Synthesis and Characterization of Poly(lactide) Functional Oligomers and Block Copolymers

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Abstract

Amphiphilic block copolymers consisting of poly(ethylene oxide) and poly(lactide) have great potential for formulating drug delivery systems. Our approach was to synthesize poly(ethylene oxide-*b*-D,L-lactide), (PEO-*b*-PDLLA), block copolymers with controlled molecular weights and good functionality on the poly(ethylene oxide) end for the design of potential core-shell delivery vehicles for HIV drugs. PEO-*b*-PDLLA block copolymer was used as a polymeric nanocarrier to encapsulate the HIV protease inhibitor, Ritonavir, within magnetite nanoparticles. Well-defined multifunctional polymeric nanoparticles with controlled sizes and size distributions were fabricated by rapid nanoprecipitation using blends of the PEO-*b*-PDLLA block copolymer with poly(L-lactide), (PLLA) homopolymer. Heterobifunctional PEO oligomers were directly prepared by initiating ethylene oxide with functional alcohols bearing vinylsilane, vinylether and maleimide moieties to provide appropriate end groups for conjugating targeting ligands. The polyethers with narrow molecular weight distributions were utilized as macroinitiators for the synthesis of poly(lactide) block. Heterobifunctional diblock copolymers possessing carboxylic acids were prepared from ene-thiol addition reaction of mercaptoacetic acid across the vinyl group on the PEO end, while preserving the hydroxyl functionality on the other end. Additionally, PDLLAs bearing maleimide functionality with controlled molecular weights were synthesized using maleimide functional initiator. End group modification was performed via

Michael addition using cysteamine hydrochloride to introduce an amino group over the vinyl bond. The resulting carboxylic acid functional PEO-*b*-PDLLA diblock copolymers, and amino functional PDLLAs are potential biocompatible polymers that can be utilized to encapsulate an array of bioactive molecules, targeting ligands.

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This thesis is dedicated to my family for their heartfelt support and in loving memory of my grandfather, Ali Kayandan.

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CHAPTER 1 : Introduction

The design of multifunctional polymeric nanoparticles is essential for delivery of therapeutics and for simultaneously monitoring their biodistribution *in vivo*. Among the different molecular architectures, amphiphilic block copolymers are commonly used for designing nanoparticles. Poly(ethylene oxide-*b*-lactide) (PEO-*b*-PDLLA) amphiphilic copolymers are attractive candidates for use in controlled delivery of anticancer drugs. PEO is a nondegradable hydrophilic polymer with very low toxicity and good biocompatibility, while PDLLA is a biodegradable, biocompatible polymer with well-documented safety. The PEO in PEO-*b*-PDLLA copolymers provides steric stability and can possess targeting ligands on its end, and hydrophobic drugs can be incorporated into the cores of micellar PEO-*b*-PDLLA assemblies. The second chapter reviews the synthetic methods of poly(lactide)s and poly(ethylene oxide)s, and their block copolymer nanoparticles in pharmaceutical preparations.

The third chapter describes the synthesis and characterization of multifunctional polylactide nanoparticles that can integrate magnetite and therapeutic agents for magnetic resonance imaging and antiretroviral therapy (in collaboration with Sharavanan Balasubramaniam, Dr. Richey M. Davis). PEO-*b*-PDLLA diblock copolymer nanocarriers encapsulating magnetite and the drug were fabricated by rapid nanoprecipitation. Additionally, poly(L-lactide) homopolymer was incorporated into the hydrophobic core to serve as a nucleating agent in the nanoparticles without the magnetite.

The fourth chapter describes the synthesis and characterization of heterobifunctional poly(ethylene oxide) oligomers, poly(ethylene oxide-*b*-lactide) block copolymers and poly(lactide) oligomers having hydroxyl group at one end and different moieties at the other chain end utilizing functional alcohol initiators. It is highly desirable to synthesize polymers

with terminal functionality, which allows the post-polymerization modifications. The heterobifunctional polyether-polyester block copolymers functionalized on the polyether end were further modified via ene-thiol addition for targeting ligand conjugation. The fifth chapter covers the conclusions drawn from the previous chapters and proposed future work of this research.

CHAPTER 2 : Literature review

2.1. Overview

This literature review will discuss areas directly related to the research topic and is mainly divided into four sections. The first section will give an overview of poly(lactide)s describing synthetic methods, properties, and applications. The second section will present synthesis, properties and functionalization of poly(ethylene oxide)s. The copolymerization of poly(lactide) and poly(ethylene oxide) and the application of their block copolymer nanoparticles in pharmaceutical preparations will be explored in the next section.

2.2. Synthesis and Properties of Poly(lactide)s

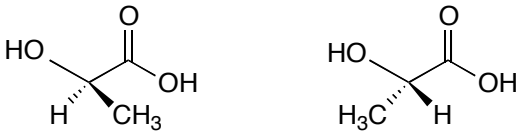
2.2.1. Introduction

Polyesters have raised increasing interest for use in biomedical applications due to their biodegradability and relative ease of synthesis.[1] Among the leading polyesters, poly(lactide) (PLA), poly(glycolide) and their copolymers have proven to be the most attractive class of synthetic aliphatic biodegradable polyesters.[2] PLA has been approved by the US Food and Drug Administration for several notable clinical uses. Due to its biocompatibility, low immunogenicity, biodegradability into non-toxic products and good mechanical properties, PLA has gained widespread application in pharmaceutical and biomedical applications as drug carriers, sutures, implants and tissue engineering scaffolds.[3-7]

2.2.2. Raw Material

Lactic acid (2-hydroxypropanoic acid), the basic building unit of PLA, is a simple 2-hydroxycarboxylic acid with an asymmetric carbon atom and exists in two optically active forms, D- and L- isomers (Figure 2.1). Lactic acid can be manufactured either biologically or chemically. While both D- and L- isomers are produced in bacterial systems, the L- isomer is

produced in humans and other mammals. The chemical process for preparing lactic acid is based on hydrolysis of lactonitrile by strong acids and yields various ratios of D- and L-isomers. However, the lactic acid obtained by fermentation is mostly stereoregular L-lactic acid. Due to the use of renewable resources instead of petrochemicals, there is more interest in the fermentative production of lactic acid. [8-11]



D-Lactic Acid L-lactic Acid

Figure 2.1 Isomeric Forms of Lactic Acid

The cyclic dimer of lactic acid is called lactide (3,6-dimethyl-1,4-dioxane-2,5-dione). The chiral nature of lactic acid results in three distinct forms of lactide, D-lactide, L-lactide and Meso-lactide (Figure 2.2). The polymerization of L-lactide produces poly(L-lactide) PLLA, whereas D-lactide produces poly(D-lactide), PDLA. Both D- and L- lactide isomers are optically active. In contrast, the racemic mixture of D- and L-lactide gives poly(D,L-lactide), PDLLA which is optically inactive. PLLA and PDLA are semi-crystalline polymers with stereoregularity of the polymer chain; PDLLA is amorphous because of its irregular structure. Since the properties of PLA are exclusively dependent on the stereochemical configurations of lactide monomers, the characteristics of PLA can be controlled with polymer stereochemistry.[8, 12] Reeve *et al.* reported that the degree of crystallinity of PLA dramatically decreased as L content decreased in the range from 100% to 92%. It was found that the degree of crystallinity of PLA with 85% L content was amorphous.[13]

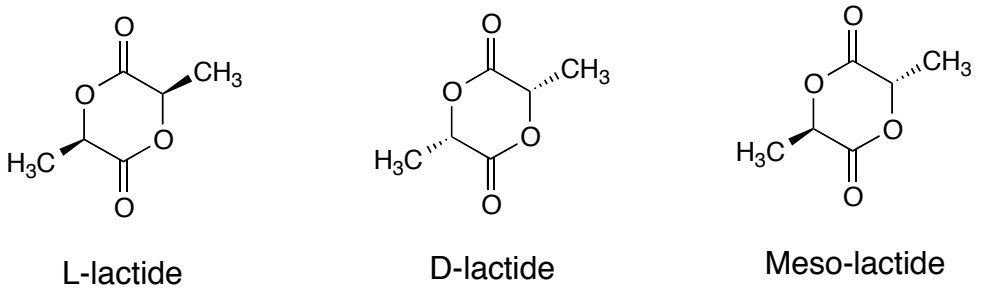


Figure 2.2 Three different isomers of lactides

2.2.3. Polymerization Methods

There are two main routes for the synthesis of PLA: (1) Polycondensation (condensation polymerization) of lactic acids and (2) ring opening polymerization of lactides. Polymers produced from lactic acid by polycondensation are sometimes referred to as poly(lactic acid) whereas the polymers manufactured by ring opening polymerization are referred to as poly(lactide)s. [2]

2.2.3.1. Polycondensation

PLA can be synthesized via direct polycondensation of lactic acid. An aqueous solution of lactic acid can be concentrated, then cyclization and polycondensation are initiated with removal of water. After addition of a transesterification catalyst, the reaction proceeds at elevated temperature without isolation of reaction intermediates.[14] Even though it is a simple and inexpensive method, there have several limitations. It is difficult to control the molecular weight, molecular weight distribution, and end groups. The polymer obtained by polycondensation has a low molecular weight. Since one molecule of water is produced at each step of polymerization, the polymer chain is degraded and yields low molecular weight PLA.[15, 16] Yamaguchi *et al.* synthesized high molecular weight PLA using molecular sieves to remove water by direct polycondensation. However, long reaction times and high

temperatures were required. Therefore, the polycondensation method for PLA synthesis is applicable if low molecular weight PLA is desired.[17]

2.2.3.2. Ring Opening Polymerization

Although direct polycondensation of lactic acid is a simple and inexpensive method for PLA polymerization, ring-opening polymerization of a lactide is favored commercially. The ring-opening polymerization of lactide was first demonstrated by Carothers in 1932. However, only low molecular weight polymer was obtained until the purification techniques of lactide were improved by DuPont in 1954.[18] Due to the ease of control of chemistry and properties, the ring-opening polymerization of lactide was studied by many researchers over the past decades.

The thermodynamic driving force for the ring-opening polymerization is ring strain (ΔH = -22.1 kJ/mol f), which is an important parameter for the cyclic ester monomers.[19] Ring strain increases with ring size from five to seven for the lactones. [20] The ring-opening polymerization of lactides can be classified by four different reaction mechanisms: (1) anionic polymerization, (2) cationic polymerization, (3) coordination-insertion mechanisms and (4) enzymatic polymerization. The first three mechanisms will be discussed here.

2.2.3.2.1. Anionic Ring Opening Polymerization

The anionic ring opening polymerization is started when the nucleophilic anion of an initiator attacks the carbonyl group of the lactide and breaks the bond between carbonyl carbon and endocyclic oxygen. This step produces a new anion, so chain growth continues with alkoxide reactive species (Figure 2.3).[21]

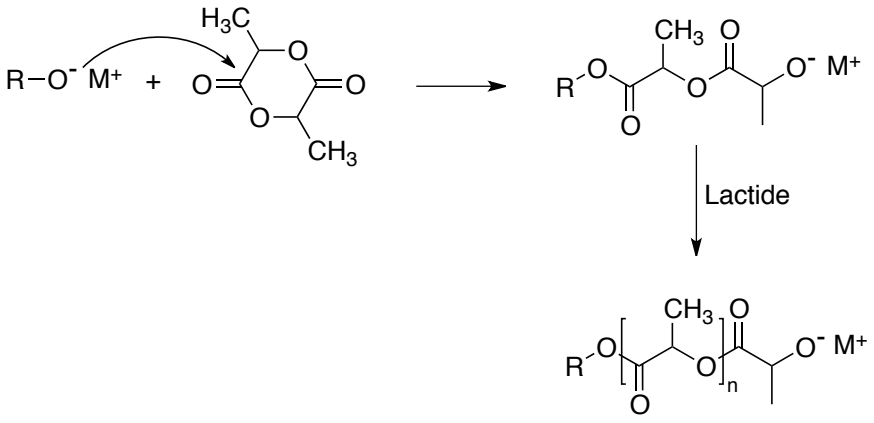


Figure 2.3 Anionic Ring-Opening Polymerization

Kricheldorf *et al*. studied different anionic initiators for lactide polymerizations. They reported that strong nucleophilic initiators are required for lactide initiation. Weak bases such as potassium phenoxide, zinc stearate, and potassium benzoate do not initiate at low temperatures, but they can initiate at high temperatures. Stronger nucleophilic initiators can deprotonate the monomer, and result in racemization. At high temperatures in bulk back biting as well as racemization can occur and these may prevent chain propagation (Figure 2.4).[2, 22, 23] Thus, it is difficult to produce high molecular weight. In contrast, Jedlinski *et al*. studied the anionic ring-opening polymerization of lactides in the presence of potassium methoxide. They showed that when the reaction was initiated with potassium methoxide in THF at 20 °C, they were able to obtain high yield with well-defined polymers. Only 5% of racemization was observed.[24, 25] When the results at different temperatures were considered, it was observed that high reaction temperatures were an important factor promoting side reactions of polymerization of lactides.

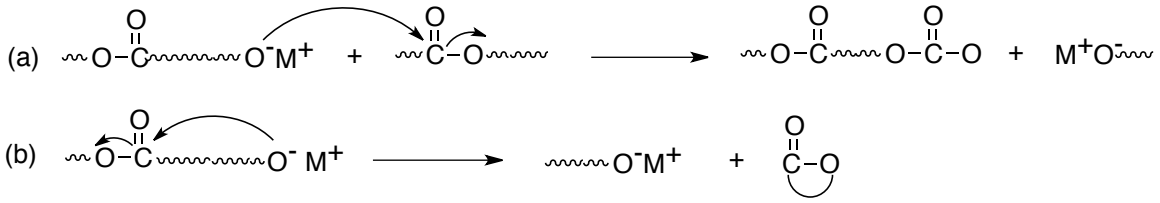


Figure 2.4 Back-biting reactions (a) Intermolecular, (b) Intramolecular

2.2.3.2.2. Cationic Ring Opening Polymerization

Cationic ring-opening polymerization of lactides can be carried by using carbenium ions, and strong acids such as boron trifluoride or trifluoroacetic acid. The initiation step starts when the carbonyl oxygen atom (exocyclic oxygen) of the lactide is alkylated or protonated by the initiator. This electrophilically activates the O-CH bond, which becomes positively charged. Nucleophilic attack of another monomer cleaves this bond and creates another electrophilic carbenium ion (Figure 2.5). This propagation process continues with additional monomers until the polymerization is terminated with monofunctional nucleophile.[26, 27] However, racemization occurs at high temperatures in cationic polymerizations since there is a nucleophilic substitution at the chiral carbon. Kricheldorf *et al*. reported that optically pure poly(L-lactide) can be produced at low temperatures, ≤ 50 °C. However, even though racemization is minimized at low temperatures, the rate of reaction is very slow and the process yields low molecular weight polymers.[27]

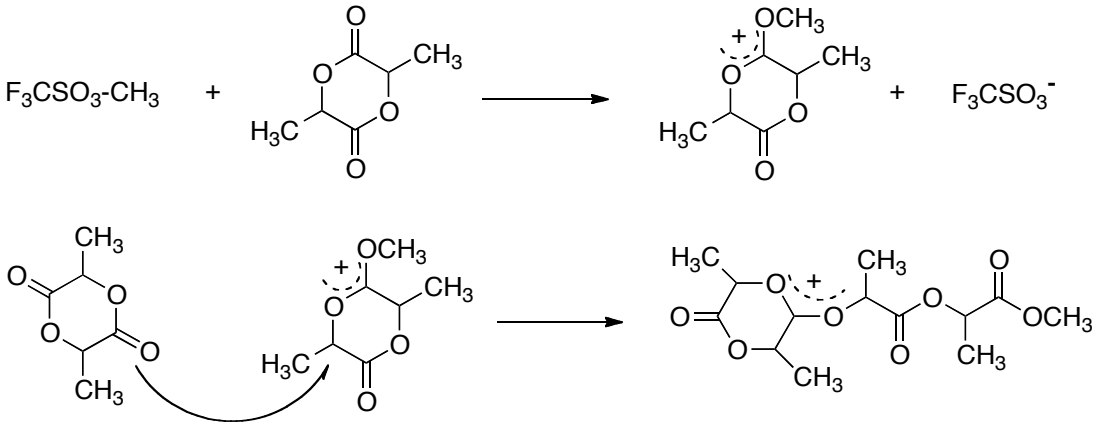


Figure 2.5 Cationic Ring-Opening Polymerization

2.2.3.2.3. Coordination-Insertion Ring-Opening Polymerization

The coordination-insertion mechanism is the most commonly used method for the synthesis of PLA. Metal alkoxide catalysts are usually used for this route. The alkoxides of Mg, Zn, Sn, Al, Ti, Zr which contain free p or d orbitals, have a covalent metal-oxygen bond and function as weak Lewis acids.[28-30] In literature, there are different proposed coordination-insertion mechanisms. The main method is to use an alcohol initiator with a coordination catalyst and the molecular weight of the polymer chain can be controlled based on the monomer to alcohol initiator ratio (Figure 2.8). Another proposed coordination-insertion mechanism involves the reaction of a monomer and a catalyst. This coordination-insertion mechanism starts when one of the exocylic oxygens of lactide coordinates with the metal atom of the initiator.[31-33] Since the nucleophilicity of the alkoxide part of the initiator and electrophilicity of the CO- group of lactide increase, the insertion of the lactone into the metal O-bond can occur.[26] In the next step, the bond between the carbonyl group and endocyclic oxygen of the lactide is broken and the lactide chain is inserted into the metal-oxygen bond of the initiator. The lactide monomers continuously are opened and inserted into the bond between the metal atom and its adjacent oxygen atom until the alkoxide end of the initiator becomes a dead chain end (Figure 2.6).[2, 26] The non-reacting chain end group can be

differed by reacting the initiator with alcohol or phenol, which can become an ester end group. Hence, bioactive molecules such as drugs, hormones, and vitamins having at least one hydroxyl group can be initiators for the polymer chain. [26, 34]

By varying the monomer to initiator ratio, the coordination-insertion mechanism allows the control of molecular weights over a broad range. Due to the lower possibility of side reactions compared to ionic initiators, higher molecular weights can be obtained.[26] Nijenheus *et al*. reported that they synthesized polylactides having number average molecular weights above 200,000 Da.[35, 36] Because of the covalent nature of the initiators; there is a lower probability of racemization at high temperatures. Therefore, the coordination-insertion mechanism is preferable for synthesis of high molecular weight polylactides.

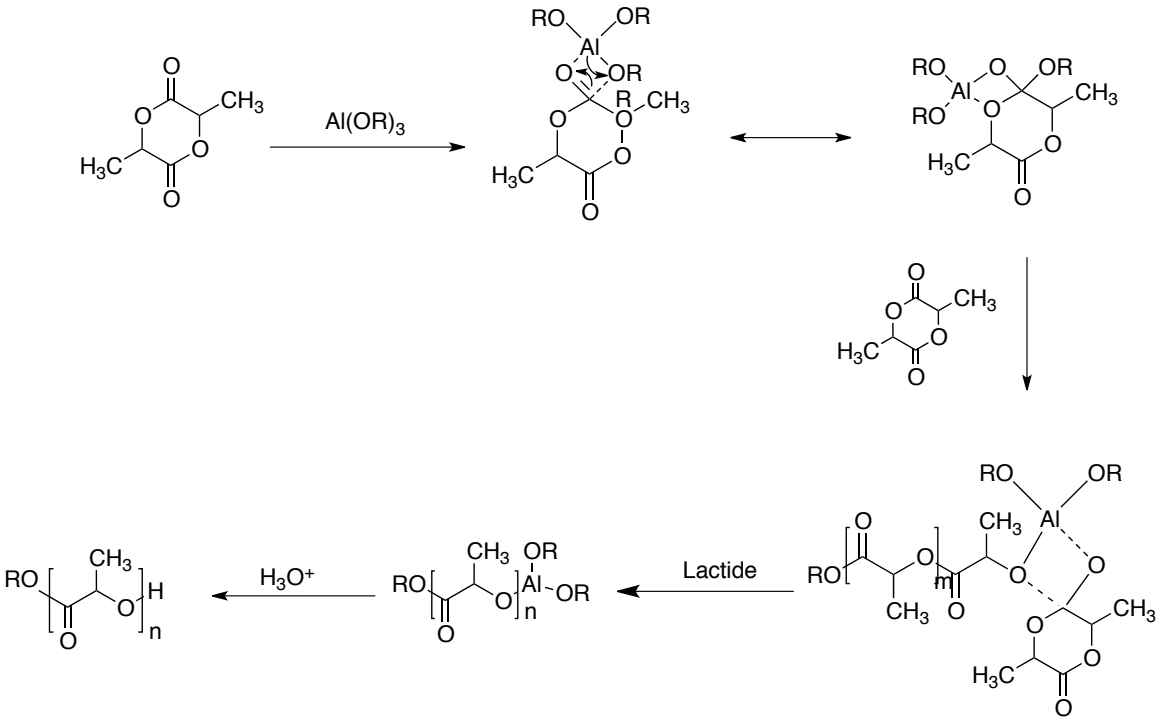


Figure 2.6 Coordination-insertion Ring-Opening Polymerization

Among the large number of catalysts, which have been studied for the polymerization of lactide, aluminum and tin alkoxides are primarily used. Both can produce controllable

molecular weights with narrow distributions.[37, 38] However, tin catalysts are better transesterification catalysts rather than aluminum alkoxides. Additionally, they are more hydrolytically stable than aluminum, so they are easier to handle.[37, 39]

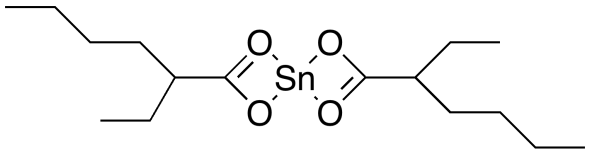


Figure 2.7 Structure of tin (II) 2-ethylhexanoate ($Sn(Oct)_2$)

Tin complexes as catalysts, in combination with alcohol initiators, for the ring-opening polymerization of lactides and lactones have been studied since the 1960's.[40] The most widely used catalyst for the production of well-defined aliphatic polyesters of controllable molecular weights and minimum racemization is tin (II) 2-ethylhexanoate, $Sn(Oct)_2$ (Figure 2.7).[2] Due to its high solubility in organic solvents, stability on storage and high catalytic activity, $Sn(Oct)_2$ is commonly used. Furthermore, $Sn(Oct)_2$ has a much lower toxicity than heavy metal salts, and it has been approved by the FDA for several biomaterial processes.[9] Since this catalyst has gained attention for the synthesis of polyesters for food packaging and medical applications, purification of the polymers is of great importance.

There are several studies concerning the mechanism of $Sn(Oct)_2$-catalyzed ring-opening polymerization, but this is still not fully understood. Nijenhuis *et al*. first reported that cationic polymerization played a role in the lewis acid catalyzed transesterification reaction between an activated lactone and a hydroxyl group.[35] Nevertheless, these authors did not demonstrate detailed evidence of the mechanism for the polymerization of lactide. Since then, two mechanisms have been reported, coordination-insertion and an activated monomer mechanism. Even though the reaction mechanism remains controversial, it is generally accepted that there is a co-initiation of $Sn(Oct)_2$ with a hydroxyl group (Figure 2.8).[41-44] In the

activated monomer mechanism, a lewis acid complex is formed with coordination of the tin atom to the lactide monomer. It is followed by nucleophilic attack of a hydroxyl-containing group, so the carbonyl-acyl bond of the activated monomer is cleaved. [45]

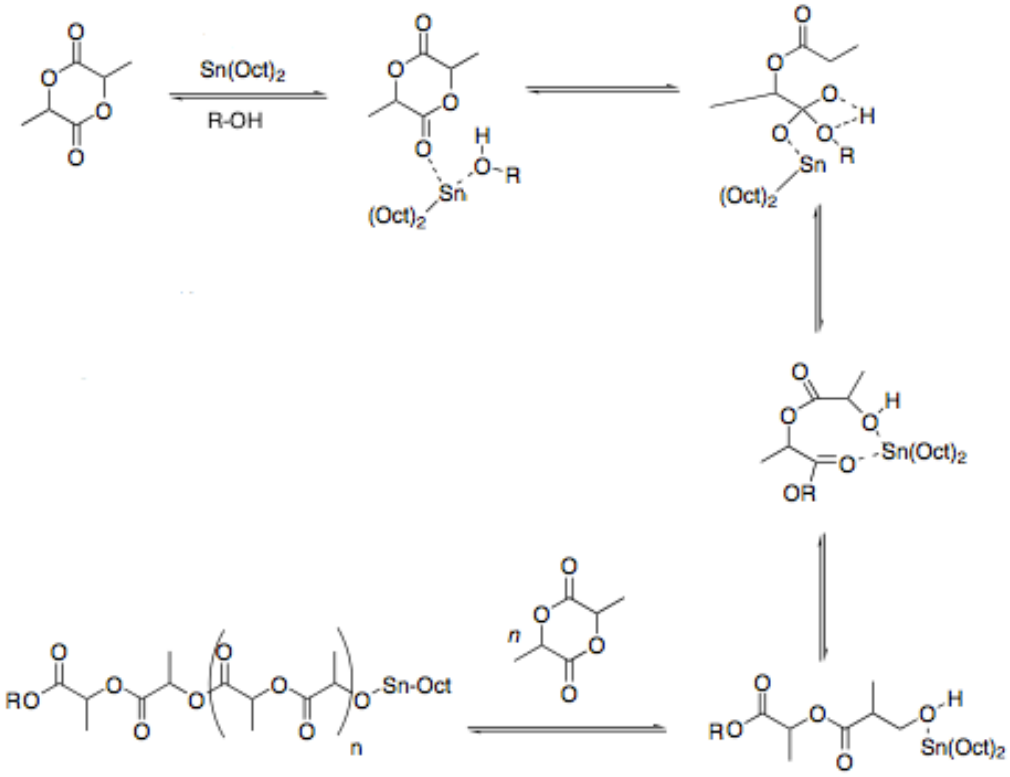


Figure 2.8 Coordination-insertion mechanism of lactide

Kricheldorf and Kowalski postulated that the reaction pathway was based on a coordination-insertion mechanism rather than a cationic or activated-monomer mechanism.[46, 47] It was proposed that $Sn(Oct)_2$ reacts with a hydroxyl group and tin alkoxide is formed as the initiating species. Ring opening begins with formation of the tin-alkoxide complex.[47, 48] This mechanism was demonstrated by $Sn(Oct)_2$ catalyzing the ring opening polymerization of ε-caprolactone in the presence of water or alcohol as a co-initiator.[49] By studying MALDI-

TOF mass spectrometry, it was observed that macromolecules existed with tin atoms in the chains such as $Bu[O(O)C(CH_2)_5]_nOSnOct$ and $[O(O)C(CH_2)_5]_nOSn$ cyclics. This suggested that the polymerization continued with an "active chain end" mechanism, since Sn-alkoxide bonds were present at the chain ends. Hence, the authors proposed that $Sn(Oct)_2$ and other metal carboxylates need to be converted to an alkoxide before the initiation step of the reaction.[49]

At high reaction temperatures (> 180 °C) in the presence of large amounts of catalyst, it was found that transesterification reactions occurred during the polymerization of lactide.[50, 51] High catalyst concentration led to an increase in the number of sites for ester interchange reactions whereas high polymerization temperatures caused alkyl-oxygen bond breakage and racemization. There are two sites where ester bond cleavage can occur on the polymer chain (Figure 2.9). [52]

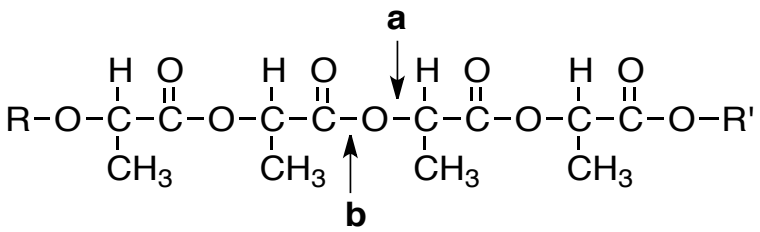


Figure 2.9 Ester bond breakage through transesterification

a) Racemization occurs and b) No racemization occurs

There are two types of transesterification reactions, which broaden the molecular weight distribution (Figure 2.10). Intermolecular transesterification reactions cause rearrangement of chain sequences. Intramolecular transesterification reactions result in back-biting with rearrangement. [53]

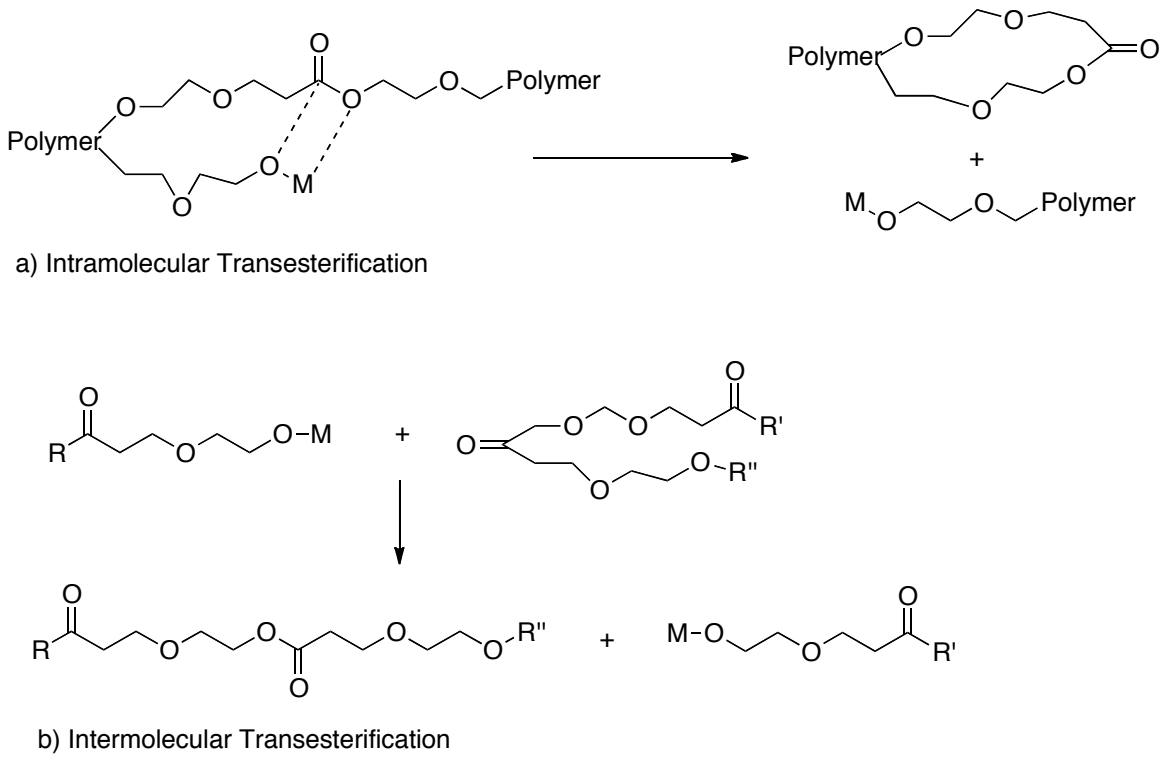


Figure 2.10 Transesterification Reactions

With different metals, the reactivity of the initiator changes. It can be more or less reactive toward side reactions, i.e transesterification reactions. Reactivities of the various metal alkoxides have been reported as $Bu_2Sn(OR)_2$>Bu_3SnOR>$Ti(OR)_4$>$Zn(OR)_2$>$Al(OR)_3$.[54] Furthermore, the configuration of lactide has an impact on the possibility of the transesterification reactions. It was shown that D,L-lactide had relatively higher probability to undergo transesterification reactions. [55]

2.2.4. Properties of Poly(lactide)s

The properties of poly(lactide)s vary due to the stereochemistry of pendent methyl groups on the alpha carbon atoms. Two asymmetric carbons of lactide monomer yield optically active L-, D- and the racemic D,L isomers. The wide range of physical, thermal, and

mechanical properties and degradation rates of PLA and its copolymers are dependent on isomer composition, molecular weight and molecular weight distribution. By controlling the stereochemical nature of the lactide monomer, PLA with different chemical and physical properties can be obtained that are suitable for targeted applications.

Poly(L-lactide), PLLA, is comprised of the most common isomer derived from nature which is L-lactide. An optically pure PLLA has crystallinity of approximately 37%. PLLA and PDLA are semi-crystalline materials due to the stereoregular chain structures. Both of them have glass transition temperatures between 55-65 °C and equilibrium melting points in the range of 175-185°C. The melting points depend on the molecular weight and the size of the crystallites.[56] PLLA is a relatively hard material, so when the brittleness of PLLA is decreased, it can be used as a tough engineering material for applications such as internal fixation of bone structures and orthopedics. [26] In contrast poly(D,L-lactide), PDLLA, is an amorphous polymer which has a glass transition temperature in the range of 50-60 °C. The semi-crystalline versus amorphous morphologies of PLLA versus PDLLA impact their practical applications. PDLLA is used in applications such as drug delivery systems. Since the glass transition temperature of PDLLA is above body temperature, the polymer matrix can be stiff with low elasticity in the body and relatively brittle at room temperature. [57] Therefore, both the glass transition temperature and melting point are important to determine the use temperatures for desired applications. Amorphous PLA is a viscous fluid above the glass transition temperature. In contrast, PLA acts as a glass below the glass transition temperature until cooled down to its β transition temperature, around -45 °C, while it becomes brittle under this temperature. [44]

The reported glass transition temperatures of PLA in the literature vary because of the effect of different molecular weights, physical aging, molecular architecture, and the degree

of crystallinity or plasticizers. Baker *et al.* studied the relationship between the glass transition temperature of PLA and the polymer structure. [58]

a) Sample History: There can be several variations in the measurement of the glass transition temperature because of the sample history. During isolation or purification processes of the polymer, the morphology of the material can be changed. The polymer can undergo thermo- or solvent-induced changes. Therefore, the same polymer sample can exhibit different thermal behavior. [58]

b) Tacticity: It is well established that the tacticity of the polymer chains has an impact on the chain conformations and so the glass transition temperature of the polymer can be affected. For example, the glass transition temperature of syndiotactic poly(methyl methacrylate) (PMMA) is 160 °C whereas isotactic PMMA has a glass transition temperature at 43 °C.[59] As noted earlier, lactide monomer has three different isomeric forms because of the chiral structure of lactyl unit. The polymerization of either optically active pure D- or L-lactide yields isotactic PLA that has stereogenic carbons with the same absolute configuration.

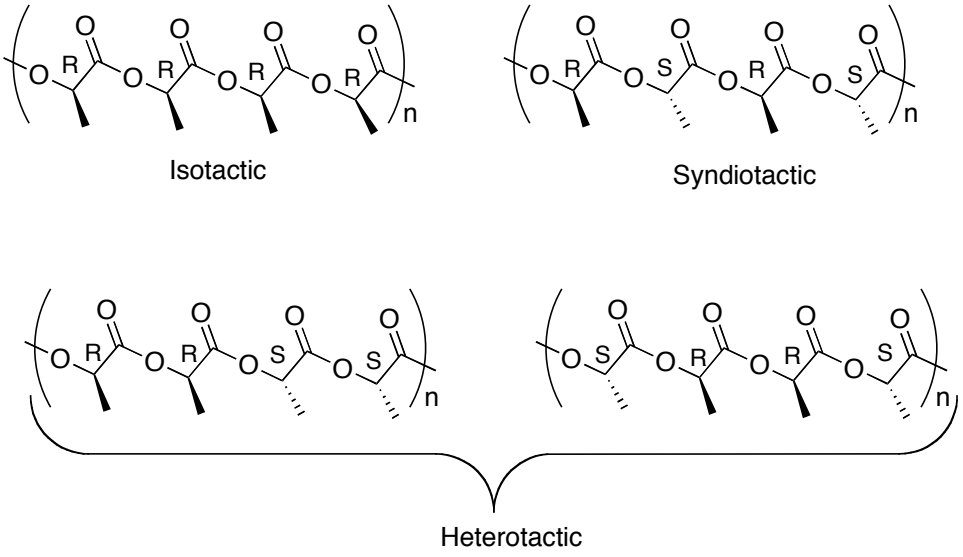


Figure 2.11 Different microstructures of PLA

On the other hand, PLA having alternating configurations of the sequential stereocenters is syndiotactic while the random configurations of stereocenters is atactic (Figure 2.11).[60] It was demonstrated that the tacticity of PLA can be determined by ^{1}H NMR by focusing on the methine hydrogen.[61] Coates *et al*. demonstrated polymerization of syndiotactic PLA using stereoselective catalysts.[62] The glass transition temperature of syndiotactic PLA was reported as 34 °C while it was 49 °C for heterotactic PLA.[62, 63]

c) Molecular weight: The Flory-Fox equation relates the glass transition temperature, T_g, and the number average molecular weight, M_n of the polymer. (Equation 2.1)

$$T_g = T_{g,\infty} - \frac{K}{M_n} \qquad \text{Equation 2.1}$$

$T_{g,\infty}$ is the glass transition temperature at infinite molecular weight and K is an empirical constant related to the free volume in the polymer.[64] In the literature, predictions by the Flory-Fox equation have been confirmed for many polymers. The glass transition temperatures of low molecular weight PLA samples have also confirmed the theoretical prediction of Flory-Fox. Steendam *et al*. reported the glass transition temperatures of dry and hydrated PDLLA samples with different molecular weights (Figure 2.12). The glass transition temperature of PDLLA increased from 35.7 °C (M_v 12.5x10^3) to 50.2 °C (M_v 69x10^3). In the lower M_v region, the glass transition temperature of PLA rapidly increased, while it was observed that there was a slight change in the glass transition temperature resulting in a plateau value in the higher M_v region around 50 °C. [65]

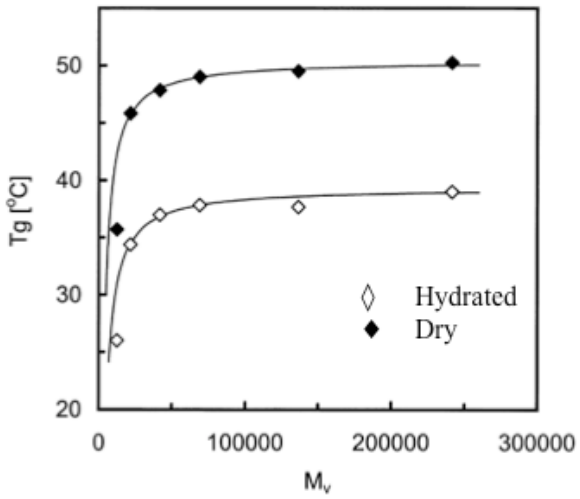


Figure 2.12 Effect of molecular weight of PDLLA on the glass transition temperature

d) Crystallinity: Since the crystalline part limits mobility of the chains in the amorphous phase, the glass transition temperature of the polymer increases with an increase in crystallinity. Uryama examined the thermal properties of PLA with different L- and D,L- unit sequences. The polymers prepared by copolymerization of L- with D,L-lactide monomers, had 75-100% L-content. By increasing the D,L- units, the crystallinity decreased as expected and it was observed that the polymers with less than 85% L-units were amorphous. PLA chains with higher L-unit contents had a helical structure causing higher glass transition temperature and lower density. [66]

e) Polymer architecture: The different polymer architectures of PLAs including block copolymers, random copolymers, combs, and stars can be synthesized to tailor their properties. As expected, the polymer architecture has an impact on the glass transition temperature of PLA. By using different multifunctional initiators such as dextran, cellulose acetate, or polyvinyl alcohol, the comb-like PLAs have been synthesized. The glass transition temperatures of these polymers, which had similar molecular weights, ranged between 20 and 60 °C. [58, 67-69]

f) Plasticizers: Plasticizers are additives, which can provide flexibility, improve the resilience of the polymers and lower the glass transition temperature. Plasticizers can be monomers, low molecular weight species or residual solvent from the processing or synthesis of polymers. [58] In order to make PLA as a flexible polymer, a wide range of plasticizers has been studied. For example, poly(ethylene glycol), poly(3-methyl-1,4-dioxan-2-one) and fatty acid esters have been used to improve the flexibility of PLAs. [70, 71] The efficiency of plasticizers has been investigated by evaluating decreases in the glass transition temperature. Martin reported that the glass transition temperature of PLA decreased from 58 to 12°C with 20% of poly(ethylene glycol) with M_n of 1,500 g mol^{-1}.[71] It was also proposed that water can act as a plasticizer. Blasi examined the relationship of thermal behavior and water content of polylactide-*co*-polyglycolide and showed that 5% water depressed the glass transition temperature by 15 °C.[72]

The mechanical properties of PLA are highly dependent on the molecular weight. PLA can be varied from a soft, elastic plastic to a stiff, high strength plastic. [2] As the molecular weight of PDLLA was increased from 47.5 to 114k, the tensile and flexural strength increased from 49-53 to 84-88 MPa. [73] The mechanical properties of PLA showed higher tensile strength and modulus compared to other polyesters, but it was still a brittle material. Since the toughness is poor with less than 10% elongation at break, the applications of PLA are limited.[74] In order to improve its toughness and broaden its applications, PLA has been modified by copolymerization, and blending with other polymers and plasticizers.[16] For example, polymer blending has been widely used to modify PLA-based materials to enhance their properties. A wide range of materials such as plasticizers, olefin polymers/fiber, inorganic materials, natural polymers, and biodegradable polyesters have been used to blend with PLA. Broz *et al*. reported the structure and mechanical properties of poly(D,L-

lactide)/poly(e-caprolactone) blends by varying mass fraction across the range of compositions. It was observed that the mechanical properties of the blends are highly dependent on the composition. The modulus and ultimate tensile strength increased almost linearly as a function of PLA composition above a threshold PLA mass fraction of 0.4.[75]

Another method to enhance the mechanical properties as well as the thermal resistance and the resistance to the hydrolysis of PLA is stereocomplex formation between enantiomeric PLA. This occurs when PLLA and PDLA are mixed in equimolar concentrations and co-crystallize into a new arrangement due to the strong interaction between L-lactyl and D-lactyl unit sequences that result in more dense chain packing.[16, 44] Ikada *et al.* reported that stereocomplexes of PLA had different properties than PDLA and PLLA. The melting temperature of the stereocomplex was 40-50 °C higher than the melting temperature of either PDLA or PLLA.[76] Mechanical properties including tensile strength, Young's modulus and elongation at break were enhanced by stereocomplex formation of enantiomeric PLA of either low or high molecular weight.[77] Furthermore, the activation energy for thermal degradation of PLA stereocomplexes were reported as 82-110 kJ/mol which is higher than both PLLA and PDLA.[78] Stereocomplex formation of PLA can be traced by methods such as differential scanning calorimetry, wide-angle and small angle X-ray scattering, infrared spectroscopy, and ^{1}H and ^{13}C NMR spectroscopy. [12]

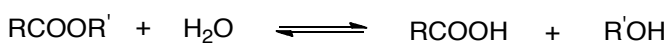
Polymer solubility in commonly used organic solvents is an important factor for the fabrication and processing of polymers. The solubility of PLA depends on the molecular weight, the degree of crystallinity, and any co-monomers.[2] In general, PLA is soluble in chlorinated or fluorinated organic solvents including dichloromethane, chloroform and 1,1,2-trichloroethane, acetonitrile, dioxane, and ethyl acetate. PLA is sparingly soluble in toluene, tetrahydrofuran, and ethyl benzene at room temperature, but is more soluble at higher

temperatures. Non-solvents for PLA are water and some alcohols such as methanol and ethanol, and in hydrocarbons such as hexane or heptane. [11, 21]

2.2.5. Degradation of PLA

Polymer degradation occurs through scission of the main chains or side chains of polymers. Factors such as thermal activation, hydrolysis, biological activity, oxidation or photolysis can cause polymer degradation.[79] Different mechanisms, chemical or biological, can be involved in degradation of biodegradable polyesters. The biodegradation of PLA has been shown to depend on parameters including: (1) first-order structure (chemical structure, molecular weight and molecular weight distribution), (2) higher order structure (glass transition temperature, melting temperature, crystallinity), and (3) surface conditions (surface area, hydrophobicity, hydrophilicity, purity).[80, 81]

PLA degradation depends on the environment to which it is exposed. The biodegradation of PLA mainly occurs hydrolytically in human or animals and soluble oligomers are metabolized by cells.[81] When PLA is exposed to water, water molecules penetrate into the polymer matrix and lead to hydrolysis of ester groups. Thus, long polymer chains are degraded into shorter ones, including low molecular weight water-soluble oligomers.[2, 82] The ester hydrolysis reaction can be shown as follows:



This hydrolytic reaction can be catalyzed by both acids and bases. The degradation can be autocatalytic due to the reaction product, RCOOH, which promotes further ester hydrolysis. [83] Other factors that can accelerate the degradation are incorporation of more hydrophilic monomers, more hydrophilic, acidic end groups, more hydrolytically reactive group in the backbone and less crystallinity.[82] Pitt *et al*. examined *in vivo* degradation of films of different

polyesters including PLA. They demonstrated that the first stage of degradation was limited to a molecular weight decrease due to random hydrolytic ester cleavage promoted by carboxyl end groups, and the second stage was characterized by the onset of weight loss and a decrease in the rate of chain scission.[84]

In the hydrolytic degradation, the rate of water penetration relative to hydrolytic scission of the chains is an important factor. If water molecules penetrate rapidly into the polymer matrix (i.e. hydrophilic polymers), degradation occurs rapidly, mostly within the bulk. In contrast, more hydrophobic polymers are degraded slowly and from the surface.[83] Furthermore, the polymer morphology has a significant effect on degradation. Since the crystalline regions are more resistant to water permeability, degradation of semi-crystalline PLA is much slower than in amorphous PLA. The hydrolytic degradation of PLA first occurs in the amorphous domain, which has a higher water uptake.[85] Additionally, the stereocomplex structures of PLA have been reported to have a more resistance to hydrolytic degradation than either PDLA or PLLA polymers.[77]

The properties of PLA (i.e. molecular weight, morphology, mechanical properties) are altered during degradation. In order to avoid hydrolytic degradation during processing, it is highly important to remove moisture from the polymer. Otherwise, the presence of moisture during processing can deteriorate the desired polymer properties.[82]

PLA has been studied extensively as a biocompatible polymer in a wide range of biomedical applications due to its non-toxic degradable products. However, low hydrophilicity and the absence of functional groups limit the applications of PLA. The copolymerization of PLA with different polymers has been investigated as a useful method to overcome its limitations and tailor its desirable properties. Among various polymers, incorporation of poly(ethylene oxide)s can enhance the properties of PLA.

2.3. Synthesis and Properties of Poly(ethylene oxide)

2.3.1. Introduction

Amphiphilic polymers have gained considerable attention in pharmaceutics and biomedical applications.[86] PLA has been successfully implemented in many applications, but its broader utilization as a biomaterial is limited due to its hydrophobicity and difficulties with encapsulation of significant loadings of polar drugs.[3] A common method to overcome these limitations and improve the hydrophilicity of the hydrophobic polymers is copolymerization with a hydrophilic block such as poly(ethylene oxide) (PEO).[87] PEO is a linear or branched polyether that is usually terminated with hydroxyl groups. For biomedical applications, PEO has desirable characteristics such as (1) low immunogenicity, (2) rapid renal clearance *in vivo,* (3) high flexibility upon hydration of the main chain, (4) good solubility in both aqueous and organic media, and (5) resistance to biodegradation.[88] The repeating units of PEO do not contain functional groups, but the ends can be functionalized with bioactive molecules. Through the copolymerization with PEO, the hydrophilicity of PLA can be enhanced to facilitate drug loading, increase degradation rate, and functionality can be added.[89] Thus, PLA-PEO amphiphilic block copolymers show great potential for pharmaceutical applications.

2.3.2. Synthesis of PEO

PEO is generally synthesized via anionic ring-opening polymerization of ethylene oxide. Ring-opening polymerization is a common method to synthesize homopolymers with well-defined structures or end groups as well as copolymers with different architectures, such as block or graft copolymers.[53] Anionic ring-opening polymerization of ethylene oxide typically produces PEO that has narrow molecular weight distribution with a polydispersity less than 1.1.[90] Ethylene oxide can be initiated by a number of different species including

alkoxides, hydroxides, oxides and metal alkyls and aryls. The reaction is initially carried out by S_N2 nucleophilic attack of the initiator on the ethylene oxide methylene, which opens the ring to form the propagating species. The reaction mechanism of PEO is shown in the Figure 2.13.

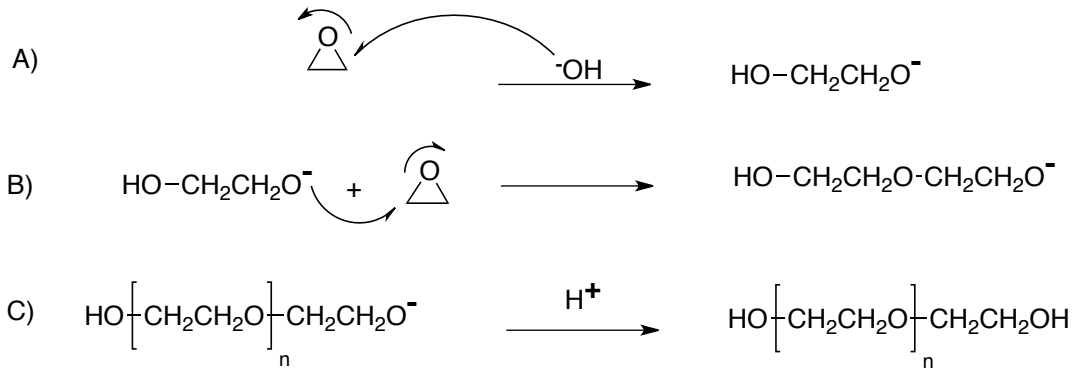


Figure 2.13 Reaction mechanism of anionic polymerization of ethylene oxide: A) initiation, B) propagation, C) termination

To synthesize heterobifunctional PEO, two methods are commonly used. In the first method, a heterobifunctional anionic initiator is used to control the molecular weight and the polymerization is terminated with an appropriate terminating agent. Thus, heterobifunctional PEO oligomers can be synthesized with different end groups such as maleimide, methacryloyl, carboxyl groups by introducing protected or deprotected functional initiators.[91-93] Furthermore, the hydroxyl terminus of PEO can be tailored for modifications to obtain more complex heterobifunctional polymers.[94] The polymerization of PEO needs to be conducted under anhydrous reaction conditions to obtain the pure, targeted heterobifunctional product. Otherwise, ethylene oxide can be initiated by water, which leads to side products and uncontrolled molecular weight of the resulting polymer.[95] Another method to obtain heterobifunctional PEO is to use derivatizations of PEO diols. In this method, the terminal hydroxyl groups of α,ω-dihydroxy-poly(ethylene oxide) (PEO diol) are changed by the series of the reactions and this is followed by tedious isolation of heterobifunctional PEO

oligomers.[96] However, as the molecular weight of PEO diols increase, the chemical and physical differences of the mono-, di- and unsubstituted products decrease during the polymerization. Therefore, the isolation of the desired product becomes complicated.[97] In order to enable the isolation of the polymer, heterobifunctional PEOs that are synthesized by this method are usually low molecular weight oligomers or they have ionizable end groups such as amines, which can allow help with separation via ion exchange chromatography.

2.3.3. Functionalization of Poly(ethylene glycol)

Polymeric carriers, which covalently conjugate molecules of interest, have an essential role in modern polymer therapeutics.[88] In designing polymer-based drug entities, the potential of the drug or biomolecule is advanced by (1) increasing water solubility, (2) targeting delivery of the drugs to specific sites in the body, and (3) improving stability against degrading enzymes.[98] Ringsdorf introduced a model for pharmacologically active polymers in 1975. A rational concept of his model consisted of several components: (1) polymer as carrier, (2) drug, peptide or protein as a biological active entity, and (3) a spacer segment that allowed for hydrolysis of the biomolecule or targeting moiety (Figure 2.14).[99,102]

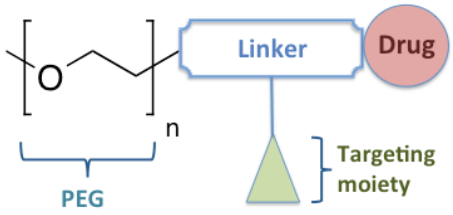


Figure 2.14 Schematic presentation for PEG-based prodrug model

PEO is the most widely used nonionic polymer in pharmaceutical and biomedical applications. Commonly available forms consist of ethylene oxide units with one hydroxyl

terminus and one methoxy end group (mPEG), or a linear polymer with two terminal hydroxyl groups.[100] Since PEG has good solubility in common organic solvents, end-group modifications are not difficult. Additionally, the low toxicity and high water solubility make PEG a versatile candidate for polymer-based drug delivery systems.[88] Covalent conjugation of PEG with biologically active molecules such as proteins, small drugs, and targeting moieties has been a valuable tool for polymer therapeutics.[101] Conjugation of PEG with a biomolecule depends on the chemical structure, molecular weight, steric hindrance and the reactivity of the both the biomolecule and polymer. The bioactive molecule as well as polymer needs to have a reactive or functional end group such as –COOH, -OH, -SH, or –NH_2 to synthesize a bioconjugate. Chemical conjugation of polymers with drugs or biomolecules can yield stable bonds like amides, esters or disulfides. In order to eliminate drug release during its transport, the bond linkage needs to be stable. Covalent bonds such as ester and amide are relatively stable bonds and can deliver drug to its targeted site in the body.[102]

In order to conjugate a molecule to PEG, it is essential to derivatize PEG such that one or both end groups are able to react with the molecule of interest. For example, a route for conjugation of proteins to PEG is to modify PEG with appropriate functional groups that can react with lysine and N-terminal amino acid groups. Because of the labile molecules used in such conjugations with PEG, mild reaction conditions are required. Some different PEG derivatives for amino conjugation are shown in Figure 2.15. The coupling reactions may have drawbacks such as lacking of selectivity on modification, unstable linkages, and can be limited to low molecular weights. The succunimidyl carbonate (3a) in figure 2.15 is an example of conjugation through acylation. While it is able to react with a reactive amino acid group such as lysine to form a carbamate linkage, it can react with histidine to form a hydrolytically unstable imidazole-carbamate linkage.[103]

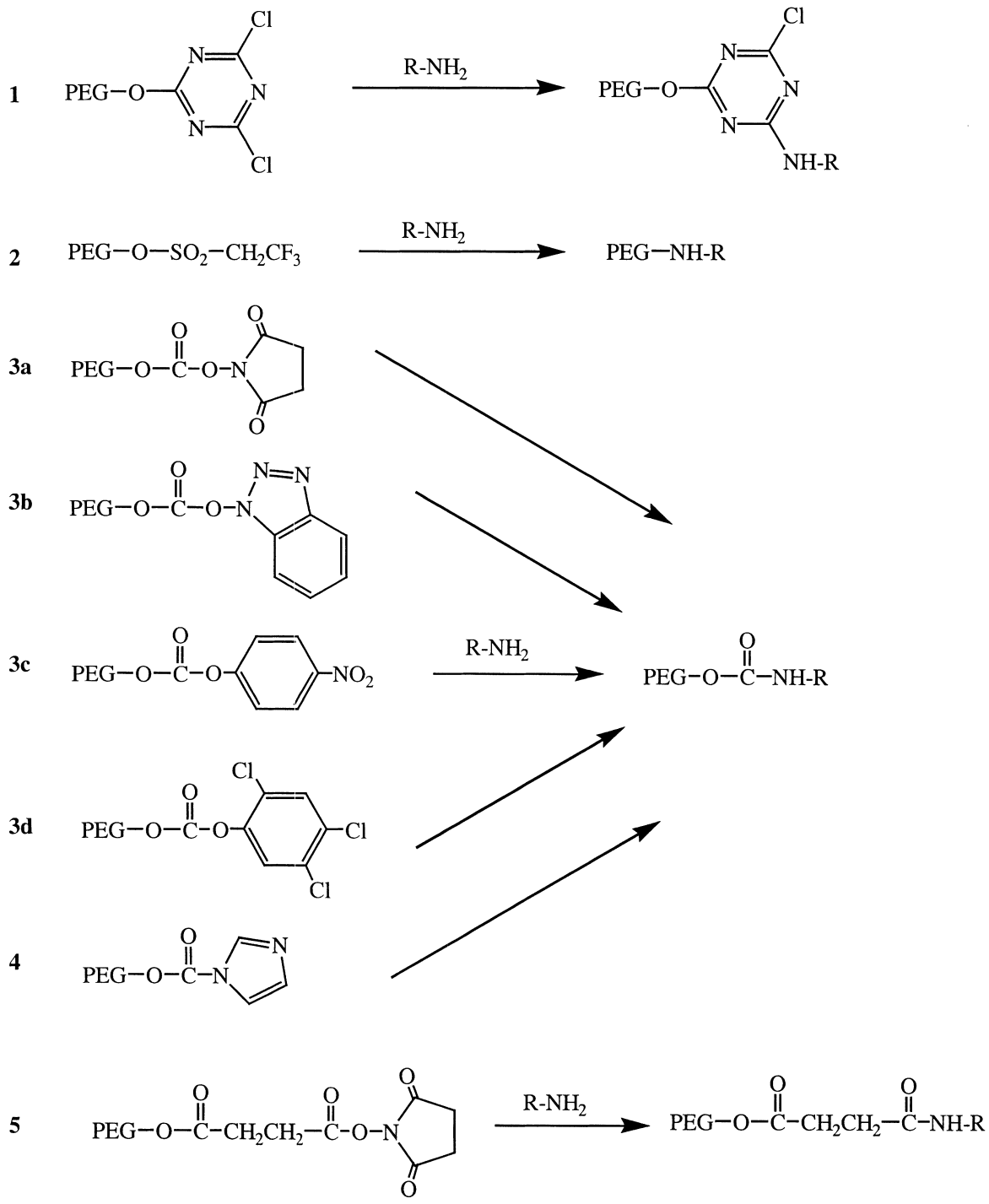


Figure 2.15 PEG derivatives for Amine Conjugation[103] (Used under fair use, 2012)

Weak linkages can be advantageous for controlled release formulations, but they can be a limitation if conjugate stability is highly desired.

PEO can be functionalized with terminal carboxylic acid groups via a number of synthetic methods.[104-106] Zhang *et al.* investigated a synthetic route to heterobifunctional PEO containing both amino and carboxylate end groups. It was proposed that ethylene oxide was initially polymerized by anionic polymerization using cyanomethyl potassium as the initiator. After converting the end-hydroxyl group to a primary amino group, the carboxylate group was obtained via the hydrolysis of cyano group.[94]

2.4. Copolymerization of Poly(ethylene oxide) with Poly(lactide)

Amphiphilic block copolymers consisting of PEO and PLA have great potential for formulating drug delivery systems. Copolymers of PLA and PEO with different PLA and PEO ratios and molecular weights can be synthesized for a number of biomedical applications.[107] Block copolymers of PEO and PLA can be obtained by using lactide as a monomer and PEO as an initiator and the copolymerization can yield diblock, triblock, multi block or star block copolymers.[87, 108-110] PEO and PLA can be copolymerized by polycondensation, anionic polymerization or ring opening polymerization methods. Otsuka *et al*. synthesized an α-acetal-PEG-PLA block copolymer via anionic ring-opening polymerization of ethylene oxide with 3,3-diethoxy-potassium propanol as an initiator at room temperature under argon, then used lactide as a monomer to grow the second block.[111] Cohn *et al.* reported the synthesis of PLA and PEO triblock copolymers via polycondesation of lactic acid with the composition of 20-84 mole % with PEO in the 600-6000 molecular weight range using antimony trioxide, Sb_2O_3, as a catalyst.[112]

The ring-opening polymerization of lactide monomer by using PEO as an initiator is a widely utilized method for synthesis of PEO-PLA copolymers. While PEG-PLA diblock copolymers can be synthesized using poly(ethylene glycol) methyl ether (mPEG) to initiate lactide monomer, PLA-PEG-PLA triblock copolymer can be synthesized using dihydroxy

PEG as an initiator. Kricheldorf *et al*. demonstrated the synthesis of ABA triblock copolymers of L-lactide and PEG by conducting ring-opening polymerization in bulk using metal oxides and stannous octoate as catalysts. It was shown that among the catalysts which were tested, stannous octoate yielded the highest conversion with minimum racemization.[113] Zhang *et al*. reported a paclitaxel conjugate in which paclitaxel was covalently connected to a mPEG-PLA diblock copolymer. The polymer formed micelles with the PLA block in the core and mPEG in the shell. The diblock was synthesized by the ring-opening polymerization of L-lactide in the presence of mPEG with $Sn(Oct)_2$ as a catalyst. The lactide reaction was conducted at 110 °C for 24 hours in toluene (Figure 2.16). The terminal hydroxyl group was converted to a carboxyl group for further reaction with paclitaxel.[110]

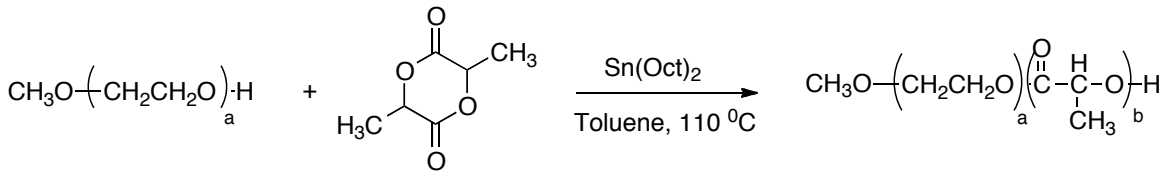


Figure 2.16 Synthesis scheme of mPEG-*b*-PLA block copolymer

In the last decade, polymeric nanoparticles have been investigated as versatile materials for delivery of therapeutics which are able to incorporate drugs and targeting and imaging agents.[114] Therefore, the careful design of these polymeric nanoparticles is important for their versatility for potential treatment of a wide range of diseases including cancer and cardiovascular diseases.[115] PEO-PLA amphiphilic block copolymers, with their biodegradability and good biocompatibility, are good candidates for nanoparticle formation. PEO-PLA block copolymers can enhance drug loading and encapsulation efficiency of hydrophobic drugs, decrease particle sizes and prolong blood circulation time.[89] For these reasons, PEO-PLA nanoparticles have great potential in drug delivery systems and imaging applications.

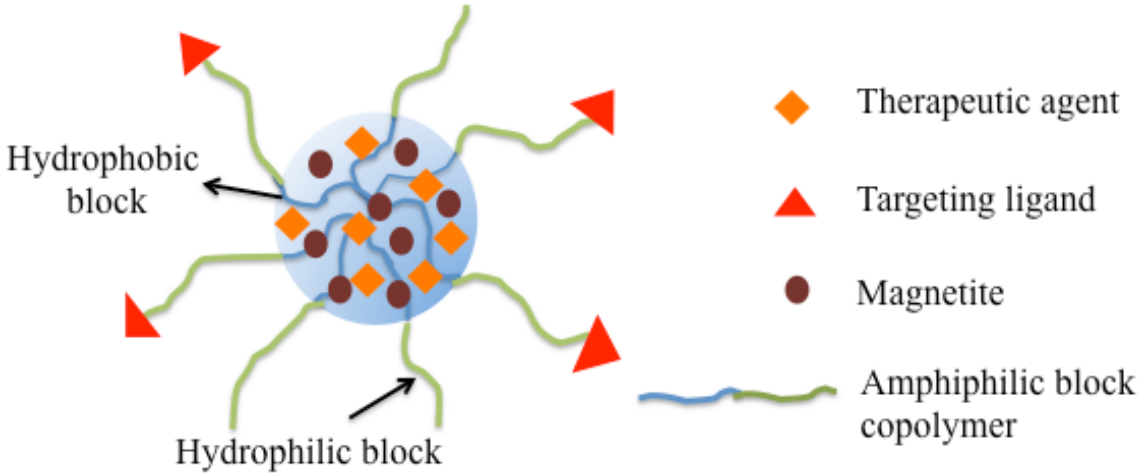


Figure 2.17 Schematic representation of multifunctional nanoparticle

Polymeric nanoparticles can be prepared from PEO-PLA block copolymers with a PLA block in the molecular weight range of 2k to 110k and a PEO block with a range of 750 to 20k g/mole.[116, 117] In general, the core-shell structure of block copolymer nanoparticles can enhance the solubility of poorly water-soluble drugs and surround hydrophobic imaging agents. To this end, PLA blocks, which can be either amorphous or semi-crystalline, can form a hydrophobic core and encapsulate drug and magnetite, while PEO blocks form a solvated, hydrophilic corona that provide colloidal stability and regulate interactions with proteins and cells (Figure 2.17).[114, 118] Thus, drugs can be incorporated into the hydrophobic region of the nanoparticles and can be released by a diffusion mechanism.[117]

CHAPTER 3 : Multifunctional Polylactide Nanoparticles for Magnetic Resonance Imaging and Antiretroviral Therapy

3.1. Synopsis

This chapter discusses the synthesis and characterization of multifunctional polylactide nanoparticles that can integrate magnetite and therapeutic agents for magnetic resonance imaging and antiretroviral therapy. The amphiphilic biocompatible block copolymers are widely used for the design of nanoparticles for drug delivery and imaging applications. Poly(ethylene glycol-*b*-D,L-lactide) block copolymer was synthesized by ring-opening polymerization of D,L-lactide in the presence of poly(ethylene oxide) methyl ether using stannous octoate as the catalyst. The diblock copolymer was used as a polymer nanocarrier to encapsulate the HIV protease inhibitor Ritonavir within magnetite nanoparticles. Magnetite-copolymer and drug-magnetite-copolymer nanoparticles were fabricated by rapid nanoprecipitation in a multi-inlet vortex mixer. Addition of poly(L-lactide) homopolymer (PLLA) to the drug-copolymer nanoparticles in the nanoprecipitation step produced monomodal size distributions as measured by dynamic light scattering. Alternatively, an amorphous copolyester (TMCBD-CHDC) comprised of a cycloaliphatic ester, dimethyl-1,4-cyclohexane dicarboxylate and the aliphatic diol, 2,2,4,4-tetramethyl-1,3-cyclobutanediol was synthesized by melt polycondensation using dibutyltin oxide as the catalyst. Hence, PLLA and TMCBD-CHDC were used to make well-defined nanoparticles without magnetite.

3.2. Experimental

3.2.1. Materials

D,L-lactide and L-lactide were obtained from Purac and recrystallized from dry ethyl acetate twice. Poly(ethylene oxide) methyl ether (mPEO) with a molar mass of ~5000 g mol^{-1} was obtained from Aldrich and vacuum-dried at room temperature for 18 h before use. Iron (III) acetylacetonate, tin (II) 2-ethylhexanoate (stannous octoate), benzyl alcohol (>98%), oleic acid (90%, technical grade) and tetrahydrofuran (anhydrous) were purchased from Aldrich and used as received. Ethyl ether (anhydrous) and ethyl acetate (anhydrous) were purchased from Fisher Scientific and used as received. Toluene (Fischer Scientific) was stirred over calcium hydride and distilled. Dimethyl-1,4-cyclohexane dicarboxylate (DMCD) and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (CBDO) were donated by Eastman Chemical Co. Dibutyltin (IV) oxide was purchased from Sigma Aldrich.

3.2.2. Synthesis of a Poly(ethylene glycol-*b*-D,L-lactide) Copolymer (mPEO-*b*-PDLLA)

A mPEO-*b*-PDLLA block copolymer with a targeted M_n of 5000-1000 was synthesized by ring-opening polymerization of D,L-lactide initiated by of mPEO-OH using stannous octoate as the catalyst.[110, 116] D,L-lactide (4.44 g, 0.03 mol), mPEO (2.22 g, 4.44 x 10^{-4} mol) and 17 mL toluene were charged to a 250-mL flame-dried, round-bottom flask equipped with a magnetic stir bar. The flask was placed in an oil bath at 80 °C to dissolve the monomer and initiator. Stannous octoate catalyst solution (0.98 mL, 0.012 g/mL) in toluene was added to the flask and the temperature of the oil bath was raised to 105 °C. The polymerization was allowed to proceed for 48 h. The polymer was isolated by precipitation into cold diethyl ether and collected by vacuum filtration. The product was vacuum dried at 40 °C for 40 h and 5.68 g of polymer was obtained.

3.2.3. Synthesis of Poly(L-lactide) Homopolymer (PLLA)

A PLLA homopolymer with a targeted M_n of 11,000 g mol^{-1} was prepared with benzyl alcohol as the initiator. L-lactide (2.05 g, 0.01 mol) and 6.5 mL toluene were charged into a 100-mL round-bottom flask equipped with a stir bar and condenser.[119] Benzyl alcohol (0.36 mL of a 0.51 M solution in toluene, 0.18 mmol) was added to the stirring reaction via syringe. Stannous octoate catalyst solution (0.31 mL, 0.012 g/mL) in toluene was added to the flask. The polymerization was conducted at toluene reflux for 48 h. The polymer was isolated by precipitation into cold diethyl ether and collected by vacuum filtration. The product was vacuum dried at 40 °C for 40 h and 1.65 g of polymer was obtained.

3.2.4. Synthesis of Poly(oxy-2,2,4,4-tetramethyl-1,3-cyclobutanediyloxy-1,4-cyclohexanedicarbonyl) (TMCBD-CHDC)

The amorphous copolyester, TMCBD-CHDC, was synthesized by melt polycondensation from the cycloaliphatic ester, DMCD, and the cycloaliphatic diol, CBDO, with dibutyltin oxide as the catalyst.[120, 121] CBDO (14.4 g, 0.1 mol) and DMCD (20.0 g, 0.1 mol), dibutyltin oxide (29 mg, 500 ppm) were charged into a 100-mL, one-neck, round-bottom flask. The reaction flask was immersed into a molten Belmont metal bath, which was heated to 190 °C. The reaction temperature was gradually increased. Initially, the reaction mixture was stirred at 190 °C for 2 h, and this was followed by heating at 220 °C for 2 h and at 250 °C for 2 h. The temperature was raised to 275 °C and held for 45 min under high vacuum (~0.1 mm Hg) was applied, then the reaction was kept at 275 °C for 1 h. The vacuum was released and nitrogen was purged through the reaction flask. After the metal-bath was removed, the reaction flask was allowed to cool to room temperature. The polymer was dissolved in dichloromethane and precipitated into methanol. Then, the polymer was vacuum-filtered and dried under vacuum at 60 °C.

3.2.5. Preparation of Ritonavir-loaded polymer nanoparticles

Ritonavir-loaded nanoparticles were prepared by rapid nanoprecipitation in a multi-inlet vortex mixer (MIVM). The following procedure is for a targeted composition of 20 wt% RTV. The diblock copolymer PEO(5k)-PDLLA(10k) and TMCBD-CHDC(2.5k) homopolymer and RTV were dissolved in THF at a concentration of 33, 11 and 11 mg mL^{-1}, respectively. The THF solution was passed through a 1 μm PTFE filter and fed into the MIVM at 9.99 mL min^{-1} using a computer-controlled syringe pump (New Era Pump Systems, Farmingdale, New York) along with three streams of de-ionized water at 33.3 mL min^{-1} each, controlled by a PHD 4000 programmable syringe pump (Harvard Apparatus, Holliston, Massachusetts). The nanoparticle suspension exiting the mixer was dialyzed against de-ionized water (100X) for 24 h using a Spectra/Por dialysis bag (25,000 MWCO, Spectrum Laboratories, Inc.) with four changes of dialysate. The dialyzed suspension was freeze-dried for 72 h (0.021 mBar, -52 °C) and stored as a solid product. On the other hand, magnetite nanoparticles were also synthesized by reducing Fe(III) acetylacetonate in benzyl alcohol at 205°C using a method adapted from the procedure of Pinna *et al*.[122]

3.3. Characterization

^{1}H NMR spectra were obtained on a Varian Unity 400 MHz NMR spectrometer operating at 400 MHz or a Varian Inova 400 NMR operating at 399.97 MHz. The NMR parameters included a pulse width of 28.6° and a relaxation delay of 1.000 s at ambient temperature. The samples were dissolved in *d*-$CHCl_3$ for obtaining the spectra.

Size exclusion chromatography (SEC) was carried out to investigate the molecular weight and molecular weight distributions of homo and block copolymers. The chromatograms were obtained in HPLC grade chloroform at 30 °C on a Waters Alliance model 2690 chromatograph equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel

column set. A Viscotek viscosity detector and a refractive index detector were utilized with polystyrene calibration standards to generate a universal molecular weight calibration curve for absolute molecular weight analyses. Samples were prepared by dissolving 20–25 mg in 10 mL of HPLC grade chloroform.

Particle sizes were measured by dynamic light scattering at 25 °C on a Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd., Worcestershire, U. K.) equipped with a 4 mW He-Ne laser (λ = 633 nm) and backscatter detection. Lyophilized samples were suspended in de-ionized water at a concentration of 0.1 mg mL^{-1} and sonicated in a water bath sonicator (Model 8890, Cole Parmer) for 10 min before measurements. Monomodal size distributions and narrow polydispersities were obtained as shown in Figure 3.1.

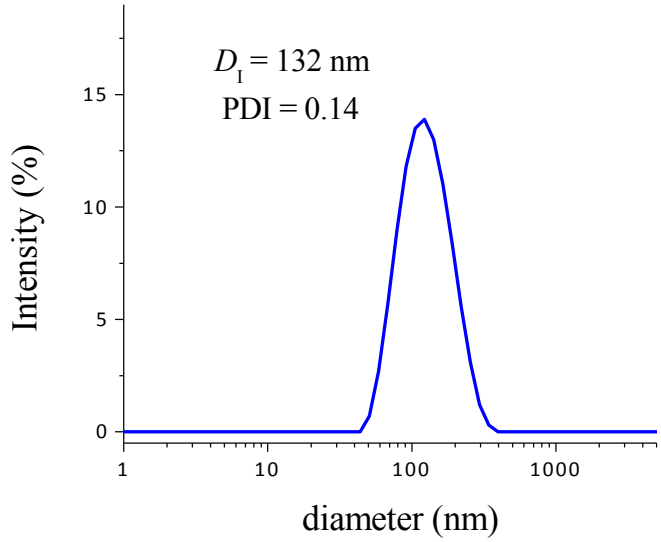


Figure 3.1 A representative DLS curve of an amorphous copolyester (TMCBD-CHDC) with monomodal size distribution

The iron contents of nanoparticles containing magnetite were determined by thermogravimetric analysis (Q5000 TGA, TA Instruments). Samples (10-15 mg) were heated in a nitrogen atmosphere up to 700 °C at a rate of 10 °C/min and the weight remaining taken as the amount of magnetite in the sample.

3.4. Results and Discussion

3.4.1. Synthesis and Characterization Poly(ethylene glycol-*b*-D,L-lactide) Copolymer (mPEO-*b*-PDLLA)

Poly(ethylene oxide-*b*-lactide) copolymers (PEO-*b*-PLA) are attractive candidates for use in controlled delivery of anticancer drugs. PEO-PLA block copolymers are amphiphilic with good dispersion stability in vivo. PEO is a nondegradable hydrophilic polymer with very low toxicity and good biocompatibility. The PEO in PEO-*b*-PLA copolymers provides steric dispersion stability of the polymers in aqueous media, and hydrophobic drugs can be incorporated into the cores of micellar PEO-*b*-PLA assemblies.[89]

The most widely used method to synthesize the PEO-PLA diblock copolymers is by ring-opening polymerization using coordination catalysts. By varying the monomer to initiator ratio, the coordination-insertion mechanism allows control of molecular weights over a broad range.[110] In this case, a mPEO-PDLLA diblock copolymer was synthesized by ring-opening polymerization of D,L-lactide using mPEO as an initiator and stannous octoate as catalyst in toluene. Figure 3.2 depicts the synthesis scheme. The length of lactide chain was controlled by changing the proportion of lactide monomer and mPEO whose hydroxyl groups initiated the ring-opening of the monomer.

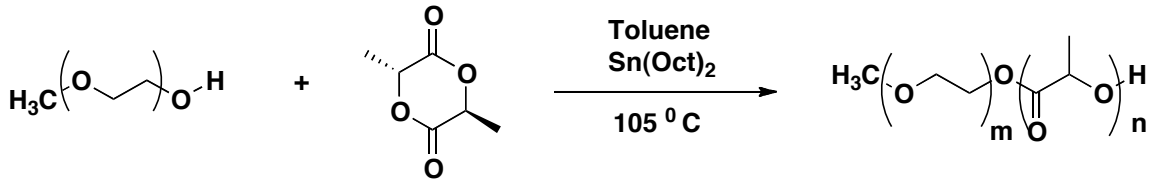


Figure 3.2 Synthetic reaction scheme for mPEO-*b*-PDLLA

^{1}H NMR was utilized to analyze the composition of the copolymer (Figure 3.3).

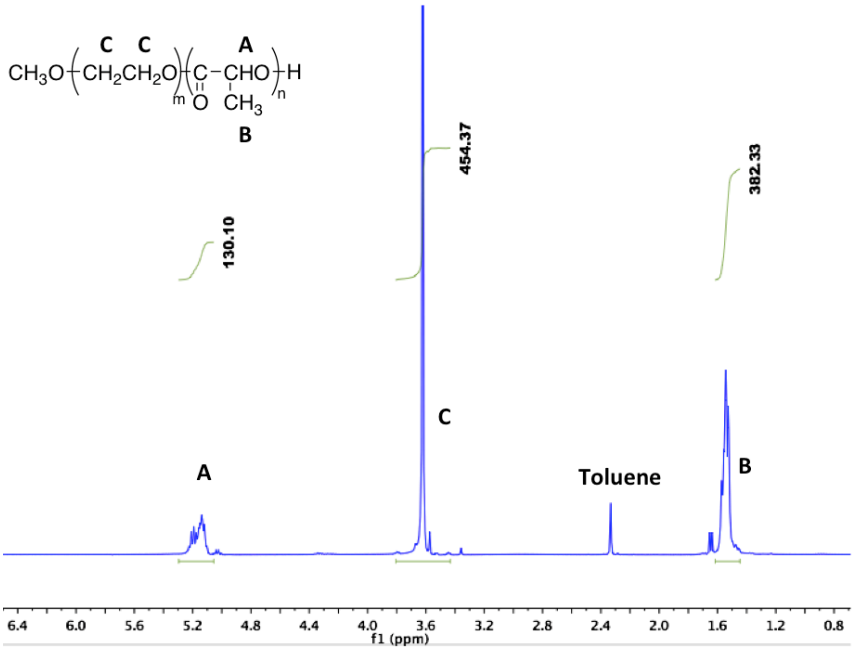


Figure 3.3 ^{1}H NMR spectrum of mPEO-*b*-PDLLA

The peaks at 1.65 and 5.20 ppm correspond to the methine (-CH) and methyl protons (-CH_3) of the PLA block, whereas the peak at 3.65 ppm belongs to the methylene proton (-CH_2) of the PEO block. Integrations of the proton peaks on the ^{1}H NMR spectrum indicates that the mPEO-*b*-PDLLA block copolymer was as expected.

Both ^{1}H NMR and SEC were used to examine the molecular weight of the mPEO-*b*-PDLLA copolymer. From ^{1}H NMR, the number average molecular weight of the mPEO-*b*-PDLLA was calculated by comparing the integrated area of the peak at 5.20 ppm (-CH in PLA) relative to the peak at 3.65 ppm (-CH_2 in PEO), assuming the number average molecular weight of the PEO segment was 5000 g mol^{-1}. From the ratio of these two peaks, the number average molecular weight was calculated to be 14,360 g mol^{-1}. Figure 3.4 shows a representative SEC curve of a 5000-10000 M_n mPEO-*b*-PDLLA. A single, monomodal

peak with the polydispersity of 1.07 demonstrated the well-controlled molecular weight distribution of the diblock copolymer.

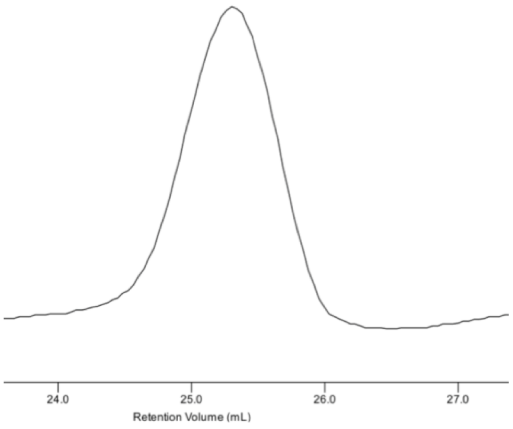


Figure 3.4 Size Exclusion Chromotogram of a mPEO-*b*-PDLLA copolymer

3.4.2. Synthesis and Characterization of Poly(L-lactide) Homopolymer (PLLA)

Synthesis of a poly(L-lactide) homopolymer (PLLA) was conducted using benzyl alcohol as the initiator with stannous octoate as the catalyst. The method follows a similar procedure as described above for preparing the diblock copolymer (Figure 3.5). The reaction mixture was refluxed at 120 °C for 48 h.

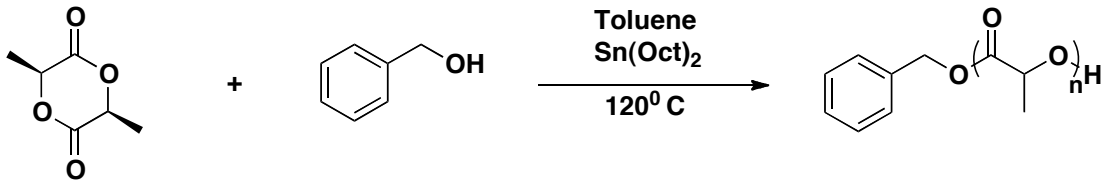


Figure 3.5 Synthetic reaction scheme for a PLLA homopolymer

The structure of the product was analyzed by ^{1}H NMR. Figure 3.6 shows the ^{1}H NMR spectrum of PLLA homopolymer initiated by benzyl alcohol. According to ^{1}H NMR, the number average molecular weight of the PLLA was calculated to be 9,800 g mol^{-1}.

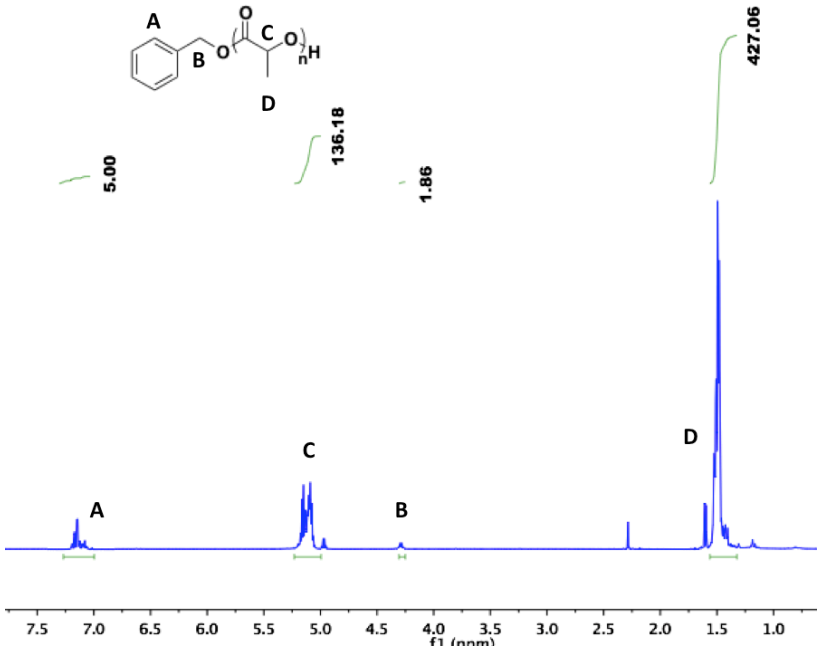


Figure 3.6 ^{1}H NMR spectrum of a PLLA homopolymer

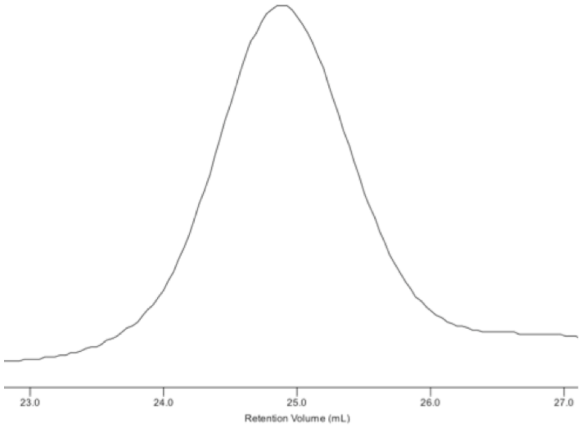


Figure 3.7 Size Exclusion Chromotogram of PLLA

Figure 3.7 depicts a representative SEC curve showing a monomodal peak with a polydispersity of 1.05 for an 11,000 g mol^{-1} PLLA. The data presented in Table 3.1 indicates that number average molecular weights achieved were quite close to the targeted molecular weights. The polydispersity indices (PDI) of the both the homopolymer and diblock copolymer of PLA were narrow and the chromatograms were monomodal.

Table 3-1 A summary of molecular weight and molecular weight distributions of homo and diblock copolymers of PLA

Polymer	**Target Mn[a] (g mol^{-1})**	**Mn[b] (g $mol^{-1)}$**	**Mn[c] (g $mol^{-1)}$**	**PDI[c]**
mPEO-*b*-PDLLA	5,000-10,000	14,400	17,900	1.07
PLLA	11,000	9,800	10,700	1.05

[a] Calculated from monomer to initiator ratio. [b] Calculated from ^{1}H NMR. [c] Determined via SEC.

3.4.3. Synthesis and Characterization of Poly(oxy-2,2,4,4-tetramethyl-1,3-cyclobutanediyloxy-1,4-cyclohexanedicarbonyl) (TMCBD-CHDC)

Utilization of an amorphous, partially cycloaliphatic polyester in the nanoparticles was investigated as a route for improving the stabilities of the nanoparticles at physiological temperature. It was hypothesized that the rates of release of drugs from such materials would be slower if the glass transition temperature of the nanoparticle core was above 37 °C. Melt

polycondensation is a common method to synthesize step-growth polyesters at elevated temperatures under vacuum. High vacuum and a suitable catalyst are also required for melt transesterification.

Kelsey *et al.* demonstrated transesterification of CBDO and a linear diol with dimethyl terephthalate using the dibutyltin oxide at 220-250 °C. After the methanol was removed by distillation, the excess diol was removed by heating at 250-260 °C using high vacuum. The authors proposed that dibutyltin oxide was a more effective catalyst for this transesterification than other typical tin and lead catalysts.[120] In the synthesis of TMCBD-CHDC, CBDO and DMCD were used with dibutyltin oxide following a similar procedure to that described above. The reaction scheme of TMCBD-CHDC is shown in Figure 3.8.

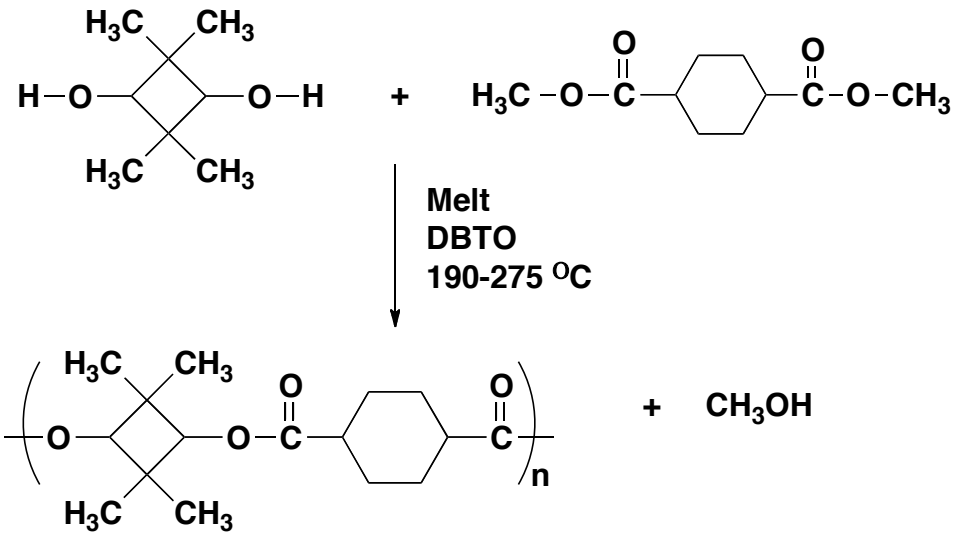


Figure 3.8 Synthetic scheme for TMCBD-CHDC

The molecular structure of the product was examined by ^{1}H NMR in chloroform (Figure 3.9) and shows the expected resonances. No other peaks were detected in the spectrum.

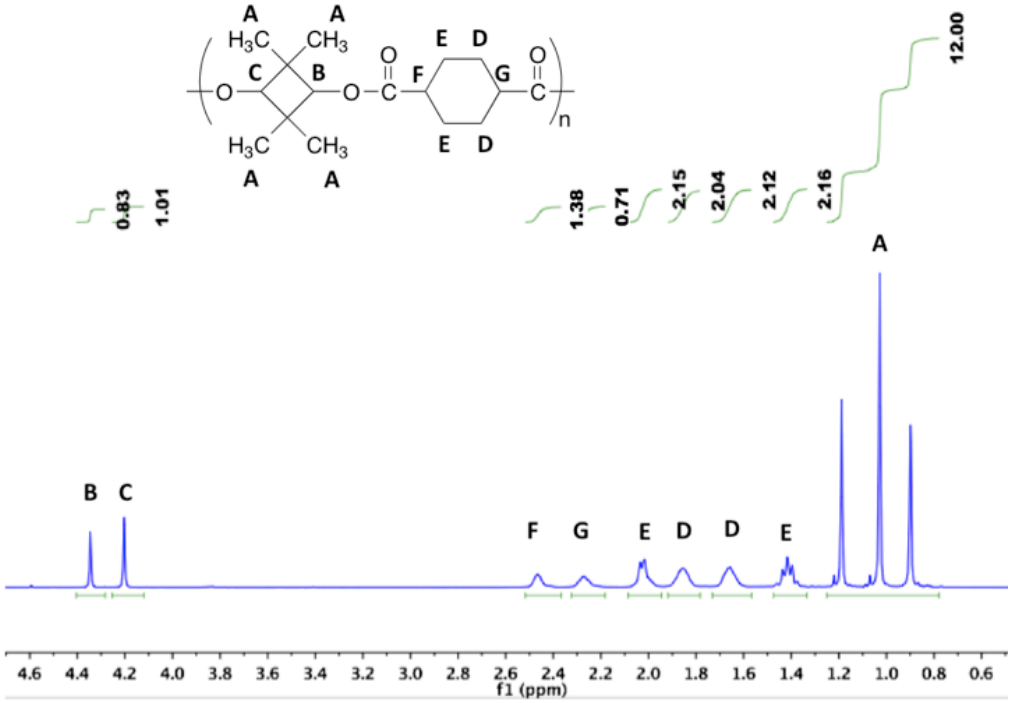


Figure 3.9 ^{1}H NMR spectrum of TMCBD-CHDC

From ^{1}H NMR, the cis/trans ratio of CHDC was also determined by comparing the integrated area of the peak at 2.5 ppm (cis isomer) relative to the peak at 2.3 ppm (trans isomer). The molecular weight was determined to be 2,500 g mol^{-1} by SEC. A monomodal peak with a polydispersity of 1.65 suggests that a well-controlled polymerization occurred (Figure 3.10).

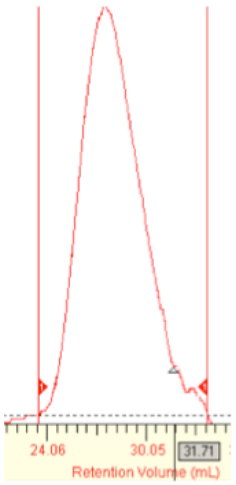


Figure 3.10 Size Exclusion Chromatogram of TMCBD-CHDC

3.4.4. Preparation of Ritonavir-loaded polymer nanoparticles

Multifunctional polymeric nanoparticles containing magnetite hold promise as versatile materials for delivery of therapeutics and for simultaneously monitoring their biodistribution *in vivo* due to their stability, particle sizes and drug loading capacity.[114] Among the different molecular architectures, amphiphilic block copolymers are commonly used for designing nanoparticles. The core-shell structure of amphiphilic block copolymers can encapsulate hydrophobic drugs and imaging agents, while the hydrophilic shell can provide steric dispersion stability and present targeting ligands.[114, 123] In recent decades, PEO-PLA block copolymers and nanoparticles have attracted considerable interest since they have potential advantages such as high bioavailability of the drugs, enhanced drug loading and encapsulation efficiency, and low toxicity.[89, 124]

To this end, the amphiphilic diblock copolymer, mPEO-*b*-PDLLA was utilized to encapsulate the drug along with magnetite. The diblock copolymer nanocarriers encapsulating magnetite and the drug were fabricated by rapid nanoprecipitation. In the fabrication of theranostic nanoparticles, Ritonavir, RTV was used as a model drug for treating HIV infection. RTV is an antiviral medication, and functions as a protease inhibitor. It has low water solubility, 1 μg mL^{-1}. Figure 3.11 shows the chemical structure of RTV.

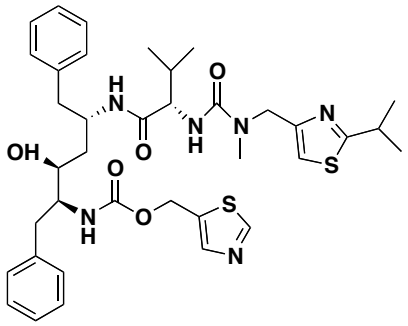


Figure 3.11 Chemical structure of RTV

The hydrophobic polyester core of the nanoparticles encapsulates RTV and/or oleic acid coated magnetite (OA- Fe_3O_4), while the PEO segment forms a hydrated shell that provides steric stability. Nanoparticles with hydrodynamic sizes in the range of 100-160 nm, narrow size distributions and controlled compositions of magnetite and drug were fabricated by a rapid precipitation method first described by Prud'homme *et al*.[125] A four-jet multi-inlet vortex mixer was utilized to rapidly mix the drug, magnetite, and block copolymer at supersaturated concentrations and this lead to nucleation of copolymer stabilized multifunctional nanoparticles.

This flash nanoprecipitation technique was used for fabricating diblock nanocarriers containing magnetite only, RTV only, and both components. Additionally, a PLLA homopolymer was incorporated into the hydrophobic core to serve as a nucleating agent when the nanoparticles were prepared without the magnetite. To investigate the effect of the polyester core chemistry on drug release and encapsulation efficiency, the biodegradable, amorphous polyester TMCBD-CHDC, which had a higher glass transition temperature and higher hydrophobicity than PLLA, was compared as a component of the nanoparticles. Table 3.2 shows the measured loadings of drug and magnetite together with their hydrodynamic sizes and the nanoparticle polydispersities as measured by dynamic light scattering.

Table 3-2 Composition and Size of Drug- and Magnetite loaded Polymeric Nanoparticles

	Composition Analysis				Size Analysis	
	% Fe_3O_4 (targeted)	**% RTV (targeted)**	**% Fe_3O_4 (measured)**	**% RTV (measured)**	**D_I (nm)**	**PDI**
OA-Fe_3O_4 / PEO(5k)-PDLLA(10k)	20	---	21	---	117	0.12
RTV/ OA-Fe_3O_4/ PEO(5k)-PDLLA(10k)	20	20	23	13	159	0.16
RTV/PLLA(11k)/ PEO(5k)-PDLLA(10k)	---	20	---	7.6	143	0.15
RTV/TMCBD-CHDC (2.5k)/PEO(5k)-PDLLA(10k)*	---	20	---	16	132	0.14

* The loading of RTV was determined by ^{1}H NMR instead of HPLC

Particle sizes were measured by dynamic light scattering while the iron oxide content of the nanoparticles containing magnetite was determined by thermogravimetric analysis. It was shown that the multifunctional nanoparticles had sizes ranging between 100-160 nm and narrow size distributions. Magnetite loadings were quantitative and sufficiently high to serve as an effective MRI imaging agent. For the PEO-PDLLA diblock copolymer and drug-loaded polymer nanoparticles, addition of either PLLA or TMCBD-CHDC in the nanoprecipitation step lead to low polydispersities with monomodal size distributions. The drug content of RTV-loaded TMCBD-CHDC nanoparticles was measured by ^{1}H NMR, while drug contents in the RTV-loaded PLLA and PEO-PDLLA diblock copolymer nanoparticles were determined by HPLC. It was observed that the RTV loading of TMCBD-CHDC/PEO-PDLLA diblock copolymer nanoparticles was higher than the RTV loading in the PLLA/PEO-PDLLA nanoparticles. Even RTV loadings are not quantitative; they are high enough to be therapeutically useful.

In summary, quantitative incorporation of magnetite, therapeutically useful drug loadings, and well-defined polymeric nanoparticles with controlled size and size distributions make these nanoparticles potentially suitable for HIV therapeutics. For these reasons, these

potential multifunctional polylactide nanoparticles could be used for the development of the treatment of HIV.

CHAPTER 4 : Synthesis and Characterization of Heterobifunctional Poly(ethylene oxide)-Poly(lactide) Block Copolymers

4.1. Synopsis

This chapter discusses the synthesis and characterization of heterobifunctional poly(ethylene oxide) oligomers, poly(ethylene oxide-*b*-lactide) block copolymers and poly(lactide) oligomers having hydroxyl group at one end and different moieties at the other chain end. Two methods were attempted for preparing poly(ethylene oxide) oligomers with vinylsilane end group utilizing heterobifunctional initiator. Double metal cyanide coordination catalyst and potassium naphthalide were separately used to polymerize ethylene oxide initiated by 3-hydroxylpropyldimethylvinylsilane. Then, well-defined, base-catalyzed poly(ethylene oxide) oligomer with narrow molecular weight distribution was used as a macroinitiator to initiate D,L-lactide with base catalysis. Heterobifunctional poly(ethylene oxide-*b*-lactide) block copolymers bearing carboxylic acid group on one end were prepared from thiol-ene addition reactions of mercaptoacetic acid across the vinyl group. Poly(ethylene oxide) oligomer possessing another moiety, maleimide group at one end was prepared using N-(2-hydroxylethyl)maleimide as an initiator via coordination catalyzed reaction because of the sensitivity of the initiator toward base. Since broad molecular weight distributions were obtained in this polymerization, no further polymerization reactions of ethylene oxide with this initiator were conducted. On the other hand, well-defined poly(D,L-lactide) oligomers with maleimide functional group was synthesized utilizing N-(2-hydroxylethyl)maleimide as the initiator, and stannous octoate as a catalyst. Cysteamine hydrochloride was used to introduce an amino group across the maleimide double bond.

4.2. Experimental

4.2.1. Materials

Tetrahydrofuran (THF, 99.5%, EM Science) was refluxed over sodium with benzophenone until the solution was deep purple. It was fractionally distilled into a flame-dried, round-bottom flask prior to each reaction. Vinylmagnesium bromide (Aldrich, 1.0 M in THF) and 3-chloropropylchlorodimethylsilane (Gelest, MW 171.14 g mol^{-1}, d 1.043 g mL^{-1}, BP 179 °C) were used as received. Sodium iodide (NaI, Aldrich, 99%, MW 149.85 g mol^{-1}) was dried under vacuum at 110°C overnight prior to use. Hexamethylphosphoramide (HMPA, MW 179.20 g mol^{-1}, d 1.030 g mL^{-1}, MP 7 °C, BP 230-232 °C), sodium bicarbonate ($NaHCO_3$, 99%, MW 85 g mol^{-1}, 50 °C), and magnesium sulfate ($MgSO_4$, MW 120.36 g mol^{-1}), furan (99%), ethanolamine (99%) were purchased from Aldrich and used as received. The Impact 3 zinc hexacyanocobaltate catalyst was kindly provided by Bayer, Inc., and was utilized as received. Naphthalene (Aldrich, MW 128.17 g mol^{-1}) was purified by sublimation. Potassium (Aldrich, 98 %, MW 39.098 g mol^{-1}) was stored in mineral oil. Ethylene oxide (Aldrich, 99.5 %, MW 44.05 g mol^{-1}, d 0.882 g mL^{-1}, MP -111 °C, BP 10.7 °C) was obtained in pressurized 227 g stainless steel lecture bottles and used as received. D,L-lactide monomer was purchased from Purac and recrystallized in anhydrous ethyl acetate twice. Toluene (Fischer Scientific, 99 %, MW 92.14 g mol^{-1}, d 0.865 g mL^{-1}, MP -63 °C, BP 110 °C) was stirred over calcium hydride and distilled. Stannous octoate (Aldrich, 95 %) was used as received. Mercaptoacetic acid (97 %, MW 92.12 g mol^{-1}, d 1.325 g mL^{-1}, MP -16 °C, BP 96 °C/5mm Hg), 2,2'-azobisisobutyronitrile (AIBN, 98 %, MW 164.21 g mol^{-1}, MP 103-105 °C, and acetic acid (99.9 %, MW 60.05 g mol^{-1}, d 1.049 g mL^{-1}, MP 16.2 °C, BP 117 – 118 °C) were purchased from Aldrich and used as received. Diethyl ether (anhydrous, MW 74.12 g mol^{-1}, d 0.706 g mL^{-1}, MP -116 °C, BP 34.6 °C), acetone (99.6 %, MW 58.08 g mol^{-1}, d 0.791 g mL^{-1}, MP -94 °C, BP 56 °C) and dichloromethane (99.6 %, MW 86.93 g mol^{-1}, d

1.325 g mL^{-1}, MP -97 °C, BP 39.8 –40 °C) were obtained from Fischer Scientific and used as received.

4.2.2. Synthesis of 3-chloropropyldimethylvinylsilane[104]

3-Chloropropyldimethylchlorosilane (10.0 g, 0.06 mol) was transferred via syringe into a clean, flame-dried, two-neck, round-bottom flask equipped with a stir bar under a N_2 purge. The reaction flask was cooled to 0 °C using an ice-bath. A 1.0 M solution of vinylmagnesium bromide (64.0 mL, 0.064 mol) in THF was slowly added to the flask via a syringe in eight equal aliquots of 8 mL over 30 minutes. The reaction was allowed to reach room temperature, then was stirred for 24 h. The reaction mixture was diluted with dichloromethane (100 mL), transferred to a separatory funnel, and washed with a saturated aqueous ammonium chloride solution (150 mL). The organic layer was collected and washed with aqueous sodium chloride (3 X 150 mL). To remove residual water, magnesium sulfate was added to the organic layer, then the mixture was filtered under vacuum. The solvents were removed under vacuum and the product was distilled at 100 ^{o}C, 0.8 Torr, yielding 3-chloropropyldimethylvinylsilane (7.11 g, 0.04 mol) (3-CPMVS). The final product was a clear, colorless liquid. ^{1}H NMR confirmed the quantitative addition of vinyl groups.^{1}H NMR ($CDCl_3$): δ 0.65 ppm (2 H), δ 1.78 ppm (2 H), δ 3.45 (2 H), and δ 5.6-6.2 ppm (3 H).

4.2.3. Synthesis of 3-iodopropyldimethylvinylsilane[104]

3-CPMVS (7.11 g, 0.04 mol) was charged into a 250-mL round-bottom flask equipped with a stir bar and condenser. Sodium iodide (12.1 g, 0.08 mol) was dissolved in acetone (50 mL) in another flame-dried flask, the sodium iodide solution was added to the 3-CPMVS, and the reaction mixture was heated at 60 ^{o}C for 48 h. The resulting solution was filtered to remove salt by-products. The acetone was removed by rotary evaporation and the reaction mixture was dissolved in dichloromethane (100 mL). The excess of NaI and salt by-

products were removed by vacuum filtration. Dichloromethane was removed under vacuum and the product was distilled at 60 ^{o}C, 0.8 Torr, yielding 3-iodopropyldimethylvinylsilane (3-IPMVS, 11.10 g, 0.04 mol). The product was a clear liquid. ^{1}H NMR ($CDCl_3$): δ 0.60 ppm (2 H), δ 1.72 ppm (2 H), δ 3.21 (2 H), and δ 5.6-6.2 ppm (3 H).

4.2.4. Synthesis of 3-hydroxypropyldimethylvinylsilane[104]

3-IPMVS (11.10 g, 0.04 mol) was placed in a 250-mL round-bottom flask equipped with a stir bar and condenser. Hexamethylphosphoramide (20 mL), sodium bicarbonate (7.0 g, 0.08 mol), and de-ionized water (6 mL) were added to the reaction flask. The reaction was stirred at 100 ^{o}C for 24 h. The reaction mixture was cooled to room temperature and transferred to a separatory funnel. It was washed with de-ionized water to remove the excess sodium bicarbonate. Then, magnesium sulfate was added to the organic layer and the mixture was filtered under vacuum. The product was distilled at 90 ^{o}C, 0.8 Torr, yielding 3-hydroxypropyldimethylvinylsilane (3-HPMVS, 5.2 g, 0.04 mol). ^{1}H NMR showed the conversion of the alkyl iodide to an alcohol. The final product was a clear, colorless liquid. ^{1}H NMR ($CDCl_3$): δ 0.55 ppm (2 H), δ 1.58 ppm (2 H), δ 3.60 (2 H), and δ 5.6-6.2 ppm (3 H).

4.2.5. Attempted Synthesis of poly(ethylene oxide) with a vinyldimethylsilyl propoxy group at one end and a hydroxyl group at the other end via double-metal cyanide catalyzed ring-opening polymerization

An exemplary procedure for synthesizing a 2,990 g mol^{-1} vinyldimethylsilyl-PEO-OH is provided. 3-HPMVS (0.722 g, 5.0 mmol) was syringed into a flame-dried 50-mL round-bottom flask and 10 mL of THF was added. A zinc hexacyanocoboltate catalyst solution (0.75 mL of 3.04 mg mL^{-1} in THF) was added into the flask, and the initiator mixture was stirred for 18 h prior conducting the reaction. A 300-mL, high pressure Series 4561 Parr reactor, equipped with a thermocouple, mechanical stirrer and valve-controlled gas inlets and outlets was cooled to -42 °C using a dry-ice bath. Ethylene oxide (15.1 g, 0.34 mol) was

charged from a lecture bottle into the pressure reactor under 20 psi of nitrogen. The initiator solution was added to the reactor via syringe and this was followed by adding 5 mL of THF to wash the syringe. The reaction vessel was heated to 105 ^{o}C. A maximum pressure of 160 psi was noted followed by a temperature spike of 35 ^{o}C and a gradual pressure decrease of 75 psi over 45 minutes. When the equilibrium pressure was achieved, the reactor was allowed to cool to room temperature. The contents were diluted with 200 mL of dichloromethane. The mixture was filtered through Celite® twice to remove the heterogeneous catalyst using dichloromethane as the eluent. The solution was concentrated and the polymer was precipitated into cold diethyl ether. After collecting the product by vacuum-filtration, the polymer was dried under vacuum at 40 °C for 24 hours yielding 13.4 g of vinyldimethylsilyl-PEO-OH. It should be noted that this procedure yielded a polymer with a very broad molecular weight distribution, but it did retain the desired functional end groups.

4.2.6. Preparation of a potassium naphthalide standard base solution in THF

A potassium naphthalide solution was prepared by initially weighing naphthalene (1.29 g, 0.01 mol) into a 50-mL, flame-dried, round-bottom flask equipped with a stir bar. Dry THF (10 mL) was syringed into the flask to dissolve the naphthalene. Potassium metal (0.391 g, 0.01 mol) was cut and mineral oil was removed by blotting on a Kimwipe, then the potassium was added to the solution quickly. The reaction was purged with nitrogen for one hour, then was covered with aluminum foil and stirred for 24 h at room temperature. The final solution was titrated against a standardized 1 N HCl solution to determine the exact molarity of the potassium naphthalide solution, which was found to be 0.95 M.

4.2.7. Synthesis of poly(ethylene oxide) with a vinyldimethylsilyl propoxy group at one end and a hydroxyl group at the other end via base-catalyzed ring-opening polymerization

A representative procedure for synthesizing a 2,105 g mol^{-1}

vinyldimethylsilypropoxy-terminated poly(ethylene oxide) is provided. A 300-mL, high-pressure Series 4561 Parr reactor, equipped with a thermocouple, mechanical stirrer, and valve-controlled gas inlets and outlets was cooled to -55 ^{o}C using an isopropanol-dry ice bath. EO (12.5 g, 0.28 mol) was distilled from a lecture bottle into the pressure reactor under a 20 psi head of nitrogen. The lecture bottle was weighed on an analytical balance before and after the addition to determine the amount of EO transferred. 3-Hydroxypropyldimethylvinylsilane initiator (0.855 g, 5.94 mmol) and 10 mL of THF that had been purged with nitrogen were added into a flame-dried, septum-sealed round bottom flask. Potassium naphthalide (5.64 mmol, 1 mol initiator: 0.95 mol base, 5.9 mL of a 0.95 M solution in THF) was added into the initiator solution to form the alkoxide initiator. The color of the solution changed from dark green to yellow. The initiator solution was added to the stirring reaction mixture in the Parr reactor with an additional 10 mL of THF via syringe. The cooling bath was removed, and the reaction mixture was allowed to reach room temperature and stirred for 24 h. The reaction progress was monitored by observing a drop in pressure from 30 to 20 psi. The polymerization was terminated by adding acetic acid (1.0 mL of 0.34 g mL^{-1} solution in THF, 5.66 mmol) to the pressure reactor via syringe to neutralize the polymer. The Parr reactor was purged with N_2 for 1 h, and opened. Its contents were transferred to a 250-mL round-bottom flask. THF (20 mL) was added to the reactor to rinse it, then this was added to the polymer solution. The solution was transferred to a separatory funnel and diluted with 200 mL of dichloromethane. The product was washed twice with deionized water, then the aqueous layer was also extracted with dichloromethane to ensure that the product was recovered. The polymer solution was concentrated by removing ~85% of the dichloromethane via rotary evaporator. The PEO was precipitated into cold diethyl ether and dried under vacuum at 40 ^{o}C overnight.

4.2.8. Synthesis of poly(ethylene oxide)-*b*-(D,L-lactide) with a vinyldimethylsilyl propoxy group at the PEO terminus and a hydroxyl group at the other end

An exemplary procedure for preparing a diblock copolymer with a 1,760 g mol^{-1} vinyldimethlysilylpropoxy-terminated PEO and a 8,100 g mol^{-1} PDLLA block is provided. D,L-lactide was polymerized using the vinyldimethylsilylpropoxy-terminated PEO as the macroinitiator. D,L-lactide (6.90 g, 0.05 mol) and 13 mL of THF were charged to a flame-dried, septum-sealed round-bottom flask with a magnetic stir bar to dissolve the monomer. The vinyldimethlysilylpropoxy-functional PEO (1.50 g, 0.85 mmol) was placed into another flame-dried, round-bottom flask with 3 mL of THF and sonicated for 30 minutes to dissolve the PEO. Then, potassium naphthalide (0.81 mmol, 1 mol initiator: 0.95 mol base, 0.85 mL of a 0.95 M solution in THF) was added into the initiator solution to form the alkoxide initiator. After the D,L-lactide monomer was completely dissolved, the initiator solution was added into the flask via syringe. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched with acetic acid (0.15 mL of 0.34 g mL^{-1} solution in THF, 0.85 mmol) to neutralize the polymer. The polymer solution was transferred to a separatory funnel and 100 mL of dichloromethane was added. It was washed twice with deionized water and the aqueous phase was extracted with dichloromethane to recover the polymer from the aqueous phase. The resulting polymer solution was concentrated via rotary evaporator by removing ~85% of the solvent. The product was isolated by precipitation into cold diethyl ether, then vacuum-filtered. The copolymer was vacuum-dried at 40 ^{o}C overnight.

4.2.9. Functionalization of the vinyldimethylsilylpropoxy-functional poly(ethylene oxide)-*b*-(D,L-lactide) with mercaptoacetic acid

A representative procedure for carboxylic acid addition across the vinyl terminus via a thiol-ene reaction is provided. A 9,860 g mol^{-1} vinyldimethylsilylpropoxy-terminated poly(ethylene oxide)-*b*-(D,L-lactide) was functionalized with the addition of mercaptoacetic

acid. The copolymer with one vinylsilyl group at one end (1g, 0.10 mmol) was added to a flame-dried, septum-sealed round-bottom flask equipped with a magnetic stir bar and dissolved in 3 mL of deoxygenated toluene. Mercaptoacetic acid (0.16 mL of a 0.16 g mL^{-1} solution in toluene, 0.28 mmol), was added into the flask via syringe and this was followed by addition of AIBN (8.3 mg, 0.05 mmol) and 1 mL of THF. The reaction mixture was deoxygenated by purging with N_2 for one hour. The reaction flask was placed into an oil bath at 80 ^oC for 24 h. The reaction mixture was dissolved in 150 mL of dichloromethane and transferred to a separatory funnel. In order to remove the excess mercaptoacetic acid, it was washed with de-ionized water three times. The polymer solution was concentrated by removing dichloromethane via rotary evaporation. The concentrated polymer was precipitated into cold diethyl ether. The product was vacuum filtered and dried under vacuum at 40 ^oC for 18 hours to yield 0.87 g of the carboxylic acid functional diblock copolymer.

4.2.10. Synthesis of poly(ethylene oxide) with an ethylene glycol vinyl ether group at one end and a hydroxyl group at the other end

The preparation of ethylene glycol vinyl ether-functional poly(ethylene oxide) follows a similar procedure as described above for vinyldimethylsilypropoxy-terminated poly(ethylene oxide). The ethylene glycol vinyl ether was used as an initiator instead of 3-hydroxypropyldimethylvinylsilane to initiate ethylene oxide monomer. A characteristic procedure for the synthesis of a 7,100 g mol^{-1} poly(ethylene oxide) with an ethylene glycol vinyl ether group at the PEO terminus and a hydroxyl group at the other end is provided. EO (21.2 g, 0.48 mol) was distilled from a lecture bottle into the 300-mL Parr pressure reactor, which was equipped with a mechanical stirrer, thermocouple and valve-controlled gas inlets and outlets cooled to -40 ^oC using an isopropanol-dry ice bath. The lecture bottle was weighed on the analytical balance before and after the addition to determine the amount of EO transferred. Ethylene glycol vinyl ether (0.264 g, 3.0 mmol) and 10 mL THF that had

been deoxygenated with nitrogen were added into a flame-dried, septum-sealed round-bottom flask. Potassium naphthalide (2.85 mmol, 1 mol initiator: 0.95 mol base, 2.94 mL of a 0.97 M solution in THF) was added into the initiator solution to form the alkoxide initiator. The color of the initiator solution turned yellow. The initiator solution was syringed into the Parr reactor with an additional 10 mL of THF while the reactor was stirring. The cooling bath was removed, and the reactor was allowed to warm to room temperature and was stirred for 24 h. The polymerization was terminated by adding acetic acid (0.34 mL of 0.45 g mL^{-1} solution in THF, 2.63 mmol) to the reactor via syringe to neutralize the polymer. The Parr reactor was purged with N_2 for 1 h, then opened. Its contents were transferred to a separatory funnel and 200 mL of dichloromethane was added. The product was washed twice with deionized water, and the aqueous layer was also extracted with dichloromethane to ensure that the product was recovered. The polymer solution was concentrated by evaporating ~85% of dichloromethane via rotary evaporator. The PEO was precipitated into cold diethyl ether and dried under vacuum at 40 ^{o}C overnight.

4.2.11. Synthesis of poly(ethylene oxide)-*b*-(D,L-lactide) with an ethylene glycol vinyl ether group at the PEO terminus and a hydroxyl group at the other end

The synthesis of a diblock copolymer with an ethylene glycol vinyl ether group at the PEO terminus follows a similar procedure to that described above for vinyldimethlysilylpropoxy-terminated PEO as the macroinitiator. An exemplary procedure for the synthesis of a diblock copolymer with a 7,100 g mol^{-1} ethylene glycol vinyl ether-terminated PEO and a 10,000 g mol^{-1} PDLLA block is provided. D,L-lactide (2.11 g, 0.01 mol) and 4 mL of THF were charged to a flame-dried, septum-sealed round-bottom flask with a magnetic stir bar to dissolve the lactide monomer. The ethylene glycol vinyl ether-functional PEO (1.5 g, 0.21 mmol) was placed into another flame-dried, round bottom flask with 3 mL of THF and sonicated for 30 minutes to dissolve the PEO. Then, potassium

naphthalide (0.20 mmol, 1 mol initiator: 0.95 mol base, 0.21 mL of a 0.97 M solution in THF) was added into the initiator solution to form the alkoxide initiator. After the D,L-lactide monomer was completely dissolved, the initiator solution was added into the flask via syringe. The reaction mixture was stirred at room temperature for 24 h. The reaction was quenched with acetic acid (0.05 mL of 0.20 g mL^{-1} solution in THF, 0.17 mmol) to neutralize the polymer. The polymer solution was transferred to a separatory funnel and 100 mL of dichloromethane was added. It was washed twice with deionized water and the aqueous phase was extracted with dichloromethane to recover the polymer from the aqueous phase. The resulting polymer solution was concentrated via rotary evaporator by removing ~85% of solvent. The product was isolated by precipitation into cold diethyl ether and vacuum-filtered. The copolymer was vacuum-dried at 40 ^{o}C overnight.

4.2.12. Functionalization of the ethylene glycol vinyl ether-functional poly(ethylene oxide)-*b*-(D,L-lactide) with mercaptoacetic acid

A 17,100 g mol^{-1} ethylene glycol vinyl ether-terminated poly(ethylene oxide)-*b*-(D,L-lactide) was functionalized with mercaptoacetic acid by a similar procedure to that described in 4.2.9. The copolymer with the vinyl ether group at the PEO terminus (1.2 g, 0.07 mmol) was added to a flame-dried, septum-sealed round bottom flask equipped with a magnetic stir bar and dissolved in 3 mL of deoxygenated toluene. Mercaptoacetic acid (0.08 mL of 0.16 g mL^{-1} solution in toluene, 0.14 mmol)) was added into the flask via syringe followed by the addition of AIBN (5.74 mg, 0.04 mmol) and 1 mL of toluene. The reaction mixture was deoxygenated by purging with N_2 for one hour. The reaction flask was placed into an oil bath at 80 ^{o}C for 24 h. The reaction mixture was dissolved in 150 mL of dichloromethane and transferred to a separatory funnel. It was washed with deionized water three times to remove excess mercaptoacetic acid. The polymer solution was concentrated by removing dichloromethane via rotary evaporation. The concentrated polymer was precipitated into cold

diethyl ether. The product was vacuum filtered and dried under vacuum at 40 ^{o}C for 18 hours to yield 1.23 g of the carboxylic acid functional diblock copolymer.

4.2.13. Diels-Alder addition of furan to maleic anhydride: Synthesis of 4,7,7-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione [126]

Maleic anhydride (30.0 g, 0.306 mol) was added to a flame-dried 500-mL round-bottom flask equipped with a magnetic stir bar, and this was followed by adding 150 mL of toluene. The solution was heated to 80 °C. Furan (33.4 mL, 0.459 mol) was added to the reaction flask via syringe and stirred at 80 °C for 18h. Then, the white crystals were collected via vacuum filtration and washed with 35-mL of hexane three times.

4.2.14. Reaction of ethanolamine with the anhydride: Synthesis of 4,7-Epoxyisobenzofuran-1,3-dione-4,7-Epoxy-1H-isoindole-1,3 (2H)-dione[126]

4,7,7-Tetrahydro-4,7-epoxyisobenzofuran-1,3-dione (15.05 g, 0.09 mol) was added to a flame-dried, three neck, 250-mL round-bottom flask equipped with a reflux condenser and a magnetic stir bar. Ethanolamine (5.5 mL, 0.90 mol) was dissolved in 30 mL of methanol, transferred to the reaction flask, and the solution was stirred for 30 min at room temperature, then refluxed for 18h. The reaction mixture was cooled to room temperature and the product crystallized from the solution. The reaction mixture was stored at 4 ^{o}C overnight. The crystals were collected via vacuum filtration and washed with 30 mL portions of methanol three times.

4.2.15. Reverse Diels-Alder reaction to yield the maleimide-functional initiator: Synthesis of *N*-(2-Hydroxyethyl)maleimide[126]

7-Epoxyisobenzofuran-1,3-dione-4,7-epoxy-1H-isoindole-1,3(2H)-dione (7.5 g, 0.03 mol) and 50 mL of toluene were added into a three neck, 250-mL, round-bottom flask equipped with a Dean Stark trap and a magnetic stir bar. The reaction mixture was refluxed

and stirred for 6 h. Toluene (10 mL) was removed from the Dean Stark trap and allowed to refill. The reaction mixture was hot filtered and the product crystallized from solution upon cooling. The crystals were collected via vacuum filtration.

4.2.16. Attempted Synthesis of PEO with a maleimide group at one end and a hydroxyl group at the other end (maleimide-PEO-OH)

A representative procedure for the synthesis of a 5,350 g mol^{-1} maleimide-PEO-OH using N-(2-hydroxyethyl)maleimide as the inititator is provided. N-(2-hydroxyethyl)maleimide (0.424 g, 3.0 mmol) was added into a 50-mL, flame-dried round-bottom flask equipped with a magnetic stir bar. The zinc hexacyanocoboltate (Impact 3) catalyst (0.60 mL of a 2.4 mg mL^{-1} dispersion) and 10 mL of THF were added to the flask and the initiator solution was stirred for 18 h prior to conducting the polymerization reaction. A 300-mL, high pressure Series 4561 Parr reactor, equipped with a thermocouple, mechanical stirrer and valve-controlled gas inlets and outlets was cooled to -50 °C using a dry-ice bath. Ethylene oxide (16.1 g, 0.36 mol) was distilled from a lecture bottle into the pressure reactor under 20 psi of nitrogen. The initiator solution was added to the reactor via syringe and this was followed by 5 mL of THF to wash the syringe. The pressure reactor was heated to 90 °C and the polymerization was allowed to proceed until a decrease in pressure was no longer observed (~3h). Then, the reaction was cooled to room temperature and purged with nitrogen for half an hour. The reactor was opened and its contents were transferred to a 400-mL beaker and diluted with 200 mL of dichloromethane. The resulting mixture was filtered through Celite® two times using dichloromethane as the eluent. The polymer solution in dichloromethane was concentrated via rotary evaporator and precipitated into cold diethyl ether. After collecting the product by vacuum-filtration, the polymer was dried under

vacuum at 40 °C for 24 hours yielding 14.4 g of maleimide-PEO-OH. Please note that this procedure yielded a PEO oligomer with a very wide molecular weight distribution, but the maleimide endgroup was retained.

4.2.17. Synthesis of PDLLA with a maleimide group at one end and a hydroxyl group at the other end (maleimide-PDLLA)

A representative procedure for synthesis of a 4,000 g mol^{-1} maleimide-terminated PDLLA utilizing N-(2-hydroxyethyl)maleimide as the initiator is provided. D,L-lactide (8.07 g, 0.06 mol) and 22 mL dry toluene were charged to a 250-mL flame-dried, round-bottom flask equipped with a magnetic stir bar. The flask was placed in an oil bath at 80 °C to dissolve the monomer. Then, N-(2-hydroxyethyl)maleimide (0.282 g, 2 mmol) was added into the flask. 1 mL of stannous octoate catalyst solution (6.2 mg/mL) in toluene was added to the flask and the temperature of the oil bath was raised to 100 °C. The polymerization was allowed to proceed for 24 h. The polymer was isolated by precipitation into cold diethyl ether and collected by vacuum filtration. The product was vacuum dried at 40 °C for 40 h and 7.10 g of polymer was obtained.

4.2.18. Functionalization of the maleimide-terminated PDLLA with cysteamine hydrochloride

A representative procedure of amino group addition across the double bond of the maleimide-terminated PDLLA via Michael addition is provided. A 4,000 g mol^{-1} maleimide-PDLLA was functionalized with the addition of cysteamine hydrochloride. Maleimide-PDLLA (1.07 g, 0.27 mmol) was added to a flame-dried, septum-sealed round-bottom flask equipped with a magnetic stir bar and dissolved in 3 mL of deoxygenated DMF. Cysteamine hydrochloride (0.091 g, 0.80 mmol), which was dissolved in 1 mL of DMF was added into the flask via syringe. The reaction flask was stirred at room temperature for 24 h. Then,

sodium hydroxide (0.032 g, 0.80 mmol) dissolved in 0.5 mL of de-ionized water was added into the flask and stirred for 20 minutes to neutralize the acid. 10 mL of de-ionized water was added to the reaction mixture and the polymer was extracted into methylene chloride (80 mL) to remove the excess cysteamine. The extraction was performed for three times to ensure that the polymer was extracted from the water and cysteamine hydrochloride was removed. The polymer solution was concentrated by removing dichloromethane via rotary evaporation. The concentrated polymer was precipitated into cold diethyl ether. The product was vacuum filtered and dried under vacuum at 40 ^{o}C for 18 hours to yield 0.77 g of the product.

4.3. Characterization

All ^{1}H NMR spectra were obtained on a Varian Unity 400 MHz NMR spectrometer operating at 400 MHz. The NMR parameters included a pulse width of 28.6° and a relaxation delay of 1.000 sec at ambient temperature. Samples were prepared by dissolving 50-60 mg in 0.8 mL of *d*-$CHCl_3$. 64 scans were acquired for obtaining the spectra.

Size exclusion chromatograms were obtained in HPLC grade chloroform at 30 °C on a Waters Alliance model 2690 chromatograph equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set. A Viscotek viscosity detector and a refractive index detector were utilized with polystyrene calibration standards to generate a universal molecular weight calibration curve for absolute molecular weight analyses. Samples were prepared by dissolving 20–25 mg in 10 mL of HPLC grade chloroform for obtaining the spectra.

4.4. Results and Discussion

4.4.1. Synthesis and characterization of 3-chloropropyldimethylvinylsilane

The heterobifunctional initiator, 3-HPMVS was prepared using a modified procedure originally developed by Vadala *et al*.[104, 127] Functional organosilanes have been prepared with different synthetic methods. Chlorosilanes are usually used as intermediates to prepare

organosilanes. Since silicon is more electropositive than carbon, nucleophilic substitution at silicon is facile. Therefore, chlorosilanes can be used to react with Grignard reagents or other organometallics to yield substitution at the silicon atom.[128]

In the first step of the initiator synthesis, vinyl magnesium bromide as a Grignard reagent was utilized to react with 3-chloropropyldimethylchlorosilane by a nucleophilic substitution for chlorine on the silicon atom (Figure 4.1).

Figure 4.1 Preparation of 3-chloropropyldimethylvinylsilane

A stoichiometric amount of vinylmagnesium bromide to silyl chloride was used for the first step of the reaction. Due to the high reactivity of vinylmagnesium bromide, the reaction was conducted at 0 °C, and it was continued for 24 hours to ensure complete substitution of the silyl chlorides. It was important to remove any unreacted Grignard reagent after the reaction was completed. Therefore, the reaction mixture was washed with saturated ammonium chloride solution. Any unreacted Grignard reagent would react with water, and the ammonium chloride salt facilitated separation of the two phases.

^{1}H NMR was used to determine the molecular structure of the product. The 3-chloropropyldimethylvinylsilane had proton integral ratios of 3:2:2:2:6 for the labeled protons (Figure 4.2).

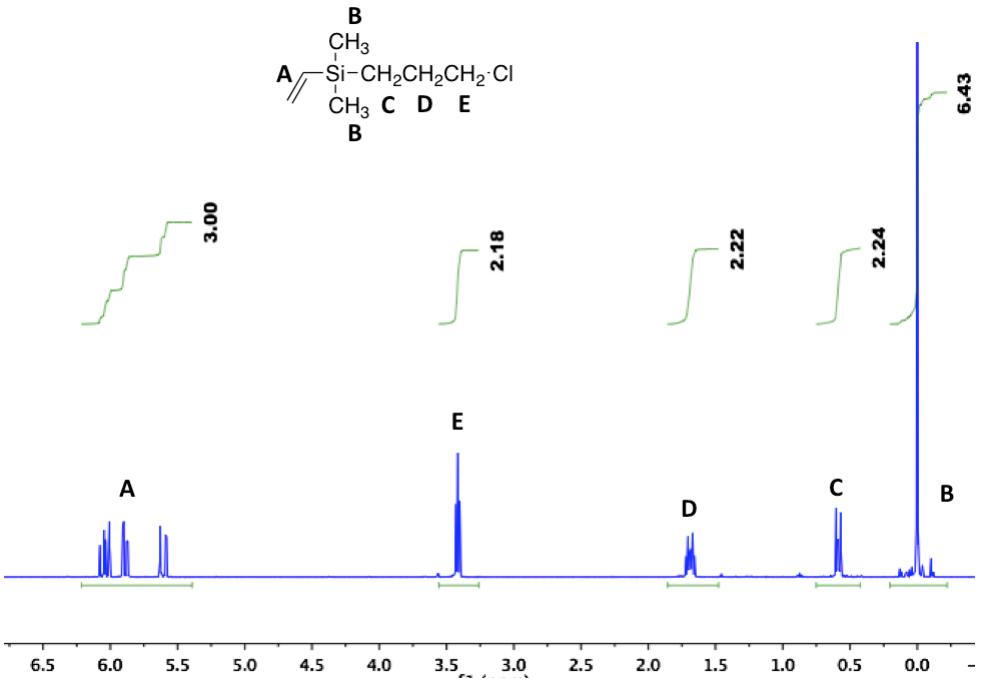


Figure 4.2 ^{1}H NMR spectrum of 3-chloropropyldimethylvinylsilane

4.4.2. Synthesis and characterization of 3-iodopropyldimethylvinylsilane

The second step of the synthesis of the vinylsilane initiator was transformation of an alkyl chloride to an alkyl iodide through S_N2 nucleophilic substitution. Due to the nucleophilic strength of the leaving group, which is the chloride anion, this reaction can be hindered. In comparing nucleophilic strength to basicity, it is observed that chloride has higher nucleophilicity than iodide. The chloride anion has a pK_b of 6.3 x 10^{-17} and iodide anion has a pK_b of 6.3 x 10^{-20}. Additionally, the C-I bond has a bond strength of 53 kcal mol^{-1} which is much weaker than the C-Cl bond with a bond strength 80 kcal mol^{-1}, so iodide is a better leaving group.[129]

The alkyl chloride was transformed to the alkyl iodide to obtain a more active product than the alkyl chloride. Acetone is a good solvent for sodium iodide, while it is a poor solvent for sodium chloride. Hence, when the reaction is carried out in acetone, the reaction equilibrium is shifted to the alkyl iodide. In order to ensure the substitution of iodide, excess

NaI was used. The reaction scheme of the conversion of the alkyl chloride to alkyl iodide is shown in Figure 4.3.

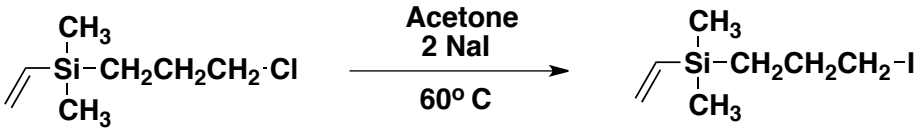


Figure 4.3 Preparation of 3-iodopropyldimethylvinylsilane

^{1}H NMR was used to monitor the substitution reaction. There was an increase in the integral ratio of the peak at 3.2 ppm corresponding to the methylene attached to iodine, relative to the peak at 3.6 ppm corresponding to the methylene bonded to the chlorine. The molecular structure of the product was confirmed by examining the protons based on the integral ratio of vinyl protons to methylenes. 3-Iodopropyldimethylvinylsilane has the proton integral ratios as 3:2:2:2:6 for the labeled protons (Figure 4.4).

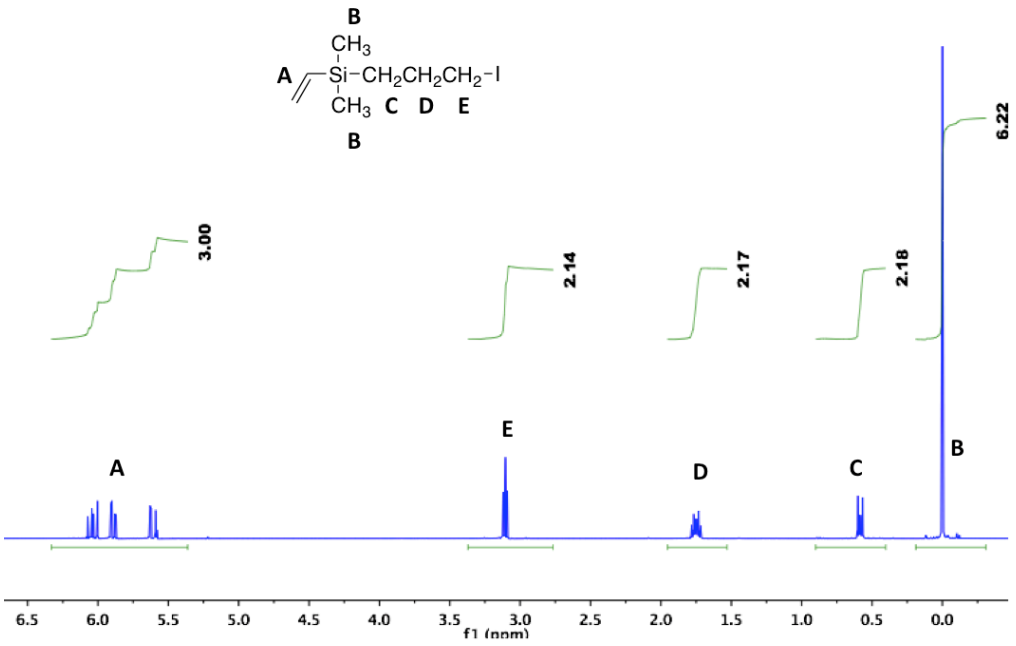


Figure 4.4 ^{1}H NMR spectrum of 3-iodopropyldimethylvinylsilane

4.4.3. Synthesis and characterization of 3-hydroxypropyldimethylvinylsilane

In the last step, 3-iodopropyldimethylvinylsilane was converted to 3-hydroxypropyldimethylvinylsilane through an S_N2 reaction. This was utilized as the initiator for the synthesis of PEO. There are several methods for conversion of alkyl halides to alcohols. Hutchins *et al.* conducted the synthesis of alcohols from alkyl halides, such as alkyl chlorides and alkyl iodides. It was shown that when they combined a polar aprotic solvent such as NMP or HMPA with sodium bicarbonate, it could act as a good source of nucleophilic oxygen. They also reported that 1-iodooctane was successfully converted to the alcohol in the presence of HMPA with a conversion higher than 90%, while 1-chlorooctane reached a conversion less than 50% conversion.[129] For that reason, 3-chloropropyldimethylvinylsilane was initially converted to 3-iodopropyldimethylvinylsilane and then that was transformed to 3-hydroxypropyldimethylvinylsilane. The reaction was conducted in HMPA at 100 °C. Sodium bicarbonate served as an acid scavenger to neutralize the HI by-product. It should be noted that the sodium bicarbonate is not soluble in the reaction medium in the beginning. As the reaction continues, it becomes soluble due to the production of HI in situ. The reaction scheme is shown in Figure 4.5.

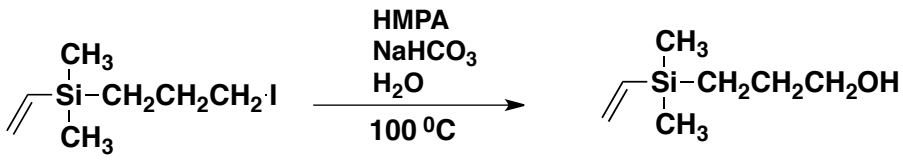


Figure 4.5 Preparation of 3-hydroxypropyldimethylvinylsilane

The molecular structure of the product was analyzed by ^1H NMR. After the substitution, the methylene peak next to the iodine at 3.2 ppm was shifted to 3.6 ppm. The integral ratios of

the product closely matched the theoretical proton ratios of 3:2:2:2:6 (Figure 4.6).

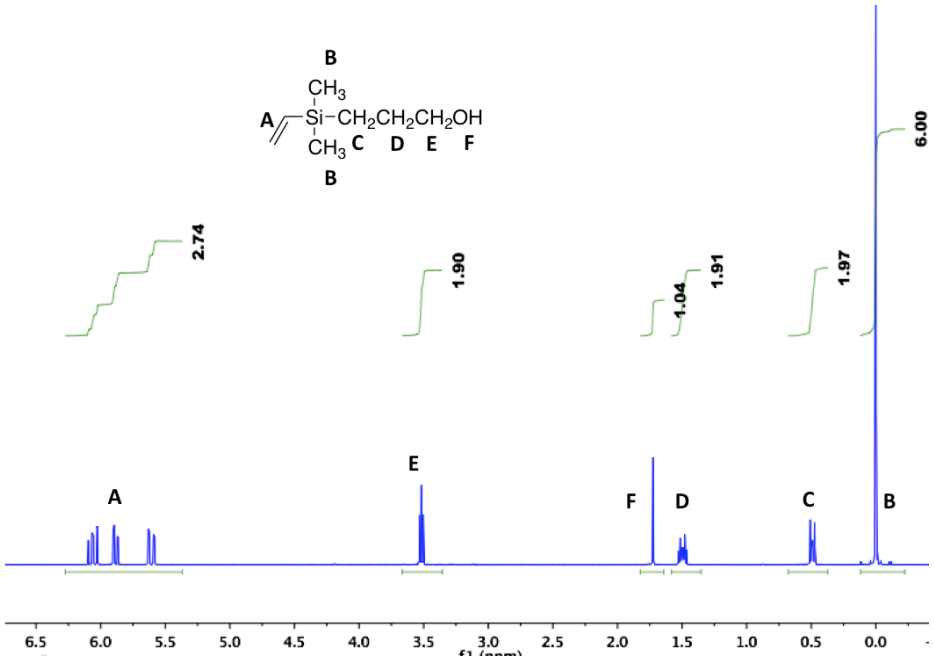


Figure 4.6 ^{1}H NMR spectrum of 3-hydroxypropyldimethylvinylsilane

4.4.4. Synthesis and characterization of vinyldimethylsilylpropoxy-functional poly(ethylene oxide) oligomers

It is highly desirable to synthesize polymers with terminal functionality, which allows the post-polymerization modifications. Ethylene oxide can be initiated by a number of different initiators such as alkoxides, hydroxides, oxides, and metal alkyls and aryls. In this case, 3-hydroxypropyldimethylvinylsilane, which allows the post-addition of a variety of functional groups, was utilized as the initiator for the polymerization of ethylene oxide. Two methods were attempted for preparing vinyldimethylsilylpropoxy-functional PEO.

In one method, a coordination catalyst, zinc hexacyanocobaltate, (a double metal cyanide heterogeneous catalyst), $Zn_3[Co(CN)_6]_2$ was utilized to polymerize ethylene oxide (Figure 4.7). This non-basic double metal cyanide coordination catalyst has been established to be good for the synthesis of poly(propylene oxide) with low unsaturation, and fast rates of propagation with low catalyst concentrations.[130] The polymerization was conducted at 105 ^{o}C.

Before the precipitation of the polymer into diethyl ether, it was important to filter the polymer solution through celite to remove the catalyst.

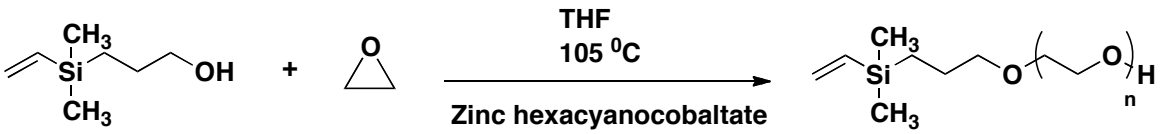


Figure 4.7 Synthesis of vinyldimethylsilypropoxy-PEO-OH via coordination polymerization

As determined from ^{1}H NMR, the composition was very close to the targeted composition based on the monomer to initiator ratio (Figure 4.8). The molecular weight calculated from ^{1}H NMR was 2,860 g mol^{-1}, while the targeted molecular weight was 2,990 g mol^{-1}.

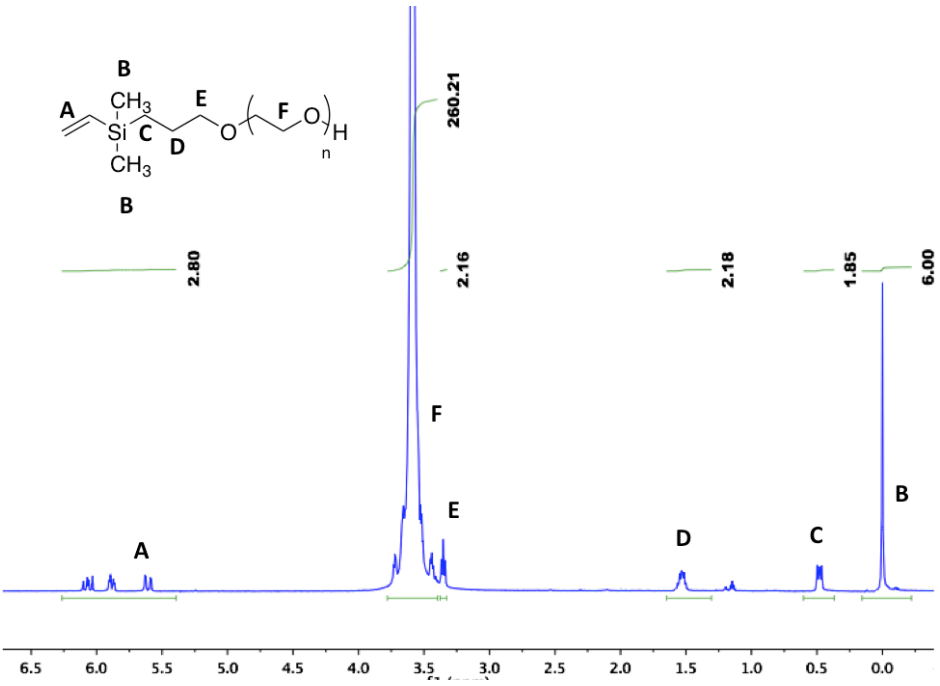


Figure 4.8 ^{1}H NMR spectrum of vinyldimethylsilylpropoxy-PEO-OH via coordination polymerization

SEC was utilized to determine the molecular weight distribution of the polymer.

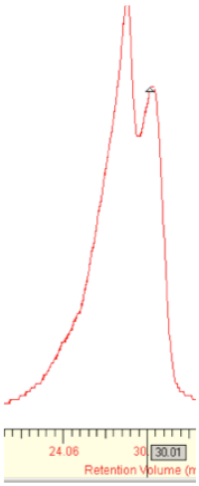


Figure 4.9 SEC curve of vinyldimethylsilylpropoxy-PEO-OH via coordination polymerization

However, SEC analysis revealed a bimodal molecular distribution. The relatively broad molecular weight distributions obtained utilizing this double metal cyanide coordination catalyst can likely be attributed to a combination of the heterogeneous nature of the catalyst and few active chains in the initial stages of the polymerization. These conditions combined with the fast rate of propagation could lead to the broad molecular weight distributions.[131]

Anionic ring-opening polymerization of ethylene oxide typically yields PEO with narrow molecular weight distributions. The reaction scheme of the synthesis of a heterobifunctional PEO oligomer with a vinylsilyl terminus and a hydroxyl end group is shown in Figure 4.10.

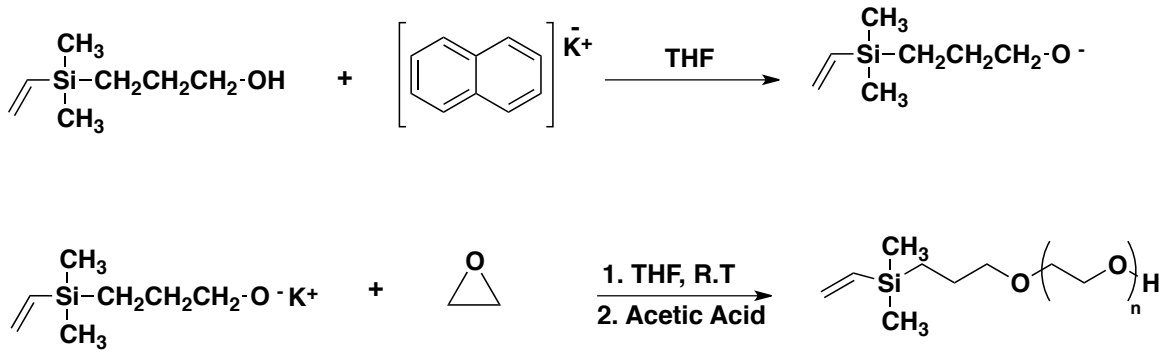


Figure 4.10 Synthesis of vinyldimethylsilylpropoxy-PEO-OH via anionic ring-opening polymerization

Potassium naphthalide was utilized to react with the initiator to form an alkoxide in order to initiate ethylene oxide. The reaction was conducted with a small deficiency of potassium naphthalide relative to alcohol to ensure that only alkoxide would initiate the ethylene oxide. The initiator to base ratio was 1:0.95 mole ratio. The polymerization was conducted under 30 psi at room temperature. When almost all of the ethylene oxide was polymerized, the pressure dropped to around 20 psi. It is important to note that the polymerization was quenched with acetic acid before opening the reactor to avoid any oxidative side reactions, then, the polymer solution in dichloromethane was washed with water for two times to remove the potassium acetate.

End group analysis was performed via ^{1}H NMR to ensure that the end groups were preserved during the polymerizations. The ratio of end group protons closely matched with theoretical values. Figure 4.11 shows that the end groups remained intact under the basic reaction conditions.

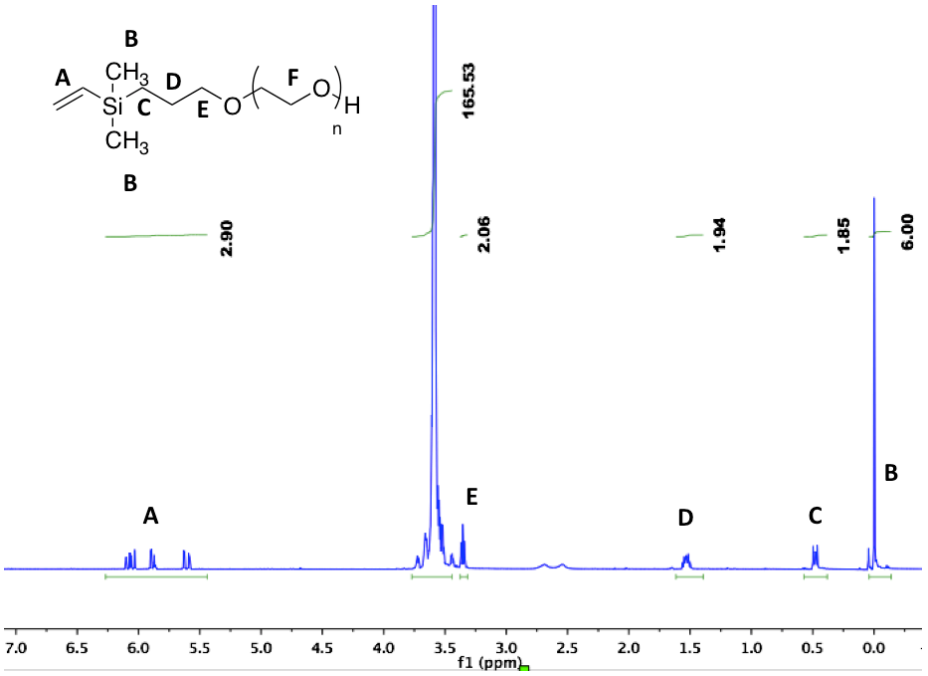


Figure 4.11 ^{1}H NMR of vinyldimethylsilylpropoxy-PEO-OH via anionic polymerization

Both ^{1}H NMR and SEC were utilized to examine the molecular weight of the heterobifunctional PEO oligomers. The number average molecular weight was determined by using end group resonances via ^{1}H NMR. While the targeted molecular weight was 2,105 g mol^{-1}, the molecular weight calculated from ^{1}H NMR was 1,820 g mol^{-1}. Moreover, the molecular weight determined by SEC was 1,710 g mol^{-1}, which was consistent with targeted molecular weight. The SEC yielded a monomodal peak with a narrow molecular weight distribution (Figure 4.12). A series of vinyldimethylsilylpropoxy-PEO-OH oligomers were synthesized by this method (Table 4.1). Good agreement in molecular weight was obtained by both methods of analysis.

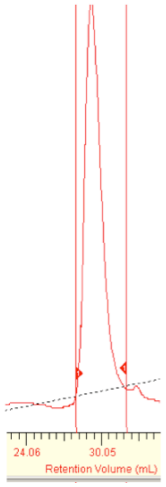


Figure 4.12 SEC chromatogram of a vinyldimethylsilylpropoxy-PEO oligomer showing a M_n of 2,105 g mol^{-1} and a polydispersity of 1.07

Table 4-1 A summary of molecular weights and molecular weight distributions of vinyldimethylsilylpropoxy-PEO-OH oligomers

Target M_n [a] (g/mol)	M_n [b] (g/mol)	M_n [c] (g/mol	PDI [c]
2100	1800	1700	1.07
5000	5600	4300	1.16
7000	6300	6100	1.08
7900	7000	6300	1.09

[a]Calculated from monomer to initiator ratio. [b]Calculated from ^{1}H NMR.
[c]Determined via SEC

4.4.5. Synthesis and characterization of vinyldimethylsilylpropoxy-poly(ethylene oxide-*b*-D,L-lactide)

Amphiphilic block copolymers consisting of PEO and PLA have great potential for a number of biomedical applications. They can be obtained using lactide as a monomer and PEO as a macroinitiator and the copolymerization can yield di- or tri-block copolymers. In this particular case, heterobifunctional PEO with one vinyl terminus and a hydroxyl terminus was used as the macroinitiator to initiate lactide monomer.

The synthesis of vinyldimethylsilylpropoxy-poly(ethylene oxide-*b*-D,L-lactide) copolymer follows a similar procedure as described for the synthesis of vinyldimethylsilylpropoxy-PEO-OH oligomers. The reaction was conducted with a small deficiency of potassium naphthalide relative to the PEO macroinitiator, so only alkoxide was available to initiate chains. It is also noteworthy that the potassium naphthalide base solution was prepared just prior to the polymerization reaction to avoid gradual contamination with moisture. After transferring the initiator solution into the reaction flask, the polymerization was conducted at room temperature for 24 hours. Figure 4.13 shows the reaction scheme for preparing the heterobifunctional diblock copolymer.

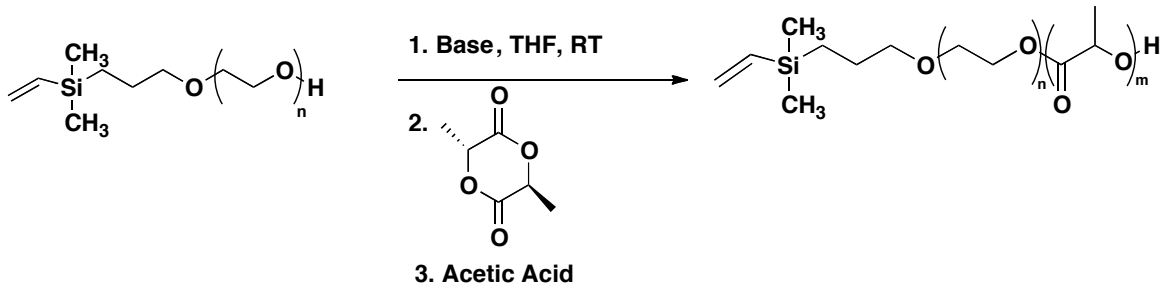


Figure 4.13 The polymerization of D,L lactide using a vinyldimethylsilylpropoxy functional PEO as the macroinitiator

End group analysis was performed via ^{1}H NMR to analyze the molecular structure of the products and calculate the molecular weights. Figure 4.20 depicts a ^{1}H NMR spectrum of vinyldimethylsilylpropoxy-functional PEO-*b*-PDLLA. The ratio of the end groups closely matched the theoretical values. The number average molecular weights of the copolymers were calculated according to the proton ratio of the end group to backbone. According to ^{1}H NMR, the molecular weight of a PLA block was calculated to be 7,560 g mol^{-1} and PEO block was 1,770 g mol^{-1}, while the targeted molecular weight of the PLA block was 8,100 g mol^{-1}. Figure 4.14 depicts the SEC chromatogram of this vinyldimethylsilylpropoxy-functional PEO-*b*-PDLLA copolymer showing a M_n of 10,000 g mol^{-1}.

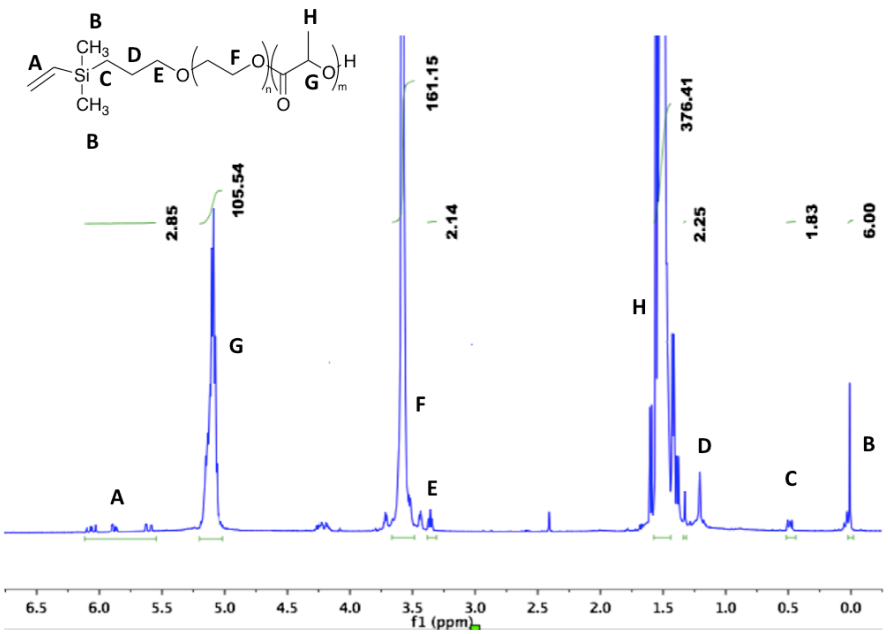


Figure 4.14 [1]H NMR spectrum of a vinyldimethylsilylpropoxy-functional PEO-*b*-PDLLA

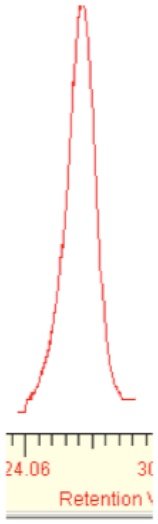


Figure 4.15 SEC chromatogram of a vinyldimethylsilylpropoxy-functional PEO-*b*-PDLLA

4.4.6. Synthesis and Characterization of vinyldimethylsilylpropoxy-poly(ethylene oxide-*b*-D,L-lactide) copolymers with carboxylic acid at one end

Ene-thiol addition was used to introduce a carboxylic acid onto the vinyldimethylsilylpropoxy-functional PEO-*b*-PDLLA under free radical conditions. Since the

vinylsilyl functionality does not polymerize readily, it is a good moiety for ene-thiol post-modification reactions.

Utilizing an excess of the thiol relative to vinylsilane ensures quantitative addition of the carboxylic acid. The reaction was conducted at 80 °C in toluene using AIBN as the free radical initiator (Figure 4.16). To avoid inhibition of the free radical process due to oxygen, it was important to deoxygenate the reaction system before starting the reaction.

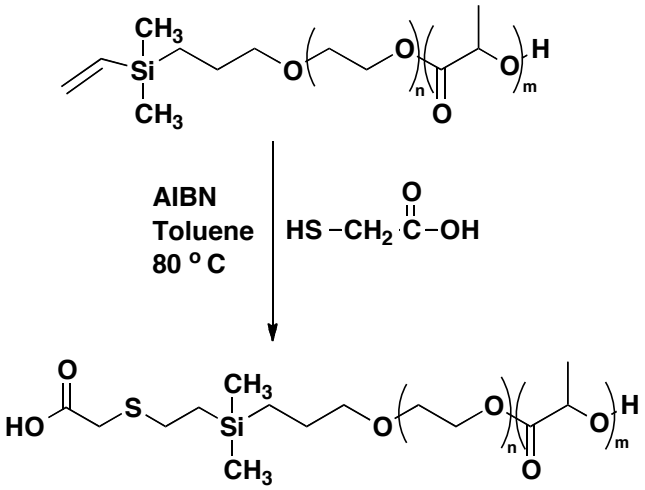


Figure 4.16 Functionalization of vinylsilylpropoxy-functional PEO-*b*-PDLLA with mercaptoacetic acid

^{1}H NMR was utilized to monitor addition of mercaptoacetic acid to the diblock copolymer across the vinyl end group by following the disappearance of vinyl protons at around 6 ppm. At the same time, the appearance of new methylene peaks confirmed the addition of mercaptoacetic acid. Figure 4.17 shows a ^{1}H NMR spectrum of a HOOC-PEO-*b*-PDLLA block copolymer after the addition of mercaptoacetic acid.

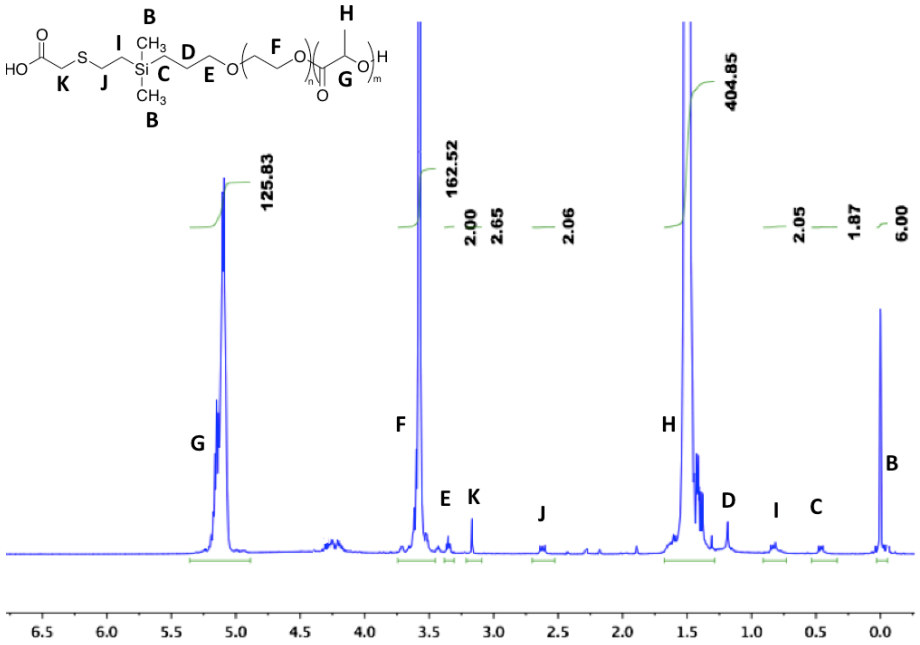


Figure 4.17 Ene-thiol addition of mercaptoacetic acid to a vinyldimethylsilylpropoxy-functional PEO-*b*-PDLLA diblock copolymer

4.4.7. Synthesis and Characterization of poly(ethylene oxide-*b*-D,L-lactide) copolymers with an ethylene glycol vinyl ether group at one end and a hydroxyl group at the other end

The synthesis of heterobifunctional PEO oligomers with a vinyl end group can also be synthesized by using commercially available ethylene glycol vinyl ether as an initiator. The preparation of a 7,100 g mol^{-1} PEO oligomer utilizing ethylene glycol vinyl ether follows a similar procedure to that described in 4.2.7. The reaction was conducted at room temperature for 24 hours, and quenched with acetic acid (Figure 4.18).

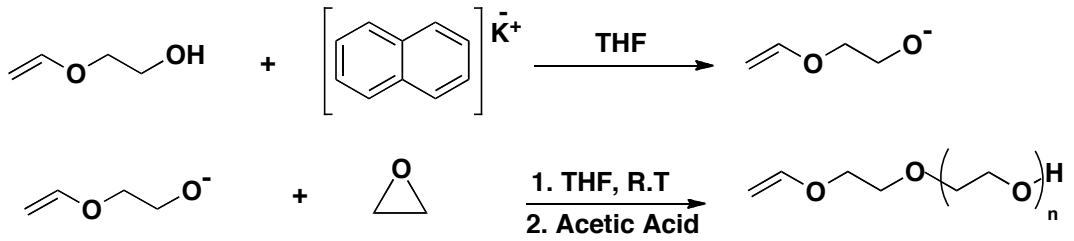


Figure 4.18 Synthesis of ethylene glycol vinyl ether initiated-PEO via anionic ring-opening polymerization

End group analysis via ^{1}H NMR showed that the end group remained intact and the molecular weight was well controlled (Figure 4.19).

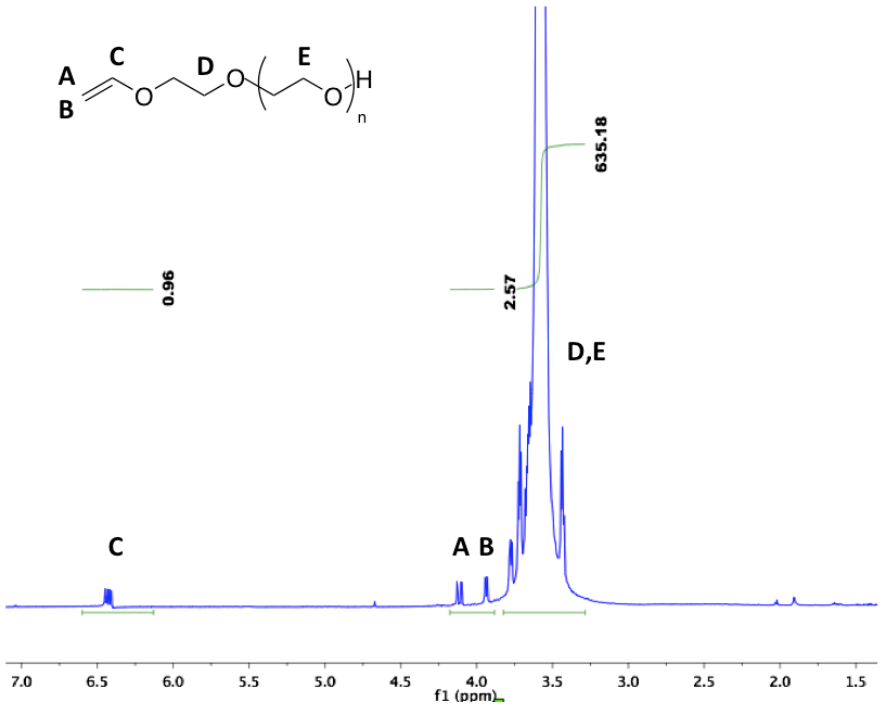


Figure 4.19 ^{1}H NMR spectrum of a 7,100 g mol^{-1} ethylene glycol vinyl ether-initiated PEO

Synthesis of a PEO-*b*-PDLLA diblock copolymer with vinyl ether group at the PEO terminus was performed utilizing ethylene glycol vinyl ether-functional PEO as a macroinitiator (Figure 4.20). End group analysis via ^{1}H NMR showed that the molecular

weight was 16,460 g mol^{-1} (Figure 4.27). Additionally, SEC showed a molecular weight distribution of 1.3 and indicated a number average molecular weight of 15,100 g mol^{-1}.

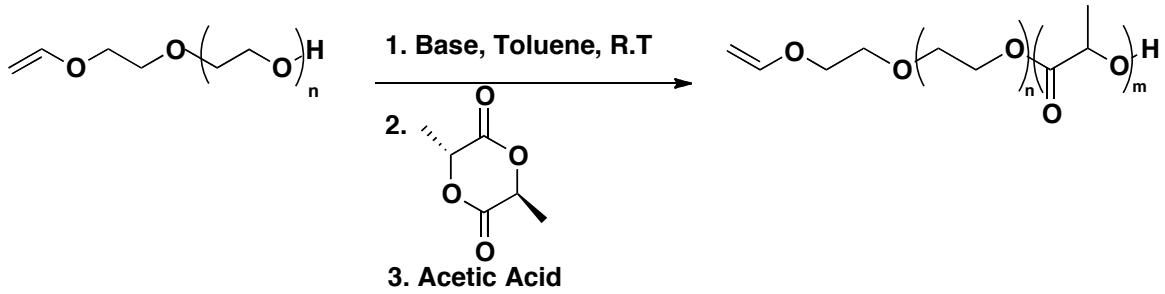


Figure 4.20 Polymerization of D,L-lactide using ethylene glycol vinyl ether-functional PEO as the macroinitiator

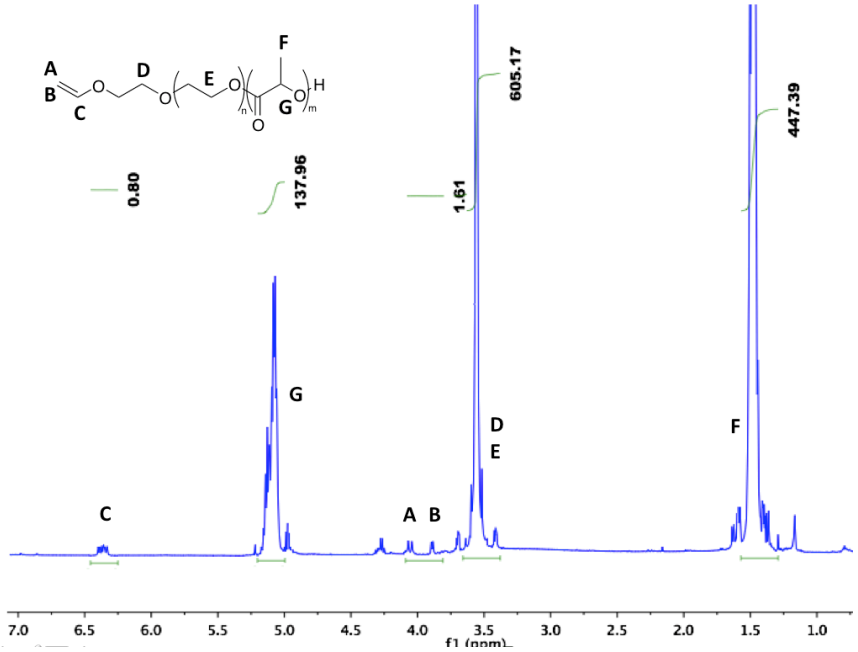


Figure 4.21 ^{1}H NMR spectrum of ethylene glycol vinyl ether-functional PEO-*b*-PDLLA

4.4.8. Synthesis and Characterization of ethylene glycol vinyl ether- functional poly(ethylene oxide-b-D,L-lactide) copolymers with carboxylic acid at one end

Ene-thiol chemistry was also utilized to functionalize the vinyl ether terminus of the PEO-*b*-PDLLA diblock copolymer. A carboxylic acid was added across the vinyl group at

the PEO terminus by adding mercaptoacetic acid in the same manner as described in 4.2.9 (Figure 4.22).

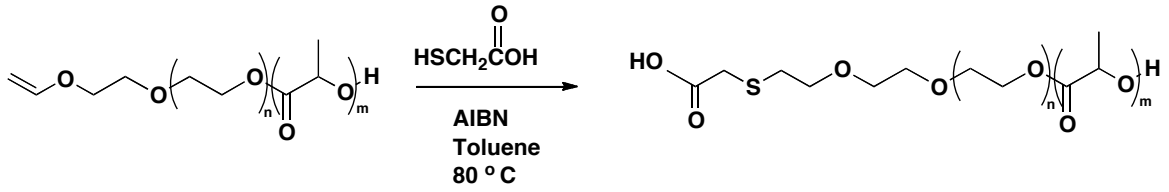


Figure 4.22 Functionalization of vinyl ether-functional PEO-*b*-PDLLA with mercaptoacetic acid

^{1}H NMR was utilized to monitor the addition of mercaptoacetic acid to the diblock copolymer by following the disappearance of the vinyl protons around 6 ppm and the appearance of the new methylene peaks.

4.4.9. Synthesis of N-(2-Hydroxyethyl)maleimide

The heterobifunctional initiator, N-(2-hydroxyethyl)maleimide, was synthesized in three steps (Figure 4.23). First, the double bond of maleic anhydride was protected through a Diels-Alder reaction with furan.[126] In the second step, ethanolamine was added to the anhydride and dehydrated to form the imide. The reaction was conducted under anhydrous conditions to avoid any side reactions with moisture. The product crystallized from the solution and was collected via vacuum-filtration, whereas unreacted reagents remained in the methanol solvent. In the last step, deprotection of the double bond was carried out in toluene. Since the protected alcohol was insoluble in toluene, the protected starting material was removed through hot-filtration of the reaction mixture. Then, the deprotected product was crystallized from the resulting solution, yielding the initiator to utilize for polymerization of ethylene oxide.[132] ^{1}H NMR was used to examine the structure of the product and confirmed that the initiator had been successfully prepared (Figure 4.24).

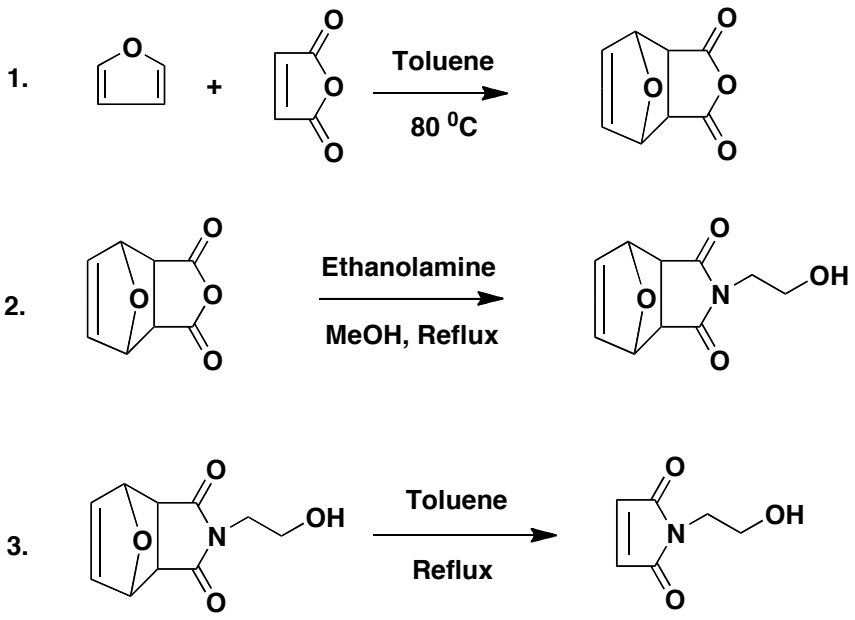


Figure 4.23 Synthesis of N-(2-hydroxyethyl)maleimide

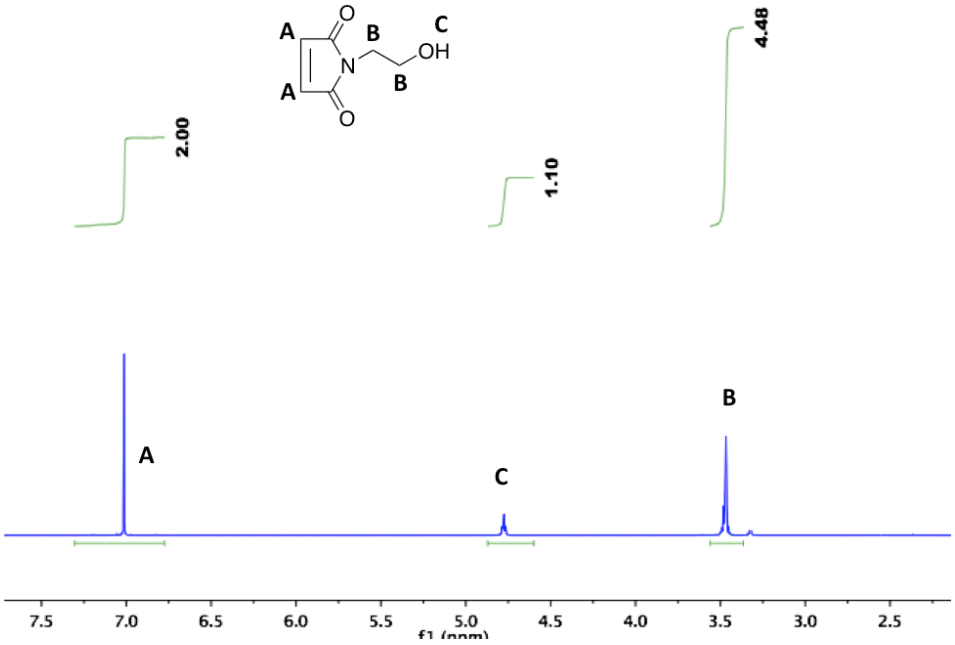


Figure 4.24 ^{1}H NMR Spectrum of N-(2-hydroxyethyl)maleimide

4.4.10. Synthesis and Characterization of PEO with a maleimide group at one end and a hydroxyl group at the other end (maleimide-PEO-OH)

Conjugation of biomolecules to the hydrophilic end of amphiphilic block copolymers is often desirable. It is well established that maleimides react under mild conditions with a variety of functional groups such as thiols in water.[133-135] Therefore, PEO oligomers which have a maleimide group at one end and another functional group at the other end have been used in targeting drug delivery applications.[136] To this end, we attempted to use the N-(2-hydroxyethyl)maleimide as an initiator for ethylene oxide.

A double metal cyanide zinc hexacyanocobaltate catalyst was used to prepare heterobifunctional PEO with a maleimide group at one end and a hydroxyl group at the other end.[137] Because of the sensitivity of the N-(2-hydroxyethyl)maleimide initiator towards base, it was reasoned that the coordination catalyzed reaction would be preferred to retain the maleimide functionality.

The initiator was combined with the catalyst and allowed to adsorb onto the heterogeneous catalyst particles for approximately 24 hours at room temperature before the polymerization.[131] The polymerization was conducted in a Parr pressure reactor at 90 °C until a decrease in pressure was no longer observed (~3 hours). Figure 4.25 depicts the synthetic scheme.

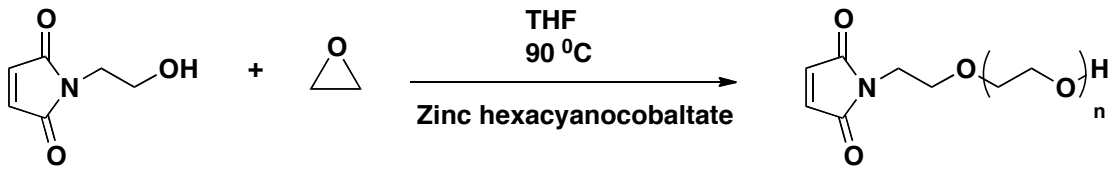


Figure 4.25 Synthesis of Maleimide-Functional PEO

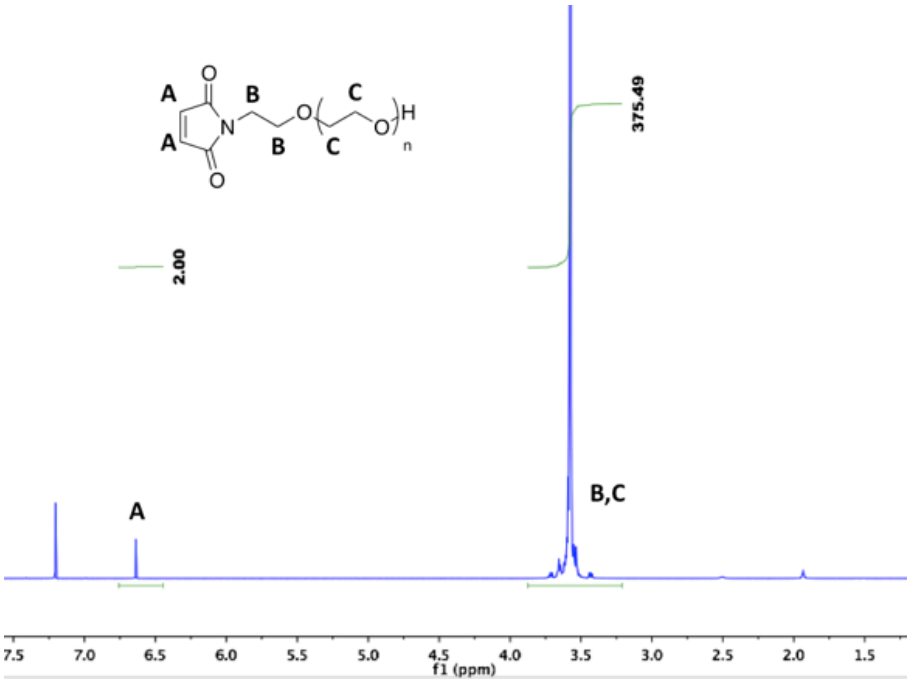


Figure 4.26 ^{1}H NMR Spectrum of Maleimide-Functional PEO

The structure of the product was examined by ^{1}H NMR. It was confirmed that the maleimide end group withstood the polymerization (Figure 4.26). According to ^{1}H NMR analysis, the molecular weight was calculated to be 4,130 g mol^{-1}, while the targeted molecular weight based on to monomer to initiator ratio was 5,350 g mol^{-1}. Unfortunately, the SEC chromatogram showed a multimodal distribution. Figure 4.27 depicts the SEC curve showing the broad molecular weight distribution of the resulting polymer.

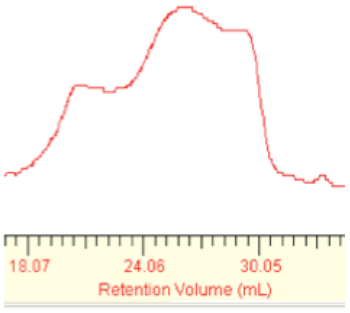


Figure 4.27 Size Exclusion Chromatogram of Maleimide-Functional PEO

The broad molecular weight distribution obtained in this polymerization could be a result of the same reasons described in 4.4.4. The heterogeneous nature of the catalyst as well as the fast rate of propagation relative to initiation could lead to broad molecular weight distribution. Since base-catalyzed reaction of ethylene oxide using N-(2-hydroxyethyl)maleimide as the initiator is not a desirable method, no further polymerization reactions of ethylene oxide with this initiator were conducted.

4.4.11. Synthesis and Characterization of PDLLA with a maleimide group at one end and a hydroxyl group at the other end (maleimide-PDLLA)

Poly(lactide) bearing a maleimide functional group was synthesized by ring-opening polymerization of lactide using the heterobifunctional initiator, N-(2-hydroxyethyl)maleimide catalyzed by stannous octoate (Figure 4.28). The polymerization was conducted under anhydrous conditions to avoid any side reactions with the esters caused by moisture.

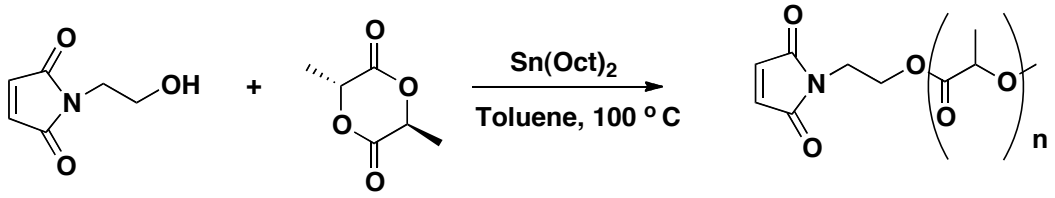


Figure 4.28 Synthesis of Maleimide-terminated PDLLA

End group analysis was performed via ^{1}H NMR to analyze the structure of the product and calculate the molecular weight (Figure 4.29). The ^{1}H NMR spectra confirmed that the maleimide end group was retained during the polymerization. The molecular weights of the polymers were consistent with the targeted values based on the monomer to initiator ratio, not the monomer to catalyst ratio.

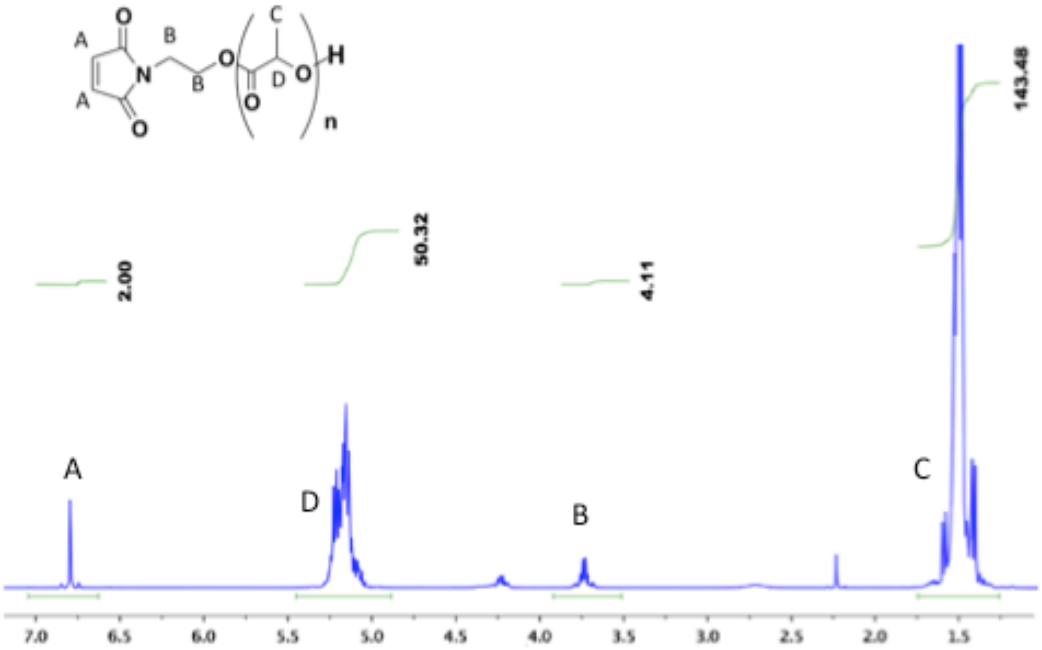


Figure 4.29 ^{1}H NMR Spectrum of Maleimide-PDLLA

The molecular weight calculated by ^{1}H NMR was 4,300 g mol^{-1}, while the targeted molecular weight was 4,000 g mol^{-1}. Furthermore, SEC yielded a symmetric monomodal peak with the number average molecular weight of 4,000 g mol^{-1} and a poldispersity index of 1.12 (Figure 4.30). The fact that both methods of analysis showed good agreement on molecular weight suggests that these polymerizations were well controlled.

Figure 4.30 SEC Chromatogram of Maleimide-PDLLA showing a M_n of 4000 g mol^{-1}

Table 4-2 A summary of molecular weights and molecular weight distributions of maleimide-functional PDLLA oligomers

Target M_n [a] (g/mol)	M_n [b] (g/mol)	M_n [c] (g/mol	PDI [c]
2000	2300	2100	1.11
4000	4300	4000	1.12
10000	9300	10000	1.16

[a]Calculated from monomer to initiator ratio. [b]Calculated from ^{1}H NMR [c]Determined via SEC

4.4.12. Synthesis and Characterization of PDLLA with a an amino group at the other end through modification of the maleimide end group

Post-polymerization modification of polymers provides a method for introduction of different functional groups. Maleimides offer efficient methods for functionalization via 1,4-addition of a thiol over the vinyl bond. The selective nature of these synthetic methods has led to maleimide bearing polymers being used in a wide range of biological applications to conjugate biomolecules.[133] To this end, end group modification was performed via Michael addition. Cysteamine hydrochloride was utilized as a thiol to introduce an amino group

across the maleimide double bond (Figure 4.31). An excess of thiol relative to maleimide was used for these reactions to ensure quantitative addition.

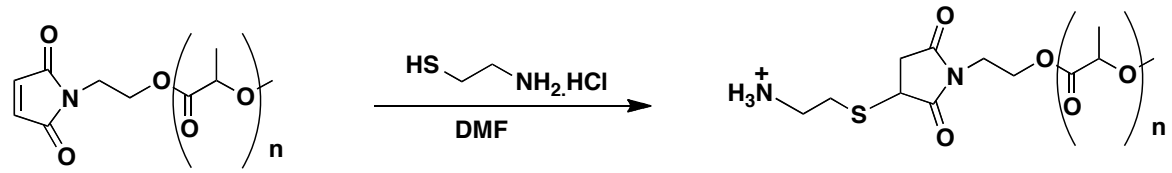


Figure 4.31 Cysteamine Addition to Maleimide-PDLLA

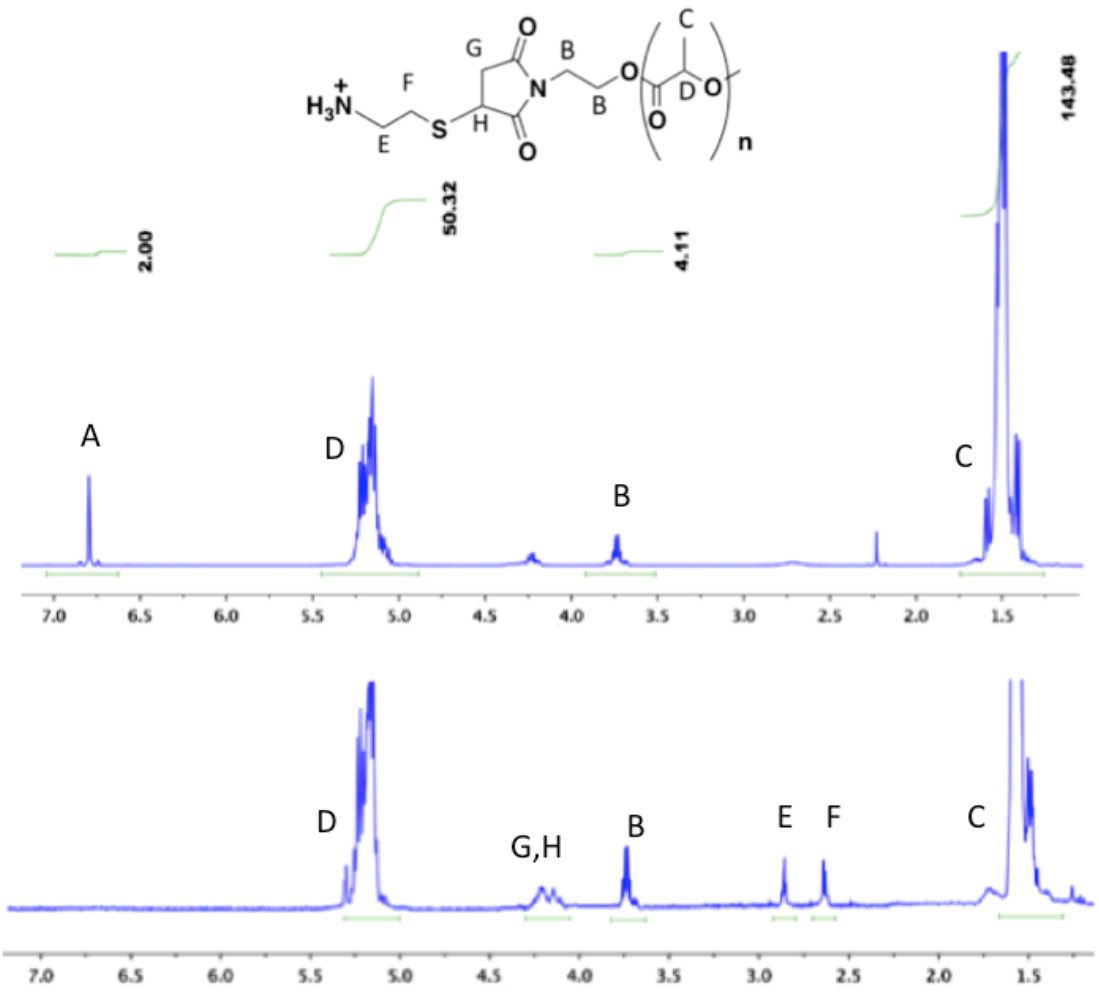


Figure 4.32 ^{1}H NMR of (A) Maleimide-PDLLA, and (B) Cysteamine Addition to Maleimide-PDLLA

^{1}H NMR was used to monitor the addition of cysteamine hydrochloride onto the maleimide end group of maleimide-functional PDLLA by observing the disappearance of the peak around 6.8 ppm, and appearances of methylene peaks between 2.5-3.0 ppm (Figure 4.32). The reaction between cysteamine hydrochloride and maleimide-PDLLA was demonstrated to be a quantitative and mild method for end group modification of these polymers due to the high reactivity of the maleimide group.

CHAPTER 5 : Conclusions and Recommendations for Future Work

Well-defined poly(lactide) homopolymers and poly(ethylene oxide-*b*-lactide) copolymers with controlled molecular weights in each block were prepared through sequential ring-opening polymerizations. Their characterization was described via ^{1}H NMR and SEC.

Chapter 3 discussed the synthesis and characterization of multifunctional polylactide nanoparticles that can integrate magnetite and therapeutic agent for magnetic resonance imaging and antiretroviral therapy. PEO-*b*-PDLLA copolymer was used as a polymer nanocarrier to encapsulate the HIV protease inhibitor, Ritonavir as a model drug within magnetite nanoparticles. Additionally, PLLA homopolymer was incorporated into the drug-copolymer nanoparticles in the nanoprecipitation step to serve as a nucleating agent. It was hypothesized that the rates of release of drugs from hydrophobic core would be slower if the glass transition temperature of the nanoparticle core was above 37 °C. In order to investigate the effect of polyester core chemistry on drug release, an amorphous, partially cycloaliphatic copolyester, TMCBD-CHDC that had a higher glass temperature and higher hydrophocity than PLLA was prepared. Particle sizes were measured by dynamic light scattering, while the iron oxide content of nanoparticles was determined by thermogravimetric analysis. It was shown that the multifunctional nanoparticles had sized ranging between 100-160 nm and narrow size distributions. The drug loading of TMCBD-CHDC/PEO-*b*-PDLLA copolymer nanoparticles was higher than the drug loading in PLLA/PEO-*b*-PDLLA nanoparticles. The quantitative incorporation of magnetite, and therapeutically useful drug loadings, and well-defined polymeric nanoparticles make these multifunctional nanoparticles potentially suitable for HIV therapeutics. Since PDLLA and PLLA are steroisomeric, these core-shell delivery

vehicle is an ideal system to investigate the polymer morphology, drug binding and release. Further research will be required to understand more of the parameters involved in particularly the rate of drug release from the hydrophobic core.

Chapter 4 described the synthesis and characterization of heterobifunctional oligomers, which were prepared utilizing functional alcohols bearing vinylsilane, vinylether, and maleimide moieties. Two methods were attempted for preparing vinylsilane-functional PEO. In one method, a double metal cyanide coordination catalyst was used for the synthesis of PEO with vinylsilane end group at one end, while potassium naphthalide was utilized to react with the initiator to initiate the ethylene oxide in another method. Since the coordination catalyst produced PEO oligomers with broad molecular weight distributions, base-catalyzed PEO oligomers were utilized as macroinitiator for the synthesis of heterobifunctional PEO-*b*-PDLLA diblock copolymers via anionic ring-opening polymerization. The amphiphilic diblock copolymers bearing vinylsilane and vinyl ether end group were functionalized with mercaptoacetic acid via ene-thiol addition reactions. Thus, these carboxylic acid functional diblock copolymers could be potential to complex to magnetite nanoparticles. On the other hand, because of the sensitivity of the maleimide-functional initiator toward base, the coordination-catalyzed reaction was preferred to synthesize PEO oligomers with a maleimide end group. However, no further polymerization reactions of ethylene oxide with this initiator were conducted, since the broad molecular weight distributions were obtained with the same catalyst. This indicates that the heterogeneous nature of the coordination catalyst and the fast rate of propagation could lead to broad molecular weight distributions. Maleimide termini could potentially be used to bind a variety of biomolecules via thiol-containing cysteine residues. Therefore, the synthesis of well-defined PEO oligomers bearing a maleimide group at one end will need to be synthesized utilizing protected maleimide initiator via anionic ring-opening polymerization to retain maleimide functionality. Further study will be to utilize the

carboxylic acid functional PEO-*b*-PDLLA diblock copolymers and amino functional PDLLAs for conjugating targeting moieties and encapsulation of an array of bioactive molecules.

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