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Synthesis of Self-Immolative Monomers and Their Applications in Polymeric Materials

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Graduate Program in Chemistry

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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SYNTHESIS OF SELF-IMMOLATIVE MONOMERS AND THEIR APPLICATIONS IN POLYMERIC MATERIALS

(Spine title: Self-Immolative Monomers and Polymers)

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by

Matthew A. DeWit

Graduate Program in Chemistry

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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The thesis by

Matthew Alexander DeWit

entitled:

Synthesis of Self-Immolative Monomers and Their Applications in Polymeric Materials

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____________________  _______________________
Date                    Chair of the Thesis Examination Board
Abstract

Self-immolative spacers are a unique class of molecules employed in a variety of applications, particularly in the biomedical field. Most commonly, they are molecules containing two reactive termini with a capping group at one terminus and a substrate of interest at the other. Upon removal of the capping group, the spacer undergoes an intramolecular reaction that results in its removal from the molecule and liberation of the substrate. These spacers have been extensively studied in monomeric form within prodrugs, as well as to form dendrimers that have been used for applications such as signal amplification, molecular logic gates and amplified drug release. Their use to form polymeric systems, however, remains largely unexplored and undeveloped. The work described in this thesis serves to expand this particular area of research, exploring the use of amine-based self-immolative spacers in the context of self-immolative polymers.

Two new polymeric systems were developed using N,N-dimethylethlenediamine in conjunction with first 4-hydroxybenzyl alcohol and then 2-mercaptoethanol. In the first example, when a poly(ethylene glycol) end cap was used a block copolymer was formed that self-assembled in an aqueous environment and was capable of encapsulating a hydrophobic molecule and releasing it upon polymer degradation. The second polymeric system was the first fully aliphatic self-immolative polymer, and when a disulfide end cap was employed polymer degradation could be triggered by the addition of dithiothreitol.

Following this work, a series of novel self-immolative spacers derived from 4-aminobutyric acid were developed in efforts to gain access to more rapidly cyclizing amine-based spacers. Carrying out modifications to the N and α positions, a series of spacers were developed with half-lives of cyclization ranging from 2 – 39 s. Lastly, these spacers were then combined with 4-hydroxybenzyl alcohol and 2’-hydroxyhydrocinnamic acid in efforts to develop novel 4-aminobutyric acid-based self-immolative polymers.

Keywords

Self-immolative, monomer, polymer, block copolymer, spacer, cyclization, electronic rearrangement, degradation, synthesis, kinetics.
Co-Authorship Statement

The work described in this thesis contains contributions from the author as well as coworkers Andrew D. Wong and Annelise Beaton and supervisor Dr. Elizabeth Gillies. The exact contributions of each are described below.

Chapter 1 is composed of two portions, the first written by the author and the second containing portions of a review article written by Andrew D. Wong, Dr. Elizabeth Gillies and the author. The first portion comprises all of the sections on degradable and responsive polymers, while the sections on self-immolative materials were adapted from the review article. The sections on self-immolative spacers and self immolative oligomers were written by Andrew Wong, the section on self-immolative dendrimers was written by the author and the section on self-immolative polymers was written by Dr. Elizabeth Gillies.

Chapter 2 describes a project proposed by Dr. Elizabeth Gillies, with all experimental work carried out by the author under the supervision and guidance of Dr. Gillies. The manuscript was prepared by both Dr. Gillies and the author.

The work described in Chapter 3 was a collaboration between the author and Annelise Beaton, a fourth year undergraduate student supervised by the author. The project proposal was a joint effort between the author and Dr. Elizabeth Gillies, and the initial synthetic route was developed by the author. Preliminary synthetic studies were carried out by the author, but thereafter larger scale synthesis of all intermediates and polymers were carried out by Annelise Beaton under the supervision of the author. Degradation studies were carried out primarily by Annelise Beaton. Following Annelise’s departure from the lab, a final synthesis and degradation study of the polymer was carried out by the author. Manuscript preparation was carried out by both Dr. Gillies and the author.

Chapter 4 describes a project proposed by the author, and all experimental work was carried out by the author under the supervision and guidance of Dr. Gillies. The manuscript was first drafted by the author and Dr. Gillies provided assistance with editing and final preparations.
Chapter 5, likewise, describes a project proposed by the author with all experimental work being carried out by the author under the supervision of Dr. Gillies. Non-linear regression analysis of the cyclization kinetics of modified monomer $5.36$ was carried out by Ryan McBride. A draft was written by the author with Dr. Gillies providing assistance with editing and final preparations.
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<td>acetyl</td>
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<tr>
<td>Alloc</td>
<td>allyloxycarbonyl</td>
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</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>Dox</td>
<td>doxorubicin</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>ePTFE</td>
<td>expanded polytetrafluoroethylene</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isocyanate</td>
</tr>
<tr>
<td>Fmoc</td>
<td>fluorenylmethoxycarbonyl</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HMPA</td>
<td>N-(2-hydroxypropyl)-methacrylamide</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-resolution mass spectrometry</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
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<td>hv</td>
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</tr>
<tr>
<td>IC50</td>
<td>inhibitory concentration-50</td>
</tr>
<tr>
<td>iPr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LCST</td>
<td>lower critical solution temperature</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>LG</td>
<td>leaving group</td>
</tr>
<tr>
<td>LHMDS</td>
<td>lithium hexamethyldisilylazide</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>matrix-assisted laser desorption ionization - time of flight</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>Mn</td>
<td>number average molecular weight</td>
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<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>Mw</td>
<td>weight average molecular weight</td>
</tr>
</tbody>
</table>
MWCO  molecular weight cutoff
NIPAm  N-isopropylacrylamide
NIR    near infrared
NMR    nuclear magnetic resonance
Nu     nucleophile
PBD    polybutadiene
PBS    phosphate buffered saline
PCC    pyridinium chlorochromate
PCL    polycaprolactone
PDI    polydispersity index
PDLLA  poly(D/L-lactic acid)
PDS    polydioxanone
PEG    poly(ethylene glycol)
PEO    poly(ethylene oxide)
PG     protecting group
PGA    poly(glycolic acid)
Ph     phenyl
Piv    pivaloyl
PLA    poly(lactic acid)
PLGA   poly(D/L-lactic-coglycolic acid)
PLLA   poly(L-lactic acid)
PNIPAm poly(N-isopropylacrylamide)
PPHA   polypythtalaldehyde
PTA    phosphotungstic acid
PVA    poly(vinyl alcohol)
Py     pyridine
RA     retinoic acid
RT     room temperature
s      singlet
SEC    size exclusion chromatography
t      triplet
Tax    Taxol®
TBAF   tetrabutylammonium fluoride
TBDPS  tert-butylidiphenylsilyl
TBS    tert-butylmethyisilyl
iBu    tert-butyl
TEGMME triethylene glycol monomethyl ether
TEM    transmission electron microscopy
TFA    trifluoroacetic acid
THF    tetrahydrofuran
TLC    thin layer chromatography
UV     ultraviolet
Chapter 1
Biodegradable and Self-ImmOLative Materials in Medical Applications

1.1 General Introduction

Biodegradable polymers are an important class of materials with applications in diverse areas. With uses from bulk commercial items such as biodegradable plastics to highly specialized drug delivery systems for the treatment of cancer, these polymers represent one of the most broadly applicable classes of materials. Predominantly, they are polyesters such as polycaprolactone (PCL), poly(D/L-lactic acid) (PDLLA), poly(L-lactic acid) (PLLA), or poly(glycolic acid) (PGA) (Figure 1.1), owing to their ease of synthesis, often in a single step through a ring-opening polymerization of readily available monomers (Scheme 1.1). Additionally, other polyesters derived from diacids and diols have been employed, along with other backbones such as polyamides, polyanhydrides, polyphosphazenes, and more specialized polydisulfides, polyacetals, and poly(ortho ester)s (Figure 1.2). Of particular interest to this thesis is their use in biomedical applications, including stents and sutures, tissue engineering and particularly as drug delivery vehicles. Recent advancements in the use of biodegradable polymers in each of these applications will be highlighted below.

![Chemical structures of the most common biodegradable polyesters.](image)

**Figure 1.1.** Chemical structures of the most common biodegradable polyesters.

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1.2 Biodegradable Polymers in Medical Applications

Poly(hydroxyacid)s have received substantial attention for applications in biomedical devices. They possess numerous desirable properties, such as biocompatibility, maintenance of tensile strength over time, and eventual breakdown into nontoxic degradation products. Additionally, each polymer has unique physical properties, such as polymer morphology, glass transition temperature and melting point, as well as chemical properties such as degradation kinetics. Furthermore, by developing copolymers of different monomeric units, the physical and chemical properties can be fine-tuned to fit a host of potential applications.

Common polymers used in medical devices include PCL, PGA, PLLA, PDLLA, and more recently polydioxanone (PDS), as well as various combinations of these in
copolymers such as poly(D/L-lactic-coglycolic acid) (PLGA). *In vivo* degradation times of these polymers range from 1 – 2 months for 50/50 PLGA up to greater than two years for PCL and PLLA, with homopolymers generally having longer lifetimes than copolymers.\(^1\) This degradation timeline makes these materials well suited to a variety of medical applications such as sutures or stents, as the extended timeline allows the polymer to maintain tensile strength throughout the healing process while eventually degrading into small molecules, eliminating the need for additional procedures to remove the device.

Numerous polyesters, both homopolymers and copolymers, have already been commercialized as degradable sutures. These include products such as Vicryl\(^\circledR\) and Monocryl\(^\circledR\), copolymers of PGA-PLA and PCL-PLA, respectively, Dexon\(^\circledR\), a glycolic acid homopolymer, and PDS II\(^\circledR\), made from polydioxanone.\(^2\) In a report highlighting the development of Monocryl\(^\circledR\), the authors found that the material maintained approximately 25% breaking strength after two weeks compared to greater than 80% retention for PDS II\(^\circledR\). Analyzing the degradation, Monocryl\(^\circledR\) was found to undergo complete degradation in approximately 4 months.

In 2008, Pektok and coworkers evaluated PCL nanofiber as a vascular graft material, comparing it to the more conventionally used expanded polytetrafluoroethylene (ePTFE).\(^3\) They found that PCL nanofibers were superior to ePTFE in healing capability, as faster endothelialization and extracellular matrix formation was observed. Degradation studies proved that the polymer was being broken down, with approximately 20% reduction in molecular weight being observed after 24 weeks.

Taking advantage of the healing capabilities and prolonged degradation times of these polymers, researchers have also investigated their potential application in scaffold-based tissue engineering. Scaffold-based tissue engineering is a technique used to repair a wide variety of damaged tissues in the body. It involves the use of a biocompatible and degradable scaffold on which cells of a desired type can be seeded and then grown. Upon maturation, the cells are implanted into the body at the site of the injury, proliferate
and in turn heal the damaged site. Throughout this process the polymer gradually degrades, resulting in its complete removal from the matrix.

![Chemical structures](https://example.com/chemistry.png)

**Figure 1.3.** Biodegradable polymers used in different tissue engineering applications.

The types of tissues engineered by this process have included soft tissue, bone, smooth muscle, as well as cardiovascular tissues. Each of these tissues requires scaffolds with different biocompatibilities, physical properties, and degradation timelines. To this end, numerous biodegradable polymers have been developed which possess the properties desirable for each application. With their elastic properties, PGA/PCL copolymers were found to be ideally suited to smooth muscle tissue engineering. For soft tissue engineering, a polymer with a lower tensile strength is desired and thus poly(ester urethane)ureas and similar polymers have been employed. Conversely, for bone tissue engineering, materials with much higher tensile strength are required, and materials such as poly(propylene fumarate) and a polycarbamate derived from lysine diisocyanate and glucose have been employed (Figure 1.3).
1.3 Drug Delivery Systems

A very important and exciting application of biodegradable polymers is their use as drug delivery vehicles. These vehicles are designed to increase drug bioavailability and effectiveness, particularly pertaining to more hydrophobic drugs, as well as reduce toxicity, and help control the dosage and release rate of the drug. A number of polymeric systems have been developed to achieve this task, which can be divided into two major groups, both of which will be discussed in greater detail below. The first group involves nonspecific biodegradable polymers, examples of which include polyesters, polyphosphazenes, and polyanhydrides. The second group is stimuli responsive polymers, including polyketals, poly(ortho esters), disulfide containing polymers, polyelectrolytes and a variety of others. This second group can be further subdivided into two categories, degradable and non-degradable. Degradable stimuli responsive polymers will break down into smaller molecules upon triggering, while non-degradable polymers, often referred to as bioresponsive polymers, will lose any kind of supramolecular assembly upon triggering, but the polymer chains themselves remain intact. There are many types of triggering mechanisms, but those used in the context of drug delivery are primarily changes in pH and redox potential.

1.3.1 Biodegradable Homopolymers

Scheme 1.2. Encapsulation & release from a biodegradable polymer particle.

Within the first class of nonspecific biodegradable polymers, there have been primarily two approaches to using these as drug delivery agents. The first approach involves the
synthesis of a homopolymer of the degradable material and its assembly into a nanoparticle or microparticle loaded with the drug to be delivered. This is an attractive approach, as the polymers are formed in a single step from inexpensive starting materials, and numerous methods exist to create particles of various sizes. A general method for particle formation and drug release is shown in Scheme 1.2, where the polymer and the drug are mixed together and then the particle is formed with the drug encapsulated. Additionally, the exterior of the particles can be coated with different materials for applications such as improved targeting or to promote cell adhesion. Once the particles have been prepared, they can be injected into the body whereupon the polymer gradually degrades, releasing its payload.

Particles of various sizes and compositions have been employed for a variety of applications. For example, Benoit and coworkers demonstrated the capability of PCL microparticles as agents for oral vaccine delivery.\textsuperscript{32} Under various conditions they were able to synthesize particles ranging from 5 – 10 μm, a suitable size for oral delivery. Using bovine serum albumin (BSA) as a water-soluble model antigen, they demonstrated that the protein could be taken up inside the microparticle with loading efficiencies ranging from 1 – 36%. Carrying out polymer degradation and release studies \textit{in vitro}, they found that there was an initial burst release of approximately 25% of the protein, followed by the gradual release of a further 10% over the course of 80 h, leading to a maximum release of approximately 35%. Analyzing the BSA released from the particles, they found it to be unchanged throughout the course of encapsulation and release. While the authors did not investigate their system \textit{in vivo}, their \textit{in vitro} analyses demonstrated the proof of concept that their microparticles could be used for oral vaccine administration.

Demonstrating that dual functions could be achieved, Newman and McBurney developed porous PLA/PGA microspheres for the uptake and delivery of cells for tissue engineering.\textsuperscript{18} In their study, they synthesized microspheres in the presence of retinoic acid (RA), a molecule used to induce cell differentiation into neurons. Following this, the microspheres were coated with laminin to promote cell adhesion and then added to P19 embryonal carcinoma cell culture, a model of embryonic stem cells. They found that
after one day, cells had begun to attach themselves to the surface of the microspheres, and by day four cells had encompassed many of the microspheres. They observed that the cells exhibited a slight improvement in neuron formation when compared to cells cultured in the presence of free RA. In studying biodegradation of the microspheres, the percent release of RA was monitored over a course of 125 h, and was found to be 50 – 60%.

Similar materials have also been investigated for the treatment of vascular injury. In a study performed by Chandy and coworkers, they demonstrated the ability of PLA/PCL microspheres to encapsulate cisplatin and slowly release the drug over the course of 30 days. They synthesized PLA/PCL microspheres in the presence of cisplatin, encapsulating the drug, and then coated the microspheres with either poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), chitosan or alginate. These coatings were added to improve circulation time, as well as to slow the degradation time of the particle, thus slowing the release of the drug. Carrying out release studies, they found that there was an initial burst release of between 20 – 30% of the drug within the first day with a more gradual release being observed over the remaining 29 days of the study. They found that PVA-coated microspheres displayed the fastest release of cisplatin, with greater than 90% of the drug released after 30 days. The remaining three coatings were similar, exhibiting approximately 75 – 85% release over the same time period, with PEG-coated microspheres showing the slowest release profile. Based on these findings, the authors demonstrated that PEG and alginate-coated PLA/PCL microspheres were indeed viable candidates for cisplatin drug delivery vehicles.

While the above examples have demonstrated the effectiveness of micron-sized particles for drug delivery applications, there has also been significant investigation of similar materials on the nanoscale. Nano-sized particles have been found to be ideally suited to traverse cellular membranes, making them extremely valuable as potential drug delivery agents. Mu and Feng have demonstrated their potential in use in anti-cancer therapy. In their study, they synthesized PLA/PGA nanoparticles loaded with Taxol® (Tax), and demonstrated its controlled release from the nanoparticle. Under optimized conditions, they were able to encapsulate Tax within the nanoparticles with 84%
efficiency. In *in vitro* release studies, the results observed were similar to those of the microparticle studies described above. There was an initial burst release of approximately 20% in the first day, and by day thirty 50 – 70% of the drug had been released from the carrier.

![Diagram of polyphosphazenes](image)

**Figure 1.4.** Various steroid-substituted polyphosphazenes.

Polyphosphazenes have also been investigated as different class of potential drug delivery vehicles.\(^{35}\) Due to the possibility of varying the substituents on the phosphorus, the degradation kinetics of these polymers can be readily tuned by incorporating hydrophilic or hydrophobic substituents. Additionally, there exists the possibility of incorporating the drug molecule directly onto the polymer backbone. Provided the linkage is hydrolytically sensitive, this should allow for its gradual release from the polymer as the polymer breaks down. This proof of concept was first demonstrated by Allcock and Fuller in 1980, when they synthesized a series of polyphosphazenes with various steroidal substituents incorporated on the backbone (Figure 1.4).\(^{34}\) The steroids were linked either through aryloxy or alkoxy groups to the phosphorus, and they found that while aryloxy-based phosphazenes were stable, the alkoxy-substituted ones were not. The authors did not carry out a thorough investigation of the release of the steroids upon degradation, but they did demonstrate this as a possible mechanism for drug delivery.
Figure 1.5: Polyphosphazenes synthesized for drug delivery.

Allcock and Fuller also later reported imidazole and methylphenoxy co-substituted polyphosphazene 1.21 for release of noncovalent drug molecules. They varied the ratio of the two substituents to contain 20, 45 and 80% imidazole substitution, and found that 20% imidazole content showed the best release kinetics.\(^{36}\) Carrying out \textit{in vitro} release studies on different compounds such as 4-nitroaniline, progesterone as well as BSA, they observed that contrary to polyester-based drug carriers discussed thus far, little or no burst release of the drug was observed. The timeline of release was also found to be much faster than polyester-based delivery vehicles. 4-Nitroaniline was completely released within approximately 10 days, while progesterone was fully released within 30 days. While the 30-day timeline was the same that from the polyester carriers previously discussed, in those cases the drug continued to be released and the experiment was simply terminated, whereas in the polyphosphazene case the drug had been fully released from the polymer. The only exception was with BSA, which was found to have much slower and incomplete release, as it began to level off at around 50% release after 3 weeks. These results have shown that polyphosphazenes represent a viable alternative to polyester-based drug delivery systems, and have great potential for further applications.

1.3.2 Biodegradable Block Copolymer Assemblies for Drug Delivery

Self-assemblies are the arrangements of material, in this case polymers, into ordered supramolecular structures. While there are numerous driving forces for these assemblies, such as hydrogen bonding or charge-charge interactions, the driving force for the assemblies to be detailed in this section is primarily entropic in nature and relies on the hydrophobic and hydrophilic characteristics of the block copolymers. By preparing polymers composed of a relatively hydrophobic polyester block connected to a more hydrophilic block such as PEG and then immersing them in water, the polymers
spontaneously arrange themselves in order to minimize contact of the hydrophobic blocks with water. While these self-assemblies can vary in nature and size, to include spherical and worm-like micelles, vesicles, toroids, and others, only spherical micelles and vesicles (Figure 1.6) will be discussed here, as they have been most widely investigated for drug delivery applications. A spherical micelle is an assembly where the hydrophobic chains of the polymers aggregate at the core while the hydrophilic chains surround it on the exterior, making up the corona. A polymer vesicle, also referred to as a polymersome, is the macromolecular analogue of the liposome, an assembly composed of phospholipid surfactants that forms the basis for the membranes in living cells. In this case, the hydrophobic chains make up the interior of the membrane, while the hydrophilic block contacts the water on both the interior and exterior of the vesicle. In general, the ability of an amphiphilic block copolymer to form micelles, vesicles, or other assemblies can be predicted based on the volume fractions of the hydrophobic and hydrophilic block, although the structure of the polymer, its rigidity, and crystallinity can also play a role. By selecting different polymers, varying the ratio between the hydrophilic and hydrophobic blocks, and employing different assembly methods, researchers are can obtain control over both the type of assembly formed, as well as its size.

**Figure 1.6.** Spherical micelle and vesicle.
1.3.2.1 Biodegradable Micelles for Drug Delivery

**Figure 1.7.** Encapsulation & release of drug from micelle.

**Figure 1.8.** Chemical structure of Doxorubicin.

Polymer micelles are ideally suited for the delivery of hydrophobic drugs. As shown in Figure 1.7, the drug molecules can be encapsulated within the hydrophobic core of the micelle and slowly leach out over time. This increases the bioavailability of these drugs, which otherwise exhibit poor water solubility, and therefore limited efficacy within the body. This is particularly relevant in anti-cancer therapy, as a number of drugs currently employed in treatments are quite hydrophobic and therefore have poor bioavailability on their own. Of particular interest is the anti-cancer drug doxorubicin (Dox), shown in Figure 1.8. Several recent reports have highlighted the possibility of using PCL-PEG block copolymer micelles as delivery vehicles for Dox.²⁰,²¹,²³
Lang and coworkers have recently reported a modified PCL-PEG micelle for Dox delivery.\textsuperscript{21} In addition to using ε-caprolactone (CL), they incorporated γ-(carbamic acid benzyl ester)-ε-caprolactone (CABCL) into the hydrophobic block in either 10:1 or 2.5:1 CL:CABCL ratio (Figure 1.9). CABCL was a monomer they had recently developed for the purpose of encapsulating Dox, as it was found to have superior interactions with the drug compared to conventional ε-caprolactone. Having synthesized their block copolymers, they assembled them into micelles in the presence of Dox via dialysis and observed loading efficiencies of 47 and 69% for 10:1 and 2.5:1 polymer blends, respectively. Examining release kinetics, they obtained a slow release profile of Dox from the micelles, with 10–15% of the payload being released over a course of 3 days compared to a 30% release of Dox from unmodified PCL-PEG micelles. No substantial burst release was observed. Their findings demonstrated that the incorporation of CABCL into the polymer backbone improved Dox internalization, making these potential candidates for slow release treatments.

In a second example, Diao and coworkers tested their Dox-loaded PCL-PEG micelles for treatment of resistant K562 tumor cells.\textsuperscript{23} Dox-loaded micelles were prepared in this case through ultrasonication of a mixed organic-aqueous solution followed by evaporation of the organic solvent, and a loading efficiency of 49% was obtained. \textit{In vitro} release studies showed increased release relative to Lang and coworkers, with approximately 70% of encapsulated Dox being released after 4 days following an initial burst of approximately 20%. Most importantly, when they tested their loaded micelles against Dox-resistant K562 tumor cells, they found that at dosages
of both 6 and 12 µg/mL, approximately 80% of the cells had been killed after 3 days of incubation, relative to just 50% when free Dox was used. Their work has effectively demonstrated that the use of drug delivery vehicles is a potentially useful treatment option for drug-resistant in tumor cells.

Figure 1.10. Folate-terminated star-shaped polycaprolactone-poly(ethylene glycol) micelles.

Figure 1.11. Folate-terminated micelles bind to a receptor and release a drug payload.

Hsieh and coworkers have very recently developed a method to improve the targeting of tumor cells with Dox-loaded PCL-PEG star-shaped micelles. This may minimize the
harmful side effects often associated with chemotherapy. Attaching folic acid to the end of the PEG block (Figure 1.10), they sought to take advantage of the fact that several tumor cell lines overexpress folate receptor proteins, whereas these proteins are expressed to much less extent in healthy tissue. Thus, the folic acid-terminated micelles should preferentially bind to cancerous cells, resulting in Dox being primarily administered to these cells (Figure 1.11). Dox-loaded micelles were formed by dialysis in a similar fashion to Lang’s work. Once formed, release studies showed that a significant amount of the Dox was lost within the first hour, with approximately 30% release being observed. An additional 10% leached out over the next 12 h, at which point a maximum release of 40% was obtained, and held constant over the course of 7 days. To test the targeting efficiency of folic acid on the micelle, they incubated MCF-7 cells for 48 h in the presence of free Dox, Dox-loaded folic acid-terminated micelles, Dox-loaded hydroxy-terminated micelles, and finally Dox-loaded folic acid-terminated micelles in the presence of free folic acid. They found that their folic acid-terminated micelles showed the best cytotoxicity, with an observed cell viability of below 20%. They also found that free folic acid indeed inhibited binding, with cell viability rising to approximately 25%. Micelles lacking targeting groups resulted in approximately 35% viability. These findings verify that the addition of targeting groups can improve the delivery of anticancer drugs to tumor cells, thus improving the potency of the drug.

1.3.2.2 Biodegradable Vesicles for Drug Delivery

Vesicles possess an advantage over micelles, in that they are capable of not only encapsulating hydrophobic molecules within the vesicle membrane, but also hydrophilic drugs in the aqueous core. This allows greater flexibility in the choice of drug to be administered, and also opens the possibility of combination therapy, simultaneously releasing multiple drug molecules.

In a recent example by Lee and Feijen, a variety of biodegradable polymer vesicles were synthesized and the hydrophobic membranes were loaded with fluorescein-labeled Tax. Making use of different polyester groups as hydrophobic blocks, they
synthesized four different vesicles containing PEG with either PCL, PDLLA, PCL-b-PDLLA or a random PCL/PDLLA copolymer acting as the hydrophobic block (Figure 1.12). Using a solvent injection method to generate the vesicles, they were able to achieve Tax loading efficiencies of 75 – 80%. They analyzed the release over the course of 4 weeks, and found significant differences depending on the hydrophobic block. PCL had the most rapid and complete release, resulting in nearly 100% release by the end of the study. The random PCL/PDLLA copolymer released approximately 70% of its payload, while the block PCL-PDLLA and PDLLA on their own exhibited the slowest release rates, with approximately 50% of Tax release over 4 weeks in each case. These findings demonstrate that the nature of the hydrophobic block plays a key role in the release profile of the drug, and offers insight into the potential to tune these systems to obtain controlled release of a drug.

![Structures of block copolymers with varying hydrophobic groups](image)

**Figure 1.12.** Structures of block copolymers with varying hydrophobic groups, which were used for vesicle formation.

Hammer, Therien, and coworkers have also investigated the potential of PCL-PEO (PEO = poly(ethylene oxide)) vesicles for drug delivery. Their vesicles were formed through thin-film rehydration, followed by Dox incorporation into the core using an ammonium sulfate gradient. Testing their release *in vitro*, they carried out studies at both pH 7.4 and 5.5. In both cases an initial burst of approximately 20% was observed, after which time the release slowed substantially at pH 7.4, reaching a maximum of approximately 50%. At pH 5.5, release was found to be both more rapid and more complete, with a maximum of approximately 70% release observed. They also analyzed
the rate of Dox release, measured in % initial load/h. The authors found two distinct release profiles, depending on the pH, and proposed two different release mechanisms, shown in Scheme 1.3. At pH 7.4, a rapid release was observed initially, after which time the remaining contents slowly leaked out. Throughout these domains, two different release rate constants were observed. To account for this, they suggested that there was an initial leaching out of the drug through the intact vesicle walls, after which the remaining drug release occurred as a result of vesicle wall rupture as the polyester component began to hydrolyze. At pH 5.5, a more steady release curve was observed, with a rate constant similar to that of the second phase of release in pH 7.4. Therefore, the authors suggested that ester hydrolysis had occurred much more rapidly at pH 5.5, and all of the observed release was as a result of vesicle wall rupture.

Scheme 1.3. pH dependence on release mechanism from polymer vesicles.

As previously mentioned, the use of polymer vesicles allows for the possibility of utilizing a single drug carrier for combination therapy. This potential was effectively demonstrated by Discher and coworkers.\textsuperscript{13} They synthesized vesicles from a mixture of PLA-PEO and polybutadiene (PBD)-PEO through thin film rehydration, after which they loaded them with Dox using a pH gradient method. Tax was then incorporated by injecting a solution of Tax in MeOH into a buffered solution containing the vesicles, after which dialysis removed any unencapsulated drug. They carried out release studies and
found that over the course of 3 days nearly 100% of the Dox had been released, while 80% of Tax was released. More importantly, they carried out \textit{in vivo} studies in mice models and compared their drug-loaded vesicles to free Dox and Tax. They noted that after 3 days the tumor volumes were reduced by 60% relative to untreated tumors and did not increase in size for the remainder of the study. Comparatively, free Dox and Tax reduced tumor size by approximately 45% after 3 days, but thereafter tumor volume began to increase.

1.3.3 Advantages and Limitations of Polyesters in Drug Delivery

These past examples have highlighted how the use of biodegradable drug delivery vehicles can offer significant advantages in therapeutic applications. Their effectiveness at controlling dosage has been highlighted in multiple cases, and by using different polyester blocks, further control over the release kinetics can be obtained. In therapeutic applications, the use of drug delivery vehicles has proven to be more effective than using the free drug, and in fact has even been shown to overcome resistance to a drug by different cell lines. Lastly, the use of targeting groups on the periphery has also been shown to be effective in increasing localized dosage, maximizing therapeutic effect while minimizing side effects. Despite these numerous advantages, there is a major limitation inherent to the use of these materials, namely that they all rely on uncontrolled hydrolysis of the polymer backbone. While the overall rate can be affected by such parameters as pH or the length and nature of the polymer chain, there is still no way to control when and where hydrolysis occurs along the backbone.

1.3.4 Stimuli Responsive Polymers

To overcome the limitations of ester hydrolysis as a mechanism for the breakdown of materials and the release of drugs, over the past couple of decades much research has focused on the development of stimuli-responsive polymers. These polymers are synthesized with specific functional groups incorporated that are sensitive to certain
changes in the environment. Upon exposure to these changes, the functional groups react, triggering the breakdown of the structure and causing release of the payload (Scheme 1.4). This section will discuss recent advances in this field, with the following subsections focusing on prominent types of triggering mechanisms.

**Scheme 1.4.** Stimuli responsive polymers in drug delivery.

### 1.3.4.1 pH Responsive Polymers

The design of polymers responsive to changes in pH represents an important advancement for drug delivery, as tumors and inflammatory tissues, as well as other tissues, have been reported to be mildly acidic (ie. pH 5.8–7.4). The best types of treatments are those that deliver the drug molecule selectively to the damaged tissue while leaving healthy tissue untouched. Therefore, any significant differences between healthy and damaged tissue can potentially be exploited for selective drug delivery. A pH gradient is one such property. Because certain damaged tissues are moderately acidic relative to healthy tissue, there exists the potential to utilize materials that break down more rapidly in acidic medium for drug delivery applications. Much focus has been placed on exploiting this difference in pH, and some of the findings both in the areas of degradable and non-degradable materials will be highlighted below.
1.3.4.1.1 Acid Degradable Polymers

Scheme 1.5. Polyacetals as acid sensitive drug delivery vehicles.

Figure 1.13. Series of polyamidoamines containing acid-sensitive acetals and ketals.

The most widely used functional groups for degradable pH-sensitive materials are acetals and ketals. These groups are well known to be stable under neutral or basic media, but readily undergo hydrolysis under acidic conditions. Therefore, they are ideally suited for drug delivery applications, as they remain stable under physiological conditions but can break down upon exposure to the more acidic medium encountered within a tumor cell, triggering polymer degradation and release of the payload (Scheme 1.5). This concept was demonstrated by Fréchet and coworkers, through the synthesis of polymers containing acetal, dimethylketal, or aromatic ketals (Figure 1.13). Comparing relative hydrolysis rates at pH 7.4 and pH 5.0, they observed marked enhancements at lower pH. The half-life of polymer 1.31 dropped from 161 to 81 days, while that of 1.32 went from 6 to 0.03 days, and lastly 1.33 decreased from 15 to 3 days at pH 5.0 relative to pH 7.4. This demonstrates that the use of a ketal linkage within the backbone can provide significant enhancement in degradation rates.
In a more recent example from the same group, crosslinked polyacrylamide 1.34, shown in Figure 1.14, was investigated for use in protein delivery. Acrylamide and a benzaldehyde acetal-containing bis(acrylamide) were copolymerized in the presence of BSA, forming BSA-loaded crosslinked microparticles. To evaluate the release kinetics, they carried out experiments at both pH 5.0 and 7.4. At pH 5.0 they observed complete release of BSA after 7 h, while at pH 7.4 less than 20% release had occurred over a period of 20 h, effectively demonstrating their potential applications in drug delivery.

**Scheme 1.6.** Particle formation and release from polyketal 1.35.

In another example, Murthy and coworkers designed a ketal-containing polymer as a drug delivery vehicle for the treatment of inflammatory diseases. They synthesized polymers with the general structure 1.35 containing varying ratios of 1,4-
cyclohexanediol and 1,5-pentanediol linked via dimethylketal groups, and prepared microparticles from these polymers. Testing their degradation rates, they found that the ideal ratio was 2.5:1 1,4-cyclohexanediol:1,5-pentanediol, and used this polymer for *in vitro* and *in vivo* tests. Beginning with *in vitro* release of rhodamine B, they observed complete release of the molecule within 4 days at pH 4.5, compared to approximately 65% at pH 7.4. For *in vivo* studies, they tested mice suffering from liver damage by treating them with imatinib-loaded microparticles, using alanine aminotransaminase (ALT) levels in the mice to evaluate the therapeutic effects. They found that at low concentrations the drug-loaded particles offered no improvement over free imatinib, but as concentrations rose the drug-loaded particles became extremely effective, reducing ALT to almost zero at the highest dosage, thus demonstrating the potential of this strategy for further treatments.

![Figure 1.15. Acid-sensitive poly(ortho ester amide) 1.39.](image)

While the previous examples have focused on acetal and ketal linkages as acid-sensitive groups, there has also been some focus on utilizing ortho esters for this same role. Wang and coworkers demonstrated this possibility by developing a series of poly(ortho ester amides) (Figure 1.15). Following polymer synthesis, they prepared hydrogels containing FITC-labeled dextran, and tested the release kinetics. They measured both polymer mass loss as well as dextran release at pH 5.0 and 7.4. They found that at pH 5.0, complete mass loss was observed after 10 days, corresponding to full dextran release along the same timeline, while at pH 7.4 only approximately 25% mass loss and 25% dextran release was observed. Thus, they showed that poly(ortho esters) are also viable candidates for pH sensitive drug delivery.
1.3.4.1.2 Non-Degradable pH Responsive Polymers

Kataoka and coworkers designed a polymer for drug delivery that itself is stable to pH, but contains drug molecules covalently bound to the polymer via acid sensitive linkages.\textsuperscript{25} Utilizing a PEG-poly(aspartic acid) block copolymer, they modified the carboxylate sidechain and covalently bound Dox through a hydrazone linkage. At physiological pH, Dox remains bound to the block copolymer micelle, but upon exposure to mild acid, the hydrazone was cleaved, releasing Dox from the micelle (Scheme 1.7). To verify this, they incubated their micelles at a range of pHs from 7.5 down to 3.0, and measured the release of Dox over a period of 3 days. At pH 7.5, no release was observed throughout the course of the experiment, while at pH 3 they observed 100% release. At the more relevant pH ranges of 4.5 – 6.5, the amount of released Dox ranged from 5 – 45% as the pH decreased. This demonstrates a highly effective and innovative technique for anti-cancer treatment, as near complete selectivity was achieved for release in more acidic environment versus in conditions mimicking those of healthy tissues.

\textbf{Scheme 1.7.} Covalent binding of Dox to block copolymer \textbf{1.40} via hydrazone linkage followed by acid catalyzed hydrolysis.
Figure 1.16. a) poly(2-(dialkylamino)ethyl) methacrylate structure and b) poly(2-(dimethylamino)ethyl)-b-(2-(diethylamino)ethyl) methacrylate.

A number of other examples that make use of non-degradable polymers as pH responsive drug delivery vehicles have also been reported. For these systems, basic functional groups have typically been employed. One of the most extensively investigated classes of non-degradable pH responsive polymers is poly((dialkylamino)ethyl methacrylate)s 1.42 (Figure 1.16a).41,43 For example, Armes and coworkers developed the first block copolymer of 2-(dimethylamino)ethyl methacrylate and 2-(diethylamino)ethyl methacrylate, shown in Figure 1.16b.43 They performed a series of experiments at varying pHs and noted a significant drop in surface tension of the solution in the pH range 6 – 8, corresponding to a change in polymer morphology. Additionally, they found that the hydrodynamic size reached a maximum at pH 9.5, indicating micellization of the block copolymer.

Figure 1.17. Block copolymer of poly(2-vinylpyridine) and poly(ethylene oxide).

Another example by Webber and coworkers made use of poly(2-vinylpyridine) and PEO as a pH-sensitive block copolymer (Figure 1.17).44 In their study, they found that micellization occurred at approximately pH 5, while below that the polymer chains existed as unimers. While no further drug release studies were carried out, this example indeed shows great promise. At physiological pH, the block copolymer exists primarily as micelles. However, if exposed to acidic tissue, the pyridine groups protonate in the
mildly acidic medium, making them hydrophilic and rupturing the micelle, releasing its contents.

1.3.4.2 Reduction Sensitive Polymers

Scheme 1.8. Disulfide-containing polymers as drug delivery vehicles.

Reduction sensitive polymers have also been developed, and typically involve disulfide linkages. This strategy takes advantage of the fact that the extracellular environment is typically oxidizing with glutathione concentrations on the order of micromolar, while the interior of a cell is reducing with millimolar concentrations of glutathione. Therefore, the use of a disulfide-containing polymer offers stability of the delivery vehicle outside of the cell, limiting the risk of unwanted degradation. Once inside the cell, the disulfide bond will be reduced, breaking down the polymer and releasing the payload entirely within the cell (Scheme 1.8).

Figure 1.18. Disulfide-containing polyamidoamine 1.45.

A common backbone utilized for these polymers has been a disulfide-linked polyamidoamine. In a study by Ferruti and coworkers, a polymer containing disulfide-linked cysteine groups and piperazine dicarboxylates, 1.45, was synthesized and its breakdown in both the presence and absence of 2-mercaptoethanol as a reducing agent was evaluated. They found that the polymer was susceptible to hydrolysis in buffered
media, with 10 – 20% molecular weight (MW) reduction observed after 1 day and complete degradation occurring within a week. However, this degradation rate was substantially improved in the presence of 2-mercaptoethanol, with a 50% MW reduction within a day, and 90% MW reduction occurring within 2 days.

![Figure 1.19. Disulfide-containing poly(amido ethyleneimine) 1.46.](image)

Kim and coworkers sought to apply this general polymer backbone for gene delivery, utilizing poly(amido ethyleneimine) 1.46 (Figure 1.19). Taking advantage of the cationic ethyleneimine portion of the polymer backbone, they tested its ability to complex DNA, and found that complete complexation occurred when a 1.5:1 or higher w/w ratio of polymer:DNA was employed. Testing complex stability using gel electrophoresis, they found them to be stable in buffered solution in the absence of any reducing agents. Upon addition of dithiothreitol (DTT), complete release of the DNA was observed across all polymer:DNA ratios. Based on these results, polymer 1.46 has shown good promise for applications in intracellular gene delivery.

![Figure 1.20. Poly(ethylene glycol)-polyaspartate copolymer linked via disulfide.](image)

Looking at a different polymeric structure, Kataoka and coworkers designed block copolymer micelles from PEG and diethylenetriamine-substituted poly(aspartic acid) containing a disulfide group as the linker between the two blocks (Figure 1.20).
Similar to Kim’s work, they utilized the amino groups to form complexes with the DNA, synthesizing a series of complexes with varying N/P ratios. They found that for N/P ratios greater than 2:1 they achieved complete complexation of DNA. Carrying out \textit{in vitro} degradation studies, they found that at sufficiently low concentrations of DTT (10 \(\mu\)M), the micelle structure remained stable. At 10 mM DTT, complete rupture occurred within 1 h. They also carried out cell transfection studies, and found that gene delivery began to occur within 11 h and 16 h for HeLa and 293T cells, respectively. This result effectively demonstrates the possibility of these systems for use in gene therapy.

1.3.4.3 Thermoresponsive polymers

![Diagram of Thermoresponsive Polymers](image)

\textbf{Scheme 1.9.} a) Polymer in solution below and above LCST. b) Thermoresponsive polymer used for drug delivery.

![Chemical Structure](image)

\textbf{Figure 1.21.} Chemical structure of poly(N-isopropylacrylamide).

The third and final major class of stimuli responsive polymers utilized for drug delivery systems is thermoresponsive polymers. These polymers have the unique property of possessing a lower critical solution temperature (LCST) in water. The lower critical solution temperature is the maximum temperature at which the polymer is soluble, above which it precipitates out of solution (Scheme 1.9a). This property has been exploited by
using polymers possessing LCSTs at or near physiological temperatures in water for drug delivery applications, with the basic principle being that the polymer-drug complex is formed at room temperature when the polymer is soluble, and then after injection into the body and upon warming, the polymer precipitates out and the drug contents are released (Scheme 1.9b). The most thoroughly investigated thermoresponsive polymer, for drug delivery as well as a host of other applications, has been poly(N-isopropylacrylamide) (PNIPAm) (Figure 1.21). More recently, however, additional methacrylate-based polymers have also been investigated, and found to have similar thermoresponsive properties.

Ge and coworkers studied a crosslinked PNIPAm network for drug delivery applications. Following the synthesis of crosslinked networks by two methods, conventional radical polymerization and frontal polymerization, they tested the products of each for drug loading capacities and drug release kinetics. Using aspirin as a model drug, they found a 30% increase in drug loading capacity using frontal polymerization. To evaluate release kinetics, they studied both polymers at 25 °C and 37 °C, and found that the polymer produced from frontal polymerization exhibited both a more rapid and smoother release profile at 25 °C, reaching 100% release after 12 h versus 48 h for the conventional polymer. Interestingly, the release slowed down drastically upon heating to 37 °C, with 3 weeks being required for both polymers to release 90% of their payloads. While surprising, they attribute this slow release primarily to hydrophobic effects keeping the drug trapped within the polymer network.
Scheme 1.10. Conjugation and release of Dox from poly(N-isopropylacrylamide) hydrogel.

NIPAm has also been combined with other monomers to form multifunctional polymeric structures that are both thermo- and pH-responsive. This strategy was employed effectively by Zhao and coworkers, who prepared a crosslinked copolymer composed of NIPAm, methacrylic acid, and N,N’-methylenebisacrylamide as a crosslinker. Using this, they conjugated Dox to the acid groups through an acid-sensitive hydrazone linkage (Scheme 1.10). In the evaluation of these Dox-conjugated nanogels, they noted significant thermoresponsivity, with the size dropping from 375 nm at 25 °C to 135 nm at 37 °C. This did not result in substantial release of Dox, as only 20% release was observed over a course of 6 days. When the pH was decreased to 5.3, however, greater than 70% release of Dox was noted. They further tested their drug delivery system in cell viability assays at pH 7.4 and 6.8 at 37 °C and 43 °C. It was found that changing one of the variables did not lead to any significant changes, whereas lowering the pH and heating the solution to 43 °C resulted in cell viability dropping to
65%. While they did not carry out cell studies at lower pH, based on the release profile they proposed that this would be an effective drug delivery vehicle for hyperthermal cancer treatment.

![Thermoresponsive polymer](image)

**Figure 1.22.** Thermoresponsive polymer **1.52**, a proposed alternative to poly(N-isopropylacrylamide).

In addition to PNIPAm, other acrylate-based polymers have also been studied for thermoresponsivity. Lutz and coworkers have recently developed methacrylate polymer **1.52**, a random copolymer of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate (Figure 1.22). Comparing its physical characteristics directly to PNIPAm, they found that it was superior to PNIPAm in a variety of aspects of its LCST behaviour. First, they found it had a smaller temperature gradient and hysteresis between heating and cooling cycles. Additionally, it was found to consistently maintain a lower LCST than NIPAm in a variety of scenarios, such as changing salt concentration, polymer concentration, as well as polymer chain length. From these findings, the authors suggest polymethacrylate **1.52** as a viable alternative to PNIPAm, though as of yet no application studies have been carried out.

### 1.3.4.4 Polymers Responsive to Other Stimuli

While pH, reduction and thermosensitive polymers represent the bulk of stimuli responsive materials studied for biological applications, there are some noteworthy examples of polymers responsive to other stimuli being employed for biological applications. The groups of Morris and Zhao have developed a light sensitive block
copolymer micelle composed of PEG and polymethacrylate with photosensitive pendant groups\textsuperscript{26}. Upon exposure to either UV (365 nm) or NIR (794 nm) light, the ester group is cleaved, making the methacrylate portion of the block copolymer hydrophilic and thus rupturing the micelle (Scheme 1.11). Using Nile Red and a coumarin dye as model drugs, they monitored the decrease in fluorescence over time upon exposure to both wavelengths. Exposure to UV light resulted in much faster release, with the fluorescence decreasing by 60% within 40 min. When the same copolymer micelle was exposed to NIR light, the fluorescence decreased by 60% within 5 h.

Scheme 1.11. Release of coumarin dye 1.55 from polymer 1.53 upon irradiation with UV or NIR light.

In another example, Kataoka and coworkers have developed a thermoresponsive polymer that is highly sensitive to glucose concentrations for the treatment of diabetes (Scheme 1.12).\textsuperscript{24} Incorporating a small amount of phenylboronic acid-substituted acrylamide into PNIPAm, they found that glucose concentration played a dramatic role in determining the LCST of the polymer. In the absence of glucose, the LCST was approximately 22 °C. As the concentration of glucose was gradually increased up to 5 g/L, the LCST correspondingly increased to 35 °C. They then tested the polymer gel’s potential for insulin delivery by carrying out release studies at 28 °C and pH 9.0 with varying glucose concentration. With no glucose, approximately 10% insulin release was observed after 24 h. At 1 g/L of glucose, 30% was released over the same time period. Finally, at 3 g/L 80% was released. While the parameters for these tests were outside of physiological temperature and pH, they still effectively demonstrate the possibility for a system of this nature to be employed for diabetes treatment.
Scheme 1.12. Complexation of 1.56 with glucose and resultant precipitation.

1.3.4.5 Advantages and Limitations of Stimuli Responsive Polymers

The examples described above highlight the effectiveness of stimuli responsive polymers for biological applications. Their main advantage is their ability to release their therapeutic payload in response to changes in conditions, such as pH, reduction potential, temperature or other stimuli. This can potentially allow for a more localized release, providing enhanced efficacy in treatments, and minimal side effects. However, they do possess limitations. For example, in the case of pH, there is only a small pH gradient that exists within the body so it is difficult to synthesize materials that can be highly sensitive within such a small range. While the research thus far has indeed been promising and substantial increases in release rates have been obtained by slight decreases in pH, there is invariably uncontrolled release occurring. While disulfide-containing polymers address the issue of nonspecific degradation to some degree, as degradation outside of a cell is minimized, an additional targeting group is often required to preferentially deliver drug payloads to diseased cells. Lastly, with body temperature remaining constant at 37 °C, it is difficult to employ thermoresponsive polymers in vivo, unless their LCSTs can be modulated by other chemical factors, such as was demonstrated with glucose-sensitive thermoresponsive hydrogels.
1.4 Introduction to Self-Immolative Materials

To address a number of the above challenges and to further develop the field of polymeric materials in biomedical applications, there has been a recent focus on a new class of degradable polymers called self-immolative polymers. Composed of a unique class of monomers called self-immolative spacers that are capable of undergoing intramolecular cleavage reactions, they are a new class of stimuli-responsive polymers that require just a single triggering event to induce complete depolymerization. Containing an end cap at one terminus, these polymers maintain their stability as long as the end cap remains attached. Once the end cap is removed, a cascade of intramolecular cleavage events ensues, resulting in stepwise, end-to-end degradation of the polymer. By using different functional groups as an end cap, depolymerization can be triggered under a variety of conditions or in response to a variety of different molecules present in solution. Additionally, because only a single triggering event is required, the sensitivity to stimuli is greatly increased, with only trace amounts of an effector being required to induce depolymerization.

While the field of self-immolative polymers is relatively new and unexplored, that of self-immolative spacers is much more widely studied. Beginning as a capping group for prodrugs, their usage has gradually expanded to include short oligomeric species, dendrimers, and most recently linear self-immolative polymers, for a variety of applications such as drug delivery, signal amplification and logic gates. A review of these areas is presented in the following sections.

1.5 Self-Immolative Spacers

A class of molecules termed self-immolative spacers has been a principle component in the design of all self-immolative materials. Originally developed for the field of prodrug chemistry, such linkers were designed to overcome steric limitations by increasing the physical distance between the parent drug and the cleavage site as well as to alter the inherent stability of this linkage. In the absence of a self-immolative spacer, prodrugs
could be composed of a specifier conjugated directly to the parent drug by an enzyme-labile linkage.\textsuperscript{49-52} Unfortunately, due to the demanding steric requirements of the enzymatic cleavage step, such prodrugs were found to be relatively ineffective.\textsuperscript{53-55} Introduction of a spacer (Figure 1.24a) capable of undergoing a spontaneous intramolecular reaction to release the drug in its parent, active form, increased the accessibility of the cleavage site, enhancing drug release.\textsuperscript{56}

![Diagram of prodrug structure](image)

**Figure 1.23.** a) Schematic of a prodrug comprising a specifier/trigger, spacer and drug; b) general structure of electronic cascade spacers; and c) general structure of cyclization spacers.

The first self-immolative spacer introduced by Katzenellenbogen and coworkers in 1981 was based on 4-aminobenzyl alcohol.\textsuperscript{48} Commonly referred to as a 1,6-elimination spacer, it is an example of an electronic cascade spacer, where unmasking of an aromatic amine,\textsuperscript{48} hydroxyl,\textsuperscript{57} or thiol\textsuperscript{58} moiety allows these chemical functionalities to become electron-donating, initiating an electronic cascade leading to release of a free drug or other leaving group, often following a subsequent decarboxylation reaction (Figure 1.23b). As shown in Figure 1.23c, spacers based on cyclization reactions have also been introduced, whereby the unmasking of a nucleophilic functional group permits cyclization.\textsuperscript{59,60} In general, electronic cascade spacers react more rapidly than the cyclization spacers. Since their introduction, a variety of spacers based on both electronic
cascades and cyclization reactions have been developed and they have been applied in various prodrug, sensor, and drug delivery systems.

1.6 Self-Immolative Oligomers

Figure 1.24. a) Spacers based on extended aromatic systems such as naphthyl and biphenyl moieties that did not undergo the desired electronic cascade eliminations; b) Spacers based on multiple 1,6-elimination spacers in sequence; and c) Spacers based on multiple 1,6-elimination spacers followed by a cyclization spacer (PG = protecting group, D = drug).

Self-immolative oligomers were introduced by Scheeren and coworkers in 2001 as potential improvements to the spacers previously employed in prodrug systems. It was hypothesized that the use of elongated linkers might enhance the cleavage rates by further separating the cleavage site from the sterically bulky parent drug. Linkers based on
extended aromatic systems such as naphthyl and biphenyl moieties (Figure 1.24a) were prepared. However, these systems failed to undergo the electronic cascade leading to drug release, a result that was attributed to the high energetic cost of dearomatization associated with the required 1,8- and 1,10-elimination reactions. In addition, the repulsion of ortho hydrogens in the biphenyl system would disfavor the planar intermediate structure. In contrast, the combination of multiple 1,6-elimination spacers in sequence (Figure 1.24b) linking a plasmin sensitive specifier and the drug Tax or Dox, led to significantly enhanced drug release rates in the presence of the enzyme, when compared with analogous systems containing only one linker. In addition, it was demonstrated that a diamine cyclization spacer (Figure 1.24c) could be incorporated into the sequence of linkers, also resulting in enhanced enzymatic cleavage rates relative to the single spacer system. In this work, the prodrugs containing only electronic cascade spacers released the free drug more rapidly than those incorporating the cyclization spacers. However, the diamine cyclization spacer provided the opportunity to conjugate hydroxyl-containing parent drugs via stable carbamate linkages instead of the less stable carbonate linkages that would be generated using the 1,6-elimination spacers alone. While none of the above systems resulted in an amplification of the biological stimulus, the groundwork was laid for the synthesis of future linear and dendritic systems. It was also demonstrated that the electronic cascade and cyclization reactions utilized in self-immolative spacers could be extended to multimeric systems. Since then, several other examples of oligomeric combinations of self-immolative spacers have been reported and demonstrated to undergo reaction cascades to release reporter molecules.65,74,79,90-93

The first example of a self-immolative oligomer that led to chemical amplification was reported several years later by Warnecke and Kratz.94 This molecule, termed a "linear self-eliminating system", was composed of a sequence of three 1,6-elimination spacers (Scheme 1.13). In contrast to the above oligomers, which only carried a drug payload at one terminus of the oligomer, amplification was achieved in this case by the conjugation of a model drug to aromatic moieties such that it could be released by a 1,4-elimination reaction. Kinetic studies revealed that cleavage of the oligomer backbone by the sequence of 1,6-elimination reactions was the faster process, while the release of drugs via the 1,4-elimination occurred more slowly. In this system, tryptamine was used
simultaneously reported the first examples of self-immolative dendrimers\textsuperscript{99-101}. While similar in design, each dendrimer was unique in its chemical structure, release trigger and potential applications.

\textbf{Scheme 1.14.} a) A \textsuperscript{2nd} generation dendrimer that fragments from the periphery to the focal point by a sequence of three 1,6-elimination reactions upon cleavage of the allyl ether, leading to the release of 4-nitrophenol; and b) A \textsuperscript{2nd} generation dendrimer that
fragments from the focal point to the periphery upon allyl ether cleavage, resulting in the release of four molecules of 4-nitrophenol.

McGrath and coworkers described up to 2\textsuperscript{nd} generation "linearly disassembling" benzyl(aryl ether) dendrimers based on 4-hydroxybenzyl alcohol derivatives.\textsuperscript{100} In the case of the 2\textsuperscript{nd} generation dendrimer (Scheme 1.14a), cleavage of a peripheral 4-allyloxybenzyl ether group initiated a sequence of three 1,6-elimination reactions leading to the release of a 4-nitrophenol reporter at the dendrimer's periphery. Under optimized conditions for removal of the allyl group, the release of the 4-nitrophenol was complete in approximately 15 min and no significant generational differences were observed. Interestingly, the fragmentation was much slower in THF with complete release of the reporter requiring on the order of 10 h. A new UV absorbance band was observed during degradation, and was used to monitor the reaction. This band increased rapidly upon the dendrimer’s subjection to degradation conditions, and gradually decreased as the experiment proceeded. The authors attributed this absorbance band to phenoxide intermediates, supporting the proposed fragmentation mechanism, as opposed to simple cleavage of the ether linkages.

While the above linearly disassembling dendrimers did not technically lead to signal amplification as only a single reporter molecule was released, this work was followed up with a new series of polyether dendrimers based on 2,4-bis(hydroxymethyl)phenol.\textsuperscript{102} In this case, cleavage of an allyl ether at the dendrimer's focal point initiated a cascade of both 1,4- and 1,6-elimination reactions leading to fragmentation of the dendrimer backbones and release of 4-nitrophenol from the dendrimer peripheries (Scheme 1.14b). Generation 0, 1, and 2 dendrimers released 1, 2, and 4 reporters respectively, demonstrating the utility of the dendrimer backbone for signal amplification. In subsequent work, it was demonstrated that the chemically cleavable allyl ether at the dendrimer's focal point could be replaced with a photolabile 2-nitrobenzyl derivative providing an alternative triggering mechanism.\textsuperscript{103}
Scheme 1.15. A 2nd generation dendrimer fragmenting by a sequence of cyclization and 1,4-elimination reactions upon cleavage of a focal point photochemical trigger, to release four molecules of aminomethylpyrene.

The self-immolative dendrimers reported by Shabat and coworkers in 2003 were polycarbamates based on the branching unit 2,6-bis(hydroxymethyl)-4-cresol and the cyclization spacer $N,N'$-dimethylethylene diamine (Scheme 1.15). In the 1st and 2nd generation dendrimers, cleavage of a photolabile 2-nitrobenzyl derivative at the focal point enabled cyclization of the diamine. Subsequent 1,4-elimination-decarboxylation reactions fragmented the branching units, regenerating the amines for cyclization and thus the next phase of degradation. Two molecules of aminomethylpyrene were released from the 1st generation dendrimer and four were released from the 2nd generation dendrimer as detected by HPLC. Contrary to the results of McGrath and coworkers with polyether dendrimers, these polycarbamate dendrimers exhibited significant differences in release kinetics between dendrimer generations. Characterization of the intermediates allowed the authors to determine that cyclization of the diamine spacer was the rate...
limiting step, and thus as more of these spacers were incorporated, the time required for complete degradation correspondingly increased.

While use of the diamine spacer reduced steric crowding in the dendrimer backbone, allowing for the preparation of the 3rd generation dendrimer backbone, it was found that steric crowding at the dendrimer periphery prevented the conjugation of eight aminomethylpyrene reporters. Therefore, the reporter was changed to the smaller molecule 4-nitroaniline. Using a chemically cleavable t-butyloxycarbonyl (Boc) group at the focal point, this 3rd generation dendrimer was found to degrade as expected upon removal of the Boc group by trifluoroacetic acid (TFA). While neither of the above examples explicitly demonstrated the applicability of their systems in drug delivery applications, using model systems they were able to demonstrate the potential of self-immolative dendrimers in signal amplification and were able to identify degradation intermediates that supported their proposed degradation pathways.

![Scheme 1.16](image)

Scheme 1.16. A 1st generation dendrimer that fragments via 1,8-elimination-decarboxylation reactions upon focal point reductions to release two molecules of Tax.

The 2003 report of de Groot and coworkers was the first to explicitly demonstrate the potential use of self-immolative dendrimers for amplified drug release. The backbone of their dendrimer was the branched monomer 2-(4-aminobenzylidene)propane-1,3-diol. A nitro group was used to mask the focal point
aniline in oxidized form and two molecules of Tax were conjugated to the periphery of the 1\textsuperscript{st} generation dendrimer (Scheme 1.16), while 4 molecules of 4-nitrophenol were conjugated to the periphery in the 2\textsuperscript{nd} generation dendrimer. Upon reduction of the nitro group to the corresponding aniline with zinc metal in acetic acid, each branched monomer underwent two 1,8-elimination-decarboxylation reactions, leading to dendrimer fragmentation and release of the dendrimer's drug molecules or reporters. As only electronic cascade reactions were involved in this degradation process, the kinetics were similar to those observed by McGrath and coworkers, with release of Tax occurring over a period of 30 min as detected by NMR spectroscopy.

When carbonate-bound Tax was subjected to the nitro-reducing conditions, no degradation of the material was observed, illustrating that the conditions for the reduction of the nitro group did not effect cleavage of the carbonates by some other mechanism. Thus, they demonstrated that release of Tax occurred only after the nitro group was reduced to the corresponding amine and the electronic cascade reaction had taken place. As this system was designed for prodrug therapy, the degradation product 2-(4-aminobenzylidene)propane-1,3-diol was assayed for cytotoxicity in human tumor cell lines, and found to have negligible adverse effects.

With the groundwork for self-immolative dendrimers laid in these seminal contributions, emphasis shifted towards improvements, modifications, and applications of these dendrimers. McGrath and coworkers recently reported improvements to their methods for synthesizing the linearly disassembling benzyl(aryl ether) dendrimers, and were able to prepare 1\textsuperscript{st}, 2\textsuperscript{nd}, and 3\textsuperscript{rd} generation dendrimers on a multigram scale\textsuperscript{104}. An allyl ether was used at the dendrimer periphery, and its cleavage led to release of 4-nitrophenol at the dendimer focal point. Using improved degradation conditions, the disassembly was shown to proceed smoothly and more rapidly than previously reported.

McGrath and coworkers also reported a new convergent synthesis strategy towards their signal amplifying dendrimers based on 2,4-bis(hydroxymethyl)phenol\textsuperscript{105}. Previous self-immolative dendrimer syntheses were divergent (i.e. synthesized outward from the focal point), and in fact, a synthetic approach working from the periphery
towards the core was not possible for other dendrimers due to the tendency of the self-immolative units to disassemble. McGrath’s group was able to use a copper-mediated coupling reaction to generate these unique ether linkages without revealing a free phenol, which made a convergent synthesis feasible. Using this strategy, they were able to synthesize 0\textsuperscript{th}, 1\textsuperscript{st}, and 2\textsuperscript{nd} generation dendrimers containing an allyl ether focal point and multiple peripheral 2,4-xylenol molecules that could be released in an amplified manner. However, the use of these dendrimers for drug delivery applications has not been explored to this point.
Figure 1.25. a) A 1st generation dendrimer that releases anticancer drugs Dox and camptothecin via a series of cyclization and elimination-decarboxylation reactions upon focal point cleavage by a catalytic antibody; and b) a similar dendrimer for the release of Dox, camptothecin and etoposide by the same mechanism as in a).
Following their initial report on self-immolative dendrimers, Shabat and coworkers have made several significant advancements in the application of their self-immolative dendrimers for amplified detection and drug release. It was demonstrated that a trigger sensitive to the catalytic antibody 38C2, which catalyzes a sequence of retro-aldol, retro-Michael cleavage reactions could be introduced at the focal point of a 1st generation dendrimer, while the periphery could be used to conjugate Dox and/or camptothecin, providing both homodimeric and heterodimeric enzyme sensitive prodrugs (Figure 1.25a).\textsuperscript{106} Cell-growth inhibition assays in MOLT-3 leukemia cells with a fixed antibody concentration demonstrated that in each case, and particularly for the heterodimer, the dimeric prodrug was more active than the corresponding monomeric produg or combination thereof. This result was attributed mainly to the dendritic amplification effect.

In an extension of this work, a modified branching unit capable of undergoing three elimination-decarboxylation reactions was used with the same catalytic antibody 38C2 substrate as the focal point trigger to provide the nearly simultaneous release of three different chemotherapeutic drugs, camptothecin, Dox, and etoposide, in response to a single enzymatic cleavage event (Figure 1.25b).\textsuperscript{107} In a modified system, three equivalents of the chemotherapeutic drug melphalan were conjugated to an enzyme-labile trigger through purely elimination spacers. The use of a trigger and branching unit that did not utilize a slow cyclization step for signal transmission allowed for rapid release of all three drug molecules, leading to enhanced drug toxicity in the presence of the enzyme penicillin-G-amidase.\textsuperscript{108} The disassembly rate of the system based on 2,6-bis(hydroxymethyl)-4-cresol could also be enhanced by replacement of the methyl substituent on the aromatic ring with a more electron-withdrawing ethylcarboxy-ester at the sacrifice of one branching position.\textsuperscript{109} Most recently, a first generation dendrimer fragmenting by the 1,6-elimination-decarboxylation cascade was used to conjugate Tax to \textit{N}(2-hydroxyproyl)-methacrylamide (HPMA) via a focal point oligopeptide spacer sensitive to cathepsin B.\textsuperscript{110} Cleavage of this peptidic spacer initiated the dendrimer disassembly, resulting in the release of three equivalents of Tax. This new polymer-drug conjugate exhibited enhanced cytotoxicity to murine prostate adenocarcinoma (TRAMP-
C2) cells in comparison with the classic monomeric polymer-drug conjugate, again demonstrating the advantage of dendritic amplification.

Figure 1.26. Chemical structure of a self-immolative dendrimer with water solubilizing PEO moieties and camptothecin on the periphery, with a penicillin-G-amidase-sensitive focal point.

While the above approaches were successful in demonstrating amplified enzymatically-triggered drug release from 1st generation dendrimers, the enzymatic activation generally failed for 2nd generation dendrimers. This was attributed to the high hydrophobicity of the larger dendritic structures, which resulted in aggregation of the molecules in aqueous solution and prevented access of the enzyme to its substrate. This
problem was addressed by the conjugation of PEO chains to the dendrimer periphery. Using this strategy, 2nd generation dendrimers were synthesized that could be activated at their focal point by the enzyme penicillin-G-amidase to release four molecules of camptothecin (Figure 1.26). Assays in MOLT-3, JURKAT, and HEK-293 cells revealed that the IC₅₀ values for this prodrug were 2 – 3 orders of magnitude lower in the presence of the enzyme, suggesting that high concentrations of prodrug could be applied in chemotherapy with decreased side effects. An alternative approach to addressing the aqueous solubility issue was the use of ionizable reporter groups. For example, the reporter 5-amino-2-nitrobenzoic acid was utilized to improve solubility, and it was possible to activate 0th to 2nd generation dendrimers with penicillin-G-amidase without the need for PEO. However, this approach is limited in scope compared to the use of PEO chains, as the structures of chemotherapeutic compounds cannot be altered as easily as those of reporter molecules.

**Figure 1.27.** Schematics of dendrimer designs: a) Cleavage of either one of the two different triggers leads to the release of a reporter/drug molecule (molecular OR logic trigger); b) Cleavage of a single trigger leads to the release of multiple reporters (amplifier); c) A receiver-amplifier dendrimer with high sensitivity and gain, whereby a low intensity signal can be amplified via the release of multiple reporters.

In addition to the triggered release of drug molecules, self-immolative dendrimers are also highly promising as sensor molecules, due to their ability to transmit and amplify one or more signals. For example, by using two peripheral groups sensitive to different
stimuli, and a self-immolative spacer designed to fragment towards the focal point, it was possible to transmit a signal from the cleavage of either moiety to a reporter group at the focal point (Figure 1.27a).\textsuperscript{113} When the moiety released from the focal point was a drug molecule, the approach was referred to as “prodrug activation by a molecular OR logic trigger”.\textsuperscript{114} While these dendrimers resembled antennae more than amplifiers, it is also possible to provide amplification by transmitting a signal from the focal point to the dendrimer periphery. For example, using a branching unit capable of undergoing six 1,8-elimination-decarboxylation reactions, it was possible to release six tryptophan reporter molecules in response to a single enzymatic cleavage event (Figure 1.27b).\textsuperscript{115} Dual output dendrimers have also been synthesized, wherein two types of reporters are conjugated to the dendrimer periphery. Such structures facilitate the use of orthogonal spectroscopic techniques such as fluorescence and absorbance, and could be potentially useful in cases where one signal is unreliable; for example in a case where fluorescence is quenched.\textsuperscript{116} In an interesting combination of the antennae-amplifier approach, it was possible to transmit a signal from the periphery of one “receiver” dendrimer to its focal point following a single cleavage event, then out to all of the peripheral units of a dendritic “amplifier”, leading to the release of multiple reporter molecules and providing a response with high sensitivity and gain (Figure 1.27c).\textsuperscript{117}

The most recent examples demonstrating the use of dendritic amplification for detection purposes were sensors capable of detecting H\textsubscript{2}O\textsubscript{2}. Triacetone triperoxide is an illicit explosive that decomposes to release H\textsubscript{2}O\textsubscript{2} upon treatment with acid. Thus an H\textsubscript{2}O\textsubscript{2} sensor could potentially provide a means of detecting triacetone triperoxide. To this end, a self-immolative dendrimer containing an arylboronate ester at the focal point was synthesized. Reaction of this boronate ester with H\textsubscript{2}O\textsubscript{2} led to a new borate that was readily hydrolyzed to produce a phenol, initiating elimination-decarboxylation reactions to release multiple fluorescent reporters from the dendrimer periphery (Scheme 1.17).\textsuperscript{118}
Scheme 1.17. Chemical structure of a H$_2$O$_2$ sensor. Decomposition of a borate ester by H$_2$O$_2$ leads to fragmentation of the self-immolative dendrimer and the amplified release of reporter chromophores 1.99.

Following this work, Shabat and coworkers introduced an approach to exponentially amplify diagnostic signals using a dendritic chain reaction.$^{119}$ Using a phenylboronic acid at the focal point, H$_2$O$_2$ triggered the generation of the focal point phenol followed by disassembly of the dendrimer by elimination-decarboxylation reactions (Scheme 1.18). However, in this case the system was designed to release one reporter and two molecules of choline from the dendrimer periphery. The released choline reacted with choline oxidase in situ to produce four molecules of H$_2$O$_2$, which were able to activate four additional dendrimers. Thus in principle, a single molecule of H$_2$O$_2$ could initiate a chain reaction leading to disassembly of all dendrimers in the solution and release of all reporter groups. At high analyte concentrations, this system behaved similar to a conventional probe; however at lower analyte concentrations the chain reaction led to a 53-fold increase in signal due to the exponential amplification
provided by the dendritic chain reaction. It was also demonstrated that this approach could be applied to the detection of proteases. In this case, penicillin-G-amidase cleaved a substrate, resulting in the release of choline and subsequent activation of the dendritic chain reaction. The H₂O₂ sensor was later improved via the replacement of choline with methanol. The decreased acidity of methanol (pKₐ 15.5) relative to choline (pKₐ 13.9) resulted in a more stable carbonate, and thus reduced spontaneous carbonate hydrolysis that had previously resulted in a relatively high background signal and decreased sensitivity. Generation of amplified concentrations of H₂O₂ in this system was achieved via the oxidation of methanol by alcohol oxidase.

Scheme 1.18. A dendrimer system capable of exponential signal amplification. Cleavage of the focal point trigger by H₂O₂ releases a reporter molecule and choline molecules that are converted to additional H₂O₂ via an enzymatic reaction with choline oxidase.

The dendritic chain reaction approach was also later extended to a two component system wherein a dendritic amplifier moiety capable of releasing choline was combined with a separate molecule containing a chromogenic or fluorescent reporter. Both components were triggered by H₂O₂ such that some molecules of H₂O₂ generated from the oxidation of choline could activate additional dendritic amplifiers while others could activate the reporter. This two-component approach led to simplification of the synthetic
strategy required for preparation of the dendrimers. While all the above sensor approaches focused on the detection of \( \text{H}_2\text{O}_2 \) and used this molecule in the signal amplification cascade, it was also recently demonstrated that a similar system could be generated using thiols.\(^{122}\) A dendrimer with a thiol sensitive benzoquinone trigger at its focal point was designed to release two new thiol molecules upon self-immolative disassembly. The combination of this dendron with a chromogenic reporter having a benzoquinone trigger allowed for amplified sensing of the thiols using the two-component strategy. This demonstrated the versatility of the dendritic chain reaction with respect to the incorporation of different chemistries.

As demonstrated by the examples above, due to their branched structures, dendrimers can provide an ideal framework for amplified drug release and sensing. Their step-wise synthesis provides well-defined molecules that are attractive for *in vivo* applications and are ideal for detailed fundamental physical studies in molecular sensing. However, these same attributes have also introduced some limitations. The step-wise syntheses of the complicated dendritic structures used in the aforementioned applications are tedious and likely expensive to perform on a large scale. In addition, the use of large peripheral moieties such as drugs and chromophores has introduced significant steric hindrance at the dendrimer peripheries. While the latter has been addressed to some extent by the use of larger branching units and linkers, the vast majority of dendrimers described above were limited to the 1st or 2nd generations, thus limiting the degree of signal amplification that could be obtained from a single molecule. Nevertheless, as demonstrated by the examples above, self-immolative dendrimers possess a great deal of potential for a variety of applications. They can be effectively used for enhanced drug delivery, both for increased dosage delivery as well as the potential to release a drug cocktail from a single source. Their effectiveness in biosensing has also been demonstrated, with the detection of a specific biomolecule triggering the release of multiple reporters. Lastly, their role in trace analyte detection was demonstrated by making use of dendritic chain reactions to heighten probe sensitivity.
1.8 Self-Immolative Linear Polymers

Following dendrimers and oligomers, the investigation of self-immolative linear polymers was the next logical step in the evolution of self-immolative materials. While linear polymers are not monodisperse like dendrimers and oligomers, with the appropriate monomers in hand they are synthesized in a single step, making their preparation both practical and scalable. In addition, as polymers are inherently composed of many monomer units, the cleavage of the trigger has the potential to provide a higher degree of amplification than the oligomeric or dendritic structures. This amplification can potentially be achieved by fragmentation of the linear polymer backbone leading directly to the release of drug molecules or reporters. Alternatively, as linear copolymers are known to assemble into a variety of supramolecular structures such as micelles, vesicles, and nanoparticles capable of encapsulating drug molecules, the disassembly of the polymer backbone in response to a stimulus can potentially lead to the controlled release of drugs from these assemblies.

The first self-immolative linear polymer was reported in 2008 by Shabat and coworkers (Scheme 1.19). An end cap sensitive to BSA was incorporated at the polymer terminus such that upon cleavage, a series of 1,6-elimination-decarboxylation reactions resulted in depolymerization of the polycarbamate backbone. Depolymerization was readily detected by the release of a fluorescent monomeric 4-aminobenzyl alcohol derivative whereas its fluorescence was significantly reduced when the amine was masked as a carbamate. The polymer backbone comprised approximately 15 – 20 units, providing significant amplification for each cleavage event.
Scheme 1.19. A self-immolative linear polymer that depolymerizes upon cleavage of the terminal end cap by BSA, releasing many fluorescent reporters, thus providing amplified sensing of BSA activity.

The same polymer backbone was also later used by the same group for the activity-linked labeling of enzymes. The concept behind this work was that if an enzyme successfully cleaved the end cap of the polymer, depolymerization would generate reactive azaquinone methide intermediates that could be trapped by proximal nucleophilic moieties on the protein's amino acid side chains, resulting in labeling of the active enzyme with fluorophores. Penicillin-G-amidase and the catalytic antibody 38C2 were successfully labeled in this study. It was found that while penicillin-G-amidase did not exhibit a significant reduction in its enzymatic activity as a result of the labeling, antibody 38C2 did show diminished catalytic activity post-labeling, which was attributed to labeling of the active site lysine ϵ-amine. Interestingly, in a comparison between self-immolative polymers, oligomers, and monomers as labeling agents, it was demonstrated that the polymeric agents could provide enhanced levels of labeling, while preserving higher levels of enzymatic activity. This was explained by the breakdown of the polymeric species over a period from seconds to minutes, allowing the depolymerization to release azaquinone methide species farther from the active site where they could be trapped without affecting activity. Despite this potential for polymer diffusion, it was shown that when the labeling was performed in the presence of an activating and a nonactivating protein in solution, the labeling of the activating protein was more effective than the non-activating protein by a factor of 8.
Scheme 1.20. A water-soluble self-immolative polymer that depolymerizes by 1,6-elimination-decarboxylation reactions following activation by penicillin-G-amidase to release model drug 1.102 via additional 1,6-elimination-decarboxylation reactions at a vinylogous benzylic site on the monomer.

While specific choices of monomer units can lead to the direct generation of reporter molecules upon depolymerization, Shabat and coworkers have also reported a water soluble linear polymer where backbone depolymerization occurs via a series of 1,6-elimination-decarboxylation reactions, with an additional 1,6-elimination-decarboxylation at a vinylogous 2-benzyl position to release a reporter group (Scheme 1.20). This polymer provided signal amplification in response to the cleavage of the end cap by penicillin-G-amidase via the release of non-monomeric reporters. Although 4-nitroaniline was used in this case, the reporter could be replaced with a drug molecule allowing end cap cleavage to trigger amplified drug release.
Scheme 1.21. The use of self-immolative polymers to prepare crosslinked microcapsules that rupture and release their contents upon cleavage of the polymeric end caps.

Expanding on the concept of amplified release of non-covalently encapsulated molecules from self-immolative materials, Moore and coworkers used a self-immolative polycarbamate backbone\textsuperscript{129} to prepare microcapsules (Scheme 1.21).\textsuperscript{132} This was achieved by the cross-linking of the linear polymers using interfacial polymerization conditions. Removal of Boc or fluorenlymethyloxycarbonyl (Fmoc) end caps stimulated the release of the microcapsule contents via depolymerization and capsule rupture. Although these capsules were not specifically targeted towards drug delivery applications, it was suggested that they might be useful for the release of healing agents in autonomous repair systems.

An alternative approach not technically involving a self-immolative polymer backbone was the introduction of self-immolative spacers throughout a linear polymer by Almutairi and coworkers (Figure 1.28).\textsuperscript{133} In this case, cleavage of a 4,5-dimethoxy-2-nitrobenzylcarbamate using either a one- or two-photon process led to unmasking of an amine followed by cyclization and 1,4-elimination reactions to cleave the polymer
backbone. The polymers were used to prepare nanoparticles by a single emulsion method. It was demonstrated that encapsulated Nile Red was released from the particles following irradiation at 350 nm or 750 nm. The initial burst, followed by slower release suggested that the mechanism of release likely involved both the rapid change in hydrophilicity of the nanoparticles resulting from removal of the hydrophobic photochemical trigger, as well as a slower breakdown of the nanoparticles resulting from chain cleavage.

**Figure 1.28.** Self-immolative spacers lead to cleavage of a polymer backbone upon removal of a trigger moiety photochemically. The polymer was used to prepare photoresponsive nanoparticles.

**Figure 1.29.** General structure of polyphthalaldehyde.

In addition to 4-aminobenzyl alcohol spacers used thus far, Phillips and coworkers have recently explored polyaldehydes as self-immolative polymers (Figure 1.29). Utilizing polyphthalaldehyde (PPHA) capped with different functional groups, they developed stimuli-responsive patterned plastics as well as self-powered microscale pumps. In the first scenario, they synthesized allyloxycarbonyl (Alloc)- and tert-butyldimethylsilyl (TBS)-capped polymers sensitive to Pd$^0$ and F$^-$, respectively,
and cast them onto a thin film surrounded by nondegradable polymer. Exposure to stimuli resulted in rapid degradation of the polymer, with complete erosion of the film occurring within 5 min following exposure of Alloc-terminated PPHA to 0.4 eq of Pd(PPh₃)₄, and within 1 min after exposing TBS-capped PPHA to 0.5 eq tetrabutylammonium fluoride (TBAF). Utilizing the same TBS-capped scaffold they generated a thin film and measured velocity of the tracers following exposure to cleavage conditions, and were able to generate average flow rates of 1.0 µm/s in the presence of TBAF, and 12 µm/s in pH 14 solution.

All of the above oligomers, dendrimers, and linear polymers used the electronic cascade reactions in their backbones. These reactions have been attractive for the development of self-immolative materials due to their rapid degradation rates. In addition, they are relatively straightforward to work with synthetically as they are unreactive when the aromatic amines or alcohols are masked. However, the biocompatibility of the quinone methide and azaquinone methide intermediates generated during the disassembly process is currently debated, making the potential of these systems for in vivo applications questionable. While studies of some prodrug systems involving electronic cascade reactions have suggested that the byproducts are nontoxic, other studies have demonstrated toxicity of quinone methides. The work by Shabat and coworkers on the activity-linked labeling of enzymes by self-immolative polymers demonstrated that azaquinone methides can react with proteins in their vicinity and such reactivity certainly raises concerns.

While linear polymers do not possess the well-defined structures of dendrimers or oligomers, as demonstrated above they exhibit other advantages such as simplified synthetic approaches. In addition, they can provide a high degree of signal amplification due to a high reporter to trigger ratio. This amplification has been achieved thus far by use of the polymer backbone monomers as reporters, or by the conjugation of reporters along the polymer backbone that are released through the disassembly process. Alternatively, the non-covalent encapsulation of materials in nanoparticles or capsules composed of self-immolative polymers can provide amplified release upon stimulus-triggered depolymerization. Considering that the first examples of self-immolative linear
polymers were reported only recently, significant progress has already been made and it is likely that there will be considerable progress in the application of these materials for a variety of drug delivery, sensing, and materials science applications over the years to come.

1.9 Scope of This Thesis

When work began on this thesis, no prior publications on self-immolative linear polymers had been reported. We, alongside Shabat and other research groups, sought to develop this new class of materials. In addition to employing 4-aminobenzyl alcohol derivatives, focus was placed on utilizing additional spacers previously used in small molecule prodrugs and self-immolative dendrimers in an effort to build up a library of these polymers with unique physical characteristics, cleavage mechanisms and degradation profiles. The goal in doing so was to develop materials for a wide array of applications, including drug delivery vehicles responsive to many potential stimuli and possessing a broad range of release profiles, as well as more materials-based applications such as tissue engineering. The general goal was to design and synthesize polymers from self-immolative spacers either previously reported in the literature or developed within our lab, and attach them to a variety of end caps. Once synthesized, the polymers were then tested for self-assembly properties, if applicable, and then study the kinetics of depolymerization and release of analytes.

Chapter two focuses on the development of a self-immolative polymer derived from \(N,N\)-dimethylethylenediamine and 4-hydroxybenzyl alcohol. By attaching a PEG end cap, this polymer will be shown to self-assemble in aqueous solution and to encapsulate and release a model drug molecule.

Chapter three describes the synthesis of a self-immolative polymer derived from the same diamine spacer in conjunction with 2-mercaptoethanol. Possessing a disulfide end cap, this polymer will be shown to be stable to buffered solution in an oxidizing
environment, but exposure to a reducing agent cleaves the disulfide and triggers polymer degradation.

Chapter four discusses the development of 4-aminobutyric acid derivatives as a new class of rapidly cyclizing self-immolative amine-based spacers as an alternative to the previously employed diamine. Results will highlight the effect of various substituents at the $N$ and $\alpha$ positions on the observed cyclization rates.

Chapter five builds upon the results of chapter four and discusses the development of self-immolative polymers derived from 4-aminobutyric acid. Two polymers were developed utilizing different phenolic spacers in conjunction with 4-aminobutyric acid, and the different strategies and synthetic routes to make these polymers will be discussed. Lastly the degradation kinetics of the monomer will be reported.

1.10 References

Chapter 2

A Self-Immolative Polymer Based on Alternating Cyclization and Elimination Reactions

2.1 Introduction

In recent years there has been significant interest in the development of biodegradable polymers and in their application to areas as diverse as food sciences, medical devices, drug delivery, and tissue engineering. In particular, polyesters such as PLA, PGA, and PCL have been extensively used in biomedical applications and have also been proposed as environmentally friendly replacements for traditional plastics such as polyethylene. While the biodegradation rates of these polymers can be controlled to some extent by tuning their composition, solubility and processing, they typically degrade by random hydrolytic cleavages throughout the backbone, a process that is relatively unregulated. For many applications, it would be desirable to use polymers that can be degraded in a controlled manner in a specified environment or in response to a stimulus. To address this, polymers with many acetal or disulfide linkages in their backbones have been developed. These polymers have been demonstrated to degrade under mildly acidic and reducing conditions respectively, but the mechanism of degradation still involves random chain scissions throughout the polymer backbone, and many environmentally mediated cleavage events are required to completely degrade the polymer.

Another interesting class of molecules that is under development is stimuli responsive polymers. For example, polymers based on NIPAM or oligo(ethylene glycol) methacrylates have been demonstrated to be thermally responsive, with high aqueous solubilities below their LCST and precipitation above the LCST. Polymers

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containing pendant amines or carboxylic acids are typically responsive to pH, being soluble within specified pH ranges. In addition, hydrophobic groups and drugs have been appended to polymer backbones by pH sensitive acetals, hydrazones, or photochemically cleavable linkages such that the solubilities of the polymers are significantly altered upon the removal of these groups. Other systems responsive to stimuli such as sugar concentration and redox potential have also been reported. These stimuli responsive polymers have increasingly been used in recent years to prepare assemblies such as micelles, vesicles, and nanoparticles that are capable of releasing molecules in response to changes in environmental conditions. However, the design of systems capable of responding within the relatively narrow range of biologically accessible conditions is still a significant challenge.

A new and attractive concept for the design of materials that are both degradable and stimuli responsive is end capped self-immolative polymers. As illustrated in Figure 2.1, these polymers comprise a backbone that is stable when the end cap is intact, but upon removal of the end cap via a single bond cleavage, a functionality is revealed at the polymer terminus that initiates a cascade of intramolecular reactions leading to complete depolymerization from end to end. This concept was initially introduced in dendritic systems that upon removal of a focal point group were demonstrated to degrade by an intramolecular cascade, releasing multiple molecules from the dendrimer periphery. Such systems were then further developed to provide for the simultaneous release of multiple different drug molecules, the incorporation of tumor targeting groups, and focal point groups that were sensitive to reducing conditions or enzymes.

Figure 2.1. Schematic of a self-immolative linear polymer, where removal of a stabilizing end cap initiates a cascade of reactions leading to depolymerization.
The application of this concept to linear self-immolative polymers has the potential to dramatically expand their utility. For example, such materials may be used for the assembly of supramolecular aggregates such as micelles, vesicles, and nanoparticles or for the fabrication of medical devices or tissue engineering scaffolds, where they can impart several unique and advantageous properties. For example, the use of end caps responsive to different conditions could allow the degradation of a single polymer to be triggered under a wide range of conditions, while the composition of the polymer backbone itself would determine the rate of degradation. By tuning the length of the polymer backbone, the time required for polymer degradation can potentially be controlled, as a longer polymer should take longer to completely depolymerize than a shorter polymer. In addition, due to the end-to-end degradation mechanism resulting in a controlled and gradual reduction of polymer molecular weight, the physical properties of the polymer should be retained longer during the degradation process in comparison with a traditional degradable polymer such as a polyester, where a single cleavage event may decrease the molecular weight by up to 50%. Despite these attractive features, only one self-immolative linear polymer backbone has been reported to date.\textsuperscript{54,55} This backbone was a polycarbamate based on 4-aminobenzyl alcohol derivatives, which degrades entirely by intramolecular 1,6-elimination reactions via azaquinone methide intermediates. It has been demonstrated that by using enzyme sensitive end caps this backbone can be used as an amplifying sensor,\textsuperscript{54} or to release multiple model drugs conjugated along the polymer backbone.\textsuperscript{55} The development of alternative backbones and cascade degradation mechanisms will be necessary in order to tune the properties and degradation rates, opening the way for new applications of this class of materials.

Inspired by the incorporation of alternating cyclization and 1,6-elimination spacers into a dendritic system,\textsuperscript{52} reported here is the first example of a linear self-immolative polymer that degrades by alternating elimination and cyclization reactions. The degradation rate and mechanism were studied using NMR spectroscopy and size exclusion chromatography (SEC) and it was demonstrated that the incorporation of a monomer that induces depolymerization by a cyclization mechanism provides an effective means of tuning the degradation rate. Furthermore, by conjugating PEG to the terminus of the self-immolative polymer as an end cap, an amphiphilic block copolymer
was obtained, which assembled into nanoparticles in aqueous solution. Hydrolysis of the ester linkage between the blocks initiated the cascade degradation process under physiological conditions. These nanoparticles were found to encapsulate a hydrophobic dye and release it upon depolymerization, thus demonstrating for the first time the utility of this class of molecules in the development of functional polymer assemblies and nanomaterials.

2.2 Results and Discussion

2.2.1 Design

The use of monomers such as hydroxybenzyl alcohol and aminobenzyl alcohol derivatives in the development of self-immolative polymers is attractive as the presence of the free aromatic alcohol or amine functionalities that normally trigger quinone methide mediated elimination reactions can be masked when they are activated, thus providing relatively stable polymerization monomers and synthetic intermediates. Another potential class of monomers for self-immolative polymers is those capable of undergoing intramolecular cyclization reactions. One example is a carbamate derivative of \(N,N'\)-dimethylethylenediamine, which spontaneously cyclizes to form \(N,N'\)-dimethylimidazolidinone, releasing the alcohol. This spacer has been incorporated into some of the previously reported self-immolative dendrimers, where protecting groups can be carefully manipulated during the step-wise dendrimer synthesis; however, the synthesis of linear polymers based on this class of monomers presents a significant challenge, as the requisite activation of the monomer for polymerization introduces the possibility for an intramolecular cyclization to occur much more rapidly than the intermolecular polymerization (Figure 2.2a). For this work, it was proposed that by incorporating alternating \(N,N'\)-dimethylethylenediamine units with 4-hydroxybenzyl alcohol units linked via carbamates as shown in Figure 2.2b, the site of the monomer activation for polymerization could be moved distal to the diamine, thus significantly slowing the intramolecular cyclization, allowing effective polymerization of the activated heterodimer. Upon removal of the end cap from the amine polymer terminus, cyclization
of the diamine should occur, releasing \( N,N' \)-dimethylimidazolidinone and revealing the phenol. The phenol should then undergo a 1,6-elimination, ultimately releasing 4-hydroxybenzyl alcohol, \( \text{CO}_2 \), and another amine terminus, from which the cascade can continue until depolymerization is complete.

![Diagram](image)

**Figure 2.2.** a) A competing intramolecular cyclization makes the incorporation of cyclizing monomers into a self-immolative linear polymer challenging. b) By using alternating monomers, the activating leaving group can be moved distal to the cyclization monomer, thus facilitating polymerization. End cap removal then allows for depolymerization by alternating cyclization and 1,6-elimination reactions.

### 2.2.2 Monomer Synthesis

The synthesis of the target monomer began by the reaction of 4-hydroxybenzyl alcohol with *tert*-butoxydimethylchlorosilane in the presence of imidazole to provide compound **2.6**, with selective protection on the aliphatic alcohol as shown in Scheme 2.1. The phenol of **2.6** was then activated using 4-nitrophenyl chloroformate to provide the activated carbonate **2.8**. Tri(ethylene glycol) monomethyl ether (TEGMME) was added
to the reaction mixture upon completion of the activation in order to quench the remaining chloroformate, which was otherwise inseparable from 2.8. \textit{N,N’-}Dimethylethylenediamine (2.9) was converted to the mono Boc-protected derivative 2.10 by reaction with di-\textit{tert}-butyldicarbonate, and then the other amine was reacted with the activated carbonate 2.8 in the presence of 4-(dimethylamino)pyridine (DMAP) and \textit{N,N-}diisopropylethylamine (DIPEA) to provide the carbamate 2.11. Removal of the TBS protecting group was accomplished with 1 vol\% HCl in EtOH, conditions under which the Boc protecting group remained intact, to give 2.12. Finally, the protected polymerization monomer 2.13 was prepared by activation of 2.12 with 4-nitrophenylchloroformate.

\textbf{Scheme 2.1.} Synthesis of Boc-protected active monomer 2.13.
2.2.3 Polymer Synthesis

Removal of the Boc protecting group from compound 2.13 using TFA provided the monomer 2.2a·TFA, capable of self-condensing via reaction of the amine with the activated carbonate to form a polycarbamate, releasing 4-nitrophenol (Scheme 2.2). When isolated as the TFA salt, the amine was sufficiently stable that as long as the deprotection was performed within hours prior to the polymerization reaction it could be isolated and transferred to the polymerization conditions without premature polymerization or cyclization. Compound 2.13 with the Boc-capped amine also served as a convenient end cap in the synthesis of the initial model polymer. Although a Boc group cannot be readily cleaved under any known physiological conditions, it can be easily cleaved with TFA under non-aqueous conditions, allowing the depolymerization process to be studied independently of the end cap cleavage. Thus, monomer 2.2a was reacted with 0.05 equivalents of the end cap 2.13 in the presence of DMAP and DIPEA to provide the polymer 2.3a. Based on the synthesis of 2.11 from 2.8 it had already been determined that the reaction of the secondary amines with 4-nitrophenyl carbonates was a rapid and very high yielding process, thus favoring its use as the key polymerization reaction. Indeed the crude polymer was isolated in 92% yield following extraction to remove the 4-nitrophenol, DIPEA, and DMAP, and less than 5% of the monomer had been converted into the cyclic urea byproduct as determined by $^1$H NMR spectroscopy. This indicated that the polymerization reaction successfully competed with potential intramolecular cyclizations under the reaction conditions. It is also noteworthy that although each monomer had a 4-nitrophenyl end group at the beginning of the polymerization, no 4-nitrophenyl groups were observed in the polymer product. This indicates that this group was lost during the polymerization, in the reaction medium upon completion of the polymerization, or during workup, leaving a benzylic alcohol terminus. This is not surprising considering the high reactivity of this group under these conditions.
Scheme 2.2. Deprotection and polymerization of monomer 2.13.

SEC analysis of polymer 2.3a showed a relatively broad distribution of molecular weights and some low MW oligomers (see appendix), some of which might be cyclic oligomers, consistent with the expected result for a condensation type polymerization. Therefore, in order to facilitate the degradation studies, the higher MW fraction was isolated in 45% yield by preparative SEC in DMF to provide a monomer to end cap ratio of approximately 16:1 based on $^1$H NMR analysis. This value corresponds to a number average molecular weight ($M_n$) of approximately 4600. A number average molecular weight of 17 000 and polydispersity index (PDI) of 1.6 was determined based on SEC relative to polystyrene standards (Figure 2.3). The overestimation of the MW by SEC using a conventional calibration can be attributed to the contracted conformation of polystyrene relative to polymer 2.3a in DMF as DMF is a relatively poor solvent for polystyrene.\textsuperscript{59} Polymer 2.3a did not provide a light scattering signal of sufficient intensity to allow for absolute MW determination by multi-angle light scattering, likely due to its relatively low MW, but the $^1$H NMR analysis should provide an accurate measurement of the average degree of polymerization. Preparative SEC could be easily performed on 200 mg batches of polymer, which was suitable for the current study, but for scale up it is anticipated that dialysis using a membrane with an appropriate MW cut-off can likely be used.
Figure 2.3. Size exclusion chromatograms of polymer 2.3a prior to degradation (after preparative SEC), after 24 h of degradation and after 4 days of degradation (eluent = DMF with 10 mM LiBr and 1% (v/v) NEt₃; detection by differential refractive index).

2.2.4 Degradation Kinetics for Polymer 2.3a

While the rate of the 1,6-elimination reaction has previously been reported and was found to be very rapid, the diamine cyclization was expected to be much slower. As this was anticipated to be the rate-limiting step in the polymer degradation it was important to investigate its rate under the same conditions to be used in the polymer degradation study in order to gain insight into the time scale expected for the depolymerization. To accomplish this, the Boc protecting group was removed from compound 2.12 by treatment with 1:1 TFA:CH₂Cl₂, and the product was incubated in a pH 7.4 0.1 M phosphate buffer:acetone (3:2) at 37 °C. The appearance of N,N'-dimethylimidazolidinone and 4-hydroxybenzyl alcohol were quantified by ¹H NMR spectroscopy. Mass spectrometry was used to further support the identities of the proposed degradation products (see appendix). As shown in Figure 2.4a, the cyclization was complete after approximately 3 h, and fitting of the data to a first order rate law provided a half-life of approximately 35 min, very close to that measured by Saari et al. in a fully aqueous system. It is noteworthy that although quantitative kinetic studies were not carried out in other solvents, it was found that the cyclization was generally
slower in less polar solvents. The pH 7.4 0.1 M aqueous phosphate buffer:acetone (3:2) mixture was selected for the above kinetic study as it was found to be the most polar one capable of fully dissolving polymer 2.3a.

**Figure 2.4.** a) Kinetics of cyclization of compound 2.12 following Boc group removal, as measured by $^1$H NMR spectroscopy in pH 7.4 0.1 M phosphate buffer (D$_2$O):acetone-d$_6$ (3:2) at 37 °C, following removal of the Boc protecting group. b) Kinetics of depolymerization of polymer 2.3a, as measured by $^1$H NMR spectroscopy in pH 7.4 0.1 M phosphate buffer (D$_2$O):acetone-d$_6$ (3:2), following removal of the Boc end cap.

To study the degradation of polymer 2.3a, the polymer was treated with 1:1 TFA:CH$_2$Cl$_2$ to remove the Boc end cap, and then the polymer was incubated in pH 7.4 0.1 M phosphate buffer:acetone (3:2). The degree of degradation was quantified by $^1$H NMR spectroscopy. As shown in Figure 2.5, peaks corresponding to 4-hydroxybenzyl alcohol and $N,N'$-dimethylimidazolidinone emerged as the degradation progressed. This supports that the degradation occurred by the proposed depolymerization mechanism as a degradation mechanism based primarily on random chain scission of the carbamate linkages in the polymer backbone would generate primarily $N,N'$-dimethylethylenediamine rather than the cyclic urea. As predicted based on the half-life for the cyclization, and the approximate length of the polymer, the degradation was 50% complete in less than one day, and complete degradation was observed after 4 – 5 days (Figure 2.4b). In comparison, a control sample of polymer 2.3a in which the Boc end cap was left intact exhibited only trace levels of degradation after 4 days (see appendix).
fact that the depolymerization reached completion also indicates that there was no significant amount of nondegradable cyclic polymers in this high MW fraction that was subjected to degradation. The degradation was also monitored by SEC and it was found that after 24 h of degradation, as shown in Figure 2.3, there was a significant increase in the retention time of the polymer, corresponding to a decrease in the polymer MW. After 4 days, no polymeric material could be detected by SEC.

**Figure 2.5.** $^1$H NMR spectra of a) polymer 2.3a following end cap removal in pH 7.4 0.1 M phosphate buffer (D$_2$O):acetone-d$_6$ (3:2) at t = 0 in the degradation process; and b) the same polymer solution after 5 days at 37 °C, showing complete depolymerization into small molecules.

### 2.2.5 Synthesis of a PEG End Cap

The development of an end cap based on PEG was motivated by the possibility of forming an amphiphilic block copolymer capable of assembling into self-immolative aggregates in aqueous solution. In this study, an ester linkage that could readily be hydrolyzed under neutral physiological conditions was selected for the conjugation of the PEG block to the terminus of the self-immolative block, but this concept could readily be
extended to pH or redox sensitive linkages in order to initiate the degradation cascade under a wide range of conditions. In contrast to a traditional polyester, only one ester cleavage should be required to initiate the degradation cascade.

As shown in Scheme 2.3, the synthesis of the PEG end cap began by reaction of the commercially available acid terminated PEG monomethyl ether 2.14 having a MW of approximately 5000 g/mol, with 4-nitrophenyl chloroformate to provide the active ester 2.15. This active ester was reacted with the phenol 2.6 in the presence of triethylamine to provide the ester 2.16. The TBS protecting group of 2.16 was then removed by treatment with 1 vol% HCl in a 3:1 mixture of EtOH:CH₂Cl₂ and the resulting alcohol 2.17 was activated with 4-nitrophenyl chloroformate to provide the activated end cap 2.18.

Scheme 2.3. Synthesis of poly(ethylene glycol) end cap 2.18.

2.2.6 Synthesis and Assembly of the PEG-Polycarbamate Block Copolymer

Polymerization of the deprotected monomer 2.2a with the end cap 2.18 was carried out under the same polymerization conditions described above to provide the block copolymer 2.3b in 76% yield (Scheme 2.4). Like polymer 2.3a, this polymer was further purified by preparative SEC for kinetic studies. ¹H NMR analysis in CDCl₃ indicated that the ratio of monomer to end cap in the resulting polymer was approximately 15:1, corresponding to an Mₙ of 9100, while an Mₙ of 28 700 and a PDI of 1.2 was measured by SEC relative to polystyrene standards. When polymer 2.3b was added to pure water or pH 7.4 0.1 M phosphate buffer, the polymer did not immediately dissolve, but with
sonication it readily dispersed. Transmission electron microscopy (TEM) performed using phosphotungstic acid as a stain revealed the presence of nearly spherical aggregates with a relatively broad distribution of diameters ranging from less than 100 nm to a few hundred nm (Figure 2.6). The light corona surrounding the darker core suggests that the hydrophobic aromatic polycarbamate block forms the cores of the nanoparticles while the hydrophilic PEG coats their surfaces. TEM performed using osmium tetroxide to stain the polycarbamate block also revealed the presence of spherical aggregates (see appendix). Assemblies in water were also detected by dynamic light scattering (see appendix). The extreme broadness and very low intensities of the peaks corresponding to the hydrophobic polycarbamate block of 2.3b in its $^1$H NMR spectrum in D$_2$O is additional evidence of the polymer's assembly (Figure 2.7a).

Scheme 2.4. Synthesis of poly(ethylene glycol)-capped polymer 2.3b.

Figure 2.6. Transmission electron microscopy image of nanoparticles formed by the assembly of polymer 2.3b in water. Staining was performed with phosphotungstic acid. Scale bar = 100 nm.
2.2.7 Degradation Kinetics for Polymer 2.3b.

Prior to evaluating the degradation rate of the end capped polymer 2.3b, it was of interest to determine the rate at which the ester linkage of the end cap would be hydrolytically cleaved under physiological conditions, as this was the essential first step in initiating the degradation cascade. Thus, the PEG derivative 2.17 was incubated in pH 7.4 0.1 M phosphate buffered D$_2$O at 37 °C, and the release of the hydrolysis product 4-hydroxybenzyl alcohol was quantified by $^1$H NMR spectroscopy. As shown in Figure 2.8a, the hydrolysis occurred over a period of approximately 1 – 2 days, with a calculated half-life of 15 h.

![Figure 2.7](Image)

**Figure 2.7.** $^1$H NMR spectra of a) polymer 2.3b in pH 7.4 0.1 M phosphate buffered D$_2$O; and b) the same polymer solution after 29 days at 37 °C, showing complete depolymerization.
Figure 2.8. a) Ester hydrolysis kinetics for compound 2.17 in pH 7.4 0.1 M phosphate buffered D$_2$O at 37 °C as measured by $^1$H NMR spectroscopy. b) Kinetics of depolymerization of polymer 2.3b in pH 7.4 0.1 M phosphate buffered D$_2$O at 37 °C as measured by $^1$H NMR spectroscopy.

To measure the rate of degradation of the assemblies formed from polymer 2.3b, the polymer was dispersed using sonication in pH 7.4 0.1 M phosphate buffered D$_2$O and incubated at 37 °C. The depolymerization was monitored by $^1$H NMR spectroscopy. As described above for polymer 2.3a, peaks corresponding to 4-hydroxybenzyl alcohol and N,N'-dimethylimidazolidinone emerged as the degradation progressed (Figure 2.7b) and the kinetic data are shown in Figure 2.8b. As shown, the depolymerization rate is clearly slower than that observed for polymer 2.3a. This can likely be attributed to the formation of the above-described nanoparticles from polymer 2.3b in aqueous solution. While the depolymerization of 2.3a was carried out in a water:acetone mixture in which the polymer was fully dissolved, the depolymerization of 2.3b occurred at the hydrophobic core of the nanoparticles. A slowing of reactions with polar transition states in the hydrophobic cores of micelles has been previously reported$^{32,60}$ and is consistent with the observation that the diamine cyclization was quite dependent on the polarity of the solvent. These results indicate that the rate of the cascade degradation can be modulated not only by incorporating new monomers with different depolymerization rates, but also by modulating the hydrophobicity of the materials and controlling their assembly into nanoaggregates.
It is also of interest to note that the depolymerization kinetics for both polymer 2.3a and 2.3b are significantly slower than those of the previously reported backbone based entirely on 1,6-elimination spacers. While a rapid depolymerization is a definite asset for the previously reported application of amplified sensing, the availability of a polymer degrading in a controlled manner but at a slower rate opens up many new potential applications such as drug carrier systems or biomedical devices where more a prolonged degradation or release of molecules is required.

2.2.8 Evaluation of Controlled Release Properties

![Graph](image)

**Figure 2.9.** Fluorescence decrease of Nile Red corresponding to its release from nanoparticles comprising polymer 2.3b, as a function of time incubated in pH 7.4 0.1 M phosphate buffer at 37 °C.

To evaluate the potential utility of this new polymer system for encapsulation and controlled release applications, the dye Nile Red was encapsulated and its release was studied. Nile Red was chosen as a hydrophobic dye because its fluorescence is negligible in aqueous solutions but is known to increase substantially in the hydrophobic compartments of polymer assemblies. The encapsulation was performed by sonicating the polymer in pH 7.4 0.1 M phosphate buffer in the presence of insoluble
Nile Red. As the nanoparticles formed, Nile Red was taken up as evidenced by a ~20 fold increase in the fluorescence of the solution in comparison with the minimal fluorescence of Nile Red which was sonicated in phosphate buffer alone. The loaded nanoparticles were then incubated in this buffer at 37 °C, allowing depolymerization of the polymers to occur. Over a time period of two weeks, the fluorescence of the dye relative to a control solution of Nile Red, decreased to less than 15% of its initial fluorescence, consistent with its release from the nanoparticles (Figure 2.9). The time scale of this release was similar to that of the polymer degradation. This experiment therefore suggests that nanoparticles comprising self-immolative polymers can provide well-controlled release properties that depend on the rate of depolymerization.

2.3 Conclusions

In conclusion, a new approach was developed for the incorporation of spontaneously cyclizing monomers into linear polymers, providing a new class of self-immolative linear polymers. This approach was based on the use of activated heterodimers as polymerization monomers, leading to polymers degrading by alternating elimination and cyclization reactions. Kinetics studies were carried out on both the monomer and corresponding polymer and the data supported the proposed route of degradation via end-to-end depolymerization. This work therefore demonstrated that cyclization spacers could be incorporated to control the rate of degradation, and as a number of different cyclization spacers have previously been reported, this approach should allow for the depolymerization rate to be readily tuned according to the desired application, while the choice of the end cap can be used to determine under which conditions the degradation will be initiated. In addition, using a PEG end cap, an amphiphilic block copolymer was developed and was demonstrated to assemble into self-immolative nanoparticles in aqueous solution. It was possible to encapsulate the hydrophobic fluorescent dye Nile Red into these nanoparticles and to subsequently release it over the time scale of the depolymerization. This ability of the block copolymers to form functional
nanoassemblies in aqueous solution is highly promising as it opens up many new and exciting applications of the materials.

2.4 Experimental

General procedures and materials:

All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Anhydrous DMF and CH$_2$Cl$_2$ were obtained from a solvent purification system. Pyridine and NEt$_3$ were distilled from CaH$_2$. Unless otherwise stated, all reactions were performed under a N$_2$ atmosphere using flame or oven dried glassware. Column chromatography was performed using silica gel (0.063-0.200 mm particle size, 70-230 mesh). Thin layer chromatography was performed using Macherey-Nagel Polygram® SIL G/UV$_{254}$ plates. $^1$H NMR spectra were obtained at 400 MHz and $^{13}$C NMR spectra were obtained at 100 MHz using a Varian Mercury or Varian Inova spectrometer. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signal of CDCl$_3$ ($\delta$ 7.27, 77.00). Coupling constants (J) are expressed in Hertz (Hz). Infrared spectra were obtained as films from CH$_2$Cl$_2$ on NaCl plates using a Bruker Tensor 27 instrument. High-resolution mass spectrometry (HRMS) was performed using a Finnigan MAT 8400 electron impact (EI) or a Micromass LCT electrospray ionization time-of-flight (ESI) mass spectrometer. Matrix assisted laser desorption ionization time of flight (MALDI-TOF) MS was performed on a Bruker Reflex IV instrument using trans-3-indoleacrylic acid as a matrix. Size exclusion chromatography (SEC) was carried out at a flow rate of 1 mL/min in $N,N$-dimethylformamide (DMF) with 10 mM LiBr and 1% (v/v) NEt$_3$ at 85 °C using a Waters 2695 separations module equipped with a Waters 2414 differential refractometer and two PLgel 5 µm mixed-D (300 mm × 7.5 mm) columns from Polymer Laboratories connected in series. The calibration was performed using polystyrene standards. Preparative size exclusion chromatography was performed in DMF at 3 mL/min using a Waters 515 pump equipped with an Optilab Rex refractive index detector from Wyatt Technology and 100 Å and 500 Å PLGel columns from Polymer Laboratories.
Synthesis of compound 2.6: tert-Butyldimethylsilyl chloride (3.38 g, 22.4 mmol) and imidazole (3.01 g, 44.3 mmol) were dissolved in DMF (20 mL), and the solution was stirred for 10 min. 1.97 (2.50 g, 20.1 mmol) was then added, and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and then the crude material was partitioned between CH$_2$Cl$_2$ and 0.1 M HCl. The aqueous layer was further extracted with CH$_2$Cl$_2$ and the combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The product was purified by column chromatography (9:1 hexanes:EtOAc followed by 1:1 hexanes:EtOAc), yielding 2.6 (3.49 g, 71%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.20 (d, J = 8.1, 2H), 6.79 (d, J = 8.6, 2H), 4.95 (s, 1H), 4.67 (s, 2H), 0.94 (s, 9H), 0.10 (s, 6H). $^{13}$C NMR (CDCl$_3$): $\delta$ 154.8, 133.9, 128.0, 115.3, 65.0, 26.2, 18.7, -5.0. IR (cm$^{-1}$): 3405, 2955, 2855. HRMS: calcd [M]$^+$ (C$_{13}$H$_{22}$O$_2$Si): 238.1389. Found: (EI) 238.1385.

Synthesis of compound 2.8: To a flask containing CH$_2$Cl$_2$ (45 mL) was added the phenol 2.6 (2.0 g, 8.5 mmol) in 5 mL of anhydrous CH$_2$Cl$_2$, followed by freshly distilled NEt$_3$ (6.0 mL, 43 mmol). 4-Nitrophenyl chloroformate (3.4 g, 17 mmol) was added slowly to the reaction flask, and the solution was stirred until the reaction was complete as determined by thin layer chromatography (TLC) (30 min). To consume the excess 4-nitrophenyl chloroformate and facilitate product isolation, distilled TEGMME (2.0 mL, 13 mmol) was added and the reaction was stirred until all 4-nitrophenyl chloroformate was consumed as determined by TLC (20 min). The solution was then diluted with CH$_2$Cl$_2$ and washed with 1 M HCl. The organic phase was dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The crude product was purified with column chromatography (9:1 CH$_2$Cl$_2$:hexanes), yielding 2.8 (2.5 g, 75%) as a white powder. $^1$H NMR (CDCl$_3$): $\delta$ 8.33 – 8.30 (m, 2H), 7.51 – 7.47 (m, 2H), 7.41 – 7.37 (m, 2H), 7.26 – 7.21 (m, 2H), 4.75 (s, 2H), 0.96 (s, 9H), 0.11 (s, 6H). $^{13}$C NMR (CDCl$_3$): $\delta$ 155.3, 151.1, 149.5, 145.6, 140.1, 127.2, 125.4, 121.7, 120.4, 64.2, 25.9, 18.4, -5.3. IR (cm$^{-1}$): 2925, 2856, 1770, 1637, 1527. HRMS: calcd [M]$^+$ (C$_{20}$H$_{25}$NO$_6$Si): 404.1524 Found: (ESI) 404.1516.
**Synthesis of compound 2.10:** Under air atmosphere, a flask containing diamine 2.9 (4.06 g, 46.0 mmol) in 7:1 MeOH:NEt₃ (120 mL) was cooled to 0 °C in an ice bath. A solution of di-tert-butyl dicarbonate (10.04 g, 46.0 mmol) in MeOH (20 mL) was added dropwise to the reaction over approximately 1 h via a dropping funnel. The reaction mixture was allowed to warm to room temperature and the reaction was stirred overnight. The solvent was removed, and the resulting residue was purified by column chromatography (10:3:87 MeOH:NEt₃:EtOAc followed by 20:3:77 MeOH:NEt₃:EtOAc), yielding 2.10 (3.34 g, 39%) as a yellow oil. $^1$H NMR (CDCl₃): $\delta$ 3.33 (t, J = 6.2, 2H), 2.87 (s, 3H), 2.72 (t, J = 6.5, 2H), 2.45 (s, 3H), 1.44 (s, 9H). $^{13}$C NMR (CDCl₃): $\delta$ 155.8, 79.2, 70.5, 48.4, 36.2, 34.6, 28.3. IR (cm$^{-1}$): 3410, 2975, 1650. HRMS: calcd [M]$^+$ (C₉H₂₀N₂O₂): 188.1525 Found: (EI) 188.1524.

**Synthesis of compound 2.11:** Nitrophenyl carbonate 2.8 (2.53 g, 6.28 mmol) was dissolved in toluene (20 mL). Amine 2.10 (1.42 g, 7.53 mmol) was added, followed by DIPEA (1.64 mL, 9.42 mmol) and DMAP (0.077 g, 0.63 mmol), and the reaction mixture was stirred at room temperature for 24 h. The solution was then diluted with EtOAc, and washed with 1 M HCl, followed by 1 M Na₂CO₃. The organic layer was dried over MgSO₄, filtered, and the solvent was removed in vacuo. The resulting pale yellow oil was used in the next step without further purification. $^1$H NMR (CDCl₃): $\delta$ 7.30 (d, J = 7.6, 2H), 7.05 (d, J = 8.2, 2H), 4.71 (s, 2H), 3.63 – 3.40 (m, 4H), 3.12 & 3.03 (2 s, 3H total (rotamers)), 2.95 – 2.86 (m, 3H (rotamers)), 1.50 – 1.42 (m, 9H (rotamers)), 0.93 (s, 9H), 0.09 (s, 6H).

**Synthesis of compound 2.12:** Compound 2.11 (2.84 g, 6.28 mmol) was dissolved in a solution of 1 vol% 12M HCl in EtOH (20 mL) and the solution was stirred for 1 h at room temperature under air atmosphere. The solution was then diluted with CH₂Cl₂ and washed with saturated NaHCO₃ solution. The organic phase was dried over MgSO₄, filtered, and the solvent was removed in vacuo. The crude material was purified by column chromatography (3:2 hexanes:EtOAc followed by 3:1 EtOAc:hexanes), yielding 2.12 (1.97 g, 93% over two steps) as a colourless oil. $^1$H NMR (CDCl₃): $\delta$ 7.35 (d, J = 8.2, 2H), 7.09 (dd, J = 2.3 & 8.6, 2H), 4.66 (s, 2H), 3.62 – 3.39 (m, 4H), 3.12 & 3.03 (2 s, 3H total (rotamers)), 2.94 – 2.87 (m, 3H (rotamers)), 1.76 (br. s, 1H), 1.50 – 1.42 (m, 9H).
(rotamers)). $^{13}$C NMR (CDCl$_3$): $\delta$ 155.5, 154.7, 150.7, 138.4, 127.9, 127.6, 79.8, 64.2, 46.8, 35.8, 28.3. IR (cm$^{-1}$): 3430, 2927, 2243, 1677. HRMS: calcd [M]$^+$ (C$_{17}$H$_{26}$N$_2$O$_5$): 338.1842 Found: (ESI) 338.1838.

**Synthesis of compound 2.13:** Compound 2.12 (1.09 g, 3.22 mmol) was dissolved in a mixture of CH$_2$Cl$_2$ (10 mL) and freshly distilled pyridine (0.80 mL, 9.9 mmol). 4-Nitrophenyl chloroformate (1.29 g, 6.41 mmol) was added and the reaction mixture was stirred for 2 h. The solution was then diluted with CH$_2$Cl$_2$ and washed with 1 M HCl. The organic layer was dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. Column chromatography (CH$_2$Cl$_2$ followed by 1:1 EtOAc:CH$_2$Cl$_2$) was used to purify the material, yielding 2.13 (1.48 g, 91%) as a pale yellow oil. $^1$H NMR (CDCl$_3$): $\delta$ 8.30 – 8.26 (m, 2H), 7.44 (d, J = 8.6, 2H), 7.41 – 7.35 (m, 2H), 7.19 – 7.12 (m, 2H), 5.27 (s, 2H), 3.61 – 3.42 (m, 4H), 3.13 & 3.04 (2 s, 3H total (rotamers)), 2.94 – 2.87 (m, 3H (rotamers)), 1.50 – 1.42 (m, 9H (rotamers)). $^{13}$C NMR (CDCl$_3$): $\delta$ 155.5, 154.3, 152.3, 151.9, 145.3, 131.2, 129.9, 125.2, 122.2, 122.0, 121.8, 79.6, 70.3, 46.8, 45.5, 35.1, 34.5, 28.4. IR (cm$^{-1}$): 2914, 1775, 1720, 1689, 1525. HRMS: calcd [M]$^+$ (C$_{24}$H$_{29}$N$_3$O$_9$): 503.1904 Found: (EI) 503.1909.

**Synthesis of polymer 2.3a and general polymerization procedure:** The protected monomer 2.13 (1.04 g, 2.07 mmol) was dissolved in a 1:1 mixture of CH$_2$Cl$_2$:TFA (5 mL) under air atmosphere and the solution was stirred for 2 h. The solvent was removed in vacuo, and CH$_2$Cl$_2$ was successively added and evaporated to remove residual TFA, to provide the deprotected monomer 2.2a-TFA. The end cap 2.13 (0.055, 0.109 mmol) was then added, and the resulting mixture was dissolved in anhydrous toluene (10 mL). To this was added freshly distilled NEt$_3$ (1.55 mL, 11.1 mmol) and DMAP (0.0254 g, 0.208 mmol), and the reaction was stirred for 24 h at room temperature. The solution was diluted with CH$_2$Cl$_2$ and washed once with 1 M HCl, then twice with 1 M Na$_2$CO$_3$. The organic layer was dried over MgSO$_4$ and the solvent was removed in vacuo to provide 0.643 g (91% crude yield) of 2.3a as a yellow solid. The crude polymer was then further purified using preparative SEC to remove lower molecular weight oligomers for the degradation study. An optimized yield for the preparative SEC was approximately 45%. $^1$H NMR (CDCl$_3$): $\delta$ 7.35 (br s, 2H), 7.12 – 7.00 (m, 2H), 5.15 – 5.03 (m, 2H), 3.66 –
3.41 (m, 4H), 3.15 – 2.83 (m, 6H), 1.50 – 1.40 (m, 0.5H). SEC: \( M_n = 17\,000, M_w = 26\,900, \) PDI = 1.58.

**Synthesis of compound 2.15:** Acid terminated poly(ethylene glycol) methyl ether (2.01 g, 0.401 mmol) with a peak molecular weight of 5017 g/mol (n = 113) as determined by MALDI-TOF MS was dissolved in CH\(_2\)Cl\(_2\) (20 mL), and freshly distilled pyridine (0.65 mL, 8.04 mmol) was added. 4-Nitrophenyl chloroformate (0.805 g, 3.99 mmol) was added and the reaction was stirred for 24 h. The product was then partitioned between 1 M HCl and CH\(_2\)Cl\(_2\) and the aqueous solution was extracted twice with CH\(_2\)Cl\(_2\). The combined organic layers were dried over MgSO\(_4\), and the volume was reduced in vacuo to approximately 5 mL. The product was precipitated in 200 mL of cold Et\(_2\)O, yielding 2.15 (1.87 g, 90%) as a white powder. \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 8.28 (d, J = 9.2, 2H), 7.33 (d, J = 9.2, 2H), 4.47 (s, 2H), 3.85 – 3.47 (m, 450H), 3.37 (s, 3H). IR (cm\(^{-1}\))): 2880, 2692, 1765, 1650, 1590. MS calcd. for \([M+Na]^+\) based on conjugation to the starting polymer with a peak MW of 5017 g/mol (n = 113): 5160. Found (MALDI-TOF): 5162.

**Synthesis of compound 2.16:** Phenol 2.6 (0.839 g, 3.52 mmol) was dissolved in CH\(_2\)Cl\(_2\) (20 mL) and freshly distilled NEt\(_3\) (0.95 mL, 6.83 mmol) was added. To this, activated PEG derivative 2.15 (1.80 g, 0.351 mmol) was slowly added and the reaction mixture was stirred at room temperature for 24 h. The product was then partitioned between 1 M HCl and CH\(_2\)Cl\(_2\) and the aqueous solution was extracted twice with CH\(_2\)Cl\(_2\). The combined organic layers were dried over MgSO\(_4\), and the volume was reduced in vacuo to approximately 5 mL. Precipitation into 150 mL of cold Et\(_2\)O yielded 2.16 (1.40 g, 77%) as a white powder. \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 7.33 (d, J = 8.8, 2H), 7.07 (d, J = 8.6, 2H), 4.73 (s, 2H), 4.42 (s, 2H), 3.86 – 3.42 (m, 450H), 3.38 (s, 3H), 0.94 (s, 9H), 0.10 (s, 6H). IR (cm\(^{-1}\))): 2880, 2692, 1757, 1615. MS calcd. for \([M+Na]^+\) based on conjugation to the starting polymer with a peak MW of 5017 g/mol (n = 113): 5260. Found (MALDI-TOF): 5259.

**Synthesis of compound 2.17:** Under air atmosphere, 2.16 (1.53 g, 0.292 mmol) was dissolved in a solution of 1 vol% 12 M HCl in 3:1 EtOH:CH\(_2\)Cl\(_2\) (20 mL) and the reaction mixture was stirred for 1 h. The resulting mixture was then partitioned between
CH$_2$Cl$_2$ and saturated NaHCO$_3$ solution, and the aqueous layer was further extracted with CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, and the volume was reduced in vacuo to approximately 5 mL. Precipitation of the CH$_2$Cl$_2$ solution into 150 mL cold Et$_2$O yielded 2.17 (1.15 g, 77%) as a white powder. $^1$H NMR (CDCl$_3$): $\delta$ 7.38 (d, J = 8.8, 2H), 7.10 (d, J = 8.8, 2H), 4.68 (s, 2H), 4.41 (s, 2H), 3.84 – 3.45 (m, 450H), 3.37 (s, 3H). IR (cm$^{-1}$): 3550, 2880, 2692, 2240, 1760, 1625. MS calcd. for [M+Na]$^+$ based on conjugation to the starting polymer with a peak MW of 5017 g/mol (n = 113): 5146. Found (MALDI-TOF): 5146.

Synthesis of compound 2.18: 2.17 (1.12 g, 0.220 mmol) was dissolved in CH$_2$Cl$_2$ (15 mL). To this was added freshly distilled pyridine (0.36 mL, 4.5 mmol), followed by 4-nitrophenyl chloroformate (0.446 g, 2.21 mmol), and the solution was stirred for 24 h. The resulting mixture was then partitioned between CH$_2$Cl$_2$ and 1 M HCl, and the aqueous layer was further extracted with CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, and the volume was reduced in vacuo to approximately 5 mL. Precipitation of the CH$_2$Cl$_2$ solution into 150 mL of cold Et$_2$O yielded 2.18 (0.412 g, 79%) as a white powder. $^1$H NMR (CDCl$_3$): $\delta$ 8.28 (d, J = 9.2, 2H), 7.48 (d, J = 8.8, 2H), 7.39 (d, J=9.2, 2H), 7.17 (d, J = 8.8, 2H), 5.29 (s, 2H), 4.44 (s, 2H), 3.89 – 3.43 (m, 450H), 3.38 (s, 3H). IR (cm$^{-1}$): 2870, 1760, 1640. MS calcd. for [M+Na]$^+$ based on conjugation to the starting polymer with a peak MW of 5017 g/mol (n = 113): 5311. Found (MALDI-TOF): 5312.

Synthesis of Polymer 2.3b: This polymer was prepared by the same procedure described above for the synthesis of polymer 2.3a, except 2.18 was used as the end cap. Yield = 76%. $^1$H NMR (CDCl$_3$): $\delta$ 7.34 (br s, 2H), 7.12 – 7.01 (m, 2H), 5.16 – 5.05 (m, 2H), 4.42 (s, 0.1H), 3.85 – 3.41 (m, 71H), 3.38 (s, 0.3H), 3.15 – 2.89 (m, 6H). SEC: $M_n$ = 28 700, $M_w$ = 35 000, PDI = 1.21.

Degradation studies

Buffer Preparation: NaH$_2$PO$_4$·H$_2$O (0.069 g, 0.5 mmol) was dissolved in D$_2$O (5 mL). To this, a saturated solution of NaOH in D$_2$O was added dropwise with stirring, while monitoring with a pH meter until the desired pH of 7.4 was obtained.
Degradation of deprotected monomer 2.12: Under air atmosphere, monomer 2.12 (0.020 g, 0.059 mmol) was dissolved in 1:1 TFA:CH₂Cl₂ (1 mL), and the solution was stirred for 2 h at room temperature. The solvent was removed in vacuo, and then the material was taken up in CH₂Cl₂ and washed with saturated NaHCO₃ solution to remove all residual TFA. The organic layer was dried over MgSO₄, filtered, and the solvent was removed in vacuo. The product was then taken up in pH 7.4 0.1 M phosphate buffer (D₂O):acetone-d₆ (3:2) (1 mL), and the solution was incubated at 37 °C. The extent of cyclization was quantified using ¹H NMR by integrating the benzylic methylene peak on the liberated 4-hydroxybenzyl alcohol relative to that on the starting material.

Degradation of polymer 2.3a: Under air atmosphere, polymer 2.3a (0.030 g) was dissolved in 1:1 TFA:CH₂Cl₂ (1 mL), and the solution was stirred for 2 h at room temperature. The solvent was removed in vacuo, and then the material was taken up in CH₂Cl₂ and washed with saturated NaHCO₃ solution to remove all residual TFA. The organic layer was dried over MgSO₄, filtered, and the solvent was removed in vacuo. The product was then taken up in pH 7.4 0.1 M phosphate buffer (D₂O):acetone-d₆ (3:2) (1 mL), and the solution was incubated at 37 °C. The extent of depolymerization was quantified using ¹H NMR by integrating the methylene peak of the N,N'-dimethylimidazolidinone degradation product relative to residual DMF in the sample.

Hydrolysis of PEG derivative 2.17: Compound 2.17 (0.040 g, 7.6 µmol) was dissolved in pH 7.4 0.1 M phosphate buffer (D₂O) (1 mL) and was incubated at 37 °C. The extent of hydrolysis was quantified using ¹H NMR by integrating the benzylic methylene peak on the liberated 4-hydroxybenzyl alcohol relative to that on the ester.

Degradation of polymer 2.3b: Polymer 2.3b (0.040 g) was dissolved in pH 7.4 0.1 M phosphate buffer (D₂O) (1 mL), and the solution was sonicated for 30 s. The solution was then incubated at 37 °C, and the extent of depolymerization was quantified using ¹H NMR by integrating the methylene peak of the N,N'-dimethylimidazolidinone degradation product relative to residual DMF in the sample.

Transmission electron microscopy (TEM)
**Sample preparation:** Polymer 2.3b (0.2 mg) was dissolved in CH₂Cl₂, and the solution was passed through a 0.2 μm syringe filter. The solvent was removed in vacuo, 0.5 mL of H₂O was filtered into the flask, and the mixture was sonicated for 30 s. A single drop of solution was added to a carbon formvar grid and was left for 1 min. The excess solution was then removed using a kimwipe. The grid was stained with either phosphotungstic acid (PTA) or OsO₄.

**PTA Staining:** Immediately following removal of the excess polymer 2.3b solution from the TEM plate, a drop of 2% aqueous PTA solution was added and left for between 1 and 2 min. The excess solution was then removed using a kimwipe.

**OsO₄ Staining:** The sample grid containing polymer 2.3b was stored in a sealed container, exposing it to the vapors of aqueous OsO₄, and after 7 h the sample was removed.

**Imaging:** Images were obtained using a Phillips CM10 microscope operating at 80kV with a 40 μm aperture.

**Encapsulation and release of Nile Red:** Polymer 2.3b (2 mg) and Nile Red (0.2 mg) were dissolved in 1 mL of CH₂Cl₂ and mixed thoroughly. The solvent was then removed, first under a stream of nitrogen then under vacuum. pH 7.4 0.1 M phosphate buffer solution (2 mL) was added, and the mixture was sonicated for 2 h. The fluorescence was measured immediately after sonication, after which the sample was incubated at 37 °C. A second sample, containing only Nile Red, was treated in exactly the same manner as the polymer solution to obtain a background fluorescence value for Nile Red in phosphate buffer. A control sample of Nile Red was also prepared by dissolving 0.1 mg in THF (1 mL) and diluting by 1500x to obtain a fluorescence value similar to that of the polymer. The fluorescence of the degrading sample relative to the control sample was measured daily to determine the percentage of the initial fluorescence while correcting for instrumental fluctuations. The fluorescence spectra were obtained on a QM-4 SE spectrometer from Photon Technology International (PTI), equipped with double excitation and emission monochromators. An excitation wavelength of 550 nm was used for Nile Red and the emission spectra were recorded from 565 and 700 nm.
2.5 References


Chapter 3
A Reduction Sensitive Self-Immolative Linear Polymer*

3.1 Introduction

Biodegradable polymers have been of significant interest in recent years for a wide range of applications. For example, they can serve as environmentally friendly substitutes for nondegradable polymers in materials such as food and beverage containers. They have also been developed for biomedical materials such as sutures, stents, and tissue engineering scaffolds, thus allowing the materials to degrade during the natural healing or tissue regeneration process, preventing the need for further interventions to remove the foreign material. Furthermore, their incorporation into drug delivery systems such as micelles, worms, vesicles and nanoparticles facilitates the release of encapsulated drug molecules throughout the degradation process. Thus far, significant progress has been made in these areas using polymers such as PCL, PLA, and PGA. However, the ability to "turn on" the degradation of these polymers under specific physiological conditions has not been demonstrated as these polymer backbones exhibit gradual degradation under most physiological conditions.

The ability to trigger the degradation of a polymer backbone under specified conditions such as photochemical or enzymatic stimuli, or changes in pH or redox potential offers the possibility to utilize polymer backbones that will be stable for extended periods but that will degrade under the desired conditions, resulting in a controlled disintegration of biomedical materials or release of drug molecules from the drug delivery system. Thus far, several polymer backbones containing acetal or disulfide linkages have been developed to degrade under mildly acidic or reducing

conditions respectively. However, the mechanisms of degradation for these polymers involve random chain scissions throughout the polymer backbone, and many environmentally mediated cleavage events are required to completely degrade the polymer.

Inspired by elegant work on dendrimer systems that were designed to degrade by a cascade of reactions upon removal of a single trigger moiety, the group of Shabat as well as our group have recently developed end capped self-immolative linear polymers. These polymers comprise backbones that are stable when the end cap is intact, but upon removal of the end cap via a single bond cleavage, a functionality is revealed at the polymer terminus that initiates a cascade of intramolecular reactions leading to complete depolymerization from end to end. Like the dendrimer systems, both of these systems have used self-immolative linkers previously developed for prodrugs. Shabat's group reported the use of a polycarbamate based on 4-aminobenzyl alcohol derivatives, that depolymerized to fluorescent monomers via a series of rapid 1,6-elimination reactions in response to an enzyme mediated end cap cleavage, thus serving as a sensor for the enzyme. Our group developed a polycarbamate that degraded by alternating cyclization and 1,6-elimination reactions and incorporated this polymer into an amphiphilic block copolymer by using a PEG derivative as an end cap. It was demonstrated that the cyclization reaction could be used to control the overall rate of depolymerization and also that the block copolymer could be assembled into nanoparticles in aqueous solution. These nanoparticles were capable of encapsulating and releasing a model drug molecule in a controlled manner, thus demonstrating the promise of self-immolative linear polymers in drug delivery applications.

In order to fully exploit this new class of polymers, it will be necessary to develop a series of polymer backbones with different depolymerization rates and also a series of end caps that can be removed under different conditions. This will allow for the selection of the appropriate backbone and end cap combination for the desired application. Furthermore, it has been suggested that the quinone methide intermediates involved in the 1,6-elimination reactions can potentially lead to toxicity, so it would be desirable to develop new backbones that do not involve hydroxybenzyl alcohol or aminobenzyl
alcohol. Towards this goal we report here the first example of a self-immolative linear polymer that degrades entirely by cyclization reactions. Furthermore, we describe the first incorporation of a disulfide end cap that can be cleaved under mildly reducing conditions. Such conditions can be encountered in hypoxic tumor tissue\(^54\) where the concentration of the reducing agent glutathione is at least 4-fold higher than in normal tissues\(^55\) or within the intracellular environment where the concentration of glutathione is approximately 0.5 - 10 mM relative to 2 - 20 µM in the extracellular environment.\(^56,57\)

### 3.2 Results and Discussion

#### 3.2.1 Design

A diverse array of intramolecular cyclization reactions have been reported, potentially allowing the rate of depolymerization to be controlled by the choice of the cyclization reaction.\(^48,59,60\) In this particular work, the cyclizations of 2-mercaptoethanol derivatives to the corresponding cyclic thiocarbonate (Figure 3.1a) were of interest as they have been recently reported as components of traceless self-immolative spacers in fluorescent protease sensors.\(^59\)

The development of monomers capable of undergoing polymerization to form self-immolative polymers requires careful design. In particular, in the preparation of polymers designed to degrade by cyclization mechanisms, cyclization of the activated monomer (Figure 3.1b) must be avoided. In our previous work, it has been found that the synthesis and polymerization of activated dimers is an effective approach, as the activated leaving group is distant from the nucleophilic moiety such that the resulting ring size is not particularly favourable for cyclization.\(^44\) This allows polymerization to be a highly competitive reaction at high concentrations. In particular, the use of alternating monomers, and thus the preparation of activated heterodimers as polymerizable "monomers" has been found to be an effective strategy for overcoming the challenges associated with the synthesis of both the activated monomers and their corresponding polymers.\(^44\) Therefore, an activated heterodimer based on 2-mercaptoethanol and \(N,N'-\)
dimethylethlenediamine units was proposed. Carbamate derivatives of \(N,N'-\)
dimethylethylenediamine are known to spontaneously cyclize to form \( N,N' \)-dimethylimidazolidinone\(^{49} \) and this spacer has been incorporated into our previously reported linear self-immolative polymer\(^{44} \) as well and some of the previously reported self-immolative dendrimers.\(^{32,36,39,40} \) As shown in Figure 3.1c, polymerization of this activated heterodimer in the presence of an end cap should lead to a polymer based on 2-mercaptoethanol and \( N,N' \)-dimethylethylenediamine with alternating carbamate and thiocarbamate linkages. Removal of an end cap would lead to alternating cyclization reactions resulting in end to end depolymerization with the release of \( N,N' \)-dimethylimidazolidinone and 1,3-oxathiolan-2-one.

**Figure 3.1.** Design of the self-immolative polymer: a) cyclization of 2-mercaptoethanol derivatives to 1,3-oxathiolan-2-one; b) undesired cyclization of an activated 2-mercaptoethanol-based monomer prohibits polymerization; c) proposed polymerization of an activated heterodimer and depolymerization of the resulting polymer; d) proposed disulfide-based end cap.
The end cap selected for the target polymer was a disulfide (Figure 3.1d). Disulfide linkages are known to be cleaved by biological reducing agents such as glutathione, and it has been shown that the incorporation of disulfide linkages into gene and drug delivery systems can provide a selective release of the cargo under the reducing conditions within cells, leading to enhanced therapeutic efficacy. In addition, because of the incorporation of the 2-mercaptoethanol cyclization reaction in the degradation cascade, the disulfide was a natural choice for an end cap as the thiol moiety can be readily converted to a disulfide which upon cleavage can directly initiate the depolymerization cascade.

3.2.2 Boc-Capped Polymer Synthesis

As shown in Scheme 3.1, the synthesis of the target activated heterodimer began by the selective protection of the alcohol group on 2-mercaptoethanol (3.8) using tert-butylidiphenylchlorosilane in the presence of imidazole to provide the tert-butylidiphenylsilyl (TBDPS) protected derivative 3.9. The thiol of 3.9 was then treated with 4-nitrophenylchloroformate to provide the activated thiocarbonate 3.10. The mono Boc protected derivative of \(N,N'\)-dimethylethylenediamine\(^{44}\) (2.10) was reacted with 3.10 using DMAP as a catalyst and DIPEA as a base to give the thiocarbamate 3.11, and then the TBDPS protecting group was removed using TBAF in THF to provide the alcohol 3.12. The alcohol was then converted to the activated carbonate 3.13 by reaction with 4-nitrophenyl chloroformate, providing the protected version of the polymerization monomer.

Prior to incorporating the desired disulfide end cap, it was prudent to investigate polymer synthesis and degradation using a model Boc end cap, provided by active monomer 3.13. This allowed the polymerization procedure to be tested and optimized with materials already on hand prior to investing synthetic efforts into the more valuable disulfide end cap. Furthermore, the Boc group can be cleanly removed by treatment with TFA, allowing the kinetics of depolymerization to be assessed independently. To this end, compound 3.13 was first deprotected in a mixture of TFA and CH\(_2\)Cl\(_2\), affording
**3.4a** as its TFA salt. While relatively stable in its salt form, upon deprotonation compound **3.4a** readily undergoes self-condensation via attack of the amine on the nitrophenyl carbonate moiety, forming the desired polycarbamate. This process was triggered by addition of NEt₃ and catalytic DMAP to a solution of **3.4a-TFA** in toluene, with 0.05 equivalents of **3.13** added to serve as an end cap. Following reaction workup, purification of the crude polymer mixture was carried out using preparative SEC in DMF to remove lower MW oligomers and small molecule byproducts. Following purification, analytical SEC in THF revealed an $M_n$ of 1650 g/mol, an $M_w$ of 2200 g/mol (relative to polystyrene standards), and a resultant PDI of 1.3.

**Scheme 3.1.** Synthesis of active monomer **3.13**.

**Scheme 3.2.** Deprotection and polymerization of monomer **3.13**.
3.2.3 Polymer Degradation

To study the kinetics of depolymerization, polymer 3.5a was first deprotected using 1:1 TFA:CH₂Cl₂, and the resulting material was incubated at 37 ºC in pH 7.4 0.1M phosphate (D₂O):acetone-d₆ (3:2). The progress of degradation was monitored using NMR spectroscopy, measuring the integration of peaks arising from N,N'-dimethylimidazolidinone. As shown in Figure 3.2, depolymerization was monitored over a course of 7 days, resulting in approximately 60% degradation. While the study was not followed to completion, it demonstrated that depolymerization indeed occurred as predicted, and furthermore it gave an approximate timeline for future studies with a more relevant end cap.

![Figure 3.2. Kinetics of depolymerization of polymer 3.5a as determined by ¹H NMR spectroscopy in pH 7.4 0.1 M phosphate (D₂O):acetone-d₆ (3:2).](image)

3.2.4 Disulfide-Capped Polymer Synthesis

For the synthesis of the target end cap, the alcohol group of the previously reported thiopyridyl derivative 3.14 was treated with 4-nitrophenyl chloroformate to provide the activated carbonate 3.7a as shown in Scheme 3.3. This activated carbonate allows for incorporation of the end cap onto the polymer. TEGMME was used to quench the excess
chloroformate in this reaction as it was otherwise chromatographically inseparable from the product.

Scheme 3.3. Synthesis of disulfide end cap 3.7a.

Polymerization of 3.13 with end cap 3.7a was carried out in an analogous fashion to formation of 3.5a, except that the monomer:end cap ratio was modified to 98:2. To this end, 3.13 was first deprotected with TFA, after which the solvent was removed and the material redissolved in toluene. NEt₃ and catalytic DMAP were then added to initiate polymerization in the presence of 2 mol% of 3.7a.

Scheme 3.4. Polymerization of 3.13 utilizing disulfide end cap 3.7a.

The resulting polymer 3.5b was purified by dialysis in DMF using a regenerated cellulose membrane with a MW cutoff of 3500 g/mol to remove small molecule byproducts. The material isolated from the dialysis was pure and free of low MW impurities as determined by ¹H NMR and SEC. In the analysis of the dialysate, cyclization products were not detected (see appendix), indicating that the cyclization of the monomer was not a competing reaction during the polymerization. In addition, no monomer was detected, indicating that the polymerization proceeded to completion. However, some polymeric material was lost into the dialysate. It should be noted that the MW cutoff of 3500 g/mol is an estimate as it depends on the macromolecule's size and
shape. In addition, the MW cutoff corresponds to aqueous conditions and is likely lower in DMF due to the decreased swelling of the membrane in DMF. However, we have routinely observed that linear polymers above the MW cut-off can pass through the membrane. Although the yield for this polymer following dialysis was relatively low, approximately 35%, this method is much less labour intensive than preparative SEC, which was previously used to purify our self-immolative linear polymers, including 3.5a. Using $^1$H NMR spectroscopy a ratio of end cap to monomer of approximately 35:1 was determined, corresponding to an $M_n$ of 7800 (see appendix). Using SEC the polymer was found to have an $M_n$ of 1800 g/mol, an $M_w$ of 2950 g/mol, and a PDI of 1.6 relative to polystyrene standards.

### 3.2.5 Polymer degradation

To study the depolymerization initiated by end cap cleavage, polymer 3.5b was dissolved in pH 7.4 0.1M phosphate (D$_2$O):acetone-d$_6$ (3:2). DTT was added to cleave the disulfide end cap, thus initiating the degradation cascade and the sample was incubated at 37 °C. The reducing conditions were maintained by periodic additions of fresh DTT, as gradual oxidation of the DTT within the buffered solution was observed. The degradation was monitored by $^1$H NMR spectroscopy. As shown in Figure 3.3, over time, characteristic peaks appeared corresponding to $N,N'$-dimethylimidazolidinone at 2.8 and 3.4 ppm and 1,3-oxathiolan-2-one at 3.2 and 3.7 ppm. The presence of these products is a strong indicator that the degradation proceeds by the proposed cascade of cyclization reactions as random chain scissions would lead to $N,N'$-dimethylthelylenediamine and 2-mercaptoethanol, products that were not detected in the NMR spectra. It is noteworthy that in addition to the thiol cyclizing to form 1,3-oxathiolan-2-one, a competing cyclization reaction to form thiirane could also be occurring, as evidenced by the apparent lower integration of 1,3-oxathiolan-2-one relative to $N,N'$-dimethylimidazolidinone. In addition, a control sample incubated under the same conditions except in the absence of DTT did not reveal the appearance of any degradation products, thus indicating the end cap cleavage was required to initiate the degradation (see appendix). Furthermore, an additional control polymer having a Boc end cap was
also prepared and was demonstrated to undergo depolymerization in pH 7.4 0.1 M phosphate (D$_2$O):acetone-d$_6$ (3:2) following prior treatment with 1:1 TFA:CH$_2$Cl$_2$ to remove the Boc group. This confirmed that the polymer degradation could not be attributed to random polymer backbone cleavage by the DTT (see appendix).

![Diagram](image)

**Figure 3.3.** $^1$H NMR spectra of polymer 3.5b and its degradation products in pH 7.4 0.1 M phosphate (D$_2$O):acetone-d$_6$ (3:2) at different time points following the addition of DTT: a) immediately following DTT addition; b) after 4 days; c) after 8 days.

The percentage of degradation was determined by the relative integration of the peak at 4.3 ppm assigned to the methylene group adjacent to the oxygen in the polymer and the peak at 3.4 ppm corresponding to the methylene unit of $N,N'$-dimethylimidazolidinone. As shown in Figure 3.4, the degradation reached 80% completion after 10 – 14 days. However, no significant further degradation was
observed, even after 30 days. Size exclusion chromatograms were also obtained at different time points during the degradation process. As shown in Figure 3.5, prior to degradation, the chromatogram exhibited a peak at an elution volume of 16.7 mL as well as a distinct shoulder at 18.5 mL. As the degradation progressed, the peak at 16.7 mL decreased in intensity, consistent with the degradation progress observed by $^1$H NMR spectroscopy. On the other hand, no change in the intensity of the peak at 18.5 mL was observed. Therefore, it is possible that the peak at 18.5 mL corresponds to cyclic polymers. These cyclic species would not be end capped and thus degradation would only be initiated by a random chain scission of the polymer backbone. As observed for the control sample such cleavages are extremely slow under the degradation conditions. This may explain why the depolymerization did not reach 100% completion according to $^1$H NMR spectroscopy.

![Figure 3.4. Kinetics of depolymerization of polymer 3.5b, as measured by $^1$H NMR spectroscopy in pH 7.4 0.1 M phosphate (D$_2$O):acetone-δ$_6$ (3:2), following addition of DTT.](image)

It should also be noted that the cyclic polymers would not be distinguishable from the linear end capped polymers by NMR spectroscopy. Therefore, based on the degradation plateau at 80% completion, it is possible that the ratio of monomer to end cap in the linear polymers is closer to 30:1 than the 35:1 ratio mentioned above. Altering the concentration of the polymerization reaction did not appear to change the content of the
possible cyclic species significantly, nor the polymer MW. Interestingly, such nondegradable species were not observed in our previously reported self-immolative polymers based on N,N'-dimethylethylenediamine and 4-hydroxybenzyl alcohol. This may be explained by the increased rigidity imparted by the aromatic groups of 4-hydroxybenzyl alcohol, making cyclization less favourable. Nevertheless, this somewhat unexpected result provides additional evidence of the polymer backbone’s inherent stability and the specificity of the degradation process mediated by end cap cleavage. It is possible that in the future, the extent of the possible cyclic species could be decreased by tuning the reactivity of the activated carbonate in the polymerization monomer.

![Size exclusion chromatograms of polymer 3.5b before degradation and after 1 day, 4 days, and 8 days of incubation in pH 7.4 0.1M phosphate (D$_2$O):acetone-d$_6$ (3:2) in the presence of DTT (detection by refractive index).](image)

**Figure 3.5.** Size exclusion chromatograms of polymer 3.5b before degradation and after 1 day, 4 days, and 8 days of incubation in pH 7.4 0.1M phosphate (D$_2$O):acetone-d$_6$ (3:2) in the presence of DTT (detection by refractive index).

### 3.3 Conclusions

In conclusion, a new polymer designed to degrade by a cascade of intramolecular cyclization reactions was prepared for the first time. A disulfide end cap was incorporated such that the degradation could be selectively initiated under reducing
conditions. The degradation was initiated by the addition of DTT, a known thiol-based reducing agent and was monitored by $^1$H NMR and size exclusion chromatography. The data supported the proposed degradation mechanism, and also suggested that the polymer likely contained approximately 20% of a proposed cyclic species that did not degrade as they were lacking the labile linkage to the end cap. Overall, this new class of polymers offers a high degree of control over the degradation process as the polymer backbone is very stable under physiological conditions (pH 7.4 buffer) in the absence of the trigger required to cleave the end cap. In addition, the degradation mechanism of this polymer avoids the potentially undesirable quinone methide species generated in the degradation of the previously reported self-immolative linear polymers. Future work on this polymer will focus on its biological properties and applications in biomedical materials.

3.4 Experimental

General Procedures and Materials:

Solvents used were anhydrous and obtained from a solvent purification system. Chemicals were obtained from Sigma Aldrich and Alfa Aesar and were used without further purification unless otherwise noted. Unless otherwise stated, all reactions were performed under a N$_2$ atmosphere using flame or vacuum-dried glassware. Silica used for column chromatography was 70-230 mesh, 0.063-0.200mm particle size. $^1$H NMR spectra were obtained at 400 MHz and $^{13}$C NMR spectra were obtained at 100 MHz using a Varian Mercury or Varian Inova spectrometer. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signal of CDCl$_3$ (δ 7.27, 77.00). Chemical shifts are reported in ppm. Coupling constants (J) are reported in Hz. Infrared spectra were obtained as films from CH$_2$Cl$_2$ on NaCl plates using a Bruker Tensor 27 instrument. ESI mass spectrometry was performed using a PE-Sciex API 365 triple quadrupole instrument. Analytical size exclusion chromatography was performed using a Waters 515 HPLC pump, equipped with Wyatt miniDawnTREOS and Wyatt Optilab Rex detectors, and two ResiPore 300x7.5mm, 3µm particle size columns from Polymer Laboratories. The eluent used was THF and the calibration was performed using
polystyrene standards. Preparative size exclusion chromatography was carried out in DMF at 3 mL/min using a Waters 515 pump equipped with an Optilab Rex refractive index detector from Wyatt Technology and 100 Å and 500 Å PLGel columns from Polymer Laboratories.

**Synthesis of compound 3.9:** To a solution of imidazole (3.84 g, 56.3 mmol) in DMF (40 mL) was added a solution of tert-butyldiphenylchlorosilane (7.75 g, 28.2 mmol) in DMF (30 mL) and the resulting solution was stirred for 10 min. 2-Mercaptoethanol (2.04 g, 26.1 mmol, 1.00 eq.) in DMF (8 mL) was then added and the reaction mixture was stirred at room temperature for 24 h. The DMF was then removed in vacuo and the crude product was taken up in CH₂Cl₂, filtered, and washed with H₂O to remove the imidazole. The organic layer was dried with MgSO₄, filtered, and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (97:3 hexanes:EtOAc) to provide 3.9 (7.74 g, 94%) of as a clear colourless oil. ¹H NMR (CDCl₃): δ 7.71 – 7.67 (m, 4H), 7.48 – 7.37 (m, 6H), 3.79 (t, J = 6.35 Hz, 2H), 2.68 (dt, J = 8.30 & 6.30 Hz, 2H), 1.60 (t, J = 8.30 Hz, 1H), 1.08 (s, 9H). ¹³C NMR (CDCl₃): δ 135.2, 133.1, 129.4, 127.4, 65.3, 26.8, 26.5, 18.9. IR (cm⁻¹): 3070, 2970, 1930, 1770, 1685, 1650, 1520. HRMS: calc’d [M-H]⁺ (C₁₈H₂₄OSSi): 315.1239 Found: (ESI) 315.1230.

**Synthesis of compound 3.10:** Compound 3.9 (1.01 g, 3.20 mmol) was dissolved in CH₂Cl₂ (40 mL). NEt₃ (2.2 mL, 15 mmol) was added followed by 4-nitrophenyl chloroformate (1.30 g, 6.39 mmol) and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then poured onto 1 M HCl and the product was extracted twice into CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed in vacuo. The product was purified by silica gel chromatography (1:1 CH₂Cl₂:hexanes) to provide 3.10 (1.52 g, 99%) as a clear, colourless oil. ¹H NMR (CDCl₃): δ 8.28 (d, J = 9.4 Hz, 2H), 7.69 (dd, J = 7.9 & 1.5 Hz, 4H), 7.37 – 7.50 (m, 6H), 7.32 (d, J = 9.4 Hz, 2H), 3.91 (t, J = 6.1 Hz, 2H), 3.18 (t, J = 6.1 Hz, 2H), 1.06 – 1.11 (m, 9H). ¹³C NMR (CDCl₃): δ 169.5, 155.6, 135.6, 133.1, 129.8, 127.7, 125.2, 122.0, 62.2, 34.3, 26.8, 19.2. IR (cm⁻¹): 3050, 2970, 1930, 1770, 1685, 1650, 1520. HRMS: calc’d [M+H]⁺ (C₂₅H₂₇NO₅SSi): 482.1457 Found: (ESI) 482.1461.
Synthesis of compound 3.11: To a solution of compound 3.10 (3.17 g, 6.58 mmol) in toluene (60 mL) was added DMAP (0.077 g, 0.63 mmol), DIPEA (1.72 g, 13.3 mmol), and diamine 2.10 (1.81 g, 9.63 mmol). The reaction mixture was heated at reflux for 5 h. The reaction mixture was then washed 1 M HCl, followed by two washes with saturated Na₂CO₃ solution. The organic layer was dried with MgSO₄, filtered, and the solvent was removed in vacuo to provide 3.11 (3.48 g, 99%) as a clear pale yellow oil. ¹H NMR (CDCl₃): δ 7.68 (dd, J = 7.9 & 1.7Hz, 4H), 7.47 – 7.34 (m, 6H), 7.30 – 7.14 (m, 3H), 3.82 (m, 2H), 3.57 – 3.32 (m, 4H, (rotamers)), 3.18 – 3.08 (m, 2H), 3.02 (br s, 3H), 2.94 – 2.81 (m, 3H), 1.46 (d, J = 8.40 Hz, 9H), 1.06 (s, 9H, CH₃). ¹³C NMR (CDCl₃): δ 168.2 (rotamer), 167.5 (rotamer), 155.7, 155.4, 135.5, 133.6, 129.6, 127.6, 125.3, 115.6, 79.7, 63.3, 47.6 (rotamer), 46.7 (rotamer), 45.8 (rotamer), 35.7 (rotamer), 35.3 (rotamer), 34.8 (rotamer), 34.6 (rotamer), 32.99 (rotamer), 32.93 (rotamer), 28.4, 26.8, 19.2. IR (cm⁻¹): 3060, 2925, 2850, 1690, 1650. HRMS: calc’d [M+H]⁺ (C₂₈H₄₂N₂O₄SSi): 531.2707 Found: (ESI) 531.2691.

Synthesis of compound 3.12: To a solution containing 3.11 (1.18 g, 2.22 mmol) in THF (20 mL) was added TBAF (1.0 M solution in THF, 4.43 mL, 4.43 mmol) and the reaction mixture was stirred at room temperature for 2 h. The solvent was then removed in vacuo and the resulting residue was purified by silica gel chromatography (85:15 hexanes:EtOAc) to provide 3.5 (0.50 g, 78%) as a clear, pale yellow oil. ¹H NMR (CDCl₃): δ 3.75 (t, J = 5.8 Hz, 2H), 3.54 – 3.33 (m, 4H (rotamers)), 3.05 (t, J = 5.6 Hz, 2H), 2.99 (br s, 3H), 2.90 – 2.78 (m, 3H), 2.00 (s, 1H), 1.42 (s, 9H). ¹³C NMR (CDCl₃): δ 168.9 (rotamer), 168.7 (rotamer), 155.5, 80.0 (rotamer), 79.7 (rotamer), 79.5 (rotamer), 62.1 (rotamer), 61.8 (rotamer), 48.1 (rotamer), 47.8 (rotamer), 47.4 (rotamer), 47.4 (rotamer), 46.7 (rotamer), 46.5 (rotamer), 45.6 (rotamer), 35.7 (rotamer), 35.4 (rotamer), 35.1 (rotamer), 34.9 (rotamer), 34.7 (rotamer), 34.4 (rotamer), 33.2 (rotamer), 32.8, 28.2. IR (cm⁻¹): 3400, 2960, 2930, 2850, 1680, 1650. HRMS: calc’d [M]+ (C₁₂H₂₄N₂O₄S): 292.1457 Found: (ESI) 292.8123.

Synthesis of compound 3.13: To a solution containing 3.12 (0.45 g, 1.5 mmol) in CH₂Cl₂ (10 mL) was added pyridine (0.37 mL, 4.6 mmol), followed by 4-nitrophenyl chloroformate (0.62 g, 3.1 mmol), and the reaction mixture was stirred for 20 h. The
reaction mixture was then washed with 1 M HCl, then the organic layer was dried over MgSO₄, filtered, and the solvent was removed in vacuo. The resulting residue was purified by silica gel chromatography (1:1 CH₂Cl₂:EtOAc) to provide 3.13 (0.60 g, 85%) as a clear, pale yellow oil. ¹H NMR (CDCl₃): δ 8.33 – 8.25 (m, 2H), 7.46 – 7.37 (m, 2H), 4.42 (t, J = 6.5 Hz, 2H) 3.61 – 3.50 (m, 2H (rotamer)), 3.40 (d, J = 6.1 Hz, 2H), 3.27 (d, J = 5.5 Hz, 2H (rotamer)), 3.04 (s, 3H), 2.89 (br s, 3H), 1.47 (br. s. 9H). ¹³C NMR (CDCl₃): δ 167.0 (rotamer), 166.8 (rotamer), 166.2 (rotamer), 155.6 (rotamer), 155.4, 155.2 (rotamer), 152.1, 145.3, 125.1, 121.7, 79.7 (rotamer), 79.52 (rotamer), 79.45 (rotamer), 88.0, 53.4, 47.9 (rotamer), 47.5 (rotamer), 46.7 (rotamer), 46.4 (rotamer), 45.6 (rotamer), 35.5 (rotamer), 35.2 (rotamer), 34.7 (rotamer), 34.3 (rotamer), 28.5 (rotamer), 28.3 (rotamer). IR (cm⁻¹): 3105, 3070, 2964, 2920, 1770, 1690, 1650. HRMS: calc’d [M+H]⁺ (C₁₉H₂₇N₃O₈S): 458.1597 Found: (ESI) 458.1599.

**Synthesis of polymer 3.5a and general polymerization procedure:** Under air atmosphere, compound 3.13 (1.9 g, 4.2 mmol) was dissolved in 1:1 CH₂Cl₂:TFA (20 mL) and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated in vacuo, and then CH₂Cl₂ was added and evaporated five times to remove residual TFA, providing 3.4a·TFA. The residue was dissolved in toluene (40 mL) and NEt₃ (2.9 mL, 21 mmol), DMAP (0.056 g, 0.46 mmol) and end cap 3.13 (0.096 g, 0.21 mmol) were added. The resulting solution was stirred at room temperature for 18 h. The reaction was quenched with 1 M HCl, and the product was extracted three times into CH₂Cl₂. The organic layers were dried over MgSO₄, filtered, and the solvent was removed. Purification via preparative SEC afforded polymer 3.5a (0.44 g, 45%). ¹H NMR (CDCl₃): δ 4.27 – 4.13 (m, 2H), 3.60 – 3.33 (m, 4H), 3.22 – 3.10 (m, 2H), 3.02 (s, 3H), 2.97 – 2.89 (m, 3H), 1.46 (s, 0.5H). SEC: Mₙ = 1650 g/mol, Mₘ = 2200 g/mol, PDI = 1.3.

**Synthesis of compound 3.7a:** Compound 3.14 (0.24 g, 1.3 mmol) was dissolved in CH₂Cl₂ (6 mL) and pyridine (0.30 mL, 3.8 mmol), then 4-nitrophenyl chloroformate (0.51 g, 2.5 mmol) was added and the reaction mixture was stirred for 4 h. NEt₃ (0.34 mL, 2.5 mmol) and TEGMME (0.30 mL, 1.9 mmol) were then added and the reaction mixture was stirred for an additional 10 min. The reaction mixture was then poured into
1 M HCl and the product was extracted twice into CH₂Cl₂. The combined organic layers were dried with MgSO₄, filtered, and the solvent was removed. The residue was purified by silica gel chromatography (9:1 hexanes:EtOAc) to provide 3.7a (0.33 g, 74%) as a pale yellow oil. ¹H NMR (CDCl₃): δ 8.53 - 8.48 (m, 1H), 8.32 - 8.26 (m, 2H), 7.70 - 7.63 (m, 2H), 7.42 - 7.36 (m, 2H), 7.15 - 7.12 (m, 1H), 4.57 (t, J = 6.4, 2H), 3.17 (t, J = 6.4, 2H). ¹³C NMR (CDCl₃): δ 159.1, 155.3, 152.1, 149.8, 145.4, 137.1, 125.3, 121.7, 121.1, 120.2, 66.6, 36.7. IR (cm⁻¹): 3115, 3080, 3045, 2960, 2855, 1760, 1615, 1590, 1570, 1520. HRMS: calc’d [M]⁺ (C₁₄H₁₂N₂O₅S₂): 352.0188 Found: (ESI) 352.0184.

Synthesis of polymer 3.5b: This polymer was prepared by the same procedure described above for the synthesis fo polymer 3.5a except that 3.7a was used as the end cap. The polymer was purified by dialysis against DMF (200 mL, 1 solvent change) using a regenerated cellulose membrane (Spectrum Laboratories Spectra/Por, 3500 MW cutoff). The DMF was then removed in vacuo to provide polymer 3.5a (0.10 g, 34%). ¹H NMR (CDCl₃): δ 8.48 (d, J = 4.7 Hz, 0.2H), 8.28 (d, J = 9.0 Hz, 0.2H) 7.74 – 7.61 (m, 0.4H), 7.41 (d, J = 9.0 Hz, 0.2H), 7.16 (br s, 0.2H), 4.39 – 4.28 (m, 2H), 4.27 – 4.07 (m, 2H), 3.61 – 3.34 (m, 4H), 3.23 – 3.10 (m, 2H), 3.02 (br S, 3H), 2.98 – 2.89 (m, 3H). SEC: Mₙ = 1800 g/mol, Mₚ = 2950 g/mol, PDI = 1.6.

Degradation Study

Buffer Preparation: NaH₂PO₄·H₂O (0.069 g, 0.5 mmol) was dissolved in D₂O (5 mL). To this, a saturated solution of NaOH in D₂O was added dropwise with stirring, while monitoring with a pH meter until the desired pH of 7.4 was obtained.

Degradation of polymer 3.5a: Under air atmosphere, 12 mg of polymer 3.5a was dissolved in 1:1 TFA:CH₂Cl₂ (1 mL) and the solution was stirred 2 h. The solvent was removed, and then CH₂Cl₂ was added and removed five times to azeotrope residual TFA. The material was then dissolved in pH 7.4 0.1 M phosphate (D₂O):acetone-d₆ (3:2) (1 mL), and the solution was incubated at 37 °C. The extent of depolymerization was quantified using ¹H NMR by integrating the methylene peak corresponding to the N,N'-dimethylimidazolidinone a degradation product (3.4 ppm) relative to the peak corresponding to the methylene group adjacent to the oxygen in the polymer (4.3 ppm).
A control sample with the Boc group still attached was monitored under the same conditions as above.

**Degradation of polymer 3.5b:** Under air atmosphere, 15 mg of polymer 3.5b was dissolved in pH 7.4 0.1 M phosphate buffered D₂O:acetone-d₆ (3:2) (1 mL), and the solution was incubated at 37 °C. 3 mg of DTT was added at the beginning and subsequently every 7 days to maintain reducing conditions. Extent of depolymerization was monitored as described above for degradation of 3.5a. A control sample was monitored under the same conditions as above, but without DTT. For SEC samples, a 0.25 mL aliquot was dried and the resulting residue was taken up in THF. The salts were removed by filtration through a 0.2 µm filter.

### 3.5 References

Chapter 4

Design, Synthesis and Cyclization of 4-Aminobutyric Acid Derivatives: Potential Candidates as Self-Immolative Spacers

4.1 Introduction

Chemical moieties capable of undergoing rapid and spontaneous intramolecular reactions in response to the cleavage of a capping or triggering unit are commonly referred to as self-immolative spacers. In their typical form, these moieties comprise two reactive termini with a capping group or trigger as one terminus and the substrate of interest, such as a drug, fluorophore, or an additional spacer on the other terminus. Removal of the capping group results in an intramolecular reaction that ultimately results in the liberation of the substrate. As shown in Figure 4.1, these intramolecular reactions generally involve electronic rearrangements such as 1,4, 1,6, or 1,8 elimination reactions or cyclizations to form highly favored five- or six-membered rings.

In recent years, the interest in self-immolative spacers has grown significantly as their application in various prodrug, sensor, and drug delivery systems has been explored. For example, the conjugation of self-immolative spacers to drug molecules has created inactive prodrugs that are converted to the free and active drugs by cleavage of the trigger upon exposure to an external stimulus. They have also been used in the linkage of drug molecules to small molecule or antibody targeting moieties. Sensors have been developed by using self-immolative spacers to conjugate reporter molecules such as fluorophores or imaging agents to peptide or enzyme sensitive triggers. The use of these linkers in dendrimeric and oligomeric systems has also been explored, leading to an amplified release of drugs or reporter molecules.

* This chapter contains work that has been published: Dewit, M. A.; Gillies, E. R. Org. Biomol. Chem. 2011, 9, 1846-1854. See Co-Authorship Statement for specific contributions from each author.
Recently, our group and others have explored the development of linear polymers comprising self-immolative spacers. Shabat and coworkers have explored polymers based entirely on 1,6-elimination reactions as amplified reporters, enzyme sensors, and enzyme labels. Moore and coworkers have prepared microcapsules based on cross-linked versions of similar polymers. Our group has introduced cyclization spacers in alternation with elimination spacers as a means of controlling the polymer degradation rate, and have demonstrated that amphiphilic block copolymers such as 2.3b (Figure 4.2) comprising one self-immolative block are capable of assembling into nanoparticles that degrade in a controlled manner to release their cargo. Furthermore, we have also developed linear polymers such as 3.5b, capable of degrading entirely by cyclization reactions in order to address the potential toxicity of the quinone methide intermediates that are produced during the 1,6-elimination reaction.

In order to fully exploit self-immolative spacers in these materials and other new applications, it is essential to have access to spacers that react at different rates. For example, the N,N’-dimethylethylenediamine spacer used in both polymers 2.3b and 3.5b cyclized slowly at pH 7.4, which resulted in polymer degradation over a period of days to weeks. While several self-immolative spacers based on cyclization reactions have been reported, there are very few that cyclize rapidly under mild conditions. To address

**Figure 4.1.** a) Schematic of a self-immolative spacer; b) example of a 1,6 elimination reaction; c) example of a cyclization reaction.
this need and to develop a spacer that could potentially replace the $N,N'$-dimethylethlenediamine spacer in polymers such as 2.3b or 3.5b, we have investigated 4-aminobutyric acid derivatives as a potential new class of rapidly cyclizing self-immolative spacers. The recent incorporation of a 4-aminobutyric acid unit into an enzymatic detection probe suggested that this class of molecules may serve as rapidly cyclizing spacers. However, there has not been a versatile synthetic strategy developed for the preparation of various analogues, nor a comprehensive study of their cyclization rates. Thus, described here is the design and synthesis of eleven different derivatives of 4-aminobutyric acid, and studies of their cyclizations.

**Figure 4.2.** Chemical structures of previously reported self-immolative polymers incorporating cyclization spacers.44,45

4.2 Results and discussion

4.2.1 Design

**Figure 4.3.** Target 4-aminobutyric acid derivatives for kinetic studies.
The targets shown in Figure 4.3 were designed with several aspects in mind. First, a phenyl ester was selected, as it would serve as a good model system for future applications. Many dyes, such as fluorescein, Hoechst stain and umbelliferone, contain phenolic groups, along with the chemotherapy drug Topotecan. In addition, many other self-immolative spacers involve phenols, thus allowing the new spacer to be readily alternated with other spacers, and incorporated into polymers analogous to 2.3b. Secondly, some N-methylated derivatives were targeted as this has previously been reported to significantly enhance the cyclization rate in the case of ethylenediamine derivatives. Finally, substitution at the α position allowed us to examine the Thorpe-Ingold effect and/or the reactive rotamer effect on these compounds. It was also expected to slow any competing ester hydrolysis. To test the scope of these effects, the substitution pattern and the size of the substituents were varied from unsubstituted to an α,α-dibenzyl derivative. A single cyclopentyl ring was also incorporated to test the effect of a conformationally locked system. The 2-hydroxy derivatives were designed to provide insight into inductive effects.

### 4.2.2 Synthesis

![Scheme 4.1. Synthesis of α-unsubstituted derivatives.](image)

The synthesis of targets 4.1a-j began with the previously reported Boc-protected 4-aminobutyric acid 4.2. As shown in Scheme 4.1, the phenyl ester 4.4a was obtained by coupling the acid 4.2 to phenol using N,N'-dicyclohexylcarbodiimide (DCC) in the
presence of DMAP. The \( N \)-methyl derivative was prepared by treating 4.2 with MeI and NaH, immediately followed by hydrolysis of the resulting methyl ester with LiOH. The resulting free acid 4.3 was then coupled to phenol using DCC and DMAP to obtain the desired Boc-protected phenyl ester 4.4b.

**Scheme 4.2.** Synthesis of \( \alpha \)-monosubstituted derivatives.

To prepare the \( \alpha \)-monosubstituted compounds, the acid was first converted to a \( t \)-butyl ester 4.6a using \( t \)-tert-butyl-2,2,2-trichloroacetimidate in the presence of BF\(_3\)Et\(_2\)O, and then \( N \)-methylation was performed as described above using MeI and NaH to provide 4.6b (Scheme 4.2). Monoalkylated \( t \)-butyl esters 4.7a-c were obtained by treatment of 4.6b with lithium hexamethyldisilylazide (LHMDS) in the presence of LiCl at -78 °C, followed by the addition of the alkyl halide. In marked contrast to the formation of the benzyl and allyl derivatives which suffered from partial over-alkylation, monomethylation was cleanly achieved even when a large excess of both LHMDS and MeI were used, simplifying purification and increasing its yield relative to the other compounds. The substituted \( t \)-butyl esters were then converted to the free acids by first removing both the Boc group and the ester using 1:1 TFA:CH\(_2\)Cl\(_2\), and then reinstalling the Boc group on the amine. This process worked very efficiently for all substrates, with all products being obtained with yields in excess of 90%. The final step was formation of the phenyl ester. When DCC was used as described above for the preparation of 4.4a and 4.4b, unsatisfactory yields of the desired products were obtained, likely due to steric hindrance at the \( \alpha \) carbon. To circumvent this problem, the acid was converted to a
mixed anhydride using pivaloyl chloride, and this anhydride was then treated with phenol. This afforded phenyl esters 4.9a-c in good yields, ranging from 78 – 86%.

Scheme 4.3. Synthesis of α,α-disubstituted derivatives.

The α,α-dialkylated compounds were similarly derived from the intermediate 4.6b (Scheme 4.3). Diallyl and dibenzyl tert-butyl esters 4.10b & 4.10c were synthesized directly from 4.6b by treatment first with 1 eq of LHMDS and then the alkyl halide followed an hour later by 2 eq of each, which gave clean conversion to the disubstituted products. Synthesis of the cyclopentyl ring was done similarly except with a single addition of the alkyl dihalide. Following the same protocol as allylation and benzylation to generate 4.10a did not prove successful, and the products obtained were a mixture of the mono and disubstituted compounds. Resubjection of this material also did not prove successful, even after removal of byproducts. Similarly, 4.7a could not be further methylated under these conditions. However, when the base was switched to lithium diisopropylamide (LDA), the second methylation occurred cleanly, affording 4.10a in very good yield. Curiously, when LDA was used on 4.6b, a mixture of mono and dialkylated products was once again obtained, so it appears for this particular substrate the dimethyl tert-butyl ester could only be obtained by doing successive methylations of 4.6b and then 4.7a, using LHMDS for the first methylation and LDA for the second.

At this point a global deprotection and reinstallation of the Boc group was carried out, again producing the N-Boc acids in very good yields. To install the phenyl ester, it
was evident that a mixed pivaloyl anhydride would be ineffective as there would be little steric differentiation between the two carbonyls of the anhydride. Therefore, the best option appeared to be conversion of the acid to an acid chloride. As the acid sensitivity of the Boc groups was incompatible with conventional methods for generating acid chlorides, the Ghosez reagent, 1-chloro-N,N,2-trimethylpropenylamine, which generates the acid chloride with no acidic byproducts, was used. This method proved quite effective, providing the desired phenyl esters in yields ranging from 60 – 90% after isolation.

Scheme 4.4. Synthesis of α-hydroxy substituted derivatives.

As shown in Scheme 4.4, the synthesis of the α-hydroxy targets began with Boc-protected (S)-4-amino-2-hydroxybutyric acid \(^{54} 4.14\). This acid was treated with MeI in the presence of Cs\(_2\)CO\(_3\) to afford the desired methyl ester \(^{4.15}\) in very good yield. The next step was protection of the 2-hydroxy group, for which we selected a second Boc group. This group was chosen because it could be attached easily in high yields, and cleavage could occur simultaneously with the N-Boc group, thus removing an additional deprotection step from the reaction sequence. This Boc group was installed using Boc\(_2\)O in the presence of catalytic DMAP, affording di-Boc protected compound \(^{4.16a}\) in 90% yield. To generate the target lacking an N-methyl group, \(^{4.16a}\) was treated with LiOH to cleave the methyl ester, and the corresponding acid was converted to phenyl ester \(^{4.17a}\) in 70% yield using the mixed anhydride method described above. The N-methyl derivative was synthesized by first methylating \(^{4.16a}\) using MeI and NaH, and then following the same procedure as above to obtain the phenyl ester \(^{4.17b}\).
4.2.3 Kinetics

**Figure 4.4.** Normalized UV-visible absorption spectra of phenol and a representative phenyl ester 4.1a·TFA.

UV-visible spectroscopy was evaluated as a possible analytical tool for measuring the cyclization kinetics. The UV-visible spectra of phenol and a representative phenyl ester 4.1a·TFA are shown in Figure 4.4. While there is some overlap between the two spectra, at 276 nm the phenol is strongly absorbing, with a molar extinction coefficient of 1100 M⁻¹cm⁻¹ while the phenyl ester is only weakly absorbing, with a molar extinction coefficient of just 65 M⁻¹cm⁻¹. Therefore, it was possible to perform kinetic studies measuring the phenol released upon cyclization by monitoring the absorbance at 276 nm, as the change in absorbance at this wavelength throughout the course of the reaction was expected to be due almost exclusively to the increase in concentration of phenol while the decreasing phenyl ester concentration was expected to have negligible impact on the absorbance.

**Scheme 4.5.** Deprotection and subsequent cyclization of 4-aminobutyric acid derivatives.
All of the target molecules were stored in their Boc-protected forms and were deprotected immediately prior to kinetic studies by treatment with TFA as shown in Scheme 4.5. Removal of the Boc group was verified by $^1$H NMR spectroscopy. In most cases, protonation of the amine in the form of the TFA salt was sufficient to inhibit cyclization prior to and during NMR spectroscopy, but occasionally some cyclization was observed (see appendix). The kinetic studies were performed by dissolving the deprotected substrates in iPrOH, then diluting this solution ten-fold with pH 7.4 0.1 M phosphate buffer. The measurements were carried out at the physiological temperature of 37 °C. The pH was verified after cyclization, and no change was observed. Due to the rapid rates of cyclization observed for certain spacers, it was found to be necessary to begin monitoring the change in absorbance at 276 nm immediately prior to addition of phosphate buffer. To compensate for initial mixing effects, the point at which a steady increase in the absorbance at 276 nm began was taken to be $t = 0$. The linearity of $\ln[A]_0/[A]$ versus time graphs where [A] is the concentration of the starting material suggested that the cyclizations followed first order or pseudo first order kinetics (see appendix). Rate constants were calculated as the slopes of these graphs. The reported rate constants are the average of those obtained over a minimum of 3 experimental runs (Table 4.1). Reported errors correspond to the calculated standard deviations of these runs. From the average rate constant, the half-life for each spacer was also calculated. In each case, the structure of the cyclized product was verified by NMR spectroscopy and mass spectrometry (see appendix). There was no evidence of background ester hydrolysis for any of the substrates.

All of the spacers cyclized quite rapidly with the half-lives ranging from 2 to 39 s. When comparing 4.1a and 4.1b, it is clear that methylating the amine had a dramatic effect on the rate, with the half-life being reduced by a factor of approximately four. Consistent with the Thorpe-Ingold$^{50,51}$ and reactive rotamer effects,$^{52}$ α substitution further increased the rate of cyclization, as all of the monosubstituted spacers 4.1c, 4.1d, and 4.1e reacted faster than the unsubstituted spacer 4.1b and cyclized at similar rates. The α,α-disubstituted spacers 4.1f and 4.1g cyclized at similar rates to their monosubstituted analogues. However, the α,α-dibenzyl substituted spacer 4.1h exhibited a nearly 4-fold decrease in rate, suggesting that steric crowding proximal to the ester
impedes cyclization. In contrast, the α-cyclopentyl substituted compound 4.1i exhibited the fastest rate of cyclization, indicating that conformational rigidity can play a role. Finally, both of the α-hydroxy substituted spacers 4.1j and 4.1k cyclized faster than the α-aliphatic substituted derivatives. Interestingly, the dramatic increase in rate caused by N-methylation that was observed for 4.1a versus 4.1b was not noted for 4.1j versus 4.1k. The rate did increase, indicating that there was still an effect, but it appears that the most significant contribution was from the α-hydroxy substituent.

Table 4.1. Rate constants and corresponding half-lives for the intramolecular cyclizations of 4.1a-k.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rate Constant (s(^{-1}))</th>
<th>Half-life (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1a</td>
<td>0.018 ± 0.004</td>
<td>39</td>
</tr>
<tr>
<td>4.1b</td>
<td>0.071 ± 0.009</td>
<td>9.8</td>
</tr>
<tr>
<td>4.1c</td>
<td>0.12 ± 0.01</td>
<td>6.0</td>
</tr>
<tr>
<td>4.1d</td>
<td>0.13 ± 0.02</td>
<td>5.5</td>
</tr>
<tr>
<td>4.1e</td>
<td>0.10 ± 0.02</td>
<td>6.7</td>
</tr>
<tr>
<td>4.1f</td>
<td>0.13 ± 0.01</td>
<td>5.2</td>
</tr>
<tr>
<td>4.1g</td>
<td>0.13 ± 0.01</td>
<td>5.2</td>
</tr>
<tr>
<td>4.1h</td>
<td>0.028 ± 0.002</td>
<td>25</td>
</tr>
<tr>
<td>4.1i</td>
<td>0.35 ± 0.01</td>
<td>2.0</td>
</tr>
<tr>
<td>4.1j</td>
<td>0.17 ± 0.02</td>
<td>4.0</td>
</tr>
<tr>
<td>4.1k</td>
<td>0.23 ± 0.01</td>
<td>3.0</td>
</tr>
</tbody>
</table>
It was also of interest to investigate the effect of pH on the cyclization rate. This was of interest in considering the potential application of these new spacers in areas such as drug delivery. For example, certain drug delivery targets such as tumors,\textsuperscript{55,56} inflamed tissues,\textsuperscript{57} and intracellular compartments such as endosomes and lysosomes\textsuperscript{58} are known to exhibit mildly acidic pHs. It has been established that aminolysis of esters is a pH dependent process due to the basicity of the amine functionality.\textsuperscript{59,60} At lower pH the equilibrium between the protonated (inactive) and deprotonated (active) states favours the protonated state and therefore hinders the cyclization. Thus, it was of importance to test the effect of pH on the rate of intramolecular cyclization. To explore this, the cyclization of spacer \textbf{4.1i} was investigated at pHs 4.0, 5.0, 6.0 and 7.0 following the method previously described. As shown in Table 4.2, there was indeed a pronounced decrease in the cyclization rate with decreasing pH. Nevertheless, the cyclization was still rapid even at pH 4.0 with a half-life of 76 s. In comparison, the \textit{N},\textit{N}-dimethylethylenediamine spacer previously employed\textsuperscript{44,45} has a reported half-life of greater than 15 days at pH 4.2 and 37 °C.\textsuperscript{7} Therefore, these 4-aminobutyric acid spacers appear to be more promising for a wider range of physiological environments.

**Table 4.2.** Rate constants and corresponding half-lives for the intramolecular cyclizations of \textbf{4.1i} at different pHs.

<table>
<thead>
<tr>
<th>pH</th>
<th>Rate Constant (s\textsuperscript{-1})</th>
<th>Half-life (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>0.35 ± 0.01</td>
<td>2.0</td>
</tr>
<tr>
<td>7.0</td>
<td>0.18 ± 0.02</td>
<td>3.8</td>
</tr>
<tr>
<td>6.0</td>
<td>0.062 ± 0.009</td>
<td>11</td>
</tr>
<tr>
<td>5.0</td>
<td>0.020 ± 0.001</td>
<td>35</td>
</tr>
<tr>
<td>4.0</td>
<td>0.0091 ± 0.0012</td>
<td>76</td>
</tr>
</tbody>
</table>
4.3 Conclusion

A new series of self-immolative spacers derived from 4-aminobutyric acid was developed. A modular synthetic approach was used for the preparation of eleven different derivatives. These derivatives allowed the effects of N-methylation and α-substitution to be explored. As expected, N-methylation led to enhanced cyclization rates. α-Substitution led to enhanced cyclization rates when the substituents were not too bulky but large groups such as benzyl slowed the cyclization. Electron withdrawing groups or conformationally restricted groups at the α position accelerated the rate. Overall, all of the target compounds exhibited rapid cyclization kinetics with half lives of less than one minute at pH 7.4 and 37 °C. In addition, cyclization still occurred rapidly at mildly acid pH. This suggests that these spacers should be of great utility in systems where a rapid release of the substrate even at acidic pHs is required. Furthermore, the versatile synthetic approach should allow the introduction of additional functionalities and also for their incorporation into a range of chemical systems, allowing for many new applications.

4.4 Experimental

General procedures and materials:

All reagents were purchased from commercial sources and used without further purification unless otherwise noted. Anhydrous DMF and THF were obtained from a solvent purification system. Anhydrous CH₂Cl₂ and NEt₃ were distilled over CaH₂. Diisopropylamine was distilled over MgSO₄. Unless otherwise stated, all reactions were performed under a N₂ atmosphere using flame or vacuum-dried glassware. Column chromatography was performed using silica gel (0.063–0.200 mm size, 70–230 mesh). ¹H NMR spectra were obtained at 400 MHz and ¹³C NMR spectra were obtained at 100 MHz. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals of CDCl₃ (δ 7.27, 77.00). Coupling constants are expressed in Hertz (Hz).
Infrared spectra were obtained as films from CH$_2$Cl$_2$ on NaCl plates using a Bruker Tensor 27 instrument. High-resolution mass spectrometry (HRMS) was performed using a Finnigan MAT 8400 electron impact (EI) or a Micromass LCT electrospray ionization time-of-flight (ESI) mass spectrometer. UV-visible spectrscopy experiments were carried out using a Varian Cary 300 Bio UV-visible spectrophotometer.

**Synthesis of compound 4.3:** To a flask containing 4.2$^{53}$ (0.250 g, 1.23 mmol), DMF (12 mL) and MeI (0.23 mL, 3.69 mmol) were added, and the solution was cooled to 0 °C. NaH (0.128 g, 3.21 mmol) was suspended in DMF (1 mL) and the suspension was added dropwise to the reaction mixture. The resulting mixture was stirred for 4 h. A second portion of MeI (0.10 mL, 1.61 mmol) was then added followed by additional NaH (0.070 g, 2.92 mmol) suspended in DMF (0.5 mL) and the reaction mixture was stirred for 2 h. The solvent was removed in vacuo, and the crude material was taken up in CH$_2$Cl$_2$ and poured into H$_2$O. The product was extracted with CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The resulting oil was taken up in 1:1 THF:H$_2$O (12 mL), LiOH·H$_2$O (0.104 g, 2.48 mmol) was added and then the solution was stirred overnight under air atmosphere. The solution was poured into 1 M HCl, and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. The crude product was purified by column chromatography (3:2 cyclohexane:EtOAc), yielding 4.3 (0.195 g, 73%) as a clear, colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 11.08 (br s, 1H), 3.35 – 3.22 (m, 2H), 2.85 (s, 3H), 2.36 (t, $J$ = 7.2, 2H), 1.85 (quintet, $J$ = 6.8, 2H), 1.45 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 178.1, 155.9, 79.6, 47.9 & 47.5 (rotamers), 34.0, 31.0 & 30.8 (rotamers), 28.2, 22.7. IR (cm$^{-1}$): 3205, 2980, 2937, 1738, 1698, 1490, 1457, 1401, 1367. HRMS: calc’d [M]+ (C$_{10}$H$_{19}$NO$_4$): 217.1314. Found: (EI) 217.1318.

**Synthesis of compound 4.4a and general DCC mediated esterification procedure:** Compound 4.2$^{53}$ (0.409 g, 2.01 mmol) was dissolved in CH$_2$Cl$_2$ (20 mL). Phenol (0.228 g, 2.45 mmol), DCC (0.623 g, 3.02 mmol) and DMAP (0.0236 g, 0.193 mmol) were added, and the solution was stirred for 1 h. The precipitate was filtered off and rinsed with CH$_2$Cl$_2$. The filtrate was poured into 1 M NaOH and the product was extracted three times into CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered,
and the solvent was removed in vacuo. The crude material was purified by column chromatography (85:15 cyclohexane:EtOAc), affording 4.2a (0.536 g, 95%) as a white solid. 

$^{1}$H NMR (CDCl$_3$): $\delta$ 7.42 – 7.35 (m, 2H), 7.27 – 7.22 (m, 2H), 7.12 – 7.07 (m, 1H), 4.67 (br s, 1H), 3.27 (quartet, $J$ = 6.6 Hz, 2H), 2.62 (t, $J$ = 7.4, 2H), 1.95 (quintet, $J$ = 7.0 Hz, 2H), 1.46 (s, 9H). 

$^{13}$C NMR (CDCl$_3$): $\delta$ 171.7, 155.9, 150.5, 129.2, 125.7, 121.4, 79.1, 39.7, 31.5, 28.3, 25.2. IR (cm$^{-1}$): 3080, 3068, 3010, 2981, 2936, 2866, 1762, 1697, 1596, 1523, 1494, 1483, 1367. HRMS: calc’d [M+H]$^+$ (C$_{15}$H$_{22}$NO$_4$): 280.1543. Found: (EI) 280.1549.

**Synthesis of compound 4.4b:** The same procedure described above for the preparation of compound 4.4a was followed except that 4.3 was used as a starting material. The product was purified by column chromatography (9:1 cyclohexane:EtOAc) to provide 4.4b (0.128 g, 79%) as a clear, colorless oil. 

$^{1}$H NMR (CDCl$_3$): $\delta$ 7.42 – 7.36 (m, 2H), 7.26 – 7.21 (m, 2H), 7.12 – 7.07 (m, 1H), 3.35 (t, $J$ = 6.6 Hz, 2H), 2.89 (s, 3H), 2.58 (t, $J$ = 7.4 Hz, 2H), 1.97 (quintet, $J$ = 7.0 Hz, 2H), 1.48 (s, 9H). 

$^{13}$C NMR (CDCl$_3$): $\delta$ 171.6, 155.7, 150.6, 129.3, 125.7, 121.4, 79.4, 48.0 & 47.4 (rotamers), 34.1, 31.3, 28.4, 23.0 & 22.8 (rotamers). IR (cm$^{-1}$): 3103, 3074, 3045, 3014, 2979, 2937, 2873, 1762, 1697, 1596, 1494, 1477, 1396, 1366. HRMS: calc’d [M+H]$^+$ (C$_{16}$H$_{24}$NO$_4$): 294.1705. Found: (EI) 294.1711.

**Synthesis of compound 4.6a:** Compound 4.2$^{53}$ (1.00 g, 4.92 mmol) was dissolved in CH$_2$Cl$_2$ (15 mL). tert-Butyl-2,2,2-trichloroacetimidate (1.76 mL, 9.83 mmol) and BF$_3$·Et$_2$O (0.100 mL, 0.707 mmol) were added and the reaction mixture was stirred for 45 min. The solution was then filtered to remove the precipitate and the precipitate was rinsed with CH$_2$Cl$_2$. The filtrate was poured into 1 M Na$_2$CO$_3$, and the product was extracted three times into CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The material was taken up in cyclohexane and filtered a second time. The solvent was removed in vacuo, yielding 4.6a (1.22 g, 96%) as a clear, colourless oil. 

$^{1}$H NMR (CDCl$_3$): $\delta$ 4.73 (br s, 1H), 3.10 (quartet, $J$ = 6.3 Hz, 2H), 2.22 (t, $J$ = 7.4 Hz, 2H), 1.73 (quintet, $J$ = 7.0 Hz, 2H), 1.40 (s, 9H), 1.39 (s, 9H). 

$^{13}$C NMR (CDCl$_3$): $\delta$ 172.6, 155.7, 80.3, 79.0, 39.9, 32.8, 28.3, 28.0,
Synthesis of compound 4.6b: To a flask containing 4.6a (1.11 g, 4.28 mmol) were added DMF (20 mL) and MeI (0.29 mL, 4.66 mmol), and the solution was cooled to 0 °C. NaH (0.103 g, 4.31 mmol) was suspended in DMF (2 mL) and added dropwise to the reaction mixture. The solution was stirred for 1 h, then a second equivalent each of MeI and NaH were added as above, and the solution was stirred overnight. The solution was poured into 1:1 H₂O:saturated brine, and the product was extracted three times into CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed in vacuo. Purification by column chromatography (19:1 cyclohexane:EtOAc followed by 9:1 cyclohexane:EtOAc) yielded 4.6b (0.990 g, 84%) as a thin, colourless oil. ¹H NMR (CDCl₃): δ 3.23 (t, J = 7.4 Hz, 2H), 2.85 (s, 3H), 2.22 (t, J = 7.6 Hz, 2H), 1.79 (quintet, J = 7.6 Hz, 2H), 1.46 (s, 9H), 1.45 (s, 9H). ¹³C NMR (CDCl₃): δ 172.4, 155.6, 80.1, 79.1, 48.1 & 47.6 (rotamers), 34.0, 32.5, 28.3, 28.0, 23.3 & 22.9 (rotamers). IR (cm⁻¹): 2979, 2937, 1731, 1700, 1482, 1458, 1395, 1367. HRMS: calc’d [M]⁺ (C₁₄H₂₇NO₄): 273.1940. Found: (EI) 273.1947.

Synthesis of compound 4.7a: Compound 4.6b (0.188 g, 0.687 mmol) was dissolved in THF (2 mL), and then LiCl (0.044 g, 0.104 mmol) was added and the solution was cooled to -78 °C. MeI (0.43 mL, 6.91 mmol) was added, followed by dropwise addition of LHMDS (1.0 M solution in THF, 2.00 mL, 2.00 mmol). The resulting solution was stirred for 30 min at -78 °C, then warmed to RT and stirred an additional 90 min. The reaction mixture was then poured into 1 M HCl and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed in vacuo. The crude material was purified by column chromatography (19:1 cyclohexane:EtOAc) to afford 4.7a (0.174 g, 88%) as a pale yellow oil. ¹H NMR (CDCl₃): δ 3.37 – 3.11 (m, 2H), 2.84 (s, 3H), 2.31 (sextet, J = 7.0 Hz, 1H), 1.88 (sextet, J = 7.0 Hz, 1H), 1.63 – 1.51 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.15 (d, J = 7.0 Hz, 3H). ¹³C NMR (CDCl₃): δ 175.5, 155.6, 80.0, 79.2, 46.9 & 46.5 (rotamers), 37.9, 34.1, 31.7 & 31.2 (rotamers), 28.4, 28.0, 17.1. IR (cm⁻¹): 2979, 2937,
Synthesis of compound 4.7b and general monoalkylation procedure: Compound 4.6b (0.204 g, 0.747 mmol) was dissolved in THF (3 mL), and then LiCl (0.048 g, 1.128 mmol) was added and the solution was cooled to -78 °C. LHMDS (1.0 M solution in THF, 0.93 mL, 0.93 mmol) was added slowly, and the solution was stirred 30 min. Allyl bromide (0.068 mL, 0.786 mmol) was then added dropwise, and the solution was stirred for 30 min at -78 °C, then warmed to RT and stirred for an additional 90 min. The reaction mixture was then poured into 1 M HCl, and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed in vacuo. Purification by column chromatography (99:1 cyclohexane:EtOAc followed by 19:1 cyclohexane:EtOAc) to provide 4.7b (0.162 g, 69%) as a thick, colorless oil. ¹H NMR (CDCl₃): δ 5.81 – 5.68 (m, 1H), 5.15 – 4.98 (m, 2H), 3.39 – 3.06 (m, 2H), 2.84 (s, 3H), 2.40 – 2.17 (m, 3H), 1.88 – 1.76 (m, 1H), 1.69 – 1.57 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H). ¹³C NMR (CDCl₃): δ 174.1, 155.5, 135.2, 116.7, 80.3, 79.2, 47.0 & 46.4 (rotamers), 43.3, 36.4, 34.1, 29.6 & 29.3 (rotamers), 28.3, 28.0. IR (cm⁻¹): 2978, 2935, 1729, 1700, 1482, 1458, 1394, 1367. HRMS: calc’d [M]+ (C₁₇H₂₉NO₄): 287.2097. Found: (EI) 287.2085.

Synthesis of compound 4.7c: The same procedure described above for the preparation of compound 4.7b was followed except that benzyl bromide was used as the alkyl halide and only 1.2 eq of LHMDS was used. The product was purified by column chromatography (99:1 cyclohexane:EtOAc followed by 19:1 cyclohexane:EtOAc) to provide 4.7c (0.188 g, 70%) as a thick, colorless oil. ¹H NMR (CDCl₃): δ 7.32 – 7.24 (m, 2H), 7.23 – 7.15 (m, 3H), 3.43 – 3.08 (m, 2H), 2.99 – 2.67 (m, 2H), 2.81 (s, 3H), 1.93 – 1.79 (m, 1H), 1.73 – 1.60 (m, 1H), 1.54 – 1.38 (m, 9H), 1.38 – 1.28 (m, 9H). ¹³C NMR (CDCl₃): δ 174.2, 155.5, 139.1, 128.9, 128.2, 126.2, 126.2, 80.3, 79.2, 47.2, 45.6, 38.5, 34.1, 29.9, 28.3, 27.9. IR (cm⁻¹): 3090, 3066, 3031, 3006, 2978, 2932, 2892, 1727, 1699, 1483, 1456, 1394, 1367. HRMS: calc’d [M]+ (C₂₁H₃₃NO₄): 363.2410. Found: (EI) 363.1924.
Synthesis of compound 4.8a and general deprotection-N-Boc reprotection procedure: Under an air atmosphere, a flask was charged with 4.7a (0.108 g, 0.376 mmol), and then 1:1 TFA:CH₂Cl₂ (2 mL) was added and the solution was stirred for 2 h. The solvent was then removed in vacuo, and CH₂Cl₂ was added and removed 3 times to help further remove residual TFA. After thorough drying, the material was taken up in 1:1 dioxane:0.5 M NaOH solution (2 mL), and the pH was adjusted to approximately 12 using 1 M NaOH. Di-tert-butyldicarbonate (0.103 g, 0.470 mmol) was added, and the solution was stirred overnight. The material was poured into 1:1 1 M HCl:saturated brine, and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed in vacuo. The crude material was purified by column chromatography (85:15 cyclohexane:EtOAc followed by 70:30 cyclohexane:EtOAc) to provide 4.8a (0.0795 g, 91%) as a clear, colorless oil.

¹H NMR (CDCl₃): δ 10.24 (br s, 1H), 3.52 – 3.11 (m, 2H), 2.85 (s, 3H), 2.46 (sextet, J = 7.0 Hz, 1H), 2.02 – 1.82 (m, 1H), 1.71 – 1.55 (m, 1H), 1.46 (s, 9H), 1.23 (d, J = 7.4 Hz, 3H).

¹³C NMR (CDCl₃): δ 181.3, 156.0, 79.9, 46.7, 36.7, 34.0, 31.2, 28.3, 17.0. 3472, 3237, 2979, 2941, 1701, 1687, 1488, 1467, 1403, 1368. IR (cm⁻¹): 3472, 3237, 2979, 2941, 1701, 1687, 1488, 1467, 1403, 1368. HRMS: calc’d [M]⁺ (C₁₁H₂₁NO₄): 231.1471. Found: (EI) 231.1464.

Synthesis of compound 4.8b: The same procedure described above for the preparation of compound 4.8a was followed except that 4.7b was used as a starting material. The product was purified by column chromatography (5:1 cyclohexane:EtOAc) to provide 4.8b (0.122 g, 99%) as a thick, colorless oil.

¹H NMR (CDCl₃): δ 10.77 (s, 1H), 5.80 – 5.67 (m, 1H), 5.14 – 5.10 (m, 2H), 3.40 – 3.16 (m, 2H), 2.81 (s, 3H), 2.46 – 2.35 (m, 2H), 2.33 – 2.22 (m, 1H), 1.90 – 1.78 (m, 1H), 1.75 – 1.64 (m, 1H), 1.43 (s, 9H). IR (cm⁻¹): 3450, 2980, 2941, 1700, 1670, 1489, 1457, 1401, 1368. HRMS: calc’d [M]⁺ (C₁₃H₂₃NO₄): 257.1627. Found: (EI) 257.1634.

Synthesis of compound 4.8c: The same procedure described above for the preparation of compound 4.8a was followed except that 4.7c was used as a starting material. The product was purified by column chromatography (3:1 cyclohexane:EtOAc) to provide
4.8c (0.143 g, 96%) as a thick, colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 10.90 (s, 1H), 7.34 – 7.15 (m, 5H), 3.45 – 3.19 (m, 2H), 3.14 – 3.00 (m, 1H), 2.84 – 2.73 (m, 1H), 2.77 (s, 3H), 2.73 – 2.64 (m, 1H), 1.94 – 1.82 (m, 1H), 1.78 – 1.67 (m, 1H), 1.54 – 1.34 (m, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 180.2, 156.3, 138.6, 128.8, 128.4, 126.5, 80.5, 47.1 & 46.6 (rotamers), 44.4, 38.0, 34.1, 29.0, 28.2. IR (cm$^{-1}$): 3092, 3180, 3067, 3032, 2980, 2938, 1735, 1700, 1667, 1488, 1456, 1404, 1368. HRMS: calc’d [M]$^+$ (C$_{17}$H$_{25}$NO$_4$): 307.1784. Found: (EI) 307.1783.

Synthesis of compound 4.9a and general pivaloyl chloride-mediated esterification procedure: Acid 4.8a (0.181 g, 0.783 mmol) was dissolved in CH$_2$Cl$_2$ (8 mL). Freshly distilled NEt$_3$ (0.27 mL, 1.94 mmol) and pivaloyl chloride (0.12 mL, 0.975 mmol) were added and the solution was stirred for 30 min. Phenol (0.115 g, 1.22 mmol) and DMAP (0.012 g, 0.0982 mmol) were added and the solution was stirred overnight. The solution was poured into 1 M HCl and the product was extracted three times into CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. The crude material was purified by column chromatography (9:1 cyclohexane:EtOAc) to provide 4.9a (0.187 g, 78%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.39 (dd, J = 7.8 & 8.2 Hz, 2H), 7.23 (dd, J = 7.0 & 7.4 Hz, 1H), 7.09 (d, J = 7.8 Hz, 2H), 3.37 – 3.23 (m, 2H), 2.88 (s, 3H), 2.70 (sextet, J = 7.0 Hz, 1H), 2.16 – 2.03 (m, 1H), 1.80 – 1.67 (m, 1H), 1.47 (s, 9H), 1.36 (d, J = 7.0 Hz, 3H). $^{13}$C NMR (CDCl$_3$): $\delta$ 174.9, 155.6, 150.7, 129.3, 125.6, 121.4, 79.3, 46.7 & 46.3 (rotamers), 37.0, 31.6 & 31.0 (rotamers), 28.3, 17.1. IR (cm$^{-1}$): 3040, 2979, 2940, 1758, 1695, 1495, 1481, 1398, 1366. HRMS: calc’d [M+H]$^+$ (C$_{17}$H$_{26}$NO$_4$): 308.1856. Found: (EI) 308.1860.

Synthesis of compound 4.9b: The same procedure described above for the preparation of compound 4.9a was followed except that 4.8b was used as a starting material. The product was purified by column chromatography (93:7 cyclohexane:EtOAc) to provide 4.9b (0.128 g, 86%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.37 (t, J = 7.4 Hz, 2H), 7.22 (t, J = 7.4 Hz, 1H), 7.08 (d, J = 7.8 Hz, 2H), 5.94 – 5.80 (m, 1H), 5.22 – 5.08 (m, 2H), 3.48 – 3.21 (m, 2H), 2.87 (s, 3H), 2.75 – 2.65 (m, 1H), 2.60 – 2.39 (m, 2H), 2.04 (sextet, J = 7.4 Hz, 1H), 1.80 (sextet, J = 7.4 Hz, 1H), 1.46 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 173.4, 155.6, 150.6, 134.7, 129.3, 125.7, 121.5, 117.5, 79.5, 47.0 & 46.4 (rotamers), 42.5, 36.5,
Synthesis of compound 4.9c: The same procedure described above for the preparation of compound 4.9a was followed except that 4.8c was used as a starting material. The product was purified by column chromatography (93:7 cyclohexane:EtOAc) to provide 4.9c (0.146 g, 82%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.33 – 7.26 (m, 4H), 7.25 – 7.19 (m, 3H), 7.15 (t, $J = 7.4$ Hz, 1H), 6.85 (d, $J = 7.4$ Hz, 2H), 3.52 – 3.18 (m, 2H), 3.13 – 2.84 (m, 3H), 2.81 (s, 3H), 2.04 (sextet, $J = 7.8$ Hz, 1H), 1.89 – 1.73 (m, 1H), 1.52 – 1.35 (m, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 173.5, 155.6, 150.4, 138.5, 129.2, 129.0, 128.4, 126.6, 125.7, 121.4, 79.4, 47.1 & 46.3 (rotamers), 45.0, 38.5, 34.1, 29.8, 28.3. IR (cm$^{-1}$): 3091, 3068, 3033, 2993, 2978, 2931, 2867, 1756, 1696, 1594, 1494, 1481, 1396, 1367. HRMS: calc’d [M$^+$] (C$_{19}$H$_{27}$NO$_4$): 333.1940. Found: (EI) 333.1932.

Synthesis of compound 4.10a: A flask was charged with freshly distilled NH(iPr)$_2$ (0.25 mL, 1.77 mmol) and THF (1 mL), and the solution was cooled to -78 °C. Butyllithium (2.5 M solution in hexane, 0.69 mL, 1.73 mmol) was added and the solution was stirred for 15 min. This solution was then transferred via canula to a flask containing 4.7a (0.165 g, 0.574 mmol) and LiCl (0.041 g, 0.967 mmol) in THF (2 mL) and the solution was stirred for 15 min. MeI (0.22 mL, 3.53 mmol) was added dropwise and the solution was stirred overnight. The reaction mixture was then poured into 1 M HCl, and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The product was further purified by column chromatography (19:1 cyclohexane:EtOAc) to afford 4.10a (0.165 g, 95%) as a pale yellow oil. $^1$H NMR (CDCl$_3$): $\delta$ 3.25 – 3.12 (m, 2H), 2.84 (s, 3H), 1.75 – 1.66 (m, 2H), 1.46 (s, 9H), 1.45 (s, 9H), 1.60 (s, 6H). $^{13}$C NMR (CDCl$_3$): $\delta$ 176.6, 155.5, 79.9, 79.1, 45.4, 41.2, 37.8, 33.9, 28.4, 27.9, 25.1. IR (cm$^{-1}$): 2979, 2953, 1700, 1654, 1480, 1458, 1394, 1367. HRMS: calc’d [M$^+$] (C$_{16}$H$_{31}$NO$_4$): 301.2253. Found: (EI) 301.2246.

Synthesis of compound 4.10b and general dialkylation procedure: Compound 4.6b (0.232 g, 0.849 mmol) was dissolved in THF (4 mL) and the solution was cooled to -78
°C. LHMDS (1.0 M solution in THF, 0.85 mL, 0.85 mmol) was added slowly and the solution was stirred for 15 min. Allyl bromide (0.074 mL, 0.855 mmol) was added dropwise, and the solution was stirred 1 h. A second addition of LHMDS (1.70 mL, 1.70 mmol) and allyl bromide (0.13 mL, 1.73 mmol) was performed and the solution was stirred overnight. The solution was poured into 1 M HCl and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed in vacuo. Further purification by column chromatography (97:3 cyclohexane:EtOAc) afforded 4.10b (0.280 g, 93%) as a clear, colorless oil. ¹H NMR (CDCl₃): δ 5.83 – 5.66 (m, 2H), 5.15 – 5.05 (m, 4H), 3.27 – 3.10 (m, 2H), 2.82 (s, 3H), 2.30 (d, J = 7.4 Hz, 4H), 1.76 – 1.68 (m, 2H), 1.46 (s, 18H). ¹³C NMR (CDCl₃): δ 174.4, 155.4, 133.4, 118.3, 80.6, 79.3, 47.7, 44.6 & 44.2 (rotamers), 39.0, 34.0, 32.2 & 31.8 (rotamers), 28.4, 28.0. IR (cm⁻¹): 3081, 2979, 2936, 1723, 1483, 1458, 1393, 1367. HRMS: calc’d [M]⁺ (C₂₀H₃₅NO₄): 353.2566. Found: (EI) 353.3557.

Synthesis of compound 4.10c: The same procedure described above for the preparation of compound 4.10b was followed except that benzyl bromide was used as the alkyl halide. The product was purified by column chromatography (93:7 cyclohexane:EtOAc) to provide 4.10c (0.384 g, 93%) as a thick, colorless oil. ¹H NMR (CDCl₃): δ 7.27 – 7.14 (m, 10H), 3.50 – 3.30 (br m, 2H), 3.02 (d, J = 14.1 Hz, 2H), 2.83 (d, J = 14.1 Hz, 2H), 2.66 (s, 3H), 1.78 – 1.65 (br m, 2H), 1.45 (s, 9H), 1.34 (s, 9H). ¹³C NMR (CDCl₃): δ 174.5, 155.5, 137.3, 130.4, 128.0, 126.4, 81.1, 79.5, 50.2, 44.6 & 43.9 (rotamers), 42.0, 33.6, 31.1 & 30.4 (rotamers), 28.5, 27.9. IR (cm⁻¹): 3088, 3065, 3032, 3006, 2978, 2935, 1698, 1496, 1482, 1455, 1395, 1366. HRMS: calc’d [M]⁺ (C₂₈H₃₉NO₄): 453.2879 Found: (EI) 453.2866.

Synthesis of compound 4.10d: Compound 4.6b (0.142 g, 0.519 mmol) was dissolved in THF (25 mL) and the solution was cooled to -78 °C. LHMDS (1.0 M soln in THF, 0.84 mL, 0.84 mmol) was added and the solution was stirred for 15 min. 1,4-Dibromobutane (0.070 mL, 0.586 mmol) was added dropwise and the solution was stirred 30 min. A second equivalent of LHMDS was added and the solution was stirred overnight. The reaction mixture was poured into 1 M HCl and the product was extracted three times into EtOAc. The combined organic layers were dried over MgSO₄, filtered, and the solvent
was removed in vacuo. Purification by column chromatography (98:2 cyclohexane:EtOAc followed by 97:3 cyclohexane:EtOAc) yielded 4.10d (0.078 g, 46%) as a clear, colorless oil. 

\[ \text{\textsuperscript{1}H NMR (CDCl}_3\text{)}: \delta 3.22 - 3.08 \text{(m, 2H), 2.83 \text{(s, 3H), 2.14 - 2.02 \text{(m, 2H), 1.86 - 1.74 \text{(m, 2H), 1.69 - 1.58 \text{(m, 4H), 1.53 - 1.39 \text{(m, 2H), 1.46 \text{(s, 9H), 1.45 \text{(s, 9H).}}} \]

\[ \text{\textsuperscript{13}C NMR (CDCl}_3\text{)}: \delta 176.3, 155.4, 79.8, 79.0, 52.7, 46.2 \& 45.9 \text{(rotamers), 35.6, 36.1, 34.0, 28.4, 27.9, 24.8. IR (cm}\text{-1\text{)}: 2976, 2953, 2875, 1724, 1701, 1482, 1456, 1393, 1367. HRMS: calc’d [M+H]\text{+} (C\text{18H}_34\text{NO}_4): 328.2482. Found: (EI) 328.2482.

### Synthesis of compound 4.11a:

The same procedure described above for the preparation of compound 4.8a was followed except that 4.10a was used as a starting material. The product was purified by column chromatography (85:15 cyclohexane:EtOAc) to provide 4.11a (0.166 g, 97%) as a thick, colorless oil. 

\[ \text{\textsuperscript{1}H NMR (CDCl}_3\text{)}: \delta 3.24 \text{(br t, J = 7.8 Hz, 2H), 2.83 \text{(s, 3H), 1.82 - 1.74 \text{(m, 2H), 1.46 \text{(s, 9H), 1.25 \text{(s, 6H).}}} \]

\[ \text{\textsuperscript{13}C NMR (CDCl}_3\text{)}: \delta 182.9, 155.6, 132.6, 118.8, 80.3, 47.6, 38.7, 34.1, 32.1, 28.3. IR (cm\text{-1\text{)}: 3430, 3270, 3080, 3010, 2980, 2935, 1730, 1700, 1488, 1454, 1404, 1368. HRMS: calc’d [M]\text{+} (C\text{12H}_23\text{NO}_4): 245.1627. Found: (EI) 245.1618. \]

### Synthesis of compound 4.11b:

The same procedure described above for the preparation of compound 4.8a was followed except that 4.10b was used as a starting material. The product was purified by column chromatography (5:1 cyclohexane:EtOAc) to provide 4.11b (0.092 g, 90%) as a thick, colorless oil. 

\[ \text{\textsuperscript{1}H NMR (CDCl}_3\text{)}: \delta 5.84 - 5.70 \text{(m, 2H), 5.19 - 5.11 \text{(m, 4H), 3.25 \text{(br t, J = 7.4 Hz, 2H), 2.82 \text{(s, 3H), 2.38 \text{(d, J = 7.4 Hz, 4H), 1.84 - 1.76 \text{(m, 2H), 1.46 \text{(s, 9H).}}} \]

\[ \text{\textsuperscript{13}C NMR (CDCl}_3\text{)}: \delta 181.0, 155.6, 132.6, 118.8, 80.3, 47.6, 38.7, 34.1, 32.1, 28.3. IR (cm\text{-1\text{)}: 3430, 3270, 3080, 3010, 2980, 2935, 1730, 1700, 1488, 1454, 1404, 1368. HRMS: calc’d [M]\text{+} (C\text{16H}_27\text{NO}_4): 297.1940. Found: (EI) 297.1949. \]

### Synthesis of compound 4.11c:

The same procedure described above for the preparation of compound 4.8a was followed except that 4.10c was used as a starting material. The product was purified by column chromatography (85:15 cyclohexane:EtOAc) to provide 4.11c (0.139 g, 94%) as a sticky white solid. 

\[ \text{\textsuperscript{1}H NMR (CDCl}_3\text{)}: \delta 7.33 - 7.19 \text{(m, 10H), 3.55 - 3.31 \text{(m, 2H), 3.13 \text{(d, J = 13.7 Hz), 2.93 \text{(d, J = 14.0 Hz), 2.69 \text{(s, 3H), 1.80 - 1.70 \text{(m, 1H).}}} \]
(m, 2H), 1.47 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 181.2, 155.9, 136.8, 130.2, 128.2, 126.8, 80.1, 50.3, 44.8 & 43.9 (rotamers), 42.1, 43.7, 30.6 & 29.4 (rotamers), 28.5. IR (cm$^{-1}$): 3460, 3210, 3090, 3067, 3031, 2979, 2935, 1697, 1665, 1497, 1456, 1404, 1368. HRMS: calc’d [M]$^+$ (C$_{24}$H$_{31}$NO$_4$): 397.2253. Found: (EI) 397.2241.

**Synthesis of compound 4.11d:** The same procedure described above for the preparation of compound 4.8a was followed except that 4.10d was used as a starting material. The product was purified by column chromatography (85:15 cyclohexane:EtOAc) to provide 4.11d (0.095 g, 88%) as a thick, colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 3.30 – 3.13 (m, 2H), 2.83 (s, 3H), 2.22 – 2.10 (m, 2H), 1.92 – 1.80 (m, 2H), 1.76 – 1.63 (m, 4H), 1.62 – 1.50 (m, 2H), 1.45 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 182.4, 155.5, 79.3, 51.7, 46.2 & 45.7 (rotamers), 36.4, 36.1, 34.0, 28.3, 24.9. IR (cm$^{-1}$): 3470, 2975, 2876, 1698, 1674, 1468, 1454, 1403, 1368. HRMS: calc’d [M]$^+$ (C$_{14}$H$_{25}$NO$_4$): 271.1784. Found: (EI) 271.1776.

**Synthesis of compound 4.13a and general Ghosez reagent-mediated esterification procedure:** Compound 4.11a (0.153 g, 0.624 mmol) was dissolved in CH$_2$Cl$_2$ (6 mL). 1-Chloro-$N,N,2$-trimethylpropenylamine (0.12 mL, 0.907 mmol) was added and the solution was stirred for 1 h. Phenol (0.118 g, 1.25 mmol) and distilled NEt$_3$ (0.18 mL, 1.29 mmol) were added and the solution was stirred overnight. The reaction mixture was poured into 1 M HCl and the product was extracted three times into CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. Purification by column chromatography (97:3 cyclohexane:EtOAc) yielded 4.13a (0.181 g, 90%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.39 (dd, J = 7.8 & 8.2 Hz, 2H), 7.25 (dd, J = 8.2 & 7.4 Hz, 1H), 7.07 (d, J = 7.8 Hz, 2H), 3.37 – 3.29 (m, 2H), 2.88 (s, 3H), 1.97 – 1.87 (m, 2H), 1.47 (s, 9H), 1.38 (s, 6H). $^{13}$C NMR (CDCl$_3$): $\delta$ 175.7, 155.4, 150.8, 129.3, 125.6, 121.4, 79.3, 45.3 & 45.0 (rotamers), 41.1, 37.8 & 37.0 (rotamers), 34.1, 28.4, 25.1. IR (cm$^{-1}$): 3107, 3078, 3073, 2978, 2934, 2898, 1752, 1699, 1594, 1494, 1485, 1471, 1428, 1394, 1368. HRMS: calc’d [M+H]$^+$ (C$_{18}$H$_{28}$NO$_4$): 322.2013. Found: (EI) 322.2013.

**Synthesis of compound 4.13b:** The same procedure described above for the preparation of compound 4.13a was followed except that 4.11b was used as a starting material. The
product was purified by column chromatography (97:3 cyclohexane:EtOAc) to provide 4.13b (0.085 g, 74%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.38 (t, $J = 7.4$ Hz, 2H), 7.23 (t, $J = 7.4$ Hz, 1H), 7.06 (br d, $J = 7.4$ Hz, 2H), 5.94 – 5.78 (m, 2H), 5.26 – 5.16 (m, 4H), 3.42 – 3.26 (m, 2H), 2.86 (s, 3H), 2.52 (d, $J = 7.8$ Hz, 4H), 1.98 – 1.87 (m, 2H), 1.47 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 174.0, 155.5, 150.7, 132.8, 129.4, 125.8, 121.6, 119.1, 79.6, 48.0, 44.7 & 44.3 (rotamers), 39.1, 34.1, 32.2 & 31.9 (rotamers), 28.5. IR (cm$^{-1}$): 3080, 2979, 2934, 1752, 1697, 1642, 1594, 1494, 1457, 1396, 1367. HRMS: calc’d [M]$^+$ (C$_{22}$H$_{31}$NO$_4$): 373.2253. Found: (EI) 373.2267.

**Synthesis of compound 4.13c:** The same procedure described above for the preparation of compound 4.13a was followed except that 4.11c was used as a starting material. The product was purified by column chromatography (97:3 cyclohexane:EtOAc) to provide 4.13c (0.092 g, 60%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.44 – 7.26 (m, 12H), 7.23 (t, $J = 7.4$ Hz, 1H), 6.88 (d, $J = 7.4$ Hz), 3.66 – 3.44 (m, 2H), 3.28 (d, $J = 13.7$ Hz, 2H), 3.05 (d, $J = 14.0$ Hz, 2H), 2.73 (s, 3H), 2.02 – 1.86 (m, 2H), 1.49 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 173.9, 155.5, 150.5, 136.8, 130.3, 129.3, 128.3, 126.9, 125.8, 121.4, 79.6, 50.8, 44.7 & 43.7 (rotamers), 42.3, 36.7, 30.5 & 29.5 (rotamers), 28.5. IR (cm$^{-1}$): 3091, 3066, 3032, 2980, 2934, 2874, 1750, 1693, 1594, 1495, 1456, 1398, 1367. HRMS: calc’d [M]$^+$ (C$_{30}$H$_{35}$NO$_4$): 473.2566. Found: (EI) 473.2557.

**Synthesis of compound 4.13d:** The same procedure described above for the preparation of compound 4.13a was followed except that 4.11d was used as a starting material. The product was purified by column chromatography (98:2 cyclohexane:EtOAc followed by 97:3 cyclohexane:EtOAc) to provide 4.13d (0.135 g, 76%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.38 (t, $J = 7.8$ Hz, 2H), 7.22 (t, $J = 7.4$ Hz, 1H), 7.07 (br d, $J = 7.4$ Hz, 2H), 3.38 – 3.24 (m, 2H), 2.87 (s, 3H), 2.38 – 2.26 (m, 2H), 2.00 (br t, $J = 7.8$ Hz, 2H), 1.83 – 1.60 (m, 6H), 1.46 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 175.8, 155.5, 150.9, 129.3, 125.3, 121.4, 79.4, 52.3, 46.3 & 45.9 (rotamers), 36.6 & 36.2 (rotamers), 36.4, 34.2, 28.4, 25.0. IR (cm$^{-1}$): 3103, 3095, 3047, 2974, 2934, 2878, 1750, 1699, 1597, 1494, 1458, 1399, 1367. HRMS: calc’d [M+H]$^+$ (C$_{20}$H$_{30}$NO$_4$): 348.2169. Found: (EI) 348.2169.
Synthesis of compound 4.15: Compound 4.14\textsuperscript{54} (3.31 g, 15.1 mmol) was dissolved in DMF (150 mL). Cs\textsubscript{2}CO\textsubscript{3} (6.17 g, 18.9 mmol) was added, and then MeI (0.99 mL, 15.9 mmol) was added slowly and the solution was stirred for 90 min. The reaction mixture was poured into 1:1 H\textsubscript{2}O:saturated brine, and the product was extracted three times into CH\textsubscript{2}Cl\textsubscript{2}. The combined organic layers were dried over MgSO\textsubscript{4}, filtered, and the solvent was removed in vacuo. Column chromatography (7:3 cyclohexane:EtOAc followed by 1:1 cyclohexane:EtOAc) yielded 4.15 (3.05 g, 87%) as a yellow oil.

\[ ^{1}H\text{ NMR (CDCl}_3\text{): } \delta 4.89 (\text{br s, 1H}), 4.26 (\text{dd, } J = 3.9 \text{ & } 8.1 \text{ Hz, 1H}), 3.79 (\text{s, 3H}), 3.43 \text{ – } 3.20 (\text{m, 2H}), 2.07 \text{ – } 1.96 (\text{m, 1H}), 1.89 \text{ – } 1.77 (\text{m, 1H}), 1.44 (\text{s, 9H}). \]

\[ ^{13}C\text{ NMR (CDCl}_3\text{): } \delta 174.9, 156.2, 79.1, 68.4, 52.1, 36.5, 33.7, 28.1. \]

IR (cm\textsuperscript{-1}): 3390, 2980, 2952, 1754, 1697, 1527, 1455, 1394, 1368. HRMS: calc’d [M+H]\textsuperscript{+} (C\textsubscript{10}H\textsubscript{20}NO\textsubscript{5}): 234.1336. Found (EI) 234.1335.

Synthesis of compound 4.16a: Compound 4.15 (0.491 g, 2.11 mmol) was dissolved in THF (20 mL). Di-tert-butyldicarbonate (0.577 g, 2.64 mmol) and DMAP (0.030 g, 0.25 mmol) were added and the solution was stirred for 1 h. The solution was then poured into 1 M HCl and the product was extracted three times into CH\textsubscript{2}Cl\textsubscript{2}. The combined organic layers were dried over MgSO\textsubscript{4}, filtered, and the solvent was removed in vacuo. Purification by column chromatography (9:1 cyclohexane:EtOAc) afforded 4.16a (0.635 g, 91%) as a thick, colorless oil.

\[ ^{1}H\text{ NMR (CDCl}_3\text{): } \delta 4.90 (\text{br s, 1H}), 4.80 (\text{dd, } J = 5.0 \text{ & } 7.0 \text{ Hz, 1H}), 3.64 (\text{s, 3H}), 3.23 \text{ – } 3.04 (\text{m, 2H}), 2.02 \text{ – } 1.83 (\text{m, 2H}), 1.37 (\text{s, 9H}), 1.31 (\text{s, 9H}). \]

\[ ^{13}C\text{ NMR (CDCl}_3\text{): } \delta 170.3, 155.4, 152.5, 82.7, 78.9, 72.0, 52.0, 36.2, 31.1, 28.1, 27.3. \]

IR (cm\textsuperscript{-1}): 3408, 2982, 2958, 1749, 1718, 1521, 1458, 1369. HRMS: calc’d [M+H]\textsuperscript{+} (C\textsubscript{15}H\textsubscript{28}NO\textsubscript{7}): 334.1866. Found: (EI) 334.1874.

Synthesis of compound 4.16b: Compound 4.16a (0.133 g, 0.399 mmol) was dissolved in DMF (2 mL), and the solution was cooled to 0 °C. MeI (0.25 mL, 4.01 mmol) was added, and NaH (0.010 g, 0.429 mmol) suspended in DMF (0.5 mL) was added dropwise to the reaction mixture. The solution was stirred for 1 h and then a second equivalent of NaH was added as above and the solution was stirred a second hour. The reaction mixture was then poured into 1:1 1 M HCl:saturated brine, and the product was extracted three times into CH\textsubscript{2}Cl\textsubscript{2}. The combined organic layers were dried over MgSO\textsubscript{4}, filtered, and the solvent was removed in vacuo. Further purification by column chromatography
(96:4 cyclohexane:EtOAc) yielded 4.16b (0.103 g, 74%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 4.81 (dd, $J = 4.3 \& 8.6$ Hz, 1H), 3.71 (s, 3H), 3.56 – 3.31 (m, 1H), 3.26 – 3.15 (m, 1H), 2.80 (s, 3H), 2.14 – 1.89 (m, 2H), 1.44 (s, 9H), 1.40 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 170.4, 155.4, 152.7, 82.9, 79.5, 72.0, 52.2, 44.9, 34.5 & 34.2 (rotamers), 29.5 & 29.1 (rotamers), 28.2, 27.5. IR (cm$^{-1}$): 2981, 2937, 1748, 1700, 1483, 1460, 1396, 1369. HRMS: calc’d [M+H]$^+$ (C$_{16}$H$_{30}$NO$_7$): 348.2017. Found: (EI) 348.2015.

**Synthesis of compound 4.17a and general procedure for conversion from a methyl to phenyl ester:** Under an air atmosphere, a flask was charged with 4.16a (0.168 g, 0.503 mmol) and the material was dissolved in 1:1 THF:H$_2$O (5 mL). LiOH·H$_2$O (0.0264 g, 0.629 mmol) was added, and the solution was stirred overnight. The reaction mixture was then poured into 1:1 1 M HCl:saturated brine and the product was extracted three times into CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The flask was then fully evacuated, refilled with N$_2$, and the material was dissolved in CH$_2$Cl$_2$ (5 mL). Pivaloyl chloride (0.077 mL, 0.626 mmol) and NEt$_3$ (0.18 mL, 1.29 mmol) were added and the solution was stirred for 30 min. Phenol (0.0724 g, 0.769 mmol) and DMAP (0.0073 g, 0.060 mmol) were then added and the solution was stirred overnight. The reaction mixture was poured into 1 M HCl and the product was extracted three times into CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The crude material was purified by column chromatography (9:1 cyclohexane:EtOAc) to yield 4.17a (0.140 g, 70%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.37 (t, $J = 7.4$ Hz, 2H), 7.23 (t, $J = 7.4$ Hz, 1H), 7.11 (d, $J = 7.8$ Hz, 2H), 5.11 (dd, $J = 5.1 \& 7.4$ Hz, 1H), 4.84 (br s, 1H), 3.45 – 3.27 (m, 2H), 2.31 – 2.13 (m, 2H), 1.51 (s, 9H), 1.44 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 168.8, 155.7, 152.8, 150.1, 129.4, 126.1, 121.2, 83.3, 79.4, 72.3, 36.5, 31.3, 28.3, 27.6. IR (cm$^{-1}$): 3460, 2983, 2962, 1747, 1695, 1653, 1521, 1495, 1369. HRMS: calc’d [M+H]$^+$ (C$_{20}$H$_{30}$NO$_7$): 396.2017. Found: (EI) 396.2016.

**Synthesis of compound 4.17b:** The same procedure described above for the preparation of compound 4.17a was followed except that 4.16b was used as a starting material. The product was purified by column chromatography (93:7 cyclohexane:EtOAc) to provide 4.17b (0.096 g, 58%) as a colorless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.38 (t, $J = 7.8$ Hz, 2H),
7.23 (t, J = 7.4 Hz, 1H), 7.11 (d, J = 7.8 Hz, 2H), 5.05 (dd, J = 4.3 & 8.6 Hz, 1H), 3.64 – 3.48 (m, 1H), 3.43 – 3.29 (m, 1H), 2.89 (s, 3H), 2.38 – 2.13 (m, 2H), 1.51 (s, 9H), 1.46 (s, 9H). $^{13}$C NMR (CDCl$_3$): δ 168.7, 155.6, 152.8, 150.2, 129.5, 126.1, 121.2, 83.3, 79.8, 72.2, 45.0, 34.5, 29.7, 28.4, 27.7. IR (cm$^{-1}$): 3120, 3084, 3024, 3008, 2982, 2938, 2898, 1778, 1747, 1698, 1593, 1493, 1476, 1396, 1369. HRMS: calc’d [M+H]$^+$ (C$_{21}$H$_{31}$NO$_7$): 410.2173. Found: (EI) 410.2173.

**Kinetic Studies:** Absorption spectra for phenol and 4.1a-TFA were obtained by preparing a 0.1 mg/mL solution of each in 1 M HCl and measuring the absorbance between 320 and 230 nm, and the molar extinction coefficients were calculated using the Beer-Lambert equation, $A = \varepsilon c\ell$. To measure the cyclization rate, the Boc protected compound was dissolved in 1:1 TFA:CH$_2$Cl$_2$ (approximately 4 mL) and the resulting solution was stirred for 2 h. The solvent was removed in vacuo, and CH$_2$Cl$_2$ was added and removed three times to remove residual TFA, after which the flask was fully evacuated. The material was then suspended in H$_2$O, frozen in liquid nitrogen, and dried on a lyophilizer. 1.5 mg of the target 4.1a-k was then dissolved in iPrOH (0.2 mL) and the solution was transferred to the cuvette and preheated within the spectrometer to 37 °C. To this solution was then added, with stirring, pH 7.4 0.1 M phosphate buffer solution (1.8 mL), preheated to 37 °C. The change in absorbance at 276 nm with respect to time was measured. Due to the rapid rates of cyclization observed for certain spacers, the measurement of absorbance values at 276 nm was started immediately prior to the addition of phosphate buffer solution, and was continued throughout the course of the reaction. The value of $t = 0$ was taken to be the point of onset of steady increase of absorbance, and this time was taken as 0% conversion, while the absorbance value after the absorbance had stabilized was taken as 100% conversion (verified by NMR). From this, the % conversion with respect to time was calculated. To obtain the first order rate constants, ln[A]$_0$/[A] versus time was plotted where [A]$_0$/[A] effectively corresponds to 100/(100-% conversion). Phosphate buffers (0.1 M) were used for pHs 6.0, 7.0, and 7.4. Acetate buffers (0.1 M) were used for pHs 4.0 and 5.0.
4.5 References

Progress Toward Rapidly Degrading Self-Immolative Polymers Based on 4-Aminobutyric Acid

5.1 Introduction

The development of self-immolative polymers is a new and promising field of research.\textsuperscript{1-8} Originally inspired by self-immolative dendrimers that fragmented via a series of intramolecular reactions, resulting in the release of molecules on the dendrimer periphery,\textsuperscript{9-14} these polymers are the most recent advancement in the study and application of self-immolative spacers. Self-immolative polymers offer numerous unique and attractive features, making them valuable synthetic targets. First, they can be accessed in an efficient manner, with commercially available materials often requiring just a few functional group manipulations to be converted into polymerizable monomers. Once prepared, these monomers can then be polymerized in a single step to afford the desired target. This offers a significant improvement over stepwise, iterative dendrimer synthesis, which can be costly and time-consuming. Second, it has been demonstrated that it is possible to obtain supramolecular structures,\textsuperscript{4,6,7} such as microcapsules or micelles with these materials.\textsuperscript{4,6} These structures could then be non-covalently loaded with a small molecule payload, which could be subsequently released upon polymer degradation.\textsuperscript{4,6} Third, a variety of end caps have been reported,\textsuperscript{1-8} which allow polymer degradation to be initiated in a variety of ways, such as in acidic or basic media,\textsuperscript{1,3,6,8} via enzymatic or hydrolytic cleavage,\textsuperscript{2,4} or under reducing conditions.\textsuperscript{5} Such diversity allows the researcher to tailor the polymer to best suit the desired application.

While such diversity exists in the design and incorporation of end caps, there is surprisingly little variety in the self-immolative spacers that have been employed in self-immolative polymers. By using different spacers, one can change the physical properties, such as the physical form of the polymer (crystalline, globular, etc), solubility in different media, and the possible formation of supramolecular structures. In addition, differences in monomer degradation kinetics should allow access to a wide range of polymer
degradation timelines. The majority of reports thus far have utilized derivatives of 4-aminobenzyl alcohol.\textsuperscript{2,3,6} While these spacers have been modified to form cross-linked microcapsules,\textsuperscript{6} release reporter molecules\textsuperscript{3} or act as fluorescent probes themselves,\textsuperscript{2} the core aminobenzyl alcohol structure, and therefore the depolymerization mechanism, remain the same.

In an effort to explore this dimension and gain access to a rich variety of polymer backbones, our group has focused on the design and syntheses of heterodimeric repeat units as polymerizable monomers. Two examples have been reported thus far that use \(N,N'\)-dimethylethylenediamine in conjunction with first 4-hydroxybenzyl alcohol\textsuperscript{4} and then 2-mercaptoethanol (Chapters 2 and 3).\textsuperscript{5} This led to two polymeric structures with distinctly different physical properties, as well as degradation profiles. Although these polymers possessed numerous attractive features, they both suffered from the limitation of relying on the relatively slow cyclization of the diamine spacer, which resulted in degradation times ranging from 6 – 10 days in a mixed acetone/aqueous phosphate buffer solvent system and up to a month at the core of a degradable micelle in pure phosphate buffer.

While these are certainly useful for slow release applications, it was desirable to gain access to more rapidly degrading polymers. To this end, our lab developed and tested a series of self-immolative spacers based on 4-aminobutyric acid (Chapter 4).\textsuperscript{15} By introducing substituents at the amine and at the \(\alpha\) position, 11 different spacers were synthesized and demonstrated to cyclize on a phenyl ester very rapidly, with half-lives ranging from 2 – 39 s (Figure 5.1a). When compared to \(N,N'\)-dimethylethylenediamine, reported to cyclize on a 4-methoxyphenyl carbamate with a half-life of 36 min in pH 7.4 phosphate at 37 °C (Figure 5.1b),\textsuperscript{16} and found in our previous work (Chapter 2) to have a similar rate when derivatized with a hydroxymethyl carbamate and studied in a mixed acetone-phosphate solution (Figure 5.1c), the 4-aminobutyric acid derivatives exhibited between 55 and over 1000-fold increases in cyclization rates. This was a very promising result, and based on this it was hypothesized that these would be excellent candidates as spacers in new, rapidly degrading polymeric systems.
Figure 5.1. Comparison of half-lives of cyclization for a) 4-aminobutyric acid phenyl ester derivatives, and b) and c) dimethylethlenediamine aryl carbamates.

With these spacers in hand, two new general polymeric structures were envisioned comprising heterodimeric repeat units with a 4-aminobutyric acid linked to a second self-immolative spacer. The first is based on 4-hydroxybenzyl alcohol (Figure 5.2a), previously employed in our lab, and the second would employ a 2’-hydroxyhydrocinnamic acid spacer designed to undergo spontaneous cyclization (Scheme 5.1) that had been previously reported by Amsberry and Borchardt in 1990 (Figure 5.2b).\(^\text{17}\) This spacer contains a so called “trimethyl lock” system, referring to the methyl group on the phenol and the two methyl groups on the sidechain, which bias the conformation of the molecule to favour cyclization. This conformational bias proved extremely effective, as the authors demonstrated its capability to cyclize on amides with a half-life of 65 s in pH 7.5 0.3 M PBS:acetonitrile (5:1) at 30 °C, compared to a half-life of greater than 19 days for the non-methylated substrate. Based on this information, it was proposed that this 2’-hydroxyhydrocinnamic acid spacer would be an ideal partner for a 4-aminobutyric acid derivative in the development of a polymeric system. Described here is work towards the synthesis and degradation studies of these target polymers.
Figure 5.2. Proposed polymeric structures containing 4-aminobutyric acid with a) 4-hydroxybenzyl alcohol; and b) 2'-hydroxyhydrocinnamic acid.

Scheme 5.1. Degradation mechanism of polymer 5.5.

5.2 Results and Discussion

5.2.1 Design

In contrast to the heterodimers described in Chapters 2 and 3, which polymerized smoothly via nucleophilic attack of the aliphatic amine of $N,N'$-dimethylethlenediamine on the 4-nitrophenyl carbonate, there was concern that the more rapidly cyclizing 4-aminobutyric acid spacer would favour intramolecular monomer fragmentation to form the corresponding γ-lactam and hydroxybenzyl alcohol, thus preventing polymerization (Scheme 5.2). With this in mind, a flexible approach was taken toward the synthesis of both polymeric systems.
**Scheme 5.2.** Two possible scenarios where a) intermolecular substitution is faster than intramolecular cyclization, leading to polymer formation; or b) intramolecular cyclization is faster than intermolecular substitution leading monomer degradation.

**Figure 5.3.** Target monomers for alternative strategies to polymer 5.4 utilizing a) nucleophilic nitrogen and electrophilic oxygen and b) electrophilic nitrogen and nucleophilic oxygen.

Figure 5.3 illustrates the two potential monomer units envisioned for the synthesis of polymer 5.4. First, following our previous polymerization strategy, both a 4-nitrophenyl carbonate and a chloroformate were considered as activating groups for the oxygen. The nitrophenyl carbonate was chosen as this activating group has been successfully employed in our lab for polymerization, and its synthesis was expected to be straightforward. The chloroformate was proposed as a more electrophilic activating group should the nitrophenyl carbonate prove to be unable to induce polymerization in preference to the competing intramolecular cyclization reaction to form the \(\gamma\)-lactam. Alternatively, the amine could be converted to an electrophilic functionality such as a phenyl carbamate or chloroformamide, which could react with a free benzyl alcohol to generate the polymer.
For polymer 5.5, two possibilities were also considered. First, the heterodimer could be formed via an amide bond, and polymerization would then follow via esterification reactions (Scheme 5.3a). Alternatively, the ester could be made first and polymerization could be carried out via amide bond formation reactions (Scheme 5.3b).

Scheme 5.3. Alternative synthetic strategies to access polymer 5.5 utilizing a) amide heterodimer; or b) ester heterodimer.
5.2.2 Polymer 5.4

5.2.2.1 First Generation Synthesis

![Chemical structure of 5.4a]

**Figure 5.4.** First generation target polymer 5.4a.

Our target 5.4 involved the use of unsubstituted 4-aminobutyric acid in conjunction with 4-hydroxybenzyl alcohol. The rationale behind this was twofold. First, our previous studies had demonstrated that 4-aminobutyric acid can cyclize rapidly enough to be potentially useful in a polymeric system, and second, the number of synthetic steps required to access the target polymer is minimized. The synthesis, outlined in Scheme 5.4, commenced with the DCC-mediated coupling of previously reported acid 4.2 and phenol 2.6 to afford ester 5.17. Following this, the TBS protecting group was removed in acidic isopropanol, and the resultant alcohol was treated with 4-nitrophenyl chloroformate in the presence of pyridine to provide carbonate 5.9a. While model studies had revealed that a 4-nitrophenyl carbonate was less reactive than the corresponding chloroformate and would therefore suffer from increased competition from intramolecular cyclization, its facile synthesis made it a good test substrate, which could be further improved upon if necessary.

The final step prior to polymerization was removal of the Boc protecting group, achieved by treatment of 5.9a with 1:1 TFA:CH₂Cl₂. Following this, 0.05 equivalents of Boc-protected 5.9a was added to serve as an end cap, and then the material was polymerized with the addition of DIPEA and catalytic DMAP.
Scheme 5.4. First generation synthesis of polymer 5.4a.

While the results of the polymerization were not ideal, analysis of the reaction mixture proved quite instructive. As expected, there was a substantial amount of intramolecular cyclization to form γ-lactam 4.18a, demonstrating that a 4-nitrophenyl carbonate was not electrophilic enough to induce preferential intermolecular attack of the amine at that site over intramolecular attack on the ester, yet there was still a small amount of polymeric material formed. However, this material proved to be insoluble in a wide array of solvents tested. While the material obtained was not useful, it proved that polymerization was indeed possible, and with slight modification to the 4-aminobutyric acid spacer polymer 5.4 should be obtainable.

5.2.2.2 Second Generation Synthesis

Figure 5.5. Second generation target polymer 5.4b.
The first modification carried out was methylation of the amine. This methyl group was introduced with the aim of preventing hydrogen bonding, which was hypothesized to be the cause of polymer insolubility. As an added benefit, based on the cyclization kinetics described in Chapter 4, this would increase the rate of cyclization and therefore lead to faster polymer degradation times. The synthesis of the modified polymer, outlined in Scheme 5.3, was analogous to the first generation synthesis. The N-methylated acid derivative 4.3 was coupled to phenol 2.6 using DCC and catalytic DMAP to afford ester 5.19. TBS removal proceeded as previously described, revealing the benzylic alcohol 5.20. Due to the previous polymerization results with a nitrophenyl carbonate, combined with the fact that N-methyl-4-aminobutyric acid was found to cyclize substantially faster than unfunctionalized 4-aminobutyric acid, it was decided to convert the hydroxyl group into the more reactive chloroformate in an effort to increase the electrophilicity of this site and therefore bias the reaction to favour polymerization instead of the previously observed cyclization on the phenyl ester.

![Scheme 5.5. Second generation synthesis of unactivated monomer 5.20.](image)

While in theory a straightforward transformation, installation of the chloroformate proved particularly challenging with substrate 5.20, as highlighted in Scheme 5.6. Initially, standard conditions of triphosgene (5.21) and pyridine were employed, but the sole product isolated was the benzyl chloride rather than the desired chloroformate. When the reaction was carried out in the absence of base, the chloroformate was obtained as the major product along with a small amount of the undesired chloride and some starting material, but attempts to purify the material via column chromatography proved detrimental as substantial conversion to the benzyl chloride occurred. While polymerization was attempted on the reaction mixture, only oligomeric products were
obtained, likely due to the low monomer purity. Further attempts to optimize the synthesis and purification of chloroformate 5.9b proved unsuccessful.

Scheme 5.6. Attempted formation of chloroformate 5.9b.

Having been unable to produce polymer 5.4 utilizing a nucleophilic amine and electrophilic alcohol derivative, attention was turned to developing a heterodimer possessing an electrophilic amine derivative in conjunction with a nucleophilic benzyl alcohol. While previous reports involving this methodology made use of a phenyl carbamate as an electrophilic amine derivative, this approach is applicable to primary amines only, as secondary amines cannot undergo the requisite elimination to form the isocyanate. Therefore, an alternative electrophilic group was required, and a chloroformamide was selected as it had been demonstrated to be compatible with secondary amines.

The approach to this activated heterodimer began with the TBS protected ester 5.19. This material was first treated with TFA to remove the Boc group, which simultaneously removed the TBS ether and replaced it with a trifluoroacetate group. While not initially anticipated, this trifluoroacetate proved advantageous, as it allowed the next step, treatment of the amine with trichloroethylene to install the chloroformamide, to proceed without risk of competitive chloroformate formation. Following this, the trifluoroacetate was removed using NaHCO₃ in a mixture of THF and H₂O.
Unfortunately, attempts to isolate this material proved unsuccessful, as the reaction sequence led to the formation of a number of byproducts, which proved to be inseparable from the desired product on a column, and additional methods of purification led to hydrolysis of the sensitive chloroformamide functionality.

Scheme 5.7. Synthesis of chloroformamide monomer 5.10a.

Having unsuccessfully attempted two methods to generate \( N \)-methylated polymer 5.4b, this strategy was abandoned in favour of pursuing an alternative solubilizing group.

5.2.2.3 Third Generation Synthesis

Figure 5.6. Third generation target polymer 5.4c.

The third variant of polymer 5.4 involved using (S)-4-amino-2-hydroxybutyric acid as the starting material and functionalizing the 2-hydroxy group. To this end, the synthetic route outlined in Scheme 5.8 was developed. Beginning from previously reported methyl ester 4.15, the secondary alcohol was converted to the corresponding \( N,N \)-diethyl carbamate 5.25 in two steps, first by forming active carbonate 5.24 and then displacing 4-nitrophenol with diethylamine. It should be noted that while a diethyl carbamate seems
like an unusual choice for a solubilizing group, several different possible solubilizing groups including various ethers and a pivalate ester were evaluated initially and proved unsuitable. The methyl ester was then removed using LiOH to reveal the free acid, which was subsequently coupled with phenol 2.6 via a mixed anhydride to afford ester 5.27.

Scheme 5.8. Synthesis of monomer 5.10b.

In order to provide an electrophilic amine derivative and nucleophilic free alcohol, TBS protected ester 5.27 was first treated with 1:1 TFA:CH₂Cl₂ to remove the Boc group while simultaneously converting the silyl ether to a trifluoroacetate. A phenyl carbamate was then installed using phenyl chloroformate with excess NEt₃, and finally the trifluoroacetate was cleaved under mildly basic conditions using NaHCO₃ in a mixture of THF and H₂O to afford monomer 5.10b.

Before polymerization could be carried out, an end cap needed to be prepared, and for initial studies a Boc group was selected. To this end, free alcohol 5.29 was synthesized by treatment of 5.27 with dilute HCl in iPrOH to remove the silyl protecting group. With this material in hand, polymerization of phenyl carbamate 5.7b was carried
out using catalytic dibutyltin dilaurate (DBTL) in refluxing dioxane with 0.05 equivalents of 5.29 serving as the Boc end cap source (Scheme 5.9).

![Scheme 5.9](image)

**Scheme 5.9.** a) Deprotection of 5.27 to form Boc-protected end cap 5.29; and b) polymerization of monomer 5.10b in the presence of end cap 5.29.

Polymerization of the material was indeed successful; however, an unexpected product was also formed in the course of the reaction. Analysis of the $^1$H NMR spectrum revealed that in addition to the expected aromatic peaks corresponding to the ester-functionalized hydroxybenzyl alcohol, a new set of aromatic protons of approximately equal intensity were present that resembled unsubstituted hydroxybenzyl alcohol (Figure 5.7a). When the sample was doped with 4-hydroxybenzyl alcohol, however, the two signals did not overlap, indicating that the peaks observed in the polymer arise from a different, unidentified compound (Figure 5.7b). Repeated dialyses with increasing molecular weight cutoffs were carried out (Figure 5.7c), but it was found that the additional aromatic peaks persisted, indicating that this species had been incorporated into the polymeric backbone. Carrying out preliminary degradation studies, the additional aromatic peaks persisted, as shown by the overlapping doublets near 7 ppm, suggesting that this phenolic species was covalently bound to the 4-aminobutyric acid backbone in such a way that either prevented cyclization or was unaffected by it. Unfortunately, as a number of different routes to the polymer had already been unsuccessfully explored and that the source of the problem was not evident, this polymer was not explored further.
Figure 5.7. $^1$H NMR spectra of the aromatic region of polymer 5.4c a) following dialysis with 3500 MWCO; b) following addition of hydroxybenzyl alcohol; c) following dialysis with 6000-8000 MWCO; and d) after 24 h in pH 7.4 0.1 M phosphate (D$_2$O):methanol-d$_4$ (1:1) (spectrum taken in pH 7.4 0.1 M phosphate (D$_2$O):methanol-d$_4$ (1:1)).

5.2.3 Polymer 5.5

5.2.3.1 Synthesis

As described above (Scheme 5.3), two strategies were envisioned to synthesize target polymer 5.5. Initially it was thought that the strategy depicted in Scheme 5.3a would be the more successful route, as Amsberry and Borchardt had successfully employed this strategy in their syntheses of prodrugs containing a 2'-hydroxyhydrocinnamic acid self-immolative spacer. However, it was found that while the two components could be coupled, deprotection of the phenol resulted in spontaneous lactonization, with no intermediate phenol observed in the reaction mixture (Scheme 5.10). Based on this subsequent efforts were then focused on the strategy depicted in Scheme 5.3b.
Scheme 5.10. Deprotection of heterodimer 5.13 followed by spontaneous lactonization.

The synthesis of carboxylic acid 5.33, outlined in scheme 5.11, followed a similar protocol to that used by Greenwald and coworkers.\textsuperscript{18} It began with the EDC-mediated coupling of Boc protected acid 4.2 and previously reported phenol 5.15a (EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide).\textsuperscript{18} TBS deprotection of the resulting ester was achieved using 3:1:1 HOAc:THF:H\textsubscript{2}O. The free alcohol was then oxidized to a carboxylic acid in a two-step process, first converting it to aldehyde 5.32 with pyridinium chlorochromate (PCC), and then completing the oxidation with Oxone\textsuperscript{60}, affording acid 5.33.

Scheme 5.11. Synthesis of acid 5.33.
Scheme 5.12. Activation and polymerization of acid 5.33.

In order to complete the polymerization, the acid had to be first converted to an active ester. A brief screening of potential candidates revealed that a pentafluorophenyl ester was the most viable option and thus was selected for the polymerization. This group was readily installed using an EDC coupling with pentafluorophenol to form active ester 5.34. Following Boc group removal with TFA, polymerization was carried out at 0 °C in toluene with excess DIPEA, catalytic DMAP, and 0.05 equivalents of Boc protected 5.34 as an end cap. The final polymer was then purified via dialysis using a 3500 MWCO membrane.

Analysis of the purified polymer was carried out using NMR and SEC. NMR revealed that the desired end cap-to-repeat unit ratio of approximately 1:20 had been obtained, corresponding to an $M_n$ of 5900, and SEC in DMF indicated an $M_n$ of 4500, $M_w$ of 6100 and a resulting PDI of 1.4 relative to polystyrene standards.

5.2.3.2 Degradation Studies

The increased hydrophobic character of polymer 5.5b rendered it insoluble in the previously used degradation conditions of pH 7.4 0.1 M phosphate:acetone (3:2), and therefore alternative conditions needed to be employed. A mixture of pH 7.4 acetone:0.1 M HEPES buffer (3:2) was found to be the most suitable. Due to the substantial change in solvent composition relative to what was previously used for 4-aminobutyric acid cyclization studies, it was deemed prudent to perform monomer degradation kinetics under these conditions to determine what effect this had on the rate of cyclization. Additionally, it was not inherently obvious which of the cyclization reactions would be rate limiting. In order to probe this, modified monomer 5.36 was prepared by coupling
acid 5.33 with 4-aminobutyric acid methyl ester 5.12a·HCl. Once prepared, the monomer was deprotected using TFA and the resulting material was dissolved in the buffered solution and incubated at 37 °C (Scheme 5.13). Reaction progress was monitored by $^1$H NMR, measuring the formation of lactone 5.7. The results are shown in Figure 5.8.

Scheme 5.13. a) Synthesis of modified monomer 5.36; b) deprotection and degradation of monomer 5.36 into lactam 4.18a, lactone 5.7 and free amine 5.12a.
Figure 5.8. Plot of conversion of monomer 5.36 into lactone 5.7 over time.

While the data highlighted in Figure 5.8 effectively shows the timeline for complete monomer degradation, it is in fact an oversimplification of the reaction process. As shown in Scheme 5.9b, monomer 5.36 fragments into three different components, lactam 4.18a, lactone 5.7 and amine 5.12a. It is noteworthy that while in theory amine 5.12a could cyclize to generate additional 4.18a, previous studies in our lab on similar substrates have revealed this process to be slow enough that negligible conversion would be expected to occur throughout the course of this study. Based on this, the assumption was made that the percent composition of amine 5.12a would be equal to that of lactone 5.7.

To more accurately probe the reaction and follow the course of events leading to formation of the final degradation products (Scheme 5.14), a more thorough analysis of the aromatic region of the spectra was carried out, the results of which are highlighted in Figure 5.9. At t = 0, two singlets are present, arising from deprotected monomer 5.37. As the reaction progressed, two new sets of singlets began to emerge, first one set higher upfield, followed by a second set in between those assigned to 5.37. The first set, higher upfield than the other two, was presumed to from an intermediate species produced from the first cyclization event. The second set, which emerged in between the two singlets assigned to the monomer, was assigned to lactone 5.7. As shown in Figure 5.10b, after
10 h the major component in the reaction mixture was lactone 5.7, but there was still a significant amount of 5.37 as well as a small amount of phenol 5.38 present in solution. By 28 h (Figure 5.8c), only trace amounts of starting material and intermediate were detected, with the vast majority of the material having converted to the final lactone.

**Figure 5.9.** Aromatic region of the $^1$H NMR spectra of monomer degradation reaction after a) 4 min; b) 10 h; and c) 28 h showing conversion of 5.37 into phenolic intermediate 5.38 and then lactone 5.7.

**Scheme 5.14.** Degradation pathway of deprotected monomer 5.37, with the first cyclization event generating lactone 4.18a and intermediate phenol 5.38 which then undergoes a second cyclization event to form lactone 5.7 and amine 5.12a.
An analysis of the percent composition of the degradation products over time is shown in Figure 5.10. What is immediately apparent is that the cyclizations of both 4-aminobutyric acid and 2’-hydroxyhydrocinnamic acid were significantly slower than in previously reported examples. As shown, the cyclizations required more than 24 h to proceed to completion, versus just minutes for the same spacers to cyclize under previously reported conditions, namely pH 7.4 0.1 M phosphate:iPrOH (9:1) at 37 °C for 4-aminobutyric acid and pH 7.5 PBS:acetonitrile (4:1) at 30 °C for 2’-hydroxyhydrocinnamic acid. While the substrates under investigation in this experiment were not identical to the previous examples and therefore were expected to have somewhat different reaction rates, the processes themselves are fundamentally quite similar (the amine cyclizing on a phenyl ester and the phenol cyclizing on an amide), and therefore would be expected to occur at a somewhat similar rate. Because of the orders of magnitude in difference for the observed cyclizations in this study relative to previous work, it is apparent that the higher organic content in the solvent mixture played a key role in suppressing the cyclization rate of these spacers.

**Figure 5.10.** Change in percent composition of the degradation products over the course of the reaction.
Kinetic analysis of the reaction was carried out using nonlinear regression. The two rate constants were solved using the concentration of intermediate phenolic species 5.38, according to the equation \[ [\text{B}] = [\text{A}]_0 \left( \frac{k_1}{k_2} - k_1 \right) (e^{k_1 t} - e^{k_2 t}) \], where \( \text{B} \) is intermediate 5.38, \( \text{A} \) is ester 5.37, \( k_1 \) is the rate constant corresponding to the initial cyclization to form lactam 4.18a and phenol 5.38 and \( k_2 \) is the rate constant corresponding to the second cyclization to form 5.7 and 5.12a. Additionally, based on NMR data obtained for the reaction, the assumptions were made that the starting concentration of 5.38 was zero, and \( k_1 \neq k_2 \). The rate constant \( k_1 \) was calculated to be \( 3.03 \times 10^{-5} \text{s}^{-1} \), corresponding to a half-life of 6.4 h. The second rate constant was \( 8.08 \times 10^{-5} \text{s}^{-1} \), with a corresponding half-life of 2.4 h. From these values, it has been shown that the two cyclization reactions do indeed occur on a similar timeline, as was expected, and furthermore the first cyclization was demonstrated to be the rate limiting step in the overall monomer degradation process.

Due to the relatively slow kinetics of cyclization observed for monomer 5.37, it was predicted that polymer 5.5b would require a significant amount of time to degrade. A thorough kinetic study of the degradation of polymer 5.5b was not undertaken and will be the subject of ongoing investigation in the laboratory.

### 5.3 Conclusions

In conclusion, significant progress has been made toward the synthesis of self-immolative polymers containing 4-aminobutyric acid spacers. Two general polymeric structures, 5.4 and 5.5, were targeted. While 5.4 proved to be very challenging to prepare, much valuable insight was gained into the potential synthesis of this polymer, as well as other similar polymeric structures. Polymer 5.5b was successfully synthesized, and investigation of the kinetics of monomer cyclization revealed that monomer 5.37 underwent conversion to lactam 4.18a and lactone 5.7. In the solvent required for the dissolution of polymer 5.5b, the rates observed for each cyclization event were significantly slowed relative to previous studies due to the increased organic content of the solvent medium. An investigation of the kinetics of polymer degradation was not undertaken, but will be the focus of future work.
5.4 Experimental

All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Anhydrous DMF, toluene and dioxane were obtained from a solvent purification system. CH$_2$Cl$_2$, pyridine and NEt$_3$ were distilled from CaH$_2$. Unless otherwise stated, all reactions were performed under an argon atmosphere using flame or oven dried glassware. Column chromatography was performed using silica gel (0.063-0.200 mm particle size, 70-230 mesh). Thin layer chromatography was performed using Macherney-Nagel Polygram$^\text{®}$ SIL G/UV$_{254}$ plates. $^1$H NMR spectra were obtained at 400 MHz and $^{13}$C NMR spectra were obtained at 100 MHz using a Varian Inova spectrometer. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signal of CDCl$_3$ (δ 7.27, 77.00) or D$_2$O (δ 4.75). Coupling constants (J) are expressed in Hertz (Hz). Infrared spectra were obtained as films from CH$_2$Cl$_2$ on NaCl plates using a Bruker Tensor 27 instrument. High-resolution mass spectrometry (HRMS) was performed using a Finnigan MAT 8400 electron impact (EI) or a Micromass LCT electrospray ionization time-of-flight (ESI) mass spectrometer. Size exclusion chromatography was carried out at a flow rate of 1 mL/min in N,N-dimethylformamide (DMF) with 10 mM LiBr and 1% (v/v) NEt$_3$ at 85 °C using a Waters 2695 separations module equipped with a Waters 2414 differential refractometer and two PLgel 5 μm mixed-D (300 mm × 7.5 mm) columns from Polymer Laboratories connected in series. The calibration was performed using polystyrene standards.

**Synthesis of compound 5.17 and general DCC-mediated ester synthesis:** Acid 4.2 (0.980 g, 4.11 mmol) and phenol 2.6 (1.03 g, 5.06 mmol) were dissolved in CH$_2$Cl$_2$ (40 mL). DCC (1.28 g, 6.20 mmol) and DMAP (0.100 g, 0.819 mmol) were added and the solution was stirred 90 min. The precipitate was filtered off and rinsed with CH$_2$Cl$_2$. The solution was then poured into 1 M Na$_2$CO$_3$, and the product was extracted three times into CH$_2$Cl$_2$. The organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. Purification via column chromatography (90:10 cyclohexane:EtOAc for 600 mL followed by 80:20 cyclohexane:EtOAc) afforded 5.17 (1.58 g, 91%) as a colourless
oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.33 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 8.6 Hz, 2H), 4.73 (s, 2H), 4.67 (br s, 1H), 3.27 (quart, J = 6.6 Hz, 2H), 2.61 (t, J = 7.0 Hz, 2H), 1.94 (quint, J = 7.0 Hz, 2H), 1.46 (s, 9H), 0.94 (s, 9H), 0.10 (s, 6H). $^{13}$C NMR (CDCl$_3$): $\delta$ 171.8, 155.9, 149.4, 138.9, 126.9, 121.1, 79.2, 64.3, 39.8, 31.6, 28.3, 25.9, 25.2, 18.3, -5.4. IR (cm$^{-1}$): 2959, 2933, 2883, 2860, 1760, 1702, 1676, 1508, 1463, 1367. HRMS: calc’d [M+H]$^+$ (C$_{22}$H$_{38}$NO$_5$Si): 424.2514. Found (EI): 424.2519.

Synthesis of compound 5.18 and general HCl/iPrOH-mediated TBS deprotection:
Under air atmosphere, ester 5.17 (1.44 g, 3.40 mmol) was dissolved in 1 vol% 12 M HCl in iPrOH (30 mL), and the solution was stirred for 2 h. The reaction was quenched with saturated NaHCO$_3$ solution, and the product was extracted three times with CH$_2$Cl$_2$. The organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. Column chromatography (80:20 cyclohexane:EtOAc followed by 1:1 cyclohexane:EtOAc) afforded 5.18 (0.957 g, 91%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.39 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H), 4.70 (s, 2H), 4.66 (br s, 1H), 3.27 (quart, J = 6.6 Hz, 2H), 2.62 (t, J = 7.4 Hz, 2H), 1.95 (quint, J = 7.0 Hz, 2H), 1.72 (br s, 1H), 1.46 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 171.9, 156.0, 149.8, 138.7, 127.9, 121.4, 79.3, 64.3, 39.7, 31.5, 28.3, 25.1. IR (cm$^{-1}$): 3450, 2985, 2940, 2873, 1756, 1703, 1509, 1367. HRMS: calc’d [M+H]$^+$ (C$_{16}$H$_{24}$NO$_3$): 310.1649. Found (EI): 310.1647

Synthesis of compound 5.9a: Ester 5.18 (0.422 g, 1.36 mmol) was dissolved in CH$_2$Cl$_2$ (13 mL). Pyridine (0.33 mL, 4.08 mmol) and 4-nitrophenyl chloroformate (0.552 g, 2.74 mmol) were added, and the solution was stirred 3 h. 1 M HCl was added, and the product was extracted three times into CH$_2$Cl$_2$. The organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. Column chromatography (90:10 cyclohexane:CH$_2$Cl$_2$ followed by 1:1 CH$_2$Cl$_2$:EtOAc) afforded 5.9a (0.617 g, 95%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 8.29 (d, J = 9.4 Hz, 2H), 7.47 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 9.4 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H), 5.29 (s, 2H), 4.72 (br s, 1H), 3.28 (quart, J = 6.6 Hz, 2H), 2.64 (t, J = 7.0 Hz, 2H), 1.95 (quint, J = 7.0 Hz, 2H), 1.47 (s, 9H). $^{13}$C NMR (CDCl$_3$): $\delta$ 171.5, 155.9, 155.3, 152.2, 151.0, 145.3, 131.7, 129.9, 125.1, 121.9, 121.7, 79.1, 70.1, 39.6, 31.4, 28.3, 25.1. IR (cm$^{-1}$): 2980, 2935, 2873, 1765, 1703,
Synthesis of compound 5.19: The same general DCC-mediated coupling procedure was followed except acid 4.3 (2.28 g, 10.5 mmol) was used as the starting material. Purification via column chromatography (95:5 cyclohexane:EtOAc followed by 85:15 cyclohexane:EtOAc) afforded 5.19 (3.27 g, 86%) as a colourless oil. \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.33 (d, \(J = 8.2\) Hz, 2H), 7.05 (d, \(J = 8.6\) Hz, 2H), 4.73 (s, 2H), 3.34 (t, \(J = 6.6\) Hz, 2H), 2.88 (s, 3H), 2.57 (t, \(J = 7.4\) Hz, 2H), 1.97 (quintet, \(J = 7.4\) Hz, 2H), 1.47 (s, 9H), 0.94 (s, 9H), 0.10 (s, 9H). \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 171.6, 155.7, 149.4, 138.8, 126.8, 121.1, 79.3, 64.3, 48.0, 34.0, 31.3, 28.3, 25.8, 23.0, 18.3, -5.4. IR (cm\(^{-1}\)): 2957, 2934, 2858, 1761, 1699, 1508, 1471, 1394, 1365. HRMS: calc’d \([\text{M}+\text{H}]^+\) (C\(_{23}\)H\(_{47}\)N\(_2\)O\(_9\)): 475.1711. Found (EI): 475.1712.

Synthesis of compound 5.20: The same procedure was followed for general HCl/iPrOH-mediated deprotection except ester 5.19 (0.582 g, 1.33 mmol) was used as the starting material. Column chromatography (85:15 cyclohexane:EtOAc followed by 1:1 cyclohexane:EtOAc) afforded 5.20 (0.396 g, 92%) as a colourless oil. \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.39 (d, \(J = 8.6\) Hz, 2H), 7.09 (d, \(J = 8.6\) Hz, 2H), 4.70 (s, 2H), 3.35 (t, \(J = 6.6\) Hz, 2H), 2.88 (s, 3H), 2.58 (t, \(J = 7.4\) Hz, 2H), 1.97 (quintet, \(J = 7.4\) Hz, 2H), 1.47 (s, 9H). \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 171.7, 155.8, 149.8, 138.7, 127.8, 121.4, 79.5, 64.3, 48.0, 34.0, 31.3, 28.3, 23.0. IR (cm\(^{-1}\)): 3450, 2976, 2934, 2876, 1757, 1693, 1508, 1398, 1367. HRMS: calc’d \([\text{M}+\text{H}]^+\) (C\(_{17}\)H\(_{26}\)NO\(_5\)): 324.1805. Found (EI): 324.1809.

Synthesis of compound 5.24: Ester 4.15 (2.02 g, 8.64 mmol) was dissolved in CH\(_2\)Cl\(_2\) (60 mL). Pyridine (2.10 mL, 26.0 mmol) and 4-nitrophenyl chloroformate (3.48 g, 17.3 mmol) were added, and the solution was stirred 90 min. The reaction was quenched with 1 M HCl, and then the aqueous phase was extracted three times with CH\(_2\)Cl\(_2\). The organic layers were dried over MgSO\(_4\), filtered, and the solvent was removed. Purification via column chromatography (CH\(_2\)Cl\(_2\) followed by 3:1 CH\(_2\)Cl\(_2\):EtOAc) afforded carbonate 5.24 (3.23 g, 94%) as a white solid. \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 8.29 (d, \(J = 9.0\) Hz, 2H), 7.44 (d, \(J = 9.0\) Hz, 2H), 5.13 (dd, \(J = 6.4 & 5.9\) Hz, 1H), 4.72 (br. s, 1H),
3.83 (s, 3H), 3.48 - 3.36 (m, 1H), 3.34 – 3.24 (m, 1H), 2.25 – 2.13 (m, 2H) 1.45 (s, 9H).

$^{13}$C NMR (CDCl$_3$): $\delta$ 169.4, 155.7, 155.1, 151.7, 145.2, 125.0, 121.5, 79.1, 73.7, 52.5, 35.9, 31.1, 28.0. IR (cm$^{-1}$): 3435, 2980, 2935, 1763, 1757, 1709, 1528, 1350. HRMS: calc’d [M+H]$^+$ (C$_{17}$H$_{23}$N$_2$O$_9$): 399.1404. Found (EI): 399.1544.

**Synthesis of compound 5.25:** Carbonate 5.24 (5.04 g, 12.6 mmol) was dissolved in toluene (60 mL). Diethylamine (4.00 mL, 38.5 mmol) and DMAP (0.161 g, 1.32 mmol) were added, and the solution was stirred 24 h. The solvent was removed in vacuo, and then the crude material was taken up in CH$_2$Cl$_2$. 1 M HCl was added, and the aqueous phase was extracted three times with CH$_2$Cl$_2$. The combined organic layers were then washed with 1 M Na$_2$CO$_3$, and the aqueous layer was further extracted twice with CH$_2$Cl$_2$. The organic phases were dried over MgSO$_4$, filtered, and the solvent was removed. The product was purified via silica gel chromatography (65:35 Cyclohexane:EtOAc), affording carbamate 5.25 (4.09 g, 97%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 5.11 (dd, J = 4.3 & 7.8 Hz, 1H), 4.81 (br. s, 1H), 3.75 (s, 3H), 3.40 – 3.24 (m, 5H), 3.23 – 3.12 (m, 1H), 2.14 – 1.96 (m, 2H), 1.44 (s, 9H), 1.19 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 7.0 Hz, 3H). $^{13}$C NMR (CDCl$_3$): $\delta$ 170.7, 155.1, 154.2, 78.3, 69.9, 51.4, 41.3, 40.8, 36.0, 30.9, 27.6, 13.3, 12.7. IR (cm$^{-1}$): 3373, 2978, 2937, 1759, 1711, 1522, 1479, 1433, 1367. HRMS: calc’d [M]$^+$ (C$_{15}$H$_{28}$N$_2$O$_6$): 332.1947. Found (EI): 332.1953.

**Synthesis of compound 5.26:** Under an air atmosphere, compound 5.25 (4.09 g, 12.3 mmol) was dissolved in 1:1 THF:H$_2$O (100 mL). LiOH·H$_2$O (0.778 g, 18.5 mmol) was added, and the solution was stirred for 24 h. The solution was acidified with 1 M HCl, and the solution was extracted four times with CH$_2$Cl$_2$. The organic layers were dried with MgSO$_4$, filtered, and the solvent was removed to provide acid 5.26 (3.63 g, 92%) as a thick, colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 5.10 (dd, J = 5.5 & 6.4 Hz, 1H), 4.90 (br. s, 1H), 3.45 – 3.17 (m, 6H), 2.37 – 1.90 (m, 2H), 1.44 (s, 9H), 1.19 (t, J = 7.4 Hz, 3H), 1.14 (t, J = 7.4 Hz, 3H). $^{13}$C NMR (CDCl$_3$): $\delta$ 173.4, 155.9, 155.0, 79.1, 70.5, 41.8, 41.3, 36.4, 31.2, 28.1, 13.6, 13.0. IR (cm$^{-1}$): 3369, 2980, 2937, 1699, 1527, 1481, 1435, 1367. HRMS: calc’d [M]$^+$ (C$_{14}$H$_{26}$N$_2$O$_6$): 318.1791. Found (EI): 318.1781.
Synthesis of compound 5.27: Acid 5.26 (3.60 g, 11.3 mmol) was dissolved in CH₂Cl₂ (50 mL). NEt₃ (4.00 mL, 28.7 mmol) and pivaloyl chloride (1.67 mL, 13.6 mmol) were added and the solution was stirred for 15 min. Phenol 2.6 (3.78 g, 15.8 mmol) was dissolved in CH₂Cl₂ (10 mL) and added via syringe to the reaction mixture, which was then stirred for 24 h. The solution was poured into 1 M HCl, and the product was extracted three times into CH₂Cl₂. The organic layers were dried over MgSO₄, filtered, and the solvent was removed. Column chromatography (9:1 cyclohexane:EtOAc followed by 3:1 cyclohexane:EtOAc) afforded ester 5.27 (5.38 g, 88%) as a colourless oil. ¹H NMR (CDCl₃): δ 7.43 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.6 Hz, 2H), 5.30 (dd, J = 4.7 & 7.8 Hz, 1H), 4.87 (br. s, 1H), 4.73 (s, 2H), 3.52 – 3.23 (m, 6H), 2.30 – 2.13 (m, 2H), 1.45 (s, 9H), 1.20 (t, J = 7.0 Hz, 3H), 1.18 (t, J = 7.0 Hz, 3H). ¹³C NMR (CDCl₃): δ 169.6, 155.7, 154.8, 149.0, 139.2, 126.9, 121.0, 79.2, 70.6, 64.3, 42.1, 41.5, 36.6, 31.5, 28.3, 18.3, 13.9, 13.3, -5.4. IR (cm⁻¹): 3371, 2959, 2934, 1776, 1711, 1508, 1431, 1367. HRMS: calc’d [M+H]+ (C₂₇H₄₇N₂O₇): 539.3147. Found (EI): 539.3133.

Synthesis of compound 5.10b: Under an air atmosphere, 5.27 (1.01 g, 1.87 mmol) was dissolved in 1:1 TFA:CH₂Cl₂ (10 mL) and the solution was stirred 2 h. The solvent was removed, and CH₂Cl₂ was then added and removed three times to remove residual TFA. The flask was then fully evacuated and refilled with Ar. CH₂Cl₂ (9 mL) was added, and the solution was cooled to 0°C. Phenyl chloroformate (0.35 mL, 279 mmol) was added, followed by NEt₃ (1.00 mL, 7.19 mmol), and the solution was stirred for 1 h. The reaction was quenched with 1 M HCl and the aqueous phase was extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered, and the solvent was removed. The material was then taken up in 2:1:1 THF:H₂O:1 M NaHCO₃ (16 mL), and the solution was stirred for 2 h under an air atmosphere. Saturated NaHCO₃ solution was then added, and the product was extracted three times into CH₂Cl₂. The organic layers were dried over MgSO₄, filtered, and the solvent was removed. Purification via column chromatography (2:1 cyclohexane:EtOAc followed by 1:1 cyclohexane:EtOAc) afforded 5.10b (0.673 g, 81%) as a thick, colourless oil. ¹H NMR (CDCl₃): δ 7.39 (d, J = 8.8 Hz, 2H), 7.36 (t, J = 8.2 Hz, 2H), 7.20 (t, J = 7.4 Hz 1H), 7.15 – 7.12 (m, 4H), 5.43 (t, J = 5.9 Hz, 1H), 5.40 (dd, J = 4.7 & 8.2 Hz, 1H), 4.70 (d, J = 5.3 Hz, 2H), 3.64 – 3.57 (m, 1H), 3.47 – 3.29 (m, 5H), 2.40 – 2.34 (m, 1H), 2.33 – 2.25 (m,
1H), 1.67 (t, J = 5.3 Hz, 1H), 1.22 (t, J = 7.0 Hz, 3H), 1.17 (t, J = 7.0 Hz, 3H). $^{13}$C NMR (CDCl$_3$): $\delta$ 169.5, 169.1, 154.8 & 154.7 (rotamers), 149.3 & 149.2 (rotamers), 139.2 & 139.0 (rotamers), 129.2, 127.9, 125.2, 121.4, 121.1 & 121.0 (rotamers), 70.5 & 70.4 (rotamers), 64.12 & 64.08 (rotamers), 42.2, 41.6, 37.2 & 35.9 (rotamers), 31.2 & 30.6 (rotamers), 13.9, 13.2. IR (cm$^{-1}$): 3350, 3074, 2980, 2937, 2880, 1772, 1709, 1699, 1483, 1435, 1383. HRMS: calc’d [M+H]$^+$ (C$_{23}$H$_{29}$N$_2$O$_7$): 445.1979. Found (EI): 445.1979.

**Synthesis of compound 5.29:** The same procedure was followed for general HCl/iPrOH-mediated deprotection except ester 5.27 (0.129 g, 0.240 mmol) was used as the starting material. Purification via column chromatography (3:1 cyclohexane:EtOAc followed by 1:1 cyclohexane:EtOAc) afforded 5.29 (0.0905 g, 89%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 7.39 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 8.6 Hz, 2H), 5.30 (dd, J = 4.7 & 7.8 Hz, 1H), 4.86 (br s, 1H), 4.70 (d, J = 5.9 Hz, 2H), 3.50 – 3.24 (m, 6H), 2.32 – 2.14 (m, 2H), 1.70 (t, J = 5.9, 1H), 1.45 (s, 9H), 1.20 (t, J = 7.0 Hz, 3H), 1.16 (t, J = 7.0 Hz, 3H). $^{13}$C NMR (CDCl$_3$): $\delta$ 169.6, 155.7, 154.8, 149.4, 139.0, 127.9, 121.2, 79.4, 70.7, 64.3, 42.1, 41.6, 36.6, 31.5, 28.3, 13.9, 13.3. IR (cm$^{-1}$): 3440, 2978, 2936, 1772, 1695, 1503, 1433, 1367. HRMS: calc’d [M+H]$^+$ (C$_{21}$H$_{32}$N$_2$O$_7$): 425.2286. Found (EI): 425.2286.

**Synthesis of compound 5.16a:** A flask was charged with acid 4.2 (1.57 g, 4.88 mmol) and previously reported phenol 5.15a (1.98 g, 9.72 mmol). CH$_2$Cl$_2$ (20 mL) was added, followed by EDC-HCl (2.80 g, 14.6 mmol) and DMAP (4.16 g, 34.0 mmol), and then the solution was stirred for 24 h. The solution was poured into saturated NaHCO$_3$ solution, and the product was extracted twice into CH$_2$Cl$_2$. The organic extracts were then washed with 1 M HCl, and the aqueous phase was further extracted twice with additional CH$_2$Cl$_2$. The combined organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. Purification of the crude material by column chromatography (19:1 cyclohexane:EtOAc followed by 9:1 cyclohexane:EtOAc) afforded 5.16a (2.09 g, 85%) as a colourless oil. $^1$H NMR (CDCl$_3$): $\delta$ 6.81 (s, 1H), 6.55 (s, 1H), 4.72 (br. s, 1H), 3.48 (t, J = 7.4 Hz, 2H), 3.26 (quartet, J = 6.6 Hz, 2H), 2.59 (t, J = 7.4 Hz, 2H), 3.52 (s, 3H), 2.23 (s, 3H), 2.02 (t, J = 7.4 Hz, 2H), 1.94 (quintet, J = 7.0 Hz, 2H), 1.46 (s, 15H), 0.85 (s, 9H), -0.02 (s, 6H). $^{13}$C NMR (CDCl$_3$): $\delta$ 172.3, 155.9, 149.6, 138.3, 135.8, 134.0, 132.2, 123.0, 79.1, 60.9, 45.9, 39.8, 39.0, 32.2, 31.8, 28.4, 25.9, 25.2, 24.9, 20.1, 18.2,
5.4. IR (cm$^{-1}$): 3380, 2959, 2930, 2858, 1759, 1718, 1520, 1474, 1365. HRMS: calc’d [M+H]$^+$ (C$_{28}$H$_{50}$NO$_5$Si): 508.3453. Found (EI): 508.3459.

**Synthesis of compound 5.31:** Under an air atmosphere, 5.16a (2.08 g, 4.10 mmol) was dissolved in 3:1:1 HOAc:THF:H$_2$O (20 mL), and the solution was stirred for 2 h. Removal of the solvent and purification by column chromatography (3:1 cyclohexane:EtOAc followed by 1:1 cycohexane:EtOAc) afforded 5.31 (1.48 g, 91%) as a colourless oil. $^1$H NMR (CDCl$_3$): δ 6.83 (d, J = 1.6 Hz, 1H), 6.56 (d, J = 1.6 Hz, 2H), 4.73 (br. s, 1H), 3.54 (t, J = 7.4 Hz, 2H), 2.61 (t, J = 7.4 Hz, 2H), 2.53 (s, 3H), 2.24 (s, 3H), 2.05 (t, J = 7.4 Hz, 2H), 1.94 (quint, J = 7.0 Hz, 2H), 1.49 (s, 6H), 1.45 (s, 9H). $^{13}$C NMR (CDCl$_3$): δ 172.2, 155.9, 149.2, 137.8, 135.4, 133.4, 131.8, 122.7, 78.5, 59.3, 45.2, 39.2, 38.4, 31.6, 31.4, 28.0, 24.8, 24.4, 19.7. IR (cm$^{-1}$): 3410, 2976, 2934, 1753, 1697, 1524, 1456, 1366. HRMS: calc’d [M+H]$^+$ (C$_{22}$H$_{36}$NO$_5$): 394.2588. Found (EI): 394.2603.

**Synthesis of compound 5.32:** Under an air atmosphere, 5.31 (1.45 g, 3.69 mmol) was dissolved in CH$_2$Cl$_2$ (40 mL), and PCC (1.79 g, 8.32 mmol) was added. The solution was stirred for 2 h, at which time the material was filtered through a silica plug, and the product was eluted using Et$_2$O. Removal of the solvent afforded aldehyde 5.32 (1.26 g, 87%) as a brown oil. $^1$H NMR (CDCl$_3$): δ 9.54 (t, J = 2.7 Hz, 1H), 6.85 (s, 1H), 6.60 (s, 1H), 4.69 (br. s, 1H), 3.27 (quart, J = 6.6 Hz, 2H), 2.82 (d, J = 2.7 Hz, 2H), 2.60 (t, J = 7.4 Hz, 2H), 2.54 (s, 3H), 2.25 (s, 3H), 1.94 (quint, J = 7.0 Hz, 2H), 1.56 (s, 6H), 1.46 (s, 9H). $^{13}$C NMR (CDCl$_3$): δ 201.6, 171.3, 155.3, 148.5, 136.8, 135.7, 131.7, 131.6, 122.5, 77.9, 55.6, 38.8, 37.1, 31.3, 30.6, 27.5, 24.8, 24.4, 19.3. IR (cm$^{-1}$): 3410, 2978, 2932, 1751, 1718, 1522, 1366. HRMS: calc’d [M]$^+$ (C$_{22}$H$_{33}$NO$_5$): 391.2359. Found (EI): 391.2359.

**Synthesis of compound 5.33:** Under an air atmosphere, 5.32 (1.33 g, 3.40 mmol) was dissolved in DMF (35 mL). Oxone® (2.64 g, 4.28 mmol) was added and the solution was stirred for 24 h. The solution was poured into 1:1 H$_2$O:saturated brine, and the product was extracted four times into CH$_2$Cl$_2$. The organic layers were dried over MgSO$_4$, filtered, and the solvent was removed. Purification via column chromatography (8:2
cyclohexane:EtOAc followed by 6:4 cyclohexane:EtOAc afforded acid 5.33 (1.26 g, 91%) as a pale yellow oil. \( ^1H \text{NMR (CDCl}_3\): } \delta 6.83 (d, J = 2.0 Hz, 1H), 6.58 (d, J = 2.0 Hz, 1H), 4.77 (br. s, 1H), 3.24 (br. s, 2H), 2.82 (s, 2H), 2.61 (t, J = 7.0 Hz, 2H), 2.55 (s, 3H), 2.23 (s, 3H), 1.93 (quint, J = 7.0 Hz, 2H), 1.58 (s, 6H), 1.46 (s, 9H). \( ^{13}C \text{NMR (CDCl}_3\): } \delta 176.3, 172.1, 156.0, 149.2, 137.8, 135.9, 133.3, 132.2, 122.8, 79.1, 47.3, 39.6, 38.4, 32.0, 31.1, 28.2, 25.0, 24.7, 20.0. IR (cm\(^{-1}\)): 3385, 2978, 2934, 1755, 1713, 1522, 1367. HRMS: calc’d [M+H]\(^+\) (C\(_{22}\)H\(_{33}\)NO\(_6\)): 407.2381. Found (EI): 408.2378.

**Synthesis of compound 5.34:** 5.33 (1.18 g, 2.89 mmol) was dissolved in CH\(_2\)Cl\(_2\) (15 mL). Pentafluorophenol (0.64 g, 3.46 mmol), EDC-HCl (0.69 g, 3.62 mmol) and pyridine (0.46 g, 5.81 mmol) were then added and the solution was stirred for 24 h. The solution was poured into 1 M HCl and the product was extracted three times into CH\(_2\)Cl\(_2\). The organic layers were dried over MgSO\(_4\), filtered, and the solvent was removed. The crude material was purified via column chromatography (9:1 cyclohexane:EtOAc followed by 3:1 cyclohexane:EtOAc), affording ester 5.34 (1.57 g, 95%) as a pale yellow oil. \( ^1H \text{NMR (CDCl}_3\): } \delta 6.84 (d, J = 2.0 Hz, 1H), 6.62 (d, J = 2.0 Hz, 1H), 4.68 (br s, 1H), 3.27 (quart, J = 6.6 Hz, 2H), 3.20 (s, 2H), 2.65, (t, J = 7.4 Hz, 2H), 2.57 (s, 3H), 2.24 (s, 3H), 1.96 (quint, J = 7.0 Hz, 2H), 1.64 (s, 6H), 1.45 (s, 9H). \( ^{13}C \text{NMR (CDCl}_3\): } \delta 172.1, 167.3, 156.1, 149.4, 142.4, 139.8, 138.9, 137.7, 136.6, 132.40, 132.36, 124.9, 123.1, 79.0, 46.7, 39.7, 38.9, 32.1, 31.0, 28.2, 25.03, 24.97, 20.0. IR (cm\(^{-1}\)): 2981, 2934, 2898, 1792, 1763, 1716, 1620, 1521, 1368. HRMS: calc’d [M+H]\(^+\) (C\(_{28}\)H\(_{33}\)F\(_5\)NO\(_6\)): 574.2223. Found (EI): 574.2242.

**Synthesis of polymer 5.5b:** Under an air atmosphere, 5.34 (1.41 g, 2.46 mmol) was dissolved in 1:1 TFA:CH\(_2\)Cl\(_2\) (16 mL), and the solution was stirred for 2 h. The solvent was removed, and CH\(_2\)Cl\(_2\) was then added and removed three times to remove residual TFA. Additional 5.34 (0.0717 g, 0.133 mmol) was added and the flask was fully evacuated and refilled with Ar. Toluene (5 mL) was added and the solution was cooled to 0 °C. DIPEA (1.80 mL, 10.3 mmol) and DMAP (63.2 mg, 0.517 mmol) were added, and the solution was stirred 24 h. The solution was poured into 1 M HCl, and the product was extracted three times into CH\(_2\)Cl\(_2\). The organic layers were dried over MgSO\(_4\), filtered, and the solvent was removed. The crude material was dialyzed against DMF.
(200 mL, 1 solvent change) using a regenerated cellulose membrane (Spectrum Laboratories Spectra/Por, 3500 MW cutoff). The recovered polymer was then dissolved 1:1 dioxane:H₂O (1 mL) and fully dried on a lyophilizer to afford 5.5b (0.132 g, 17%) as a powdery white solid. 

**Synthesis of compound 5.12a-HCl:** This compound was prepared following a literature procedure.²⁰ Under an air atmosphere, 5.35 (1.00 g, 9.73 mmol) was dissolved in MeOH (50 mL). SOCl₂ (3.50 mL, 49.5 mmol) was added, and the solution was heated to reflux and stirred for 24 h. The solution was cooled to RT and then the solvent was removed, affording 5.12a-HCl (1.50 g, 99%) as a white crystalline solid. 

**Synthesis of compound 5.36:** Acid 5.33 (0.0728 g, 0.179 mmol) was dissolved in CH₂Cl₂ (1 mL). Pivaloyl chloride (0.030 mL, 0.244 mmol) and NEt₃ (0.10 mL, 0.718 mmol) were added and the solution was stirred for 15 min. 5.12a-HCl (0.0566 g, 0.368 mmol) was added, and the solution was stirred for 24 h. The reaction mixture was then poured into 1 M HCl, and the product was extracted three times into CH₂Cl₂. The organic layers were dried over MgSO₄, filtered, and then the solvent was removed. Column chromatography (3:1 cyclohexane:EtOAc followed by 1:1 cyclohexane:EtOAc) afforded 5.36 (0.0511 g, 56%) as a colourless oil.

**Synthesis of compound 5.35:** Acid 5.33 (1.00 g, 9.73 mmol) was dissolved in MeOH (50 mL). SOCl₂ (3.50 mL, 49.5 mmol) was added, and the solution was heated to reflux and stirred for 24 h. The solution was cooled to RT and then the solvent was removed, affording 5.12a-HCl (1.50 g, 99%) as a white crystalline solid.
Kinetics Studies

Buffer preparation: HEPES (0.119 g, 0.500 mmol) was dissolved in D$_2$O (5.0 mL). To this, a saturated solution of NaOH in D$_2$O was added dropwise with stirring, while monitoring with a pH meter until the desired pH of 7.4 was obtained.

NMR Kinetics Study: Compound 5.36 (7.5 mg) was dissolved in 1:1 TFA:CH$_2$Cl$_2$ (1 mL), and the solution was stirred for 2 h under an air atmosphere. The solvent was removed, and CH$_2$Cl$_2$ was added and removed three times to remove residual TFA. The material was then taken up in H$_2$O and dioxane (approximately 2:1 H$_2$O:dioxane, 2 mL) and freeze-dried to remove all solvents. The material was then dissolved in pH 7.4 acetone-d$_6$:0.1 M HEPES (D$_2$O) (3:2) and incubated at 37 °C. Reaction progress was monitored using $^1$H NMR spectroscopy by comparing the relative integrations of the aromatic peaks arising from ester 5.37, intermediate phenol 5.38 and lactone 5.7.

5.5 References


Chapter 6
Conclusions and Future Directions

The work described in this thesis represents a significant expansion of the relatively new but rapidly growing field of self-immolative polymers. The primary focus was on the development of amine-based self-immolative spacers for use in heterodimers to form novel polymeric structures with unique degradation profiles. In the first study, it was found that \( \text{N,N'} \)-dimethylethylenediamine could be combined with the aromatic spacer 4-hydroxybenzyl alcohol to form a linear self-immolative polycarbamate. An initial model polymer containing a Boc group as an end cap was shown to undergo end-to-end depolymerization following end cap removal in the predicted manner, generating \( \text{N,N'} \)-dimethylimidazolidinone and 4-hydroxybenzyl alcohol as the sole detectable degradation products. When the polymer was studied under the same conditions without prior end cap removal, minimal degradation was observed, indicating that depolymerization was a specific process and required end cap removal in order to occur. This was the first example involving the incorporation of cyclization spacers into self-immolative linear polymers and demonstrated that they could be used to alter the depolymerization rate.

Utilizing the same polymer backbone with a PEG end cap, an amphiphilic block copolymer was formed, which was found to undergo self-assembly into nanoparticles in an aqueous environment. These nanoparticles were capable of encapsulating a hydrophobic molecule and gradually releasing it as degradation progressed, demonstrating the potential for drug delivery applications. This was the first example
involving the development of supramolecular assemblies of self-immolative linear polymers.

The $N,N'$-dimethylethylenediamine spacer was also combined with 2-mercaptoethanol to generate a linear self-immolative polymer that degraded entirely via intramolecular cyclization reactions. This was of interest due to the potential toxicity of the quinone methide degradation intermediates that would be generated from the depolymerization of the polymers comprising 4-hydroxybenzyl alcohol. By incorporating a disulfide end cap, polymer degradation was initiated under reducing conditions. Indeed, when the polymer was incubated in a phosphate buffered solution in the presence of DTT as a reducing agent, polymer degradation occurred over the course of 10 days, producing $N,N'$-dimethylimidazolidinone and 1,3-oxathiolan-2-one. Analyzing this process using SEC revealed a gradual decrease in the molecular weight of the polymer, further supporting the proposed end-to-end degradation mechanism. When the polymer was studied under the same conditions in the absence of a reducing agent, no degradation was observed, indicating that depolymerization was once again an intramolecular process requiring end cap removal.

At this stage, it was of interest to demonstrate that self-immolative linear polymers with a broad range of depolymerization rates could be developed. If this could be achieved, it would eventually allow different polymer backbones, and thus degradation rate to be selected according to their desired applications. While the backbones based on 4-aminobenzyl alcohol depolymerized very rapidly, the above described backbones degraded slowly over periods of days. To access polymer backbones with different depolymerization rates, it would be necessary to develop new monomers. To further broaden the scope of self-immolative spacers and gain access to more rapidly cyclizing amine-based monomers, a series of 4-aminobutyric acid derivatives were developed. Varying substituents were incorporated at the $N$- and $\alpha$-positions to probe the effects of steric, rigidity and electronics on the rate of cyclization of the amine on a phenyl ester. The findings suggested that 4-aminobutyric acid was indeed a viable candidate as a self-immolative spacer, with half-lives of cyclization in the range of 2 – 39 s. Furthermore, substitution was found to have a positive effect on the
rate, as N-alkylated substrates cyclized more rapidly, and further substitution at the \( \alpha \)
position provided greater enhancement of cyclization rate. Additionally, further tests were carried out under moderately acidic conditions utilizing the most rapidly cyclizing spacer. As expected, the cyclization rate slowed as pH decreased, but even at pH 4.0, well outside the pH range found within the body, the half-life of cyclization was still found to be 76 s. Based on this result, it was hypothesized that polymeric systems based on this spacer could be developed for applications in moderately acidic environments.

Building off of these results, two new polymeric systems were developed utilizing 4-aminobutyric acid in conjunction with either 4-hydroxybenzyl alcohol or 2’-hydroxyhydrocinnamic acid. Multiple routes toward the first polymer were investigated employing a variety of methodologies as well as variations to the 4-aminobutyric acid backbone. While no usable polymer was ultimately obtained, much groundwork was laid for future investigations. In the most promising approach, polymeric material was indeed obtained. However, throughout the course of the polymerization an unexpected and as of yet unknown side reaction occurred, rendering the polymer unusable. Efforts toward the second polymer were more fruitful, as usable polymer was obtained. This polymer was found to be substantially more hydrophobic than those previously studied, and thus mandated that degradation studies be carried out in a predominantly organic, rather than aqueous, environment. This modification had a profound effect on the rate of cyclization, as monomer studies demonstrated that cyclization of the amine occurred orders of magnitude slower with a half-life of greater than 8 h. Due to this prolonged timeline of monomer degradation, no investigation of polymer degradation was undertaken at this point.

Future investigations in this area of study involve the continuing development of 4-aminobutyric acid-based polymers. First, a thorough kinetic investigation of the degradation of polymer 5.5b is required. Following that, it would be desirable to increase the hydrophilic character of such a polymer, first to improve the rate of depolymerization, but also for potential use in other applications such as drug delivery. To this end, a solubilizing group such as a triethylene glycol chain could be incorporated into the 4-aminobutyric acid backbone. Alternatively, the polymer could be combined with PEG to
generate a block copolymer capable of self-assembly in aqueous environment. By synthesizing novel PEG end caps with varying functionality, it would be possible to initiate degradation through alternative mechanisms other than ester hydrolysis.

Additionally, it would be desirable to employ the methodologies developed toward polymeric structure 5.4 to obtain usable polymeric material. Each method employed showed significant potential but suffered from a flaw that could not be readily overcome. In two of the cases, purity of the activated monomer proved to be the limiting factor, so if alternative purification procedures could be developed it is likely that these routes could be used to access polymeric material. In the final case, polymeric material was obtained but an unwanted side reaction occurred during polymerization. Further probing of the reaction and further characterization of the polymer obtained and its degradation products may provide further insight into this side reaction and potentially prevent it from occurring. Finally, the synthetic routes developed can potentially lay the groundwork to the synthesis of other 4-aminobutyric acid-based polymers incorporating alternative spacers.

Over the longer term, the controlled degradation of these self-immolative polymers makes them of significant interest for biomedical applications where it is desirable to trigger the breakdown of a biomaterial in order to release drug molecules or perform other functions. Such applications may involve the use of these polymers as bulk materials or the preparation of new block copolymers that will assemble into different nanostructures such as micelles or vesicles in aqueous solution. Thus, future work will involve further exploration of these areas as well as investigations into the toxicity and biocompatibilities of these materials in different applications.
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Appendix 2: NMR spectra for compounds 2.6, 2.8, 2.10 – 2.13, 2.15 – 2.18, polymers 2.3a & 2.3b, control degradation experiment of polymer 2.3a, SEC chromatogram for crude polymer 2.3a, TEM image of polymer 2.3b stained with OsO₄, DLS spectrum of polymer 2.3b, MS data for degradation products 1.72 and 1.97.
Figure A2.1. $^1$H NMR of compound 2.6 (400 MHz, CDCl$_3$).

Figure A2.2. $^{13}$C NMR of Compound 2.6 (100 MHz, CDCl$_3$).
Figure A2.3. $^1$H NMR of compound 2.8 (400 MHz, CDCl$_3$).

Figure A2.4. $^{13}$C NMR of Compound 2.8 (100 MHz, CDCl$_3$).
Figure A2.5. $^1$H NMR of compound 2.10 (400 MHz, CDCl$_3$).

Figure A2.6. $^{13}$C NMR of Compound 2.10 (100 MHz, CDCl$_3$).
Figure A2.7. $^1$H NMR of compound 2.11 (400 MHz, CDCl$_3$).
Figure A2.8. $^1$H NMR of Compound 2.12 (400 MHz, CDCl$_3$).

Figure A2.9. $^{13}$C NMR of Compound 2.12 (100 MHz, CDCl$_3$).
Figure A2.10. $^1$H NMR of Compound 2.13 (400 MHz, CDCl$_3$).

Figure A2.11. $^{13}$C NMR of Compound 2.13 (100 MHz, CDCl$_3$).
Figure A2.12. $^1$H NMR of Compound 2.15 (400 MHz, CDCl$_3$).

Figure A2.13. $^1$H NMR of Compound 2.16 (400 MHz, CDCl$_3$).
Figure A2.14. $^1$H NMR of Compound 2.17 (400 MHz, CDCl$_3$).

Figure A2.15. $^1$H NMR of Compound 2.18 (400 MHz, CDCl$_3$).
Figure A2.16. $^1$H NMR of Compound 2.3a (400 MHz, CDCl$_3$).

Figure A2.17. SEC trace of Compound 2.3a prior to preparative SEC purification.
Figure A2.18. \(^1\)H NMR spectrum of polymer 2.3a without end cap removal in pH 7.4 0.1 M phosphate buffer (D\(_2\)O):acetone-d\(_6\) (3:2) after 4 days at 37 °C, showing only trace degradation into small molecules.

Figure A2.19. \(^1\)H NMR of Compound 2.3b (400 MHz, CDCl\(_3\)).
Figure A2.20. Transmission electron microscopy image of nanoparticles formed by the assembly of polymer 2.3b in water. Staining was performed with OsO₄. Scale bar = 100 nm.

Figure A2.21. Size distribution of nanoparticles of polymer 2.3b in water as measured by dynamic light scattering (1 mg/mL, Zetasizer Nano ZS from Malvern Instruments).
Figure A2.22. Mass spectrometry data further supporting the structures of the proposed degradation products.
Appendix 3: NMR spectra for compounds 3.7a, 3.9 – 3.13, polymers 3.5a & 3.5b, 3.5b dialysate, control degradation experiments for polymer 3.5a & 3.5b, SEC chromatogram for polymer 3.5a.
Figure A3.1. $^1$H NMR of Compound 3.9 (400 MHz, CDCl$_3$).

Figure A3.2. $^{13}$C NMR of Compound 3.9 (100 MHz, CDCl$_3$).
Figure A3.3. $^1$H NMR of Compound 3.10 (400 MHz, CDCl$_3$).

Figure A3.4. $^{13}$C NMR of Compound 3.10 (100 MHz, CDCl$_3$).
Figure A3.5. $^1$H NMR of Compound 3.11 (400 MHz, CDCl$_3$).

Figure A3.6. $^{13}$C NMR of Compound 3.11 (100 MHz, CDCl$_3$).
Figure A3.7. $^1$H NMR of Compound 3.12 (400 MHz, CDCl$_3$).

Figure A3.8. $^{13}$C NMR of Compound 3.12 (100 MHz, CDCl$_3$).
Figure A3.9. $^1$H NMR of Compound 3.13 (400 MHz, CDCl$_3$).

Figure A3.10. $^{13}$C NMR of Compound 3.13 (100 MHz, CDCl$_3$).
Figure A3.11. $^1$H NMR of Compound 3.7a (400 MHz, CDCl$_3$).

Figure A3.12. $^{13}$C NMR of Compound 3.7a (100 MHz, CDCl$_3$).
Figure A3.13. $^1$H NMR of Polymer 3.5a (400 MHz, CDCl$_3$).

Figure A3.14. SEC trace of Polymer 3.5a.
Figure A3.15. $^1$H NMR of Polymer 3.5b (400 MHz, CDCl$_3$).

Figure A3.16. $^1$H NMR spectrum of dialysate from polymer purification showing the presence of polymeric species along with reaction byproducts 4-nitrophenol and triethylammonium salts.
**Figure A3.17.** $^1$H NMR spectrum of a degradation control sample in which the Boc end cap was not removed from Polymer 3.5a, but the polymer was incubated for 5 days in pH 7.4 0.1 M phosphate buffered D$_2$O:acetone-d$_6$ (3:2). Degradation was not observed.

**Figure A3.18.** $^1$H NMR spectrum of a degradation control sample in which Polymer 3.5b was incubated for 14 days in pH 7.4 0.1 M phosphate buffered D$_2$O:acetone-d$_6$ (3:2) in the absence of DTT. No detectable degradation was observed.
Appendix 4: NMR spectra for 4.3 – 4.4b, 4.6a – 4.11d, 4.13a-d, 4.15 – 4.17b, 4.1a-k TFA, 4.18a-k, graphs of UV-Vis spectra and ln plots for cyclization of 4.1a-k.
Figure A4.1. $^1$H NMR of Compound 4.3 (400 MHz, CDCl$_3$).

Figure A4.2. $^{13}$C NMR of Compound 4.3 (100 MHz, CDCl$_3$).
Figure A4.3. $^1$H NMR of Compound 4.4a (400 MHz, CDCl$_3$).

Figure A4.4. $^{13}$C NMR of Compound 4.4a (100 MHz, CDCl$_3$).
Figure A4.5. $^1$H NMR of Compound 4.4b (400 MHz, CDCl$_3$).

Figure A4.6. $^{13}$C NMR of Compound 4.4b (100 MHz, CDCl$_3$).
Figure A4.7. $^1$H NMR of Compound 4.6a (400 MHz, CDCl$_3$).

Figure A4.8. $^{13}$C NMR of Compound 4.6a (100 MHz, CDCl$_3$).
Figure A4.9. $^1$H NMR of Compound 4.6b (400 MHz, CDCl$_3$).

Figure A4.10. $^{13}$C NMR of Compound 4.6b (100 MHz, CDCl$_3$).
Figure A4.11. $^1$H NMR of Compound 4.7a (400 MHz, CDCl$_3$).

Figure A4.12. $^{13}$C NMR of Compound 4.7a (100 MHz, CDCl$_3$).
**Figure A4.13.** $^1$H NMR of Compound 4.7b (400 MHz, CDCl$_3$).

**Figure A4.14.** $^{13}$C NMR of Compound 4.7b (100 MHz, CDCl$_3$).
Figure A4.15. \(^1\)H NMR of Compound 4.7c (400 MHz, CDCl\(_3\)).

Figure A4.16. \(^{13}\)C NMR of Compound 4.7c (100 MHz, CDCl\(_3\)).
Figure A4.17. $^1$H NMR of Compound 4.8a (400 MHz, CDCl$_3$).

Figure A4.18. $^{13}$C NMR of Compound 4.8a (100 MHz, CDCl$_3$).
Figure A4.19. $^1$H NMR of Compound 4.8b (400 MHz, CDCl$_3$).

Figure A4.20. $^{13}$C NMR of Compound 4.8b (100 MHz, CDCl$_3$).
Figure A4.21. $^1$H NMR of Compound 4.8c (400 MHz, CDCl$_3$).

Figure A4.22. $^{13}$C NMR of Compound 4.8c (100 MHz, CDCl$_3$).
Figure A4.23. $^1$H NMR of Compound 4.9a (400 MHz, CDCl$_3$).

Figure A4.24. $^{13}$C NMR of Compound 4.9a (100 MHz, CDCl$_3$).
Figure A4.25. $^1$H NMR of Compound 4.9b (400 MHz, CDCl$_3$).

Figure A4.26. $^{13}$C NMR of Compound 4.9b (100 MHz, CDCl$_3$).
Figure A4.27. $^1$H NMR of Compound 4.9c (400 MHz, CDCl$_3$).

Figure A4.28. $^{13}$C NMR of Compound 4.9c (100 MHz, CDCl$_3$).
Figure A4.29. $^1$H NMR of Compound 4.10a (400 MHz, CDCl$_3$).

Figure A4.30. $^{13}$C NMR of Compound 4.10a (100 MHz, CDCl$_3$).
Figure A4.31. $^1$H NMR of Compound 4.10b (400 MHz, CDCl$_3$).

Figure A4.32. $^{13}$C NMR of Compound 4.10b (100 MHz, CDCl$_3$).
Figure A4.33. $^1$H NMR of Compound 4.10c (400 MHz, CDCl$_3$).

Figure A4.34. $^{13}$C NMR of Compound 4.10c (100 MHz, CDCl$_3$).
Figure A4.35. $^1$H NMR of Compound 4.10d (400 MHz, CDCl$_3$).

Figure A4.36. $^{13}$C NMR of Compound 4.10d (100 MHz, CDCl$_3$).
Figure A4.37. $^1\text{H}$ NMR of Compound 4.11a (400 MHz, CDCl$_3$).

Figure A4.38. $^{13}\text{C}$ NMR of Compound 4.11a (100 MHz, CDCl$_3$).
Figure A4.39. $^1$H NMR of Compound 4.11b (400 MHz, CDCl$_3$).

Figure A4.40. $^{13}$C NMR of Compound 4.11b (100 MHz, CDCl$_3$).
Figure A4.41. $^1$H NMR of Compound 4.11c (400 MHz, CDCl$_3$).

Figure A4.42. $^{13}$C NMR of Compound 4.11c (100 MHz, CDCl$_3$).
Figure A4.43. $^1$H NMR of Compound 4.11d (400 MHz, CDCl$_3$).

Figure A4.44. $^{13}$C NMR of Compound 4.11d (100 MHz, CDCl$_3$).
Figure A4.45. $^1$H NMR of Compound 4.13a (400 MHz, CDCl$_3$).

Figure A4.46. $^{13}$C NMR of Compound 4.13a (100 MHz, CDCl$_3$).
Figure A4.47. $^1$H NMR of Compound 4.13b (400 MHz, CDCl$_3$).

Figure A4.48. $^{13}$C NMR of Compound 4.13b (100 MHz, CDCl$_3$).
Figure A4.49. $^1$H NMR of Compound 4.13c (400 MHz, CDCl$_3$).

Figure A4.50. $^{13}$C NMR of Compound 4.13c (100 MHz, CDCl$_3$).
Figure A4.51. $^1$H NMR of Compound 4.13d (400 MHz, CDCl$_3$).

Figure A4.52. $^{13}$C NMR of Compound 4.13d (100 MHz, CDCl$_3$).
Figure A4.53. $^1$H NMR of Compound 4.15 (400 MHz, CDCl$_3$).

Figure A4.54. $^{13}$C NMR of Compound 4.15 (100 MHz, CDCl$_3$).
Figure A4.55. $^1$H NMR of Compound 4.16a (400 MHz, CDCl$_3$).

Figure A4.56. $^{13}$C NMR of Compound 4.16a (100 MHz, CDCl$_3$).
Figure A4.57. $^1$H NMR of Compound 4.16b (400 MHz, CDCl$_3$).

Figure A4.58. $^{13}$C NMR of Compound 4.16b (100 MHz, CDCl$_3$).
Figure A4.59. $^1$H NMR of Compound 4.17a (400 MHz, CDCl$_3$).

Figure A4.60. $^{13}$C NMR of Compound 4.17a (100 MHz, CDCl$_3$).
Figure A4.61. $^1$H NMR of Compound 4.17b (400 MHz, CDCl$_3$).

Figure A4.62. $^{13}$C NMR of Compound 4.17b (100 MHz, CDCl$_3$).
Figure A4.63. $^1$H NMR of Compound 4.1a·TFA (400 MHz, CDCl$_3$).

Figure A4.64. $^1$H NMR of Compound 4.18a (400 MHz, CDCl$_3$).
Figure A4.65. $^1$H NMR of Compound 4.1b-TFA (400 MHz, CDCl$_3$).

Figure A4.66. $^1$H NMR of Commercial NMP and compound 4.18b (400 MHz, CDCl$_3$).
Figure A4.67. $^1$H NMR of Compound 4.1c-TFA (400 MHz, CDCl$_3$).

Figure A4.68. $^1$H NMR of Compound 4.18c (400 MHz, CDCl$_3$).
Figure A4.69. $^1$H NMR of Compound 4.1d·TFA (400 MHz, CDCl$_3$).

Figure A4.70. $^1$H NMR of Compound 4.18d (400 MHz, CDCl$_3$).
Figure A4.71. $^1$H NMR of Compound 4.1e-TFA (400 MHz, CDCl$_3$).

Figure A4.72. $^1$H NMR of Compound 4.18e (400 MHz, CDCl$_3$).
Figure A4.73. $^1$H NMR of Compound 4.1f-TFA (400 MHz, CDCl$_3$).

Figure A4.74. $^1$H NMR of Compound 4.18f (400 MHz, CDCl$_3$).
Figure A4.75. $^1$H NMR of Compound 4.1g·TFA (400 MHz, CDCl$_3$).

Figure A4.76. $^1$H NMR of Compound 4.18g (400 MHz, CDCl$_3$).
Figure A4.77. $^1$H NMR of Compound 4.1h·TFA (400 MHz, CDCl$_3$).

Figure A4.78. $^1$H NMR of Compound 4.18h (400 MHz, CDCl$_3$).
Figure A4.79. $^1$H NMR of Compound 4.1i·TFA (400 MHz, CDCl$_3$).

Figure A4.80. $^1$H NMR of Compound 4.18i (400 MHz, CDCl$_3$).
Figure A4.81. $^1$H NMR of Compound 4.1j·TFA (400 MHz, CDCl$_3$).

Figure A4.82. $^1$H NMR of Compound 4.18j (400 MHz, CDCl$_3$).
Figure A4.83. $^1$H NMR of Compound 4.1k·TFA (400 MHz, CDCl$_3$).

Figure A4.84. $^1$H NMR of Compound 4.18k (400 MHz, CDCl$_3$).
Figure A4.85. Cyclization kinetics for compounds 4.1a and 4.1b.

Figure A4.86. Determination of first order rate constant by \( \ln[A]_0/[A] \) vs t graph for compounds 4.1a and 4.1b.
Figure A4.87. Cyclization kinetics for compounds 4.1c, 4.1d and 4.1e.

Figure A4.88. Determination of first order rate constant by ln[A]₀/[A] vs t graph for compounds 4.1c, 4.1d, and 4.1e.
Figure A4.89. Cyclization kinetics for compounds 1.4f, 1.4g and 1.4i.

Figure A4.90. Determination of first order rate constant by \( \ln[A]_0/[A] \) vs t graph for compounds 3f, 3g, and 3i.
Figure A4.91. Cyclization kinetics for compound 4.1h.

Figure A4.92. Determination of first order rate constant by ln\([A]_0/[A]\) vs t graph for compound 4.1h.
Figure A4.93. Cyclization kinetics for compounds 4.1j and 4.1k.

Figure A4.94. Determination of first order rate constant by $\ln[A]/[A]$ vs t graph for compounds 4.1j and 4.1k.
Figure A4.95. Cyclization rate of compound 4.1i at pH 7.0 and 6.0.

Figure A4.96. Determination of first order rate constant by ln[A]₀/[A] vs t graph for compound 4.1i at pH 7.0 and 6.0.
**Figure A4.97.** Cyclization of compound 4.1i and pH 5.0 and 4.0.

**Figure A4.98.** Determination of first order rate constant by $\ln[A]_0/[A]$ vs t graph for compound 4.1i at pH 5.0 and 4.0.
Appendix 5: NMR spectra for compounds 5.9a, 5.10b, 5.12a, 5.16a – 5.20, 5.24 – 5.27, 5.29, 5.31 – 5.34, 5.36, polymer 5.5b, SEC chromatogram for polymer 5.5b.
Figure A5.1. $^1$H NMR of Compound 5.17 (400 MHz, CDCl$_3$).

Figure A5.2. $^{13}$C NMR of Compound 5.17 (100 MHz, CDCl$_3$).
Figure A5.3. $^1$H NMR of Compound 5.18 (400 MHz, CDCl$_3$).

Figure A5.4. $^{13}$C NMR of Compound 5.18 (100 MHz, CDCl$_3$).
Figure A5.5. $^1$H NMR of Compound 5.9a (400 MHz, CDCl$_3$).

Figure A5.6. $^{13}$C NMR of Compound 5.9a (100 MHz, CDCl$_3$).
Figure A5.7. $^1$H NMR of Compound 5.19 (400 MHz, CDCl$_3$).

Figure A5.8. $^{13}$C NMR of Compound 5.19 (100 MHz, CDCl$_3$).
Figure A5.9. $^1$H NMR of Compound 5.20 (400 MHz, CDCl$_3$).

Figure A5.10. $^{13}$C NMR of Compound 5.20 (100 MHz, CDCl$_3$).
Figure A5.11. $^1$H NMR of Compound 5.24 (400 MHz, CDCl$_3$).

Figure A5.12. $^{13}$C NMR of Compound 5.24 (100 MHz, CDCl$_3$).
Figure A5.13. $^1$H NMR of Compound 5.25 (400 MHz, CDCl$_3$).

Figure A5.14. $^{13}$C NMR of Compound 5.25 (100 MHz, CDCl$_3$).
**Figure A5.15.** $^1$H NMR of Compound 5.26 (400 MHz, CDCl$_3$).

**Figure A5.16.** $^{13}$C NMR of Compound 5.26 (100 MHz, CDCl$_3$).
Figure A5.17. $^1$H NMR of Compound 5.27 (400 MHz, CDCl$_3$).

Figure A5.18. $^{13}$C NMR of Compound 5.27 (100 MHz, CDCl$_3$).
Figure A5.19. $^1$H NMR of Compound 5.10b (400 MHz, CDCl$_3$).

Figure A5.20. $^{13}$C NMR of Compound 5.10b (100 MHz, CDCl$_3$).
Figure A5.21. $^1$H NMR of Compound 5.29 (400 MHz, CDCl$_3$).

Figure A5.22. $^{13}$C NMR of Compound 5.29 (100 MHz, CDCl$_3$).
Figure A5.23. $^1$H NMR of Compound 5.16a (400 MHz, CDCl$_3$).

Figure A5.24. $^{13}$C NMR of Compound 5.16a (100 MHz, CDCl$_3$).
Figure A5.25. $^1$H NMR of Compound 5.31 (400 MHz, CDCl$_3$).

Figure A5.26. $^{13}$C NMR of Compound 5.31 (100 MHz, CDCl$_3$).
Figure A5.27. $^1$H NMR of Compound 5.32 (400 MHz, CDCl$_3$).

Figure A5.28. $^{13}$C NMR of Compound 5.32 (100 MHz, CDCl$_3$).
Figure A5.29. $^1$H NMR of Compound 5.33 (400 MHz, CDCl$_3$).

Figure A5.30. $^{13}$C NMR of Compound 5.33 (100 MHz, CDCl$_3$).
Figure A5.31. $^1$H NMR of Compound 5.34 (400 MHz, CDCl$_3$).

Figure A5.32. $^{13}$C NMR of Compound 5.34 (100 MHz, CDCl$_3$).
Figure A5.33. $^1$H NMR of Polymer 5.4b (400 MHz, CDCl$_3$).

Figure A5.34. SEC trace of Polymer 5.4b.
Figure A5.35. $^1$H NMR of Compound 5.12a·HCl (400 MHz, D$_2$O).
Figure A5.36. $^1$H NMR of Compound 5.36 (400 MHz, CDCl$_3$).

Figure A5.37. $^{13}$C NMR of Compound 5.36 (100 MHz, CDCl$_3$).
Curriculum Vitae for Matthew A. DeWit

EDUCATION

• 09/2007 – 04/2012 The University of Western Ontario, Canada, Ph.D. in Organic Chemistry
  Research advisor: Prof. E.R. Gillies

• 09/2003 – 04/2007 The University of Western Ontario, Canada, H.B.Sc. in Chemistry
  Thesis title: Direct Fluorination of Peptides by Incorporating an Unnatural Amino Acid
  Undergraduate honours supervisor: Dr. L.G. Luyt

AWARDS and HONOURS

• 2010 NSERC Graduate Scholarship PGS-D2
• 2007 Graduate Tuition Scholarship in Science (Western Ontario)
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• 2003 Western Scholarship of Excellence (Western Ontario)

RESEARCH EXPERIENCE

• The University of Western Ontario, Department of Chemistry (2007)
  Summer research assistant
  Research advisor: Dr. E.R. Gillies
  Project: Cascade biodegradable polymers

• The University of Western Ontario, Department of Chemistry (2006)
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  Project: Side-chain modification of truncated ghrelin analogues

TEACHING EXPERIENCE

• Undergraduate Student Mentor, University of Western Ontario
  NSERC USRA student Brian Malbrecht                May – August 2009
  Chemistry 4490 student Annelise Beaton            Sept. 2008 - April 2009

• Teaching Assistant, University of Western Ontario
  Laboratory instructor and help room assistant for
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2007 – present

PUBLICATIONS

Articles submitted to refereed journals:


Non-refereed contributions:

• DeWit, Matthew A.; Beaton, Annelise; Gillies, Elizabeth R. “Design and Synthesis of Cascade Degradable Linear Block Polymers” *Polymer Preprints (American Chemical Society, Division of Polymer Chemistry)* **2010**, 51(1), 311-312.

• Knight, Darryl K.; Atkins, Katelyn M.; DeWit, Matthew A.; Wang, Zixi; Lopez, David; Mequanint, Kibret; Gillies, Elizabeth R. “Functionalized Poly(ester amide)s as Scaffolds for Vascular Tissue Engineering.” *Polymer Preprints (American Chemical Society, Division of Polymer Chemistry)* **2010**, 51(1), 37-38.

**CONFERENCE PRESENTATIONS**


• DeWit, Matthew A.; Beaton, A.; Gillies, E.R.; *Development of Self-Immolative Polymers: A New Class of Biodegradable Materials*; 94th Annual Canadian Chemistry Conference and Exhibition, Montreal, Ontario (2011), *Oral Presentation*

• DeWit, Matthew A.; Gillies, E.R.; *A New Class of Self-Immolative Spacers Based on 4-Aminobutyric Acid*; 93rd Annual Canadian Chemistry Conference and Exhibition, Toronto, Ontario (2010), *Oral Presentation*
• DeWit, Matthew A.; Gillies, E.R.; A Novel Cascade Biodegradable Polymer Based on 4-Hydroxybenzyl Alcohol and N,N'-dimethylethylenediamine; 92nd Annual Canadian Chemistry Conference and Exhibition, Hamilton, Ontario (2009), Oral Presentation

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