Chromatography-free Solid Phase Oligosaccharide Synthesis

An Honors Thesis for the Department of Biology

Undertaken in the lab of Dr. Clay Bennett in the Department of Chemistry

John Cao

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Abstract

To fill a demand for synthetic oligosaccharides for biochemical study, new methods of synthesis must fill standard in both purity and efficiency. Eliminating the need for chromatography purification during synthesis lessens the manual work needed to synthesize each sequence and allows a greater degree of automation.

This thesis describes a new cap-and tag solid phase oligosaccharide synthesis. Completed sequences were tagged with an alkyne linker-tag and captured onto azide functionalized catch resin using "click" chemistry. Filtration separated these desired sequences from incomplete "truncated" sequences. A mannose monomer and a trimannose sequence were synthesized on the solid phase to demonstrate proof of concept.

This method allows synthesis of oligosaccharides without the use of chromatography and is designed for ease with automated parallel synthesis.

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Chapter 1

Introduction

1.1 Introduction to Carbohydrate Chemistry

Carbohydrates play a central part in biological function. They provide structure and protection to cells and tissue, mediate cellular recognition, and are important signaling pathway messengers. They are also responsible for disease mechanisms and provide epitopes for immune recognition [1].

The significance of carbohydrates lies in being one of the three main types of biopolymers found in living systems, alongside nucleic acids and proteins. Biopolymers play a fundamental role in nature by encoding biological information through their structure[1].

Our advancement in understanding and synthesis of proteins and nucleic acids has been impressive. Synthetic technology has given us faster and more efficient methods of genetic sequencing [2]. Synthetic nucleic acids are used as primers for PCR reactions and are utilized on arrays to detect the presence of specific genes. Synthetic proteins mimicking the functions of natural proteins [3] have been created and evaluated. Our understanding of sugars, however, has been comparatively limited. A major impediment has been the lack of sufficient methods for synthesis.

In the past three decades, a variety of technologies have been developed to aid in the synthesis of oligosaccharides. They have allowed the study of previously inaccessible structures. However, those sugars currently available for study still represent a small fraction of the population of potentially biologically relevant oligosaccharides [4]. The ability to efficiently synthesize and purify oligosaccharides has huge implications in drug

research and development, as well as insight into cell processes. This thesis will present a methodology that can be used for large scale parallel synthesis of oligosaccharides.

1.2 Biological Roles of Oligosaccharides

The biological function of glycosylation and roles of oligosaccharides, like that of other biological macromolecules like peptides, are diverse and complex. Oligosaccharides are commonly found in the body linked to proteins, lipids, and small molecules. The biological roles of oligosaccharides can be divided into two general groups: structure and recognition [5]. The functions of structure and recognition frequently overlap for many biologically active glycans [6]. The latter of the functions, recognition, can be further classified into endogenous (self) recognition and exogenous (non-self) recognition. Glycans cover the surfaces of most cells: they present a cell's "identity" to the outside environment. Trafficking of lysosomal enzymes to specific cells (self) as well as immune system recognition of bacteria and viruses (non-self) are both dependant on oligosaccharides. Oligosaccharides also play key roles in biosynthesis and function of proteins [7,8,9], maintaining cellular structure [6] and binding of sperm to egg [10]. Furthermore, cancer cells display different patters of glycosylation compared to normal cells [1]. Advances in carbohydrate chemistry would further our understanding of cancer and in identification of tumor markers.

One of the earliest clinical discoveries on the importance of glycans is the human ABO blood groups. The surfaces of human erythrocytes are covered with oligosaccharide chains that project into the bloodstream. These chains are the basis of the ABO blood groups. If an erythrocyte carries a surface oligosaccharide profile different from that of one's own blood cells, the immune system will attack the foreign erythrocyte. The necessity of matching blood groups for transfusions was realized through devastating

immune rejection and fatalities in early blood recipients, While Landsteiner [11] realized by 1931 that it was carbohydrates on blood cells that are the basis of the ABO classification, it wasn't until 1978 that the actual glycan structures were worked out.

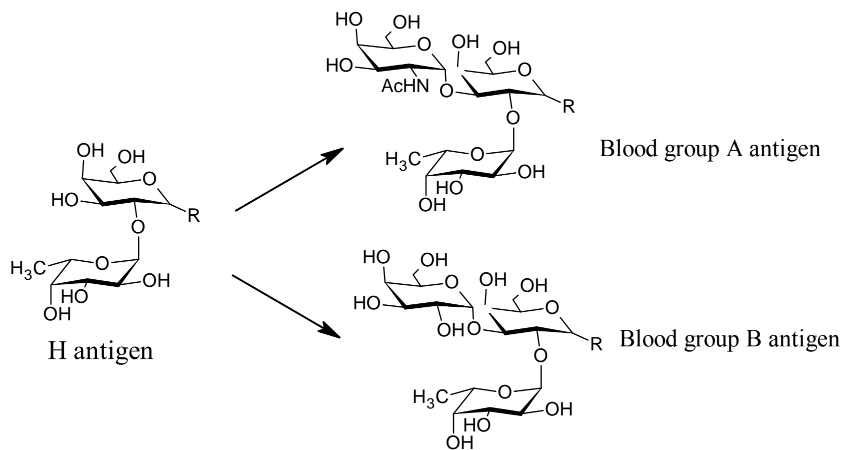


Figure 1. Human blood group antigens

Erythrocyte cell surface antigens are composed of a core H antigen, comprising of fucose (Fuc) and galactose (Gal) residues [12]. In individuals with the A blood group, the enzyme N-acetylaminogalactosyltransferase (GTA) adds a *N*-acetylgalactosamine unit to the end of the core to form the blood group A antigen expressed on the erythrocyte surface. Individuals with the B blood group possess the alleles for a mutated form of the glycaltransferase enzyme (GTB) that instead transfers a galactose unit to the end of the core, producing on the erythrocyte surface the blood type B antigen. They will possess antibodies against the *N*-acetylgalactosamine- terminal A surface antigen, but not the galactose- terminal B antigen, and vice versa for those with blood group A. Individuals

possessing both GTA and GTB alleles will produce both antigens on their blood cells, and thus lack antibodies against either. Loss of function mutation of the erythrocyte surface glycotransferase enzyme results in the unmodified H-antigen core being expressed. These blood group O individuals would have in their blood antibodies against both the A and B antigens, while the other blood groups would not produce antibodies against the H antigen because it is the precursor glycan. In the interplay of the ABO blood groups, we can see the importance of oligosaccharide structure in the body.

1.3 Uses of Synthetic Oligosaccharides

While it is easy to see the biological importance of carbohydrates, very little is understood about the molecular basis of their function. Such knowledge is critical for developing glycan-based vaccines as well as developing new therapeutics. Synthetic oligosaccharides provide us with a basis in which to study molecular interactions and screen for possible therapies. Due to the roles carbohydrates have in the body and in disease, chemical syntheses of oligosaccharides have been entwined with medicine. Chemical synthesis allows researchers to design specific structures, making invaluable to studying of molecular pathways and drug discovery.

An example of how synthesis can be utilized to elucidate the structure-activity relationships of clinically relevant biomolecules is the story of heparin. Heparin was discovered in 1916 by Mclean to be a powerful anticoagulant [13]. A heavily sulfonated glycosaminoglycan, heparin inhibits blood coagulation by binding to and activating the protease inhibitor antithrombin III, which in turn blocks thrombin and factor Xa in the

blood coagulation pathway. The drug is naturally isolated as a mixture of polysaccharides of different lengths averaging a molecular weight of 15 kDa [14], and is associated with a plethora of side effects such as thrombocytopenia, osteoporosis, bleeding, and allergic reactions[15]. Purified from the heparin mixture, fractions of low molecular weight heparins (LMWH) averaging a 4.5 kDa molecular weight were found to have the effective anticoagulant effects of unfractionated heparin with less of the undesirable side effects [123]. Further studies revealed that a specific pentasaccharide sequence of heparin is responsible for binding to and activating antithrombin[16]. The successful chemical synthesis of this pentasaccharide have allowed elucidation of the sequence's structural functions and specific interactions with antithrombin, as well as opening the door to the creation of synthetic heparin variants [17]. Fondaparinux (Arixtra), a synthetic analogue of the active heparin pentasaccharide, have been marketed for treatment of deep vein thrombosis and pulmonary embolism, and has an extended half-life of 17 hours compared to heparin's 1 hour [18]. Effective synthetic routes to heparin analogs have permitted the construction of analogs with improved pharmacokinetic profiles. For example, Idraparinux, another synthetic analogue of the heparin pentasaccharide, is under clinical trials with a half-life of 80 hours [19].

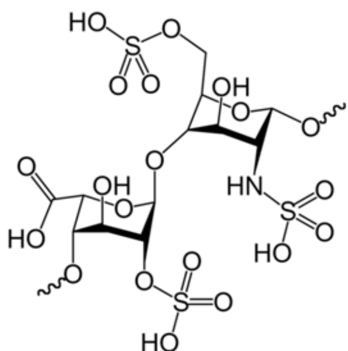


Figure 2: A subunit of heparin

Much of the infectivity and pathogenicity of viruses, bacteria, and parasites are mediated by glycan interactions. Entry of viruses into cells requires carbohydrate-protein interactions [5]. Furthermore, infected cells often have altered patterns of oligosaccharide expression [20].Access to synthetic oligosaccharide structures allows the development of carbohydrate based vaccines against infectious disease. The protozoan parasite responsible for malaria, P. falciparum, expresses surface glycosylphosphatidylinositols responsible for severe symptoms associated with malarial infection [21]. This glycolipid toxin activates a powerful inflammatory response, including endothelial activation and the release of a high level of TNF-a and IL-2 from macrophages [22, 23]. The inflammatory response triggered by GPI has been linked with lethality in malaria infections [24]. A non-toxic chemically synthesized GPI conjugated to protein has been successfully shown to induce anti-GPI antibodies in mice. The immunized mice, when challenged with malarial infection, showed increase survival rate (25% mortality vs. 100% for control nice). The anti-GPI antibodies induced by the synthetic oligosaccharide neutralized parasite toxicity and prevented the pulmonary edema, blood acidosis, and cerebral malaria normally associated with infection [25].

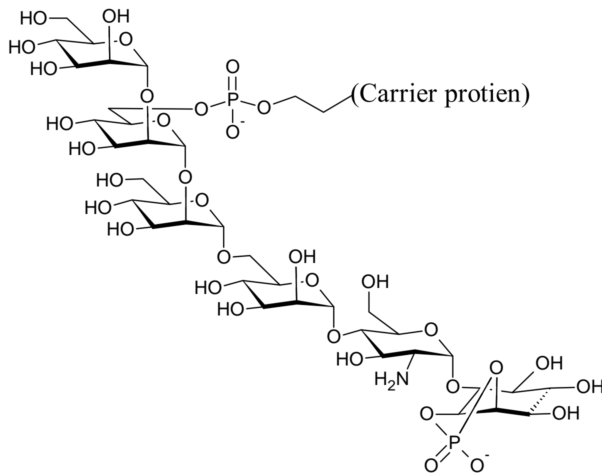


Figure 3. Synthetic GPI anchor used to raise antibodies against malarial GPI toxin.

gp120 is a glycoprotein expressed on the surface of the HIV envelope that binds to, and in a complex with gp41, allows the virus entry into CD4 lymphocytes [26]. gp120 is covered by complex oligosaccharide chains that confound detection by the immune system- 50% of the 120kDa weight of this glycoprotein is comprised of N-linked carbohydrates. The monoclonal antibody 2G12 was been shown to respond to an oligomannose epitope on gp120 (Sanders) and neutralize HIV-1 in vitro [27, 28]. Further study on the specificity of 2G12 shows that the antibody binds to α-(1→2)-trimannose arm of a Man9GlcNAc2 unit [29]. Synthetic oligosaccharide immunogens containing this 2G12 epitope could elicit production of potential HIV neutralizing antibodies in patients.

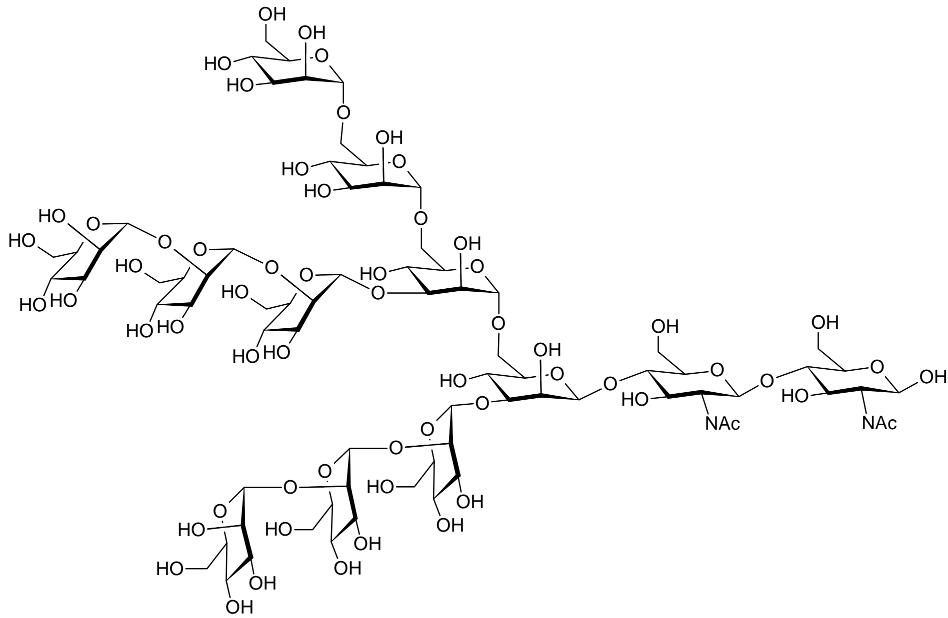


Figure 4. HIV gp120 high mannose

1.4 Synthetic Strategies

Given the role of oligosaccharides in investigating biological processes and therapies, there is a great demand for carbohydrates for biochemical study. The study of carbohydrates has unfortunately been hindered by difficulties in obtaining specific and structurally defined material [30]. Unlike nucleic acids and proteins, oligosaccharide synthesis in living systems is not template controlled. As a consequence, natural sources often produce carbohydrates as complex mixtures which are difficult, if not impossible, to separate on a preparative scale [31]. In the rare examples where a homogeneous carbohydrate has been isolated from a biological sample, the process is very time-consuming and tedious. For example, the extraction of sialylglycans from hen eggs required fresh eggs less than 12 hours old. 115 eggs were required to extract materials of 10g, and the extraction involved six chromatography procedures [32]. As a consequence, synthesis is often the only avenue available for the production of homogeneous oligosaccharides.

Formation of a glycosidic linkage by chemical glycosylation involves the coupling of a glycosyl donor sugar to a glycosyl acceptor. The glycosyl donor bears a suitable leaving group at the 1 position. Activation of the donor eliminates the leaving group and forms an electrophilic oxocarbenium intermediate, which is then attacked by the nucleophilic glycosyl acceptor to form a glycosidic linkage.

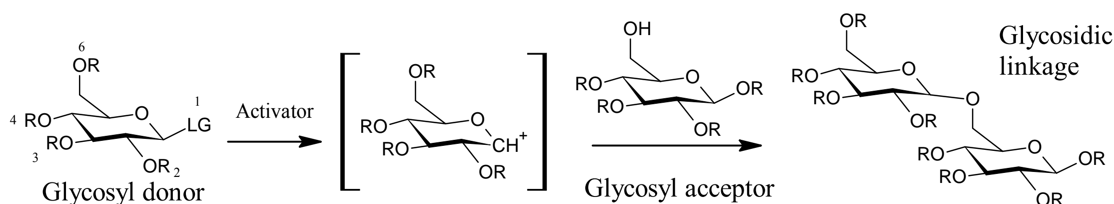


Figure 5. Chemical formation of glycosidic linkage

The synthesis of carbohydrates is an extremely challenging endeavor. Whereas nucleic acids and amino acids have two possible directions for chain elongation, each carbohydrate monomer contain up to 5 different points for glycosylation. This allows branched structures, adding a level of complexity not commonly seen in oligopeptides and oligonucleotides

	Length	Composition	Number of possible configurations	
			Peptide, Nucleic acid	Carbohydrate
Homogeneous sequence	monomer	A1	1	1
	dimer	A2	1	11
	trimer	A3	1	120
	tetramer	A4	1	1424
	pentamer	A5	1	17,872
Heterogeneous sequence	monomer	A	1	1
	dimer	AB	2	20
	trimer	ABC	6	720
	tetramer	ABCD	24	34,560
	pentamer	ABCDE	120	2,144,640

Table 1. Isomeric possibilities of biopolymers [33]

Additionally, every glycosidic linkage can exist as one of two possible diastereomeric forms. After activation of the glycosyl donor, the glycosyl acceptor can attack from two possible positions. As shown in the figure below, glycosylation creates a new stereogenic center, and thus such linkages can exist in either alpha or beta anomers. This complexity is also not present in the synthesis of nucleic acids or amino acids.

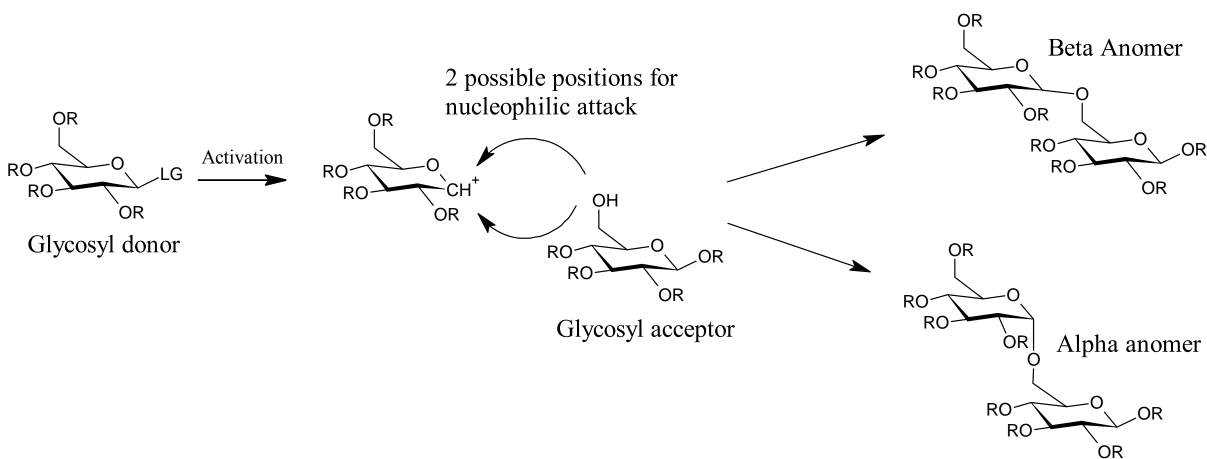


Figure 6. Formation of new stereogenic center during glycosylation

Figure 7. Example of two possible glycosidic linkages

The result of these properties is that compared to peptide and nucleic acid synthesis, oligosaccharide synthesis is often a very involved process. To address these issues, a

number of chemical and chemoenzymatic approaches to carbohydrate synthesis have been developed.

Enzymatic Synthesis

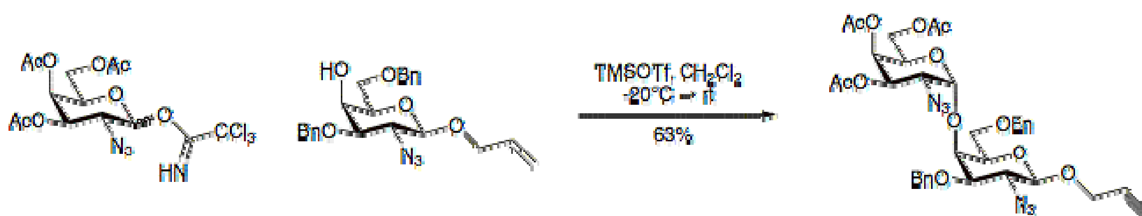
In vivo, glycosidic linkages are catalyzed by specialized enzymes. The use of these natural enzymes has allowed oligosaccharide synthesis with unprotected building blocks and with high specificity. Glycotransferases catalyze regio and stereospecific synthesis of oligosaccharide [34]. However, the use of glycotransferases is limited due to limited availability and high cost [35].Glycosidases can also be used for enzymatic synthesis, and are more available than glycotransferases. By altering the reaction equilibrium so that condensation rather than hydrolysis occurs, glycosidases can be used to create glycosidic linkages. However, the product acts as a substrate for the enzyme, and yields are low. More recently glycosynthases, mutant glycosidases that efficiently synthesizes glycosidic bonds without breaking them [35] have been used. A second major limitation of enzymatic synthesis is due to its substrate specificity. In enzymatic synthesis, the ability to use novel saccharides with unusual or unnatural sugars as substrates is limited [37].

Both extraction from natural sources and enzymatic synthesis are limited to the carbohydrates occurring in nature. However, biochemical study and drug development often requires the synthesis of glycans and glycosidic linkages not found naturally.

Chemical synthesis is more flexible than extraction from natural sources or enzymatic synthesis, and allows access to a wide range of structures. Protecting groups on the monomeric building blocks are required to specify direction of synthesis, and catalysts and specialized leaving groups and required to control anomeric linkages. Traditional

solution phase oligosaccharide synthesis generally follows a basic deprotection/coupling order. A glycosyl acceptor's hydroxyl group is deprotected to allow it to attack an activated glycosyl donor to form the glycosidic linkage. That donor is then deprotected to allow it to become the glycosyl acceptor in the next glycosylation. In order to specify at what position the acceptor is exposed, the sugar must be orthogonally protected.

The first glycosylation reactions used glycosyl halides as donors (referred to as the Koenigs-Knorrs method), however these compounds proved to be very reactive and thus unstable [38]. Since then, commonly used glycosyl donors include trichloroacetimidates and thioglycosides [39]. Trichloroacetimidates, first described by Schmidt in 1980, have the advantages of ease of formation, stable at room temperature, and high glycosylation yields [40, 41].



Scheme 1. Glycosylation with trichloroacetimidate donor [42]

Chemical oligosaccharide synthesis is a difficult and time consuming practice [43, 44, 45]. Time consuming chromatography purification must be done after each reaction to separate the product from reactants in order to prevent side reactions during the next synthetic step. A portion of the desired product is also inevitably lost during chromatography, and over the course of a multi-step synthesis yields would be

significantly impacted. In order to allow efficient chemical synthesis, methods that bypass the chromatography purification between reactions have been developed.

"One Pot" method

The "one pot" synthesis method relies on the relative reactivity of a series of donor and acceptors to control the direction of synthesis. One pot synthesis uses what is known as an armed/disarmed method to control reaction order. Sugars protected with electron withdrawing groups such as acetyl groups, are "disarmed," and when reacted with sugars protected with electron donating benzyl groups ("armed" sugars), the disarmed sugars acts exclusively as the glycosyl acceptor in reaction, which prevents self-glycosylation. In disarmed sugars, electron withdrawing groups destabilizes the oxocarbenium cation intermediate, preventing the sugar from acting as glycosyl donors. Only the more reactive armed sugars act as donors. This eliminates the need for temporary protecting groups during synthesis

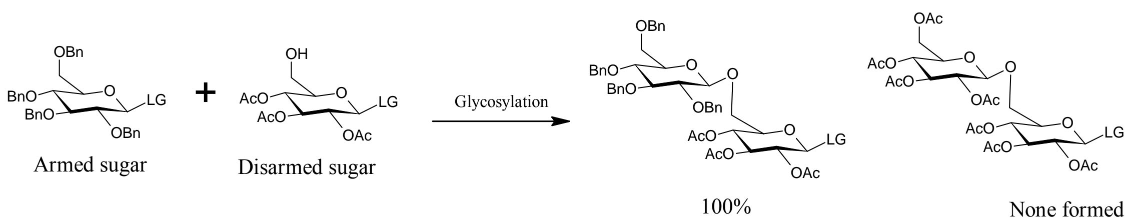


Figure 8. Example of glycosylation reaction between armed and disarmed sugars. The disarmed sugar does not act as a glycosyl donor.

Strategy for One-Pot Synthesis of Linear and Branched Oligosaccharides[a]

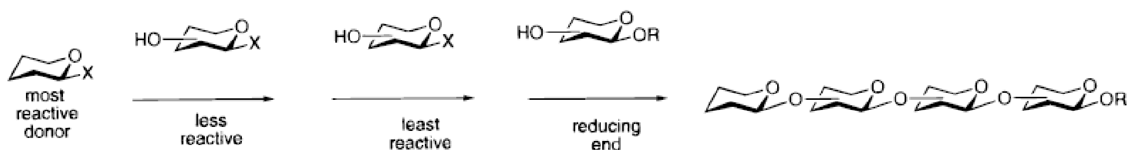
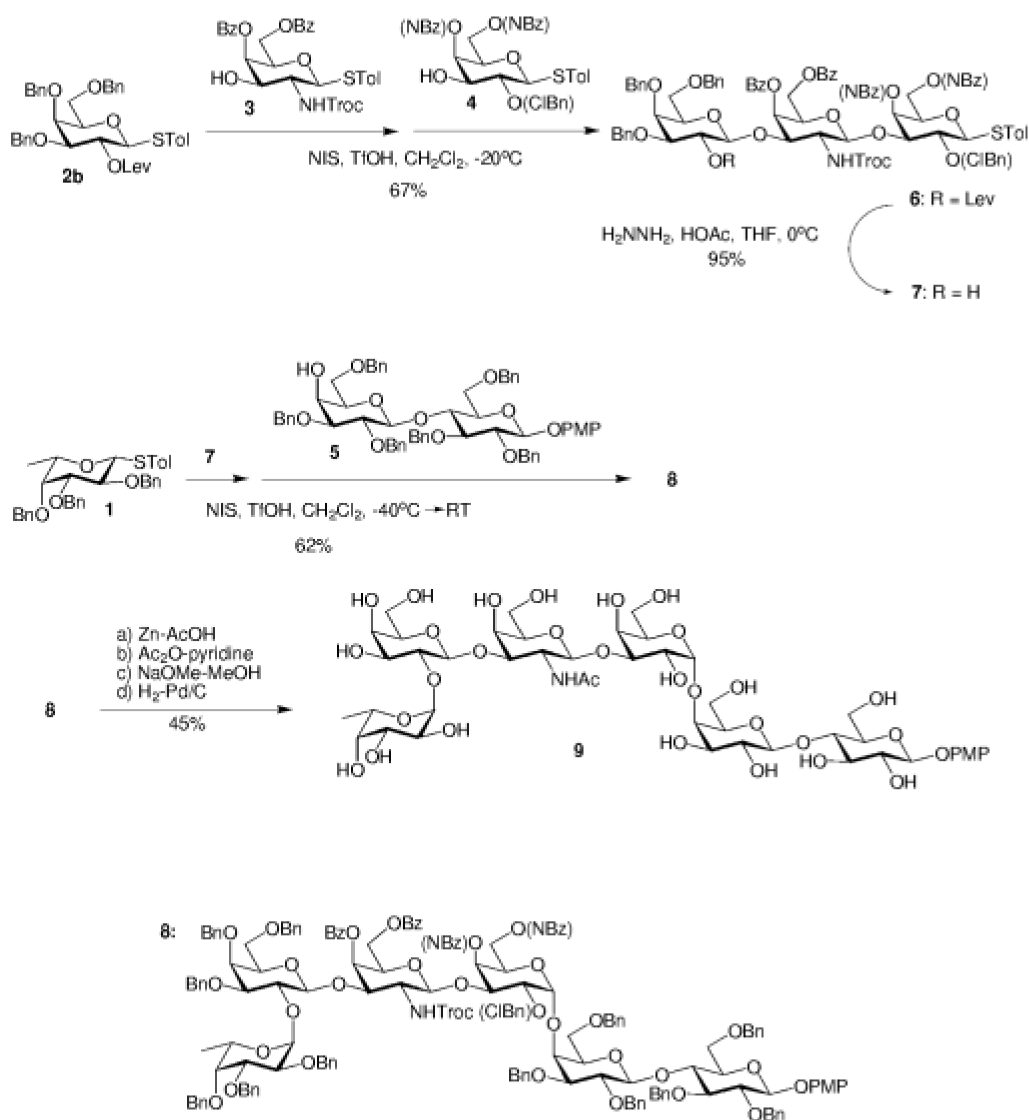


Figure 9. General one-pot strategy [46]

In order to use the armed/disarmed strategy in complex oligosaccharide synthesis, oligosaccharides must be synthesized with donors in order of decreasing reactivity. Chi-Huey Wong's Optimer system [46] uses this one-pot approach to synthesis oligosaccharides. Relative reactivity profiles are first constructed for over 100 thioglycosyde donors. Synthesis is then planned using a computer database of the profiles. The applicability of one-pot synthesis has been demonstrated with the synthesis of the Globo-H hexasaccharide [47. 48] and oligomannose 2G12 epitope mimic [49].



Scheme 2. One-pot synthesis of Globo-H hexasaccharide

The position and nature of protecting group, glycosyl donor, and glycosyl linkages all effect a sugar’s reactivity. Thus, a main drawback of one-pot synthesis is the need to determine the reactivity of each potential reactant, including any new sugars synthesized or building block not in database. This is done by competition reactions, a time

consuming process. Existing databases of donor reactivity, such as Optimer are proprietary information. Such aspects thus limit the use of one-pot synthesis.

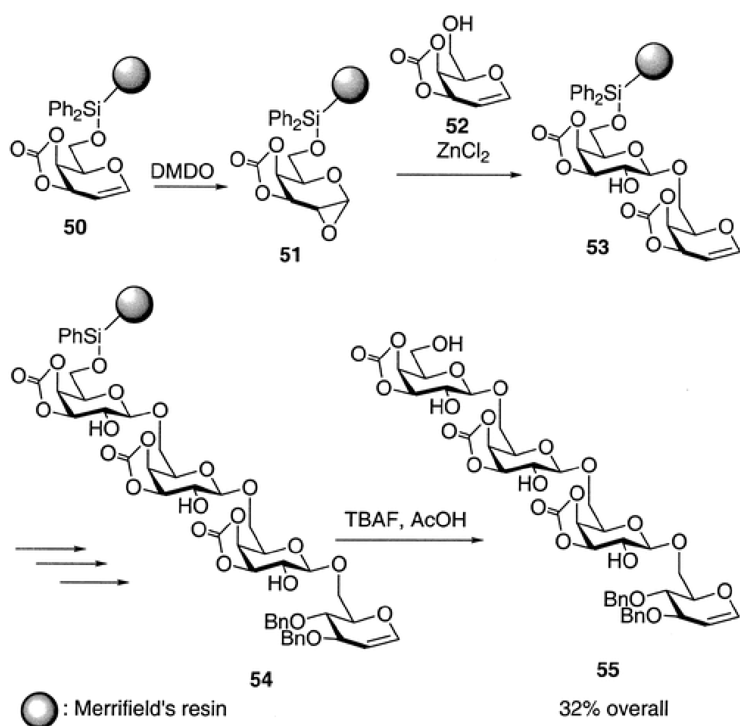
1.5 Solid Phase Oligosaccharide Synthesis

The principle of solid phase synthesis is that synthesis on an insoluble solid support allows simple filtration to remove excess reagents and byproduct. This way, there is no need for time-consuming chromatography between reactions. Large excess of reagents can be used to drive coupling to completion, and is washed off at the end of each cycle. These advantages of solid phase synthesis have greatly improved the speed and efficiency of biopolymer synthesis. Since its introduction and application to peptide synthesis in 1959 by Merrifield, solid phase synthesis has been commonly used in peptide and oligonucleotide synthesis [50, 51].

Another major advantage of solid phase synthesis is that it lends itself well to automation [52]. Automated synthesis has allowed greater access of oligonucleotides and oligopeptides to biochemical research groups. Automated synthesis has also opened the doors to combinatorial studies- automated synthesizers working in parallel can quickly synthesize a library of molecules for bioassay study [53.]

Solid phase synthesis of carbohydrates has been studied since 1971 [54]. Some of the key aspects of solid phase synthesis on oligosaccharides include the nature of the tether attaching glycan to solid support, and the need for both temporary and permanent protecting groups during synthesis. The temporary protecting groups prevent donor-donor glycosylation, while the permanent protecting groups dictate the position of glycosylation.

There are two general solid phase synthesis strategies: donor bound, where the glycosyl donor is attached to solid support and acceptor bound, where the acceptor is tethered. Donor bound synthesis uses polymer-bound glycals as glycosyl donors [55]. A drawback to the donor bound strategy is loss of yield due to side reactions of the donor [48].

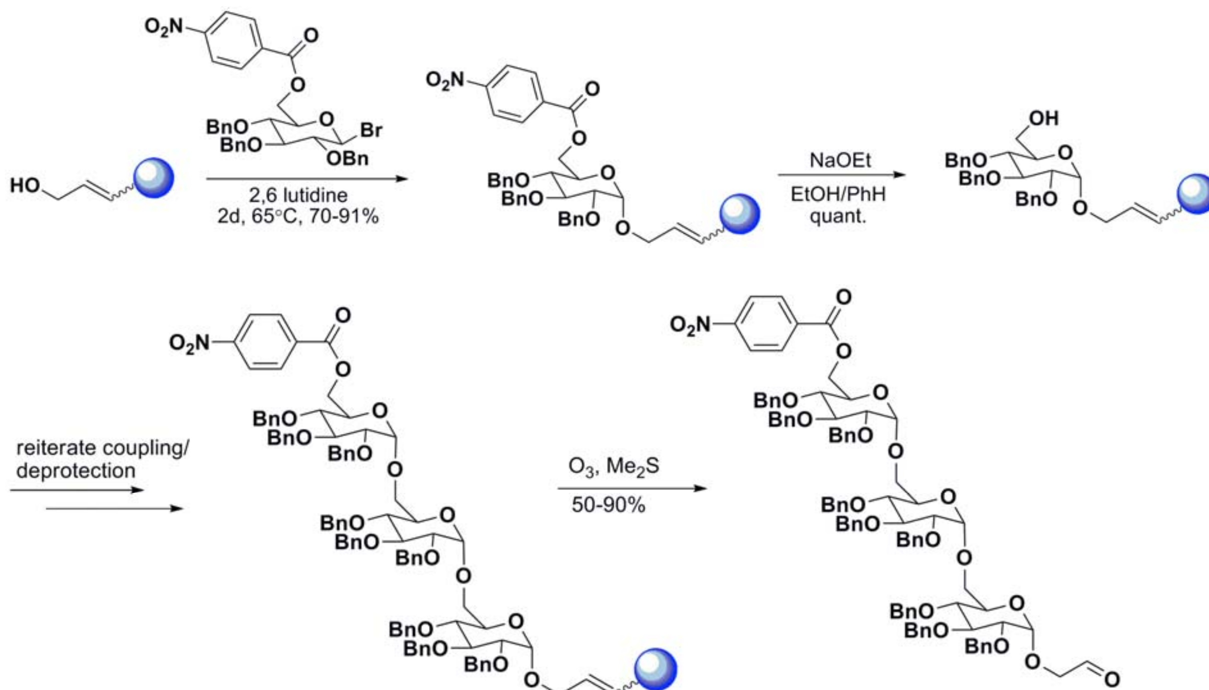


Scheme 3. Donor bound synthesis: glycal assembly [55].

The acceptor bound approach uses a functionalized support polymer as the first glycosyl acceptor. An excess of glycosyl donor can be used during glycosylation to drive the reaction, for a greater glycosylation yield, and the excess donor, as well as any side products, is washed away during filtration.

After the tethering of the initial sugar unit to the solid support, a typical synthesis cycle involves 1) the removal of the temporary protecting group to expose a free

hydroxyl group for nucleophilic attack and 2) activation of glycosyl donor and coupling to the growing oligosaccharide. The product sequence, on resin, is isolated by filtration after each reaction. After completion of synthesis, the product is cleaved from the resin.



Scheme 4. Acceptor bound strategy [54]. Merrifield's resin is used as solid support. Allyl alcohol served as the glycosyl acceptor for attachment to resin. In the first step, 2,6 lutidine was used to activate the donor, a glycosyl bromide. Then, sodium ethoxide was used to remove the p-nitrobenzoate ester temporary protecting group. This cycle was reiterated until the sequence is complete, then the product was cleaved from the solid support by ozonolysis.

The tether that attaches the growing oligosaccharide to the polymer support must be stable to reaction conditions during synthesis (removal of temporary protecting group and activation of glycosyl donor), as well as being cleavable once synthesis complete to release the completed oligosaccharide from the solid support.

Frechet's original acceptor bound solid phase synthesis used allyl alcohol functionalized Merrifield's resin as solid support, glycoside bromides as donors, and used ozonolysis to cleave from resin. Subsequent advancements in solid phase oligosaccharide synthesis include the investigation of different solid support materials [56, 57], different glycans-resin linkages, including thioglycosidic [58], ester [59] and those cleaved by olefin metathesis [60, 30], as well as more stable donors such as trichloroacetimidates [39].

The repetitive nature of solid phase oligosaccharide synthesis makes it ideal for automation. The filtration purification of solid phase synthesis can be reliably done by machine, in contrast to traditional purification methods such as chromatography, which requires at least a certain level of human supervision. Cycles of deprotection and glycosylations with prepared donors can be thus completely automated, saving manpower the on these often long synthesis and otherwise tedious synthesis.

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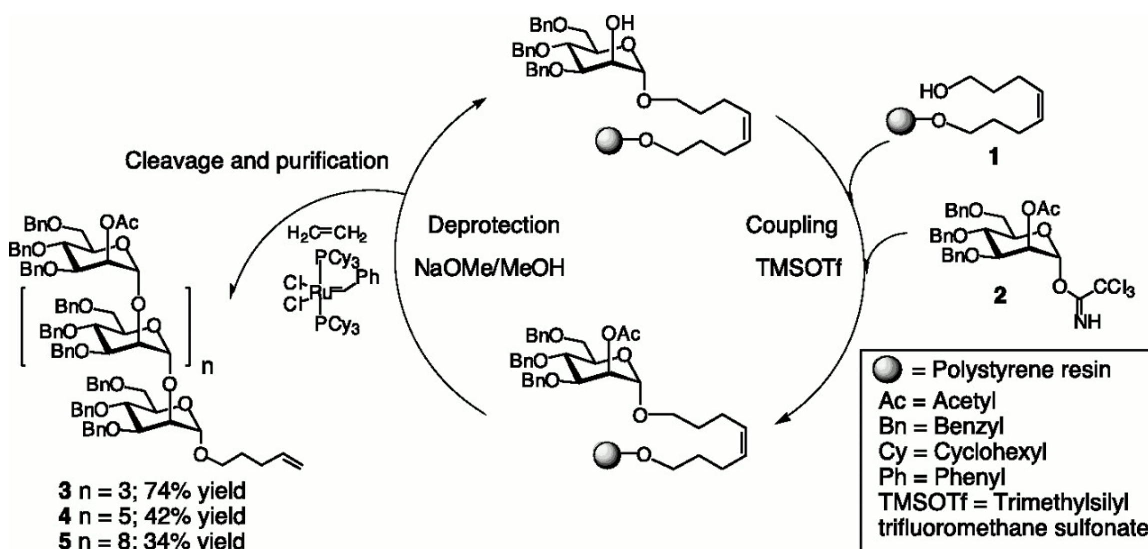
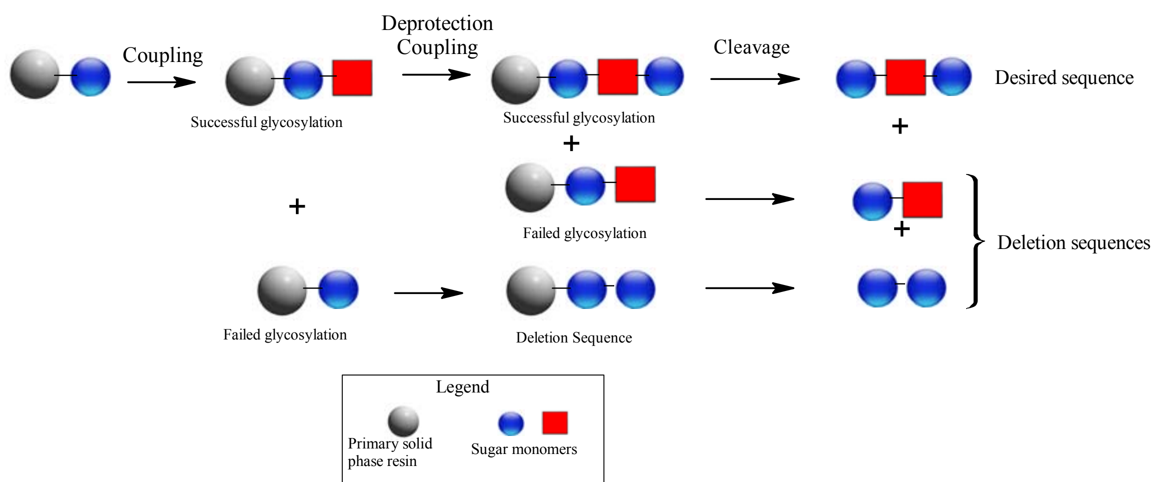


Scheme 5. Seeberger's automated synthesis cycle. Trichloroacetimidate donors were activated with TMSOTf. After glycosylation, the acetyl temporary protecting group was removed with methoxide to expose the glycosyl acceptor for the next round of synthesis [61].

Seeberger's lab first carried out automated synthesis using a modified peptide synthesizer [61]. Octenediol functionalized Merrifield's resin was used as the solid support, mannose trichloroacetimidates as donors, acetyl groups as temporary protecting groups, and benzyl groups as permanent protecting groups. Cleavage from the resin was done by olefin cross-metathesis. The time it took to synthesize a heptamannoside with automated synthesizer is 20 hours, compared to 14 days for manual synthesis, with favorable yield (42% compared to 9% yield for manual synthesis). The applicability of automated solid phase synthesis was further demonstrated with synthesis of Globo-H [62]. While the 30% reported yield is lower than the 45% yield reported for synthesis of Globo-H by one-pot

synthesis [47], the solid phase synthesis time of 25 hours without the need for human interaction demonstrates the utility of solid phase synthesis.

In spite of the introduction of powerful glycosyl donors, one of the weaknesses of solid phase oligosaccharide synthesis is the lack of quantitative glycosylation. Yields of o-glycosylation, (50-90%) are usually lower than peptide coupling (quantitative) [63]. While the glycosylation yield can be improved by reiteration and using excess of donor, there is no guarantee of quantitative glycosylation at each step. A single incomplete glycosylation during synthesis would lead to the formation of (n-1) “deletion” sequences, which are difficult to separate from completes sequences after cleavage from the solid support. Accumulation of these “deletion” sequences, which, during the course of a multi-step synthesis could greatly outnumber the desired product, would lead to a difficult purification. The difficulty of the final purification negates the distinct advantage of easy purification in solid phase synthesis. Newer strategies in solid phase synthesis use a “capping” method to get around this shortcoming, to offer a more streamlined synthesis.



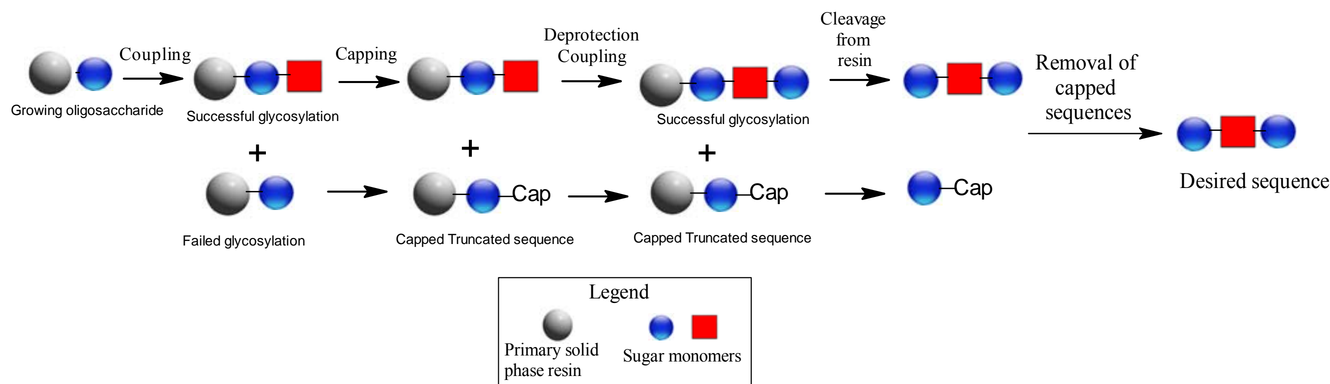
Scheme 6. Formation of (n-1) deletion sequences during solid phase synthesis.

1.6 Capping and purification strategies

To deal with the problem of (n-1) deletion sequences, capping reagents [64, 65] were introduced into the carbohydrate solid phase synthetic cycle. Caps serve as a permanent protecting group to block unreacted acceptors (free hydroxyl groups) on the resin-bound oligosaccharide from further glycosylations. This way, (n-1) deletion sequences would not be formed. The capping reaction is done after the glycosylation step but before deprotection for the next cycle. The capping conditions must be orthogonal to all existing temporary and permanent protecting groups on the sugar, and must proceed in quantitative yield so as to block all future glycosylations on the incomplete sequence. In addition, the cap must be stable to all reaction conditions in future deprotection, glycosylation, and cleavage steps, and itself chemically inert. After cleavage from solid support, the products of a cap-synthesis are capped "truncated" sequences along with the desired complete sequences. Examples of capping reagents used in solid phase oligosaccharide synthesis include acetic anhydride and benzoyl isocyanate [66].

Cap-tag

The cap on truncated sequences can be designed additionally as an affinity tag to remove these sequences from solution. This is known as the cap-tag approach: the cap used to prevent a sequence from further glycosylation doubles as the tag for purification.



Scheme 7. Cap-tag solid phase synthesis. Incomplete sequences are capped to prevent them from taking place in any future glycosylations.

Scavenger resins contain a functional group that reacts with a reactant bound to the truncated sequences in solution, linking the truncated sequence to the solid resin and allowing its removal from solution . Scavenger resins have been used to remove hydrolyzed glycosyl donor and unreacted acceptors in solution phase carbohydrate synthesis [67]. In cap-tag solid phase synthesis, the scavenger resin's functional group would react with the cap-tag on truncated sequences. The cap tagged truncated sequences would be attached to the resin and removed, while the uncapped desired sequences would remain in solution [68].

Due to the properties of fluorous phase interactions, compounds capped with a fluorous tag can be separated from non-fluorinated compounds by chromatography on fluorous silica gel [69]. This can also be used to isolate capped truncated sequences. Fluorous cap-tagging has been investigated in solid phase peptide synthesis [114].

Seeberger's group investigated cap-tag solid phase carbohydrate syntheses with both scavenger resin and fluorous tag [70]. The A tag, introduced during capping as an anhydride, contains an inert azide functional group and acted to stop subsequent glycosylation on incomplete sequences. After cleavage of sugars from resin, the azide moiety was converted into an amine by treatment with tributylphosphane. An isocyanate scavenger resin was then used to attach to the amines. Removal of the scavenger resin removed the capped truncated sequence in solution. The F-tag capped sequences with a fluorous functional group instead. Fluorous phase chromatography was used to remove these capped sequences from solution.

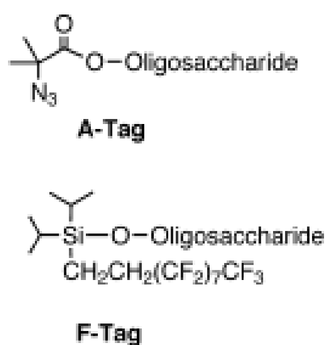
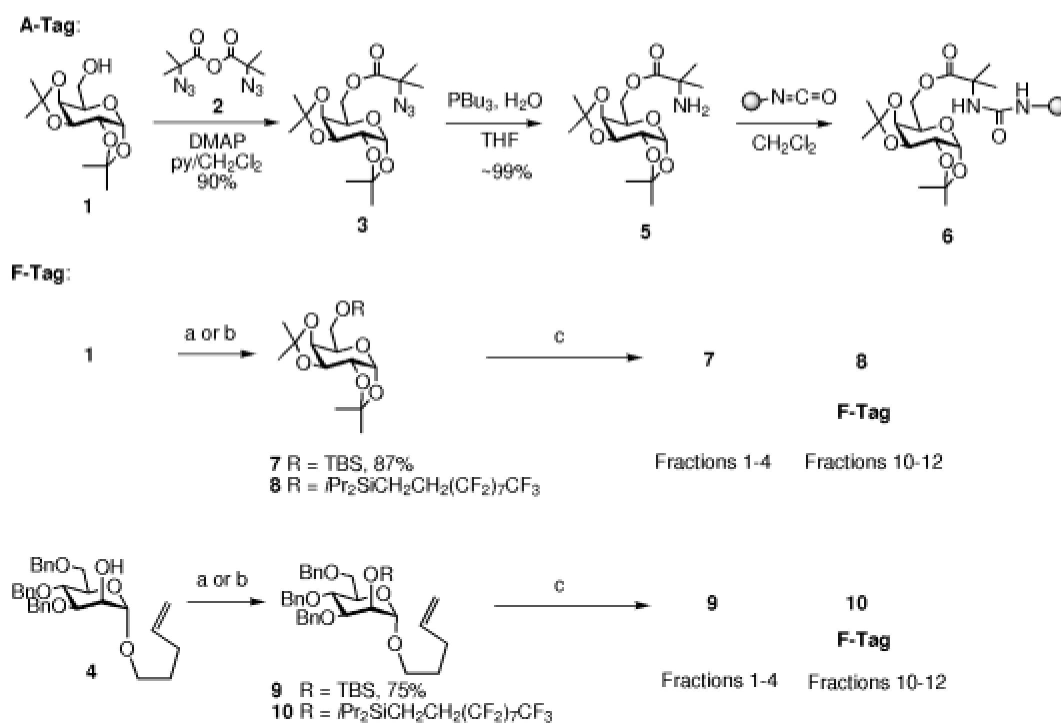


Figure 10. Two different cap-tags. [70].



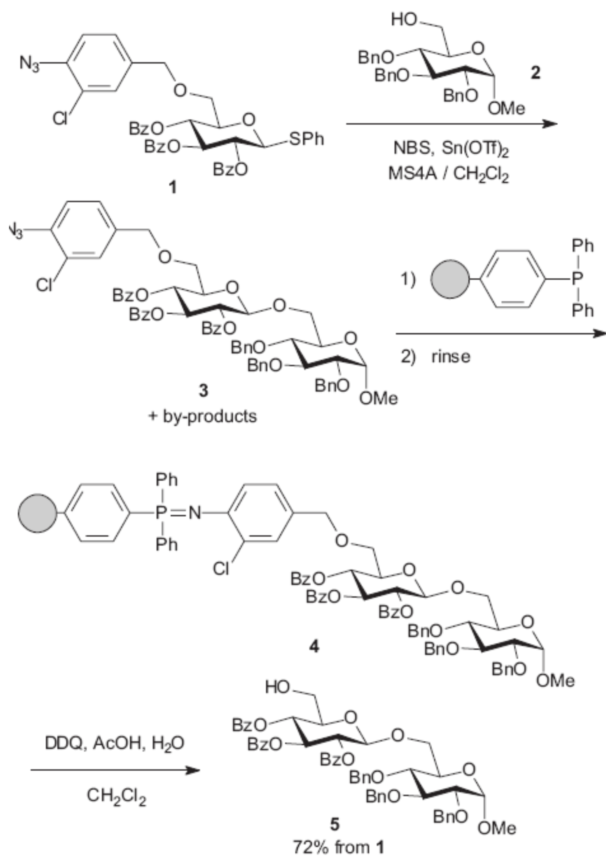
Scheme 8. Cap-tag synthesis using A and F-tags [70]. After conversion of the nonreactive azide group into an amine, the A tag allowed removal of capped sequenced by scavenger resin, while the F-tag allowed removal by fluorous phase chromatography.

While the cap-tag synthetic approach was successful in the removal of most of the tagged truncated sequences, it did not result in furnishing a pure, complete oligosaccharide. The weakness in the cap-tag approach lies in the dependence of a)coupling of the cap-tag and b) the attachment of the cap-tag to the scavenger resin. If any truncated sequences fail to attach to the cap-tag, or any capped sequences fail to attach to scavenger resin, these truncated sequences would be left in solution with the desired sequences. Chromatographic separation is still necessary to completely purify the desired sequence.

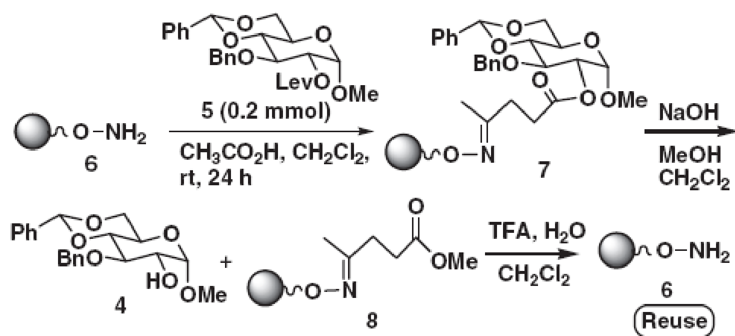
Capture and release

The capture and release method of solid phase synthesis instead puts a tag onto the completed oligosaccharides, as opposed to the truncated sequences. The tagged complete sequence is then linked to a catch resin, allowing any impurities, including incomplete sequences, to be washed away. The solid phase isolation of the complete sequences instead of the truncated sequences puts less dependence on the attachment of tag to resin- if incomplete capture occurs, the yield will be effected but not the purity of the final product.

Capture and release purification on solution phase synthesis was described by Fukase's research group [71]. An oligosaccharide was assembled using "one pot" strategy. The last glycosyl donor in the synthesis, however, was protected with a 4-Azido-3-chlorobenzyl (ClAzB) protecting group, which functioned as the tag. After synthesis of the oligosaccharide chain, the ClAzb tags were captured on phosphine functionalized resin by Staudinger reaction between the tag azido and resin phosphine group, and impurities were washed away. Cleavage of the ClAzb group and attached resin yielded pure desired product. Fukase additionally demonstrated the use of the levulinyl group as a tag with aminoxy catch resin [72].

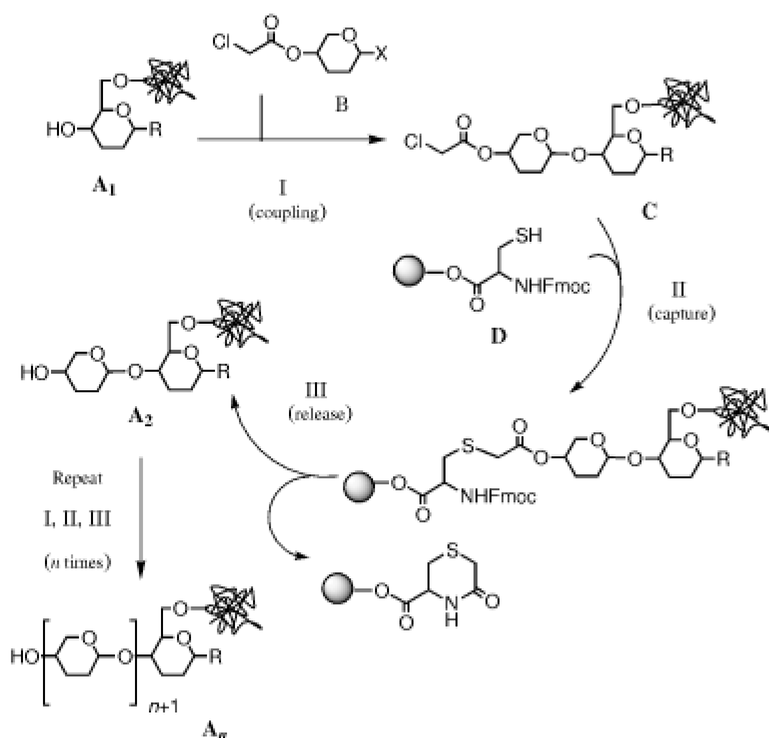


Scheme 9. Capture and release with ClAzB tag [71]



Scheme 10. Capture and release with levulinyl tag [72]

Ito's group applied the capture and release strategy to polymer-supported synthesis [73]. Synthesis was carried out with the growing oligosaccharide linked to a poly(ethylene)glycol support, a polymer which can be either soluble or insoluble depending on the solvent. Glycosylation was done with the donor containing a chloroacetyl protecting group. After glycosylation, excess donor was removed by filtration through silica gel, as per standard solid phase synthesis. Then, the chloroacetyl groups were captured onto a thiol functionalized solid phase resin. Filtration, in which the PEG support stayed in solution, removed the sequences not bound to the resin. Cleavage from the capture resin resulted in pure product. This cycle was repeated for each monomer in the sequence.



Scheme 11. Capture and release synthesis with chloroacetyl tag [73].

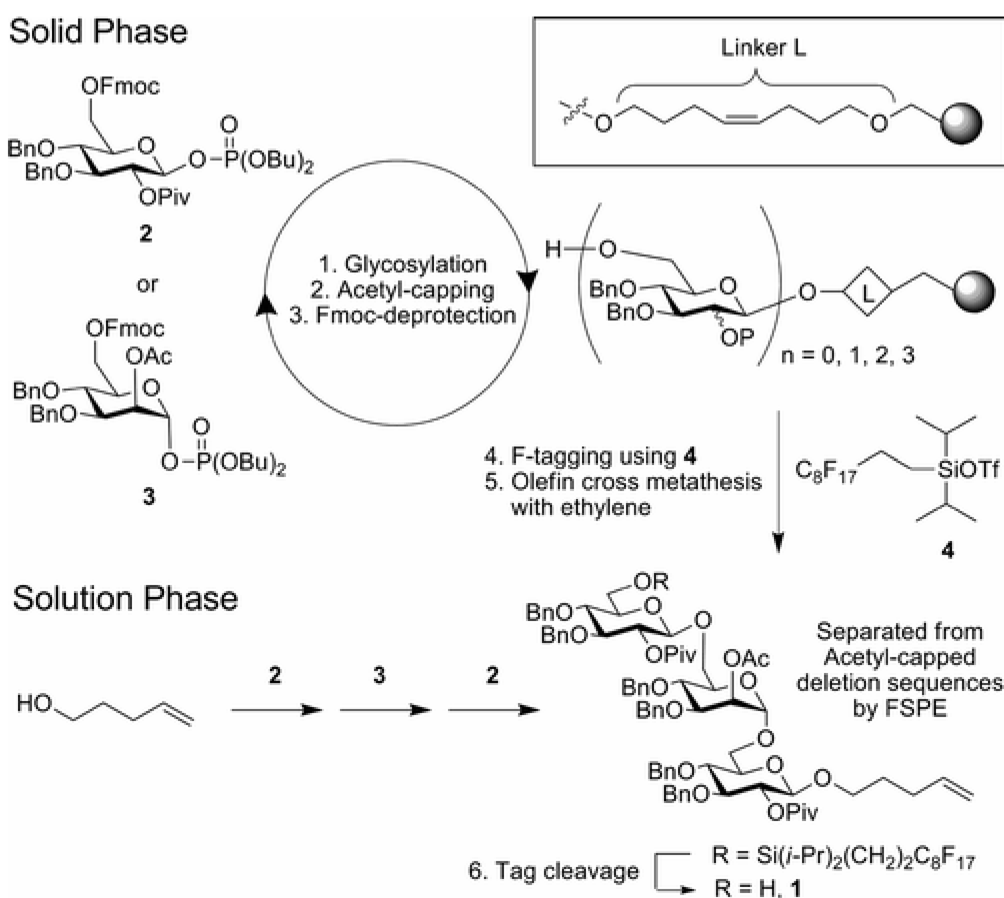
This strategy requires capture and release reactions for every glycosylation. Over the synthesis of long oligosaccharide chain, this will add a significant amount of complexity to synthesis. This method is also limited to using chloroacetyl protecting groups, which might not be compatible with certain protecting group strategies. The major benefit of capture and release synthesis is that there is no need for chromatography step in purification. Cleavage from the capture resin furnishes a pure product.

Cap and tag

A “cap and tag” strategy described by Seeberger [74] incorporates capping reagents and a product-linked tag for purification in solid phase synthesis. Incomplete glycosylations are capped with acetyl groups each cycle to prevent further reaction. After the last sugar monomer is incorporated, the protecting group on the last chain is removed and a fluorous tag is coupled to the acceptor site. As the coupling is done on solid phase, an excess of the tag may be used to drive reaction to completion, and the excess is washed away. Because the truncated sequences are capped, they will not couple to the tag. The use of capping reagent eliminates the need to purify the oligosaccharide after each cycle. After cleavage from the solid support the fluorous tagged complete sequences can be separated from the truncated sequences by fluorous solid phase extraction (FSPE). Cleavage of the fluorous tag by TBAF yields a pure desired sequence.

Compared to the fluorous cap-tag method, tagging of the completed sequences (cap *and* tag) puts less dependence on the coupling yield; if incomplete coupling occurs, the yield will be effected but not the purity of the product. The cap and tag approach allows capping with acetyl groups, put on by acetic anhydride, rather than more complex cap-

tags. The capping step needs to be reiterated every glycosylation cycle and requires a large excess of the capping reagent to drive to completion. While cap-tags are often expensive or require synthesis, acetyl capping with acetic anhydride is simple, inexpensive, high yielding, and is stable with removal conditions of common temporary protection groups such as Fmoc.



Scheme 12. Cap and tag solid phase synthesis. Tagging the desired sequence with fluorous tag allows facile separation from truncated sequences [74].

While gradient fluorous solid phase extraction of a fluorous tagged sequence allows an easier separation than traditional chromatography purification, it is still a variation of chromatography. It is neither as simple nor as applicable to automated synthesis as purification by filtration. In addition, the fluorous silica gel used in FSPE is expensive ($2250.00/kg vs $165/kg for regular silica gel) [75].

Cap, Tag and Capture-release

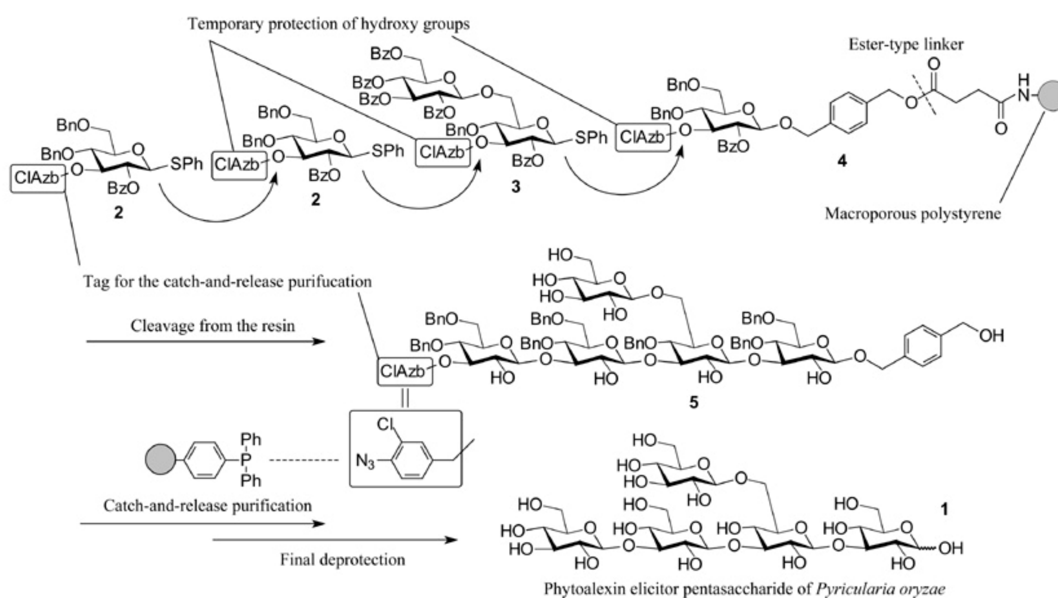
Going back to the primary principle of solid phase synthesis, that attachment to a resin allows simple filtration purification, we propose a methodology for solid phase oligosaccharide synthesis that does not require any chromatography steps whatsoever. By tagging the final completed oligosaccharide before cleavage from solid phase, we provide a handle to isolate the completed sequences. Instead of utilizing the tag for chromatographic separation, we will instead use this tag for capture-release purification. Following cleavage from the primary solid phase resin, a mixture of tagged desired sequence and capped truncated sequences would be left in solution. We will use a secondary resin to capture the tagged sequences onto solid phase, allowing removal of the truncated sequenced by washing. We will also describe a solid phase procedure for deprotection of the synthesized sugar. (see Chapter 3.5)

The goal of this project is to design a chromatography free synthesis from monomeric building blocks to a complete, deprotected sugar sequence that would be ready for bioassay. The benefit of chromatography free synthesis is its application to automated synthesis. Current automated synthesis extends only so far as the synthesis of complete, protected sequences. The time consuming purification and deprotection of the

synthesized oligosaccharides still must be done manually. A chromatography-free synthesis consists entirely of reactions and filtrations, which can be reliably done by machine.

We envision a future which automated synthesizers permit the parallel synthesis of large variety of oligosaccharide structures for study. Synthesis of combinatorial oligosaccharide library would empower key technologies like glycans microarrays. A streamlined, automated synthesis, combined with an increasing availability of sugar monomers [115], would allow access of oligosaccharides to non-specialists in the field.

Capture and release purification following solid phase synthesis been evaluated by Fukase et al. [76]. Thioglycosides bearing ClAzb temporary protecting groups were used as glycosyl donors. Deprotection of the ClAzb revealed a free acceptor for the next cycle of glycosylation. When the final donor in the sequence was put on, the ClAzb group of the last donor was not removed so that the completed sequences were tagged with the ClAzb group. All sequences were then cleaved from the solid support. A phosphine functionalized catch resin was used to capture sequences with ClAzb protecting groups. The incomplete sequences that were not captured were washed away, and the complete sequences were cleaved from resin with 2,3-dichloro-5,6-dicyanobenzoquinone.

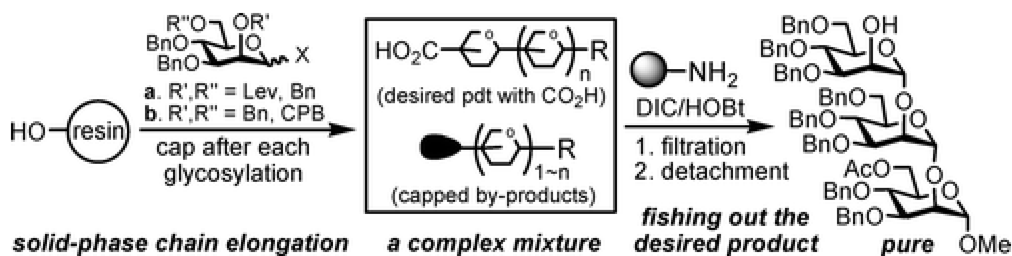


Scheme 13. Solid phase synthesis followed by capture on phosphine resin [76]

A problem reported with the ClAzb-phosphine tag system is hydrolysis of the iminophosphorane linking the resin with sugar. Some of captured sequences are lost during washing. Another potential problem is that capping was not done in between reactions, thus unreacted glycosyl acceptors may participate in subsequent chain elongation.

Another paper describes the use of cap and tag solid phase synthesis with capture-release purification [77]. Solid phase synthesis was carried out with trichloroacetimidate donors and levulinoyl protecting group. Capping was done with acetic anhydride. The final sugar monomer contained *p*-(5-carboxypentyloxy)benzyl (CPB) group as a linker-tag to the capture resin. Capture was done by coupling the carboxyl group on the CPB linker to an amine functionalized resin with DIC/Hob as the coupling agent. After

filtration, the sugar was cleaved from the CPB linker and resin with trifluoroacetic acid (TFA).



Scheme 14. Cap and tag synthesis followed by capture on resin by formation of amide [77].

The use of TFA for cleavage from the capture resin in this approach might not be compatible with certain sequences: oligosaccharides would be prone to hydrolysis under the strong acid.

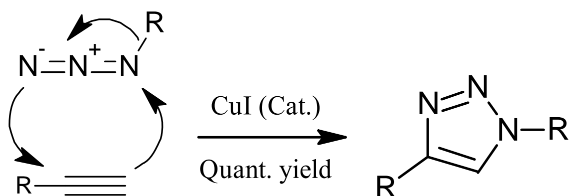
Both strategies rely upon a special protecting group on the last glycosyl donor to act as a handle for capture. If we can instead install this handle to the acceptor site during solid phase synthesis, we would be able to accept a wider variety of glycosyl donors for synthesis. Tagging the completed sequence after glycosylation and deprotection would allow the use of general building block donors instead of having to prepare specialized ones.

As shown by hydrolysis of the iminophosphorane resin during the capture of product on resin, current tag-resin linking systems could be improved upon. In order to make cap, tag, capture and release solid phase synthesis a more robust system, we investigated more reliable methods of linking resin to tagged sugar.

1.7 Introduction to Click Chemistry

In searching for a method of reliably linking tagged glycans sequence to a capture resin, we came across the field of click chemistry. "Click chemistry", first described by K. Barry Sharpless is a modular chemical approach for reliably joining molecules together [78]. The requirements for a reaction to be a "click reaction" include versatility, high yielding, and non-chromatographic removal of by-products. These properties of click chemistry would prove ideal in our need to attach an oligosaccharide chain to a purification resin.

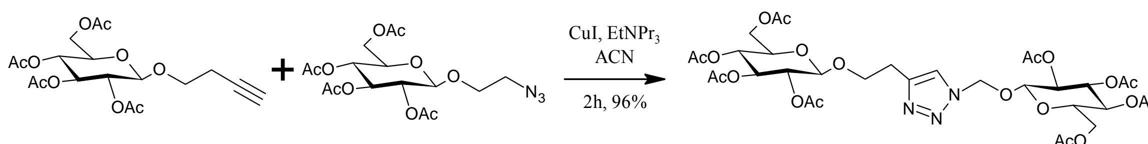
The 1,3 Huisgen cycloaddition is often referred to as the "ideal" click reaction: the reaction has the properties of being quantitative, robust, insensitive, and general [79]. The Huisgen reaction describes a cycloaddition reaction between an alkyne and an azide to form a 1,3 triazole. Catalysis by Cu(I) greatly increases the reaction rate (700% rate increase) [78, 80] and allows the reaction to occur at room temperature. The mechanism of the alkyne-azide cycloaddition reaction [81] and the copper catalysis [79] has been well described in literature. The reaction proceeds in a variety of solvents, pH values and temperatures [81], and is high yielding (97%+) as well.



Scheme 15. Copper catalyzed huisgen 1,3-dipolar cycloaddition.

Click chemistry, and specifically the 1,3 cycloaddition, is recently used prominently in drug discovery [82], polymer chemistry [83], and cellular labeling [84]. Azides and alkynes are inert to most biological and organic conditions [81]. Alkyne-azide cycloaddition has been successfully used for molecular labeling in live mice with no apparent toxicity [85].

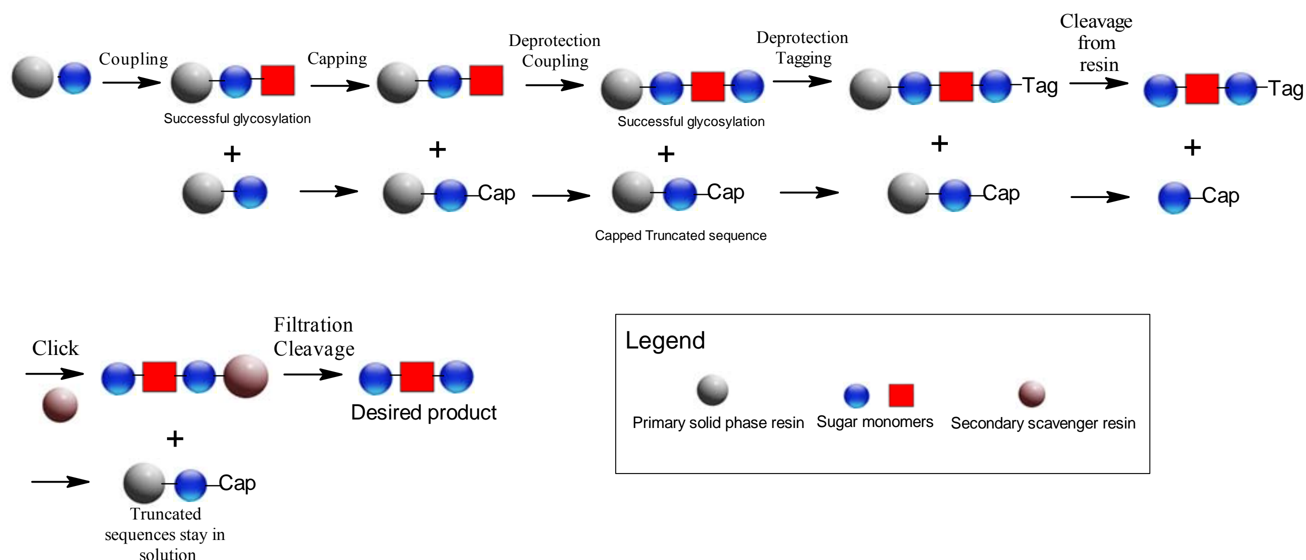
Click chemistry's utility in glycobiology has been well studied [86]. The versatile triazole cycloaddition reaction has been used to introduce novel functional groups on sugars, link carbohydrates to aglycone [87], and join carbohydrate monomers together to form pseudo-oligosaccharides [88, 89]. The 1,3 Huisgen cycloaddition was found to be compatible with various protecting groups such as Fmoc and benzyl groups, both used in carbohydrate synthesis.



Scheme16. Joining of carbohydrate monomers by click chemistry [88]

These studies establish the applicability of the Cu(I) catalyzed 1,3 Huisgen cycloaddition to solid phase carbohydrate synthesis. The non-reactive alkyne or azide group could be introduced during solid phase synthesis and be inert during the cleavage from the resin. After cleavage, a resin functionalized with the complimentary functional group will be used to capture the tagged sequence, allowing filtration. The properties of the 1,3 Huisgen cycloaddition reaction, such as robustness, inertness to side reactions, and high yield makes it an ideal candidate for linking tagged oligosaccharide to capture resin during capture-release purification.

In this thesis, will combine click chemistry with both cap and tag solid phase synthesis and capture-release oligosaccharide purification to design a chromatography-free method of assembling oligosaccharides. By tagging the final completed oligosaccharide with an alkyne tag before cleavage from solid phase, we provide a "click chemistry" handle to isolate the completed sequences. Following cleavage from the primary solid phase resin, a mixture of tagged desired sequence and capped truncated sequences would be left in solution. We will use a secondary azide functionalized capture resin to capture the tagged sequences by alkyne-azide cycloaddition, allowing removal of the truncated sequenced by washing. Due to the excellent properties of the cycloaddition "click" reaction, it will be an improvement on current methods of linking sugar to capture resin. We expect this methodology to be highly applicable to parallel automated synthesis and facilitate the creation of carbohydrate libraries for biochemical analysis.



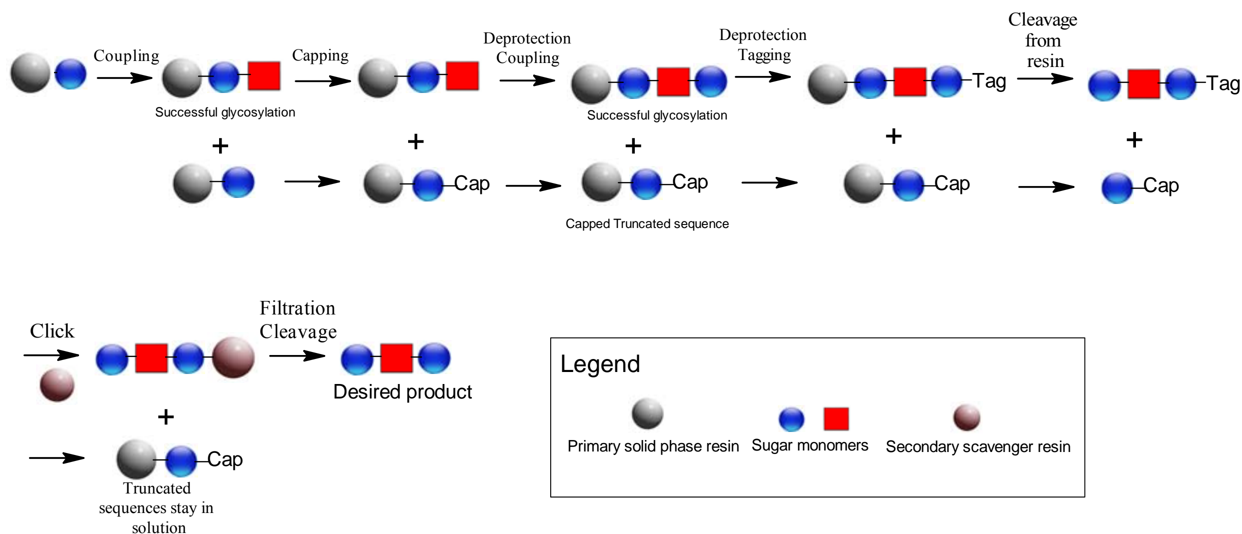
Proposed scheme for chromatography free solid phase oligosaccharide synthesis.

Chapter 2

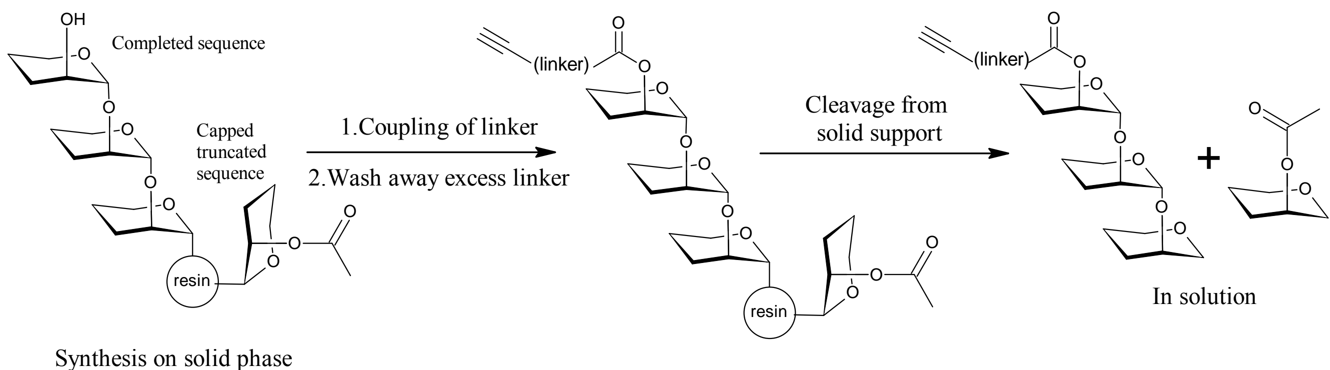
Capture- release with Click chemistry

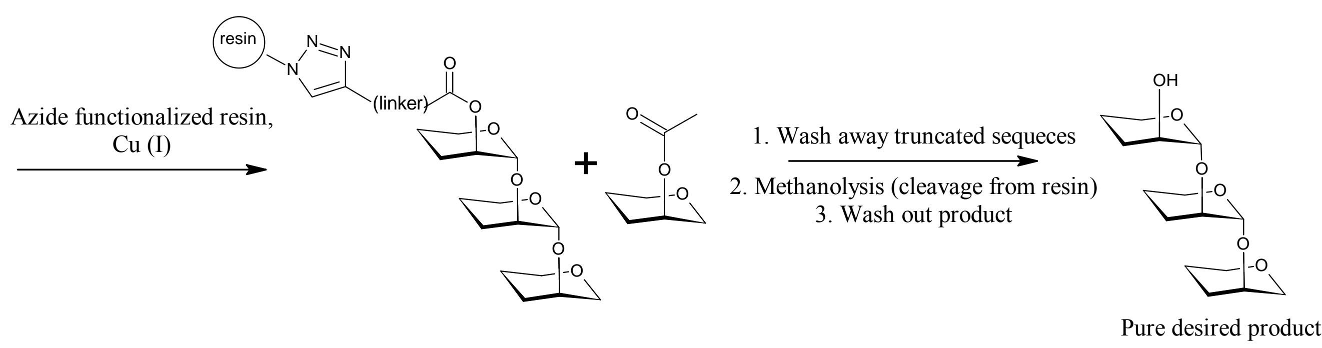
2.1 Design of Linker-Tags for Click Chemistry

In order to introduce a handle for capture-and-release, we need to put a linker-tag on the final completed oligosaccharide sequence during solid phase synthesis. To optimize conditions for coupling of tag to glycosyl acceptor, we prepared a series of potential tags for screening. This tag should be able to couple to a free hydroxyl group on the sugar as well as provide a handle where which we can "click" on the capture resin. By introducing a cleavable tag with a click functional group (alkyne/azide) onto the final, complete oligosaccharide, we could "click" that desired sequence onto the capture resin and wash away all the undesired, incomplete sequences. After purification, we would cleave off the tag and attached resin, giving us the pure sugar.



Scheme 17. Scheme for cap, tag, and click capture-release solid phase oligosaccharide synthesis.





Scheme 18: Tagging of completed sequence, capture onto resin, and cleavage from resin.

The requirements for a tag are thus of the following:

i) ease and yield of coupling to a sugar

ii) incorporation of a functional group for click chemistry

iii) non-reactive during cleavage of sugar from solid support

iv) easily cleaved from the sugar

In satisfying requirements i and iv, a carboxylic acid can be coupled to a free hydroxyl group of a sugar via an ester linkage, and likewise can be cleaved by addition-elimination reaction, principally methanolysis.

Since the synthesis of azide functionalized resins is well documented [90] and alkyne functionalized carboxylic acids are commercially available, we decided to design the tag and catch resin with azide on resin and alkyne on tag.

Acylating agents **1**,**2**, and **4** were prepared for screening. Each contains a carboxylic acid end for coupling to sugar and an alkyne end for reaction with capture resin. Synthesis of tag 5 was attempted, but ultimately not successful.

Acyl halides react readily with alcohols in forming esters. They tend to be more reactive than carboxylic acids, however they are less stable and prone to hydrolysis. Commercially available chloroformate **6** will also be evaluated as a potential tag. In addition, any of the prepared carboxylic acid tags can be converted into acyl halides by reaction with thionyl chloride. The acyl halide derivatives 1a,2a, and 4a can be synthesized if the present tags are deemed not suitable for the role.

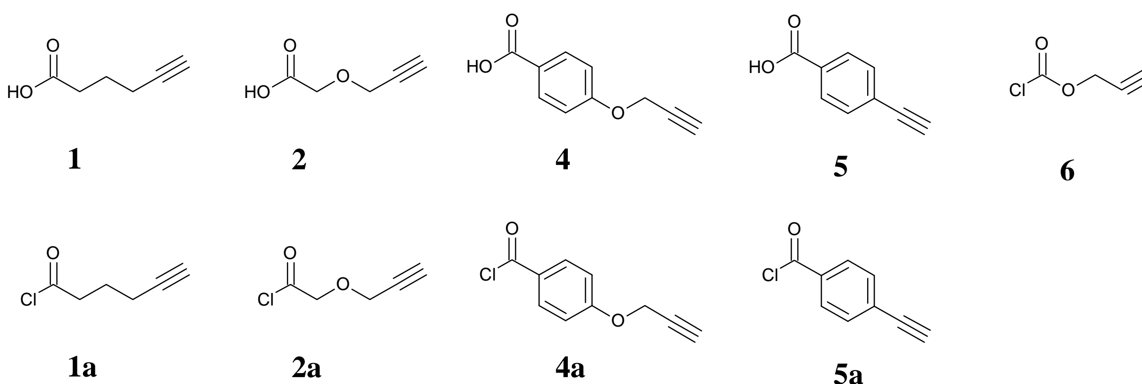
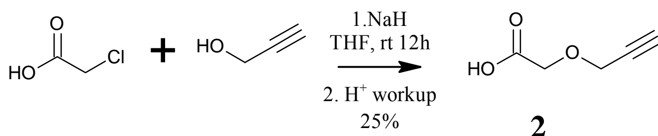


Figure 11. Proposed alkyne functionalized tags.

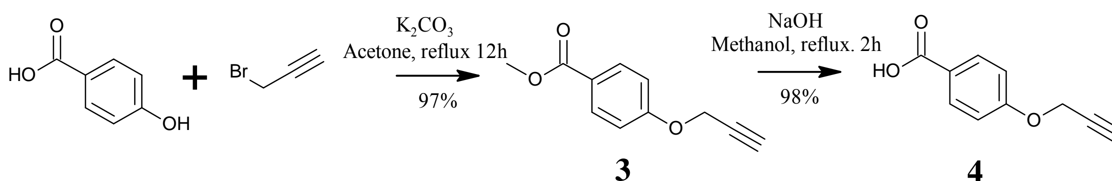
Tag **1**, pentynoic acid, was found to be commercially available. Tag **2** was prepared from chloroacetic acid as described in literature [91]. 2-Propyn-1-ol was deprotonated by NaH, and proceeded to mount a nucleophilic attack on chloroacetic acid to give compound **2** in

25% yield after chromatography. Tag **2**, unlike the other tags, is an oil, so it will be more difficult to measure out when setting up reactions.



Scheme 19. Preparation of tag **2**.

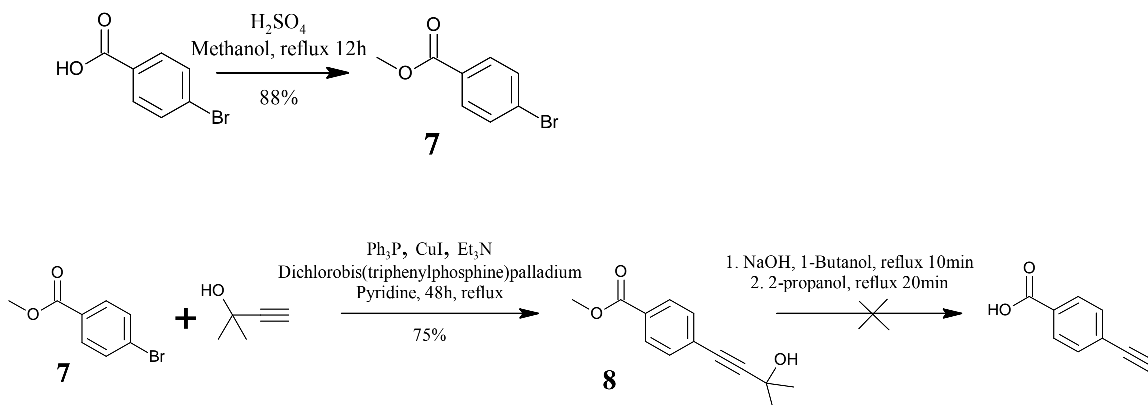
For the synthesis of tag **4**, Methyl 4-hydroxybenzoate was stirred in acetone with 3-bromo-1-propyne under reflux conditions. The product was dissolved in water and extracted by chloroform and dried to give compound 3 in 97% yield [92]. Benzoate **3** was subsequently refluxed with methoxide and NaOH, extracted, and acidified to carboxylic acid to give tag **4** in 98% yield [93].



Scheme20. Preparation of tag **4**.

Synthesis of tag **6** was attempted using Sonagashira reaction, a coupling reaction of terminal alkynes to aryl halide. First, 4-bromobenzoic acid was converted to 4-bromobenzoate **7** by reflux in sulfuric acid and methanol (88% yield) [94]. Compound **7** was reacted with methyl p-bromobenzoate to give product **8** by Sonagashira reaction (75% yield) [95]. Removal of an acetone by reflux in base was however, not successful.

Reflux conditions led to the formation of a black solid revealed by TLC to be a large mixture of products. The reflux temperature and acid has destroyed the compound. After two unsuccessful attempts at synthesizing tag **5**, we decided to first screen the available tags **1**, **2**, **4**, and **6**.

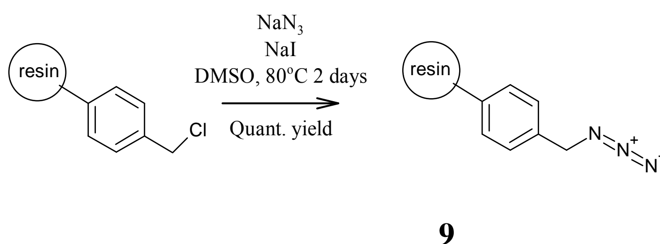


Scheme 21. Preparation of tag **5**. The final step in removing an acetone group from **8** was not successful.

2.2 Design of Click Purification Resin

Once we determined to place the alkyne moiety on the tag, it was clear that we needed a suitable capture resin containing the azide moiety. Merrifield's resin, composed of polystyrene cross-linked with divinylbenzene, is a popular solid support choice because of its high loading capacity, compatibility with many reaction conditions, durability, and low price. In order to access all of the loading sites in Merrifield's resin, it needs to be swollen in nonpolar solvent [30]. Copper catalyzed 1, 3 Huisgen cycloaddition is insensitive to reaction conditions [79]. Because an oligosaccharide completed in solid phase synthesis will contain hydrophobic protecting groups, we will choose to run the click reaction in nonpolar solvent, which should be suitable with Merrifield's resin.

Alkyne functionalized Merrifield resin was prepared by shaking Merrifield resin with an equimolar amount of sodium azide in DMSO at 80°C. The reaction was carried out inside a Plexiglas shield, as sodium azide is capable of explosive decomposition into nitrogen gas. Nucleophilic substitution of the chlorine by azide yielded compound **9** in quantitative yield. Azide functionalization was detected on the resin by the presence of an IR peak at 2300cm^{-1}.



Scheme 22. Synthesis of azide functionalized resin **9**

2.3 Design of model deactivated sugar to screen tags

The success of our tag-click purification procedure depends on the coupling of the tag to the sugar. If the tag does not couple well to the sugar, subsequent click purification will not result in a satisfactory yield.

Testing the efficacy of the synthesized tags requires a model sugar to be coupled to. In order to screen for a versatile tag and tag coupling conditions capable of attaching to a broad range of sugars, we prepared a model sugar that is strongly deactivated.

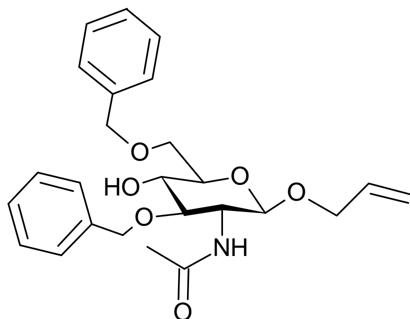


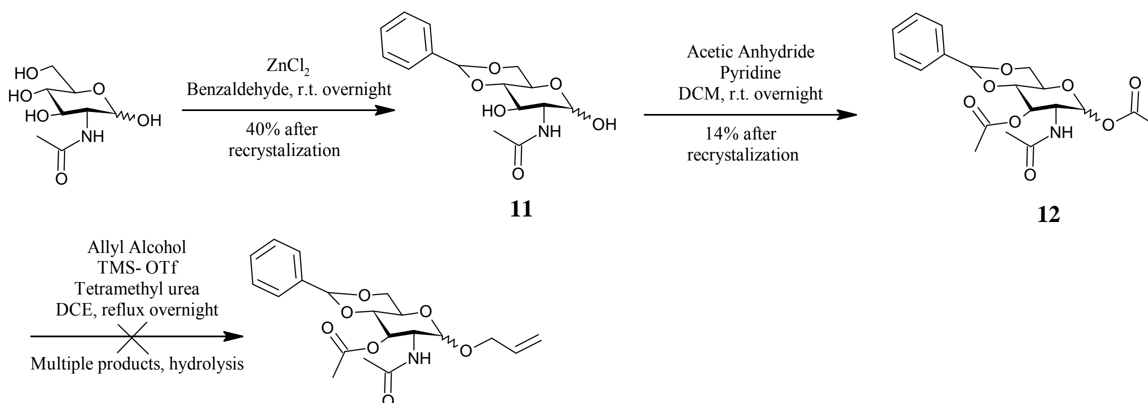
Figure 12. Hindered model sugar **10**.

Protected N-acetyl glucosamines have been shown to be especially non-reactive as glycosyl acceptors [96, 97]. The hydroxyl groups have experimentally been proven to be especially poor nucleophiles [98]. It has been proposed that intermolecular hydrogen bonding between the amide and hydroxyl groups is what is responsible for the hydroxyl's lack of reactivity [96].

Sugar **10**, whose synthesis is described in literature [99] contains benzyl ethers on the 3 and 6 positions that sterically hinder the 4-position hydroxyl acceptor site in between. This further hinders the hydroxyl as a nucleophile on this glucosamine derivative. This

deactivated sugar is thus an attractive target for us to screen. If a potential tag is able to couple with acceptable yield to this sugar, it will likely couple well to a wide range of glycosyl acceptors.

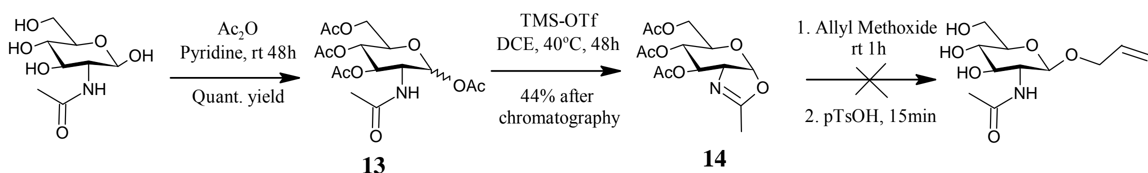
We started synthesis of this model deactivated sugar from N-acetylglucosamine, the monomer subunit of chitin. Our original synthetic strategy started off with the formation of a 4-6-O-benylidine acetal (**11**) and then acetyl protection (**12**) of the remaining hydroxyls, as described in literature [99]. However, the reflux condition with strong acid used in substituting the anomeric position acetyl group of sugar **12** with allyl alcohol proved to be too harsh for the molecule. After workup, thin-layer chromatography revealed a mixture of decomposition products.



Scheme 23. Initial synthetic steps towards sugar **10**.

Seeing the conditions for allyl alcohol substitution to be too strong, we decided to use an alternate method of introducing the allyl group to the sugar [100]. Global acetylation is done by acetic anhydride to give **13** in quantitative yield. The fully acetylated glucosamine is treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) to form oxazoline **14** (44% yield). We had issues with the oxazoline ring opening on the acidic

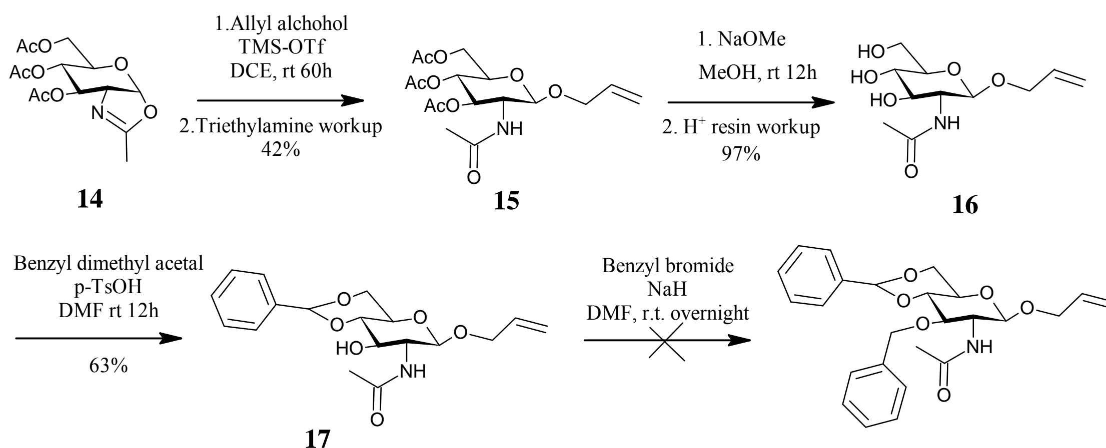
silica gel during column chromatography purification, however addition of 1% triethylamine to the chromatography solution solved this problem. The acetyl groups was then removed by treatment with NaOAllyl in allyl alcohol. After one hour, the solution was acidified with p-toluenesulfonic acid to attempt glycosylation of the oxazoline with allyl alcohol, but the reaction was not successful.



Scheme 24. Oxazoline ring formation

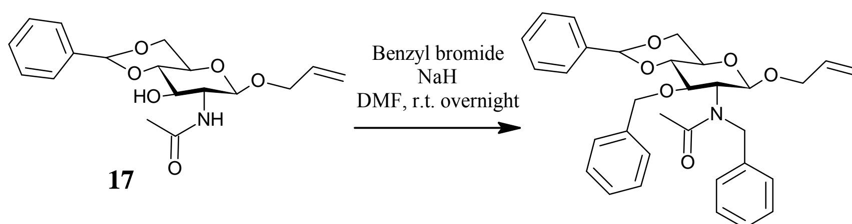
Seeing a one-pot approach to deacytylation and allyl glycosylation did not work, we decided on a step by step approach to formation of allyl protected N-acetylglucosame. Re-synthesized oxazoline **14** was opened by reaction with allyl alcohol with TMSOTf, forming allyl glycoside **15**. Because the reaction conditions are sensitive to water (leading to hydrolysis), we originally used molecular sieves to dry the reaction. However, we found that the yield after chromatography (25%) quite low. Suspecting that incomplete drying of the molecular sieves actually introduced water into the reaction, we ran the reaction without sieves and achieved a better yield (42%). After opening of the oxazoline, we used Zemplen deacetylation conditions to successfully remove the o-acetyl protecting groups to yield sugar 16 (97% yield) after work-up by Amberlight acidic resin. P-TsOH catalyzed acetal exchange was used to protect the 4 and 6 positions with

benzylidine acetal. Compared to acetal formation from benzaldehyde, acetal exchange allows milder acidic reaction conditions [101]. The reaction was originally done in a rotary evaporator at 50°C in order to drive the reaction to completion by removing the methanol byproduct. However, we found the solvent DMF being evaporated as well. Future benzylidene formations (to make sugar **17**) were carried out in a flask under argon overnight. Running the reaction for longer periods of time without evaporating off methanol gave us better yields (63% versus 20% for sugar **17**)



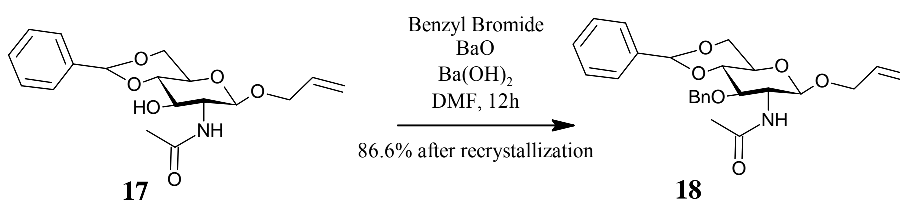
Scheme 25. Allyl and acetal protection

After protection of the 4 and 6 positions, we attempted to protect the 3 position of the sugar with a benzyl protecting group. Sugar **17** was benzylated with benzyl bromide and NaH overnight. NMR analysis after chromatographic purification of the product revealed a different structure than what we desired, however. Analysis of the NMR spectra revealed that the acetamino group has been benzylated along with the 3 position hydroxyl, giving us an unwanted product



Scheme 26. Global benzylation

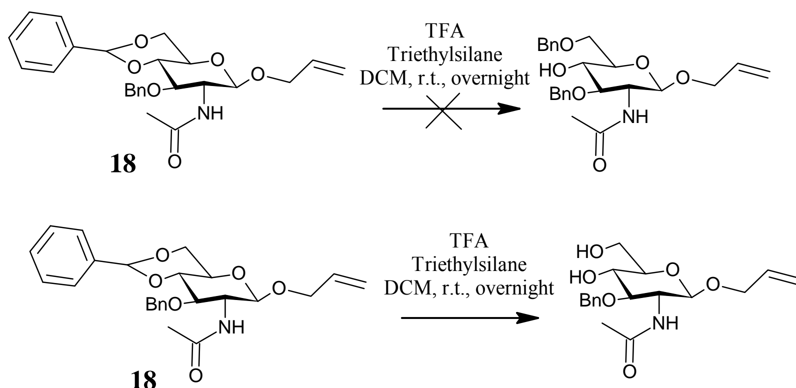
As benzylation by NaH was shown to be too strong, we found a milder procedure to achieve selective benzylation just the exposed hydroxyl of **17**. Re-synthesized sugar **17**, barium oxide, barium hydroxide, and benzyl bromide reacted at $0^{o}C$ to successfully yield benzyl protected sugar **18** in 87% yield [102].



Scheme 27. Milder benzylation conditions

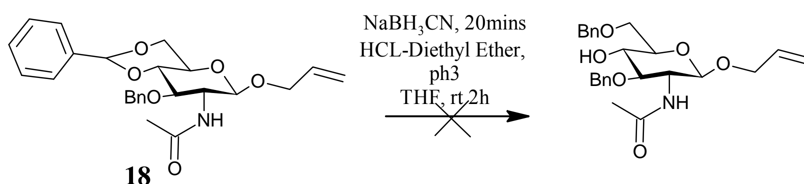
The final synthetic step to target sugar **10** was opening of the benzylidene ring to reveal a 4 position hydroxyl for attachment of the tag. Benzylidene ring opening requires acidic conditions, however, and some decomposed hydrolysis side products would be expected to form. We first investigated reductive ring opening using trifluoroacetic acid and triethylsilane [103, 104] We discovered that treatment with TFA gave us only hydrolysis products- instead of opening the benzylidene ring, it removed the acetal. This product is

not desired because it has two adjacent unprotected hydroxyl groups, and there is no know way to selectively protect only one of them.



Scheme 28. Attempted ring opening by TFA

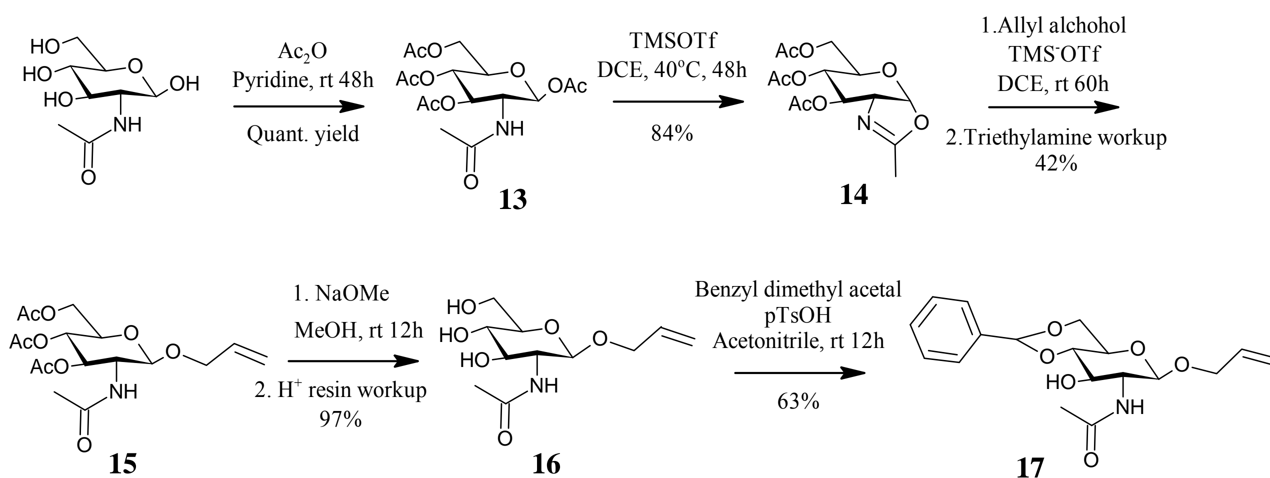
Next, we attempted benzylidiene ring opening on **18** with sodium cyanoborohydrate in acidic conditions [99, 102]. TLC revealed a mixture of products, as we would expect hydrolysis to occur alongside ring opening. After separation of the products by chromatography, we were unable to isolate any desired product.



Scheme 29. Attempted ring opening by $NaBH_3CN$

Following the disappointing results of benzylidene ring opening, we decided to use sugar **17** to screen tags. Sugar **17** provides a single free hydroxyl on the 3 position and as a protected glucosamine, is likely to be similarly deactivated to coupling as our original

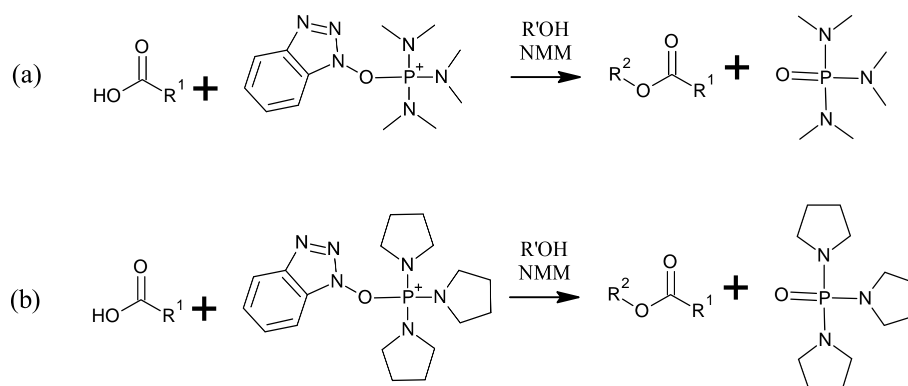
target sugar **9**. We worked on re-synthesizing a sufficient quantity of **17** to screen tags with. We had some problems with putting on the acetal during the synthesis of compound **16**; it was later discovered the reaction did not proceed because the solution was basic from unquenched methoxide leftover from Zemplen deacetylation (formation of compound **16**). Adding p-toluenesulfonic acid until the solution was acidic solved this problem and allowed the acetal exchange to proceed (with 63% yield). 2.7g of pure product **17** was obtained and ready for coupling to linker-tag.



Scheme 30. Synthesis of model hindered sugar 17 for screening of linker coupling.

2.4 Coupling of Proposed Linkers to Model Sugar

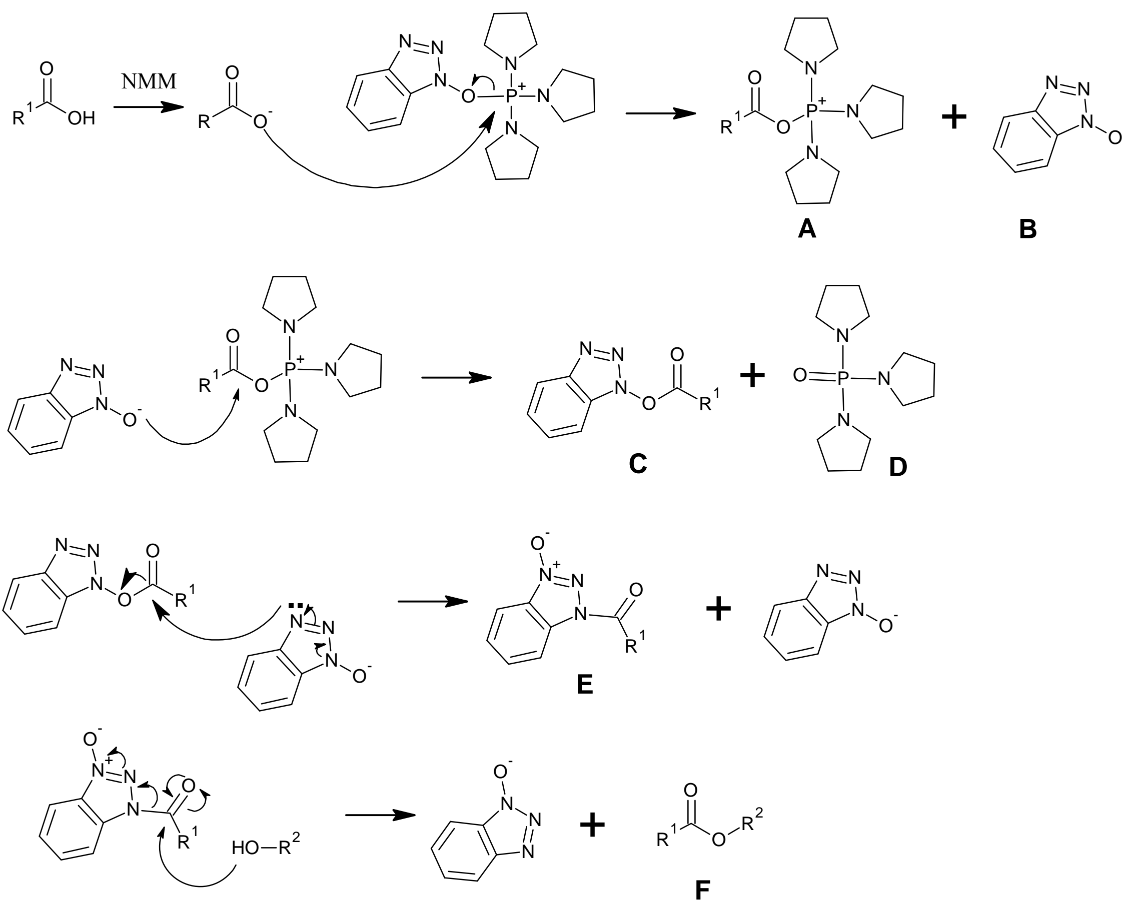
Having prepared potential linkers 1,2,4,6 and model deactivated sugar 17, we proceeded to screen conditions for coupling of linker to sugar. Coupling of the carboxylic acid linker to the sugar require the formation of an ester bond. PyBop [105] is a coupling reagent designed to promote difficult carboxylic acid couplings. It is an alternative to the coupling agent BOP, which produces the highly carcinogenic HMPA as a side product [106].



Scheme 31. Coupling of carboxylic acid with (a) Bop and (b)PyBop. The side product hexamethylphosphoramide (HMPA) of Bop coupling is a potent carcinogen. The side product of PyBop coupling, tris(pyrrolidino)phosphine oxide, is not as toxic.

The mechanism of PyBop coupling is complex [107]: First, an organic base (N-methylmorpholine) deprotonates the carboxylic acid. The carboxylate attacks the phosphate group on PyBop, producing BtO^-(B). BtO^- attacks ester A, and addition elimination reaction gives benzotriazole ester C and side product D. Then, another BtO^- molecule attacks the benzotriazole ester to form a benzotriazole amide with both + and – charges (E), and regenerates BtO^- . An excess of BtO^- drives this step. Then, in the

coupling step, the alcohol attacks the carbonyl of compound E. Addition-elimination gives the product ester E and regenerates BtO$^-$. The quenching of the positive charge of E is what drives the coupling.

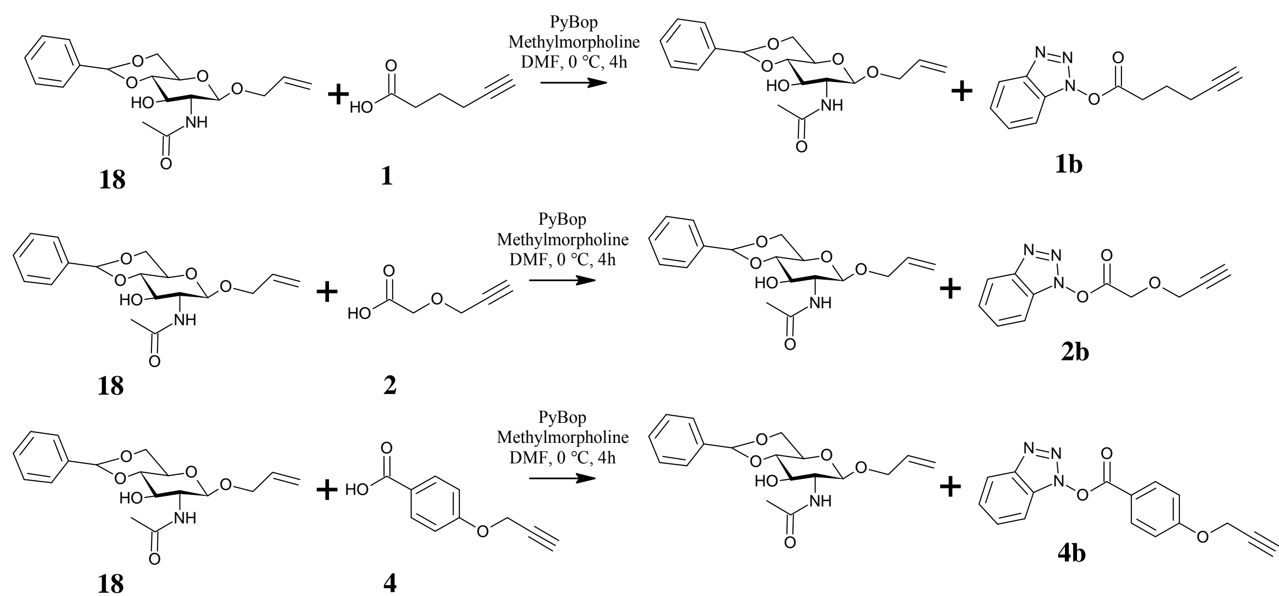


Scheme 32. The mechanism of PyBop coupling.

While PyBop is typically used for peptide coupling, forming an amide, peptide coupling agents are also often used for ester formation [108], so we reason that PyBop

would be suitable in linking a linker-lag to our model sugar. We have found in literature conditions in which PyBop is used to couple carboxylic acid with hydroxyl group [109].

We ran the coupling of linkers **1**,**2**, and **4** to model sugar **16**. We originally carried out the reaction with four fold molar excess of linker and PyBop. We monitored the reactions by TLC, looking for disappearance of starting material **16**. However, after the literature reaction completion time of four hours, the sugar starting material spot did not disappear from TLC. The reaction did not seem to be complete after 24 hours and even after several days, however, it seems that a new product has formed. After chromatographic purification of the products, NMR analysis showed that 1b,2b, and 4b remained in solution and failed to couple. These appeared to be reaction intermediate C in the PyBop coupling mechanism. This indicates that the reaction conditions were not sufficient to push through with the coupling. Since the reaction is partly driven by the concentration of BtO^-, we reasoned that a greater excess of PyBop could introduce more BtO^- into the reaction and drive it to completion. However, after repeating the couplings with a larger, eight fold excess of PyBop as well as at room temperature, we still did not get successful coupling of linker to sugar.

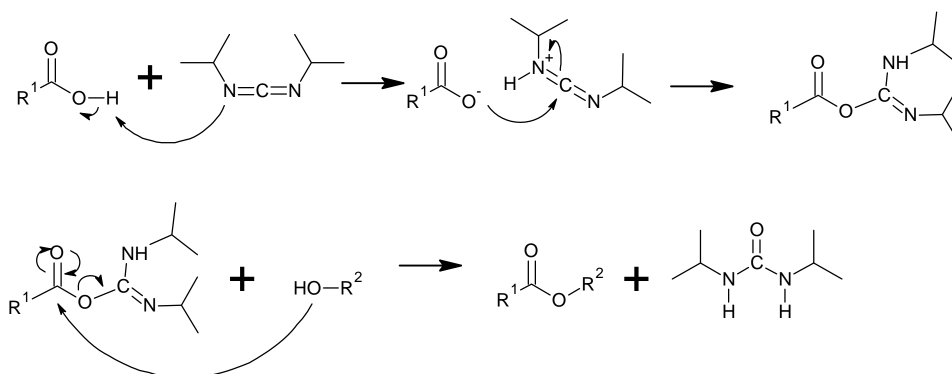


Scheme 33. Failed coupling by PyBop.

Following the failure of PyBop to couple linker to sugar, we explored other coupling agents capable of activating carboxylic acid to nucleophilic attack.

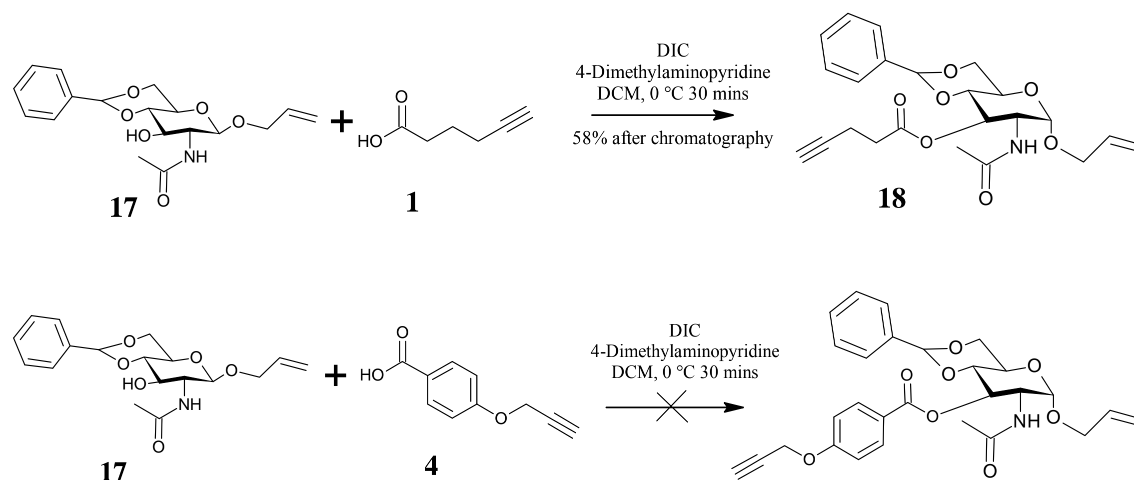
Carbodiimides are commonly used to activate carboxylic acids towards amide and ester formation [108, 110]. Diisopropyl carobidiimide (DIC) is commonly used as a coupling agent in solid synthesis because the side product, N,N'-diisopropylurea, is soluble in organic solvents and can be washed away.

In the coupling reaction with DIC, the carbodiimide first takes a proton from the carboxylic acid. The carboxylate then attacks the carbodiimide, forming O- acylisourea, which is activated to nucleophilic attack. The alcohol then attacks to form the ester product and N,N'-diisopropylurea side product.



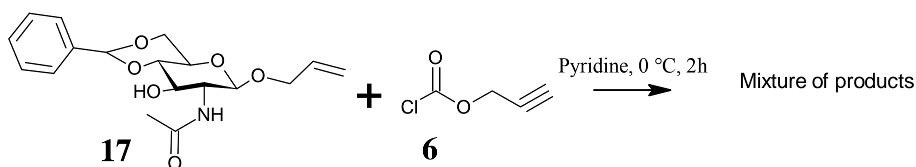
Scheme 34. Mechanism of DIC coupling.

Following conditions used for coupling of carboxylic acids to sugars [111], we used DIC to couple linkers **1** and **4** to model sugar **17**. We did not investigate linker 2 because it was an oil and thus more difficult to measure out. At first, none of the couplings were successful. While literature reports coupling to be complete after 20 minutes, in our reaction TLC revealed only the starting materials, even after 24 hours and beyond. We suspected that the DIC we used has been compromised, as the bottle was old. DIC decomposes into nonreactive N,N'-diisopropylurea over time and with exposure to moisture. After shipment of a new bottle of DIC, we attempted the coupling of linker to sugar again. Linker **1** successfully coupled to the sugar to give ester **18**. After a challenging chromatography procedure, pure **18** was obtained in 58% yield and characterized by NMR. There were more of the product **18** in impure chromatography fractions- collecting these fraction would greatly improve the overall yield. Linker **4**, however, failed to couple to the model hindered sugar. This is possibly due to steric effects- the phenyl ring in linker **4** makes it bulkier compared to linker **1**.



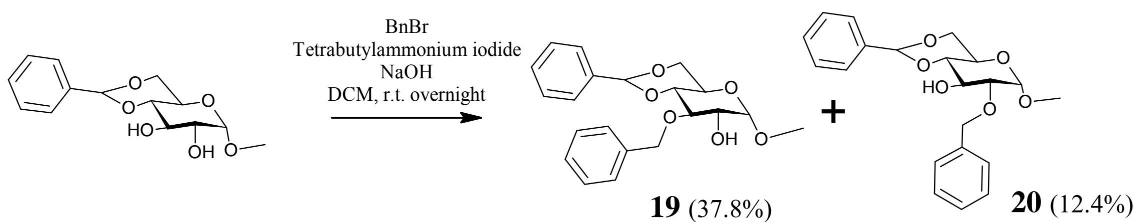
Scheme 35. Coupling of linkers to sugar with DIC.

We also investigated coupling acyl chloride **6** to hindered model sugar **17**. TLC analysis during reaction revealed a mixture of products, which would be difficult to isolate in chromatography. It is possible that hydrolysis products formed, as acyl chloride is water sensitive. Due to the success of DIC coupling of linker **1** to sugar, products were not purified and analyzed and other linkers were not investigated. A direction for future study would be an in-depth comparison of all the proposed alkyne functionalized linkers in coupling to sugar.



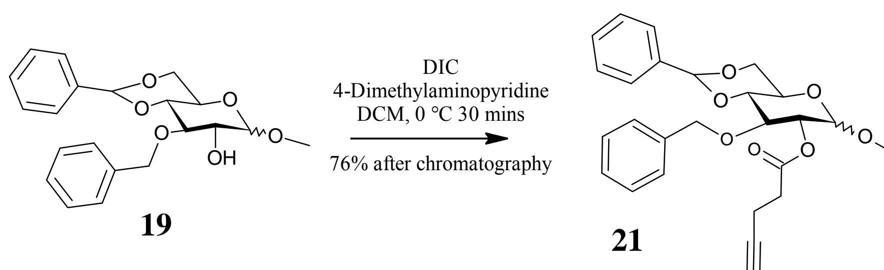
Scheme 36. Coupling of acyl chloride 6.

To test the DIC coupling of linker **1** to different sugars, protected sugars **19** and **20** were synthesized from methyl 4,6-O-(phenylmethylene)- α-D-glucopyranoside [112]. In this reaction, tetrabutylammonium iodide (TBAI) was used to generate the more reactive benzyl iodide in situ to facilitate benzyl ether formation. The reaction furnished compounds **19** (38%) and **20** (12%). Separation of the two compounds was done by flash chromatography.



Scheme 37. Synthesis of protected sugars 18 and 19.

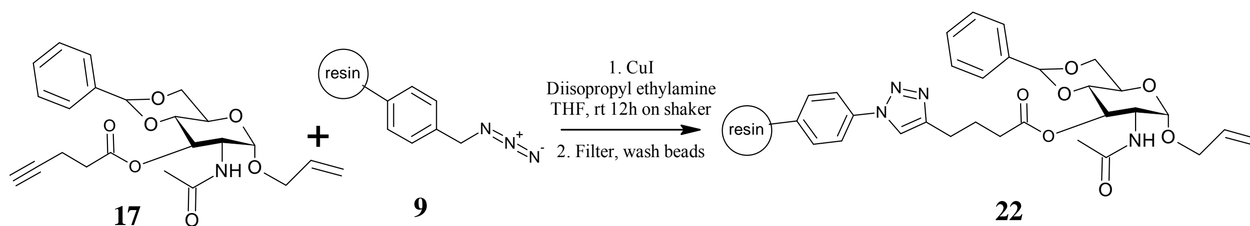
Linker **1** successfully coupled to sugar **19** using the same DIC coupling conditions as before. 76% was recovered after chromatography, and the product was verified by NMR. The successful coupling of linker **1** to sugars **17** and **19** established the feasibility of coupling an alkyne functionalized linker-tag to a sugar, including those with hindered acceptor sites. With a sugar now attached to an alkyne functionalized handle, we can investigate capture and release purification using click chemistry.



Scheme 38. DIC coupling of linker to sugar 19

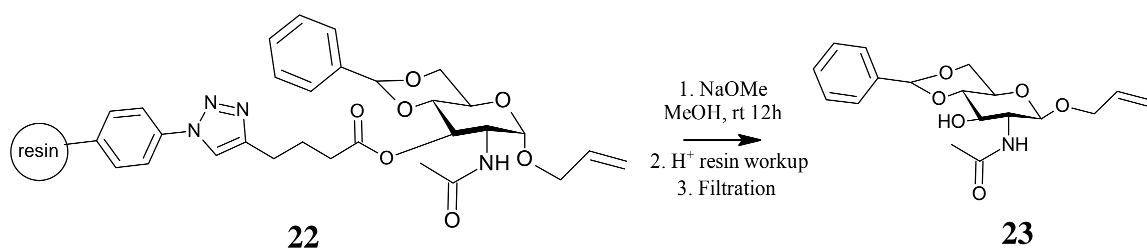
2.5 Click purification and Evaluation

Having established the feasibility of coupling an azide functionalized linker-tag (**1**) onto the free hydroxyl of a sugar (**16**), we examined the ability of this linker to capture the sugar azide functionalized capture resin **9**. To this end alkyne tagged sugar **17** was shaken overnight at room temperature with azide functionalized resin **9**, catalytic amount of copper iodide, and diisopropylethylamine, which was experimentally shown to accelerate the click reaction [113]. The solvent, THF, was chosen for ability to solvate the reagents and being able to swell the Merrifield resin, so its azide loading sites could be accessed. The progress of clicking onto resin was monitored by TLC. A disappearance of sugar **17** indicated successful linkage to the resin.



Scheme 39. Clicking of alkyne tagged sugar to azide functionalzed catch resin.

The resin was then filtered over a fritted filter and washed successively with THF (3x3 mL), methanol (3x3 ml), dichloromethane (3x3 ml), and diethyl ether (3x3 ml) to remove the reagents used in the click reaction, including the copper. After washing of the resin and captured sugar, methanolysis was used to release the sugar from the linker-resin complex. After workup with Amberlite acidic ion exchange resin, the cleavage product **23** was dried in vacuo to afford product **23** in 96% yield from **17**.



Scheme 40. Release of sugar from capture resin.

Comparison of the NMR characterization of **22** with that of **16** showed they are identical compounds. This indicates that we have successfully gotten the original sugar **16** after click chemistry capture and release.

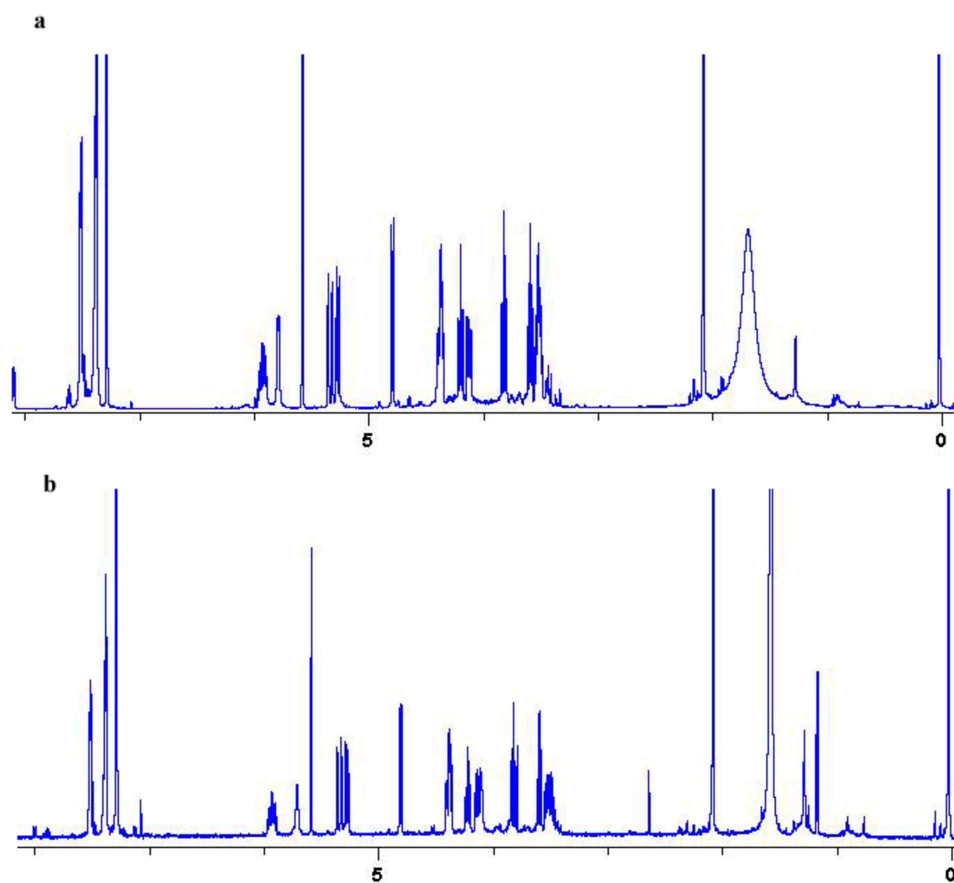


Figure 13. NMR spectra of a) compound 16 and b) compound 22

2.6 Conclusion

The high yield (96%) and purity of the sugar after cleavage proves the applicability of click chemistry to capture and release purification of sugars. It indicates that a) the linker-tag specifically “clicked” onto the capture resin with high yield and b) most of the product was successfully released from the resin by methanolysis.

In this chapter we have demonstrated proof of principle of using click chemistry for capture and release of sugar. We described the synthesis of a model deactivated sugar and the coupling of an alkyne functionalized linker-tag onto the sugar. We described the synthesis of an azide functionalized capture resin and the capture-release of the tagged sugar with copper catalyzed 1,3 Huisgen cycloaddition, or “click” chemistry.

With proven conditions for linker tag attachment, click capture, and release, we continued on to demonstrate proof of principle of click chemistry based capture-release on solid phase oligosaccharide synthesis.

Chapter 3

Cap, tag, and click Solid Phase Synthesis

3.1 Design of Orthogonally Protected Mannose for Solid Phase Studies

After demonstrating the feasibility of a tag, capture, and release purification method utilizing click chemistry, we evaluated this chromatography-free purification method on solid phase oligosaccharide synthesis,

As a target for solid phase synthesis, we chose α-linked trimannose. As mentioned in the introduction, this oligosaccharide appears is part of the gp120 high mannose structure, and appears to be a recognition epitope for the antibody 2G12 [26]. A practical reason for choosing this sequence as a target is that so we would not have to worry about the complexities of anomeric linkages during synthesis. Mannose prefers an α linkage: -linked mannosides requires special procedures to create [116]. While anomeric control of glycosidic linkages is a topic our lab is investigating, this project is focused on a chromatography free solid phase synthesis method. Advances in anomeric control of glycosylation alongside a streamlined synthesis procedure would work in synergy to allow access to a wide variety of sequences.

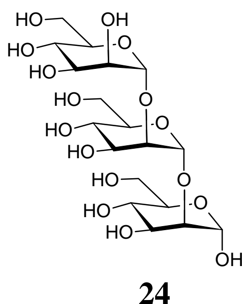
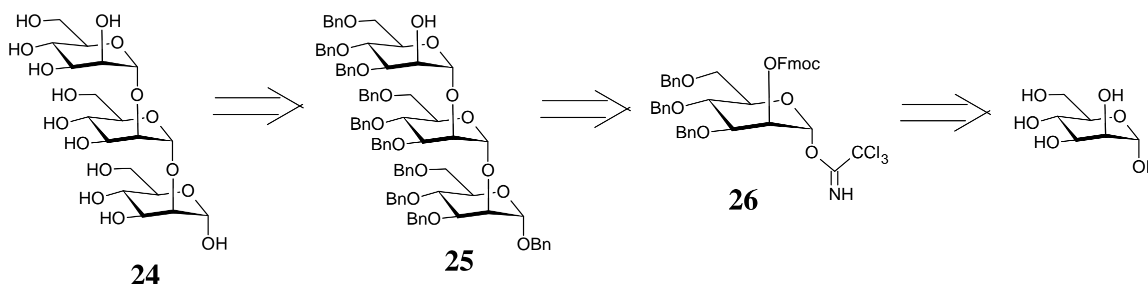


Figure 14. α-linked trimannose.

Retrosynthetic analysis show that our target oligosaccharide 24 can be made from **25**. Protected trimannose **25** will be our target for solid phase synthesis.



Scheme 41. Retrosynthetic analysis of trimannose.

Oligosaccharide **25** can be made in solid phase synthesis by three rounds of glycosylation with trichloroacetimidate donor **26**. Trichloroacetimidates, first described by Schmidt in 1980, have the advantages of ease of formation, stable at room temperature, and high glycosylation yields [40,41]. As mentioned in Chapter 1, trichloroacetimidates are activated by catalytic amounts of Lewis acid, frequently trimethylsilyl trifluoromethanesulfonate.

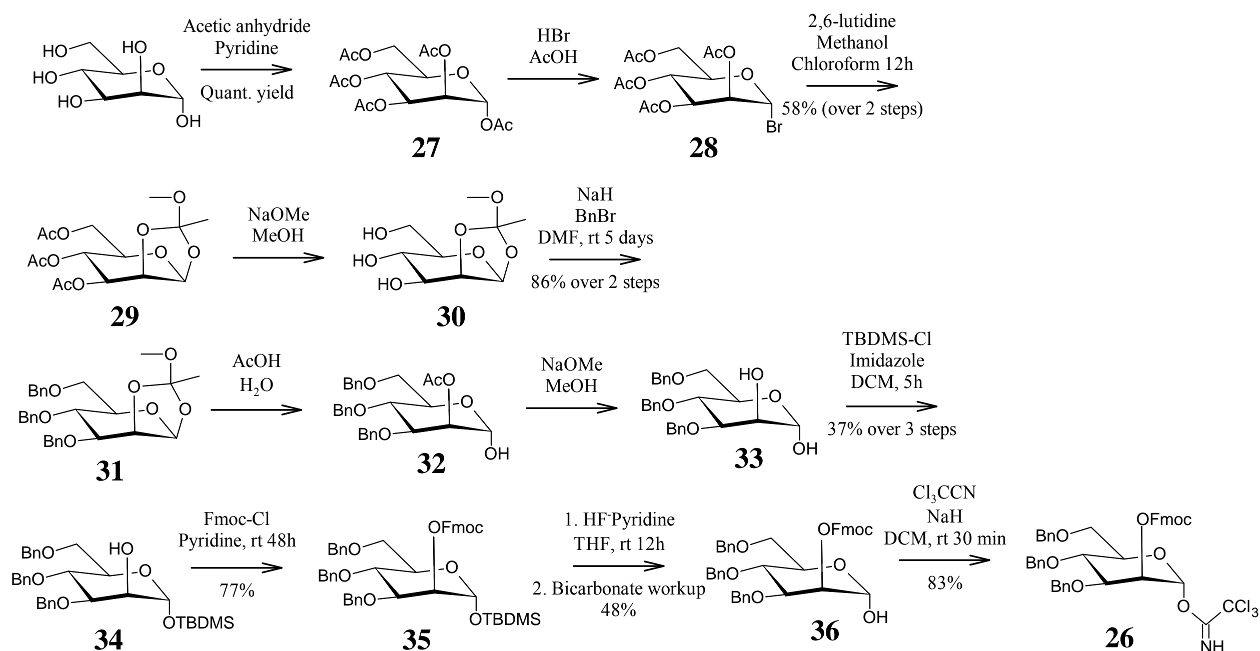
Fmoc is a base-labile temporary protecting group commonly used in peptide and oligosaccharide synthesis [30]. Fmoc allows us to quantify the glycosylation steps by spectroscopic measurement of the piperidine-dibenzofulvene adduct released during its cleavage [117].This way, we could follow incomplete glycosylation and formation of truncated sequences during solid phase synthesis if concentration of piperidine-dibenzofulvene in leftover cleavage solution decreases after each glycosylation cycle.

Benzyl groups are base- stable and thus orthogonal to Fmoc as a protecting group. Stable to acid as well, benzyl groups are a commonly used permanent protecting group. They are removed by hydrogenolysis after synthesis is complete.

The strategy for synthesizing protected mannose trichloroacetimidate **26** is to first temporarily protect the 1 and 2 positions with an orthoester ring, then globally benzylate the unprotected 3,4, and 6 positions. After opening the orthoester, position 1 can be temporarily protected while the 2 position protecting group is put on. After taking off the temporary protecting group, the sugar can be converted into the desired trichloroacetimidate.

Synthesis of the trichloroacetimidate **26** started off with the global acetylation of mannose to form **27** in quantitative yield [118]. Excess pyridine is removed from the viscous oil product by repeated co-evaporations with toluene, until NMR confirmed a pure **27** without traces of pyridine. Treatment with HBr in acetic acid converted the protected mannose into mannosyl bromide **28**, which is then treated with 2,6-lutidine in methanol to form orthoester **29**. Compound **29** was purified by precipitation from solution. The acetyl groups on orthoester **29** were removed by treatment with potassium carbonate in methanol to give **30**. Originally, compound **30** was taken into the next reaction without purification. However, we found that benzyation of unpurified **30** resulted in a mixture of products that was very difficult to separate. This was a result of both incomplete deacetylation and incomplete benzylation. To prevent incomplete deacetylation, **29** was subjected to the stronger Zemplen deacetylation conditions using sodium methoxide in methanol. Benzylation once again resulted in a complex mixture. We solved this problem by purifying compound **30** by column chromatography before the next reaction. This was

a challenging column due to the scale in which it was carried out (10g of material). Such scales were necessary to ensure sufficient product at the end of a multi-step synthesis. The most challenging part of this synthesis was maximizing product yield while ensuring a purity. Chromatography presumably removed side products that were left over from precipitation of compound **28**. Benzylation of purified **30** resulted in an easier to separate mixture. Incomplete benzylation was minimized by running the reaction over a longer period of time (5 days) and by adding benzyl bromide over the course of the reaction to drive it to completion. Chromatographic purification yielded benzyl protected orthoester **31** in 86% yield. Opening of the orthoester was done with acetic acid in water to form sugar **32** [119]. After removal of water by co-evaporation with toluene, the sugar was deacetylated to give compound **33**. A temporary tert-butyldimethylsilyl (TBDMS) protecting group was put on the 1 position with TBDMS-Cl and imidazole [120]. The product was purified by chromatography to give TBDMS protected sugar **34** in 37% yield. TBDMS adds preferentially to the 1 position of the sugar because the 1 position hydroxyl is more activated. With the 1 position protected by TBDMS, Fmoc was added to the 2 position with Fmoc-Cl in pyridine to give compound **35** (77% yield after chromatography). The TBDMS group was then removed with HF-pyridine to give compound **36** (48% yield after chromatography). The base labile Fmoc group was not effected by this acidic deprotection. Compound **36** was treated with trichloroacetonitrile and NaH (86% after chromatography) to give trichloroacitimdate donor **26,** ready for solid phase synthesis.



Scheme 42. Synthesis of protected glycosyl donor

3.2 Design of Solid Phase Linker Resin

As explained in the introduction, a variety of solid supports have been used in solid phase synthesis. We chose to investigate our chromatography-free synthesis on Merrifield resin because it is well-documented in solid phase synthesis and inexpensive as well. The solvents we will use during glycosylation and linker tag coupling (DCM) is nonpolar and thus able to swell the resin to maximize loading.

The tether that attaches the growing oligosaccharide to the polymer support must be stable to reaction conditions during synthesis (removal of temporary protecting group and activation of glycosyl donor), as well as being cleavable once synthesis complete to release the completed oligosaccharide from the solid support. We will use a thiol functionalization to link the growing oligosaccharide to solid resin. Thiol functionalized Merrifield's resin have been reported to give good yields by glycosylation with

trichloroacetimidates [121]. The sulfur linkage can be activated and cleaved with a thiophilic compound to release the sugar from resin [121]. Importantly, our lab is investigating this thiol activation for direct transfer of glycans from resin to aglycone. By developing our solid phase synthesis procedure with a thiol functionalized resin, we will be able to do such a transfer after synthesis once these conditions are worked out. This technology would allow us to easily create glycans linked compounds such as glycoproteins for biochemical study.

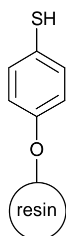
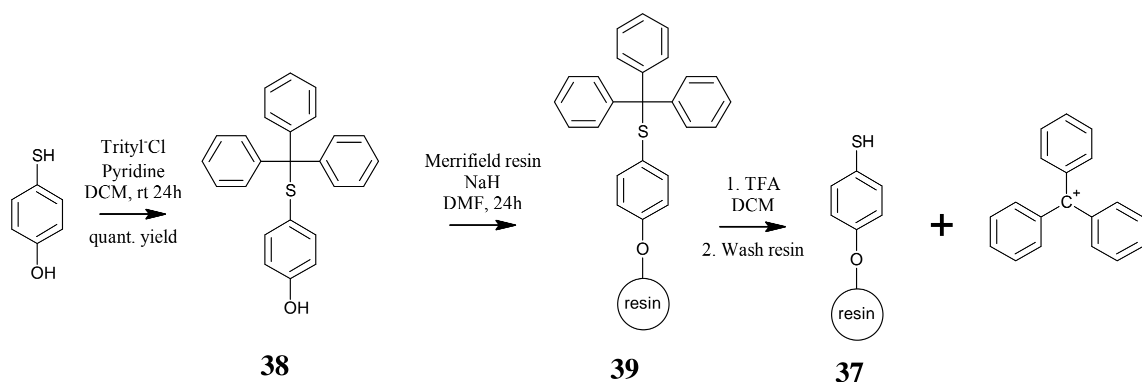


Figure 15. Thiol functionalied Merrifield resin.

Thiolphenol was protected with trityl group in pyridine to give compound [38] in quantitative yield. That compound was functionalized to Merrifield's resin by reaction with NaH to give trityl protected resin 39. Cleavage of the trityl group by trifluoroacetic acid (TFA) gave us thiol functionalized resin 37, as well as trityl cation released into solution with which we quantified the thiol functionalization of the resin.



Scheme 43. Synthesis of thiol functionalized resin

Quantification of thiol resin loading:

The thiol functionalized resin's loading was determined by the amount of trityl cation found in solution after cleavage. 0.25ml of the solution after cleavage and washing was added to 3ml of 10% TFA in DCM. The trityl cation was detected by UV-Vis spectroscopy and quantified using Beer's law.

Beer's law: A=ebc

e= molar extinction coefficient = 35,300 (L mol^{-1} cm^{-1})for trityl cation at 410 nm (Guzei JOC 2004)

b=path length = 1 cm

Absorbance at 410nm: 0.31238

C=8.85E-6 mol L^{-1}

Sample was diluted 12x, so

8.85E-6 x 12 = 0.001062 mol L^{-1}

0.001062 mol L-1 x 9ml = 0.009558 mmol total cation in solution

0.009558mmol/0.2g of resin

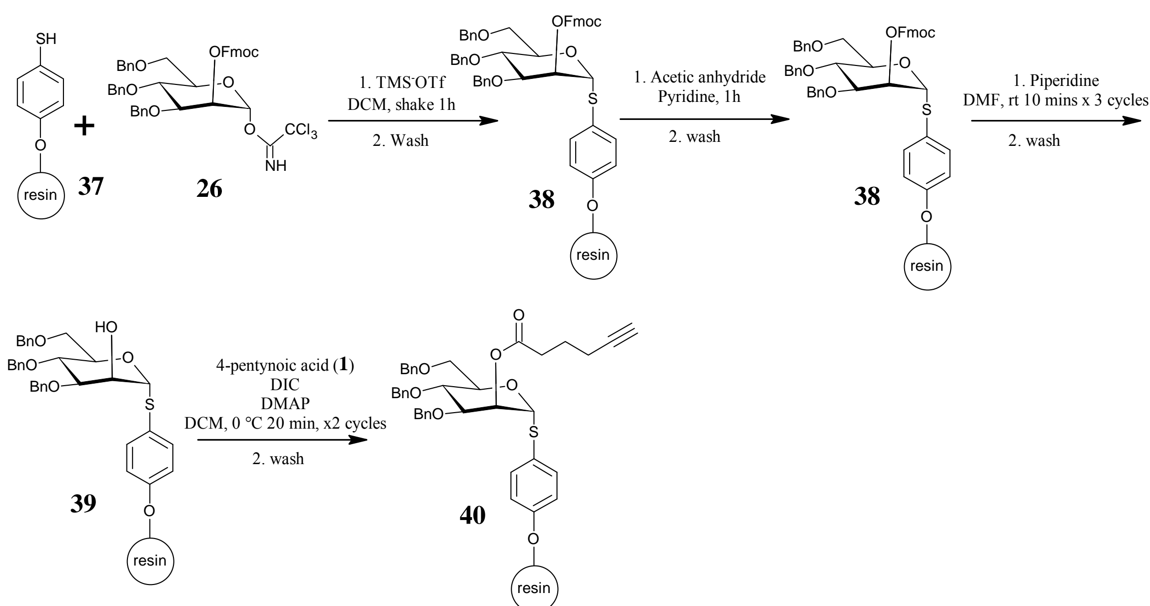
=0.0478 mmol/g

Calculated loading of thiol functionalized resin: 0.0478 mmol/g

3.3 Solid Phase Synthesis and Click Purification

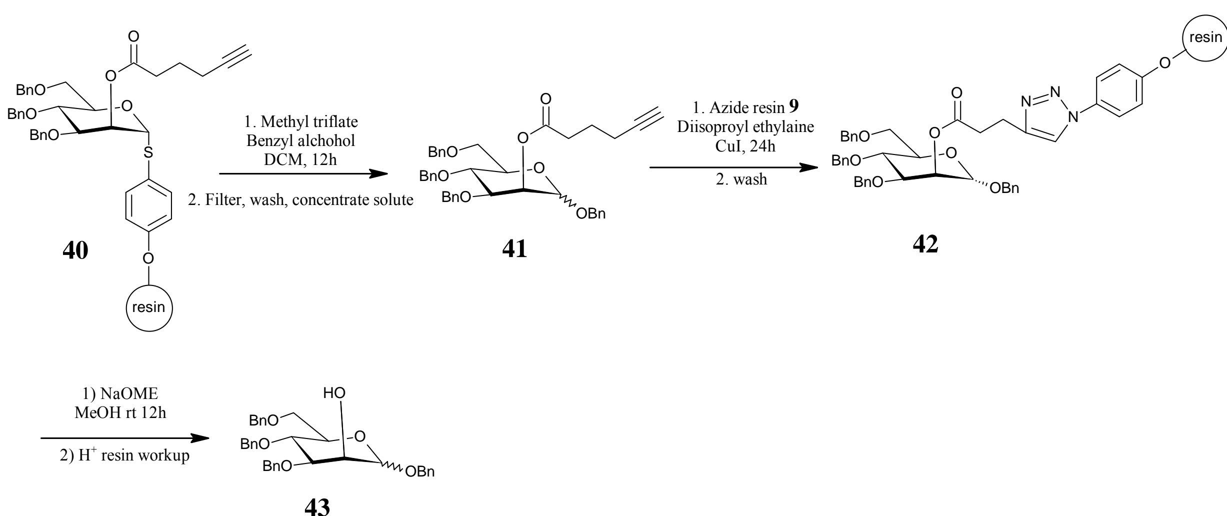
To test the click chemistry capture release strategy on solid phase, we carried out the solid phase synthesis and click capture-release purification on a single mannose unit.

Donor **26** was loaded onto thiol resin **37** by activation with trimethylsilyl trifluoromethanesulfonate (TMSOTf). After shaking for one hour, the resin was filtered and washed with, DCM (3x3 ml), THF (3x3 ml), MeOH (3x3 ml) and DCM (3x3 ml). Any unreacted loading sites on the resin were then capped with acetic anhydride in pyridine, and shaken one hour, followed by the standard washing sequence. Then, the Fmoc protecting group was removed by treatment with 4:1 DCM:Piperidine. The reaction mixture was shaken for 10 minutes and washed. The deprotection and washing procedures were repeated three times to ensure deprotection of the sugar. Following deprotection, linker-tag **1** was coupled with DIC to the exposed hydroxide **39**, as described in Chapter 2. The coupling reaction was carried out twice to maximize coupling. Washing of the resin would save us product [40] on resin.



Scheme 44. Conjugation of linker to sugar on solid phase.

The sugar **40** was then cleaved from the resin by activation methyl triflate in benzyl alcohol. Benzyl alcohol was used as acceptor for the activated sulfur, forming **41**. The cleaved product was washed into solution. We then used azide functionalized capture resin **9** to capture the tagged sugar. The capture resin, Cu(I), and DIC were shaken in the cleavage solution for one day, then the resin was washed to give us the sugar captured on resin. The ester bond between the sugar and the linker-tag was cleaved by methanolysis to give product **43**.



Scheme 45. Capture and release with click chemistry.

TLC analysis showed two spots after release from the capture resin, which we would expect because the linkage to benzyl alcohol would result in two anomers. 0.0023g of product was collected. Comparison of NMR spectra of product **43** with sugar **33** showed many of the peaks in common. We would not expect matching NMR spectra because **43** was been conjugated to an additional, anomeric benzyl group. We saw a heavy amount of grease (large peaks at the lower chemical shifts) in the spectra. This is likely to come from sources such as plastics dissolved in solvent. The very small amount of product isolated would make the grease peaks look large on NMR. Product **43** was run through a silica gel “plug” to attempt to wash out the grease to allow better characterization, but this was not successful. Nevertheless, the similar peaks of the spectra of the two compounds showed that we were successful in obtaining the product **43** after tagging on solid phase, cleavage from solid support, click capture , and cleavage from capture resin. This demonstrates the feasibility of our procedure in the solid phase.

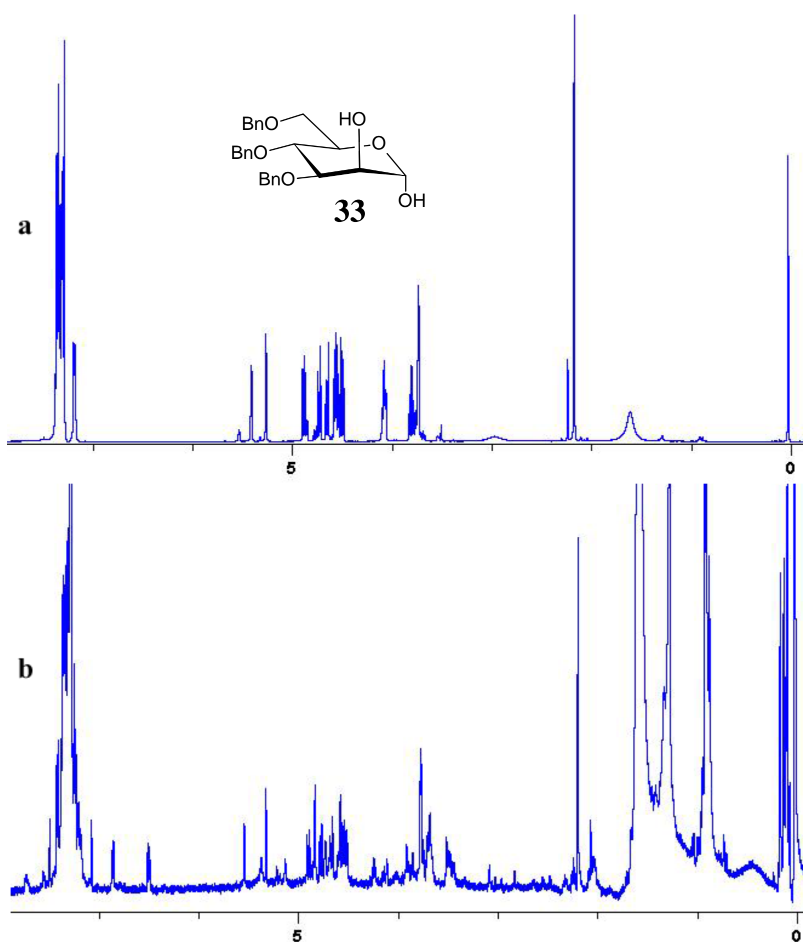


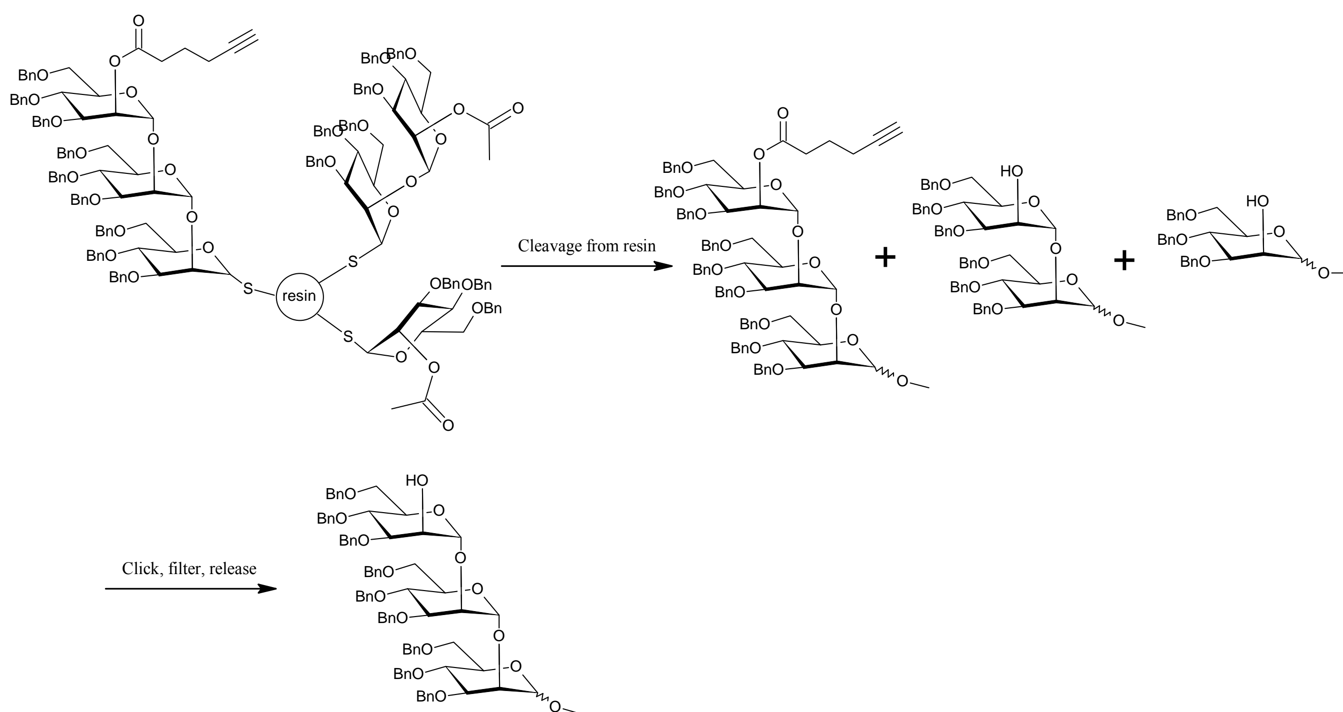
Figure 16. NMR spectra of a) sugar **33** and b) synthetic product **43**

3.4 Solid Phase Synthesis and Click Purification of Trimannose

After testing our cap, tag, and click solid phase synthesis procedure on a single mannose unit, we used the procedure to synthesize our target oligosaccharide **25**.

After loading onto resin, we used only 0.75 equivalents of donor at each glycosylation in order to purposely create truncated sequences. Measurements of piperidine-dibenzofulvene adducts after each cleavage step allows us to quantify how many successful glycosylations and how many truncated sequences there are on resin.

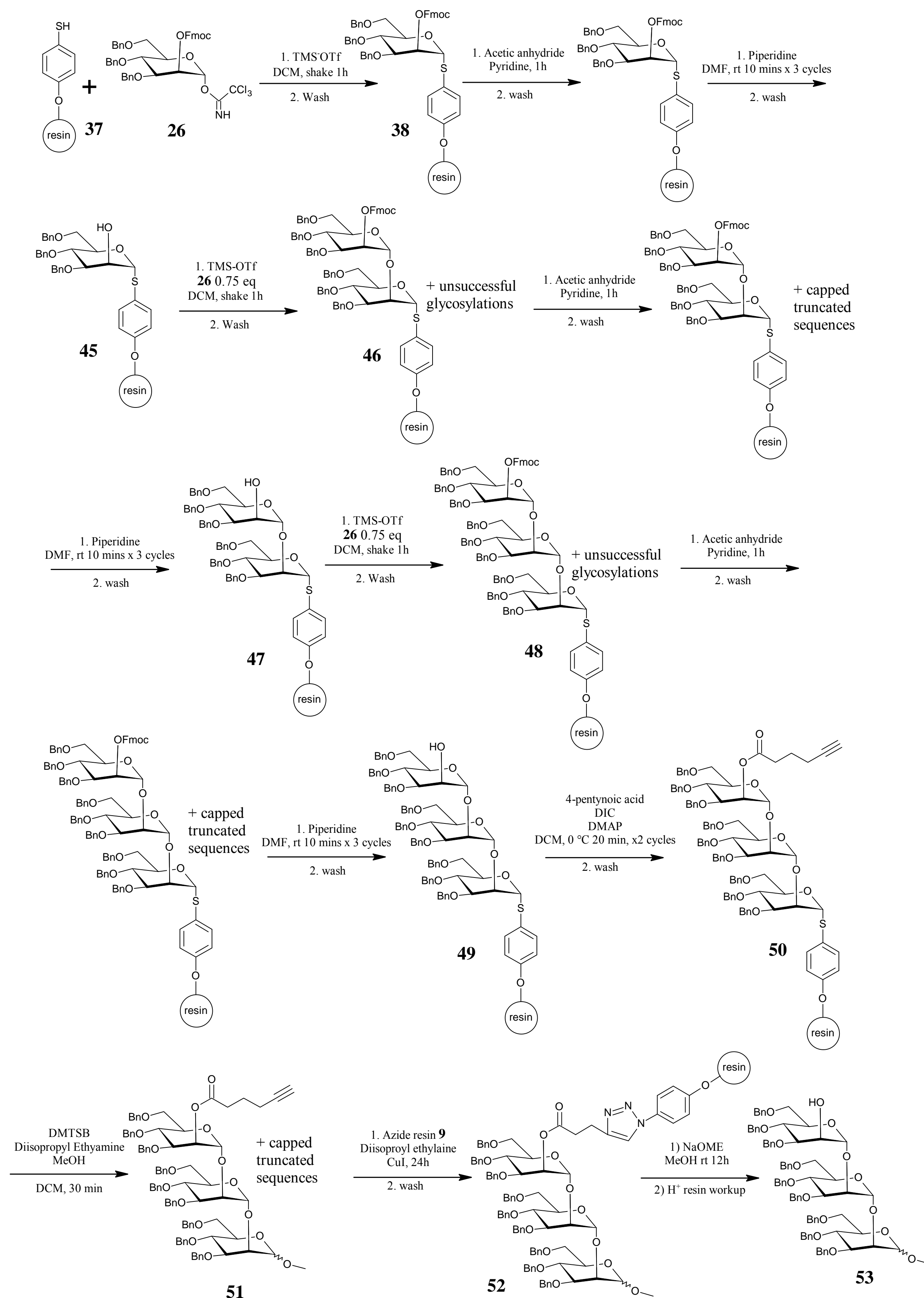
These truncated sequences will be capped. Following cleavage from resin, we would have tagged complete sequences and capped truncated sequences in solution. The scavenger resin will click on the completed sequences, and the truncated sequences will be washed away, leaving us with the desired sequence at then end of the procedure.



Scheme 46. Formation of truncated sequences and isolation of desired sequence with click chemistry based capture and tag.

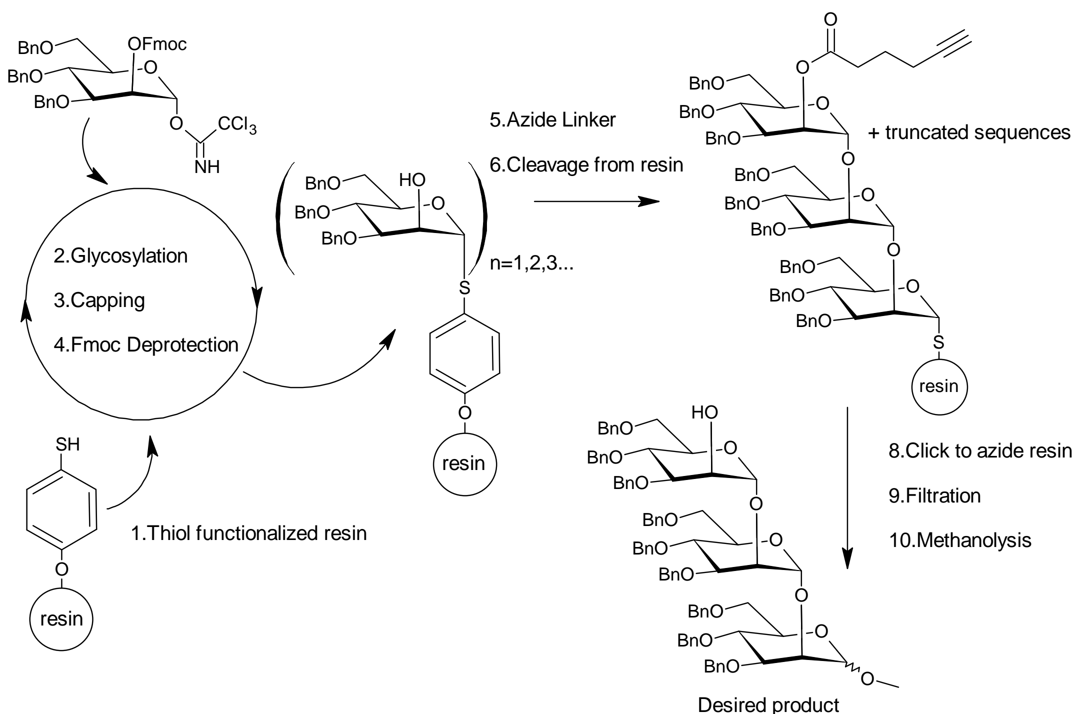
Donor **26** was loaded onto thiol resin **37** by activation with trimethylsilyl trifluoromethanesulfonate (TMSOTf). After shaking for one hour, the resin was filtered

and washed 10 minutes each with DCM (3x3 mL), THF (3x3 ml), DCM (3x3 ml), MeOH (3x3 ml) and DCM (3x3 ml). We washed the resins excessively to ensure impurities were washed off completely. Any unreacted loading sites on the resin was then capped with acetic anhydride and in pyridine, and shaken for one hour, followed by the standard washing sequence. Then, the Fmoc protecting group was removed by treatment with 4:1 DCM:Piperidine. The reaction mixture was shaken for 10 minutes and washed. The deprotection and washing was repeated three times to ensure deprotection of the sugar. The washings containing piperidine-dibenzofulvene adduct was saved for later analysis. After deprotection to get glycosyl accepter **45**, glycosylation was carried out with 0.75 equivalents of donor **26** activated by TMSOTf. Then, any glycan acceptors that failed to glycosylation were capped by acetic anhydride. The Fmoc was removed by a second round of 4:1 DCM:Piperidine. The washings from the second deprotection were once again saved. This cycle of glycosylation/capping/deprotection was carried out one more time to give trimannose **48**, along with capped truncated sequences, linked to resin. Following deprotection, linker-tag **1** was coupled to the exposed hydroxide on **49**. We decided to use dimethyl (methylthio) sulfonium triflate (DMTST), a thiol activator, to cleave the sugar from resin. DMTST is milder than methyl triflate for cleavage [122]. Methanol was used as an accepter, giving alkyne tagged trimannose **51** alongside capped truncated sequences in solution. The tagged sequences were captured onto the azide functionalized resin by copper catalyzed 1,3 Huisgen cycloaddition to give resin bound triazole **52**. After washing away reactants and truncated sequences, methanolysis was used to cleave the completed sugar from the resin-triazole complex



SH
O
resin
37
OFmoc
BnO
BnO
BnO
O
O
CCl3
NH
26
1. TMS-OTf
DCM, shake 1h
2. Wash
S
38
1. Acetic anhydride
Pyridine, 1h
2. wash
1. Piperidine
DMF, rt 10 mins x 3 cycles
2. wash
HO
45
1. TMS-OTf
26 0.75 eq
DCM, shake 1h
2. Wash
46
+ unsuccessful glycosylations
1. Acetic anhydride
Pyridine, 1h
2. wash
+ capped truncated sequences
1. Piperidine
DMF, rt 10 mins x 3 cycles
2. wash
47
1. TMS-OTf
26 0.75 eq
DCM, shake 1h
2. Wash
48
+ unsuccessful glycosylations
1. Acetic anhydride
Pyridine, 1h
2. wash
+ capped truncated sequences
1. Piperidine
DMF, rt 10 mins x 3 cycles
2. wash
49
4-pentynoic acid
DIC
DMAP
DCM, 0 °C 20 min, x2 cycles
2. wash
50
DMTSB
Diisopropyl Ethyamine
MeOH
DCM, 30 min
51
+ capped truncated sequences
1. Azide resin 9
Diisoproyl ethylaine
CuI, 24h
2. wash
N
52
1) NaOME
MeOH rt 12h
2) H+ resin workup
53

Scheme 47. Solid phase synthesis of trimannose using cap, tag, click, and release Procedure



Scheme 48. Synthetic cycle for cap, tag-click-capture synthesis

Compound [46] was determined to be one spot on TLC, indicating a pure compound. After drying in vacuo, it was analyzed by NMR. The NMR spectra did not show evidence of the presence of truncated sugars, indicating the synthesis strategy worked. However, we still see prominent amounts of grease in the spectra. This is explained by a low amount of product that we obtained, in part because of the initial solid phase resin's low loading capacity, as shown in section 3.2.

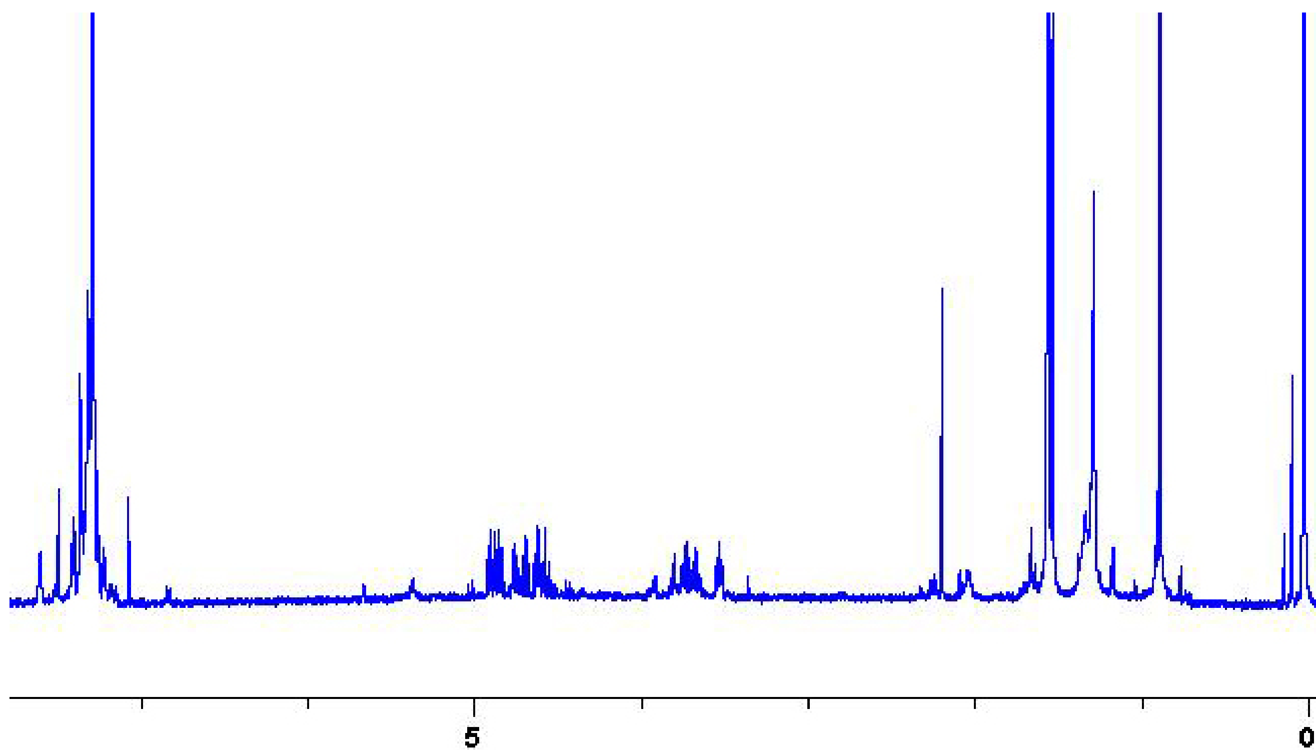


Figure 17. NMR spectra of the product of chromatography-free solid phase synthesis.

To determine the identity of the product, we used matrix-assisted laser desorption/ionization (MALDI) to analyze a sample of it. Negative-ion MALDI reported a prominent peak at molecular mass of 1327, which is a weight we could expect for an ionized form of sugar **53**.

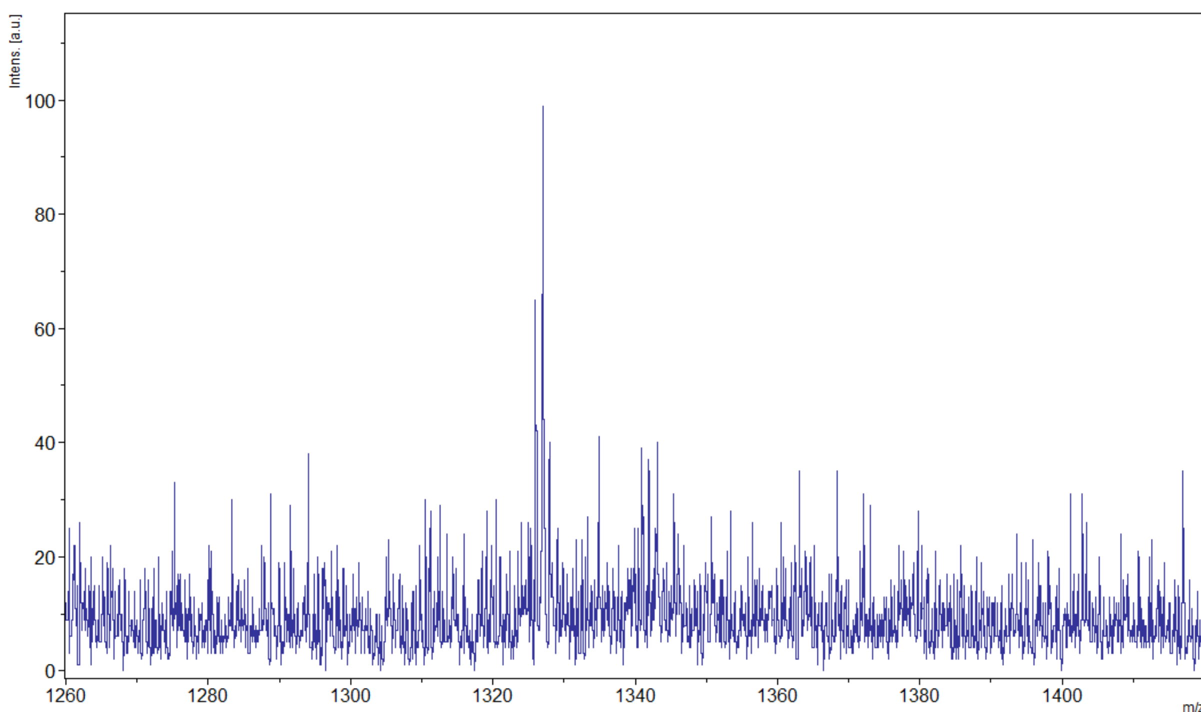


Figure 18. MALDI of product 53.

The amount of glycosylation that occurs during each cycle is measured indirectly by the concentration of dibenzofulvene measured by UV spectrometer. Successful glycosylation incorporates an Fmoc projecting group on the resin, which is cleaved off during deprotection.

e= molar extinction coefficient = 7800 (L mol^{-1} cm^{-1})for dibenzofulvene at 301 nm (seebach 2000)

b=path length = 1 cm

A=Absorbance at 301nm:

C=concentration in solution (in mol L^{-1})

Total moles= C x 0.022L

% successful glycosylation = # moles/#moles during first deprotection

Glycosylation	A	E	path length	C	total moles in solution	% successful glycosylation
1st	0.095	7800	1 cm	1.2179E-05	2.67949E-07	
2nd	0.054	7800	1 cm	6.9231E-06	1.52308E-07	0.568421053
3rd	0.04	7800	1 cm	5.1282E-06	1.12821E-07	0.421052632

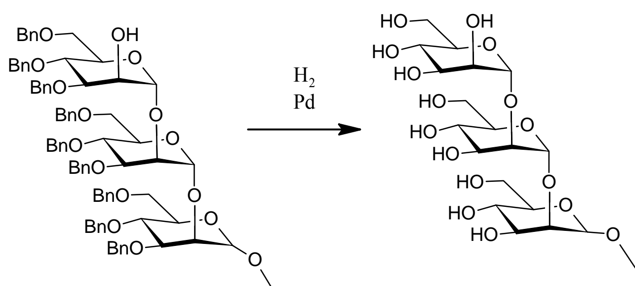
Table 2. UV quantification of glycosylation

After three glycosylations, 42% of sequences are the full length oligosaccharide while the rest are truncated sequences.

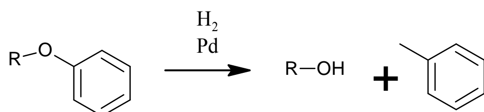
Judging from the NMR spectrum, the product of our synthesis method seems devoid of these truncated sequences

3.5 Removal of Benzyl ethers by treatment with Encapsulated Palladium.

After solid phase oligosaccharide synthesis, the product we obtain would still contain protecting groups. A final deprotection step is necessary to obtain a sugar that would be ready for bioassays. Benzyl ethers are commonly used as protecting groups in solid phase synthesis because they are stable to reaction conditions during solid phase and to conditions used to cleave temporary protecting groups. Stable to both basic and acidic cleavage conditions, benzyl ethers are instead cleaved by palladium transfer hydrogenation (quai). Palladium catalyzed transfer hydrogenation cleaves benzyl ethers into alcohol and toluene.



Scheme 49. Deprotection of synthesized oligosaccharide



Scheme 50. Hydrogenation of benzyl ether.

During the hydrogenation, some palladium nanoparticles will leech into the solution with the sugar. This is problematic because palladium is often not compatible with biological screening. For example, palladium is shown to have anti-microbial activities (graham 1979). Its presence in a bioassay could complicate an experiment to test the effects of a sugar. Thus, oligosaccharide synthesis requires another chromatography step after isolation of the sugar to get rid of palladium nanoparticles.

Pd Encat uses polyurea to encapsulate palladium. This retains the catalytic properties of palladium while minimizing palladium leaching and allowing simple cleanup by filtration. In a sense, it allows deprotection of benzyl groups on the "solid phase".

After deprotecting the sugar, the PdEncat catalyst can be removed via filtration. Toluene side product can be removed in vacuo. Combining benzyl ether deprotection with encapsulated palladium with chromatography-free solid phase synthesis results in a

streamlined procedure for oligosaccharide synthesis from donor building blocks to a pure, deprotected sugar ready for biochemical analysis.

Hydrogenolysis of compound **53** under hydrogen gas and catalyzed by PdEncat produced a compound that, based on its NMR spectra, still seemed to contain phenyl groups. While our current results from Pd-Encat hydrogenolysis have not been encouraging, we will need to obtain more material to test different conditions for hydrogenolysis.

3.6 Conclusion

In conclusion, we have introduced and demonstrated proof of principle of the "cap tag, and click" method of solid phase oligosaccharide synthesis. We used the cap and tag solid phase synthesis method to attach a linker functionalized with an alkyne group to the completed sequence. Then, we used an azide functionalized resin to selectively capture the tagged complete sequences by click chemistry, followed by cleavage to obtain a pure, complete sequence. By this method, oligosaccharide **53** has been synthesized without any chromatography at all.

With our method, purification at each step is done on the "solid phase" by filtration and washing instead of chromatography. This strategy is especially practical and useful for automated synthesis, as it would allow large scale parallel synthesis with minimal human labor.

Future Directions:

To improve the synthetic yield, we will need to investigate different materials for both solid phase support and the catch resin. As shown by our synthetic results and UV

quantification, the loading of the phenol functionalized Merrifield resin is quite low. Future experiments will use more resin relative to sugar in order to obtain more product. Also, our conditions for cleavage of the linker-tag and capture resin by methanolysis is run in methanol, were Merrifleds resin does not have the best swelling properties. It is possible that some products are "trapped" in the collapsed resin and is unable to be released. We will investigate other solid support resins such as Polyethylene glycol resins (PEGs), which are designed to swell in a wider variety of solvents. Janjajel in particular has been reported to have good swelling and loading properties [76].

In the near future we plan on synthesizing **53** on a different solid support resin using our solid phase synthesis strategy. With enough material, will then further investigate the use of encapsulated palladium to deprotect the synthesized product, and evaluate the final sugar for purity and suitability for biological screening.

3.7 References

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