Studies Directed Towards the Synthesis of Immunologically Relevant Carbohydrates

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Abstract

Tumor cells often express an over abundance of unique carbohydrate structures. It is believed that vaccines targeting these structures could elicit an immune response capable of degrading tumors and eliminating micrometastases. Paramount to this endeavor is the ability to design and synthesize vaccines that can induce an immune response against carbohydrates. This is often difficult for a number of reasons, including: (i) the inherent low immunogenicity of carbohydrates, (ii) challenges associated with isolating and purifying large quantities of naturally occurring polysaccharides to homogeneity, and (iii) the challenges presented by complex carbohydrate synthesis.

This study focuses on the α-Gal epitope, a unique, highly immunogenic carbohydrate that, once incorporated into a vaccine, has the potential ability to stimulate an immune response against a structurally linked tumor-associated carbohydrate antigen. Studies directed towards a one-pot synthesis of the α-Gal epitope were carried out with unsatisfying results. The underlying reasons for the difficulty of this synthesis were elucidated. This led to the development of a Fmoc-based strategy of oligosaccharide synthesis that afforded the pure α-Gal epitope in high overall yield. The synthesized α-Gal epitope derivative was converted into a glycoamino acid and initial studies on the synthesis of a glycopeptide-based antitumor vaccine were performed.

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The work presented here is the culmination of a long, arduous, and more often-than-not frustrating process, one that I would not have been able to complete without the love and support of my family and friends. I'd like to thank my family for their continued love and support, and for the occasional baked good-laden care-package. I'd like to thank the DuValls for opening their home to me and providing me with a beach house to escape to during the summer months. I would also like to thank my friends, "the nerd-herd": Courtney Scherting, Danielle Thompson, Dr. Brandon Thompson, and Dr. Tristan Butler. Lastly, and most importantly, I'd like to thank my soon to be wife, Dr. Jacquelyn A. DuVall, for her unconditional love and support, for helping me enjoy the good

times and for providing me with the encouragement to get through the worst of times.

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Abbreviations

Ab(s)	antibody(s)
Ac	acetyl
AcOH	acetic acid
AgOTf	Silver trifluoromethanesulfonate
aq.	aqueous
Arg	arginine
Asp	aspartate
BCR(s)	B cell receptor
Bn	benzyl
BSP	1-benzenesulfinyl piperidine
^{t}Bu	*tert*-butyl
Bz	benzoyl
^{o}C	degrees Celsius
Cbz	benzyl carbamate
δ	chemical shift in ppm (NMR)
DCM	dichloromethane

DIPEA	*N,N*-diisopropylethylamine
DIC	*N,N'*-diisopropylcarbodimide
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMTST	dimethyl(methylthio)sulfonium trifluoromethanesulfonate
DTBMP	2,6-di-*tert*-butyl-4-methyl pyridine
equiv	equivalents
Fmoc	9-fluorenylmethyl carbonate
g	gram(s)
Gal	galactose
α-Gal	α-Galili
Glu	glucose
h	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*,-tetramethyluronium hexafluorophosphate
HOAt	1-Hydroxy-7-azabenzotriazole
HRMS	high resolution mass spectrometry
Hz	hertz
IgG	immunoglobin G
IgM	immunoglobin M
IR	infrared
J	coupling constant (NMR)
KLH	Keyhole limpet hemocyanin
M	molar (moles per liter)
MeCN	acetonitrile
MHz	megahertz

min	minute(s)
mL	milliliter(s)
mol	mole(s)
mmol	millimole(s)
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
Ph	phenyl
Pro	proline
Pth	pthalimide
Pyr.	pyridine
R_f	retention factor (in chromatography)
RT	room temperature
S_N2	bimolecular nucleophilic substitution
Su	succinimide
TACA	tumor associated carbohydrate antigen
TBDPS	*tert*-butyl-di-methyl silyl
TCA	trichloroacetate
TCR(s)	T cell receptor
Tf	trifluoromethanesulfonate (triflate)
Tf_2O	trifluoromethanesulfonic anhydride (triflic anhydride)
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Troc	trichloroethoxycarbonyl

UV	ultraviolet
Z	carbobenzyloxy

Chapter 1

Introduction

Section 1.1. Vaccination

Vaccination began in the 7th century when Buddhist monks drank snake venom in order to become immune to its effects. Several centuries later, in 1798, Edward Jenner published the results of his studies on inoculation with the cowpox virus as a means of preventing smallpox. Since then, vaccines have become one of the most important methods of improving public health, with only the expansion of access to safe drinking water having had a larger, positive effect on reducing the global mortality rate. [1]

The eradication of polio is an excellent demonstration of the efficacy of vaccines. As recently as 1988, polio paralyzed more than 1000 children globally per day. However, due to vaccination efforts, there are now only three polio endemic countries and the incidence of polio worldwide has dropped by 99%.[2] The measles vaccine is a similar success story. Prior to the development of the measles vaccine, virtually every child contracted measles, leading to ~500,000 cases each year in the US. After the introduction of the measles vaccine in the US in 1963, the incidence of measles decreased tremendously, and in 2000, measles was declared eradicated from the Americas.[3]

Vaccines modulate the immune system to focus it on specific pathogens. To have a discussion about the design and synthesis of vaccines, it is pertinent to first discuss the components and functions of the human immune system.

Section 1.2. Innate Immunity

The innate immune system serves as the human body's first defense against microbial invasion. It is comprised of, among other things, circulating leukocytes, epithelial barriers, and pattern-recognition receptors located in the plasma membranes of normal human tissues (Figure 1.1). While these elements

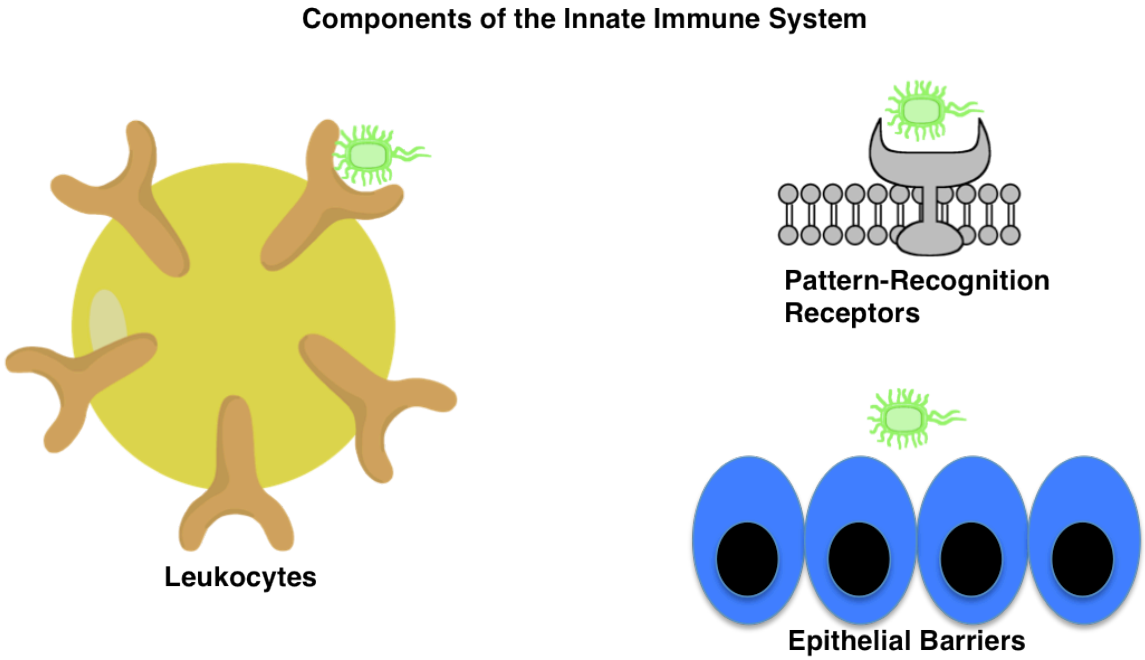


Figure 1.1

recognize motifs that are commonly associated with broad classes of pathogens, they cannot recognize antigens or motifs unique to one specific pathogen. The molecular patterns that are recognized by the innate immune system are genetically determined and, as a result, the innate immune system is limited to recognizing only about 1000 pathogen-associated patterns[4]. Repeated exposure of the innate immune system to an antigen is found to neither increase nor

decrease the magnitude of the immune response generated towards it. Consequently, the innate immune system is not an attractive target for vaccines; a more appealing target for vaccine intervention is found in the adaptive immune system.

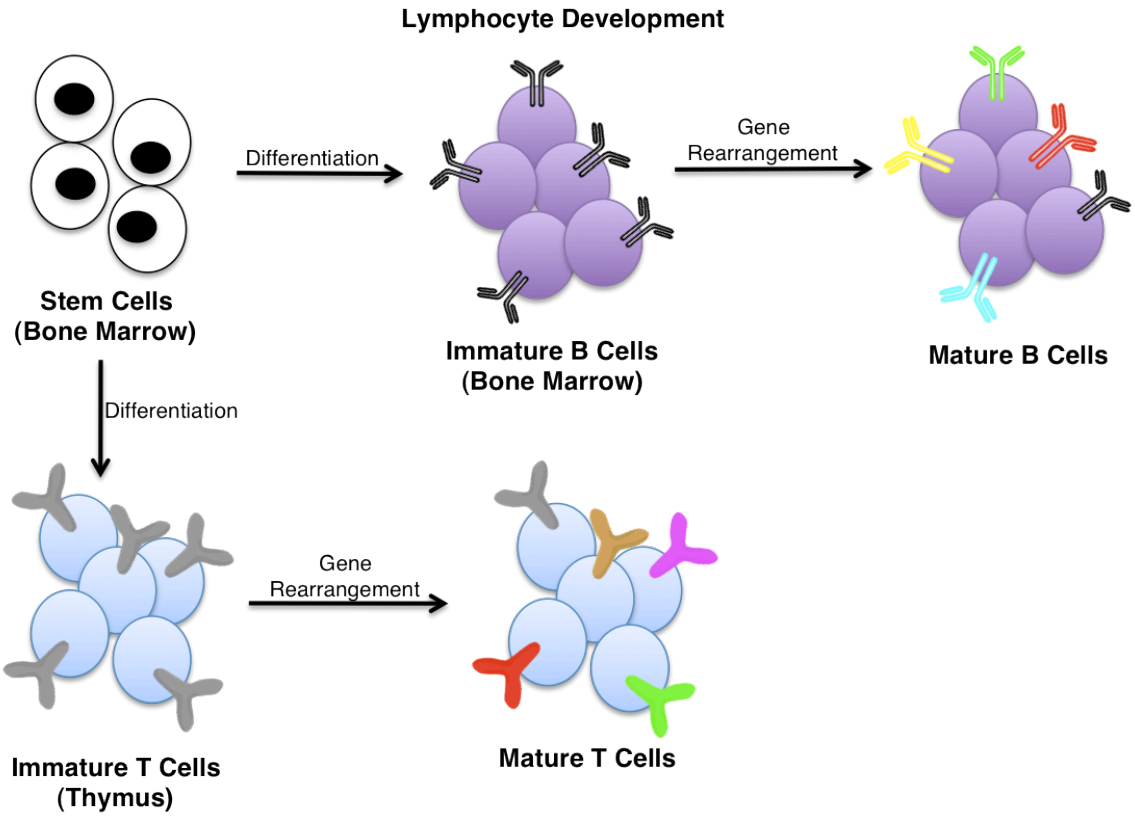
Section 1.3. Adaptive Immunity

The adaptive, (also called the acquired or specific) immune system allows the human body to respond and eliminate new microbial threats that are not recognized by the innate immune system. It is comprised primarily of circulating leukocytes that secrete antigen specific antibodies (Abs). In contrast to the innate immune system, the components of the adaptive immune system are selective enough to be able to detect a specific pathogen. This specificity is derived from antibodies that bind to specific antigens; the portion of the antigen bound by the antibody being the epitope. The adaptive immune response is long lasting and stimulates the growth of memory B cells that confer immunity against a pathogen for the duration of their lifetime. Creation of memory B cells against a specific pathogen is called immunological memory. Before maturing into memory B cells, immature lymphocytes follow a specific development pathway that is crucial to the generation of a long lasting adaptive immune response.

Section 1.4. Lymphocyte Development and The Adaptive Immune Response

The adaptive immune response is relatively slow, typically beginning a few days after exposure to a pathogen, and can confer immunity against a pathogen for months to years. For an adaptive immune response to result in the creation of immunological memory, antigens must be processed through what is known as the T cell-dependent pathway.

Both B and T lymphocytes begin as hematopoietic stem cells in the bone marrow, but while B cells mature in the bone marrow, cells designated to mature into T cells circulate to the thymus where they complete their maturation (Scheme 1.1). During the course of their maturation, both B and T cells undergo a



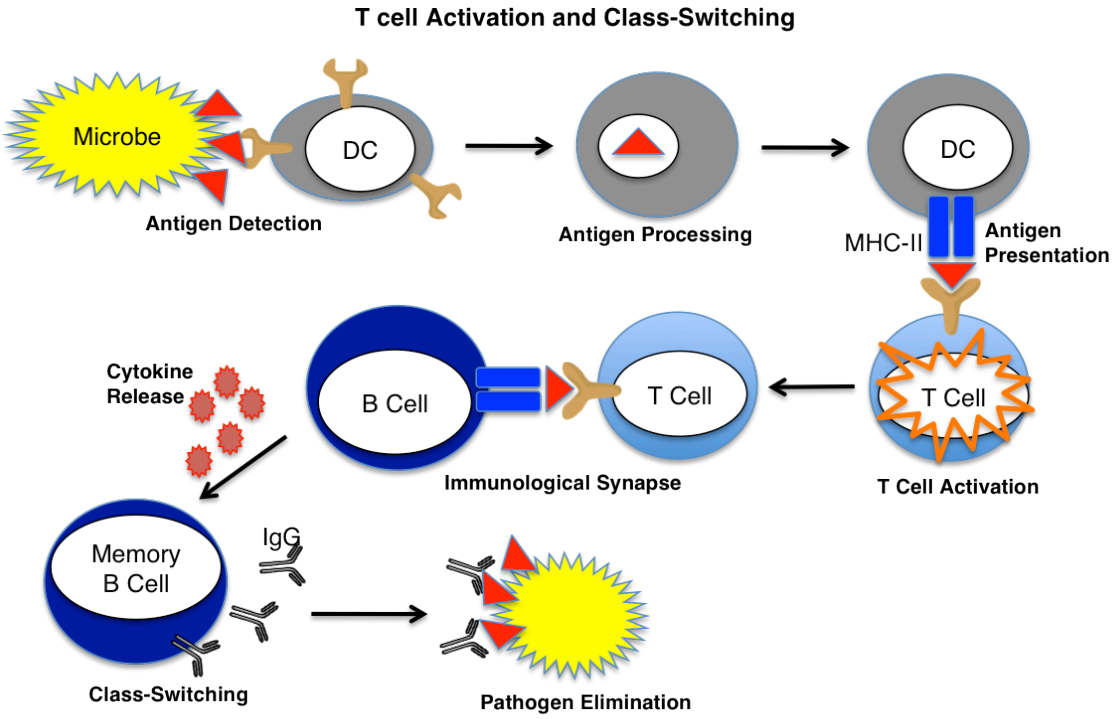
Scheme 1.1

sequence of gene rearrangements that produce many different isoforms of antigen receptors that are displayed on the cell surface. In T cells, these are called T cell receptors (TCRs), and in B Cells, they are termed B cell receptors (BCRs). This process produces up to 10^7 unique B cell and T cell variants that can each bind to a different antigen. Once fully matured, lymphocytes migrate from either the bone marrow or thymus to various lymphatic organs and await activation.

Following migration to the lymphatic organs, B cells await exposure to an antigen that binds their BCRs. The binding of an antigen to a BCR triggers a process in which B cells begin to proliferate and secrete antigen-specific Abs. These activated B cells, termed plasma B cells, have short life spans, and secrete low-affinity IgM Abs. IgM Abs consist as pentamers of five identical antibodies. The individual antibody subunits of an IgM antibody exhibit a low affinity for their target antigen, but together they confer an IgM Ab with a high avidity for its antigen. Consequently, IgM Abs are well suited to recognizing antigens that are clustered or expressed in repeated patterns, but they lack the ability to detect an isolated copy of an antigen on the surface of a pathogen. In contrast, high affinity IgG antibodies are capable of detecting even one single copy of an antigen. Hence, IgG antibodies are necessary for a strong and lasting immune response. Plasma B cells do not express IgG Abs, so they cannot confer immunological memory against a specific antigen. In order to stimulate the

creation of immunological memory, plasma B cells must differentiate into memory B cells, which can secrete IgG Abs. For this to occur, they require further activation by helper T cells.

In contrast to B cells, T cells cannot be activated simply by exposure to their antigen. Instead, T cells require a class of cells known as antigen presenting cells (APCs) for activation (Scheme 1.2). APCs, typically B cells, macrophages, or most commonly, dendritic cells, detect and endocytose specific antigens; once endocytosed, antigens are processed into linear peptides and presented on the surface of the APC by a protein called major hisotcompatability complex II (MHC-II)[5]. Following presentation on the surface of the APC, TCRs can recognize and



Scheme 1.2

bind to the processed antigen. Binding to the MHC-antigen complex causes T cells to become activated; activated T cells go on to stimulate and further enhance the adaptive immune response.

After activation, in addition to proliferating and secreting IgM antibodies, plasma B cells process and present their activating antigen on MHC-II molecules. If the antigen presented on the MHC-II molecule is bound by an activated T-helper cell, an immunological synapse is formed. Upon formation of the immunological synapse, the T-helper cell begins to secrete chemical compounds, known as cytokines, which stimulate the differentiation of plasma B cells into memory B cells and induce the process of class-switching. Class-switching is the process by which the class of antibody secreted by a B cell changes from IgM to IgG. It is this T-cell dependent pathway that vaccines aim to stimulate. Vaccines, including those focused on carbohydrates, are designed with the end goal of having their targeted antigen displayed on MHC-II molecules allowing for the activation of T cells and induction of class-switching and immunological memory.

Section 1.5. Carbohydrates as Vaccine Targets

Many unique carbohydrates are expressed abundantly on the surface of pathogens (Figure 1.2). Additionally, unique carbohydrates can be indicative of the progression of certain disease states; making them intriguing targets for vaccines.[6] The expression of cell surface oligosaccharides is not directly

Carbohydrate Antigens

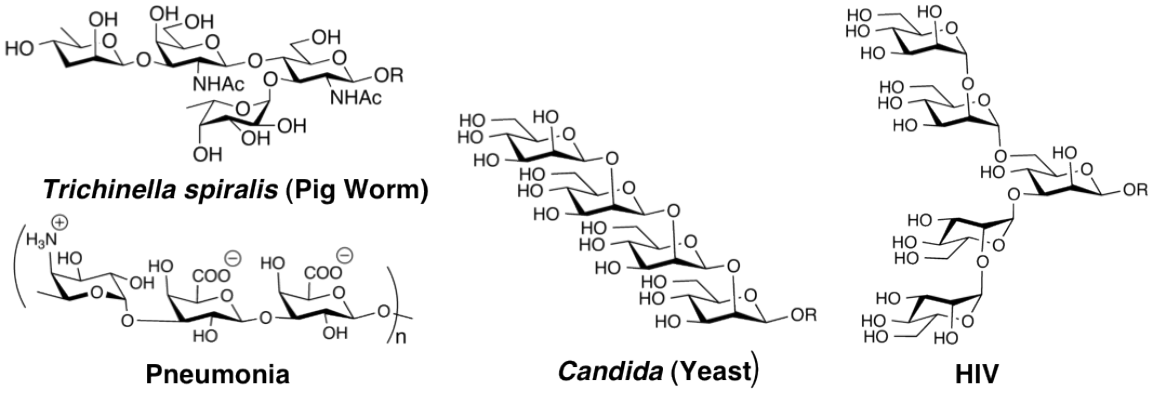


Figure 1.2

genetically controlled, consequently, many microbes cannot adapt to evade a vaccine that targets a specific carbohydrate structure.[7] In fact, vaccines targeting carbohydrates have been successfully developed against *Streptococcuss pneumoniae,*[8] *Haemophilus influenzae* type b, and *Neisseria meningitides,*[9] among others. The success of carbohydrate-based vaccines in the prevention of microbial disease has raised the possibility that these vaccines could be utilized to treat a more complex disease, cancer.

Tumor associated carbohydrate antigens (TACAs), (Figure 1.3), possess, in addition to the advatntages previously described for pathogen-associated carbohydrates, unique traits that make them attractive as vaccine targets: (i) the glycosylation patterns of cancerous cells can vary significantly from healthy cells,[10a,b] (ii) TACAs often play a role in tumor progression making it difficult for tumor cells to shed them and evade immune detection,[11] (iii) a large percentage

of tumor specific Abs are TACA specific,[12] and (iv) the presence of TACA specific Abs correlates positively with increased patient survival.[13] While offering several promising advantages over other vaccine antigens, carbohydrates have a low inherent immunogenicity, making their incorporation into vaccines challenging.[14]

Tumor Associated Carbohydrate Antigens

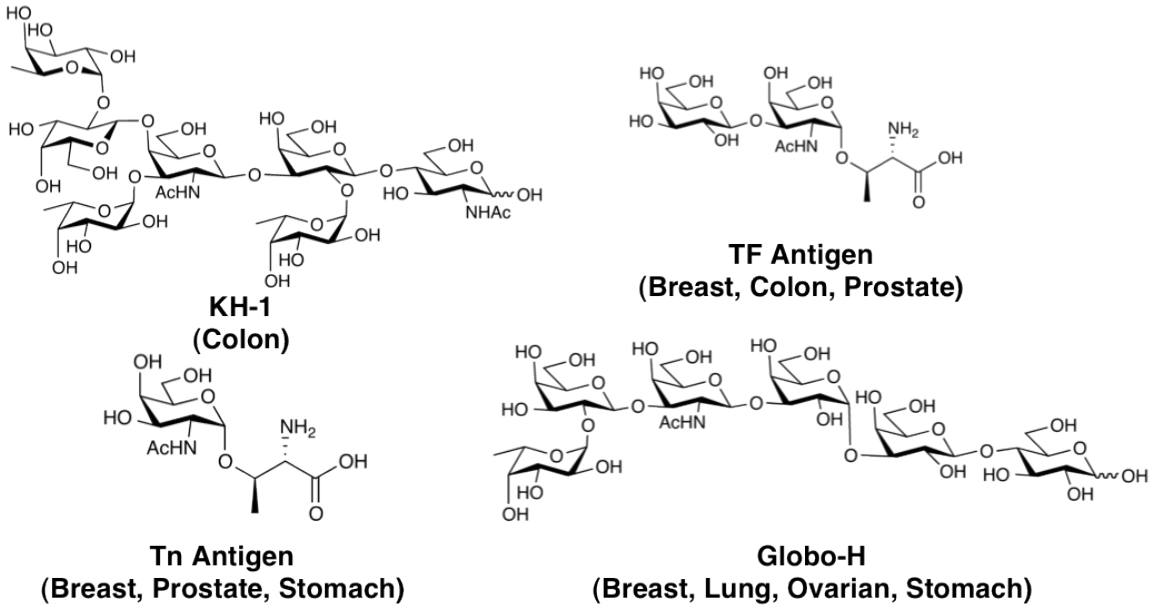


Figure 1.3

Section 1.6. Carbohydrate Immunology

The innate immune system contains pattern recognition receptors, known as C-lectins, which bind to specific pathogen-associated polysaccharides. C-lectins are very efficient at detecting pathogen-associated carbohydrates and tagging their associated pathogen for degradation, but often times this innate immune response does not trigger an adaptive immune response that leads to

the creation of immunological memory.

There are, however, some saccharides that are recognized by the adaptive immune system directly; among them are the blood group antigens, bacterial zwitterionic polysaccharides, α-Rhamnose, and the α-Gal epitope (Figure 1.4). These select carbohydrates can bind to BCRs and elicit a plasma B

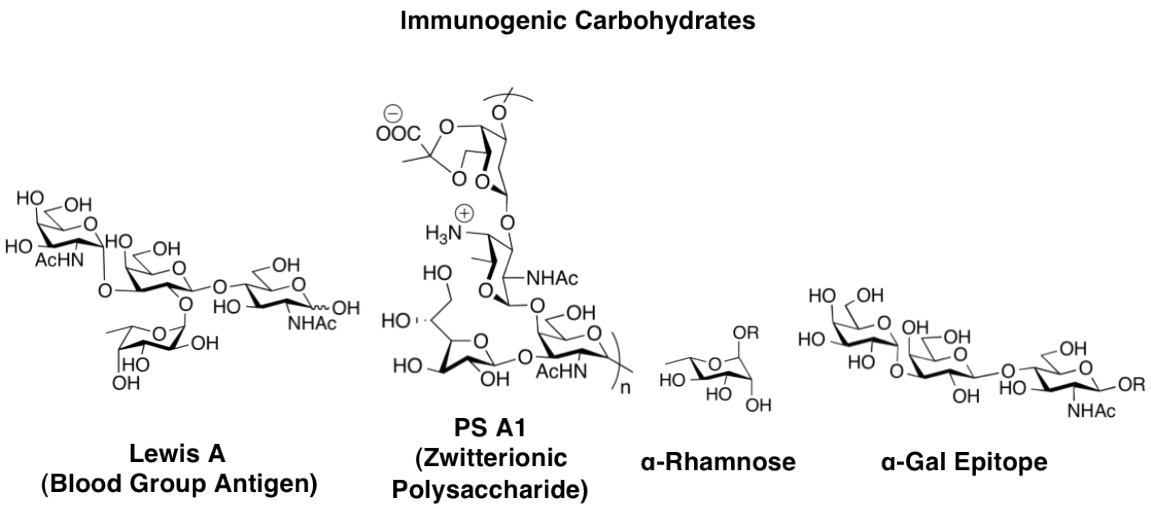


Figure 1.4

cell response, but carbohydrate specific plasma B cells do not get processed through the T cell-dependent pathway. MHC-II molecules, with rare exception,[15a,b] bind to linear peptides,[16] as a result, plasma B cells lack the ability to present carbohydrates on MHC-II. Therefore, carbohydrate specific plasma B cells cannot be bound by T-helper cells and cannot differentiate into memory B cells or undergo the process of class-switching. Consequently, the B cell response to carbohydrate antigens is short lived and does not confer immunological memory[17a,b]. Hence unique strategies are needed to increase the immunogenicity of carbohydrate antigens.

Section 1.7. Augmenting Carbohydrate Immunogenicity

A successful vaccine stimulates a T cell-dependent response against an antigen. Carbohydrates do not stimulate a T cell-dependent response and, therefore, cannot be administered as vaccines in their native form. Instead carbohydrate antigens require conjugation to a helper epitope that can elicit a T cell-dependent response.[18] Typically, these helper epitopes are large carrier proteins such as keyhole limpet hemocyanin (KLH),[19] tetanus toxoid,[20] or diptheria toxin.[21] The Danishefsky group has synthesized several KLH-TACA conjugate vaccines (Figure 1.5).[10,21,22] Higher levels of anti-TACA IgG Abs were detected in the blood sera of mice after inoculation with these KLH-TACA conjugates demonstrating the ability of large carrier proteins to induce class-switching.[23] While carbohydrate-protein conjugates are effective at eliciting a strong T cell-dependent response, it has been demonstrated that the immune

Anti-Lung Cancer Vaccine (KLH Conjugate)[22]

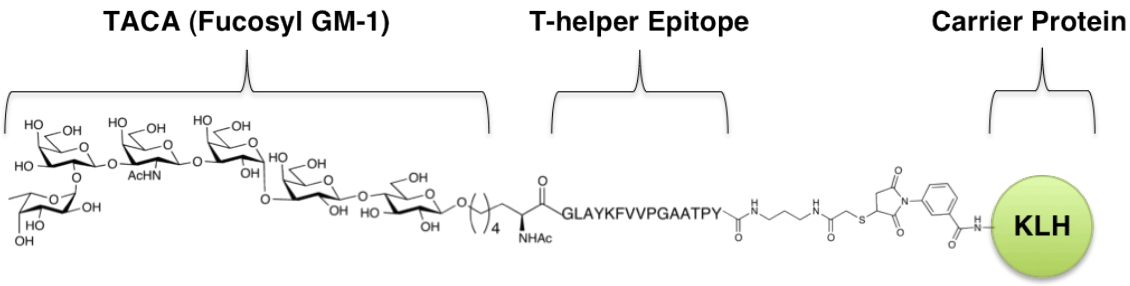


Figure 1.5

response to the carrier protein or even to the connecting linker moiety, can overshadow and suppress the immune response towards the carbohydrate antigen.[24] Hence, novel strategies of inducing a T cell-dependent response have been developed.

One approach that has been employed to circumvent the disadvantages of carrier proteins mentioned above is the use of virus-like particles (VLPs). The laboratory of M.G. Finn has recently synthesized bacteriaphage Qβ capsids that were conjugated to a TACA, the Tn antigen, using click chemistry (Figure 1.6). Subsequent mouse studies revealed that this vaccine could elicit the formation of anti-Tn IgG Abs that were highly specific for the Tn antigen on human leukemia cells.[25] Another strategy that has been developed is the use of subunit vaccines. A subunit vaccine is defined as a vaccine that is composed only of the bare minimum components necessary to elicit the desired immune response.[26] Typical subunit vaccines contain the carbohydrate antigen of interest, a peptide that can activate T-helper cells, and an adjuvant that stimulates the initial immune response. G.J. Boons and coworkers synthesized a subunit vaccine that contained an immunogenic lipid, (Pam_2CysSK_4), that can interact with Toll-like receptors on dendritic cells, a short MHC-II binding peptide, and the Tn antigen.[27] This subunit vaccine was successful in generating anti-Tn IgG Abs in mouse models. The group of Prof. Sucheck recently synthesized and tested a subunit vaccine, also targeting the Tn antigen, that contained a helper peptide isolated

from *Neisseria meningitides*, and an α-Rhamnose moiety as the immunostimulant.[28] The α-Rhamnose residue could be detected by circulating anti-Rham IgG Abs[29], allowing for uptake by APCs, stimulating a T cell-dependent immune response that resulted in the production of anti-Tn IgG Abs.

Alternative Approaches for Inducing Class-Switching

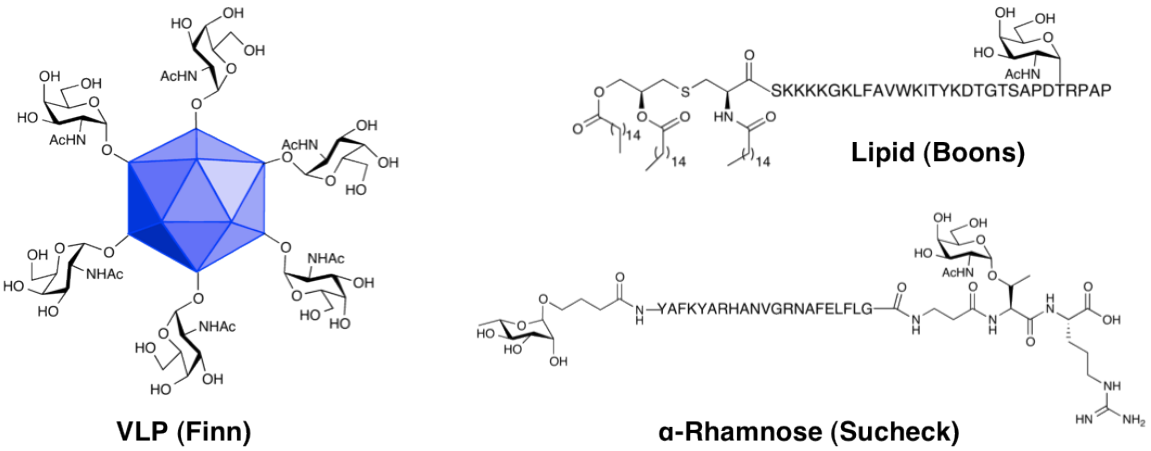


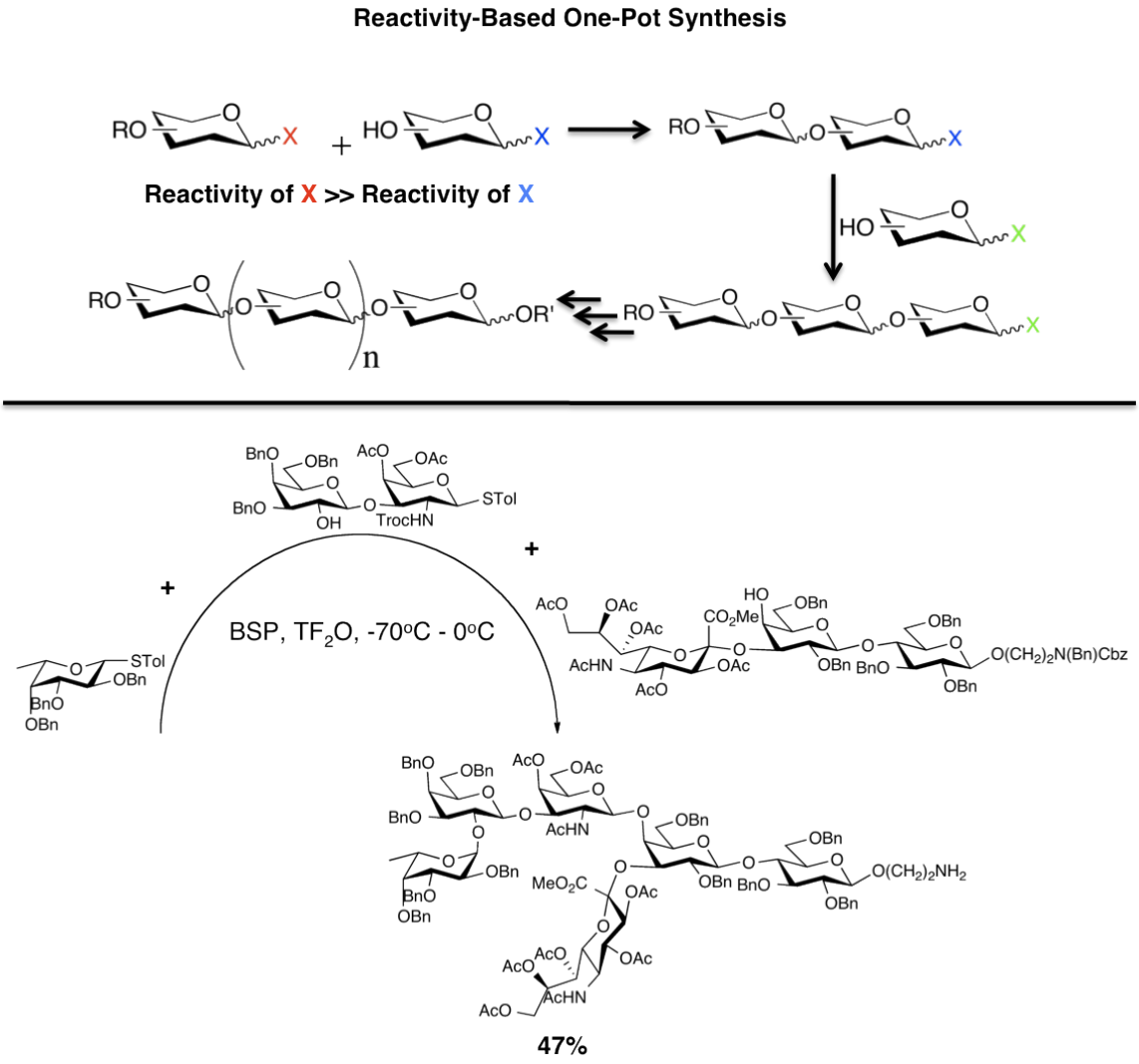
Figure 1.6

Section 1.8. Complex Oligosaccharide Synthesis

Subunit vaccines possess the advantage of being completely synthetic, making them more homogenous and structurally defined than whole cell vaccines or protein conjugates in which the conjugation chemistry can be difficult to control. Importantly, however, synthesis of subunit vaccines requires significant amounts of highly pure carbohydrate antigen. While in theory, carbohydrate antigens can be isolated from natural sources, in practice this is difficult because cell surface carbohydrates are often expressed in low concentrations and in

heterogeneous forms, making them challenging to isolate and purify.[30] Additionally, the naturally occurring form of a carbohydrate may not necessarily allow for the conjugation chemistry required for incorporation into a subunit vaccine. Chemical synthesis has the potential to overcome this problem, but offers numerous challenges, including regioselective protection of hydroxyl groups[31] and stereocontrol of the glycosylation reaction.[32] Despite these hurdles, recent advances in the study of chemical carbohydrate synthesis continue to yield more efficient and complex total syntheses than ever before.

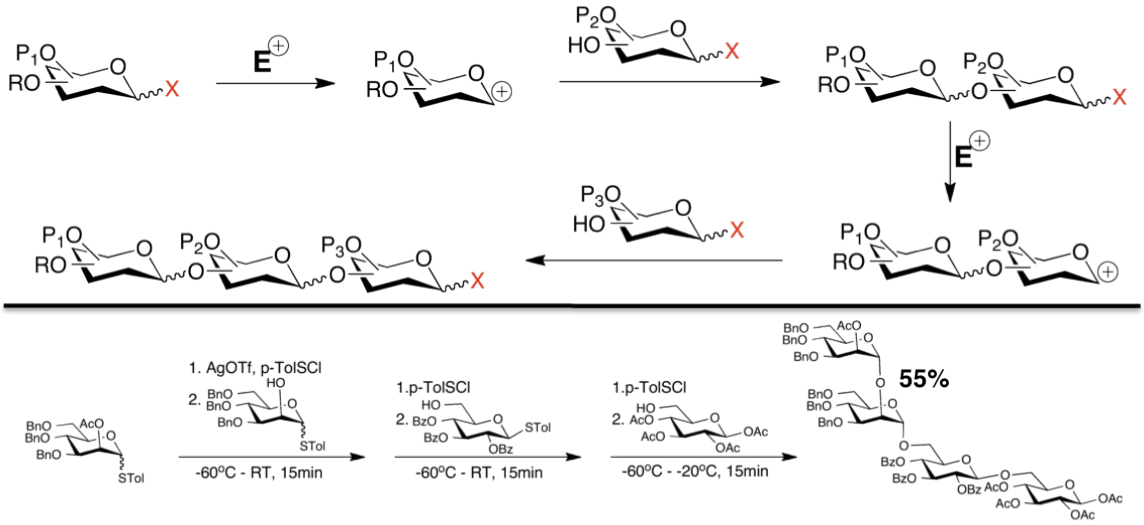
One strategy that has been employed to simplify the process of oligosaccharide synthesis is the use of one-pot methodologies. These strategies eliminate the need for difficult and time-consuming intermediate purification steps. Wong and coworkers measured the relative rates of reactivity (RRVs) of the anomeric position of hundreds of monosaccharides, and using these RRVs, they were able to design and execute chemoselective one-pot synthesis of many complex polysaccharides.[33] This reactivity-based approach was successfully employed to synthesize the Fucosyl-GM_1 antigen,[34] and the Globo-H antigen (Scheme 1.3). The Globo-H antigen was then incorporated onto a microarray that analyzed the antibodies present in blood serum of breast cancer patients.[35] However, because this strategy relies on the diminishing reactivity of carbohydrate building blocks, it is limited in how many sequential glycosylation steps can be carried in one-pot. In order to alleviate this problem while still



Scheme 1.3

maintaining the advantages of a one-pot reaction, Ye and Huang pioneered the iterative pre-activation strategy (Scheme 1.4).[36] In this method, a monosaccharide is converted to its oxocarbenium form first, then a sugar nucleophile is added to it, making the relative reactivities of the two sugars irrelevant to the reaction. To its detriment, this strategy often relies on performing the reaction at low temperatures and requires the use of powerful

Iterative Pre-Activation One-Pot Synthesis[43]

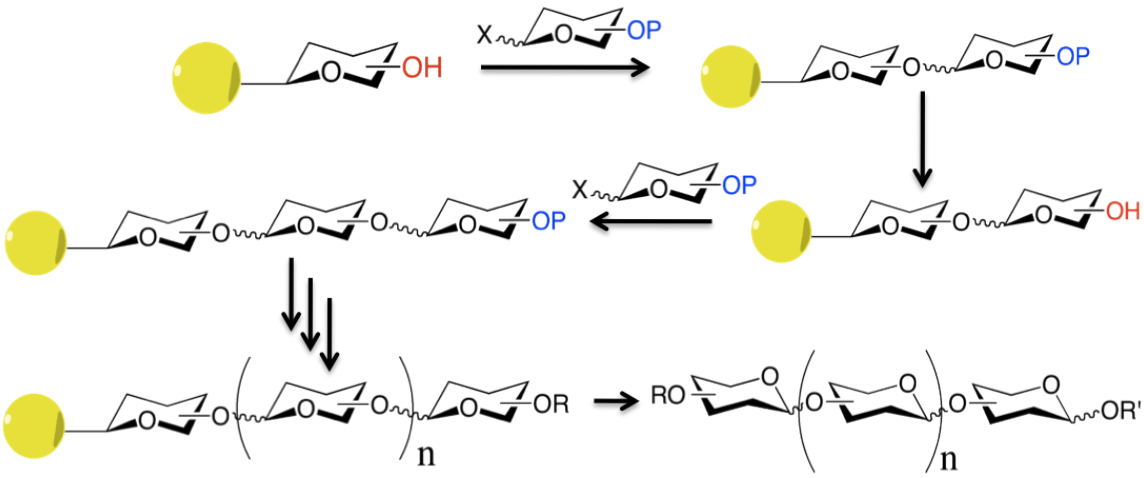


Scheme 1.4

electrophilic reagents that can react with and degrade the furnished oligosaccharide.[37]

A different approach to simplifying carbohydrate synthesis is the use of automated solid-phase oligosaccharide synthesis (Scheme 1.5). While nucleotides and peptides are routinely synthesized by automated solid-phase synthesis, the branched and asymmetric nature of oligosaccharides makes them difficult to synthesize by this method.[38] The Seeberger lab uses monosaccharide building blocks that are regioselectively protected, allowing orthogonal deprotection that yields branched oligosaccharides. They have also developed methods of controlling the stereochemistry of the glycosylation in high-yield.[39] Additionally, because this method relies on a solid-phase resin, with washings between each glycosylation reaction, only one purification step at the end is

Automated Solid-Phase Oligosaccharide Synthesis



Scheme 1.5

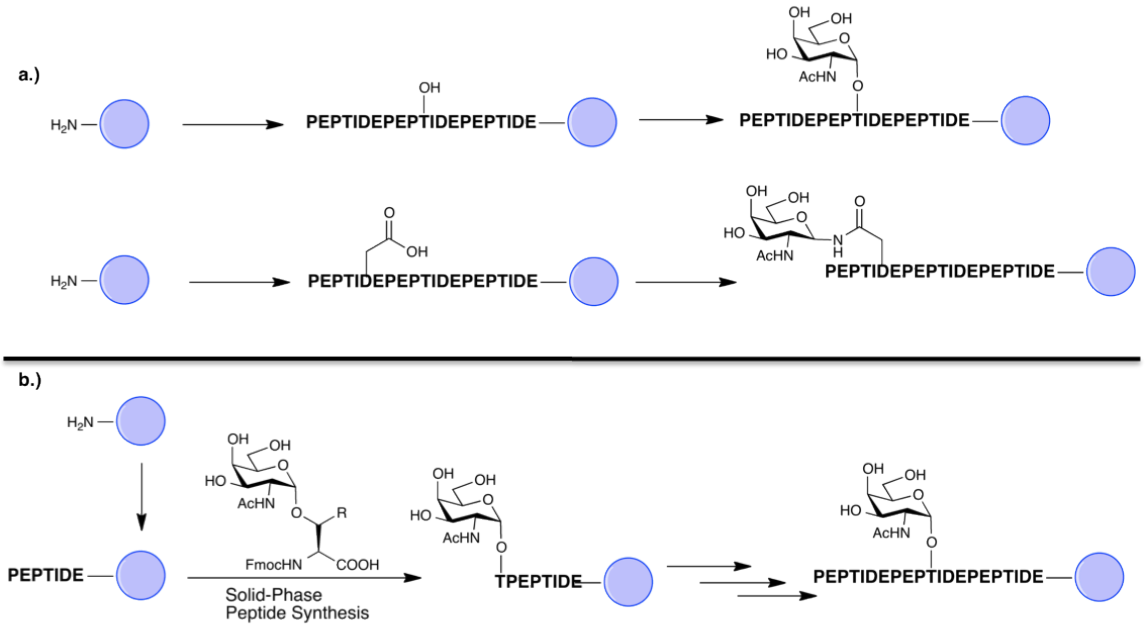
required.[40] However, solid-phase synthesis requires large excesses of monosaccharide building blocks to drive each reaction to completion, and is limited in scale by the loading capacity of the solid-phase resin, illustrating that there is not yet any universal strategy for complex oligosaccharide synthesis, and that novel strategies are needed.

Section 1.9. Glycopeptide Synthesis

In addition to a requirement for chemically pure oligosaccharides, construction of subunit vaccines requires the ability to chemically link the different components of the vaccine. Often this is done using solid-phase peptide synthesis (SPPS); however, much like complex oligosaccharide synthesis, the synthesis of these glycopeptides is fraught with challenges. There are two main strategies used to assemble glycopeptides (Scheme 1.6). In the first method,

assembly of the peptide backbone is followed by chemical or enzymatic glycosylation of specific sequences.[41a-b] This method has been demonstrated to be efficient, but it requires expensive enzymes and UDP-sugars, and this approach is hindered by the limited number of glycosyl linkages that glycosyl transferases can catalyze. Chemical glycosylation of a peptide avoids the problems associated with enzymatic synthesis, but glycosylation of a peptide by chemical means is often low yielding (Scheme 1.6, **a**).[42]

Methods of Glycopeptide Assembly



Scheme 1.6

The second method commonly employed in glycopeptide synthesis is the use of glycosylated amino acid building blocks. This strategy avoids the problems associated with chemoenzymatic synthesis, but it to suffers significant shortcomings. First, glycosylated amino acids are not commercially available,

creating a requirement that complex carbohydrate synthesis be performed in order to access them. As a result, the challenges associated with complex oligosaccharide synthesis are transferred to glycopeptide synthesis.[43] Additionally, coupling of glycosylated amino acids requires prolonged reaction times, and shows enhanced epimerization[44] and β-elimination[45] when compared to coupling of non-glycosylated amino acids. Despite this, the use of glycosylated amino acids offers the most promising approach to accessing chemically pure glycoproteins.[46] Thus the need for efficient syntheses of novel glycoamino acids is highly desirable.

Section 1.10. The α-Gal Epitope

A highly immunogenic carbohydrate, the α-Gal epitope, (Figure 1.7), has the unusual ability to elicit a strong immune response. Discovered in 1984,[47] the α-Gal epitope is found on the tissues of all mammals except for humans, apes, and old world monkeys.[48] It is bound by the anti-Gal Ab, which is found in all humans and constitutes up to 1% of all circulating Abs.[49] It is believed that

The α-Gal Epitope

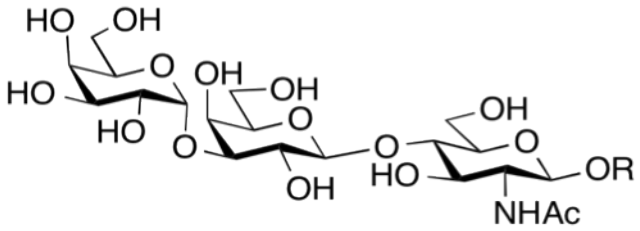


Figure 1.7

immunity to the α-Gal epitope evolved as a method of controlling enterobacteria in the human gut. Polysaccharides on strains of bacteria in human flora provide constant stimulation of the adaptive immune system leading to continual production of the anti-Gal Ab by circulating B cells.[50]

The unique immunogenic properties of the α-Gal epitope have been harnessed in a variety of applications in cancer immunotherapy. Yoshimura and coworkers demonstrated that human pancreatic cancer cells that were genetically altered to express the α-Gal epitope, were susceptible to antibody-dependent cell-mediated cytotoxicity.[51] The laboratory of Uri Galili has demonstrated that α-Gal-containing miscelles can spontaneously insert lipid conjugates of the α-Gal epitope into the membranes of tumor cells, tagging them for destruction by the immune system.[52] Recently, it was demonstrated that α-Gal knockout mice, that can express the anti-Gal Ab, showed an increase in serum levels of anti-BSA IgGs, after being inoculated with α-Gal-BSA conjugates.[53] This illustrated the ability of the α-Gal epitope to augment the natural immune response towards a normally poorly immunogenic protein, BSA. Despite the powerful immunological properties of the α-Gal epitope, there have been, to date, no reported subunit vaccines that feature the α-Gal epitope as a built in adjuvant. The work presented in this dissertation encompasses efforts to develop a novel synthesis of an α-Gal epitope glycoamino acid for use in the synthesis of glycopeptide-based subunit vaccines.

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Chapter 2

One-Pot Syntheses of the α-Gal Epitope

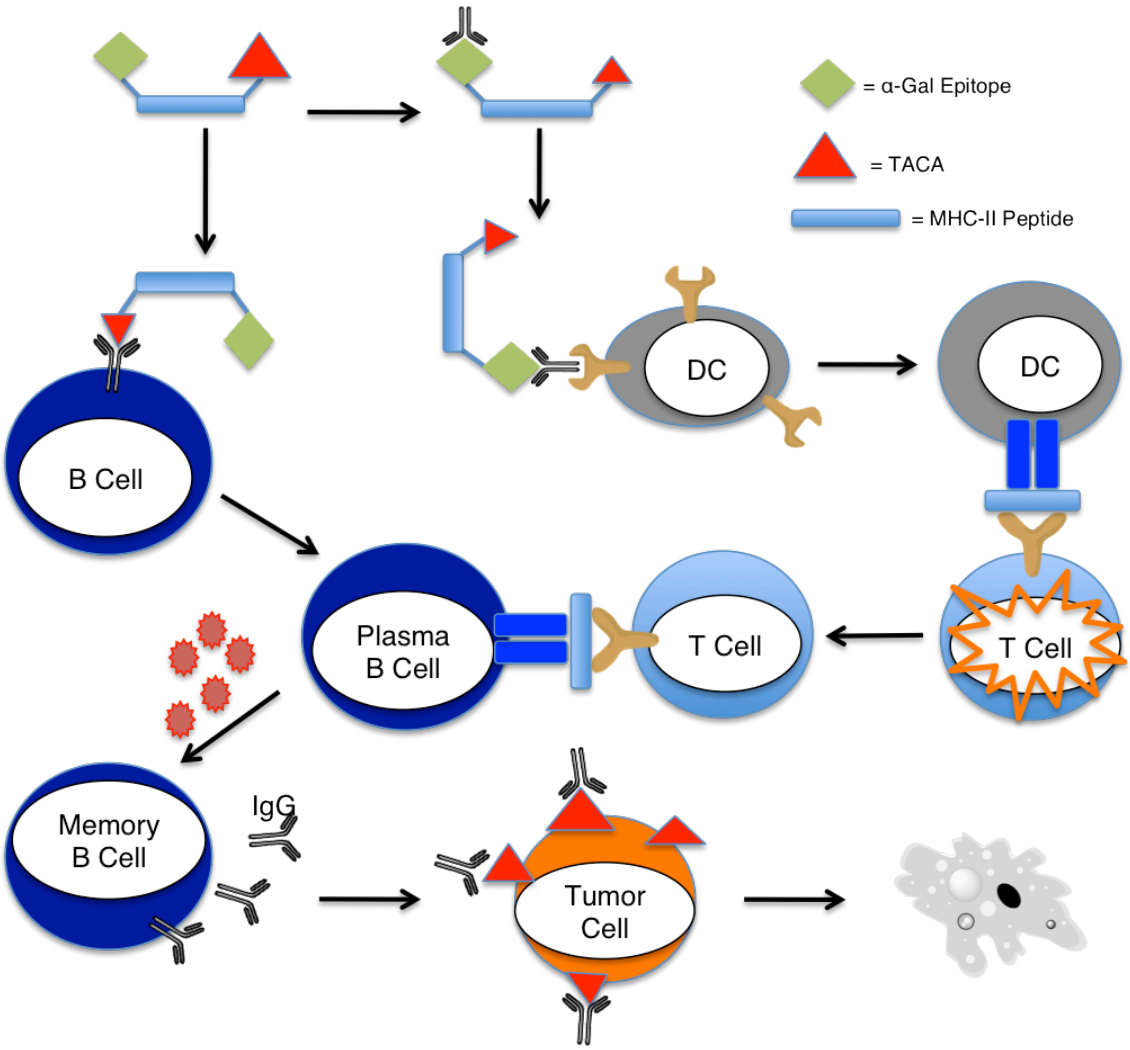
Section 2.1. Introduction

The study of subunit vaccines relies on the ability to synthesize glycopeptides. Consequently, it suffers from the same limitations as glycopeptide synthesis, most significantly, the time consuming and often challenging synthesis of complex carbohydrates. Therefore, novel syntheses of immunologically relevant carbohydrates and glycoamino acids are needed in order to facilitate a greater array of subunit vaccine designs. One such carbohydrate is the α-Gal epitope. Its unique immunological properties make it a logical candidate for use in a subunit vaccine as the built-in adjuvant. The α-Gal epitope can be bound by circulating anti-Gal Abs, which are then detected by Fcγ receptors on the surface of dendretic cells (DCs).[1] A subunit vaccine bearing the α-Gal epitope would, in theory, be detected in this manner leading to uptake by DCs and subsequent presentation of its MHC-II binding peptide to a naïve T cell (Scheme 2.1). This would trigger a T cell-dependent response against the target antigen of the subunit vaccine.[2] However, this subunit vaccine design cannot be tested because there are currently no reports of the synthesis of a glycoamino acid bearing the α-Gal epitope. Thus a synthesis of this glycoamino acid would be valuable.

Section 2.2. Definitions

To facilitate the discussion of carbohydrate synthesis that follows, it is worth defining here, a few terms employed by carbohydrate chemists: glycosyl donor, glycosyl acceptor, activation, armed, and disarmed (Scheme 2.2). The

Potential Anti-TACA Subunit Vaccine Bearing α-Gal as a Built-in Adjuvant

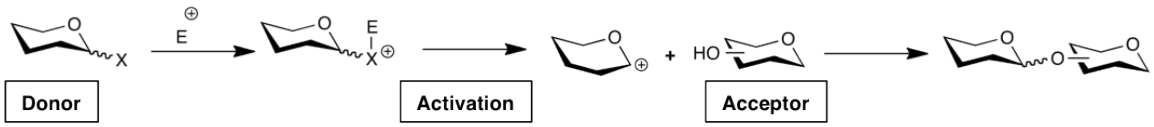


Scheme 2.1

"glycosyl donor" is a carbohydrate that has an anomeric leaving group and forms an oxocarbenium ion (Lewis acid) during a glycosylation reaction. "Glycosyl acceptor" refers to the nucleophile (Lewis base) in the glycosylation reaction. The activation of a glycosyl donor is the complexing of an electrophilic promoter to an anomeric leaving group and the subsequent formation of an oxocarbenium ion. Lastly, "armed" refers to glycosyl donors that have electron-donating protecting

groups. Armed donors tend to be more susceptible to activation. “Disarmed” donors possess electron withdrawing protecting groups and tend to be less reactive.

Terms in Carbohydrate Chemistry

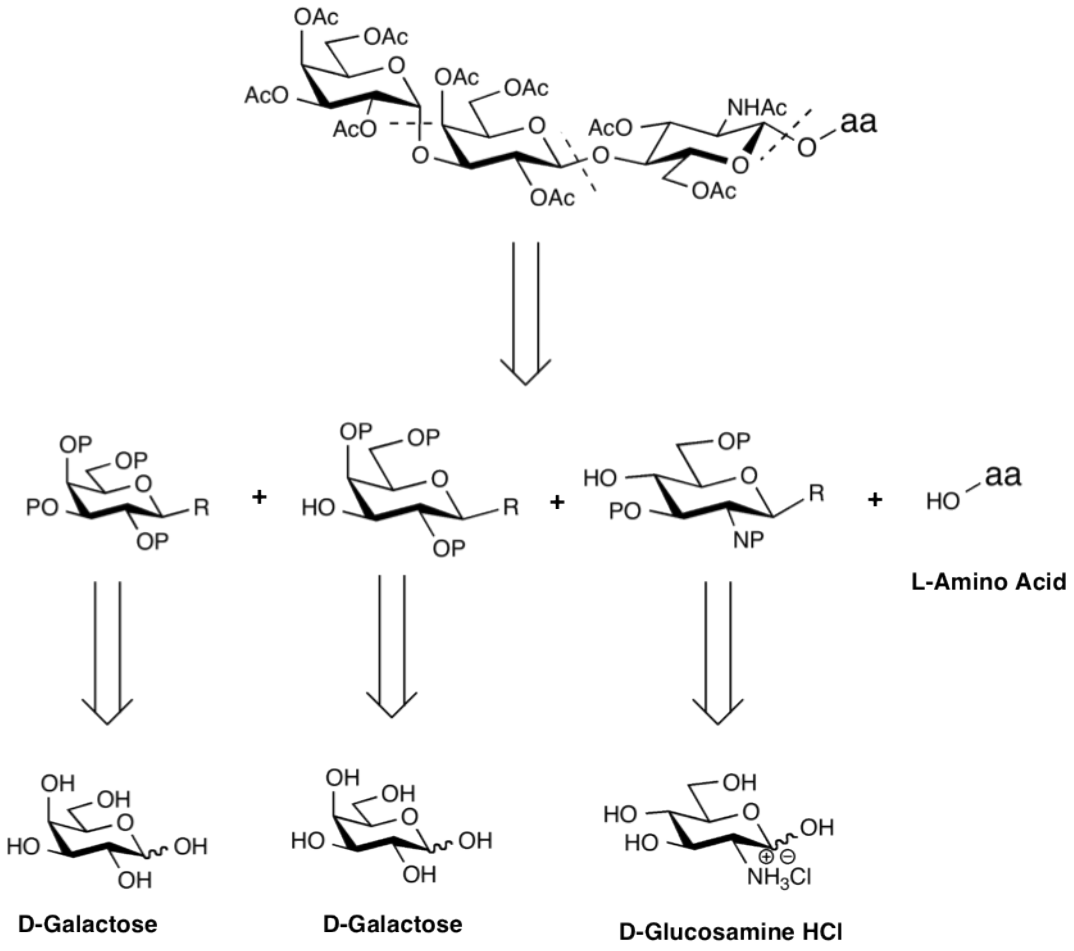


Scheme 2.2

Section 2.3. Synthetic Planning

Synthesis of an α-Gal glycoamino acid requires the synthesis of the α-Gal trisaccharide. A retrosynthetic analysis of an α-Gal glycoamino acid is shown in Scheme 2.3. The α-Gal epitope can be synthesized from two protected galatosyl residues, a protected glucosamine residue, and a suitably protected amino acid. The two galatosyl residues can be synthesized from D-galactose, while the glucosamine residue can be realized from D-glucosamine HCl. While regioselective protection of the monosaccharide building blocks may be difficult and time consuming, the most challenging aspect of a synthesis of the α-Gal epitope is the formation of the three glycosidic bonds: Gal-α(1-3)-Gal, Gal-β(1-4)-Gln, and Gln-β(1)-amino acid. The difficulty in forming these glycosidic bonds is two fold: high yields are often elusive, and control of the stereochemistry of the reaction can be demanding.

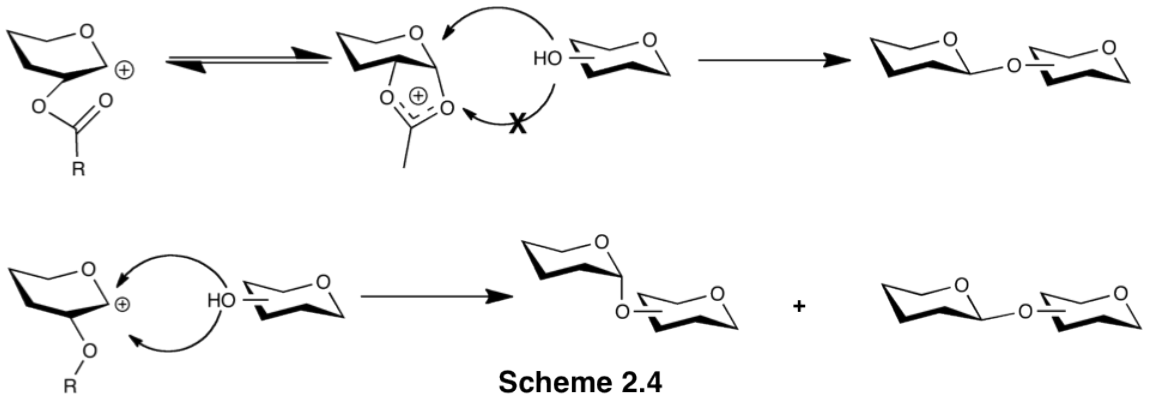
Retrosynthesis of an α-Gal Glycoamino Acid



Scheme 2.3

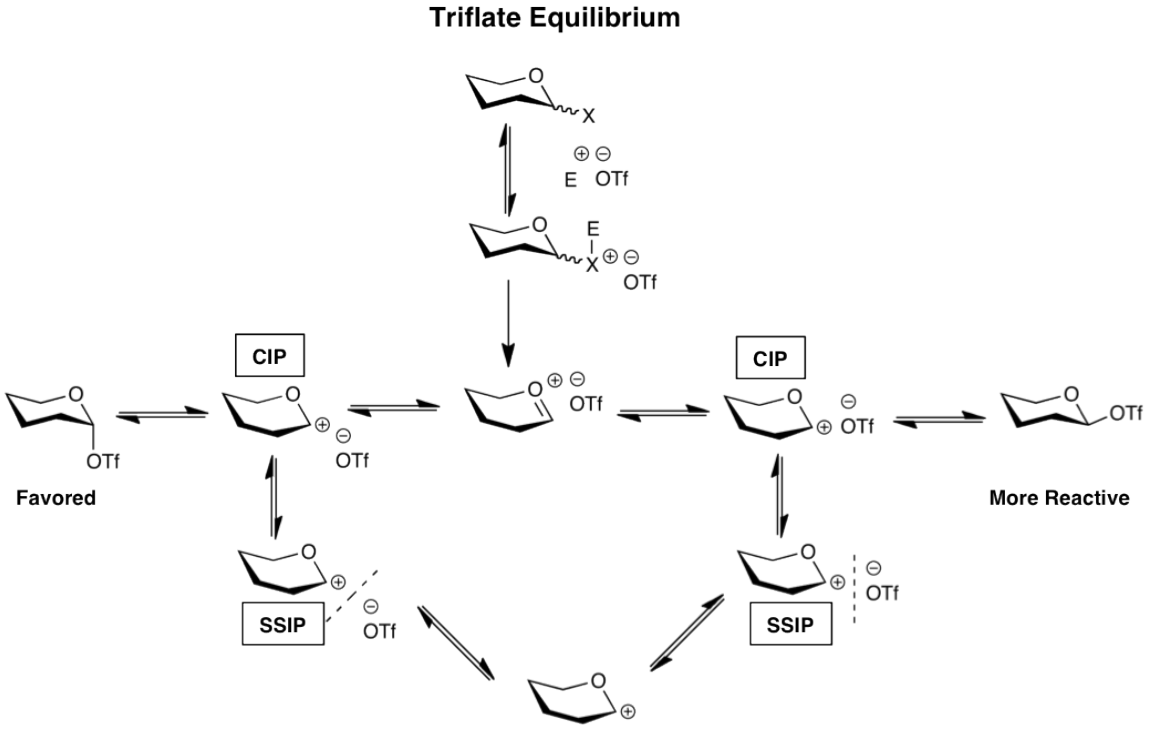
The stereoselectivity of the glycosylation reaction can be controlled as shown in Scheme 2.4. An acyl protecting group at the C2 position, through the formation of a dioxalenium ion, can shield the α-face of the oxocarbenium ion, leading selectively to the formation of the β-glycoside.[3] Hence, the galactosyl donor involved in the formation of the Gal-β(1-4)-Gln bond as well as the

Stereocontrol In the Glycosylation Reaction



Scheme 2.4

glucosamine residue must possess an acyl protecting group at C2 to favor the formation of the desired β-glycosides. Formation of an α-glycosidic bond cannot be controlled as easily. Oxocarbenium ions with a non-participating protecting group at C2 cannot form a dioxalenium ion; instead thermodynamic and kinetic forces dictate the stereoselectivity of the reaction. When a glycosyl donor is activated, it forms an oxocarbenium ion. If a glycosyl acceptor is present in solution when the donor is activated, it can attack either the α or β face of the oxocarbenium ion. Attack of the α-face is favored kinetically, and, due to the anomeric effect, the resulting α-glycoside is also the thermodynamically favored product. Activation of a glycoside in the absence of a nucleophile, leads to the establishment of the equilibrium shown in Scheme 2.5. In this equilibrium, the oxocarbenium ion and a triflate counter-ion equilibrate between an α and β-close ion pair (CIP), an α and β-solvent separated ion pair (SSIP), an α and β-covalent triflate, and a free oxocarbenium ion.[4] Due to the anomeric effect, the equilibrium favors the α-triflate. When a nucleophile is introduced into the reaction, it can

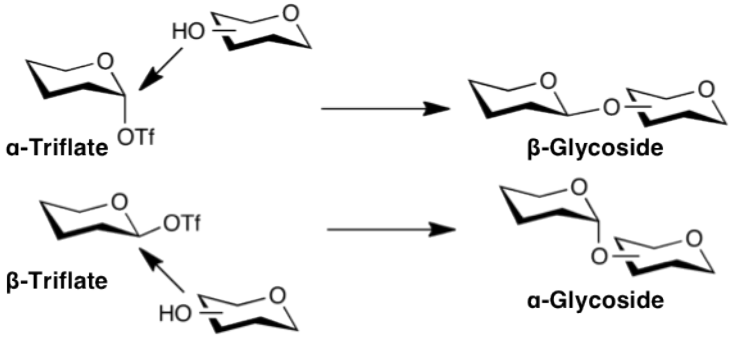


Scheme 2.5

displace the triflate of the CIP, SSIP, or covalent triflate through a S_N2-like mechanism. When an α-oriented triflate species is displaced through a S_N2-like mechanism, it leads to the formation of a β-glycoside. The β-oriented triflate species, conversely, when displaced through a S_N2-like mechanism give an α-glycoside (Scheme 2.6). The anomeric bond of the β-triflate is less thermodynamically stable than that of the α-triflate, as a result, the β-triflate can be nucleophilicly displaced more easily. It follows then that despite the presence of a larger amount of the α-triflate at equilibrium, the β-triflate selectively reacts, leading to the formation of mainly the α-glycoside.[5] However, this pathway is not as α-selective as the pathway in which the oxocarbenium ion is intercepted by a

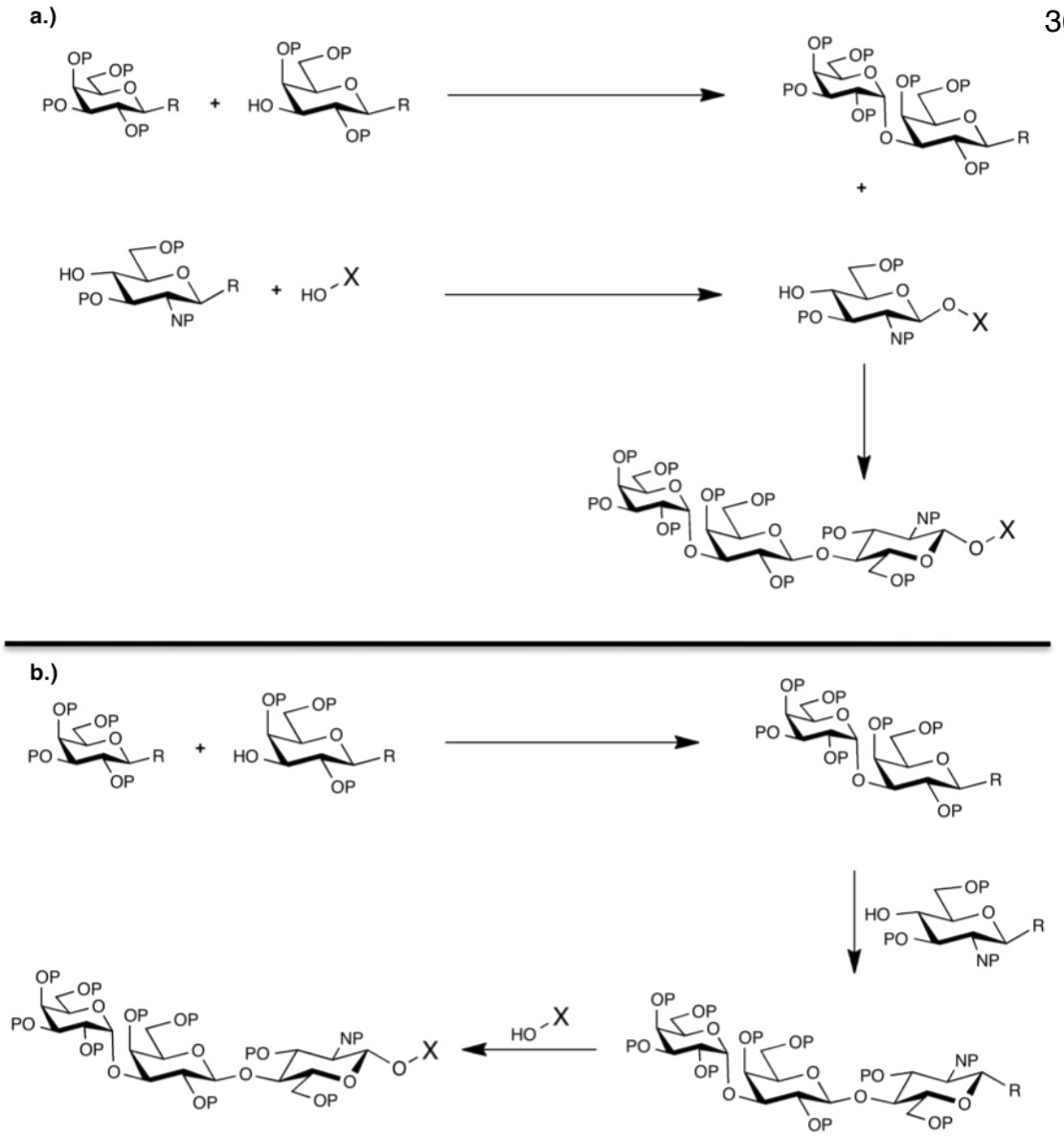
glycosyl acceptor before the establishment of the triflate equilibrium. Thus the galactosyl donor used in the formation of the Gal-α(1-3)-Gal bond needs to possess a non-participating protecting group at the C2 position, and it must be activated in the presence of a glycosyl acceptor, to favor the formation of the α-glycoside.

S_N2-Like Displacement of Glycosyl Triflates



Scheme 2.6

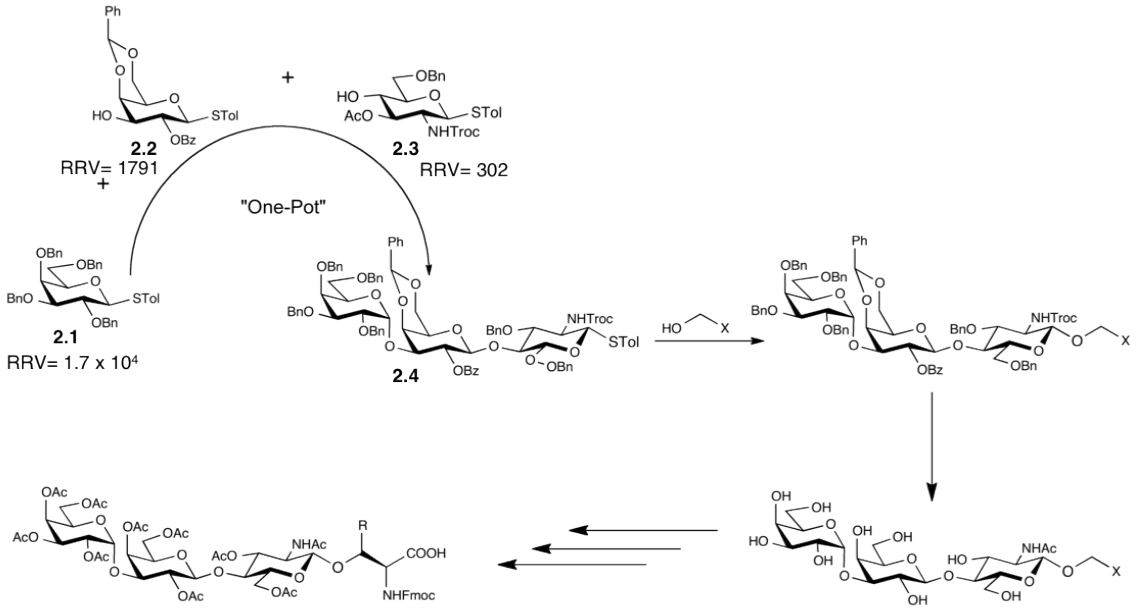
There were three criteria that were desired in a novel synthesis of the α-Gal epitope: (i) efficiency, (ii) scalability, and (iii) the ability to allow for a variety of amino acids to be conjugated. It was thought that the third criteria would be advantageous in glycopeptide synthesis. Although there were previously reported syntheses of the α-Gal epitope, they had all been convergent syntheses (Scheme 2.7, **a**).[6] It was hypothesized that a linear synthesis of the α-Gal epitope (Scheme 2.7, **b**), would more readily meet the desired criteria as it had the potential to be more efficient, and significantly, it would more easily allow for variability of the amino acid residue. With this in mind, an initial synthetic plan



Scheme 2.7

was designed in which residues **2.1**, **2.2**, and **2.3**, would, in one-pot, be assembled into trisaccharide **2.4** (Scheme 2.8). Monosaccharides **2.1**, **2.2**, and **2.3** were chosen because their RRVs decrease successively, allowing for a reactivity-based one-pot synthesis.[7] Additionally, **2.2** and **2.3** have acyl protecting groups at their C2 position, allowing for the formation of β-glycosidic bonds, while **2.1** had a non-participating C2 group, which favors the formation of an

Initially Planned Synthesis of α-Gal Amino Acid



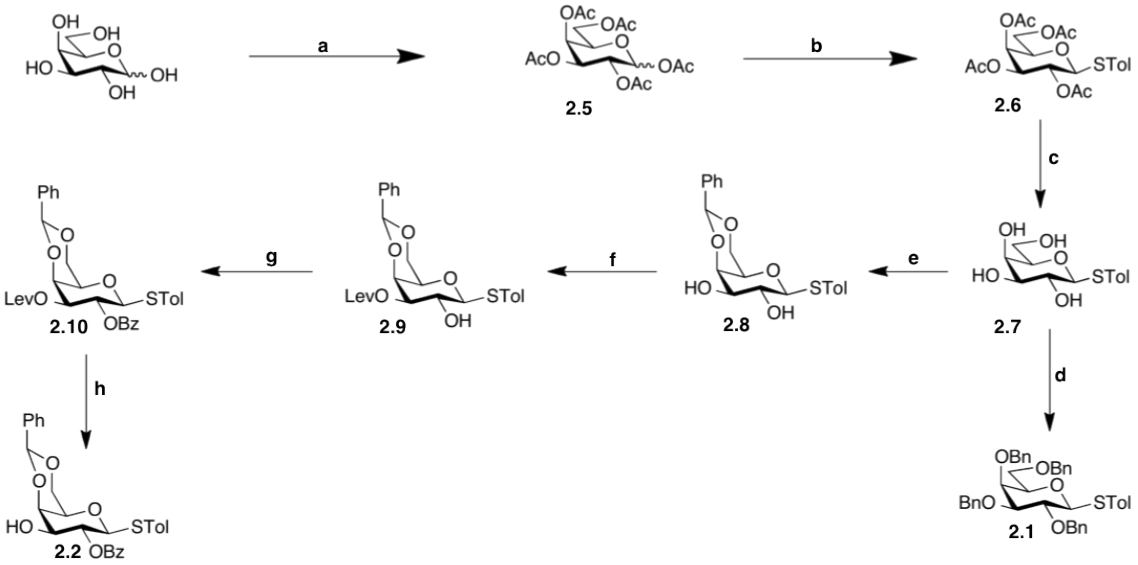
Scheme 2.8

α-glycosidic bond. Following the one-pot synthesis of **2.4**, an amino acid would be incorporated. Lastly, this glycoamino acid would be deprotected and reacetylated to yield an α-Gal glycoamino acid that could be used in the synthesis of a subunit vaccine.

Section 2.4. Synthesis of Monosaccharides 2.1, 2.2, and 2.3

The synthesis of **2.1**, and **2.2** began with the acetylation of D-galactose, followed by substitution of the anomeric acetate by p-toluenethiol (Scheme 2.9).[8] The acetates were removed under Zémplen conditions, and for **2.1**, this was followed by benzylation with benzyl bromide and sodium hydride.[7] For **2.2**, deacetylated thiogalactoside **2.7** was transformed into benzylidene acetal **2.8**

Synthesis of Galactosyl Building Blocks 2.1 and 2.2

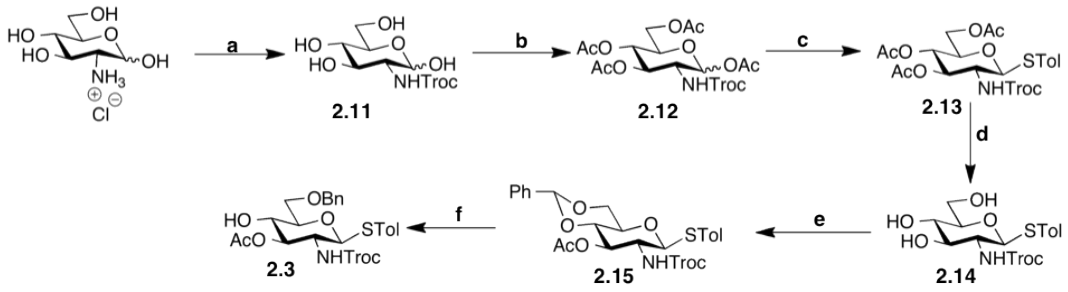


Reagents and conditions: a. Ac_2O, Pyr. (Quant).; b. TolSH, BF_3-OEt_2, DCM, rt (78%); c. NaOMe, MeOH, rt; d. NaH, BnBr, DMF, 0°C-rt, (82% 2 steps); e. $PhCH_2(OMe)_2$, MeCN, rt, (80%); f. LevOH, DIC, 4-DMAP, DCM, rt (50%); g. BzCl, Pyr., DCM, rt (85%); h. AcOH (cat.), NH_2NH_2-H_2O, DCM, MeOH, rt (86%)

Scheme 2.9

under standard conditions, followed by regioselective protection of the C3 hydroxyl group with levulinic acid and DIC.[9] This was followed by benzoylation of the C2 position and selective deprotection of the levulinate ester to furnish **2.2**.[7] Following the selective protection of the C2 nitrogen of D-glucosamine HCl, **2.11** was acetylated and p-toluenethiol was substituted in at the anomeric position (Scheme 2.10). This was followed by acetate removal, benylidene acetal formation, acetylation of the C3 position and lastly regioselective ring opening to yield **2.3**.[7]

Synthesis of Glucosamine Building block 2.3



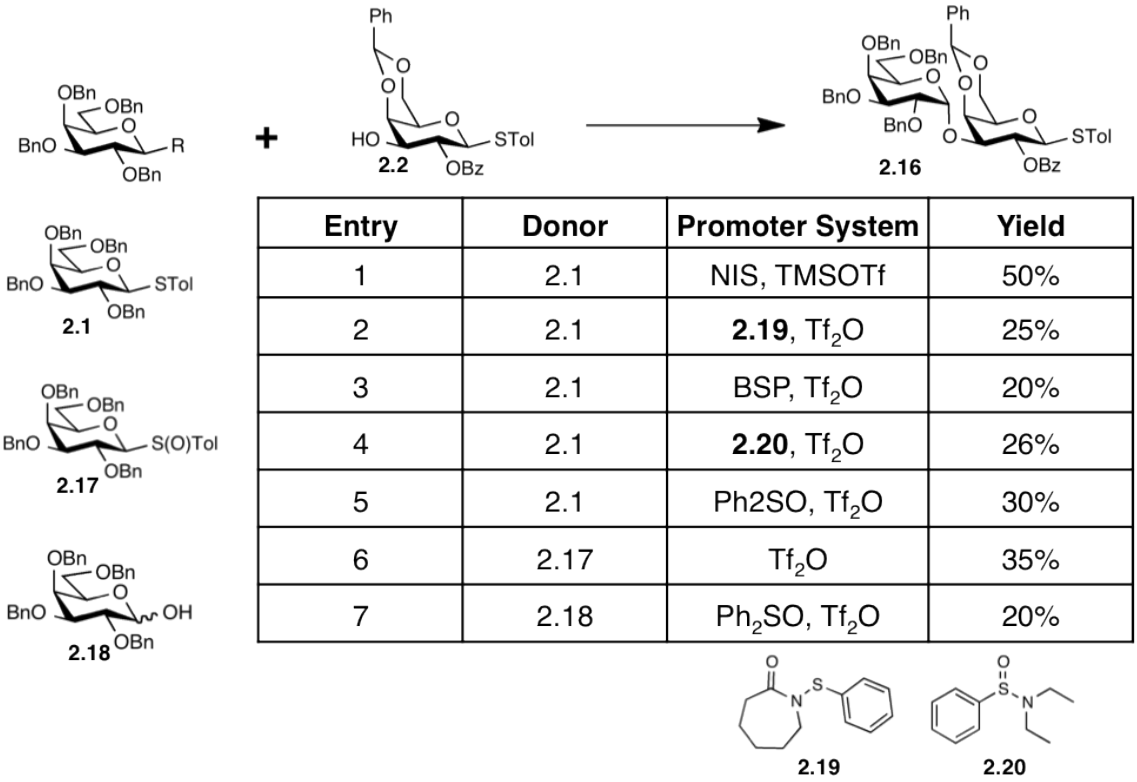
Reagents and conditions: a. TrcoCl, $NaHCO_3$, H_2O, (85%); b.. Ac_2O, Pyr.; c. TolSH, BF_3-OEt_2, DCM, rt (72% 2 steps); c. NaOMe, MeOH, rt; d. $PhCH_2(OMe)_2$, MeCN, rt,; e. Ac_2O, Et_3N, (65% 2 steps); f. TFA, Et_3SiH, DCM, 0°C, (60%)

Scheme 2.10

Section 2.5. Optimization of Glycosylation Reactions

The desired building blocks now in hand, a plan was made to first optimize each glycosylation step individually, and then to combine the optimized steps together into a one-pot reaction. To this end, **2.1** was used to glycosylate **2.2** under a variety of conditions, (Table 2.1, Entries 1-5). However conditions were not found that led to a satisfactory yield. It was possible that the low yields were the result of some unanticipated property of glycosyl donor **2.2**. To determine if this was the case, **2.17,**[10] and **2.18,**[11] were synthesized and used to glycosylate **2.2**. Unfortunately, using these glycosyl donors did not lead to higher yields (Table 2.1, Entries 6 and 7). Knowing that donor **2.2** most likely was not the cause of the disappointing yields, it was reasoned that using different glycosyl acceptors would increase the yield of this step. To this end, glycosyl acceptors

Synthesis of Disaccharide 2.16



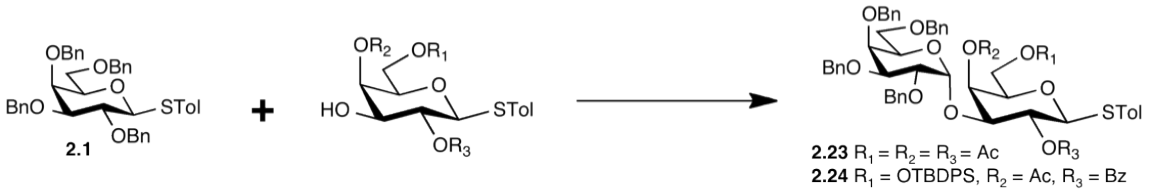
Entry	Donor	Promoter System	Yield
1	2.1	NIS, TMSOTf	50%
2	2.1	**2.19**, Tf_2O	25%
3	2.1	BSP, Tf_2O	20%
4	2.1	**2.20**, Tf_2O	26%
5	2.1	Ph2SO, Tf_2O	30%
6	2.17	Tf_2O	35%
7	2.18	Ph_2SO, Tf_2O	20%

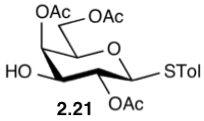
Table 2.1

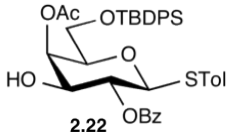
2.21,[12] and **2.22**,[13] were synthesized and glycosylated with donor **2.2**. Glycosylation of **2.21**, and **2.22** proceeded in significantly higher yields (Table 2.2, Entries 2-5). While interesting, a cause for the discrepancy in yield between the glycosylations of **2.21**, and **2.22**, and the glycosylation of **2.2** was not immediately apparent, especially because **2.2** had been used in previously reported high yielding glycosylations.[6b,7a,14]

With a suitable synthesis of the Gal-α(1-3)-Gal disaccharide, attention was then turned to optimizing the glycosylation of glucosamine acceptor **2.3**. Since disaccharide **2.23** was isolated in the highest yield in initial tests, it was used in

Synthesis of Disaccharides 2.23 and 2.24







Entry	Acceptor	Promoter System	Yield
1	2.21	**2.19**, Tf_2O	55%
2	2.21	Ph_2SO, Tf_2O	59%
3	2.21	NIS, TMSOTf	80%
4	2.21	AgOTf, pNO_2SCl	74%
5	2.22	DMTST	60%
6	2.22	AgOTf, pNO_2SCl	27%
7	2.22	NIS, TMSOTf	45%

Table 2.2

the initial attempted glycosylations of **2.3**. This approach did not yield trisaccharide **2.25,** (Table 2.3). Because the RRV of **2.23** was unknown, it was hypothesized that the difference in reactivity between **2.23** and **2.3** was not great enough to allow chemoselective activation of **2.23**. To eliminate the possibility of coactivation glycoamino acid **2.27** was synthesized from **2.3**, and **2.26**, (Scheme

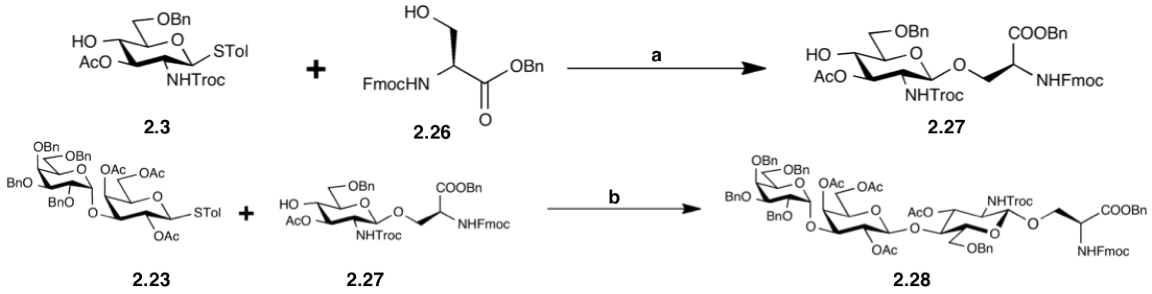
Synthesis of Trisaccharide 2.25

Entry	Promoter	Yield
1	NIS, TMSOTf	NR
2	Ph_2SO, Tf_2O	NR
3	AgOTf, pNO_2SCl	NR

Table 2.3

2.11). Glycosylation of **2.27** by **2.23** was higher yielding (48%), but not gratifyingly so. Next, it was thought that the electron withdrawing nature of the acetyl protecting groups of **2.23** were severely disarming it, leading to only partial activation of **2.23**.[5] With this thought in mind, **2.23** was converted to trichloroimidate **2.29**. Glycosyl trichloroimidates are more readily activated

Synthesis of Glycoamino Acid 2.27 and Trisaccharide 2.28



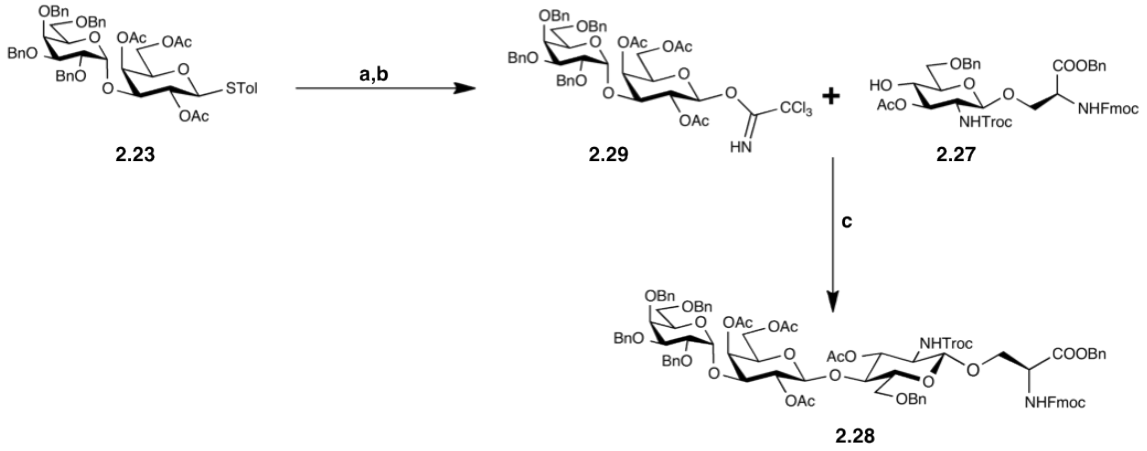
Reagents and conditions: a. NIS, TMSOTf, DCM, -40°C, (74%), b. AgOTf, pNO_2SCl, DTBMP, DCM, -45°C, (48%)

Scheme 2.11

than thioglycosides, so it was postulated that even with the same acetyl protecting groups, activation of **2.29** would be more facile than **2.23**, leading to a higher yield of **2.28.** Glycosylation of **2.27** with **2.29** went smoothly in 66% yield (Scheme 2.12).

The success of the glycosylation of **2.27** with **2.29** demonstrated that the electron withdrawing properties of the acetyl groups of **2.23** were lowering the yield of the reaction between **2.23** and **2.27**. This effect could be alleviated by using a disaccharide donor with fewer electron withdrawing (disarming) protecting groups and more electron donating (arming) protecting groups. The

Synthesis of Donor 2.29 and Trisaccharide 2.28

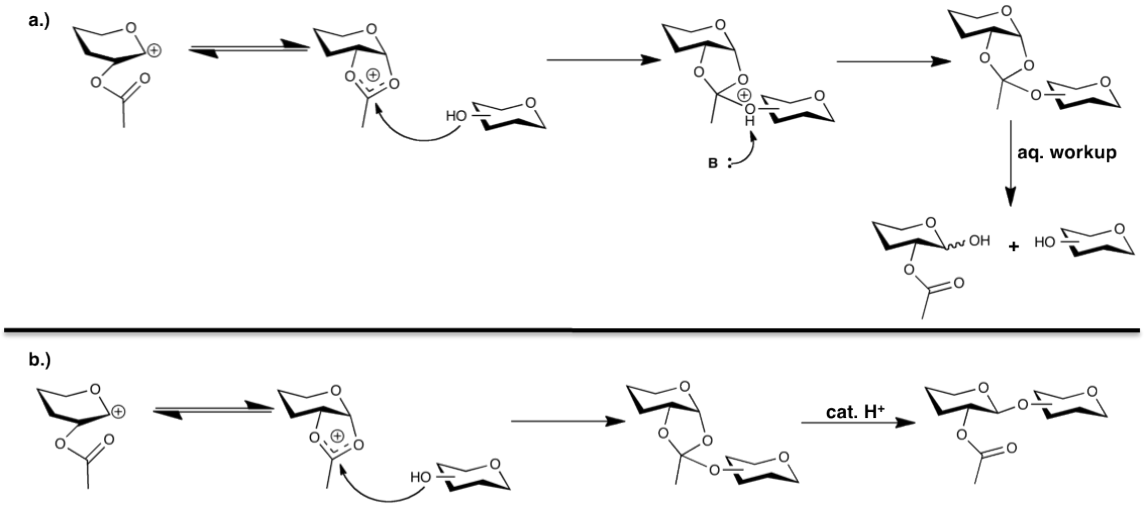


Reagents and conditions: a. NBS, H_2O, Acetone, rt.; b. $CsCO_3$, Cl_3CCN,DCM, 0°C 50% (2 steps); c. TMSOTf, DCM, 0°C (66%)

Scheme 2.12

success of **2.29** also raised the possibility that orthoester formation was hindering the formation of **2.28**. In glycosylations where an acyl protecting group is used at the C2 position, an orthoester can form as the kinetic product (Scheme 2.13). A mild base used to buffer the reaction mixture can then trap the orthoester; this orthoester can then hydrolyze upon aqueous work-up leading to lower yields. In the case of trichloroimidates, such as **2.29**, where a catalytic amount of acid is required for activation, the catalytic acid present can catalyze the rearrangement of any orthoester formed during the course of the reaction to the β-glycoside.[15] The yield of the glycosylation of **2.27** was increased by the use of trichloroimidate donor **2.29**. This suggests that orthoester formation could also be a possible cause of the low yields encountered in glycosylation reactions with donor **2.23**.

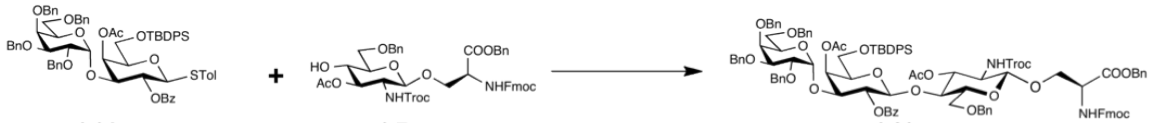
Orthoester Formation (a) and Rearrangement (b)



Scheme 2.13

In light of this, attention was turned to disaccharide **2.24**. The disarming acetyl group at C6 was replaced by an electron donating *tert*-butyl-di-methyl silyl group and it was anticipated that the increased steric bulk of the benzoyl group at C2 would suppress orthoester formation.[16] As expected, glycosylation of **2.27** with **2.24** went smoothly and in higher yield than the glycosylations involving **2.23** (Table 2.4).

Synthesis of Trisaccharide 2.30



2.24 **2.7** **2.30**

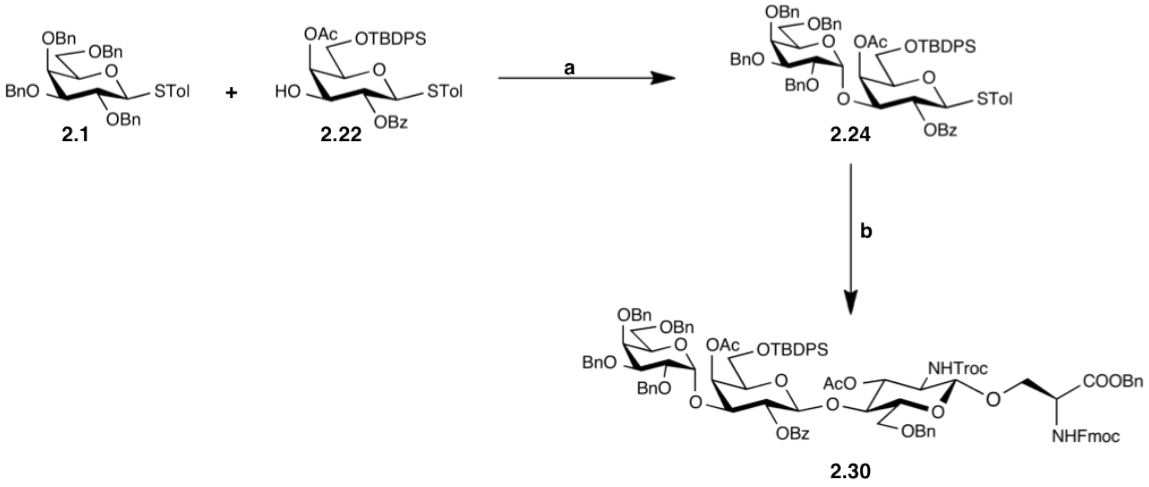
Entry	Promoter	Yield
1	AgOTf, pNO2SCl[a]	74%
2	DMTST	50%

Table 2.4

Section 2.6. Reactivity Based One-Pot Syntheses

With optimized conditions for the two glycosylation reactions in hand, attention was turned to combining them into a one-pot reaction. Galactosyl donor **2.1**, galactosyl acceptor **2.22**, and glycoamino acid **2.27** were chosen as the monosaccharide building blocks to be used in the attempted one-pot reaction. The success of disaccharide donor **2.24,** led to the decision to use **2.22** as the second galactosyl residue. Glycoamino acid **2.27** was chosen to eliminate the possibility of coactivation of its p-toluenethiol residue. In the event that the one-pot reaction was successful, a one-pot reaction with glucosamine residue **2.3** would then be attempted. To avoid yield-lowering side reactions, reactivity based one-pot syntheses require that the promoter system used in each step be the same. For this reason, DMTST was chosen as the promoter system as it had given an acceptable yield of disaccharide **2.24** and a moderate yield of **2.30**. There are literature reports of the overall yield of a glycosylation sequence increasing when the individual glycosylation steps were combined into one-pot.[6a,b] It was hoped that despite the moderate yields of each glycosylation step when DMTST was used as the promoter, the overall yield of the one-pot sequence would be higher than the overall yield of the step-wise synthesis. However, the yield of the one-pot reaction using DMTST was a disappointing 28%, which almost exactly matched the overall yield of the step-wise synthesis, (Scheme 2.14).

One-Pot Synthesis of 2.30



Reagents and conditions: a. DMTST, DTBMP, DCM, 0°C; b. DMTST, DTBMP, **2.27**, DCM, 0°C, 28% (2 steps)

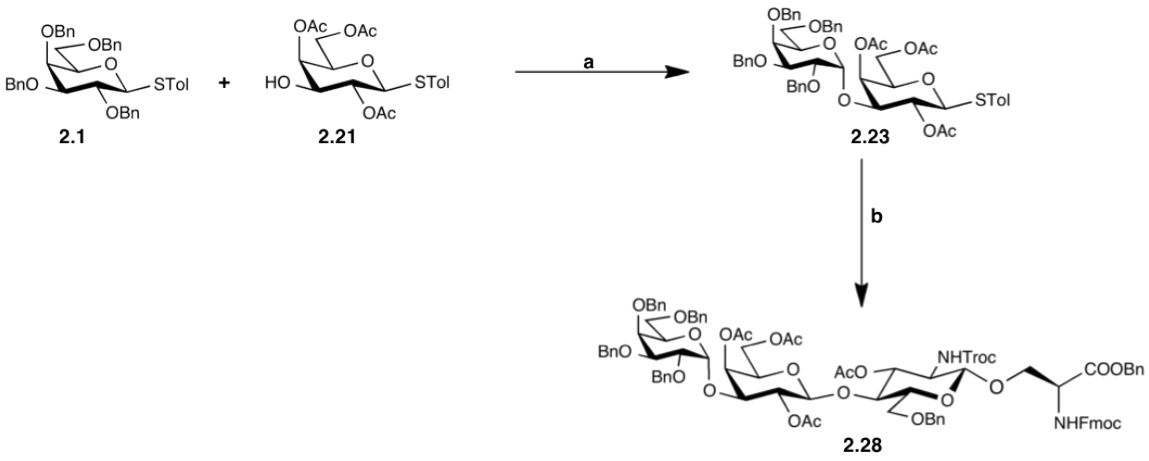
Scheme 2.14

It was then thought that galatosyl residue **2.21** might lead to a higher yielding one-pot reaction because the yields obtained in the formation of disaccharide **2.23** were up to 20% greater than those of disaccharide **2.24**. The AgOTf/pNO_2SCl promoter system was chosen as it had given good to moderate yields in both glycosylation steps. Unfortunately, the yield of this one-pot reaction was again low (33%) and showed almost no increase over the overall yield of the step-wise synthesis (Scheme 2.15).

Section 2.7. Conclusions

Monosaccharide building blocks **2.1**, **2.2**, **2.22**, and **2.23**, were synthesized from D-galactose. These monosaccharides were then subjected to a variety of reaction conditions to find the highest yielding reaction of Gal-α(1-3)-

Synthesis of Trisaccharide 2.28



Reagents and conditions: a. AgOTf, pNO_2SCl, DTBMP, DCM, -45°C; b. AgOTf, pNO_2SCl, DTBMP, **2.27**, DCM, -45°C, 33% (2 steps)

Scheme 2.15

Gal disaccharide. It was found that the use of acceptor **2.22** gave the highest yields of this glycosylation. **2.3** was synthesized from D-glucosamine HCl and attempts were made to glycosylate it to yield an α-Gal trisaccharide. It was found that conversion of **2.3** into glycoamino acid **2.27** led to higher yields and that the use of more armed disaccharide donor **2.24** also led to an increase in yield of the this glycosylation reaction. Based on these results, two one-pot syntheses of an α-Gal glycoamino acid were attempted; but both were low yielding.

The development of the one-pot syntheses disclosed here cannot be considered a success, as the syntheses do not meet the initially identified criteria. They are not efficient, it is unknown if they are scalable, and because they rely on the use of glycoamino acid **2.27**, they do not have the desired ability to easily vary the amino acid conjugate. In order to devise a synthesis that meets these

criteria, further experimentation was needed to fully understand the underlying reasons why these specific glycosylation reactions were low-yielding.

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Chapter 3

Elucidation of Glycosylation Side Reactions

Section 3.1. Introduction

Following the disappointing results of the one-pot syntheses of the α-Gal trisaccharide, it was decided that a new approach to the synthesis of the α-Gal epitope was needed. To determine exactly what approach this would be, an analysis of the prior results was performed.

The first question raised was why the glycosylations of glucosamine acceptors **2.3** and **2.27** (Figure 3.1), were consistently low-yielding. It was hypothesized that this was due to the low nucleophilicty of the glucosamine acceptors.[1] The Crich group previously demonstrated that hydrogen bonding

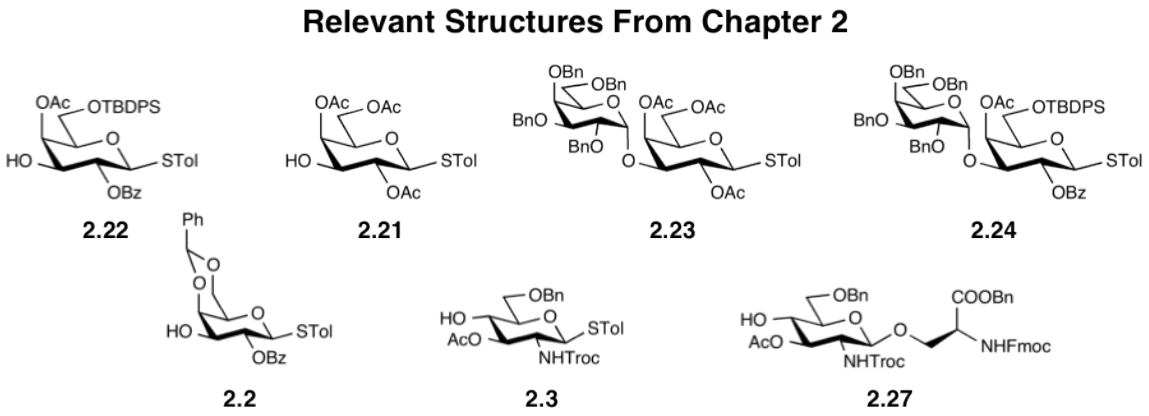


Figure 3.1

interactions with the amide hydrogen cause the hydroxyl group at C4 to be more weakly nucleophilic than that of a typical glycosyl acceptor. There were two postulated methods to increase the yield of these glycosylations: (i) replace the electron-withdrawing acyl protecting group at C3 with a more electron rich protecting group, and (ii) use a disaccharide donor that was more highly armed

than either **2.23** or **2.24**.[2] The use of a more electron donating protecting group at C3 would not necessarily disrupt the hydrogen-bonding network that was purported to cause the low yields of the glycosylations, so this investigation was not pursued. This meant that a more armed disaccharide donor would need to be synthesized to test for increased glycosylation yields. Due to the stabilizing effect of its electron donating protecting groups, less energy is required to displace the covalent triflate formed from an armed donor than to displace the covalent triflate formed from a disarmed donor. Consequently, a weak nucleophile such as a glucosamine acceptor, should more readily displace the covalent triflate of an armed glycosyl donor, leading to higher glycosylation yields. However, testing of this theory was complicated by the fact that of the three glycosyl acceptors tested, **2.2**, **2.21**, and **2.22**, acceptor **2.2** was the most armed, but its use as a glycosyl acceptor led to meager yields of the Gal-α(1-3)-Gal disaccharide. In order to optimize the glycosylation of the terminal glucosamine residue it became necessary to first discover what factors contributed to the low yields of the glycosylation of **2.2**.

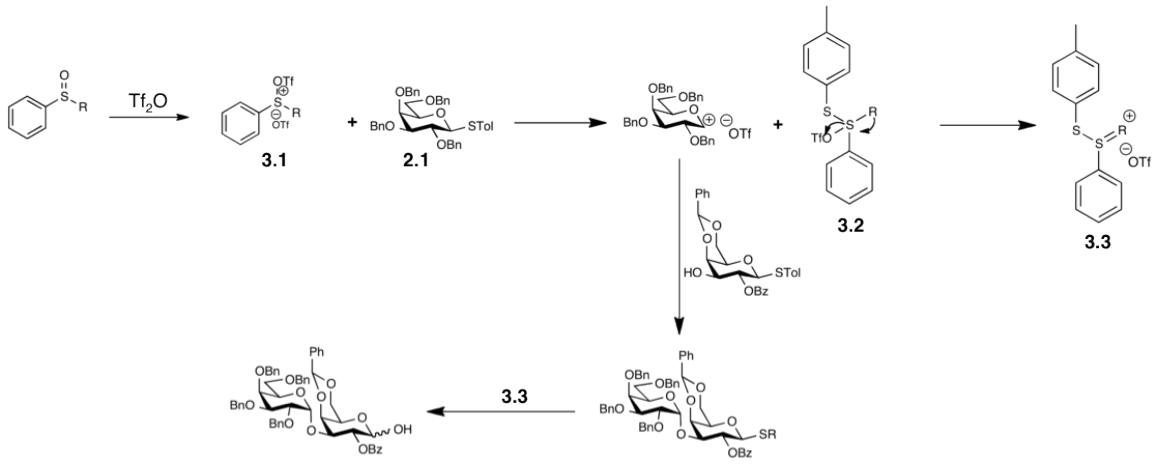
Section 3.2. Elucidation of Reagent-Based Side Reactions in Gal-α(1-3)-Gal Disaccharide Synthesis

One of the more surprising results of the initial studies in the synthesis of the α-Gal epitope were the low yields of the glycosylation of **2.2**. With only one disarming benzoyl group, and the C4 and C6 hydroxyl groups protected by an electron rich benzylidene acetal, it was believed that the hydroxyl group of **2.2**

would be sufficiently nucleophilic to give high yields of disaccharide **2.16**. However, the opposite result was observed; glycosylations of **2.2** were low yielding and the use of less electron rich galactosyl acceptors **2.21** and **2.22** led to higher yields. Interestingly, the most electron poor acceptor, **2.21**, was the most easily glycosylated.

Compound **2.2** was protected by a benzylidene acetal at its C4 and C6 positions, whereas **2.22** and **2.23** bore an acyl protecting group on at least one of those positions. It was reasoned that the arming effect of the benzylidene acetal would stabilize the oxocarbenium ion formed from **2.2**, making a reaction occurring at the anomeric residue of **2.2** more thermodynamically favorable than a reaction occurring at the anomeric residues of **2.22** or **2.23**. This led to the notion that a side reaction involving the anomeric residue, or aglycon, of **2.2,** was occurring. The results from earlier studies of the glycosylation of **2.2** indicated that the use of powerful sulfonium based promoter systems (BSP, Ph_2SO, **2.19**, **2.20**) gave lower yields than the use of the more mild iodine based NIS/TMSOTf system. Based on these results, and the armed nature of **2.2**, it was postulated that the reaction shown in Scheme 3.1 was occurring.[3] In this reaction pathway sulfonium species **3.1** activates the glycosyl donor, forming activated donor and species **3.2**. Intermediate **3.2** can then rearrange to give **3.3**. After the activated donor glycosylates the acceptor and forms a new disaccharide, electrophilic

Side Reaction at the Acceptor Aglycon

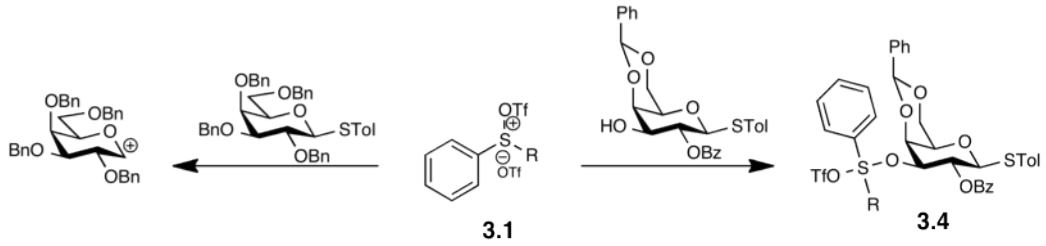


Scheme 3.1

species **3.3** can activate the aglycon of the newly formed disaccharide, degrading it, and lowering the yield of the reaction. Coddée and coworkers demonstrated that this side reaction only occurs at higher temperatures, (> -20°C), and that the electrophilic species can be quenched by the addition $(OEt)_3P$ before it reacts with the newly formed carbohydrate.[4] Following these guidelines, keeping the temperature below -20°C and quenching with $(OEt)_3P$, doubled the yield of **2.16**, (Table 3.1, Entry 2), but this only increased it to a yield of 40%.

Next, attention was turned to a possible reaction occurring at the hydroxyl group of the glycosyl acceptor. It was believed that the inductive effects of the protecting groups on **2.2** would make its hydroxyl group relatively nucleophilic. Thus a side reaction taking place at the hydroxyl of **2.2** might not occur at the less nucleophilic hydroxyl groups of **2.22** and **2.23.** Specifically, it was thought that the side reaction shown in Scheme 3.2**,** was leading to lower yields. In this side reaction, the electrophilic species **3.1** reacts with the hydroxyl of the glycosyl

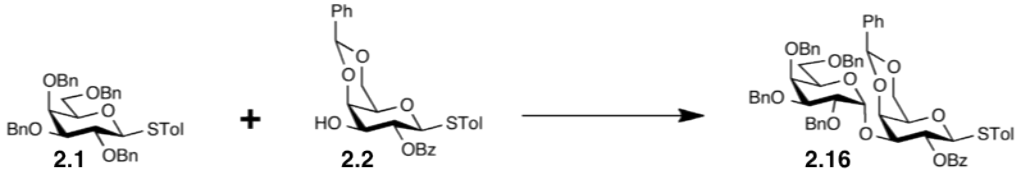
Reaction of Acceptor Hydroxyl and 3.1



Scheme 3.2

acceptor.[4a] This prevents **3.1** from activating the glycosyl donor, and inhibits the glycosylation of the acceptor. These two effects combine to lower the yield of the glycosylation. This problem can be avoided by following a preactivation procedure in which the glycosyl donor is activated first and then the glycosyl acceptor is added to the reaction mixture. Following this procedure ensures that the promoter reacts only with the glycosyl donor. Utilization of this procedure, combined with a $(OEt)_3P$ quench increased the yield of **2.16** to 55% (Table 3.1, Entry 3). Lastly, it was reasoned that if species **3.3**, (Scheme 3.1), can also activate thioglycoside donors, then only 0.5 equivalents of the promoter were

Synthesis of Disaccharide 2.12



Entry	Conditions	Yield
1	Premixing	20%
2	Premixing, $(EtO)_3P$ quench	40%
3	Preactivation, $(EtO)_3P$ quench	55%

Reagents and conditions: BSP, Tf_2O, DTBMP, CH_2Cl_2, -60°C- 0°C

Table 3.1

necessary for full activation. Species **3.1** would first activate 0.5 equivalents of the glycosyl donor, then **3.3** would activate the remaining half. In theory, this would yield no leftover **3.3** that could then react with the newly formed disaccharide.[5] This procedure, however, yielded no disaccharide. It is possible that at the higher temperatures required for **3.3** to activate the glycosyl donor, the generated electrophilic species reacts with more than just the donor aglycon, hindering the formation of the desired disaccharide.

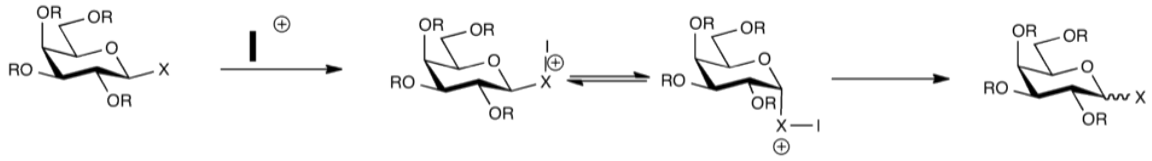
While the use of a $(OEt)_3P$ quench and a preactivation procedure increased the yield of disaccharide **2.16** substantially, it was still only increased to 55%. This was disappointing, and not a high enough yield if this reaction was to eventually be condensed into a one-pot synthesis. As a result, attention was turned to mechanistic-based side reactions.

Section 3.3. Aglycon Transfer

In several of the trials in the synthesis of **2.16** it was noted that a substantial amount, (≥ 40%), of an α/β mixture of the glycosyl donor was recovered. It was first thought that this was due to the inability of the promoter systems to fully activate the glycosyl donor. The observed racemization of the donor aglycon that occurred could be rationalized by the process shown in Scheme 3.3. Here the promoter, in this example iodine, forms a complex with the donor aglycon and equilibrates it prior to activation. It has been shown that this process occurs in the presence of iodine based promoter systems.[6] It was

expected

Iodonium Promoted Aglycon Racemization



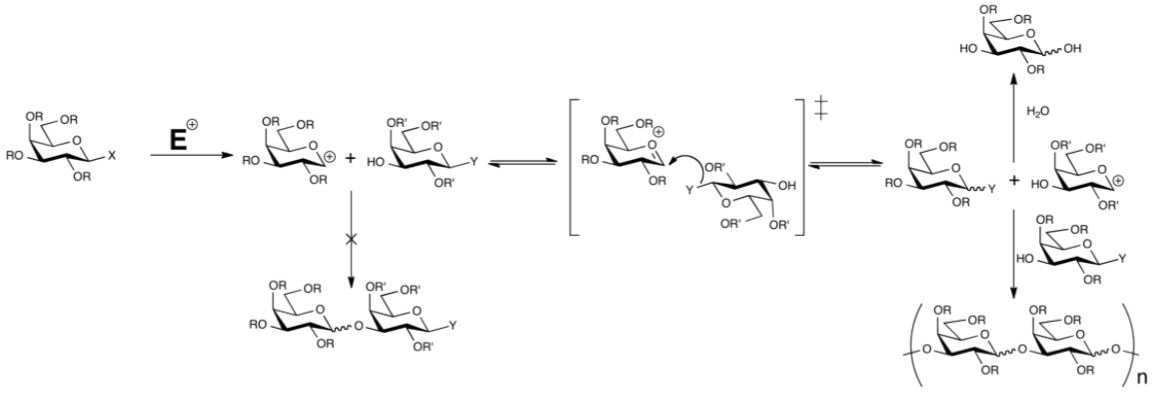
Scheme 3.3

however, that the more powerful sulfonium promoter species would activate the glycosyl donor fully and irreversibly; hence racemization of the donor aglycon would not be detected. The fact that glycosyl donor with a racemized aglycon was consistently recovered even when powerful promoter systems were used, indicated that a reaction known as aglycon transfer, could be taking place.

Aglycon transfer is the establishment of an equilibrium during the course of a glycosylation reaction, in which the aglycon of the glycosyl acceptor, rather than its free hydroxy group, acts as a Lewis base and attacks the oxocarbenium ion of the glycosyl donor. In this way the acceptor aglycon is transferred to the glycosyl donor (Scheme 3.4).[7] The end result of this is the regeneration of the glycosyl donor and formation of activated glycosyl acceptor that can either react with another equivalent of glycosyl acceptor and polymerize, or, upon aqueous work-up, it can hydrolyze. Given that this is an equilibrium process, the relative energy levels of the oxocarbenium ions formed dictate the extent to which this side reaction occurs. Galactosyl donor **2.1** has four electron donating benzyl protecting groups, while **2.2** has one electron donating benzylidene protecting

group and one electron withdrawing benzoyl group. Acyl protecting groups at the

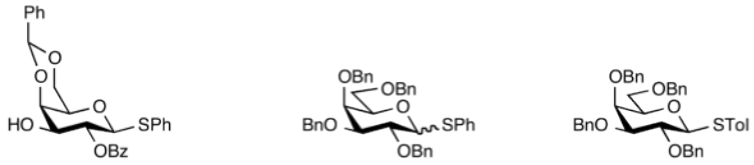
Aglycon Transfer



Scheme 3.4

C2 position have been shown to have a significant destabilizing effect on glycosyl oxocarbenium ions.[8] Based on this, it was reasoned that the oxocarbenium ion formed from donor **2.1** would be more thermodynamically stable than that of **2.2**, therefore the aglycon transfer equilibrium would favor the donor oxocarbenium ion and hence, the formation of the desired disaccharide. However, the observation that significant amounts of glycosyl donor were recovered indicated that this might not be the case. To determine if aglycon transfer was occurring, acceptor **3.4**[9] was synthesized. **3.4** and **2.1** had structurally different aglycons (Figure 3.2). Thus, if the aglycon of **3.4** was transferred to **2.1** during the course

Compounds 3.4, 3.5, and 2.1

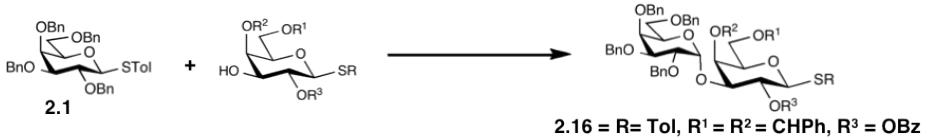


3.4 **3.5** **2.1**

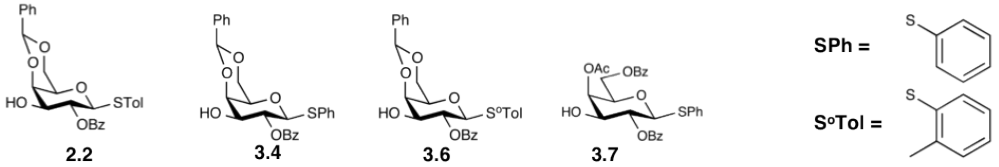
Figure 3.2

of the reaction, it would be easy to detect. When **3.4** was glycosylated with **2.1,** glycosyl donor **2.1** was not recovered. This indicated that donor **2.1** was being fully activated under these glycosylation conditions and as a result, incomplete donor activation could be eliminated as a possible cause for the low glycosylation yields. Additionally, the aglycon transfer product **3.5**[10] was recovered, demonstrating that aglycon transfer was a competing side reaction in this glycosylation reaction (Table 3.2, Entry 2). This was a surprising result, but it can be rationalized in two ways shown in Scheme 3.5. First, it is possible that the benzoyl group stabilizes the oxocarbenium ion of **2.2** via the formation of a dioxalenium ion, and that the stabilization effect of this outweighs the

Aglycon Transfer Suppression



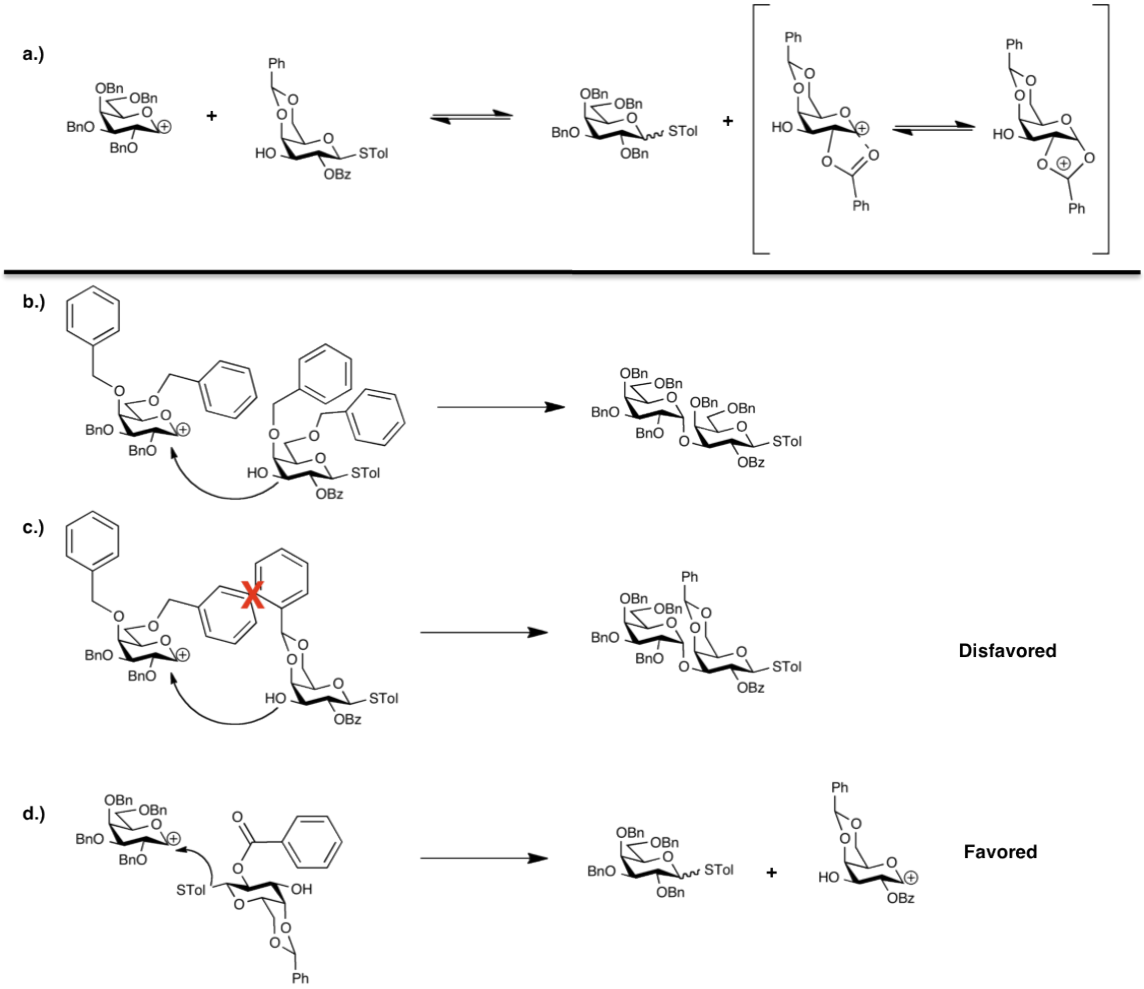
2.16 = R= Tol, $R^1 = R^2$ = CHPh, R^3 = OBz
3.8[9] = R= Ph, $R^1 = R^2$ = CHPh, R^3 = OBz
3.9 = R= ^{o}Tol, $R^1 = R^2$ = CHPh, R^3 = OBz
3.10 = R= Ph, $R^1 = R^3$ = OBz, R^2 = OAc



Entry	Acceptor	Promoter	Solvent	Aglycon Transfer	Donor Activation	Yield
1	2.2	PhSOTf	DCM	Y	Unknown	30%
2	3.4	PhSOTf	DCM	Y	Full	40%
3	3.6	PhSOTf	DCM	N	Full	65%
4	3.6	NIS/TMSOTf	DCM	N	Full	70%
5	3.7	PhSOTf	DCM	N	Full	50%
6	3.7	NIS/TMSOTf	DCM	N	Full	62%

Table 3.2

Possible Explanations for Aglycon Transfer Between 2.1 and 2.2

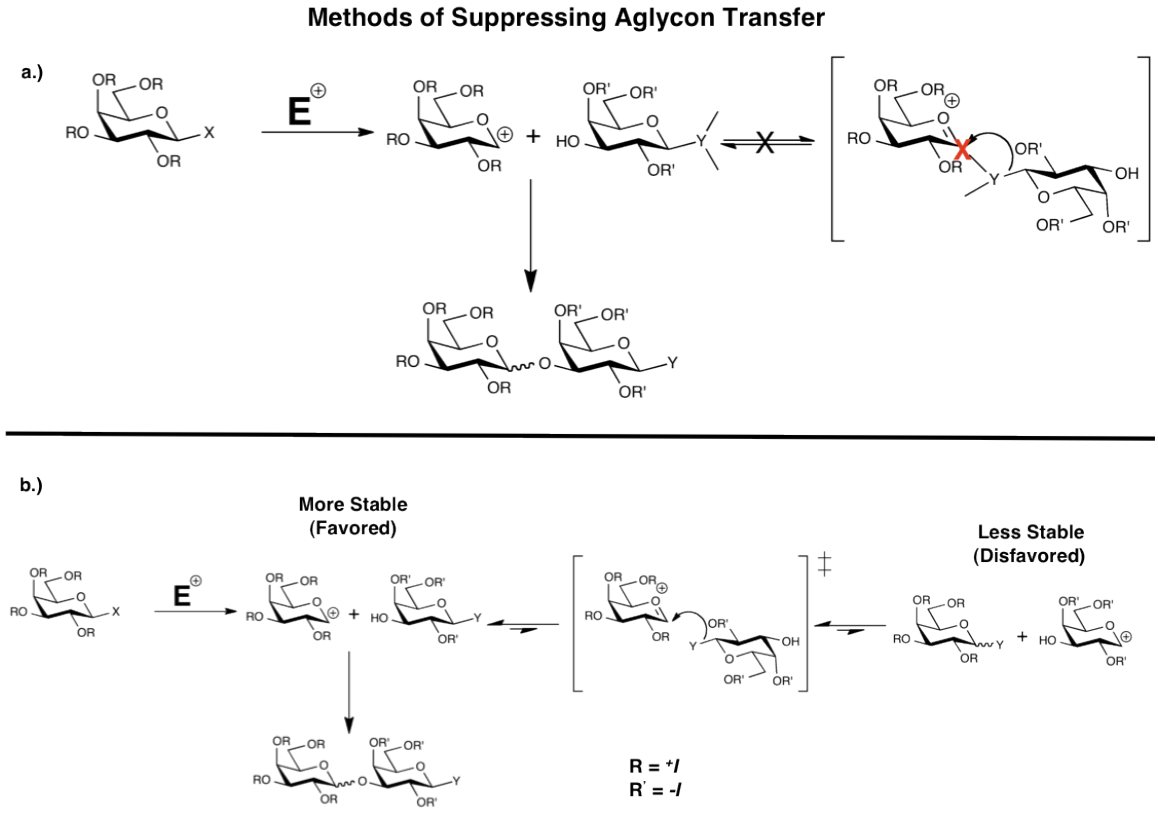


Scheme 3.5

destabilizing effect of the electron withdrawing nature of the benzoyl group (Scheme 3.5, **a**). Second, steric repulsion between the C6 benzyl group of **2.1** and the benzylidene acetal of **2.2** may have an effect. The benzylidene acetal of **2.2** is conformationally restricted and does not have the mobility to avoid steric interactions in the way that a glycosyl acceptor with separate protecting groups on C4 and C6 could (Scheme 3.5, **b** and **c**). This could make the formation of the desired disaccharide less favorable than the aglycon transfer reaction, leading to the selective formation of the aglycon transfer product. Taken together or

separately, both of these mechanistic theories could explain the unexpected amount of aglycon transfer occurring in the glycosylation of **2.2**.

There are two known methods of suppressing aglycon transfer, the first is the use of a sterically hindered aglycon.[7] The increased steric repulsion introduced into the reaction by a bulky aglycon will severely limit the ability of the aglycon to be transferred from the glycosyl acceptor to the activated glycosyl donor, (Scheme 3.6, **a**).[11] The second method is to use a disarmed glycosyl acceptor (Scheme 3.6, **b**). As discussed previously, this will shift the equilibrium towards the donor oxocarbenium ion, leading to the formation of the desired carbohydrate.[12] To further illustrate that aglycon transfer was in fact occurring,



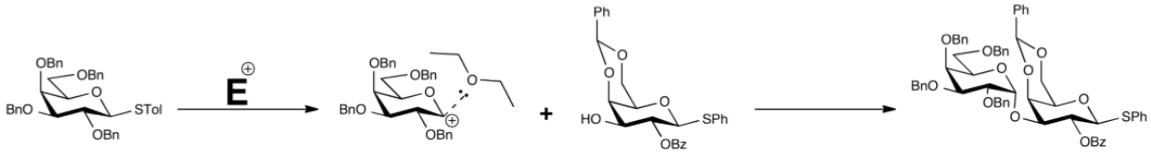
Scheme 3.6

and that suppressing it would lead to higher yields, an effort was made to use these methods to suppress any aglycon transfer occurring in the glycosylation of **2.2**. To this end, galactosyl acceptors **3.6**, and **3.7**[13] were synthesized. Galactosyl acceptor **3.6** featured a sterically hindered o-toluenethiol aglycon,[8] and **3.7** was extremely disarmed. Glycosylations of these acceptors with donor **2.1** proceeded in higher yield than the glycosylation of **2.2** (Table 3.2, Entries 3-6), demonstrating that aglycon transfer was occurring and that when suppressed, yields could be improved.

With this evidence that aglycon transfer was in fact an ongoing problem in the formation of the Gal-α(1-3)-Gal disaccharide, it was decided that exploring potential novel methods of suppressing aglycon transfer would be useful. The two known methods of aglycon transfer suppression shift the equilibrium towards the formation of the desired carbohydrate by destabilizing the acceptor oxocarbenium ion or by raising the activation energy of the transfer reaction. However, neither of these methods shifts the equilibrium in favor disaccharide formation by lowering the energy of the donor oxocarbenium ion. It was postulated that a suitable method could be found to stabilize the donor oxocarbenium ion that would shift the equilibrium in its favor, thereby lessening the effect of aglycon transfer. It is possible to do this by using a glycosyl donor with an increased number of electron donating protecting groups. The most arming protecting group commonly employed in carbohydrate synthesis is the benzyl group. Since donor **2.1** was already fully benzyl protected, it was not

possible to stabilize its oxocarbenium ion via protecting group manipulation. Instead, it was hypothesized that the oxocarbenium ion could be stabilized by the use of a participating solvent. Ethereal solvents are known to participate in glycosylation reactions by way of coordination to the oxocarbenium ion. They preferentially coordinate to the β-face of the activated glycoside, and lead to the selective formation of an α-glycoside.[14] It was believed that coordination of an ethereal solvent to the donor oxocarbenium ion would stabilize it enough to significantly suppress aglycon transfer (Scheme 3.7). With this in mind the

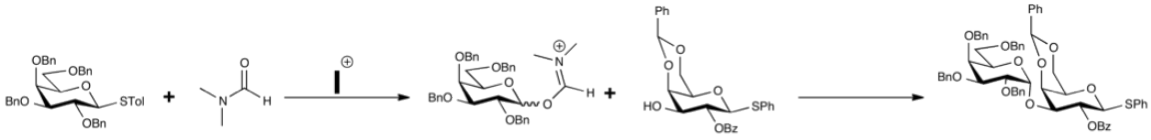
Postulated Ether Based Aglycon Transfer Mitigation



Scheme 3.7

glycosylation of **3.4** was carried out in diethyl ether. However aglycon transfer was still observed and only a moderate increase in yield was measured, (Table 3.3, Entry 2). Next attention was turned to the use N,N-dimethylformamide (DMF) as a cosolvent. The laboratory of T.K. Mong has demonstrated that when DMF is included in the glycosylation reaction mixture, it can nucleophilicly attack the activated donor, forming an imidate (Scheme 3.8).[15] This imidate interconverts between the α and β forms, with the β imidate being displaced preferentially by the glycosyl acceptor, leading to the selective formation of an α-glycoside. The glycosyl imidate formed in this reaction pathway is more stable than either a glycosyl triflate or a oxocarbenium ion, consequently it was hypothesized that it

Aglycon Transfer Inhibition by DMF

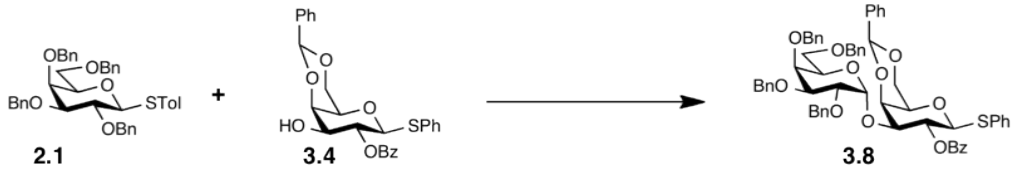


Scheme 3.8

would be selectively displaced by the hydroxyl group of the glycosyl acceptor leading to higher yields of the desired disaccharide and reducing the impact of aglycon transfer. When the glycosylation of **3.4** was performed in the presence of DMF no aglycon transfer was observed, and the yield of disaccharide **2.16** increased by 20%, (Table 3.3, Entry 3). This result indicated that stabilizing the activated donor species in a glycosylation reaction was a viable strategy for mitigating the effects of aglycon transfer.

Section 3.4. Use of Optimized Disaccharides in the Glycosylation of 3.13

Novel Methods of Aglycon Transfer Reduction



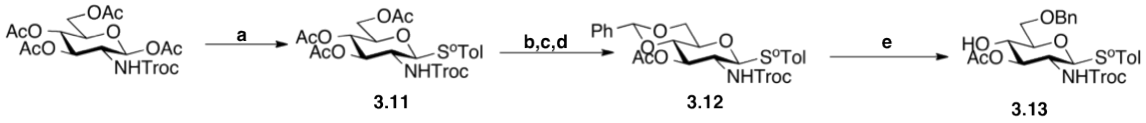
2.1 **3.4** **3.8**

Entry	Promoter	Solvent	Recovered Donor	Donor Activation	Yield
1	PhSOTf	DCM	Y	Full	20%
2	PhSOTf	Et_2O	Y	Full	45%
3	NIS/TMSOTf	DCM/DMF	N	Full	60%

Table 3.3

With a strategy to suppress aglycon transfer it was now possible to synthesize armed disaccharide donors **2.16**, and **3.10** in high yield. These armed disaccharides could be used to test if using a more armed donor would increase the glycosylation yield of a glucosamine residue. To mitigate any aglycon transfer that was occurring in the glycosylation of the glucosamine residues while also allowing for the possibility of further manipulation of the desired trisaccharide, o-toluenethiol glucosamine acceptor **3.13** was synthesized and used as the glycosyl acceptor in these tests (Scheme 3.9). The initial belief was that a

Synthesis of 3.13



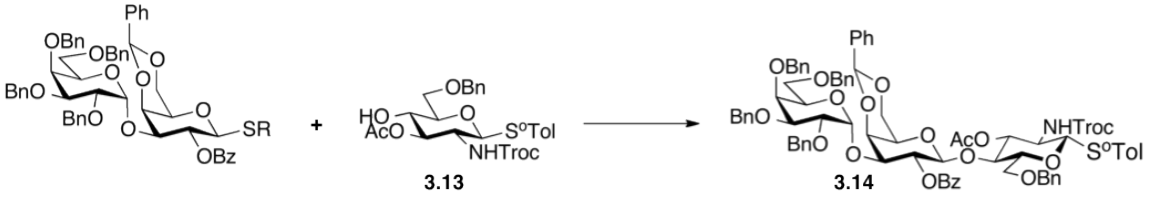
Reagents and conditions: a.) BF_3OEt_2, HS^{o}Tol, DCM, RT, 60%, b.) NaOMe, MeOH, DCM, RT, c.) $Cu(OTf)_2$, benzaldehyde dimethyl acetal, MeCN, RT. d.) acetic anhydride, MeCN, RT. 72% (3 steps) e.) TFA, Et_3SiH, DCM, 0^{o}C, 80%

Scheme 3.9

disaccharide donor that was benzylidene acetal protected at C4 and C6 of the second galactosyl residue would be more armed than a disaccharide that bore an acyl protecting group at one or both of those positions. The more highly armed disaccharide donor would then glycosylate the glucosamine acceptors in higher yield. Of the benzylidene acetal protected disaccharides synthesized in this study, **3.9** was synthesized in the highest yield so it was chosen to be the glycosyl donor in the glycosylation of **3.13**. Disappointingly, it was observed that glycosylations with **3.9** were low yielding, (Table 3.4, Entries 1 and 2). It is speculated that this is due to the sterically hindered nature of the o-toluenethiol aglycon. While its increased steric bulk suppressed aglycon transfer, it is

plausible that it also raises the energy required to activate disaccharide **3.9.** This notion was supported by the observation that temperatures as high as -20°C were required for activation, whereas activation of p-toluenethiol donor **2.16** took place at -60°C. At higher temperatures, side reactions that degrade the activated disaccharide donor can proceed.[16] If these side reactions proceed faster than the rate of nucleophilic attack by the weakly nucleophilic glucosamine acceptor, it would inhibit formation of trisaccharide **3.14**. Likewise, the glycosylation of **3.13** by disaccharide donor **2.16**, with DMF as a cosolvent, did not lead to the desired high yield of **3.13**. It is believed that this was due to inability of the poorly nucleophilic glucosamine acceptor to displace the glycosyl imidate that is formed in the DMF-mediated reaction.

Attempted Synthesis of 3.14 with Suppressed Aglycon Tranfer Conditions



Entry	R	Promoter	Yield
1	S°Tol	NIS, TMSOTf	NR
2	S°Tol	PhSOTf	44%
3	STol	NIS, TMSOTf, DMF	30%

Table 3.4

Section 3.5. Conclusions

Studies designed to elucidate, and mitigate side reactions in the synthesis of the α-Gal trisaccharide were performed. It was discovered that side reactions

involving the electrophilic promoters used in the synthesis of the initial disaccharide were lowering the yield and that procedures that eliminated the causes of these side reactions increased the yield. More significantly, it was demonstrated that a mechanistic effect, aglycon transfer was also lowering the glycosylation yields. Two established methods were used to decrease the effects of aglycon transfer, leading to higher yields in the synthesis of the α-Gal disaccharide. A novel method of aglycon transfer suppression was also found. In this method DMF can trap the oxocarbenium ion, leading to relief from the detrimental effects of aglycon transfer.

The initial hypothesis was that if it was possible to synthesize armed disaccharide donors in good yield, these armed donors would in turn lead to higher glycosylation yields of the glucosamine acceptors. With the discovery and mitigation of various side reactions, it became possible to synthesize more highly armed donors. These armed donors did not lead to higher yields. Instead, it became apparent that the same procedures utilized to allay the effects of aglycon transfer also decreased the glycosylation yields of glucosamine acceptors below previous levels. These observations led to the conclusion that a new strategy for the synthesis of the α-Gal epitope is necessary. This novel strategy should offer two key features: (i) assembly of the trisaccharide in such a way that aglycon transfer is not a possible side reaction, and (ii) increased yields for the glycosylation yield of glucosamine acceptors.

Section 3.6. Literature Cited

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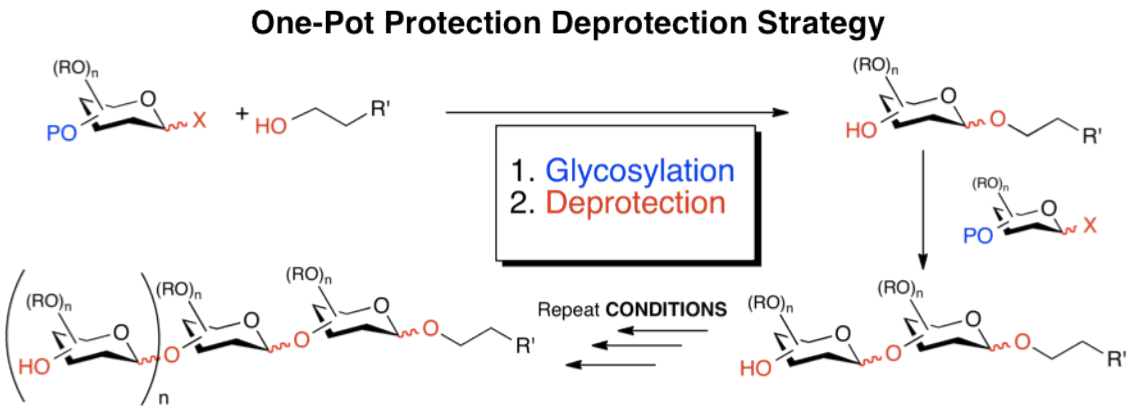
Chapter 4

Fmoc-Based Syntheses of the α-Gal Epitope

Section 4.1. Introduction

After the failure to significantly improve the synthesis to the α-Gal epitope by reducing the impact of side reactions, a new synthetic strategy was sought. In a glycosylation reaction, if the glycosyl acceptor is *O*-linked at its anomeric position, then aglycon transfer with an activated donor species cannot occur. Hence, it was postulated that a synthetic strategy in which an *O*-linked acceptor was glycosylated in each step would eliminate the possibility of aglycon transfer and lead to higher yields. To this end, a one-pot glycosylation-deprotection strategy was envisioned (Scheme 4.1). In this strategy a glycosyl donor bearing a temporary protecting group would be used to glycosylate an *O*-linked glycosyl acceptor. The temporary protecting group of the glycosyl donor would then be



Scheme 4.1

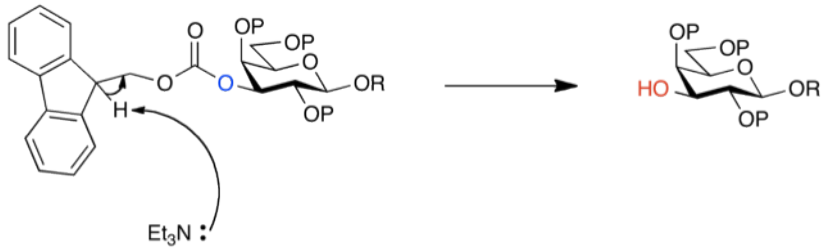
removed, yielding a sugar alcohol that could be used as a glycosyl acceptor in the next step of the reaction sequence. While this eliminated the possibility of a one-pot glycosylation sequence, it was thought that the increased efficiency of

this new approach would outweigh the hindrances of intermediate purification steps.

Section 4.2. The Fmoc Group in Carbohydrate Synthesis

This newly devised strategy required the use of a temporary protecting group that could be selectively removed in high yield. There are many commonly employed protecting groups in carbohydrate synthesis that can be selectively removed: p-methoxybenzyl ethers (DDQ oxidation),[1] levulinyl esters (H_2NH_2N, cat. H^+),[2] chloroacetyl acetates (thiourea, $NaHCO_3$),[3] silyl ethers (F^-),[4] and allyl ethers ($PdCl_2$)[5] among others, but all of these moieties require removal conditions that are not compatible with standard glycosylation conditions. For this reason, the use of any of these protecting groups would require an additional deprotection step between each glycosylation, lowering the efficiency of this approach. It was thought, however, that the use of the 9-fluorenylmethoxycarbonyl (Fmoc) group would allow for a one-pot glycosylation-deprotection. Glycosylation reactions often require quenching of the reaction by triethylamine. It was postulated that this triethylamine quench could quench the reaction *and* remove the Fmoc group simultaneously (Scheme 4.2). This would eliminate the need for a separate deprotection step after each glycosylation, thus increasing the efficiency of this strategy. While used extensively in solid-phase peptide synthesis and solid-phase oligosaccharide synthesis,[6] the Fmoc group has only found limited use in solution phase carbohydrate synthesis[7] and no literature reports of it being used in an iterative one-pot glycosylation-

Fmoc Deptotection



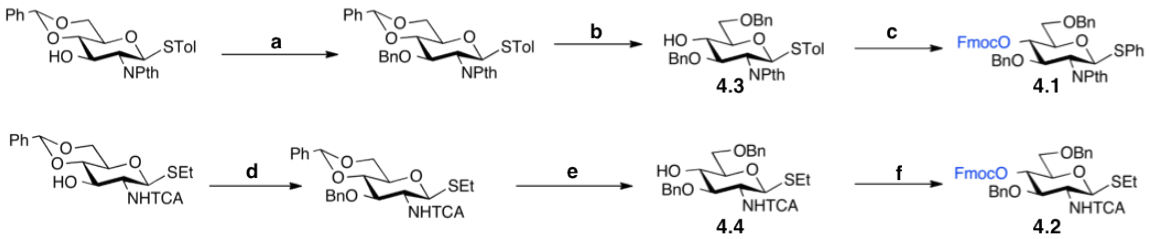
Scheme 4.2

deprotection strategy could be found.

Section 4.3. Synthesis of Fmoc Protected Monosaccharides

In addition to a novel synthetic strategy it was thought that the use of two new glucosamine residues would improve the yields of the reaction sequence (Scheme 4.3). Glucosamine derivative **4.1** replaced the C3 acetyl group of **2.3** with an electron donating benzyl group and the C2 nitrogen was pthalimide protected. The pthalimide group does not possess an amide hydrogen that that can participate in the hydrogen bonding interactions that were believed to have lowered the glycosylation yields of **2.3** and **2.27**. **4.2** also featured a benzyl group

Synthesis of 4.1 and 4.2



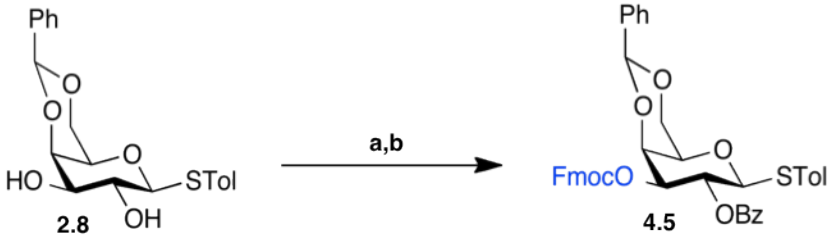
Reagents and conditions: a. NaH, BnBr, DMF, 0°C, 80%, b. TFA, Et_3SiH, DCM, RT, 65%,c. FmocCl, Pyr., RT, 85%, d. NaH, BnBr, DMF, -20°C, 65%, e. TFA, Et_3SiH, DCM, RT, 60%, f., Pyr., RT, 90%

Scheme 4.3

at C3, but a trichloroacetyl group was used to protect the C2 nitrogen of this monosaccharide. The trichloroacetyl group, while similar in structure to the previously used Troc protecting group, was chosen because it was stable to the harsh basic conditions required to benzylate the C3 position. **4.3**[8] and **4.4**[9] were synthesized according to known procedures and then Fmoc protected with Fmoc-Cl and pyridine in good yield (90%).

As shown in Scheme 4.4, the synthesis of **4.5** was quite efficient. It was found that selective Fmoc protection of the C3 hydroxyl followed by benzoylation

Synthesis of 4.5



Reagents and conditions: a. FmocCl, DCM/Pyr. 1:1, 0°C – RT, b. BzCl, DCM/Pyr. 1:1, 0°C – RT, 60% (2 steps)

Scheme 4.4

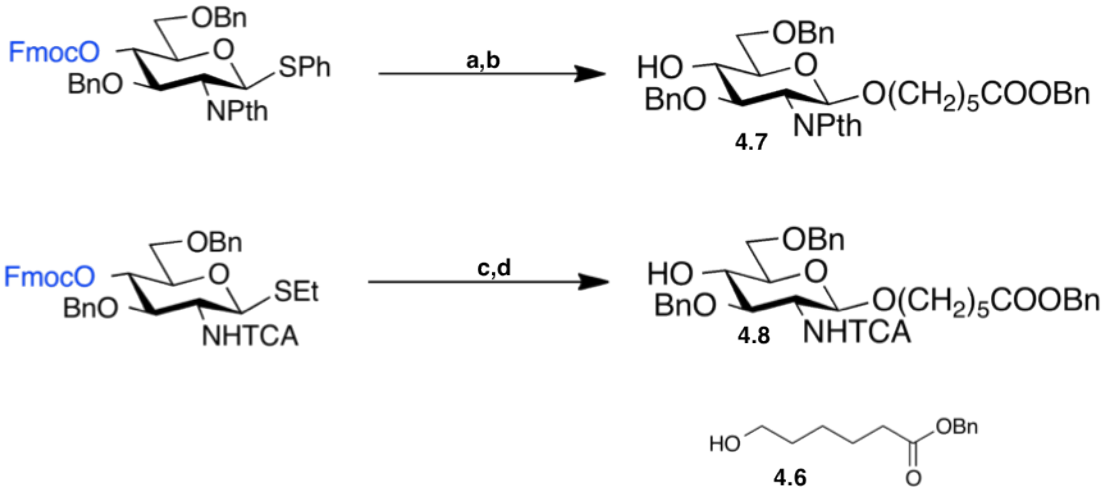
could be carried out in good yield in one-pot. This was a significant improvement in efficiency over the previously used levulinyl ester-based synthesis used to access acceptor **2.2**.

Section 4.4. Fmoc Based Syntheses of the α-Gal Epitope

With Fmoc protected donors **4.1**, **4.2**, and **4.5**, in hand, attention was turned to assembling them into the α-Gal trisaccharide. First, **4.1** and **4.2** were

used to glycosylate 6-hydroxy-1-benzylhexanoic acid (**4.6**)[10] (Scheme 4.5). It was planned that after the synthesis and deprotection of the α-Gal trisaccharide, the carboxyl terminus of **4.6** could be coupled to an amino acid to yield a glycoamino acid that could be used in glycopeptide synthesis. The ICl/AgOTf[11] system was chosen to promote these glycosylations. Both a 1.0 M ICl solution and AgOTf are commercially available and, unlike other common promoter systems, neither requires synthesis or prepurification prior to being used to promote a

Synthesis of 4.7 and 4.8



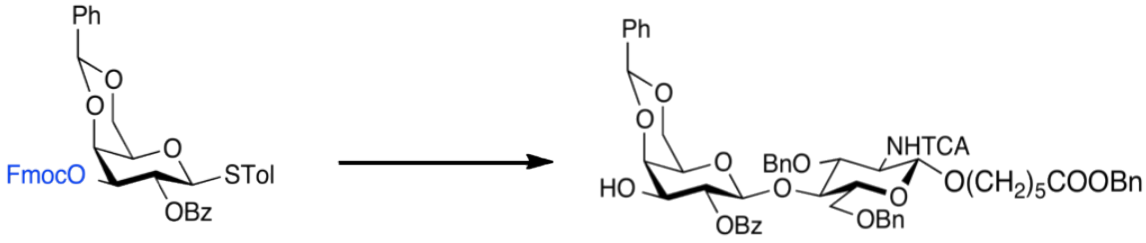
Reagents and conditions: a. **4.6**, ICl, AgOTf, DCM, -20°C, b. Et_3N, RT, 72% (2 steps) c. **4.6**, ICl, AgOTf, DCM, -45°C, d. Et_3N, RT, 93%

Scheme 4.5

glycosylation. It was hoped that the use of these reagents would streamline the process of performing the planned glycosylation reactions. When **4.1** was used to glycosylate **4.6**, the glycosylation proceeded in good yield, but when **4.6** was glycosylated with **4.2**, the reaction produced **4.8** in an excellent 93% yield. Next **4.8** was glycosylated with **4.5** in an efficient 84% yield (Scheme 4.6). Following

this, **2.1** was used to glycosylate **4.9** in a moderate 65% yield. This yield was lower than anticipated, based on the results of the previous two steps, and the desired trisaccharide was isolated as a disappointing 8:1 α:β anomeric mixture (Table 4.1, Entry 1). This was surprising as the stereocontrol in the formation of an α-glycoside using donor **2.1**, had not previously been a problem.

Synthesis of 4.9



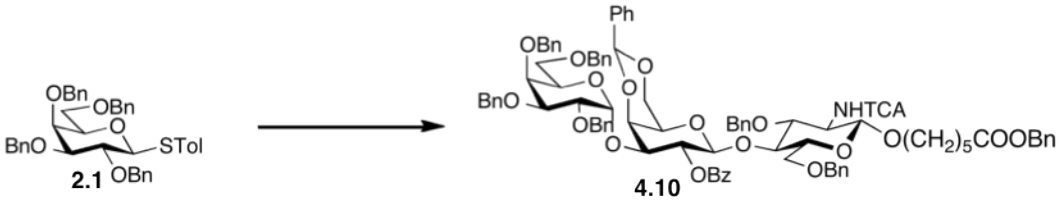
4.5 **4.9**

Reagents and consitions: a. **4.8,** Icl, AgOTf, DCM, -45^{0}C, b. ET_3N, RT, 84% (2 steps)

Scheme 4.6

When the temperature of the reaction was increased to -45^{o}C from -60^{o}C, the stereoselectivity worsened, giving a 6:1 α:β ratio (Table 4.1, Entry 2). When the number of molar equivalents of the glycosyl donor used was increased from 1.5 to 2 equivalents, the α:β increased to 20:1, (Table 4.1, Entry 3). When 3 molar equivalents of donor were used, the ratio increased to 1:0. Additionally, increasing the number of molar equivalents of donor in the reaction mixture correlated with an increase in yield (Table 4.1, Entry 4). However, because using 3 equivalents of glycosyl donor would waste a large amount of donor, especially when these reactions were performed on a gram-scale, future glycosylations were carried out using 2 equivalents of glycosyl donor.

Synthesis of 4.10

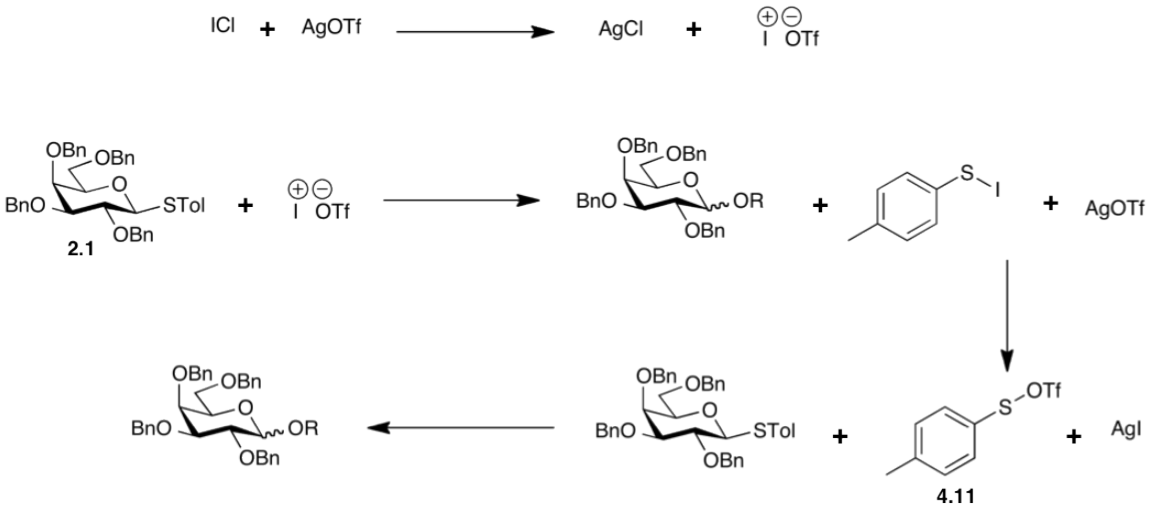


Entry	Donor Equiv.	Temp. (°C)	Yield	α:β
1	1.5	-60	65	8:1
2	1.5	-45	72	6:1
3	2	-60	82	20:1
4	3	-60	85	1:0

Table 4.1

The observation that temperature and molar equivalents of glycosyl donor used had a significant impact on the yield and stereoselectivity of the reaction could be rationalized, in part, based on the mechanism of activation by the ICl/AgOTf promoter system. Activation of a thioglycoside by ICl/AgOTf is shown in Scheme 4.7,[12] AgOTf and ICl react to form iodonium triflate and AgCl, the latter of which precipitates from the reaction mixture. The cationic iodine species can then activate the thioglycoside donor, which reacts with the glycosyl acceptor leading to the formation of a new glycosidic bond and one equivalent of a sulfenyl iodide species. The sulfenyl iodide reacts with another equivalent of AgOTf to yield sulfenyl triflate **4.11**, and AgI, which precipitates out of solution. The sulfenyl triflate can then activate another equivalent of glycosyl donor, leading to the formation of another equivalent of the desired glycoside. As a result, for every mol of new glycosidic bond formed, only 0.5 equivalents of ICl is required. However, the mechanisms of thioglycoside activation by the iodonium species

Thioglycoside Activation by ICl/AgOTf System

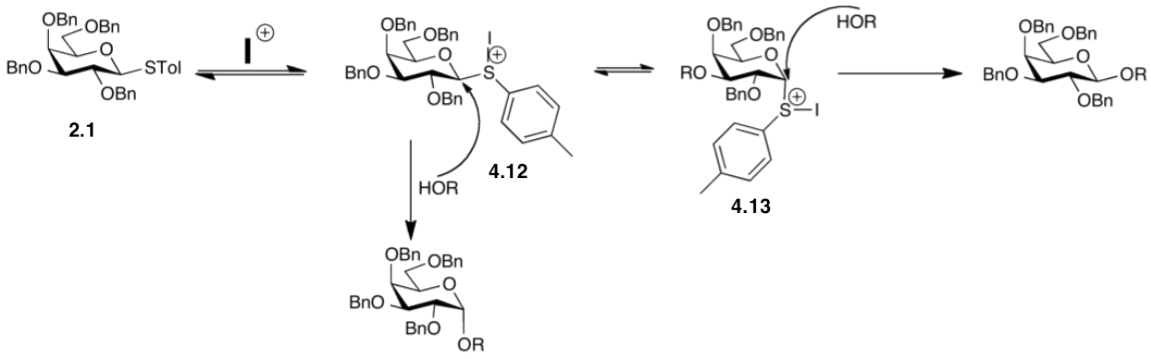


Scheme 4.7

and sulfenyl triflate are different, and these competing mechanisms have a significant impact on the stereoselectivity of the reaction.

In iodine-mediated thioglycoside activation, the iodonium ion and the donor aglycon react reversibly, to form glycosyl sulfenyl iodide intermediates **4.12** and **4.13** (Scheme 4.8). It is postulated, that much like β-glycosyl triflates the β intermediate can be more easily displaced in a S_N2 fashion than the α intermediate. This leads to the predominant formation of the α-glycoside. Hence the α-glycoside is the kinetic product. It was observed that lower temperatures favored the formation of the α-glycoside, indicating that the stereocontrol of this glycosylation is derived kinetically, rather than thermodynamically. This explains the observed erosion of stereoselectivity when the reaction was carried out at warmer temperatures.

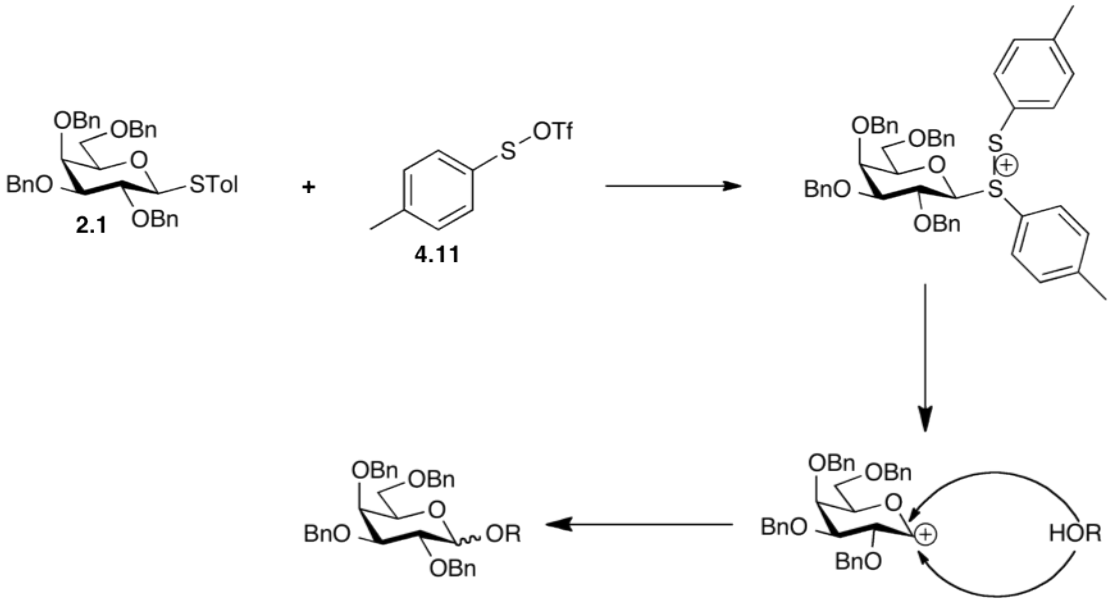
Thioglycoside Activation by Iodonium Cation



Scheme 4.8

In contrast, when the donor thioglycoside is activated by the sulfenyl triflate species, it reacts irreversibly to form an oxocarbenium ion, allowing for nucleophilic attack of the glycosyl acceptor on both the α and β face (Scheme 4.9). In theory, formation of the α-glycoside is favored kinetically and thermodynamically, but it is hypothesized that this pathway is less stereoselective than the iodine-mediated pathway. Following this logic, increasing the amount of glycoside that is formed through the iodine-mediated pathway should increase the stereoselectivity of this glycosylation reaction. By increasing the molar equivalents of glycosyl donor present in the reaction mixture, the amount of glycosyl sulfenyl iodide species that is initially formed is also increased. Because the glycosyl sulfenyl iodide species must react first in order to form the sulfenyl triflate, increasing the amount of glycosyl sulfenyl iodide species initially present in the reaction mixture increases the amount of new glycoside that is formed through the iodine-mediated pathway, increasing the stereoselectivity of this glycosylation reaction. In this way, the observation that

Thioglycoside Activation by 4.11

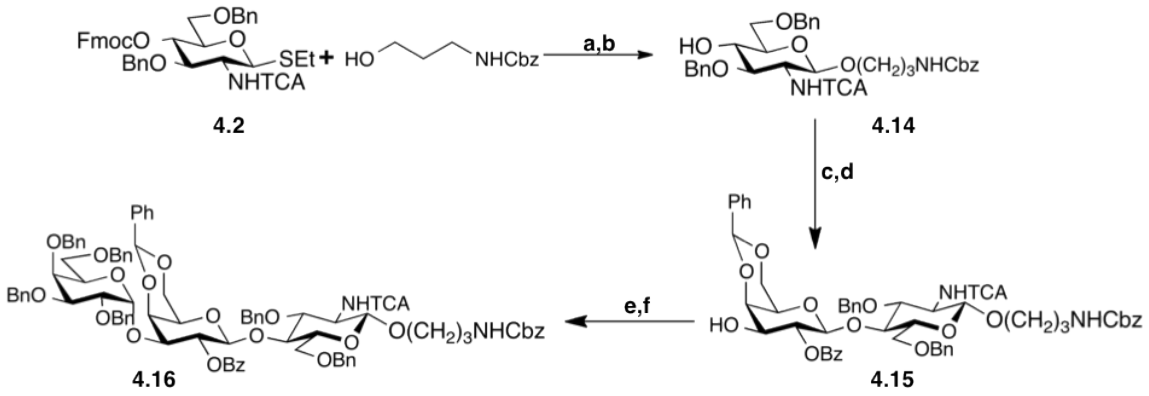


Scheme 4.9

increasing the number of molar equivalents of glycosyl donor present in the reaction mixture correlated with an increase in the stereoselectivity of the reaction is rationalized.

After synthesis of α-Gal trisaccharide **4.10**, α-Gal trisaccharide **4.16** was synthesized using the same optimized reaction conditions in 65% overall yield (Scheme 4.10). Additionally, this synthesis was scaled up to gram scale with the same result. The syntheses of **4.10** and **4.16** demonstrated that this Fmoc based approach to the synthesis of the α-Gal epitope met the three initial criteria: (i) it was efficient, (ii), it was scalable, and (iii) it offered the ability to easily vary the terminal conjugate.

Synthesis of Trisaccharide 4.16



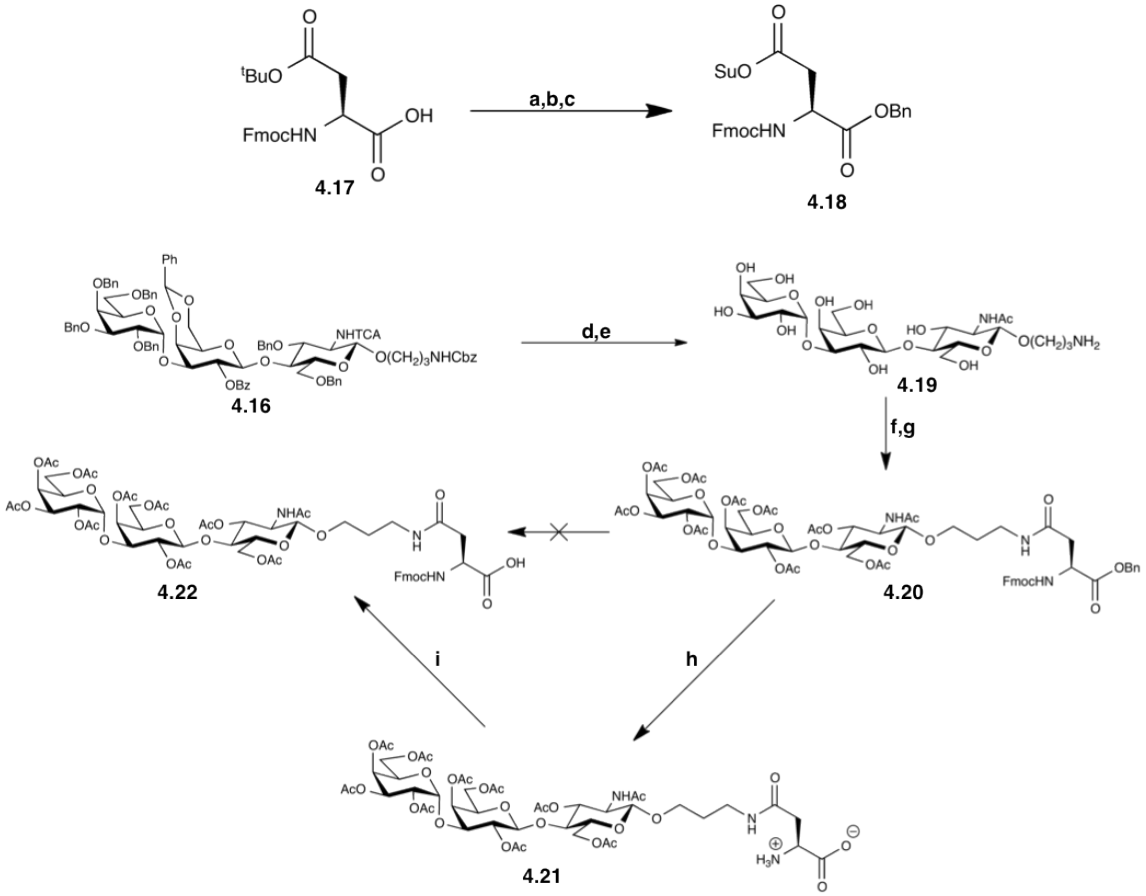
Reagents and conditions: a. ICl, AgOTf, DCM, -45°C, b. Et_3N, RT, 95%, (2 steps), c..**5**, ICl, AgOTf, DCM, -45°C, d. Et_3N, RT, 85%, (2 steps), e. **2.2**, ICl, AgOTf, DCM, -60°C, f. Et_3N, RT, 82%, (2 steps)

Scheme 4.10

Section 4.5. Synthesis of Glycoamino Acids

With trisaccharide **4.16** in hand, attention was turned to conversion of this protected oligosaccharide into a glycoamino acid that could be used in glycopeptide synthesis. It was thought that once fully deprotected, the terminal amino group could be easily coupled to a protected aspartate residue to yield a glycoamino acid. To this end, commercially available aspartate derivative **4.17** was converted to succinimidyl ester **4.18** in good yield.[13] Trisaccharide **4.16** was deprotected in two steps to furnish **4.19** in 55% yield. The coupling of **4.18** to **4.19**, and subsequent acetylation produced **4.20** in 54% yield. Frustratingly, Pd-catalyzed hydrogenation of the benzyl ester did not yield desired glycoamino acid **4.22**. Instead it led to the formation of what appeared to be an impure mixture comprised mostly of compound **4.21**. Fortunately, treatment of this mixture with Fmoc-Cl and $NaHCO_3$ furnished **4.22** (Scheme 4.11)**.**

Synthesis of Glycoamino Acid 4.22



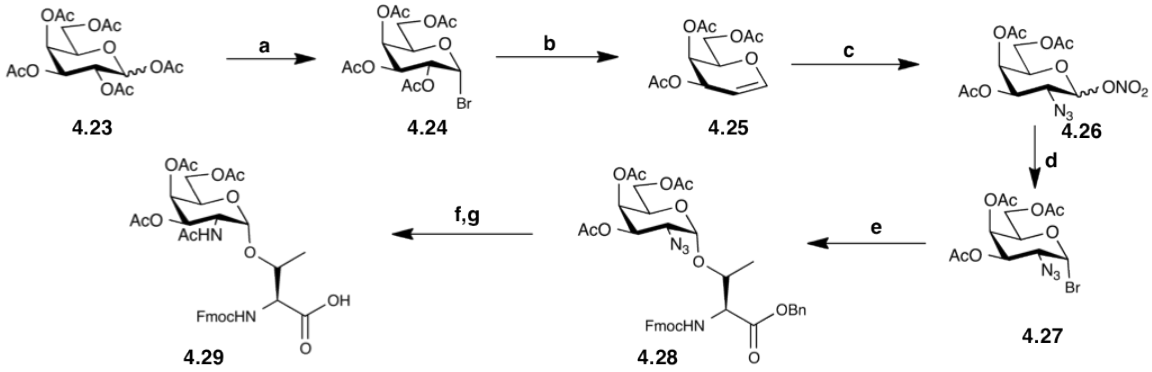
Reagents and conditions: a. Cs_2CO_3, BnBr, DMF, RT, b. DCM/TFA 4:1, RT, c. HOSu, DIC,DCM, RT, 60% (3 steps), d. NaOMe, DCM/MeOH 1:1, RT, e.10% Pd/C, H_2, HCl, EtOAc/ MeOH/H_2O 1:1:0.2, 55% (2 steps), f. **4.18**, $NaHCO_3$, DMF/H_2O 1:1, RT, g. Ac_2O, Pyr., RT,54% (2 steps), h. 10% Pd/C, H_2, MeOH, RT, i. FmocCl, $NaHCO_3$, DCM/MeOH 1:1, RT, 31% (2 steps)

Scheme 4.11

After the synthesis of **4.22**, and anticipation of its subsequent use in a subunit antitumor vaccine, a tumor-associated carbohydrate antigen, the Tn antigen, was synthesized. Penta-acetyl-D-galatcose was converted to the corresponding galactosyl bromide, which was reduced with Zn^0 to yield galactal **4.25**. This compound was converted to azidobromide **4.27**, using standard conditions.[14] **4.27** was then coupled to N-Fmoc-L-threonine benzyl ester using

the reaction conditions set forth by Danishefsky and coworkers.[15] Lastly, **4.28** was deprotected in two steps to furnish glycoamino acid **4.29** (Scheme 4.12).

Synthesis of Tn Antigen 4.29



Reagents and conditions: a. Ac_2O, HBr/AcOH, RT, b. Zn^0, $CuSO_4$, Et_2O/AcOH/H_2O 1:1:1, 0°C, 68% (2 steps), c. CAN, NaN_3, MeCN, -15°C, d. LiBr, MeCN, RT, 36% (2 steps), e. N-Fmoc-L-threonine benzyl ester, $AgClO_4$, DCM, RT, f. AcSH, Pyr., RT, 50% (2 steps), g. 10% Pd/C, H_2, MeOH, RT, 75%

Scheme 4.12

Section 4.6. Preliminary Studies in Glycopeptide Synthesis

Following the syntheses of glycoamino acids **4.22** and **4.29**, it appeared that the time to synthesize an α-Gal based subunit vaccine was at hand. The envisioned subunit vaccine is shown in Figure 4.1, it is comprised of a built-in adjuvant (α-Gal), a MHC-II binding peptide (PADRE peptide),[16] and a tumor antigen (MUC-1). Recent studies have demonstrated that the Tn antigen elicits a stronger immune response when presented as part of the MUC-1 glycopeptide.[17] Consequently, the Tn antigen is incorporated into this subunit vaccine design as part of a five amino acid sequence found in the MUC-1 glycopeptide rather than

being incorporated on its own. It is believed that this glycopeptide can be assembled using standard solid-phase peptide procedures and that the use of

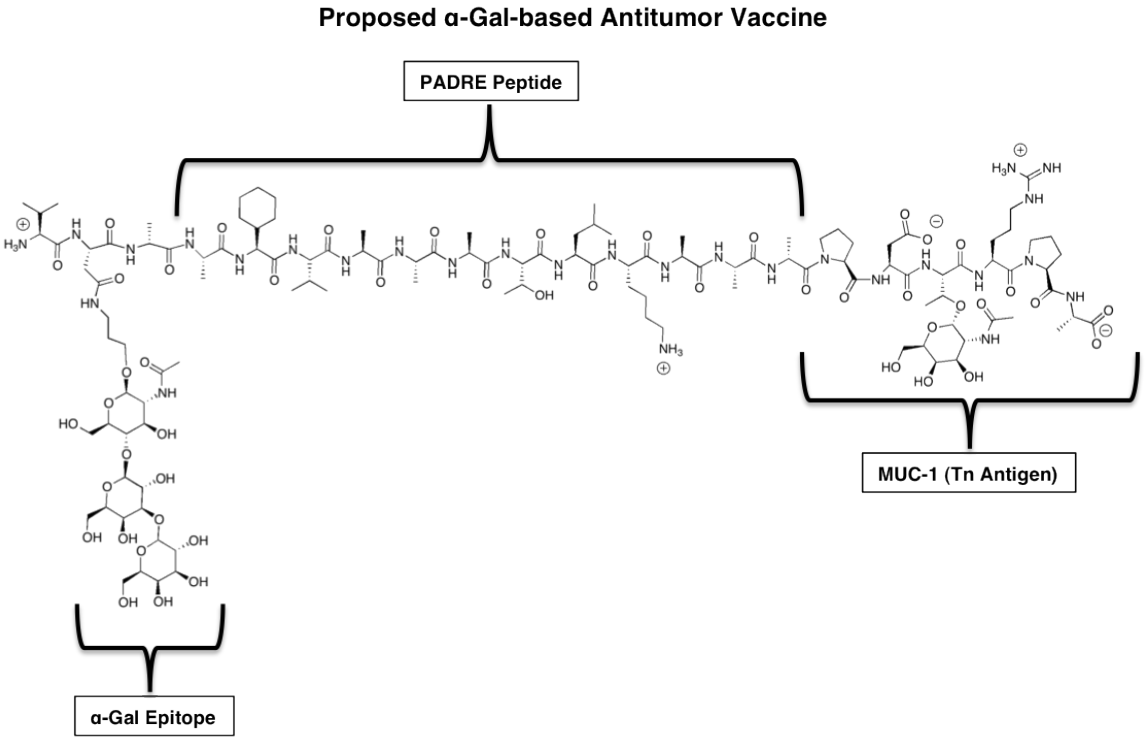
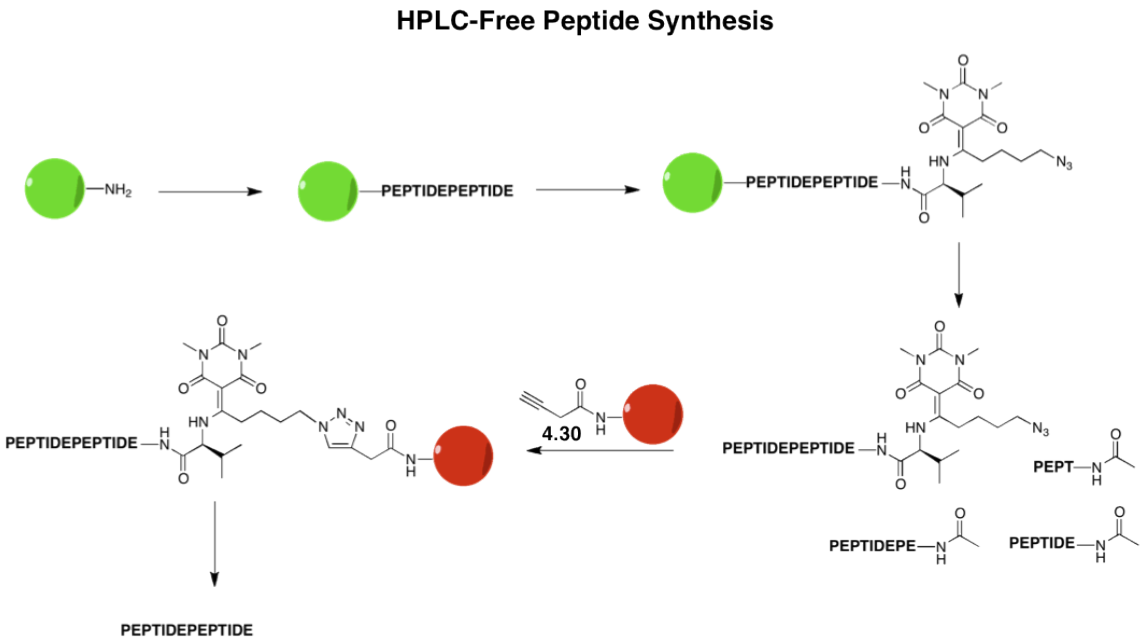


Figure 4.1

the HPLC-free purification procedure of Galibert and coworkers[18] will enhance the efficiency of this synthesis. The HPLC-free purification method relies on an azide tag that is coupled to the peptide following elongation using SPPS procedures (Scheme 4.13). After cleavage from the resin, copper-catalyzed Huisgen 1,3-dipolarcycloaddition is used to couple the azide-tagged peptide to a propargylated resin. Washing of the resulting resin removes peptide deletion sequences. This is followed by cleavage of the peptide from the resin furnishing

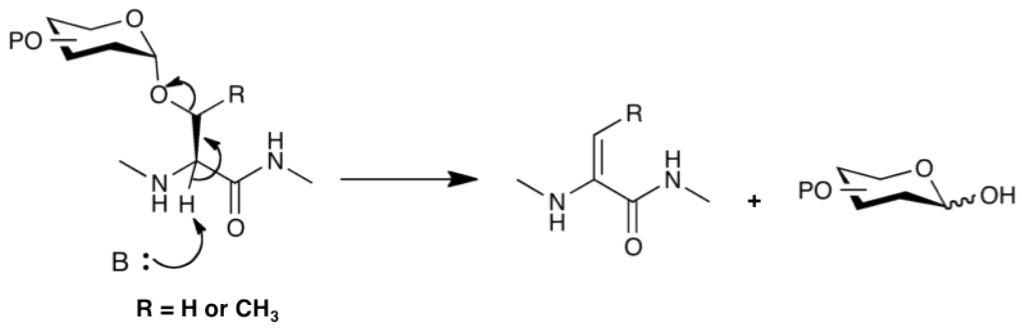


Scheme 4.13

the desired peptide in high-purity without the need for time-consuming and scale-limiting preparative HPLC purification.

A common problem in the synthesis of glycopeptides is the removal of the glycosyl acetates. These acetates are required during glycopeptide synthesis to reduce the likelihood of hydrolysis of the glycosyl residues during acidic cleavage of the glycopeptide from the solid-phase resin. However, the basic conditions used to remove acetates can lead to β-elimination (Scheme 4.14). The HPLC-free purification method uses a 1.0 M solution of NH_4OH to cleave the purified peptide from the propargylated resin. It was anticipated that these mild conditions would also remove the glycosyl acetates, and that the mild basicity of NH_4OH would reduce possibility of β-elimination. The MUC-1 glycopeptide was chosen as a model glycopeptide to gauge the applicability of this approach to glycopeptide synthesis without having to waste any of the α-Gal glycoamino acid,

β-elimination of Glycoamino Acids



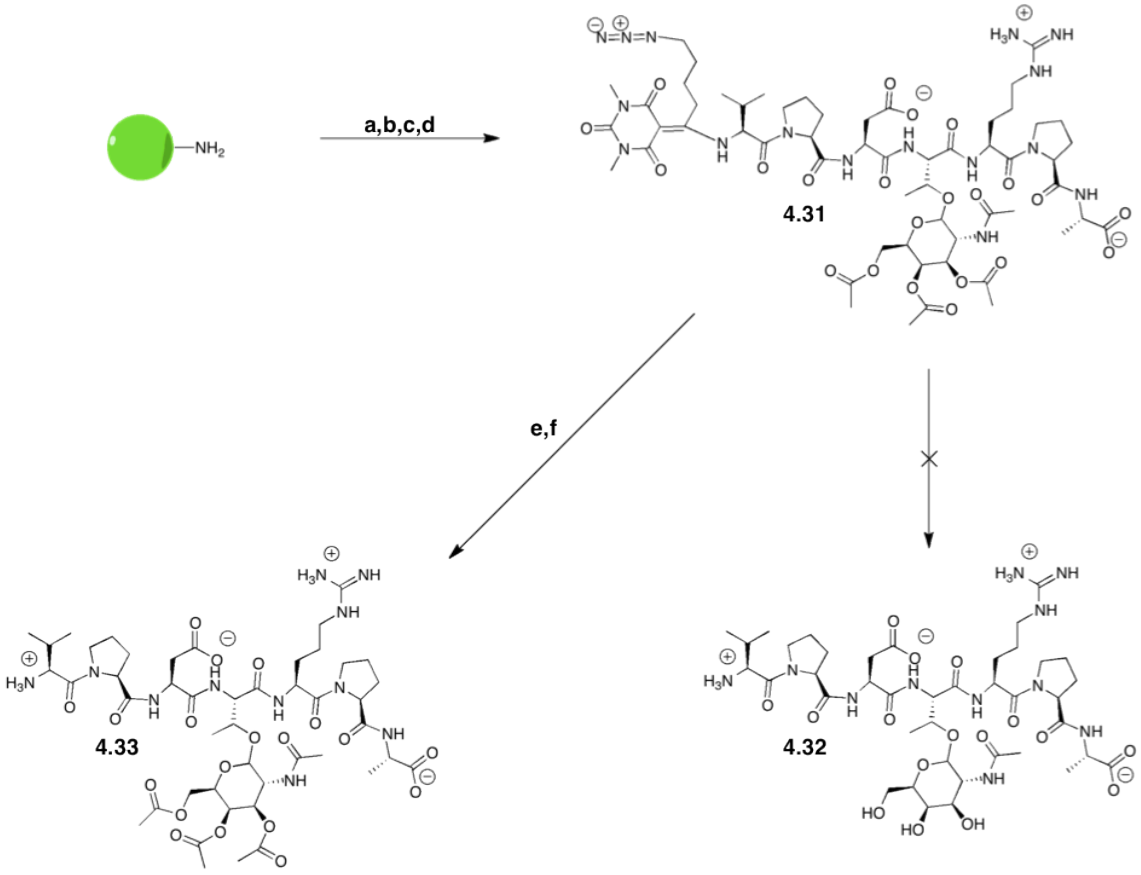
Scheme 4.14

which was a sufficiently valuable commodity. The desired MUC-1 glycopeptide was isolated using this procedure. However, as shown in Scheme 4.15, the conditions used to cleave the purified peptide from the propargylated resin did not concurrently remove the glycosyl acetates. Instead it led to a mixture of products with varying degrees of deprotection. Hence further work is required to optimize this HPLC-free procedure for the synthesis of glycopeptides before its application in the synthesis of the envisioned α-Gal based antitumor vaccine.

Section 4.7. Conclusions

After repeated failures in the synthesis of the α-Gal trisaccharide, a new strategy was devised. In this strategy, a Fmoc-protected glycosyl donor is coupled to a non-reducing glycosyl acceptor and the Fmoc group is then removed in one-pot. This one-pot glycosylation/deprotection strategy eliminates the possibility of aglycon transfer occurring. When applied to the synthesis of the

Attempted Synthesis MUC-1 Glycopeptide 4.32



Reagents and conditions: a. N-Fmoc-L-amino acid, HATU, HOAt, DIPEA, DMF, RT, 30 min, b. Ac_2O, DIPEA, DMF, RT, 10 min, c. 20% Piperidine in DMF, RT, 20 min, d. Reagent B, RT, 3 h, e. **4.28**, $CuSO_4$, Na Ascorbate, THPTA, Amino Guanidine HCl, HEPES buffer/DMF 6:1, RT, f. 1.0 M NH_4OH, RT

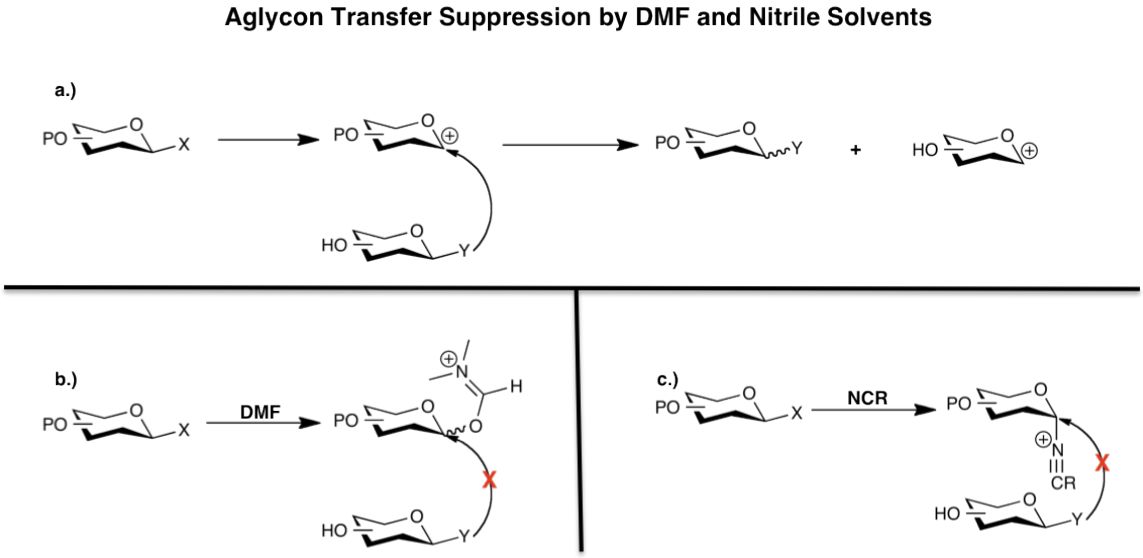
Scheme 4.15

α-Gal trisaccharide, this strategy led to excellent overall yields of two protected derivatives of the α-Gal epitope. Subsequent deprotection of trisaccharide **4.16**, coupling to succinimidyl ester **4.18** and acetylation furnished glycoamino acid **4.20**. The hydrogenation of this residue gave a mixture of products, but treatment of this mixture with Fmoc-Cl led to the formation of desired glycoamino acid **4.22**. Further experimentation is needed to increase the efficiency of the coupling and deprotection steps of this glycoamino acid synthesis. Following the synthesis of

4.22, the Tn antigen was synthesized and used in the synthesis of a tumor-associated antigen, the MUC-1 glycopeptide. A HPLC-free purification of this glycopeptide was attempted, but it failed to remove the glycosyl acetates of the Tn antigen.

Section 4.8. Future Directions.

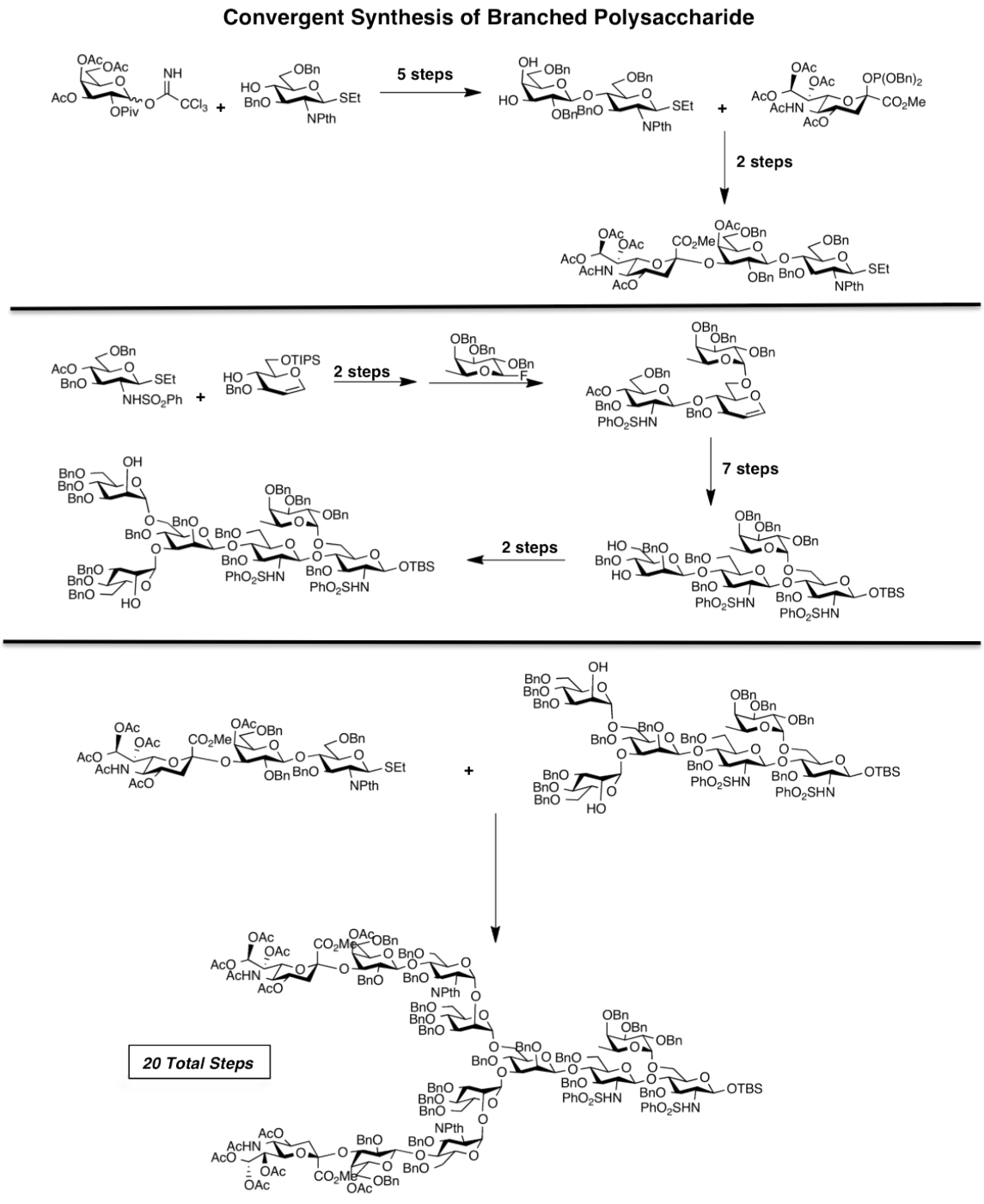
The results disclosed in this work present several opportunities for further exploration. The discovery that aglycon transfer was having a significant negative impact on the synthesis of the α-Gal trisaccharide highlights the need for novel methods to mitigate this side reaction. It was demonstrated that the use of DMF as a cosolvent could effectively reduce the impact of aglycon transfer on a glycosylation reaction. Further work is necessary to probe the limits of this method. Additionally, because a DMF mediated glycosylation favors the formation of an α-glycoside, this method is not applicable to glycosylation reactions in which the formation of a β glycosidic bond is desired. The use of nitrile solvents presents an alternative to the DMF mediated suppression of aglycon transfer. Nitrile solvents can coordinate to the α face of oxocarbenium ions, favoring the formation of β-glycosides.[19] It is hypothesized that this effect could be harnessed to suppress aglycon transfer while favoring the formation of a β-glycoside, presenting a complimentary method to the DMF approach (Scheme 4.16). In this way there would be made available two stereoselective methods with which to mitigate the impact of aglycon transfer.



Scheme 4.16

The Fmoc-based glycosylation strategy disclosed here furnished a highly efficient synthesis of the α-Gal epitope, but further work is required to probe the limits of this strategy. It is proposed that this strategy could increase the efficiency of the synthesis of large, branched oligosaccharides. As shown in Scheme 4.17 in the current state of the art, syntheses of these large polysaccharides rely on a convergent strategy in which di- and trisaccharides are assembled first, and then these saccharides are coupled to yield the desired polysaccharide. The linear strategy presented in this work could potentially streamline this process. A comparison of these two approaches is shown in Scheme 4.17[20] and 4.18.

Lastly, the α-Gal glycoamino acid synthesis presented here allows for the synthesis and evaluation of an α-Gal based subunit vaccine; a potential design for this vaccine and work towards its synthesis were presented in this work,

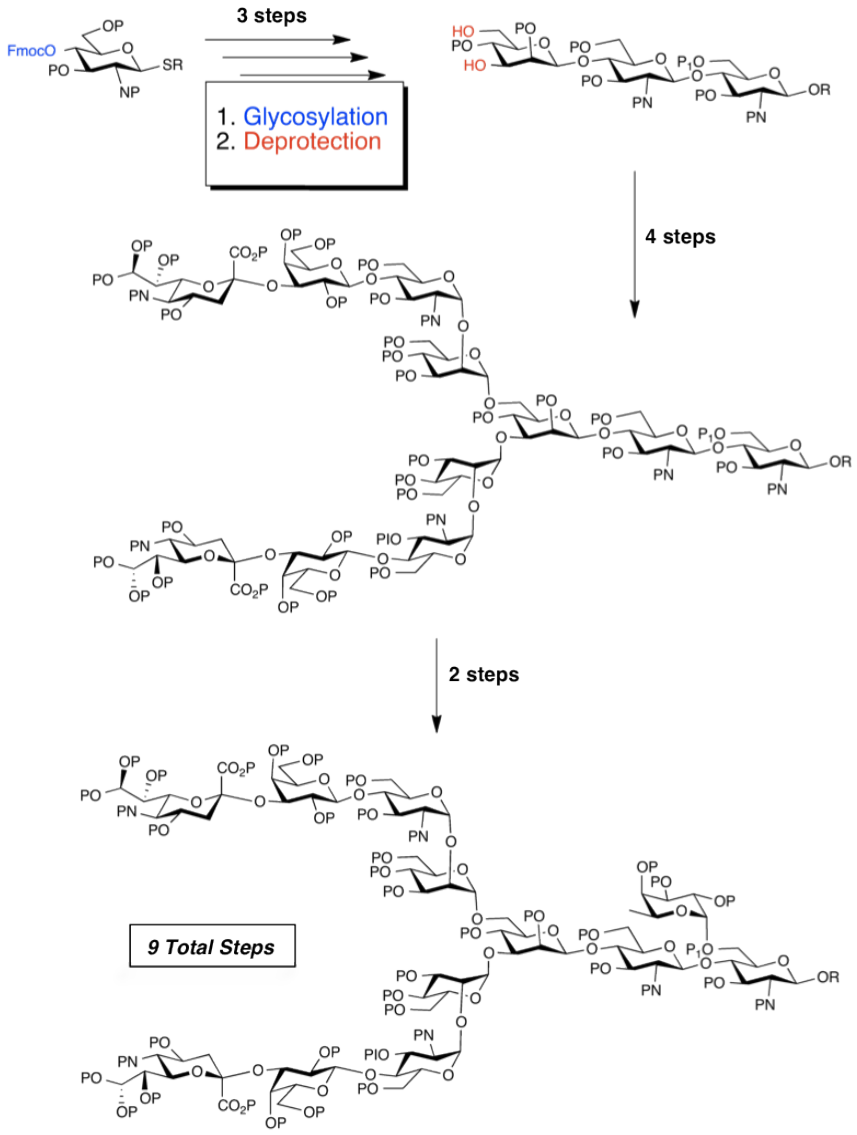


Scheme 4.17

but further work optimizing the HPLC-free purification conditions are needed. Aqueous hydrazine has been used in previous glycopeptide syntheses to remove the glycosyl acetates without inducing β-elimination.[21] It is speculated that it

could replace the NH_4OH solution used cleave the purified glycopeptide from the propargylated resin. This would allow for concurrent cleavage and deprotection of the desired glycopeptide vaccine.

Proposed Fmoc-Based Synthesis of a Branched Oligosaccharide



Scheme 4.18

Following synthesis of an α-Gal based subunit vaccine, further biological evaluation of its immunological effects would be required. This can be done using α-Gal knockout mice. These mice, like humans, express anti-Gal antibodies.[22] Exposure of these mice to the α-Gal subunit vaccine followed by analysis of their blood serum would reveal if the α-Gal epitope is capable of conferring immunity against the tumor associated MUC-1 glycopeptide. If successful, this would raise the possibility that the α-Gal epitope could confer immunity against a wide variety of antigens: microbial, fungal, and cancerous. Thus, a wide variety of α-Gal based subunit vaccines could be synthesized and screened, leading to a clinically viable vaccine.

Section 4.9. Literature Cited

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Chapter 5

Experimental Protocols for Selected Compounds

Section 5.1. General Materials and Methods

All non-aqueous reactions were performed under an argon atmosphere passed over Drierite® in oven dried glassware with a Teflon coated magnetic stir bar and magnetic stirring unless otherwise stated. Dry CH_2Cl_2, was obtained by allowing the solvent to sit over 4 Å molecular sieves under an inert atmosphere for at least 24 h and up to 7 days. Dry MeCN, and MeOH were obtained by allowing the solvents to sit over 3 Å molecular sieves under an inert atmosphere for at least 24 h and up to 7 days. All sieves were oven dried (>160^{o}C) overnight and cooled *in vacuo* before use. Dry DMF and pyridine were obtained in Acroseal® bottles and used without any further purification. Triflic anhydride was distilled over phosphorous pentoxide prior to use. Triethylamine was distilled over NaOH and stored over KOH. N-Iodosuccinimde was recrystallized from carbon tetrachloride and 1,4-dioxane. All other reagents were used as purchased from Sigma (St. Louis, MO), Acros (Pittsburg, PA), Aldrich (Milwaukee, WI), and Novabiochem (La Jolla, CA).

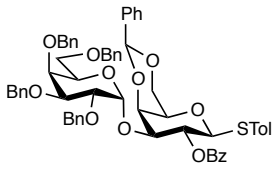
Reaction progress was monitored by analytical thin layer chromatography on 250 µm thick glass-backed silica plates (MF254, Agela). Developed plates were visualized by UV (254 nm) and/or staining with iodine, or Hanessian's stain (acidic cerium ammonium molybdate). Column chromatography was carried out using 60Å silica gel (400-600 mesh, Agela). Soild-Phase extraction was conducted using Supelco® C_{18} cartidges (500 mg, Fluka), or Chromabond® C_8

cartridges (500 mg, Sorbtech). Yields reported are for spectroscopically and chromatographically pure compounds unless otherwise reported.

Infared spectra were obtained from a Shimadzu IRAffinity-1S and reported in cm^{-1} (%T). All ^{1}H and ^{13}C spectra were taken on either a Varian Inova 500 (125), or a Varian Inova 600 (150) spectrometer. Chemical shifts are reported in ppm (δ) using the residual ^{1}H (7.26 ppm) and ^{13}C (77.23 ppm) resonances from $CDCl_3$ as internal standards. ^{1}H NMR data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets), coupling constant, integration. Diastereomeric ratios were obtained by ^{1}H NMR analysis of crude mixtures. High-resolution mass spectra (HRMS) were measured by the Mass Spectrometry Laboratory at the University of Illinois, Urbana-Champaign.

Section 5.2. Experimental Information for Compounds in Chapter 2

Compound 2.16



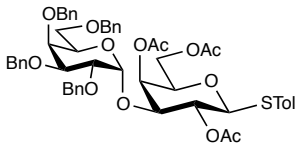
A 2-necked flask was charged with powdered 4Å molecular sieves (200 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.1** (48 mg, 0.074 mmol, 1.0 equiv.),

2.2 (32 mg, 0.067 mmol, 0.9 equiv.), 1-benzenesulfenyl piperidine (17.2 mg, 0.082 mmol, 1.1 equiv.), and 2,6-di-*tert*-butyl-4-methyl pyridine (30 mg, 0.148 mmol, 2 equiv.) were added. Dry CH_2Cl_2 (2 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. After cooling to -78^oC and the reaction mixture was stirred for 5 min. At this time, triflic anhydride (14.90 μL, 0.088 mmol, 1.2 equiv), was added dropwise to the reaction mixture. The resultant was allowed to slowly warm to room temperature over the course of 2 h. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1-2:1) as the eluent, to give desired product **2.16** (9.5 mg, 20%), as a clear oil. Analytical data was in agreement with the literature.[1]

R_f (hexane:EtOAc 2:1) = 0.45

^{1}H NMR (500 MHz, $CDCl_3$) δ ^{1}H NMR 8.04 (d, *J* = 7.0, 2H), 7.51-7.01 (m, 32H), 5.56 (t, *J* = 9.8 Hz, 1H), 5.39 (s, 1H), 5.06 (d, *J* = 3.5 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.73 (d, *J* = 9.8 Hz, 1H), 4.63 (d, *J* = 11.7 Hz, 1H), 4.57 (d, *J* = 11.6 Hz, 1H), 4.47 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.7 Hz, 1H), 4.32 (dd, *J* = 3.4, 1.1 Hz, 1H), 4.26 (d, *J* = 11.8 Hz, 1H), 4.01 (dd, *J* = 9.8, 3.3 Hz, 1H), 3.98 (d, *J* = 1.7 Hz, 1H), 3.95 (t, *J* = 2.3 Hz, 1H), 3.82 (td, *J* = 6.6, 1.6 Hz, 1H), 3.64 (dd, *J* = 10.1, 2.9 Hz, 1H), 3.42 (dq, *J* = 4.6, 1.3 Hz, 2H), 3.27-3.17 (m, 2H), 2.33 (s, 3H).

Compound 2.23



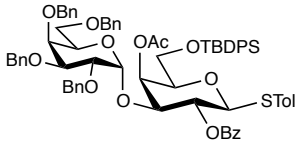
A 2-necked flask was charged with powdered 4Å molecular sieves (200 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.1** (50 mg, 0.077 mmol, 1.0 equiv.), **2.21** (28.7 mg, 0.069 mmol, 0.9 equiv.), and N-Iodosuccinimide (20.8 mg, 0.092 mmol, 1.2 equiv.) were added. Dry CH_2Cl_2 (2 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. After cooling to 0^oC and the reaction mixture was stirred for 5 min. At this time, trimethylsilyl triflate (1.4 µL, 0.008 mmol, 0.1 equiv), was added dropwise to the reaction mixture. The resultant was stirred 30 min. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (2:1) as the eluent, to give **2.23** (51.5 mg, 80%), as a clear oil.

R_f (hexane:EtOAc 2:1) = 0.3

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42-7.21 (m, 22H), 7.11 (d, *J* = 7.9 Hz, 2H), 5.50-5.46 (m, 1H), 5.23 (t, *J* = 9.9 Hz, 1H), 5.07 (d, *J* = 3.4 Hz, 1H), 4.92 (d, *J* = 11.3

Hz, 1H), 4.83 (d, *J* = 11.8 Hz, 1H), 4.73-4.64 (m, 3H), 4.54-4.44 (m, 3H), 4.38 (d, *J* = 11.8 Hz, 1H), 4.12-4.07 (m, 2H), 4.00 (dd, *J* = 9.4, 3.4 Hz, 1H), 3.93-3.86 (m, 1H), 3.86-3.76 (m, 2H), 3.70 (t, *J* = 6.5 Hz, 1H), 3.50 (d, *J* = 6.4 Hz, 2H), 2.34 (s, 3H), 2.05 (d, *J* = 1.1 Hz, 3H), 1.99 (s, 2H), 1.81 (s, 2H).

Compound 2.24



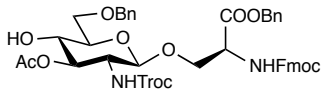
A 2-necked flask was charged with powdered 4Å molecular sieves (100 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.1** (48 mg, 0.075 mmol, 2.0 equiv.), **2.22** (25 mg, 0.038 mmol, 1.0 equiv.), and 2,6-di-*tert*-butyl-4-methyl pyridine (15.4 mg, 0.075 mmol, 2 equiv.) were added. Dry CH_2Cl_2 (1 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. At this time, the reaction mixture was cooled to 0^{o}C, and dimethyl(methylthio)sulfonium triflate (20.6 mg, 0.08 mmol, 2.1 equiv), was added, as a solid, to the reaction mixture. The resultant was stirred at 0^{o}C for 30 min and then removed from ice-bath and stirred at room temperature for 45 min. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using

hexanes/EtOAc (7:1-6:1) as the eluent, to give desired **2.24** (27 mg, 80%), as a clear oil.

R_f (hexane:EtOAc 6:1) = 0.4

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.5 Hz, 2H), 7.70-7.61 (m, 4H), 7.50 (td, *J* = 7.4, 1.4 Hz, 1H), 7.45-7.20 (m, 29H), 7.15-7.09 (m, 2H), 7.03 (d, *J* = 7.8 Hz, 2H), 5.66-5.61 (m, 1H), 5.55-5.47 (m, 1H), 5.28-5.23 (m, 1H), 4.73 (d, *J* = 10.1 Hz, 2H), 4.67-4.57 (m, 3H), 4.52 (dd, *J* = 11.8, 1.5 Hz, 1H), 4.36 (dt, *J* = 11.9, 1.5 Hz, 2H), 4.31-4.27 (m, 1H), 4.13-4.05 (m, 1H), 3.94-3.85 (m, 2H), 3.80-3.74 (m, 1H), 3.70-3.62 (m, 2H), 3.52 (dt, *J* = 10.1 Hz, 1H), 3.46 (t, *J* = 7.5, Hz, 1H), 3.17 (q, *J* = 5.3, Hz, 2H), 2.31 (s, 3H), 1.75 (d, *J* = 1.6 Hz, 3H), 1.06 (d, *J* = 1.7 Hz, 9H).

Compound 2.27



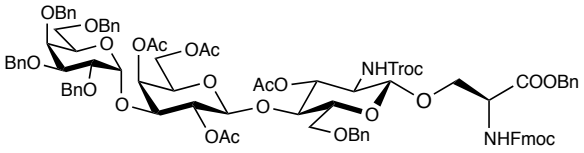
A 2-necked flask was charged with powdered 4Å molecular sieves (500 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.3** (248 mg, 0.418 mmol, 1.0 equiv.), and N-Fmoc-L-serine benzyl ester (262 mg, 0.627 mmol, 1.5 equiv.), Dry CH_2Cl_2 (2 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. At this time, the reaction mixture was cooled to -45^{o}C, and N-

Iodosuccinimide (112.5 mg, 0.50 mmol, 1.2 equiv.) was added to the solution. The resulting mixture was stirred for 5 min. Trimethylsilyl triflate (7.0 µL, 0.04 mmol, 0.1 equiv), was added dropwise to the reaction mixture. The resultant was then allowed to warm to -20^{o}C over 0.5 h. Dry triethylamine (0.2 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by radial chromatography on silica gel using hexanes/EtOAc (3:2) as the eluent, to give the desired product (288 mg, 78%), as a clear oil.

R_f (hexane:EtOAc 3:2) = 0.2

^{1}H NMR (500 MHz, $CDCl_3$) δ^{1}H NMR 7.77 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.37-7.26 (m, 13H), 5.90 (d, *J* = 8.0 Hz, 1H), 5.32 (d, *J* = 9.4 Hz, 1H), 5.25-5.15 (m, 2H), 5.06-4.98 (m, 1H), 4.76 (d, *J* = 9.7 Hz, 1H), 4.61-4.45 (m, 8H), 4.31-4.19 (m, 3H), 3.88 (dd, *J* = 10.4, 3.3 Hz, 1H), 3.66-3.56 (m, 1H), 3.48 (dt, *J* = 9.5, 4.7 Hz, 1H), 2.10 (s, 4H).

Compound 2.28



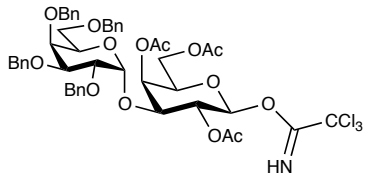
A 2-necked flask was charged with powdered 4Å molecular sieves (150 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.27** (28.4 mg, 0.032 mmol, 1.5

equiv.), and **2.23** (20 mg, 0.021 mmol, 1.0 equiv.), Dry CH_2Cl_2 (1.5 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. At this time, the reaction mixture was cooled to -78^oC, and AgOTf (16.4 mg, 0.064 mmol, 3 equiv.) was added to the solution. The resulting mixture was stirred for 5 min. p-Nitrophenylsulfenyl chloride (6.0 mg, 0.032 mmol, 1.5 equiv), was then added to the reaction mixture. The resultant was then allowed to warm to 0^oC over 1.25 h, and then stirred at 0^oC for 2h. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (2:1-1:1) as the eluent, to give the desired product (17 mg, 48%), as a clear oil.

R_f (hexane:EtOAc 2:1) = 0.3

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.5, 2.6 Hz, 2H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.43-7.21 (m, 40H), 5.91 (d, *J* = 8.4 Hz, 1H), 5.38 (d, *J* = 3.5 Hz, 1H), 5.20 (s, 2H), 5.04 (d, *J* = 10.4 Hz, 2H), 4.99 (d, *J* = 3.4 Hz, 1H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.82 (d, *J* = 11.8 Hz, 1H), 4.71 (t, *J* = 12.0 Hz, 2H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.50 (d, *J* = 11.3 Hz, 1H), 4.44 (d, *J* = 11.9 Hz, 1H), 4.38 (d, *J* = 11.8 Hz, 2H), 4.34 (d, *J* = 8.1 Hz, 1H), 4.23 (d, *J* = 5.1 Hz, 1H), 4.04-3.97 (m, 3H), 3.90 (t, *J* = 8.8 Hz, 1H), 3.87-3.78 (m, 4H), 3.66 (d, *J* = 11.9, Hz, 3H), 3.55-3.47 (m, 3H), 3.40 (dt, *J* = 9.4, 3.0 Hz, 1H), 2.18 (s, 1H), 2.08 (s, 3H), 2.01 (s, 3H), 1.89 (s, 3H), 1.82 (s, 3H).

Compound 2.29



To a stirred solution of **2.23** (40 mg, 0.042 mmol, 1.0 equiv), in a mixture of acetone and water (15:1 v/v, 1.6 ml), at 0°C, was added N-bromosuccinimide (10 mg, 0.051 mmol, 1.2 equiv.). The resulting solution as stirred for 1.0 h at 0°C, and then additional N-bromosuccinimide (10 mg, 0.051 mmol, 1.2 equiv.) was added. The resultant was then stirred for an additional hour at 0°C. At this time, the solution was removed from the ice-bath and stirred for an additional 30 min at room temperature. The solvent was removed under reduced pressure and the resulting residue was dissolved in CH_2Cl_2 (15 ml). The CH_2Cl_2 solution was then washed with satd. aq. sodium thiosulfate (3 x 10 mL), and brine (1 x 10 mL). The solution was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure and the resulting oil was used in the next step without further purification.

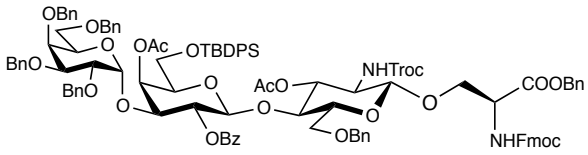
To the flask containing the hemiacetal intermediate was added a stir-bar and cesium carbonate (8 mg, 0.026 mmol, 0.5 equiv.). The flask was evacuated and flushed with dry argon, and dry CH_2Cl_2 (2 ml) was added. The resultant was cooled to 0°C, at which time trichloroacetonitrile (52 µL, 0.53 mmol, 10 equiv.) was added dropwise. The resulting turbid solution was stirred for 30 min at 0°C.

At this time, the reaction mixture was removed from the ice-bath and diluted with CH_2Cl_2 (10 ml). The resulting solution was washed with water (1 x 5 ml) and brine (1 x 5 ml). The solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel hexanes/EtOAc (2:1) as the eluent, to yield the desired product (25.7 mg, 50%) as an oil.

R_f (hexane:EtOAc 2:1) = 0.75

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.46-7.08 (m, 20H), 6.61 (d, *J* = 3.6 Hz, 1H), 5.62 (dd, *J* = 3.4, 1.3 Hz, 1H), 5.30 (d, *J* = 10.6, Hz, 1H), 5.15 (d, *J* = 3.5 Hz, 1H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.83 (d, *J* = 11.8 Hz, 1H), 4.74 (s, 1H), 4.69 (q, *J* = 11.5, 10.5 Hz, 3H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.44-4.34 (m, 2H), 4.35-4.29 (m, 2H), 4.15 (dd, *J* = 11.5, 6.0 Hz, 1H), 4.07-4.00 (m, 3H), 3.94 (m, 1H), 3.86 (dd, *J* = 10.1, 2.8 Hz, 1H), 3.59 (t, *J* = 8.6 Hz, 1H), 3.41 (dd, *J* = 8.5, 5.0 Hz, 1H), 2.03 (s, 3H), 1.90 (s, 3H), 1.85 (s, 3H).

Compound 2.30



A 2-necked flask was charged with powdered 4Å molecular sieves (200 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.23** (35 mg, 0.029 mmol, 1.0 equiv.), and **2.27** (23.4 mg, 0.026 mmol, 0.9 equiv.), Dry CH_2Cl_2 (2.0 ml) was introduced

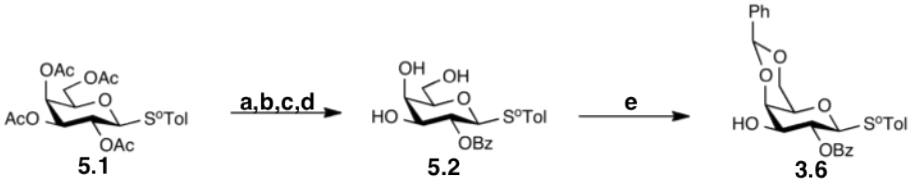
and the resulting solution was stirred for 0.5 h at ambient temperature. At this time, the reaction mixture was cooled to -50^{o}C, and AgOTf (18.6 mg, 0.073 mmol, 2.5 equiv.) was added to the solution. The resulting mixture was stirred for 5 min. p-Nitrophenylsulfenyl chloride (6.6 mg, 0.035 mmol, 1.2 equiv), was then added to the reaction mixture. The resultant was then allowed to warm to 0^{o}C over 1.5 h. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (2:1) as the eluent, to give the desired product (37 mg, 74%), as a clear oil.

R_f (hexane:EtOAc 2:1) = 0.2

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00-7.95 (m, 2H), 7.76 (d, *J* = 7.6 Hz, 2H), 7.65-7.57 (m, 4H), 7.50-7.09 (m, 45H), 5.86 (d, *J* = 8.6 Hz, 1H), 5.68 (d, *J* = 3.2 Hz, 1H), 5.37 (dd, *J* = 10.2, 8.0 Hz, 1H), 5.30 (d, *J* = 3.4 Hz, 1H), 5.14 (s, 2H), 4.97 (t, *J* = 9.6 Hz, 1H), 4.74 (d, *J* = 11.4 Hz, 2H), 4.70 (d, *J* = 11.8 Hz, 1H), 4.66-4.59 (m, 3H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.50-4.45 (m, 3H), 4.39-4.33 (m, 3H), 4.31 (d, *J* = 10.6 Hz, 1H), 4.26 (d, *J* = 8.1 Hz, 2H), 4.21 (d, *J* = 15.0 Hz, 5H), 4.01 (dd, *J* = 10.2, 3.2 Hz, 1H), 3.93 (d, *J* = 10.1 Hz, 1H), 3.90-3.82 (m, 2H), 3.71 (dd, *J* = 10.4, 3.6 Hz, 1H), 3.67-3.65 (m, 1H), 3.57-3.51 (m, 5H), 3.49-3.43 (m, 3H), 3.26-3.21 (m, 2H), 3.20-3.17 (m, 1H), 1.76 (s, 3H), 1.73 (s, 3H), 1.07 (s, 9H).

Section 5.3. Experimental Information for Compounds in Chapter 3

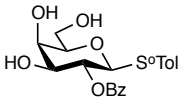
Synthesis of 3.6



Reagents and conditions: a. NaOMe, MeOH, CH_2Cl_2, RT, b. CSA, 2,2-dimethoxypropane, RT, c. Et_3N, 4-DMAP, BzCl, CH_2Cl_2, RT, d. AcOH, H_2O, RT, 44% (4 steps) e. CSA, benzaldehyde dimethyl acetal, MeCN, RT (79%)

Scheme 5.1

Compound 5.2



Compound **5.1**[2] (1.5 g, 3.3 mmol, 1.0 equiv), was added to flame dried flask, and the flask was evacuated and flushed with dry argon gas. To this flask was added a mixture of CH_2Cl_2 and MeOH (1:1 v/v, 15 ml). After stirring at ambient temperature for 5 min, a 0.5 M solution of NaOMe in MeOH (0.66 ml, 0.33 mmol, 0.1 equiv.), was introduced to the reaction mixture. The resultant was stirred for 1.0 h at room temperature. At this time the reaction was quenched by the addition of Amberlite IR-120 Resin (H^+ form) and vigorous stirring until pH paper indicated that homogeneous solution had been neutralized. The resulting mixture was filtered and the solvent was removed under reduced pressure. The

flask containing the resulting residue was coevaporated with toluene (3 x 10 ml), and put on high vacuum for 3 h.

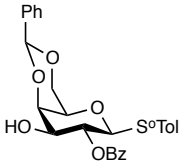
At this time, camphorsulfonic acid (77 mg, 0.33 mmol, 0.1 equiv) was added to the flask and the flask was evaporated and flushed with dry argon. 2,2,-dimethoxypropane (20 mL), was introduced to the flask, and the resulting solution was stirred for 18 h at room temperature. To the resultant was added dry triethylamine (1.83 ml, 13.2 mmol, 4 equiv.), and the result was stirred for 5 min at ambient temperature. Dry CH_2Cl_2 (20 mL), and 4-dimtheylaminopyridine (40.3 mg, 0.33 mmol, 0.1 equiv) were then introduced to the reaction mixture and the resultant was stirred for 10 min. At this time, BzCl (0.6 ml, 4.95 mmol, 1.5 equiv) was added to the solution and the resultant mixture was stirred for 3 h at ambient temperature. CH_2Cl_2 (60 ml) was added and the resulting solution was washed with 1.0 M HCl (1 x 40 ml), water (1 x 40 ml), and brine (1 x 40 ml). The resultant organic solution was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure to yield a yellow oil.

To the flask containing the impure residue was added a mixture of water and AcOH (2:8 v/v, 15 ml), the resulting solution was stirred for 3 h at room temperature. To this solution was added toluene (50 ml) and the resulting solution was evaporated under reduced pressure. The resulting oil was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as the eluent, to yield compound **5.2** (570 mg, 44%) as a white solid.

R_f (CH_2Cl_2:MeOH 10:1) = 0.5

^{1}H NMR (500 MHz, CD_3OD) δ 8.08 (d, *J* = 8.3, Hz, 2H), 7.67-7.58 (m, 2H), 7.50 (t, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 5.0 Hz, 3H), 5.43 (t, *J* = 9.8 Hz, 1H), 4.85 (s, 1H), 4.01 (d, *J* = 3.3 Hz, 1H), 3.89 (d, *J* = 9.4 Hz, 1H), 3.81 (qd, *J* = 11.4, 6.0 Hz, 2H), 3.69 (d, *J* = 6.9 Hz, 1H), 2.20 (s, 3H).

Compound 3.6

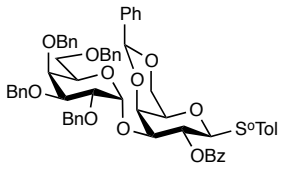


Compound **5.2** (570 mg, 1.46 mmol, 1.0 equiv), and copper(II) triflate (26 mg, 0.073 mmol, 0.05 equiv.) were added to a flame-dried flask. The flask was evacuated and flushed with dry argon. Dry MeCN (10 mL), followed by benzaldehyde dimethyl acetal (0.26 ml, 1.75 mmol, 1.2 equiv.) were then introduced to the flask. The resulting solution was stirred for 2 h at room temperature. Dry triethylamine (0.1 ml) was added to the reaction mixture, and the solvent was removed under reduced pressure to yield a yellow solid. The impure solid was dissolved in CH_2Cl_2 (20 ml) and titrated with hexanes to yield **3.7** (550 mg, 79%), as a white solid.

R_f (hexane:EtOAc 1:1) = 0.3

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11-8.04 (m, 2H), 7.72 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.61-7.54 (m, 1H), 7.51-7.35 (m, 8H), 7.25-7.15 (m, 2H), 7.11 (t, *J* = 7.6 Hz, 1H), 5.56 (s, 1H), 5.36 (t, *J* = 9.7 Hz, 1H), 4.84 (d, *J* = 9.8 Hz, 1H), 4.40 (d, *J* = 12.4 Hz, 1H), 4.28 (d, *J* = 3.7 Hz, 1H), 4.07 (dd, *J* = 12.4, 1.7 Hz, 1H), 3.90 (ddd, *J* = 10.9, 9.5, 3.7 Hz, 1H), 3.59 (s, 1H), 2.62 (d, *J* = 10.9 Hz, 1H), 2.29 (s, 3H).

Compound 3.9



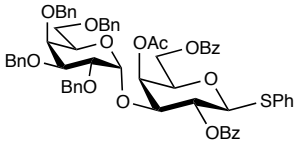
A 2-necked flask was charged with powdered 4Å molecular sieves (200 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.1** (80.85 mg, 0.125 mmol, 1.2 equiv.), **3.6** (50 mg, 0.1 mmol, 1.0 equiv.), and 2,6-di-*tert*-butyl-4-methyl pyridine (61.6 mg, 0.30 mmol, 3.0 equiv.) were added as solids. Dry CH_2Cl_2 (2.0 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. At this time, the reaction mixture was cooled to -78^{o}C, and AgOTf (77 mg, 0.30 mmol, 3.0 equiv.) was added to the solution. The resulting mixture was stirred for 5 min. Freshly distilled phenylsulfenyl chloride[3] (14.5 µL, 0.125 mmol, 1.2 equiv), was then added to the reaction mixture. The resultant was then allowed to warm to 0^{o}C over 2.0 h. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent

was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc/CH_2Cl_2 (3:1:1) as the eluent, to give the desired product (67.3 mg, 65%), as a clear oil.

R_f (hexane:EtOAc:CH_2Cl_2 3:1:1) = 0.35

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.2 Hz, 2H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.55-7.50 (m, 3H), 7.37-7.05 (m, 29H), 5.72 (t, *J* = 9.8 Hz, 1H), 5.44 (s, 1H), 5.11 (d, *J* = 3.5 Hz, 1H), 4.82 (d, *J* = 11.5 Hz, 2H), 4.66 (d, *J* = 11.8 Hz, 1H), 4.61 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 11.8 Hz, 1H), 4.40-4.35 (m, 4H), 4.28 (d, *J* = 11.8 Hz, 1H), 4.05 (dd, *J* = 9.7, 3.3 Hz, 1H), 4.01-3.96 (m, 2H), 3.87 (td, *J* = 6.4, 1.4 Hz, 1H), 3.69 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.46 (dd, *J* = 2.8, 1.4 Hz, 1H), 3.43-3.40 (m, 1H), 3.25 (qd, *J* = 9.4, 6.4 Hz, 2H), 2.27 (s, 3H).

Compound 3.10



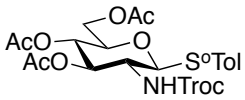
A 2-necked flask was charged with powdered 4Å molecular sieves (350 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.1** (50 mg, 0.075 mmol, 1.0 equiv.), and **3.7** (36.5 mg, 0.07 mmol, 0.9 equiv.), Dry CH_2Cl_2 (3.6 ml) was introduced and the resulting solution was stirred for 10 min at ambient temperature. At this time, the reaction mixture was cooled to 0^{o}C, and N-Iodosuccinimide (17 mg,

0.075 mmol, 1.0 equiv.) was added to the solution. The resulting mixture was stirred for 5 min. Trimethylsilyl triflate (1.4 µL, 0.008 mmol, 0.1 equiv), was added to the reaction mixture. The resultant was then stirred at 0°C for 3.5 h. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1) as the eluent, to give the desired product (45.4 mg, 62%), as a clear oil.

R_f (hexane:EtOAc 3:1) = 0.2

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 8.3, 1.4 Hz, 2H), 8.02 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.53-7.43 (m, 5H), 7.36-7.10 (m, 28H), 5.65 (d, *J* = 3.3 Hz, 1H), 5.59 (t, *J* = 9.9 Hz, 1H), 5.19 (d, *J* = 3.4 Hz, 1H), 4.78 (d, *J* = 10.1 Hz, 1H), 4.74 (d, *J* = 11.5 Hz, 1H), 4.63 (s, 2H), 4.50-4.44 (m, 2H), 4.41 - 4.35 (m, 2H), 4.32 (dd, *J* = 14.6, 11.6 Hz, 2H), 4.13 (dd, *J* = 9.8, 3.2 Hz, 1H), 3.95-3.90 (m, 2H), 3.88 (t, *J* = 6.8 Hz, 1H), 3.54 (dd, *J* = 10.2, 2.8 Hz, 1H), 3.42 (dd, *J* = 9.6, 7.1 Hz, 1H), 3.21 (s, 1H), 3.17 (dd, *J* = 9.6, 5.1 Hz, 1H), 1.89 (s, 3H).

Compound 3.11



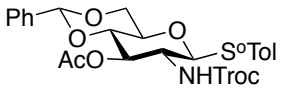
To a flask containing **2.12** (4.1 g, 7.84 mmol, 1.0 equiv.), under argon, was added dry CH_2Cl_2 (40 mL). The resulting solution was stirred for 5 min, and then

ortho-toulenethiol (1.4 mL, 11.77 mmol, 1.5 equiv.) and BF_3-OEt_2 (1.16 mL, 9.4 mmol, 1.2 equiv.) were introduced. The resultant was stirred overnight at room temperature. At this time, the reaction mixture was diluted with CH_2Cl_2 (100 mL). The result was washed with satd. $NaHCO_3$ (3 x 50 mL), and brine (1 x 50 mL). The organic solution was dried over sodium sulfate, and filtered. The solvent was removed under reduced pressure to yield a yellow oil. The resulting oil was purified by column chromatography on silica gel using hexanes/EtOAc (2:1) as the eluent, to furnish **3.11** (2.76 g, 60%) as a white foam.

R_f (hexane:EtOAc 2:1) = 0.4

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58-7.51 (m, 1H), 7.23-7.12 (m, 3H), 5.28 (dd, *J* = 10.3, 9.2 Hz, 1H), 5.20 (d, *J* = 9.1 Hz, 1H), 5.06 (dd, *J* = 10.1, 9.3 Hz, 1H), 4.84 (d, *J* = 10.4 Hz, 1H), 4.75 (q, *J* = 12.0 Hz, 2H), 4.22 (dd, *J* = 12.2, 5.7 Hz, 1H), 4.14 (dd, *J* = 12.3, 2.4 Hz, 1H), 3.77 (q, *J* = 10.0 Hz, 1H), 3.71 (ddd, *J* = 10.3, 5.7, 2.4 Hz, 1H), 2.41 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H).

Compound 3.12



Compound **3.11** (1.38 g, 2.35 mmol, 1.0 equiv), was added to flame dried flask, and the flask was evacuated and flushed with dry argon gas. To this flask was added a mixture of CH_2Cl_2 and MeOH (1:1 v/v, 15 ml). After stirring at ambient temperature for 5 min, a 0.5 M solution of NaOMe in MeOH (0.47 ml,

0.23 mmol, 0.1 equiv.), was introduced to the reaction mixture. The resultant was stirred for 1.0 h at room temperature. At this time the reaction was quenched by the addition of Amberlite IR-120 Resin (H^+ form) and vigorous stirring until pH paper indicated that homogeneous solution had been neutralized. The resulting mixture was filtered and the solvent was removed under reduced pressure. The flask containing the resulting residue was coevaporated with toluene (3 x 10 ml), and put on high vacuum for 3 h.

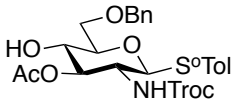
Copper (II) triflate (42.5 mg, 0.12 mmol, 0.05 equiv) was added to the flask and the flask was evaporated and flushed with dry argon. Dry MeCN (25 mL) was then introduced to the flask, followed by benzaldehyde dimethyl acetal (0.42 mL, 2.82 mmol, 1.2 equiv.). The reaction mixture was stirred for 3 h at ambient temperature. At this time, acetic anhydride (1.11 mL, 11.75 mmol, 5 equiv.) was added, and the resultant was stirred for an additional 18 h at ambient temperature. Triethylamine (0.1 mL) was added to quench the reaction and the solvent was removed under reduced pressure to yield a yellow solid. The solid was dissolved in CH_2Cl_2 (20 mL), and titrated with hexanes to yield **3.12** (1.0 g 72%) as a white solid.

R_f (hexane:EtOAc 2:1) = 0.6

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52-7.48 (m, 1H), 7.46-7.31 (m, 5H), 7.25-7.14 (m, 3H), 5.51 (s, 1H), 5.31 (t, *J* = 9.4 Hz, 2H), 4.84-4.71 (m, 3H), 4.32 (dd, *J* = 10.6,

5.0 Hz, 1H), 3.89 (q, *J* = 10.0 Hz, 1H), 3.80 (t, *J* = 10.3 Hz, 1H), 3.73 (t, *J* = 9.5 Hz, 1H), 3.52 (td, *J* = 9.7, 5.0 Hz, 1H), 2.40 (s, 3H), 2.07 (s, 3H).

Compound 3.13

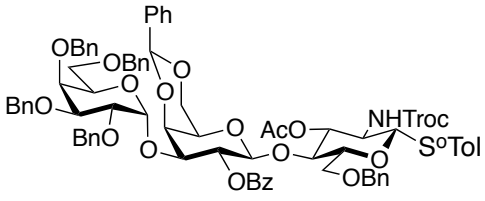


Compound **3.12**, was added to a flame-dried flask, and CH_2Cl_2 (20 mL), was added. After cooling to 0°C, trifluoroacetic anhydride (42 µL, 0.30 mmol, 0.2 equiv.) was introduced; this was followed by Et_3SiH (2.43 mL, 15.23 mmol, 10 equiv.) and TFA (1.17 mL, 15.23 mmol, 10 equiv.). The reaction mixture was stirred at 0°C for 1 h. At this time, the solution was diluted with CH_2Cl_2 (20 mL), and washed with satd. $NaHCO_3$ (3 x 20 mL), and brine (1 x 20 mL). The solution was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure to yield a clear oil, which was purified by column chromatography on silica gel using hexanes/EtOAc/CH_2Cl_2 (2:1:1), to give **3.13** (720 mg, 80%), as a clear oil.

R_f (hexane:EtOAc/ CH_2Cl_2 2:1:1) = 0.2

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56-7.50 (m, 1H), 7.39-7.27 (m, 5H), 7.20-7.06 (m, 3H), 5.23 (d, *J* = 9.5 Hz, 1H), 5.08-5.02 (m, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.75-4.70 (m, 2H), 4.6-4.53 (m, 2H), 3.86-3.73 (m, 5H), 3.54 (dt, *J* = 9.7, 4.9 Hz, 1H), 2.99-2.90 (bs, 1H), 2.39 (s, 3H), 2.10 (s, 3H).

Compound 3.14



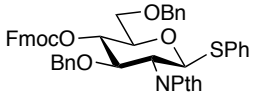
A 2-necked flask was charged with powdered 4Å molecular sieves (150 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compounds **3.9** (50 mg, 0.05 mmol, 1.5 equiv.), **3.13** (19.6 mg, 0.033 mmol, 1.0 equiv.), and 2,6-di-tert-butyl-4-methyl pyridine (20.5 mg, 0.099 mmol, 3.0 equiv.) were added. Dry CH_2Cl_2 (1.5 ml) was introduced and the resulting solution was stirred for 0.5 h at ambient temperature. At this time, the reaction mixture was cooled to -45^oC, and AgOTf (25.7 mg, 0.10 mmol, 3.0 equiv.) was added to the solution. The resulting mixture was stirred for 5 min. Phenylsulfenyl chloride (7.0 µL, 0.057 mmol, 1.6 equiv), was then added to the reaction mixture. The resultant was then allowed to warm to 0^oC over 1.5 h. Dry triethylamine (0.1 ml) was then added to quench the reaction mixture and the solution was filtered through a celite plug. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (1:1) as the eluent, to give the desired product (21.3 mg, 44%), as a clear oil.

R_f (hexane:EtOAc 1:1) = 0.4

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.50-7.43 (m, 4H), 7.37-7.07 (m, 30H), 7.04-6.98 (m, 1H), 5.49 (dd, *J* = 10.1, 7.9 Hz, 1H), 5.34 (s, 1H), 5.24 (d, *J* = 9.7 Hz, 1H), 5.09-5.03 (m, 2H), 4.79 (d, *J* = 11.5 Hz, 1H), 4.75 (d, *J* = 5.4 Hz, 1H), 4.66-4.53 (m, 4H), 4.53-4.48 (m, 2H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.39-4.21 (m, 7H), 4.01-3.80 (m, 6H), 3.67 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.62 (dd, *J* = 11.2, 3.9 Hz, 1H), 3.57-3.47 (m, 2H), 3.35 (d, *J* = 9.6 Hz, 1H), 3.25 (dd, *J* = 9.2, 6.8 Hz, 1H), 3.21-3.10 (m, 3H), 2.35 (s, 3H), 2.04 (s, 3H).

Section 5.4. Experimental Information for Compounds in Chapter 4

Compound 4.1



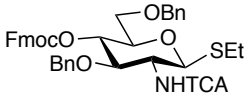
A 2-necked flask was charged flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **4.3** (2.34 g, 4.02 mmol, 1.0 equiv.), and a mixture of dry CH_2Cl_2 and dry pyridine (2:1 v/v, 12 ml) was introduced and the resulting solution was stirred for 10 min at 0^{o}C. At this time 9-fluorenylmethoxycarbonyl chloride (1.56 g, 6.03 mmol, 1.5 equiv.) was added, as a solid, to the solution. The resulting mixture was stirred for 30 min at 0^{o}C. At this time, the reaction mixture was removed from the ice bath and stirred at ambient temperature for an additional 30 min. CH_2Cl_2 (75 ml) was added to the reaction mixture and the resultant was washed with water (1 x 30 ml), satd. aq. $NaHCO_3$ (2 x 30 mL), and brine (1 x 30 mL). The organic layer was dried over

sodium sulfate and filtered. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1) as the eluent, to give the desired product (2.75 g, 85%), as a yellow foam.

R_f (hexane:EtOAc 3:1) = 0.2

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (d, *J* = 7.1 Hz, 1H), 7.75 (dd, *J* = 12.7, 7.6 Hz, 1H), 7.73-7.65 (m, 3H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.46-7.18 (m, 13H), 7.00 (d, *J* = 7.4 Hz, 1H), 6.91-6.84 (m, 3H), 5.62 (dd, *J* = 10.6, 1.7 Hz, 1H), 5.05 (ddd, *J* = 10.2, 9.0, 1.6 Hz, 1H), 4.63 (d, *J* = 12.2 Hz, 1H), 4.59-4.57 (m, 2H), 4.55 (t, *J* = 10.2, Hz, 1H), 4.49-4.38 (m, 3H), 4.32 (d, *J* = 12.3 Hz, 1H), 4.19 (t, *J* = 7.0 Hz, 1H), 3.96-3.92 (m, 1H), 3.77-3.74 (m, 2H).

Compound 4.2



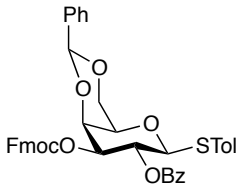
A 2-necked flask was charged flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **4.4** (3.0 g, 5.47 mmol, 1.0 equiv.), and a mixture of dry CH_2Cl_2 and dry pyridine (2:1 v/v, 18 ml) was introduced and the resulting solution was stirred for 10 min at 0^{o}C. At this time 9-fluorenylmethoxycarbonyl chloride (2.12 g, 8.21 mmol, 1.5 equiv.) was added, as a solid, to the solution. The resulting mixture was stirred for 30 min at 0^{o}C. At this time, the reaction mixture was removed from the ice bath and stirred

at ambient temperature for an additional 30 min. CH_2Cl_2 (100 ml) was added to the reaction mixture and the resultant was washed with water (1 x 50 ml), satd. aq. $NaHCO_3$ (2 x 50 mL), and brine (1 x 50 mL). The organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1) as the eluent, to give the desired product (3.79 g, 90%), as a white foam. Analytical data was in agreement with the literature.[4]

R_f (hexane:EtOAc 3:1) = 0.15

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77-7.71 (m, 2H), 7.56 (dt, *J* = 7.6, 0.9 Hz, 1H), 7.51 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.38 (td, *J* = 7.6, 1.1 Hz, 2H), 7.34-7.16 (m, 13H), 6.90 (dd, *J* = 7.8, 2.8 Hz, 1H), 5.03 (d, *J* = 10.3 Hz, 1H), 4.93 (t, *J* = 9.5 Hz, 1H), 4.63 (s, 2H), 4.56-4.48 (m, 2H), 4.34-4.26 (m, 2H), 4.23 (dd, *J* = 10.0, 8.9 Hz, 1H), 4.10 (t, *J* = 7.2 Hz, 1H), 3.75 (ddd, *J* = 10.0, 5.2, 3.7 Hz, 1H), 3.68-3.60 (m, 3H), 2.80-2.64 (m, 2H), 1.28 (t, *J* = 7.4 Hz, 3H).

Compound 4.5



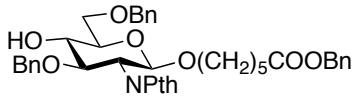
A 2-necked flask was charged flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and compound **2.8** (4.5 g, 12.01 mmol, 1.0 equiv.), and a mixture of dry CH_2Cl_2 and dry pyridine (4:5 v/v,

100 ml) was introduced and the resulting solution was stirred for 10 min at 0°C. At this time 9-fluorenylmethoxycarbonyl chloride (3.42 g, 13.22 mmol, 1.1 equiv.) was added, as a solid, to the solution. The resulting mixture was stirred for 30 min at 0°C. At this time, the reaction mixture was removed from the ice bath and stirred at ambient temperature for an additional 30 min. At this point, the reaction mixture was cooled to 0°C, and BzCl (4.2 mL, 36.0 mmol, 3 equiv.), was added dropwise to the mixture. The resultant was stirred an additional 3 h at 0°C. CH2Cl2 (400 ml) was added to the reaction mixture and the resultant was washed with water (1 x 50 ml), satd. aq. $NaHCO_3$ (2 x 50 mL), and brine (1 x 50 mL). The organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel using hexanes/EtOAc (3:1-2:1) as the eluent, to give the desired product (5.05 g, 60%), as a white foam.

R_f (hexane:EtOAc 2:1) = 0.5

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.3 Hz, 1H), 7.67 (dd, *J* = 7.0, 6.0 Hz, 1H), 7.59-7.28 (m, 16H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 5.66-5.59 (m, 1H), 5.52 (s, 1H), 5.05 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.86 (d, *J* = 9.8 Hz, 1H), 4.50 (d, *J* = 3.4 Hz, 1H), 4.44-4.40 (m, 1H), 4.24 (d, *J* = 7.5 Hz, 2H), 4.10-4.05 (m, 2H), 3.66 (d, *J* = 1.3 Hz, 1H), 2.34 (s, 3H).

Compound 4.7

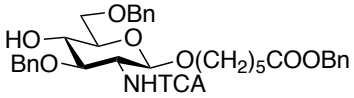


A 2-necked flask was charged with powdered 4Å molecular sieves (350 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and **4.6** (60 mg, 0.27 mmol, 1.5 equiv.) and **4.1** (144.7 mg, 0.18 mmol, 1.0 equiv) were added to the flask. Dry CH_2Cl_2 (3.6 ml) was then introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -20^{o}C, the result was stirred for 5 min. AgOTf (139 mg, 0.54 mmol, 3.0 equiv) was then introduced as a solid and the resultant heterogeneous mixture was stirred an additional 10 min at -20^{o}C. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (0.1 ml, 0.099 mmol, 0.55 equiv) was added dropwise over 1 min. The result was stirred for 30 min at -20^{o}C. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (1.75 ml) was then added to the reaction. The reaction mixture was removed from cold-bath and stirred at room temperature for 1 h. EtOAc (4 ml) was added to the solution to precipitate an undesired brown impurity. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc (3:2-1:1) as the eluent, to give the desired residue (89.9 mg, 72%) as a clear oil.

R_f (hexane:EtOAc 3:2) = 0.2

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86-7.59 (m, 4H), 7.40-7.28 (m, 10H), 7.09-7.03 (m, 2H), 6.99-6.91 (m, 3H), 5.16-5.09 (m, 2H), 5.04 (s, 2H), 4.75 (d, *J* = 12.3 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.23 (dd, *J* = 10.8, 8.3 Hz, 1H), 4.14 (dd, *J* = 10.8, 8.3 Hz, 1H), 3.86-3.72 (m, 4H), 3.67-3.59 (m, 1H), 3.36 (dt, *J* = 9.9, 6.6 Hz, 1H), 2.38 (t, *J* = 7.5 Hz, 1H), 2.08-1.92 (m, 2H), 1.75-1.64 (m, 1H), 1.45-1.31 (m,4H), 1.11-1.00 (m, 2H).

Compound 4.8



A 2-necked flask was charged with powdered 4Å molecular sieves (1.2 g) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and **4.2** (2.0 g, 2.6 mmol, 1.5 equiv.) and **4.6** (385 mg, 1.73 mmol, 1.0 equiv) were added to the flask. Dry CH_2Cl_2 (35 ml) was then introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -45^{o}C, the result was stirred for 5 min. AgOTf (1.34 g, 5.20 mmol, 3.0 equiv) was then introduced as a solid and the resultant heterogeneous mixture was stirred an additional 10 min at -45^{o}C. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (1.43 ml, 1.43 mmol, 0.825 equiv) was added dropwise over 1 min. The result was stirred for 1.25 h at -45^{o}C. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (17 ml) was then

added to the reaction. The reaction mixture was removed from cold-bath and stirred at room temperature for 1.5 h. EtOAc (40 ml) was added to the solution to precipitate an undesired brown impurity. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc (2:1-1:1) as the eluent, to give the desired residue (1.14 g, 93%) as a clear oil.

R_f (hexane:EtOAc 3:2) = 0.5

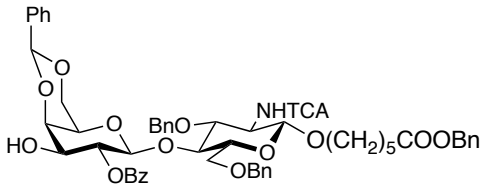
IR (neat): 3307.98, 3033.11, 2931.85, 2866.27, 1705.10, 1686.78, 1532.47, 1058.94, 838.08, 817.83 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38-7.24 (m, 15H), 7.06 (t, 1H, *J* =8.3Hz), 5.09 (s, 2H), 4.83 (d, 1H, *J*, = 8.3Hz), 4.79 (d, 1H, *J* = 11.7Hz), 4.75 (d, 1H, *J* = 11.7Hz), 4.60 (d, 1H, *J* = 11.3Hz), 4.55 (d, 1H, *J* = 11.3Hz), 4.00 (t, 1H, *J* = 8.3Hz), 3.87-3.81 (m, 1H), 3.77-3.72 (m, 2H), 3.69 (t, 1H, *J* = 9.5Hz), 3.54-3.47 (m, 2H), 3.46-3.41 (m, 1H), 2.92 (bs, 1H), 2.32 (t, 2H, *J* = 7.5Hz), 1.65-1.49 (m, 4H), 1.41-1.29 (m, 4H)

^{13}C NMR (150 MHz, $CDCl_3$) δ 173.50, 161.82, 138.03, 137.57, 136.00, 128.57, 128.54, 128.49, 128.19, 128.16, 128.08, 127.97, 127.89, 127.78, 99.30, 92.53, 79.61, 74.66, 73.74, 73.67, 73.52, 70.49, 69.66, 66.13, 58.35, 34.10, 29.12, 25.50, 24.59

ESI-HRMS: calcd for $C_{35}H_{40}Cl_3NO_8$ (M+H): 708.1820, found 708.1898

Compound 4.9



A 2-necked flask was charged with powdered 4Å molecular sieves (1.2 g) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and **4.5** (988.1 mg, 1.41 mmol, 2.0 equiv.) and **4.8** (500 mg, 0.705 mmol, 1.0 equiv) were added to the flask. Dry CH_2Cl_2 (14 ml) was then introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -45^{o}C, the result was stirred for 5 min. AgOTf (724.3 mg, 2.82 mmol, 3.0 equiv) was then introduced as a solid and the resultant heterogeneous mixture was stirred an additional 10 min at -45^{o}C. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (0.78 ml, 0.78 mmol, 1.1 equiv) was added dropwise over 1 min. The result was stirred for 1.5 h at -45^{o}C. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (7 ml) was then added to the reaction. The reaction mixture was removed from cold-bath and stirred at room temperature for 1.5 h. EtOAc (40 ml) was added to the solution to precipitate an undesired brown impurity. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc/CH_2Cl_2 (2:1:1-1:1:1) as the eluent, to give the desired residue (630 mg, 84%) as a clear oil.

R_f (hexane:EtOAc:CH_2Cl_2 1:1:1) = 0.25

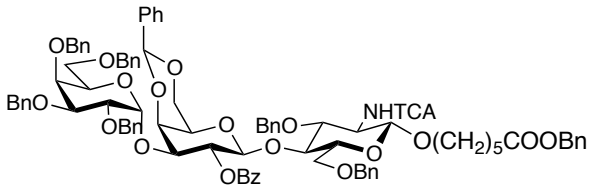
IR (neat):3335.94, 3034.08, 2929.92, 2864.34, 1725.36, 1699.32, 1527.65, 1451.46, 1265.32, 1051.22, 819.76 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (d, 2H, *J* = 10.6Hz), 7.57 (t, 1H, *J* = 8.3Hz), 7.47-7.16 (m, 25H), 6.94 (d, 1H, *J* = 8.3Hz), 5.53 (s, 1H), 5.32 (t, 1H, *J* = 8.3Hz), 5.12 (d, 1H, *J* = 9.5Hz), 5.07 (s, 2H), 4.72-4.66 (m, 3H), 4.64 (d, 1H, *J* = 10.6Hz), 4.39 (d, 1H, *J* = 12.8Hz), 4.26 (d, 1H, *J* = 12.5Hz), 4.16 (d, 1H, *J* = 3.0Hz), 4.07 (t, 1H, *J* = 8.3Hz), 4.01 (t, 1H, *J* = 8.3Hz), 3.97 (d, 1H, *J* = 11.7Hz), 3.79-3.71 (m, 2H), 3.66 (d, 1H, *J* = 8.3Hz), 3.61 (d, 1H, *J* = 11.7Hz), 3.53 (q, 1H, *J* = 8.3Hz), 3.36-3.25 (m, 2H), 3.22 (s, 1H), 2.29 (t, 2H, *J* = 7.6Hz), 1.62-1.43 (m, 4H), 1.35-1.22 (m, 4H)

^{13}C NMR (150 MHz, $CDCl_3$) δ 173.45, 165.81, 161.63, 138.17, 137.35, 136.02, 133.28, 129.76, 129.63, 129.26, 128.52, 128.51, 128.46, 128.45, 128.28, 128.23, 128.15, 127.95, 127.91, 127.57, 126.41, 101.52, 100.07, 99.42, 92.48, 78.17, 76.73, 75.48, 74.92, 74.84, 73.48, 73.19, 71.57, 69.52, 68.68, 68.0, 66.61, 66.09, 57.82, 34.09, 29.06, 25.46, 24.58

ESI-HRMS: calcd for $C_{55}H_{58}Cl_3NO_{14}$ (M-H): 1060.2923, found 1060.2833

Compound 4.10



A 2-necked flask was charged with powdered 4Å molecular sieves (660 mg) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and **4.8** (350 mg, 0.33 mmol, 1.0 equiv.) and **2.1** (427 mg, 0.66 mmol, 2.0 equiv) were added to the flask. Dry CH_2Cl_2 (14 ml) was then introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -60^oC, the result was stirred for 5 min. AgOTf (340 mg, 1.32 mmol, 4.0 equiv) was then introduced as a solid and the resultant heterogeneous mixture was stirred an additional 10 min at -60^oC. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (0.36 ml, 0.36 mmol, 1.1 equiv) was added dropwise over 1 min. The result was stirred for 2.0 h at -60^oC. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (1.0 ml) was then added to the reaction. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc (2:1) as the eluent, to give the desired residue (430 mg, 82%) as a clear oil.

$\mathbf{R_f}$ (hexane:EtOAc 2:1) = 0.2

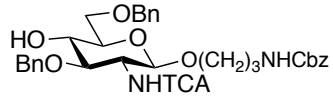
IR (neat):3307.98, 3063.98, 3031.19, 2919.31, 2854.70, 1729.21, 1690.64, 1532.47, 1495.82, 1453.39, 1262.43, 1051.22, 819.76 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (d, 2H, *J* = 8.3Hz), 7.51-7.44 (m, 3H), 7.36-7.09(m, 50H), 6.96 (d, 1H, *J* = 7.9Hz), 5.61 (t, 1H, *J* = 8.9Hz), 5.35 (s, 1H), 5.08 (s, 2H,), 5.06 (m, 2H), 4.8 (d, 1H, *J* = 11.2Hz), 4.69 (d, 1H, *J* = 8.3Hz), 4.67-4.59 (m, 4H), 4.57 (d, 1H, *J* = 11.5Hz), 4.52 (d, 1H, *J* = 11.5Hz), 4.45 (d, 1H, *J* = 12.1Hz), 4.38 (d, 1H, *J* = 11.5Hz), 4.36-4.30 (m, 2H), 4.24-4.17 (m, 3H), 4.07 (t, 1H, *J* = 7.7Hz), 4.00-3.96 (m, 2H), 3.89 (t, 1H, *J* = 6.8Hz), 3.86-3.81 (m, 2H), 3.73-3.66 (m, 3H), 3.63-3.57 (m, 2H), 3.54 (s, 1H), 3.39-3.35 (m, 1H), 3.29 (t, 1H, *J* = 7.4Hz), 3.23-3.17 (m, 1H), 3.06 (s, 1H), 2.29 (t, 2H, *J* = 6.8Hz), 1.60-1.42 (m, 4H), 1.33-1.22 (m, 4H)

^{13}C NMR (150 MHz, $CDCl_3$) δ 173.44, 164.81, 161.64, 138.71, 138.59, 138.56, 138.23, 138.16, 138.09, 137.66, 136.02, 133.12, 129.74, 129.71, 128.78, 128.51, 128.46, 128.39, 128.35, 128.33, 128.22, 128.20, 128.16, 128.15, 128.11, 128.10, 128.03, 127.81, 127.78, 127.73, 127.66, 127.60, 127.47, 127.44, 127.39, 127.354, 127.31, 126.33, 100.99, 100.39, 99.53, 95.69, 92.47, 78.57, 78.35, 76.29, 75.87, 75.07, 75.05, 74.95, 74.68, 74.63, 73.28, 73.26, 73.14, 72.44, 72.19, 71.09, 69.87, 69.45, 68.08, 68.71, 68.33, 66.65, 66.08, 57.40, 34.09, 29.06, 25.44, 24.58

ESI-HRMS: calcd for $C_{89}H_{92}Cl_3NO_{19}$ (M-H): 1582.5329, found 1582.5215

Compound 4.14



A 2-necked flask was charged with powdered 4Å molecular sieves (1.2 g) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and 3- (benzyloxycarbonylamino)-1-propanol (126.6 mg, 0.605 mmol, 1 equiv.) and **4.2** (700 mg, 0.908 mmol, 1.5 equiv) were added to the flask. Dry CH_2Cl_2 (12 ml) was then introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -45^{o}C, the result was stirred for 5 min. AgOTf (466.3 mg, 1.82 mmol, 1.5 equiv) was then introduced as a solid and the resultant heterogeneous mixture was stirred an additional 10 min at -45^{o}C. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (0.45 ml, 0.45 mmol, 0.50 equiv) was added dropwise over 1 min. The result was stirred for 30 min at -45^{o}C. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (6 ml) was then added to the reaction. The reaction mixture was removed from cold-bath and stirred at room temperature for 1 h. EtOAc (10ml) was added to the solution to precipitate an undesired brown impurity. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc (3:2-1:1) as the eluent, to give the desired residue (385mg, 91%) as a clear oil.

R_f (hexane:EtOAc 1:1) = 0.4

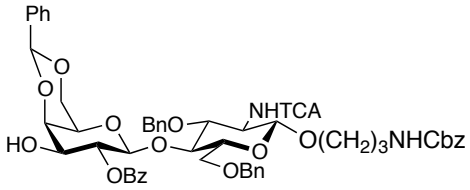
IR (neat): 3307.98, 3031.19, 2941.49, 2869.16, 1698.14, 1530.54, 1260.50, 735.66 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, 1H, N*H*COCCl$_3$, *J* = 8.7Hz), 7.37-7.24 (m, 15H), 5.29 (t, 1H, N*H*COOBn J = 7.5Hz), 5.07 (s, 2H, -OCOOC*H*$_2$Ph), 4.80 (d, 1H, *J* = 10.6Hz), 4.77 (d, 1H, *J* = 10.6Hz), 4.67 (d, 1H, H1, *J* = 8.7Hz), 4.56 (d, 1H, *J* = 10.6Hz), 4.52 (d, 1H, *J* = 10.6Hz), 3.87-3.6 (m, 6H), 3.5-3.44 (m, 2H), 3.4-3.33 (m, 1H), 3.2-3.3 (bs, 1H, -OH), 3.17-3.09 (m, 1H), 1.77-1.59 (m, 2H)

^{13}C NMR (150 MHz, $CDCl_3$) δ 162.29, 156.69, 138.02, 137.69, 136.65, 128.53, 128.52, 128.46, 128.10, 128.03, 127.93, 127.84, 127.75, 100.20, 92.67, 80.91, 74.66, 74.16, 73.59, 72.67, 70.18, 67.00, 66.62, 57.45, 37.57, 29.71

ESI-HRMS: calcd for $C_{33}H_{37}Cl_3N_2O_8$ (M+H): 695.1615, found 695.1700

Compound 4.15



A 2-necked flask was charged with powdered 4Å molecular sieves (2.9 g) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and **4.14** (1.0 g, 1.44 mmol, 1.0 equiv.) and **4.5** (1.51 g, 2.15 mmol, 1.5 equiv) were added to the flask. Dry CH_2Cl_2 (29 ml) was then

introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -20°C, the result was stirred for 5 min. AgOTf (1.11 g, 4.3 mmol, 3.0 equiv.) was then introduced as a solid and the resultant heterogeneous mixture was stirred an additional 10 min at -20°C. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (1.12 ml, 1.12 mmol, 0.77 equiv) was added dropwise over 1 min. The result was stirred for 30 min at -20°C. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (15 ml) was then added to the reaction. The reaction mixture was removed from cold-bath and stirred at room temperature for 1.5 h. EtOAc (30ml) was added to the solution to precipitate an undesired brown impurity. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc (2:1-1:2) as the eluent, to give the desired residue (385mg, 91%) as a clear oil.

R_f (hexane:EtOAc 1:1) = 0.1

IR (neat): 3326.30, 3031.19, 2869.16, 1697.39, 1520.90, 1452.42, 1261.47, 1051.22, 818.80 cm^{-1}.

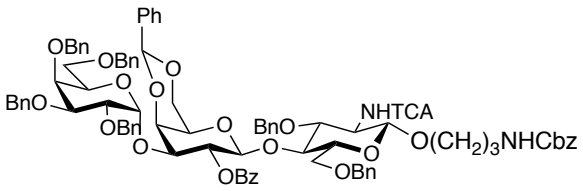
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (d, 2H, *J* = 8.3Hz), 7.58 (t, 1H, *J* = 8.3Hz), 7.48-7.16 (m, 25H), 5.52 (s, 1H), 5.32 (t, 1H, *J* = 8.3Hz), 5.15-5.08 (m, 2H), 5.03 (s, 2H), 4.69-4.62 (m, 2H), 4.56 (d, 1H, *J* = 8.3Hz), 4.34 (d, 1H, *J* = 12.1Hz), 4.23 (d, 1H, *J* = 12.1Hz), 4.14 (d, 1H, *J* = 4.5Hz), 4.06 (t, 1H, *J* = 8.3Hz), 3.94 (d, 1H, *J*

= 12.1Hz), 3.84 (t, *J* = 8.3Hz), 3.81-3.53 (m, 5H), 3.38-3.25 (m, 3H), 3.21 (s, 1H), 3.17-3.09 (m, 1H), 2.55 (d, 1H, *J* =10.2Hz), 1.73-1.58 (m, 2H)

^{13}C NMR (150 MHz, $CDCl_3$) δ 165.79, 161.89, 156.47, 138.15, 137.36, 136.70, 133.32, 129.75, 129.60, 129.25, 128.49, 128.46, 128.26, 128.22, 128.02, 127.98, 127.93, 127.53, 126.40, 101.48, 100.16, 99.92, 92.52, 78.79, 76.42, 75.45, 74.89, 74.79, 73.42, 73.20, 71.55, 68.65, 67.97, 66.89, 66.60, 66.54, 57.30, 37.69, 29.50

ESI-HRMS: calcd for $C_{53}H_{55}Cl_3N_2O_{14}$ (M+H): 1049.2719, found 1049.2797

Compound 4.16



A 2-necked flask was charged with powdered 4Å molecular sieves (3.5 g) and flame-dried under vacuum. After cooling to room temperature, the flask was flushed with dry argon, and **4.15** (1.1 g, 1.05 mmol, 1.0 equiv.) and **2.1** (1.36 g, 2.1 mmol, 2.0 equiv) were added to the flask. Dry CH_2Cl_2 (35 ml) was then introduced and the mixture was stirred for 0.5 h at room temperature. After cooling to -60^{o}C, the result was stirred for 5 min. AgOTf (1.08 g, 4.2 mmol, 4.0 equiv) was then introduced as a solid and the resultant homogeneous mixture was stirred an additional 10 min at -60^{o}C. At this time, a 1.0 M solution of ICl in CH_2Cl_2 (1.15 ml, 1.15 mmol, 1.1 equiv) was added dropwise over 1 min. The

result was stirred for 2.0 h at -60°C. During this period, the solution gradually changed color from dark red to light yellow. Dry triethylamine (1.0 ml) was then added to the reaction. The reaction mixture was filtered through a celite plug and washed several times with EtOAc. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography on silica gel using hexanes/EtOAc (2:1-1:1) as the eluent, to give the desired residue (1.35 g, 82%) as a clear oil.

R_f (hexane:EtOAc 3:2) = 0.4

IR (neat): 3326.30, 3031.19, 2870.13, 1696.42, 1526.68, 1453.39, 1263.40, 1051.22, 819.76 cm^{-1}.

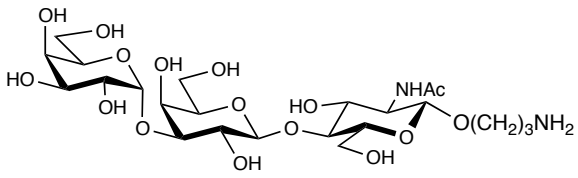
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (d, 2H, *J* = 7.3Hz), 7.52 (t, 1H, *J* = 7.9Hz), 7.48 (d, 2H, *J* = 7.3Hz), 7.39-7.11 (m, 50H), 5.62 (dd, 1H, ^1J = 11.1Hz, ^2J = 7.9Hz), 5.35 (s, 1H), 5.10-5.04 (m, 4H), 4.82 (d, 1H, *J* = 11.5Hz), 4.70-4.61 (m, 4H), 4.54 (d, 2H, *J* = 11.5Hz), 4.47 (d, 1H, *J* = 11.5Hz), 4.39 (d, 1H, *J* = 11.5Hz), 4.35-4.30 (m, 2H), 4.19 (d, 1H, *J* = 12.1Hz), 4.06 (t, 1H, *J* = 7.9Hz), 3.99 (dd, 1H, ^1J = 9.8Hz, ^2J = 3.6Hz), 3.90 (t, 1H, *J* = 6.4Hz), 3.87-3.80 (m, 3H), 3.77-3.65 (m, 4H), 3.59-3.55 (m, 2H), 3.33-3.27 (m, 4H), 3.22-3.11 (m, 2H), 3.07 (s, 1H), 1.74-1.59 (m, 2H)

^{13}C NMR (150 MHz, $CDCl_3$) δ 164.96, 162.02, 156.60, 138.86, 138.75, 138.71, 138.29, 138.27, 138.24, 137.82, 136.86, 133.32, 129.88, 129.84128.92, 128.65, 128.61, 128.52, 128.49, 128.38, 128.36, 128.30, 128.26, 128.26, 128.18, 128.15,

127.96, 127.91, 127.82, 127.75, 127.76, 127.58, 127.53, 127.51, 127.47, 126.47, 101.11, 100.63, 100.10, 95.93, 92.64, 78.99, 78.75, 76.43, 76.03, 75.29, 75.18, 75.09, 74.84, 74.75, 73.41, 73.40, 73.28, 72.62, 72.35, 71.25, 70.04, 68.93, 68.84, 68.42, 67.06, 66.80, 66.66, 57.10, 37.92, 29.62

ESI-HRMS: calcd for $C_{87}H_{89}Cl_3N_2O_{19}$ (M+H): 1571.5125, found 1571.5203

Compound 4.19



To a flask, under argon, containing **4.16** (300 mg, 0.19 mmol, 1.0 equiv.), was added dry CH_2Cl_2 (3 ml) and dry MeOH (3 mL). To this solution was added a 0.5 M solution of NaOMe in MeOH (0.95 ml, 0.47 mmol, 2.5 equiv). The resultant was stirred for 18 h at room temperature. At this time, the NaOMe was neutralized with Amberlite IR-120 resin (H^+ form) and vigorous stirring. The heterogeneous solution was filtered and the solvent was removed under reduced pressure, to yield a white solid. The solid was dissolved in a mixture of EtOAc/MeOH/H_2O (5 ml, 5:5:1). To the resulting solution was added 10% Pd/C (200 mg, 0.19 mmol, 1.0 equiv.), and 1.0 M HCl (0.2 ml, 0.2 mmol, 1.1 equiv). The resultant was placed under a hydrogen atmosphere (balloon) and stirred for 18 h at ambient temperature. At this time, the heterogeneous mixture was filtered though a celite plug and washed several time with water. The solvent was

removed under reduced pressure to yield a yellow solid. The yellow solid was purified by dissolving it in water (2 mL), and passing it through a C_{18} solid-phase extraction cartridge. The cartridge was washed with water (2 x 2 mL); the water fractions were combined and the solvent was removed under reduced pressure to yield **4.19** (63.0 mg, 55%) as a white solid. Analytical values were in accordance with the literature[5]

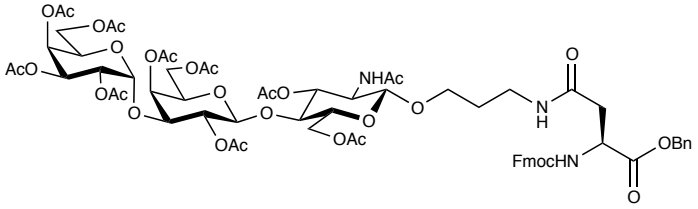
IR (neat): 3272.29 (b), 2939.57, 2889.42, 1647.24, 1553.69, 1375.27, 946.40 cm^{-1}.

^{1}H NMR (600 MHz, D_2O) δ 5.15 (d, 1H, *J* = 3.9Hz), 4.54 (t, 1H, *J* = 7.4Hz), 4.2-4.16 (m, 2H), 4.05-3.59 (m, 18H), 3.09 (t, 2H, *J* = 6.0Hz), 1.96 (m, 2H)

^{13}C NMR (150 MHz, D_2O) δ 175.89, 104.00, 102.35, 96.63, 79.91, 78.39, 76.25, 75.88, 73.47, 72.03, 70.78, 70.47, 70.33, 69.38, 69.12, 66.01, 62.19, 62.13, 61.27, 56.22, 38.80, 27.85, 23.37,

ESI-HRMS: calcd for $C_{23}H_{42}N_2O_{16}$ (M+H): 603.2534, found 603.2613

Compound 4.20



4.19 (20 mg, 0.03 mmol, 1 equiv.), and $NaHCO_3$ (11.1 mg, 0.132 mmol, 4 equiv.), were dissolved in deionized water (1 mL), and stirred at room

temperature for 10 in. At this time a solution of **4.18** (32.0 mg, 0.06 mmol, 2.0 equiv.) in DMF (1 mL) was added to the reaction mixture. The resultant was stirred 18 h at room temperature. The reaction mixture was neutralized by careful addition of 1.0 M HCl until pH neutral. The solution was concentrated under reduced pressure and coevaporated with toluene (2 x 5 mL), to remove all water from the solution. The resultant solution was then added dropwise to ice-cold diethyl ether (20 mL) to yield a white precipitate. This heterogeneous solution was centrifuged (15 min, 3300 rpm), to concentrate the precipitate into a white pellet. The supernatant was then decanted away. CH_2Cl_2 (15 mL) was then added to the precipitate, and the centrifuge tube was shaken until dispersion of the precipitate throughout the solution. The heterogeneous mixture was cooled in an ice water bath for 30 min, and then centrifuged (20 min, 3300 rpm). After decanting of the supernatant, this yielded a white solid. To this solid was added pyridine (2.0 mL) and acetic anhydride (0.75 mL, 7.9 mmol, 264 equiv.). The resultant was stirred overnight at ambient temperature. At this time, the reaction mixture was diluted with toluene (5 mL), and concentrated under reduced pressure. The resulting residue was coevaporated with toluene (3 x 5 mL), to yield a yellow oil, which was purified by column chromatography on silica gel using CH_2Cl_2 (95:5) as the eluent. This afforded **4.20** (20.0 mg, 55%) as a white film.

R_f (CH_2Cl_2:MeOH 95:5) = 0.5

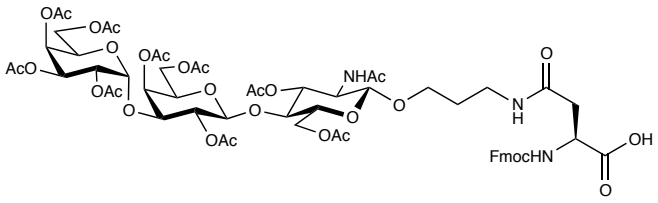
IR (neat): 3335.94, 2939.57, 1745.61, 1371.41, 1219.03, 1043.51 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.7 Hz, 2H), 7.59 (dd, *J* = 14.6, 7.5 Hz, 2H), 7.42 (t, *J* = 7.6 Hz, 2H), 7.34 (q, *J* = 7.0 Hz, 2H), 7.26 (m, 4H), 7.16 (dd, *J* = 14.4, 7.3 Hz, 1H), 6.28-6.21 (m, 1H), 5.84 (d, *J* = 7.0 Hz, 1H), 5.44 (d, *J* = 3.3 Hz, 1H), 5.31 (d, *J* = 3.0 Hz, 1H), 5.28-5.22 (m, 2H), 5.15 (dd, *J* = 10.3, 7.8 Hz, 1H), 5.09 (dd, *J* = 10.9, 3.1 Hz, 1H), 4.99 (t, *J* = 9.0 Hz, 1H), 4.49-4.35 (m, 4H), 4.27 (d, *J* = 8.2 Hz, 3H), 4.23-4.14 (m, 2H), 4.05 (dt, *J* = 16.2, 6.1 Hz, 4H), 3.89 (dd, *J* = 11.2, 6.3 Hz, 1H), 3.82 (dd, *J* = 10.3, 3.0 Hz, 1H), 3.80-3.70 (m, 3H), 3.52 (d, *J* = 13.2 Hz, 1H), 3.44 (s, 1H), 3.35-3.28 (m, 1H), 3.02 (dd, *J* = 18.1, 9.2 Hz, 1H), 2.86-2.79 (m, 1H), 2.13 (s, 3H), 2.12 (s, 6H), 2.05 (s, 14H), 2.03 (s, 3H), 1.94 (s, 6H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.40, 170.38, 170.31, 170.17, 170.12, 170.06, 169.88, 169.69, 168.95, 143.54, 141.27, 128.99, 128.18, 127.92, 127.89, 127.17, 127.13, 124.97, 124.91, 120.11, 120.07, 101.19, 100.94, 93.34, 75.86, 73.23, 72.71, 72.63, 70.64, 69.74, 67.65, 67.30, 67.28, 67.14, 66.82, 66.41, 64.52, 62.22, 61.24, 60.89, 60.83, 50.27, 46.92, 35.88, 27.13, 23.16, 20.88, 20.75, 20.72, 20.64, 20.63, 20.61, 20.57, 20.54, 20.50

ESI-HRMS: calcd for $C_{67}H_{81}N_3O_{30}$ (M-OBn): 1300.2243 found 1300.4408

Compound 4.22



To a flask containing **4.20** (10 mg, 0.007 mmol, 1.0 equiv.) was added 10% Pd/C (~1 mg), and the solids were dissolved in MeOH (5 mL). The resulting solution was placed under a hydrogen atmosphere (balloon), with stirring, for 3 h at room temperature. At this time, the heterogeneous solution was filtered through a celite plug and the celite plug washed with MeOH (2 x 3 mL). The solvent was removed under pressure to yield impure **4.21** as a thin yellow film. The yellow residue was dissolved in a mixture of CH_2Cl_2 and MeOH (1:1 v/v, 3 mL), and $NaHCO_3$ (2.5 mg, 0.028 mmol, 4 equiv.) was added, and the resulting heterogeneous solution was stirred for 5 min. 9-fluorenylmethylcarbonyl chloride (4.0 mg, 0.014 mmol, 2.0 equiv) was added, and the reaction mixture was stirred for 2 h at room temperature. At this time, the solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (95:5) as the eluent, to give **4.22** (3.0 mg, 31%) as a white film.

R_f (CH_2Cl_2:MeOH 95:5) = 0.5

IR (neat): 3359.09, 2927.99, 1745.61, 1706.07, 1524.76, 1396.48, 1219.03, 1043.51 cm^{-1}.

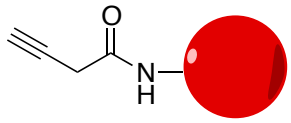
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.61 (dt, *J* = 14.0, 7.3 Hz, 2H), 7.42 (q, *J* = 9.5, 8.2 Hz, 2H), 7.38-7.28 (m, 2H), 6.20 (d, *J* = 9.1 Hz, 1H), 5.74 (d, *J* = 6.9 Hz, 1H), 5.48-5.42 (m, 1H), 5.34-5.23 (m, 4H), 5.16 (d, *J* = 9.9 Hz, 1H), 5.10 (d, *J* = 11.0 Hz, 1H), 5.02-4.97 (m, 1H), 4.43-4.36 (m, 2H), 4.27 (d,

J = 8.0 Hz, 3H), 4.23-4.15 (m, 3H), 4.09-4.02 (m, 5H), 3.90 (s, 1H), 3.83 (dt, *J* = 10.1, 2.5 Hz, 1H), 3.80-3.71 (m, 4H), 3.53 (d, *J* = 13.4 Hz, 2H), 3.45 (s, 1H), 3.32 (d, *J* = 10.9 Hz, 1H), 3.04 (dd, *J* = 18.9, 9.5 Hz, 1H), 2.83 (d, *J* = 18.0 Hz, 1H), 2.14 (s, 3H), 2.13 (s, 6H), 2.05 (s, 12H), 2.04 (s, 3H), 1.95 (s, 3H), 1.95 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 170.52, 170.37, 170.30, 170.17, 170.12, 170.06, 169.87, 169.68, 168.93, 167.06, 141.33, 141.29, 129.00, 128.19, 127.94, 127.91, 127.18, 127.13, 120.13, 120.08, 101.19, 100.97, 93.37, 72.72, 72.65, 70.68, 69.88, 69.73, 67.66, 67.37, 67.34, 67.17, 66.83, 66.42, 66.35, 64.53, 62.23, 61.25, 60.84, 50.31, 29.68, 27.15, 23.19, 20.89, 20.76, 20.73, 20.66, 20.64, 20.62, 20.59, 20.55, 20.51.

ESI-HRMS: calcd for $C_{60}H_{75}N_3O_{30}$ (M-OH_2): 1300.2243 found 1300.4408

Resin 4.30



To a 5 mL polypropylene syringe that had been fitted with a frited glass disk was added Amino PEGA resin (0.135 g, 0.046 mmol, 1.0 equiv). The resin was washed with MeOH (1 x 4 mL), CH_2Cl_2 (2 x 4 mL), and DMF (3 x 4 mL). HATU (85.7 mg, 0.23 mmol, 5.0 equiv.), HOAt (31.3 mg, 0.23 mmol, 5.0 equiv.), and 4-pentynoic acid (23 mg, 0.23 mmol, 5.0 equiv.) were dissolved in DMF (2.0 mL). This solution was then added to the resin by suction and the syringe was

shaken for 10 sec. DIPEA (80 µL, 0.46 mmol, 10.0 equiv.) in DMF (1.0 mL) was then added to the resin my suction, and the syringe was shaken vigorously for 1 h at room temperature. At this time, the resin was removed from the reaction mixture by vacuum filtration and washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), and DMF (2 x 4 mL) to give resin **4.30**. Completion acylation of the amino residues was confirmed by the Kaiser test.[6]

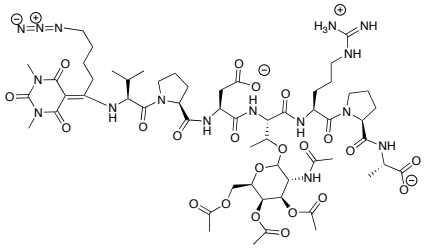
Standard Solid-Phase Peptide Synthesis Procedures:

Deprotection: To the resin is added a 20% solution of piperidine in DMF (3 mL). The reaction vessel is then shaken for 1 min. The reaction mixture is then removed from the resin by vacuum filtration and washed with DMF (1 x 4 mL). A solution of 20% solution of piperidine in DMF (3 mL) is then added to the resin and the reaction vessel and the vessel is shaken at room temperature for 20 min. At this point, the resin is separated from the reaction mixture by vacuum filtration and washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), and DMF (2 x 4 mL).

Coupling: N-Fmoc-L-amino acid (5.0 equiv), HATU (4.9 equiv), and HOAt (5.0 equiv.) are dissolved in DMF (2 mL), and added to the resin (1.0 equiv). DIPEA (10.0 equiv.) in DMF (1 mL) is then added to the resin and the reaction vessel is shaken for 30 min at ambient temperature. The resin is then separated by the reaction mixture by vacuum filtration, and washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), and DMF (2 x 4 mL). This procedure is repeated once, and completion of the coupling reaction is assessed by the Kaiser test.

Acetylation: To the resin is added a solution of acetic anhydride/DIPEA/DMF (1:2:7 v/v/v, 3 mL), and the reaction vessel is shaken for 15 min at room temperature. The reaction mixture is the separated from the resin by vacuum filtration, and the resin is washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), and DMF (2 x 4 mL).

Compound 4.31



Fmoc-Ala Wang resin (250 mg, 0.1 mmol, 1.0 equiv) was added to a 5 ml polypropylene syringe that had been fitted with a glass frit. CH_2Cl_2 (5 mL) was drawn into the syringe and the vessel was shaken for 30 min to swell the resin. The resin was then washed with DMF (2 x 4 mL), and the Fmoc group removed using the standard deprotection procedure. Fmoc-Pro-OH, and Fmoc-Arg(Pbf)-OH were used to elongate the peptide using the standard coupling, acetylation and coupling procedures. At this time, **4.29** (100.6 mg, 0.15 mmol, 1.5 equiv.), HATU (53 mg, 0.14 mmol, 1.4 equiv.), and HOAt (20.4 mg, 0.15 mmol, 1.5 equiv) were dissolved in DMF (1 mL) and added to the reaction vessel. DIPEA (52 µL, 0.3 mmol, 3.0 equiv.) in DMF (0.5 mL) was then added to the reaction mixture. The reaction vessel was shaken overnight at room temperature. The resin was then removed from the reaction mixture by vacuum filtration. The resulting resin

was washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), and DMF (2 x 4 mL), and completion of the coupling reaction was confirmed by the Kaiser test. Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, and amino acid **5.3**[7] (Figure 5.1) were then coupled to the growing peptide chain using the standard procedures. At this point, the resin was treated with a mixture of TFA/H_2O/Phenol/TIS (88/5/5/2, 3 mL), and the reaction vessel was shaken at room temperature for 3 h. The solution that contained the desired peptide was separated from the resin by filtration and the resin was washed with TFA (2 x 2 mL). The cleavage cocktail and TFA washings were combined and concentrated to ½ the original volume by a gentle stream of air. The resultant was added dropwise to ice-cold diethyl ether (15 ml) to precipitate the crude peptide. The resulting solution was centrifuged (15 min, 3300 rpm), and the supernatant was decanted. The resulting residue was treated with more diethyl ether (15 ml), and shaken until dispersion of the precipitate. The heterogeneous solution was cooled in an ice-bath for 20 min and centrifuged (20 min, 3300 rpm) to yield crude **4.31** (120 mg, 89%) as a white powder.

Compound 5.3

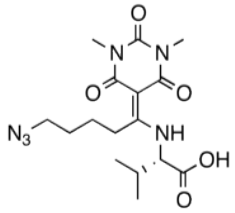
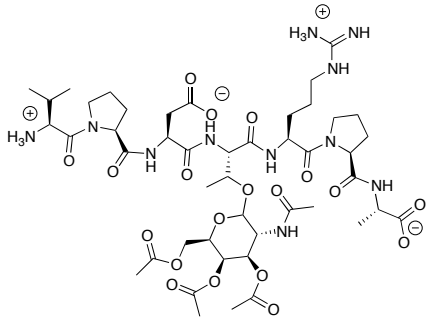


Figure 5.1

MS: calcd for $C_{57}H_{85}N_{16}O_{22}$ (M+H): 1347.37 found 1347.56

Compound 4.33



4.31 (34 mg, 0.023 mmol, 1.0 equiv), $CuSO_4$ (11.5 mg, 0.046 mmol, 2.0 equiv.), and THPTA (100 mg, 0.23 mmol, 10.0 equiv.), were dissolved in a mixture of DMF/1.0 HEPES buffer solution (1:4 v/v, 2 mL). This solution was then added to resin **4.30** (0.046 mmol, 2.0 equiv.), in a 5 mL polypropylene syringe that had been fitted with a glass frit. Amino guanidine HCl (2.5 mg, 0.023 mmol, 1.0 equiv.), and Na Ascorbate (18.23 mg, 0.092 mmol, 4 equiv.), in 1.0 M HEPES buffer solution (1 mL) were added to the reaction mixture, and the reaction vessel was shaken overnight at room temperature. At this time, the resin was removed from the reaction mixture by vacuum filtration, and the resin was washed with DMF (2 x 4 mL), CH_2Cl_2 (2 x 4 mL), MeOH (2 x 4 mL), and water (2 x 4 mL). A 1.0 M solution of NH_4OH (3 mL), was added to the reaction vessel, and it was shaken for 1 h at ambient temperature. The solution containing the desired peptide was removed from the resin by filtration, and the resin was washed with water (2 x 2 mL). The peptide solution and water washes were combined and the solvent was removed by a gentle stream of air to yield a yellow residue. This residue was dissolved in 0.1% aq. TFA (1 mL) and added a C_8 solid-phase

extraction cartridge. The extraction cartridge was then washed with 0.1% aq. TFA (2 x 2 mL), a mixture of 0.1% aq. TFA/MeCN (1:1 v/v, 2 x 2 mL), and MeCN (2 x 4 mL). The 0.1% aq. TFA/MeCN fractions were concentrated under a gentle stream of air to give **4.33** (18 mg, 72%) as a white solid.

MS: calcd for $C_{46}H_{73}N_{11}O_{19}$ (M+H): 1084.13 found 1084.37

Section 5.5. Literature Cited

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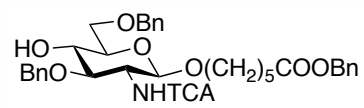
Chapter 6

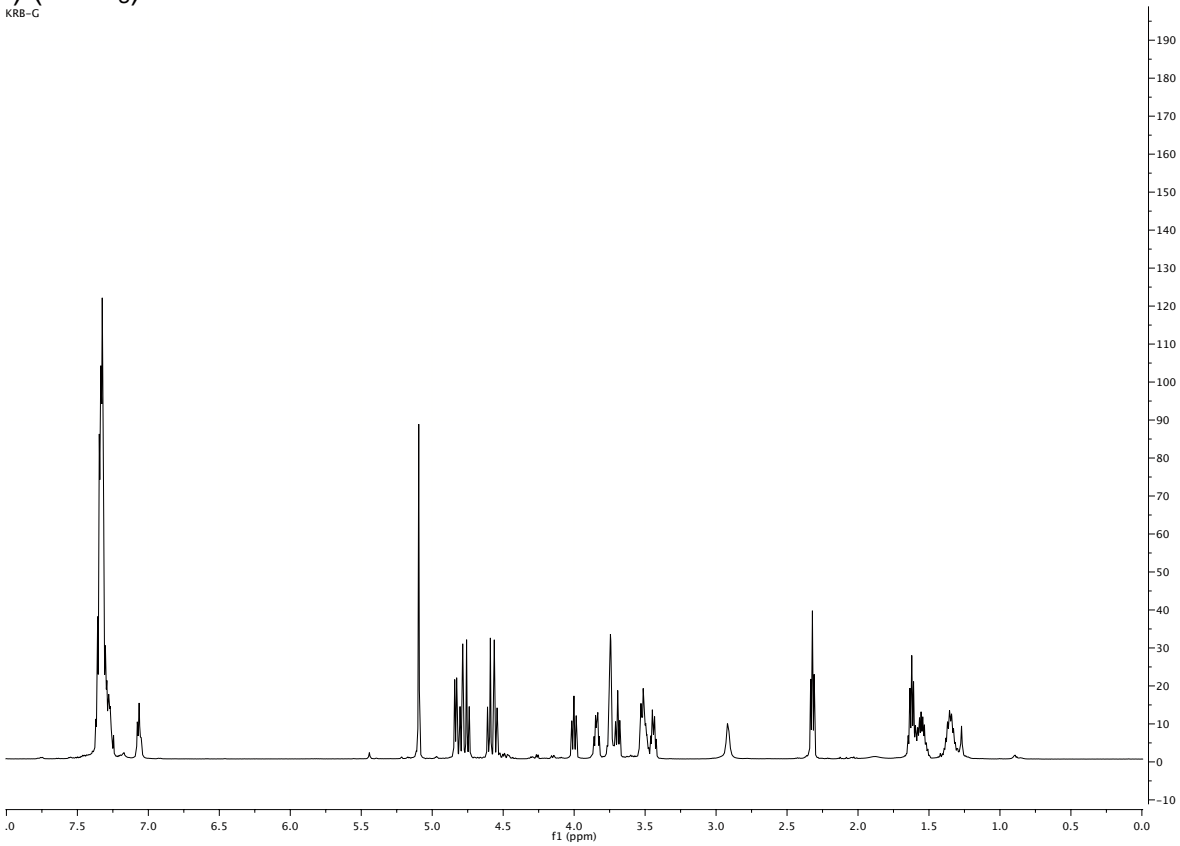
Spectra of Selected Compounds

Compound 4.8

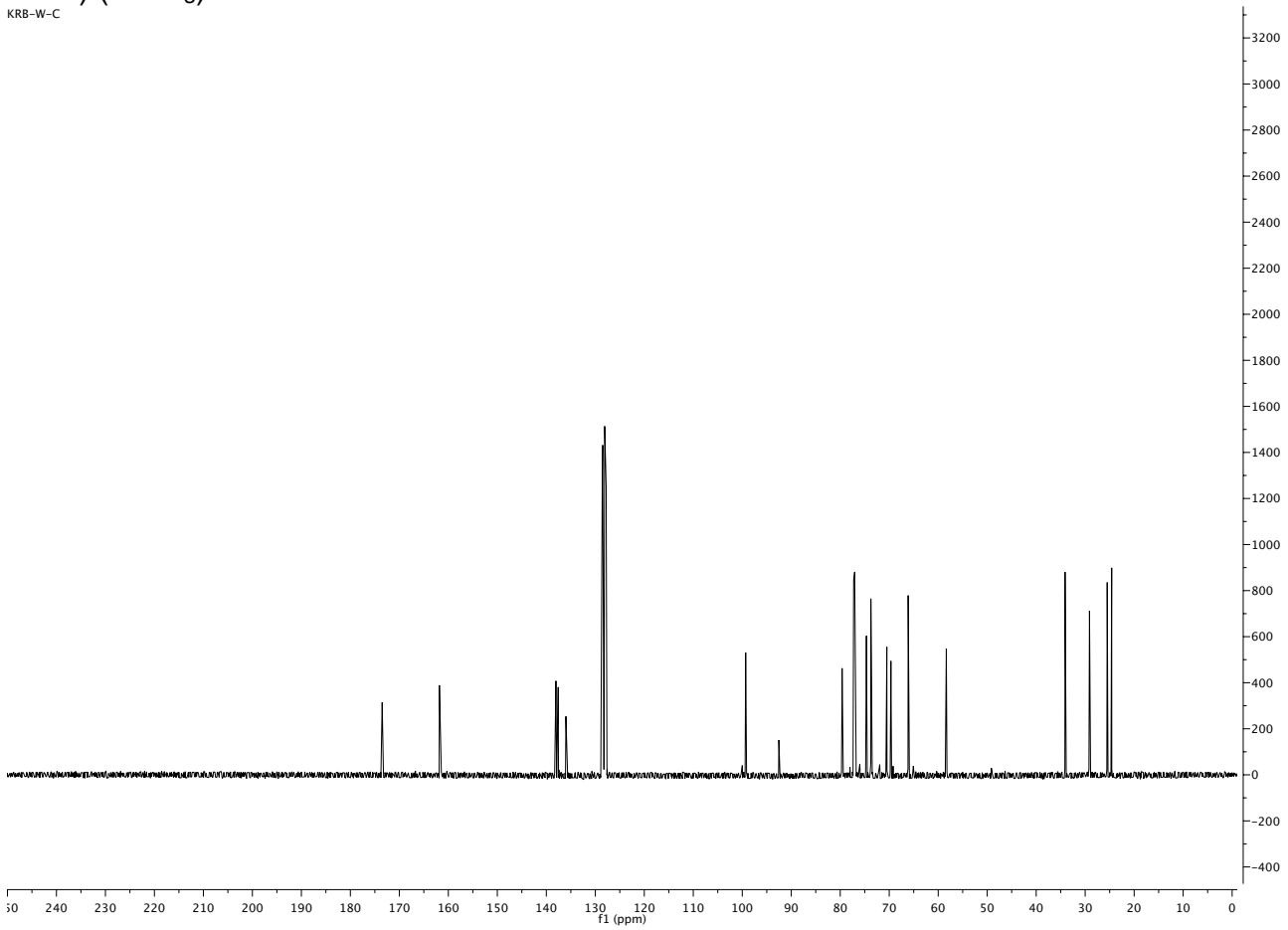


^{1}H NMR (600 MHz) ($CDCl_3$)

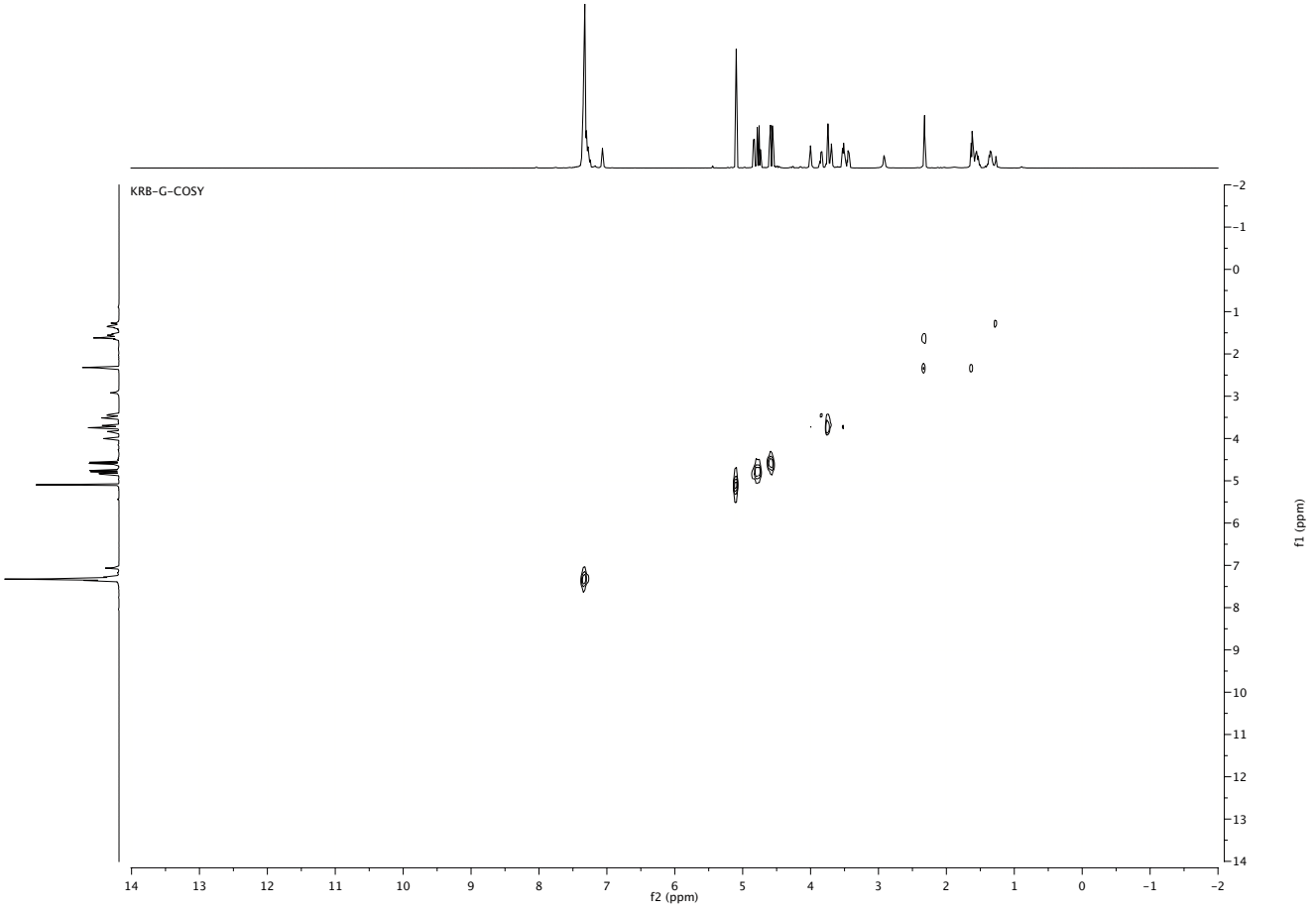
KRB-G



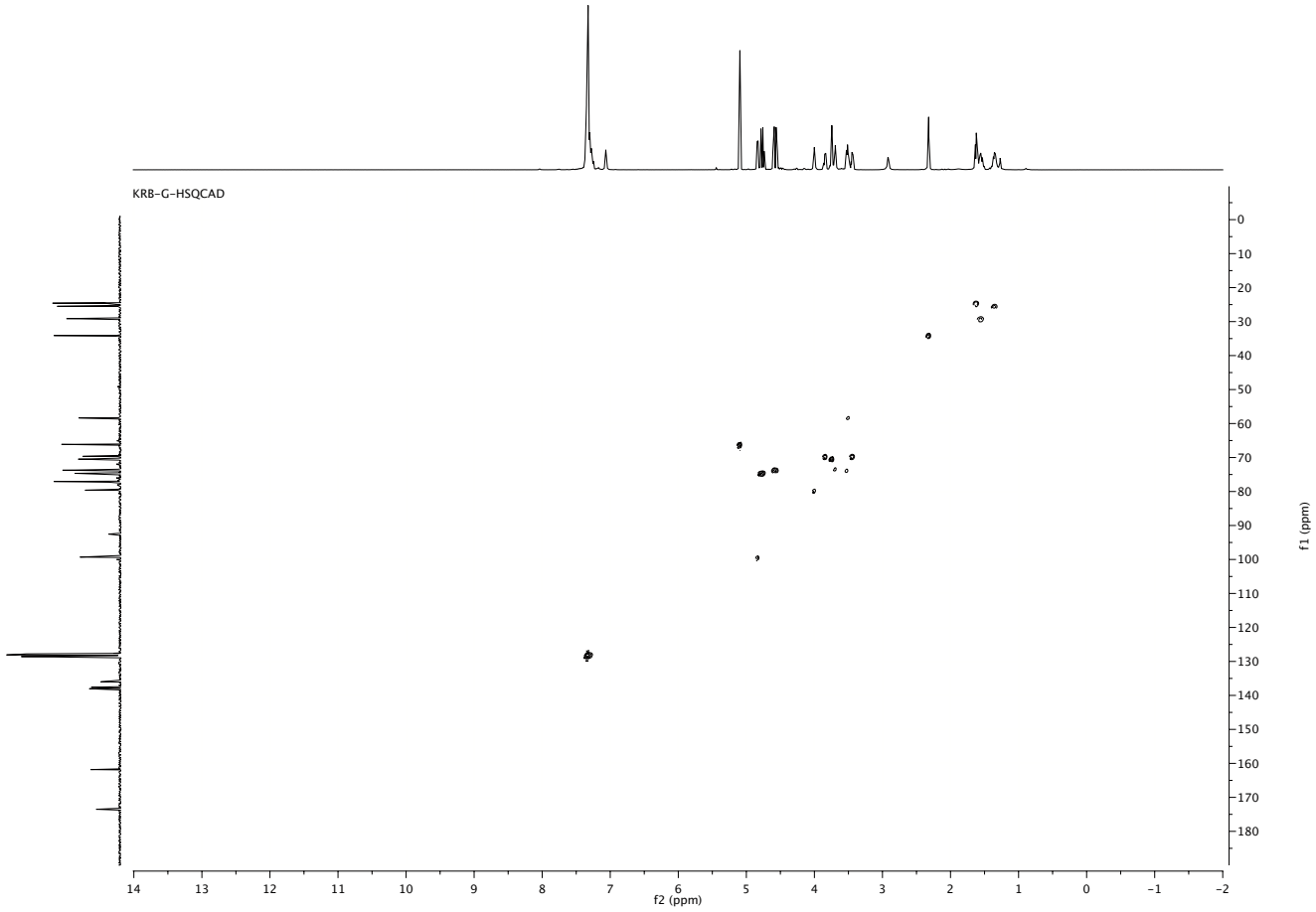
^{13}C NMR (150 MHz) ($CDCl_3$)



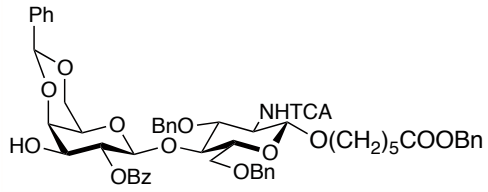
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



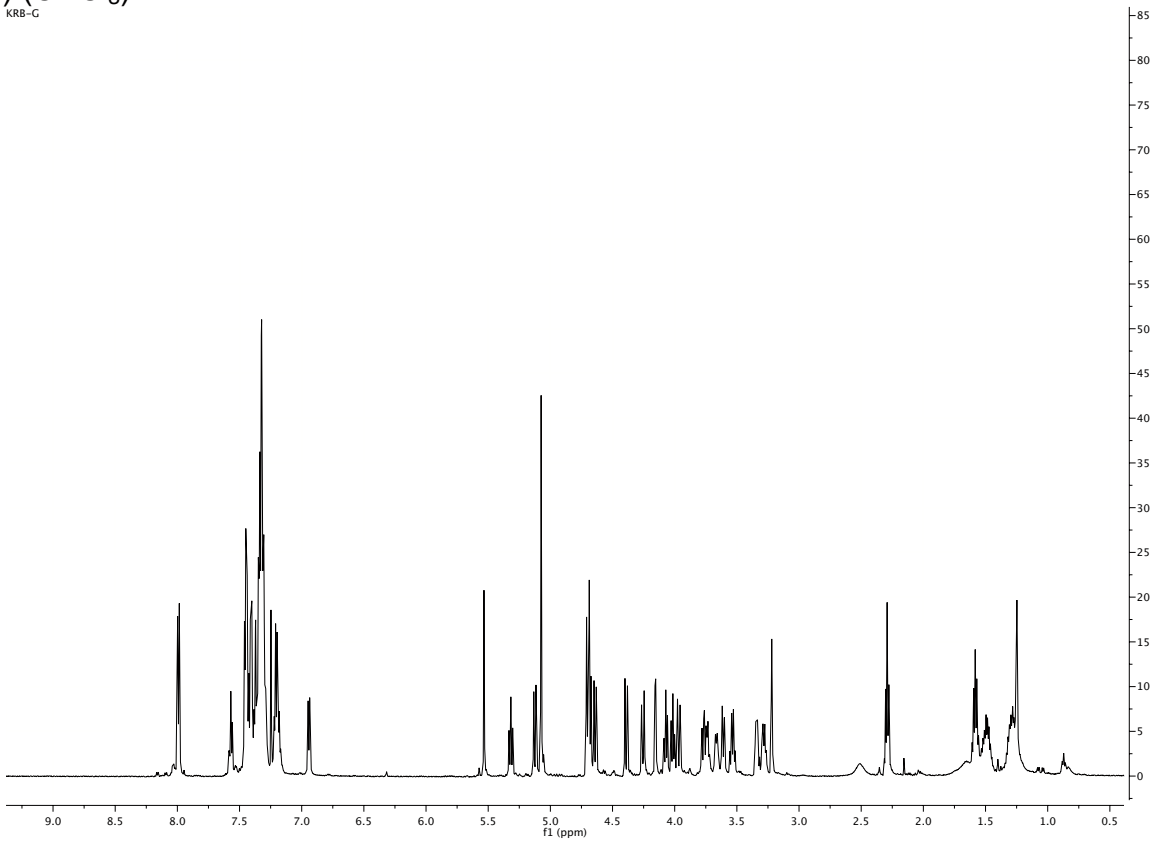
^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)



Compound 4.9

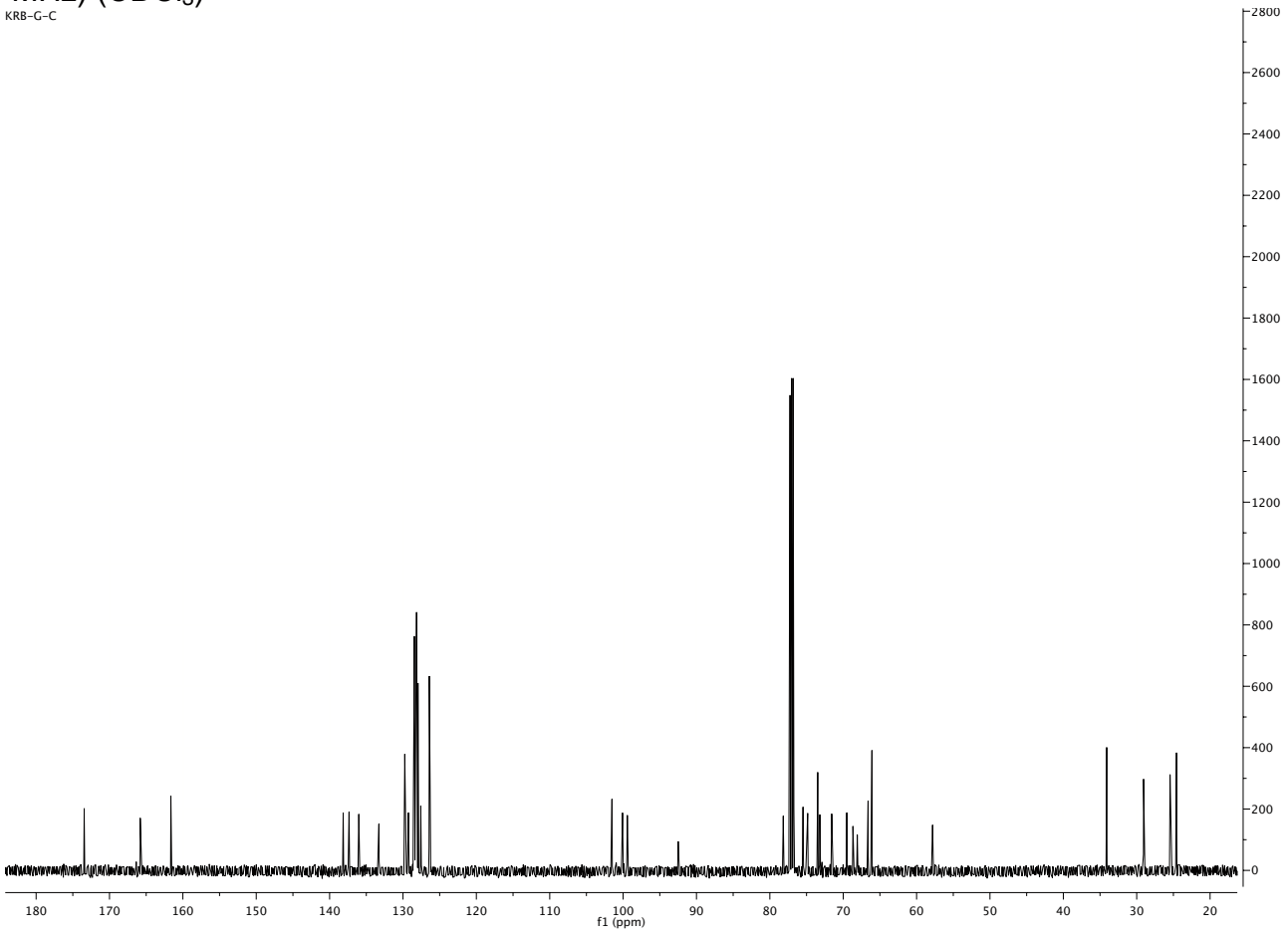


^{1}H NMR (600 MHz) ($CDCl_3$)

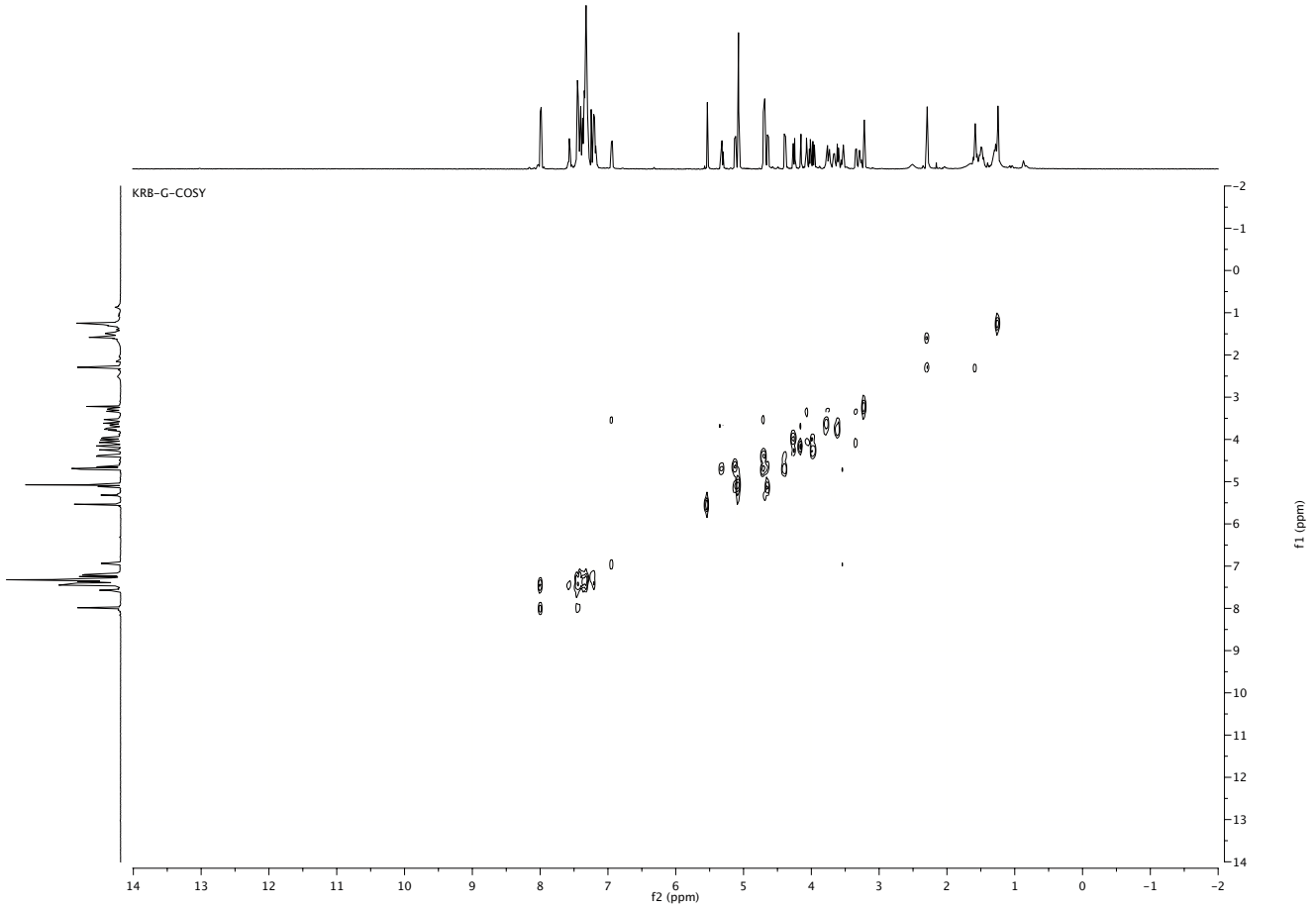


^{13}C NMR (150 MHz) ($CDCl_3$)

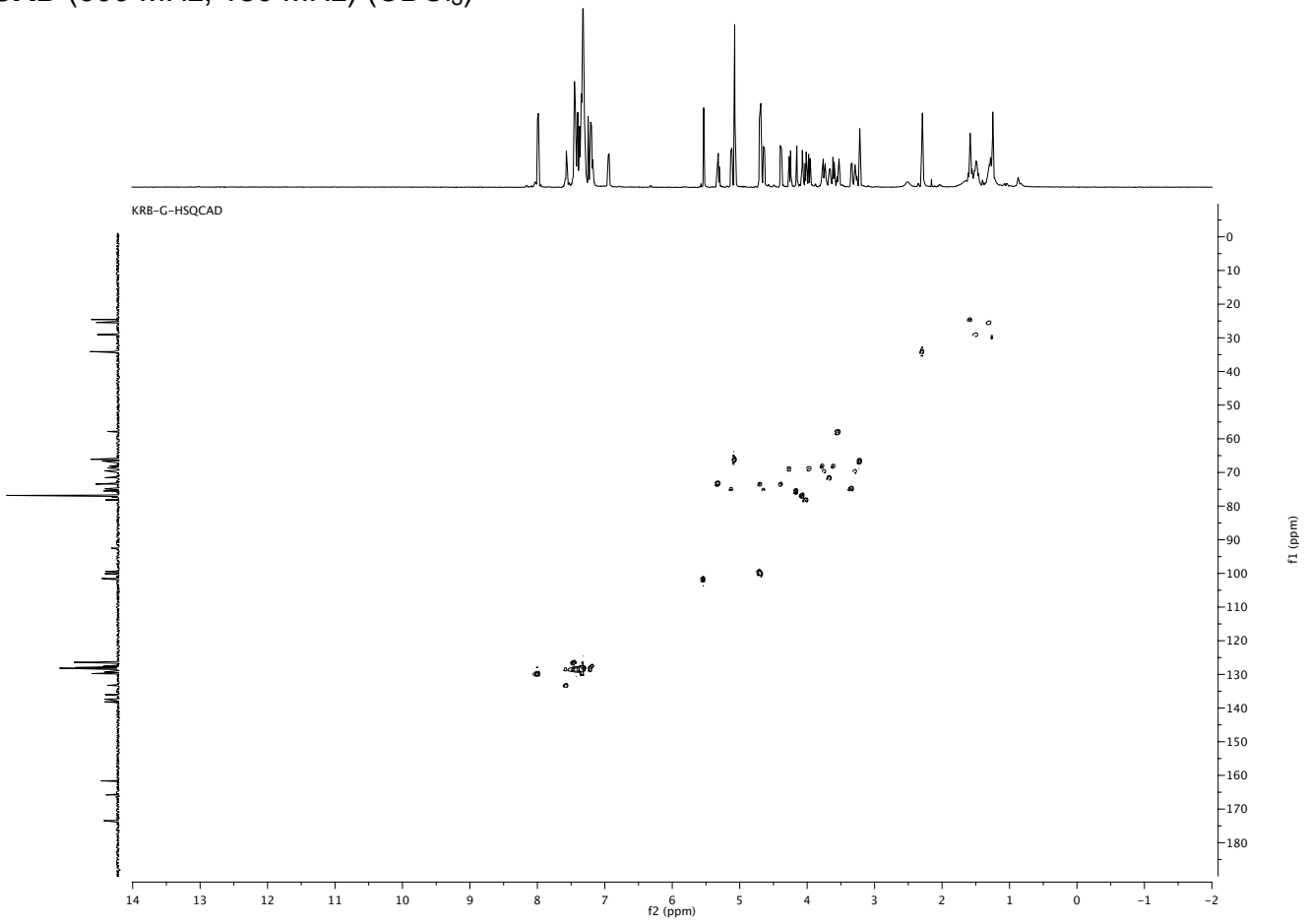
KRB-G-C



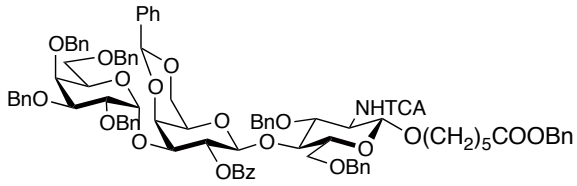
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)

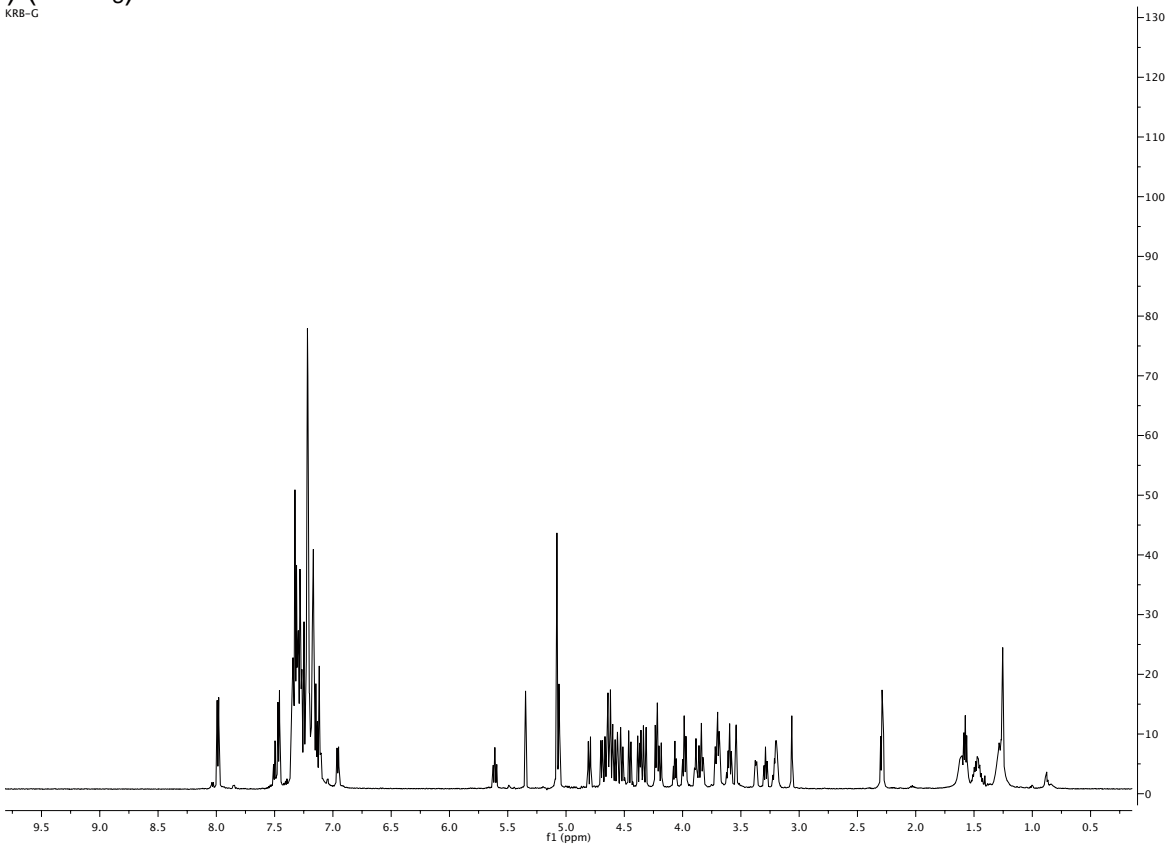


Compound 4.10



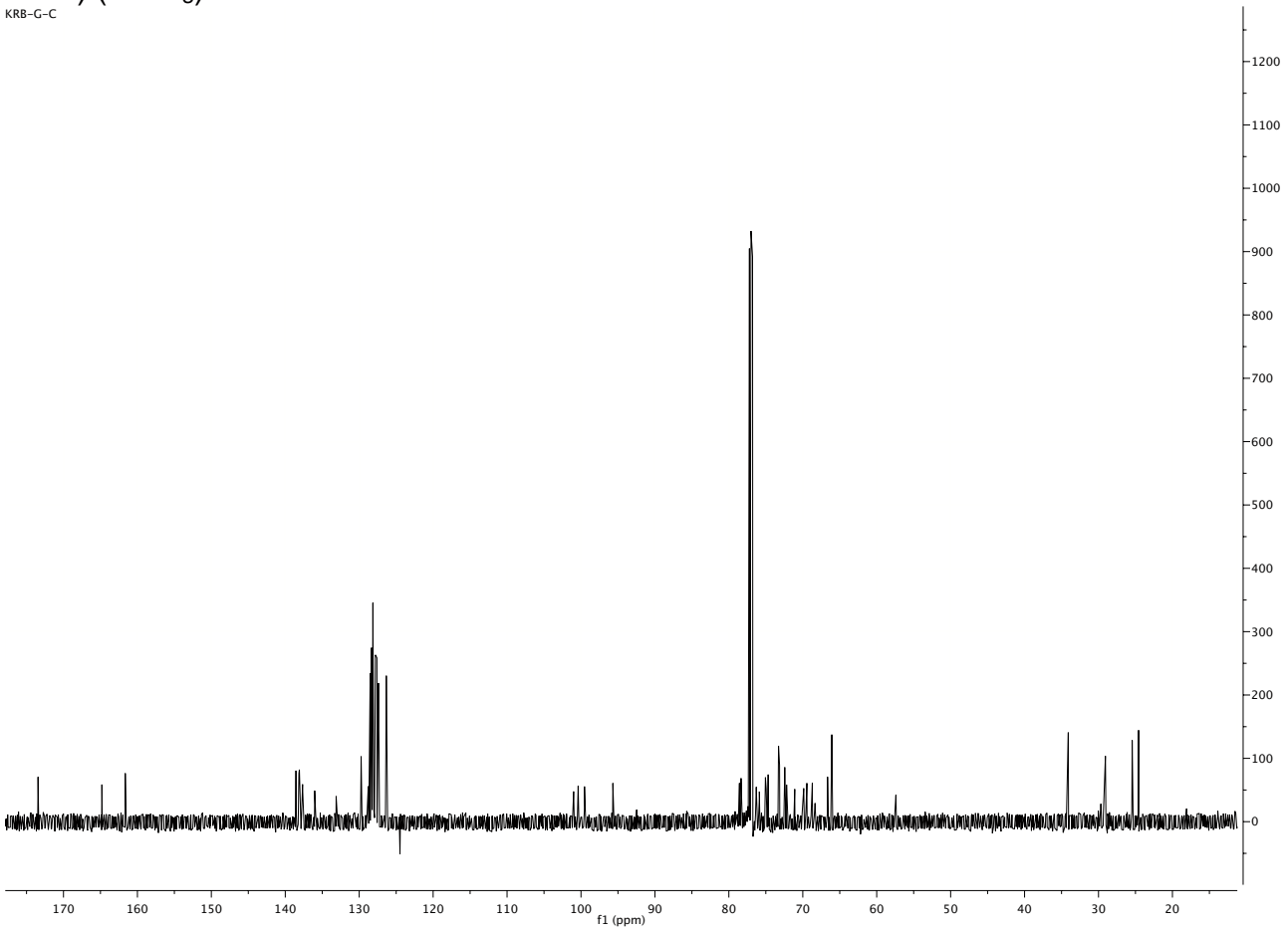
^{1}H NMR (600 MHz) ($CDCl_3$)

KRB-G

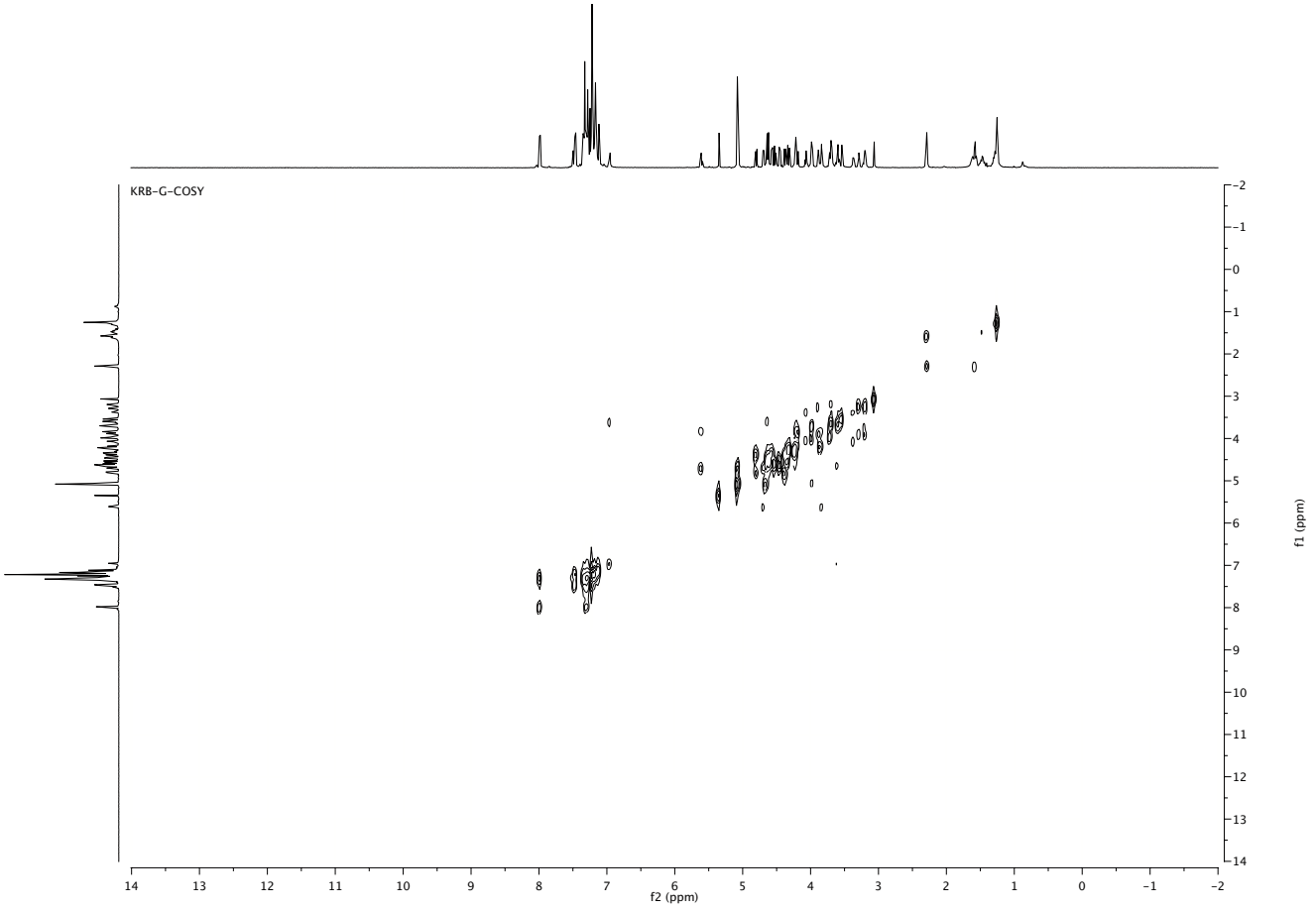


^{13}C NMR (150 MHz) ($CDCl_3$)

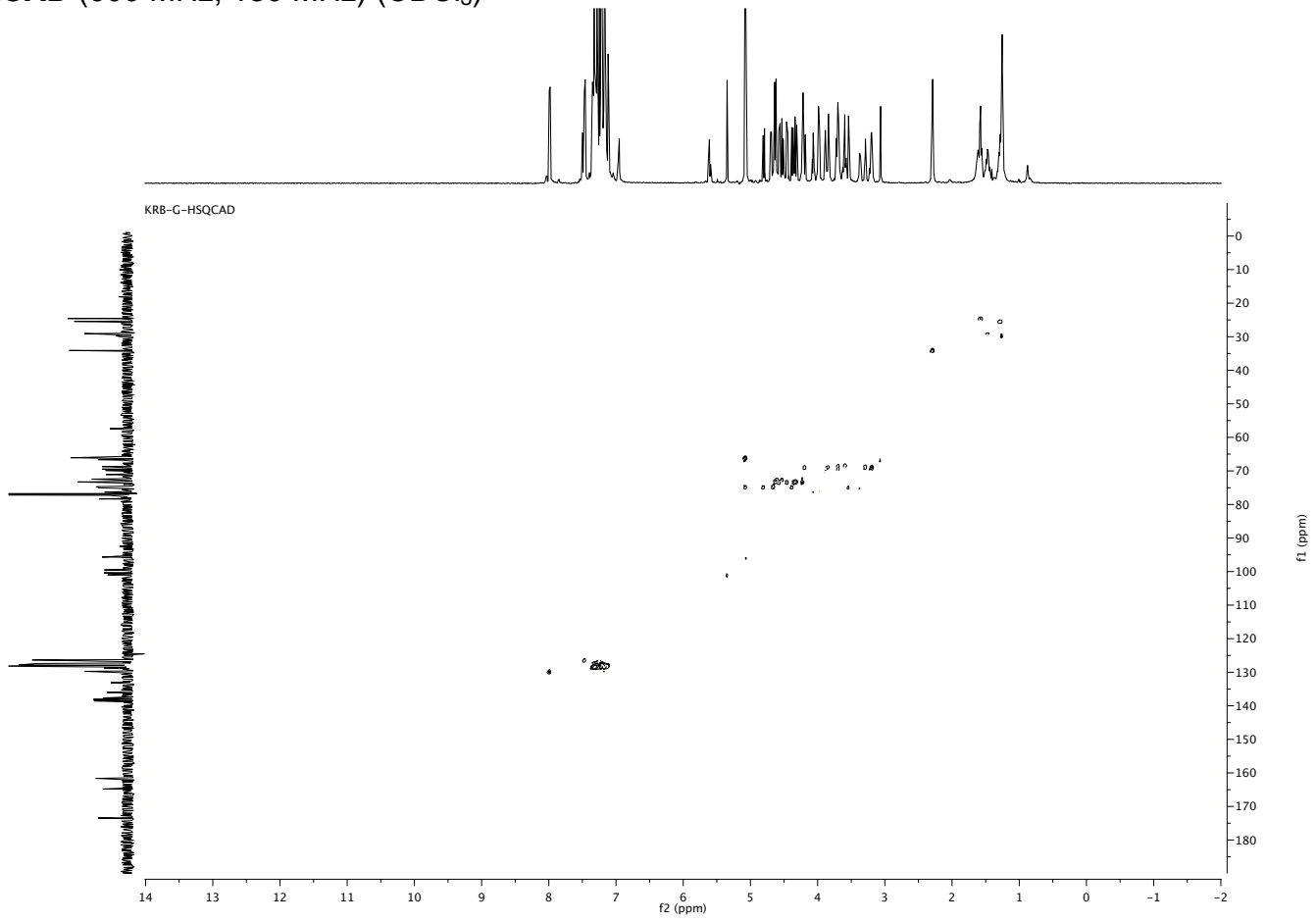
KRB-G-C



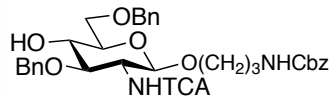
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)

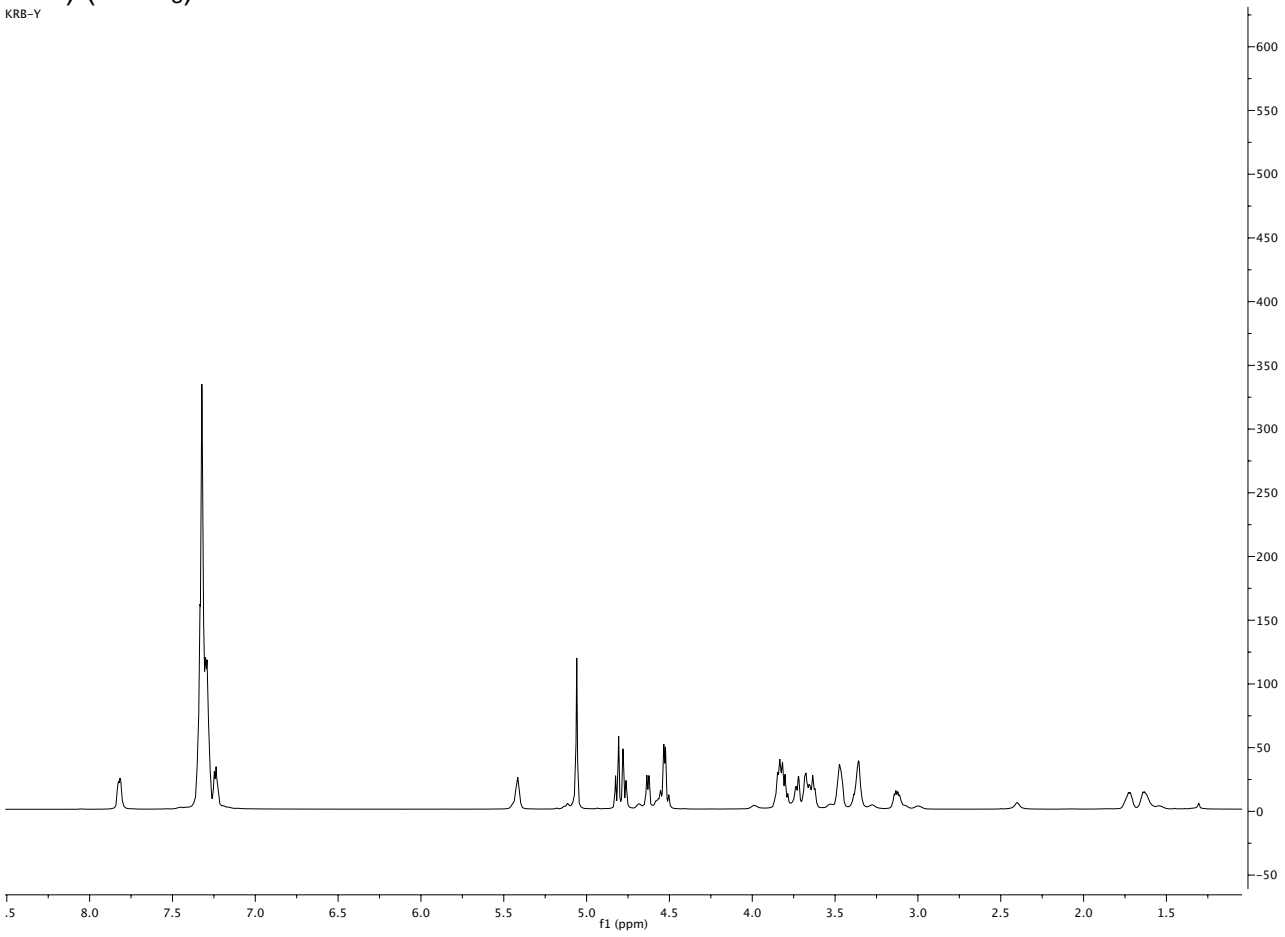


Compound 4.14



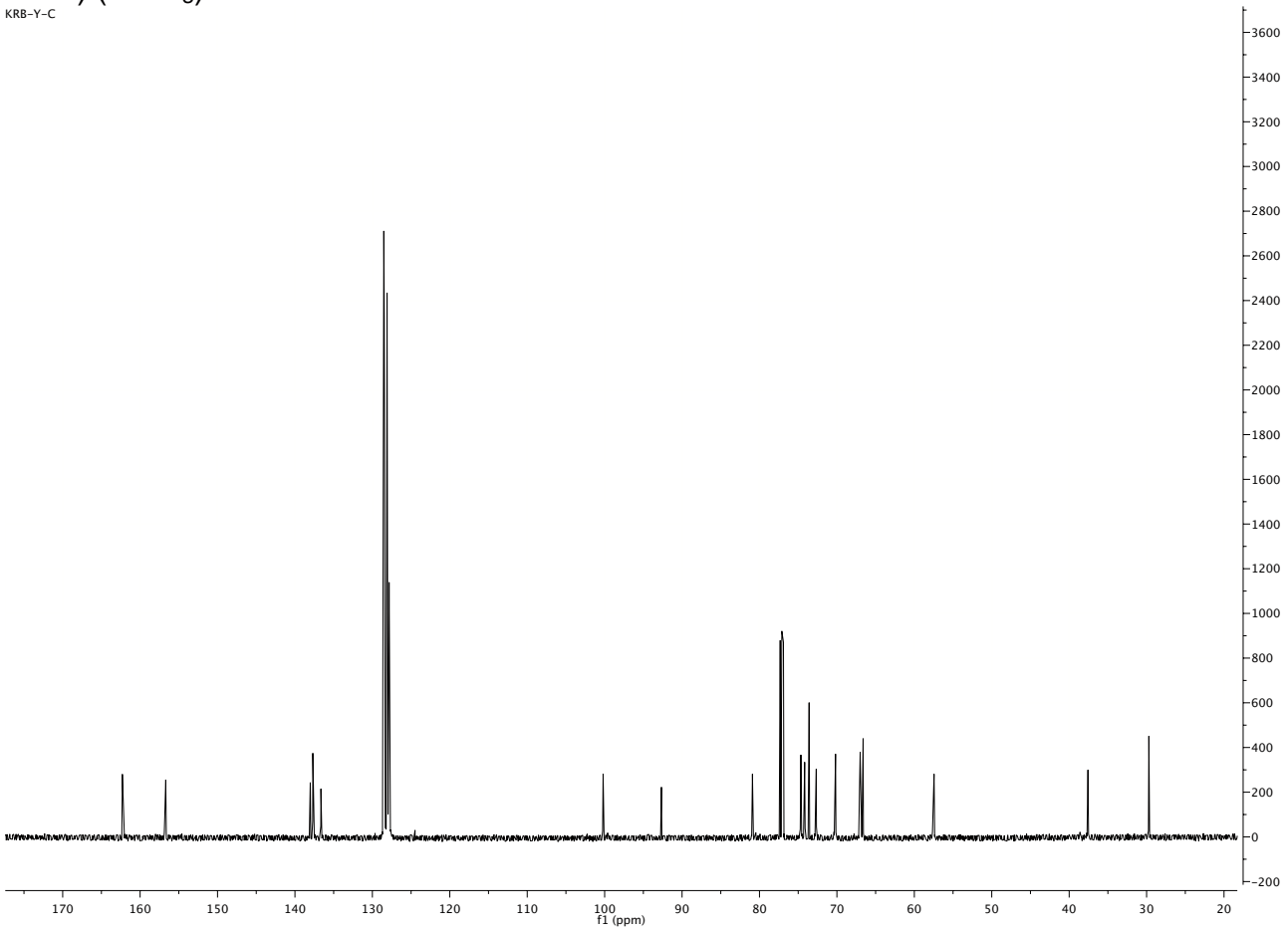
^{1}H NMR (600 MHz) ($CDCl_3$)

KRB-Y

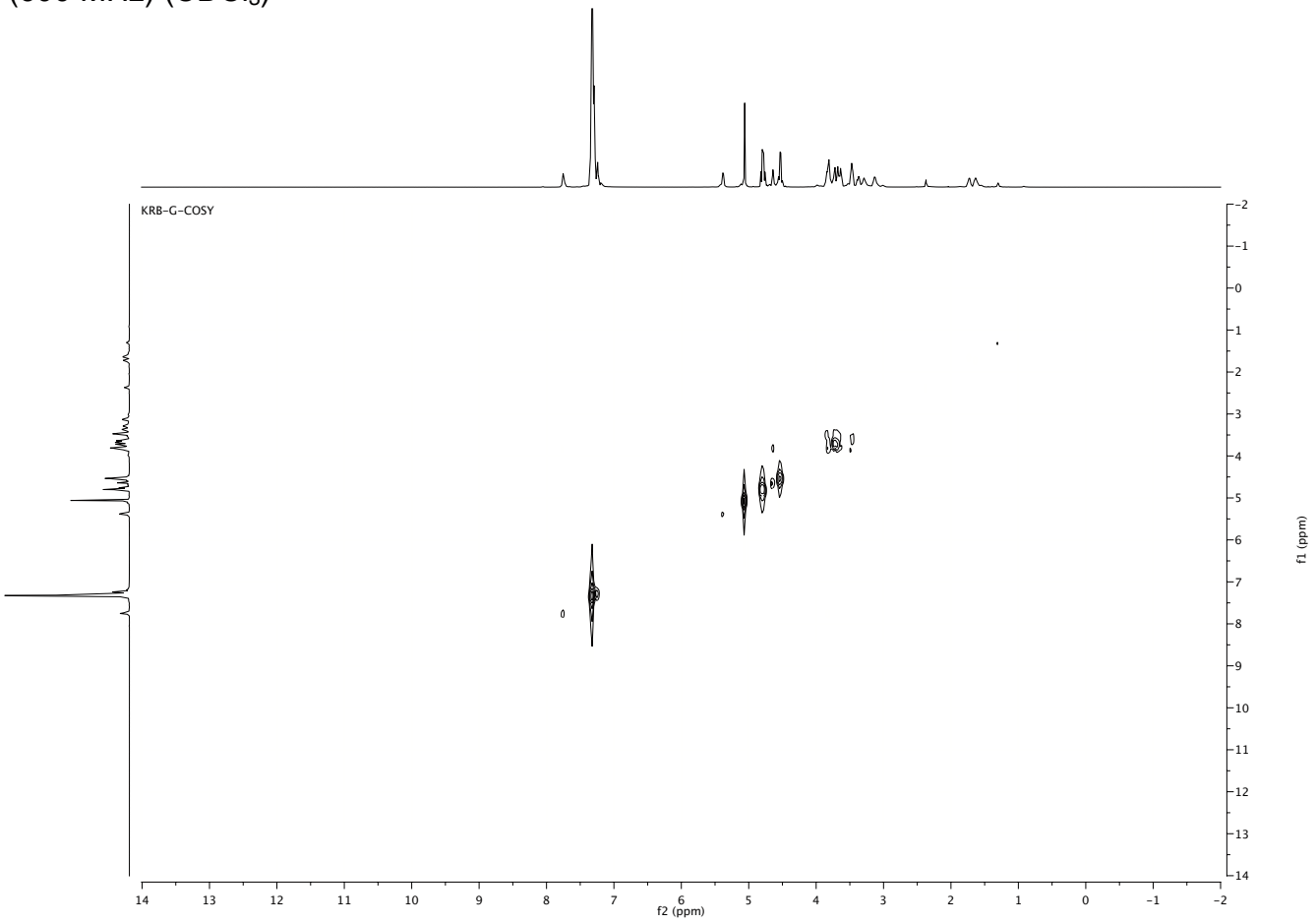


^{13}C NMR (150 MHz) ($CDCl_3$)

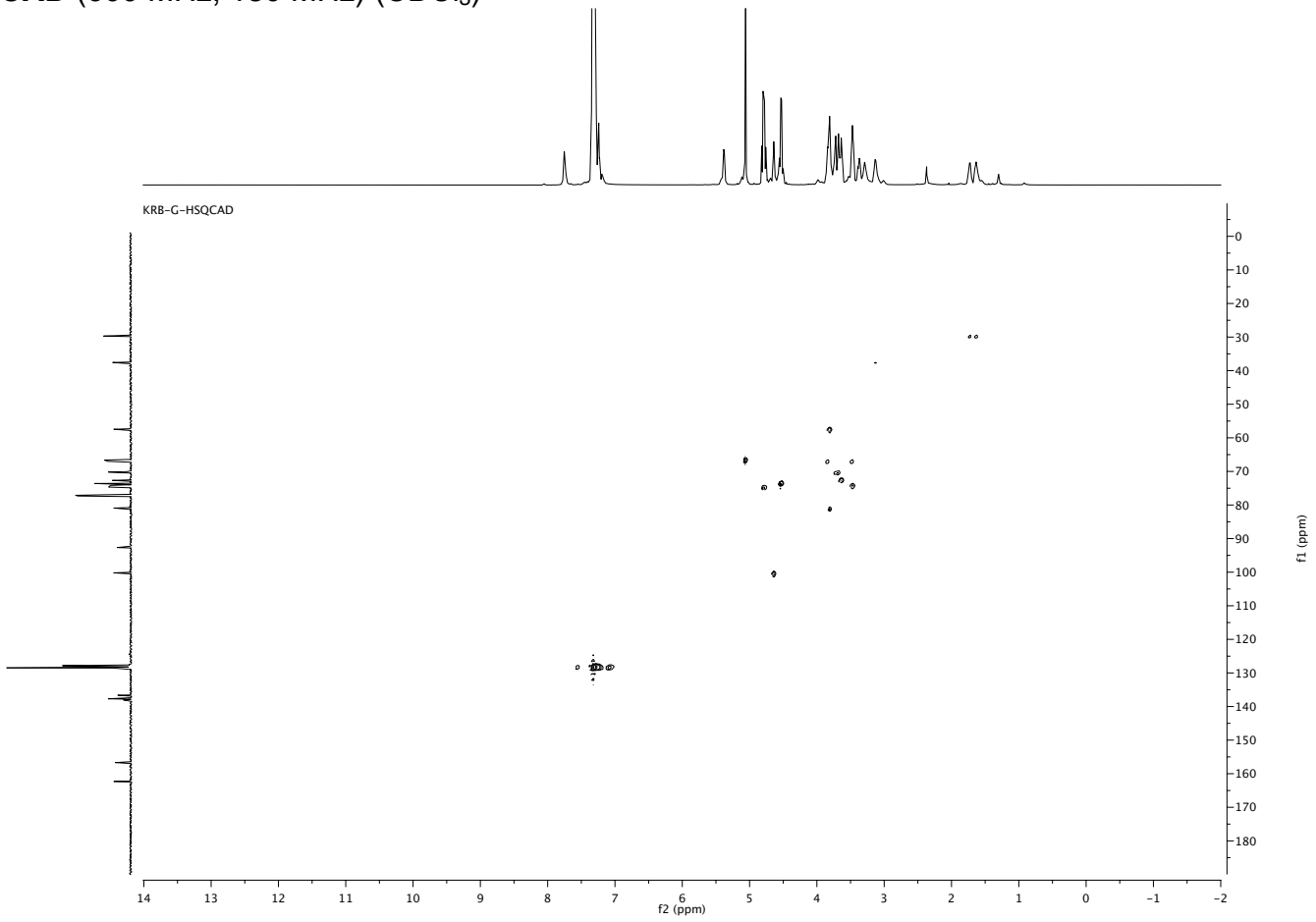
KRB-Y-C



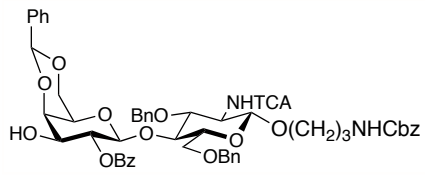
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)

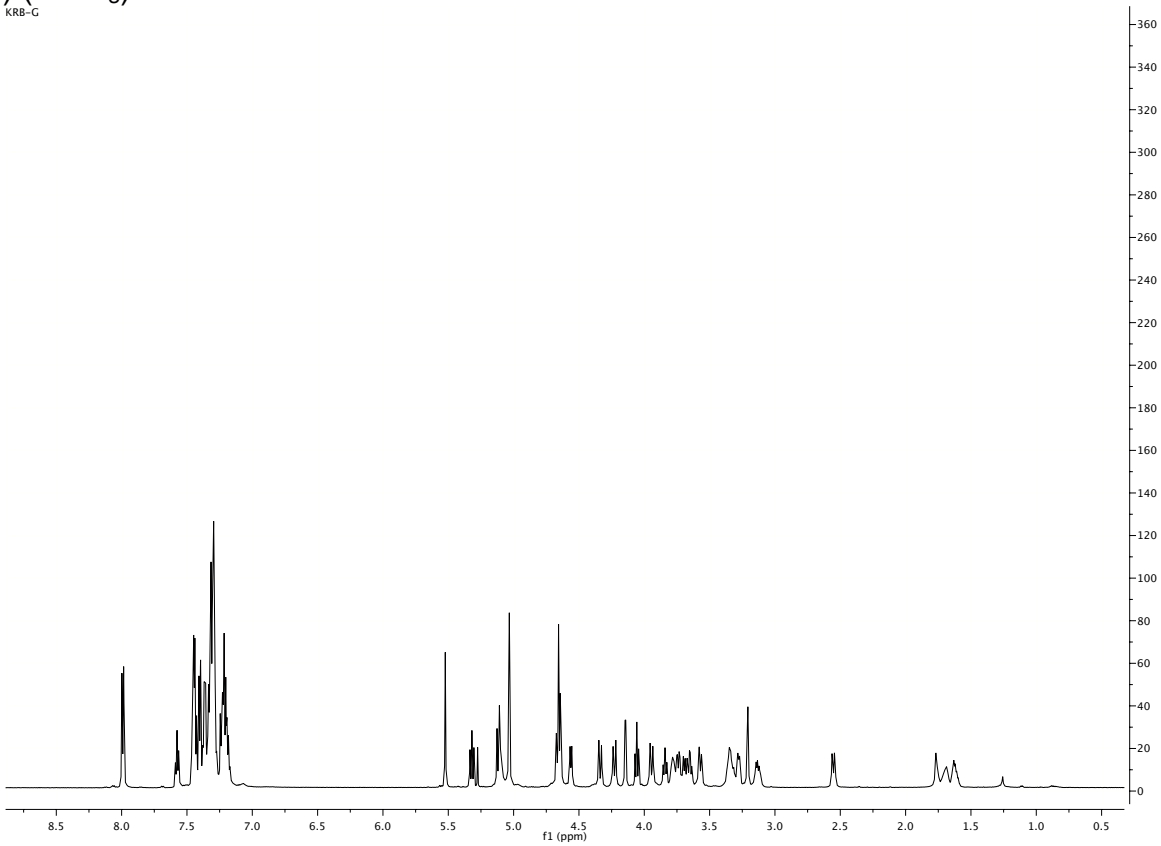


Compound 4.15

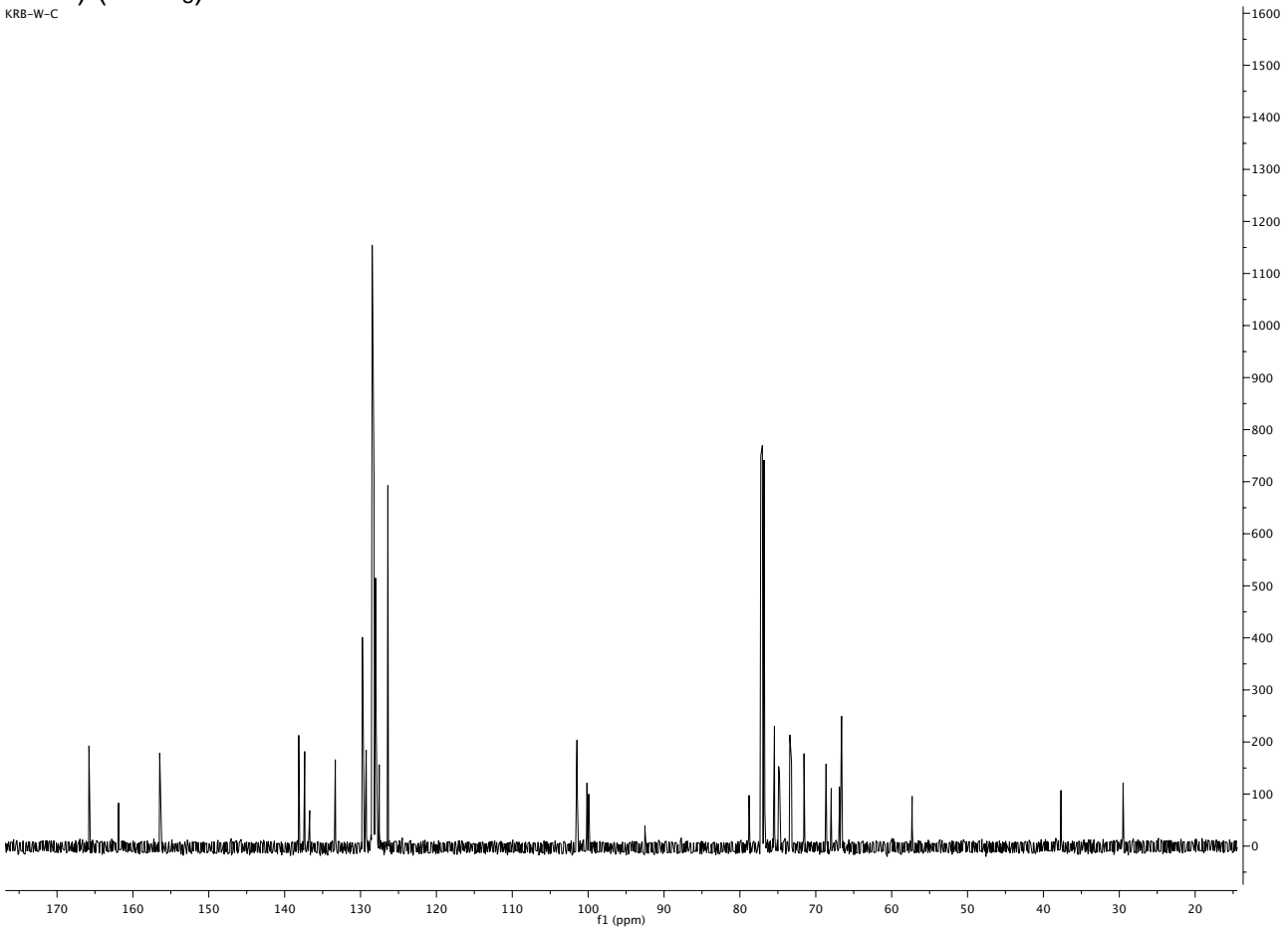


^{1}H NMR (600 MHz) ($CDCl_3$)

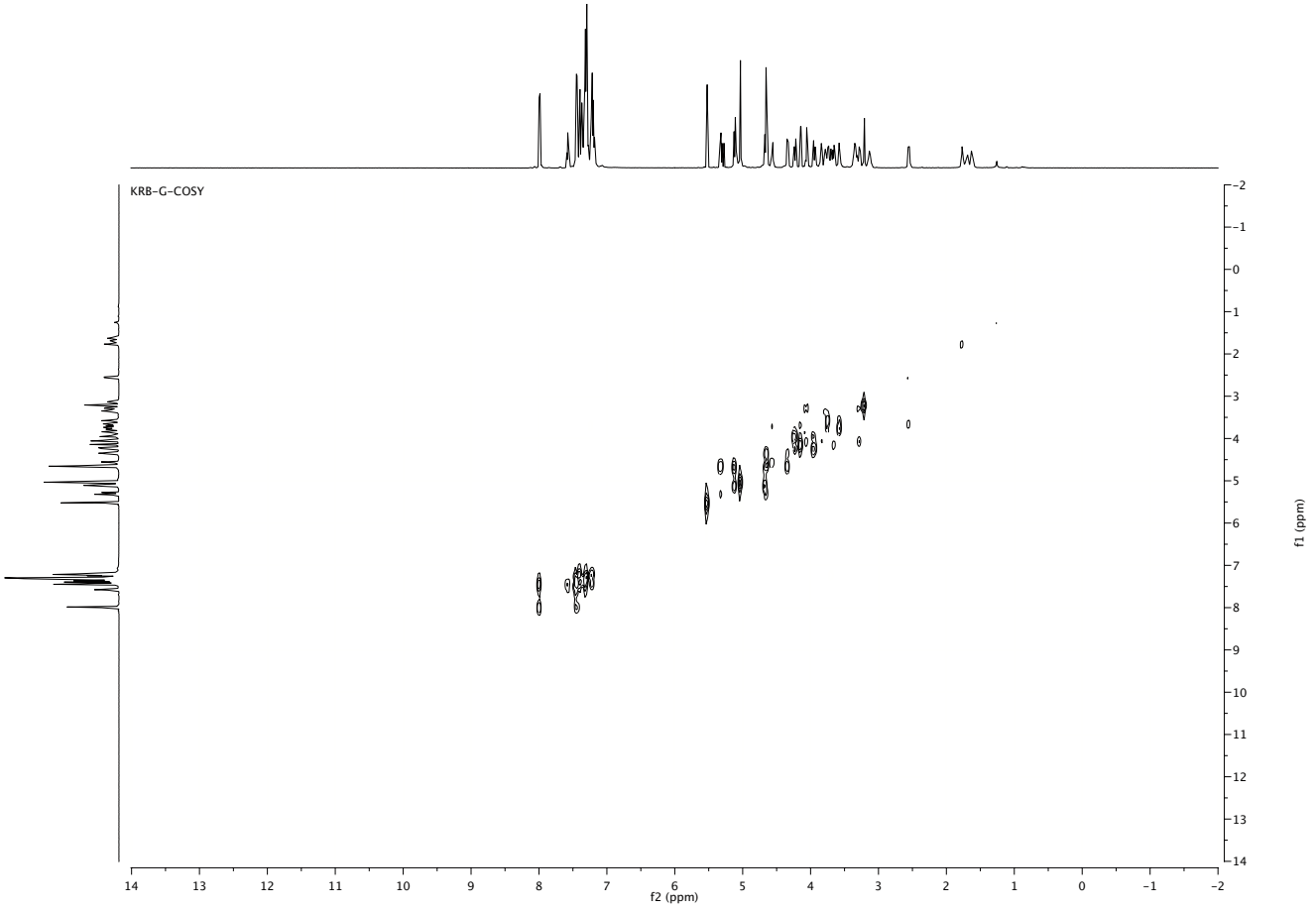
KRB-G



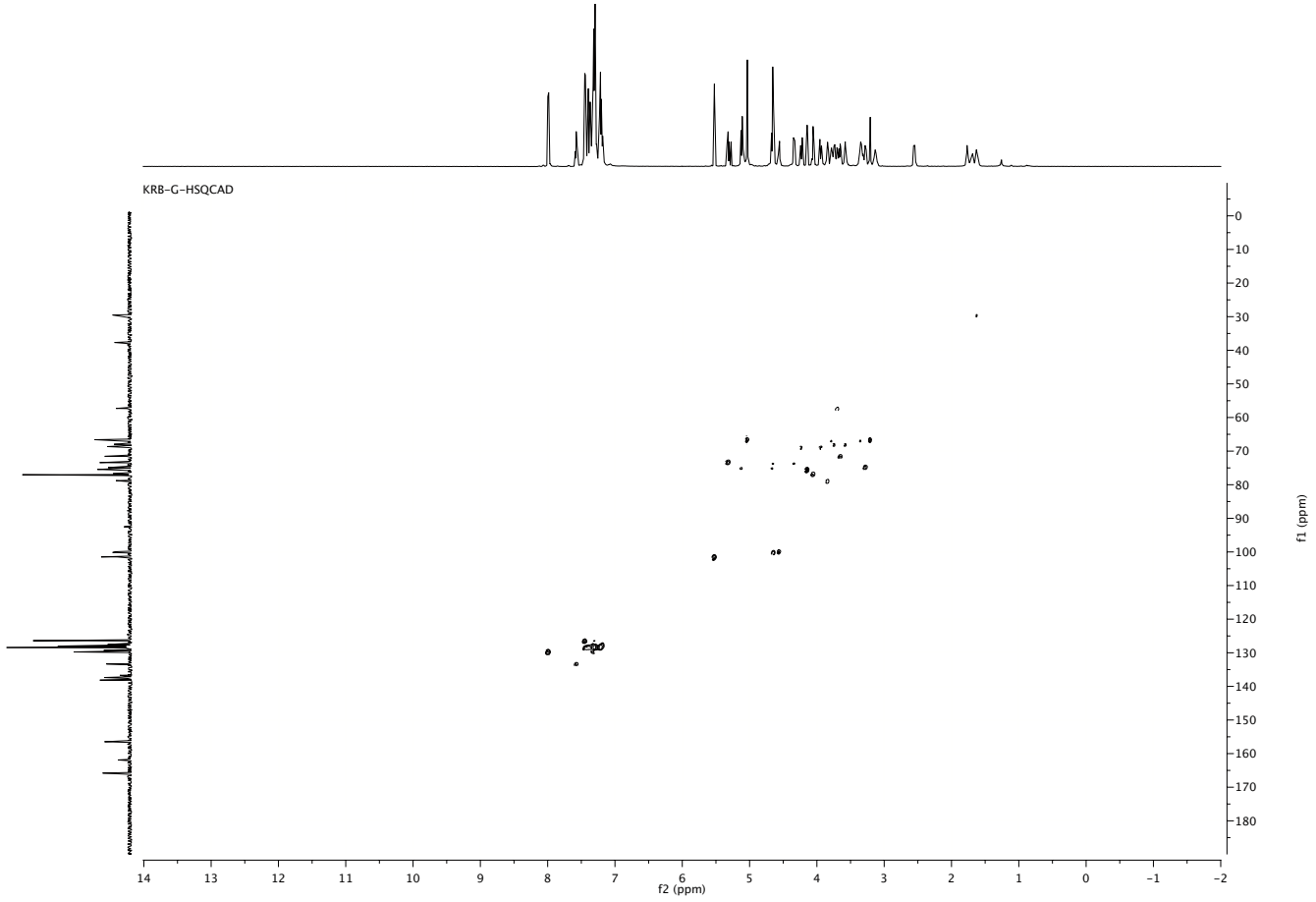
^{13}C NMR (150 MHz) ($CDCl_3$)



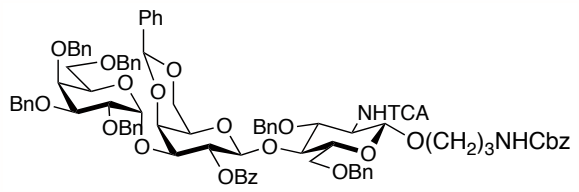
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



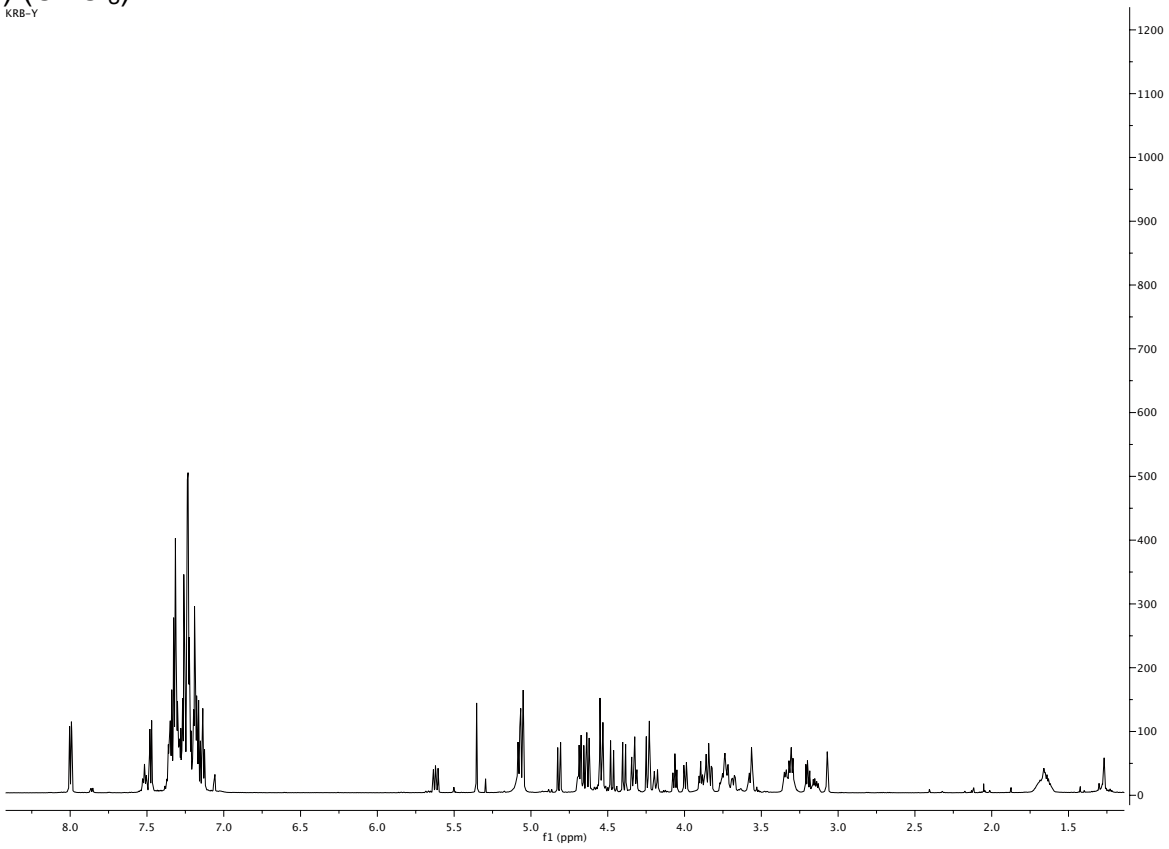
^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)



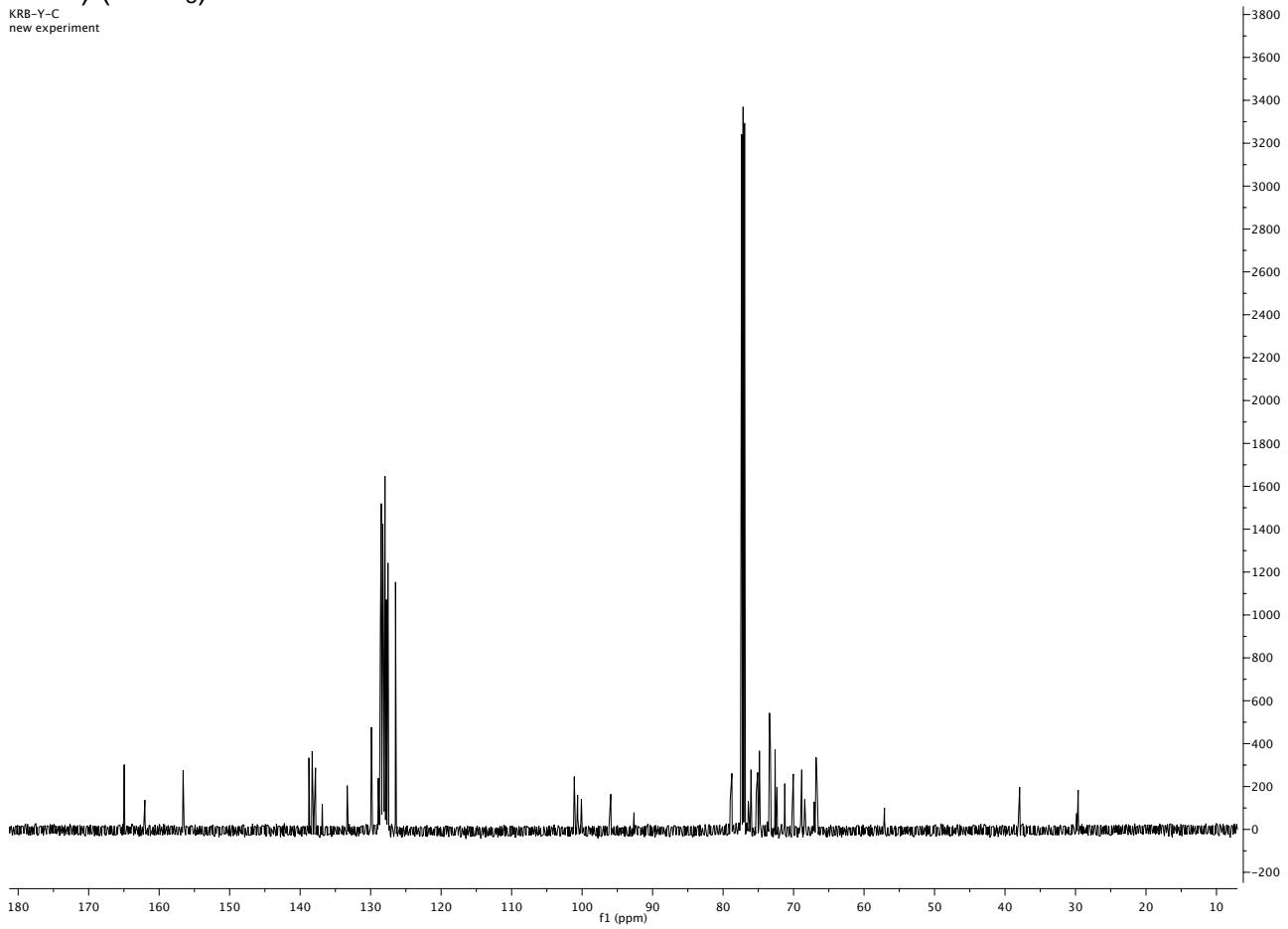
Compound 4.16



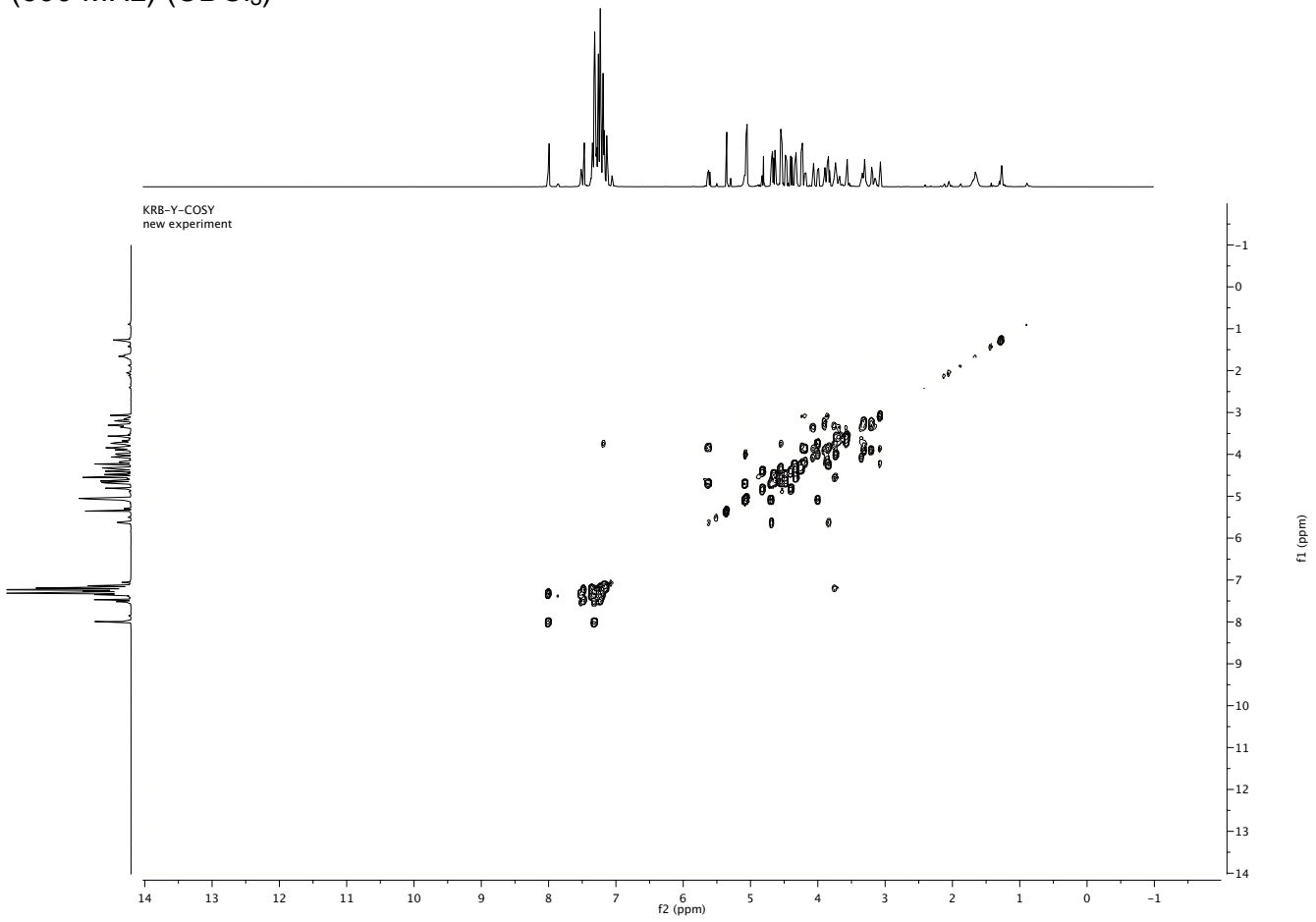
^{1}H NMR (600 MHz) ($CDCl_3$)



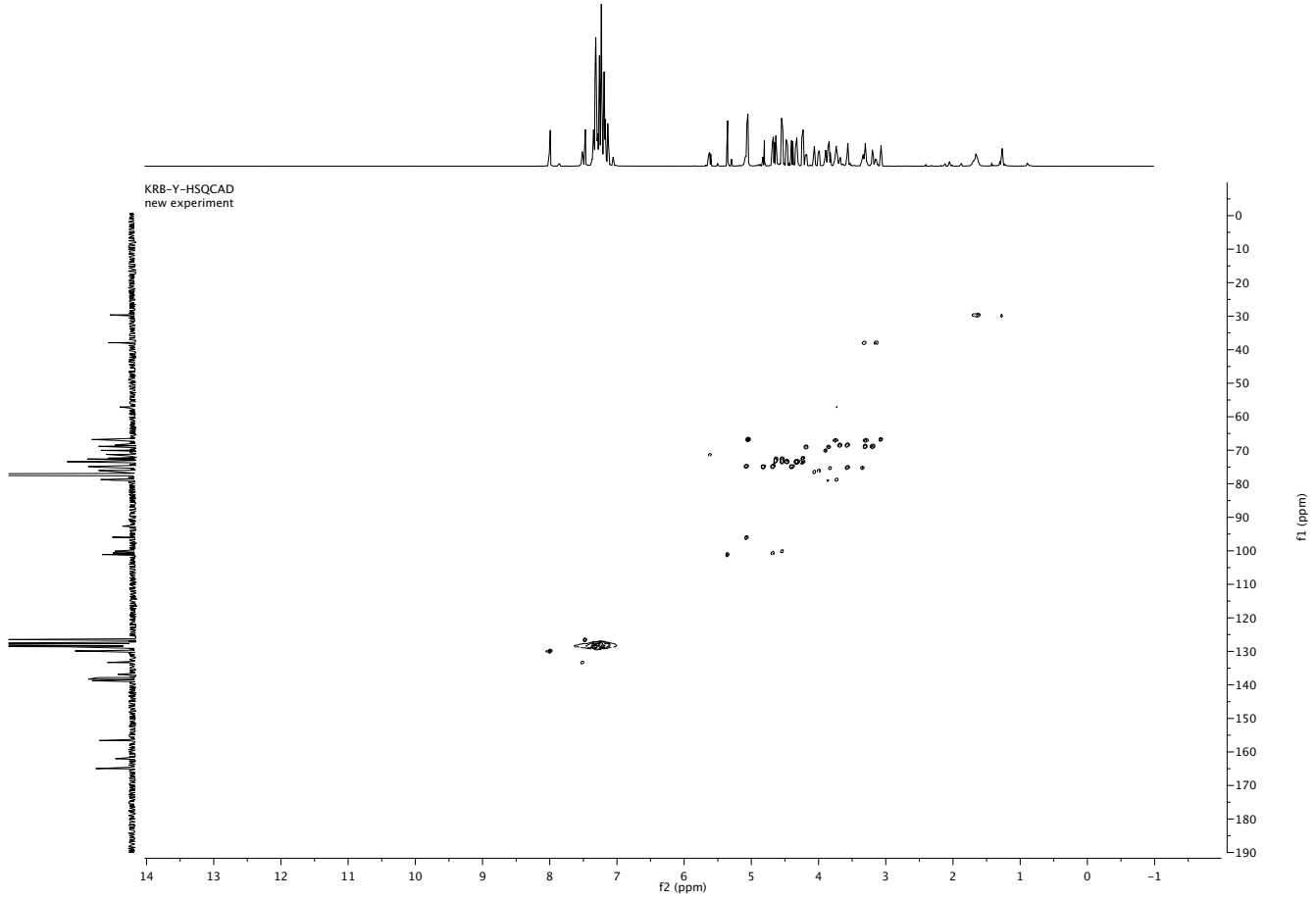
^{13}C NMR (150 MHz) ($CDCl_3$)



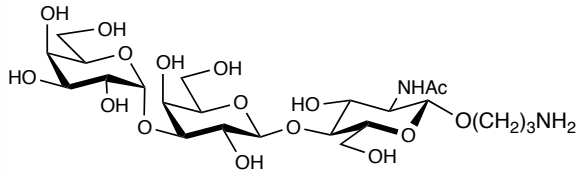
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)

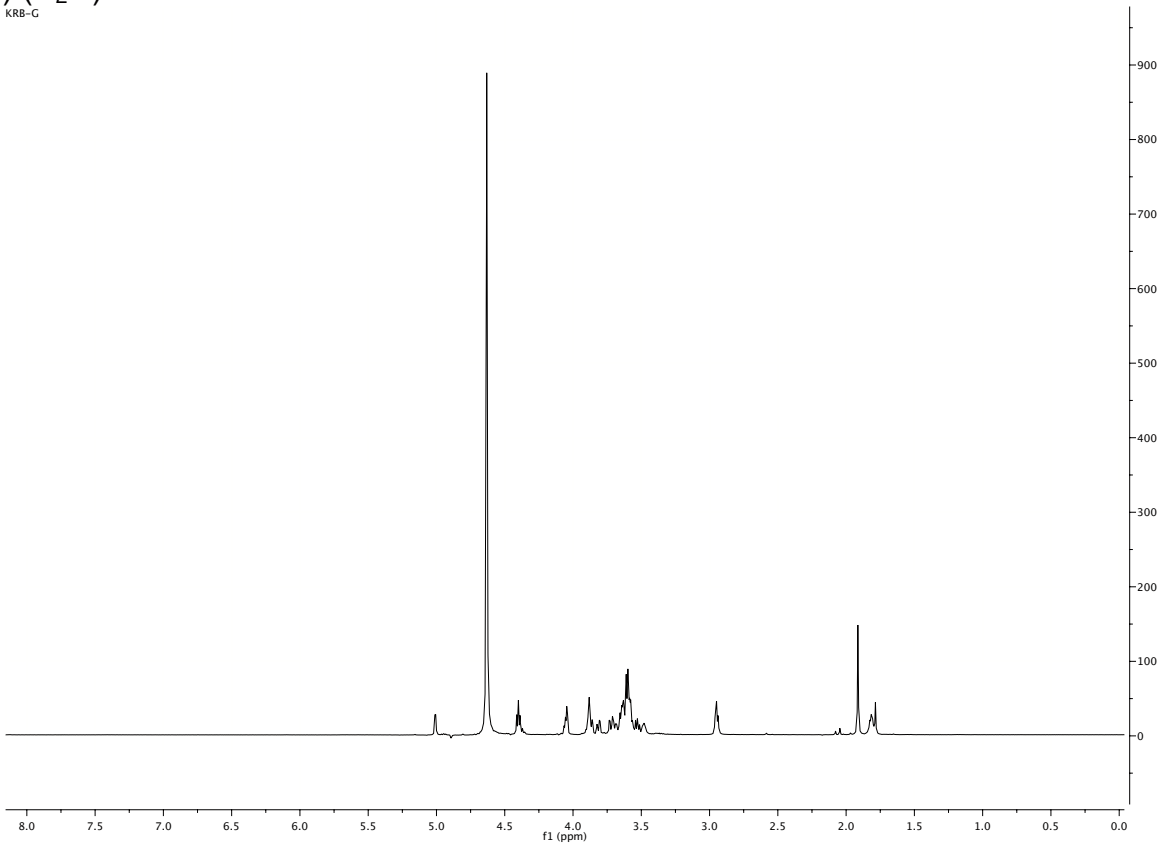


Compound 4.19



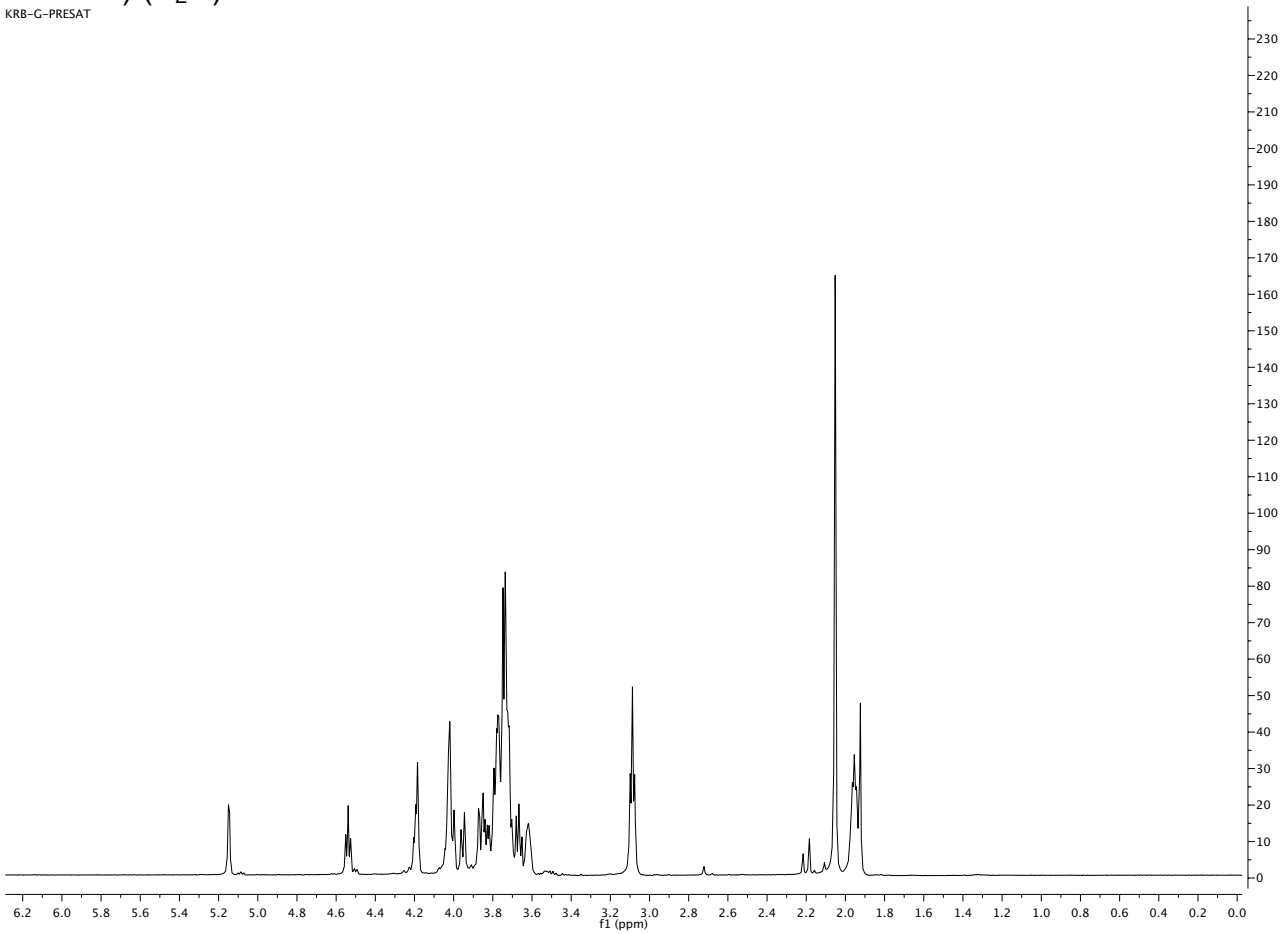
^{1}H NMR (600 MHz) (D_2O)

KRB-G



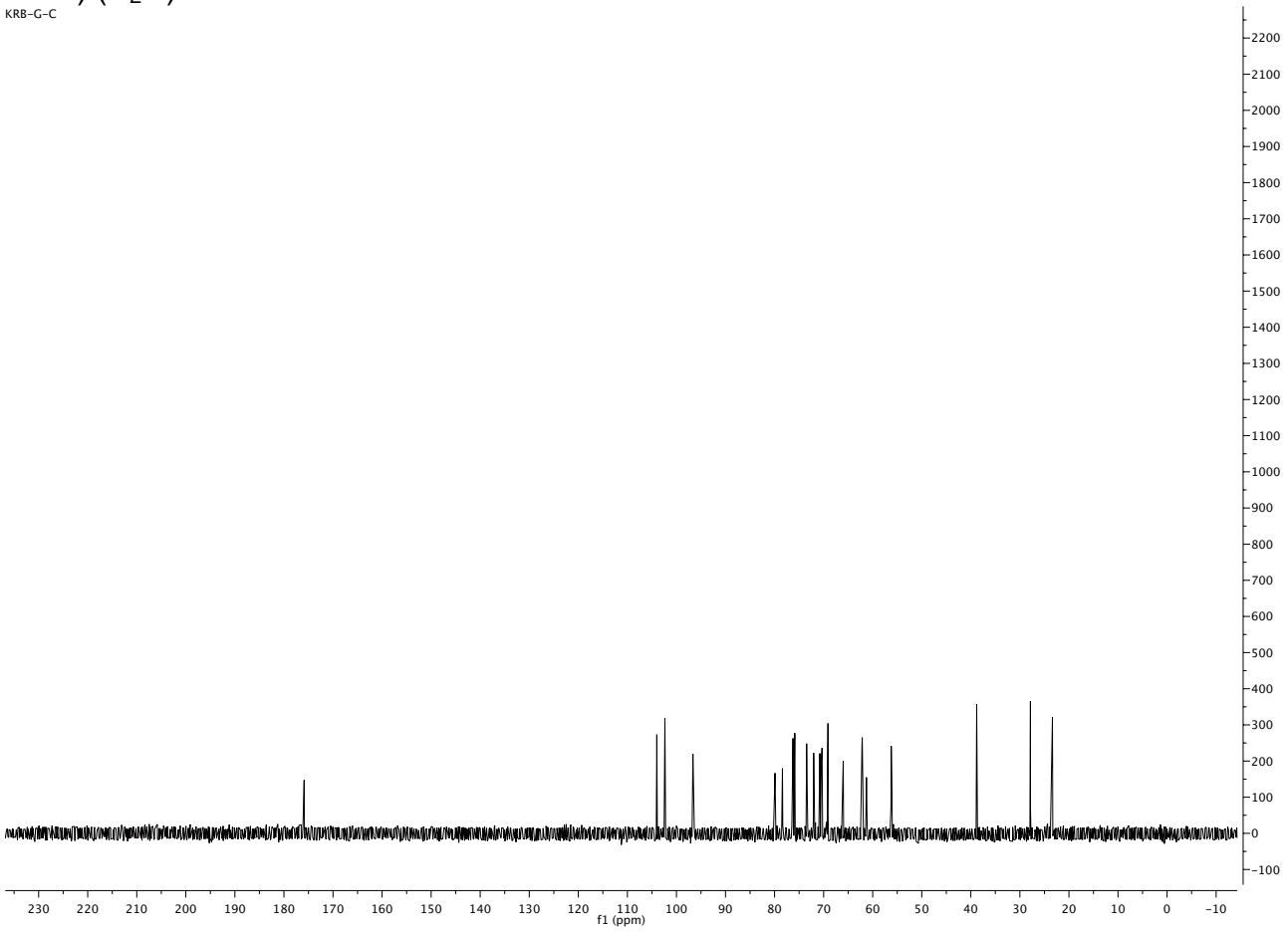
^{1}H PRESAT (600 MHz) (D_2O)

KRB-G-PRESAT

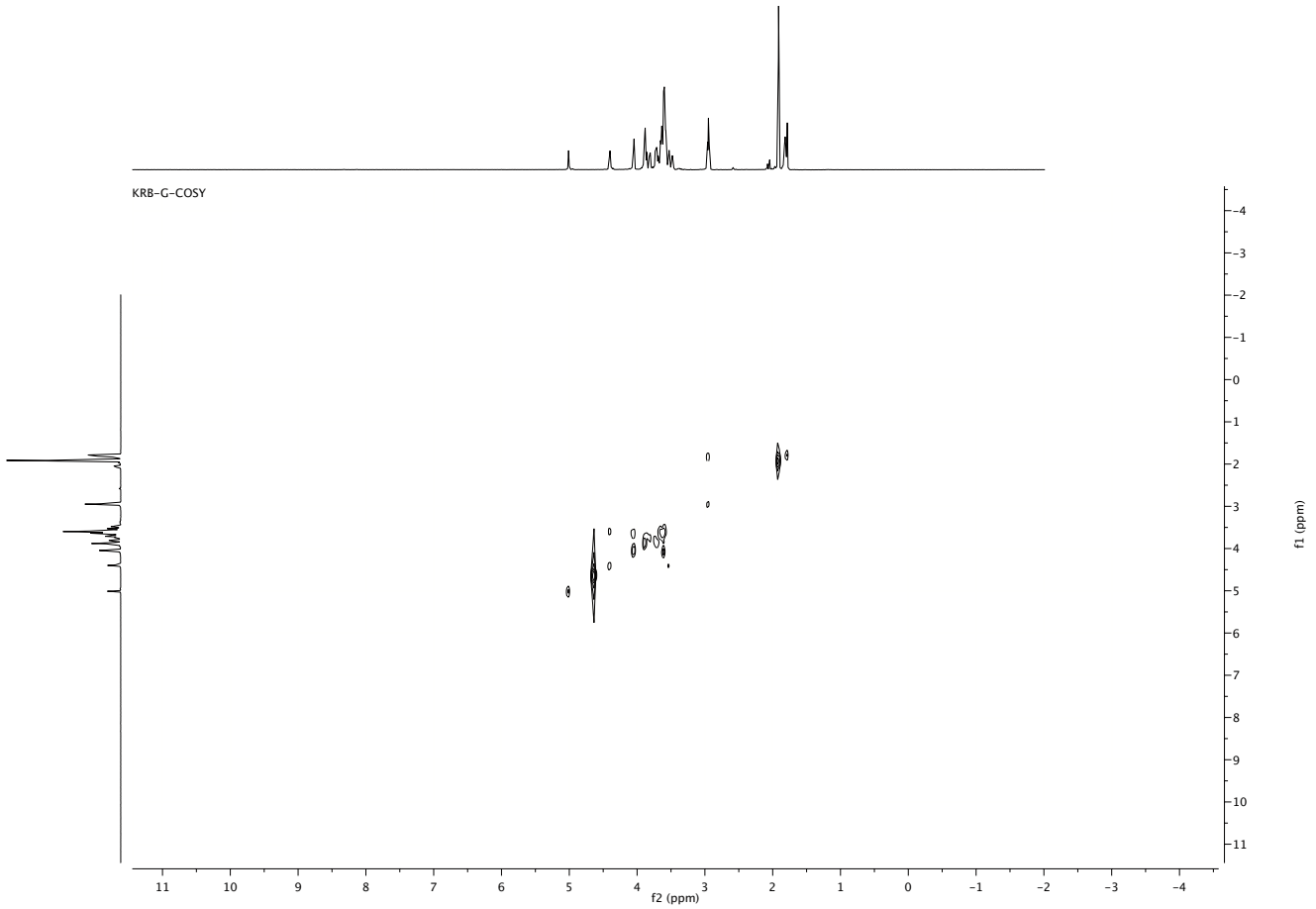


^{13}C NMR (150 MHz) (D_2O)

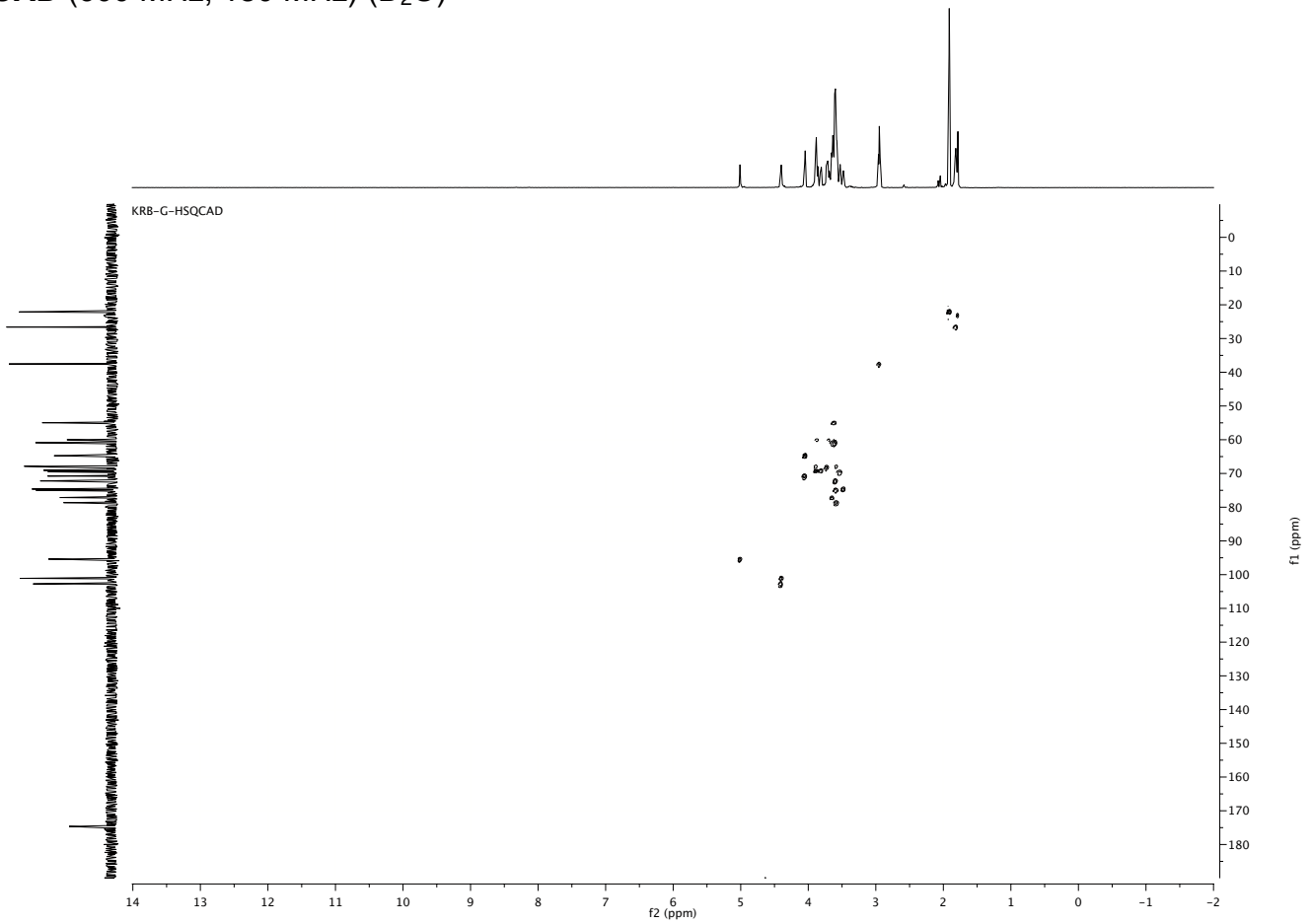
KRB-G-C



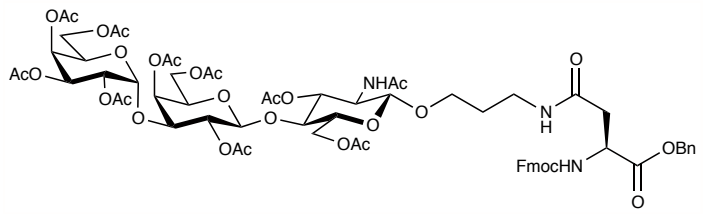
^{1}H - ^{1}H COSY (600 MHz) (D_2O)



^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) (D_2O)

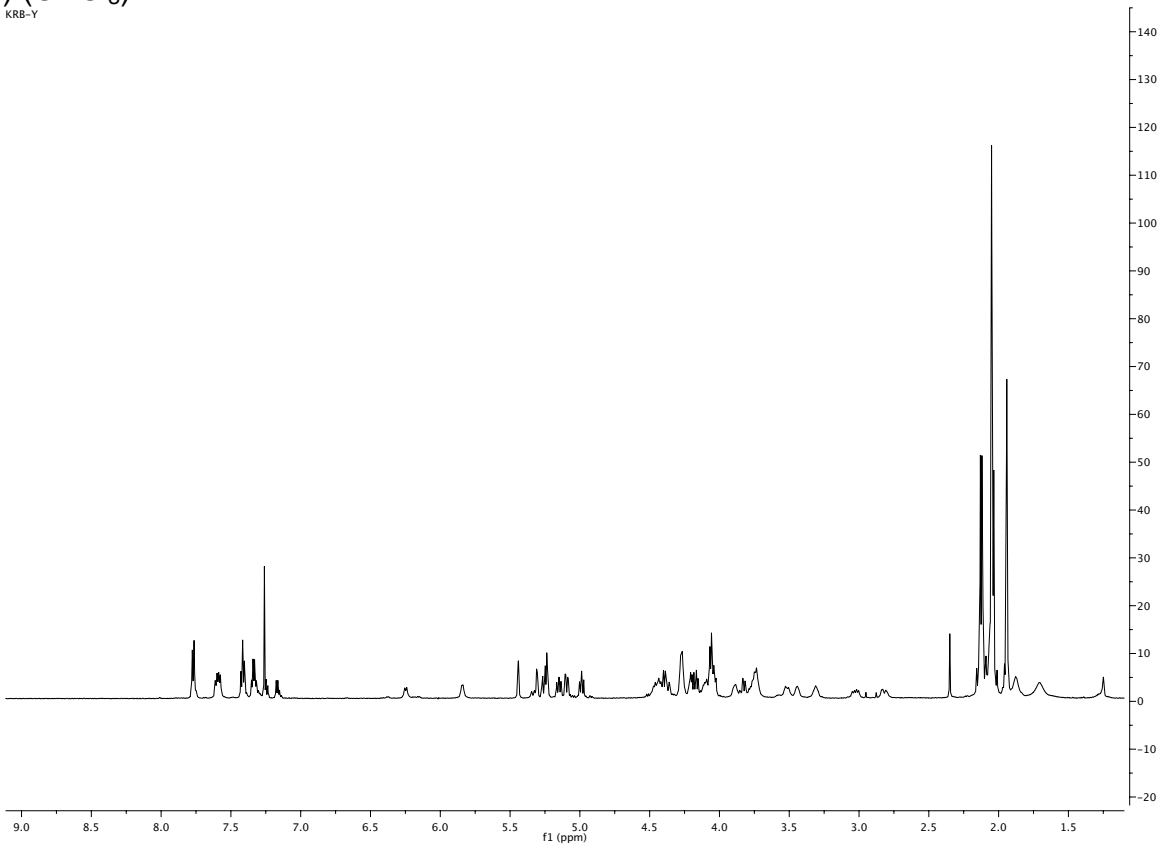


Compound 4.20

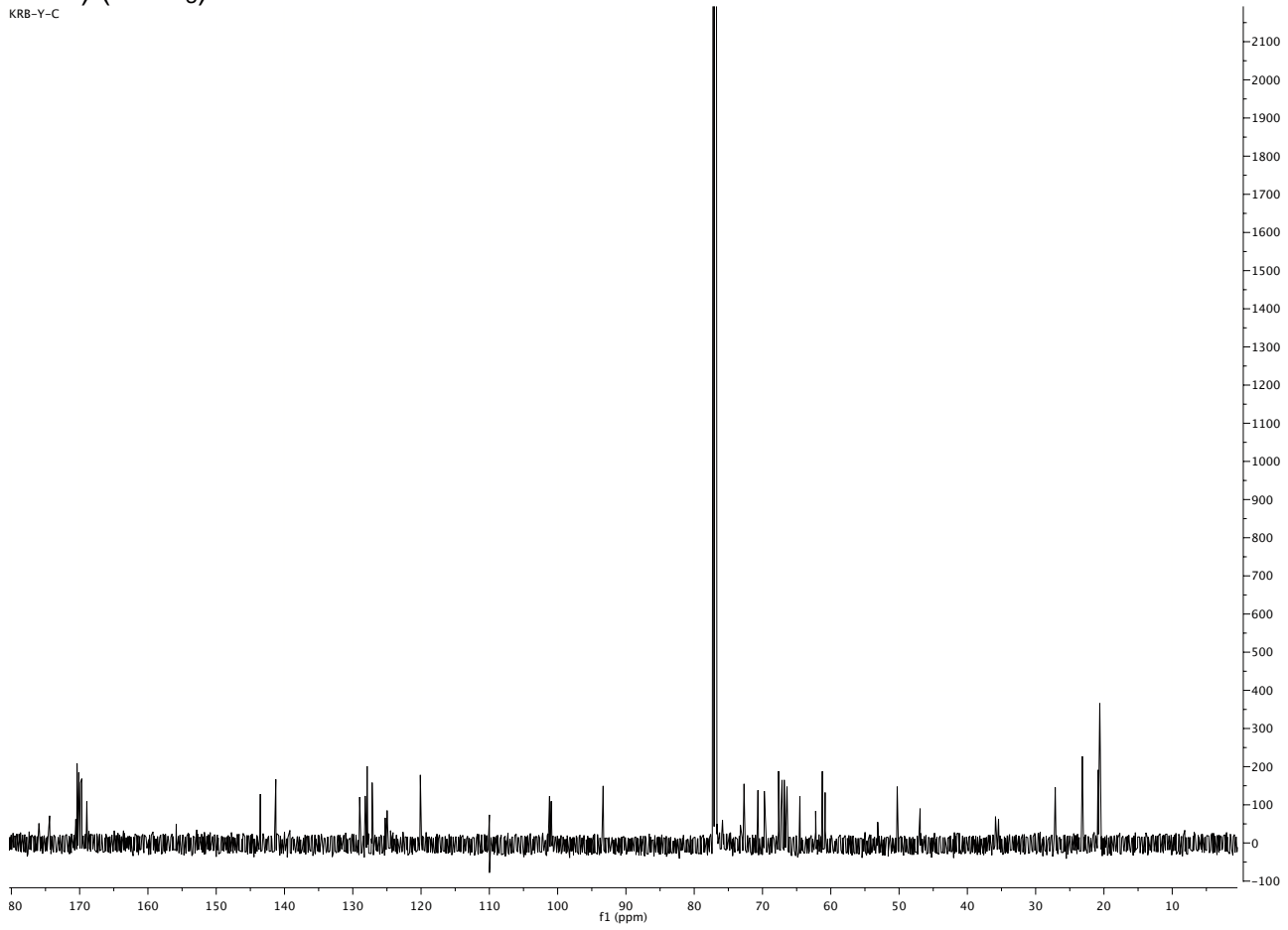


^{1}H NMR (600 MHz) ($CDCl_3$)

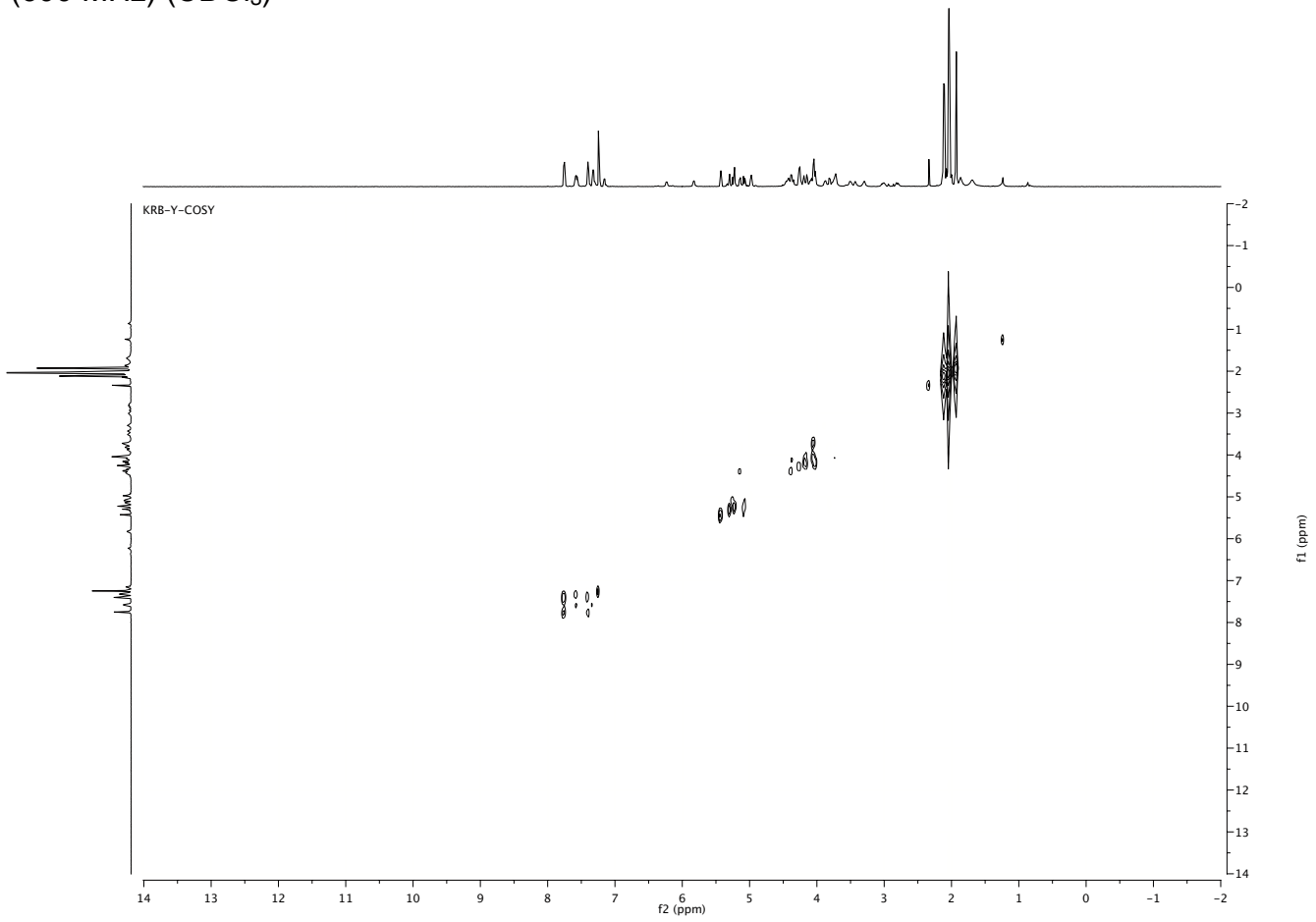
KRB-Y



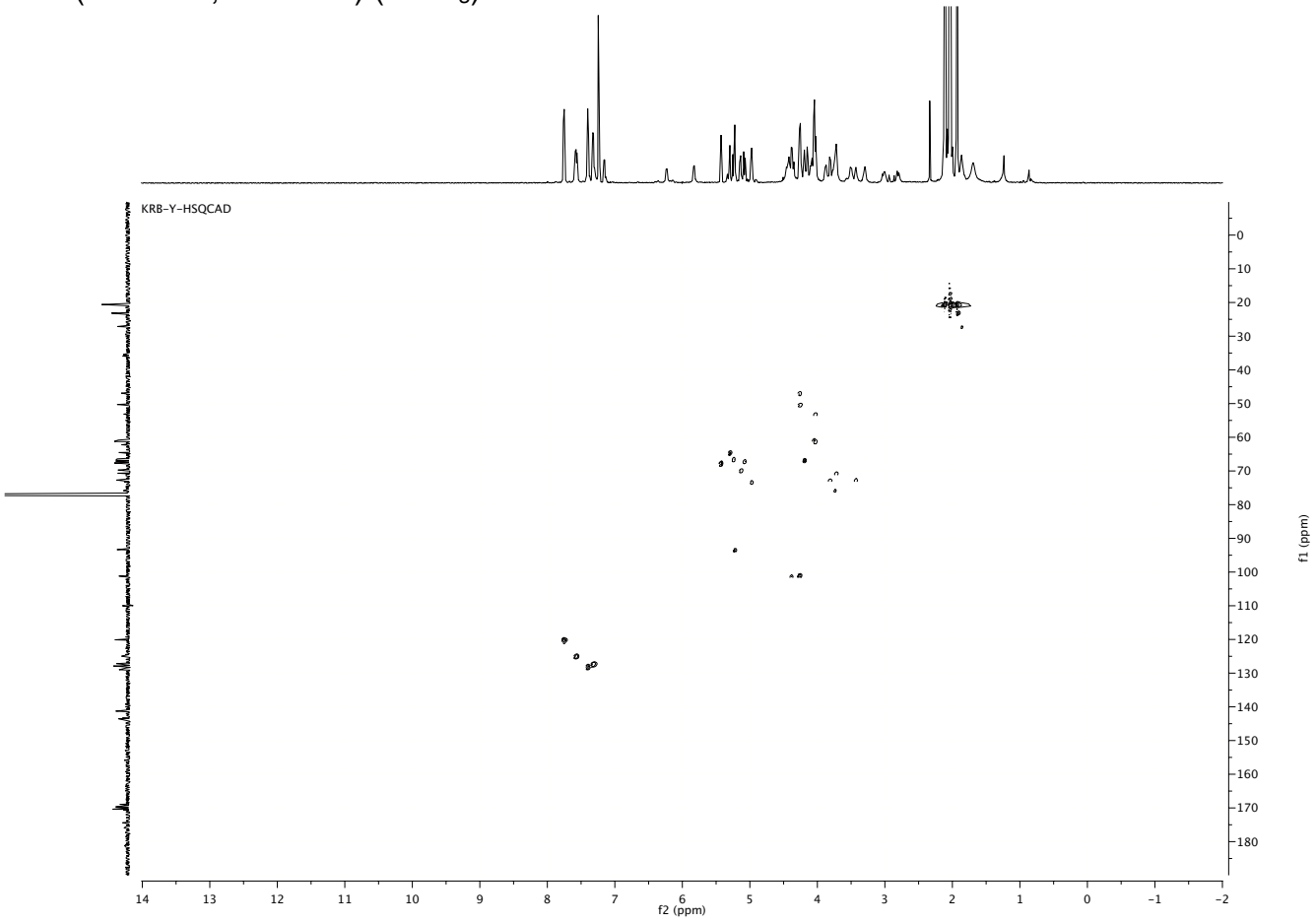
^{13}C NMR (150 MHz) ($CDCl_3$)



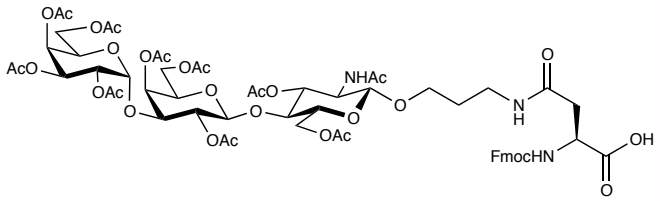
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



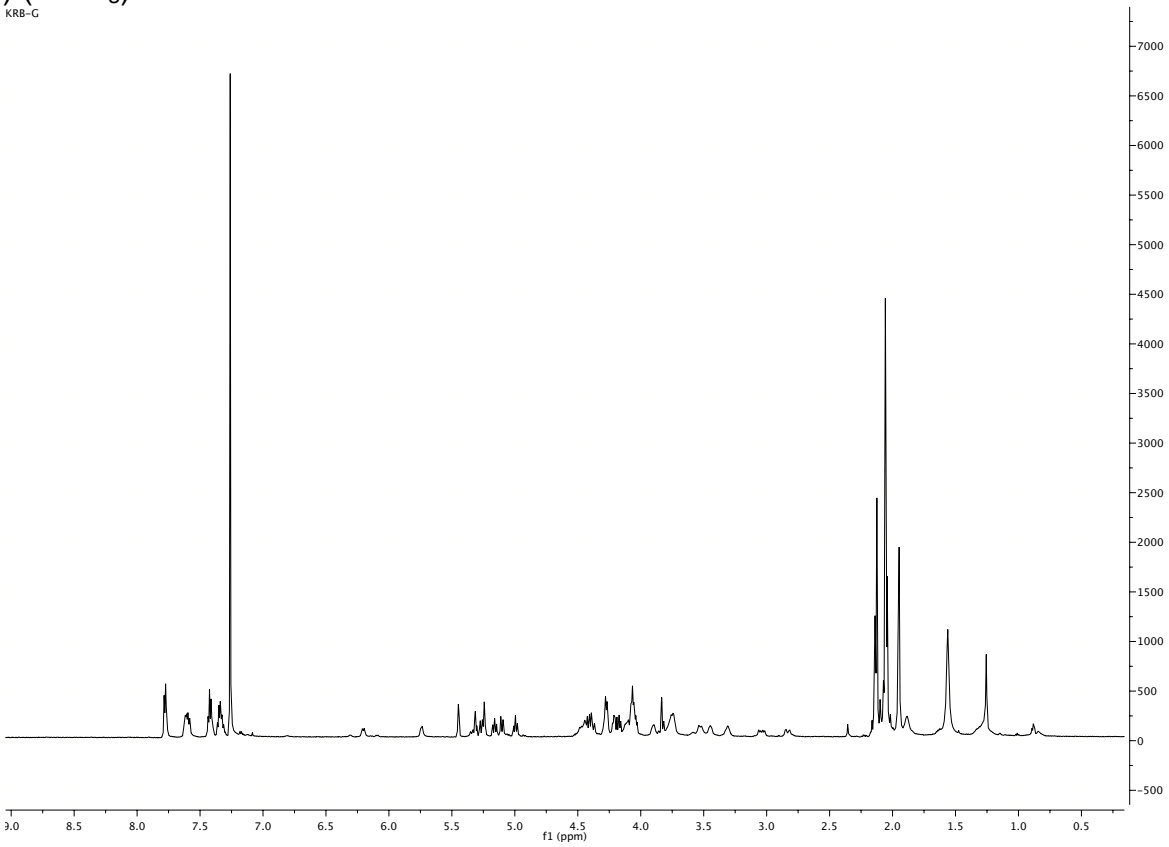
^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)



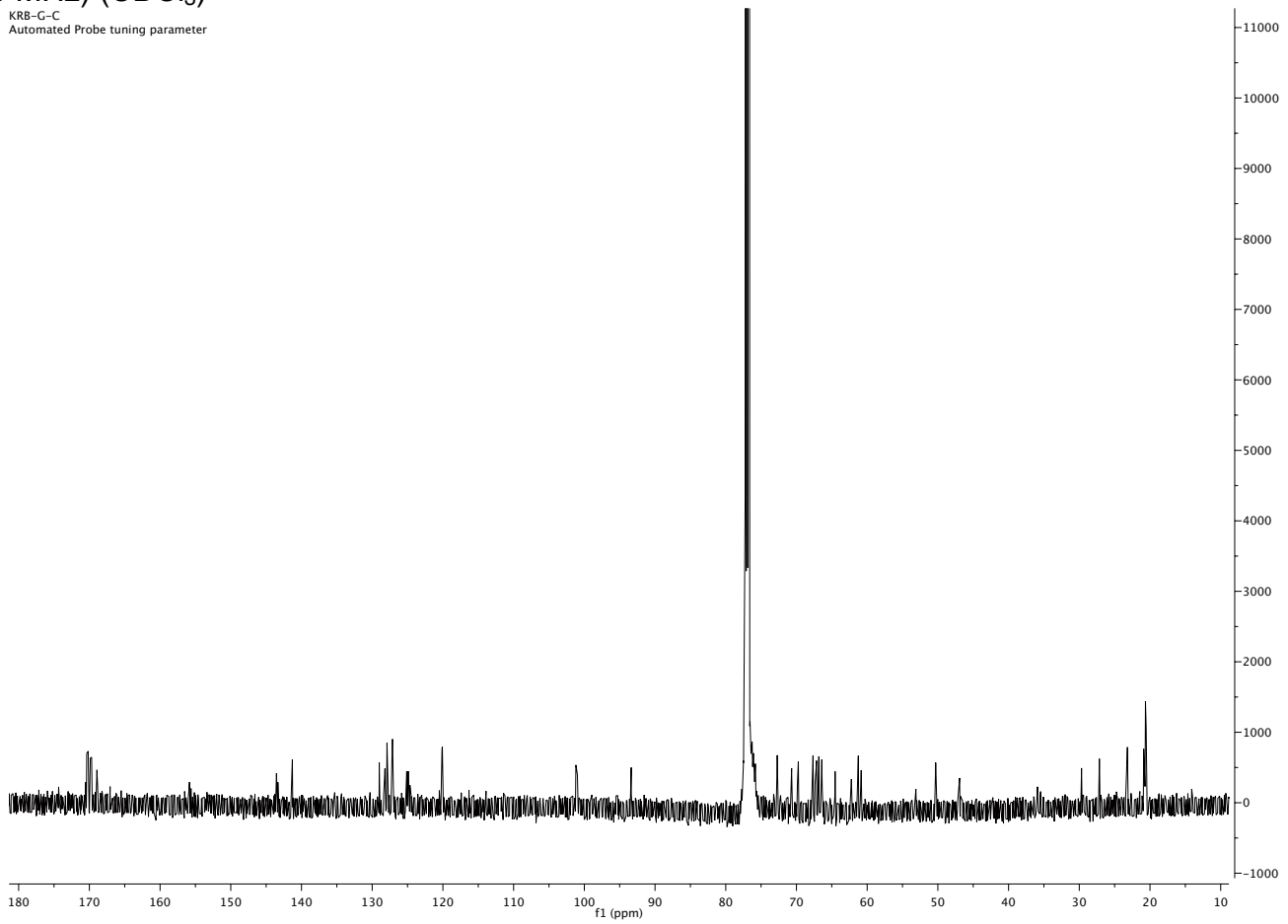
Compound 4.22



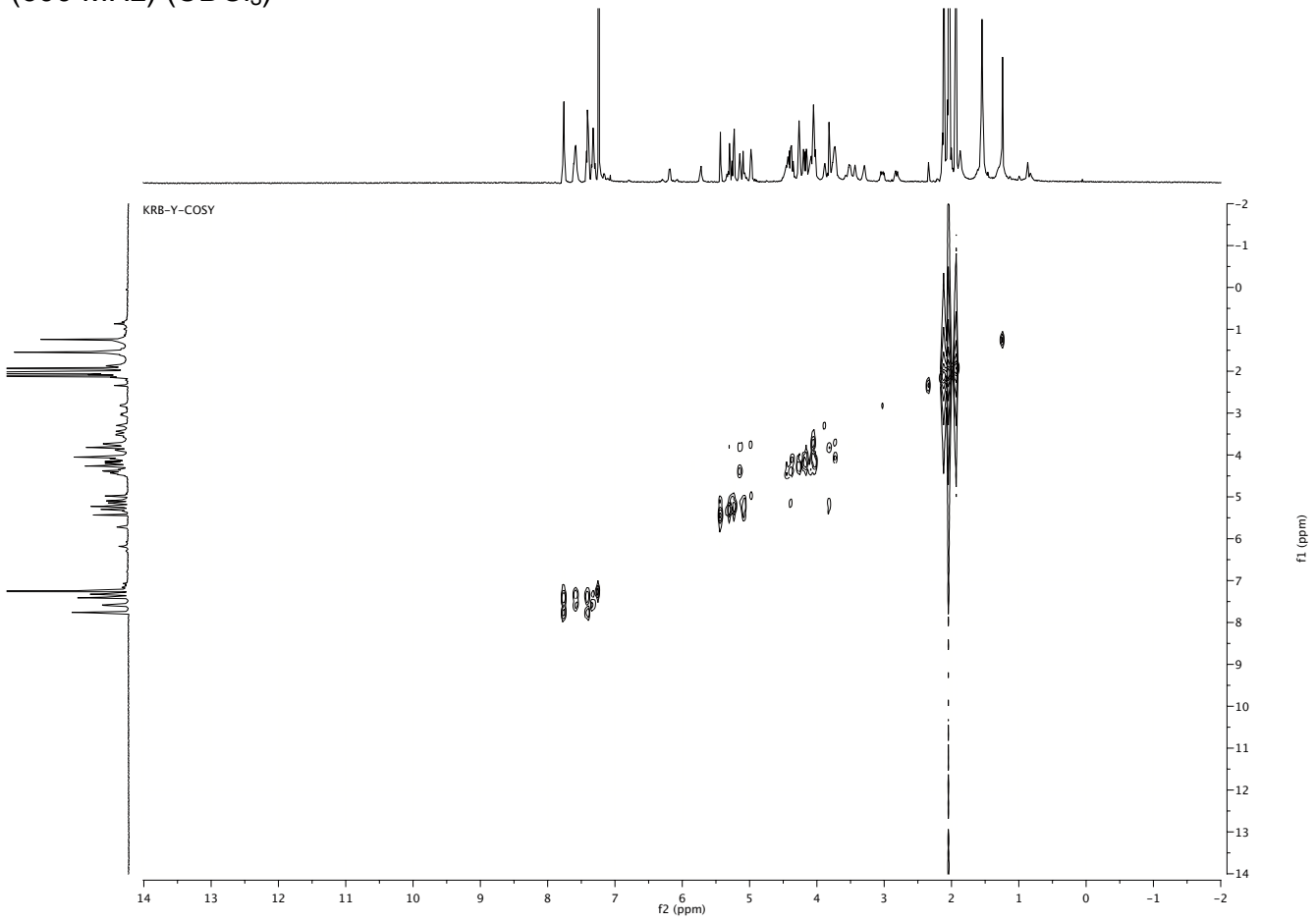
^{1}H NMR (600 MHz) ($CDCl_3$)



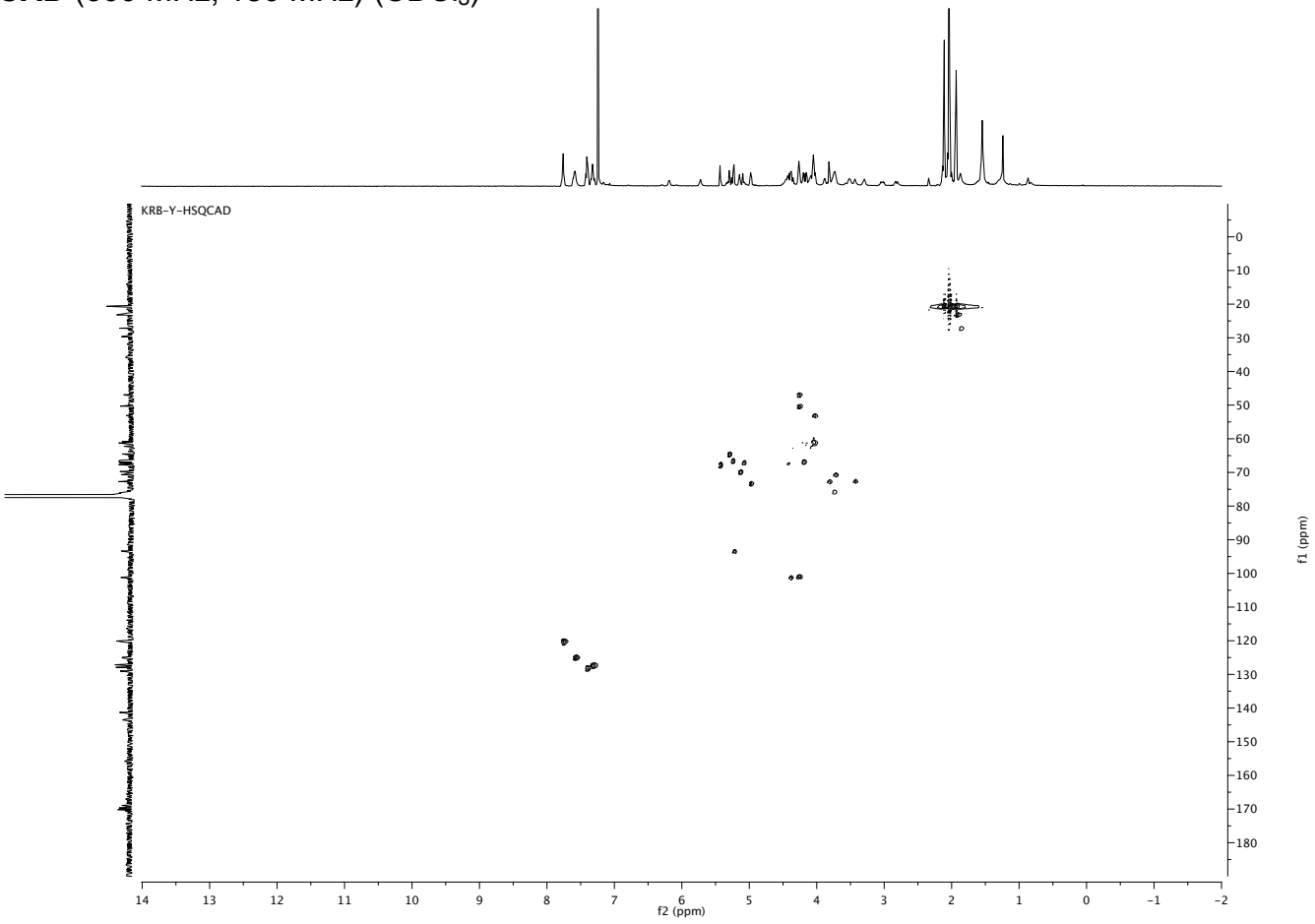
^{13}C NMR (150 MHz) ($CDCl_3$)



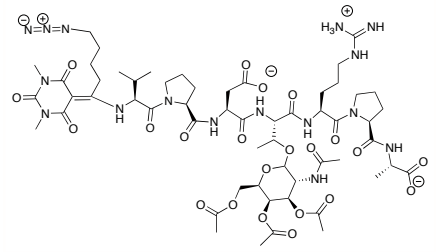
^{1}H - ^{1}H COSY (600 MHz) ($CDCl_3$)



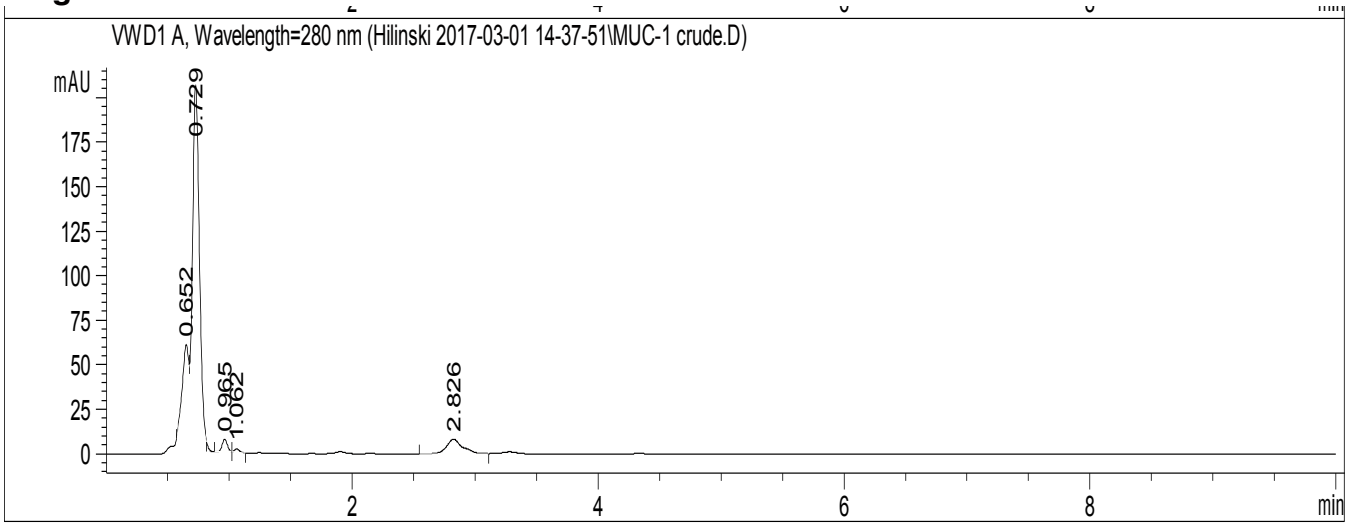
^{1}H – ^{13}C HSQCAD (600 MHz, 150 MHz) ($CDCl_3$)



Compound 4.31

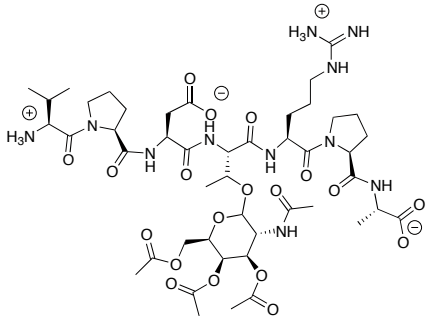


HPLC Chromatogram

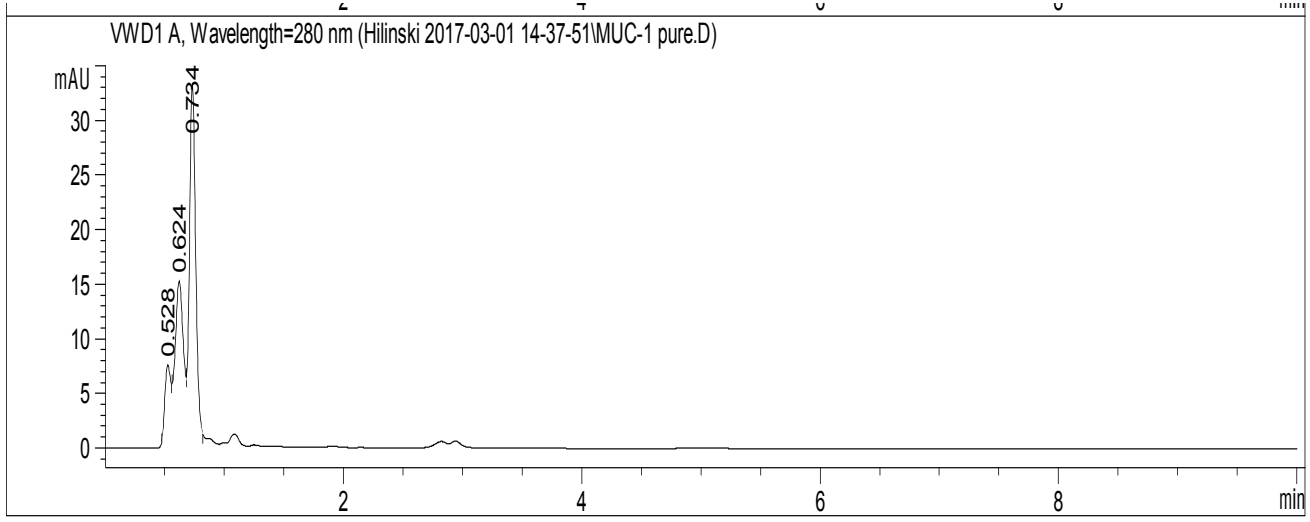


Gradient: 30%-905 MeCN in H_2O over 30 min

Compound 4.33



HPLC Chromatogram



Gradient: 30%-905 MeCN in H_2O over 30 min