SYNTHESIS AND STRUCTURE-PROPERTY CORRELATIONS OF
\(\gamma\)-SUBSTITUTED PYRROLIDONE-BASED POLYMERS

by

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Abstract of thesis

Synthesis and Structure-Property Correlations of γ-Substituted Pyrrolidone-Based Polymers

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Synthesis and characterization of novel γ-substituted pyrrolidone-based polymers and PEG-PPS based polymers have been reported. In this thesis, pyrrolidone-based polymers have been explored with an interest to study the influence of substituents on the γ-position. Synthesis of 5-methoxy-2-pyrrolidone was carried out using anodic decarboxylation of pyroglutamic acid. Acid catalyzed alkoxy/thioate exchange of 5-methoxy-2-pyrrolidone, derivatization with acryloyl moiety and subsequent polymerization of the monomers, yielded pyrrolidone-based polymers that varied in their γ-substituents. Polymerization was initially carried out via conventional free radical polymerization but later on was carried out via reversible addition-fragmentation transfer (RAFT) polymerization in order to get molecular weight control and narrower dispersity. The glass transition temperature $T_g$ was found to be significantly influenced by both substituent structure (e.g., saturated linear aliphatic vs cyclic aliphatic vs aromatic) and chemical class.

Inspired by these findings, amphiphilic block copolymers comprising of pyrrolidone-based polymers as the hydrophilic and hydrophobic blocks were synthesized. These block copolymers comprised of poly(MeOEtONP) as the hydrophilic block and poly(N-acryloyl-2-pyrrolidone) (NP), poly(N-acryloyl-5-ethoxy-2-pyrrolidone) (EtONP) or poly(N-acryloyl-5-ethylthiolate-2-pyrrolidone) (EtSNP) as the hydrophobic block.
Studies were conducted to assess the influence of the length of hydrophilic block and the chemical class of bridging unit in the hydrophobic block. Critical micelle concentration, turbidimetry and hydrodynamic radii studies indicate an increase in hydrophobicity on addition of a $\gamma$-substituent on the pyrrolidone ring. Drug-encapsulation and release studies conducted on the block copolymers suggested that the structure of the hydrophobic block in the copolymer plays an important role in determining the performance of these polymeric nanocarriers.

In a collaborative project with Prof. Robert K. Prud’homme of Princeton University, amphiphilic poly(ethylene glycol)-$b$-poly(propylene sulfide) (PEG-PPS) block copolymers of variable lengths were synthesized via anionic initiation method for flash nanoprecipitation of drug-loaded polymeric nanoparticle. PEG-PPS based block copolymers of variable lengths were synthesized and thermal properties were analyzed through differential scanning calorimetry and thermal gravimetric analysis. Initial studies conducted in the Prud’homme laboratory indicate a significant change in polymeric nanoparticle size upon subjecting it to oxidizing conditions. This indicates promising applications of the synthesized polymers in the field of drug-delivery.
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<th>Description</th>
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<tbody>
<tr>
<td>2D</td>
<td>2- Dimensional</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celcius</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublet</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom(s)</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>Approx.</td>
<td>Approximately</td>
</tr>
<tr>
<td>AROP</td>
<td>Anionic ring opening polymerization</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom-transfer radical polymerizations</td>
</tr>
<tr>
<td>BDTB</td>
<td>Benzyl dithiobenzoate</td>
</tr>
<tr>
<td>COSY</td>
<td>Homonuclear correlation spectroscopy</td>
</tr>
<tr>
<td>D</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DLC</td>
<td>Drug loading content</td>
</tr>
<tr>
<td>DLE</td>
<td>Drug loading efficiency</td>
</tr>
<tr>
<td>DPₙ</td>
<td>Degree of polymerization</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>DMA</td>
<td>Dimethyl acrylamide</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified Eagle’s medium</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DOX. HCl</td>
<td>Doxorubicin hydrochloride</td>
</tr>
<tr>
<td>Equiv.</td>
<td>Equivalent</td>
</tr>
<tr>
<td>FNP</td>
<td>Flash nanoprecipitation</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>K$_2$CO$_3$</td>
<td>Potassium carbonate</td>
</tr>
<tr>
<td>LiBr</td>
<td>Lithium bromide</td>
</tr>
<tr>
<td>LCST</td>
<td>Lower critical solution temperature</td>
</tr>
<tr>
<td>M</td>
<td>Molarity</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>$\bar{M}_n$</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>$\bar{M}_w$</td>
<td>Weight average molecular weight</td>
</tr>
<tr>
<td>mmol.</td>
<td>Millimoles</td>
</tr>
<tr>
<td>NMP</td>
<td>nitroxide-mediated polymerization</td>
</tr>
</tbody>
</table>
NMR          Nuclear magnetic resonance
PEG          Poly (ethylene glycol)
PGA          Pyroglutamic acid
PPS          Poly (propylene sulfide)
PNIPAAm      Poly (N-isopropylacrylamide)
PLA          Polylactide
PSt          Polystyrene
PVP          Poly (vinylpyrrolidone)
RAFT         Reversible addition fragment chain transfer
Rf           Retention factor
ROS          Reactive oxidative species
s            Singlet
t            Triplet
T\text{dec}  Decomposition temperature
T\text{g}    Glass transition temperature
T\text{m}    Melt transition temperature
THF          Tetrahydrofuran
UV-Vis       Ultraviolet-Visible spectrum
wt           Weight
CHAPTER 1
Introduction

1.1 Poly(N-vinylpyrrolidone)

In early 20th century, I. G. Farben dominated the world market in the production of synthetic aromatic dyes.\textsuperscript{1} Its gradual expansion into acetylene chemistry in the early 1930s was considered a risky venture due to the explosive nature of the small molecule. Despite the potential dangers, these pioneering investigations paved the way for a variety of acetylene-based products.\textsuperscript{2}

![Poly(N-vinylpyrrolidone)](image)

**Figure 1-1** Poly(N-vinyl pyrrolidone) or PVP

Dr. Walter Reppe, a German chemist employed with I.G. Farben, was one of the few chemists conducting research on the safe-handling of acetylene under high pressure. Pioneered by Reppe, acetylene research had a far-reaching impact on the development of many processes and the synthesis of numerous products including the production of synthetic rubber.\textsuperscript{3} During the course of his work, Walter Reppe and his research team would synthesize \textit{N}-vinyl pyrrolidone (NVP) (Scheme 1-1).\textsuperscript{4} He subsequently polymerized it to create poly(\textit{N}-vinyl pyrrolidone) (PVP) or povidone, with a patent filed in 1939.\textsuperscript{5} The water-soluble PVP was found to be completely soluble in buffered saline solution at a concentration of 3.5 per cent, making it nearly isotonic with blood plasma. Consequently,
it was administered to large number of injured soldiers and civilians during the ensuing war years in Germany, Europe and United States.\(^6\)

**Scheme 1-1** Synthesis of \(N\)-vinyl pyrrolidone from acetylene.

PVP is a multipurpose, water-soluble polymer with a broad scope of utility\(^7\) due to its excellent biocompatibility\(^8\) and strong coordination ability.\(^9\) Moreover, the chemical structure of PVP enables it to form several complexes with low molecular-weight compounds of pharmaceutical significance\(^10\) making drug delivery one of its first sought after applications.\(^11\) For instance, owing to the ability of PVP to complex iodine, PVP-I\(_2\) solutions contain much lower free iodine rendering it a non-irritating, milder antiseptic alternative to standard aqueous I\(_2\) solutions.\(^12\) In addition to its use as drug carriers,\(^13,14\) PVP has also been used as nanoparticle stabilizers,\(^15\) dispersing agents,\(^16\) adhesives,\(^17\) and as surface ligand immobilizers where the modified PVP polymer is adsorbed on colloidal silica nanoparticle that enables terminal groups to be accessible for reactions.\(^18\) But despite its industrial utility, PVP lacks functionality along the polymer chain thus limiting its potential in the field of biotechnology. To circumvent this issue, chemists have tried: i) copolymerizing it with different monomers, ii) introducing functionalities on the
pyrrolidone moiety, and/or iii) introducing a spacer between pyrrolidone ring and vinyl group.

The difference in reactivities between NVP and other related monomers such as methacrylates leads to small amount of NVP that is incorporated into the copolymer chain during radical copolymerization.\textsuperscript{19} In order to overcome this issue and integrate more pyrrolidone functionality into the copolymer, the NVP monomers have been modified with carboxylic acid,\textsuperscript{20} (Figure. 1-2a), thiol, or hydroxyl groups (Figure. 1-2b,c).\textsuperscript{21} Subsequent copolymerization of these derivatives with NVP gave rise to uniform random copolymers with pendant functional groups along the backbone.\textsuperscript{22} Inspired by the preliminary attempts to modify the pyrrolidone ring, Ritter \textit{et al.}\textsuperscript{23} and Li \textit{et. al.}\textsuperscript{24} synthesized PVP modified at the \(\alpha\)-position with an ethyl group (C\(_2\)-PVP). In addition to its water solubility, they also observed that C\(_2\)-PVP (Figure 1-2-d) displayed a reversible lower critical solution temperature (LCST). That is, a temperature where the polymer chains in solution undergo coil-to-globule transformation, a stimuli-responsive phenomenon to be explained further in section 1.3. of this chapter. Inspired by these findings, we set forth to modify the pyrrolidone ring at the gamma position with the hope of altering the physiochemical property of the polymers bearing variety of alkoxy, aryloxy, thiolate or ether groups, results of which are explained in detail in Chapter 2.
1.2 Polymerization Techniques

Polymerization methods are broadly classified into two categories: a) step-growth polymerization and b) chain-growth polymerization. Step-growth polymerization refers to a technique in which bifunctional or multifunctional monomers add to each other simultaneously involving multiple sites of initiation and propagation (Figure 1-3). During the course of this polymerization, small molecules such as water, gas, or HCl are released. Examples of polymers prepared in this style include polyesters, polyamides, and polyethers as illustrated in Fig. 1-4a, b and c.25

![Diagram of Polymerization Techniques](image)

**Figure 1-3** Schematic diagram of step-growth and chain growth polymerization.
Chain growth polymerization is a technique where monomers are added sequentially to the active site of the polymer chain,\textsuperscript{26} with the addition of each unit creating a new active site (\textit{e.g.}, radical or charged species) that attacks the next monomer (Figure. 1-3). Unlike step-growth polymerization where monomer consumption is rapid in the initial period of synthesis, only few macromolecules are active at a certain time in case of chain growth polymerization. In this thesis, conventional free-radical, reversible addition-fragmentation chain-transfer (RAFT), and anionic ring-opening polymerizations were used to prepare the (co)polymers described herein. Few examples of polymers prepared \textit{via} chain growth polymerization are enlisted in Fig. 1-4 d, e and f.

\textbf{Figure 1-4} Examples of polymers prepared through step-growth and chain growth polymerization.

\textbf{1.2.1 Conventional Free-radical polymerization}

Conventional free-radical polymerization involves the sequential addition of monomer units. The mechanism of the free-radical polymerization consists of distinct steps that include initiation, propagation, and termination, along with some side reactions (\textit{e.g.} chain-transfer) that cause an increase in polymer dispersity. Generally, monomers undergoing free-radical polymerization contain an unsaturation in the molecule.\textsuperscript{27} The first
report of ethylene molecule undergoing free radical polymerization by using mercury as an initiator was observed in 1926 by Olsen and Meyers. Over the course of years, azo compounds and peroxides were seen to be more effective as initiators.

In the first step, the initiator molecules are thermally or photochemically decomposed to generate a free radical that can attack a carbon-carbon double bond in another molecule to create a new radical, starting a chain reaction. Thermal decomposition is also a common method of initiation where an appropriate compound is heated until the bond is homolytically cleaved to generate two sets of radical initiators. This method is commonly employed for the initiation of peroxide radicals and azo compounds such as azoisobisbutryonitrile (AIBN) (Figure 1-5).

![Figure 1-5](image)

**Figure 1-5** Examples of initiators used in conventional free-radical polymerizations.

In the propagation step, the newly generated "active species" adds to another monomer in the same manner as in the initiation step. This step is repeated until all the monomer is consumed. The factors affecting the polymerization include polarity of the bond, solvent polarity, and steric influences of the monomer. Termination of the reaction can occur via coupling of radical species or disproportionation. Due to the notoriety of the side reactions like chain transfer, the polymers synthesized via free radical polymerizations have a broad dispersity, therefore, controlled radical polymerizations such
as reversible addition-fragmentation chain transfer (RAFT) polymerization are often employed to afford polymers with lower dispersity.

**Scheme 1-2** Mechanism of conventional free-radical polymerization.

1.2.2 Reversible Addition-Fragmentation Chain Transfer (RAFT)

The advantages of RAFT polymerization over conventional free-radical polymerization are numerous and include the ability to produce polymers with controlled molecular weight, narrow dispersity, and tailored end groups. Moreover, this polymerization is tolerant to a number of functional groups. RAFT chain transfer agents are usually comprised of thiocarbonyl, thiolate, and an alkyl or aryl group as illustrated in Figure 1-6. The dithioate group is mainly responsible for achieving the reversible step of radical formation through the addition of the polymer chain, thus giving it a living nature.
The mechanistic pathway of RAFT polymerization is illustrated in Scheme 1-2. In the first step, an initiator molecule is thermally or photochemically decomposed in order to generate an active species.\textsuperscript{35} Initiators can be disulfides,\textsuperscript{36} peroxides, or azo compounds.\textsuperscript{37} The polymerization is set in motion by the attack of the initiator species on the monomer molecule. Chain growth takes place by the repetitive attack of the polymer chain-end on the monomer molecules. The rapid equilibrium of the propagating polymeric radical and the former dormant polymer on the dithioate compounds is what’s responsible for the narrow polymer dispersity as it gives equal opportunity for all the polymer chains to grow.\textsuperscript{38} RAFT has been successfully applied for the preparation of polymeric materials derived from a wide variety of monomer classes, including (co)polymer with ionic functionalities without compromising polymerization control.\textsuperscript{39} The versatility of the reaction along with its tolerance to a variety of reaction conditions, clearly makes RAFT one of the most superior polymerization techniques. As such, we chose to employ RAFT for the polymerization of several monomers described in this thesis.

**Figure 1-6 Examples of RAFT agents**

![Chemical structures of RAFT agents](image)
Before the discovery of RAFT polymerization, the living radical polymerization for the synthesis of block copolymers, had to be carried out by successive polymerization of two or more monomers without the intermediate purification. However, this method often led to irregularities in the monomer consumption due to the reactivity differences and thus was strongly limited to few monomers with similar physicochemical properties. Although nitroxide-mediated polymerization (NMP) and atom-transfer radical polymerizations (ATRP) have been employed in the past successfully for different monomers, they appear to lack tolerance for various functionalities such as carboxylic acid. ATRP products are also generally contaminated by metal ions as they are a part of initiation process. These processes can be mitigated by appropriate choice of solvents and conditions but clearly, RAFT polymerization is the superior choice for the ease of polymer synthesis.
1.2.3 Anionic Ring-Opening Polymerization (AROP)

Anionic ring-opening polymerization proceeds via nucleophilic attack by an anion on a cyclic monomer. The mechanism of AROP is outlined in scheme 1-3. Initiators employed for anionic ring-opening polymerizations are usually comprised of organometallic compounds such as organolithium,\textsuperscript{45} Grignard reagents,\textsuperscript{46} metal amides,\textsuperscript{47} or metal alkoxides.\textsuperscript{48} In presence of an electronegative atom in the ring, the attack of the nucleophile takes place especially on the carbon-heteroatom bond causing the cleavage of the bond.\textsuperscript{49} In case of unsymmetrical monomers, the attack of the nucleophile can take place in two ways as given in Fig. 1-7. End-group analysis and careful observation of stability of the intermediate anion can reveal the preferred pathway for the preferred structure of polymer.\textsuperscript{50}

![Figure 1-7 Nucleophilic ring opening in unsymmetrical heterocycles.](image)

Propagation of the polymerization takes place by the repetitive attack of the nucleophilic end of the growing polymer chain on the electron-deficient carbon of the monomer. The termination of the growing polymer chain can occur either by abstraction of a proton from solvent or water\textsuperscript{51} or by the addition of external quenching agent.\textsuperscript{52} The mechanism of anionic ring-opening polymerization is outlined in scheme 1-4. The first reports of anionic ring-opening polymerization dates back to 1906 when Leuchs reported on the synthesis of a polypeptide by ring-opening a \( N \)-caboxyanhydride derivative of an amino acid.\textsuperscript{53} Subsequently, mechanistic pathways of AROP were established through: i) studies on anhydrous sugars that provided polysaccharides after AROP and ii) calculations.
of the free energy of polymerization of cycloparaffins.\textsuperscript{54} Indeed, cyclic compounds that have highly polarizable bonds or bonds between carbon and a heteroatom are the most susceptible to anionic polymerization due to the easier attack of the nucleophile on the electrophilic carbon.\textsuperscript{55} Enthalpy also plays an important role in AROP. That is, three-to-eight membered rings polymerize readily due to the loss of enthalpy associated with complete loss of ring strain.\textsuperscript{56} Introducing moieties such as sulfides, silicon, or carbonate lead to an increase in rotational freedom of these groups resulting in an increase in entropy, the chief motive for ring-opening polymerization. \textsuperscript{57}

\textbf{Initiators}

\begin{align*}
\text{R-Li} & \rightarrow \text{R + Li} \\
\text{R-MgX} & \rightarrow \text{R + MgX} \\
\text{NaNH}_2 & \rightarrow \text{Na + NH}_2 \\
\text{R-ONa} & \rightarrow \text{R-O + Na}
\end{align*}

\textbf{Propagation}

\textbf{Termination}

\textbf{Scheme 1-4} Mechanism of anionic ring opening polymerization (AROP).

One of the most important products of anionic ring-opening polymerization is the Nylon-6 prepared by the AROP of \(\varepsilon\)-caprolactam\textsuperscript{58} (Figure 1-8a). Nylon-6 was first synthesized by Paul Schlack\textsuperscript{59} in late 1930s. Due to its high crystallinity and the tensile
strength, it was immediately employed in the manufacturing of stockings, parachutes, and toothbrush bristles. Other examples of heterocycles that undergo AROP are given in Fig 1-8. For instance, poly(ethylene oxide) derived from the ring-opening polymerization of ethylene oxide (Figure 1-8b) is a versatile, water-soluble polyether with low toxicity that is widely used in the pharmaceutical industry.

![Diagram of cyclic monomers for AROP](image)

**Figure 1-8** Examples of common cyclic monomers for AROP

Finally, episulfides are a class of compounds that are structurally similar to epoxides and as a result, can be polymerized through anionic ring-opening polymerization. Polymerization of propylene sulfide (Figure 1-8e), has gained a lot of attention in the past few decades with research being dedicated to investigating the mechanism of polymerization and application. For instance, block copolymers comprised of poly(propylene sulfide) blocks have been studied for a variety of applications such as photolithography, nanoparticles for chemisorption on gold, and in the field of drug-delivery. The end group of the polymer could be fashioned to bear reactive functional groups such as acrylates for chain-extension of the polymer, nitroso groups for bioactivity, or Michael-addition type of reactions for the purpose of surface modification of nanoparticles.
Anionic ring-opening of propylene sulfide can be carried out with thiols as an initiator due to the mild nature of thiolates.\(^7\) Owing to the versatility of poly(propylenesulfide) (PPS) and the oxidation-responsive nature, PPS is emerging as a new stimuli-responsive polymer that could be modified for drug-delivery studies.\(^7\) A more thorough investigation on the ring-opening synthesis of propylene sulfide is discussed in Chapter 4.

1.3 Stimuli-responsive polymers

Stimuli-responsive polymers are polymers that undergo large and abrupt physical or chemical changes in response to changes in its environment.\(^7\) The nature of these stimuli could be physical, such as temperature, magnetic field, or mechanical stress; or chemical which include changes in pH, ionic strength, and oxidation state.\(^7\) Poly(\(N\)-isopropylacrylamide) (PNIPAAm) is a popular thermo-responsive polymer that shows a distinct phase separation in aqueous solution owing to the coil-to-globule transformation at 32°C.\(^7\) Some systems are even designed to incorporate more than one stimulus (for instance, both pH and temperature) in order to impart a dual response in nanocarriers for unloading a hydrophobic payload.\(^7\) As such, stimuli-responsive polymers are emerging as an innovative means of delivering pharmaceutical agents in the form of nanocarriers,\(^7\) bioconjugates\(^7\) and hydrogels.\(^7\) In this thesis we have prepared thermo-responsive polymers and oxidation-stimuli based polymers for drug delivery applications.

1.3.1 Thermoresponsive Polymers

One of the most commonly employed stimuli for inducing a phase change in stimuli-responsive materials is temperature. Variation and control of temperature is not only relatively easy, but also applicable in both \textit{in vitro} and \textit{in vivo} environments.\(^8\) The
most interesting characteristic of all thermoresponsive polymers is their ability to undergo a phase change in solution with variation in temperature, known as a critical solution temperature.\textsuperscript{73} When the homogenous polymer solution undergoes an abrupt phase change, \textit{i.e.} polymer precipitates out upon increasing temperature, it is said to have a \textit{lower critical solution temperature} (LCST) (Figure. 1-9a, b). The reason for this phase is because of the favorable increase in the entropy of the system.\textsuperscript{81} That is, an increase in temperature induces the dehydration of the polymeric chains by breaking the hydrogen-bonds between the polymer and solvent molecules (in most cases, the solvent is water), thus causing a phase separation.\textsuperscript{82} Some polymers dissolve in aqueous solutions upon increasing the solution temperature.\textsuperscript{83} These polymers possess an \textit{upper critical solution temperature} (UCST) (Figure 1-8a, c). It is important to note that a USCT phase separation is enthalpically driven effect rather than entropically driven as in the case of polymers with LCST.\textsuperscript{84} However, LCST-based polymer systems are relatively more popular in the field of drug-delivery as the formulations can be prepared with ease when the polymer solution is homogenous. \textsuperscript{67d}
Figure 1-9 A) Phase diagrams for upper critical solution temperature and lower critical solution temperature (solid lines) and the overlapping area of UCST and LCST (dotted lines) (Figure adapted from reference 72 (b)). B) Phase transition of PNIPAAm showing LCST in aqueous solution (Figure adapted from reference 86 (b)) C) Phase transition of poly(N-propionyl-aspartic acid/ethylene glycol) displaying UCST in aqueous solution (Figure adapted from reference 80 (f)).

There are several polymers that are used towards the synthesis of thermoresponsive materials. Some of the more popular examples that possess an LCST are illustrated in Figure 1-10. Along with PNIPAAm, poly(N-vinyl caprolactone) (PVCL),\textsuperscript{85} poly(N-(dl)-(1-hydroxymethyl) propylmethacrylamide), \textsuperscript{(p(dl)-HMPMA)}\textsuperscript{86} and poly(N,N'-diethylacrylamide) (PDEAAm)\textsuperscript{87} are also temperature-responsive, with LCSTs of \textit{ca.} 32°C, 37°C, and 33°C, respectively. It is interesting to note that although the LCST of PDEAAm is very close to that of PNIPAAm, the phase transition of the polymer heavily depends on the tacticity of the polymer.\textsuperscript{88}
As mentioned earlier, PNIPAAm is a well-known polymer that is extensively used in the synthesis of biomaterials as it exhibits a reversible coil-to-globule transition at a temperature (32°C) close to that of the human body. The attractive thermoresponsive properties of PNIPAAm were reported in 1968 and paved the way for exploration of the material for potential biomedical applications. One of the main uses of thermoresponsive polymers is in the field of drug-delivery. The biocompatibility, water-solubility, and the reversible phase transition of the polymer at a temperature close to human body makes PNIPAAm a very versatile polymer for controlled drug-release. Although the LCST of PNIPAAm is independent of molecular weight and concentration, it can be tuned by copolymerizing the monomer with hydrophobic or hydrophilic monomers (Figure 1-11a, b) or hydrophilic monomers (Figure. 1-11, c, d).
1.3.2 Oxidation-responsive polymers

Reactive oxygen species (ROS) like hydrogen peroxide, superoxides, hydroxyl radicals, peroxynitrite, and hypochlorite play vital roles in cell signaling pathways.\textsuperscript{95} In healthy tissue, ROS are produced at low levels throughout the electron-transport pathway during aerobic respiration.\textsuperscript{96} However, oxidative species released in higher concentration often indicate a pathological situation and may lead to oxidative stress that has far reaching consequences like impairment of cell components such as proteins, lipids and even DNA.\textsuperscript{97} Moreover, macrophages tend to accumulate in tissues with inflammations and cancerous cells, leading to increased levels of ROS. In this context, oxidation-responsive polymers are an obvious choice for stimuli-induced drug release and have recently drawn considerable attention due to their ability to respond to ROS generated in the cell.\textsuperscript{98} This also represents their potential use as biosensors in drug delivery and as protective barriers against oxidative stress.\textsuperscript{99}

Promising candidates for oxidation-responsive drug carriers are polymers that bear seleno-ethers,\textsuperscript{100} thioketals,\textsuperscript{101} phenyl boronic acids/ester,\textsuperscript{102} and thioether functional
groups\textsuperscript{72} as illustrated in the Table 1-1. Of all the candidates studied, the most investigated oxidation-sensitive materials are thioether-based polymers\textsuperscript{104}. In a pioneering study by Hubbell and co-workers, a block copolymer of poly(ethylene glycol) (PEG) and poly(propylene sulfide) (PPS) was reported \textit{via} ring-opening of propylene sulfide by the attack of deprotected PEG-thiolate on the monomer, propylene sulfide\textsuperscript{105}. The synthetic procedure reported was particularly promising for generating polymeric amphiphiles, especially as drug carriers, as it does not require complicated purifications. The synthetic procedure was seen to tolerate common polar groups such as alcohols, esters, amides and carboxylic acids\textsuperscript{106}.

As an extension of this work, the oxidation-response by polymeric vesicles based on the ABA block polymers of poly(ethylene glycol) (PEG) and poly(propylene sulphide) (PPS) was also studied in detail\textsuperscript{72} (Figure. 1-12 b). They observed that owing to the low dipole moment of PPS that imparts more hydrophobicity to the polythioether unit than its oxygen-containing analogue, \textit{i.e.}, PEG, the copolymers self-assemble into unilamellar vesicles. Moreover, the vesicles formed in solution were seen to rapidly undergo oxidation of the PPS block in presence of hydrogen peroxide. The rapid oxidation of thioether moiety

\textbf{Figure 1-12} A) Structure of amphiphilic PEG-PPS block copolymer (reference 102) B) Oxidation of PEG-PPS-PEG triblock copolymer in presence of hydrogen peroxide (reference 69)
of the block copolymer to sulfoxides and ultimately to sulfones, increases the polarity of
the polymer leading to an abrupt phase change. These studies indicate that the block
copolymer vesicles were sensitive to oxidation and could undergo similar disruption in the
vesicular structure in the presence of peroxide, the most prevalent ROS in biological
system. Consequently, these vesicles can be loaded with drug for the release at the
appropriate site of inflammation or tumor by the application of oxidation-stimulus.

Table 1-1 Examples of reactive-oxygen species (ROS) polymers.

<table>
<thead>
<tr>
<th>ROS-responsive polymers</th>
<th>Chemical structure and oxidation of polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenoethers</td>
<td>$\text{Se} \xrightarrow{\text{O}} \text{Se} + \text{Se}$</td>
</tr>
<tr>
<td>Phenylboronic acids/esters</td>
<td>$\text{Ph} \xrightarrow{\text{H}_2\text{O}_2} \text{B-OH} + \text{Ph}$</td>
</tr>
<tr>
<td>Thioketals</td>
<td>$\text{} \xrightarrow{\text{O}} \text{} + \text{SH}$</td>
</tr>
<tr>
<td>Thioethers</td>
<td>$\xrightarrow{\text{H}_2\text{O}_2} \xrightarrow{\text{H}_2\text{O}_2} \text{S}$</td>
</tr>
</tbody>
</table>
1.4 Structure modification of stimuli-responsive block copolymers for drug-delivery

Drug encapsulation is a phenomenon that is dependent upon multiple block copolymer characteristics such as hydrophobicity, structure, and polymer-drug interaction. One of the most popular water-soluble thermoresponsive polymers that has been studied for stimuli-responsive drug-delivery applications is PNIPAAm. In the past few decades, there has been a surge in the synthesis and modification of PNIPAAm-based block copolymers for studying the relationship between drug encapsulation and block copolymer structure. For instance, Chung et al. investigated the thermo-responsive behavior of polymeric micelles for thermal-induced drug release as a function of hydrophobic block structure. Block copolymer micelles comprised of PNIPAAm coronae and either poly(butyl methacrylate) (PBMA) or polystyrene (PSt) cores (Figure. 1-13) were prepared in aqueous media using the dialysis method.

![Figure 1-13](image)

**Figure 1-13** Block copolymers of PNIPAAm employed for drug release in reference 105.

The micelles showed reversible intermicellar dispersion/aggregation in response to temperature cycles at 32.5°C as observed by turbidimetric experiments. Upon heating above the LCST, PNIPAAm–PBMA micelles were seen to exhibit an abrupt increase in micropolarity and an abrupt decrease in microrigidity as sensed by the chemical probe pyrene and 1,3-bis(1-pyrenyl)propane (PC₃P), respectively. This was hypothesized to be a result of change in the microenvironment of pyrene due to the collapse of outer PNIPAAm
shell. On the contrary, PNIPAAm–PSt micelles maintained constant values with lower micropolarity and higher microrigidity than those of PNIPAAm–PBMA micelles over the temperature range 20 to 40°C. This indicates that regardless of heating or cooling of PNIPAAm outer shells, the inner cores comprising of PSt segments do not readily permit a structural change during the phase transition. Drug encapsulation studies on these systems were conducted using Adriamycin as a pharmaceutical agent. Upon raising the temperature of the polymeric system with the loaded drug, only PNIPAAm–PBMA micelles released the drug, whereas the thermo-responsive drug release capability was absent in the micelles prepared by PNIPAAm–PSt. It is clear that since the outer shell of both polymeric systems was comprised of PNIPAAm, the disparity in thermo-responsive drug release capability originates from the design of the hydrophobic block. Thus, the nature of hydrophobic segments comprising the micelle inner core offers an important control point for thermo-responsive drug release by the thermo-responsive polymeric micelle.

In another example, thermo-responsive micelles were synthesized by employing poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide) (PNIPAAm-co-DMA) with a lower critical solution temperature (LCST) around 40 °C, while biodegradable poly(D,L-lactide) (PLA), poly(ε-caprolactone) (PCL), or poly(D,L-lactide-co-ε-caprolactone) (PLA-co-CL) was used as the hydrophobic block (Figure 1-14). Variation of both the block lengths of the poly(D,L-lactide)-containing block copolymers was seen to influence the physical parameters such as hydrodynamic radii and critical micelle concentration.
Figure 1-14 PNIPAAm-co-DMA-based thermoresponsive block copolymers mentioned in reference 120.

While the polymeric micelles of consisting of (PNIPAAm-co-DMA)-block-PCL did not show a thermoresponsive drug release at temperatures higher than the LCST, the polymeric micelle comprised of (PNIPAAm-co-DMA)-block- PLA and (PNIPAAm-co-DMA)-block- (PLA-co-CL) successfully exhibited thermoresponsive drug release at 41°C. The cumulative drug release of (PNIPAAm-co-DMA)-block- (PLA-co-CL) was observed to be 84% in ten hours as compared to (PNIPAAm-co-DMA)-block-PLA that showed 15% total drug release after 42.5 hours, thus emphasizing the importance of inner core modification.

It is interesting to note that in the examples mentioned above, while relationships between drug-encapsulating ability and copolymer structure have been studied to date, few experimental studies have examined drug loading and thermoresponsive release profiles as a function of hydrophilic block length and hydrophobic block residue class. Moreover, in past reports, micelles were comprised of hydrophobic repeat units with diverse chemical architectures and as a result, structure/property correlations could not be adequately addressed. Therefore, there remains a need for comparative analyses that evaluate
thermoresponsive block copolymer micelles with only modest differences in their hydrophobic interior in order to assess the dependence of drug loading and release phenomena on the nature of the polymeric core as well as the length of the hydrophilic block. This is critical to establishing design criteria for micellar drug delivery vehicles with efficient encapsulation and release profiles. Indeed, this topic is related to the research described in Chapter 3 where thermoresponsive block copolymer micelles comprised of hydrophilic and hydrophobic pyrrolidone-based polymer blocks are described, along with their physicochemical properties and drug-encapsulation and release capabilities as a function of hydrophilic block length and hydrophobic core structure.

1.5 Flash nanoprecipitation

Approximately, 30–40% of pharmaceutically active ingredients have poor aqueous solubility, which presents a problem when developing aqueous formulations. In order for large drug molecules to be soluble in water, the molecular structure of the drug could be modified to incorporate more hydroxyl, carboxyl, or ionic groups. Unfortunately, modification of the drugs can lead to loss of bioactivity, increase in crystallinity (decreasing solubility) and decrease in bioavailability. Amphiphilic polymers are widely being employed in micellar, vesicular, or liposomal forms as nanocarriers for drug-delivery. With an average size of ca. 10-100 nm these types of nanocarriers are ideal for stability and long circulation times in the body. The block copolymers that are generally employed for this application are amphiphilic in nature and rely on differences in hydrophobic character between both blocks to spontaneously self-assemble in aqueous media. Vesicles are formed when amphiphilic polymer molecules assemble into a bilayered nanoparticle with hydrophilic exterior and interior (Figure. 1-15A)
Phospholipid-based self-assembly in aqueous solutions are referred to as liposomes. Liposomes are vesicles that consist of hydrophilic interior enveloped by lipid bilayers.\textsuperscript{123} Contrary to vesicles and liposomes, micelles (Figure 1-15B) are simpler structures with hydrophobic interiors and a stabilizing hydrophilic exterior. The hydrophobic microenvironment of these nanoparticles enables loading of the hydrophobic drug while the hydrophilic coronae shields the core from unwanted interaction in the system.\textsuperscript{124}

\textbf{Figure 1- 15} A) Cross section of a single-walled liposome consisting of an amphiphilic bilayer surrounding an aqueous core. B) Structure of a polymeric micelle. ●Hydrophilic head; Hydrophobic tail.

However, the typical drug loading of these systems is low (ca. 10-20\%\textsuperscript{125}) One way to circumvent this problem is to produce carriers that can efficiently entrap the drug compound in kinetically stabilized nanoparticles via the process of flash nanoprecipitation (FNP). Pioneered by Prof. Prud’homme of Princeton University, flash nanoprecipitation (FNP) is a simple and scalable process that uses rapid micromixing of the drug and polymer molecules to create supersaturated conditions leading to the encapsulation of hydrophobic drugs in a stabilized polymer based nanoparticle.\textsuperscript{126}
Figure 1- 16 Schematic representation of impingement mixer forming block copolymer-protected nanoparticles (2D view of cross section) adapted from reference 135c.

Flash nanoprecipitation uses either a four-stream multi inlet vortex (MIV) mixer\textsuperscript{127} or as represented in Fig 1-16, two-stream confined impingement jet mixer (CIJ).\textsuperscript{128} The lipophilic organic drug is dissolved along with the amphiphilic block copolymers in a water miscible organic solvent. The organic solvent stream and water is mixed vigorously for a minute amount of time to induce supersaturation, which initiates precipitation of the dissolved hydrophobic components. The hydrophobic core of the block copolymer nanoparticle enables the lipophilic pharmaceutical to be dispersed in the core while the hydrophilic coronae sterically stabilize the particles to prevent aggregation. This process yields very high drug-encapsulation capacities on the order of 40-50 wt\%.$^{135a}$ FNP has been used to produce nanoparticles loaded with a number of hydrophobic molecules including bifenthrin,$^{129}$ paclitaxel prodrugs,$^{130}$ β-carotene,$^{135}$ vitamin E,$^{131}$ nitric oxide prodrugs,$^{132}$ curcumin,$^{133}$ and even beauty products like sunscreen agents.$^{134}$ In Chapter 4
of this thesis, we developed poly(ethylene glycol)-block-poly(propylenesulfide)-based amphiphilic block copolymers that would be utilized in Prof. Prud’homme’s lab for the development of oxidation-responsive nanocarriers that would release a hydrophobic payload upon exposure to aqueous solutions of hydrogen peroxide.
1.6 References


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CHAPTER 2

Synthesis and Characterization of Novel γ-Substituted Pyrrolidone-based Polymers

2.1 Introduction

Owing to the amphiphilic, non-ionic, and biocompatible nature of PVP and its extensive use in the pharmaceutical, textile, and cosmetic industries, there has been growing interest in preparing functionalized pyrrolidone-based polymers with tunable physicochemical properties.\(^1\) Towards this end, modified pyrrolidone-based polymers have been targeted by placing substituents on the pyrrolidone moiety or by inserting a spacer between the ring and the polymerizable group (Figure. 2-1).

![Figure 2-1 Strategies towards synthesizing functionalized pyrrolidone-based monomers.](image)

Following Strategy 1 (Figure 2-1, a), both Trenlenkamp and coworkers\(^{1b}\) and Chen, G-T and coworkers\(^{1a, 2}\) synthesized modified PVP polymers where the pyrrolidone ring was

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functionalized at the α-position with alkyl groups (Figure 2-2 a-c). These modified PVP polymers were seen to possess a lower critical solution temperature (LCST) that is absent in PVP. Moreover, the results showed that the polymer LCST could be tuned by modifying the alkyl substituents on the pyrrolidone ring, a property that can be exploited for the synthesis of thermoresponsive biomaterials.³

Another approach towards the synthesis of pyrrolidone-based polymers is to insert spacers between the pyrrolidone ring and the polymer backbone (Figure 2-1 b). Cai and his coworkers were one of the pioneers who synthesized pyrrolidone-based polymers with hydrophobic spacers and to examine their influence on the physicochemical properties of the polymer.⁴ Following Strategy 2, Sun. J et. al. synthesized a series of thermoresponsive polymers with hydrophobic spacers varying in structure (Figure 2-2 d-f). They observed that not only did the LCST of the polymers decrease exponentially with increase in polymer concentration, but also that the LCST of polymer solutions depended heavily on the structure of the hydrophobic spacer.⁴a

![Figure 2-2 Examples of modified pyrrolidone polymers.](image-url)
While the majority of reports on pyrrolidone-based polymers focus on the synthesis and characterization of polymers bearing α-substituted pyrrolidones, systematic studies on γ-substituted derivatives have received comparatively little attention. Since the γ-substituent on the pyrrolidone moiety is positioned much closer to the polymer backbone compared to α-substituents, we hypothesize that it should be easier to manipulate the properties of the polymer by tailoring the structure of the residues (Figure 2-3). To the best of our knowledge, γ-substituted poly(N-acryloyl-2-pyrrolidone)s have not been reported. As such, detailed knowledge of structure-property correlations is essential to establishing design criteria if our targets are to have far reaching potential as functional materials, particularly in the field of drug delivery where glass transition temperature, hydrophobicity, and secondary interactions such as π-π stacking or hydrogen-bonding play a critical role in both drug encapsulation and release.

Figure 2-3 γ and α substituted N-acryloyl-2-pyrrolidones.

In this chapter, we report the synthesis and polymerization of a series of PGA-derived, N-acryloyl-2-pyrrolidone monomers using conventional free radical and reversible atom-fragment transfer (RAFT) polymerization techniques. We also report on the relationships between the identity of the γ-substitutent and polymer glass-transition tempertaure ($T_g$), thermal decomposition temperature ($T_{dec}$), and thermoresponsivity
Selection of the monomer substituents was rationalized at two levels that encompass: i) structure (*i.e.* saturated linear/cyclic aliphatic, aryl, ether, and cyclic ether moieties) and ii) chemical classification (*i.e.* alkoxy (RO-) *vs* thiolate (RS-)). Conventional free radical polymerization was initially used to polymerize the monomers with methoxy, ethoxy, butoxy and methoxyethoxy residues. (Co)polymers prepared using azobisisobutyronitrile (AIBN) as an initiator had broader dispersities (D) which is characteristic of conventional free radical polymerization. For subsequent studies, reversible addition fragment chain transfer (RAFT) polymerization was employed given the versatility and control of this method. Since pyrrolidone-based polymers are well suited for biomedical applications (due to the pyrrolidone functionality that imparts both coordination ability and biocompatibility to the system), it is anticipated that knowledge gleaned from this work will be used towards the design and application of poly(N-acryloyl-2-pyrrolidone)s as potential nanoscale drug-delivery platforms with encapsulating and release capabilities that are made tunable through adjustments to T<sub>g</sub>, hydrophobicity, and intermolecular substrate-polymer interactions.

2.2 Results and discussion

2.2.1 γ-Substituted poly(N-acryloyl-2-pyrrolidone)s Prepared by Conventional Free-Radical Polymerization

Pyrrolidone-based Homopolymers

In light of the recent success that some have had in preparing polymers from bio-derived resources, we were inspired to develop a facile route to functionalized pyrrolidone-based polymers from pyroglutamic acid (PGA), a bio-derived resource prepared from glutamic acid. The synthetic route to γ-substituted pyrrolidone-based monomers is outlined in Scheme 2-1. 5-Methoxy-2-pyrrolidone is prepared via anodic
decarboxylation of pyroglutamic acid (PGA) in methanol using graphite plates as the anode and cathode.\textsuperscript{14} Subsequent derivatization of the lactam scaffold is achieved by stirring 5-methoxy-2-pyrrolidone with the appropriate alcohol over Amberlyst\textsuperscript{®} 15, a heterogeneous cation exchange resin catalyst.\textsuperscript{15} While our preliminary studies focused on monomers bearing simple alkoxy substituents (methoxy, ethoxy, butoxy and propargyloxy) in principle, any alcohol can be used in the exchange reaction provided it is available in excess, dissolves 5-methoxy-2-pyrrolidone, and is stable to the cation-exchange resin. In the final step, monomers are prepared by treating the respective \(\gamma\)-substituted pyrrolidone precursors with \(n\)-butyl lithium (\(n\)-BuLi) followed by addition of acryloyl chloride. \textsuperscript{1}H NMR spectra of the monomers are consistent with their structure (Figure 2-4) showing three distinct sets of doublets of doublets in the regions ca. \(\delta\) 7.40, 6.50, and 5.85 that are characteristic of two-bond and three-bond coupling of the vinylic protons (Figure 2-32 to 2-36)

**Scheme 2- 1** Synthesis and conventional polymerization of N-acryloyl-5-alkoxy-2-pyrrolidone monomers.

**Poly(MeONP), poly(EthONP), poly(BuONP) and poly(MeOEtONP)** were prepared by conventional free-radical polymerization using 1 mol\% azoisobisbutyronitrile (AIBN)
as an initiator at 75°C for 18 h in anhydrous tetrahydrofuran (THF). All polymers where isolated as hydroscopic white powders except for poly(PrgONP) which was an insoluble glassy solid, a result that is attributed to extensive crosslinking by the propargyl residues that are known to act as chain transfer agents during free radical polymerization.\(^\text{16}\) The \(^1\)H NMR spectra of poly(MeONP), poly(EthONP), poly(BuONP) and poly(MeOEtONP) are shown in Figure 2-4.

![Figure 2-4](image)

Figure 2-4 \(^1\)H NMR spectra (500 MHz, CDCl\(_3\), 25°C) of a) poly(MeONP), b) poly(EthONP), c) poly(BuONP) and d) poly(MeOEtONP).

All spectra possess a resonance at \(ca. \delta = 5.54\)\(^\text{-}\)5.62 ppm that is assigned to the pyrrolidone ring hydrogen (\(\omega\)) adjacent to the 5-alkoxy substituent. The integral ratio of these resonances to the \(\theta\)-resonances \(I_{3.36}\) (-OCH\(_3\), Figure 2-4a), \(I_{1.13}\) (-OCH\(_2\)CH\(_3\), Figure 2-4b), \(I_{0.88}\) (-OCH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\), Figure 2-4c) and \(I_{3.33}\) (-OCH\(_2\)CH\(_2\)OCH\(_3\), Figure 2-4d) is 1:3:3:3:3, confirming that the targeted pyrrolidone polymers are intact with the appropriate alkoxy residues. Moreover, absence of a doublet of doublet signal at \(ca. \delta = 6.49\) ppm
suggests that monomeric impurities could not be detected, indicating the high purity of the samples.

Table 2-1 Characterization of homopolymers\(^{(a)}\)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Yield ([%])</th>
<th>(M_n) (^{(b)}) Daltons</th>
<th>(D_M) (^{(b)})</th>
<th>(T_{dec}) (^{(c)}) ([^\circ C])</th>
<th>(T_g) (^{(d)}) ([^\circ C])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(MeONP)</td>
<td>79</td>
<td>11600</td>
<td>2.9</td>
<td>274</td>
<td>120</td>
</tr>
<tr>
<td>Poly(EtONP)</td>
<td>98</td>
<td>26500</td>
<td>4.03</td>
<td>278</td>
<td>113</td>
</tr>
<tr>
<td>Poly(BuONP)</td>
<td>57</td>
<td>27300</td>
<td>2.17</td>
<td>270</td>
<td>72</td>
</tr>
<tr>
<td>Poly(MeOEtONP)</td>
<td>63</td>
<td>32300</td>
<td>2.75</td>
<td>255</td>
<td>60</td>
</tr>
</tbody>
</table>

\(^{(a)}\) [M]/[AIBN] = 100, [M] = 2.0 M in THF, 65\(^\circ\)C, 24 h. \(^{(b)}\) Determined by GPC (relative to polystyrene in THF). \(^{(c)}\) Decomposition temperature, onset, determined by TGA. \(^{(d)}\) Glass transition temperature, onset, second heating curve, determined by DSC.

The number average molecular weights \((M_n)\) of our polymers (Table 2-1) were estimated by GPC analysis to be in the range from 11-32kDa while the polymer dispersities \((D_M, M_w/M_n)\) were broad, typical of polymers prepared from conventional free-radical polymerization (Figure 2-77 – 2-80). The polymers possessed excellent long-term stability at ambient conditions as confirmed by the identical \(^1\)H NMR spectra and GPC traces of each sample taken eight weeks apart. The thermal properties of poly(MeONP), poly(EtONP), poly(BuONP), poly(PrgONP) and poly(MeOEtONP) were analyzed by thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The glass
transition temperatures determined by DSC (Figure 2-109 – 2-112) revealed high $T_g$’s that are dependent on both the length and structure of the alkoxy residues on the pyrrolidone moieties (Table 2-1). Comparative analysis revealed that the glass transition temperatures decreased as the length of hydrophobic residue increased and when -CH$_2$ was replaced with an oxygen atom as in poly(MeOEtONP). The polymers were also observed to be thermally stable as confirmed by the high decomposition temperatures that exceed ca. 250°C (Figure 2-95 – 2-99). Basic solubility tests revealed that poly(MeONP), poly(EthONP), poly(BuONP) are readily soluble in common organic solvents such as THF, chloroform, dimethylformamide, and dichloromethane while only poly(MeOEtONP) was found to be soluble in water.

Interestingly, turbidimetric experiments revealed that aqueous solutions of poly(MeOEtONP) possess remarkable thermoresponsive sensitivity and reversibility. An LCST of 42 °C was measured as the point at which the aqueous solution of poly(MeOEtONP) exhibited 50% transmittance at 500 nm (Figure 2-5a). Above the LCST, the transparent solution turned opaque indicating a phase transition whereby the polymer chains undergo a coil-to-globule transition. Moreover, this transition was found to be reversible over eight cycles in a 2h period when the temperature of the aqueous poly(MeOEtONP) solution was cycled between 37 and 45 °C at 1°C/min (Figure 2-5b), indicating high reversibility of the transition with a low hysteresis. Polymers with lower critical solution temperatures of ca. 40°C in water are particularly attractive for drug-delivery applications when a phase transition slightly above the temperature of the human body is required. 17
Random Copolymers with Tunable Thermoresponsive Properties

We next explored the formation of random copolymers as a means of tuning the thermoresponsivity of our materials through macromolecular design. The random copolymers were synthesized according to the Scheme 2-2 with molecular weights and disperities summarized in Table 2-2. The ratio of the hydrophilic to hydrophobic units in the random copolymer was targeted to be ca. 75:25, respectively, with actual values confirmed by comparing appropriate integral ratios in the $^1$H-NMR spectrum.

Scheme 2-2 Synthesis of random copolymers with tunable LCST.
All random copolymers synthesized according to Scheme 2-2 exhibited reversible thermoresponsivity. It was observed that the LCSTs of these PGA-derived random copolymers bearing methoxy, ethoxy and butoxy residues were found to be highly dependent on the length of the residue that defined the hydrophobic pyrrolidone repeat unit (Table 2-2, Figure 2-6). For instance, poly(MeOEtONP)-co-(BuONP) possesses the longest hydrophobic alkoxy chain moiety (i.e., butoxy) and lowest LCST (i.e., 9 °C) of all the thermoresponsive (co)polymers studied in this chapter. The LCSTs of both poly(MeOEtONP)-co-EthONP and poly(MeOEtONP)-co-MeONP were measured to be 21 and 31 °C respectively. We attribute this result to an overall decrease in hydrophilic character as the lengths of the alkoxy moieties of the hydrophobic repeat units in the copolymer increase. Moreover, poly(MeOEtONP)-co-(MeONP), and poly(MeOEtONP)-co-(EthONP) were found to exhibit remarkable thermoresponsive reversibility (Figure 2-74 and 2-75, respectively), similar to that observed for homopolymer poly(MeOEtONP). On the contrary, poly(MeOEtONP)-co-(BuONP) exhibited a large hysteresis (Figure 2-4, Figure 2-76) during thermal cycling, a feature that is also observed for poly(N-isopropylacrylamide) and attributed to an irreversible coil-to-globule transition. 18
Figure 2-6 A plot of poly(MeOEtONP) (5mg/mL, 500nm, forward scan - ▲ -; reverse scan - ▼ -), poly(MeOEtONP)-co-MeONP (5mg/mL, 500nm, forward scan - ▲ -; reverse scan - ▼ -), poly(MeOEtONP)-co-EthONP (5mg/mL, 500nm, forward scan - ▲ -; reverse scan - ▼ -), poly(MeOEtONP)-co-BuONP (2.5mg/mL, 500nm, forward scan - ▲ -; reverse scan - ▼ -).

Table 2- 2 Characterization of thermoresponsive copolymers.

<table>
<thead>
<tr>
<th>(Co)Polymer</th>
<th>% of MeOEtONP (a)</th>
<th>Mn (b)</th>
<th>D_M</th>
<th>LCST [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly-MeOEtONP</td>
<td>100</td>
<td>32300</td>
<td>2.75</td>
<td>42</td>
</tr>
<tr>
<td>poly-MeEtONP-co-MeONP</td>
<td>(c)</td>
<td>47500</td>
<td>3.31</td>
<td>31</td>
</tr>
<tr>
<td>poly-MeEtONP-co-EthONP</td>
<td>71</td>
<td>28200</td>
<td>4.12</td>
<td>21</td>
</tr>
<tr>
<td>poly-MeEtONP-co-ButONP</td>
<td>76</td>
<td>10500</td>
<td>4.36</td>
<td>9</td>
</tr>
</tbody>
</table>

a) [M]/[AIBN]=100, [M] = 2.0 M in THF, 65 °C, 24 h. Monomer feed: 75% MeOEtONP, 25% MeONP, EthONP, or BuONP; b) Determined by ¹H NMR in CDCl₃; c) Determined by GPC (relative to polystyrene in THF); d) % of MeOEtONP in poly(MeOEtONP)-co-MeONP could not be determined by spectroscopic methods.
2.2.2 Structure-Property Correlation of γ-Substituted Monomers

The results of our previous investigation indicate that the physicochemical properties of γ-substituted polymers correlate strongly to the molecular architecture of the constituent repeat units. To have a thorough understanding of the effect of hydrophobic residues on the polymer properties, it is essential to examine the effect of different substituents based on structure and chemical class. Although both belonging to the chalcogen group of the periodic table, sulfur and oxygen-containing congeners possess different physicochemical properties and reactivity due to the differences in size, hydrophobicity, and electronegativity between O and S. Hence, we decided to explore the differences in properties between the γ-substituted pyrrolidone-based polymers bearing alkoxy and thiolate residues. Moreover, we wanted to perform a more exhaustive structure-property correlation study based on residue structure by examining residues that encompass: i) linear vs. cyclic aliphatic vs. aromatic hydrocarbons, and ii) linear vs cyclic ethers (Scheme 2-3).

Scheme 2-3 Synthesis and RAFT polymerization of N-acryloyl-5-alkoxy/thiolate-2-pyrrolidones.
All monomers and polymers are prepared as per Scheme 2-3 starting with the anodic decarboxylation of PGA to 5-methoxy-2-pyrrolidone. Subsequently, acid-catalyzed alkoxy-exchange with the appropriate residue occurs by either stirring 5-methoxy-2-pyrrolidone in: i) excess alcohol over Amberlyst®-15 (Method A) or ii) dichloromethane in the presence of 1.5 molar equivalents of alcohol and p-toluenesulfonic acid (1 mol%) (Method B). Due to the temperature sensitivity of γ-substituted pyrrolidones, method B is preferred when high-boiling point alcohols (e.g. cyclohexanol bp = 160°C) are employed to reduce the chance of decomposition during the removal of residual alcohols. Due to the greater nucleophilicity of thiols over their alcohol congeners, all thiolated pyrrolidones are prepared according to Method A with the exception that only a single molar equivalent of thiol (in THF) is required for near quantitative conversion. All monomers were prepared by deprotonation with n-butyl lithium (1.6M in hexanes) followed by the addition of N-acryloyl chloride. All monomers were stored cold in inert atmospheres to prevent autopolymerization.

**Monomer Characterization**

$^1$H-NMR spectra of EthONP, EthSNP, and N-acryloyl-2-pyrrolidone (NP) monomers are shown in Fig. 2-7 a-c and share common acryloyl group resonances at $\delta$ 7.48, 6.51 and 5.84 ppm. The presence of a γ-substituent on the pyrrolidone ring creates diastereotopic environments across the cyclic scaffold as observed by both the chemical shifts and complex coupling patterns that are assigned to the $a$ and $b$ protons of both monomers EthONP and EthSNP (via 2D NMR (COSY) Spectroscopy, (Figures 2-72 and 2-73), features that are absent in the spectrum of NP. Interestingly, we noticed that the differences in chemical shift between the diastereotopic $b$ protons are more pronounced in EthSNP.
compared to the ethoxy congener. We hypothesize that the greater steric demand imposed by the sulfur atom and/or the more acute R’-X-R angle associated with the sulfide residue (ca. 105°)\textsuperscript{21} may generate a distinct diastereotopic environment that is responsible for the differences in chemical shift and coupling patterns.

Figure 2-7. \textsuperscript{1}H NMR spectra (500 MHz, CDCl\textsubscript{3}, 25°C) of a) NP, b) EthONP, c) EthSNP, d) poly(NP), e) poly(EthONP), and f) poly(EthSNP).

All monomers were isolated as viscous oils with the exception of StSNP that is a solid at room temperature. To gain further insight into monomer structure, single-crystal X-ray diffraction studies were performed on StSNP (Figure 2-8) after crystallizing the monomer in a 1:1 mixture of acetonitrile and hexane at room temperature. The \(\gamma\)-substituted \(N\)-
acryloyl-2-pyrrolidone was found to pack into the monoclinic P2₁/c space group with four independent molecules in the asymmetric unit. Weak hydrogen-bonding interactions between the adjacent monomer units stabilized the crystal packing, while the stearyl chains on the pyrrolidone moieties arranged in a non-penetrating bilayer structure. In line with dipole moment and spectroscopic data obtained from N-acetyl lactams, StSNP adopts an (E,Z) configuration with Csp²-Nsp² (1.404(8) and 1.394(9) Å) and Csp²-Osp² (1.225(9) and 1.213(9) Å) bond lengths that are consistent with the imide functional group. As expected, the N atom adopts a trigonal planar geometry with the sum of bond angles around N equal to ca. 360°, however the O=C-N-C(O) torsion angles show a deviation from planarity [C(4)-N(1)-C(5)-O(2) = 165.3° and C(5)-N(1)-C(4)-O(1) = -164.9] that may be caused by steric congestion from the acryloyl and the bulky thiolate groups of the monomer.

![Figure 2-8](image-url) A single molecule representation of the X-ray crystal structure of StSNP as 50% thermal ellipsoids (except H atoms). Key: C = white, N = blue, O = red, S = yellow.
Indeed, the envelope conformation of the pyrrolidone ring and the closeness of the thiolate residue (R-S-R’ bond angle, \textit{ca.} 105.5°) and acryloyl moiety provide an impetus for making modest adjustments to residue structure in order to modify the physicochemical properties of the polymer.

**Polymer Synthesis and Characterization**

In the section 2.2.1., we synthesized (co)polymers using the conventional free-radical polymerization method with AIBN as the initiator. As a consequence of the uncontrolled free radical polymerization, the (co)polymers possessed large dispersities (Figures 2-77 to 2-83). In order to reduce the polymer dispersity and have reasonable control over the length of the polymer chain, we employed the reversible addition fragment chain transfer (RAFT) polymerization technique. Using benzyl dithiobenzoate (BDTB) as the RAFT agent, polymers were prepared by using a molar feed ratio of \([\text{Monomer}]_0:[\text{BDTB}]_0:[\text{AIBN}]_0 = 100:1:0.1\) at 70°C for 20 h. GPC traces of the polymers (Figures 2-84 to 2-94) are typically monomodal with dispersities in the range of 1.4-1.6, values that are considerably lower than those discussed earlier in the chapter (\textit{ca.} 2.2-4.0). All \(^1\)H NMR spectra (Figures 2-61 to 2-68) exhibit resonances and integral ratios that are consistent with the targeted polymer structures with common features at \(\delta\) 5.60 and 3.60 assigned to the pyrrolidone \(\gamma\)-hydrogen and -CH\(_2\)CH- proton of the polymer backbone respectively (Figure 2-7 d-f, protons \(c\) and \(f\)). In the case of alkoxy-functionalized congeners, the latter is buried beneath the OCH\(_3\) resonance (Figure 2– 7e, proton \(f\)) as confirmed by 2D NMR (COSY) studies on a small molecule model (Figure 2-71). In line with our earlier work, all homopolymers are soluble in a variety of organic solvents (\textit{e.g.} dichloromethane, tetrahydrofuran, chloroform, ethylacetate) with only polymers
poly(MeOEtONP) and poly(FurONP) showing solubility in water. Interestingly, poly(NP) was found to be completely insoluble in water despite possessing the pristine pyrrolidone moieties known to impart water solubility in materials like PVP, indicating that the carbonyl spacer group that comprises the imide functionality has a tremendous influence on the hydrophilicity of the polymer.

Since glass transition temperature can be of particular importance in assessing the processability and performance of a polymeric material, differential scanning calorimetry (DSC) was used to probe polymer thermal transitions as a function of substituent structure. As shown in Figure 2-113, both poly(BuONP) and poly(BuSNP) possess lower $T_g$s than their ethyl-containing analogs (i.e. poly(EthONP) and poly(EthSNP), respectively), results that arise from an increase in polymer free volume as the linear aliphatic moiety is extended. Moreover, a reduction in $T_g$ is also observed on replacing a -CH$_2$- group of the butoxy(thiolate) substituent with an oxygen atom as seen in polymers poly(MeOEthONP) and poly(MeOEthSNP). Alternatively, polymers bearing cyclohexyl or phenyl groups (e.g., poly(CyONP), poly(CySNP), and poly(PhSNP)) possess the highest $T_g$s (Figure 2-114) among the polymers described here, results that are attributed to both the bulky and rigid nature of these substituents that restrict backbone mobility. Poly(StSNP) possesses the lowest $T_g$ (ca. 30°C) and is the only semi-crystalline polymer with a melt transition temperature $T_m$ of 42°C (Figure 2-116). A comparative analysis between the alkoxy- and thiolate congeners reveals that irrespective of residue structure, a reduction in $T_g$ is observed (by ca. 8-26°C) when sulfur is used in lieu of oxygen. This effect can be attributed to a combination of the larger van der Waals radius of sulfur and/or its poor hydrogen-bond
acceptor ability\textsuperscript{25} that is expected to weaken cohesive intra- and inter-polymer chain interactions.

The thermal stability of polymers was assessed by thermogravimetric analysis. The thermograms clearly show that all alkoxy-functionalized polymers exhibit a defined, multi-step decomposition process (Figures 2-101 to 2-105) than those observed in the thiolate congeners. However, the alkoxy-functionalized polymers were all found to possess lower thermal decomposition temperatures ($T_{\text{dec}}$) indicating that $\gamma$-substituted poly(N-acryloyl-2-pyrrolidone)s bearing thiolate residues are more thermally stable than their alkoxide-bearing analogs regardless of substituent structure.

**Table 2-3** Characterization of pyrrolidone-based homopolymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$\textsuperscript{b)}</th>
<th>$D_M$\textsuperscript{b)}</th>
<th>$T_g$ (°C)\textsuperscript{c)}</th>
<th>$T_{\text{dec}}$ (°C)\textsuperscript{d)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(NP)</td>
<td>12600\textsuperscript{c)}</td>
<td>1.4</td>
<td>120</td>
<td>150</td>
</tr>
<tr>
<td>poly(EtONP)</td>
<td>5000</td>
<td>1.4</td>
<td>108</td>
<td>172</td>
</tr>
<tr>
<td>poly(EtSNP)</td>
<td>4700</td>
<td>1.5</td>
<td>89</td>
<td>213</td>
</tr>
<tr>
<td>poly(ButONP)</td>
<td>6500</td>
<td>1.2</td>
<td>71</td>
<td>186</td>
</tr>
<tr>
<td>poly(ButSNP)</td>
<td>6000</td>
<td>1.2</td>
<td>56</td>
<td>320</td>
</tr>
<tr>
<td>poly(CyONP)</td>
<td>14800</td>
<td>1.5</td>
<td>129</td>
<td>180</td>
</tr>
<tr>
<td>poly(CySNP)</td>
<td>13100</td>
<td>1.4</td>
<td>103</td>
<td>310</td>
</tr>
<tr>
<td>poly(PhSNP)</td>
<td>14000</td>
<td>1.6</td>
<td>110</td>
<td>308</td>
</tr>
<tr>
<td>poly(MeOEONP)</td>
<td>9200</td>
<td>1.4</td>
<td>56</td>
<td>170</td>
</tr>
<tr>
<td>poly(MeOEtSNP)</td>
<td>10200</td>
<td>1.6</td>
<td>48</td>
<td>317</td>
</tr>
<tr>
<td>poly(FurONP)</td>
<td>5300</td>
<td>1.5</td>
<td>75</td>
<td>263</td>
</tr>
<tr>
<td>poly(StSNP)</td>
<td>16600</td>
<td>1.5</td>
<td>30</td>
<td>311</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} [M]:[BDTB]:[AIBN] = 100:1:0.2, [M] = 2.0 M in THF, 70 °C, 20 h. \textsuperscript{b)} Determined by GPC (relative to polystyrene in THF). \textsuperscript{c)} Glass transition temperature, second heating curve, determined by DSC. \textsuperscript{d)} Decomposition temperature, onset, determined by TGA. \textsuperscript{e)} Determined by GPC (relative to poly(methylmethacrylate) in DMF).
Thermoresponsivity of Water Soluble Polymers

In section 2.2.1 we discussed the synthesis of the thermoresponsive homopolymer $\text{poly}(\text{MeOEtONP})$ via conventional free radical polymerization that showed a LCST of $42^\circ C$. Tailoring the LCST was achieved by copolymerizing $\text{MeOEtONP}$ with hydrophobic monomers bearing methoxy, ethoxy or butoxy substituents. In order to further explore the thermoresponsive behavior of $\gamma$-substituted poly($N$-acryloyl pyrrolidone)s, we synthesized a pyrrolidone-based polymer ($\text{poly}(\text{FurONP})$) with tetrahydrofurfuryl groups tethered to the $\gamma$-position of the pyrrolidone scaffolds. The thermoresponsive behavior of $\text{poly}(\text{MeOEtONP})$ and $\text{poly}(\text{FurONP})$ was measured by turbidimetry and the results illustrated in Figure 2-9a. Both polymers are completely soluble in deionized water (0.2 mg/mL) resulting in near perfect optical transmittance at 500 nm. Upon approaching the LCST, the solutions turn opaque (a consequence of chain aggregation in solution) with the LCST measured as the temperature at which 50% of the original transmittance is lost. Interestingly, polymers $\text{poly}(\text{NP})$ and $\text{poly}(\text{MeOEtSNP})$ are insoluble in deionized water which indicates that the thermoresponsive behavior of $\text{poly}(\text{MeOEtONP})$ and $\text{poly}(\text{FurONP})$ must arise from the substituent, specifically, its ether-like topology and alkoxy class.

The main reason why we selected the tetrahydrofurfuryl residue as the side chain was because the parent alcohol is miscible with water.\textsuperscript{26} Quite surprisingly, $\text{poly}(\text{FurONP})$ exhibits an LCST ($\text{ca. } 15^\circ C$) that is $32^\circ C$ lower than that of $\text{poly}(\text{MeOEtONP})$ ($\text{ca. } 47^\circ C$). This difference in the LCST is significant, with its origin a likely combination from both: i) the steric hindrance imposed by the polymer chain on the hydrophilic tetrahydrofurfuryl moieties and ii) the rigid nature of the substituent, contributing factors that are expected to
weaken intermolecular polymer/solvent interactions that are responsible for polymer dissolution.\textsuperscript{4a} The latter is in line with DSC data and explains why the $T_g$ of poly(FurONP) ($\text{ca. } 75^\circ\text{C}$) is greater than that of poly(MeOEtONP) ($\text{ca. } 56^\circ\text{C}$). Indeed, both polymers redissolve upon cooling below their respective LCST confirming highly reversible thermoresponsive behavior. Moreover, the heating/cooling curve of poly(MeOEtONP) exhibits a narrow hysteresis compared to that of poly(FurONP), a phenomenon that is anticipated in more hydrophilic polymers that possess only H-bond donors in the macromolecular structure.\textsuperscript{27}

![Figure 2-9](image)

**Figure 2-9** a) Plot of transmittance (%) as a function of temperature in deionized water of poly(MeOEtONP) and poly(FurONP) (0.2 mg/mL). b) Plot of measured cloud points in deionized water and PBS solution as a function of polymer concentration; poly(MeOEtONP), deionized water, $\bullet$; poly(MeOEtONP), PBS solution, $\square$, poly(FurONP), deionized water, $\circ$; poly(FurONP), PBS solution, $\blacksquare$. CP values are measured as the inflection points of the heating cycles

Since the thermoresponsive behavior of (co)polymers can be influenced by the presence of salts,\textsuperscript{27a,28} the LCSTs of poly(MeOEtONP) and poly(FurONP) were measured (Figure. 2-9a) in phosphate buffered saline (PBS, $[\text{KH}_2\text{PO}_4] = 1.06 \text{ mM}$, $[\text{NaCl}])$
\[ [\text{Na}_2\text{HPO}_4-7\text{H}_2\text{O}] = 2.97 \text{ mM}, \] a solution often used in biological research as a diluent and additive to cell culture media. The results show that at polymer concentrations of 0.2 mg/mL, a subtle salting-out effect was observed for \textit{poly(MeOEtONP)} (\textit{ca.} LCST = 45°C), whereas \textit{poly(FurONP)} exhibits a slightly higher LCST (\textit{ca.} 16°C) in PBS, consistent with a salting-in effect. As was the case in deionized water, LCST values decreased by \textit{ca.} 9°C and 10°C for \textit{poly(MeOEtONP)} and \textit{poly(FurONP)} respectively over the same concentration range (\textit{e.g.} 0.2 - 1.0 mg/mL, Figure 2-9b, Table 2-4). In sum, the results, the thermoresponsivity of these systems is relatively insensitive to changes in environmental conditions with LCSTs varying only a few degrees between deionized water and PBS formulations.

\textit{In Vitro Cytotoxicity}

Materials that exhibit thermoresponsivity in aqueous media at temperatures approaching that of the human body (\textit{ca.} 38°C) are highly desirable for biotechnology applications. With the help of Dr. Pei-Chin Tsai of the Michniak-Kohn group in Rutgers-University, New Jersey Center of Biomaterials lab, the cytotoxicity of \textit{poly(MeOEtONP)} was evaluated at 37°C using human dermal fibroblasts (HDF) and MCF-7 breast cancer cells. MCF-7 and HDF were cultured with Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum, 100 units/mL penicillin, 100 µg/mL of streptomycin, and 0.25 µg/mL of amphotericin B at 37°C/5% CO\textsubscript{2}. Individual wells from 96 well plates were seeded with 5,000 cells/well and incubated for 24 hours prior to experimentation. Before testing, wells of MCF-7 and HDF cells were treated with 200 µL of DMEM polymer solution varying in concentration (1000, 100, 10, 1, 0.1 µg/mL), for 24 hours at 37°C. Upon completion of an experiment, cells were washed with phosphate buffer saline (PBS) three
times prior to adding 100 µL of DMEM containing 10% AlamarBlue®. After incubating at 37°C for 3 hrs, fluorescence intensity was measured at 590 nm (excitation wavelength, 560 nm) using a microplate reader. Cell viability was expressed as a percentage by normalizing the fluorescence intensity of the experimental group relative to DMEM media treated cells done in triplicate. The results of this investigation are plotted in Figure 2-10 and indicate that poly(MeOEtONP) exhibits high cell viability in the concentration range of 0.1 - 100 µg/mL, a desirable attribute for drug delivery platforms that rely on a thermal stimulus to release a therapeutic payload to an affected area without compromising the health of non-targeted cells.

**Figure 2-10.** *In vitro* cytotoxicity of poly(MeOEtONP) at 37°C. MCF-7 and HDF cells were incubated with polymer for 24h, washed and measured for viability after 3h. Data are expressed in mean cell viability (%) with error bars indicating standard deviation. n=3

### 2.3 Conclusion

In summary, we have created a new family of pyrrolidone-based polymers derived from pyroglutamic acid, a bio-derived resource. The pyrrolidone-based monomers are
fascinating owing to the synthetic ease through which they can be prepared from PGA and structurally tuned to bear appropriate alkoxy residues. Indeed, the results presented in this work suggest that minor adjustments to both the length and chemical nature of these residues can significantly influence the physical properties of the subsequent polymers. Highlights of this work include the homopolymer \textit{poly(MeOEtONP)} that was found to exhibit a highly reversible thermoresponsive phase transition with an LCST of 42 °C in water. Furthermore, the LCSTs of PGA-derived copolymers were found to be highly sensitive to the length of the alkoxy residues on the hydrophobic repeat units suggesting that the thermoresponsivity of these polymers can be tuned by adjusting polymer structure. Inspired by these findings, a more thorough investigation into the structure/property correlations on \(\gamma\)-substituted poly(\(N\)-acryloyl-2-pyrrolidone)s was pursued. When compared against their alkoxy congeners of comparable molecular weight, the thiolated polymers possess higher thermal stability and lower \(T_g\)s regardless of substituent structure. The results also show that glass transition temperature can be modified over a broad temperature range by making subtle modifications to the substituent structure. This feature of altering the glass transition of polymeric chains through substituent variation could be attributed to steric congestion between the pyrrolidone \(\gamma\)-substituent and polymer backbone as inferred from a single crystal x-ray diffraction study on monomer StSNP. All the polymers prepared in this work were soluble in common organic solvents but only \textit{poly(MeOEtONP)} and \textit{poly(FurONP)} were soluble in deionized water as well. \textit{Poly(MeOEtONP)} and \textit{poly(FurONP)} exhibited a lower critical solution temperature at 47°C and 15°C (0.2 mg/mL) respectively, a thermoresponsive behavior that is also concentration dependent over the range of 0.2-1.0 mg/mL. At fixed polymer
concentrations, the LCST values in deionized water are comparable to those measured in PBS solution indicating that there is very little salting effect on the thermoresponsivity. Moreover, polymers poly(NP) and poly(MeOEtSNP) were found to be insoluble in aqueous media indicating that the thermoresponsive behavior of poly(FurONP) and poly(MeOEtONP) arises solely from the ether moiety and the oxygen atom that tethers it to the lactam ring. Finally, in vitro cytotoxicity studies using both MCF-7 and HDF cells revealed that poly(MeOEtONP) is largely noncytotoxic over the polymer concentration range of 0.1-100 µg/mL. In spite of high demand for the use of polymeric nanoparticles as drug-delivery vehicles, there is a strict control imposed by Food and Drug Administration over their use due to their physiological and toxicological fate along with the design constraints associated with particle size, biocompatibility and overall cytotoxicity. The information obtained from this study provides evidence for the potential utility of γ-substituted poly(N-acryloyl-2-pyrrolidone)s as they can be tailored to adjust: i) hydrophobic or hydrophilic character, ii) thermoresponsivity, iii) hydrogen-bonding (alkoxy vs thiolate substituents) / π-π stacking (cyclohexyl vs phenyl moieties) capability, and iv) Tg, without complex modifications to the macromolecular scaffold. Systems such as these are highly desirable for biomedical applications, particularly as drug delivery platforms that are designed to load and release therapeutic payloads in a controlled manner. Compelled by the negligible toxicity of poly(MeOEtONP), block copolymer micelles with poly(MeOEtONP) coronae and γ-substituted poly(N-acryloyl-2-pyrrolidone)s cores were examined to study the influence of substituent structure and chemical class on the physicochemical properties, drug-loading efficiency, and thermoresponsive release (if any)
among this novel class of pyrroldione-based polymer. The results of which are explained in detail in the next chapter.

2.4 Experimental

Materials and Equipment. Sodium, ethanol, butanol, propargyl alcohol, and Amberlyst®15 were purchased from Aldrich. Methanol and ethyl acetate were purchased from Pharmco-AAPER. D, L-Pyroglutamic acid was purchased from TCI-America. 2-Methoxyethanol was purchased from Acros. Deuterated solvents were purchased from Cambridge Isotopes Laboratories, Inc. All purchased chemicals were used without further purification. All electrochemical reactions were conducted in a 1L Chemglass Life Sciences jacketed beaker (Figure 2-117) using a Metrohm USA Inc. AUTOLAB PGSTAT302N potentiostat/galvanostat. Medium extruded graphite plates (purchased from graphitestore.com) were used as the anode and cathode. $^1$H NMR spectra were recorded on a Varian INOVA 500 MHz and Bruker ACEND 500MHz spectrometer and calibrated to the residual protonated solvent peaks at δ 7.24 for deuterated chloroform (CDCl$_3$) and δ 5.32 for deuterated dichloromethane (CD$_2$Cl$_2$). $^{13}$C NMR spectra were calibrated at δ 77.23 for CDCl$_3$ and δ 54.00 for CD$_2$Cl$_2$. UV/vis spectra were recorded on a Cary-100 spectrophotometer equipped with a peltier heated multi-cell holder with a Cary temperature controller and probe. All GC-MS experiments were conducted on an Agilent Technologies HP6890 GC system and 5973A MSD. The standard method involved an initial oven temperature of 70 °C (held for 1 min) followed by a 10 °C min$^{-1}$ ramp to 250 °C. Molecular weights ($M_n$ and $M_w$) and polydispersity indices ($M_w/M_n$) were determined by gel permeation chromatography (GPC) using a Malvern Viscotek TDAmax chromatograph with tetrahydrofuran as the mobile phase at 30 °C. The chromatograph was equipped with
two PLC mixed columns and one PLD mixed column. Output was detected with a Viscotek TDA 305-055 Tetra Detector Array (PDA+RI+Visc+LALS/RALS) using an eluent flow rate of 1 mL/min and a 60 µL injection loop. Molecular weights were determined from a 10-point calibration curve created using polystyrene standards purchased from Polymer Laboratories. For poly(NP), GPC analysis was performed in DMF/0.01 M LiBr (0.5 mL/min) using a Waters Empower system equipped with a 717plus autosampler, a 1525 binary HPLC pump, a 2487 dual λ absorbance detector, and a 2414 refractive index detector. Two styrage PLC mixed columns (column heater, 50 °C) were used for separation. Molecular weights were determined from a 12-point calibration curve using poly(methyl methacrylate) standards. Differential scanning calorimetry (DSC) was performed on a TA Instruments Discovery differential scanning calorimeter at a scan rate of 10 °C/min. DSC data for the polymers synthesized by RAFT technique were recorded from the second heating scans. Thermal gravimetric analyses were performed on a TA Instruments Discovery thermogravimetric analyzer at a scan rate of 20 °C/min up to 700 °C. Differential scanning calorimetry (DSC) measurements for polymers synthesized by free radical technique were performed on a Perkin-Elmer differential scanning calorimeter Pyris 1 at a scan rate of 10 °C/min. All DSC data were recorded from the second heating scans. Thermal gravimetric analysis measurements were performed on a Perkin-Elmer Pyris 1 thermogravimetric analyzer at a scan rate of 20 °C/min up to 900 °C.

**Single Crystal X-Ray Analysis.** A suitable crystal of StSNP was selected and mounted on a Bruker-AXS SMART APEX II CCD diffractometer at 100(1)K. The cell dimensions and the intensities were collected with Cu-Kα radiation (α = 1.54178 Å). Data processing, Lorentz-polarization, and face-indexed numerical absorption corrections were performed.
using SAINT, APEX, and SADABS computer programs. The structure was solved by direct methods and refined by full-matrix least-squares methods on F^2, using the SHELXTL V 6.14 program package. All non-hydrogen atoms were refined anisotropically. All the H atoms in all of the structures were found in electron-density difference maps.

The methyl H atoms were put in ideally staggered positions with C--H distances of 0.98 Å and U_{iso}(H) = 1.5U_{eq}(C). The methine, methylene, and pyrrolidone Hs were all placed in geometrically idealized positions and constrained to ride on their parent C atoms with C--H distances of 0.93, 0.97, and 0.98 Å, respectively, and U_{iso}(H) = 1.2U_{eq}(C).

**Anodic Oxidation Procedure.** 5-Methoxy-2-pyrrolidone was prepared using a procedure reported by Iwasaki *et al.* with the following modifications. A mixture of D,L-pyroglutamic acid (16 g, 0.124 mol) in sodium methoxide solution (1.6 g sodium in 480 mL methanol) was placed in a 1 L water-cooled jacketed beaker to maintain a temperature below 25 °C. Anodic oxidation was carried out at a constant current of 750 mA using graphite anode and cathode electrodes (45 mm x 50 mm x 6 mm) placed 2 mm apart. After all the starting material was consumed (according to TLC), the reaction mixture was evaporated to dryness (below 25 °C) and the white solid stirred over ethyl acetate (480 mL) for 10 minutes followed by filtration through a sintered glass filter. The filtrate was evaporated to dryness and the crude product recrystallized from cold ethyl acetate /hexanes (70/30, 0 °C) to afford white crystals (8.90 g, 62% yield). All analytical data were consistent to those reported in the literature. \[^1^H\text{NMR (CDCl}_3, 500 MHz): δ 7.73 (1H, s), 4.86 (1H, m), 3.28 (3H, s), 2.50 (1H, m), 2.23 (1H, m), 2.04 (1H, m).\]
General Procedure for Preparation of \(\gamma\)-substituted 2-pyrrolidones

**Method A.** A solution of 5-methoxy-2-pyrrolidone (2.0 g, 17 mmol, Scheme 2-2) in 20 mL of appropriate absolute alcohol was stirred over Amberlyst\textsuperscript{®}15 at 25 °C for 5 h. The solution was filtered over a pad of celite and excess alcohol removed under reduced pressure. The crude products were taken up in a minimum volume of ethyl acetate and recrystallized overnight at ca. -28 °C to afford white crystals. *Note:* 5-alkoxy-2-pyrrolidones are thermally sensitive and must be stored at ca. 0 °C.

**Method B.** Over a period of 15 min, a solution of 5-methoxy-2-pyrrolidone (2.0 g, 17 mmol) in 5 mL of dichloromethane was added dropwise with constant stirring to a solution of an appropriate absolute alcohol (1.5 molar equiv.) and \(p\)-toluenesulfonic acid (1 mol\%) in 5 mL of dichloromethane. After 24 h, the solvent was removed and the residue crystallized by dissolving it in a minimum amount of ethyl acetate and layering with hexanes at -28 °C. All 5-alkoxy-2-pyrrolidones were prepared according to a literature procedure.\textsuperscript{34}

**General Procedure for Preparation of \(N\)-(acryloyl)-\(\gamma\)-substituted-2-pyrrolidones.**

Over a period of 30 min, \(n\)-butyllithium (1.6 M in hexanes, 2.5 mmol) was added dropwise to a solution of 5-alkoxy-2-pyrrolidone (2.5 mmol) in dry THF (ca. 70 mL) at -78 °C. At this temperature, the reaction mixture was stirred for 1.5 h followed by the dropwise addition of acryloyl chloride (3.0 mmol). After stirring for an additional 5 h at -78 °C, the reaction was quenched with saturated aqueous \(\text{NH}_4\text{Cl}\) (ca. 5 mL) and warmed to room temperature. A drop of \textit{tert}-butyl catechol solution (15 mM in acetone) was added to the reaction mixture and the solvent removed by reduced pressure. The residue was taken up
in ethyl acetate (ca. 20 mL) and water (ca. 10 mL) and the solution extracted with ethyl acetate (2x 10 mL). The combined organic phases were washed with saturated NaHCO₃ (ca. 10 mL) and brine (ca. 10 mL), and dried over anhydrous Na₂SO₄. After filtering the mixture, a second drop of inhibitor was added to the filtrate and the solvent removed under reduced pressure to afford a yellow opaque oil. The crude product was purified by column chromatography (silica, ethyl acetate/hexanes, 1:1).

**General Procedure for Conventional Free-Radical Polymerization.** Prior to polymerization, *N*-({acrlyoyl}-5-alkoxy-2-pyrrolidone (7 mmol) was dissolved in diethyl ether and passed through a short neutral alumina plug to remove inhibitor. In the absence of light, the solvent was removed by reduced pressure and redissolved in dry THF (ca. 3.5 mL) containing AIBN (0.07 mmol). The solution was sparged with N₂ for 30 min, sealed, and placed in a preheated oil bath at 65 °C for 24 h. The polymer was precipitated out of solution by dropwise addition to rapidly stirred cold hexanes (30 mL, 0 °C). The polymer was then redissolved in a minimum volume of THF and precipitated in cold hexanes (3x).

**General Procedure for RAFT Polymerization.**

Prior to polymerization, all monomers were passed through an alumina plug (using THF as the eluent) to remove inhibitor. Monomer, benzyl benzodithioate (BDTB), and 2,2′azobis(2-methylpropionitrile) (AIBN) (100:1:0.2) were placed in a polymerization tube followed by the addition of anhydrous THF such that monomer concentration was ca. 2.0 M. The solution was subjected to freeze-pump-thaw cycles (3x) and the tube backfilled with dry dinitrogen gas before being placed in an oil bath at 70°C for 20 h. The solution was cooled to ambient temperature and added dropwise into rapidly stirring cold hexanes.
causing a pink solid to precipitate. The polymer was isolated by filtration, dissolved in THF, and reprecipitated in hexanes (2x), followed by drying in vacuo. Note: poly(StSNP) was precipitated in cold (ca. 5 °C) methanol.

**Model compound.** Over a period of 30 min, n-butyllithium (1.6 M in hexanes, 13.76 mmol) was added drop-wise to a solution of 5-methoxyethoxy-2-pyrrolidone (12.5 mmol) in anhydrous THF (ca. 50 mL) at -78 °C. The reaction mixture was stirred at same temperature for 2.5 h followed by the addition of acryloyl chloride (2.3mL, 16.5 mmol). The solution was allowed to warm up to room temperature overnight. The next morning the solution was quenched with saturated aqueous NH₄Cl (ca. 5 mL). The solvent was removed by reduced pressure and the residue extracted with ethyl acetate (3 x 50 mL). The organic phases were combined and washed with brine (ca. 10mL) and dried over anhydrous Na₂SO₄. After filtering the mixture, the solvent was removed under reduced pressure to afford a yellow opaque oil. The crude product was purified twice by column chromatography (silica followed by alumina, ethyl acetate/hexanes, 1:1) to afford the product. Yield, 70%. ¹H NMR (500 MHz, CDCl₃): δ 5.78 (d, ³JHH = 4.78 Hz, 1H), 3.90 – 3.71 (m, 2H), 3.68 – 3.56 (m, 1H), 3.51 (t, ³JHH = 4.63 Hz, 2H), 3.37 (s, 3H), 2.91 (m, 1H), 2.47 (m ,1H), 2.09 (m, 2H), 1.71 (m, 2H), 1.62 – 1.39 (m, 2H), 0.92 (t, ³JHH = 7.43 Hz, 3H), 0.88 (t, ³JHH = 7.42 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 177.82, 175.30, 87.49, 72.00, 69.37, 58.97, 46.78, 31.83, 26.23, 24.54, 24.05, 11.62, 11.36. GC/MS: m/z (%): 228 (1%), 198 (3%), 182 (45%), 174 (3%), 153 (8%), 126 (1%), 115 (1%), 98 (58%), 84 (100%), 71 (48%), 59 (16%), 43 (8%).

**MeONP.** Yield, 31%. Rf = 0.55. ¹H NMR (CDCl₃, 500 MHz): δ 7.41 (1H, dd, ³JHH = 17.02 Hz, ³JHH = 10.45 Hz), 6.50 (1H, dd, ³JHH = 17.03 Hz, ³JHH = 1.80 Hz), 5.85 (1H, dd, ³JHH
= 10.46 Hz, \( ^3J_{HH} = 1.78 \) Hz), 5.65 (1H, m), 3.41 (3H, s), 2.83 (1H, m), 2.46 (1H, m), 2.05 (2H, m). \( \delta \) \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) 175.66, 165.94, 131.52, 129.24, 87.92, 57.09, 31.49, 25.84. GC/MS: m/z (%): 169 (8%) \([M^+]\), 154 (13%), 139 (90%), 84 (37%), 55 (100%).

**poly(N-(acryloyl)-5-methoxy-2-pyrrolidone).** \(^1\)H NMR (CDCl\(_3\), 500 MHz): \( \delta \) 5.54 (1H, br s), 3.75 (1H, br s), 3.36 (3H, s), 3.00-1.25 (6H, m)

**5-Ethoxy-2-pyrrolidone.** Yield, 66%. Analytical data consistent with reported data.\(^{35}\) \(^1\)H NMR (CDCl\(_3\), 500 MHz): \( \delta \) 7.65 (1H, s), 4.94 (1H, m), 3.52 (1H, m), 3.40 (1H, m), 2.50 (1H, m), 2.22 (2H, m), 2.04 (1H, m), 1.19 (3H, t, \( ^3J_{HH} = 6.90 \) Hz).

**EtONP.** Yield, 25%. \( R_f = 0.60 \). \(^1\)H NMR (CDCl\(_3\), 500 MHz): 7.41 (1H, dd, \( ^3J_{HH} = 17.08 \) Hz, \( ^3J_{HH} = 10.49 \) Hz), 6.49 (1H, dd, \( ^3J_{HH} = 17.04 \) Hz, \( ^3J_{HH} = 1.54 \) Hz), 5.84 (1H, dd, \( ^3J_{HH} = 10.44 \) Hz, \( ^3J_{HH} = 1.55 \) Hz), 5.73 (1H, m), 3.63 (2H, m), 2.87 (1H, m), 2.45 (1H, s), 2.05 (1H, s) 1.16 (3H, t, \( ^3J_{HH} = 7.01 \) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) 175.78, 165.92, 131.35, 129.38, 86.60, 65.27, 31.61, 26.48, 15.39. GC/MS: m/z (%): 183 (6%) \([M^+]\), 154 (17%), 111 (45%), 84 (37%), 55 (100).

**poly(N-(acryloyl)-5-ethoxy-2-pyrrolidone).** \(^1\)H NMR (CDCl\(_3\), 500 MHz): \( \delta \) 5.62 (1H, s), 3.85-3.25 (3H, m), 2.90-1.30 (6H, m), 1.13 (3H, s).

**5-Butoxy-2-pyrrolidone.** Yield, 22%. The crude product was recrystallized from hexanes. Analytical data consistent with reported data.\(^{36}\) \(^1\)H NMR (CDCl\(_3\), 500 MHz): \( \delta \) 7.56 (1H, m), 4.91 (1H, m), 3.45 (1H, m), 3.32 (1H, m), 2.49 (1H, m), 2.21 (2H, m), 2.03 (1H, m), 1.52 (2H, m), 1.34 (2H, m), 0.88 (3H, t, \( ^3J_{HH} = 7.37 \) Hz).
BuONP. Yield, 25%. Rf = 0.87. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.41 (1H, dd, $^3$J$_{HH}$ = 16.99 Hz, $^3$J$_{HH}$ = 10.43 Hz), 6.49 (1H, dd, $^3$J$_{HH}$ = 16.99 Hz, $^3$J$_{HH}$ = 1.67 Hz), 5.84 (1H, dd, $^3$J$_{HH}$ = 10.45 Hz, $^3$J$_{HH}$ = 1.66 Hz), 5.72 (1H, m), 3.57 (2H, m), 2.86 (1H, m), 2.45 (1H, m), 2.05 (2H, m), 1.50 (2H, m), 1.32 (2H, m), 0.88 (3H, t, $^3$J$_{HH}$ = 7.36 Hz). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 175.95, 166.08, 131.48, 129.49, 86.93, 69.78, 32.07, 31.76, 26.52, 19.40, 14.01. GC/MS: m/z (%): 211 (1%) [M$^+$], 154 (14%), 138 (100%), 111 (28%), 84 (78%), 55 (100%).

poly(N-(acryloyl)-5-butoxy-2-pyrrolidone). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 5.60 (1H, s), 3.52 (3H, m), 2.90-1.10 (10H, m), 0.88 (3H, s).

5-propargyloxy-2-pyrrolidone Yield, 44%. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.83, (1H, s), 5.14 (1H, m), 4.16 (2H, m), 2.48 (2H, m), 2.29 (1H, m), 2.19 (1H, m), 2.08 (1H, m). $^{13}$C NMR (CD$_2$Cl$_2$, 100 MHz): 179.66, 85.70, 79.90, 75.08, 55.15, 28.75, 28.60.

PrgONP. Yield, 22%. Rf = 0.83. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.40 (1H, dd, $^3$J$_{HH}$ = 16.98 Hz, $^3$J$_{HH}$ = 10.43 Hz), 6.52 (1H, dd, $^3$J$_{HH}$ = 17.02 Hz, $^3$J$_{HH}$ = 1.73 Hz), 5.85 (3H, m), 4.34 (2H, m), 2.88 (1H, m), 2.46 (2H, m), 2.14 (2H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 175.65, 166.31, 132.01, 129.19, 86.79, 79.77, 74.78, 57.90, 31.54, 26.74. GC/MS: m/z (%): 166 (17%), 84 (100%).

5-methoxyethoxy-2-pyrrolidone Yield, 38%. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.20 (1H, s), 4.97 (1H, m), 3.46-3.63 (4H, m), 3.36 (3H, s), 2.49 (1H, m), 2.30 (1H, m), 2.19 (1H, m), 2.07 (1H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz): 178.95, 87.06, 72.51, 67.68, 59.27, 28.62, 28.52.
MeOEtONP. Yield, 13%. $R_f = 0.53$. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.39 (1H, dd, $^3J_{HH} = 17.00$ Hz, $^3J_{HH} = 10.44$ Hz), 6.48 (1H, dd, $^3J_{HH} = 17.01$ Hz, $^3J_{HH} = 1.69$ Hz), 5.83 (1H, dd, $^3J_{HH} = 10.45$ Hz, $^3J_{HH} = 1.69$ Hz), 5.74 (1H, m), 3.78 (1H, m), 3.48 (2H, t, $^3J_{HH} = 4.73$ Hz), 3.33 (3H, s), 2.87 (1H, m), 2.44 (1H, m), 2.09 (2H, m). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 175.92, 166.22, 131.61, 129.42, 87.53, 72.12, 69.51, 59.17, 31.67, 26.55. GC/MS: m/z (%): 168 (3%), 154 (19%), 139 (30%), 138 (100%), 127 (6%), 84 (60%), 55 (100%), 45 (15%).

poly(N-(acryloyl)-5-methoxyethoxyxy-2-pyrrolidone). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 5.60 (1H, s), 4.00-3.40 (5H, m) 3.33 (3H, s), 2.90-1.10 (6H, m).

5-Ethylthio-2-pyrrolidone. Method A. Yield, 70%. Analytical data consistent with reported data.$^{37}$ $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.13 (br, s, 1H), 4.83 (m, 1H), 2.64 (q, $^3J_{HH} = 7.40$ Hz, 2H), 2.52 (m, 2H), 2.33 (m, 1H), 2.09 (m, 1H), 1.30 (t, $^3J_{HH} = 7.40$ Hz, 3H).$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 178.24, 58.89, 29.75, 28.58, 24.35, 14.79.

EthSNP. Yield, 25%. $R_f = 0.54$. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.44 (dd, $^3J_{HH} = 16.95$ Hz, $^3J_{HH} = 10.47$ Hz, 1H), 6.52 (d, $^3J_{HH} = 16.91$ Hz, 1H), 5.87 (d, $^3J_{HH} = 10.27$ Hz, 1H), 5.67 (d, $^3J_{HH} = 7.38$ Hz, 1H), 2.88 (m, 2H), 2.73 (m, 1H), 2.55 (m, 1H), 2.45 (m, 1H), 2.14 (m, 1H), 1.30 (t, $^3J_{HH} = 7.31$ Hz, 3H).$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 174.79, 165.28, 131.33, 129.13, 61.34, 32.53, 27.54, 26.38, 14.88. GC/MS: m/z (%): 199 (6%) [M$^+$], 138 (90%), 84 (23%), 55 (100%), 28 (12%).

poly(EthSNP). Yield, 76%. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.66 (br, s, 1H), 3.69 (br, s, 1H), 2.83-2.52 (br, m, 4H), 2.01 – 1.51 (br, m, 4H), 1.27(s, 3H).
5-Butylthio-2-pyrrolidone. Method A. Yield, 40%. Analytical data consistent with reported data.\(^{38}\) \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 6.51 (s, 1H), 4.78 (dd, \(\^3J_{\text{HH}} = 7.16\) Hz, \(\^3J_{\text{HH}} = 3.52\) Hz, 1H), 2.52 (m, 4H), 2.30 (m, 1H), 2.09 (m, 1H), 1.57 (m, 2H), 1.39 (m, 2H), 0.90 (t, \(\^3J_{\text{HH}} = 7.32\) Hz, 3H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta\) 178.24, 59.17, 31.69, 29.93, 29.75, 28.64, 22.02, 13.63. GC/MS: m/z (%): 128 (100%), 98 (49%), 90 (3%), 83 (9%), 68 (5%), 55 (9%), 45 (33%), 28 (24%).

BuSNP. Yield, 53%. \(R_f = 0.61.\) \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.43 (dd, \(\^3J_{\text{HH}} = 16.96\) Hz, \(\^3J_{\text{HH}} = 10.46\) Hz, 1H), 6.55 – 6.48 (m, 1H), 5.90 – 5.83 (m, 1H), 5.63 (d, \(\^3J_{\text{HH}} = 7.46\) Hz, 1H), 2.92 (m, 1H), 2.81 (m, 1H), 2.70 (m, 1H), 2.55 (m, 1H), 2.44 (m, 1H), 2.15 (m, 1H), 1.60 (m, 2H), 1.41 (m, 2H), 0.92 (t, \(\^3J_{\text{HH}} = 7.25\) Hz, 3H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta\) 174.82, 165.25, 131.31, 129.15, 61.67, 32.52, 31.98, 31.94, 27.59, 21.99, 13.65.

poly(BuSNP). Yield, 25%. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 5.49 (s, 1H), 3.63-3.47 (m, 1H), 1.38 (s, 2H), 0.89 (s, 3H).

5-Phenylthio-2-pyrrolidone. Method A. Yield, 86%. Analytical data consistent with reported data.\(^{39}\) \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.58 – 7.39 (m, 2H), 7.42 – 7.28 (m, 3H), 6.14 (s, 1H), 5.03 (d, \(\^3J_{\text{HH}} = 7.3\) Hz, 1H), 2.56 (m, 1H), 2.31 – 2.04 (m, 3H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta\) 177.71, 134.54, 131.53, 129.39, 128.78, 62.27, 29.11, 28.22.

PhSNP. Yield, 21%. \(R_f = 0.38.\) \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.59-7.55 (m, 2H), 7.47 (dd, \(\^3J_{\text{HH}} = 17.00\) Hz, \(\^3J_{\text{HH}} = 10.45\) Hz, 1H), 7.39 (m, 3H), 6.57 (dd, \(\^3J_{\text{HH}} = 17.00\) Hz, \(\^3J_{\text{HH}} = 1.64\) Hz, 1H), 5.91 (dd, \(\^3J_{\text{HH}} = 10.44\) Hz, \(\^3J_{\text{HH}} = 1.64\) Hz, 1H), 5.74 (d, \(\^3J_{\text{HH}} = 7.30\) Hz, 1H), 2.86 – 1.72 (m, 4H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta\) 174.75, 164.60, 135.58, 131.74,
131.06, 129.34, 129.23, 128.99, 64.07, 31.95, 26.35. GC/MS: m/z (%) : 247 [M+] (3%), 138 (98%), 109 (12%), 84 (10%), 55 (100%), 28 (20%).

**poly(PhSNP).** Yield, 35% $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.51 (s, 2H), 7.27 (s, 3H), 5.67 (s, 1H), 3.66 (s, 1H), 2.38 – 1.32 (br m, 6H).

**5-Stearylthio-2-pyrrolidone.** Method A. Yield, 74%, $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 6.84 (br, s, 1H), 4.81 (dd, $^3$J$_{HH}$ = 7.37 Hz, $^3$J$_{HH}$ = 3.52 Hz, 1H), 2.59 (t, $^3$J$_{HH}$ = 7.42, 2H), 2.52 (m, 2H), 2.33 (m, 1H), 2.11 (m, 1H), 1.60 (m, 2H), 1.38 (m, 2H), 1.26 (s, 28H), 0.88 (t, $^3$J$_{HH}$ = 6.93Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 174.87, 165.28, 131.42, 129.12, 61.69, 32.55, 32.35, 31.93, 29.89, 29.71, 29.67, 29.60, 29.51, 29.38, 29.21, 28.92, 27.60, 22.71, 14.15.

**StSNP.** Yield, 22%, $R_f$ = 0.67. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.43 (dd, $^3$J$_{HH}$ = 16.96, 10.46 Hz, 1H), 6.52 (m, 1H), 5.86 (d, $^3$J$_{HH}$ = 10.41 Hz, 1H), 5.62 (d, $^3$J$_{HH}$ = 7.42 Hz, 1H), 2.91 (m, 1H), 2.80 (m, 1H), 2.68 (m, 1H), 2.54 (m, 1H), 2.43 (m, 1H), 2.14 (m, 1H), 1.59 (m, 2H), 1.38 – 1.33 (m, 2H), 1.25 (s, 28H), 0.87 (t, $^3$J$_{HH}$ = 6.7 Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 174.87, 165.28, 131.42, 129.12, 61.69, 32.55, 32.35, 31.93, 29.89, 29.71, 29.60, 29.51, 29.38, 29.21, 28.92, 27.60. GC/MS: m/z (%): 286 (14%), 252 (14%), 224 (5%), 196 (2%), 182 (2%), 168 (3%).

**poly(StSNP).** Yield, 44%, $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.60 (s, 1H), 3.51 (s, 1H), 2.71 (br d, 4H), 1.99 (s, 2H), 1.59 (s, 2H), 1.29 (s, 32H), 0.91 (t, $^3$J$_{HH}$ = 6.73 Hz, 3H).

**5-Cyclohexylthio-2-pyrrolidone.** Method A. Yield, 77% $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 6.50 (br s, 1H), 4.90 (dd, $^3$J$_{HH}$ = 7.36, $^3$J$_{HH}$ = 4.09 Hz, 1H), 2.79 (m, 1H), 2.53 (m, 2H), 2.33 (m, 1H), 2.09 (m, 1H), 1.97 (m, 2H), 1.79 (m, 2H), 1.64 (m, 1H), 1.46 – 1.21 (m, 5H).
$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 177.42, 57.81, 43.40, 34.30, 34.20, 29.50, 29.43, 26.03, 25.91, 25.59.

**CySNP.** Yield, 36%, $R_f = 0.38$. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.45 (dd, $^3J_{HH} = 17.01$, 10.45 Hz, 1H), 6.53 (d, $^3J_{HH} = 17.01$ Hz, $^3J_{HH} = 1.76$ Hz, 1H), 5.88 (dd, $^3J_{HH} = 10.46$ Hz, $^3J_{HH} = 1.76$ Hz, 1H), 5.68 (d, $^3J_{HH} = 7.32$ Hz, 1H), 5.50 (s, 1H), 5.00 (m, 1H), 4.00 (m, 1H), 3.50 (s, 1H), 3.00 (m, 1H), 2.00 (m, 1H), 1.90 (m, 1H), 1.75 (m, 1H), 1.55 (m, 1H), 1.40 (m, 1H), 1.30 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 174.87, 165.17, 131.21, 129.24, 60.50, 44.48, 34.68, 33.48, 32.49, 28.47, 25.70 ppm. GC/MS: m/z (%): 253 (9%) [M$^+$], 170 (4%), 138 (100%), 84 (18%), 55 (78%).

**poly(CySNP).** Yield, 74%, $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.55 (s, 1H), 3.52 (s, 1H), 3.21 – 2.28 (m, 6H), 1.97 (m, 4H), 1.76 – 1.29 (m, 7H).

**5-Methoxyethanethio-2-pyrrolidone.** Method A. Yield, 70%, $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.06 (br s, 1H), 4.85 (dd, $^3J_{HH} = 7.52$ Hz, $^3J_{HH} = 4.50$ Hz, 1H), 3.68 (m, 1H), 3.60 (m, 1H), 3.41 (s, 3H), 2.83 (m, 2H), 2.53 (m, 2H), 2.36 (m, 1H), 2.02 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 176.79, 73.77, 60.69, 58.87, 32.50, 29.94, 28.48.

**MeOEtSNP.** Yield, 16%, $R_f = 0.23$. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.45 (dd, $^3J_{HH} = 17.00$, 10.45 Hz, 1H), 6.53 (dd, $^3J_{HH} = 17.00$, 1.71 Hz, 1H), 5.89 (dd, $^3J_{HH} = 10.45$, 1.70 Hz, 1H), 5.71 (d, $^3J_{HH} = 7.51$ Hz, 1H), 3.62 (m, 2H), 3.39 (s, 3H), 3.08 (m, 1H), 2.93 (m, 2H), 2.63 – 2.40 (m, 2H), 2.20 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 174.78 , 165.42 , 131.44 , 129.12 , 72.20 , 62.16 , 58.80 , 32.46 , 32.14 , 27.64. GC/MS: m/z (%): 229 (2%) [M$^+$], 197 (13%), 170 (9%), 138 (100%), 84 (24%), 55 (80%).
**poly(MeOEtSNP).** Yield, 61% $^1$H NMR (500 MHz, CDCl$_3$): δ 5.59 (br, s, 1H) 3.64 (br, m, 3H), 3.39 (s, 3H), 3.04 - 2.05 (br m, 5H), 2.04 – 1.25 (br m, 3H).

**5-Tetrahydrofurfuryloxy-2-pyrrolidone.** Method B. Yield, 22%. $^1$H NMR (500 MHz, CDCl$_3$): δ 7.23 (br, d, 1H), 4.99 (m, 1H), 3.99 (m, 1H), 3.86 (m, 1H), 3.76 (m, 1H), 3.62 - 3.54 (m, 1H), 3.41 (m, 1H), 2.49 (m, 1H), 2.24 (m, 2H), 2.05 (m, 1H), 1.90 (m, 4H), 1.58 – 1.47 (m, 1H). $^{13}$C NMR (126 MHz, Chloroform-$d$): δ 177.48, 176.98, 85.30, 84.54, 77.62, 76.33, 76.01, 69.34, 68.01, 66.44, 66.39, 66.19, 62.79, 26.58, 26.49, 26.36, 26.26, 25.95, 25.24, 23.97, 23.61, 23.58.

**FurONP:** Yield, 13% R$_f$ = 0.12. $^1$H NMR (500 MHz, CDCl$_3$): δ 7.44 (dd, $^3$J$_{HH}$ = 17.00, 10.45 Hz, 1H), 6.51 (d, $^3$J$_{HH}$ = 17.01 Hz, 1H), 5.88 (m, 1H), 5.80 (d, $^3$J$_{HH}$ = 5.28 Hz, 1H), 4.00 (m, 1H), 3.86 (m, 1H), 3.80 (m, 1H), 3.66 (m, 2H), 2.91 (m, 1H), 2.48 (m, 1H), 2.11 (m, 2H), 1.99 – 1.78 (m, 3H), 1.56 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$): 175.78, 175.74, 166.04, 166.02, 131.37, 129.27, 87.63, 87.35, 78.06, 77.73, 76.82, 73.08, 72.53, 68.46, 68.32, 31.51, 27.94, 27.78, 26.47, 26.42, 25.72, 25.55. GC/MS: m/z (%): 169 (11%), 138 (69%), 111 (6%), 84 (74%), 71 (100%), 55 (70%), 43 (22%), 27 (9%).

**poly(FurONP).** Yield, 34% $^1$H NMR (500 MHz, CDCl$_3$): δ 5.68 (s, 1H); 4.01 (s, 2H), 3.87-3.60 (m, 4H); 2.78- 1.61 (br, m, 10H).

**5-Cyclohexanoxy-2-pyrrolidone.** Method B. Yield, 40%. $^1$H NMR (500 MHz, CDCl$_3$): 6.87 (br, m, 1H), 5.12 (d, $^3$J$_{HH}$ = 6.10Hz, 1H), 3.37 (m, 1H), 2.55 (m, 1H), 2.33 (m, 1H), 2.23 (m, 1H), 2.05 (m, 1H), 1.87 (m, 2H), 1.76 (m, 2H), 1.56 (m, 1H), 1.37-1.21 (m, 5H). $^{13}$C NMR (126 MHz, CDCl$_3$): δ 178.88, 83.62, 75.44, 33.36, 32.36, 29.16, 28.35, 25.55, 24.15, 24.06.
**CyONP.** Yield, 26%. $R_f = 0.62$ $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.47 (dd, $^3J_{HH} = 17.00$ Hz, $^3J_{HH} = 10.45$ Hz, 1H); 6.55 (d, $^3J_{HH}$ 17.02 Hz, 1H); 5.88 (d, $^3J_{HH}$ 10.47 Hz, 1H), 5.85 (d, $^3J_{HH}$ 5.4 Hz, 1H), 3.70 (m, 1H), 2.93 (m, 1H), 2.48 (m, 1H) 2.15 (m, 1H); 2.03 (m, 1H); 1.96 (m, 1H) 1.88 (m, 1H) 1.74 (m, 2H); 1.55 (m, 1H); 1.3 (m, 5H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 175.75, 165.86, 131.14, 129.47, 85.13, 32.93, 32.72, 31.56, 27.20, 25.61, 24.14, 24.06 GC/MS: m/z (%): 207 (1%), 138 (100%), 111 (9%), 84 (32%), 55 (100%), 28 (20%).

**poly(CyONP).** Yield, 65%. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 5.70 (br, s, 1H), 3.65 (br, m, 2H), 2.77 (br, m, 3H), 2.17 (br m, 2H), 1.86 (br, m, 3H), 1.70 (br, s, 1H), 1.51 (br, s, 2H), 1.27 (br, m, 5H).

**poly(PNP).** Yield, 68% $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 3.74 (br, m, 3H), 2.48 (br, m, 2H), 1.97 (s, 2H), 1.71 - 1.38 (br, m, 2H).

![Figure 2-11](image-url). $^1$H NMR spectrum of **5-propargyloxy-2-pyrrolidone** (500 MHz, CDCl$_3$).
Figure 2-12. $^{13}$C NMR spectrum of 5-propargyloxy-2-pyrrolidone (100 MHz, CDCl$_3$).

Figure 2-13. $^1$H NMR spectrum of 5-methoxyethoxy-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-14. $^{13}$C NMR spectrum of 5-methoxyethoxy-2-pyrrolidone (100 MHz, CDCl$_3$).

Figure 2-15 $^1$H NMR spectrum of 5-ethylthio-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-16. $^{13}$C NMR spectrum of 5-ethylthio-2-pyrrolidone (126 MHz, CDCl$_3$).

Figure 2-17. $^1$H NMR spectrum of 5-butylthio-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-18. $^{13}$C NMR spectrum of 5-butylthio-2-pyrrolidone (126 MHz, CDCl$_3$).

Figure 2-19. $^1$H NMR spectrum of 5-cyclohexyloxy-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-20. $^{13}$C NMR spectrum of 5-cyclohexyloxy-2-pyrrolidone (126 MHz, CDCl$_3$).

Figure 2-21. $^1$H NMR spectrum of 5-cyclohexylthio-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-22. $^{13}$C NMR spectrum of 5-cyclohexylthio-2-pyrrolidone (126 MHz, CDCl$_3$).

Figure 2-23. $^1$H NMR spectrum of 5-phenylthio-2-pyrrolidone (500 MHz, CDCl$_3$).
**Figure 2-24.** $^{13}$C NMR spectrum of 5-phenylthio-2-pyrrolidone (126 MHz, CDCl$_3$).

**Figure 2-25.** $^1$H NMR spectrum of 5-methoxyethylthio-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-26. $^{13}$C NMR spectrum of 5-methoxyethylthio-2-pyrrolidone (126 MHz, CDCl$_3$).

Figure 2-27. $^1$H NMR spectrum of 5-tetrahydrofurfuryloxy-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-28. $^{13}$C NMR spectrum of 5-tetrahydrofurfuryloxy-2-pyrrolidone (126 MHz, CDCl$_3$).

Figure 2-29. $^1$H NMR spectrum of 5-stearylthio-2-pyrrolidone (500 MHz, CDCl$_3$).
Figure 2-30. $^{13}\text{C}$ NMR spectrum of 5-stearylthio-2-pyrrolidone (126 MHz, CDCl$_3$).
Figure 2-31. $^1$H NMR spectrum of MeONP (500 MHz, CDCl$_3$).

Figure 2-32 $^{13}$C NMR spectrum of MeONP (100 MHz, CDCl$_3$).
Figure 2-33. $^1$H NMR spectrum of EtONP (500 MHz, CDCl$_3$).

Figure 2-34. $^{13}$C NMR spectrum of EtONP (100 MHz, CDCl$_3$).
Figure 2-35. \(^1\)H NMR spectrum of BuONP (500 MHz, CDCl\(_3\)).

Figure 2-36. \(^{13}\)C NMR spectrum of BuONP (100 MHz, CDCl\(_3\)).

Figure 2-37. \(^1\)H NMR spectrum of PrgONP (500 MHz, CDCl\(_3\)).
Figure 2-38. $^{13}$C NMR spectrum of PrgONP (100 MHz, CDCl$_3$).

Figure 2-39. $^1$H NMR spectrum of MeOEtONP (500 MHz, CDCl$_3$).
Figure 2-40. $^{13}$C NMR spectrum of MeOEtONP (100 MHz, CDCl$_3$).

Figure 2-41. $^1$H NMR spectrum of EthSNP (500 MHz, CDCl$_3$).
Figure 2-42. $^{13}$C NMR spectrum of EthSNP (126 MHz, CDCl$_3$).

Figure 2-43. $^1$H NMR spectrum of BuSNP (500 MHz, CDCl$_3$).
Figure 2-44. $^{13}$C NMR spectrum of BuSNP (126 MHz, CDCl$_3$).

Figure 2-45. $^1$H NMR spectrum of CyONP (126 MHz, CDCl$_3$).
Figure 2-46. $^{13}$C NMR spectrum of CyONP (126 MHz, CDCl$_3$).
Figure 2-47. $^1$H NMR spectrum of CySNP (500 MHz, CDCl$_3$).

Figure 2-48. $^{13}$C NMR spectrum of CySNP (126 MHz, CDCl$_3$)
Figure 2-49. $^{1}$H NMR spectrum of PhSNP (500 MHz, CDCl$_3$).

Figure 2-50. $^{13}$C NMR spectrum of CySNP (126 MHz, CDCl$_3$).
Figure 2-51. $^1$H NMR spectrum of MeOEthSNP (500 MHz, CDCl$_3$).

Figure 2-52. $^{13}$C NMR spectrum of MeOEthSNP (126 MHz, CDCl$_3$).
Figure 2-53. $^1$H NMR spectrum of FurONP (500 MHz, CDCl$_3$).

Figure 2-54. $^{13}$C NMR spectrum of FurONP (126 MHz, CDCl$_3$).
Figure 2-55. $^1$H NMR spectrum of StNP (500 MHz, CDCl$_3$).

Figure 2-56. $^{13}$C NMR spectrum of StSNP (126 MHz, CDCl$_3$).
Figure 2-57. $^1$H NMR spectrum of poly(MeEtONP-co-MeONP) (500 MHz, CDCl$_3$).

Figure 2-58. $^1$H NMR spectrum of poly(MeEtONP-co-EtONP) (500 MHz, CDCl$_3$).
Figure 2-59. $^1$H NMR spectrum of poly(MeEtONP-co-BuONP) (500 MHz, CDCl$_3$).

Figure 2-60. $^1$H NMR spectrum of poly(NP) (500 MHz, CDCl$_3$).
Figure 2-61. $^1$H NMR spectrum of poly(EthSNP) (500 MHz, CDCl$_3$).

Figure 2-62. $^1$H NMR spectrum of poly(BuSNP) (500 MHz, CDCl$_3$).
Figure 2-63. $^1$H NMR spectrum of poly(CyONP) (500 MHz, CDCl$_3$).

Figure 2-64. $^1$H NMR spectrum of poly(CySNP) (500 MHz, CDCl$_3$).
Figure 2-65. $^1$H NMR spectrum of poly(PhSNP) (500 MHz, CDCl$_3$).

Figure 2-66. $^1$H NMR spectrum of poly(MeOEtSNP) (500 MHz, CDCl$_3$).
Figure 2-67. $^1$H NMR spectrum of poly(FurONP) (500 MHz, CDCl$_3$).

Figure 2-68. $^1$H NMR spectrum of poly(StSNP) (500 MHz, CDCl$_3$).
Figure 2-69. $^1$H NMR spectrum of model compound (500 MHz, CDCl$_3$).

Figure 2-70. $^{13}$C NMR spectrum of model compound (500 MHz, CDCl$_3$).
Figure 2-71. 2D COSY NMR spectrum of model compound (500 MHz, CDCl₃).
Figure 2-72. 2D COSY NMR spectrum of EthONP (500 MHz, CDCl₃).
Figure 2-73. 2D COSY NMR spectrum of EthSNP (500 MHz, CDCl$_3$).
Figure 2-74. %Transmittance vs time plot of poly(MeEtONP-co-MeONP) dissolved in water (5 mg/mL, 500 nm). The temperature was cycled between 26 °C and 34 °C at a rate of 1 °C/min.

Figure 2-75. %Transmittance vs time plot of poly(MeEtONP-co-EtONP) dissolved in water (2.5 mg/mL, 500 nm). The temperature was cycled between 16 °C and 24 °C at a rate of 1 °C/min.
Figure 2-76. %Transmittance vs time plot of poly(MeEtONP-co-EtONP) dissolved in water (2.5 mg/mL, 500 nm). The temperature was cycled between 1 °C and 18 °C at a rate of 1 °C/min.

Figure 2-77. GPC trace of poly(MeONP) (synthesized via free radical).
Figure 2-78. GPC trace of poly(EtONP) (synthesized via free radical).

Figure 2-79. GPC trace of poly(BuONP) (synthesized via free radical).
Figure 2-80. GPC trace of poly(MeEtONP) (synthesized via free radical).

Figure 2-81. GPC trace of poly(MeEtONP-co-MeONP) (synthesized via free radical).
**Figure 2-82.** GPC trace of poly(MeEtONP-co-EtONP) (synthesized via free radical).

**Figure 2-83.** GPC trace of poly(MeEtONP-co-BuONP) (synthesized via free radical).
Figure 2-84. GPC trace of poly(EthONP) (synthesized via RAFT).

Figure 2-85. GPC trace of poly(EthSNP) (synthesized via RAFT).
Figure 2-86. GPC trace of poly(BuONP) (synthesized via RAFT).

Figure 2-87. GPC trace of poly(BuSNP) (synthesized via RAFT).
Figure 2-88. GPC trace of poly(CyONP) (synthesized via RAFT).

Figure 2-89. GPC trace of poly(CySNP) (synthesized via RAFT).
Figure 2-90. GPC trace of poly(PhSNP) (synthesized via RAFT).

Figure 2-91. GPC trace of poly(MeOEthONP) (synthesized via RAFT).
Figure 2-92. GPC trace of poly(MeOEthSNP) (synthesized via RAFT).

Figure 2-93. GPC trace of poly(FurONP) (synthesized via RAFT).
Figure 2-94. GPC trace of poly(StSNP) (synthesized via RAFT).

Figure 2-95. TGA trace of poly(MeONP) (synthesized via free radical) (20 °C/min).
Figure 2-96. TGA trace of poly(EtONP) (synthesized via free radical) (20 °C/min).

Figure 2-97. TGA trace of poly(BuONP) (synthesized via free radical) (20 °C/min).
Figure 2-98. TGA trace of poly(PrgONP) (synthesized via free radical) (20 °C/min).

Figure 2-99. TGA trace of poly(MeEtONP) (synthesized via free radical) (20 °C/min).
Figure 2-100. TGA thermograms of poly(NP).

Figure 2-101. TGA thermograms of poly(EthONP) and poly(EthSNP).
Figure 2-102. TGA thermograms of poly(BuONP) and poly(BuSNP).

Figure 2-103. TGA thermograms of poly(CyONP) and poly(CySNP).
Figure 2-104. TGA thermograms of poly(MeOEthONP) and poly(MeOEthSNP).

Figure 2-105. TGA thermograms of poly(PhSNP) and poly(StSNP).
Figure 2-106. TGA trace of poly(MeEtONP-co-MeONP) (20 °C/min).

Figure 2-107. TGA trace of poly(MeEtONP-co-EtONP) (20 °C/min).
Figure 2-108. TGA trace of poly(MeEtONP-co-BuONP) (20 °C/min).
Figure 2-109. DSC trace of poly(MeONP) (10 °C/min).

Figure 2-110. DSC trace of poly(EtONP) (20 °C/min).
Figure 2-111. DSC trace of poly(BuONP) (20 °C/min).

Figure 2-112. DSC trace of poly(MeEtONP) (20 °C/min).
Figure 2-113. DSC traces of poly(EthONP) (red, solid), poly(EthSNP) (red, dash), poly(BuONP) (black, solid), poly(BuSNP) (black, dash). Second scan, ramp rate: 10 °C/min.

Figure 2-114. DSC traces of poly(CyONP) (black, solid), poly(CySNP) (black, dash), poly(PhSNP) (red, solid). Second scan, ramp rate: 10 °C/min.
Figure 2-115. DSC traces of poly(FuONP) (black, solid), poly(NP) (black, dash), poly(MeOEthONP) (red, solid) and poly(MeOEthSNP) (red, dash). Second scan, ramp rate: 10 °C/min.

Figure 2-116. DSC traces of poly(StSNP). Second scan, ramp rate: 10 °C/min.
Table 2-4. Cloud point temperatures of poly(MeOEthONP) and poly(FurONP). \(^a\)

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<th>Polymer</th>
<th>Conc. (mg/mL)</th>
<th>Medium</th>
<th>CP (°C)</th>
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<tr>
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\(^a\) Cloud points were measured by turbidimetry (\(\lambda = 500\) nm) and taken as the temperature at which the solution lost 50\% of its original optical transmission during the heating scan.
Figure 2-117. Anodic decarboxylation apparatus. (Adapted from Bhat, R.; Pietrangelo, A. Macromol. Rapid Commun. 2013, 34, 447-451)
2.5 References


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CHAPTER 3

Pyrrolidone-Based Block Copolymer Micelles for Drug-Loading and Controlled Release

3.1 Introduction

Amphiphilic block copolymers are comprised of hydrophobic and hydrophilic blocks in the polymer structure. The difference between the hydrophobicity of the two segments results in spontaneous self-assembly into core-shell architectures in aqueous media. These structures, known as micelles, provide a hydrophobic solubilizing microenvironment for lipophilic pharmaceuticals that are otherwise poorly soluble in aqueous media. Block copolymer micelles are studied extensively for their encapsulating abilities that are attractive for nanoscale drug-delivery applications. As colloidal aggregates, the micellar scaffolds also shield therapeutic drugs from unwanted interactions with healthy tissues and increase blood residence times by reducing the rate of body clearance facilitated by the reticuloendothelial system. Moreover, the chemical flexibility of the block copolymer permits: 1) customization of the hydrophobic interior to improve drug-loading capacity and 2) surface modification to the hydrophilic exterior to enhance target efficiency and specificity of tissue targeting.

Drug encapsulation is a complex phenomenon that relies on multiple related mechanisms such as the hydrophobic effect, polymer/drug miscibility, electrostatic complexation, and/or secondary interactions such as π-π stacking or hydrogen-bonding. To date, few experimental studies have examined drug-loading and

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a This chapter is adapted from a manuscript in preparation. Authors’ contributions are specified in results and discussions section.
thermoreponsive release profiles as a function of block copolymer structure. Moreover, in past reports, micelles were comprised of hydrophobic repeat units with exceedingly diverse chemical architectures (e.g., poly(styrene) vs. poly(butyl methacrylate),\textsuperscript{24} or, poly(lactide) vs. poly(ε-caprolactone)\textsuperscript{33}), hence structure-property correlations cannot be adequately addressed. As such, there remains a need for comparative analyses that evaluate thermoreponsive block copolymer micelles with only modest differences in their hydrophobic interior in order to assess the influence that core-structure has on drug-loading and release phenomena, information that is critical to establishing design criteria for micellar drug delivery vehicles with efficient encapsulation and release profiles.

3.1.1 Block Copolymer Micelles with Thermoreponsive Drug Release

Currently, there is interest in preparing \textit{smart} micellar drug-delivery vehicles that expel pharmaceuticals in both a spatially and temporally controlled manner upon application of external stimuli such as pH\textsuperscript{25,26,27}, magnetic field,\textsuperscript{28,29} or temperature.\textsuperscript{30,31,32} The latter is particularly appealing since the application of heat to an affected area is both convenient and toxicologically safe.\textsuperscript{33} To impart this mode of activation, many micellar models employ poly(N-isopropylacrylamide) (PNIPAAm) as the hydrophilic thermosensitive block that undergoes a phase transition upon exceeding its LCST.\textsuperscript{34} During this phase transition, the PNIPAAm blocks become hydrophobic resulting in collapse of the micellar corona, increased intermicellar aggregation, and expulsion of drug from the hydrophobic cores (Figure. 3-1).\textsuperscript{35,36,37,38,39,40,41,42,43,44,45}
In the previous chapter, we described the synthesis of novel pyrrolidone-based polymers and elucidated some of their physicochemical properties that were affected by modest modifications to the hydrophobic residues on the pyrrolidone moiety. Inspired by the results of this investigation, our group has prepared block copolymer micelles with thermoresponsive PNIPAAm coronae and poly(N-acryloyl-2-pyrrolidone) cores as potential drug-delivery agents given the structural tunability of the pyrrolidone scaffold and the biocompatibility and coordination ability that it lends to polymers of similar structure. 46, 47, 48, 49, 50, 51, 52, 53, 54
In this work performed by Dr. Xiao-Li Sun et. al. in the Pietrangelo group, block copolymer self-assembly, intermicellar aggregation, drug loading efficiency (DLE), and thermoresponsive drug release were examined using three sets of block copolymers that are distinguishable only by the pyrrolidone moiety (i.e., 2-pyrrolidone, 5-methoxy-2-pyrrolidone, and 5-butoxy-2-pyrrolidone) or hydrophobic block length. The synthesis of these block copolymers is outlined in the Scheme 3-1. The performance of these systems as drug carriers was evaluated using the anthracycline chemotherapeutic agent doxorubicin$^{55}$ as the hydrophobic payload and MCF-7 breast cancer cells as the biological target. The goal of this work was to identify how both the addition and lengthening of simple aliphatic alkoxy residues (i.e., MeO and BuO) tethered to the pyrrolidone moieties influence the micellar physicochemical properties and their ability to serve as thermoresponsive drug carriers.

In sum, Dr. Xiao-Li Sun observed that critical micelle concentrations (CMCs) decreased by two orders of magnitude in the order of PNIPAAm-PNP, PNIPAAm-PMNP, and PNIPAAm-PBNP, indicating that the alkoxy residue on the pyrrolidone scaffold significantly increases the overall hydrophobic character of the pyrrolidone-based polymer block. Moreover, in the long chain block copolymer, the hydrodynamic radii were seen to
decrease in the order of PNIPAAm-PBNP, PNIPAAm-PMNP and PNIPAAm-PNP whereas the trend was reversed in the short chain polymers. These structural modifications were also seen to influence the intermicellar aggregation above the lower critical solution temperature (LCST) and this was found to vary depending on the nature of the hydrophobic residue.

While the thermoresponsivity of a micelle arises from the hydrophilic corona, its drug-loading capacity is a direct consequence of noncovalent interactions between the hydrophobic and the lipophilic drug (Doxorubicin, Fig 3-2). Doxorubicin belongs to the Class 1 anthracycline family of chemotherapeutic agents, used effectively for the treatment of breast cancer, hematological malignancies, and soft tissue sarcomas.\textsuperscript{55}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{doxorubicin.png}
\caption{Doxorubicin drug}
\end{figure}

Using doxorubicin as a therapeutic hydrophobic payload, drug loading efficiencies were found to increase significantly in the order of PNIPAAm-PNP, PNIPAAm-PMNP, and PNIPAAm-PBNP a trend attributed to enhanced cohesive forces (i.e. London dispersion forces) between DOX and core as the latter becomes more hydrophobic. This indicates that drug encapsulation can be improved with only modest adjustments to macromolecular structure.

Surprisingly, when heated above the LCST, the thermoresponsive DOX release decreases in the order of PNIPAAm-PNP, PNIPAAm-PMNP, and PNIPAAm-PBNP
(Table 3-1), indicating that release processes are hindered by the cohesive forces responsible for efficient encapsulation. This trend brings to light a fundamental issue that must be addressed if efficient loading and release is to be achieved. Finally, cytotoxicity assays performed above the LCST revealed that DOX-loaded micelles are less cytotoxic than the free drug in formulations where DOX concentrations are equivalent. This is expected as we observe that drug-release from the micelles is incomplete.

**Table 3-1 Drug encapsulation profiles of thermoresponsive block copolymers**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Copolymer</th>
<th>DLE&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>DLC&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>Release&lt;sup&gt;c&lt;/sup&gt; 37°C (%)</th>
<th>Release&lt;sup&gt;d&lt;/sup&gt; &lt;20°C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PNIPAAm&lt;sub&gt;72&lt;/sub&gt;-PBNP&lt;sub&gt;73&lt;/sub&gt;</td>
<td>82.4±1.9</td>
<td>28.7±2.1</td>
<td>22.7±2.0</td>
<td>4.7±1.2</td>
</tr>
<tr>
<td>2</td>
<td>PNIPAAm&lt;sub&gt;72&lt;/sub&gt;-PBNP&lt;sub&gt;26&lt;/sub&gt;</td>
<td>74.9±3.2</td>
<td>25.9±2.0</td>
<td>24.9±0.2</td>
<td>6.0±0.4</td>
</tr>
<tr>
<td>3</td>
<td>PNIPAAm&lt;sub&gt;72&lt;/sub&gt;-PMNP&lt;sub&gt;78&lt;/sub&gt;</td>
<td>72.4±2.7</td>
<td>25.3±1.5</td>
<td>25.2±1.0</td>
<td>6.5±0.6</td>
</tr>
<tr>
<td>4</td>
<td>PNIPAAm&lt;sub&gt;72&lt;/sub&gt;-PMNP&lt;sub&gt;29&lt;/sub&gt;</td>
<td>65.9±2.3</td>
<td>24.1±1.7</td>
<td>31.1±1.4</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>5</td>
<td>PNIPAAm&lt;sub&gt;72&lt;/sub&gt;-PNP&lt;sub&gt;79&lt;/sub&gt;</td>
<td>60.6±7.5</td>
<td>21.5±3.6</td>
<td>33.1±0.8</td>
<td>4.8±0.3</td>
</tr>
<tr>
<td>6</td>
<td>PNIPAAm&lt;sub&gt;72&lt;/sub&gt;-PNP&lt;sub&gt;29&lt;/sub&gt;</td>
<td>56.8±5.7</td>
<td>20.4±3.4</td>
<td>39.9±3.1</td>
<td>5.7±0.2</td>
</tr>
</tbody>
</table>

a) Drug loading efficiency (DLE) is defined as the mass ratio of loaded drug to drug in the feed solution. Values are expressed as a mean with standard deviation (n = 3). b) Drug loading content (DLC) is defined as the mass ratio of the loaded drug to drug-loaded micelle. Values are expressed as a mean with standard deviation (n = 3). c, d) Values are expressed as a mean with standard deviation (n = 3).

Although DOX is approved by the Food and Drug Administration,<sup>56</sup> it is limited by the toxicity it expresses in non-cancerous cells of body. As a consequence, the development of novel drug delivery systems for a sustained drug release with low cytotoxicity towards non-cancerous cells is constantly being explored.<sup>57</sup> In the previous study, we employed PNIPAAm as the thermoresponsive block and varied the length and nature of the hydrophobic core. Most studies dealing with the preparation of polymeric micelles for drug delivery employ poly(ethylene glycol) (PEG)<sup>58,59</sup> or PNIPAAm<sup>60,61</sup> blocks as the
hydrophilic block constituting the shell of the micelle. As such, the limited scope for water-soluble and thermoresponsive polymers discourages the modification of micellar systems through the hydrophilic block, compelling research efforts to focus more on hydrophobic block manipulation. To the best of our knowledge, studies on the drug encapsulation profiles of micelles bearing novel pyrrolidone-based polymers as both the core and coronae are virtually unexplored.

3.1.2 Pyrrolidone-based block copolymer micelles

In Chapter 2, we discussed the synthesis and characterization of γ-substituted homopolymers that varied in terms of residue structure and chemical class. The comparative study provided evidence for the potential utility of γ-substituted poly(N-acryloyl-2-pyrrolidone)s as they can be tailored to adjust: i) hydrophobic or hydrophilic character, ii) thermoresponsivity, iii) hydrogen-bonding capability (alkoxy vs thiolate substituents), iv) π-π stacking capability (cyclohexyl vs phenyl moieties), and v) $T_g$, without complex modifications to the macromolecular scaffold. As an extension of this work, this chapter discusses the results of an investigation into understanding how the physicochemical properties and drug-loading and release capabilities of pyrrolidone-based block copolymer micelles are influenced by similar structural modifications. Specifically, we set out to elucidate the structure-property correlations in these systems by synthesizing two sets of block copolymers that differ by residue structure (i.e. $R = H$, vs $XCH_2CH_3$), chemical class (i.e. $R = OCH_2CH_3$ vs $SCH_2CH_3$), and by the length of the hydrophilic segment (i.e., degree of polymerization, $DP_n 25$ vs $50$). We anticipated that since sulfur-containing compounds and their oxygen-containing congeners exhibit different physicochemical properties due to the differences in size, hydrophobicity and
electronegativity between the heteroatom(s), the chemical class of our hydrophobic micelles would influence the physicochemical properties of our micellar systems.

3.2 Results and discussion

3.2.1 Synthesis and Characterization

Scheme 3-2 Synthesis of block copolymer with hydrophilic segment.

All block polymers were synthesized according to Scheme 3-2. All macroinitiators were synthesized via RAFT polymerization of EtONP, EtSNP or NP monomers tailored to a degree of polymerization ($DP_n$) of ca. 25. The macroinitiators were then used for chain-extending polymerizations with the hydrophilic $N$-acryloyl-5-methoxyethoxy-2-pyrrolidone monomer. In this chapter, block copolymers with hydrophobic segments prepared from $N$-acryloyl-2-pyrrolidone (NP), $N$-acryloyl-5-ethoxy-2-pyrrolidone (EtONP), and $N$-acryloyl-5-ethylthio-2-pyrrolidone (EtSNP) are designated as MeOEtO–NP, MeOEtO–EtONP, and MeOEtO–EtSNP respectively. For each block copolymer type, two block copolymers that are distinguishable only by the length of the hydrophilic segment were prepared and are designated herein as short chain or long chain.
All $^1$H-NMR spectra (Figure. 3-3 and Fig 3-9 to 3-17) exhibit resonances (e.g. $\alpha$, $\beta$, $\theta$, $\omega$ and $\omega'$ Fig. 3-3) and relative integral ratios that are consistent with those observed from homopolymer samples of each segment. The degree of polymerization was calculated by measuring the integral ratios of peaks for methyl protons in the methoxyethoxy side chain ($\theta$) and the ethyl side chain ($\alpha$) ($I_\theta : I_\alpha$). Due to the distinct environment of the pyrrolidone hydrogen(s), $\omega$ and $\omega'$ protons were taken in to consideration for calculating the block length of MeOEtONP-NP polymer chains. The polymers were designated as MeOEtO$_{24}$–NP$_{22}$, MeOEtO$_{48}$–NP$_{22}$, MeOEtO$_{28}$–EtONP$_{28}$, MeOEtO$_{49}$–EtONP$_{28}$ and MeOEtO$_{22}$–EtSNP$_{24}$, MeOEtO$_{48}$–EtSNP$_{24}$.

![Figure 3-3 $^1$H NMR spectra (500MHz, CDCl$_3$, 25 °C) of (a) MeOEtO$_{28}$–EtONP$_{28}$, (b) MeOEtO$_{22}$–EtSNP$_{24}$, and (c) MeOEtO$_{24}$–NP$_{22}$](image-url)

All GPC traces were predominantly monomodal and with dispersities ranging between 1.2-1.5 indicating that polymer initiation was efficient for all polymerizations. As anticipated, reductions in retention volumes were observed in chromatograms for each
block copolymer type upon extension of the hydrophilic pyrrolidone-based polymer block (Figure. 3-4, Fig 3-18,19). This phenomenon is attributed to an increase in hydrodynamic volume as the length of the copolymer is extended.

![Graph showing GPC traces](image)

**Figure 3-4** GPC traces of EtSNP-CTA (black dashed), MeOEtO\textsubscript{22}–EtSNP\textsubscript{24} (black solid), MeOEtO\textsubscript{48}–EtSNP\textsubscript{24} (red solid). Relative to polystyrene standards in THF.

3.2.2 **Effect of Residue Class on Critical Micelle Concentration and Micelle Size.**

Block copolymer self-assembly was investigated in order to examine how both the presence of a pyrrolidone residue and residue class (i.e., alkoxy vs thiolate) influence the critical micelle concentration and size of the micelle using pyrene as a fluorescent probe.\textsuperscript{63} As micelles with poly(MeOEtONP) coronae and NP, EtSNP and EtONP core were assembled with increasing polymer concentration, the partitioning of pyrene into the hydrophobic core of micelles caused a red shift in its excitation spectrum. As such, association behavior was monitored by measuring the pyrene intensity ratio \(I_{337}/I_{334}\) as a function of copolymer concentration in water. From the appropriate plot (Figure. 3-5 and...
3-38 to 3-42), a CMC value was estimated as the point of intersection between two linear lines of regression.

Figure 3-5 The intensity ratio $I_{337}/I_{334}$ obtained from pyrene excitation spectra of block copolymer solutions vs. block copolymer concentration. MeOEtONP$_{22}$-EtSNP$_{24}$ (●), MeOEtONP$_{28}$-EtONP$_{28}$ (▲), MeOEtONP$_{24}$-NP$_{22}$ (♦). Studies were done in triplicate (Figure 3-38 to 3-42).

Among the short chain block polymers, the CMC values were seen to be similar for blocks bearing ethyl thiolate and ethoxy side chains (0.8 ± 0.1 mg/L and 0.8 ± 0.1 mg/L, respectively, n = 3) whereas the block with NP showed a much higher CMC (28.0 ± 4.3 mg/L) indicating that the latter is much more hydrophilic in character. As expected, the block copolymers with longer hydrophilic blocks, MeOEtONP$_{48}$-EtSNP$_{24}$, MeOEtONP$_{49}$-EtONP$_{28}$, MeOEtONP$_{48}$-NP$_{22}$ were found to have higher CMC values (ca. 2.1 ± 0.7 mg/L, 3.5 ± 0.4 mg/L, 40.6 ± 4.3 mg/L, respectively, n = 3) than their corresponding shorter blocks. Indeed, the results of this investigation shows that while the residues greatly increase the overall hydrophobic character of the block copolymer, the CMC value is not so much effected by the residue class (i.e. whether the residue is a thiolate or alkoxy...
moiety). Moreover, the block copolymers exhibit excellent micellar stability at low polymer concentrations making them attractive for drug delivery vehicles that must be diluted upon entering the body’s bloodstream to sustain longer drug release and have low inherent toxicity.

In addition to low CMCs, ideal nanocarriers must adopt a suitable size (10-100 nm) for sufficient stability and longer circulation time \textit{in vivo}. All the micelles prepared in this work assembled into a size-range that is optimal for being employed as nanocarriers in drug delivery (Figure. 3-6b, c, d and Fig 3-20 to 3-37). Micelles were prepared by dialyzing block copolymer/DMF solutions (ca. 0.4 mg/mL) against deionized water. Size differences among the short chain block copolymer micelles were found to be similar for MeOEtONP$_{28}$-EtONP$_{28}$ and MeOEtONP$_{22}$-EtSNP$_{24}$, but significantly smaller than MeOEtONP$_{24}$-NP$_{22}$ (ca. 55.2 ±5.3 nm, 56.7 ±5.4 nm and 90.1 ±2.9 nm respectively, n = 3). Based on the CMC data, the decrease in the hydrodynamic radius can be attributed to the enhanced hydrophobic character of the pyrrolidone-based polymer blocks upon addition of the thiolate or alkoxy residues, a phenomenon that is expected to increase attractive hydrophobic interfacial forces at the hydrophobic/hydrophilic block interface resulting in micelles with smaller surface areas. Interestingly, among the long chain block copolymers micelles $D_h$ was seen to decrease in the order of MeOEtONP$_{48}$-NP$_{22}$, MeOEtONP$_{49}$-EtONP$_{28}$, MeOEtONP$_{48}$-EtSNP$_{24}$ (81.5 ± 5.9 nm, 35.3 ± 2.3 nm, 28.6 ± 2.3 nm, respectively, n = 3). While the origin of this trend is not understood at this time, the results suggest that the influence of the pyrrolidone residue on micelle size is block-length dependent.
3.2.3 Thermal-induced Deformation and Aggregation

Since poly(MeOEtONP) is known to be thermoresponsive, the thermoresponsive behavior of our block copolymer micelles was investigated by turbidimetry and dynamic light scattering (DLS). Although the micelles did not show significant turbidity in deionized water above the critical point temperature, aggregation in phosphate buffer solution (7.4 pH) was extensive. Micellar LCSTs (defined as the onset temperature of decrease in optical transmittance) in PBS were measured to be in the range of ca. 27 to 39 °C (Table 3-2).

Table 3-2 Characterization data for pyrrolidone-based block copolymers

<table>
<thead>
<tr>
<th>Entry</th>
<th>Copolymers</th>
<th>(\bar{M}_n) \text{NMR} \text{a}</th>
<th>(D_m) \text{a}</th>
<th>CMC (mg/L)</th>
<th>(D_h) (nm) \text{c}</th>
<th>LCST \text{d} [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOEtONP\textsubscript{22}-EtSNP\textsubscript{24}</td>
<td>10500</td>
<td>1.3</td>
<td>0.8 mg/L±0.1</td>
<td>56.7± 5.3</td>
<td>27.6±0.3</td>
</tr>
<tr>
<td>2</td>
<td>MeOEtONP\textsubscript{48}-EtSNP\textsubscript{24}</td>
<td>15500</td>
<td>1.5</td>
<td>2.1 mg/L±0.7</td>
<td>28.6±2.3</td>
<td>36.9±1.3</td>
</tr>
<tr>
<td>3</td>
<td>MeOEtONP\textsubscript{28}-EtONP\textsubscript{28}</td>
<td>10900</td>
<td>1.5</td>
<td>0.8mg/L±0.1</td>
<td>55.2±5.2</td>
<td>32.5±0.5</td>
</tr>
<tr>
<td>4</td>
<td>MeOEtONP\textsubscript{49}-EtONP\textsubscript{28}</td>
<td>15800</td>
<td>1.4</td>
<td>3.5mg/L±0.4</td>
<td>35.3±2.3</td>
<td>36.8±0.3</td>
</tr>
<tr>
<td>5</td>
<td>MeOEtONP\textsubscript{24}-NP\textsubscript{22}</td>
<td>8400\textsuperscript{b}</td>
<td>1.2</td>
<td>27.9mg/L±4.3</td>
<td>90.1±2.9</td>
<td>27.8±1.3</td>
</tr>
<tr>
<td>6</td>
<td>MeOEtONP\textsubscript{48}-NP\textsubscript{22}</td>
<td>13500\textsuperscript{b}</td>
<td>1.2</td>
<td>40.6mg/L±4.1</td>
<td>81.5±5.9</td>
<td>32.8±0.3</td>
</tr>
</tbody>
</table>

a) Determined by GPC (relative to polystyrene) in THF. b) Determined by GPC (relative to poly(methyl methacrylate) in 0.01M LiBr in DMF. c) Determined by DLS. d) Values are expressed as a mean (number (%) or temperature (°C)) with standard deviation (n = 3). Sample preparation: [polymer] = 0.4 mg/mL in PBS.

Specifically, all short chain block copolymer micelles undergo non-reversible thermal-induced aggregation upon exceeding the LCST in PBS as made evident by the large reductions in optical transmittance as more efficient light scatterers are formed (Figure. 3-6a, y1-axis). This phenomenon was confirmed by variable-temperature DLS.
where particle sizes (Figure 3-6b, c and d) increase significantly over the temperature range of 25 to 40°C, and is consistent with data collected from the long chain block copolymers. The micelle size of the MeOEtONP$_{24}$-NP$_{22}$ and MeOEtONP$_{22}$-EtSNP$_{24}$ increase significantly (Figure 3-6b and d, respectively) at 37°C as compared to MeOEtONP$_{28}$-EtONP$_{28}$ (Figure 3-6c). This result is attributed to the higher LCST of MeOEtONP$_{28}$-EtONP$_{28}$ (ca. 32.5±0.5°C) that results in a gradual increase in hydrodynamic radii as compared to the ethylthiolate and pyrrolidone congeners with lower LCST (ca. 27.6±0.3°C and 27.8±1.3°C, respectively).

**Figure 3-6** a) LCST profiles for MeOEtONP$_{24}$-NP$_{22}$ (dashed - • - y2 axis), MeOEtONP$_{22}$-EtSNP$_{24}$ (solid - • - ), and MeOEtONP$_{28}$-EtONP$_{28}$ (solid - • - ) micellar solutions determined by transmittance at 500 nm, [polymer] = 0.4 mg/mL in phosphate buffer solution. Size distribution of b) MeOEtONP$_{24}$-NP$_{22}$ at ca. 25 °C ( ● ), 35 °C ( ■ ), 40 °C ( ♦ ), c) MeOEtONP$_{28}$-EtONP$_{28}$ at ca. 25 °C ( ● ), 35 °C ( ■ ), 40 °C ( ♦ ), and d) MeOEtONP$_{22}$-EtSNP$_{24}$ at ca. 25 °C ( ● ), 35 °C ( ■ ), 40 °C ( ♦ ).
3.2.4 DOX Loading and Cumulative drug release

DOX-loaded micelles were prepared by dialyzing an N-ethylacetamide solution of ethylthiolate and ethoxy-functionalized block copolymers, DOX.HCl, and triethylamine against deionized water under sink conditions. A 1:1 mixture of DMF and N-ethylacetamide was used to prepare the polymers with unsubstituted pyrrolidone block due to solubility issues. It should be noted that the use of N-ethylacetamide was critical to micelle formation as initial attempts using DMF or dimethylsulfoxide resulted in copolymer aggregation and precipitation during dialysis. The hydrodynamic diameters of the drug-loaded micelles were measured by DLS and found to be significantly shorter than the drug-free micelles for the short chain block copolymers, suggesting that intermolecular interactions between the drug and pyrrolidone-based polymer block are strong resulting in the formation of dense micellar coronae upon self-assembly.

Drug-loading efficiencies were measured using spectroscopic methods and found to be higher for MeOEtONP$_{28}$-EtONP$_{28}$ (ca. 64.7±2.3 %) compared to MeOEtONP$_{24}$-EtSNP$_{22}$ (ca. 57.9±4.1 %) and pyrrolidone (ca. 51.1±4.8 %) block copolymer micelles respectively. We attribute this to enhanced attractive hydrogen-bonding forces between DOX and the ethoxy residues present in the hydrophobic core. This trend was also observed among micelles prepared from MeOEtONP$_{49}$-EtONP$_{28}$ and MeOEtONP$_{48}$-EtSNP$_{22}$, where DOX encapsulation is greater for the block copolymer bearing ethoxy side chain than the ethylthiolate congener (ca. 67.6±2.8 %, 55.5 ± 1.7 %, respectively). Interestingly, the DOX loaded MeOEtONP$_{48}$-NP$_{24}$ block polymer was unstable during dialysis resulting in micelle aggregation and precipitation. The drug loading contents (DLCs) of all the
polymers were calculated to be in the range of 10-17% showing good payload capacities and excellent drug encapsulation.

Table 3-3 Drug encapsulation and release data of pyrrolidone block copolymers

<table>
<thead>
<tr>
<th>Entry</th>
<th>Copolymers</th>
<th>DLE&lt;sup&gt;d&lt;/sup&gt;</th>
<th>DLC&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Release at 37°C&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Release at 20°C&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOEtONP&lt;sub&gt;22&lt;/sub&gt;-EtSNP&lt;sub&gt;24&lt;/sub&gt;</td>
<td>57.9±4.1</td>
<td>13.5±0.5</td>
<td>21.1±1.0</td>
<td>18.9±0.9</td>
</tr>
<tr>
<td>2</td>
<td>MeOEtONP&lt;sub&gt;48&lt;/sub&gt;-EtSNP&lt;sub&gt;24&lt;/sub&gt;</td>
<td>55.5±1.7</td>
<td>12.9±1.6</td>
<td>23.4±1.3</td>
<td>16.5±0.9</td>
</tr>
<tr>
<td>3</td>
<td>MeOEtONP&lt;sub&gt;28&lt;/sub&gt;-EtONP&lt;sub&gt;28&lt;/sub&gt;</td>
<td>64.7±2.3</td>
<td>15.8±1.5</td>
<td>18.2±1.2</td>
<td>11.5±0.1</td>
</tr>
<tr>
<td>4</td>
<td>MeOEtONP&lt;sub&gt;49&lt;/sub&gt;-EtONP&lt;sub&gt;28&lt;/sub&gt;</td>
<td>67.6±2.8</td>
<td>16.4±2.0</td>
<td>19.7±0.7</td>
<td>12.9±0.3</td>
</tr>
<tr>
<td>5</td>
<td>MeOEtONP&lt;sub&gt;24&lt;/sub&gt;-NP&lt;sub&gt;22&lt;/sub&gt;</td>
<td>51.1±4.8</td>
<td>9.9±1.5</td>
<td>30.4±1.9</td>
<td>20.1±0.9</td>
</tr>
<tr>
<td>6</td>
<td>MeOEtONP&lt;sub&gt;48&lt;/sub&gt;-NP&lt;sub&gt;22&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>d</sup> Drug loading efficiency (DLE) is defined as the mass ratio of loaded drug to drug in the feed solution. Values are expressed as a mean with standard deviation (n = 3). <sup>e</sup>Drug loading content (DLC) is defined as the mass ratio of the loaded drug to drug-loaded micelle. Values are expressed as a mean with standard deviation (n = 3). <sup>f</sup> Values are expressed as a mean with standard deviation (n = 3).

Time-dependent cumulative DOX release by the short-chain DOX-loaded micelles in PBS solution was evaluated at ca. 37 °C and 20 °C and the results illustrated in Fig. 3-7. All the polymers showed increased drug release at 37°C as compared to 20 °C. Interestingly, the overall percentage of drug release decreased in the order of MeOEtONP<sub>24</sub>-NP<sub>22</sub>, MeOEtONP<sub>22</sub>-EtSNP<sub>24</sub>, MeOEtONP<sub>28</sub>-EtONP<sub>28</sub>. (ca. 30.4±1.9, 21.1±1.0 and 18.2±1.2%), despite the increasing order of DLE. This trend was also observed in MeOEtONP<sub>48</sub>-EtSNP<sub>24</sub> and MeOEtONP<sub>49</sub>-EtONP<sub>28</sub> while the MeOEtONP<sub>49</sub>-NP<sub>22</sub> was unstable and precipitated in the dialysis bag during the loading. The enhancement of drug release at elevated temperatures is attributed to the increase in the hydrophobicity
of the micellar coronae leading to deformation of the micellar architecture. The trend in the drug encapsulation and release suggests that the mechanisms responsible for improving drug-loading efficiency prevents efficient release upon thermal activation due to increased hydrophobic interactions. Moreover, the differences in release characteristics between the ethylthiolate- and ethoxy-group containing polymers may be due to the hydrogen-bonding capability of the latter which could impede the release of DOX when the formulation temperature is elevated about the LCST.

![Graph](image)

Figure 3-7 DOX release from a) MeOEtONP$_{24}$-NP$_{22}$ at 37°C (●, solid) and 20°C (■, solid), MeOEtONP$_{22}$-EtSNP$_{24}$ (●, solid) at 37°C and 20°C (■, solid), and MeOEtONP$_{48}$-EtSNP$_{24}$ at 37°C (●, dashed) and 20°C (■, dashed), and MeOEtONP$_{28}$-EtONP$_{28}$ at 37°C (●, solid) and 20°C (■, dashed), and MeOEtONP$_{49}$-EtONP$_{28}$ (●, dashed) and 20°C (■, dashed). Data points are plotted as a mean with standard deviation (n = 3)

3.2.5 In vitro Cytotoxicity

On the basis of these reports, the cytotoxic activity of our DOX-loaded micelles ([DOX], ca. 30 µg/mL) was evaluated in vitro against both free DOX ([DOX], ca. 30 µg/mL) and blank (i.e. drug-free) micelles at ca. 37°C by Dr. Pei-Chin Tsai of the
Michniak-Kohn group in the Rutgers-University, New Jersey Center of Biomaterials. MCF-7 breast cancer cells were incubated with the exogenous substrates for 3 h at the appropriate temperature then washed and evaluated for viability after 24 h. The results of this investigation show that at 37°C, the cytotoxic activity in DOX-loaded micelle formulations is greater than their blank micelle controls adjusted for polymer concentration. Taken together with the observation that DOX-loaded micelles are less cytotoxic than DOX in its free form, the results are indicative of a sustained and incomplete drug release process during cell incubation that is consistent with the thermoresponsive release data illustrated in Fig. 3-8. The blank micellar controls showed inherent cytotoxicity at higher concentrations of 0.1-0.2mg/mL. In order to eliminate the possibility of cytotoxicity arising from the block copolymer micelles, the polymer concentration was adjusted to approx. 50-65 µg/mL for all the DOX-loaded micelles.

**Figure 3-8** In vitro cytotoxicity of blank micelles, free DOX, and DOX loaded micelles at 37°C. [DOX] = 30 µg/mL. [Polymer]≈ 0.067 mg/mL. MCF-7 cells were incubated with the exogenous substrates for ca. 3 h, washed, and measured for viability after 24 h. Data are expressed in mean cell viability (%).
3.3 Conclusion

Two series of pyrrolidone-based block copolymer micelles were synthesized and both their physicochemical properties and drug encapsulation profiles were examined as a function of hydrophobic residue class. Consistent with earlier results published by our group on PNIPAAm-P(B, M)NP systems, the results of our findings show that the addition of a single ethoxy residue to the hydrophobic pyrrolidone scaffold can increase the overall hydrophobic character of the block resulting in very low CMCs. This feature is comparable among the ethylthiolate congeners as well and is very desirable for nanocarriers that are diluted upon entry into the blood stream. Residue class was also found to affect block copolymer self-assembly and intermicellar aggregation below and above the LCST respectively. Using DOX as a therapeutic hydrophobic payload, drug loading efficiencies were found to increase significantly in the order of, MeOEtONP-EtONP, MeOEtONP-EtSNP and MeOEtONP-NP indicating that drug encapsulation can be improved with only modest adjustments to macromolecular structure. Time-dependent drug release studies revealed that cumulative DOX release is greater when the drug-loaded micelles are heated above the LCST, a feature that is much less pronounced when compared to block copolymer micelles with PNIPAAm thermoresponsive hydrophilic block studied previously in our group. Moreover, the cumulative release was found to decrease in the order of MeOEtONP-EtONP, MeOEtONP-EtSNP and MeOEtONP-NP suggesting that the mechanisms responsible for improving encapsulation also impede efficient release. We hypothesize that, the pendant ethoxy groups must be interacting with the pharmaceutical agent by forming hydrogen bonds thus decreasing the total drug-release in spite of higher encapsulation. Finally, all blank micellar solutions showed cytotoxicity at higher
concentrations but the micellar solutions could be adjusted to lower concentration to eliminate the possibility of *in vitro* cytotoxicity arising from blank micelles at 37 °C. To the best of our aware, we are the pioneers in the synthesis of pyrrolidone-based micellar systems that have competitive drug-encapsulation abilities. On the basis of the work reported in this chapter, we can conclude that although the drug-release is not as prominent as PNIPAAm, we definitely observe a significant effect of the residues on the physicochemical property of the polymeric micellar system. Subsequent studies would be geared towards better understanding of the interaction of the drug in the micelle core and the synthesis of better micellar models by block copolymerization with thermoresponsive polymers.

3.4 Experimental

**Materials and Equipment.** All polymerizations were performed in an inert atmosphere. Azobis(isobutyronitrile) (AIBN) was purchased from Aldrich and recrystallized from methanol prior to use. THF was dried and collected from a PureSolv MD solvent purification system (Innovative Technology Inc.) equipped with two activated alumina columns. All other solvents and reagents were used as received. Benzyl dithiobenzoate,68,69 N-acryloyl-5-ethoxy-2-pyrrolidone (EtONP), and N-acryloyl-5-ethylthio-2-pyrrolidone (EtSNP)70 were prepared according to literature procedures. 1H NMR spectra were recorded on a Varian INOVA 500 and Bruker ASCEND 500MHz spectrometer and calibrated to the residual protonated solvent peak at δ = 7.24 for deuterated chloroform (CDCl3). UV/vis spectra were recorded on a Cary-100 spectrophotometer equipped with a peltier heated multi-cell holder and Cary temperature controller and probe. Excitation and emission spectra were measured on a Varian Cary
Eclipse fluorescence spectrophotometer. GPC analyses were performed in DMF/0.01 M LiBr (0.5 mL/min) using a Waters Empower system equipped with a 717plus autosampler, a 1525 binary HPLC pump, a 2487 dual λ absorbance detector, and a 2414 refractive index detector. Two styragel columns (Polymer Laboratories; 5 μm Mix-C, column heater, 50 °C) were used for separation. Molecular weights were determined from a 12-point calibration curve using poly(methyl methacrylate) standards. GPC of MeOEtONP$_{28}$-EtONP$_{28}$ and MeOEtONP$_{49}$-EtONP$_{28}$ were carried out using a Malvern Viscotek TDAmax chromatograph with tetrahydrofuran as the mobile phase at 30 °C. The chromatograph was equipped with two PLC mixed columns and one PLD mixed column. Output was detected with a Viscotek TDA 305-055 Tetra Detector Array (PDA+RI+Visc+LALS/RALS) using an eluent flow rate of 1 mL/min and a 60 μL injection loop. Molecular weights were determined from a 10-point calibration curve created using polystyrene standards purchased from Polymer Laboratories. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano-ZS instrument, equipped with a 4 mW, 633 nm HeNe laser and an Avalanche photodiode detector at an angle of 173°.

**Synthesis of (EtO, EtS, N) P-CTA.** A pressure vessel (equipped with a sidearm and stir bar) was charged with N-acryloyl-5-ethoxy-2-pyrrolidone or N-acryloyl-5-ethylthio-2-pyrrolidone or N-acryloyl-2-pyrrolidone (0.01 mol), benzyl dithiobenzoate (0.4 mmol), AIBN (0.11mmol), and THF (ca. 12 mL). The solution was degassed using freeze-pump-thaw techniques (3 cycles) and immersed in a preheated oil bath at 75°C for 16 hours. The reaction was quenched by cooling the solution in a liquid nitrogen bath followed by precipitation in diethyl ether. The polymer was isolated by filtration and dried under
vacuum to afford a pink solid. A degree of polymerization ($D_{P_n}$) was calculated by end-group analysis using $^1$H NMR spectroscopy.

**Synthesis of Block Copolymers.** All block copolymers were synthesized using EtONP, EtSNP or NP, as the macromolecular chain-transfer agent and AIBN as the initiator. All block copolymers were prepared in THF with the exception of MeOEtONP$_{24}$-NP$_{22}$ and MeOEtONP$_{48}$-NP$_{22}$ that were prepared in anhydrous dimethylformamide (DMF). The feed ratios of (EtO, EtS, N) P CTA: MeOEtONP were adjusted to achieve different chain lengths of MeOEtONP block. In general, a pressure vessel was charged with solvent (ca. 1.5 mL), MeOEtONP monomer, (EtO, EtS, N) P-CTA (ca. 200 mg), and AIBN using the following molar ratios: 1:1:0.2 for the equal length block polymer; 2:1:0.2 for the longer block of MeOEtONP. The solution was degassed using freeze-pump-thaw techniques (3 cycles) and immersed in a preheated oil bath at 80°C for ca. 2 hours. The reaction was quenched by cooling the solution in a liquid nitrogen bath followed by precipitation in diethyl ether. The polymer was isolated by filtration and dried under vacuum to afford a pink solid.

**Preparation of Block Copolymer Micelles.** In a typical experiment, 1 mL of block copolymer solution (4 mg/mL, DMF) was added to 8 mL of deionized water under vigorous stirring at a rate of 0.1 mL/min. The mixture was then placed in a dialysis bag (MWCO = 3.5 kDa) and dialyzed against 1000 mL deionized water or phosphate buffer solution for 48 h. Deionized water or phosphate buffer solution was changed every 24 h.

**Fluorescence Measurements for CMC Determination.** CMC data were collected according to literature procedures. Aliquots (ca. 1 mL) of a pyrene stock solution (3.0 x $10^{-6}$ M in acetone) were dispensed into vials and stored in the absence of light until the
solvent completely evaporated. Aliquots (ca. 5 mL) of aqueous micelle solutions over a broad concentration range were added to vials, agitated and stored at room temperature for 24 h. The excitation spectra of these solutions were recorded between 250 and 360 nm at λ_em = 390 nm. The intensity ratio (I_{337}/I_{334}) of the bands at 337 nm and 334 nm were plotted as a function of block copolymer concentration using a logarithmic scale. CMC values were determined as the point of intersection between two logarithmic lines of regression generated by MS Excel.

**Preparation of DOX-Loaded Block Copolymer Micelles.** A solution of DOX-HCl (ca. 2.5 mg, 0.0043 mmol), triethylamine (10 μL, 0.072 mmol) and N-ethylacetamide (ca. 1 mL) was stirred at room temperature for 3 h for complete neutralization of the hydrochloride. The block copolymer (ca. 10mg) was added to this solution and stirred for 12 hours at room temperature. The solution was added to 10 mL of deionized water at a rate of 0.1 mL/min and stirred for an additional 24 h. The solution was transferred to a dialysis bag (MWCO =6800) and dialyzed against deionized water (ca. 300 mL) for 20 h (using a fresh dialysis bag and water after 10 h). The amount of DOX loaded into the micelles was determined by fluorescence spectroscopy. During the dialysis procedure, an aliquot (100 μL) of the micelle solution was removed periodically and diluted with DMF into a 10 mL volumetric flask. Emission spectra of the aliquot-DMF solutions were recorded between 500 to 650 nm at λ_ex = 483 nm. With the use of a calibration curve, the mass of DOX in the dialysis bag was calculated from the emission intensity at 592 nm. The weight of DOX loaded into the micelles was determined once the concentration of DOX in the dialysis bag no longer decreased with time. Finally, the polymer solutions were
diluted to *ca.* 0.4 mg/mL with deionized water and stored at 4°C in the absence of light for subsequent *in vitro* drug-release and cytotoxicity experiments.

Drug Loading Efficiency (DLE) and Drug Loading Content (DLC) were as follows:

\[
\text{DLE (\%) = \frac{\text{mass of loaded DOX}}{\text{mass of DOX in the feed}} \times 100%}
\]

\[
\text{DLC (\%) = \frac{\text{mass of loaded DOX}}{\text{mass of DOX-loaded micelles}} \times 100%}
\]

**Drug Release at 20°C and 37°C.** A 50 mL volumetric flask was charged with 5 mL of DOX-loaded micelle solution (polymer concentration, 0.4 mg/mL) and diluted with phosphate buffered saline (PBS) solution (0.01 M, pH = 7.4). The content of this flask was distributed equally into ten vials (*i.e.*, 5 mL/vial, polymer concentration, 0.04 mg/mL) and placed in a single water bath at 20°C or 37°C. Over the course of several hours, a vial was removed periodically and placed in a centrifuge at *ca.* 4.4k rpm for 15 min. The supernatant was analyzed by DLS to ensure the absence of micelles. Emission spectra of the solutions (diluted in PBS) were recorded in the range of 500 to 650 nm at \(\lambda_{ex} = 483\) nm. With the use of a calibration curve, the mass of DOX was calculated from the emission intensity at 592 nm.

**In Vitro Cytotoxicity.** *In vitro* cytotoxicity studies were conducted using the MCF-7 breast cancer cell line. MCF-7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 \(\mu\)g/mL of streptomycin, and 0.25 \(\mu\)g/mL of amphotericin B at 37°C and 5% CO\(_2\). Individual wells (96 well plates) were seeded with 5,000 cells/well and incubated for 24 hours prior to experimentation. To evaluate cytotoxicity, wells of MCF-7 cells were treated with 200 \(\mu\)L of free DOX solution, blank micelles, or DOX-loaded micelles (*approx.* 50 \(\mu\)g/mL) for 3 hours at 37°C. The cells were then washed free of DOX/micelle reagents with PBS and
incubated at 37°C for an additional 24 hours. Cell viability was expressed as a percentage by normalizing the fluorescence intensity of the experimental group relative to DMEM media treated cells; each experimental group was repeated in triplicate. Block copolymer concentrations were similar in all formulations used to compare cytotoxicity between DOX-loaded and blank micelles. For experiments that compared cytotoxicity between DOX-loaded micelles and free DOX, polymer concentrations were adjusted to ensure that DOX concentration were identical in all formulations. Experiments conducted with DOX-loaded micelles with different DOX content (ca. 30 and 15µg/mL) were the only ones which showed some amount of cytotoxicity and are hence included in the thesis.

![Figure 3-9](image)

**Figure 3-9** $^1$H-NMR spectrum of EtONP$_{28}$-macroinitiator (500MHz CDCl$_3$, 25°C)
Figure 3-10 $^1$H-NMR spectrum of MeOEtONP$_{28}$-EtONP$_{28}$ polymer (500MHz CDCl$_3$, 25°C)

Figure 3-11 $^1$H-NMR spectrum of MeOEtONP$_{49}$-EtONP$_{28}$ polymer (500MHz, CDCl$_3$, 25°C)
Figure 3-12 $^1$H-NMR spectrum of EtSNP$_{24}$-macroinitiator (500MHz CDCl$_3$, 25°C)

Figure 3-13 $^1$H-NMR spectrum of MeOEtONP$_{24}$-EtSNP$_{24}$ polymer (500MHz, CDCl$_3$, 25°C)
Figure 3-14 $^1$H-NMR spectrum of MeOEtONP$_{48}$-EtSNP$_{24}$ polymer (500MHz CDCl$_3$, 25°C)

Figure 3-15 $^1$H-NMR spectrum of NP$_{22}$-macroinitiator (500MHz CDCl$_3$, 25°C)
Figure 3-16 $^1$H-NMR spectrum of MeOEtONP$_{48}$- NP$_{22}$ polymer (500MHz CDCl$_3$, 25°C)

Figure 3-17 $^1$H-NMR spectrum of MeOEtONP$_{24}$- NP$_{22}$ polymer (500MHz CDCl$_3$, 25°C)
Figure 3-18 GPC trace of EtONP\textsubscript{28}-macroinitiator (Black, dashed), MeOEtONP\textsubscript{49}-EtONP\textsubscript{28} (Red, solid), MeOEtONP\textsubscript{28}-EtONP\textsubscript{28} (Black solid)

Figure 3-19 GPC trace of NP\textsubscript{22}-macroinitiator (Black, dashed), MeOEtONP\textsubscript{48}-NP\textsubscript{22} (Red, solid), MeOEtONP\textsubscript{24}-NP\textsubscript{22} (Black solid)
Figure 3-20 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{49}$-EtONP$_{28}$ micelles at 25 °C (●), 35 °C (○), 40 °C (●)- Run 1

Figure 3-21 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{39}$-EtONP$_{28}$ micelles at 25 °C (●), 35 °C (○), 40 °C (●)- Run 2
Figure 3-22 Hydrodynamic diameter ($D_{h}$) distribution (Volume (%)) of MeOEtONP$_{49}$-EtONP$_{28}$ micelles at 25 °C ($\bullet$), 35 °C ($\bigcirc$), 40 °C ($\triangle$)- Run 3

Figure 3-23 Hydrodynamic diameter ($D_{h}$) distribution (Volume (%)) of MeOEtONP$_{48}$-EtSNP$_{24}$ micelles at 25 °C ($\bullet$), 35 °C ($\bigcirc$), 40 °C ($\triangle$)- Run 1
Figure 3-24 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{48}$-EtSNP$_{24}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 2

Figure 3-25 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{48}$-EtSNP$_{24}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 3
Figure 3- 26 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{22}$-EtSNP$_{24}$ micelles at 25 °C (●), 35 °C (■), 40 °C (▲)- Run 1

Figure 3- 27 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{22}$-EtSNP$_{24}$ micelles at 25 °C (●), 35 °C (■), 40 °C (▲)- Run 2
Figure 3-28 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{22}$-EtSNP$_{24}$ micelles at 25 °C (●), 35 °C (■), 40 °C (▲) - Run 3

Figure 3-29 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{28}$-EtONP$_{28}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●) - Run 1
Figure 3-30 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{28-}$EtONP$_{28}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 2

Figure 3-31 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{28-}$EtONP$_{28}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 3
Figure 3-32 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{24}$-NP$_{22}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●) - Run 1

Figure 3-33 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{24}$-NP$_{22}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●) - Run 2
Figure 3-34 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{24}$-NP$_{22}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 3

Figure 3-35 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{24}$-NP$_{22}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 1 $I_{337}/I_{334}$
Figure 3-36 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{24}$-NP$_{22}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 2

Figure 3-37 Hydrodynamic diameter ($D_h$) distribution (Volume (%)) of MeOEtONP$_{24}$-NP$_{22}$ micelles at 25 °C (●), 35 °C (●), 40 °C (●)- Run 3
Figure 3-38 The intensity ratio $I_{337}/I_{334}$ obtained from pyrene excitation spectra of block copolymer solutions vs block copolymer concentration MeOEtONP$_{48}$-NP$_{22}$ micelles (●), MeOEtONP$_{49}$-EtONP$_{28}$ (●), MeOEtONP$_{48}$-EtSNP$_{24}$ (●), Run-1

Figure 3-39 The intensity ratio $I_{337}/I_{334}$ obtained from pyrene excitation spectra of block copolymer solutions vs block copolymer concentration MeOEtONP$_{48}$-NP$_{22}$ micelles (●), MeOEtONP$_{49}$-EtONP$_{28}$ (●), MeOEtONP$_{48}$-EtSNP$_{24}$ (●), Run-2
Figure 3-40 The intensity ratio $I_{337}/I_{334}$ obtained from pyrene excitation spectra of block copolymer solutions vs block copolymer concentration MeOEtONP$_{48}$-NP$_{22}$ micelles (●), MeOEtONP$_{49}$-EtONP$_{28}$ (●), MeOEtONP$_{48}$-EtSNP$_{24}$ (●), Run-3

Figure 3-41 The intensity ratio $I_{337}/I_{334}$ obtained from pyrene excitation spectra of block copolymer solutions vs block copolymer concentration; MeOEtONP$_{24}$-NP$_{22}$ (●), MeOEtONP$_{28}$-EtONP$_{28}$ (●), MeOEtONP$_{22}$-EtSNP$_{24}$ (●), Run-1
Figure 3-42 The intensity ratio $I_{337}/I_{334}$ obtained from pyrene excitation spectra of block copolymer solutions vs block copolymer concentration; MeOEtONP$_{24}$-NP$_{22}$ (●), MeOEtONP$_{28}$-EtONP$_{28}$ (●), MeOEtONP$_{22}$-EtSNP$_{24}$ (●), Run-2

Figure 3-43 Transmittance versus temperature plot of MeOEtONP$_{49}$-EtONP$_{28}$ micellar solution. Run 1 (●), run 2(●), run 3 (●). (0.4mg/mL, PBS)
Figure 3-44 Transmittance versus temperature plot of MeOEtONP$_{28}$-EtONP$_{28}$ micellar solution. Run 1 (●), run 2 (●), run 3 (●). (0.4mg/mL, PBS)

Figure 3-45 Transmittance versus temperature plot of MeOEtONP$_{48}$-EtSNP$_{24}$ micellar solution. Run 1 (●), run 2 (●), run 3 (●). (0.4mg/mL, PBS)
Figure 3-46 Transmittance versus temperature plot of MeOEtONP$_{22}$-EtSNP$_{24}$ micellar solution. Run 1 (●), run 2 (●), run 3 (●) (0.4mg/mL, PBS)

Figure 3-47 Transmittance versus temperature plot of MeOEtONP$_{48}$-NP$_{22}$ micellar solution. Run 1 (●), run 2 (●), run 3 (●) (0.4mg/mL, PBS)
Figure 3-48 Transmittance versus temperature plot of MeOEtONP_{24-NP_{22} micellar solution. Run 1 (●), run 2 (●), run 3 (●) (0.4mg/mL, PBS)

Figure 3-49 In vitro cytotoxicity of blank micelles, free DOX, and DOX loaded micelles of copolymer MeOEtONP_{48-EtSNP_{24} at 37°C. [DOX] = 30 µg/mL and 15 µg/mL. MCF-7 cells were incubated with the exogenous substrates for ca. 3 h, washed, and measured for viability after 24 h. Data are expressed in mean cell viability (%)
Figure 3-50 In vitro cytotoxicity of blank micelles, free DOX, and DOX loaded micelles of copolymer MeOEtONP_{49}-EtONP_{28} at 37°C. [DOX] = 30 µg/mL and 15 µg/mL. MCF-7 cells were incubated with the exogenous substrates for ca. 3 h, washed, and measured for viability after 24 h. Data are expressed in mean cell viability (%)

Figure 3-51 In vitro cytotoxicity of blank micelles, free DOX, and DOX loaded micelles at 37°C. [DOX] = 15 µg/mL. [Polymer]≈ 0.033 mg/mL. MCF-7 cells were incubated with the exogenous substrates for ca. 3 h, washed, and measured for viability after 24 h. Data are expressed in mean cell viability (%).
3.5 References


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CHAPTER 4

Synthesis and Characterization of PEG-PPS Block Copolymers for Flash Nanoprecipitation

4.1 Introduction

In Chapter 3, amphiphilic copolymers were introduced as promising candidates for producing nanoparticles as drug-delivery vehicles. These particles possess diameters in the range of 1-100 nm and can carry the lipophilic drug by way of encapsulation in the particle core, adsorption onto the particle surface, or interspersion within the particle polymer matrix. Owing to the solubility, anti-fouling nature, and biocompatibility of polyethylene glycol (PEG, often employed as a non-toxic and water-soluble dispersant for cosmetics, toothpastes, eye drops and laxatives), it is often used as the hydrophilic block in amphiphilic copolymers used for drug delivery. Although exploration of colloidal nanoparticles for drug delivery initiated forty years ago, PEGylated nanoparticles only found their way into formulations in the 1990s when Doxil® (PEGylated liposomal delivery vehicle for doxorubicin) and oncospar (PEG-Lasparaginase) became the first FDA-approved NP therapeutics. Indeed, the incorporation of PEGylated liposomes were seen to increase the bioavailability of doxorubicin by 90-fold along with the increase in the half-life of in vitro circulation. These results clearly indicate that the PEG-based polymeric nanoparticles hold tremendous potential as nanocarriers.

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*This chapter is adapted from a manuscript in preparation. Authors’ contributions are specified in results and discussions section.*
Extensive investigations have been carried out on PEG-based block copolymers that include PEG-\textit{b}-poly(propylene glycol) di- and triblock copolymers,\textsuperscript{12} poly-(ethylethylene)-\textit{b}-PEG,\textsuperscript{13} and poly(styrene)-\textit{b}-PEG.\textsuperscript{14} In this chapter we report on the synthesis and characterization of amphiphilic block copolymers comprised of hydrophilic polyethylene glycol and hydrophobic polypropylene sulfide (PPS) segments (PEG-\textit{b}-PPS) to be used as nanocarriers for pharmaceutically active agents. The latter belongs to a family of compounds known as sulfides that are susceptible to oxidation in the presence of oxidizers/catalysts such as peroxides,\textsuperscript{15} metal oxides\textsuperscript{16} and metal salts.\textsuperscript{17} Upon oxidation, hydrophobic sulfides are converted to highly water-soluble sulfoxides and sulfones, giving PPS its inherent oxidative-responsivity. This property is highly desirable for stimuli-responsive drug release as it has been observed that tissues with activated macrophages,\textsuperscript{18} solid tumors,\textsuperscript{19} or general inflammation\textsuperscript{20} tend to release oxidative oxygen species. That is, drug-loaded nanoparticles comprised of PEG-PPS can be destabilized in oxidative environments where tissues are under stress, releasing the pharmaceutical agent at the target site of interest.

In a pioneering work by the Hubbell group (Figure. 4-1), monomodal low-molecular weight PEG-PPS-PEG based triblock copolymer vesicles were synthesized and subjected to oxidative denaturation to study the effect on the structure of the vesicles.\textsuperscript{21} On treatment with aqueous H\textsubscript{2}O\textsubscript{2} (0.03\%\textit{vol}), they report a gradual decrease in optical density (OD) in turbidimetric experiments. The decrease in OD confirms the disruption of vesicular structure to worm-like and spherical micelles that afford a clear solution after ten hours compared to a control where peroxide was absent. The \textsuperscript{1}H-NMR spectrum of the peroxide treated polymer in D\textsubscript{2}O revealed that the broad, featureless peaks of methyl protons of
pristine PPS at ca. δ 1.41 are replaced by sharp doublets in at δ 1.53 that represent the methyl at the β-position to the -SOx- moieties of the oxidized PPS block. Taken together, the results suggest that the PPS blocks had undergone a significant change in structure while the characteristic resonances of the PEG-blocks remained unchanged, confirming their integrity. Finally, cryo-TEM showed definite a decrease in the vesicular structures upon oxidation as would be expected if the PPS-blocks became hydrophilic, a feature that is highly desirable for drug-delivery applications.

![Figure 4-1 Oxidative transformation of sulphide to sulfone of PEG-PPS polymer](image)

As an extension of the previous work, Hubbell and coworkers investigated the drug encapsulation abilities of their PEG-PPS block copolymer micelles.28 Cyclosporin-A (CsA) was selected as the hydrophobic payload and loaded into the copolymer micelles by the co-solvent evaporation method. Upon dissolving the drug and copolymer together in water, they observed that the poorly soluble CsA (23µg/mL water) was soluble up to 1.915mg/mL in water. Drug encapsulation efficiency of the loaded micelles was calculated to be 70% by fluorescence spectroscopy, while in vivo release under sink conditions was observed to occur over 9-12 days. This slow release was attributed to the strong interaction
of the hydrophobic block and drug leading to the increased stability\textsuperscript{22} and low mobility of the micelle core.\textsuperscript{23}

Taken together, the results show that PEG-PPS block copolymers are highly attractive candidates as nanocarriers. The block copolymers described herein will be used for nanoparticle (NP) formation by way of \textit{flash nanoprecipitation} (FNP), a procedure that was performed and pioneered by our collaborator, Professor Robert K. Prud’homme of Princeton University.\textsuperscript{24} Flash nanoprecipitation affords particles with higher drug-loading contents (DLC) compared to micelles that are thermodynamically self-assembled in aqueous suspensions, giving rise to non-equilibrium systems that lead to aggregation from high interfacial tension.\textsuperscript{25} In the case of FNP, the rapid precipitation of drug-loaded nanoparticles is stabilized by using block copolymers that provide steric stabilization and limit aggregation due to Ostwald ripening.\textsuperscript{26} Moreover, the size distribution is primarily governed by the nucleation and growth time of a hydrophobic molecule under supersaturated conditions that should be matched with the aggregation time of the stabilizing amphiphilic molecule. In our preliminary investigations in collaboration with the Prud’homme group, PEG-PPS copolymers of varying molecular weight were synthesized and subjected to flash nanoprecipitation using polystyrene and poly(propylene sulfide) hydrophobic payloads to examine how oxidative conditions influence NP decomposition and payload release.

4.2 Results and discussion

Synthesis and Characterization
Hubbell and coworkers first reported on the synthesis of monomodal low-molecular-weight PEG\textsubscript{750-}b-PPS\textsubscript{1900} where propylene sulfide was ring-open polymerized anionically using PEG-thioacetate as a macroinitiator\textsuperscript{27}. Due to the mild nature of the propagating species (thiolates), this type of anionic polymerization did not require extremely anhydrous conditions, however, attempts to reproduce these experiments in our laboratory failed when higher molecular weight polymers were targeted. Consequently, we were tasked with developing an optimized set of conditions such that copolymers with controlled molecular weights and predominantly monomodal distributions could be isolated. The target molecular weights of these polymers were PEG\textsubscript{5000-}PPS\textsubscript{2500}, PEG\textsubscript{5000-}PPS\textsubscript{5000}, PEG\textsubscript{2000-}PPS\textsubscript{2000} and PEG\textsubscript{2000-}PPS\textsubscript{4000}

Scheme 4-1 Synthesis of PEG-PPS block copolymers

All block copolymers were synthesized according to Scheme 4-1 using a modified version of previously reported procedure.\textsuperscript{28} Monomethyl poly(ethylene glycol) ($M_n = ca. 5kDa$ and $2kDa$) was dissolved in toluene and refluxed using a Dean-Stark apparatus for 3 hours under constant flow of nitrogen in order to remove trace amounts of water. The solution was then cooled to room temperature and 1.8 equivalents thionyl bromide was added carefully. It should be noted that for PEG5k, it was essential that one equivalent of triethylamine be added for the complete conversion of hydroxyl groups to bromide. The solution was then filtered and the filtrate refluxed for 2 hours before cooling overnight.
Substituting the bromide end-group with thioacetate was carried out in anhydrous dichloromethane in the presence of potassium carbonate and thioacetic acid distilled prior to use. After quenching the excess acid with DOWEX® cationic-exchange resin, the solution was concentrated and the polymer precipitated upon addition to stirring diethylether. It should be emphasized that the steps requiring freshly distilled thioacetic acid and DOWEX® cationic-exchange resin are critical for the complete conversion of this reaction.

To activate the PEG macroinitiators, thioacetate end-groups were deprotected with sodium methoxide to form thiolate anions. Subsequent addition of propylene sulfide monomer afforded the poly(propylene sulfide) block. In this work, the termination of the polymerization was carried out by adding a ten-fold excess of iodoacetamide to completely quench the living polymerization. Otherwise, the growing polymer chain thiolate endgroups dimerize when exposed to air giving a mixture of (PEG-PPS)₂ and PEG-PPS as confirmed by GPC.
Figure 4.2 $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of a) poly(ethylene) glycol thioacetate (MW=5000kDa) and b) PEG$_{5000}$-PPS$_{2500}$ block copolymer

$^1$H NMR spectroscopy was used to calculate the degree of polymerization ($DP_n$) in these studies. Specifically, the $DP_n$ of the PEG macroinitiator was estimated by the ratio of integrals $I_a/I_b$, where $a$ and $b$ correspond to the OCH$_3$ protons and ethylene protons in the backbone, respectively. The absence of the characteristic triplet corresponding to the terminal -OH group in deuterated DMSO confirmed the successful conversion of -OH to -Br. The successful substitution of bromide with thioacetate (CO-CH$_3$) was also confirmed by $^1$H NMR spectroscopy according to the presence of the thioacetate peak at ca. 2.36 ppm. Due to the difference in electronegativity between sulfur and oxygen, protons on the methylene group adjacent to the thioacetate group resonate at a lower frequency and appear as a triplet at ca. 3.11 ppm. The methylene groups in the poly(ethylene) glycol backbone resonate around 3.48-3.75 ppm giving rise to a broad peak that is characteristic of protons in the polymer. As the anionic polymerization of propylene sulfide is carried out by the
deprotection of the thioacetate, the disappearance of the characteristic peak at 2.36 ppm of the methyl protons of thioacetate confirmed the complete initiation. As the polymerization proceeds, the monomer is sequentially added to the “living” end of the polymer.

The end-capped polymer was analyzed by proton NMR to confirm successful initiation and the required degree of polymerization. The length of the polypropylene sulfide block was determined by comparing the integral ratios, $I_b:I_c$, of PEG (CH$_2$-CH$_2$) protons and the protons in the methylene protons in the propylene sulfide block (CH$_2$-CH(CH$_3$)-S-). The integral ratios of the $d$, $e$ and $f$ protons were observed to be 2:1:3 confirming the integrity of the propylene sulfide block in the polymer. The protons from the end-group of the amide are represented by broad peaks around 6.82 ppm and 6.31 ppm whereas the $g$ and $c$ protons from the polymer chain were buried under the signals from PPS and PEG respectively.

**Gel Permeation Chromatography of polymers**

The living end of the PPS block is known to be susceptible to oxidation. It was observed that the unreacted diblock copolymer readily dimerizes when exposed to air. Therefore, a mixture of diblock PEG-PPS-amide and disulfide linked “dimeric” (PEG-PPS)$_2$ was detected in samples with incomplete end-capping with iodoacetamide. With the complete end capping of polymers, only diblock PEG-PPS-amide was observed by GPC.
All GPC traces of the block copolymers were multimodal indicating that initiation was inefficient in case of PEG₅₀₀₀ macroinitiator. The macroinitiator with PEG₂₀₀₀ displayed multimodal peaks but complete initiation in GPC and NMR (Figure. 4-16, 4-13, 4-14). As anticipated, reductions in retention times were observed in chromatograms for each block copolymer type upon addition of the PPS polymer block (Figure. 4-3 and Figure 4-16), a phenomenon that is attributed to an increase in hydrodynamic volume as the length of the copolymer is extended. The low polydispersity (ca. 1.1-1.28) of both these block copolymers (Table 4-1) proved that the polymerization was not only living but also controlled.

Table 4-1 Characterization of (co)polymers

<table>
<thead>
<tr>
<th>Block copolymer</th>
<th>$M_n$[Da]ᵃ</th>
<th>$D_M$ᵇ</th>
<th>$T_g$[°C]ᶜ</th>
<th>$T_c$[°C]ᵈ</th>
<th>$T_{dec}$[°C]ᵉ</th>
</tr>
</thead>
</table>

ᵃ The number-average molecular weight $M_n$ in Daltons.
ᵇ The molecular weight distribution $D_M$.
ᶜ The glass transition temperature $T_g$.
ᵈ The crystallization temperature $T_c$.
ᵉ The decomposition temperature $T_{dec}$. 

Figure 4-3 GPC traces of PEG₅₀₀₀ macroinitiator (black, solid), PEG₅₀₀₀-PPS₅₀₀₀ (blue, solid) and PEG₅₀₀₀-PPS₂₅₀₀ in DMF solvent.
PEG₅₀₀₀-PPS₂₅₀₀  7400  1.23  -42.4  52  286
PEG₅₀₀₀-PPS₅₀₀₀  9400  1.21  -41.7  52  256
PEG₂₀₀₀-PPS₂₀₀₀  4200  1.19  -42.8  -  247
PEG₂₀₀₀-PPS₄₀₀₀  5700  1.34  -42.5  -  216
PPS₅₀₀₀  5300  1.18  -39.0  -  175

a) Determined through NMR  b) Determined through GPC in THF as solvent using polystyrene standards  c) Determined through second heating curve of DSC with half height of tangents at inflection point  d) Determined through second heating curve of DSC with peak height being taken as the crystallization temperature  e) Determined through TGA (temperature ramp 30-900°C at 10°C/min)  f) Determined through GPC in DMF as solvent using polymethylmethacrylate standards.

Thermal properties of the polymer

The glass transition temperatures of our (co)polymers were studied by thermal gravimetric analysis (Figure 4-4 and 4-17). The homopolymer and the block copolymers were subjected to two cycles of heating from -70°C to 100°C at the rate of 10°C/min. The homopolymer PPS₅₀₀₀ showed a distinct glass transition at -39.0°C that is consistent to previously reported $T_g$ (ca. -40°C) for PPS₆₀₀₀. Interestingly, the PEG-PPS block copolymers showed a glass transition temperature that was around -42°C. Also, the block copolymers, PEG₅₀₀₀-PPS₂₅₀₀ and PEG₅₀₀₀-PPS₅₀₀₀ were seen to possess a distinct crystallization temperature ($T_c$). The decrease in the glass transition temperature and introduction of distinct crystallization temperature at 52°C can be attributed to the incorporation of PEG moiety, a well-known plasticizer for decreasing $T_g$ and promoting crystallinity of the polymer. Surprisingly, this crystallinity is absent in PEG₂₀₀₀-PPS₂₀₀₀ and PEG₂₀₀₀-PPS₄₀₀₀. While the reason for this loss of crystalline nature of lower molecular weight block polymers is not known, it is evident that the chain length of PPS block in the polymer does affect the thermal transitions of the PEG-based copolymer. In other words,
the glass transition temperature of PPS is dependent on the molecular weight of the polymer. The plasticizing effect of PEG and the lower molecular weight of PPS in the block copolymers, PEG\textsubscript{2000}-PPS\textsubscript{2000} and PEG\textsubscript{2000}-PPS\textsubscript{4000}, together give rise to glass transition temperature which are relatively lower than PPS\textsubscript{5000}, PEG\textsubscript{5000}-PPS\textsubscript{2500} and PEG\textsubscript{5000}-PPS\textsubscript{5000}.

![DSC curves (second heating) of PEG\textsubscript{5000}-PPS\textsubscript{2500} (red, solid) and PEG\textsubscript{5000}-PPS\textsubscript{2500} (black, solid)](image)

**Figure 4- 4** DSC curves (second heating) of PEG\textsubscript{5000}-PPS\textsubscript{2500} (red, solid) and PEG\textsubscript{5000}-PPS\textsubscript{2500} (black, solid)

The thermal stability of the polymers was examined by thermal gravimetric analysis (Figure 4-5) by subjecting them to a temperature range from 30-900°C at 20°C/min. All the polymers showed a distinct decomposition step that was dependent on the length of the polymers. The homopolymer PPS\textsubscript{5000} was seen to decompose at 175°C; whereas, the PEG\textsubscript{5000}-PPS\textsubscript{5000} block copolymer exhibited the decomposition temperature of *ca.* 256°C. The block copolymer PEG\textsubscript{5000}-PPS\textsubscript{2500} was observed to have the highest decomposition temperature (286°C) implying that the incorporation of poly(ethylene glycol) unit
increased the thermal stability of the copolymer. The thermal stability was observed to decrease as the length of PPS in the block increased. Thus, we can conclude that the decomposition temperature of PEG-PPS block is influenced by the incorporation of PEG moiety and the length of PPS unit.

![TGA curves of block copolymers and PPS5000](image)

**Figure 4-5** TGA curves of block copolymers and PPS\textsubscript{5000}

**Nanoparticle synthesis with block copolymers**

Block co-polymers were subjected to flash nanoprecipitation in the Prud’homme laboratory. The nanoparticles were composed of the polystyrene core and the block copolymer in the ratio of 1:1 core: copolymer shell by mass. In order to test their stability in a non-oxidizing environment, synthesized particles were left in the dark for 150h. In order to have a reference for the oxidative response of the nanoparticles, a control with poly(styrene)-PEG\textsubscript{5000} (PS-PEG\textsubscript{5k}) based nanoparticle with a polystyrene core was subjected to similar conditions as the nanoparticle under observation.
The oxidation of nanoparticles (both PS-PEG-PS$_{4.6k}$ along with PPS$_{2500}$-PEG$_{5000}$-PS$_{4.6k}$) was conducted by bubbling in (10\% by vol) H$_2$O$_2$ solution while particle size was monitored over a course of 160h. It was evident that the nanoparticles formed from PPS$_{2500}$-PEG$_{5000}$ showed a distinct decrease in the size as compared to the control PS-PEG (Figure 4-6). This reduction in size of the nanoparticles is hypothesized to be the result of the oxidation of the PPS core, leading to destabilization of the polymer. These studies prove the potential of PEG-PPS-based nanoparticles for drug-delivery upon oxidation stimulus. Studies for evaluating the stability of the polymeric nanoparticles of PEG$_{5000}$-PPS$_{5000}$ PEG$_{2000}$-PPS$_{2000}$ and PEG$_{2000}$-PPS$_{4000}$ are in progress in Prof. Prud’homme’s laboratory.

4.3 Conclusion

In this work we have synthesized four block copolymers and one homopolymer using a modified synthetic methodology for targeting the required $DP_n$ and narrow dispersity, however, polymers with a monomodal distribution have not been prepared at this time.
The bromination reaction of hydroxyl-terminated PEG was sensitive to moisture and vigorous refluxing of monomethyl PEG in toluene was necessary prior to the addition of thionyl bromide. The macroinitiator was synthesized by the attack of thioacetatic acid on brominated PEG in presence of a base. It was imperative to distill thioacetic acid prior to the use due to the presence of disulfides that introduce colored impurities in the macroinitiator. The anionic polymerization for the synthesis of the block copolymers mandated anaerobic conditions although the reaction was otherwise robust to the presence of water. A ten-fold excess of end-capping agent, iodoacetamide, was absolutely necessary for the complete quenching of the polymerization, otherwise incompletely quenched reactions gave rise to dimer formation characterized by bimodal peaks in GPC. Although all the polymers showed no evidence of thioacetate peak in the NMR, the multimodal GPC curve of PEG5000 polymers suggests a mixture of polymers in the sample. The polymers PEG2000-PPS2000 and PEG2000-PPS4000 did not show any evidence of macroinitiator but did display multimodal curves in GPC indicating mixture of polymers of different molecular weight. The thermal stability of our (co)polymers revealed that decomposition temperatures were affected by: i) the length of the PPS block in the copolymer and ii) the presence of the PEG block. Similarly, the glass transition temperatures were also seen to be affected by the plasticizing effect of PEG as observed by the reduction of $T_g$ of the block copolymers. Indeed, the preliminary studies conducted in the Prud’homme lab indicate that the block copolymer-based nanoparticles do undergo reduction in particle diameter upon oxidation suggesting that the PPS block is responsive to oxidation-stimulus. In future, efforts will be dedicated to synthesis of monodisperse polymers with uniform chain length
by modifying the reaction conditions and changing the initiator system to get a more efficient initiation.

4.4 Experimental

Materials and Equipment. All polymerizations were performed in an inert atmosphere. THF and toluene were dried and collected from a PureSolv MD solvent purification system (Innovative Technology Inc.) equipped with two activated alumina columns. DOWEX resin and propylene sulfide were used as purchased. \(^1\)H NMR spectra were recorded on a Varian INOVA 500 and Bruker ASCEND 500MHz spectrometer and calibrated to the residual protonated solvent peak at \(\delta = 7.24\) for deuterated chloroform (CDCl\(_3\)). For PEG\(_{5000}\) block, GPC analyses were performed in DMF/0.01 M LiBr (0.5 mL/min) using a Waters Empower system equipped with a 717plus autosampler, a 1525 binary HPLC pump, a 2487 dual \(\lambda\) absorbance detector, and a 2414 refractive index detector. Two styragel columns (Polymer Laboratories; 5 \(\mu\)m Mix-C, column heater, 50 °C) were used for separation. Molecular weights were determined from a 12-point calibration curve using poly(methyl methacrylate) standards. For the PEG\(_{2000}\) block, GPC analyses were performed in THF, using Waters Empower system equipped with 717plus autosampler a 1525 binary HPLC pump, a 2487 dual \(\lambda\) absorbance detector, and a 2414 refractive index detector. Two styragel columns (Polymer Laboratories; 5 \(\mu\)m Mix-C, D column heater, 50 °C) were used for separation. Molecular weights were determined from a 10-point calibration curve using poly(styrene) standards Differential scanning calorimetry (DSC) was performed on a TA Instruments Discovery differential scanning calorimeter at a scan rate of 10 °C/min. DSC data for the polymers were recorded from the second heating scans.
Thermal gravimetric analyses were performed on a TA Instruments Discovery thermogravimetric analyzer at a scan rate of 20 °C/min up to 700 °C.

**General procedure for the synthesis of monomethyl PEG\textsubscript{5000-Br}:** 10 gm of monomethyl PEG (5000 g/mol, 0.002 mol) was dissolved in dry toluene and the apparatus was equipped with Dean-Stark condenser and refluxed over 4 hours. Upon cooling down to room temperature, 1.2 equivalents of triethyl amine (0.31 mL, 0.0024 mol) was added and stirred for 5 mins. Thionyl bromide (1.8eq, 0.46 mL) was added slowly. After complete addition, the brown solution was refluxed for 1 hour under nitrogen. The reaction mixture was cooled to room temperature and then filtered to remove salts. The brown solution was then stirred overnight. The solution was concentrated the next day and precipitated twice in diethyl ether to yield a white powder. \textsuperscript{1}H-NMR was recorded in DMSO to check for unreacted PEG-OH peak at δ 4.53. Yield = 85% \textsuperscript{1}H-NMR (DMSO, 500MHz): δ 3.24 (s, 3H), δ 3.38-3.75 (broad, 448H).

**General procedure for the synthesis of monomethyl PEG\textsubscript{2000-Br}:** Synthesis was carried out according to reported procedure \textsuperscript{27}

**General procedure for the synthesis of monomethyl PEG-Thioacetate:** 5 gm PEG-Br was purged with nitrogen for 10 minutes and was dissolved in 50 mL dry DMF. To this solution, 5 equivalents of K\textsubscript{2}CO\textsubscript{3} and 0.9mL of previously distilled thioacetic acid was added. The solution was purged with nitrogen and was stirred overnight at room temperature. To quench the excess of thioacetic acid, 1gm Dowex\textregistered resin was added the next day and was stirred for an hour. The reaction mixture was then concentrated and precipitated twice in diethyl ether.
PEG_{2000}-thioacetate (macroinitiator): Yield = 85%; $^1$H-NMR (CDCl$_3$, 500MHz): $\delta$ 2.35 (s, 3H), $\delta$ 3.11 (t, 2H, $J_{HH} = 6.19$Hz), $\delta$ 3.39 (s, 3H), $\delta$ 3.48-3.64 (broad PEG backbone, 188H)

PEG$_{5000}$-thioacetate (macroinitiator): Yield = 92%; $^1$H-NMR (CDCl$_3$, 500MHz): $\delta$ 2.36 (s, 3H), $\delta$ 3.11 (t, 2H, $J_{HH} = 6.19$Hz), $\delta$ 3.40 (s, 3H), $\delta$ 3.48-3.64 (broad PEG backbone, 450H).

PEG-PPS block polymers: A Schlenk flask was charged with 1gm of PEG-thioacetate and evacuated and back-filled with nitrogen gas. To this, 40mL of dry and distilled THF was added. After the dissolution of all solids, 1.1 equivalent of freshly prepared sodium methoxide in dry methanol (0.5N) was added. The solution was sealed and allowed to stir at room temperature for 45 minutes. Propylene sulfide (34eq., 53eq., for PEG$_{5000}$-PPS$_{2500}$ and PEG$_{5000}$-PPS$_{5000}$ or 27eq., 68 eq. for PEG$_{2000}$-PPS$_{2000}$ and PEG$_{2000}$-PPS$_{4000}$, respectively) was added to this and the reaction mixture was stirred for an hour at 50°C after which, a 10-fold excess of iodoacetamide in 2mL THF was added as an end capping agent. After 24 hours, the reaction mixture was concentrated and precipitated three times in cold diethyl ether. Filtration and vacuum drying of the solid yielded polymer as a pale yellow solid in case of PEG$_{5000}$-PPS$_{5000}$ and PEG$_{5000}$-PPS$_{2500}$. Whereas, the product was obtained as a dark and viscous oil in case of PEG$_{2000}$-PPS$_{2000}$, PEG$_{2000}$-PPS$_{4000}$.

PEG$_{5000}$-PPS$_{2500}$: Yield = 60% ($^1$H-NMR CDCl$_3$, 500MHz): $\delta$ 1.39 (br, m, 100H), $\delta$ 2.62-2.68 (br, m, 33H), $\delta$ 2.76 (m, 2H), $\delta$ 2.87-2.92 (br, m, 66H), $\delta$ 3.29 (m, 4H), $\delta$ 3.40 (s, 3H), $\delta$ 3.57-3.82 (m, 450H)
**PEG<sub>5000</sub>-**<br>**PPS<sub>5000</sub>:** Yield = 60% \((^1H\text{-NMR CDCl}_3, 500\text{MHz})\) \(\delta\) 1.40 (br, m, 100H), \(\delta\) 2.64-2.68 (br, m, 33H), \(\delta\) 2.76 (m, 2H), \(\delta\) 2.87-2.94 (br, m, 66H), \(\delta\) 3.29 (m, 4H), \(\delta\) 3.40 (s, 3H), \(\delta\) 3.50-3.74 (m, 450H)

**PEG<sub>2000</sub>-**<br>**PPS<sub>2000</sub>:** Yield = 40% \((^1H\text{-NMR CDCl}_3, 500\text{MHz})\) \(\delta\) 1.38 (br, m, 100H), \(\delta\) 2.62-2.66 (br, m, 29H), \(\delta\) 2.75 (m, 2H), \(\delta\) 2.87-2.91 (br, m, 59H), \(\delta\) 3.28 (m, 4H), \(\delta\) 3.38 (s, 3H), \(\delta\) 3.50-3.74 (m, 184H)

**PEG<sub>2000</sub>-**<br>**PPS<sub>4000</sub>:** Yield = 50% \((^1H\text{-NMR CDCl}_3, 500\text{MHz})\) \(\delta\) 1.40 (br, m, 100H), \(\delta\) 2.64-2.68 (br, m, 58H), \(\delta\) 2.75 (m, 2H), \(\delta\) 2.89-2.95 (br, m, 120H), \(\delta\) 3.28 (m, 4H), \(\delta\) 3.41 (s, 3H), \(\delta\) 3.50-3.74 (m, 184H)

**General Procedure for the synthesis of**<br>**PPS<sub>5000</sub>:** Thiophenol (23µL, 0.025mol) was dissolved in 15mL of dry tetrahydrofuran in a schlenk flask equipped with a stirbar. The solution was purged gently with nitrogen for 10 minutes. Freshly prepared sodium methoxide (0.5N in methanol) (3 equivalents, 0.15mL, 0.075) was added via micropipette in to the solution. The mixture was allowed to stir for an hour under sealed conditions. Propylene sulfide (1gm, 0.015mol) was added to this solution and stirred for 1.5 hours. A 10-fold excess of iodoacetamide was added to the solution and was stirred overnight. Concentration and precipitation of the solution twice in hexanes yielded the polymer. (Yield = 0.4g, 40%). **PPS<sub>5000</sub>** \((^1H\text{-NMR CDCl}_3, 500\text{MHz})\): Yield = 50% \(\delta\) 1.39 (br, m, 222H), \(\delta\) 2.64-2.68 (br, m, 75H), \(\delta\) 2.90-2.94 (br, m, 154H), \(\delta\) 3.28 (m, 2H), \(\delta\) 7.22 (t, 1H, \(J_{HH}=6.3\text{Hz}\)), 7.32 (dd, 2H, \(J_{HH}=5.62\text{Hz}\)), 7.38 (dd, 2H, \(J_{HH}=5.81\text{Hz}\))
Figure 4-7 $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of poly(propylene) sulfide$_{5000}$ (PPS$_{5000}$)

Figure 4-8 $^1$H NMR (500 MHz, DMSO, 25 °C) of poly(ethylene glycol)$_{5000}$-bromide (PEG5K-Br).
**Figure 4-9** $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of poly(ethylene glycol)$_{2000}$-thioacetate.

**Figure 4-10** $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of poly(ethylene glycol)$_{5000}$-poly(propylenesulfide)$_{5000}$ (PEG$_{5000}$-PPS$_{5000}$) polymer
Figure 4- 11 $^1$H NMR (500 MHz, DMSO, 25 °C) of poly(ethylene glycol)$_{2000}$-bromide (PEG2K-Br).

Figure 4- 12 $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of poly(ethylene glycol)$_{2000}$-thioacetate (PEG2K-SAc).
Figure 4-13 $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of Poly(ethylene glycol)$_{2000}$-poly(propylenesulfide)$_{2000}$ (PEG$_{2000}$-PPS$_{2000}$) polymer.

Figure 4-14 $^1$H NMR (500 MHz, CDCl$_3$, 25 °C) of poly(ethylene glycol)$_{2000}$-poly(propylenesulfide)$_{4000}$ (PEG$_{2000}$-PPS$_{4000}$) polymer.
Figure 4-15 GPC trace of poly(propylene sulfide)$_{5000}$ homopolymer

Figure 4-16 GPC trace of PEG$_{2000}$-macroinitiator (black, solid), PEG$_{2000}$-PPS$_{2000}$, (red, solid) PEG$_{2000}$-PPS$_{4000}$. 
Figure 4-17 DSC curves (second heating) of PPS_{5000} (black, solid), PEG_{2000}-PPS_{4000} (red, solid) and PEG_{2000}-PPS_{2000} (blue, solid)
4.5 References


8] Illum, L. Drug delivery system. 1990, US 4904479 A


CHAPTER 5
Conclusion

5.1 General Conclusion

This thesis describes the synthesis, characterization, and application of a novel family of \(\gamma\)-substituted pyrrolidone-based (co)polymers derived from pyroglutamic acid. Initially, pyrrolidone-based polymers bearing methoxy, ethoxy, propargyl, butoxy and methoxethoxy were synthesized via conventional free radical polymerization. Thermal analyses determined that both polymer glass transition and decomposition temperatures were affected by the length of the \(\gamma\)-substituents. Moreover, the homopolymer, \text{poly(MeOEtONP)}\), was found to exhibit a highly reversible thermoresponsive phase transition with a LCST of 42 °C in water, a feature that could be tuned by copolymerizing \text{MeOEtONP} with comonomers bearing methoxy, ethoxy or butoxy substituents. Inspired by these findings, a more thorough investigation into the structure/property correlations of \(\gamma\)-substituted poly(N-acryloyl-2-pyrrolidone)s (synthesized \textit{via} RAFT) was pursued. The \(\gamma\)-substituents of these polymers were selected based on differences in: i) aliphatic length ii) structure (aliphatic \textit{vs} cyclic \textit{vs} aromatic) and iii) chemical class (alkoxide \textit{vs} thiolate). The results of our investigation revealed how these \(\gamma\)-substituent characteristics influenced their glass transition temperatures, thermal stability, and water-solubility. Single crystal diffraction studies on the monomer \text{StSNP} confirmed the steric congestion between pyrrolidone \(\gamma\)-substituent and the polymer backbone that may be the reason for the sensitivity of the polymer physiochemical properties to minute changes in the substituent structure. Of all the polymers prepared in this thesis, only \text{poly(MeOEtONP)} and \text{poly(FurONP)} were soluble in water and exhibited a lower critical solution temperature
at 42°C and 15°C respectively. Since poly(MeOEtSNP) was insoluble in water, we conclude that both the γ-substituent structure and class contribute to the polymer’s thermoresponsivity. Interestingly, in vitro cytotoxicity studies revealed that poly(MeOEtONP) is largely nontoxic over the polymer concentration range of 0.1-100 μg/mL, a feature that makes these materials attractive for thermoresponsive drug delivery applications.

The results of the findings in Chapter 2 inspired us to synthesize block copolymer micelles employing poly(MeOEtONP) as the hydrophilic block and poly(NP), poly(EtSNP), and poly(EtONP) as hydrophobic blocks in order to examine how the structure of blocks influenced the hydrophobic drug uptake and thermoresponsive release. Specifically, the block copolymers differed by residue structure (i.e. R = H, vs XCH2CH3), chemical class (i.e. R = OCH2CH3 vs SCH2CH3), and by the length of the hydrophilic segment (i.e., degree of polymerization, DPn 25 vs 50). The results of our comparative studies revealed an increase in hydrophobicity on addition of a γ-substituent on the hydrophobic block indicated by decrease in the critical micelle concentration as compared to the poly(NP) containing block. Moreover, the residue class affected both block copolymer self-assembly and intermicellar aggregation below and above the lower critical solution temperature LCST. Drug-encapsulation studies conducted using DOX as a hydrophobic payload were found to decrease in the order of MeOEtONP-EtONP, MeOEtONP-EtSNP, and MeOEtONP-NP whereas the time-dependent cumulative DOX-release was found to increase in the same order. The cumulative drug release of the chemotherapeutic agent, Doxorubicin, was found to be higher at temperatures above the LCST of the polymers than at lower temperatures, although not as pronounced as in
PNIPAAm-block copolymer solutions. These results indicate that the cohesive forces responsible for encapsulation may be inhibiting the polymer to release the drug entirely.

In chapter 4, we describe the synthesis of oxidation-responsive amphiphilic block copolymers that were subjected to flash nanoprecipitation in the Prud’homme laboratory at Princeton University. The polymers PEG$_{5000}$-PPS$_{5000}$ and PEG$_{5000}$-PPS$_{2500}$ displayed multimodal peaks in GPC indicating inefficient initiation whereas the PEG$_{2000}$ macroinitiator provided polymers with multimodal peaks but with complete initiation. The thermal stability of PEG$_{5000}$-PPS$_{2500}$ was analyzed through thermogravimetric analysis and was found to be higher than PEG$_{5000}$-PPS$_{5000}$, PEG$_{2000}$-PPS$_{2000}$, PEG$_{2000}$-PPS$_{4000}$, whereas PPS$_{5000}$ showed the lowest thermal stability. The glass transition temperatures of the polymers were seen to increase in the order of PEG$_{5000}$-PPS$_{5000}$ < PEG$_{5000}$-PPS$_{2500}$ < PEG$_{2000}$-PPS$_{2000}$ < PEG$_{2000}$-PPS$_{4000}$. PPS$_{5000}$ displayed relatively higher glass transition. Polymer-coated nanoparticles were prepared using a polystyrene core in the Prud-homme laboratory using the PEG$_{5000}$-PPS$_{2500}$ block copolymer. On subjecting them to oxidative conditions of 10% peroxide, the nanoparticles showed a remarkable decrease in particle size indicating PPS block oxidation and release from the polystyrene core. These findings confirm their potential in drug-delivery as oxidation-sensitive nanoparticles. In the future research efforts will be dedicated to synthesizing high-molecular weight monomodal block copolymers for nanoprecipitation by modifying the reaction conditions and using better initiators.

5.2 Suggestions for future work

In the future, we hope to synthesize stimuli-responsive poly (aspartic acid)-block-poly (propylene sulfide) block copolymers for drug delivery applications. Poly(aspartic
acid) is a biocompatible water-soluble polymer consisting of carboxylic acid residues that is expected to impart pH-responsivity to the corresponding amphiphilic PAsp-PPS block copolymer. As an extension of the work described in Chapter 4, synthesis of PAsp-PPS would be carried out for preparing nanoparticles by flash nanoprecipitation in Prud’homme laboratory.

**Scheme 5-1** Synthesis of PAsp(OBn)-PPS block copolymer.

The synthetic scheme for the polymerization of benzyl-protected aspartic acid (asp(OBn)-NCA) initiated by amine terminated macroinitiator PPS-NH$_2$ is outlined in Scheme 5-1. The PPS-NH$_2$ macroinitiator was synthesized according to the procedure reported for PPS$_{5000}$ (Chapter 4) with the modification of quenching with allyl amine instead of iodoacetamide. We found that although the polymerization of asp(OBn)-NCA with PPS-NH$_2$ proceeded with relative ease, efforts to deprotect the carboxyl group have failed so far. The deprotection of the benzyl group was attempted with 1M trifluoromethanesulfonic acid (TFMSA)/trifluoroacetic acid (TFA)/thioanisole, according to literature procedures. However, it was found that the fluorinated reagents that are capable of cleaving many protective groups also reacted with the PPS block of the copolymer. Moreover, we hypothesized that the scavenging nature of thioanisole was suppressed due to the presence of PPS block that led to unwanted side reactions. Deprotection of the carboxyl group was also attempted with hydrobromic acid but unfortunately failed as it led to the degradation of the PPS-block as well. In a final attempt
to cleave the benzyl group, sodium hydroxide solution was added to the block copolymer solution in tetrahydrofuran and stirred overnight. We observed that the poly (aspartic acid) block was completely hydrolyzed leaving only the PPS block intact as confirmed by $^1$H-NMR.

In order to navigate through these synthetic obstacles, future efforts will be dedicated to isolating the target PAsp-PPS block copolymers. One route that will be pursued is to couple PPS and PAsp blocks using the Diels-Alder reaction between diene and dienophile end-groups. Specifically, the polymerization of the benzyl protected monomer, aspartic acid, could be carried out by the initiator furfuryl amine in order to yield a diene-terminated polymer. Deprotection of the benzyl group of PAsp(OBn) can be carried out by the aforementioned method$^2$ to yield the required polymer. Polypropylene sulfide can be fashioned with maleimide which can act as a dienophile. The two blocks designed with these functionalities could then be simply, allowed to react to undergo Diels-Alder of the terminal groups as represented in Scheme 5-2.

**Scheme 5-2** Synthesis of poly(aspartic acid)-b-poly(propylenesulfide) *via* Diels-Alder
Another approach in synthesizing the PAspA-\textit{b}-PPS copolymer would be based on a thiol-ene “click” reaction. The “click” type of reactions were first highlighted by Kolb. \textit{et. al.},$^5$ and were designed for the synthesis of complex and functional molecules. The reaction of thiols with an unsaturated group specifically, maleimide, has been long studied as Michael-type additions or namely, “click” reactions.$^6$ The electron-withdrawing group and the ring-strain associated with the maleimide group coupled with the nucleophilic nature of the thiolates provide the necessary driving force in thiol-maleimide reactions.$^7$ Due to the reliability, stereospecificity$^8$ and efficiency of “click” reaction, they have been utilized extensively as primary means of bioconjugation$^9$ and more recently for polymer and material synthesis.$^{10}$ The general mechanism of the thiol-maleimide reaction is outlined in scheme 5-3.

\begin{center}
\textbf{Scheme 5-3} Thiol-maleimide “click” reaction mechanism.
\end{center}
For synthesizing the targeted polymers with lengths PAsp$_{2000}$-PPS$_{2000}$, PAsp$_{5000}$-PPS$_{7000}$, PAsp$_{5000}$-PPS$_{15000}$, the poly(aspartic acid) of the required length (2000 or 5000 Da) will be fashioned with a maleimide group by using the $N$-$\gamma$-maleimidobutryryl-oxosuccinimide (GMBS) crosslinker as the quenching agent during the polymerization. The polymerization of propylene sulfide will be carried out as mentioned before in Chapter 4 with thiophenol. The click reaction between the propagating thiolate of the propylene sulfide unit with the maleimide-terminated polyaspartic acid in a calculated stoichiometry is predicted to yield the required block copolymer of the appropriate length and dispersity (Scheme 5-4).

![Scheme 5-4](image)

**Scheme 5-4** Synthesis of poly(aspartic acid)-b-poly(propylenesulfide) via click reaction
5.3 References


