SYNTHESIS AND CHARACTERIZATION OF MANNOSE-GRAFTED
PEPTIDE-LIKE POLYESTERS

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SYNTHESIS AND CHARACTERIZATION OF MANNOSE-GRAFTED PEPTIDE-LIKE POLYESTERS

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Thesis

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ABSTRACT

Glycoproteins play significant roles in many physiological as well as pathological cell functions and they are potential for the development of therapeutics, diagnostics, and vaccines. However, the function of glycoproteins is not fully understood due to the challenge to obtain them in a homogeneous form. Therefore, different types of synthetic analogues of glycoprotein have been prepared in literature to mimic the properties of natural glycoproteins.

In this work, mannose-grafted copolyesters were designed as polymeric analogues of glycoprotein, which contain a peptide-like polyester backbone and mannose-based polyacrylate side chain. The graft copolymers were then prepared by “grafting to”, “grafting through” and “grafting from” method. The number of carbohydrates in the polymer was determined by sulfuric acid assay. The interaction between these mannose-grafted polyesters and Concanavalin A (Con A) was then studied by turbidimetry binding assay.
First and foremost, I truly appreciate my advisor, Dr. Abraham Joy, for providing me this interesting project. During the investigation of this project, he supported me lots of helpful suggestions on how to thinking, how to solve problems and, most importantly, taught me to enjoy my work.

Also, I would like to thank Dr. Yu Zhu who read my thesis patiently and generously attend my bachelor defense. I sincerely appreciate it.

What’s more, I would like to thank all the members from Joy’s lab, especially Chao Peng who is my mentor for this project. He not only taught me basic experimental skills, but also gave me various fantastic ideas in this research project.

In the end, I would like to thank all the support, concern and love from my family, friends.
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CHAPTER I
INTRODUCTION

1.1 Graft Copolymer

Graft copolymer belongs to branched copolymer which consisted of a linear backbone and a relatively long side chains that attach to each other covalently (shown in Figure 1.1). Graft copolymer has unique properties due to the confined and compact structure\(^1\), which gives graft copolymers great attention and various applications. Generally, three methods (shown in Scheme 1.1) have been utilized to synthesize graft copolymer: “grafting to”, “grafting through”, and “grafting from”.

a) “Grafting to” method\(^2\)

In this method, the linear backbone and the side chains are synthesized separately. Generally, living free-radical polymerization or anionic polymerization is utilized to synthesize the side chains, which yield the side chains with reactive end group. After that, the end functionalized side chains are attached to the backbone by coupling reaction. Through this method, more controlled graft length can be obtained; however, this method requires highly effective coupling reaction to achieve high conversion.
b) “Grafting through” method

In “grafting trough” method, macromonomer is first prepared, and then it was polymerized by various polymerization techniques including ring-opening metathesis polymerization (ROMP), ring-opening polymerization (ROP), livingcontrolled radical polymerization, and living anionic polymerization. This method has advantages of controlled side chain length and density of the repeating unit in backbone.

c) “Grafting from” method

In “grafting from” method, polymers with initiating pendant groups are first synthesized, and they are utilized to initiate polymerizations from the side chain, which can be achieved by different polymerization technique such as ring-opening polymerization or living radical polymerization. “Grafting from” method owns prominent advantage of low steric hindrance of coupling reaction compared to “grafting through” and “grafting to” methods.
Scheme 1.1 Synthesis of graft copolymers a) “grafting to” method b) “grafting through” method c) “grafting from” method

1.2 Copper-catalyzed azide–alkyne cycloaddition (CuAAC)

Scheme 1.2 Copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction

In 1893, as shown in Scheme 1.2, A. Michael first explored azide–alkyne cycloaddition (CuAAC) reaction which was the reaction of phenyl azide with diethyl acetylene-dicarboxylate. Then attributed to the thorough investigation by Huisgen and his coworkers, the content of CuAAC was fully understood. The azide–alkyne cycloaddition was initially limited by the low reaction rate due to the high activation barrier for this reaction even after rising the reaction temperature; for instance, the activation barrier of methyl azide and propyne is nearly 25 kcal mol\(^{-1}\). Thereafter,
the utilization of catalyst, especially copper catalysts, dramatically changed the imperfection of the reaction. With the assistance of copper(I), C-N bond will form between the nucleophilic vinylidene-like β-carbon of copper(I) acetylide and the electrophilic nitrogen of the coordinated organic azide, which improve the reaction to a series of successive steps and form the eventual 5-triazolyl copper intermediate. The details of the mechanism are shown in Figure 1.2. Various copper(I) catalysts can be utilized in the reaction including copper(I) salts such as copper(I) chloride, copper(I) bromide, and copper(I) iodide, as well as coordination complexes such as [Cu(CH$_3$CN)$_4$]PF$_6$. However, the use of copper(I) iodide might decrease the reactivity of the reaction because iodide can act as a ligand and coordinate with effective 5-triazolyl copper intermediate. Besides, the utilizing of copper(I) chloride may be deleterious due to the high concentration of chloride. Therefore, copper(I) bromide is effectively favored and commonly used.$^8$

Due to the high efficiency of the copper-catalyzed azide-alkyne cycloaddition, this reaction was usually used for post-polymerization modification of polymers and the synthesis of different polymer architectures, such as graft copolymers, star copolymers and block copolymers.
1.3 Reversible addition-fragmentation chain transfer (RAFT) polymerization

Reversible addition-fragmentation chain transfer (RAFT) polymerization is a type of controlled radical polymerizations. In conventional radical polymerization, termination often occurs due to the high radical concentration. Therefore, uncontrolled molecular weight and broad polydispersity were usually obtained. The mechanism of RAFT polymerization is shown in Figure 1.3. In RAFT polymerization, a chain transfer agent with high chain transfer constant is used to control the chain growth. During the polymerization, there is a rapid equilibrium that the propagating radical can react with the chain transfer agent to form a dormant polymer chain and lose a leaving group which is able to reinitiate the polymerization. This process lowers the radical concentration and provides equal opportunities for all the chains to grow during the polymerization, therefore results in a controlled molecular weight and low polydispersity. In RAFT polymerization, dithioester or trithiocarbonate
derivatives are usually used as chain transfer agent, because of their high chain transfer constants.

**Initiation**

$\text{Initiator} \rightarrow I^* \rightarrow M \rightarrow M \rightarrow P_n^*$

**Reversible chain transfer**

$P_n^* + S \rightarrow S-R \xrightarrow{k_{\text{add}}} P_n^*-S-S-R \xrightarrow{k_\beta} P_n^*-S-S + R^*$

$M \xrightarrow{k_p} P_n^*$

**Reinitiation**

$R^* \xrightarrow{k_i} R-M^* \rightarrow M \rightarrow M \rightarrow P_m^*$

**Chain equilibration**

$P_m^* + S \rightarrow S-P_n \xrightarrow{k_{\text{add}}} P_m^*-S-S-P_n \xrightarrow{k_{\text{addp}}} P_m^*-S-S + P_n^*$

$M \xrightarrow{k_p} P_n^*$

**Termination**

$P_n^* + P_m^* \rightarrow \text{Dead polymer}$

Figure 1.3 The mechanism of Reversible addition-fragmentation chain transfer (RAFT)

### 1.4 Carbohydrate

Carbohydrates are compound that commonly exist in the nature, which mainly consist of carbon, hydrogen, and oxygen. The empirical formula of carbohydrates is $C_m(H_2O)_n$. It has been known that carbohydrates are basic compound of food and the major plant martials. There are four kinds of carbohydrates—monosaccharide, disaccharides, oligosaccharides, and polysaccharides—defined by the number of fundamental units of sugars in the compound. Monosaccharides are usually called...
“simple sugars” which is the basic unit of all carbohydrate molecules. Table 1.1 showed common monosaccharides found in living systems.

Table 1.1 Nice common monosaccharides found in living systems

1.5 Lectin

Lectins are saccharide-binding proteins, which bind to saccharides through saccharide recognition domains (CRDs). There are four types of animal lectins: C-type, P-type, S-type (galectins) and others. a) C-type lectins are the most important animal lectins, which include selectins, mannose receptors and glycoprotein receptors. They require the assistance of calcium ion for binding, which helps bind the protein and saccharide by interacting with the hydroxyl groups on the saccharide. C-type lectins have functions such as cell-cell adhesion, immune response to foreign bodies and cell-cell destruction. b) S-type lectins (galectins) are soluble proteins that
lack of the requirement for calcium ion. They rely on disulfide bonds for stability and binding to saccharides, which play an various roles in mediation of cell–cell interactions, cell–matrix adhesion and transmembrane signaling, respectively.\(^{14}\) c) P-type lectins contain a phosphate group. They have vital function in the generation of functional lysosomes within the cells.\(^{15}\) *Concanavalin A* (ConA) is a C-type of lectin which mostly obtained from *Jack Bean*. ConA consists of four sub-units whose molecular weight is 26.5 kDa, and it has one binding site in each sub-unit that can specifically bind to glucose and mannose through carbohydrate-protein interaction. The stimulation structure of ConA and the structure of a binding site of ConA are shown in Figure 1.4.

![Stimulation structure of ConA and structure of a binding site of ConA](image)

1.6 Carbohydrate-protein interaction

Carbohydrate-protein interaction is a complex interaction mainly include hydrogen bond interaction, saccharide-aromatic interaction, ionic bond interaction, and coulomb interaction.\(^{17}\) In fact, carbohydrates play significant roles in vivo biological process including communication within cells, host-pathogen recognition, cancer
metastasis, fertilization and Inflammation. These processes largely involve the carbohydrate-protein interaction which can be divided into carbohydrate-lectin interaction and carbohydrate-antibody interaction, and the carbohydrate complexes participate in them usually exist as glycoproteins, peptidoglycans or glycolipids.

1.7 Multivalency

In biological process, both monovalency and multivalency are reversible, non-covalent interaction which largely participate activity between molecular interface. Compared to monovalent interaction that usually has weak strength, the multivalency interaction has much stronger binding between ligands and receptors due to its multiple interacted binding molecules. Figure 1.5 showed general event of multivalency. The general binding event can be concluded by three equilibriums.

\[
L + R \rightleftharpoons LR
\]

\[
R_0 = R + LR
\]

\[
L_0 = L + LR
\]

In which:

M = Free receptor
L = Free ligand
LR = Complex of ligand and receptor
R_0 = Initial ligand
L_0 = Initial receptor
Thus, the dissociation constant $K_d$ is:

$$K_d = \frac{[L] \times [R]}{[LR]} = \frac{[L]_{eq} \times [R]_{eq}}{[LR]_{eq}}$$

In most calculations, the concentration of complex of ligand and receptor at equilibrium ($[LR]$) is measured or calculated instead of measuring the concentration of free ligand ($[L]$). That is, the initial concentration of ligand ($[L_0]$) is close to the initial concentration of receptor ($[R_0]$).

So that,

$$K_d = \frac{[L] \times [R]}{[LR]} = \frac{[R_0] - [LR] \times [L_0] - [LR]}{[LR]}$$

$$\Rightarrow [LR]K_d = ([R_0] - [LR]) \times ([L_0] - [LR])$$

$$\Rightarrow [LR]K_d = [L_0][R_0] - ([L_0][LR] - [R_0][LR] + [L + R]^2$$

The equation is similar to quadratic form, as a result,

$$[RL] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$[RL] = \frac{([R_0] + [L_0] + K_d) - \sqrt{([R_0] + [L_0] + K_d)^2 - 4[M_0][L_0]}}{2}$$

Based on this equation, $[ML]$ can be calculated from known, and $K_d$.22
1.8 Glycoprotein

Glycoproteins consist of oligosaccharide chains and polypeptide, and they are covalently attached. There are various glycoproteins existing in cell membrane, which play a key role in human physiological activity including lubricating, transporting, immunity, cell adhesion and recognition etc.\textsuperscript{23} There are two kinds of glycoprotein: N-linked glycoproteins and O-linked glycoproteins. N-linked glycoprotein is formed between sugars and nitrogen of the side chain of asparagine. O-linked glycoprotein is formed between sugars and oxygen of the side chain of serine or threonine. Common glycoproteins and their functions were listed in Table 1.2.
Table 1.2 Common glycoproteins and their functions

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<td>Interact with specific carbohydrates</td>
<td>Lectins, selectins (cell adhesion lectins), antibodies</td>
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<td>Lubricant and protective agent</td>
<td>Mucins</td>
</tr>
<tr>
<td>Transport molecule</td>
<td>Transferrin, ceruloplasmin</td>
</tr>
<tr>
<td>Immunologic molecule</td>
<td>Immunoglobins, histocompatibility antigens</td>
</tr>
<tr>
<td>Hormone</td>
<td>Human chorionic gonadotropin (HCG), thyroid-stimulating hormone (TSH)</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Various, e.g., alkaline phosphatase, patatin</td>
</tr>
<tr>
<td>Cell attachment-recognition site</td>
<td>Various proteins involved in cell–cell (e.g., sperm–oocyte), virus–cell, bacterium–cell, and hormone–cell interactions</td>
</tr>
<tr>
<td>Antifreeze protein</td>
<td>Certain plasma proteins of coldwater fish</td>
</tr>
<tr>
<td>Affect folding of certain proteins</td>
<td>Calnexin, calreticulin</td>
</tr>
</tbody>
</table>

1.9 Previous work on the synthetic polymeric glycoprotein mimics

Glycoproteins are potential for the development of therapeutics or vaccines, however, their structures and functions are still not clear. Because of the complicated purification process, it is difficult to obtain pure glycoproteins and study their functions. Therefore, different research groups have been working on the synthesis of polymeric glycoprotein analogues using different methodologies.
1.9.1 Solid-phase peptide synthesis (SPPS) method

SPPS technique has been successfully used to prepare glycoprotein mimics (shown in Scheme 1.3).\textsuperscript{26} By this method, well-defined sequence of amino acids can be obtained. The sugar unit can be incorporated by either using a glycosylated amino acid or introducing the glycan by chemical modification. However, the disadvantage of this method is that the size of the peptide sequence is limited to around 50 residues. Also, the yield decreases with the number of the amino acid.

![Scheme 1.3 Synthesis of glycoprotein mimics by solid-phase peptide synthesis (SPPS) technique\textsuperscript{26}](image)

1.9.2 Ring-opening polymerization (ROP) of N-carboxyanhydride method

Another type of glycoprotein mimics was glycopolypeptides, which are synthesized by ROP of N-carboxyanhydride. For example, Kramer\textsuperscript{27} reported the synthesis of glycopolypeptides by ROP of a glycosylated N-carboxyanhydrides (shown in Scheme 1.4). Also, the glycan can be introduced into the polymer \textit{via} post-polymerization modification. For example, Sun\textsuperscript{19} prepared glycopolypeptide using alkene functionalized polypeptide, in which a thiol functionalized glycan was attached to the polymer via thiol-ene click chemistry (shown in Scheme 1.5).
There are also other interesting structures in which oligosaccharide-based glycopolypeptides are attached to the end of a synthetic polypeptide to mimic the structure of glycoproteins. For example, Bonduelle synthesized polypeptides which are end functionalized with oligosaccharide via azide-alkyne cycloaddition to provide either linear or tree-like glycoprotein mimics (shown in Scheme 1.6).
CHAPTER II
EXPERIMENTAL

2.1 Materials

6-Bromohexanoic acid (99.2%) and D(+)-mannose (99.7%) were purchased from Chem-Impex. Sodium azide (99.7%), sodium chloride (99%), copper(I) bromide (98%), 2,2’-Azobis(2-methylpropionitrile) (98%), 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) and Concanavalin A (from Jack bean Type VI) were purchased from Sigma-Aldrich. Magnesium sulfate (98%), potassium carbonate (99%) and 2-hydroxyethyl acrylate (97%) were purchased from Fisher. Boron trifluoride diethyl etherate (98%) was purchased from Alfa Aesar. Thionyl chloride (98%) was purchased from Tokyo Chemical. Anhydrous sodium sulfate (99%) was purchased from EMD Millipore. N-(2-Hydroxyethyl)acrylamide (98%) was purchased from TCI America. 2,2’-Azobis(2-methylpropionitrile) (AIBN) (Aldrich, 98%) was recrystallized multiple times from methanol before use. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid and 4-(dimethylamino) pyridinium p-toluenesulfonate (DPTS) were synthesized according to reported procedures.29,30

2.2 Instrumentation

The chemical structure of the monomers and polymers was confirmed by $^1$H NMR spectroscopy using Varian NMRS 300. The NMR spectra were processed by ACD NMR
processor. Size exclusion chromatography (SEC) analysis in DMF was performed on a HLC-8320 GPC from TOSOH equipped with RI and UV detectors using PS as standard.

The determination of total carbohydrates number and binding efficiency was studied on UV−Vis spectroscopy (Shimadzu).

2.3 Purification of copper(I) bromide

1 g of copper (I) bromide was washed by acetic acid (25 mL) three times. After that, the residual was collected via vacuum filtration. The filtrate was washed by ethanol (25 mL) three times and diethyl ether (25 mL) three times. Then the residual was collected by vacuum filtration and dried under vacuum overnight.

2.4 Synthesis of RAFT agent

The propargyl functionalized chain transfer agent (CTA) was synthesized in two steps. A carboxyl functionalized trithiocarbonate was first synthesized. Then, the carboxyl group was converted to a propargyl group by reacting with propargyl alcohol.

2.4.1 Synthesis of 2-(Dodecylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (carboxyl functionalized RAFT agent)

Sodium hydroxide (1.76 g, 0.044 mol) was added into 60 mL acetone in a round bottom flask. The temperature was maintained at 10 °C. Then, dodecane thiol (4.00 g, 19.76 mmol) was added dropwise, and the mixture was stirred for 10 minutes. Then carbon disulfide (4.10 g, 53.85 mmol) was added dropwise, and the solution turned bright yellow. After stirring for extra 10 minutes, 2-bromo-2-methylpropionic acid (3.00 g, 17.96 mmol) was added. The reaction was carried out at room temperature for 24 hours.
After the reaction, acetone was removed under reduced pressure. The compound was dissolved in dichloromethane (50 mL) and washed with water (50 mL) three times, followed dried over anhydrous magnesium sulfate. The product was purified by column chromatography (10% ethyl acetate in hexane, \( R_f = 0.5 \)) and dried in vacuum give a yellow solid. (3.01 g, 47.3%) \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) (ppm) 0.89 (t, 3H), 1.25-1.53 (m, 18H), 1.73 (s, 8H), 3.35 (t, 2H).

2.4.2 Synthesis of prop-2-yn-1-yl 2-(((ethylthio)carbonothioyl)thio)-2-methylpropanoate (propargyl functionalized RAFT agent)

In a round bottom flask, EDC (0.784 g, 4.1 mmol), DMAP (0.50 g, 4.0 mmol) and 2-[(Dodecylsulfanyl)carbonothioyl]sulfanyl-2-methylpropanoic acid (1.00 g, 2.74 mmol) were dissolved in anhydrous dichloromethane (16 mL). The mixture was stirred for 10 minutes in nitrogen atmosphere. Then propargyl alcohol (0.50 g, 8.9 mmol) was added dropwise and the mixture was stirred overnight at room temperature. After that, the product was purified by column chromatography (20% ethyl acetate in hexane, \( R_f = 0.9 \)) and dried in vacuum give a yellow solid. (0.997 g, 90.3%) \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) (ppm) 0.87 (t, \( J = 0.68 \) Hz, 3H), 1.23~1.36 (m, 20H), 1.68 (s, 6H), 2.44 (s, 1H), 3.26 (t, \( J = 6.4 \) Hz, 2H), 4.67 (s, \( J = 4.8 \) Hz, 2H).

2.5 Synthesis of Methyl 6-bromohexanoate

6-bromohexanoic acid (4.90 g, 25.1 mmol) and anhydrous methanol (50 mL) were added in a round-bottom flask equipped with a magnetic stir bar. The flask was cooled in an ice bath and thionyl chloride (3.34 g, 2 mL, 28.1 mmol) was added dropwise. The ice
bath was removed after 10 min and the reaction was carried out at room temperature for 24 hours. After that, the reaction was concentrated under reduced pressure. The compound was dissolved in ethyl acetate (35 mL) and washed in order with water (15 mL), saturated sodium bicarbonate (15 mL), water (15 mL), brine (15 mL) and dried over anhydrous magnesium sulfate. The solvent was then removed under reduced pressure to yield a yellow oil (4.03 g, 76.7%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 1.49 (m, 2H) 1.66 (m, 2H) 1.88 (m, 2H) 2.34 (t, J = 7.5 Hz, 2H) 3.41 (t, J = 6.9 Hz, 2H) 3.68 (s, 3H).

2.6 Synthesis of Methyl 6-azidohexanoate

Methyl 6-bromohexanoate (4.0 g, 19.1 mmol) and sodium azide (3.73 g, 57.4 mmol) was dissolved in anhydrous DMF (16 mL) in a round-bottom flask equipped with a magnetic stir bar. Then the reaction was carried out at 80 ºC overnight. After that, salt was removed by filtration. The filtrate was then poured into dichloromethane (100 mL) followed by washing with water (4*150 mL). The organic phase was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give a yellow oil (3.31 g, 90.1%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm) 1.42 (m, 2H) 1.64 (m, 4H) 2.33 (t, J = 7.4 Hz, 2H) 3.28 (t, J = 6.9 Hz, 2H) 3.68 (s, 3H)

2.7 Synthesis of 6-azido-N,N-bis(2-hydroxyethyl)hexanamide

Methyl 6-azidohexanoate (2.2 g, 12.8 mmol) and 2.75 g of diethanolamine (2.75 g, 26.2 mmol) were mixed in a round-bottom flask equipped with a magnetic stir bar. Then the mixture was stirred at 80 ºC overnight. The product was purified by column chromatography (10% ethyl acetate in hexane, R$_f$= 0.5) to give a slightly yellow oil (1.61
$g$, 51.4%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$(ppm) 1.42-1.49(m, 2H), 1.60-1.72(m, 4H), 2.41-2.46(m, 2H), 3.27-3.31(m, 4H), 3.50-3.59(m, 4H), 3.78-3.89(m, 4H).

2.8 Synthesis of azide functionalized polyester

6-Azido-N, N-bis(2-hydroxyethyl)hexanamide (1.6146 g, 6.61 mmol), succinic acid (0.7805 g, 6.61 mmol) and 2-(Dimethylamino)pyridinium p-toluenesulfonate (0.7728 g, 2.64 mmol) were added into a 50 mL round-bottom flask. The flask was evacuated and backfilled with nitrogen three times. Then anhydrous dichloromethane (6.6 mL) was added to the flask and the mixture was cooled in an ice bath. Then diisopropylcarbodiimide (2.50 g, 3.1 mL, 19.83 mmol) was added dropwise and the reaction was carried out at room temperature for 48 h. The polymer was purified by precipitation in methanol (Mn= 12.1K, PDI= 1.67).

2.9 Synthesis of 1,2,3,4,6-penta-O-acetyl-α-D-mannose

Sulfuric acid (2 drops) was added to a stirred mixture of acetic anhydride (27 mL) and D-mannose (4.98 g, 27.8 mmol) at 0 ºC. The mixture was stirred for 10 minutes at 0 ºC and then allowed to warm up to room temperature and stirred for a further 30 minutes. The mixture was then diluted with ice–water (100 mL), and extracted with ethyl acetate (100 mL). The extract was washed with water (3*100 mL) and then saturated aqueous sodium bicarbonate (100 mL). The organic phase was dried by anhydrous magnesium sulfate and concentrated under reduced pressure to yield the white gel-like product. (8.68 g, 80.4%) $^1$H NMR (300 MHz, CDCl$_3$): $\delta$(ppm) 1.98 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 3.07-3.12(m, 6H), 3.27-3.31(m, 4H), 3.50-3.59(m, 4H), 3.78-3.89(m, 4H).
2.15 (s, 3H), 2.19 (s, 3H), 3.99–4.05 (m, 1H), 4.07 (dd, J = 12.4 Hz), 4.26 (dd, J = 12.4 Hz, 1H), 5.23 (dd, J = 3.1 Hz, 1H), 5.31–5.34 (m, 2H), 6.06 (d, J = 2.0 Hz, 1H).

2.10 Synthesis of acrylate 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside

1,2,3,4,6-penta-O-acetyl-α-D-mannose (1 g, 5.56 mmol) and 2-hydroxyethyl acrylate (1.29 g, 11.11 mmol) were dissolved in 10 mL of dry dichloromethane. The mixture was placed in an ice bath and boron trifluoride etherate (3.9 g, 27.78 mmol) was added dropwise over 15 minutes. The mixture was kept in an ice bath for an hour and then continued at room temperature overnight. The mixture was poured into 15 mL ice water and the two phases were separated. The aqueous phase was extracted with dichloromethane (3*20 mL) and the organic phases were combined and washed in order with water (20 mL), saturated aqueous sodium bicarbonate (20 mL), water (20 mL), and dried by anhydrous magnesium sulfate and the filtrate was concentrated under vacuum and then isolated by silica gel chromatography (30% ethyl acetate in hexane, Rf = 0.3), and concentrated under vacuum again to give a white gel product. (0.69 g, 61.3%) 1H NMR (300 MHz, CDCl3): δ(ppm) 6.43 (d, J = 17.3 Hz, 1H), 6.13 (m, 1H), 5.86 (d, J = 10.4 Hz, 1H), 5.28 (m, 3H), 4.86 (s, 1H), 4.14 (m, 6H), 3.83 (m, 2H), 2.14 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H).

2.11 Synthesis of propargyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside

To a solution of 1,2,3,4,6-penta-O-acetyl-α-D-mannose (0.50 g, 1.28 mmol) in dry dichloromethane (10 mL) was added boron trifluoride etherate (0.3 mL, 2.38 mmol)
dropwise in ice bath under nitrogen. The mixture was allowed to warm up and stirred at room temperature for 4 hours. Propargyl alcohol (0.3 mL, 5.12 mmol) was added and the mixture was stirred at room temperature for 36 hours. The mixture was washed successively with water (20 mL), saturated aqueous sodium bicarbonate (20 mL), water (20 mL), and dried by anhydrous magnesium sulfate and the filtrate was concentrated under vacuum and then isolated by silica gel chromatography (20% ethyl acetate in hexane, R_f=0.3), and concentrated under vacuum again to give a white gel product. (0.34 g, 64.7%) ^1H NMR (300 MHz, CDCl_3): δ(ppm) 5.35 (dd, J=10.0 Hz, 1H), 5.26 (t, J=10.0 Hz, 1H), 5.23 (dd, J=3.3 Hz, 1H), 5.01 (d, J=1.6 Hz, 1H), 4.32-4.26 (m, 3H), 4.11 (dd, J=12.1 Hz, 1H), 4.00 (ddd, J=5.2 Hz, 1H), 2.48 (t, J=2.4 Hz, 1H), 2.16 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H).

2.12 Synthesis of mannose-functionalized N-substituted polyester

The mannose-functionalized polyester was synthesized in two steps. A mannose functionalized diol was first synthesized. Then, the diol was polymerized by DIC/DPTS mediated polycondensation.

2.12.1 Synthesis of mannose functionalized diol by azide-alkyne click reaction

Diethanolamine (0.751 g, 3.070 mmol), propargyl 2,3,4,6-tetra-O-acetyl-mannopyranoside (1.304 g, 3.377 mmol) and copper (I) bromide (24 mg, 0.168 mmol) were added to dichloromethane (10 mL). Then the mixture was added into a Schlenk Flask. The flask was subjected to three freeze-pump-thaw cycles. Then the flask was backfilled with nitrogen and TMEDA (1 mL) was quickly added to the flask while the solvent was frozen. After that, the flask was subjected to two freeze-pump-thaw cycles.
Then the reaction was carried out at room temperature overnight. After that, the solution was passed through an alumina column and the monomer was then purified by column chromatography (10% menthol in dichloromethane, \( R_f = 0.4 \)) and dried in vacuum to give a slight-yellow solid. (1.497 g, 80.9%)

2.12.2 Synthesis of mannose-functionalized N-substituted polyester by polycondensation

Mannose functionalized diol monomer (245.6 mg, 0.389 mmol), succinic acid (45.9 mg, 0.389 mmol) and 2-(Dimethylamino)pyridinium p-tolenesulfonate (45.5 mg, 0.156 mmol) were added into a 50 mL round-bottom flask. The flask was evacuated and backfilled with nitrogen three times. Then anhydrous dichloromethane (4.0 mL) was added to the flask and the mixture was cooled in an ice bath. Then diisopropylcarbodiimide (147.4 mg, 0.18 mL, 1.168 mmol) was added dropwise and the reaction was carried out at room temperature for 48 h. The polymer was purified by precipitation in ethyl ether (\( M_n = 4.7K \), \( PDI = 1.67 \)).

2.13 Synthesis of protected-mannose-grafted copolymer (pMGCP) by grafting to method

The synthesis of graft copolymers by “grafting to” method involved two steps. First, the side chain was synthesized by RAFT polymerization. Then, the side chain was attached to the polymer backbone by click chemistry.

2.13.1 Synthesis of propargyl end functionalized mannose-based polyacrylate by RAFT polymerization

Acrylate 2,3,4,6-tetra-O-acetyl-\( \alpha \)-D-mannopyranoside (741.1 mg, 1.66 mmol), RAFT agent (133.7 mg, 0.33 mmol) and AIBN (5.4 mg, 0.033 mmol) was dissolved in dioxane
(3.3 mL). The solution was transferred to a Schlenk flask. The flask was subjected to three freeze-pump-thaw cycles. Then the reaction was carried out at 70 °C for 24 hours. The polymer was purified by dialysis in methanol to give a dark-yellow polymer. (M_n= 2.7K PDI= 1.11)

2.13.2 Synthesis of pMGCP by azide alkyne cycloaddition

Azide functionalized polyester (25.4 mg, 0.074 mmol) and propargyl end functionalized mannose-based polyacrylate (140.1 mg, 0.082 mmol) was dissolved in dichloromethane (8.0 mL). Then Copper(I) bromide (0.98 mg, 0.003 mmol) was added to the solution and the mixture was transferred to a Schlenk flask. The flask was subjected to three freeze-pump-thaw cycles. Then the flask was backfilled with nitrogen and TMEDA (100 µL) was quickly added to the flask while the solvent was frozen. After that, the flask was subjected to two freeze-pump-thaw cycles. The reaction was carried out at room temperature overnight. Then the solution was passed through an alumina column and the polymer was purified by dialysis in methanol to give a yellow solid. (M_n= 21.8K, PDI= 1.96)

2.14 Synthesis of pMGCP by grafting through method

The synthesis of graft copolymers by “grafting through” method basically involved two steps. First, the side chain was attached to an azide functionalized diol. Then, the diol was polymerized by DIC/DPTS mediated polycondensation.
2.14.1 Synthesis of propargyl end functionalized mannose-based polyacrylate by RAFT polymerization

Acrylate 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (741.1 mg, 1.66 mmol), RAFT agent (133.7 mg, 0.33 mmol) and AIBN (5.4 mg, 0.033 mmol) was dissolved in dioxane (3.3 mL). The solution was transferred to a Schlenk flask. The flask was subjected to three freeze-pump-thaw cycles. Then the reaction was carried out at 70 ºC for 24 hours. The polymer was purified by dialysis in methanol to give a dark-yellow material. ($M_n=2.7K, PDI=1.11$)

2.14.2 Synthesis of RAFT functionalized diol by azide-alkyne cycloaddition

Diethanolamine (59.9 mg, 0.245 mmol), propargyl end functionalized RAFT agent (378.5 mg, 0.223 mmol) and copper (I) bromide (1.5 mg, 0.012 mmol) were added to dichloromethane (2.5 mL). Then the mixture was added into a Schlenk Flask. The flask was subjected to three freeze-pump-thaw cycles. Then the flask was backfilled with nitrogen and TMEDA (150 µL) was quickly added to the flask while the solvent was frozen. After that, the flask was subjected to two freeze-pump-thaw cycles. Then the reaction was carried out at room temperature overnight. After that, the solution was passed through a alumina column and the polymer was then purified by dialysis in methanol to give a slight-yellow material. ($M_n=2.9K, PDI=1.09$)

2.14.3 Synthesis of pMGCP by polycondensation

Macromonomer (0.284 g, 0.105 mmol), succinic acid (0.0124 g, 0.105 mmol) and 2-(Dimethylamino)pyridinium p-toluenesulfonate (0.013 g, 0.042 mmol) were added into a
50 mL round-bottom flask. The flask was evacuated and backfilled with nitrogen three times. Then anhydrous dichloromethane (1.5 mL) was added to the flask and the mixture was cooled in an ice bath. Then diisopropylcarbodiimide (0.015 g, 0.04 mL, 0.049 mmol) was added dropwise and the reaction was carried out at room temperature for 48 h. The polymer was purified by precipitation in ethyl ether (Mn= 4.7K, PDI=1.67).

2.15 Synthesis of pMGCP by grafting from method

The synthesis of graft copolymers by “grafting from” method involved two steps. First, trithiocarbonate functionalized polyester was synthesized as a macro-RAFT agent. Then, the macro-RAFT agent was used to synthesize the graft copolymers.

2.15.1 Synthesis of RAFT functionalized polyester by azide-alkyne cycloaddition

Azide functionalized polyester (120.1 mg, 0.316 mmol), propargyl functionalized RAFT agent (127.6 mg, 0.348 mmol) and copper (I) bromide (5 mg, 0.015 mmol) were added to dichloromethane (3.0 mL). Then the mixture was added into a Schlenk Flask. The flask was subjected to three freeze-pump-thaw cycles. Then the flask was backfilled with nitrogen and TMEDA (500 µL) was quickly added to the flask while the solvent was frozen. After that, the flask was subjected to two freeze-pump-thaw cycles. Then the reaction was carried out at room temperature overnight. After that, the solution was passed through a alumina column and the polymer was then purified by dialysis in methanol to give a dark-yellow material. (M_n= 26.4K, PDI= 1.51)
2.15.2 Synthesis of pMGCP by RAFT polymerization

Acrylate 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (166.12 mg, 0.372 mmol), macro-RAFT agent (55.6 mg, 0.074 mmol) and AIBN (1.22 mg, 0.0074 mmol) were dissolved in dioxane (1.5 mL). Then the solution was transferred to a Schlenk flask. The flask was subjected to three freeze-pump-thaw cycles. Then the reaction was carried out at 70 °C for 24 hours. The polymer was purified by dialysis in methanol to give a yellow solid. (M_n=53.3K, PDI= 1.76)

2.16 Synthesis of (mannose-co-phenyl) copolyester

Polymers with different sugar density were synthesized by copolymerization with phenyl functionalized diol to study the influence of sugar density on the carbohydrate-protein interactions.

2.16.1 Synthesis of N,N-bis(2-hydroxyethyl) benzene propanamide

Methyl 3-phenylpropanoate (1.51 g, 9.21 mmol) and diethanolamine (1.92 g, 18.27 mmol) were mixed in a round bottom flask equipped with a magnetic stir bar. Then the mixture was stirred at 80 °C overnight. The product was purified by column chromatography (10% ethyl acetate in hexane, R_f= 0.5) to give a white solid. (1.61 g, 74.4%) ¹H NMR (300 MHz, CDCl₃): δ(ppm) 7.28–7.26 (m, 2H), 7.20–7.19 (m, 3H), 3.82 (t, J = 4.6 Hz, 2H), 3.70 (t, J = 4.7 Hz, 2H), 3.54 (t, J = 4.5 Hz, 2H), 3.41 (t, J = 4.6 Hz, 2H), 2.95 (t, J = 7.7 Hz, 2H), 2.71 (t, J = 7.8 Hz, 2H).
2.16.2 Synthesis of (azide-co-phenyl) copolyester by polycondensation

Azide functionalized diol monomer (494.3 mg, 2.022 mmol), N,N-bis(2-hydroxyethyl)benzenepropanamide (720.2 mg, 3.034 mmol), succinic acid (597.4 mg, 5.055 mmol) and 2-(Dimethylamino)pyridinium p-toluenesulfonate (5.9 mg, 2.021 mmol) were added into a 50 mL round-bottom flask. The flask was evacuated and backfilled with nitrogen three times. Then anhydrous dichloromethane (5.0 mL) was added to the flask and the mixture was cooled in an ice bath. Then diisopropylcarbodiimide (1753.1 mg, 2.41 mL, 15.71 mmol) was added dropwise and the reaction was carried out at room temperature for 48 h. The polymer was purified by precipitation in ethyl ether (Mn= 25.5 K, PDI= 1.71).

2.16.3 Synthesis of (mannose-co-phenyl) copolymer by grafting from method

The synthesis of graft copolymers by “grafting from” method involved two steps. First, trithiocarbonate functionalized polyester was synthesized as a macro-RAFT agent. Then, the macro-RAFT agent was used to synthesize the graft copolymers.

2.16.3.1 Synthesis of RAFT functionalized copolyester by azide-alkyne cycloaddition

(Azide-co-phenyl) copolyester (235.6 mg, 0.7209 mmol), propargyl functionalized RAFT agent (107.6 mg, 0.237 mmol) and copper (I) bromide (5 mg, 0.015 mmol) were added to dichloromethane (3.5 mL). Then the mixture was added into a Schlenk Flask. The flask was subjected to three freeze-pump-thaw cycles. Then the flask was backfilled with nitrogen and TMEDA(200 µL) was quickly added to the flask while the solvent was frozen. After that, the flask was subjected to two freeze-pump-thaw cycles. Then the reaction was carried out at room temperature overnight. After that, the solution was
passed through an alumina column and the polymer was then purified by dialysis in methanol to give a dark-yellow material. ($M_n=30.7$ K, PDI= 1.69)

2.16.3.2 Synthesis of (mannose-co-phenyl) polyesters by RAFT polymerization

Acrylate 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (465.95 mg, 1.403 mmol), macro-co-RAFT agent (85.3 mg, 0.2088 mmol) and AIBN (3.4 mg, 0.026 mmol) were dissolved in dioxane (2.0 mL). Then the solution was transferred to a Schlenk flask. The flask was subjected to three freeze-pump-thaw cycles. Then the reaction was carried out at 70 °C for 24 hours. The polymer was purified by dialysis in methanol to give a yellow solid. ($M_n=53.6$K, PDI=1.64)

2.17 General deprotection of pMGCP

Polymer (10 mg) was dissolved in methanol (5 mL). Then potassium carbonate (8.3 mg, 0.06 mmol) was added to the solution and the reaction mixture was stirred at room temperature until disappearance of starting material. After that, the solution was concentrated and filtered. The product was purified by precipitation in diethyl ether to give a yellow solid. (8.4 mg, 100%)

2.18 Determine the total carbohydrate number

The mannose standard stock solution and glycopolymer solution was prepared by dissolving the sample in deionized water. Then, 1 mL aliquot of solution is rapidly mixed with 3 mL of concentrated sulfuric acid in a vail and vortexed for 30 seconds. Next, the solution was cooled in ice for 2 min to bring it to room temperature. Finally, UV light absorption at 315 nm is recorded using UV spectrophotometer.
2.19 Turbidimetry binding assay

This assay was carried out following a previously described procedure by Stenzel et al.\textsuperscript{33} Con A was dissolved in 0.01 M HEPES buffer at pH 7.4 to form a 32μM solution. Then, 0.5 mL of this solution was transferred to a 1.0 mL masked quartz crystal cuvette and placed on the sample holder of the UV–vis spectrophotometer. A baseline was taken after 1 min. Next, the solution of glycopolymers in HEPES buffer was added and mixed thoroughly for 30 seconds. Finally, the absorbance at 420 nm was recorded over a period of 160 minutes.
CHAPTER III
RESULT AND DISCUSSION

3.1 Synthesis of propargyl functionalized RAFT agent

To accurately control the number of mannose in the side chain, RAFT polymerization was utilized to synthesize the mannose-based polyacrylate side chain, which can provide controlled molecular weight and low polydispersity index. The synthetic route for the RAFT agent used in this work is shown in Scheme 3.1. First, carboxyl functionalized RAFT agent was synthesized by reaction of 1-dodecanethiol, carbon disulfide and α-Bromoisobutyric acid in the presence of $K_3PO_4$ in acetone. Then carboxyl functionalized RAFT agent was further functionalized by reacting with propargyl alcohol catalyzed by EDC/DMAP in $CH_2Cl_2$ to yield a propargyl functionalized RAFT agent. The propargyl group can be used for the post-polymerization modification via azide-alkyne cycloaddition “click” chemistry. The structure of propargyl functionalized RAFT agent was confirmed by $^1H$ NMR spectroscopy (shown in Figure 3.1) and IR-spectroscopy (shown in Figure 3.2). As shown in Figure 3.1, the signal at 2.47 ppm corresponds to the proton on the propargyl group, and the signal at 4.70 ppm corresponds to the two protons
on the methylene group next to the propargyl group. The signals from 0.87 to 1.72 ppm correspond to the protons on the C\textsubscript{12}H\textsubscript{25} hydrocarbon chain. From the IR spectra, the C-H stretching around 3300 cm\textsuperscript{-1} confirmed the presence of propargyl group.

Figure 3.1 $^1$H NMR spectrum of propargyl functionalized chain transfer agent

Figure 3.2 IR-spectroscopy of propargyl functionalized chain transfer agent
3.2 Synthesis of azide functionalized polyester

To facilitate post-polymerization modification, azide functionalized polyester was chosen for the main chain. The azide functional group allows for azide-alkyne cycloaddition “click” chemistry, which is efficient and widely used for the modification of polymers.

The diol monomer was synthesized by the amidation reaction between diethanolamine and methyl 6-azidohexanoate as outlined in Scheme 3.2. The structure and purity of the monomer was confirmed by \(^1\)H NMR spectroscopy (shown in Figure 3.3). The four peaks around 3.51, 3.56, 3.79 and 3.86 ppm correspond to the four methylene groups between
the amide group and the hydroxyl groups. The broad peak at 3.38 ppm corresponds to the hydroxyl groups. All the peaks in the NMR spectrum were labelled and all the integration accord with the desired structure. Besides, the structure of azide functionalized diol was confirmed by IR-spectroscopy (shown in Figure 3.4). The peak around 3400 cm\(^{-1}\) corresponding to OH stretching of hydroxyl group, the peak around 2050 cm\(^{-1}\) corresponding to N-N-N antisymmetric stretching of azide group, and the peak around 1650 cm\(^{-1}\) corresponding to C-O stretching of amide group.

![Figure 3.3 ¹H NMR spectrum of azide functionalized diol](image)

![Figure 3.4 IR-spectroscopy of azide functionalized diol](image)
The azide functionalized polyester was synthesized by DIC/DPTS mediated polycondensation of the azide functionalized diol and succinic acid as outlined in Scheme 3.3. To obtain a decent molecular weight, the amount of the diol and the diacid was weighed very carefully in order to get a 1:1 molar ratio. The structure of azide functionalized polyester was confirmed by $^1$H NMR (Figure 3.5). It can be seen that the three peaks around 4.24, 3.62 and 2.62 ppm correspond to the six methylene groups in the polymer backbone. The peak at 3.29 ppm corresponds to the methylene group next to the azide group. Also, the structure of azide functionalized polyester was confirmed by IR-spectroscopy (Figure 3.6), which the peak around 2050 cm$^{-1}$ corresponding to N-N-N antisymmetric stretching of azide group, the peak around 1750 cm$^{-1}$ corresponding to C-O stretching of ester group, and the peak around 1650 cm$^{-1}$ corresponding to C-O stretching of amide group.

The number-average molecular weight determined by SEC was 12.1K ($M_n=12.1K$, $M_w=20.4K$, PDI=1.67). It is worth noting that the polyester need to be stored in a dry condition or the moisture would lead to the degradation of the polymer.

![Figure 3.5 $^1$H NMR spectrum of azide functionalized polyester](image)

Figure 3.5 $^1$H NMR spectrum of azide functionalized polyester
3.3 Synthesis of acrylate and propargyl functionalized mannose

The side chain of the graft copolymers was formed by mannose-based polyacrylate via RAFT polymerization. Therefore, mannose-based acrylate monomer was first synthesized by a two-step procedure.

In the first step, the hydroxyl groups of mannose were protected by acetyl groups using acetic anhydride and the reaction reached more than 80% in 40 minutes (Scheme 3.4). The structure of the compound was confirmed by $^1$H NMR spectroscopy (shown in Figure 3.7). The signals around 2 ppm correspond to the protons on the acetyl groups.

![Figure 3.6 IR-spectroscopy of azide functionalized polyester](image)

**Figure 3.6 IR-spectroscopy of azide functionalized polyester**

Scheme 3.4 Synthesis of 1,2,3,4,6-penta-O-acetyl-$\alpha$-D-mannopyranoside
Figure 3.7 $^1$H NMR spectrum of 1,2,3,4,6-penta-O-acetyl-α-D-mannopyranoside

Scheme 3.5 Synthesis of acrylate 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside

Scheme 3.6 Synthesis of propargyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside
After protection of hydroxyl group, the desired compounds were synthesized by reacting 1,2,3,4,6-penta-O-acetyl-α-D-mannopyranoside with 2-hydroxyethyl acrylate or propargyl alcohol in the presence of boron trifluoride diethyl etherate (Scheme 3.5, Scheme 3.6). Since the boron trifluoride diethyl etherate is unstable and can be hydrolyzed by moisture in air to form toxic hydrogen fluoride, the compound was handled very carefully. Boron trifluoride diethyl etherate was added using a constant pressure dropping funnel with a rubber septum in case of the release of hydrogen fluoride. Also, the adding rate of boron trifluoride diethyl etherate by constant pressure dropping funnel was required to be very slow.

![Chemical structure](image)

Figure 3.8 $^1$H NMR spectra of acrylate 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside

In addition, the presence of water in dichloromethane may decrease the conversion of these two reactions, therefore dry dichloromethane was freshly distilled and the reaction was carried out under nitrogen atmosphere. The two compounds were both purified by column chromatography to get about 60% yield. The structure of the compounds was
confirmed by $^1$H NMR spectroscopy (shown in Figure 3.8, Figure 3.9). For example, in Figure 3.8, the peaks around 5.86, 6.17 and 6.42 ppm correspond to the three protons on the vinyl group. The signals for the acetyl protecting group in the mannose were shown around 2 ppm.

Figure 3.9 $^1$H NMR spectra of acetyl protected mannose acrylate monomer

3.4 Synthesis of protected-mannose-grafted copolymer (pMGCP) via “grafting to” method

“Grafting to” method was first attempted to synthesize the graft copolymers, which was achieved by two steps as outlined in scheme 3.7 and 3.8. Basically, a propargyl end functionalized graft was synthesized by RAFT using a propargyl functionalized CTA. Then this polymer was attached to the azide functionalized polyesters via azide-alkyne cycloaddition click chemistry.
3.4.1 Synthesis of propargyl end-functionalized mannose-based polyacrylate by RAFT polymerization

Scheme 3.7 Synthesis of propargyl end functionalized mannose-based polyacrylate by RAFT polymerization

To mimic the structure of glycoprotein, short mannose graft (5-10 units) was targeted. Therefore, three different [M]: [CTA]: [I] ratio were chosen for the RAFT polymerization - 3:1:0.1, 5:1:0.1 and 10:1:0.1. The [M]:[CTA] ratio decides the number of repeating unit. The [CTA]:[I] ratio influences the reaction rate and the living character of the polymerization. The reaction was carried out for 24 hours and aliquot was taken to determine the monomer conversion by $^1$H NMR spectroscopy. The ratio of the integration of $-\text{CH}$ peak in the polymer to the integration of $=\text{CH}$ peak in the monomer was used to determine the monomer conversion.

$$Conversion = \frac{I(-\text{CH}^b)_{\text{polymer}}}{I(-\text{CH}^b)_{\text{polymer}} + I(=\text{CH}^a)_{\text{monomer}}} \times 100\%$$

Based on the conversion calculated from the NMR, the theoretical molecular weight was calculated using the following equation:

$$M_n^{\text{calc}} = \frac{[M]_0 \times MW_m \times Conversion}{[\text{CTA}]_0} + MW_{\text{CTA}}$$
The experimental molecular weight was determined by SEC. And the results were shown in Table 3.1. These results showed that the molecular weight of propargyl end functionalized mannose-based polyacrylate can be well controlled by RAFT polymerization.

Table 3.1 Polymerization Conditions and SEC Analysis

<table>
<thead>
<tr>
<th>[M]_0:[CTA]_0:[I]_0</th>
<th>Time (h)</th>
<th>M_n,SEC (kDa)</th>
<th>PDI (M_w/M_n)</th>
<th>Conv. (%)</th>
<th>M_n,theory (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:1:0.1</td>
<td>24</td>
<td>1.8</td>
<td>1.08</td>
<td>100</td>
<td>1.7</td>
</tr>
<tr>
<td>5:1:0.1</td>
<td>24</td>
<td>2.7</td>
<td>1.11</td>
<td>100</td>
<td>2.6</td>
</tr>
<tr>
<td>10:1:0.1</td>
<td>24</td>
<td>4.8</td>
<td>1.13</td>
<td>100</td>
<td>4.8</td>
</tr>
</tbody>
</table>

The structure of the polymer was confirmed by \(^1\)H NMR spectroscopy (shown in Figure 3.10). The broad signals around 2 ppm correspond to the protons on the acetyl groups. The peaks around 0.98 ppm correspond to the protons on the CH\(_3\) at the chain end. and 2.07 ppm correspond to the protons on the polymer backbone. By comparing the integration of the proton in mannose ring signal and that of the methyl group in the chain end, the theoretical molecular weight can be calculated.
Figure 3.10 $^1$H NMR spectrum of propargyl end-functionalized mannose-polyacrylate

3.4.2 Synthesis of pMGCP by azide-alkyne cycloaddition

The Cu(I)Br catalyzed azide-alkyne cycloaddition requires oxygen-free condition to prevent the oxidation of CuBr, and a Schlenk flask was used for degassing. Basically, azide functionalized, CTA and Cu(I)Br were added to dichloromethane (DCM), and the mixture was subjected to three freeze-pump-thaw cycles to remove the oxygen in the system. Since Cu(I)Br is not soluble in DCM, TMEDA (ligand) is required to solubilize
the Cu(I)Br. Thus, after degassing a small amount of TMEDA was quickly added to the Schlenk flask while the mixture in the flask was frozen and protected by nitrogen. Then the flask was sealed and subjected to two freeze-pump-thaw cycles to remove the oxygen that entered the flask during the addition of TMEDA. After the reaction was done, the reaction solution was passed through a short alumina column to remove the Cu(I)Br in the system and the polymer was purified by dialysis in methanol to give a yellow polymer.

The NMR spectrum of pMGCP is shown in figure 3.11. The conversion of this reaction is only 35% based on the average integration from NMR spectrum. Also, the molecular weight determined by SEC is low (Table 3.2). These two results indicated that azide-alkyne cycloaddition between the two polymers was not complete, which is probably due to the steric hindrance.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>M_{n,SEC} (kDa)</th>
<th>PDI (M_w/M_n)</th>
<th>Conv. (%)</th>
<th>M_{n,theory} (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1v3</td>
<td>12</td>
<td>20.3</td>
<td>1.88</td>
<td>14</td>
</tr>
<tr>
<td>1v5</td>
<td>12</td>
<td>21.8</td>
<td>1.96</td>
<td>5</td>
</tr>
<tr>
<td>1v10</td>
<td>18</td>
<td>46.5</td>
<td>1.76</td>
<td>25</td>
</tr>
</tbody>
</table>
3.5 Synthesis of pMGCP via “grafting through” method

Due to the low conversion of grafting to method, grafting through method was then attempted to prepare the desired structure, which may ensure the complete attachment of side chain. Similar to the grafting to method, propargyl end functionalized mannose-based polyacrylate was synthesized by RAFT polymerization. Then, the graft was attached to the azide functionalized diol monomer instead of the azide functionalized polyester, and the product acted as a macro-monomer. After that, the macromonomer was polymerized by polycondensation (Scheme 3.9 and 3.10).

3.5.1 Synthesis RAFT functionalized diol by azide-alkyne cycloaddition

In a typical experiment, a propargyl end functionalized mannose-based polyacrylate with five repeating unit was reacted with the azide functionalized diol to give the macromonomer. The structure of the macro-monomer was confirmed by $^1$H NMR
spectroscopy (shown in Figure 3.12). Also, SEC results showed the monomer before and after conjugation was different, which was 2.7k and 2.9k, respectively. The increase in molecular weight further confirmed the successful synthesis of the macromonomer.

Scheme 3.9 Synthesis RAFT functionalized diol by azide-alkyne cycloaddition

Figure 3.12 $^1$H NMR spectrum of RAFT functionalized diol
3.5.2 Synthesis pMGCP by polycondensation

The graft copolymer pMGCP was then synthesized by DIC/DPTS mediated polycondensation of the macro-monomer and succinic acid (Scheme 3.10). However, the molecular weight determined by SEC was unexpectedly only 4.7K (PDI=1.67), which is probably caused by the polydispersed molecular weight of the macro-monomer.

3.6 Synthesis of pMGCP via “grafting from” method

“Grafting from” method was finally employed to synthesize the graft copolymers, which was achieved by two steps as outlined in scheme 3.11 and scheme 3.12. Basically, a trithiocarbonate RAFT agent was first attached to the side chain of the polyester via Cu(I)Br catalyzed azide-alkyne cycloaddition. After that, the trithiocarbonate pendant group was used as a RAFT initiating group for the synthesis of the graft by RAFT polymerization.
3.6.1 Synthesis trithiocarbonate-functionalized polyester by azide-alkyne cycloaddition

![Scheme 3.11 Synthesis of trithiocarbonate functionalized polyester](image)

The trithiocarbonate-functionalized polyester was prepared by azide-alkyne cycloaddition of azide functionalized polyester and a propargyl functionalized chain transfer agent (CTA). And the NMR spectrum of the polymer was shown in Figure 3.13. Compared to the NMR spectrum of the azide functionalized polymer, additional peaks at 0.87, 1.25, 1.67, 1.94 and 3.23 ppm were observed, which correspond to the signal for the trithiocarbonate. The signal at 7.59 corresponds to the one proton in the triazole ring. It was found that the peak at 3.30 ppm in the azide functionalized polyester of NMR spectrum (which corresponds to the methylene group next to the azide) was disappeared completely, also as shown in Figure 3.14, the peak around 2050 cm\(^{-1}\) corresponding to N-N-N stretching of azide is completely disappear, which indicated the conversion was complete.
3.6.2 Synthesis pMGCP by using protected mannose acrylate

To synthesize the graft copolymers, the trithiocarbonate functionalized polyester was used as a macro RAFT agent so that the graft will grow from the main chain. The [M]:[CTA]:[I] used was 5:1:0.5, 10:1:0.5, and 15:1:0.5. The reaction was carried out for 24 hours and aliquot was taken to determine the monomer conversion by $^{1}H$ NMR spectroscopy. The molecular weight determined by SEC, the molecular weight calculated
from NMR, and the molecular weight calculated based on the [M]:[CTA]:[I] ratio were shown in (Table 3.3). The large difference of the molecular weight is probably because MGCPs are graft polymers and they have very different architecture compared to the linear polystyrene SEC standard. The polymer structure was confirmed by $^1$H NMR (shown in Figure 3.15). The peak around 4.90 ppm corresponds to the proton in the 6-member ring of mannose, and the integration ratio of the peak of three pMGCP (with different side chain repeating unit 5, 10, 15) is approximately 1:2:3, which proves that SEC using linear polystyrene as the standard may not be the ideal method to determine the molecular weight of the described graft copolymers.

![Scheme 3.12 Synthesis of pMGCP by using protected mannose acrylate](image-url)
Figure 3.15 $^1$H NMR spectrum of pMGCP via “graft-from” method

<table>
<thead>
<tr>
<th>[M]$_0$:[CTA]$_0$:[I]$_0$</th>
<th>Time</th>
<th>$M_n$SEC (kDa)</th>
<th>PDI</th>
<th>Conv. (%)</th>
<th>$M_n$theory (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1:0.1</td>
<td>24</td>
<td>53.3</td>
<td>1.76</td>
<td>100</td>
<td>104.1</td>
</tr>
<tr>
<td>10:1:0.1</td>
<td>24</td>
<td>64.0</td>
<td>1.69</td>
<td>100</td>
<td>182.2</td>
</tr>
<tr>
<td>15:1:0.1</td>
<td>24</td>
<td>61.5</td>
<td>1.88</td>
<td>100</td>
<td>260.2</td>
</tr>
</tbody>
</table>
3.7 Synthesis of (mannose-co-phenyl) copolyester

To study the influence of mannose density on binding efficiency, (mannose-co-phenyl) copolyester was synthesized by “graft from” method. Generally, (Azide-co-phenyl) copolyester was synthesized first by polycondensation of phenyl functionalized diol, azide functionalized diol, and succinic acid. Then, propargyl functionalized RAFT agent was attached to the main chain by aizde-alkyne cycloaddition, and the product was then used as macro-RAFT agent for the synthesis of graft copolymers.

3.7.1 Synthesis of phenyl functionalized diol monomer N,N-bis(2-hydroxyethyl) benzenepropanamide

![Scheme 3.13 Synthesis of N,N-bis(2-hydroxyethyl) benzenepropanamide](image)

The synthesis of N,N-bis(2-hydroxyethyl) benzenepropanamide is similar to the synthesis azide functionalized diol monomer, and the synthesis route was shown in Scheme 3.13. The structure and purity of the monomer was confirmed by $^1$H NMR spectroscopy (shown in Figure 3.16). The two peaks around 2.70 and 2.94 ppm correspond to the four methylene groups between the amide group and the phenyl groups. The broad peak at around 3.89-3.41 ppm corresponds to the proton in the backbone and hydroxyl groups. The signal around 7.29 ppm corresponds to the proton in phenyl group. All the peaks in the NMR spectrum were labelled and the integration accord with the desired structure.
Figure 3.16 $^1$H NMR spectrum of N,N-bis(2-hydroxyethyl) benzenepropanamide

3.7.2 Synthesis (azide-co-phenyl) copolyester by polycondensation

![Scheme 3.14 Synthesis (azide-co-phenyl) copolyester by polycondensation](image)

The synthesis scheme is shown in Scheme 3.14, and it is the basic DIC/DPTS mediated copolycondensation of the azide functionalized diol, phenyl-functionalized and succinic acid. The structure and purity of the polymer was confirmed by $^1$H NMR spectroscopy (shown in Figure 3.17). The three peaks around 4.20, 3.60, and 2.66 ppm correspond to the methylene groups in the azide functionalized polyester block. The four peaks around 3.51, 3.56, 3.79 and 3.86 ppm correspond to the four methylene groups between the
amide group and the hydroxyl groups. The two peaks around 2.70 and 2.94 ppm correspond to the four methylene groups between the amide group and the phenyl groups. All the peaks in the NMR spectrum were labelled and all the integration accord with the desired structure. The reaction was carried on room temperature for 48 hours, and the molecular weight determined by SEC is 25.5K and polydispersity index is 1.71.

Figure 3.17 $^1$H NMR spectrum of (azide-co-phenyl) copolyester
3.8.3 Synthesis of (mannose-co-phenyl) copolymer through “grafting from” method

Scheme 3.15 Synthesis of (mannose-co-phenyl) copolymer through grafting from method

This synthesis route is similar to the synthesis of pMGCP via “grafting from” method. Trithiocarbonate pendant group was first introduced into (azide-co-phenyl) copolyester by azide-alkyne cycloaddition, and then the product was used as Macro-RAFT agent for RAFT polymerization of mannose-based acrylate. The structure and purity of the polymer was confirmed by $^1$H NMR spectroscopy (shown in Figure 3.18). The two peaks around 2.70 and 2.94 ppm correspond to the four methylene groups between the amide group and the phenyl groups. The two peaks around 5.30 and 4.90 ppm correspond to the proton in 6-member ring of mannose. All the peaks in the NMR spectrum were labelled and all the integration accord with the desired structure. The molecular weight determined by SEC is 53.4K and PDI is 1.64.
Figure 3.18 $^1$H NMR spectrum of (mannose-co-phenyl) copolymer

3.8 Synthesis of mannose-functionalized N-substituted polyester

Scheme 3.16 Synthesis mannose-functionalized N-substituted polyester

Mannose-functionalized N-substituted polyester was synthesized as the positive control to compare to the MGCP and study the relationship between number carbohydrates in the sides and binding efficiency. This method is similar to “grafting through” method, a propargyl functionalized mannose was reacted with the azide functionalized diol prior to the polymerization. After that, the mannose functionalized
diol was polymerized by DIC/DPTS mediated polycondensation. The structure of the compounds was confirmed by $^1$H NMR spectroscopy (Figure 3.19 and 3.20). Figure 3.19 shows the $^1$H NMR spectra of the mannose functionalized diol. For example, the signal around 2.01 ppm correspond to the protons on the acetyl groups, the signal around 7.60 ppm correspond to the proton on the triazole ring, and the signal around 4.95 ppm correspond to the proton on the mannose ring. Figure 3.20 shows the $^1$H NMR spectra of the mannose functionalized polyester. The peak around 2.61 ppm corresponds to the protons on the backbone. The molecular weight of mannose-functionalized N-substituted polyester is 40.1K and polydispersity index is 1.47.

![Figure 3.19 $^1$H NMR spectrum of mannose-functionalized diol](image-url)
3.9 General deprotection

The acetyl protecting group was then removed using potassium carbonate in methanol. The reaction time was about 45 minutes to two hours and the conversion of the reaction was monitored by TLC during the reaction. Figure 3.17 shows a general $^1$H NMR spectra of the deprotected graft copolymers. From the NMR spectra, the characteristic peaks for acetyl protecting groups around 2 ppm were disappeared completely, which indicated that the polymer was fully deprotected.
Figure 3.21 \(^1\)H NMR spectrum of mannose-grafted copolyester (MGCP)

3.10 Determine the total carbohydrate number

Total carbohydrate number analysis is based on the phenol-sulfuric acid assay which is known as Molisch's test. It is a rapid, simple, and reliable measurement in which carbohydrates react with concentrated sulfuric acid and convert to furfural derivatives, and the derivatives then react with phenol to form the products with purple color that have absorbance at 490 nm (the scheme is shown in Scheme 3.18).

However, phenol-sulfuric acid assay has latent dangerous due to large amount concentrated sulfuric acid is required to be added and the healthy problems caused by introducing phenol. Improvement of this assay was carried out by Ghezzehei and his co-workers.\(^{32}\) Since furfural derivatives naturally have absorbance at 315 nm, after relatively small amount of concentrated sulfuric acid is added, the furfural derivatives can be directly measured by UV spectrophotometer at absorbance at 315 nm.
Scheme 3.18 The principle of determine the total carbohydrate number\textsuperscript{32}

The concentration of mannose standard solution is shown as following:

Table 3.4 Preparation of standard mannose solution

<table>
<thead>
<tr>
<th>concentration (ug/ml)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml mannose stock</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>ml dd water</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>
The absorbance of mannose standard solution is shown as following:

Table 3.5 The result of mannose standard solution from analysis

<table>
<thead>
<tr>
<th>C (mg/ml)</th>
<th>Absorbance(test 1)</th>
<th>Absorbance(test 2)</th>
<th>Absorbance(average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.092</td>
<td>0.077</td>
<td>0.085</td>
</tr>
<tr>
<td>0.02</td>
<td>0.240</td>
<td>0.222</td>
<td>0.231</td>
</tr>
<tr>
<td>0.03</td>
<td>0.188</td>
<td>0.175</td>
<td>0.182</td>
</tr>
<tr>
<td>0.04</td>
<td>0.369</td>
<td>0.361</td>
<td>0.365</td>
</tr>
<tr>
<td>0.05</td>
<td>0.539</td>
<td>0.519</td>
<td>0.529</td>
</tr>
<tr>
<td>0.06</td>
<td>0.557</td>
<td>0.561</td>
<td>0.559</td>
</tr>
<tr>
<td>0.07</td>
<td>0.672</td>
<td>0.657</td>
<td>0.665</td>
</tr>
<tr>
<td>0.08</td>
<td>0.788</td>
<td>0.797</td>
<td>0.793</td>
</tr>
<tr>
<td>0.09</td>
<td>0.820</td>
<td>0.822</td>
<td>0.821</td>
</tr>
<tr>
<td>0.1</td>
<td>0.804</td>
<td>0.822</td>
<td>0.813</td>
</tr>
</tbody>
</table>
Thus, the standard curve is:

\[ y = 9.9662x - 0.0248 \]
\[ R^2 = 0.99718 \]

Figure 3.22 Standard curve derived from analysis

The absorbance of MGCP is shown as following:

Table 3.6 The result of MGCP solution from analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance(test 1)</th>
<th>Absorbance(test 2)</th>
<th>Absorbance(average)</th>
<th>Concentration(mg/ml)</th>
<th>Theoretical $M_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1vs5</td>
<td>0.542</td>
<td>0.544</td>
<td>0.543</td>
<td>0.19</td>
<td>75.0k</td>
</tr>
<tr>
<td>1vs10</td>
<td>0.349</td>
<td>0.303</td>
<td>0.326</td>
<td>0.08</td>
<td>124.0k</td>
</tr>
<tr>
<td>1vs15</td>
<td>0.465</td>
<td>0.486</td>
<td>0.476</td>
<td>0.08</td>
<td>173.0k</td>
</tr>
</tbody>
</table>
From the standard curve, the relationship between absorbance and concentration of mannose is:

\[
\text{Absorbance} = 9.9662 \times C(\text{mannose}) - 0.0248 \quad (1)
\]

And the relationship between concentration of mannose and number of total mannose is:

\[
\text{Number of mannose} = \frac{C(\text{mannose}) \times V(\text{mannose})}{M(\text{mannose})} \div \frac{C(\text{polymer}) \times V(\text{polymer})}{M(\text{polymer})} \quad (2)
\]

Combine (1) and (2):

\[
C \text{ (mannose, 1vs5)} = \frac{(0.543+0.0248)/9.9662}{0.057 \text{mg/ml}}
\]

\[
N \text{ (mannose, 1vs5)} = \frac{0.057 \text{ mg/\text{mL} \times mL}}{180.156 \text{ g/mol}} \div \frac{0.19 \text{ mg/\text{mL} \times mL}}{75000 \text{ g/mol}} = 148
\]

\[
C \text{ (mannose, 1vs10)} = \frac{(0.326+0.0248)/9.9662}{0.0352 \text{mg/ml}}
\]

\[
N \text{ (mannose, 1vs10)} = \frac{0.0352 \text{ mg/\text{mL} \times mL}}{180.156 \text{ g/mol}} \div \frac{0.08 \text{ mg/\text{mL} \times mL}}{124000 \text{ g/mol}} = 327
\]

\[
C \text{ (mannose, 1vs15)} = \frac{(0.476+0.0248)/9.9662}{0.0502 \text{mg/ml}}
\]

\[
N \text{ (mannose, 1vs15)} = \frac{0.0502 \text{ mg/\text{mL} \times mL}}{180.156 \text{ g/mol}} \div \frac{0.08 \text{ mg/\text{mL} \times mL}}{173000 \text{ g/mol}} = 597
\]
<table>
<thead>
<tr>
<th>Sample</th>
<th>Total mannose number (experimental)</th>
<th>Total mannose number (theoretical)</th>
<th>Mannose number in each side chain (experimental)</th>
<th>Mannose number in each side chain (theoretical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1vs5</td>
<td>148</td>
<td>175</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1vs10</td>
<td>327</td>
<td>350</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>1vs15</td>
<td>597</td>
<td>525</td>
<td>17</td>
<td>15</td>
</tr>
</tbody>
</table>

This result shows that the experimental mannose number in each side chain is close to the theoretical number, which proves the complete conversion of the RAFT polymerization via “grafting from” method.

3.11 Determination of the binding efficiency

Various tools were introduced to study the binding events between ligands and receptors, such as: surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) which determine the altering of surface after binding events, isothermal titration calorimetry (ITC) which measure the kinetics changing among binding process, and turbidimetry binding assay (turbidity assay) which study the transmittance and absorbance at 420 nm. In this research work, turbidimetry binding assay was used to study the binding events between MGCP and Con A. In this assay, Con A plays as a cross linker and thus precipitation will be formed due to the binding between MGCP and Con A. The absorbance intensity at 420 nm was monitored with time. Although this method may cause an uncertainty originated during the thoroughly mixing of the existent Con A and MGCP, the result is still able to give us a general idea of the binding behavior. For each mannose containing polyester, the assay was repeated three times.
The binding curve of 1vs5 polyester (polyester with five mannoses in the side chain via grafting from method) is shown in Figure 3.23, 1vs10 polyester (polyester with ten mannoses in the side chain via grafting from method) is shown in Figure 3.24, 1vs15 polyester (polyester with fifteen mannoses in the side chain via grafting from method) is shown in Figure 3.25, grafting to polyester (polyester with ten mannoses in the side chain via grafting to method) is shown in Figure 3.26, Mannose(1vs5)-co-phenyl polyester (polyester with five mannoses in the side chain and phenyl via grafting from method) is shown in Figure 3.27, and also 1vs1 polyester (polyester with single mannose in the side chain) is shown in Figure 3.28. The binding events between Macro-RAFT agent and Con A, as well as grafting to polyester and bovine serum albumin (BSA) were studied by turbidity assay which is shown in Figure 3.29.

![Figure 3.23 The binding curve of 1vs5 polyester from turbidity assay](image-url)
Figure 3.24 The binding curve of 1vs10 polyester from turbidity assay

Figure 3.25 The binding curve of 1vs15 polyester from turbidity assay
Figure 3.26 The binding curve of grafting to polyester from turbidity assay.

Figure 3.27 The binding curve of Mannose(1vs5)-co-phenyl polyester from turbidity assay.
Figure 3.28 The binding curve of 1vs1 polyester from turbidity assay

Figure 3.29 The binding curves of Macro-RAFT agent with ConA and grafting to polyester with bovine serum albumin (BSA) from turbidity assay
In general, the binding curve is as following:

![Absorbance at 420nm over time](image)

**Figure 3.30** The general binding curve of five polyesters from turbidity assay

**Table 3.8 Half maximum binding time and initial binding rate from turbidity assay**

<table>
<thead>
<tr>
<th>Mannose-based Glycopolymer</th>
<th>Number of Mannose</th>
<th>$t_{1/2}$ (min)</th>
<th>$k_i$ ($10^5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1vs1</td>
<td>54</td>
<td>8.5±0.68</td>
<td>5.45±1.12</td>
</tr>
<tr>
<td>1vs5</td>
<td>140</td>
<td>41.5±1.94</td>
<td>7.64±0.79</td>
</tr>
<tr>
<td>1vs10</td>
<td>315</td>
<td>43.5±1.06</td>
<td>29.63±4.87</td>
</tr>
<tr>
<td>1vs15</td>
<td>595</td>
<td>41.2±1.67</td>
<td>12.20±1.16</td>
</tr>
<tr>
<td>1vs10 (GraftingTo)</td>
<td>85</td>
<td>28.2±1.43</td>
<td>91.75±6.79</td>
</tr>
<tr>
<td>1vs5-co-phenyl</td>
<td>52</td>
<td>&gt;60</td>
<td>6.68±1.47</td>
</tr>
</tbody>
</table>

From turbidity assay, half maximum binding time ($t_{1/2}$) and initial binding rate ($k_i$) can be obtained, half maximum binding time is the half time to achieve maximal binding, the initial binding rate was calculated from the beginning of binding events and the results is shown in Table 3.8. It was found that half maximum binding time of most polymers were beyond 25 minutes, and initial binding rate increase with the number of mannose.
However, the initial binding rate will reach a maximum when the number of mannose increase to ten and after that the binding rate will slightly decrease. Similar result was also reported by Stenzel,\textsuperscript{33} who showed that star-shape glycopolymers with medium molecular weight has higher binding efficiency.

Interestingly, the polymer made by grafting to method has the highest binding rate compared to those made by grafting from method. There are two hypotheses for this result. First, high mannose density polymer compared to low mannose density polymer which may actually inhibit availability of inner mannose and decrease the binding efficiency, thus the binding rate will be lower than polymer with low mannose density (as shown in Scheme 3.19). Second, the polymer made by grafting to method is less hydrophilic compared to the one made by grafting from method due to the lower incorporation of mannose, which may precipitate out more easily.

What’s more, the binding between MGCP and BSA as well as Macro-RAFT agent and Con A were investigated by this assay (shown in Figure 3.29). From the result, it clearly shows that there are no binding events between MGCP and BSA, and Macro-RAFT agent and Con A, which proves that Con A is specifically binds to mannose.

\begin{center}
\includegraphics[width=\textwidth]{scheme319.png}
\end{center}

Scheme 3.19 Hypothesis for the three types binding events
CHAPTER IV
CONCLUSIONS

In this work, mannose-based graft copolymers were synthesized via “grafting to”, “grafting through”, and “grafting from” methods to mimic the properties of the natural glycoproteins. The “grafting to” method was achieved by the azide-alkyne cycloaddition of the azide functionalized polyester and the mannose-based polyacrylate end-functionalized with a propargyl group. In “grafting through” method, macro-monomer was synthesized first by azide-alkyne cycloaddition and then it was polymerized by polycondensation. In “grafting from” method, trithiocarbonate pendant groups were introduced into the polyester, which were subsequently used as initiating group for the RAFT polymerization of mannose-based acrylate. The structure of monomer and polymer was confirmed by $^1$H NMR spectroscopy. By comparison, it was found that “grafting from” method was the most ideal method to synthesize the described graft copolymers.

Carbohydrate-protein interaction was studied by turbidimetry binding assay. Firstly, it was found that the binding between most polymers and Con A reach $t_{1/2}$ after 25 minutes. Secondly, the binding rate increased with the number of mannose initially and it would decrease after reaching a certain number. Lastly, high carbohydrates density actually decreases the efficiency of binding events.
REFERENCES


30. G. M. Wutsa. Synthetic Communications 1980, 11, 139-140.


Figure S1. $^1$H NMR spectrum of methyl 6-bromohexanoate

Figure S2. $^1$H NMR spectrum methyl 6-azidohexanoate
Figure S3. $^1$H NMR spectrum

2-(Dodecylsulfanyltiocarbonylsulfanyl)-2-methylpropionic acid

Figure S4. GPC trace of mannose-based polyacrylate by RAFT polymerization (1:3)
Figure S5. GPC trace of mannose-based polyacrylate by RAFT polymerization (1:5)

Figure S6. GPC trace of mannose-based polyacrylate by RAFT polymerization (1:10)
Figure S7. GPC trace of p-MGCP by grafting to method (1:3)

Figure S8. GPC trace of p-MGCP by grafting to method (1:5)
Figure S9. GPC trace of p-MGCP by grafting to method (1:10)

Figure S10. GPC trace of Macro-monomer
Figure S11. GPC trace of p-MGCP by grafting through method

Figure S12. GPC trace of azide functionalized polyester
Figure S13. GPC trace of Macro-RAFT agent

Figure S14. GPC trace of p-MGCP by grafting from method (1:5)
Figure S15. GPC trace of p-MGCP by grafting from method (1:10)

Figure S16. GPC trace of p-MGCP by grafting from method (1:15)