CONTROLLED DEGRADATION OF POLY(ESTER AMIDE)S VIA CYCLIZATION
OF PENDANT FUNCTIONAL GROUPS

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by

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THE UNIVERSITY OF WESTERN ONTARIO
School of Graduate and Postdoctoral Studies

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The thesis by

José Samuel Mejia

entitled:

Controlled Degradation of Poly(ester amide)s via Cyclization of Pendant Functional Groups

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Date   Chair of the Thesis Examination Board

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Abstract

Polyesters have been used for many biomedical applications ranging from sutures to drug delivery vehicles. However, their bulk degradation results in an accumulation of acidic byproducts, which is detrimental to the human body. In order to circumvent this problem, as well as to impart new properties and functions to polymers for biomedical applications, poly(ester amide)s (PEAs) have been proposed as a different class of biodegradable polymers. However, up to date, there exists no way to stimulate the degradation of these polymers.

The Gillies research group has previously incorporated self-immolative spacers into polymers and has been able to stimulate their degradation by adding the appropriate trigger. The objective of this thesis was to incorporate amino acids capable of 1,5-cyclization into the PEA backbone such that upon activation of the functional moiety, a 1,5-cyclization was induced, leading to degradation of the PEA backbone. PEAs containing *L*-2,4-diaminobutyric acid and *DL*-homocysteine were synthesized and their degradation was monitored in solution, and in films. It was found that the polymers containing the self-immolative spacers degraded faster than their controls under specific triggers (i.e. change in pH, reducing conditions, UV light), thereby allowing polymer degradation to be accelerated under these specific conditions.

Keywords

Acknowledgments

Firstly, I would like to thank the Lord for giving me the strength, the persistence, and the knowledge to bring you the thesis which is before you.

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for pushing me forward, for supporting me and being there during those long nights, and for brightening up those dreary days. I could not have done this without your love and support. Te amo, mi vida. So much.

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List of Abbreviations

Ar   aromatic

Boc   *tert*-butyloxycarbonyl

BSA   bovine serum albumin

CI   chemical ionization

d   doublet

DAB   2, 4-diaminobutyric acid

DBU   1,8-Diazobicyclo[5.4.0]undec-7-ene

DCC   dicyclohexylcarbodiimide

DCU   dicyclohexylurea

DIPEA   *N,N*-diisopropylethylamine

DMAP   *N*, *N*-dimethylaminopyridine

DMF   *N,N*-dimethylformamide

DMSO   dimethyl sulfoxide

DNA   deoxyribonucleic acid

DOX   doxorubicin

DPTS   4-(dimethylamino)pyridinium *p*-toluenesulfonate

DTT   dithiothreitol

EPR   enhanced permeation and retention effect

ESI   electrospray ionization

FDA	food and drug administration
Fmoc	9-fluorenylmethoxycarbonyl
GA	glycolide
HCASMC	human coronary artery smooth muscle cells
HCC	hepatocellular carcinoma
Hcy	homocysteine
HRMS	high resolution mass spectrometry
$h\nu$	light
IR	infrared spectroscopy
LA	lactide
m	multiplet
MACE	major adverse cardiac events
M_n	number average molecular weight
MWCO	molecular weight cutoff
MW	molecular weight
M_w	weight average molecular weight
NIR	near infrared
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
PEA	poly(ester amide)

PEEA	poly(ether ester amide)
PEG	polyethylene glycol
PGA	poly (glycolic acid)
Ph	phenyl
Phe	phenylalanine
PDI	polydispersity index
PDLLA	poly (D/L-lactic acid)
PLGA	poly (lactic-co-glycolic acid)
PLA	poly (L-lactic acid)
Psi	pounds per square inch
ROP	ring opening polymerization
s	singlet
SEC	size exclusion chromatography
SEM	scanning electron microscopy
t	triplet
TCPS	tissue culture polystyrene
T_g	glass transition temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromotography

TVR   target vessel revascularization

UV   ultraviolet

VEGF   vascular endothelial growth factor

Chapter 1

Biodegradable and stimuli-responsive polymers for biomedical applications

1.1   General introduction

Polymers that degrade under biological conditions have become a part of our daily lives. Due to their biocompatibility and their structural properties they have found many uses and have even achieved FDA approval in many cases. They have found applications in areas such as packaging (both medical and commercial), [1,2] medical implants (such as stents and internal fixation devices), [2] drug delivery, [2] and have even been used as scaffolds for tissue engineering. [1,2] Among the polymers used are the polyesters, whose structures are shown (Figure 1.1).

1.1
Poly(glycolic acid)
(PGA)

1.2
Poly(D/L-lactic acid)
(PDLLA)

1.3
Poly(L-lactic acid)
(PLA)

1.4
Poly(lactic-co-glycolic acid)
(PLGA)
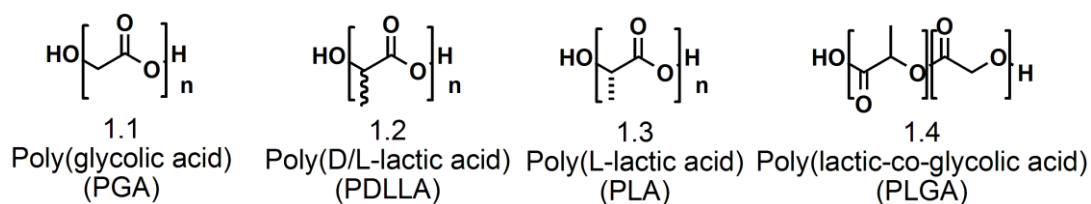
Figure 1.1 Chemical structures of common polyesters.

Another class of polymers that has also emerged for biomedical applications is poly(ester amide)s (PEAs) (Figure 1.2). These polymers, as well as other polymers which are stimuli-responsive are also shown in Figure 1.2 and shall be of interest to this thesis. Their advancements in the areas of drug delivery, tissue engineering, and biomedical applications such as stents shall also be reviewed.

R$_1$= aliphatic chain,aromatic moiety,
unsaturated chain
R$_2$= amino acid
R$_3$,R$_4$= aliphatic chain
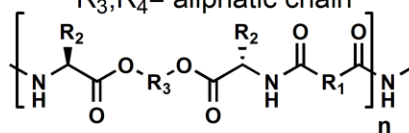
1.5
Poly (ester amide)
PEA
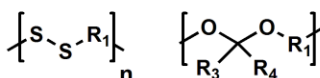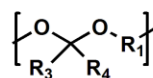
1.6   1.7
Polydisulfide   Polyacetal

Figure 1.2 Chemical structures of other biodegradable polymers.

1.2   Biomedical applications: Tissue engineering & drug delivery

Tissue engineering is a multi-disciplinary field which aims to apply the principles of engineering and the life sciences in order to develop biological materials that can restore, maintain, or improve tissue functions. Its main focus is to overcome the lack of tissue donors, while at the same time avoiding the immune response between host and guest. In this process, cells are cultured onto a scaffold which is then placed in the non-functioning part of the patient. In some cases, the culturing on the scaffold may be skipped altogether such that the scaffold (or the scaffold containing cells), is placed *in vivo* directly and the host's body acts as a bioreactor to construct new tissues.[1,2]

Ideally, a scaffold should not only meet the bulk mechanical and structural requirement of the target tissue; it should also enable molecular interactions with cells that promote healing. To this end, synthetic polymers are viable candidates owing to their material properties that are more flexible than those of natural materials. Their mechanical and chemical properties may also be adjusted accordingly and the materials are readily prepared and in some cases, inexpensive to produce.[2]

Drug delivery is another application in which polymers may be utilized. The hydrophobicity of some drugs hampers their ability to be administered and can result in their rapid clearance from the body. Synthetic polymers not only increase the solubility of drugs in water, but they also provide numerous advantages. Among them is the protection of bioactive drugs from degradation by the host, protection of the host from the toxicity of the drug thus increasing the therapeutic index of the drug, targeting of cells and organs, delivery of drugs at a predetermined rate and avoiding the need for repeated administrations. [3] Due to their ability to degrade over time, polyesters have found applications as packaging, both medical and commercial.[3,4,5]

1.3 Polyesters for biomedical applications

Among the polymers that have been used most for biomedical applications are the poly (hydroxyacid)s. These polymers have been chosen due to their ease of synthesis (Scheme 1.1), biocompatibility, and because their degradation byproducts are broken down into water and carbon dioxide via the citric acid cycle.[1,6,7] The polymers most commonly used for biomedical applications are: PGA, PLA, PLGA, and PDLLA. As these polymers contain hydrolytically labile ester linkages along their backbones, they are susceptible to hydrolysis. Each has different rates of degradation due to their differing hydrophilicities and solid state structures.
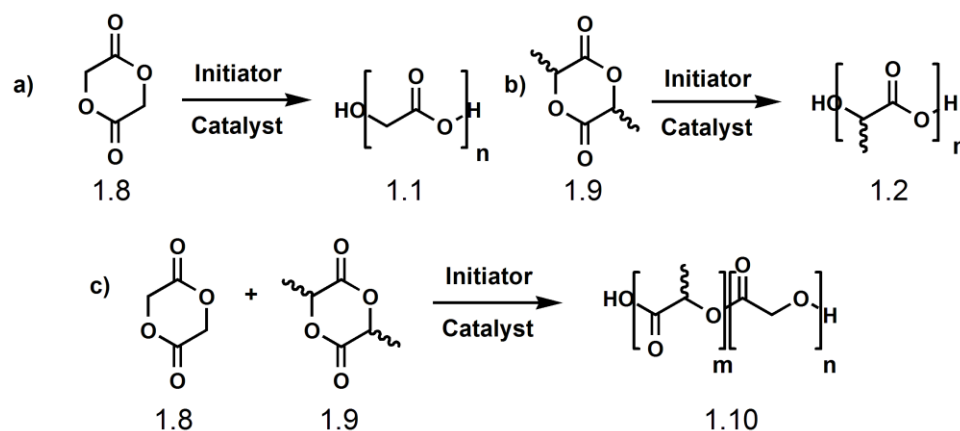
Scheme 1.1 Synthesis of polyester from a) glycolide (GA) b) racemic lactide (LA) c) glycolide and lactide.

As PGA is a highly crystalline polymer, it has a high tensile strength and low solubility in organic solvents. As a result of its high crystallinity, it has excellent mechanical properties which prompted its use as the bone internal fixation device,[1] Biofix®. PGA was also one of the first polymers to be approved as a resorbable suture in 1969[1,5] under the trademark name DEXON®. This particular polymer has been known to degrade in 6-12 months.

PLA is another polymer that has garnered much attention. Due to the monomer's optical activity, PLA comes in two forms: the *D* and *L* isomer, with the *L* isomer being more prevalent. Like PGA it is also crystalline, which results in a high tensile strength and a high modulus which makes it ideal for load-bearing devices such as orthopaedic devices. It can also be made into a high strength fiber and as a result, was chosen as a more effective suture over DEXON® in 1971. Recently, an injectable form of PLLA (Sculptra®), has received FDA approval for the restoration or correction of facial fat loss and lipoatrophy in patients suffering from the human immunodeficiency virus.[1] Due to its more hydrophobic nature (owing to its methyl group), PLA has a longer degradation time and has been known to take 2-6 years to degrade.[3,8] In contrast, stereoisomeric PDLLA containing a mix of *D* and *L* monomers has been known to degrade in a period of 12-16 months, thereby highlighting the effect of stereochemistry on degradation.[2,9] Other factors that affect polyester degradation are T_g, crystallinity, MW, PDI, tacticity, as well as the sequence of monomers.[2,4,9,10,11] As a result, in order to maximize the degradation of polyesters, various copolymers containing lactide and glycolide have been synthesized to exploit the characteristics of each monomer.

Various mixtures of *L*-lactide and glycolide have been used over the years and it has been found that the more lactide incorporated, the slower it degrades. As a consequence, PLGAs have been known to degrade in a short period of 2-3 months, upward to a year. A co-polymer containing 90% glycolide and 10% lactic acid has been used for the development of a multifilament suture, Vicryl®. PANACRYL® is another commercially available suture containing the same monomers, only with a higher lactide ratio in order to decrease the degradation.[1] PLGA also exhibit good cell adhesion and proliferation, thereby making them an excellent candidates for tissue engineering. PLGAs

have been used to synthesize the tissue engineered skin graft (Dermagraft®), using Vicryl mesh® as the scaffolding structure; Vicryl mesh® was made from the PLGA co-polymer.[1]

Another application for this polymer and other polyesters is in the area of drug eluting stents. Drug eluting stents have been accepted by interventional cardiologists over bare metal stents due to their lower restenosis rate.[12] In the last couple of years, the focus has been shifted to stents that can not only release drugs, but also degrade over the treatment period. This would be ideal in that it would omit another surgical step to remove the stent from the patient. Among the candidates is the Conor stent which elutes paclitaxel (Figure 1.3). In a 1700 patient study, it was found to not only have a greater target vessel revascularization (TVR) than the commercially available Taxus stent, but it was also found to have less major adverse cardiac events (MACE) as well.[12] In a recent follow up of a ten year study; it was found that the first fully bioabsorbable stent, known as the Igaki-Tamai stent (made entirely out PLLA), had MACE rates similar to that of bare metal stents.[13]

1.11
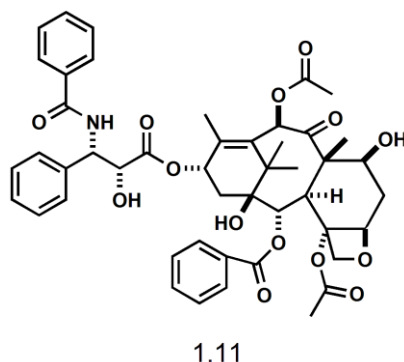
Figure 1.3 Paclitaxel (taxol).

1.4   Polyesters for drug delivery

Tumors that reside in the body need a tremendous amount of oxygen and nutrients in order to sustain their growth. They release growth factors such as VEGF (vascular endothelial growth factor) in order to facilitate the growth of new blood vessels. Unlike regular blood vessels, these vessels contain cell junctions that are not as tight as normal ones. As a result, anything that enters these vessels remains trapped therein.[14] Many drug delivery systems have taken advantage of this phenomenon (otherwise known as the

enhanced permeation and retention effect; EPR for short), including those that contain polyesters.

      Of the ways to deliver a drug to the body, drug delivery using self-assemblies has garnered much attention. The driving force behind these assemblies relies on the hydrophobic and hydrophilic characteristics of block copolymers. When a polymer containing a relatively hydrophobic block (such as polyester), is connected to a more hydrophilic block such as (polyethylene glycol (PEG)) and is immersed in water, the polymers arrange themselves such that the hydrophobic blocks minimize contact with water. Of the various shapes formed, the spherical micelle shall be discussed herein; it is also widely studied in the area of drug delivery (Figure 1.4).
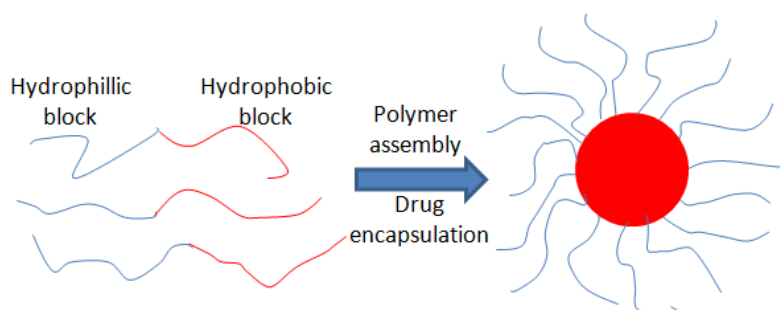
Figure 1.4 Formation of a spherical micelle.

      The hydrophobic core of the micelle provides an opportunity for hydrophobic drugs such as doxorubicin (Figure 1.5), which would otherwise exhibit limited solubility in the body to be delivered to their target. This in turn increases the bioavailability of the drug, thereby increasing its efficacy. There have been various reports utilizing this drug delivery type system.[15-17]

1.12
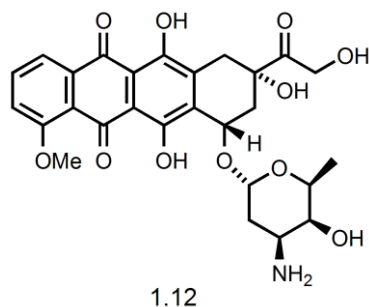
Figure 1.5 Doxorubicin (DOX).

One of the major disadvantages to incorporating hydrophobic drugs in the core of micelles is that they exhibit poor loadings and they release their drugs over a shorter period of time. In order to circumvent this, Park and coworkers conjugated the terminal hydroxyl group of the PLGA by first activating it with *p*-nitrophenyl chloroformate, which was later used to conjugate doxorubicin (Scheme 1.2).[15]
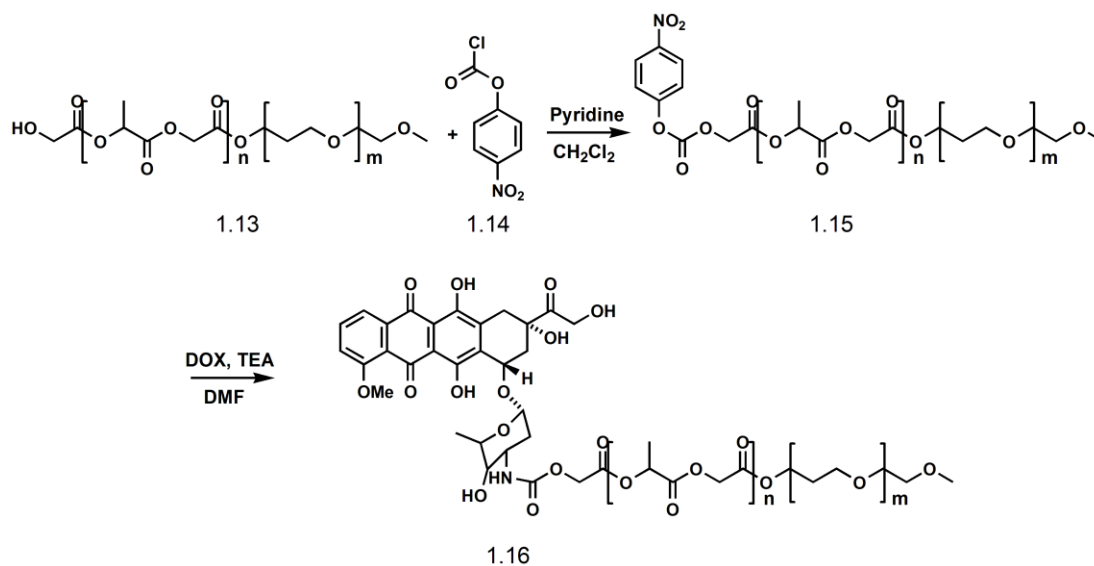
Scheme 1.2 Synthetic route to DOX-PLA-PEG conjugate.

Once the DOX-PLA-PEG conjugate was synthesized, micelles were formed via the dialysis method. It was found that the DOX-PLA-PEG conjugate exhibited superior drug loadings then the control, non conjugated DOX-PLA-PEG conjugate (2.18% compared to 0.51%). This is attributed to the carbamate linkage which forces the hydrophobic drug into the core, whereas if it had been free, it would have escaped during the self- assembly process. When the micelles were incubated in a phosphate buffer, the

DOX-PLA-PEG conjugate showed a sustained release profile with only 50% of the drug released over a period of two weeks. In contrast, the non-conjugated micelle exhibited a burst release in 3 days. The sustained profile of the conjugated DOX-PLA-PEG micelle can be attributed to the carbamate linkage which takes longer to degrade than the PLA backbone. Cell studies were also undertaken which showed that not only are the conjugated micelles taken up more by cells (presumably via endocytotic transport), they are also more cytotoxic to cancer cells than free doxorubicin.

Recently, target specific micelles have been the focus of many studies. In order to increase therapeutic efficiency and to reduce the side effects from chemotherapy, Du and co-workers devised a strategy where they decorated polymeric carriers with a moiety, such as an antibody for active targeting.[17] The antibody used in this study was HAb18 F (ab′) 2, which was specific for the hepatocellular carcinoma (HCC) tumor, the 3rd leading cause of death from cancer. The micelles were synthesized utilizing the same method as the Park group; however, once the micelles were synthesized, the antibodies were then attached. Although they exhibited lower drug loading efficiencies than naked micelles (2.19% as opposed to 2.36%), they were taken up more efficiently by cancer cells and were more cytotoxic towards them compared to naked micelles. This can be attributed to receptor mediated endocytosis, owing to the antibody moiety on the micelle. From the *in vivo* studies, they discovered that the targeted micelles accumulated in the tumor, rather than accumulating in the organs such as the liver or heart, which is the case in drugs that are not targeted. This provides an exciting opportunity for drug delivery systems in that it reduces cardiac toxicity, which is one of the leading side effects of DOX chemotherapies.

Polyesters have also found applications as micro- and nanoparticles, as well. Due to FDA approval, PLGA has found utilization in many drug delivery systems. A human growth hormone delivery system under the trademark name Lupron Depot® was the first FDA approved implantable PLGA microparticle system.[4,18] Since then, there have been reports of microparticles for use in the treatment of asthma[19] and they have even showed promise as a promising drug carrier for local sustained inhalation therapy of pulmonary diseases.[20]

Polyesters have also found usage at the nano-scale level. Owing to their small size, nanoparticles have been shown to have improved cellular penetration. Throughout the years, there have been studies in which nanoparticles have been used in many preclinical trials for treatment of cerebral diseases, osteoporosis, cardiovascular diseases, diabetes, as well as for regenerative medicine and vaccinations.[18] One such example involving the treatment of endometrial carcinoma utilized the folate receptor (which has been shown to be over expressed in tumor cells)[14] to enhance uptake by cancerous cells. It was found that not only were the nanoparticles containing the folate receptor more likely to be taken up by cancer cells, but they were also less toxic to the other organs.[21]

1.5 Poly(ester amide)s

PEAs are a promising class of biodegradable polymers. They have excellent high temperature stability, excellent mechanical strength (a result of the H bonding of the polymer backbone) and crystallinity in some cases. In addition to these qualities, PEAs also exhibit the high degradability and versatility of the polyesters.[22-24] PEAs commonly consist of three naturally occurring and non-toxic building blocks: α-amino acids, fatty diacids and diols (Figure 1.6).
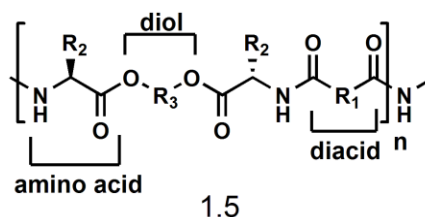
Figure 1.6 Poly(ester amide) PEA.

Due to the vast accessibility and choices of these building blocks, many different types of PEAs have been synthesized which exhibit a wide range of thermal, physical, mechanical, chemical and biodegradation properties. Also, because they degrade via a surface erosion type mechanism, they have found usage as drug eluting stents,[24-25] as commercial skin grafts (PhagoBioderm®)[26] and have shown promise for future applications in the area of drug delivery,[27-30] transfection agents,[31-33] tissue engineering[34-]

[36] and as possible candidates for processing of high performance engineering plastics and photoelectric materials.[37]

PEAs containing functional groups are advantageous in that their functional handles allow for the covalent attachment of drug molecules for drug delivery or growth factors and adhesion molecules, both important components of medical implants and tissue engineering scaffolds. As a result, there are many examples in the literature that aim to add functionality to these polymers. They shall be discussed in the following section, along with their potential applications.

1.5.1 Unsaturated PEAs

One way of adding functionality to a PEA is by incorporating double bonds in the polymer backbone, or incorporating them as pendant groups. In the first example, Chu and co-workers used the double bond on the polymer backbone as a reactive site to conjugate an amine, or a carboxylic acid via a thiol-ene reaction.[38] The polymer was synthesized via a solution polycondensation of the unsaturated monomer, di-*p*-nitrophenol fumarate with either the di-*p*-toluenesulfonic acid salts of *L*-phenylalanine butanediol-1,4-diester, or the di-*p*-toluenesulfonic acid salts of *L*-phenylalanine triethylene glycol diesters. With the polymer synthesized, the thioether was then formed by reacting the unsaturated PEA or poly(ether ester) amide (PEEA), with either 3-mercaptopropionic acid, or cysteamine (Scheme 1.3).
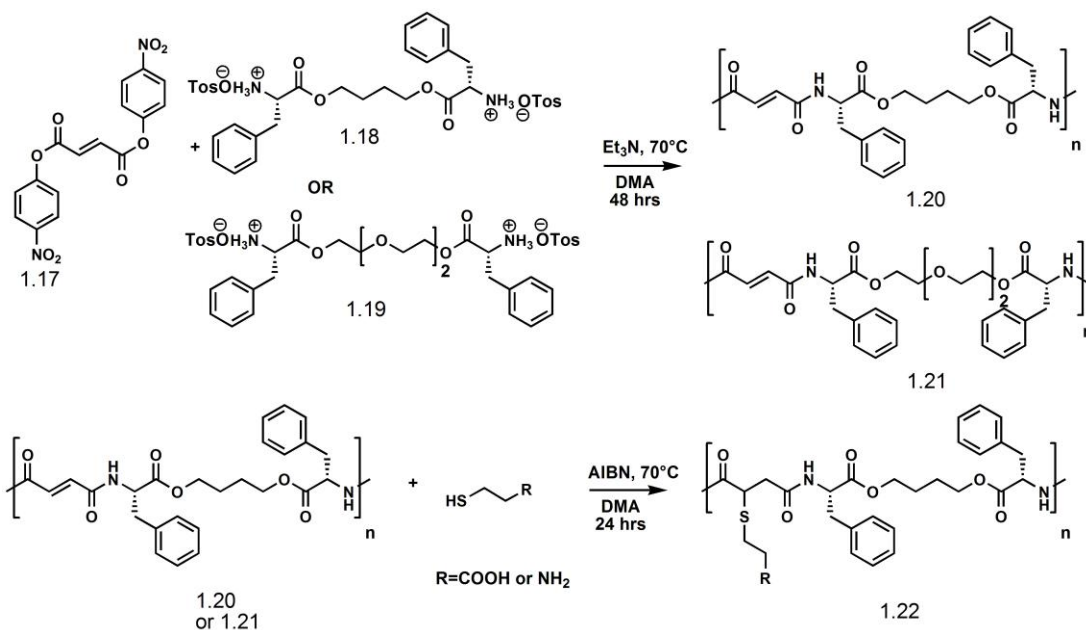
Scheme 1.3 Synthesis of functional PEA or PEEA with pendant functional groups.

The ether linkage was used as a replacement for the fully aliphatic diol because in previous work, it was found that the fumarate monomer led to polymers with high rigidity and crystallinity.[39-40] Therefore, the ether linkage was used to increase the hydrophilicity, flexibility, and biodegradability of the polymers. Thermal properties indicated that incorporation of the functional groups lowered the glass transition temperature.

Another way to add functionality using the unsaturated approach has been postulated by the same group. Instead of using the backbone as the reactive site, the pendant groups were used to conjugate molecules; this has been accomplished by using the *DL*-2-allyglycine monomer which contains a pendant double bond.[41] The polymers were synthesized by a solution polycondensation utilizing the allylglycine monomer, the di-*p*-toluenesulfonic acid salts of *L*-phenylalanine butane-1,4-diester and the di-*p*-nitrophenyl diesters, each with varying aliphatic groups and monomer ratios. The functionality was then added by conjugating the pendant alkene group with the corresponding functional thiol to form the carboxylic acid, amine, or sulfonate moiety on the backbone (Scheme 1.4). The polymers were then cast into films and mass loss studies were undertaken to determine the effect the length of the chain and the monomer ratio

had on the degradation. An increase in the methylene chain length was found to accelerate the degradation of the polymers when the appropriate enzyme was present, while an increase in the allylglycine monomer lowered the degradation rate. This was thought to be attributed to the hydrophobicity of the polymer; an increase in the chain length made it more hydrophobic and thus led to better affinity towards the enzyme. In contrast, adding more of the allylglycine monomer led to less of the phenylalanine monomer being incorporated, thereby causing the polymer to be less hydrophobic. As a result, the degradation rate decreased.
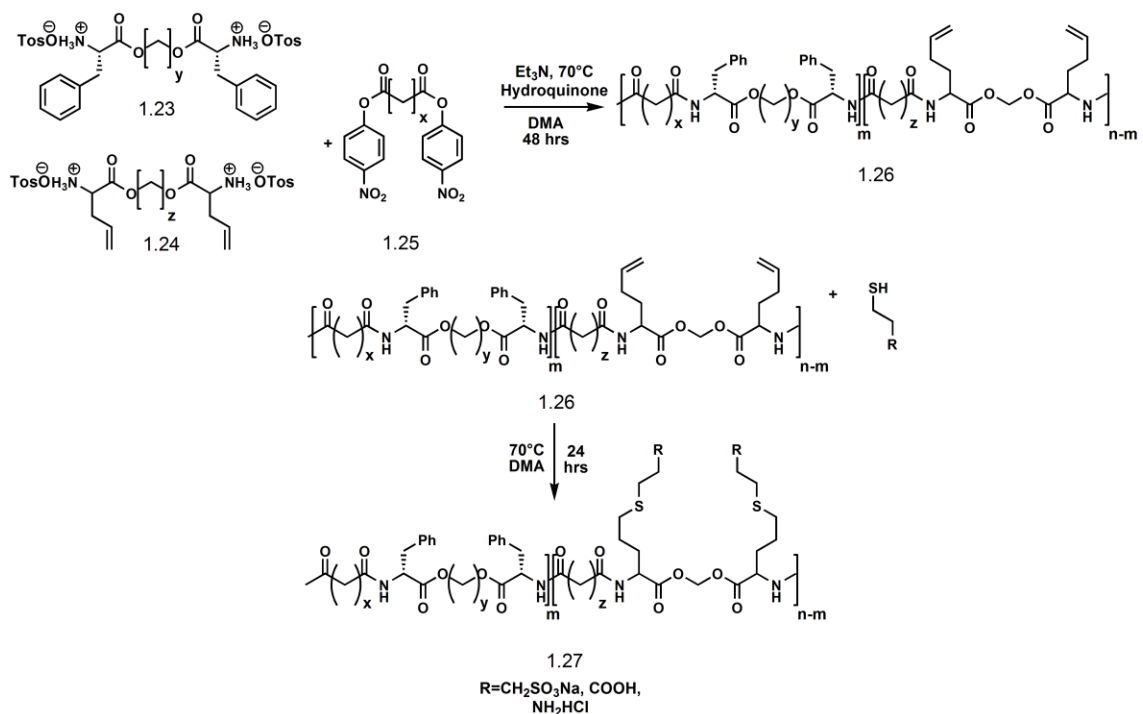
Scheme 1.4 Synthesis of functional PEAs using a pendant double bond approach.

1.5.2 PEAs using the copolymer approach

Another approach to add functionality to the PEA backbone is by copolymerization with functional monomers, an approach used by many groups with much success.[30-31,33,36-37, 41, 42-43] Recent work by Knight et al. have shown that by utilizing an interfacial polycondensation (as opposed to a solution condensation), the polymers that were synthesized had higher molecular weights and significantly reduced reaction times.[36] In

brief, the di-*p*-toluenesulfonic acid salt monomer containing a varying amount of methylene groups in the diol chain was copolymerized with bis-N-ε-*t*-Boc-*L*-lysine diester and sebacoyl chloride (Scheme 1.5). Previous work indicated that only a small percentage of the functional groups was required to impart functionality to the PEA.[42-43] As a result, the same percentage was used in this work.

The interactions of these poly(ester amide)s with human coronary artery smooth muscle cells (HCASMCs) were then studied and it was found that all PEAs not only supported HCASMC attachment, they also led to cell spreading which is indicative of a proliferative phenotype. Also, in some cases, cell viability was higher than the tissue culture polystyrene (TCPS), which served as the control. Out of all the PEAs studied, the one containing the deprotected lysine moiety was the most attractive as it could be used in future applications to conjugate molecules that regulate cell growth, differentiation and signaling pathways.
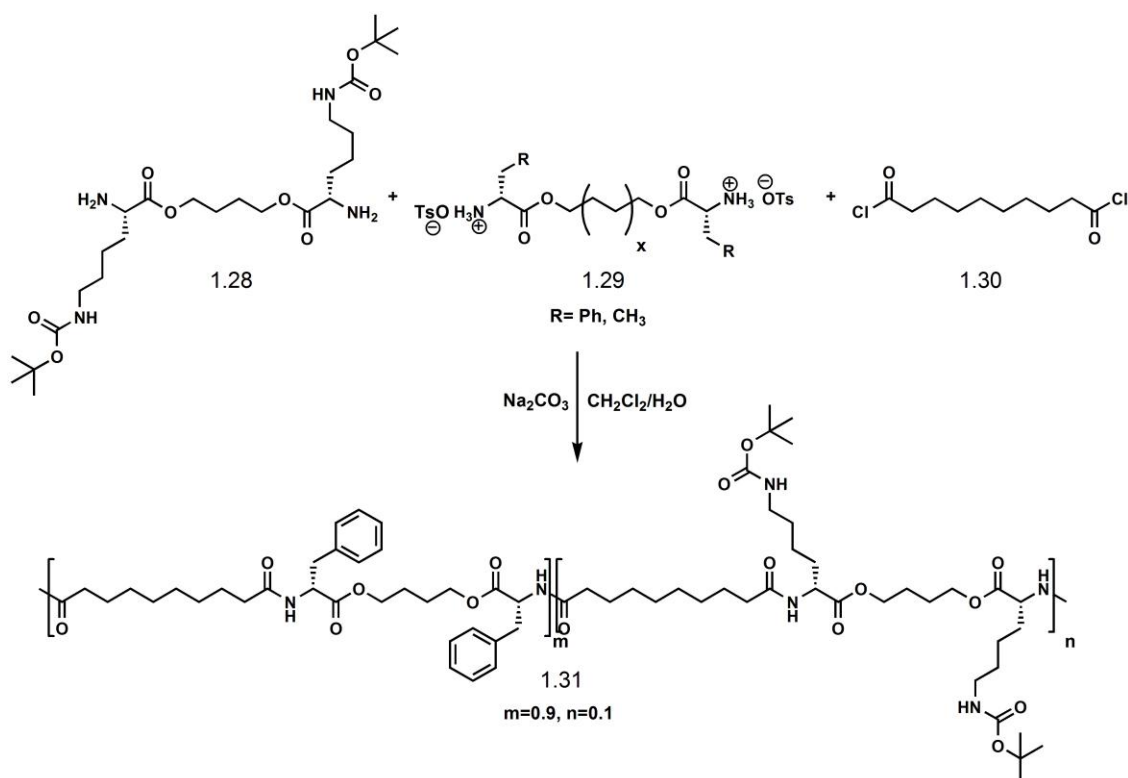
Scheme 1.5 Synthesis of functional PEA via the interfacial polycondensation method.

PEAs have also found applications in gene delivery. For example, PEAs containing the bis-*L*-arginine ester monomer (synthesized from oligo ethylene glycols), were synthesized and nanoparticles were formed to transfect cells with DNA.[33] In this particular study, the oligo(ethylene glycol) linker was substituted for the diol to not only increase its solubility, but to also improve transfection compared to previous results.[31-32] Solubility was enhanced remarkably, as polymers containing the ether linkage had better solubilities than their diol counter-parts. Mobility shift assays indicated that an increase in the methylene chain unit allowed for better DNA complexation. A similar result was found with the ether linkage to a degree, presumably because the charge density between two adjacent arginine moieties was too far apart. With respect to transfection efficiency, the newly synthesized PEAs not only had higher transfection efficiencies, but in some cases, achieved better transfection efficiencies at a lower cytotoxicity levels than Lipofectamine2000® (a commercial transfection agent). Subsequent cells studies indicated that compared to other transfection agents, these newly synthesized PEAs were less toxic, even at a higher dosage.

1.6 PEAs for drug delivery

Another exciting application for PEAs is in the area of drug delivery. There have been many ways in which drugs have been encapsulated and delivered over the years and these different methods shall be the focus of this section.

Throughout the years, dendrimers have garnered a lot of interest for biomedical applications due to their regular and highly branched three-dimensional globular structure, low viscosity, high solubility, abundance of functional end groups and internal cavities. Also, their size and structure can be tuned to ensure biocompatibility and biodegradability. Dendrimers can also be seen as the analogs of enzymes, proteins, viruses and antibodies.[44] As a result, they are attractive as drug delivery vehicles. However, a major drawback is their tedious and complex multistep synthesis which results in expensive products for limited usage in large-scale industrial applications.[27] To this end, hyperbranched polymers can be seen as viable alternatives to dendrimers.

Hyperbranched polymers consist of highly branched, polydisperse macromolecules with a treelike topology and a large number of functional groups. In cases where structural perfection is not a necessity, hyperbranched polymers may be substituted. Unlike dendrimers, their synthesis is rapid owing to their one-step reactions which results in economically promising products for small and large-scale industrial applications. Hyperbranched PEAs have been prepared and have already found applications as drug delivery vehicles, the most noted example being Hybrane® (Figure 1.7). This polymer was synthesized via a polycondensation between a cyclic anhydride and a diisopropanol amine to afford the hyperbranched structure.[45] Hybrane® has also found other applications outside of drug delivery such as in additives in polypropylene fibers to allow dyeability, suppression of gas hydrates to aid in transportation of gas or oil at low temperatures and as a paper coating to improve printing speeds.[28]

1.32
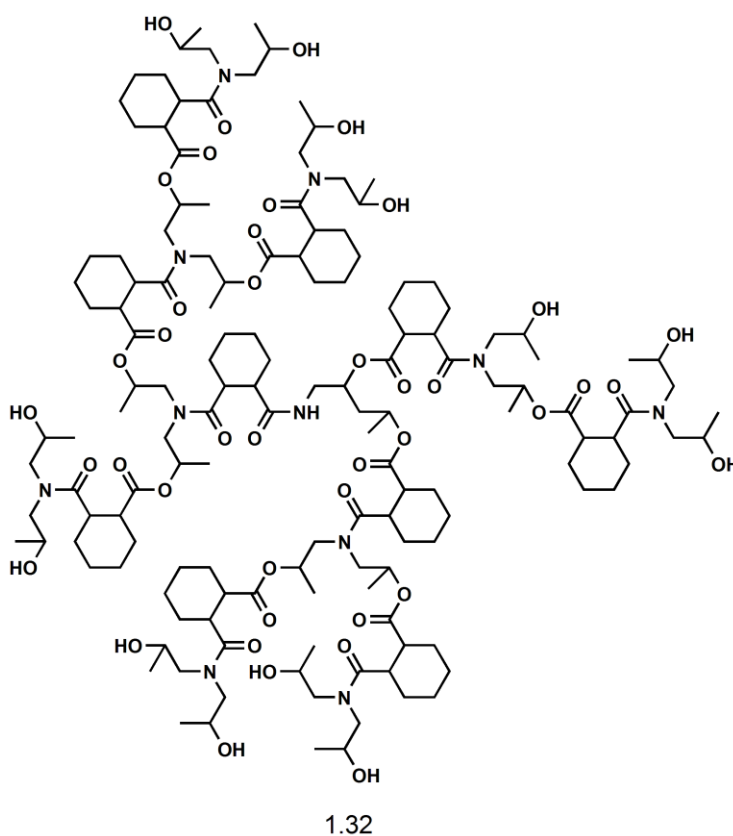
Figure 1.7 Structure of commercially available hyperbranched poly(ester amide), Hybrane®.

PEA microparticles have been reported for the physical encapsulation of drugs,[46] but in general, a problem with microparticles is their large size which causes them to exhibit rapid uptake by the liver and spleen. To address this, Zilinskas et al. sought to synthesize the first self-assembled PEA micelle by grafting a hydrophilic block (via the pendant amine group), in order to form an amphiphilic block copolymer (Scheme 1.6).[30] The hydrophilic block grafted was PEG, chosen for its high water solubility, known biocompatibility in drug delivery applications and its stealthy properties. Micelles were formed using the nanoprecipitation method and the sizes were suitable for *in vivo* studies (<100 nm). Nile red was then used as a model drug and was encapsulated. Nile red was found to be released over a period of 15 hr at physiological pH, whereas an accelerated release occurred at pH 5; both rates were reasonable for drug delivery applications. The enhanced release at the acidic pH was thought to be attributed to the protonation of the residual pendant amine groups in the micelle core, which increased the hydrophilicity of the core, thereby favoring drug release. Also, protonation of the drug at acidic pH would also favor release of the protonated drug molecule into the aqueous environment. Cell toxicity studies indicated that the micelles were not toxic to HeLa cells *in vitro*, even at a high 2 mg/mL concentration.
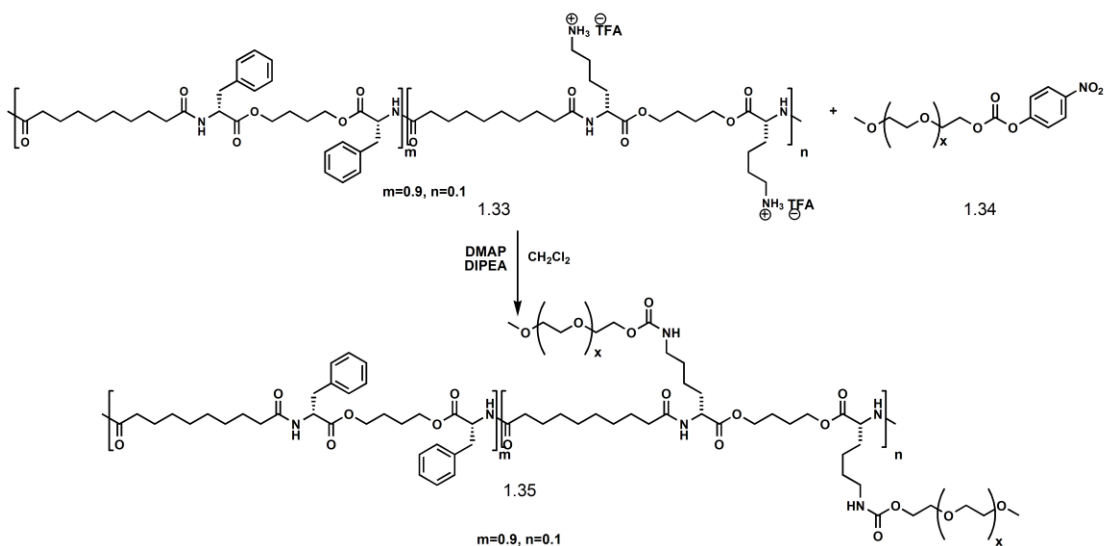
Scheme 1.6 Formation of amphiphilic block copolymer for self-assembly of PEAs into micelles.

1.7 Limitations of Polyesters and PEAs

Although polyesters have been used in many drug delivery applications, they suffer from an initial burst release in which a considerable amount of drug is released at the start of the degradation; this burst release may be harmful for *in vivo* applications. The burst release is a result of their bulk erosion degradation mechanism,[1,9,47] resulting in accumulation of acidic byproducts in the system.[2,9] These acidic byproducts are detrimental to cell growth and limit the usage of polyesters for tissue engineering.

To circumvent these problems, PEAs have been seen as a viable alternative. As PEAs often degrade via a surface erosion mechanism,[47-50] they are more attractive for drug delivery systems because they should lead to a sustained drug release over time. Also, because the degradation byproducts are often natural building blocks, they are expected to be relatively non-toxic.[22,26,51]Although their degradation is accelerated in the presence of enzymes,[48-50,52] their degradation in neutral buffer is extremely slow.[26,41] Also, one should note that in the biological world, in order for enzymes to be present; the correct cells must also be present, an idea often overlooked when designing biodegradable polymers.[3] To date, there exists no way to initiate the degradation of PEAs; they degrade via random uncontrolled hydrolysis of the polymer backbone.

1.8 Stimuli responsive polymers

Over the years there have been an increasing number of examples in the literature that aim to instigate the degradation of a polymer using a trigger. These polymers contain specific functional groups that are sensitive to specific stimuli (i.e. light, change in pH or redox potential). Upon encountering these stimuli, the functional groups react or change in some manner, releasing the payload (Scheme 1.7). The following section shall discuss recent advancements in this area, as well as different types of stimuli responsive polymers.
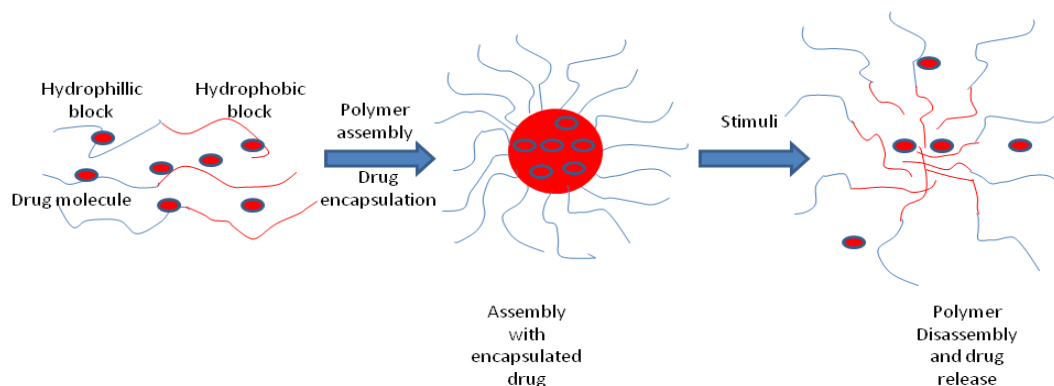
Scheme 1.7 Self-assembly, encapsulation of drug and subsequent release.

1.8.1 Acid sensitive polymers

Change in pH is a particularly useful type of stimulus for drug delivery agents, as there are numerous pH gradients in both normal and diseased states. It has been well documented that the extracellular pH of tumors is lower than that of normal tissue, with an average pH of 7.0, as opposed to the normal physiological pH of 7.4.[53] The pH drops to about 5.5-6.0 in the endosomes and is close to 5.0 in the lysosome.[53] As a result, drug carriers may exploit this gradient to deliver their payload to a specific area without harming nearby tissues. There have been numerous examples that have utilized this pH gradient and they shall be the focus of this section.

One of the ways in which a polymer may be sensitive to acidic pH is by incorporating an acid degradable linkage in the hydrophobic part of the backbone.[53-56] Upon forming a self-assembly and encountering an acidic environment, the acid sensitive linkage is cleaved which leads to disruption of the micelle core and release of any payload, if present. Chen and coworkers have synthesized polycarbonates (Scheme 1.8),[56]

containing different electron donating groups that were able to form micelles. At physiological pH there was negligible degradation. However, the half-lives at pH 4 and 5 were 1 and 6.5 hrs, respectively. It was found that the polymer containing the two methoxy groups degraded faster, presumably because of their electron donating properties. The drugs paclitaxel and doxorubicin were then loaded with efficiencies of 65 and 60% respectively; release studies were then undertaken. *In vitro* studies showed that release was accelerated at pH 4 and 5, as opposed to 7.4. For example, within 48 hr the release of both drugs were 98, 89, and 44% at pHs 4, 5, and 7.4, respectively.
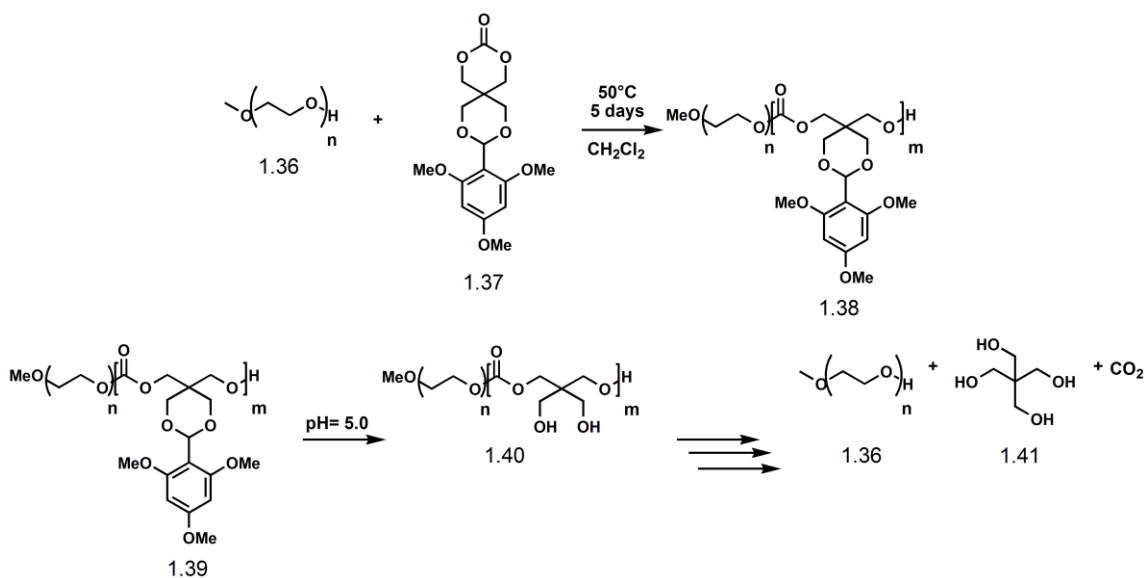
Scheme 1.8 Synthesis of polycarbonate and subsequent degradation.

In some cases, a drug may be conjugated to a copolymer by an acid-labile linker[57-59], so that the polymer backbone remains intact. This is beneficial in that no further modification of the drug is required and can be generalized to different drugs without changing the linkage. In a particular example,[59] doxorubicin was conjugated to a double hydrophilic block copolymer of poly(ethylene glycol)-*hyperbranched*-polyglycerol (PEG-*hb*-PG) by a hydrazone linker (Figure 1.8). Micelles were formed and it was found that the release was accelerated at pH 5, as opposed to pH 7.4 (71% compared to 53.6% after 48 hr). Laser fluorescence microscopy proved that the micelles were internalized within the cell and cell viability studies indicated that the micelles were not cytotoxic to HeLa cells, due to the drug.

1.42
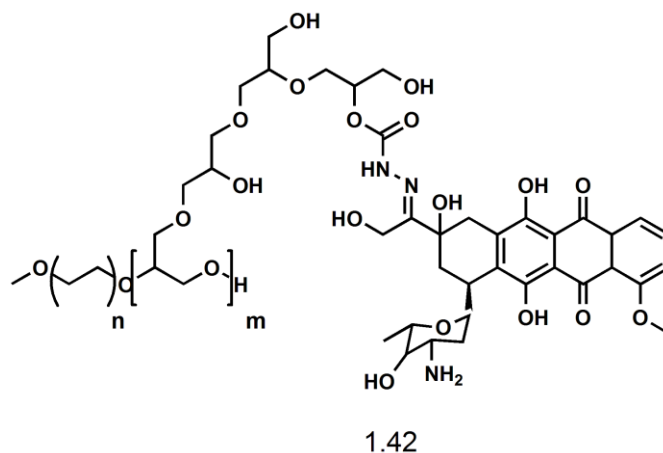
Figure 1.8 Doxorubicin conjugated to PEO-*hb*-PG by a hydrazone linkage.

1.8.2 Reduction sensitive polymers

There exists a large difference in reducing potential between the intracellular and extracellular environments of the cell.[60] It has also been proven that tumor tissues are highly reducing.[61-62] As a result, there have been many drug delivery vehicles that aim to use this reduction potential as a trigger to initiate degradation. The linkage that is sensitive to this trigger is the disulfide bond, which can either be incorporated into the main chain, or may be used as a cross-linker.

A disulfide bond may be incorporated into the polymer backbone so that upon reduction, the polymer chain is cleaved, thereby commencing degradation. A class of polymers that incorporates the disulfide into the polymer backbone is the poly(amido amine)s, which have gained usage as transfection agents due to their buffering capacities.[63-65] In a particular example,[63] Zhong and co-workers synthesized a series of reducible poly(amido amine)s in order to compare their buffering capacities (Scheme 1.9). Buffering capacity is thought to play a role in endosomal escape of polyplexes ("proton sponge theory").[66] These polymers were synthesized by a Michael addition between various amines to the disulfide-containing cysteamine bisacrylamide. It was found that the derivatives containing the dimethylamino as the pendant group were the least effective, mainly because protonation of the tertiary nitrogen on the backbone was suppressed.

Scheme 1.9 Derivatives of reducible poly (amido amine)s and their degradation.

Using the ABOL derivative as an example, the degradation was monitored in non-reducing conditions and there was only a 5% decrease in M_w after 3 days and up to 60% decrease after 20. In contrast, when dithiothreitol (DTT, a well known reducing agent) was added, the polymer completely degraded within 5 minutes. The polymer was found to retain DNA effectively and was also able to release it once DTT was added. Cell toxicity studies indicate that the reducible poly(amido amine)s have better cell viabilities (~100%) than the control, polyethyleneimine (~20%). These polymers were also found to have better transfection efficiencies than the control.

Another way to add reducing functionality to a polymer is by adding the reducing linkage in the linker.[67-69] Using a recent example,[67] a new gene carrier composed of poly(*L*-lysine) and PEG was synthesized containing two labile linkages - one responsive to a decrease in pH and the other responsive to a change in reduction potential (Figure 1.9). It was reasoned that by adding a reduction labile PEG shell (which would aid in intracellular uptake), the acid linker would disintegrate in the endosome and aid in release of genetic payload to the cytosol. Although the buffering capacity of this catiomer (mPEG-SS-PLL$_{15\text{-Star}}$) was reduced compared to the control polyethyleneimine, the cell viability profiles were enhanced for this novel polymer. The catiomer was also able to not only condense DNA, it was also able to release it upon applying either one of the stimuli (reduction in pH, change in reduction potential).

Figure 1.9 Dual stimuli responsive catiomer, mPEG-SS-PLL$_{15\text{-Star}}$.

1.8.3 Photochemically sensitive polymers

Light as a stimulus is attractive because it is non-invasive and can be applied from an external source for a short period of time with high spatial and temporal precision.[70] Recently, near infrared (NIR) light has achieved much attention due to its ability to penetrate deeper into the tissue and because it is less harmful to cells.[70-74] Functional groups that are sensitive to light have been implemented into polymers to stimulate their degradation. These functional groups are *o*-nitrobenzyl[70,72, 75-79] and derivatives of bromocoumarin.[70-71,73-74,77]

One example incorporating the *o*-nitrobenzyl moiety is illustrated by Dong and coworkers.[79] In this particular example, a new polypeptide was synthesized by the ring opening polymerization (ROP) of a photoresponsive S-(*o*-nitrobenzyl)-*L*-cysteine-*N*-carboxyanhydride to form the corresponding poly(S-(*o*-nitrobenzyl)-*L*-cysteine)-*b*-poly(ethylene glycol) (pNBC-b-PEG) (Scheme 1.10). The copolymers were found to form spherical micelles and irradiation for 20-40 minutes at 365 nm was required to completely remove the photolabile groups, depending on the polarity of the solution. Upon encapsulation of DOX and irradiating for different time periods (i.e. 2, 5 and 10

min), it was found that DOX release became faster with increasing irradiation time; this demonstrates that DOX can be released in a controlled manner by varying irradiation time.
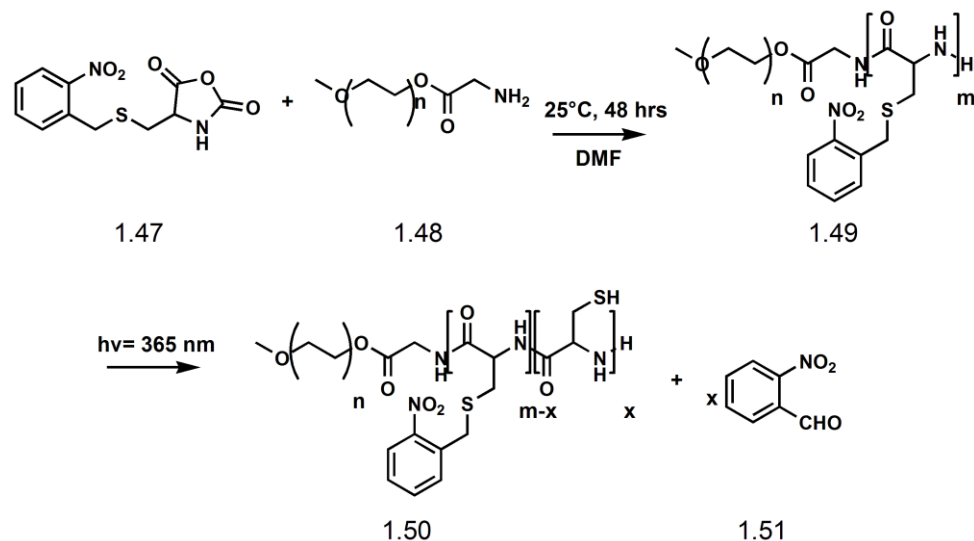
Scheme 1.10 Synthesis and photo-cleavage of pNBC-b-PEO.

NIR irradiation presents another way to photochemically degrade a polymer. In order to make their polymers sensitive to NIR light, Zhao et al. added coumarin moieties to the glutamic acid monomers in order to increase its hydrophobicity and thus assist micelle formation in an aqueous solution.[73] It was reasoned that upon self-assembly of the block copolymer and subsequent irradiation with NIR light, the hydrophilic-hydrophobic balance of the micelle would be disrupted, leading to destabilization of micelles in solution (Scheme 1.11). When these micelles were irradiated at 794 nm in solution, it was found that the absorbance corresponding to the coumarin groups disappeared after 220 minutes of irradiation. Two different drugs were encapsulated and then released with varying times. In the case of the rifampicin antibiotic drug, there was ~75% release after 55 hours, compared to ~10% in a sample that was not irradiated. For paclitaxel, the release was found to be 50% after 145 hours, with neglible release from the control. The decreased release is thought to be attributed to the hydrophobic nature of paclitaxel, compared to rifampicin which is amphiphilic.
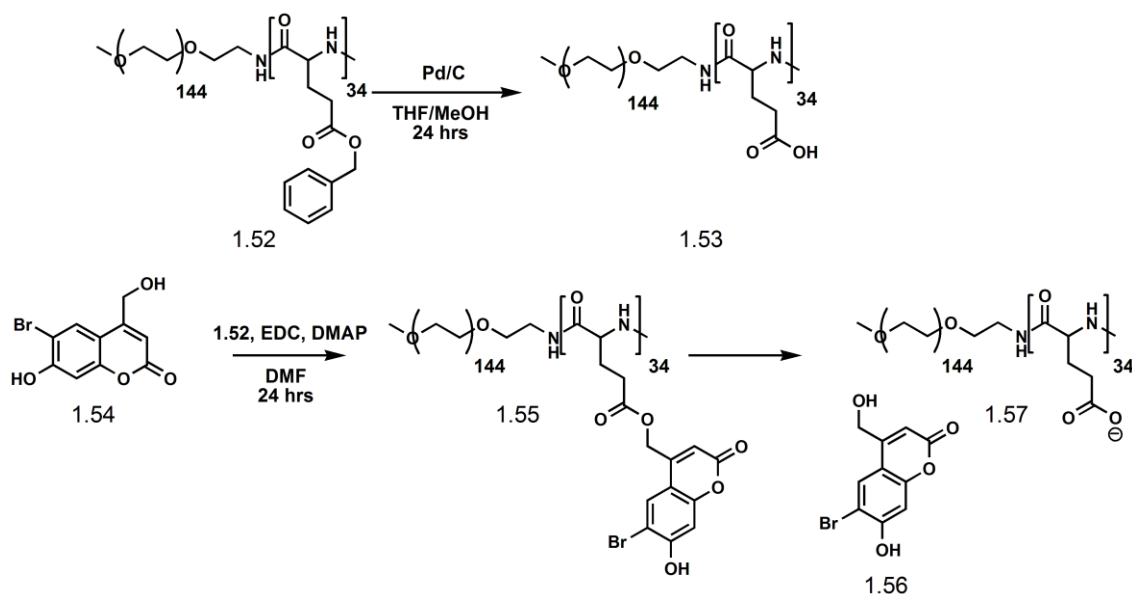
Scheme 1.11 Synthesis and photo-cleavage of PEG-*b*-p(LGA-*co*-COU)**.**

1.9 Self-immolative spacers

Self-immolative spacers have been involved in prodrug synthetic strategies since the early 1980s. It was during this time that Katzenellenbogen and coworkers hypothesized that adding a linker in between the drug and the specifier would not only prevent premature release of the drug, it would also increase accessibility of the cleavage site, thereby enhancing release (Figure 1.10a) .[80] As a result, the first ever self-immolative spacer was created which was based on 4-aminobenzyl alcohol. Unmasking of the aromatic amine began a 1,6-elimination which led to an electronic cascade, resulting in the release of CO_2, followed by the free drug or other leaving group (Figure 1.10b). Other examples of self-immolative spacers are the ones capable of cyclization. In the first example, the functional group undergoes 1,5-cyclization to form the corresponding lactone (Figure 1.10c),[81] whereas in the second example, due to three interlocking methyl groups; cyclization forms the corresponding coumarin (Figure 1.10d).[82] The applications of these spacers are diverse and they range from applications in prodrug strategies,[83-85] signal amplifiers,[86-87] and as latent fluorophores.[88-89] Their applications in linear polymers shall be discussed herein.
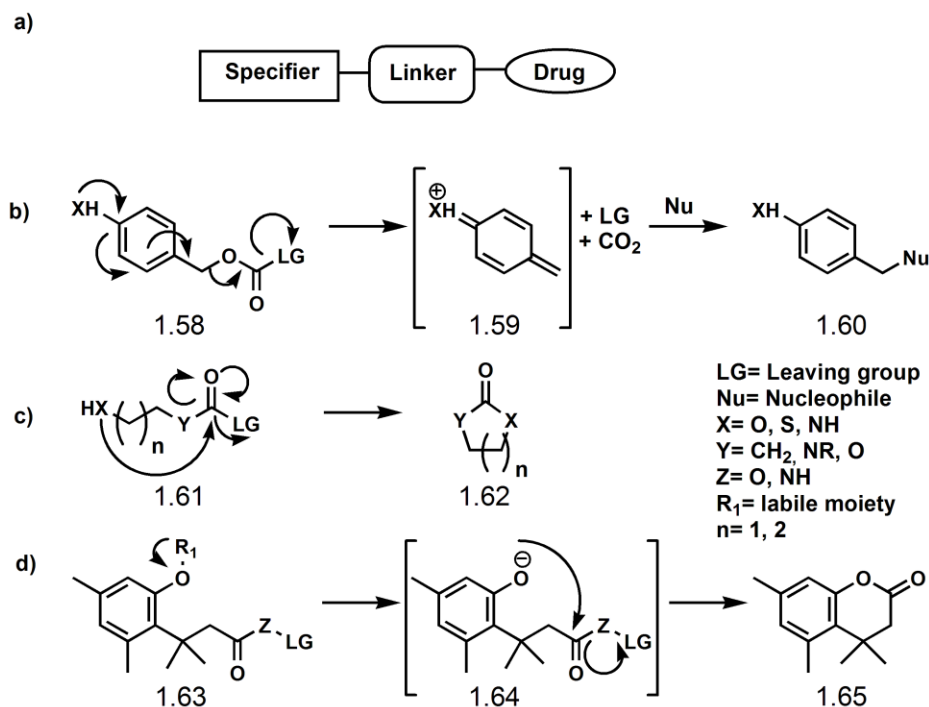
Figure 1.10 a) Schematic of a prodrug consisting of: specifier (trigger), spacer and drug; b) general structure of electronic cascade spacers; c) general structure of cyclization spacers; d) Rapid reaction of a trimethyl lock acid to form a δ-lactone.

1.9.1 Self-immolative linear polymers

When self-immolative spacers were first introduced, they were incorporated into dendritic systems for drug delivery applications with much success. They were advantageous in that only one single event was required to release multiple drug molecules; a signal amplification of sorts.[90-94] However, the number of groups that can be incorporated on a dendritic periphery is limited by steric hindrance and the higher generations require additional synthetic steps.[95] As a result, the first linear self-immolative polymer was created by the Shabat group.[96] The polymer was created by polymerizing a phenyl carbamate derivative and then capping it with an enzyme-labile trigger, 4-hydroxy-2-butanone. The protein BSA then catalyzed the removal of the trigger and the florescent molecule was emitted over a period of ten hours (Scheme 1.12). Since it has been proven that the cyclization mechanism is slower than the elimination mechanism, cyclic spacers have been incorporated by the Gillies group into linear

polymers to adjust the rate of degradation. For example, polymer **1.69** (Scheme 1.13) degrades by alternating cyclization and elimination reactions at a slower rate than polymer **1.66**.[97]
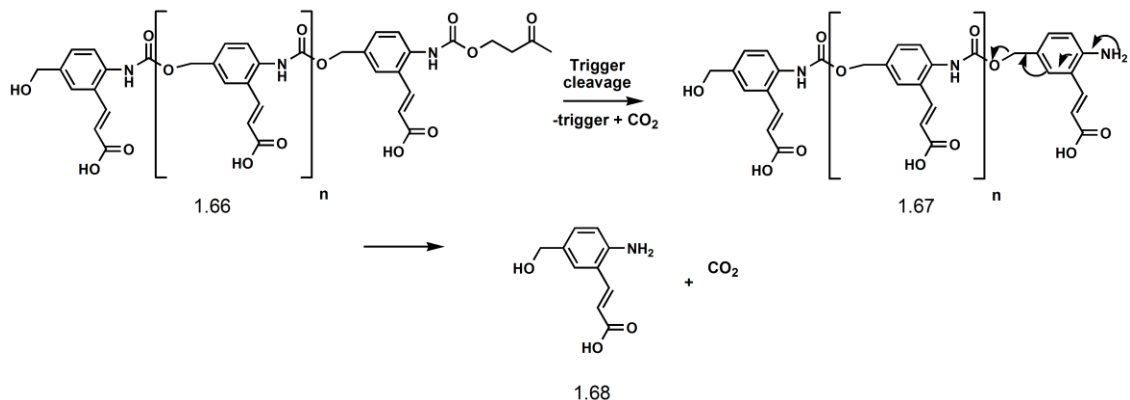
Scheme 1.12 Disassembly of linear self-immolative polymer.
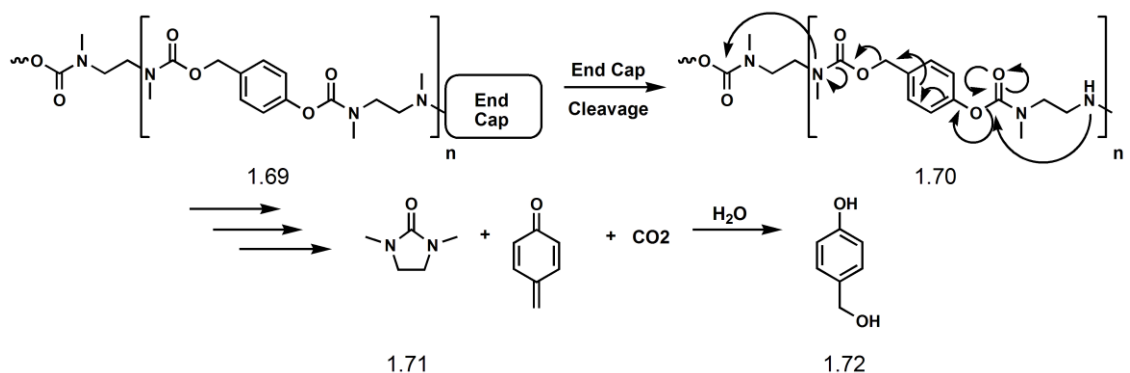
Scheme 1.13 Disassembly of self-immolative polymer by alternating and 1,6-elimination reactions.

Previous studies that require light as a trigger have involved long irradiation times, and as a result, do not seem to have practical applications in the biological realm. In order to evade this issue, Almutairi and coworkers designed a system that was sensitive to brief irradiation.[71] Using polymer **1.69** from the Gillies group as a starting point, they capped the polymer with a trigger that was sensitive to either UV (*o*-nitrobenzyl), or NIR (4-bromo-7-hydroxycoumarin) light to compare the effect each had on degradation rate. Degradation was monitored by SEC and it was found that the polymer triggered under UV light for 10 min was fully degraded after 24 days. In

contrast, the polymer triggered under near IR light for 10 min was 70% degraded after 4 days. Nanoparticles were created from these polymers and florescence dropped to 65% and 40% after 1 and 5 minutes of irradiation. The polymers were also subjected to toxicity studies and it was revealed that the polymers and degradation products were tolerated as well as the FDA approved polymer, PLGA.

As there have been studies that indicate that quionone methides can contribute to toxicity,[98-99] Gillies and coworkers synthesized a self-immolative polymer consisting entirely of cyclization spacers.[100] The spacers that were chosen for this particular study consisted of 2-mercaptoethanol and *N,N'*-dimethylethylenediamine. These spacers cyclized to their corresponding cyclic thiocarbonate and cyclic urea respectively (Scheme 1.14). Degradation experiments were carried out and the polymer was found to be 80% degraded after 10-14 days, with the control polymer (with no reducing agent added), hardly exhibiting degradation over the same time period. Related cyclization spacers based on 4-aminobutyric acid were also developed by Gillies and coworkers with the aim of obtaining more rapid cyclization kinetics. However these have not yet been incorporated into linear polymers (Figure 1.11.) .[101]
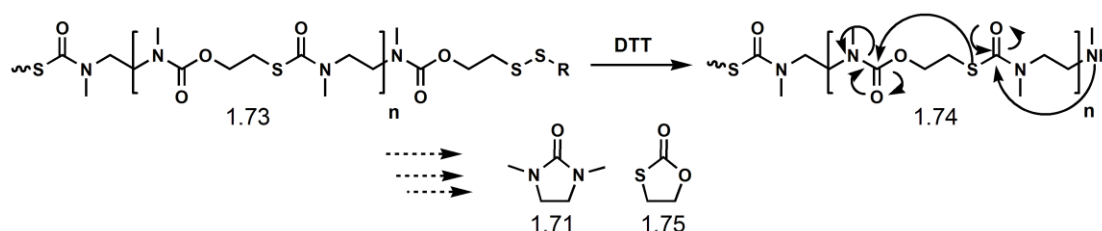
Scheme 1.14 Disassembly of a reduction sensitive self-immolative polymer.

1.76

R= Ph, allyl, OH, aliphatic chain
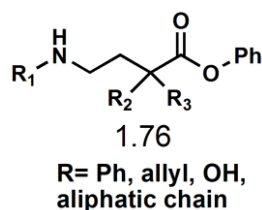
Figure 1.11 4-aminobutyric acid.

Although there have been many applications in which the so called "trimethyl lock" spacer has been used in prodrug applications,[102-103] their use in the development of cleavable polymers has also been recently reported.[104] Redox-sensitive nanoparticles were synthesized that had the trimethyl-locked benzoquinone moiety monomer. This monomer was polymerized with adipoyl chloride and nanoparticles were subsequently formed using the emulsion method (Scheme 1.15). It was reasoned that upon reduction of the benzoquinone moiety, the functional amine groups would be revealed, causing chemical changes in the nanoparticles which would lead to swelling or dissolution at physiological pH. Paclitaxel was loaded into these polymers at an efficiency of 77.9% and redox-triggered drug release was carried out *in vitro*.
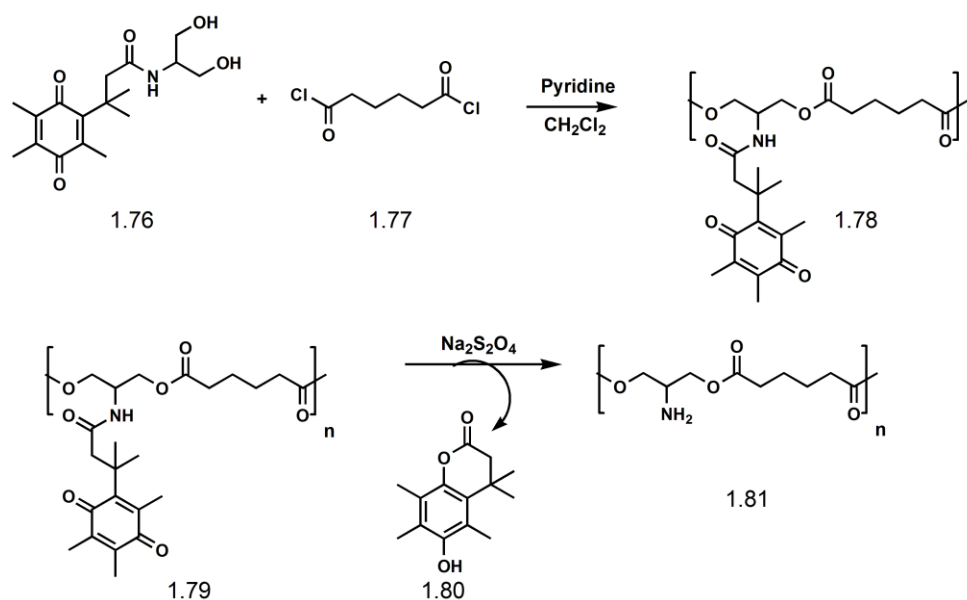
Scheme 1.15 Synthesis and subsequent reduction of redox-sensitive polymer based on trimethyl-locked benzoquinone.

1.10 Objective of this thesis

The aim of this thesis was to combine the structures of PEAs bearing pendant functional groups with the concepts of self-immolative spacers to develop PEAs for which the degradation of the backbone can be triggered by an external stimulus. As described above, the Gillies group has previously demonstrated that amino acids, such as lysine and

aspartic acid bearing protected pendant functional groups can be incorporated into PEAs. For this thesis it was proposed that by the use of amino acids such as *L*-2,4-diaminobutyric acid and *DL*-homocysteine into the PEA backbone, it would be possible to trigger the cleavage of the PEA backbone upon cleavage of a protecting group or "trigger" on the amine or thiol moiety respectively (Scheme 1.16). Described in the following sections are studies to demonstrate that the 4-amine on an aliphatic ester derivative of *L*-2,4-diaminobutyric acid (DAB) does undergo cyclization to the corresponding lactam when the free 4-amine is revealed and that similarly, the thiol on *DL*-homocysteine (Hcy) cyclizes to form the thiolactone when the thiol is revealed. The synthesis of the target polymers is also described, followed by studies of their degradation in solution and in thin films. In addition, the development of a photoresponsive version of the polymer containing DAB is also described, followed by studies of its degradation.
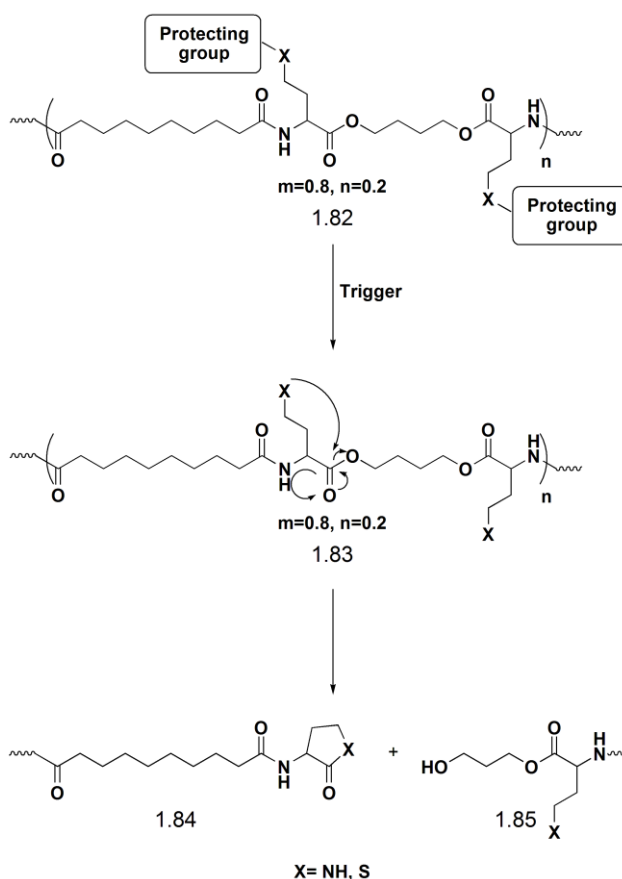
Scheme 1.16 Degradation of PEA via a triggered cyclization reaction

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Chapter 2
Controlled degradation of PEAs via cyclization of pendant functional groups

2.1   Introduction

Polymers that degrade under biological conditions have become a part of our daily lives due to their utilization as sutures,[1,2] stents,[3,4] drug delivery vehicles,[5,6-9] packaging for medical and non-medical applications [2,5,10] and as scaffolds for tissue engineering.[1,11] Of the different types of the polymers used for these applications, the poly(hydroxyacid)s have garnered the most attention; receiving FDA approval in some cases.[1,2,9,10] Although widely used in many applications, their bulk degradation results in many acidic byproducts,[1,11-13] resulting in inflammation which is undesirable in many medical applications. Also, their lack of functionality and inability to exhibit stimuli-mediated degradation are other limitations of this class of polymers.

PEAs have been proposed as an alternative class of materials to polyesters. They are composed of amide and ester bonds which offer the thermal and mechanical properties derived from polyamides, while giving the degradability and flexibility of polyesters.[14-16] In addition, their monomers can be selected from simple metabolic intermediates such as amino acids and dicarboxylic acids, such that their degradation by surface erosion results in non-toxic, non-inflammatory byproducts.[14,17-18] Another advantage is that by using amino acid monomers with functional handles, PEAs with functional groups can be prepared. These functional handles may be used for the conjugation of drug molecules in delivery systems, cell signaling molecules in tissue engineering scaffolds and as a means to tune the properties of the polymer.[19-23] As a result, there have been many instances of PEAs being used for biomedical applications such as drug delivery,[24-27] gene carriers[28-30] and as scaffolds for tissue engineering.[19-20,31] However, much like polyesters, their degradation is relatively uncontrolled and although they have been known to degrade under enzymatic conditions *in vitro*, their degradation under neutral conditions is still slow.

The ability to trigger degradation under conditions such as photochemical, or changes in pH or redox potential is advantageous in that a polymer backbone may be stable to for extended periods, but degrades once the appropriate stimuli is applied; resulting in a controlled degradation. As a result, there have been many polymers that have been synthesized that are sensitive to stimuli such as light,[32-41] changes in pH,[42-49] or redox potential.[50-56] Another way to impart controlled degradation to a polymer has been through the incorporation of self-immolative spacers. The first self-immolative spacers were based on 1,6-elimination reactions.[57] They first found use in prodrug strategies where unmasking of an aromatic alcohol, amine, or thiol allowed it to become electron-donating, initiating an electronic cascade leading to release of a free drug or other leaving group. This strategy has since been applied to drug delivery applications.[58-62] Although initial work in the field of dendrimers was successful, the additional synthetic steps for the higher generations prevented its use for future applications, thereby inspiring the use of linear self-immolative polymers.[63] It was later demonstrated by our group that by incorporating self-immolative spacers capable of cyclization, the rate of degradation could be adjusted.[64] As there have been studies that indicate that the quinone methide intermediated formed during the elimination reaction can result in toxicity, [65-66] the Gillies group also synthesized a self-immolative polymer consisting entirely of cyclization spacers.[67] Although the degradation required several days due to the slow rate of the diamine cyclization, related cyclization spacers based on 4-aminobutyric acid have since been developed by Gillies and coworkers with the aim of obtaining more rapid cyclization kinetics.[68] However, these have not yet been incorporated into linear polymers.

The aim of this work is to combine the structures of PEAs bearing pendant functional groups, with the concepts of self-immolative spacers, to develop PEAs for which the degradation can be triggered by an external stimulus. Since previous work from our group has demonstrated that amino acids such lysine,[20,23,27] and aspartic acid[69] can be incorporated into the PEA backbone and subsequently deprotected following polymerization, for this work we propose incorporating amino acids such as *L*-2,4-diaminobutyric acid and *DL*-homocysteine into the PEA backbone. These amino acids would allow triggering of the degradation of the PEA backbone following deprotection of

a thiol, or amine moiety, respectively. Described in the following sections are studies to demonstrate that the 4-amine on an aliphatic ester derivative of *L*-2,4-diaminobutyric acid (DAB) does undergo cyclization to the corresponding lactam when the free 4-amine is revealed and that similarly, the thiol on *DL*-homocysteine (Hcy) cyclizes to form the thiolactone when the thiol is revealed. The synthesis of the target polymers is also described, followed by studies of their degradation in solution and in thin films. In addition, the development of a photoresponsive version of the polymer containing DAB is also described, followed by studies of its degradation.

2.2   Results and discussion

2.2.1 Molecular design

The choice of pendant functionalities that would induce cyclization, and thus backbone cleavage of the PEAs was based on previous work in the Gillies group. DeWit and Gillies recently demonstrated that the amine moiety of 4-aminobutyric acid derivatives cyclized rapidly upon phenyl esters to provide the corresponding 5-membered lactam.[68] Recent work by Chen et al. showed that a thiol moiety could be more nucleophilic than the amine at the neutral pH of 7.4, and thus undergo cyclization to the corresponding 5-membered ring more rapidly.[70] Based on these data, DAB and Hcy were the target amino acids for incorporation into the PEA backbone.

Following previous synthetic protocols for PEAs, it should be possible to prepare diamine-diester derivatives of these amino acids with aliphatic diols and polymerize these with aliphatic dicarboxylic acid derivatives by solution or interfacial routes. The interfacial route was selected as we have found that it leads to higher molecular weight (MW) polymers with shorter reaction times.[20,26] This dictates the use of sebacoyl chloride as the dicarboxylic acid derivative as the shorter chain derivatives are too soluble in the aqueous phase of the CH_2Cl_2-water mixture that is used for the interfacial polymerization, resulting in premature monomer hydrolysis. 1,4-Butanediol was chosen as the aliphatic diol because it is easy to work with synthetically. In principle, it would be feasible to polymerize sebacoyl chloride with the diamine-diester derivative of DAB or Hcy (having protected side chain moieties). However, as with other PEAs containing

pendant functional groups, these DAB and Hcy monomers are relatively expensive to prepare, and to impart degradability to the target PEAs, it is not necessary to have a self-immolative spacer at every repeat unit. Thus the functional DAB and Hcy monomers were diluted with an *L*-phenylalanine (Phe)-1,4-butanediol diester-diamine to provide 20% of the DAB or Hcy monomer is the resulting polymer. This Phe monomer was selected due to its ease of synthesis and for the thermal and mechanical properties of the resulting polymer which enhance the polymer's processability in melt.[71] Thus, the specific target polymers **2.1** and **2.2** containing DAB and Hcy are shown in Figure 2.1.

A t-butyloxycarbonyl (Boc) protecting group was selected for the DAB monomer. Although this Boc group cannot be cleaved under physiological conditions, it can be readily cleaved with trifluoroacetic acid (TFA), allowing the degradation process to be readily studied. It can later be replaced with a more specific trigger as described in section 2.2.7 of this thesis. A dithiopyridyl moiety was selected for the Hcy monomer as it can be cleaved by biological reducing agents such as glutathione, which are present at elevated levels in the intracellular environment as well as in hypoxic tissues such as tumors.[72-74] In addition to the target polymers, two small molecule derivatives **2.3** and **2.4** of DAB and Hcy were targeted in order to study the cyclization rate upon removal of the side chain protecting group and to verify that the cyclization was faster than background hydrolysis of the ester.
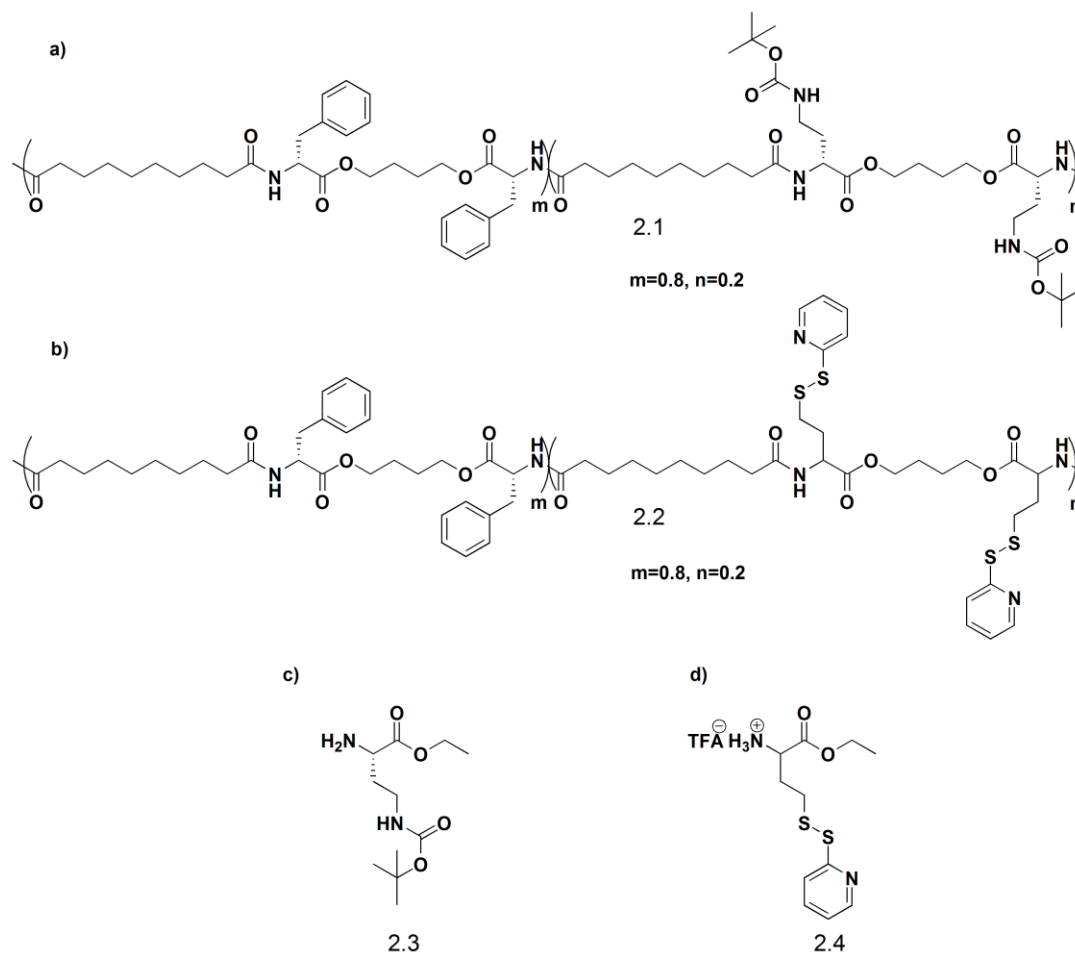
Figure 2.1 Chemical structures of proposed PEAs incorporating a) DAB monomer, b) Hcy monomer, c) DAB ester with Boc protecting group, d) Hcy ester with dithiopyridyl protecting group.

2.2.2 Synthesis of small molecule model compounds 2.3 and 2.4

The preparation of the model compounds **2.3** and **2.4** was necessary because the rates of the specific cyclization reactions to be utilized in this work had not been explicitly studied and it was important to understand their rates in the context of the future polymer degradation experiments. While phenyl ester derivatives of 4-aminobutyric acid have been previously reported by our group, an ethyl ester was selected for this work as it more closely mimics the aliphatic ester in the target polymer. The synthesis of this DAB ester was investigated using two different routes. In the first approach (the direct-method approach, Scheme 2.1), a Fischer esterification was attempted. The reaction conditions

involving sulfuric acid proved unsuccessful, presumably due to the residual water in the acid. Another attempt was made using dry hydrochloric acid formed from calcium chloride; [75] however, this method proved to be more labor-intensive as acid had to be constantly replenished and finally, neutralized with base once the reaction was complete.
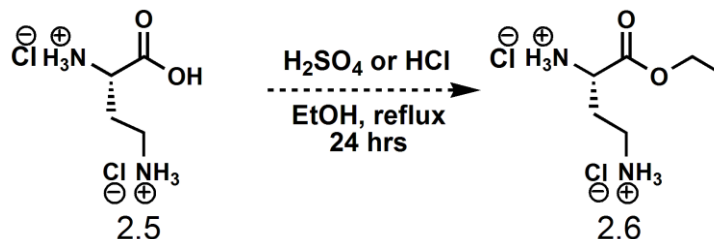
Scheme 2.1 Unsuccessful synthesis of DAB ethyl ester using direct-method approach.

As the direct-method approach was unsuccessful, the synthesis was performed using the commercially available starting material, (9-fluorenylmethoxycarbonyl) Fmoc-DAB(Boc)-OH that was in hand for preparation of the polymerization monomer as a starting point (Scheme 2.2). First, the ethyl ester was synthesized using a *N,N'*-dicyclohexylcarbodiimide (DCC)-mediated coupling with catalytic amounts of 4-(dimethylamino) pyridine (DMAP) and 4-(dimethylamino)-pyridinium-4-toluenesulfonate (DPTS) to afford **2.9**. With the ester in hand, the protecting group was removed in order to make the ester soluble in the phosphate buffer. The Fmoc group was removed using a catalytic amount of 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) and excess 1-octanethiol, providing **2.3**.
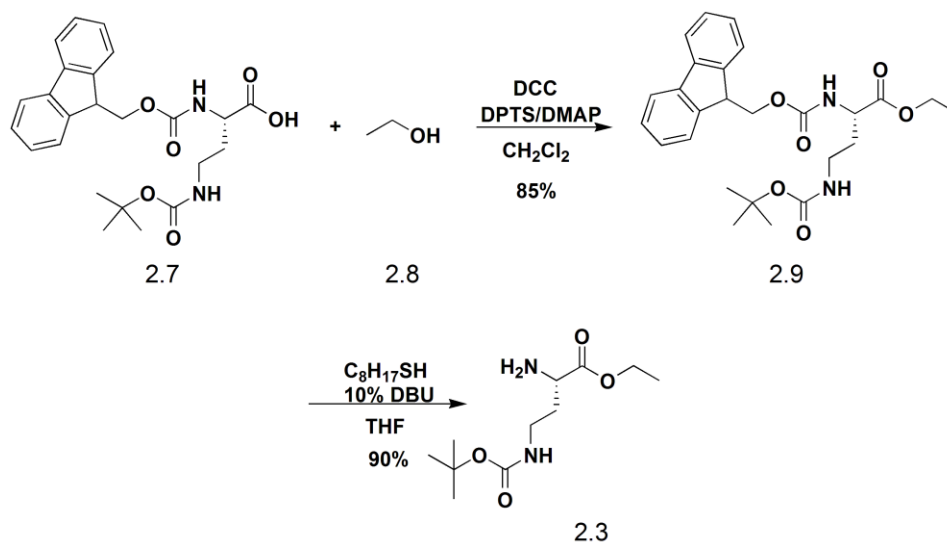
Scheme 2.2 Formation of DAB ethyl ester.

As shown in Scheme 2.3, the reduction sensitive Hcy ethyl amino acid was first synthesized by protecting the hydrochloric acid salt of thiolactone Hcy derivative **2.10** with Boc to form **2.11** and later ring opening of the thiolactone with lithium hydroxide to give **2.12**. The disulfide bond was installed by reacting **2.12** with 2,2'- dipyridyl disulfide and a catalytic amount of acetic acid to give **2.14**. The ethyl ester was then formed by coupling ethanol to **2.14** using DCC with catalytic amounts of DMAP and DPTS.
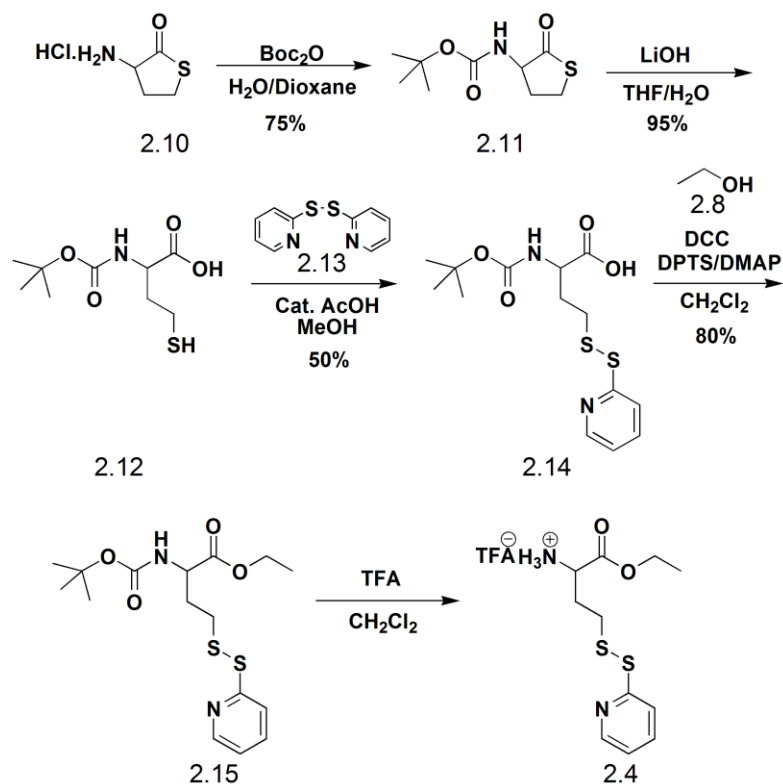
Scheme 2.3 Formation of Hcy ethyl ester.

2.2.3 Kinetic studies on small molecule model compounds

The cyclization of the ethyl ester derivatives of DAB and Hcy were first investigated in pH 7.4 phosphate buffered D_2O. In the case of the DAB derivative **2.3**, the Boc protecting group was removed immediately prior to the study, providing the salt **2.16** (Scheme 2.4 a).However, the Boc protected compound **2.13** was also studied as a control compound in order to determine the rate of background ester hydrolysis. In the case of the Hcy derivative **2.4**, DTT was added to cleave the disulfide bond in the Hcy ester, providing **2.17** (Scheme 2.4 b). A control without DTT addition was also performed for compound **2.4**. The rates were determined by quantifying the amount of ethanol in comparison to ethyl ester by integration of their respective peaks in the ^{1}H NMR spectrum.
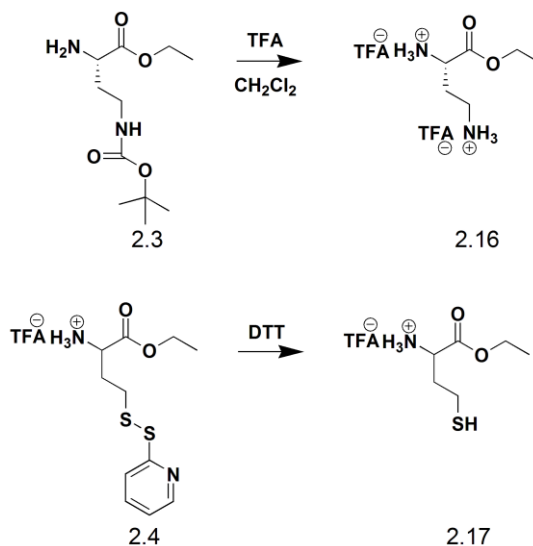
Scheme 2.4 a) Deprotection of Boc group, b) reduction of dithiopyridyl moiety.

As shown in Figures 2.2 and 2.3 by comparing the rates of ethanol evolution for compounds **2.16** and **2.17** with their respective control compounds **2.3** and **2.4** having protecting groups on the side chains, the rate of cyclization is faster than that of background ester hydrolysis. Fitting of the data to a first order rate law (appendix) gave half-lives of 6 hr and 2 hr for the DAB and Hcy monomers, respectively. In contrast, the half-lives for the controls **2.3** and **2.4** were 42 and 53 hr, respectively. As shown in Figure 2.4 for the DAB derivative **2.16**, the NMR spectroscopic analysis of the products was consistent with a majority of the expected cyclization product along with a small amount of background hydrolysis product. As shown in Figure 2.5 for the Hcy derivative, the expected cyclization product was also predominant in the case of this molecule. Assignment of these spectra was facilitated by comparison with the spectra of pure DAB and Hcy in the same buffer (appendix).
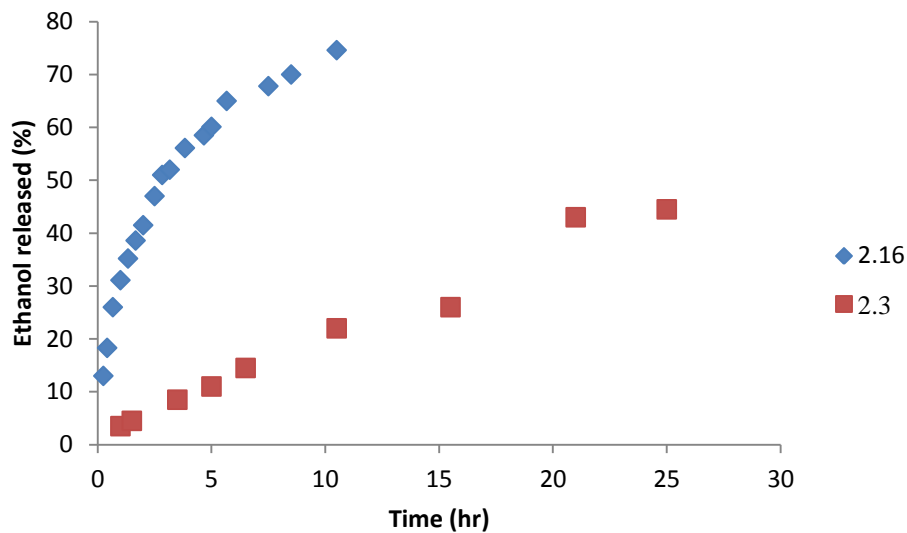
Figure 2.2 Kinetics of degradation for compound **2.16** and background hydrolysis of **2.3** in a 0.1 M, pH 7.4 phosphate buffer.
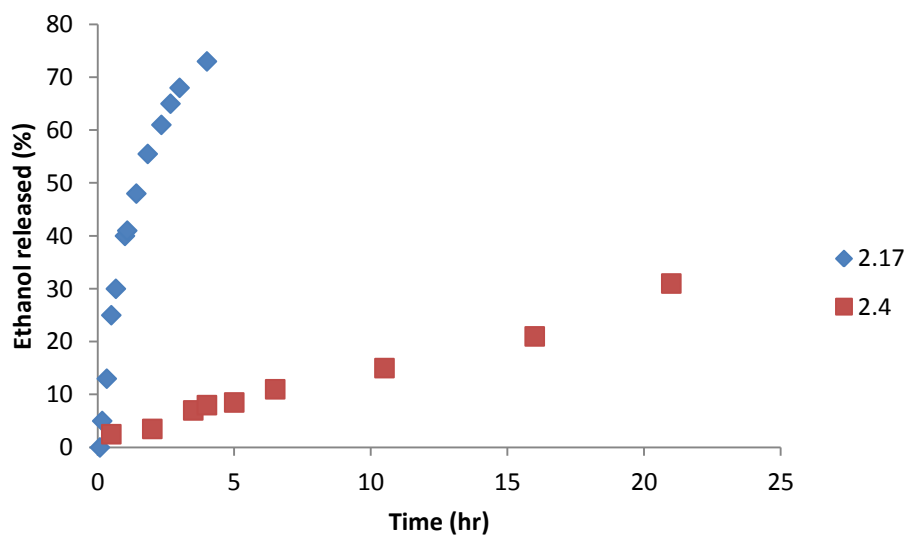
Figure 2.3 Kinetics of degradation for compound **2.17** and background hydrolysis of **2.4** and background hydrolysis in a 0.1 M, pH 7.4 phosphate buffer.
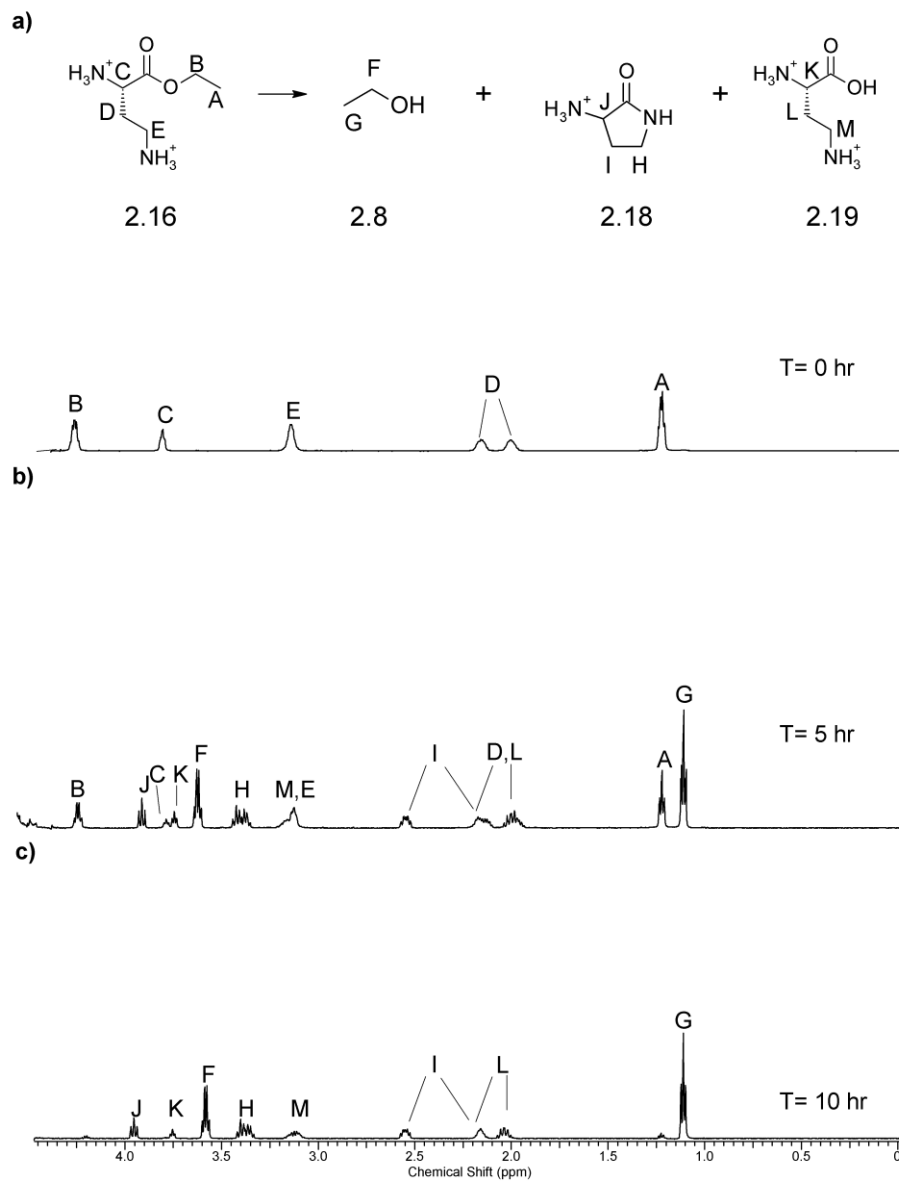
Figure 2.4 ^{1}H NMR spectra in 0.1 M, pH 7.4 phosphate buffered D_2O of a) DAB derivative **2.16** immediately following dissolution in the buffer. b) After 5 hr in the same buffer. c) After 10 hr in the same buffer.
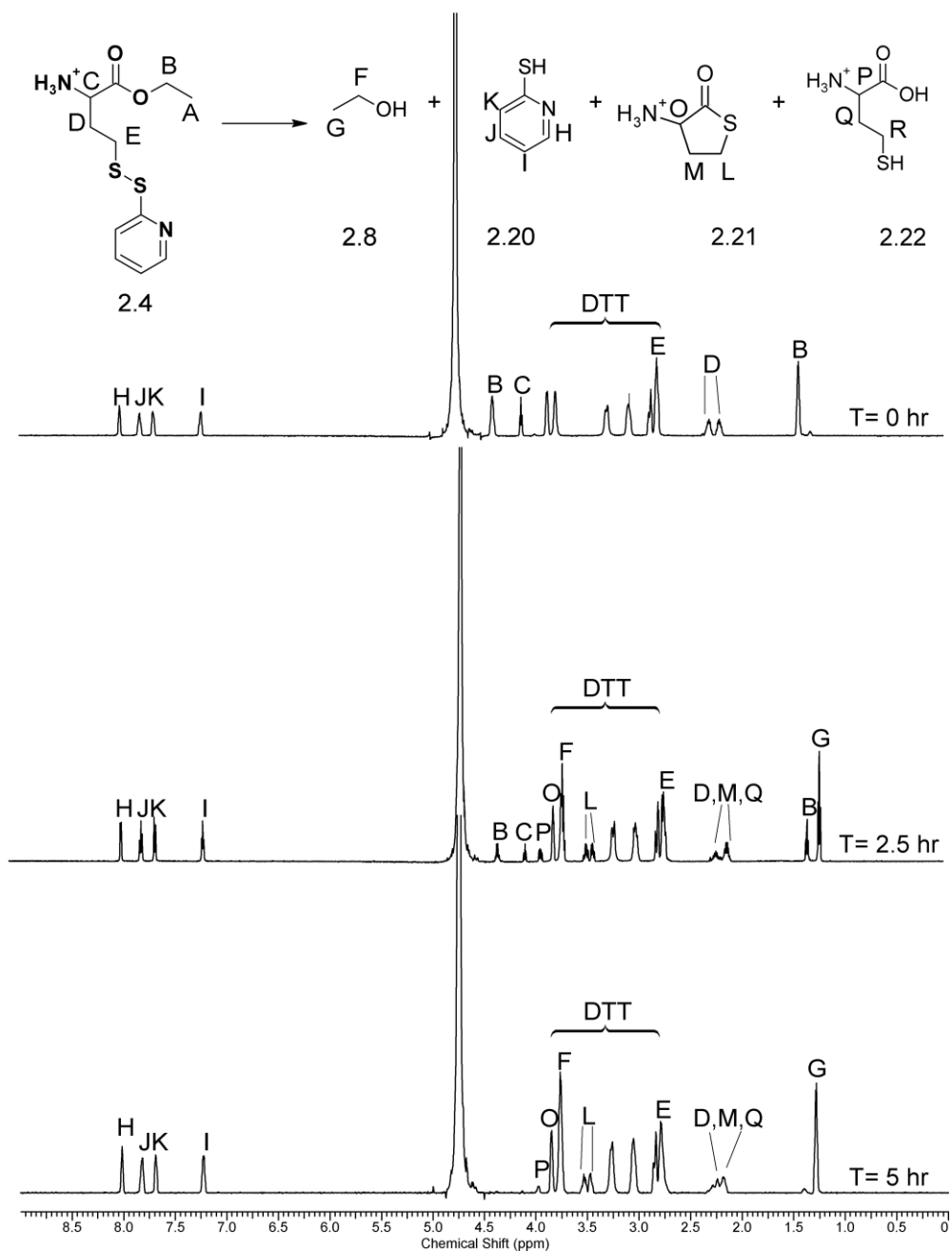
Figure 2.5 [^1]H NMR spectra in 0.1 M, pH 7.4 phosphate buffered D_2O of a) Hcy derivative **2.17** immediately following dissolution in the buffer. b) After 5 hr in the same buffer. c) After 10 hr in the same buffer.

For comparison purposes, spectra of the time dependent background hydrolysis of the control molecules **2.3** and **2.4** are shown in the appendix. The DAB ethyl ester **2.3** undergoes clean hydrolysis to form DAB and ethanol as expected. On the other hand, in the absence of DTT addition, the Hcy derivative **2.4** appears to undergo another reaction in addition to simple hydrolysis. As the disulfide bond, and in particular the activated dithiopyridyl group, is susceptible to attack by a variety of nucleophiles, it is hypothesized that the α-amine attacks this bond to generate a five membered sulfenamide (Scheme 2.5).
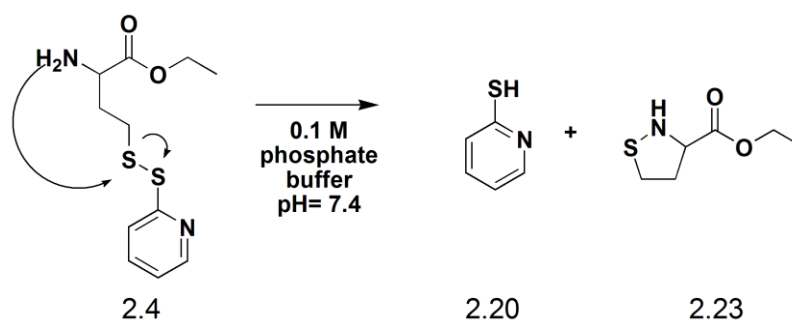
Scheme 2.5 Formation of five membered sulfenamide.

This functional group has recently found usage in polymers that are quite stable to basic and neutral conditions, but degrade once the pH decreases.[76-77] This side reaction was not observed for model compound **2.17** due to the rapid cleavage of the dithiopyridyl group and would not be expected in the target polymer as the α-amines would be tied up as amides in the polymer backbone. Thus, despite this unexpected side reaction of the control compound, these kinetic studies showed that the cleavage of the side chain protecting groups could induce rapid cyclization reactions in DAB and Hcy derivatives, showing the promise of these monomers for application in the triggered degradation of PEAs.

As it has been established by our group and others that the choice of solvent affects the rates of cyclization of self-immolative spacers,[33,78-79] the cyclization kinetics for the small molecule model compounds were also evaluated in 7:2:1 ratio of DMSO: acetone $-D_6$: 0.1 M, pH 7.4 phosphate buffered D_2O. This solvent mixture was selected as it was found to be one of only a few aqueous solvent mixtures that could dissolve the

target polymers **2.1** and **2.2**. Initially, studies were performed at 37°C and NMR spectroscopy was used to evaluate the degree of ethanol evolution over time for compounds **2.3**, **2.4**, **2.16**, and **2.17**. However, preliminary experiments indicated that the rate of ethanol evolution was extremely slow for all compounds. This is consistent with our observations that the cyclization rate is much slower in aqueous solvent mixtures with increasing organic content. In order to accelerate the kinetics to occur over a more readily measurable time scale, the temperature was increased to 70 °C. The rates of ethanol evolution for the DAB derivative **2.16** and the protected control compound **2.3** are shown in Figures 2.6 and their respective half-lives were found to be 3 and 14 days respectively.

The kinetics for the Hcy derivative **2.17** are shown in Figure 2.7 and the corresponding half-life was found to be 52 days. Unfortunately it was not possible to evaluate the control compound **2.4** in this experiment as the dithiopyridyl group was rapidly cleaved from this molecule, likely by the same sulfenamide mechanism described above. In addition, examination of the ^{1}H NMR spectra of **2.17** throughout the experiment revealed that it was converted to an unexpected intermediate byproduct that subsequently reacted to release ethanol. Although the nature of this intermediate was not identified conclusively, it is possible that as described above for control compound **2.4**, conversion to a cyclic sulfenamide occurred, in this case via disulfides of **2.4** with itself or with DTT, that might exist transiently throughout the reaction. This sulfenamide could then hydrolyze to reveal the thiol, capable of cyclizing or alternatively undergo simple hydrolysis to release ethanol. It was difficult to distinguish between these pathways due to the complexity of the NMR spectra (appendix) but either mechanism would explain the slow kinetics. It appears that this side reaction does not occur when the thiol cyclization is rapid as for **2.17** in pure aqueous buffer, but when the reaction is slowed by the organic solvent there is an opportunity for it to occur. This would explain the reversal of relative rates with the Hcy monomer faster in pure buffer and the DAB monomer faster in 7:2:1 DMSO:acetone:buffer. Again, this side reaction would not be expected to occur during the degradation of the polymer.
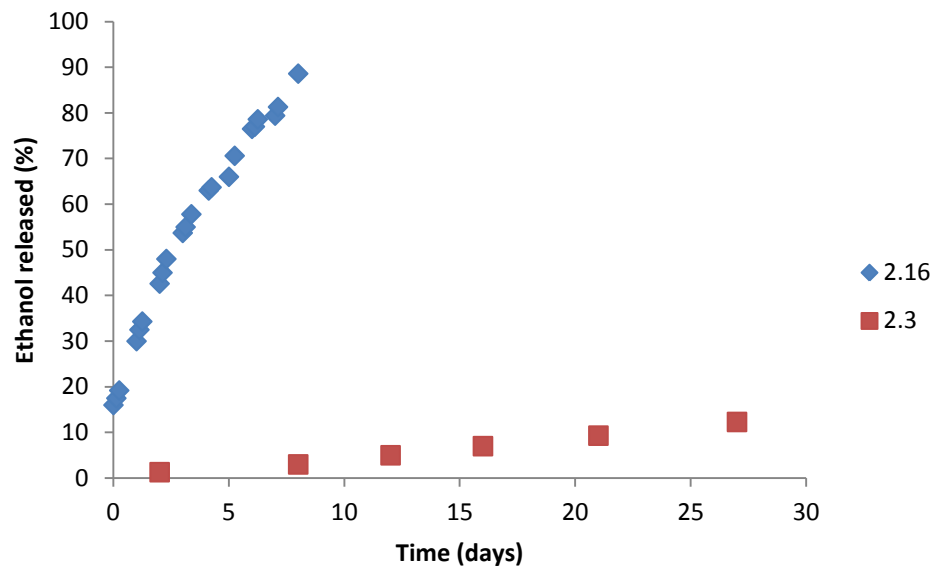
Figure 2.6 Kinetics of degradation for compound **2.16** and background hydrolysis of **2.3** in a DMSO: acetone –D_6: 0.1 M, pH 7.4 phosphate buffer (D_2O).
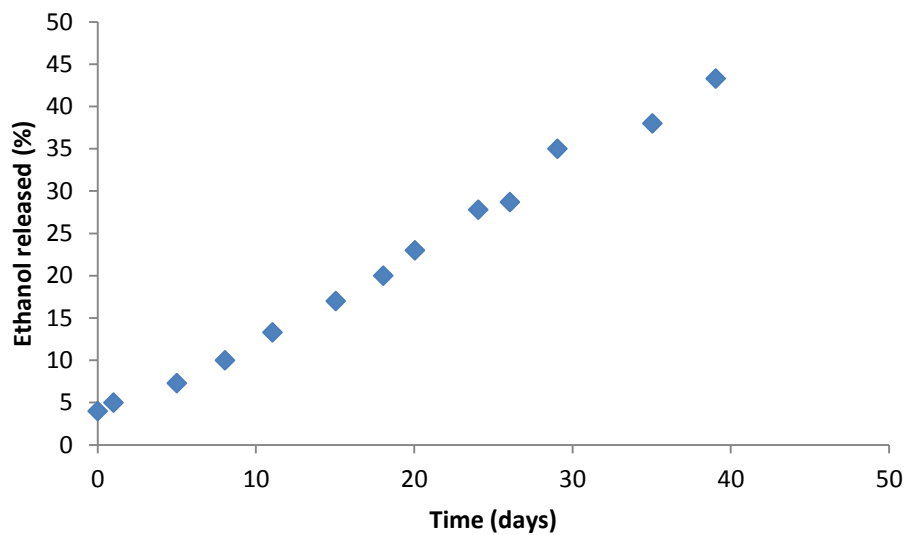
Figure 2.7 Kinetics of degradation for compound **2.17** in a DMSO: acetone –D_6: 0.1 M, pH 7.4 phosphate buffer (D_2O).

2.2.4 Synthesis of polymerization monomers

In order to prepare the target polymer **2.1**, it was necessary to prepare a diamine-diester derivative of DAB with 1,4-butanediol having free α-amines and protected γ-amines. As shown in Scheme 2.6, the synthesis began with the commercially available orthogonally protected amino acid, **2.7**. The diester was synthesized by coupling **2.7** to 1,4-butanediol using a DCC, DMAP and DPTS to afford **2.25**. The Fmoc group was then removed using a catalytic amount of DBU and an excess of 1-octanethiol.
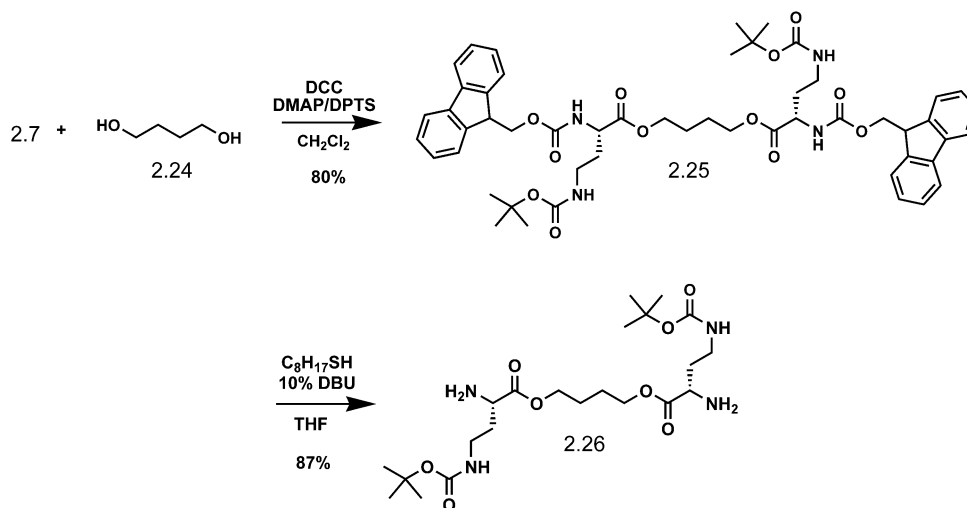
Scheme 2.6 Synthesis of DAB monomer**.**

The synthesis of the diamine-diester derivative of Hcy was performed by first coupling the protected Hcy **2.14** with 1,4-butanediol using DCC, DMAP and DPTS to provide **2.27**. The target monomer was then obtained by removing the Boc groups with TFA to give **2.28** (Scheme 2.7).
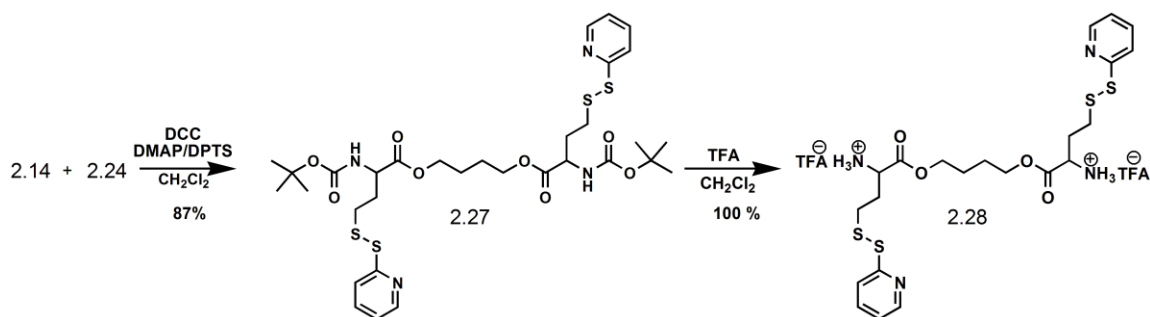
Scheme 2.7 Synthesis of Hcy monomer.

2.2.5 Synthesis of polymers

As described above, the target polymers were prepared by an interfacial polymerization in CH_2Cl_2/water. For the preparation of target polymer **2.1** and **2.2**, sebacoyl chloride (1.0 equiv.) was dissolved in CH_2Cl_2 and the solution was added drop wise to a solution of the diesters **2.26** or **2.28** (0.2 equiv.) and **2.29**[80] (0.8 equiv.) in aqueous Na_2CO_3 (Scheme 2.8 and Scheme 2.9). The reaction mixtures were then stirred for 24 hr, after which the polymers were then purified by dialysis in *N,N*-Dimethylformamide (DMF). A control polymer **2.31**[20] without any DAB or Hcy monomer was also prepared (Scheme 2.10). SEC (table 2.1) in DMF indicated that the polymers had M_n's ranging from 91,800 – 155,500 g/mol relative to polystyrene standards and polydispersity indices (PDIs) from 1.22-1.24. The low PDIs and relatively high M_n's can be attributed to the dialysis step which removes lower MW polymers in addition to low MW byproducts. The Boc protecting groups could be removed from polymer **2.1** by treatment with TFA, providing polymer **2.30**. A reduction in M_n was observed as a result of the deprotection process which has been reported by our group,[20,81] as well as others.[16,19] This is in part a result of the interaction of the amino groups of the polymer with the column during SEC.
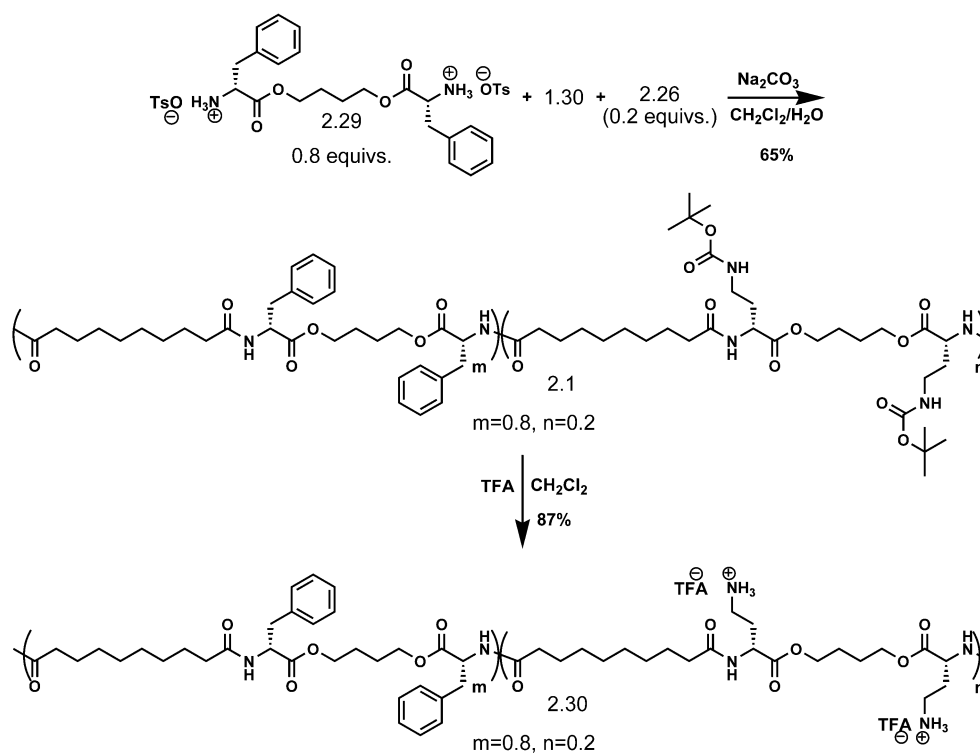
Scheme 2.8 Synthesis of and deprotection of PEA incorporating 20% DAB.
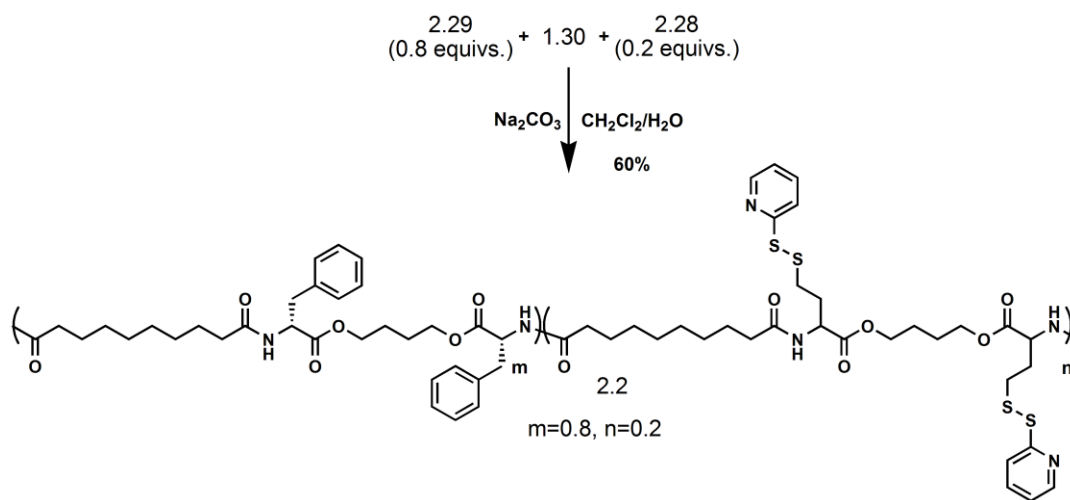
Scheme 2.9 Synthesis of PEA incorporating 20% of Hcy.

2.29 + 1.30 → 78%
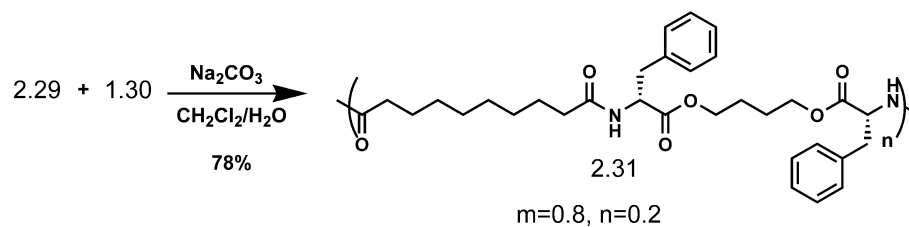
Scheme 2.10 Synthesis of control polymer.

Polymer	Yield (%)	M_n (g/mol)	M_w/M_n (PDI)
2.1	65	155, 500	1.23
2.2	60	98, 300	1.24
2.30	87	91, 839	1.22
2.31	78	63, 600	2.65

Table 2.1 Yields and molecular weight data for functional PEAs and non-functional PEAs.

Incorporation of the DAB and Hcy monomers into the PBSe backbone was verified by ^{1}H NMR spectroscopy. As shown in Figure 2.8a, in comparison to the control polymer **2.31**, in polymer **2.1** a new peak arises at 1.36 ppm which corresponds to the methyls of the Boc group. Another peak appears at 4.21 ppm, which belongs to the α-proton of the DAB monomer. In addition, there is a peak at 6.78 ppm corresponding to the carbamate N-H of the Boc group that, upon addition of TFA to form **2.30**, disappears (Figure 2.8 b) along with the peak attributed to the methyls of the Boc group. In the case of polymer **2.2**, the new peaks arise in the aromatic region that corresponds to the pyridyl moiety of the Hcy monomer, as well as another at 4.39 ppm that corresponds to the α-proton of this monomer (Figure 2.9).
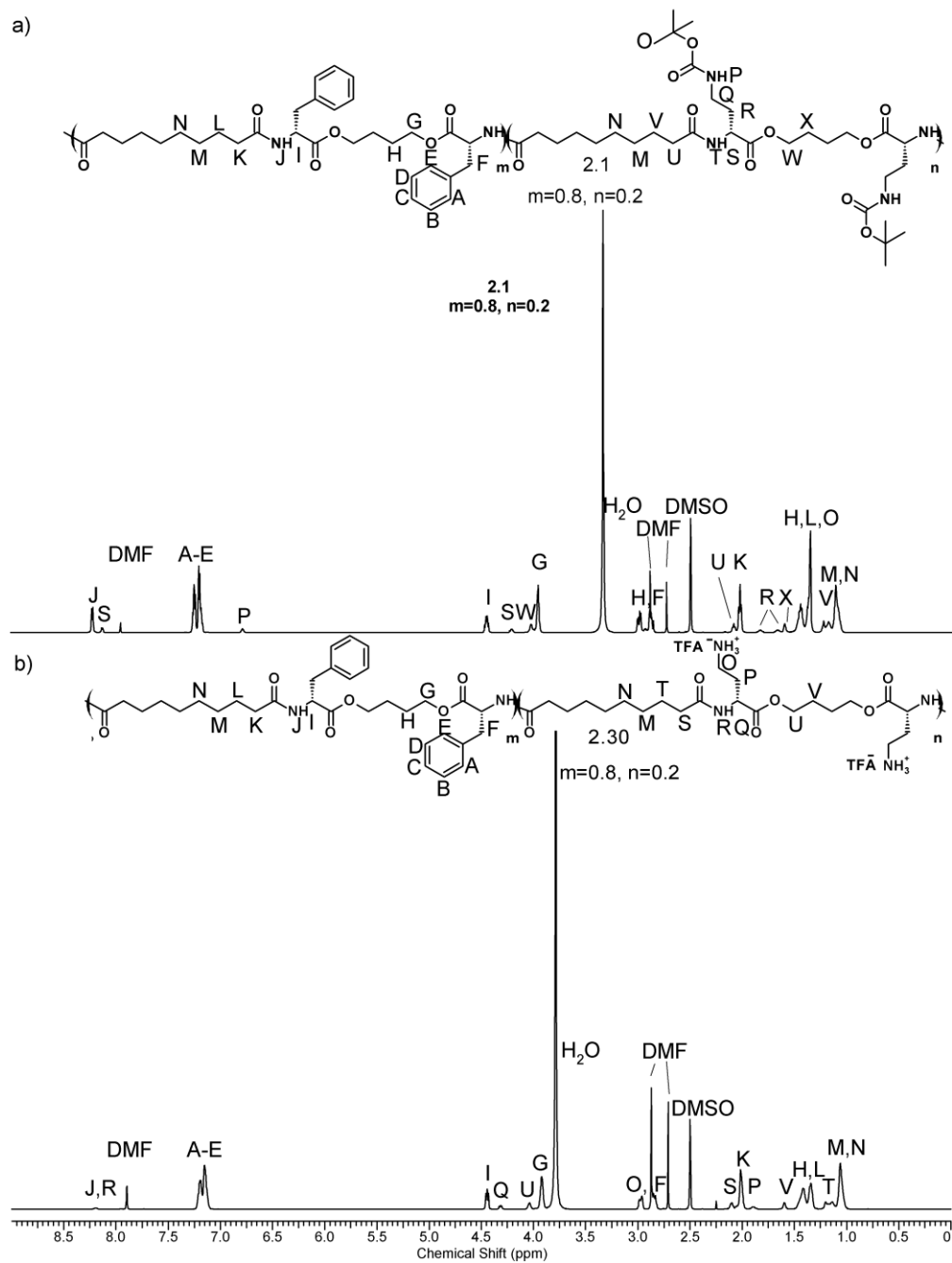
Figure 2.8 ^{1}H NMR (DMSO-D$_6$, 600 MHz) spectra of a) PBSe incorporated with 20% DAB monomer, protected version b) PBSe incorporated with 20% DAB monomer, deprotected version.
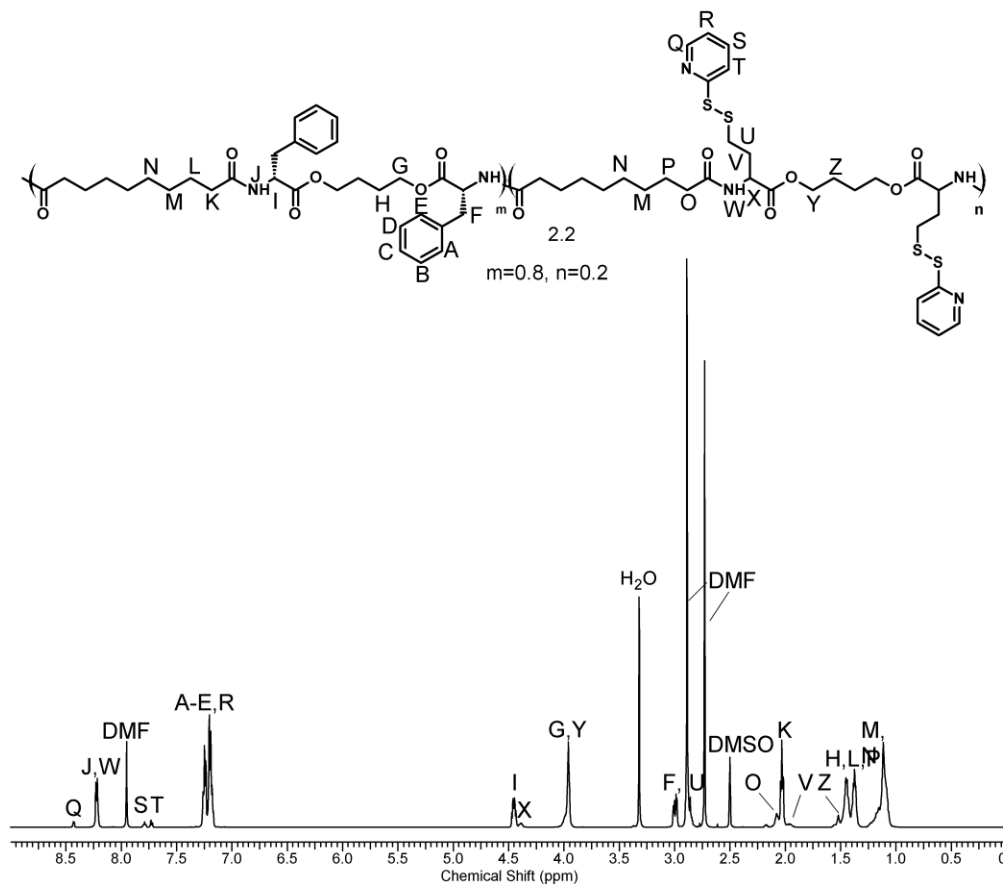
Figure 2.9 ^{1}H NMR (DMSO-D$_6$, 600 MHz) spectra of PBSe incorporated with 20% Hcy monomer.

2.2.6 Solution degradation of polymers

Due to the hydrophobic nature of the polymers **2.1**, **2.2**, and **2.30**, they were soluble primarily in organic solvents such as chloroform, CH$_2$Cl$_2$, DMSO and DMF. In order to degrade the polymers, varying ratios of different organic solvents with 0.1 M, pH 7.4 phosphate buffers were experimented with. For the study of the polymer degradation in solution, it was found that the mixture that gave the best solubility was a 7:2:1 ratio of DMSO: acetone–D$_6$: 0.1 M, pH 7.4 phosphate buffered D$_2$O. Deuterated solvents were chosen with the goal of monitoring the degradation process by NMR spectroscopy. Having already determined from the monomer cyclization kinetics that the cyclization reactions would be significantly slowed due to the high organic solvent content, the degradation study was performed at 70°C for a period of twelve days. In the case of

polymer **2.2**, DTT was added to cleave the pendant disulfides and initiate degradation of this polymer. Polymer **2.30** was used as is, because the Boc protecting group had already been removed, making the pendant amine capable of cyclization. The Boc protected polymer **2.1** was used as a control for these studies, whereas the control polymer **2.31** was insoluble under these conditions and could not be included in the solution degradation study.

The degradation of the 3 polymers was monitored by ^{1}H NMR spectroscopy. The emergence of new peaks was observed (appendix). However, the complexity of the spectra and overlapping peaks made it impossible to quantify the degree of degradation. Therefore, the degradation was monitored by SEC. This was done by removing aliquots of the solution periodically, removing the solvent and then dissolving the material in DMF for SEC analysis. As shown in Figure 2.10, the deprotected polymers showed a much more rapid decrease in MW than the protected polymer. For example, whereas polymer **2.1** exhibited a 50% reduction in M_n over two weeks, polymer **2.30** showed a 90% decrease in M_n over the same period. The same trends were observed when examining changes in M_w (Figure 2.11). In the case of polymer **2.2**, molecular weight decreased by as much as 94% in 12 days, which demonstrates the faster rate of cyclization of the thiol group over the amine; this enhanced rate was also seen in the model ethyl ester studies in pure phosphate buffer, suggesting that the side reaction, and resulting increased cyclization half-life observed in the organic solvent mixture was indeed likely the result of the free α-amine that was present in the model compound but not in the polymer. Figure 2.12 and 2.13 show the evolution of the SEC traces over time for polymer **2.1** and **2.30** respectively. Overall, these results show that the rates of polymer degradation are much faster for the polymers upon cleavage of the moiety protecting the pendant groups, confirming that the strategy should be a viable approach to creating stimuli responsive PEAs.
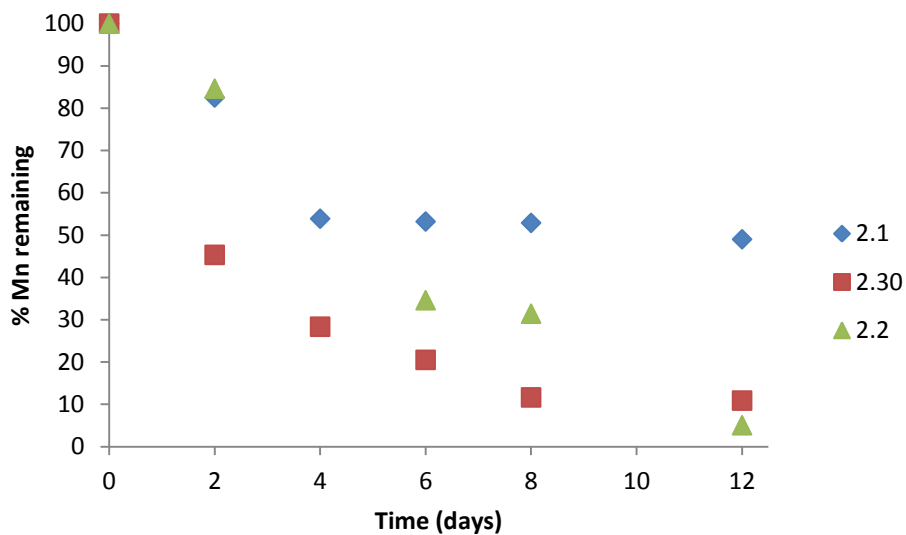
Figure 2.10 Molecular weight data for protected and deprotected PEAs.
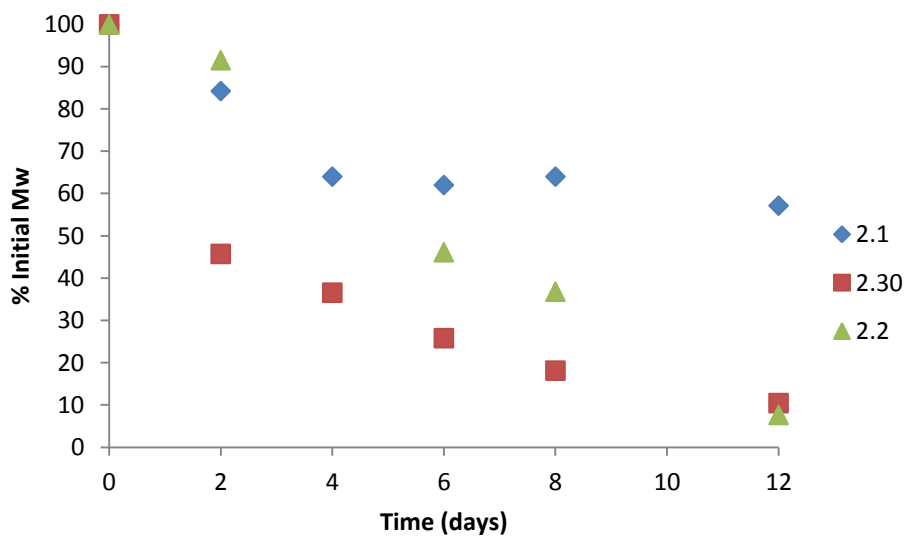
Figure 2.11 Molecular weight data for protected and deprotected PEAs.
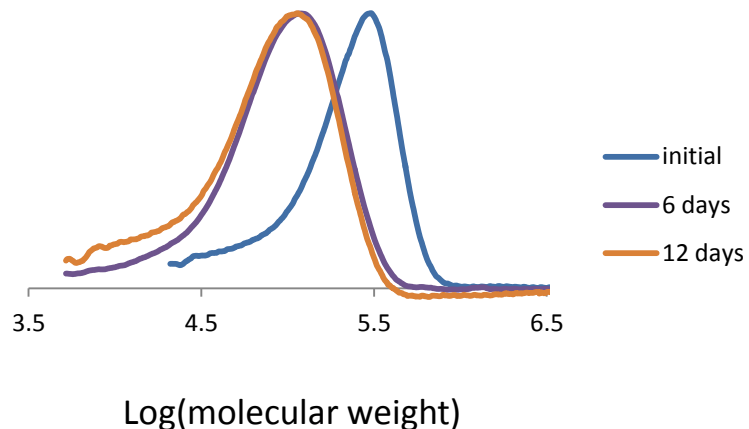
Figure 2.12 Size exclusion chromatograms of polymer **2.1** plotted with respect to Log (molecular weight) prior to degradation, as well as after different time points in the degradation process (eluent = DMF with 10 mM LiBr and 1% (v/v) NEt3; detection by differential refractive index).
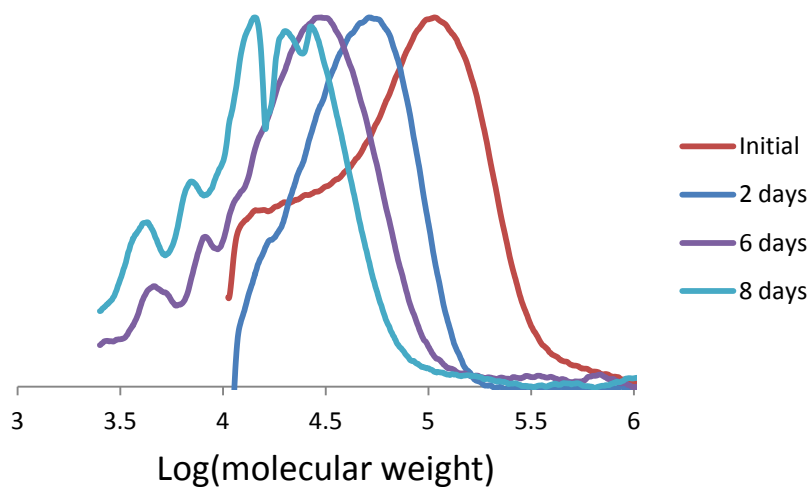
Figure 2.13 Size exclusion chromatograms of polymer **2.30** plotted with respect to Log (molecular weight), prior to degradation, as well as after different time points in the degradation process (eluent = DMF with 10 mM LiBr and 1% (v/v) NEt3; detection by differential refractive index).

2.2.7 Mass loss studies of polymers

The solution degradation properties described above for the PEAs were of interest for applications in which the PEAs would be present in solution such as in a micellar carrier.[19-20,31] However, there is also substantial interest in the use of PEAs in applications such as tissue engineering where they would be used as a coating or scaffold material.[34-36] Here they offer potential advantages over polyesters, which typically erode via bulk degradation,[1,13] rapidly releasing harmful acidic byproducts.[11,12] PEAs have been proposed to undergo surface erosion mechanisms,[13,82-84] which limit the release of acidic byproducts.[14,17-18] Although there have been many reports of PEAs degrading in the presence of enzymes, [71,82-84] their degradation in buffer solution in the absence of specific enzymes is quite slow.[17,22] Therefore, it was of interest to determine how the cleavage of the pendant side chain protecting groups could trigger the degradation of PEA films via trigger-initiated cyclization. Polymers **2.1**, **2.2**, and **2.30** and were melt pressed to form 1 mm thick films, and circular disks, each weighing approximately 5-8 mg, were punched from these films. In this case, the control polymer **2.31** was also included to investigate the effect of pendant group incorporation. The films were placed into vials containing pH 7.4 phosphate buffer, along with DTT in the case of polymer **2.2**. As preliminary studies showed that degradation was very slow at 37 °C (eg. only 10% mass loss over 2 weeks in the case of polymer **2.2**), the films were incubated at 70°C in order to accelerate degradation to a more easily measured rate. At various time points, the films were removed from the buffer solution, washed thoroughly with pure water, and then dried to a constant mass.

As shown in Figure 2.14, the control polymer **2.31** exhibited only ~10% mass loss over 8 weeks. In comparison, the Boc protected polymer **2.1** exhibited almost 30% mass loss over 8 weeks, an enhancement likely attributable to its increased polarity relative to control **2.31**, and/or disruption of solid phase packing of the polymer, allowing enhanced penetration of water into the film. This has been previously observed by our group for polymers containing polar pendant groups, even in the absence of cyclization reactions.[64] Polymer **2.2** exhibited more than 30% mass loss over 8 weeks, likely due to the cyclization of the pendant thiols upon cleavage by DTT. The polymer containing pendant

amines degraded fastest, likely due to the propensity of the amines to cyclize as well as their high polarity as they would be largely protonated at pH 7.4. As can be seen in Figure 2.14, the almost linear plot for each polymer suggests a surface erosion mechanism. This was also supported by SEC analyses as it was found that there were no measurable changes in the MW characteristics of the polymers comprising the residual films (see appendix).
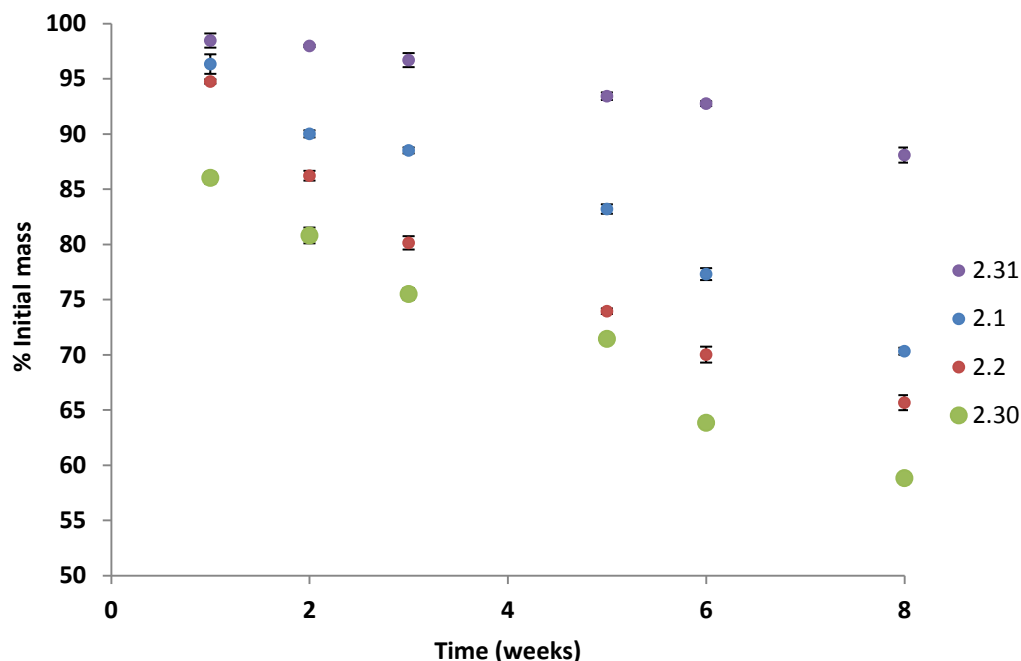
Figure 2.14 Mass loss studies of deprotected and protected polymers.

Scanning electron microscopy (SEM) was performed on the films throughout the degradation process in order to further investigate the surface erosion process. SEM images of the films before degradation are shown in Figure 2.15 a-d. The surfaces of the polymers appear relatively smooth prior to degradation. Following 2 weeks in pH 7.4 phosphate buffer, small pore formation is evident in polymer **2.31** (Figure 2.15e). More surface erosion is evident for polymer **2.1** (the Boc protected), consistent with the results of the mass loss studies. Extensive surface erosion was observed for polymers **2.30** (Figure 2.12 h) and **2.2** (Figure 2.12 g), again consistent with the results of the mass loss studies. Overall, the mass loss, SEC, and SEM studies of these films indicate that as

designed, these polymers undergo surface erosion processes at rates that are dependent on the unmasking of the pendant amines or thiols.
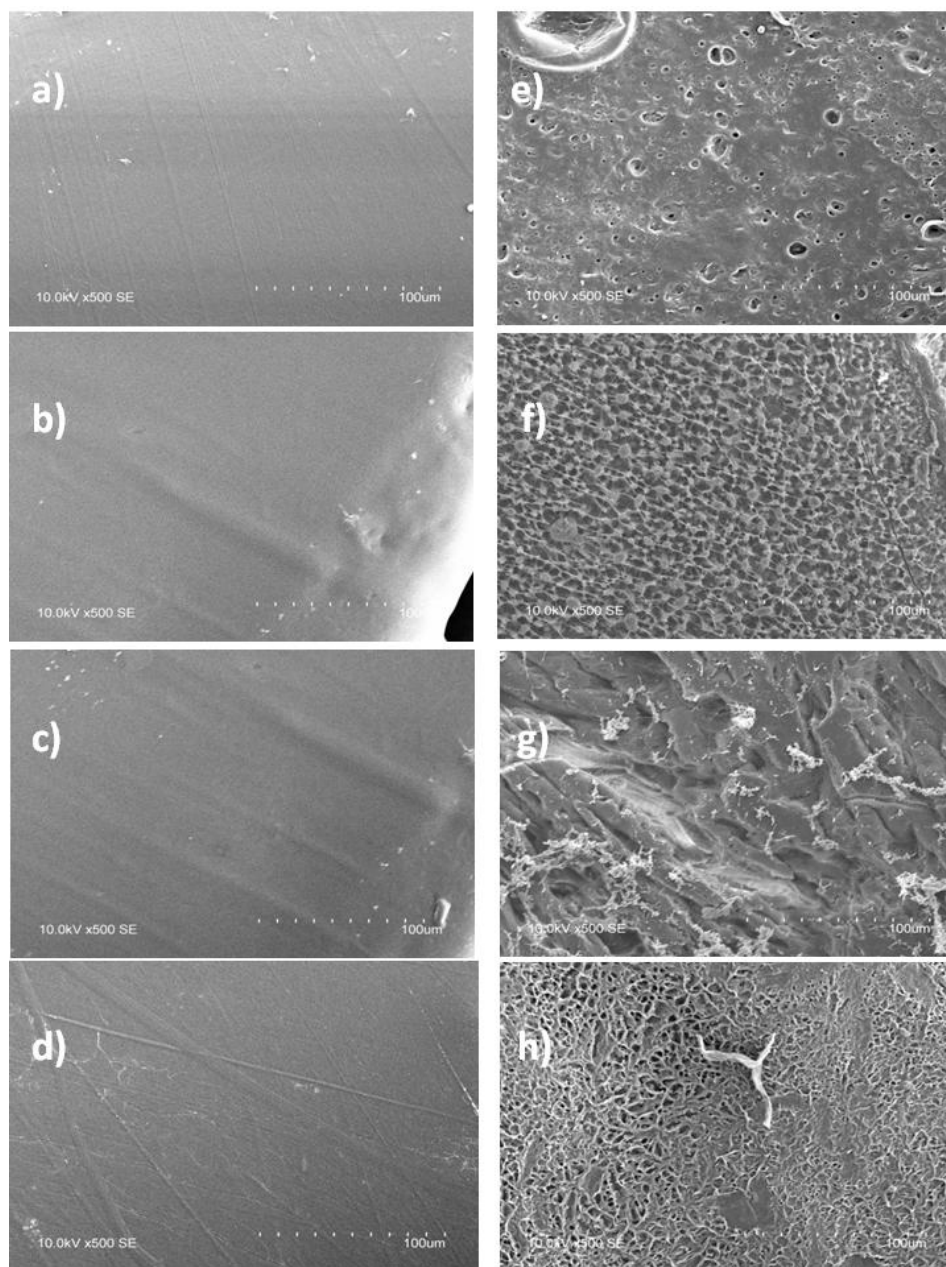
Figure 2.15 Scanning electron microscopy images (x500 magnification) of thin films of polymer (a) **2.31** prior to incubation, (b) **2.1** prior to incubation, (c) **2.30** prior to incubation, (d) **2.2** prior to incubation, (e) **2.31** following 2 week incubation in PBS (70 °C), (f) **2.1** following 2 week incubation in PBS (70 °C), (g) **2.30** following 2 week incubation in PBS (70 °C), (h) **2.2** following 2 week incubation in PBS (70 °C).

2.2.8 Development of photochemically responsive PEA

2.2.8.1 Design

While the specific cleavage of the disulfide bond is possible in vivo in reducing environments such as within cells or in hypoxic tissues such as tumours,[72-74] it should be noted that there is no method to remove a Boc group under biological conditions. As the degradation properties of the DAB polymer **2.30** appeared highly promising, an approach allowing for a more relevant amine unmasking strategy that could eventually be applied in vivo was sought. Light as a stimulus is attractive because it is non-invasive and can be applied for a short period of time with high spatial and temporal precision.[32] One of the most widely used groups for these applications is the *o*-nitrobenzyl moiety, widely chosen for its clean removal.[32,34,37-41] Due to its favorable properties, 4,5-dimethoxy-2-nitrobenzyl alcohol was chosen as the photo-labile group for the DAB polymer. Although the short wavelength of light (~350 nm) required for cleavage of this *o*-nitrobenzyl group is not directly applicable in vivo, it would be relatively simple to later extend the chemistry to moieties that are responsive in the near-infrared range where tissue is much more transparent.[32-36,39]

2.2.8.2 Synthesis of a photochemically responsive PEA

The commercially available *o*-nitrobenzyl alcohol (**2.32**) was coupled to nitrophenyl chloroformate under basic conditions and with a catalytic amount of DMAP to afford **2.34** (Scheme 2.11). The target polymer was then formed by coupling the amines of the deprotected polymer **2.30** to this nitrophenyl carbonate to give the final product, **2.35**, which was purified by dialysis. SEC analysis revealed a unimodal distribution corresponding to an M_n of 72,200 g/mol and a PDI of 1.41. ^{1}H NMR spectroscopy verified the incorporation of the *o*-nitrobenzyl groups onto the polymer backbone and as can be seen in Figure 2.16 a, a peak at 5.30 ppm corresponding to the benzylic protons verifies incorporation of the photochemically responsive monomer. Other peaks corresponding to the dimethoxy nitrobenzyl group also further verify the incorporation. NMR was also used to determine the time scale needed to remove the dimethoxy nitrobenzyl moiety. In brief, the polymer was dissolved in the same mixed organic

solvent mixture (7:2:1 ratio of DMSO: acetone –D$_6$: 0.1 M, pH 7.4 phosphate buffer D$_2$O) and irradiated with a medium–pressure Hg lamp (λ=350 nm) for different time periods. It was found that an irradiation time of two hours was sufficient enough to cleave the photo labile group (Figure 2.16 b).
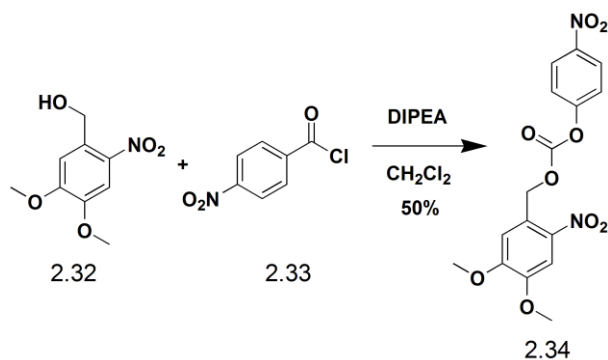
Scheme 2.11 Synthesis of 4-nitrophenyl carbonate derivative **2.34**.
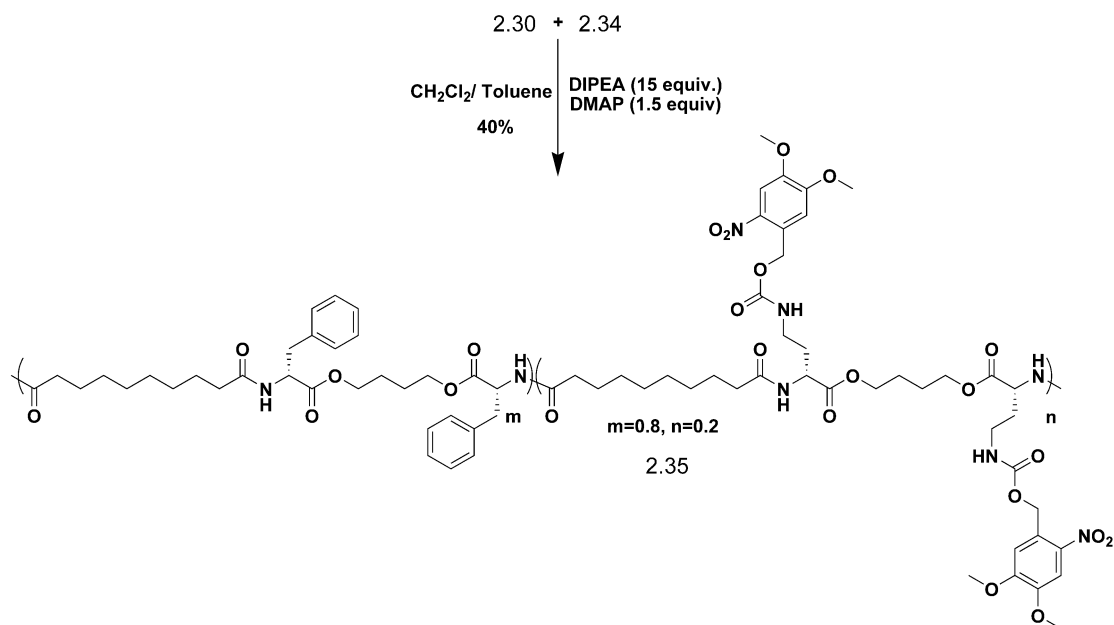
Scheme 2.12 Synthesis of photochemically responsive PEA **2.35**.
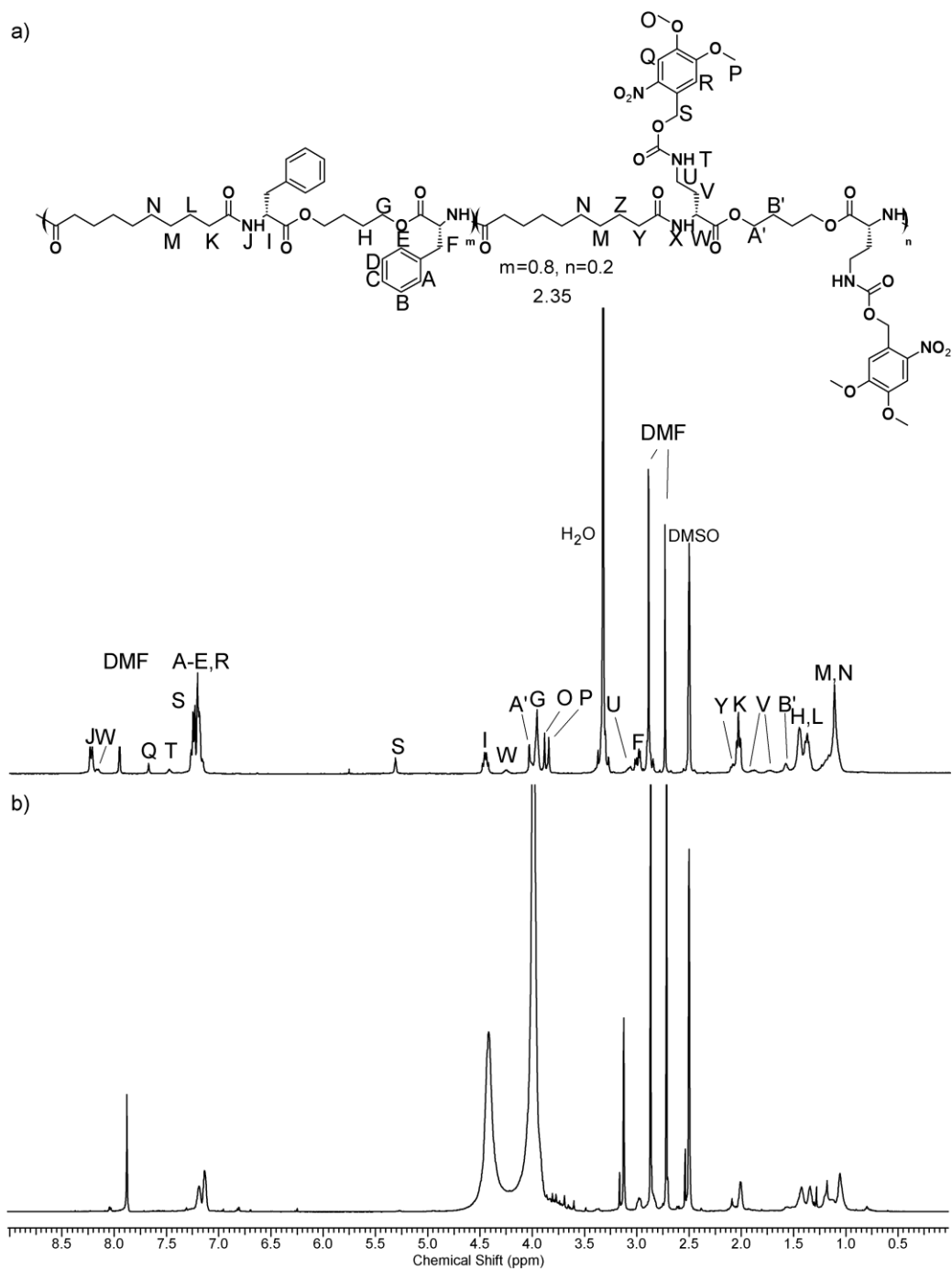
Figure 2.16 [^1]H NMR spectra of a) polymer **2.35** in DMSO:acetone–D$_6$: 0.1 M pH 7.4 phosphate buffered D$_2$O b) polymer **2.35** in DMSO:acetone–D$_6$: 0.1 M pH 7.4 phosphate buffered D$_2$O following 2 hr of irradiation.

2.2.8.3 Degradation of photochemically responsive PEA

In a similar fashion to the other stimuli responsive PEAs, two samples of polymer **2.35** were dissolved in DMSO:acetone–D_6: 0.1 M, pH 7.4 phosphate buffered D_2O solvent system. One sample was irradiated, while the other sample was not subjected to irradiation. The solutions were incubated at 70°C and aliquots were submitted for SEC analysis. The changes in M_n and M_w over time are shown in Figure 2.17 and 2.18. Similar to polymer **2.30**, described above, the irradiated sample exhibited a 97% reduction in M_n during the first ten days. In contrast, the non-irradiated sample exhibited only a 68% reduction in M_n over the sample time period.
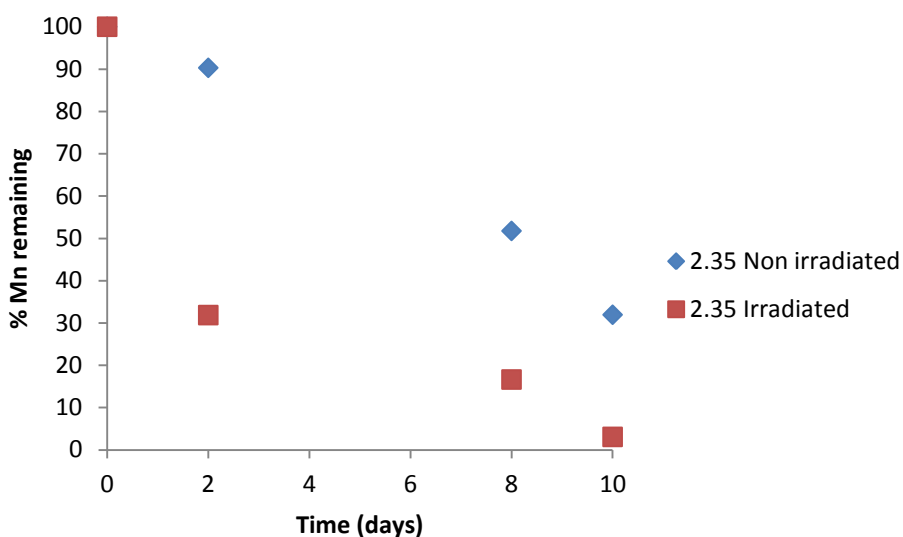
Figure 2.17 Molecular weight data for non irradiated and irradiated samples.
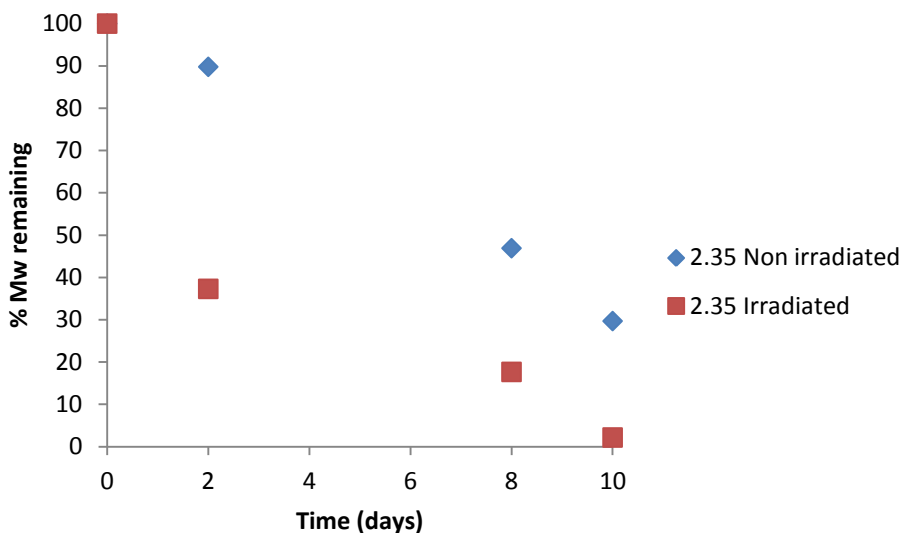
Figure 2.18 Molecular weight data for non irradiated and irradiated samples.

As for the polymers described above, it was also of interest to investigate the degradation of films of polymer **2.35** in the response to photochemical irradiation. In this case, spin coating rather than melt pressing was used to prepare the films as thinner films were required to achieve light penetration throughout the film. In order to determine the time scale required for the irradiation process, UV-visible spectroscopy was undertaken to determine the decrease in absorbance over time of the characteristic peak corresponding to the *o*-nitrobenzyl group.[32,34,37] Based on the results shown in Figure 2.19, it was found that an irradiation time of 1 hour was enough to cleave the photo labile groups in the thin films. To investigate the thin film degradation in response to the photochemical stimulus, samples of films were either irradiated, or not, then placed in a pH 7.4 phosphate buffer solution and incubated at 70 °C. Due to the requirement for thin films, the quantities of materials were not sufficient for mass loss or SEC measurements, but the film erosion was investigated by SEM imaging. As shown in Figures 2.20a, the film is smooth prior to degradation. The non irradiated is intact after 2 days in the buffer solution (Figure 2.17b). In contrast degradation of the irradiated sample is very rapid, with only small fragments of the film remaining after 2 days (Figure 2.17c). Based on these SEM results, it is clear that photochemical cleavage does trigger accelerated degradation relative to the non-irradiated sample, suggesting that these DAB-based polymers are versatile backbones for

the incorporation of various trigger moieties that can potentially be cleaved under biologically accessible conditions, leading to stimuli-responsive PEAs.
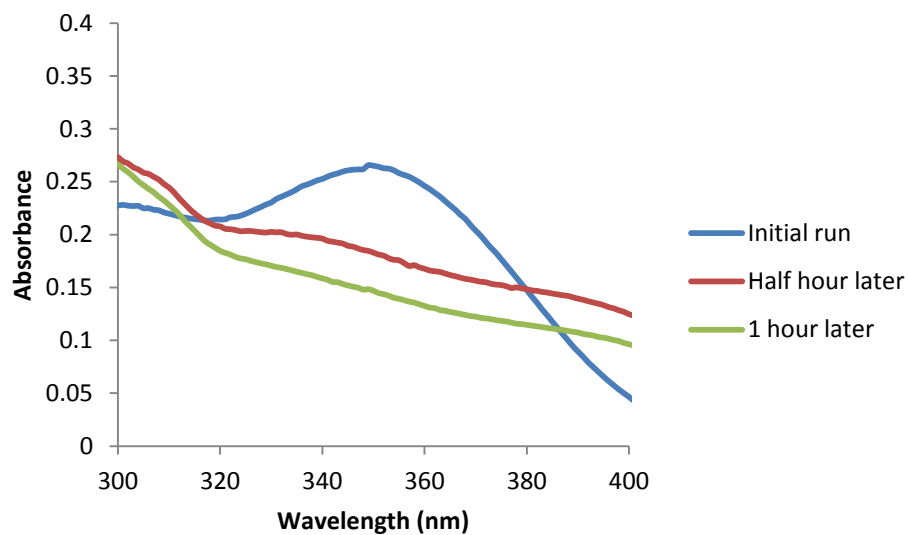
Figure 2.19 Irradiation of thin films.
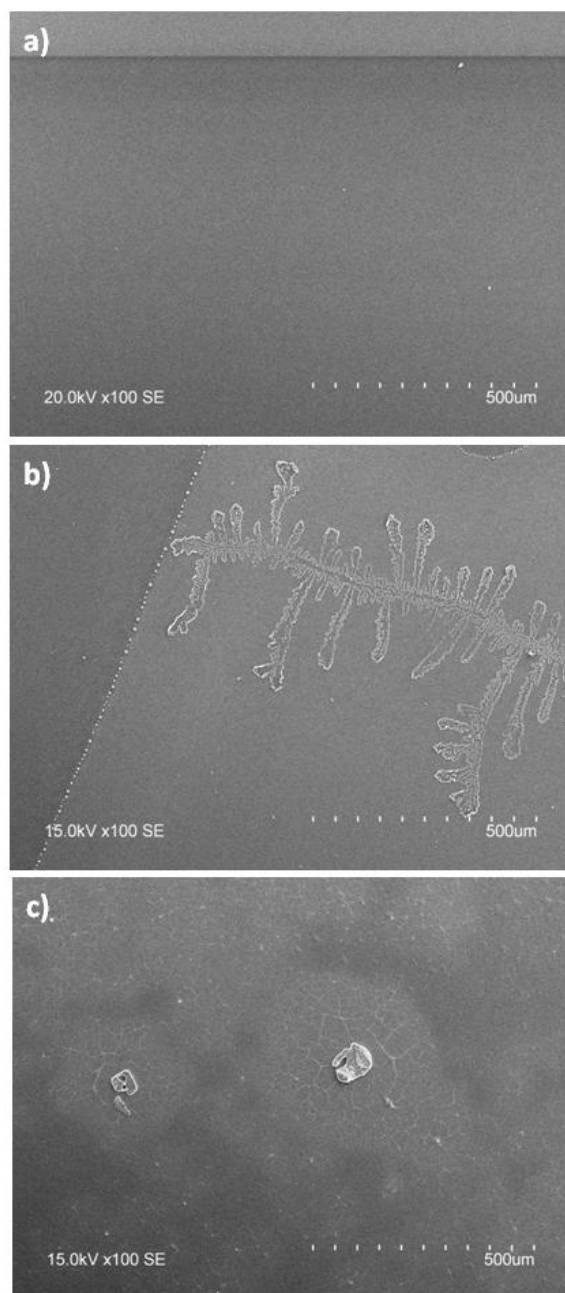
Figure 2.20 Scanning electron microscopy images (x100 magnification) of thin films of polymer (a) **2.35** prior to irradiation and incubation in PBS (70 °C), (b) **2.35** (non irradiated), following 24 hr incubation in PBS (70 °C), (c) **2.35** (irradiated), following 24 hr incubation in PBS (70 °C).

2.3   Conclusions

In conclusion, the first stimuli responsive PEAs were synthesized. This was accomplished by incorporating monomers with pendant groups capable of cyclization. Upon cleavage of trigger moieties protecting these pendant groups, in response to stimuli such as changes in redox potential, acid or light into the backbone, the monomers underwent 1,5-cyclizations, leading to backbone cleavage and thus degradation of the PEAs. To support the design, kinetic studies were performed on small molecule models and they supported the proposed route of degradation via 1,5-cyclization. The polymers were then synthesized by interfacial polymerization reactions. Polymer degradation studies were also carried out in solution using SEC analysis and on thin films using mass loss, SEC, and SEM and these studies suggested that as designed, the polymers could be cleaved by these 1,5-cyclization reactions more rapidly than the background rate of ester hydrolysis. In addition, a photoresponsive PEA was synthesized showing that various triggers could be incorporated, thereby demonstrating the potential for this degradation mechanism to be applied for biomedical applications. Overall, the results show that the concepts of self-immolative spacers could be applied to PEAs in order to trigger their degradation under specified conditions.

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_2Cl_2 were obtained from a solvent purification system based on aluminum oxide columns. 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 Macherney-Nagel Polygram® SIL G/UV$_{254}$ plates. ^{1}H NMR spectra were obtained at 400 or 600 MHz and ^{13}C NMR spectra were obtained at 100 MHz or 150 MHz using a Varian Mercury or Varian Inova spectrometer. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals of

CDCl$_3$ (δ 7.27, 77), DMSO-d$_6$ (δ 2.50, 40), CD$_3$OD (δ 3.31, 49), and D$_2$O (δ 4.79). 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 mass spectrometer using either electron impact (CI) or a Micromass LCT electrospray ionization time-of-flight (ESI) mass spectrometer 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. Dialysis of the polymers was performed using Spectra/Por regenerated cellulose membranes with an 8000 - 12,000 g/mol molecular weight cutoff (MWCO).

Synthesis of compound 2.9: An excess of dry ethanol was added to a flame-dried flask containing *N*-α-Fmoc-*N*-*ε*-t-Boc-*L*-2, 4-diaminobutyric acid (1.00 g, 2.27 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (0.934 g, 4.53 mmol), 4-(dimethylamino)pyridine (DMAP) (0.0462 g, 0.378 mmol), and 4-(dimethylamino)pyridinium-4-toluenesulfonate (DPTS) (0.222, 0.755 mmol), dissolved in distilled CH$_2$Cl$_2$ (30 mL). The solution was stirred until completion, as measured by TLC (0.5 hr). The reaction mixture was then diluted with CH$_2$Cl$_2$ and filtered over cotton to remove dicyclohexylurea (DCU). The solvent was removed and the crude product was then purified via column chromotography (70:30 cyclohexane:ethyl acetate) to give 0.543 g (1.16 mmol) of compound **2.9** as a white powder. Yield: 85%. ^{1}H NMR (400 MHz, CDCl$_3$): 7.78 (d, J = 7.4, 2H, Ar-*H*), 7.62 (d, J= 7.4, 2H, Ar-*H*), 7.42 (t, J = 7.4, 2H, Ar-*H*), 7.31-7.35 (m, 2H, Ar-*H*), 5.62 (d, J= 7.82, 1H, Fmoc-N*H*-CαH-), 5.05 (s, 1H, Boc-N*H*-CH$_2$-), 4.42-4.44 (m, 3H, Ar$_2$-CH-C*H*$_2$-CO$_2$-NH-CαH-, Ar$_2$-C*H*-CH$_2$- Boc-NH-), 4.20-4.25 (m, 3H, -CO$_2$-C*H*$_2$-CH$_3$, Boc-NH-(CH$_2$)$_2$-Cα*H*-), 3.33-3.36 (m, 1H, (diastereotopic), -CαH-CH$_2$-C*H*$_2$-NH-Boc), 2.97-3.01 (m, 1H, (diastereotopic), -CαH-CH$_2$-C*H*$_2$-NH-Boc), 2.08-2.09 (m, 1H, (diastereotopic), -CαH-C*H*$_2$-CH$_2$-NH-Boc), 1.76-1.78 (m, 1H, (diastereotopic), -CαH-C*H*$_2$-CH$_2$-NH-Boc), 1.46 (s, 9H, Boc), 1.30 (t, J= 7.03, 3H, -CO$_2$-CH$_2$-C*H*$_3$-). ^{13}C NMR (CDCl$_3$): *δ* 172.23, 156.37, 143.82, 141.30 127.73, 127.08, 125.06, 120.00, 79.35, 67.03,

61.73, 51.53, 47.17, 36.49, 33.27, 28.42, 14.14. IR (cm^{-1}): 3345, 2977, 2933, 1711, 1521, 1510, 1045, 1168. HRMS: calc [M]$^+$ (C$_{26}$H$_{32}$N$_2$O$_6$): 468.2260 Found: (CI) 468.2237.

Synthesis of compound 2.3: Compound **2.5** (0.314 g, 0.670 mmol) was dissolved in tetrahydrofuran (THF) (4.0 mL). To this was added 1-octanethiol (1.16 mL, 6.70 mmol) and 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) (0.010 mL, 67.0 µmol). The solution was stirred for 3 hr, and then the solvent was removed. The crude mixture was purified via column chromatography (70:25:5 ethyl acetate:cyclohexane:triethylamine), affording 0.132 g (0.536 mmol) of compound **2.6** as a slightly yellow oil. Yield: 80%. ^{1}H NMR (400 MHz, D$_2$O): 4.13 (q, J= 7.03, 2H, -CO$_2$-C*H$_2$*-CH$_3$), 3.46-3.51 (m, 1H, -Cα*H*-(CH$_2$)$_2$-NH-Boc), 3.09-3.14 (m, 2H, -CαH-CH$_2$-C*H$_2$*-NH-Boc), 1.77-1.84 (m, 2H, -CαH-C*H$_2$*-CH$_2$-NH-Boc), 1.35 (s, 9H, Boc), 1.21 (t, J= 7.03, 3H, -CO$_2$-CH$_2$-C*H$_3$*). ^{13}C NMR (CDCl$_3$): 175.32, 155.60, 78.73, 60.67, 52.57, 37.49, 34.03, 28.02, 13.81. IR (cm^{-1}): 3374, 2978, 1711, 1251, 1027, 1174, 781. HRMS: calc [M]$^+$ (C$_{11}$H$_{22}$N$_2$O$_4$): 246.1580 Found: (CI) 247.1650

Synthesis of compound 2.11: NaHCO$_3$ (8.19 g, 97.5 mmol) was placed in a 1:1 mixture of H$_2$O:dioxane (100 mL). To this stirring solution, *DL*-homocysteine thiolactone·HCl (5.00 g, 32.5 mmol) was added over a period of 30 min. Finally, di-*tert*-butyl dicarbonate (10.64 g, 48.75 mmol) was added and the reaction mixture was stirred overnight. The reaction mixture was then diluted with ethyl acetate, washed twice with saturated NaHCO$_3$, and then twice with brine. The organic phase was dried over MgSO$_4$, filtered, and the solvent was removed to yield 8.69 g (36.9 mmol) compound **2.11** as a white solid. Characterization data agreed with that previously reported in the literature.[85]

Synthesis of compound 2.12: To a stirring solution of LiOH (3.0 g, 71.3 mmol) in a 1:1 mixture of H$_2$O:THF (90 mL) was added compound **2.11** (5.50 g, 23.8 mmol). The solution was stirred until the reaction was complete (3 hr) as detected by TLC. The solution was then diluted with ethyl acetate and washed three times with 1 M HCl. The organic phase was then dried over MgSO$_4$, filtered, and the solvent was removed to give 5.03 grams (21.4 mmol) of **2.12** as an amber colored oil. Yield: 90%. ^{1}H NMR (400 MHz, CD$_3$OD): 4.89 (s, 1 H, -N*H*-Boc-Cα*H* -CO$_2$H), 4.23-4.26 (m, 1H, -Cα*H* -CO$_2$H),

2.48- 2.58 (m, 2H, -CαH-CH_2-CH$_2$-), 1.89-2.02 (m, 2H, -CαH-CH_2-CH$_2$-), 1.41 (s, 9H, Boc). ^{13}C NMR (CD$_3$OD): δ 175.90, 158.24, 80.68, 53.53, 37.27, 28.85, 21.71. IR (cm^{-1}): IR (cm^{-1}): 3361, 2925, 2867, 1656, 1510. HRMS: calc [M]$^+$ (C$_9$H$_{17}$NO$_4$ S$_1$): 235.0878 Found: (CI) 236. 0950.

Synthesis of compound 2.14: 2,2'-Dipyridyl disulfide, **2.13** (9.56 g, 43.4 mmol) was dissolved in methanol (53 mL) and acetic acid (0.71 mL). To this, compound **2.12** (5.11 g, 21.7 mmol) dissolved in methanol (10.0 mL) was added dropwise via an addition funnel. The solution was stirred until reaction completion as determined by NMR (3 hr). The reaction mixture was then diluted with ethyl acetate and washed 3 times with 0.5 M pH 5 citrate buffer. The organic phase was dried over MgSO$_4$, filtered, and the solvent was removed in vacuo. The crude product was purified with column chromotography (70:30 cyclohexane:ethyl acetate, then 60:40 cyclohexane:ethyl acetate), yielding 4.27 g (12.4 mmol) of compound **2.14** as a white powder. Yield: 57%. ^{1}H NMR (400 MHz, CDCl$_3$): 8.51-8.52 (m, 2H, Ar-H), 7.78-7.80 (m, 1H, Ar-H), 7.70-7.72 (m, 1H, Ar-H), 7.15-7.18 (m, 1H, Ar-H), 5.45-5.47 (s, 1H, Boc-NH-), 4.45-4.46 (m, 2H, -NH-CαH-CO$_2$H-), 2.85-2.90 (m, 2H, -CαH-CH$_2$-CH_2-S-S-), 2.39 (s, 1H, (diastereotopic), -CαH-CH_2-CH$_2$-S-S-), 2.05-2.10 (m, 1H, (diastereotopic), -CαH-CH_2-CH$_2$-S-S-), 1.44 (s, 9H, Boc). ^{13}C NMR (CDCl$_3$): δ 175.13, 160.11, 155.94, 149.11, 138.18, 121.36, 120.88, 80.38, 52.75, 35.12, 32.50, 28.57. IR (cm^{-1}): 3356, 2978, 2932, 1712, 1574, 1518, 1250, 1120, 510. HRMS: calc [M]$^+$ (C$_{14}$H$_{20}$N$_2$O$_4$ S$_2$): 244.0864 Found: (CI) 244.0334.

Synthesis of compound 2.15: An excess of dry ethanol was added to a flame-dried flask containing **2.14** (0.700 g, 2.03 mmol), DCC (1.05 g, 5.07 mmol), DMAP (0.0517 g, 0.423 mmol), and DPTS (0.249 g, 0.845 mmol) dissolved in distilled CH$_2$Cl$_2$ (20 mL). The solution was stirred until completion by TLC (1.5 hr). The reaction mixture was then diluted with CH$_2$Cl$_2$ and filtered over cotton to remove DCU. The solvent was removed, and the crude product was then purified via column chromotography (60:40 cyclohexane: ethyl acetate) to give 0.502 g (1.35 mmol)of compound **2.15** as a clear, pale green oil. Yield: 80%. ^{1}H NMR (400 MHz CDCl$_3$): 8.46-8.49 (m, 1H, Ar-H), 7.64-7.67 (m, 2H, Ar-H), 7.08-7.12 (m, 1H, Ar-H), 5.28 (s, 1H, Boc-NH-CαH-CO$_2$-), 4.38-4.39 (m, 1H, -NH-CαH-CO$_2$-CH$_2$-), 4.11-4.20 (m, 2H, -CO$_2$-CH_2-CH$_2$-), 2.82-2.88 (m, 2H, -CαH-CH$_2$-

CH_2-S-S-), 2.25-2.28 (m, 1H, (diastereotopic), -CαH-CH_2-CH$_2$-S-S-), 2.00-2.06 (m, 1H, (diastereotopic), -CαH-CH_2-CH$_2$-S-S-), 1.43 (s, 9H, Boc),1.24 (t, 3H, J =7.23, -CO$_2$-CH$_2$-CH_3). ^{13}C NMR (CDCl$_3$): δ 172.33, 160.14, 155.68, 149.97, 137.29, 121.01, 120.11, 80.29, 61.86, 52.92, 35.07, 32.49, 28.57, 14.41. IR (cm^{-1}): 3356, 2978, 2932, 1712, 1574, 1518, 1250, 1118, 500. HRMS: calc [M+H]$^+$ (C$_{16}$H$_{24}$N$_2$O$_4$ S$_2$): 373. 1257 Found: (CI) 373. 0734.

Synthesis of compound 2.4: Compound **2.15** (0.115 g, 0.309 mmol) was dissolved in a 1:1 mixture of TFA:CH$_2$Cl$_2$ and stirred for 2 hr. The solvent was then removed to give compound **2.4** as a slightly yellow oil in quantitative yield. ^{1}H NMR (400 MHz D$_2$O): 8.31-8.33 (m, 1H, Ar-H), 7.67-7.74 (m, 2H, Ar-H), 7.21-7.23 (m, 1H, Ar-H), 4.14-4.18 (m, 3H, -CO$_2$-CH_2-CH$_3$-, -NH-CαHαH- CO$_2$-CH$_2$), 2.85-2.87 (m, 2H, -CαH-CH$_2$-CH_2-S-S-), 2.23-2.25 (m, 2H, -CαH-CH_2-CH$_2$-S-S-), 1.09-1.13 (t, J=7.23, 3H, -CO$_2$-CH$_2$-CH_3-). ^{13}C NMR (CD$_3$OD): δ 170.11, 160.36, 150.33, 139.81, 122.29, 122.09, 63.97, 52.90, 34.87, 30.85, 14.42. IR (cm^{-1}): 3443, 1636, 1204, 1258, 723. HRMS: calc [M+H]$^+$ (C$_{11}$H$_{16}$N$_2$O$_2$ S$_2$): 273. 0733 Found: (CI) 273. 0734.

Synthesis of 2.25:1,4-butanediol (0.160 mL, 1.82 mmol), was added to a flame-dried flask containing DAB (2.00 g, 4.54 mmol), DCC (1.13 g, 5.46 mmol), DMAP (0.0556 g, 0.455 mmol), and DPTS (0.134, 0.455 mmol), dissolved in distilled CH$_2$Cl$_2$ (40 mL). The solution was stirred until completion by TLC (0.5 hr). The reaction mixture was then diluted with CH$_2$Cl$_2$ and filtered over cotton to remove DCU. The solvent was removed, and the crude product was then purified via column chromotography (70:30cyclohexane: ethyl acetate, then 60:40cyclohexane: ethyl acetate) to give 1.37 g (1.46 mmol) of compound **2.25** as a white powder. Yield: 80%.^{1}H NMR (400 MHz CDCl$_3$): 7.78 (d, J= 7.42, 4H, Ar-H), 7.61 (d, J= 7.42, 4H, Ar-H), 7.41 (t, J= 7.42, 4H, Ar-H), 7.33 (m, 4H, Ar-H), 5.68 (d, J= 7.82, 2H Fmoc-NH-CαH-), 5.10 (s, 2H, Boc-NH-CH$_2$-), 4.40-4.46 (m, 6H, Ar$_2$-CH-CH_2-CO$_2$-NH-CαH and Ar$_2$-CH-CH$_2$-Boc-NH-), 4.12-4.18 (m, 6H, -CO$_2$-CH_2-CH$_3$ and Boc-NH-(CH$_2$)$_2$-CαHH-), 3.36-3.39 (m, 2H, (diastereotopic), -CαH-CH$_2$-CH_2-NH-Boc), 2.97-3.01 (m, 2H, (diastereotopic), -CαH-CH$_2$-CH_2-NH-Boc), 2.03-2.09 (m, 2H, (diastereotopic), -CαH-CH_2-CH$_2$-NH-Boc), 1.73 (m, 2H, (diastereotopic), -CαH-CH_2-CH$_2$-NH-Boc, 4H, CO$_2$-CH$_2$-CH_2-), 1.46 (s, 18H, Boc). ^{13}C NMR (CDCl$_3$): δ

172.28, 156.39, 143.81, 141.29, 127.73, 125.06, 120.00, 79.40, 67.06, 64.93, 51.53, 47.15, 36.53, 33.94, 28.42, 25.07. IR (cm^{-1}): 3245, 2987, 2953, 1741, 1532, 1510, 1218, 1066, 1200. HRMS: calc [M]$^{+}$ ($C_{51}H_{60}N_4O1_2$):934.4364 Found: (ESI) 934. 4364.

Synthesis of compound 2.26: Compound **2.26** (0.285 g, 0.305 mmol) was dissolved in 4 mL of THF. To this was added 1-octanethiol (0.529 mL, 3.05 mmol) and DBU (0.0456 µL, 0.0148 µmol). The reaction was stirred for 3hr, and then the solvent was removed. The crude mixture was then redissolved in chloroform, then precipitated from a cold solution of hexanes (20 mL), affording 0.0633 g of compound **2.26** as a yellow gel 0.105 g (0.214 mmol). Yield: 70%. The product was carried on towards the next step without further purification. ^{1}H NMR (400 MHz CDCl$_3$): 5.19 (br s, 2H, Boc-N*H*-(CH$_2$)$_2$-CαH), 4.15 (s, 4H, -CO$_2$-C*H*$_2$-CH$_3$), 3.48 (br s, 2H, -Cα*H*-CH$_2$-CH$_2$-NH-Boc) 3.35-3.37 (m, 2H, (diastereotopic),-CαH-CH$_2$-C*H*$_2$-NH-Boc), 3.22-3.25 (m, 2H, (diastereotopic), -CαH-CH$_2$-CH$_2$-NH-Boc), 1.94-1.97 (m, 2H, (diastereotopic), -CαH-C*H*$_2$-CH$_2$-NH-Boc), 1.44 (s, 18H, Boc).

Synthesis of compound 2.27: 1,4-butanediol (0.103 mL, 1.16 mmol), was added to a flame-dried flask containing **2.14** (1.00 g, 2.90 mmol), DCC (0.718 g, 3.48 mmol), DMAP (0.0354 g, 0.290 mmol), and DPTS (0.170 g, 0.580 mmol), dissolved in distilled CH$_2$Cl$_2$ (15 mL). The solution was stirred until completion by TLC (2.0 hr). The reaction mixture was then diluted with CH$_2$Cl$_2$ and filtered over cotton to remove DCU. The solvent was removed, and the crude product was then purified via column chromotography (60:40cyclohexane: ethyl acetate, then 50:50cyclohexane: ethyl acetate) to give 0.689 g (0.928 mmol) of compound **2.27** as a cloudy, pale green gel. Yield: 80%. ^{1}H NMR (400 MHz CDCl$_3$): 8.47-8.49 (m, 2H, Ar-*H*), 7.62-7.68 (m, 4H, Ar-*H*), 7.08-7.10 (m, 2H, Ar-*H*), 5.40 (s, 2H, Boc-N*H*-CαH-CO$_2$-), 4.39-4.40 (m, 2H, -NH-Cα*H*-CO$_2$-CH$_2$-), 4.12 (s, 4H, -CO$_2$-C*H*$_2$-CH$_2$-), 2.83-2.88 (m, 4H, -CαH-CH$_2$-C*H*$_2$-S-S-), 2.27-2.29 (m, 2H, (diastereotopic), -CαH-C*H*$_2$-CH$_2$-S-S-), 2.02-2.06 (m, 2H, (diastereotopic), -CαH-C*H*$_2$-CH$_2$-S-S-), 1.43 (s, 18H, Boc). ^{13}C NMR (CDCl$_3$): δ 172.29, 159.98, 155.66, 149.91, 137.28, 121.00, 120.05, 80.25, 65.04, 52.87, 5.01, 32.21, 28.54, 25.29. IR (cm^{-1}): 3374, 2977, 2965, 1712, 1520, 1576, 1049, 1166, 510. HRMS: calc [M]$^{+}$ ($C_{32}H_{46}N_4O_8S_4$): 742.2198 Found: (ESI) 742. 2198.

Synthesis of compound 2.28: Compound **2.28** (0.200 g, 0.268 mmol) was dissolved in a 1:1 mixture of TFA:CH_2Cl_2 and stirred for two hours. The solvent was then removed to give compound **2.28** as a yellow viscous oil in quantitative yield. ^{1}H NMR (400 MHz CD_3OD): 8.44-8.45 (m, 2H, Ar-*H*), 7.81-7.83 (m, 4H, Ar-*H*), 7.26-7.29 (m, 2H, Ar-*H*), 4.23- 4.30 (m, 1H, -NH-Cα*H*-CO_2-CH_2-, 4H, -CO_2-C*H*$_2$-CH_2-), 2.96-3.01 (m, 4H, -CαH-CH_2-C*H*$_2$-S-S-), 2.31-2.40 (m, 4H, -CαH-C*H*$_2$-CH_2-S-S-), 1.71-1.73 (br s, 4H, -CO_2-CH_2-C*H*$_2$-). ^{13}C NMR (CD_3OD): δ 169.96, 159.88, 149.56, 140.34, 123.05, 122.30, 67.03, 52.66, 34.72, 30.63, 25.88. IR (cm^{-1}): 3443, 1636, 1204, 1142, 723. HRMS: calc [M]$^+$ ($C_{22}H_{30}N_4O_4 S_4$): 542. 1150 Found: (ESI) 542. 1150.

Synthesis of compound 2.34: Freshly distilled *N,N*-diisopropylethylamine (DIPEA) (0.0113 mL, 11.3 mmol) was added slowly to a reaction flask containing the nitrobenzyl alcohol **2.32** (2.00 g, 9.38 mmol) and 4-nitrophenyl chloroformate (2.28 g, 11.3 mmol) in distilled CH_2Cl_2 (94 mL) . The solution was stirred until the reaction was complete as determined by thin layer chromatography (3 hr). The solution was then diluted with CH_2Cl_2 and washed with 1 M HCl, then with 1M $NaHCO_3$. The organic phase was dried over MgSO4, filtered, and the solvent was removed in vacuo. The crude product was purified with column chromatography (80:20cyclohexane: ethyl acetate, then 60:40cyclohexane: ethyl acetate), yielding **2.34** (1.70 g, 4.50 mmol) as an off white powder. Yield: 48 %. ^{1}H NMR (400 MHz $CDCl_3$): 8.30-8.32 (m, 2H, Ar-*H*), 7.78 (s, 1H, Ar-*H*), 7.41-7.44 (m, 2H, Ar-*H*), 7.12 (s, 1H, Ar-*H*), 5.72 (s, 2H, Ph-C*H*$_2$-CO_2-Ph), 4.0-4.04 (s, 9H, C*H*$_3$-Ph). ^{13}C NMR ($CDCl_3$): δ 154.97, 153.32, 151.68, 148.42, 145.14, 139.62, 124.98, 121.35, 110.26, 108.01, 67.30, 56. 20. HRMS: calc [M]$^+$ ($C_{16}H_{14}N_2O_9$): 378.0699 Found: (CI) 378.0699.

Synthesis of polymer 2.1: The di-p-toluenesulfonic acid salt monomer **2.29**, whose synthesis has been previously reported elsewhere,[80] (1.71g, 2.35 mmol) and sodium carbonate (0.654 g, 6.17 mmol) were dissolved in distilled water (20 mL). Diester **2.26** (0.288 g, 0.587 mmol) was dissolved in dichloromethane (20 mL) and added to the aqueous phase and allowed to mix for 30 min. Sebacoyl chloride (0.628 mL, 2.94 mmol) diluted in anhydrous dichloromethane (5 mL), was added drop wise over 30 min to the biphasic solution and was allowed to react for 24 hr. Upon completion of the reaction,

solvent was removed in vacuo. The resulting polymer was redissolved in DMF, permitting filtration of the insoluble salts. The filtrate was then dialysed against DMF for 24 hrs, with at least one dialysate change over this time period. The purified PEA was precipitated in water (5 mL) and lyophilized for 24 hrs to give an off white solid (1.31 g, 1.91 mmol). Yield: 65%. ^{1}H NMR (600 MHz DMSO): 8.22 (d, J= 7.63, 1.6H, -CO-NH-C$_\alpha$H-CH$_2$-Ph), 8.13 (d, J=7.63, 0.4H, -CO-NH-C$_\alpha$H-CO$_2$-), 7.17-7.26 (m, 8H, Ph) 6.78 (s, 0.4H, Boc-NH-(CH$_2$)$_2$-C$_\alpha$H-), 4.43-4.47 (m, 1.6H, -CO-NH-C$_\alpha$$H$-CH$_2$-Ph), 4.18-4.21 (m, 0.4H, Boc-NH-(CH$_2$)$_2$-C$_\alpha$$H$-), 4.03(s, 0.8H, Boc-NH-(CH$_2$)$_2$-C$_\alpha$H-CO$_2$-CH_2-), 3.96 (s, 1.6H, Ph-CH$_2$-C$_\alpha$H-CO$_2$-CH_2-), 2.87-3.01 (m, 4H, -C$_\alpha$H-CH_2-Ph, -C$_\alpha$H-CH$_2$-CH_2-NH-Boc), 2.09 (b s, 0.8H, -NH-CO-CH_2-), 2.02-2.04 (m, 3.2H, -NH-CO$_2$-CH_2-), 1.83-1.84 (m, 0.4H, (diastereotopic), -CαH-CH$_2$-CH_2-NH-Boc), 1.63-1.69 (m, 0.4H, (diastereotopic), -CαH-CH_2-CH$_2$-NH-Boc), 1.60 (s, 0.8H, -CO$_2$-CH$_2$-CH_2-), 1.43 (b s, 3.2H, -CO$_2$-CH$_2$-CH_2-), 1.36 (b s, 3.2H, -NH-CO-CH$_2$-CH_2-), 1.32 (s, 3.6H, Boc), 1.20 (b s, 0.8H, -NH-CO-CH$_2$-CH_2-), 1.11 (b s, 8H, -NH-CO-CH$_2$-CH$_2$-CH_2-, -NH-CO-CH$_2$-CH$_2$-CH$_2$-CH_2-). IR (cm^{-1}): 3442, 2930, 2875, 1647, 1523, 1180, 1201, 700. SEC: M$_n$ =155,500; M$_w$ = 126,400; PDI = 1.23.

Synthesis of polymer 2.2: The di-p-toluenesulfonic acid salt monomer **2.29**,[80] (3.85 g, 5.28 mmol) and sodium carbonate (1.47 g, 13.9 mmol) were dissolved in distilled water (33 mL). Diester **2.28** (1.02 g, 1.32 mmol) was dissolved in mixture containing dichloromethane (23 mL) and THF (2 mL), then added to the aqueous phase and allowed to mix for 30 min. Sebacoyl chloride (1.41 mL, 6.6 mmol) diluted in anhydrous dichloromethane (8 mL), was added drop wise over 30 min to the biphasic solution and was allowed to react for 24 hr. Upon completion of the reaction, solvent was removed in vacuo. The resulting polymer was purified as described above for polymer **2.2** to give an off-white solid (2.34 g, 3.3 mmol). Yield: 50 %. ^{1}H NMR (600 MHz DMSO): 8.47-8.49 (s, 0.4H, Ar-H) 8.23 (d, J= 7.63, 1.6H, -CO-NH-C$_\alpha$H-CH$_2$-Ph), 7.72-7.79 (m, 0.4H, Ar-H), 7.17-7.26 (m, 8.4H, Ph, Ar-H), 4.43-4.47 (m, 1.6H, -CO-NH-C$_\alpha$$H$-CH$_2$-Ph), 4.38-4.39 (m, 0.4H, Pyr-S-S-(CH$_2$)$_2$-C$_\alpha$$H$-), 3.96 (s, 4H, Pyr-S-S-(CH$_2$)$_2$-C$_\alpha$H-CO$_2$-CH_2-, Ph-CH$_2$-C$_\alpha$H-CO$_2$-CH_2-), 2.87-3.01 (m, 4H, -C$_\alpha$H-CH_2-Ph, Pyr-S-S-CH_2-CH$_2$-), 2.16-2.17(m, 0.4H, (diastereotopic), Pyr-S-S-CH$_2$-CH_2-), 2.07 (m, 0.8H, -NH-CO$_2$-CH_2-), 2.02-2.04 (m, 3.2H, -NH-CO-CH_2-), 1.94-1.98 (m, 0.4H, diastereotopic (Pyr-S-S-CH$_2$-

CH_2-), 1.52 (s, 0.8H, -CO$_2$-CH$_2$-CH_2-), 1.44 (b s, 3.2H, -CO$_2$-CH$_2$-CH_2-), 1.36 (b s, 4H, -NH-CO-CH$_2$-CH_2-, -NH-CO-CH$_2$-CH_2), 1.11 (s, 8H, -NH-CO-CH$_2$-CH$_2$-CH_2-, -NH-CO-CH$_2$-CH$_2$-CH$_2$-CH_2-). IR (cm^{-1}): 3443, 2926, 2850, 1644, 1455, 1510, 1180, 1200, 700. SEC: M_n = 98,300; M_w = 79,300; PDI = 1.24.

Synthesis of compound 2.30: Compound **2.1** (0.300g, 0.457 mmol) was dissolved in a 1:1 mixture of TFA:CH$_2$Cl$_2$ and stirred for 2 hr. The solvent was then removed to give compound **2.30** as slightly brown oil in quantitative yield. The polymer was precipitated into cold water yielding polymer **2.30** as a white solid (2.35 g, 3.43 mmol). Yield: 75%. ^{1}H NMR (400 MHz DMSO): 8.19 (s, 1.6H, -CO-NH-C$_\alpha$H-CH$_2$-Ph), 7.15-7.19 (m, 8H, Ph), 4.43-4.47 (m, 1.6H, CO-NH-C$_\alpha$$H$-CH$_2$-Ph), 4.30-4.33 (m, 0.4H, Boc-NH-(CH$_2$)$_2$-C$_\alpha$$H$-), 4.04 (s, 0.8H, Boc-NH-(CH$_2$)$_2$-C$_\alpha$H-CO$_2$-CH_2-), 3.92 (s, 1.6H, Ph-CH$_2$-C$_\alpha$H-CO$_2$-CH_2-), 2.87-3.01 (m, 4H, -C$_\alpha$H-CH_2-Ph, -C$_\alpha$H-CH$_2$-CH_2-NH$_3$$^+TFA^-$), 2.10 (b s, 0.8H, -NH-CO-CH_2-), 2.02-2.04 (m, 3.2H, -NH-CO-CH_2-), 1.87-1.90 (m, 0.8H, -CαH-CH_2-CH$_2$-NH$_3$$^+TFA^-$), 1.60 (s, 0.8H, -CO$_2$-CH$_2$-C$H_2$-), 1.42 (b s, 3.2H, -CO$_2$-CH$_2$-C$H_2$-), 1.34 (b s, 3.2H, -NH-CO-CH$_2$-CH_2), 1.20 (b s, 0.8H, -NH-CO-CH$_2$-CH_2-), 1.11 (b s, 8H, -NH-CO-CH$_2$-CH$_2$-CH_2-, -NH-CO-CH$_2$-CH$_2$-CH$_2$-CH_2-). SEC: M_n =91, 800; M_w = 75, 400 PDI = 1.22

Synthesis of compound 2.35: Compound **2.34** (0.503 g, 1.33 mmol) and DMAP (0.121 g, 0.998 mmol) was dissolved in a mixture containing toluene (4 mL) and CH$_2$Cl$_2$ (7 mL). To this reaction flask was added **2.30** (0.384 g, 0.562) dissolved in CH$_2$Cl$_2$ (5 mL), then freshly distilled DIPEA (2 mL, 9.98 mmol).The reaction mixture was then stirred for 3 days. The solution was then diluted with CH$_2$Cl$_2$ and washed with 1 M HCl, then with 1 M NaHCO$_3$.The organic layer was dried over MgSO4, filtered, and the solvent was removed in vacuo. The resulting polymer was purified as described above for polymer **2.1** and **2.2** to give an amber colored solid The purified PEA was precipitated in water (5 mL) and lyophilized for 24 hrs to an amber colored solid (0.229 g, 0.253 mmol). Yield: 45%. ^{1}H NMR (400 MHz DMSO): 8. 23 (d, J= 7.80, 1.6H, -CO-NH-C$_\alpha$H-CH$_2$-Ph), 8.16 (d, J=7.63, 0.4H, -CO-NH-C$_\alpha$H-CO$_2$-), 7.67 (s, 0.4H, Ar-H), 7.47 (s, 0.4H, -CO$_2$-NH-(CH$_2$)$_2$-), 7.16-7.25 (m, 8.4H, Ph, Ar-H), 5.31 (s, 0.8H, Ar-CH_2-CO$_2$-NH-) 4.42-4.48 (m, 1.6H, -CO-NH-C$_\alpha$$H$-CH$_2$-Ph), 4.21-4.24 (m, 0.4H, -CH$_2$-CO$_2$-NH-(CH$_2$)$_2$-C$_\alpha$$H$-), 4.03 (s,

0.8H, -NH-(CH$_2$)$_2$-C$_\alpha$H-CO$_2$-CH$_2$-), 3.96 (s, 1.6H, Ph-CH$_2$-C$_\alpha$H-CO$_2$-CH$_2$-), 3.88 (s, 1.2H, CH$_3$-O-Ar), 3.84 (s, 1.2H, CH$_3$-O-Ar), 3.03-3.06 (m, 0.8H, -C$_\alpha$H-CH$_2$-CH$_2$-NH-CO$_2$-CH$_2$-Ar), 2.87-3.01 (m, 3.2H, -C$_\alpha$H-CH$_2$-Ph), 2.08 (m, 0.8H, -NH-CO-CH$_2$-), 2.02-2.04 (m, 3.2H, -NH-CO-CH$_2$-), 1.81-1.87 (m, 0.4H, (diastereotopic), -C$_\alpha$H-CH$_2$-CH$_2$-NH-CO$_2$-CH$_2$-Ar), 1.71-1.77 (m, 0.4H, (diastereotopic), -C$_\alpha$H-CH$_2$-CH$_2$-NH-CO$_2$-CH$_2$-Ar), 1.58 (s, 0.8H, -CO$_2$-CH$_2$-CH$_2$-), 1.44-1.45 (m, 3.2H, -CO$_2$-CH$_2$-CH$_2$-), 1.35-1.39 (m, 3.2H, -NH-CO-CH$_2$-CH$_2$), 1.11 (b s, 8H, -NH-CO-CH$_2$-CH$_2$-CH$_2$-, -NH-CO-CH$_2$-CH$_2$-CH$_2$-CH$_2$-). SEC: M$_n$ =72, 200; M$_w$ = 101, 900; PDI = 1.41.

Small molecule compound cyclization studies. In the case of compound **2.3**, the Boc group was removed by placing the compound in a 1:1 ratio of TFA:CH$_2$Cl$_2$. Compounds **2.3**, **2.4** were dissolved in either pH 7.4 phosphate buffered D$_2$O or 7:2:1 ratio of DMSO: acetone –D$_6$: 0.1 M, pH 7.4phosphate buffer D$_2$O. For compounds **2.16** and **2.4**, the pH was readjusted to 7.4 to account for the addition of any acid in the form of TFA salts. In the case of compound **2.4**, DTT (10 mg, 0.0648 mmol) was added. The samples were then incubated in a sand bath and ^{1}H NMR spectra were obtained at regular intervals. The degree of ethanol evolution was calculated based on the relative integrations of the peaks corresponding to the methyl group of ethanol and that of the ethyl ester. First order rate constants were determined as the slope of ln (percent remaining) vs. time (hr) and the corresponding half lives were calculated as $t_{1/2}= \ln(2)/ k$

Polymer degradation studies in solution. Polymers **2.1**, **2.2**, and **2.31** were dissolved in 7:2:1 ratio of DMSO: acetone –D$_6$: 0.1 M pH 7.4 phosphate buffer D$_2$O and incubated in a 70° C oven for two weeks. Aliquots were taken and were lyophilized for at least 24 hr, then redissolved in DMF eluent and submitted for SEC analysis

Polymer film degradation studies. Polymers **2.1**, **2.2**, **2.30** and **2.31** were melt pressed using a Carver 3851c two post hydraulic press at 500 psi and 45 °C. The samples were then immersed in a 1 mL solution of phosphate buffer (pH=7.4), for a period of 8 weeks. For polymer **2.2**, 5 mg of DTT was added. Samples were then taken out periodically, washed thoroughly in order to remove any salts, then lyophilized for 24 hr. In the case of polymer **2.35**, the polymer was first dissolved at a concentration of 20 mg/mL, then an

aliquot (10 µL) was deposited onto a glass cover slip using a Laurell WS-400Bz-6NPP-Lite spin coater, operating at 2300 RPM for 10 seconds. The thickness of the spin coated polymers was measured using a KLA Tensor P-10 profiler equipped with a tungsten stylus with a diamond on the tip. The applied force was 3 mg and scan speed was 200 µm/s. The average thickness of the films was found to be 221.23 nm. Irradiation was performed using a medium-pressure mercury lamp (Hanovia S9 PC451050/805221). UV-visible spectroscopy experiments were carried out using a Varian Cary 300 Bio UV-visible spectrophotometer. In all cases, all polymers were mounted on aluminum stubs with carbon tape (or adhesive for polymer **2.35**), then sputter coated with gold. The surface microstructure was then imaged by scanning electron microscopy (SEM) (S-2600N, Hitachi, Japan).

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Chapter 3
Conclusions

3.1   Concluding remarks and future directions

The ability to trigger the degradation of a polymer is attractive for many biological applications. For example, in the area of drug delivery, a polymeric system may circulate without decomposing and thus retain its therapeutic efficacy. However, once this polymer encounters a stimulus that triggers its degradation, it can release its payload to a specific part of the body, rather than unloading it in undesirable locations such as the heart, or the liver.

To this end, this thesis described the development of the first stimuli responsive PEAs. This was accomplished by combining the principles of self-immolative spacers and PEAs containing functional pendant groups, by incorporating monomers that are capable of 1,5-cyclization reactions into the PEA backbone. In addition, these spacers contain protecting groups sensitive to external stimuli such as light or changes in pH or redox potential, such that once cleavage occurs; the functional moiety is revealed and induces the degradation of the PEA backbone. Kinetic studies on aliphatic esters indicate that the degradation of an ester containing these spacers is accelerated, as opposed to those that do not contain it, thereby supporting the mechanism of degradation.

The polymers were synthesized and subjected to degradation studies. It was found that even in an organic solution, the polymers containing the self-immolative spacers degraded faster than their control counter-parts. In addition, studies were carried out on thin films and the result was the same; the polymers containing the self-immolative spacers exhibited more mass loss and exhibited more surface erosion than their control counterparts. The mass loss study results are attractive in that there have not been too many studies of PEAs in neutral conditions only, as most of them have been undertaken in the presence of enzymes, whose presence in physiological environments require special conditions. [1] Also, since the mass loss was gradual, this could be applied to drug delivery applications, as sustained release is attractive in these studies.

As well as synthesizing polymers sensitive to changes in pH and redox potential, a polymer that was sensitive to light was also synthesized. Since the Boc group cannot be cleaved under physiological conditions, a protecting group sensitive to light was chosen due to its ability to be applicable to biological applications. The polymer was subjected to the same degradation conditions as the other polymers (in addition to being irradiated) and it was found that compared to its control counter part (non irradiated), the polymer degraded faster. The polymers were then cast into films in order to determine if the results were applicable on the surface, as well. The polymer that was irradiated exhibited more surface erosion than its control, which was not irradiated. This study not only shows that degradation containing these spacers is accelerated; it also exhibits the robustness of the PEA backbone, able to incorporate different functional groups such that degradation can be done via different stimuli.

Due to their ability to exhibit triggered degradation and subsequently to degrade over time (as seen with the mass loss studies), these PEAs are attractive as drug delivery vehicles. They can potentially release the drug once the stimulus is applied and can release it over time due to their sustained release. However, work needs to be done to make these polymers more hydrophilic as they are quite hydrophobic. This could be circumvented by replacing the diol with ethylene glycol,[2] or by attaching a hydrophilic chain to the pendant functional group, as has been previously reported by our group to make self-assemblies.[3] Also, since the PEA backbone is able to incorporate different monomers, different ratios of different monomers containing different protecting groups could be synthesized, such that the polymer is multi responsive to different stimuli.[4,5,6]

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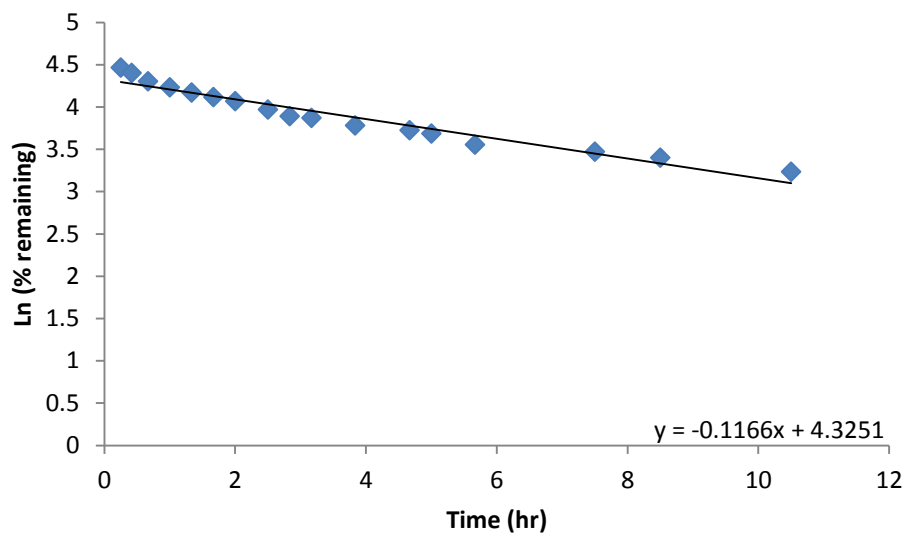
Appendix

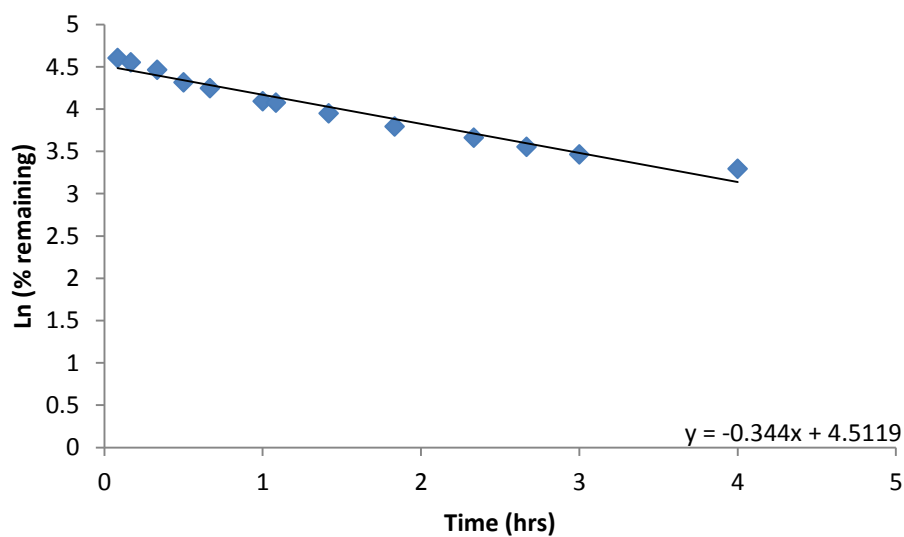**Figure A.1** First order plot of **2.16** monomer degradation.

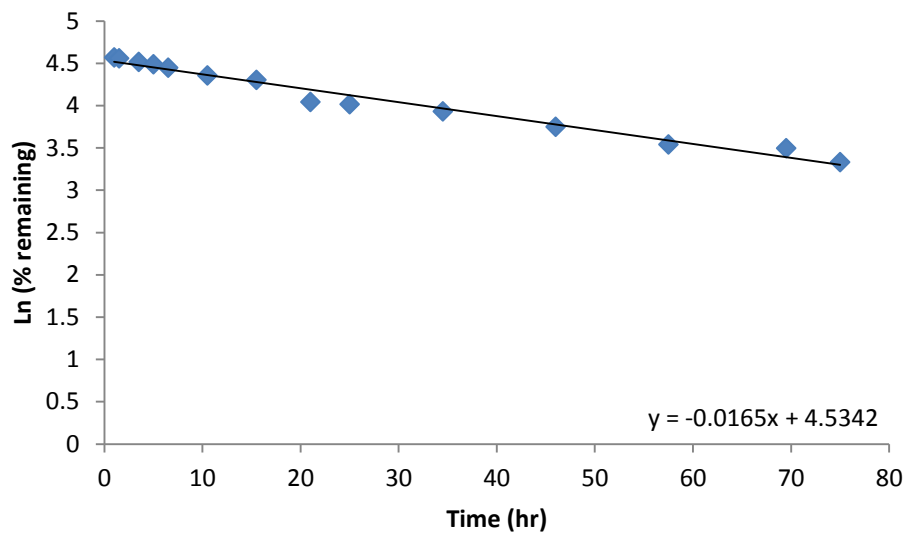**Figure A.2** First order plot of **2.17** monomer degradation.
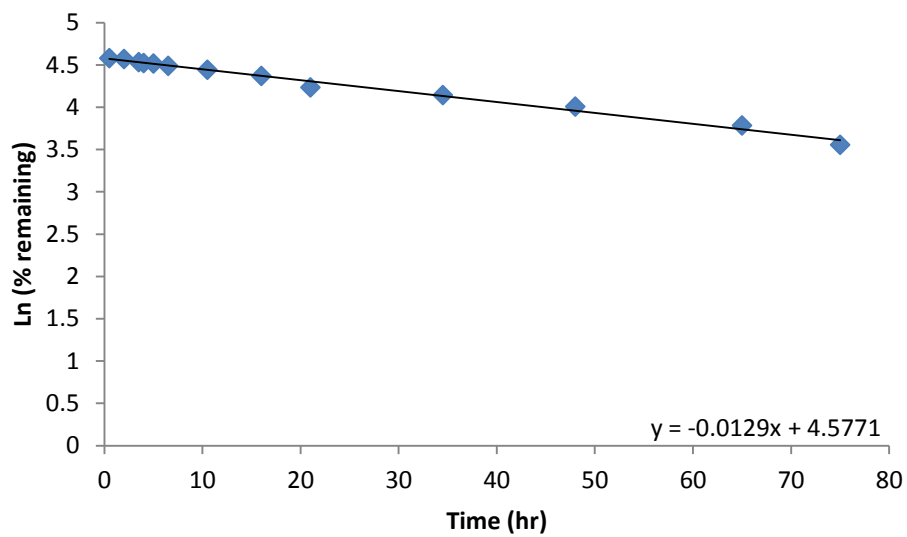
Figure A.3 First order plot of **2.3** monomer control.
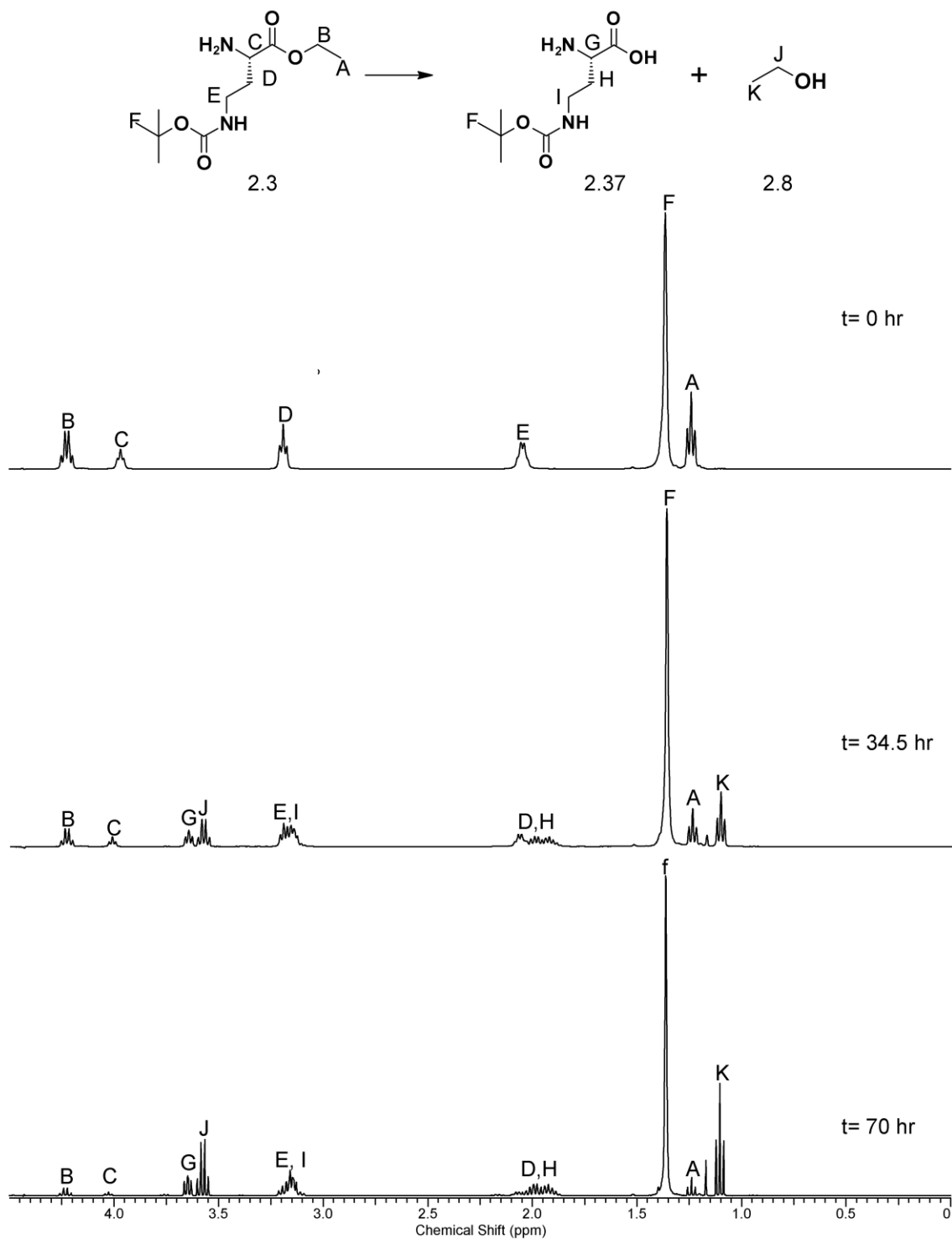

Figure A.4 First order plot of **2.17**, without DTT.

Figure A.5 ^{1}H NMR (400 MHz) spectra in pH 7.4 phosphate buffered D$_2$O of a) DAB derivative **2.3** immediately following dissolution in the buffer. b) After 34.5 hr in the same buffer. c) After 70 hr in the same buffer.
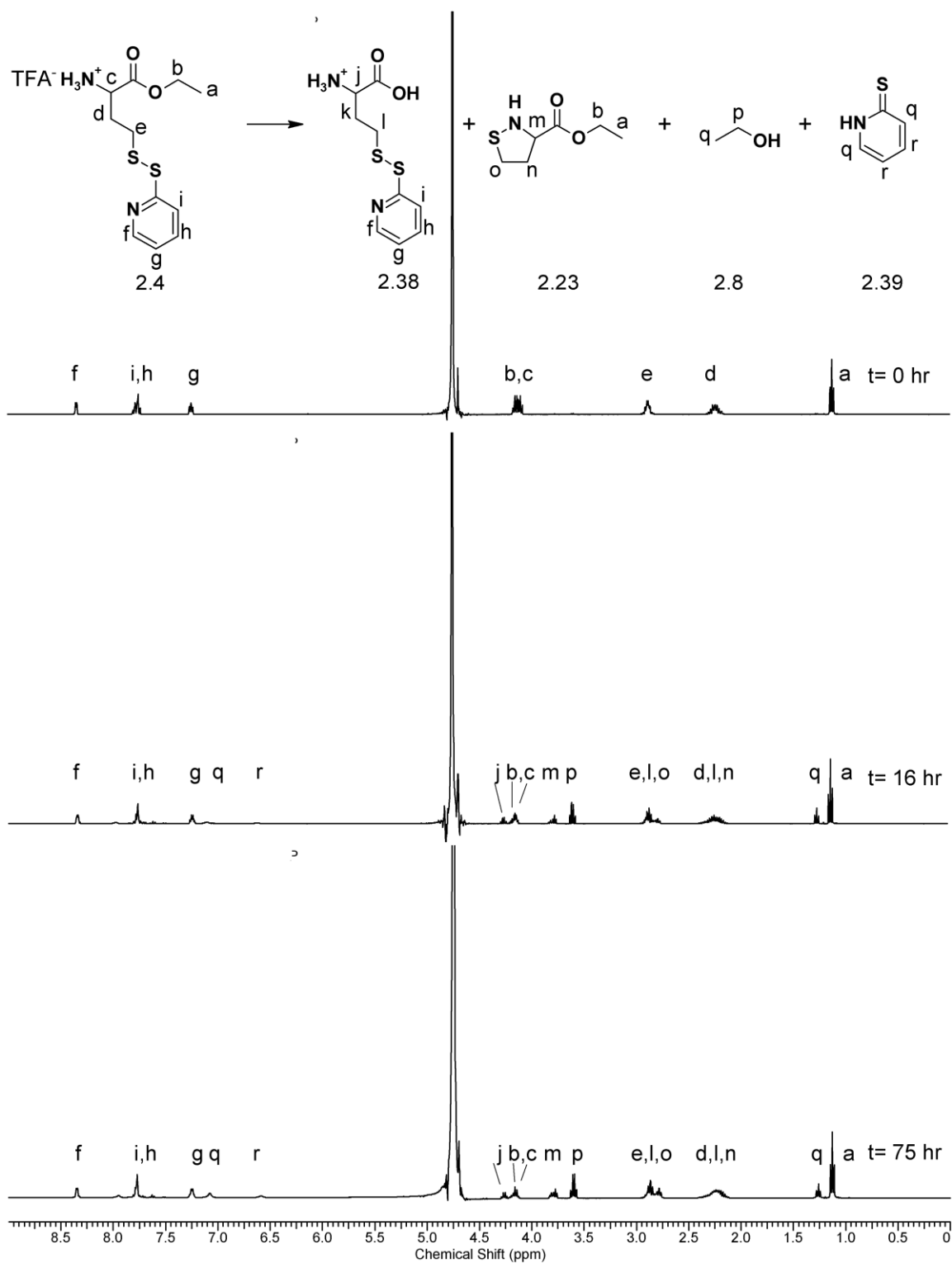
Figure A.6 ^{1}H (400 MHz) NMR spectra in pH 7.4 phosphate buffered D$_2$O of a) Hcy derivative **2.4** immediately following dissolution in the buffer. b) After 16 hr in the same buffer. c) After 75 hr in the same buffer.
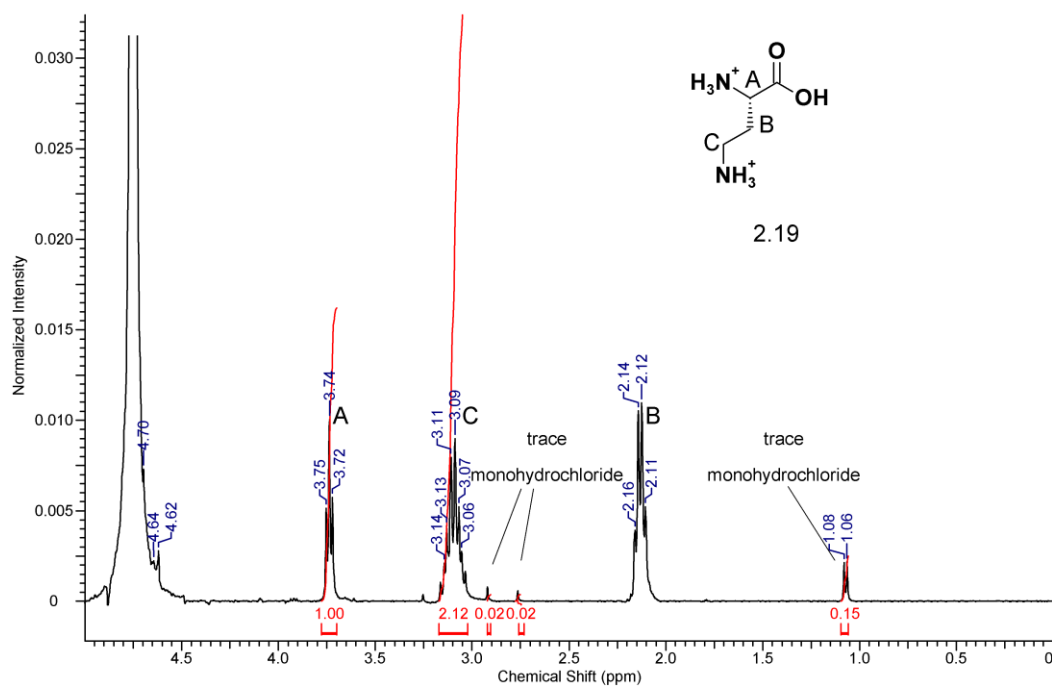
Figure A.7 ^{1}H NMR (400 MHz) spectra in pH 7.4 phosphate buffered D_2O of DAB amino acid.
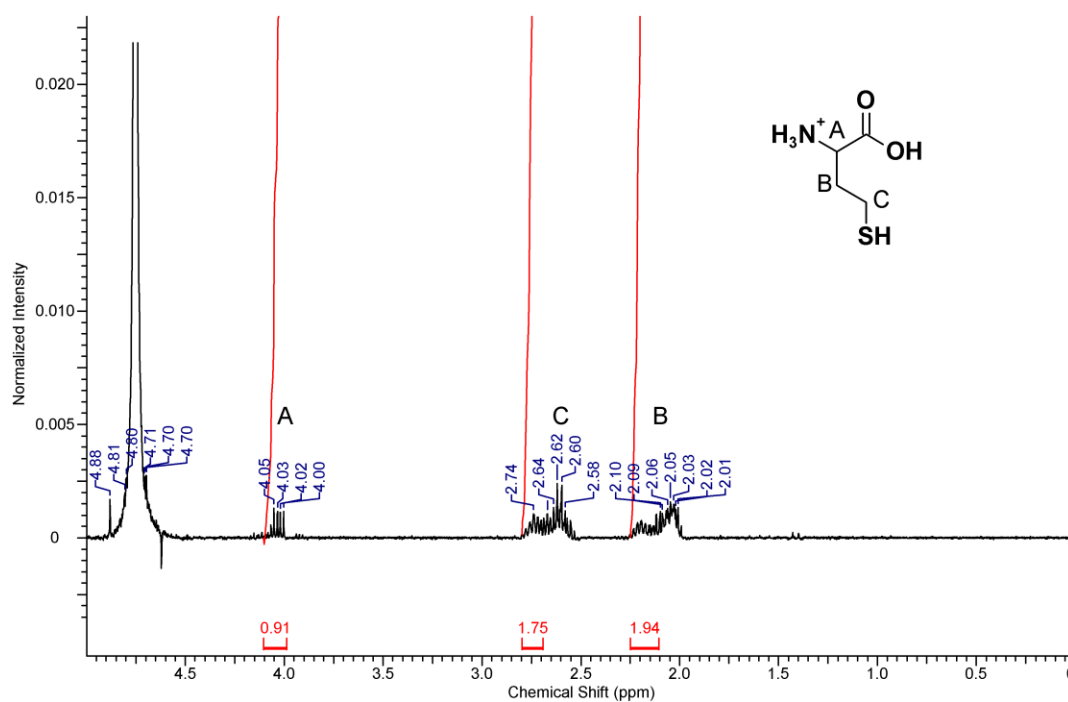
Figure A.8 ^{1}H NMR (400 MHz) spectra in pH 7.4 phosphate buffered D_2O of Hcy amino acid.
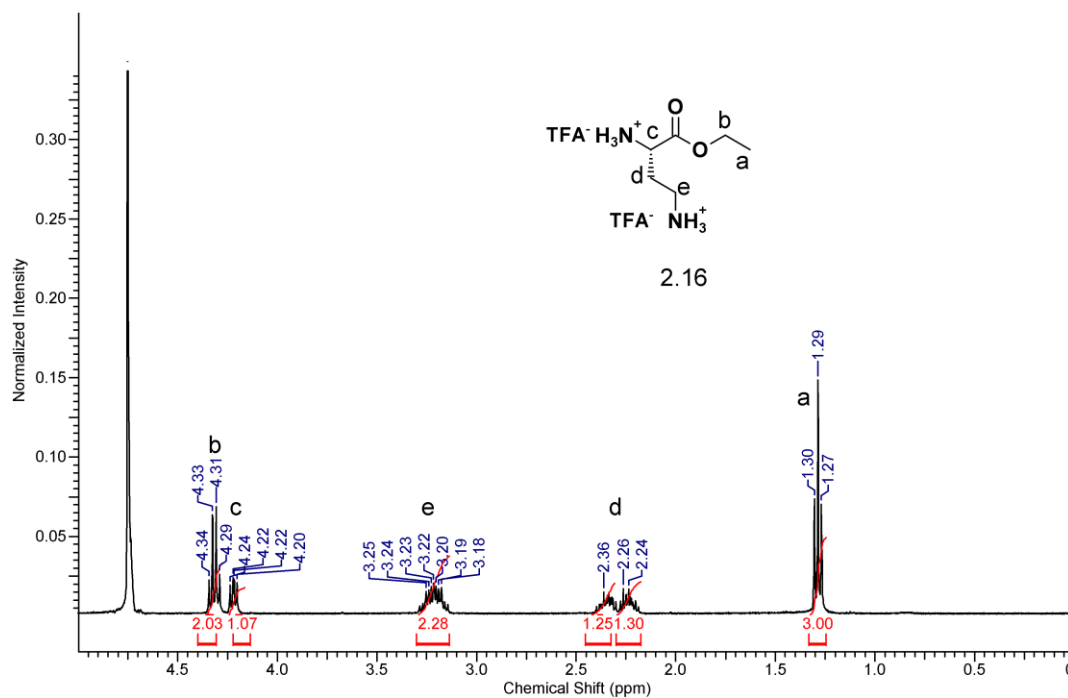
Figure A.9 ^{1}H NMR (400 MHz) spectra in D_2O of DAB amino acid prior to degradation.
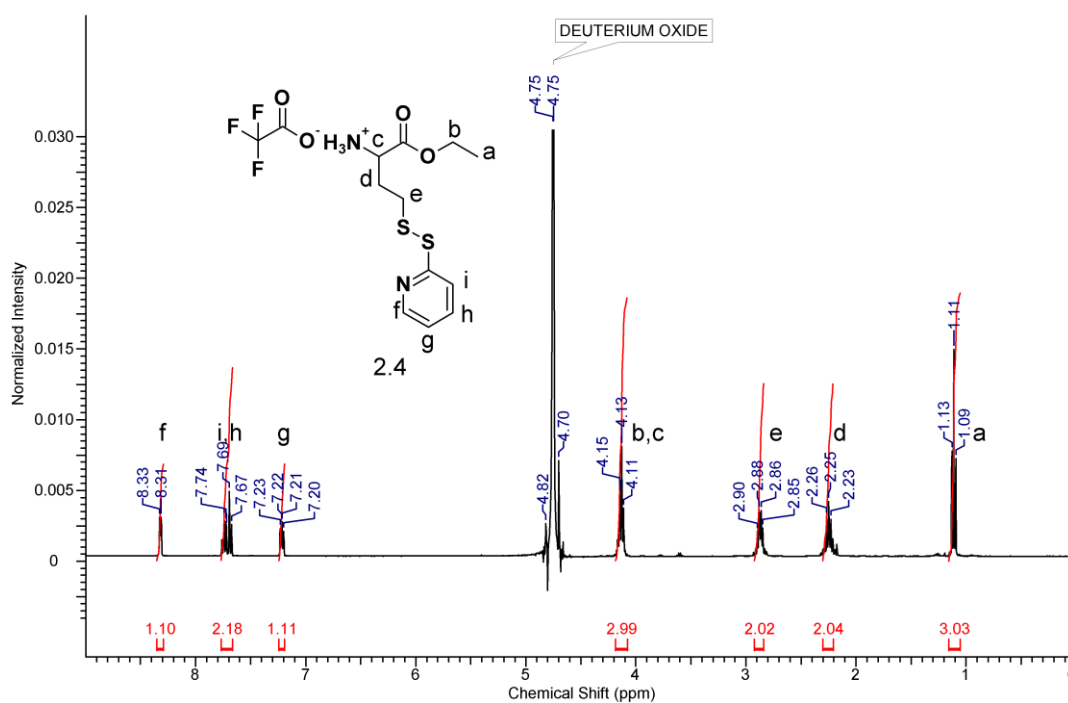
Figure A.10 ^{1}H NMR (400 MHz) spectra in D_2O of Hcy amino acid prior to DTT cleavage.
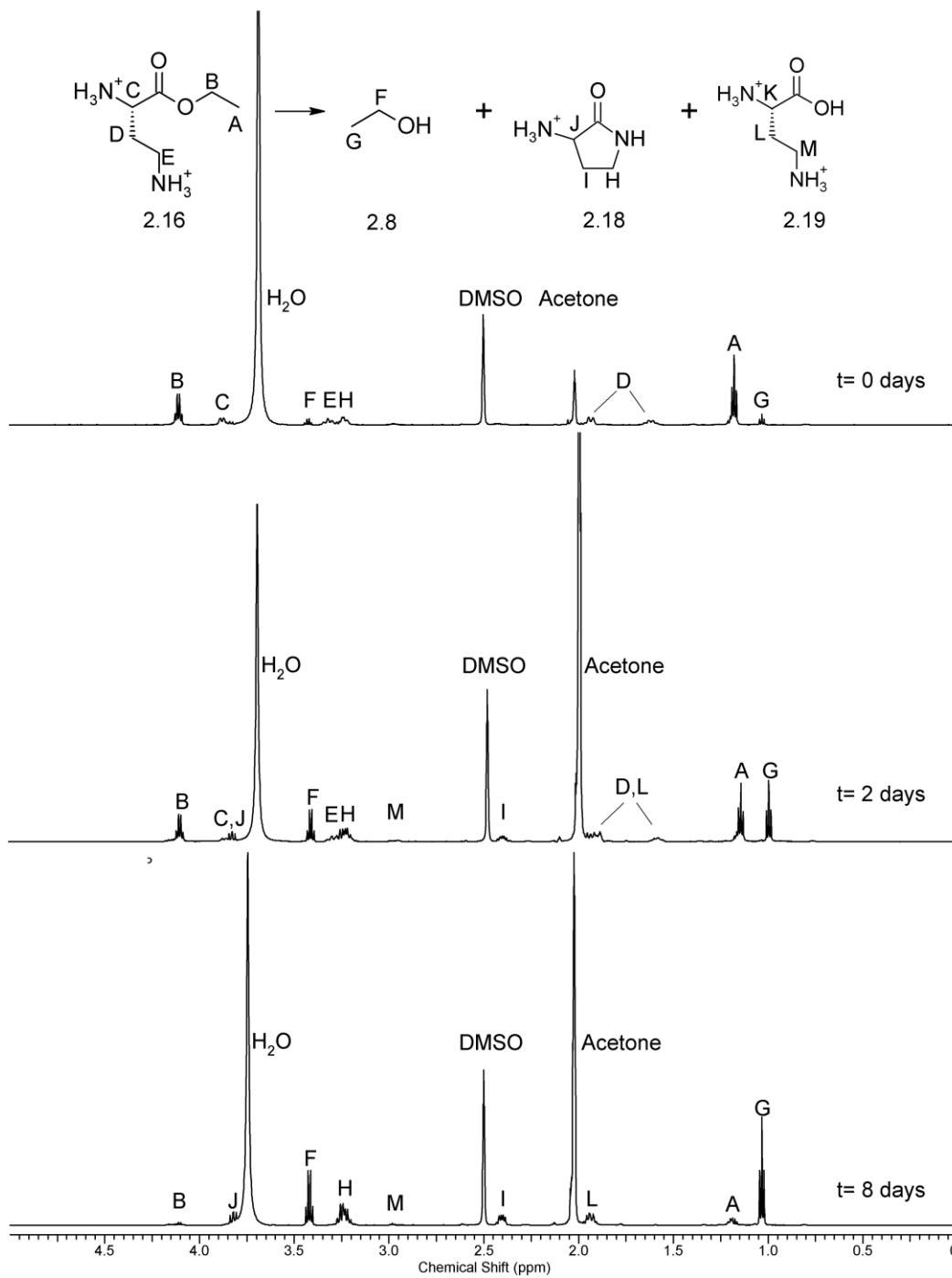
Figure A.11 ^{1}H (400 MHz) NMR spectra in 7:2:1 ratio of DMSO: acetone –D$_6$: 0.1 M, pH 7.4 phosphate buffered D$_2$O of compound a) DAB derivative **2.16** immediately following dissolution. b) After 2 days in the same solution. c) After 8 days in the same solution.
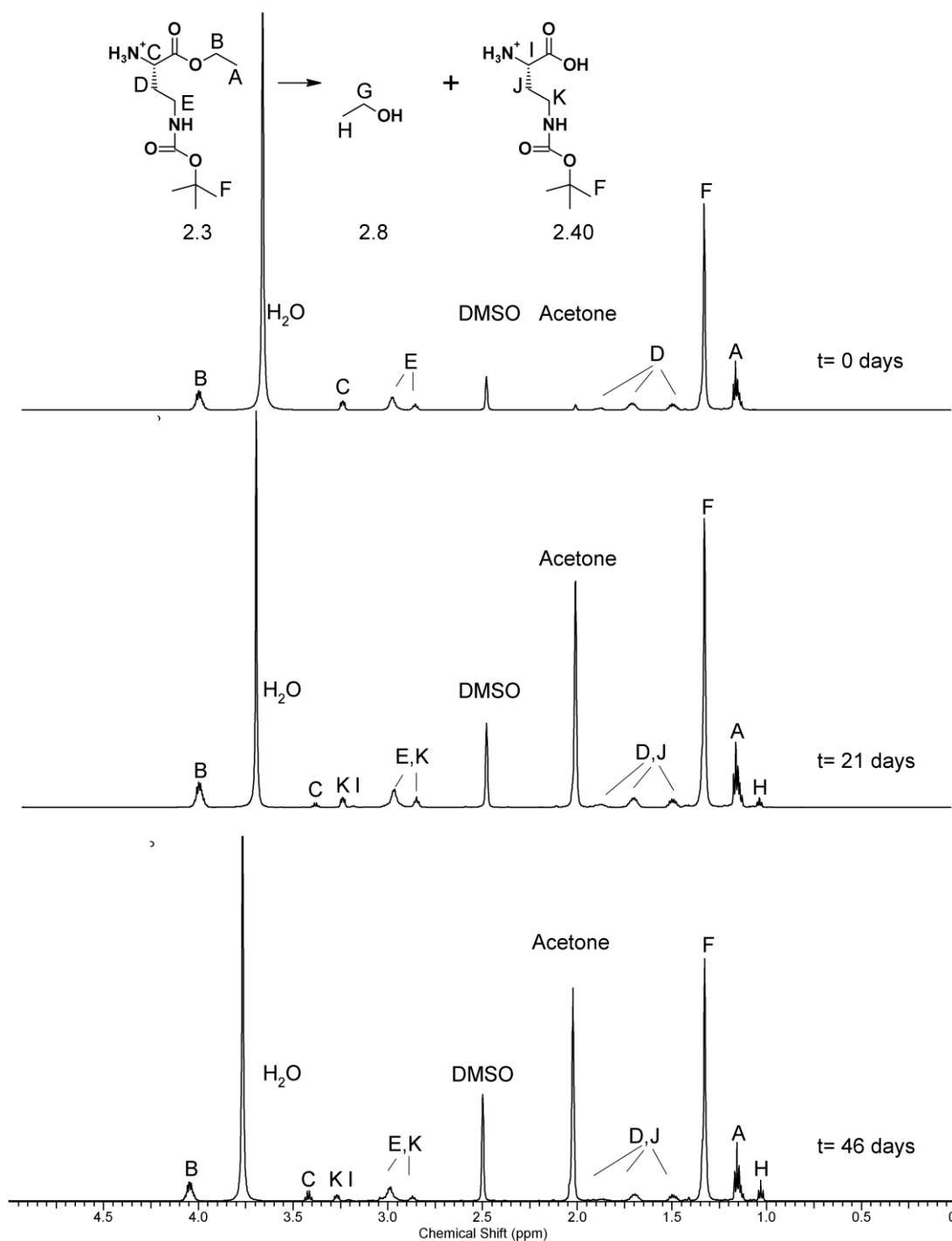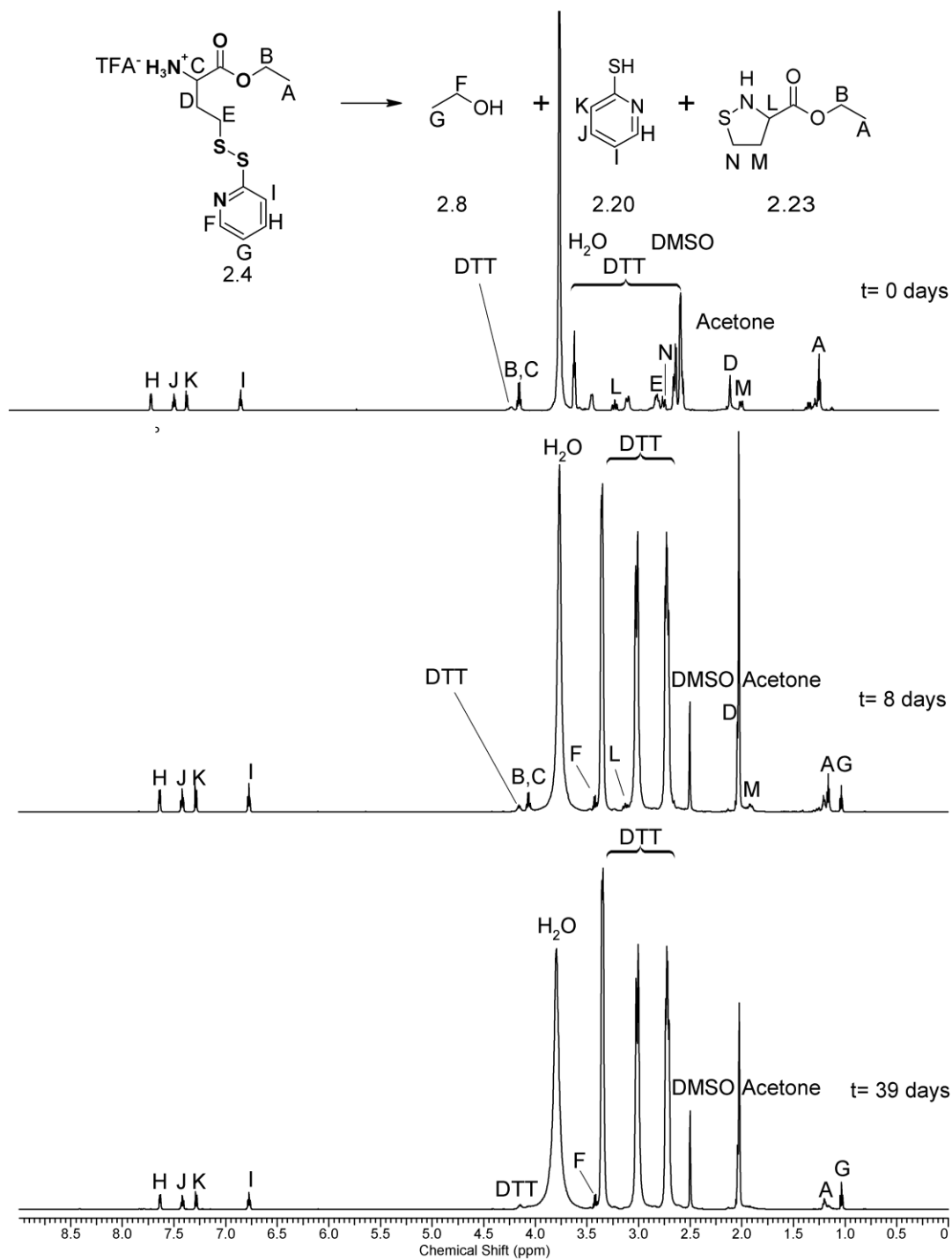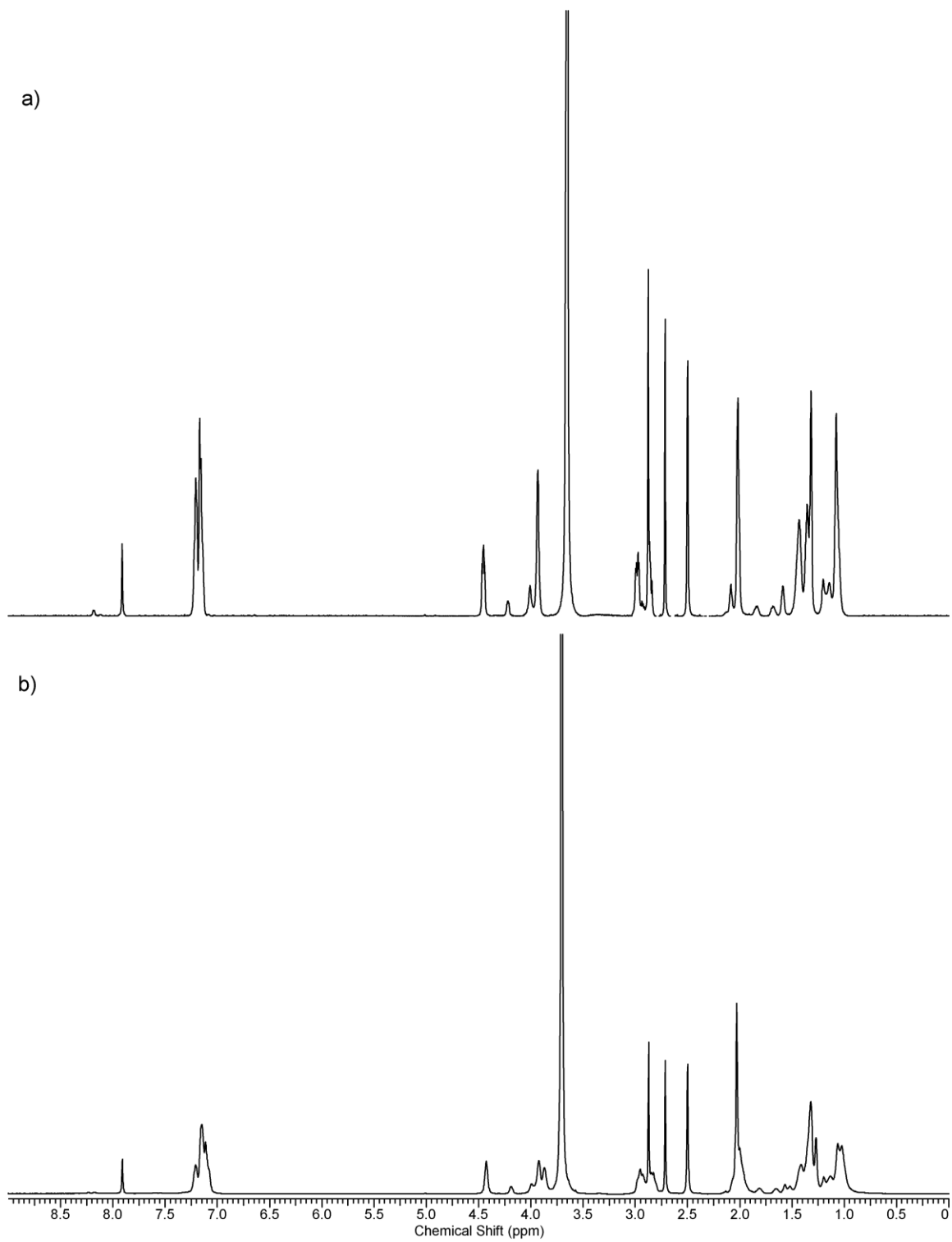
Figure A.12 ^{1}H (400 MHz) NMR spectra in 7:2:1 ratio of DMSO: acetone $-D_6$: 0.1 M, pH 7.4 phosphate buffered D_2O of compound a) DAB derivative **2.3** immediately following dissolution. b) After 21 days in the same solution. c) After 46 days in the same solution.

Figure A.13 ^{1}H (400 MHz) NMR spectra in 7:2:1 ratio of DMSO: acetone –D$_6$: 0.1 M, pH 7.4 phosphate buffered D$_2$O of compound a) Hcy derivative **2.17** immediately following dissolution. b) After 8 days in the same solution. c) After 39 days in the same solution.

Figure A.14 ^{1}H (400 MHz) NMR spectra in 7:2:1 ratio of DMSO: acetone –D$_6$: 0.1 M, pH 7.4 phosphate buffered D$_2$O of compound a) Polymer **2.1** immediately following dissolution. b) After 2 weeks in the same solution.
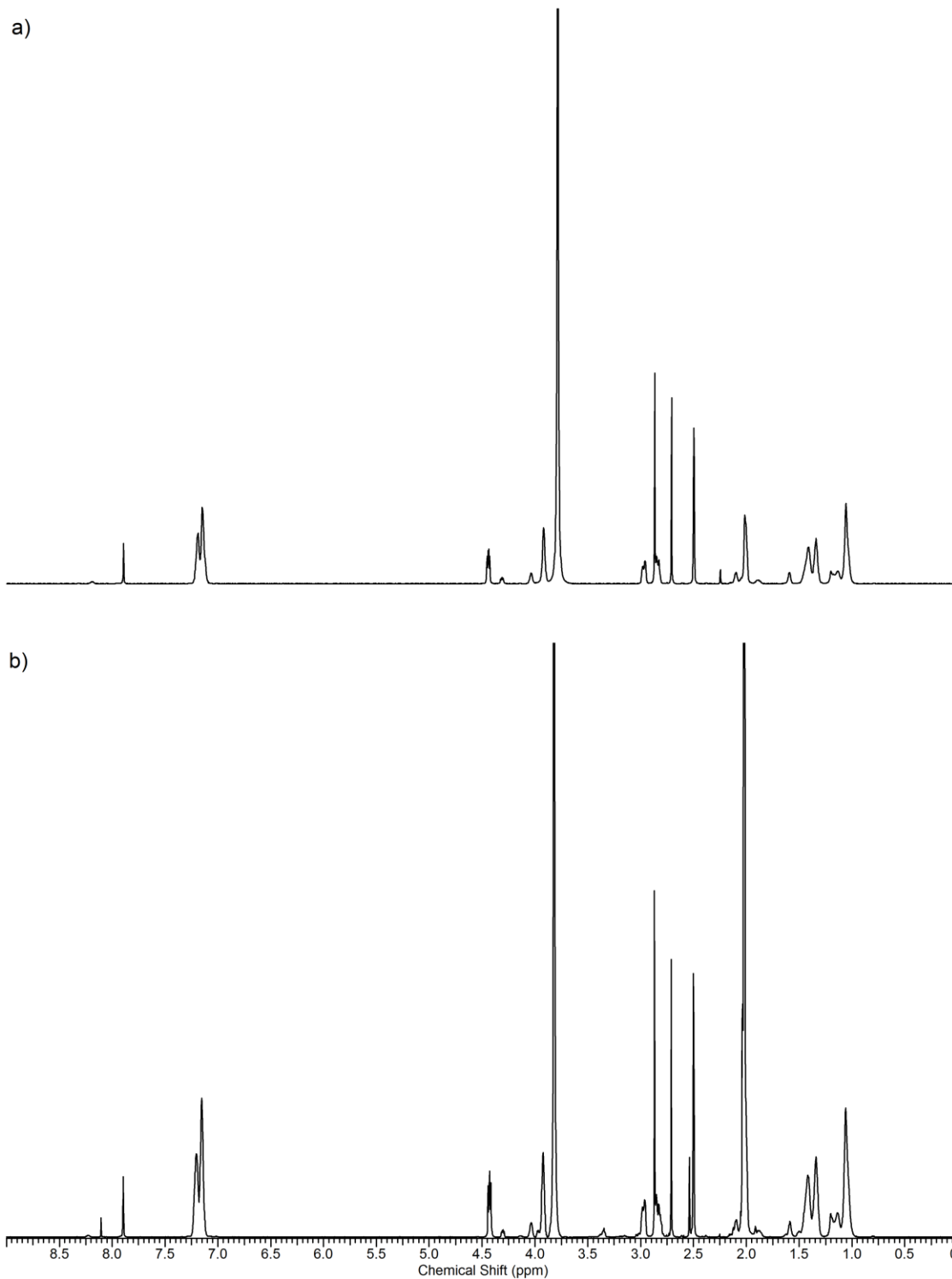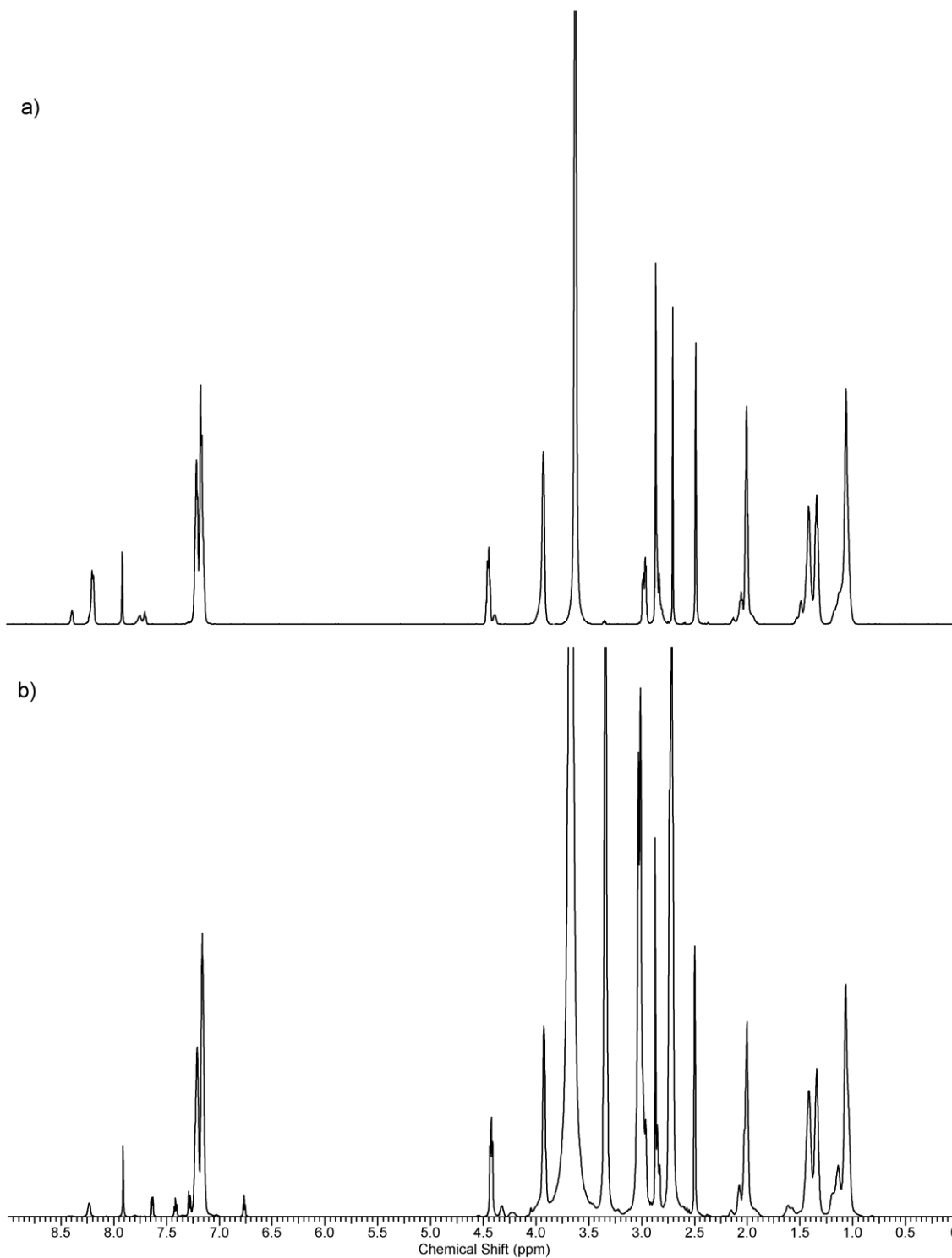
Figure A.15 ^{1}H (400 MHz) NMR spectra in 7:2:1 ratio of DMSO: acetone $-D_6$: 0.1 M, pH 7.4 phosphate buffered D_2O of compound a) Polymer **2.30** immediately following dissolution. b) After 2 weeks in the same solution.

Figure A.16 ^{1}H (400 MHz) NMR spectra in 7:2:1 ratio of DMSO: acetone $-D_6$: 0.1 M, pH 7.4 phosphate buffered D_2O of compound a) Polymer **2.2** immediately following dissolution. b) After 2 weeks in the same solution.
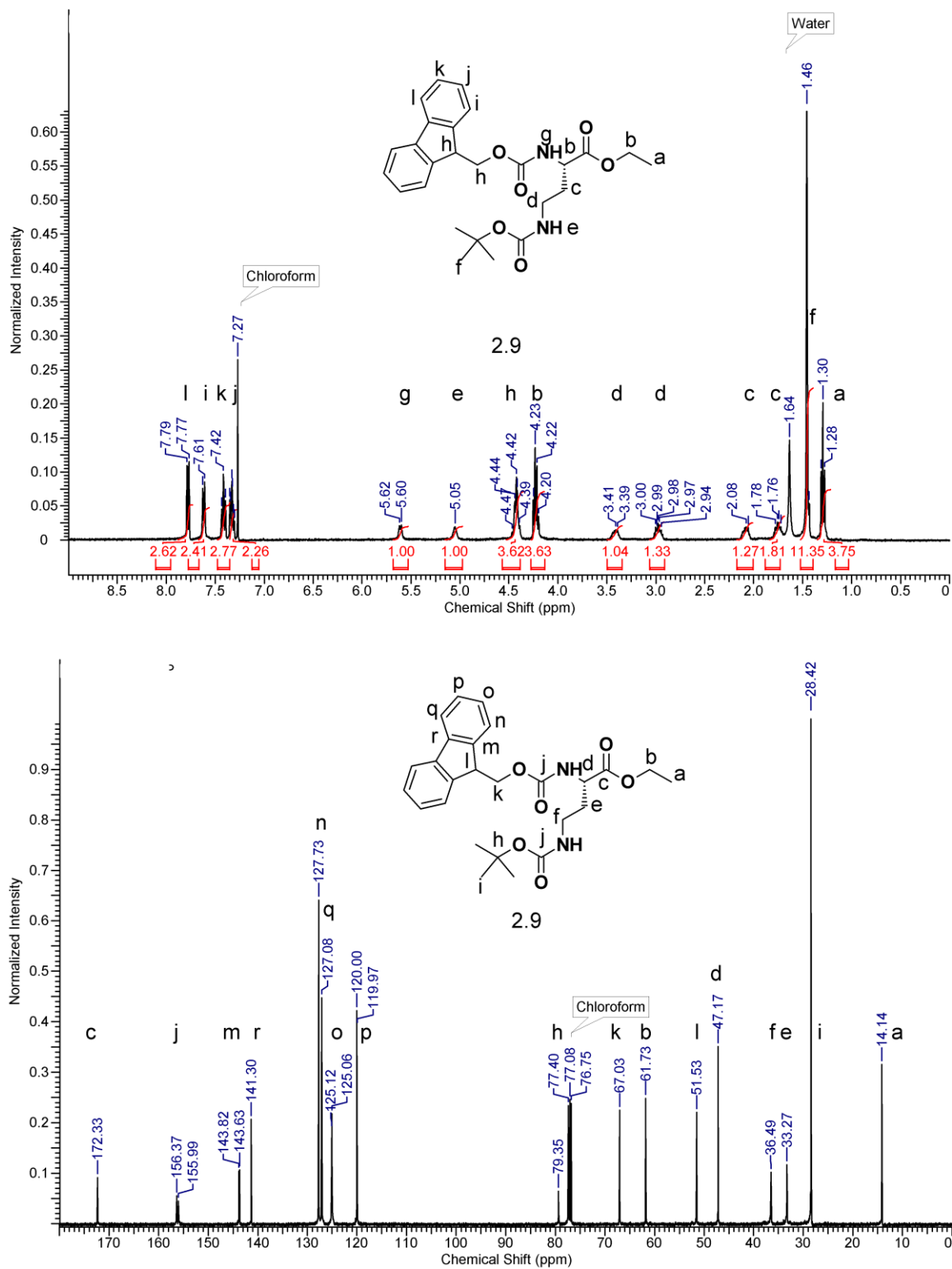
Figure A.17 ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compound **2.9** in CDCl$_3$.
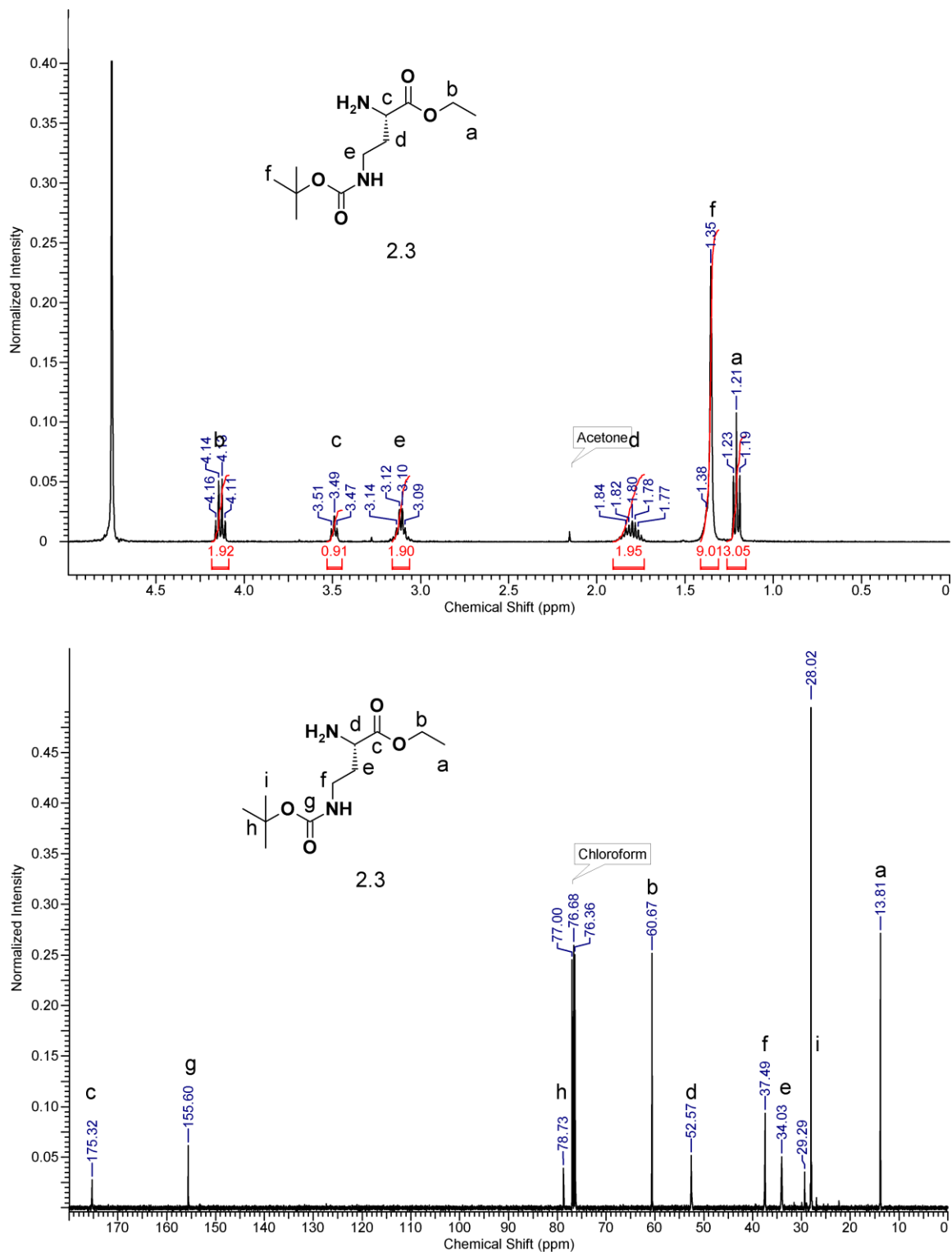
Figure A.18 ^{1}H NMR (400 MHz) in D$_2$O and ^{13}C NMR (100 MHz) spectra of compound **2.3** in CDCl$_3$.

Figure A.19 ^{1}H NMR (400 MHz) and ^{13}C NMR (150 MHz) spectra of compound **2.12** in CD$_3$OD.

Figure A.20 ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compound **2.14** in CDCl$_3$.

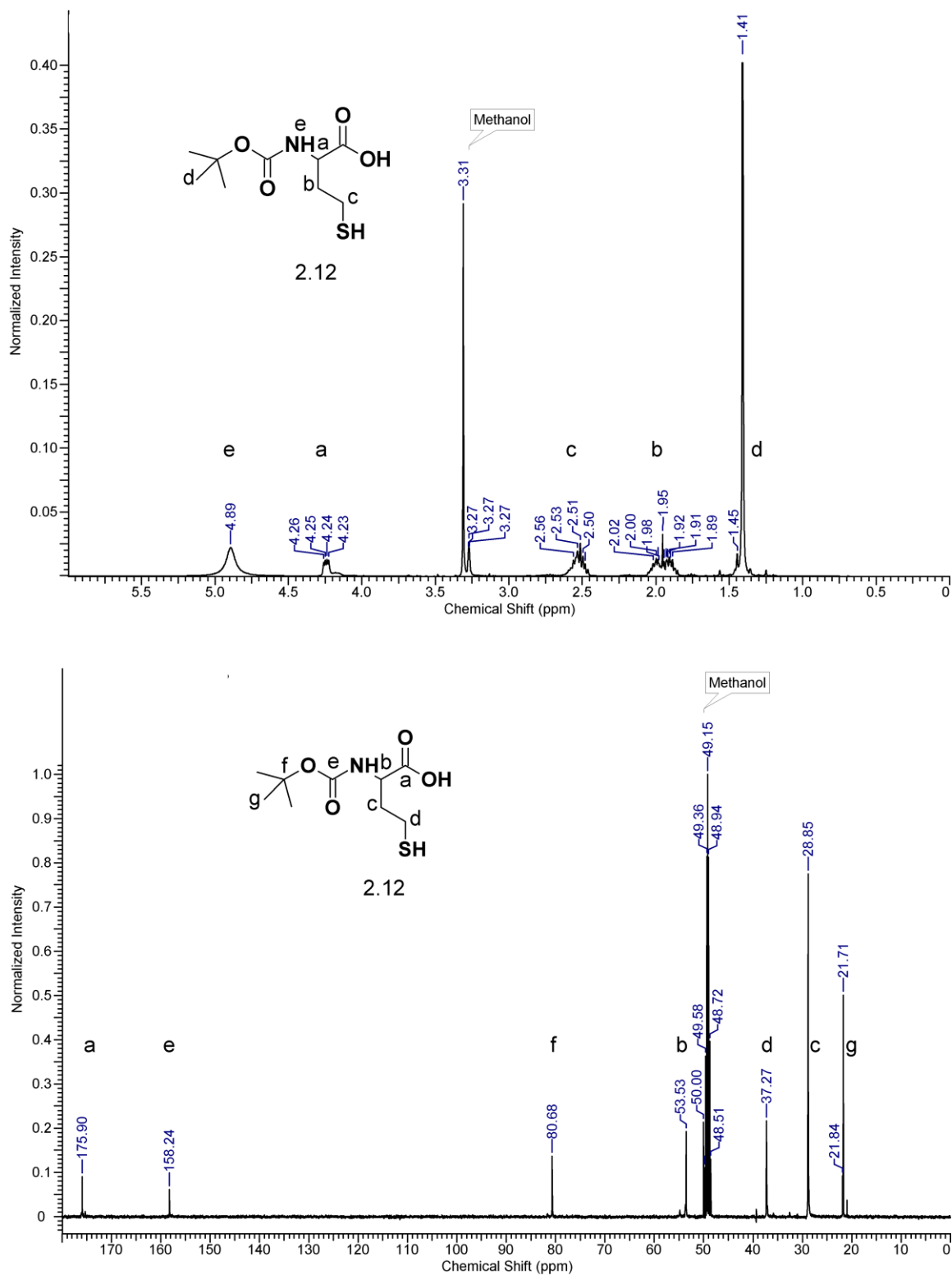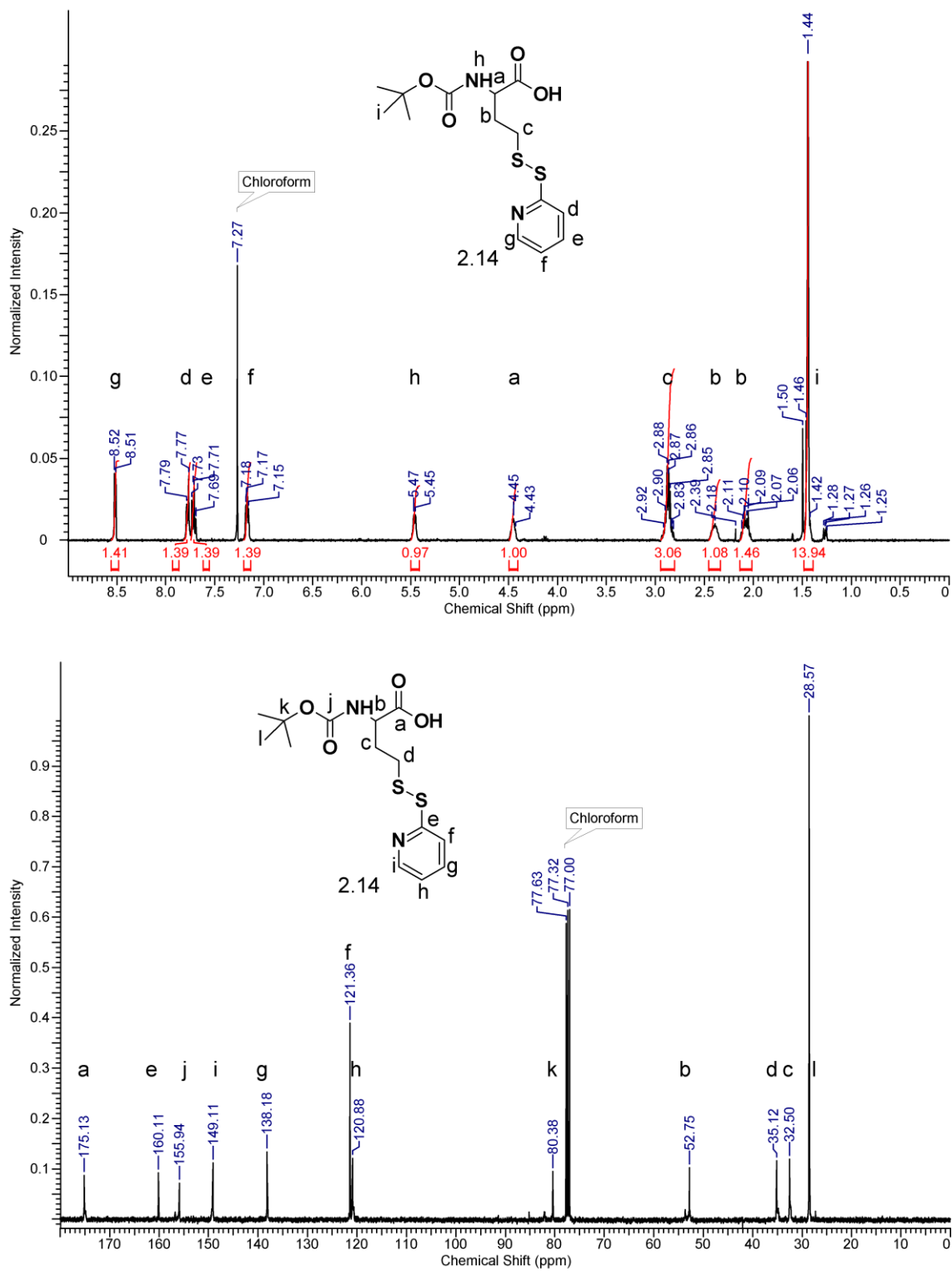**Figure A.21** ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compound **2.15** in CDCl$_3$.

Figure A.22 ^{1}H NMR (400 MHz) in D$_2$O and ^{13}C NMR (150 MHz) spectra of compound **2.4** in CD$_3$OD.

Figure A.23 ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compound **2.26** in CDCl$_3$.
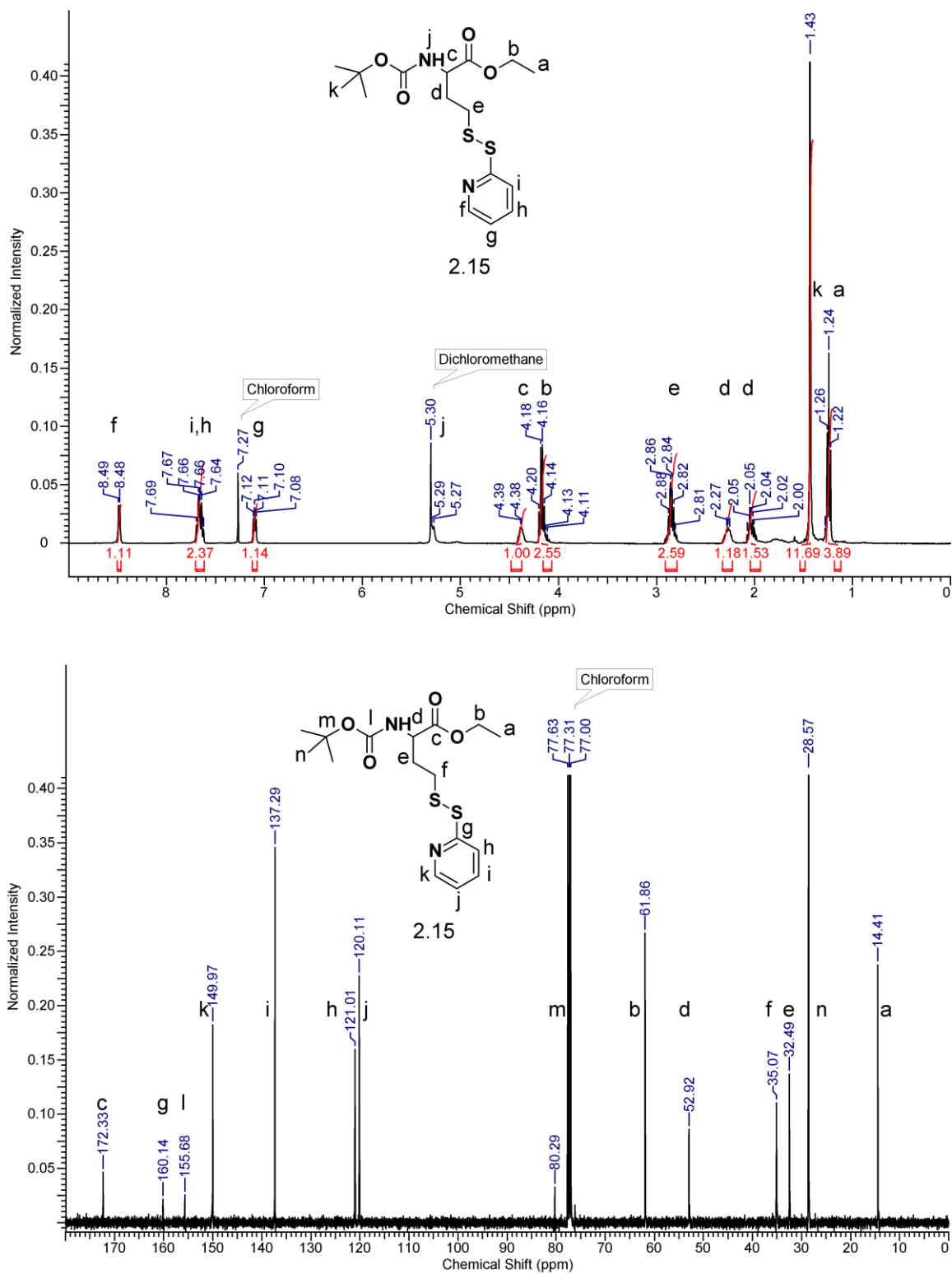
Figure A.24 ^{1}H NMR (400 MHz) of compound **2.27** in CDCl$_3$.

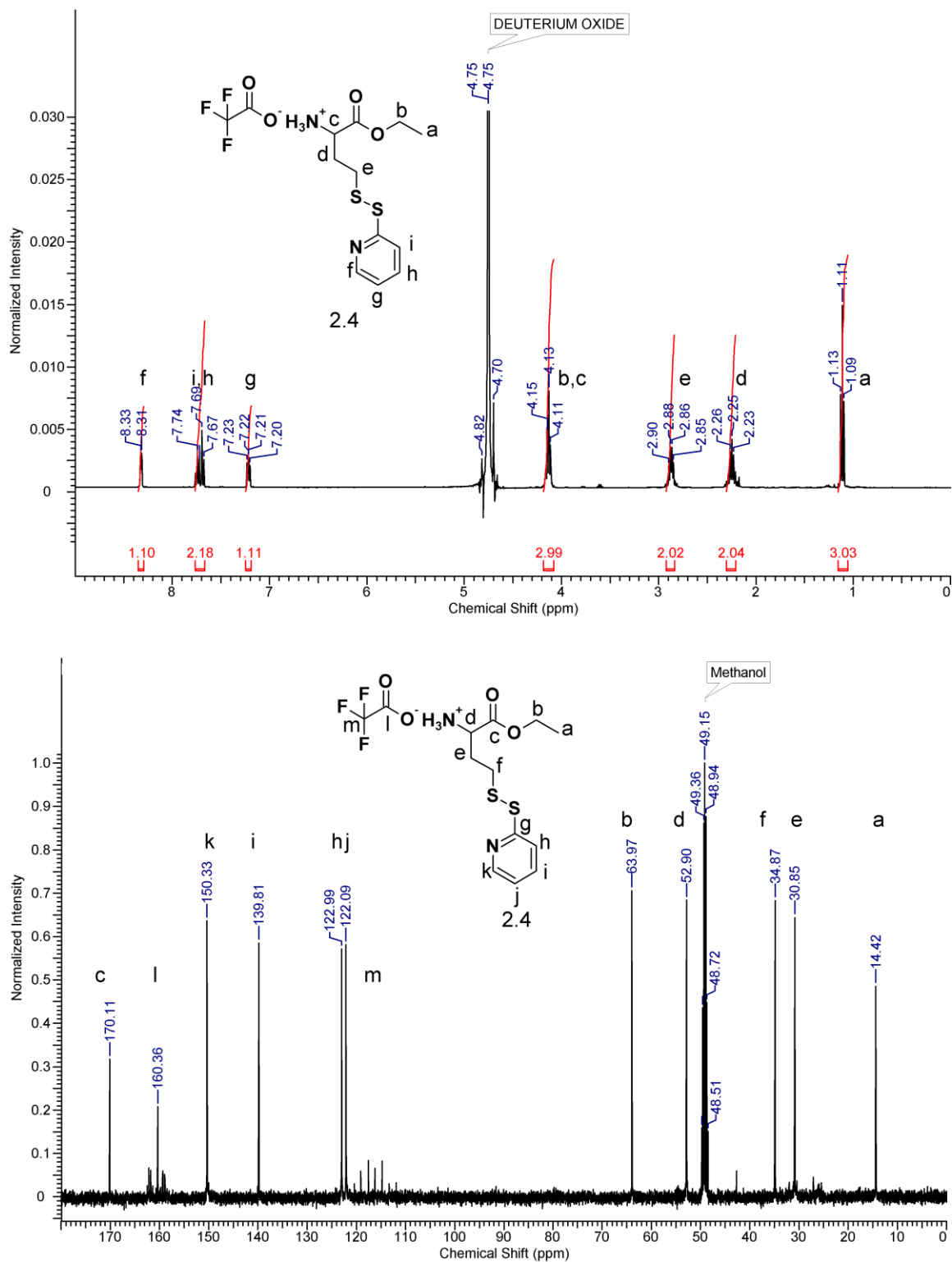**Figure A.25** ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compound **2.28** in CDCl$_3$.

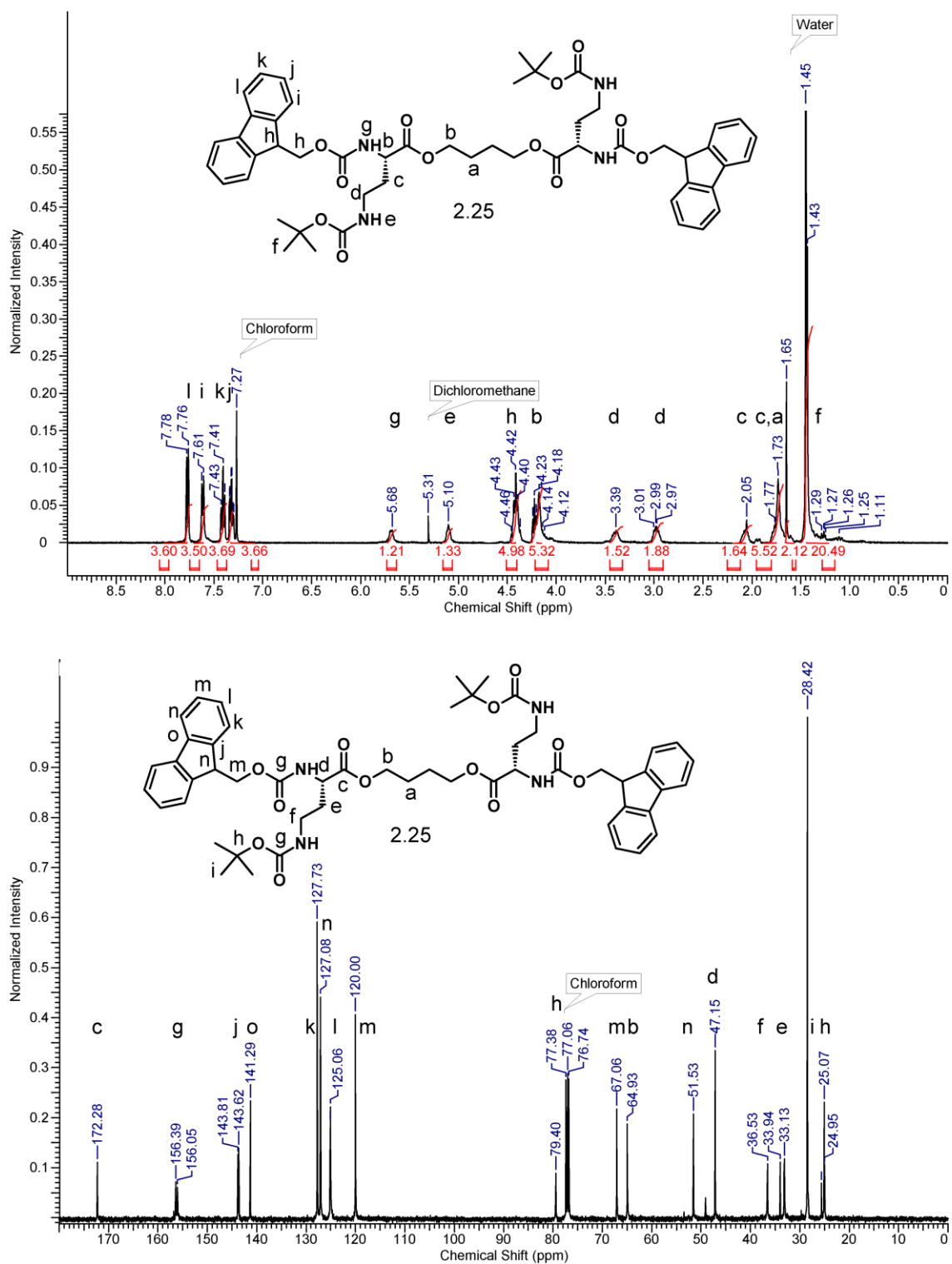**Figure A.26** ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compound **2.29** in CD$_3$OD.

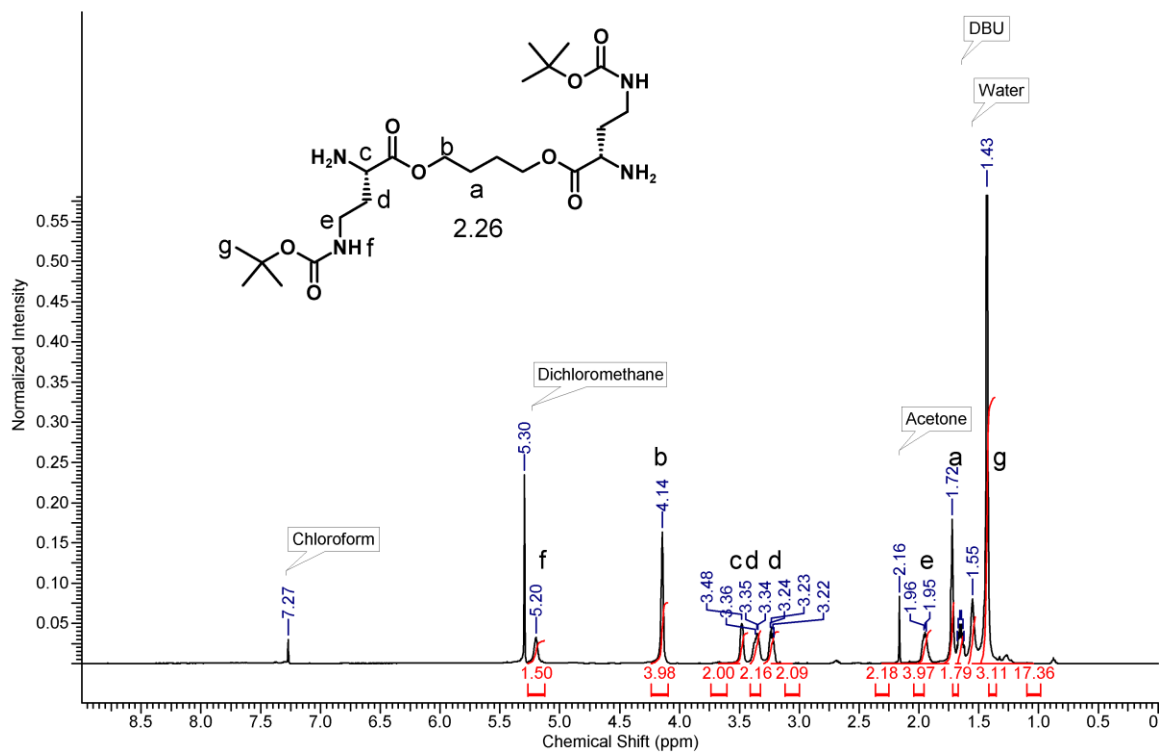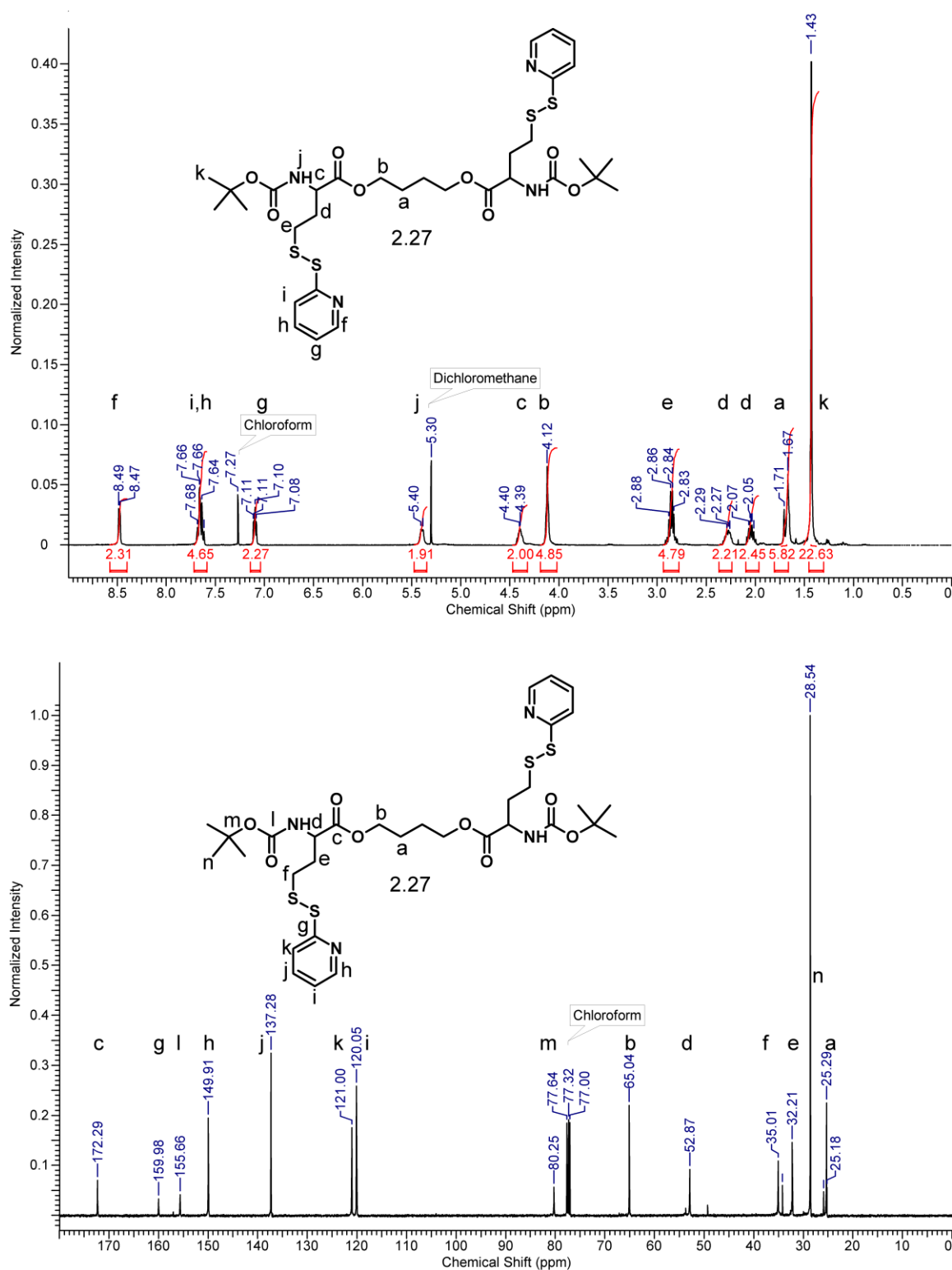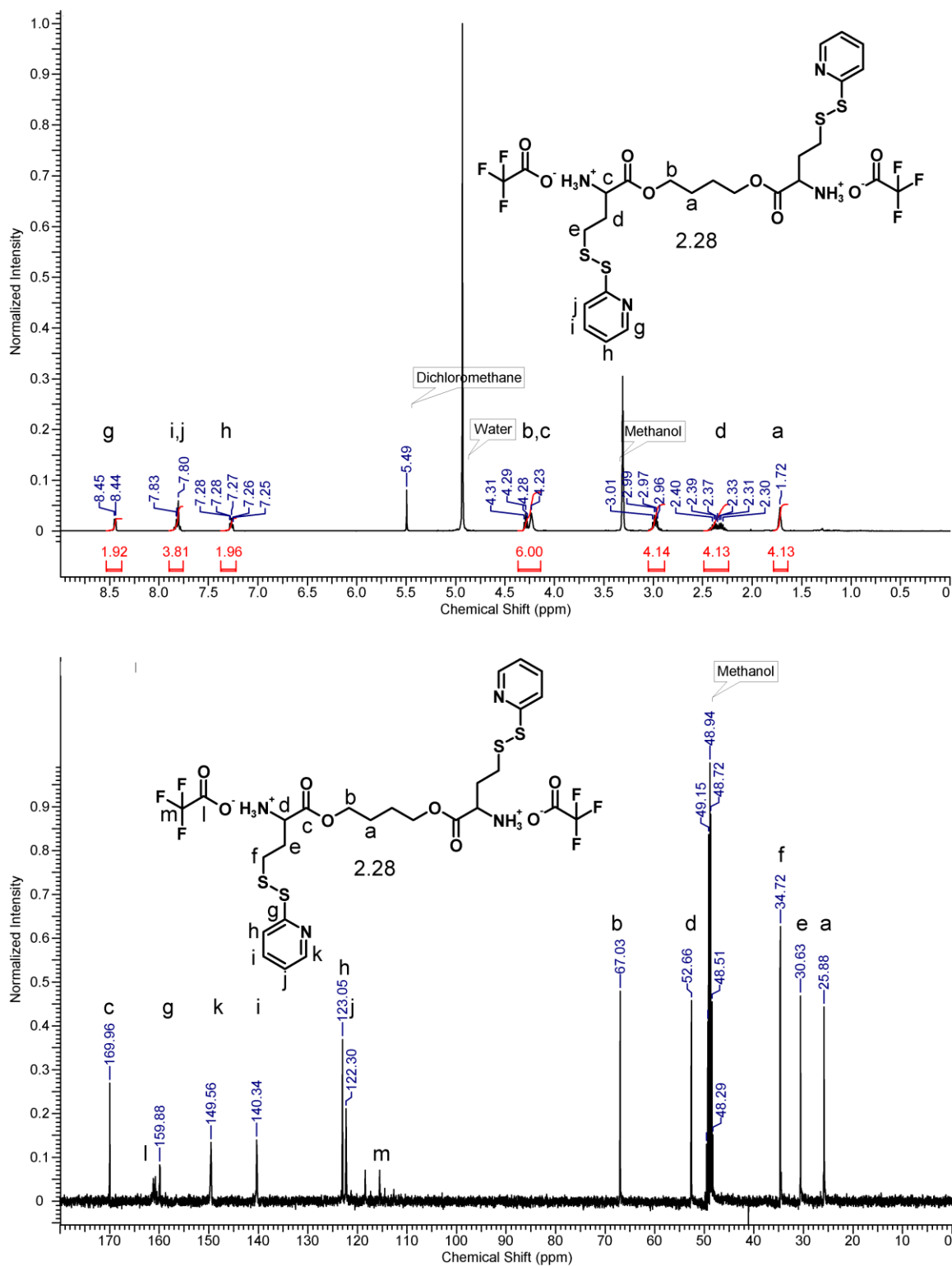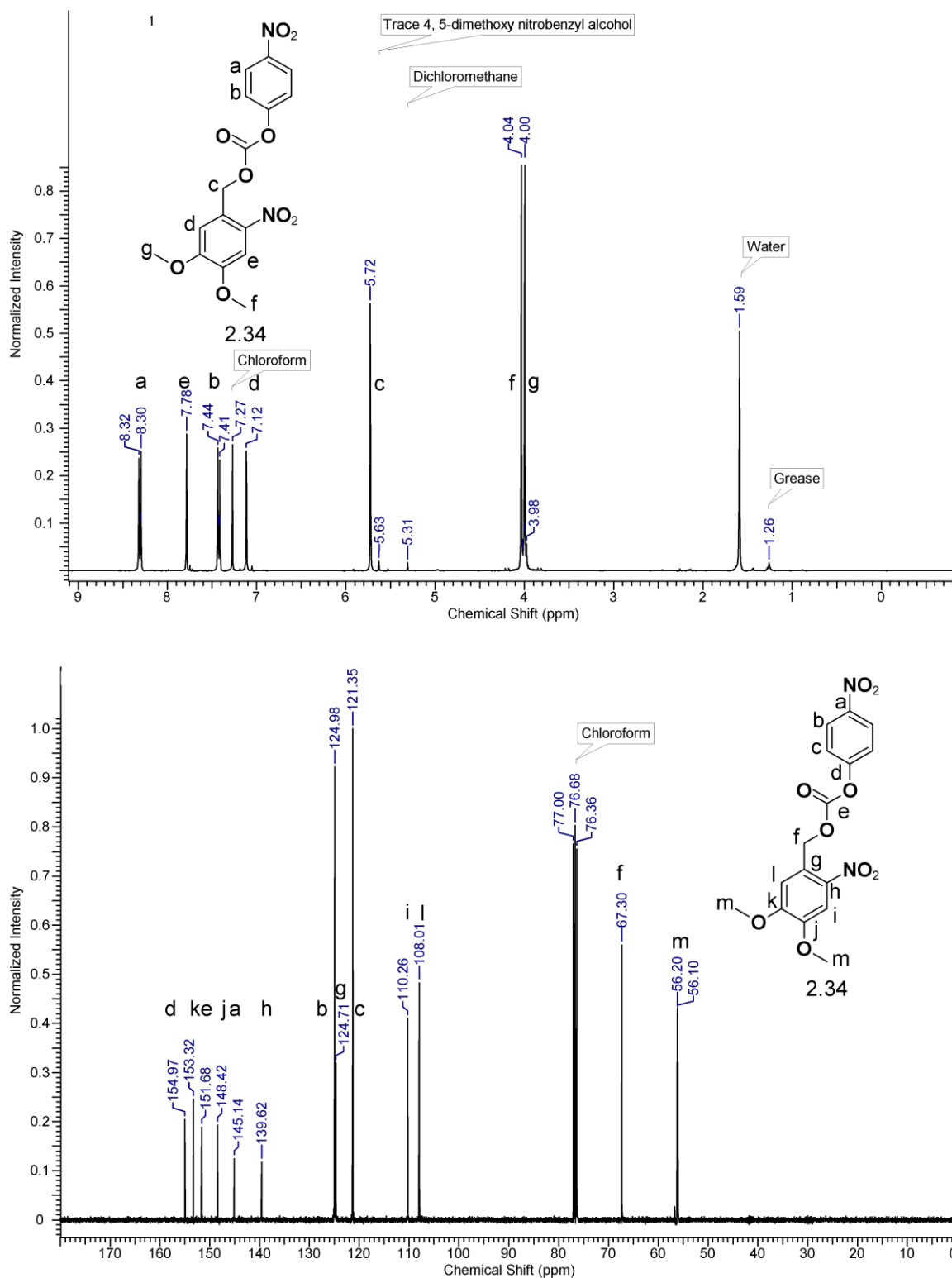**Figure A.27** ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra of compound **2.34**.

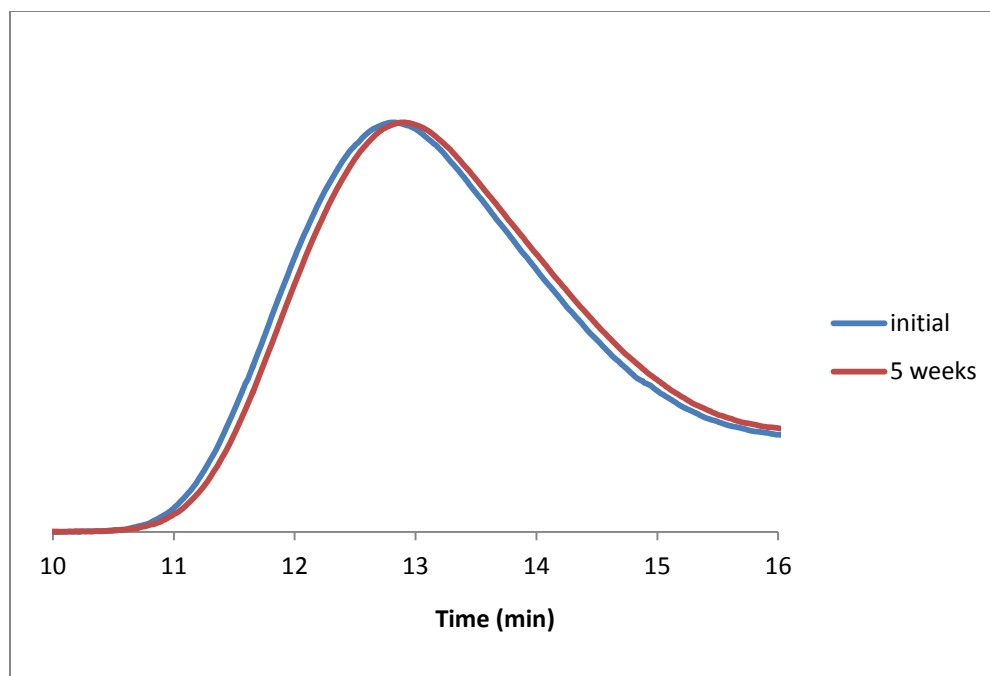**Figure A.28** SEC chromatogram of melt-pressed polymer **2.2** before and after 5 weeks of degradation.

Curriculum Vitae for José Samuel Mejia

EDUCATION

- 05/2010 – 11/2012 The University of Western Ontario, Canada, M.Sc. in Organic Chemistry
  Thesis title: Controlled Degradation of Poly(ester amide)s via cyclization of Pendant Functional Groups
  Research advisor: Dr. E.R. Gillies

- 09/2005 – 04/2009 The University of Western Ontario, Canada, H.B.Sc. in Chemistry
  Thesis title: Removal of Sample Background Buffer Ions and Myoglobin Enrichment Via a pH Junction by Discontinuous Buffers in Capillary Electrophoresis
  Undergraduate honors supervisor: Dr. K. Yeung

AWARDS and HONORS

- **2009**   Dean's Honors List
- **2008**   Dean's Honors List

Teaching Experience

- **Teaching Assistant**, University of Western Ontario
  Laboratory instructor for chemistry 2213 and 2223   Sept. 2011- April 2012

- **Outreach Assistant**, University of Western Ontario
  Instructor and participant for various outreach   Sept. 2010-June 2012
  events

Publications

- Booker, C.J.; Sun, S.; Woolsey, S.; **Mejia, J.S**.; Yeung, K.-C. "Removal of Sample Background Buffer Ions and Myoglobin Enrichment Via a pH Junction by Discontinuous Buffers in Capillary Electrophoresis". *J. Chromotogr. A* **2011**, 1218, 5705-5711.

CONFERENCE PRESENTATIONS

- **Mejia, J.S**.; Gillies, E.R. *Controlled Degradation of Poly(ester amide)s via cyclization of Pendant Functional Groups*; 35th Canadian High Polymer Forum, Gananoque, Ontario (**2012**), *Oral Presentation*