SYNTHESIS OF PEGYLATED POLY(LACTIC ACID)
VIA RADICAL COUPLING

A Thesis
Presented to
The Graduate Faculty of The University of Akron

In Partial Fulfillment
Of the Requirement for the Degree
Master of Science

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May 2015
SYNTHESIS OF PEGYLATED POLY(LACTIC ACID)
VIA RADICAL COUPLING

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Thesis

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ABSTRACT

Poly(lactic acid) (PLA) plays an important role in biodegradable polymers due to its unique properties. However, PLA only has reactive end groups; therefore it is difficult to make bulk modifications and an efficient way to functionalize the main-chain of this polyester needs to be found. 2-Bromo-3-hydroxypropanoic acid (BHPA) is a halogenated constitutional isomer of lactic acid. In this thesis, BHPA is used to prepare to make statistical copolymers with lactic acid by direct polycondensation to provide reactive sites along the polymer backbone. The halogen is distributed along the backbone of the copolymer, such as a grafting-to structure can be made. Poly(ethylene glycol) (PEG) is most often used as a hydrophilic segment because of its biodegradability and biocompatibility. The PEGylated poly(lactic acid) can serve as a polymer to which various medical and drug delivery systems can be attached. Here, mPEG side chains were formed by grafting mPEG homopolymers with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) end groups onto the polymer by a radical coupling reaction. TEMPO is one of the most widely used nitrooxide radicals due to its remarkable persistency. PEGylated poly(lactic acid) can be prepared via a radical coupling reaction of PEG-supported TEMPO with the brominated poly(lactic acid).

Key words: biodegradable polyesters, lactic acid, TEMPO, and PEG
DEDICATION

The author would like to dedicate this thesis to his girlfriend, Jiacen Ni. The author wants to thank her for waiting for him patiently for these two years. Her love and trust enable him to finish his studies and make him what he is today. Also to his parents, Ying Wu and Guiyin Zhang, who love him, believe in him and support him from his birth to this point. The author could never have done this without their support.
ACKNOWLEDGEMENTS

The author would like to thank University of Akron and East China University of Science and technology for providing the opportunity to pursue his Master’s Degree. The author also would like to thank Dr. Coleen Pugh from Department of Polymer Science, University of Akron for providing support and guiding him in research. Dr. Yang H. Yun from the Department of Biomedical Engineering is the reader and committee member. The author wants to thank him for reading the thesis and attending the formal seminar. The author would like to thank Colin Wright for introducing him to the world of polymer synthesis. Special thanks to Carolyn A. Scherger, Abby Freeman, Cesar Lopez, Zewei Wang, Xiang Yan, Bonan Yu and other group members for their assistance and help during the two years, therefore he can complete his thesis successively. Support from his roommate, Jiancheng Luo, is gratefully acknowledged. Acknowledgement is also made to National Science Foundation for general laboratory support through DMR-1006195. In the end, the author wants to thank his parents for the financial support during his studies.
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CHAPTER I

INTRODUCTION

1.1 Biodegradable polymers

Over the past decades, rapidly increasing research interest has been developed in biodegradable polymers because of their unique properties. Generally speaking, biodegradable polymers are polymers that can disappear in the body with or without enzymatic assistance. The cause of the interest is the three main advantages biodegradable polymers have compared to non-degradable polymers:¹

(1) Biodegradable polymers do not need to be removed from the human body after their aims are reached.

(2) The degradation products will not cause long-term toxicity because they are metabolized and excreted.

(3) Biodegradable polymers can provide some bio-medical functions by degradation, for example, controlled release of drugs at a certain speed or target.

Biodegradable polymers have been widely used in many applications from packing fibers,² to drug carriers in some drug delivery systems,³ and tissue-engineering scaffolds.⁴

Aliphatic polyesters, such as poly(glycolic acid) (PGA) and poly(lactic acid) (PLA), are the particularly attractive and the most widely used biodegradable polymers⁵ because they possess tunable degradation rates,⁶ good mechanical properties,⁷ low toxic
degradation products,\textsuperscript{8} and are used in many FDA (USA) approved applications for clinical use. Other commonly used biodegradable polyesters includes polycaprolactone and various copolymers, for example, poly[(lactic acid)-co-(glycolic acid)]. Figure 1.1 shows the structures of their cyclic monomers and Figure 1.2 shows the structure of the common biodegradable polymers.

\textbf{Figure 1.1. Structures of lactide, glycolide and other typical monomers}

\textbf{Figure 1.2. Structures of widely used biodegradable polymers}
1.2 Poly(lactic acid)

Poly(lactic acid) or polylactide is a thermoplastic aliphatic polyester, which can be produced from renewable resources, such as biomass, and therefore is an environmental-friendly product.9

1.2.1 Properties of Poly(lactic acid)

The chirality of lactic acid has long been recognized. Therefore, poly(lactic acid) exists in different forms, such as poly(L-lactic acid) and poly(DL-lactic acid). Natural existing PLLA is considered one of the most important polyesters due to its unique properties. Table 1 summarizes some important physical properties of PLLA.

<table>
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<th>PLLA</th>
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<th>Glass-transition Temp.</th>
<th>m.p</th>
<th>Tensile modulus</th>
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<td>PLLA</td>
<td>Around 37%</td>
<td>60-65 °C</td>
<td>173-178 °C</td>
<td>2.7-16 GPa</td>
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* Data from references 10, 11

Modified PLA can be highly heat-resistant and withstand higher temperature than conventional PLA.12 PLA can be dissolved in methylene chloride, chloroform, THF, dioxane and benzene.13 Fiber and film are two major forms of PLA processed from traditional melting spinning methods. Physical blending with PDLA can modify the physical properties of PLLA. For example, the melting temperature increases from around 175 °C to around 220 °C. And the heat deflection temperature of PLLA also had a significant increment. The crystallinity increases when blending with PDLA to form a highly regular stereocomplex. The temperature stability also increases and reaches the maximum when equal amount of PLLA and PDLA are blended. Even containing low
ratio of PDLA, the stability still greatly improves. The explanation for this phenomenon is that the small amount of PDLA plays the role of a nucleation agent, thereby increasing the crystallization rate. The higher crystallinity of PDLA leads to slower degradation rates than PLLA.

1.2.2 Preparation of PLA

Since 1845, many attempts have been made to prepare PLA.\textsuperscript{14} Bischoff and Walden first reported the production formulas to prepare PLA from lactide and in 1932 Carothers and coworkers successfully prepared low molecular weight PLA.\textsuperscript{15} DuPont\textsuperscript{TM} started to market PLA for medical use, including medical implants and drug delivery. In 2002, Kanebo Gohsen Ltd., put PLA fibers known as Lactron into commercial production in 1994. Cargill Dow LLC began commercial production of starch made PLA under the trade name of Nature Works.\textsuperscript{16} There are several routes to achieve usable PLA. As outlined in Figure 1.3, two main monomers used to prepare PLA are lactic acid and the cyclic dimer lactide.

\textbf{Figure 1.3. Production of PLA from lactide}
Figure 1.4. Synthesis of PLA by direct polycondensation of lactic acid

One of the routes to synthesize PLA is by metal catalyzed ring-opening polymerization of lactide. The reaction can be performed in solution, in the melt state, or in a suspension. The metal-catalyzed reaction tends to cause racemization of the PLA and affect its stereoregularity compared to the starting material. As shown in Figure 1.4, another commonly used method to synthesize PLA is by direct polycondensation of lactic acid monomers. Carrying out this process at above 200 °C is not favored. Above that temperature, it is prone to generate the lactide due to thermodynamics. This condensation reaction between carboxylic acid and hydroxyl group generates one equivalent of water for each step, which is not desirable because water leads to the chain-transfer reaction preventing from high molecule products. The direct condensation is thus performed in a stepwise procedure. Firstly, PLA oligomers are formed. Thereafter, further polycondensation occurs in the melt state or in solution where low molecule weight oligomers are polymerized to yield a high molecular weight polymer. Removing water by employing of a vacuum or by azeotropic distillation is critical to favor polycondensation over transesterification and to yield high molecule weight polymer. Poly-DL-lactide is usually synthesized by ring-opening polymerization of a mixture of L- and D- lactides. Heterotactic poly(lactic acid) showing crystallinity has also been successfully prepared by using catalysts with stereoselectivity. The degree of
crystallinity along with many important properties is dependent on the monomer composition to a great extent, and the catalyst variety has little effect.

1.2.3 Advantages of PLA

For PLA, there are many advantages besides the environmental-friendliness mentioned above. Since synthetic biodegradable sutures were first approved by the US Food and Drug Administration (FDA) in the 1960s, polymers prepared from lactic acid have been found useful in the medical industry.\(^{21}\) Secondly, PLA was reported to have better processibility than other biopolymers, and is therefore widely used as packing materials.\(^{22}\) Moreover, the production of PLA is also energy saving compared to petroleum-based polymers.\(^{23}\) Due to these unique properties, PLA plays a significant role in biodegradable polymers.

1.2.4 Limitations of PLA

However, there are some limitations. Firstly, the properties of PLA are limited by its structure, and broader applications of PLA therefore cannot be achieved. Many monomers can be used to prepare copolymers with lactic acid to solve such problems, such as hydroxyl acid, amino acid and polymers like PEG and cellulose. Glycolic acid is the commonly used hydroxyl acid monomer to modify the properties of poly(lactic acid). Such copolymers can be prepared as either random or block copolymers. Their properties, such as degradation rate and thermal behavior, can be tuned and improved through varying the architecture of the polymer, monomer composition, and stereochemistry of the monomers.
Although the mechanical properties have been improved, PLGA still lacks reactive sites along the backbone of the copolymer, which so far is a major limitation for a large number of applications. Therefore, it is important and necessary to find an efficient way to functionalize the main-chain of these aliphatic polyesters. In general, polyesters are prepared by polycondensation of monomers with carboxylic groups and hydroxyl groups or by ring-opening polymerization of lactones. However, the most commonly used functional groups, e.g. hydroxyl, amine and carboxylic acid, are not able to be involved in such preparation methods of polyesters because their functionality will cause cross-links and be eliminated. Different methods have been made to address this problem, e.g. protecting and deprotecting chemistry, chemoselective step-growth polymerization, and ring-opening polymerization of monomers with non-reactive functional groups. Varying degrees of success have been gained to prepare polyesters with functional groups, e.g. hydroxyl, azido, alkyne, and poly(ethylene glycol) group, but no highly universal and general strategy has yet been reported for functionalization of aliphatic polyesters.

1.2.5 Modifications of PLA

The application of graft copolymers of PLA to biodegradable polymers with desired properties has recently received increasing interests. In 2000, Chen et al. synthesized new grafting copolymer (PLA-HEC) of cellulose and lactide by ring-opening copolymerization. The copolymers, which combine cellulose derivatives with biodegradability, have potential use in tissue engineering. Dong et al. reported the PLLA-g-cellulose copolymer and studied their ability to self-assemble into polymeric micelles.
in water.\textsuperscript{25} PLLA segments are hydrophobic and become the core, while the hydrophilic cellulose segments face towards water to form the shell. The micelles showed drug-loading capacity and controlled release of drugs in water; therefore they can be used in drug delivery system. Hadano et al. reported the graft copolymer PBz-g-LA from benzylated waste pulps and lactic acid.\textsuperscript{26} PBzs were prepared from pretreated waste pulp and benzyl chloride catalyzed by quaternary ammonium salt in aq. NaOH. Starch has potential use in biodegradable plastics due to their abundance and starch-based materials have been formulated. Chen et al. prepared graft copolymers PLLA-g-starch that can stabilize the blends of PLLA and starch.\textsuperscript{27} Their mechanical properties showed better performance compared to the simple PLLA/starch blends without the compatibilizer. Chitin and chitosan are naturally abundant and potentially applicable in the biomedical field. Kim et al. synthesized biodegradable copolymers using chitin as the macroinitiator by ring-opening polymerization of L-lactide.\textsuperscript{28} There are many other examples of new graft copolymer materials based on PLA and the common way is to ring-opening polymerize lactide by a macroinitiator such as cellulose, chitin and dextran.\textsuperscript{29} Such copolymers were obtained by grafting PLA segments from the other polymer. However, grafting the other polymer onto PLA is seldom reported due to the difficulty of modifying the PLA backbone. In the thesis, graft copolymers with PLA polymer backbone are synthesized by introducing the comonomer, 2-bromo-3-hydroxypropanoic acid.

1.3 2-Bromo-3-Hydroxypropanoic Acid （BHPA）

DL-2-Bromo-3-hydroxypropionic acid (BHPA) is a brominated constitutional isomer of LA. It is a white crystalline solid with a melting point ranging from 48 ℃ to 51
°C. It can be synthesized from an amino acid termed DL-serine, which is a natural resource. The structure of BHPA is similar to lactic acid and it can provide reactive sites along the backbone of the copolymer. 2-Bromo-3-hydroxypropanoic acid (BHPA) is prepared to synthesize random copolymers with lactic acid by direct polycondensation at higher temperature and vacuum or room temperature with a linking agent and catalyst. The co-polycondensation is acid-catalyzed and driven by removing the water by high temperature and vacuum. Random copolymers are copolymers with a specific statistical distribution of their monomer sequence. Such polyesters prepared by LA with BHPA are potentially biodegradable and can be further functionalized, via nucleophilic substitution, radical addition, radical-radical coupling and electrophilic substitution.

1.4 Radical coupling

The carbon halogen bond can undergo homolytic cleavage to form a carbon centered radical under redox conditions (Figure 1.5).

![Figure 1.5. Scheme of carbon-halogen bond abstraction](image)

When the halogen is distributed along the backbone of the copolymer, grafting-to or grafting-from structures can be made via radical-radical coupling with tunable graft density. The grafting-onto approach attaches polymer branches onto the polymer backbone chain by a coupling reaction between their functional groups. Matyjaszewski
et al. reported that the concentration of macroradicals generated in ATRP can be increased by adding zerovalent metal, therefore the radicals will undergo a bimolecular termination reaction.\cite{33} Huang et al. then reported that the macroradicals would not undergo bimolecular termination if TEMPO as well as other radical scavengers existed.\cite{34} The nitroxide radicals would rapidly trap the macroradicals generated in situ. As shown in Figure 1.6, the graft copolymers poly(GTEMPO-co-EO)-g-PS were successfully prepared by this coupling reaction, which is termed atom transfer nitroxide radical coupling.

![Synthesis scheme of poly(GTEMPO-co-EO)-g-PS](image)

Figure 1.6. Synthesis scheme of poly(GTEMPO-co-EO)-g-PS
(Reprinted with permission from ref. 35. Copyright 2008, American Chemical Society)

Atom transfer nitroxide radical coupling (ATNRC) is a newly developed coupling reaction that can couple a bromo-containing polymer with a TEMPO-containing polymer at high efficiency.\cite{35} TEMPO is a useful nitroxide radical due to its remarkable persistency. Dimerization of TEMPO is very slow. However, highly reactive radicals such as alkyl radical can react with TEMPO.\cite{36} The reaction is promoted by a transition metal species such as copper bromide. The transition metal species, $M_t^n$, abstracts the halogen from the organic halide to form the oxidized species, $M_t^{n+1}X$ and generates the
carbon centered radical R'. Then the reaction between the alkyl radical and free radical of nitroxide of TEMPO results in the target product.

1.5 Polymer micelles and drug delivery

The self-assembly of polymers has been extensively studied in the past decades. Increasing research attraction has been developed in self-assembled systems for biomedical use, especially at the nanometer scale such as polymer micelles, polymersomes and nanogels. The most attractive and most studied biodegradable polymers are aliphatic polyesters. Figure 1.7 shows examples of self-assembled systems at nanometer-scale from block and graft amphiphilic polymers. There are a series of applications based on biodegradable polymers for medical use, such as drug delivery systems.\(^{37}\) Polymer micelles consist of a hydrophobic core and a hydrophilic outer shell that are formed with amphiphilic copolymers. The cause of interest in polymer micelles lies in their nano size and the unique structure, which can be used to protect drugs by their polymer shells. Drugs entrapped in such polymeric micelles can escape from renal filtration, and remain in the circulation in the human body for a longer time\(^{38}\). Yokoyama et al. firstly reported the polymeric micelle-type drug carrier poly(aspartic acid)-b-PEG loading the hydrophobic chemotherapy drug doxorubicin.\(^{39,40,41}\) Shin et al. reported the therapeutical capability of PLA-b-PEG micelles loading various hydrophobic anticancer drugs.\(^{42}\) The PLA-b-PEG micelles were able to solubilize and stabilize different anticancer agents. Ding et al. reported the block copolymer PLA-b-PEG encapsulating protoporphyrin IX residues and studied the micelle production and their potent as an effective drug delivery carrier.\(^{43}\)
1.6 Synthesis Route

In this thesis, 2-bromo-3-hydroxypropionic acid was synthesized from D,L-serine and was introduced to prepare random copolymer with lactic acid in different monomer feed ratios by direct polycondensation. Also, mPEG-supported TEMPO was synthesized in two steps. As outlined in Figure 1.8, the mesylation of mPEG was performed with methanesulfonyl chloride catalyzed by triethylamine. Then 4-hydroxy-TEMPO was used to react with mesylated PEG in the presence of NaH to synthesize the PEG-supported TEMPO. In the end, PEGylated poly(lactic acid) was prepared via radical-radical coupling between brominated poly(lactic acid) and nitroxide in the presence of copper bromide.
Also, 2-ethoxyethanol was attached to 2-bromo-3-hydroxypropionic acid in 4 steps. First, 2-ethoxyethanol was converted into 2-ethoxyethyl methanesulfonate by reacting with methanesulfonyl chloride. Then 4-hydroxy-TEMPO was used to react with 2-ethoxyethyl methanesulfonate in the presence of NaH to synthesize 2-ethoxyethyl TEMPO. This TEMPO derivative was coupled with methyl 2-bromo-3-hydroxypropionate via radical-radical coupling in the presence of copper bromide. Finally, the hydroxyl acid monomer was prepared from hydrolysis. It is polymerized by both conventional polycondensation at high temperature and with a coupling reagent.
CHAPTER II

EXPERIMENTAL SECTION

2.1 Materials

N,N'-Diisopropylcarbodiimide (Creosalus, 99%), diphenyl ether (Linton, 99%), 4-dimethylaminopyridine (Oakwood Chemicals, 99.9%), ethanol (Sigma Aldrich, 99.5%), ethyl acetate (Sigma Aldrich, 99.5%), 2-ethoxylethanol (ACROS, 99%), hydrobromic acid (Sigma Aldrich, 48%wt), 4-hydroxyl-2,2,6,6-tetramethylpiperidine-1-oxyl (Oakwood Chemicals, 98%), lactic acid (Acros, 85%), lithium hydroxide monohydrate (Sigma Aldrich, 99.99%), methanol (Sigma Aldrich, 99.5%), methylsulfonyl chloride (ACROS Organics, 99.5%), methylene chloride (Sigma Aldrich, 99.5%), potassium bromide (Alfar Aesar, 99%), sodium hydride (Aldrich, 95%), sodium nitrite (Alfa Aesar, 98%), D,L-serine (MP biomedicals, 98%) and p-toluenesulfonic acid (Sigma Aldrich, 98%) were used as received. 2,2'-Bipyridine (Sigma Aldrich, 98%) was recrystallized from hexane. Cuprous bromide (Alfa Aesar, 98%) was stirred with acetic acid, washed with diethyl ether and dried under vacuum. N,N,N',N'-Pentamethyldiethylenetriamine (Sigma Aldrich, 99%) (PMDETA) was dried over K2CO3 and distilled under reduced pressure. Reactant grade tetrahydrofuran
(THF) was distilled from purple sodium benzophenone ketyl. Triethylamine (Sigma Aldrich, 99.5%) was distilled from and stored over KOH under N₂.

All other reactants and solvents were commercially available and used as received.

2.2 Techniques

All reactions are performed under a N₂ atmosphere using a Schlenk line unless noted otherwise.

NMR: ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra (δ, ppm) were recorded on a Varian Mercury instrument. Unless noted otherwise, all spectra were recorded in CDCl₃, and the resonances were measured relative to residual solvent resonances and referenced to tetramethylsilane.

GPC: Number-average (Mₙ) and weight-average (Mₘ) molecular weights relative to linear polystyrene (GPCₚₛₜ) and polydispersities (PDI=Mₘ/Mₙ) were determined by gel permeation chromatography (GPC) from calibration curves of log Mₙ vs elution volume at 35 °C using THF (unless noted otherwise) as solvent (1.0 mL/min), a guard column and set of 50 Å, 100 Å, 10⁴Å, and linear (50-10⁴Å) Styrage 5 µm columns, a Waters 486 tunable UV/vis detector set at 254 nm, a Waters 410 differential refractometer, and Millenium Empower 2 software. The samples (~0.1 g/L) were dissolved overnight and filtered through a 0.45 µm PTFE filter.

The dialysis bags (Spectra/pro, MWCO 1000) were purchased from Spectrum Laborites Inc.

TEM: A Philips TECNAI TEM was used to study the size and distribution of the micelles on 200-mesh Cu grids with an accelerating voltage of 120 kV.
2.3.1 Synthesis of 2-Bromo-3-hydroxypropionic Acid (Scheme 3.1)

2-Bromo-3-hydroxypropionic acid was synthesized according to scheme 3.1 and resulted in 58% yield as in the following example. Sodium nitrite (79.5 g, 1.01 mol) was added in portions over 3 h to a solution of D,L-serine (52.2 g, 0.496 mol), HBr (120 mL, 48 w/w% aq, 1.0 mol) and KBr (180.8 g, 1.519 mol) in H₂O (400 mL) at approximately –10 °C. After stirring at r.t. for 20 h, the light-greenish solution was saturated with NaCl and extracted with EtOAc (5 × 100 mL). The aq layer was acidified with HCl to pH<2 and extracted five times with EtOAc (5 × 100 mL). The combined organic extracts were washed with sat. aq NaCl (5 × 100 mL) and dried over Na₂SO₄. After filtration and removing the solvent by trap-to-trap distillation, the residue was recrystallized from CH₂Cl₂ to obtain 48.68 g (58%) of 2-bromo-3-hydroxypropionic acid as a white hygroscopic solid; mp 50-53 °C. ¹H NMR (300 MHz, CDCl₃–DMSO-d₆): 3.78 (dd, CH₂HOH, J = 11.3 Hz, J = 5.9 Hz), 3.65 (dd, CH₂HOH, J = 11.3 Hz, J = 7.8 Hz), 4.22 (dd, CHBr, J = 5.9 Hz, J = 7.8 Hz), 7.19 (br s, CO₂H and OH).

2.3.2 Synthesis of Poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)] (Scheme 3.2)

Poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)] was synthesized in 44-55% yield as in the following example. A melted solution of D,L-lactic acid (0.8533 g, 9.473 mmol), 2-bromo-3-hydroxypropionic acid (0.1677 g, 0.9925 mmol), pTSA•H₂O (0.0574 g, 0.301 mmol) and diphenyl ether (1 mL) was stirred at 90 °C at atmospheric pressure for 2 h, and at 90 °C under reduced pressure (1-3 mm Hg) for 90 h. After opening the system to the atmosphere, the polymerization mixture was dissolved in
CH₂Cl₂ (5 mL) and precipitated in methanol (50 mL). The solvents were decanted, and the copolymer was reprecipitated from CH₂Cl₂ (5 mL) into methanol (50 mL) to yield 0.4028 g (55.74%) of halogenated PLA as a white solid. The product was analyzed by ¹H NMR spectroscopy and GPC. ¹H NMR: 1.7-1.4 (m, CH₃), 4.7-4.4 (m, CHBrCH₂), 5.3-5.1 (m, CHCH₃). GPC: Mₙ=1.42 x 10⁴-3.68 x 10⁴ Da, PDI=1.69-2.30.

2.3.3 ATNRC of poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)]

Attempts were made to synthesize graft copolymer by atom transfer nitroxide radical coupling chemistry. An example of ATNRC of PLB and nitroxide is as follows. In a Schlenk flask sealed with a glass stopper was mixed PLB (0.300 g, 0.375 mmol Br), 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) (0.064 g, 0.41 mmol), N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA) (0.071 g, 0.41 mmol) and ethyl acetate (5mL) under N₂. The solution was degassed four times then cuprous bromide (0.065 g, 0.45 mmol) was added under N₂. The flask was moved to an oil bath at 50 °C after degasing twice. After stirring for 24 h, the reaction was quenched by immerging into liquid N₂. The solution was washed twice by saturated NH₄Cl (10 mL ea) then dried over MgSO₄ overnight. The solvent was removed under reduced pressure after filtration. The residue was taken up by 1 mL CH₂Cl₂ then precipitated into 20 mL methanol under vigorous stirring. Solvent was decanted carefully from the precipitation. The residue was taken up by 1 mL CH₂Cl₂ then precipitated into 20 mL M hexane under vigorous stirring. Solvent was decanted carefully from the precipitation. Precipitation was dried under vacuum overnight to yield 0.124 g (34.0%) white solid. ¹H NMR: 1.7-1.4 (m, CH₃), 4.7-4.4 (m, CHBrCH₂), 5.3-5.1 (m, CHCH₃), 1.2-1.1 (m, CH₃).
2.3.4 Synthesis of Methyl 2-bromo-3-hydroxypropionate (Scheme 3.4)

Concentrated hydrobromic acid (10 drops) was added to a solution of 2-bromo-3-hydroxypropionic acid (10 g, 60 mmol) in methanol (80 ml, 2.0 mol). After refluxing the solution for 17 h, the solvent was removed using a rotary evaporator. The resulting oil was dissolved in CH₂Cl₂ (150 mL), and washed twice with aq. NaHCO₃ (75 mL ea) and once with brine (100 mL). The organic layer was dried over MgSO₄. After filtration, the solvent was removed by rotary evaporation, and the residue was distilled (105-110 °C / 4 mm Hg) to yield 9.7 g (87%) of methyl 2-bromo-3-hydroxypropionate as a yellow oil. The product was analyzed by ¹H NMR spectroscopy. ¹H NMR: 2.22 (br s, OH), 3.83 (s, CH₃), 3.95 (dd, CHOH), 4.06 (dd, CHOH, ²J = 12.1 Hz, ³J = 7.4 Hz), 4.36 (dd, CHBr, ³J = 5.6 Hz, ³J = 7.4 Hz).

2.3.5 Synthesis of 2-Ethoxyethyl methanesulfonate (Scheme 3.5)

Methanesulfonic chloride (1.51 g, 12.1 mmol) was added dropwise over 15 min into a solution of 2-ethoxyethan-1-ol (1.00g, 0.011 mol) and triethylamine (1.909 g, 18.9 mmol) in methylene chloride (10 mL) at 0 °C under N₂. After stirring for 24 h, the reaction was quenched by pouring into ice. The solution was washed twice with diluted 1 M aq HCl (10 mL ea.), aq. NaCO₃ (10 mL ea.) successively and once with brine (10 mL). The organic layer was dried over MgSO₄ overnight. After filtration, the solvent was removed by rotary evaporation and dried under vacuum to yield 2.286 g (80.1%) 2-ethoxyethyl methanesulfonate as a light yellow oil. The product was analyzed by ¹H NMR spectroscopy. ¹H NMR: 4.36 (t, CH₂OSO₂, ³J=4.5 Hz), 3.69 (t, OCH₂, ³J=4.5 Hz), 3.56 (q, CH₃CH₂, ³J=6.0 Hz), 3.06 (s, CH₃), 1.21 (t, CH₂CH₃, ³J=6.0 Hz).
2.3.6 Synthesis of 2-Ethoxyethoxy TEMPO (EETEMPO) (Scheme 3.6)

To a suspension of NaH (0.126 g, 5.24 mmol) in THF (5 mL) was added 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (0.812 g, 4.76 mmol) over 10 min at 0 °C and the resulting slurry was stirred for 1 h at room temperature under N₂. 2-Ethoxyethyl methanesulfonate (0.400 g, 2.38 mmol) in 5 mL THF was then added. The mixture was refluxed for 48 h, and then adding H₂O (10 mL) to the solution in order to terminate the reaction. THF was removed by rotary evaporation. The aqueous layer was extracted with ethyl acetate (10 mL, ea) three times. The organic layers were merged and dried over MgSO₄ overnight. After filtration, the solution was concentrated then passed through a silica gel column using hexane/ethyl acetate (10:3, v/v) as the eluent. Three fractions were collected. The solvent in fraction 2 was removed by rotary evaporation to yield 2-ethoxyethyl TEMPO (0.400 g, 68.9%) as a red oil. The product was analyzed by ¹H NMR spectroscopy. ¹H NMR: 3.40-3.81 (m, CH₂O, CH(OCH₂), 7 H), 1.55-1.67 (m, CHCH₂, 2 H), 1.92-2.04 (m, CHCH₂, 2 H), 1.29 (s, CH₃, 12 H), 1.21 (s, CH₂CH₃, 3H).

2.3.7 Synthesis of Methyl 2-(4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-yl-1-oxyl)-3-hydroxyacetate (Scheme 3.7)

Methyl 2-bromo-3-hydroxypropionate (0.210 g, 1.15 mmol), 4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-oxyl (0.310 g, 1.27 mmol) and PMDETA (0.199 g, 1.15 mmol) were mixed in a Schlenk flask sealed with a glass stopper under N₂. After three cycles of freeze-pump-thaw (5-10-5 min), Cuprous bromide (CuBr) (0.165 g, 1.15 mmol) was added to the flask as a solid. Then the flask was degassed by three cycles of freeze-pump-thaw following by stirring at 50 °C for 24 h. The reaction was quenched by liquid
N\textsubscript{2}. The reaction mixture was then washed twice with aqueous ammonium chloride (10 mL ea.) to remove the copper complex. The organic layer was dried over MgSO\textsubscript{4} overnight. After filtration, the solution was concentrated then passed through a silica gel column to get the product. The solvent was removed by rotary evaporation to give methyl 2-(4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy)-3-hydroxyacetate (0.264 g, 49.5%) as a light yellow oil. The product was analyzed by \textsuperscript{1}H NMR spectroscopy. \textsuperscript{1}H NMR: 4.50 (t, CH\textsubscript{2}OH, \textsuperscript{3}J=6.0 Hz), 4.01 (dd, CH\textsubscript{2}OH, \textsuperscript{2}J=17.2 Hz, \textsuperscript{3}J=6.0 Hz), 3.91 (dd, CH\textsubscript{2}OH, \textsuperscript{2}J=17.2 Hz, \textsuperscript{3}J=6.0 Hz), 3.71 (s, CH\textsubscript{3}), 3.40-3.81 (m, CH\textsubscript{2}O, CH\textsubscript{2}OCH\textsubscript{2}, 7 H), 1.55-1.67 (m, CH\textsubscript{2}H\textsubscript{2}, 2 H), 1.92-2.04 (m, CH\textsubscript{2}H\textsubscript{2}, 2 H), 1.21 (s, CH\textsubscript{3}, 12 H), 1.09 (t, CH\textsubscript{3}CH\textsubscript{2}, 3H).

2.3.8 Synthesis of 2-(4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy)-3-hydroxy acid (Scheme 3.8)

Methyl 2-[4-(2-ethoxyethoxy)-(2,2,6,6-tetramethylpiperidin-1-yl)-1-oxy]-3-hydroxyacetate (0.780 g, 2.34 mmol) was dissolved in 10 mL THF under N\textsubscript{2}. LiOH (0.197 g, 4.68 mmol) was added dropwise over 20 min in 20 mL deionized water at 0 °C. Then the solution was warmed to room temperature and moved to an oil bath at 50 °C. Reaction was stopped after 4 h. THF was removed under reduced pressure and aqueous solution was concentrated to 5 mL under reduced pressure. The aqueous layer was washed three times (10 mL ea.) with ethyl acetate to get rid of unreacted ester. The aqueous layer was then acidified to pH<2 and extracted three times (10 mL ea.) with ethyl acetate. The organic layers were merged and dried over MgSO\textsubscript{4} overnight. The solvent was then removed under reduced pressure after filtration to yield 2-(4-(2-
ethoxylethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy)-3-hydroxy acid (0.560 g, 74.9%) as a light yellow oil. The product was analyzed by \textsuperscript{1}H NMR spectroscopy. \textsuperscript{1}H NMR: 4.50 (t, CHCH\textsubscript{2}OH, 1H, \textsuperscript{3}J=6.0 Hz), 3.99 (dd, CHHOH, 1H, \textsuperscript{2}J=17.2 Hz, \textsuperscript{3}J=6.0 Hz), 3.92 (dd, CHHOH, 1H, \textsuperscript{2}J=17.2 Hz, \textsuperscript{3}J=6.0 Hz), 3.72 (m, CH, 1H), 3.49-3.69 (m, CH\textsubscript{2}O, 6H), 1.57-1.69 (m, CHCH\textsubscript{2}, 2 H), 1.99-2.19 (m, CHCH\textsubscript{2}, 2 H), 1.34 (s, CH\textsubscript{3}, 12 H), 1.21 (t, CH\textsubscript{2}CH\textsubscript{3}, 3H).

2.3.9 Synthesis of Poly[2-(4-(2-ethoxylethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy)-3-hydroxy acid] (Scheme 3.9)

Poly[2-(4-(2-ethoxylethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy)-3-hydroxy acid] was prepared by conventional polycondensation of 2-(4-(2-Ethoxylethoxy)-2,2,6,6-tetramethylpiperidin-1-yl-1-oxy)-3-hydroxy acid. 2-(4-(2-Ethoxylethoxy)-2,2,6,6-tetramethylpiperidin-1-yl-1-oxy)-3-hydroxy acid (0.500 g, 1.61 mmol) and pTSA (0.025 g, 0.145 mmol) were mixed in a 100 mL sealed Schlenk tube. The mixture was stirred at 90 \degree C under N\textsubscript{2} for 2 h then it was switched to vacuum for 72 h. The Schlenk tube was then removed from oil bath and opened to the atmosphere, and its contents were dissolved in 1 mL CH\textsubscript{2}Cl\textsubscript{2} then precipitated into 15 mL hexane. Hexane was carefully decanted from the precipitation, and the black oil was dried in vacuo. The product was analyzed by \textsuperscript{1}H NMR spectroscopy and GPC. \textsuperscript{1}H NMR: 4.51 (t, CHCH\textsubscript{2}OH, 1H, \textsuperscript{3}J=6.0 Hz), 3.95 (dd, CHHOH, 1H, \textsuperscript{2}J=17.2 Hz, \textsuperscript{3}J=6.0 Hz), 3.90 (dd, CHHOH, 1H, \textsuperscript{2}J=17.2 Hz, \textsuperscript{3}J=6.0 Hz), 3.78 (m, CH, 1H), 3.56 (m, CH\textsubscript{2}O, 6H), 1.57-1.69 (m, CHCH\textsubscript{2}, 2 H), 1.99-2.19 (m, CHCH\textsubscript{2}, 2 H), 1.34 (s, CH\textsubscript{3}, 12 H), 1.21 (t, CH\textsubscript{2}CH\textsubscript{3}, 3H). GPC: M\textsubscript{n}=447, PDI=1.10.
2.3.10 Synthesis of Poly[2-(4-(2-ethoxyethoxy)-(2,2,6,6-tetramethylpiperidin-1-yl)-1-oxy)-3-hydroxy acid] (Scheme 3.10)

2-(4-(2-Ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-yl-1-oxy)-3-hydroxy acid (0.500 g, 1.61 mmol) and 4-(dimethylamino)pyridinium-4-toluene sulfonate (DPTS) (69 mg, 0.32 mmol) were mixed in a rubber sealed Schlenk tube and cooled to -10 °C. N,N-Diisopropylcarbodiimide (DiPC) was then added dropwise over 30 min. The reaction was performed for 4 h at -10 °C. The mixture was dissolved in 30 mL acetone and polymer (0.140 g, 29.7%) was recovered as a light yellow solid by removing the solvent under reduced pressure after dialysis for 48 h in acetone. The product was analyzed by $^1$H NMR spectroscopy and GPC. $^1$H NMR: 4.56 (br, $CHCH_2OH$), 3.56 (br, $CH_2O$), 1.20 (br, $CH_2$, $CH_3$). GPC: $M_n$=1,679, PDI=1.25

2.3.11 Synthesis of linker-less mPEG TEMPO ether (mPEG-TEMPO)

The terminal hydroxyl functionalities in the PEG chains were converted to the corresponding mesyl esters as reported in the literature. A 100 mL, three-necked, round-bottomed flask was charged with polyethylene glycol (2.50 g, 4.54 mmol), triethylamine (1.38 g, 13.6 mmol) in 25 mL of anhydrous $CH_2Cl_2$, and was cooled to 0 °C. Methanesulfonylchloride (0.66 mL, 7.72 mmol) was then added dropwise over 20 min. And the reaction mixture was stirred at room temperature for 48 h. The suspension was filtered, and the resulting filtrate was washed twice by cold water, 10% HCl (15 mL ea), saturated NaHCO$_3$ (15 mL ea), and saturated NaCl (10 mL ea) successively. Then, the organic layer was dried over MgSO$_4$ overnight. Filtered and rotate evaporate the solvent gave mesylated mPEG as a light yellow solid. The yield was 84.27 %. The product was
analyzed by $^1$H NMR spectroscopy. $^1$H NMR: 3.06 (s, Ms-CH$_3$ 3H), 3.36 (s, -CH$_3$, 3H), 3.51-3.64 (m, -OCH$_2$-, 44 H), 3.75 (m, -OCH$_2$CH$_2$-, 2 H), 3.36 (m, -OCH$_2$CH$_2$-, 2 H).

2.3.12 General Procedure for Preparing the Linear Linker- less PEG-TEMPO Ether (mPEG-TEMPO)

To a suspension of NaH (61 mg, 2.5 mmol) in DMF (5 mL) was added 4-hydroxy-TEMPO (438 mg, 2.54 mmol) and the resulting slurry stirred for 1 h at room temperature under inert atmosphere. mPEG mesylate ($M_n$=2k Da, 0.801 g, 1.28 mmol) was then added and stirring resumed for 72 h at 70 °C. The suspension was then filtered and concentrated to 2 mL under reduced pressure. This solution was then added dropwise to diethyl ether (70 mL) that was stirred vigorously. The white precipitate (0.421 g, 52%) collected by filtration, washed twice with diethyl ether (35 mL ea.), and dried under vacuum. The product was analyzed by $^1$H NMR spectroscopy. $^1$H NMR: 3.85 (t, OCH$_2$), 3.80-3.52 (m, CH$_2$CH$_2$O), 3.39 (s, CH$_3$), 2.21-2.05 (m, CH$_2$), 2.0-1.8 (m, CH$_2$), 1.49 (s, CH$_3$), 1.37 (s, CH$_3$).

2.3.13 Micelles Preparation and Characterization

Polymer (2 mg) was dissolved in 2 ml THF. Then deionized water was added through a syringe pump dropwise over 2 h. The solution was allowed to evaporate for 6 h to remove THF. The micelles were characterized by Transmission Electron Microscopy (TEM).
CHAPTER III
RESULTS AND DISCUSSION

3.1 Synthesis of 2-Bromo-3-hydroxypropionic Acid

Scheme 3.1. Synthesis route for 2-bromo-3-hydroxypropionic acid.

Scheme 3.1 illustrates the synthesis of 2-bromo-3-hydroxypropionic acid and Figure 3.1 presents its $^1$H NMR spectrum. The integral ratio of CHBr and CH$_2$ is close to 1:2 and the NH$_2$ group was replaced with Br atom successfully. The yield was as much as reported in the literature$^{45}$ (around 50%). The product was pure according to the $^1$H NMR spectrum.

Figure 3.1. 300 MHz $^1$H NMR spectrum of 2-bromo-3-hydroxypropionic acid.
3.2 Synthesis of poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)].

Scheme 3.2 illustrates the synthesis of poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic) acid] and Figure 3.2 presents the $^1$H NMR spectra of polymers with varying monomer composition. The ratio of lactic acid units to 2-bromo-3-hydroxypropionic acid units was calculated by integrals of each peak, which agrees with the initial monomer feed ratio. The product is very pure according to the $^1$H NMR spectrum except for the small solvent peak. Table 3.1 summarizes the results of the polymerizations with varying monomer ratios. The number-average molecule weight ranges from $1.4 \times 10^4$ to $3.7 \times 10^4$ Da and polydispersity is around 2.0 as expected for a polycondensation. Figure 3.3 presents the GPC traces of polymers with different monomer compositions. As shown in the figure, the molecule weight increases when the content of lactic acid increases.

![Scheme 3.2. Synthesis route for poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)]](image)

Table 3.1. Different monomer ratios results of PLBr

<table>
<thead>
<tr>
<th>NO</th>
<th>LA %$^a$</th>
<th>BrH %$^a$</th>
<th>Time (h)</th>
<th>$M_n^b$</th>
<th>PDI$^c$</th>
<th>LA %$^c$</th>
<th>BrH %$^c$</th>
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<tr>
<td>1</td>
<td>90</td>
<td>10</td>
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<tr>
<td>2</td>
<td>70</td>
<td>30</td>
<td>90</td>
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<td>33</td>
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<td>49</td>
<td>51</td>
</tr>
</tbody>
</table>

a) Mole percentage in the monomer feed. b) Calculated by GPC c) Percentage composition in the copolymer calculated from $^1$H NMR spectrum.
Figure 3.2. 300 MHz $^1$H NMR spectrums of poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)] with different monomer ratios.

Figure 3.3. GPC traces of poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)] with different monomer ratios.
3.3 ATNRC of poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)]

Scheme 3.3 illustrates the synthesis route of TEMPO coupled PLB. As shown in Table 3.2, different reactions were performed to synthesize the product. Figure 3.4 presents the GPC results of different products obtained from stirring with different reactants. When coupling with HTEMPO using PMDETA as a ligand, nothing was obtained after working up. It was found that both HTEMPO and PMDETA could cause the degradation of PLB. However, the mechanisms for the degradation are different. HTEMPO could cause the degradation due to its hydroxyl group acting as a base. PMDETA acts as a strong base and could cause the elimination to degrade the polymer. As shown in figure 3.4, TEMPO and 2,2'-bipyridine (Bipy) did not cause the degradation of polymer. Therefore, TEMPO and Bipy were used instead of HTEMPO and PMDETA in further reactions. 2,2'-Bipyridine is a weaker base than PMDETA. However, the product still had lower molecule weight comparing to the starting material. The amount of Bipy was reduced to the minimum (10%) and it turned out to be effective in depressing elimination reaction. The coupling efficiency increased from almost 0% to 30% as the amount of Bipy increased from 5% of the amount required to 30%. The optimal condition for the coupling reaction was using CuBr and Bipy in the ratio of 1:0.6. The coupling efficiency was optimized to 30%.

Scheme 3.3. Synthesis route of TEMPO coupled poly[(lactic acid)-co-(2-bromo-3-hydroxypropionic acid)].
Table 3.2. Varying ATNRC reaction conditions and results*

<table>
<thead>
<tr>
<th>No.</th>
<th>Precursor Polymer</th>
<th>Nitroxide</th>
<th>Redox System</th>
<th>Results</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>PLB5050</td>
<td>HTEMPO</td>
<td>CuBr/PMDET A/Br=1:1:1</td>
<td>No precipitation</td>
</tr>
<tr>
<td>2</td>
<td>PLB7030</td>
<td>TEMPO</td>
<td>CuBr/PMDET A/Br=1:1:1</td>
<td>Degraded Polymer</td>
</tr>
<tr>
<td>3</td>
<td>PLB7030</td>
<td>TEMPO</td>
<td>CuBr/Bipy/Br =1:2:1</td>
<td>Degraded Polymer</td>
</tr>
<tr>
<td>4</td>
<td>PLB9010</td>
<td>TEMPO</td>
<td>CuBr/Bipy/Br =1:0.1:1</td>
<td>No degradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Very low conversion</td>
</tr>
<tr>
<td>5</td>
<td>PLB9010</td>
<td>TEMPO</td>
<td>CuBr/Bipy/Br =1:0.6:1</td>
<td>30% coupling</td>
</tr>
</tbody>
</table>

*All reactions were performed in ethyl acetate at 50 °C for 24 h. Working up procedure is precipitating into methanol or hexane.

Figure 3.4. GPC results of PLB after stirring with various reactants at 50 °C for 24 h.
3.4 Synthesis of Methyl 2-bromo-3-hydroxypropionate

Scheme 3.4 illustrates the synthesis of methyl 2-bromo-3-hydroxypropionate. Figure 3.5 presents its $^1$H NMR spectrum, which confirms the ester formation according to the appearance of methyl group and chemical shift of protons. The ratio of CHBr to CH$_2$ to CH$_3$ is close to 1:2:3. Therefore the product was successfully prepared and pure.

![Scheme 3.4. Synthesis of methyl 2-bromo-3-hydroxypropionate.](image)

Figure 3.5. 300 MHz $^1$H NMR spectrum of methyl 2-bromo-3-hydroxypropionate.

3.5 Synthesis of 2-Ethoxyethyl methansulfonate (EEMs)

Scheme 3.5 illustrates the synthesis of 2-ethoxyethyl methansulfonate and its $^1$H NMR spectrum in Figure 3.6 demonstrates the formation of product. In the figure, the
resonances at 4.37 ppm and 3.0 confirm the formation of sulfonic ester. The ratio of CH$_2$ and CH$_3$ is close to 2:3, which means the conversion is 100% and product is pure.

Scheme 3.5. Synthesis of 2-Ethoxyethyl methanesulfonate

Figure 3.6. 300 MHz $^1$H NMR spectrum of 2-Ethoxyethyl methansulfonate (EEMs)

3.6 Synthesis of 4-ethoxyethoxy-2,2,6,6-tetramethylpiperidin-1-yl)oxy (EETEMPO)

Scheme 3.6 illustrates the synthesis of 4-(2-ethoxyethoxy)-(2,2,6,6-tetramethylpiperidin-1-yl)oxy and Figure 3.7 presents the $^1$H NMR spectrum of product. The NMR spectrum was obtained by adding equal mole amount of phenylhydrazine into the NMR tube to quench the TEMPO radical. Methylenes from 2-ethoxyethyl
methanesulfonate shift and overlap at 3.6 ppm. The methyl group from sulfonic ester disappears and the ether bond forms, which demonstrates the 100% conversion and formation of TEMPO derivative.

Scheme 3.6. Synthesis of 2-Ethoxyethoxy TEMPO (EETEMPO)

Figure 3.7. 300 MHz $^1$H NMR spectrum of 4-ethoxyethoxy-2,2,6,6-tetramethylpiperidin-1-yl)oxy (EETEMPO).
3.7 Synthesis of Methyl 2-((4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxyacetate

Scheme 3.7 illustrates the synthesis of 2-((4-ethoxyethoxy2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxy acid. Figure 3.8 presents the \(^1\)H NMR spectrum of pure product. The coupling reaction is demonstrated by the chemical shift of CHCH\(_2\)OH at 4.5 ppm and the appearance of TEMPO resonances. The product was successfully prepared and pure according to the \(^1\)H NMR spectrum.

![Scheme 3.7. Synthesis of Methyl 2-(4-(2-ethoxyethoxy)-(2,2,6,6-tetramethylpiperidin-1-yl)-1-oxy)-3-hydroxyacetate](image)

![Figure 3.8. 300 MHz \(^1\)H NMR spectrum of methyl 2-((4-ethoxyethoxy-2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxyacetate](image)
3.8 Synthesis of 2-(4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy)-3-hydroxy acid

Scheme 3.8 illustrates the synthesis of monomer. Figure 3.9 presents the $^1$H NMR spectrum of product. The disappearance of the methyl group at 3.75 ppm from methyl ester demonstrates the conversion from ester to acid. The resonances around 7.0 ppm come from phenylhydrazine. The product is pure except for a small amount of ethyl acetate.

![Scheme 3.8. Synthesis of 2-[4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1-oxy]-3-hydroxy acid](image)

Figure 3.9. 300 MHz $^1$H NMR spectrum of 2-((4-ethoxyethoxy-2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxy acid
3.9 Synthesis of poly[2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxy acid]

Scheme 3.9 illustrates the polymerization of monomer catalyzed by pTSA. Figure 3.10 presents the $^1$H NMR spectrum of product. The resonances from hydroxyl carboxylic acid decrease after polymerization, which indicates that the monomer decomposes during the polymerization. The reason for the decomposition might be the high temperature used. GPC result shows only monomer or dimer after polymerization. The monomer cannot tolerate 90 °C; therefore the traditional acid catalyzed polycondensation cannot be applied to this monomer.

Scheme 3.9. Synthesis of poly[2-[4-(2-ethoxyethoxy)-2,2,6,6-tetramethylpiperidin-1- oxy]-3-hydroxy acid].

Figure 3.10. $^1$H NMR spectrum of decomposed 2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxy acid
3.10 Synthesis of poly[2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxy acid]

Scheme 3.10 illustrates the synthesis of poly[2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3-hydroxy acid] by coupling reagent. Figure 3.11 presents the $^1$H NMR spectrum of the product. The breadth of the resonance at 4.5 indicates the formation of polymer. It comes from overlapping of CH and CH$_2$O. The ratio of integral of CHCH$_2$O to CH$_2$O is close to 3:7, which agrees with the polymer structure. The polymer was recovered from dialysis in acetone for 48 because it doesn't precipitate in Methanol. The polymer is soluble in acetone, methanol, methylene chloride and insoluble in water. Figure 3.12 presents the GPC result of the polymer. The $M_n$ is 1,500 and PDi is 1.25. The reason for the low molecule weight might be low temperature and short reaction time because it is polycondensation and molecule weight increases step-wise in polycondensation.

\[
\text{Scheme 3.10. Synthesis of poly[2-[4-(2-ethoxyethoxy)-(2,2,6,6-tetramethylpiperidin-1-yl)-1-oxy]-3-hydroxy acid]}
\]
Figure 3.11. 300 MHz $^1$H NMR spectrum of poly[2-(2,2,6,6-tetramethylpiperidin-1-yl)oxy]-3-hydroxy acid]

Figure 3.12. GPC result of poly[2-(2,2,6,6-tetramethylpiperidin-1-yl)oxy]-3-hydroxy acid]
3.11 Synthesis of mesylated mPEG2000

Scheme 3.11 illustrates the synthesis of mesylated mPEG. Figure 3.13 presents the $^1$H NMR spectrum of product. The resonances at 4.36 ppm and 3.06 ppm indicate the formation of sulfonic ester. The NMR spectrum shows complete conversion of mPEG2000 as the ratio of CH$_2$O to CH$_3$ is exactly 2:3. The reaction was successfully performed and get relatively high yield (84.27%).

Scheme 3.11. Synthesis route of mesylated mPEG2000 (mPEG-Ms)

Figure 3.13. 300 MHz $^1$H NMR spectra of mesylated mPEG.

3.12 Preparation and characterization of micelles

Micelles were formed from self-assembly of the polymer by evaporation method. The formation of micelles was confirmed by TEM. Figure 3.14 presents the TEM image
of micelles. The micelles have uniform size with spherical morphology and the diameters are around 40 nm.

Figure 3.14 TEM image of PLGA-g-EE TEMPO micelles.
CHAPTER IV
CONCLUSION

2-Bromo-3-hydroxypropionic acid was prepared in bulk for the follow up reactions. Then the copolymer was successfully made and fully characterized by NMR and GPC analyses. Different monomer ratios were attempted and results agreed with Dr. Pugh’s patent while a little bit higher molecular weight was obtained. Model compound was successfully synthesized and coupled with TEMPO and TEMPO derivatives. mPEG-TEMPO was successfully prepared by using fresh sodium hydride dispersion in mineral oil. Another TEMPO derivative, 2-ethoxyethoxy TEMPO, was also synthesized by the same procedure.

The difficulty of the project lay in the radical coupling chemistry of polymer and nitroxides. It turned out that the copolymer degraded during the coupling reaction. Various conditions were attempted to avoid the degradation. Finally, TEMPO derivative coupled copolymer was made with 50% graft ratio. Also, graft copolymer PLGA-g-EETEMPO was prepared in 5 steps by polymerizing EETEMPO coupled hydroxyl acid monomer. Avoiding concurrence of the ligand PMDETA and polyester solved the elimination problem. The monomer could not be polymerized by acid-catalyzed polycondensation at 90 °C. In the end, it was polymerized by using a coupling reagent. The micelle formation was also investigated after obtaining the polymer. Micelles with uniform size and spherical morphology were obtained and confirmed by TEM.
REFERENCES


