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Synthesis Of Carbohydrate Functionalized Dendrons For Use As Multivalent Scaffold And In Self-Assembled Structures

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Graduate Program in Chemistry

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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SYNTHESES OF CARBOHYDRATE-
FUNCTIONALIZED DENDRONS FOR USE AS
MULTIVALENT SCAFFOLDS AND IN SELF-
ASSEMBLED STRUCTURES

(Thesis format: Monograph)

by

Namrata Jain

Graduate Program in Chemistry

Submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
University of Western Ontario
London, Ontario, Canada

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Abstract

Carbohydrates are implicated in a large number of biological processes ranging from cell-cell interactions to bacterial and viral infection. Lectins are carbohydrate-binding proteins that are generally specific for certain sugars. However, typical carbohydrate–lectin interactions tend to have very low monomeric binding affinities. In many cases, the binding of saccharide ligands by protein receptors can be improved significantly through the attachment of multiple saccharide residues to a common support. Dendronized polymers constitute a class of macromolecules whose nanoscale size, rigidity, and functionality can be controlled with precision by tuning their molecular architecture. It is hypothesized that due to their large size and tunable properties, carbohydrate-functionalized dendronized polymers can be designed to be highly efficient ligands for lectins. In the current work, we generated a library of dendronized polymers, with different generations of mannose-terminated dendrons conjugated to a polycaprolactone backbone to study their lectin binding abilities. This work also describes the self-assembly of amphiphilic linear-dendron hybrids synthesized from galactose-functionalized dendrons coupled with a linear polypeptide poly-γ-benzyl-L-glutamate (PBLG). Collectively, this work provides the synthesis and applications of mannose and galactose based glycodendrons and their applications in ultra-high multivalent scaffolds and self-assembled structures for carbohydrate-lectin binding studies.

Keywords

Dendron, Click chemistry, Dendronized polymers, Glycodendrons, Multivalent mannose, Multivalent galactose, Polycaprolactone
I would like to thank my supervisor, Dr. Elizabeth Gillies for providing me with the opportunity to conduct this research, and for all her help and support throughout my time here. Her dedication and encouragement has made this experience invaluable and she has been a constant guidance and inspiration throughout.

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<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>AM</td>
<td>activated monomer</td>
</tr>
<tr>
<td>Bis-MPA</td>
<td>2,2-bis(hydroxymethyl)propionic acid</td>
</tr>
<tr>
<td>Boc</td>
<td>t-buty carbamate</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CBM</td>
<td>carbohydrate-binding modules</td>
</tr>
<tr>
<td>Con A</td>
<td>concanavalin A</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
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<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EPR</td>
<td>enhanced permeability and retention</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray-ionization</td>
</tr>
<tr>
<td>HABA</td>
<td>2-(4′-hydroxybenzeneazo)benzoic acid</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
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<tr>
<td>mCPBA</td>
<td>meta-chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MSA</td>
<td>methanesulfonic acid</td>
</tr>
<tr>
<td>MWCO</td>
<td>molecular weight cutoff</td>
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<td>NaAsc</td>
<td>sodium ascorbate</td>
</tr>
<tr>
<td>Neu5Ac</td>
<td>N-acetylneuraminic acid</td>
</tr>
<tr>
<td>NKT</td>
<td>natural killer T</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<td>PAMAM</td>
<td>poly(amidoamine)</td>
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<td>PBLG</td>
<td>poly-γ-benzyl-L-glutamate</td>
</tr>
<tr>
<td>PCL</td>
<td>polycaprolactone</td>
</tr>
<tr>
<td>PDB-PEO</td>
<td>poly(butadiene-block-ethylene oxide)</td>
</tr>
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<td>PDI</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>PEG-PCL</td>
<td>poly(ethylene glycol)-block-poly(ε-caprolactone)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PLL</td>
<td>poly-L-lysine</td>
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<tr>
<td>RCA120</td>
<td><em>recinus communis agglutinin</em></td>
</tr>
<tr>
<td>ROMP</td>
<td>ring-opening metathesis polymerisation</td>
</tr>
<tr>
<td>ROP</td>
<td>ring-opening polymerization</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>Sn(Oct)$_2$</td>
<td>tin(II) 2-ethylhexanoate</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TEOC</td>
<td>trimethylsilyl ethyloxy carbonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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Chapter 1

1 Introduction:

1.1 Carbohydrates: Role in biology and medicine

1.1.1 General role of carbohydrates in biology

Polypeptides, polynucleotides and polysaccharides are the three main classes of biopolymers. Among these, polysaccharides or carbohydrates are a particularly diverse class that are made up of monosaccharide units as building blocks with a variety of stereochemical linkages, branching, as well as chain lengths among them. These carbohydrates exist in different forms in the body, for example as polysaccharides, glycopeptides, glycolipids, glycoaminoglycans, or other glycoconjugates. A vast amount of literature and experimental data exists for the study of proteins and nucleic acids and their interdependence and mutual interactions but because of the complexity, structural diversity and synthetic intricacies of higher polysaccharides, their direct applications in the field of medicine has not received as much attention as their two counterparts.1
In recent years, studies have been done to learn about the role of carbohydrates in many of the biological processes in the body. A variety of natural products have been synthetically modified to their carbohydrate derivatives and have been proven to exhibit similar biological activity to their parent compounds while simultaneously having decreased toxicity and enhanced solubility. For example, the aureolic acid group present on several compounds is considered to be an antimicrobial agent active against mycobacteria. One particular carbohydrate derivative of this group of compounds, chromomycin A₃ (figure 1.2), is used clinically as an antitumor agent, specifically as an inhibitor of DNA-dependent RNA polymerase. Degradation experiments on
this molecule suggested that the activity and the binding of this molecule with DNA decreased with decreasing numbers of sugar groups $^3$.

![Structural diagram of chromomycin A](image)

**Figure 1.2: Structure of chromomycin A**

Several proteins active in the body have acquired carbohydrate-binding active centres or ‘modules’ that perform multiple biological functions. These modules, called the carbohydrate-binding modules (CBMs) are highly carbohydrate selective and are capable of recognizing a variety of polysaccharides such as cellulose, chitin, $\beta$-glucans, starch, glycogen, inulin, pullulan, xylan, and many other different sugars such as arabinofuranose, mannan, fucose, lactose, galactose, polygalacturonic acid, $\beta$-D-galactosyl-1,4-$\beta$-D-N-acetylg glucosamine, lipopolysaccharides etc $^4$. 
Carbohydrates also play a major role in cell-cell interaction processes. Cells have complex carbohydrates coating their surfaces and with the help of receptor-ligand interactions, a variety of cell-events can be triggered. In certain cases, the carbohydrates can be recognised by other, complementary carbohydrates in order to initiate these events. For example, corneal epithelial cells express a glycoprotein bearing the glycan determinant Le\(^x\). These kinds of cell-cell interactions mediated by Le\(^x\) are responsible for mechanisms mediating corneal epithelial cell differentiation\(^5\).

Aside from cell-cell and cell-material interactions, carbohydrate literature contains many examples describing their role in reinforcing cell-microbe interactions. For example, *Vibrio cholerae* is a gram-negative, comma-shaped bacterium that releases the toxin that causes cholera. This toxin is a multisubunit, carbohydrate-binding protein that belongs to the family of AB\(_5\) toxins. Its B subunits are capable of binding to the carbohydrate part of the GM1 ganglioside on the intestinal cell surface and the A subunit internalizes the toxin, thus initiating the disease\(^6\). The approach to intercept this interaction of the toxin to the cell could be done in an anti-adhesion manner by using carbohydrates to bind with the toxin instead\(^7\).
Polysaccharides of glucose, such as glycogen and starch serve as the main form of energy storage in animals and plants respectively. In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, and functions as the secondary long-term energy storage (with the primary energy stores being fats held in adipose tissue). Muscle glycogen is converted into glucose by muscle cells, and liver glycogen is converted to glucose for use throughout the body, including the central nervous system. In addition, primary and secondary cell walls of plants are composed of high amounts of cellulose, hemicellulose and pectin.
Polysaccharides and oligosaccharides in biological systems can hence serve a variety of functions from being recognition markers for proteins to fine tuning the intrinsic properties of the proteins they’re attached to and altering their interactions with other systems\textsuperscript{11}. This necessitates further advancements in the study of carbohydrate-protein interactions.

\subsection*{1.1.2 Role of mannose in biology}

Mannose is a sugar monomer of the aldohexose series of carbohydrates. It is a C-2 epimer of glucose and is involved in many biological processes such as toxin mediation. It has also been shown to have increased binding when presented in a multivalent manner\textsuperscript{12}. Mannose-binding receptors are found in a variety of cells including alveolar and splenic macrophages, which actively participate in phagocytosis and cell migration and thus have a profound influence on the immune response of the host\textsuperscript{13}. Certain mannose receptors expressed in hepatic endothelial cells internalize glycoproteins that are terminated with mannose via receptor-mediated endocytosis\textsuperscript{14}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{mannose.png}
\caption{Chemical structure of D-mannose}
\end{figure}

The mannose receptor, a C-type Lectin, found primarily on the surface of dendritic cells and macrophages recognises terminal mannose as well as \textit{N}-acetylglucosamine and fucose residues
on glycans, and is involved in a variety of immunological processes within the body\textsuperscript{15}. The receptor recognises terminal mannose, N-acetylglucosamine and fucose residues on glycans attached to proteins\textsuperscript{16} found on the surfaces of some microorganisms, playing a role in both the innate and adaptive immune systems. It also performed additional functions such as the clearance of glycoproteins from the circulation, including sulphated glycoprotein hormones and glycoproteins to be released in response to pathological events\textsuperscript{17}. The mannose receptor recycles continuously between the plasma membrane and endosomal compartments in a clathrin-dependent manner\textsuperscript{18}.

Certain strains of the uropathogenic bacterium \textit{Escherichia coli} (\textit{E. coli}) have the ability to adhere to epithelial cells in the kidneys and lead to urinary tract infections. This ability is mediated by the hair-like appendages on the bacteria, called fimbriae, that are coated with carbohydrate-receptors on their surfaces\textsuperscript{19}. These receptors are capable of recognizing and binding to cell surfaces of the host via adhesion through the carbohydrates expressed abundantly on the uroepithelial cells. The adhesion marks the initiation of the infection process\textsuperscript{19} and can potentially be blocked, thus successfully preventing the infection, by using certain multivalent aryl\textsuperscript{20} and alkyl\textsuperscript{19} mannosides to bind to these protein receptors instead, and hence can serve as an alternative therapeutic approach against bacterial infection.

**1.1.3 Role of $\alpha$-galactose in biology**

Galactose is a C-4 epimer of glucose which exists in both open-chain and cyclic form as $\alpha$- and $\beta$-anomers. It has many roles in biological system. Approximately 20\% of the oligosaccharides on Recombinant Soluble Human CD4 (sCD4) have $\alpha$-galactose residues present on them\textsuperscript{11}. CD4
is a cell-surface glycoprotein found on the mature helper T cells and immature thymocytes, as well as on monocytes and macrophages.

![Figure 1.5: Structure of D-galactose in its open form and closed α anomer form.](image)

Certain α-galactose derivatives like galactose-α-1,3-galactose, commonly known as alpha-gal, is a carbohydrate found in most organisms’ cell membranes. However, it is not found in primates or humans, whose immune systems recognise it as a foreign body and produce xenoreactive immunoglobulin M antibodies, leading to organ rejection in cases of transplantation from non-primate mammals, such as pigs\textsuperscript{11}.

α-Galactosylceramide (α-Gal-Cer; KRN7000) is a biological response modifier which was first isolated from an extract of the marine sponge \textit{Agelas mauritianus} and consists of an α-galactose combined with a ceramide structure. α-Gal-Cer (figure 1.6) is a specific ligand for human and mouse natural killer T (NKT) cells and was found to exhibit potent anti-tumor activity in murine \textit{in vivo} experiments including a subcutaneous implanted model and metastatic models in the liver and lung\textsuperscript{21}. A majority of sugar ceramides found naturally contain β-linkages and have no such activities like α-GalCer.
Galactose is also involved in many disease processes. For example, sulfated multivalent galactose derivatives have been shown to be potential inhibitors for HIV-1 infections. α-Galactose based ligands are involved in certain bacterial infections in humans as well. The glycosphingolipid ligand, Gb3, which is present on human epithelial cell surfaces has an α-galactose terminus which is actively involved in the surface binding with various verotoxins, including Shiga and Shiga-like toxins. It is known that infection with verotoxin producing *E. coli* leads initially to hemorrhagic colitis and further to hemolytic uremic syndrome in humans. This surface binding interaction is the infection initiating step and can be blocked by availability of competing ligands with the same α-galactose functionality. This suggests further exploration in the chemical synthesis of such ligands would be a valuable pursuit.

1.2 Multivalent platforms for carbohydrates

1.2.1 Lectins as model systems for cell-material recognition

Lectins are an important class of proteins that interact with carbohydrates in a non-covalent manner. They are highly sugar-specific and are upstream initiators of a variety of biological processes such as cell adhesion, migration, phagocytosis, cell differentiation and apoptosis. They also act as recognition determinants in processes such as the clearance of glycoproteins.
from the circulatory system, control of intracellular trafficking of glycoproteins, adhesion of infectious agents to host cells, recruitment of leukocytes to inflammatory sites, as well as cell interactions in the immune system, in malignancy and metastasis. Lectins are proteins that do not have inherent catalytic, antibody-like or enzymatic roles in the body.

Figure 1.7: Lectin receptor-mediated targeting to macrophages (Reproduced with permission from reference 26)

The nature of the interactions between lectins and carbohydrates is mainly based on hydrophobic interactions and hydrogen bonds between the hydroxyl groups of the carbohydrates and the amino-acid residues of the lectin proteins. Metal-ion coordination and hydrophobic stacking of patches of sugar surface with the proteins are some other forces responsible for lectin-carbohydrate binding. The combining sites of lectins are in the form of shallow grooves on the
surface of the protein. Typically, only one or two edges or faces of the carbohydrate ligand are bound to the protein\textsuperscript{25}.

One of the striking features of the lectin-carbohydrate interaction is that their binding affinities are weak ($K_a: 10^3$-$10^4$ M$^{-1}$) when the saccharides are in their monovalent forms. In order for the interactions between lectins and carbohydrates to be biologically relevant, multiple copies of these interactions have to be made either sequentially or simultaneously. For example, an increase in binding affinity of about $10^5$ was observed in case of methyl-$\alpha$-mannoside when a synthetic polymer was prepared which displayed multiple mannose residues\textsuperscript{27}.

![Figure 1.8: The structures of a few branched oligosaccharides and their binding affinities to lectin ConA](image)

While a variety of lectins have been researched in the past, Concanavalin A (Con A) has been of major importance, especially for $\alpha$-D-mannoside residues. It also binds $\alpha$-D-glucosyl residues. It is a member of the legume lectin family. Con A exists in the form of a tetramer at near-neutral pH and has one binding site per subunit.
A variety of assays are commonly used for the evaluation of glycodendrimer binding with glycoconjugates. For example, an IC$_{50}$ can be obtained by labelling Con A with an enzyme and adding it to polystyrene wells to which a mannose derivative has been attached. On addition of the glycoconjugate, a concentration profile required to inhibit binding of this Con A to the wells can be generated giving insights into the binding affinities of the glycoconjugate$^{29}$. Receptor clustering assays, including precipitation and turbidity assays, involve the formation of insoluble aggregates of Con A with the dendritic ligand, and these assays can provide data concerning the kinetics and stoichiometry of binding$^{30}$. The hemagglutination assay is one of the widely used assays used for characterizing glycoconjugates-Con A binding. This assay can compare the relative binding affinities of various monovalent or multivalent mannose-based ligands. Con A
interacts with glycoproteins found on the surfaces of red blood cells and causes their agglutination or aggregation. This agglutination is inhibited by the above mentioned mannoside-based ligands by their binding to the lectin instead. The comparison of the minimum concentration of ligands required for the agglutination inhibition can lead to insights into their relative binding affinities.

1.2.2 Multivalency in carbohydrates

Multivalency is found widely in nature. For example, a gecko’s foot has millions of spatula-shaped setae arranged in lamellae, which help it adhere to practically any surface by creating a strong resulting attractive force from millions of Van der Waals interactions of small magnitude from each seta. In a similar fashion, multivalency in carbohydrate interactions with proteins and microbes is of major importance. It has been shown that the clustering of carbohydrates on a multivalent architecture significantly increases the binding affinity of these interactions via the mechanisms of the chelate effect as well as receptor clustering or the proximity effect, whereby the local density of the ligands increases due to clustering, resulting in efficient binding. This effect was first noted by Lee and co-workers and has found a wide range of applications in biology and medicine, proving to be an important tool for biotechnological and therapeutic purposes. By clustering ligands, their topological and spatial proximities can be tuned and the optimal molecular features can be identified, leading to increased binding affinities.
There are a variety of multivalent platforms that have been used in order to depict multivalency for carbohydrates. These include, for example, polymers, nanoparticles, dendrimers, vesicles and proteins.

**Proteins:** Carbohydrate epitopes can be attached to the surfaces of proteins through chemical or enzymatic methods with the help of reactive groups in the side chains of proteins. A number of examples\(^{32, 33}\) of carbohydrate attachment to Bovine Serum Albumin (BSA) carrier protein have been reported. In order to identify the effects of varying the number of sugar residues on the binding affinities to a lectin, a competitive inhibition assay was employed, wherein the inhibition of the binding of a chosen glycoprotein receptor to its lectin by the synthetic neoglycoprotein was tested. The studies demonstrated that the binding affinities increased with increasing sugar content in the neoglycoprotein, thereby establishing the effect of increasing valencies and the logarithmic increases in binding affinities\(^{33}\). However, in large carrier proteins, the spatial disposition of individual sugar ligands on the proteins is not very well identified because of their
overall complex nature. Additionally, the use of proteins as carriers might lead to non-carbohydrate-specific interactions during biological analysis, which essentially hinders structure-property relationship attribution.\textsuperscript{34}

**Vesicles:** Polymer vesicle is yet another architecture that has been used for sugar-scaffolding. Amphiphilic block copolymers can self-assemble in solution into vesicles with a structure analogous to that of phospholipid vesicles\textsuperscript{35}. When mixed with a suitable solvent such as water, the hydrophobic blocks of the polymer amphiphiles associate to form the core of the membrane while the more hydrophilic blocks are exposed to the interior and exterior solution. The surfaces of these vesicles can be functionalized with carbohydrates. For example, Gillies et al. functionalized poly(butadiene-block-ethylene oxide) (PDB-PEO) vesicles with dendritic mannose at the periphery\textsuperscript{35}. It was demonstrated that the functionalized vesicles showed enhancement in binding affinity to lectins by 1-2 orders of magnitude relative to the non-dendritic vesicle system. In another example, a multifunctional polymer vesicle system with polyester dendrons functionalized with $N$-Acetylneuraminic acid (Neu5Ac) was synthesized\textsuperscript{36}. It was found that while the sialodendron exhibited a 17-fold enhancement (per sialoside) relative to the small molecule, the dendritic sialopolymersomes resulted in an almost 2000-fold enhancement in binding affinity\textsuperscript{36}.

**Nanoparticles:** In order to explore various scaffolds for carbohydrate ligand representation, nanoparticles were used as carriers for a variety of sugar residues. Jain et al. developed the mannosylated gelatin nanoparticles for targeted delivery of an anti HIV drug, didanosine\textsuperscript{37}. The authors synthesized the nanoparticles by a two-step desolvation technique and mannose was coupled to the surface amino groups of gelatin nanoparticles. In cellular uptake studies mannose-coupled nanoparticles showed increased uptake by the lung, liver and lymph nodes compared to
uncoupled gelatin nanoparticles and free drug. In yet another example, gold nanoparticles with α-acetal-poly(ethylene glycol) layers were functionalized with β-D-lactopyranoside and their aggregation behavior towards *Recinus communis agglutinin* (RCA<sub>120</sub>), a bivalent lectin selective to galactose, was studied. The aggregation of the lactose-functionalized gold nanoparticles by the RCA<sub>120</sub> lectin was found to be reversible, recovering the original dispersed phase and color by addition of excess galactose<sup>38</sup>.

![Figure 1.11: Synthesis of sugar-functionalized gold nanoparticles (adapted from reference 28)](image)

**Polymers and polymer assemblies:** Polymers are considered excellent models for binding carbohydrates owing to their vast scope of pendant functionalities. They are relatively cheaper and easier to synthesize, purify and characterize on a small to large scale compared to the previously discussed models. A study involving a polymethacrylate functionalized with galactopyranoside pendant units and peanut *agglutinin* lectin showed that the glycopolymer
exhibited approximately 50 times better binding affinity when compared to the monovalent sugar\textsuperscript{39}. Recently, ring-opening polymerization (ROP) and copper-catalyzed alkyne-azide cycloaddition “click” reaction were used to prepare a series of amphiphilic block-graft (poly-α-azidecaprolactone-graft-sugar)-b-polycaprolactone polymers and their self-assembly as well as cytotoxicity was studied\textsuperscript{40}. In this context, Kiessling et al. have largely exploited ring-opening metathesis polymerization (ROMP) to generate multivalent ligands based on dextran, ficoll, or polyacrylamide derivatives with a variety of recognition elements including carbohydrates at their termini\textsuperscript{41}. Sialic acid- or neuraminic acid-appended polyvalent polymers were subjected to a series of studies. Whitesides and co-workers used polyacrylamides as scaffolds for pendant α-sialoside groups. This strategy is based on polyvalent polymeric inhibitors able to compete with the polyvalent virus-cell interaction. The studies were focused primarily on the inhibition of the viral hemagglutinin binding to the erythrocytes by synthetic polymers bearing sialosides\textsuperscript{42}. Although polymers provide tremendous multivalent functionalities, the exact quantitative characterization of sugar groups attached per mole of the carrier is rather ambiguous.

## 1.3 Dendrons and dendrimers

### 1.3.1 Introduction

Dendrimers and dendrons represent a unique class of macromolecules that has recently attracted increasing attention from both academia and industry. They are highly-branched macromolecules with well-defined, 3-dimensional architecture. As a hierarchical structure, dendrimers are made up of three structural regions: a) a core b) layers of branching repeat units comprising the backbone, each layer termed a generation, and c) end groups on the periphery (figure 1.12). The
interior part of the dendrimer structure is suitable for guest encapsulation purposes, given that it is safely protected from the surroundings by the dendritic layers. ‘Dendrons’ have a wedge-like structure and emerge from a monovalent core moiety called the focal point. In such molecules, conjugation from the periphery and/or focal-point may result. Each generation of these macromolecules results in an increase in the overall diameter as well as in the number of end groups. Since the increase in the number of end groups is exponential, different generations differ in rigidity as well as density and may assume an increasingly globular shape at higher generations\textsuperscript{43}.

Figure 1.12: Schematic representation of the architectural differences between a) linear, b) hyperbranched, and c) dendritic polymers (Reprinted with permission from reference 44. Copyright 2002 American Chemical Society)
It was in the early 1980s that dendron and dendrimer synthesis first gained significant interest when Tomalia et al.\textsuperscript{46} followed by Newkome et al.\textsuperscript{47} described successfully synthesized poly(amidoamine) (PAMAM) and arborol dendrimers respectively. Since then, over 100 different structure of dendrimers have been reported\textsuperscript{48}.

The two striking features of these macromolecules are their monodispersity and their multivalency. The synthesis of dendrimers is highly iterative and except for some minor defects in the structure in higher generations such as incomplete growth of the dendrons, the number of functionalizable end groups on the periphery can be well-controlled, giving dendrimers their unique monodispersity. In biomedical applications, such as carbohydrate-protein interactions, monodispersity is a promising tool as it gives a very good control on the number of ligands attached to the periphery, thus enabling a better understanding of the structure-property relationship of the bonded system\textsuperscript{43}. Additionally, the multivalent nature of their peripheries allows for a scaffold for these ligands, which in a variety of cases has been shown to increase the
binding affinities of ligands to their receptors via the multivalent effect\textsuperscript{35}. In addition to these properties, the covalent nature of bonds in dendrimers and dendrons eliminates the need for the achievement of thermodynamic equilibrium that is needed for self-assembled structures, making it a more stable choice for multivalency\textsuperscript{44} and their overall globular shape gives rise to interesting architecture-dependent properties in solution\textsuperscript{49}.

Because of the above quoted properties of dendrimers, they have proven their significance in boosting the solubility as well as bioavailability of drugs\textsuperscript{50}, DNA derivatives, and carbohydrates\textsuperscript{51} and have helped in increasing the \textit{in vivo} circulation times of contrast agents\textsuperscript{52}.

However, in order to tackle the toxicity challenges associated with use of dendrimers in a biological setting, the use of dendrimers with biodegradable cores and branches has been of increasing focus. The approach to the production of such dendrons is through including monomers that are metabolic products of various biological pathways. The resulting dendrimers are required to be water-soluble, non-toxic, non-immunogenic and capable of crossing appropriate biological barriers. In this regard, polyester dendritic systems are of utmost importance. Dendrimers based on 2,2-bis(hydroxymethyl)propionic acid (Bis-MPA) have proved promising in clinical trials for the delivery of doxorubicin, paclitaxel, camptothecin, and diammineplatinum (anticancer agents)\textsuperscript{43}.

### 1.3.2 Chemical synthesis of dendrimers and dendrons

There are two main strategies for the synthesis of dendrimers, the divergent and the convergent methods. The divergent method of dendrimer/dendron synthesis\textsuperscript{46} is an inside out approach wherein the synthesis is initiated at the core or focal point and the macromolecule is built up through each layer or generation to the periphery. In the convergent approach\textsuperscript{53}, the synthesis
initiates at the periphery which is bound together to the required number of generations and later coupled to the core.

The divergent approach is typically used for the large scale synthesis of dendrimers as the quantity increases with every generation. It however has the limitation of time-consuming protection and deprotection reactions that have to be done for the iterative synthesis. Moreover, in higher generations, reaction incompleteness, and hence defects in the structure of dendrimers poses a problem.

On the other hand, convergent synthesis reduces the number of defects in the structure as it involves coupling of individual pieces of the macromolecule, making it a better choice in the scenario where monodispersity is of utmost importance. The convergent approach can also potentially be used to synthesize in a controlled manner an asymmetric dendrimer structure with multiple different kinds of dendrons attached at the core. This approach, however, suffers from problems involving steric hindrance for coupling in higher generation dendrons and decrease of overall mass in each step, making it non-ideal for large scale synthesis.
1.3.3 Carbohydrate-dendrimer conjugates

The need for a detailed study of carbohydrate-protein interactions at a molecular level is essential, as evident from the multitude of scenarios where such interactions are involved, including cell adhesion, infection initiation, cancer metastasis etc. The clear understanding of such processes is only possible through development of structurally well-defined model systems and macromolecules that can be synthesized, purified, characterized and analyzed for their
biological activity\textsuperscript{34}. As described in section 1.2.2, there are a variety of scaffolds available and they have been researched upon in the context of multivalency of carbohydrates. Among these, dendrimers have emerged to be a yet another class of molecules, as in addition to improving the valency of the sugars, their well-defined structure eliminates limitations over heterogeneity of the resulting structure.

Different generations of dendrimers provide a choice in further tuning of the valency as well as the size of the macromolecule. By changing the kind of dendrimers used, their linkages to the carbohydrates can be experimented upon. A wide range of saccharides including mannose, galactose, glucose, lactose, maltose, xylose, Neu5Ac (sialic acid), and other oligosaccharides have been conjugated to various dendrimer peripheries via different linkages such as amide, hydrazide, amine, thioether, thriourea, and triazole linkages. For example, Peiters \textit{et al.} synthesized dendrimers based on 3,5-di-(2-aminoethoxy)-benzoic acid repeat unit with alkyne functional groups at their periphery and conjugated them with azide-terminal mannose with the help of a copper-catalyzed alkyne-azide cycloaddition “click” reaction to yield glycodendrimers functionalizable to a chip surface to make a microarray\textsuperscript{54}. In yet another example, Thompson and Schengrund have successfully displayed GM1 pentasaccharide ligands for the \textit{E. coli} heat-labile toxin on a poly(propylene) amine dendrimer using an amine-isothiocyanate coupling reaction\textsuperscript{55}. Cloninger and coworkers have prepared mannose functionalized PAMAM dendrimers ranging from the 1\textsuperscript{st} to 6\textsuperscript{th} generation and their binding affinities to Con A were shown to depend on the sizes and multivalencies of the bioconjugates\textsuperscript{29}. Moreover, they were able to systematically control the degree of lectin clustering and overall activity by varying the mannose/hydroxyl ratios on the surfaces of 3\textsuperscript{rd} to 6\textsuperscript{th} generation dendrimers\textsuperscript{56}. 

23
Roy et al. prepared nanometer-sized, water-soluble glycodendrimers (mannose-functionalized poly(amidoamine)) (PAMAM) generation G0 cystamine core dendrimer) coated gold nanoparticles\(^ {57}\) (figure 1.15). The binding constant of Con A to the gold-mannose interactions was found to be 100 times higher than the binding constant values obtained in the interactions with the glycodendrimer alone\(^ {57}\). Similarly, McReynolds et al. evaluated the feasibility of synthesizing six sialic acid-PAMAM glycodendrimers using unprotected sialic acid and amine terminated PAMAM dendrimer\(^ {58}\). Compounds that showed promising binding characteristics were then further assessed for inhibition of HIV-1 infection using a well-characterized luciferase reporter gene neutralization assay\(^ {58}\).
The use of glycodendrimers as ligands, although attractive in the scheme of homogeneity and multivalency, needs a thorough optimization of the choice of dendrimer backbone as well as generation. In lower generations of the dendrimer, because of low valency, the binding affinities may not vastly improve in comparison to monovalent saccharides. On the other hand, steric factors including crowding of ligands at the periphery of dendrons at higher generations may lead to their incomplete binding to the receptors. 

Figure 1.15: Gold nanoparticle functionalized with mannose terminal glycodendron (Reproduced with permission from reference 57)
1.4. Linear-dendron hybrids

Linear-dendron hybrids are a relatively new class of block copolymers that have gained significant interest in the past years. They are composed of fragments of polymers and perfectly branched dendritic entities that are fundamentally different from each other in architecture. The linear nature of the polymers contrasts with the globular architecture of dendrimers to give rise to interesting properties exclusive to neither of the two constituents.

![Diagram showing monomers, molecular interactions, and self-assembled structures.](image)

Figure 1.16: The resultant structures formed through self-assembly. (Reproduced with permission from reference 59)

There are three principal synthetic routes that are popular for synthesis of such hybrids\(^60\):

a) Coupling of individual linear and dendritic blocks.
b) Divergent growth of dendrons on the polymer

c) Use of dendrimers as macroinitiators for polymerization of linear chains.

Such amphiphilic hybrids undergo ‘microphase separation’ in solution and generate interesting self-assembled morphologies. These assemblies are driven by various weak, non-covalent forces and can effectively entrap drug molecules in their voids. The increase in entropy due to aggregation of hydrophobic part and exclusion of water plays an important role in driving these structures to form. These structures offer an attractive alternative to traditional drug carriers. They solubilize and protectively encapsulate the drugs and control their release into specific desired locations minimizing frequency and concentrations for target-specific delivery. Recently, there has been an increasing interest in such self-assembled amphiphilic structures because of their applications as drug-encapsulating materials as well as their bioactive outer membranes.

Some examples of self-assembled molecules include poly(ethylene glycol)-block-poly(ε-caprolactone)(PEG-PCL) micelles that have been used to form micelles capable of encapsulating small drugs like paclitaxel and rapamycin. In yet another example, vesicles made by using two distinct diblock copolymers were able to solubilize doxorubicin as well as paclitaxel in their aqueous lumen and hydrophobic walls respectively and the shrinkage of tumor cells in mice was more compared to when no carrier was used.

Additionally, in the field of tumor cell targeting, such nano-sized, drug-encapsulating systems can be used to address some of the major challenges of drug-delivery systems such as selectivity. In order for tumor cells to rapidly grow, they must stimulate the production of blood vessels. Due to the rapid growth and corresponding angiogenesis of tumor cells, they have leaky architecture and wide fenestrations. These cells also lack an efficient lymphatic system. Because of these properties, tumor blood vessels show high permeability to macromolecules and nano-sized
particles and their retention is prolonged by tumor cells. This phenomenon is known as Enhanced Permeability and Retention (EPR) effect.

General guidelines for designing such delivery vehicles include use of molecules that are biocompatible, have minimum cytotoxicity and are efficiently degradable by the body after delivery. In this regard, linear dendron hybrids with glycodendrons as the hydrophilic block and a polypeptide such as poly-γ-benzyl-L-glutamate (PBLG) as the hydrophobic block can be hypothesized to self-assemble into some interesting structures. These systems with carbohydrates on the surface would have an additional benefit in biorecognition for target-specific drug delivery to cells. They are structural mimics of natural glycoproteins and possess the advantage of having a well-defined secondary structure allowing ordered display of carbohydrate moieties.

1.5 Dendronized Polymers

Dendronized polymers (or denpols), as the name suggests, are analogues of the above discussed dendrimers, where these dendrimers of various types and generations are displayed on a long, linear, polymeric backbone, as grafted pendant groups. There are several aspects of dendronized polymers that can be examined:

1. Overall l/d ratio and rigidity: Denpols are made up of a one-dimensional polymeric chain combined with more globular dendritic wedges, providing these macromolecules with interesting properties. The rigidity of these structures has been hypothesized to be dependent on the density and generation of the dendrons grafted. In case of high steric repulsions between higher generation and more densely packed dendrons this leads the overall structure to be rigid and rod-like, while the chain flexibility is much more relaxed otherwise, and the structure can be bent and coiled. In both these extreme cases, the overall length to diameter ratio increases drastically with
increasing rigidity. The length of these molecules can also be tuned by choosing the backbone of appropriate size and the distance between adjacent grafted groups can be varied as well.

2. Multivalency: Owing to a large number of pendant groups on one molecule of the denpols, the scope of end-group functionality of these structures is tremendous. For example, on a polymer with 20 repeating monomeric units grafted with a dendron with 8 peripheral groups per unit, 160 sites of further end-group attachment is possible, making these molecules one of the richest sources for multivalent applications.

![Cartoon representation of a coiled polymer backbone’s stretching through the attachment of increasingly sterically demanding dendrons. Polymer backbone with a) no dendrons; b) first generation dendrons; c) second generation dendrons; d) third generation dendrons (reproduced with permission from reference 66)]
There are three main synthetic approaches to make dendronized polymers:\footnote{67}

1. In the ‘graft-to’ approach, the dendritic entity of appropriate generation and the polymer backbone are synthesized individually, and then later connected to make the denpols. This approach offers the maximum control on the dendritic functionalization, but suffers from incomplete coverage of the backbone because of steric effects during conjugation to the polymers.

2. The ‘graft-from’ approach, on the other hand, includes synthesis of the dendritic entity from the polymer backbone via a stepwise growth process. This method ensures increased density of pendant groups on the polymer comparing to the ‘graft-to’ way, but may lead to synthetic difficulties leading to defects in dendron structures.

3. The ‘macromonomer’ approach includes polymerization as the last step in synthesis, whereby the monomer is conjugated with the dendron beforehand. This approach gives the benefit of each unit having the perfect dendronization as well as full coverage of the polymer backbone. Yet another advantage is the possibility of dendrons of different types and generations grafted on the same polymeric chain. However, dendrons of higher generation suffer from low degree of polymerization and hence fewer pendant groups per polymer molecule.

Extremely long dendronized polymers with polystyrene as the backbone and two trimethlysilyl ethyloxy carbonyl (Teoc) protected peripheral amine groups dendrons of generation 4 were synthesized by Schluter et al\footnote{68} (figure 1.18), and their individualization and atomic force microscopy (AFM) visualization on graphite, and its manipulation with the AFM tip were reported. Seeberger et al. reported biocompatible glyco-dendronized poly-L-lysine (PLL) polymers that carried either three or nine mannose- or galactose-bearing dendrons\footnote{69}. These
glycodendronized polymers were capable of providing a platform for studying the carbohydrate-pathogen binding interactions as well as for biosensor applications for mannose-binding *E. coli*.

Figure 1.18: Generation 4 polystyrene dendronized polymer (Reproduced with permission from reference 68)

Although a number of examples of dendronized polymers of various polymeric backbones and different generations and kinds of dendrons have been reported, to the best of our knowledge, lectin binding properties of ultra-high multivalency glyco-denpols have not been explored.

1.6 **Scope of the thesis**

The overall goal of the thesis is the synthesis of glycodendrons consisting of a 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) backbone with peripheral α-mannose and α-
galactose derivatives, and the conjugation of these dendrons to linear polymers to form highly multivalent carbohydrate-based ligand systems.

The first goal is to conjugate glycodendrons to a functionalized linear polymer backbone to create glyco-denpols. Despite many systems having been developed as models for multivalency, dendronized polymers that combine the globular structure of dendrons with the linear structure of linear polymers have not been previously studied upon in the context of carbohydrate-protein recognition. In addition to studying the effect of multivalency, comparing different lengths and generations of dendronized polymers help with the understanding of the effect of rigidity of these systems on their binding ability, which has not previously been done for such glycodendrons.

The second goal of this thesis is to use these glycodendrons and covalently attach them to polypeptides such as PBLG to make an amphiphilic linear dendron hybrid system capable of self-assembling in aqueous solution. These self-assembled structures are novel as they combine

Figure 1.19: Second generation dendronized polymer with mannose end-groups
the effects of multivalent presentation of carbohydrates with self-assembly properties of amphiphilic copolymers all in a single scaffold, thereby avoiding the necessity of surface functionalization steps.

Figure 1.20: Model reaction scheme for synthesis of linear dendron hybrids and their self-assembly
Chapter 2

2 Results and Discussions

2.1 Synthesis of glycodendrons

2.1.1 Synthesis of Amine-functionalized Polyester Dendrons of Generations 1, 2, 3 and 4

A library of 4 generations of dendrons was developed in order to compare the effect of size and valency in the final macromolecules. Amine groups were selected for the peripheries of the dendrons as they are ideal functional handles for further derivatization with the carbohydrates. The preparation of the amine-functionalized dendrons was done in two synthesis phases wherein the respective hydroxyl-terminated dendrons were synthesized first using the previously reported procedure, and then an additional linker was added to them using protected β-alanine through a hydroxy-carboxylic acid coupling reaction.

For this synthesis, a polyester dendron based on Bis-MPA was selected due to its ease of synthesis and biocompatibility. Bis-MPA-based dendrimers have shown promise in clinical trials for the delivery of doxorubicin, paclitaxel, camptothecin, and diammineplatinum, which are all anticancer agents. They can be prepared iteratively through protection and deprotection reactions using both convergent and divergent approaches. The divergent approach was selected as the experience in the group has been that this method results in easy purification and good yields. An alkyne focal point was selected as the starting point for the dendron synthesis since it allows for the post-synthetic conjugation to various terminal azides by a copper-catalyzed alkyne-azide cycloaddition “click” reaction.
The polyester bis-MPA based anhydride 2 (scheme 2.1) was first prepared by the \textit{N,N’-}
dicyclohexylcarbodiimide (DCC) coupling reaction of the acetonide protected bis-MPA 1. The first generation dendron 4 was prepared by the coupling of propargyl alcohol to anhydride 2 using a standard coupling reaction in presence of 4-(dimethylamino)pyridine (DMAP) as a catalyst (scheme 2.1). The reaction was monitored by thin layer chromatography for the complete disappearance of the spot corresponding to propargyl alcohol. The crude dendron after removal of the catalyst and pyridine was purified by column chromatography to remove byproducts such as residual anhydride and protected bis-MPA. Deprotection of the acetonide groups with H$_2$SO$_4$/MeOH was performed to give the deprotected first generation (G1) dendron 5.

The coupling to anhydride 2 and deprotection was repeated iteratively to provide the second, third and fourth generation dendrons 7, 8 and 9 with four, eight and sixteen available sites for functionalization respectively. The reactions were monitored via $^1$H NMR spectroscopy and thin layer chromatography. It was essential that all the end-groups of the dendron undergo complete functionalization since the removal of incompletely functionalized dendrons was very difficult owing to their similarity in structure and polarity. The purification was performed at the protected dendron stage for each generation to remove unreacted starting materials and byproducts. The dendrons were very stable and could be stored in the deprotected form in the fridge at 4 °C temperature over several months with no change in their respective $^1$H NMR spectra. These hydroxyl terminated dendrons 5, 7, 8 and 9 were previously reported and their characterization data agreed with those previously reported$^{61}$. 
Scheme 2.1: Synthesis of hydroxyl-terminated dendrons of generations 1 through 4

In the second phase of the synthesis process, dendrons 5, 7, 8 and 9 with peripheral hydroxyl groups were further functionalized by introducing peripheral amine moieties. Based on previous work from our group\textsuperscript{72}, it was determined that the addition of a $\beta$-alanine shell would provide the desired amines. The carboxylic acid of $\beta$-alanine was used to functionalize the prepared dendrons. A $t$-butyl carbamate (Boc) group was used to protect the amine group of the amino
acid during the coupling reaction as it would otherwise be a competing nucleophile in the reaction. Boc groups are very common amine protecting groups because of their easy removal by trifluoroacetic acid (TFA). Hence, the peripheral groups were converted to protected amines by reacting them with Boc protected β-alanine 11 using a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling reaction in presence of DMAP to give the protected dendrons 11, 13, 15 and 17 (schemes 2.2-2.5). Previously in our group, this reaction has been done via an anhydride derivative of boc-protected β-alanine\textsuperscript{24} using DCC as the coupling agent. This approach however suffered from the difficulty in removal of the reaction by-products (N-acylurea and dicyclohexylurea) in organic solvents owing to their partial solubility. The byproducts generated by EDC (figure 2.1), however, are water soluble, because of which they are easily removable by acidic and basic water washes without the need of time-consuming chromatography procedures, thus making the synthesis more efficient and increasing yields.

\begin{center}
\includegraphics[width=\textwidth]{Fig2.png}
\end{center}

\textbf{Figure 2.1: Difference in structure of DCC and EDC coupling reagents.}
Scheme 2.2: Synthesis of the amine-terminated generation 1 dendron

Scheme 2.3: Synthesis of the amine-terminated generation 2 dendron
Scheme 2.4: Synthesis of the amine-terminated generation 3 dendron
Scheme 2.5: Synthesis of the amine-terminated generation 4 dendron

The generation-3 boc-protected dendron 15 has been previously reported\textsuperscript{72} and the spectral data of this molecule was consistent with the literature values. The generation 1, 2 and 4 dendrons (11, 13 and 17) are new molecules and have been characterized with \textsuperscript{1}H Nuclear magnetic resonance (NMR) spectroscopy, \textsuperscript{13}C NMR spectroscopy, Infrared (IR) spectroscopy and Matrix-assisted laser desorption/ionization (MALDI) spectrometry.
The quantification of linker groups attached per hydroxyl dendron could be easily done by integrating the respective peaks in the $^1$H NMR spectra. The conversion was found to be quantitative for all the four generations. The major peaks in the following MALDI spectra are consistent with the expected molecular weights of the corresponding dendrons (figure 2.2). The MALDI spectrum of the generation 4 boc-protected dendron 17 did not show any peaks near the theoretical molecular weight, possibly because of the disintegration of the molecule during
ionization. The large number of boc-groups on the surface of the molecule are highly labile, particularly in the presence of acidic MALDI matrices, and hence sensitive to degradation.

These dendrons were then deprotected using TFA in dichloromethane (DCM) to remove the boc group and provide the unprotected dendrons 12, 14, 16 and 18 respectively, which were then ready for conjugation to the desired target surface groups. Dendron 16 has been previously characterized\textsuperscript{72} and the other generations were characterized as described above. For example, figure 2.4 shows a MALDI of the generation 2 dendron 14.

![Figure 2.4: MALDI spectrum of amine functionalized dendron](image)

Calculated: [M + Na\textsuperscript{+}]: 712

\textbf{2.1.2 Synthesis of an Isothiocyanate-Functionalized Mannose Derivative}

As described above, mannose was chosen as the carbohydrate entity of interest for the preparation of the multivalent dendronized polymers. In particular, it was selected as a model biological ligand, as its multivalent binding to targets such as Con A has been extensively
investigated and a number of assays, including the hemagglutination and turbidity assays, have been used to elucidate the relative binding affinities and binding rates of different multivalent mannose ligands. Our group has previously been successful in functionalizing the peripheral groups of third generation amine-terminal polyester dendrons using isothiocyanate derivatives of sugars. Hence, an isothiocyanate functionalized mannose was targeted.

As shown in scheme 2.6, mannose was first globally protected using acetic anhydride and DMAP in pyridine to provide 20 as a 0.8:1 mixture of α and β anomers as determined by $^1$H NMR spectroscopy. In order to introduce a linker, a glycosylation reaction with an alcohol was chosen. 2-Azidoethanol was prepared separately by refluxing sodium azide in 2-bromoethanol for 2 days at 100 °C. Water and ethanol were removed by distillation and it was stored over activated molecular sieves until required. It was then reacted with the protected mannose derivative 20 in the presence of freshly distilled boron trifluoride etherate (BF$_3$·Et$_2$O) as a Lewis acid to selectively glycosylate the sugar from the bottom face through the anchimeric assistance of the C-2 acetate. The reaction was monitored by shifting of the peak corresponding to the anomeric proton in the $^1$H NMR spectrum. The rate of reaction of the two anomers of the starting material was observed to be unequal as the peak corresponding to the β-anomer was slow to disappear. Nevertheless, the reaction mixture was stirred with the addition of extra catalyst until the complete disappearance of the starting material peak.
Scheme 2.6: Synthesis of isothiocyanate-functionalized mannose derivative

The azide derivative 21 was then deacetylated in basic medium using sodium methoxide in methanol to yield the deprotected sugar 22. The azide product was finally reduced using Pd/C and H₂ in methanol to yield the amine 23.

Treating the amine with thiophosgene gave the desired isothiocyanatoethyl-α-D-mannopyranoside 24. In the early synthetic attempts for the conversion of the amine derivative 23 to the isothiocyanate derivative 24, it was noticed that the product conversion was very low. It was concluded that the reaction did not go to completion because of the acidic environment generated by the byproduct HCl and that the conversion of amine to isothiocyanate requires basic medium. A tertiary amine N,N-diisopropylethylamine (DIPEA) that does not hinder the reaction in any way while neutralizing the byproduct was selected. To avoid hydrolyzing the anomeric group of the mannose, the pH was maintained around 8.5-9 for the entire reaction time.
The reactions were primarily monitored via the shifting of the anomeric proton as well as protons corresponding to the side chain of the sugar, by \(^1\)H NMR spectroscopy. The reaction product was lyophilized to remove water; however, no further purification was done to remove the DIPEA-HCl salt and the dry product was taken to the next step directly. This product was further characterized via \(^1\)H NMR spectroscopy and electrospray-ionization (ESI) mass spectrometry to ascertain the presence of the isothiocyanate derivative in the mixture and the proton shifts and the molecular weight was consistent with the expected value. The amine derivative was stored under argon at -4 °C and converted to isothiocyanate as needed given the instability of the isothiocyanate and its tendency to dimerize.

### 2.1.3 Conjugation of Isothiocyanate-Functionalized Mannose to Dendrons

The first through fourth generation dendrons \(12, 14, 16\) and \(18\) were conjugated via an isothiocyanate-amine coupling reaction\(^35\). Excess base DIPEA was added to the reaction mixture in order to deprotonate the amine groups of the dendron as well as to neutralize any trace amount of TFA from the boc deprotection of the dendrons. 2.0 equivalents of the mannose derivative \(24\), per amine group of the dendron, were then added as a solution in dry \(N,N\)-dimethylformamide (DMF) and the reaction was performed in a moisture-free environment for 36 hours.

The first generation dendron \(25\) could not be purified using dialysis owing to the small size of the molecule. Chromatographic techniques were unsuccessful as well due to failure in establishing a solvent system that lead to an appropriate separation between the impurities and the product.

For the 2\(^{nd}\) and 3\(^{rd}\) generation dendrons \(26\) and \(27\), excess starting materials, possible urea dimers of \(24\), as well as DIPEA were removed by dialysis in DMF, using a 1000 g/mol molecular
weight cutoff (MWCO) dialysis membrane for 48 hrs and then in water for 6 hours using the same membrane. The fourth generation dendron 28 was characterized so far using $^1$H NMR and $^{13}$C NMR spectroscopy with further characterization to follow in the future work.

Scheme 2.7: Synthesis of mannose functionalized dendrons of generation 2, 3 and 4
2.1.4 Synthesis of an Alkyne functionalized Mannose Derivative

In order to compare the dendritic architecture on the dendronized polymer with a non-dendritic one, a mannose derivative that could be conjugated directly to the polymer was also synthesized. To achieve this, a glycosylation reaction was done on fully-protected mannose 29 with propargyl alcohol as the nucleophile and BF$_3$·Et$_2$O as the Lewis acid in dry conditions. The resulting products 30 and 31 have been previously reported and were characterized by $^1$H NMR spectroscopy with the data corresponding well with the reported values.

![Scheme 2.8: Synthesis of alkyne functionalized Mannose Derivative](image)

2.1.5 Conjugation of Isothiocyanate-Functionalized Galactose to Dendrons

The polyester dendrons 14, 16 and 18 with peripheral amine groups were functionalized with an α-D-galactose derivative 32, linked to a terminal isothiocyanate group. This derivative was synthesized by Dr. John Trant in the Gillies group as a C-linked derivative of galactose. A carbon-linkage to the sugar makes the glycosylated product highly stable to hydrolysis as compared to an oxygen linkage.
Figure 2.5: C-linked isothiocyanate derivative of α-D-galactose

This galactose derivative was then conjugated to the dendrons via the amine-isothiocyanate reaction described above in presence of an organic base DIPEA. As shown in scheme 2.9, second (33), third (34) and fourth (35) generation glycodendrons were isolated after dialysis.
2.9: Synthesis of galactose functionalized dendrons of generation 2, 3 and 4

Scheme 2.9: Synthesis of galactose functionalized dendrons of generation 2, 3 and 4

2.2 Applications of glycodendrons

These glycodendrons with well-defined multivalent architecture and a multiple carbohydrate groups displayed on the periphery can be further utilized for a variety of applications. Physiologically relevant affinities of lectins are generally reached only when multivalent
interactions are achieved and for this the above-mentioned glycodendrons can potentially be used to study carbohydrate-protein interactions that are implicated in many intercellular events\textsuperscript{77}. These glycodendrons are also hydrophilic in nature owing to multiple hydroxyl groups at their surface and on coupling with a hydrophobic entity (such as a polypeptide), they have the potential to generate interesting self-assembled molecules in appropriate solvents. A few applications of these glycodendrons are listed in the following sections.

2.2.1 Ultra-high multivalency dendronized polymers

In order to greatly increase the number of carbohydrate units displayed on a scaffold, these glycodendrons of different generation were grafted on to a linear polymer backbone to yield ultra-high multivalency dendronized polymers. For this purpose, a linear polycaprolactone (PCL) chain was selected. PCL is a well-known biodegradable polymer that is currently FDA approved for uses in tissue engineering\textsuperscript{78} and drug delivery\textsuperscript{79}. As an aliphatic polyester, this polymer can be hydrolytically and/or enzymatically degraded into easily metabolized products in the body, and hence is anticipated to be a biocompatible choice for biomedical applications\textsuperscript{80}.

2.2.1.1 Synthesis of poly(α-azido-ε-caprolactone)

Since the mannose binding efficiency to the lectin Con A is proposed to increase tremendously with its multivalency, a high molar mass linear polymer backbone was selected as a target. The glycodendrons were visualized to be attached to the polymer chain via an alkyne-azide cycloaddition “click” reaction owing to the high coupling efficiency of the reaction. In order for the dendronized polymer to have a sufficiently large number of functional groups, the polymer molar mass of 10,000 g/mol or more with more than 50 functionalizable pendant azide groups per chain was targeted. To compare the effect of the length of polymer chain on binding affinity,
a polymer with smaller number of repeat units was also used to synthesize a dendronized polymer.

Synthesis of the required monomer followed the previously reported strategy. A commercially obtained α-chlorohexanone was dissolved in DCM and stirred with meta-chloroperoxybenzoic acid (mCPBA) for 96 hours. The residual mCPBA and reaction byproducts were precipitated by cooling the reaction mixture to -20 °C and filtered. The filtrate was further purified by washing it with sodium metasulphite and sodium carbonate solutions to yield the monomer following evaporation of the solvent. The monomer was freshly synthesized, stored for not more than 24 hrs at low temperatures and freshly distilled before use.

![Scheme 2.10: Synthesis of α-chloro-ε-caprolactone monomer](image)

Ring-opening polymerization is the commonly used method to polymerize ε-caprolactone. To avoid chain initiation as well as chain degradation by water, the polymerization was performed under dry conditions. Oven-dried glassware was used and the starting materials were added under a constant flow of argon in a Schlenk flask.
Scheme 2.11: Synthesis of α-azido-caprolactone polymer using methanesulfonic acid as catalyst

PCL with pendant azide group has been previously prepared and reported as a homopolymer as well as copolymer with polycaprolactone using a variety of catalysts and initiators.

In the initial polymerization attempts, ring-opening polymerization with methanesulfonic acid (MSA) as the catalyst and benzyl alcohol as the initiator were selected, as these reaction conditions have previously been successfully applied in our group to prepare PEO-PCL. Acid catalyzed polymerization of ε-caprolactone proceeds by an activated monomer (AM) mechanism in a controlled/living manner. The monomer was freshly distilled over calcium hydride and the initiator over molecular sieves prior to the polymerization. The reaction times were varied from 2.5-48 hrs, and the temperature was varied from 30-60 °C, but the desired length and polydispersity of the polymer could not be obtained, possibly because of the side reactions during the polymerization which led to more than one chain per molecule of the initiator, thereby decreasing the average molar mass and broadening the molar mass distribution. The number average molar masses \(M_n\) obtained by cationic ROP using MSA were consistently between 1000-3000 g/mol, as measured by size exclusion chromatography (SEC) relative to polystyrene standards. The dialysis of polymer in DMF with a 6000-8000 MWCO membrane was useful in removing some of the oligomeric chains and narrowing down the polydispersity in some cases. Therefore, this method allowed for the preparation of a lower molar mass polymer chain (39D in table 2.1) which was deemed to be useful for evaluating the effect of chain length on lectin binding. However, a method was still needed for the preparation of the target higher molar mass backbone.
Table 2.1: Summary of attempts at polymerization using MSA as catalyst

At this stage, ring opening polymerization with tin(II) 2-ethylhexanoate [Sn(Oct)$_2$] as the catalyst was investigated as it has been previously used to make α-substituted ε-caprolactone polymers via ring-opening polymerization$^{91}$ and is a commonly used catalyst for the polymerization of ε-caprolactone$^{88,84,92}$. The product was polymerized via ring opening polymerization with benzyl alcohol as the initiator in dry toluene, as shown in scheme 2.12. As shown in table 2.2, the reaction time was varied to determine the time dependence of the conversion and the influence of transesterification and back-biting degradation. Using benzyl alcohol as initiator and Sn(Oct)$_2$ as catalyst and stirring the reaction mixture for 35 hrs at 120 °C, a polymer with an $M_n$ of 12400
and PDI of 1.5 was obtained (39L from table 2.2), which was deemed to be sufficient for further studies.

![Scheme 2.12: Synthesis of α-azido-caprolactone polymer using Sn(Oct)₂ as catalyst](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>SEC Mₙ</th>
<th>PDI</th>
<th>Temp (°C)</th>
<th>Reaction time (hrs)</th>
<th>Notes</th>
</tr>
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<td>1.5</td>
<td>120</td>
<td>16</td>
<td>Dialyzed 25,000 g/mol MWCO</td>
</tr>
<tr>
<td>39I</td>
<td>5100</td>
<td>1.8</td>
<td>120</td>
<td>48</td>
<td>Degraded (as per NMR)</td>
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<tr>
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<td>3960</td>
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<td>120</td>
<td>24</td>
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<tr>
<td>39K</td>
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<td>120</td>
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<tr>
<td>39L</td>
<td>12430</td>
<td>1.5</td>
<td>120</td>
<td>35</td>
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</tr>
</tbody>
</table>

**Table 2.2: Summary of attempts at polymerization using Sn(Oct)₂ as catalyst**

As shown in scheme 2.12, a nucleophilic substitution reaction was then performed to convert the α-chloro group of the polymer to an α-azido group by stirring the polymer with sodium azide in DMF. The salts were removed from the reaction by centrifugation in toluene to yield the azide functionalized polymer. In order to remove the shorter oligomeric units and narrow the molar mass dispersity of the polymer, DMF dialysis was performed using a 25,000 g/mol MWCO membrane. Since the solubility of the polymer in water was very low, DMF was chosen as a
solvent. It should be noted that the cellulose pores of the dialysis membrane have a tendency to shrink about 3 to 4-fold in DMF and hence at MWCO of 25,000 g/mol would only remove impurities below 6000-8000 g/mol. Dialysis, nevertheless, proved very helpful in decreasing the molecular weight distribution of the polymer to 1.5.

![Image of IR spectra comparison](image)

Figure 2.6: Comparison of IR spectra of polymers 38 and 39
2.2.1.2 Conjugation of glycodendrons to poly(α-chloro-ε-caprolactone)

In the current work, the ‘graft-to’ approach for synthesizing the dendronized polymer was selected. In order to achieve the highest degree of multivalency, the molar mass of the polymer chain was envisioned to be about 10 kg/mol. Based on the target polymer length, as well as our group’s well-established procedures\textsuperscript{35} for the divergent synthesis of dendrons with alkyne focal point moieties, this was anticipated to be the most efficient approach. In addition, the independent synthesis of the dendrons ensures that they are well-characterized and defect-free.

The copper-catalyzed alkyne-azide cycloaddition “click” reaction of azides and terminal alkynes has been established to be a highly efficient reaction for the modification of polymers\textsuperscript{93}. This particular reaction is highly selective and generally produces very high yield in either organic or aqueous media, requiring only mild experimental conditions, and hence was selected for an efficient conjugation of glycodendrons to the polymer backbone.
In order to optimize the reaction conditions for conjugation of glycodendrons to the polymer backbone, a model reaction was run using polymer 39D (from table 2.1 above). This polymer was composed of ~15 azide-functionalized caprolactone and the target was to couple it with the hydroxyl terminated dendron 7 of generation 2. Dendron 7 was chosen because it was available on large scale for trial reactions. It was envisioned that the shifting of the relevant peaks in the $^1$H NMR spectrum could be a fairly reliable estimate of the reaction completion. It was also anticipated that IR spectroscopy would provide semi-quantitative information due to the strong azide stretch at 2108 cm$^{-1}$ which should disappear upon its reaction with the alkyne.

Following a previously established procedure for such copper-catalyzed alkyne-azide cycloaddition “click” coupling reactions$^{94,72}$, 1.1 equivalents of dendron, 2.2 equivalents of copper sulfate and 4.4 equivalents of sodium ascorbate were used per azide group. Careful choice of the solvent system was required taking into account the difference of solubility of the dendron and the polymer in water, the dendron showing significantly more hydrophilicity in aqueous systems compared to the polymer which was essentially hydrophobic. In addition to that, the poor solubility and hence decreased activity of the catalysts in organic solvents necessitated a mixture of water and organic solvent as an ideal solvent system to the reaction. In this regard, a mixture of DMF and water (9:1 ratio) was chosen and the reaction mixture was stirred at 40 °C for 48 hours.
A $^1$H NMR spectrum obtained after removal of residual copper and other reagents such as excess dendron by ethylenediaminetetraacetic acid disodium salt (Na$_2$EDTA) dialysis at pH=7, followed by pure water dialysis, showed clear conversion of azide units to 1,2,3-triazole units with the attachment of dendrons through shifting of the alkyne focal point peak from 2.96 to a triazole proton peak at 8.22 (figure 2.8) and in addition, IR spectroscopy showed complete disappearance of the azide peak of the polymer after conjugation with the dendron.
Figure 2.8: $^1$H NMR spectrum of a) 7 (in CD$_3$OD), b) 39 (in CDCl$_3$), c) 40 (in CD$_3$SOCD$_3$) at 400MHz frequency

Hence, this reaction system was chosen for the conjugation of mannose-terminated dendrons 26 and 27 to the polycaprolactone backbone and this procedure was then used to prepare the target dendronized polymers of generation 2 and 3 using two polymers 39D and 39L from tables 2.1 and 2.2 above. To compare the effect of the absence of dendritic architecture on the dendronized
polymers, alkyne functionalized mannose 31 was also coupled to the polymers. 1.1 equivalents of the glycodendron (or 31) to 1 equivalent of azide on the polymer were added to a solution in DMF:water (9:1) and the reaction mixture was stirred at 40 °C for 48 hrs in all the three reactions. EDTA is a very good copper-binding ligand and the resulting complex is easily purifyable using dialysis. The resulting products were purified by dialysis against a 25,000 g/mol (6000-8000 g/mol for 31) MWCO membrane in water with EDTA to remove the residual copper from the solution, followed by dialysis in pure water to remove the EDTA.

The resulting products were characterized via infrared spectroscopy with further characterization by 1H NMR spectroscopy and SEC to be undertaken as part of future work. The IR spectrum of the generation 3 dendronized polymer 43 clearly showed the reduction in the azide peak intensity, indicating the dendron functionalization to the polymer to a good extent.
Scheme 2.14: Synthesis of dendronized polymers (D=from polymer 39D; L=from polymer 39L)
2.2.2 Linear-Dendron Hybrids

Linear-dendron hybrids are block copolymers with a dendritic unit coupled to a linear polymer. If the copolymer is amphiphilic, containing hydrophobic and hydrophilic segments, it can self-assemble into various nano-sized structures. A polypeptide PBLG conjugated with the glycodendrons 33, 34 and 35 displaying α-galactose units on the periphery was chosen to study their self-assembly behavior as well as the protein binding properties of the conjugated α-galactose.
Scheme 2.15: Synthesis of linear dendron hybrid (Reproduced from a presentation by Justine Ades, University of Bordeaux)

In this collaborative work, done with the Lecommandoux group in the University of Bordeaux, France, the dendritic entities with α-galactose on the periphery were prepared by our lab with the aim of coupling them to PBLG prepared in the Lecommandoux group. PBLG is useful in such studies, being a synthetic biomimetic polymer. It has been shown that the helical conformation of a hydrophobic PBLG is an efficient way to form and stabilize vesicles\textsuperscript{95}. Block copolymers consisting of PBLG and conjugated with β-galactose on the surface have been used to form biomimetic polymersomes\textsuperscript{96}. It is anticipated that the competing effects of dendron architecture favoring the formation of micellar assemblies and peptide rigidity favoring lamellar/vesicular morphologies will lead to interesting fundamental insights into polymer assembly with potential applications arising from the carbohydrate moieties.
2.2.2.1 Synthesis of Linear-Dendron Hybrids

Studies have been conducted in our collaborator’s laboratory towards the synthesis and self-assembly of the proposed linear-dendron hybrids. Linear PBLG with a terminal azide group and varying degrees of polymerization (table 2.3) were synthesized by the ring opening polymerization of γ-benzyl-L-glutamate N-carboxyanhydride monomer with a 1-azido-3-aminopropane initiator. Hydroxyl-terminated dendron 8 was used as a model dendron and was conjugated with azide-terminated PBLG under copper-catalyzed alkyne-azide cycloaddition “click” reaction conditions (scheme 2.15) in dimethyl sulfoxide (DMSO) using copper sulphate and sodium ascorbate as reagents. The success of this reaction was confirmed by SEC, NMR, and IR spectroscopy, so these conditions were subsequently applied to galactose dendrons of generation 2, 3 and 4 (33, 34 and 35 respectively) to prepare a small library of synthetic glycopolypeptides (table 2.3).

<table>
<thead>
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<th>Glycodendron</th>
<th>Structure</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>G2-galactose 33</td>
<td>1680</td>
</tr>
<tr>
<td>G3</td>
<td>G3-galactose 34</td>
<td>3430</td>
</tr>
<tr>
<td>G4</td>
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<td>6920</td>
</tr>
<tr>
<td>G3b</td>
<td>G3-OH 8</td>
<td>870</td>
</tr>
</tbody>
</table>

Table 2.3: Molecular characteristics of α-functionalized building blocks for click-chemistry

2.2.2.2 Self-Assembly of Linear-Dendron Hybrids

The self-assembly behavior of these linear-dendritic glycopolypeptides as well as the model compound prepared from the hydroxyl terminated dendron 8 was studied by using the
nanoprecipitation method. This involved either the rapid injection of a DMSO solution of the polymer into pure water, or the use of a syringe pump for the controlled addition of water into the DMSO solution of polymer (figure 2.10)

Figure 2.10: Nanoprecipitation method for self-assembly of linear-dendron hybrids

On injecting the solution of glycopolypeptide P28-G4 in DMSO into water, aggregates of hydrodynamic diameter of 24 nm with a relatively low polydispersity (PDI=0.25) were obtained. Transmission electron microscopy (TEM) images (figure 2.11) were consistent with the dynamic light scattering (DLS) results and showed micellar morphologies.

Figure 2.11: TEM images. Fast addition of DMSO into water of (A) P28-G4, unstained
Turbid solutions of macroscopic aggregates were obtained with the rapid injection of DMSO into water for P14-G2, P28-G2, P28-G3 and P54-G3. Thus, only the fourth generation galactose-functionalized dendron was sufficient to stabilize assemblies in water. It is suggested that the carbohydrates are strongly hydrated in solution and as a result, they induce a curvature that disfavors lamellar packing that would otherwise be favored based on the hydrophilic mass ratio. Interestingly, P54-G3b exhibiting the lowest hydrophilic weight fractions of 0.07 was able to assemble as nano-objects in solution, probably because it is less densely packed and can therefore assemble more easily. An increase in size was observed with the decrease of the flow rate when injecting water into the DMSO solution of the glycopolypeptides (while keeping a relatively low dispersity). TEM confirmed the sizes obtained by DLS. As shown in figure 2.12 (a), (b) and (c), P54-G3b self-assemble as spherical objects with increasing sizes depending on the flow rate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fast</th>
<th>64 mL/hr</th>
<th>8 mL/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>0.14</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 2.4: DLS measurement data of self-assembly of P54-G3b

(Dh = hydrodynamic diameter), (PDI= Polydispersity index)
Figure 2.12: TEM images, addition of water into the DMSO solution of P54-G3b: (a) fast addition, uranyl stained, (b) 64 mL/hr, and (c) 8 mL/hr.

2.2.2.3 Lectin binding studies

Lectin binding studies are currently underway. Preliminary results suggest that the nanoparticles formed by the assembly of P28-G4 can precipitate isolectin B₄ from *Griffonia simplicifolia* at a concentration where dendron G4 or control particles formed from P54G3b do not. *Griffonia simplicifolia* (BS1-B4) is a tetrameric legum lectin that specifically binds α-galactose residues (contrary to most lectins that lack anomeric specificity). This suggests a benefit in terms of
binding affinity arising from the high multivalency of these nanoparticles. However, in a hemagglutination assay it was found that all of these assemblies actually agglutinate red blood cells. The reason for this effect is not clear at this time, but it prevents the use of this valuable assay, which would otherwise enable the measurement of relative binding affinities.
Chapter 3

3 Conclusions and Future Work

3.1 Conclusions

In conclusion, amine functionalized dendrons of generations 1, 2, 3 and 4 were synthesized via DCC and EDC coupling reactions of hydroxyl groups to the carboxylic acid groups on Boc-protected-β alanine. All but the generation 3 system were new molecules and were characterized using $^1$H NMR, $^{13}$C NMR, IR spectroscopy and MALDI spectrometry. They are expected to serve as useful scaffolds for further chemistry both in this thesis and in the broader area of dendrimer bioconjugates. Isothiocyanate functionalized mannose was synthesized via a glycosylation reaction on a mixture of fully protected α and β anomers of mannose to yield (2,3,4,6-tetraacetyl-(2-azidoethyl))-α-D-mannopyranoside, which was then used to synthesize (2-isothioethyl)-α-D-mannopyranoside by an improved procedure. The dendrons of generation 1-4 were then coupled to the isothiocyanate functionalized carbohydrate to yield the respective glycodendrons. The glycodendrons of generation 2 and 3 were fully characterized by $^1$H NMR, $^{13}$C NMR and IR spectroscopy.

For the preparation of glyco-dendronized polymers, azide functionalized caprolactone homopolymers of two different degrees of polymerizations were synthesized using benzyl alcohol as initiator and MSA and Sn(Oct)$_2$ as catalysts respectively. The Sn(Oct)$_2$ catalyst was found to afford better results in terms of degree of polymerization. An alkyne-functionalized mannose was synthesized to couple the sugar to the polymer backbone to study the effect of dendritic structure on binding affinities of dendronized polymer. This mannose derivative, as well as the glycodendrons of generation 2 and 3, were conjugated to the polymers via copper-
catalyzed alkyne-azide alkyne-azide cycloaddition “click” reaction to yield a small library of dendronized polymers. IR spectroscopy revealed a significant extent of dendron conjugation to the polymer.

These amine functionalized dendrons were also coupled to a C-linked isothiocyanate derivative of galactose synthesized by Dr. John Trant in the Gillies group and galactose terminated glycodendrons of generation 2 to 4 were isolated and characterized. These glycodendrons as well as hydroxyl terminated dendron of generation 3 were further coupled with polypeptides PBLG of different degrees of polymerization in the Lecommandoux group in the University of Bordeaux, France to generate a small library of linear dendron hybrids. Self-assembly behavior of these hybrids was studied in the Lecommandoux group and aggregates of hydrodynamic diameter of 24 nm with a relatively low polydispersity (PDI=0.25) were obtained for generation 4 dendron coupled with PBLG with 28 repeat units. Hybrids of PBLG 54 and hydroxyl terminated generation 3 dendron self-assemble as spherical objects with increasing sizes depending on the flow rate. These aggregates were studied using light scattering and the size profile was consistent with the values measured via TEM.

3.2 Future Work

The future work will involve the further characterization of dendronized polymers with $^1$H NMR, $^{13}$C NMR and SEC in aqueous media as well as calculation of their conjugation yields. In addition, complete characterization of generation 4 mannose glycodendron will be done following which its corresponding dendronized polymers will be synthesized with different degrees of polymerization of the polymer backbone.
Lectin-binding properties of these materials will be evaluated using a variety of biological assays including the hemagglutination assay for mannose-terminal dendronized polymers, and compared to the binding properties of the non-scaffolded saccharide molecules.

In addition, the hydrophilicity of the linear dendron hybrids will be tuned by the preparation of triblock systems incorporating PBLG, poly(ethylene oxide) and the dendron, with the aim of preventing the aggregation problems that were observed for many of the linear-dendron hybrids. Further lectin binding and biological studies of the new self-assembled aggregates will follow.
Chapter 4

4 Experimental

4.1 General procedures and materials

Dendrons 5, 7, 8 and 9 were prepared as previously reported in the literature. All the reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Anhydrous DMF and toluene was obtained from a solvent purification system. Triethylamine (Et₃N), N,N-Diisopropylethylamine (DIPEA) and dichloromethane (CH₂Cl₂) were distilled from calcium hydride (CaH₂). Moisture-sensitive reactions were performed under an argon atmosphere using flame or oven-dried glassware. Column chromatography was performed using silica gel (0.063-0.200 mm particle size, 70-230 mesh). ¹H NMR spectra were obtained at 400 MHz or 600 MHz and ¹³C NMR spectra were obtained at 100 or 150 MHz using a Varian Mercury or Varian Inova spectrometer. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signal of CDCl₃ (δ7.27, 77.2), CD₃OD (δ3.31, 49.15) or CD₃SOCD₃ (δ2.50, 39.52). Infrared spectra were obtained either as films from methanol/THF, DCM or methanol on sodium chloride (NaCl) plates, or via attenuated total reflection by direct placement of the solid-state sample on a KBr crystal. Dialysis of the dendrons was performed using Spectra/Por regenerated cellulose membranes with various MWCO. Freeze-drying was carried out using a Labconco FreeZone 2.5 plus lyophilizer. SEC was performed in DMF using a Waters 2695 separations module equipped with a 2414 differential refractometer and two PLgel 5 μm mixed-D (300 mm × 7.5 mm) columns from Polymer Laboratories. Polymer MWs were calculated using the Wyatt Astra software, with dn/dc values of the polymers determined from the refractive index (RI) detector using Astra. MALDI-TOF (time of flight) mass spectrometry
data were obtained using a 4700 Proteomics Analyzer, MALDI TOF (Applied Biosystems, Foster City, CA, USA). 2-(4'-hydroxybenzeneazo)benzoic acid (HABA) was used as the matrix for the measurements. Reflectron and linear positive ion modes were used. All monomers, initiators and catalysts were dried by azeotropic distillation (×3) with dry toluene using a Schlenk line system under argon. Centrifuge VMR clinical 200

4.2 Experimental Section

Synthesis of dendron 11: Dendron 5 (2.0 g, 11.56 mmol, 1 equiv.) and boc-protected β-alanine (8.75 g, 46.24 mmol, 4 equiv.), were dissolved in distilled pyridine (4.6 mL, 5 equiv.). EDC.HCl (7.18 g, 46.24 mmol, 4 equiv.) and DMAP (5.64 g, 69.36 mmol, 4 equiv.) were added to the mixture. 40 mL of anhydrous CH$_2$Cl$_2$ was added to dissolve all the reagents and starting materials. The mixture was stirred at room temperature for 36 hours. The solution was then diluted with 100 mL of CH$_2$Cl$_2$, and washed with H$_2$O (1x 50 mL), 1M HCl (3x 50 mL), 1M Na$_2$CO$_3$ (2x 50 mL), and brine (1x 50 mL). The organic phase was isolated and dried with MgSO$_4$, filtered and solvent removed in-vacuo. It was further purified by column chromatography (2:1 hexanes/ethyl acetate) to give 4.9 g (82%) of 11. $^1$H NMR (CDCl$_3$): δ 5.19 (br s, 2H), 4.75 (d, 2H, J=2.6 Hz), 4.31- 4.21 (m, 4H), 3.37 (m, 4H), 2.53 (t, 4H, J=6.2 Hz), 1.42 (s, 18H), 1.27 (s, 3H). $^{13}$C NMR (CDCl$_3$): δ: 171.76, 155.62, 79.16, 76.92, 75.32, 64.89, 53.31, 52.52, 46.24, 35.91, 34.32, 28.19, 17.50. MALDI-TOF molar mass: 537.2. Calculated [M+Na$^+$]: 537.5

Synthesis of dendron 13: Dendron 7 (4.25 g, 10.52 mmol, 1 equiv.) and boc-protected β-alanine (15.90 g, 84.16 mmol, 8 equiv.), were dissolved in distilled pyridine (8.4 mL, 10 equiv.). EDC.HCl (13.06 g, 84.16 mmol, 8 equiv.) and DMAP (10.27 g, 84.16 mmol, 8 equiv.) were
added. 50 mL of anhydrous CH₂Cl₂ was added to dissolve all the reagents and starting materials. The mixture was stirred at room temperature for 36 hours. The solution was then diluted with 100 mL of CH₂Cl₂, and washed with H₂O (1x 50 mL), 1M HCl (3x 50 mL), 1M Na₂CO₃ (2x 50 mL), and brine (1x 50 mL). The organic phase was isolated and dried with MgSO₄, filtered and solvent removed in vacuo. It was further purified by column chromatography (1:5 hexanes/ethyl acetate) to give 9.1 g (80%) of 13. ¹H NMR(CDCl₃): δ 5.18 (br, 1H), 4.74 (d, 2H, J=2.3 Hz), 4.31-4.10 (m, 12H), 3.33 (d, 8H, J=5.8 Hz), 2.52-2.48 (t, 8H, J=6.6 Hz), 1.38 (s, 36H), 1.24 (s, 3H). ¹³C NMR (CDCl₃): δ: 171.85, 171.30, 155.66, 79.17, 76.90, 75.63, 65.49, 64.78, 60.21, 52.75, 46.52, 46.34, 35.96, 34.29, 33.80, 28.24, 20.87, 17.68, 17.35. MALDI-TOF molar mass: 1111.5. Calculated [M+Na⁺]: 1111.2

Synthesis of dendron 15: Dendron 8 (0.16 g, 0.19 mmol, 1 equiv.) and boc-protected β-alanine (0.56 g, 2.96 mmol, 16 equiv.), were dissolved in distilled pyridine (0.3 mL, 20 equiv.). EDC.HCl (0.46 g, 2.96 mmol, 16 equiv.) and DMAP (0.36 g, 2.96 mmol, 16 equiv.) were added. 10 mL of anhydrous CH₂Cl₂ was added to dissolve all the reagents and starting materials. The mixture was stirred at room temperature for 36 hours. The solution was then diluted with 15 mL of CH₂Cl₂, and washed with H₂O (1x 15 mL), 1M HCl (3x 15 mL), 1M Na₂CO₃ (2x 15 mL), and brine (1x 15 mL). The organic phase was isolated and dried with MgSO₄, filtered and solvent removed in vacuo. It was further purified by column chromatography (1:1 hexanes/ethyl acetate) to give 0.36 g (85%) of 15. Spectral data were in accordance with literature values.³⁵

Synthesis of dendron 17: Dendron 9 (0.5 g, 0.25 mmol, 1 equiv.) and boc-protected β-alanine (1.50 g, 8.18 mmol, 32 equiv.), were dissolved in distilled pyridine (8.4 mL, 40 equiv.). EDC.HCl (1.27 g, 8.18 mmol, 32 equiv.) and DMAP (1.0 g, 8.18 mmol, 32 equiv.) were added. 20 mL of anhydrous CH₂Cl₂ was added to dissolve all the reagents and starting materials. The
mixture was stirred at room temperature for 36 hours. The solution was then diluted with 20 mL of CH\(_2\)Cl\(_2\), and washed with H\(_2\)O (1x 20 mL), 1M HCl (3x 20 mL), 1M Na\(_2\)CO\(_3\) (2x 20 mL), and brine (1x 20 mL). The organic phase was isolated and dried with MgSO\(_4\), filtered and solvent removed in-vacuo. It was further purified by column chromatography (1:1 hexanes/ethyl acetate to remove by products and starting materials, pure ethyl acetate to extract product) to give 0.87 g (76\%) of x. \(^1\)H NMR (CDCl\(_3\)): δ 5.27 (br, 1H), 4.73 (d, 2H, J=2.3 Hz), 4.27-4.16 (m, 60H), 3.36 (d, 32H, J=5.8 Hz), 2.53 (t, 32H, J=6.6 Hz), 1.42 (s, 144H), 1.33 (s, 3H), 1.27 (s, 6H), 1.26 (s, 12H), 1.25 (s, 24H). \(^{13}\)C NMR: δ: 171.56, 171.37, 155.41, 79.26, 77. 21, 64.87, 53.51, 46.70, 36.12, 34.42,28.38, 17.79, 17.50

**Synthesis of dendron 12:** Dendron 11 (0.25g, 0.48mmol) was dissolved in 1mL of 1:1 TFA/CH\(_2\)Cl\(_2\) and the solution was stirred at room temperature for 1 hour. The solvent was removed in vacuo to provide 98% of product as light brownish viscous oil and stored at -4°C. \(^1\)H NMR (CD\(_3\)OD): δ: 4.76 (d, 2H, J=2.3 Hz), 4.34 (m, 4H), 3.21 (t, 4H, J=6.7 Hz), 3.02 (t, 1H, J=2.5 Hz), 2.76 (t, 4H, J=6.6 Hz), 1.31 (s, 3H). \(^{13}\)C NMR (CD\(_3\)OD): δ 173.47, 173.47, 171.82, 78.46, 76.74, 67.05, 53.79, 47.77, 36.41, 32.18, 17.94. 171.82, 78.46, 76.74, 67.05, 53.79, 47.77, 36.41, 32.18, 17.94. MALDI-TOF molar mass found: 337.1. Calculated [M+Na\(^+\)]: 337.1

**Synthesis of dendron 14:** Dendron 13 (0.25g, 0.23mmol) was dissolved in 1mL of 1:1 TFA/CH\(_2\)Cl\(_2\) and the solution was stirred at room temperature for 2 hours. The solvent was removed in vacuo to provide 98% of product as light brownish viscous oil and stored at -4°C. \(^1\)H NMR (CD\(_3\)OD) δ: 4.79 (d, 2H, J=2.3 Hz), 4.35-4.26 (m, 12H), 3.22 (t, 8H, J=6.4 Hz), 3.02 (t, 1H, J=2.3 Hz), 2.79 (t, 8H, J=7.0 Hz), 1.33 (s, 3H), 1.29 (s, 6H) \(^{13}\)C NMR (CD\(_3\)OD): δ: 173.63, 171.91, 78.59, 77.20, 67.06, 53.96, 47.83, 39.50, 36.40, 34.84, 32.24, 26.84, 26.18, 18.13. MALDI: MALDI-TOF molar mass found: 711.3. Calculated [M+Na\(^+\)]: 711.3
Synthesis of dendron 16: Dendron 15 (0.1g, 0.04 mmol) was dissolved in 1mL of 1:1 TFA/CH₂Cl₂ and the solution was stirred at room temperature for 2.5 hours. The solvent was removed in vacuo to provide 98% of product as light brownish viscous oil and stored at -4°C. Spectral data agreed with those previously reported.³⁵

Synthesis of dendron 18: Dendron 17 (0.25g, 0.05 mmol) was dissolved in 1mL of 1:1 TFA/CH₂Cl₂ and the solution was stirred at room temperature for 4 hours. The solvent was removed in vacuo to provide 94% of product as light brownish viscous oil and stored at -4°C. ¹H NMR (CD₃OD) δ: 4.80 (d, 2H, J=2.3 Hz), 4.35-4.28 (m, 60H), 3.25 (t, 32H, J=6.4 Hz), 2.85 (t, 1H, J=2.3 Hz), 2.82 (t, 32H, J=7.0 Hz), 1.37 (s, 3H), 1.34 (s, 6H), 1.31 (s, 12H), 1.29 (s, 24H). MALDI: Found (MALDI-TOF): 2955.8. Calculated [M+S->O+K⁺]: 2956.1

Synthesis of azidoethanol: 2-bromoethanol (25 g, 200 mmol, 1.0 equiv.) and sodium azide (18.2 g, 280 mmol, 1.4 equiv.) were refluxed at 100 °C for 48 hours in a solvent-free reaction. The reaction mixture was then diluted with diethyl ether and filtered under vacuum to remove sodium azide. Diethylether was removed by evaporation. Any residual ethanol was removed by evaporation under vacuum to yield the product as light yellow oil (14.9 g, 86 %), which was stored under molecular sieves until further use. Spectral data agreed with those previously reported.³⁵

Synthesis of 21 (2,3,4,6-tetraacetyl-(2-azidoethyl))- α-D-mannopyranoside: Under a nitrogen atmosphere, α, β-D-mannose pentaacetate, (3.7 g, 9.4 mmol, 1 equiv.) and azidoethanol (1.2 g, 14 mmol, 1.5 equiv.) were dissolved in anhydrous CH₂Cl₂ (12 mL) and the solution was cooled to 0°C. BF₃·OEt₂ (5.8 mL, 47 mmol, 5 equiv.) was added dropwise and the resulting solution was stirred at 0°C for one hr and then at room temperature for an additional 24 hrs. The solution
was monitored by 1H NMR for the disappearance of the peak at 6.10 ppm (anomeric peak for 1,2,3,4,6-pentaacetyl-α-D-mannopyranoside). An additional 2.5 mL BF₃·OEt₂ was added to further catalyze the reaction. After 12 hours the reaction solution was diluted with 20 mL of chloroform, washed with H₂O (3x 15 mL), and 1M aqueous NaHCO₃ (3x 15 mL) to give 2.7 g of 21 (69.5%). Spectral data agreed with those previously reported³⁵.

**Synthesis of 22 (2-azidoethyl)-α-D-mannopyranoside:** 21 (2.7 g, 6.6 mmol) was stirred with 6 mL of NaOMe/MeOH solution in 10 mL of methanol (pH 7.6-8.0) at room temperature for two hours. The solution was then neutralized with Dowex (H+), filtered and concentrated under reduced pressure. The product, 22, was isolated as an oil (1.5 g, 91%). Spectral data agreed with those previously reported³⁵.

**Synthesis of 23 (2-aminoethyl)-α-D-mannopyranoside:** 22 (1.0 g, 4.0 mmol) was dissolved in 25 mL of MeOH, flask was degassed thrice under vacuum to remove oxygen and argon was introduced into the flask. 150 mg of 10% Pd/C catalyst was added. The atmosphere was exchanged for H₂ gas and the solution was stirred vigorously at room temperature. After 6 hours, the reaction was complete and the mixture was diluted with 1 mL of H₂O (for amine solubility), filtered over celite and concentrated in vacuo to yield the product, 23 (0.80 g, 90%). Spectral data agreed with those previously reported³⁵.

**Synthesis of 24 (2-isothioethyl)-α-D-mannopyranoside:** 23 (50 mg, 0.22 mmol) was dissolved in 5 mL 80% EtOH in water and DIPEA (1.12 mmol, 195µl) was added. Thiophosgene (0.52 mmol, 40µl) was added and the reaction was stirred at room temperature for 2 hours. The solution was maintained at basic pH throughout the reaction time. Ethanol was removed by rotavap and the remaining solution was freeze dried using liquid nitrogen and a lyophilizer.
overnight to remove water. The product was used without further purification. Spectral data agreed with those previously reported\textsuperscript{35}.

**Synthesis of glycodendron 26:** 14 (0.96 g, 0.84 mmol, 1 equiv.) was dissolved in dry DMF (10 mL) and DIPEA (6 mL) was added. 24 (2.36 g, 8.4 mmol, 10 equiv.) was added as a solution in dry DMF (20 mL). The reaction mixture was stirred under argon at room temperature for 64 hours. The solvent was removed in vacuo and the crude product was dissolved in fresh DMF (5 mL) and dialyzed in DMF (500 mL) using a 1000 MWCO membrane for 48 hours and in pure water for 6 hours. The water was removed by freeze drying and product (300 mg, 20.6%) was isolated as a hygroscopic, brown powder. $^1$H NMR (CD$_3$SOCD$_3$) $\delta$: 4.98 (br, 2H), 4.73 (br, 2H), 4.71 (br, 4H), 4.62 (br, 4H), 4.55 (d, 3H, J=4.7 Hz), 4.44 (t, 4H, J=5.8 Hz), 4.11-4.24 (m, 15H), 3.66-3.56 (m,17H), 3.51 (br,4H), 3.49-3.43 (m, 10H), 3.41-3.37 (m,4H), 2.61 (t,9H, J=5.2 Hz), 1.17 (s,6H), 1.14 (s,3H). $^{13}$C NMR (CD$_3$SOCD$_3$) $\delta$:179.18, 178.57, 107.42, 85.37, 81.41, 78.37, 77.68, 74.39, 72.56, 68.64 53.51,50.51, 50.61, 43.23, 40.77, 24.64, 24.41, 18.13.

**Synthesis of glycodendron 27:** 16 (0.95 g, 0.40 mmol, 1 equiv.) was dissolved in dry DMF (10 mL) and DIPEA (6 mL) was added. 24 (2.35 g, 8.8 mmol, 22 equiv.) was added as a solution in dry DMF (20 mL). The reaction mixture was stirred under argon at room temperature for 64 hours. The solvent was removed in vacuo and the crude product was dissolved in fresh DMF (5 mL) and dialyzed in DMF (500 mL) using a 1000 MWCO membrane for 48 hours and in pure water for 6 hours. The water was removed by freeze drying and product (350 mg, 24.5%) was isolated as a hygroscopic, brown powder. Spectral data was found to be in accordance with the literature value\textsuperscript{35}.  

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Synthesis of glycodendron 28: 18 (0.95 g, 0.40 mmol, 1 equiv.) was dissolved in dry DMF (10 mL) and DIPEA (6 mL) was added. 24 (2.35 g, 8.8 mmol, 22 equiv.) was added as a solution in dry DMF (20 mL). The reaction mixture was stirred under argon at room temperature for 64 hours. The solvent was removed in vacuo and the crude product was dissolved in fresh DMF (5 mL) and dialyzed in DMF (500 mL) using a 1000 MWCO membrane for 48 hours and in pure water for 6 hours. The water by removed by freeze drying and product (350 mg, 24.5%) was isolated as a hygroscopic, brown powder. Further characterization to follow in future work.

Synthesis of alkyne functionalized mannose 30: Under a nitrogen atmosphere, α, β-D-mannose pentaacetate, (5 g, 12.88 mmol, 1 equiv.) and propargyl alcohol (1.08 g, 19.33 mmol, 1.5 equiv.) were dissolved in anhydrous CH₂Cl₂(30 mL) and the solution was cooled to 0˚C. BF₃·OEt₂ (6.4 mL, 64.4 mmol, 5 equiv.) was added dropwise and the resulting solution was stirred at 0˚C for one hour and then at room temperature for an additional 48 hours. The reaction solution was diluted with 50mL of chloroform, washed with H₂O (3x 50 mL) and 1M aqueous NaHCO₃ (3x 50 mL) to give 3.6 g of 30 (73%). Spectral data agreed with those previously reported⁷⁶.

Synthesis of alkyne functionalized mannose 31: 30 (5.7 g, 14.76 mmol) was stirred with 4 mL of NaOMe/MeOH solution in 40 mL of methanol (pH 7.6-8.0) at room temperature for 3 hours. The solution was then neutralized with Dowex (H+), filtered and concentrated under reduced pressure. The product, 31, was isolated as solid crystals (3.06 g, 94%). Spectral data agreed with those previously reported⁷⁶.

Synthesis of α-chloro-ε-caprolactone 37: Commercially obtained chlorocyclohexanone (10 gm, 75 mmol, 1 equiv.) was dissolved in dry DCM (150 mL). mCPBA (70%, 24 gm, 98 mmol, 1.3 equiv.) was added to the reaction flask. The reaction was run at room temperature for 96 hours
and monitored using NMR spectroscopy for the disappearance of starting material peaks. The solution was then cooled to -20°C and to precipitate residual mCPBA, which was filtered off. The product diluted with 150 mL of CH₂Cl₂ was then washed with saturated Na₂S₂O₅ (3x 100 mL), 1M Na₂CO₃ (3x 100 mL) and brine (1x 100 mL). The organic phase was isolated and dried with MgSO₄, filtered and solvent removed in-vacuo, and was used the same day. The spectral data was found to be in accordance with the literature values.³¹

**Synthesis of poly(α-azido-ε-caprolactone) 39D:** The monomer 37 freshly distilled over CaH₂ (6.18 g, 41.75 mmol, 90 equiv.) and initiator benzyl alcohol freshly distilled on molecular sieves (50 mg, 0.4 mmol, 1 equiv.) were added to a flame dried Schlenk flask under argon through a Schlenk line with 1 mL of dry toluene. The reaction mixture was equilibrated at 30°C for 10 min. The catalyst methanesulfonic acid (44 mg, 0.4 mmol, 1 equiv.) was added and the reaction mixture was stirred at 30°C for 2.5 hours. The polymer was then dissolved in chloroform and precipitated in cold hexanes to give 38 as a light yellow oil. This polymer 38 was then dissolved in DMF (2 ml) and sodium azide (3.5 g, 53.84 mmol, 1.2 equiv.) was added. The reaction mixture was stirred at room temperature for 24 hours. The solvent was removed by distillation under vacuum and the crude polymer was dissolved in toluene (10 mL) and centrifuged for 15 min at 6000 rpm thrice, each time with addition of fresh toluene. The toluene portions were collected and the solvent was removed in-vacuo to yield the crude product. This product was dissolved in 2 mL DMF and dialyzed in 200 mL DMF for 48 hours using a 6000-8000 g/mol MWCO. The solvent was removed in vacuo to yield polymer 39D. Spectral data agreed with those previously reported.³¹

**Synthesis of poly(α-azido-ε-caprolactone) 39L:** The monomer 37 (1.8 gm, 12.16 mmol, 90 equiv.), initiator benzyl alcohol (14 mg, 0.1 mmol, 1 equiv.) and catalyst Sn(Oct)₂ (as a 10
mg/ml solution in dry toluene) (0.14 mg, 0.00033 mmol, 1/400 equiv.) were added to a flame dried Schlenk flask under argon through a Schlenk line and azeotropically dried under vacuum thrice by addition and evaporation of toluene (1 ml) in the flask. The reaction temperature was increased to 110°C and the reaction mixture was stirred for 96 hours under dry conditions. The polymer was then cooled to room temperature, dissolved in chloroform and precipitated in cold hexanes to yield polymer 38 a brown paste. This polymer was dissolved in DMF (7 mL) and sodium azide (0.7 g, 11.35 mmol, 1.2 equiv.) was added. The reaction mixture was stirred at room temperature for 24 hours. The solvent was removed by distillation under vacuum and the crude polymer was dissolved in toluene (10 mL) and centrifuged for 15 min at 6000 rpm thrice, each time with addition of fresh toluene. The toluene portions were collected and the solvent was removed in-vacuo to yield the crude product. This product was dissolved in 2 mL DMF and dialyzed in 200 mL DMF for 48 hrs using a 25000 g/mol MWCO. The solvent was removed in vacuo to yield polymer 39L. Spectral data agreed with those previously reported81.

Synthesis of denpol 40: 39D (53 mg, 0.02 mmol, 1 equiv.) and 7 (150 mg, 0.37 mmol, 16.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (0.3 g, 1.5 mmol, 66 equiv.) was added as a solid powder. Copper sulfate (118 mg, 0.74 mmol, 33 equiv.) was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (66 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L, pH=7, 25000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 40. 1H NMR (CD3SOCD3) δ: 8.22 (br, 1H), 5.48 (br, 1H), 5.19 (br, 2H), 4.63 (br, 2H), 4.12 (br, 8H), 3.44 (br, 6H), 2.15 (br, 2H), 1.54 (br, 3H), 1.17 (s, 3H), 0.98 (s, 6H)
Synthesis of denpol 41L: 39L (40 mg, 0.0031 mmol, 1 equiv.) and 7 (63 mg, 0.29 mmol, 93.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (231 mg, 1.17 mmol, 374 equiv.) was added as a solid powder. Copper sulfate (93 mg, 0.58 mmol, 187 equiv.) was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (374 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L pH=7, 6000-8000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 41L. \(^1\)H NMR (D\(_2\)O) δ: 8.14 (br, 1H), 5.39 (br, 1H), 4.88 (br, 2H), 4.08 (br, 2H), 3.93 (br, 5H), 3.70 (br, 3H), 2.16 (s, 2H), 1.54 (br, 2H), 1.10 (br, 2H). Further characterization to follow in future work.

Synthesis of denpol 42L: 39L (10 mg, 0.0012 mmol, 1 equiv.) and 7 (126 mg, 0.10 mmol, 93.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (57 mg, 0.42 mmol, 374 equiv.) was added as a solid powder. Copper sulfate (23 mg, 0.21 mmol, 187 equiv.) was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (374 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L pH=7, 25000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 42L. Further characterization to follow in future work.

Synthesis of denpol 43L: 39L (8.7 mg, 0.0066 mmol, 1 equiv.) and 7 (223 mg, 0.06 mmol, 93.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (50 mg, 0.25 mmol, 374 equiv.) was added as a solid powder. Copper sulfate (20 mg, 0.12 mmol, 187 equiv.)
was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (374 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L pH=7, 25000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 43L. Further characterization to follow in future work.

Synthesis of denpol 41D: 39D (40 mg, 0.02 mmol, 1 equiv.) and 7 (60 mg, 0.277 mmol, 16.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (219 mg, 1.1 mmol, 66 equiv.) was added as a solid powder. Copper sulfate (88 mg, 0.55 mmol, 33 equiv.) was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (66 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L pH=7, 1000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 41D.

$^1$H NMR (D$_2$O) δ: 8.14 (br, 1H), 5.39 (br, 1H), 4.89 (br, 2H), 4.11 (br, 3H), 3.85 (br, 2H), 3.70 (br, 6H), 2.17 (s, 3H), 1.57 (br, 3H), 1.10 (br, 3H). Further characterization to follow in future work.

Synthesis of denpol 42D: 39D (7 mg, 0.002 mmol, 1 equiv.) and 7 (83 mg, 0.04 mmol, 16.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (38 mg, 0.2 mmol, 66 equiv.) was added as a solid powder. Copper sulfate (38 mg, 0.09 mmol, 33 equiv.) was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (66 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L pH=7, 2000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 42D.
g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 42D. Further characterization to follow in future work.

**Synthesis of denpol 43D:** 39D (5 mg, 0.002 mmol, 1 equiv.) and 7 (121 mg, 0.03 mmol, 16.5 equiv.) were each dissolved in DMF (2 mL) and added to a flask. Sodium ascorbate (27 mg, 0.13 mmol, 66 equiv.) was added as a solid powder. Copper sulfate (11 mg, 0.06 mmol, 33 equiv.) was added to the mixture as a solution in water (0.4 mL). Reaction mixture was stirred at 40°C for 48 hours. Additional sodium ascorbate (66 equiv.) was added to the mixture after the first 24 hours. The reaction mixture was then dialyzed first against EDTA solution (5g/L pH=7, 6000-8000 g/mol MWCO) for 12 hours with EDTA solution changed after the first 5 hours, and then against pure water overnight. The resulting aqueous mixture was lyophilized to yield the product 43D. Further characterization to follow in future work.
Chapter 5

5 References


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Chapter 6

6 Appendix

$^1$H NMR of Dendron 11

$^1$H NMR of Dendron 13
$^1$H NMR of Dendron 16

$^1$H NMR of Dendron 18

$^1$H NMR of Dendron 12
$^{1}H$ NMR of 23

$^{1}H$ NMR of glycodendron 26
\(^1\)H NMR of glycodendron 27

\(^1\)H NMR of 31
$^1$H NMR of α-chloro-caprolactone 37

$^1$H NMR of polymer 39
$^1$H NMR of denpol 40

$^{13}$C NMR of dendron 11
$^{13}$C NMR of dendron 13

$^{13}$C NMR of dendron 17

$^{13}$C NMR of dendron 12
$^{13}$C NMR of dendron 14

$^{13}$C NMR of glycodendron 26

$^{13}$C NMR of dendron 28
MALDI Spectrum of dendron 11

MALDI Spectrum of dendron 13
MALDI Spectrum of dendron 12

MALDI Spectrum of dendron 14
MALDI Spectrum of dendron 18

IR spectrum of dendron 11
IR spectrum of dendron 13

IR spectrum of dendron 17
IR spectrum of dendron 12

IR spectrum of dendron 14
IR spectrum of dendron 18

IR spectrum of glycodendron 26
IR spectrum of polymer 38

IR spectrum of polymer 39
IR spectrum of denpol 42L

IR spectrum of denpol 43L
IR spectrum of denpol 43D
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- Supramolecular Chemistry (9523T)
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