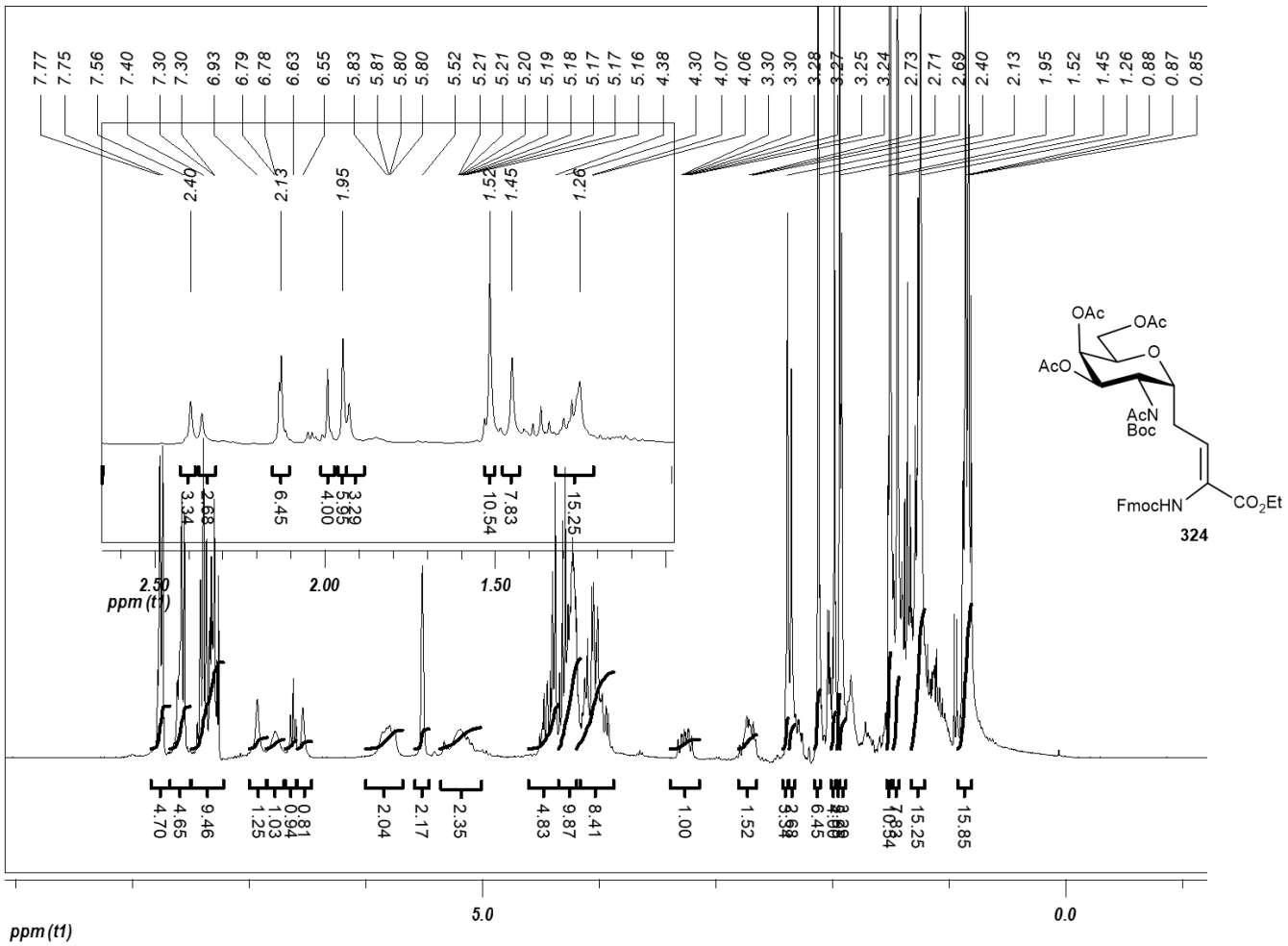
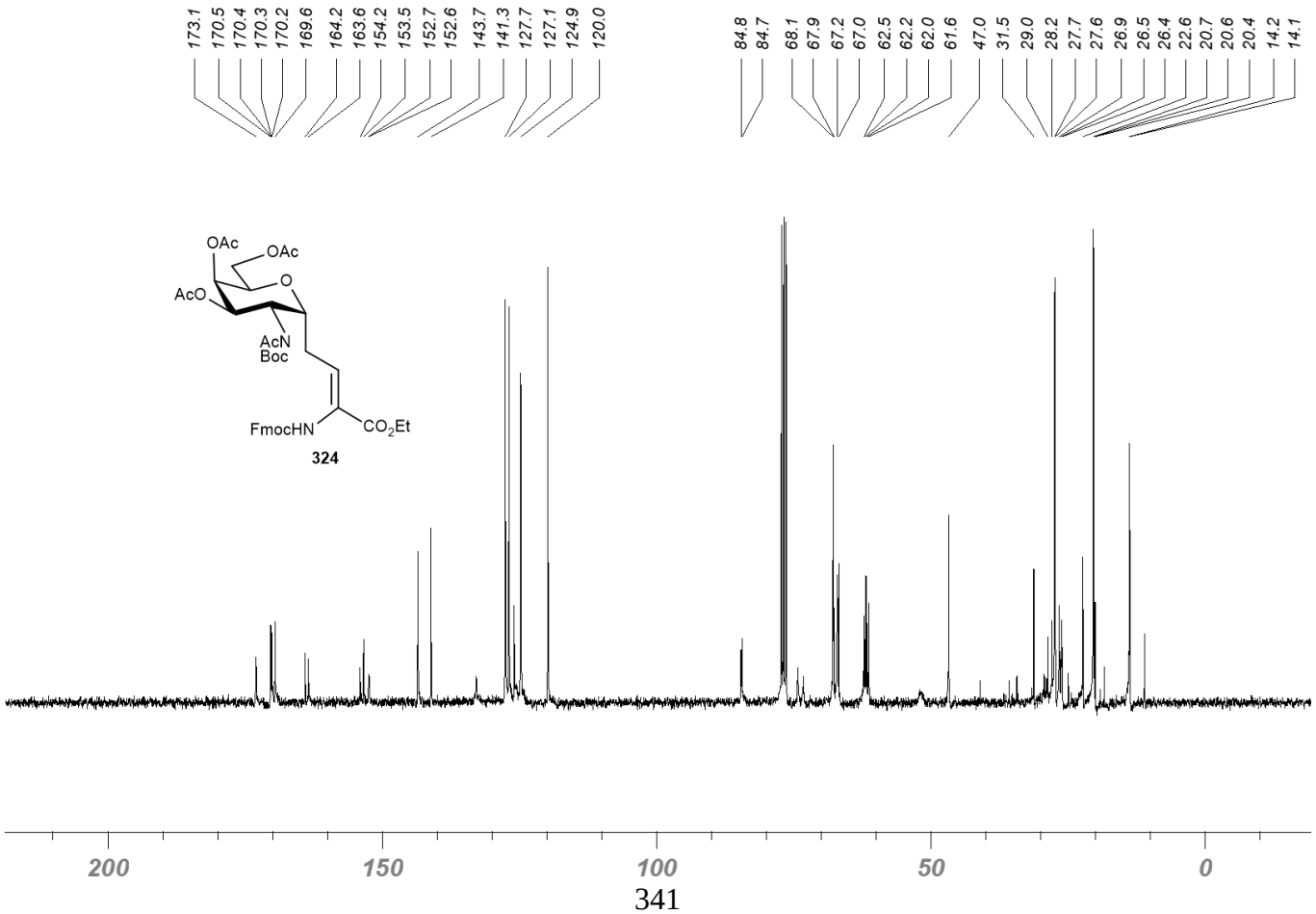
5.87
5.42
5.38
5.15
5.15
4.29
4.29
4.27
4.27
4.25
4.24
4.22
4.16
4.14
4.11
4.09
3.75
3.72
3.72
3.71
3.69
1.34
1.32
1.29
1.29
1.26
1.24
OEt
Cbz
N
H
OEt
O
321
2.65
3.48
1.50
1.40
1.30
1.20
1.10
1.00
5.27
1.00
1.09
2.25
1.61
2.48
10.0
5.0


7.36
5.62
5.60
5.19
5.14
5.13
4.92
4.89
4.85
4.82
4.29
4.27
4.19
4.18
4.16
4.16
4.15
4.15
4.14
4.14
4.13
4.12
4.12
4.11
4.09
1.35
1.34
1.34
1.33
1.32
1.31
1.31
1.30
1.29
1.28
NHCbz
(OEt)2P
CO2Et
O
322
4.88
0.89
2.17
1.00
6.43
9.16
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0


7.79
7.77
7.62
7.60
7.59
7.42
7.39
7.35
7.33
7.33
5.71
5.70
5.70
5.68
5.67
4.95
4.92
4.88
4.85
4.43
4.41
4.32
4.30
4.27
4.25
4.22
4.22
4.21
4.20
4.19
4.19
4.17
4.17
4.14
4.12
1.36
1.34
1.34
NHFmoc
(OEt)2P
CO2Et
O
323
2.29
2.23
2.34
2.43
1.00
0.95
2.31
9.28
9.15
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


166.6
155.6
155.5
143.4
141.0
127.5
126.9
124.9
124.8
119.8
67.5
63.6
63.6
62.3
53.5
51.5
46.8
16.2
16.1
13.8
NHFmoc
(OEt)2P
CO2Et
O
323
200
150
100
50


7.77 7.75 7.56 7.40 7.30 7.30 6.93 6.79 6.78 6.63 6.55 5.83 5.81 5.80 5.80 5.52 5.21 5.21 5.20 5.19 5.18 5.17 5.17 5.16 4.38 4.30 4.07 4.06 3.30 3.30 3.28 3.27 3.25 3.24 2.73 2.71 2.69 2.40 2.13 1.95 1.52 1.45 1.26 0.88 0.87 0.85
2.40 2.13 1.95 1.52 1.45 1.29
3.34 2.68 6.45 4.00 10.54 7.83 15.25
2.50 2.00 1.50
ppm (t1)
4.70 4.65 9.46 1.25 1.03 0.94 0.81 2.04 2.17 2.35 4.83 9.87 8.41 1.00 1.52 6.45 15.25 15.85
5.0 0.0
ppm (t1)
OAc OAc O AcO AcN Boc FmocHN CO2Et
324


173.1 170.5 170.4 170.3 170.2 169.6 164.2 163.6 154.2 153.5 152.7 152.6 143.7 141.3 127.7 127.1 124.9 120.0
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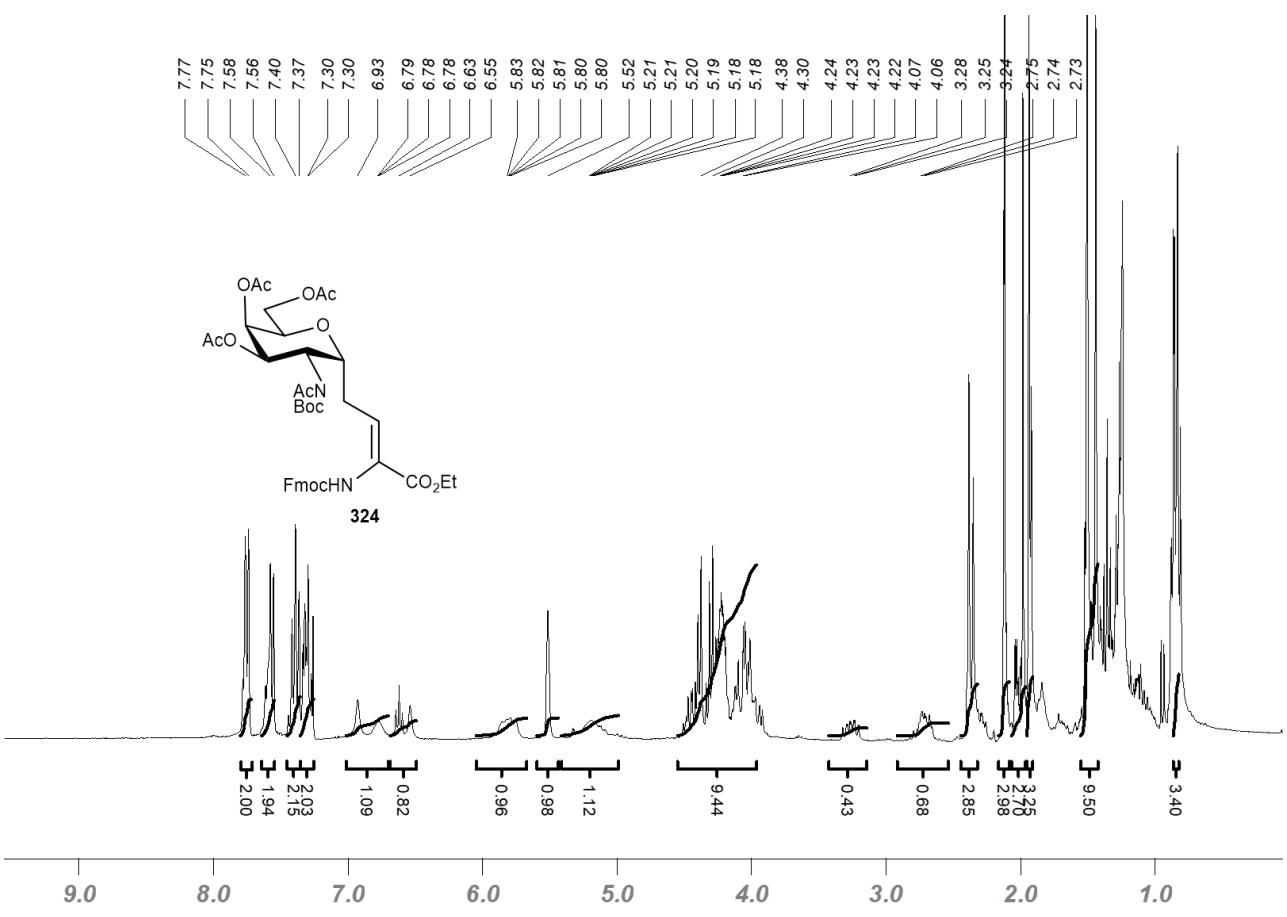
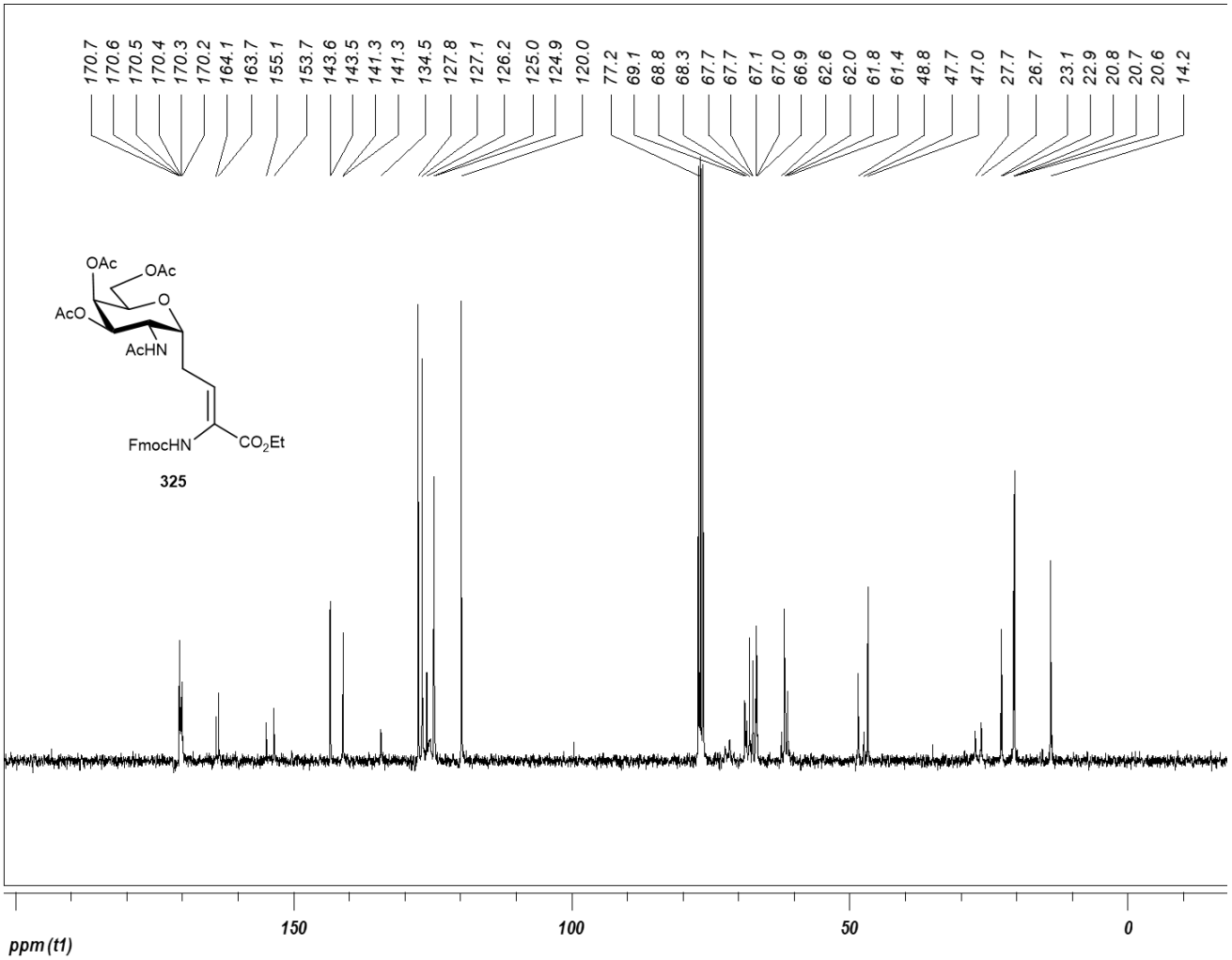
OAc OAc O AcO AcN Boc FmocHN CO2Et
324
200 150 100 50 0


7.77 7.75 7.58 7.56 7.40 7.37 7.30 7.30 6.93 6.79 6.78 6.78 6.63 6.55 5.83 5.82 5.81 5.80 5.80 5.52 5.21 5.21 5.20 5.19 5.18 5.18 4.38 4.30 4.24 4.23 4.23 4.22 4.07 4.06 3.28 3.25 3.24 2.75 2.74 2.73
OAc
OAc
O
AcO
AcN
Boc
FmocHN
CO2Et
324
2.00 1.94 2.15 2.03 1.09 0.82 0.96 0.98 1.12 9.44 0.43 0.68 2.85 2.98 3.25 9.50 3.40
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0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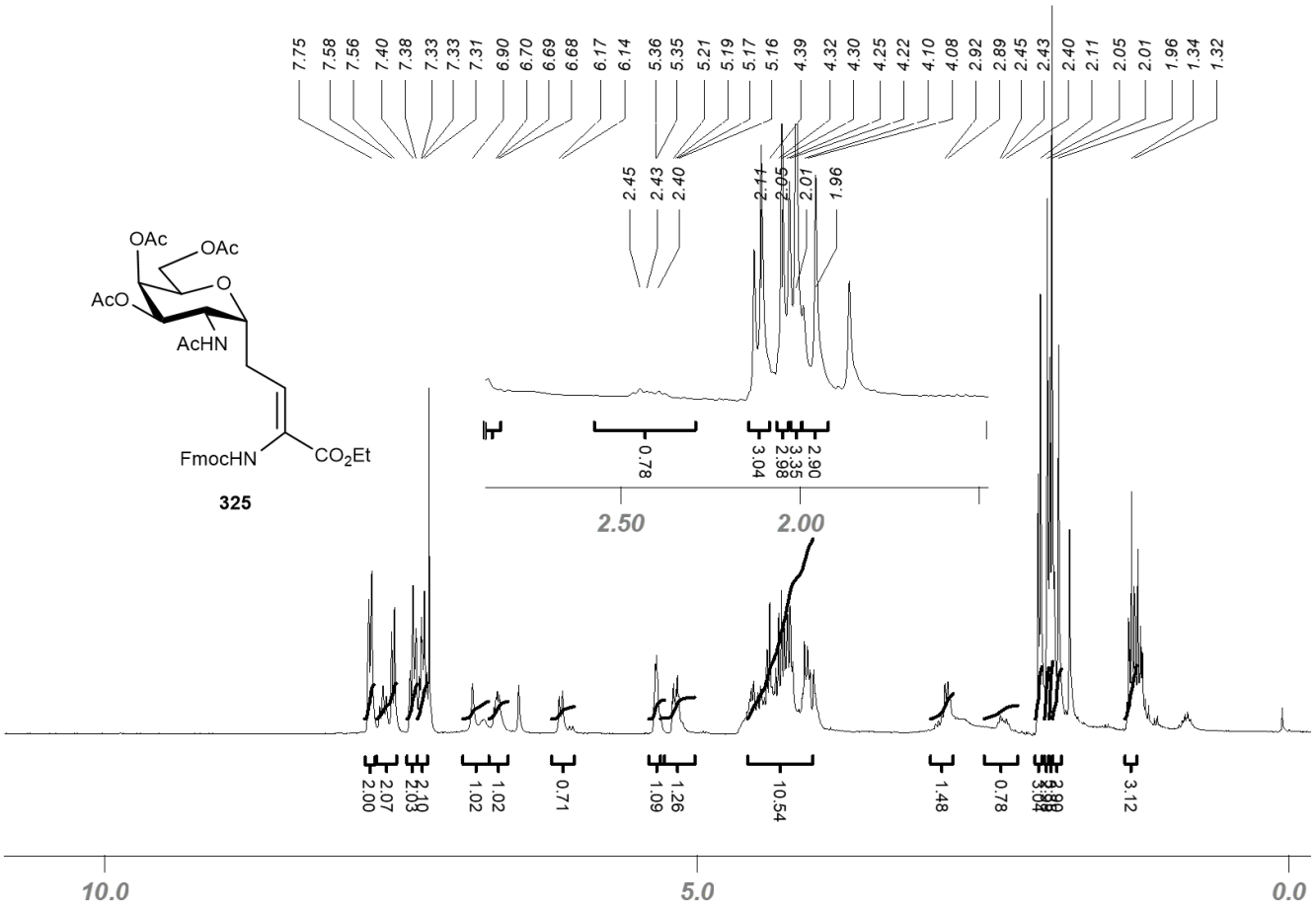
OAc
OAc
O
AcO
AcHN
FmocHN
CO2Et
325
150 100 50 0
ppm (t1)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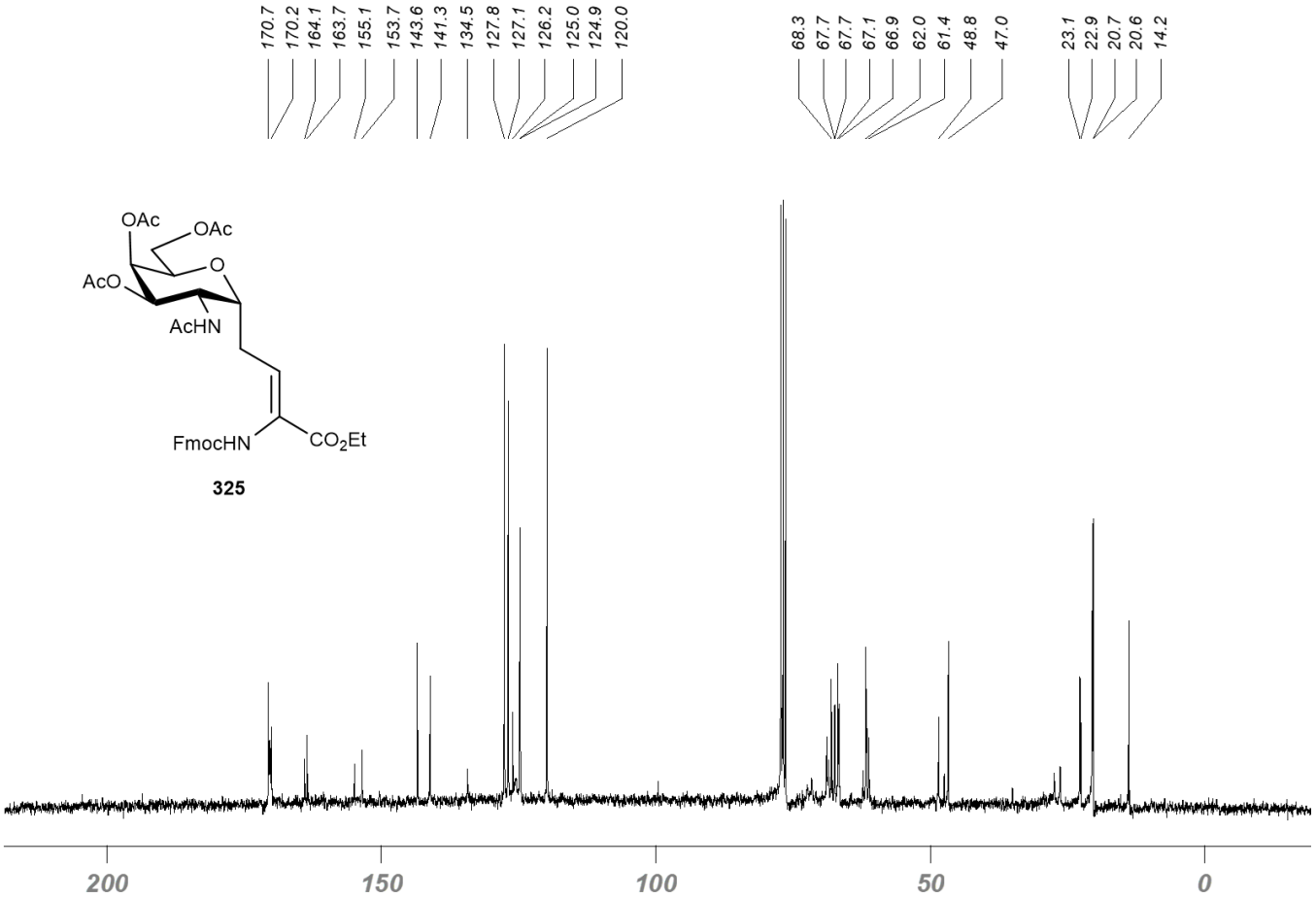
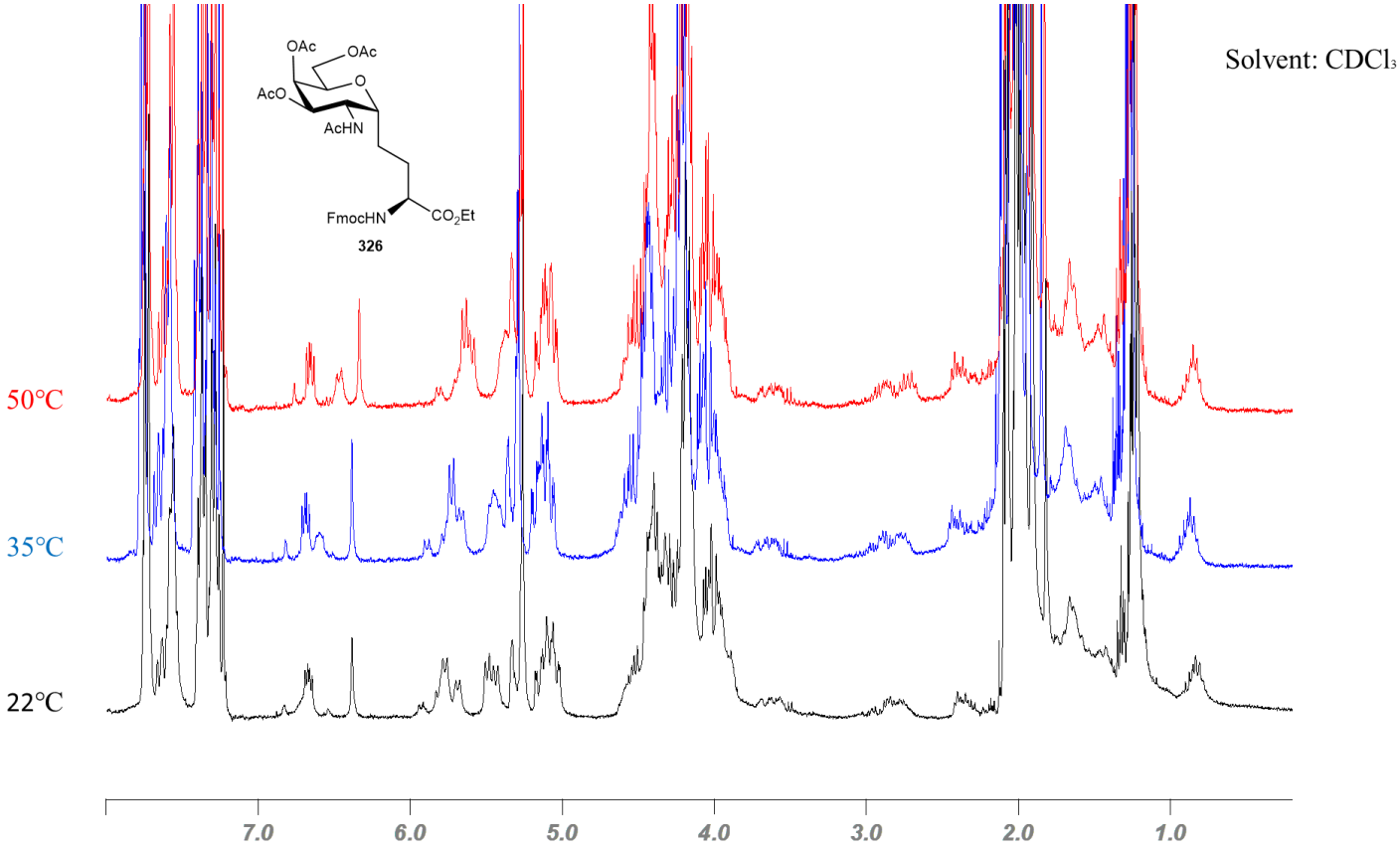
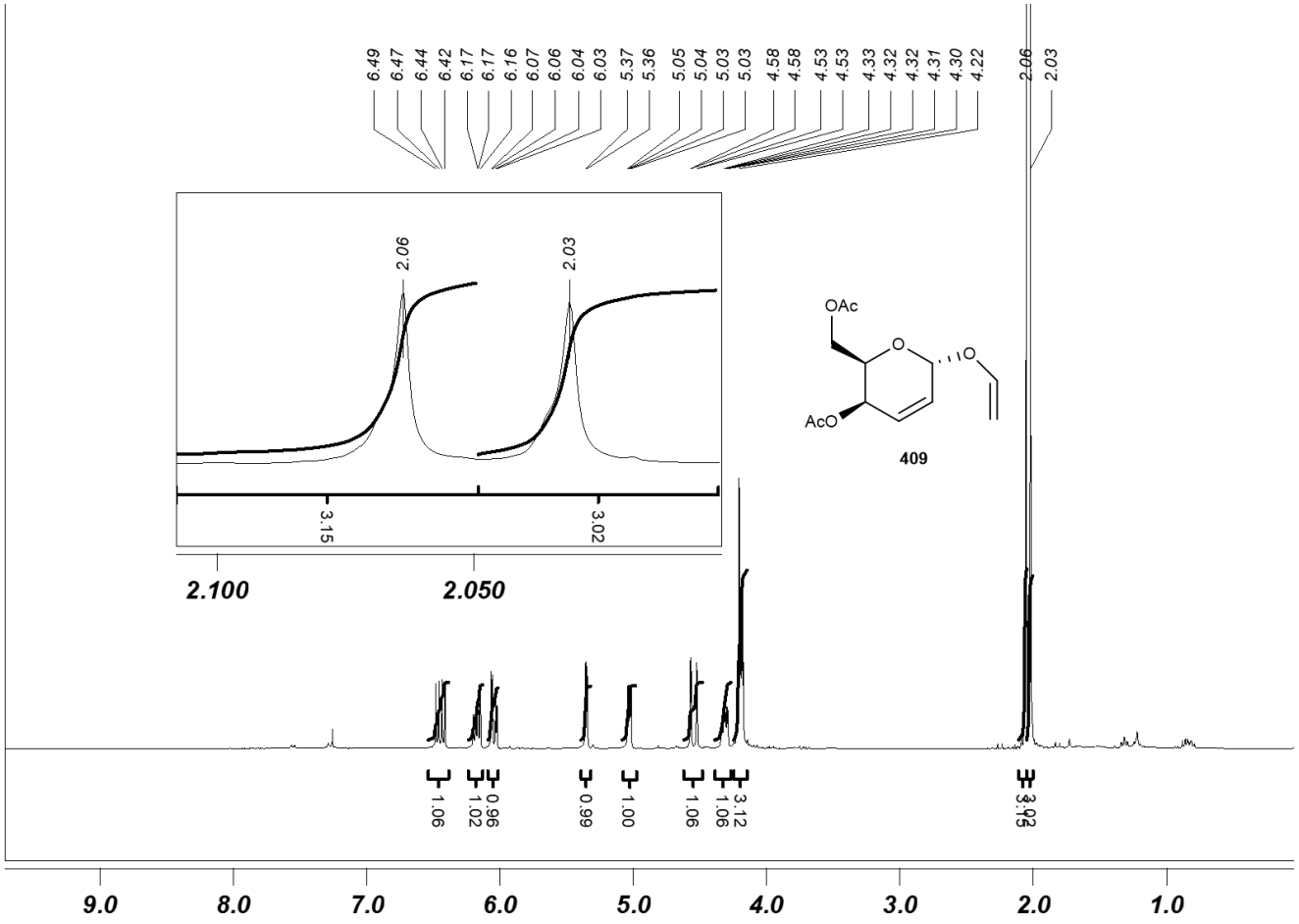
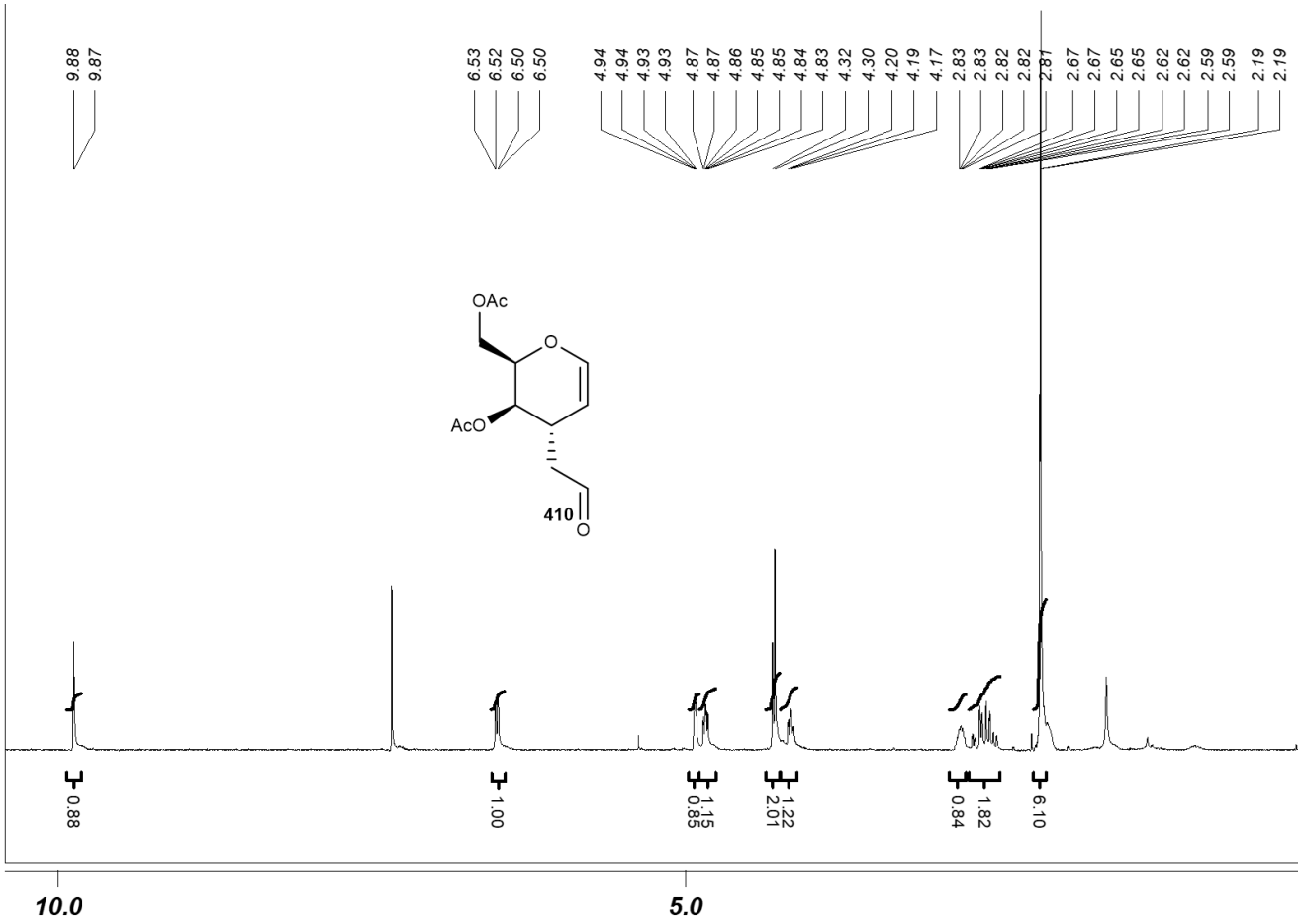
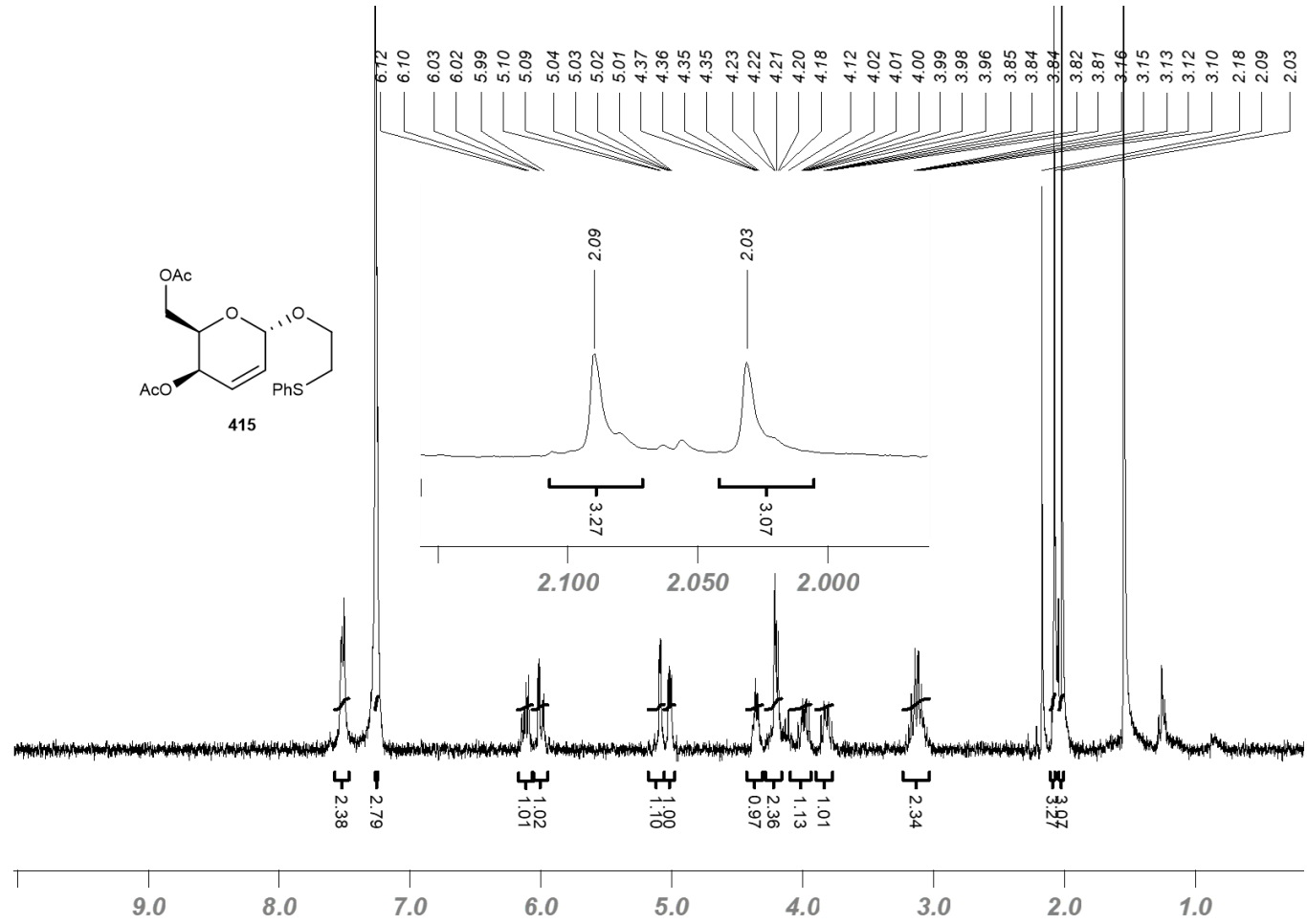
OAc OAc AcO O AcHN FmocHN CO2Et
325
2.50 2.00
10.0 5.0 0.0

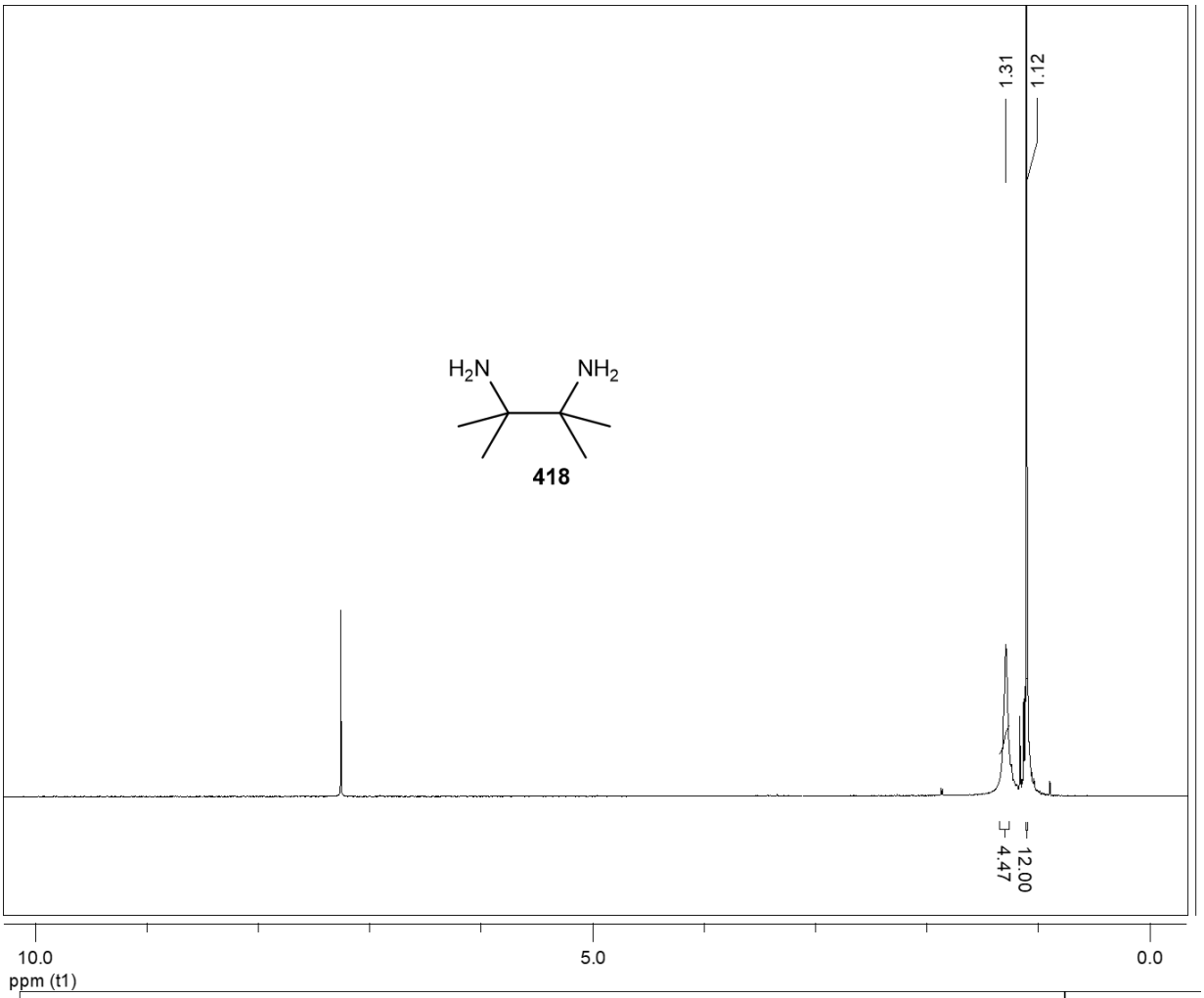

170.7 170.2 164.1 163.7 155.1 153.7 143.6 141.3 134.5 127.8 127.1 126.2 125.0 124.9 120.0
68.3 67.7 67.7 67.1 66.9 62.0 61.4 48.8 47.0
23.1 22.9 20.7 20.6 14.2
OAc OAc AcO O AcHN FmocHN CO2Et
325
200 150 100 50 0


Solvent: CDCl3
OAc
OAc
AcO
O
AcHN
FmocHN
CO2Et
326
50°C
35°C
22°C
7.0
6.0
5.0
4.0
3.0
2.0
1.0


6.49
6.47
6.44
6.42
6.17
6.17
6.16
6.07
6.06
6.04
6.03
5.37
5.36
5.05
5.04
5.03
5.03
4.58
4.58
4.53
4.53
4.33
4.32
4.32
4.31
4.30
4.22
2.06
2.03
2.06
2.03
3.15
3.02
2.100
2.050
OAc
O
O
AcO
409
1.06
1.02
0.96
0.99
1.00
1.06
1.06
3.12
3.00
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0


9.88
9.87
6.53
6.52
6.50
6.50
4.94
4.94
4.93
4.93
4.87
4.87
4.86
4.85
4.85
4.84
4.83
4.32
4.30
4.20
4.19
4.17
2.83
2.83
2.82
2.82
2.81
2.67
2.67
2.65
2.65
2.62
2.62
2.59
2.59
2.19
2.19
OAc
AcO
O
410
O
0.88
1.00
1.15
0.85
1.22
2.01
0.84
1.82
6.10
10.0
5.0


6.12
6.10
6.03
6.02
5.99
5.10
5.09
5.04
5.03
5.02
5.01
4.37
4.36
4.35
4.35
4.23
4.22
4.21
4.20
4.18
4.12
4.02
4.01
4.00
3.99
3.98
3.96
3.85
3.84
3.84
3.82
3.81
3.16
3.15
3.13
3.12
3.10
2.18
2.09
2.03
OAc
AcO
O
O
PhS
415
2.09
2.03
3.27
3.07
2.100
2.050
2.000
2.38
2.79
1.02
1.01
1.00
1.10
2.36
0.97
1.13
1.01
2.34
3.27
3.27
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0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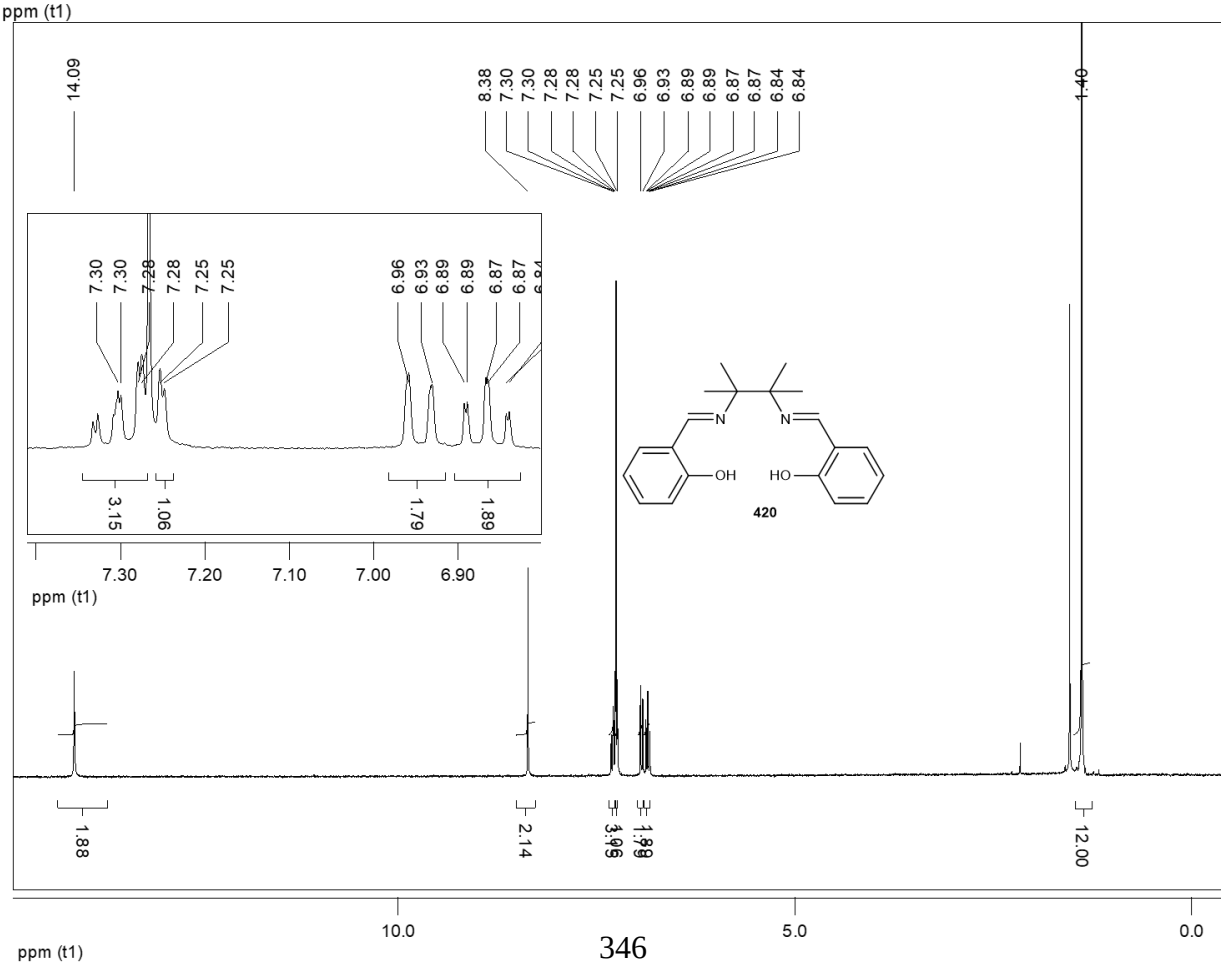
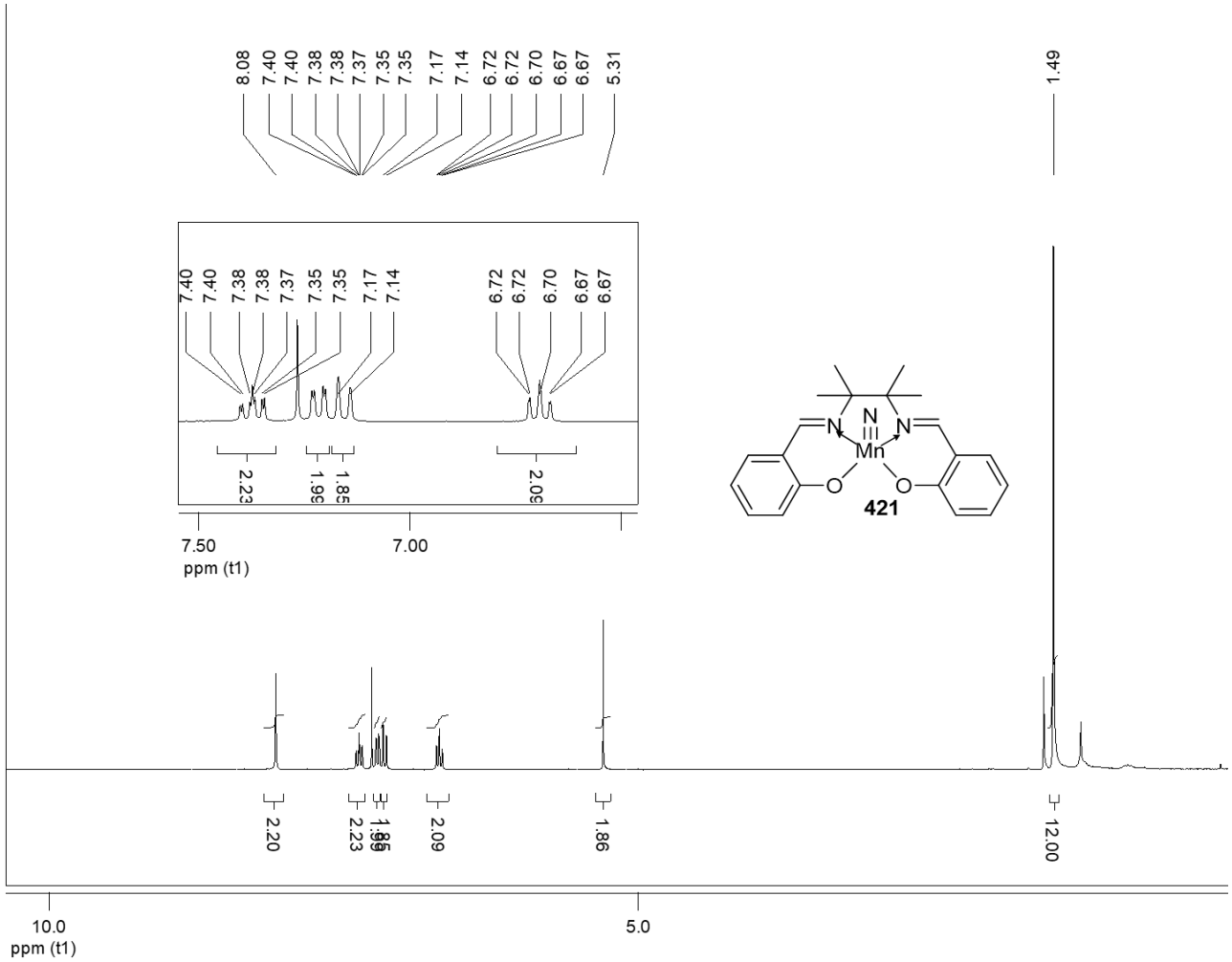
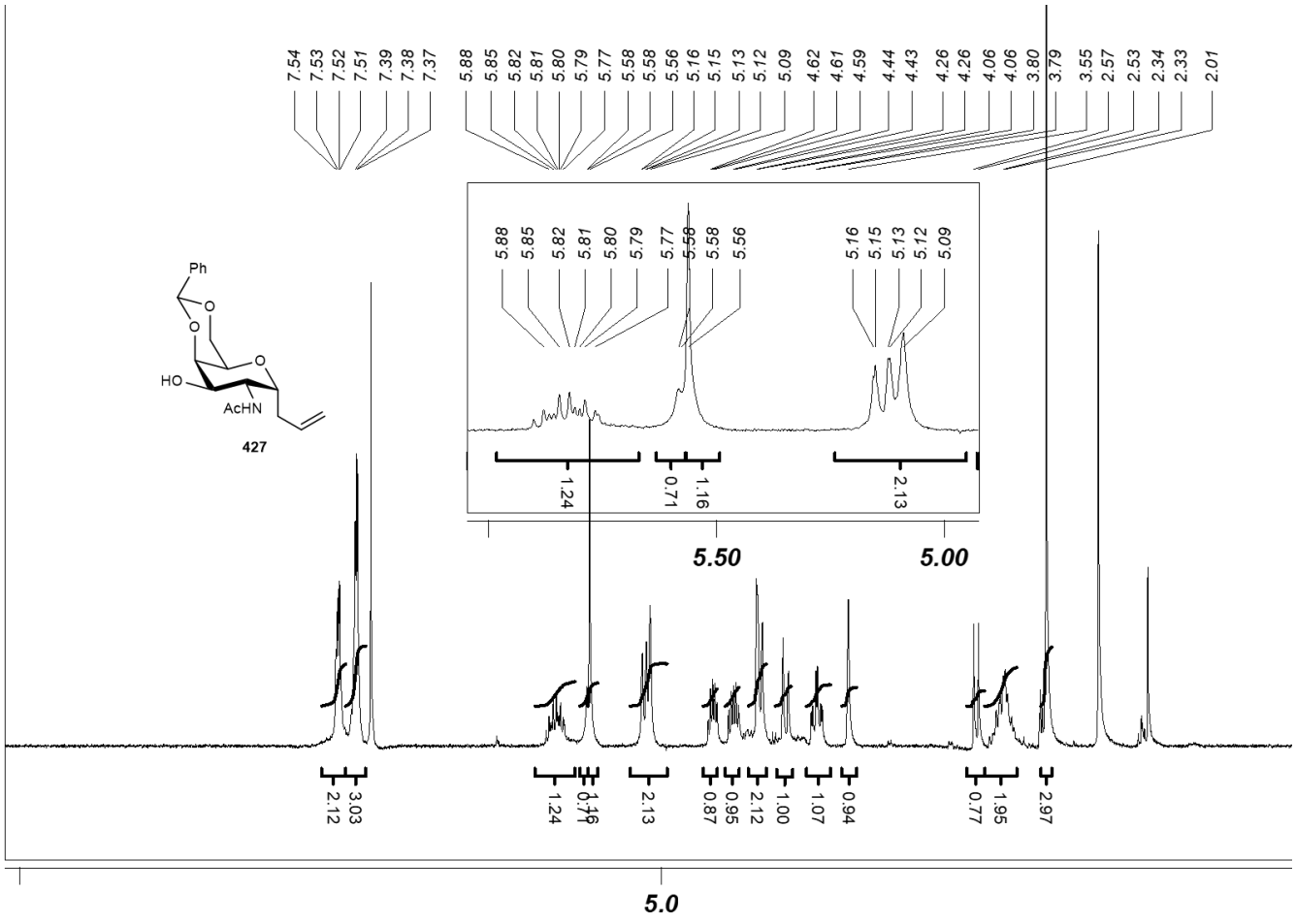
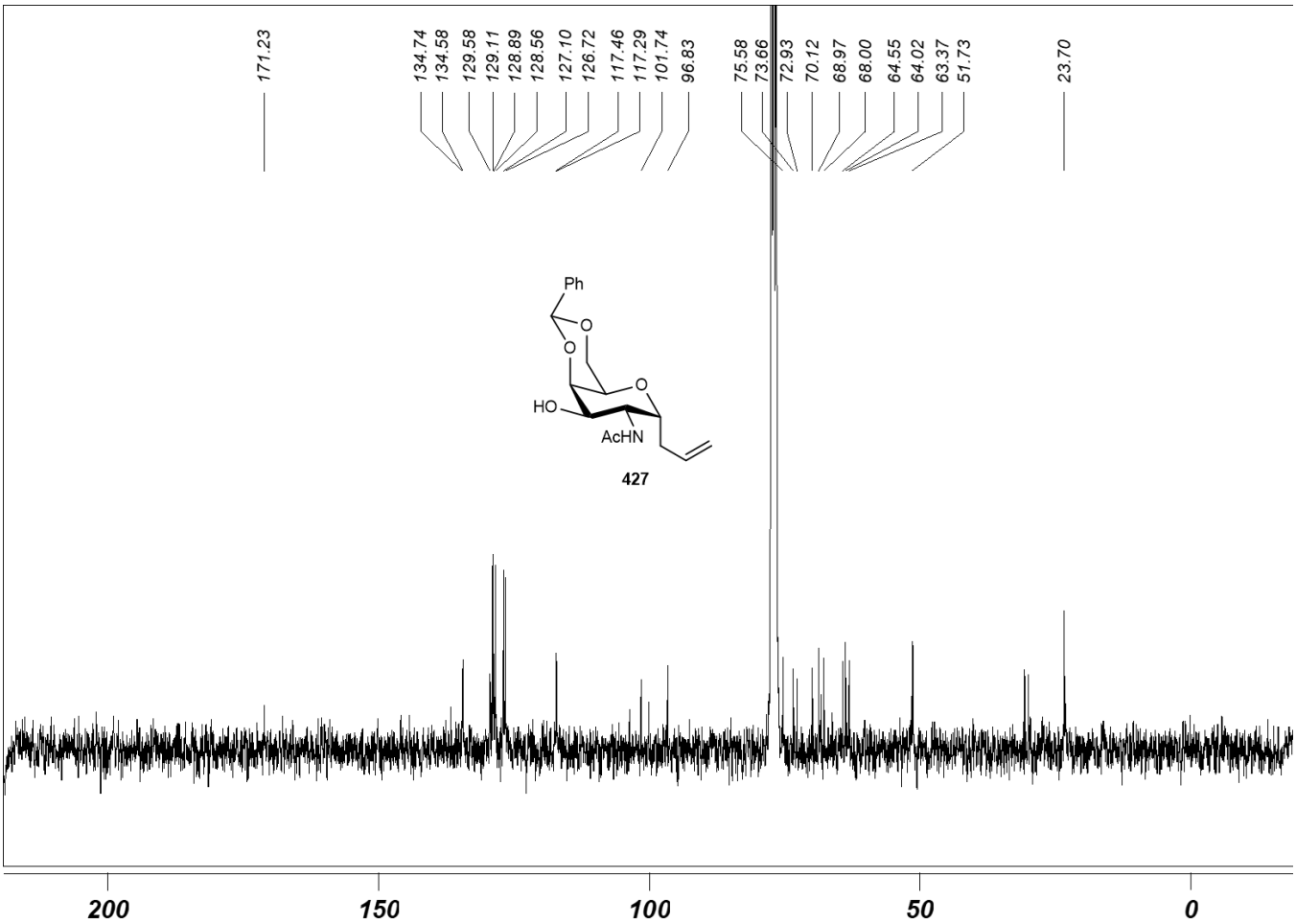
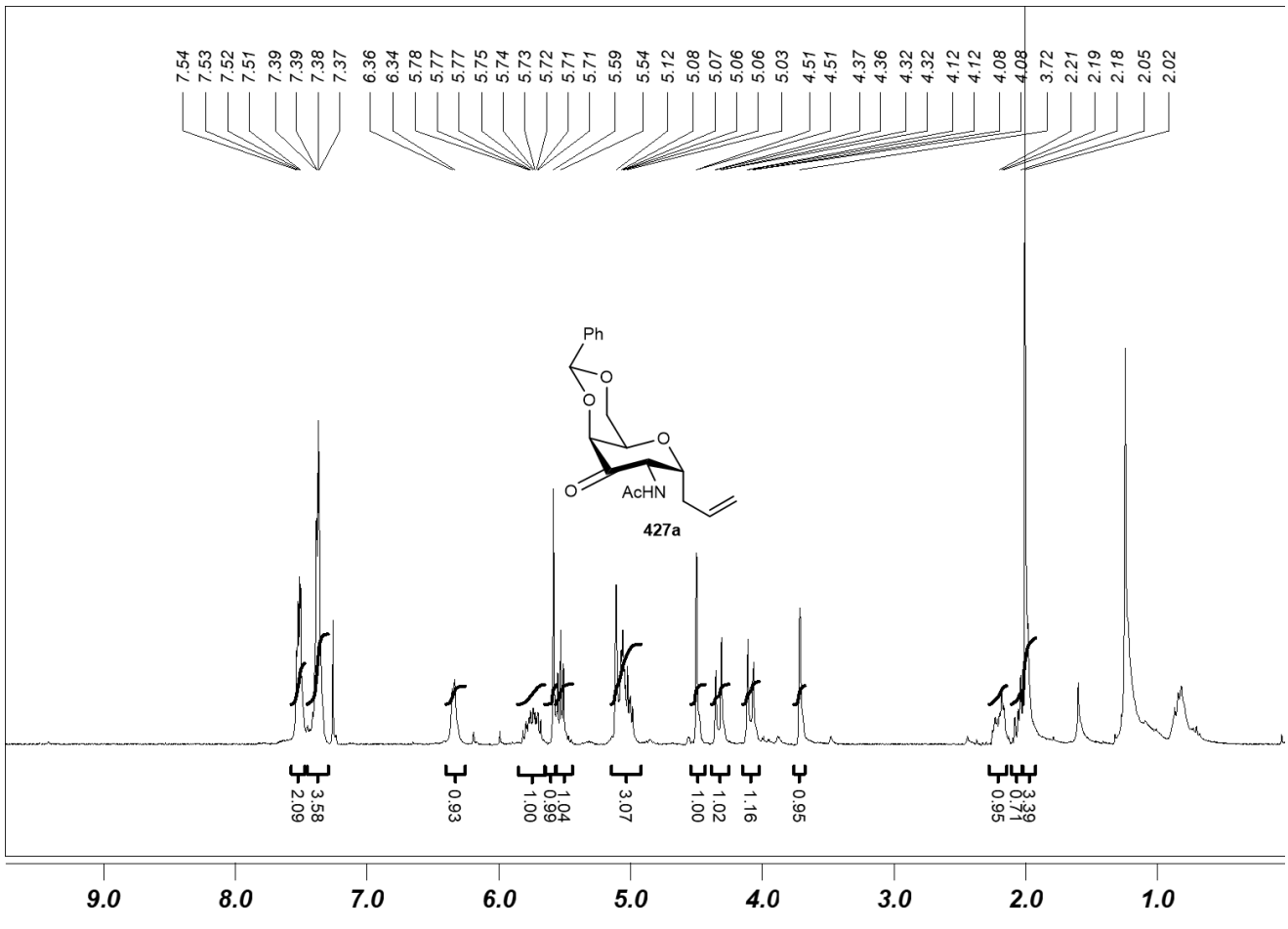
1.12
H2N
NH2
418
4.47
12.00
10.0
5.0
0.0
ppm (t1)

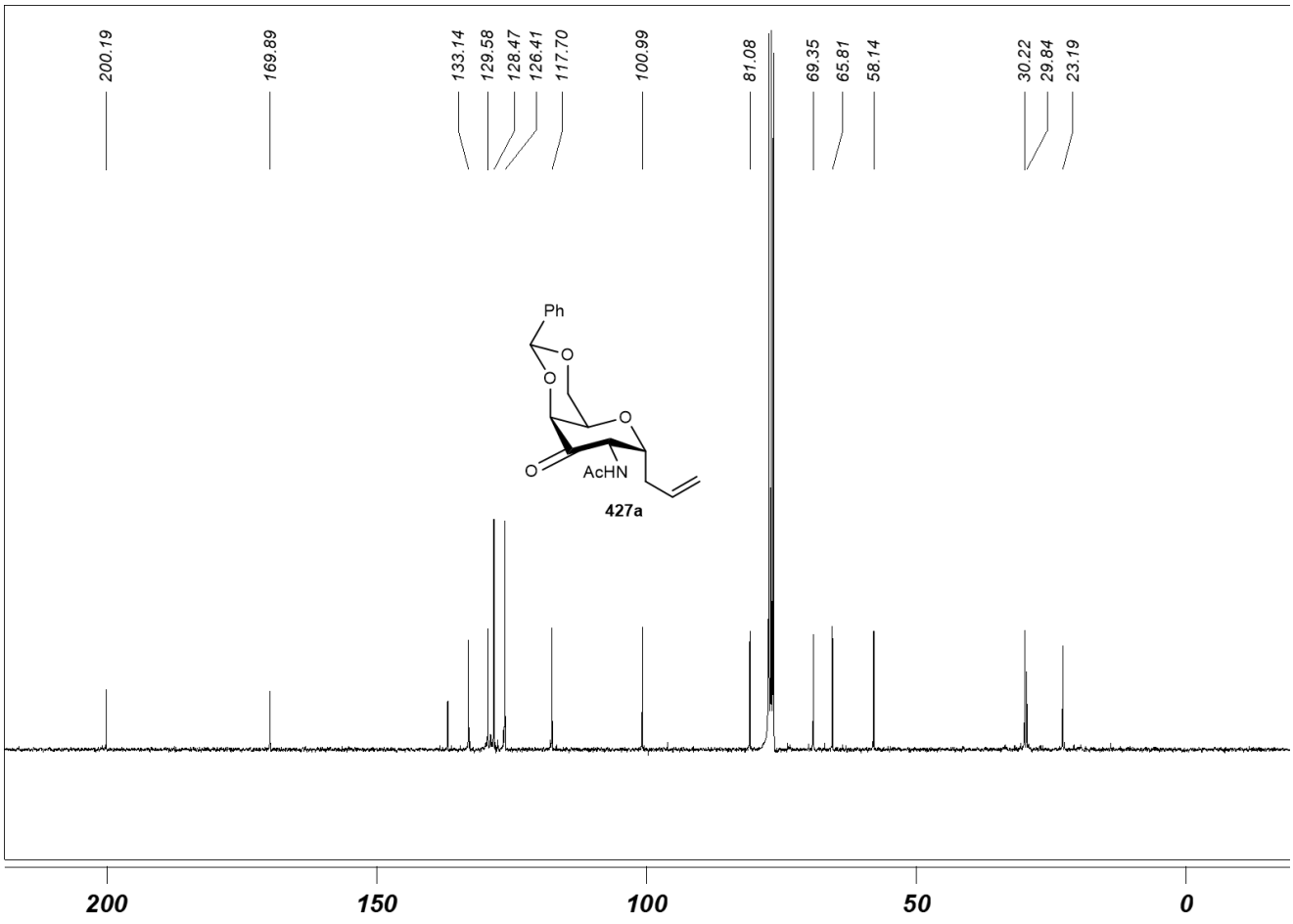

14.09
8.38
7.30
7.30
7.28
7.28
7.25
7.25
6.96
6.93
6.89
6.89
6.87
6.87
6.84
6.84
1.00
3.15
1.06
1.79
1.89
7.30
7.20
7.10
7.00
6.90
ppm (t1)
N
N
OH
HO
420
1.88
2.14
3.96
1.89
12.00
10.0
5.0
0.0
ppm (t1)

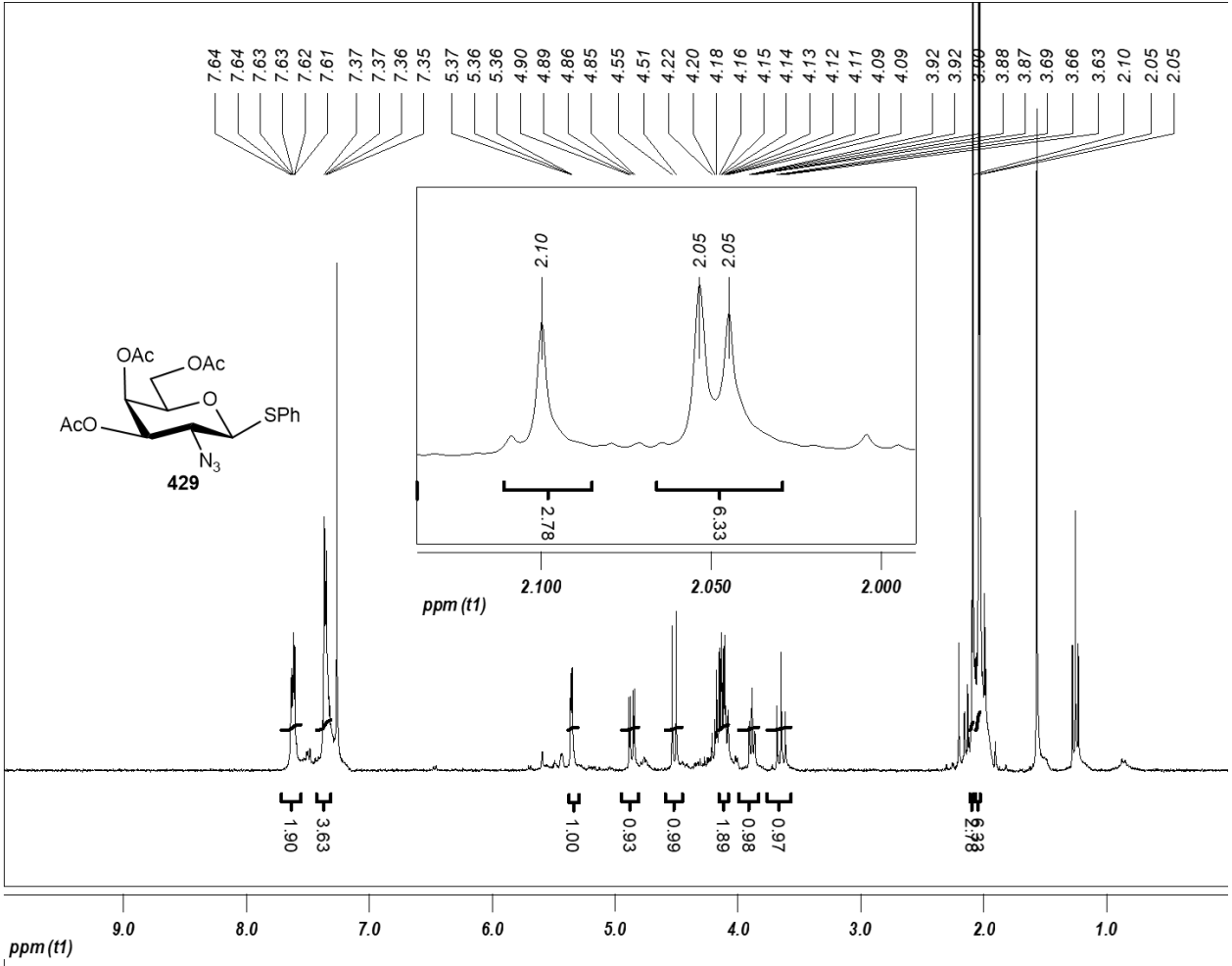

8.08
7.40
7.40
7.38
7.38
7.37
7.35
7.35
7.17
7.14
6.72
6.72
6.70
6.67
6.67
5.31
1.49
2.23
1.96
1.85
2.03
7.50
7.00
ppm (t1)
N
N
N
Mn
O
O
421
2.20
2.23
2.09
1.86
12.00
10.0
5.0
ppm (t1)

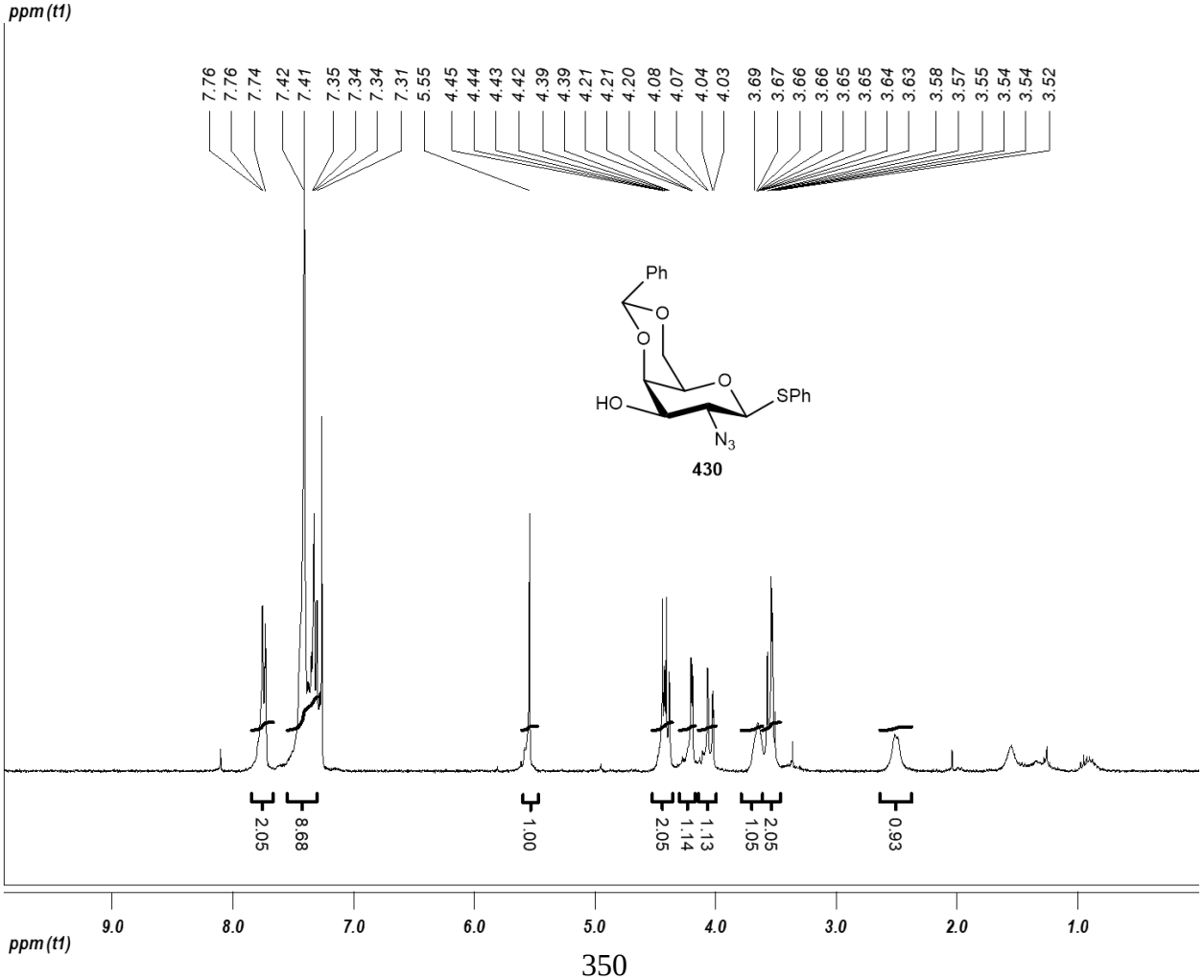

7.54 7.53 7.52 7.51 7.39 7.38 7.37
5.88 5.85 5.82 5.81 5.80 5.79 5.77 5.58 5.58 5.56 5.16 5.15 5.13 5.12 5.09
4.62 4.61 4.59 4.44 4.43 4.26 4.26 4.06 4.06 3.80 3.79 3.55 2.57 2.53 2.34 2.33 2.01
Ph
O
O
O
HO
AcHN
427
1.24
0.71
1.16
2.13
5.50
5.00
2.12 3.03 1.24 0.71 2.13 0.87 0.95 2.12 1.00 1.07 0.94 0.77 1.95 2.97
5.0

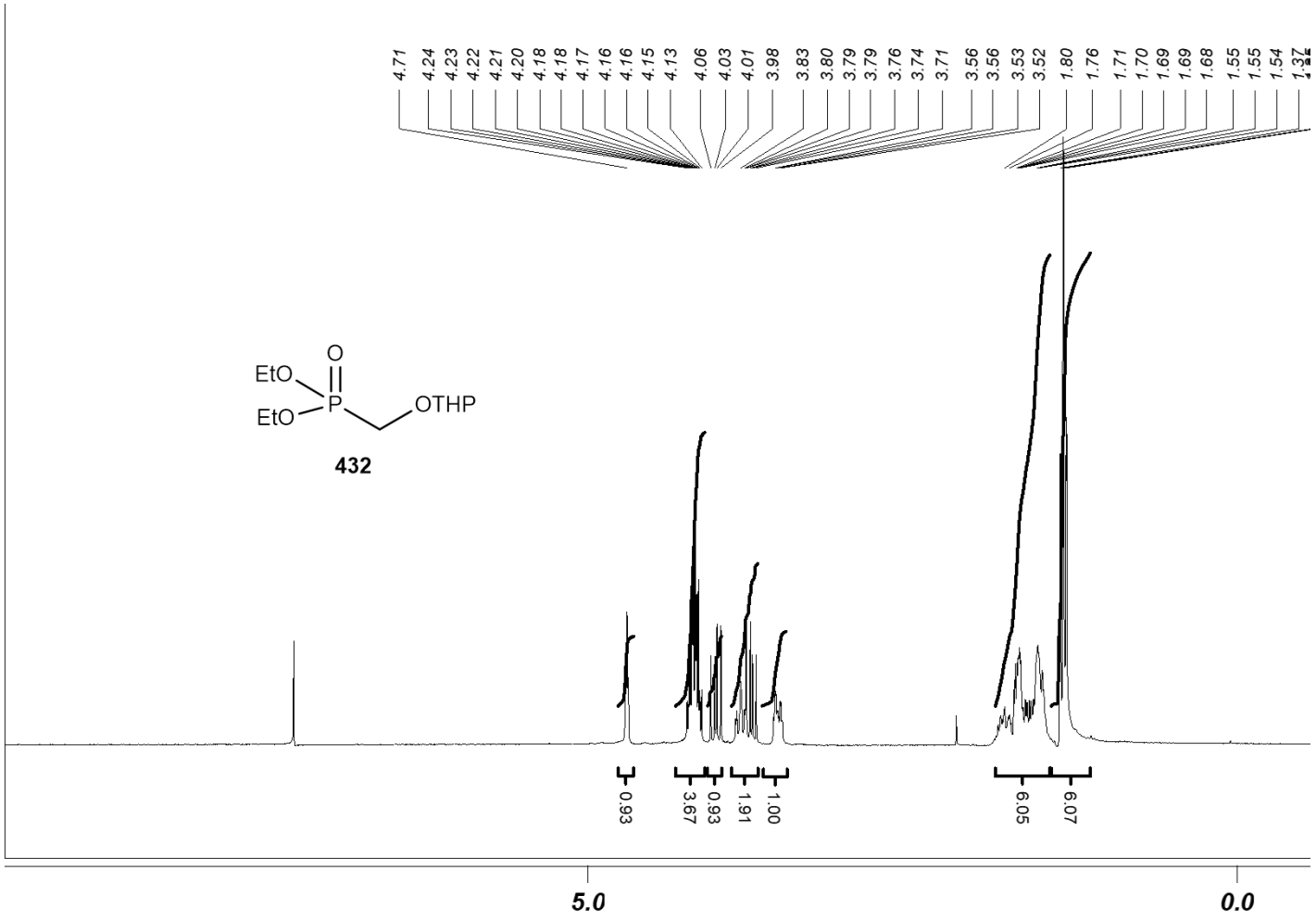

171.23
134.74 134.58 129.58 129.11 128.89 128.56 127.10 126.72 117.46 117.29 101.74 96.83
75.58 73.66 72.93 70.12 68.97 68.00 64.55 64.02 63.37 51.73
23.70
Ph
O
O
O
HO
AcHN
427
200
150
100
50
0


7.54
7.53
7.52
7.51
7.39
7.39
7.38
7.37
6.36
6.34
5.78
5.77
5.77
5.75
5.74
5.73
5.72
5.71
5.71
5.59
5.54
5.12
5.08
5.07
5.06
5.06
5.03
4.51
4.51
4.37
4.36
4.32
4.32
4.12
4.12
4.08
4.08
3.72
2.21
2.19
2.18
2.05
2.02
Ph
O
O
O
O
AcHN
O
427a
2.09
3.58
0.93
1.00
0.99
1.04
3.07
1.00
1.02
1.16
0.95
0.96
1.71
3.39
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

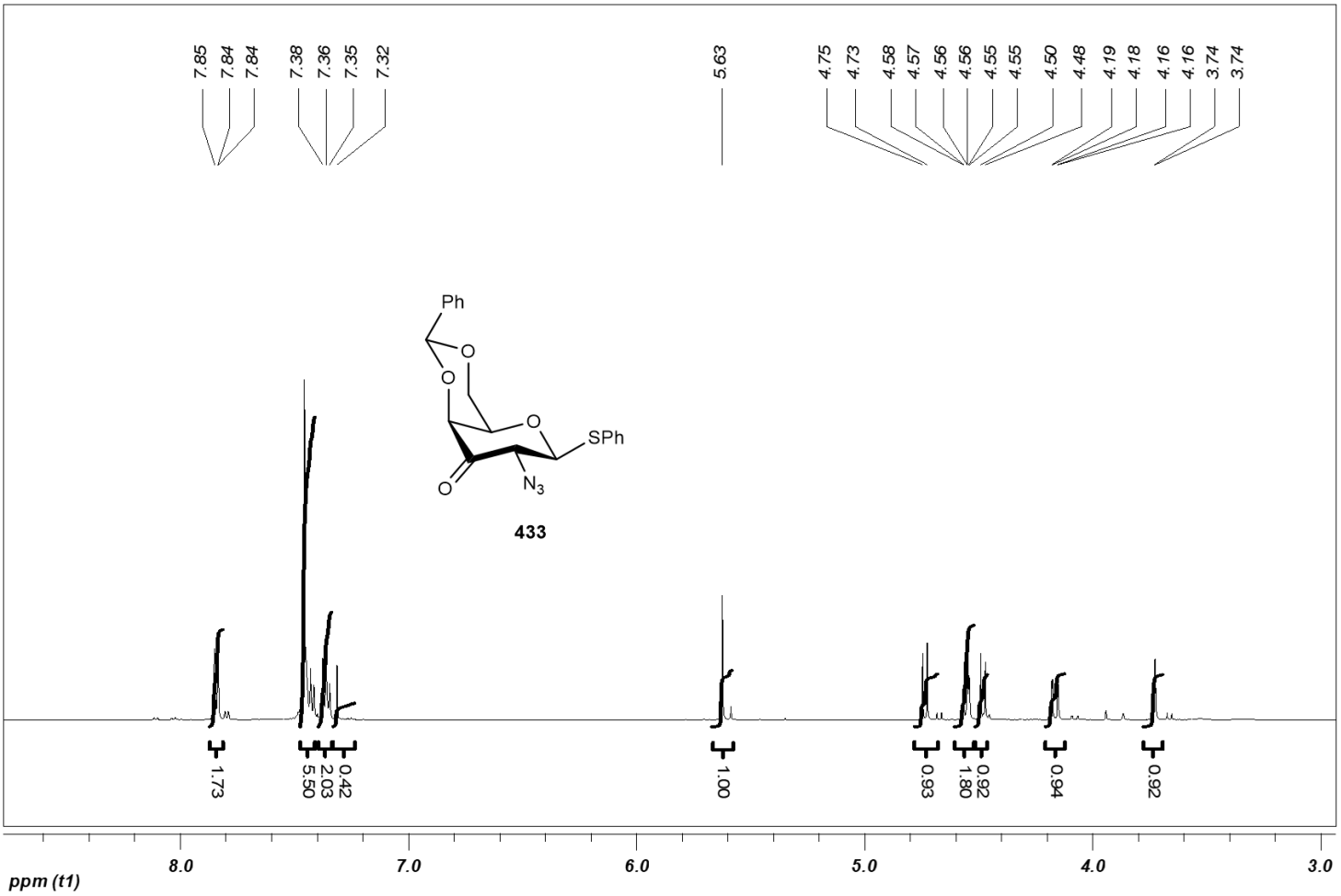

200.19
169.89
133.14
129.58
128.47
126.41
117.70
100.99
81.08
69.35
65.81
58.14
30.22
29.84
23.19
Ph
O
O
O
O
AcHN
O
427a
200
150
100
50
0

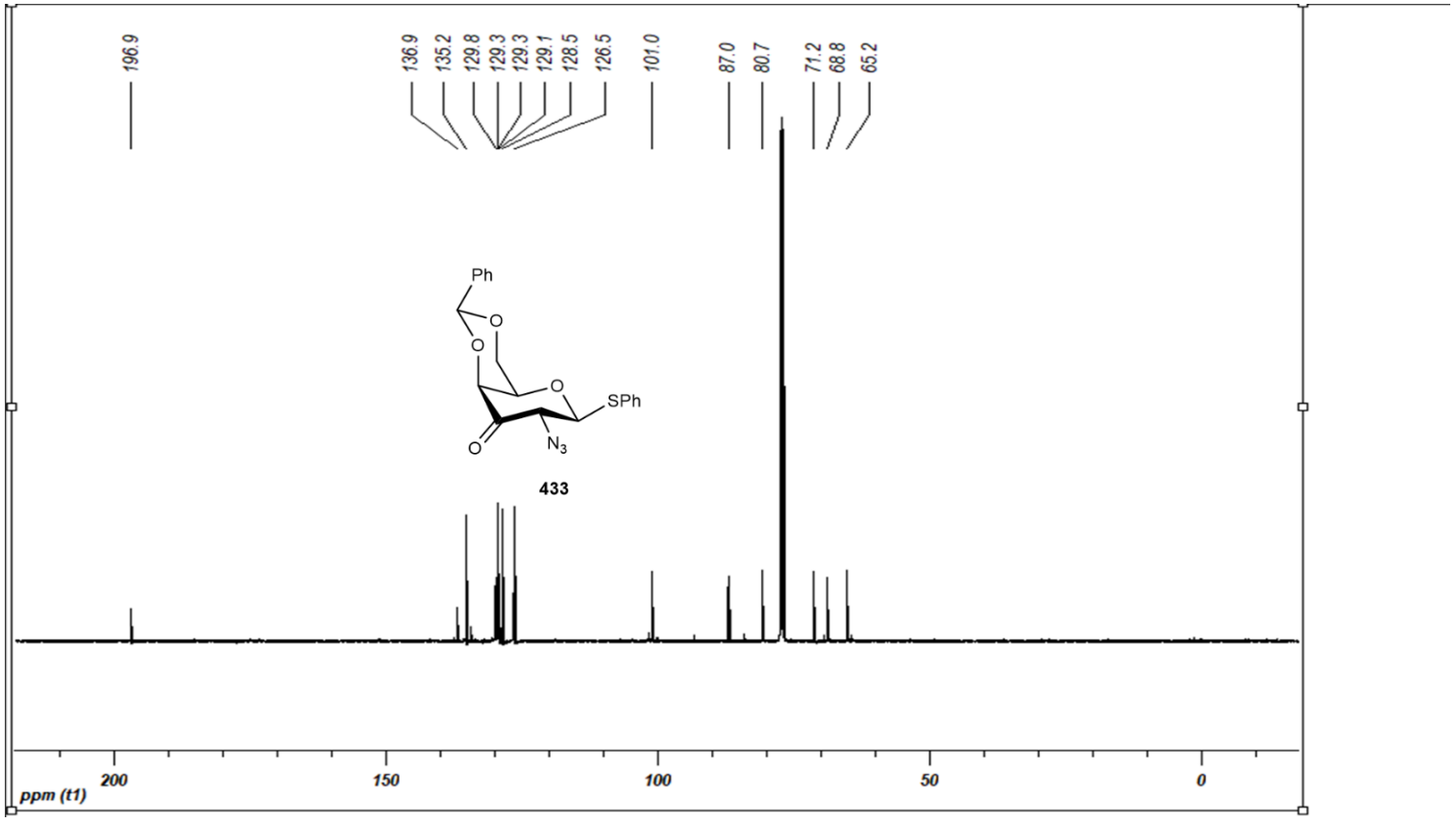

7.64 7.64 7.63 7.63 7.62 7.61 7.37 7.37 7.36 7.35 5.37 5.36 5.36 4.90 4.89 4.86 4.85 4.55 4.51 4.22 4.20 4.18 4.16 4.15 4.14 4.13 4.12 4.11 4.09 4.09 3.92 3.92 3.90 3.88 3.87 3.69 3.66 3.63 2.10 2.05 2.05
OAc
OAc
O
AcO
SPh
N3
429
2.10
2.05
2.05
2.78
6.33
2.100
2.050
2.000
ppm (t1)
1.90
3.63
1.00
0.93
0.99
1.89
0.98
0.97
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

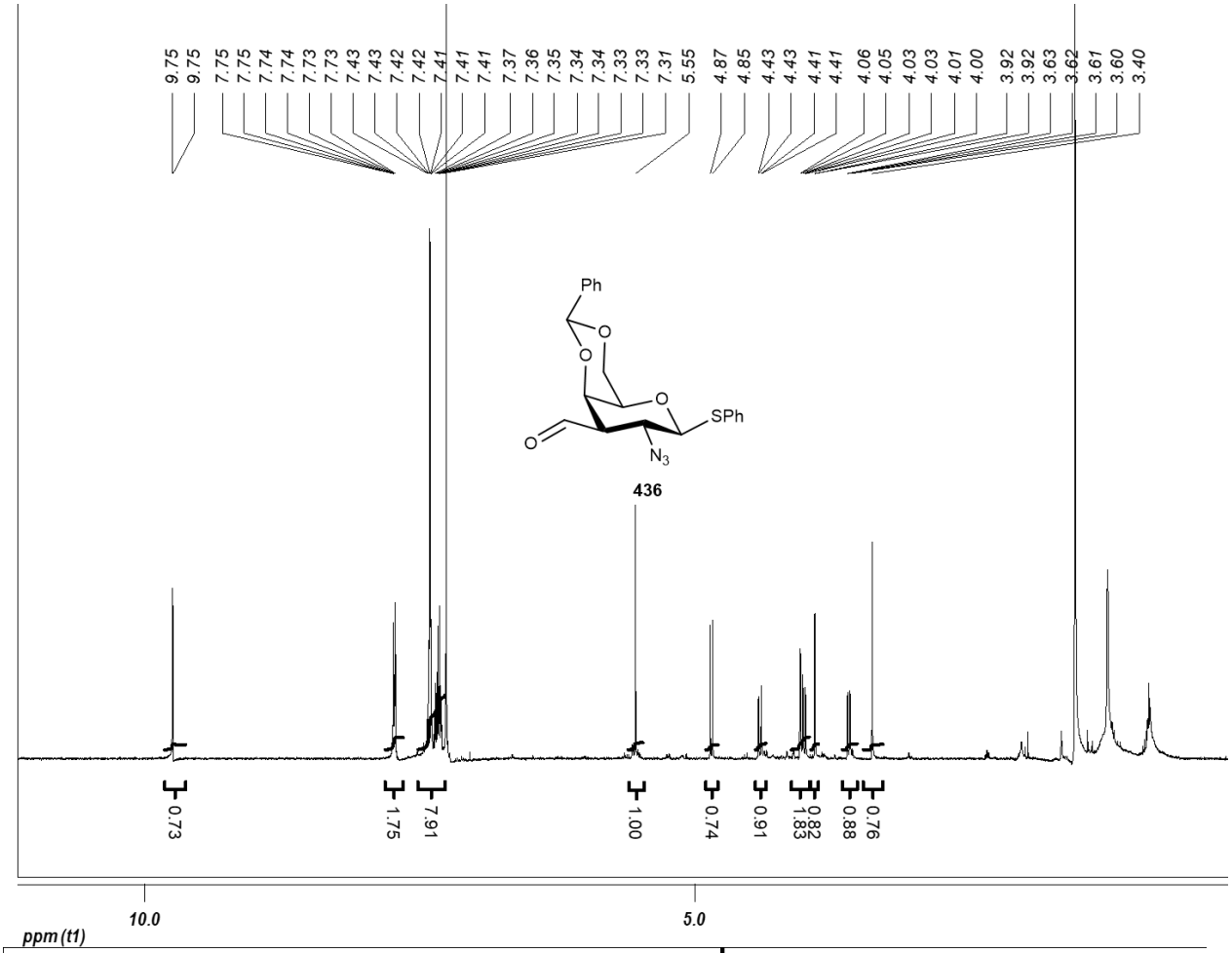

7.76 7.76 7.74 7.42 7.41 7.35 7.34 7.34 7.31 5.55 4.45 4.44 4.43 4.42 4.39 4.39 4.21 4.21 4.20 4.08 4.07 4.04 4.03 3.69 3.67 3.66 3.65 3.65 3.64 3.63 3.58 3.57 3.55 3.54 3.54 3.52
Ph
O
O
O
HO
SPh
N3
430
2.05
8.68
1.00
2.05
1.14
1.13
2.05
1.05
0.93
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)


4.71
4.24
4.23
4.22
4.21
4.20
4.18
4.18
4.17
4.16
4.16
4.15
4.13
4.06
4.03
4.01
3.98
3.83
3.80
3.79
3.79
3.76
3.74
3.71
3.56
3.56
3.53
3.52
1.80
1.76
1.71
1.70
1.69
1.69
1.68
1.55
1.55
1.54
EtO
O
P
EtO
OTHP
0.93
3.67
0.93
1.91
1.00
6.05
6.07
5.0
0.0

432


7.85
7.84
7.84
7.38
7.36
7.35
7.32
5.63
4.75
4.73
4.58
4.57
4.56
4.56
4.55
4.55
4.50
4.48
4.19
4.18
4.16
4.16
3.74
3.74
Ph
O
O
O
SPh
O
N3
433
1.73
5.50
2.03
0.42
1.00
0.93
1.80
0.92
0.94
0.92
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)


196.9
136.9
135.2
129.8
129.3
129.3
129.1
128.5
126.5
101.0
87.0
80.7
71.2
68.8
65.2
Ph
O
O
O
SPh
O
N3
433
200
150
100
50
0
ppm (t1)


9.75 9.75 7.75 7.75 7.74 7.74 7.73 7.73 7.43 7.43 7.42 7.42 7.41 7.41 7.41 7.37 7.36 7.35 7.34 7.34 7.33 7.33 7.31 5.55 4.87 4.85 4.43 4.43 4.41 4.41 4.06 4.05 4.03 4.03 4.01 4.00 3.92 3.92 3.63 3.62 3.61 3.60 3.40
Ph
O
O
O
SPh
O
N3
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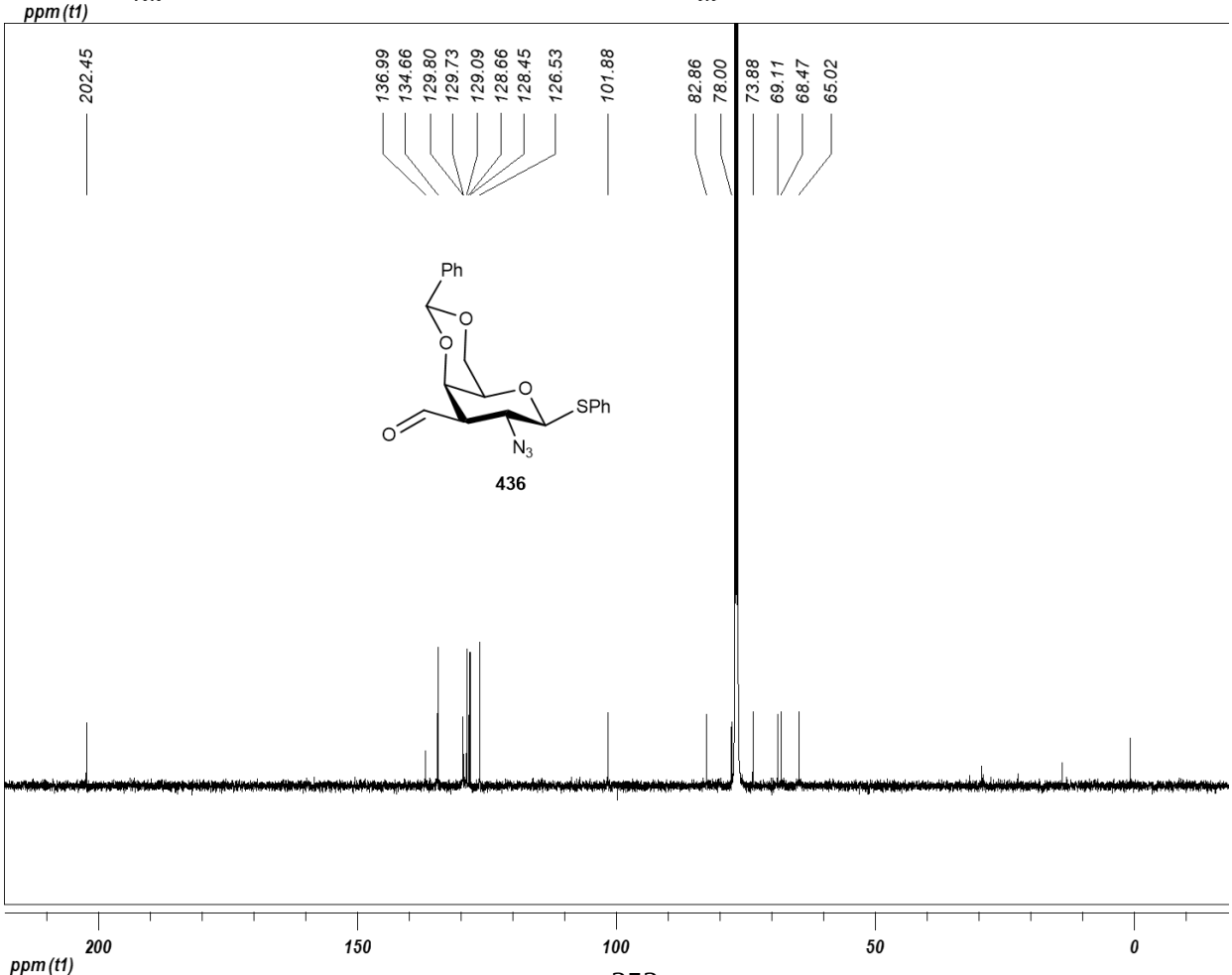
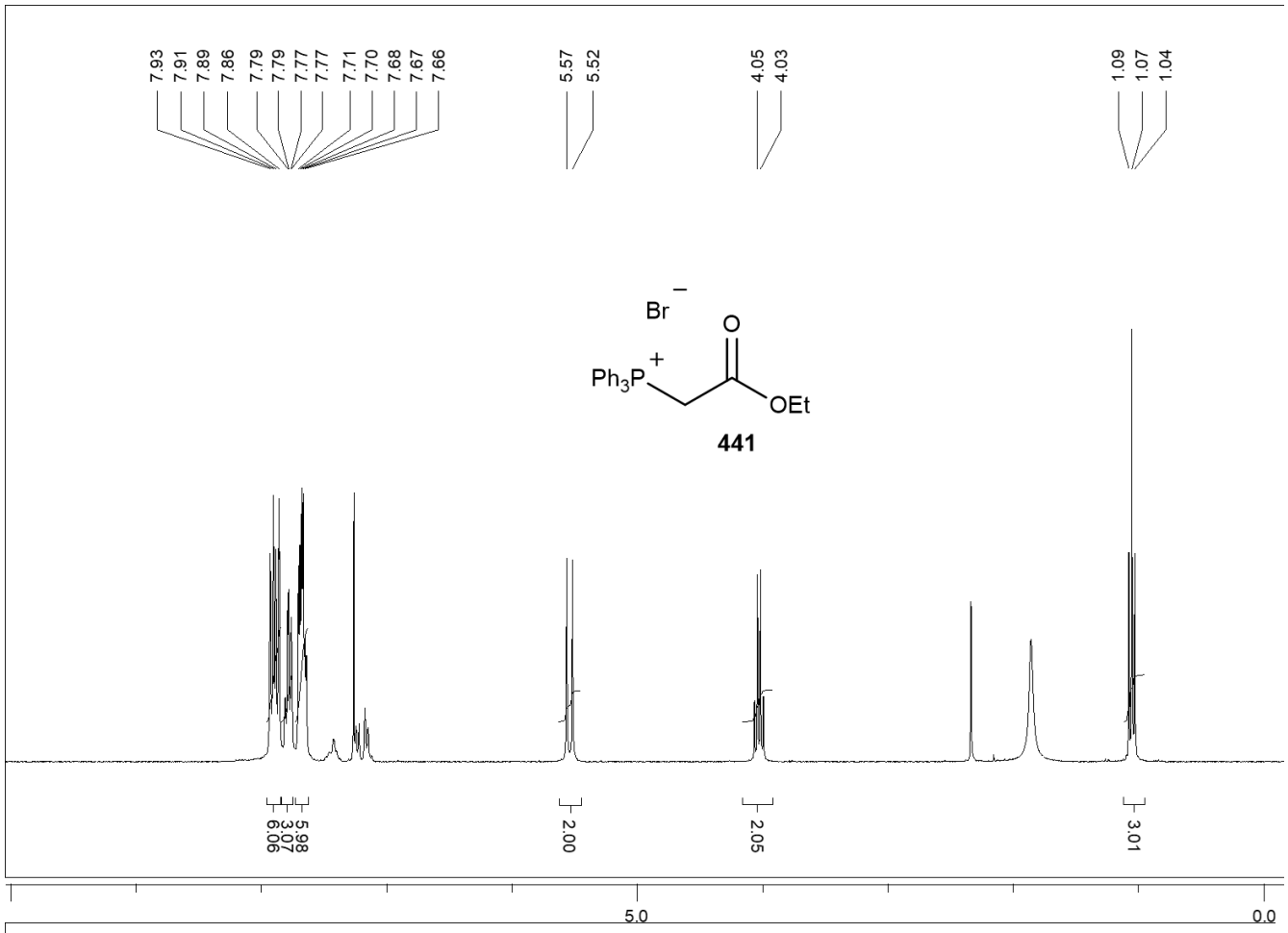
10.0
5.0
ppm (t1)


202.45
136.99 134.66 129.80 129.73 129.09 128.66 128.45 126.53
101.88
82.86 78.00 73.88 69.11 68.47 65.02
Ph
O
O
O
SPh
O
N3
436
200
150
100
50
0
ppm (t1)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7.91
7.89
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7.77
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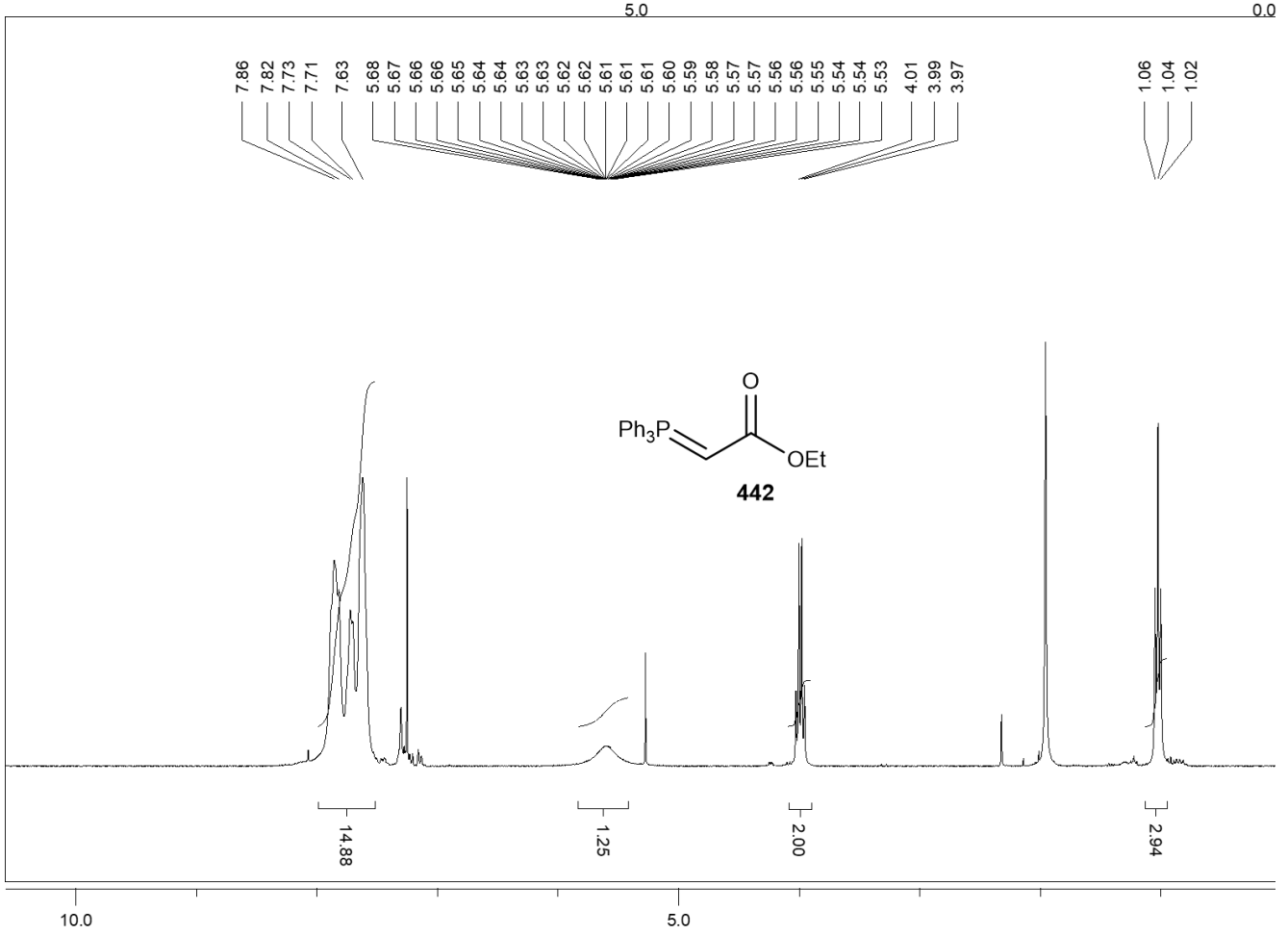
7.70
7.68
7.67
7.66
5.57
5.52
4.05
4.03
1.09
1.07
1.04
Br
O
Ph3P
OEt
441
6.06
3.07
5.98
2.00
2.05
3.01
5.0
0.0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5.67
5.66
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5.65
5.64
5.64
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5.63
5.62
5.62
5.61
5.61
5.61
5.60
5.59
5.58
5.57
5.57
5.56
5.56
5.55
5.54
5.54
5.53
4.01
3.99
3.97
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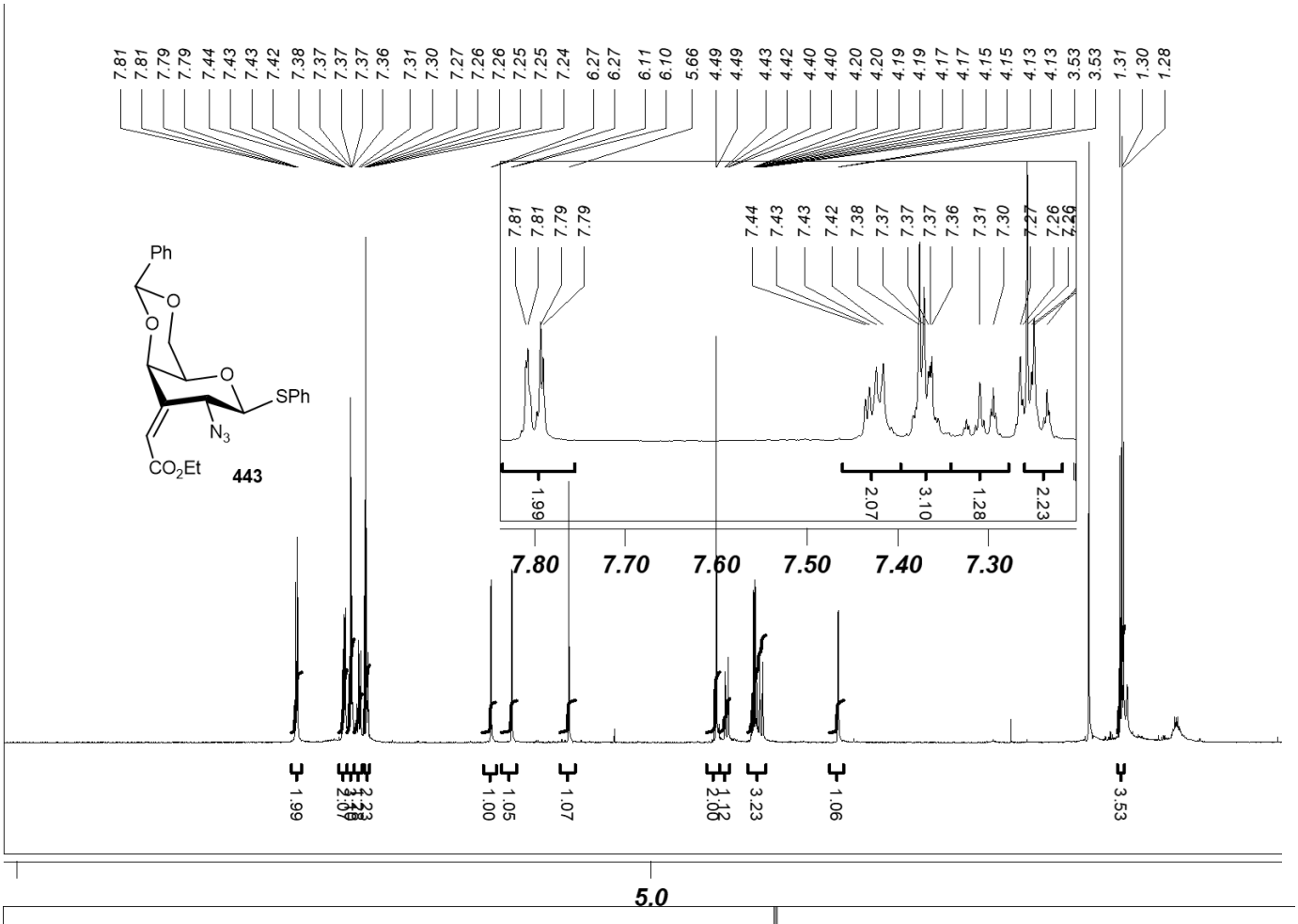
1.04
1.02
O
Ph3P
OEt
442
14.88
1.25
2.00
2.94
10.0
5.0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Ph
O
O
O
SPh
N3
CO2Et
443
1.99
2.07
3.10
1.28
2.23
7.80
7.70
7.60
7.50
7.40
7.30
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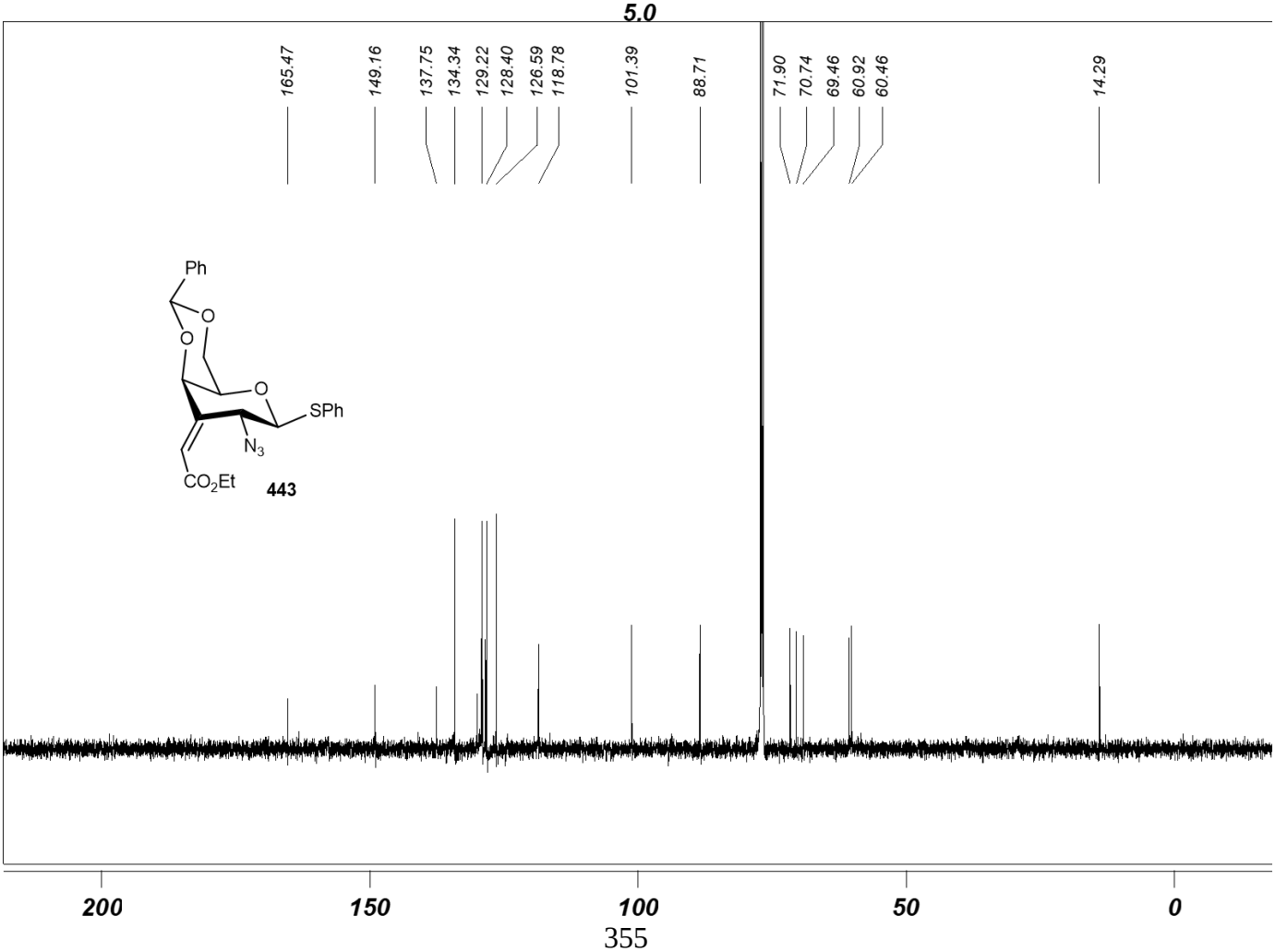
1.00
1.05
1.07
2.00
1.12
3.23
1.06
3.53
5.0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137.75
134.34
129.22
128.40
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118.78
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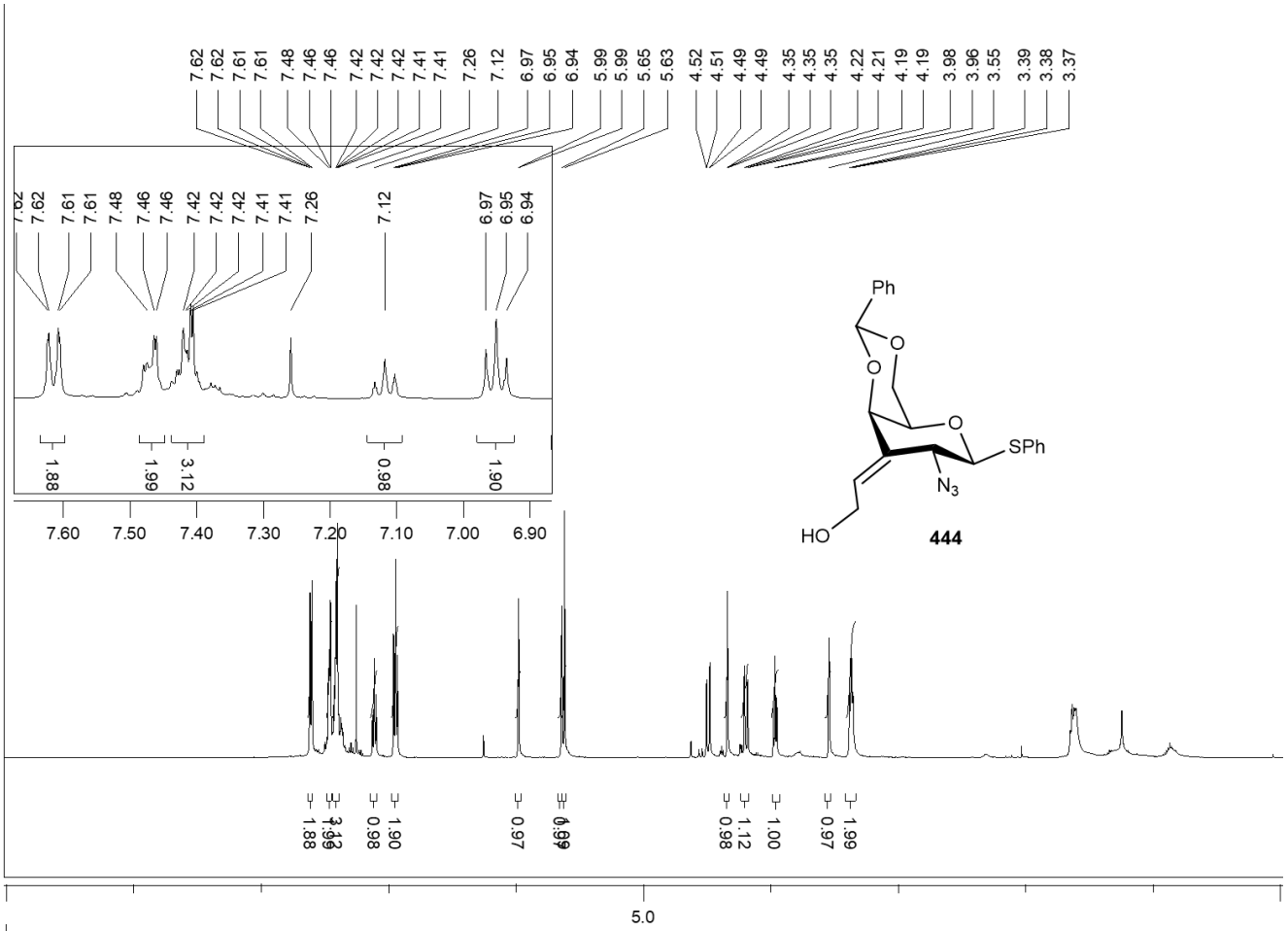
88.71
71.90
70.74
69.46
60.92
60.46
14.29
Ph
O
O
O
SPh
N3
CO2Et
443
200
150
100
50
0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7.46
7.46
7.42
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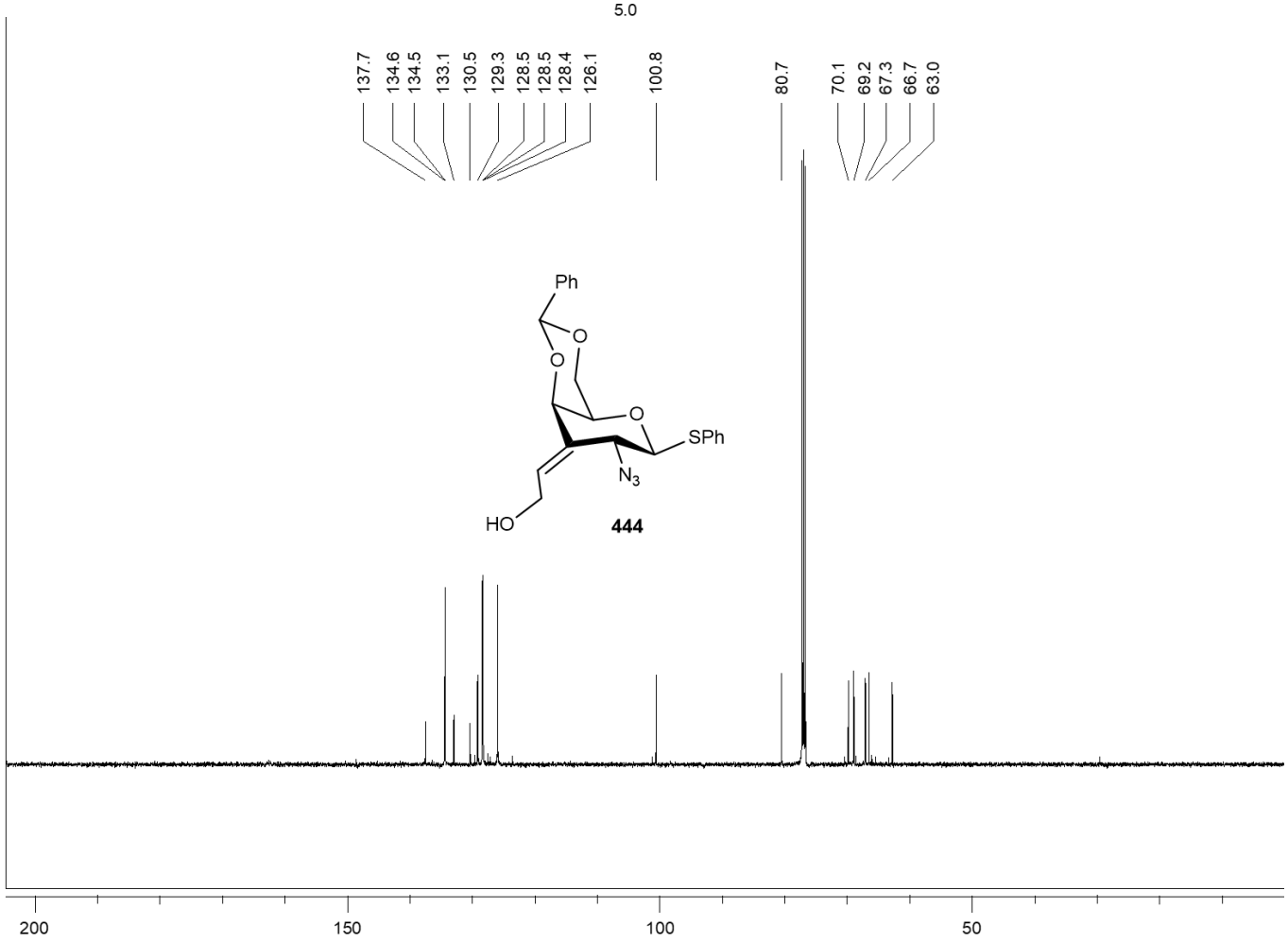
7.42
7.41
7.41
7.26
7.12
6.97
6.95
6.94
5.99
5.99
5.65
5.63
4.52
4.51
4.49
4.49
4.35
4.35
4.35
4.22
4.21
4.19
4.19
3.98
3.96
3.55
3.39
3.38
3.37
1.88
1.99
3.12
0.98
1.90
7.60
7.50
7.40
7.30
7.20
7.10
7.00
6.90
Ph
O
O
O
SPh
N3
HO
444
1.88
1.99
3.12
0.98
1.90
0.97
0.98
1.12
1.00
0.97
1.99
5.0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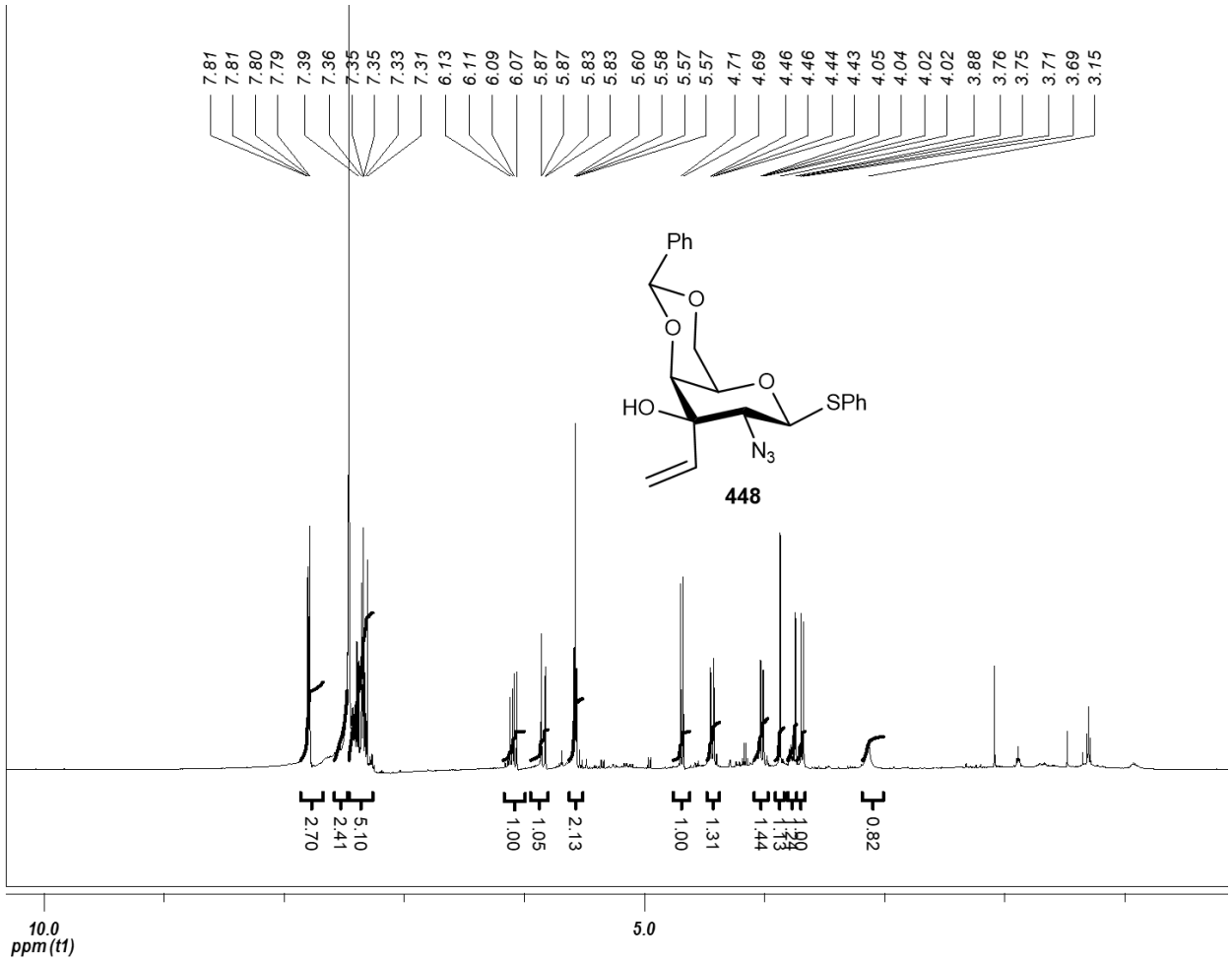
134.6
134.5
133.1
130.5
129.3
128.5
128.5
128.4
126.1
100.8
80.7
70.1
69.2
67.3
66.7
63.0
Ph
O
O
O
SPh
N3
HO
444
200
150
100
50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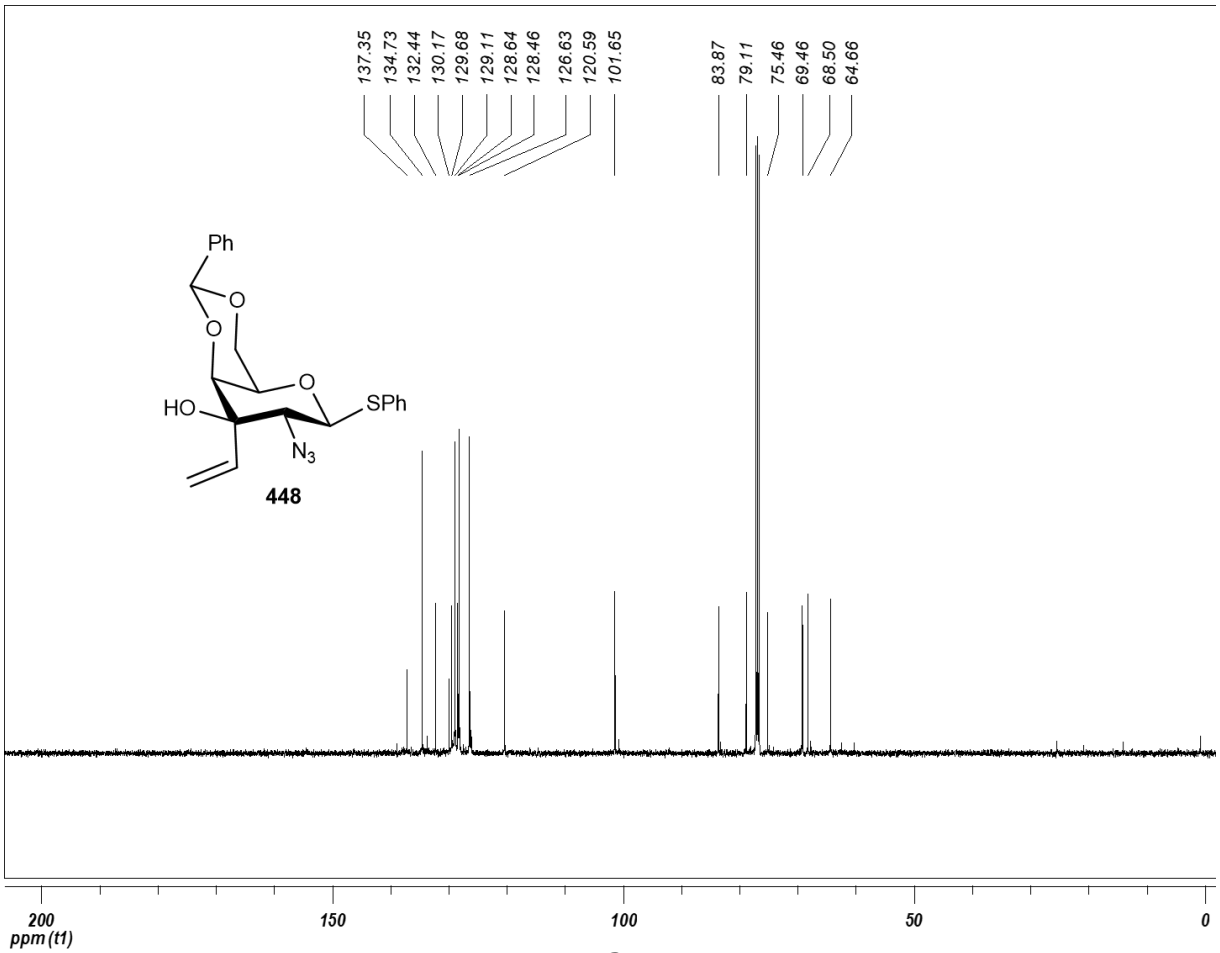
7.81
7.80
7.79
7.39
7.36
7.35
7.35
7.33
7.31
6.13
6.11
6.09
6.07
5.87
5.87
5.83
5.83
5.60
5.58
5.57
5.57
4.71
4.69
4.46
4.46
4.44
4.43
4.05
4.04
4.02
4.02
3.88
3.76
3.75
3.71
3.69
3.15
Ph
O
O
O
HO
SPh
N3
448
2.70
2.41
5.10
1.00
1.05
2.13
1.00
1.31
1.44
0.82
10.0
ppm (t1)
5.0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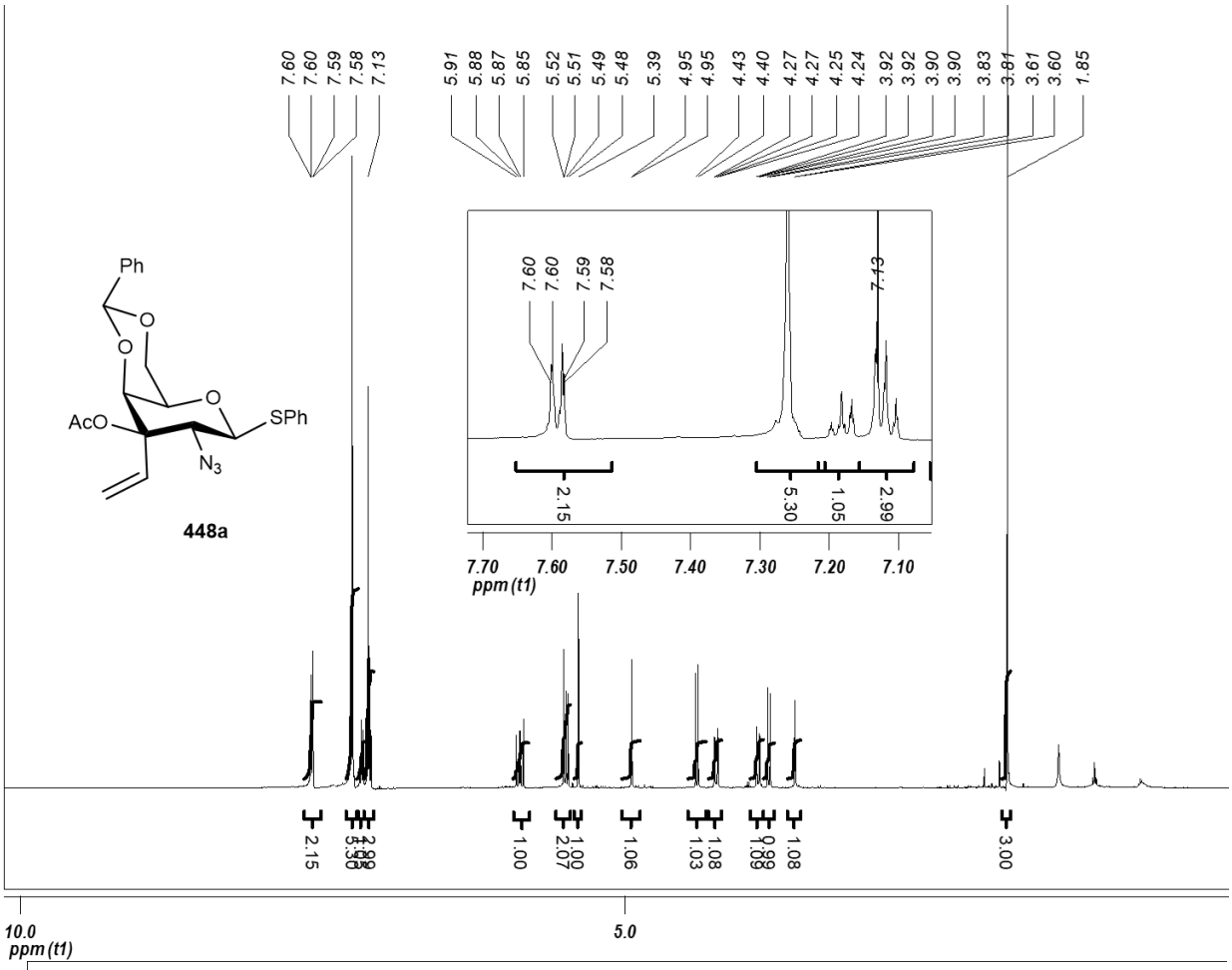
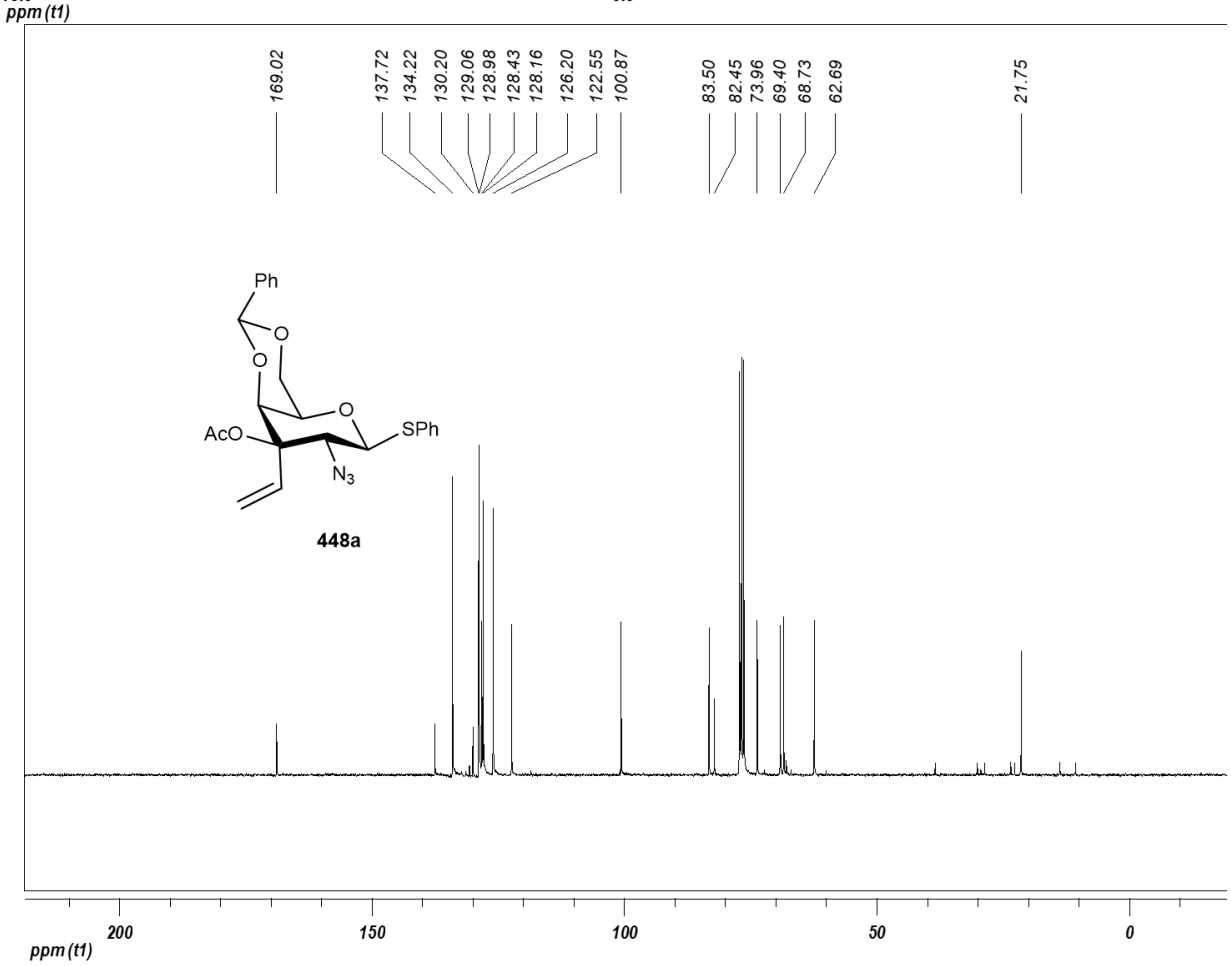
134.73
132.44
130.17
129.68
129.11
128.64
128.46
126.63
120.59
101.65
83.87
79.11
75.46
69.46
68.50
64.66
Ph
O
O
O
HO
SPh
N3
448
200
ppm (t1)
150
100
50
0


7.60
7.60
7.59
7.58
7.13
5.91
5.88
5.87
5.85
5.52
5.51
5.49
5.48
5.39
4.95
4.95
4.43
4.40
4.27
4.27
4.25
4.24
3.92
3.92
3.90
3.90
3.83
3.81
3.61
3.60
1.85
Ph
O
O
O
AcO
SPh
N3
448a
2.15
5.30
1.05
2.99
7.70
7.60
7.50
7.40
7.30
7.20
7.10
ppm (t1)
2.15
1.00
2.07
1.06
1.03
1.08
1.08
3.00
10.0
5.0
ppm (t1)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130.20
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128.98
128.43
128.16
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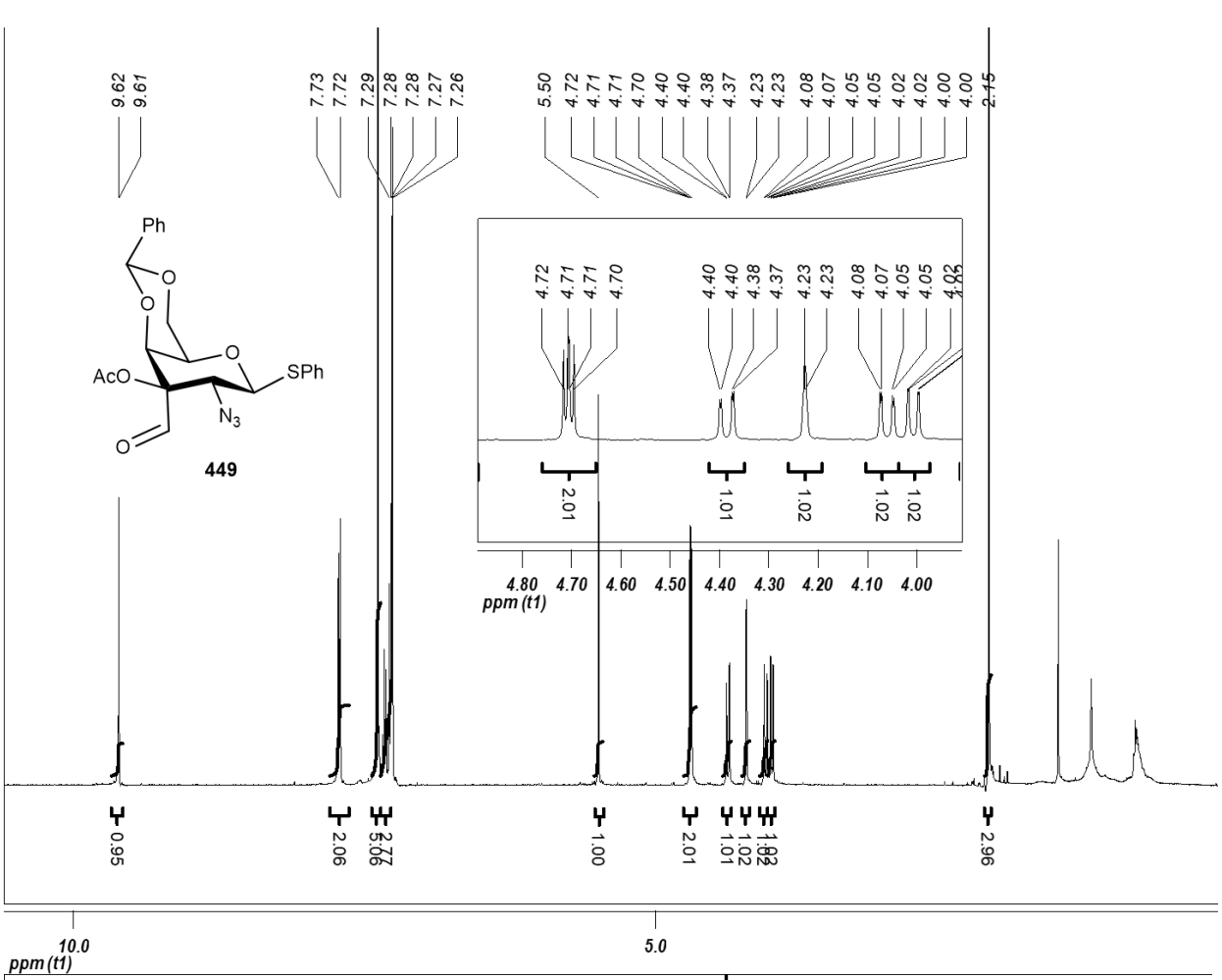
122.55
100.87
83.50
82.45
73.96
69.40
68.73
62.69
21.75
Ph
O
O
O
AcO
SPh
N3
448a
200
150
100
50
0
ppm (t1)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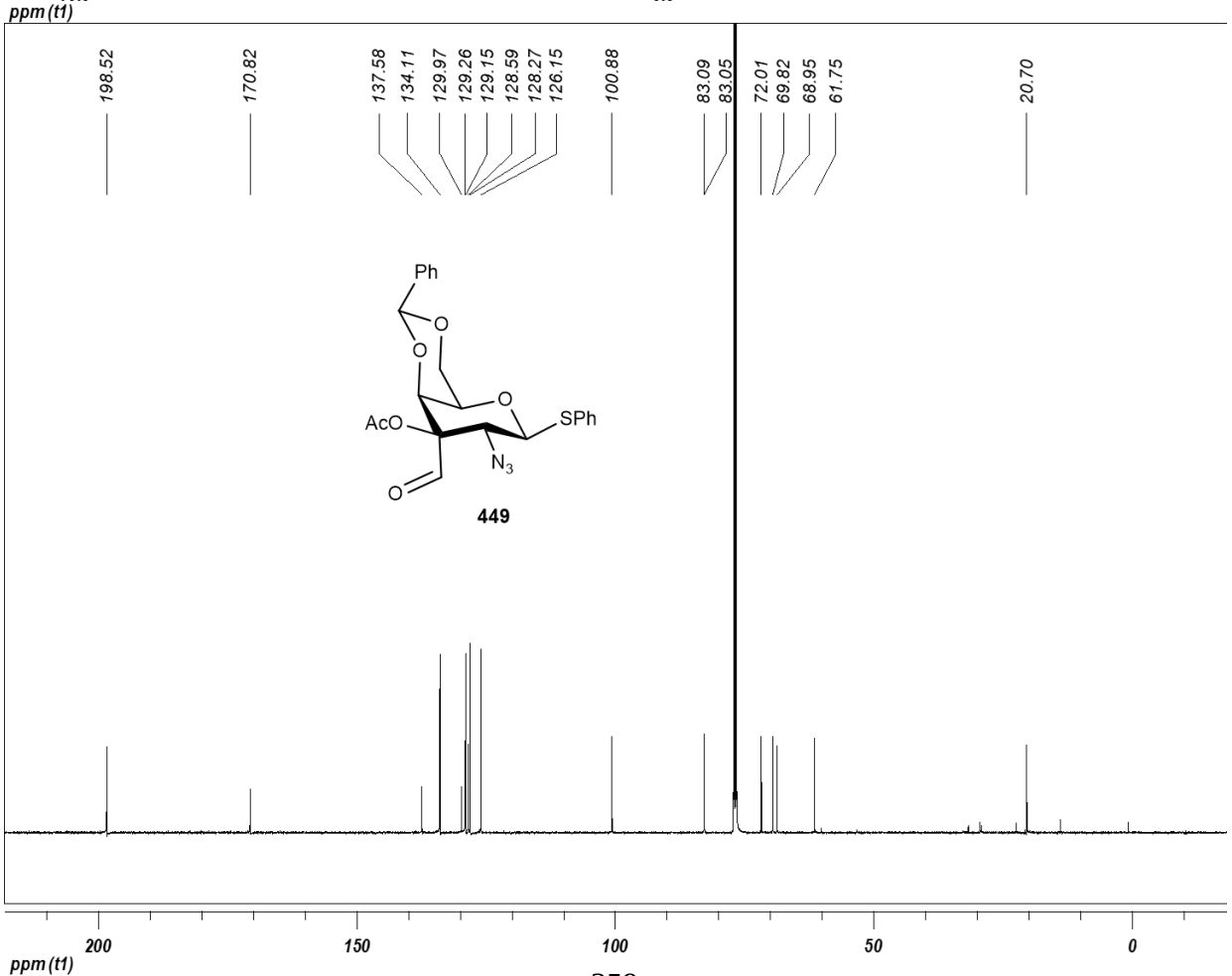
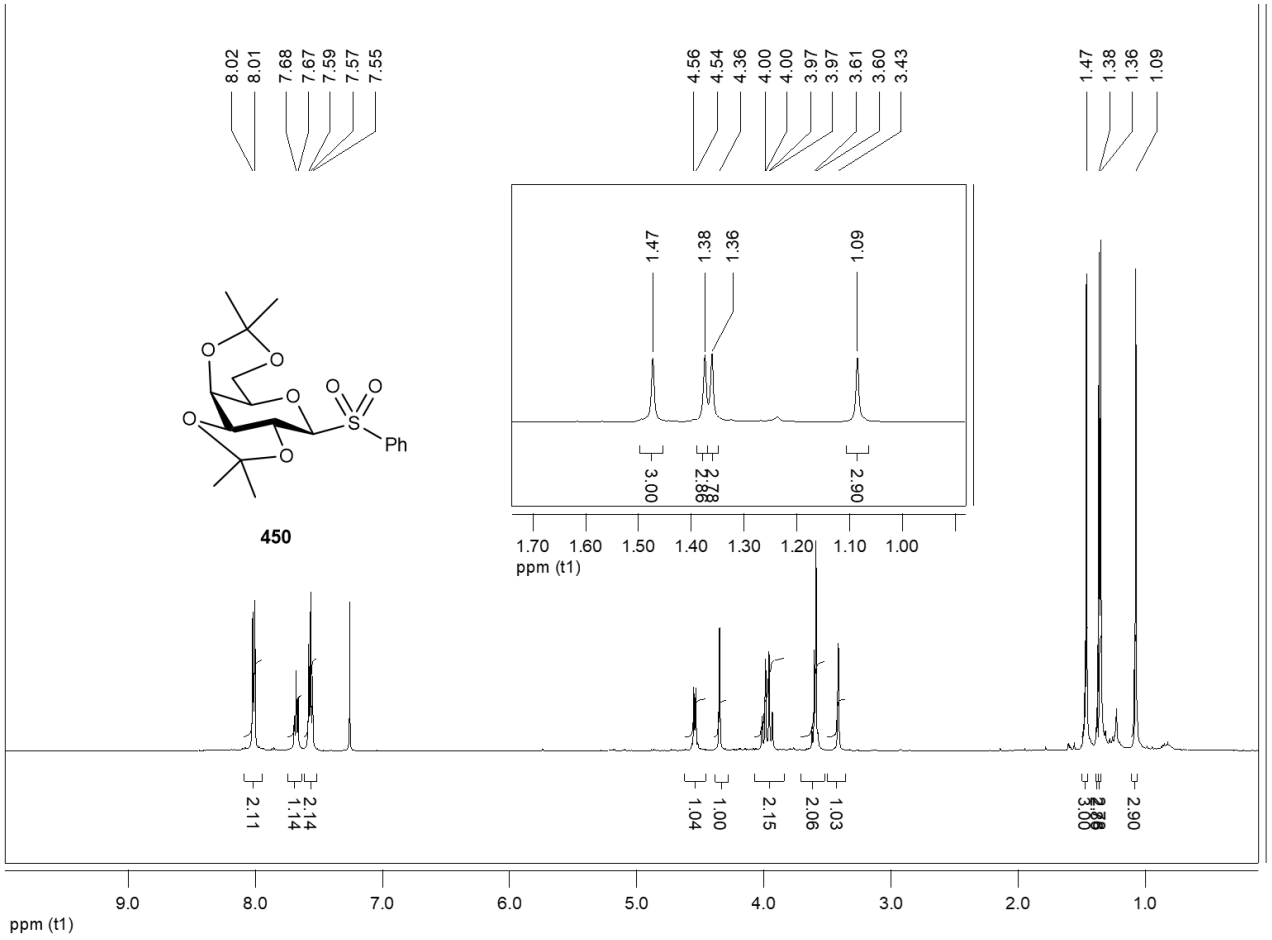
9.61
7.73
7.72
7.29
7.28
7.28
7.27
7.26
5.50
4.72
4.71
4.71
4.70
4.40
4.40
4.38
4.37
4.23
4.23
4.08
4.07
4.05
4.05
4.02
4.02
4.00
4.00
2.15
Ph
O
O
O
AcO
SPh
N3
O
449
4.80
4.70
4.60
4.50
4.40
4.30
4.20
4.10
4.00
ppm (t1)
0.95
2.06
5.06
2.77
1.00
2.01
1.01
1.02
1.02
1.02
2.96
10.0
5.0
ppm (t1)


198.52
170.82
137.58
134.11
129.97
129.26
129.15
128.59
128.27
126.15
100.88
83.09
83.05
72.01
69.82
68.95
61.75
20.70
Ph
O
O
O
AcO
SPh
N3
O
449
200
150
100
50
0
ppm (t1)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8.01
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7.67
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7.57
7.55
4.56
4.54
4.36
4.00
4.00
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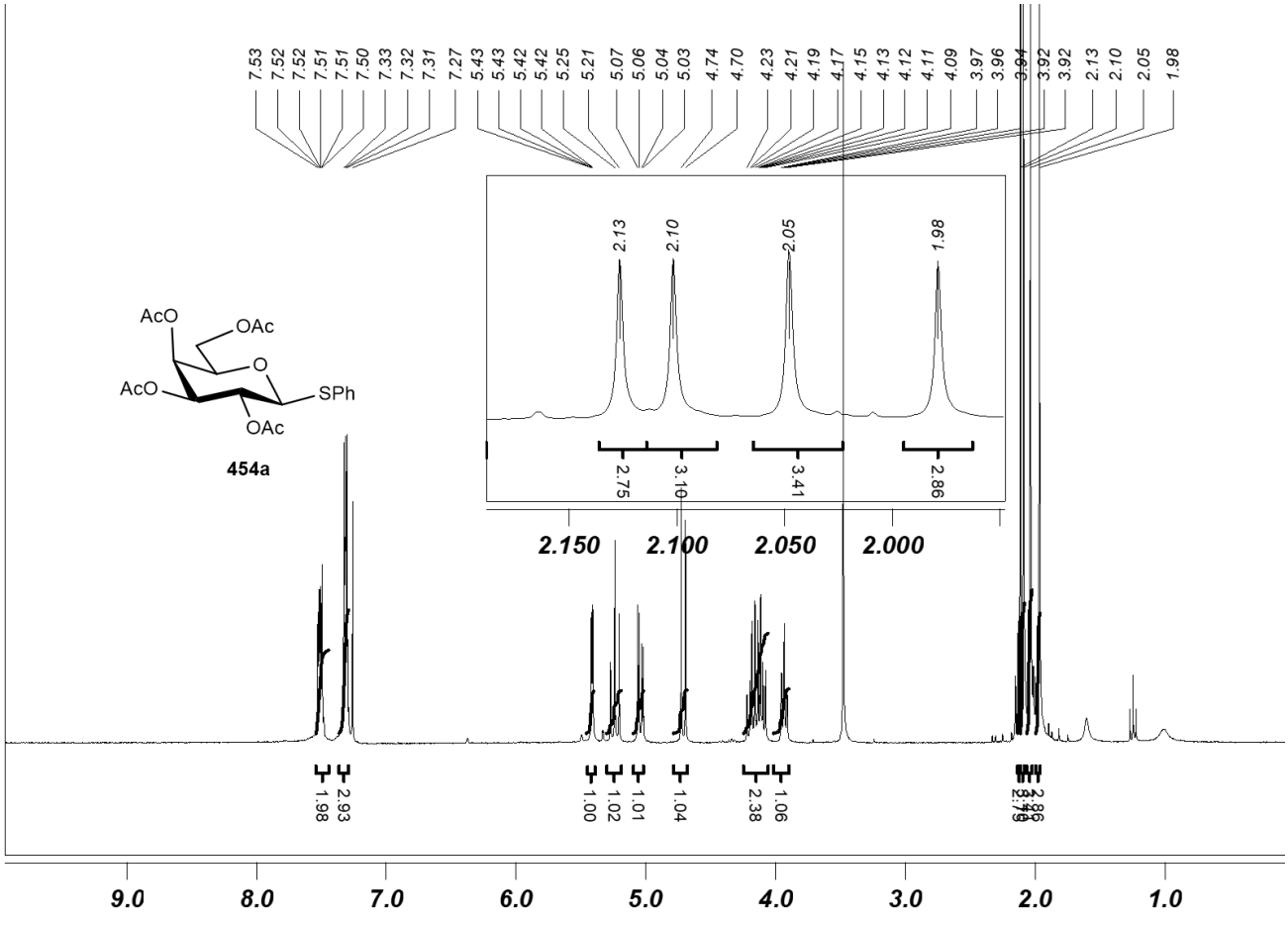
3.97
3.61
3.60
3.43
1.47
1.38
1.36
1.09
1.47
1.38
1.36
1.00
3.00
2.88
2.90
1.70 1.60 1.50 1.40 1.30 1.20 1.10 1.00
ppm (t1)
O
O
O
O
O
O
S
Ph
450
2.11
1.14
2.14
1.04
1.00
2.15
2.06
1.03
3.00
2.90
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
ppm (t1)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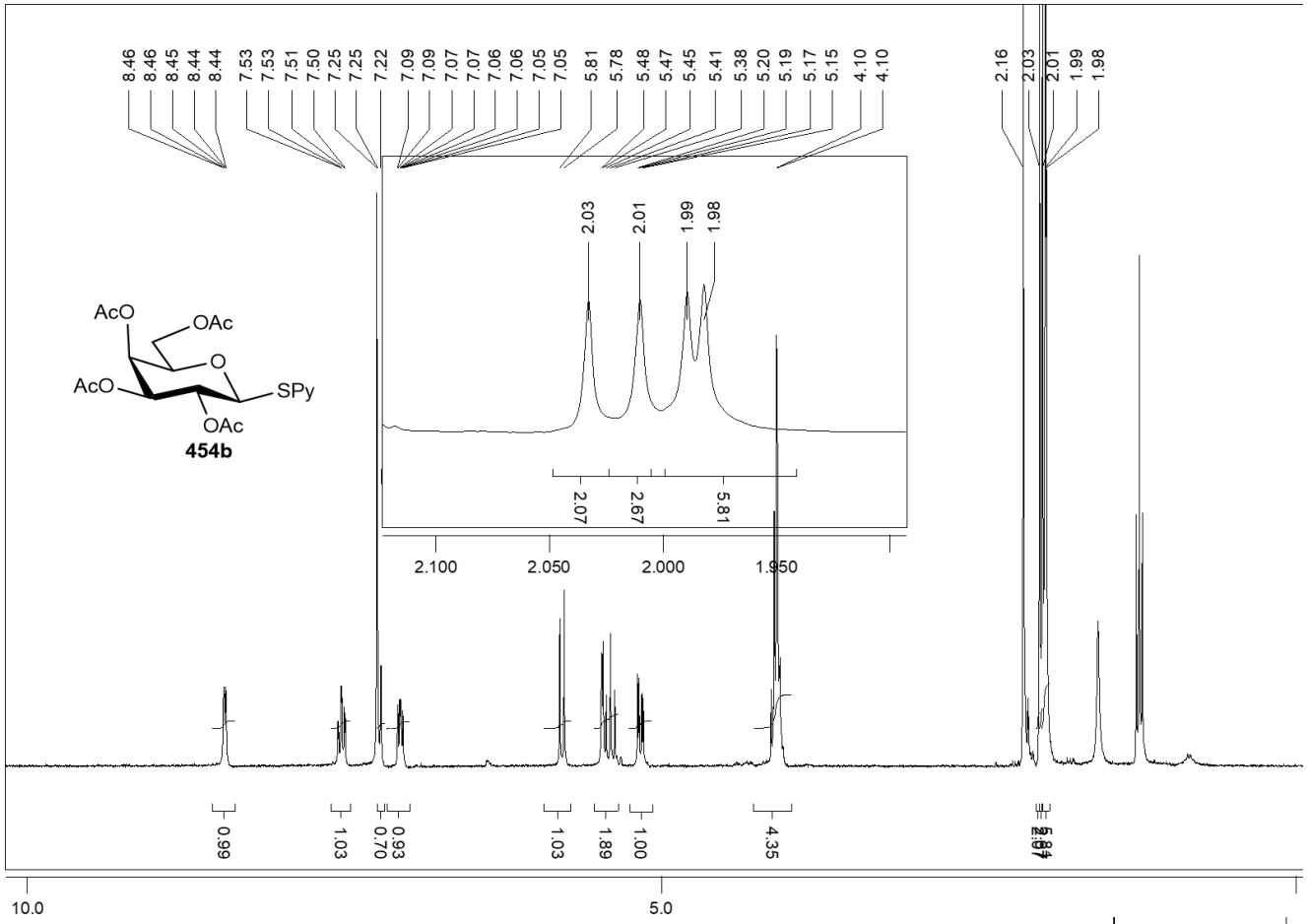
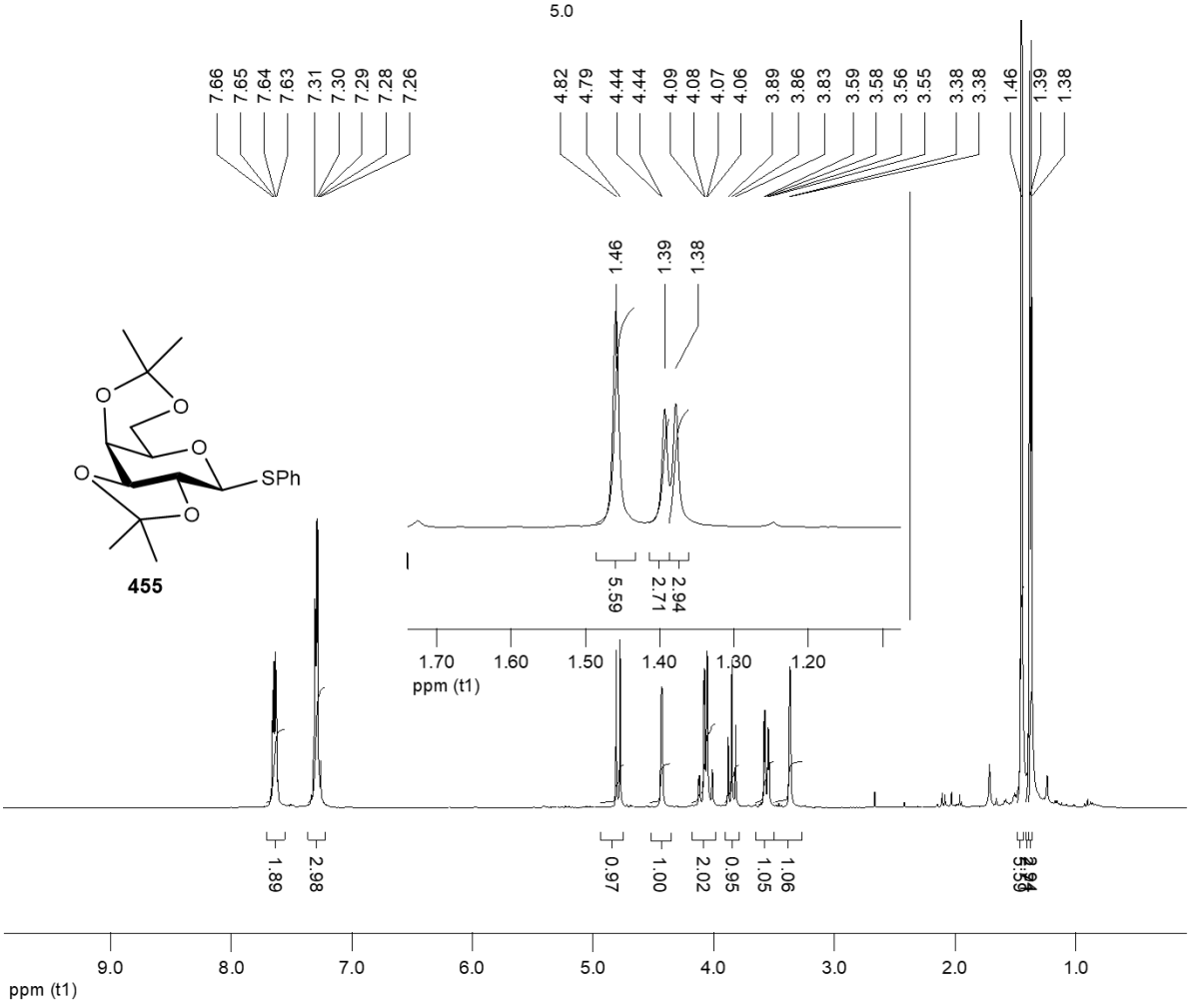
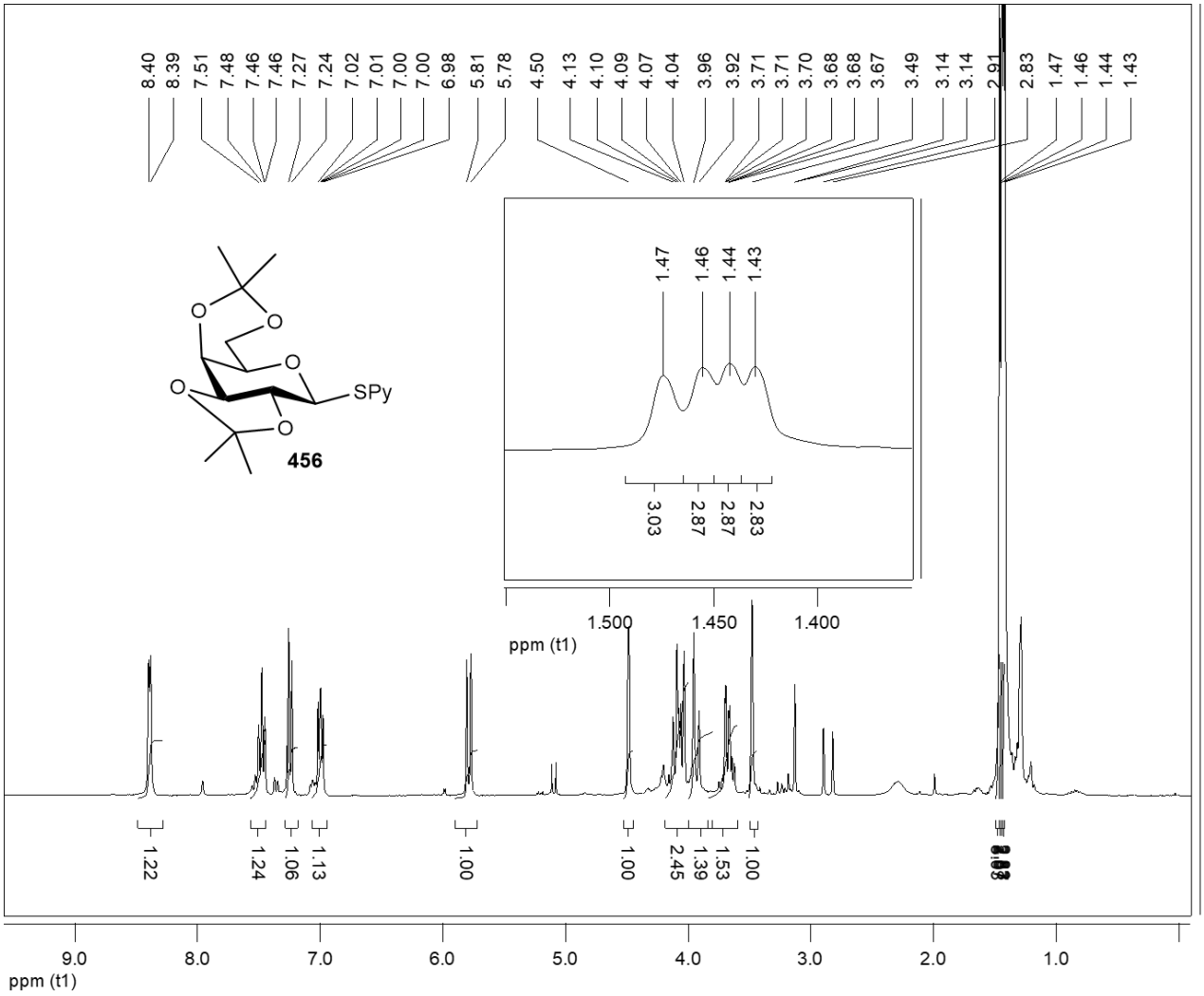
7.31
7.27
5.43
5.43
5.42
5.42
5.25
5.21
5.07
5.06
5.04
5.03
4.74
4.70
4.23
4.21
4.19
4.17
4.15
4.13
4.12
4.11
4.09
3.97
3.96
3.94
3.92
3.92
2.13
2.10
2.05
1.98
2.13
2.10
2.05
1.98
2.75
3.10
3.41
2.86
2.150
2.100
2.050
2.000
AcO
OAc
O
AcO
SPh
OAc
454a
1.98
2.93
1.00
1.02
1.01
1.04
2.38
1.06
2.86
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0


AcO
OAc
O
AcO
SPy
OAc
454b
8.46 8.46 8.45 8.44 8.44 7.53 7.53 7.51 7.50 7.25 7.25 7.22 7.09 7.09 7.07 7.07 7.06 7.06 7.05 7.05 5.81 5.78 5.48 5.47 5.45 5.41 5.38 5.20 5.19 5.17 5.15 4.10 4.10 2.16 2.03 2.01 1.99 1.98
2.03 2.01 1.99 1.98
2.07 2.67 5.81
2.100 2.050 2.000 1.950
0.99 1.03 0.70 0.63 1.03 1.89 1.00 4.35
10.0 5.0


7.66 7.65 7.64 7.63 7.31 7.30 7.29 7.28 7.26
4.82 4.79 4.44 4.44 4.09 4.08 4.07 4.06 3.89 3.86 3.83 3.59 3.58 3.56 3.55 3.38 3.38 1.46 1.39 1.38
1.46 1.39 1.38
5.59 2.71 2.94
1.70 1.60 1.50 1.40 1.30 1.20
ppm (t1)
O
O
O
O
O
SPh
455
1.89 2.98 0.97 1.00 2.02 0.95 1.05 1.06
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
ppm (t1)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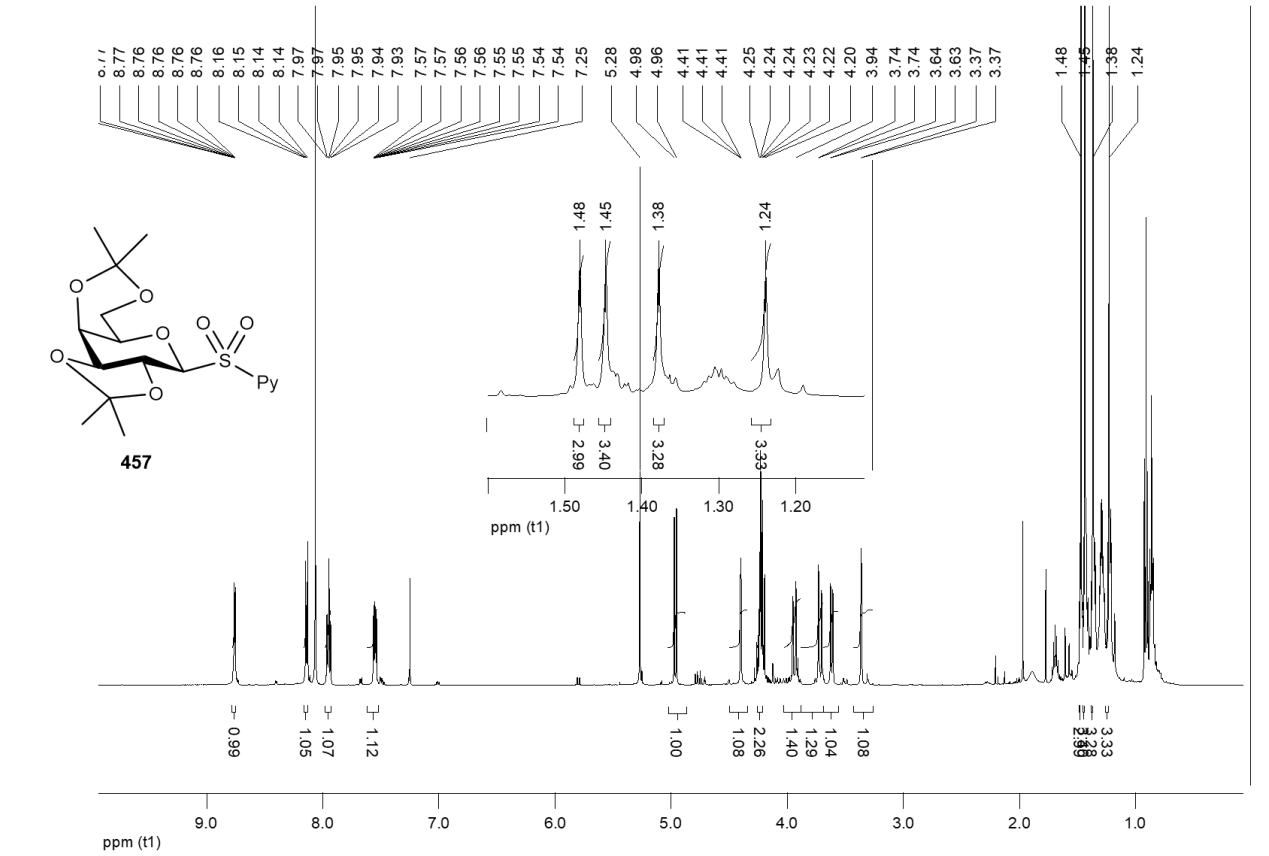
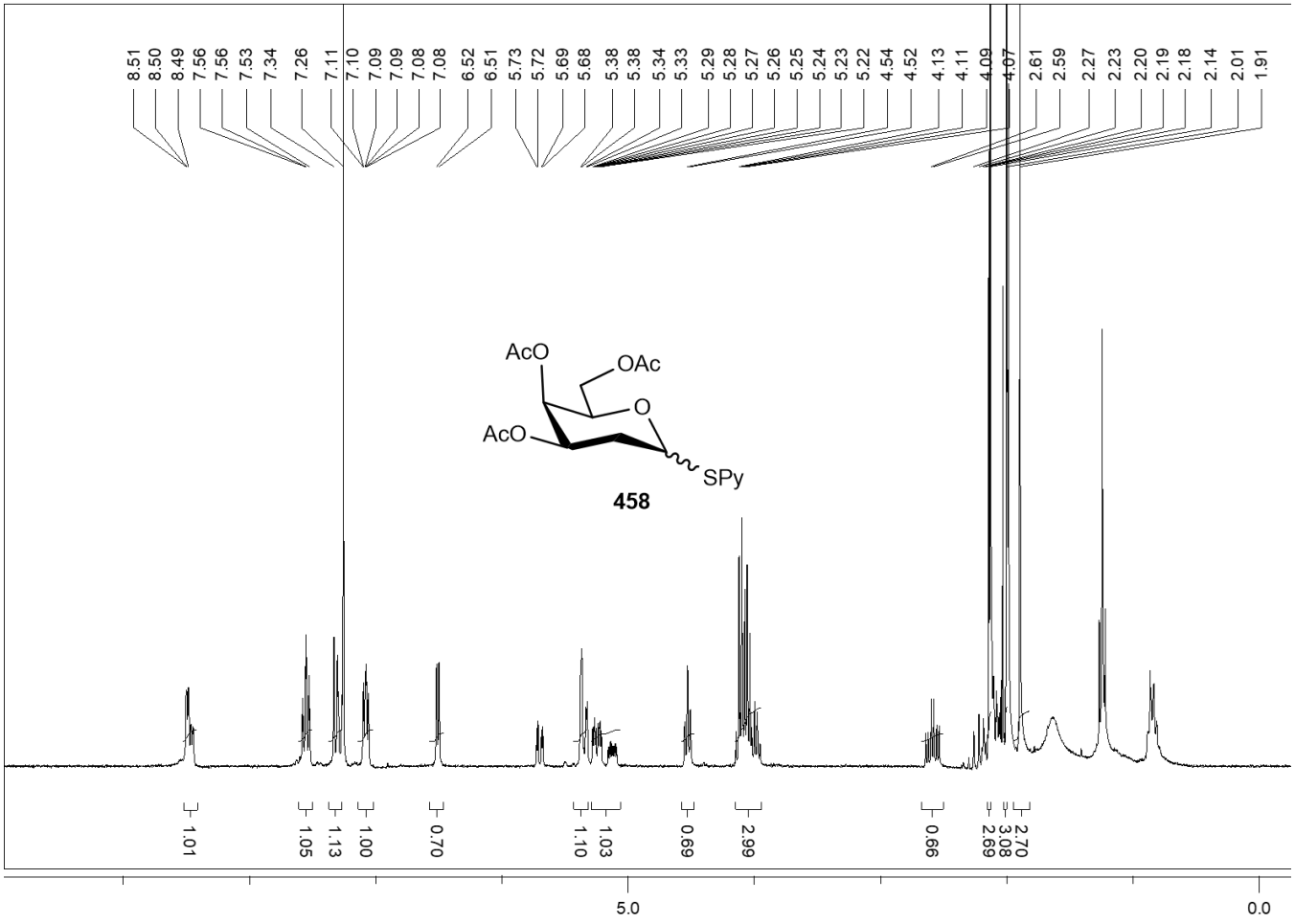
SPy
456
1.47 1.46 1.44 1.43
3.03 2.87 2.87 2.83
1.500 1.450 1.400
ppm (t1)
1.22 1.24 1.06 1.13 1.00 1.00 2.45 1.39 1.53 1.00
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
ppm (t1)


8.77 8.76 8.76 8.76 8.76 8.16 8.15 8.14 8.14 7.97 7.97 7.95 7.95 7.94 7.93 7.57 7.57 7.56 7.56 7.55 7.55 7.54 7.54 7.25 5.28 4.98 4.96 4.41 4.41 4.41 4.25 4.24 4.24 4.23 4.22 4.20 3.94 3.74 3.74 3.64 3.63 3.37 3.37 1.48 1.45 1.38 1.24
Py
457
1.48 1.45 1.38 1.24
2.99 3.40 3.28 3.33
1.50 1.40 1.30 1.20
ppm (t1)
0.99 1.05 1.07 1.12 1.00 1.08 2.26 1.40 1.04 1.29 1.08 3.33
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
ppm (t1)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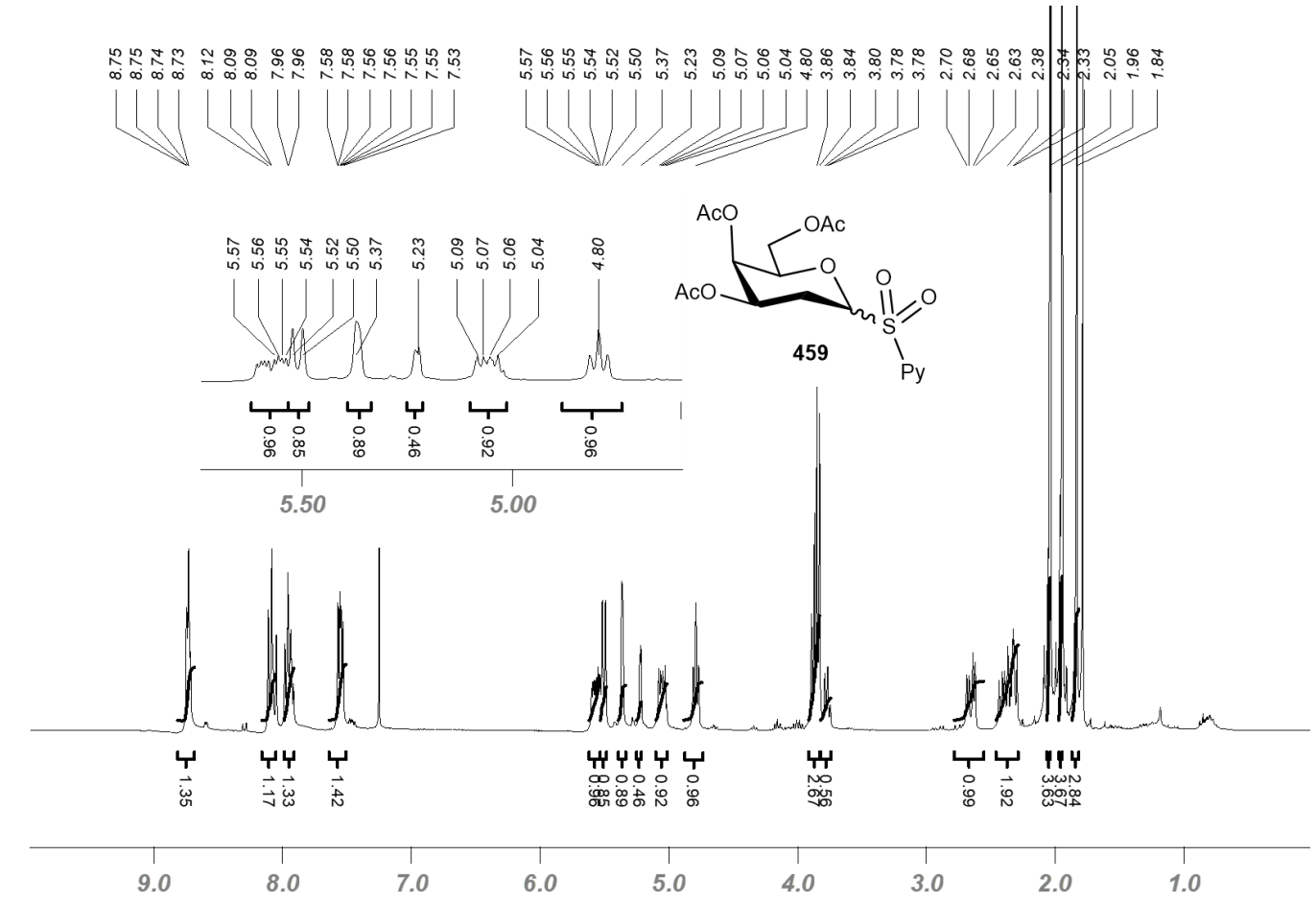
7.56
7.56
7.53
7.34
7.26
7.11
7.10
7.09
7.09
7.08
7.08
6.52
6.51
5.73
5.72
5.69
5.68
5.38
5.38
5.34
5.33
5.29
5.28
5.27
5.26
5.25
5.24
5.23
5.22
4.54
4.52
4.13
4.11
4.09
4.07
2.61
2.59
2.27
2.23
2.20
2.19
2.18
2.14
2.01
1.91
AcO
OAc
O
AcO
SPy
458
1.01
1.05
1.13
1.00
0.70
1.10
1.03
0.69
2.99
0.66
2.69
3.08
2.70
5.0
0.0


8.75 8.75 8.74 8.73 8.12 8.09 8.09 7.96 7.96 7.58 7.58 7.56 7.56 7.55 7.55 7.53
5.57 5.56 5.55 5.54 5.52 5.50 5.37 5.23 5.09 5.07 5.06 5.04 4.80 3.86 3.84 3.80 3.78 3.78
2.70 2.68 2.65 2.63 2.38 2.34 2.33 2.05 1.96 1.84
AcO OAc AcO O O O S Py
459
0.96 0.85 0.89 0.49 0.92 0.96
5.50 5.00
1.35 1.17 1.33 1.42 0.85 0.89 0.46 0.92 0.96 2.87 0.69 1.92 3.07 3.63 2.84
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
169.9 169.8 169.6 169.4 155.1 154.4 150.5 150.2 137.8 127.6 124.7 123.9
85.7 85.4 75.3 71.1 68.2 65.6 65.2 65.2 62.0 61.2
23.0 21.3 20.6 20.4 20.3
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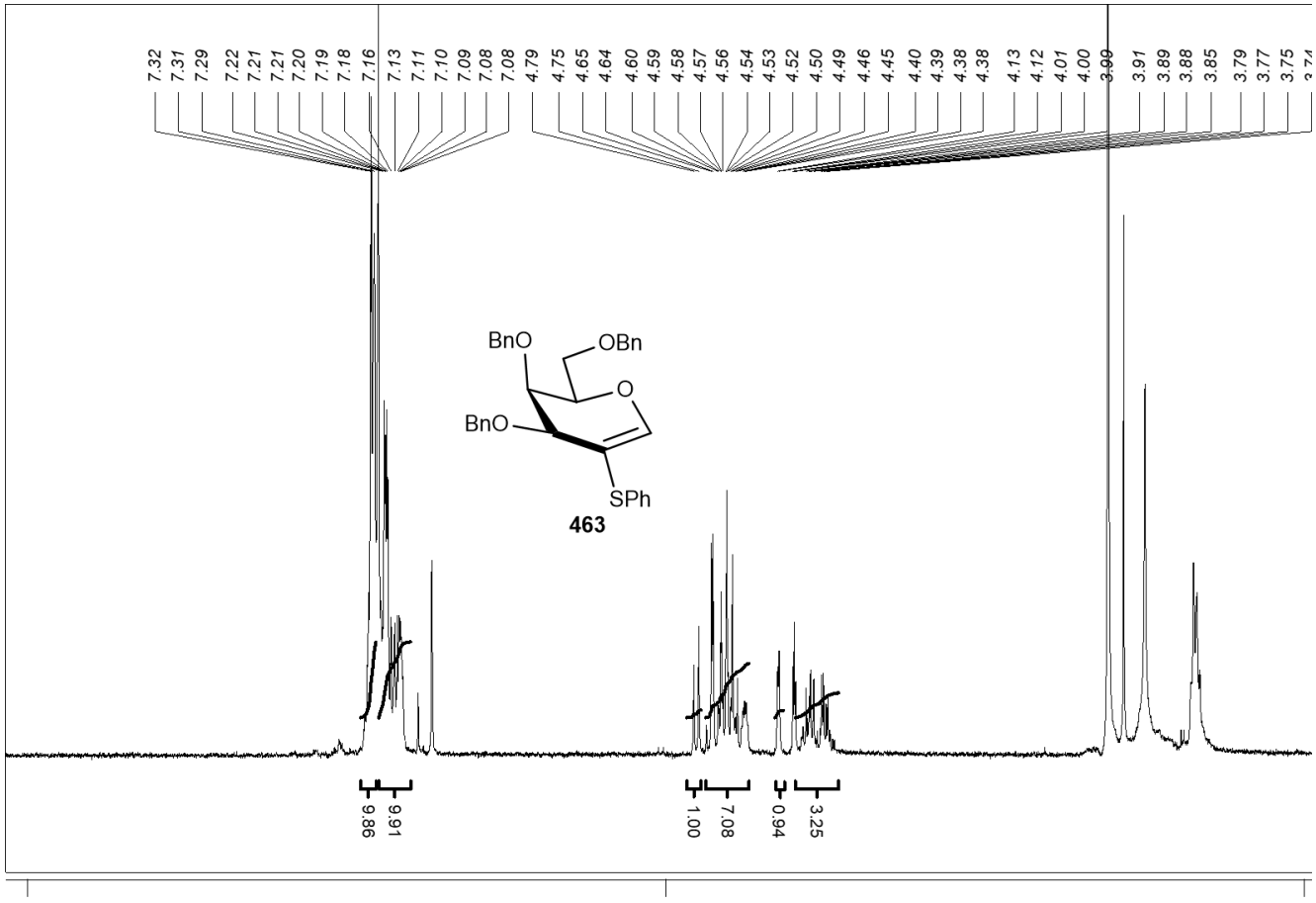
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BnO
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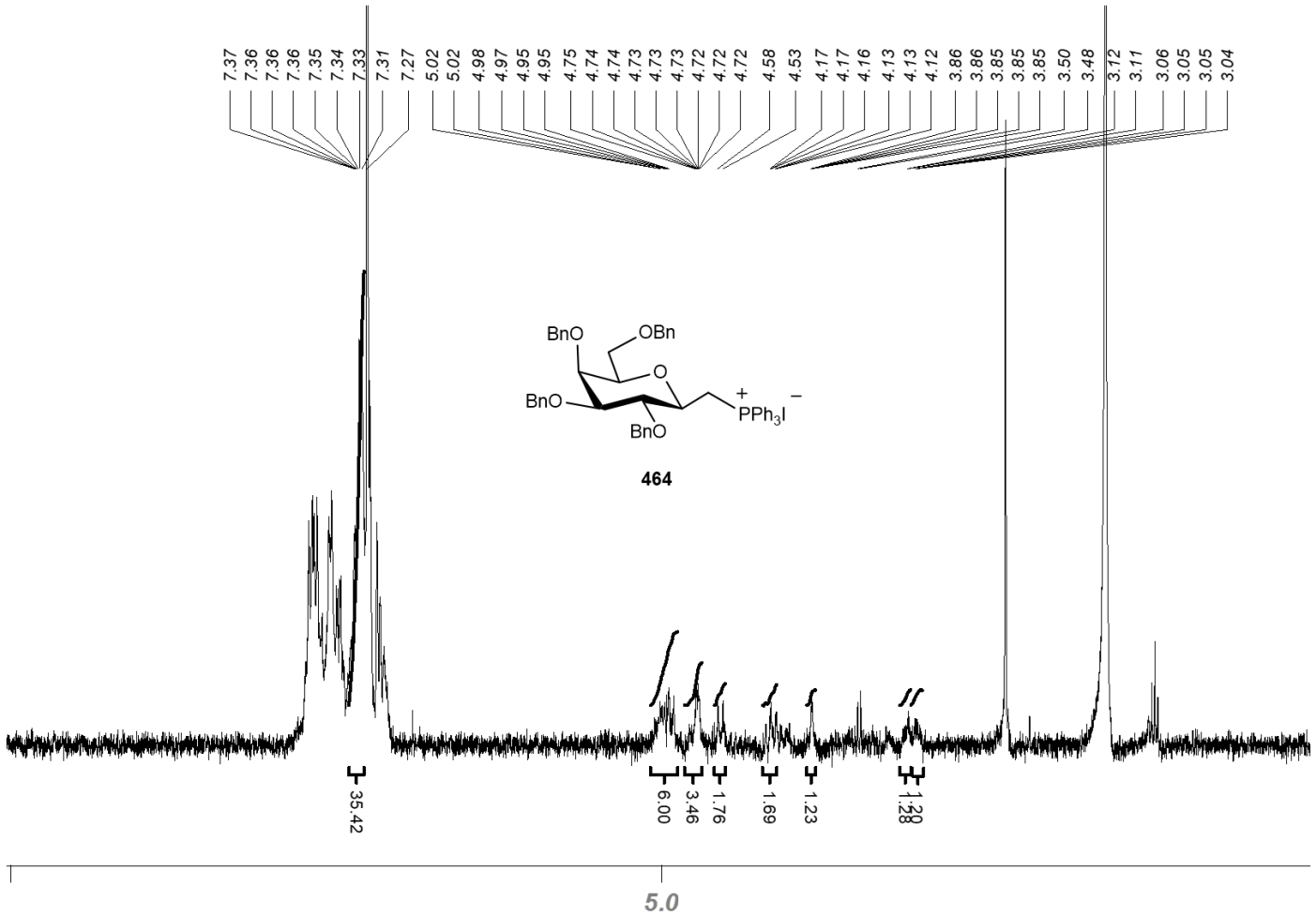
5.0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BnO
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+
PPh3I
–
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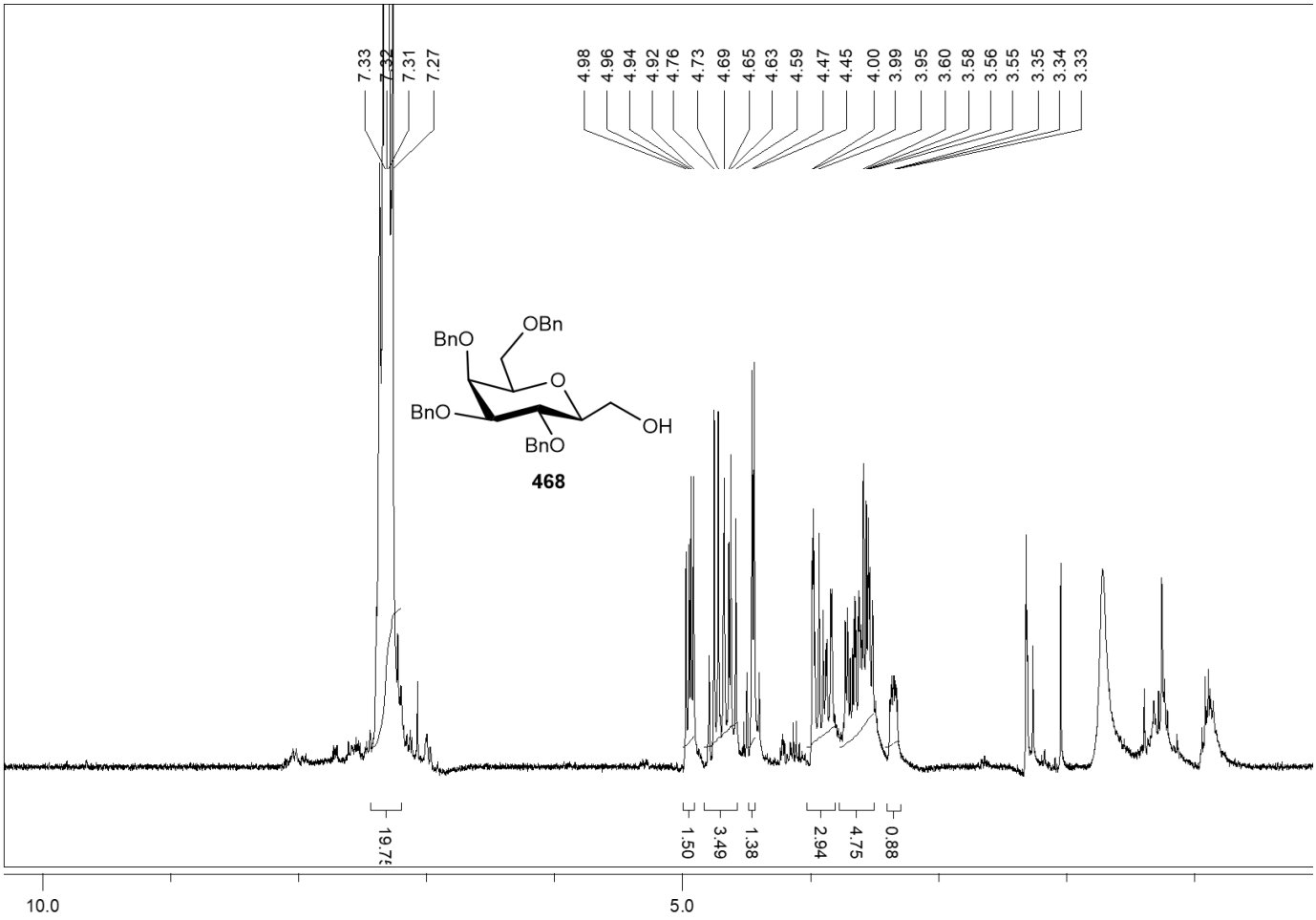
5.0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4.59
4.47
4.45
4.00
3.99
3.95
3.60
3.58
3.56
3.55
3.35
3.34
3.33
BnO
OBn
O
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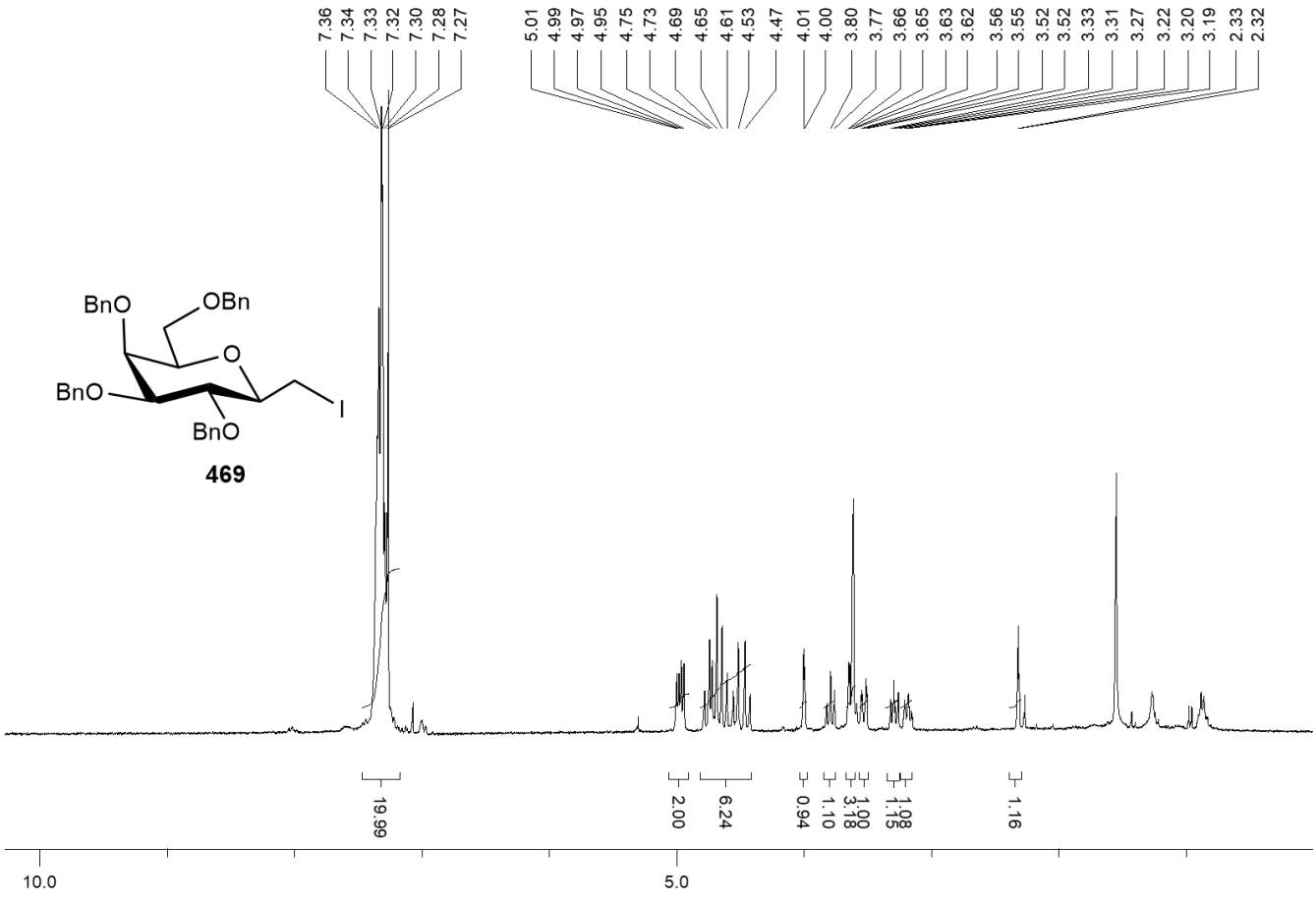
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3.65
3.63
3.62
3.56
3.55
3.52
3.52
3.33
3.31
3.27
3.22
3.20
3.19
2.33
2.32
BnO
OBn
O
BnO
I
BnO
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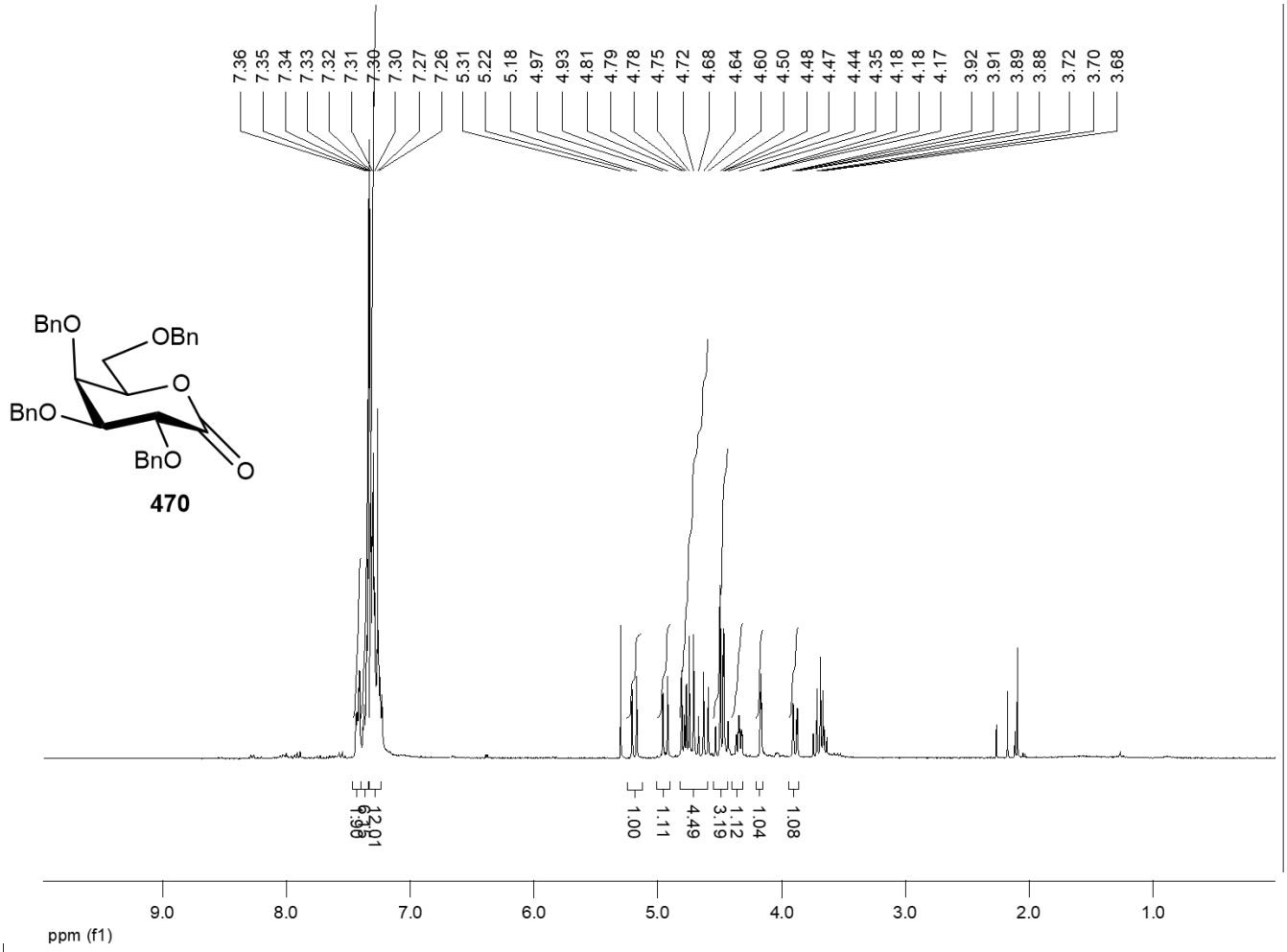
5.0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4.47
4.44
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3.89
3.88
3.72
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3.68
BnO
OBn
O
BnO
BnO
O
470
12.01
1.00
1.11
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1.12
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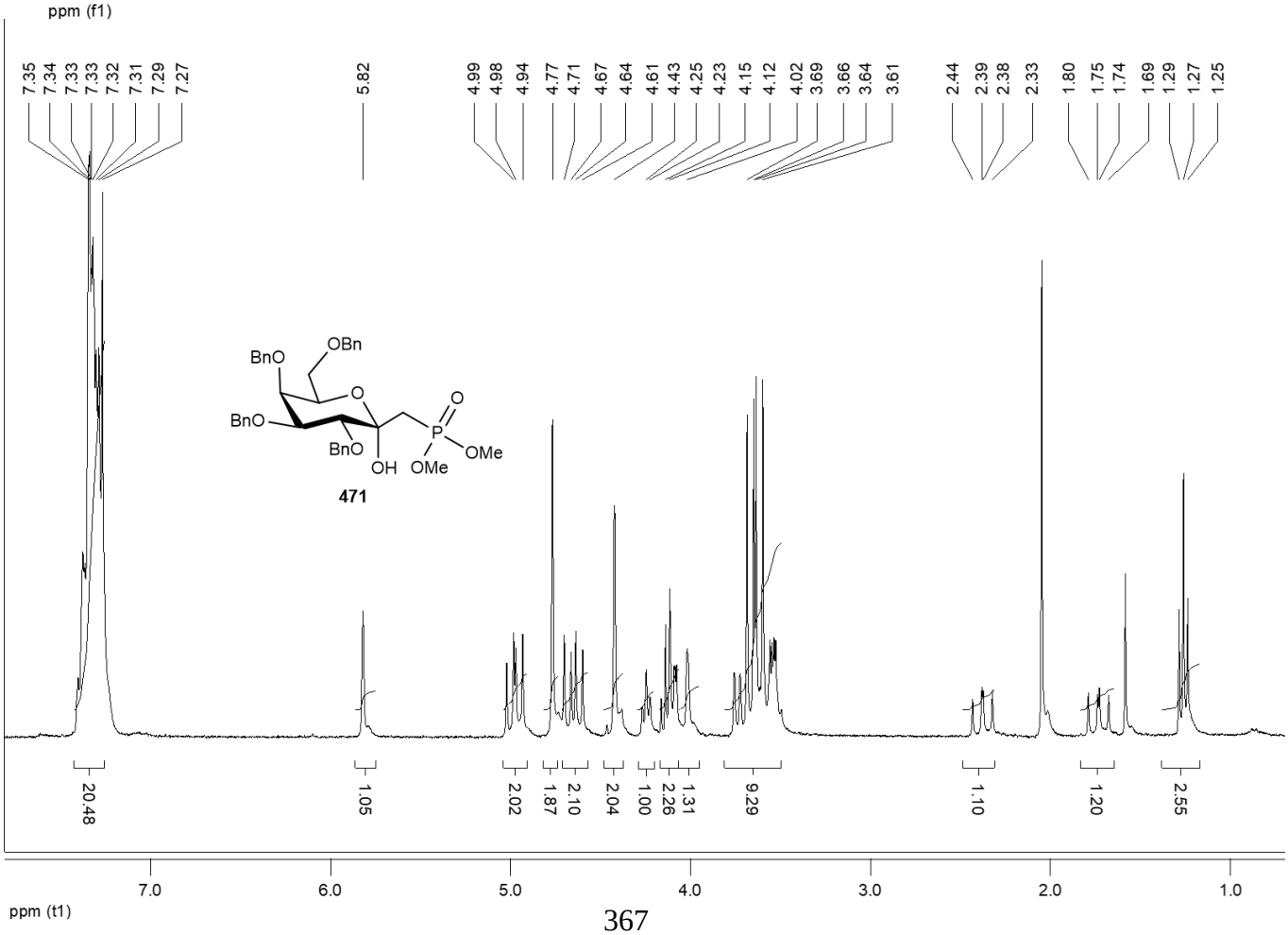
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4.98
4.94
4.77
4.71
4.67
4.64
4.61
4.43
4.25
4.23
4.15
4.12
4.02
3.69
3.66
3.64
3.61
2.44
2.39
2.38
2.33
1.80
1.75
1.74
1.69
1.29
1.27
1.25
BnO
OBn
O
BnO
BnO
OH
P
O
OMe
OMe
471
20.48
1.05
2.02
1.87
2.10
2.04
1.00
2.26
1.31
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1.10
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2.55
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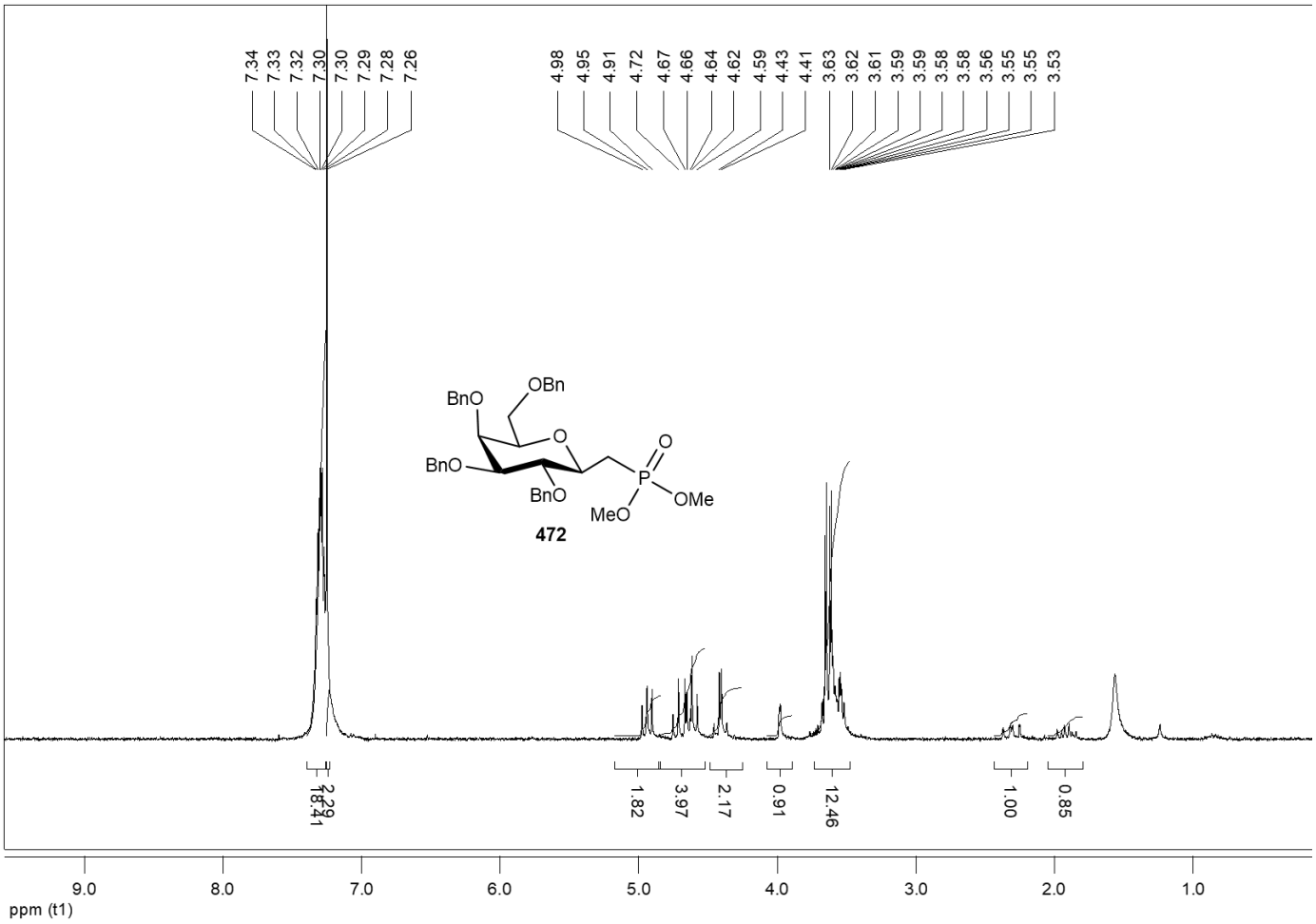
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)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4.95
4.91
4.72
4.67
4.66
4.64
4.62
4.59
4.43
4.41
3.63
3.62
3.61
3.59
3.59
3.58
3.58
3.56
3.55
3.55
3.53
BnO
OBn
O
BnO
BnO
P
O
OMe
MeO
472
2.29
18.41
1.82
3.97
2.17
0.91
12.46
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8.0
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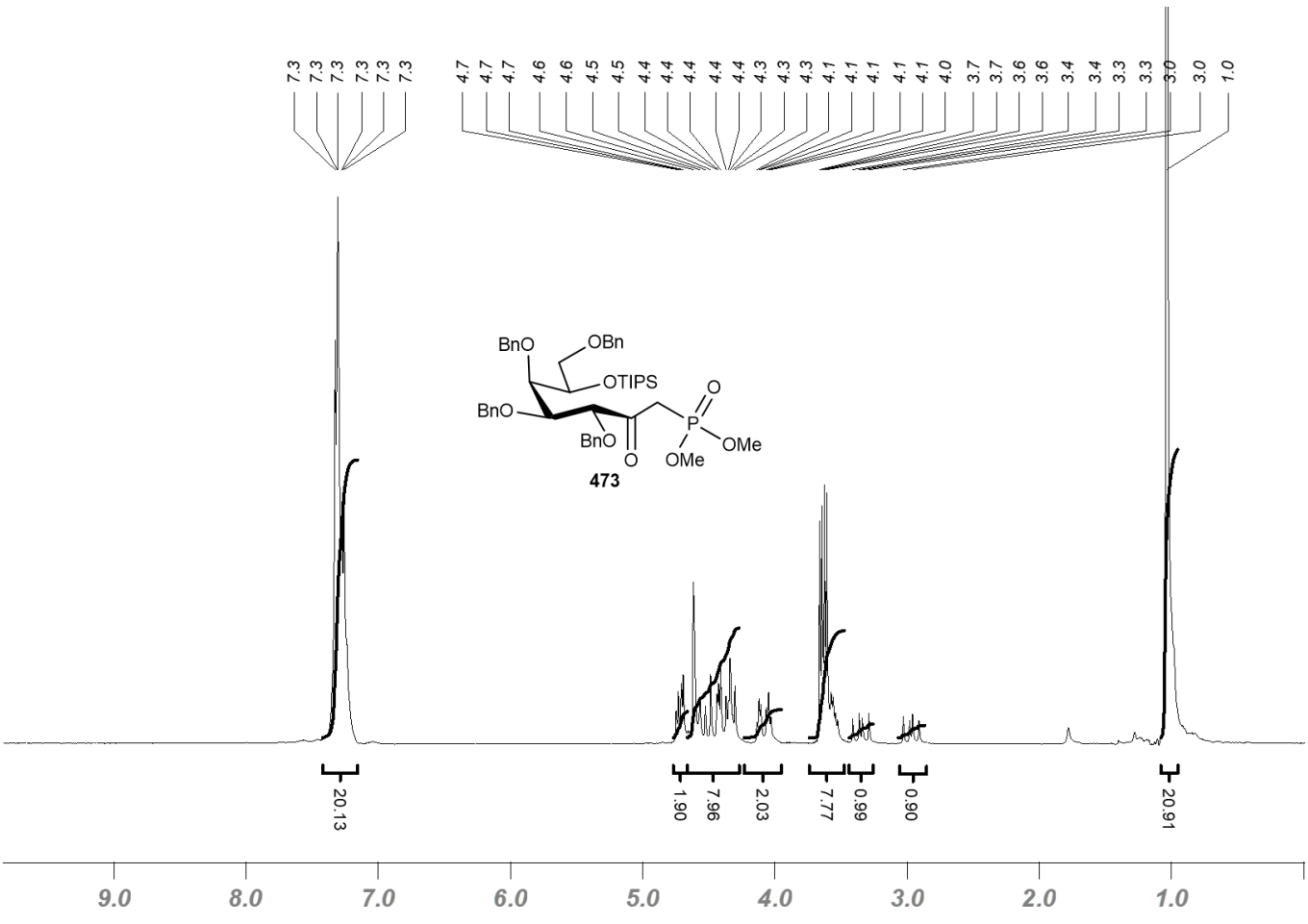
3.0
2.0
1.0
ppm (t1)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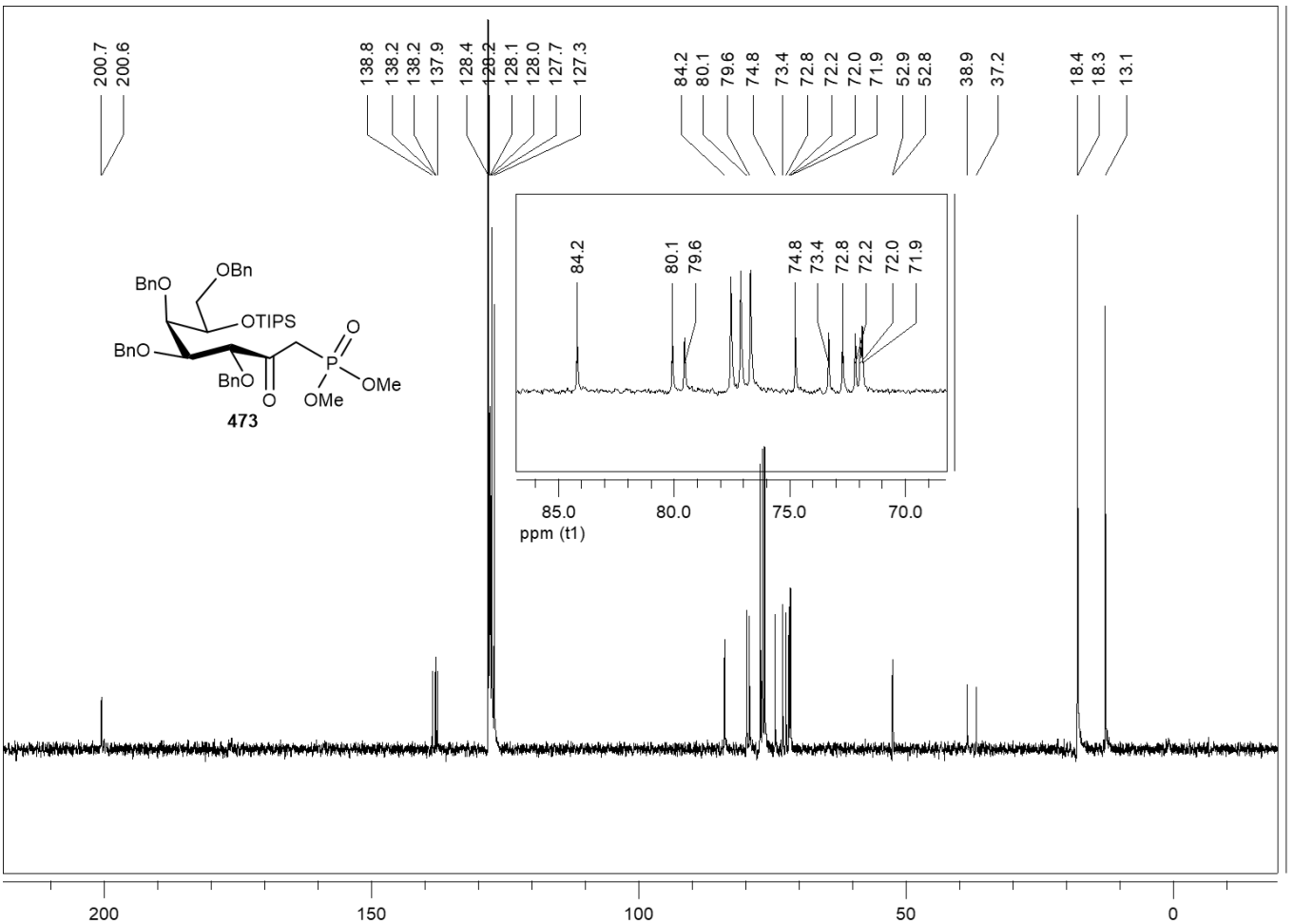
4.7 4.7 4.7 4.6 4.6 4.5 4.5 4.4 4.4 4.4 4.4 4.4 4.3 4.3 4.3 4.1 4.1 4.1 4.1 4.0 3.7 3.7 3.6 3.6 3.4 3.4 3.3 3.3 3.0 3.0 1.0
BnO
OBn
OTIPS
BnO
BnO
O
P
OMe
OMe
O
473
20.13
1.90
7.96
2.03
7.77
0.99
0.90
20.91
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0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84.2 80.1 79.6 74.8 73.4 72.8 72.2 72.0 71.9
52.9 52.8
38.9 37.2
18.4 18.3 13.1
BnO
OBn
OTIPS
BnO
BnO
O
P
OMe
OMe
O
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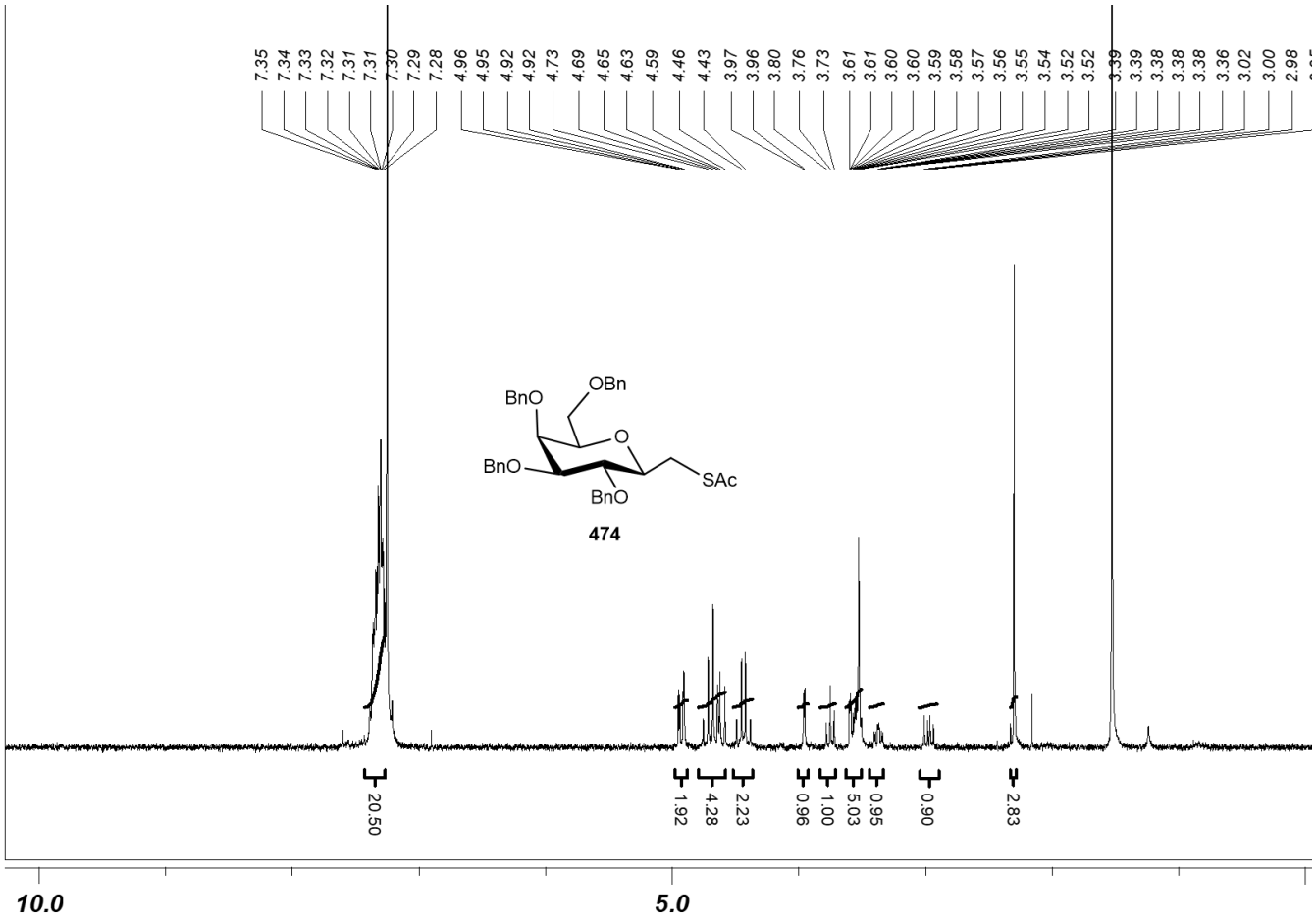
85.0
80.0
75.0
70.0
ppm (t1)
200
150
100
50
0
ppm (t1)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4.96 4.95 4.92 4.92 4.73 4.69 4.65 4.63 4.59 4.46 4.43
3.97 3.96 3.80 3.76 3.73 3.61 3.61 3.60 3.60 3.59 3.58 3.57 3.56 3.55 3.54 3.52 3.52
3.39 3.39 3.38 3.38 3.38 3.36 3.02 3.00 2.98
BnO
OBn
O
BnO
SAc
BnO
474
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4.28
2.23
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1.00
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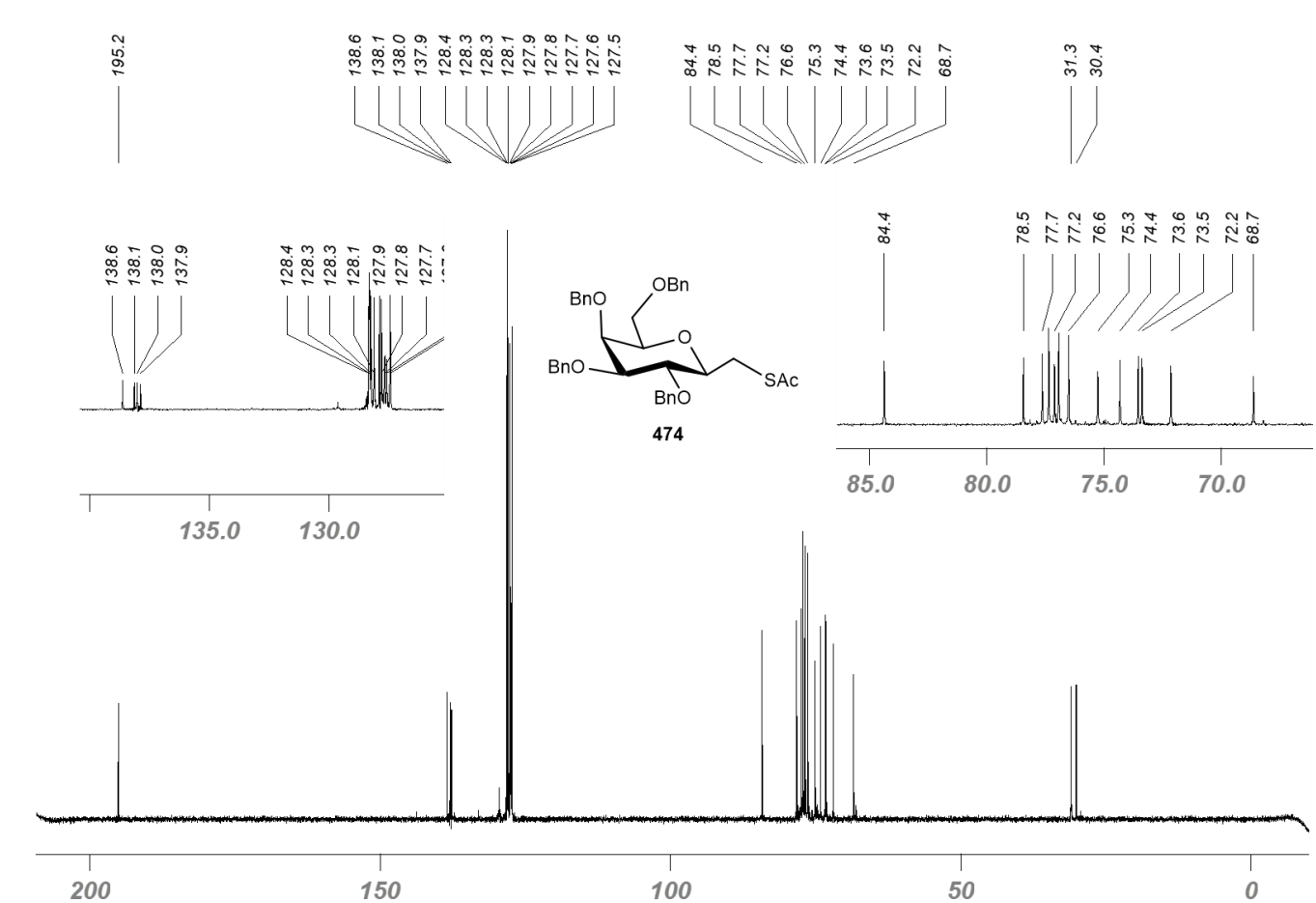
10.0
5.0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84.4 78.5 77.7 77.2 76.6 75.3 74.4 73.6 73.5 72.2 68.7
31.3 30.4
BnO
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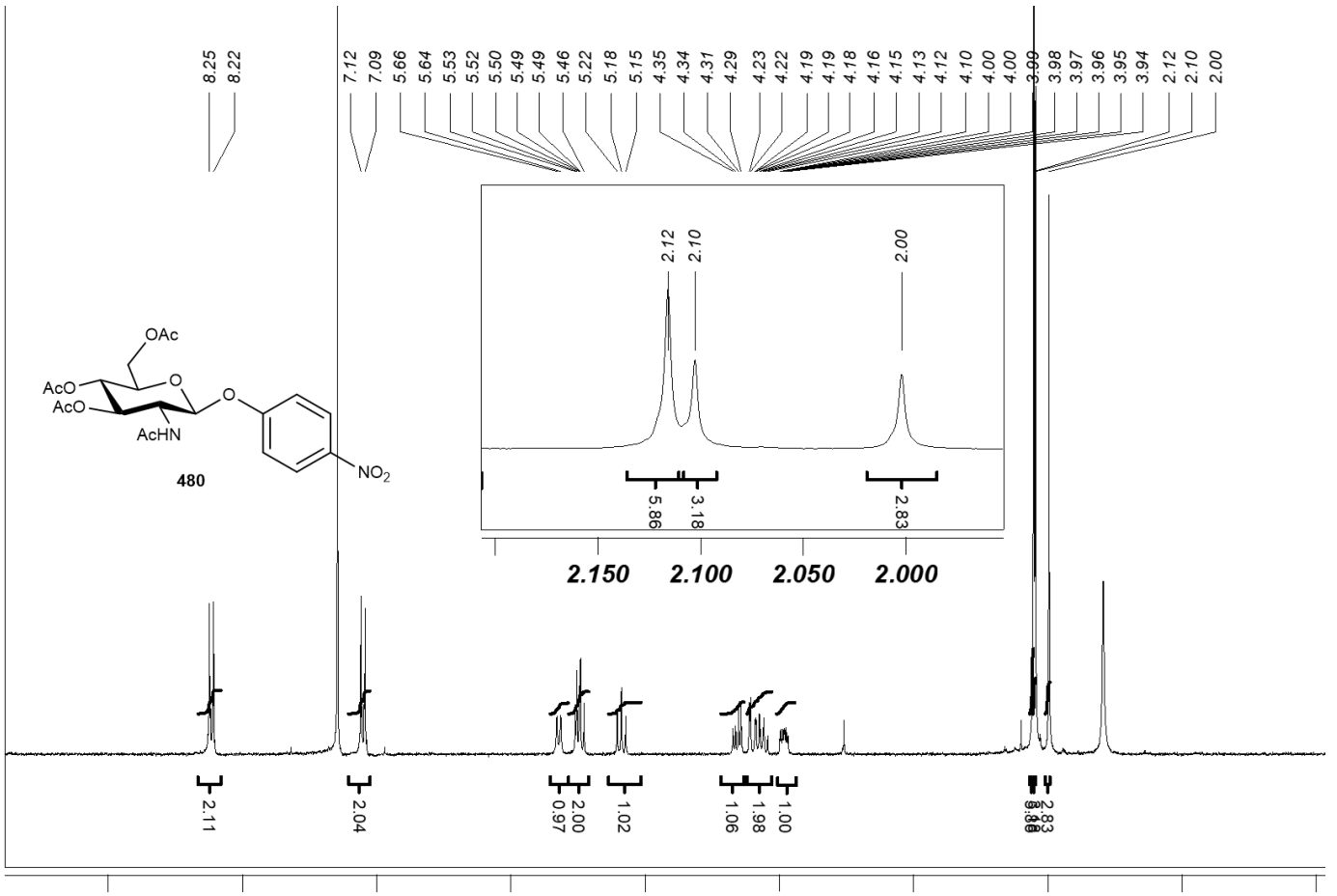
O
BnO
SAc
BnO
474
135.0 130.0
85.0 80.0 75.0 70.0
200 150 100 50 0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5.49
5.46
5.22
5.18
5.15
4.35
4.34
4.31
4.29
4.23
4.22
4.19
4.19
4.18
4.16
4.15
4.13
4.12
4.10
4.00
4.00
3.99
3.98
3.97
3.96
3.95
3.94
2.12
2.10
2.00
OAc
AcO
AcO
O
O
AcHN
NO2
480
2.12
2.10
2.00
5.86
3.18
2.83
2.150
2.100
2.050
2.000
2.11
2.04
0.97
2.00
1.02
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2.0
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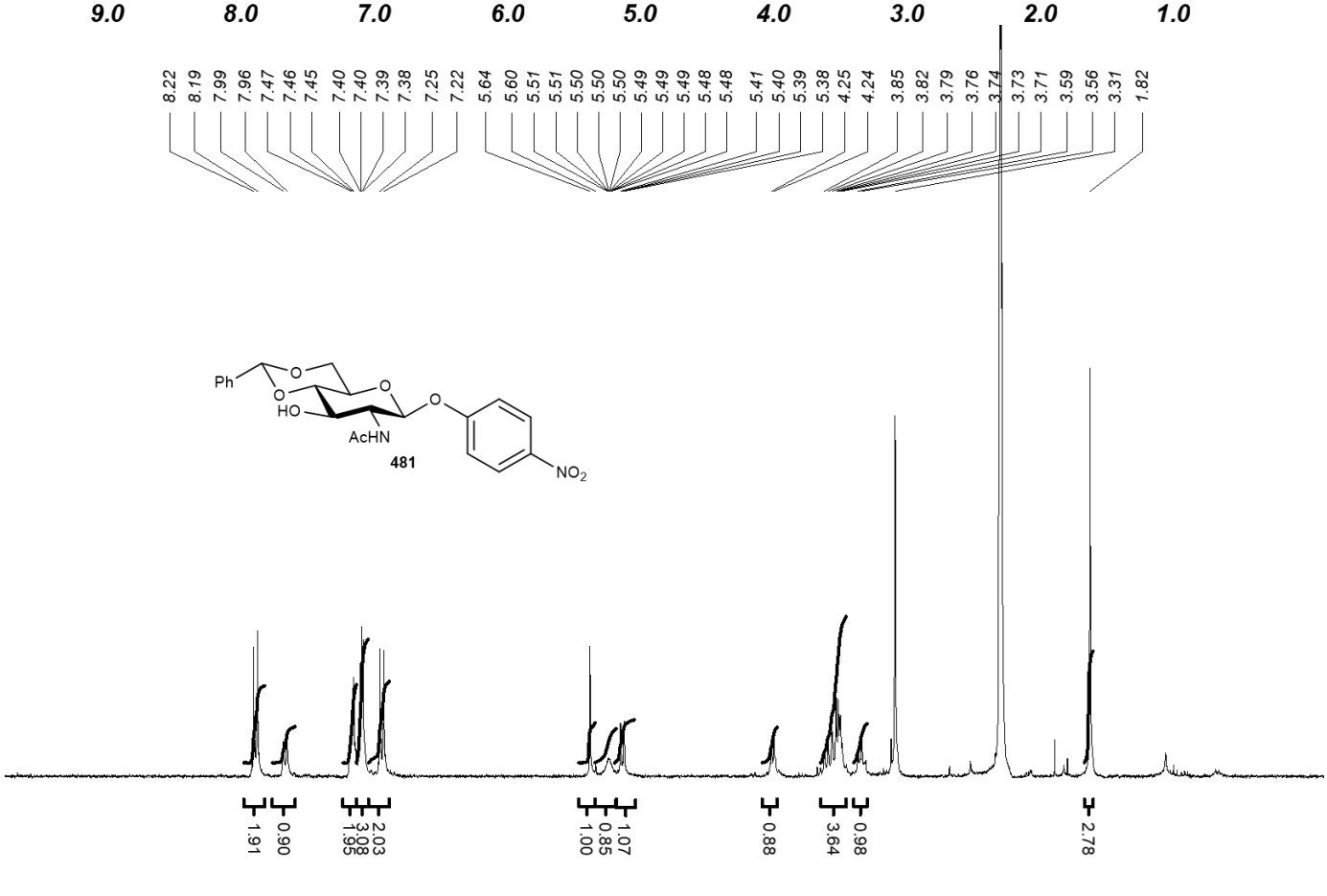
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7.39
7.38
7.25
7.22
5.64
5.60
5.51
5.51
5.50
5.50
5.50
5.49
5.49
5.49
5.48
5.48
5.41
5.40
5.39
5.38
4.25
4.24
3.85
3.82
3.79
3.76
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3.71
3.59
3.56
3.31
1.82
Ph
O
O
HO
O
O
AcHN
NO2
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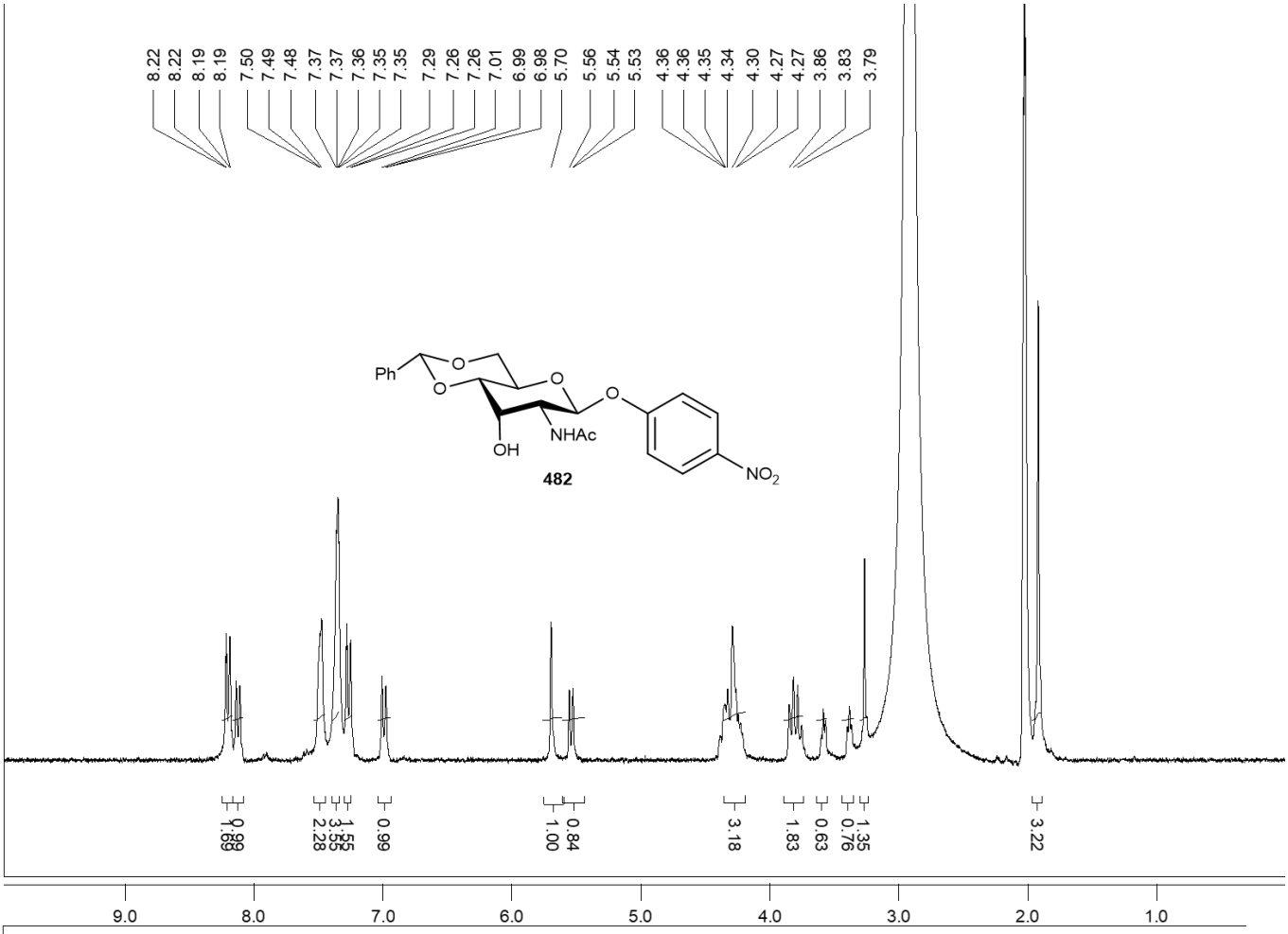
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7.37
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7.35
7.35
7.29
7.26
7.26
7.01
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5.70
5.56
5.54
5.53
4.36
4.36
4.35
4.34
4.30
4.27
4.27
3.86
3.83
3.79
Ph
O
O
O
O
NHAc
OH
NO2
482
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1.69
2.28
3.55
1.55
0.99
1.00
0.84
3.18
1.83
0.63
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7.0
6.0
5.0
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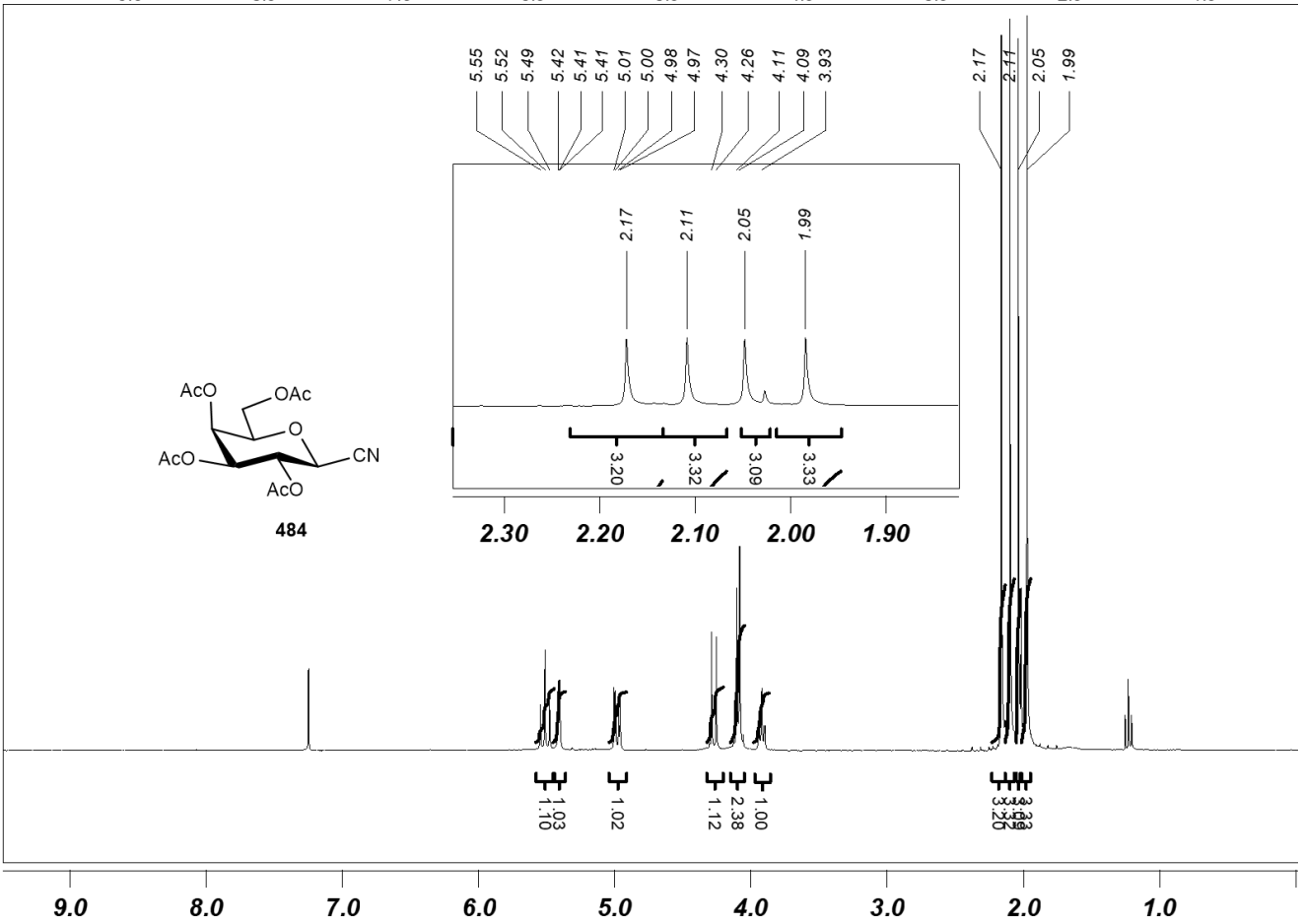
3.0
2.0
1.0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5.41
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5.00
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4.97
4.30
4.26
4.11
4.09
3.63
2.17
2.11
2.05
1.99
AcO
OAc
O
AcO
CN
AcO
484
3.20
3.32
3.09
3.33
2.30
2.20
2.10
2.00
1.90
1.03
1.10
1.02
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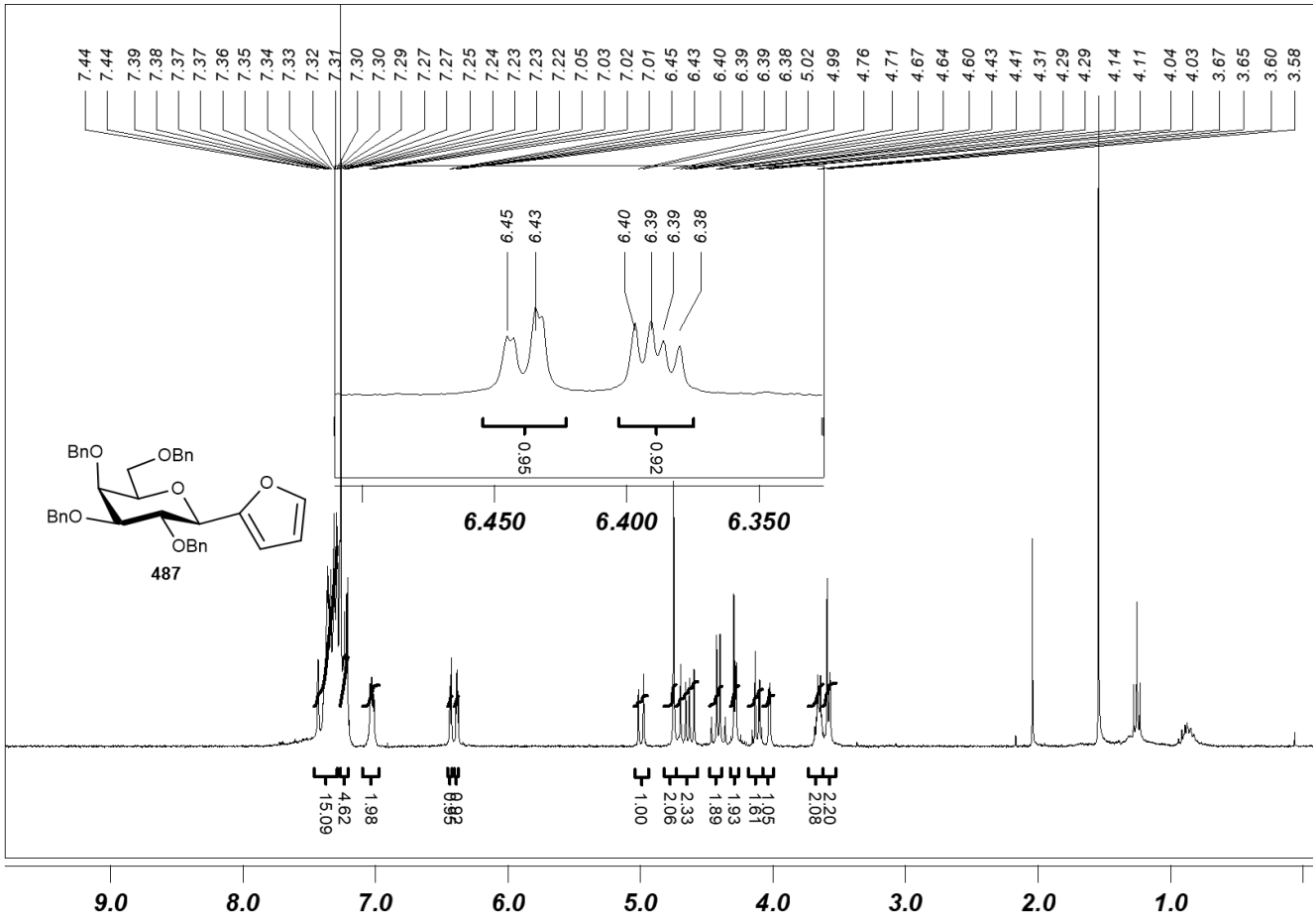
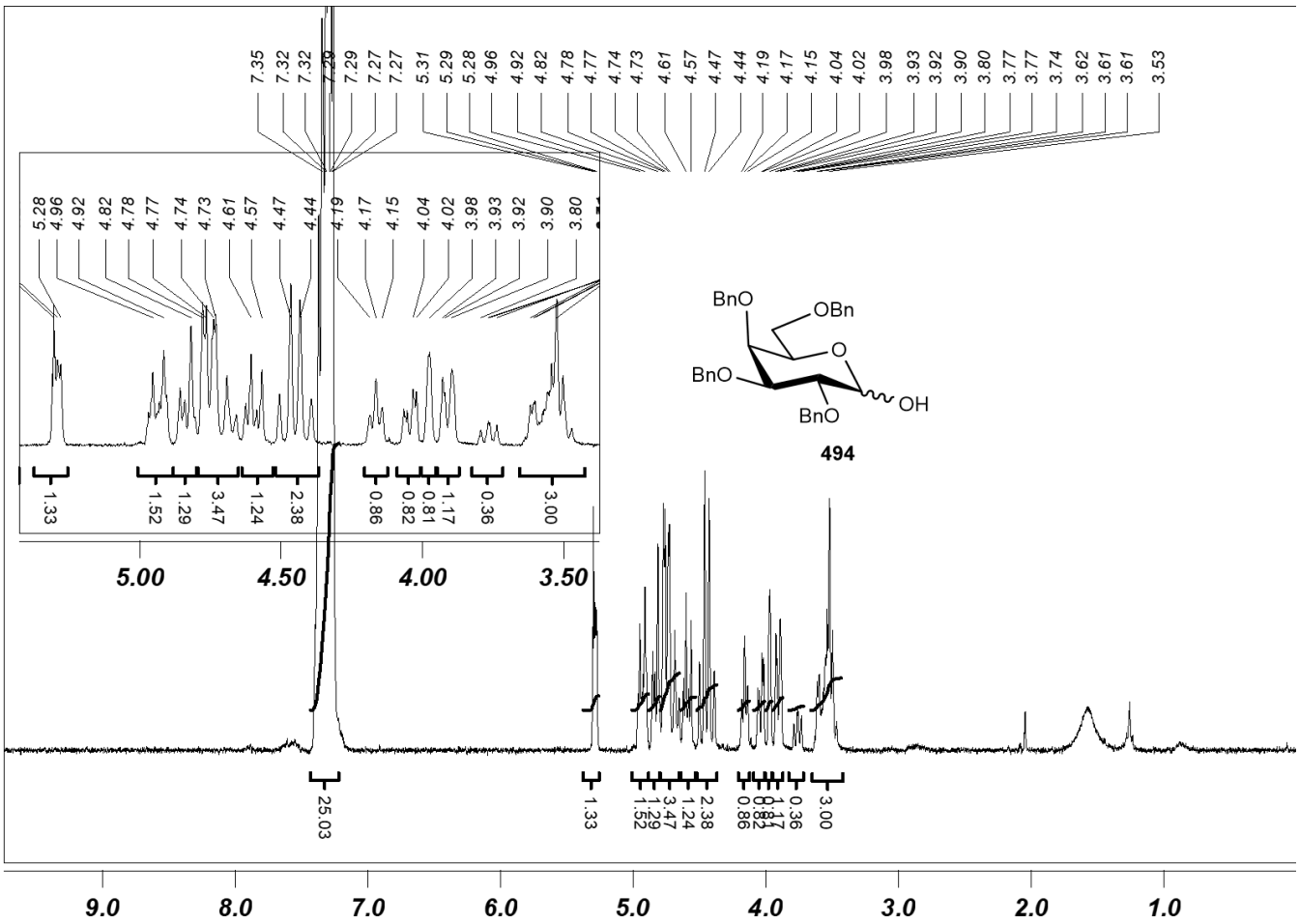
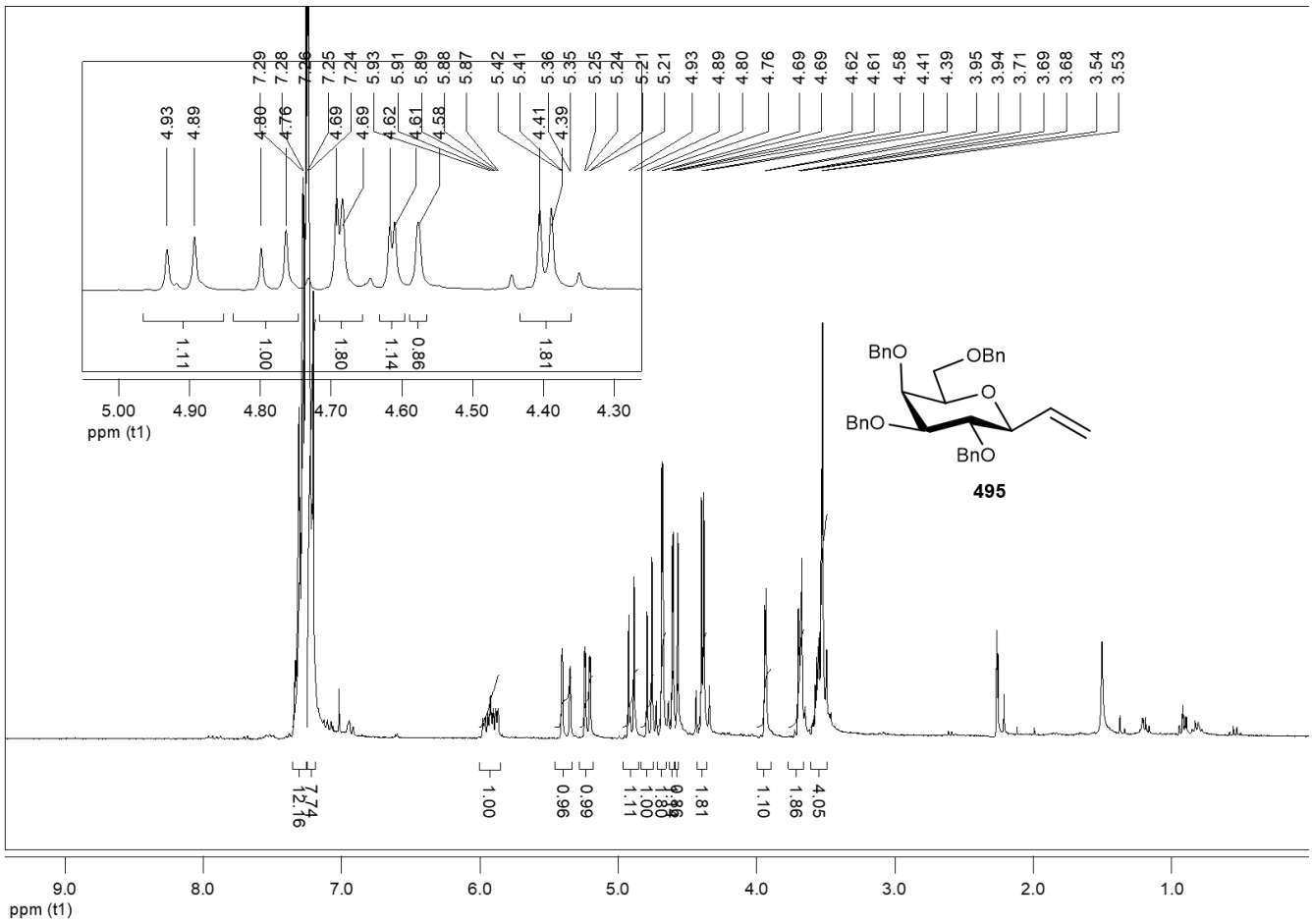
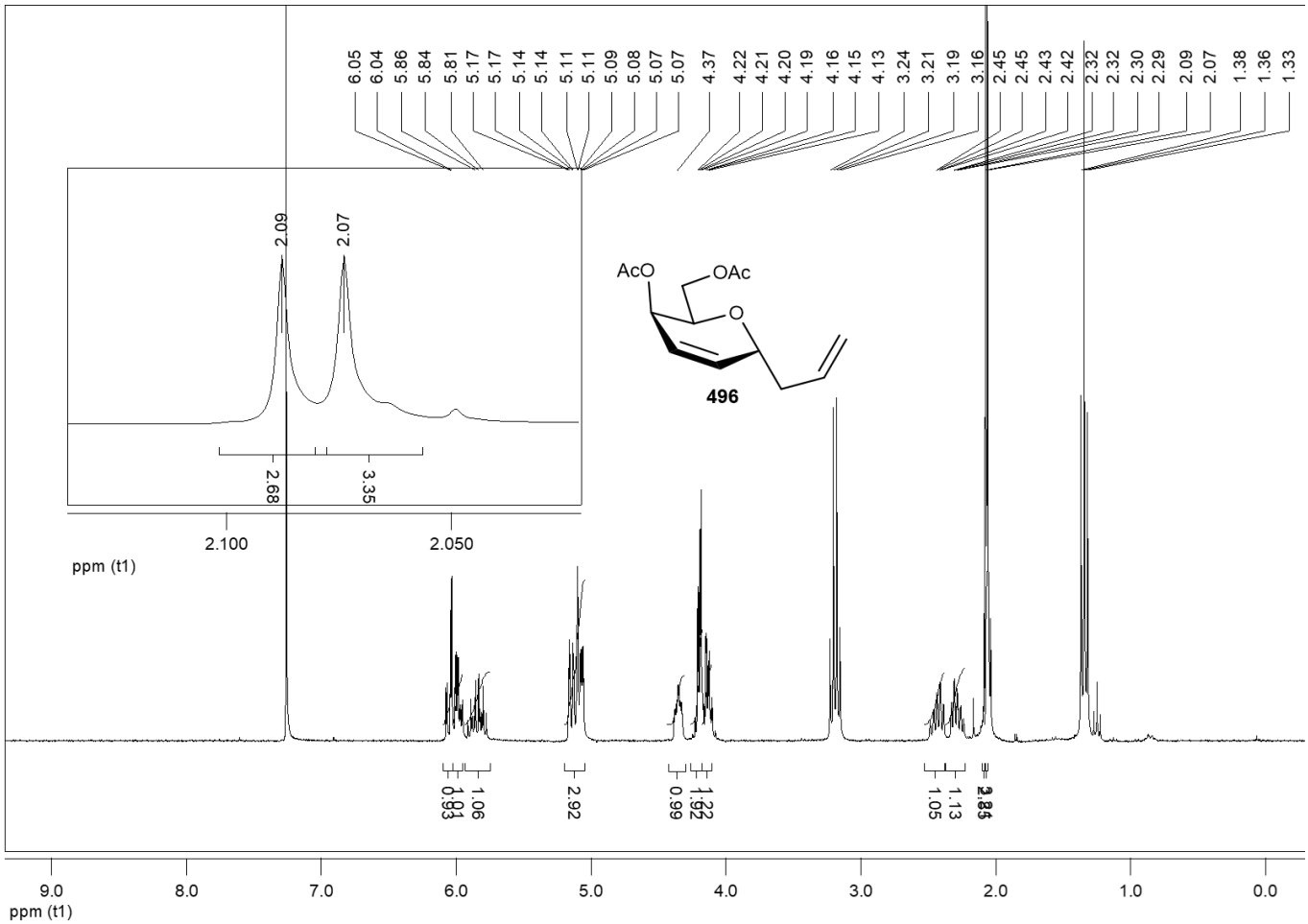
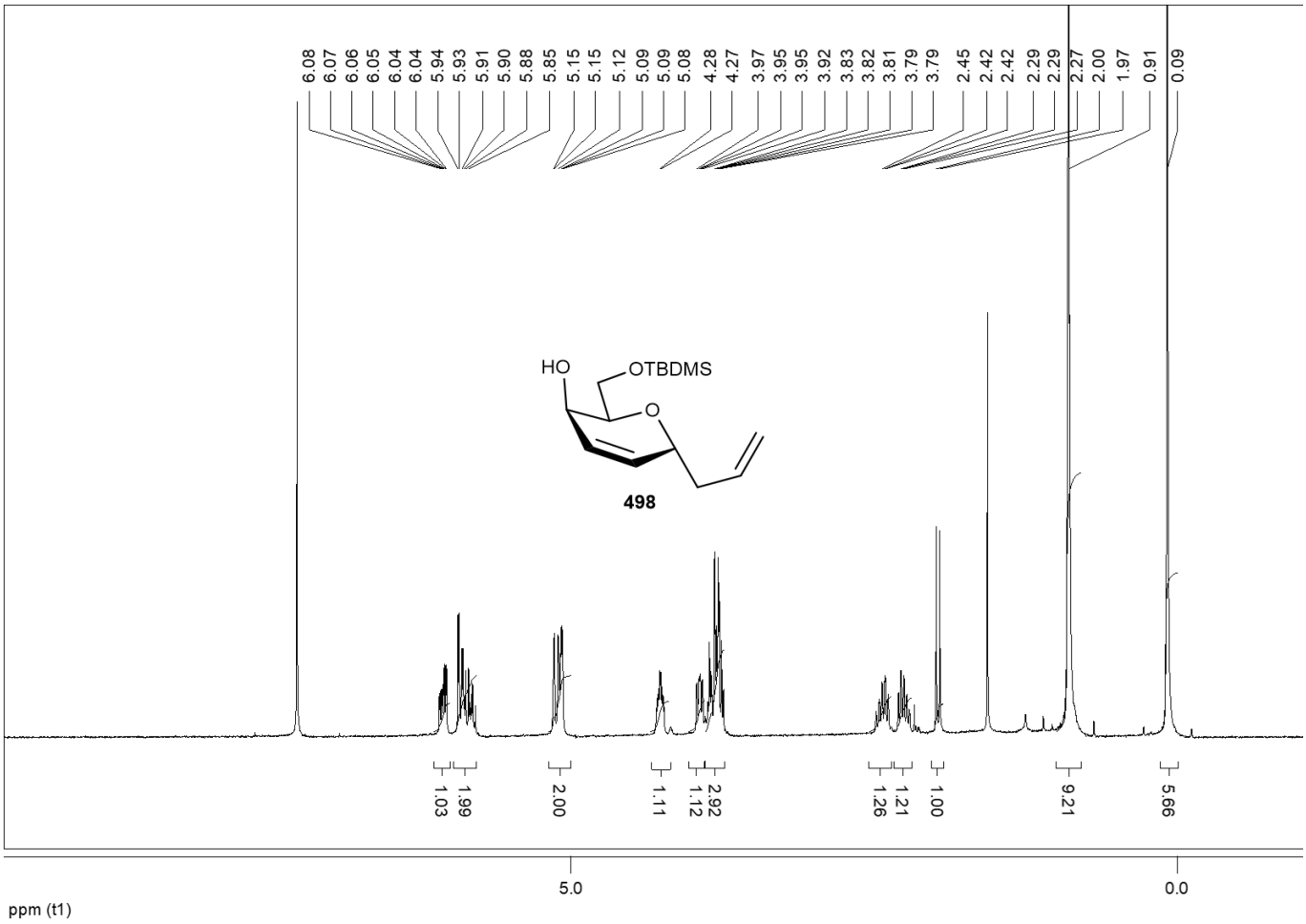

BnO
OBn
O
BnO
OBn
O
487


BnO
OBn
O
BnO
BnO
OH
494


BnO
OBn
O
BnO
BnO
495
ppm (t1)


AcO
OAc
O
496
ppm (t1)


6.08
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6.04
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5.90
5.88
5.85
5.15
5.15
5.12
5.09
5.09
5.08
4.28
4.27
3.97
3.95
3.95
3.92
3.83
3.82
3.81
3.79
3.79
2.45
2.42
2.42
2.29
2.29
2.27
2.00
1.97
0.91
0.09
HO
OTBDMS
O
498
1.03
1.99
2.00
1.11
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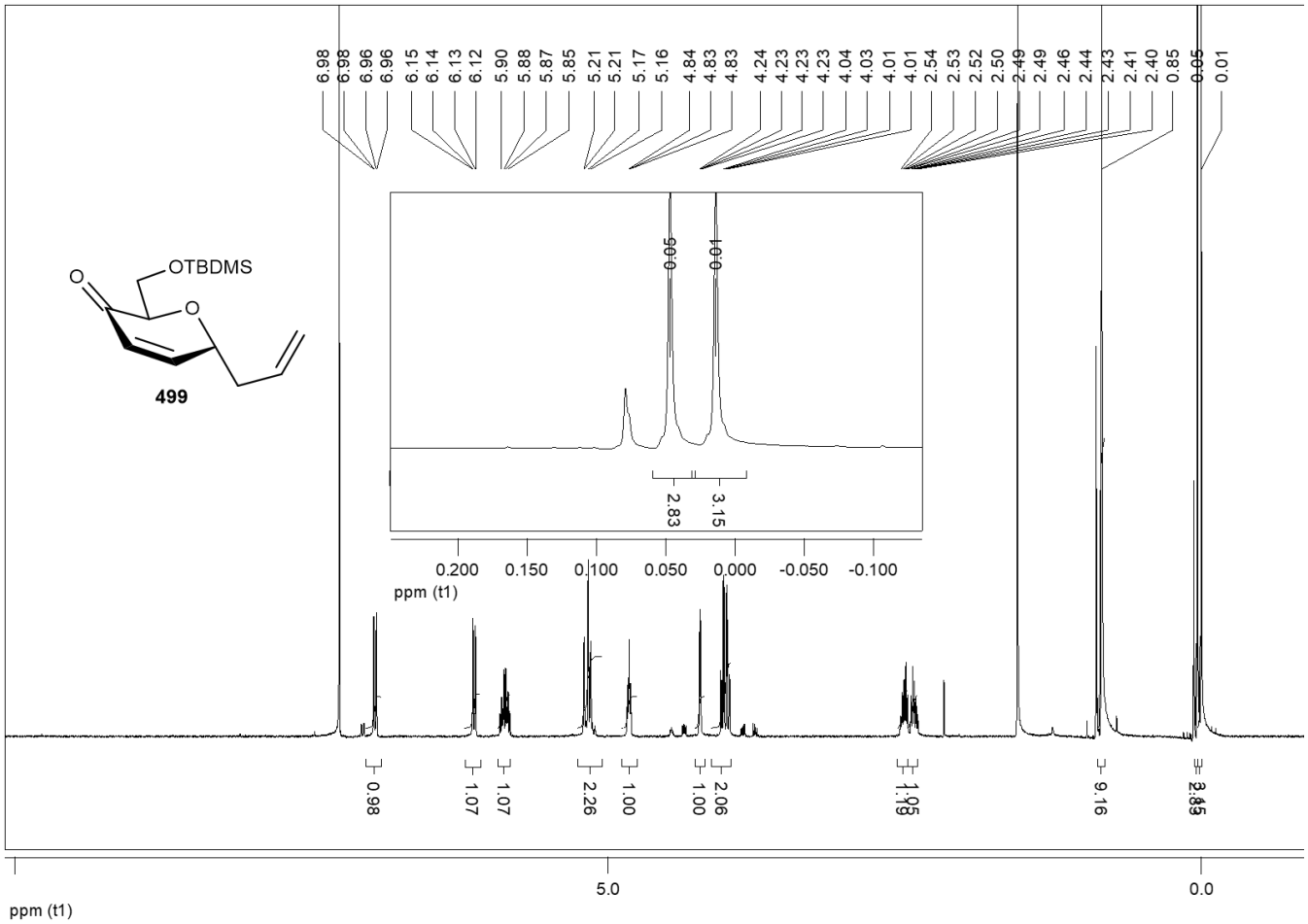
2.92
1.26
1.21
1.00
9.21
5.66
5.0
0.0
ppm (t1)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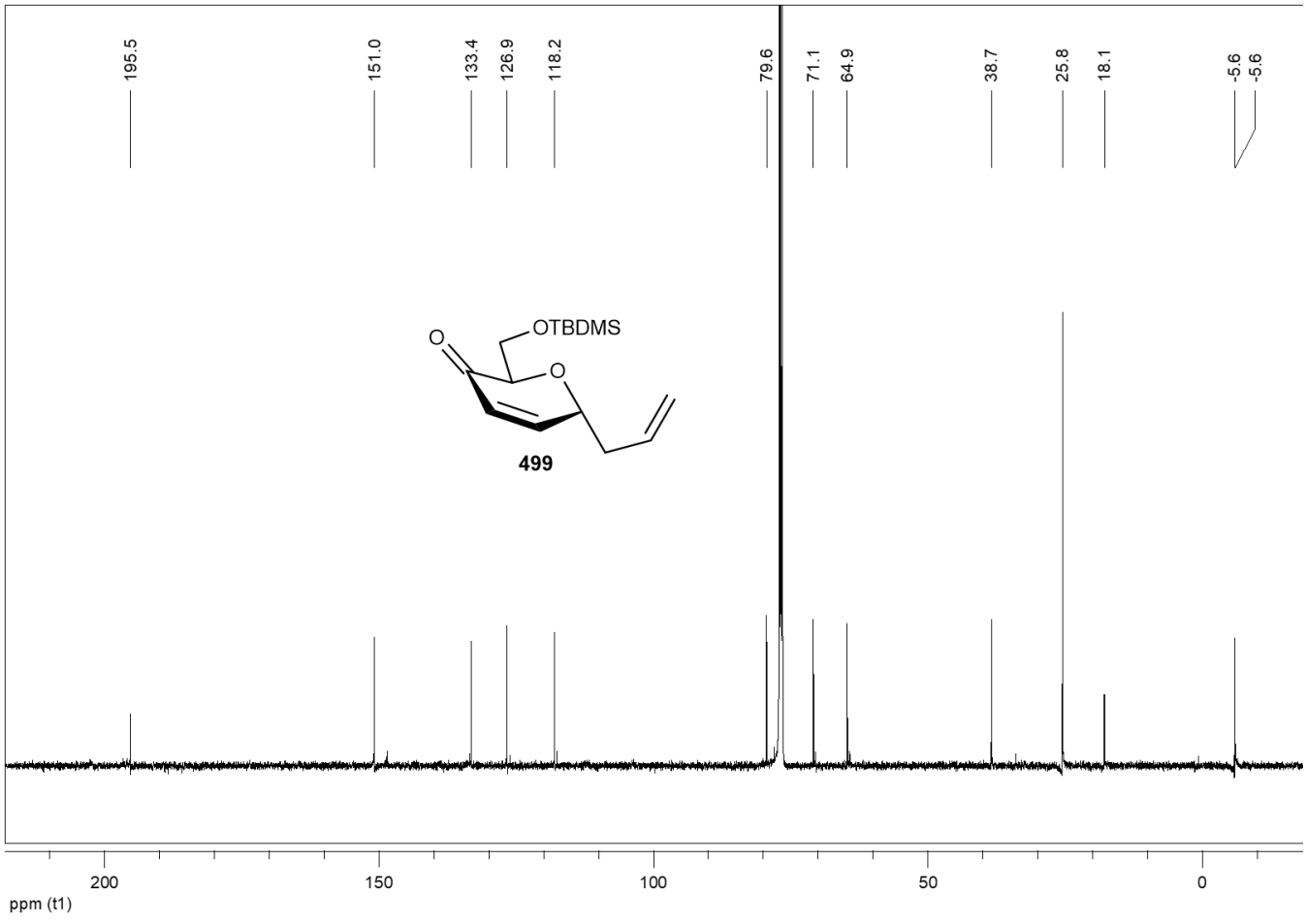
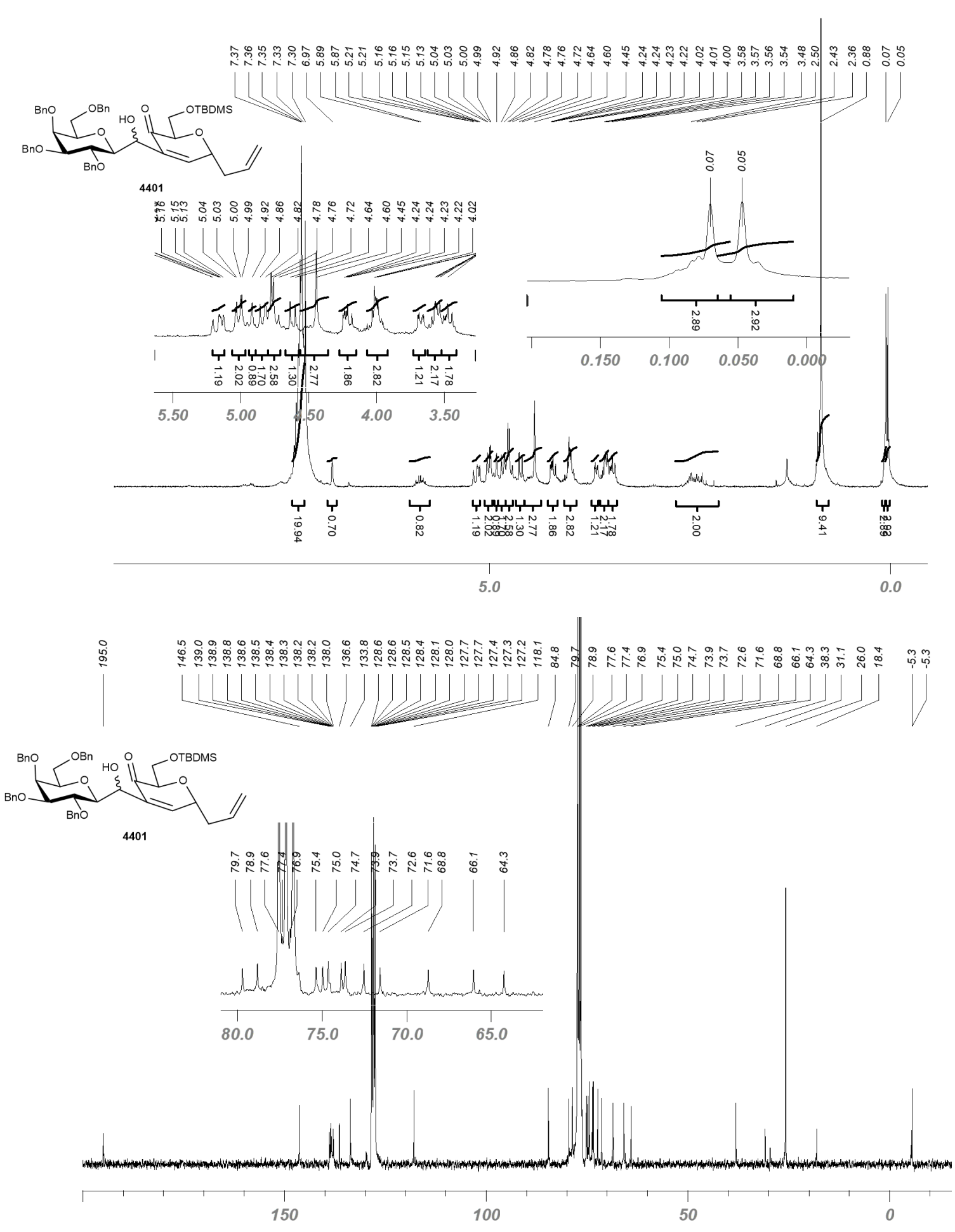
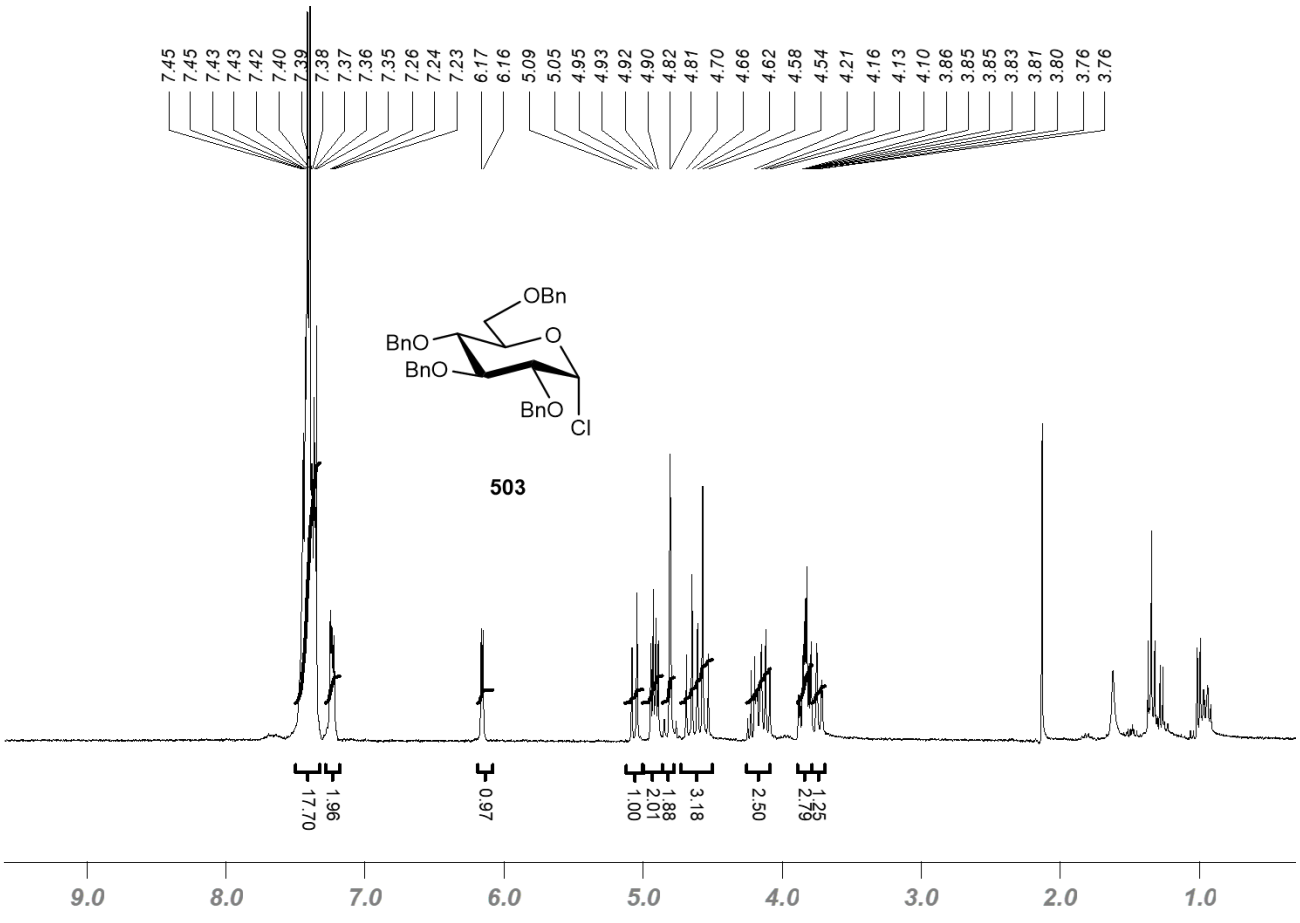
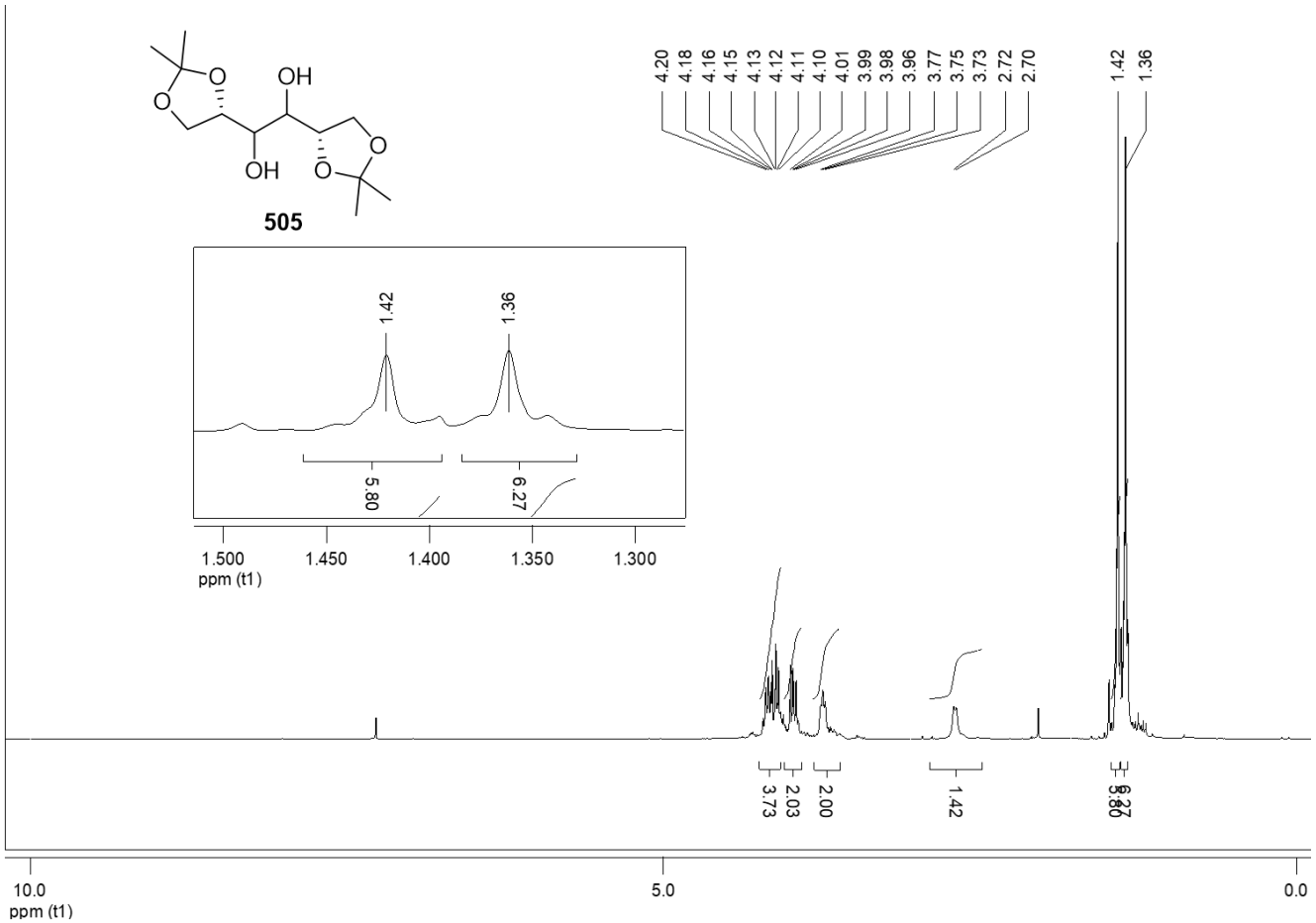
OTBDMS
O
O
499
1.00 1.01
2.83 3.15
0.200 0.150 0.100 0.050 0.000 -0.050 -0.100
ppm (t1)
0.98 1.07 1.07 2.26 1.00 1.00 2.06 1.05 9.16 2.85
5.0 0.0
ppm (t1)

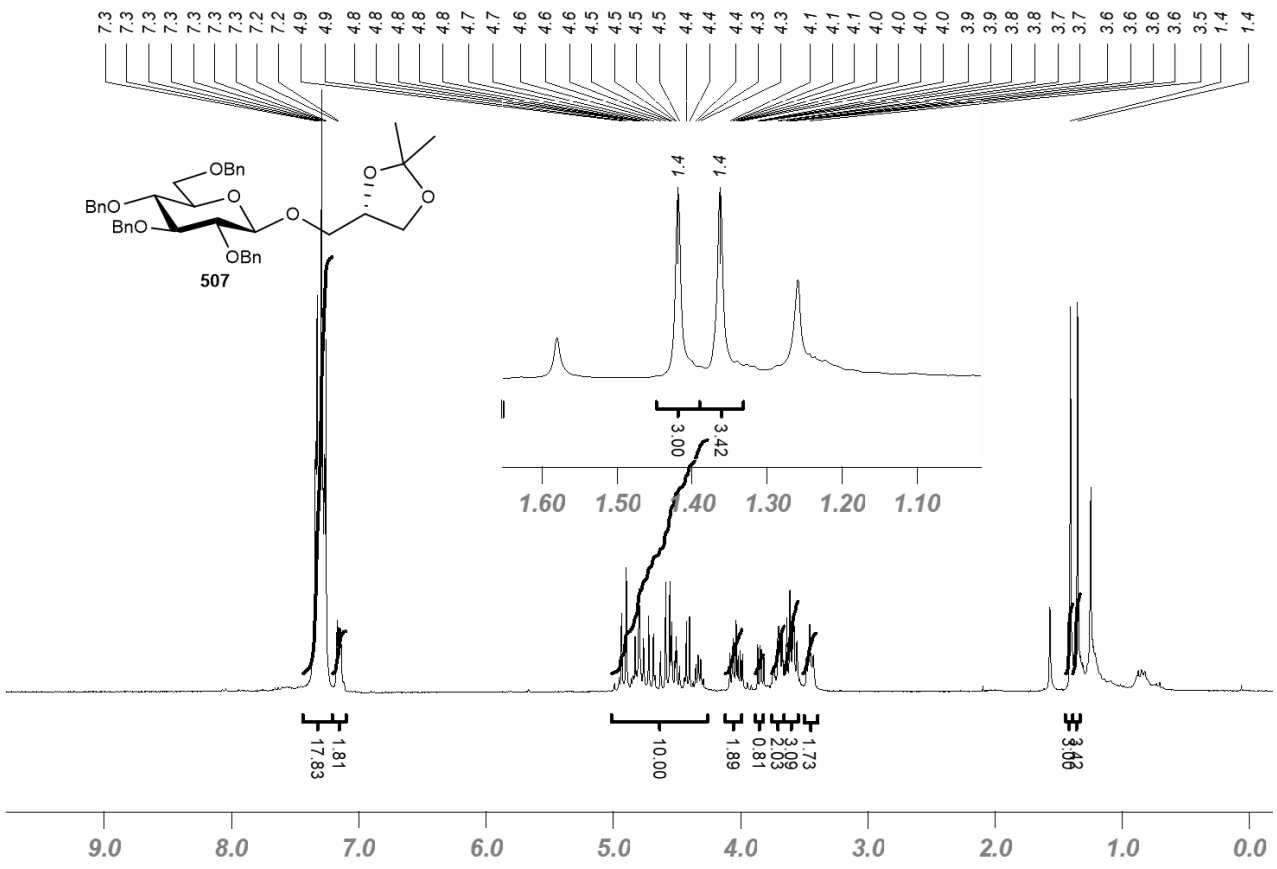

195.5 151.0 133.4 126.9 118.2 76.7 71.1 64.9 38.7 25.8 18.1 -5.6 -5.6
OTBDMS
O
O
499
200 150 100 50 0
ppm (t1)

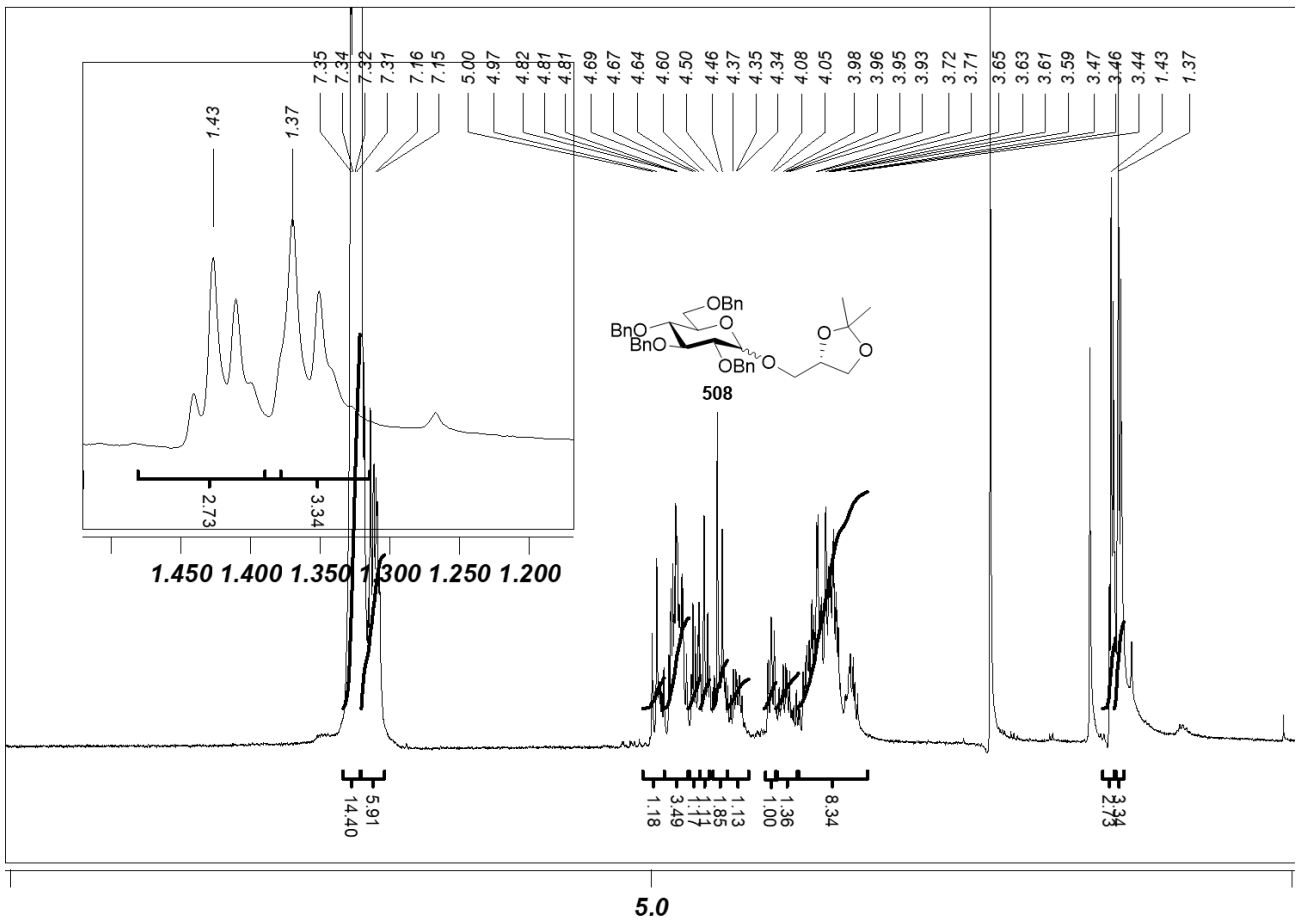

BnO
OBn
HO
O
OTBDMS
BnO
BnO
4401

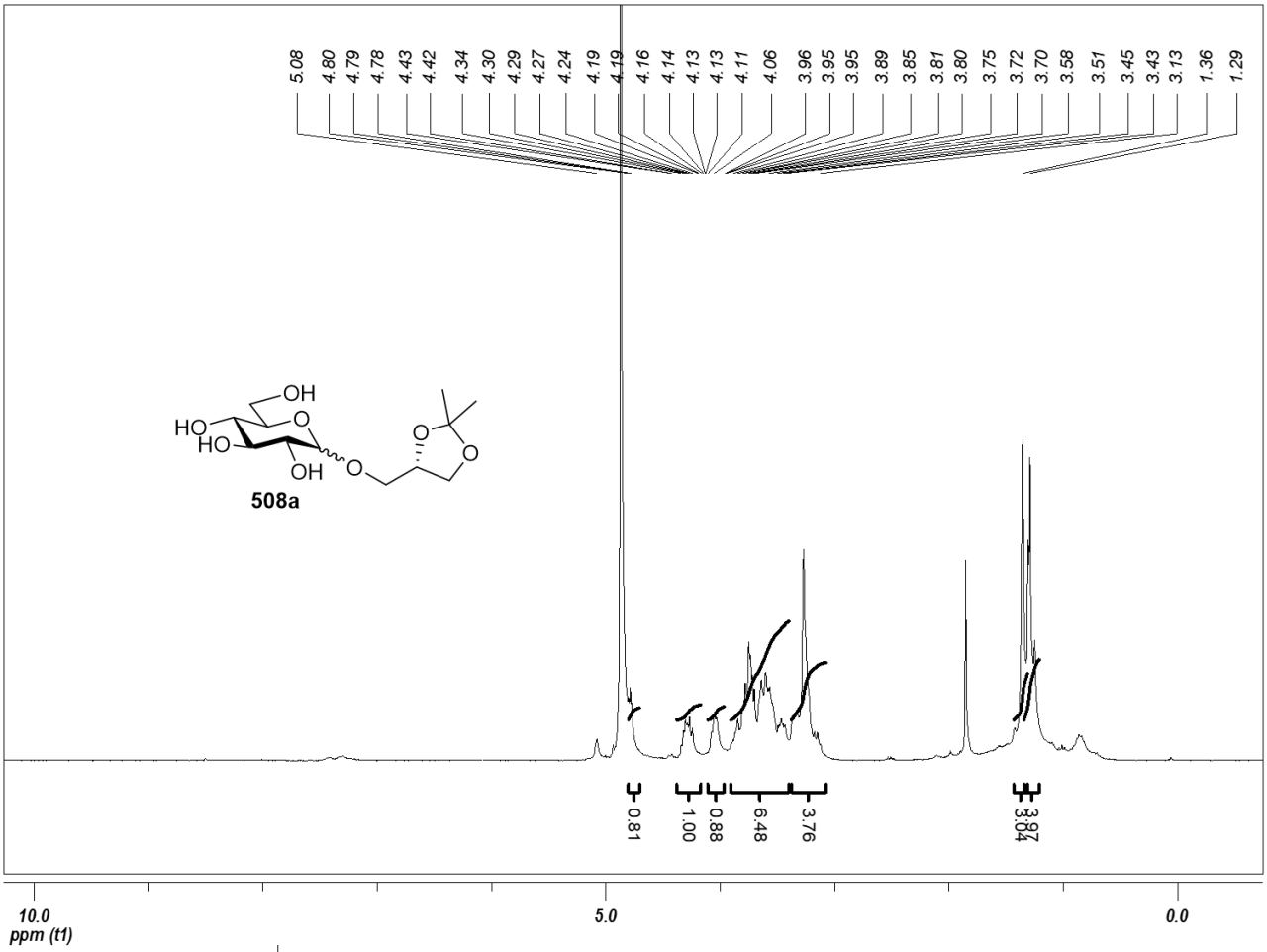
BnO
OBn
HO
O
OTBDMS
BnO
BnO
4401

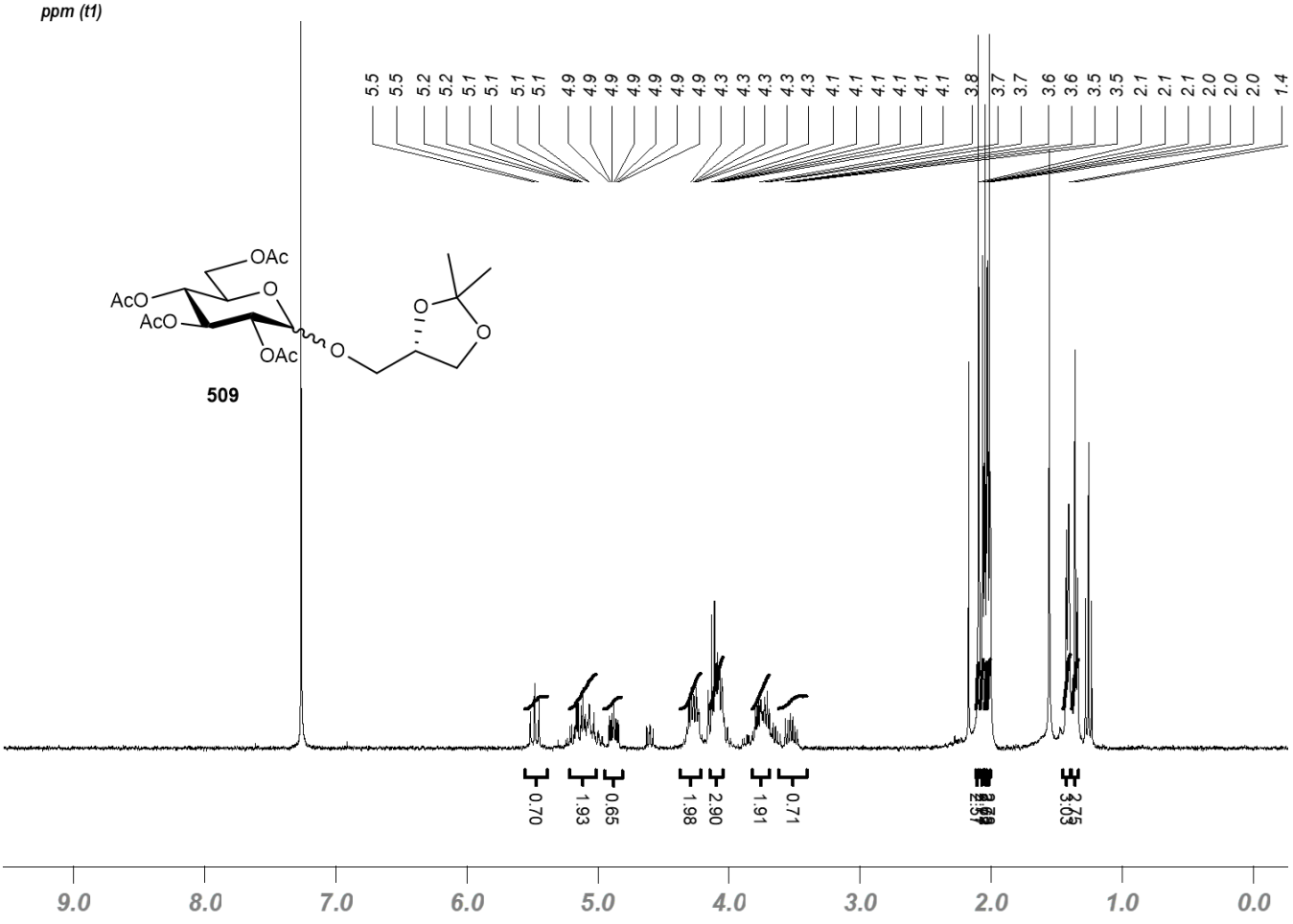

7.45 7.45 7.43 7.43 7.42 7.40 7.39 7.38 7.37 7.36 7.35 7.26 7.24 7.23 6.17 6.16 5.09 5.05 4.95 4.93 4.92 4.90 4.82 4.81 4.70 4.66 4.62 4.58 4.54 4.21 4.16 4.13 4.10 3.86 3.85 3.85 3.83 3.81 3.80 3.76 3.76
OBn
BnO
BnO
BnO
O
Cl
503
17.70
1.96
0.97
1.00
2.01
1.88
3.18
2.50
2.79
1.25
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

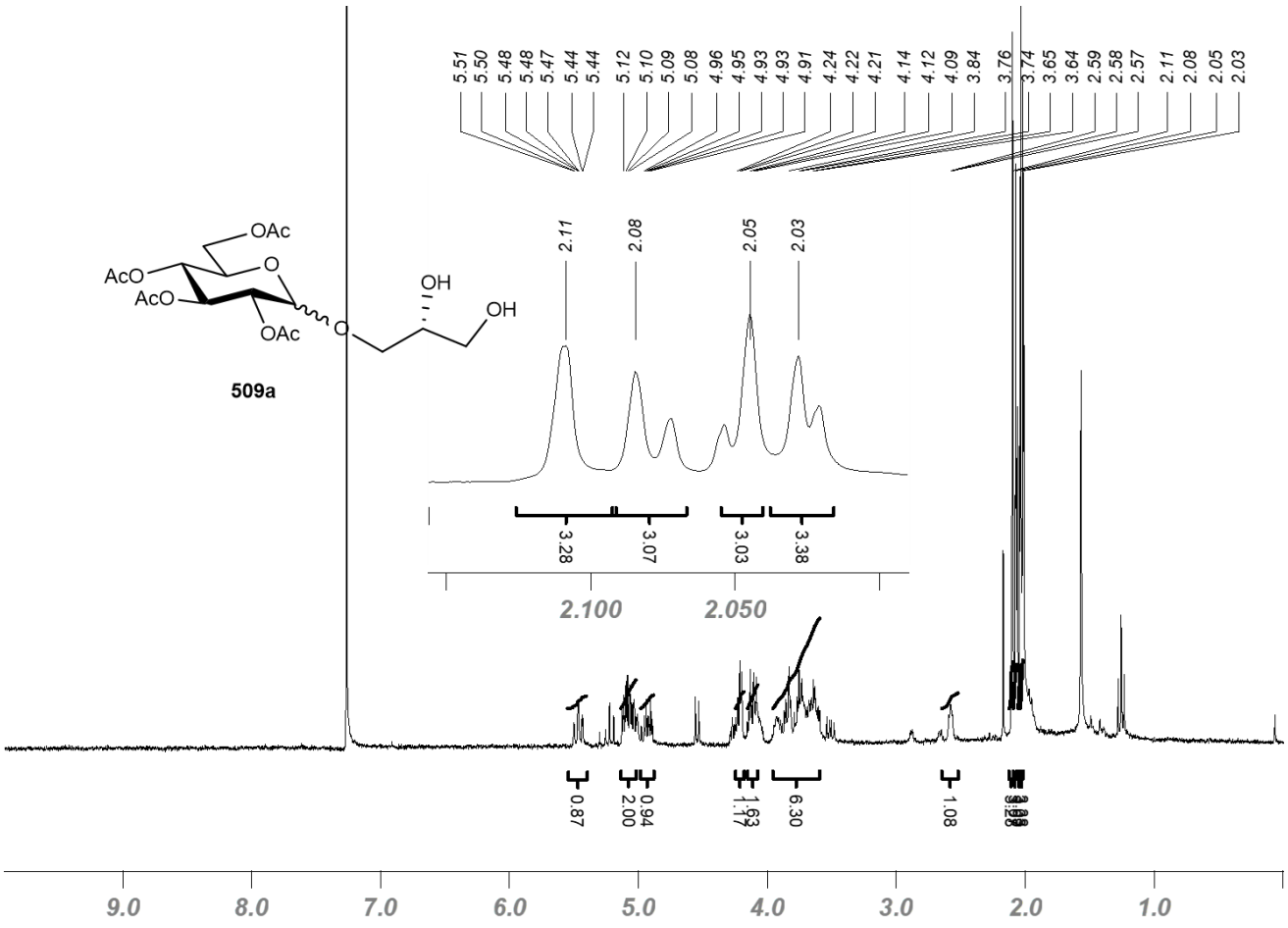

OH
OH
505
4.20 4.18 4.16 4.15 4.13 4.12 4.11 4.10 4.01 3.99 3.98 3.96 3.77 3.75 3.73 2.72 2.70
1.42 1.36
1.42
1.36
5.80
6.27
1.500
1.450
1.400
1.350
1.300
ppm (t1)
3.73
2.03
2.00
1.42
5.80
6.27
10.0
5.0
0.0
ppm (t1)

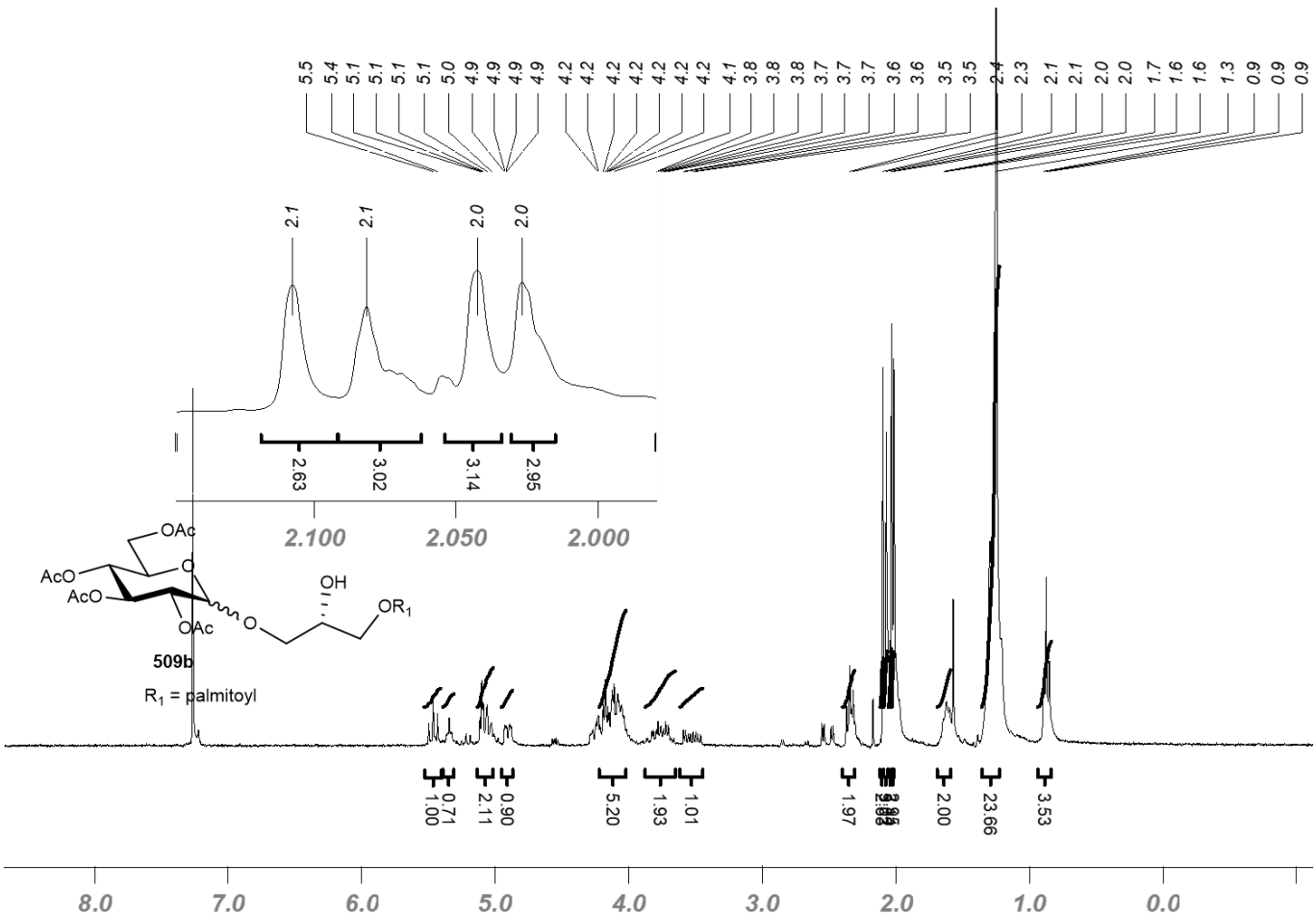

OBn
BnO
BnO
OBn
O
O
O
507
1.60
1.50
1.40
1.30
1.20
1.10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

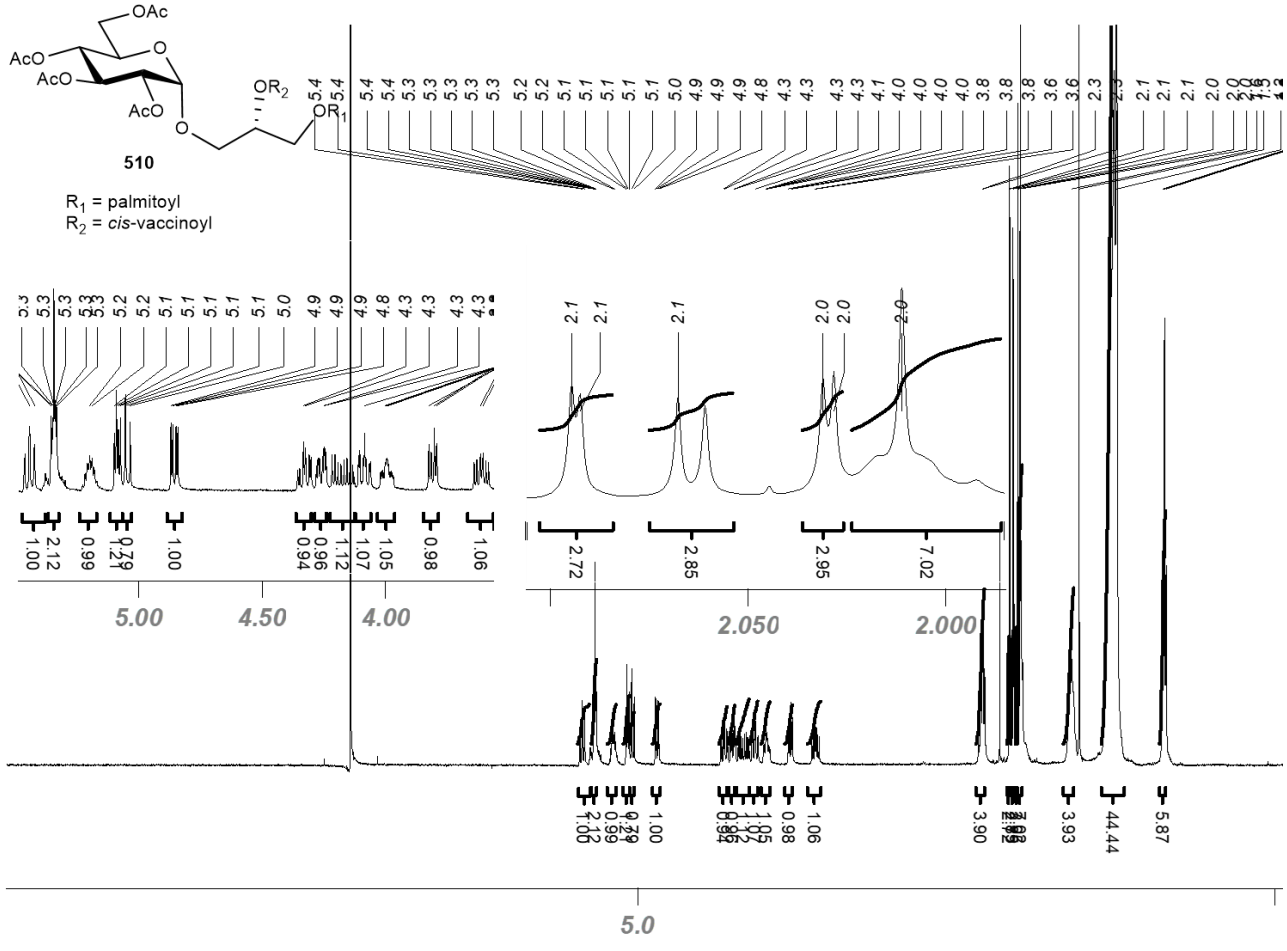

OBn
BnO
BnO
OBn
O
O
508
1.450 1.400 1.350 1.300 1.250 1.200
5.0

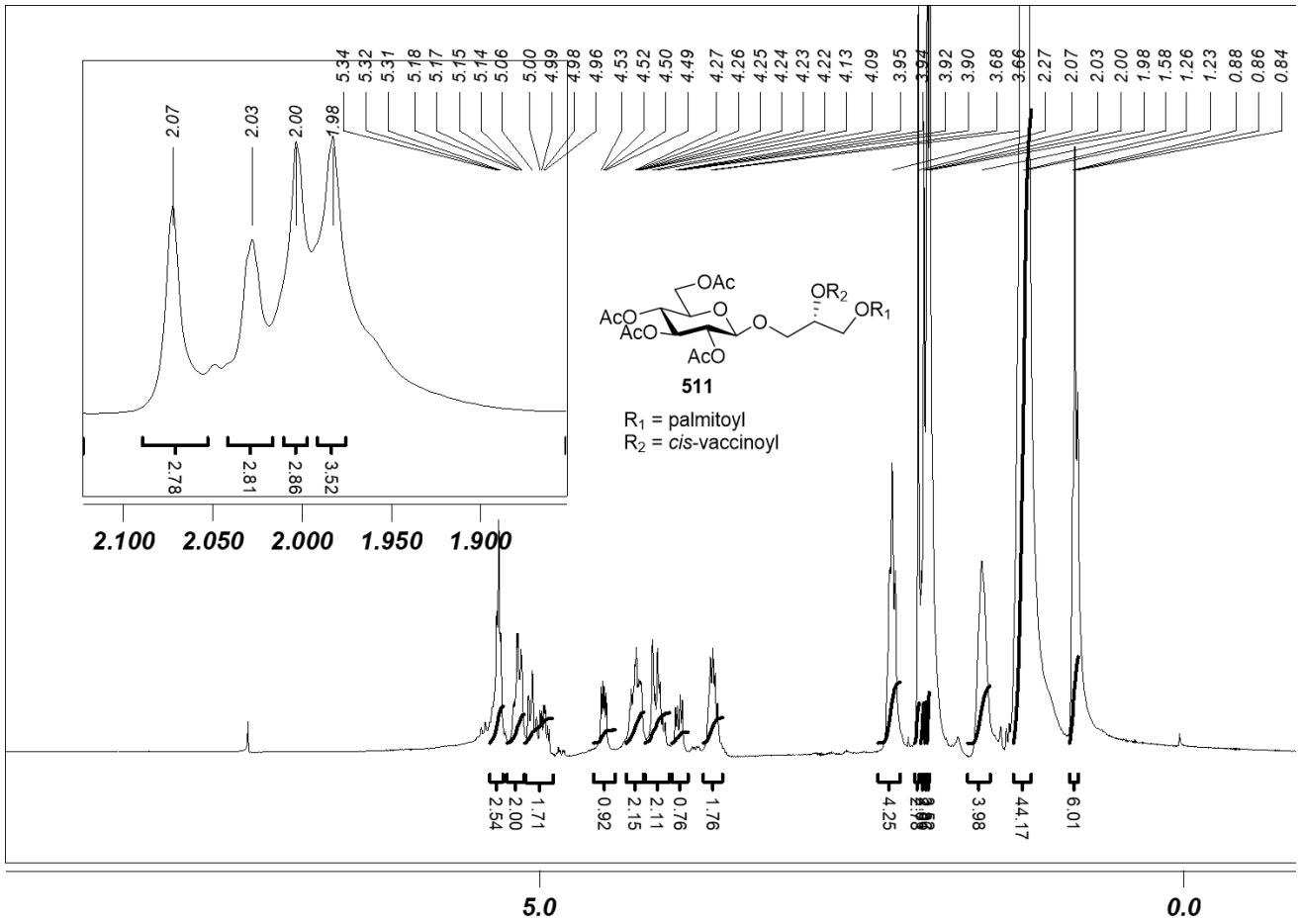

5.08 4.80 4.79 4.78 4.43 4.42 4.34 4.30 4.29 4.27 4.24 4.19 4.19 4.16 4.14 4.13 4.13 4.11 4.06 3.96 3.95 3.95 3.89 3.85 3.81 3.80 3.75 3.72 3.70 3.58 3.51 3.45 3.43 3.13 1.36 1.29
OH
HO
HO
OH
O
O
O
O
508a
0.81 1.00 0.88 6.48 3.76 3.87 3.84
10.0
5.0
0.0
ppm (t1)

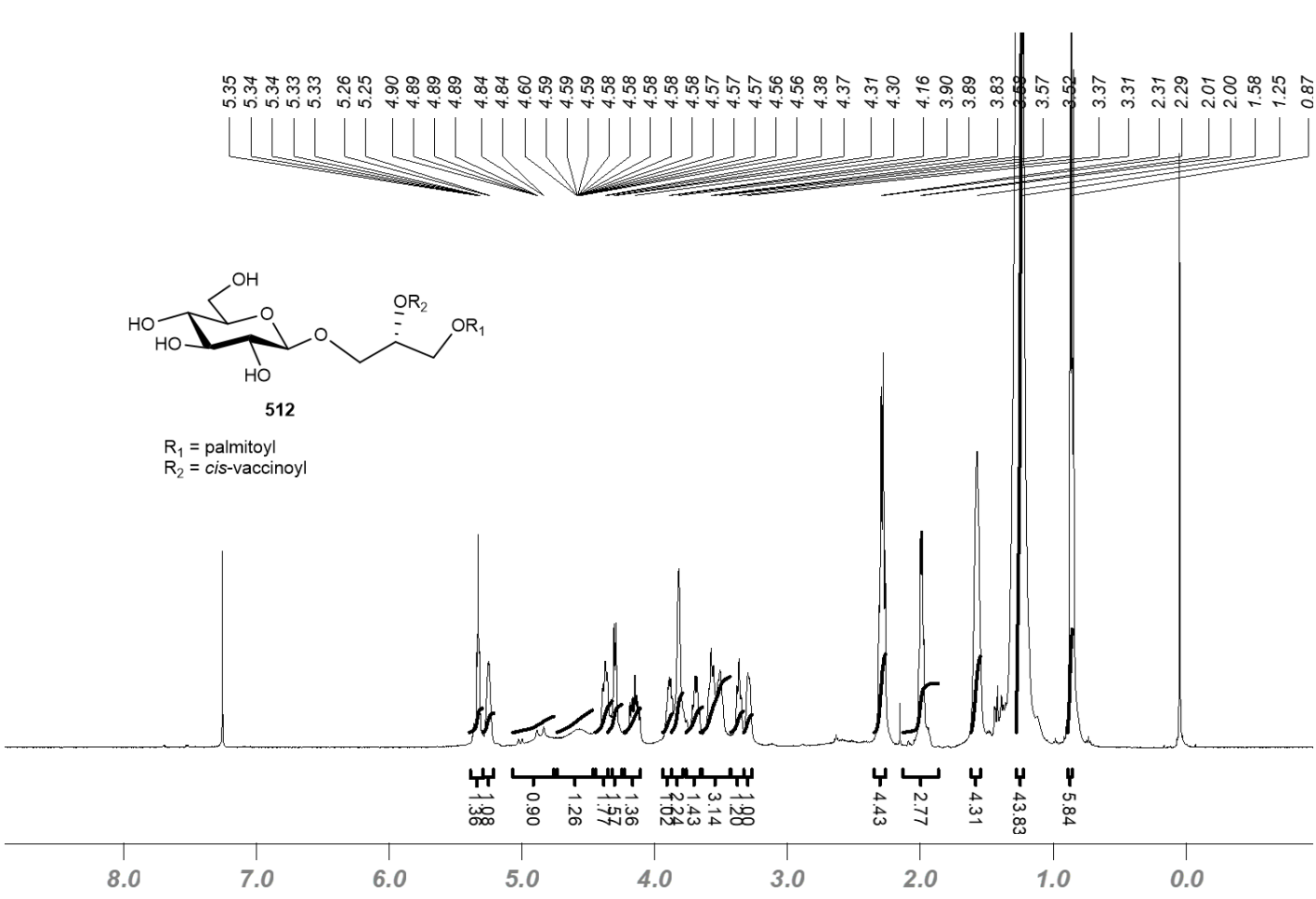

5.5 5.5 5.2 5.2 5.1 5.1 5.1 5.1 4.9 4.9 4.9 4.9 4.9 4.9 4.3 4.3 4.3 4.3 4.1 4.1 4.1 4.1 4.1 3.8 3.7 3.7 3.6 3.6 3.5 3.5 2.1 2.1 2.1 2.0 2.0 2.0 1.4
OAc
AcO
AcO
OAc
O
O
O
O
509
0.70 1.93 0.65 1.98 2.90 1.91 0.71 3.03
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

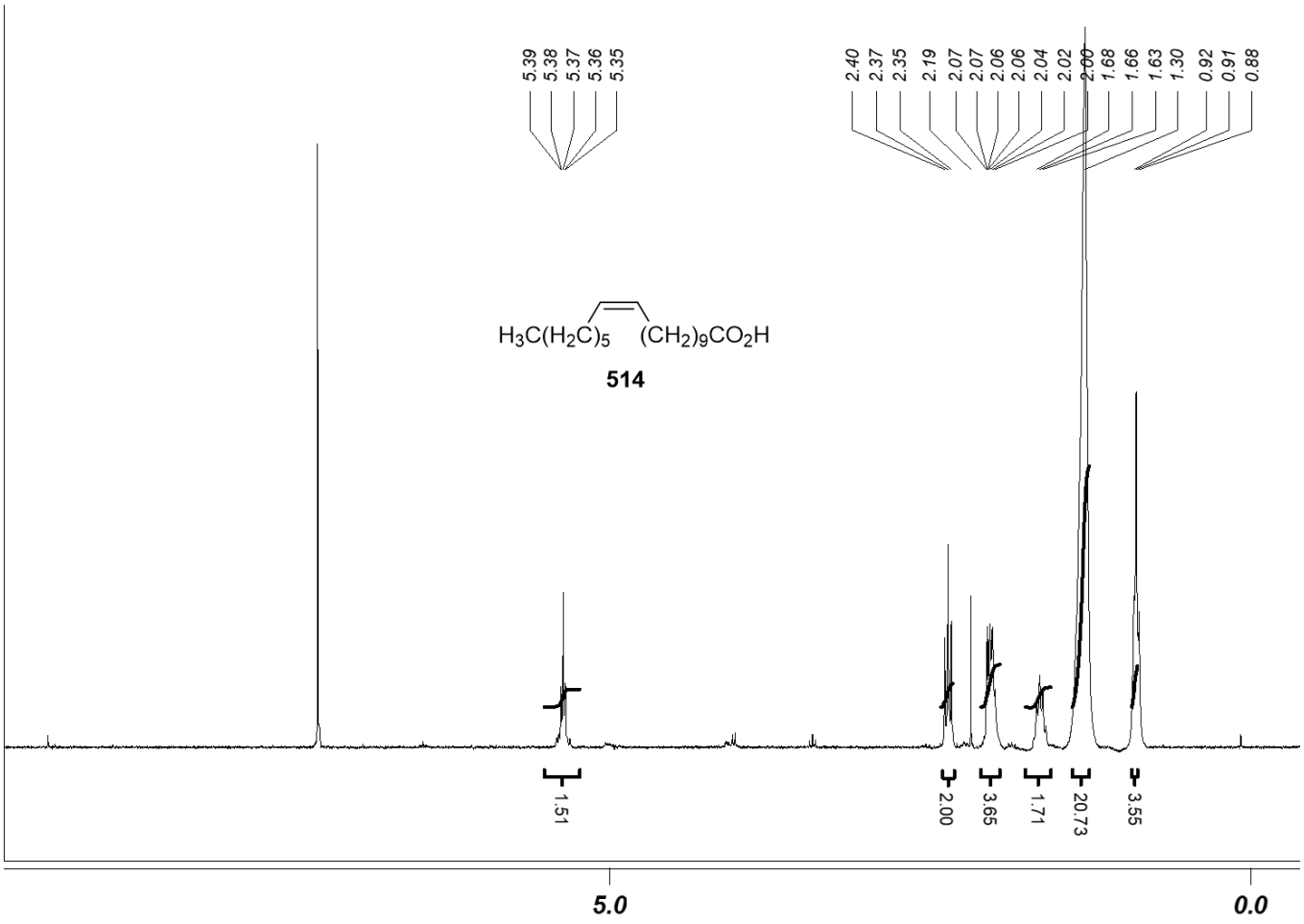

509a

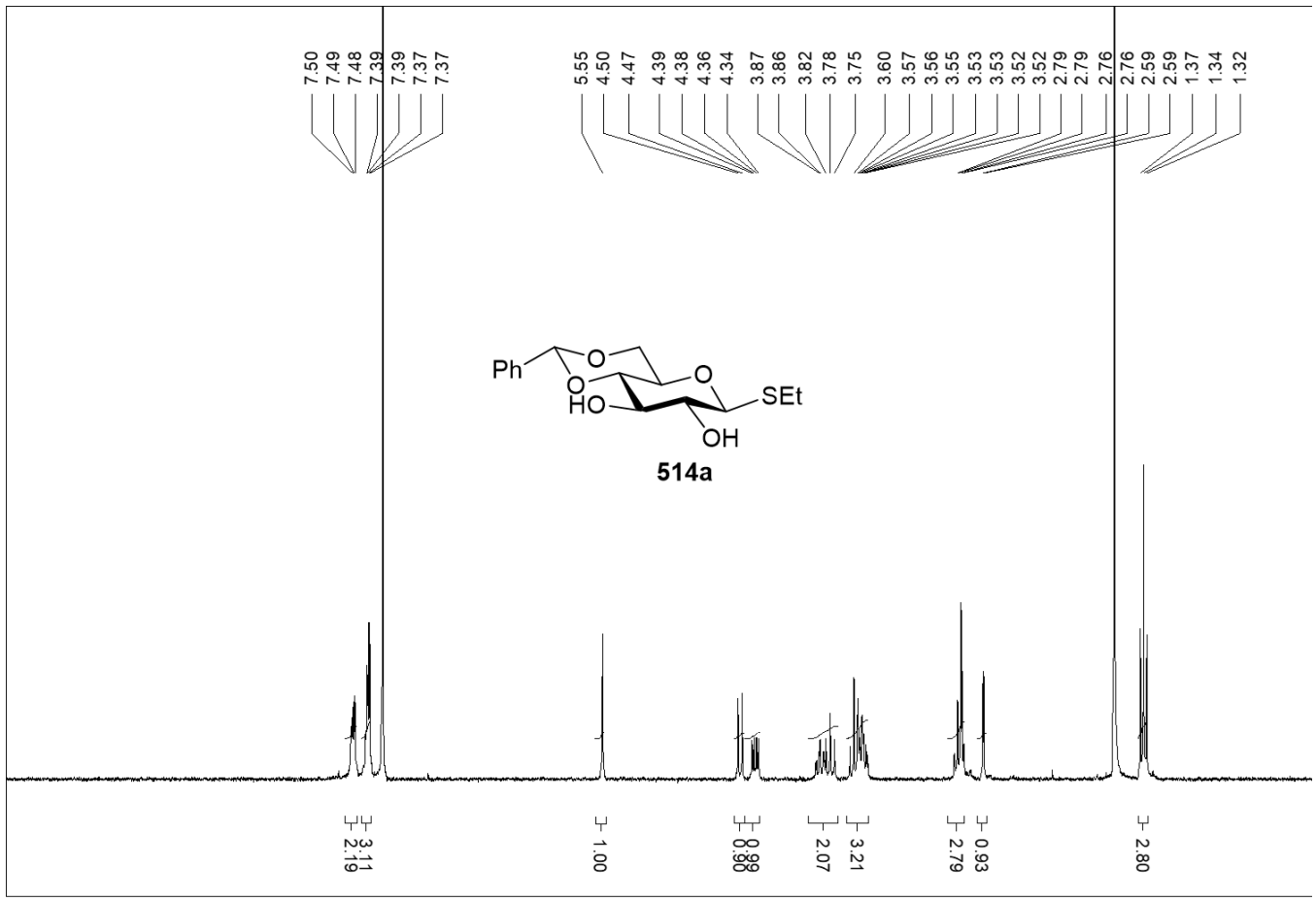

509b
R1 = palmitoyl

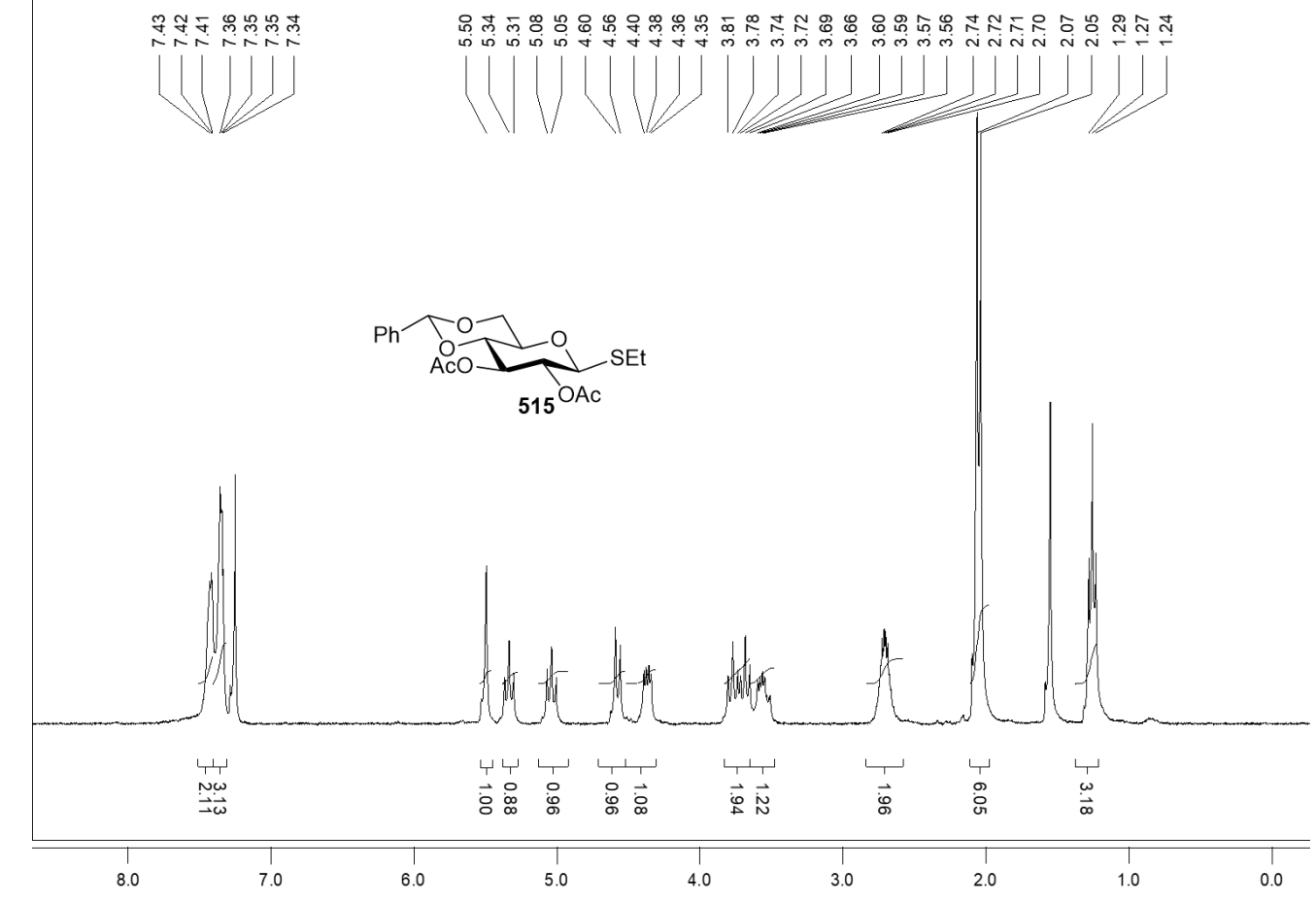

OAc
AcO
AcO
AcO
O
OR2
OR1
510
R1 = palmitoyl
R2 = cis-vaccinoyl
5.00
4.50
4.00
2.050
2.000
5.0

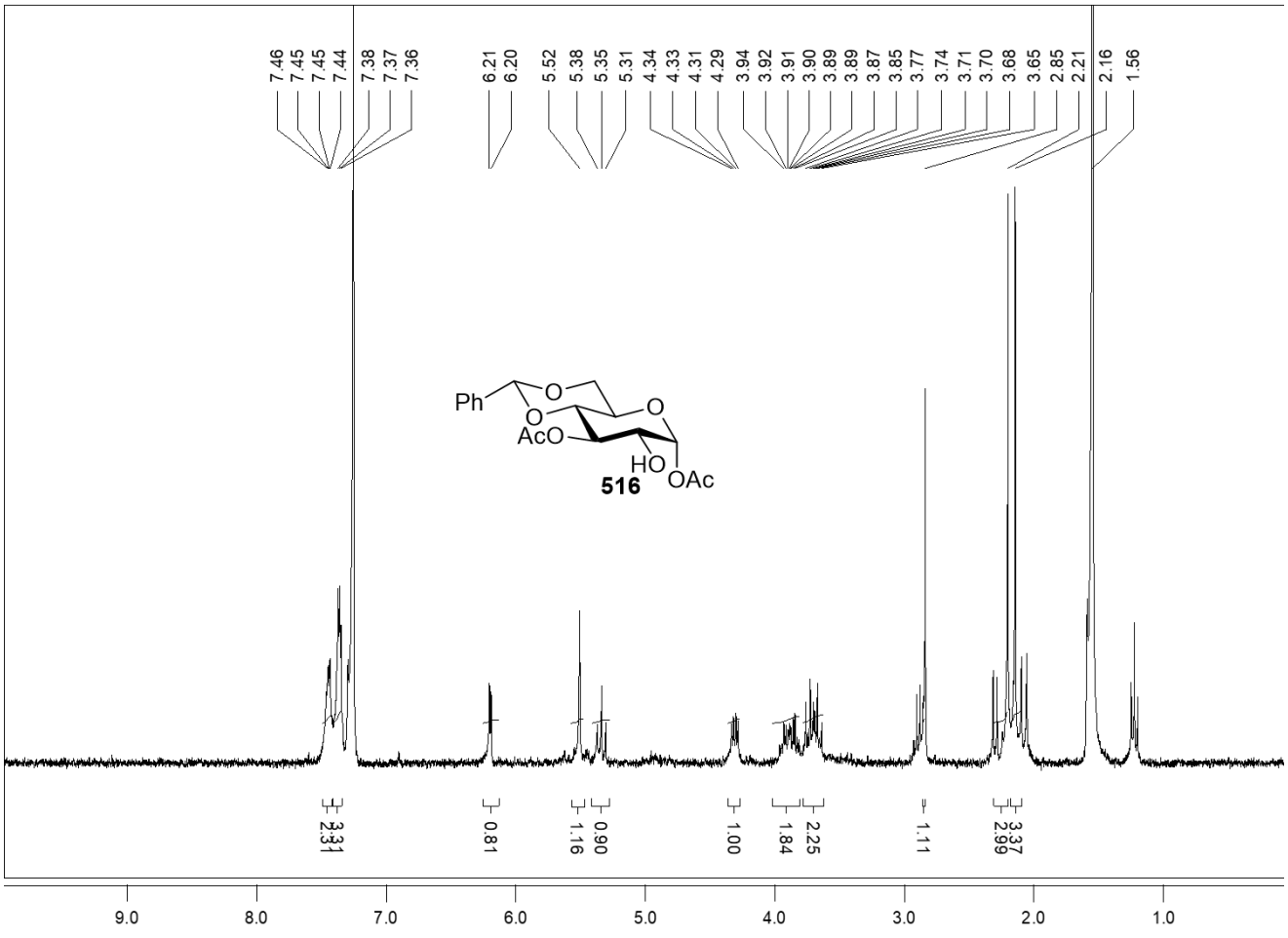

2.07
2.03
2.00
1.98
OAc
AcO
AcO
AcO
O
OR2
OR1
511
R1 = palmitoyl
R2 = cis-vaccinoyl
2.100
2.050
2.000
1.950
1.900
5.0
0.0

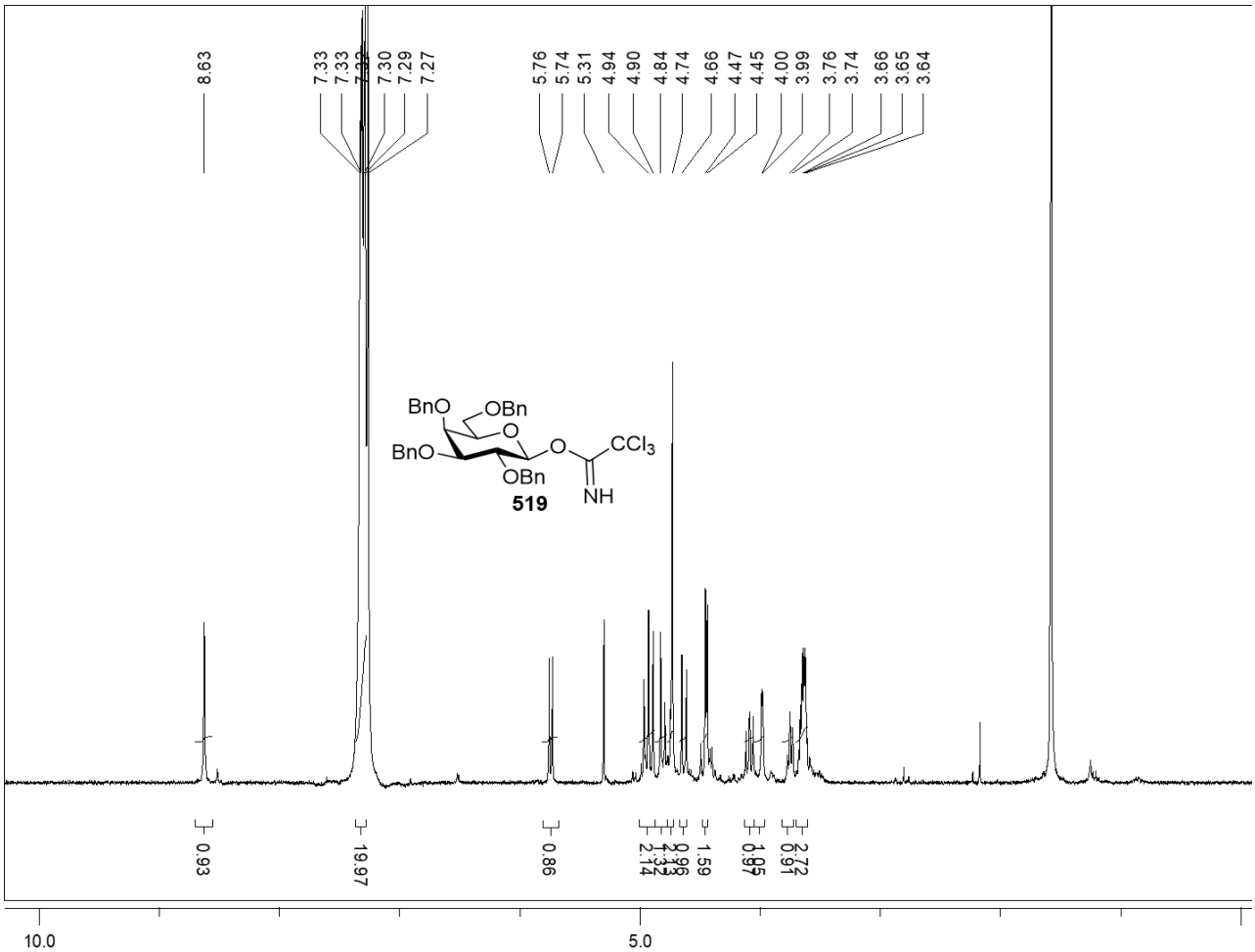

5.35 5.34 5.34 5.33 5.33 5.26 5.25 4.90 4.89 4.89 4.89 4.84 4.84 4.60 4.59 4.59 4.59 4.58 4.58 4.58 4.58 4.58 4.57 4.57 4.57 4.56 4.56 4.38 4.37 4.31 4.30 4.16 3.90 3.89 3.83 3.57 3.52 3.37 3.31 2.31 2.29 2.01 2.00 1.58 1.25 0.87
OH
HO
HO
HO
O
O
OR2
OR1
512
R1 = palmitoyl
R2 = cis-vaccinoyl
1.38 1.08 0.90 1.26 1.77 1.57 1.36 4.02 4.24 1.43 3.14 1.20 1.00 4.43 2.77 4.31 43.83 5.84
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0

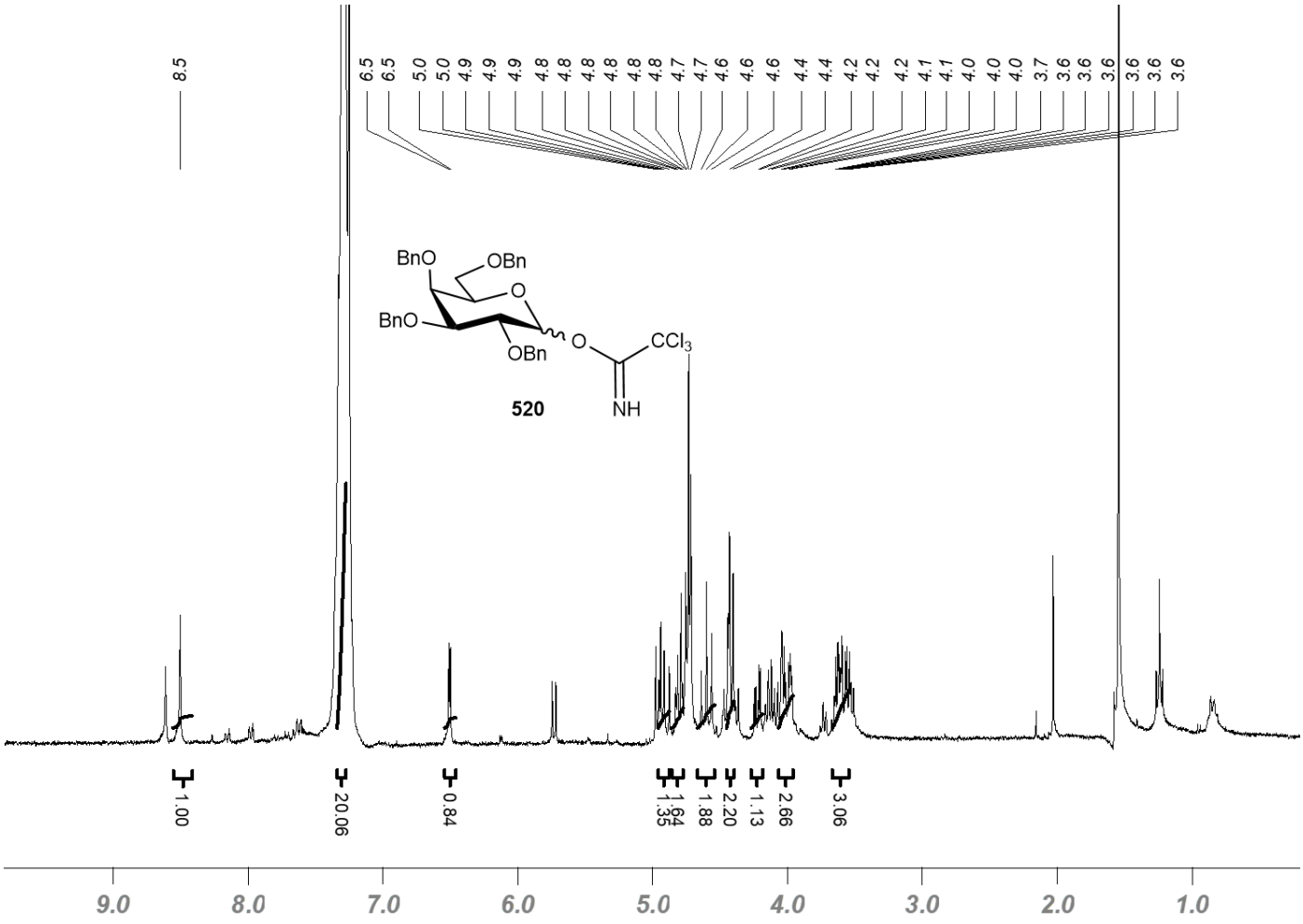

5.39 5.38 5.37 5.36 5.35
2.40 2.37 2.35 2.19 2.07 2.07 2.06 2.06 2.04 2.02 2.00 1.68 1.66 1.63 1.30 0.92 0.91 0.88
H3C(H2C)5 (CH2)9CO2H
514
1.51 2.00 3.65 1.71 20.73 3.55
5.0 0.0

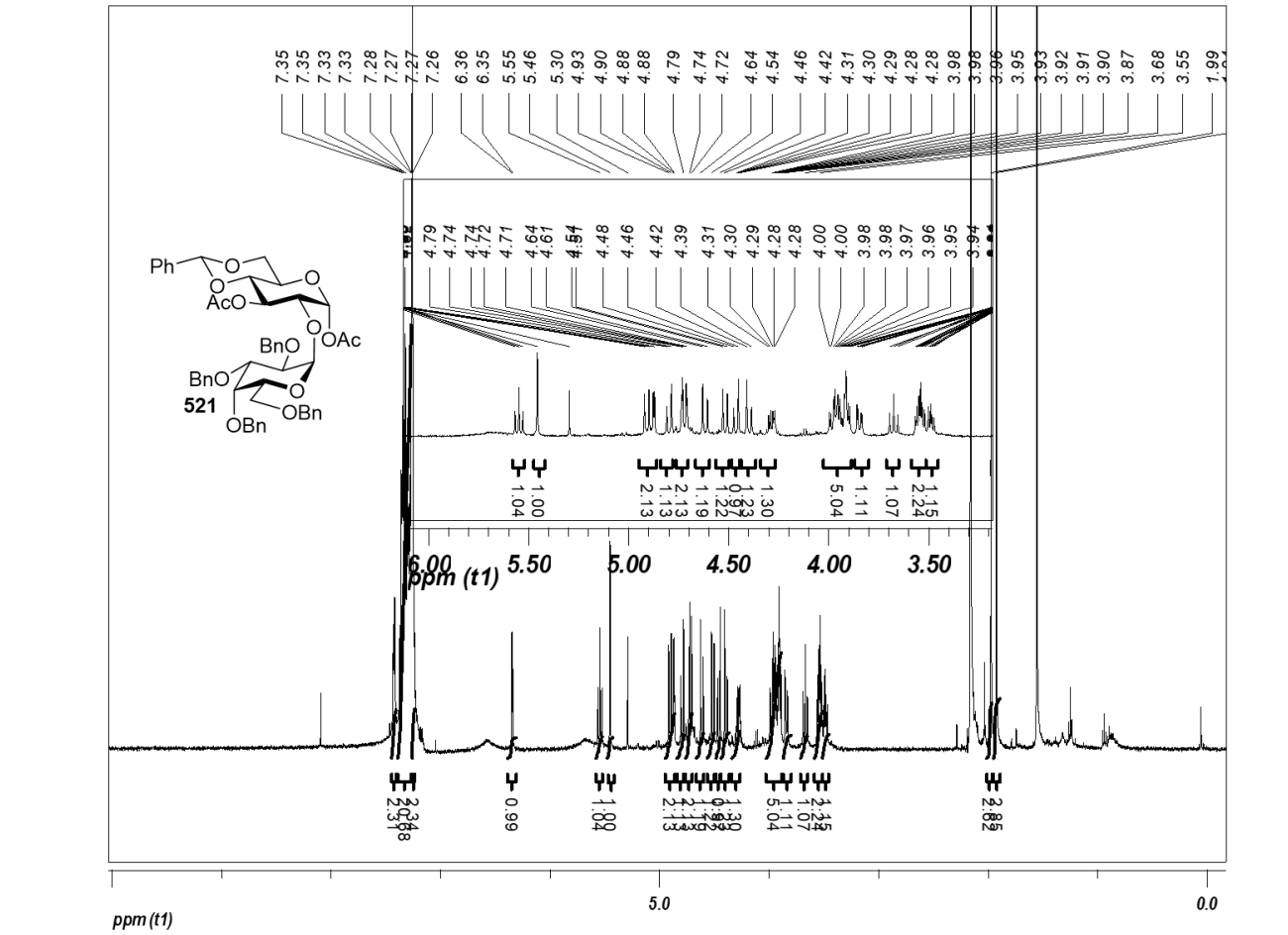

7.50
7.49
7.48
7.39
7.39
7.37
7.37
5.55
4.50
4.47
4.39
4.38
4.36
4.34
3.87
3.86
3.82
3.78
3.75
3.60
3.57
3.56
3.55
3.53
3.53
3.52
3.52
2.79
2.79
2.76
2.76
2.59
2.59
1.37
1.34
1.32
Ph
O
O
HO
O
SEt
OH
514a
3.11
2.19
1.00
0.90
0.90
2.07
3.21
2.79
0.93
2.80
10.0
5.0

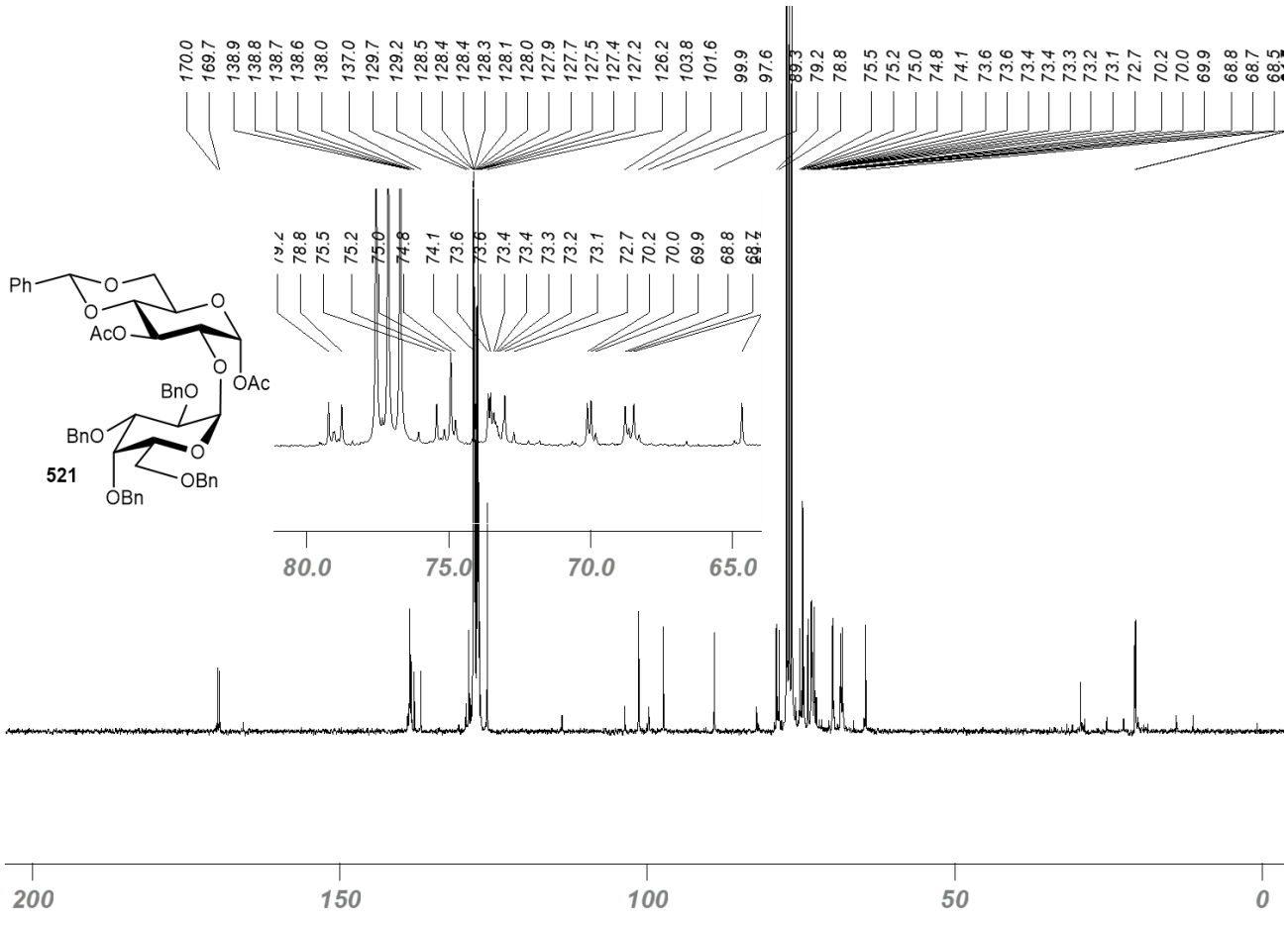

7.43
7.42
7.41
7.36
7.35
7.35
7.34
5.50
5.34
5.31
5.08
5.05
4.60
4.56
4.40
4.38
4.36
4.35
3.81
3.78
3.74
3.72
3.69
3.66
3.60
3.59
3.57
3.56
2.74
2.72
2.71
2.70
2.07
2.05
1.29
1.27
1.24
Ph
O
O
AcO
O
SEt
OAc
515
2.11
3.13
1.00
0.88
0.96
0.96
1.08
1.64
1.22
1.96
6.05
3.18
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

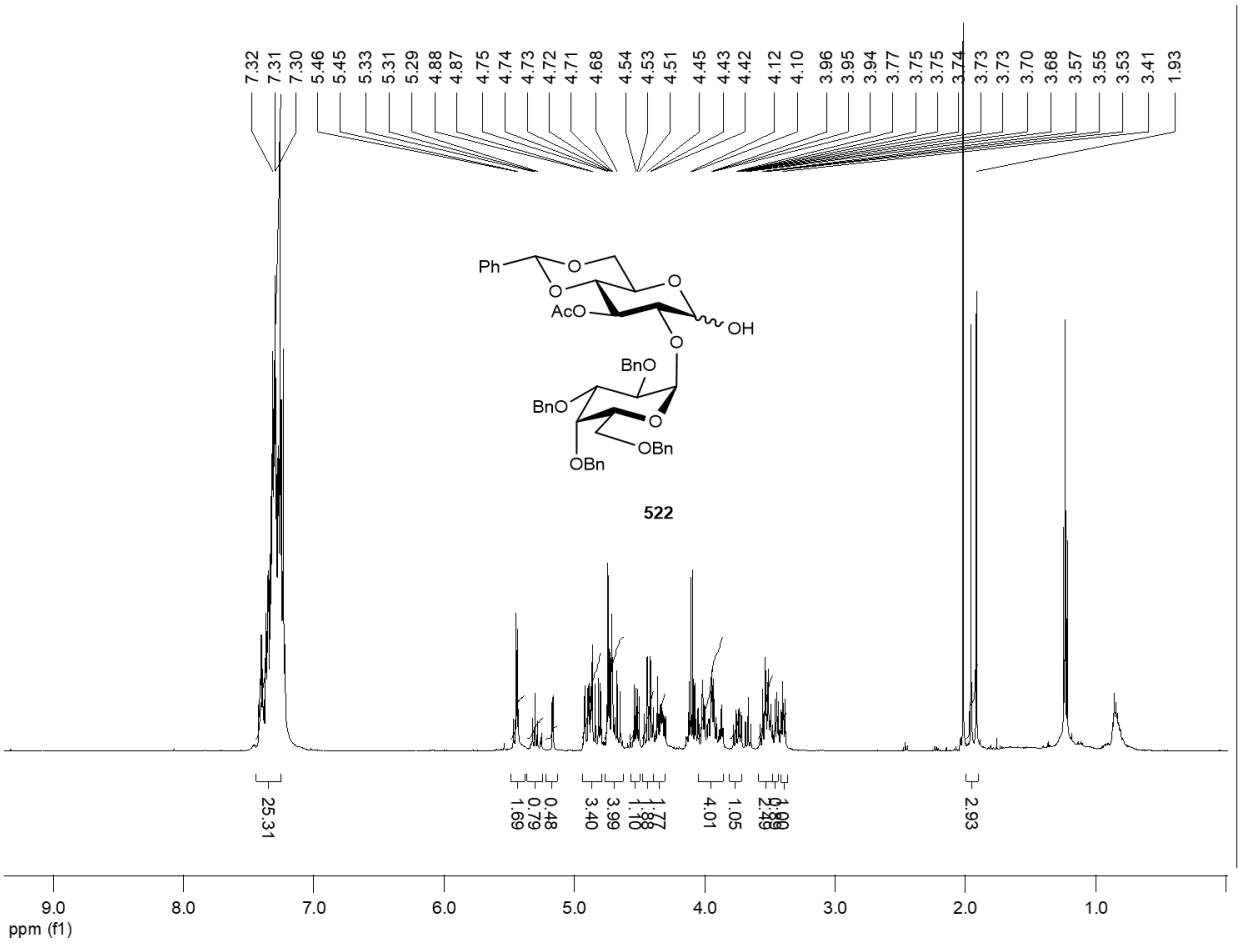

7.46
7.45
7.45
7.44
7.38
7.37
7.36
6.21
6.20
5.52
5.38
5.35
5.31
4.34
4.33
4.31
4.29
3.94
3.92
3.91
3.90
3.89
3.89
3.87
3.85
3.77
3.74
3.71
3.70
3.68
3.65
2.85
2.21
2.16
1.56
Ph
O
O
AcO
O
HO
OAc
516
3.31
0.81
1.16
0.90
1.00
1.84
2.25
1.11
2.99
3.37
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

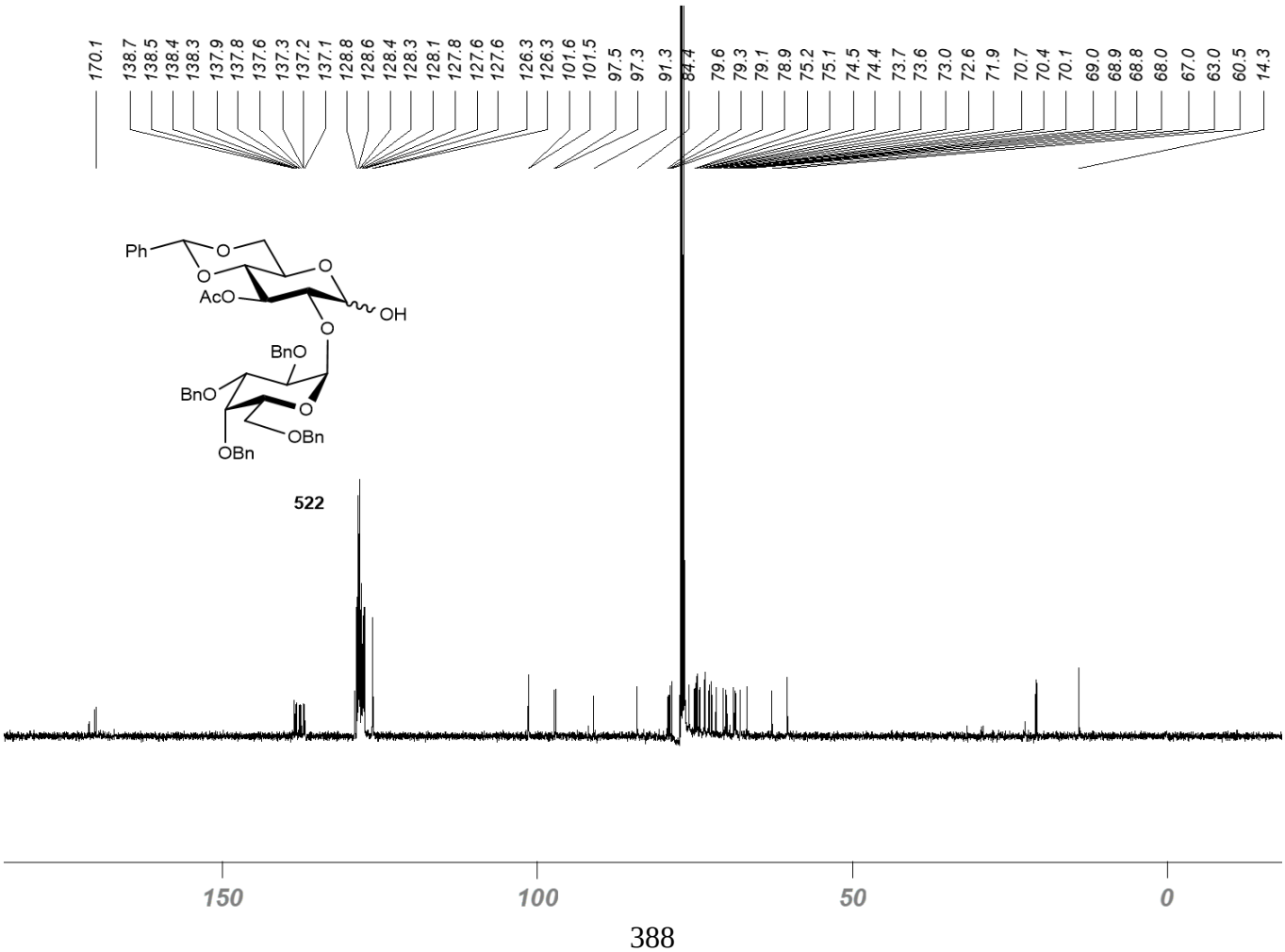

8.63
7.33
7.33
7.30
7.29
7.27
5.76
5.74
5.31
4.94
4.90
4.84
4.74
4.66
4.47
4.45
4.00
3.99
3.76
3.74
3.66
3.65
3.64
BnO
OBn
O
BnO
O
CCl3
OBn
NH
519
0.93
10.97
0.86
2.14
1.59
1.05
0.91
10.0
5.0

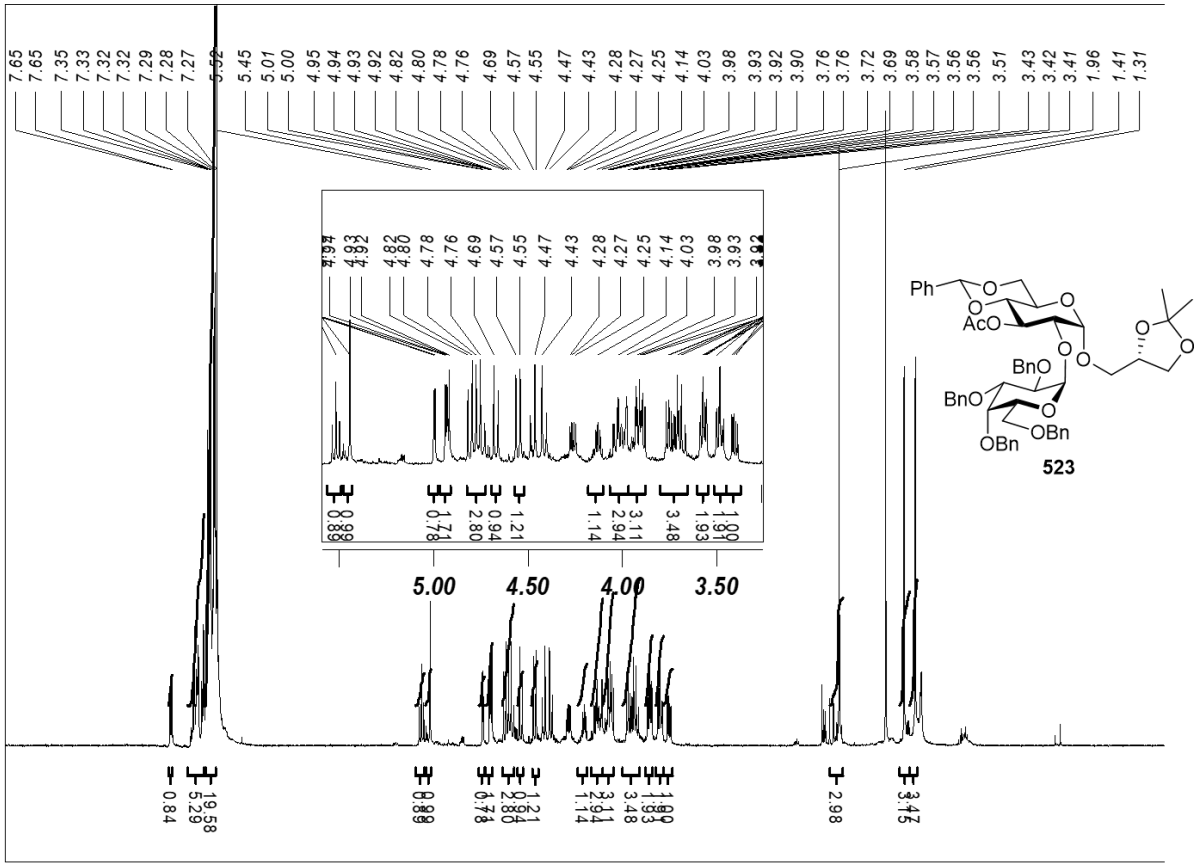

8.5
6.5 6.5 5.0 5.0 4.9 4.9 4.9 4.8 4.8 4.8 4.8 4.8 4.7 4.7 4.6 4.6 4.6 4.4 4.4 4.2 4.2 4.2 4.1 4.1 4.0 4.0 4.0 3.7 3.6 3.6 3.6 3.6 3.6
BnO
OBn
O
BnO
OBn
O
CCl3
NH
520
1.00
20.06
0.84
1.35
1.64
1.88
2.20
1.13
2.66
3.06
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

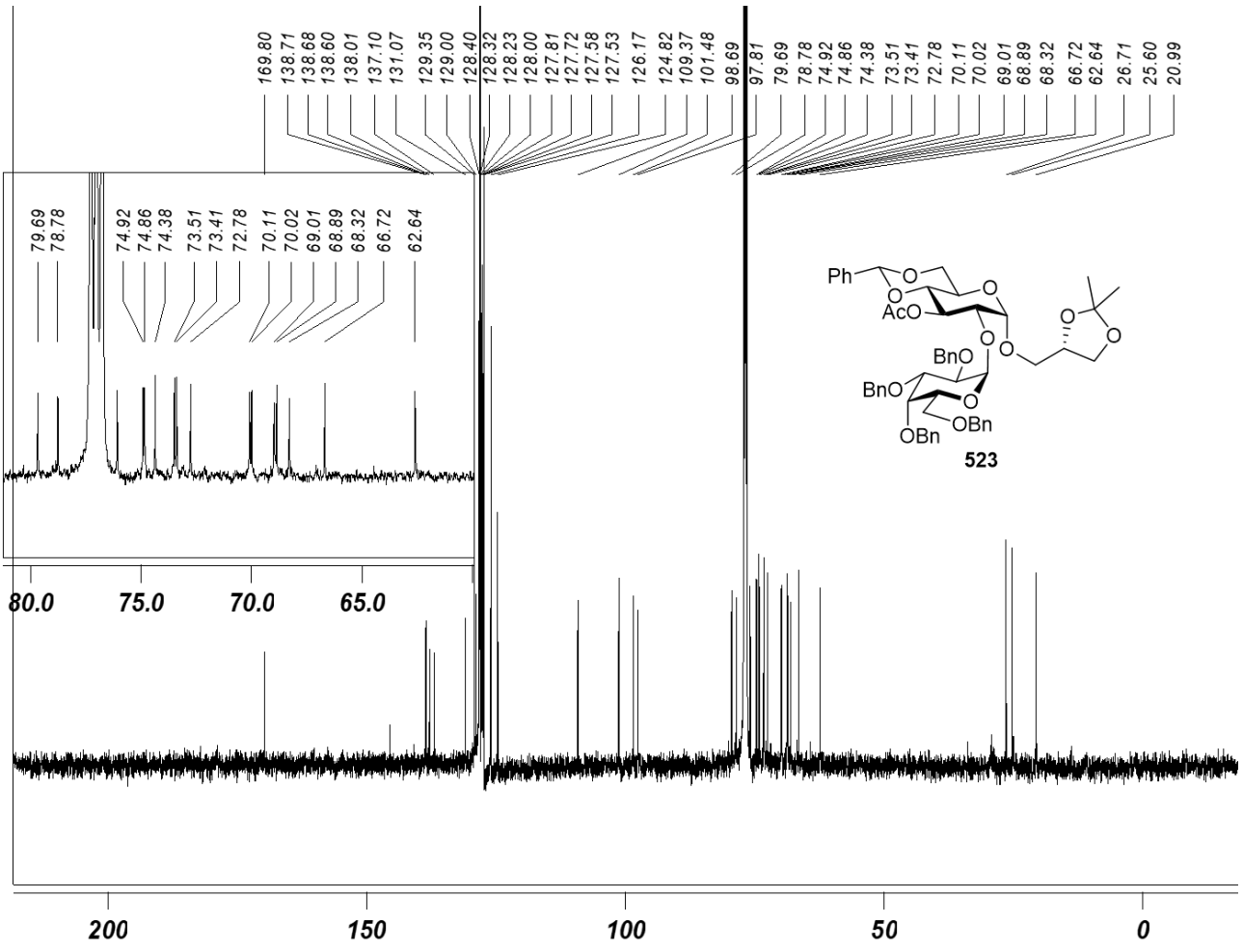

Ph
AcO
BnO
OAc
BnO
521
OBn
OBn
ppm (t1)
6.00
5.50
5.00
4.50
4.00
3.50
5.0
0.0

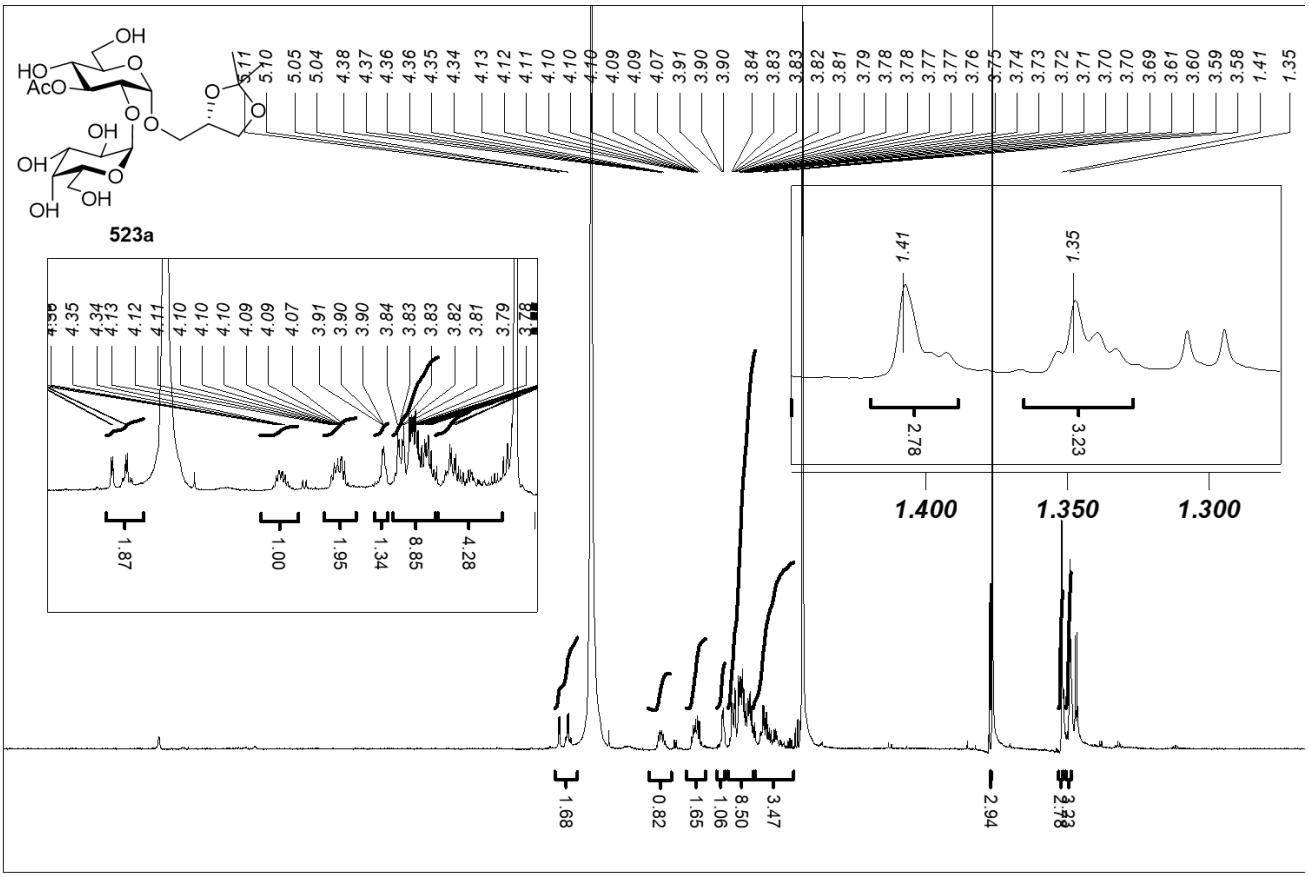

Ph
AcO
BnO
OAc
BnO
521
OBn
OBn
80.0
75.0
70.0
65.0
200
150
100
50
0

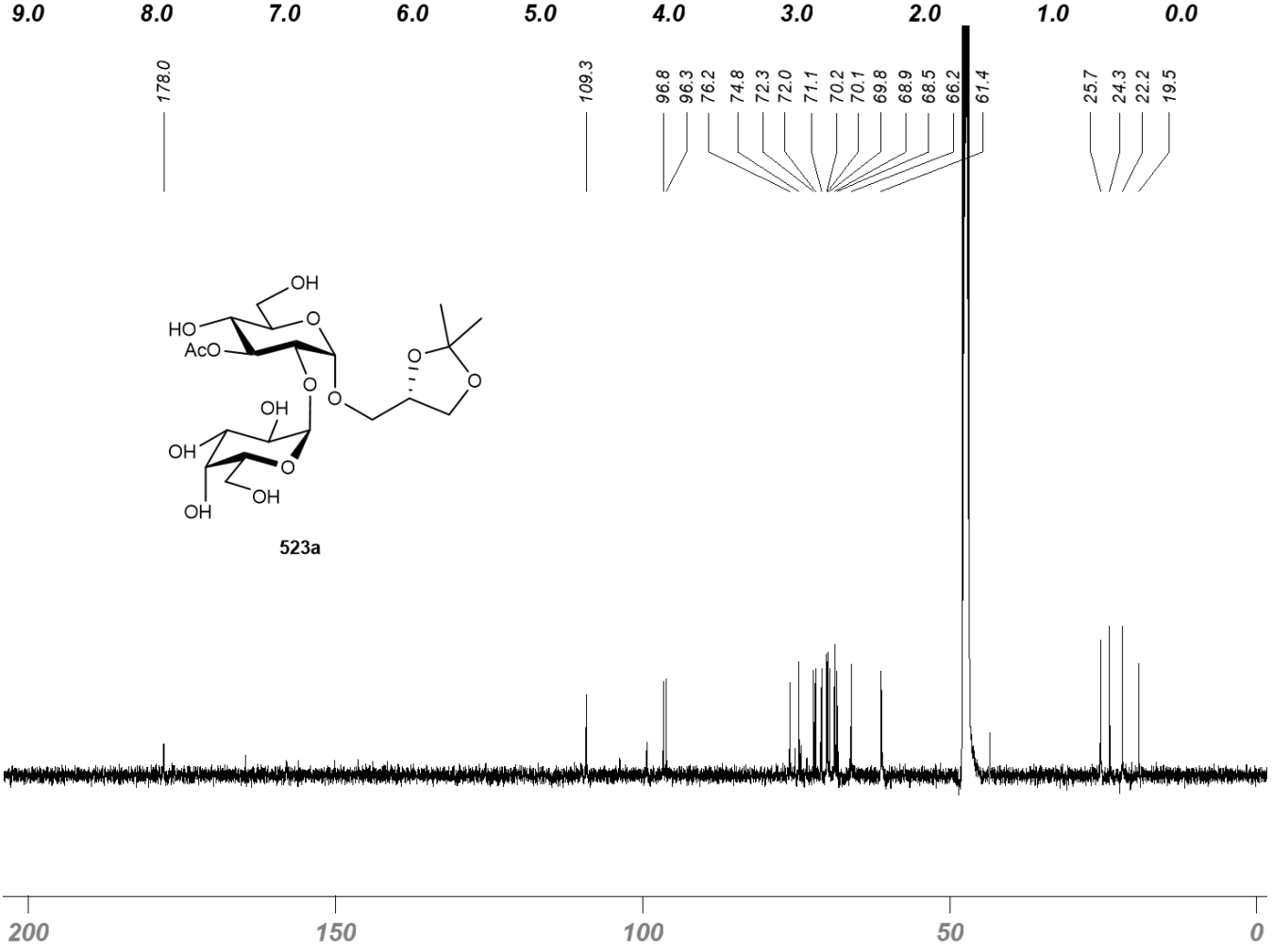

7.32
7.31
7.30
5.46
5.45
5.33
5.31
5.29
4.88
4.87
4.75
4.74
4.73
4.72
4.71
4.68
4.54
4.53
4.51
4.45
4.43
4.42
4.12
4.10
3.96
3.95
3.94
3.77
3.75
3.75
3.74
3.73
3.73
3.70
3.68
3.57
3.55
3.53
3.41
1.93
Ph
AcO
OH
BnO
BnO
OBn
OBn
522
25.31
1.69
0.79
0.48
3.40
3.66
1.10
1.88
1.77
4.01
1.05
2.93
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

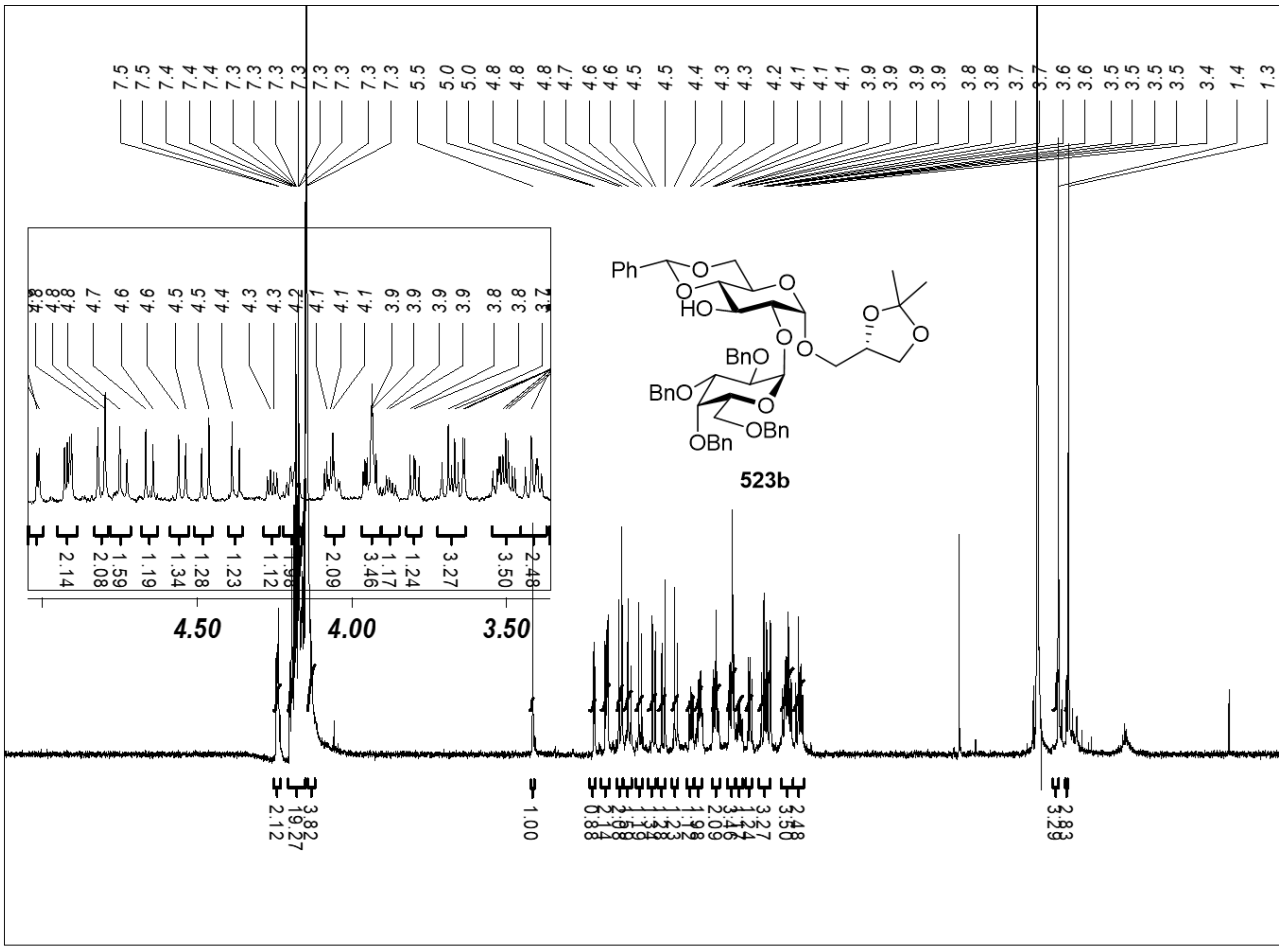

170.1
138.7
138.5
138.4
138.3
137.9
137.8
137.6
137.3
137.2
137.1
128.8
128.6
128.4
128.3
128.1
127.8
127.6
127.6
126.3
126.3
101.6
101.5
97.5
97.3
91.3
84.4
79.6
79.3
79.1
78.9
75.2
75.1
74.5
74.4
73.7
73.6
73.0
72.6
71.9
70.7
70.4
70.1
69.0
68.9
68.8
68.0
67.0
63.0
60.5
14.3
Ph
AcO
OH
BnO
BnO
OBn
OBn
522
150
100
50
0

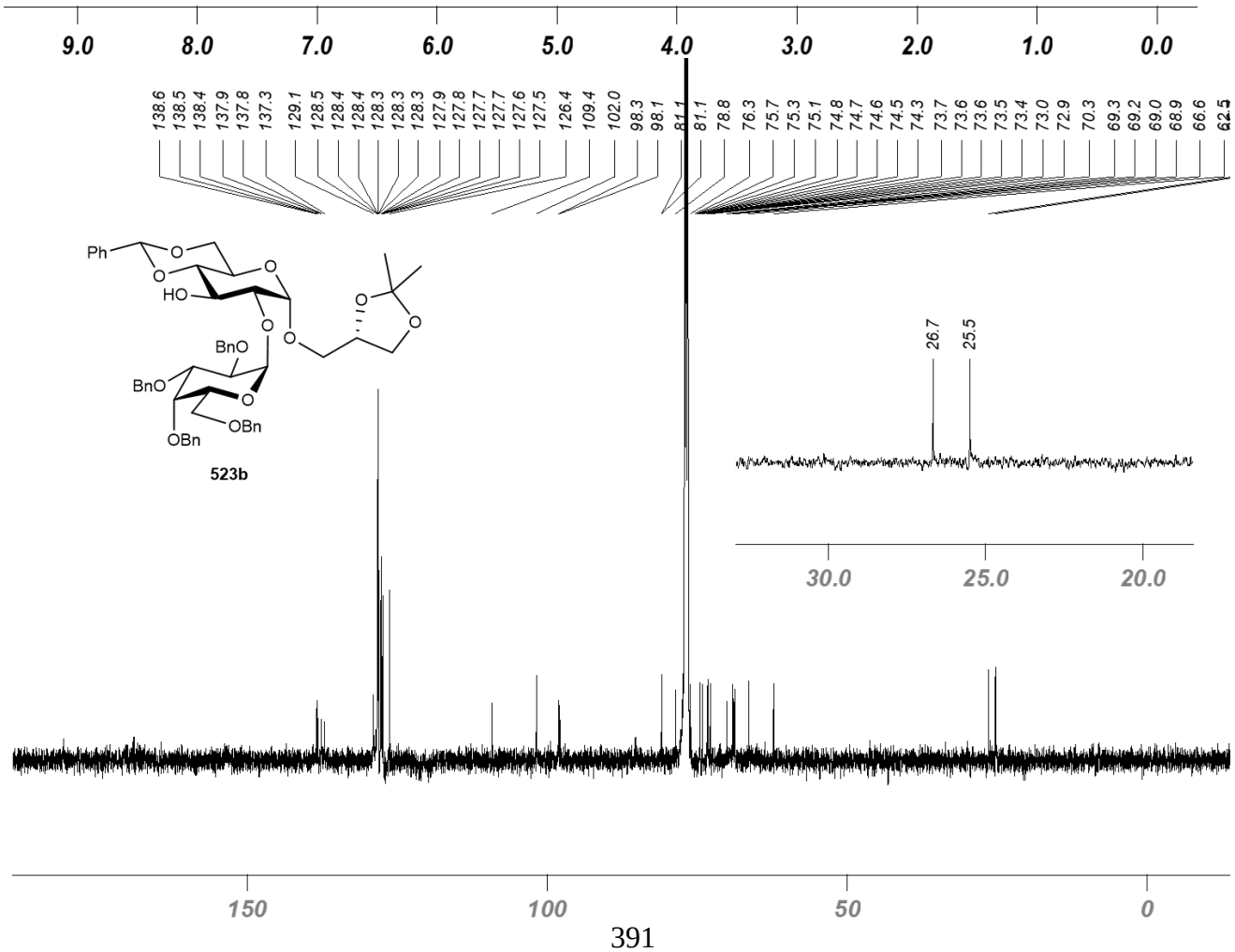

Ph
AcO
BnO
BnO
OBn
OBn
523

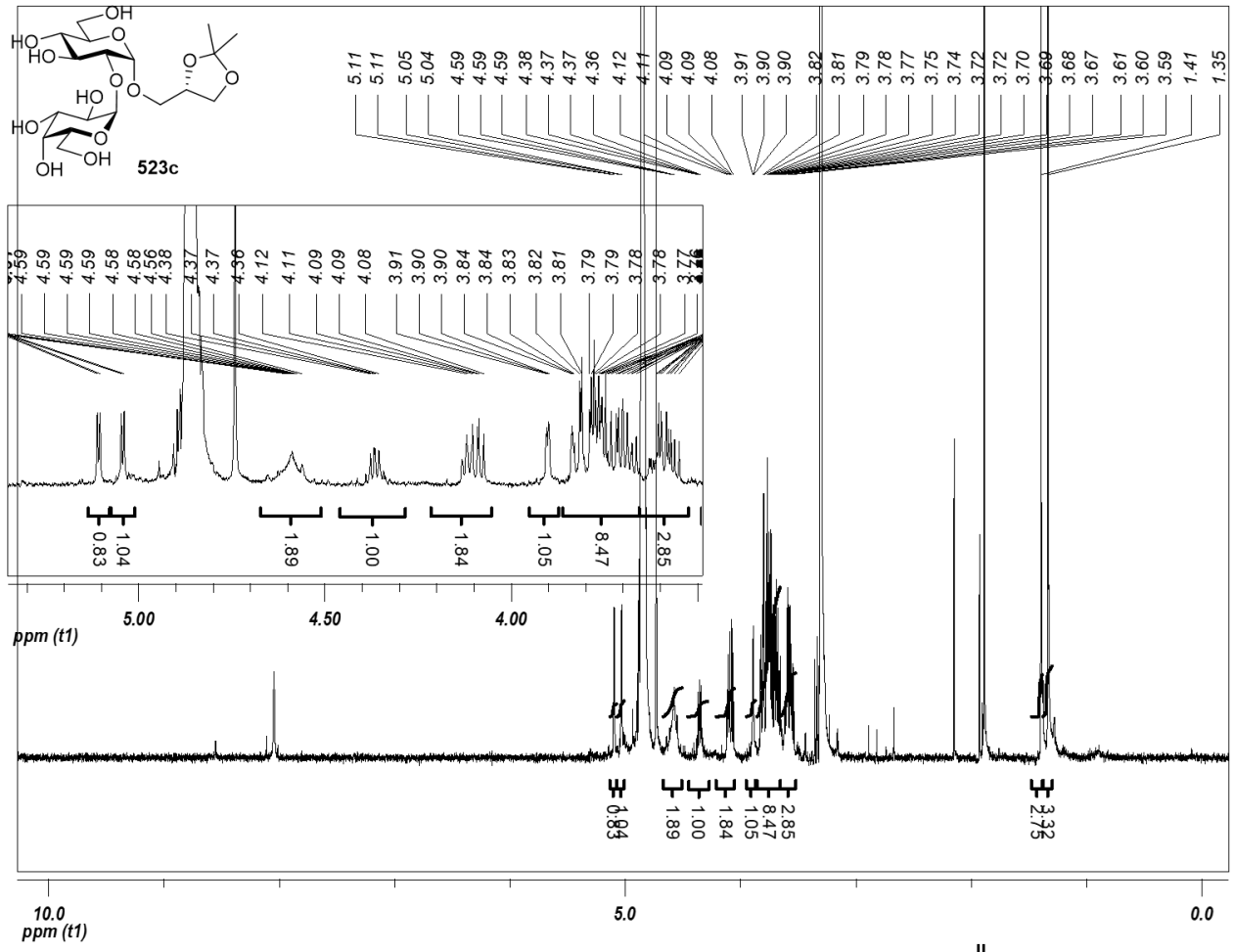

Ph
AcO
BnO
BnO
OBn
OBn
523

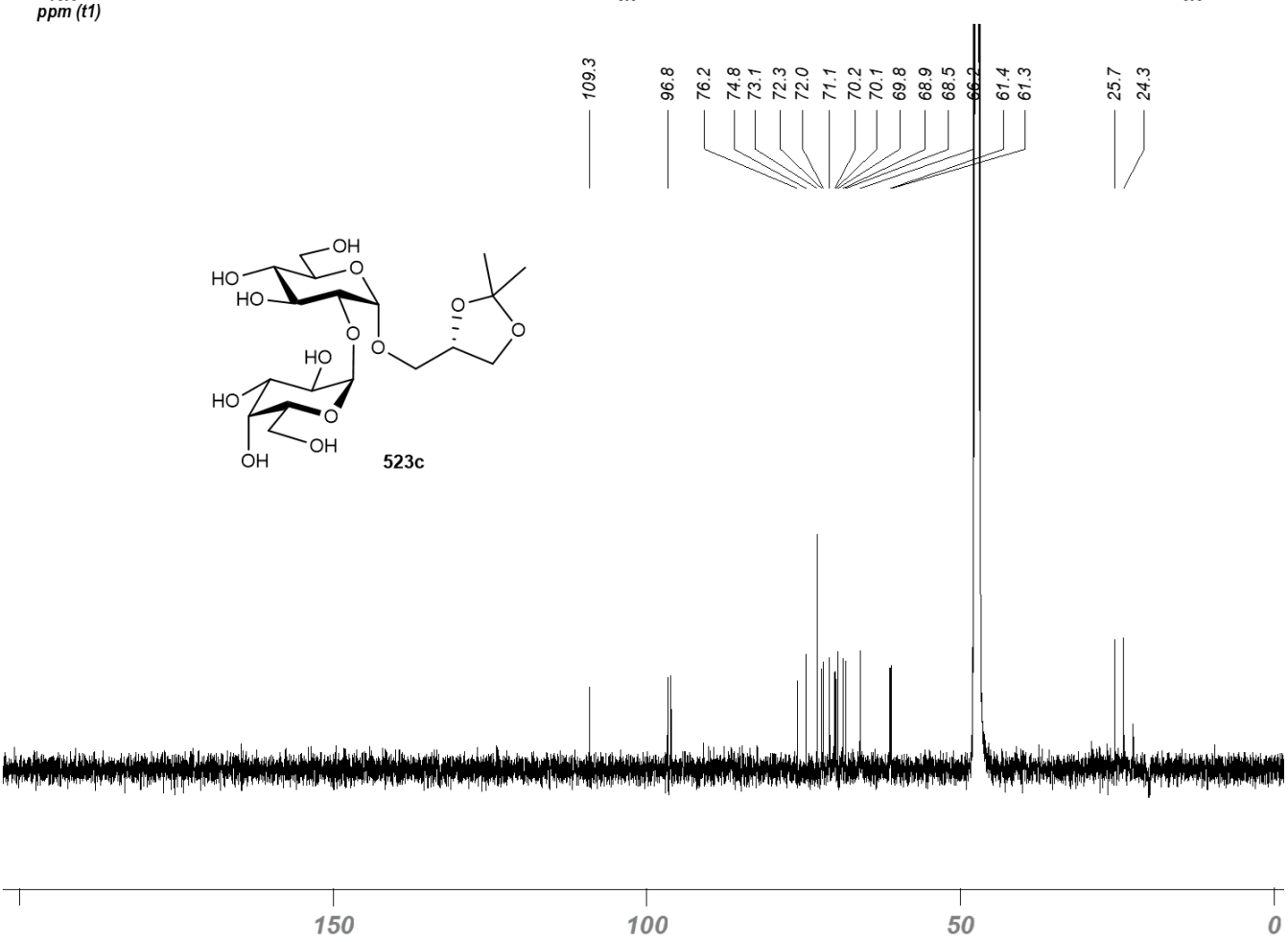

523a
1.87
1.00
1.95
1.34
8.85
4.28
2.78
3.23
1.400
1.350
1.300
1.68
0.82
1.65
1.06
8.50
3.47
2.94
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

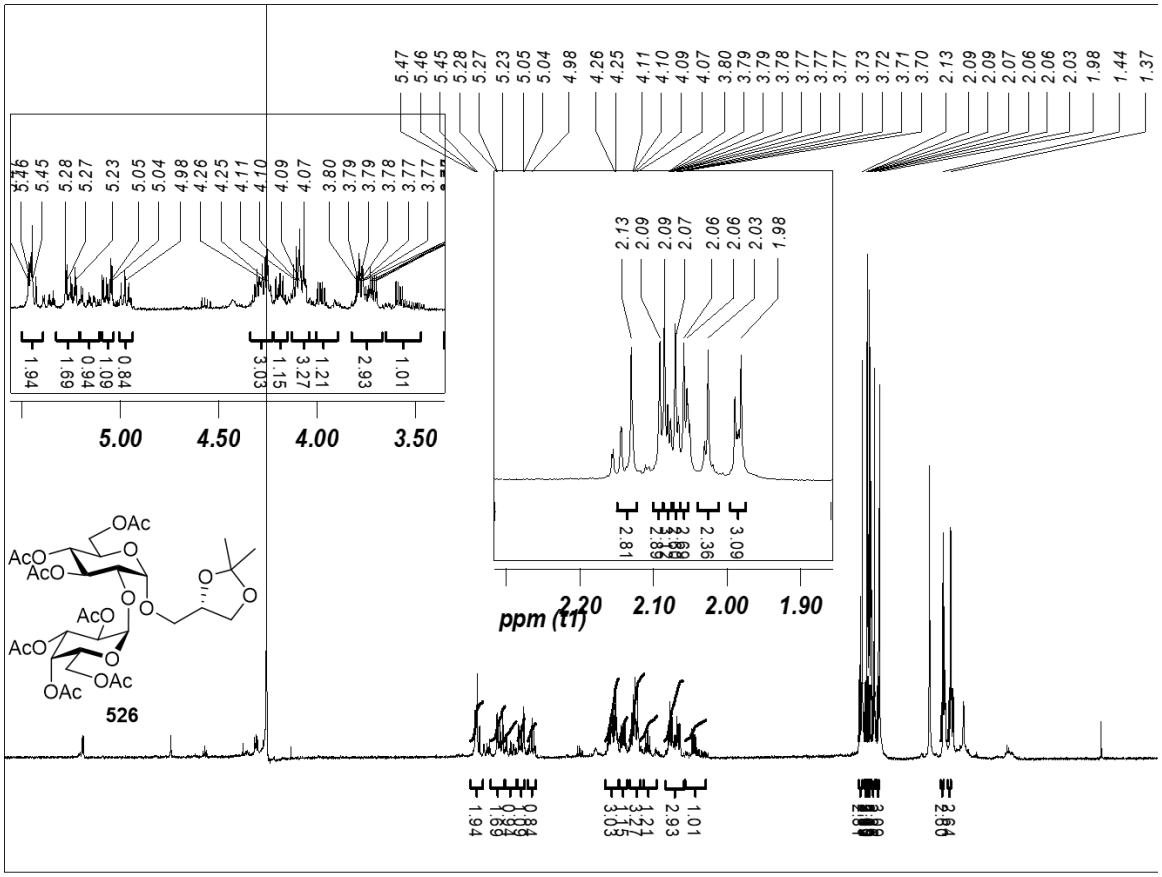

178.0
109.3
96.8
96.3
76.2
74.8
72.3
72.0
71.1
70.2
70.1
69.8
68.9
68.5
66.2
61.4
25.7
24.3
22.2
19.5
523a
200
150
100
50
0

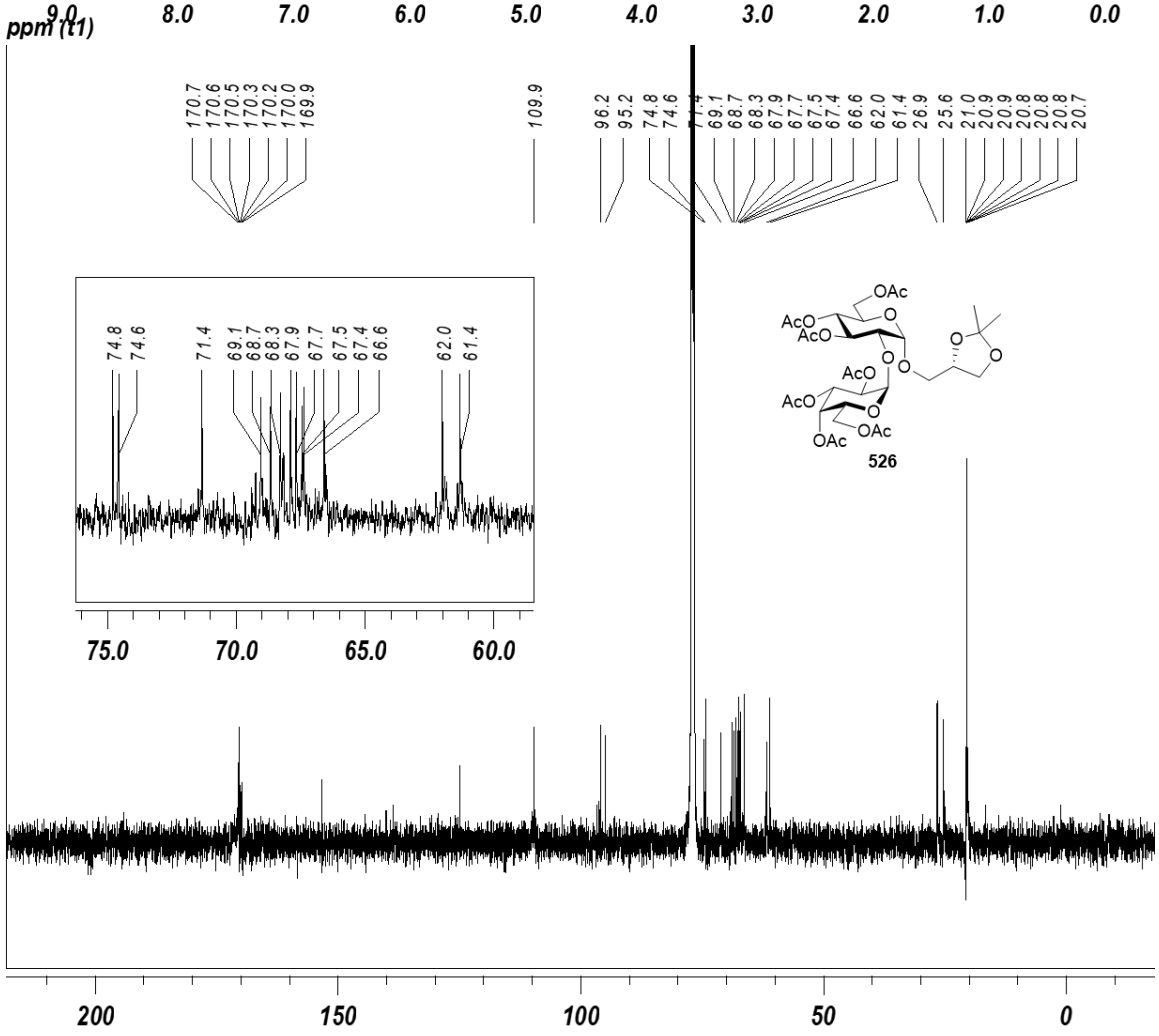

523b
4.50
4.00
3.50

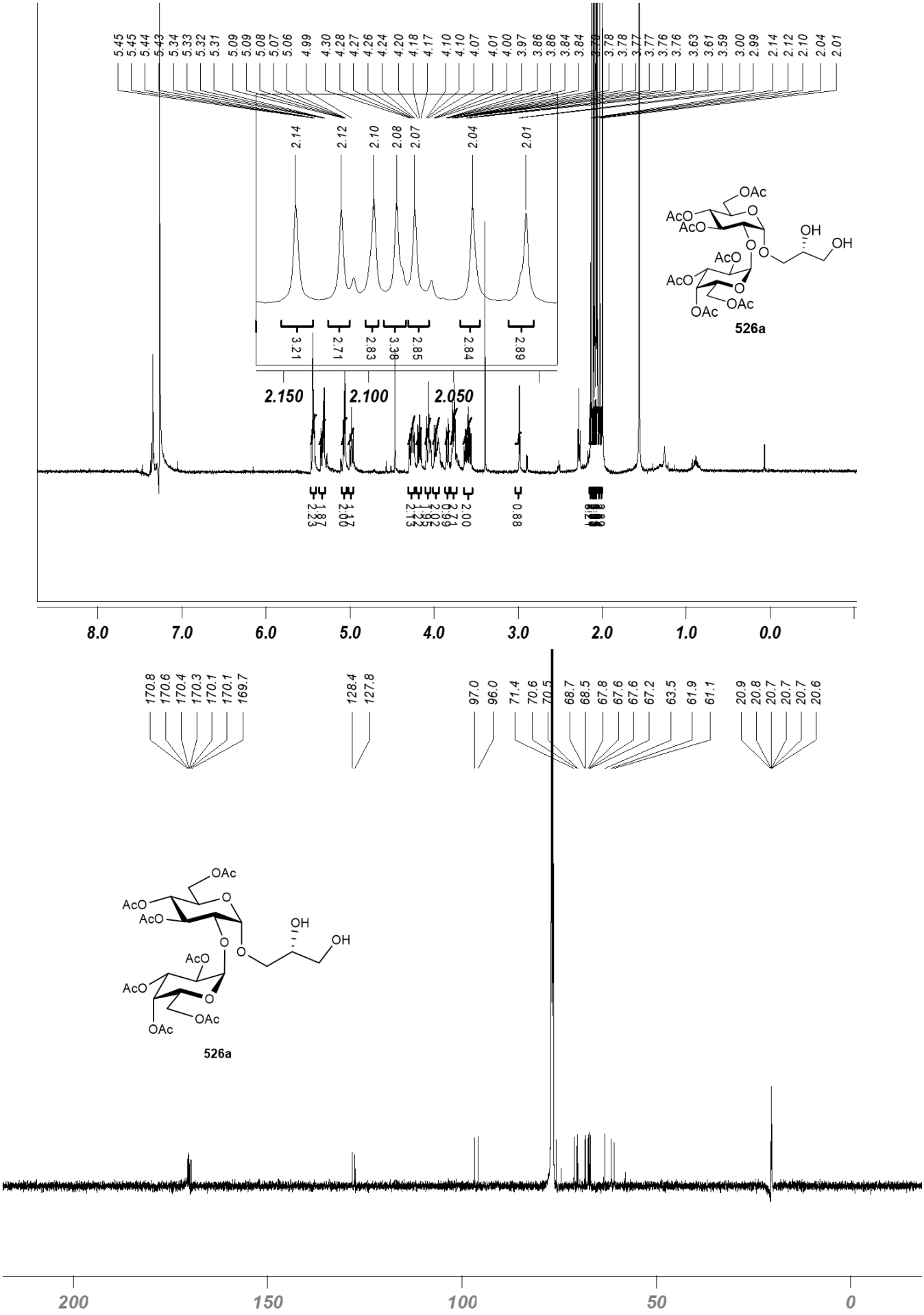

523b
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
30.0
25.0
20.0
150
100
50
0

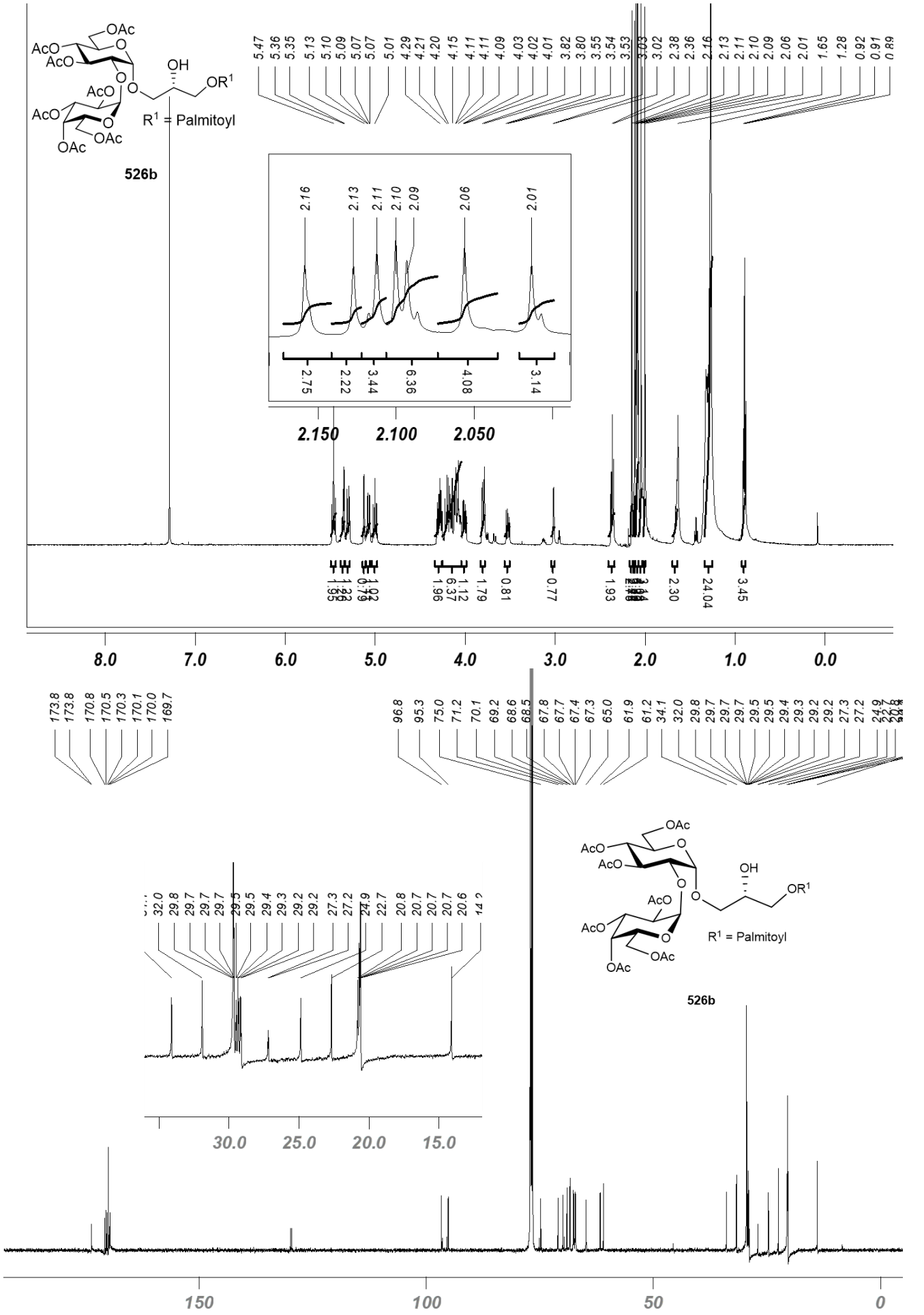

523c
5.11 5.11 5.05 5.04 4.59 4.59 4.59 4.38 4.37 4.37 4.36 4.12 4.11 4.09 4.09 4.08 3.91 3.90 3.90 3.82 3.81 3.79 3.78 3.77 3.75 3.74 3.72 3.72 3.70 3.69 3.68 3.67 3.61 3.60 3.59 1.41 1.35
0.83 1.04 1.89 1.00 1.84 1.05 8.47 2.85 3.12
5.00 4.50 4.00
ppm (t1)
10.0 5.0 0.0
ppm (t1)

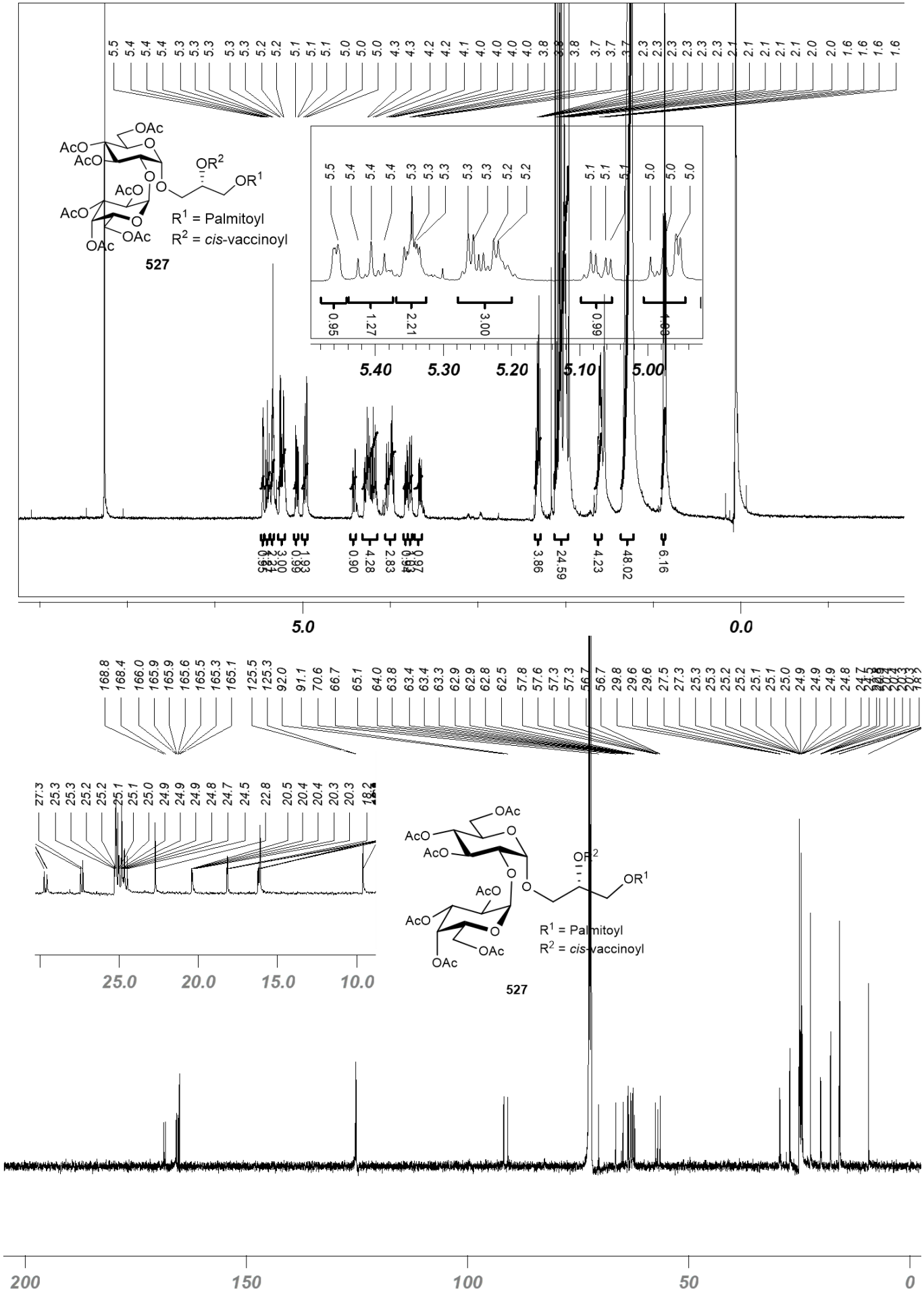

109.3 96.8 76.2 74.8 73.1 72.3 72.0 71.1 70.2 70.1 69.8 68.9 68.5 66.2 61.4 61.3 25.7 24.3
523c
150 100 50 0

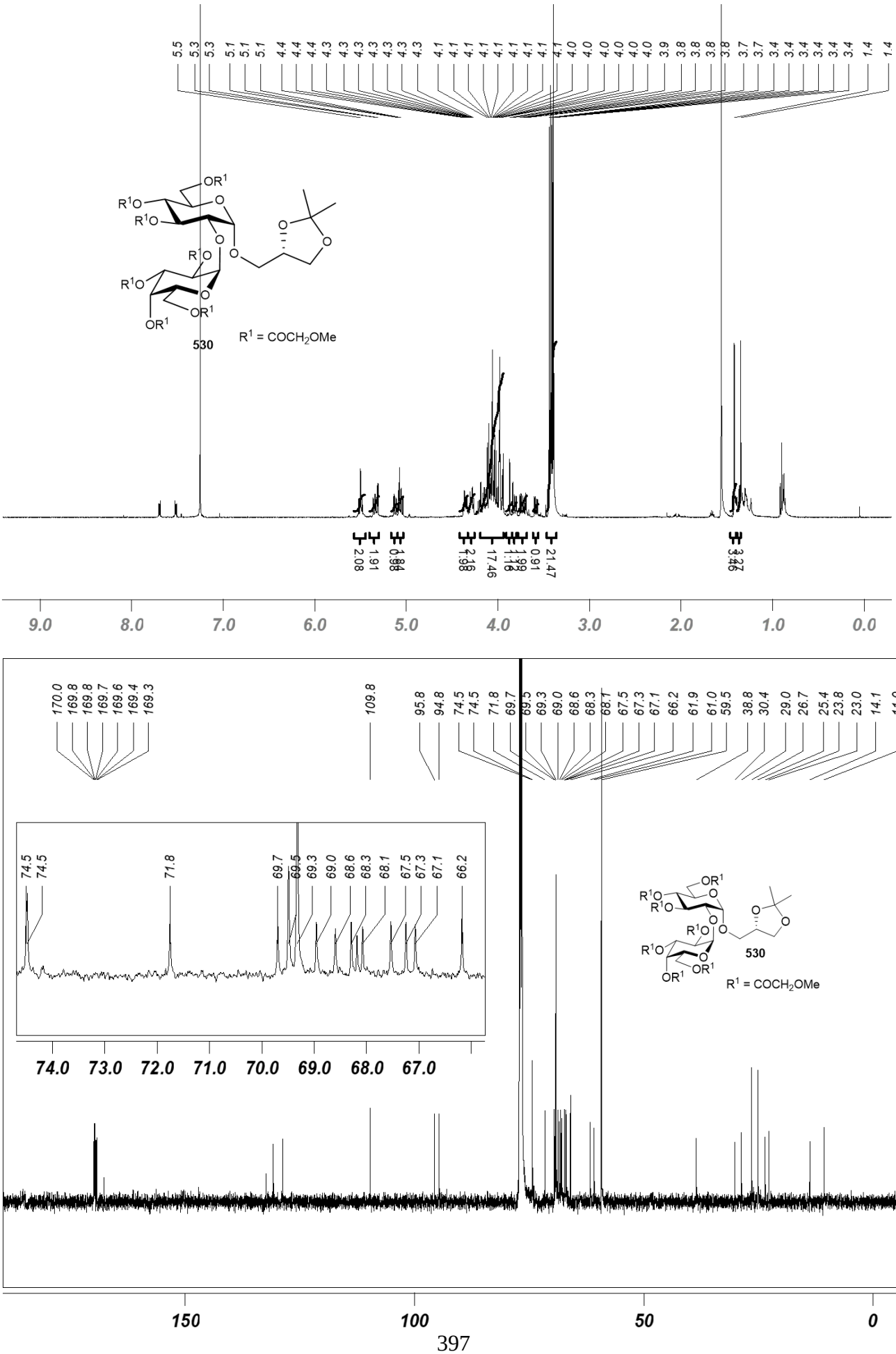

526
ppm (t1)

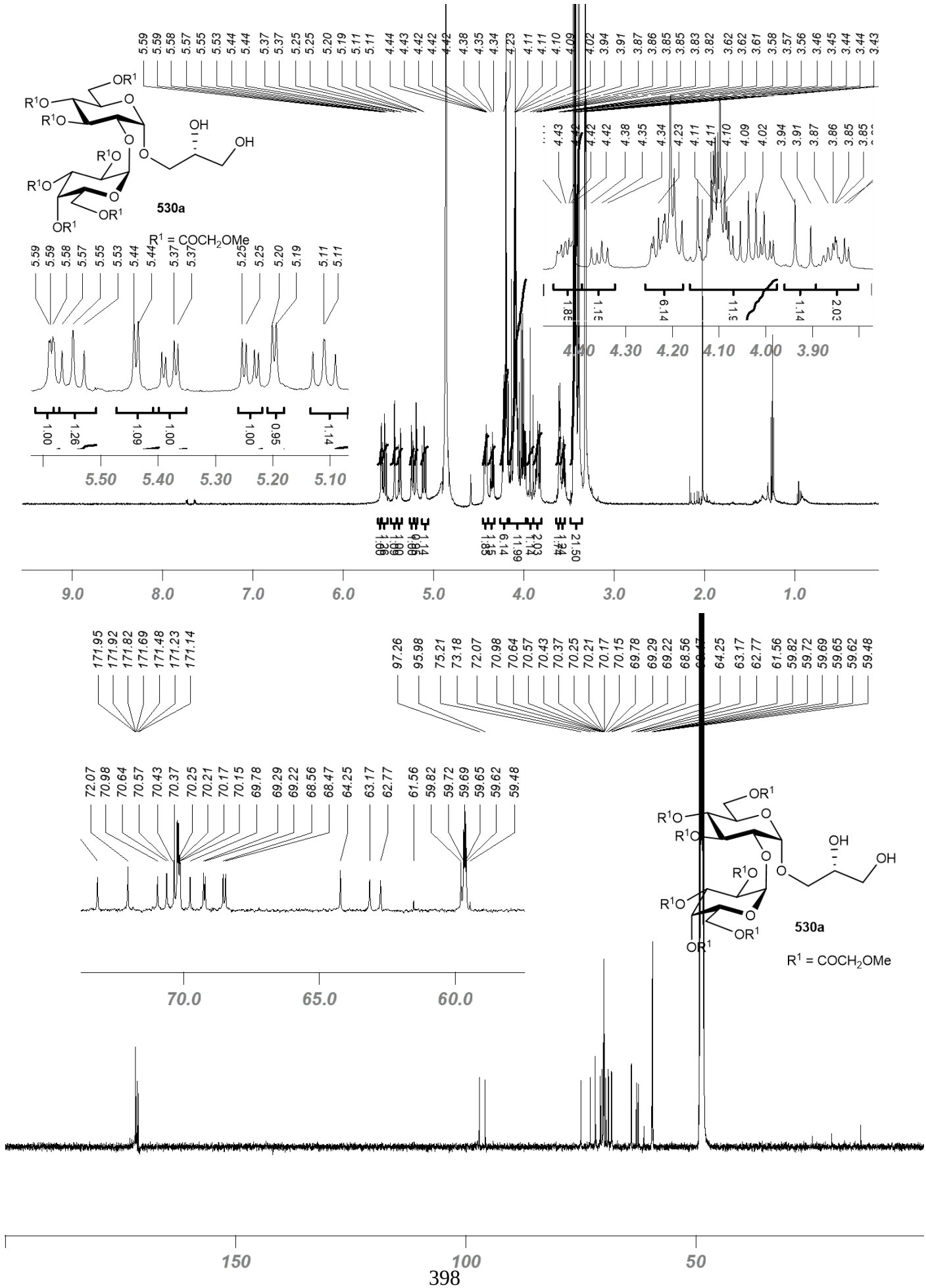

526
ppm (t1)

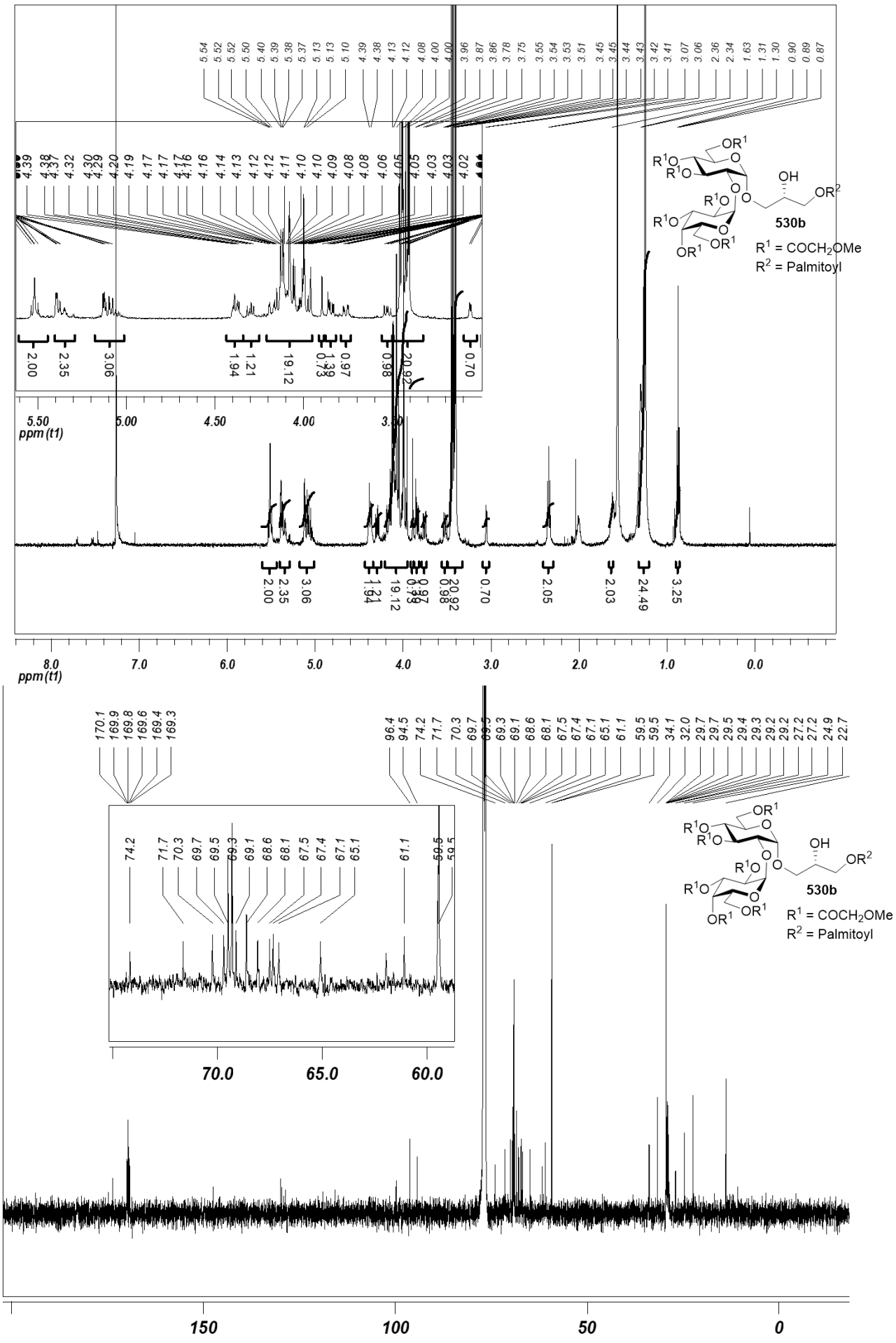

526a
526a

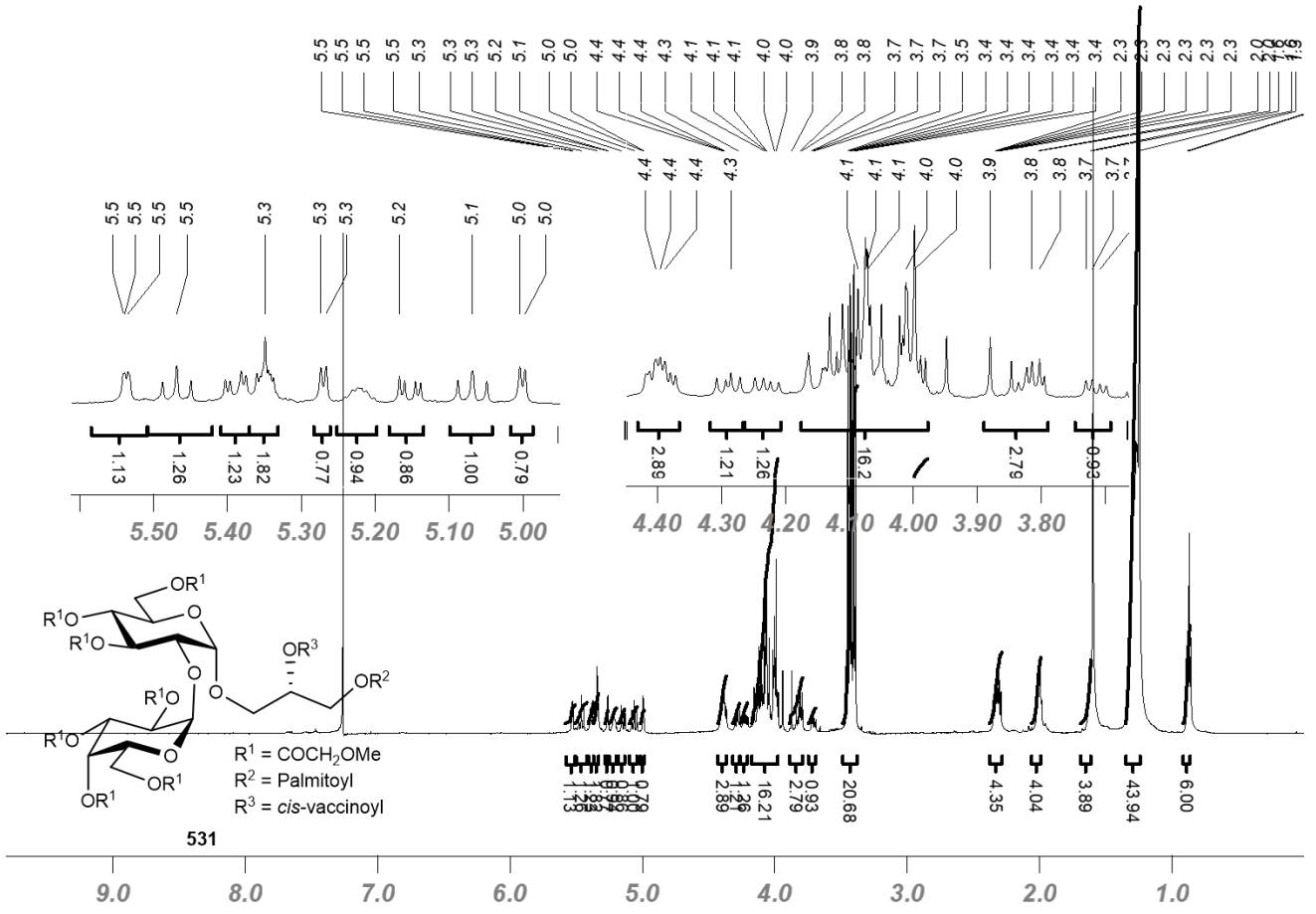

OAc
AcO
AcO
OH
OR1
AcO
AcO
OAc
OAc
R1 = Palmitoyl
526b
5.47 5.36 5.35 5.13 5.10 5.09 5.07 5.07 5.01 4.29 4.21 4.20 4.15 4.11 4.11 4.09 4.03 4.02 4.01 3.82 3.80 3.55 3.54 3.53 3.03 3.02 2.38 2.36 2.16 2.13 2.11 2.10 2.09 2.06 2.01 1.65 1.28 0.92 0.91 0.89
2.16 2.13 2.11 2.10 2.09 2.06 2.01
2.75 2.22 3.44 6.36 4.08 3.14
2.150 2.100 2.050
1.96 6.37 1.12 1.79 0.81 0.77 1.93 2.30 24.04 3.45
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
173.8 173.8 170.8 170.5 170.3 170.1 170.0 169.7
96.8 95.3 75.0 71.2 70.1 69.2 68.6 68.5 67.8 67.7 67.4 67.3 65.0 61.9 61.2 34.1 32.0 29.8 29.7 29.7 29.7 29.5 29.5 29.4 29.3 29.2 29.2 27.3 27.2
32.0 29.8 29.7 29.7 29.7 29.5 29.5 29.4 29.3 29.2 29.2 27.3 27.2 24.9 22.7 20.8 20.7 20.7 20.7 20.6
30.0 25.0 20.0 15.0
150 100 50 0

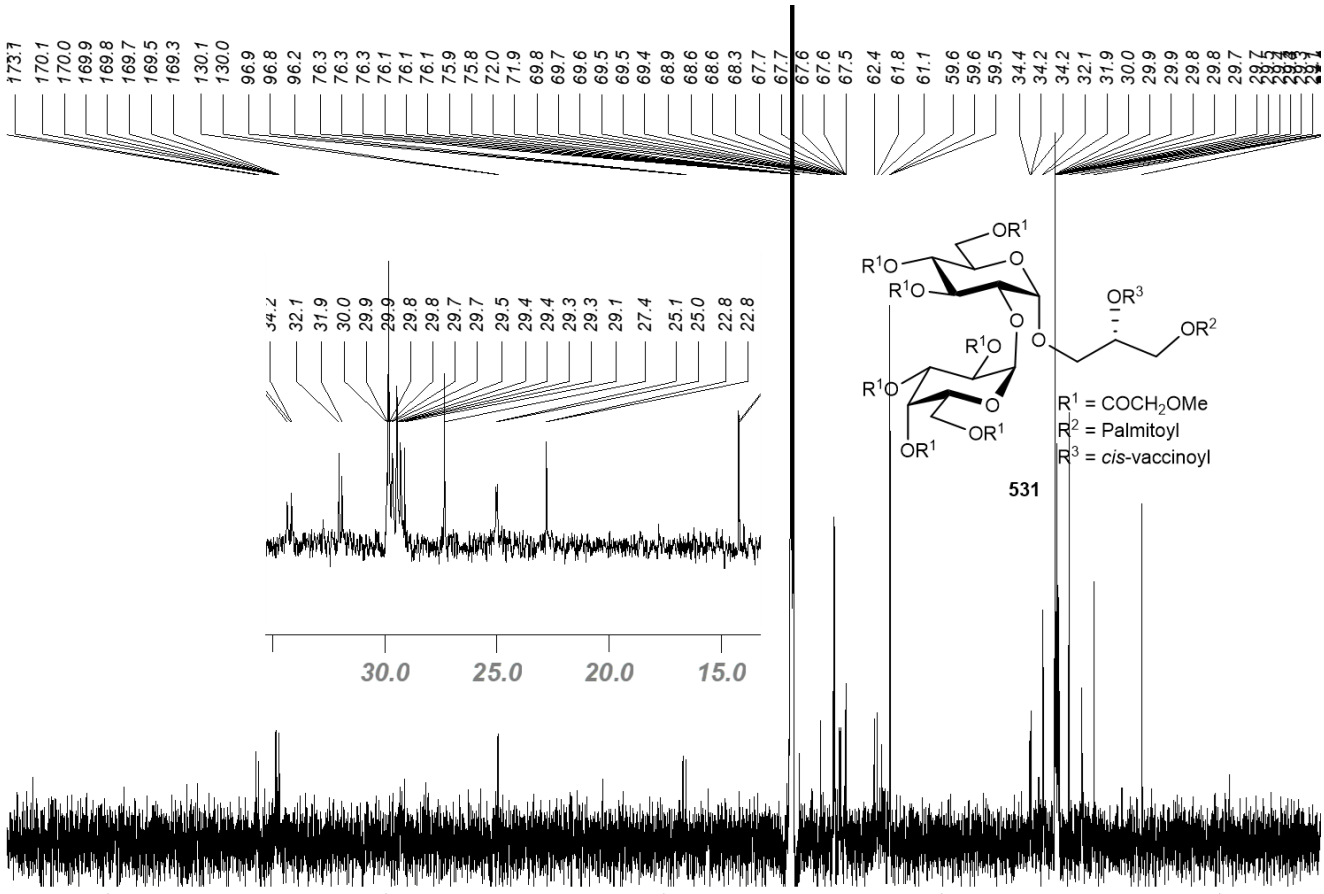

AcO
AcO
OAc
OR2
OR1
AcO
AcO
OAc
OAc
R1 = Palmitoyl
R2 = cis-vaccinoyl
527
5.40
5.30
5.20
5.10
5.00
5.0
0.0
25.0
20.0
15.0
10.0
200
150
100
50
0

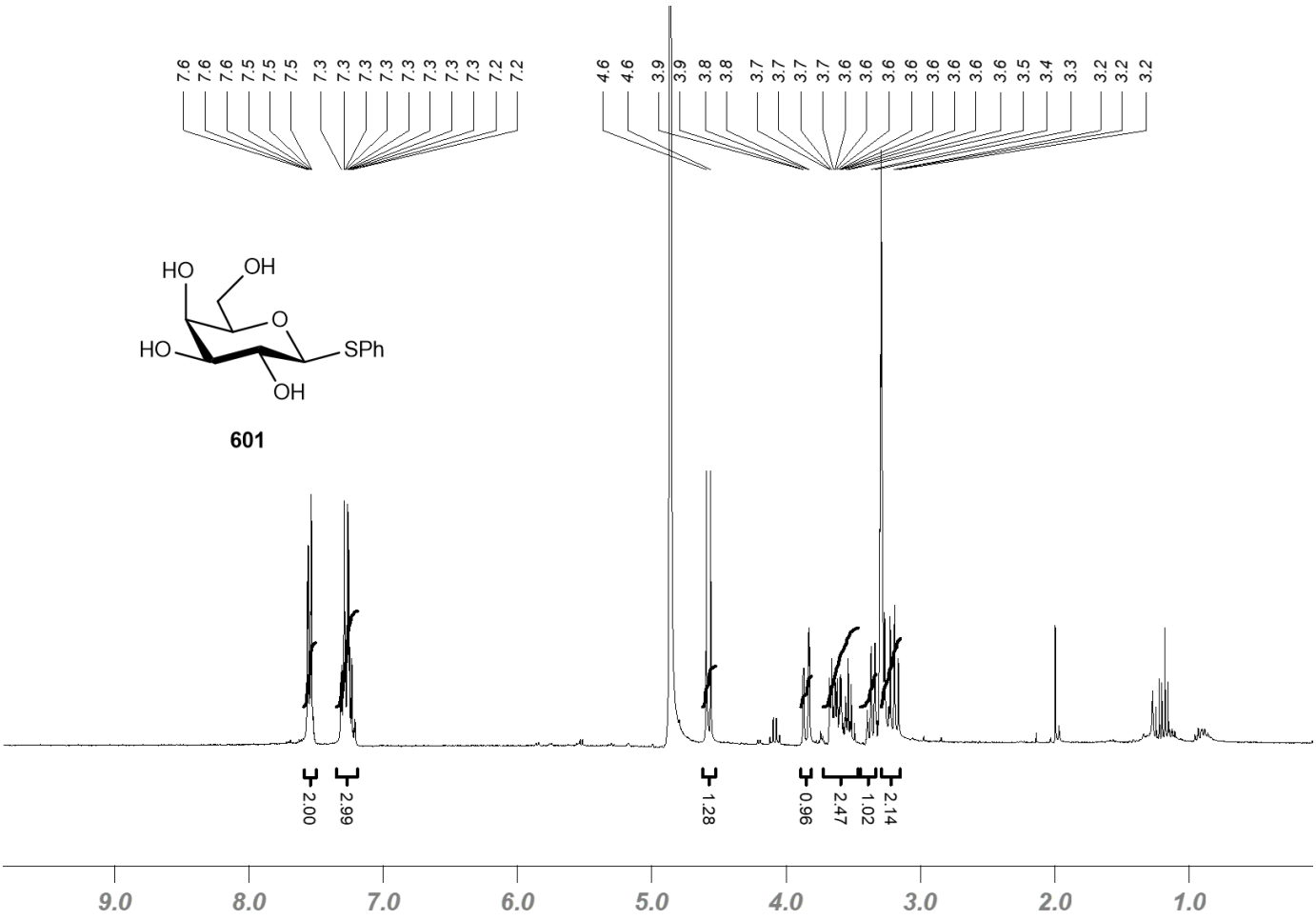

530
R1 = COCH2OMe
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
150
100
50
0
74.0
73.0
72.0
71.0
70.0
69.0
68.0
67.0

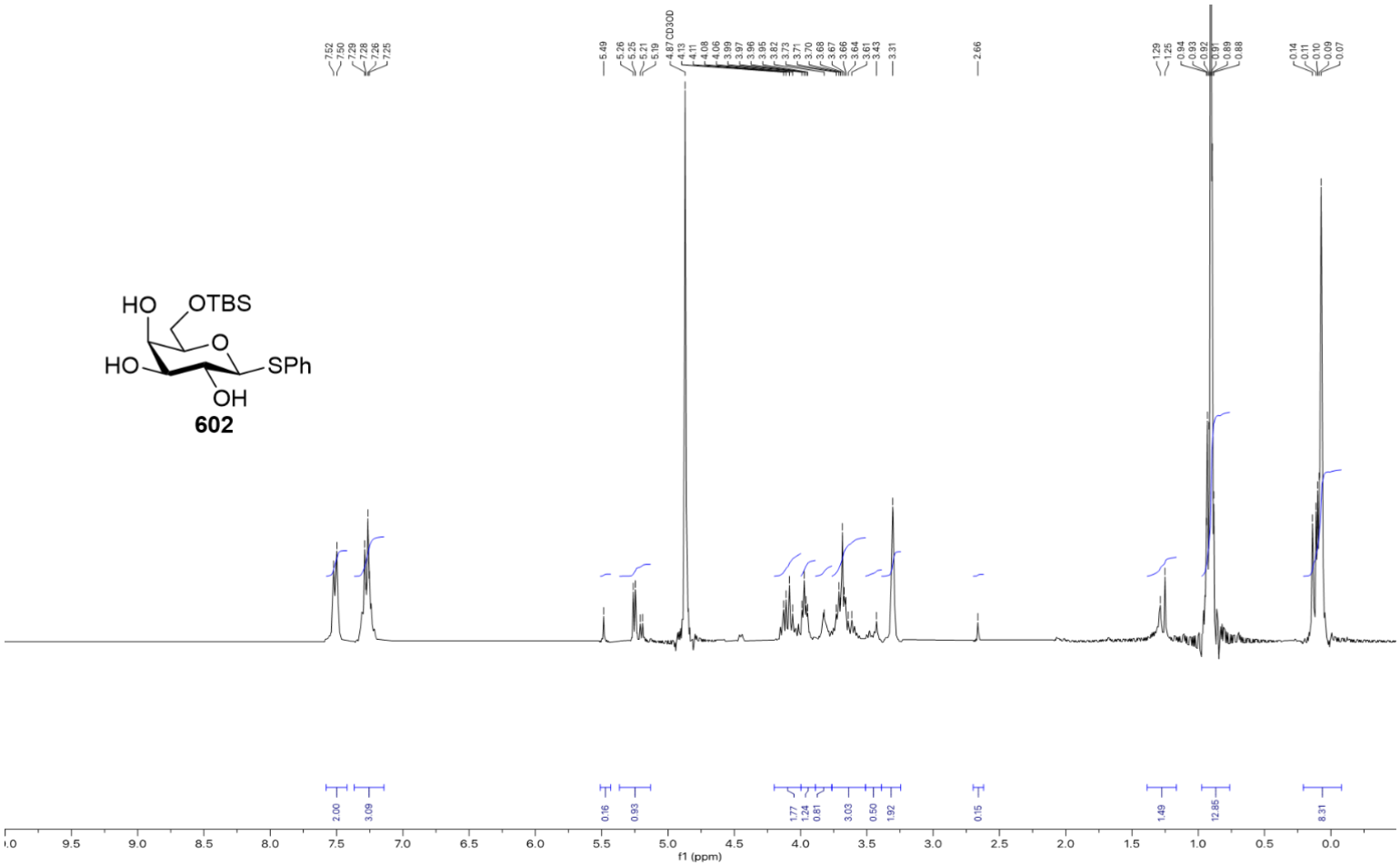

530a
R1 = COCH2OMe

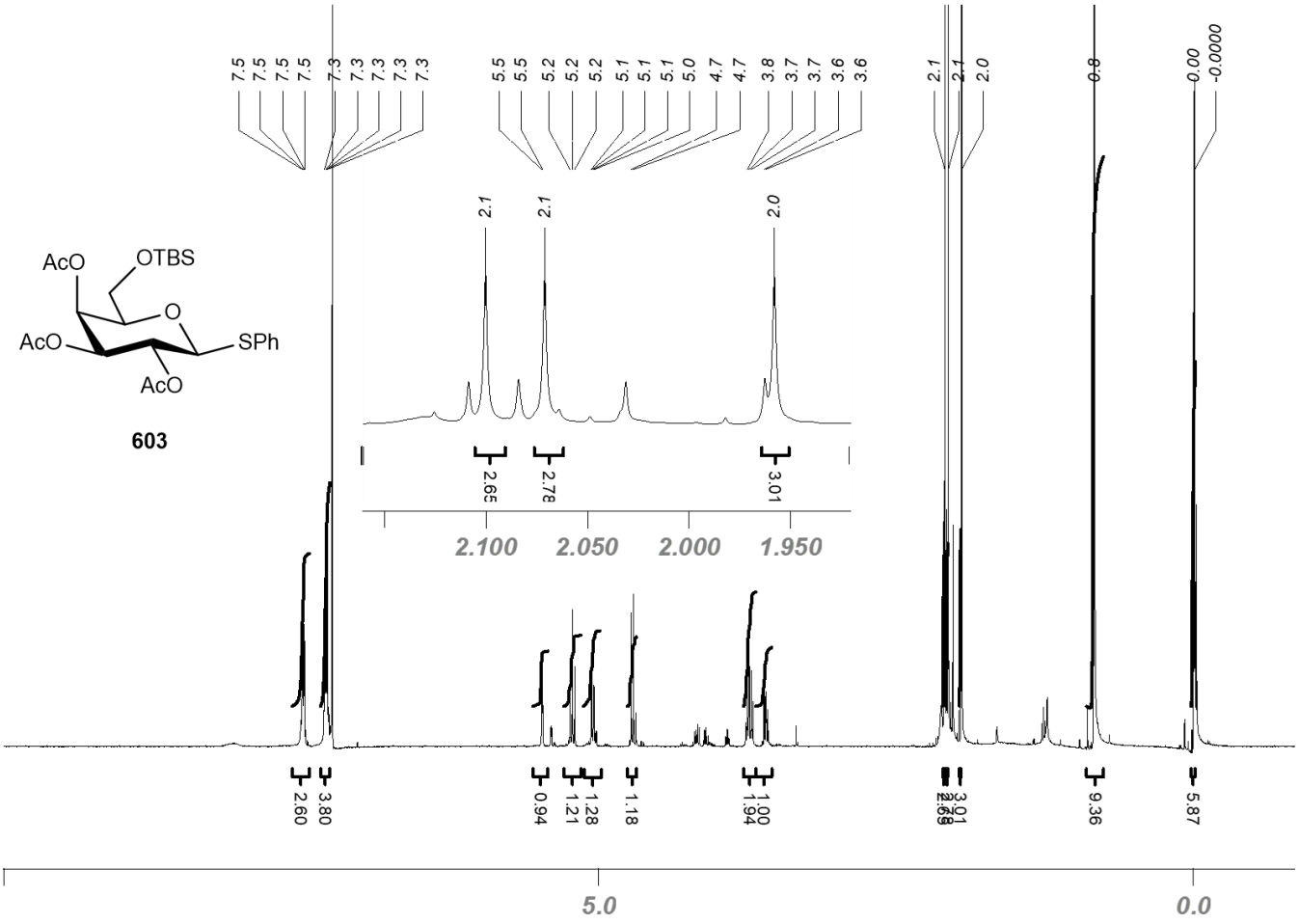

530b
R1 = COCH2OMe
R2 = Palmitoyl
ppm (t1)
530b
R1 = COCH2OMe
R2 = Palmitoyl
ppm (t1)

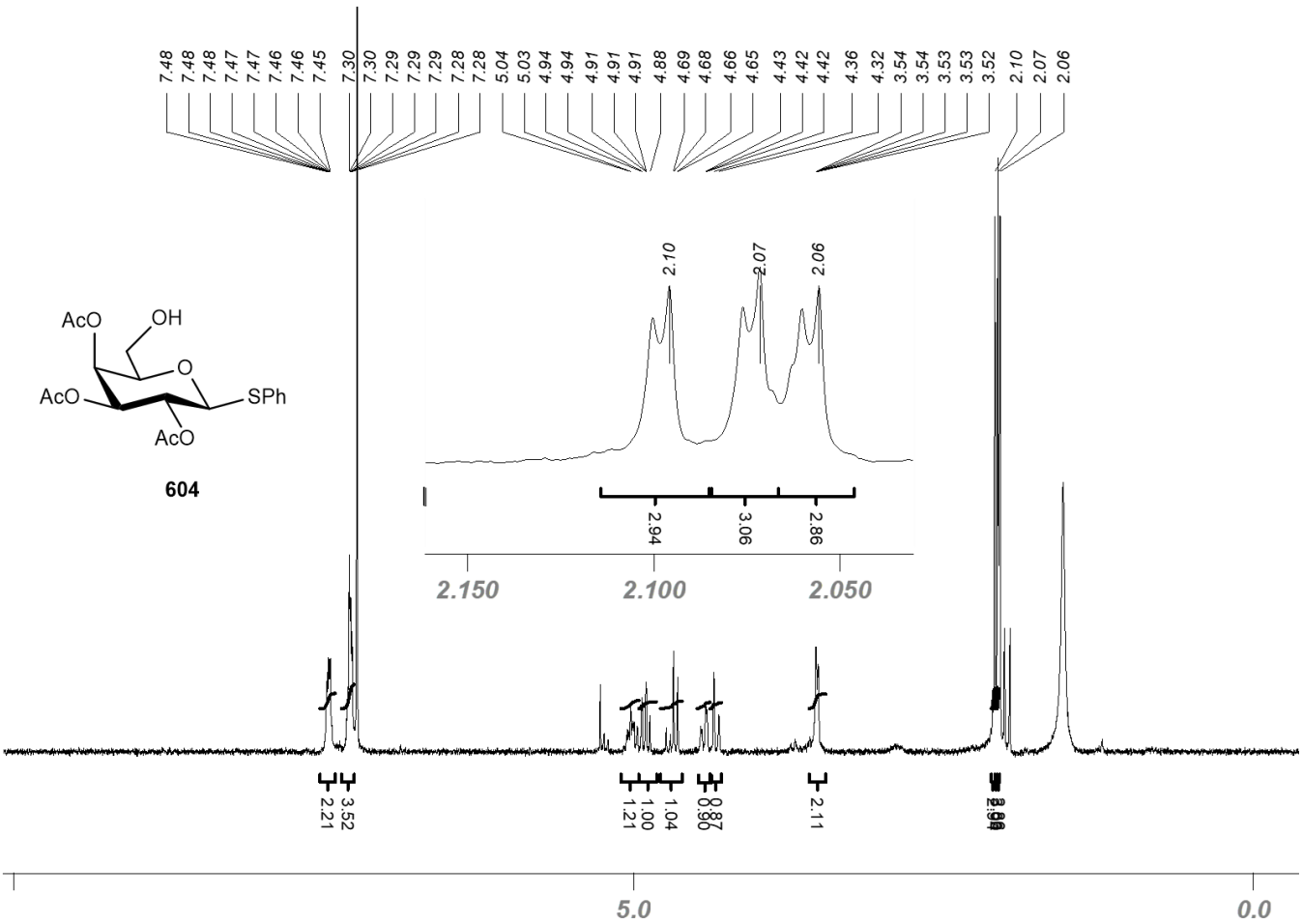

R1 = COCH2OMe
R2 = Palmitoyl
R3 = cis-vaccinoyl
531

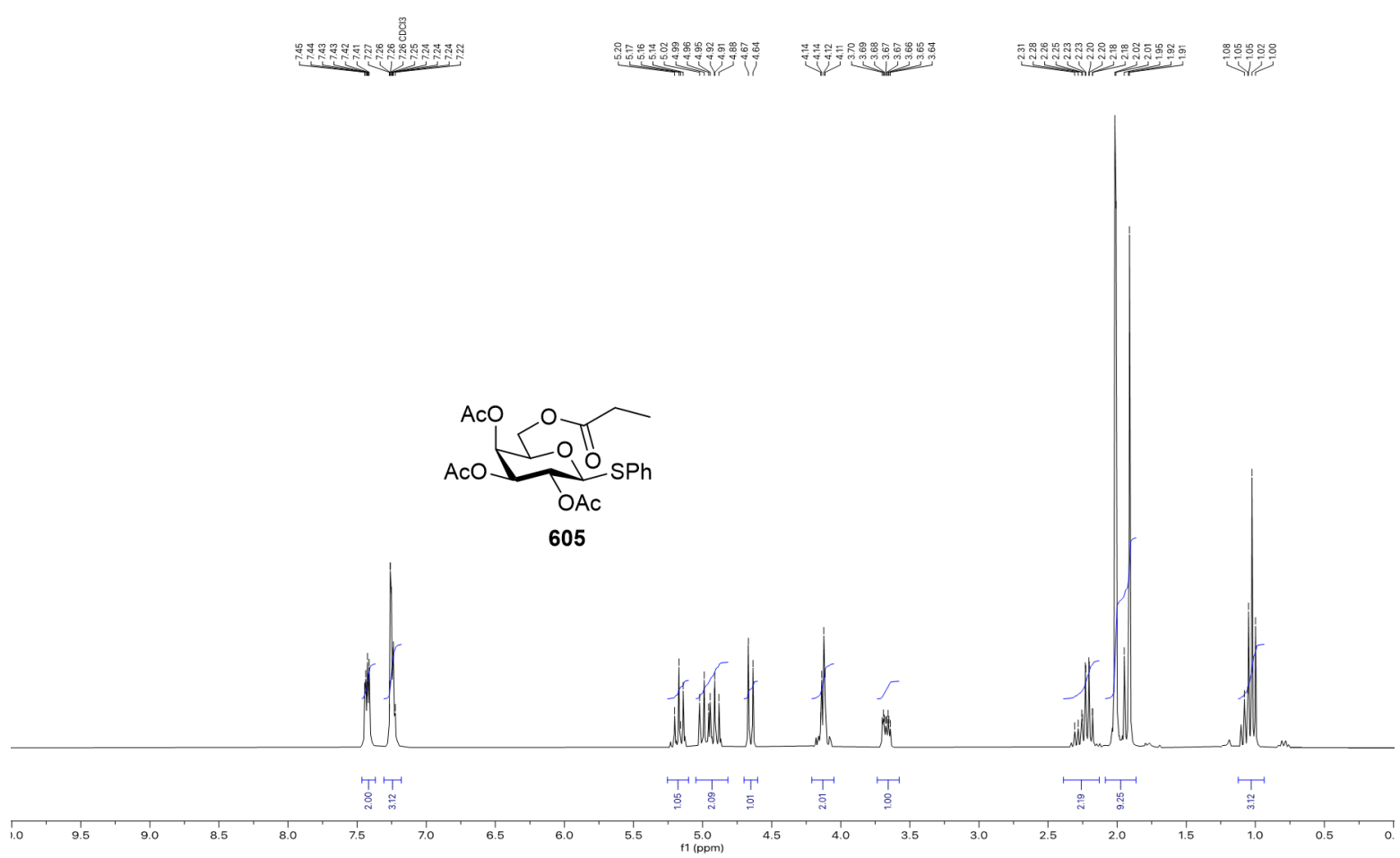

R1 = COCH2OMe
R2 = Palmitoyl
R3 = cis-vaccinoyl
531

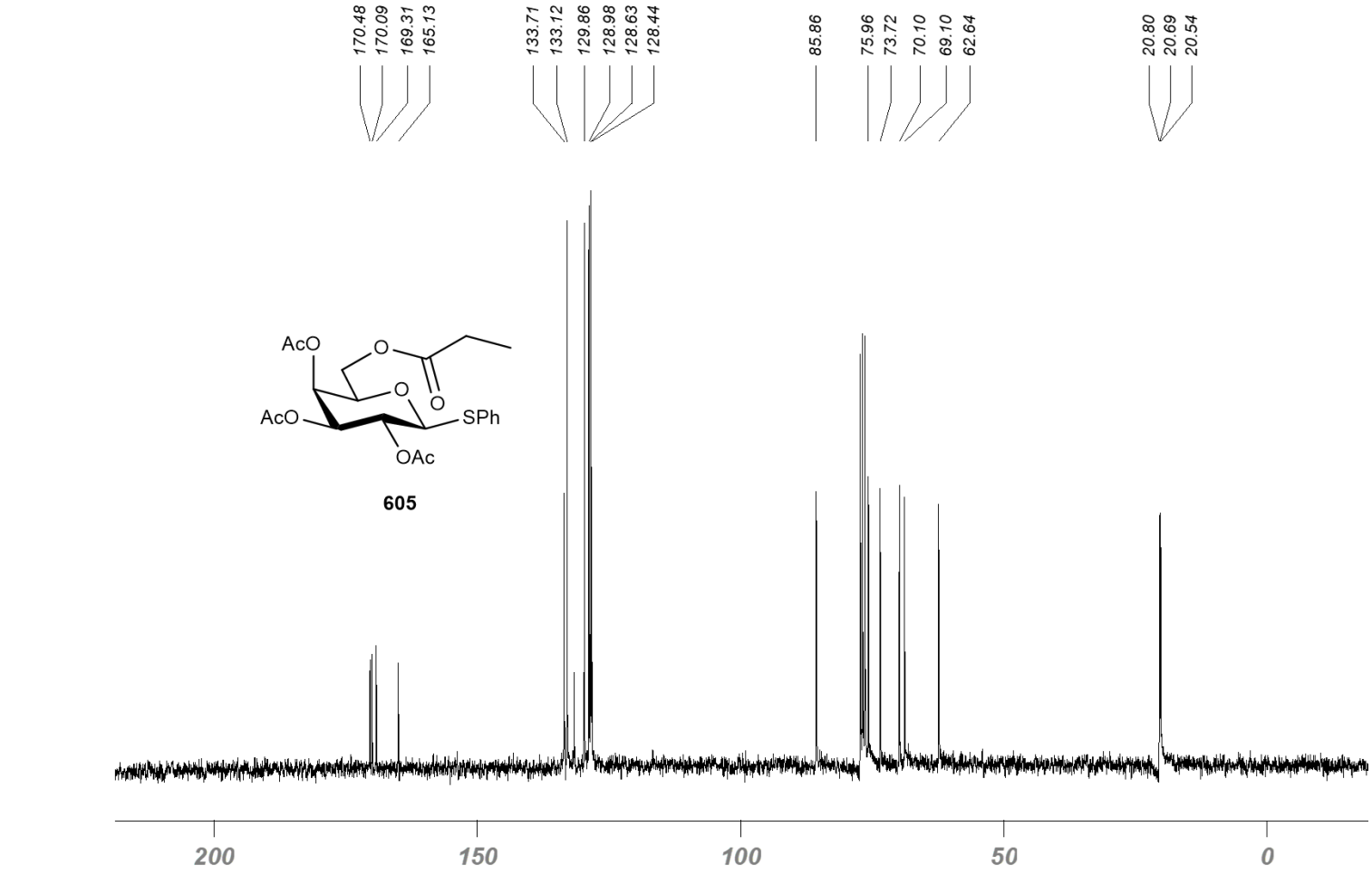

HO
OH
O
HO
SPh
OH
601
2.00
2.99
1.28
0.96
2.47
1.02
2.14
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

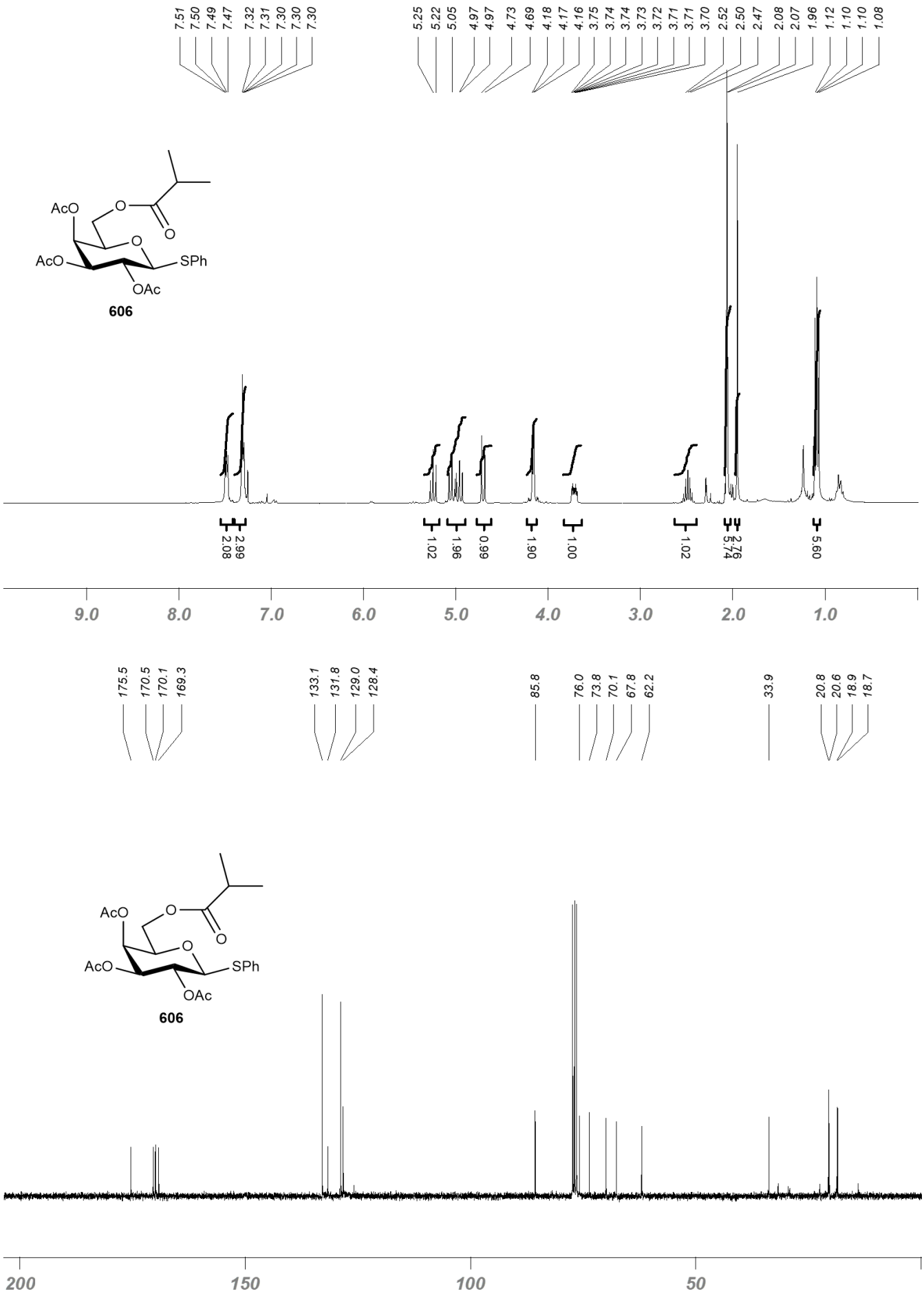

HO
OTBS
O
HO
SPh
OH
602
f1 (ppm)

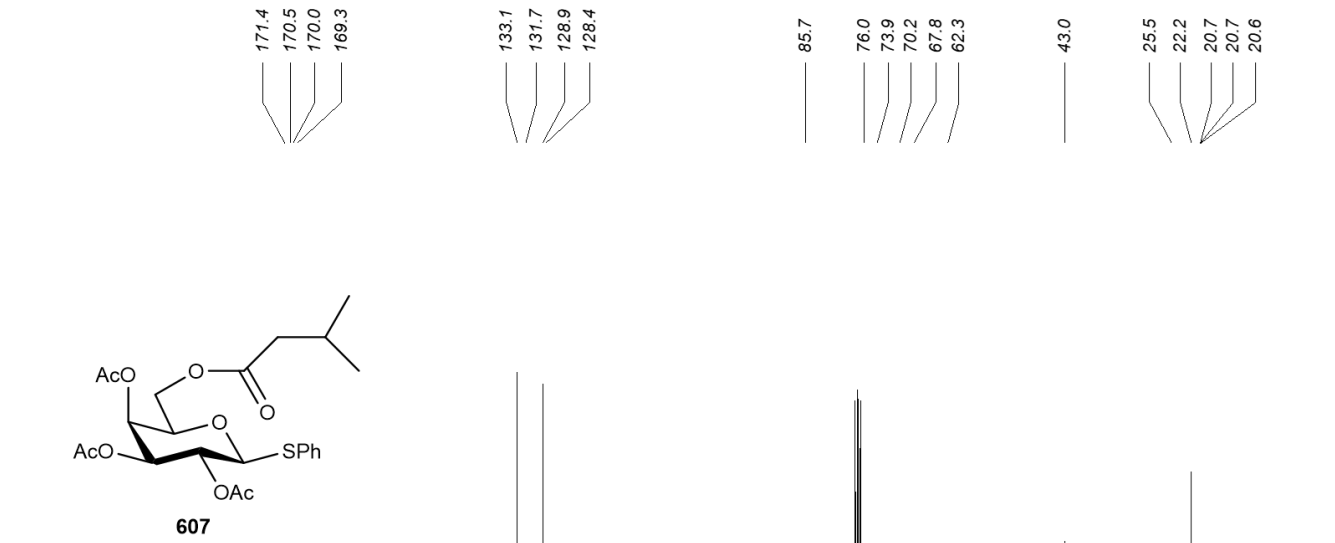

AcO
OTBS
O
AcO
SPh
AcO
603
2.100
2.050
2.000
1.950
5.0
0.0

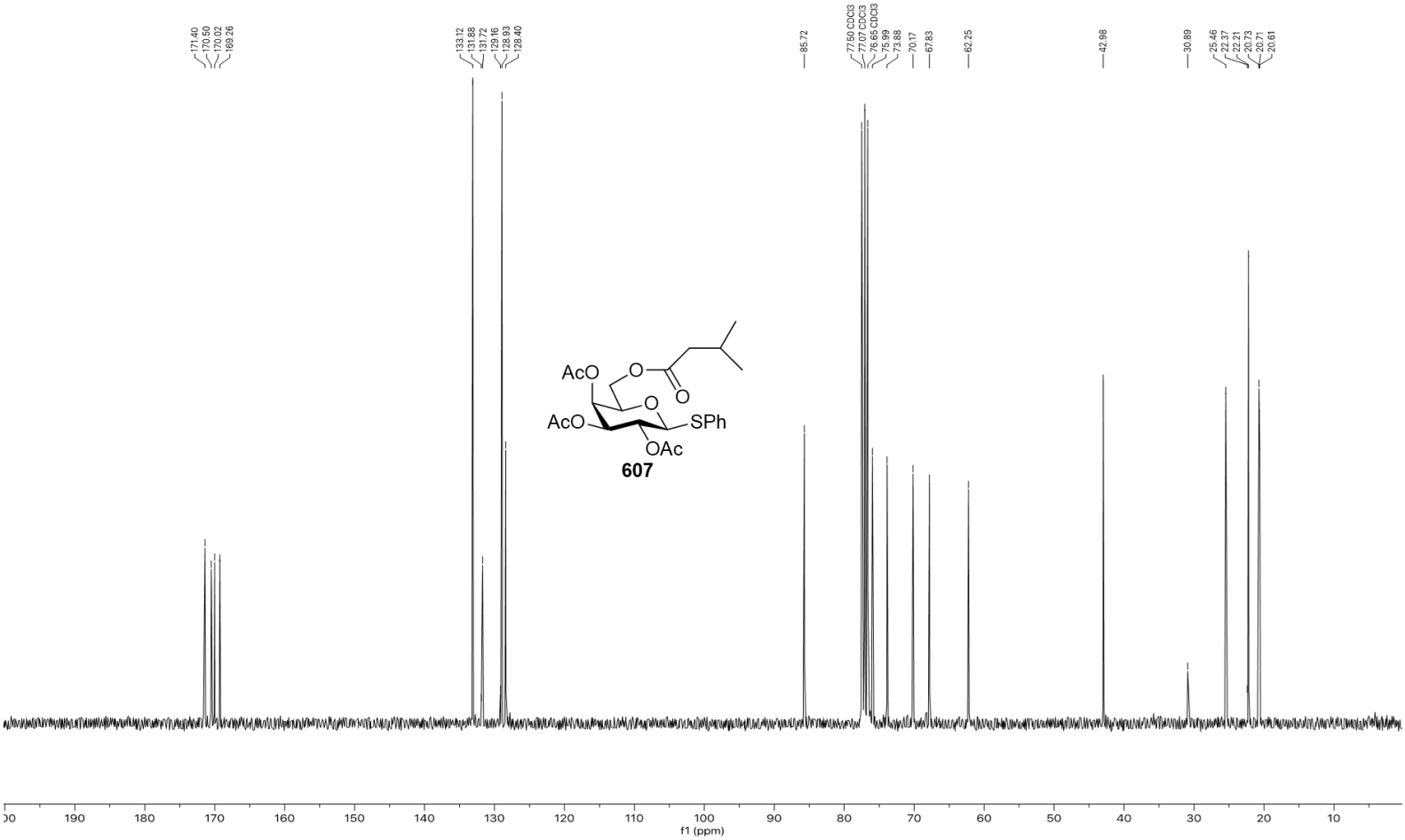

AcO
OH
O
AcO
SPh
AcO
604
2.150
2.100
2.050
5.0
0.0

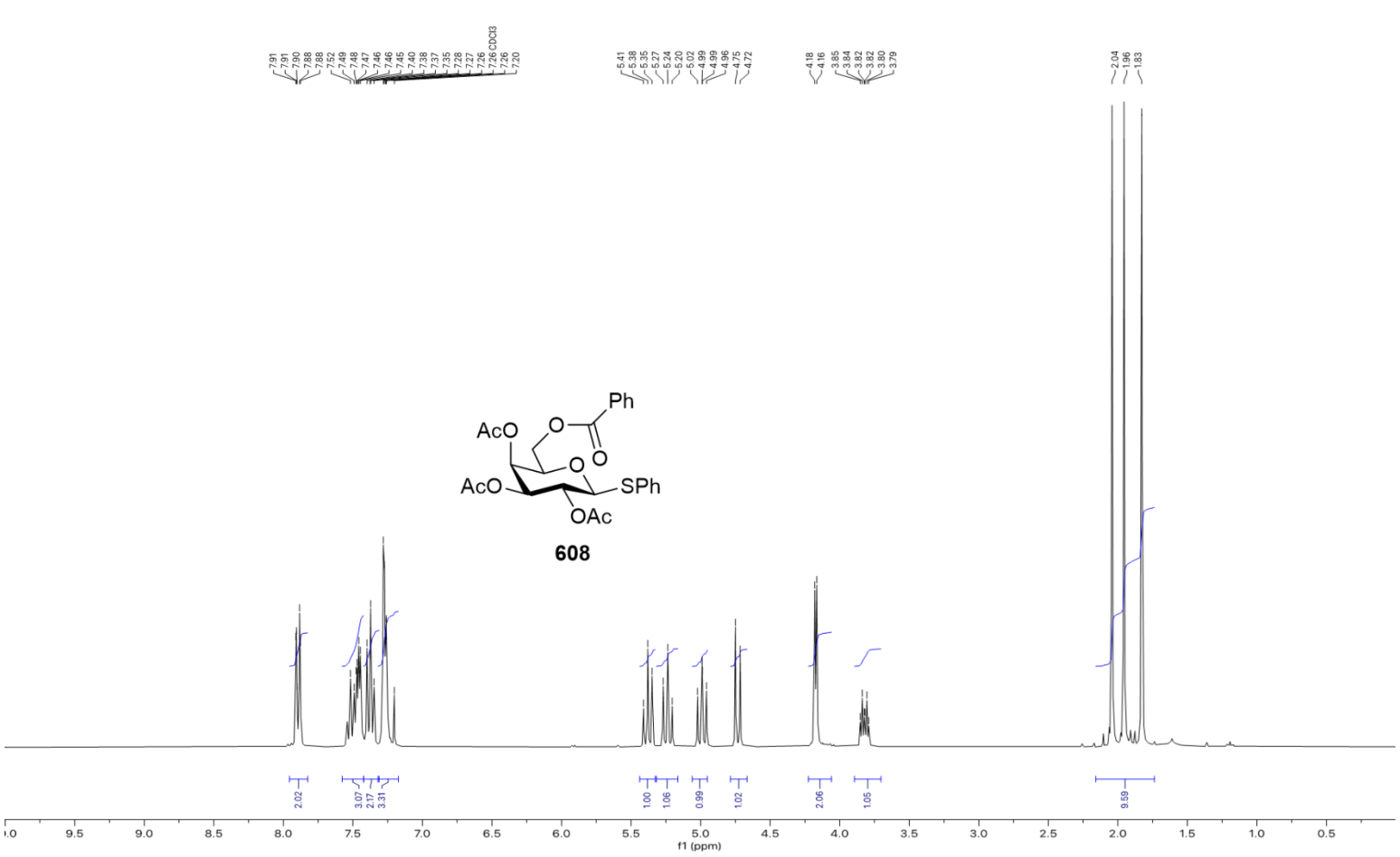

AcO
AcO
OAc
SPh
605
f1 (ppm)

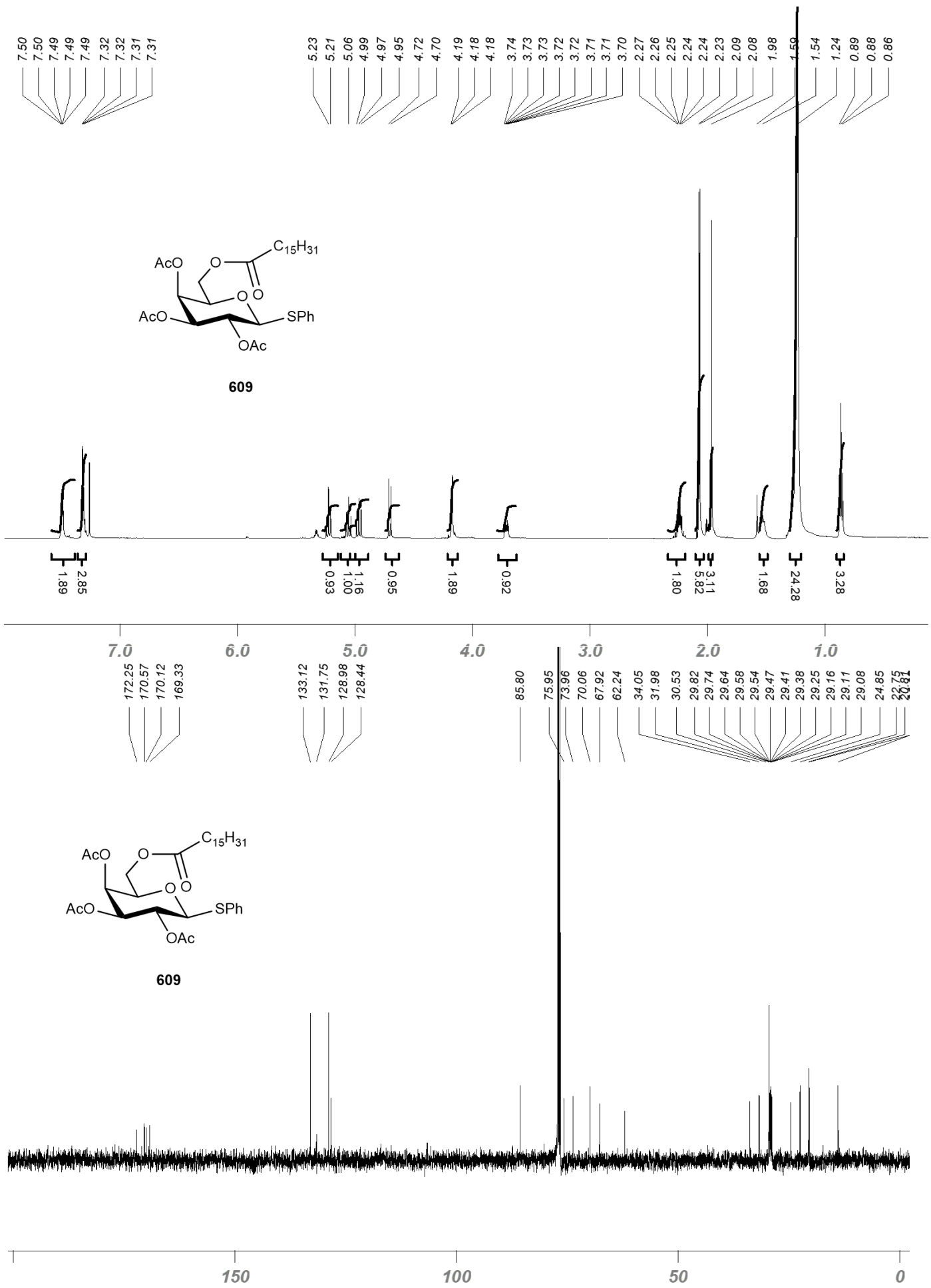

AcO
AcO
OAc
SPh
605

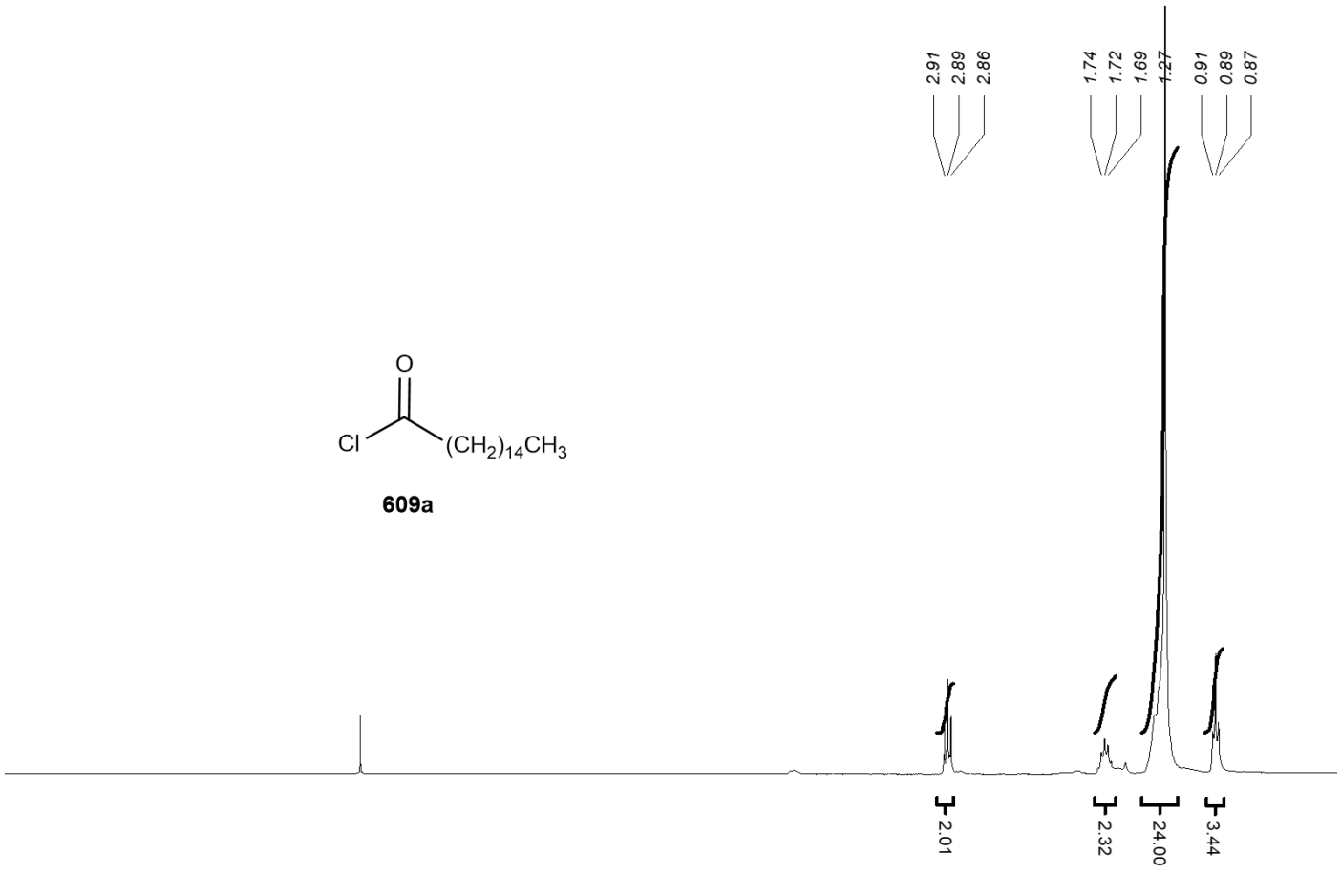

7.51
7.50
7.49
7.47
7.32
7.31
7.30
7.30
7.30
5.25
5.22
5.05
4.97
4.97
4.73
4.69
4.18
4.17
4.16
3.75
3.74
3.74
3.73
3.72
3.71
3.71
3.70
2.52
2.50
2.47
2.08
2.07
1.96
1.12
1.10
1.10
1.08
AcO
O
O
AcO
SPh
OAc
606
2.08
2.99
1.02
1.96
0.99
1.90
1.00
1.02
2.76
5.74
5.90
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
175.5
170.5
170.1
169.3
133.1
131.8
129.0
128.4
85.8
76.0
73.8
70.1
67.8
62.2
33.9
20.8
20.6
18.9
18.7
AcO
O
O
AcO
SPh
OAc
606
200
150
100
50


171.4
170.5
170.0
169.3
133.1
131.7
128.9
128.4
85.7
76.0
73.9
70.2
67.8
62.3
43.0
25.5
22.2
20.7
20.7
20.6
AcO
O
O
AcO
O
SPh
OAc
607
200
150
100
50
0


171.40
170.50
170.02
169.26
133.12
131.88
131.72
129.16
128.93
128.40
85.72
77.50 CDCl3
77.17 CDCl3
76.65 CDCl3
75.99
73.88
70.17
67.83
62.25
42.98
30.89
25.46
22.37
22.21
20.73
20.71
20.61
AcO
O
O
AcO
O
SPh
OAc
607
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

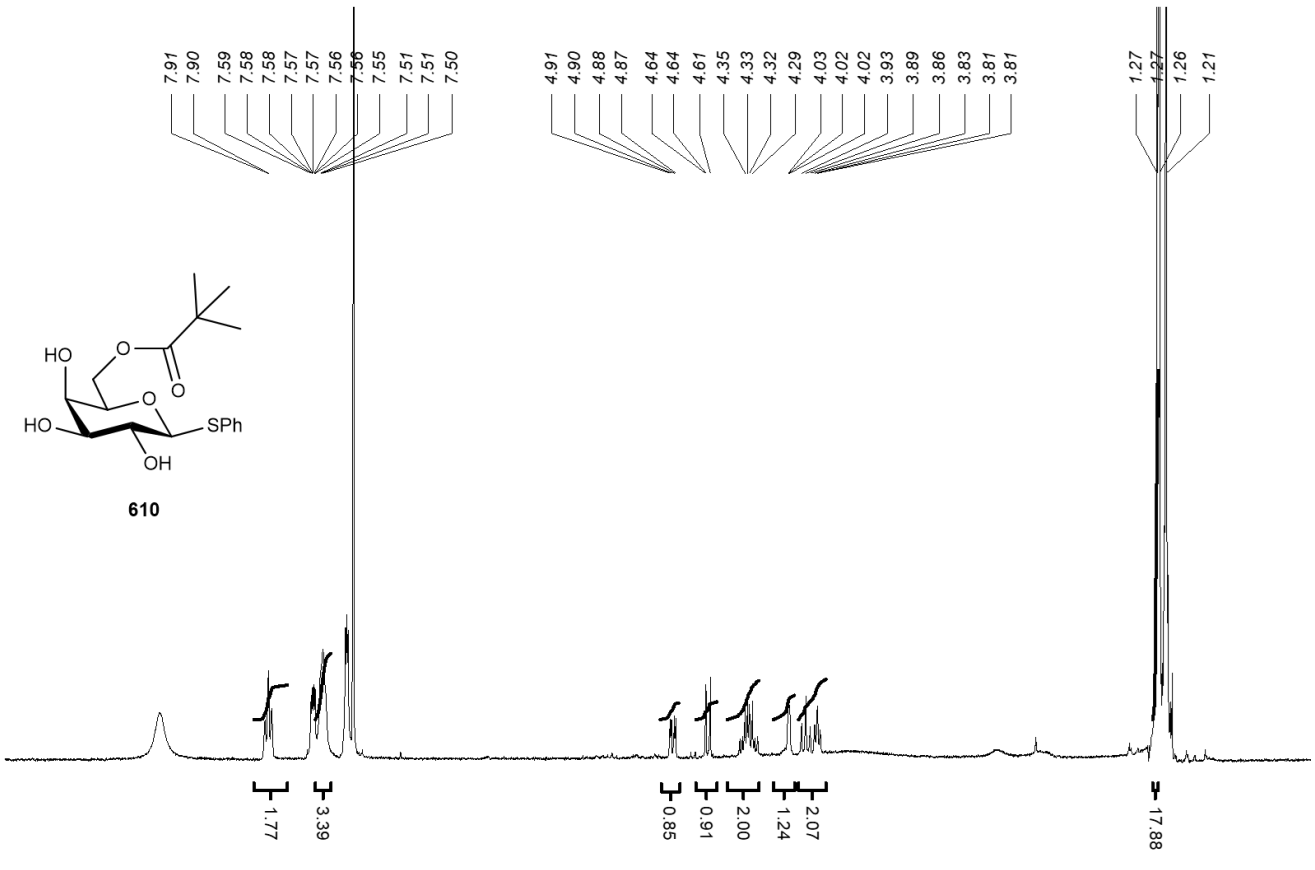

7.91
7.91
7.90
7.88
7.88
7.52
7.49
7.48
7.47
7.46
7.46
7.45
7.40
7.38
7.37
7.35
7.28
7.27
7.26
7.26 CDCl3
7.20
5.41
5.38
5.35
5.27
5.24
5.20
5.02
4.99
4.99
4.96
4.75
4.72
4.18
4.16
3.85
3.84
3.82
3.80
3.79
2.04
1.96
1.83
Ph
AcO
O
O
O
AcO
SPh
OAc
608
2.02
3.07
2.17
3.31
1.00
1.06
0.99
1.02
2.06
1.05
9.59
f1 (ppm)


7.50 7.50 7.49 7.49 7.49 7.32 7.32 7.31 7.31
5.23 5.21 5.06 4.99 4.97 4.95 4.72 4.70 4.19 4.18 4.18 3.74 3.73 3.73 3.72 3.72 3.71 3.71 3.70 2.27 2.26 2.25 2.24 2.24 2.23 2.09 2.08 1.98 1.59 1.54 1.24 0.89 0.88 0.86
AcO
O
C15H31
O
O
AcO
SPh
OAc
609
1.89 2.85 0.63 1.00 1.16 0.95 1.89 0.92 1.80 5.82 3.11 1.68 24.28 3.28
7.0 6.0 5.0 4.0 3.0 2.0 1.0
172.25 170.57 170.12 169.33 133.12 131.75 128.98 128.44 85.80 75.95 73.96 70.06 67.92 62.24 34.05 31.98 30.53 29.82 29.74 29.64 29.58 29.54 29.47 29.41 29.38 29.25 29.16 29.11 29.08 24.85
AcO
O
C15H31
O
O
AcO
SPh
OAc
609
150 100 50 0

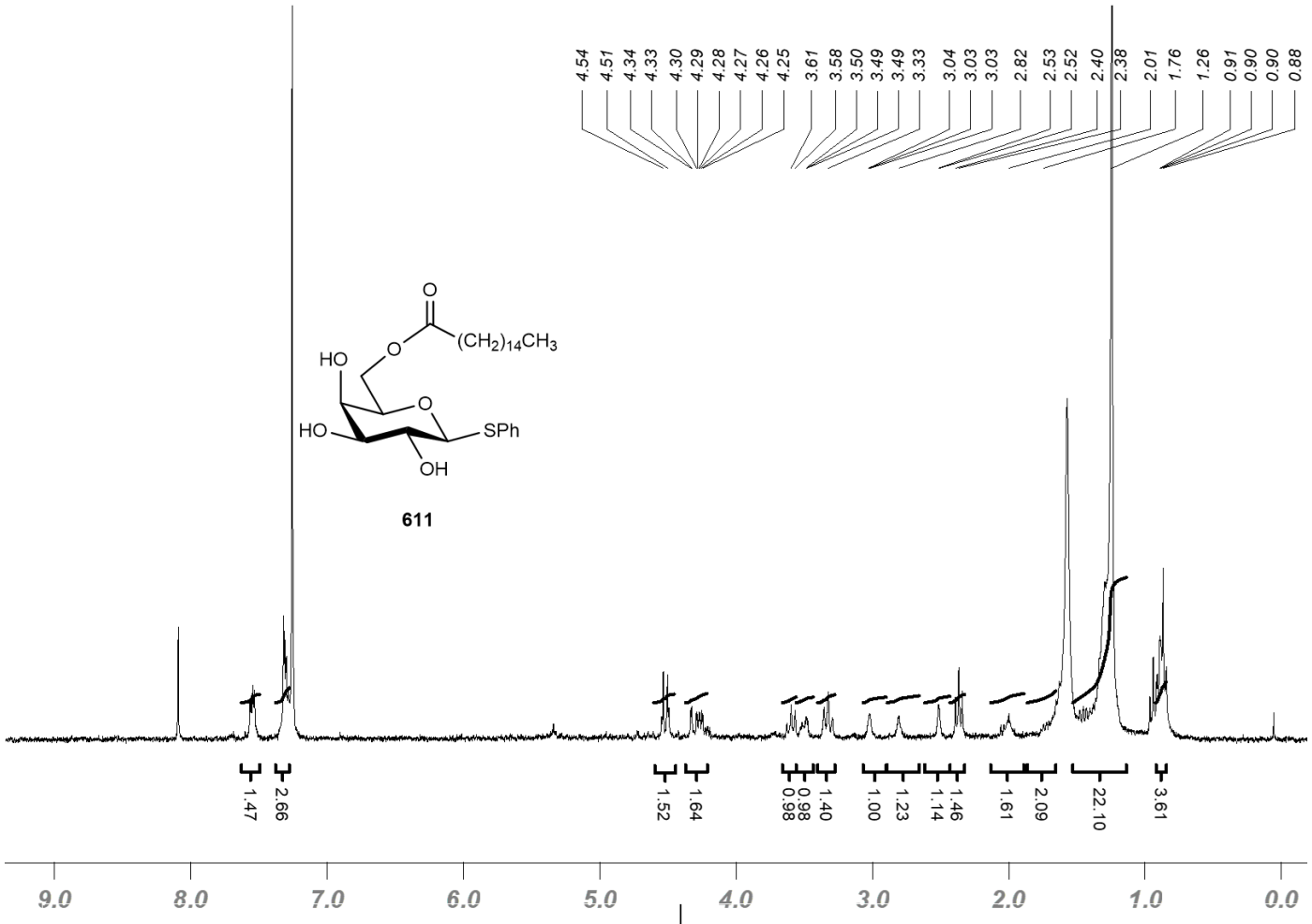

2.91
2.89
2.86
1.74
1.72
1.69
1.27
0.91
0.88
0.87
2.01
2.32
24.00
3.44
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

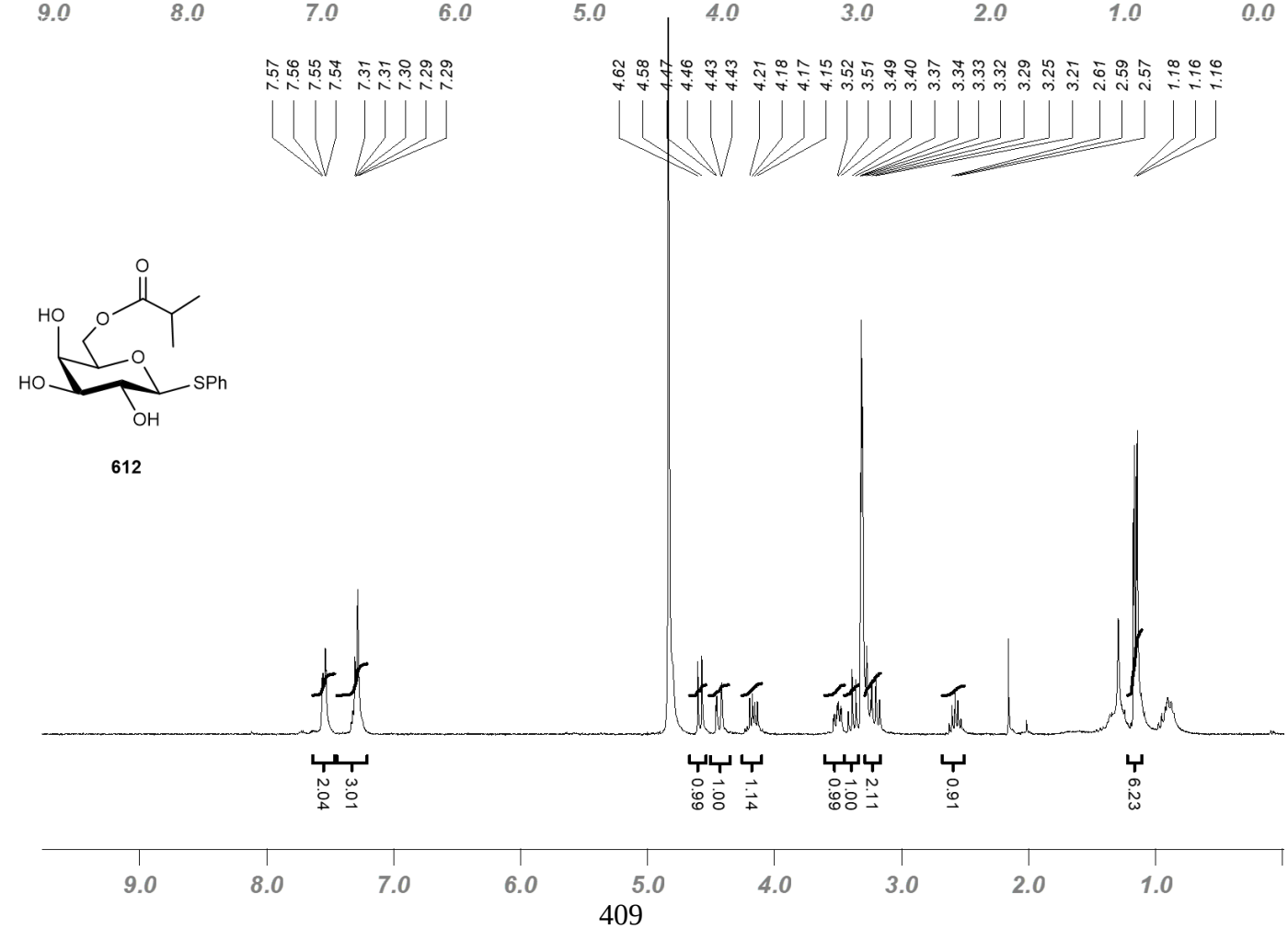
O
Cl
(CH2)14CH3

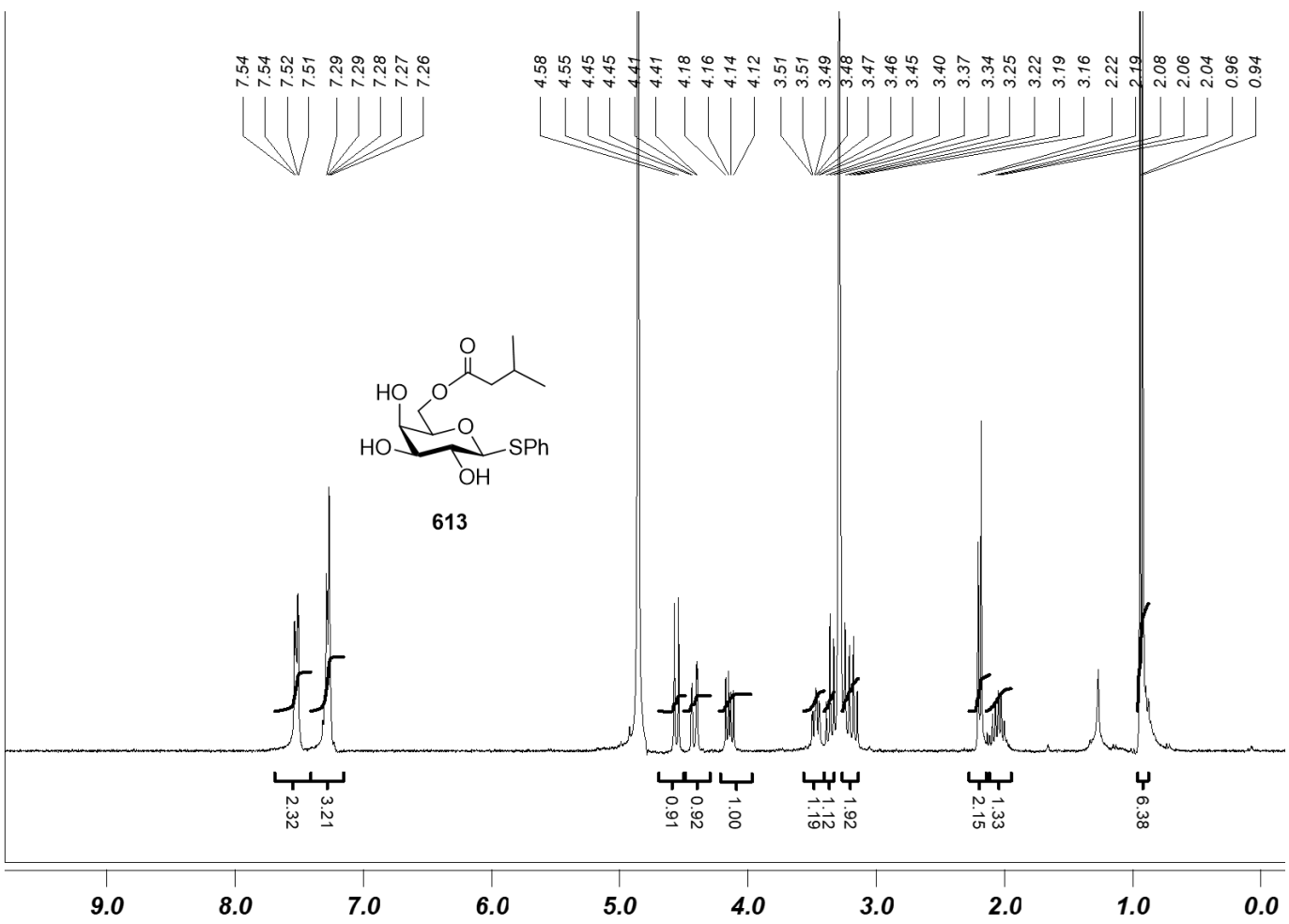
609a


7.91
7.90
7.59
7.58
7.58
7.57
7.57
7.56
7.56
7.55
7.51
7.51
7.50
4.91
4.90
4.88
4.87
4.64
4.64
4.61
4.35
4.33
4.32
4.29
4.03
4.02
4.02
3.93
3.89
3.86
3.83
3.81
3.81
1.27
1.26
1.21
HO
O
O
HO
SPh
OH
1.77
3.39
0.85
0.91
2.00
1.24
2.07
17.88
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

610


4.54 4.51 4.34 4.33 4.30 4.29 4.28 4.27 4.26 4.25 3.61 3.58 3.50 3.49 3.49 3.33 3.04 3.03 3.03 2.82 2.53 2.52 2.40 2.38 2.01 1.76 1.26 0.91 0.90 0.90 0.88
O
(CH2)14CH3
HO
O
HO
SPh
OH
611
1.47 2.66 1.52 1.64 0.98 0.98 1.40 1.00 1.23 1.14 1.46 1.61 2.09 22.10 3.61
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0


7.57 7.56 7.55 7.54 7.31 7.31 7.30 7.29 7.29
4.62 4.58 4.47 4.46 4.43 4.43 4.21 4.18 4.17 4.15 3.52 3.51 3.49 3.40 3.37 3.34 3.33 3.32 3.29 3.25 3.21 2.61 2.59 2.57 1.18 1.16 1.16
O
HO
O
HO
SPh
OH
612
2.04 3.01 0.99 1.00 1.14 0.99 1.00 2.11 0.91 6.23
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0


7.54
7.54
7.52
7.51
7.29
7.29
7.28
7.27
7.26
4.58
4.55
4.45
4.45
4.41
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3.37
3.34
3.25
3.22
3.19
3.16
2.22
2.19
2.08
2.06
2.04
0.96
0.94
HO
O
O
HO
SPh
OH
613
2.32
3.21
0.91
0.92
1.00
1.12
1.19
1.92
2.15
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2.0
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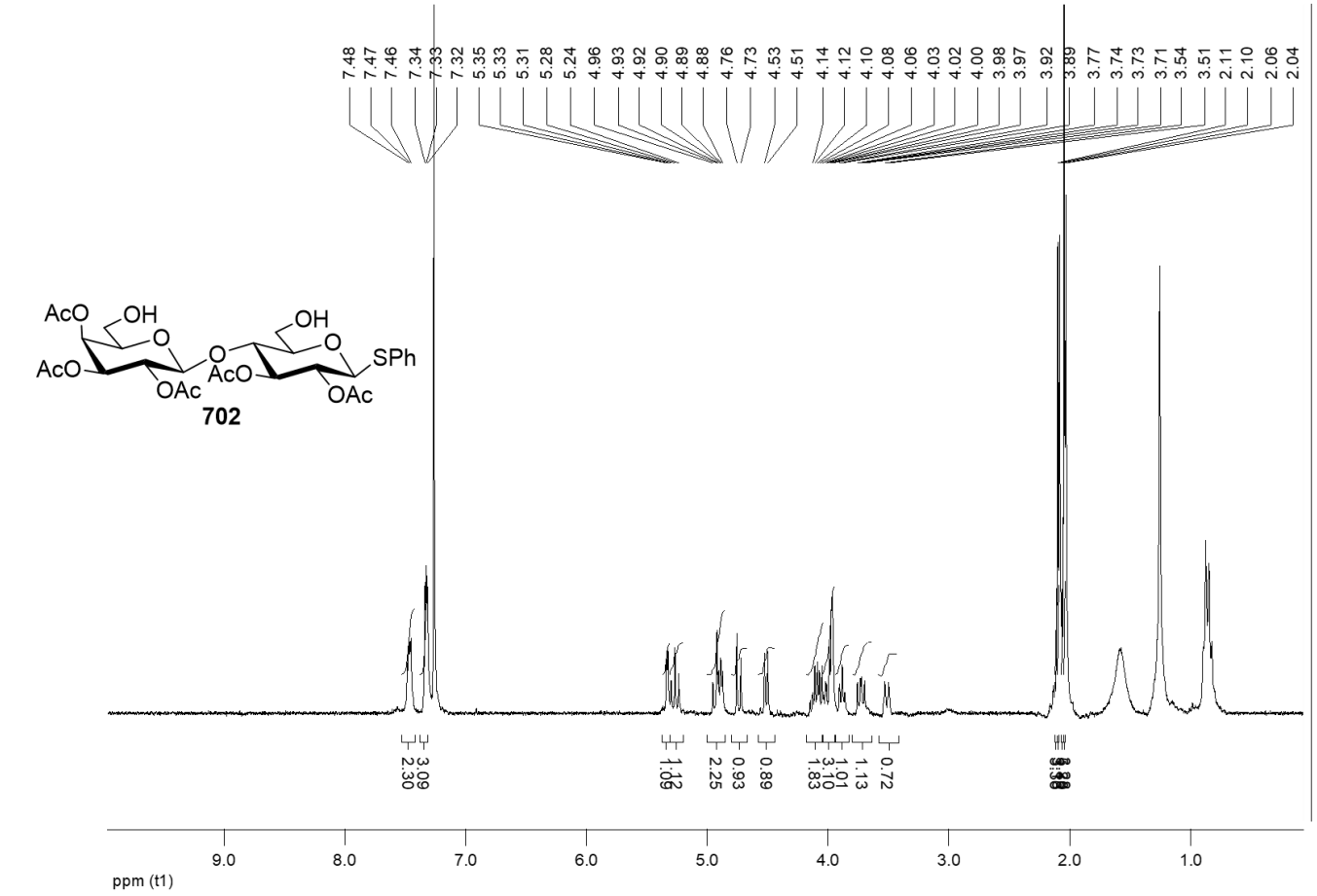
0.0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7.47
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4.93
4.92
4.90
4.89
4.88
4.76
4.73
4.53
4.51
4.14
4.12
4.10
4.08
4.06
4.03
4.02
4.00
3.98
3.97
3.92
3.89
3.77
3.74
3.73
3.71
3.54
3.51
2.11
2.10
2.06
2.04
AcO
OH
O
AcO
OAc
O
AcO
OH
O
SPh
OAc
702
2.30
3.09
1.09
2.25
0.93
0.86
1.83
3.10
1.01
1.13
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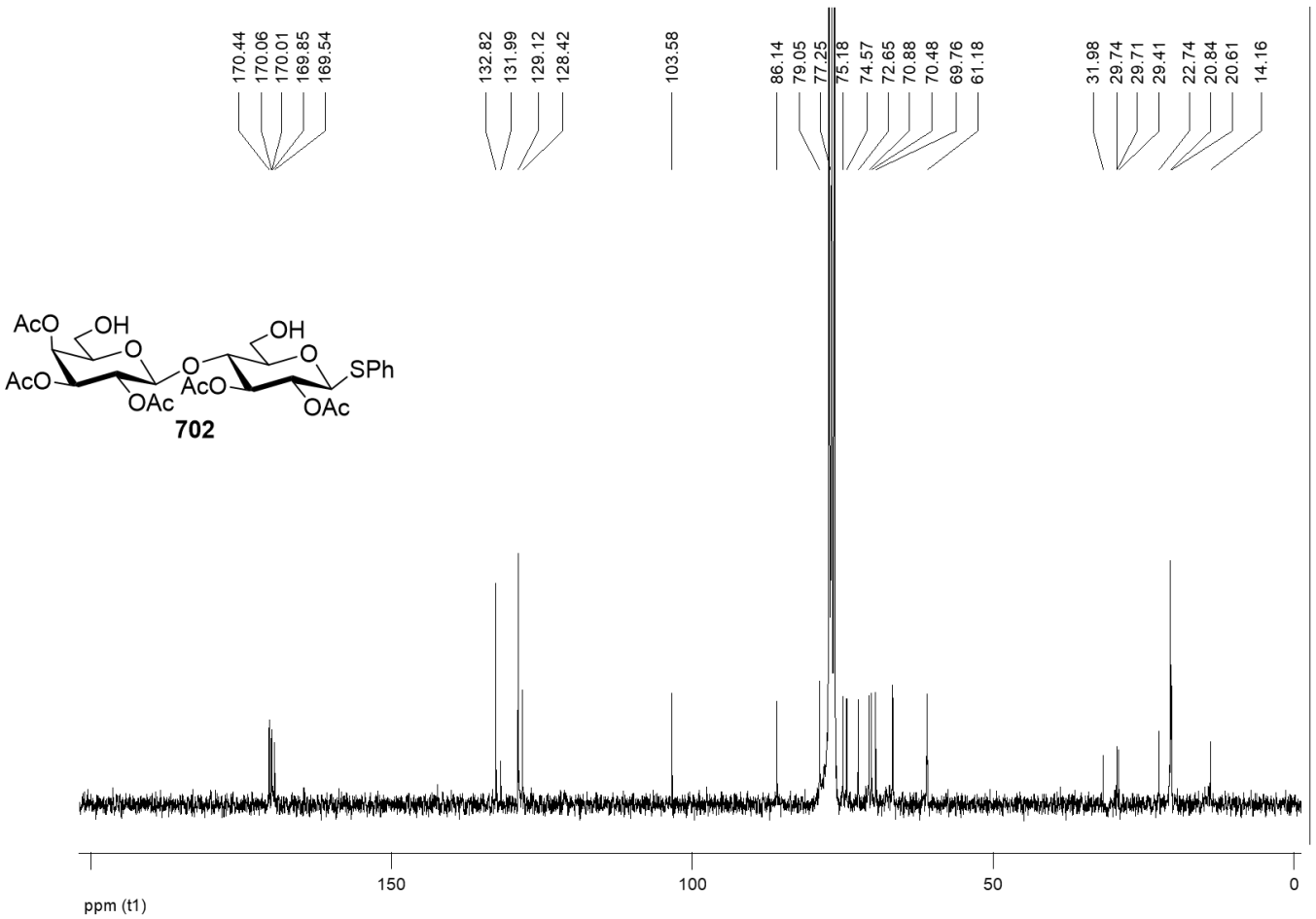
ppm (t1)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103.58
86.14
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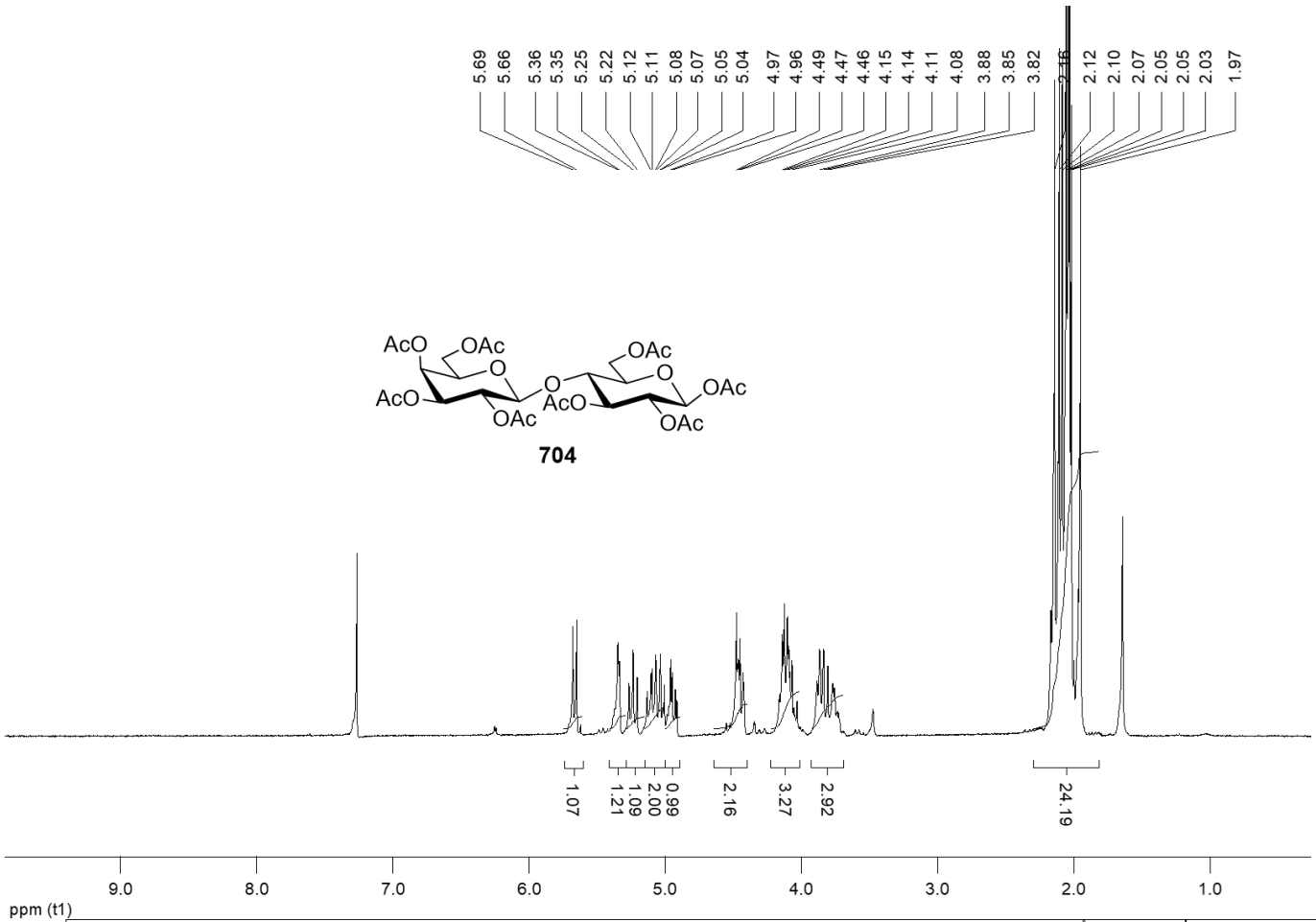
77.25
75.18
74.57
72.65
70.88
70.48
69.76
61.18
31.98
29.74
29.71
29.41
22.74
20.84
20.61
14.16
AcO
OH
O
AcO
OAc
O
AcO
OH
O
SPh
OAc
702
150
100
50
0
ppm (t1)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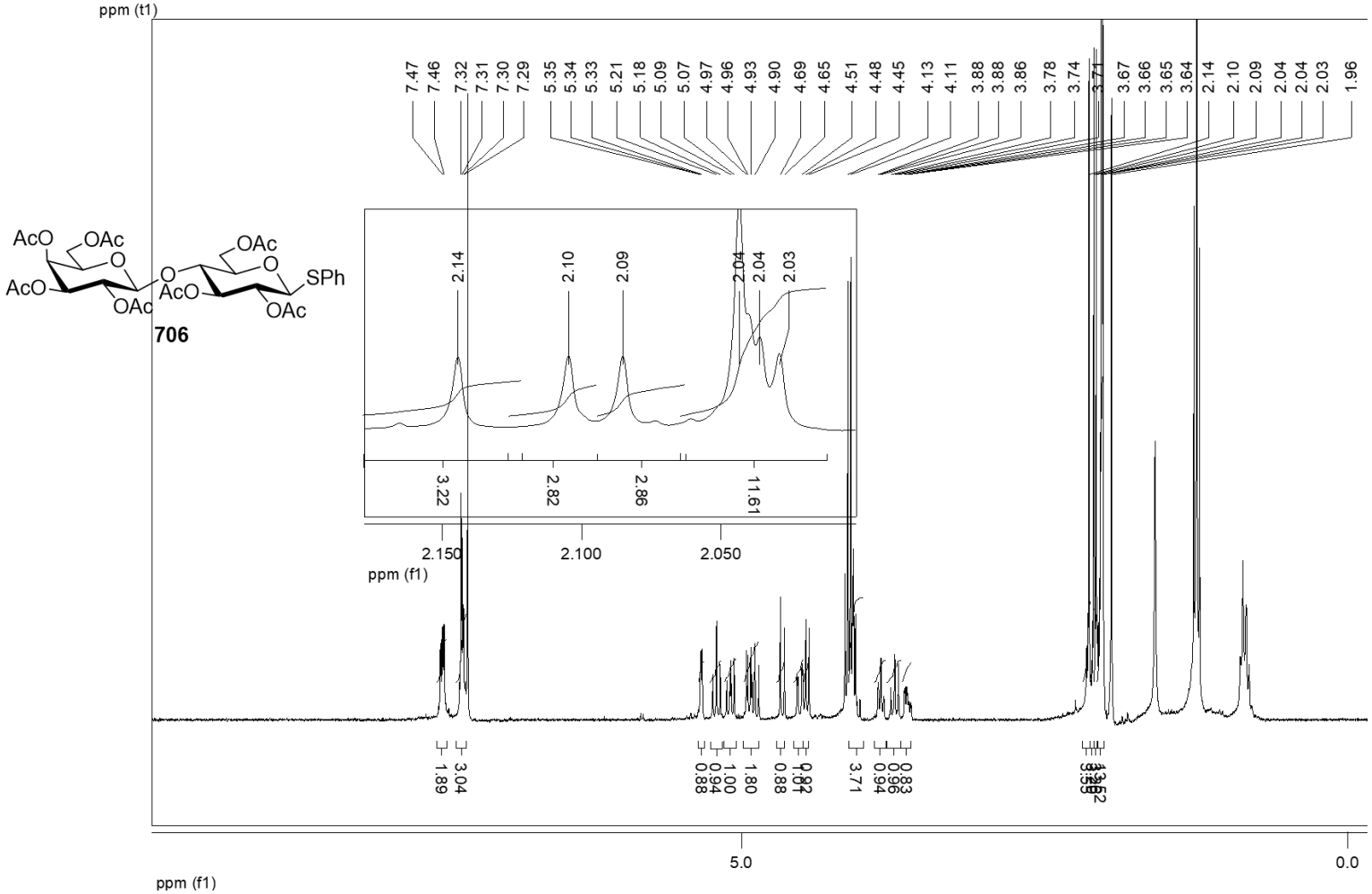
AcO OAc O O OAc AcO OAc AcO OAc OAc
704
1.07 1.21 1.09 2.00 0.99 2.16 3.27 2.92 24.19
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
ppm (t1)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AcO OAc O O OAc AcO OAc AcO OAc SPh
706
2.14 2.10 2.09 2.04 2.04 2.03
3.22 2.82 2.86 11.61
2.150 2.100 2.050
ppm (f1)
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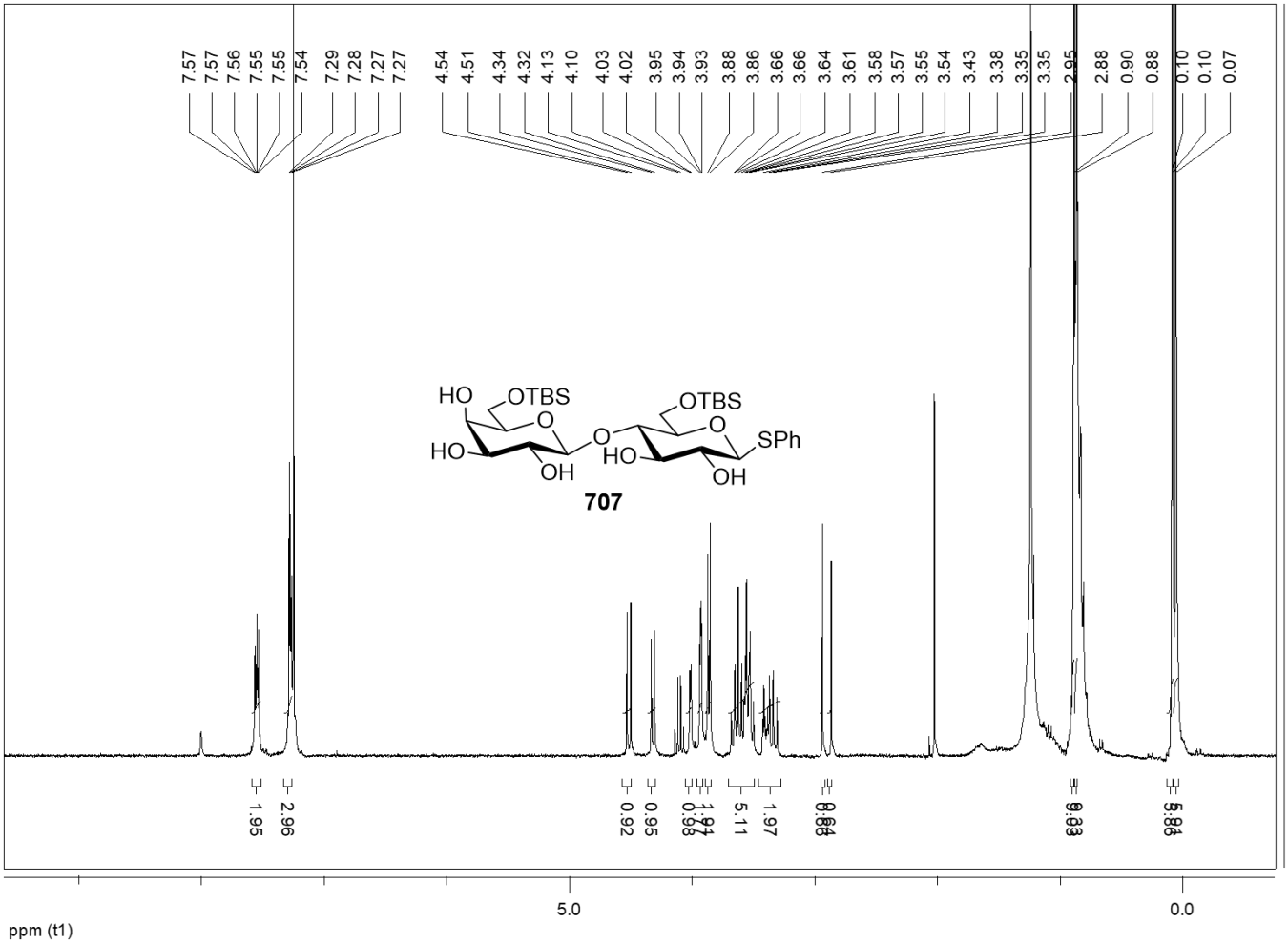
5.0 0.0
ppm (f1)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3.64
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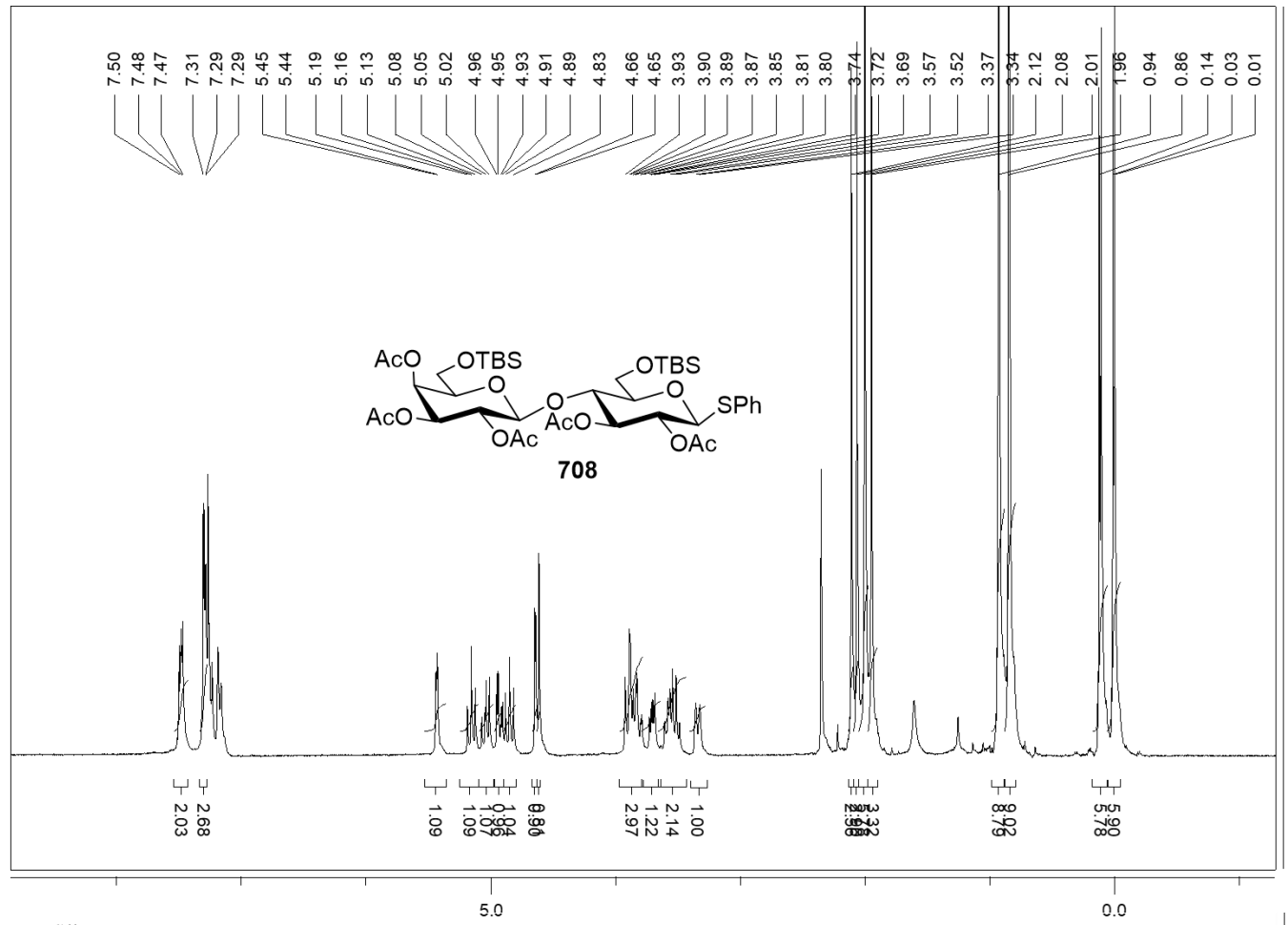
3.58
3.57
3.55
3.54
3.43
3.38
3.35
3.35
2.95
2.88
0.90
0.88
0.10
0.10
0.07
HO
OTBS
O
HO
OH
O
OTBS
O
HO
SPh
OH
707
1.95
2.96
0.92
0.95
1.97
5.11
1.97
9.03
5.86
5.0
0.0
ppm (t1)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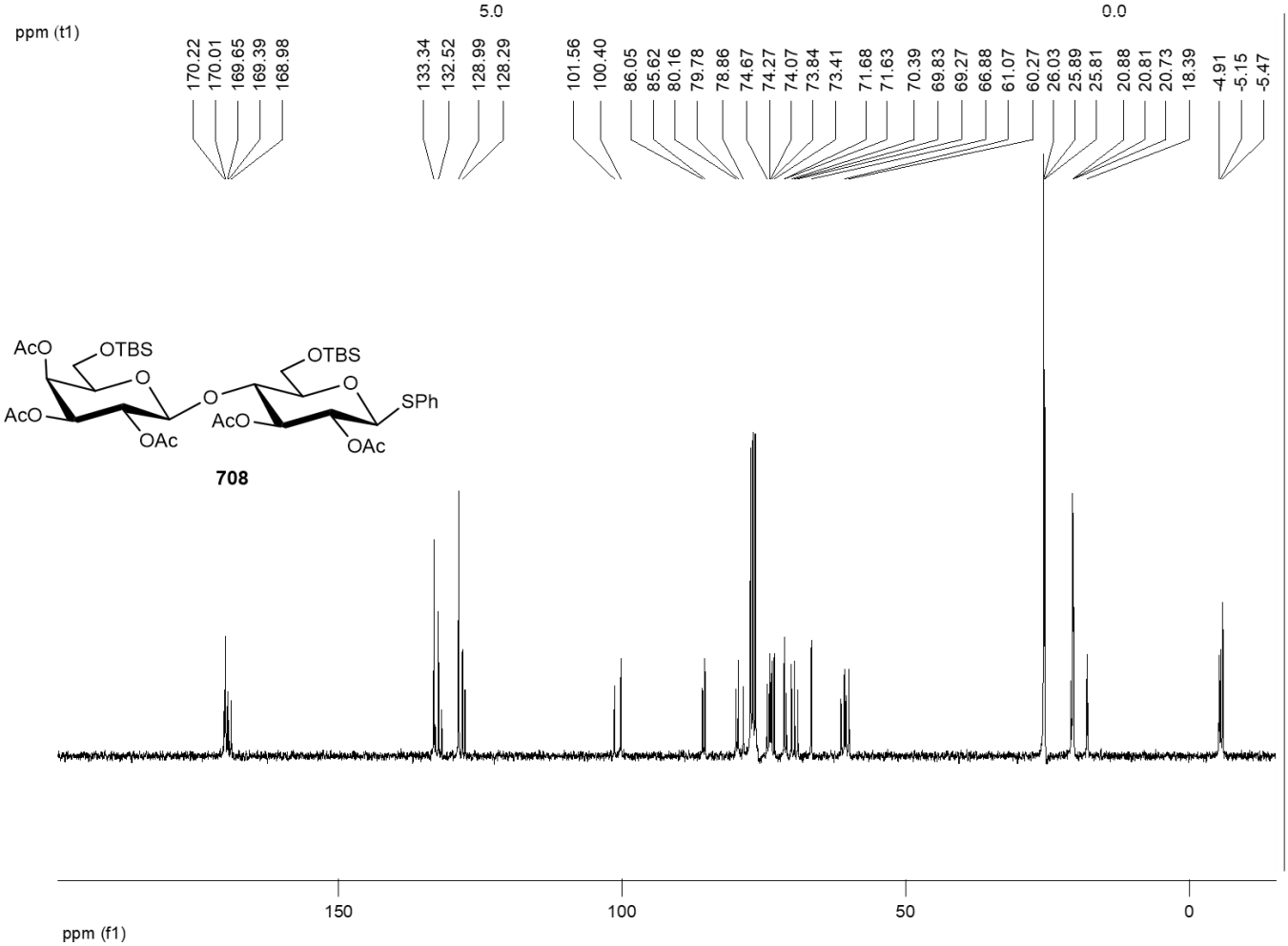
AcO OTBS O OTBS O SPh AcO AcO OAc AcO OAc
708
2.03 2.98 1.09 1.09 1.07 1.04 0.84 0.84 2.97 1.22 2.14 1.00 8.79 9.02 5.78 5.90
5.0 0.0
ppm (t1)


170.22 170.01 169.65 169.39 168.98 133.34 132.52 128.99 128.29 101.56 100.40 86.05 85.62 80.16 79.78 78.86 74.67 74.27 74.07 73.84 73.41 71.68 71.63 70.39 69.83 69.27 66.88 61.07 60.27 26.03 25.89 25.81 20.88 20.81 20.73 18.39 -4.91 -5.15 -5.47
AcO OTBS O OTBS O SPh AcO AcO OAc AcO OAc
708
150 100 50 0
ppm (f1)

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