

# Synthesis of Polylactide-*block*-Poly(L-Lysine) Block Copolymers

by  
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## **Author's Declaration**

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## Abstract

Poly lactide (PLA) is an important biodegradable, biocompatible and renewable material that has been studied for many years for biomedical applications such as tissue scaffolding and surgical sutures. The research project described in this Thesis focused on the synthesis of poly lactide-*block*-polylysine (PLA-*b*-PLy) amphiphilic block copolymers designed for use in targeted drug delivery systems. Block copolymers with a cleavable, redox-sensitive disulfide bond at the block junction, as well as non-cleavable block copolymers were synthesized for comparison. The molecular weight of the PLA and the PLy blocks in the copolymers was varied, so as to control their hydrophobic/hydrophilic balance, and ultimately their self-assembly behavior. A novel redox-sensitive initiator, *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate, was used to generate the redox-sensitive copolymers, whereas (3-(Boc-amino)-1-propanol served for the non-cleavable systems. The hydroxyl group of these compounds was activated with the non-metallic catalyst 1,8-diazabicyclo[5.4.0]undec-7-ene/benzoic acid (DBU/BA) to initiate the ring-opening polymerization (ROP) of lactide. After the polymerization of lactide and removal of the Boc protecting group at the end of the PLA chain, the free primary amine functionality was used to initiate the ring-opening polymerization of N<sup>6</sup>-carbobenzoxy-L-lysine N-carboxyanhydride (Z-protected L-lysine NCA monomer). Sample analysis by size exclusion chromatography (SEC) and proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy confirmed that the block copolymers had low polydispersity indices (PDI) and the expected molecular weights. Deprotection of the Z-lysine units of the copolymers was accomplished with HBr, but degradation of the PLA segment could not be avoided under the reaction conditions investigated. It is suggested that future investigations either rely on a different deprotection method for the Z-group, or that a different protecting group be used in the NCA monomer serving to build the PLy block.

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# Dedication

I dedicate this work to my parents and my friends,  
for their endless and consistent support and encouragement,  
and all the people that helped and warmed me through the cold winters in Canada.

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## List of Abbreviations

$^1\text{H}$ NMR	Proton nuclear magnetic resonance
AcOH	Acetic acid
A. U.	Arbitrary units
b	Broad peak in NMR spectrum
BA	Benzoic acid
bm	Broad multiplet peak in NMR spectrum
Boc	<i>tert</i> -Butyloxycarbonyl group
Cat	Catalyst
$\text{CDCl}_3$	Chloroform- <i>d</i>
CMC	Critical micelle concentration
cpp	Critical packing parameter
d	Doublet peak in NMR Spectrum
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
$\text{DMSO-}d_6$	Dimethyl sulfoxide- <i>d</i> <sub>6</sub>
DRI	Differential refractive index
$\text{Et}_2\text{O}$	Diethyl ether
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
HBr	Hydrobromic acid
DOX	Doxorubicin
H-Lys(Z)-(OH)	2-Amino-6-(phenylmethoxycarbonylamino)hexanoic acid

LA	Lactic acid
m	Multiplet peak in NMR spectrum
MeOH	Methanol
$M_n$	Number-average molecular weight
$M_w$	Weight-average molecular weight
meq	Milliequivalent (0.001 mol functional groups)
NCA	N-Carboxyanhydride
PDI	Polydispersity index ( $= M_w/M_n$ )
PLA	Polylactide
PLA <sub>n</sub> -Boc	Polylactide with <i>tert</i> -butyloxycarbonyl end-group
PLA <sub>n</sub> - <i>b</i> -PLy <sub>m</sub> Z	Polylactide- <i>block</i> -polylysine (non-cleavable) copolymer, protected with carboxybenzyl (Z) groups
PLA <sub>n</sub> -NH <sub>2</sub>	Polylactide with amino end-group
PLA <sub>n</sub> -SS-Boc	Polylactide with disulfide bond and amino end-group protected with a <i>tert</i> -butyloxycarbonyl functionality
PLA <sub>n</sub> -SS-NH <sub>2</sub>	Polylactide with a disulfide bond and an amino end-group
PLA <sub>n</sub> -SS-PLyZ <sub>m</sub>	Polylactide- <i>block</i> -polylysine copolymer with (cleavable) disulfide bond, protected with carboxybenzyl (Z) groups
PLy	Polylysine
R	DBU/BA residue
RBF	Round-bottomed flask
ROP	Ring-opening polymerization
RT	Room temperature

s	Singlet peak in NMR spectrum
SEC	Size exclusion chromatography
t	Triplet peak in NMR spectrum
TFA	Trifluoroacetic acid
Z	Carboxybenzyl group

**CHAPTER 1**  
**INTRODUCTION**



## **1.1 Brief Introduction to Polymer Chemistry**

When the word polymer first came into existence in 1833, the Swedish chemist Jöns Jacob Berzelius used it to describe compounds having identical empirical formulae but differing in terms of properties and molecular mass.<sup>1</sup> This is very different from the definition given by the International Union of Pure and Applied Chemistry (IUPAC) nowadays, namely a substance composed of macromolecules.<sup>2</sup> This leads to the definition of a macromolecule (also from the IUPAC), referring to molecules of high relative molecular mass, the structure of which essentially comprises multiple repeating units derived, actually or conceptually, from molecules with a low relative molecular mass.<sup>3</sup>

The uses of polymers in our daily lives dates back to much before the existence of macromolecules was conceived. For example, as early as 1600 B.C., indigenous people in Central America played a game using balls made of natural rubber, a polymer still having an important role as an industrial raw material in the modern world. While the modern concept of polymers was first introduced by Hermann Staudinger in 1920,<sup>4</sup> synthetic polymers can be found almost everywhere around us: From simple kitchenware to car tires, the surface of rockets in outer space, to the sonar system of submarines in the deepest seas. This is the result of huge technology leaps over less than a century. The tremendous speed of development of polymer science is a clear indication of its irreplaceable role in our modern world. The necessity of studying and developing polymer science therefore goes without saying.

## **1.2 Classification of Polymerization Reactions**

To obtain polymers with desired functions and properties, the ways in which polymers are formed must be considered. The most common way to classify polymerization reactions is into two

categories, according to the changes happening to the structure of the monomers. Usually, when there is no loss of atoms or fragments from the monomer during the reaction, the reaction is called an addition polymerization, whereas if low molecular weight by-products (often water) are eliminated in the reaction, it is defined as a condensation polymerization. Condensation polymerization reactions typically involve functional groups and lead to the formation of linkages like amide -NHCO-, ester -OCO- or ether -O- bonds within the polymer backbone. However, some reactions also display the characteristics of addition polymerization by not yielding by-products, even though their polymerization mechanism corresponds to a condensation reaction. This is the case of the reaction of diisocyanates with diols, yielding polyurethanes without elimination. Consequently, another approach suggested to classify polymerization reactions is according to their mechanism, namely as either chain-growth or step-growth reactions. The essential differences between chain-growth and step-growth polymerization lies in whether monomer is present throughout the whole polymerization process, and whether the chains formed remain reactive. In step-growth polymerization, most of the monomer is consumed to form oligomers relatively early in the reaction, and there is no termination of the chains, while in chain-growth polymerization high molecular weight polymer chains and the monomer coexist, and chain termination yields essentially unreactive chain ends.<sup>5</sup>

### **1.2.1 Living Polymerization**

As stated earlier, polymers are mixtures of macromolecules with different molecular weights, and the molecular weight of polymers can have a major influence on the physical and chemical properties of these materials. To achieve better control over the physical properties of polymers, or to obtain polymers with specific chemical functionalities along the chains or at the chain ends, the synthesis of controlled molecular weight polymers, and particularly polymers with a narrow

molecular weight distribution, can be targeted. Because of the nature of step-growth polymerization reactions, the products obtained have a high dispersity (also known as the polydispersity index,  $PDI = M_w/M_n$ , the ratio of the number-average to the weight-average molecular weights), typically around 2.

In the case of chain-growth polymerization, if termination and chain transfer reactions can be avoided during the polymerization process, and the rate of initiation is faster than the rate of propagation, all the polymer chains can start growing at almost the same time, yielding polymer chains of uniform length. This leads to a low dispersity ( $PDI < 1.10$  in many cases). Furthermore, in the absence of termination and chain transfer processes, the degree of polymerization can be controlled by adjusting the molar ratio between the initiator and the monomer, which means that polymers with precisely controlled molecular weights can be obtained.

### **1.2.2 Living Anionic Polymerization**

Among the different families of living polymerization reactions developed, several techniques have been more widely applied including living cationic polymerization, living anionic polymerization and living radical polymerization. Living anionic polymerization will be discussed in detail here, as it is closely related to the research done in this project.

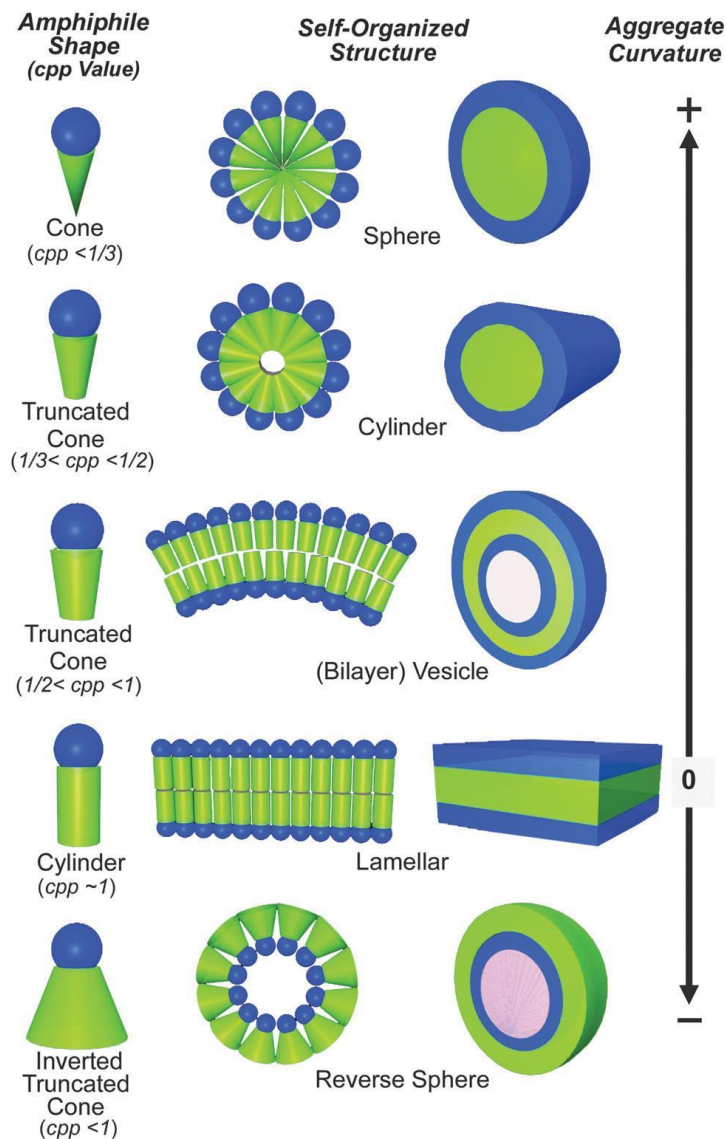
As the name implies, living anionic polymerization refers to a chain-growth reaction whose active species are anions in the propagation process, without interference from termination nor chain transfer reactions. This type of reaction was reported by Szwarc and coworkers in 1956 for the first time.<sup>6</sup> To achieve this, nucleophilic initiators are used to trigger the addition reaction of unsaturated monomers with (ideally) electron-deficient groups, usually electron-withdrawing atoms or functional groups. Because the active species in the polymerization are highly reactive

anions, rigorous purification of the solvent and monomers is necessary to remove protic impurities such as water. Since anhydrous reaction conditions are required, this can be best achieved with high vacuum and inert atmosphere techniques, and flaming of the glassware.

### **1.2.3 Block Copolymers**

The absence of termination in living polymerization gives the opportunity to add another monomer to the living chains after the first monomer has reached full conversion.<sup>6</sup> The addition of another monomer means that not only the molecular weight of the polymers can be increased as needed, but also that block copolymers can be created for different purposes. Furthermore, polymers with more complicated structures, such as star-shaped and telechelic polymers, can be synthesized by the living polymerization techniques.<sup>7,8</sup>

Since block copolymers have more than one segment within the polymer chain with different compositions, polymer segments with very different properties (e.g. hydrophobic and hydrophilic characters) can be combined. These amphiphilic block copolymers are able to self-assemble in solution into different nanostructures such as spherical micelles, wormlike micelles and polymeric vesicles (polymersomes).<sup>9</sup> When the concentration of the amphiphile is well above its critical micelle concentration (CMC), the formation of self-assembled structures is predicted by different models. For example, one model is based on the curvature of the amphiphilic structures at interfaces, and another one is based on the architecture of the macromolecules.<sup>10</sup> The molecular structure of amphiphiles is related to the types of superstructures they prefer to form, and relates to aggregate curvature as shown in Figure 1-1.<sup>10</sup> The self-assembly is defined in terms of the critical packing parameter (cpp), which is the ratio of the tail volume over the head area and the tail length of the amphiphile.



**Figure 1-1.** Superstructures formed by amphiphiles in relation to the molecular structure and aggregate curvature (cpp: critical packing parameter).<sup>10</sup>

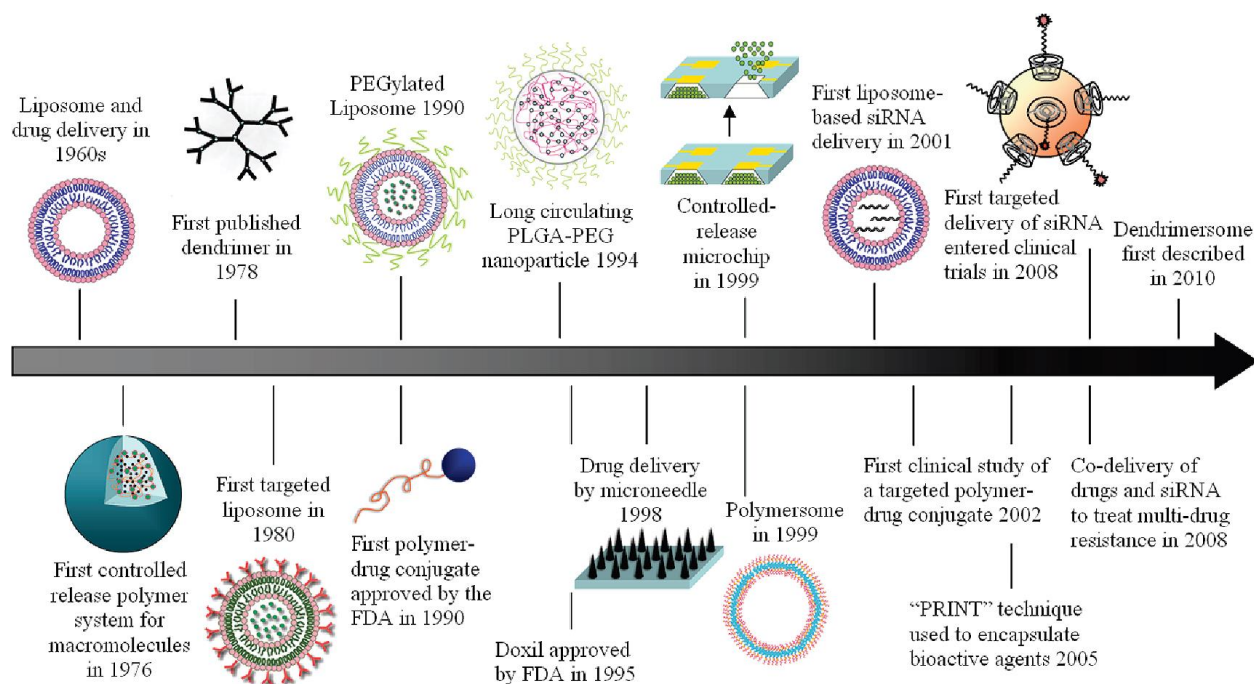
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### 1.3 Polymers for Drug Delivery Systems

Cancer is one of the biggest threats to modern life. Different ways have been developed to fight it such as radiation therapy, chemotherapy and surgery, but none of these methods are 100% efficient,

and there is a need for a method able to not only kill cancer cells, but also to minimize damage to healthy body parts arising from the treatment. One of the alternate methods being developed to fight cancer is using the self-assembly of amphiphilic block copolymers, to serve as drug carriers.<sup>11,12</sup> This approach is thought to help prevent the deactivation of therapeutic drugs such as proteins, antibodies and nucleic acids during their travel through the bloodstream.<sup>13</sup> Once the carriers arrive at the target disease site, the copolymers must be able to release the drug under the influence of a stimulus. Depending on the types of functional groups used and the external stimuli present, different response mechanisms may come into play including pH-sensitive, thermosensitive, redox-sensitive, enzyme-sensitive, photosensitive release, and so on.<sup>14</sup>

It has now been roughly six decades since the beginning of investigations on drug delivery systems. As can be seen in Figure 1-2,<sup>15</sup> over that timeline, this approach has evolved from being a scientific curiosity to becoming the topic of intense field testing. Undoubtedly, this method has a promising future, so the need to invest much effort into drug delivery research goes without saying. It has become a key element in the administration of drugs to a patient and contributes to saving millions of lives in the fight against cancer.



**Figure 1-2.** Timeline of developments in drug delivery research.<sup>15</sup>

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### 1.3.1 Monomer Selection for Drug Delivery Applications

Our body is a highly sophisticated system, hence the selection of monomers to synthesize polymers for drug delivery applications should take different aspects into account. First, the materials must of course be non-toxic and safe to our body and organs. Second, biocompatibility needs to be considered: If the polymer is recognized as a foreign component by the body, a rejection reaction caused by the immune system can lead to acute reactions with disastrous consequences. Third, the material ought to be biodegradable and excretable from the body. Furthermore, it should be economical and affordable, so that the cost of targeted drug delivery systems used in clinical

treatments on a large scale remains accessible. Consequently, easy access to the raw materials used in their synthesis also needs to be taken into account.

In consideration of these different points, lactide has attracted a fair bit of attention for the preparation of drug delivery systems and other materials for biomedical applications. The polymer derived from this monomer, polylactide (PLA), is a biodegradable and biocompatible aliphatic thermoplastic polyester, which can be derived from renewable resources such as corn starch, tapioca roots or sugarcane. It is also approved by the FDA (the American Food and Drug Administration) for biomedical applications. Polylactide is already widely used to manufacture tissue engineering scaffolds, surgical sutures as well as functional nanoparticles, because of its biodegradability under human physiological conditions.

Considering the advantages of lactide and polylactide listed above, that material was selected to form the hydrophobic segment of the amphiphilic block copolymers used in this project. As for the hydrophilic part, lysine also matches the different requirements discussed above. Polylysine is also positively charged under normal physiological conditions (pH 7.4), which can help micelles built from that material to penetrate cell membranes, that are negatively charged. Moreover, lysine plays a vital role in body muscle building, calcium absorption and injury recovery, and serves in the process of hormone, antibody and enzyme production. Unfortunately, lysine is not produced by the human body and must be recovered from ingested food. This makes it a perfect candidate as a monomer to build amphiphilic block copolymers for drug delivery: After enzymatic degradation of the micelles, lysine can also serve as a nutriment and help the body to recover from the illness faster.



### **1.3.2 Degradation of Polyesters**

It is well-known that polyesters such as PLA can be degraded through hydrolysis of the ester bonds within the polymer chains under both acidic and alkaline conditions. For example, the ester carbonyl group is easily protonated under acidic conditions, which can lead to cleavage of the carbon–oxygen bond along the polymer chain. The rate of hydrolysis of polyesters is largely dependent on the structure around the ester bond as well as the side chains, because these may affect properties of the polymer such as the level of water absorption, the chain stiffness, as well as many other aspects that can affect the rate of degradation.<sup>16,17</sup>

In the specific case of PLA, the methyl group on the polymer chain is small and the distance between successive ester linkages is rather short, which make it highly susceptible to hydrolytic degradation. Apart from hydrolysis, the degradation of polyesters can be facilitated by endo- and exo-enzymes.

### **1.3.3 Trigger Mechanisms for Drug Release**

For the past few years, drug delivery systems have ranked high as a research topic in polymer science, and that field is evolving quickly. From the very beginning, researchers relied on the biodegradability of PLA under physiological conditions to trigger the release of drugs, however this process is slow and relatively difficult to control.<sup>18</sup> Consequently, the concept of stimulus-responsive degradation (SRD) was introduced to enhance the rate as well as the precision of targeted drug release. To achieve SRD, stimulus-responsive polymers (typically copolymers) are required. Most of these fall into two main categories: In response to a stimulus, either a physical transition like the molecular volume or a conformation change, or a chemical transformation take

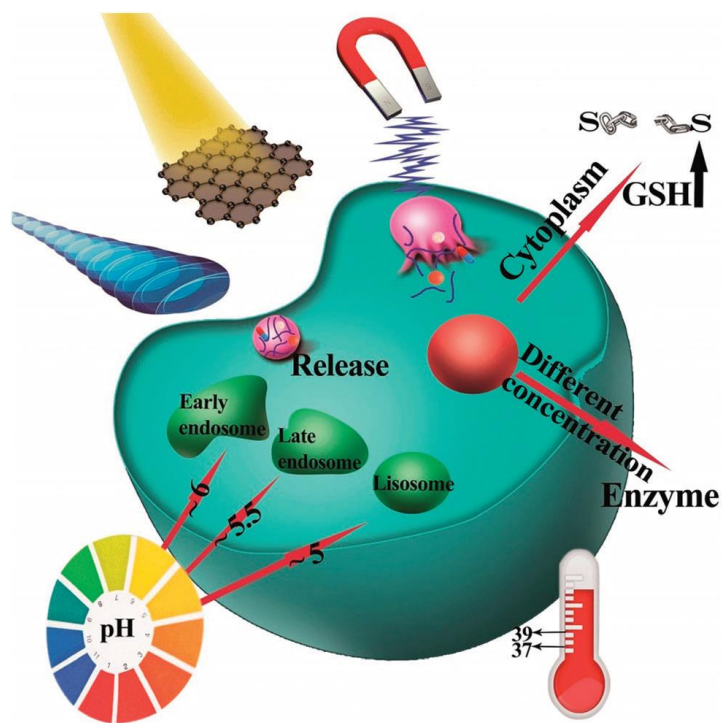
place. Physical stimuli used to trigger drug release include pH variations, magnetic and electric fields, as well as temperature differences. Chemical stimulus-triggered drug release is a bit more complicated and usually involves the cleavage of covalent bonds. To achieve good release, specific linkages need to be designed within the polymers to respond to the different stimuli. Typical linkages include reduction-responsive, acid-labile, enzyme-responsive and photocleavable linkages,<sup>18</sup> as shown in Figure 1-3.<sup>19</sup>

Among the different SRD strategies mentioned, pH-responsive, redox-responsive and thermoresponsive systems have attracted the most attention, because they do not require any external trigger, which makes them more patient-friendly and economical. As compared to normal tissues, diseased tissues display slight differences in terms of temperature, pH and other factors. One drawback of SRD systems not requiring external stimuli (such as a magnetic field or an illumination source) is that the differences between healthy tissues and lesion areas can be very minute. For example, the temperature of lesion sites is usually only one-half to one degree higher, and the pH around 0.5 pH unit lower than for healthy tissue. This requires SRD polymers to be highly sensitive to variations, to be able to react to stimuli quickly. One notable exception concerns the use of glutathione as a trigger for drug release. The concentration of glutathione (an antioxidant found in the human body) can be several times higher in tumor tissues than in healthy tissues. This provides an opportunity for redox-responsive polymers to play a key role in controlled drug delivery systems.

Consequently, an amphiphilic polylactide-*block*-polylysine copolymer system was designed with a disulfide linker at the block junction, which can be cleaved by reducing agents like L-cysteine or glutathione. Under normal conditions, the concentration of glutathione in the human blood plasma is very low, about 1-10 mM,<sup>20</sup> but the cytosolic concentration of glutathione at tumor sites

can be seven times higher than in healthy tissues.<sup>21</sup> Consequently, once a micellar drug carrier consisting of an amphiphilic block copolymer with a disulfide block junction reaches a diseased site, the high concentration of glutathione can cleave the hydrophilic chain segments from the micelles. This leaves the bare hydrophobic core of the micelles, which spontaneously forms large aggregates that remain trapped within the tumor tissues and release the drug slowly. Through this mechanism, the targeted release of drugs in tumor tissues can be achieved.

The synthesis and the characterization of block copolymers with different hydrophobic (polylactide) and hydrophilic (polylysine) segment lengths are the topic of this Thesis.



**Figure 1-3.** Various stimuli used to trigger stimulus-responsive polymers.<sup>19</sup>

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## 1.4 Thesis Outline

The first goal of this project was to synthesize polylactide by the ring-opening polymerization of D,L-lactide, to obtain the hydrophobic portion of the block copolymer. The synthesis of PLA usually involves a metal complex catalyst, mostly tin (II) octoate, which is a health hazard due to its high toxicity. To avoid this problem, a recently reported<sup>22</sup> non-metallic bifunctional catalyst system of 1,8-diazabicyclo[5.4.0]undec-7-ene and benzoic acid (DBU/BA salt) was used to obtain PLA having low PDI values and controlled molecular weights. In the second part of the project, a lysine N-carboxyanhydride derivative was prepared as a monomer precursor for the hydrophilic block. The PLA segment served as macroinitiator for the polymerization of the lysine derivative, to yield the block copolymer of interest for drug delivery applications.

A novel initiator with a cleavable disulfide bond, *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate, was synthesized to obtain the block copolymers with a redox-sensitive block junction. Samples with different hydrophobic and hydrophilic segment lengths were prepared in view of an investigation of self-assembly. For comparison, conventional amphiphilic block copolymers without a cleavable block junction were also synthesized using (3-(Boc-amino)-1-propanol as initiator. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy as well as size exclusion chromatography (SEC) analysis were used to characterize the samples and to provide information about the structure, the composition and the molecular weight of the copolymers synthesized.

The thesis is organized into different chapters describing the synthetic strategy selected and the objectives of the work (Chapter 2), followed by the detailed procedures used for the synthesis of the cleavable and non-cleavable block copolymer systems (Chapter 3). The results obtained in the project are discussed in Chapter 4 and summarized in Chapter 5, together with the main

conclusions drawn from the work. Finally, suggestion for further work are supplied in Chapter 6, before the list of references cited throughout the thesis (Chapter 7).

**CHAPTER 2**  
**OBJECTIVES**

## **2.1 Overview**

The main goal of this research project was to synthesize PLA-based amphiphilic block copolymers of different molecular weights for use in drug delivery. The reaction conditions were optimized to obtain block copolymers with targeted molecular weights and mole ratios of the two blocks, using poly(L-lysine) as a hydrophilic block. For comparison, two series of block copolymers were synthesized: One with a regular (non-cleavable) block junction, and one with a cleavable disulfide block junction. To obtain the block copolymers with a cleavable block junction, a novel initiator containing a disulfide bond was synthesized and used to initiate the polymerization of lactide.

In order to determine the optimal ratio between the hydrophilic and hydrophobic portions of the block copolymers to form spherical micelles suitable as drug carriers, different block copolymers with varying hydrophilic and hydrophobic chain lengths were synthesized. <sup>1</sup>H NMR spectroscopy and SEC analysis were used to study these samples after their synthesis.

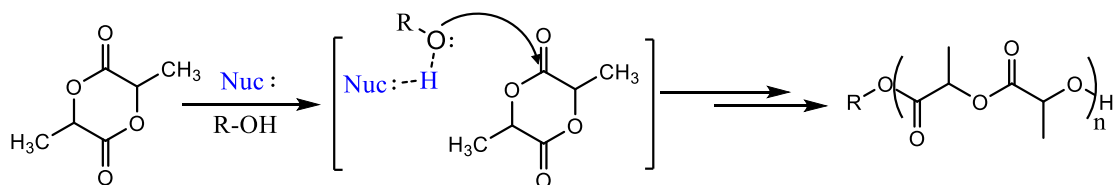
## **2.2 Synthesis of Polylactide**

As a sophisticated system, the human body can sometimes be very fragile, especially when it faces attacks from within itself, such as from tumors in organs. For that reason, the selection of monomers used in the construction of drug delivery systems should be considered very carefully, by taking into account their biocompatibility, but also the toxicity of all the materials used in their synthesis, so as to minimize their use. While the polymerization catalyst should be non-toxic, it should nevertheless provide polymers with designed molecular weights and narrow molecular weight distributions. The cost of the catalyst should be likewise minimized, if at all possible.

The techniques commonly used for the ROP of lactide rely upon metal complexes with organic ligands. Even though these techniques are quite mature, and multiple metals and organic ligands

have been tested, the PDI obtained is relatively high in many cases, especially when the molecular weight of PLA is below 5000 g/mol.<sup>23-26</sup> Another problem to take into consideration is that the metal ions, which are often toxic, cannot be removed completely after the reactions – there are always residues remaining. Another drawback of these metal complex catalysts is their price, which can be very high in some cases.

To overcome the problems listed above, nucleophilic organocatalysts such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) can be used to activate initiating hydroxyl groups, and the propagating centers, through hydrogen bonding as illustrated in Scheme 2-1. This approach was used in this project.

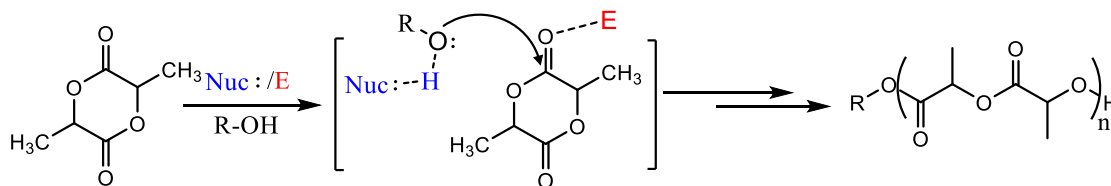


**Scheme 2-1.** Use of a nucleophilic catalyst to activate the ROP of lactide.<sup>22</sup>

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Increasing the nucleophilicity of the catalyst usually leads to an increase in the polymerization rate, but at the same time it can also cause unwanted transesterification reactions between the activated hydroxyl groups and the polymer chain carbonyl groups. To ensure that the nucleophilicity is not too high, i.e. just sufficient for activation of the hydroxyl group, but not enough to attack the polymer chain carbonyl groups, a DBU/ BA bifunctional catalyst system was introduced that led to minimal transesterification side reactions.<sup>22</sup> The mechanism of bifunctional activation is shown in Scheme 2-2.





**Scheme 2-2.** Use of bifunctional catalyst for the polymerization of lactide.<sup>22</sup>

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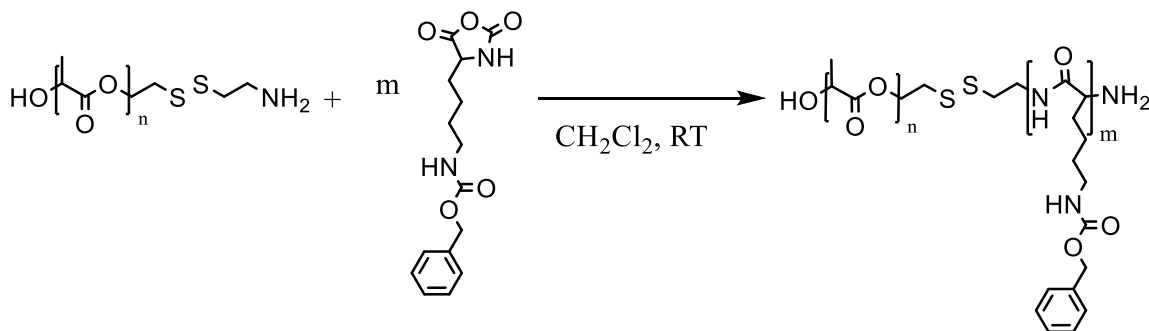
The synthesis of PLA was one of the main goals of this project. In the presence of the DBU/BA catalyst, PLA samples with targeted molecular weights and low polydispersities were obtained.

### 2.3 Synthesis of PLA-Based Block Copolymer Amphiphiles

As discussed above, biodegradable functional block copolymer nanoparticles can be applied as drug delivery vehicles.<sup>27</sup> Lately, some research groups have reported new developments in that area.<sup>28-33</sup> A considerable portion of the recent work reported in the literature focused on the development of pH-sensitive micelles formed by PLA-based amphiphiles as carriers for doxorubicin (DOX), an anticancer drug. The cytotoxicity of drug-carrying nanoparticles was studied, and enhanced efficacy and control over the release of DOX were observed for these systems as compared to hydrophobic DOX-loaded polymersomes.<sup>31</sup> This demonstrated the potential of this kind of PLA-based self-assembled nanoparticle platform as a modular delivery vehicle. These methods are promising enough to be generalized to a wider range of chemotherapeutic agents, to be applied to various kinds of diseases.<sup>29</sup>

PLA samples prepared with targeted molecular weights were used as macroinitiators in this project, as shown in Scheme 2-3 (using the cleavable disulfide macroinitiator as an example), to initiate

the polymerization of an N-carboxyanhydride (NCA) derivative of lysine with a carboxybenzyl (Z) protecting group on its side-chain. The length of the polylactide and polylysine segments was varied to control the hydrophilic-hydrophobic balance of the amphiphilic block copolymers.



**Scheme 2-3.** Synthesis of  $\text{PLA}_n\text{-SS-PLyZ}_m$  block copolymers.

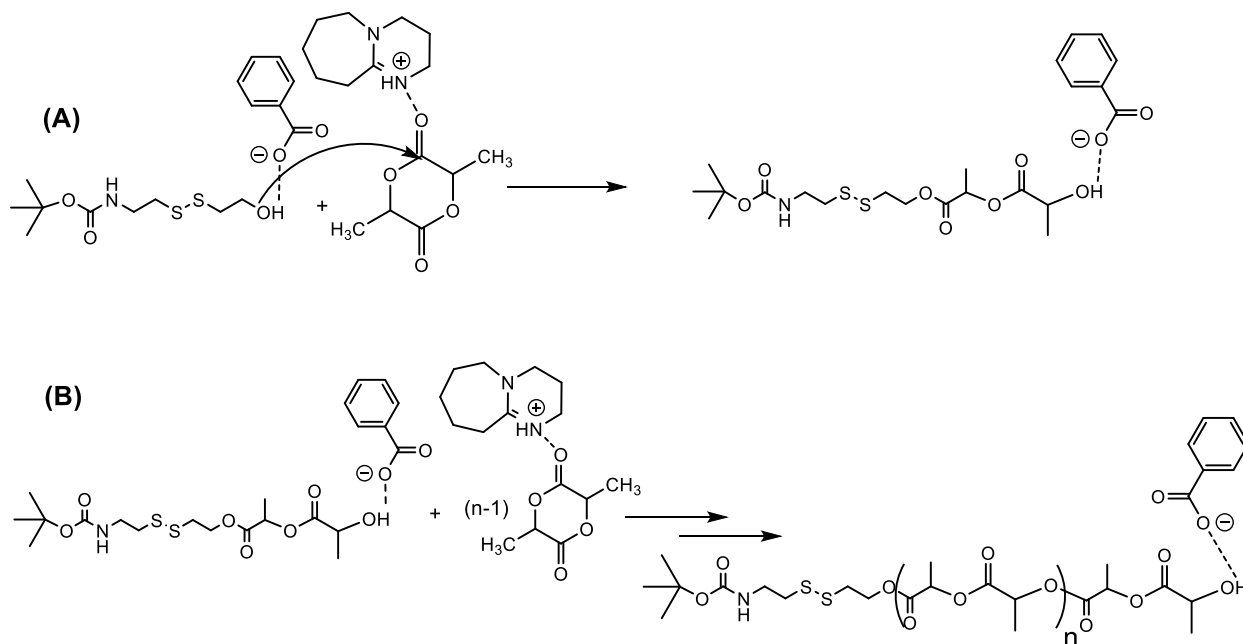
## 2.4 Side Reactions

The main side reactions in the polymerization of lactide and the lysine NCA are linked to the presence of water or other protic impurities, which can cause chain transfer reactions or chain termination. Thus, impurity removal before each reaction should be carefully carried out by recrystallization. In addition, the DBU and BA used to catalyze the polymerization of lactide should be strictly in a mole ratio of 1:1; an excess of DBU causes an acceleration of the polymerization and a high polydispersity, while an excess of BA leads to a low reaction rate or even termination of the polymerization.<sup>22</sup>

## 2.5 Improvements Based on Previous Research

In previous research,<sup>27,34-37</sup> the polydispersity index of PLA and PLA-based block copolymers was deemed to be acceptable but nevertheless not very low (usually above 1.10 for PLA), especially

when the molecular weight of the polymers was below 5000 g/mol. Low polydispersity indices are kinetically favored under conditions where the rate of initiation is fast vs. the rate of propagation (Scheme 2-4). Consequently, achieving low PDI values was one of the top priorities in this project.



**Scheme 2-4.** Polymerization reaction of lactide with DBU/BA: (A) Initiation, (B) Propagation.

As mentioned above, many research teams synthesized pH-responsive copolymers and used them as drug carriers. One of the problems encountered with these systems is that the differences between normal tissue and lesion areas *in vivo* are not so large. For example, the pH of tumors is typically about 0.5 pH unit lower, and the temperature ca. 1.0 °C higher than in healthy tissues. Thus, to achieve precise control over drug release performance, external assistance is often required to enhance a triggering signal. To simplify this situation, this research project focused on the synthesis of redox-responsive PLA-based amphiphiles. Introducing a disulfide linker at the

block junction of the copolymers would be one option to do this,<sup>38</sup> due to its high sensitivity to reduction. Indeed the concentration of glutathione, a disulfide bond-breaking agent, can be many times higher in lesions tissue than in normal tissue,<sup>39</sup> and the controlled drug release mechanism of related systems has been studied.<sup>40</sup> Consequently, this was the approach used in the current research project.

**CHAPTER 3**  
**EXPERIMENTAL PROCEDURES**

### 3.1 Reagent Purification

Ethyl acetate ( $\geq 99.7\%$ , Sigma-Aldrich) was dried by stirring and distillation over calcium hydride ( $\text{CaH}_2$ ; Sigma-Aldrich, 95%) under nitrogen. Lactide (3,6-dimethyl-1,4-dioxane-2,5-dione; D,L-isomer mixture, Sigma-Aldrich) was purified by recrystallization from a 20% w/v solution in dry ethyl acetate (recovery yield 69%). The purified lactide was stored under nitrogen in a freezer at  $-10^\circ\text{C}$ . Dichloromethane (DCM; Sigma-Aldrich,  $\geq 99.8\%$ ) was purified by distillation after drying over  $\text{CaH}_2$  overnight. It was then stored in a round-bottomed flask (RBF) under nitrogen over 4A molecular sieves (EMD, 1/16" pellets) in a refrigerator at  $4^\circ\text{C}$ . Benzoic acid (BA; J. T. Baker Chemicals, ACS grade) was dried in a vacuum oven at  $80^\circ\text{C}$  overnight immediately before use. Diazabicyclo[5.4.0]-undec-7-ene (DBU; Alfa Aesar, 99%), anhydrous diethyl ether (Sigma-Aldrich,  $\geq 99.0\%$ ), *tert*-butyl N-(3-hydroxypropyl)carbamate (3-(Boc-amino)-1-propanol; Aldrich, 97%), (2S)-2-amino-6-(phenylmethoxy-carbonylamino)hexanoic acid (H-Lys(Z)-(OH); Aldrich,  $\geq 99.0\%$ ), triphosgene (Aldrich, 98%), hexane (Sigma-Aldrich,  $\geq 98.5\%$ ), trifluoroacetic acid (TFA; Sigma-Aldrich,  $\geq 99\%$ ), sodium bicarbonate ( $\text{NaHCO}_3$ , BDH, ACS grade), anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ , EMD, ACS grade), hydrogen bromide solution (HBr; Sigma-Aldrich, 33 wt. % in acetic acid), 2,2'-dipyridyl disulfide (Aldrich, 98%), glacial acetic acid (Sigma-Aldrich,  $\geq 99.5\%$ ), 2-(Boc-amino)ethanethiol (Aldrich, 97%), 2-mercaptoethanol (Sigma-Aldrich,  $\geq 99.0\%$ ), calcium hydride (Sigma-Aldrich, reagent grade, 95%), hydrochloric acid (Sigma-Aldrich, 37% in water) and methanol (Sigma-Aldrich,  $\geq 99.8\%$ ) were all used as received.

### 3.2 Sample Characterization

All the polymer samples were analyzed by proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectroscopy on a Bruker 300 MHz NMR instrument at room temperature, to determine their number-average degree of polymerization ( $X_n$ ) and molecular weight ( $M_n$ ), at a concentration of

5% w/v in either CDCl<sub>3</sub> or DMSO-*d*<sub>6</sub>. The deprotection of the terminal Boc group on the PLA chains and of the Z group on the lysine units of the block copolymers was also confirmed by <sup>1</sup>H NMR analysis.

SEC analysis served to determine the molecular weight and the polydispersity index of the polymer samples. The system used consisted of a Viscotek VE 2001 sample module with a Model 305 triple detector array including refractive index (RI), viscosity, low-angle and right-angle laser light scattering (7° and 90°, λ = 670 nm) detectors. The system was operated with a set of three PolyAnalytik mixed bed columns, PAS-103-L, PAS-104-L and PAS-105-L, each with dimensions of 8 mm (ID) × 300 mm (L), using tetrahydrofuran (THF) as eluent at a flow rate of 1 mL/min, and a polystyrene standards calibration curve.

### 3.3 Preparation of DBU/BA Salt<sup>22</sup>

Dry benzoic acid (1.00 g, 8.18 mmol) was dissolved in 20.0 mL of anhydrous diethyl ether by stirring under nitrogen in a flame-dried 100 mL RBF sealed with a rubber septum. When DBU (1.22 mL, 8.18 mmol) was added with a syringe to the stirred solution, a white precipitate formed immediately. Stirring was stopped after one hour and the product was washed with dry diethyl ether (3 × 80 mL). The removal of diethyl ether was completed with a stream of dry nitrogen, after adding another needle to the septum. The product was then dried under vacuum overnight at ambient temperature. Yield: 2.18 g, 97%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ ppm: 7.99-8.13 (bm, 2H), 7.25-7.38 (bm, 3H), 3.43-3.54 (t, 2H), 3.28-3.42 (bm, 4H), 2.80-3.00 (bm, 2H), 1.85-2.03 (bm, 2H), 1.65-1.80 (bm, 4H), 1.54-1.64 (bm, 2H).

### 3.4 Synthesis of N6-Carbobenzoxy-L-lysine N-Carboxyanhydride (Lysine(Z)-NCA)<sup>41</sup>

**NOTE:** Because of the high toxicity of triphosgene, this entire procedure must be carried out in a well-ventilated fume hood. In a 1-L oven-dried RBF, 10.0 g (35.7 mmol) of H-Lys(Z)-(OH) was

suspended in 300 mL of dry ethyl acetate and heated to reflux under nitrogen atmosphere. Triphosgene (4.23 g, 14.3 mmol) was then added to the RBF and refluxing was continued for 4 hours, by which time the solution became completely clear. The solution was allowed to cool to room temperature before transferring the stoppered flask to a -10°C freezer for one hour. The chilled reaction mixture was transferred to a cold 1-L separatory funnel, washed with 100 mL of ice-cold water, and then with 100 mL of 0.5% ice-cold aqueous NaHCO<sub>3</sub> solution. The (upper) organic layer was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before concentration on a rotary evaporator to 100-120 mL in a 500 mL RBF. An equal volume of ice-cold hexane was added to the solution to induce crystallization of the product. The RBF was stored in a -10 °C freezer for 12 hours, and the product was recovered in a Schlenk funnel purged with nitrogen. The product was dried under vacuum overnight and stored under nitrogen atmosphere in a -80 °C freezer. Yield: 7.98 g, 73%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ ppm: 7.27-7.43 (s, 5H), 6.82-6.95 (s, 1H), 5.05-5.16 (s, 2H), 4.85-4.98 (bm, 1H), 4.20-4.30 (bm, 1H), 3.10-3.28 (bm, 2H), 1.88-2.03 (bm, 2H), 1.70-1.87 (bm, 2H), 1.33-1.63 (bm, 4H).

### **3.5 Synthesis of *tert*-Butyl(2-((2-hydroxyethyl)disulfanyl)ethyl) Carbamate<sup>42</sup>**

#### **3.5.1 Synthesis of 2-(pyridin-2-yl)disulfanyl)ethan-1-ol (Compound 1 in Scheme 3-1)**

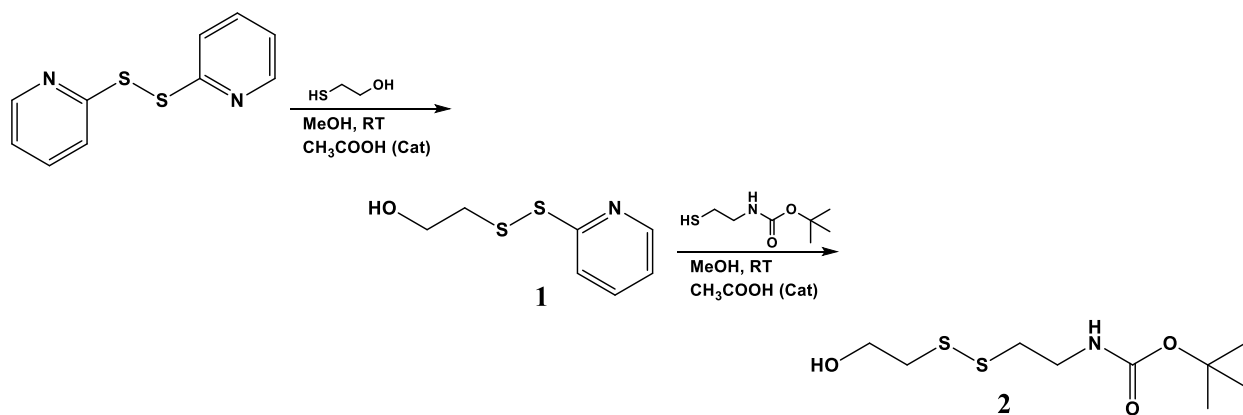
Aldrithiol-2 (25.0 g, 0.113 mol) was dissolved in 150 mL of methanol in an oven-dried 500 mL RBF, and glacial acetic acid (1.0 mL) was added. A solution of 2-mercaptoethanol (6.45 g, 0.083 mol) in 60 mL of methanol was then added drop-wise with vigorous stirring to the mixture at ambient temperature. The solution was stirred further at room temperature for 6 hours before the solvent was removed on a rotary evaporator, to obtain the crude product as a yellowish oil. This product was purified on a 15 mm ID × 400 mm column by flash chromatography (Figure 3-1) with



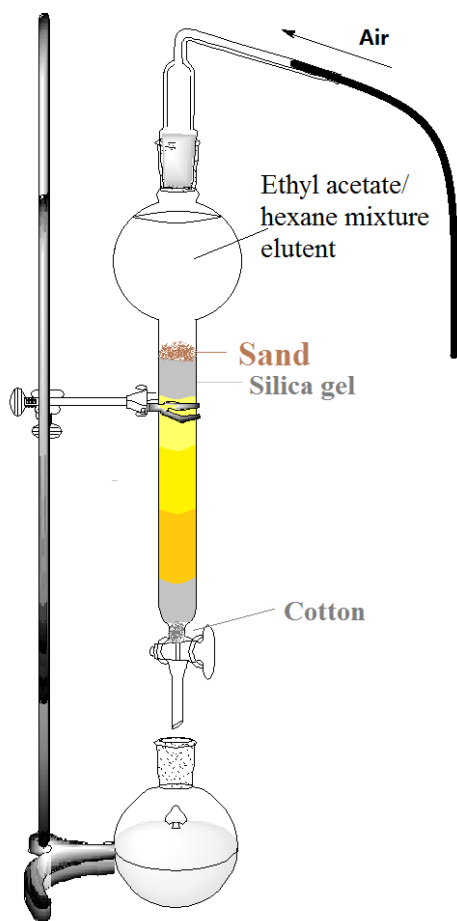
silica gel (35 cm bed height), using ethyl acetate/hexane mixtures as eluent. Excess Aldrithiol-2 was eluted first with 15:85 vol/vol ethyl acetate:hexane; afterwards the polarity of the eluent was gradually increased to 30:70 ethyl acetate:hexane to elute the product, obtained as a colorless oil after removal of the solvents. Yield: 8.10 g, 52%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ ppm: 8.42-8.53 (d, 1H), 7.52-7.62 (m, 1H), 7.33-7.42 (d, 1H), 7.10-7.18 (m, 1H), 5.60-5.74 (t, 1H), 3.72-3.85 (m, 2H), 2.88-2.98 (t, 2H).

### 3.5.2 Synthesis of *tert*-butyl (2-((2-hydroxyethyl)disulfanyl) ethyl) carbamate (Compound 2 in Scheme 3-1)

Compound 1 (8.00 g, 0.043 mol) was dissolved in 70.0 mL of methanol in an oven-dried 250 mL RBF, and 1.0 mL of glacial acetic acid was added. A solution of 2-(Boc-amino)ethanethiol (7.98 g, 0.045 mol) in 30.0 mL of methanol was then added drop-wise with vigorous stirring to this mixture at room temperature. The solution was stirred further at room temperature for 6 hours. The solvent was evaporated to obtain the crude product as a yellowish viscous liquid, which was purified by flash column chromatography as described above. Yield: 6.07 g, 56%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ ppm: 4.83-5.13 (b, 1H), 3.77-3.88 (t, 2H), 3.32-3.46 (m, 2H), 2.79-2.87 (t, 2H), 2.70-2.78 (t, 2H), 2.33 (bm,1H), 1.39 (s, 9H).



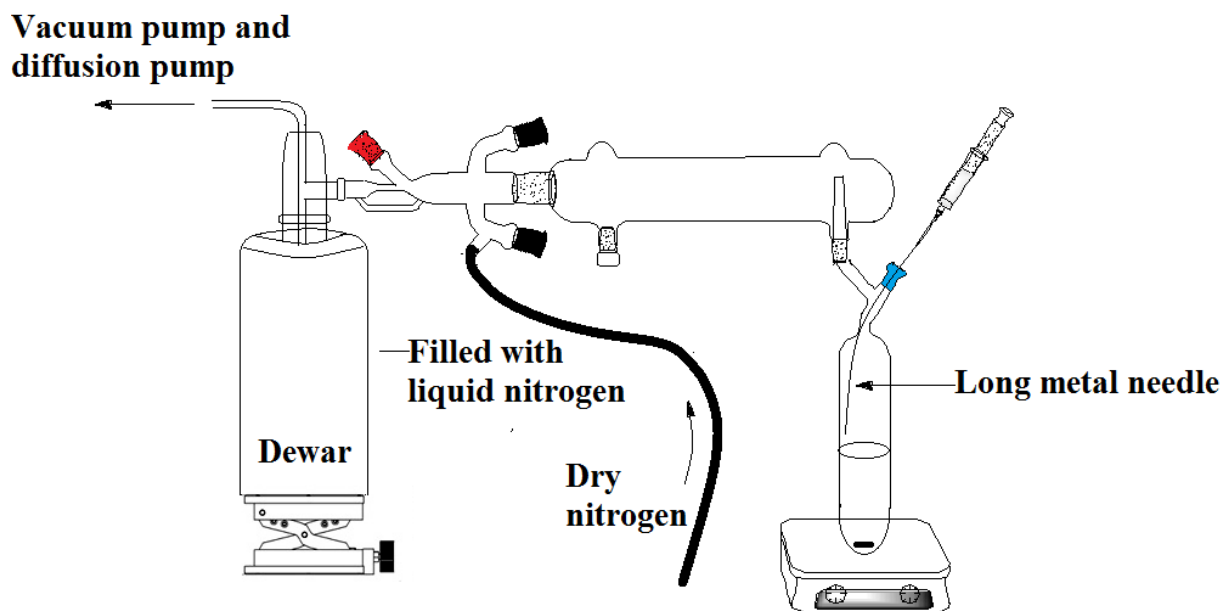
**Scheme 3-1.** Synthesis of *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate.



**Figure 3-1.** Product purification by column flash chromatography.

### 3.6 Synthesis of Boc-protected Polylactide (PLA<sub>n</sub>-Boc)<sup>43</sup>

The synthesis of block copolymers started with the polymerization of lactide using 3-(Boc-amino)-1-propanol as initiator. The synthesis of PLA<sub>17</sub>-Boc (target  $M_n = 2.6 \times 10^3$  g/mol, 17 lactide units/34 lactic acid units per polymer chain) is provided as an example. The DBU/BA salt (0.11 g, 0.40 mmol) was added to a flame-dried ampoule containing purified lactide (1.00 g, 6.94 mmol) under nitrogen atmosphere in 3 mL of DCM. The initiator 3-(Boc-amino)-1-propanol (0.07 g, 0.41 mmol) was dissolved in dry DCM (4.00 mL), and the solution was added to the ampoule using a syringe with a long oven-dried needle to initiate the polymerization (Figure 3-2). Stirring was continued for 48 hours before the polymerization was terminated by adding two drops of a mixture of 2 drops of HCl and five drops of deionized water in 5 mL of methanol. The polymer was recovered by precipitation with stirring in chilled 2-propanol, giving a white solid that was isolated by decantation. The product was dried with air and residual solvent was removed under vacuum overnight. The dried PLA<sub>17</sub>-Boc product was a fluffy white solid. Yield: 0.90 g, 84%.  $M_n$  [NMR] =  $2.6 \times 10^3$  g/mol,  $M_n$  [SEC] =  $3.06 \times 10^3$  g/mol,  $M_w$  [SEC] =  $3.33 \times 10^3$  g/mol, PDI = 1.09, <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 5.04-5.29 (bm, 1H  $\times$  34), 4.64-4.75 (bm, 1H), 4.15-4.22 (t, 2H), 3.11-3.21 (t, 2H), 1.95-2.06 (m, 2H), 1.50-1.64 (bm, 3H  $\times$  34), 1.42 (S, 9H).



**Figure 3-2.** Addition of the initiator to the monomer solution in an ampoule to initiate the polymerization of lactide under nitrogen.

### 3.7 Deprotection of Boc-protected Polylactide (PLA<sub>n</sub>-NH<sub>2</sub>)<sup>44</sup>

A polymer with a free amine chain end was generated by adding a large excess of TFA (0.58 mL, 7.6 mmol) to a solution of PLA<sub>17</sub>-Boc (0.50 g, 0.19 mmol chains) in DCM (6.3 mL) and stirring for 4 hours at room temperature.<sup>45</sup> The TFA was removed by extraction with 50 mL of a 5% aqueous NaHCO<sub>3</sub> solution, followed by washing with deionized water until the pH reached 6-7. The solution was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated by purging with air before precipitation in chilled 2-propanol. The polymer was recovered by decantation, evaporation of the residual solvent under vacuum, and drying for 12 hours under vacuum to provide a fluffy white solid. Yield: 0.43 g, 89%.  $M_n$  [NMR] =  $2.5 \times 10^3$  g/mol,  $M_n$  [SEC] =  $3.03 \times 10^3$  g/mol,  $M_w$  [SEC] =

$3.30 \times 10^3$  g/mol PDI = 1.09,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 5.08-5.26 (bm, 1H  $\times$  34), 4.30-4.40 (m, 1H), 4.18-4.27 (m, 2H), 2.74-2.84 (m, 2H), 1.74-1.84 (m, 2H), 1.49-1.67 (bm, 3H  $\times$  34).

### 3.8 Synthesis of Polylactide-*block*-Poly(Z-Lysine) Copolymer ( $\text{PLA}_n$ -*b*- $\text{PLyZ}_m$ )

To generate a block copolymer, the  $\text{PLA}_n$ - $\text{NH}_2$  generated in the previous step was used as macroinitiator for the polymerization of the H-lysine(Z)-NCA monomer. The synthesis of  $\text{PLA}_{17}$ -*b*- $\text{PLyZ}_{68}$ , initiated with  $\text{PLA}_{17}$ - $\text{NH}_2$ , is provided as an example. Lysine(Z)-NCA (1.00 g, 3.26 mmol) was dissolved in dry DCM (20 mL) under nitrogen in a flame-dried ampoule. A solution of  $\text{PLA}_{17}$ - $\text{NH}_2$  ( $M_n = 2.5 \times 10^3$  g/mol, 0.12 g, 0.05 mmol chains) was prepared in 20 mL of dry DCM and added to the stirred H-Lysine(Z)-NCA solution using a syringe with a long oven-dried needle. The polymerization was allowed to proceed at 0 °C for the first 10 hours, and continued at room temperature for another 48 hours. The block copolymer was purified by precipitation into ice-cold diethyl ether, isolated by decantation, and dried under vacuum for 12 hours. The final appearance of the Z-protected block copolymer was a white rigid plastic-like solid. Yield: 0.92 g, 94%.  $M_n$  [NMR] =  $1.86 \times 10^4$  g/mol,  $M_n$  [SEC] =  $2.89 \times 10^4$  g/mol,  $M_w$  [SEC] =  $3.58 \times 10^4$  g/mol PDI = 1.24,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.10-7.33 (bm, 5H  $\times$  61 + 1H  $\times$  61), 5.32-5.53 (b, 1H  $\times$  61), 5.10-5.24 (bm, 1H  $\times$  34), 4.89-5.08 (b, 2H  $\times$  61), 3.60-4.08 (b, 1H  $\times$  61), 2.96-3.30 (b, 2H  $\times$  61), 1.25-2.35 (bm, 3H  $\times$  34 + 6H  $\times$  61).

### 3.9 Deprotection of the Z Group<sup>45,46</sup>

The block copolymer  $\text{PLA}_{17}$ -*b*- $\text{PLyZ}_{68}$  (1.00 g, target  $M_n = 2.03 \times 10^4$  g/mol, actual  $M_n = 1.86 \times 10^4$  g/mol, 61 meq Z-lysine units) was dissolved in TFA (10 mL, 0.13 mol), and 33% HBr/acetic acid (0.30 mL, 1.65 mmol) solution was added with stirring at room temperature. After 4 hours the polymer was recovered by precipitation in diethyl ether, isolated by decantation, and dried under vacuum for 12 hours. Yield: 0.78 g, 93%.  $M_n$  [NMR] =  $1.51 \times 10^4$  g/mol,  $^1\text{H}$  NMR (300 MHz,

DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 7.64-8.23 (bm, 3H  $\times$  61 + 1H  $\times$  61), 5.10-5.25 (bm, 1H  $\times$  31), 4.01-4.38 (bm, 1H  $\times$  61), 2.67-2.84 (bm, 2H  $\times$  61), 1.07-1.85 (bm, 3H  $\times$  31 + 6H  $\times$  61).

### 3.10 Synthesis of Boc-protected Polylactide with Cleavable Disulfide Bond (PLA<sub>n</sub>-SS-Boc)

In replacement of 3-(Boc-amino)-1-propanol, *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate was used to initiate the polymerization of lactide and provide a redox-sensitive block copolymer. The synthetic steps were otherwise identical to the procedures described above. For example, to synthesize PLA<sub>17</sub>-SS-Boc (target  $M_n = 2.7 \times 10^3$  g/mol, 17 lactide units/34 lactic acid units per polymer chain), DBU/BA salt (0.11 g, 0.40 mmol) and lactide (1.00 g, 6.94 mmol) were added to a dried ampoule in 3 mL of DCM. A solution of *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate (0.10 g, 0.41 mmol) in 4 mL of DCM was then injected into the ampoule to start the polymerization. The reaction was allowed to proceed for 2 days and the product was recovered by precipitation in cold 2-propanol, also yielding a white fluffy solid after drying. Yield: 0.98 g, 89%.  $M_n$  [NMR] =  $2.7 \times 10^3$  g/mol,  $M_n$  [SEC] =  $2.98 \times 10^3$  g/mol,  $M_w$  [SEC] =  $3.24 \times 10^3$  g/mol, PDI = 1.09, <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 5.07-5.27 (bm, 1H  $\times$  33), 4.33-4.41 (bm, 2H), 3.36-3.46 (bm, 2H), 2.83-2.93 (t, 2H), 2.73-2.83 (t, 2H), 1.48-1.62 (bm, 3H  $\times$  33), 1.42 (s, 9H).

### 3.11 Deprotection of Boc-protected Polylactide with Cleavable Disulfide Bond (PLA<sub>n</sub>-SS-NH<sub>2</sub>)

PLA<sub>17</sub>-SS-Boc (0.50 g, 0.20 mmol chains) was dissolved in DCM (6.3 mL) and treated with TFA (0.58 mL, 7.57 mmol) for 4 hours. The reaction was stopped by adding aqueous NaHCO<sub>3</sub> solution and washing with deionized water until the solution became neutral (or slightly acidic, because of CO<sub>2</sub> dissolved in the deionized water). After recovery and drying, the polymer was a fluffy white solid. Yield: 0.45 g, 93%.  $M_n$  [NMR] =  $2.5 \times 10^3$  g/mol,  $M_n$  [SEC] =  $2.87 \times 10^3$  g/mol,  $M_w$  [SEC] =

$3.13 \times 10^3$  g/mol, PDI = 1.09,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 5.07-5.27 (bm, 1H  $\times$  33), 4.33-4.41 (bm, 2H), 2.97-3.06 (bm, 2H), 2.83-2.93 (t, 2H), 2.73-2.83 (t, 2H), 1.48-1.62 (bm, 3H  $\times$  33).

### **3.12 Synthesis of Cleavable Polylactide-*block*-Poly(Z-Lysine) Copolymer (PLA<sub>n</sub>-SS-PLyZ<sub>m</sub>)**

Under nitrogen atmosphere, PLA<sub>17</sub>-SS-NH<sub>2</sub> ( $M_n = 2.5 \times 10^3$  g/mol, 0.12 g, 0.05 mmol chains) was dissolved in 20 mL of dry DCM and added to a solution of 1.00 g Lysine(Z)-NCA (3.26 mmol) in 20 mL of DCM in a flame-dried ampoule. The polymerization was allowed to proceed for 2 days. Cold diethyl ether was used to recover the polymer as a white rigid solid after overnight drying. Yield: 0.96 g, 98%.  $M_n$  [NMR] =  $1.17 \times 10^4$  g/mol,  $M_n$  [SEC] =  $2.73 \times 10^4$  g/mol,  $M_w$  [SEC] =  $3.58 \times 10^4$  g/mol, PDI = 1.31,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.08-7.38 (bm, 5H  $\times$  36 + 1H  $\times$  36), 5.34-5.59 (b, 1H  $\times$  36), 5.10-5.26 (bm, 1H  $\times$  33), 4.84-5.09 (b, 2H  $\times$  36), 3.64-4.12 (b, 1H  $\times$  36), 2.93-3.30 (b, 2H  $\times$  36), 1.28-2.35 (bm, 3H  $\times$  33 + 6H  $\times$  36).

### **3.13 Deprotection of the Z Group of Copolymers with a Cleavable Disulfide Bond**

TFA (10 mL, 0.13 mol) was used to dissolve the block copolymer PLA<sub>17</sub>-SS-PLyZ<sub>68</sub> (1.00 g, target  $M_n = 2.04 \times 10^4$  g/mol, actual  $M_n = 1.17 \times 10^4$ , 36 meq Z-lysine units) before adding 33% HBr/acetic acid (0.30 mL, 1.65 mmol) solution. After 4 hours, the polymer was recovered using chilled diethyl ether and dried to provide a white powder. Yield: 0.81 g, 95%.  $M_n$  [NMR] =  $8.46 \times 10^3$  g/mol,  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  ppm: 7.57-8.17 (bm, 3H  $\times$  36 + 1H  $\times$  36), 5.10-5.25 (bm, 1H  $\times$  13), 4.07-4.35 (bm, 1H  $\times$  36), 2.65-2.84 (bm, 2H  $\times$  36), 1.17-1.80 (bm, 3H  $\times$  13 + 6H  $\times$  36). Partial degradation of the polylactide block was observed under these conditions, as discussed in the following Chapter.

### 3.14 Optimization Attempts for Deprotection of the Z Group

To minimize the degradation of the PLA segment, different reaction conditions were investigated for the deprotection of the Z group. This included different concentrations of HBr, reaction times and temperatures.

In the first attempt, rather than using 0.30 mL of HBr/acetic acid solution per gram of PLA<sub>17</sub>-SS-PLYZ<sub>68</sub> sample as in the previous procedure, 0.10 mL HBr/acetic acid was used for a 0.4 g sample (0.25 mL per gram PLA<sub>17</sub>-SS-PLYZ<sub>68</sub>). After 1 hour, the polymer was recovered by precipitation in chilled diethyl ether. The peaks assigned to protons on the aromatic ring of Z group still appeared in the <sup>1</sup>H NMR spectrum at 7.15- 7.55 ppm, meaning that the sample was only partially deprotected. Under these conditions, the M<sub>n</sub> [NMR] would be an average of the molecular weight of protected PLA<sub>17</sub>-SS-PLYZ<sub>68</sub> and deprotected PLA<sub>17</sub>-SS-PLY<sub>68</sub>-NH<sub>3</sub><sup>+</sup>Br<sup>-</sup> units produced in the polymer. M<sub>n</sub> [NMR] = 1.38×10<sup>4</sup> g/mol, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 7.57-8.17 (bm, 3H × 58 + 1H × 58), 7.15-7.55 (bm, 5H × 58) 5.10-5.25 (bm, 1H × 23), 4.07-4.35 (bm, 1H × 58), 2.65-2.84 (bm, 2H × 58), 1.17-1.80 (bm, 3H × 23 + 6H × 58).

Using the same concentration of HBr/acetic acid solution (0.25 mL for a 1.0 g PLA<sub>17</sub>-SS-PLYZ<sub>68</sub> sample), the reaction time was increased from 1 to 6 hours. A small peak was still visible in the <sup>1</sup>H NMR spectrum at 7.15-7.55 ppm, and M<sub>n</sub> [NMR] = 1.30×10<sup>4</sup> g/mol was determined for the partially deprotected block copolymer. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 7.57-8.17 (bm, 3H × 58 + 1H × 58), 7.15-7.55 (bm, 5H × 58) 5.10-5.25 (bm, 1H × 12), 4.07-4.35 (bm, 1H × 58), 2.65-2.84 (bm, 2H × 58), 1.17-1.80 (bm, 3H × 12 + 6H × 58).

In a third attempt, the concentration of HBr/acetic acid solution was increased to 0.38 mL per gram of PLA<sub>17</sub>-SS-PLYZ<sub>68</sub> (0.15 mL for a 0.4 g sample) and the reaction time was set to 3 hours. A trace amount of protected block copolymer was still observed in the <sup>1</sup>H NMR spectrum, and M<sub>n</sub> [NMR]



=  $1.31 \times 10^4$  g/mol was determined.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  ppm: 7.57-8.17 (bm,  $3\text{H} \times 58 + 1\text{H} \times 58$ ), 7.15-7.55 (bm,  $5\text{H} \times 58$ ) 5.10-5.25 (bm,  $1\text{H} \times 14$ ), 4.07-4.35 (bm,  $1\text{H} \times 58$ ), 2.65-2.84 (bm,  $2\text{H} \times 58$ ), 1.17-1.80 (bm,  $3\text{H} \times 14 + 6\text{H} \times 58$ ).

In a fourth attempt, the concentration of HBr/acetic acid solution was maintained at 0.15 mL for a 0.4 g sample (0.38 mL per gram) but the reaction time was increased to 5 hours. This time the sample was completely deprotected, as confirmed by NMR analysis.  $M_n$  [NMR] =  $1.30 \times 10^4$  g/mol,  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  ppm: 7.57-8.17 (bm,  $3\text{H} \times 58 + 1\text{H} \times 58$ ), 5.10-5.25 (bm,  $1\text{H} \times 13$ ), 4.07-4.35 (bm,  $1\text{H} \times 58$ ), 2.65-2.84 (bm,  $2\text{H} \times 58$ ), 1.17-1.80 (bm,  $3\text{H} \times 13 + 6\text{H} \times 58$ ).

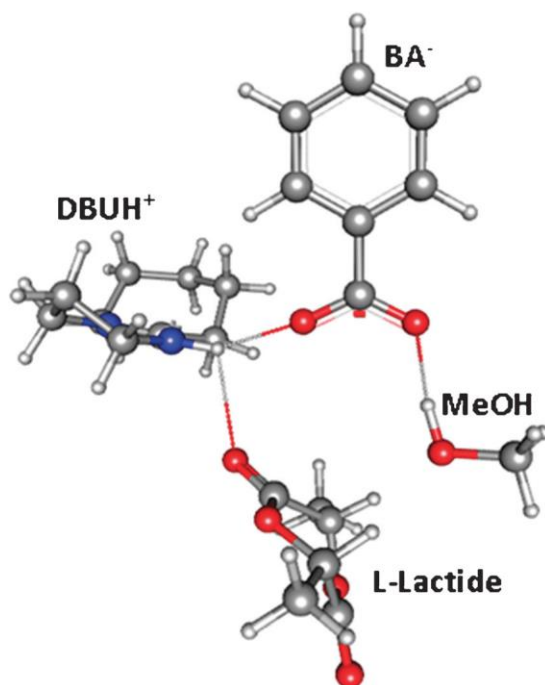
The influence of temperature in the deprotection was also considered. Attempt five used 0.30 mL of HBr/acetic acid solution per gram of  $\text{PLA}_{17}\text{-}b\text{-PLyZ}_{68}$ , but the reaction temperature was maintained at  $0^\circ\text{C}$  for 4 hours before recovery. Complete deprotection was also achieved in this case.  $M_n$  [NMR] =  $1.44 \times 10^4$  g/mol,  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  ppm: 7.57-8.17 (bm,  $3\text{H} \times 61 + 1\text{H} \times 61$ ), 5.10-5.25 (bm,  $1\text{H} \times 24$ ), 4.07-4.35 (bm,  $1\text{H} \times 61$ ), 2.65-2.84 (bm,  $2\text{H} \times 61$ ), 1.17-1.80 (bm,  $3\text{H} \times 24 + 6\text{H} \times 61$ ).

**CHAPTER 4**  
**RESULTS AND DISCUSSION**

PLA has been used increasingly since the 1980's as a biodegradable and renewable thermoplastic. The biocompatibility and biodegradability of PLA make it an interesting material for biomedical applications. The most common procedure to synthesize high molecular weight PLA is utilizing metal-containing catalysts for ring-opening polymerization, most commonly tin compounds such as tin dioctoate. However, no matter how carefully the products are purified, toxic metal residues are left and contaminate the polymer. The polymers synthesized in this investigation are designed to serve as carriers for targeted drug delivery applications. To avoid heavy metal contamination, a recently reported non-metallic DBU/BA bifunctional catalyst system developed by Coady et al.<sup>22</sup> was employed to synthesize PLA with not only a low PDI, but also as a cleaner and safer building block. It should be noted that, since the lactide monomer is essentially a cyclic dimer of lactic acid, the degree of polymerization ( $X_n$ ) of the polymer can be reported either in terms of lactide or lactic acid units in the chain. For the sake of consistency, however, and since the monomer used was lactide, the  $X_n$  values reported herein are expressed exclusively in terms of lactide units.

#### **4.1 Preparation of DBU/BA Salt**

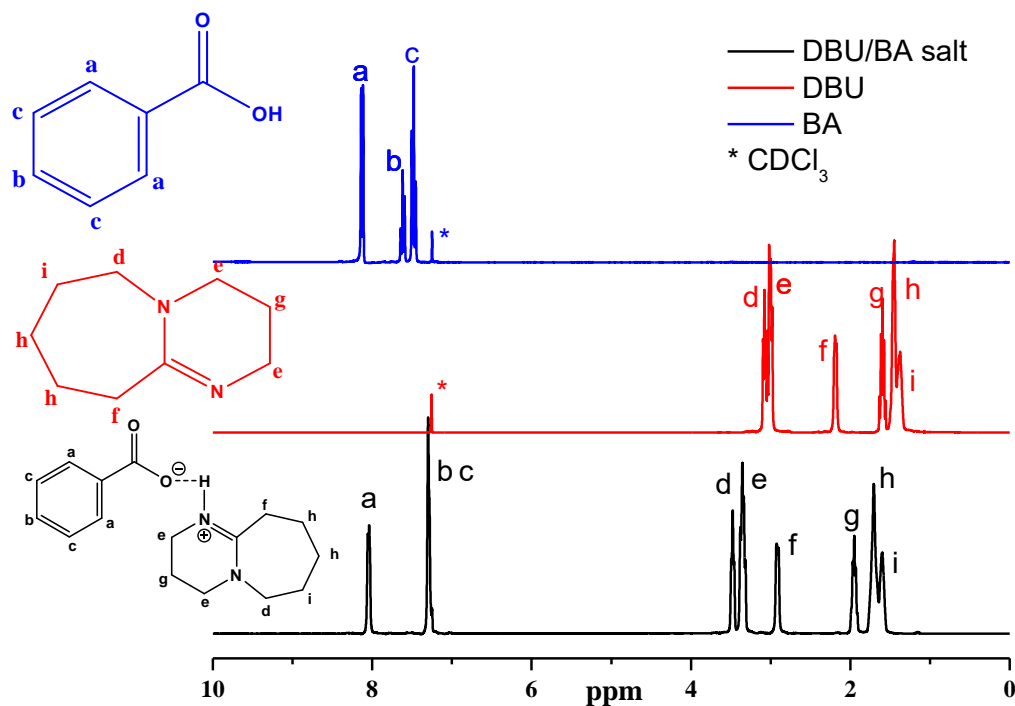
The polymerization mechanism with the DBU/BA salt relies upon the formation of a complex between these two components, whereby the amine group of DBU is protonated by the carboxylic proton of BA. The ammonium ion thus formed on DBU can electrostatically interact with the carbonyl groups of lactide, providing electrophilic activation for further hydroxyl group additions in the propagation step. The negatively charged carboxylate group can also interact with the alcohol used as initiator, as shown in Figure 4-1.<sup>22</sup>



**Figure 4-1.** The DBU/BA salt providing electrophilic activation to the lactide and interacting with the alcohol.<sup>22</sup>

*Reprinted from Ref. 22, with permission from the Royal Society of Chemistry.*

The <sup>1</sup>H NMR spectra obtained for pure BA, DBU and the DBU/BA salt are compared in Figure 4-2. The peaks at 7.42-7.55 ppm (peak b) and 7.57-7.68 ppm (peak c) are assigned to the protons on the aromatic ring of BA. These merged for the salt, and the peaks for the protons on DBU (peaks d through i) all shifted slightly, while the integrals for all the peaks remained unchanged after combining the two compounds to form the salt. This confirms that DBU reacted with BA to form a DBU/BA salt, instead of just forming a mixture.



**Figure 4-2.**  $^1\text{H}$  NMR spectra (300 MHz, in  $\text{CDCl}_3$ ) for (from top to bottom) BA, DBU and the DBU/BA salt, confirming that the DBU/BA salt was formed.

#### 4.2 Synthesis and Characterization of PLA Macroinitiators Initiated with (3-(Boc-amino)-1-propanol and *tert*-Butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate)

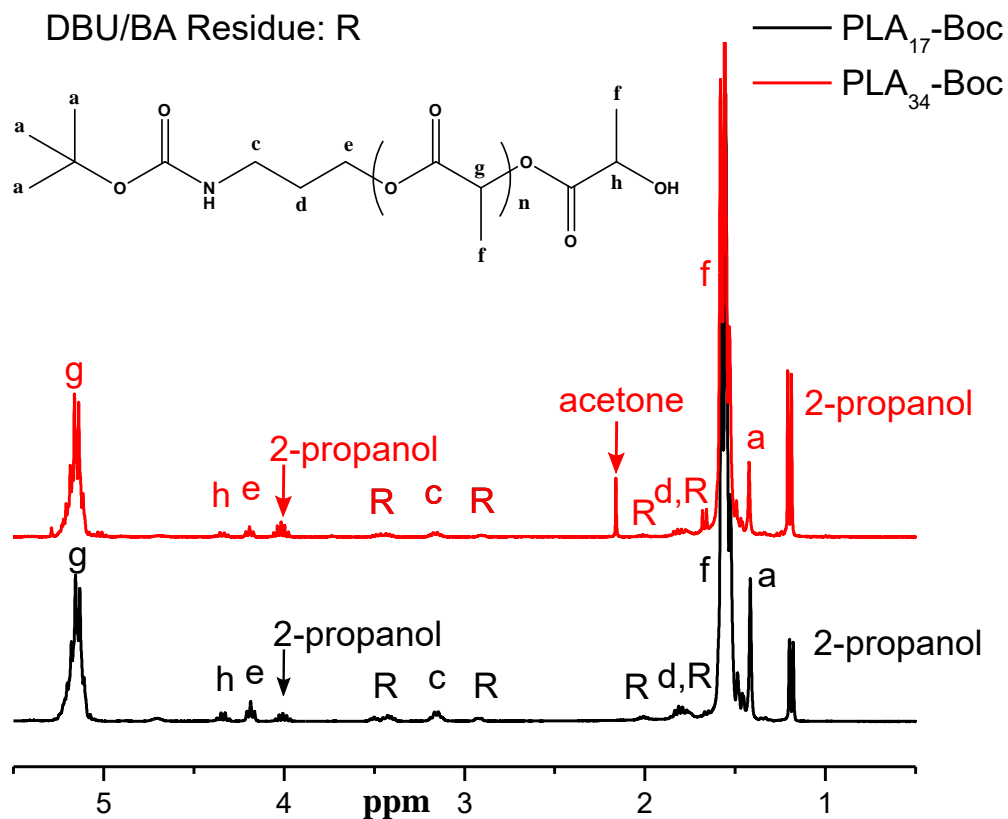
The first step in this project was the ring-opening polymerization of D,L-lactide in the presence of the DBU/BA salt, under nitrogen atmosphere, initiated with 3-(Boc-amino)-1-propanol. All the samples obtained were characterized by  $^1\text{H}$  NMR spectroscopy. The NMR spectrum obtained for a polylactide sample with a Boc-protected amino end group ( $\text{PLA}_{17}\text{-Boc}$ ) is shown in Figure 4-3. The peaks in the spectrum are assigned as indicated. The ratio of the integrals for the peak at 1.42 ppm (peak a, assigned to protons on *tert*-butyloxycarbonyl protecting group) and the peaks at 1.50-

1.64 ppm (peak f, for the methyl group on the polymer backbone) is 1:11.3. Since there are 9 protons on the Boc protecting group and but three methyl proton per lactic acid unit, this means that the chains contained  $(11.3 \times 9) / 3 = 34$  lactic acid repeating units per initiator fragment. This corresponds to a number-average degree of polymerization  $X_n = 17$  in terms of lactide units, or a number-average molecular weight  $M_n = 2.6 \times 10^3$  g/mol, matching exactly the theoretical (target) value  $M_n = 2.6 \times 10^3$  g/mol. The ratio of integrals for the peaks at 4.18 ppm (peak e, assigned to two protons on the methylene group on the initiator, but closest to the lactic acid structural units in the chain) and peak f at 1.50-1.64 ppm was 1 to 48.9, further confirming there were  $48.9 / 3 \times 2 \approx 34$  lactic acid units per polymer chain. The integral for peak c at 3.15 ppm provides the same result. However the integration of peak d (from 1.72-1.88 ppm, signal from the two protons in the middle methylene group on the initiator) and peak g is only 1 to 13.5, while ideally the ratio should be around 1 to 51 (like the ratio of peak g to peak e or peak c). This is attributed to catalyst residues, since peaks from the DBU/BA salt also appear around this area and would increase the integral value for peak d.

The number-average molecular weight ( $M_n$ ), weight-average molecular weight ( $M_w$ ) and the polydispersity index ( $PDI = M_w/M_n$ ) of the polymers were determined by SEC analysis using a polystyrene standards calibration curve. The SEC trace obtained for sample PLA<sub>17</sub>-Boc is provided in Figure 4-4. The values obtained for that sample were  $M_n = 3.06 \times 10^3$  g/mol and  $M_w = 3.33 \times 10^3$  g/mol, somewhat higher than the target  $M_n = 2.60 \times 10^3$  g/mol. This is not unexpected, since the instrument used in the project was calibrated with polystyrene standards and the molecular weights obtained by SEC analysis are therefore only apparent (polystyrene-equivalent) values. While the instrument used had a light scattering detector, potentially allowing the determination of the absolute molecular weight of the samples, the light scattering signal was unfortunately too noisy

to allow such analysis. The apparent PDI obtained by SEC analysis, 1.09, was nevertheless lower than for typical metal-mediated lactide polymerization, which are usually above 1.15.<sup>47</sup>

In view of the planned collaboration to study the self-assembly behavior of PLA-*block*-PLy copolymers, to allow the determination of the optimal weight ratio between the hydrophobic and hydrophilic segments, PLA-Boc macroinitiators with different molecular weights were synthesized. The molar ratio between the initiator and the lactide monomer was thus increased from 1:17 to 1:34, corresponding to an increase in the target  $M_n$  from  $2.50 \times 10^3$  to  $5.00 \times 10^3$  g/mol. For the second PLA-Boc sample the ratio of integrals for peaks a and g (Figure 4-3) was 1 to 6.89, close to the expected value of 7.55. Because there are 9 protons on the *tert*-butyloxycarbonyl protecting group (peak a), this yields a ratio of 62 lactic acid units per initiator residue ( $X_n = 31$  in terms of lactide units, close to the target value of 34) and almost doubled as compared to the PLA-Boc sample with  $X_n = 17$ . The integration of peaks c and e similarly yielded 58 and 60 lactic acid repeating units per chain, respectively, which is also reasonable. Due to the increased length of the polymer chain, the signals characteristic for protons on the initiator fragment decreased proportionally, so integration errors for small peaks are expected to be larger. The SEC analysis provided  $M_n = 4.48 \times 10^3$  g/mol and  $M_w = 4.85 \times 10^3$  g/mol, with a PDI of 1.09.



**Figure 4-3.**  $^1\text{H}$  NMR spectra (300 MHz, in  $\text{CDCl}_3$ ) for PLA<sub>17</sub>-Boc and PLA<sub>34</sub>-Boc samples initiated with 3-(Boc-amino)-1-propanol, using the DBU/BA salt as catalyst.

To further improve the drug delivery efficiency, block copolymers were designed with a cleavable disulfide bond at the junction between the PLA and PLY blocks, thus providing redox-responsive character to the block copolymer. As can be seen in the  $^1\text{H}$  NMR spectrum obtained for the redox-sensitive initiator *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate (Figure 4-5), the protons of the Boc group still appear around 1.40 ppm. Setting the integral value for this peak to 9.00 (9 protons on the Boc group), the integration of peaks b, e, f, c and d (assignments provided

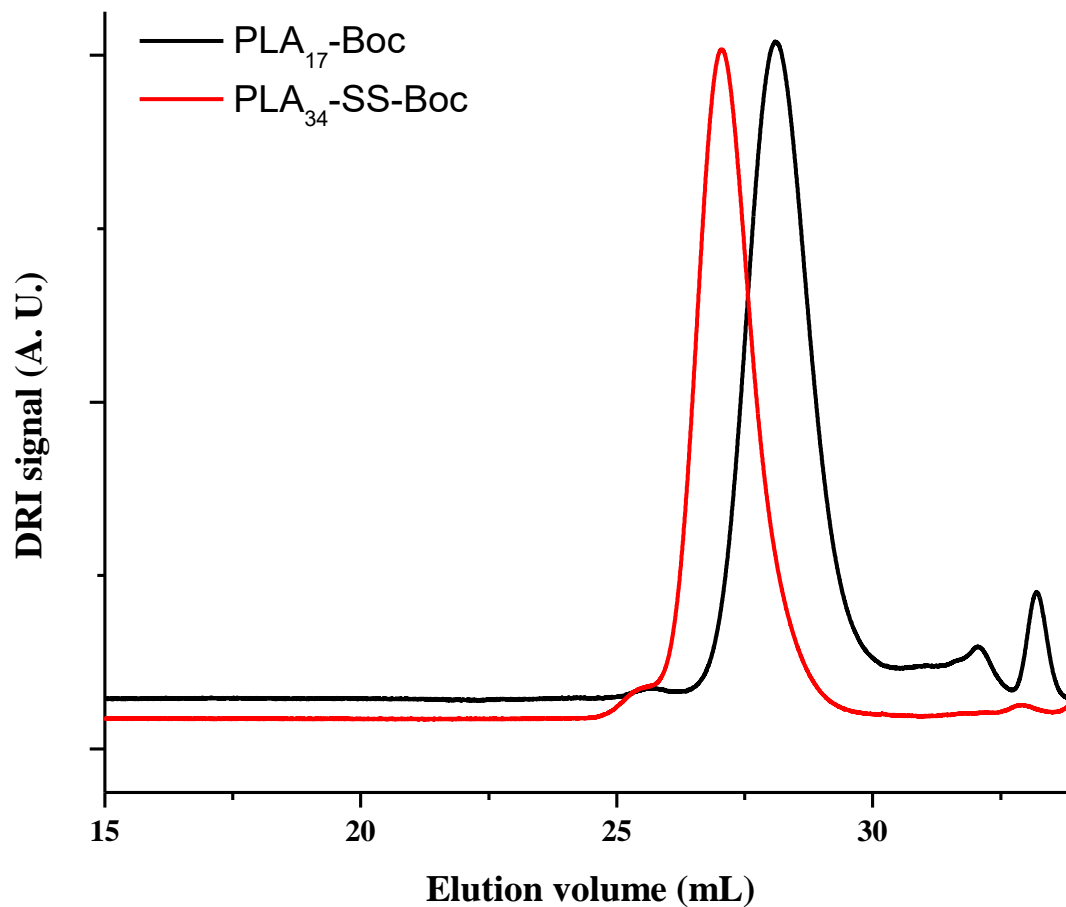


on Figure 4-5) gives values of 1.98, 2.01, 0.98 and 4.97, respectively, matching closely the theoretical values of 2, 2, 1 and 5.

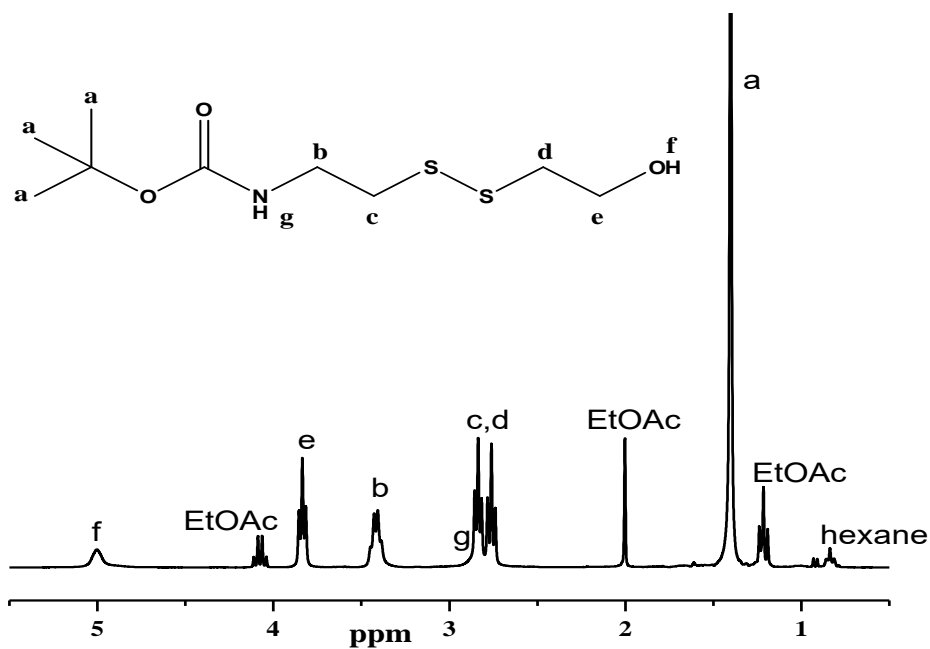
In Figure 4-6 the  $^1\text{H}$  NMR spectrum for sample  $\text{PLA}_{17}\text{-SS-Boc}$ , with a cleavable disulfide bond, is almost identical to the PLA samples initiated with 3-(Boc-amino)-1-propanol and shown in Figure 4-3. This is of course because the only difference between these two PLA samples is in the initiator fragment. Peak a (for the Boc group), at 1.42 ppm, was also used as the reference peak to calculate the  $X_n$  of the sample. Setting the integral value for that peak to 9, peaks g and h (assigned to protons on the methine and methyl groups of the PLA units) provided integral values of 33.2 and 99.6, respectively, corresponding to 33 lactic acid repeating units on the polymer chains, so  $X_n = 17$  in terms of lactide units, and  $M_n = 2.70 \times 10^3$  g/mol on the basis of  $^1\text{H}$  NMR analysis. The peak integral values for peaks c, f + i, and d + e were 2.11, 3.38 and 3.99, respectively, close to the expected values of 2, 3 and 4, considering uncertainties in the integration procedure. The molecular weights obtained from SEC analysis were  $M_n = 2.98 \times 10^3$  g/mol and  $M_w = 3.24 \times 10^3$  g/mol, again somewhat larger than  $M_n$  from  $^1\text{H}$  NMR (and for the 3-(Boc-amino)-1-propanol-initiated samples), because of the use of a polystyrene standards calibration curve. The PDI = 1.09 was likewise low.

A higher molecular weight PLA sample with a cleavable disulfide bond was also prepared. The  $^1\text{H}$  NMR spectrum obtained for the  $\text{PLA}_{34}\text{-SS-Boc}$  sample was very similar to the  $\text{PLA}_{17}\text{-SS-Boc}$  sample shown in Figure 4-6, except that the integral of the characteristic peaks gave the result  $M_n = 5.00 \times 10^3$  g/mol ( $X_n = 33$ ). Value of  $M_n = 4.92 \times 10^3$  g/mol,  $M_w = 5.35 \times 10^3$  g/mol and PDI = 1.09 were obtained by SEC analysis. The SEC traces for samples  $\text{PLA}_{17}\text{-Boc}$  and  $\text{PLA}_{34}\text{-SS-Boc}$  are compared in Figure 4-4. The peak for  $\text{PLA}_{34}\text{-SS-Boc}$  eluted at 27.0 min, at a retention volume

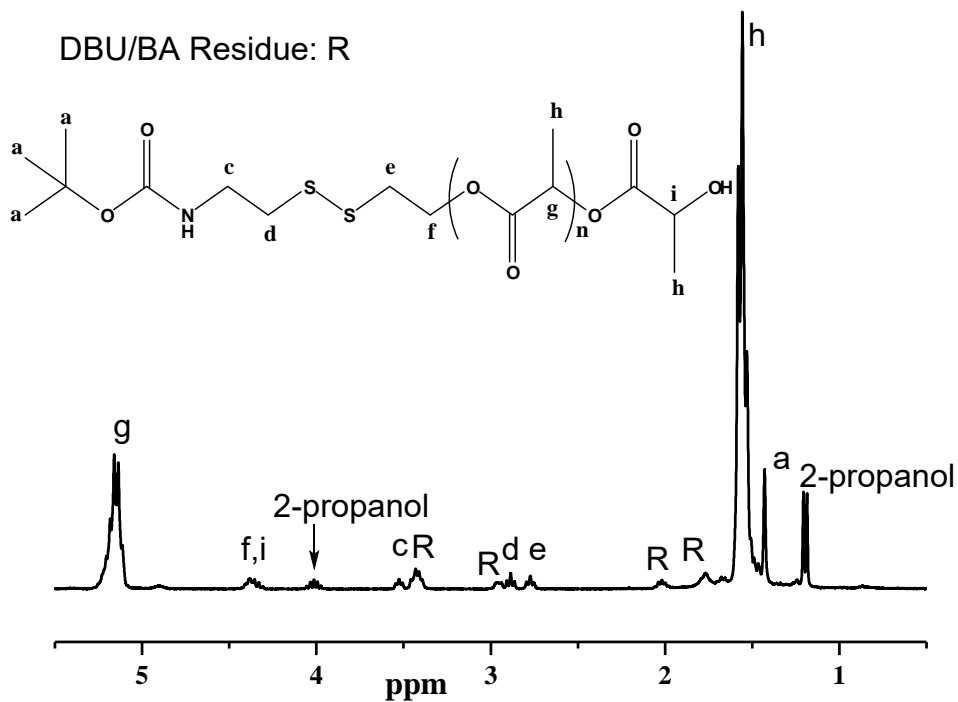
about 1 mL lower than PLA<sub>17</sub>-Boc, at 28.1 mL. This makes perfect sense, since smaller molecules are delayed further as they travel through the SEC column packed with porous beads.



**Figure 4-4.** SEC traces for samples PLA<sub>17</sub>-Boc and PLA<sub>34</sub>-SS-Boc.



**Figure 4-5.** <sup>1</sup>H NMR spectrum (300 MHz, in CDCl<sub>3</sub>) for the redox-responsive disulfide initiator.



**Figure 4-6.** <sup>1</sup>H NMR spectrum (300 MHz, in CDCl<sub>3</sub>) for sample PLA<sub>17</sub>-SS-Boc.

The characterization information obtained for the PLA samples synthesized with the two different initiators are summarized in Table 4-1. It can be seen that the  $M_n$  obtained by  $^1\text{H}$  NMR analysis for the higher ( $M_n \approx 5 \times 10^3$  g/mol) PLA samples are slightly lower than the theoretical values. This is likely caused by incomplete monomer conversion in the polymerization process, since the polymerization rate is expected to decrease with decreasing propagating center concentration in the reaction, leading to shorter polymer chains. This situation may also occur in the synthesis of the PLA-*block*-PLy copolymers, due to their larger target molecular weights. As for the different  $M_n$  and  $M_w$  values obtained from SEC analysis, that are larger than the targeted molecular weights, this is due to the different hydrodynamic volume of the PLA samples as compared to the polystyrene standards. The different functional groups and chain stiffness of PLA and polystyrene, and their different interactions with the solvent used as the mobile phase in the SEC analysis (THF), would necessarily lead to differences in hydrodynamic volume, and therefore in elution volume for samples of the two polymers having the same absolute molecular weight. This is why the  $M_n$  and  $M_w$  values derived from SEC analysis are all apparent molecular weights, relatively to the polystyrene standards used to calibrate the instrument, rather than absolute molecular weights.

**Table 4-1.**  $^1\text{H}$  NMR and SEC characterization results for  $\text{PLA}_n\text{-Boc}$  and  $\text{PLA}_n\text{-SS-Boc}$  samples.

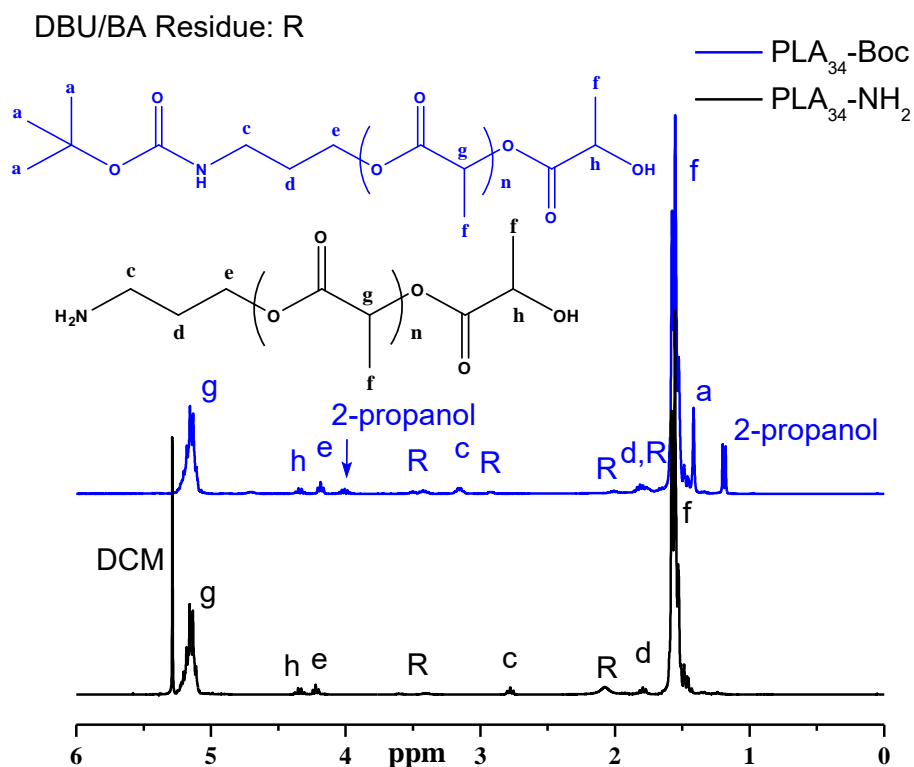
Sample Name	Target $M_n$ (g/mol)	$M_n$ [NMR] (g/mol)	$M_n$ [SEC] (g/mol)	$M_w$ [SEC] (g/mol)	PDI [SEC]
$\text{PLA}_{17}\text{-Boc}$	$2.60 \times 10^3$	$2.60 \times 10^3$	$3.06 \times 10^3$	$3.33 \times 10^3$	1.09
$\text{PLA}_{34}\text{-Boc}$	$5.10 \times 10^3$	$4.70 \times 10^3$	$4.48 \times 10^3$	$4.85 \times 10^3$	1.09
$\text{PLA}_{17}\text{-SS-Boc}$	$2.70 \times 10^3$	$2.60 \times 10^3$	$2.98 \times 10^3$	$3.24 \times 10^3$	1.09
$\text{PLA}_{34}\text{-SS-Boc}$	$5.15 \times 10^3$	$5.00 \times 10^3$	$4.92 \times 10^3$	$5.35 \times 10^3$	1.09

### 4.3 PLA Boc Group Deprotection ( $\text{PLA}_n\text{-NH}_2$ and $\text{PLA}_n\text{-SS-NH}_2$ )

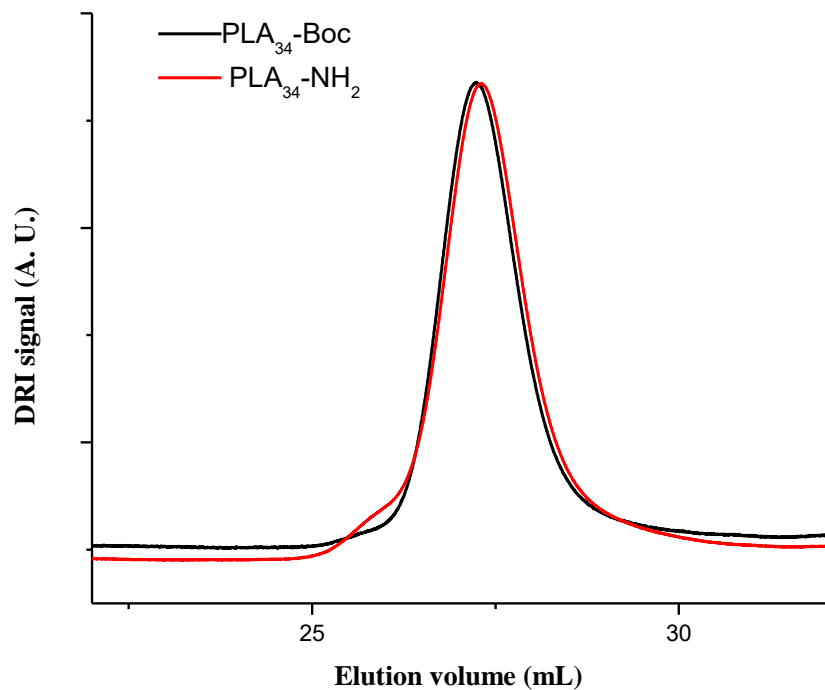
Trifluoroacetic acid was used to cleave the Boc group from  $\text{PLA}_n\text{-Boc}$  and  $\text{PLA}_n\text{-SS-Boc}$  and regenerate a free primary amine group from the protected initiator. It was determined, by  $^1\text{H}$  NMR and SEC analyses, that removal of the Boc group was complete and there was no degradation of the PLA chain. As can be seen in Figure 4-7, the peak at 1.42 ppm (peak a, for the protons in the Boc group before deprotection) completely disappeared after the TFA treatment. Integration of the remaining peaks matched the integration results before deprotection, indicating that no significant degradation of the PLA chain occurred. As for SEC analysis, it can be seen from Figure 4-8 that after removal of the Boc group, the peak only shifted slightly to the right of the original Boc-protected sample, which is consistent with a slight molecular weight decrease. The shape of the curve also remained the same (not broadened), further confirming that no degradation happened during the deprotection step. Correspondingly, the  $M_n$ ,  $M_w$  and PDI values remained essentially unaffected for all the samples (Table 4-2).

The  $^1\text{H}$  NMR spectra for  $\text{PLA}_n\text{-SS-NH}_2$  (Figure 4-7) and  $\text{PLA}_n\text{-NH}_2$  (Figure 4-9) are very similar, and the differences between the two samples (due to the initiator fragments) become

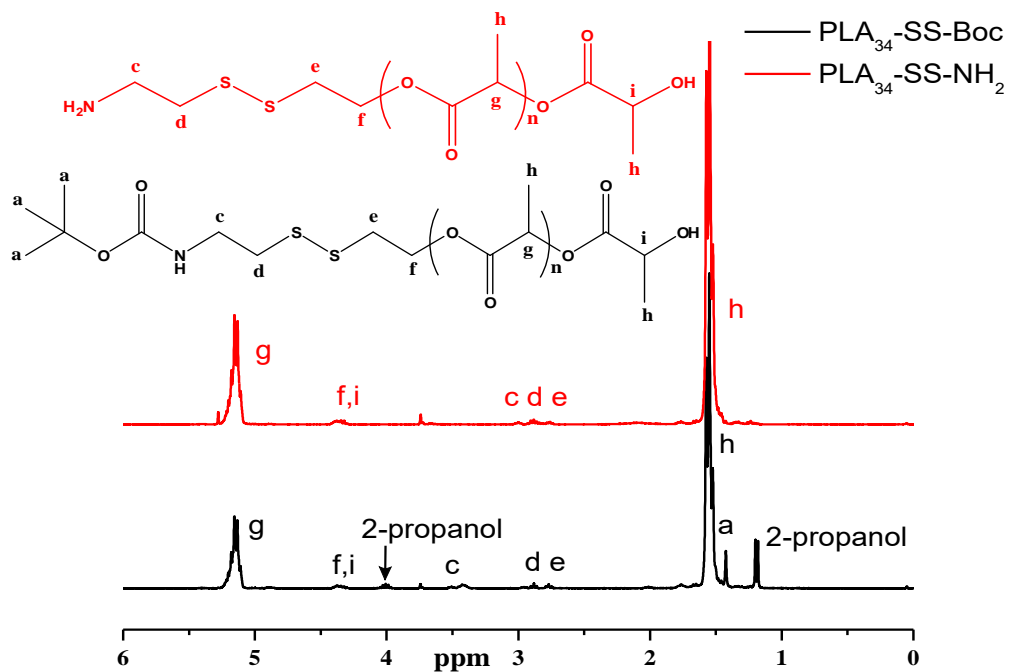
indistinguishable for the longer chain samples. Consequently,  $^1\text{H}$  NMR spectra for polymers initiated with either 3-(Boc-amino)-1-propanol or *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate will be used interchangeably in the following discussion.



**Figure 4-7.**  $^1\text{H}$  NMR spectra (300 MHz, in  $\text{CDCl}_3$ ) for PLA before ( $\text{PLA}_{34}\text{-Boc}$ ) and after ( $\text{PLA}_{34}\text{-NH}_2$ ) Boc group removal, confirming complete deprotection.



**Figure 4-8.** SEC traces for PLA<sub>34</sub>-Boc and PLA<sub>34</sub>-NH<sub>2</sub>.



**Figure 4-9.** <sup>1</sup>H NMR spectra (300 MHz, in CDCl<sub>3</sub>) for sample PLA<sub>34</sub>-SS-Boc before and after deprotection.

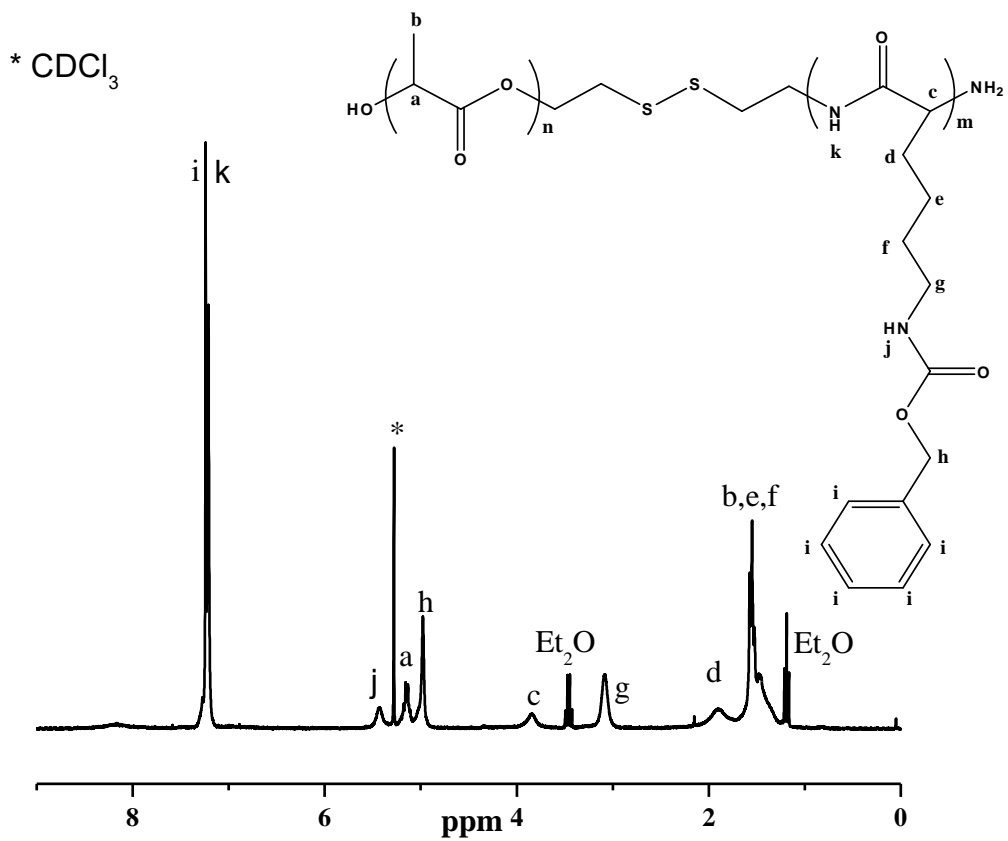
**Table 4-2.**  $^1\text{H}$  NMR and SEC analysis results for PLA samples after removal of the Boc group.

Sample Name	Theoretical $M_n$ (g/mol)	$M_n$ [NMR] (g/mol)	$M_n$ [SEC] (g/mol)	$M_w$ [SEC] (g/mol)	PDI [SEC]
PLA <sub>17</sub> -NH <sub>2</sub>	$2.50 \times 10^3$	$2.50 \times 10^3$	$3.03 \times 10^3$	$3.30 \times 10^3$	1.09
PLA <sub>34</sub> -NH <sub>2</sub>	$5.00 \times 10^3$	$4.60 \times 10^3$	$4.42 \times 10^3$	$4.84 \times 10^3$	1.09
PLA <sub>17</sub> -SS-NH <sub>2</sub>	$2.60 \times 10^3$	$2.50 \times 10^3$	$2.87 \times 10^3$	$3.13 \times 10^3$	1.09
PLA <sub>34</sub> -SS-NH <sub>2</sub>	$5.05 \times 10^3$	$4.90 \times 10^3$	$4.88 \times 10^3$	$5.34 \times 10^3$	1.09

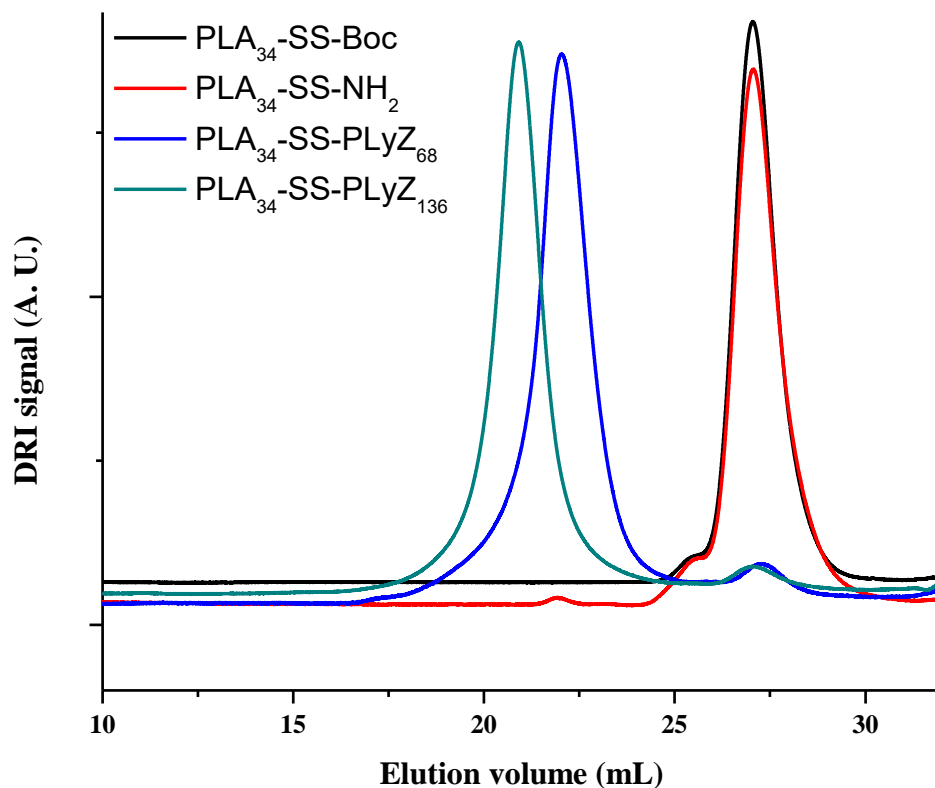
#### 4.4 Synthesis and Characterization of Poly(lactide-*block*-Poly(Z-Lysine) Copolymers (PLA<sub>n</sub>-*b*-PLyZ<sub>m</sub> and PLA<sub>n</sub>-SS-PLyZ<sub>m</sub>)

The deprotected PLA<sub>n</sub>-NH<sub>2</sub> and PLA<sub>n</sub>-SS-NH<sub>2</sub> samples generated in the previous step were used as macroinitiators to polymerize H-Lysine(Z)-NCA, thus yielding the block copolymers PLA<sub>n</sub>-*b*-PLyZ<sub>m</sub> and PLA<sub>n</sub>-SS-PLyZ<sub>m</sub>, respectively. Since NCA monomers are water-sensitive, the reactions were carried out in dry DCM under nitrogen for 48 h. Formation of the desired products was confirmed by  $^1\text{H}$  NMR analysis (Figure 4-10), and the apparent molecular weights were determined by SEC analysis (Figure 4-11). The  $^1\text{H}$  NMR spectrum for sample PLA<sub>34</sub>-SS-PLyZ<sub>68</sub> is provided as an example in Figure 4-10, together with the peak assignments. The signal for the methyl protons on the PLA backbone (peak b) appears to overlap with peaks for protons d, e and f, between 1.25-2.35 ppm. Setting the integral value for peak a to 1 (1 proton on the methine group of the lactic acid units), the integration of peaks i + k, j, h, c, g, b + d + e + f (assignments displayed as above on Figure 4-10, from left to right) gave values of 7.48, 0.96, 1.95, 0.99, 1.99, 8.81, respectively, so the mole ratio of lactic acid to lysine structural units was determined to be 1.00 to 1.03, since the PLA segment consisted of 66 lactic acid units (33 lactide units), this corresponds to 67 lysine units added to the PLA block.





**Figure 4-10.** <sup>1</sup>H NMR spectrum (300 MHz, in CDCl<sub>3</sub>) for PLA<sub>34</sub>-SS-PLyZ<sub>68</sub>.

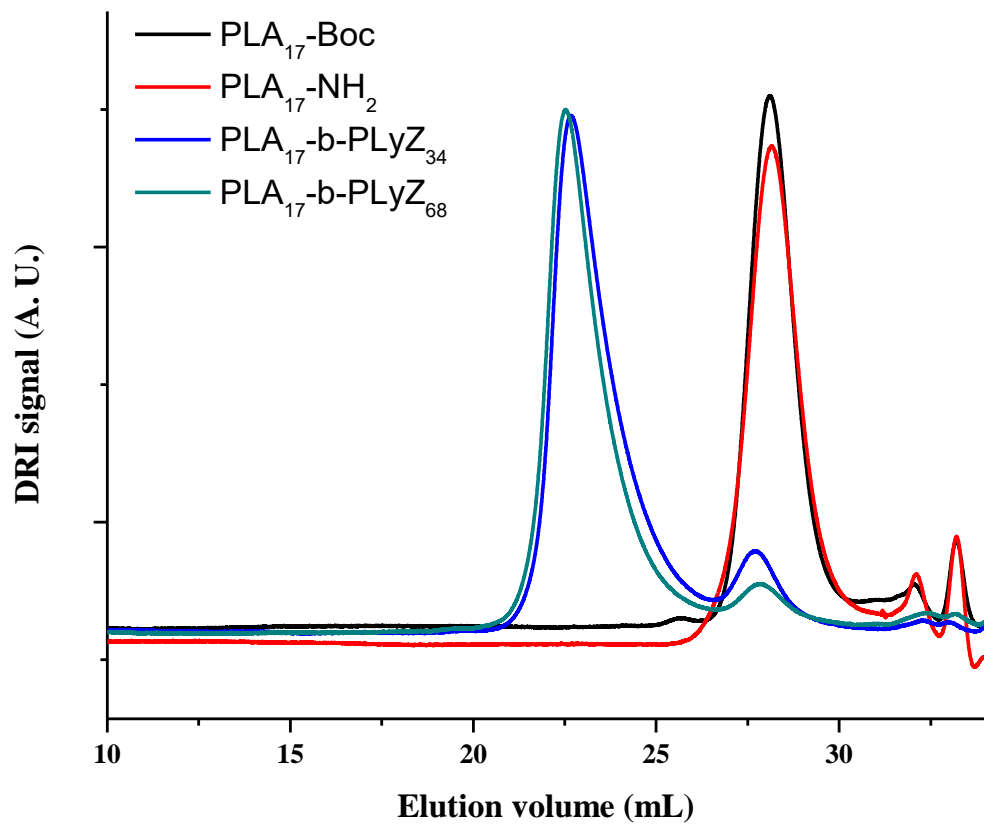


**Figure 4-11.** SEC traces for PLA<sub>34</sub>-SS-PlyZ<sub>m</sub> and their PLA precursors.

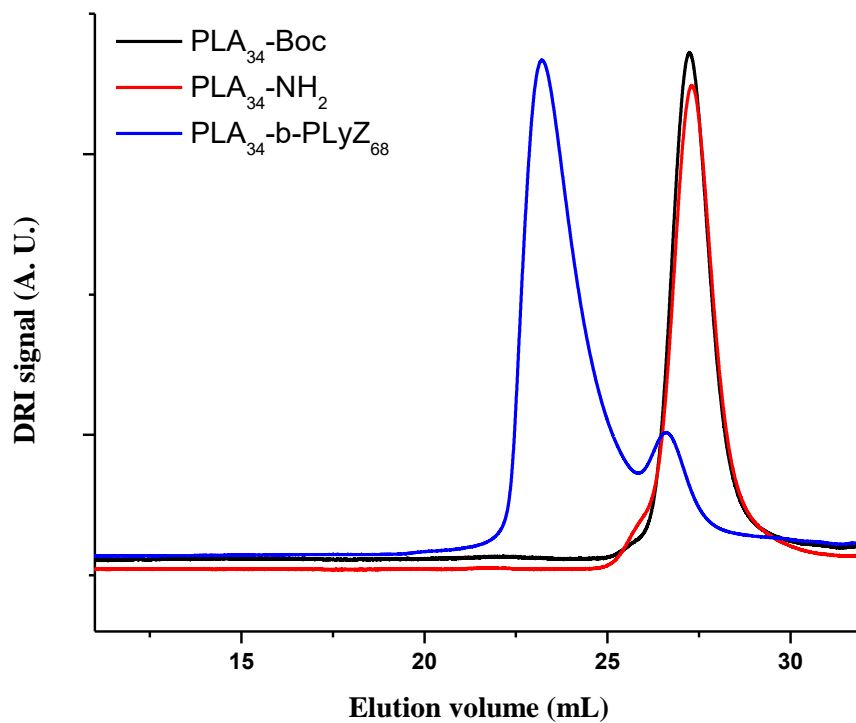
The SEC traces obtained for the block copolymer samples derived from the deprotected PLA<sub>34</sub>-SS-NH<sub>2</sub> macroinitiator (Figure 4-11) are clearly shifted to the left, which means that they elute much earlier than their PLA precursor, or that the molecular weight of the samples increased significantly in the copolymerization process. As expected, the elution volume is lower for the copolymer with the longer PLYZ segment (PLA<sub>34</sub>-SS-PLYZ<sub>136</sub>) in comparison to sample PLA<sub>34</sub>-SS-PLYZ<sub>68</sub>. It is also clear that not all the PLA<sub>34</sub>-SS-NH<sub>2</sub> macroinitiator was chain-extended to give the PLA<sub>34</sub>-SS-PLYZ<sub>68</sub> block copolymer, since there was a small detector response for the block copolymer at an elution volume corresponding to the macroinitiator ( $V_e = 27.0$  mL). This is the same in the case of sample PLA<sub>34</sub>-SS-PLYZ<sub>136</sub>, as a small peak is still visible at  $V_e = 27.0$  mL. This suggests that a small amount of macroinitiator did not react. If the area of the peaks in the chromatogram is assumed to be proportional to the amount of each component eluted, the amount

of macroinitiator contaminant in the block copolymer can be calculated. Since the area (in arbitrary units) of the macroinitiator peak is 9.1 units while the block copolymer peak has an area of 564 units, the macroinitiator content is  $9.1/(9.1+564) \times 100\% = 1.6\%$ ; for sample PLA<sub>34</sub>-SS-PLyZ<sub>68</sub>, the result is 3.0%. This calculation is only approximate however, since the refractive indices of PLA and PLyZ are different, and so the response to each component in the DRI detector is likewise different. The most likely explanation for the presence of macroinitiator contaminant in sample PLA<sub>34</sub>-SS-PLyZ<sub>136</sub> is the presence of impurities in the copolymerization reaction, leading to the early termination of a small amount of chains. This contaminant could be eliminated from the product by further precipitation of the sample or by dialysis. However given the small amount present, it is unlikely to influence the behavior of the block copolymer in processes such as self-assembly for the preparation of polymeric micelles used in drug delivery.

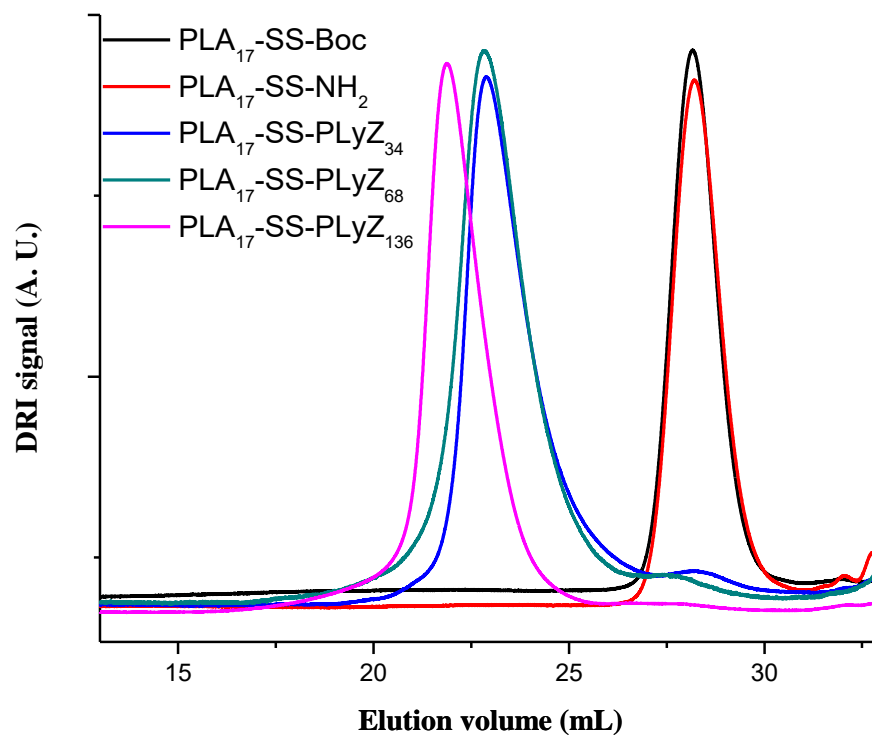
The SEC traces obtained for all the PLA<sub>n</sub>-*b*-PLyZ<sub>m</sub> samples synthesized are provided in Figures 4-12–4-14, and the characterization results are summarized in Table 4-3.



**Figure 4-12.** SEC traces for PLA<sub>17</sub>-b-PlyZ<sub>m</sub> and its PLA precursor.



**Figure 4-13.** SEC traces for  $\text{PLA}_{34}\text{-}b\text{-PlyZ}_{68}$  and its PLA precursor.



**Figure 4-14.** SEC traces for  $\text{PLA}_{17}\text{-SS-PlyZ}_m$  and its PLA precursor.

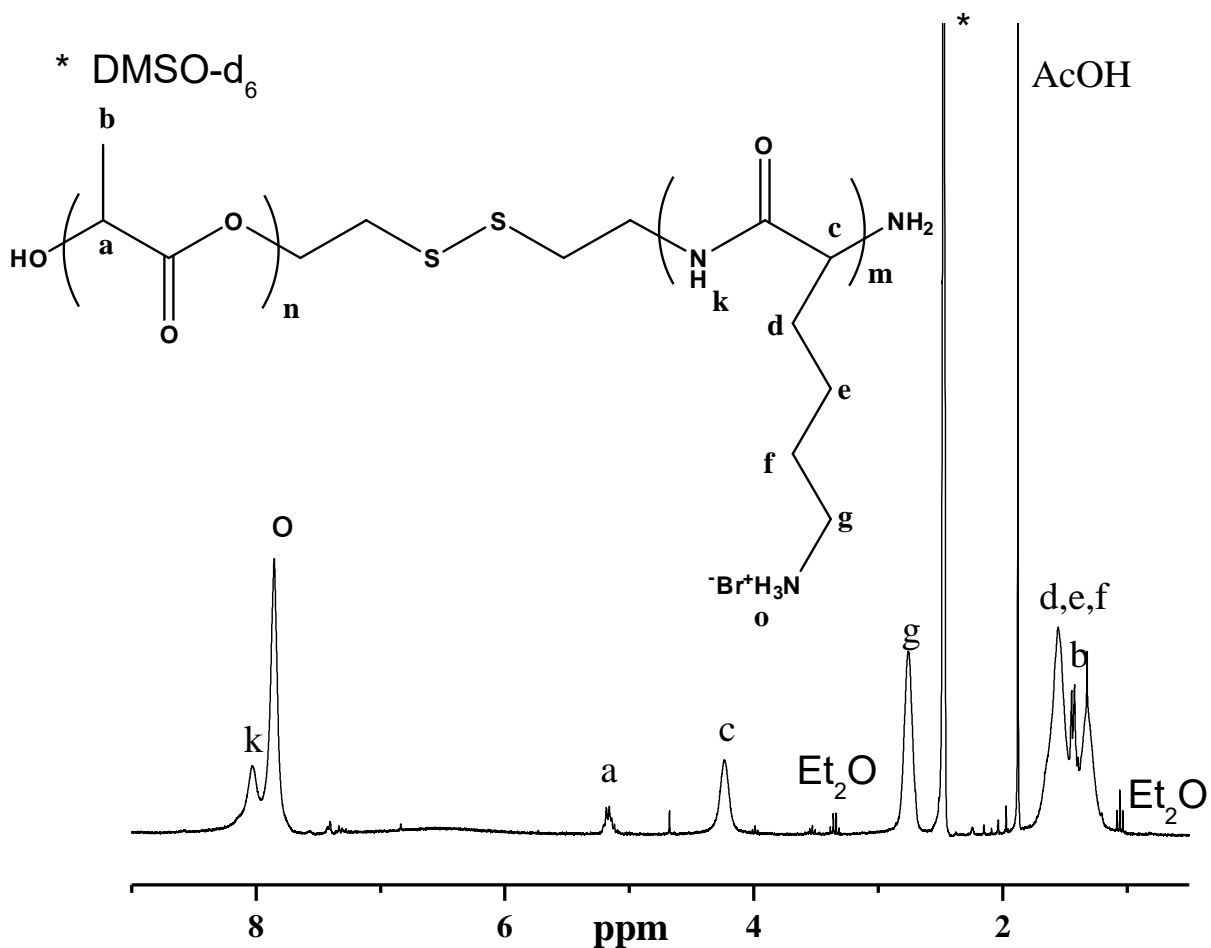
**Table 4-3.** Characterization results for the PLA<sub>n</sub>-*b*-PLyZ<sub>m</sub> and PLA<sub>n</sub>-SS-PLyZ<sub>m</sub> samples.

Sample Name	Theoretical M <sub>n</sub> (g/mol)	M <sub>n</sub> [NMR] (g/mol)	M <sub>n</sub> [SEC] (g/mol)	M <sub>w</sub> [SEC] (g/mol)	PDI [SEC]	Unreacted PLA (%)
PLA <sub>17</sub> - <i>b</i> -PLyZ <sub>34</sub>	1.14×10 <sup>4</sup>	1.80×10 <sup>4</sup>	2.58×10 <sup>4</sup>	3.21×10 <sup>4</sup>	1.24	8.7
PLA <sub>17</sub> - <i>b</i> -PLyZ <sub>68</sub>	2.03×10 <sup>4</sup>	1.86×10 <sup>4</sup>	2.89×10 <sup>4</sup>	3.58×10 <sup>4</sup>	1.24	5.2
PLA <sub>34</sub> - <i>b</i> -PLyZ <sub>68</sub>	2.28×10 <sup>4</sup>	1.69×10 <sup>4</sup>	2.15×10 <sup>4</sup>	2.59×10 <sup>4</sup>	1.20	16.6
PLA <sub>17</sub> -SS-PLyZ <sub>34</sub>	1.15×10 <sup>4</sup>	1.15×10 <sup>4</sup>	2.30×10 <sup>4</sup>	2.95×10 <sup>4</sup>	1.28	2.6
PLA <sub>17</sub> -SS-PLyZ <sub>68</sub>	2.04×10 <sup>4</sup>	1.17×10 <sup>4</sup>	2.73×10 <sup>4</sup>	3.58×10 <sup>4</sup>	1.31	1.6
PLA <sub>17</sub> -SS-PLyZ <sub>136</sub>	3.82×10 <sup>4</sup>	1.87×10 <sup>4</sup>	4.67×10 <sup>4</sup>	6.35×10 <sup>4</sup>	1.36	0
PLA <sub>34</sub> -SS-PLyZ <sub>68</sub>	2.29×10 <sup>4</sup>	2.20×10 <sup>4</sup>	4.91×10 <sup>4</sup>	6.97×10 <sup>4</sup>	1.42	3.0
PLA <sub>34</sub> -SS-PLyZ <sub>136</sub>	4.07×10 <sup>4</sup>	2.30×10 <sup>4</sup>	9.36×10 <sup>4</sup>	1.30×10 <sup>5</sup>	1.39	1.6

#### 4.5 Characterization of Block Copolymers after Z Group Deprotection (PLA<sub>n</sub>-*b*-PLy<sub>m</sub> and PLA<sub>n</sub>-SS-PLy<sub>m</sub>)

After removal of the Z group, the solubility of the copolymers in common solvents such as chloroform and THF was very poor. Consequently, DMSO-*d*<sub>6</sub> was used for the NMR analysis of the deprotected block copolymers. An example of a <sup>1</sup>H NMR spectrum obtained for sample PLA<sub>17</sub>-SS-PLy<sub>34</sub> is provided in Figure 4-15. Because a hydrogen bromide solution (33 wt% in acetic acid) was used in this process, the -NH<sub>2</sub> side chains on the lysine units were protonated to their -NH<sub>3</sub><sup>+</sup>Br<sup>-</sup> form. Taking sample PLA<sub>17</sub>-SS-PLy<sub>34</sub> as an example, the integral ratio for peak a to peak c decreased from 1.00:1.00 before deprotection to 0.48:1.00 after deprotection. Assuming that there is no degradation of PLy during removal of the Z group, the number of lysine units should stay the same as 34. The decrease in the peak ratio observed after Z group deprotection therefore shows

that the number of lactic acid units in the block copolymer decreased from 34 (1.00:1.00) to 16 (0.48:1.00). In the absence of degradation reactions, that integral ratio should have remained constant. This means that roughly half of the PLA block remained in that sample on average. Partial degradation of the PLA block was observed to different extents for PLA<sub>17</sub>-*b*-PLY<sub>68</sub> and all the PLA<sub>*n*</sub>-SS-PLY<sub>*m*</sub> samples, as shown in Table 4-4.



**Figure 4-15.** <sup>1</sup>H NMR spectrum (300 MHz, in DMSO-*d*<sub>6</sub>) for PLA<sub>17</sub>-SS-PLY<sub>34</sub>.

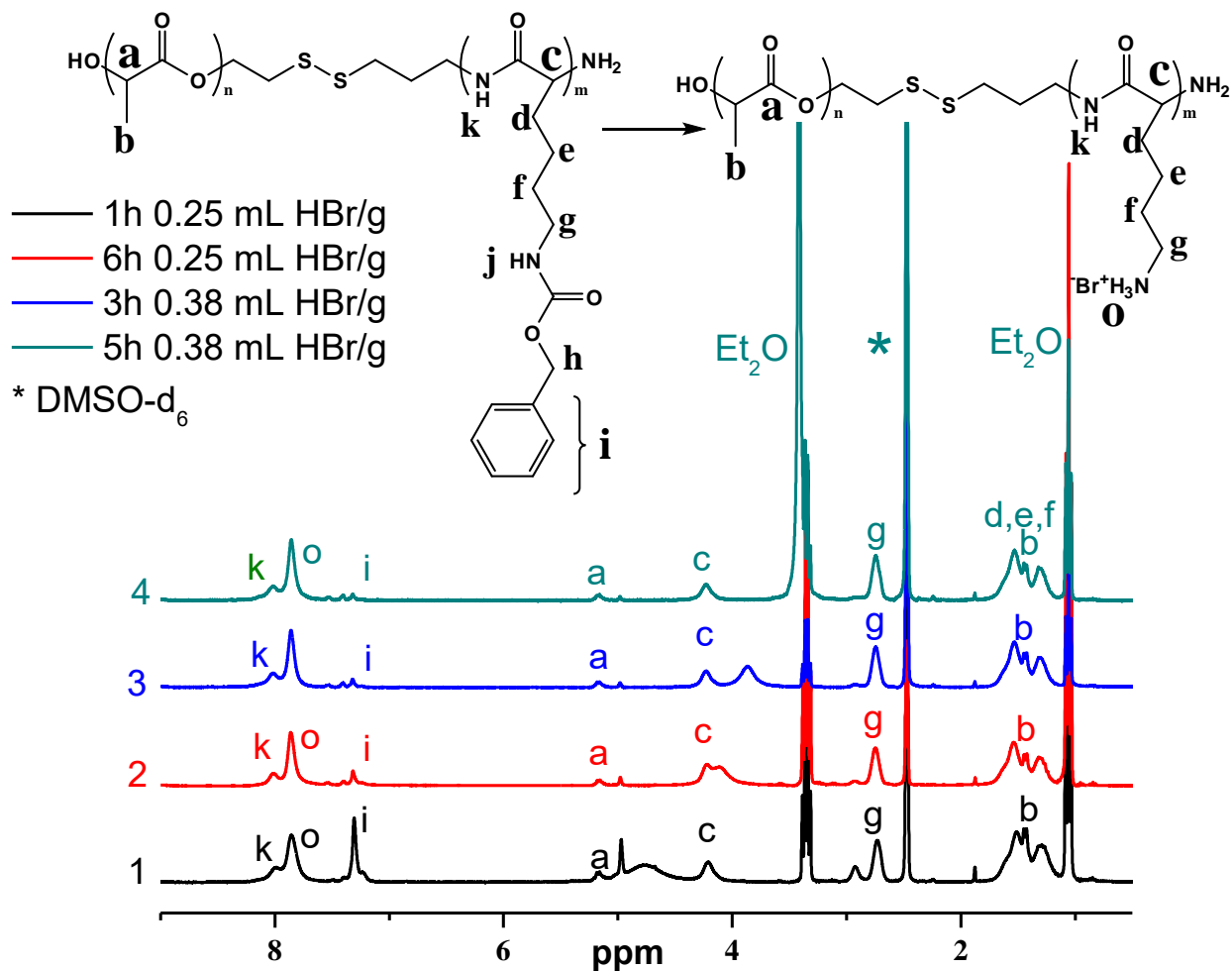
**Table 4-4.** Characterization results for the block copolymers before and after deprotection. The length of the PLA block (expressed as the number of lactic acid repeating units) after deprotection was calculated on the assumption that the polylysine block remained unaffected.

<b>Sample Name</b>	<b>Theoretical M<sub>n</sub> (g/mol)</b>	<b>M<sub>n</sub> [NMR] before (g/mol)</b>	<b>M<sub>n</sub> [NMR] after (g/mol)</b>	<b># LA units before</b>	<b># LA units after</b>
PLA <sub>17</sub> - <i>b</i> -PLy <sub>68</sub>	1.67×10 <sup>4</sup>	1.86×10 <sup>4</sup>	1.51×10 <sup>4</sup>	34	31
PLA <sub>17</sub> -SS-PLy <sub>34</sub>	9.71×10 <sup>3</sup>	1.15×10 <sup>4</sup>	8.41×10 <sup>3</sup>	34	16
PLA <sub>17</sub> -SS-PLy <sub>68</sub>	1.68×10 <sup>4</sup>	1.17×10 <sup>4</sup>	8.46×10 <sup>3</sup>	33	13
PLA <sub>17</sub> -SS-PLy <sub>136</sub>	3.10×10 <sup>4</sup>	1.87×10 <sup>4</sup>	1.33×10 <sup>4</sup>	34	8
PLA <sub>34</sub> -SS-PLy <sub>68</sub>	1.93×10 <sup>4</sup>	2.20×10 <sup>4</sup>	1.46×10 <sup>4</sup>	66	14
PLA <sub>34</sub> -SS-PLy <sub>136</sub>	3.34×10 <sup>4</sup>	2.30×10 <sup>4</sup>	1.62×10 <sup>4</sup>	66	23

To minimize degradation of the PLA block, attempts were made to optimize the reaction conditions in the Z-group deprotection step. This included changing the concentration of HBr, the reaction time as well as the reaction temperature. As can be seen in Figure 4-16, when four samples of PLA<sub>17</sub>-SS-PLy<sub>68</sub> were treated with different amounts of HBr solution for different times, the outcome of the reaction varied. By integrating the peaks at 7.15-7.55 ppm (peak i, five protons from the aromatic ring of the Z group) for comparison with peak c (methine proton on the lysine units) and peak a (methine proton on the lactic acid units), the extent of deprotection and PLA block degradation could be monitored. On Figure 4-16, spectrum 1 was obtained using 0.25 mL HBr/g polymer for 1 hour in the deprotection step. The integrals for peaks i and c indicate that 29% of the lysine units in the PLy block were still protected with the Z group. By integrating peaks a and c, it can be determined that after deprotection for one hour, the number of lactic acid repeating



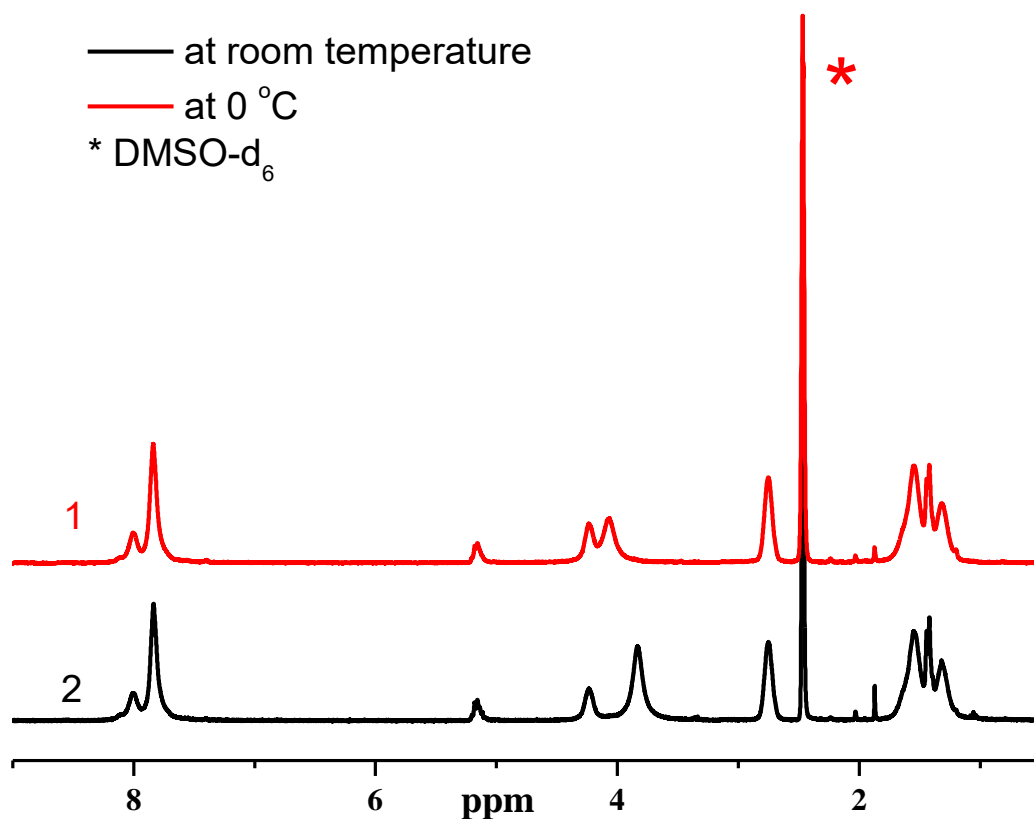
units decreased from 33 to 23. Spectrum 2 is for a sample obtained using the same amount of HBr solution, but a longer reaction time (6 hours). A small amount (13%) of Z groups still remained, yet an obvious signal decrease for peak a was observed, and integration showed that the sample lost 20 lactic acid repeating units after deprotection, leaving only 12 units on the chain. Even though the concentration of HBr in the solution was increased to 0.38 mL HBr/g for spectrum 3 and the reaction time was shortened, the peak for the Z group was still observed (10% from the integration result) after 3 hours of reaction, while 18 lactic acid units were lost during the process, leaving 14 units remain after deprotection. Finally, when the reaction time was increased to 5 hours under the same conditions (spectrum 4), deprotection was almost complete (less than 5% residual Z groups). Unfortunately, integration showed that the ratio between the number of lactic acid units and lysine units also decreased from 0.55:1.00 before deprotection, to 0.22: 1.00 after deprotection, meaning that only 13 lactic units remained on the polymer chain, or that the degradation of PLA worsened under these conditions.



**Figure 4-16.** <sup>1</sup>H NMR spectra (300 MHz, in DMSO-*d*<sub>6</sub>) for PLA<sub>17</sub>-SS-PLY<sub>68</sub> deprotected at room temperature with different amounts of HBr solution and for different reaction times.

The influence of temperature on the Z-group deprotection and PLA degradation reactions was also investigated by comparing <sup>1</sup>H NMR spectra for sample PLA<sub>17</sub>-*b*-PLY<sub>68</sub> before and after deprotection (Figure 4-17). After 4 hours of treatment with HBr at 0°C and room temperature, respectively, the integration of peaks a and c yielded decreased ratios between the PLA and PLY blocks for both samples, namely to 0.40:1.00 (spectrum 1, 0°C) and 0.41:1.00 (spectrum 2, room

temperature). This clearly demonstrates that temperature is not the main factor affecting the degradation of the PLA segment. Considering that degradation of the PLA block could not be avoided in any of the procedures investigated in this work, alternate approaches to be considered for the deprotection of the PLy segment in future investigations will be discussed in the following chapter.



**Figure 4-17.** <sup>1</sup>H NMR spectra (300 MHz, in DMSO-*d*<sub>6</sub>) for sample PLA<sub>17</sub>-*b*-PLyZ<sub>68</sub> deprotected at 0°C and at room temperature, using the same amount of HBr solution (0.3 mL/g polymer) for 4 hours. The ratio of lactic acid to lysine units was identical in both cases, showing that temperature had no significant influence on PLA degradation.

The deprotection of the Z group with HBr invariably induced the degradation of the PLA block in the PLA<sub>n</sub>-SS-PLyZ<sub>m</sub> samples to some extent. Some of the reaction parameters were varied, namely the concentration of HBr, the temperature and the reaction time, in an attempt to optimize the reaction. In spite of this, it was impossible to achieve complete deprotection of the Z groups without partial degradation of the PLA block. This represents the most significant setback in the current project. It should be pointed out that, while the deprotection of PLA-*b*-PLyZ copolymers with non-cleavable block junctions with HBr, under conditions similar to the ones used in this work, has been reported in the literature before,<sup>41</sup> no occurrence of PLA degradation was mentioned. The results obtained herein show that, while this information was either overlooked or omitted in the literature, degradation of the PLA block should, in all likelihood, also have occurred in these cases.

**CHAPTER 5**  
**CONCLUSIONS**

Techniques for the metal-free synthesis of PLA homopolymers, and of non-cleavable PLA-*b*-PLy and redox-cleavable PLA-SS-PLy block copolymers have been developed. While the synthesis of PLA-Ply block copolymers has been reported in the literature before,<sup>41</sup> the path reported herein for the synthesis of the redox-sensitive initiator *tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate is novel and simpler. It is also the first time that a combination of metal-free PLA synthesis and redox-sensitive block junction is used for the synthesis of these types of materials geared towards biomedical applications.

In all cases, the PLA samples had number-average molecular weights close to the target (theoretical) values  $M_n = 2500$  or  $5000$  g/mol, and narrow molecular weight distributions were obtained, as demonstrated by the low apparent polydispersity values (PDI = 1.09) obtained by SEC analysis. Deprotection of the BOC group at the end of the chains, introduced by the bifunctional initiator used in the reaction (*tert*-butyl (2-((2-hydroxyethyl)disulfanyl)ethyl) carbamate for the redox-sensitive copolymers, (3-(Boc-amino)-1-propanol for the non-cleavable copolymers), was cleanly achieved with TFA while leaving the molecular weight distribution of the PLA block unchanged (PDI = 1.09). The amine-terminated PLA samples were used as macroinitiators for the polymerization of Z-protected lysine N-carboxyanhydride, to grow a PLyZ segment and yield the corresponding block copolymers, both in their cleavable and non-cleavable forms. These copolymers also had low PDI values and molecular weights close to the target values, albeit significant amounts of residual macroinitiator were present in some cases (5-17%). It is interesting that this was the case for the non-cleavable block copolymers, while for all the cleavable block copolymers, which were the main target of this research project, the residual macromonomer content was negligible (0-3%). This points out that the protocol for the synthesis of the non-cleavable block copolymers may have weaknesses needing to be improved. Potential solutions to

that problem will be discussed in the next chapter. Unfortunately, degradation of the PLA block of the copolymers was observed in all cases during deprotection of the Z-group, when the block copolymers were treated with HBr. While this is a standard deprotection method for carboxybenzyl groups, this represents rather harsh conditions when it comes to avoiding the degradation of the acid-sensitive ester bonds present in the PLA block. Again in this case, alternate approaches to the deprotection of the PLy block, while minimizing or eliminating the degradation of the PLA segments, will be discussed in the next chapter.

In comparison to the molecular weights obtained by SEC analysis, the results derived from  $^1\text{H}$  NMR spectra were much closer to the target molecular weights. This was attributed to the fact that the SEC instrument used was calibrated with polystyrene standards. The apparent values obtained at least confirmed that the polymers synthesized had narrow molecular weight distributions (low PDI values) in most cases.

**CHAPTER 6**  
**SUGGESTIONS FOR FUTURE WORK**



Two main hurdles were encountered in this project, namely contamination of the non-cleavable block copolymers by unreacted macroinitiator, and degradation of the PLA chain segments during deprotection of the Z-lysine units in the block copolymers.

The presence of unreacted macroinitiator was confirmed by SEC analysis of the non-cleavable block copolymers, while it was negligible in the case of the cleavable systems. Since the synthetic protocol used for both systems was essentially identical, this suggests that the protocol for the non-cleavable copolymer syntheses requires further optimization. The contamination issue could be related to the purity of any of the components used in the reaction, namely the (3-(Boc-amino)-1-propanol initiator, the macromonomers, or the N-carboxyanhydride monomer. While the Boc-protected initiator was used as received from the supplier, it could be purified by vacuum distillation before use. Further purification of the PLA-NH<sub>2</sub> macromonomers could be attempted by dialysis before the chain extension reaction, and the Z-lysine NCA monomer could be purified by recrystallization.

The main challenge in this project appears to be avoiding the degradation of the PLA block in the copolymers. This is critical, since the PDI of the PLA block should have increased due to the occurrence of random chain cleavage in the presence of HBr (although this could not be confirmed by SEC analysis, due to insolubility of the deprotected products in THF). A higher PDI for the hydrophobic PLA block, in turn, may influence the self-assembly behavior of the copolymers in solution. There may be several potential solutions to this issue. For an example, rather than HBr, hydrogenolysis in the presence of palladium on carbon can also be used to deprotect the Z group. Another option could be to use a different protecting group for the amine functionality on lysine, such as Boc or trifluoroacetyl groups. The Boc group is easily cleaved by addition of TFA, as it was done in this project for the Boc-capped PLA macroinitiators. The trifluoroacetyl group is easily

displaced with potassium carbonate. In both cases, deprotection of the PLA segment should be avoided or minimized, given the much milder conditions required for deprotection.

Finally, besides the lysine N-carboxyanhydride derivative, other monomers could be considered such as ethylene oxide to introduce a different hydrophilic block. Once the synthetic work is completed, the focus of this project will change to the investigation on the self-assembly behavior, to determine which block copolymers are best suited as micellar drug delivery systems. Degradation of the block copolymers under reducing conditions will also need to be investigated in the presence of glutathione. If this approach works, release of the drug can be controlled without the use of external stimuli, which would greatly simplify the implementation of these systems as drug delivery vehicles.

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