I, Leeanne R Taylor, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Chemistry.

It is entitled:
Rational design of glycosaminoglycan mimics using N-alkyl-N,N-linked urea oligomer containing polymers

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Rational design of glycosaminoglycan mimics using $N$-alkyl-$N,N$-linked urea oligomer containing polymers

A dissertation submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

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Abstract of dissertation

This dissertation details the synthesis and characterization of N-alkyl-N,N-linked urea oligomers and their incorporation into glycosaminoglycan mimicking polymers. N-alkyl-N,N-linked urea oligomers are oligomers that can incorporate a wide variety of functional side chains and are prepared through standard organic chemistry techniques. Polymers in this thesis were synthesized using reversible addition-fragmentation chain transfer (RAFT) polymerization. RAFT polymerization is a controlled/“living” radical polymerization technique that can be used to prepare (co)polymers of controlled molecular weights and narrow polydispersities. The N-alkyl-N,N-linked urea oligomer containing polymers have been designed to mimic the properties of the naturally occurring anticoagulant GAG molecule heparin through incorporation of sulfonated carbohydrates as N-alkyl side groups. Heparin is a naturally occurring heterogeneous, sulfated, anionic sugar macromolecule that is commonly used as a therapeutic anticoagulant. In the second chapter, N-alkyl-N,N-linked urea oligomers containing terminal alkyne units were modified with monomeric saccharides. In third chapter, copper catalyzed azide-alkyne cycloaddition (CuAAC) or “click” chemistry is used to synthesize a universal N-alkyl-N,N-linked urea oligomer that allows for the quick and simple synthesis of several oligomers bearing different monomeric saccharides. In the fourth chapter, more complex saccharide units are incorporated and sequence specificity is built in to the universal N-alkyl-N,N-linked urea oligomer of chapter 3 by utilizing simple protecting group chemistry to allow for the step-wise modification of the N-alkyl urea peptoid oligomer backbone.
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Chapter 1. General Introduction

1.1 Glycosaminoglycans

Glycosaminoglycans (GAGs) are naturally occurring polydisperse, linear polysaccharides with a molecular weight range of 10-1000 kDa and are highly negatively charged due to the deprotonation of the carboxylic acid and sulfate groups on the carbohydrates at physiological pH. GAGs can be separated into two main classifications: non-sulfated or sulfated. The non-sulfated GAGs include hyaluronic acid which acts as a lubricant for synovial joints and joint movement. The sulfated GAGs include chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), heparin and heparan sulfate (HS).

All GAGs are composed of repeating disaccharide units which are comprised of either D-glucuronic acid or L-iduronic acid and an amino sugar. Chondroitin sulfate and dermatan sulfate contain galactosamine, further classifying them as galactosaminoglycans whereas heparin and heparan sulfate contain glucosamine, making them glucosaminoglycans. The difference between the structure of heparin and heparan sulfate is difficult to determine and is usually based upon sulfate content since over 80% of the D-glucosamine residues are N-sulfated in heparin. The biggest distinction between heparin and heparan sulfate is their location in the body. Heparan sulfate is primarily linked to a core protein and is found on cell surfaces or in the extracellular matrix (ECM). Heparin is found in the connective tissue type mast cells and is released in the ECM without a core protein.

The interactions between GAGs and proteins are very complex and specific due to the flexibility of the polysaccharide chains and the patterns of repeating disaccharide units and their modifications along the polysaccharide backbone. GAGs participate in many biological
processes through protein binding and protein regulation. In addition to cushioning and lubricating the joints, hyaluronic acid also plays roles in signaling during embryonic morphogenesis, pulmonary and vascular diseases, and wound healing. GAGs have also been found to play major roles in signaling and development, angiogenesis, axonal growth, tumor progression, metastasis, and anti-coagulation. They are a common factor in many amyloids, indicating that they may play an important role in amyloid diseases such as Alzheimer’s disease, type-2 diabetes, Parkinson’s disease and prion diseases. The role of sulfated GAGs in anti-coagulation is one of the most studied.

1.2 Heparin

Heparin and heparan sulfate are sulfated glycosaminoglycans that are well known for their interaction with antithrombin III (ATIII) and factor Xa to disrupt the coagulation pathway. Heparin was first discovered in 1917 and was found to prolong blood clotting. Heparan sulfate, which is located in the ECM, interacts with and regulates proteins such as growth factors, chemokines, and cytokines. Heparin is produced in connective tissue type mast cells, where it is biosynthesized as heparin proteoglycan and stored in the cytoplasmic secretory granules as non-covalent complexes with basic proteases. It binds to the same proteins as heparan sulfate, often with a higher affinity but less specificity. Heparin and heparan sulfate interact with the natural inhibitor of thrombin, antithrombin III. Heparin is used as a therapeutic agent in the treatment of thrombosis, thrombophlebitis, and embolism. Therapeutic heparin is sourced from bovine lung or porcine intestine.

1.2.1 Structure of heparin
Heparin is a right-handed helical structure with 1.65-1.73 nm repeating tetrasaccharide sequence along the chain axis. It consists of an uronic acid-(1→4)-D-glucosamine repeating disaccharide unit with variable patterns of substitution with N-sulfate, O-sulfate, and N-acetyl groups (Figure 1). Heparin is biosynthesized in three parts: (1) chain initiation; (2) polymerization; and (3) polymer modification. Chain initiation occurs from the stepwise addition of a xylose, two galactose, and glucosamine residues to specific serines of the core protein to form a tetrasaccharide linker.\(^\text{16,20}\) Polymerization then occurs through the addition of alternating N-acetyl glucosamine and glucuronic acid residues to the tetrasaccharide linker.

**Figure 1.** Carbohydrate building blocks for heparan sulfate and heparin. \(^1\)
Polymer modification occurs through sequential $N$-deacetylase/$N$-sulfotranferase, epimerase, and $O$-sulfotransferase-catalyzed reactions. However, due to the incomplete nature of the initial $N$-deacetylation reactions, the heparin chains are complex and heterogeneous.$^{16,20}$ The polymer modification begins with the $N$-deacetylation/$N$-sulfation of the $N$-acetyl glucosamine residues. This occurs at random sites along the heparin proteoglycan chain and continues until an isolated $N$-acetyl glucosamine residue is encountered. This is then followed by C5 epimerase-catalyzed conversion of glucuronic acid to iduronic acid. The C5 epimerase-catalyzed conversion occurs specifically when the glucuronic acid is attached to the reducing end of the $N$-sulfated glucosamine residues. $O$-Sulfotransferase then occurs on C2 of the iduronic acid residues and C6 of the $N$-sulfated glucosamine residues. $O$-Sulfation is possible at other positions but take place to a much smaller extent.$^{21-23}$ The modified polysaccharide chains of the heparin proteoglycan are then cleaved at some of the glucuronic acid residues by an $\textit{endo-}\beta$-$\text{D}$-glucuronidase to give shorter free heparin chains. The resulting polymer chains are polydisperse due to the unequal distribution of glucuronic acid throughout the heparin chain and also because cleavage does not occur at every residue.

\section*{1.2.2 Role heparin and heparan sulfate in blood coagulation}

Blood coagulation is a complex series of linked precursor-serine protease transformations that result in formation of fibrin, as shown in Figure 2.$^{20,24}$ Under normal conditions a small amount of coagulation proteins are present at any given time and this process is regulated by natural anticoagulation serine protease inhibitors such as antithrombin III.$^{24}$ Antithrombin III inhibits most serine proteases in the coagulation pathway, including thrombin and factors IXa and Xa in 1:1 complexes.$^{24}$ By itself antithrombin III is relatively inactive, however upon binding to circulating heparin or heparan sulfate on the surface of the endothelium, a conformational change
occurs that facilitates the formation of a ternary complex of antithrombin III with thrombin and factor Xa. After the formation of the ternary complex, the heparin or heparan sulfate loses its affinity for binding to the antithrombin-protease complex, releasing it to circulate.\textsuperscript{20,25-27}

**Figure 2.** Coagulation pathway shown here illustrates the complex cascade that leads to fibrin (clot) formation. The extrinsic pathway is triggered by damage to a blood vessel that releases tissue factor and the intrinsic pathway is triggered by damage to a surface from injury that activates factor XII. Factors indicated in red provide a negative feedback in the pathway, while factors indicated in green provide a positive feedback in the pathway.

Antithrombotic activity of heparin comes mainly from the binding of anti-thrombin III with a unique pentasaccharide sequence (Figure 3) in heparin.\textsuperscript{28-30} It has been found that the \textit{N}-sulfate groups, the 6-\textit{O}-sulfate groups of the glucosamine residues, and the 3-\textit{O}-sulfate groups of the internal glucosamine residue (bolded in Figure 3) are all important to the biological activity of
heparin.\textsuperscript{31-33} Roughly one-third of the heparin chains in pharmaceutical grade heparin contain this sequence.\textsuperscript{34}

\begin{center}
\includegraphics[width=\textwidth]{heparin_structure.png}
\end{center}

\textbf{Figure 3.} Pentasaccharide unit of heparin involved in binding to ATIII. The bolded sulfate residues have been shown to be important for the biological activity of the pentasaccharide unit.\textsuperscript{20}

Heparin is utilized in three forms for therapeutic use: unfractionated heparin, low molecular weight heparin (LMW), and the synthetic ultra-low molecular weight heparin (ULMW) pentasaccharide, Arixtra. Unfractionated heparin has an approximate molecular weight of $\sim14000$ dalton (Da) and is typically used for patients undergoing surgery or kidney dialysis due to its relatively short half-life.\textsuperscript{35} LMW and ULMW heparins, such as Arixtra are becoming increasingly more prevalent because of their more predictable doses, enhanced subcutaneous bioavailability and longer half-lives when compared to heparin.\textsuperscript{16,34} LMW heparins are prepared through the systematic chemical and enzymatic depolymerization of heparin and typically have a molecular weight average of 6000 Da. ULMW heparins typically consist of the pentasaccharide unit (molecular weight of 1508.3 Da for Arixtra) and are made through complicated chemical or enzymatic synthesis techniques. Due to the small chain size of LMW and ULMW heparins they inhibit the blood coagulation pathway mainly through factor Xa rather than antithrombin III. A longer sequence, $\geq 18$ sugar residues, including the pentasaccharide unit is required for the inactivation of thrombin allowing antithrombin III and thrombin to form a tighter complex.\textsuperscript{36}
The heparin binding area of antithrombin III appears to be a composite site consisting of peptide sequences mainly from the N-terminal domain.\textsuperscript{36} Residues that are essential for anticoagulant activity have been determined by NMR and include histidine, arginine, and lysine. The exact residues have been identified and conformational changes have been studied through crystal structures.\textsuperscript{37,38}

### 1.2.3 Problems associated with heparin

Heparin is a mammalian sourced therapeutic agent which is most commonly obtained from porcine intestine or bovine lung tissue.\textsuperscript{3} However, since the outbreak of bovine spongiform encephalopathy, only porcine sourced heparin can be used in the United States and Europe to reduce the possible risk of pathogenic contamination.\textsuperscript{39} Heparins obtained from the tissues of different species have differences in their structure and activity that affect their affinity for antithrombin III.\textsuperscript{34} There are several risks associated with the use of therapeutic heparin, including heparin-induced thrombocytopenia and contamination.

Heparin-induced thrombocytopenia (HIT) is a possible complication associated with the use of therapeutic heparin in anticoagulation. It occurs within the first few weeks of treatment and is an immunological response that activates antibody-mediated platelets and subsequently generates thrombin. This leads to the formation of blood clots that can cause limb gangrene or death.\textsuperscript{40,41} HIT occurs when an antibody binds to an epitope on the platelet factor 4 (PF4)-heparin complex. The antibody-PF4-heparin complex can then bind to FcyRII inducing platelet activation and aggregation which triggers the blood coagulation pathway.\textsuperscript{40,41} If this occurs in a patient, heparin treatment must be stopped and the use of a different anticoagulant therapy must take place to prevent further thrombosis.
One notable incident of contamination occurred in 2008. A marked increase in adverse reactions were noted in patients undergoing dialysis with unfractionated heparin, causing rashes, fainting, racing hearts and in some cases, death. The rapid onset of symptoms suggested an anaphylactic response that resulted in over 100 deaths. This crisis brought together an international consortium of laboratories to collaborate with the FDA and pharmaceutical industry to identify the contaminant. After an exhaustive effort, researchers analyzing the contaminated batches of heparin by optical rotation spectroscopy, capillary electrophoresis, \( ^1 \text{H-NMR} \) spectroscopy, and other analytical techniques were able to determine the contaminant to be the “heparin like” carbohydrate oversulfated chondroitin sulfate (OSCS). OSCS was first synthesized by Linhardt and Toida in 1998 and is made by the chemical sulfonation of chondroitin sulfate, which is an inexpensive carbohydrate. OSCS directly activated the kinin-kallikrein pathway in human plasma, which can generate bradykinin, a potent vasoactive mediator. OSCS also caused the generation of C3a and C5a, which are anaphylatoxins derived from complement proteins.

1.3 Synthetic therapeutics derived from heparin

Concern over the safety of the mammalian sourced unfractionated and LMW heparins instigated the search for new approaches for preparing heparin in a synthetic and cost effective manner. However, due to the heterogeneous nature of heparin and heparan sulfate it is difficult to produce a similar structure through traditional chemical means.

To synthesize the pentasaccharide unit of heparin requires multiple protection and deprotection steps to afford the correct stereochemistry of the glycosidic linkages, as well as the regioselective introduction of sulfate groups. This synthesis was first done by Sinay and coworkers in 60 steps.
with a yield of \( \sim 0.5\% \).\textsuperscript{45,46} In 2002 Sanofi introduced the synthetic heparin pentasaccharide drug called Arixtra, Figure 4.\textsuperscript{34,43} Arixtra is a specific anti-FXa agent that does not have many of the important pharmacological properties of heparin. Unlike heparin, Arixtra has a longer half-life that can cause an excess of anticoagulation activity that cannot be mediated by protamine. Additionally, Idraparinux, which is the pegylated form of Arixtra, exhibits a high risk bleeding effect due to its even longer half-life.\textsuperscript{47}

**Figure 4.** Structure of the pharmaceutical drug Arixtra.\textsuperscript{34} The structure is different from the pentasaccharide unit by the \( O \)-methylation on the terminus.

An enzymatic approach to the synthesis of a ULMW heparin was investigated by Kuberan et al.\textsuperscript{48} To achieve this, the researchers needed to clone and express all of the enzymes and isoforms involved in heparan sulfate biosynthesis. Beginning with a nonsulfated polysaccharide from E. coli strain K5, they were able to transform the polysaccharide in six steps into an antithrombin III binding pentasaccharide at \( \sim 1.1\% \) yield (Figure 5). The reaction was performed on a microgram scale, however the researchers believed that it could be scaled up 1000-fold. While this approach reduced the number of synthetic steps, it was still relatively low yielding.
Xu and coworkers further improved on the overall yield of the ULMW heparin by using a chemoenzymatic approach that relied on a series of heparan sulfate biosynthetic enzymes to mimic the synthesis of heparin and heparan sulfate. They targeted the synthesis of two ULMW heparins as shown in Figure 6. Compound 1 most closely resembles the antithrombin III binding domain in human heparin and compound 2 has the same structure as Arixtra with the exception of the methyl group on the terminal saccharide allowing for the direct comparison of their analogs. Through backbone elongation steps and saccharide modification, the researchers were
able to obtain compound 1 with an overall 45% yield and compound 2 with an overall 37% yield of product.

**Figure 6.** Chemoenzymatic synthesis of the ULMW heparin.$^{35}$

To assess the anticoagulant activity of their two compounds, they looked at the in vitro anticoagulation properties and in vivo pharmacokinetics compared to Arixtra. The binding affinities with antithrombin III were $5.2 \pm 0.2 \text{ nM}$ and $9.1 \pm 0.2 \text{ nM}$ for compound 1 and 2 respectively, which is very similar to the $5.9 \pm 1.5 \text{ nM}$ that was measured for Arixtra.$^{35}$

### 1.4 Biomaterials

In addition to their use in coagulation therapy, heparin and heparan sulfate can also be employed to modify the surface of blood contacting materials to improve biomaterials.$^{49-52}$ Biomaterials are materials that are designed for implantation or incorporation into living tissue.$^{52}$ There are a wide variety of biomaterials that can come in contact with blood including catheters, blood vessel grafts, stents, artificial heart valves, circulatory support devices, extracorporeal tubing,
hemodialysis, hemapheresis and oxygenator membranes. The materials chosen for these biomaterials were selected for their mechanical properties, stability, permeability, processing price, ease of sterilization and non-toxicity. If these materials exhibit inadequate hemocompatibility, it will impair their function and safety by activating the blood coagulation and immune response pathways. To improve the hemocompatibility of these materials it is necessary to modify the physical and chemical properties of the material to increase hydrophilicity and also better mimic the naturally occurring endothelium.

Ideal hemocompatible materials should not activate the blood coagulation pathway or attract or alter platelets or leukocytes and is dependent on surface charge, surface free energy, chemical group distribution, heterogeneity, surface texture, porosity, smoothness and flow conditions. Current materials that are used include polydimethyl siloxane, cellulose acetate, polyacrylonitrile, polytetrafluoroethylene (PTFE), nylon, polycarbonate, polyurethane, poly(methyl methacrylate) (PMMA), and pyrolytic and low-temperature isotropic carbons. A few of the approaches used to modify biomaterial surfaces for hemocompatibility include coating with heparin or heparin derivatives, coating with sulfated polysaccharides, synthetic sulfated polymers, or synthetic polymer containing sulfated carbohydrates.

1.4.1 Biomaterials modified with sulfated polysaccharides

Naturally occurring polysaccharides such as chitosan, hyaluronic acid, dextran, acharan, and others non-mammalian sourced polysaccharides have been investigated for improving the hemocompatibility of biomaterials. These polysaccharides are widely available and can be easily modified to contain sulfate or carboxylate groups.
Chitosan is one of the most promising polysaccharide for biomaterial applications due to its physicochemical and biological properties. Chitosan is a polysaccharide that is made through the thermochemical deacetylation of chitin and is composed of $N$-acetyl-2-amino-2-deoxy-$\alpha$-glucopyranose and 2-amino-2-deoxy-$\alpha$-glucopyranose linked by $(1\rightarrow4)$-$\beta$-glycosidic bonds (Figure 7). Chitin is often considered a close structural analog to heparin, which makes it a commonly studied analog.\textsuperscript{55,56} Chitosan is sourced from the shell waste of shrimp, lobster, krills and crab.

![Figure 7. Chitin and Chitosan.\textsuperscript{55}](image)

Fasl et al. modified polyethylene terephthalate (PET) surfaces using sulfated chitosan to improve its hemocompatibility.\textsuperscript{55} PET is a commonly used biomaterials and is often used for cardiovascular implants due to its mechanical properties, however it only has moderate blood compatibility.\textsuperscript{55} Fasl et al. prepared a highly sulfated chitosan using $\text{SO}_3$/pyridine complex and a low sulfate chitosan using chlorosulfonic acid and compared the hemocompatibility with heparin and dextran sulfate. To evaluate the materials performance the researchers used an optimized free hemoglobin method using fresh whole blood to determine the in vitro compatibility of glass, two PET surfaces, and the PET surfaces coated with the sulfated polysaccharides. Other the influences such as shaking rates, pH and the added blood temperature were investigated during the optimization procedure. A drop of blood was placed on the surfaces and the amount of free hemoglobin was assessed at different time points. After 20 minutes, 90% of the hemoglobin was detected from the heparin coated surface and 20% of the hemoglobin was detected from the glass
surface, showing the positive and negative controls of the assay. The PET surfaces that were unmodified had ~ 50% free hemoglobin after 20 minutes and the chitosan surface released 40% of the free hemoglobin which was comparable to the unmodified PET. All of their sulfated chitosan materials improved upon the performance of the PET surfaces and the chitosan coated PET. They found that the sulfated chitosan with 16.05% sulfur content performed most comparably to heparin (10.5 % sulfur). By using a polysaccharide that is considered a structure analog of heparin, the researchers were able to modify a material to improve its hemocompatibility using a widely available source and without the use of mammalian sourced materials.

Another naturally occurring polysaccharide being used to modify blood contacting materials is hyaluronic acid (HA). For example, Xu and coworkers designed a polyurethane material that incorporated hyaluronic acid as block copolymers. By covalently bonding a blood compatible element to the polymer they reduced the risk of the active portion eluting from the material. They synthesized a polyurethane-hyaluronic acid copolymer with a hyaluronic acid content of 0.33-5.4 wt%, shown in Figure 8, that was macroscopically indistinguishable from the polyurethanes that did not contain HA. The incorporation of the HA yielded a highly crosslinked copolymer that possessed a higher elastic molduli than the polyurethanes alone.
The researchers used static platelet and red blood cell (RBC) adhesions tests to measure hemocompatibility. The platelet and RBC adhesion was almost completely inhibited by all polyurethane-HA material compositions, with the 0.33% showing a 20-fold reduction in adhesion from the polyurethanes alone. These polyurethane-HA materials have built in antithrombotic activity and the added advantage of promoting endothelialization without further modification.
1.4.2 Biomaterials modified with synthetic sulfated polymers and glycopolymers

Another approach to impart hemocompatibility onto materials is to make polymers that either contain completely synthetic monomeric units or contain saccharide bearing monomeric units and still possess the function of heparin. This typically entails the use of polymers that possess or can be modified to possess carboxylic acid and sulfate functional groups.

Fougnot and coworkers designed heparin-like materials made from a polyethylene-polystyrene copolymer, where the polystyrene moieties were modified with sulfonate and aspartic acid sulfonamide groups. While these materials do not physically resemble the carbohydrate structure of heparin and heparan sulfate, they still possess hemocompatibility based on prolonged clotting time. Pellethane polyurethane blended with 1% or 5% (w/w) polytetramethylene oxide (PTMO) (Figure 9) has been evaluated as a hemocompatible material by Herbert et al. PTMO is a polyether soft segment and protein and platelet adsorption differs between hard and soft segments.

![Polytetramethylene oxide-methylene bis(p-phenyl isocyanate)-butanediol copolymer](image)

Figure 9. Polytetramethylene oxide-methylene bis(p-phenyl isocyanate)-butanediol copolymer.

The addition of the 1% and 5% PTMO caused a decrease in the adhesion of the platelets and thrombi with a 49% and 71% reduction in thrombi in the respective PTMO compositions when compared to the unmodified polyurethanes.
Ahmed et al. incorporated carbohydrates into a synthetic hyperbranched glycopolymers by synthesizing the methacrylamide derivatives 3-gluconamidopropylmethacrylamide and 2-lactobionamidoethylmethacrylamide.\textsuperscript{63} The two methacrylamide derivatives where then polymerized under reversible addition-fragmentation chain transfer (RAFT) control with a crosslinking agent to generate the hyperbranched structure, Figure 10.\textsuperscript{63}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure10.png}
\caption{Crosslinking scheme shown with 2-lactobionamidoethylmethacrylamide.\textsuperscript{63}}
\end{figure}

The hemocompatibility of these structures where then evaluated my measuring the activation of the coagulation and complement systems, as well as platelet activation and the interaction with RBCs. The coagulation activation was measured by looking at the prothrombin time (PT) and the activated partial thromboplastin time (APTT) and where shown to not prolong or decrease coagulation time. The hyperbranched glycopolymers also did not promote platelet activation at any molecular weight. Overall the hyperbranched glycopolymers were found to be hemocompatible in the remaining assays, as well as biocompatible through cytotoxicity assays.

\textbf{1.5 Conclusions}
Heparin and heparan sulfate are complex polysaccharides that are widely used as a therapeutic anticoagulant. However, it is becoming more apparent a synthetic replacement for heparin would be advantageous since heparin is biologically sourced, can have adverse side effects, and can be contaminated during processing. While there has been moderate success in synthesizing structural analogs through complex chemical and enzymatic techniques, they are often expensive and low yielding. Other approaches to mimicking the activity of heparin and heparan sulfate include using non-mammalian source carbohydrates, synthetic polymer with greater hydrophilicity or that can be sulfonated, or polymers that contain carbohydrate residues.

While all of these approaches have improved hemocompatibility of materials they have focused only on charge and hydrophilicity rather than the complex interactions that occur during the sequence of events in which heparin inhibits the coagulation. The work summarized in this thesis outlines a strategy to utilize \(N\)-alkyl-\(N, N'\)-linked urea oligomers (\(N\)-alkyl urea peptoids) and reversible addition-fragmentation chain transfer (RAFT) polymerization to build well-defined glycosaminoglycan mimics, specifically of heparin. \(N\)-alkyl-\(N, N'\)-linked urea oligomers have the synthetic versatility to allow us to explore the structure–property relationships of a wide variety of carbohydrates and determine which yield anticoagulant activity.

1.6 References


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Chapter 2. Synthesis of a glycosaminoglycan polymer mimetic using an N-alkyl-N,N-linked oligomer containing glucose as pendant groups

2.1 Abstract

In this chapter the synthesis of a glycosaminoglycan polymer mimetic is detailed. An isopropylidene protected glucose methacrylate monomer was copolymerized under reversible addition fragmentation chain transfer (RAFT) polymerization control with an azido-containing comonomer to a molecular weight of 29,000 g/mol with polydispersity of 1.21. The comonomer ratio was determined to be 15:1 based on $^1$H NMR spectroscopy. This copolymer was coupled to sugar-functionalized N-alkyl-N,N-linked urea oligomers using a copper catalyzed alkyne/azide cycloaddition (CuAAC) reaction. The reaction efficiency was 100% as monitored by $^1$H NMR spectroscopy. The isopropylidene protecting groups on the polymer and N-alkyl-N,N-linked urea oligomer were removed using acid hydrolysis to give the final polysaccharide mimetic. It is expected that these polymer systems will have applications in a variety of potential future therapeutic applications.

2.2 Introduction

Glycosaminoglycans (GAGs) are carbohydrate biopolymers of remarkable heterogeneity that possess multiple important regulatory roles in biology. For example, GAGs are involved in processes such as cell signaling and development, angiogenesis, axonal growth, tumor progression, and metastasis. Similarly, sulfated GAGs are a factor in the pathology of amyloid diseases including Alzheimer’s disease and prion diseases. GAGs are present on cell surfaces, and some are known to bind and regulate a number of proteins, including chemokines, cytokines, growth factors, morphogens, enzymes and adhesion molecules. Therefore, a vast scientific effort
has been deployed to characterize the chemical structure of GAGs and understand the role this plays in the function of individual GAGs. However, for the most part, the primary sequence of GAGs has not been found to correlate in any straightforward way with activity or binding. Instead it appears that the combination of overall conformation, charge distribution, and flexibility exhibited by a particular GAG will determine the activity. Generically, GAGs are linear, negatively charged polysaccharides that possess molecular weights of ca. 10-100 kDa. Non-sulfated GAGs include hyaluronic acid, while sulfated GAGs include chondroitin sulfate, dermatan sulfate, keratin sulfate and heparin and heparan sulfate. However, the heterogeneity of GAGs is quickly revealed. For example, the sequence-diversity displayed by heparan sulfate results in more than 5 x 10^6 potential octasaccharide oligomers.

It is therefore unsurprising that few synthetic macromolecular GAG-mimics exist. Currently, synthetic analogues of GAGs consist mainly of sulfated polymers and sulfated polysaccharides. These analogues are able to mimic some of the properties of GAGs and have been shown to promote angiogenesis and myogenic differentiation, bind different biologic cofactors and enzymes, and impart a degree of hemocompatibility onto surfaces. In contrast to the relatively few reports on synthesis of GAG-mimicking polymers, there is a large volume of literature describing synthetic polymers containing carbohydrate residues for other applications or end-uses. Many of these have been prepared using controlled-polymerization techniques. One type of controlled radical polymerization mechanism that has proven ideal for synthesizing sugar-containing polymers is reversible addition fragmentation chain transfer (RAFT) polymerization. RAFT polymerization is beneficial as it is simple to perform, tolerant to a wide range of functional groups, and the polymerization can be conducted in either aqueous or organic media. For example, Narain and coworkers synthesized a hyperbranched glycopolимер

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via RAFT polymerization that contained 3-gluconamidopropylmethacrylamide (GAPMA) and 2-lactobionamidoethylmethacrylamide (LAEMA) to enhance the biocompatibility of drug carriers. Blanazs and coworkers also used RAFT polymerization in the synthesis of well-defined glycopolymer that contained 2-(β-D-galactosyloxy)ethyl methacrylate (GalEMA) and methyl 6-O-methacryloyl-α-D-glucoside (MAMGlc), as well as blocks of hydrophilic and hydrophobic monomers to study their behavior in aqueous solutions. The synthesis of well-defined pH-sensitive block copolymers containing poly(2-(diethylamino)ethyl methacrylate (poly(DEAEMA)) and poly(3-O-methacryloyl-α,β-D-glucopyranose) (poly(MAGlc)) was obtained by RAFT polymerization by Wang and coworkers showed lectin binding capabilities with Concanavalin A.

Our group has previously demonstrated the copolymerization of an isopropylidene protected glucose methacrylate monomer, 3-O-methacryloyl-1,2:5,6-di-O-isopropylidene-D-glucofuranose (MAIpGlc) under RAFT control with an azide-containing comonomer. The carbohydrate-based monomer has been used elsewhere in surface initiated polymerizations, modifying polymer membranes, synthesis of liquid CO\textsubscript{2} amphiphiles, and the functionalization of carbon nanotubes. The azide-containing repeat units in our copolymer were coupled to sequence-specific \( N \)-alkyl-\( N,N \)-linked urea oligomers using a copper catalyzed azide/alkyne cycloaddition (CuAAC) reaction, often referred to as “click” chemistry. These oligomers can be thought of as hybrids of two well-known classes of sequence specific oligomers, urea oligomers and \( N \)-acyl glycines, or “peptoids”. Oligomeric ureas have been shown to adopt well defined folded and helical conformations that are created through hydrogen bonding between urea groups, and also possess increased resistance to enzymatic degradation than corresponding peptide oligomers, making these intriguing for potential therapeutic
applications. Peptoids have recently been used increasingly in polymer synthesis and materials chemistry, and Zhang has published several papers detailing the mechanism and properties of synthetic polypeptoids. While the N-acyl glycines do not possess the innate hydrogen bonding found in peptides, they have been shown to be able to fold into helices, self-assemble into sheets, and segregate as block copolymers depending upon their design parameters. Furthermore, longer peptoid sequences of up to 50 residues are synthetically accessible. The N-alkyl-N,N-linked urea oligomers prepared in our lab have the potential for hydrogen bonding through the urea group, are prepared through simple reactions, and allow the incorporation of many different functional side groups, e.g. carbohydrates. Also, due to the different sites of chemical functional groups in the oligomers, they are readily incorporated into synthetic macromolecules. This combination of advantageous properties positions the use of N-alkyl-N,N-linked urea oligomers for the preparation of soft materials and the study of structure-property relationships, including the synthesis and examination of GAG-mimicking oligomers and polymers.

In this work the synthesis of glucose-containing potential GAG mimicking polymer was accomplished using RAFT polymerization of MAIpGlc and 6-azidohexyl methacrylate (AzHMA), and conjugating the polymer to glucose functionalized N-alkyl-N,N-linked urea oligomers. Our oligomers are tetramers containing three isopropylidene protected glucose units and a terminal alkyne. By virtue of using the same protecting group in the oligomer and polymer we are able to perform a single deprotection to yield the final polymer GAG-mimetic.

2.3 Experimental Section
All starting materials were purchased from Aldrich at the highest purity available and used as received unless specified otherwise. \(N\)-(2-nitrobenzenesulfonyl)-2-imidazolidone was prepared according to the method of Wilson and Nowick.\(^{63}\) \(N\)-(2-(3,3-diethylureido)ethyl)-2-nitrobenzenesulfonamide was synthesized as previously reported.\(^{25}\) The RAFT agent cumyl dithiobenzoate (CDB) was synthesized according to literature procedures.\(^{64,65}\) The sugar functionalized monomer 3-\(O\)-methacryloyl-1,2:5,6-di-\(O\)-isopropylidene-\(\alpha\)-\(D\)-glucofuranose (MAIpGlc) was synthesized according to literature procedures.\(^{11}\) The azide-containing monomer 6-azidoethyl methacrylate (AzHMA) were synthesized by modifying a published protocol.\(^{66}\)

### 2.3.1 Modification of glucose for side group attachment

**Synthesis of 3-\(O\)-(4'-Chlorobutyl)-1,2:5,6-di-\(O\)-isopropylidene-\(\alpha\)-\(D\)-glucofuranose (Chlorobutyl-IpGlc).\(^{67}\)** 1,2:5,6-di-\(O\)-isopropylidene-\(\alpha\)-\(D\)-glucofuranose (3.04g, 0.012 mol) and 12.5 M NaOH (1.6 mL) in 10 mL of dimethyl sulfoxide were added to a 100 mL round bottom flask equipped with a stir bar. 1-bromo-4-chlorobutane (2.0 mL, 0.017 mol) in 10 mL of dimethyl sulfoxide was added slowly while stirring at room temperature. The reaction was allowed to stir at room temperature overnight before the addition of deionized water (10 mL) and allowed to stir for an additional 20 min. The product was extracted three times with diethyl ether. The ether layers were combined and washed with once with a saturated solution of sodium chloride and then dried over sodium sulfate. The solution was concentrated by rotary evaporation to yield 4.03 g of clear, colorless oil. Yield: 98%. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 5.97-5.88 \text{ (d, 1H)}, 3.55-3.45 \text{ (m, 11H)}, 2.1-1.6 \text{ (m, 4H)}, 1.55-1.3 \text{ (m, 12H)} \text{ ppm.} \) \(^{13}\)C\({}^{\text{\(^1\)H}}\) NMR (100 MHz, CDCl\(_3\)): \(\delta = 112.01, 109.23 \text{ [2 x C(CH}_3\text{)\_2]}, 105.5 \text{ (C-1)}, 82.69 \text{ (C-2)}, 82.39 \text{ (C-3)}, 81.47 \text{ (C-4)}, 72.60 \text{ (C-5)}, 67.64 \text{ (C-6)}, 69.80 \text{ (-OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}), 44.98 \text{ (-OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}), 29.50,
27.23 (−OCH₂CH₂CH₂CH₂Cl), 27.05, 26.45, 25.58, 25.34 (4 x CH₃) ppm. MS (TOF MS ESI): M_theo = 350.8360 g/mol. M+Na⁺ = 371.1376 g/mol.

2.3.2 Synthesis of the glucose N-alkyl-N,N-linked urea oligomer

**Compound 2.** N-(2-(3,3-diethylureido)ethyl)-2-nitrobenzenesulfonamide (Compound 1) (3.0 g, 8.7 mmol), K₂CO₃ (3.01 g, 21.8 mmol) and chlorobutyl-IpGlc (9.18 g, 26.1 mmol) were dissolved in 30 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at 60 °C overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH₂Cl₂ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:methanol (4:1 v/v) as the mobile phase. The product was dried in vacuum to afford 3.0 g of oil. Yield: 96%. ¹H NMR (400 MHz, CDCl₃): δ = 1.10 (t, 6 H, 2 x CH₃CH₂N), 1.28-1.45 (m, 12H, 2 x -C(CH₃)₂), 3.17-3.48 (m, 13 H, 2 x CH₃C₂H₂N, CH₂CH₂NH-Ns, CH₂CH₂NHCH₂), 4.89 (bs, 1 H, NH), 5.81, 5.82 (d, 1 H, acetal), 7.60-7.68 (m, 3 H, aromatic H), 8.11-8.14 (m, 1 H, aromatic H) ppm.

¹³C{¹H} NMR (100 MHz, CDCl₃): δ = 13.74 (2 x CH₃CH₂N), 25.07, 25.33, 25.53, 26.16, 26.78, 29.32 (4 x −CH₃, −OCH₂CH₂CH₂CH₂NNs), 41.06 (CH₂CH₂NH-Ns), 41.10 (OCNHCH₂CH₂NNs), 44.22 (2 x CH₂CH₂N), 47.44 (−OCH₂CH₂CH₂CH₂NNs), 53.49 (OCNHCH₂CH₂NNs), 69.68 (−OCH₂CH₂CH₂CH₂NNs), 67.09 (C-6), 72.46 (C-5), 81.03 (C-4), 82.12 (C-3), 82.34 (C-2), 105.21 (C-1), 108.86, 111.67 (2 x C(CH₃)₂), 125.25, 131.07, 132.75, 133.66, 133.75, 147.99 (aromatic CH), 157.18 (CONH). MS (TOF MS ESI): M_theo = 658.7640 g/mol. M+Na⁺ = 681.2726 g/mol.

**Compound 3.** Compound 2 (3.0 g, 4.6 mmol) and K₂CO₃ (2.14 g, 15.5 mmol) were dissolved in 20 mL of DMF. The reaction was purged with N₂ and benzenethiol (1.95 mL, 19.0 mmol) was
added. The reaction was allowed to stir at 60 °C overnight. The DMF was removed by vacuum distillation. The residue was dissolved in CH₂Cl₂ and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with CH₂Cl₂:MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product dried in vacuum to yield 1.59 g. Yield: 71%. ¹H NMR (400 MHz, CDCl₃): δ = 1.10-1.50 (m, 22H, 2 x C₆H₃CH₂N, 2 x -C(CH₃)₂, -NHCH₂CH₂CH₂CH₂O-), 2.87-4.85 (m, 18 H, furanose ring, linker, backbone), 5.84(s, 1 H, acetal). ¹³C{¹H} NMR (100 MHz, CDCl₃): δ = 13.74 (2 x C₆H₃CH₂N), 25.07, 25.33, 25.53, 26.16, 26.78, 29.32 (4 x –CH₃, -OCH₂CH₂CH₂CH₂NNs), 41.06 (CH₂CH₂NH-Ns), 41.10 (OCNHCH₂CH₂NNs), 44.22 (2 x CH₃CH₂N), 47.44 (-OCH₂CH₂CH₂CH₂NNs), 53.49 (OCNHCH₂CH₂NNs), 69.68 (-OCH₂CH₂CH₂CH₂NNs), 67.09 (C-6), 72.46 (C-5), 81.03 (C-4), 82.12 (C-3), 82.34 (C-2), 105.21 (C-1), 108.86, 111.67 (2 x C(CH₃)₂), 125.25, 131.07, 132.75, 133.66, 133.75, 147.99 (aromatic CH), 157.18 (CONH). MS (TOF MS ESI): Mₜheo = 473.6110 g/mol. M+H⁺ = 474.3102 g/mol.

Compounds 4 and 5. Compounds 4 and 5 were prepared using the experimental protocols described above. Complete synthetic details and characterization are provided in supplemental information. The final product was isolated as colorless oil (0.8 g). Overall yield after compound 5 = 34%. ¹H NMR (400 MHz, CDCl₃): δ = 1.10-1.12 (s, 6H 2 x CH₃CH₂N ), 1.32-1.50 (m, 48H, 6 x -C(CH₃)₂, 3 x -NHCH₂CH₂CH₂CH₂CH₂O-), 5.87 (s, 3 H, acetal). MS (TOF MS ESI): Mₜheo = 1273.7520 g/mol. M+H⁺ = 1274.7676 g/mol

Compound 6. N-(2-Nitrobenzenesulfonyl)-2-imidazolidone (0.12 g, 0.04 mmol) and (dimethylamino)pyridine (DMAP) (0.22 g, 0.18 mmol) were added to a solution of Compound 5 (0.40 g, 0.31 mmol) in 5 mL of pyridine. The reaction flask was sealed with a rubber septum,
purged with \( \text{N}_2 \) for 30 min, and immersed in preheated oil bath at 60°C overnight. The solvent was removed using a rotary evaporator, the residue dissolved in \( \text{CH}_2\text{Cl}_2 \), washed with 0.5 M aqueous HCl and dried over \( \text{Na}_2\text{SO}_4 \). The product was dried in a vacuum oven to afford 4.65 g of white solid. Yield = 43%. \(^1\text{H} \) NMR (400 MHz, \( \text{CDCl}_3 \)): \( \delta = 1.12 \) (t, 6 H, 2 x \( \text{CH}_3\text{CH}_2\text{N} \)), 1.32-1.50 (m, 48H, 6 x \(-\text{C(CH}_3)_2\)), 3 x \(-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}-\)), 5.87 (s, 3 H, acetal), 8.01 (m, 1 H, aromatic H) ppm. MS (TOF MS ESI): \( M_{\text{theo}} = 1544.7783 \) g/mol. \( M+\text{H}^+ = 1545.7573 \) g/mol

The isolated compound (0.16 g, 0.11 mmol), propargyl bromide (0.038g, 0.32 mmol) and \( \text{K}_2\text{CO}_3 \) (0.04 g, 0.29 mmol) in 2 mL of DMF were added. The reaction was allowed to stir at 60 °C overnight. The DMF was removed by vacuum distillation and the afforded residue dissolved in \( \text{CH}_2\text{Cl}_2 \) and passed through Celite. The solution was concentrated and the resultant product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:methanol (4:1 v/v) as the mobile phase. The product was dried in vacuum to afford 0.11 g of an oil. Yield: 65% \(^1\text{H} \) NMR (400 MHz, \( \text{CDCl}_3 \)): \( \delta = 1.09 \) (t, 6 H, 2 x \( \text{CH}_3\text{CH}_2\text{N} \)), 1.32-1.46 (m, 48H, 6 x \(-\text{C(CH}_3)_2\)), 3 x \(-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}-\)), 2.21, 2.40 (m, 1H, \(-\text{CCH}\)), 5.84 (s, 3 H, acetal), 7.97 (m, 2 H, aromatic H), 8.08 (m, 1H, aromatic H) ppm. MS (TOF MS ESI): \( M_{\text{theo}} = 1582.7939 \) g/mol. \( M+\text{H}^+ = 1583.8379 \) g/mol

2.3.3 Carbohydrate containing polymers

**Polymerization of MAIpGlc.** MAIpGlc, CDB, and 2,2’-azobis(2-methylpropionitrile) (AIBN), (100:1:0.2-0.33) were added to 4 mL of anhydrous anisole (Solvent:Monomer = 4:1 v/v). Air was removed from the reaction flasks through three consecutive freeze-pump-thaw cycles and the flasks were back-filled with nitrogen. The polymerizations were transferred to an oil bath at the desired temperature and allowed to react for 18 h. Samples were taken every hour using a
degassed syringe and quenched by exposure to air and rapid cooling. All polymerizations were quenched by exposure to air and rapid cooling and purified by precipitation into cold hexane to yield a pale pink solid.

**Poly(MAlpGlc-co-AzHMA).** A 50 mL air-free round bottom flask was charged with MAIpGlc (1.903 g, 5.80 mmol), AzHMA (0.107 g, 0.58 mmol), CDB (0.017 g, 0.062 mmol), and AIBN (0.0035 g, 0.021 mmol) in 8 mL of anhydrous anisole. Air was removed from the flask by three consecutive freeze-pump-thaw cycles and the polymerization was performed for 9 h at 70 °C before being quenched by exposure to air and rapid cooling. The polymer was precipitated twice from hexane and dried in vacuum to afford 1.5 g of pale pink solid. Isolated yield: 75%. \(^1\)H NMR (CDCl\(_3\)): δ (ppm) 1.08-1.61 (m, 576 H, -CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)N\(_3\), isopropylidene protecting groups, all CH\(_3\), protons from polymer backbone and end group), 3.32 (bs, 2 H, CH\(_2\)N\(_3\)), 3.83-4.90 (m, 100H, 98 OCH, 1 OCH\(_2\)CH\(_2\)CH\(_2\)N\(_3\)), 5.84 (15 H, OCHO). FT-IR: 2986.5 cm\(^{-1}\) CH stretch, 2098.5 cm\(^{-1}\) N\(_3\) 1729.0 cm\(^{-1}\) CO stretch.

**Synthesis of the N-alkyl-N,N-linked oligomer-polymer conjugate.**\(^{25}\) Poly(MAlpGlc-co-AzHMA) (0.2 g, 0.03 mmol of azide), CuBr (2.1 mg, 0.015 mmol), and compound 6 (0.060 g, 0.038 mmol) were mixed in 5 mL of CH\(_2\)Cl\(_2\) and purged with dry N\(_2\) gas for 30 min. The ligand N\(_N\),N',N”-pentamethyldiethylenetriamine (PMDETA) (2.49 mg, 0.014 mmol) was added to the solution using a syringe, and the solution immediately turned blue. The reaction was stirred overnight at room temperature. After this time the reaction solution was passed through a short silica column to remove the copper complex using CH\(_2\)Cl\(_2\)/MeOH = 20:1 (v/v) as the mobile phase. The solvent was removed using a rotary evaporator, the obtained oligomer/polymer conjugate dissolved in a minimum amount of CH\(_2\)Cl\(_2\) and precipitated from hexane, and dried in vacuum for 2 days. \(^1\)H NMR (CDCl\(_3\)): δ (ppm) 0.80-1.98 (m, 1810 H, -CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)N\(_3\),
all CH₃, protons from polymer backbone and end group, and CH₂CH₂N, -C(CH₃)₂, -NHCH₂CH₂CH₂O from peptoid), 5.85 (103 H, acetal proton), 7.69 (m, 18 H, aromatic H), 7.99 (m, 6 H, aromatic H) ppm. FT-IR: 2985.6 cm⁻¹ CH stretch, 1730.5 cm⁻¹ CO stretch.

**Removal of isopropylidene protecting groups.**²⁵ The N-alkyl-N,N-linked oligomer-polymer conjugate (0.15 g) and a solution of 20% trifluoroacetic acid, TFA, in 5 mL dichloromethane was stirred in a 10 mL round-bottom flask for 30 min at room temperature. The TFA and solvent removed under a stream of air to isolate the final product. ^1^H NMR (d₄-MeOH): δ (ppm) 1.00-2.00 (576, -CH₂CH₂CH₂CH₂CH₂N₃, polymer backbone and end group), 7.69-8.15 (m, 30 H, aromatic H from Ns group and triazole H). FT-IR: 3397.8 cm⁻¹ (-OH stretch), 2917.2 and 2848.8 cm⁻¹ (-CH stretch), 1782.5 and 1710.8 cm⁻¹ (C=O stretches).

**2.4 Characterization**

^1^H and ^13^C{¹H} NMR spectroscopy were performed in CDCl₃ with Si(CH₃)₄, MeOH-d₄, and D₂O with 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid, sodium salt as internal standards using a Bruker Ultrashield 400 MHz (100 MHz for ^13^C{¹H}-NMR). The ^1^H and ^13^C-NMR spectra were processed using UXNMR version 2.5 and MestReNova Lite. Molecular weights and polydispersities were determined by gel permeation chromatography (GPC). Poly(MAIpGlc-co-AzHMA) copolymers were characterized with an Agilent 1100 series HPLC equipped with a PSS SDV Lux column (5 µm) guard column and two PSS SDV Linear XL Lux Columns (5 µm) (linear range of MW = 100 - 3x10⁶ g/mol) with filtered tetrahydrofuran (THF) containing 200 ppm 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) mobile phase at a flow rate of 1.0 mL/min at ambient temperature and a miniDAWN TREOS light scattering (60 mW GaAs linearly polarized laser, 658 nm) calibrated against a 30 000 g/mol polystyrene standard (Wyatt
Technology Corp.) and Optilab rEX differential refractometer (light source = 658 nm) detectors. Poly(MAlpGlc-co-AzHMA) copolymers and N-alkyl-N,N-linked oligomer-poly(MAlpGlc-co-AzHMA) conjugates were characterized with an Agilent 1200 series HPLC equipped with a PSS Gram (10 μm) guard column and 2 PSS Gram columns (10 μm) (linear range of MW = 100 – 1x10^6 g/mol) with filtered dimethyl formamide (DMF) with 0.1% LiBr w/v mobile phase at a flow rate of 0.5 mL/min at 70°C and a Optilab rEX differential refractometer (light source = 658 nm) detector calibrated against poly(methyl methacrylate) standards (850 Da – 2,000,000 Da). ASTRA software v. 6.1.0 was used to determine polymer characteristic values. Fourier transform infrared (FT-IR) spectra were collected on a Nicolet 6700 spectrometer and analyzed with OMNIC32 software.

2.5 Results and Discussion

The synthesis of the glucose functionalized N-alkyl-N,N-linked urea oligomer was performed following procedures previously developed by our group\(^2^5\) from initial work by Nowick’s group.\(^6^3\) The synthesis is simple to perform and involves solution-phase iterative reaction cycles. Three steps are used: (1) Main chain extension using a N-(2-nitrobenzene sulfonyl)-2-imidazolidone, (2) side group attachment following Fukuyama’s procedure\(^6^8\) to introduce the N-alkyl group, and (3) removal of the 2-nitrobenzene sulfonyl group using thiophenol to afford a new secondary amine used in the next iterative cycle. The side group attachment uses alkyl halides, where the alkyl group of the halide becomes the N-alkyl functional group in the growing urea oligomer. Therefore, considering the number of alkyl halides commercially available or synthetically accessible, this results in a vast diversity of potential oligomers. Moreover, the identity of the side groups incorporated into the N-alkyl-N,N-linked urea oligomers will partially control the physical properties of the oligomers, and the choice of side groups will dictate what
applications the oligomers are ultimately used for. We designed oligomers containing glucose carbohydrate residues as $N$-alkyl side groups for the polymer mimics of glycosaminoglycans in this work. We accomplished this by modifying an isopropylidene protected glucose molecule with an alkyl chloride group. We chose to use 1,2:5,6-di-$O$-isopropylidene-$\alpha$-$D$-glucofuranose as the isopropylidene protecting groups are resistant to the basic conditions of the oligomer synthesis protocols, unlike the acetyl groups that are commonly used and are not compatible with our synthesis route. Isopropylidene protected glucose was reacted with 1-bromo-4-chlorobutane to produce 3-$O$-(4'-Chlorobutyl)-1,2:5,6-di-$O$-isopropylidene-$\alpha$-$D$-glucofuranose (Chlorobutyl-IpGlc) following literature procedures (Figure 1).^67

![Figure 1. Synthetic scheme for the synthesis of 3-$O$-(4'-Chlorobutyl)-1,2:5,6-di-$O$-isopropylidene-$\alpha$-$D$-glucofuranose (Chlorobutyl-IpGlc).](image)

We used chlorobutyl-IpGlc to synthesize an $N$-alkyl-$N,N$-linked urea oligomer tetramer that contained three protected sugar side groups and a terminal alkyne, Compound 6. The synthetic scheme of the preparation of Compound 6 is shown in Figure 2. The terminal alkyne group is used to couple the oligomer to an azide-containing copolymer using the copper catalyzed azide/alkyne cycloaddition (CuAAC) reaction. The percentage yields shown on Figure 3 relate to the yield of each compound from its predecessor, not the running total yield from the first step. As can be seen, the synthesis of Compound 6 suffered from low yields in several steps that limited the amount of the final oligomer obtained. However, we still obtained 110 mg of Compound 6 from 3.0 g of Compound 1 after a ten-step synthesis where the product after each
step was isolated and characterized. Furthermore, each step is able to be performed using traditional organic-chemistry laboratory techniques, negating the need for expensive resin-based synthetic techniques and their accompanying purification and characterization equipment requirements.
Figure 2. Synthetic scheme of the sugar containing \( N \)-alkyl-\( N,N \)-linked urea oligomer.

We previously reported the synthesis of a statistical copolymer of an isopropylidene protected glucose monomer (MAlpGlc) and 3-azido-propylmethacrylate (AzPMA) under reversible addition fragmentation chain transfer (RAFT) polymerization conditions and its coupling to \( N \)-
alkyl-\(N,N\)-linked urea oligomers with simple alkyl side-groups.\(^{25}\) However, it was observed that the polymer synthesized in that work had a significantly higher number-average molecular weight \(\langle M_\text{n} \rangle\) (101 100 g/mol) than the number-average molecular weight predicted by theory using Equation 1 (32 200 g/mol). In a RAFT polymerization it is possible to predict the theoretical molecular weight at a particular conversion using Equation 1, where \([M]_0\) is the initial monomer concentration; \([CTA]_0\) is the initial CTA concentration; \(M\text{repeat}\) is the molecular weight of the repeat unit; \(p\) is the conversion; and \(M_{CTA}\) is the molecular weight of the CTA.

\[
M_{n(\text{theo})} = \left( \frac{[M]_0 \times M_{\text{repeat}} \times p}{[CTA]_0} \right) + M_{CTA}
\]

\textbf{Equation 1.} Calculation for the theoretical molecular weight of a polymer obtained using RAFT polymerization.

Considering this lack of controlled behavior, in this work we explored the polymerization conditions to prepare polymers where the observed molecular weight more closely agreed with that predicted by theory. Based on the dramatically higher observed molecular weight in the earlier paper, we decided to slow the rate of polymerization by using a dithioester RAFT chain transfer agent instead of the trithiocarbonate chain transfer agent (S-1-dodecyl-S’-(\(\alpha\`\alpha\`\)-dimethyl-\(\alpha``\)-acetic acid)trithiocarbonate (DDMAT)) used in our previous work. Our hypothesis was that dithioester RAFT agents with stabilizing Z-groups should result in longer intermediate radical lifetimes and therefore a slower observed rate of polymerization.\(^{69,70}\) We selected cumyl dithiobenzoate (CDB) as the dithioester RAFT chain transfer agent as CDB possesses a stabilizing benzyl Z-group compared to the 12-carbon chain in DDMAT. The pseudo-first order kinetic plot of the polymerization using CDB shows an inhibition period of approximately 120 minutes, greatly reduced from approximately 400 minutes using DDMAT (Figure 3a). Once the
polymerization began, the kinetics were first-order over the next 500 minutes to approximately 60 % conversion. The molecular weight of the polymer increased smoothly with increasing conversion in much better agreement with the molecular weight predicted by theory, although the observed \( M_n \) was still higher than that predicted by theory (Figure 3b). The polydispersity (PDI) remained approximately 1.2 over the course of the polymerization.

![Figure 3](image)

**Figure 3.** a. Pseudo-first order kinetic plot of MAIpGlc at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70 °C. b. Number average molecular weight \( (M_n) \) versus conversion of MAIpGlc at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70 °C.

We copolymerized MAIpGlc with 6-(azidohexyl) methacrylate (AzHMA) using the M:CTA:I ratio of 100:1:0.33 with a monomer ratio of MAIpGlc:AzHMA of 10:1. \(^1\)H-NMR spectroscopy revealed that the actual ratio of MAIpGlc to AzHMA incorporated into the polymer was 15:1. The copolymer molecular weight was determined to be \( 2.9 \times 10^4 \) g/mol with a relatively large PDI of 1.21 using gel permeation chromatography with light scattering and refractive index detectors. In our previous work, the copolymerization of 3-(azidopropyl) methacrylate, AzPMA and MAIpGlc showed a PDI of 1.07, however, as we stated earlier the conditions in that polymerization were not ideally controlled and the molecular weight of the polymer was much
higher.\textsuperscript{25} Benicewicz\textsuperscript{71} studied the homopolymerization of AzHMA under RAFT control, they observed PDI values between 1.2 and 1.31 at 30\% conversion using free and silica bound 4-cyanopentanoic acid dithiobenzoate, while Matyjaszewski\textsuperscript{66} obtained a PDI of 1.33 for the homopolymerization of AzPMA by ATRP to 75\% conversion. Lui’s group studied more complex block copolymer that consisted of poly(N,N-dimethylacrylamide)-b-poly(N-isopropylacrylamide-co-3-azidopropylacrylamide) and found that the PDI of the final polymer was 1.23.\textsuperscript{72} We are still exploring the conditions for these polymerizations with the goals of preparing glucose-containing copolymers with well-controlled molecular weights and narrow polydispersities.

We used the copper catalyzed azide/alkyne cycloaddition (CuAAC) reaction to couple the \textit{N}-alkyl-\textit{N},\textit{N}-linked oligomers with the copolymer (Figure 4). The CuAAC reaction is used as an orthogonal coupling chemistry and has had a major impact in synthetic chemistry, including polymer chemistry. This has been illustrated by the large number of papers and reviews published using the CuAAC reaction.\textsuperscript{26-29}
We coupled the glucose-containing oligomer to the copolymer as indicated by $^1$H-NMR by comparing the integration of the proton of the triazole ring with the acetal proton on the sugar residues. Characterization of the $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugate was performed using GPC, however good-quality chromatography was not obtained, presumably due to the interaction of the polymer with the column packing material. Comparing the FT-IR spectrum of poly(MAlpGlc-co-AzHMA) with that of the oligomer-polymer conjugate showed a clear decrease in the intensity of the peak from the -N$_3$ moiety at 2098 cm$^{-1}$, further corroborating the successful coupling reaction. The FT-IR spectra are shown in Figure 6.

Following the synthesis of the $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugate the isopropylidene protecting groups present on both the methacrylate monomers and the urea oligomers were removed in one step to form the final, polysaccharide mimetic. The
isopropylidene protecting groups were removed using acid hydrolysis by stirring the \( N \)-glucose functionalized oligomer-polymer conjugate in a 20% solution of TFA at room temperature for 5 hours (Figure 5).

![Reaction scheme](image)

**Figure 5.** Reaction scheme for the deprotection of the \( N \)-alkyl-\( N,N \)-linked oligomer-polymer conjugate. “Oligomer-OR” and “Oligomer-OH” in Figure 5 refers to the \( N \)-alkyl-\( N,N \)-linked urea oligomers before and after removal of the isopropylidene protecting groups in the oligomers respectively.

The deprotected \( N \)-alkyl-\( N,N \)-linked oligomer-polymer conjugate was characterized using FT-IR and \(^1\)H NMR spectroscopy. The FT-IR spectrum showed the appearance of a broad band at 3500 cm\(^{-1}\) indicating the presence of hydroxyl groups arising from the deprotected glucose moieties on the polymer conjugate. The FT-IR spectra of the poly(MAlpGlc-\( co \)-AzHMA), polymer conjugate and final, deprotected polymer conjugate are overlaid in Figure 6.
We used the $^1$H NMR spectrum of the final $N$-alkyl-$N,N$-linked urea oligomer/polymer conjugate to quantify the extent of the deprotection reaction. The deprotection reaction was found to be quantitative by comparing the relative integrations of the peaks due to the nitrobenzene-sulfonamide and triazole protons to the peaks due to the aliphatic protons in the spectrum from the deprotected poly/$N$-alkyl-$N,N$-linked urea oligomer conjugate with those in the spectrum from the isopropylidene protected parent polymer/oligomer conjugate and accounting for the degree of polymerization of MAIpGlc and AzHMA determined from GPC (Figure 7).
2.6 Conclusions

This work demonstrates the synthesis of a polysaccharide biomimetic by using carbohydrate functionalized $N$-alkyl-$N,N$-linked urea oligomers with polymers prepared under RAFT control. The afforded polymer conjugate will be used as a baseline model for a variety of studies in future publications in comparison with other $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugates and naturally occurring sugar-based macromolecules. Indeed, we believe that the synthesis strategy presented here will enable easy comparison of design parameters including the identity of the sugar residue used as the urea oligomer side group, the molecular weight of the polymer, and the...
polymer architecture (e.g. blocks, stars, etc.) with respect to the macromolecules GAG-mimicking abilities. These experiments are ongoing, and will be the focus of future publications. Furthermore, by using a controlled radical polymerization technique we can eliminate polydispersity effects from future assays. This work is an extension from our previous papers where we focused on proof-of-concept systems, towards a functional polymer with potential biomedical and therapeutic applications.

2.7 References


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Chapter 3. Synthesis of glycosaminoglycan polymer mimetics using an N-alkyl-N,N-linked urea oligomer and copper catalyzed azide/alkyne cycloaddition chemistry

3.1 Abstract

The syntheses of glycosaminoglycan mimetic polymers are reported. Acetate protected glucose- and mannose-methacrylate monomers were copolymerized under reversible addition fragmentation chain transfer (RAFT) polymerization control with an azide-containing comonomer 3-azidopropyl methacrylate (AzPMA). Polymers of 39 000 g/mol and polydispersity index (PDI) of 1.14 for the glucose copolymer and 72 000 g/mol and PDI = 1.17 for the mannose copolymer were obtained. The comonomer ratios were determined to be 3:1 and 7:1 for the glucose and mannose polymers respectively based on $^1$H NMR spectroscopy. These copolymers were coupled to sugar-functionalized N-alkyl-N,N-linked urea oligomers using the copper catalyzed alkyne/azide cycloaddition (CuAAC) reaction. The reaction efficiency was 100% as monitored by $^1$H NMR spectroscopy. The acetate protecting groups on the polymer and N-alkyl-N,N-linked urea oligomers were removed using Zemplén deacetylation followed by the sulfonation to obtain the final GAG mimetic. It is anticipated that these N-alkyl-N,N-linked urea oligomers-polymer conjugates may have uses as therapeutics for preventing blood clotting similar to heparin and heparin-derived products.

3.2 Introduction

Glycosaminoglycans (GAGs) are heterogeneous sugar macromolecules that are involved in many biological processes, such as wound healing, angiogenesis, amyloid diseases, and anticoagulation. Due to their involvement in so many biological functions, there has been great effort to characterize the structure of GAGs and their individual functions. However, it is
difficult to determine the exact structure of many GAGs, and at present there does not appear to be a direct correlation of their structure to their activity. Generally, GAGs are linear polysaccharides that have a molecular weight range of 10-1000 kDa and are negatively charged due to carboxylate and sulfonate groups. There are two main classes of GAGs, non-sulfated and sulfates. Hyaluronic acid is a non-sulfate GAG, while chondroitin sulfate, dermatan sulfate, keratin sulfate, heparin and heparan sulfate are sulfated GAGs.

The role of heparin and heparan sulfate in inhibiting blood coagulation is one of the most studied biological roles of GAGs. Heparin is sourced from bovine lung and porcine intestine and is currently used as an anticoagulant in therapeutic treatments of thrombosis, thrombophlebitis, and embolism. However, there are several risks involved in the use of heparin, such as pathogenic contamination and heparin-induced thrombocytopenia. Due to these risks there have been many attempts to mimic the activity of heparin by preparing synthetic derivatives or using non-mammalian sourced polysaccharides and sulfated polymers.

Many synthetic derivatives of GAGs are synthesized using controlled-polymerization techniques including reversible addition fragmentation chain transfer (RAFT) polymerization. RAFT polymerization has proven to be an excellent method for polymerization of carbohydrate-containing monomers due to its simplicity and tolerance to a wide variety of functional groups. For example, Guo and coworkers used RAFT polymerization to prepare polymers that contained lactose residues. The researchers synthesized an acetate protected lactose monomer by reacting an acetatylated lactose molecule with 2-hydroxyethylmethacrylate. Using cumyl dithiobenzoate (CDB) they were able to generate well-defined polymers that contain lactose pendant groups that can then be deprotected and used as a novel media for the separation and analysis of biologics. Liu and coworkers used RAFT polymerization to synthesize well-
defined pH-sensitive copolymers that contained poly(2-(diethylamino)ethyl methacrylate (poly(DEAEMA)) and poly(3-O-methacryloyl-α,β-D-glucopyranose) (poly(MAGlc)). These copolymers were able to bind Concanavalin A, which is a common lectin binding protein.\(^{28}\)

Abdelkader et al. used aqueous RAFT polymerization to generate well-defined clickable glycopolymers. They used novel carbohydrate based acrylamides that had an azide at C-2 or C-6 and determined that they could be polymerized under RAFT control.\(^{20}\) Narain and coworkers used RAFT polymerization that contained 3-gluconamidopropylmethacrylamide (GAPMA) and 2-lactobionamidoethylmethacrylamide (LAEMA) to synthesize a hyperbranched glycopolymer to enhance the biocompatibility of drug carriers.\(^{16}\)

We previously demonstrated the copolymerization of an isopropylidene protected glucose methacrylate monomer, 3-O-methacryloyl-1,2:5,6-di-O-isopropylidene-β-D-glucofuranose (MAIpGlc) under RAFT control with an azide-containing comonomer.\(^{29,30}\) The incorporation of azide containing monomer have been shown to be a useful synthetic tool in the post modification of polymers.\(^{31-34}\) Sequence-specific N-alkyl-N,N-linked urea oligomers were coupled to the azide-containing repeat units using a copper catalyzed azide/alkyne cycloaddition (CuAAC) reaction, often referred to as “click” chemistry.\(^{35-37}\) We consider the N-alkyl-N,N-linked urea oligomers hybrids of urea oligomers\(^{38,39}\) and N-acyl glycine oligomers or “peptoids”\(^{40,41}\). The N-alkyl-N,N-linked urea oligomers made by our lab are prepared through a series of simple synthetic reactions and can incorporate a wide variety of functional groups.\(^{29,30,42}\) These properties enable N-alkyl-N,N-linked urea oligomers to be used in structure-property relationship studies, including the study of oligomers and polymers with the potential of mimicking the activity of heparin.\(^{30,43}\)
In this work the synthesis of a glucose- and mannose-containing polymers was accomplished using RAFT polymerization of 2-(2',3',4',6'-tetra-O-acetyl-β-D-glucosyloxy)ethyl methacrylate (AcGluEMA) or 2-(2',3',4',6'-tetra-O-acetyl-β-D-mannosyloxy)ethyl methacrylate (AcManEMA) and 3-azidopropyl methacrylate (AzPMA), and conjugating the polymers to the corresponding glucose or mannose functionalized N-alkyl-N,N-linked urea oligomers. In our previous work we used isopropylidene protected glucose residues and added these to the N-alkyl-N,N-linked urea oligomer as N-alkyl side group. While this was successful, the overall reaction suffered from low yields and difficult purifications. In this work we aimed to simplify the synthesis of the N,N-linked urea oligomer by including terminal alkyne units as the N-alkyl groups allow for simple post-modification of the oligomers to investigate the properties of several carbohydrates with greater ease. We used copper catalyzed click chemistry to attach azide containing acetate protected glucose or mannose residues, prior to adding the final terminal alkyne. By using the same protecting group on the polymer and oligomer, we are able to perform a single deprotection to yield a final polymer GAG-mimetic.

3.3 Experimental Section

All starting materials were purchased from Aldrich at the highest purity available and used as received unless specified otherwise. N-(2-Nitrobenzenesulfonyl)-2-imidazolidone was prepared according to the method of Wilson and Nowick. N-(2-(3,3-diethylureido)ethyl)-2-nitrobenzenesulfonamide was synthesized as previously reported. The RAFT agent cumyl dithiobenzoate (CDB) was synthesized according to literature procedures. The synthesis of 3-azidopropyl methacrylate was performed according to literature. The synthesis of the acetylated sugar residues followed standard carbohydrate chemistry techniques.
3.3.1 Synthesis of alkyne containing N-alkyl-N,N-linked urea oligomer

**Compound 2.** N-(2-(3,3-diethylureido)ethyl)-2-nitrobenzenesulfonamide (Compound 1) (7.43 g, 21.6 mmol), K$_2$CO$_3$ (3.01 g, 21.8 mmol) and propargyl chloride (3.21 g, 43.1 mmol) were dissolved in 50 mL of N,N-dimethylformamide (DMF) and stirred at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH$_2$Cl$_2$ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The product was dried in vacuum to afford 8.0 g of light yellow oil. Yield: 97%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.13$ (t, $J = 8$ Hz, 6H, 2x CH$_3$CH$_2$N), 2.20 (s, $\text{HCCCH}_2$), 3.25 (q, $J = 8$, 16 Hz, 4 H, 2x CH$_3$CH$_2$N), 3.48-3.59 (m, 4 H, NHCH$_2$CH$_2$NNs), 4.27 (s, NCH$_2$CCH), 4.80 (bs, CONH), 7.64-7.71 (m, 3 H, aromatic H) 8.04-8.06 (m, 1 H, aromatic H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 13.70$ (2 x CH$_3$CH$_2$N), 36.84, (CH$_2$CCH), 37.93 (NHCH$_2$-CH$_2$NNs), 41.19 (2x CH$_3$CH$_2$), 46.90 (NHCH$_2$CH$_2$NNs), 53.40 (CH$_2$CCH), 74.06 (CH$_2$CCH), 124.16, 130.87, 131.73, 132.57, 133.79, 148.10 (aromatic), 157.19 (C=O). MS (TOF MS ESI): $M_{\text{theo}} = 382.1311$ g/mol. M+H$^+$ = 383.1378 g/mol.

**Compound 3.** Compound 2 (8.0 g, 20.9 mmol) and K$_2$CO$_3$ (8.65 g, 62.6 mmol) were dissolved in 50 mL of DMF. The reaction was purged with N$_2$ and benzenethiol (4.11 mL, 40.0 mmol) was added. The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation. The residue was dissolved in CH$_2$Cl$_2$ and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with CH$_2$Cl$_2$:MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product dried in vacuum to yield 3.94 g of a light yellow oil. Yield: 96%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.13$ (t, $J = 8$ Hz, 6 H, 61
2 x CH$_3$CH$_2$N), 2.25 (s, 1 H, CH$_2$CCH), 2.43 (s, 2 H, CH$_2$CCH), 2.86 (t, $J = 16$ Hz, 2 H, NHCH$_2$CH$_2$NHCH$_2$CCH), 3.26 (q, $J = 4$, 12 Hz, 4 H, 2 x CH$_3$CH$_2$N), 3.34 (t, 2 H, NHCH$_2$-CH$_2$NHCH$_2$CCH). 5. 03 (bs, CONH); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 13.70 (2 x CH$_3$CH$_2$N), 37.56 (CH$_2$CCH), 37.93 (NHCH$_2$CH$_2$NHCH$_2$CCH), 41.01 (2 x CH$_3$CH$_2$), 48.00 (NHCH-CH$_2$NHCH$_2$CCH), 71.57 (CH$_2$CCH), 81.61 (CH$_2$CCH), 157.46 (CONH). MS (TOF MS ESI): $M_{\text{theo}} = 197.1528$ g/mol. M+H$^+$ = 198.1613 g/mol.

**Compound 4.** Compound 3 (3.94 g, 19.9 mmol), N-(2-nitrobenzenesulfonyl)-2-imidazolidone (7.04 g, 25.9 mmol) and (dimethylamino)pyridine (DMAP) (1.22 g, 9.9 mmol) were dissolved in 20 mL of pyridine. The reaction flask was sealed with a rubber septum, purged with N$_2$ for 30 min, and then immersed in preheated oil bath at 60 °C for 6 h. The solvent was removed using a rotary evaporator, the residue dissolved in CH$_2$Cl$_2$, washed with 0.5 M aqueous HCl and then dried over Na$_2$SO$_4$. The solved was removed and the product was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The solvent was removed and the product was dried in a vacuum to yield 8.87 g of a pale yellow solid. Yield: 95%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 1.16 (t, $J = 8$ Hz, 6 H, 2 x CH$_3$CH$_2$N), 2.21 (s, 1 H, HCCCH$_2$), 3.26-3.38 (m, 12 H, all CH$_2$ on backbone), 4.12 (s, 2 H, HCCCH$_2$N), 4.92 (s, NH) 7.06 (s, NH), 7.71-7.77 (m, 3 H, aromatic), 8.11 (s, 1 H, aromatic). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 13.57 (2 x CH$_3$CH$_2$N), 36.61 (CH$_2$CCH), 39.94 (NHCH-CH$_2$N), 40.67 (OCNHCH$_2$CH$_2$NCO), 41.23 (2 x CH$_3$CH$_2$N), 44.15 (NHCH$_2$CH$_2$N), 45.45 (OCNHCH$_2$CH$_2$NCO), 71.54 (CH$_2$CCH), 80.39 (CH$_2$CCH), 124.88, 130.88, 132.45, 133.24, 134.20, 147.95 (aromatic), 157.60 (C=O), 158.20 (C=O). MS (TOF MS ESI): $M_{\text{theo}} = 468.1791$ g/mol. M+H$^+$ = 469.1871 g/mol.
**Compound 5.** Compound 4 (11.44 g, 24.36 mmol), K₂CO₃ (8.42 g, 60.9 mmol) and propargyl chloride (3.63 g, 48.7 mmol) were dissolved in 50 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH₂Cl₂ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:methanol (4:1 v/v) as the mobile phase. The product was dried in vacuum to afford 11.54 g of oil. Yield: 94%. 

**1H NMR (400 MHz, CDCl₃):** δ = 1.12 (t, J = 8 Hz, 6H, 2 x C₂H₅N), 2.20 (s, 1 H, HCCCH₂), 2.26 (s, 1 H, HCCCH₂), 3.22-3.61 (m, 14 H, all C₂H₂ on backbone), 4.08 (s, 2 H, NC₂H₂CCH), 4.33 (s, 2 H, -NC₂H₂CCH), 7.65-7.70 (m, 3 H, aromatic H) 8.05 (m, 1 H, aromatic H). 

**13C NMR (100 MHz, CDCl₃):** δ = 13.71 (2 x C₂H₅N), 36.48, 36.98 (2 x C₂H₅CCH), 37.88 (NHCH₂CH₂NNs), 39.76 (OCNHCH₂CH₂NCO), 41.09 (2 x C₂H₅N), 46.67 (NHCH₂CH₂NNs), 47.05 (OCNHCH₂CH₂NCO), 56.80 (NsNCH₂CCH), 72.05 (CH₂CCH), 74.03 (CH₂CCH), 82.25 (CH₂CCH), 124.15, 130.80, 131.73, 131.73, 133.63, 147.95 (aromatic), 157.74 (C=O), 157.81 (C=O). MS (TOF MS ESI): M⁻theo = 506.1948 g/mol. M⁺Na⁺ = 529.1807 g/mol.

**Compound 6.** Compound 5 (11.54 g, 22.78 mmol) and K₂CO₃ (9.44 g, 68.3 mmol) were dissolved in 50 mL of DMF. The reaction was purged with N₂ and benzenethiol (4.87 mL, 45.6 mmol) was added. The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation. The residue was dissolved in CH₂Cl₂ and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with CH₂Cl₂:MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product was dried in vacuum to yield 6.42 g of oil. Yield: 88%. 

**1H NMR (400 MHz, CDCl₃):** δ = 1.13 (t, J = 8 Hz, 6 H, 2 x
CH₁CH₂N), 2.25 (s, 2 H, 2 x HCCCH₂), 2.63-2.90 (m, 5 H, NHCH₂CCH + NHCH₂CCH) 3.22-3.46 (m, 12 H, CH₂ on backbone), 6.01 (bs, 1 H, CONH), 6.66 (bs, 1 H, CONH); ¹³C NMR (100 MHz, CDCl₃): δ = 13.66 (2 x CH₃CH₂N), 36.61, 37.63 (2 x CH₂CCH), 39.74 (OCNHCH₂CCH₂NH), 40.23 (OCNHCH₂CH₂NCO), 41.09 (2 x CH₃CH₂N), 45.66 (OCNHCH₂CH₂NH), 46.55 (OCNHCH₂CH₂NCO), 46.82, 48.11 (NHCH₂CCH), 71.82 (CH₂CCH), 71.89 (CH₂CCH), 80.10 (CH₂CCH), 81.19 (CH₂CCH), 157.85 (C=O), 158.13 (C=O). MS (TOF MS ESI): M_theo = 321.2165 g/mol. M+H⁺ = 322.2187 g/mol.

**Compound 7.** Compound 6 (6.42 g, 19.9 mmol), N-(2-nitrobenzenesulfonyl)-2-imidazolidone (6.5 g, 24 mmol) and (dimethylamino)pyridine (DMAP) (1.22 g, 9.99 mmol) were dissolved in 25 mL of pyridine. The reaction flask was sealed with a rubber septum, purged with N₂ for 30 min, and then immersed in preheated oil bath at 60 °C for 6 h. The solvent was removed using a rotary evaporator, the residue dissolved in CH₂Cl₂, washed with 0.5 M aqueous HCl and then dried over Na₂SO₄. The solved was removed and the product was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The solvent was removed and the product was dried in a vacuum to yield 8.53g of a white solid. Yield: 72%. ¹H NMR (400 MHz, CDCl₃): δ = 1.14 (t, J = 8 Hz, 6 H, 2 x CH₃CH₂N), 2.19 (s, 1 H, HCCCH₂N), 2.27 (s, 1 H), 3.23-3.43 (m, 16 H, all CH₂ on backbone), 4.11-4.20 (m, 4 H, 2 x NCH₂CCH), 7.70-7.76 (m, 3 H, aromatic), 8.12 (s, 1 H, aromatic). ¹³C NMR (100 MHz, CDCl₃): δ = 13.77 (2 x CH₃CH₂N), 36.59 (NHCH₂CH₂NNs), 39.27 (2 x OCNHCH₂CH₂NCO), 40.68(OCNHCH₂CH₂NCO), 41.17 (2 x CH₃CH₂N), 44.34, 46.22 (NHCH₂CH₂NNs), 71.40 (CH₂CCH), 71.45 (CH₂CCH), 80.50 (CH₂CCH), 81.02 (CH₂CCH), 124.89, 128.90, 131.08, 132.56, 133.33, 134.11 (aromatic), 157.57 (C=O), 159.12 (C=O), 159.83 (C=O). MS (TOF MS ESI): M_theo = 592.2428 g/mol. M+H⁺ = 593.2487.
3.3.2 Modification of carbohydrates for CuAAC reactions

**(2-bromoethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside**. 1,2,3,4,6-penta-O-acetyl-α,β-D-glucopyranose (7.0 g, 18 mmol) and 2-bromoethanol (1.52 mL, 21.4 mmol) were dissolved in 70 mL dry CH$_2$Cl$_2$. The reaction was purged with nitrogen and BF$_3$·Et$_2$O (12.71 mL, 102.9 mmol) was added dropwise while stirring on ice. The reaction was allowed to stir at room temperature overnight. The reaction was quenched by pouring over ice water while stirring. The organic layer was washed with saturated NaHCO$_3$ and brine solutions, dried over Na$_2$SO$_4$, and the solvent was removed from rotary evaporation. The product was recrystallized from ethyl acetate:isoctane (1:1 v/v) to obtain 5.7 g of a light brown solid. Yield 70%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 2.02-2.19 (m, 12 H, 4 x COOC$_3$H$_3$), 3.47-3.53 (m, 2H, -OCH$_2$CH$_2$Br), 3.71-3.84 (m, 2 H, H$_5$, -OCCH$_2$CH$_2$Br), 4.03-4.29 (m, 3 H, H$_6$, -OCCH$_2$CH$_2$Br), 4.58 (d, $J =$ 8 Hz, 1 H, H$_1$), 5.00-5.22 (m, 3 H, H$_2$, H$_3$, H$_4$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 20.45, 20.59, 20.74, 20.88 (4 x COOCH$_3$), 29.89 (CH$_2$CH$_2$Br), 61.82 (C$_6$), 63.81 (CH$_2$CH$_2$Br), 68.29 (C$_4$), 69.79 (C$_5$), 70.99 (C$_5$), 72.58 (C$_3$), 101.00 (C$_1$), 169.39 (C=O), 170.19 (C=O), 170.24 (C=O), 170.63 (C=O). MS (TOF MS ESI): $M_{\text{theo}}$ = 454.0475 g/mol. M+Na$^+$ = 477.0415 g/mol.

**(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside**. (2-Bromoethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (5.7 g, 13 mmol) and 20 mL of 0.5 M NaN$_3$ in DMSO were added to a round bottom flask and allowed to stir overnight. The reaction was quenched with 20 mL of water and extracted with ether several times. The combined organic layers were washed with water and brine solution and dried over MgSO$_4$. The solvent was removed to yield 3.51 g of a light brown solid. Yield 67%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 1.99-2.18 (m, 12 H, 4 x COOCH$_3$), 3.29-3.52 (m, 2H, -OCH$_2$CH$_2$N$_3$), 3.72-3.76 (m, 2 H, H$_5$, -OCH$_2$CH$_2$N$_3$), 4.03-4.29 (m, 3 H, H$_6$, -OCH$_2$CH$_2$N$_3$), 4.63 (d, $J =$ 8 Hz, 1 H, H$_1$), 5.00-5.25 (m, 3 H, H$_2$, H$_3$, H$_4$). $^{13}$C
NMR (100 MHz, CDCl\textsubscript{3}): \(\delta = 20.12, 20.27, 20.36, 20.41\) (4 x COOCH\textsubscript{3}), 50.19 (CH\textsubscript{2}CH\textsubscript{3}N\textsubscript{3}), 61.53 (CH\textsubscript{2}CH\textsubscript{2}N\textsubscript{3}), 68.00 (C\textsuperscript{6}), 68.31 (C\textsuperscript{4}), 70.75 (C\textsuperscript{2}), 71.57 (C\textsuperscript{5}), 72.46 (C\textsuperscript{3}), 100.32 (C\textsuperscript{1}), 169.06 (C=O), 169.10 (C=O), 169.88 (C=O), 170.29 (C=O). MS (TOF MS ESI): \(M_{\text{theo}} = 417.1383\) g/mol. \(M+Na^+ = 440.1255\) g/mol. FT-IR (cm\textsuperscript{-1}): 1736.6 (C=O), 2105.7 (N\textsubscript{3}).

\((2\text{-bromoethyl})\text{-2,3,4,6-tetra-O-acetyl-}\beta\text{-D-mannopyranoside.}\) 1,2,3,4,6-penta-O-acetyl-\(\alpha,\beta\text{-D-mannopyranose (53.53 g, 0.137 mol) and 2-bromoethanol (11.61 mL, 0.164 mol) were dissolved in 600 mL dry CH\textsubscript{2}Cl\textsubscript{2}. The reaction was purged with nitrogen and BF\textsubscript{3}.Et\textsubscript{2}O (97.14 mL, 0.787 mol) was added dropwise while stirring on ice. The reaction was allowed to stir at room temperature overnight. The reaction was quenched by pouring over ice water while stirring. The organic layer was washed with saturated NaHCO\textsubscript{3} and brine solutions and then dried over Na\textsubscript{2}SO\textsubscript{4}. The product was purified by triturating with hexane:ethyl acetate (1:1 v/v) and the solvent was reduced to obtain 26.35 g of a light brown solid. Yield 42%. \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta = 2.00 - 2.18\) (m, 12 H, 4 x COOCH\textsubscript{3}), 3.52 (t, \(J = 6\) Hz, 2 H, OCH\textsubscript{2}CH\textsubscript{2}Br), 3.89-3.97 (m, 2 H, (OCH\textsubscript{2}CH\textsubscript{2}Br), 4.12-4.29 (m, 3 H, H\textsuperscript{6}, H\textsuperscript{5}), 4.88 (d, \(J = 8\) Hz, 1 H, H\textsuperscript{1}), 5.28-5.37 (m, 3 H, H\textsuperscript{2}, H\textsuperscript{3}, H\textsuperscript{4}). \(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta = 20.65, 20.69, 20.73, 20.85\) (4 x COOCH\textsubscript{3}), 29.58 (CH\textsubscript{2}CH\textsubscript{2}Br), 62.38 (C\textsuperscript{6}), 65.97 (CH\textsubscript{2}CH\textsubscript{2}Br), 68.45 (C\textsuperscript{4}), 68.91 (C\textsuperscript{2}), 68.99 (C\textsuperscript{5}), 69.40 (C\textsuperscript{3}), 97.72 (C\textsuperscript{1}), 169.58 (C=O), 169.72 (C=O), 169.83 (C=O), 169.99 (C=O). MS (TOF MS ESI): \(M_{\text{theo}} = 454.0475\) g/mol. M+Na\textsuperscript{+} = 477.1215 g/mol.

\((2\text{-azidoethyl})\text{-2,3,4,6-tetra-O-acetyl-}\beta\text{-D-mannopyranoside.}\) (2-Bromoethyl)-2,3,4,6-tetra-O-acetyl-\(\beta\text{-D-mannopyranoside (26.35 g, 0.0579 mol) and 100 mL of 0.5 M NaN\textsubscript{3} in DMSO were added to a round bottom flask and allowed to stir overnight. The reaction was quenched with 100 mL of water and extracted with ether several times. The combined organic layers were washed with water and brine solution and dried over MgSO\textsubscript{4}. The solvent was removed to yield 16.19 g

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of a light brown solid. Yield 70%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 2.00-2.18 (m, 12 H, 4 x COOCH$_3$), 3.8 (t, $J$ = 8 Hz, 2 H, OCH$_2$CH$_2$N$_3$), 3.67-3.88 (m, 2 H, (OCH$_2$CH$_2$N$_3$), 4.06-4.32 (m, 3 H, H$^6$, H$^7$), 4.88 (s, 1 H, H$^1$), 5.23-5.37 (m, 3 H, H$^2$, H$^3$, H$^4$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 20.29, 20.33, 20.37, 20.50 (4 x COOCH$_3$), 49.93 (CH$_2$CH$_2$N$_3$), 62.03 (C$^6$), 63.56 (CH$_2$CH$_2$N$_3$), 65.53 (C$^4$), 66.66 (C$^2$), 68.41 (C$^5$), 69.95 (C$^3$), 97.31 (C$^1$), 169.40 (C=O), 169.66 (C=O), 170.27 (C=O), 170.36 (C=O). MS (TOF MS ESI): $M_{\text{theo}}$ = 417.1383 g/mol. M+Na$^+$ = 440.1214 g/mol. FT-IR (cm$^{-1}$), 1739.5 (C=O), 70.36 (C=O). 

3.3.3 Copper catalyzed azide/alkyne cycloaddition reactions

(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-$\beta$-D-glucopyranoside to compound 7. (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-$\beta$-D-glucopyranoside (2.1 g, 5.1 mmol), Compound 7 (1.0 g, 1.7 mmol) and CuBr (0.072 g, 0.502 mmol) in dichloromethane were added to a round bottom flask equipped with a stir bar. The flask was purged with N$_2$ for 30 min and 0.1 mL of N,N,N’,N”’,N”’-pentamethyldiethylenetriamine (PMDETA) was added and the reaction was allowed to stir at room temperature overnight. The solution was then passed through basic alumina using a gradient elution of dichloromethane and 10:1 dichloromethane:methanol to obtain 1.98 g of compound 8A. Yield 83%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 1.09-1.11 (t, 6 H, 2 x CH$_3$CH$_2$N), 1.99-2.09 (m, 24 H, 4 x COOCH$_3$), 3.22-3.40 (m, 16 H, all CH$_2$ on backbone), 4.00-4.57 (m, 16 H, 2 x NCH$_2$CCN, 2 x H$^5$, 2 x -OCH$_2$CH$_2$N, 2 x H$^1$, 4 x H$^6$), 4.94-5.17 (m, 6 H, 2 x H$^2$, 2 x H$^3$, 2 x H$^4$), 7.70-7.76 (m, 4 H, aromatic, triazole H), 8.10 (s, 1 H, aromatic). MS (TOF MS ESI): $M_{\text{theo}}$ = 1426.5195 g/mol. M+Na$^+$ = 1540.9104 g/mol.

(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-$\beta$-D-mannopyranoside to compound 7. (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-$\beta$-D-mannopyranoside (2.1 g, 5.1 mmol), Compound 7 (1 g, 1.68 mmol)
and CuBr (0.072 g, 0.502 mmol) in dichloromethane were added to a round bottom flask equipped with a stir bar. The flask was purged with N$_2$ for 30 minutes and then 0.1 mL of PMDETA was added and the reaction was allowed to stir at room temperature overnight. The solution was then passed through basic alumina using a gradient elution of dichloromethane and 10:1 dichloromethane:methanol to obtain 1.79 g of compound 8B. Yield 75%. $^1$H NMR (400 MHz, CDCl$_3$): δ = 1.09-1.11 (t, 6 H, 2 x CH$_3$CH$_2$N), 1.99-2.18 (m, 24 H, 4 x COOCH$_3$), 3.22-3.40 (m, 16 H, all CH$_2$ on backbone), 4.01-4.57 (m, 16 H, 2 x NCH$_2$CCN, 2 x H$_5$, 2 x -OCH$_2$CH$_2$N, 2 x H$^1$, 4 x H$^6$), 4.80-5.25 (m, 6 H, 2 x H$^2$, 2 x H$^3$, 2 x H$^4$), 7.72-7.77 (m, 4 H, aromatic, triazole H), 8.10 (s, 1 H, aromatic). MS (TOF MS ESI): $M_{theo}$ = 1426.5195 g/mol. M+Na$^+$ = 1540.5406 g/mol.

**Compound 9A.** Compound 8A (1.98 g, 1.39 mmol), K$_2$CO$_3$ (0.48 g, 3.47 mmol) and propargyl chloride (0.207 g, 2.78 mmol) were dissolved in 10 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH$_2$Cl$_2$ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:methanol (4:1 v/v) as the mobile phase. The product was dried in vacuum to afford 1.32 g of light yellow oil. Yield: 65%. $^1$H NMR (400 MHz, CDCl$_3$): δ = 1.08-1.10 (t, 6 H, 2 x CH$_3$CH$_2$N), 1.99-2.18 (m, 20 H, 4 x COOCH$_3$), 3.22-3.70 (m, 16 H, all CH$_2$ on backbone), 3.98-4.57 (m, 18 H, 1 x NCH$_2$CCH, 2 x NCH$_2$CCN, 2 x H$_5$, 2 x -OCH$_2$CH$_2$N, 2 x H$^1$, 4 x H$^6$), 4.95-5.31 (m, 6 H, 2 x H$^2$, 2 x H$^3$, 2 x H$^4$), 7.64-7.77 (m, 4 H, aromatic, triazole H), 7.99 (s, 1 H, aromatic). MS (TOF MS ESI): $M_{theo}$ = 1464.5351 g/mol. M+H$^+$ = 1465.5431 g/mol.
**Compound 9B.** Compound 8B (1.79 g, 1.25 mmol), K$_2$CO$_3$ (0.43 g, 3.11 mmol) and propargyl chloride (0.188 g, 2.52 mmol) were dissolved in 10 mL of $N,N$-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH$_2$Cl$_2$ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:methanol (4:1 v/v) as the mobile phase. The product was dried in vacuum to afford 1.1 g of light yellow oil. Yield: 60%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 1.06-1.12 (t, 6 H, 2 x CH$_3$CH$_2$N), 1.99-2.19 (m, 20 H, 4 x COOC$_3$H$_7$), 3.22-3.70 (m, 16 H, all CH$_2$ on backbone), 3.91-4.59 (m, 18 H, 1 x NCH$_2$CCH, 2 x NCH$_2$CCN, 2 x H$_5$, 2 x -OCH$_2$CH$_2$N, 2 x H$^1$, 4 x H$^6$), 4.93-5.27 (m, 6 H, 2 x H$^2$, 2 x H$^3$, 2 x H$^4$), 7.64-7.79 (m, 4 H, aromatic, triazole H), 7.99 (s, 1 H, aromatic). MS (TOF MS ESI): $M_{\text{theo}}$ = 1464.5351 g/mol. M+Na$^+$ = 1465.5239 g/mol.

### 3.3.4 Carbohydrate containing polymers

**Synthesis of 2-(2',3',4',6'-tetra-O-acetyl-β-D-glucosyloxy)ethyl methacrylate (AcGluEMA)**. $^{47}$ 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (50.00 g, 0.108 mol), 2-hydroxyethyl methacrylate (18.6 mL, 0.154 mol) and 175 mL of anhydrous dichloromethane were added to a round bottom flask equipped with a constant pressure addition funnel. BF$_3$Et$_2$O (81.16 mL, 0.64 mol) was added to the addition funnel and the reaction was flushed with nitrogen for 30 minutes in an ice bath. The BF$_3$Et$_2$O was then added dropwise over 1 hour while stirring on ice and then allowed to come to room temperature overnight. The reaction was quenched with 100 mL of water and the organic layer was isolated. The organic layer was then washed with saturated Na$_2$CO$_3$ and brine solutions and dried over Na$_2$SO$_4$ and reduced. An excess of pyridine and acetic anhydride was then added and allowed to react under nitrogen
overnight to remove any unreacted 2-hydroxyethyl methacrylate. This was then washed with saturated NaHCO₃, 1 M HCl and brine solutions and then dried over Na₂SO₄ and reduced. The resulting oil was recrystallized from 1.2:1 hexane:ethyl acetate to obtain 15.93 g of a white solid. Yield 27%. ¹H NMR (400 MHz, CDCl₃): δ = 1.96-2.16 (m, 15 H, 4 x COOC₃H₇, -CH₃), 3.77-3.81 (m, 1 H, H₃), 3.90-3.94 (m, 1 H, H₇a), 4.04-4.11 (m, 2 H, H₇b, H₆a), 4.26-4.36 (m, 3 H, H₆b, H₈), 4.88 (s, 1H, H₁), 5.26-5.37 (m, 3 H, H², H³, H⁴), 5.61 (s, 1H, C=CH₂), 6.14 (s, 1H, C=CH₂).

¹³C NMR (100 MHz, CDCl₃): δ = 18.58, 20.96, 20.98, 21.15 (5 x COOC₃H₇), 62.64 (CH₂CH₂CO), 63.40 (C⁶), 66.15 (CH₂CH₂CO), 66.33 (C⁴), 68.85 (C⁵), 68.19 (C⁵), 69.67 (C³), 97.77 (C¹), 126.35 (C=CH₂), 136.20 (C=CH₂), 167.37, (C=O), 169.98 (C=O), 170.15 (C=O), 170.30 (C=O), 170.91 (C=O). MS (TOF MS ESI): Mᵢ₆ₑₒ = 460.1583 g/mol. M+Na⁺ = 483.1463 g/mol.

Synthesis of 2-(2’,3’,4’,6’-tetra-O-acetyl-β-D-mannosyloxy)ethyl methacrylate (AcManEMA). 1,2,3,4,6-penta-O-acetyl-α,β-D-mannopyranose (63.76 g, 0.138 mol), 2-hydroxyethyl methacrylate (20.8 mL, 0.172 mol) and 250 mL of anhydrous dichloromethane were added to a round bottom flask equipped with a constant pressure addition funnel. BF₃-Et₂O (103.5 mL, 0.817 mol) was added to the addition funnel and the reaction was flushed with nitrogen for 30 minutes in an ice bath. The BF₃-Et₂O was then added dropwise over 1 hour while stirring on ice and was then allowed to come to room temperature overnight. The reaction was quenched with 100 mL of water and the organic layer was isolated. The organic layer was then washed with saturated Na₂CO₃ and brine solutions and then dried over Na₂SO₄ and reduced. An excess of pyridine and acetic anhydride was added and allowed to react under nitrogen overnight to remove any unreacted 2-hydroxyethyl methacrylate. This was then washed with saturated NaHCO₃, 1 M HCL and brine solutions and then dried over Na₂SO₄ and reduced. The resulting oil was then recrystallized from 1.2:1 hexane:ethyl acetate to obtain 18.65 g of a white solid.
Yield 26%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 2.01-2.10 (m, 15 H, 4 x COOCH$_3$, -CH$_3$), 4.08-4.15 (m, 3 H, H$_5$, H$_{7a}$, H$_{7b}$), 4.28-4.36 (m, 3 H, H$_6$, H$_8$), 5.08-5.17 (m, 3 H, H$_2$, H$_3$, H$_4$), 5.61 (s, 1H, C=CH$_2$), 6.13 (s, 1H, C=CH$_2$) 6.34 (s, 1H, H$_1$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 18.20, 20.37, 20.49, 20.61, 20.79 (5 x COOCH$_3$), 61.39 (CH$_2$CH$_2$CO), 62.05 (C$_6$), 62.36 (CH$_2$CH$_2$CO), 67.41 (C$_4$), 67.81 (C$_5$), 69.12 (C$_5$), 69.75 (C$_5$), 88.98 (C$_1$), 125.96 (C=CH$_2$), 135.88 (C=CH$_2$), 167.01, (C=O), 168.67 (C=O), 169.32 (C=O), 169.57 (C=O), 170.12 (C=O). MS (TOF MS ESI): $\text{M}_{\text{theo}}$ = 460.1583 g/mol. $\text{M+Na}^+$ = 483.1412 g/mol.

**Polymerization of AcGluEMA.** AcGluEMA, CDB, and 2,2’-azobis(2-methylpropionitrile) (AIBN), (100:1:0.33) were added to 4 mL of anhydrous anisole (Solvent:Monomer = 2:1 v/v). Air was removed from the reaction flasks through three consecutive freeze-pump-thaw cycles and the flasks were back-filled with nitrogen. The polymerizations were transferred to an oil bath at the desired temperature and allowed to react for 10 h. Samples were taken every hour using a degassed syringe and quenched by exposure to air and rapid cooling. All polymerizations were quenched by exposure to air and rapid cooling and purified by precipitation into cold hexane to yield a pale pink solid.

**Synthesis of poly(AcGluEMA-co-AzPMA).** A 50 mL air-free round bottom flask was charged with AcGluEMA (1.929 g, 0.0042 mol), AzPMA (0.0709 g, 0.419 mmol), CDB (0.0114 g, 0.048 mmol), and AIBN (0.00229 g, 0.0139 mmol) in 4 mL of anhydrous anisole. Air was removed from the flask by three consecutive freeze-pump-thaw cycles and the polymerization was performed for 10 h at 70 °C before being quenched by exposure to air and rapid cooling. The polymer was precipitated twice from hexane and dried in vacuum to afford 1.62 g of pale pink solid. Isolated yield: 81%. $M_w$ = 39,710 g/mol. AcGluEMA:AzPMA = 3:1. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 1.96-2.16 (m, 50 H, 4 x COOCH$_3$, -CH$_3$, OCH$_2$CH$_2$CH$_2$N$_3$), 3.76-4.29 (m, 23 H,
OCH₂CH₂CH₂N₃, CHs of sugar), 4.61 (s, 3 H, H¹), 5.26-5.00-5.23 (m, 9 H, H², H³, H⁴). FT-IR: N₃ 1729.0 cm⁻¹ CO stretch.

**Polymerization of AcManEMA.** AcManEMA, CDB, and 2,2’-azobis(2-methylpropionitrile) (AIBN), (100:1:0.33) were added to 4 mL of anhydrous anisole (Solvent:Monomer = 2:1 v/v). Air was removed from the reaction flasks through three consecutive freeze-pump-thaw cycles and the flasks were back-filled with nitrogen. The polymerizations were transferred to an oil bath at the desired temperature and allowed to react for 10 h. Samples were taken every hour using a degassed syringe and quenched by exposure to air and rapid cooling. All polymerizations were quenched by exposure to air and rapid cooling and purified by precipitation into cold hexane to yield a pale pink solid.

**Synthesis of poly(AcManEMA-co-AzPMA).** AcGluEMA, CDB, and 2,2’-azobis(2-methylpropionitrile) (AIBN), (100:1:0.33) were added to 4 mL of anhydrous anisole (Solvent:Monomer = 2:1 v/v). Air was removed from the reaction flasks through three consecutive freeze-pump-thaw cycles and the flasks were back-filled with nitrogen. The polymerizations were transferred to an oil bath at the desired temperature and allowed to react for 10 h. Samples were taken every hour using a degassed syringe and quenched by exposure to air and rapid cooling. All polymerizations were quenched by exposure to air and rapid cooling and purified by precipitation into cold hexane to yield 1.89 g of a pale pink solid. Isolate yield: 95%. Mw= 72,650 g/mol. AcManEMA:AzPMA = 7:1.¹H NMR (400 MHz, CDCl₃): δ = 1.96-2.16 (m, 110 H, 4 x COOCH₃, -CH₃, OCH₂CH₂CH₂CH₂N₃), 3.76-4.29 (m, 50 H, OCH₂CH₂CH₂CH₂N₃, CHs of sugar), 4.88 (s, 7 H, H¹), 5.26-5.00-5.23 (m, 21 H, H², H³, H⁴). FT-IR: N₃ 1729.0 cm⁻¹ CO stretch.
3.3.5 Synthesis of N-alkyl-N,N-linked urea oligomers-polymer conjugates

**Compound 10A.** Compound 9A (0.6 g, 0.41 mmol), poly(AcGluEMA-co-AzPMA) (0.53 g, 0.025 mmol) and CuBr (0.0049 g, 0.034 mmol) in dichloromethane were added to a round bottom flask equipped with a stir bar. The flask was purged with N₂ for 30 minutes and then 0.007 mL of PMDETA was added and the reaction was allowed to stir at room temperature overnight. The conjugate was precipitated three times into cold methanol to obtain 0.39 g of a pale off-white solid (10A). Yield 39%. ¹H NMR (400 MHz, CDCl₃): δ = 1.96-2.16 (m, 98 H, 4 x COOCH₃, -CH₃, OCH₂CH₂CH₂N), 3.22-4.29 (m, 57 H, OCH₂CH₂CH₂N, CHs of sugar, CH₃s of oligomer), 4.88 (s, 7 H, H¹), 5.26-5.00-5.23 (m, 15 H, H², H³, H⁴), 7.67 (m, 6 H, aromatic, 3 H of triazole), 7.91 (m, 1 H, aromatic).

**Compound 10B.** Compound 9B (0.3 g, 0.20 mmol), poly(AcManEMA-co-AzPMA) (0.66 g, 0.009 mmol) and CuBr (0.0024 g, 0.017 mmol) in dichloromethane were added to a round bottom flask equipped with a stir bar. The flask was purged with N₂ for 30 minutes and then 0.004 mL of PMDETA was added and the reaction was allowed to stir at room temperature overnight. The conjugate was precipitated three times into cold methanol to obtain 0.72 g of a pale off-white solid (10B). Yield 79%. ¹H NMR (400 MHz, CDCl₃): δ = 1.98-2.15 (m, 158 H, 4 x COOCH₃, -CH₃, OCH₂CH₂CH₂N), 3.22-4.29 (m, 84 H, OCH₂CH₂CH₂N, CHs of sugar, CH₃s of oligomer), 4.88 (s, 9 H, H¹), 5.26-5.00-5.23 (m, 27 H, H², H³, H⁴), 7.63-7.79 (m, 6 H, aromatic, 3 H of triazole), 7.93 (m, 1 H, aromatic).

**Deprotection of N-alkyl-N,N-linked urea oligomers-polymer conjugates.** Compounds 10A and 10B were added to separate round bottom flasks equipped with stir bars. They were dissolved 10 mL in dry methanol and 100 μL of 1 M sodium methoxide in methanol was added
and the reaction was allowed to stir at room temperature for 30 minutes. The reactions were then stirred with Dowex 50WX8 hydrogen form exchange resin. The reaction mixtures were filtered to remove the resin, reduced using a rotary evaporator, and the polymers where precipitated into cold hexane to obtain the final products Compound 11A: 0.098 g, yield 36%. \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 1.96-2.16\) (m, 16 H, OCH\(_2\)CH\(_2\)CH\(_2\)N), 3.22-4.29 (m, 57 H, OCH\(_2\)CH\(_2\)CH\(_2\)N, CHs of sugar, CH\(_2\)s of oligomer), 4.47 (s, 7 H, H\(^1\)), 7.91 (m, 1 H, aromatic). FT-IR: OH = 3355.3 cm\(^{-1}\). Compound 11B: 0.13 g, yield 27%. \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 1.98-2.15\) (m, 22 H, OCH\(_2\)CH\(_2\)CH\(_2\)N\(_3\)), 3.19-4.19 (m, 84 H, OCH\(_2\)CH\(_2\)CH\(_2\)N, CHs of sugar, CH\(_2\)s of oligomer), 4.85 (s, 9 H, H\(^1\)), 7.64 (m, 1 H, aromatic). FT-IR: OH = 3346.4 cm\(^{-1}\).

**Sulfonation of N-alkyl-N,N-linked urea oligomers-polymer conjugates.** Compounds 11A and 11B were added to separate round bottom flasks equipped with stir bars. Two equivalents of sulfur trioxide pyridine complex per hydroxyl group were added to the round bottom flask and dissolved in 20 mL of pyridine. This was allowed to react overnight at 90ºC. The solvent was then removed by rotary evaporation and the product dissolved in saturated NaHCO\(_3\) solution and the pH was adjusted to 8.0. The polymers were isolated through dialysis and lyophilization to give off white powders. Compound 12A: 0.13 g Compound 12B: 0.077 g.

### 3.4 Characterization

\(^1\)H and \(^{13}\)C\(^{\{1\}H\}\) NMR spectroscopy were performed in CDCl\(_3\) with Si(CH\(_3\))\(_4\), MeOH-d\(_4\), and D\(_2\)O with 3-(trimethylsilyl)propionic-2,2,3,3-d\(_4\) acid, sodium salt as internal standards using a Bruker Ultrashield 400 MHz (100 MHz for \(^{13}\)C\(^{\{1\}H\}\)-NMR). The \(^1\)H and \(^{13}\)C-NMR spectra were processed using UXNMR version 2.5 and MestReNova Lite. Molecular weights and polydispersities were determined by gel permeation chromatography (GPC). Poly(AcGluEMA-
co-AzPMA) and poly(AcManEMA-co-AzPMA) copolymers were characterized with an Agilent 1200 series HPLC equipped with a PSS SDV Lux column (5 µm) guard column and two PSS SDV Linear XL Lux Columns (5 µm) (linear range of MW = 100 - 3×10^6 g/mol) with filtered tetrahydrofuran (THF) containing 200 ppm 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) mobile phase at a flow rate of 1.0 mL/min at ambient temperature and a miniDAWN TREOS light scattering (60 mW GaAs linearly polarized laser, 658 nm) calibrated against a 30 000 g/mol polystyrene standard (Wyatt Technology Corp.) and Optilab rEX differential refractometer (light source = 658 nm) detectors. ASTRA software v. 6.1.0 was used to determine polymer characteristic values. Fourier transform infrared (FT-IR) spectra were collected on a Nicolet 6700 spectrometer and analyzed with OMNIC32 software.

3.5 Results and Discussion

The synthesis of glucose and mannose functionalized N-alkyl-N,N-linked urea oligomers was performed following procedures developed by our group from work by Nowick. The synthesis of N-alkyl-N,N-linked urea oligomers is a simple and iterative solution-phase synthesis. There are three steps: (1) Main chain extension using a N-(2-nitrobenzene sulfonyl)-2-imidazolidone, (2) side group attachment following Fukuyama’s procedure, and (3) removal of the 2-nitrobenzene sulfonyl group using thiophenol to afford a new secondary amine used in the next cycle. We designed oligomers that contained terminal alkynes as N-alkyl side groups resulting in an oligomer that could easily be modified with alkyl azides to synthesize potential GAG mimics with different carbohydrates (Figure 1).
Figure 1. Reaction scheme for the synthesis of alkyne containing N-alkyl-N,N-linked urea oligomer.

The synthesis of the N-alkyl-N,N-linked urea dimer was performed by completing two iterative cycles followed by the ring opening of N-(2-nitrobenzene sulfonyl)-2-imidazolidone to obtain compound 7. Each step of the reaction is performed using traditional organic chemistry techniques, which eliminates the use of expensive resin-based synthetic techniques and their accompanying purification and characterization requirements. The percent yield after each step ranged between 72-97% and 8.53 g of the final product was obtained. We attached glucose and mannose residues to compound 7 using CuAAC chemistry (“click chemistry”). This was accomplished by modifying acetylated glucose and mannose residues through a Fisher glycosidation reaction with 2-bromoethanol followed by the conversion of the halide to an azide (Figure 2). The CuAAC reactions are an orthogonal coupling technique widely used in synthetic and polymer chemistry.35-37,49,50
Figure 2. Reaction scheme for the synthesis of an (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside. Similar reaction conditions were utilized in the synthesis of an (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside.

We coupled the carbohydrates to the N-alkyl-N,N-linked urea dimers with nearly 100% efficiency as determined by $^{1}$H-NMR spectroscopy by comparing the integration of the proton of the triazole ring with the signals of the acetate protecting groups and the terminal –CH$_3$ residues of the N-alkyl-N,N-linked urea dimer (Figure 3).
Figure 3. i. Reaction scheme for the CuAAC reaction to attach sugar residues to \(N\)-alkyl-\(N,N\)-linked urea oligomer to obtain compound 8. \(N_3\) Sugar can be (A) (2-azidoethyl)-2,3,4,6-tetra-\(O\)-acetyl-\(\beta\)-\(D\)-glucopyranoside or (B) (2-azidoethyl)-2,3,4,6-tetra-\(O\)-acetyl-\(\beta\)-\(D\)-mannopyranoside. ii. \(^1\)H-NMR of compound 8A that contains the protected glucose residues. iii. \(^1\)H-NMR of compound 8B that contains the protected mannose residues.

The final step in the synthesis of \(N\)-alkyl-\(N,N\)-linked urea oligomers is the addition of a terminal alkyne to compounds 8A and 8B to obtain a 65% yield of compound 9A and a 60% yield compound 9B. The lower yield of this reaction is attributed to the partial deprotection of the
glucose and mannose residues and subsequent side reactions under the basic conditions that are removed upon purification by column chromatography (Figure 4).

**Figure 4.** Reaction scheme for the addition of the final alkyne onto compound 8 to obtain compound 9. 

$\text{N}_3$ Sugar can be (A) (2-azidoethyl)-2,3,4,6-tetra-$O$-acetyl-$\beta$-D-glucopyranoside or (B) (2-azidoethyl)-2,3,4,6-tetra-$O$-acetyl-$\beta$-D-mannopyranoside.

In our previous work, we reported the synthesis of the a statistical copolymer of isopropylidene protected glucose monomer (MALpGlc) and 6-azido-hexylmethacrylate (AzHMA) under reversible addition fragmentation chain transfer (RAFT) polymerization conditions and its coupling to $N$-alkyl-$N,N$-linked urea oligomers with glucose containing alkyl side-groups.\(^{30}\) In this work we synthesized statistical copolymers of acetate protected carbohydrate monomers and 3-azidopropyl methacrylate (AzPMA), by using the same protecting groups on the polymer we are able to do a single deprotection step after conjugating the $N$-alkyl-$N,N$-linked urea oligomers to the polymer. We used a procedure adapted from Ambrose *et al* to synthesize the acetate protected carbohydrate monomers 2-(2’,3’,4’,6’-tetra-$O$-acetyl-$\beta$-D-glucosyloxy)ethyl methacrylate (AcGluEMA) and 2-(2’,3’,4’,6’-tetra-$O$-acetyl-$\beta$-D-mannosyloxy)ethyl methacrylate (AcManEMA).
methacrylate (AcManEMA). Prior literature examples have polymerized these monomers and 2-(2’,3’,4’,6’-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2’,3’,6’-tri-O-acetyl-β-D-glucopyranosyl)ethyl methacrylate (AcLacEMA) have been polymerized by free radical polymerization, atom transfer radical polymerization and RAFT. Before synthesizing the statistical copolymers in our work, we investigated the conditions to prepare polymers under RAFT conditions using cumyl dithiobenzoate. Both polymerizations were conducted in anisole at 70°C using 100:1:0.33 monomer:CDB:AIBN with a 1:2 w/v of monomer to solvent ratio. The pseudo-first order kinetic plot for the polymerization of the AcGluEMA with CDB shows inhibition period of 180 minutes before the kinetics are first-order over the next 400 minutes up to ~75% conversion (Figure 5A). The molecular weight of the polymer increased smoothly with increasing conversion in agreement with the predicted molecular weight (Figure 5B). For the polymerization of the AcManEMA with CDB, the pseudo-first order kinetic plot shows little inhibition and first order kinetics over 600 min up to ~80% conversion (Figure 5C). The molecular weight of the polymer increased with conversion, however it was higher than predicted by theory (Figure 5D). In a RAFT polymerization it is possible to predict the theoretical molecular weight at a particular conversion using Equation 1, where \([M]_0\) is the initial monomer concentration; \([CTA]_0\) is the initial CTA concentration; \(M_{\text{repeat}}\) is the molecular weight of the repeat unit; \(p\) is the conversion; and \(M_{CTA}\) is the molecular weight of the CTA.

\[
M_{n(\text{theo})} = \left( \frac{[M]_0}{[CTA]_0} \times M_{\text{repeat}} \times p \right) + M_{CTA}
\]

**Equation 1.** Calculation for the theoretical molecular weight of a polymer obtained using RAFT polymerization.
Figure 5. A. Pseudo-first order kinetic plot of AcGluEMA at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70°C. B. Number average molecular weight ($M_n$) versus conversion of AcGluEMA at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70°C. Red squares are the theoretical molecular weights. C. Pseudo-first order kinetic plot of AcManEMA at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70°C. D. Number average molecular weight ($M_n$) versus conversion of AcManEMA at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70°C. Red squares are the theoretical molecular weights.

We copolymerized the acetate protected carbohydrate monomers with 3-azidopropyl methacrylate (AzPMA) using same conditions as the homopolymerization with a monomer feed ratio of the carbohydrate-functionalized monomer to AzPMA of 10:1. $^1$H-NMR spectroscopy was used to determine the monomer ratio in the polymer; for poly(AcGluEMA-co-AzPMA) it was 3:1 and for poly(AcManEMA-co-AzPMA) it was 7:1. The molecular weights of both
copolymers were determined using gel permeation chromatography with light scattering and refractive index detectors. The molecular weight of poly(AcGluEMA-co-AzPMA) was $3.9 \times 10^4$ g/mol with a PDI of 1.14 and the molecular weight of poly(AcManEMA-co-AzPMA) $7.2 \times 10^4$ g/mol with a polydispersity of 1.17.

We again used CuAAC chemistry to couple the $N$-alkyl-$N,N$-linked urea oligomers (9A and 9B) to their respective copolymers to obtain 10A and 10B (Figure 6). We coupled the $N$-alkyl-$N,N$-linked urea oligomers to their respective copolymers with nearly 100% efficiency as determined by $^1$H-NMR spectroscopy by comparing the integration of the protons of the triazole ring and Ns group with the signals of the anomeric proton of the carbohydrates. The oligomer-polymer conjugates were not characterized by GPC due to the partial deprotection of the oligomer from the previous step. Comparing the FT-IR spectrum of the poly(AcGluEMA-co-AzPMA) and poly(AcManEMA-co-AzPMA) with their respective conjugates showed a clear decrease in the intensity of the $\sim N_3$ peak around 2100 cm$^{-1}$, further showing a successful coupling reaction (Figure 7).
Figure 6. Synthetic scheme for the CuAAC reaction to couple the glucose containing $N$-alkyl-$N$,$N$-linked oligomer (9A) to the poly(AcGluEMA-co-AzPMA) to obtain 10A. Similar reaction conditions were used to obtain the mannose containing analog.
After the synthesis of the $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugate the remaining acetate protecting groups were removed in one step to form the final GAG mimetic. The acetate protecting groups were removed using catalytic sodium methoxide in methanol and stirring at room temperature for 20 minutes. The reaction was then stirred with Dowex 50WX8 hydrogen form exchange resin. The reaction was then filtered to remove the resin, the solvent reduced and then precipitated into cold hexane. $^1$H-NMR was used to quantify the extent of the deprotection reaction. The deprotection reaction for both $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugates was found to be quantitative by comparing the relative integrations of the peaks of the Ns and triazole protons to the peaks of the aliphatic protons in the spectrum. The deprotected $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugates were also characterized using FT-IR (Figure 7). The spectrum showed the appearance of a broad band at 3500 cm$^{-1}$ indicating the presence of hydroxyl groups that came from the deprotection of the carbohydrate moieties.

The final step to obtain the heparin mimetic is to sulfonate the deprotected $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugates (Figure 8). This was done by reacting the $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugates with two equivalents of sulfur trioxide pyridine complex per hydroxyl group at 90ºC overnight. The pyridine was then removed by rotary evaporation and product was redissolved in saturated sodium bicarbonate solution. The product was then purified and isolated using dialysis and lyophilization to obtain 0.5 g of the glucose containing $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugate and 0.6 g of the mannose $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugate.
Figure 8. Synthetic scheme for the deprotection and sulfonation of the glucose containing \( N \)-alkyl-\( N,N \)-linked urea oligomer-polymer conjugates. Similar reaction conditions were also done for deprotection and sulfonation the mannose \( N \)-alkyl-\( N,N \)-linked urea oligomer-polymer conjugate.

3.6 Conclusions

We have prepared a heparin-mimicking polymer using an \( N \)-alkyl-\( N,N \)-linked urea oligomer that is capable of adding to a variety of carbohydrate structures by using the CuAAC “click” reaction. Specifically, glucose and mannose decorated \( N \)-alkyl-\( N,N \)-linked urea oligomers were synthesized and added to copolymers prepared under RAFT control. The afforded \( N \)-alkyl-\( N,N \)-linked urea oligomer-polymer conjugates will enable structure/property studies to be performed with these heparin mimics with respect to their therapeutic potential. Moreover, the chemistry developed can be modified to generate a library of carbohydrate containing \( N \)-alkyl-\( N,N \)-linked urea oligomer-polymer conjugates, for example lactose-based or glucosamine-based macromolecules.

3.7 References


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Chapter 4. Synthesis of a sequence specific glycosaminoglycan polymer mimetics using an N-alkyl-N,N-linked urea oligomer and copper catalyzed “click” chemistry.

4.1 Abstract

The synthesis of a glycosaminoglycan mimetic polymer disaccharides and sequence permutations along the $N$-alkyl-$N,N$-linked urea oligomer is presented. An acetate protected lactose monomer was copolymerized under reversible addition-fragmentation chain transfer polymerization (RAFT) control with the alkyne containing monomer, trimethysilyl methacrylate (TMS-MA), to a molecular weight of 47 000 g/mol with a polydispersity index (PDI) of 1.6. The comonomer feed ratio was 10:1 and the actual ratio was determined to be 10:1 by $^1$H-NMR spectroscopy. Two different sugar-functionalized $N$-alkyl-$N,N$-linked urea oligomers were coupled to the copolymer using copper catalyzed azide/alkyne cycloaddition (CuAAC) reactions. There are two $N$-alkyl-$N,N$-linked urea oligomer used in this work. The first consists of two terminal alkynes as the $N$-alkyl side group and was modified using CuAAC reactions to contain the disaccharide lactose. The second was synthesized to allow sequence permutations along the $N$-alkyl-$N,N$-linked urea oligomer through the use of protecting group chemistry of the terminal alkynes. The CuAAC reactions were monitored by $^1$H-NMR spectroscopy and the efficiency was determined to be 100%. The acetate protecting groups on the polymer and the $N$-alkyl-$N,N$-linked urea oligomer were removed using a Zemplén deacetylation reaction to give the final polysaccharide mimetic that was then sulfonated. It is anticipated that $N$-alkyl-$N,N$-linked urea oligomers-polymer conjugates will have uses in therapeutic applications as glycosaminoglycan mimetics.

4.2 Introduction
Glycosaminoglycans (GAGs) are heterogeneous, linear polysaccharides that have a wide variety of roles in biological processes. Many GAGs are highly anionic due to the presences of carboxylate and sulfate groups along the polysaccharide backbone.\(^1\) There are two main types of GAGs, non-sulfated and sulfated, with heparin and heparan sulfate being important sulfated GAGs.

Heparin and heparan sulfate are involved in the inhibition of blood coagulation and they are the one of the more studied GAGs.\(^2\) Heparin is currently used in the treatment of thrombosis, thrombophlebitis, and embolisms and is obtained from mammalian sources.\(^1\) There are several risks associated with the use of heparin such as pathogenic contamination and heparin-induced thrombocytopenia and because of this there is a push to mimic the anti-thrombotic activity of heparin.

In previous work we synthesized \(N\)-alkyl-\(N, N\)-linked urea oligomers that contained isopropylidene protected glucose residues\(^3\) and alkyne side groups that allowed for the post modification of the \(N\)-alkyl-\(N, N\)-linked urea oligomers with carbohydrate residues as potential GAG mimetics. \(N\)-alkyl-\(N, N\)-linked urea oligomers are a hybrid of urea oligomers\(^4\)–\(^6\) and \(N\)-acyl glycines or “peptoids”.\(^7,8\) \(N\)-alkyl-\(N, N\)-linked urea oligomers are synthesized using simple organic chemistry techniques, are easily purified and it is possible to incorporate a wide variety of side groups.\(^3,9\)–\(^12\) In this work, we wanted to build upon the complexity of \(N\)-alkyl-\(N, N\)-linked urea oligomers by incorporating disaccharide residues and sequence control to the oligomer. To build sequence control into our oligomer we included a trimethyl silane protected terminal alkyne as a side group in addition to the unprotected terminal alkyne. This allows for the modification of the \(N\)-alkyl-\(N, N\)-linked urea oligomer at specific locations along the oligomer giving control over the final sequence. Baradel and coworkers took a similar approach in the
synthesis of single-chain sugar arrays.\textsuperscript{13} In their work, $N$-substituted maleimides were incorporated into polystyrene chains by time-controlled monomer addition. They synthesized three derivatives of $N$-propargylmaleimide that contained protecting groups, trimethyl silyl (TMS), triethylsilyl (TEP) and triisopropylsilyl (TIPS) that could be selectively deprotected. Through selective deprotection and CuAAC reactions these were post-modified to control the sequence of carbohydrates.\textsuperscript{13}

Many GAG mimicking polymers contain carbohydrate residues and are synthesized using controlled-polymerization techniques with RAFT polymerization being a common choice.\textsuperscript{14-22} RAFT polymerization is a robust polymerization technique that is tolerant of several media types and functional groups.\textsuperscript{23,24} We have previously demonstrated the copolymerization of isopropylidene protected glucose methacrylate monomer, 3-$O$-methylacyloyl-1,2:5,6-di-$O$-isopropylidene-$\alpha$-glucopyranosyl (MAIpGlc) and acetate protected carbohydrate monomers, 2-(2',3',4',6'-tetra-$O$-acetyl-$\beta$-$\alpha$-galactopyranosyl)ethyl methacrylate (AcGluEMA) or 2-(2',3',4',6'-tetra-$O$-acetyl-$\beta$-$\alpha$-mannosyloxy)ethyl methacrylate (AcManEMA), with azide-containing monomers under RAFT control.

In this work, the synthesis of lactose-containing polymer is accomplished using RAFT polymerization of 2-(2',3',4',6'-tetra-$O$-acetyl-$\beta$-$\alpha$-galactopyranosyl-(1-4)-2',3',6'-tri-$O$-acetyl-$\beta$-$\alpha$-glucopyranosyl)ethyl methacrylate (AcLacMEA) with trimethylsilylpropargyl methacrylate (TMS-MMA). TMS-MMA has been used in atom transfer radical polymerizations (ATRP) and RAFT polymerizations in the synthesis of glycopolymers by the Haddleton group.\textsuperscript{25-27} This allows us to use CuAAC reactions to couple the $N$-alkyl-$N,N$-linked urea oligomers to the polymer. We are able to deprotect the conjugated in a single step prior to sulfonation by using
acetate protecting groups on the urea oligomer and the polymer, we are able to obtain the final GAG mimetic.

4.3 Experimental Section

All starting materials were purchased from Aldrich at the highest purity available and used as received unless specified otherwise. \(N\)-(2-Nitrobenzenesulfonyl)-2-imidazolidone was prepared according to the method of Wilson and Nowick.\(^9,28\) \(N\)-(2-(3,3-diethyleureido)ethyl)-2-nitrobenzenesulfonamide was synthesized as previously reported.\(^9\) The RAFT agent cumyl dithiobenzoate (CDB) was synthesized according to literature procedures.\(^29\) The protected side group (3-bromoprop-1-ynyl) trimethyl silane was synthesized from a procedure adapted from Lutz et al.\(^13\) The synthesis of a trimethylsilyl (TMS) protected propargyl methacrylate monomer was synthesized as reported.\(^25\)

4.3.1 Synthesis of alkyne containing \(N\)-alkyl-\(N\)-linked urea oligomer

**Compound 2.** \(N\)-(2-(3,3-diethyleureido)ethyl)-2-nitrobenzenesulfonamide (Compound 1) (7.43 g, 21.6 mmol), \(K_2CO_3\) (3.01 g, 21.8 mmol) and propargyl chloride (3.21 g, 43.1 mmol) were dissolved in 50 mL of \(N,N\)-dimethylformamide (DMF) and stirred at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in \(CH_2Cl_2\) and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The product was dried in vacuum to afford 8.0 g of light yellow oil. Yield: 97%. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 1.13\) (t, \(J = 8\) Hz, 6H, 2 CH\(_3\)CHN), 2.20 (s, \(HCCCH_2\)), 3.25 (q, \(J = 8\), 16 Hz, 4 H, 2x CH\(_3\)CH\(_2\)N), 3.48-3.59 (m, 4 H, NHCH\(_2\)CH\(_2\)NNs), 4.27 (s, NCH\(_2\)CCH), 4.80 (bs, CONH), 7.64-7.71 (m, 3 H, aromatic H) 8.04-8.06 (m, 1 H, aromatic
$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.13$ (t, $J = 8$ Hz, 6 H, 2 x CH$_3$CH$_2$N), 2.25 (s, 1 H, CH$_2$CH), 2.43 (s, 2 H, CH$_2$CCH), 2.86 (t, $J = 16$ Hz, 2 H, NHCH$_2$CH$_2$NHCH$_2$CCH), 3.26 (q, $J = 12$ Hz, 4 H, 2 x CH$_3$CH$_2$N), 3.34 (t, 2 H, NHCH$_2$-CH$_2$NHCH$_2$CCH). 5.03 (bs, CONH); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 13.70$ (2 CH$_3$CH$_2$N), 37.56 (CH$_2$CCH), 37.93 (NHCH$_2$CH$_2$NHCH$_2$CCH), 41.01 (2 x CH$_3$CH$_2$), 48.00 (NHCH-CH$_2$NHCH$_2$CCH), 71.57 (CH$_2$CCH), 81.61 (CH$_2$CCH), 157.46 (CONH). MS (TOF MS ESI): $M_{\text{theo}} = 197.1528$ g/mol. M+H$^+$ = 198.1613 g/mol.

**Compound 3.** Compound 2 (8.0 g, 20.9 mmol) and K$_2$CO$_3$ (8.65 g, 62.6 mmol) were dissolved in 50 mL of DMF. The reaction was purged with N$_2$ and benzenethiol (4.11 mL, 40.0 mmol) was added. The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation. The residue was dissolved in CH$_2$Cl$_2$ and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with CH$_2$Cl$_2$:MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product dried in vacuum to yield 3.94 g of a light yellow oil. Yield: 96%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.13$ (t, $J = 8$ Hz, 6 H, 2 x CH$_3$CH$_2$N), 2.25 (s, 1 H, CH$_2$CH), 2.43 (s, 2 H, CH$_2$CCH), 2.86 (t, $J = 16$ Hz, 2 H, NHCH$_2$CH$_2$NHCH$_2$CCH), 3.26 (q, $J = 12$ Hz, 4 H, 2 x CH$_3$CH$_2$N), 3.34 (t, 2 H, NHCH$_2$-CH$_2$NHCH$_2$CCH). 5.03 (bs, CONH); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 13.70$ (2 CH$_3$CH$_2$N), 37.56 (CH$_2$CCH), 37.93 (NHCH$_2$CH$_2$NHCH$_2$CCH), 41.01 (2 x CH$_3$CH$_2$), 48.00 (NHCH-CH$_2$NHCH$_2$CCH), 71.57 (CH$_2$CCH), 81.61 (CH$_2$CCH), 157.46 (CONH). MS (TOF MS ESI): $M_{\text{theo}} = 197.1528$ g/mol. M+H$^+$ = 198.1613 g/mol.

**Compound 4.** Compound 3 (3.94 g, 19.9 mmol), N-(2-nitrobenzenesulfonyl)-2-imidazolidone (7.04 g, 25.9 mmol) and (dimethylamino)pyridine (DMAP) (1.22 g, 9.9 mmol) were dissolved in 20 mL of pyridine. The reaction flask was sealed with a rubber septum, purged with N$_2$ for 30 min, and then immersed in preheated oil bath at 60 °C for 6 h. The solvent was removed using a rotary evaporator, the residue dissolved in CH$_2$Cl$_2$, washed with 0.5 M aqueous HCl and then
dried over Na$_2$SO$_4$. The solution was removed and the product was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The solvent was removed and the product was dried in a vacuum to yield 8.87 g of a pale yellow solid. Yield: 95%. $^1$H NMR (400 MHz, CDCl$_3$): δ = 1.16 (t, $J$ = 8 Hz, 6 H, 2 x CH$_3$CH$_2$N), 2.21 (s, 1 H, HCCCH$_2$), 3.26-3.38 (m, 12 H, all CH$_2$ on backbone), 4.12 (s, 2 H, HCCCH$_2$N), 4.92 (s, NH) 7.06 (s, NH), 7.71-7.77 (m, 3 H, aromatic), 8.11 (s, 1 H, aromatic).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ = 13.57 (2 x CH$_3$CH$_2$N), 36.61 (CH$_2$CCH), 39.94 (NHCH$_2$CCH), 40.67 (OCNHCH$_2$CH$_2$NCO), 41.23 (2 x CH$_3$CH$_2$N), 44.15 (NHCH$_2$CH$_2$NNs), 45.45 (OCNHCH$_2$CH$_2$NCO), 71.54 (CH$_2$CCH), 80.39 (CH$_2$CCH), 124.88, 130.88, 132.45, 133.24, 134.20, 147.95 (aromatic), 157.60 (C=O), 158.20 (C=O). MS (TOF MS ESI): M$^{+}$ = 468.1791 g/mol. M+H$^+$ = 469.1871 g/mol.

**Compound 5.** Compound 4 (11.44 g, 24.36 mmol), K$_2$CO$_3$ (8.42 g, 60.9 mmol) and propargyl chloride (3.63 g, 48.7 mmol) were dissolved in 50 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH$_2$Cl$_2$ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:methanol (4:1 v/v) as the mobile phase. The product was dried in vacuum to afford 11.54 g of oil. Yield: 94%. $^1$H NMR (400 MHz, CDCl$_3$): δ = 1.12 (t, $J$ = 8 Hz, 6H, 2 x CH$_3$CH$_2$N), 2.20 (s, 1 H, HCCCH$_2$), 2.26 (s, 1 H, HCCCH$_2$), 3.22-3.61 (m, 14 H, all CH$_2$ on backbone), 4.08 (s, 2 H, NCH$_2$CCH), 4.33 (s, 2 H, -NCH$_2$CCH), 7.65-7.70 (m, 3 H, aromatic H) 8.05 (m, 1 H, aromatic H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ = 13.71 (2 x CH$_3$CH$_2$N), 36.48, 36.98 (2 x CH$_2$CCH), 37.88 (NHCH$_2$CH$_2$NNs), 39.76 (OCNHCH$_2$CH$_2$NCO), 41.09 (2 x CH$_3$CH$_2$N), 46.67 (NHCH$_2$CH$_2$NNs), 47.05 (OCNHCH$_2$CH$_2$NCO), 56.80 (NsNCH$_2$CCH), 72.05
(CH₂CCH), 74.03 (CH₂CCH), 82.25 (CH₂CCH), 124.15, 130.80, 131.73, 131.73, 133.63, 147.95 (aromatic), 157.74 (C=O), 157.81 (C=O). MS (TOF MS ESI): M\textsubscript{theo} = 506.1948 g/mol. M+Na\textsuperscript{+} = 529.1807 g/mol.

**Compound 6.** Compound 5 (11.54 g, 22.78 mmol) and K\textsubscript{2}CO\textsubscript{3} (9.44 g, 68.3 mmol) were dissolved in 50 mL of DMF. The reaction was purged with N\textsubscript{2} and benzenethiol (4.87 mL, 45.6 mmol) was added. The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation. The residue was dissolved in CH₂Cl₂ and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with CH₂Cl₂:MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product dried in vacuum to yield 6.42 g of an oil. Yield: 88%. \( ^1\)H NMR (400 MHz, CDCl\textsubscript{3}): \( \delta = 1.13 \) (t, \( J = 8 \) Hz, 6 H, 2 x CH₃CH₂N), 2.25 (s, 2 H, 2 x HCCCH₂), 2.63-2.90 (m, 5 H, NHCH₂CCH + NHCH₂CCH) 3.22-3.46 (m, 12 H, CH₂ on backbone), 6.01 (bs, 1 H, CONH), 6.66 (bs, 1 H, CONH); \( ^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}): \( \delta = 13.66 \) (2 x CH₃CH₂N), 36.61, 37.63 (2 x CH₂CCH), 39.74 (OCNHCH₂CH₂NH), 40.23 (OCNHCH₂CH₂NCO), 41.09 (2 x CH₃CH₂N), 45.66 (OCNHCH₂CH₂NH), 46.55 (OCNHCH₂CH₂NCO), 46.82, 48.11 (NHCH₂CCH), 71.82 (CH₂CCH), 71.89 (CH₂CCH), 80.10 (CH₂CCH), 81.19 (CH₂CCH), 157.85 (C=O), 158.13 (C=O). MS (TOF MS ESI): M\textsubscript{theo} = 321.2165 g/mol. M+Na\textsuperscript{+} = 322.2187 g/mol.

**Compound 7.** Compound 6 (6.42 g, 19.9 mmol), N-(2-nitrobenzenesulfonyl)-2-imidazolidone (6.5 g, 24 mmol) and (dimethylamino)pyridine (DMAP) (1.22 g, 9.99 mmol) were dissolved in 25 mL of pyridine. The reaction flask was sealed with a rubber septum, purged with N\textsubscript{2} for 30 min, and then immersed in preheated oil bath at 60 °C for 6 h. The solvent was removed using a rotary evaporator, the residue dissolved in CH₂Cl₂, washed with 0.5 M aqueous HCl and then
dried over Na₂SO₄. The solution was removed and the product was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The solvent was removed and the product was dried in a vacuum to yield 8.53 g of a white solid. Yield: 72%. \(^1\)H NMR (400 MHz, CDCl₃): \(\delta = 1.14 \, (t, \, J = 8 \, \text{Hz}, \, 6 \, \text{H}, \, 2 \times \text{CH}_3\text{CH}_2\text{N}), \, 2.19 \, (s, \, 1 \, \text{H}, \, \text{HCCH}_2\text{N}), \, 2.27 \, (s, \, 1 \, \text{H}), \, 3.23-3.43 \, (m, \, 16 \, \text{H}, \, \text{all CH}_2 \, \text{on backbone}), \, 4.11-4.20 \, (m, \, 4 \, \text{H}, \, 2 \times \text{NCH}_2\text{CCH}), \, 7.70-7.76 \, (m, \, 3 \, \text{H}, \, \text{aromatic}), \, 8.12 \, (s, \, 1 \, \text{H}, \, \text{aromatic}). \(^{13}\)C NMR (100 MHz, CDCl₃): \(\delta = 13.77 \, (2 \times \text{CH}_3\text{CH}_2\text{N}), \, 36.59 \, (\text{NHCCH}_2\text{CH}_2\text{NNs}), \, 39.27 \, (2 \times \text{OCNHCH}_2\text{CCH}), \, 40.68 \, (\text{OCNHCH}_2\text{CH}_2\text{NCO}), \, 41.17 \, (2 \times \text{CH}_3\text{CH}_2\text{N}), \, 44.34, \, 46.22 \, (\text{NHCH}_2\text{CH}_2\text{NNs}), \, 71.40 \, (\text{CH}_2\text{CCH}), \, 71.45 \, (\text{CH}_2\text{CCH}), \, 80.50 \, (\text{CH}_2\text{CCH}), \, 81.02 \, (\text{CH}_2\text{CCH}), \, 124.89, \, 128.90, \, 131.08, \, 132.56, \, 133.33, \, 134.11 \, (\text{aromatic}), \, 157.57 \, (\text{C=O}), \, 159.12 \, (\text{C=O}), \, 159.83 \, (\text{C=O}). \) MS (TOF MS ESI): \(M_{\text{theo}} = 592.2428 \, \text{g/mol}. \) \(M+H^+ = 593.2487 \, \text{g/mol}.\)

**Compound 8.** Compound 7 (2.45 g, 4.13 mmol), K₂CO₃ (1.43 g, 10.3 mmol) and 1-bromo-4-chlorobutane (0.95 mL, 8.2 mmol) were dissolved in 10 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH₂Cl₂ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate as the mobile phase. The product was dried in vacuum to afford 2.47 g of a pale yellow solid. Yield: 88%. \(^1\)H NMR (400 MHz, CDCl₃): \(\delta = 1.11-1.14 \, (t, \, 6 \, \text{H}, \, 2 \times \text{CH}_3\text{CH}_2\text{N}), \, 1.74 \, (m, \, 4 \, \text{H}, \, \text{OCNNsCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}), \, 2.23 \, (m, \, 2 \, \text{H}, \, \text{NCH}_2\text{CCH}), \, 3.22-3.46 \, (m, \, 20 \, \text{H}, \, 2 \times \text{CH}_3\text{CH}_2\text{N}, \, 2 \times \text{OCNCH}_2\text{CH}_2\text{NCO}, \, \text{OCNCH}_2\text{CH}_2\text{NNs}, \, \text{OCNNsCH}_2\text{CH}_2\text{CH}_2\text{Cl}), \, 4.08-4.15 \, (m, \, 4 \, \text{H}, \, 2 \times \text{NCH}_2\text{CCH}), \, 7.61-7.70 \, (m, \, 3 \, \text{H}, \, \text{aromatic}), \, 8.01-8.03 \, (m, \, 1 \, \text{H}, \, \text{aromatic}). \(^{13}\)C NMR (100 MHz, CDCl₃): \(\delta = 13.97 \, (2 \times \text{CH}_3\text{CH}_2\text{N}), \, 25.54 \, (\text{OCNNsCH}_2\text{CH}_2\text{CH}_2\text{Cl}), \, 29.58 \, (\text{OCNNsCH}_2\text{CH}_2\text{CH}_2\text{Cl}), \, 31.64 \)
(OCNNsCH₂CH₂CH₂CH₂Cl), 36.49 (NCH₂CCH), 36.74 (OCNNsCH₂CH₂CH₂CH₂Cl), 39.36 (OCNHCH₂CH₂NNs), 39.86 (OCNHCH₂CH₂NCO), 49.94 (OCNHCH₂CH₂NCO), 41.31 (2 x CH₃CH₂N), 44.61 (OCNHCH₂CH₂NCO), 45.64 (OCNHCH₂CH₂NCO), 47.01 (OCNHCH₂CH₂NNs), 71.88 (CH₂CCH₂), 72.12 (CH₂CCH), 80.47 (CH₂CCH), 81.35 (CH₂CCH), 124.33, 131.00, 132.06, 133.34, 133.67, 148.17 (aromatic), 158.00, 158.24, 158.78 (C=O). MS (TOF MS ESI): M_{theo} = 682.2664 g/mol. M+H⁺ = 683.2203 g/mol.

4.3.2 Synthesis of alkyne containing N-alkyl-N,N-linked urea oligomer with sequence control

**Synthesis of compound 2’.** N-(2-(3,3-diethylureido)ethyl)-2-nitrobenzenesulfonamide

(Compound 1) (14.09 g, 40.90 mmol), K₂CO₃ (14.14 g, 102.3 mmol) and (3-bromoprop-1-ynyl) trimethyl silane (9.0 g, 6.82 mmol) were dissolved in 50 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH₂Cl₂ and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (3:1 v/v) as the mobile phase. The product was dried in vacuum to afford 18.48 g of a pale yellow solid. Yield: 99%. ¹H NMR (400 MHz, CDCl₃): δ = 0.15 (s, 9 H, -Si(CH₃)₃), 1.24 (t, J = 8 Hz, 6 H, 2 x CH₃CH₂N), 3.35 (q, J = 6 Hz, 4 H, 2 x CH₃CH₂N), 3.64 (dt, J = 8, 28 Hz, 4 H, OCNCH₂CH₂NNs), 4.37 (s, 2 H, OCNCH₂CCSi(CH₃)₃), 7.74-7.85 (m, 3 H, aromatic), 8.16-8.18 (d, J = 8 Hz, 1 H, aromatic).

¹³C NMR (100 MHz, CDCl₃): δ = -0.06 (Si(CH₃)₃), 14.03 (2 x CH₃CH₂N), 37.94 (CH₂CCSi(CH₃)₃), 38.20 (OCNHCH₂CH₂NH-Ns), 41.47 (2 x CH₃CH₂N), 47.09(OCNHCH₂CH₂NHNs), 91.59(NCH₂CCSi(CH₃)₃), 98.41 (NCH₂CCSi(CH₃)₃), 124.35,
131.14, 131.90, 132.92 133.95 (aromatic), 157.50 (C=O). MS (TOF MS ESI): \( M_{\text{theo}} = 454.1706 \) g/mol. \( M+H^+ = 455.1725 \) g/mol.

**Compound 3’**. Compound 2’ (18.48 g, 40.65 mmol) and K\(_2\)CO\(_3\) (16.85 g, 121.9 mmol) were dissolved in 100 mL of DMF. The reaction was purged with \( N_2 \) and benzenethiol (1.71 mL, 9.07 mmol) was added. The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation. The residue was dissolved in \( CH_2Cl_2 \) and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with \( CH_2Cl_2\):MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product dried in vacuum to yield 6.84 g. Yield: 62%. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 0.16 \) (s, 9 H, -Si(CH\(_3\))\(_3\)), 1.14 (t, \( J = 8 \) Hz, 6 H, 2 x CH\(_3\)CH\(_2\)N), 1.64 (s, 1 H, NHCH\(_2\)CCSi), 2.84 (t, \( J = 8 \) Hz, 2 H, OCNHCH\(_2\)CH\(_2\)NH), 3.24-3.36 (m, 6 H, OCNHCH\(_2\)CH\(_2\)NH, 2 x CH\(_3\)CH\(_2\)N), 3.44 (s, 2 H, NCH\(_2\)CCSi(CH\(_3\))\(_3\)). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = -0.17 \) (Si(CH\(_3\))\(_3\)), 14.06 (2 x CH\(_3\)CH\(_2\)N), 38.96 (CH\(_2\)CCSi(CH\(_3\))\(_3\)), 40.23 (OCNHCH\(_2\)CH\(_2\)NH), 41.32 (2 x CH\(_3\)CH\(_2\)N), 48.32(OCNHCH\(_2\)CH\(_2\)NH), 88.20 (NCH\(_2\)CCSi(CH\(_3\))\(_3\)), 104.40 (NCH\(_2\)CCSi(CH\(_3\))\(_3\)), 157.69 (C=O). MS (TOF MS ESI): \( M_{\text{theo}} = 269.1923 \) g/mol. \( M+H^+ = 270.1958 \) g/mol.

**Compound 4’**. Compound 3’ (6.84 g, 25.4 mmol) \( N\)-(2-Nitrobenzenesulfonyl)-2-imidazolidone (8.95 g, 33.0 mmol) and (dimethylamino)pyridine (DMAP) (1.55 g, 12.7 mmol) were dissolved in 35 mL of pyridine. The reaction flask was sealed with a rubber septum, purged with \( N_2 \) for 30 min, and then immersed in preheated oil bath at 60 °C for 6 h. The solvent was then removed using a rotary evaporator, the residue dissolved in \( CH_2Cl_2 \), washed with 0.5 M aqueous HCl and then dried over Na\(_2\)SO\(_4\). The solved was removed and the product was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with ethyl
acetate:hexane (2:1 v/v) as the mobile phase. The solvent was removed and the product was
dried in a vacuum to yield 12.3 g of a white solid. Yield: 98%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$
0.15 (s, 9 H, -Si(CH$_3$)$_3$), 1.15 (t, $J = 8$ Hz, 6 H, 2 x CH$_3$CH$_2$N), 3.27-3.37 (m, 12 H, 2 x
CH$_3$CH$_2$N, OCNCH$_2$CH$_2$NCO, OCNCH$_2$CH$_2$NNs), 4.14 (s, 2 H, NCH$_2$CCSi(CH$_3$)$_3$), 7.10 (s, 1
H, NHCO), 7.29 (s, 1 H, NHCO), 7.71-7.78 (m, 3 H, aromatic), 8.10-8.13 (m, 1 H, aromatic).
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ -0.10 (Si(CH$_3$)$_3$), 13.84 (2 x CH$_3$CH$_2$N), 37.61
(CH$_2$CCSi(CH$_3$)$_3$), 40.21 (OCNHCH$_2$CH$_2$NH-Ns), 40.97 (OCNHCH$_2$CH$_2$NCO), 41.45 (2 x
CH$_3$CH$_2$N), 44.49 (OCNHCH$_2$CH$_2$NCO), 46.54 (OCNHCH$_2$CH$_2$NNs), 88.39
(NCH$_2$CCSi(CH$_3$)$_3$), 102.58 (NCH$_2$CCSi(CH$_3$)$_3$), 125.10, 131.09, 132.70, 133.52, 134.45,
148.25 (aromatic), 157.87, 158.47 (C=O). MS (TOF MS ESI): $M_{theo} =$ 540.2168 g/mol. M+H$^+$ =
541.2304 g/mol.

**Compound 5'.** Compound 4' (12.3 g, 22.7 mmol), K$_2$CO$_3$ (7.99 g, 57.8 mmol) and propargyl
cloride (3.35 mL, 46.3 mmol) were dissolved in 50 mL of N,N-dimethylformamide (DMF).
The reaction was allowed to stir at room temperature overnight. The DMF was removed by
vacuum distillation and the residue dissolved in CH$_2$Cl$_2$ and passed through Celite. The solution
was concentrated and the product isolated by column chromatography using silica gel (silica gel
60 Å, 70-230 mesh) with ethyl acetate as the mobile phase. The product was dried in vacuum to
afford 5.73 g of a pale yellow solid. Yield: 43%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 0.15 (s, 9 H, -
Si(CH$_3$)$_3$), 1.12 (t, $J = 8$ Hz, 6 H, 2 x CH$_3$CH$_2$N), 2.31 (s, 1 H, NCH$_2$CCH), 3.26 (q, $J = 6$ Hz, 4
H, 2 x CH$_3$CH$_2$N), 3.34-3.35 (m, 4 H OCNCH$_2$CH$_2$NCO), 3.60 (t, $J = 4$ Hz, 4 H,
OCNCH$_2$CH$_2$NNs), 4.10 (s, 2 H, NCH$_2$CCH), 4.35 (s, 2 H, NCH$_2$CCSi(CH$_3$)$_3$), 6.73 (s, 1 H,
NHCO), 7.70-7.77 (m, 3 H, aromatic), 8.4 (d, $J = 8$ Hz,1 H, aromatic). $^{13}$C NMR (100 MHz,
CDCl$_3$): $\delta =$ -0.26 (Si(CH$_3$)$_3$), 13.64 (2 x CH$_3$CH$_2$N), 36.51 (NCH$_2$CCH), 37.74 (NCH-
2CCHSi(CH₃)₃, 37.74 (OCNHCH₂CH₂NNs), 38.09 (OCNHCH₂CH₂NCO), 38.71 (OCNHCH₂CH₂NCO), 40.93 (2 x CH₃CH₂N), 46.50 (OCNHCH₂CH₂NNs), 53.34 (CH₂CCH), 74.17(CH₂CCH), 91.59(NCH₂CCSi(CH₃)₃), 98.41 (NCH₂CCSi(CH₃)₃), 124.35, 131.14, 131.90, 132.92 133.95 (aromatic), 157.50 (C=O).

MS (TOF MS ESI): M₁₇₀ = 578.2343 g/mol. M+H⁺ = 578.23 g/mol.

**Compound 6'.** Compound 5' (5.73 g, 9.91 mmol) and K₂CO₃ (4.11 g, 29.7 mmol) were dissolved in 20 mL of DMF. The reaction was purged with N₂ and benzenethiol (2.03 mL, 19.8 mmol) was added. The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation. The residue was dissolved in CH₂Cl₂ and passed through Celite. The solvent was removed to afford the crude secondary amine which was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with CH₂Cl₂:MeOH (10:1 v/v) as the mobile phase. The solvent was removed and the product dried in vacuum to yield 2.45 g. Yield: 63%.

¹H NMR (400 MHz, CDCl₃): δ = 0.16 (s, 9 H, -Si(CH₃)₃), 1.13 (t, J = 8 Hz, 6 H, 2 x CH₃CH₂N), 2.26 (s, 1 H, NCH₂CCH), 2.87 (s, 1 H, NHCH₂CCSi), 3.35 (q, J = 6 Hz, 4 H, 2 x CH₃CH₂N), 3.35-3.46 (m, 10 H OCNHCH₂CH₂NCO, OCNHCH₂CH₂NNs, NCH₂CCH), 4.12 (s, 2 H, NCH₂CCSi(CH₃)₃), 5.47 (s, 1 H, NHCO), 6.48 (s, 1 H, NHCO); ¹³C NMR (100 MHz, CDCl₃): δ = -0.02 (Si(CH₃)₃), 13.85 (2 x CH₃CH₂N), 37.68 (NCH₂CCH), 37.88 (NCH₂CCSi(CH₃)₃), 39.97 (OCNHCH₂CH₂NNs), 40.55 (OCNHCH₂CH₂NCO), 41.32 (2 x CH₃CH₂N), 46.66 (OCNHCH₂CH₂NCO), 48.34 (OCNHCH₂CH₂NNs), 72.07 (CH₂CCH), 81.57 (CH₂CCH), 91.59(NCH₂CCSi(CH₃)₃), 101.92 (NCH₂CCSi(CH₃)₃), 124.35, 131.14, 131.90, 132.92 133.95 (aromatic), 157.50 (C=O). MS (TOF MS ESI): M₁₇₀ = 393.2560 g/mol. M+H⁺ = 394.2599 g/mol.
**Compound 7'**. Compound 6' (2.45 g, 6.22 mmol), N-(2-Nitrobenzenesulfonyl)-2-imidazolidone (2.91 g, 8.07 mmol) and (dimethylamino)pyridine (DMAP) (0.38 g, 3.1 mmol) were dissolved in 10 mL of pyridine. The reaction flask was sealed with a rubber septum, purged with N\textsubscript{2} for 30 min, and then immersed in preheated oil bath at 60 °C for 6 h. The solvent was then removed using a rotary evaporator, the residue dissolved in CH\textsubscript{2}Cl\textsubscript{2}, washed with 0.5 M aqueous HCl and then dried over Na\textsubscript{2}SO\textsubscript{4}. The solved was removed and the product was purified using column chromatography on silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1 v/v) as the mobile phase. The solvent was removed and the product was dried in a vacuum to yield 3.97 g of a white solid. Yield: 96%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ = 0.17 (s, 9 H, -Si(CH\textsubscript{3})\textsubscript{3}), 1.13 (t, J = 6 Hz, 6 H, 2 x CH\textsubscript{3}CH\textsubscript{2}N), 2.19 (s, 1 H, NCH\textsubscript{2}CCH), 3.23-3.44 (m, 16 H, 2 x CH\textsubscript{3}C\textsubscript{H}\textsubscript{2}N, 2 x OCNCH\textsubscript{2}CH\textsubscript{2}NCO, OCNCH\textsubscript{2}CH\textsubscript{2}NNs), 4.12-4.19 (m, 4 H, NCH\textsubscript{2}CCSi(CH\textsubscript{3})\textsubscript{3}, NCH\textsubscript{2}CCH), 7.14 (s, 1 H, N=CO), 7.41 (s, 1 H, N=CO), 7.70-7.76 (m, 3 H, aromatic), 8.11-8.14 (m, 1 H, aromatic); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): δ = -0.13 (Si(CH\textsubscript{3})\textsubscript{3}), 14.03 (2 x CH\textsubscript{3}CH\textsubscript{2}N), 36.85 (NCH\textsubscript{2}CCH), 37.75 (NCH\textsubscript{2}CCHSi(CH\textsubscript{3})\textsubscript{3}), 39.48 (OCNHCH\textsubscript{2}CH\textsubscript{2}NNs), 40.29 (2 x OCNHCH\textsubscript{2}CH\textsubscript{2}NCO), 41.38 (2 x CH\textsubscript{3}CH\textsubscript{2}N), 44.62 (2 x OCNHCH\textsubscript{2}CH\textsubscript{2}NCO), 46.49 (OCNHCH\textsubscript{2}CH\textsubscript{2}NNs), 71.70 (CH\textsubscript{2}CCH), 80.76 (CH\textsubscript{2}CCH), 125.15, 131.32, 132.80, 133.58, 134.40, 148.26 (aromatic), 157.78, 158.32, 159.39 (C=O). MS (TOF MS ESI): M\textsubscript{theo} = 664.2823 g/mol. M+Na\textsuperscript{+} = 687.2667 g/mol.

**Compound 8'**. Compound 7' (3.97 g, 5.97 mmol), K\textsubscript{2}CO\textsubscript{3} (2.06 g, 14.9 mmol) and 1-bromo-4-chlorobutane (1.39 mL, 12.06 mmol) were dissolved in 20 mL of N,N-dimethylformamide (DMF). The reaction was allowed to stir at room temperature overnight. The DMF was removed by vacuum distillation and the residue dissolved in CH\textsubscript{2}Cl\textsubscript{2} and passed through Celite. The solution was concentrated and the product isolated by column chromatography using silica...
gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate as the mobile phase. The product was dried in vacuum to afford 4.32 g of a pale yellow solid. Yield: 96%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 0.16 (s, 9 H, -Si(CH$_3$)$_3$), 1.14 (t, $J =$ 8 Hz, 6 H, 2 x CH$_3$CH$_2$N), 1.76 (m, 4 H, NCH$_2$CCSi(CH$_3$)$_3$), 3.23-3.53 (m, 20 H, 2 x CH$_3$C$_6$H$_2$N, 2 x OCNCH$_2$CH$_2$NCO, OCNCH$_2$CH$_2$NNs, OCNNsCH$_2$CH$_2$CH$_2$Cl), 4.12-4.17 (m, 4 H, NC$_6$H$_2$CCSi(CH$_3$)$_3$, NC$_6$H$_2$CCH), 5.02 (s, 1 H, NHCO), 6.88 (s, 1 H, NHCO), 7.63-7.70 (m, 3 H, aromatic), 8.02-8.04 (m, 1 H, aromatic). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 0.13 (Si(CH$_3$)$_3$), 14.02 (2 x CH$_3$CH$_2$N), 26.63 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 29.65 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 31.69 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 36.56 (NCH$_2$CCH), 36.76 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 37.54 (NCH$_2$CCHSi(CH$_3$)$_3$), 39.39 (OCNHCH$_2$CH$_2$NNs), 39.90 (OCNHCH$_2$CH$_2$NCO), 40.03 (OCNHCH$_2$CH$_2$NCO), 41.41 (2 x CH$_3$CH$_2$N), 44.65 (OCNHCH$_2$CH$_2$NCO), 46.44 (OCNHCH$_2$CH$_2$NCO), 47.01 (OCNHCH$_2$CH$_2$NNs), 71.86 (CH$_2$CCH), 124.35, 131.15, 132.07, 133.63, 133.79, 148.22 (aromatic), 158.00, 158.37, 158.81 (C=O). MS (TOF MS ESI): $M_{theo} =$ 754.3059 g/mol. M+H$^+$ = 755.3199 g/mol.

4.3.3 Synthesis of azide containing carbohydrate moieties

(2-bromoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside. 1,2,3,4,6-penta-O-acetyl-α,β-D-mannopyranose (53.53 g, 0.137 mol) and 2-bromoethanol (11.61 mL, 0.164 mol) were dissolved in 600 mL dry CH$_2$Cl$_2$. The reaction was purged with nitrogen and BF$_3$.Et$_2$O (97.14 mL, 0.787 mol) was added dropwise while stirring on ice. The reaction was allowed to stir at room temperature overnight. The reaction was quenched by pouring over ice water while stirring. The organic layer was washed with saturated NaHCO$_3$ and brine solutions and then dried over Na$_2$SO$_4$. The product was purified by triturating with hexane:ethyl acetate (1:1 v/v) and the solvent was reduced to obtain 26.35 g of a light brown solid. Yield 42%. $^1$H NMR (400 MHz,
CDCl₃): δ = 2.00-2.18 (m, 12 H, 4 x COOCH₃), 3.52 (t, J = 6 Hz, 2 H, OCH₂CH₂Br), 3.89-3.97 (m, 2 H, (OCH₂CH₂Br), 4.12-4.29 (m, 3 H, H⁶, H⁵), 4.88 (d, J = 8 Hz, 1 H, H¹), 5.28-5.37 (m, 3 H, H², H³, H⁴). ¹³C NMR (100 MHz, CDCl₃): δ = 20.65, 20.69, 20.73, 20.85 (4 x COOCH₃), 29.58 (CH₂CH₂Br), 62.38 (C₆), 65.97 (CH₂CH₂Br), 68.45 (C⁴), 68.91 (C²), 68.99 (C⁵), 69.40 (C³), 97.72 (C¹), 169.58 (C=O), 169.72 (C=O), 169.83 (C=O), 169.99 (C=O). MS (TOF MS ESI): Mthèo = 454.0475 g/mol. M+Na⁺ = 477.1215 g/mol.

(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside. (2-Bromoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside (26.35 g, 0.0579 mol) and 100 mL of 0.5 M NaN₃ in DMSO were added to a round bottom flask and allowed to stir overnight. The reaction was quenched with 100 mL of water and extracted with ether several times. The combined organic layers were washed with water and brine solution and dried over MgSO₄. The solvent was removed to yield 16.19 g of a light brown solid. Yield 70%. ¹H NMR (400 MHz, CDCl₃): δ = 2.00-2.18 (m, 12 H, 4 x COOCH₃), 3.8 (t, J = 8 Hz, 2 H, OCH₂CH₂N₃), 3.67-3.88 (m, 2 H, (OCH₂CH₂N₃), 4.06-4.32 (m, 3 H, H⁶, H⁵), 4.88 (s, 1 H, H¹), 5.23-5.37 (m, 3 H, H², H³, H⁴). ¹³C NMR (100 MHz, CDCl₃): δ = 20.29, 20.33, 20.37, 20.50 (4 x COOCH₃), 49.93 (CH₂CH₂N₃), 61.03 (C⁶), 63.56 (CH₂CH₂N₃), 65.53 (C⁴), 66.66 (C²), 68.41 (C⁵), 69.95 (C³), 97.31 (C¹), 169.40 (C=O), 169.66 (C=O), 170.27 (C=O), 170.36 (C=O). MS (TOF MS ESI): Mthèo = 417.1383 g/mol. M+Na⁺ = 440.1214 g/mol. FT-IR (cm⁻¹), 1739.5 (C=O), 2104.8 (N₃).

Synthesis of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-bromoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside. 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (18.0 g, 26.5 mmol) and 2-bromoethanol (9.4 mL, 0.13 mol) were dissolved in 200 mL dry CH₂Cl₂. The reaction was purged with nitrogen and BF₃.Et₂O (22 mL, 0.18 mol) was added dropwise while stirring on ice. The reaction was allowed to stir at room
temperature overnight. The reaction was quenched by pouring over ice water while stirring. The organic layer was washed with saturated NaHCO$_3$ and brine solutions and then dried over Na$_2$SO$_4$. The product was purified by column chromatography on silica gel (60 Å, 70-230 mesh) with ethyl acetate:hexane (2:1) as the mobile phase. The solvent was removed to yield 6.74 g of brown solid. Yield 34%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 1.97-2.19 (m, 21 H, 7 x COOCH$_3$), 3.28-5.49 (m, 18 H, lactose rings, OCH$_2$CH$_2$Br). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 20.71, 20.85, 20.88, 21.00, 21.03, 21.06, 21.15 (7 x COOCH$_3$), 61.02 (CH$_2$CH$_2$Br), 61.68 (CH$_2$CH$_2$Br), 62.06 (C$_{12}$), 66.81 (C$_6$), 66.33 (C$_{10}$), 69.63 (C$_5$), 69.81 (C$_{11}$), 69.99 (C$_8$), 70.92 (C$_3$), 71.20 (C$_9$), 76.00 (C$_2$), 76.37 (C$_4$), 101.27(C$_7$), 101.41 (C$_1$), 169.13 (C=O), 169.33 (C=O), 169.80 (C=O), 170.13 (C=O), 170.25 (C=O), 170.34 (C=O), 170.49 (C=O). MS (TOF MS ESI): $M_{\text{theo}}$ = 743.1321 g/mol. M+Na$^+$ = 767.1619 g/mol.

**Synthesis of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside.** 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-bromoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl acetate (3 g, 4.25 mmol) and 10 mL of a 0.5 M NaN$_3$ in DMSO were added to a round bottom flask and allowed to stir overnight. The reaction was quenched with 20 mL of water and extracted with ether several times. The combined organic layers were washed with water and brine solution and dried over MgSO$_4$. The solvent was removed to yield 1.85 g of a light brown solid. Yield 65%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 1.91-2.19 (m, 21 H, 7 x COOCH$_3$), 3.25-5.49 (m, 18 H, lactose rings, OCH$_2$CH$_2$N$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 20.09, 20.23, 20.30, 20.36, 20.43, 20.53, 20.64 (7 x COOCH$_3$), 50.55 (CH$_2$CH$_2$N$_3$), 60.35 (CH$_2$CH$_2$N$_3$), 61.00 (C$_{12}$), 66.13 (C$_6$), 68.27 (C$_{10}$), 68.63 (C$_5$), 70.23 (C$_{11}$), 70.54 (C$_8$), 71.00 (C$_3$), 72.24 (C$_9$), 72.33 (C$_2$), 75.35 (C$_4$), 99.99 (C$_7$), 100.75 (C$_1$), 168.71 (C=O), 169.20 (C=O), 169.35 (C=O), 169.52 (C=O), 169.65 (C=O), 169.72 (C=O), 169.94.
(C=O). MS (TOF MS ESI): $M_{\text{theo}} = 705.2229$ g/mol. $M+Na^+ = 728.2040$ g/mol. FT-IR (cm$^{-1}$): 1738.7 (C=O), 2106.4 (N$_3$).

### 4.3.4 Copper catalyzed azide/alkyne cycloaddition reactions

#### 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside to compound 8

2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (2.49 g, 4.17 mmol), compound 8 (0.95, 1.4 mmol), and CuBr (0.059 g, 0.411 mmol) in dichloromethane were added to a round bottom flask equipped with a stir bar. The flask was purged with N$_2$ for 30 min and 0.1 mL of PMDETA was added and the reaction was allowed to stir at room temperature overnight. The solution was then passed through basic alumina using a gradient elution of dichloromethane and 10:1 dichloromethane:methanol to obtain 1.29 g of compound 9. Yield 44%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.11-1.14$ (t, 6 H, 2 x CH$_3$CH$_2$N), 1.70 (m, 4 H, OCNNsCH$_2$CH$_2$CH$_2$CH$_2$Cl), 1.97-2.15 (m 42 H, 14 x -OCH$_3$), 7.69 (m, 5 H, 2 H triazole, 3 H aromatic), 8.00 (m, 1 H, aromatic). MS (TOF MS ESI): $M_{\text{theo}} = 2092.7121$ g/mol. $M+2H^+ = 2094.4558$ g/mol.

#### Compound 10

Compound 9 (1.29 g, 0.615 mmol) and 10 mL of a 0.5 M NaN$_3$ in DMSO were added to a round bottom flask and allowed to stir overnight. The reaction was quenched with 20 mL of water and extracted with ether several times. The combined organic layers were washed with water and brine solution and dried over MgSO$_4$. The solvent was removed to yield 1.02 g of an off-white solid. Yield 72 %. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.07-1.14$ (t, 6 H, 2 x CH$_3$CH$_2$N), 1.63 (m, 4 H, OCNNsCH$_2$CH$_2$CH$_2$CH$_2$Cl), 1.97-2.20 (m 42 H, 14 x -OCH$_3$), 7.63-7.70 (m, 5 H, 2 H triazole, 3 H aromatic), 7.98 (m, 1 H, aromatic). FT-IR: N$_3 = 2097.6$ cm$^{-1}$. MS (TOF MS ESI): $M_{\text{theo}} = 2101.0390$ g/mol. $M+H^+ = 2102.7852$ g/mol.

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(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside to compound 8’. (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside (2.39 g, 5.73 mmol), Compound 8’ (1.25 g, 1.91 mmol) and CuBr (0.082 g, 0.572 mmol) in dichloromethane were added to a round bottom flask equipped with a stir bar. The flask was purged with N₂ for 30 min and 0.12 mL of PMDETA was added and the reaction was allowed to stir at room temperature overnight. The solution was then passed through basic alumina using a gradient elution of dichloromethane and 10:1 dichloromethane:methanol to obtain 2.09 g of compound 9’. Yield 93%. ¹H NMR (400 MHz, CDCl₃): δ = 0.16 (s, 9 H, -Si(CH₃)₃), 1.11-1.14 (t, J = 8 Hz, 6 H, 2 x CH₃CH₂N), 1.74 (m, 4 H, OCNNsCH₂CH₂CH₂CH₂Cl), 1.99-2.17 (m, 12 H, 4 x COOC₃H₃), 3.24-3.52 (m, 24 H, 2 x CH₃C₃H₂N, 2 x OCNCH₂CH₂Cl), 4.02-4.30 (m, 7 H, NC₃H₂CCH, NC₃H₂CCN, H⁶, H⁵), 4.81 (1 1 H, H¹), 5.19-5.27 (m, 3 H, H₂, H₃, H₄), 7.66 (m, 1 H, triazole H), 7.67-7.71 (m, 3 H, aromatic), 8.02 (m, 1 H, aromatic). ¹³C NMR (100 MHz, CDCl₃): δ = 0.11 (Si(CH₃)₃), 14.02 (2 x C₃H₃CH₂N), 20.94, 20.97, 21.02, 21.11 (4 x COOCH₃), 25.32 (OCNNsCH₂CH₂CH₂CH₂Cl), 29.60 (OCNNsCH₂CH₂CH₂CH₂Cl), 30.34 (OCNNsCH₂CH₂CH₂CH₂Cl), 36.60 (NCH₂CCH), 36.76 (OCNNsCH₂CH₂CH₂CH₂Cl), 37.22 (NCH₂CCNCHSi(CH₃)₃), 37.53 (OCNHCH₂CH₂NNs), 38.75 (OCNHCH₂CH₂NCO), 40.00 (OCNHC₂CH₂CH₂NCO), 41.37 (2 x CH₃CH₂N), 44.65 (OCNHCH₂CH₂NCO), 46.77 (OCNHCH₂CH₂NCO), 47.05 (OCNHCH₂CH₂NNs), 49.27 (CH₂CHN), 53.72 (CH₂CH₂N), 62.59 (C⁶), 65.74 (C⁴), 66.57 (C²), 68.02 (C⁵), 69.13 (C³), 69.42 (CH₂CCN), 82.70 (CH₂CCN), 97.85 (C¹), 124.38, 130.93, 132.01, 133.59, 133.93, 148.20 (aromatic), 158.14, 158.43, 158.86 (NCONH), 160.21 (C=O), 170.76 (C=O), 170.90 (C=O), 172.31 (C=O). MS (TOF MS ESI): M₉theo = 1171.4443 g/mol. M+H⁺ = 1172.4678 g/mol.
Compound 10'. Compound 9' (2.09 g, 1.78 mmol) was dissolved in tetrahydrofuran. K$_2$CO$_3$ (5.17 g, 37.4 mmol) was dissolved in 18 mL of methanol:water (2:1 v/v) and added to compound 9' and allowed to stir at room temperature for 30 minutes. The solvent was removed by rotary evaporation and the product isolated by column chromatography using silica gel (silica gel 60 Å, 70-230 mesh) with ethyl acetate as the mobile phase to obtain 0.82 g of a pale yellow solid. Yield 49%. $^1$H NMR (400 MHz, CDCl$_3$): δ = 1.10-1.13 (t, 6 H, 2 x CH$_3$CH$_2$N), 1.72 (m, 4 H, OCNNsCH$_2$CH$_2$CH$_2$Cl), 3.16-3.51 (m, 24 H, 2 x CH$_3$C$_2$N, 2 x OCNCH$_2$CH$_2$NCO, OCNCH$_2$CH$_2$NNs, OCNNsCH$_2$CH$_2$CH$_2$Cl, OCH$_2$CH$_2$N, OCH$_2$CH$_2$N), 3.99-4.15 (m, 7 H, NCH$_2$CCH, NCH$_2$CCN, H$^6$, H$^5$), 4.75 (s, 1 H, H$^1$), 4.97-5.10 (m, 3 H, H$^2$, H$^3$, H$^4$), 7.60 (m, 1 H, triazole H), 7.68-7.70 (m, 3 H, aromatic), 8.00 (m, 1 H, aromatic). $^{13}$C NMR (100 MHz, CDCl$_3$): δ = 13.90 (2 x CH$_3$CH$_2$N), 25.35 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 29.52 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 31.81 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 35.43 (NCH$_2$CCH), 36.78 (OCNNsCH$_2$CH$_2$CH$_2$Cl), 37.36 (NCH$_2$CCH), 38.57 (OCNHCH$_2$CH$_2$NNs), 39.07 (OCNHCH$_2$CH$_2$NCO), 39.69 (OCNHCH$_2$CH$_2$NCO), 41.34 (2 x CH$_3$CH$_2$N), 44.61 (OCNHCH$_2$CH$_2$NCO), 47.04 (OCNHCH$_2$CH$_2$NCO), 47.14 (OCNHCH$_2$CH$_2$NNs), 49.87 (CH$_2$CHN), 52.99 (CH$_2$CH$_2$N), 61.09 (C$^6$), 65.20 (C$^4$), 66.43 (C$^5$), 67.21 (C$^3$), 69.51 (C$^3$), 69.73 (CH$_2$CCN), 82.72 (CH$_2$CCN), 97.68 (C$^1$), 124.37, 130.77, 132.20, 133.48, 133.81, 148.08 (aromatic), 158.23, 158.85, 158.74 (NCONH). MS (TOF MS ESI): M$^{+}$theo = 1099.4047 g/mol. M+H$^+$ = 954.2928 g/mol.

2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside to compound 10'. Compound 10' (0.82 g, 0.87 mmol), 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (1.86 g, 2.64 mmol) and CuBr (0.037 g, 0.258 mmol) in dichloromethane were added to a round bottom flask
equipped with a stir bar. The flask was purged with N\textsubscript{2} for 30 min and 0.1 mL of PMDETA was added and the reaction was allowed to stir at room temperature overnight. The solution was then passed through basic alumina using a gradient elution of dichloromethane and 10:1 dichloromethane:methanol to obtain 0.49 g of compound 11’. Yield 27\%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ = 1.06-1.10 (t, 6 H, 2 x CH\textsubscript{3}CH\textsubscript{2}N), 1.71 (m, 4 H, OCN\textsubscript{N}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 1.97-2.15 (m, 21 H, 7 x OCH\textsubscript{3}), 7.60-7.72 (m, 5 H, 2 x triazole H, 3H aromatic), 8.00 (m, 1 H, aromatic). MS (TOF MS ESI): M\textsubscript{theo} = 1804.6276 g/mol. M+Na\textsuperscript{+=} = 1821.0601 g/mol.

**Compound 12’.** Compound 11’ (0.49 g, 0.35 mmol) and 10 mL of a 0.5 M NaN\textsubscript{3} in DMSO were added to a round bottom flask and allowed to stir overnight. The reaction was quenched with 20 mL of water and extracted with ether several times. The combined organic layers were washed with water and brine solution and dried over MgSO\textsubscript{4}. The solvent was removed to yield 0.45 g of an off-white solid. Yield 92\%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ = 1.06-1.10 (t, 6 H, 2 x CH\textsubscript{3}CH\textsubscript{2}N), 1.52-1.62 (m, 4 H, OCN\textsubscript{N}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 1.97-2.15 (m, 21 H, OCH\textsubscript{3}), 7.69-7.73 (m, 5 H, 2 x triazole H, 3H aromatic), 7.88 (m, 1 H, aromatic). FT-IR: OH = 3330.7 cm\textsuperscript{-1}, N\textsubscript{3} = 2097.6 cm\textsuperscript{-1}. MS (TOF MS ESI): M\textsubscript{theo} = 1811.6680 g/mol. M+H\textsuperscript{+=} = 1814.6745 g/mol.

4.3.4 Carbohydrate containing polymers

**Synthesis of 2-(2’,3’,4’,6’-tetra-O-acetyl-\beta-\textsubscript{D}galactopyranosyl-(1-4)-2’,3’,6’-tri-O-acetyl-\beta-\textsubscript{D}glucopyranosyl)ethyl methacrylate (AcLacEMA).**\textsuperscript{29} 2,3,4,6-tetra-O-acetyl-\beta-\textsubscript{D}-galactopyranosyl(1-4)-2,3,6-tri-O-acetyl-\beta-\textsubscript{D}-glucopyranosyl acetate (30.0 g, 0.04 mol), 2-hydroxyethyl methacrylate (6.37 mL, 0.042 mol) and 200 mL of anhydrous dichloromethane were added to a round bottom flask equipped with a constant pressure addition funnel. BF\textsubscript{3}Et\textsubscript{2}O (28.32 mL, 0.229 mol) was added to the addition funnel and the reaction was flushed with
nitrogen for 30 minutes in an ice bath. The BF$_3$Et$_2$O was then added dropwise over 1 hour while stirring on ice and then allowed to come to room temperature overnight. The reaction was quenched with 100 mL of water and the organic layer was isolated. The organic layer was then washed with saturated Na$_2$CO$_3$ and brine solutions and dried over Na$_2$SO$_4$ and reduced. An excess of pyridine and acetic anhydride was then added and allowed to react under nitrogen overnight to remove any unreacted 2-hydroxyethyl methacrylate. This was then washed with saturated NaHCO$_3$, 1 M HCL and brine solutions and then dried over Na$_2$SO$_4$ and reduced. The resulting oil was recrystallized from methanol acetate to obtain 20.99 g of a white solid. Yield 64%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.64$ (s, 3 H, CH$_3$), 1.97-2.19 (m, 21 H, OCH$_3$), 3.82-5.13 (m, 18 H, ring H, and CH$_2$s), 5.36 (s, 1 H, C=CH$_2$), 6.25 (s, 1 H, C=CH$_2$). MS (TOF MS ESI): $M_{theo} = 748.2426$ g/mol. $M$+Na$^+$ = 771.2335 g/mol.

**Polymerization of AcLacEMA.** AcLacEMA, CDB, and 2,2’-azobis(2-methylpropionitrile) (AIBN), (100:1:0.33) were added to 8 mL of anhydrous anisole (Solvent:Monomer = 4:1 v/v). Air was removed from the reaction flasks through three consecutive freeze-pump-thaw cycles and the flasks were back-filled with nitrogen. The polymerizations were transferred to an oil bath at the desired temperature and allowed to react for 10 h. Samples were taken every hour using a degassed syringe and quenched by exposure to air and rapid cooling. All polymerizations were quenched by exposure to air and rapid cooling and purified by precipitation into cold hexane to yield a pale pink solid.

**Synthesis of poly(AcLacEMA-co-TMS-MMA).** A 50 mL air-free round bottom flask was charged with AcLacEMA (1.947 g, 2.6 mmol), TMS-MMA (0.050 g, 0.26 mmol), CDB (0.007 g, 0.026 mmol), and AIBN (0.0014 g, 0.0085 mmol) in 8 mL of anhydrous anisole. Air was removed from the flask by three consecutive freeze-pump-thaw cycles and the polymerization
was performed for 10 h at 70 °C before being quenched by exposure to air and rapid cooling. The polymer was precipitated twice from hexane and dried in vacuum to afford 1.62 g of pale pink solid. Isolated yield: 81%. $M_w = 47,280$ g/mol. AcLacEMA:TMS-MMA = 10:1. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.19$ (s, 9 H, Si(CH$_3$)$_3$), 5.49 (s, 30 H, acetal H), 6.26 (s, 30 H, acetal H).

**Deprotection of TMS-MA in poly(AcLacEMA-co-TMS-MMA).** Poly (AcLacEMA-co-TMS-MMA) was dissolved in tetrahydrofuran. K$_2$CO$_3$ (5.17 g, 37.4 mmol) was dissolved in 18 mL of methanol:water (2:1 v/v) and added and the reaction was allowed to stir at room temperature for 30 minutes. The solvent was removed by rotary evaporation and the product isolated by precipitation into cold diethyl ether to obtain 1.36 g of pale pink solid. Yield 100%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 5.48$ (s, 30 H, acetal H), 6.29 (s, 30 H, acetal H). FT-IR (cm$^{-1}$): 3605 (OH).

### 4.3.5 Synthesis of $N$-alkyl-$N,N$-linked urea oligomers-polymer conjugates

$N$-alkyl-$N,N$-linked urea oligomers (10 and 12’) were added to separate round bottom flasks with 0.2 g of poly(AcLacEMA-co-alkyne-MMA) and CuBr in dichloromethane. The flasks were purged with N$_2$ for 30 minutes and then PMDETA was added and the reaction was allowed to stir at room temperature overnight. The conjugate was precipitated three times into cold methanol to obtain the products. Conjugate A (10): 0.16 g of an off-white solid. Conjugate B (12’): 0.23 g of an off-white solid.

**Deprotection of N-alkyl-N,N-linked urea oligomers-polymer conjugates.** Conjugates A and B were added to separate round bottom flasks equipped with stir bars. They were dissolved 10 mL in dry methanol and 100 μL of 1 M sodium methoxide in methanol was added and the reaction was allowed to stir at room temperature for 30 minutes. The reactions were then stirred with
Dowex 50WX8 hydrogen form exchange resin. The reaction mixtures were filtered to remove the resin, reduced using a rotary evaporator, and the polymers where precipitated into cold hexane to obtain the final products Conjugate A.1: 0.1 g of an off-white solid. Conjugate B.1: 0.2 g of an off-white solid.

**Sulfonation of N-alkyl-N,N-linked urea oligomers-polymer conjugates.** Conjugates A.1 and B.1 were added to separate round bottom flasks equipped with stir bars. Two equivalents of sulfur trioxide pyridine complex per hydroxyl group were added to the round bottom flask and dissolved in 20 mL of pyridine. This was allowed to react overnight at 90ºC. The solvent was then removed by rotary evaporation and the product dissolved in saturated NaHCO₃ solution and the pH was adjusted to 8.0. The polymers were isolated through dialysis and lyophilization to give off white powders. Conjugate A.2: 0.08 g of an off-white solid. Conjugate B.2: 0.10 g of an off-white solid.

### 4.4 Characterization

$^1$H and $^{13}$C-$^1$H NMR spectroscopy were performed in CDCl₃ with Si(CH₃)₄, MeOH-d₄, and D₂O with 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid, sodium salt as internal standards using a Bruker Ultrashield 400 MHz (100 MHz for $^{13}$C-$^1$H-NMR). The $^1$H and $^{13}$C-NMR spectra were processed using UXNMR version 2.5 and MestReNova Lite. Molecular weights and polydispersities were determined by gel permeation chromatography (GPC). All polymers were characterized with an Agilent 1200 series HPLC equipped with a PSS SDV Lux column (5 µm) guard column and two PSS SDV Linear XL Lux Columns (5 µm) (linear range of MW = 100 - 3x10⁶ g/mol) with filtered tetrahydrofuran (THF) containing 200 ppm 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) mobile phase at a flow rate of 1.0 mL/min at ambient
temperature and a miniDAWN TREOS light scattering (60 mW GaAs linearly polarized laser, 658 nm) calibrated against a 30 000 g/mol polystyrene standard (Wyatt Technology Corp.) and Optilab rEX differential refractometer (light source = 658 nm) detectors. ASTRA software v. 6.1.0 was used to determine polymer characteristic values. Fourier transform infrared (FT-IR) spectra were collected on a Nicolet 6700 spectrometer and analyzed with OMNIC32 software.

4.5 Results and Discussion

4.5.1 Synthesis of a $N$-alkyl-$N,\text{N}$-linked urea oligomer with dissacharide groups

The synthesis of the $N$-alkyl-$N,\text{N}$-linked urea oligomers were performed following procedures developed by our group$^{29}$ from work by Nowick.$^{44}$ The synthesis of the urea oligomers is a simple and iterative solution-phase techniques. There are three steps: (1) Main chain extension using a $N$-(2-nitrobenzene sulfonyl)-2-imidazolidone, (2) side group attachment following Fukuyama’s procedure$^{48}$, and (3) removal of the 2-nitrobenzene sulfonyl group using thiophenol to afford a new secondary amine used in the next cycle. We designed oligomers that contained both protected terminal alkynes and unprotected terminal alkynes as $N$-alkyl side groups resulting in an oligomer that could easily be modified with alkyl azides to synthesize potential
GAG mimics with different carbohydrates (Figure 1).

![Reaction scheme](image)

**Figure 1.** Reaction scheme for the synthesis of alkyne containing $N$-alkyl-$N,N$-linked urea oligomer.

The synthesis of the alkyne containing $N$-alkyl-$N,N$-linked urea oligomer was performed by completing two iterative cycles followed by the ring opening of $N$-(2-nitrobenzene sulfonyl)-2imidazolidone. An alkyl chloride side group was then added (compound 8, Figure 1) so that we could later introduce a terminal azide onto the urea oligomer. Each step of the reaction is performed using traditional organic chemistry techniques, which eliminates the use of expensive resin-based synthetic techniques and their accompanying purification and characterization requirements. The percent yield after each step ranged between 72-97% and 2.47 g of the final product was obtained.
To synthesize the azide containing carbohydrate residues we synthesized an 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (N₃etAcLac) and (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside (N₃etAcMan) through a Fisher glycosidation reaction of 2-bromoethanol with acetate protected lactose and mannose residues. The bromine was then converted to an azide so that it could be used in copper catalyzed azide/alkyne cycloaddition (CuAAC) coupling of the carbohydrate to the oligomer (Figure 2). CuAAC reactions are an orthogonal coupling technique widely used in synthetic and polymer chemistry.³⁵⁻³⁷,⁴⁹,⁵⁰

![Figure 2](image.png)

**Figure 2.** Reaction scheme for the synthesis of an 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside. Similar reaction conditions were used for the synthesis of (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside.

The efficiency of the coupling reaction between the carbohydrate and the oligomer determined to be 100% by ¹H-NMR spectroscopy by comparing the integration of the proton of the triazole ring with the signals of the acetate protecting groups and the terminal –CH₃ residues of the N-alkyl-N,N-linked urea oligomer (Figure 3 ii).
Figure 3. i. Reaction scheme for the CuAAC reaction to attach sugar residues to \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomer to obtain compound 8. ii. \(^1\)H-NMR of compound 9. iii. FT-IR of Compound 10.

The final step in the synthesis of \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomers is the conversion of the alkyl chloride to an alkyl azide. This was done by stirring the lactose containing \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomer with 0.1 M NaN\(_3\) in DMSO overnight to obtain 1.01 g of compound 10 (Figure 3 iii).

4.5.2 Synthesis of a \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomer with side groups that allow for sequence specific modifications
The synthesis of a $N$-alkyl-$N,N$-linked urea oligomer that allows for sequence specific reactions was done by completing two iterative cycles followed by the ring opening of $N$-(2-nitrobenzene sulfonyl)-2-imidazolidone and addition of an alkyl chloride chain to obtain compound 8$. We incorporated a trimethylsilyl (TMS) protect terminal alkyne in oligomer 8$ allowing the selective attachment of azide containing carbohydrates at the unprotected alkyne. The percent yield after each step of the reaction ranged from 43-99% and 4.34 g of the final product was isolated (Figure 4).

**Figure 4.** Reaction scheme for the synthesis of alkyne containing $N$-alkyl-$N,N$-linked urea oligomer that contains a TMS-protected terminal alkyne.
We attached a modified mannose residue, (2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside, at the unprotected terminal alkyne of the N-alkyl-N,N-linked urea oligomer to obtain compound 9’ (Figure 3). The efficiency of the coupling reaction was determined to be nearly 100% as determined by $^1$H-NMR spectroscopy by comparing the integration of the proton of the triazole ring and Ns protecting group with the signals of the acetate protecting groups and terminal –CH$_3$ residues of the N-alkyl-N,N-linked urea oligomer.

The trimethylsilyl protecting group was removed from the oligomer by dissolving the oligomer in 20 mL of THF and adding a solution of K$_2$CO$_3$ dissolved 18 mL of a 2:1 MeOH:H$_2$O at room temperature for 30 min. The product was then purified by column chromatography to obtain 0.72 g of compound 10’ (Figure 5). The reaction was quantified using $^1$H-NMR spectroscopy by noting the complete disappearance of the proton signal at 0.15 ppm from the trimethylsilyl group. The conditions used for the removal of the trimethylsilyl group also caused the partial deprotection of the mannose residue on the oligomer as seen in the $^1$H-NMR.

The modified lactose residue, 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl(1-4)-(2-azidoethyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside, was then added to the newly formed terminal alkyne of the N-alkyl-N,N-linked urea oligomer to obtain compound 11’. The reaction was quantified using $^1$H-NMR and the efficiency was determined to be 100%. Finally, the chloride was then converted to an azide to obtain 1.29 g of compound 12’. FT-IR confirms the addition of the azide with a strong peak at 2097 cm$^{-1}$ evident in the spectrum (Figure 5 iii).
Figure 5. i. Reaction scheme for the synthesis of the $N$-alkyl-$N,N$-linked urea oligomer with a controlled carbohydrate sequence. ii. $^1$H-NMR of Compound 12'. iii. FT-IR of compound 12'.

4.5.3 Synthesis of carbohydrate containing polymers and copolymers

In our previous work, we reported the synthesis of statistical compolymers of isopropylidene and acetate protected carbohydrate monomers with azide containing monomers under reversible addition-fragmentation chain transfer (RAFT) polymerization conditions and their coupling to $N$-
alkyl-N,N-linked urea oligomers containing corresponding carbohydrates. In this work we synthesized statistical copolymers of acetate protected carbohydrate monomers with trimethylsilyl propargyl methacrylate. This was done so that we could incorporate the azide into the urea oligomer instead of the copolymer which allowed us to eliminate problematic side reactions in the synthesis of the urea oligomers.

We used procedures from Ambrosi and Guo to synthesize the acetate protected carbohydrate monomer, 2-(2’,3’,4’,6’-tetra-O-acetyl-β-D-galactopyranosyl-(1-4)-2’,3’,6’-tri-O-acetyl-β-D-glucopyranosyl)ethyl methacrylate (ALacEMA). Prior to synthesizing the statistical copolymers, we verified the controlled nature of the polymerization by preparing homopolymers of AcLacEMA under RAFT conditions. The polymerization was conducted in anisole at 70°C using a 100:1:0.33 monomer:CDB:AIBN ratio with a 1:4 w/v ratio of monomer to solvent. The pseudo-first order kinetic plot for the polymerization of the AcLacEMA with CDB shows an inhibition period of 300 minutes before the first-order kinetics over the next 500 minutes to 80% conversion (Figure 4A). The number average molecular weight of the polymer increased linearly with increasing conversion, however they did not agree with the theoretical molecular weight. The final molecular weight of the polymer was 168 000 with a PDI of 1.13 (Figure 4B). In RAFT polymerization it is possible to predict the theoretical molecular weight at a given conversion using Equation 1, where \([M]_0\) is the initial monomer concentration; \([CTA]_0\) is the initial CTA concentration; \(M_{\text{repeat}}\) is the molecular weight of the repeat unit; \(p\) is the conversion; and \(M_{\text{CTA}}\) is the molecular weight of the CTA. The difference in the actual molecular weight could be due to the loss of propagating radicals over the course of the reaction leading to few growing polymer chains.
Equation 1. Calculation for the theoretical molecular weight of a polymer obtained using RAFT polymerization.

\[
M_{n(\text{theo})} = \left( \frac{[M]_0}{[CTA]_0} \times M_{\text{repeat}} \times p \right) + M_{CTA}
\]

**Figure 6.** Pseudo-first order kinetic plot of AcLacEMA at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70°C. B. Number average molecular weight (M_n) versus conversion of AcLacEMA at a M:CTA:I ratio of 100:1:0.33, using AIBN as the free radical initiator at 70°C. Red squares are the theoretical molecular weights.

We then copolymerized the AcLacEMA with trimethylsilyl propargyl methacrylate using the same polymerization conditions as the homopolymer of AcLacEMA with a monomer feed ratio of AcLacEMA:TMS-MA of 10:1. The resulting copolymer had a number average molecular weight of 4.8x10^4 g/mol and a PDI of 1.6 determined using gel permeation chromatography with light scattering and refractive index detectors and a comonomer ratio of 10:1 as determined by \(^1\)H-NMR. The copolymer was dissolved in 20 mL of THF and a solution of K₂CO₃ dissolved 18 mL of a 2:1 MeOH:H₂O was added. The reaction was stirred at room temperature for 30 min. The polymer was purified by precipitation in to cold hexane and isolated. \(^1\)H-NMR spectroscopy was used to determine that the trimethylsilyl protecting group had been removed and it was also...
observed that the acetate protecting groups were removed under these conditions too, the disappearance of the acetate signal in the $^1$H-NMR spectrum.

We again used CuAAC chemistry to couple the $N$-alkyl-$N$,$N$-linked oligomers 10 and 12' to the poly(LacEMA-co-alkyne-MA) (Figure 5) to obtain conjugate A and conjugate B respectively. The coupling efficiency was determined to be 100% efficient by using $^1$H-NMR spectroscopy by comparing the integration of the signal from the triazole ring and Ns group with the signals of the anomeric protons of the carbohydrates.
Figure 7. Synthetic scheme for the CuAAC reaction to couple compounds 10 and 12' to the statistical copolymer of poly(LacEMA-co-alkyne-MMA).

The oligomer-polymer conjugates were purified by dialysis to remove excess copper prior to deprotection of the remaining acetate protecting groups. The acetate protecting groups were removed using catalytic sodium methoxide in methanol and stirring at room temperate for 20 min. The purified polymer was precipitated into cold hexane and the deprotection reaction
quantified using $^1$H NMR spectroscopy. The deprotection reaction for the $N$-alkyl-$N, N$-linked urea oligomer-polymer conjugates was found to be quantitative by comparing the relative integrations of the peaks of the Ns and triazole protons to the peaks of the aliphatic protons in the spectrum.

The final step to obtain the heparin mimetic is to sulfonate the conjugates (Figure 8). This was achieved by reacting the deprotected $N$-alkyl-$N, N$-linked urea oligomer-polymer conjugates with two equivalents of sulfur trioxide pyridine complex per hydroxyl group at 90ºC overnight. The product was purified and isolated by dialysis and lyophilization to obtain conjugate 0.08 g of conjugate A.2 and 0.10 g of conjugate B.2.

**Figure 8.** Synthetic scheme for the deprotection and sulfonation of the glucose containing $N$-alkyl-$N, N$-linked urea oligomer-polymer conjugates. Similar reaction conditions were also used for deprotection and sulfonation the mannose $N$-alkyl-$N, N$-linked urea oligomer-polymer conjugate.

### 4.6 Conclusions
This work shows the synthesis of a $N$-alkyl-$N,N$-linked urea oligomer-polymer conjugate that incorporated a disaccharide and another that allows for the control of the sequence of the carbohydrates along the oligomer backbone. We were able to combine simple alkyne protecting group chemistry in the $N$-alkyl side groups to enable us to control where the carbohydrates were added to the $N$-alkyl-$N,N$-linked urea oligomer. This opens up the possibility of investigating the effect of larger carbohydrate units and different combinations of carbohydrates effect on the structure-activity relationship of the conjugates compared with naturally occurring sugar-based macromolecules.

4.7 References


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Chapter 5. Dissertation conclusion

This dissertation summarizes our syntheses of well-defined \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomer-polymer conjugates that mimic the naturally occurring glycosaminoglycan heparin. To accomplish this we combined the robust and versatile synthesis of \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomers, reversible addition-fragmentation chain transfer (RAFT) polymerization, carbohydrate chemistry and copper catalyzed azide/alkyne cycloaddition (CuAAC) reactions.

We have shown that using \(N\)-alkyl-\(N,\)\(N\)-linked urea oligomers is a versatile technique to synthesize well-defined oligomers by employing strategies including using isopropylidene protected glucose residues as \(N\)-alkyl side groups, and protected and unprotected alkynes as \(N\)-alkyl side groups. The oligomers that contained the isopropylidene protected glucose residues demonstrated the successful concept that is the foundation of this thesis. We were able to couple the oligomers to a polymer prepared under RAFT control using a CuAAC reaction. RAFT polymerization is a controlled radical polymerization technique that allows us to control molecular weights and prepare polymers that have low polydispersity. By using the same carbohydrate protecting groups on both the oligomer and polymer, we were able to deprotect all the carbohydrates in a single step to yield the final glycosaminoglycan mimic.

The oligomers containing alkyne functional groups as \(N\)-alkyl side groups allow post-synthesis modification of the oligomers. This simplifies the synthesis of the urea oligomers for exploring the structure/activity relationship of our glycosaminoglycan mimics compared with heparin. We synthesized three alkyne containing urea oligomers. The first two contained two terminal alkynes that were coupled to azide containing mono- and di-saccharides. The second urea oligomer contained a protected and an unprotected alkyne, which allowed us to incorporate carbohydrates
of different types. These two approaches allow us to synthesize a single oligomer that can be used to make multiple carbohydrate containing urea oligomers. The oligomers were modified using CuAAC reactions and azide containing carbohydrate residues. The first oligomers contained either acetate protected glucose or mannose residues and were coupled to a copolymer of a complimentary acetate protected carbohydrate and an azide containing monomer. The subsequent oligomers contained the disaccharide lactose or a combination of lactose and mannose. These were then coupled to a copolymer of acetate protected lactose and a protected alkyne monomer. We were able to deprotect the conjugates in a single step by using acetate protecting groups on all components of the oligomer-polymer conjugates. All four conjugates were then sulfonated and purified and isolated using dialysis and lyophilization to obtain final heparin mimetic macromolecules.

The strategies outlined in this thesis will enable future studies of structure-activity relationships. We will be able to compare the carbohydrate side group, the molecular weight of the polymer and the polymer architecture of the oligomer-polymer conjugates with respect to their heparin mimicking abilities. This work adds to the heparin mimetics in novel ways. Current research efforts have largely focused only on charge or hydrophilicity in the design of heparin mimetics. Our goal is to look more deeply at complex interactions that occur due to charge, hydrophilicity and structural conformation of carbohydrate residues and determine which best mimic the biological action of heparin. By building a set of well-defined carbohydrate containing N-alkyl-N,N-linked urea oligomer-polymer conjugates we can apply the best performing macromolecules as biomaterials and improve blood compatibility of blood contacting materials.