Addressing the Issues of Drug Delivery via Advanced Macromolecular Design

Brooks Allen Abel

University of Southern Mississippi

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ADDRESSING THE ISSUES OF DRUG DELIVERY
VIA ADVANCED MACROMOLECULAR DESIGN

by

Brooks Allen Abel

A Dissertation
Submitted to the Graduate School
and the School of Polymers and High Performance Materials
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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May 2016
ABSTRACT

ADDRESSING THE ISSUES OF DRUG DELIVERY VIA ADVANCED MACROMOLECULAR DESIGN

by Brooks Allen Abel

May 2016

The work described in this dissertation focuses on the development of synthetic approaches toward novel polymer architectures that specifically address the issues of in vivo drug delivery. Successful implementation of the synthetic methodologies described herein required fundamental investigations into the underlying chemistries in ways that now provide greater insights into the nature of these chemical reactions.

In Section I, the synthesis of tunable pH- and CO$_2$-responsive sulfonamide-containing polymers using reversible addition-fragmentation chain transfer (RAFT) polymerization is described. Initially, poor polymerization control of methacryloyl sulfonamide (MSA) monomers was observed using traditional RAFT polymerization conditions. Ultimately, reducing the polymerization temperature to 30 °C afforded polymers of controlled molecular weights and low dispersities. A library of sulfonamide-containing polymers was subsequently synthesized and their tunable pH-responsive and reversible CO$_2$-responsive aqueous solution properties investigated.

The work in Section II provides mechanistic understanding of the limited molecular weight control observed during the RAFT polymerization of MSAs at 70 °C (from Section I). This work demonstrates the unique influence of N-arylmethacrylamide substitution on trithiocarbonate chain-end degradation during RAFT polymerization at elevated temperatures. Detailed kinetic and structural analysis of RAFT polymer small...
molecule analogs showed trithiocarbonate chain-end degradation occurs by N-5 nucleophilic attack on the terminal thiocarbonyl by the ultimate methacrylamide unit. Ongoing work regarding the development of a mechanistic and kinetic theory aimed at explaining the unique influence of N-aryl substitution on amide nucleophilicity is further discussed in Appendix B.

In section III we investigate deleterious side reactions that occur during “one-pot” aminolysis/thiol-maleimide end group modification of RAFT polymers. Commonly employed thiol-ene Michael catalysts including amines, amidines, and phosphines were demonstrated to initiate the anionic polymerization of maleimide in a range of organic solvents, resulting in reduced RAFT polymer end group functionalization efficiency. Additionally, thiols and thiol-maleimide adducts were shown to initiate maleimide polymerization in polar solvents in the presence of triethylamine (TEA). Reaction conditions which favor rapid and quantitative end group functionalization of RAFT polymers using “one-pot” aminolysis/thiol-maleimide chemistry were ultimately identified.

Section IV details a new “grafting through” synthetic route towards molecular brushes capable of intracellular-induced disassembly. RAFT polymer-derived macromonomers were synthesized using “one-pot” aminolysis/thiol reactions with maleimide- or methanethiosulfonate-functional oxanorbornenes. Subsequent ring opening metathesis polymerization (ROMP) of the resulting macromonomers afforded molecular brushes with RAFT polymer side chains attached to a polyoxanorbornene backbone via either permanent thioether linkages or reversible disulfide linkages. Molecular brushes
comprised of disulfide linkages were shown to undergo reduction-induced disassembly and show promise as a new class of stimuli-responsive polymer therapeutics.
ACKNOWLEDGMENTS

I would like to extend my sincerest thanks to my research advisor, Dr. Charles L. McCormick, for his patience, support, and mentorship throughout my graduate career. I am particularly grateful to Dr. McCormick for granting me the intellectual freedom to pursue projects of my interest and to “dig deep” into the unknown. I would also like to thank my graduate committee, Dr. Robson F. Storey, Dr. Derek L. Patton, and Dr. Daniel A. Savin for their efforts and guidance. Dr. William J. Jarrett is gratefully acknowledged for his extensive assistance in performing many of the NMR experiments in this work.

I am thankful for all past and current McGroup members for their camaraderie. I especially thank Dr. Deedee Smith, Dr. Wenming Wan, Dr. Marco Oliveira, Dr. Christopher Holley, Keith Parsons, Phil Pickett, Michael Sims, David Siefker, and Aaron Spahr. I am forever indebted to Dana Froelich for her years of dedication and friendship.

With too many names to list, I am especially grateful for the many friends I have made throughout my graduate career. They have been the source of innumerable memories that I will cherish forever.

I would also like to acknowledge the financial support for this research provided by the National Science Foundation Graduate Research Fellowship Program (GM004636/GR04355) and National Science Foundation’s EPSCoR (IIA1430364).

And lastly but not least, my most heartfelt thanks go to my best friend and wife Emily for her love, companionship, and understanding. She has made these years especially worthwhile.
DEDICATION

This work is dedicated to mom and dad for their limitless love, sacrifice, and support of any and all pursuits that I have chosen to partake in.
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<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>AIBN</td>
<td>azobisisobutyronitrile</td>
</tr>
<tr>
<td>aRAFT</td>
<td>aqueous reversible addition-fragmentation chain transfer</td>
</tr>
<tr>
<td>ATRP</td>
<td>atom transfer radical polymerization</td>
</tr>
<tr>
<td>BIC</td>
<td>block ionomer complex</td>
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<tr>
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<td>Camptothecin</td>
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EPR  enhanced permeation and retention
ESI-MS  electrospray ionization mass spectrometry
EtOAc  ethyl acetate
EtOH  ethanol
Fab  fragment antigen binding
ΔG  Gibbs free energy
GC-MM  growth-then-coupling macromonomer
GSH  Glutathione
ΔH  change in enthalpy
H₂O  water
HCl  hydrogen chloride
HexAM  hexylamine
HSAB  hard and soft Lewis acids and bases
IPEC  interpolyelectrolyte complex
Kₐ  acid dissociation constant
LAM  less activated monomers
LCST  lower critical solution temperature
Mal  maleimide
MALLS  multi angle laser light scattering
MAM  more activated monomers
MeCN  acetonitrile
MeOH  methanol

xviii
<table>
<thead>
<tr>
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<tr>
<td>MM</td>
<td>macromonomer</td>
</tr>
<tr>
<td>$M_n$</td>
<td>number average molecular weight</td>
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<tr>
<td>MSA</td>
<td>methacryloyl sulfonamide</td>
</tr>
<tr>
<td>MTS</td>
<td>methanethiosulfonate</td>
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<tr>
<td>$M_w$</td>
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<td>NIPAM</td>
<td>N-isopropylacrylamide</td>
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<td>ring opening metathesis polymerization</td>
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<tr>
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<tr>
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<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
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<td>siRNA</td>
<td>small interfering ribonucleic acid</td>
</tr>
<tr>
<td>SMUI</td>
<td>single monomer unit insertion</td>
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<tr>
<td>TBP</td>
<td>tributylphosphine</td>
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<td>TEA</td>
<td>triethylamine</td>
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<td>trifluoroacetic acid</td>
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<td>trimethylphosphite</td>
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<tr>
<td>TTC</td>
<td>thithiocarbonate</td>
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<tr>
<td>UCST</td>
<td>upper critical solution temperature</td>
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<tr>
<td>UV</td>
<td>ultraviolet</td>
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<tr>
<td>UV-Vis</td>
<td>ultraviolet-visual</td>
</tr>
<tr>
<td>V-501</td>
<td>azobis(4-cyanovaleric acid)</td>
</tr>
<tr>
<td>V-70</td>
<td>2, 2’-azobis(4-methoxy-2-4-dimethyl valeronitrile)</td>
</tr>
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CHAPTER I

INTRODUCTION

Pharmacologically Active Polymers

Barriers to Polymer-Facilitated Drug/Gene Delivery

The “depot” model (Figure 1.1), first proposed by Helmut Ringsdorf in 1975, has been the basis of design for many polymer therapeutics. According to this model, Ringsdorf proposed the use of a modifiable polymer scaffold that would incorporate four major components including i) a water-soluble and biocompatible polymer backbone, ii) targeting moiety/diagnostic agent, iii) therapeutic agent, and iv) a degradable spacer for reversible attachment of the therapeutic agent. Various interpretations of this approach to polymer-facilitated drug delivery have since yielded many new solutions to addressing the issues associated with drug delivery. However, successful implementation of the idealized “depot” model has proven difficult, largely due to synthetic challenges and the complex nature of in-vivo drug delivery.\(^2\)

Figure 1.1. Ringsdorf “depot” model of idealized polymer-based therapeutic.
There are numerous barriers associated with polymer-facilitated cytosolic delivery of chemo- and gene-based therapeutics that must be overcome in order for polymer-based therapeutics to be a viable treatment option \textit{in-vivo} \cite{3-9}. These barriers include i) therapeutic agent degradation, ii) vascular circulation, iii) cell-specific targeting, iv) cellular internalization, v) endosomal escape, vi) controlled release of the therapeutic agent in its active form, and vii) polymer clearance/elimination from the body. The drug delivery “vehicle” must also be simultaneously biocompatible and non-immunogenic. Numerous polymer-based approaches have been developed to specifically address each of these issues with regard to drug/gene delivery but with varying degrees of success owing to the complex nature of drug delivery. Fully understanding each barrier to drug delivery is crucial to designing successful polymer therapeutics.

\textit{Therapeutic Agent Protection and Enhanced Vascular Circulation}

Small molecule drug use \textit{in-vivo} is largely inefficient due to rapid renal clearance in the kidneys and/or liver. \cite{10,11} The reduced circulation half-lives of small molecule drugs necessitate high initial dosages and frequent administration which can result in unintended drug-induced side effects. \textit{In-vivo} drug degradation also results in reduced therapeutic efficacy and may result in complete inhibition of therapeutic activity. For example, the extracellular half-life of unprotected small interfering ribonucleic acid (siRNA) is 3-5 minutes, making direct delivery of “naked” siRNA therapeutics completely ineffective. \cite{12} It is therefore desirable to extend the circulation half-life of small molecule and nucleotide-based therapeutics by preventing degradation and increasing vascular retention.
Some of the earliest polymeric delivery vehicles encapsulated or sequestered therapeutic agents inside liposomes or micellar-like structures which simultaneously protected the therapeutic agent from degradation while also increasing circulation time before ultimate renal clearance.\textsuperscript{13–15} However, these approaches did not specifically target cells, but rather relied upon non-specific cellular uptake and the enhanced permeation and retention (EPR) effect to direct the delivery vehicle to tumoral tissues.\textsuperscript{16–20} As illustrated in Figure 1.2, the EPR effect enhances polymeric drug accumulation in tumoral tissue due to the combination of a leaky vascular system and poorly formed lymphatic drainage system, which are characteristic of rapidly growing tumors. In contrast, polymer therapeutics tend not to accumulate in healthy tissues due to tight endothelial junctions and improved lymphatic drainage compared to tumoral tissue.

![Figure 1.2. Enhanced permeation and retention (EPR) effect.\textsuperscript{21}](image)

Interpolyelectrolyte complex (IPEC) formation is a commonly used strategy to electrostatically bind negatively charged oligonucleic acids to cationic polymers, effectively shielding the nucleic acid from enzymatic degradation.\textsuperscript{22–24} Traditionally, an excess of cationic charge is used to maintain water solubility of IPECs, but the overall
positive charge results in non-specific cellular uptake and high \textit{in-vitro} and \textit{in-vivo} cytotoxicity.\textsuperscript{25-27} Recently, overall neutral block ionomer complexes (BICs) comprised of neutral-block-cationic copolymers and siRNA have shown adequate protection of siRNA while allowing for cell-specific uptake when used with appropriate targeting methods.\textsuperscript{24,28}

Other approaches, aimed at improving therapeutic agent circulation time involve functionalization of nanoparticles or polymeric scaffolds with biocompatible water-soluble polymers such as poly(N-(2-hydroxypropyl)methacrylamide) (pHMPMA) or poly(ethylene glycol) (PEG).\textsuperscript{29-32} This effectively shields the drug-containing core of the delivery vehicle from eliciting a potential immune response while simultaneously protecting the therapeutic agent from degradation.

The ultimate goal of limiting therapeutic agent degradation while increasing the circulation half-life of the delivery vehicle is to allow for suitable time to reach the site of intended therapeutic action. To this end, the EPR effect can be exploited as a passive tumor targeting mechanism; however, this approach will not specifically target any cell of interest when well-defined tumors are not present and does not facilitate cell-specific internalization. Therefore, to improve drug delivery specificity and avoid therapeutic accumulation in healthy tissue, a cell-specific and preferably modifiable targeting strategy is desired.

\textit{Cell-Specific Targeting}

Polymeric drug carriers can be modified to contain targeting moieties through end group functionalization, incorporation along the polymer backbone, or side chains.\textsuperscript{33} Availability of the targeting moiety on the surface of the polymer-drug conjugate is necessary to ensure appropriate contact with surface receptors expressed on the exteriors
of targeted cells. Common targeting moieties include antibodies and antibody fragments, peptides, oligosaccharides, and folate among others.\textsuperscript{34–41} Antibodies represent a versatile targeting agent since it is possible to produce an antibody that can uniquely bind to a corresponding antigen expressed on any cell-type. Then, by conjugating the antibody to the polymer therapeutic, the delivery vehicle can be specifically targeted to the desired cell-type. It is also possible to cleave and isolate the variable regions of the antibody known as the fragment antigen binding (Fab) regions, which reduces the entire size of the targeting moiety from \(~150\) kDa per antibody to \(~50\) kDa per Fab.\textsuperscript{42,43} Kopecek and coworkers have shown that Fab-targeted polymer therapeutics promote a 3-fold reduction in tumor size compared to the non-targeted therapeutics that relied exclusively upon the EPR effect for passive tumor targeting.\textsuperscript{44} Despite their specificity, antibodies and Fab fragments are often recognized as being foreign biological molecules and can induce an immunological response that results in rapid elimination/degradation of the targeting moiety and the attached polymer therapeutic.

The use of oligosaccharides represents another attractive targeting option since all cells express saccharide-binding proteins on their surfaces called lectins.\textsuperscript{45} Due to the large number of unique possible combinations of sugar residues in oligosaccharides, specific cellular targeting is possible if a unique lectin-oligosaccharide binding interaction is known for a particular cell-type. Although lectins exhibit high specificity of recognition, not all lectins induced cellular internalization of bound oligosaccharides such that an additional means of cellular internalization would be needed.

Folate-targeted polymer therapeutics have been used extensively \textit{in-vitro} and \textit{in-vivo}.\textsuperscript{22,46,47} Folic acid is an essential vitamin required by cells and several cancer cell-
types are known to over-express folic acid receptors. Furthermore, folate is readily available compared to specifically targeted antibodies and other biomacromolecules which are more difficult to prepare and isolate. Despite the targeting method used, the polymer therapeutic must also be internalized by the cell. In some instances, the mode of targeting can result in cellular internalization, known as “piggy-back endocytosis,” whereas other routes require an additional strategy to induce cell entry.

Cellular Internalization and Endosomal Escape

Cellular internalization is most often achieved via endocytosis, the process by which a portion of the cell membrane invaginates and separates from the rest of the cell membrane, subsequently internalizing extracellular compounds (Figure 1.3). Endocytosis is usually triggered in response to an external stimulus, such as binding of a biomolecule to a particular cellular receptor. This type of cellular entry is known as receptor-mediated endocytosis and is a readily exploited method to internalize polymer therapeutics that target cellular surface receptors. Following endocytosis, the internalized polymer therapeutic and extracellular media are isolated in endosomes, accompanied by a reduction in pH from physiological (7.4) to early endosomal (pH 6-6.5) to late endosomal (pH 5-6). Within approximately 30 minutes of endocytosis, the endosomes fuse with or transform into lysozomes, which contain degradative enzymes that are particularly detrimental to most small molecule and nucleic acid-based therapeutics. Furthermore, endosomes can fuse with the cell membrane via a process known as exocytosis, subsequently releasing their contents outside of the cell. For this reason, endosomal escape is often cited as the “bottle neck” of intracellular drug delivery thus making
escape of polymer-based therapeutics from the endosome prior to lysozomal fusion or exocytosis crucial for the successful application of polymer therapeutics.

Figure 1.3. Cellular internalization of polymer therapeutics by endocytosis.¹

Several methods of endosomal release have been developed that disrupt the structural integrity of the endosomal membrane, thus facilitating the release of its contents into the cytoplasm. One approach utilizes osmotic swelling of the endosomal vesicle by promoting ion influx into the endosome by a process known as the proton sponge effect.⁵² High buffer capacity polymers such as polyethylene imine (PEI), contain a large number of unprotonated amines and can act as “proton sponges” when exposed to the acidified environment of the endosome.⁵³ The resulting influx of counterions is further accompanied by an influx of water, subsequently swelling the endosome which results in either compromised membrane integrity or complete endosomal rupture, facilitating release of its contents, polymer therapeutic included. pH-responsive peptides and polymers that become hydrophobic at endosomal pH (5.0-6.5) have also been used to
destabilize the amphiphilic endosomal membrane facilitating the release of the endosomal contents. Ultimately, the goal of endosomal escape is to release the polymer therapeutic into the cytoplasm, where triggered release of the therapeutic agent can elicit the desired pharmacological response.

*Therapeutic Release and Ultimate Polymer Fate*

The final role of a polymer therapeutic is to release the therapeutic agent upon cellular internalization. This is typically desired following escape from the endosome to avoid unwanted degradation by endosomal and lysosomal enzymes. For example, when delivering siRNA, release is preferred in the cytoplasm where it can then directly enter into the RNA interference (RNAi) pathway. Therefore, a number of approaches have been developed to promote release of therapeutic agents at a desired location within the cell.

A common strategy for intracellular-induced therapeutic release is to conjugate the therapeutic agent to a polymer scaffold using a degradable linkage. The most common types of degradable linkages used for intracellular drug release cleave in response to changes in pH, redox potential, or enzymatic degradation. Acid-labile linkages include esters, carbonates, carbamates, acetals, and hydrazones among others, while enzymatically-degradable linkers comprised of specific ester- or peptide-containing sequences are selectively cleaved by esterases or peptidases, respectively, facilitating intracellular therapeutic release. Disulfide linkages are the primary example of a chemical linkage that cleaves upon experiencing a change in redox potential. Glutathione, the most abundant non-protein peptide in mammalian cells, is an efficient reducing agent meant to maintain the redox potential inside of the cell by
reducing reactive oxygen species but also has been shown to effectively reduce most forms of disulfide bonds.\textsuperscript{70} This type of bond cleavage occurs readily and almost exclusively inside of the cytoplasm, which would promote therapeutic release from the polymeric carrier only after escaping the endosome. Also, higher levels of glutathione are observed in a number of cancer cell types compared to concentrations present in healthy tissues, which further promotes the likelihood of disulfide bond cleavage in these cancer cells.\textsuperscript{71}

After the polymer “vehicle” has delivered its therapeutic payload to the appropriate location within the cell, it must be reabsorbed or eliminated from the body to avoid toxic \textit{in vivo} accumulation. This can be accomplished using a polymer therapeutic in which the polymer component is partially or entirely degradable, or small enough to be excreted whole by the body. Examples of biodegradable polymers include poly(L-lactide/glycolide),\textsuperscript{72} polypeptides,\textsuperscript{73} and polysaccharides.\textsuperscript{74} These polymers are capable of being completely degraded, or can be used as part of larger polymeric scaffolds which are reduced in size upon partial degradation of lactide or glycolide linkages, thus facilitating polymer clearance from the body. Polymers that are to be secreted based upon size are removed by renal filtration in the kidneys and typically must have hydrodynamic diameters less than \textasciitilde15 nm for adequate removal without renal accumulation.\textsuperscript{75} Thus, adequate control over the size of the delivery vehicle is necessary to avoid both rapid clearance from the body and to prevent perpetual accumulation by being too large for long term elimination to occur.
Reversible Addition-Fragmentation Chain Transfer Polymerization

The development of reversible-deactivation radical polymerization (RDRP) techniques including nitroxide-mediated polymerization (NMP),\textsuperscript{76,77} atom transfer radical polymerization (ATRP),\textsuperscript{78–81} and reversible addition-fragmentation chain transfer polymerization (RAFT)\textsuperscript{82–87} has made possible the synthesis of polymers with precise compositions, predetermined molecular weights, and advanced architectures comprised of monomers possessing a wide variety of functional groups. RAFT polymerization is arguably the most versatile of the RDRP techniques owing to the increased tolerance of protic functional groups and solvents allowing for the polymerization of styrenics, (meth)acrylates, (meth)acrylamides, acrylonitriles, vinyl esters, and vinyl amides, in organic or aqueous media under homogeneous or heterogeneous conditions. In particular, the development of aqueous RAFT (aRAFT) polymerization has allowed for the direct synthesis of polymers containing biologically relevant functional groups such as amines, carboxylates, phosphates, sulfonates, and betaines among others.\textsuperscript{88–98} For these reasons, RAFT is an ideal polymerization method for the synthesis of advanced polymer-based therapeutics.

The RAFT Mechanism

The mechanism of RAFT polymerization is fundamentally different from the mechanisms of NMP and ATRP in that the main active/dormant equilibrium is established by degenerative chain transfer between active (propagating) and dormant chain ends rather than reliance upon the persistent radical effect. Successful degenerative chain transfer is achieved when the product of chain transfer is also a chain transfer agent of equal or similar reactivity. Operating under these conditions, RAFT polymerization
exhibits similarities to pseudo-living polymerizations in that narrow molecular weight distributions can be obtained while molecular weight progresses linearly with monomer conversion and high chain-end fidelity is maintained throughout the reaction such that sequential monomer addition results in the formation of block copolymers.

**Scheme 1.1. Proposed mechanism of RAFT polymerization.**
Like classical free radical polymerization, the first stage in the RAFT process involves decomposition of initiator into radicals (Scheme 1.1). However, the fate of initiator-derived radicals differs significantly during RAFT polymerization due to the presence of a thiocarbonylthio-containing chain transfer agent (CTA). The initiation/pre-equilibrium stage of RAFT consists of multiple kinetically distinct chain transfer reactions. During this period, initiator-derived radicals \( \text{1} \) can either initiate polymerization by direct addition to monomer to form the propagating species \( \text{3} \), or add directly to CTA \( \text{2} \) to afford the radical intermediate \( \text{4} \). Although the formation of \( \text{4} \) is also reversible, eventual fragmentation of the R-group yields a new initiator-derived CTA \( \text{9} \) and R-group-derived radical \( \text{7} \) capable of adding to monomer to form the propagating species \( \text{8} \). Similarly, the initiator-derived propagating species \( \text{3} \) can add to CTA \( \text{2} \) with a rate constant \( k_{\text{add}} \) to yield the radical intermediate \( \text{5} \). This intermediate can fragment in the reverse direction with rate constant \( k_{-\text{add}} \) or in the forward direction with rate constant \( k_{\beta} \) to form the macro-CTA \( \text{6} \) and the R-group-derived radical \( \text{7} \), which can add to monomer forming the propagating species \( \text{8} \). The pre-equilibrium period is complete when CTA \( \text{2} \) has been converted into the corresponding macro-CTA \( \text{6} \). Similar to the initiation stage of other controlled/"living" polymerizations where \( R_1 > R_p \) must be met to achieve narrow molecular weight distributions, it is important that the duration of the pre-equilibrium period during RAFT be suitably short relative to the timescale of propagation such that the majority of polymer chains start growing at the same time.

Prolonged periods of little or no monomer conversion have often been observed during the pre-equilibrium stage of RAFT polymerizations.\(^9^9\) Like conventional radical polymerizations, inhibition of RAFT is possible due to the presence of oxygen or other
impurities (e.g. thiol impurities originating from the CTA) that consume radicals early in the polymerization. Extended pre-equilibrium or inhibition periods in RAFT can also occur for certain CTA-monomer combinations because of slow reinitiation by R· due to preferred addition to the CTA thiocarbonyl (high $k_\beta$) rather than addition to monomer (low $k_i$). The cause of this initialization phenomena was first elucidated by McCleary, Klumperman, and coworkers using in situ $^1$H NMR analysis during the 2-cyano-2-propyl dithiobenzoate-mediated polymerization of styrene. As seen in Figure 1.4, complete conversion of the initial dithiobenzoate species (AD) to the corresponding single monomer unit adduct (ASD) was required before reaching the main RAFT equilibrium and thus observed as a period of minimal monomer conversion. Suppression or elimination of prolonged initialization periods can be accomplished by selection of CTA R- and Z-groups which favor fragmentation and reinitiation by R· (i.e. R-groups that are structurally similar to the propagating radical $P_n$).
Figure 1.4. Percentage of dithiobenzoate species present during the AIBN-initiated polymerization of styrene in the presence of 2-cyano-2-propyl dithiobenzoate (AD).$^{106}$

Propagation during the main RAFT equilibrium occurs as it does during conventional free radical polymerization where the rate of propagation ($R_p$) is expected to be first order with respect to both the concentration of monomer [M] and propagating radicals [$P_n$·] while independent of [CTA] as seen in equation 1. RAFT polymerizations often exhibit pseudo-first-order kinetic behavior when a steady state radical concentration is reached where $R_i \approx R_t$, allowing for simplification of equation 1 to equation 2 where $k_{app}$ is the apparent propagation rate constant equal to $k_p[P_n$·$]$.  

$$R_p = k_p [P_n$·$][M]$$  

(1)
\[ R_p = k_{app}[M] \]  

Degenerative chain transfer during RAFT polymerization allows for the total number of polymer chains to greatly exceed the number of propagating radicals. This is a cornerstone feature of any RDRP technique since reducing \([P_n\cdot]\) by a factor of 10 will result in a 10-fold reduction in \(R_p\) but a 100-fold reduction in the rate of bimolecular radical termination \(R_t\) due to the second order dependence of \(R_t\) on \([P_n\cdot]\) (equation 3).

\[ R_t = k_t[P_n\cdot]^2 \]

In theory, the introduction of CTA (RAFT agent) to a conventional free radical polymerization should have no effect on \(R_p\) as seen in equation 1. However, in practice \(R_p\) during RAFT polymerization is significantly reduced compared to the analogous free radical polymerization conducted in the absence of CTA with much debate over the exact cause of rate retardation\(^{107-110}\). One possible explanation for the differences in observed polymerization rates between conventional and RAFT polymerizations is due to extended lifetimes of the intermediate radical \(^1\), such that \([P_n\cdot]\) is lower than what would be predicted based upon the total radical concentration resulting from initiator-derived radicals.\(^{111}\) While the intermediate radical \(^1\) has been observed by electron spin resonance (ESR) spectroscopy, the observed concentrations were too low to completely explain the reduction in \(R_p\) for RAFT polymerizations.\(^{112-115}\) Radical trapping or “sink” theories which suggest intermittent and reversible coupling of the transient species \(^1\) with other radical species including \(P_n\cdot\), I\(\cdot\), and \(^1\) have also been proposed with some spectroscopic evidence provided for the formation of these intermediates using electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance
(NMR) spectroscopy, however, there is still much debate as to the exact cause of propagation rate retardation in RAFT polymerizations.116–118

**Molecular Weight Control in RAFT Polymerization**

In RAFT polymerization, the theoretical number average degree of polymerization ($X_{n,th}$) can be determined based upon the molar ratio of consumed monomer to the number of polymer chains formed during polymerization such that:

$$X_{n,th} = \frac{[M]_0 \rho}{[CTA]_0 + 2f[I]_0(1 - e^{-k_d t})}$$  \hspace{1cm} (4)

where $[M]_0$ is the initial monomer concentration, $\rho$ is the fractional monomer conversion, $[CTA]_0$ is the initial CTA concentration, $f$ is the initiator efficiency, $[I]_0$ is the initial initiator concentration, $k_d$ is the initiator dissociation constant, and $t$ is the reaction time.

In a typical RAFT polymerization, the $[CTA]_0: [I]_0$ is suitably high such that the total number of initiator-derived polymer chains is negligible allowing for omission of the second term in the denominator of equation 4.110 The theoretical number average molecular weight ($M_{n,th}$) can then be calculated according to:

$$M_{n,th} = X_{n,th} M_{MW} + CTA_{MW} = \frac{[M]_0 \rho}{[CTA]_0} M_{MW} + CTA_{MW}$$  \hspace{1cm} (5)

where $M_{MW}$ and $CTA_{MW}$ are the molecular weights of monomer and CTA respectively. It is worth noting that equation 5 assumes 100% of CTA results in formation of a polymer chain and has been shown to underestimate $M_{n,th}$ compared to experimentally determined $M_n$ ($M_{n,exp}$) during RAFT polymerizations where incomplete consumption of CTA occurs during the pre-equilibrium period or due to CTA degradation during polymerization.119
RAFT Agent Design

Chain transfer agents possessing thiocarbonylthio functional groups (RAFT agents) have been utilized to mediate the polymerization of a wide variety of monomer types including styrenics, (meth)acrylates, (meth)acrylamides, acrylonitriles, vinyl esters, and vinyl amides among others. As shown in Figure 1.5, the RAFT agent consists of a reactive C=S double bond capable of addition to propagating radicals, a weak S-R bond to promote rapid fragmentation and reinitiation by the R-group, and Z-group that influences the stability of the intermediate radical. The R-group is only involved during the initialization period while the Z-group is present on the ω-terminus of dormant RAFT chains throughout the entire polymerization. Consequently, the Z-group has the greatest influence over polymerization control and must be chosen in accordance with monomer type. For this reason a number of Z-groups including dithioesters,\textsuperscript{82} trithiocarbonates,\textsuperscript{120} xanthates,\textsuperscript{121} and dithiocarbamates\textsuperscript{122} have been utilized to polymerize a range of monomers from “more activated monomers” (MAM) such as methyl methacrylate to “less activated monomers” (LAM) such as vinyl acetate. Furthermore, “universal” or “switchable” RAFT agents based upon 4-pyridinyl dithiocarbamates (Figure 1.5) have been designed to polymerize a wider range of monomers.\textsuperscript{123–125} In the presence of acid, the pyridine ring is protonated and electron withdrawing, allowing for control over polymerization of MAMs. Alternatively, use of 4-pyridinyl dithiocarbamates in the electron donating neutral form facilitates polymerization control of LAMs. General guides for RAFT agent R- and Z-group selection for a given monomer-type are also now available as shown in Figure 1.6.
End Group Functionalization of RAFT Polymers

It is often desirable or necessary to control polymer end group functionality. Inherent to polymers synthesized by RAFT is retention of the RAFT agent R-group on the α-terminus and the thiocarbonylthio moiety and Z-group on the ω-terminus (Figure 1.7). Accordingly, preparation of functional telechelic RAFT polymers is readily accomplished by incorporating the desired functionalities onto the RAFT agent R- and Z-groups. However, precise control over end group functionality not directly
obtainable through R- and Z-group selection necessitates the use of post-polymerization end group transformations. Extensive efforts have thus been made toward end group functionalization of RAFT polymers by exploiting the latent reactivity of the thiocarbonylthio moiety.\textsuperscript{130–137}

\[
\begin{array}{c}
\text{R} \overset{\text{S}}{\smile} \overset{\text{Z}}{\text{Z}} \\
\text{M} \\
\text{telechelic RAFT polymer}
\end{array}
\]

*Figure 1.7.* R/Z-group approach toward the synthesis of \(\alpha,\omega\)-telechelic polymers by RAFT.

While the reactive thiocarbonylthio \(\omega\)-terminus of RAFT polymers functions as a useful chemical “handle” for subsequent end group transformations, the instability of this group may be undesirable depending upon the end-use of the RAFT polymer. Bulmus and coworkers recently studied the effect of RAFT agent type on cytotoxicity of the biocompatible polymer pHMPA. *In-vitro* cell viability assays showed that dithiobenzoate-functional pHMPA was significantly more toxic than analogous trithiocarbonate-terminated pHMPA.\textsuperscript{138} This is likely due to the hydrolytic instability of dithiobenzoates relative to trithiocarbonates, with hydrolysis byproducts responsible for the decreased cell viability. A number of methods now exist whereby the reactive thiocarbonylthio moiety can be converted to a benign end group to minimize unintended consequences of RAFT agent reactivity.\textsuperscript{139–142}

**Stimuli-Responsive Polymers**

Of particular interest in the field of polymeric drug carriers is the ability to design stimuli-responsive behavior into the delivery “vehicle” such that autonomous functions can be performed *in-vivo* that elicit a therapeutic effect. For example, the acidic
environment of tumoral tissue may be used as an environmental trigger for polymer-bound drug release.\textsuperscript{143} Stimuli-responsive polymers in solution can exhibit changes in chain conformation, size, or solubility in response to biologically relevant stimuli such as changes in pH, redox potential, temperature, ionic strength, or specific molecular interactions.\textsuperscript{144} Furthermore, externally applied stimuli such as electromagnetic radiation, changing magnetic fields, and mechanical forces (sonication) have also been used to elicit changes in polymer physical and chemical properties.\textsuperscript{145} Such stimuli-responsive polymer properties can be exploited to passively/actively target polymeric drug carriers, promote cell-specific internalization, endosomal escape, site-specific drug release, trigger drug carrier degradation/elimination from the body, and allow polymers to function as combined therapeutic/diagnostic (theranostic) agents. It is therefore becoming ever more pertinent to design multipli-responsive polymeric carriers that can exhibit rapid and reversible changes in physical and chemical properties in response to discrete changes in orthogonal biologically relevant stimuli.

\textit{pH-Responsive Polymers}

Polymers comprised of weakly acidic or basic functional groups (polyacids or polybases) make up a class of polyelectrolytes that exhibit pH-dependent aqueous solution properties and have been used extensively in a variety of applications including drug delivery.\textsuperscript{97,146–157} A change in solution pH results in a corresponding change in the degree of ionization of the polyelectrolyte and subsequently its hydration state. This can ultimately lead to aggregation or self assembly of polyelectrolyte-containing polymers. Polyacids exist predominantly in the ionized form when the solution pH (pH\textsubscript{sol}) is greater than the pK\textsubscript{a} of the poly acid. Accordingly, polyacids are protonated and therefore neutral
(or nearly neutral) in charge and less hydrophilic when \( pH_{sol} < pK_a \). Conversely, polybases are ionized and therefore water soluble when \( pH_{sol} < pK_a \) and neutrally charged and less water soluble when \( pH_{sol} > pK_a \). It is therefore possible to dictate whether increasing or decreasing the solution pH will increase or decrease polymer solubility depending upon whether the polymer is a weak polyacid or polybase. Figure 1.8 shows some of the more common acid- and base-functional acrylic monomers that are readily polymerizable by RDRP techniques such as RAFT to afford the corresponding pH-responsive polymers.

*Figure 1.8. Common pH-responsive monomers polymerizable by RDRP techniques.*

**Temperature-Responsive Polymers**

Temperature-responsive polymers are those which exhibit a volume phase transition at a critical temperature. In solution, the influence of temperature on polymer solubility can be explained by the mutual influences of the enthalpy (\( \Delta H \)) and entropy (\( \Delta S \)) of mixing on the total free energy of mixing (\( \Delta G \)) according to equation 6.
which exhibit a decrease in solubility in a given solvent upon heating possess a lower critical solution temperature (LCST), above which polymer-solution phase separation occurs. For these systems, $\Delta S$ is negative and an increase in temperature results in a corresponding increase in $\Delta G$ with the temperature at which $\Delta G = 0$ defined as the LCST.

Polymers that exhibit increased solubility in a given solvent upon heating possess an upper critical solution temperature (UCST) owing to a positive $\Delta S$ of mixing. In aqueous systems, entropic effects are driven by the ordering or disordering of water molecules at the polymer-water interface whereas the enthalpy of mixing is influenced by hydrogen bonding, dipole interactions, and hydrophobic effects.

$$\Delta G = \Delta H - T \Delta S$$ (6)

Figure 1.9 shows the more commonly used temperature-responsive monomers that have been polymerized by RAFT. The most extensively studied temperature-responsive polymers for biological applications are those derived from N-isopropylacrylamide (NIPAM) owing to the LCST of poly(NIPAM) ($\sim32$ °C) being near that of physiological temperature (37 °C).158–161 A number of other N-substituted (meth)acrylamides have also been polymerized by RAFT to afford the corresponding temperature-responsive polymers and have seen applications in polymer-facilitated drug delivery.146,162–166
**Redox-Responsive Polymers**

Polymers exhibiting redox-responsive chemical and physical properties are actively being explored as stimuli-responsive drug carriers. Glutathione (GSH), the most abundant non-protein peptide in mammalian cells, is largely responsible for the reductive intracellular environment within cells.\(^{71,167}\) The intracellular concentration of GSH (5-10 mM) is significantly higher than that outside of the cell (2-20μM) resulting in a difference in redox potential that can be exploited as an intracellular-specific stimulus. Redox-responsive polymers for drug delivery are almost exclusively based upon disulfide linkages which are chemically stable in the oxidative extracellular environment while being rapidly reduced to the corresponding thiols by GSH upon cellular internalization.\(^{46,168-174}\) Consequently, redox-responsive polymers differ from pH- and temperature-responsive polymers in that the “response” is cleavage of covalent disulfide linkages rather than a thermodynamic change in polymer hydration/solubility. This...
makes possible the triggered release of covalently bound therapeutics such as thiol-functional siRNA as was recently demonstrated by York et al.\textsuperscript{46} There are a number of reviews that address the potential applications of redox-responsive disulfide-containing polymer therapeutics.\textsuperscript{168,171,172,174–176}

**Advanced Macromolecular Architectures**

While imparting stimuli-responsive behavior into polymer-drug conjugates through careful selection of monomer-type and end group functionality has the potential to address many of the barriers to drug delivery in a single delivery “vehicle,” it is evident that multipli-responsive polymer therapeutics will likely be needed thus necessitating the use of branched or block copolymers where discrete blocks of the polymer are designed to respond to different stimuli. Furthermore, issues such as therapeutic agent protection, vascular circulation, tumoral accumulation, and ultimate polymer clearance from the body can be accomplished by controlling polymer architecture. Upon the advent of controlled/”living” polymerization techniques, a number of polymer architectures are now possible including alternating/statistical/gradient/multiblock copolymers, functional telechelics, stars, hyperbranched polymers, dendrimers, and molecular brushes (Figure 1.10).\textsuperscript{177–185}
Highly branched architectures with tailorable shapes and sizes are of particular interest for polymer therapeutics. Compared to analogous linear polymers, highly branched polymers have been shown to exhibit increased vascular circulation and tumoral tissue accumulation.\textsuperscript{186} While self-assembled polymer structures such as micelles and vesicles share many of the benefits of branched polymers, \textit{in-vivo} usage is limited due to dilution-induced disassembly caused by polymer concentrations falling below the critical micelle concentration (CMC).\textsuperscript{89,187} Consequently, branched polymer architectures that are assembled using permanent or reversible covalent linkages are desired.

\textit{Molecular Brush Copolymers}

Molecular brush or “bottle-brush” copolymers are comprised of polymeric side-chains attached to a polymer backbone and can exhibit highly branched spherical or cylindrical morphologies with minimal intermolecular chain entanglement owing to the volume-exclusion interactions between sterically crowded polymeric side-chains.\textsuperscript{188,189}
Molecular brush architectures are of particular interest as drug delivery “vehicles” due to their unique branched topology and readily tailorable dimensions. As stated previously, large, highly branched, and cylindrical polymer architectures exhibit increased *in-vivo* circulation times, enhanced accumulation in tumoral tissue due to the EPR effect, and improved biocompatibility compared to analogous linear polymers. Molecular brush copolymers therefore represent an attractive polymer architecture that has the potential to simultaneously address numerous issues associated with drug delivery.

In general, molecular brush synthesis is accomplished by one of three routes referred to as “grafting from,” “grafting onto,” and “grafting through” (Figure 1.11). “Grafting from” molecular brush synthesis involves initial preparation of a multifunctional backbone (macroinitiator) followed by side chain growth from initiator side groups. Controlled polymerization techniques such as RAFT, ATRP, NMP, and ROP have been used to prepare molecular brush backbones, which typically require additional post-polymerization modification in order to attach the desired initiator groups along the backbone. Side chain growth is most often accomplished using RDRP techniques such as ATRP. Limited use of RAFT polymerization to afford side chain growth is due in part to difficulties in attaching the RAFT agent to the brush backbone by post-polymerization modification and also because of the fragmentation-chain transfer nature of RAFT requiring that either a polymer side chain fragment from the backbone in order to propagate or necessitating the unlikely collision of sterically restricted polymer end groups in order for degenerate chain transfer to occur. While there are some limitations regarding the synthesis of molecular brushes using “grafting from”
approaches, purification of molecular brushes synthesized in this fashion is typically simpler since the resulting molecular brush is the only polymeric product of the reaction.

Figure 1.11. “Grafting from,” “grafting onto,” and “grafting through” synthetic routes toward molecular brush synthesis.

“Grafting onto” methods allow for preparation of molecular brush back bone and side chains separately. Side chains are then grafted onto the brush backbone using efficient coupling or “click” type reactions to ensure high side chain grafting density. This approach makes possible the synthesis of well-defined side chains and backbones first, such that molecular brushes with predetermined backbone and side chain lengths are easier to prepare. In addition to covalent means of “grafting onto” molecular brush synthesis, supramolecular brushes based upon noncovalent interactions have been demonstrated which rely upon coordination bonding, hydrogen bonding, or ionic
interactions between side chains and backbones. This type of supramolecular brush assemblage occurs naturally in proteoglycan aggregates found in cartilage tissues which are comprised of a positively charged polypeptide backbone electrostatically bound to glycosaminoglycan side chains. While practical, the primary drawback to “grafting onto” methods is the near impossibility of achieving 100% grafting density of the molecular brush backbone due to kinetic and thermodynamic restrictions resulting from steric exclusion of side chains as grafting density increases.

The third and arguably most versatile molecular brush synthetic route is referred to as “grafting through.” This method relies upon first using a controlled polymerization technique to synthesize macromonomers (MMs) which possess polymerizable end groups that can then be reacted by a second polymerization technique to afford the corresponding molecular brush. “Grafting through” is the only approach that affords molecular brushes with 100% side chain graft density since each brush backbone repeat unit is inherently attached to a polymer side chain. However, this method often results in kinetically limited reactions with low MM conversions due to the steric constraints arising from the use of MMs and the low concentration of polymerizable end groups typically ranging from 0.01-0.1 M.

To overcome these issues, ring opening metathesis polymerization (ROMP) has recently been used to successfully synthesize molecular brushes from norbornene-functional MMs while achieving near quantitative conversion of norbornene end groups owing to the high reactivity and functional group tolerance of the current generation of ruthenium-based metathesis catalysts (Figure 1.12). This approach has since been used to synthesize a variety of molecular brushes with block-backbone and block-
side chain subarchitectures from a wide range of MMs derived from controlled polymerization techniques. While ROMP of MMs overcomes the previous limitations associated with “grafting through” molecular brush synthetic routes, the facile synthesis of norbornene-functional MMs still represents an ongoing challenge.

**Figure 1.12.** Ruthenium-based metathesis catalysts.

Synthesis of MMs can be accomplished by either “direct-growth” (DG-MM) or “growth-then-coupling” (GC-MM) methods as described recently by Xia and coworkers (Figure 1.13).\(^{218}\) DG-MM synthesis has been accomplished through the use of norbornene-functional initiators during ROP, NMP, ATRP, and through the use of norbornene-functional chain transfer agents during RAFT polymerization.\(^{202,219-226}\) The use of reversible deactivation radical polymerization techniques to synthesize MMs is particularly advantageous due to the wide variety of vinyl monomers available and ease of controlling end group functionality. However, DG-MM synthesis by RDRP techniques requires polymerization optimization for a given monomer to minimize radical addition to the norbornene olefin during polymerization.\(^{214}\) Furthermore Xia and coworkers have demonstrated that even trace amounts of difunctional macromonomer impurities resulting from bimolecular radical coupling of α-norbornene-functional polymers can result in
undesired molecular brush branching and broadened molecular weight distributions.\textsuperscript{218}

Therefore, facile and efficient growth-then-coupling methods to synthesize RDRP-based MMs are needed.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure13.png}
\caption{Norbornene-functional macromonomer synthesis by direct-growth (DG-MM) and growth-then-coupling (GC-MM) synthetic routes.}
\end{figure}

Due to recent improvements in molecular brush synthetic methods, a number of drug delivery applications have been developed that readily exploit the discrete sizes and highly branched morphologies of molecular brushes. Johnson, Grubbs and coworkers recently demonstrated the synthesis of drug-loaded, bivalent molecular brush copolymers that were synthesized using “grafting through” ROMP methodologies developed in the same lab (Figure 1.14).\textsuperscript{212} Molecular brushes were comprised of polynorbornene backbones and PEG side chains with anticancer drugs doxorubicin (DOX) and camptothecin (CT) attached to the brush backbone using a photocleavable nitrobenzyloxycarbonyl linker. Subsequently, photolysis of the degradable linkages using
365 nm UV light afforded rapid release of DOX and CT with 50% drug release observed in 3.1 min and 10 min for each drug respectively. When irradiated with UV light, the molecular brush-cancer drug conjugates were shown to have similar half-maximum inhibitory concentrations to the free drugs whereas the PEG-based drug-bound molecular brushes showed minimal influence on cell viability due to steric shielding of the covalently attached anticancer drugs.

Figure 1.14. Drug-loaded, bivalent molecular brush polymers synthesized using “grafting through” ROMP of norbornene-functional macromonomers.\textsuperscript{212}
CHAPTER II

OBJECTIVES OF RESEARCH

The original “depot” model proposed by Ringsdorf in 1975 was the first attempt at addressing the issues of drug delivery using a single macromolecular architecture. Initially, limitations in the available synthetic methods prevented the synthesis of well defined polymers comprised of functionally diverse monomers and advanced architectures such as block, star, and molecular brush copolymers. Upon the advent of controlled/”living” polymerization techniques such as reversible-deactivation radical polymerization (RDRP), facile preparation of (co)polymers with tailorable architectures, predetermined molecular weights, and of narrow molecular weight distributions has made possible the synthesis of a wide variety of interpretations of the Ringsdorf “depot” model. However, limitations still exist regarding the facile synthesis of polymers aimed at specifically addressing the issues of drug delivery.

The main work described in this dissertation focuses on the development of synthetic approaches toward novel stimuli-responsive polymers and advanced polymer architectures that specifically address the issues of \textit{in vivo} drug delivery. Particular attention is given to understanding the fundamentals of the underlying chemistry used to synthesize the polymers discussed in this work.

The specific objectives of this research are to:

1. Synthesize a library of methacryloyl sulfonamide (MSA) monomers with pK\textsubscript{a} values in the biologically relevant regime.

2. Develop conditions to control the RAFT polymerization of MSA monomers.
3. Investigate the tunable pH- and CO₂-responsive aqueous solution properties of MSA RAFT polymers.

4. Investigate temperature-dependent trithiocarbonate chain-end degradation observed during RAFT polymerization of N-arylmethacrylamides.

5. Synthesize RAFT polymer small molecule analogs by single monomer unit insertion in order to study the byproducts of trithiocarbonate degradation.

6. Use in situ ¹H NMR analysis to study the mechanism of N-arylmethacrylamide promoted trithiocarbonate chain-end degradation.

7. Develop complimentary mechanistic and kinetic models to explain the observed effects of N-arylmethacrylamide substitution on amide nucleophilicity.

8. Systematically investigate the effects of nucleophilic catalyst, reducing agent, and solvent on “one-pot” aminolysis/thiol-maleimide end-group functionalization of RAFT polymers.

9. Identify and prevent detrimental nucleophile-promoted thiol-maleimide side reactions.

10. Elucidate the mechanism(s) of base catalyzed maleimide polymerization initiated by thiols and thiol-maleimide adducts.

11. Optimize “one-pot” aminolysis/thiol-maleimide end-group functionalization reaction conditions to afford selective and quantitative end group functionalization efficiency of RAFT polymers with N-substituted maleimides.

13. Synthesize ω-oxanorbornenyl macromonomers by end-group modification of RAFT polymers with thiol-reactive oxanorbornenes.

14. Develop polymerization conditions for the controlled ROMP of RAFT-polymer derived macromonomers.

15. Demonstrate reduction-induced disassembly of disulfide-containing molecular brushes.

The work addressing these objectives is divided into four main sections. The first section is concerned with the synthesis of a novel class of tunable pH-responsive polymers by RAFT polymerization. These polymers are to be synthesized using a library of sulfadrug-based methacryloyl sulfonamide monomers possessing pK_a values in the range of the early-to-late endosomes (pH = 5-7). Future work regarding these polymers is aimed at developing pH-responsive endolytic polymers that could be incorporated into polymer therapeutics to aid in endosomal escape, which is often cited as the “bottle neck” of drug delivery.

Section 2 is concerned with investigating the poor RAFT polymerization control of methacryloyl sulfonamide monomers despite using “standard” methacrylamide polymerization conditions. Consequently, work in this section aims to provide an understanding of the factors influencing RAFT agent degradation during MSA polymerization by systematically studying the effects of methacrylamide structure and polymerization temperature on the rates and mechanisms of RAFT polymer chain-end degradation. In addition, on-going work regarding the development of a mechanistic and kinetic theory aimed at explaining the unique influence of N-arylsubstitution on amide nucleophilicity is further discussed in Appendix B.
Control over polymer end group functionality is typically necessary when synthesizing highly branched polymer architectures such as molecular brushes. Section 3 details the identification and prevention of deleterious side reactions that we observed during “one-pot” aminolysis/thiol-maleimide end group functionalization reactions of RAFT polymers. The effects of solvent, nucleophilic catalyst, reducing agent, and reagent order of addition on RAFT polymer end-group functionalization efficiency are investigated. Particular attention is also given to finding suitable reagents and reaction conditions that favor quantitative end group functionalization of RAFT polymers with N-substituted maleimides with minimal side reactions.

Section 4 is concerned with developing a facile synthetic route towards molecular brush copolymers derived from RAFT polymers for potential use as drug delivery “vehicles.” This work is particularly focused on synthesizing RAFT polymer-derived macromonomers using optimized end group functionalization techniques as discussed in Section 3. Furthermore, this work aims to develop a facile synthetic route towards molecular brushes capable of intracellular-induced disassembly via the incorporation of disulfide linkages between the molecular brush backbone and side chain. Macromonomer polymerization control by ROMP and reduction-induced molecular brush disassembly are discussed.
CHAPTER III

EXPERIMENTAL SECTION

Materials

Methacryloyl chloride (Aldrich, 97%), N,N-dimethylacrylamide (Aldrich, 99%), styrene (Aldrich, 99%), aniline (Aldrich, 99%), benzylamine (Aldrich, 98%), and furan (Aldrich, 99%) were vacuum distilled and stored under N₂ at -10 °C prior to use. 4,4-Azobis(4-cyanovaleric acid) and azobisisobutyronitrile (AIBN) (Aldrich, 98%) were recrystallized from methanol and stored at -10 °C. N,N’-dimethylformamide (Acros, extra dry w/ sieves) was stirred under vacuum at room temperature for 60 min prior to use in order to remove traces of dimethylamine. The following were used as received: 2, 2’-Azobis(4-methoxy-2,4-dimethyl valeronitrile) (V-70) (Wako, 96%); sulfacetamide (Aldrich, >98%); sulfamethazine (Aldrich, >99%); sulfamethizole (Aldrich, >99%); sulfadimethoxine (Aldrich, >98.5%); sulfadoxine (Aldrich, >95%); sulfabenzamide (TCI, >98%); trimesic acid (Aldrich, 95%); 1-dodecanethiol (Aldrich, 98%); ethane thiol (Aldrich, 97%); carbon disulfide (Aldrich, 99.9%); 2-bromoisobutryl bromide (TCI, 98%); triethylamine (Aldrich, 99.5%); NaH (Aldrich, 95%); maleic anhydride (Aldrich, 99%); acetic anhydride (Fisher, 99.2%); sodium acetate (Fisher, anhydrous); N-methylmaleimide (Aldrich, 97%); ethyl 2-mercaptopropionate (Aldrich, >95%); benzyl mercaptan (Fluka, >99%); hexylamine (Aldrich, 99%); 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Aldrich >99.0%); triethylamine (Aldrich, >99.5%); tributylphosphine (Aldrich, 97%); trimethylphosphite (Aldrich, >99%); 2nd generation Grubbs catalyst (Aldrich); ethylenediamine (Aldrich > 99.5%); di-tert-butyl dicarboante (Aldrich, >99%); maleimide (Aldrich, 99%); methylchloroformate (Aldrich, 99%); 4-
methylmorpholine (Aldrich, redist., > 99.5%); trifluoroacetic acid (Aldrich, 99%); 3-bromopropylamine hydrobromide (Aldrich, 98%); sodium methane sulfinate (Alfa Aesar, 95%); 0.1 N NaOH (Alfa Aesar, standardized); and 0.05 N HCl (Alfa Aesar, standardized); CDCl₃ (Cambridge Isotopes, 99.8%); acetone-d₆ (Cambridge Isotopes, 99.9%); N, N’-dimethylformamide-d₇ (Cambridge Isotope, 99.5%); dimethylsulfoxide-d₆ (Cambridge Isotopes, 99.9%); acetonitrile-d₃ (Cambridge Isotopes, 99.8%); methylene chloride-d₂ (Cambridge Isotopes, 99.8%); Ethanol-D (Cambridge Isotopes, D 99%, <6% D₂O); and Deuterium Oxide (Aldrich, 99.9%). All other chemicals were purchased from Aldrich in the highest available purity and used as received.

Characterization

NMR spectra for structural analysis and monomer conversions were obtained using a Varian INOVA 300 MHz NMR spectrometer. Polymer molecular weights and molecular weight distributions (Mₘ/Mₙ) were determined by size exclusion chromatography (SEC) using DMF 20 mM LiBr as the eluent at a flow rate of 1.0 mL/min in combination with two Agilent PolarGel-M columns heated to 50 °C and connected in series with a Wyatt Optilab DSP interferometric refractometer and Wyatt DAWN EOS multangle laser light scattering (MALLS) detector (λ = 633 nm). Absolute molecular weights and Mₘ/Mₙ were calculated using a Wyatt ASTRA SEC/LS software package. The dn/dc values for each polymer derivative in the above eluent at 35 °C were determined offline using a Wyatt Optilab DSP interferometric refractometer and Wyatt ASTRA dn/dc software.
Dithiobenzoic acid 21

Magnesium (1.22 g, 50.0 mmol) was washed in anhydrous diethyl ether and dried in an oven prior to addition to a 250 mL round bottomed flask equipped with stir bar. The flask was flame dried under vacuum and backfilled with argon followed by the addition of anhydrous THF (25 mL). Bromobenzene (5.0 mL, 47.8 mmol) was then added dropwise via syringe over 15 min, upon which 75 mL of THF was added and the resulting Grignard solution transferred via cannula to a flame-dried 3-neck round bottomed flask equipped with condenser and heated to reflux (40 °C). Carbon disulfide (2.89 mL, 47.8 mmol) was then added dropwise over 15 min and the reaction stirred at 40 °C for an additional 2 h. Ice cold 0.5 N HCl (50 mL) was then added to the reaction followed by removal of approximately 75% of the THF/Et₂O by rotary evaporation. The pH of the aqueous solution was then adjusted to pH < 2 with 12 N HCl and transferred to a separatory funnel and extracted with diethyl ether (2 x 100 mL). The light pink aqueous layer was discarded and the dark pink/red ether layer kept in the separatory funnel, to which was added ice cold 10 wt. % NaOH (100 mL). Upon formation of distinct phases, the aqueous layer was removed and further washed with Et₂O (3 x 100 mL). The aqueous layer was again acidified (pH < 2) with 12 N HCl and the dithiobenzoic acid extracted into ether (2 x 100 mL) while discarding the aqueous layer. The Et₂O layer was dried over MgSO₄ and the solvent removed via rotary evaporation to give 21 as a purple oil (3.47 g, 47%).
Bis(thiobenzoyl)disulfide 22

Dithiobenzoic acid 21 (3.47 g, 22.5 mmol) was dissolved in 2 N NaOH (150 mL) and transferred to a 1 L round bottomed flask equipped with stir bar. A solution of K₃FeCN₆ (15 g, mmol) in H₂O (300 mL) was added dropwise over 1 h followed by stirring at room temperature for an additional hour. The resulting solids were removed by filtration and the solvent removed by rotary evaporation. 22 was further purified by recrystallization from EtOH to give deep purple crystals (1.71 g, 57%).

4-Cyanopentanoic acid dithiobenzoate 23

A solution of bis(thiobenzoyl)disulfide 22 (1.71 g, 5.6 mmol) and 4,4’-azobis(4-cyanopentanoic acid) (2.35 g, 8.4 mmol) in EtOAc (100 mL) was prepared in a 3-necked round bottomed flask equipped with stir bar and condenser. The reaction was degassed by purging with N₂ for 40 min prior to heating to reflux (77 °C) for 18 h. The reaction was quenched by exposure to air and freezing in liquid N₂ followed by solvent removal via rotary evaporation. The crude RAFT agent was purified by column chromatography (59:40:1 EtOAc:hexanes:AcOH). To remove residual AcOH, column fractions containing 23 were combined and transferred to a separatory funnel and washed with 0.05 N HCl (2 x 150 mL), brine (1 x 150 mL) and dried over MgSO₄ followed by solvent removal by
rotary evaporation to give 23 as a purple solid (2.22 g, 71%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.91 (d, 2H), 7.57 (t, 1H), 7.40 (t, 2H), 2.77-2.40 (m, 4H), 1.94 (s, 3H).

Sodium ethyl trithiocarbonate 24

A suspension of NaH (2.11 g, 83.5 mmol) in anhydrous diethyl ether (150 mL) was cooled to 0 °C using an ice bath; ethane thiol (5.73 g, 92.3 mmol) was then added dropwise over 15 min accompanied by vigorous evolution of hydrogen gas. The reaction was stirred for an additional 15 min at 0 °C followed by dropwise addition of CS$_2$ (7.03 g, 92.3 mmol) over 5 min and the reaction mixture stirred for an additional 60 min at room temperature and then diluted with pentane (100 mL). The resulting yellow precipitate was isolated by vacuum filtration before drying in-vacuo yielding 24 (12.07 g, 90%) as a hygroscopic yellow solid. $^1$H NMR (300 MHz, D$_2$O): $\delta$ 3.15 (q, 2H), 1.27 (t, 3H). $^{13}$C NMR $\delta$: 35.36, 12.39.

Bisethyl trithiocarbonate 25

Sodium ethyl trithiocarbonate 24 (7.74 g, 48.3 mmol) was dissolved in H$_2$O (50 mL) in a 250 mL round bottomed flask equipped with magnetic stir bar. A solution of I$_2$ (13.49 g, 53.1 mmol) and potassium iodide (8.82 g, 53.1 mmol) in H$_2$O (100 mL) was subsequently added dropwise over 30 min to the stirred solution of sodium ethyl trithiocarbonate. The reaction mixture was stirred for an additional 60 min and transferred to a separatory funnel and extracted with ether (3 x 75 mL). The ether layers
were combined and washed with H$_2$O (2 x 150 mL), 5% Na$_2$S$_2$O$_3$ (2 x 150 mL) and brine (1 x 150 mL) before drying over MgSO$_4$ followed by solvent removal by rotary evaporation to give 25 as a yellow oil (6.29 g, 95%). $^1$H NMR (300 MHz, CDCl$_3$): δ 3.30 (q, 4H), 1.35 (t, 6H).

4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid 26

A solution of bisethyl trithiocarbonate 25 (5.00 g, 18.2 mmol) and 4,4'-azobis(4-cyanopentanoic acid) (7.66 g, 27.3 mmol) in EtOAc (250 mL) was prepared in a 500 mL 3-necked flask equipped with stir bar and condenser. The solution was purged with N$_2$ for 40 min prior to heating at reflux for 18 h. The reaction was then quenched via exposure to air and cooled to room temperature. The solvent was removed by rotary evaporation and the crude RAFT agent purified by column chromatography (60:35:5 hexanes:EtOAc:AcOH). To remove residual AcOH, column fractions containing 26 were combined and transferred to a separatory funnel and washed with 0.05 N HCl (2 x 150 mL), brine (1 x 150 mL) and dried over MgSO$_4$ followed by solvent removal by rotary evaporation to give 26 as a yellow solid (7.10 g, 74%). $^1$H NMR (300 MHz, CDCl$_3$): δ 3.37 (q, 2H), 2.70 (t, 2H), 2.60-2.30 (m, 2H) 1.89 (s, 3H), 1.36 (t, 3H).

**General Procedure for Methacryloyl Sulfonamide Synthesis**

Using a modified procedure, sulfa drug (40.0 mmol) was dissolved in 160 mL of a 1:1 (v:v) mixture of acetone and 0.5 N aqueous NaOH and stirred while cooling in an ice bath. Methacryloyl chloride (4.10 mL, 42.0 mmol) was then added dropwise over 30 min followed by removing the ice bath and stirring the reaction at room temperature for an
additional 60 min. The acetone was removed by rotary evaporation, followed by adjusting the solution to pH = 2 with 6 N HCl. The resulting solids were isolated using vacuum filtration and washed with 100 mL of dilute HCl (0.01 N) prior to drying in-vacuo for 48 h, yielding the desired monomers as colorless to off-white solids. The synthesis of methacryloyl sulfadoxine 30 required the use of 240 mL of a 1:2 (v:v) mixture of acetone and 0.5 N aqueous NaOH.

*Methacryloyl sulfacetamide 27*

![Chemical structure of Methacryloyl sulfacetamide 27](image)

Yield: (10.29 g, 91%); mp 203-205 °C dec. $^1$H NMR (300 MHz, DMSO-d$_6$): δ 11.99 (s, 1H), 10.20 (s, 1H), 8.11 – 7.65 (m, 4H), 5.84 (s, 1H), 5.58 (s, 1H), 1.93 (s, 3H), 1.89 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-d$_6$) δ 168.69, 167.34, 143.72, 139.99, 133.13, 128.67, 121.03, 119.50, 23.22, 18.63.

*Methacryloyl sulfabenzamide 28*

![Chemical structure of Methacryloyl sulfabenzamide 28](image)
Yield: (12.89 g, 94%); mp 228-229 °C dec. $^1$H NMR (300 MHz, DMSO-d$_6$): δ 12.46 (s, 1H), 10.22 (s, 1H), 8.02 – 7.87 (m, 4H), 7.83 (d, $J = 7.2$ Hz, 2H), 7.60 (t, $J = 7.4$ Hz, 1H), 7.47 (t, $J = 7.6$ Hz, 2H), 5.84 (s, 1H), 5.58 (s, 1H), 1.93 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-d$_6$) δ 167.36, 165.38, 143.79, 139.99, 133.24, 133.15, 131.54, 128.93, 128.61, 128.40, 121.03, 119.50, 18.62.

*Methacryloyl sulfamethazine 29*

![Methacryloyl sulfamethazine 29](image)

Yield: (13.39 g, 97%); mp 234-235 °C dec. $^1$H NMR (300 MHz, DMSO-d$_6$): δ 11.47 (s, 1H), 10.10 (s, 1H), 7.86 (dd, $J = 28.3$, 8.6 Hz, 4H), 6.74 (s, 1H), 5.81 (s, 1H), 5.55 (s, 1H), 2.23 (s, 6H), 1.92 (s, 3H). $^{13}$C NMR (75 MHz, DMSO) δ 167.61, 156.65, 143.19, 140.43, 135.08, 129.49, 121.24, 119.42, 113.97, 23.37, 19.04.

*Methacryloyl sulfamethizole 30*

![Methacryloyl sulfamethizole 30](image)
Yield: (12.42 g, 91%); mp 215-217 °C dec. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 13.90 (s, 1H), 10.12 (s, 1H), 7.77 (dd, $J = 37.4, 8.4$ Hz, 4H), 5.82 (s, 1H), 5.56 (s, 1H), 2.44 (s, 3H), 1.92 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 167.80, 167.20, 154.46, 142.65, 140.03, 136.05, 126.71, 120.83, 119.70, 18.65, 16.10.

*Methacryloyl sulfadimethoxine* 31

Yield: (14.72 g, 97%); mp 216-218 °C dec. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.50 (s, 1H), 10.17 (s, 1H), 7.86 (m, 4H), 5.92 (s, 1H), 5.82 (s, 1H), 5.57 (s, 1H), 3.77 (s, 3H), 3.73 (s, 3H), 1.92 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 171.67, 167.30, 164.26, 159.90, 143.41, 139.98, 133.68, 128.30, 120.96, 119.64, 84.57, 54.54, 53.81, 18.59.

*Methacryloyl sulfadoxine* 32
Yield (14.10 g, 93%); mp 198-199 °C. \( ^1H \) NMR (300 MHz, DMSO-\( d_6 \)): \( \delta \) 11.09 (s, 1H), 10.17 (s, 1H), 8.12 (s, 1H), 5.84 (s, 1H), 5.59 (s, 1H), 3.90 (s, 3H), 3.70 (s, 3H), 1.95 (s, 3H). \( ^{13}C \) NMR (75 MHz, DMSO-\( d_6 \)) \( \delta \) 167.32, 161.63, 150.43, 143.14, 140.06, 134.61, 129.88, 128.61, 127.21, 129.92, 119.40, 60.28, 54.08, 18.64.

**General Procedure for RAFT Polymerization of Methacryloyl Sulfonamides**

Briefly, methacryloylsulfonamide (5.0 x 10\(^{-3}\) mol, 150 equiv), CTA (23 or 26) (3.3 x 10\(^{-5}\) mol, 1 equiv), initiator (V-70 or V-501) (6.7 x 10\(^{-7}\) mol, 0.2 equiv), and trimesic acid (50 mg, \( ^1H \) NMR internal standard) were combined in a 10 mL graduated cylinder and DMF added to bring the final solution volume to 5.0 mL ([M]\(_0\) = 1 M) or 6.0 mL (0.83 M) depending upon monomer solubility as indicated in Table 1. The solution was then transferred to a 10 mL test tube equipped with a magnetic stir bar and rubber septum followed by purging with \( N_2 \) for 40 mins. An initial aliquot (200 \( \mu \)L) was taken prior to heating the reaction vessel at the indicated temperature with subsequent aliquots taken at timed intervals and analyzed by \( ^1H \) NMR (DMSO-\( d_6 \)) to determine monomer conversion by comparing the relative integral areas of the trimesic acid aromatic protons (8.64 ppm, 3H) to the monomer vinyl proton (5.84 ppm, 1H). SEC-MALLS (95% DMF/5% CH\(_3\)COOH, 20 mM LiBr) was used to monitor the progression of molecular weight and molecular weight distribution (\( M_w/M_n \)) throughout each polymerization. Polymers isolated for solubility studies were purified by precipitating the reaction mixture into a 10-fold excess of MeOH followed by isolating the resulting solids by ultracentrifugation. The isolated polymers were precipitated a total of three times from DMF into MeOH before drying overnight *in-vacuo*. 
**Methacryloyl Sulfonamide Monomer Titrations**

Monomer stock solutions (1mM) were prepared by weighing each MSA (0.1 mmol) into separate 100 mL volumetric flasks, followed by the addition of 2.00 mL of 0.1 N NaOH (0.2 mmol) to each flask. Once the monomers were completely dissolved, DI H2O (18.2 MΩ resistance) was added to each volumetric flask to achieve a final volume of 100 mL. Twenty-five mL of each stock solution was transferred to a 100 mL beaker containing a stir bar and titrated against 0.05 N HCl in volume increments of 5 μL at 25 °C using a Metrohm 848 Titrino Plus autotitrator. All titrations were performed in triplicate.

**pH-dependent Polymer Solubility**

Polymer solutions were first prepared by dissolving each pMSA derivative (1 eq. sulfonamide, 2.5 x 10⁻⁵ mol sulfonamide functional groups) in 1.00 mL 0.05 N NaOH (2 eq., 5 x 10⁻⁵ mol)) followed by dilution with DI H₂O (18.2 MΩ resistance) to a final volume of 2.50 mL ([SO₂NH] = 10 mM). The polymer solution was transferred into a quartz cuvette and the solution pH adjusted incrementally by adding 1-10 μL of 0.2 N HCl followed by measuring the % transmittance at λ = 500 nm using a UV-Vis spectrophotometer.

**CO₂-dependent Polymer Solubility**

In a 20 mL vial equipped with magnetic stir bar and pierceable cap, pMSA (1 eq. sulfonamide, 2.0 x 10⁻⁵ mol sulfonamide functional groups) was dissolved in 400 μL of 0.05 N NaOH (1.25 eq., 2.5 x 10⁻⁵ mol) and subsequently diluted to a final volume of 3.00 mL ([SO₂NH] = 6.7 mM) with DI H₂O (18.2 MΩ resistance). CO₂-dependent polymer solubility was examined between purge cycles by transferring the solutions to a
quartz cuvette and measuring the percent transmittance at 500 nm. Purge cycles consisted of purging the solution with CO$_2$ for 10 s or N$_2$ for 25 min.

Chapter IV Section 2 Experimental

*N-Phenylmethacrylamide* 33

Methacryloyl chloride (11.83 mL, 121 mmol) was added dropwise over 15 min to a stirred solution of aniline (12.00 g, 121 mmol) and triethylamine (12.86 g, 127 mmol) in CH$_2$Cl$_2$ (250 mL) that was previously cooled using an ice bath. Upon complete addition of methacryloyl chloride, the reaction was stirred at 0 °C for 30 min followed by stirring at room temperature for an additional 60 min. The reaction mixture was then transferred to a separatory funnel and washed with 0.1 N HCl (1 × 200 mL), saturated NaHCO$_3$ (1 × 200 mL), and saturated NaCl (brine) (1 × 200 mL) before drying over MgSO$_4$. The solvent was removed via rotary evaporation and the isolated solids recrystallized from hot hexanes:THF (95:5) to yield 33 (17.52 g, 90%) as colorless needle-like crystals. mp 80–81 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.60 (s, 1H), 7.57 (d, 2H), 7.32 (t, 2H), 7.11 (t, 1H), 5.78 (s, 1H), 5.44 (s, 1H), 2.05 (s, 3H). $^{13}$C NMR (CDCl$_3$): $\delta$ 166.87, 141.05, 137.95, 129.15, 124.57, 120.24, 120.04, 18.96.

*N-Benzylmethacrylamide* 34

![](image1.png)

![](image2.png)
A synthetic procedure analogous to that described for 33 was used to prepare N-benzylmethacrylamide. The product was recrystallized from hot hexanes:THF (90:10) to yield 34 (17.88 g, 91%) as colorless needle-like crystals. mp 78-79 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.30 (m, 5H), 6.13 (s, 1H), 5.71 (s, 1H), 5.34 (s, 1H), 4.50 (d, 2H), 1.97 (s, 3H). $^{13}$C NMR (CDCl$_3$): δ 168.43, 140.03, 138.41, 128.87, 127.97, 127.67, 119.89, 43.86, 18.89.

*Sodium dodecyl trithiocarbonate 35*

![Image of sodium dodecyl trithiocarbonate 35]

1-Dodecanethiol (15.0 g, 74.1 mmol) was added dropwise over 30 min to a stirred suspension of NaH (1.68 g, 70.0 mmol) in anhydrous diethyl ether (350 mL) resulting in slow evolution of hydrogen gas. The reaction mixture was vented and stirred overnight (12 h) at room temperature after which carbon disulfide (5.64 g, 74.1 mmol) was added dropwise over 10 min, followed by stirring at room temperature for 60 min. The reaction mixture was subsequently diluted with pentane (100 mL) and the solids isolated by vacuum filtration and further dried *in-vacuo* to yield 35 (18.55 g, 83%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-d$_6$): δ 2.97 (t, 2H), 1.49 (m, 2H), 1.23 (b, 18H), 0.85 (t, 3H). $^{13}$C NMR (DMSO-d$_6$): δ 31.79, 29.55, 29.51, 29.27, 29.21, 29.14, 22.59, 14.45.

*Bisdodecyl trithiocarbonate 36*

![Image of bisdodecyl trithiocarbonate 36]
To a suspension of sodium dodecyl trithiocarbonate 35 (18.55 g, 61.7 mmol) in diethyl ether (200 mL) at room temperature was added solid I$_2$ (8.62 g, 34.0 mmol) over 5 min. The reaction was stirred for 60 min at room temperature followed by removal of the precipitated NaI salts by vacuum filtration. The filtrate was transferred to a separatory funnel and washed with 5% Na$_2$S$_2$O$_4$ (1 × 150 mL), H$_2$O (1 × 150 mL), and brine (1 × 150 mL) before drying over MgSO$_4$. The solvent was removed via rotary evaporation to yield 36 (16.36 g, 96%) as a yellow oil that solidified upon cooling to -10 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ 3.28 (t, 4H), 1.68 (m, 4H), 1.24 (b, 36H), 0.87 (t, 6H). $^{13}$C NMR (CDCl$_3$): δ 221.74, 38.52, 32.13, 29.85, 29.77, 29.64, 29.57, 29.31, 29.16, 27.57, 22.91, 14.36.

2-Cyano-2-propyl dodecyl trithiocarbonate 37

![Structure of 37](image)

Bisdodecyl trithiocarbonate 36 (7.88 g, 14.2 mmol) and AIBN (2.33 g, 14.2 mmol) were dissolved in EtOAc (250 mL) and the solution purged with N$_2$ for 40 min before heating to 77 °C. After 12 h, a degassed solution of AIBN (2.33 g, 14.2 mmol) in EtOAc (100 mL) was subsequently added and the reaction stirred for an additional 12 h at 77 °C. Purification by column chromatography (95:5 Hexanes:EtOAc) yielded 37 (7.36 g, 75%) as a yellow oil that solidified upon cooling to 0 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ 3.32 (t, 2H), 1.86 (s, 6H), 1.68 (m, 2H), 1.25 (b, 18H), 0.87 (t, 3H). $^{13}$C NMR (CDCl$_3$): δ 217.96, 120.66, 42.54, 37.13, 32.11, 29.82, 29.74, 29.62, 29.54, 29.27, 29.12, 27.91, 27.25, 22.90, 14.35.

2-(Ethylthiocarbonylthio)-2-methylpropionic acid 38
Ethane thiol (12.4 g, 0.200 mol), acetone (96.4 g, 1.66 mol), and trioctylmethylammonium chloride (aliquat 336) (3.23 g, 8.00 mmol) were charged into a 500 mL round bottomed flask equipped with magnetic stir bar and the solution cooled to 0-5 °C using an ice bath. 50 wt. % NaOH (16.8 g, 0.210 mol) was then added dropwise over 20 min, followed by the dropwise addition of a solution of carbon disulfide (15.2 g, 0.200 mol) in acetone (20.0 g, 0.344 mol) over 20 min. The reaction mixture was stirred at 0 °C for 20 min, upon which chloroform (35.8 g, 0.300 mol) was added in a single portion immediately followed by the dropwise addition of 50 wt. % NaOH (80.0 g, 1.00 mol) over 30 min. The ice bath was then removed and the reaction mixture stirred overnight (18 h) at room temperature, after which the remaining acetone was removed by rotary evaporation. The resulting viscous residue was diluted with 250 mL H₂O and cooled with an ice bath before acidification with 12 N HCl to pH = 2. Upon acidification, the aqueous component was transferred to a separatory funnel and extracted with hexane (4 x 250 mL) and the aqueous layer discarded. The combined hexane layers were subsequently washed with 0.1 N HCl (1 x 250 mL), brine (1 x 250 mL), and dried over Na₂SO₄ before isolating the crude product by rotary evaporation. Purification by column chromatography (60:38:2 Hexanes:EtOAc:AcOH, Rᵢ = 0.33) yielded a dark yellow oil that was further purified by recrystallization from hot hexanes to give 38 (16.01 g, 36%) as bright yellow crystals. ¹H NMR (300 MHz, CDCl₃) δ 3.31, 1.73, 1.34.

2-Bromoisobutyranilide 39
2-Bromoisobutyryl bromide (15.00 mL, 124 mmol) was added dropwise over 15 min to a stirred solution of aniline (12.03 g, 124 mmol) and triethylamine (12.28 g, 124 mmol) in CH$_2$Cl$_2$ (500 mL) that was previously cooled using an ice bath. Upon complete addition of 2-bromoisobutyryl bromide, the reaction was stirred at 0 °C for 30 min followed by stirring at room temperature for an additional 60 min. The reaction mixture was then transferred into a separatory funnel and washed with 0.1 N HCl (1 × 400 mL), saturated NaHCO$_3$ (1 × 400 mL), and brine (1 × 400 mL) before drying over MgSO$_4$ and removal of the solvent by rotary evaporation. The isolated solids were recrystallized from hot hexanes to yield 39 (29.13 g, 97%) as colorless needle-like crystals. mp 82-23 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ 8.45 (s, 1H), 7.55 (d, 2H), 7.35 (t, 2H), 7.14 (t, 1H), 2.04 (s, 6H). $^{13}$C NMR (CDCl$_3$): δ 170.12, 137.53, 129.24, 125.06, 120.11, 63.37, 32.75.

$N$-phenyl-2-(ethylsulfanylthiocarbonylsulfanyl)propanamide 40

A solution of sodium ethyl trithiocarbonate 24 (1.32 g, 8.3 mmol), 2-bromoisobutyrylanilide 39 (2.00 g, 8.3 mmol), and NaI (0.124 g, 0.83 mmol) in absolute EtOH (10 mL) was prepared and stirred at 22 °C for 48 h. The reaction mixture was then precipitated twice into water (100 mL) and the precipitate isolated by vacuum filtration and further purified by recrystallization from absolute EtOH resulting in large needle-like crystals. The product, isolated by recrystallization, was determined to be 5,5-dimethyl-3-
phenyl-2-thioxothiazolidin-4-one (40). Conducting the reaction at 60 °C for 12 h allowed for isolation of 40 in higher yields for use in additional studies. mp 108-110 °C.

$^1$H NMR (300 MHz, CDCl$_3$): δ 7.50 (m, 3H), 7.22 (d, 2H), 1.79 (s, 6H). $^{13}$C NMR (CDCl$_3$) δ 199.80, 179.64, 135.43, 129.82, 129.70, 128.54, 55.59, 27.55.

**Synthesis of 41**

![Synthesis of 41](image)

A solution of 2-cyano-2-propyl dodecyl thriothiocarbonate 37 (3.41 g, 9.9 mmol), 33 (1.59 g, 9.9 mmol), and V-70 (0.609 g, 2.0 mmol) in DMF (35 mL) was prepared in a round bottomed flask equipped with magnetic stir bar and the reaction degassed via three freeze-pump-thaw cycles and backfilled with argon. The reaction mixture was heated at 30 °C in an oil bath for 48 h, followed by exposure to air and freezing in liquid N$_2$. The reaction mixture was then diluted with EtOAc (250 mL) and transferred to a separatory funnel and washed with 75% brine (1 × 200 mL), H$_2$O (1 × 200 mL), brine (1 × 200 mL), and dried over MgSO$_4$. The crude product was then purified by column chromatography (8:2 Hexanes:EtOAc, R$_f$ = 0.30) yielding 41 (0.511 g, 10%) as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$): δ 8.44 (s, 1H), 7.45 (d, 2H), 7.32 (t, 2H), 7.12 (t, 1H), 3.26 (t, 2H), 2.55 (q, 2H), 2.02 (s, 3H), 1.64 (m, 2H), 1.47 (s, 3H), 1.46 (s, 3H), 1.23 (b, 18H), 0.87 (t, 3H). $^{13}$C NMR (CDCl$_3$): δ 219.02, 169.43, 137.45, 129.02, 124.83, 120.51, 60.48, 45.13, 37.33, 31.91, 30.45, 29.62, 29.54, 29.42, 29.35, 29.10, 29.07, 28.90, 28.69, 27.62, 23.81, 22.70, 14.15.

**Synthesis of 42**
A solution of 2-cyano-2-propyl dodecyl thio dicarbonate 37 (2.00 g, 5.8 mmol), 34 (1.01 g, 5.8 mmol), and AIBN (0.190 g, 1.2 mmol) in DMF (20 mL) was prepared in a round bottomed flask equipped with magnetic stir bar and degassed via three freeze-pump-thaw cycles and backfilled with argon. The reaction mixture was heated at 60 °C in an oil bath for 24 h, followed by exposure to air and freezing in liquid N2. The reaction mixture was then diluted with EtOAc (150 mL) and transferred to a separatory funnel and washed with 75% brine (1 × 150 mL), H2O (1 × 150 mL), brine (1 × 150 mL), and dried over MgSO4. The crude product was first purified by column chromatography (8:2 Hexanes:EtOAc, Rf = 0.25) followed by recrystallization from MeOH:H2O (98:2) at -10 °C yielding 42 (0.475 g, 16%) as yellow crystals. 

\[
{^1}H \text{ NMR (300 MHz, CDCl}_3\text{): } \delta \ 7.31 \text{(m, 5H), 6.79 (t, 1H), 4.42 (q, 2H), 3.25 (t, 2H), 2.56 (q, 2H), 1.96 (s, 3H), 1.64 (m, 2H), 1.47 (s, 3H), 1.45 (s, 3H), 1.27 (b, 18H), 0.89 (t, 3H).}
\]

\[
{^{13}}C \text{ NMR (CDCl}_3\text{): } \delta \ 219.32, 171.33, 137.62, 128.87, 128.38, 127.84, 125.04, 60.16, 45.04, 37.28, 32.12, 30.66, 29.84, 29.76, 29.64, 29.55, 29.47, 29.29, 29.14, 28.72, 27.87, 24.16, 22.90, 14.35.
\]

**RAFT Polymerization of 33 and 34**

Briefly, monomer (33 or 34) (10.0 mmol, 200 equiv), 37 (5.0 × 10⁻⁵ mol, 1 equiv), initiator (V-70 or V501) (1.0 × 10⁻⁵ mol, 0.2 equiv), and trimesic acid (50 mg, \(^1\)H NMR internal standard) were combined in a 10 mL graduated cylinder and DMF added to bring the final solution volume to 5.0 mL ([M]₀ = 2 M). The solution was then transferred to a 10 mL test tube equipped with a magnetic stir bar and rubber septum, degassed via three
freeze-pump-thaw cycles, and backfilled with argon. An initial aliquot (200 μL) was taken prior to heating the reaction vessel at the indicated temperature with subsequent aliquots taken at timed intervals and analyzed by $^1$H NMR (DMSO-d$_6$) to determine monomer conversion by comparing the relative integral areas of the trimesic acid aromatic protons (8.64 ppm, 3H) to the vinyl proton of 33 (5.86 ppm, 1H) or 34 (5.79 ppm, 1H). SEC-MALLS (DMF 20 mM LiBr) analysis of aliquots was used to monitor molecular weight and molecular weight distribution progression throughout each polymerization.

**Trithiocarbonate Degradation Analysis by UV-Vis Spectroscopy**

Reactions (final volume = 2500 μL) were performed using $[37]_0 = 5 \times 10^{-3}$ M and $[M]_0:[37]_0:[V501]_0 = 10:1:0.2$ in DMF. A typical procedure was as follows: 34 (250 μL of an 87.6 mg/mL stock soln. in DMF, 10 equiv), 37 (250 μL of a 17.3 mg/mL stock soln. in DMF, 1 equiv), V501 (25 μL of a 28.0 mg/mL stock soln. in DMF, 0.2 equiv), and DMF (1975 μL) were combined in a 4 mL test tube equipped with magnetic stir bar and rubber septum. The reaction was then degassed via 4 freeze-pump-thaw cycles and backfilled under argon. An initial aliquot (50 μL) was taken using an argon-purged gastight syringe and subsequently diluted into a quartz cuvette containing 2500 μL of acetonitrile before measuring the absorbance at $\lambda = 320$ nm using a Lambda 35 UV-vis spectrometer. Subsequent aliquots (50 μL) were taken and analyzed in the same manner.

**In situ $^1$H NMR Analysis**

Samples of 41 and 42 ($2 \times 10^{-2}$ M) in DMF-d$_7$ were prepared immediately prior to analysis by first adding DMF-d$_7$ (0.60 mL) into an NMR tube equipped with pierceable rubber septum and the solvent degassed by two freeze-pump-thaw cycles to remove
possible traces of dimethyl amine. The appropriate amount of 41 or 42 was then added as a solid directly into the NMR tube containing the previously degassed DMF-d$_7$ and the resulting solution degassed by two additional freeze-pump-thaw cycles and backfilled with argon. $^1$H NMR spectra were acquired at 70 °C using a Bruker Ascend™ 600 MHz spectrometer.

Chapter IV Section 3 Experimental

Reactions of N- and P-based Nucleophiles with N-Methylmaleimide

A solution of N-methylmaleimide (25.0 mg, 2.23 × 10$^{-4}$ mol, 10 eq.) and CH$_2$Cl$_2$ (10 μL, $^1$H NMR internal standard) in DMSO-d$_6$ (1.00 mL) was prepared in an NMR tube in the presence of air. An initial $^1$H NMR spectrum was taken upon which the appropriate nucleophile (2.23 × 10$^{-5}$ mol, 1 eq.) was added to the NMR tube and the solution mixed by inverting three times. Subsequent spectra were taken at timed intervals and the fractional change in maleimide concentration measured by comparing the relative integrated peak areas of the maleimide olefin protons (DMSO-d$_6$, 7.02 ppm, 2H) to the CH$_2$Cl$_2$ protons (DMSO-d$_6$, 5.76 ppm, 2H).

Reaction of Ethyl 2-Mercaptopropionate with N-Methylmaleimide

A solution of N-methylmaleimide (25.0 mg, 2.23 × 10$^{-4}$ mol, 10 eq.), triethylamine (3.13 μL, 2.23 × 10$^{-5}$ mol 1.0 eq.) and CH$_2$Cl$_2$ (10 μL, $^1$H NMR internal standard) in DMSO-d$_6$ (1.00 mL) was prepared in an NMR tube in the presence of air. An initial $^1$H NMR spectrum was taken upon which ethyl 2-mercaptopropionate () was added to the NMR tube and the solution mixed by inverting three times. Subsequent spectra were taken at timed intervals and the fractional change in maleimide concentration
measured by comparing the relative integrated peak areas of the maleimide olefin protons (DMSO-d$_6$, 7.02 ppm, 2H) to the CH$_2$Cl$_2$ protons (DMSO-d$_6$, 5.76 ppm, 2H).

*Synthesis of 3-Benzylsulfonyl-1-Methylmaleimide 43*

An initially colorless solution of benzylmercaptan (2.64 g, 21.2 mmol) and N-methylmaleimide (2.36 g, 21.2 mmol) in MeCN (50 mL) was first prepared at room temperature followed by the addition of TEA (0.281 mL, 2.12 mmol) via syringe. The resulting red solution was stirred at room temperature for 30 min followed by quenching with acetic acid (1.0 mL) resulting in the solution becoming colorless. The solvent was removed by rotary evaporation and the crude reaction mixture redissolved in diethyl ether (100 mL) and washed with 0.1 M HCl (100 mL), H$_2$O (100 mL), and saturated NaCl (100 mL). The product was further purified by column chromatography (65:35 hexanes:EtOAc, $R_f = 0.35$), yielding 43 (4.55 g, 91%) as a colorless viscous oil that solidified into a waxy solid after 7 days; mp 47-52 °C. $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 7.31 (m, 4H), 7.24 (m, 1H), 3.93 (dd, $J = 62.3$, 13.1 Hz, 2H), 3.76 (dd, $J = 9.0$, 3.9 Hz, 1H), 3.06 (dd, $J = 18.5$, 9.0 Hz, 1H), 2.78 (s, 3H), 2.45 (dd, $J = 18.5$, 3.9 Hz, 1H).

*Reaction of 43 with N-Methylmaleimide.*

A solution of N-methylmaleimide (25.0 mg, $2.23 \times 10^{-4}$ mol, 10 eq.), 43 (5.29 mg, $2.23 \times 10^{-5}$ mol, 1.0 eq.), and CH$_2$Cl$_2$ (10 μL, $^1$H NMR internal standard) in DMSO-d$_6$ (1.00 mL) was prepared in an NMR tube in the presence of air. An initial $^1$H NMR spectrum was taken upon which TEA (3.13 μL, $2.23 \times 10^{-5}$ mol, 1 eq) was added to the NMR tube and the solution mixed by inverting three times. Subsequent spectra were
taken at timed intervals and the fractional change in maleimide concentration measured by comparing the relative integrated peak areas of the maleimide olefin protons (DMSO-d$_6$, 7.02 ppm, 2H) to the CH$_2$Cl$_2$ protons (DMSO-d$_6$, 5.76 ppm, 2H).

*Hydrogen-Deuterium Exchange Kinetics of 43*

NMR Spectra were recorded with a Bruker Ascend™ 600 MHz spectrometer at 23 °C. Briefly, a solution of 7 (10.0 mg, 4.25 × 10$^{-5}$, 1 eq) and D$_2$O (100 μL, 5.54 mmol, 130 eq) in DMSO-d$_6$ (0.600 mL) was prepared in an NMR tube in the presence of air and an initial $^1$H NMR spectrum was acquired (t = 0 min). TEA (5.93 μL, 1 eq) was then added to the NMR tube and the solution mixed by inverting three times. Subsequent spectra were acquired at timed intervals and the fractional change in peak area ($A_t/A_0$) of protons H$_a$ (3.80 – 3.75 ppm, 1H), H$_b$ (3.10 – 3.00 ppm, 1H), and H$_c$ (2.50 – 2.40 ppm, 1H) were measured relative to the peak area of the benzylsulfanyl aromatic protons (7.30 - 7.20 ppm, 5H).

*2-cyano-2-propyl benzodithioate 44*

A solution of bis(thio benzoyl)disulfide 22 (1.00 g, 3.3 mmol) and AIBN (1.07 g, 6.6 mmol) in EtOAc (40 mL) was prepared in a 3-necked round bottomed flask equipped with stir bar and condenser. The reaction was degassed by purging with N$_2$ for 40 min prior to heating to reflux (77 °C) for 18 h. The reaction was quenched by exposure to air and freezing in liquid N$_2$ followed by solvent removal via rotary evaporation. The crude RAFT agent was purified by column chromatography (9:1 hexanes:EtOAc, R$_f$ = 0.25) to
give 44 as a dark red oil that solidified upon cooling to -10 °C (0.87 g, 60%). $^1$H NMR (200 MHz, CDCl$_3$): δ 7.90 (d, 2H), 7.57 (t, 1H), 7.40 (t, 2H), 1.94 (s, 6H).

**Synthesis of pDMA-CPDB 45**

N,N-dimethylacrylamide (28.0 g, 282 mmol), 2-cyano-2-propyl benzodithioate 44 (298.0 mg, 1.34 mmol), AIBN (44.1 mg, 0.27 mmol), and benzene (100 mL) were combined in a 250 mL round bottomed flask equipped with magnetic stir bar and sealed with a rubber septum before purging with N$_2$ for 45 mins. The reaction vessel was then heated in an oil bath at 60 °C for 5 h, upon which the reaction was quenched via exposure to air and freezing in liquid nitrogen. The solvent was removed by rotary evaporation and the polymer precipitated 4 times into pentane, redissolving in a minimal amount of CH$_2$Cl$_2$ between precipitations. The final product 45 was dried overnight *in-vacuo* before characterizing via $^1$H NMR (D$_2$O) and SEC-MALLS (DMF 20 mM LiBr). $M_n$(NMR) = 3220 g/mol, $M_n$(SEC) = 3360 g/mol, $\bar{D} = 1.06$.

**N-Benzylmaleimide 46**

A solution of maleic anhydride (20.00 g, 204 mmol) in anhydrous diethyl ether (250 mL) was first prepared at room temperature in a 3-necked 1L round bottom flask equipped with magnetic stir bar, condenser, and addition funnel. A solution of
benzylamine (21.86 g, 204 mmol) in anhydrous diethyl ether (100 mL) was added dropwise via addition funnel over 30 mins such that the exothermic reaction produced a mild reflux of the solvent. The reaction mixture was stirred for 1 h at room temperature before isolating the resulting solids by vacuum filtration followed by washing with anhydrous diethyl ether (100 mL). The resulting N-benzylmaleamic acid intermediate was dried in-vacuo and used without further purification (40.70 g, 97%).

N-benzylmaleamic acid (40.70 g, 198 mmol) was added as a solid to a stirred solution of acetic anhydride (90.00 g, mmol) and anhydrous sodium acetate (13.00 g, mmol) and the reaction heated at 100 °C for 30 min resulting in formation of a dark brown homogenous solution. The reaction mixture was then poured into a vigorously stirred solution of ice cold water (600 mL) and stirred for 30 min. The resulting brown precipitate was isolated by vacuum filtration and washed with water (3 × 100 mL). The solids were resuspended in water (500 mL) and stirred vigorously for 30 min before isolation again by vacuum filtration. The crude compound was further purified by recrystallization from EtOH:H₂O (2:1, v:v) to afford **46** (27.02 g, 73%) as fine beige crystals; mp 67-69 °C. ¹H NMR (300 MHz, CDCl₃): δ 7.22 (b, 5H), 6.64 (s, 2H), 4.61 (s, 2H). ¹³C NMR (CDCl₃): δ 170.41, 136.17, 134.19, 128.69, 128.38, 127.86, 41.42.

**Simultaneous “One-Pot” Aminolysis/Thiol-Maleimide End Group Modification of 45 with 46 (Method 1)**

A representative procedure is as follows: **45** (100.0 mg, 3.10 × 10⁻⁵ mol, 1 eq) and N-benzylmaleimide (29.0 mg, 1.55 × 10⁻⁴ mol, 5 eq) were measured into a 5 mL test tube equipped with rubber septum and dissolved in 1.00 mL of DMSO. The reaction mixture was degassed via three freeze-pump-thaw cycles and backfilled with argon. 100 μL of a
solution of hexylamine in DMSO (102 μL/mL, 7.75 × 10⁻⁵ mol, 2.5 eq) and 100 μL of a
solution of DBU in DMSO (40.0 μL/mL, mol, 1 eq) were then added via gas tight syringe
and the reaction stirred for 12 h at room temperature (23 °C). End-modified pDMA was
purified by precipitation into diethyl ether (3 × 50 mL) and dried overnight in-vacuo. End
group analysis was performed using ¹H NMR (D₂O) by comparing the integrated peak
area of the benzyl aromatic protons (7.50 - 7.15 ppm, 5H) to the integrated peak area of
the pDMA N,N-dimethyl side chain and methyne backbone protons (3.30 – 2.20 ppm,
213.22H). NMR samples were filtered through a 0.20 μm Millex PTFE filter prior to
analysis.

Sequential “One-Pot” Aminolysis/Thiol-Maleimide End Group Modification of 45 with
46 (Method 2)

A representative procedure is as follows: 45 (100.0 mg, 3.10 × 10⁻⁵ mol, 1 eq) and
trimethylphosphite (18.3 μL, 1.55 × 10⁻⁴ mol, 5 eq) were measured into a 5 mL test tube
equipped with rubber septum and dissolved in 1.00 mL of DMSO. The reaction mixture
was degassed via three freeze-pump-thaw cycles and backfilled with argon. 100 μL of a
solution of hexylamine in DMSO (102 μL/mL, 7.75 × 10⁻⁵ mol, 2.5 eq) was then added
via gas tight syringe and the reaction stirred for 30 min at room temperature (23 °C) upon
which a previously degassed solution of 46 (29.0 mg, 1.55 × 10⁻⁴ mol, 5 eq) in DMSO
(0.5 mL) was added and the reaction stirred for 12 h at room temperature. End-modified
45 was purified by precipitation into diethyl ether (3 × 50 mL) and dried overnight in-vacuo. End group analysis was performed using ¹H NMR (D₂O) by comparing the
integrated peak area of the benzyl aromatic protons (7.50 - 7.15 ppm, 5H) to the
integrated peak area of the 45 N,N-dimethyl side chain and methyne backbone protons
NMR samples were filtered through a 0.20 μm Millex PTFE filter prior to analysis.

Chapter IV Section 4 Experimental

tert-butyl 2-aminoethylcarbamate 47

To a 1 L flask equipped with a magnetic stir bar was added 1,2-diaminoethane (7.7 mL, 114 mmol) and dichloromethane (450 mL). The solution was cooled using an ice bath, upon which a solution of di-tert-butyl dicarbonate (5.00 g, 22.9 mmol) in dichloromethane (50 mL) was added dropwise via addition funnel over 2 h. Following complete addition, the ice bath was removed and the reaction stirred at room temperature for 12 h, after which the solution was filtered of solids and the filtrate concentrated via rotary evaporation to ~250 mL. The filtrate was then washed with half saturated brine (3 x 150 mL), brine (1 x 150 mL), dried using MgSO₄, and the solvent removed via rotary evaporation to yield the desired compound 47 as a colorless oil (3.25 g, 89%). ¹H NMR (300 MHz, CDCl₃): δ 3.12 (dd, J = 11.6, 5.8 Hz, 2H), 2.75 (t, J = 5.9 Hz, 2H) 1.40 (s, 9H).

N-(methoxycarbonyl) maleimide 48

A solution of maleimide (7.00 g, 72.1 mmol) in dichloromethane (250 mL) was prepared and cooled using an ice bath. A solution of N-methylmorpholine (7.92 mL, 72.1 mmol) in dichloromethane (50 mL) was added dropwise to the solution of maleimide
over 10 min while stirring. Subsequently, a solution of methylchloroformate (6.69 mL, 86.5 mmol) in dichloromethane (50 mL) was added dropwise over 1 h, upon which the ice bath was removed and the reaction stirred for 1 h at room temperature. The heterogeneous solution was then poured into a separatory funnel and washed with 1 x 250 mL each of saturated NaHCO₃, water, and brine, before drying over MgSO₄. The solvent was removed via rotary evaporation and the product further dried in vacuo to yield 48 as an off white solid (10.26 g, 92%). ¹H NMR (300 MHz, CDCl₃): δ 6.80 (s, 2H), 3.88 (s, 3H).

tert-Butyl N-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamate 49

A solution of 47 (3.25 g, 20.3 mmol) in saturated NaHCO₃ (120 mL) was prepared and stirred at room temperature for 10 min before vacuum filtering to remove the resulting solids. The filtrate was subsequently cooled using an ice bath followed by the solid addition of finely ground N-(methoxycarbonyl) maleimide 48 (3.15 g, 20.3 mmol). After 30 min, the ice bath was removed and the reaction stirred at room temperature for an additional 30 min. The resulting precipitate was filtered, washed with 100 mL water, and dried overnight in vacuo to yield the desired compound 49 as a white solid (3.87 g, 79%). ¹H NMR (300 MHz, CDCl₃): δ 6.69 (s, 2H), 3.78 – 3.53 (m, 2H), 3.31 (dd, J = 11.1, 5.8 Hz, 2H), 1.38 (s, 9H).

tert-Butyl N-{2-[(2R,6S)-3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.0²⁶]dec-8-en-4-yl]ethyl} carbamate 50
A round bottomed flask equipped with a magnetic stir bar and condenser was charged with 49 (3.87 g, 16.1 mmol), freshly distilled furan (20 mL, 275 mmol), and benzene (80 mL) and heated to reflux for 12 h, upon which the reaction was concentrated via rotary evaporation and further dried in vacuo resulting in isolation of 50 as a white solid that required no further purification (4.65 g, mmol, 96%). ¹H NMR (300 MHz, CDCl₃): δ 6.49 (s, 2H), 5.24 (s, 2H), 3.75 – 3.51 (m, 2H), 3.28 (dd, J = 11.1, 5.3 Hz, 2H), 2.83 (s, 2H), 1.40 (s, 9H).

2-[(2R,6S)-3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.0²⁶]dec-8-en-4-yl]ethan-1-amonium trifluoroacetate 51

N-bocethyl oxanorbornene 50 (4.60 g, 14.9 mmol) was dissolved in dichloromethane (40 mL) and cooled using an ice bath. Trifluoroacetic acid (10 mL) was then added via syringe over 10 min followed by stirring of the reaction overnight (12 h) at room temperature. Diethyl ether (250 mL) was then added to the reaction and the heterogeneous solution cooled using an ice bath for 30 min before isolating the resulting precipitate by vacuum filtration. The isolated solids were further dried in vacuo, yielding 51 as a white solid (4.64 g, 97%). ¹H NMR (300 MHz, D₂O): δ 6.64 (s, 2H), 5.36 (s, 2H), 3.84 (t, J = 5.8 Hz, 2H), 3.23 (t, J = 5.9 Hz, 2H), 3.19 (s, 2H).
(2R,6S)-4-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]-10-oxa-4-azatricyclo[5.2.1.0\(^2,6\)]dec-8-ene-3,5-dione 52

Oxanorbornene ethylamine TFA 51 (4.72 g, 14.6 mmol) was dissolved in saturated NaHCO\(_3\) (200 mL) and cooled using an ice bath. The solution was stirred while purging with N\(_2\) for 10 min at 0 °C to dispel any dissolved CO\(_2\). Subsequently, finely ground N-(methoxycarbonyl) maleimide 48 (2.27 g, 14.6 mmol) was then added as a solid to the solution of 51, followed by stirring for 30 min at 0 °C, upon which the ice bath was removed and the reaction stirred for an additional 3 h at room temperature. The resulting precipitate was isolated via vacuum filtration and the filtrand washed with water (50 mL) before drying overnight in vacuo to yield the desired compound 52 as an off-white solid (2.09 g, 48%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta 6.65\) (s, 2H), \(6.47\) (d, \(J = 0.7\) Hz, 2H), \(5.19\) (t, \(J = 0.8\) Hz, 2H), \(3.84 - 3.59\) (m, 4H), \(2.81\) (s, 2H).

N-(3-bromopropyl)maleimide 53

3-Bromopropylamine hydrobromide (2.00 g, 9.1 mmol) was dissolved in saturated NaHCO\(_3\) (100 mL) and cooled using an ice bath. The solution was allowed to stir for 10 min at 0 °C while purging with N\(_2\) to dispel any dissolved CO\(_2\). Subsequently, finely ground N-(methoxycarbonyl) maleimide 48 (1.42 g, 9.1 mmol) was added as a solid to the solution of 3-bromopropylamine, followed by stirring for 30 min at 0 °C,
upon which the ice bath was removed and the reaction stirred for an additional 1 h at room temperature. The resulting precipitate was isolated via vacuum filtration and the filtrand washed with water (50 mL) before drying overnight in vacuo to yield the desired compound \( \text{53} \) as a white solid (1.23 g, 62%). Note: after ~1 hr, additional \( \text{53} \) formed in the filtrate as colorless plate-like crystals. This additional product was isolated by vacuum filtration followed by drying in vacuo. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 6.70 (s, 2H), 3.65 (t, \( J = 6.9 \) Hz, 2H), 3.34 (t, \( J = 6.6 \) Hz, 2H), 2.15 (p, \( J = 6.7 \) Hz, 2H).

\((2R,6S)-4-(3\text{-bromopropyl})-10\text{-oxa-4-azatricyclo[5.2.1.0\text{2,6}]dec-8-ene-3,5-dione} \text{54}\)

A round bottomed flask equipped with a magnetic stir bar and condenser was charged with 3-bromopropyl maleimide \( \text{53} \) (5.16 g, 23.7 mmol), freshly distilled furan (30 mL, 413 mmol), and benzene (150 mL) and heated to reflux for 12 h, upon which the reaction was concentrated via rotary evaporation and further dried in vacuo resulting in isolation of \( \text{54} \) as a white solid that required no further purification (6.16 g, 91%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 6.49 (s, 2H), 5.25 (s, 2H), 3.61 (t, \( J = 6.9 \) Hz, 2H), 3.31 (t, \( J = 6.7 \) Hz, 2H), 2.83 (s, 2H), 2.12 (p, \( J = 6.8 \) Hz, 2H).

\textit{Sodium Methanethiosulfonate} \textit{55}

Sodium methanesulfinate (5.00 g, 49 mmol) and elemental sulfur (1.57 g, 49 mmol) were combined in anhydrous methanol (500 mL) and heated to reflux. After 30 min, a homogenous solution was observed and the reaction cooled to room temperature,
followed by solvent removal via rotary evaporation. The resulting crude solid was triturated with absolute ethanol (2 x 30 mL) and further dried in vacuo yielding 55 as a white crystalline solid (6.27 g, 95%) m.p. 271-272 °C (lit. 272-273 °C) 1H NMR (300 MHz, D2O): δ 3.36 (s, 3H).

(2R,6S)-4-[3-(methanesulfonylsulfanyl)propyl]-10-oxa-4-azatricyclo[5.2.1.0^2,6]dec-8-ene-3,5-dione 56

Oxanorbornene propylbromide 54 (5.24 g, 18.3 mmol) and sodium methanethiosulfonate 55 (3.69 g, 27.5 mmol) were dissolved in DMF (40 mL) and heated at 50 °C for 18 h. Most of the solvent was removed via rotary evaporation and the crude product was dissolved in EtOAc (100 mL) and washed with water (2 x 50 mL) and brine (1 x 50 mL) before drying over MgSO4. The solvent was then removed in vacuo to yield the product 56 as a yellow solid (5.00 g, 86%). 1H NMR (300 MHz, CDCl3): δ 6.50 (s, 2H), 5.25 (s, 2H), 3.61 (t, J = 6.5 Hz, 2H), 3.31 (s, 3H), 3.07 (t, J = 7.3 Hz, 2H), 2.84 (s, 2H), 2.15 – 1.99 (m, 2H).

Synthesis of 57

Styrene (15.00 g, 120 eq.), 44 (246.0 mg, 1 eq.), and AIBN (29.6 mg, 0.15 eq.) were combined in a 25 mL Schlenk tube equipped with magnetic stir bar and sealed
before degassing by three freeze-pump-thaw cycles and backfilled under argon. The reaction vessel was then heated in an oil bath at 65 °C for 5.25 h, upon which the reaction was quenched via exposure to air and freezing in liquid nitrogen. The polymer was precipitated 3 times in to methanol, redissolving in a minimal amount of CH₂Cl₂ between precipitations. The final product 57 was dried overnight in-vacuo before characterizing via NMR and DMF SEC-MALLS. \( M_n(NMR) = 3090 \, \text{g/mol} \), \( M_n(SEC) = 2980 \, \text{g/mol} \), \( D = 1.03 \), \( dn/dc = 0.160 \, \text{mL/g} \).

Synthesis of 58

\[
\begin{align*}
\text{N,N-dimethylacrylamide (28.0 g, 282 mmol), 44 (298.0 mg, 1.34 mmol), AIBN (44.1 mg, 0.27 mmol), and benzene (100 mL) were combined in a 250 mL round bottomed flask equipped with magnetic stir bar and sealed with a rubber septum before purging with N₂ for 45 mins. The reaction vessel was then heated in an oil bath at 60 °C for 6 h, upon which the reaction was quenched via exposure to air and freezing in liquid nitrogen. The solvent was removed by rotary evaporation and the polymer precipitated 4 times into pentane, redissolving in a minimal amount of CH₂Cl₂ between precipitations. The final product 58 was dried overnight in-vacuo before characterizing via } ^1\text{H NMR (D}_2\text{O)} \text{ and SEC-MALLS (DMF 20 mM LiBr). } M_n(NMR) = 3700 \, \text{g/mol} \), \( M_n(SEC) = 3630 \, \text{g/mol} \), \( D = 1.05 \), \( dn/dc = 0.086 \, \text{mL/g} \).\\
\end{align*}
\]

End group functionalization of 57 with 52 (59)
57 (600 mg, $1.94 \times 10^{-4}$ mol, 1 eq), THF (2.00 mL) and trimethylphosphite (114.4 μL, $9.69 \times 10^{-4}$ mol, 5 eq) were measured into a 10 mL test tube equipped magnetic stir bar and sealed with a rubber septum. The reaction mixture was degassed via three freeze-pump-thaw cycles and backfilled with argon. Propylamine (39.9 μL, $4.85 \times 10^{-4}$ mol, 2.5 eq) was then added via gas tight syringe and the reaction stirred for 60 min at room temperature upon which a previously degassed solution of 52 (280.0 mg, $9.69 \times 10^{-4}$ mol, 5 eq) in THF (4.0 mL) was added and the reaction stirred for 12 h at room temperature. 59 was purified by precipitation into MeOH (3 × 50 mL) and dried overnight in-vacuo. End group analysis was performed using $^1$H NMR (acetone-d$_6$) by comparing the integrated peak area of the norbornene bridgehead methyne protons (5.25 – 4.95 ppm, 2H) to the integrated peak area of the polystyrene aromatic side chain protons (7.50 – 6.25 ppm, 137.92 H).

*End group functionalization of 57 with 56 (60)*

57 (500 mg, $1.6 \times 10^{-4}$ mol, 1 eq), 56 (307 mg, $9.7 \times 10^{-4}$ mol, 6 eq), and THF (2 mL) were combined in a 5 mL test tube equipped with magnetic stir bar and sealed with a rubber septum. The reaction mixture was degassed via three freeze-pump-thaw cycles and backfilled with argon. Propylamine (200 μL, $2.4 \times 10^{-3}$ mol, 15 eq) was then added
by gas-tight syringe and the reaction stirred for 12 h at room temperature. 60 was purified by precipitation into MeOH (3 x 50 mL) and dried overnight in-vacuo. End group analysis of 60 was performed in analogous fashion to that described for analysis of 58.

*End group functionalization of 58 with 52 (61)*

![Chemical Structure](attachment:image)

58 (500 mg, 1.35 x 10^-4 mol, 1 eq), THF (2.00 mL) and trimethylphosphite (79.5 μL, 7.74 x 10^-4 mol, 5 eq) were measured into a 10 mL test tube equipped magnetic stir bar and sealed with a rubber septum. The reaction mixture was degassed via three freeze-pump-thaw cycles and backfilled with argon. Hexylamine (35.4 μL, 4.85 x 10^-4 mol, 2.0 eq) was then added via gas tight syringe and the reaction stirred for 30 min at room temperature upon which a previously degassed solution of 52 (194.0 mg, 7.74 x 10^-4 mol, 5 eq) in THF (4.0 mL) was added and the reaction stirred for 12 h at room temperature. The reaction mixture was then dialyzed against water (3 x 200 mL), changing the water every hour. The aqueous solution was reclaimed from the dialysis bag and filtered through a 0.2 uM Millipore filter and then further dialyzed against MeOH (5 x 100 mL), changing the solvent every hour. 61 was subsequently isolated by rotary evaporation as a colorless solid. End group analysis was performed using 1H NMR (D2O) by comparing the integrated peak area of the norbornenyl olefin protons (6.65 – 6.55 ppm, 2H) to the integrated peak area of the pDMA N,N-dimethyl side chain and methyne backbone protons (3.30 – 2.20 ppm, 246.26H).
End group functionalization of \(58\) with \(56\) (62)

\[
\begin{array}{c}
\text{NC} \\
N \quad \text{O} \\
\text{S} \\
N \quad \text{O} \\
\end{array}
\]

\(58\) (500 mg, \(1.6 \times 10^{-4}\) mol, 1 eq), \(56\) (307 mg, \(9.7 \times 10^{-4}\) mol, 6 eq), and THF (2 mL) were combined in a 5 mL test tube equipped with magnetic stir bar and sealed with a rubber septum. The reaction mixture was degassed via three freeze-pump-thaw cycles and backfilled with argon. Propylamine (200 μL, \(2.4 \times 10^{-3}\) mol, 15 eq) was then added by gas-tight syringe and the reaction stirred for 12 h at room temperature. The reaction mixture was then dialyzed against water (3 x 200 mL), changing the water every hour. The aqueous solution was reclaimed from the dialysis bag and filtered through a 0.2 μM Millipore filter and then further dialyzed against MeOH (5 x 100 mL), changing the solvent every hour. \(62\) was isolated by rotary evaporation as an off-white solid. End group analysis of \(62\) was performed in analogous fashion to that described for analysis of \(61\).

Synthesis of 3rd Generation Grubbs Catalyst 19

Grubbs 2nd generation catalyst (500 mg, 0.12 mmol) was weighed into a scintillation vial containing a small magnetic stir bar followed by the addition of pyridine
(0.474 mL, 5.88 mmol) in the presence of air. After 5 min, pentane (20 mL) was added to the vial resulting in precipitation of a bright green solid. The vial was placed in the refrigerator (5 °C) overnight upon which the green 3rd generation Grubbs catalyst 19 was isolated by vacuum filtration and washed with pentane (20 mL) before drying in-vacuo. 19 was subsequently stored under argon in the dark at 5 °C. Yield: 400 mg, 93%.

**ROMP of RAFT-Derived Macromonomers 58, 59, 60, or 61**

A representative procedure is as follows: A stock solution of 19 (1 eq.) in CH₂Cl₂ (degassed by 4 x freeze-pump-thaw cycles and backfilled with argon) was measured into a vial that was previously degassed with argon. A separate vial containing 59 (100.0 mg, 50 eq.) was evacuated and backfilled with argon. Degassed CH₂Cl₂ (200 uL) was added to the vial of 59, upon which the solution was transferred by gas tight syringe to the vial of 19. The reaction was stirred for 6 h at room temperature before being terminated via the addition of one drop of ethyl vinyl ether followed by precipitation in MeOH.
CHAPTER IV
RESULTS AND DISCUSSION

Section 1. Tunable pH- and CO₂-Responsive Sulfonamide-Containing Polymers by RAFT Polymerization

Overview

Lately, extensive research efforts have been directed toward the synthesis of well-defined (co)polymers capable of rapid and reversible changes in solubility and/or conformation in response to external stimuli including pH,\textsuperscript{144,228,229} temperature,\textsuperscript{230,231} or ionic strength,\textsuperscript{232} among others.\textsuperscript{233–235} Of particular interest are “smart” nanocarriers for drug and gene delivery that exploit discrete changes in physiological pH to elicit the desired therapeutic effect.\textsuperscript{236–240} Designing such polymeric systems requires that the morphological transitions occur over a very narrow designated pH range. Commonly, this specificity is achieved by the selection of a monomer with a pK\textsubscript{a} at or near the target transition pH; however, polymer design is accordingly restricted by the limited choice in monomers and their respective pK\textsubscript{a} values. Consequently, a facile method of specifically tuning polymer pH-responsiveness while maintaining a narrow transition range is needed.

Scheme 4.1. pH-dependent solubility of pMSAs.
A number of attempts have been made to systematically vary the pH-responsiveness of polymers. One versatile approach towards modification of polymer pK$_a$ was reported by Ringsdorf in seminal work in which a library of sulfonamide-containing polymers derived from sulfa drugs was synthesized by classical free radical or Michael-addition techniques. Variation of the sulfonamide R-group afforded facile, tunable control over polymer pK$_a$ and subsequent pH-dependent solubility (Scheme 4.1). Recently, Bae and coworkers further demonstrated this versatility in pK$_a$ selection for a variety of polymer-based therapeutic applications. However, until now the uncontrolled nature of the polymerization methods used to prepare such polymers has limited the ability to attain well-defined polymer architectures with the specific molecular weights and narrow molecular weight distributions required for responsive nanotherapeutics.

Reversible-deactivation radical polymerization (RDRP) techniques such as nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT) polymerization have made possible the synthesis of (co)polymers of a wide variety of architectures with predictable molecular weights and narrow molecular weight distributions. In particular, RAFT has been used to directly polymerize a variety of cationic, anionic, and other functional monomers in organic or aqueous media without the necessity of protecting group chemistries or post-polymerization modification. The facility of polymerization and excellent functional group tolerance of RAFT polymerization have driven our current objectives of synthesizing sulfonamide-containing polymers in a controlled fashion.
In this chapter we report, to our knowledge, the first controlled RAFT polymerization of a library of methacryloyl sulfonamide (MSA) monomers possessing pKₐ values in the biologically relevant regime (pH = 4.5-7.4). In this work we show that temperature has a significant influence on the polymerization of MSAs, with lower reaction temperatures affording improved molecular weight control and functional chain end retention. Varying the sulfonamide R-group is shown to be an effective means of adjusting monomer pKₐ and subsequently the pH-dependent solubility of the resulting polymethacryloyl sulfonamides (pMSAs). During our study of the weakly acidic/basic nature of the MSA derivatives chosen, we found a remarkably facile and reversible CO₂-induced solubility transition in aqueous solutions. The demonstrated control over RAFT polymerization of MSAs now allows new routes for the synthesis of advanced polymer architectures with tunable pH- and CO₂-responsive properties for ultimate use in biological and therapeutic applications.

**RAFT Polymerization of Methacryloyl Sulfonamides (MSAs) at 70 °C**

The MSA monomers 27-32 (R-groups shown in Scheme 4.2) were targeted for this work based upon their respective pKₐ values (Table 4.3) that reside within the biologically relevant pH range of 4.5-7.4. Utilizing a modified literature procedure, high monomer yields (>90%) were obtained from the reaction of methacryloyl chloride and the appropriate sulfa drug precursor, as outlined in the experimental section.

Achieving controlled RAFT polymerization of a given monomer requires appropriate choice of CTA and polymerization conditions. Previously, our group successfully utilized the trithiocarbonate 4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (26) and the dithioester 4-
cyanopentanoic acid dithiobenzoate (23) to polymerize a wide variety of (meth)acrylamide monomers in aqueous or organic media in a controlled fashion.\textsuperscript{153,248}

Based on that work, we have investigated the RAFT polymerization of MSAs using 26 and 23 as outlined in Scheme 4.2. It is worth noting that although these monomers are water soluble, polymerizations were conducted in DMF in order to avoid CTA hydrolysis or aminolysis.\textsuperscript{119}

\textbf{Scheme 4.2.} Synthetic pathway for the 23- or 26-mediated RAFT polymerization of MSAs in DMF.

Initially, 26- and 23-mediated RAFT polymerizations of methacryloyl sulfacetamide (27) were carried out at 70 °C in DMF using V-501 as the initiator at molar ratios of [M]₀:[CTA]₀:[I]₀ = 150:1:0.2. As illustrated in Figure 4.1, a near linear pseudo-first-order kinetic plot is observed for the polymerization of 27 with 26 at 70 °C. After an initialization period of approximately 30 min, monomer conversion reached 81% after 600 min. The 23-mediated polymerization of 27 at 70 °C under analogous conditions was significantly slower, reaching only 12% monomer conversion after 600 min. Retardation in rate of dithiobenzoate-mediated polymerizations as compared to analogous reactions mediated by trithiocarbonates has been observed previously for styrenics, acrylates, and acrylamides with some monomers failing to polymerize in the presence of a dithiobenzoate RAFT agent.\textsuperscript{107,249}
Despite near ideal linear pseudo-first-order kinetic behavior, the 26-mediated polymerization of 27 at 70 °C produced polymers with $M_w/M_n$ of 1.27 or higher (Table 4.1). Similarly, the polymerization of 27 with 23 yielded polymers with $M_w/M_n > 1.20$. The increased conversions achieved during the 26-mediated polymerization of 27 prompted our use of this CTA to polymerize each monomer derivative in order to ascertain what influences the sulfonamide R-group might have on conversion, molar mass, and molecular weight distribution (Table 4.1). As with the 26-mediated polymerization of 27 at 70 °C, each substituted monomer derivative also yielded moderately broad molecular weight distributions, typically increasing with conversion, and indicative of limited polymerization control.

*Figure 4.1.* Kinetic plots for the 23- and 26-mediated RAFT polymerization of 27 at 70 °C in DMF ([M]₀:[CTA]₀:[I]₀ = 150:1:0.2).
Table 4.1

*Conversion, molar mass, and molecular weight distribution data for the RAFT polymerization of MSAs in DMF at 70 °C.*

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<th>conv.</th>
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<td>25 900</td>
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</tr>
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<td>600</td>
<td>67</td>
<td>38 500</td>
<td>27 100</td>
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</table>
Sulfonamide monomers were polymerized at 70 °C in DMF ([M]<sub>0</sub>:[CTA]<sub>0</sub>:[I]<sub>0</sub> = 150:1:0.2) using V-501 as the initiator.

Conversions were determined by <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) by comparing the relative integral areas of trimesic acid (internal standard) aromatic protons (8.64 ppm, 3H) to the vinyl proton of the sulfonamide monomer (5.84 ppm, 1H). Theoretical number average molecular weights were calculated according to the following equation: \( M_n = \rho \cdot MW_{mon} \cdot [M]/[CTA] + MW_{CTA} \), where \( \rho \) is the fractional monomer conversion, \( MW_{mon} \) is the molecular weight of the monomer, and \( MW_{CTA} \) is the molecular weight of the CTA.

As determined by SEC-MALLS (95:5 (v:v) DMF:CH<sub>3</sub>COOH 20 mM LiBr).

**Chain Extension of P<sub>27</sub> macroCTA at 70 °C**

The degree of “living” chain end retention was investigated by synthesizing and isolating a P<sub>27</sub> macroCTA using 26 (P<sub>27</sub>-26) (\( M_n = 7300 \text{ g/mol, } M_w/M_n = 1.35 \)), followed by chain extension with 27 to yield the corresponding chain extended polymer (P<sub>27-b-P<sub>27</sub>-26</sub>). Figure 4.2 shows the SEC traces of both the initial monomodal P<sub>27</sub>-26 macroCTA and the corresponding P<sub>27-b-P<sub>27</sub>-26</sub> polymer after chain extension with 27. The latter exhibits multimodality and broad molecular weight distribution, indicating extensive loss of “living” polymer chain ends during the initial polymerization of the P<sub>27</sub> macroCTA. Loss of “living” polymer chains is most often attributed to irreversible radical termination, undesirable chain transfer events, or degradation of the thiocarbonylthio chain ends. During the 23- and 26-mediated polymerizations of MSAs at 70 °C, we observed a loss of the characteristic color of 23 (pink) and 26 (yellow) after extended polymerization times, qualitatively indicating degradation of the dithiobenzoate and trithiocarbonate moieties, respectively.
RAFT Polymerization of Methacryloyl Sulfonamides at 30 °C

Hypothesizing that a deleterious side reaction was competing with chain extension during the CTA-mediated polymerization, we lowered the reaction temperature. Such approaches have been previously successful in RAFT polymerizations, yielding well-defined copolymers that maintained a high degree of chain end functionality. 158,250,251 Figure 4.3 shows the comparative SEC chromatograms of the 26-mediated polymerizations of 27 at 70 °C and 30 °C under the polymerization conditions outlined in Table 4.1. It should be noted that the 30 °C reaction utilized the low decomposition temperature initiator 2,2’-azobis(4-methoxy-2-4-dimethyl valeronitrile) (V-70). While both reactions produced polymers with similar number average molecular weights, the resulting molecular weight distribution of the polymer synthesized at 30 °C (58 % conversion, M_n = 29 700 g/mol, M_w/M_n = 1.05) was substantially lower than the polymer prepared at 70 °C (67% conversion, M_n = 26 400 g/mol, M_w/M_n = 1.41).
Figure 4.3. DMF SEC RI traces of P27 polymerized at 30 °C and 70 °C using V-70 and V-501, respectively.

Figure 4.4a shows the kinetic plots for the respective 26- and 23-mediated polymerizations of 27 at 30 °C. The former exhibited a longer pre-equilibrium (initialization) period (~60 min) as compared to polymerization at 70 °C; however, linear pseudo-first-order kinetic behavior was observed up to 600 min. Deviation from linearity at longer times in this particular case is possibly due to the reduced radical flux observed as the initiator concentration decreases substantially at prolonged reaction times, as we have previously reported.95 Figure 4.4b shows the SEC chromatogram overlay at specified times during the 30 °C polymerization of 27 with 26. The progression of the polymer traces to lower elution volumes with corresponding increases in RI intensity, without high molecular weight shouldering, is indicative of controlled polymerization behavior and thus maintenance of thiocarbonylthio functionality. This is further indicated by the narrow molecular weight distributions (Figure 4.4c) and linear progression of M_n vs. monomer conversion (Figure 4.4d) observed for the 30 °C polymerization of 27.
While $M_n$ increases in a linear fashion during the RAFT polymerization of 27 at 30 °C, experimentally determined molecular weights ($M_{nexp}$) are marginally higher than those theoretically predicted ($M_{ntheory}$) based upon monomer conversion. The higher than expected molecular weights determined by MALLS directly of aliquots taken from the polymerization could be indicative of irreversible coupling of CTA intermediate radicals during the initialization stage.\textsuperscript{106,252,253}

Table 4.2 summarizes the conversion, molar mass, and molecular weight distribution data for the RAFT polymerization of each MSA derivative in DMF at 30 °C using either 26 or 23 as the RAFT agent and V-70 as the initiator. Reducing the polymerization temperature to 30 °C results in Mₘₜ/Mₙ values typically below 1.10 for all monomer derivatives. Mₙ values determined by DMF SEC-MALLS are in reasonable agreement with theoretical values calculated from monomer conversion; however, Mₙexp exceeds Mₙtheory in a similar manner to that discussed earlier. Furthermore, all polymerizations conducted at 30 °C maintained the characteristic color of the parent CTA, indicating limited degradation as compared to that at 70 °C.

The 23-mediated polymerization of 27 conducted at 30 °C resulted in 34% monomer conversion after 710 min and narrow molecular weight distributions even after 1500 min of polymerization (53% conversion, Mₙ = 20 500 g/mol, Mₘₜ/Mₙ = 1.03) (Table
4.2) with the $M_n$ values determined by DMF SEC-MALLS agreeing well with the theoretical values. The analogous reaction conducted at 70 °C yielded 12% monomer conversion after 600 min and relatively broad molecular weight distributions ($M_n = 6200$ g/mol, $M_w/M_n = 1.27$) (Table 4.1). The strikingly higher rate of polymerization observed for the 23-mediated polymerization of 27 performed at 30 °C as compared to 70 °C is consistent with effectively minimizing (though not completely eliminating) competing dithioester degradation and limiting the accumulation of potentially rate-retarding degradation byproducts.

Table 4.2

<table>
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<th>conv. (%)</th>
<th>$[M]_0$ (mol/L)</th>
<th>$M_{\text{theory}}$ (g/mol)</th>
<th>$M_{\text{exp}}$ (g/mol)</th>
<th>$M_w/M_n$</th>
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Table 4.2 (continued).

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<th>conv.(^b) (%)</th>
<th>[M](_0) (mol/L)</th>
<th>M(_{\text{theory}})^c (g/mol)</th>
<th>M(_{\text{exp}})^d (g/mol)</th>
<th>M(_w)/M(_n)^d</th>
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<td>25 000</td>
<td>30 100</td>
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</tbody>
</table>

\(^a\)Sulfonamide monomers were polymerized at 30 °C in DMF ([M]\(_0\):[CTA]:[I] = 150:1.0:0.2) using V-70 as the initiator.

\(^b\)Conversions were determined by 1H NMR (DMSO-\(d_6\)) by comparing the relative integral areas of trimesic acid (internal standard) aromatic protons (8.64 ppm, 3H) to the vinyl proton of the sulfonamide monomer (5.84 ppm, 1H). \(^c\)Theoretical number average molecular weights were calculated according to the following equation: M\(_{\text{theory}}\) = (\(p\cdot\text{MW}_{\text{mon}}\)\[M]/[CTA]) + \(\text{MW}_{\text{CTA}}\) where \(p\) is the fractional monomer conversion, \(\text{MW}_{\text{mon}}\) is the molecular weight of the monomer, and \(\text{MW}_{\text{CTA}}\) is the molecular weight of the CTA.

\(^d\)As determined by SEC-MALLS (95:5 (v:v) DMF-CH\(_3\)COOH 20 mM LiBr).

**Chain Extension of P27macroCTA at 30 °C**

To further demonstrate the controlled RAFT polymerization of MSAs at low temperatures, a P27macroCTA was prepared at 30 °C using V-70 as the initiator and isolated before chain extending with additional 27 at 30 °C. Figure 4.5 shows the SEC chromatogram of the P27 macroCTA (M\(_n\) = 25 100 g/mol, M\(_w\)/M\(_n\) = 1.09) and a distinct decrease in elution volume of the chain-extended polymer (P27-b-P27) (M\(_n\) = 49 600 g/mol, M\(_w\)/M\(_n\) = 1.07). The monomodal SEC chromatogram and absence of low molecular weight tailing at higher elution volumes of the chain extended polymer is additional evidence of improved chain end retention during the polymerization of MSAs at 30 °C as compared to the analogous chain extension conducted at 70 °C (Figure 4.2).
**Figure 4.5.** SEC traces of P27 macroCTA (M_n = 25 100 g/mol, M_w/M_n = 1.09) and P27-b-P27 (M_n = 49 600 g/mol, M_w/M_n = 1.07) after chain extension in DMF. Both polymerizations were conducted at 30 °C.

*Methacryloyl Sulfonamide Monomer pK_a Studies*

MSA monomer titrations were performed to determine the pK_a of each monomer derivative after converting the respective sulfa drug precursors into the corresponding methacrylamides. The pK_a of the sulfonamide (SO_2NH) group of each monomer derivative was determined by equation 7 where pH_{EP1/2} is the pH corresponding to the half equivalence point (EP_{1/2}) of the titration curve. The volume of HCl titrant required to reach the EP_{1/2} (Vol_{EP1/2}) was determined by equation 8 where Vol_{EP} is the volume of HCl titrant required to reach the equivalence point of the titration curve, [SO_2NH] is the sulfonamide concentration, [HCl] is the concentration of HCl titrant used, and Vol_{sol} is the initial volume of the monomer solution being titrated. Figure 4.6 shows the positions of EP and EP_{1/2} on the titration curve for P27.
\[ pK_a = \text{pH}_{EP1/2} \]  

(7)

\[ V_{EP1/2} = V_{EP} + \frac{1}{2} \frac{[SO_2NH]}{[HCl]} V_{sol} \]  

(8)

Table 4.3 contains the pK\(_a\) values for each monomer calculated using equation 1 along with the literature reported pK\(_a\) values for the corresponding sulfa drug precursors. A general trend is observed whereby the pK\(_a\) of the MSA is lower than that of the sulfa drug precursor which is consistent with the decrease in pK\(_a\) observed upon acetylation of the \(p\)-amino group of sulfa drugs.\(^{254}\)

\[ \text{Vol}_{EP} \]

\[ \text{Vol}_{EP1/2} \]

\[ \text{Vol}_{HCl} \text{(mL)} \]

\[ \text{pH} \]

\[ V_{EP} \]

\[ \text{EP} \]

\[ \text{EP}_{1/2} \text{(pK\(_a\))} \]

\[ V_{EP1/2} \]

\[ V_{sol} \]

\[ 1 \quad 2 \]

\[ [SO_2NH] \]

\[ [HCl] \]

\[ 0.0 \quad 0.2 \quad 0.4 \quad 0.6 \quad 0.8 \quad 1.0 \quad 1.2 \]

\[ 3 \quad 4 \quad 5 \quad 6 \quad 7 \quad 8 \quad 9 \quad 10 \quad 11 \quad 12 \]

\[ \text{Figure 4.6. EP and EP}_{1/2} \text{ locations on the titration curve of 27 (1 mM) titrated against HCl (0.05 N) at 25 °C using a Metrohm 848 Titrino Plus autotitrator.} \]

\[ \text{pH-dependent Solubility of Poly(methacryloyl sulfonamides)} \]

The titration curves (Figure 4.7) demonstrate the facility by which the pH-dependent solubility of pMSAs can be “tuned” by simply varying the sulfonamide R-group of the monomer. The changes in polymer solubility occur over a very narrow range of typically 0.5 pH units. Table 4.3 summarizes the pH-dependent solubility of each
MSA derivative. The critical onset of precipitation (pH*), is defined as the pH corresponding to 90% light transmittance. For each of the MSA derivatives, pH* of the polymer is greater than the pKₐ of the corresponding monomer. The pH* of a particular pMSA is dependent upon the monomer pKₐ and the relative hydrophobicity of the monomer derivative, both influenced by the sulfonamide R-group. The mutual influence of these two parameters is readily apparent by comparing the pH* and pKₐ values for P₂⁷ and P₂⁸ (Table 4.3). While the pKₐ of 28 (4.51) is lower than that of 27 (4.88), the pH* for P₂⁸ (5.3) is higher than that of P₂⁷ (5.1) due to the greater hydrophobicity of the benzoyl R-group.

Table 4.3

MSA monomer and polymer titration data.

<table>
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<th>polymer</th>
<th>Mₙexp (g/mol)</th>
<th>Mₙ/Mw</th>
<th>sulfadrug pKₐ</th>
<th>monomer pKₐ</th>
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<tr>
<td>P₂⁸</td>
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<td>4.51 ± 0.01</td>
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</tr>
<tr>
<td>P₂⁹</td>
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<td>5.29</td>
<td>5.19 ± 0.03</td>
<td>6.3</td>
</tr>
<tr>
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<td>5.44 ± 0.01</td>
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</tr>
<tr>
<td>P₃₁</td>
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<td>6.70</td>
<td>5.75 ± 0.01</td>
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<td>P₃₂</td>
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<td>7.49</td>
<td>7.33 ± 0.02</td>
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</tr>
</tbody>
</table>
Figure 4.7. Substituent effects on pH-dependent solubility transitions of sulfonamide-containing polymers. Percent transmittance was measured using a UV-Vis spectrophotometer (λ = 500 nm).

CO₂-dependent Solubility of Poly(methacryloyl sulfonamides)

To date, CO₂-responsive polymers rely almost exclusively upon protonation of amine or amidine functional groups by carbonic acid (produced upon dissolution of CO₂ in water) that alters polymer solubility and conformation in solution. However, there are very few examples of CO₂-responsive polymers based upon acidic functional groups. In order for acid-functional polymers to exhibit CO₂-induced changes in phase or conformation, the pKₐ of the acidic functional group and more importantly the pH* of the corresponding polymer, must be greater than the pH of the solution upon production of carbonic acid via dissolution of CO₂. Therefore, weakly acidic polyacids that exhibit pH-responsive behaviors above pH = 4 (the pH of an aqueous solution in equilibrium with 1 atm of CO₂ at 25 °C) should also exhibit similar changes in properties upon CO₂-induced solution acidification.

The weakly acidic pMSA derivatives we report here exhibit pH* values above pH = 5.0, making these ideal candidates as CO₂-responsive polymers. To demonstrate the
reversible CO$_2$-responsiveness of pMSAs, polymethacryloyl sulfamethazine (P$^{29}$) ($M_n = 34,400$ g/mol, $M_w/M_n = 1.08$) (1 eq sulfonamide functional group) was dissolved in 0.05 N NaOH (1.25 eq) and diluted with DI H$_2$O to yield a final [SO$_2$NH] = 6.7 mM and [NaOH] = 8.4 mM. The solution was purged with CO$_2$ (10 s) and then N$_2$ (25 min) and the % transmittance ($\lambda = 500$ nm) of the polymer solution measured before and after each purge cycle using a UV-Vis spectrophotometer. Figure 4.8 shows % transmittance as a function of purge cycle and illustrates the reversible CO$_2$-triggered change in aqueous solubility of P$^{29}$.

![Graph showing reversible solubility of P$^{29}$ in response to presence or absence of CO$_2$. Solutions were purged with either CO$_2$ for 10 s (shaded regions) or N$_2$ for 25 min (unshaded regions) and % transmittance measured using a UV-Vis spectrophotometer ($\lambda = 500$ nm).]
Section 2. Mechanistic Insights into Temperature-Dependent Trithiocarbonate Chain-End Degradation during the RAFT Polymerization of N-Arylmethacrylamides

Overview

The acknowledged utility of reversible-deactivation radical polymerization (RDRP) is the facile synthesis of polymers with precise compositions, predetermined molecular weights, and well-defined architectures while incorporating monomers possessing a wide variety of functional groups. The success of any RDRP technique depends greatly upon maintaining “living” chain end fidelity. In particular, the reversible addition-fragmentation chain transfer (RAFT) process must retain thiocarbonylthio end groups in order to maintain the active/dormant equilibrium necessary for polymerization control. While thiocarbonylthio reactivity has often been exploited as a facile means of post-polymerization end group modification of RAFT polymers, a number of deleterious side reactions involving the thiocarbonylthio moiety can occur including hydrolysis, aminolysis, thermolysis, oxidation, and irreversible coupling of intermediate radicals. It is therefore important to fully understand the nature of any degradative reactions involving thiocarbonylthio end groups in order to extend the current capabilities of RAFT polymerization.

Recently, we reported the RAFT polymerization of a library of pH-responsive methacryloyl sulfonamide (MSA) monomers derived from sulfa drugs. Loss of chain end functionality was observed during the polymerization of MSAs at the commonly used temperature of 70 °C as evidenced, in part, by broad molecular weight distributions ($M_w/M_n > 1.3$) and failure to successfully chain extend a poly(MSA) macro chain transfer agent (macroCTA). However, narrow molecular weight distributions and controlled
molecular weights were ultimately achieved by conducting the polymerizations at 30 °C. The poor polymerization control of MSAs at 70 °C surprisingly contrasts numerous literature reports of successful RAFT polymerizations of (meth)acrylamides in organic and aqueous media at temperatures greater than 60 °C,\textsuperscript{91,95,100,269–271} thus prompting our current investigation.

Since our initial report,\textsuperscript{97} we have conducted the RAFT polymerization of N-phenylmethacrylamide (33), an MSA analog lacking the sulfonamide functional group (vide infra). Notably, the trithiocarbonate-mediated polymerization of 33 at 70 °C also results in relatively broad molecular weight distributions ($M_w/M_n = 1.30$), indicating that the sulfonamide functional group is not the primary cause for chain end degradation during polymerization of MSAs. Previous reports regarding the atom transfer radical polymerization (ATRP) of (meth)acrylamides attributed loss of “living” chain ends to nucleophilic displacement of the terminal bromine by the penultimate amide unit.\textsuperscript{272–274} Similarly, oxazolone formation during peptide synthesis occurs by an amide “back-biting” reaction.\textsuperscript{275} There is also literature precedent for the effects of N-aryl substitution on the cyclization of (thio)carbamoyl derivatives formed during sequencing of peptides by Edman’s degradation\textsuperscript{276} as well as the cyclization of $\gamma$-bromobutyranilides.\textsuperscript{277} From these observations, we have hypothesized that a similar reaction involving nucleophilic attack on the $\omega$-thiocarbonyl by the terminal methacrylamide unit may be responsible for thiocarbonylthio degradation during the RAFT polymerization of N-arylmethacrylamides.

In this contribution we report the influences of methacrylamide structure and reaction temperature on trithiocarbonate degradation during the RAFT polymerization of
N-substituted methacrylamides. A detailed study of the trithiocarbonate-mediated polymerizations of 33 and N-benzylmethacrylamide (34) using SEC-MALLS and UV-vis spectroscopy has now provided a clear understanding of the influence of methacrylamide structure on CTA degradation. Furthermore, in situ $^1$H NMR analysis of RAFT polymer small molecule analogs, prepared by single monomer unit insertion, affords additional mechanistic insight into the specific degradation pathway.

**33 and 34 Polymerization Kinetics**

To our knowledge, there are no reports detailing the effects of N-aryl substitution on RAFT-mediated polymerization control of (meth)acrylamides. To this end, we chose to compare the RAFT polymerizations of 33 and 34 under analogous conditions (Scheme 4.3). 34 was chosen based upon its structural similarity to 33 while lacking direct N-aryl substitution.

![Scheme 4.3. Synthetic route for 37-mediated polymerization of 33 and 34 in DMF at 70 or 30 °C.](image)

Table 4.4 summarizes the conversion, molar mass, and molecular weight distribution data for the polymerizations of 33 and 34 in DMF at 70 and 30 °C, using the RAFT agent 2-cyano-2-propyl dodecyl trithiocarbonate (37). The effect of methacrylamide structure on polymerization control is initially apparent by comparing the increase in molecular weight distribution of $P_{33}$ ($M_w/M_n = 1.30$) relative to that of $P_{34}$ ($M_w/M_n = 1.15$) synthesized under identical reaction conditions. Limited molecular
weight control during polymerization of \textbf{33} at 70 °C can also be seen in the $M_n$ vs conversion plot (Figure A1, Appendix A) which shows a decrease in $M_{n,\text{exp}}$ relative to $M_{n,\text{th}}$ at higher conversions. By contrast, for the polymerization of \textbf{34} at 70 °C, $M_{n,\text{exp}}$ values mirror $M_{n,\text{th}}$ values, indicating that the number of active/dormant chain ends remain constant during polymerization (Figure A2, Appendix A).

Table 4.4

\textit{Conversion, Molar Mass, and Molecular Weight Distribution Data for the RAFT Polymerizations of \textbf{33} and \textbf{34} in DMF at 70 and 30 °C.\textsuperscript{a}}

<table>
<thead>
<tr>
<th>entry</th>
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<th>temp. (°C)</th>
<th>time (min)</th>
<th>conv.\textsuperscript{b} (%)</th>
<th>$M_{n,\text{th}}$\textsuperscript{c} (g/mol)</th>
<th>$M_{n,\text{exp}}$\textsuperscript{d} (g/mol)</th>
<th>$M_w/M_n$\textsuperscript{d}</th>
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<td>11200</td>
<td>1.04</td>
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</table>
Polymerizations were conducted at 70 or 30 °C in DMF ([M]:[CTA]:[I] = 200:1.0:0.2) using V501 or V-70 as the initiators respectively. Conversions were determined by 1H NMR (DMSO-d$_6$) by comparing the relative integral areas of trimesic acid (internal standard) aromatic protons (8.64 ppm, 3H) to the vinyl proton of 33 (5.86 ppm, 1H) or 34 (5.79 ppm, 1H). Theoretical $M_n$ values were calculated according to the equation $M_{n,th}=\rho MW_{\text{mon}}/[\text{CTA}]_0 + MW_{\text{CTA}}$ where $\rho$ is the fractional monomer conversion, $MW_{\text{mon}}$ is the molecular weight of the monomer, and $MW_{\text{CTA}}$ is the molecular weight of the CTA. Experimental $M_n$ and $M_w/M_n$ values were determined by SEC-MALLS (DMF 20 mM LiBr).

Figure 4.9 shows the kinetic plots for the 70 °C polymerizations of 33 (black) and 34 (red); each plot shows an initialization period of approximately 30 min followed by pseudo-first-order kinetic behavior up to 300 min with the differences in slope of the curves indicative of the relative propagation rate coefficients ($k_p$) for each monomer derivative. Similar initialization periods were observed previously for the trithiocarbonate-mediated polymerizations of N-aryl MSAs and are indicative of slow fragmentation/reinitiation by the RAFT agent R-group. Interestingly, the first-order kinetic plots for the homopolymerizations of 33 and 34 at 70 °C show similar minimal decreases in slope beyond 300 min despite limited molecular weight control observed during the same time period for the polymerization of 33. It might be expected that RAFT agent degradation during polymerization of 33 would result in a decrease in the slope ($k_p[P_n^*]$) of the pseudo-first-order kinetic plot due to chain transfer to thiol-containing degradation byproducts. However, efficient chain transfer can take place without influencing the rate of polymerization if the rate of reinitiation by thiol radicals is greater than the rate of propagation (i.e. $k_{IT} > k_p$). The decrease in $M_{n,exp}$ relative to $M_{n,th}$ throughout the 70 °C polymerization of 33 (Figure A1, Appendix A) is evidence of an increasing number of polymer chains resulting from efficient chain transfer.
Recently we demonstrated that improved chain end retention and narrow molecular weight distributions can be achieved during the RAFT polymerization of N-aryl MSAs by reducing the reaction temperature. Likewise, as seen in Figure 4.10, the molecular weight distribution of \( P_{33} \) synthesized at 30 °C (\( M_w/M_n = 1.02, M_n = 18800 \)) was markedly narrower than that of \( P_{33} \) synthesized under analogous conditions at 70 °C (\( M_w/M_n = 1.30, M_n = 18900 \)). Reduced polymerization temperature also afforded improved molecular weight control during the polymerization of \( 33 \) as evidenced by the linear progression of \( M_{n, \text{exp}} \) with conversion and good correlation between \( M_{n, \text{exp}} \) and \( M_{n, \text{th}} \) values (Figure A3, Appendix A). As shown in Table 4.4, narrower molecular weight distributions were also obtained during the polymerization of \( 34 \) at 30 °C (\( M_w/M_n < 1.10 \)) but the additional decrease was minimal due to already narrow \( M_w/M_n \) achieved during polymerization at 70 °C.
Figure 4.10. SEC RI chromatogram showing the effect of temperature on molecular weight distribution for the 37-mediated RAFT polymerization of 33 at 70 and 30 °C.

The kinetic plots for the 37-mediated polymerizations of 33 and 34 at 30 °C (Figure 4.11) exhibit initialization times of 100 min and 60 min respectively, while demonstrating near pseudo-first-order kinetic behavior up to at least 1380 min. The increase in initialization time with decreasing temperature for the RAFT polymerizations of 33 and 34 is consistent with observations made by McLeary et al.\textsuperscript{106} The improved linearity of the first-order kinetic plots is also consistent with previous low temperature RAFT polymerizations of (meth)acrylamides and is generally attributed to increased thiocarbonylthio chain end retention.\textsuperscript{158,250}
Figure 4.11. Kinetic plot for the \textbf{37}-mediated polymerization of \textbf{33} and \textbf{34} in DMF at 30 °C ([M]₀ = 2.0 M, [M]₀: [CTA]₀: [I]₀ = 200:1:0.2).

\textbf{Trithiocarbonate Degradation during the \textbf{37}-mediated Polymerizations of \textbf{33} and \textbf{34}}

It is evident from the molecular weight data summarized in Table 4.4 that chain end degradation is likely occurring during the 70 °C polymerization of \textbf{33}. Meanwhile, polymerization of \textbf{34} at the same temperature affords narrower molecular weight distributions with good correlation between $M_{n,\text{th}}$ and $M_{n,\text{exp}}$ values. In order to ascertain the influences of each polymerization component (i.e. solvent, monomer, and initiator) on temperature-dependent trithiocarbonate degradation, reactions were performed in DMF using combinations of \textbf{37}, monomer (\textbf{33} or \textbf{34}), and initiator (V501) at relative concentrations of $[37]_0:[M]_0:[I]_0 = 10:1.0:0.2$ as illustrated in the figures of Figure 4.12. The fractional change in total trithiocarbonate (TTC) concentration ($[\text{TTC}]/[\text{TTC}]_0$) was measured by comparing the absorbance ($\lambda = 320$ nm) of diluted aliquots taken at timed intervals to that of an initial aliquot at $t = 0$. It is worth noting that only minimal change in the molar extinction coefficient ($\varepsilon$) of \textbf{37} at $\lambda = 320$ nm occurs after covalent addition of \textbf{33} or \textbf{34}, allowing for accurate measurement of the total [TTC] during polymerization.
Figure A5 in Appendix A shows the Beer-Lambert plots and ε values at λ = 320 nm for 37 (ε_{320} = 9380 M^{-1} cm^{-1}) and the corresponding single monomer unit insertion (SMUI) adducts of 37 and 33 (41) (ε_{320} = 9560 M^{-1} cm^{-1}) and the SMUI adduct of 37 and 34 (42) (ε_{320} = 10300 M^{-1} cm^{-1}).

Examination of Figure 4.12a reveals no measureable influences of DMF (▼), 33 (▲), or V501 (●) independently on [TTC]/[TTC]₀ at 70 °C. However, a 60 % decrease in [TTC]/[TTC]₀ is observed after 12 h when 37, 33, and V501 are combined at 70 °C (■) such that monomer addition to 37 takes place. This result supports terminal monomer unit-induced degradation in which the ultimate methacrylamide unit is in a favored orientation for O-5 or N-5 nucleophilic attack on the terminal thiocarbonyl (Scheme 4.4). In this case “5” denotes the number of atoms between the amide oxygen or nitrogen and the thiocarbonyl carbon. By contrast, identical experiments performed with 34 showed minimal trithiocarbonate degradation during polymerization with only a 3 % decrease in [TTC]/[TTC]₀ observed after 12 h (Figure 4.12b).
Figure 4.12. Individual and combined influences of solvent, initiator, and monomer on the time-dependent change in $[\text{TTC}]/[\text{TTC}]_0$ as measured by UV-Vis spectroscopy ($[\text{M}]_0:[\text{CTA}]_0:[\text{I}]_0 = 10:1:0.2$). Trithiocarbonate degradation experiments were performed using (a) 33 or (b) 34 as the monomer.

Scheme 4.4. Proposed trithiocarbonate degradation by O-5 or N-5 nucleophilic attack by the terminal methacrylamide unit.

Previously, we attributed the substantially improved polymerization control of MSAs and 33 at 30 °C to increased chain end retention.97 As seen in Figure 4.13, the effect of temperature on trithiocarbonate degradation was confirmed by measuring $[\text{TTC}]/[\text{TTC}]_0$ during polymerization of 33 at 30 °C under analogous conditions used for experiments in Figure 4.12a. At 30 °C, only 8 % trithiocarbonate degradation was observed after 720 min compared to 60 % degradation for the same time period at 70 °C.
Small Molecule Analog Synthesis

In order to better study the mechanism and byproducts of N-arylmethacrylamide-promoted trithiocarbonate degradation, we attempted to synthesize a small molecule analog of trithiocarbonate-terminated poly(N-phenylmethacrylamide). According to Scheme 4.5, the desired product 40a should result from the SN$_1$ reaction of sodium ethyl trithiocarbonate (24) and 2-bromoisobutyrylanilide (39). Despite the reaction being performed at room temperature (22 °C), isolation of 40a proved unsuccessful. Recrystallization of the crude reaction mixture afforded 5,5-dimethyl-3-phenyl-2-thioxothiazolidin-4-one (40), the N-5 cyclization product of 40a (Scheme 4.5). The structure of 40 was confirmed in part by analysis of the $^1$H NMR spectrum (Figure 4.14) which is absent of ethylsulfanyl –SCH$_2$CH$_3$ and amide -N-H $^1$H resonances. The $^1$H and $^{13}$C chemical shifts of 40 also match those reported previously.$^{227}$ $^1$H NMR analysis of aliquots sampled during the reaction (Scheme 4.5) showed rapid formation of 40.
indicating that under these conditions, N-5 cyclization/elimination of the transient species 40a occurs even at temperatures below 30 °C.

Scheme 4.5. Synthetic route for small molecule analog 40a.

Figure 4.14. 1H NMR (300 MHz, CDCl3) spectrum of isolated N-5 cyclization/elimination product 40.

Small Molecule Analog Synthesis via Single Monomer Unit Insertion

Minimal degradation of 37 during the polymerization of 33 at 30 °C indicates that covalent adducts of 33 and 37 are stable at low temperatures and can be formed by free radical processes. Recently, single monomer unit insertion279,280 has become a facile method for CTA synthesis, exploiting the “initialization” phenomenon previously
described by McLeary, Klumperman, and coworkers.\(^{106}\) We found that the SMUI adduct of 37 and 33 (41) could be marginally favored by stoichiometric control of monomer, CTA, and initiator at 30 °C as outlined in Scheme 4.6. The SMUI adduct of 37 and 34 (42) was synthesized at 60 °C using AIBN as the initiator owing to the lower nucleophilicity of the N-benzylamide at higher temperatures as previously demonstrated.

The labeled \(^1\)H NMR spectra of 41 and 42 are shown in Figures 4.15a and 4.15b respectively.

Scheme 4.6. Synthesis of 41 and 42 by single monomer unit insertion.

Figure 4.15. \(^1\)H NMR (300 MHz, CDCl\(_3\)) spectra of (a) 41 and (b) 42 SMUI adducts.
In situ $^1$H NMR Analysis of 41 and 42 Degradation at 70 °C

In situ $^1$H NMR analysis was used to gain further insight into the mechanism and kinetics of 41 and 42 degradation. The labeled $^1$H NMR spectra at select time points during the degradation analysis of 41 at 70 °C in DMF-d$_7$ are shown in Figure 4.16. After 5 min, only the $^1$H resonances 41 are observed. Subsequently, new signals in the aromatic (7.4-7.7 ppm) and aliphatic (1.5-2.7 ppm) regions corresponding to degradation byproducts appear and increase in intensity with time. Comparison of the $^1$H NMR chemical shifts of the degradation byproducts (Figure 4.16) with those of 1-dodecanethiol and 5,5-dimethyl-3-phenyl-2-thioxothiazolidin-4-one (40) (Figures A6, A7 and A8, Appendix A) indicates that the byproducts of 41 degradation are those formed exclusively by N-5 cyclization/elimination. Labeled resonances corresponding to N-5 cyclization/elimination degradation byproducts are given prime designation in the NMR spectrum (Figure 4.16) obtained at 491 min.

*Figure 4.16.* $^1$H NMR (600 MHz, DMF-d$_7$) overlay following the time-dependent degradation of 41 at 70 °C.
Degradation of 42 at 70 °C (Figure 4.17) was also monitored using \textit{in situ} $^1$H NMR analysis. After 491 min, minimal degradation was observed as shown in the expanded regions of the $^1$H NMR spectrum in Figure 4.17 corresponding to the 3-benzyl-2-thioxothiazolidin-4-one methylene (peak i’) and the cyclized benzylmethacrylamide methyl (peak e’). Also visible is the characteristic methylene of 1-dodecanethiol (peak d’) which is additional evidence of degradation by N-5 cyclization/elimination.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.17.png}
\caption{$^1$H NMR (600 MHz, DMF-d$_7$) overlay following the time-dependent degradation of 42 at 70 °C.}
\end{figure}

Kinetic analysis of 41 degradation also provides additional evidence for exclusive N-5 cyclization/elimination. As shown in Scheme 4.7, intramolecular N-5 nucleophilic attack of the trithiocarbonate by adjacent methacrylamide can occur by either cyclization/elimination (pathway A) or rearrangement (pathway B) depending upon which C-S bond is cleaved. The total rate of degradation of 41 by pathways A and B is equal to the rate of change in area of the phenyl $^1$H resonances (Figure 4.16, peaks i, j, and k) as N-5 nucleophilic attack results in loss of the N-phenylamide. The exclusive rate of N-5 cyclization/elimination (pathway A) is equal to the rate of change in area of the –
**CH₂-S⁻¹H resonance** (Figure 4.16, peak d) as elimination of 1-dodecanethiol occurs. As shown in Figure 4.18a, the fractional changes in area ($A_t/A_0$) of peaks i (7.70 ppm) and d (3.39 ppm) are essentially identical throughout the 491 min experiment indicating that pathway A is responsible for the degradation of **41** at 70 °C.

**Scheme 4.7.** Possible N-5 nucleophilic attack degradation pathways.

Kinetic analysis of **42** degradation (Figure 4.18b) additionally supports the proposed degradation pathway of N-5 cyclization/elimination as shown by the comparable change in $A_t/A_0$ of peaks i (4.42 ppm) and d (3.37 ppm) (Peak assignments in Figure 4.17).
As seen in Figure 4.19, excellent agreement is observed between the time-dependent fractional changes in [TTC] measured during the 37-mediated polymerizations of 33 and 34 by UV-Vis spectroscopy (open data points) and during 41 and 42 degradation measured by in situ $^1$H NMR analysis (solid data points). The half lives ($t_{1/2}$) of 41 and 42 at 70 °C in DMF were calculated to be $t_{1/2} = 7.18$ h and $t_{1/2} = 78.5$ h respectively based upon the degradation rates measured using $^1$H NMR.
Figure 4.19. Time-dependent change in $[\text{TTC}] / [\text{TTC}]_0$ at 70 °C in DMF as measured by UV-vis spectroscopy during polymerization (open circles) and as measured by *in situ* $^1$H NMR during SMUI adduct degradation analysis (closed circles).

It is important to note that while we have determined that significant trithiocarbonate degradation occurs by N-5 cyclization/elimination during the RAFT polymerization of 33 at 70 °C, this work does not specifically address the influence of N-phenyl substitution on increased amide nucleophilicity and how this affects the observed reaction mechanism. We are currently examining the influences of N-aryl substitution on both reaction mechanism and rate of N-5 cyclization/elimination and will report this in a future manuscript.

*Influence of 3-phenyl-2-thioxothiazolidin-4-one Chain Ends on the RAFT Polymerization of 33*

Typically, RAFT agent degradation during polymerization results in the loss of active thiocabonythio chain ends affording “dead” polymer chains that can no longer participate in the RAFT process. However, we have shown that trithiocarbonate degradation by N-5 cyclization/elimination during the polymerization 33 results in
formation of a new cyclic thiocarbonylthio end group. To date, there are few examples of RAFT polymerizations mediated by cyclic thiocarbonylthio compounds.\textsuperscript{281–283} However, Zhan and coworkers recently reported the RAFT polymerization of vinyl acetate using \textit{40} as the RAFT agent, which resulted in incorporation of thiocarbonylthio functionality into the polymer backbone.\textsuperscript{227} Similarly, it is plausible that the 3-phenyl-2-thioxothiazolidin-4-one chain ends formed as a result of N-5 cyclization/elimination could participate during the RAFT polymerization of \textit{33} as shown in Scheme 4.8.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{scheme4.8.png}
\caption{Proposed mechanism for radical addition to 3-phenyl-2-thioxothiazolidin-4-one chain ends during RAFT polymerization of \textit{33} at 70 °C.}
\end{figure}

We investigated the possibility of 3-phenyl-2-thioxothiazolidin-4-one chain ends participating in the RAFT process by conducting the polymerization of \textit{33} at 70 °C in the presence of \textit{40}, a small molecule analogue of N-5 cyclized poly(N-phenylmethacrylamide) chain ends. Figure 4.20a shows the kinetic plot for the \textit{40}-mediated polymerization of \textit{33} in DMF at 70 °C. Linear pseudo-first-order kinetic behavior was observed up to 140 min with no initialization period. By contrast, the \textit{37}-mediated polymerization of \textit{33} under identical conditions exhibited a 30 min initialization period, consistent with similar results first reported by Klumperman and coworkers.\textsuperscript{106} While the linear pseudo-first-order kinetic behavior indicates a constant $k_p[P_n \cdot]$, the SEC RI overlay shown in Figure 4.20b demonstrates that molecular weight control is not achieved during the \textit{40}-mediated polymerization of \textit{33} at 70 °C. The intensities of the
SEC RI chromatograms shown in Figure 4.20b increase with conversion but the unchanging peak elution volumes, which occur at the exclusion limit of the SEC system (~11.0 mL), are representative of uncontrolled polymerization behavior where $M_n$ does not scale linearly with monomer conversion. While the polymers presented in Figure 4.20b were too large to characterize by our SEC-MALLS system, it is worth noting that a polyvinylpyridine standard of $M_n = 475,000$ g/mol ($M_w/M_n = 1.06$) elutes at a volume of 11.5 mL. These results suggest that 3-phenyl-2-thioxothiazolidin-4-one-terminated polymers do not participate in the normal CTA-mediated RAFT polymerization of 33 at 70 °C.
Section 3. “One-Pot” Aminolysis/Thiol-Maleimide End Group Functionalization of RAFT Polymers: Identifying and Preventing Michael Addition Side Reactions

Overview

Reversible addition-fragmentation chain transfer (RAFT) polymerization has made possible the synthesis of functionally-diverse polymers with predetermined molecular weights and low dispersities (D) using a wide variety of monomer types and polymerization conditions (e.g. aqueous and organic media). The versatility of RAFT in synthesizing tailor-made polymers also stems from the fidelity by which polymer end-functionality can be controlled. Such end-functionalized polymers have been used to prepare advanced macromolecular architectures including block copolymers, star copolymers, molecular brushes, and polymer bioconjugates. Telechelic RAFT polymers can be synthesized directly by controlling the RAFT agent R- and Z-group functionality or by post-polymerization end group modification. The latter approach often exploits the inherent reactivity of

Figure 4.20. (a) Kinetic plot and (b) SEC RI chromatogram overlay for the 40-mediated polymerization of 33 in DMF at 70 °C ([33]₀ = 2.0 M, [33]₀:[40]₀:[V501]₀ = 200:1.0:0.2).
the residual thiocarbonylthio moiety present on RAFT polymers, allowing for simultaneous removal and replacement of the unstable RAFT agent with a benign or functional end group.

In recent years, reduction or aminolysis of thiocarbonylthio-terminated RAFT polymers to the corresponding polymeric thiol has afforded a myriad of thiol “click” end group functionalization routes including thiol-isocyanate, thiol-epoxy, thiol-halogen, thiol-disulfide, and thiol-ene reactions. Particularly advantageous are thiol-maleimide Michael addition reactions which proceed to near quantitative conversions at room temperature in the presence of oxygen and water and typically occur much more rapidly than analogous thiol-acrylate or thiol-acrylamide reactions. Furthermore, thiol-maleimide end group modification of RAFT polymers can be performed as “one-pot” reactions without isolation of the intermediate polymeric thiol (Scheme 4.9).

Scheme 4.9. “One-pot” aminolysis/thiol-maleimide end group functionalization of RAFT polymers.

To ensure quantitative polymer end group functionalization, an excess of maleimide relative to polymeric thiol is required. It is therefore desirable to optimize the reaction conditions to favor rapid and efficient polymer conjugation with minimal excess of maleimide, especially when using costly biologic, therapeutic, or diagnostic agents. One potential way to maximize end group functionalization efficiency while minimizing
the required excess of maleimide is through the use of nucleophilic catalysts. Thiol-Michael addition reactions typically employ a base catalyst (e.g. tertiary amine) to generate the nucleophilic thiolate species. However, nucleophilic catalysts such as phosphines, amines, and amidines have been used to initiate Michael addition reactions and can increase reaction rates of these reactions by several orders of magnitude compared to analogous base-catalyzed reactions. The proposed mechanism of nucleophile-initiated thiol-maleimide Michael addition is illustrated in Scheme 4.10. Rather than direct deprotonation of thiol, conjugate addition of the nucleophile to the maleimide double bond forms the zwitterionic enolate, which in turn functions as a strong base (pK\textsubscript{a} ≈ 25) capable of generating the nucleophilic thiolate species while also forming a nucleophile-succinimide byproduct. In this regard, the nucleophile does not function as a catalyst which would be regenerated during each catalytic cycle, but rather serves as an initiator that generates the steady state enolate/thiolate concentration necessary for the thiol-ene chain transfer mechanism to operate. Subsequent propagation occurs by thiolate addition to maleimide forming the corresponding enolate, which abstracts a proton from thiol, regenerating the thiolate along with the desired thiol-maleimide Michael addition product.  

Scheme 4.10. Mechanism of nucleophile-initiated thiol-maleimide Michael addition.
Recently, while investigating the use of nucleophilic initiators to improve the efficiency of RAFT polymer end group functionalization with N-substituted maleimides, we discovered that in certain instances, nucleophilic initiators reduced the degree of end group functionalization compared to reactions performed using only a base catalyst. Reagent order of addition and solvent-type were also determined to have marked effects on end group functionalization efficiency (vide infra). These observations have prompted this study aimed at understanding the influences of nucleophile type, solvent, and reaction conditions on the efficacy of “one-pot” aminolysis/thiol-maleimide end group functionalization of RAFT polymers. Furthermore, the results discussed herein offer mechanistic insights into potentially detrimental side reactions that can occur during thiol-maleimide Michael addition reactions.

**Nucleophile-Promoted Michael Addition Side Reactions**

Preliminary efforts in our lab to catalyze the “one-pot” aminolysis/thiol-maleimide end group modification of acrylamido RAFT polymers in DMSO using the amidine 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted in i) low end group functionalization efficiencies compared to reactions performed in the absence of DBU, ii) quantitative maleimide consumption, and iii) the formation of high molecular weight impurities observable by SEC-MALLS. From these observations we hypothesize that a low polymeric thiol concentration relative to maleimide concentration in the presence of a strong nucleophile (DBU), results in anionic propagation of maleimide occurring faster than the desired thiol-maleimide Michael addition. It is also possible that other nucleophiles (e.g. amines and phosphines) commonly used during “one-pot” aminolysis/thiol-maleimide modification of RAFT polymers could promote similar side
reactions. Azechi et al. showed that 1°, 2°, and 3° amines can initiate the anionic polymerization of N-substituted maleimides in highly polar aprotic solvents (DMSO and DMF) whereas no polymerization was observed in less polar solvents (THF). Also, phosphines have been used as initiators for the anionic polymerizations of methylene malonic esters, cyanoacrylates, and N-substituted maleimides. It is therefore plausible that during “one-pot” aminolysis/thiol-maleimide modification of RAFT polymers, amine- and phosphine-initiated polymerization of maleimide could also outcompete the desired thiol-maleimide Michael reaction and account for the poor end group functionalization efficiencies observed under certain reaction conditions.

Reaction of N- and P-Based Nucleophiles with N-Methylmaleimide

A number of nucleophile types including amines (aminolysis), phosphines (reducing agent), and amidines (nucleophilic initiator) can be present during “one-pot” aminolysis/thiol-maleimide end group reactions of RAFT polymers. Accordingly, we chose to first investigate the effect of solvent on the reactions of representative N- and P-based nucleophiles with N-methylmaleimide in the absence of thiol as outlined in Figure 4.21. A stoichiometric excess of maleimide relative to nucleophile ([Nu]₀:[Mal]₀ = 1.0:10) was chosen to reflect the relative concentrations of these reagents used during “one-pot” RAFT polymer aminolysis/thiol-maleimide reactions and to make apparent whether or not maleimide polymerization is occurring. In situ \(^1\)H NMR analysis was used to monitor the time-dependent fractional change in maleimide concentration ([Mal]/[Mal]₀) by comparing the area of the maleimide olefin peak to the peak area of an internal standard (CH₂Cl₂).
Figure 4.21. Effect of solvent on the time-dependent fractional change in $[\text{Mal}]/[\text{Mal}]_0$ upon reaction of \textbf{65} with representative nucleophiles a) hexylamine (HexAM), b) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), c) tributylphosphine (TBP), and d) trimethylphosphite (TMP) as measured by \textit{in situ} $^1\text{H}$ NMR analysis.

Figure 4.21a shows the time-dependent $[\text{Mal}]/[\text{Mal}]_0$ plots for the reactions of hexylamine (HexAM) with \textbf{65} in different solvents with the dashed line ($[\text{Mal}]/[\text{Mal}]_0 = 0.9$) representing the theoretical decrease in $[\text{Mal}]/[\text{Mal}]_0$ predicted for the reaction of HexAM and \textbf{65} via single aza-Michael addition. Reactions conducted in MeCN, EtOH, and CH$_2$Cl$_2$ show decreases in $[\text{Mal}]/[\text{Mal}]_0$ to a value of 0.9, beyond which no change in $[\text{Mal}]/[\text{Mal}]_0$ is observed up to 12 h, indicating exclusive aza-Michael addition takes place in these solvents. Conversely, reaction of HexAM with \textbf{65} in DMSO results in rapid maleimide consumption, corresponding to 7.5 maleimides consumed on average per amine within the first 3 min. Subsequently, no change in $[\text{Mal}]/[\text{Mal}]_0$ is observed up to 12 h. These results are consistent with those reported by Azechi et al.\textsuperscript{313} and confirm that HexAM can initiate the anionic polymerization of \textbf{65} in polar solvents such as DMSO whereas exclusive aza-Michael addition takes place in less polar solvents. Furthermore,
the effect of solvent polarity on the reaction of HexAM with 65 is readily observed in
Figure 4.21a by noting the increase in reaction rate with increasing solvent polarity in the
order of CH$_2$Cl$_2$ ($\varepsilon = 8.93$) < EtOH ($\varepsilon = 24.5$) < MeCN ($\varepsilon = 37.5$) < DMSO ($\varepsilon = 46.7$).

The time-dependent [Mal]/[Mal]$_0$ plots for the reactions of DBU and 65 are
shown in Figure 4.21b. Reaction rate increases with increasing solvent polarity with 100
% 65 conversion reached in < 10 min in DMSO. Quantitative consumption of 65 was
also observed for reactions performed in EtOH and MeCN while 71% maleimide
conversion was measured in CH$_2$Cl$_2$ after 12 h. Interestingly, the kinetic plots in Figure
4.21b do not rapidly reach a constant value of [Mal]/[Mal]$_0$ as was observed during the
reaction of HexAM and 65 in Figure 4.21a. Unlike protic nucleophiles (e.g. 1°, 2°
amines, and thiols), aprotic nucleophiles such as DBU cannot undergo complete Michael
addition due to the lack of a transferable hydrogen from the nucleophile. Therefore, if an
active hydrogen-containing compound (e.g. thiol) is in low concentration or completely
absent, the nucleophile-derived zwitterionic enolate 61 can undergo reaction with
additional maleimide without enolate termination by proton transfer, allowing for a
“living-like” polymerization process to occur. Noteworthy is that precipitation was
observed during the reaction of DBU and 65 in CH$_2$Cl$_2$ after ~30 min. Limited solubility
of the propagating macro zwitterionic enolate in CH$_2$Cl$_2$ likely prevents the propagating
chain-end from reacting with 65 in solution, accounting for the non-quantitative
maleimide conversion obtained in this solvent. Nonetheless, these results indicate that
DBU-initiated polymerization of 65 occurs rapidly and extensively in both polar and
nonpolar solvents.
Similarly, the aprotic nucleophile tributylphosphine (TBP) was shown to initiate polymerization of 65 in all solvents tested (Figure 4.21c). Reaction rates increase with solvent polarity with 95% maleimide conversion reached within 2.5 min in DMSO. Precipitation was also observed during reactions performed in EtOH and CH₂Cl₂ after ~5 min following the addition of TBP. Poor solubility of the propagating phosphonium zwitterionic enolate is again a likely explanation for the non-quantitative maleimide conversions achieved in EtOH and CH₂Cl₂ during the time frame of these kinetic experiments.

From these results it is apparent that maleimide polymerization initiated by DBU or TBP is unavoidable, even in low polarity solvents. While DBU is optional during “one-pot” aminolysis/thiol-maleimide modification of RAFT polymers, mild reducing agents such as phosphines are generally required to prevent disulfide formation from occurring between polymeric thiols during the RAFT agent aminolysis step.²⁵⁸,³⁰¹ Initially, we explored triphenylphosphine as a more sterically hindered, and therefore less nucleophilic phosphine, but maleimide polymerization was again observed in each of the four solvents tested in this work (data not shown). Recently, Ho et al. reported on trialkylphosphites as cheaper and less toxic alternatives to trialkylphosphines as reducing agents during “one-pot” RAFT polymer aminolysis/thiol-ene reactions.³¹⁷ Trialkylphosphites can undergo conjugate addition to electron deficient olefins but are less nucleophilic than phosphines and typically require elevated temperatures (100 °C) for reaction to take place.³¹₈,³¹⁹ As seen in Figure 4.21d, the reaction of trimethylphosphite (TMP) with 65 results in no measureable change in [Mal]/[Mal]₀ in MeCN, EtOH, and CH₂Cl₂. Only after prolonged reaction times (12 h) in DMSO is a 65
% decrease in [Mal]/[Mal]₀ observed. Consequently, TMP is a suitable alternative to phosphines as a reducing agent during thiol-maleimide reactions when used in less polar solvents. It should also be noted that trace amounts of water must be present for trialkylphosphite (and phosphine) reduction of disulfides to occur.320 Therefore, rigorous anhydrous conditions should be avoided during RAFT polymer aminolysis when using trialkylphosphites as reducing agents.

Scheme 4.11. Proposed mechanisms of initiation, propagation, and termination for the nucleophile-initiated anionic polymerization of N-substituted maleimides.

The proposed mechanisms of initiation, propagation, and termination for the anionic polymerization of N-substituted maleimides initiated by protic or aprotic nucleophiles are illustrated in Scheme 4.11. Conjugate addition of the nucleophile to the maleimide double bond to form the zwitterionic enolate 61 in identical fashion to first
step of nucleophile-initiated thiol-maleimide reactions. However, if the thiol concentration is suitably low relative to maleimide concentration ([Mal] > [thiol]), propagation can occur faster than proton transfer to the enolate 61, resulting in the formation of the propagating macrozwitterionic species 66. For maleimide polymerization initiated by protic nucleophiles (e.g. 1° or 2° amines), termination can occur by proton transfer from the nucleophile to afford a terminated chain 67a. Conversely, aprotic nucleophiles do not possess a transferable proton and consequently cannot undergo “long range” Michael addition. However, termination by nucleophilic displacement of the positively charged nucleophilic residue (66d) by the enolate terminus of an adjacent chain (66c) could result in regeneration of the nucleophile while also producing a new macrozwitterionic polymer chain (67cd) equal in mass to the sum of 66c and 66d. These differences in termination mechanisms adequately describe the continued decrease in [Mal]/[Mal]₀ observed during reaction of aprotic nucleophiles with 65 whereas reaction of a protic nucleophile (HexAM) rapidly reach a constant value of [Mal]/[Mal]₀ owing to termination by proton transfer. Additional evidence of termination by nucleophilic displacement was also obtained by noting that the solution viscosity of the reaction of 65 with DBU continues to increase for several days after complete maleimide consumption, eventually leading to complete vitrification. This continued increase in solution viscosity would be expected if the macrozwitterions formed from the reaction of DBU and 65 were functioning as macro A-B monomers capable of continued step-growth polymerization resulting in a continued increase in molecular weight.
Reaction of Thiols with N-Methylmaleimide (65)

It is evident from the kinetic plots shown in Figure 4.21 that strong N- and P-based nucleophiles can react with maleimides to form either the Michael addition product or polymaleimide depending upon the nucleophile type (protic or aprotic) and solvent polarity. Therefore, it is plausible that other strong nucleophiles such as thiolates can initiate the anionic polymerization of maleimides in polar solvents. To this end, we chose to investigate the reaction of ethyl 2-mercaptopropionate (E2MP) with 65 under conditions identical to those outlined in Figure 4.21, except with the addition of TEA to generate the nucleophilic thiolate species. E2MP was chosen as a model thiol due to its structural similarity to the polymeric thiol that would be produced upon aminolysis of a polyacrylate RAFT polymer.

**Figure 4.22.** Effect of solvent on the time-dependent fractional change in \([\text{Mal}]/[\text{Mal}]_0\) during the TEA-catalyzed reaction of E2MP with 65 as measured by *in situ* $^1$H NMR analysis.
As shown in Figure 4.22, only the reaction of E2MP and 65 in CH$_2$Cl$_2$ gives the change in [Mal]/[Mal]$_0$ expected for exclusive thiol-maleimide Michael addition ([Mal]/[Mal]$_0 = 0.9$, dashed line). Meanwhile, the reactions conducted in more polar solvents (DMSO, EtOH, and MeCN) show a continued decrease in [Mal]/[Mal]$_0$ up to 12 h, with 100% maleimide conversion reached in 90 min in DMSO. Initially, we anticipated the kinetic profiles for the reactions of E2MP and 65 (Figure 4.22) to rapidly reach a constant [Mal]/[Mal]$_0$ value due to rapid enolate termination by means of proton transfer, as previously observed for the reactions of HexAM and 65 (Figure 4.21a). However, a continued decrease in [Mal]/[Mal]$_0$ up to 12 h is evidence of either slow reaction of thiol or an additional reaction pathway.

Closer inspection of the kinetic profiles for reactions conducted in EtOH and MeCN reveals an inflection point at the first time point (2.5 min) when [Mal]/[Mal]$_0 \approx 0.9$, indicating that thiol-maleimide Michael addition likely occurs faster than subsequent maleimide propagation in these solvents. Furthermore, in situ $^1$H NMR indicated 100% thiol conversion prior to the first time point (2.5 min) for reactions conducted in DMSO, MeCN, and CH$_2$Cl$_2$ (Figures A9-A11, Appendix A) whereas thiol conversion could not be measured for the reaction performed in EtOH-d$_6$ due to deuterium exchange between the solvent and thiol proton. Quantitative thiol consumption early in each reaction requires that proton transfer from thiol to enolate also occurs rapidly. Therefore, the continued decrease in [Mal]/[Mal]$_0$ observed at longer reaction times in more polar solvents is not likely attributable to slow reaction of thiol and is suggestive of an alternate maleimide reaction pathway.
One plausible explanation for the continued decrease in \([\text{Mal}] / [\text{Mal}]_0\) at longer reaction times is TEA-initiated maleimide polymerization. The time-dependent change in \([\text{Mal}] / [\text{Mal}]_0\) plots (Figure A12, Appendix A) for the reactions of TEA with \(\text{65}\) in DMSO and EtOH show a decrease in \([\text{Mal}] / [\text{Mal}]_0\) of 0.07 and 0.04 respectively after 12 h. Meanwhile, no change in \([\text{Mal}] / [\text{Mal}]_0\) was observed in MeCN and CH\(_2\)Cl\(_2\) after 12 h. Accordingly, TEA-initiated maleimide polymerization is insufficient to account for the significant decrease in \([\text{Mal}] / [\text{Mal}]_0\) that occurs within the same time period during the reactions of E2MP with \(\text{65}\) in these solvents.

Another potential explanation for the continued decrease in \([\text{Mal}] / [\text{Mal}]_0\) observed in the kinetic plots of Figure 4.22 involves deprotonation of the thiol-maleimide Michael adduct by TEA to regenerate the nucleophilic enolate species. Initially, this seems unlikely since the \(pK_a\) of TEA (10.75) is much lower than that of most succinimide-derived enolates (~25). However, enolate formation of 2-aminosuccinimide residues in peptides has been shown to occur under mildly basic conditions in aqueous media (pH = 7.4), indicating that heteroatom-substitution of the succinimide \(\alpha\)-carbon can reduce enolate \(pK_a\) compared to unsubstituted succinimides.\(^{321}\)

To test whether thiol-maleimide Michael addition products are capable of reinitiating maleimide polymerization in the presence of TEA, the Michael adduct of benzyl mercaptan and N-methylmaleimide (\(\text{43}\)) was synthesized and purified by column chromatography. The Michael adduct of E2MP and \(\text{65}\) was not synthesized due to potential complications arising from the presence of chemically distinct diastereomers and the complexity in identifying the \(^1\)H NMR chemical shifts of the resulting four
stereoisomers. Meanwhile, the synthesis of 43 from benzyl mercaptan and 65 affords a racemic mixture of chemically indistinct enantiomers.

![Chemical structure of 43 and 65](image)

**Figure 4.23.** Effect of solvent on the time-dependent fractional change in [Mal]/[Mal]₀ during the TEA-catalyzed reaction of 43 with 65 as measured by in situ ¹H NMR analysis.

Figure 4.23 shows that [Mal]/[Mal]₀ decreases with time during the TEA-catalyzed reactions of 43 and 65 in DMSO, EtOH, and MeCN whereas no change in [Mal]/[Mal]₀ is observed in CH₂Cl₂ up to 12 h. The kinetic profiles obtained in each solvent for the reactions of 43 and 65 in Figure 4.23 accurately reflect the kinetic profiles observed below [Mal]/[Mal]₀ = 0.9 (dashed line) for the analogous reactions of E2MP and 65 shown in Figure 4.22. This is not a surprising result since the dashed line in Figure 4.22 represents the change in [Mal]/[Mal]₀ predicted for formation of the thiol-maleimide Michael adduct. Any decrease in [Mal]/[Ma]₀ below a value of 0.9 in Figure
4.22 would presumably occur as a result of TEA-promoted regeneration of the nucleophilic enolate species and therefore resemble the kinetic profiles in Figure 4.23.

Also worth noting is that TEA has little influence on the time-dependent [Mal]/[Mal]₀ plots for the reaction of HexAM and 65 in DMSO as shown in Figure A13 in Appendix A. This indicates that the amine-maleimide adduct is not capable of reinitiation of maleimide polymerization under these reaction conditions and that reinitiation is likely unique to thiol-maleimide adducts.

![Diagram](image)

**Figure 4.24.** a) Time-dependent fractional change in peak area (Aₜ/A₀) for protons Hₐ, Hₐ, and Hₖ during TEA-catalyzed H-D exchange of 43 in DMSO. b) ¹H NMR spectral overlay of select time points during H-D exchange experiments with 43.
We next sought to provide direct evidence that TEA is a strong enough base to generate the enolate of 43 in polar solvents. To this end, we used in situ $^1$H NMR analysis to measure the relative rates of hydrogen-deuterium exchange (H-D) of 43 in a DMSO/D$_2$O mixture in the presence of TEA. Figure 4.24a shows the time-dependent fractional change in peak area ($A_t/A_0$) for the three chemically distinct protons of 43 which could be abstracted by TEA to form an enolate on either the $\alpha$-carbon (H$_a$) or the $\beta$-carbon (H$_b$ and H$_c$) relative to the benzylsulfanyl group. For simplicity, only the structure of the S enantiomer is shown in Figure 4.24a, whereas 43 is actually comprised of a racemic mixture of enantiomers. The kinetic plot of Figure 4.24a reveals that H-D exchange was only observed for proton H$_a$ with a 97% decrease in $A_t/A_0$ occurring by 35 min. Sigma withdrawing effects by the adjacent thioether are most likely responsible for the increased acidity of H$_a$, leading to exclusive enolate formation at the $\alpha$-carbon. Figure 4.24b shows the $^1$H NMR spectral overlay of select time points during the H-D exchange experiments with 43. The peak corresponding to H$_a$ decreases in area with time while maintaining the doublet of doublets (dd) splitting pattern that arises from spin-spin coupling with H$_b$ and H$_c$. Meanwhile, the peaks corresponding to the geminal protons H$_b$ and H$_c$ do not change in area, but rather show changes in splitting pattern from dd to d as spin-spin coupling of H$_b$ and H$_c$ with H$_a$ are diminished through deuterium exchange. Also apparent in Figure 4.24b is the significant downfield chemical shift of H$_a$ (3.78 ppm) relative to H$_b$ (3.07 ppm) and H$_c$ (2.46 ppm) that arises from the deshielding (sigma withdrawing) effects of the benzylsulfanyl group.
Scheme 4.12. Proposed reaction pathways for the TEA-catalyzed thiol-maleimide reaction in which reversible enolate formation is operational.

Scheme 4.12 shows the proposed reaction pathways for the TEA-catalyzed thiol-maleimide reaction when a stoichiometric excess of maleimide relative to thiol is employed in polar solvents. Thiolate addition to maleimide forms the β-enolate 63 which can either abstract a proton from thiol or +NH(Et)₃ to give the thiol-maleimide adduct 64, or react directly with maleimide to form the propagating species 68 when conditions favor propagation over termination (i.e. [Mal]>>[thiol]). Deprotonation of 64 by TEA forms the α-enolate 69 which can reversibly terminate by proton transfer or react with maleimide to form the propagating species 70. The propagating species 68 and 70 derived from β- and α-enolates respectively can continue to react with maleimide until termination occurs by proton transfer. Also, the termination products of 68 can in theory reinitiate maleimide polymerization by α-enolate formation. These additional reaction pathways can account for the continued decrease in [Mal]/[Mal]₀ following initial formation of the thiol-maleimide Michael adduct for the TEA-catalyzed reactions of
E2MP with 65 shown in Figures 4.23. It should also be noted that exclusive enolate formation at the α-carbon rules out the possibility of TEA-catalyzed β-elimination (retro Michael addition) as a means of regenerating the nucleophilic thiolate species after formation of the thiol-maleimide Michael adduct.

“One-pot” Aminolysis/Thiol-Maleimide End Group Functionalization of RAFT Polymers

It is apparent that many of the reagents (e.g. amines and phosphines) commonly used during “one-pot” RAFT polymer aminolysis/thiol-maleimide reactions can react with maleimides to form polymaleimide or Michael addition byproducts and potentially outcompete the desired polymeric thiol-maleimide Michael reaction. Also, we have shown that intermediate strength bases such as TEA can deprotonate thiol-maleimide Michael adducts in polar solvents to form a nucleophilic α-enolate capable of subsequent reaction with maleimide. However, the effects of these side reactions on RAFT polymer end group functionalization efficiency are not yet well understood. Also not well understood is the influence of aminolysis method on end group functionalization efficiency. The simplest method involves simultaneous aminolysis of the RAFT polymer in the presence of maleimide and does not require the use of a reducing agent to prevent disulfide formation. However, the competing amine-maleimide aza-Michael addition could prevent quantitative RAFT agent aminolysis and subsequently reduce the degree of end group functionalization. Alternatively, the sequential method allows for complete aminolysis of the RAFT agent to occur prior to the addition of maleimide in a second step. This route limits side reactions between the amine and maleimide but necessitates
the use of a reducing agent to prevent polymeric disulfide formation from occurring during the aminolysis stage.


To investigate the effects of solvent, catalyst, reducing agent, and aminolysis method on RAFT polymer end group functionalization efficiency, we first synthesized the water soluble polymer poly(N,N-dimethylacrylamide) (45) using the RAFT agent 2-cyano-2-propyl benzodithioate (44). Low dispersities (D = 1.06) and excellent agreement between the number average molecular weights determined by size exclusion chromatography (Mn(SEC) = 3360 g/mol) and by 1H NMR (Mn(NMR) = 3220 g/mol) are evident of a controlled polymerization and high dithiobenzoate chain-end fidelity. “One-pot” reactions of 45 with N-benzylmaleimide (46) were conducted at room temperature for 12 h using the initial molar ratios of [45]0:[HexAM]0:[46]0 = 1.0:2.5:5.0 as outlined in Scheme 4.13. End group analysis by 1H NMR was performed in D2O by comparing the integrated peak area of the benzyl aromatic protons (7.50 – 7.15 ppm) to the known integrated peak area of the N,N-dimethyl side chain and methyne backbone protons (3.30 – 2.20 ppm) of 45 (Figures A14-A15, Appendix A). The poor solubility of 46 and its nucleophile-initiated byproducts (i.e. poly(46)) in D2O allows for accurate quantification of only 46 that is covalently attached to 45.
Table 4.5

“One-pot” aminolysis/thiol-maleimide reactions of 45 with 46.a

<table>
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<th>CTA (%)</th>
<th>color (12 h)</th>
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<td>R</td>
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<sup>a</sup>[45]:[HexAM]:[46] = 1.0:2.5:5.0. <sup>b</sup>Method 1: simultaneous aminolysis/thiol-maleimide; Method 2: sequential aminolysis/thiol-maleimide. <sup>c</sup>[45]:[DBU] = 1.0:1.0. <sup>d</sup>[45]:[TBP or TMP] = 1.0:5.0. <sup>e</sup>Percent end group functionalization of 45 with 46 as measured by 1H NMR. <sup>f</sup>Percent CTA remaining after reaction of 45 with 46 as measured by 1H NMR. <sup>g</sup>Reaction color after 12 h: red (R), orange (O), or yellow (Y).
Table 4.5 summarizes the results of the “one-pot” aminolysis/thiol-maleimide reactions of $45$ with $46$ with the last column indicating the color exhibited by each reaction after 12 h. Entries 1a-d reflect our initial attempts to catalyze thiol-maleimide end group functionalization reactions with DBU as a catalyst and were performed using the simultaneous aminolysis method (method 1). A distinct trend of decreasing end group functionalization efficiency with increasing solvent polarity was observed with 0% functionalization achieved in DMSO. These results are relatable to the trend observed for the reactions of DBU and $65$ shown in Figure 4.21b, where the rate of DBU-initiated maleimide polymerization increases with solvent polarity. From these results, we conclude that in polar solvents, DBU-initiated maleimide polymerization is outcompeting the desired polymeric thiol-maleimide reaction.

Table 4.5 entries 2a-d show the effect of simultaneous aminolysis/thiol-maleimide Michael addition in the absence of DBU on end group functionalization efficiency. Incomplete aminolysis was observed in all solvents except CH$_2$Cl$_2$ (2a) with reactions performed in DMSO (2d) and EtOH (2c) retaining 46% and 90% respectively of the original dithiobenzoate functionality. Accordingly, only 31% and 8% end group functionalization was observed in DMSO EtOH respectively. These results show that the amine-maleimide aza-Michael addition can occur faster than CTA aminolysis in more polar solvents and are consistent with the effect of solvent polarity on the reaction rates of HexAM with $65$ shown in Figure 1a.

The reactions performed in Table 4.5 entries 3a-d were identical to those performed in entries 2a-d except the HexAM was allowed to react with $45$ for 30 min prior to the addition of $46$ (Method 2). CTA aminolysis was qualitatively confirmed to
occur within 30 min by noting the change in color that takes place as the dithiobenzoate end groups (orange) are aminolyzed to the corresponding N-hexylthiobenzamide (yellow). Entries 3a-d show that 100% CTA aminolysis was achieved in all solvents while end group functionalization efficiencies were significantly improved compared to reactions conducted using the simultaneous aminolysis method (entries 2a-2d). High end group functionalization (95%) was achieved in the less polar aprotic solvents CH$_2$Cl$_2$ (3a) and MeCN (3b) while moderate degrees of functionalization were obtained in EtOH (84%, 3c) and DMSO (72%, 3d). While these results are promising, it is well known that disulfide coupling of polymeric thiols can occur during CTA aminolysis, resulting in both reduced end group functionalization efficiencies and high molecular weight impurities, thus necessitating the use of a reducing agent.$^{258}$

Table 4.5 entries 4a-d show the use of TBP as a reducing agent results in decreased end group functionalization efficiencies for reactions performed in CH$_2$Cl$_2$ (4a) and MeCN (4b) compared to analogous reactions conducted without TBP (entries 3a and 3b respectively). Meanwhile, no effect of TBP was observed on the functionalization efficiencies of reactions performed in EtOH and DMSO (entries 4c and 4d respectively). From these results and the kinetic plots in Figure 4.21c, we conclude that trialkylphosphines are not suitable reducing agents during thiol-maleimide end group modification of RAFT polymers due to competing phosphine-initiated maleimide polymerization. Alternatively, using the less nucleophilic TMP as a reducing agent affords substantially increased degrees of end group functionalization in all solvents as seen in Table 4.5 entries 5a-d with 98% and 99% end group functionalization achieved in CH$_2$Cl$_2$ (5a) and MeCN (5b) respectively.
Stoichiometric Considerations

Efficient and quantitative end group functionalization of RAFT polymers using aminolysis/thiol-maleimide chemistry also requires consideration of the reactant feed ratios. Ideally, minimal excess of maleimide should be used relative to polymeric thiol to limit the waste of potentially costly maleimide compounds. However, inevitable side reactions such as amine-maleimide aza-Michael addition must be taken into account when choosing reactant stoichiometry such that \([\text{Mal}]_0 > [\text{P}_n\text{SH}] + [\text{amine}]; [\text{P}_n\text{SH}]\) and \([\text{amine}]\) are the polymeric thiol and unreacted amine concentrations respectively after complete RAFT agent aminolysis has occurred. In this work, we found that aminolysis of \(\text{45}\) with HexAM using a molar ratio of \([\text{45}]_0:[\text{HexAM}]_0 = 1.0:2.5\) results in complete loss of dithiobenzoate end groups within 30 min. However, other work conducted by our group (not reported herein) has shown that dithiobenzoate-functional polystyrene synthesized by RAFT requires several hours for complete aminolysis to occur using the same dithiobenzoate to amine ratio. Therefore, the reactant feed ratios reported herein should be considered as a starting point for stoichiometric optimization of different RAFT polymer systems.

The type of RAFT agent being aminolyzed must also be considered when choosing reactant stoichiometry. Dithiobenzoate-terminated polymers react with one equivalent of amine to yield polymeric thiol and thiobenzamide byproducts in equimolar amounts. Conversely, trithiocarbonate-terminated polymers can react with two equivalents of amine to give the polymeric thiol, Z-group derived thiol, and thiourea byproduct in equimolar amounts. In this case, the reactant stoichiometry must allow for
[Mal]_0 > [P_nSH] + [amine] + [ZSH] where [ZSH] is the concentration of small molecule Z-group derived thiol.

Section 4. Growth-Then-Coupling Method of Molecular Brush Synthesis from RAFT Polymers and Thiol- Reactive Oxanorbornenes

Overview

In recent years, extensive efforts have been made towards developing synthetic methodologies to prepare functionally diverse and structurally complex molecular brush copolymers. Molecular brush or “bottle-brush” copolymers are comprised of polymeric side-chains attached to a polymer backbone and exhibit highly branched spherical or cylindrical morphologies with minimal intermolecular chain entanglement owing to the volume-exclusion interactions between sterically crowded polymeric side-chains. Consequently, molecular brush (co)polymers have been used as polymer nanotherapeutics, rheological modifiers, surfactants, and as discrete nanostructures. The increased interest in molecular brushes has driven current efforts towards developing facile and versatile synthetic routes.

Molecular brush copolymers can be synthesized using grafting-to, grafting-from, grafting-through, or more recently by transfer-to routes. Of these approaches, grafting-through ensures the highest grafting density with 100% of polymer backbone repeat units bearing a polymeric side-chain. Lately, the strong thermodynamic driving force of ring opening metathesis polymerization (ROMP) of norbornene-functional macromonomers (MMs) has been demonstrated as an effective method of overcoming the issues of steric hindrance and low concentration of polymerizable end groups typically associated with grafting-through synthetic approaches.
Norbornene-functional macromonomers can be synthesized using either “direct-growth” (DG-MM) or “growth-then-coupling” (GC-MM) methods as reviewed recently by Xia and coworkers. DG-MM synthesis has been accomplished using norbornene-functional initiators during ring opening polymerization (ROP) and atom transfer radical polymerization (ATRP). In addition, norbornene-functional chain transfer agents have been used to prepare macromonomers using RAFT polymerization. Synthesizing MMs by RAFT is particularly advantageous due to the wide variety of vinyl monomers available and ease of controlling end-group functionality. However, DG-MM synthesis by RDRP techniques such as RAFT requires polymerization optimization for a given monomer-type to minimize radical addition to the norbornene olefin during polymerization. Furthermore Xia and coworkers have demonstrated that trace amounts of difunctional macromonomer impurities resulting from radical-radical coupling of α-norbornene-functional polymers can result in undesired molecular brush branching and broadened molecular weight distributions. Consequently, facile and efficient GC-MM synthetic routes are desired.

New Synthetic Route Toward Norbornene-Functional RAFT Polymer Macromonomers

We envisioned a GC-MM synthetic route that exploits the latent reactivity of thiocarbonylthio-terminated RAFT polymers. In Chapter IV Section III we demonstrated the efficient and quantitative end group functionalization of RAFT polymers using “one-pot” aminolysis/thiol-maleimide chemistry. Accordingly, norbornene end-functional RAFT polymers should be accessible using thiocarbonylthio end-functional RAFT polymers and a maleimide-functional oxanorbornene as illustrated in Scheme 4.14. Furthermore, it should be possible to end-functionalize RAFT polymers using a
methanethiosulfonate (MTS)-functional oxanorbornene (56) to give the corresponding disulfide-containing macromonomer. Molecular brushes comprised of side chains attached by disulfide linkages are of particular interest for drug delivery applications where selective intracellular reduction of disulfides would trigger molecular brush disassembly and favor polymer clearance in vivo.

Scheme 4.14. “One-pot” end group functionalization of RAFT polymers with thiol-reactive oxanorbornenes 52 and 56.

Synthesis of Thiol-Reactive oxaNorbornenes.

Maleimide-functional (52) and MTS-functional (56) oxanorbone derivatives were synthesized according to Schemes 4.15 and 4.16 respectively. Substituted oxanorbornedicarboximides are readily synthesized from the Diels-Alder reaction of furan and N-substituted maleimides. However, subsequent chemical reactions of oxanorbornene intermediates must be conducted at temperatures below 100 °C in order to avoid the furan-maleimide retro Diels-Alder reaction. Consequently, low temperature imide forming reactions were utilized for the synthesis of 52 and 56. Using the method developed by Keller and Rudinger, reaction of N-methoxycarbonylmaleimide (48) with primary amines in saturated NaHCO₃ at 0-23 °C affords the corresponding N-substituted maleimides which precipitated out of the aqueous reaction mixture in moderate to high yields and required no additional purification.
Reaction of sodium methanethiosulfonate (55) with primary bromide-functional oxanorbornenedicarboximide (54) affords in good yield the MTS-functional product 56. In contrast to symmetric disulfides which undergo thiol-disulfide exchange to form a mixture of mixed and homo disulfide products, reaction of MTS activated disulfides with thiols gives exclusively the mixed disulfide owing to the excellent leaving group ability and poor nucleophilicity of the methanesulfinic acid group. Consequently, this selectivity has been extensively used to prepared disulfide end-functional RAFT polymers.\textsuperscript{296}

Scheme 4.15. Synthetic route for 52.

Scheme 4.16. Synthetic route for 56.
"One-pot" Synthesis of RAFT Polymer Macromonomers from Thiol-Reactive oxanorbornenes

We first synthesized dithiobenzoate-terminated poly(styrene) (57) and poly(N,N-dimethylacrylamide) (58) as representative RAFT polymers for end group functionalization with thiol-reactive oxanorbornenes. Low dispersities and excellent agreement between the molecular weights determined by NMR and SEC-MALLS for 57 ($M_n$(NMR) = 3090 g/mol, $M_n$(SEC) = 2980 g/mol, $D$ = 1.03) and 58 ($M_n$(NMR) = 3710 g/mol, $M_n$(SEC) = 3630 g/mol, $D$ = 1.05) are indicative of controlled RAFT polymerization and high dithiobenzoate chain end fidelity.

The work in Chapter IV Section III of this dissertation shows that quantitative end group functionalization of RAFT polymers using "one-pot" aminolysis/thiol-maleimide chemistry is attainable using sequential aminolysis/maleimide addition, less polar solvents (e.g. CH₂Cl₂), and mild reducing agents (e.g. trimethylphosphite) while avoiding the use of aprotic nucleophilic catalysts such as amidines and phosphines. Similar reaction conditions were used for the reactions of 57 or 58 with 52 as outlined in the experimental section. Figure 4.25 shows the $^1$H NMR spectra of 57 before end group modification (Figure 4.25c) and after reaction with 52 (Figure 4.25b). Complete aminolysis of the dithiobenzoate end groups of 57 is observed by noting the disappearance of the aromatic protons at 8.0 – 7.3 ppm (Figure 4.25b). Ultimately, 98% end functionalization of 57 is achieved with the characteristic oxanorbornene peaks a, b, and c visible in Figure 4.25b. Similarly, reaction of the RAFT polymer 58 with 52 results in complete dithiobenzoate aminolysis and near-quantitative (>99%) end group functionalization efficiency as determined by $^1$H NMR (Figure 4.26). Minimal change in
M_0(SEC) and D of end-modified polymers (59 and 61) compared to the starting RAFT polymers (57 and 58) indicates little or no polymeric disulfide coupling (Table 4.6).

Figure 4.25. \(^1\)H NMR (300 MHz, acetone-d\textsubscript{6}) end group analysis of 57, 59, and 60.

End group functionalization of RAFT polymers 57 or 58 with 56 were also performed as “one-pot” reactions. RAFT polymer aminolysis was conducted in the presence of 56 using relative ratios of \([57/58]_0:[56]_0:[\text{amine}]_0 = 1.0:6.0:10\). \(^1\)H NMR end
group analysis of 56-modified RAFT polymers (60 and 62 respectively) confirms quantitative aminolysis of dithiobenzoate end groups along with the appearance of the characteristic oxanorbornene peaks. Reactions of 57 or 58 with 62 afforded moderately high end group functionalization efficiencies (95% and 94% respectively) with no significant change in Mₙ or D compared to the parent RAFT polymers indicating little or no polymeric disulfide coupling (Table 4.6).

Table 4.6

Summary of end group functionalization of RAFT polymers 57 and 58 with thiol-reactive oxanorbornenes 52ᵃ and 56ᵇ.

<table>
<thead>
<tr>
<th>entry</th>
<th>RAFT polymer</th>
<th>Mₙ(NMR)ᶜ (g/mol)</th>
<th>Mₙ(SEC)ᵈ (g/mol)</th>
<th>Dᵈ</th>
<th>norb. deriv.</th>
<th>funct.ᵉ</th>
<th>Mₙ(SEC)ᶠ (g/mol)</th>
<th>Dᶠ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (59)</td>
<td>57</td>
<td>3090</td>
<td>2980</td>
<td>1.03</td>
<td>52</td>
<td>98</td>
<td>3290</td>
<td>1.06</td>
</tr>
<tr>
<td>2 (60)</td>
<td>57</td>
<td>3090</td>
<td>2980</td>
<td>1.03</td>
<td>56</td>
<td>96</td>
<td>3360</td>
<td>1.05</td>
</tr>
<tr>
<td>3 (61)</td>
<td>58</td>
<td>3710</td>
<td>3630</td>
<td>1.05</td>
<td>52</td>
<td>&gt;99</td>
<td>3950</td>
<td>1.07</td>
</tr>
<tr>
<td>4 (62)</td>
<td>58</td>
<td>3710</td>
<td>3630</td>
<td>1.05</td>
<td>56</td>
<td>94</td>
<td>3930</td>
<td>1.05</td>
</tr>
</tbody>
</table>

ᵃ[57/58]c:[52]₀:[amine]₀ = 1.0:5.0:2.5.ᵇ[57/58]c:[56]₀:[amine]₀ = 1.0:6.0:10.ᶜ Mₙ(NMR) was determined by comparing the integral area of the dithiobenzoate aromatic protons (5H) to either the aromatic protons of 57 or the N,N-dimethyl and methyne protons of 58. ᵈTheoretical Mₙ values were calculated according to the equation Mₙ,th = ρMWₘ₀[M]₀/[CTA]₀ + MW₁ where ρ is the fractional monomer conversion, MWₘ₀ is the molecular weight of the monomer, and MW₁ is the molecular weight of the CTA. ᵉDetermined by ¹H NMR. ᶠMₙ(SEC) and D values of 57 and 58 were determined by SEC-MALLS (DMF 20 mM LiBr). ɢDetermined after end group modification.

Ring Opening Metathesis Polymerization of oxanorbornene End-Functional RAFT Polymers

![Diagram of RAFT Polymers](image)
Scheme 4.17. ROMP of $\omega$-oxanorbornenyl-functionalized macromonomers (59–62) into the corresponding molecular brushes (P59–P62).

The $\omega$-oxanorbornenyl-functionalized macromonomers (59–62) were next polymerized by ROMP using the 3rd Generation Grubbs catalyst (19) (Scheme 4.17). Polymerizations were conducted in CH$_2$Cl$_2$ using stoichiometric ratios of [MM]$_0$:[19]$_0 = 50:1.0$ and allowed to proceed for 6 h prior to termination via the addition of ethyl vinyl ether. SEC-MALLS analysis of aliquots taken during preliminary experiments confirmed that all reactions reached maximal MM conversion within 6 h. Figure 4.27a shows the RI traces for dithiobenzoate-terminated poly(styrene) RAFT polymer before (57) and after (59) end-functionalization with 52. 91% MM conversion was reached during the ROMP of 59, affording the corresponding molecular brush (P59) of low dispersity and with reasonable agreement between $M_{n_{th}}$ and $M_{n_{exp}}$ ($M_{n_{exp}} = 159,600$ g/mol, $D = 1.06$) (Table 4.7). Similarly, ROMP of the disulfide-containing MM (60) resulted in 87% MM conversion and produced the molecular brush P60 of low dispersity ($M_n = 198,200$ g/mol, $D = 1.04$) (Table 4.7). Worth noting is the bimodal shape of the residual MM peak following ROMP of 59 and 60 as shown in Figures 4.27a and 4.27b respectively. The residual MM peak appearing at longer elution volumes corresponds well with the peak elution volume of the respective MM. Meanwhile, the peak exhibiting a lower elution volume corresponds well with the high molecular weight shoulder exhibited by the RAFT polymer 57 and the resulting MMs (59 and 60). Consequently, the higher elution volume peak is likely due to coupled polymer resulting from termination by radical-radical coupling during RAFT polymerization. Such coupled polymer would not retain the RAFT agent end group and would not be functionalizable by 52 or 56. Nonetheless, these results show that macromonomers derived from 57 and thiol-reactive oxanorbornene...
derivatives 52 and 56 can be polymerized by ROMP to give the corresponding molecular brush of low dispersity and of relatively high MM conversion.

![SEC RI traces](image)

**Figure 4.27.** a) SEC RI traces of the parent RAFT polymer (▬ 57), 52-functional macromonomer (▬ 59), and corresponding molecular brush (▬ P59). b) SEC RI traces of the parent RAFT polymer (▬ 57), 56-functional macromonomer (▬ 60), and corresponding molecular brush (▬ P61).

SEC RI traces of macromonomers (61 and 62) synthesized from the water soluble RAFT polymer poly(N,N-dimethylacrylamide) (58) are shown in Figures 4.28a and 4.28b respectively. In contrast to the ROMP of poly(styrene)-based MMs (59 and 60), ROMP of 61 and 62 exhibited limited MM conversion and yielded molecular brushes of broad dispersities as shown in Table 4.7. Despite >99% oxanorbornenyl end group...
and 94% functionalization of 62. ROMP of 61 and 62 only reached 57% and 76% conversion respectively in 6 h. Furthermore, extending the reaction time beyond 6 h (data not shown) was determined to have no influence on MM conversion for the ROMP of 61 or 62. From these observations we hypothesize that catalyst 19 is likely degrading due to residual oxygen or as a result of some unforeseen degradation reaction. It is well known that polar solvents and functional groups can coordinate with Ru-based metathesis catalysts, consequently competing with monomer for the catalyst binding site and slowing the rate of ROMP. Indeed, the rate of polymerization measured during the ROMP of 61 and 62 is slower than that measured for the ROMP of the poly(styrene)-based MMs 59 and 60 (data not shown). Therefore it is plausible that the dimethylacrylamide side chains coordinate with 19 and slow the rate of polymerization enough for degradation of 19 (e.g. oxidation) to occur before complete consumption 61 or 62.
Figure 4.28. a) SEC RI traces of the parent RAFT polymer (▬ 58), 52-functional macromonomer (▬ 61), and corresponding molecular brush (▬ P61). b) SEC RI traces of the parent RAFT polymer (▬ 58), 56-functional macromonomer (▬ 62), and corresponding molecular brush (▬ P62).

Table 4.7

ROMP of RAFT-derived macromonomers summary.

<table>
<thead>
<tr>
<th>entry</th>
<th>MM</th>
<th>MM conv.(^a) (%)</th>
<th>M(_{\text{nth}})^b (g/mol)</th>
<th>M(_{\text{exp}})^c (g/mol)</th>
<th>D(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>91</td>
<td>135,600</td>
<td>159,600</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>87</td>
<td>129,600</td>
<td>198,200</td>
<td>1.04</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>57</td>
<td>103,500</td>
<td>161,000</td>
<td>1.24</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>76</td>
<td>137,900</td>
<td>141,700</td>
<td>1.23</td>
</tr>
</tbody>
</table>

\(^a\)Macromonomer conversion was determined from the relative integral areas of the SEC RI traces at t = 0 min and t = 6 h. \(^b\)Theoretical M\(_n\) values were calculated according to the equation M\(_{\text{nth}}\) = (\(\rho\)M\(_{\text{mol}}\)[MM] / [I\(_0\)]) where \(\rho\) is the fractional macromonomer conversion and M\(_{\text{mol}}\) is the molecular weight of the macromonomer. \(^c\)Experimental M\(_n\) and M\(_w\)/M\(_n\) values were determined by SEC-MALLS (DMF 20 mM LiBr).

Reduction-Induced Molecular Brush Disassembly

Polymer therapeutics have made extensive use of disulfide reduction as an intracellular specific bond-breaking reaction in order to trigger therapeutic agent release.
and polymer disassembly/degradation following cellular internalization.\textsuperscript{171} Similarly, we envision the use of disulfide-containing molecular brushes as highly branched “delivery vehicles” which would exhibit extended circulation times \textit{in vivo} and undergo reduction-induced disassembly upon cellular internalization to promote subsequent polymer clearance from the body. Accordingly, we investigated the reduction-induced disassembly of P\textsuperscript{62}, which is comprised of water soluble poly(N,N-dimethylacrylamide) side chains attached to a poly(oxanorbornenedicarboximide) backbone via disulfide linkages, under physiological conditions (pH = 7.4 PBS buffer) (Scheme 4.18). DL-dithiothreitol (DTT) was used as a representative reducing agent at [DTT] = 5 mM in accordance with the natural concentration range of glutathione found in mammalian cells (1-10 mM). Residual macromonomer present after ROMP of 62 (Figure 4.28) was removed by dialysis against water (MWCO = 15 kDa) followed by lyophilization. Figure 4.29 shows the monomodal SEC RI trace of P\textsuperscript{62} after dialysis (black trace).

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme.png}
\caption{DTT reduction of P\textsuperscript{62} in pH = 7.4 PBS buffer (10 mM) at 23 °C.}
\end{scheme}

DTT reduction of P\textsuperscript{62} was monitored by SEC-MALLS (DMF 20 mM LiBr) by injecting aliquots at timed intervals using an autosampler. A solution of P\textsuperscript{62} in pH = 7.4 PBS was initially prepared in a GPC vial equipped with piercable cap to give a final disulfide concentration of 1 mM. Subsequently, a stock solution of DTT in PBS was added to the GPC vial to give a final [DTT] = 5 mM. The first aliquot, which was injected 5 min after addition of DTT, is shown in Figure 4.29 (red trace). Remarkably,
disappearance of the peak corresponding to \textbf{P62} is observed by the first aliquot. The appearance of a new peak at \( \sim 17.5 \text{ mL} \) is nearly equivalent to the elution volume of \textbf{62} (elution volume = 17.4 mL). The second new peak with elution volume = 20.2 mL is that of the PBS buffer salts. Also worth noting is that precipitation was observed in the GPC vial following the addition of DTT and is likely due to the insolubility of the poly(oxanorbornenedicarboximide) backbone (Scheme 4.18) in PBS.

![SEC RI trace](image)

\textit{Figure 4.29.} SEC RI trace of the disulfide-containing molecular brush after purification by dialysis (--- \textbf{P62}) and SEC RI trace (---) of \textbf{P62} 5 min after the addition of DTT (5 eq.). \textbf{P62} molecular brush reduction reactions were performed in pH = 7.4 phosphate buffered saline (10 mM) at 23 °C.
CHAPTER V

CONCLUSIONS

Section 1. Tunable pH- and CO₂-Responsive Sulfonamide-Containing Polymers by RAFT Polymerization

A series of pMSA polymers with tunable, pH-dependent solubility in aqueous media has been synthesized by RAFT polymerization. Initially, polymerizations conducted in DMF at 70 °C gave polymers with broad molecular weight distributions, but upon reducing the polymerization temperature to 30 °C and employing the low decomposition temperature initiator V-70, polymers of narrow molecular weight distribution and increased thiocarbonylthio chain end functionality were obtained. Selection of the sulfonamide R-group of MSA monomers is a facile means of adjusting pKₐ and ultimately the critical onset of precipitation pH (pH*) of the corresponding pMSA. Thus it is possible to “fine tune” pH-dependent polymer solubility in the biologically relevant regime (pH = 4.5-7.4). Additionally, we demonstrated, the reversible CO₂-responsiveness of pMSAs in aqueous media, further indicating the potential of pMSAs in biological and nanotherapeutic applications.

Section 2. Mechanistic Insights into Temperature-Dependent Trithiocarbonate Chain-End Degradation during the RAFT Polymerization of N-Arylmethacrylamides

Methacrylamide-induced trithiocarbonate degradation during RAFT polymerization has been investigated. N-phenyl-promoted nucleophilic attack of the terminal trithiocarbonate by the ultimate methacrylamide unit was shown to occur by N-5 cyclization/elimination, resulting in rapid loss of active chain ends in DMF at 70 °C. The 3-phenyl-2-thioxothiazolidin-4-one RAFT polymer chain ends resulting from N-5 cyclization/elimination were shown to have little direct influence on the RAFT process.
and thus function as “dead” chain ends. Suppression of methacrylamide-induced trithiocarbonate degradation during the RAFT polymerization of N-arylmethacrylamides can be achieved by reducing the reaction temperature to 30 °C. Work is currently underway in our labs to study the influence of N-aryl amide substitution on both the reaction mechanism and rate of N-5 cyclization/elimination.

Section 3. “One-Pot” Aminolysis/Thiol-Maleimide End Group Functionalization of RAFT Polymers: Identifying and Preventing Michael Addition Side Reactions

In this work we have elucidated a number of deleterious nucleophile-promoted side reactions that occur during the “one-pot” aminolysis/thiol-maleimide end group modification of RAFT polymers. Nucleophilic thiol-ene Michael addition catalysts including amines, amidines, and phosphines were shown to initiate the anionic polymerization of N-methylmaleimide in a range of solvents with the rate of reaction increasing with solvent polarity. We also demonstrated that in more polar solvents, thiols can initiate the anionic polymerization of maleimides when [Mal] > [thiol]. Mechanistic evidence of TEA-catalyzed enolate formation of the thiol-maleimide adduct was acquired using proton-deuterium exchange experiments of thiol-maleimide adducts and used to prove that thiol-maleimide adducts were capable of direct initiation of maleimide polymerization in the presence of a weak base. Ultimately, optimal reaction conditions for the selective and near quantitative “one-pot” end group modification of RAFT polymers using thiol-maleimide chemistry was identified
Section 4. Growth-Then-Coupling Method of Molecular Brush Synthesis from RAFT Polymers and Thiol-Reactive Oxanorbornenes

This section details a new “grafting through” synthetic route towards molecular brushes capable of intracellular-induced disassembly. RAFT polymer-derived macromonomers were synthesized using “one-pot” aminolysis/thiol reactions with maleimide- or methanethiosulfonate-functional oxanorbornenes. Subsequent ring opening metathesis polymerization (ROMP) of the resulting macromonomers afforded molecular brushes with RAFT polymer side chains attached to a polyoxanorbornene backbone via either permanent thioether linkages or reversible disulfide linkages. Molecular brushes comprised of disulfide linkages were shown to undergo reduction-induced disassembly and show promise a new class of stimuli-responsive polymer therapeutics.
Figure A1. $M_n$ versus % conversion for 37-mediated polymerization of 33 at 70 °C.

Figure A2. $M_n$ versus % conversion for 37-mediated polymerization of 34 at 70 °C.
Figure A3. $M_n$ versus % conversion for 37-mediated polymerization of 33 at 30 °C.

Figure A4. $M_n$ versus % conversion for 37-mediated polymerization of 34 at 30 °C.
Figure A5. Beer-Lambert plot and molar extinction coefficients ($\varepsilon$) for 37, 41, and 42 in acetonitrile measured using a Lambda 35 UV-vis spectrometer ($\lambda = 320$ nm).
Figure A6. (a) $^1$H NMR (600 MHz, DMF-d$_7$) spectrum of 1-dodecanethiol, (b) $^1$H NMR (600 MHz, DMF-d$_7$) spectrum acquired at $t = 491$ min during the in situ degradation analysis of 41 at 70 °C, (c) $^1$H NMR (600 MHz, DMF-d$_7$) spectrum of 40. Peaks of interest corresponding to degradation byproducts formed during in situ degradation analysis (Figure A6b) are colored red and blue and correspond well with key peaks of analogous compounds 1-dodecanethiol (red) and 40 (blue) (Figures A6a and A6c respectively).
Figure A7. Expanded region (3.00 - 0.5 ppm) of the $^1$H NMR (600 MHz, DMF-d$_7$) spectrum acquired at t = 5 min during in situ degradation analysis of 41 at 70 °C.

Figure A8. Expanded region (3.00 - 0.5 ppm) of the $^1$H NMR (600 MHz, DMF-d$_7$) spectrum acquired at t = 491 min during in situ degradation analysis of 41 at 70 °C. Overlapping peaks are colored red or blue for improved visualization.
Figure A9. a) $^1$H NMR spectrum of E2MP in DMSO-d$_6$ at T = 0 min. b) $^1$H NMR spectrum during the reaction of E2MP and 65 in DMSO-d$_6$ at T = 3 min. Disappearance of the thiyl (peak c) and methyne (peak d) protons of E2MP confirm quantitative thiol conversion by 3.0 min.

Figure A10. a) $^1$H NMR spectrum of E2MP in MeCN-d$_3$ at T = 0 min. b) $^1$H NMR spectrum during the reaction of E2MP and 65 in MeCN-d$_3$ at T = 2.3 min. Disappearance of the thiyl (peak c) and methyne (peak d) protons of E2MP confirm quantitative thiol conversion by 2.3 min.
Figure A11. a) $^1$H NMR spectrum of E2MP in CD$_2$Cl$_2$ at $T = 0$ min. b) $^1$H NMR spectrum during the reaction of E2MP and 65 in CD$_2$Cl$_2$ at $T = 2.5$ min. Disappearance of the thyl (peak c) and methyne (peak d) protons of E2MP confirm quantitative thiol conversion by 2.5 min.
Figure A12. Effect of solvent on the time-dependent fractional change in $[\text{Mal}]/[\text{Mal}]_0$ during the reaction of TEA with 65 as measured by in situ $^1$H NMR analysis.

Figure A13. Effect of solvent on the time-dependent fractional change in $[\text{Mal}]/[\text{Mal}]_0$ during the TEA-catalyzed reaction of HexAM with 65 as measured by in situ $^1$H NMR analysis.
Figure A14. $^1$H NMR (300 MHz, D$_2$O) spectrum of 45.

Figure A15. Representative $^1$H NMR (300 MHz, D$_2$O) spectrum of 46-functionalized 45 (Table 4.5 entry 1a).
Figure A16. $^1$H NMR spectrum (300 MHz, CDCl$_3$) of 52.

Figure A17. $^1$H NMR spectrum (300 MHz, CDCl$_3$) of 56.
APPENDIX B

N-ARYLAMIDE NUCLEOPHILICITY

Overview

Previously, we described the unique influence of N-phenyl methacrylamide substitution on the limited RAFT polymerization control of methacryloylsulfonamides (27-32)\(^\text{97}\) and N-phenylmethacrylamide (33)\(^\text{330}\) at 70 °C in DMF. We showed that chain end degradation during the trithiocarbonate-mediated polymerization of 33 at elevated temperatures occurs by N-5 nucleophilic attack of the terminal thiocarbonyl moiety by the ultimate methacrylamide unit. Although mechanistic insights were gained into the specific degradation pathway (N-5 cyclization/elimination) as discussed in Chapter IV Section II, the nature of the true nucleophilic species involved in N-5 cyclization and the influence of N-phenyl amide substitution is still not understood. The purpose of this work is to better understand the influences of N-phenyl substitution on amide nucleophilicity and develop a fundamental understanding of how substituents influence the mechanism of amide-based nucleophilic reactions.

\[
\text{Scheme A1. Influence of ionized (A2) or conjugate base (A4) resonance forms on amide nucleophilicity.}
\]

\[
\text{O-nucleophilic attack}
\]

\[
\text{N-nucleophilic attack}
\]
The structures and reactivity of neutral amides (A1) are greatly influenced by the ionized resonance form (A2) whereby the lone pair on nitrogen is delocalized onto the carbonyl oxygen, increasing the double bond character of the C-N bond and increasing the electron density of the carbonyl oxygen atom (Scheme A1). Consequently, N-substituted amides exhibit planar structures due to restricted C-N bond rotation and react with electrophiles via the oxygen atom to afford the O-substituted product (A3). Alternatively, amide anions derived from protic amides and strong bases (e.g. NaH) (A4), react with electrophiles almost exclusively via the nitrogen atom to give the N-substituted product (A5). Selective reaction of amide anions at the nitrogen atom has been rationalized using the “hard and soft Lewis acids and bases” (HSAB) theory which attempts to predict/explain the outcomes of substitution reactions involving ambident nucleophiles (i.e. nucleophiles with two potential nucleophilic sites). HSAB theory can be applied to ambident nucleophiles such as amide anions by way of Kornblum’s rule which states:

“… hard acids prefer hard bases and soft acids prefer soft bases. In an SN1 mechanism, the nucleophile attacks a carbocation, which is a hard acid. In an SN2 mechanism, the nucleophile attacks the carbon atom of a molecule, which is a softer acid. The more electronegative atom of an ambident nucleophile is a harder base than the less electronegative atom. Therefore, as the character of a given reaction changes from SN1 to SN2 like, an ambident nucleophile becomes more likely to attack with its less electronegative atom.”331
In other words, SN$_2$ reactions involving amide anions will proceed via the less electronegative nitrogen atom ($\chi = 3.0$) rather than more electronegative oxygen atom ($\chi = 3.5$), where $\chi$ is the Pauling electronegativity value. This adequately describes the selectivity in forming the N-substituted product $A_5$ upon reaction of amide anion $A_4$ with an electrophilic species by an SN$_2$ mechanism as shown in Scheme A1. However, according to these theories, the neutral amide of $41$ should react via the oxygen atom to give O-5 cyclization/elimination products ($41B$) rather than the observed N-5 cyclization/elimination products ($41A$) as shown in Scheme A2. The key to understanding this mechanism and its products must therefore lie in the role of N-aryl substitution on amide nucleophilicity.

Scheme A2. Proposed degradation of $41$ by N-5 cyclization/elimination (pathway A).

In Chapter IV Section II we show that i) N-phenyl methacrylamide substitution increases the observed rate of trithiocarbonate degradation of $41$ compared to N-benzyl substitution ($42$) and ii) degradation of $41$ proceeds by N-5 cyclization/elimination. We can rationalize these observations if N-phenyl substitution promotes amide N-H dissociation to give the amide anion (and solvated proton) where the amide anion is the true nucleophilic species. As shown in Scheme A3, dissociation of an N-arylamide ($A_6$) affords the corresponding amide anion with O- and N-based resonance structures $A_7$ and
respectively. However, direct N-aryl substitution provides additional resonance forms \( \text{A9-A11} \) that can further stabilize the amide anion. These additional resonance forms should reduce the pK\(_a\) of \( \text{A6} \) by stabilizing the conjugate base and consequently shift the equilibrium towards the dissociated amide anion species ([CON\(^-\)Ph]) compared to an analogous amide with N-alkyl substituents. Indeed, comparison of the literature pK\(_a\) values of N-methylacetamide (pK\(_a\) = 25.9, DMSO\(^{332}\)) and N-phenylacetamide (pK\(_a\) = 21.5, DMSO\(^{333}\)) confirms the influence of N-aryl substitution on increased amide acidity in polar organic solvents. From these observations, we hypothesize that N-aryl substitution decreases the pK\(_a\) of \( \text{A1} \) and consequently increases [CON\(^-\)Ph], accounting for the increased rate of degradation by N-5 cyclization/elimination as compared to \( \text{A2} \).

\[ \begin{align*} 
\text{A8} & \quad \text{respectively. However, direct N-aryl substitution provides additional resonance forms} \quad \text{A9-A11 that can further stabilize the amide anion. These additional resonance forms} \\
& \quad \text{should reduce the pK\(_a\) of A6 by stabilizing the conjugate base and consequently shift the} \\
& \quad \text{equilibrium towards the dissociated amide anion species ([CON\(^-\)Ph]) compared to an} \\
& \quad \text{analogous amide with N-alkyl substituents. Indeed, comparison of the literature pK\(_a\) values of N-methylacetamide (pK\(_a\) = 25.9, DMSO\(^{332}\)) and N-phenylacetamide (pK\(_a\) = 21.5, DMSO\(^{333}\)) confirms the influence of N-aryl substitution on increased amide acidity in polar organic solvents. From these observations, we hypothesize that N-aryl substitution decreases the pK\(_a\) of A1 and consequently increases [CON\(^-\)Ph], accounting for the increased rate of degradation by N-5 cyclization/elimination as compared to A2.} 
\end{align*} \]

\[ \text{Scheme A3. Possible N-arylamide conjugate base resonance forms.} \]
To test this hypothesis, we have synthesized single monomer unit insertion adducts $A_{12}$ and $A_{13}$ which possess either electron withdrawing p-chloro or electron donating p-methoxy groups respectively (Figure A18). According to our theory, the electron withdrawing p-chloro substituent of $A_{12}$ should decrease the amide $pK_a$ and consequently increase the amide anion concentration ([CON PhCl]) resulting in an increased rate of degradation by N-5 cyclization/elimination as compared to 41. Conversely, electron donating p-methoxy substitution should increase the amide $pK_a$ of $A_{13}$ relative to 41 and decrease the rate of degradation by N-5 cyclization/elimination as compared to 41. Meanwhile we have already shown in Chapter IV Section II that 41 degrades significantly faster than 42 by N-5 cyclization/elimination. It should therefore hold true that $pK_a 41 < pK_a 42$. Indirect evidence of the relative $pK_a$ values of 41 and 42 can be inferred by noting the relative chemical shifts of the amide N-H protons in Figures 4.16 and 4.17 respectively (Chapter IV Section II). The N-H chemical shift (600 MHz, DMF-$d_7$) of 41 (9.64 ppm) is significantly downfield shifted compared to the N-H chemical shift of 42 (8.34 ppm). Greater deshielding of the N-H proton of 41 relative to 42 is indicative of increased acidity (lower $pK_a$).

Figure A18. Proposed inverse relationship between amide $pK_a$ and apparent amide nucleophilicity.
In this work, we will study the influence of amide substituent, solvent polarity, temperature, and acid/base catalysis on the kinetics of N-5 cyclization/elimination of the SMUI adducts 41, 42, A12, and A13 in order to test our theory of N-arylamide nucleophilicity. This work is currently on-going and therefore the following results and conclusions are incomplete. However, the results reported herein thus far support our current theory of N-arylamide nucleophilicity.
Experimental

4-Chlorophenylmethacrylamide **A14**

Methacryloyl chloride (5.83 mL, 59.7 mmol) was added dropwise over 15 min to a stirred solution of 4-chloroaniline (7.25 g, 56.8 mmol) and triethylamine (8.32 mL, 59.7 mmol) in CH$_2$Cl$_2$ (150 mL) that was previously cooled using an ice bath. Upon complete addition of methacryloyl chloride, the reaction was stirred at 0 °C for 30 min followed by stirring at room temperature for an additional 60 min. The reaction mixture was then transferred to a separatory funnel and washed with 0.1 N HCl (1 × 150 mL), saturated NaHCO$_3$ (1 × 150 mL), and saturated NaCl (brine) (1 × 150 mL) before drying over MgSO$_4$. The solvent was removed via rotary evaporation and the isolated solids recrystallized from hot hexanes:THF (90:10) to yield **A14** (10.05 g, 90%) as colorless needle-like crystals. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.52 (s, 1H), 7.43 (d, 2H), 7.23 (d, 2H), 5.72 (s, 1H), 5.40 (s, 1H), 1.98 (s, 3H).

4-Methoxyphenylmethacrylamide **A15**

Methacryloyl chloride (5.83 mL, 59.7 mmol) was added dropwise over 15 min to a stirred solution of p-anisidine (7.00 g, 56.8 mmol) and triethylamine (8.32 mL, 59.7 mmol) in CH$_2$Cl$_2$ (150 mL) that was previously cooled using an ice bath. Upon complete addition of methacryloyl chloride, the reaction was stirred at 0 °C for 30 min followed by stirring at room temperature for an additional 60 min. The reaction mixture was then transferred to a separatory funnel and washed with 0.1 N HCl (1 × 150 mL), saturated NaHCO$_3$ (1 × 150 mL), and saturated NaCl (brine) (1 × 150 mL) before drying over MgSO$_4$. The solvent was removed via rotary evaporation and the isolated solids recrystallized from hot hexanes:THF (90:10) to yield **A15** (10.05 g, 90%) as colorless needle-like crystals. $^1$H NMR (300 MHz, CDCl$_3$): δ 7.52 (s, 1H), 7.43 (d, 2H), 7.23 (d, 2H), 5.72 (s, 1H), 5.40 (s, 1H), 1.98 (s, 3H).
addition of methacryloyl chloride, the reaction was stirred at 0 °C for 30 min followed by stirring at room temperature for an additional 60 min. The reaction mixture was then transferred to a separatory funnel and washed with 0.1 N HCl (1 × 150 mL), saturated NaHCO₃ (1 × 150 mL), and saturated NaCl (brine) (1 × 150 mL) before drying over MgSO₄. The solvent was removed via rotary evaporation and the isolated solids recrystallized from hot hexanes:THF (90:10) to yield A15 (7.60 g, 70%) as colorless needle-like crystals. ¹H NMR (300 MHz, CDCl₃): δ 7.38 (d, 3H), 6.79 (d, 2H), 5.71 (s, 1H), 5.37 (s, 1H), 3.73 (s, 2H), 1.99 (s, 3H).

Synthesis of A12

A solution of 27 (3.41 g, 9.9 mmol), A14 (1.93 g, 9.9 mmol), and V-70 (0.609 g, 2.0 mmol) in DMF (35 mL) was prepared in a round bottomed flask equipped with magnetic stir bar and the reaction degassed via three freeze-pump-thaw cycles and backfilled with argon. The reaction mixture was heated at 30 °C in an oil bath for 48 h, followed by exposure to air and freezing in liquid N₂. The reaction mixture was then diluted with EtOAc (150 mL) and transferred to a separatory funnel and washed with 75% brine (1 × 150 mL), H₂O (1 × 150 mL), brine (1 × 150 mL), and dried over MgSO₄. The crude product was then purified by column chromatography (85:15 Hexanes:EtOAc, Rₜ = 0.20) yielding A12 (0.427 g, 8%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃): δ 8.41 (s, 1H), 7.32 (d, 2H), 7.23 (d, 2H), 3.20 (t, 2H), 2.47 (s, 2H), 1.95 (s, 3H), 1.56 (b, 2H), 1.41 (d, 6H), 1.18 (b, 18H), 0.81 (t, 3H).
Synthesis of \( \text{A13} \)

A solution of \( \text{27} \) (3.41 g, 9.9 mmol), \( \text{A15} \) (1.89 g, 9.9 mmol), and V-70 (0.609 g, 2.0 mmol) in DMF (35 mL) was prepared in a round bottomed flask equipped with magnetic stir bar and the reaction degassed via three freeze-pump-thaw cycles and backfilled with argon. The reaction mixture was heated at 30 °C in an oil bath for 48 h, followed by exposure to air and freezing in liquid N\(_2\). The reaction mixture was then diluted with EtOAc (150 mL) and transferred to a separatory funnel and washed with 75% brine (1 × 150 mL), H\(_2\)O (1 × 150 mL), brine (1 × 150 mL), and dried over MgSO\(_4\). The crude product was then purified by column chromatography (75:25 Hexanes:EtOAc, \( R_f = 0.28 \)) yielding \( \text{A13} \) (0.452 g, 9%) as a yellow solid. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \)

- 8.26 (s, 1H), 7.26 (d, 2H), 6.78 (d, 2H), 3.72 (s, 3H), 3.21 (t, 2H), 2.48 (s, 2H), 1.96 (s, 3H), 1.58 (b, 2H), 1.40 (d, 6H), 1.18 (b, 18H), 0.81 (t, 3H).

In situ \(^1\)H NMR Analysis

A representative procedure is as follows: A solution of \( \text{41} \) (2 × 10\(^{-2}\) M) in DMSO-d\(_6\) (0.6 mL) was prepared in an NMR tube equipped with pierceable rubber septum and the solvent degassed by two freeze-pump-thaw cycles followed by backfilling the NMR tube with argon. \(^1\)H NMR spectra were acquired at predetermined temperatures using a Bruker Ascend™ 600 MHz spectrometer.
Results and Discussion

If the rate determining step of N-5 cyclization/elimination of 41 is dependent upon [CON\text{-}Ph], a non-nucleophilic base should catalyze the reaction by promoting the formation of the nucleophilic CON\text{-}Ph species. To this end, we examined the effect of triethylamine (TEA) (pK\text{a} = 9.00, DMSO)\textsuperscript{334} as a base catalyst for N-5 cyclization/elimination of 41 in DMSO-d\text{6} at room temperature (23 °C). As shown in Figure A19, 100% degradation of 41 was observed within the time required to obtain the first spectrum (5 min) with peaks corresponding to the degradation byproducts given prime designation. In contrast, the half life of 41 in DMF-d\text{7} at 70 °C in the absence of TEA was measured to be $t_{1/2} = 7.18$ h (Chapter IV Section II).

![Figure A19. $^1$H NMR (300 MHz, DMSO-d\text{6}) overlay following the TEA-catalyzed degradation of 41 at room temperature.](image)

Base-catalyzed degradation of 41 is evidence of the rate determining step of N-5 cyclization/elimination being dependent upon [CON\text{-}Ph]. Consequently, we can develop a kinetic theory based upon Scheme A2 where the rate of irreversible trithiocarbonate...
(TTC) degradation due to intramolecular nucleophilic attack by an adjacent amide can be expressed as follows:

\[
Rate = -\frac{d[TTC]}{dt} = k_{app}[CONHR]_{tot}
\]  \hspace{1cm} (A1)

where [TTC] is the concentration of 41, [CONHR]_{tot} is the total concentration of associated and dissociated amide, and \( k_{app} \) is the apparent rate constant for intramolecular nucleophilic attack. Assuming that nucleophilic attack (pathway A and/or B, Scheme A2) is the rate determining step, the total rate of trithiocarbonate degradation can be expressed as the sum of the rates of degradation by both N-5 and O-5 intramolecular nucleophilic attack:

\[
-\frac{d[TTC]}{dt} = k_1[CON^-R] + k_2[CONHR]
\]  \hspace{1cm} (A2)

where [CON^-R] is the concentration of amide anion, [CONHR] is the concentration of neutral amide, and \( k_1 \) and \( k_2 \) are the first order rate constants for N-5 and O-5 nucleophilic attack respectively. We have shown that degradation of 41 occurs exclusively by N-5 cyclization such that \( k_2 = 0 \). This allows for simplification of Eq A2 to the following:

\[
-\frac{d[TTC]}{dt} = k_1[CON^-R]
\]  \hspace{1cm} (A3)

The quantity [CON^-R], which cannot be measured directly, can be determined from the measurable quantity [CONHR] and the dissociation constant (\( K_a \)) according to the equilibrium shown in Eq A4.

\[
[CONHR] \rightleftharpoons [CON^-R][H^+]
\]  \hspace{1cm} (A4)
\[ K_a = \frac{[\text{CON}^-\text{R}][H^+]}{[\text{CONHR}]} \]  

(A5)

In the absence of external acid or base, \([\text{CON}^-\text{R}] = [\text{H}^+]\) such that:

\[ [\text{CON}^-\text{R}] = K_a^{\frac{1}{2}}[\text{CONHR}]^{\frac{1}{2}} \]  

(A6)

Substitution of Eq A6 into Eq A3 gives:

\[ -\frac{d[TTC]}{dt} = k_1 K_a^{\frac{1}{2}}[\text{CONHR}]^{\frac{1}{2}} \]  

(A7)

The total trithiocarbonate concentration \([\text{TTC}]\), which is equal to the total amide concentration (\([\text{CONHR}]_{\text{tot}}\)) can be expressed as:

\[ [\text{TTC}] = [\text{CONHR}]_{\text{tot}} = [\text{CONHR}] + [\text{CON}^-\text{R}] \]  

(A8)

When \(K_a\) is small and \([\text{CONHR}] \gg [\text{CON}^-\text{R}]\), Eq A8 simplifies to \([\text{TTC}] = [\text{CONHR}]\)

and can be substituted into Eq A7 to give:

\[ -\frac{d[TTC]}{dt} = k_1 K_a^{\frac{1}{2}}[\text{TTC}]^{\frac{1}{2}} \]  

(A9)

Integration of Eq. A9 using the integral form of an \(n\)th order rate equation (Eq A10) gives the final integrated rate equation of trithiocarbonate degradation by N-5 cyclization (Eq. A11).

\[ \frac{1}{[A]^{n-1}} = \frac{1}{[A]_0^{n-1}} + (n-1)kt \]  

(A10)
According to Eq A11, plotting [TTC]^{1/2} versus time will give the y-intercept = [TTC]_0^{1/2} and slope = -(1/2)k_1K_a^{1/2}.

Figure A20. [TTC]^{1/2} vs time plots for the degradation of 41 and 42 in DMF-d7 at 70 °C ([TTC]_0 = 0.0329 M).

We next used Eq A11 to replot the kinetic data obtained previously for the degradation reactions of 41 and 42 from Figures 4.18a and 4.18b respectively (Chapter IV Section II). As shown in Figure A20, the graph of [TTC]^{1/2} vs time yields linear plots for the degradation of 41 and 42 in DMF-d7 at 70 °C. The k_1K_a^{1/2} values for 41 and 42 in DMF at 70 °C were calculated from the slopes of the linear plots in Figure A20 to be 2.22 \times 10^{-4} \text{ M}^{1/2} \text{ s}^{-1} and 1.86 \times 10^{-5} \text{ M}^{1/2} \text{ s}^{-1} respectively. However, in order for k_1 to be determined, K_a for the amide must be known.
While $K_a$ for the amides of interest in this study (41, 42, A12, A13) could be measured by potentiometric or photometric titrations in DMSO using a strong base such as sodium dimsyl, rapid thioacetate degradation would likely occur due to nucleophilic attack of the thioacetate by the dimsyl anion and also as a result of base-catalyzed N-5 cyclization. Therefore an alternative method of determining $K_a$ in situ is desired. This can be accomplished by disturbing the amide dissociation equilibrium (Eq 4) via the addition of a known amount of strongly dissociating “super acid,” such as bis trifluoromethanesulfonimide, and measuring the effect of $[H^+]$ on $k_{\text{app}}$ (Eq 1) which is a function of both $k_1$ and $K_a$. If the dissociation constant of the acid ($K_{a,\text{acid}}$) is suitably large compared to $K_a$ of the amide, $[H^+] \approx [HX]$ where $[HX]$ is the concentration of externally added acid. The relationship between $[HX]$ and $[\text{CON}^-\text{R}]$ can then be expressed as follows:

$$[\text{CON}^-\text{R}] = \frac{K_a[\text{CONHR}]}{[HX]}$$ (A12)

Combination of Eq A1, Eq A3, and Eq A12 gives:

$$k_{\text{app}}[\text{TTC}] = \frac{k_1K_a[\text{CONHR}]}{[HX]}$$ (A13)

Recalling that $[\text{TTC}] \approx [\text{CONHR}]$, Eq A13 can be simplified to:

$$k_{\text{app}} = \frac{k_1K_a}{[HX]}$$ (A14)

Plotting $k_{\text{app}}$ vs. $[HX]^{-1}$ would yield a slope $= k_1K_a$. $K_a$ can then be determined by combining the slopes from Eq A11 and Eq A14:
\[ k_1 = \frac{-2slope_1}{K_a^2} \text{ (from Eq 11)} \]  
\[ k_1 = \frac{slope_2}{K_a} \text{ (from Eq 14)} \]  
\[ K_a = \frac{1}{4} \left( \frac{slope_2}{slope_1} \right)^2 \]

where \(slope_1\) is the slope of \([TTC]^{1/2}\) vs. time plot (Eq A11) and \(slope_2\) is the slope of \(k_{app}\) vs. \([HX]^{-1}\) plot (Eq A14).

Despite not yet knowing the \(K_a\) values for the amides of interest in this study, we can make the following predictions regarding the influence of amide substituents on the values of \(k_1\) and \(K_a\) for uncatalyzed N-5 cyclization/elimination reactions.

1) Electron withdrawing substituents will *increase* the value of \(K_a\).

Electron withdrawing substituents will stabilize the conjugate base of the amide and thus shift the equilibrium towards the dissociated amide anion thus increasing the value of \(K_a\).

2) Electron withdrawing substituents will *reduce* the value of \(k_1\).

At first this seems counterintuitive since we predict that electron withdrawing substituents will increase the rate of degradation by N-5 cyclization/elimination. However, \(k_1\) is a function of the relative nucleophlicity of the amide anion. Electron withdrawing substituents will increase the stability of the amide anion relative to electron donating substituents and consequently reduce the nucleophlicity (and reduce \(k_1\)) of the amide anion. Accordingly, we predict that electron withdrawing substituents will cause
$K_a^{1/2}$ to increase more than $k_1$ will decrease, such that the product of $k_1K_a^{1/2}$, which is directly proportional to the rate of degradation by N-5 cyclization, will increase. In other words, the increase in concentration of the nucleophilic species [CON$^-\text{Ph}$] more than compensates for the reduced nucleophilicity of that species.

We can initially evaluate these predictions if we assume the $pK_a$ values of 41 and 42 in DMF-d$_7$ are similar to the $pK_a$ values of N-phenylacetamide ($pK_a = 21.5$, DMSO) and N-methylacetamide ($pK_a = 25.9$, DMSO) respectively. Using these estimated $pK_a$ values for 41 and 42 along with the $k_1K_a^{1/2}$ values determined previously from the slopes of the kinetic plots in Figure A20, we calculated the $k_1$ values for degradation by N-5 cyclization/elimination of 41 and 42 to be $1.25 \times 10^7$ M$^{1/2}$ s$^{-1}$ and $1.66 \times 10^8$ M$^{1/2}$ s$^{-1}$ respectively. As predicted, the $k_1$ value of the more acidic amide (41) is lower (by ~1 order of magnitude) than the $k_1$ value for the less acidic amide (42) despite the rate of N-5 cyclization/elimination of 41 being greater than that of 42.

**Conclusions**

We have estabilised a mechanistic theory in order to explain the influence of N-aryl substitution on the observed relative rates of N-5 cyclization/elimination of 41 and 42 at elevated temperatures. In addition, we have developed a kinetic model based upon the “amide dissociation mechanism” which thus far fits the previously obtained kinetic data. We also show that degradation by N-5 cyclization/elimination is base catalyzed and we are currently investigating the influence of electron withdrawing/donating substituents and solvent polarity on the N-5 cyclization/elimination rate constants ($k_1$) and dissociation constants ($K_a$) of select amides. Ultimately, this work has the potential to provide greater insights into the nucleophilic character of amides.
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