

**Enabling Synthesis Toward the Production of Biocompatible Magnetic
Nanoparticles With Tailored Surface Properties**

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Enabling Synthesis Toward the Production of Biocompatible Magnetic Nanoparticles With Tailored Surface Properties

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Abstract

Amphiphilic tri- and penta-block copolymers containing a polyurethane central block with pendant carboxylic acid groups flanked by hydroxyl functional polyether tails were synthesized. Our intention was to investigate the activities of these copolymers as dispersants for magnetite nanoparticles in biological media. A benzyl alkoxide initiator was utilized to prepare poly(ethylene oxide) (BzO-PEO-OH), poly(propylene oxide) (BzO-PPO-OH) and poly(ethylene oxide-b-propylene oxide) (poly(BzO-EO-b-PO-OH)) oligomeric tail blocks with varying lengths of PEO and PPO. The oligomers had a hydroxyl group at the terminal chain end and a benzyl-protected hydroxyl group at the initiated end. The polyether oligomers were incorporated into a block copolymer with a short polyurethane segment having approximately three carboxylic acid groups per chain. The block co-polyurethane was then hydrogenated to remove the benzyl group and yield primary hydroxyl functionality at the chain ends. End group analysis by ^1H NMR showed the targeted ratio of PEO to PPO demonstrating control over block copolymer composition. Number average molecular weights determined by both ^1H NMR and GPC were in agreement and close to targeted values demonstrating control over molecular weight. Titrations of the pentablock copolymers showed that the targeted value of approximately three carboxylic acid groups per chain was achieved.

Heterobifunctional poly(ethylene oxide) (PEO) and poly(ethylene oxide-*b*-propylene oxide) (PEO-*b*-PPO) copolymers were synthesized utilizing heterobifunctional initiators to yield polymers having a hydroxyl group at one chain end and additional moieties at the other chain end. For PEO homopolymers, these moieties include maleimide, vinylsilane, and carboxylic acid functional groups. Heterobifunctional PEO oligomers with a maleimide end group were synthesized utilizing a double metal cyanide coordination catalyst to avoid side reactions that occur with a basic catalyst. PEO oligomers with vinylsilane end groups were synthesized via alkoxide-initiated living ring-opening polymerization, and this produced polymers with narrow molecular weight distributions. Heterobifunctional PEO-*b*-PPO block copolymers were synthesized in two steps where the double metal cyanide catalyst was used to polymerize propylene oxide (PO) initiated by 3-hydroxypropyltrivinylsilane. The PPO was then utilized as a macroinitiator to polymerize ethylene oxide (EO) with base catalysis. Heterobifunctional PEO and PEO-*b*-PPO block copolymers possessing carboxylic acid functional groups on one end were synthesized by reacting the vinyl groups with mercaptoacetic acid via an ene-thiol addition.

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*"Research is what I'm doing when I don't know what I'm doing."
- Wernher Von Braun (1912-1977)*

*In loving memory of my grandfathers,
Joseph Vernell Balance Sr. and James Lowe Thompson.*

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Abbreviations

AFM	atomic force microscopy
AFP	anti-human α -fetoprotein
AIBN	2,2'-azobis(2-methylpropionitrile)
APTMS	(3-aminopropyl)trimethoxysilane
BHMPA	Bis(hydroxymethyl)propionic acid
BPMI	4-maleimidobenzophenone
BPMI	4-maleimidobenzophenone
BPOH	4-hydroxybenzophenone
BSA	bovine serum albumin
CBL	chlorambucil
Cbz	benzyl chloroformate
DBTDL	Dibutyltin dilaurate
DCC	dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DMPE	1,2-bis(myristoylphosphatidyl)ethanolamine
DPPE	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine
DSC	disuccinimidyl carbonate
DSS	disuccinimidyl succinate
DST	disuccinimidyl tartrate
EDC	N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide
EO	ethylene oxide
FITC	fluorescein isothiocyanate
Fmoc	fluorenylmethyl chloroformate
FTIR	Fourier transform infrared spectroscopy
GA	glutaraldehyde
GPC	gel permeation chromatography
HAS	human serum albumin
HMPA	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HTEMPO	4-hydroxyl-2,2,6,6-tetramethyl-1-piperidinyloxy
IPDI	Isophorone diisocyanate
LL1	anti-CD74 antibody
MAC	methacryloyl chloride
MPEO	monomethoxy-poly(ethylene oxide)
Ms	methane sulfonyl
NHS	N-hydroxysuccinimide
NTA	nitrilotriacetic acid
PDA	potassium 4-(diethoxymethyl)benzylalkoxide
PDI	polydispersity index
PEO	poly(ethylene oxide)
PO	propylene oxide
PPO	poly(propylene oxide)

PVA	Poly(vinyl alcohol)
rhG-CSF	recombinant human granulocyte-colony stimulating factor
RSA	rat serum albumin
SAM	self-assembled monolayer
SEC	size exclusion chromatography
SMRFM	single molecule recognition force microscopy
SPDP	N-succinimidyl-3-(2-pyridyldithio)propionate
TBAF	tetra-n-butyl ammonium fluoride
TBDMS	tert-butyldimethylsilyl
TDI	toluene diisocyanate
TDMA	N-2-(2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentyl)-ethylmethyl amine
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
TEMPO _{Na}	4-oxy-2,2,6,6-tetramethyl-1-piperidinyloxy
Tf	transferrin
TNF- α	recombinant human necrosis factor alpha (TNF- α)
VBA	vinylbenzyl alcohol
VBC	vinylbenzyl chloride
VPO	vapor pressure osmometry
XPS	X-ray photoelectron spectrum

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1. CHAPTER 1: Dissertation Overview

A major goal in the design of new polymeric biomaterials is to control the interactions of biomolecules and living cells with biomaterial surfaces. Site-specific drug delivery is one example of advanced biomaterial applications that requires such control.^{1,2} Major drawbacks of injecting hydrophobic particles into the bloodstream include the uncontrolled adsorption of proteins onto the surface as well as the uptake of these particles by phagocyte cells. It has been shown that particles with poly(ethylene oxide) surfaces have great potential as long circulating systems after intravenous administration³⁻⁷

Block copolymers containing functional groups at specific positions along the backbone of the copolymer and the ability to control the number of functional groups present in the polymer have been reported previously.⁸ In our initial work, the triblock copolymers consisted of a polyurethane anchor block containing carboxylic acid functional groups flanked by PEO tail blocks having a hydrophobic terminal methyl or tert-butyl group. The goal of this dissertation is to discuss the synthesis of biocompatible polymeric dispersion stabilizers consisting of poly(ethylene oxide) and poly(ethylene oxide-b-propylene oxide) with a free hydroxyl functionality to modify the surface properties of magnetite nanoparticles. It is believed the free hydroxyl functionality will be a key feature in covalently attaching biologically active ligands to the dispersion stabilizers.

The second chapter is a review on the synthesis and applications of heterobifunctional poly(ethylene oxide) oligomers that will be submitted for publication in Polymer. This

review discusses many of the synthetic techniques that are utilized for the synthesis of heterobifunctional poly(ethylene oxide) where a majority of the work cited is focused on preparing bioconjugates. The third chapter covers the initial attempts at producing biocompatible dispersion stabilizers utilizing tri- and pentablock copolymers consisting of a polyurethane anchor block containing carboxylic acid functional groups flanked by poly(ethylene oxide) or poly(ethylene oxide-b-propylene oxide) tail blocks. The fourth chapter will discuss a more simplified second generation of biocompatible dispersion stabilizers utilizing heterobifunctional polyethers. The second generation of dispersion stabilizers eliminates the need for a polyurethane central block by incorporating multiple carboxylic acid groups onto the polyether chain end while retaining the hydroxyl functionality at the other chain end. In our work to synthesize heterobifunctional polyethers to be utilized as dispersion stabilizers we also began to explore other heterobifunctional polyethers with biologically relevant functionalities such the maleimide moiety. It is believed that the heterobifunctional polyethers containing maleimide, vinylsilane, and carboxylic acid functional groups discussed in chapter four will also be of great interest in the field of bioconjugate chemistry. The fifth chapter discusses future research and applications of the dispersion stabilizers.

2. CHAPTER 2: Review: Synthesis and Applications of Heterobifunctional Poly(ethylene oxide) Oligomers

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2.1. Abstract

Poly(ethylene oxide) (PEO) oligomers are employed extensively in pharmaceutical and biomedical arenas mainly due to their excellent physical and biological properties, including solubility in water and organic solvents, lack of toxicity, and absence of immunogenicity.^{4,9-13} PEO can be chemically modified and reacted with, or adsorbed onto, other molecules and surfaces.¹⁴⁻¹⁸ Sophisticated applications for PEO have increased the demand for PEO oligomers with tailored functionalities, and heterobifunctional PEOs are often needed. This review discusses the synthesis and application of heterobifunctional poly(ethylene oxide) oligomers possessing amine, carboxylate, mercapto, and maleimide functional groups.

Keywords: Poly(ethylene oxide), PEO, PEG, polyethylene glycol, polyether

2.2. Introduction

Heterobifunctional PEOs have the structure X-PEO-Y, where X and Y are different functional groups. They can serve as hydrophilic, flexible, biocompatible, inert spacers with defined lengths connecting two components. Applications include linking of macromolecules to surfaces, site-specific targeting of drugs and liposomes, and functionalization of nanoparticles for bioassays and biorecognition.¹⁹⁻²⁷ When bound to other molecules, PEO typically increases their solubility in aqueous media and yields improved circulation times in vivo.^{10,28-31} PEO-modified surfaces also display increased hydrophilicity as well as suppressed protein adsorption, platelet adhesion and macrophage attachment.³²⁻³⁴

There have been several reviews discussing PEO chemistry, most of which have focused on monofunctional PEO, with only limited treatment of heterobifunctional polymers.³⁵⁻³⁹ This review concentrates on synthetic pathways to achieve heterobifunctional oligomers and applications of these materials.

2.3. Synthesis and Properties of PEO

PEO is a linear or branched polyether often terminated with hydroxyl groups that are derived from neutralization of the terminal ether repeat unit in the chain. PEO oligomers are most commonly synthesized via anionic ring-opening polymerization of ethylene oxide (EO). Ring-opening polymerization is a widely used route to homopolymers as well as random and block copolymers with well-defined molecular weights and architectures. One desirable characteristic of PEO is the relatively narrow molecular weight distribution that can be achieved compared with many other polymers. PEO prepared by anionic ring-opening polymerization generally has a polydispersity (M_w/M_n) less than 1.1.⁴⁰

Anionic ring-opening polymerization of EO can be living in nature due to the stability of the propagating species. Most often polymerizations of EO are carried out with a hydroxide or alkoxide initiator (Figure 2.1). The reactions take place via nucleophilic attack on an EO methylene to open the ring and form the propagating species.

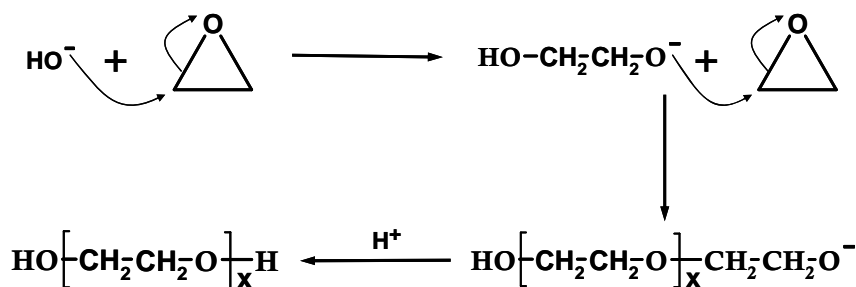


Figure 2.1. Anionic ring-opening polymerization of EO initiated by hydroxide

Epoxide polymerizations can also be conducted utilizing coordination catalysts, usually in conjunction with an alcohol initiator. Double metal cyanide catalysts are commonly employed to polymerize propylene oxide (PO) and sometimes EO. These catalysts are well known for their activities in syntheses of high quality poly(propylene oxide) (PPO) with low unsaturation.⁴¹⁻⁴³

The side reaction that occurs in PO polymerizations is caused by hydrogen abstraction from the methyl sidechain of the monomer by a basic catalyst can be avoided. This class of catalysts was originally discovered by workers at General Tire Inc., with improvements made by other companies including ARCO, Shell, Asahi Glass, and Bayer.⁴⁴⁻⁴⁷ One example of a double metal cyanide catalyst, $\text{Zn}_3[\text{Co}(\text{CN})_6]_2$, was utilized by Huang et al. to synthesize random copolymers of EO and PO.⁴⁸ Huang and coworkers obtained copolymers with various EO/PO compositions and with unimodal molecular weight distributions in the range of 1.21 to 1.55.

PEO is clear, colorless, odorless, inert to many chemical agents, stable against hydrolysis, and nontoxic. Biocompatibility and lack of immunogenicity make PEO an important polymer for biomedical applications.^{9,49} When bound to an immunogenic substrate having a desirable function in the body, PEO tends to reduce or eliminate immune response so that the organism can tolerate the substance.³⁹ Another important property of PEO is its solubility in water as well as in many organic solvents. When bound to a water insoluble compound, the resulting PEO conjugate generally displays increased water solubility or dispersibility.^{8,10,50}

Since the repeating ether units of PEO are essentially non-reactive, these oligomers must be reacted with, or adsorbed onto, other compounds through terminal or pendant functional groups. The hydroxyl terminus of X-PEO-OH is often converted to the active N-hydroxysuccinimidyl ester when reactivity toward amines is desired. PEO with thiol or carboxylic acid end groups have also been utilized to bind to metal and metal oxide surfaces.^{8,22,51-54} Addition of maleimide functionality to the PEO chain end has also been of great interest in the formation of bioconjugates.⁵⁵⁻⁵⁷

Two broad methods are commonly employed for synthesizing heterobifunctional PEO oligomers (Figure 2.2). The most direct is the ring-opening polymerization of EO from a heterobifunctional anionic initiator, and this is followed by termination with another functional moiety. The second method involves derivatizations of PEO diols, followed by separation of the resultant statistical mixtures to isolate the target heterobifunctional oligomers.

Initiation and polymerization of EO by a heterobifunctional initiator so that one of the functional groups reacts with the EO and the other group remains intact is shown in Figure 2.2A.^{35,37,58-60} Anionic ring-opening polymerization of EO from an initiator containing a protected functional group such as potassium bis(trimethylsilyl)amide or

(cyanomethyl)potassium, and termination by acidification have been utilized to prepare heterobifunctional PEOs with a hydroxyl group at one end.⁶¹⁻⁶⁵ The hydroxyl terminus can then be derivatized to produce more complex heterobifunctional polymers such as H₂N-PEO-COOH.⁵⁸ Other heterobifunctional PEO oligomers have been synthesized with various end groups such as carboxyl, vinylbenzyl, acetal, pyridyl disulfide, maleimide, and methacryloyl moieties by utilizing an appropriate initiator and terminating agent.^{60,66-70} When synthesizing heterobifunctional PEOs, the reaction conditions must be completely anhydrous to ensure the purity of the heterobifunctional product. If water is present, then it will also initiate the EO, producing PEO diols as side products and reducing the anticipated molecular weights of the desired heterobifunctional polymers.⁶³

The second method requires alteration of the terminal hydroxyl groups of α,ω -dihydroxy-poly(ethylene oxide) (PEO diol) through a series of reactions, followed by separation of the mono-, di-, and unsubstituted components (Figure 2.2B). To obtain heterobifunctional oligomers, only a portion of the hydroxyl end groups are converted to the new functional group, so the statistics of these reactions alone lead to low yields. These synthetic approaches are complicated because most employ several reaction steps and require post-separations of chemically similar polymers that differ only in their end group structures.^{63,71,72} As the molecular weights of the starting PEO diols increase, the chemical and physical differences among the mono-, di-, and unsubstituted products become increasingly smaller and the heterobifunctional oligomers are therefore harder to isolate.⁷³ For these reasons, most heterobifunctional PEOs synthesized from PEO diols are either low molecular weight oligomers, or they possess ionizable end groups such as amines or carboxylic acids that enable separations by ion exchange chromatography.

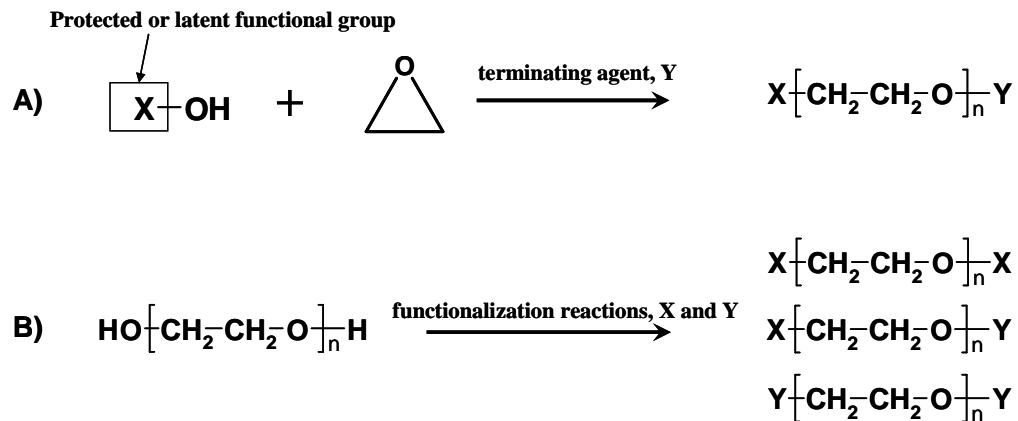


Figure 2.2. Synthetic methods to produce heterobifunctional PEO oligomers: Direct synthesis of heterobifunctional PEO (A) and end group modification of PEO diols (B)

2.4. Synthesis and Applications of X-PEO-COOH

α -Carboxy- ω -hydroxy-poly(ethylene oxide) (HOOC-PEO-OH) oligomers are important precursors to PEO bioconjugates. PEO with a carboxylic acid on one end and another functional group on the other can also be utilized as intermediates for other heterobifunctional polymers.⁷⁴⁻
⁷⁶ The carboxyl terminus of PEO can be reacted to form active esters such as the succinimidyl ester derivative. Many biologically relevant ligands have been covalently attached to PEO through amide bonds via these succinimidyl ester intermediates (Figure 2.3).^{16,17,77-79}

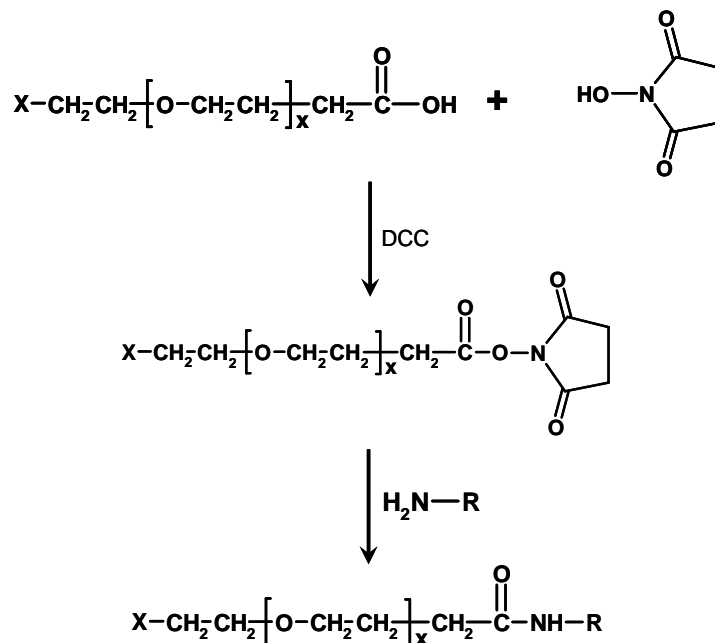


Figure 2.3. Formation of an amide linkage via the active succinimidyl ester

2.4.1. Direct Synthesis of X-PEO-COOH

2.4.1.1. Synthesis of (HOOC)₁₋₃-PEO-OH

Heterobifunctional PEO with a hydroxyl group on one chain end and multiple carboxylic acid groups (1-3) on the other chain end have been synthesized utilizing vinylsilylpropanol initiators (Figure 2.4).⁸⁰ These initiators containing one, two, or three vinyl groups were synthesized from 3-chloropropylchlorodimethylsilane, 3-chloropropyldichloromethylsilane, and 3-chloropropyltrichlorosilane, respectively, by reaction with vinylmagnesium chloride (Figure 2.5). The alkyl chlorides were then converted to the corresponding alcohols.

The alkoxide initiators were prepared by reacting the appropriate vinylsilylpropanol with potassium naphthalide in THF (1 mole of -OH:0.95 mole of potassium naphthalide). A slight deficiency of potassium naphthalide was used to ensure that the vinyl groups were preserved during alkoxide formation and polymerization. The anionic initiator was added to EO and allowed to react at room temperature, and the polymerizations were terminated with acetic acid.

The ratio of end group protons observed via ^1H NMR matched the theoretical values, (3:2:2:2, 9:2:2:2, and 6:2:2:2 for the mono-, di-, and tri-vinylsilane initiators, respectively) confirming the structures of the heterobifunctional polymers. Molecular weights obtained by ^1H NMR and SEC matched well with the targeted molecular weights based on the monomer to initiator ratios. Molecular weight distributions determined by SEC were narrow (≤ 1.13) indicating that the polymerizations proceed without significant side reactions.

Conversion of the terminal vinylsilane moieties into carboxylic acids was achieved via ene-thiol additions utilizing mercaptoacetic acid and AIBN (Figure 2.4). The ene-thiol reactions were monitored via ^1H NMR by following the disappearance of vinyl proton resonances at approximately 6.0 ppm. The NMR resonances of the products correspond with those reported by Wilson et al. for tricarboxylic acid terminated PDMS.⁸¹ A similar synthesis utilizing cysteamine hydrochloride and AIBN was also carried out to yield $(\text{H}_2\text{N})_{1-3}\text{-PEO-OH}$.⁸¹

Heterobifunctional poly(ethylene oxide-*b*-propylene oxide) (PEO-*b*-PPO) block copolymers have also been synthesized utilizing trivinylsilylpropoxide as the initiator.⁸² Even under mild reaction conditions, the anionic polymerization of PO leads to some unsaturation at the chain end due to the abstraction of a methyl proton on the PO monomer converting it to allyl alkoxide.⁸³⁻⁸⁵ This abstraction reaction is generally referred to as a chain-transfer reaction and not only increases the unsaturation in the product, but also limits the degree of polymerization. Compared with conventional base-catalyzed polymerization of PO, double metal cyanide catalysts yield high quality PPO with low amounts of unsaturation and much faster rates of polymerization even with low catalyst concentrations.^{41,48} Thus, the PPO blocks of these diblock copolymers were prepared in batch polymerizations utilizing the double metal cyanide catalyst, zinc hexacyanocobaltate (*Impact 3*, Bayer), and this yielded heterobifunctional PPO oligomers

with molecular weight distributions in the range of 1.84 to 1.91. Retention of the end groups during polymerization was confirmed by ^1H NMR indicating that the reactions proceeded without significant side reactions. These $(\text{vinyl})_3\text{-PPO-OH}$ oligomers were then utilized as macroinitiators to polymerize EO anionically, and this was followed by conversion of the vinyl groups to carboxylic acids via ene-thiol reactions as described above.

The relatively broad molecular weight distributions obtained from batch reactions utilizing double metal cyanide catalysts has been well documented.^{41,48,86} These catalysts are heterogeneous at least in the initial stages of polymerization, and they become at least somewhat solubilized as the reactions proceed. The broadened molecular weight distributions obtained with these catalysts relative to those obtained with base-initiated polymers can likely be attributed to a combination of the heterogeneous nature of the catalysts and also to the very low levels utilized (i.e., 25-100 ppm) relative to the concentrations of propagating chain ends. The initiator to catalyst ratio was 10^3 or higher for these polymerizations, which means that it is not likely that all chain ends are permanently active during polymerization. At the beginning of the reaction there are relatively few active chains coordinated with the catalyst and a relatively high concentration of monomer, these conditions combined with a fast rate of propagation relative to chain transfer could lead to the broadened molecular weight distributions for these batch type reactions.

Carboxylate anions have been widely utilized for adsorbing surfactants or polymers onto the surfaces of magnetite nanoparticles.^{8,87-89} Copolymer dispersion stabilizers have been synthesized that contain carboxylic acids at specific positions along the backbone of the copolymer, and with control over the number of functional groups in the polymer.^{8,54} Previously reported magnetite complexes with triblock copolymers consisting of a polyurethane anchor

block containing carboxylic acid functional groups flanked by PEO and PEO-*b*-PPO tail blocks have been shown to adsorb on the surface of magnetite nanoparticles, and these served as dispersion stabilizers in aqueous media.^{8,54} The synthetic approach utilizing the vinylsilylpropoxide initiators to prepare carboxylic acid containing PEO dispersion stabilizers does not require a separate anchoring block such as the urethane segment. These carboxylic acid functional oligomers can be adsorbed onto the surfaces of magnetite nanoparticles, and the resultant polyether-magnetite complexes can be dispersed in water. The nanocomplexes also offer possibilities for conjugating bioactive molecules to the free hydroxyl ends on the PEO after complexation to the magnetite surface. Post-conjugation of bioactive molecules to nanoparticles is of great interest for applications in targeted drug delivery and for biorecognition of particular cell types.

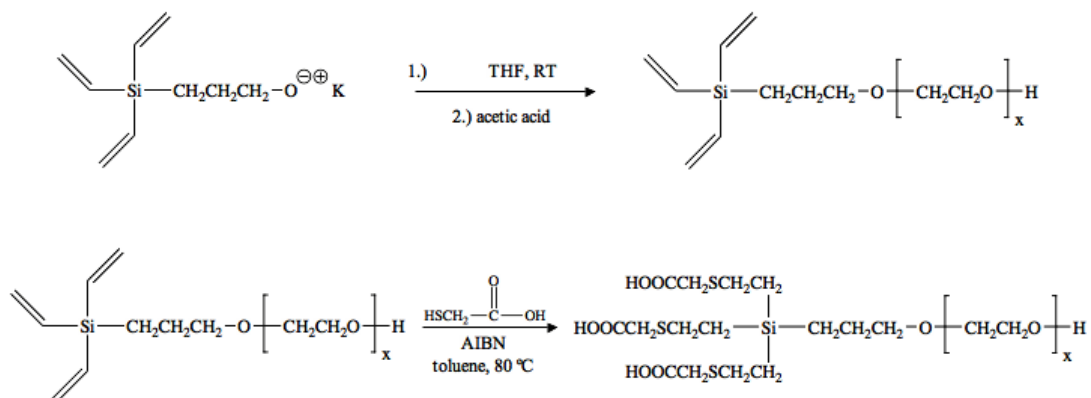


Figure 2.4 Synthesis of (HOOC)₃-PEO-OH

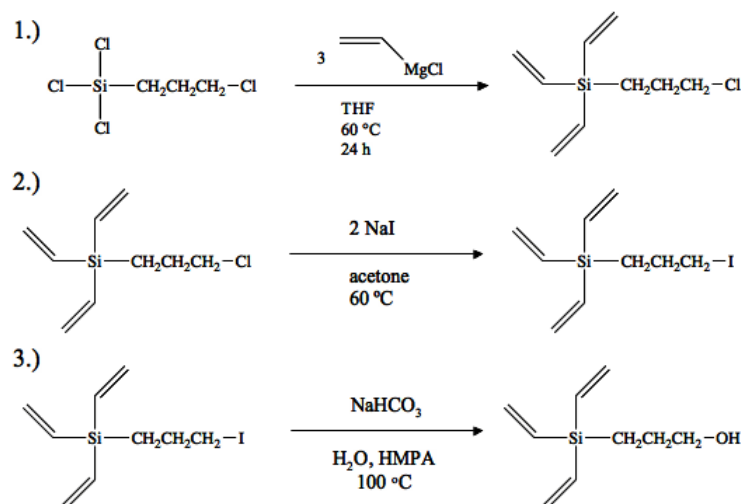


Figure 2.5. Preparation of 1.) 3-chloropropyltrivinylsilane, 2.) 3-iodopropyltrivinylsilane, and 3.) 3-hydroxypropyltrivinylsilane

2.4.1.2. Synthesis of HOOC-PEO-OH via Thiol-Initiated Anionic Polymerization

Zeng et al. investigated alkoxide and thiolate initiators containing protected or free carboxylic acid groups for synthesizing heterobifunctional PEOs with a carboxyl group at one chain end and a hydroxyl group at the other (HO-PEO-COOH).⁷⁵ One effective functional initiator was 3-mercaptopropionic acid. The heterobifunctional initiator, dipotassium-3-mercaptopropionate, was prepared by reacting 3-mercaptopropionic acid with two equivalents of potassium naphthalide. EO was added to the initiator solution and reacted at 40 °C in THF, and the reactions were terminated with hydrochloric acid to yield the heterobifunctional PEOs (Figure 2.6). Molecular weights ranging from 1000 to 25,000 g mol⁻¹ were achieved with narrow molecular weight distributions, 1.07 to 1.15. End group analysis by ¹H NMR showed the expected structure of the heterobifunctional PEOs, thus confirming that the carboxylate did not participate in the polymerizations under the conditions utilized.

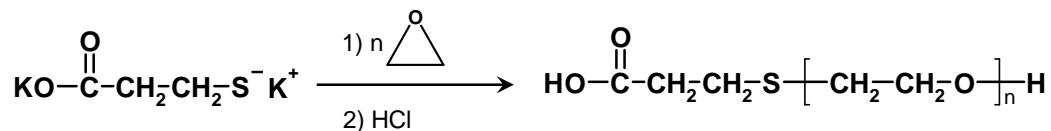


Figure 2.6. Thiolate-initiated anionic polymerization of EO in the presence of a weak carboxylate nucleophile

The HO-PEO-COOH oligomers were utilized as macroinitiators for synthesizing α -carboxy-poly(ethylene oxide-*b*- ϵ -caprolactone) copolymers via a hydrochloric acid catalyzed cationic polymerization.⁷⁵ Poly(ϵ -caprolactone) blocks were synthesized with molecular weights ranging from 2,000 to 9,000 g mol⁻¹ and the copolymers had molecular weight distributions ≤ 1.18 . Analysis of the block copolymers by SEC showed that no residual unreacted PEO macroinitiator remained.

2.4.1.3. Synthesis of NaOOC-PEO-NH₂ via (Cyanomethyl)potassium-Initiated PEO
 Polymerizations of EO by (cyanomethyl)potassium have yielded heterobifunctional PEOs with a carboxyl group at one end and an amine at the other (NaOOC-PEO-NH₂).⁵⁸ The (cyanomethyl)potassium initiator was prepared by reacting an equimolar ratio of potassium naphthalide and acetonitrile in THF at room temperature. The initiator was added to solutions of EO and 18-crown-6 in THF, and the polymerizations were conducted at 30 °C. α -Cyano- ω -hydroxy-poly(ethylene oxide) oligomers (NC-PEO-OH) were synthesized with a range of molecular weights from 400 to 5,000 g mol⁻¹ and with molecular weight distributions ≤ 1.26 . Polymer structure was confirmed by ¹H NMR end group analysis.

The hydroxy terminus of NC-PEO-OH was converted to a primary amine through a series of three reactions. The first step was activation of the hydroxyl terminus for nucleophilic displacement by reaction with toluene-4-sulfonyl chloride to yield NC-PEO-OTs. The tosyl group was displaced with potassium phthalimide, and then the phthalimide end group was

removed by reaction with hydrazine hydrate. SEC confirmed that the molecular weights of the polymers remained unaltered through this series of reactions. The degree of amination was >92 % as indicated by titrations.

The cyano group was hydrolyzed in a sodium hydroxide solution at 80 °C to yield the heterobifunctional NaOOC-PEO-NH₂ product. Hydrolysis of the cyano group was confirmed by the disappearance of the nitrile triple bond absorption at 2164 cm⁻¹ and the presence of a new absorption at 1587 cm⁻¹ corresponding to a carboxylate salt in the infrared spectra. SEC showed unimodal molecular weight distributions, and this suggested that the polymers were not degraded during hydrolysis of the cyano groups.

To develop a targeted drug delivery vehicle, Zhang et al. utilized the NaOOC-PEO-NH₂ oligomers as macroinitiators for ring-opening polymerizations of γ -benzyl-glutamate N-carboxyanhydride to produce block copolymers comprised of hydrophilic PEO and hydrophobic poly(γ -benzyl-L-glutamic acid).⁷⁴ In aqueous media these copolymers self-assembled into micelles with diameters ranging from 30-80 nm, and the aggregates had carboxylate groups on their surfaces. It was demonstrated that the carboxylate end groups could be coupled with several biologically important ligands.

2.4.1.4. Synthesis of HOOC-PEO-SH

Derivatizations of allyl groups in the presence of a radical generator to form functionalities such as amino, carboxy, hydroxy, and mercapto groups are well known.⁹⁰ Ishii et al. synthesized a heterobifunctional PEO with pyridyl disulfide at one chain end and a carboxylic acid at the other (Pyridyl-SS-PEO-COOH) utilizing allyl alcohol as the initiator (Figure 2.7).⁹¹ Allyl alcohol was reacted with potassium naphthalide to afford the potassium allyl alkoxide initiator, and this was followed by ring-opening polymerization of EO. The polymerizations

were terminated with succinic anhydride to produce a carboxylic acid end group. The allyl-PEO-COOH precursors had narrow molecular weight distributions and their molecular weights were in good agreement with the targeted values. The polymer structures were confirmed by ^1H spectra. The integral ratios of the signals assigned to the methylene protons between the two carboxyl groups and the signal assigned to the allyl methylene protons was ca. 2:4, indicating that functionalization of the chain end was quantitative.

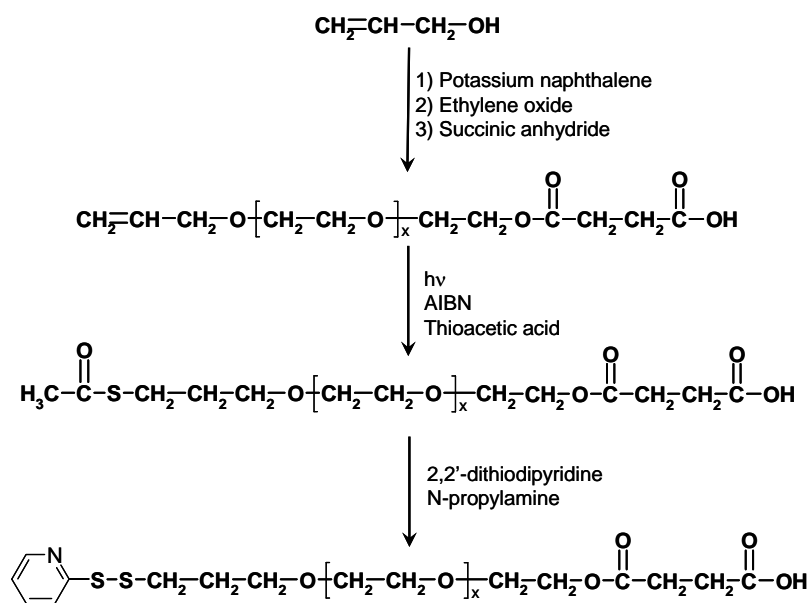


Figure 2.7. Synthesis of Pyridyl-SS-PEO-COOH

Radical addition reactions of thioacetic acid did not proceed to completion using AIBN as the initiator at 60 °C. Some coupling between chains also occurred under those reaction conditions. By contrast, when the allyl end was modified by the radical addition of thioacetic acid under UV irradiation at room temperature, the reactions proceeded to completion without any chain coupling. End group analysis via ^1H NMR, together with SEC chromatograms, showed that the functionalizations proceeded without significant side reactions. The complete disappearance of the ^1H NMR signals associated with the allyl protons combined with the

appearance of three new signals corresponding to the thio-ester end group indicated that the functionalization reaction was almost quantitative.

The thio-ester group can be cleaved under alkaline conditions to yield a mercaptan. To obtain the mercapto group without also cleaving the oxo-ester at the other chain end, reaction conditions were tailored to selectively deprotect the thio-ester. Thus, an aminolysis reaction was carried out in dry THF with a 20:1 excess of n-propylamine relative to the thio-ester. The reactions were conducted in the presence of 2,2'-dithiodipyridine and monitored by ^1H NMR. The peak intensity of the thio-ester group gradually decreased while the peak intensity associated with the carboxyl end group remained constant, indicating that selective reaction of the thio-ester was achieved. The mercapto group was then free to react with the 2,2'-dithiodipyridine to form the pyridyl-SS-PEO-COOH. The pyridyl disulfide prevented oxidation of the mercapto group during storage of the polymer. SEC confirmed that the reaction sequence proceeded without dimerization of the PEO chains. The pyridyl disulfide protecting group was cleaved with

dithiothreitol, $\text{HS-CH}_2\text{-CH}(\text{OH})\text{-CH}(\text{OH})\text{-CH}_2\text{-SH}$, to generate the free mercaptan.

A heterobifunctional PEO with the structure HS-PEO-X has been employed in biosensor chips having tethered PEO chains with functional groups at the free chain ends.⁹¹ Aldehyde-PEO-SH oligomers on the sensors were utilized to immobilize proteins via reductive amination.⁵¹ However, sufficient protein was not always bound to the surfaces via reductive amination due to the protein-repelling nature of PEO. Alternatively, HOOC-PEO-SH was tethered to the sensor surface via the SH end and the carboxyl terminus was activated with N-hydroxysuccinimide. The active ester was formed by immersing the surface with the PEO-COOH tethered chains in a solution of N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDC). Using a surface plasmon resonance sensor, the

efficiency of protein immobilization via reaction of the N-hydroxysuccinimidyl ester of the PEO tethered chains was compared to immobilization via imine formation, then reduction, of the aldehyde-ended PEO tethered chains. Ishii et al. found that the PEO surface with the active ester immobilized a much higher amount of IgG than the aldehyde-PEO surface.

Herrwerth et al. synthesized HS-PEO-COOH to functionalize the surfaces of gold substrates.⁷⁹ EO was polymerized utilizing 10-undecen-1-ol and sodium hydride as the initiator to produce an allyl-PEO-OH intermediate. The hydroxyl terminus was deprotonated with sodium hydride, then reacted with the sodium salt of chloroacetic acid to form allyl-PEO-COOH. This method of attaching a carboxylic acid via an ether linkage is more stable than the ester linkage formed from the reaction with anhydrides. Thioacetic acid was reacted with the allyl terminus utilizing UV irradiation with AIBN as the radical generator. The stability of the ether linkage allowed for subsequent hydrolysis of the thio-ester to be carried out with concentrated hydrochloric acid and ethylenediaminetetraacetic acid to afford the HS-PEO-COOH oligomer. The polymer structure was confirmed by ¹H and ¹³C NMR. Matrix assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) showed a molecular weight distribution of 1.02, indicating that this series of functionalization reactions proceeded cleanly.

The HS-PEO-COOH polymer formed a densely packed self-assembled monolayer (SAM) on gold.⁷⁹ The SAM was linked to antibodies after activation of the carboxylic acid terminus with NHS in the presence of EDC. The resistance to non-specific protein adsorption remained even after immobilization of antibodies on the surface. Even though the SAM was inert to non-specific adsorption, specific antigens could effectively bind to the immobilized receptors. The surface properties of the HS-PEO-COOH monolayer were compared to an

antibody film covalently attached to the surface via (3-aminopropyl)trimethoxysilane (APTMS) and glutaraldehyde (GA). Although the amount of surface-bound antibodies was higher on the APTMS/GA surface, the performance in complex protein solutions was significantly reduced compared to the HS-PEO-COOH SAM. Large amounts of non-specific proteins adsorbed on the APTMS/GA surface, and deactivated the binding sites of the surface-bound receptors.

2.4.2. Synthesis of X-PEO-COOH via End Group Modification of PEO Diols

2.4.2.1. Synthesis of Allyl-PEO-COOH

Völcker et al. synthesized allyl-PEO-COOH from a PEO diol in four steps.⁵⁹ First, the PEO diol was tosylated to activate the chain ends toward nucleophilic substitution (TsO-PEO-OTs). End group analysis via ¹H NMR determined that the conversion was almost quantitative, and molecular weight distributions obtained from MALDI-TOF MS and SEC remained unchanged as expected.

Selective substitution of one tosylate by an end group of higher hydrophilicity in a biphasic environment was unsuccessful. Therefore, statistically driven reaction conditions were employed, and this was followed by separation using ion exchange chromatography on DEAE Sephadex A-25. Allyl alcohol was reacted with TsO-PEO-OTs in an equimolar ratio to obtain a mixture of mono-, di-, and unsubstituted oligomers. An IR spectrum of the mixture showed characteristic absorptions corresponding to the remaining tosylates as well as the new allyl end groups. The ratio of tosyl/allyl groups, 1.16, was calculated from the ratio of ¹H NMR peak integrals corresponding to their respective end groups.

Ethyl mercaptoacetate was reacted with the remaining tosylates, and this was followed by hydrolysis of the esters to yield a mixture of allyl-PEO-allyl, allyl-PEO-COOH, and HOOC-PEO-COOH. The characteristic absorptions associated with tosyl groups were absent in the IR spectrum of the mixture and a new absorption appeared corresponding to the carboxylic acids.

^1H NMR also confirmed the absence of aromatic protons associated with the tosyl moiety. The allyl-PEO-COOH polymer was isolated from the mixture based on the amount of carboxylic acid functionality by ion exchange chromatography with an overall yield of ~35%. End group analysis via ^1H NMR determined that the ratio of allyl/carboxymethylthio groups was 1.0. Relatively narrow molecular weight distributions were obtained via MALDI-TOF MS.

2.4.2.2. Synthesis of a HOOC-PEO-OH Intermediate and Distearoylphosphatidylethanolamine-PEO-Hydrazide (DSPE-PEO-Hz)

A heterobifunctional PEO with the structure lipid-PEO-X was utilized to prepare liposomes bearing functional groups on their exterior. A heterobifunctional PEO with a DSPE moiety on one chain end and a hydrazide group at the other (DSPE-PEO-Hz) was synthesized from a PEO diol (Figure 2.8).^{78,92} First, ethyl isocyanatoacetate was utilized to partially introduce carboxyl groups via urethane linkages onto the PEO diol, and this was followed by hydrolysis of the ethyl esters. A mixture of mono- and di-functional materials was produced and some unreacted PEO diol remained. The heterobifunctional HO-PEO-COOH was separated from the mixture by ion exchange chromatography on a DEAE-Sephadex A-25 column. Titration of the carboxyl groups gave 97% of the theoretical value for the HOOC-PEO-OH polymer.

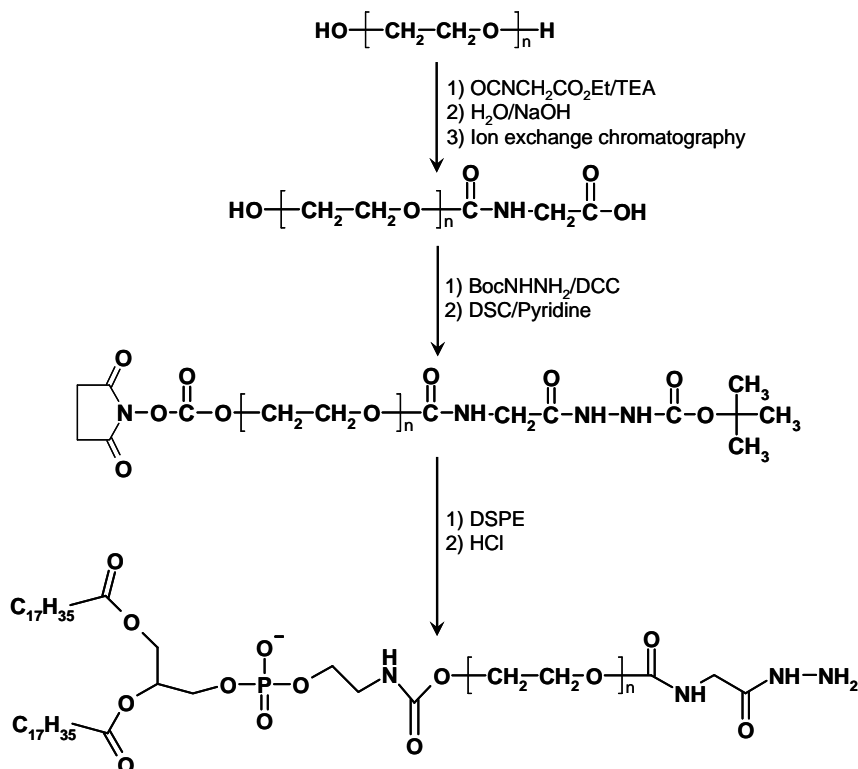


Figure 2.8. Synthesis of an amine terminated lipid-PEO copolymer (DSPE-PEO-Hz)

A boc-protected hydrazide was introduced onto the intermediate by coupling tert-butyl carbazate to the carboxyl terminus of HO-PEO-COOH in the presence of dicyclohexylcarbodiimide (DCC). The polymer structure was confirmed by both ^1H and ^{13}C NMR. The hydroxyl end group was then activated toward nucleophilic substitution with disuccinimidyl carbonate (DSC) in the presence of pyridine to yield SC-PEO-Hz-boc. Mild reaction conditions were important for introducing the succinimidyl carbonate due to the potential reactivities of the boc and hydrazide groups. Quantitative incorporation of succinimidyl carbonate was confirmed by ^1H NMR together with titrations of the active acyl groups. Finally, DSPE was conjugated to the chain end via reaction with the active acyl group in the presence of triethylamine to give DSPE-PEO-Hz-boc. The polymer was purified by dialysis against saline solution followed by deionized water and then lyophilized. Deprotection with acid

gave the target amine-functional DSPE-PEO-Hz as was confirmed by the absence of the ^1H NMR signal associated with the boc.

It has been shown that liposomes modified with methoxy-PEO-DSPE exhibited desirable pharmacokinetics and biodistributions.⁹³ A DSPE-PEO-Hz derivative was prepared to produce liposomes bearing hydrazide groups on their surfaces for introducing targeting moieties.^{78,92} It was demonstrated that DSPE-PEO-Hz could be incorporated into liposomes prepared from lecithin and cholesterol. The pharmacokinetic behavior of the hydrazide-functional liposomes was compared to their protected DSPE-PEO-Hz-boc and methoxy-PEO-DSPE analogues. The liposomes were labeled with ^{67}Ga and injected intravenously into rats, and their disappearance from the blood stream was followed by quantifying the ^{67}Ga -label. As anticipated, the pharmacokinetic behavior was essentially the same for the liposomes bearing methoxy-, hydrazide-, and boc-protected hydrazide- groups on the surface. To determine if attachment of a targeting moiety on the surface of the functional liposomes would affect their pharmacokinetic behavior, liposomes containing DSPE-PEO-Hz were covalently linked to a model ligand, IgG, through the hydrazide groups at the ends of the polymer chains.⁹² It was determined that covalent attachment of IgG to the PEO termini had no adverse effects on blood circulation times of the liposomes.

2.4.3. Additional Applications of X-PEO-COOH

2.4.3.1. SPR Sensor Chips

Surfaces coated with PEO-based materials have shown potential for preventing non-specific adsorption of proteins, and this is important for pharmaceutical applications and biomedical devices. As described previously, PEO bearing a mercapto group on one chain end can be utilized to form a SAM on gold substrates. For sensor devices, it is advantageous to have another functional group at the free PEO chain end to bind with biological vectors. A carboxylic

acid group at the free PEO chain end is typically used because it is easily activated for nucleophilic attack with NHS in the presence of a carbodiimide such as EDC. Gobi et al. produced an immunosensor based on SPR for detecting insulin utilizing a PEO with two mercapto groups on one chain end and a carboxylic acid on the other ((HS)₂-PEO-COOH) (Figure 2.9).¹⁶ The heterobifunctional PEO was used to functionalize the surface of a thin gold film on an SPR chip. The amino groups at the N-termini of the two polypeptide chains on insulin were covalently attached to the free carboxylate functional PEO chain end in the presence of NHS and EDC.

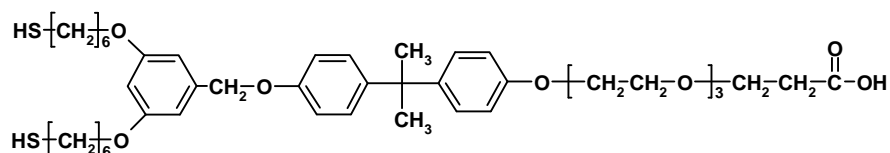


Figure 2.9. Structure of (HS)₂-PEO-COOH

A competitive immunosensing procedure was employed for detecting insulin. An anti-insulin antibody was incubated with an analyte solution before being passed over the sensor chip. In the presence of insulin, both the insulin on the sensor surface and the insulin in solution competitively adsorbed to the anti-insulin antibody. Therefore, more anti-insulin antibody detected on the SPR chip corresponded to lower concentrations of insulin in the analyte solution. This approach employing a PEO spacer produced a sensor chip with high resistance to non-specific adsorption of proteins. The chip could detect insulin concentrations as low as one and as high as 300 ng mL⁻¹. The active sensor surface could also be regenerated and reused for more than 25 cycles without significant change in sensor activity.

2.4.3.2. *Single Molecule Recognition Force Microscopy*

Single molecule recognition force microscopy (SMRFM) is an atomic force microscopy technique that can measure interaction forces on the single molecule level. The major component of SMRFM is a functionalized measuring tip that can specifically interact with a target molecule. Another key component of SMRFM is a flexible and distensible spacer for binding ligands that allows them to freely move and rotate about the tip within a restricted volume corresponding to the length of the spacer.⁹⁴ Since the PEO chain is chemically and physically inert, heterobifunctional PEOs are ideal for this application. Different functional groups at the PEO chain ends allow for their covalent reaction onto the AFM tip, and the length of the PEO defines the rotational mobility.

Riener et al. synthesized several heterobifunctional derivatives from a H₂N-PEO-COOH intermediate for tip-PEO-probe conjugation.^{17,94} These heterobifunctional PEOs possessed an NHS-activated ester on one chain end and either biotin, maleimide, vinylsulfone, or a pyridyl disulfide group at the other (Figure 2.10). Another important PEO derivative was synthesized having a pyridyl disulfide group on one chain end and a nitrilotriacetic acid (NTA) group on the other. This was successfully used to tether His₆-tagged proteins to AFM tips via noncovalent NTA-Ni²⁺-His₆ bridges.

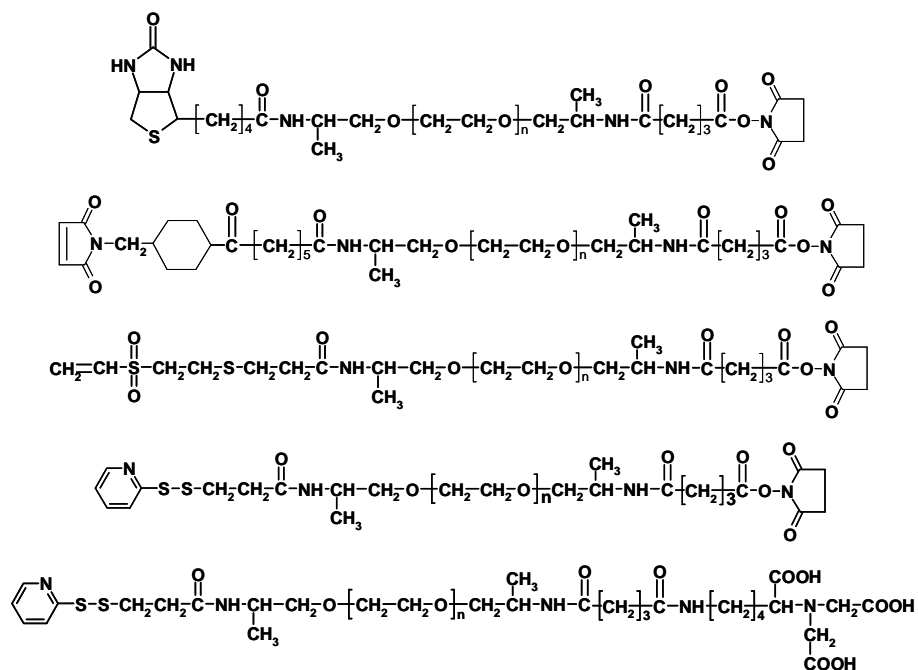


Figure 2.10. Heterobifunctional PEOs utilized in SMRFM^{17,94}

2.4.3.3. Anticoagulant Medical Devices

Prevention of blood coagulation on the surfaces of medical devices is important to help avoid complications during the clinical use of blood-contacting artificial devices. One method of reducing blood coagulation is by regulating the activity of thrombin, a key enzyme in the coagulation process, through surface modification of the biomaterial. The biocompatibility of PEO makes it an ideal candidate for surface functionalization of medical devices.

Salchert et al. utilized a heterobifunctional PEO, H₂N-PEO-COOH, and a homobifunctional PEO, HOOC-PEO-COOH, to functionalize polymer coatings with a benzamidine derivative capable of selectively binding thrombin at their free termini (Figure 2.11), and thus removing the coagulant from circulation.⁹⁵ The PEO oligomeric spacers were reacted with films of poly(octadecene-*alt*-maleic anhydride) copolymers. To attach the HOOC-PEO-COOH, the maleic anhydride-functional surface was reacted with 1,4-diaminobutane, and this was followed by coupling the polymer in the presence of EDC and the sodium salt of N-

hydroxy-sulfosuccinimide (sulfo-NHS). Attachment of the benzamidine derivative to the remaining free carboxylate groups was accomplished utilizing EDC and sulfo-NHS. When using H₂N-PEO-COOH, the amine terminus was reacted with the maleic anhydride-functional surface to form an imide, and then the benzamidine derivative was bound as described previously.

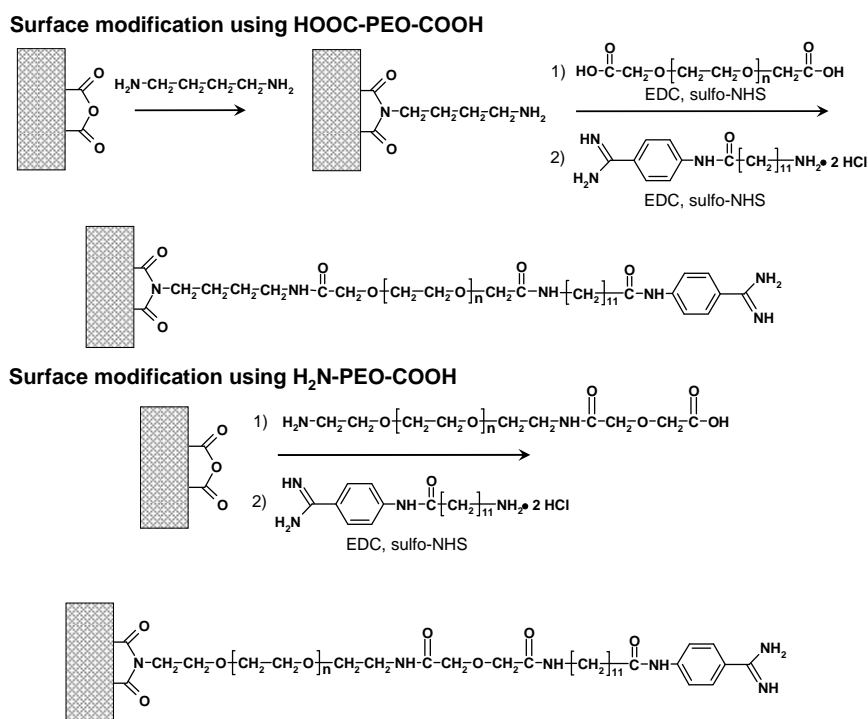


Figure 2.11. Surface functionalization with a benzamidine derivative

The benzamidine surface density was enhanced when surfaces were prepared from the heterobifunctional polymer compared to the homobifunctional polymer. The decreased activity of the homobifunctional polymer was attributed to PEO bridging, thus leading to a decreased number of free carboxyl groups on the surface. Although PEO has the propensity to resist non-specific protein adsorption, these benzamidine-modified surfaces selectively bound thrombin. The study concluded that the benzamidine-PEO functionalized surfaces had significant potential as thrombin-scavenging surfaces.

Chen et al. investigated immobilization of amine-containing biomolecules including oligopeptides, proteins, and glycosaminoglycans (e.g., heparin) on *Sylgard* polysiloxane elastomers (Dow-Corning) utilizing heterobifunctional PEO spacers.^{19,77} The siloxane surface was functionalized with Si-H groups using $(\text{MeHSiO})_n$ under acidic conditions. First, the allyl terminus of allyl-PEO-NHS was grafted to the Si-H functional surface by hydrosilation in the presence of a platinum catalyst. Amine-containing biomolecules were then covalently tethered to the surface through the PEO-NHS active ester. For comparison, surfaces with only PEO were prepared by a similar method using PEO with an allyl group at one end and an inert methoxy group at the other chain end. Heparin modified surfaces demonstrated significantly less thrombin activity as compared to the methoxy-PEO surface, while maintaining resistance to non-specific protein adsorption.¹⁹ This approach to functionalized surfaces employs a relatively simple procedure that can be utilized to tailor the surface groups to reject or attract a wide range of target molecules.

2.5. *Synthesis and Applications of H₂N-PEO-X*

The higher reactivity of primary amine-terminated PEO compared to hydroxy-terminated PEO in nucleophilic substitution reactions makes it a widely used derivative for preparing bioconjugates. Low molecular weight drugs, cofactors, peptides, glycoproteins, and biomaterials can be linked with amine-functional PEO through amide, sec-amine, urea, and other chemical bonds.⁹⁶⁻¹⁰⁰ Furthermore, amine-terminated PEO can initiate polymerizations of amino acid N-carboxyanhydrides or lactones/lactides for synthesizing biocompatible block copolymers.^{65,74,101,102}

2.5.1. Direct Synthesis of Heterobifunctional H₂N-PEO-X

2.5.1.1. Schiff Base-Initiated Polymerization of EO

Ethanolamine with a protected amine group has been utilized to initiate and polymerize EO to generate α -amino- ω -hydroxy-poly(ethylene oxide) (H₂N-PEO-OH).⁶¹ The Schiff base was prepared from benzaldehyde and ethanolamine to prevent the primary amine from reacting with the EO. The anionic initiator was prepared by reacting the Schiff base of ethanolamine with an equimolar amount of sodium. The initiator was added to EO and the polymerization was carried out at 95 °C. The benzaldehyde protecting group was removed by acidification with hydrochloric acid to yield the H₂N-PEO-OH.

It is well known that end group functionalization can be carried out by terminating the propagating species of living anionic polymerizations with a suitable terminating agent. One advantage of this method is that the polymer does not need to be separated and then functionalized in a subsequent step. Huang et al. found that when bromoacetic acid was used as a terminating agent for PEO that had been initiated with N-benzylideneaminoethoxide, the Schiff base prematurely decomposed during the termination.⁷⁶ To avoid this, a two-step process was employed to synthesize heterobifunctional PEO with a carboxylic acid at one chain end. First, a Schiff base-initiated PEO with a terminal hydroxyl group was synthesized. In the second step, the hydroxyl terminus was deprotonated with a slight molar excess of sodium metal in THF, and the mixture was cooled to 0 °C before dropwise addition of a bromoacetic acid solution in THF, and this was followed by refluxing. This produced a heterobifunctional PEO with a Schiff base-protected amine at one chain end and a carboxylic acid group at the other, with retention of the original molecular weight as determined by SEC.

To produce a heterobifunctional PEO for targeted drug delivery, a tumor cell targeting agent, sulfadiazine, was covalently bound to the carboxyl end of the PEO (Figure 2.12).⁷⁶ The

carboxylic acid was transformed to the more reactive acid chloride by reaction with benzoyl chloride, and this was followed by adding sulfadiazine. The Schiff base was then hydrolyzed with acetic acid to yield the free amine, thus producing a polymer with a targeting moiety on one chain end and an amine group at the other for attaching an anti-tumor drug. Polymer structure and molecular weight were determined by ^1H NMR and SEC.

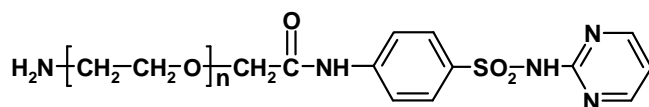


Figure 2.12. PEO with a primary amine and sulfadiazine moiety

2.5.1.2. Potassium Bis(trimethylsilyl)amide-Initiated Polymerization of EO

Potassium bis(trimethylsilyl)amide has been used as an initiator for polymerizing EO to yield heterobifunctional polymers (Figure 2.13).⁶² To monitor the polymerization, a reaction mixture was divided into portions that were terminated with acetic acid after defined intervals, then analyzed by SEC. As expected, the elution volume decreased with increasing reaction time. Once the elution volume remained constant, the reaction was assumed to be complete. The protecting group was removed by treatment with 0.1 N hydrochloric acid. The resulting polymer was purified by ion exchange chromatography with a yield of 94 %. Molecular weights obtained by SEC were in close agreement with calculated values based on the ratio of initiator to monomer, and the molecular weight distributions were ~ 1.1 . Titration of the polymer confirmed that each mole of polymer contained one mole of amine.

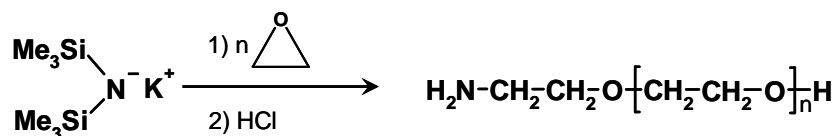


Figure 2.13. Potassium bis(trimethylsilyl)amide-initiated polymerization of EO

Tessmar et al. utilized the synthesis described above to produce amine-reactive biodegradable diblock copolymers for tissue engineering, where surface-immobilized cell adhesion peptides or growth factors are needed to control cell behavior.¹⁰³ PEO was synthesized with potassium bis(trimethylsilyl)amide to produce the macroinitiator, H₂N-PEO-OH. The block copolymer, H₂N-PEO-PLA-OH, was prepared by ring-opening polymerization of D,L-lactide using stannous 2-ethylhexanoate as catalyst. To ensure that only the hydroxyl group participated in polymerization of the D,L-lactide, the trimethylsilyl groups were either left intact or glacial acetic acid was added to the reaction mixture to convert the amine into a non-reactive ammonium salt.

The presence of the amine group was difficult to confirm by ¹H NMR due to the high molecular weight of the polymer. Thus, an amine-reactive fluorescent dye was reacted with the diblock copolymer as well as with a control diblock copolymer, methoxy-PEO-PLA, to establish that the dye would not react with the hydroxyl terminus of the PLA block. The polymer/dye mixture was analyzed using SEC with a UV detector. The methoxy-PEO-PLA and H₂N-PEO-PLA polymers showed no UV signals at low retention times. However, the H₂N-PEO-PLA/dye produced a significant UV signal indicating that those polymer chains had reacted with the dye, and this confirmed the presence of the amine terminus.

The amine chain end of the H₂N-PEO-PLA diblock copolymer was transformed into an NHS-activated ester by reaction with disuccinimidyl tartrate (DST) or disuccinimidyl succinate (DSS) (Figure 2.14). The capacity for the activated chain ends to react with amine groups was

confirmed by reaction with an amine-functional fluorescent dye, and subsequent analysis by SEC with a UV detector as previously described.

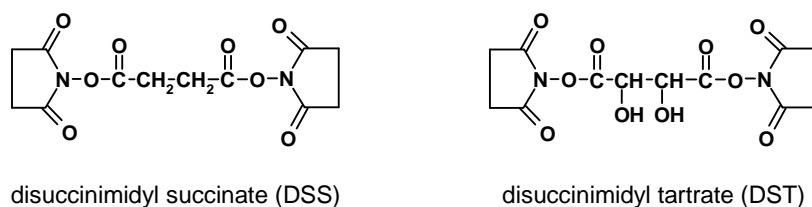
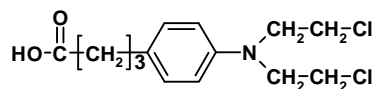
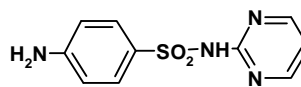


Figure 2.14. Structures of DST and DSS

Potassium bis(trimethylsilyl)amide was also utilized to synthesize a series of H₂N-PEO-OH polymers to prepare tumor-targeting drug delivery systems. These heterobifunctional PEO oligomers were used to link an anti-tumor drug, chlorambucil, with a tumor-targeting moiety, sulfadiazine (Figures 2.15-16).¹⁰⁴ Protection of the primary amine end with benzaldehyde was required before selectively coupling chlorambucil to the hydroxy terminus. The carboxylic acid group on chlorambucil was covalently reacted with the hydroxy terminus of the polymer chain using DCC as a coupling agent, and this was followed by deprotecting the amine with acetic acid to afford H₂N-PEO-CBL. The sulfadiazine moiety was covalently bound to the primary amine terminus by first reacting 2-[N¹-2-pyrimidinyl-(p-benzylidene)aminobenzenesulfonamido]ethanol with bis(trichloromethyl)carbonate. Subsequent addition of H₂N-PEO-CBL directly to the reaction mixture afforded the target compound, SD-PEO-CBL. A series of non-targeted controls having the same molecular weights as the SD-PEO-CBL polymers were also prepared by covalently coupling monomethoxy-poly(ethylene oxide) (MPEO) with chlorambucil (MPEO-CBL) using DCC.



chlorambucil (CBL)



sulfadiazine (SD)

Figure 2.15. Structure of chlorambucil and sulfadiazine

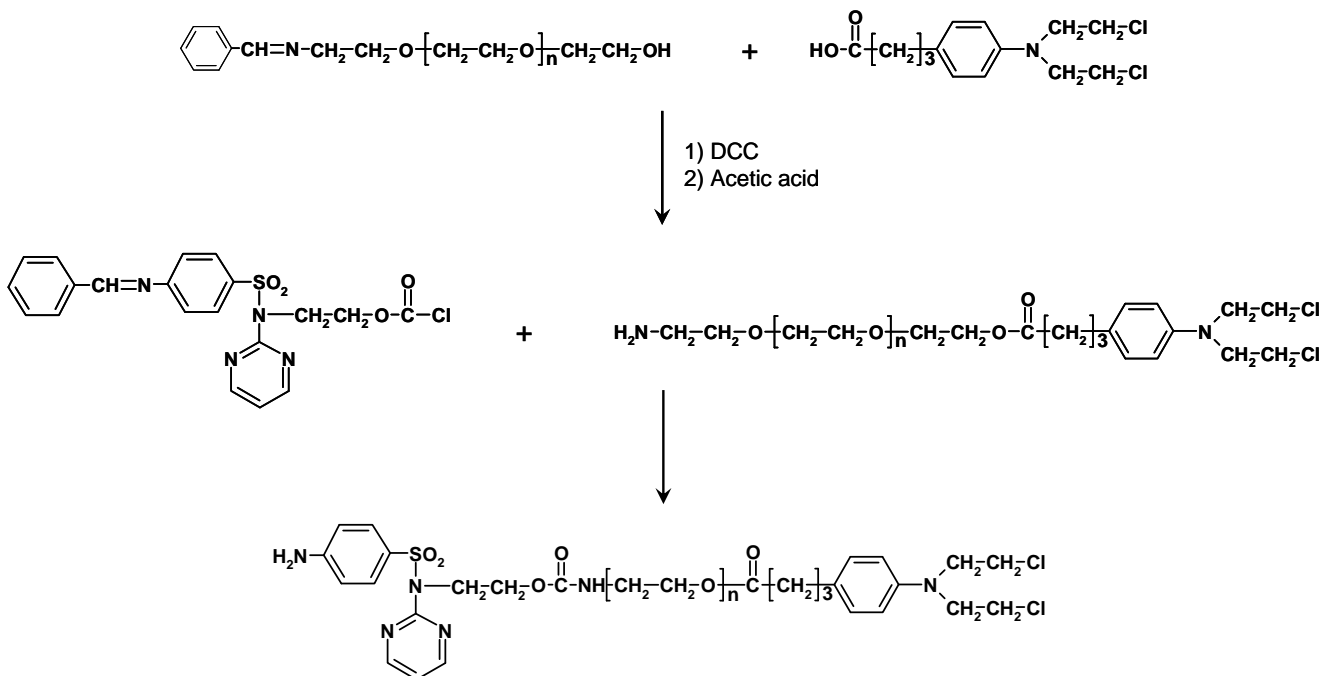


Figure 2.16. Synthesis of SD-PEO-CBL

To test the activity of chlorambucil bound to PEO chains, cytotoxic assays were performed on C6 human breast cancer cells. The IC_{50} values for MPEO-CBL, SD-PEO-CBL, and free chlorambucil were in the range of 10^{-8} - 10^{-9} mol L^{-1} , indicating that the activity of chlorambucil was preserved in vitro, and there were no significant differences among the targeted and non-targeted polymer drugs. The toxicities of the polymer drugs were tested using TA1 mice. The LD_{50} for the polymer-bound chlorambucil with or without the targeting moiety was 10-16 times higher than for the chlorambucil control.

In vivo studies were conducted using Lewis lung carcinoma implanted in mice. The anti-tumor activities of SD-PEO-CBL were higher than the corresponding MPEO-CBL controls but slightly lower than chlorambucil. Since the SD-PEO-CBL and MPEO-CBL polymer drugs showed no significant difference in activity in vitro, this suggested that the increased activity of the SD-PEO-CBL in vivo could be attributed to the targeting moiety, sulfadiazine.

2.5.1.3. Potassium TDMA-Initiated Polymerization of EO

To prepare a heterobifunctional PEO with a primary amine at one end and a hydroxyl group at the other, a protected initiator was synthesized, N-2-(2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentyl)-ethylmethylamine (TDMA) (Figure 2.17).¹⁰⁵ The polymerization was carried out by first reacting TDMA with an equimolar amount of potassium naphthalide in THF, and then the EO was polymerized at room temperature. Termination of the reaction and deprotection of the silylamine were accomplished by adding a few drops of acetic acid. The H₂N-PEO-OH polymer was obtained in almost quantitative yields. ¹H NMR spectra in DMSO-d₆ showed that the peak ratio of signals corresponding to the hydroxyl end and methylene protons adjacent to the primary amine were 1:2, indicating that the polymer had one hydroxyl and one primary amine terminus. End group analysis by ¹³C NMR also confirmed the structure of the heterobifunctional polymer. The molecular weights determined by SEC matched well with the targeted values controlled with monomer to initiator ratios, and the molecular weight distributions were relatively narrow (≤ 1.32). The results demonstrated that polymerization of EO with the TDMA initiator took place without cleavage of the protecting group. MALDI-TOF MS showed signals corresponding to the EO repeat unit plus the molecular weight of the two functional ends, thus providing evidence for purity of the heterobifunctional material.

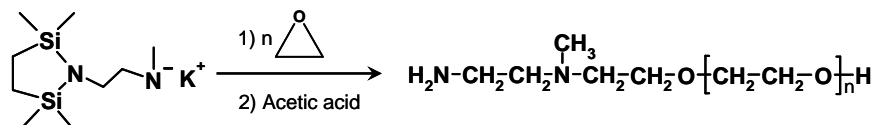


Figure 2.17. TDMA-initiated polymerization of EO

2.5.1.4. (Cyanomethyl)potassium-Initiated PEO

Heterobifunctional PEOs have also been prepared by polymerizing EO initiated with (cyanomethyl)potassium.¹⁰⁶ The synthesis of NC-PEO-OH was carried out in a similar manner as previously described.⁵⁸ Following polymerization, the cyano group was reduced with lithium aluminum hydride to afford H₂N-PEO-OH. ¹³C NMR spectra showed that the peaks corresponding to the cyano moiety completely disappeared and that new signals were present corresponding to the aminoalkyl end group.

A triblock copolymer, poly(γ -benzyl-L-glutamic acid-*b*-ethylene oxide-*b*- ϵ -caprolactone) (PBLG-PEO-PCL), was synthesized by Deng et al. with modifications to the procedure described above.⁶⁵ A nitrile-terminated block copolymer, NC-PEO-PCL, was synthesized with the (cyanomethyl)potassium initiator in THF by the sequential polymerization of EO and ϵ -caprolactone (ϵ -CL). The nitrile was converted to a primary amine by catalytic hydrogenation using palladium on carbon or Raney nickel to obtain H₂N-PEO-PCL-OH. End group analysis via ¹H NMR confirmed complete conversion of the cyano group. Catalytic hydrogenation was chosen over other methods to convert the cyano group to an amine to avoid degradation of the relatively labile PCL ester linkages. Analysis by SEC before and after hydrogenation did not show any significant changes in molecular weight or molecular weight distribution.

2.5.1.5. *Allyl-PEO-OH Intermediates for Synthesis of H₂N-PEO-OH*

Another approach to synthesizing heterobifunctional PEO that does not involve protecting and deprotecting primary amines is to utilize a heterobifunctional initiator containing hydroxyl functionality and another functional group, such as allyl, that is unreactive during the polymerization. Cammas et al. synthesized a heterobifunctional PEO utilizing an allyl alcohol initiator to yield allyl-PEO-OH with a high yield (96%) and narrow molecular weight distribution (1.1).⁶³ Molecular weights obtained from SEC and ¹H NMR were in good agreement with those targeted. These results demonstrated that polymerization of EO by an allyl alkoxide yielded a heterobifunctional polymer with an allyl group at one end and a hydroxyl group at the other.

A radical addition reaction was employed to introduce a primary amine onto the allyl terminus by reacting with 2-aminoethanethiol hydrochloride (cysteamine hydrochloride) utilizing AIBN as the radical generator. ¹H and ¹³C NMR spectra confirmed that the reaction proceeded to completion without formation of by-products. The molecular weights and molecular weight distributions determined by SEC were unaffected, indicating that the radical addition reactions took place without significant side reactions and without altering the polymer chains. This synthetic approach is extremely versatile due to the ability to convert the allyl terminus into a wide variety of functional groups through ene-thiol additions.⁹⁰

2.5.1.6. *Synthesis of Vinylbenzyl-PEO-NH₂*

Matsuya et al. constructed a core-shell fluorescent nanosphere with reactive PEO tethered chains on the surface for detecting proteins in a time-resolved fluorometric immunoassay.¹⁰⁷ A key component was a heterobifunctional PEO that could be tethered to the surface and still possess a reactive moiety for binding a biological vector. Vinylbenzyl alcohol (VBA) has been shown to be a suitable initiator for EO (Figure 2.18).¹⁰⁸ To produce an amine-

functional PEO macromonomer, the polymerization of EO was carried out utilizing VBA as the initiator. Initiation with VBA was a preferred method as opposed to obtaining the vinylbenzyl group by termination of a polymerization with vinylbenzyl bromide because it was difficult to maintain the primary amino group quantitatively with the latter approach. VBA was reacted with a stoichiometric amount of potassium naphthalide in THF to produce the alkoxide. After adding EO, the mixture was stirred at room temperature for two days. Triethylamine was added to the reaction and the mixture was poured into a solution of methanesulfonyl chloride (MsCl) in THF to yield a MsO-PEO-VBA intermediate. The methanesulfonyl ester end group activated the hydroxyl chain end toward nucleophilic substitution.

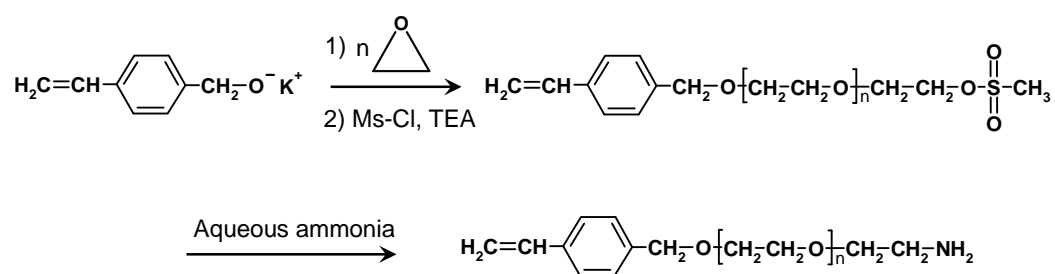


Figure 2.18. Synthesis of VBA-PEO-NH₂

A primary amine was introduced onto the activated chain end by reacting MsO-PEO-VBA with aqueous ammonia at room temperature for two days to produce the desired H₂N-PEO-VBA macromonomer. The disappearance of signals in the ¹H NMR spectra corresponding to the methylene protons adjacent to the methanesulfonyl group and appearance of new signals corresponding to the methylene protons adjacent to the primary amine provided evidence that the reactions had proceeded to completion.

Molecular weights of the macromonomers determined by SEC closely matched the calculated molecular weights based on the monomer to initiator ratios, and the molecular weight distributions were relatively narrow (e.g., 1.20). However, some high molecular weight

impurities were observed by SEC, and these were attributed to small amounts of vinyl oligomerization during formation of the alkoxide. It was reasoned that the coupled products could then initiate EO, thus leading to high molecular weight by-products.

Functionalized nanospheres were prepared in four steps. First, the H₂N-PEO-VBA was employed as a macromonomer and surfactant in a suspension radical polymerization of styrene to produce a core-shell nanosphere. Nanospheres produced by this method had PEO tethered chains on their surfaces bearing primary amino groups at the free chain ends. The second step was to incorporate fluorescent europium chelates into the nanospheres via physical entrapment. Conversion of the amino groups into thiol-reactive moieties was carried out by reacting N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate with the amine termini of the PEO chains on the nanospheres to produce maleimide-functional surfaces (Figure 2.19). Lastly, a biological vector, an anti-human α -fetoprotein (AFP) Fab' fragment, was covalently bound to the nanosphere surface via reaction of thiols with the maleimides. The fluorescent nanospheres bearing anti-human AFP Fab' fragments were utilized for an immunoassay of AFP, and zeptomolar detection was reportedly achieved. Even with the antibody bound to the surface, non-specific binding was practically negligible.

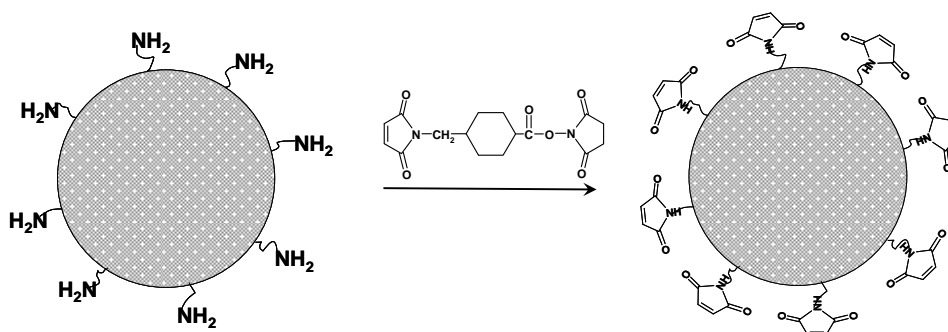


Figure 2.19 Synthesis of maleimide-functional nanospheres

2.5.2. Synthesis of H_2N -PEO- X via End Group Modification of PEO Diols

2.5.2.1. Synthesis of H_2N -PEO-COOH

Heterobifunctional PEOs were prepared from PEO diols with a carboxylic acid group on one chain end and a primary amine on the other.¹⁰⁹ A PEO diol was chlorinated with a limiting amount of thionyl chloride in refluxing toluene. Introduction of ester-protected carboxylic acid groups onto the remaining hydroxyl termini was accomplished by reaction with an excess of ethyl isocyanatoacetate. An excess of sodium azide was reacted with the chlorinated chain ends, then the carboxylate esters were deprotected by hydrolysis with an aqueous solution of sodium hydroxide to produce the carboxylic acid end groups.

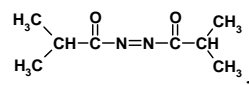
The N_3 -PEO-COOH intermediate was separated from the mixture of products based on the number of carboxylic acid groups by ion exchange chromatography on DEAE-Sephadex. It is also worth noting that PEO chains with different amounts of ionic groups could be separated by TLC on silica gel using a 10:2:1 mixture of isopropanol, concentrated aqueous ammonium hydroxide, and water. The azide was converted to a primary amine by catalytic hydrogenation to afford the target compound, H_2N -PEO-COOH. The structure was confirmed by ^{13}C NMR.

2.5.2.2. Synthesis of Aminoxy-PEO-Br

A PEO diol was converted by a series of reactions and separations to yield an α -aminoxy- ω -bromo-poly(ethylene oxide) (H_2NO -PEO-Br) spacer for bioconjugates. The ease of oxime ether formation was reported to be a superior method for targeting aldehydes and ketones compared to a reductive amination approach (Figure 2.20).¹¹⁰ This produces an oxime ether linkage that is stable at physiological pH.

A PEO diol was partially silylated by reaction with tert-butyldiphenylsilyl chloride (BPS-Cl) and the intermediate was separated by column chromatography. The remaining hydroxyl termini were then derivatized in a Mitsunobu coupling reaction with N-hydroxyphthalimide in

the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD),



, to afford the phthalimido ether. The phthalimide end group was removed via hydrazinolysis to produce the aminoxy terminated polymer.

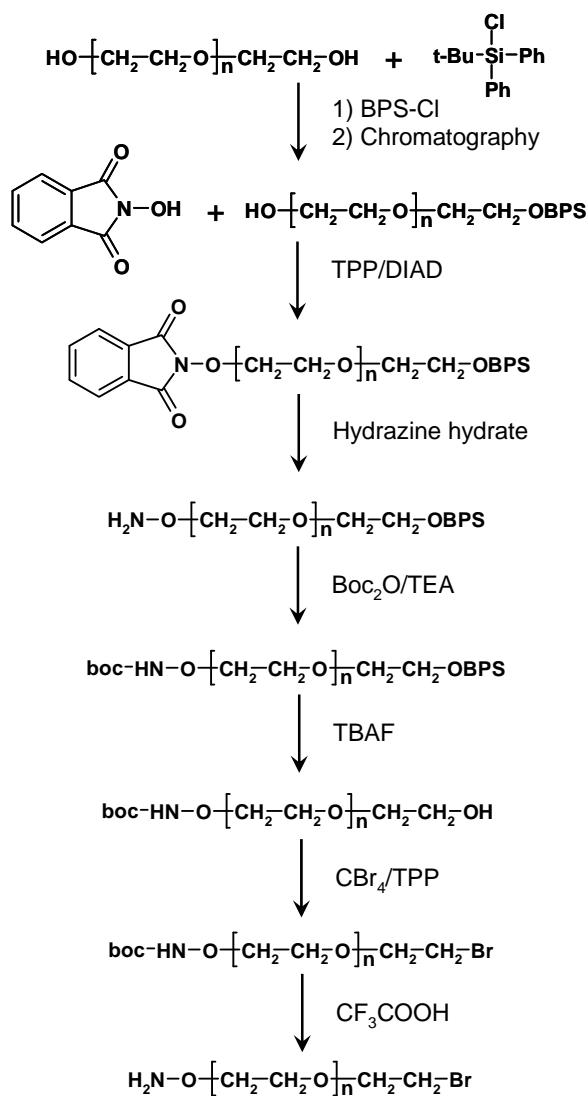


Figure 2.20. Synthesis of H₂NO-PEO-Br

Prior to conversion of the silyl ether, the aminoxy terminus was protected by reacting with di-tertbutyldicarbonate (boc₂O) in the presence of triethylamine. Introduction of the

bromide was accomplished by desilylation utilizing tetra-n-butyl ammonium fluoride (TBAF). The alcohol to bromide transformation was completed by reaction with carbon tetrabromide and triphenylphosphine at 0 °C, and this was followed by deprotecting the boc-aminooxy moiety to yield H₂NO-PEO-Br.

2.5.2.3. Synthesis of Folate-Targeted PEO Carboplatin Analogues

Carboplatin (Figure 2.21) is a chemotherapy drug that is most commonly used to treat ovarian and lung cancer but may be used to treat other types of cancer as well. Several heterobifunctional PEOs were synthesized from PEO diols to investigate the pharmacokinetic properties of PEO with a folic acid moiety (Figure 2.21) on one chain end and a carboplatin analogue on the other.³⁰ Folate-targeted PEO carriers are desirable because PEO conjugates are known to improve blood circulation times of low molecular weight drugs, and the folate terminus could also improve cell permeation by taking advantage of folate receptor-mediated endocytosis.

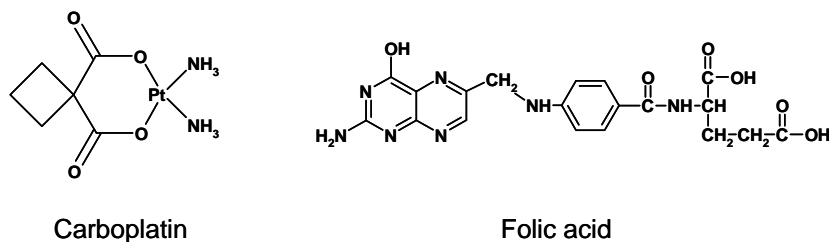


Figure 2.21. Structures of carboplatin and folic acid

The first step toward folate-targeted PEO carboplatin analogues was to synthesize a monoprotected PEO diamine. This is a versatile starting material, allowing attachment of a wide range of functional groups to either terminus via stable amide bonds. The first step was to activate the hydroxyl termini of the PEO diol by reaction with an excess of methanesulfonyl chloride to afford MsO-PEO-OMs. Then MsO-PEO-OMs was dissolved in a concentrated

solution of aqueous ammonia to displace the methanesulfonyl end groups and form the PEO diamine, H₂N-PEO-NH₂. An equimolar ratio of fluorenylmethyl chloroformate (fmoc) and PEO diamine was reacted to yield a mixture of unprotected, mono-protected, and bis-protected PEO diamines. The mixture of products was separated on a Sephadex CM-25 cation exchange column with a yield of ~30%. End group analysis by ¹H NMR showed that the ratio of signals corresponding to the methylene protons adjacent to the unprotected amine terminus and those corresponding to the methylene protons adjacent to the fmoc-protected amine terminus were 2:2. Fluorescently-labeled PEOs were prepared to determine whether the folate terminus affected accumulation of PEO in cells that had a high density of folate receptors (Figure 2.22). An active ester of folic acid (FA-NHS) was reacted with the fmoc-PEO-NH₂ to yield fmoc-PEO-FA, and this was followed by removing the fmoc protecting group by reaction with piperidine. The unprotected amine was then reacted with fluorescein isothiocyanate (FITC) to yield fluorescently-tagged FITC-PEO-FA. A non-targeted, fluorescently-tagged PEO control with a capped amine was also synthesized by reacting benzyl chloroformate (Cbz) with one amine terminus and subsequently reacting with FITC to give Cbz-PEO-FITC.

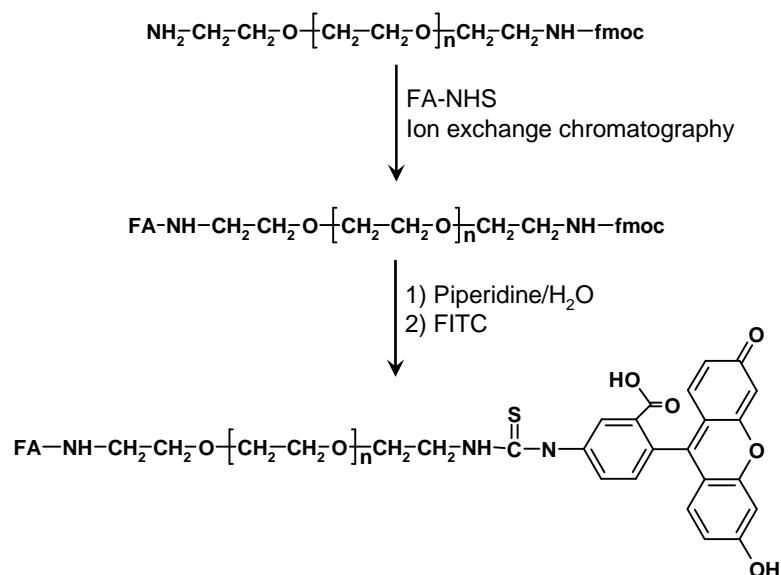


Figure 2.22. Synthesis of FITC-PEO-FA

PEO carriers that could release platinum once inside the cell were synthesized by platinating a dicarboxylate ligand on one chain end and attaching a fluorescent tag for monitoring cell uptake on the other. The carboplatin analogues were synthesized by first reacting fmoc-PEO-NH₂ with di-tert-butyl 2-(3-succinylaminopropyl)-malonate (MAL(tBu)₂). The protecting group was removed and the amine was reacted with FA-NHS as described above to give FA-PEO-MAL(tBu)₂. The tert-butyl protecting groups were removed by treatment with trifluoroacetic acid and transformed to the sodium salt by titration with a solution of sodium hydroxide to give FA-PEO-MAL(Na)₂, and this was followed by reacting with cis-[Pt(NH₃)₂(D₂O)₂]²⁺(NO₃)₂ to yield the carboplatin analogue, FA-PEO-Pt (Figure 2.23). The platination reaction was monitored ¹H NMR. Signals corresponding to the two methylenes adjacent to the malonate shifted significantly upon platination, indicating that the reaction was complete. A Cbz-PEO-Pt control was also synthesized in a similar fashion to that previously described.

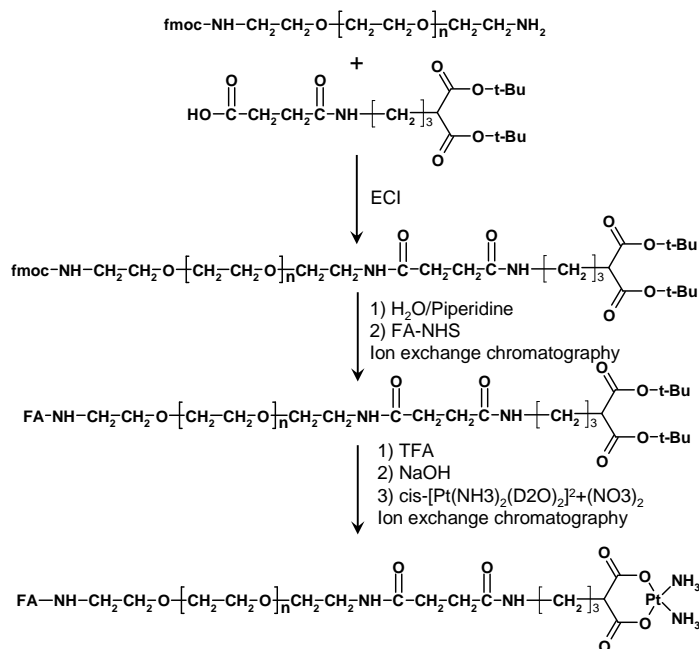


Figure 2.23. Synthesis of FA-PEO-Pt

The study showed that non-targeted Cbz-PEO-Pt was four times more effective at inhibiting cell growth than carboplatin alone and one and a half times more effective than FA-PEO-Pt. The unexpected lower activity of FA-PEO-Pt was attributed to neutralization or blocking of the Pt.

2.6. Synthesis and Applications of HS-PEO-X and Disulfide-PEO-X

PEO oligomers with a mercapto or pyridyl disulfide group at one chain end and another functional group at the other are especially useful for preparing bio-interfaces. Heterobifunctional mercapto or pyridyl disulfide-ended PEOs can be utilized for surface functionalizing of gold and silver substrates to construct reactive PEO brush layers. One such application is in the synthesis of surface-functionalized gold nanoparticles for colloidal sensor systems in biological fluids.¹¹¹ It is well known that modifying a surface with tethered PEO chains can dramatically decrease non-specific interactions of biopolymers.^{33,112-114} Wuelfing et al. found that surface functionalization of gold nanoparticles with α -methoxy- ω -mercapto-

poly(ethylene oxide) improved their dispersion stability in aqueous media due to steric repulsion of the tethered PEO. A heterobifunctional PEO was required to construct gold nanoparticles possessing both sufficient colloidal stability and biological functionality for bioassays.^{22,51}

Due to the capacity for pyridyl disulfide moieties to react with thiols, heterobifunctional PEOs bearing a pyridyl disulfide group can serve as linking agents.¹¹⁵ Another useful property of the pyridyl disulfide moiety is that it releases thiopyridone upon reaction with free mercapto groups (Figure 2.24). Thiopyridone can be used in quantitative assays to determine the number of PEO oligomers bound to a molecule or surface of interest.^{115,116}

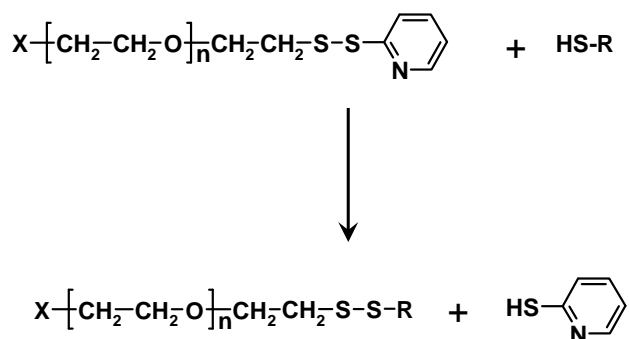


Figure 2.24. Release of thiopyridone from PEO bearing a pyridyl disulfide moiety upon reaction with a free mercapto group

2.6.1. Direct Synthesis of HS-PEO-X

2.6.1.1. Synthesis of Formyl-PEO-SH and Formyl-PEO-SS-Pyridyl

A heterobifunctional PEO containing a formyl group and a hydroxyl group was synthesized by ring-opening polymerization of EO with an initiator that contained an acetal, potassium 3,3-diethoxypropoxide.⁷⁰ The initiator was formed by reacting a stoichiometric amount of potassium naphthalide with 3,3-diethoxypropanol in THF, and this was followed by polymerizing EO. The polymer, acetal-PEO-OH, had a narrow molecular weight distribution, ~1.05, by SEC. The molecular weights obtained from ¹H NMR and SEC were in good agreement with the molecular weights calculated from the ratios of monomer to initiator. The

acetal terminus was deprotected to form the aldehyde by reaction with hydrochloric acid. Complete disappearance of the signals corresponding to the acetal and the emergence of new signals corresponding to the aldehyde terminus in the ^{13}C NMR indicated that the conversion was quantitative. End group analysis by ^1H NMR also revealed the presence of the aldehyde.

Modifications to the method described above were employed to synthesize acetal-PEO-SH and acetal-PEO-SS-pyridyl (Figure 2.25).⁶⁰ The polymerizations were terminated with methanesulfonyl chloride to produce an activated chain end. Incorporation of the methanesulfonyl moiety onto the chain end was ~98% based on end group analysis by ^1H NMR. Displacement of the methanesulfonyl ester was achieved by reaction with potassium O-ethylthiocarbonate. End group analysis via ^1H NMR showed that conversion of the methanesulfonyl ester to the ethylthiocarbonate was almost quantitative, 97%. Generation of the thiol was achieved by cleaving the dithiocarbonate terminus with n-propylamine. Transformation of the mercapto terminus to the pyridyl disulfide derivative was carried out by reacting the thiol-functional polymer with 2-pyridyl disulfide, and the conversion was 68 % as determined by ^1H NMR end group analysis. Even though the deprotection reaction to form the thiol-functional polymer appeared to proceed quantitatively via ^1H NMR, analysis of the release of thiopyridone by UV spectroscopy after reaction with 2-pyridyl disulfide (2-PDS) showed that only 85% of the PEO chains had a free mercapto group. This suggested that some thiol oxidation may have occurred during purification.

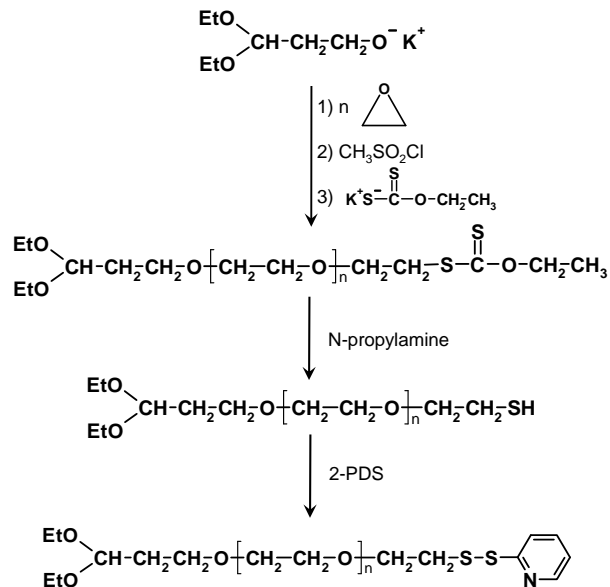


Figure 2.25. Synthesis of acetal-PEO-SH and acetal-PEO-SS-pyridyl

The heterobifunctional acetal-PEO-SH was used to construct a colloidal sensor system based on the reversible aggregation of gold nanoparticles induced by bivalent ligands.²² PEO chains were tethered to the surfaces of the gold nanoparticles, allowing the acetal ends to protrude into solution. The PEO brush layer on the surface greatly enhanced the stability of the nanoparticles in various media such as deionized water, phosphate buffer solutions, serum-containing media, and organic solvents. In order to attach a molecular probe to the distal end of the PEO-coated gold nanoparticles, the acetal terminus was converted to an aldehyde by immersion in an aqueous solution of hydrochloric acid at pH 2. *p*-Aminophenyl- β -D-lactopyranoside and *p*-aminophenyl- β -D-mannopyranoside were immobilized on the surfaces of the gold nanoparticles via reductive amination of the aldehyde at the distal end of the PEO chains.

The capacity for these particles to function in a sensor was tested by reaction with a bivalent galactose-binding lectin, *R. communis* agglutinin (RCA₁₂₀). Additions of RCA₁₂₀ to

the functionalized gold nanoparticles were monitored by UV-visible spectroscopy. The dispersed particles were red, and as the particles aggregated due to crosslinking with RCA₁₂₀, the color gradually changed from red to purple. The aggregated gold nanoparticles could be redispersed by adding D-galactose and separated from the dispersion by centrifugation. The particles could also be redispersed in buffer solution, then re-aggregated by adding RCA₁₂₀. This process was repeatable over several cycles of aggregation and redispersion. Calibration curves enabled detection of RCA₁₂₀ concentrations as low as 1 ppm.

Heterobifunctional PEOs having both mercapto and aldehyde groups were synthesized for functionalizing gold electrode surfaces on biosensors or biocatalysts.¹¹⁷ An acetal-PEO-OH precursor was synthesized as described above, then terminated with N-succinimidyl-3-(2-pyridyldithio)-propionate to produce acetal-PEO-SS-pyridyl (Figure 2.26A). The hydroxyl terminus was also converted to a thiol by condensation with an excess of thioglycolic acid to prepare acetal-PEO-SH (Figure 2.26B). End group analysis via ¹H NMR confirmed the polymer structure and incorporation of end groups. Gold electrode surfaces were coated with the heterobifunctional PEOs, and this was followed by hydrolysis of the acetal under acidic conditions to form an aldehyde.

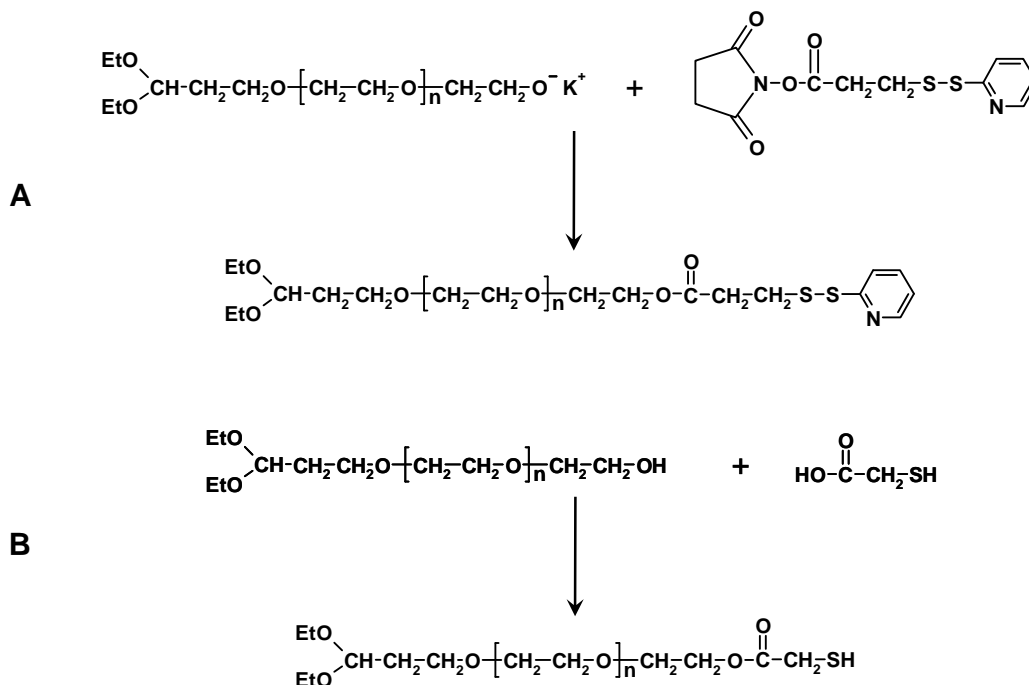


Figure 2.26. Synthesis of acetal-PEO-SS-pyridyl and acetal-PEO-SH

Immobilization of cytochrome c (cyt. c), an important electron transport protein, on a gold electrode surface was studied using aldehyde-PEO-SH and aldehyde-PEO-SS-pyridyl. The aldehyde-PEO-SH formed a monolayer on the gold electrode. The redox response of cyt. c when covalently bound to the gold electrode by the aldehyde-PEO-SH polymer was demonstrated by cyclic voltammetry. This indicated that the polymer could be utilized to form functionalized electrodes. Although no redox response was observed when the aldehyde-PEO-SS-pyridyl polymer was used to bind cyt. c to the electrode, it was observed that this polymer functioned as a good promoter for the electron transfer between cyt. c in phosphate buffer solution and a gold electrode. The lack of redox response when using aldehyde-PEO-SS-pyridyl was attributed to low surface concentrations of the PEO chains on the surfaces of the gold electrode.

2.6.1.2. Synthesis of Benzaldehyde-PEO-SS-pyridyl

Synthesis of a heterobifunctional PEO with a benzaldehyde at one chain end and a pyridyl disulfide at the other was carried out by ring-opening polymerization of EO using potassium 4-(diethoxymethyl)benzylalkoxide (PDA) as an initiator (Figure 2.27).¹¹⁸ The benzaldehyde moiety was utilized to avoid self-condensation of PEO chains due to an aldol reaction with aldehyde-functional PEO oligomers wherein the aldehyde had an α -hydrogen. Another advantage of the benzaldehyde is that the reaction of a benzaldehyde with an amine produces a sufficiently stable imine that does not require further reduction.

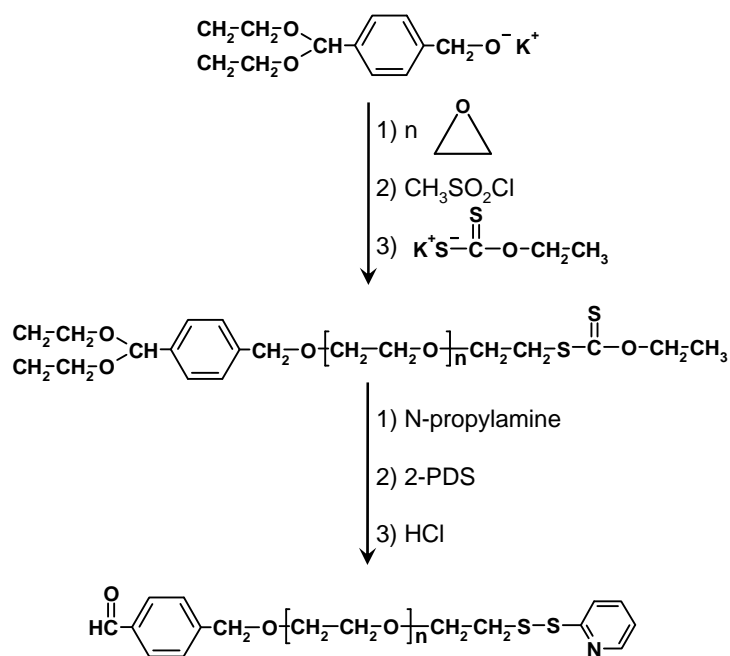


Figure 2.27. Synthesis of PEO with both a benzaldehyde and pyridyl disulfide moiety

The initiator was prepared by reducing 4-(diethoxymethyl)benzaldehyde with sodium borohydride, and this was followed by metalation of 4-(diethoxymethyl)benzyl alcohol with potassium naphthalide. Potassium 4-(diethoxymethyl)benzylalkoxide was then used as an initiator for ring-opening polymerization of EO. The reaction was terminated with methanesulfonyl chloride to produce an activated chain end. The molecular weight distribution

was narrow, 1.03, as determined by SEC, and the molecular weight closely matched the expected value.

Introduction of pyridyl disulfide onto the chain end began with quantitative displacement of the methanesulfonyl ester by reaction with O-ethyldithiocarbonate. Transformation of the ethyldithiocarbonate to the pyridyl disulfide was carried out by deprotecting the mercapto group with n-propylamine in the presence of 2-pyridyl disulfide (2-PDS). The signals in the ^1H NMR spectrum corresponding to the O-ethyldithiocarbonate moiety were no longer observed after the reaction and new signals assigned to the pyridyl disulfide were present. The ratio of integrals corresponding to the pyridyl disulfide and benzylacetal end groups in the ^1H NMR spectrum showed that the conversion of O-ethyldithiocarbonate to the pyridyl disulfide was 99%. Finally, treatment with an aqueous solution of hydrochloric acid to deprotect the benzylacetal moiety afforded the benzaldehyde-PEO-SS-pyridyl product.

2.6.2. Synthesis of HS-PEO-X via End Group Modification of Homobifunctional PEO

2.6.2.1. Synthesis of HS-PEO-OH from an Allyl-PEO-OH Intermediate

Protein-resistant SAMS were prepared on gold substrates by chemisorption of low molecular weight PEO oligomers (DP ~10) having a thiol on one end and a hydroxyl group at the other.¹¹⁹ The heterobifunctional polymer was prepared in three steps. First, reaction of 11-haloundec-1-ene and sodium hydroxide with an excess of PEO diol produced a mixture of mono, di, and unsubstituted PEO oligomers. The mixture was separated by chromatography on silica gel to obtain the allyl-PEO-OH. Free radical addition of thioacetic acid to the allyl terminus was carried out under UV irradiation with AIBN to yield PEO with a thioacetate on one chain end and a hydroxyl group at the other. The final step in preparing HS-PEO-OH was methanolysis of

the thioacetate in the presence of HCl to generate the thiol. The structure of the polymer was confirmed via end group analysis by both ^1H and ^{13}C NMR.

Ellipsometry was utilized to monitor adsorption of proteins on three different surfaces by measuring increases in thickness of the SAMs before and after exposure to separate solutions containing avidin, hexokinase, or pyruvate kinase. SAMs formed from HS-PEO-OH showed no significant increase in thickness while SAMs produced using HS-(CH₂)₁₁-CH₃ showed significant increases. Appearance of a nitrogen signal in the X-ray photoelectron spectra (XPS) confirmed that the increases in thickness were due to protein adsorption. These results indicated that the SAMs formed from HS-PEO-OH resisted non-specific protein adsorption.

2.6.2.2. Synthesis of Pyridyl-SS-PEO-NHS from a PEO Diamine

Protein-resistant SAMS were prepared on gold substrates by chemisorption of low molecular weight PEO oligomers (DP ~10) having a thiol on one end and a hydroxyl group at the other.¹¹⁹ The heterobifunctional polymer was prepared in three steps. First, reaction of 11-haloundec-1-ene and sodium hydroxide with an excess of PEO diol produced a mixture of mono, di, and unsubstituted PEO oligomers. The mixture was separated by chromatography on silica gel to obtain the allyl-PEO-OH. Free radical addition of thioacetic acid to the allyl terminus was carried out under UV irradiation with AIBN to yield PEO with a thioacetate on one chain end and a hydroxyl group at the other. The final step in preparing HS-PEO-OH was methanolysis of the thioacetate in the presence of HCl to generate the thiol. The structure of the polymer was confirmed via end group analysis by both ^1H and ^{13}C NMR.

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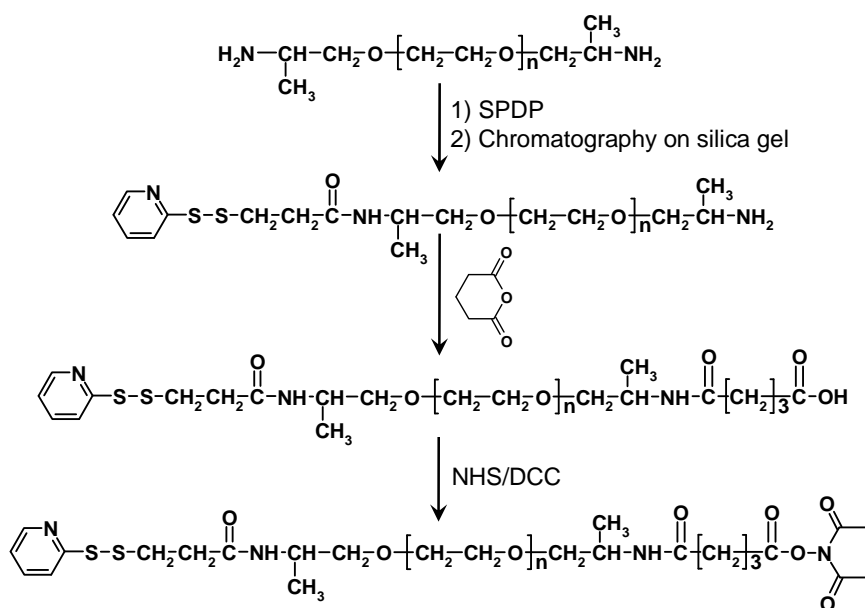


Figure 2.28. Synthesis of pyridyl-SS-PEO-NHS

Haselgrubler et al. demonstrated that these oligomers could be utilized to form liposomes with antibodies bound to their surfaces.¹¹⁵ Reactivities of the pyridyl-SS-PEO-NHS oligomers were confirmed by coupling the NHS active ester with bovine IgG, and this was followed by modifying the pyridyl disulfide end through reaction with a polyclonal sheep antibody (anti-HSA) bearing free thiol functionality. The reactivities of the heterobifunctional polymer were similar to the SPDP analogues (Figure 2.28).

Pyridyl-SS-PEO-NHS oligomers were also used to prepare liposomes with bovine IgG bound to their surfaces. An amino-functional lipid, 1,2-bis(myristoylphosphatidyl)ethanolamine (DMPE), was coupled to the NHS terminus of pyridyl-SS-PEO-NHS. Thus, liposomes bearing

pyridyl disulfide functionality were prepared from a blend of egg yolk phosphatidylcholine and the DMPE-PEO-SS-pyridyl polymer. The pyridyl disulfide groups on the surfaces of the liposomes were reacted with a mercapto-functional bovine IgG. Fluorescently-tagged anti-HSA containing a free thiol was also coupled to the surface of liposomes in a similar manner using the lipids 1-palmitoyl-2-oleoylphosphatidylcholine and asolectin. These results demonstrated the utility of heterobifunctional polymers for attaching targeting moieties to liposomes.

2.6.2.3. Synthesis of Pyridyl-SS-PEO-Biotin from PEO Diamines

To investigate binding of biotin-PEO conjugates to avidin, Kaiser et al. synthesized a heterobifunctional PEO with biotin at one chain end and a pyridyl disulfide group at the other (Figure 2.29).¹¹⁶ The pyridyl disulfide could be utilized to attach biomolecules to the biotin-PEO-SS-pyridyl, but in this study the pyridyl disulfide was used as a chromophoric marker. When it is reacted with a free thiol, the pyridyl disulfide releases the chromophoric marker, 4-thiopyridone, thus enabling quantification of biotin-PEO conjugates.

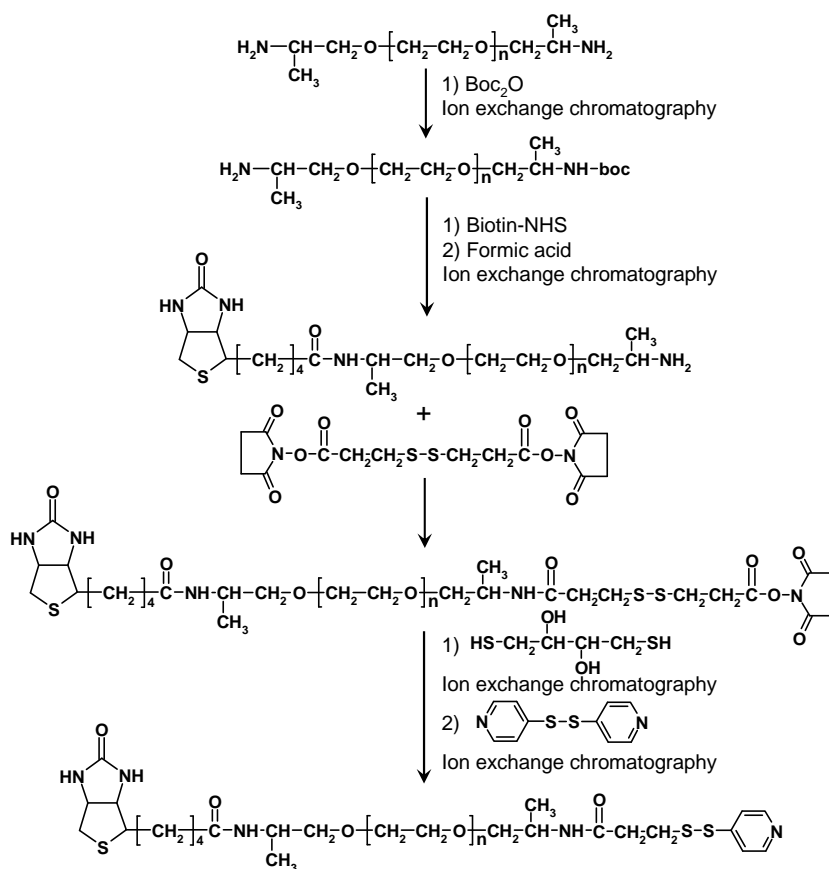


Figure 2.29. Synthesis of biotin-PEO-SS-pyridyl

A stoichiometric amount of a PEO diamine was reacted with di-tert-butylidicarbonate (boc_2O) to produce a statistical mixture of products, then the mixture was separated by column chromatography on either silica or Sephadex C-25 (depending on the length of the PEO) to obtain boc-NH-PEO-NH_2 . The free amine terminus was reacted with a biotin-NHS active ester to form an amide linkage. The boc protecting group was removed with formic acid to regenerate an amine terminus, and the polymer was purified by ion exchange chromatography.

The amine terminus was converted to a pyridyl disulfide by first reacting the oligomer with 3,3'-dithio(succinimidylpropionate). The product was reduced with an excess of 1,4-dithiothreitol to yield biotin-PEO-SH. The final product, biotin-PEO-SS-pyridyl, was obtained

by reacting the thiol terminus with 4,4'-dithiodipyridine, and the oligomer was purified by ion exchange chromatography.

The potential for conjugating avidin binding to biotin-PEO was tested by utilizing biotin-PEO-SS-pyridyl to investigate the stoichiometry and metastability of bound avidin. The avidin-biotin-PEO conjugates demonstrated dissociation kinetics and half-lives similar to those previously reported for spacers with 7-27 atoms.¹²⁰⁻¹²³ These results confirmed that biotin-PEO is a good ligand for avidin.

2.6.2.4. Polymer Supported Synthesis of Pyridyl-SS-PEO-OH from PEO Diols

A solid phase method for synthesizing heterobifunctional PEO derivatives was developed by Bettinger et al. to avoid the lengthy and often complicated separations that are usually employed when starting from homobifunctional PEOs (Figure 2.30).¹²⁴ A PEO diol was activated at one chain end before being bound to the polymer support. Diglycolic anhydride was reacted with a large excess of PEO diol to obtain mono- and unsubstituted products. Without further purification, the carboxylic acid was transformed to the active ester by reacting with NHS and DCC. After removing the urea by-product, the mixture of active and inactive PEOs was grafted onto an aminomethylated poly(styrene-co-divinylbenzene) solid phase resin. The resin was washed with dichloromethane and methanol to remove PEO diol and leave only PEO that was reacted onto the resin through amide linkages. The washing procedure was repeated after each synthetic step to ensure the purity of the resin-bound PEO. A negative Kaiser test confirmed complete capping of all amino groups. The connecting ester and amide moieties as well as the characteristic absorption bands for PEO were observed by IR spectroscopy, indicating that the active PEO chains were bound to the resin.

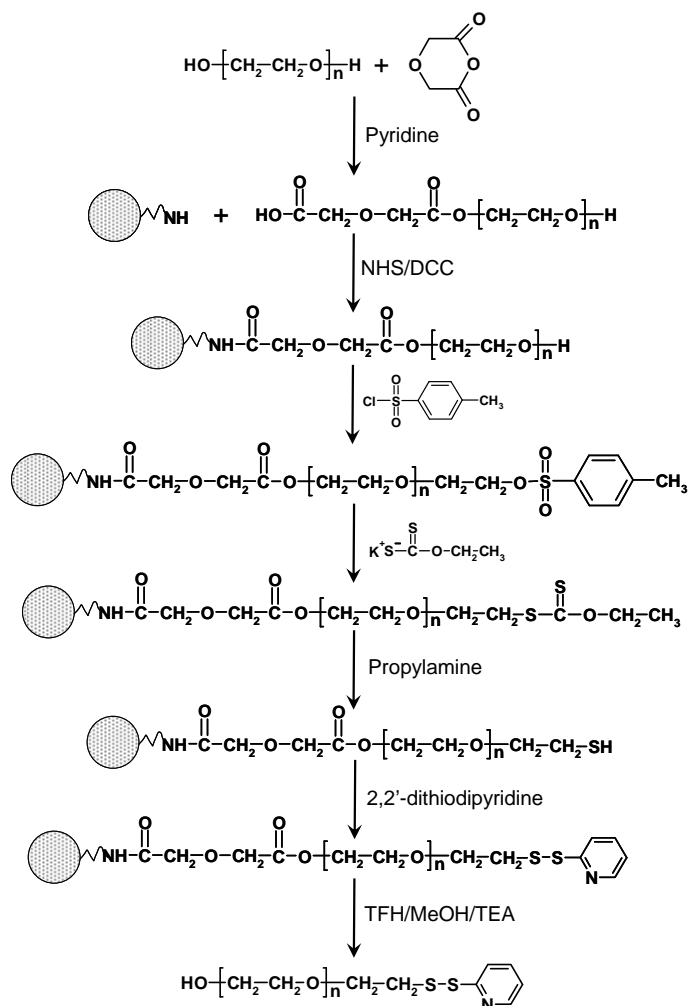


Figure 2.30. Polymer supported synthesis of pyridyl-SS-PEO-OH

The hydroxyl terminus of the resin-bound PEO (resin-PEO-OH) was activated by reacting with an excess of toluene-4-sulfonyl chloride. The resin was washed to remove excess reagents and the presence of the tosylate was confirmed by IR spectroscopy and by gel-phase ^{13}C NMR. The activated resin-PEO-OTs was stable for up to 4 months at $-20\text{ }^\circ\text{C}$. A wide variety of heterobifunctional PEOs could be synthesized from this starting material.

A thiol was introduced by reacting a stoichiometric amount of potassium O-ethyl dithiocarbonate with the resin-PEO-OTs. It should be noted that using an excess of potassium O-ethyl dithiocarbonate led to complete cleavage of the PEO from the resin. The disappearance

of signals corresponding to the tosylate end group and appearance of two new resonances corresponding to the thiocarbonate end group in the ^{13}C NMR spectra showed complete conversion of the end groups. The thiocarbonate was aminolyzed with propylamine under anhydrous conditions to avoid cleavage of the functionalized PEO from the resin. Complete conversion of the end group without oxidation to form the disulfide was confirmed by ^{13}C NMR. The thiol was protected by reacting with 2,2'-dithiodipyridine to yield resin-PEO-SS-pyridyl. The final step was cleavage of the functionalized PEO from the resin with a mildly basic polar solvent (tetrahydrofuran/methanol/triethylamine). The ^{13}C NMR spectrum of the resin revealed that all of the previously-bound PEO had been removed. The pyridyl-SS-PEO-OH was obtained in a global yield of 65%, and the structure was confirmed by ^1H and ^{13}C NMR.

2.6.2.5. *Synthesis of X-PEO-SAc from PEO Diols*

PEO diols were utilized to prepare several heterobifunctional conjugates with a protected mercapto group (thioacetate) on one chain end and either a hydroxyl, aldehyde, amine, or azide at the other chain end.⁷² The first step in these syntheses was activation of one chain end of a 1500 M_n PEO diol with a stoichiometric amount of toluene-4-sulfonyl chloride. Tosylation of both chain ends was minimized by conducting the tosylation in the presence of silver oxide with a catalytic amount of potassium iodide.¹²⁵ The protected mercapto group was introduced by nucleophilic displacement of the tosylate with potassium thioacetate to yield HO-PEO-SAc. This polymer served as an intermediate for synthesizing several heterobifunctional PEO oligomer types via derivatizations of the hydroxyl end.

Introduction of an azide moiety was achieved by activating the hydroxyl end with methanesulfonyl chloride followed by reaction with sodium azide. The hydroxyl terminus was converted to an aldehyde in two steps. Sodium hydride was used to form the alkoxide chain end,

and this was reacted with 3-bromo-1,1-dimethoxypropane. The acetal terminus was then converted to the aldehyde using Amberlyst-15 (acidic) resin. Benzylamine was reacted with the aldehyde by reductive amination while leaving the thioacetate group intact. This demonstrated that aldehyde-PEO-SAc could be selectively linked to a molecule through the aldehyde functionality, and then the mercapto group could be deprotected for later use.

A similar series of reactions was used to convert the hydroxyl terminus to an amine. The hydroxyl terminus was deprotonated with sodium hydride and reacted with fmoc-protected 3-aminopropyl bromide, and this was followed by deprotecting the amine with pyridine. The reactivity of the amine was demonstrated by adding benzaldehyde via reductive amination. The amine was also used to form a peptide bond by reacting with the active NHS ester of boc-protected lysine.

Several types of reactions were carried out to demonstrate the utility of these heterobifunctional PEOs in the field of biocompatible nanoparticles and bioconjugation. A three-stage process was employed to prepare fluorescently-labeled gold nanoparticles from HO-PEO-SAc. A fluorescent tag, coumarin isocyanate, was reacted with the hydroxy end of the PEO, and this was followed by deprotecting the thioacetate. The mercapto terminus was then coupled with freshly-prepared, 10-15 nm gold nanoparticles. The coumarin label allowed for cellular visualization assays using phase contrast microscopy. The aldehyde-PEO-SAc was covalently bound to a commercially available goat anti-mouse antibody by reductive amination. The thioacetate was hydrolyzed and the resultant thiol was reacted with a fluorescent tag, fluorescein 5-maleimide.

Even though gold nanoparticles are efficient fluorescence quenchers,¹²⁶ the coumarin-PEO-SH functionalized gold nanoparticles displayed significant emission intensity. The reduced

fluorescence quenching was attributed to the presence of the PEO spacer.¹²⁷ Coumarin-PEO-SH functionalized nanoparticles were also found to be nontoxic towards MDA-MB-231 human breast adenocarcinoma cells at concentrations up to 200 $\mu\text{g mL}^{-1}$ (higher concentrations were not tested). Tracking of the functionalized gold nanoparticles inside the cells was accomplished with a Keck 3-D Fusion microscope. Intracellular trafficking studies showed that the coumarin-PEO-SH functionalized gold nanoparticles were incorporated into the cells via non-specific endocytosis within the first few minutes of incubation. After one hour, the nanoparticles had passed through the cytosol and reached the perinuclear region. Even after 24 hours of incubation, none of the nanoparticles were observed in the nucleus. These results demonstrated that gold nanoparticles functionalized with heterobifunctional PEO spacers could be covalently linked to a variety of moieties to study cellular transport pathways.

2.6.3. SPR Sensing utilizing an Aldehyde-PEO-SH Brush Layer

Two heterobifunctional PEO oligomers with different molecular weights, having both a mercapto and an acetal group, were used to prepare SPR sensor chips.⁵¹ The acetal-PEO-SH polymers were synthesized as previously described with molecular weights of 2000 and 5000 g mol^{-1} and molecular weight distributions of 1.03 and 1.04, respectively. SAMs with acetal-PEO-SH layers were formed on SPR gold sensor chips by flowing a solution of the heterobifunctional polymer over the chip, and this was followed by washing to remove unbound polymer. The protocol was repeated several times to increase brush densities on the sensor chips. The amount of polymer bound to the sensor chip was monitored via SPR. The acetal was converted to an aldehyde by acid hydrolysis. Biotin bearing a hydrazide moiety, biocytin hydrazide, was covalently bound to the sensor chip through formation of the Schiff base with the aldehyde end of the tethered PEO (denoted as 2kPEO-B and 5kPEO-B). The capacity of the sensor chip to

bind with a solution containing only streptavidin or a mixture of streptavidin and a non-specific protein, bovine serum albumin (BSA), was monitored by SPR.

The SAM composition significantly affected performance of the SPR sensor chips. When 2kPEO-B was used to form the initial SAM followed by addition of 5KPEO, significantly reduced binding of streptavidin was observed. The decreased accessibility indicated that the longer PEO chain shielded the shorter 2kPEO-B from interacting with streptavidin. Non-specific adsorption of proteins on the sensor chip was greatly reduced when the initial SAM was formed from 5kPEO-B followed by the 2kPEO. The increased density of chains on the surface from adding the shorter 2kPEO also increased the capacity for binding the SAM with streptavidin, and this was attributed to brush extension of the 5kPEO-B. The improved binding of streptavidin and almost negligible non-specific adsorption led to SPR sensor chips with high signal to noise ratios.

2.7. Synthesis and Applications of Mal-PEO-X

Maleimide-functional PEO is of great interest for conjugating biomolecules bearing a mercapto moiety, and such oligomers have also been used to form block copolymers and modify the properties of bismaleimide resins.¹²⁸⁻¹³² The maleimide moiety reacts with mercapto groups under mild conditions to yield stable thioether linkages.¹³³ PEO oligomers with a maleimide group on one chain end and another functional group on the other (Mal-PEO-X) have been used to prepare biosensors, functionalize surfaces for enzyme immobilization, and in targeted drug delivery.^{14,134-136}

2.7.1. Direct Synthesis of Maleimide-PEO-OH

A double metal cyanide complex catalyst has been utilized to polymerize EO from a heterobifunctional initiator, N-(2-hydroxyethyl)maleimide, to yield a heterobifunctional PEO

with a maleimide group on one chain end and a hydroxyl group at the other end.⁸² Although ring-opening polymerizations of EO are usually carried out with a basic initiator,¹³⁷ the sensitivity of N-(2-hydroxyethyl)maleimide toward base prohibited this approach.^{41,48,86} The zinc hexacyanocobaltate double metal cyanide catalyst allowed for the polymerization of EO utilizing N-(2-hydroxyethyl)maleimide as the initiator with retention of the maleimide functionality at the chain end.

The heterobifunctional initiator was prepared in three steps (Figure 2.31).¹³⁸ Maleic anhydride was first protected by the Diels-Alder reaction with furan, and this was followed by reacting ethanolamine with the anhydride under anhydrous conditions. Deprotection of the double bond produced N-(2-hydroxyethyl)maleimide.

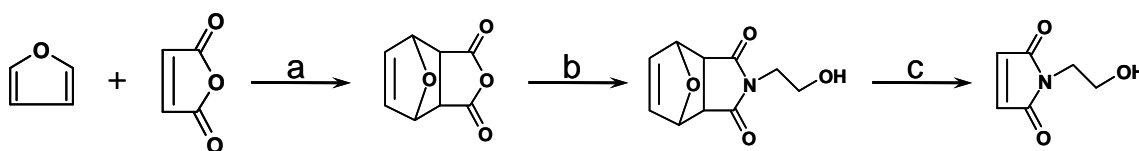


Figure 2.31. Synthesis of N-(2-hydroxyethyl)maleimide: a) toluene, RT; b) ethanolamine, MeOH, reflux; c) toluene, reflux

The N-(2-hydroxyethyl)maleimide initiator was utilized in a batch polymerization of EO in the presence of a double metal cyanide catalyst, *Impact 3* zinc hexacyanocobaltate. It has been shown that batch epoxide polymerizations activated with double metal cyanide catalysts have produced polymers with broader molecular weight distributions as compared to the base-catalyzed PPO polymers, but with essentially no unsaturation.^{41,48,86} As expected from previous studies, the molecular weight distributions obtained from these polymerizations were broad (~3.3). As determined from ¹H NMR, the molecular weights of the polymers were consistent with the targeted values based on the monomer to initiator ratios, not the monomer to catalyst ratio. Analysis via ¹H NMR confirmed that the maleimide end group was retained during

polymerization, indicating that the polymerization of EO takes place without any significant side reactions.

2.7.2. Synthesis of Maleimide-PEO-Benzophenone (Mal-PEO-BP)

A heterobifunctional PEO with a maleimide moiety on one end and a benzophenone on the other (Mal-PEO-BP) was synthesized for photo-immobilization of biomolecules on surfaces.¹³⁴ It was reasoned that these materials would be polymeric analogues of a commercially available thiol-reactive, photoactivatable linker, 4-maleimidobenzophenone (BPMI) (Figure 2.32). Biosensors were constructed by using the benzophenone to anchor the heterobifunctional PEO to the substrate and the maleimide was used to bind biomolecules of interest. It was hoped that introduction of a short PEO spacer between the maleimide and benzophenone moieties would increase accessibility of the maleimide, thereby improving sensitivity of the biosensor as compared to those derived from the commercial BPMI.

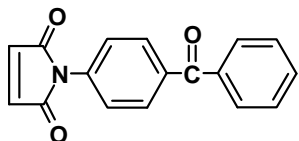


Figure 2.32. 4-Maleimidobenzophenone

Two methods were employed for synthesizing heterobifunctional Mal-PEO-BP oligomers. The first began with protecting the primary amine of 2-(2-aminoethoxy)ethanol with 9-fluorenylmethyl chloroformate (Fmoc-Cl) (Figure 2.33A). The hydroxyl group was then coupled with 4-hydroxybenzophenone (BPOH) in the presence of TPP and diethyl azodicarboxylate (DEAD), and this was followed by deprotecting the Fmoc-protected amine with pyridine. Conversion of the primary amine terminus to the maleimide was achieved by reaction with maleic anhydride in the presence of a dehydrating agent.

The second method, which did not require a heterobifunctional starting material, utilized a PEO diol (Figure 2.33B). 4-Hydroxybenzophenone was reacted with an excess of PEO diol in the presence of TPP and diisopropyl azodicarboxylate (DIAD) to produce a statistical mixture of products. The target material, BP-PEO-OH, was obtained by chromatography on silica. The hydroxyl terminus was converted to the maleimide moiety by reacting with maleimide in the present of TPP and DIAD. The structures of the desired products and all intermediates were confirmed via ^1H and ^{13}C NMR.

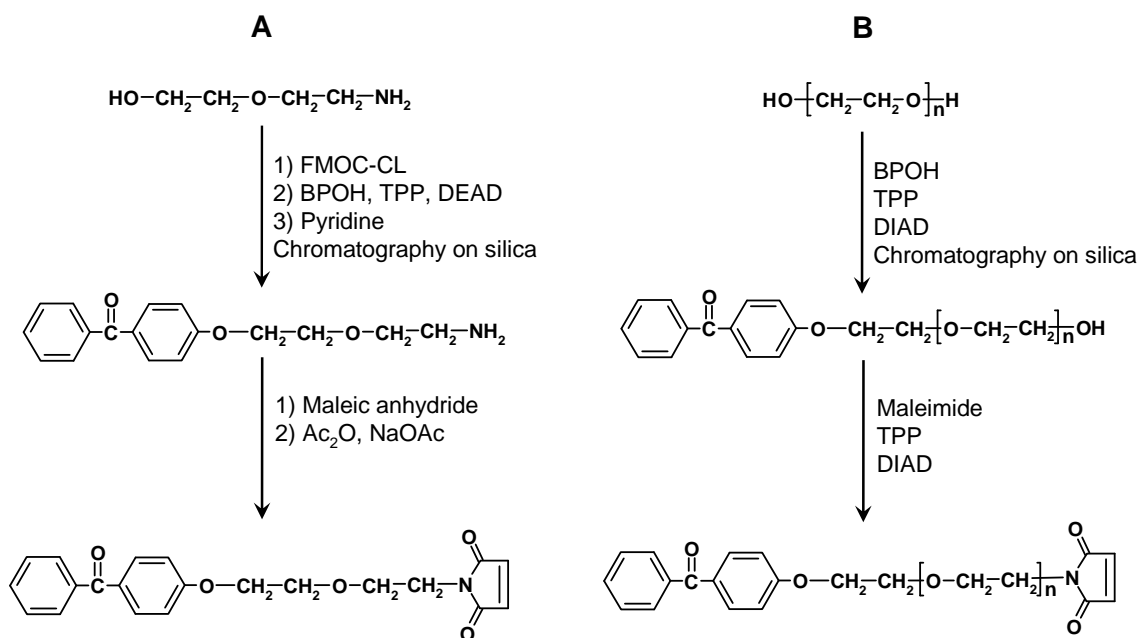


Figure 2.33. Synthesis of Mal-PEO-BP

2.7.3. Synthesis of Mal-PEO-COOH and Thiol-reactive Heterobifunctional PEO Oligomers

Three different thiol-reactive, heterobifunctional PEO oligomers were synthesized for coupling peptides to liposomes.⁹⁶ The oligomers were designed with a carboxylic acid group on one chain end for coupling to the amine of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine

(DPPE) (Figure 2.34) and either a maleimide, bromoacetamide, or pyridyl disulfide moiety on the other end for coupling to cysteine residues of peptides.

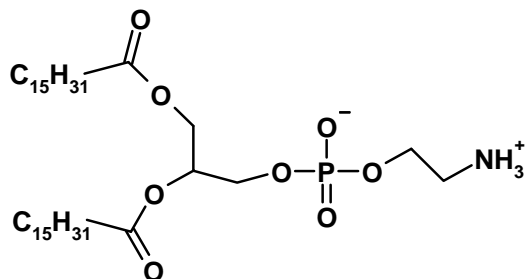


Figure 2.34. 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE)

Synthesis of the heterobifunctional PEO with a carboxylic acid and a bromoacetamide group began by reacting methanesulfonyl chloride and an equimolar amount of a PEO diol. The monosubstituted product was separated from the statistical mixture by chromatography on silica gel. Introduction of a primary amine terminus was accomplished by nucleophilic displacement of the methanesulfonyl ester with sodium azide, and this was followed by reduction to the primary amine. The primary amine terminus was converted to a bromoacetamide by reaction with the N-hydroxysuccinimidyl ester of bromoacetate. Subsequent oxidation of the hydroxy terminus using Jones reagent (chromic trioxide:sulfuric acid:water) afforded the target compound, bromoacetamide-PEO-COOH. The carboxylic acid terminus was coupled with the amine of DPPE in the presence of DCC and NHS.

A H₂N-PEO-COOH oligomer was utilized to synthesize Mal-PEO-COOH. Introduction of the maleimide functionality was accomplished by reacting the primary amine terminus with 2,5-dioxo-2,5-dihydropyrrole-1-carboxylic acid methyl ester (Figure 2.35) in the presence of bicarbonate to catalyze the cyclization reaction and produce the Mal-PEO-COOH. The amine of DPPE was then coupled to the carboxylic acid terminus by reaction with DCC and NHS.

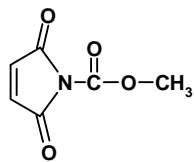


Figure 2.35. 2,5-Dioxo-2,5-dihydropyrrole-1-carboxylic acid methyl ester

Synthesis of the heterobifunctional PEO with both a carboxylic acid and pyridyl disulfide began with oxidation of a PEO oligomer that had a hydroxy group on one end and an alkyl chloride at the other using Jones reagent to afford Cl-PEO-COOH. A mercapto group was introduced by displacing the chloride with thiourea, and this intermediate was subsequently hydrolyzed with sodium hydroxide. The target compound pyridyl-SS-PEO-COOH was obtained by reacting the terminal mercaptan with 2,2'-dipyridyl disulfide. Coupling of DPPE with pyridyl-SS-PEO-COOH was carried out using (benzotriazol-1-yl-oxy)tris(dimethylamino)phosphonium hexafluorophosphate (Figure 2.36) due to the formation of byproducts that were difficult to remove when using DCC.

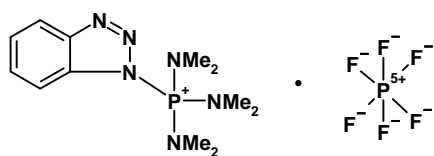


Figure 2.36. (Benzotriazol-1-yl-oxy)tris(dimethylamino)phosphonium hexafluorophosphate

Reactivities of liposomes formed with the three different thiol-reactive heterobifunctional PEOs were investigated as functions of time and pH by reactions with a peptide bearing a cysteine residue. The maleimide and pyridyl disulfide moieties produced almost quantitative conversion within two hours in the pH range of 6 to 9. However, the reactivity of the bromoacetamide-functional oligomer was significantly less than those with the maleimide and

pyridyl disulfide functionalities. Coupling with the bromoacetamide was quantitative at pH 9 within 50 minutes, but at lower pH values the reaction was incomplete even after two hours.

2.7.4. Applications of Mal-PEO-X

2.7.4.1. Improved Blood Circulation Times of rhG-CSF

A heterobifunctional PEO with a carboxylic acid on one chain end and a maleimide on the other (Mal-PEO-COOH) was used to link recombinant human granulocyte-colony stimulating factor (rhG-CSF) to rat serum albumin (RSA) and human serum albumin (HSA) to improve in vivo circulation times and increase biological response.⁹⁷ The primary amine terminus of H₂N-PEO-COOH was converted to a maleimide by reaction with N-γ-maleimidobutyryloxysuccinimide ester (Figure 2.37) in the presence of triethylamine, and this was followed by activating the carboxylic acid terminus using NHS and DCC. The amino group of rhG-CSF was coupled to the activated ester of the PEO, and either RSA or HSA was covalently reacted onto the other end via a thio-ester linkage by reaction of a mercapto group with the maleimide.

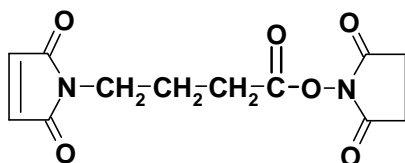


Figure 2.37. N-γ-maleimidobutyryloxysuccinimide ester

The blood circulation times of RSA-PEO-rhG-CSF, HSA-PEO-rhG-CSF, rhG-CSF, and a mixture of HSA and rhG-CSF were tested in rats. The rhG-CSF bound to either HSA or RSA showed improved blood circulation times as compared to rhG-CSF or the mixture of HSA and rhG-CSF. White blood cell response was measured to determine biological activity. The white blood cell response for the rhG-CSF linked with RSA and HSA displayed a higher peak response

than rhG-CSF, as well as a longer period of activity. It is also worth noting that rhG-CSF-PEO-RSA showed improved serum stability as compared to free rhG-CSF. These results indicated that attaching rhG-CSF to RSA and HSA via a PEO linker does not interfere with the activity of rhG-CSF. The results also suggested the potential of these materials for reducing the frequency of dosing required for patients receiving rhG-CSF treatments.

2.7.4.2. *Solid-Phase Enzyme Immobilization*

To improve the performance of enzymes bound to a solid support, a heterobifunctional PEO with a maleimide group on one chain end and an NHS activated ester on the other was used to immobilize model enzymes, β -amylase and β -galactosidase, on thiol-functional agarose beads.¹⁴ The performance of enzymes immobilized using Mal-PEO-NHS was compared to enzymes immobilized using bis-oxirane homobifunctional PEO and N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (Figure 2.38). The activity of the β -galactosidase enzyme, which acts on low molecular weight substrates, showed very little dependence on the method of immobilization. However, the activity of the β -amylase enzyme, which acts on high molecular weight substrates, was significantly improved by using the heterobifunctional PEO. Although higher immobilization yields were obtained when using the homobifunctional PEO than when using the heterobifunctional PEO, the activity of the immobilized β -amylase enzyme was higher when using the heterobifunctional PEO. This decreased activity with the homobifunctional PEO was attributed to possible crosslinking of the enzymes and a steric shielding effect from a high number of PEO chains per enzyme. The heterobifunctional PEO spacer allowed for optimizing the number of PEO chains per enzyme to maximize performance of the immobilized enzyme. The heterobifunctional PEO spacer also showed significantly higher activity than the SPDP linker due to the enzyme being more accessible to the substrate.

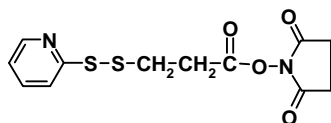


Figure 2.38. N-succinimidyl-3-(2-pyridyldithio)propionate

2.7.4.3. Targeted Drug Delivery

Lipid drug-carriers with a targeting moiety tethered to the surface were prepared via a heterobifunctional PEO spacer.¹³⁶ The PEO-lipid derivative, DPPE-PEO-Mal, was prepared by reacting DPPE (Figure 2.34) with Mal-PEO-NHS. DPPE-PEO-Mal was utilized to form liposomes and emulsions containing the fatty acid derivative of the anti-cancer drug FUdr, 3'5'-*O*-dioleoyl-FUdr (FUdr-dO), which can be liberated in cells via hydrolysis. Derivatization of the maleimide at the distal end of the PEO was carried out by reacting the free mercapto groups with the targeting moiety, an anti-CD74 antibody (LL1).

In vitro incubation with the targeted Raji B-lymphoma cells showed that ~30% of the targeted drug carriers were associated with the cells compared to only 0.6% with drug carriers without the targeting moiety. Cytotoxicities of the targeted emulsion and liposomal drug carriers compared to free FUdr were tested on Raji lymphoma cells. The activities of both the targeted emulsion and liposomes were higher than that of free FUdr.

Heterobifunctional Mal-PEO-NHS has also been used to prepare targeted drug delivery systems by coupling an anti-tumor drug, recombinant human necrosis factor alpha (TNF- α), with a targeting protein, transferrin (Tf).¹³⁵ Approximately five PEO chains were covalently attached to TNF- α through amide bonds via reaction with the NHS active ester of PEO to form PEO₅-TNF- α . Thiolated Tf was then linked to the distal end of the PEO chains through thioether bonds by reaction with the maleimide moieties. The targeted drug delivery system was prepared with an average of one Tf for every one molecule of TNF- α to form Tf-PEO₅-TNF- α .

The in vivo characteristics of the targeted drug delivery system were investigated using tumor-bearing mice. PEO₅-TNF- α and Tf-PEO₅-TNF- α significantly delayed blood clearance compared to unmodified TNF- α . It was found that Tf-PEO₅-TNF- α showed a 1.8 and 5.3 fold increase in anti-tumor activity over PEO₅-TNF- α and TNF- α , respectively. These results demonstrated that a targeting moiety can alter the in vivo characteristics of TNF- α , and they also demonstrated the potential for targeted drug delivery systems using heterobifunctional PEOs in anti-tumor therapies.

2.8. Synthesis and Applications of other Heterobifunctional PEO Oligomers

2.8.1. Heterobifunctional PEO Macroinitiators for Free Radical Polymerizations

2.8.1.1. Synthesis of TEMPO-PEO-OH

A heterobifunctional PEO containing a hydroxyl group and a 4-hydroxyl-2,2,6,6-tetramethyl-1-piperidinyloxy (HTEMPO) moiety at the chain ends was prepared and utilized as a macroinitiator for synthesizing poly(ethylene oxide-*b*-styrene) (PEO-*b*-PS) via stable free radical polymerization.¹³⁹ Potassium 2-dimethylaminoethanoate (DME-K) was prepared as an initiator by reacting 2-dimethylaminoethanol with potassium in THF at 60 °C. Polymerization of EO was carried out with DME-K in THF at 65 °C, and this was followed by termination with methanol (Figure 2.39A). Radicals were generated by UV irradiation of benzophenone in the presence of the dimethylamino-functional PEO, and they were scavenged by HTEMPO to produce a HTEMPO-PEO-OH macroinitiator (Figure 2.39B). End group analysis via ¹H NMR determined the capping efficiency to be between 85 and 90 %. Polymerization of styrene utilizing the HTEMPO-PEO-OH macroinitiator produced PEO-*b*-PS block copolymers with molecular weight distributions less than 1.5. Good correlation was found between conversion and molecular weight, suggesting that the polymerization was a living radical process.

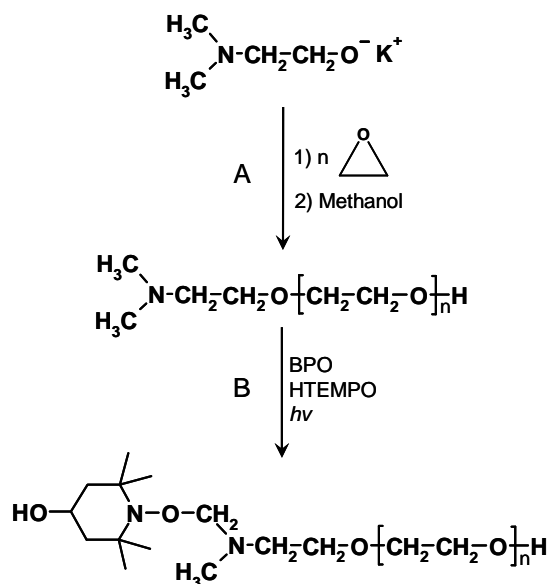


Figure 2.39. Synthesis of DME-PEO-OH and TEMPO-PEO-OH

A direct method for synthesizing a heterobifunctional PEO with a 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) moiety at one chain end and a hydroxyl group at the other has also been developed (Figure 2.40).¹⁴⁰ A 4-oxy-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPONa) initiator was prepared by reacting HTEMPO with sodium in THF at 40 °C. The ESR spectrum of TEMPONa was identical to that of HTEMPO, indicating that the stable nitroxyl radical on TEMPONa remained intact. The polymerization of EO using TEMPONa was carried out in THF at 60 °C, and this was followed by termination with methanol. Molecular weights determined by SEC and ¹H NMR matched well with the calculated values based on the monomer to initiator ratios. Molecular weight distributions were also narrow, ≤ 1.11 .

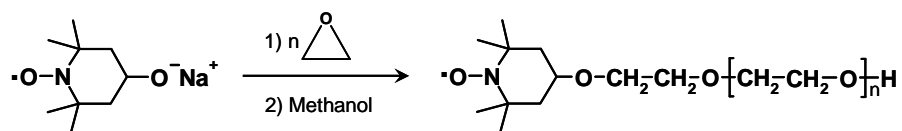


Figure 2.40. HTEMPO initiated polymerization of EO.

The TEMPO-PEO-OH macroinitiator was utilized for stable free radical polymerizations of styrene and 4-vinylpyridine to produce well-defined PEO-*b*-PS and poly(ethylene oxide-*b*-4-vinylpyridine) (PEO-*b*-PV) block copolymers.^{140,141} Both the PEO-*b*-PS and PEO-*b*-PV copolymers were prepared without any residual PS or PV homopolymer or unreacted TEMPO-PEO-OH as evidenced by the unimodal SEC traces, and the copolymer molecular weight distributions were reasonably narrow, less than 1.51. The linear dependence of molecular weight on conversion of the monomer provided evidence that the reactions had proceeded by stable free radical polymerizations. It should be noted that this method of synthesizing PEO-*b*-PS and PEO-*b*-PV block copolymers leads to reversible decomposition-combination reactions at high temperatures due to the unstable C-ON bond between the PEO and PS or PV segments.¹⁴⁰

It has also been shown that TEMPO-PEO-OH can be synthesized from a TEMPO alkoxide (Figure 2.41).¹⁴² The initiator was prepared by reacting potassium naphthalide in THF with a slight excess of TEMPO. Polymerization of EO proceeded in a controlled fashion. It is also noteworthy that polymerizations of EO using tert-butoxide in the presence of TEMPO result in polymers without any TEMPO end groups. Polymers with controlled molecular weights were obtained by tailoring the ratio of monomer to initiator and with narrow molecular weight distributions (<1.1) indicating that the polymerization reactions proceeded without significant side reactions. Polymer structure was confirmed by ¹H NMR, and molecular weights obtained via ¹H NMR end group analysis and SEC showed good correlation. The TEMPO-PEO-OH synthesized from the TEMPO alkoxide was utilized to prepare PEO-*b*-PS copolymers via stable free radical polymerization without the unstable C-ON linkage between PEO and PS as previously described.¹⁴⁰⁻¹⁴²

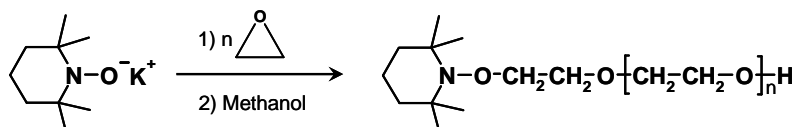


Figure 2.41. Synthesis of TEMPO-PEO-OH from TEMPO alcoholate

2.8.1.2. Synthesis of DME-PEO-Methacrylate

It has been shown that dimethylamino-functional PEO can be utilized as a macroinitiator for polymerizing styrene, and that PEO with a methacrylate end group can be copolymerized with styrene.^{139,143} A heterobifunctional PEO possessing a dimethylamino and a methacrylate end group was prepared by polymerizing EO initiated with DME-K, and terminating the polymerization with methacryloyl chloride.¹⁴⁴ The polymers had molecular weight distributions <1.14, and molecular weights determined by ¹H NMR matched closely with the calculated values based on monomer to initiator ratios. The ratio of dimethylamino to methacrylate groups was 1:1 as determined via ¹H NMR end group analysis. Photoinduced homo- and copolymerization of DME-PEO-methacrylate with methyl methacrylate in the presence of benzophenone produced crosslinked polymers.

2.8.2. Synthesis of X-PEO-Y via a Protected Hydroxyl Group

2.8.2.1. Synthesis of X-PEO-Y from a BzO-PEO-X Intermediate

Heterobifunctional PEOs were synthesized from a BzO-PEO-OH intermediate through a series of functionalization and deprotection reactions, and these procedures did not require complicated separations.¹⁴⁵ The polymerization of EO utilizing a benzyloxy anion produces a heterobifunctional PEO with a hydroxyl group on one chain end and a protected hydroxyl group at the other. The hydroxyl terminus can then be derivatized by a wide range of reactions followed by removal of the benzyl protecting group by either catalytic hydrogenation or acid-

catalyzed hydrolysis to produce heterobifunctional PEOs of the structure HO-PEO-X. Heterobifunctional PEOs of the structure X-PEO-Y can then be prepared by further derivatization of the deprotected hydroxyl group (Figure 2.42). Heterobifunctional PPOs have also been produced utilizing benzyl alcohol as the initiator in the presence of an aluminum-porphyrin catalyst.^{146,147}

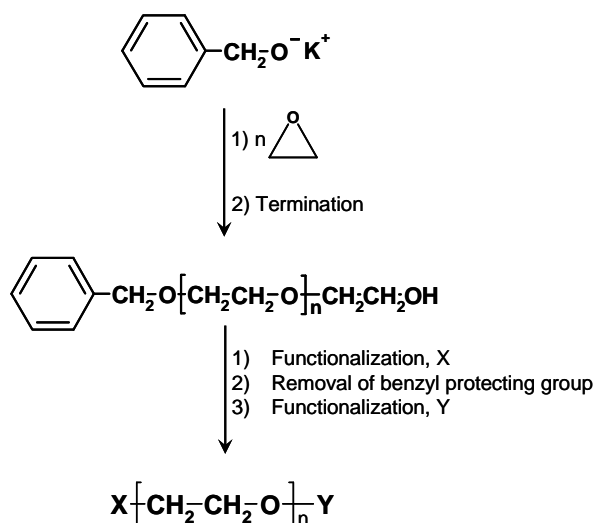


Figure 2.42. Synthesis of heterobifunctional PEOs from BzO-PEO-OH

A HO-PEO-NH₃⁺Cl⁻ polymer was prepared by first activating the hydroxyl terminus of BzO-PEO-OH by reaction with methanesulfonyl chloride in the presence of triethylamine. Introduction of a primary amine was achieved by subsequent reaction with aqueous ammonia containing ammonium chloride. Removal of the benzyl protecting group by treatment with concentrated hydrochloric acid afforded the target polymer, HO-PEO-NH₃⁺Cl⁻.

HO-PEO-COOH and Cl⁻H₃N⁺-PEO-COOH were also prepared from BzO-PEO-OH. The hydroxyl terminus of BzO-PEO-OH was deprotonated with potassium tert-butoxide and reacted with tert-butyl bromoacetate. This was followed by acid hydrolysis to yield HO-PEO-COOH. The carboxylic acid was then protected by dissolving the polymer in methanol in the

presence of sulfuric acid to form the methyl ester. The hydroxyl terminus was converted to the primary amine as described above, then the carboxylic acid was deprotected by acid-catalyzed hydrolysis to obtain the target polymer, $\text{Cl}^-\text{H}_3\text{N}^+\text{-PEO-COOH}$. The method of synthesizing heterobifunctional PEOs from BzO-PEO-OH is versatile in that it can lead to many different heterobifunctional PEO derivatives. Please refer to the patent by Bentley et al. for more examples of heterobifunctional PEOs made by this method.¹⁴⁵

2.8.2.2. *Synthesis of HO-PEO-X from TBDMS-PEO-X*

Heterobifunctional PEO macromonomers were synthesized having a hydroxyl group at one chain end and either a methacrylate or vinylbenzyl moiety at the other end.¹⁴⁸ A heterobifunctional initiator with a protected hydroxyl group, ethylene glycol tert-butyldimethylsilyl (TBDMS) ether, was utilized. The TBDMS ether was synthesized according to the procedure described by McDougal et al.,¹⁴⁹ and this was followed by reaction with potassium naphthalide to form the alkoxide. Polymerization of EO was carried out at 40 °C in THF. The polymerization was terminated by adding an excess of either vinylbenzyl chloride (VBC) or methacryloyl chloride (MAC) to afford TBDMS-PEO-VB or TBDMS-PEO-methacrylate, respectively. Desilylation was carried out with tetra-n-butylammonium fluoride with retention of the polymerizable end groups. Molecular weight distributions determined by SEC were <1.1. The molecular weights obtained by ¹H NMR and vapor pressure osmometry (VPO) also matched well with the calculated values based on the monomer to initiator ratios. This method for synthesizing heterobifunctional PEOs could potentially be utilized to prepare a wide range of heterobifunctional PEOs through a series of functionalization, deprotection, and further functionalization reactions similar to the method shown in Figure 2.42.

2.8.2.3. *Synthesis of X-PEO-OH on a Poly(vinyl alcohol) (PVA) Support*

A heterobifunctional PEO with a hydroxyl group on one terminus and a trityl-protected hydroxyl group on the other end was synthesized on a PVA support.⁷³ The function of the support was to separate the heterobifunctional PEO oligomers from the mixture, and to allow for further functionalization of the free end only. A PEO diol was reacted with trityl chloride in refluxing chloroform in the presence of triethylamine. A slight deficiency of trityl chloride relative to -OH was used (0.85:1) to ensure that only mono-, and di-substituted PEO were formed. An excess of toluene diisocyanate (TDI) was then reacted with the remaining hydroxyl groups in the mixture. Excess TDI was used to prevent coupling of PEO chains and to introduce a reactive site for coupling with PVA. The mixture of products was reacted with PVA, where only trityl-PEO-TDI could react with the PVA support. The PVA-g-PEO-trityl was separated by precipitation from DMSO into dichloromethane. The PVA-g-PEO-trityl was hydrolyzed and the regenerated support was removed by precipitation from DMSO into dichloromethane. The dichloromethane filtrate that contained the polymer was concentrated and poured into ether to precipitate the target polymer, trityl-PEO-OH. Polymer structure was confirmed by ¹H NMR, IR, MS, and elemental analysis. A variation of this method has also been used to synthesize heterobifunctional PEO oligomers with a hydroxyl group on one chain end and either an azide, amine, or benzaldehyde on the other.¹⁵⁰

2.9. *Concluding Remarks*

The unique properties of PEO have made it particularly useful in the biomedical field. Over the last few years, research and applications pertaining to heterobifunctional PEOs have grown significantly. Researchers have shown that heterobifunctional PEOs have the potential to increase drug potency, not only by prolonging circulation in vivo but also by attaching drugs to targeting moieties. Increased circulation times of drugs also have the added advantage of

decreasing the frequency of dosing for patients. It has also been demonstrated that drugs can be delivered utilizing liposomes bound to a targeting moiety through a heterobifunctional PEO spacer. Heterobifunctional PEOs have also been key components in the development of biosensors and other assay devices by tethering targeting moieties to SPR sensors and nanoparticles.

The many synthetic techniques to prepare heterobifunctional PEOs reviewed herein serve as a platform for further research on potential applications of these materials. Many commercially available heterobifunctional PEO reagents are also available for labs that do not have the capability of synthesizing these materials. With the available synthetic techniques and commercially available heterobifunctional PEOs, the current challenge for research involving heterobifunctional PEOs is the synthesis of novel initiators for the polymerization of EO, and in the application of these materials to produce improved medical devices and drug delivery vehicles.

2.10. Acknowledgements

The authors are grateful for the support of NSF projects #DMR-0312046 and DMR-0552661 for funding. The authors would also like to thank Ken McDaniel from Bayer for donation of the double metal cyanide catalyst and also for discussions regarding the polymerization of ethylene oxide and propylene oxide.

3. CHAPTER 3: Synthesis and Characterization of Amphiphilic Block Copolymer Dispersants Containing Carboxylate Binding Groups

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3.1. Abstract

Amphiphilic tri- and penta-block copolymers containing a polyurethane central block with pendant carboxylic acid groups flanked by hydroxyl functional polyether tails were synthesized. Our intention is to investigate the activities of these copolymers as dispersants for magnetite nanoparticles in biological media. Poly(ethylene oxide) (PEO), poly(propylene oxide) (PPO) and poly(ethylene oxide-*b*-propylene oxide) (poly(BzO-EO-*b*-PO-OH)) oligomeric tail blocks were prepared from a benzyl alkoxide initiator with varying lengths of PEO and PPO. The oligomers had a hydroxyl group at the terminal chain end and a benzyl-protected hydroxyl group at the initiated end. The polyether oligomers were incorporated into a block copolymer with a short polyurethane segment having approximately three carboxylic acid groups per chain. The block co-polyurethane was then hydrogenated to remove the benzyl group and yield primary hydroxyl functionality at the chain ends. End group analysis by ^1H NMR showed the targeted ratio of PEO to PPO demonstrating control over block copolymer composition. Molecular weights determined by both ^1H NMR and GPC were in agreement and close to targeted values demonstrating control over molecular weight. Titrations of the pentablock copolymers showed that the targeted value of approximately three carboxylic acid groups per chain was achieved.

3.2. Introduction

PEO is employed extensively in pharmaceutical and biomedical applications mainly due to its excellent biological and physical properties, including solubility in water and organic solvents, lack of toxicity, and absence of immunogenicity.^{4,9,10} PEO can be chemically modified and attached to other molecules or surfaces to improve the biological and physico-chemical properties of the substrates.^{6,113,151,152} The PEO oligomers are usually bound to other compounds through terminal functional groups.^{35,37}

PEO or PPO, or their block copolymers, are commonly synthesized by ring-opening polymerization of ethylene oxide initiated with an anionic initiator such as potassium hydroxide or alkoxide.¹⁵³ Such processes can be conducted without significant side reactions for PEO, but it is well established that the alkoxide propagating species promote side reactions leading to unsaturated chain ends for PPO^{83-85,154}. Coordinated double metal cyanide catalysts have been investigated extensively to avoid such side reactions in PPO polymerizations^{41,42}. The two methods that are commonly utilized for functionalizing PEO or PPO oligomers are alteration of the terminal hydroxyl group(s) through a series of reactions to yield a more reactive functional group, or polymerizations are conducted with heterobifunctional initiators so that one of the functional groups reacts with the epoxide and the other group remains intact.^{35,58,59,71,106}

This paper will discuss the synthesis of polyurethane dispersion stabilizers for magnetite nanoparticles utilizing the hydroxyl terminus of PEO or PPO initiated with benzyl alcohol and the subsequent removal of the benzyl group to yield primary hydroxyl functionality at the chain end. Block copolymers containing functional groups at specific positions along the backbone of the copolymer and the ability to control the number of functional groups present in the polymer have been reported previously.⁸ In our initial work, the triblock copolymers consisted of a polyurethane anchor block containing carboxylic acid functional groups flanked by PEO tail

blocks having a hydrophobic terminal methyl or tert-butyl group. The current work focuses on synthesizing dispersion stabilizers to tailor the surface hydrophilicity of the magnetic nanoparticles. In order to control the hydrophilicity of the particle surface, poly(ethylene oxide-*b*-propylene oxide) (poly(EO-*b*-PO)) oligomers with systematically varied compositions have been utilized to synthesize polyurethane dispersion stabilizers. In the current work, the pentablock copolymers contain hydroxyfunctional poly(EO-*b*-PO) dispersant tails. We anticipate that the terminal hydroxyl group on these nonionic stabilizers may be important for obtaining good dispersions, particularly in aqueous biological media where the ionic strengths are relatively high. Moreover, these materials have fairly short PEO end blocks, and it is reasoned that the nature of the terminal groups may thus affect the hydrophilic nature of these materials significantly.

3.3. Experimental

3.3.1. Materials

Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution was deep purple and then fractionally distilled just prior to use. Potassium (98%), benzyl alcohol (BzOH, anhydrous, 99.8%), ethylene oxide (EO, 99.5+%), propylene oxide (PO, $\geq 99\%$) were purchased from Aldrich and used as received. Naphthalide (Aldrich, 99%) was sublimed prior to use. Glacial acetic acid (Aldrich) was diluted with THF to yield a 2.5 M solution. Dimethylformamide (DMF, EMD Chemicals) was dried over CaH₂, fractionally distilled under vacuum and stored under nitrogen at 25 °C. The benzyl alcohol initiated poly(ethylene oxide-*b*-propylene oxide) (poly(BzO-EO-*b*-PO-OH)) oligomer was dried overnight at 60 °C under reduced pressure prior to incorporation into the pentablock copolymers. Isophorone diisocyanate (IPDI, Aldrich, 99.5%) was dried over CaH₂, fractionally distilled and stored under nitrogen. Bis(hydroxymethyl)propionic acid (BHMPA, Aldrich 98%) was dried

under vacuum at 60 °C for 24 h prior to use. Dibutyltin dilaurate (DBTDL, Aldrich 95%) catalyst was diluted to 10 mg mL⁻¹ in THF. Palladium, 10 wt. % on activated carbon, wet, Degussa type E101 NE/W (Aldrich) was used as received. A 300-mL high-pressure Series 4561 Parr reactor was utilized for the anionic polymerization of poly(BzO-EO-*b*-PO-OH) as well as for the catalytic hydrogenolysis of the benzyl ether protecting group. The ARCOL Impact 3 zinc hexacyanocobaltate catalyst utilized for the PPO homopolymer oligomers was kindly provided by Bayer, Inc., and was utilized as received.

3.3.2. Analysis

All ¹H 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. The samples were dissolved in either *d*-CHCl₃ or *d*₆-DMSO for obtaining the spectra. Relative molecular weights and molecular weight distributions of the polyether oligomers were measured by gel permeation chromatography (GPC) in HPLC grade THF 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 T60A 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 30-35 mg in 10 mL of HPLC grade THF.

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

Potassium naphthalide solution in THF was prepared by the method of N. D. Scott et al.¹⁵⁵ A representative procedure to prepare a 0.92 M solution of potassium naphthalide in THF is provided. Naphthalide (0.095 mol, 12.16 g) was weighed into a flame-dried flask equipped with a glass coated magnetic stir bar, sealed with a septum, and purged with nitrogen. THF (100

mL) was transferred to the flask via a glass syringe. Potassium (0.095 mol, 3.7 g) was cut, the packing oil was removed by blotting on a Kimwipe, and quickly added to the flask. The flask was resealed and purged with nitrogen. The reaction flask was covered with aluminum foil and the mixture was stirred for 24 h to form a dark green liquid. It was titrated against a standardized HCl solution to obtain the exact concentration.

3.3.4. Synthesis of a benzyl alcohol initiated poly(ethylene oxide-*b*-propylene oxide) copolymer (poly(BzO-EO-*b*-PO-OH))

A 2,100 M_n poly(BzO-EO-*b*-PO-OH) oligomer (75/25 wt/wt EO/PO) was prepared with benzyl alcohol as the initiator. EO (19.9 g, 0.452 mol) was charged from a lecture bottle into a 300-mL pressure reactor under vacuum that had been cooled with an isopropanol-dry ice bath. THF (60 mL) was added to the reactor via syringe. An initiator solution consisting of benzyl alcohol (1.47 mL, 14.2 mmol), THF (10 mL), and potassium naphthalide (1.92 mL of a 0.92 M solution, 1.77 mmol) was prepared in a separate flame-dried 100-mL roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The cooling bath was removed and the reactor was allowed to warm to room temperature. The polymerization reaction was heated to 70 °C and polymerized overnight. The reactor was cooled to room temperature and purged with nitrogen for one hour. PO (10.3 mL, 0.147 mol) was then added to the reactor via syringe, followed by 10 mL of THF. The reaction mixture was heated to 70 °C and stirred for 72 h. The reactor was allowed to cool to room temperature and purged with nitrogen for one hour. Acetic acid (5.68 mL of a 2.5 M solution in THF, 14.2 mmol) was added to terminate the reaction. The reactor was then opened and its contents were transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The dichloromethane solution was washed twice with 100 mL each of deionized water. The dichloromethane solution was

concentrated under vacuum at room temperature and precipitated in cold hexane to yield 24.4 g of product. Characterization via ^1H NMR showed a M_n of 2100 g mol^{-1} and 75/25 wt/wt EO/PO. GPC showed an M_w/M_n of 1.09. ^1H NMR (CDCl_3): 7.31 ppm (phenyl protons, 5H), 4.54 ppm ($\text{PhCH}_2\text{-O}$, 2H), 3.34-3.64 ppm ($-\text{CH}_2-$ and $-\text{CH}-$ on the PEO and PPO blocks), 1.09 ppm ($-\text{CH}_3$ on the PPO block).

3.3.5. *Synthesis of a benzyl alcohol initiated poly(propylene oxide) (poly(BzO-PO-OH))*

A 7,300 M_n poly(BzO-PO-OH) M_n homopolymer was prepared with benzyl alcohol as the initiator. Propylene oxide (30.0 g) was charged into a 300-mL pressure reactor under a 20 psi head of $\text{N}_2(\text{g})$ followed by 5 ml of THF. An initiator solution consisting of benzyl alcohol (0.47 mL, 4.54 mmol), THF (5 mL), and ARCOL (0.55 mL of a 4.17 mg/mL solution) was prepared in a separate flame-dried 100-mL roundbottom flask. The initiator solution was added to the reaction mixture via syringe followed by 5 ml of THF. The pressure reactor was heated to $90\text{ }^\circ\text{C}$. The reaction was allowed to continue until a decrease in pressure was no longer observed. The reactor was then allowed to cool to room temperature and purged with nitrogen for one hour. The reactor was then opened and its contents were transferred to a 250-mL round bottom flask. The solvent was removed under vacuum at room temperature to yield 22.0 g of product. Characterization via ^1H NMR showed a M_n of $7,300\text{ g mol}^{-1}$. GPC showed an M_w/M_n of 1.57. ^1H NMR (CDCl_3): 7.31 ppm (phenyl protons, 5H), 4.54 ppm ($\text{PhCH}_2\text{-O}$, 2H), 3.34-3.64 ppm ($-\text{CH}_2-$ and $-\text{CH}-$), 1.09 ppm ($-\text{CH}_3$).

3.3.6. Synthesis of Pentablock Copolymers Poly(BzO-EO-*b*-PO-*b*-urethane-*b*-PO-*b*-EO-OBz)

A polyurethane copolymer dispersion stabilizer with an average of three carboxylic acid groups in the central segment and 2100 g mol⁻¹ poly(BzO-EO-*b*-PO-OH) tail blocks was synthesized via a similar method to that previously described.⁸ The first reaction step involved capping monofunctional poly(BzO-EO-*b*-PO-OH) with IPDI. The dried poly(BzO-EO-*b*-PO-OH) (19.2 g, 9.14 mmol) was transferred to a flame-dried, three-neck, 250-mL, roundbottom flask equipped with a magnetic stirrer and purged with nitrogen. IPDI (4.06 g, 18.3 mmol) was syringed into the flask, and the flask was heated to 70 °C under a nitrogen atmosphere. DBTDL catalyst (2.5 mg) was added to the reaction flask via syringe. The melt reaction was monitored by Fourier transform infrared spectroscopy (FTIR) by observing the decrease of the isocyanate absorption peak at 2260 cm⁻¹. Once all of the poly(BzO-EO-*b*-PO-OH) was capped with IPDI, this material and the remaining IPDI were chain-extended by adding a solution of BHMPA (1.84 g, 13.7 mmol) dissolved in DMF (7.4 mL) and another increment of DBTDL catalyst (2.5 mg). The reaction was continued until the disappearance of the isocyanate peak was confirmed via FTIR. The copolymer was isolated by first removing most of the DMF under reduced pressure at 50 °C. The polymer was dissolved in 200 mL of dichloromethane, and the dichloromethane solution was washed twice with 100 mL each of deionized water. The copolymer was precipitated into an excess of cold hexane yielding 18.7 g of product. ¹H NMR (CDCl₃): 7.31 ppm (phenyl protons, 10H), 4.54 ppm (PhCH₂-O, 4H), 3.34-3.64 ppm (-CH₂- and -CH- on the PEO and PPO blocks), 1.09 ppm (-CH₃ on the PPO block).

3.3.7. Deprotection of the benzyl endgroups of a pentablock poly(BzO-EO-*b*-PO-*b*-urethane-*b*-PO-*b*-EO-OBz) copolymer

A poly(BzO-EO-*b*-PO-*b*-urethane-*b*-PO-*b*-EO-OBz) (15.6 g), 100 mL of 95% ethanol, and 0.75 g of catalyst (palladium, 10 wt % on activated carbon, wet Degussa type E101) were charged into the pressure reactor. The reactor was filled with 65 psi of hydrogen and stirred at 25 °C overnight. The reaction mixture was then filtered using Celite 521. The product was dried under vacuum at room temperature yielding 14.4 g of the dihydroxyfunctional pentablock copolymer. ¹H NMR (CDCl₃): 3.34-3.64 ppm (-CH₂- and -CH- on the PEO and PPO blocks), 1.09 ppm (-CH₃ on the PPO block).

3.4. Results and Discussion

The desire to investigate hydrophilic magnetite complexes and their behavior in aqueous media such as biological fluids and cell cultures has led to designing specific polyether stabilizers for magnetite nanoparticles. Carboxylic acid groups have been reported to chemisorb onto the magnetite surface via reaction with iron on the particle surface.^{156,157} The goal of this work was to prepare dispersion stabilizers with a central polyurethane anchor block that would bind to the surface of the magnetic nanoparticles, and with polyether tail blocks that would protrude out into the medium to stabilize the complexes in the dispersion (Figure 3.1). The polyether tail blocks are designed to sterically stabilize the magnetic nanoparticles, and also to possibly interact favorably with cell membranes depending on the composition of the polyether tail employed. The polyether stabilizers were designed specifically to retain the hydrophilic hydroxyl functionality at the chain end so that it might be later utilized for coupling reactions with bioactive molecules.

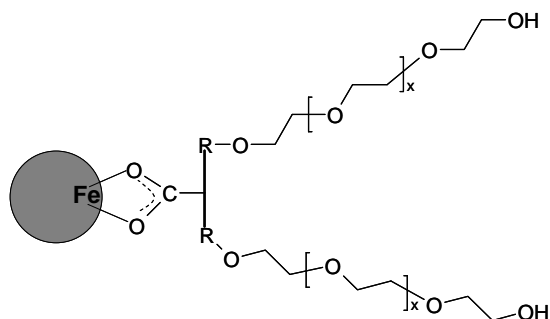


Figure 3.1. Central segment of the stabilizer anchors to the magnetite surface via the carboxylic acid groups while the polyether tail blocks extend into solution

3.4.1. *Synthesis of Benzyl Initiated Poly(EO-*b*-PO) Copolymers*

PEO and PPO are typically polymerized via anionic ring-opening polymerization techniques. These reactions proceed by nucleophilic attack of the anionic initiator on the epoxide ring of the monomer. Polyethers prepared in this manner are characterized by excellent control over the molecular weight and narrow molecular weight distributions. The living nature of this polymerization allows for the formation of well-defined block copolymers by sequential addition of the monomers to the reaction vessel. These living anionic polymerizations can result in functionality at the chain ends by incorporating heterobifunctional initiators and reactive terminating agents.^{35,37}

The synthesis of poly(EO-*b*-PO) copolymers was performed utilizing benzyl alcohol as the initiator which could later be removed by catalytic hydrogenation.¹⁵⁸ The advantage of using such an initiator is that it will allow for selectively reacting the terminal hydroxyl group that is naturally formed during the ring opening polymerization of epoxide monomer and leave the opposing chain end completely unaltered, effectively giving a protected hydroxyl group at one chain end and a labile hydroxyl group at the other end as shown in Figure 3.2.^{37,145}

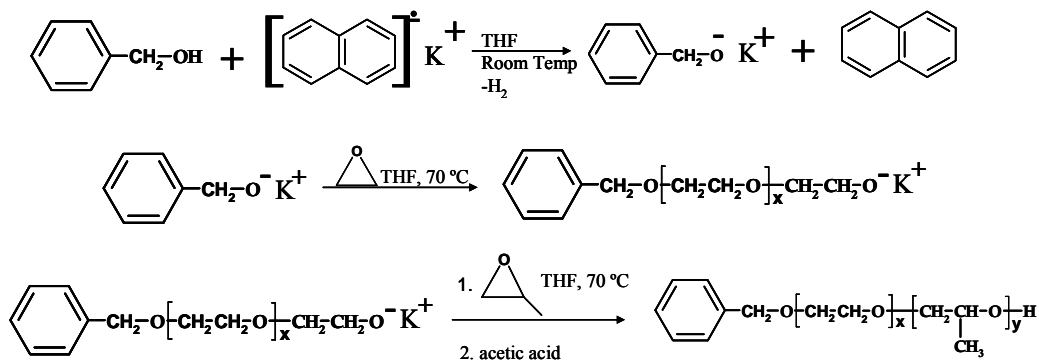


Figure 3.2. Synthesis of poly(BzO-EO-*b*-PO-OH)

These reactions were performed in a 300-mL high-pressure Parr reactor equipped with a pressure gauge so that the pressure decrease could be utilized to monitor the progress of the reaction. As the gaseous monomer was consumed and converted to polymer, the pressure in the reaction vessel decreases. When the pressure remains constant, the polymerization is terminated by addition of acetic acid. The resulting polymer is washed with water to remove any salt byproducts formed during the course of the reaction and precipitated in cold diethyl ether to remove naphthalide present from the initiation step.

These polymerizations were carried out with an 8:1 ratio of benzyl alcohol to potassium naphthalide. The polymerizations were living in nature because of the rapid proton transfer between chains relative to propagation leading to narrow molecular weight distributions. The living nature of the polymerization also allowed for the formation of block copolymers via sequential addition of monomers. Even under mild reaction conditions which require lengthy reaction times, the anionic chain end can abstract a proton from the PO monomer resulting in chain transfer and unsaturation at the chain end. For the PEO-PPO block copolymers discussed here, the unsaturation is insignificant due to the relatively small amount of PO utilized in these

reactions. For longer PPO chains, a double metal cyanide catalyst is employed to significantly decrease the amount of unsaturation in the PPO homopolymers.

End group analysis was performed via ^1H NMR to ensure that the proper molecular weight and copolymer composition could be controlled. Figure 3.3 depicts the ^1H NMR spectra obtained from the synthesis of a poly(BzO-EO-*b*-PO-OH) oligomer with a 75/25 wt/wt EO/PO composition. The ratio of end group protons closely matched the theoretical values calculated based on the targeted composition and M_n . In addition, GPC was utilized to examine the molecular weights and molecular weight distributions of these BzO-PEO-PPO-OH oligomers. The data presented in Table 3.1 indicates that the M_n 's achieved were close to their targeted values. The molecular weight distributions for these polymers were also narrow and monomodal with values approaching 1. This suggests that these polymerizations were well-controlled and living in nature.

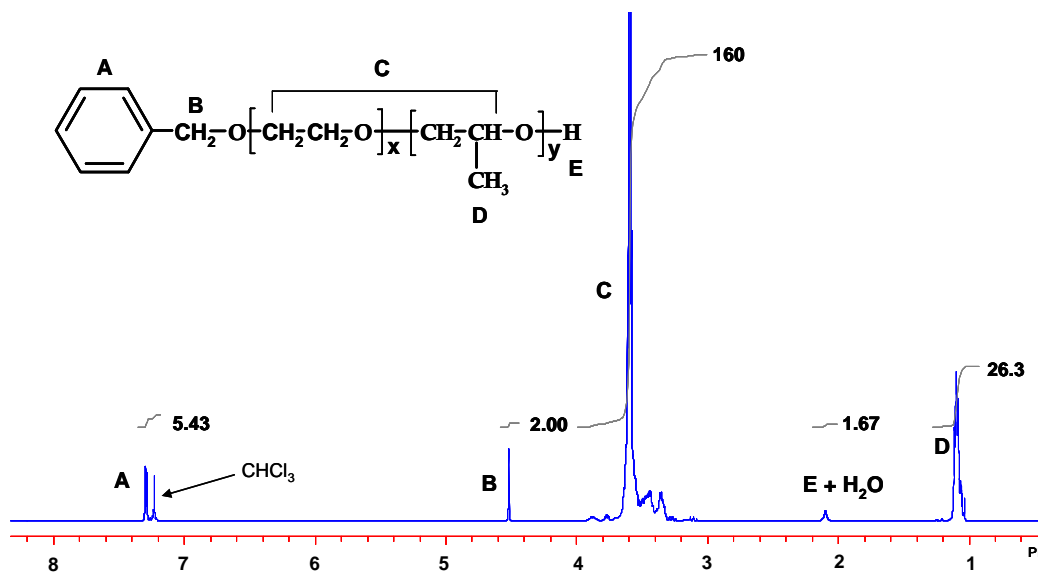


Figure 3.3. End group analysis was performed via ^1H NMR to obtain molecular weights and analyze copolymer composition

3.4.2. Synthesis of a benzyl alcohol initiated poly(propylene oxide) (poly(BzO-PO-OH))

Even under mild reaction conditions, the anionic polymerization of PO generally leads to some unsaturation at the chain end due to the abstraction of a methyl proton on the PO monomer converting it to allyl alkoxide.⁸³⁻⁸⁵ This abstraction reaction is generally referred to as a chain-transfer reaction and not only increases the unsaturation of the product but limits the degree of polymerization. Compared with conventional base-catalyzed polymerization of PO, double metal cyanide catalysts yield a high quality PPO with low amounts of unsaturation and much faster rates of polymerization even with low catalyst concentrations.^{41,86} Therefore, the double metal cyanide catalyst was utilized in order to retain the desired chain end groups that will be utilized during the synthesis of the dispersion stabilizers.

Polymerization reactions conducted utilizing the double metal cyanide catalyst showed broad molecular weight distributions as compared to the based catalyzed polymer but contained significantly less unsaturation as shown in Figure 3.4. The molecular weight of the polymers was consistent with the calculations based on the monomer to initiator ratio, not the monomer to catalyst ratio. The broadened molecular weight distribution is believed to be due to the relatively small amount of catalyst relative to initiator and the extremely fast rate of propagation relative to catalyst transfer between chains. The initiator to catalyst ratio was 10^3 or higher for these polymerizations, which means that it is not likely that all chain ends are permanently active during polymerization. At the beginning of the reaction there are relatively few active chains coordinated with the catalyst and a relatively high concentration of monomer, these conditions combined with a fast rate of propagation relative to chain transfer could lead to broadened molecular weight distribution for these batch type reactions. It is possible that the active chains grow rapidly at the start of the reaction until a significant amount of monomer is consumed so

that the rate of propagation slows and becomes closer to the rate of transfer, giving longer chains at the beginning of the reaction with shorter chains growing towards the end of the reaction.

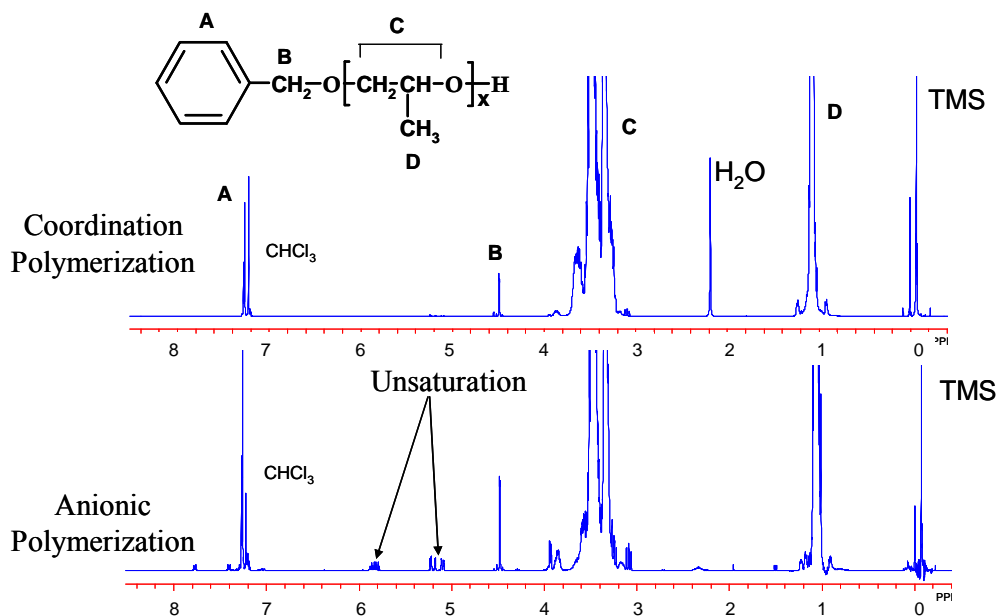


Figure 3.4. Base catalyzed polymerization of PO shows significantly more unsaturation than when the double metal cyanide catalyst it utilized

3.4.3. Synthesis of Pentablock Copolymers Poly(BzO-PEO-PPO-urethane-PPO-PEO-OBz)

Pentablock copolymers (poly(BzO-PEO-PPO-urethane-PPO-PEO-OBz)) were synthesized utilizing BzO-PEO-PPO-OH oligomers with varying weight percentages of PEO and PPO. This synthesis has been utilized previously to produce triblock copolymers having a central polyurethane block containing carboxylic acid groups and poly(ethylene oxide) tails.⁸ The synthesis was performed utilizing a two step process. First, the BzO-PEO-PPO-OH oligomer was capped using an excess of IPDI to guard against coupling of the monofunctional oligomers. Reaction progress was monitored by the decrease of the isocyanate (NCO) absorption via FTIR, as shown in Figure 3.5. Once half of the NCO had been reacted, the IPDI end capped oligomer along with the remaining IPDI was reacted with the hydroxyl functionality

of BHMPA to chain extend the polymer and provide approximately three carboxylic acid groups per polymer chain as shown in Figure 3.6. Under adequate conditions, isocyanates are known to react with carboxylic acids.¹⁵⁹ IPDI was chosen because of its relatively lower reactivity compared to aromatic isocyanates and mild reaction conditions were employed to safeguard against side reactions in order to retain the acid functionality of the BHMPA. The pentablock copolymers were titrated to determine the number of carboxylic acid groups present per chain. The values obtained from titration closely agreed with the targeted values of three carboxylic acid groups per chain, as shown in Table 3.1. The close agreement of the titration values with the theoretical number of carboxylic acid groups demonstrates that the urethane central block formed without significant side reactions involving the carboxylic acid groups.

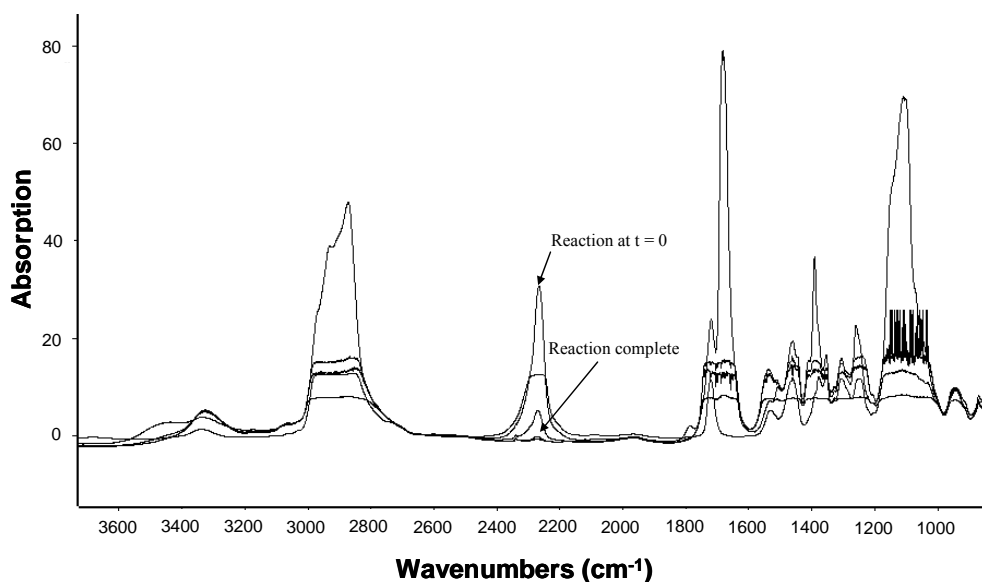


Figure 3.5. Monitoring the disappearance of the isocyanate absorption at 2260 cm^{-1} via FT-IR during the synthesis of pentablock copolymers poly(BzO-PEO-PPO-urethane-PPO-PEO-OBz)

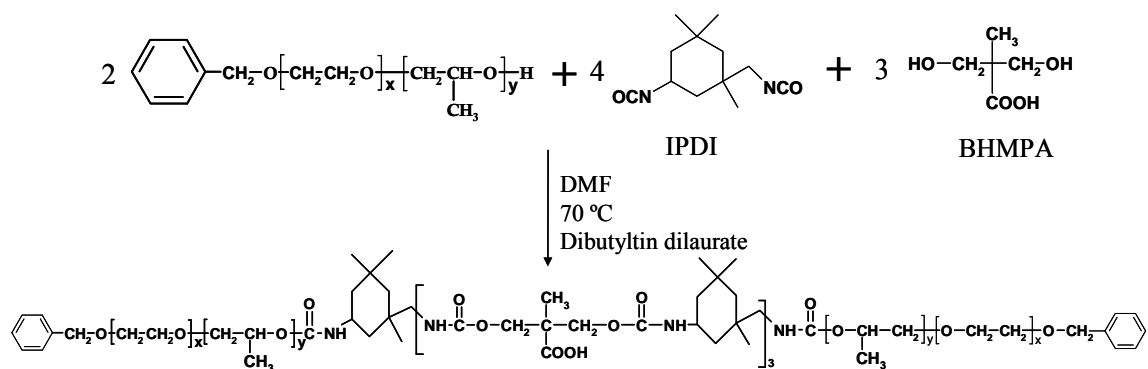


Figure 3.6. Synthesis of poly(BzO-EO-b-PO-b-urethane-b-PO-b-EO-OBz)

Table 3.1. Summary of the dispersion stabilizers

Oligomers					Dispersion Stabilizers
<u>Target Mn</u> (g/mol)	<u>M_n via GPC</u> (g/mol)	<u>M_n via ¹H NMR</u> (g/mol)	<u>EO/PO</u> (w/w %)	<u>PDI</u>	<u>COOH/mol</u>
5,000	4,200	5,300	100/00	1.05	3.03
4,000	4,500	4,200	79/21	1.07	3.01
2,000	2,500	2,100	75/25	1.09	3.07
2,000	2,600	2,200	63/37	1.08	3.26
2,000	2,600	2,100	46/54	1.10	3.15
3,300	2,900	2,900	0/100	1.91	3.07
6,600	5,700	7,300	0/100	1.57	2.80

3.4.4. Hydrogenolysis of Tri- and Penta-block Dispersants

Removal of the benzyl protecting group was performed via catalytic hydrogenolysis to yield a pentablock copolymer with terminal hydroxyl groups (HO-PEO-PPO-urethane-PPO-PEO-OH) as shown in Figure 3.7. This reaction has been utilized previously to synthesize various PEO derivatives.¹⁴⁵ The reaction was carried out in a 300 mL high-pressure Parr reactor under 65 psi of H_{2(g)} with 10 % palladium on activated carbon as the catalyst.

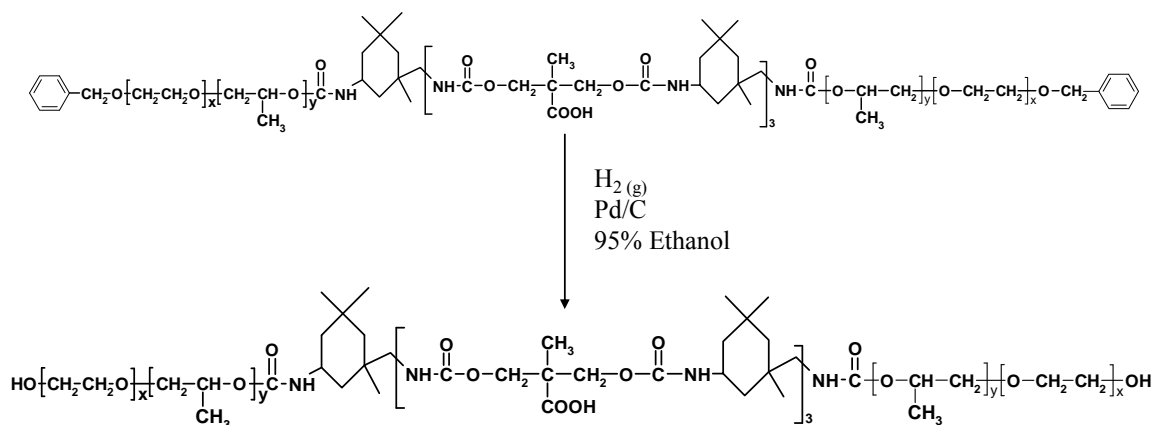


Figure 3.7. Deprotection of the benzyl endgroups via catalytic hydrogenolysis

The reaction progress was monitored via ^1H NMR by observing the disappearance of the benzyl proton resonances at approximately 7.3 ppm and the methylene proton resonances at approximately 4.5 ppm corresponding to the benzyl alcohol initiated chain end. Figure 3.8 shows a typical ^1H NMR of the benzyl and methylene proton resonances before and after catalytic hydrogenolysis. The complete disappearance of the proton resonances corresponding to the benzyl end group confirms quantitative deprotection of the chain end.

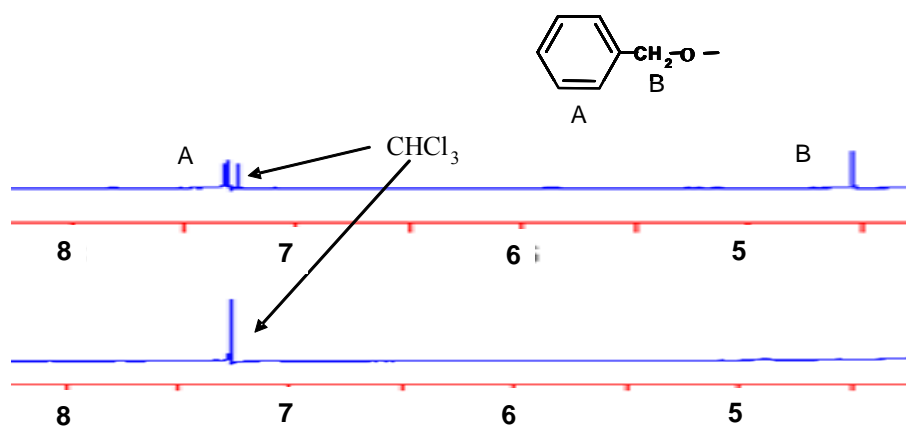


Figure 3.8. The benzyl protons are no longer evident in the ^1H NMR after catalytic hydrogenolysis

3.5. Conclusions

A series of pentablock poly(HO-PEO-PPO-urethane-PPO-PEO-OH) copolymers were synthesized with varying weight percentages of PEO and PPO and containing approximately three carboxylic acid groups per chain. It is anticipated that these carboxylic acid functional oligomers will complex to magnetite nanoparticles, and that these complexes will be dispersible in aqueous media. The ability of these surface modified magnetic nanoparticles to insert in the cell membranes will then be studied as a function of the percent of PEO and PPO in the pentablock poly(HO-PEO-PPO-urethane-PPO-PEO-OH) copolymers.

3.6. Acknowledgements

The authors are grateful for the support of NSF DMR-0312046 and DMR-0552661 for funding. The authors would also like to thank Ken McDaniel from Bayer for donation of the double metal cyanide catalyst and also for discussions regarding the polymerization of ethylene oxide and propylene oxide.

4. CHAPTER 4: Synthesis and Characterization of Heterobifunctional Poly(ethylene oxide) and Poly(ethylene oxide-*b*-propylene oxide)

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4.1. Abstract

Heterobifunctional poly(ethylene oxide) (PEO) and poly(ethylene oxide-*b*-propylene oxide) (PEO-*b*-PPO) copolymers were synthesized utilizing heterobifunctional initiators to yield polymers having a hydroxyl group at one chain end and additional moieties at the other chain end. For PEO homopolymers, these moieties include maleimide, vinylsilane, and carboxylic acid functional groups. Heterobifunctional PEO oligomers with a maleimide end group were synthesized utilizing a double metal cyanide coordination catalyst to avoid side reactions that occur with a basic catalyst. PEO oligomers with vinylsilane end groups were synthesized via alkoxide-initiated living ring-opening polymerization, and this produced polymers with narrow molecular weight distributions. Heterobifunctional PEO-*b*-PPO block copolymers were synthesized in two steps where the double metal cyanide catalyst was used to polymerize propylene oxide (PO) initiated by 3-hydroxypropyltrivinylsilane. The PPO was then utilized as a macroinitiator to polymerize ethylene oxide (EO) with base catalysis. Heterobifunctional PEO and PEO-*b*-PPO block copolymers possessing carboxylic acid functional groups on one end were synthesized by reacting the vinyl groups with mercaptoacetic acid via an ene-thiol addition.

4.2. Introduction

Poly(ethylene oxide) (PEO) is employed extensively in biomedical research and products mainly due to its excellent biological and physical properties, including solubility in water and organic solvents, lack of toxicity, and absence of immunogenicity.^{4,9,10} When PEO is bonded to biomolecules, it typically improves the solubility of the attached molecules in biological media.¹⁰ Since the backbone ether units of PEO are essentially non-reactive, these polymers are normally bound to other compounds through terminal functional groups.

Carboxylate anions have been widely utilized for adsorbing surfactants or polymers onto the surfaces of magnetite nanoparticles.^{8,87-89} Copolymer dispersion stabilizers have been synthesized that contain carboxylic acids at specific positions along the backbone of the copolymer, and with control over the number of functional groups in the polymer.^{8,54} Our laboratories previously reported magnetite complexes with triblock copolymers consisting of a polyurethane anchor block containing carboxylic acid functional groups flanked by PEO and poly(ethylene oxide-*b*-propylene oxide) (PEO-*b*-PPO) tail blocks.^{8,54} The copolymers were adsorbed onto the surfaces of magnetite nanoparticles, and the complexes formed stable dispersions in water. This paper introduces a simplified approach to synthesizing carboxylic acid containing PEO and PEO-*b*-PPO dispersion stabilizers that does not involve the urethane central block.

Maleimide-functional PEO has been of great interest for conjugating biomolecules bearing a mercapto moiety.^{15,128} Because of the high reactivity of the maleimide functionality, PEOs with terminal maleimide groups have been utilized as additives to modify bismaleimide resins and for the synthesis of block copolymers.¹³⁰⁻¹³² The maleimide reacts with mercaptans under mild conditions to yield a stable thioether linkage.¹³³ PEO oligomers with a maleimide group on one chain end and another functional group on the other end (maleimide-PEO-X) have

been used to prepare biosensors, functionalize surfaces for enzyme immobilization, and synthesize vehicles for targeted drug delivery.^{14,134-136}

Two methods that are commonly utilized for functionalizing PEO are (1) alteration of the terminal hydroxyl group through a series of reactions to yield a more reactive functional group, and (2) the polymerization of EO by heterobifunctional initiators so that one of the functional groups reacts with the monomer and the other group remains intact.³⁵ The synthetic methods for altering one hydroxyl group on the terminus of preformed PEO diols to yield heterobifunctional PEO oligomers are complicated because most employ several reaction steps and require column separations of statistical mixtures of oligomers.^{63,71} Thus, polymerization of EO utilizing a heterobifunctional initiator is the most direct route to heterobifunctional PEOs.

Ring-opening polymerization of EO from an initiator that has a protected functional group, and termination by alkylation or aqueous workup can be utilized to prepare heterobifunctional PEO.⁶¹⁻⁶⁴ The reaction conditions must be completely anhydrous to ensure the purity of the heterobifunctional product. If water is present in the reaction, then it will also initiate the EO monomer, producing α,ω -dihydroxy-poly(ethylene oxide) and reducing the anticipated molecular weight.⁶³ One example of a protected alkoxide initiator that has been used to polymerize EO is ethanolamine that has a protected amino group.⁶¹ A Schiff base was prepared from benzaldehyde and ethanolamine to prevent the primary amine from taking part in the polymerization. The polymer was acidified with hydrochloric acid to yield the α -amino- ω -hydroxy-poly(ethylene oxide).

An approach to synthesizing heterobifunctional PEO that avoids protection and deprotection reactions is to utilize a heterobifunctional initiator containing hydroxyl functionality and another functional group such as an allyl moiety that is unreactive during the polymerization

reaction. EO has been polymerized utilizing an allyl alcoholate initiator to yield α -hydroxy- ω -allyl-poly(ethylene oxide) with high yield (96%) and narrow molecular weight distribution (1.1).⁶³ An ene-thiol reaction was then utilized to convert the allyl terminus of α -hydroxy- ω -allyl-poly(ethylene oxide) into a primary amine by reaction with 2-aminoethanethiol hydrochloride (cysteamine hydrochloride). This synthetic approach is promising due to the ability to convert the allyl terminus into a wide range of functional groups, including carboxylic acid, through ene-thiol free radical addition.^{64,79,90,91,160-162}

In this paper we will expand upon previous work by utilizing heterobifunctional initiators to synthesize well-defined heterobifunctional polyethers. The polymerizations discussed herein will include methods for synthesizing heterobifunctional polyethers that possess three vinylsilane groups or a maleimide group at one chain end and a hydroxyl at the other end. Three carboxyl functionalities will be introduced onto the vinylsilane chain ends via ene-thiol addition to the vinyl groups to obtain dispersion stabilizers for magnetite nanoparticles in water. The heterobifunctional PEOs bearing maleimide functionality will be utilized as macroinitiators for synthesizing maleimide-functional biodegradable block copolymers such as poly(ethylene oxide-*b*-lactide). In a separate study, these maleimide-functional block copolymers will be investigated as a means to produce biocompatible surface-functional magnetic microspheres.

4.3. Experimental

4.3.1. Materials

Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution was deep purple and distilled just prior to use. Ethyl acetate (Fisher Scientific, 99.9%) was used as received. Potassium (98%), propylene oxide (PO, \geq 99%), maleimide (99%), furan (99%), ethanolamine (99%), mercaptoacetic acid (97%),

hexamethylphosphoramide (HMPA), sodium bicarbonate (99%), and 2,2'-azobis(2-methylpropionitrile) (AIBN, 98%) were purchased from Aldrich and used as received. Vinylmagnesium chloride (Aldrich, 1.6 M in THF) was used as received. 3-Chloropropyltrichlorosilane (Gelest, Inc.) was fractionally distilled prior to use. NaI (Aldrich) was dried under vacuum at 110 °C overnight. EO (Aldrich, 99.5 %) in a pressurized 227-g stainless steel lecture bottle was distilled directly into the Parr pressure reactor for the polymerizations. Naphthalide (Aldrich, 99%) was sublimed prior to use. Diethyl ether (EMD Chemicals) was used as received. Toluene (Burdick and Jackson, 99.9 %) was stirred over calcium hydride, distilled, and deoxygenated by sparging with nitrogen through the solution for 2 h prior to use. Glacial acetic acid (Aldrich) was diluted with THF to yield a 2.5 M solution. A 300-mL high-pressure Series 4561 Parr reactor was utilized for polymerizations of EO and PO monomers. The Impact 3 zinc hexacyanocobaltate catalyst was kindly provided by Bayer, Inc., and was utilized as received.

4.3.2. Analysis

All ^1H 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. The samples were dissolved in either *d*- CHCl_3 or *d*₆-DMSO for obtaining the spectra. Relative molecular weights and molecular weight distributions of the polyether oligomers were measured by gel permeation chromatography (GPC) in HPLC grade THF 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 T60A 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 30-35 mg in 10 mL of HPLC grade THF.

4.3.3. Preparation of a potassium naphthalide solution in THF

Potassium naphthalide solution in THF was prepared by the method of Scott et al.¹⁵⁵ A representative procedure to prepare a 0.92 M solution of potassium naphthalide in THF is provided. Naphthalide (0.095 mol, 12.16 g) was weighed into a flame-dried flask equipped with a glass-coated magnetic stir bar, sealed with a septum, and purged with nitrogen. THF (100 mL) was transferred to the flask via a glass syringe. Potassium (0.095 mol, 3.7 g) was quickly added to the flask after removing the packing oil by blotting with a Kimwipe. The flask was resealed and purged with nitrogen. The reaction flask was covered with aluminum foil and the mixture was stirred for 24 h to form a dark green liquid. It was titrated against a standardized HCl solution to obtain the exact concentration.

4.3.4. Synthesis of 3a, 4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione¹³⁸

Maleic anhydride (40.0 g, 0.41 mol) was added to a flame-dried 500-mL roundbottom flask under a nitrogen atmosphere that was equipped with a magnetic stir bar, and suspended in toluene (200 mL). Furan (30 mL, 0.41 mol) was added to the reaction flask via syringe and allowed to stir at room temperature. After 24 h, the white crystals were collected via vacuum filtration and washed with diethyl ether (3 x 50 mL).

4.3.5. Synthesis of 4,7-Epoxyisobenzofuran-1,3-dione-4,7-Epoxy-1H-isindole-1,3(2H)-dione¹³⁸

4,7,7a-Tetrahydro-4,7-epoxyisobenzofuran-1,3-dione (15.05 g, 90.5 mmol) was added under a nitrogen atmosphere to a flame-dried, three neck, 250-mL roundbottom flask equipped with a reflux condenser and a magnetic stir bar. Ethanolamine (5.53 g, 90.5 mmol) dissolved in methanol (30 mL) was quickly added to the reaction flask and the solution was stirred for 30 min

at room temperature, then refluxed for 24 h. The reaction mixture was cooled to room temperature and the product crystallized from solution. The reaction mixture was stored at 4 °C overnight. The crystals were collected via vacuum filtration and washed with methanol (3 x 20 mL).

4.3.6. Synthesis of *N*-(2-Hydroxyethyl)maleimide¹³⁸

7-Epoxyisobenzofuran-1,3-dione-4,7-epoxy-1H-isoindole-1,3(2H)-dione (5.44 g, 26.0 mmol) and toluene (50 mL) were added under a nitrogen atmosphere to a three neck, 250-mL, roundbottom flask equipped with a Dean Stark trap and a magnetic stir bar. The reaction mixture was heated to reflux 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.

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

An 8,600 M_n maleimide-PEO-OH homopolymer was prepared with *N*-(2-hydroxyethyl)maleimide as the initiator. EO (15.4 g) was charged into a 300-mL pressure reactor under a 20 psi head of $N_{2(g)}$. An initiator solution consisting of *N*-(2-hydroxyethyl)maleimide (0.36 g, 2.6 mmol), THF (5 mL), and the zinc hexacyanocobaltate (Impact 3) catalyst (0.92 mL of a 1.0 mg mL⁻¹ dispersion) was prepared in a separate flame-dried, 100-mL, roundbottom flask. The initiator solution was added to the reaction mixture via syringe followed by 5 mL of THF. The pressure reactor was heated to 90 °C, and the polymerization was conducted until a decrease in pressure was no longer observed (~2.5 h). The reactor was then allowed to cool to room temperature and purged with nitrogen for one hour. The reactor was opened and its contents were transferred to a 250-mL, roundbottom flask. The solvent was removed under vacuum at room temperature and the product was redissolved in a

minimal amount of dichloromethane (~20 mL). The polymer was precipitated into cold diethyl ether to yield 14.1 g of maleimide-PEO-OH.

4.3.8. Synthesis of 3-chloropropyltrivinylsilane

Vinylmagnesium chloride (0.14 mol, 88.47 mL of a 1.6 M vinylmagnesium chloride solution in THF) was transferred via cannula to a flame-dried, septum-sealed roundbottom flask equipped with a condenser and magnetic stir bar. 3-Chloropropyltrichlorosilane (0.047 mol, 0.14 eq chlorosilane, 7.36 mL, 10 g) was added via glass syringe in two equal aliquots to the reaction vessel. The reaction was conducted at 60 °C for 24 h. The product was concentrated by removing the THF via conventional distillation. The remaining reaction mixture was dissolved in dichloromethane (150 mL) and the salt by-products were removed by vacuum filtration. The product was vacuum distilled at 100 °C and 0.8 Torr.

4.3.9. Synthesis of 3-iodopropyltrivinylsilane

A two-fold excess of NaI (0.054 mol, 8.1 g) was charged to a roundbottom flask equipped with a magnetic stir bar. The reaction vessel was sealed with a septum and flame-dried under nitrogen. Acetone (25 mL) was added via syringe to the flask. 3-Chloropropyltrivinylsilane (0.027 mol, 5 g) was charged to the reaction vessel via syringe. The reaction was stirred at 60 °C for 48 h until 95% conversion was achieved as determined by ¹H NMR. The acetone was removed by rotary evaporation and the reaction mixture was dissolved in chloroform (150 mL). The excess NaI and the NaCl salt by-products were removed by vacuum filtration. The clear, colorless reaction product was vacuum distilled at 70 °C and 0.8 Torr.

4.3.10. Synthesis of 3-hydroxypropyltrivinylsilane

HMPA (10 mL) was added to a roundbottom flask equipped with a condenser and magnetic stir bar. Sodium bicarbonate (0.018 mol, 1.53 g) was added to the HMPA. One should note that the majority of the sodium bicarbonate remains undissolved. 3-Iodopropyltrivinylsilane (0.018 mol, 5 g) was added to the reaction vessel via syringe. Deionized water (0.17 mol, 3 mL) was added via syringe. The reaction was stirred at 100 °C for 24 h. The product was extracted twice with chloroform in a separatory funnel. The chloroform was removed by rotary evaporation and the clear, colorless reaction product was vacuum distilled at 90 °C and 0.8 Torr.

4.3.11. Synthesis of a PEO with a trivinylsilylpropoxy group at one end and a hydroxyl group at the other (trivinylsilane-PEO-OH)

A 2,800 M_n (trivinylsilane-PEO-OH) oligomer was prepared with 3-hydroxypropyltrivinylsilane as the initiator. EO (15.0 g, 0.341 mol) was charged from a lecture bottle into a 300-mL pressure reactor under vacuum that had been cooled with an isopropanol-dry ice bath followed by 30 psi of $N_{2(g)}$. An initiator solution consisting of 3-hydroxypropyltrivinylsilane (1.26 g, 7.5 mmol), THF (10 mL), and potassium naphthalide (7.5 mL of a 0.95 M solution, 7.1 mmol) was prepared in a separate flame-dried, 100-mL, roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The reactor was allowed to warm to room temperature and stirred overnight. The reaction was monitored by noting a drop in pressure from 30 to 20 psi. Acetic acid (3.0 mL of a 2.5 M solution in THF, 7.5 mmol) was added to terminate the reaction, then the reactor was purged with nitrogen for one hour. The reactor was then opened and its contents were transferred to a 250-mL, roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The dichloromethane solution was washed twice with 100 mL each of deionized water and concentrated under vacuum at room

temperature. The polymer was precipitated in cold diethyl ether to yield 13.5 g of trivinylsilane-PEO-OH.

4.3.12. Synthesis of a PPO with a trivinylsilylpropoxy group at one end and a hydroxyl group at the other end (trivinylsilane-PPO-OH)

A 5,600 M_n trivinylsilane-PPO-OH homopolymer was prepared with 3-hydroxypropyltrivinylsilane as the initiator. PO (20.0 g) was charged into a 300-mL pressure reactor under a 30 psi head of $N_{2(g)}$. An initiator solution consisting of 3-hydroxypropyltrivinylsilane (0.68 g, 4.0 mmol), THF (5 mL), and the zinc hexacyanocobaltate (Impact 3) catalyst (0.10 mL of a 2.0 mg mL⁻¹ solution) was prepared in a separate flame-dried, 100-mL, roundbottom flask. The initiator solution was added to the reaction mixture via syringe followed by 5 mL of THF. The pressure reactor was heated to 90 °C, and the reaction was continued until the pressure no longer decreased. The reactor was allowed to cool to room temperature and purged with nitrogen for one hour. The reactor was then opened and its contents were transferred to a 250-mL, roundbottom flask. The solvent was removed under vacuum at room temperature to yield 19.6 g of product. Characterization via ¹H NMR showed a M_n of 5,600 g mol⁻¹. GPC showed that the M_w/M_n was 1.91.

4.3.13. Synthesis of a PPO-b-PEO with a trivinylsilylpropoxy group at one end and a hydroxyl group at the other end (trivinylsilane-PPO-b-PEO-OH)

A 9,400 M_n trivinylsilane-PPO-*b*-PEO-OH was prepared with trivinylsilane-PPO-OH as the macroinitiator. EO (5.2 g, 0.12 mol) was charged from a lecture bottle into a 300-mL pressure reactor under vacuum that had been cooled with an isopropanol-dry ice bath, and this was followed by pressurizing the mixture with 30 psi of $N_{2(g)}$. An initiator solution consisting of a 5,600 g mol⁻¹ trivinylsilane-PPO-OH oligomer (4.96 g, 0.89 mmol), THF (10 mL), and potassium naphthalide (0.97 mL of a 0.87 M solution, 0.84 mmol) was prepared in a separate

flame-dried, 100-mL, round-bottom flask. The initiator solution was added to the stirring reaction mixture via syringe followed by an additional 5 mL of THF. The reactor was allowed to warm to room temperature and stirred for 48 h. The reaction was monitored by noting a drop in pressure. Acetic acid (0.35 mL of a 2.5 M solution in THF, 0.88 mmol) was added to terminate the reaction, then the mixture was purged with nitrogen for one hour. The reactor was then opened and its contents were transferred to a 250-mL, roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The dichloromethane solution was washed twice with 100 mL each of deionized water. The dichloromethane solution was concentrated under vacuum at room temperature and the polymer precipitated in cold diethyl ether to yield 7.0 g of trivinylsilane-PPO-*b*-PEO-OH.

4.3.14. Synthesis of polyethers with three carboxylic acids at one end and a hydroxyl group at the other end from trivinylsilane-PEO-OH and trivinylsilane-PPO-*b*-PEO-OH

A 2,800 g mol⁻¹ trivinylsilane-PEO-OH (2.0 g, 2.4 meq vinyl) was added to a clean, flame-dried, septum-sealed, nitrogen-purged flask equipped with a magnetic stir bar. Deoxygenated toluene (10 mL) was charged to the flask via syringe. AIBN (5.9 mg, 0.036 mmol, 0.015 mmol AIBN per 1 meq vinyl) was added to the reaction vessel. Mercaptoacetic acid (7.2 mmol, 0.50 mL) was added to the reaction flask via a syringe. The reaction was placed in an oil bath at 80 °C, sparged with nitrogen for 15 min, and stirred for 2 h. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The dichloromethane solution was washed twice with 100 mL each of deionized water. The dichloromethane solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether to yield 1.5 g of tricarboxyl-PEO-OH.

4.4. Results and Discussion

The high potential for nanoscale polymer therapeutics including polymer-drug and polymer-protein conjugates, polymeric micelles containing drugs, and polyelectrolyte complexes (so-called “polyplexes”) has been recognized, particularly over the past decade.^{13,163} The use of PEO oligomers bound to drugs and protein therapeutics has emerged as one of the most significant methods to increase protein solubility in biological media, to avoid rapid clearance by the RES system and thereby increase plasma circulation time, and to decrease or avoid immunogenicity. For example, PEO has been conjugated to proteins such as interferon- α -2a and interferon- α -2b for treating hepatitis C, and to recombinant methionyl human granulocyte colony stimulating factor for use as an adjunct to cancer therapy.^{12,13,163}

The desire to investigate hydrophilic magnetite complexes and their behavior in aqueous media has led to designing specific polyether stabilizers for magnetite nanoparticles. Currently, multiblock polyethers which consist of a polyurethane central segment containing carboxylate binding groups are under investigation as dispersants for magnetite in biological media (Figure 4.1B).⁵⁴ In an effort to simplify the structure of the dispersion stabilizers we have designed heterobifunctional polyethers. These polyether oligomers have carboxylic acid anchor units at one end while retaining the hydroxyl functionality at the other end (Figure 4.1A). This design is desirable so that further coupling reactions to bioactive molecules (utilizing the –OH) may be possible.

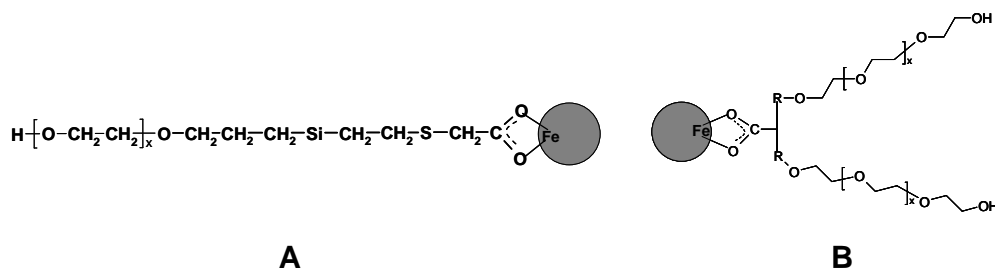


Figure 4.1. Heterobifunctional and multiblock dispersion stabilizers

Our first attempts to synthesize heterobifunctional PEO oligomers with three carboxylic acid groups on one chain end and a hydroxyl group at the other utilized triallylcarbinol for the polymerization of EO. It was reasoned that the allyl end groups could subsequently be converted to carboxylic acids by adding mercaptoacetic across the double bonds. Unfortunately, triallylcarbinol was found to be unsuitable for the ring opening anionic polymerization of EO due to degradation of the initiator once it was deprotonated.¹⁶⁴ Therefore, it was necessary to synthesize a heterobifunctional initiator, 3-hydroxypropyltrivinylsilane, for preparing the desired tricarboxylate-functional PEOs.

4.4.1. Synthesis of 3-hydroxypropyltrivinylsilane

Functional organosilanes have been prepared via a variety of synthetic methodologies. Chlorosilanes often serve as intermediates for the preparation of organosilanes. The chlorosilanes can be reacted with Grignard reagents or other organometallics yielding organic substitution at the silicon atom.^{165,166} The PEO initiators were prepared through a series of chemical modifications.

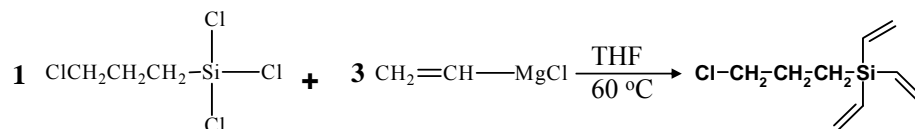


Figure 4.2. Preparation of 3-chloropropyltrivinylsilane

The first chemical transformation utilized conventional Grignard reactions. Vinylmagnesium chloride was reacted with chloropropyltrichlorosilane through nucleophilic substitution of the chlorines on the silicon (Figure 4.2). A stoichiometric amount of vinylmagnesium chloride to silyl chloride was charged to the reactions. Reactions with excess Grignard reagent lead to unwanted substitution at the 3-chloropropyl position. The exothermic nature of these reactions produced temperatures as high as 60 °C as the vinylmagnesium chloride

was charged. The reactions proceeded with immediate precipitation of MgCl_2 , but they were continued for 24 hours to ensure complete substitution of the silyl chlorides.

It was important to quantitatively remove all THF and to completely decompose any remaining Grignard reagent after the reactions were completed. The THF was removed by distillation, then the reaction mixture was exposed to air to decompose the excess Grignard reagent. Otherwise, it was observed that the THF partially polymerized, and this led to substantially decreased product yields.

The molecular structure of the chloropropyltrivinylsilane was characterized by ^1H NMR, by observing the chemical shifts of the propylene resonances as well as the peak integrals. Chloropropyltrivinylsilane has proton resonance integral ratios of 9:2:2:2 for the vinyl and methylene protons, respectively (Figure 4.3). The methylene adjacent to the silicon atom shifts to ~ 0.8 ppm after substitution of the vinyl group occurs.

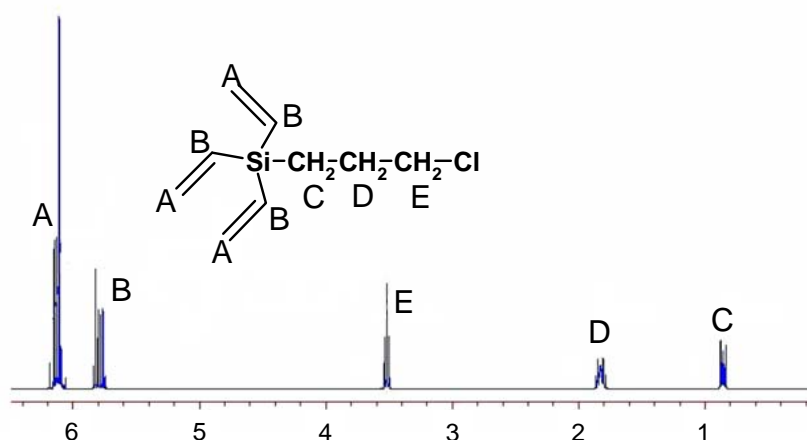


Figure 4.3. ^1H NMR of chloropropyltrivinylsilane

The alkyl chloride was transformed to an alkyl iodide via $\text{S}_{\text{N}}2$ nucleophilic substitution to produce a more active intermediate than the alkyl chloride.¹⁶⁷ This reaction is hindered by the nucleophilic strength of the chloride leaving group. However, the equilibrium is shifted toward

the alkyl iodide when the reaction is conducted in acetone, which is a solvent for sodium iodide but a non-solvent for the sodium chloride byproduct.¹⁶⁷ Figure 4.4A depicts the reaction conditions utilized in the conversion of chloropropyltrivinylsilane to the corresponding iodine containing compounds. Precipitation of NaCl was apparent within one hour. The reaction was monitored with ¹H NMR. A concurrent increase in the integral of the peak at 3.2 ppm corresponding to the methylene directly bonded to the iodine, and a decrease in the peak at 3.6 ppm corresponding to the methylene directly attached to the chlorine was observed. Figure 5 depicts a ¹H NMR spectrum of 3-iodopropyltrivinylsilane.

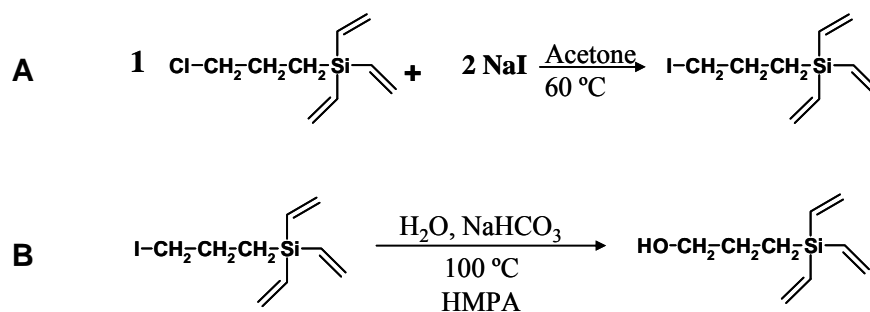


Figure 4.4. Synthesis of 3-iodopropyltrivinylsilane and 3-hydroxypropyltrivinylsilane

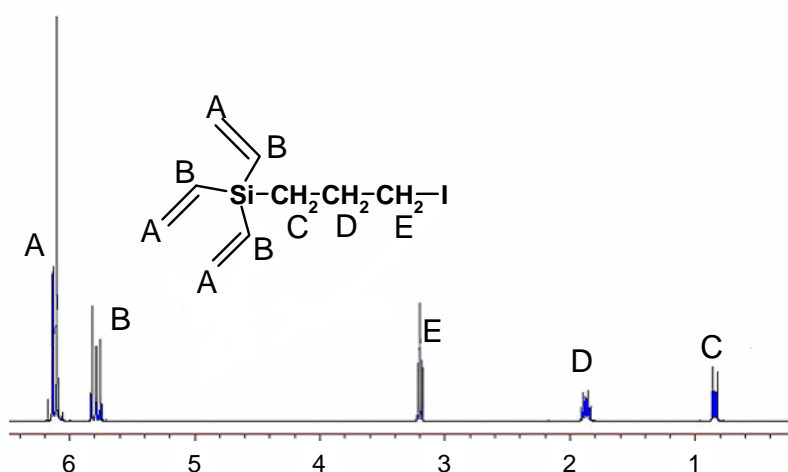


Figure 4.5. ¹H NMR of iodopropyltrivinylsilane

The 3-iodopropyltrivinylsilane was converted to the corresponding alcohol which was ultimately utilized to form the initiator for EO polymerizations. Approaches for transforming alkyl halides to alcohols have included phase transfer techniques and the use of polar aprotic solvents to enhance the rates of the reactions.¹⁶⁸⁻¹⁷⁰ Hutchins et al. probed the synthesis of alcohols from alkyl chlorides and iodides, and reported that combining a polar aprotic solvent such as NMP or HMPA with sodium bicarbonate served as a good source of nucleophilic oxygen. The research showed that 1-iodooctane was converted to the alcohol with a high yield (> 90%) in HMPA, whereas 1-chlorooctane only reached < 50% conversion under similar conditions. Moreover, the yield of alcohol from 1-chlorooctane was < 30% when the reaction was conducted in NMP.¹⁷¹

Figure 4.4B shows the synthetic schemes for the reaction of water with the 3-iodopropyltrivinylsilane. These reactions were conducted in HMPA at 100 °C. The sodium bicarbonate was added in a ratio of 1 mol iodine: 1 mol sodium bicarbonate. It is important to note that the sodium bicarbonate in these concentrations is not soluble in the reaction medium. However, the sodium bicarbonate solubilizes as the reaction proceeds.

Figure 4.6 shows a ¹H NMR of 3-hydroxypropyltrivinylsilane. The resonance of the methylene adjacent to the iodine in the starting material at 3.2 ppm shifts downfield to 3.6 after substitution. As in the case with the 3-chloro- and 3-iodopropyltrivinylsilane, the ratio of protons matched the theoretical proton ratios of 9:2:2:2 for the vinylsilane and methylene groups respectively.

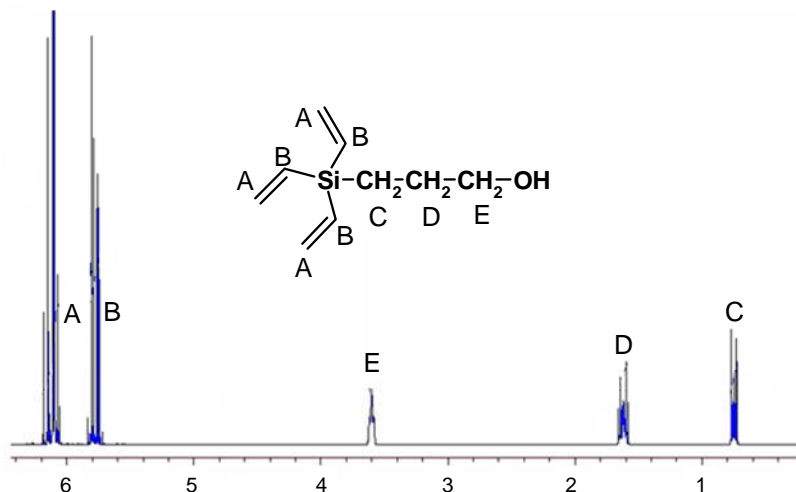


Figure 4.6. ¹H NMR of 3-hydroxypropyltrivinylsilane

4.4.2. Synthesis of *N*-(2-hydroxyethyl)maleimide

The synthesis of *N*-(2-hydroxyethyl)maleimide began with the Diels-Alder protection of the maleic anhydride double bond with furan (Figure 4.7).^{138,172,173} Figure 4.8 shows a ¹H NMR spectrum of the protected anhydride with the expected integral ratios of 2:2:2. The second step was the addition of ethanolamine to the anhydride under anhydrous conditions to minimize any side reactions that could occur in the presence of water. This reaction was carried out in a minimal amount of methanol to yield a pure product without the need for further purification steps. The product crystallizes from solution upon cooling while any unreacted reagents remain solubilized in the methanol. The structure of the second intermediate was also confirmed by ¹H NMR (Figure 4.9). The ratio of signals corresponding to B and C were shifted upfield and the ratio of A:B:C:D was 2:2:2:4, respectively.

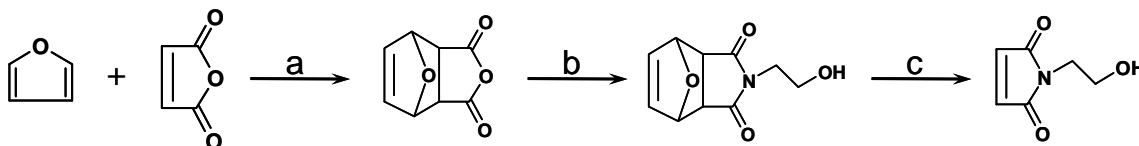


Figure 4.7. Synthesis of *N*-(2-hydroxyethyl)maleimide: a) toluene, RT; b) ethanolamine, MeOH, reflux; c) toluene, reflux

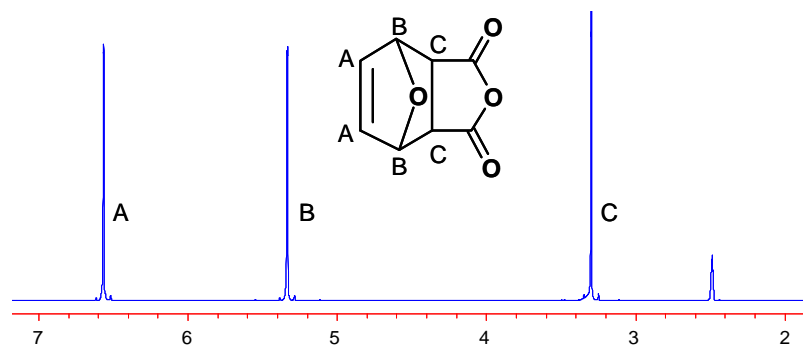


Figure 4.8. ^1H NMR of 4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione

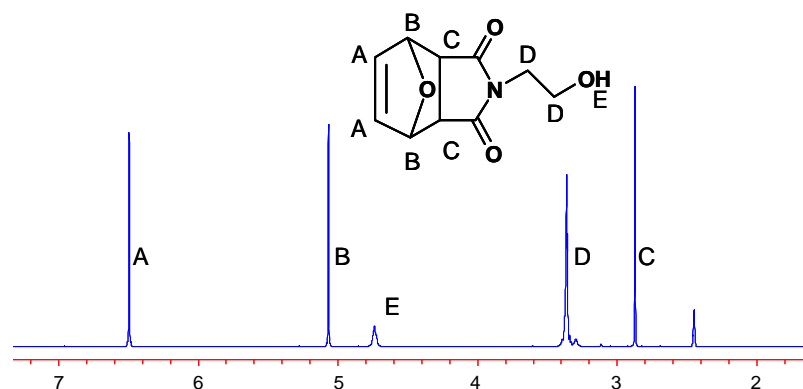


Figure 4.9. ^1H NMR of 4,7-epoxyisobenzofuran-1,3-dione-4,7-epoxy-1H-isoindole-1,3(2H)-dione

The final deprotection of the double bond was also carried out in a minimal amount of toluene to obtain the product with sufficiently high purity that further purification was not needed. The reaction was conducted with an outlet that allowed evolution of the furan byproduct. Whereas the protected alcohol is insoluble in toluene, the deprotected product dissolves as it forms. The reaction mixture was hot-filtered to remove any of the remaining protected starting material. The product crystallizes from solution upon cooling, yielding a product with sufficient purity to use as an initiator in the polymerization of EO. Analysis by ^1H NMR confirmed the structure of the final product (Figure 4.10) by observing the disappearance of signals corresponding to protons A and B and the shift of signals corresponding to protons C from 2.9 ppm to 6.95 ppm.

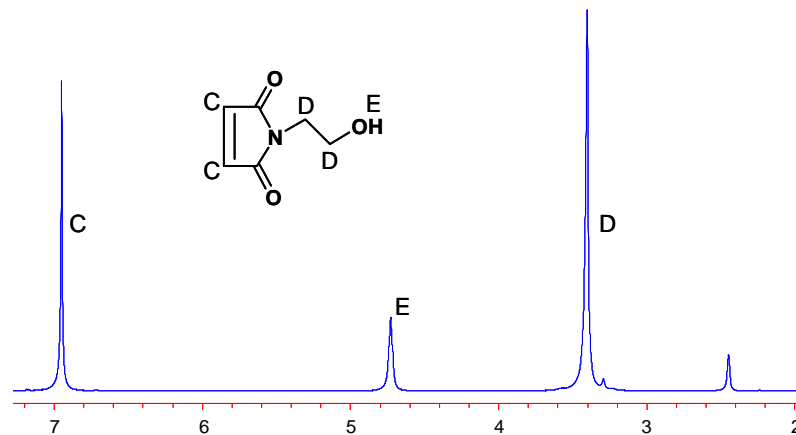


Figure 4.10. ^1H NMR of N-(2-hydroxyethyl)maleimide

4.4.3. Synthesis of polyethers utilizing a coordination catalyst: (vinyl)3-PPO-OH and maleimide-PEO-OH

Ring-opening polymerizations of EO are usually carried out with a basic initiator.¹³⁷ However, the sensitivity of N-(2-hydroxyethyl)maleimide to basic conditions required a different approach for polymerizing EO. Since non-basic double metal cyanide coordination catalysts (DMCs) have been utilized for polymerizing PO and EO/PO, it was reasoned that such an approach would be successful with the maleimide initiator.^{41,48,86}

Even under mild reaction conditions, the anionic polymerization of PO leads to some unsaturation at the chain end due to the abstraction of a methyl proton on the PO monomer converting it to allyl alkoxide.⁸³⁻⁸⁵ This abstraction reaction is generally referred to as a chain-transfer reaction and not only increases the unsaturation in the product, but also limits the degree of polymerization. Compared with conventional base-catalyzed polymerization of PO, DMC catalysts yield high quality PPO with low amounts of unsaturation and much faster rates of polymerization even with low catalyst concentrations.^{41,48} Therefore, the DMC catalyst was also utilized in the polymerization of PO to retain the desired chain end groups that will be utilized during the synthesis of the dispersion stabilizers.

Batch polymerizations conducted utilizing DMC catalysts have produced polymers with broader molecular weight distributions as compared to the base-catalyzed PPO polymers, but with essentially no unsaturation.^{41,48,86} As determined from ¹H NMR, the molecular weights of the polymers were consistent with the targeted values based on the monomer to initiator ratios, not the monomer to catalyst ratio (Table 4.1). Figure 4.11 shows that the maleimide end group is retained after the polymerization is complete. The absence of proton resonances corresponding to allyl endgroups in the ¹H NMR of trivinylsilane-PPO-OH indicate that the DMC catalyzed polymerization of PO takes place without any significant side reactions (Figure 4.12).

The broadened molecular weight distribution is believed to be due to the relatively small amount of catalyst relative to initiator, combined with the extremely fast rate of propagation relative to catalyst transfer between chains. The initiator to catalyst ratio was 10³ or higher for these polymerizations, which means that it is not likely that all chain ends are permanently active during polymerization. At the beginning of the reaction there are relatively few active chains coordinated with the catalyst and a relatively high concentration of monomer, these conditions combined with a fast rate of propagation relative to chain transfer could lead to broadened molecular weight distributions for these batch reactions (Figures 4.13 and 4.14).

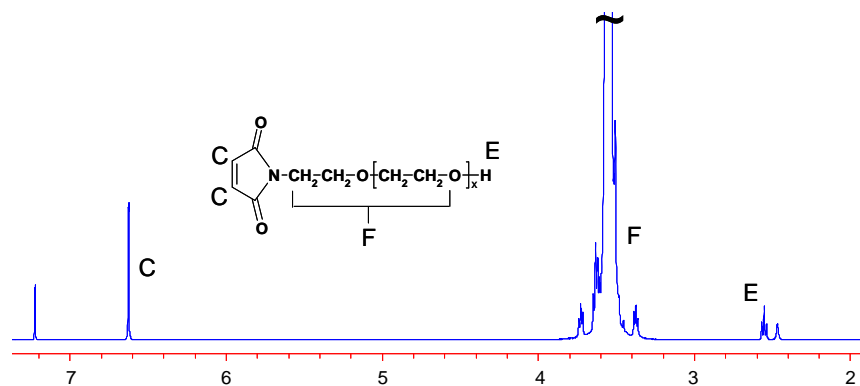


Figure 4.11. ¹H NMR of maleimide-PEO-OH

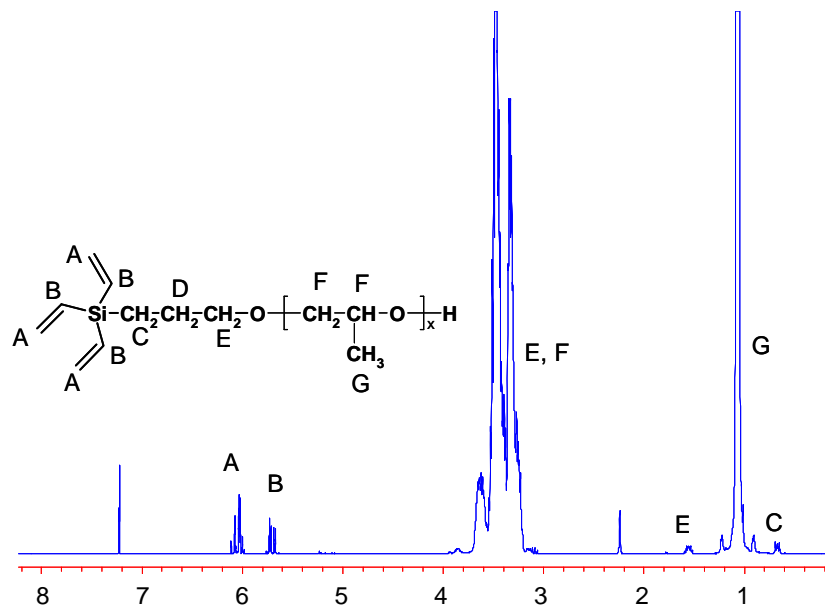


Figure 4.12. ^1H NMR of trivinylsilane-PPO-OH

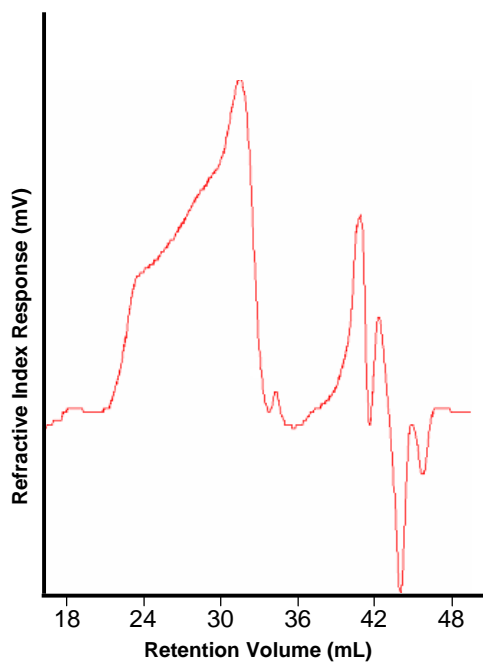


Figure 4.13. Representative GPC of maleimide-PEO-OH

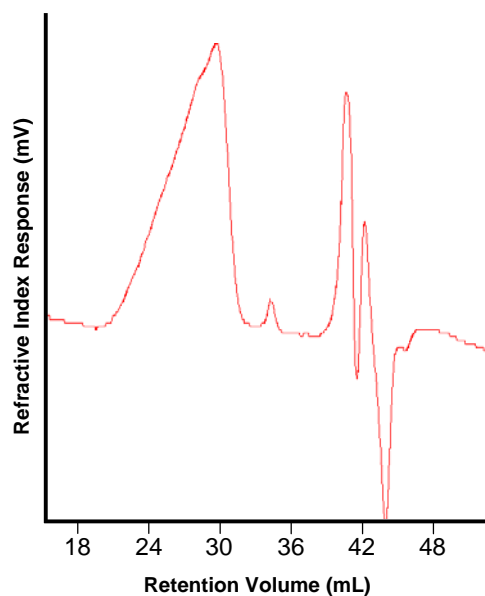


Figure 4.14. Representative GPC of trivinylsilane-PPO-OH

Table 4.1. Maleimide-PEO-OH and trivinylsilane-PPO-OH synthesized using a DMC catalyst

<u>Polymer</u>	<u>Target</u> M_n^a (g mol^{-1})	M_n^b (g mol^{-1})	M_n^c (g mol^{-1})	<u>PDI</u>^c
maleimide-PEO-OH	2,800	3,500	3,200	3.22
maleimide-PEO-OH	5,000	6,600	3,800	3.28
trivinylsilane-PPO-OH	3,000	3,300	3,400	1.84
trivinylsilane-PPO-OH	5,000	5,600	7,100	1.92
trivinylsilane-PPO-OH	7,500	8,700	8,300	1.91

^aCalculated from monomer to initiator ratio. ^bCalculated from ¹H NMR. ^cDetermined via GPC.

4.4.4. Synthesis of polyethers with trivinylsilane-PEO-OH and trivinylsilane-PPO-b-PEO-OH

PEO is typically polymerized via anionic ring-opening polymerization.¹³⁷ These reactions proceed by nucleophilic attack of the anionic initiator on the EO carbons. Initiators

include hydroxides, alkoxides, oxides, and metal alkyls/aryls such as potassium naphthalide.¹³⁷ Such polymerizations are living in nature, and are typically characterized by excellent control over the molecular weight and narrow molecular weight distributions. In this particular case, the PEO and PPO-*b*-PEO block copolymers are heterobifunctional with three vinyl groups at one terminus derived from the 3-hydroxypropyltrivinylsilane initiator and a hydroxyl terminus derived from the terminal repeat unit of EO after neutralization (Figure 4.15).

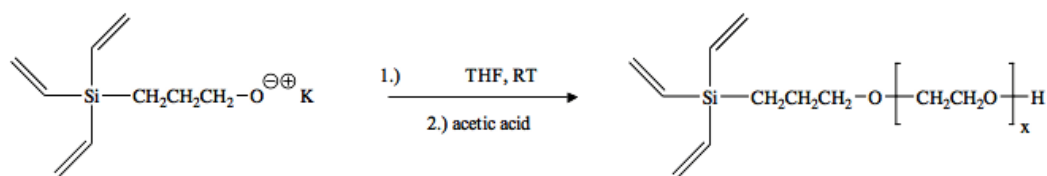


Figure 4.15. Ring-opening polymerization of EO utilizing 3-alkoxypropyltrivinylsilane as the initiator

The polymerizations were conducted under 30 psi of EO/nitrogen pressure at room temperature. As the reactions proceeded, the pressure dropped to approximately 20 psi, indicating that nearly all of the EO had polymerized. The PEO was terminated with acetic acid, then extracted with water to remove the potassium acetate byproduct.

The first step in the polymerization of EO was the reaction of 3-hydroxypropyltrivinylsilane with potassium naphthalide in THF to yield the anionic initiator. The polymerizations were conducted utilizing a slight deficiency of potassium naphthalide to –OH in preparing the initiator solution. These conditions were chosen to avoid loss of any vinyl groups during the alkoxide formation and during the polymerization. It was observed that 1 mol initiator: 0.90 mol base functioned well in preserving the vinyl moieties. Chain transfer reactions between the propagating alkoxides made it possible to utilize less than stoichiometric concentrations of base during polymerization while still maintaining living conditions.

End group analysis was performed via ^1H NMR to ensure that the end groups remained intact during the polymerization and that the desired molecular weights could be targeted and controlled. Figures 4.16 and 4.17 depict the ^1H NMR spectra obtained from the trivinylsilane-PEO-OH and trivinylsilane-PPO-*b*-PEO-OH, respectively. The ratio of signals corresponding to the 3-hydroxypropyltrivinylsilane end group protons matched the theoretical values with ratios of 9:2:2 for the vinyl and methylene protons, respectively. The methylene protons from the initiator adjacent to the hydroxyl overlap with the methylene protons on the polymer backbone.

Utilizing the end group resonances, number average molecular weight was also calculated for each block. These values correspond well with the targeted molecular weights (Table 4.2). In addition, GPC was utilized to examine the molecular weights and molecular weight distributions of these PEO oligomers. The data presented in Table 4.2 indicates that the M_n 's achieved were close to their targeted values. The molecular weight distributions for the PEO homopolymers were narrow and monomodal with values approaching 1 (Figure 4.18). Analysis of the trivinylsilane-PPO-*b*-PEO-OH via GPC showed that the molecular weight distribution became narrower after polymerization of the PEO block (Table 4.2). The high molecular weight shoulder observed in the GPC trace in figure 4.19 is believed to be due to the broad distribution of the macroinitiator. This suggests that these polymerizations were well-controlled.

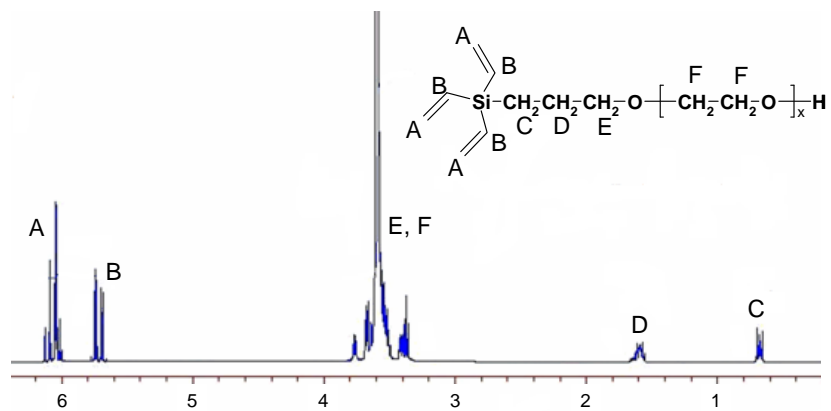


Figure 4.16. ^1H NMR of a trivinylsilane-PEO-OH

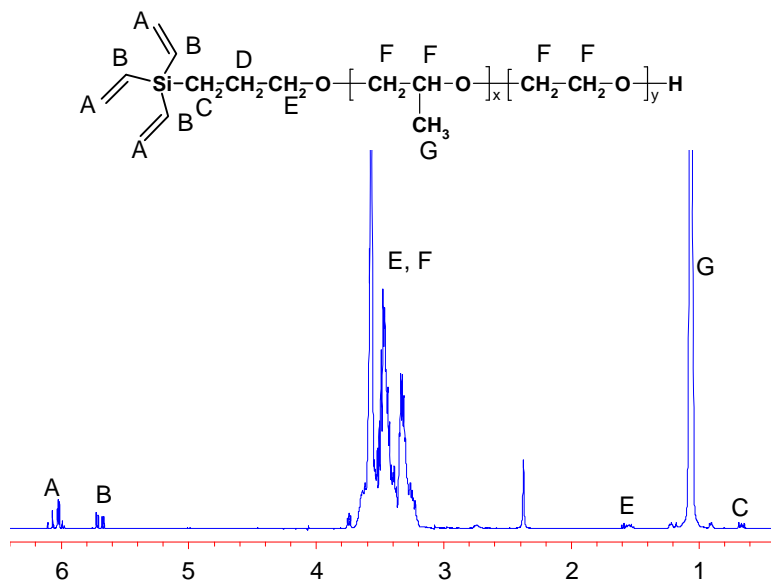


Figure 4.17. ^1H NMR of a trivinylsilane-PPO-b-PEO-OH

Table 4.2. A summary of molecular weights and molecular weight distributions for the trivinylsilane-PEO series

<u>Polymer</u>	<u>Target M_n^a</u> (<u>$g\ mol^{-1}$</u>)	<u>M_n PPO^b</u> (<u>$g\ mol^{-1}$</u>)	<u>M_n PEO^b</u> (<u>$g\ mol^{-1}$</u>)	<u>M_n^c</u> (<u>$g\ mol^{-1}$</u>)	<u>PDI^c</u>
(vinyl) ₃ -PEO-OH	3000	0	2800	2800	1.13
(vinyl) ₃ -PEO-OH	5000	0	5300	4600	1.12
(vinyl) ₃ -PEO-OH	10,000	0	14,000	14,200	1.13
(vinyl) ₃ -PPO- <i>b</i> -PEO-OH	3,000-3,300	3,300	2,600	3,800	1.39
(vinyl) ₃ -PPO- <i>b</i> -PEO-OH	3,000-6,600	3,300	4,800	4,100	1.30
(vinyl) ₃ -PPO- <i>b</i> -PEO-OH	5,000-5,600	5,600	3,800	7,800	1.88
(vinyl) ₃ -PPO- <i>b</i> -PEO-OH	5,000-11,200	5,600	7,200	8,300	1.63

^aCalculated from monomer to initiator ratio. ^bCalculated from ¹H NMR. ^cDetermined via GPC

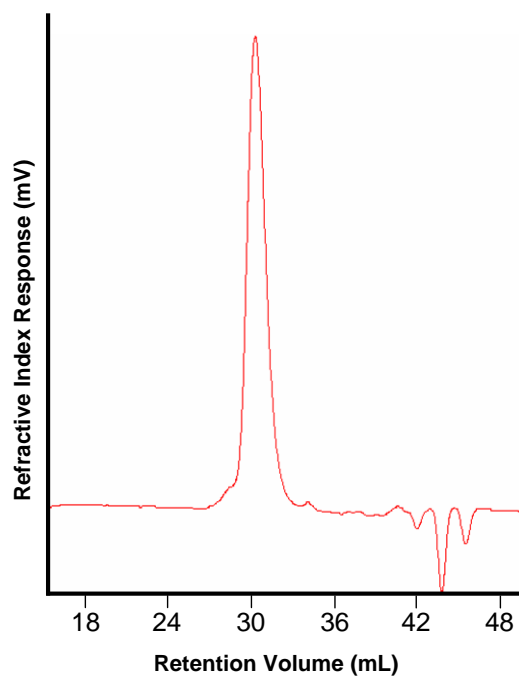


Figure 4.18. GPC trace of a trivinylsilane-PEO-OH

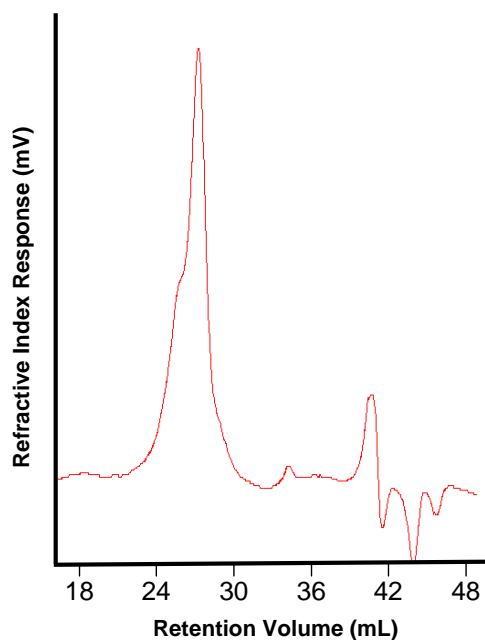


Figure 4.19. GPC trace of a trivinylsilane-PPO-*b*-PEO-OH

4.4.5. Synthesis of polyethers with three carboxylic acids at one end and a hydroxyl group at the other end from trivinylsilane-PEO-OH and trivinylsilane-PPO-*b*-PEO-OH

The series of trivinylsilane-PEO-OH and trivinylsilane-PPO-*b*-PEO-OH were chemically converted to carboxylic acids utilizing the ene-thiol addition of mercaptoacetic acid across the vinyl groups under free radical conditions. The ene-thiol reaction has been utilized previously by Chojnowski et al. to convert pendent vinyl moieties on a poly(dimethylsiloxane-*b*-methylvinylsiloxane) copolymer to carboxylic acid groups.¹⁷⁴ In addition, Wilson et al. showed that terminal trivinylsilyl groups on PDMS may be converted in a similar manner with good control over the chemistry.⁸¹

In the research presented here, carboxylic acids were introduced at one chain end utilizing AIBN as the free radical initiator (Figure 4.20). These reactions were conducted at 80 °C in toluene. It is important that the reaction system is deoxygenated prior to proceeding to ensure that oxygen does not inhibit the free radical processes.

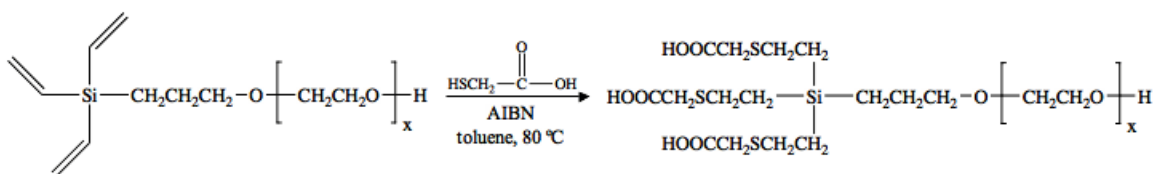


Figure 4.20. Functionalization of the terminal vinylsilyl groups utilizing the ene-thiol addition reaction

The ene-thiol reactions were monitored via ^1H NMR by following the disappearance of vinyl proton resonances at approximately 6.0 ppm. Figure 4.21 shows a representative ^1H NMR of a silane-PEO-OH before and after reaction with mercaptoacetic acid. There is an absence of vinyl peaks at 6.0 ppm and the appearance of new peaks corresponding to the methylenes A and B. These resonances correspond with previously reported values by Wilson et al. for tricarboxylic acid terminated PDMS.⁸¹

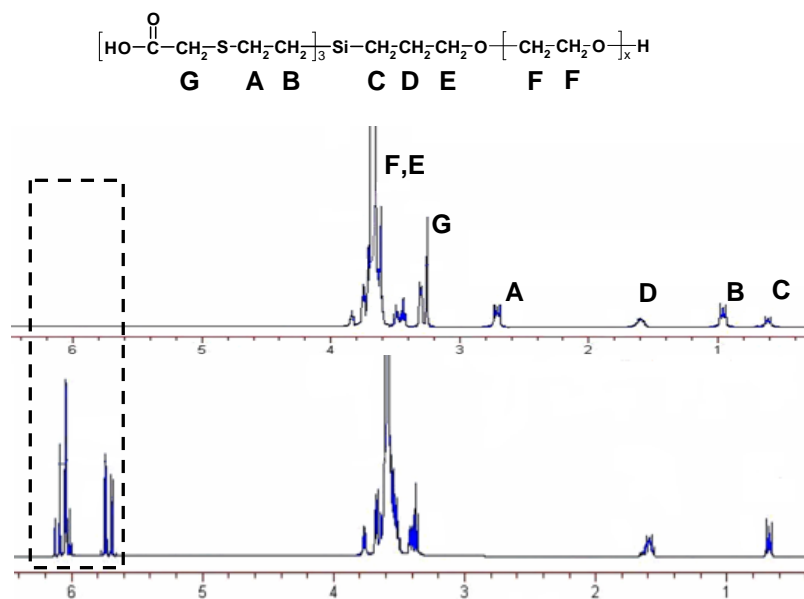


Figure 4.21. ^1H NMR reveals quantitative functionalization as evidenced by the disappearance of the vinyl resonances at 6.0 ppm and the appearance of peaks at 2.8 and 0.98 ppm

4.5. Conclusions

A series of trivinylsilane-PEO-OH, trivinylsilane-PPO-OH, and diblock trivinylsilane-PPO-*b*-PEO-OH copolymers were synthesized with varying compositions of PEO and PPO, and containing approximately three carboxylic acid groups per chain. These carboxylate-functional oligomers complex well to magnetite nanoparticles and the complexes produce stable aqueous dispersions. The ability of these surface modified magnetic nanoparticles to insert into lipid membranes will be studied as a function of the relative compositions and block lengths of PEO and PPO in the block copolymers.

Heterobifunctional PEOs with a maleimide functionality on one chain end and a hydroxyl group at the other chain end were directly synthesized from a heterobifunctional initiator utilizing a DMC catalyst. These polymers can serve as macroinitiators for the polymerization of D,L-lactide, which can be utilized to produce surface functionalized magnetic microspheres.

4.6. Acknowledgements

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5. CHAPTER 5: Recommendations for Future Work

It would be advantageous to produce microspheres with surface properties that are suitable for intravenous administration and site-specific drug delivery. These should have a biodegradable core to entrap bioactive materials. Future research is needed to produce microspheres possessing a unique surface chemistry by utilizing biodegradable/biocompatible block copolymers (Figure 5.1). It has been shown that microspheres and films formed utilizing poly(ethylene oxide-*b*-L-lactide) surface segregate, yielding a poly(ethylene oxide) modified surface.¹⁷⁵⁻¹⁸⁰ Nguyen *et al.* determined that when microspheres were formed from mixtures of poly(D,L-lactide) and poly(ethylene oxide-*b*-D,L-lactide) a protective effect was obtained and the interaction of the particles with the mononuclear phagocyte system significantly decreased.¹⁷⁷ It is believed that the hydroxyl terminus of the heterobifunctional polyethers discussed in chapter 4 could be utilized to form poly(ethylene oxide-*b*-D,L-lactide) copolymers while the maleimide or carboxylic acid functional terminus could serve to bind biologically active molecules. The hypothesis is that by combining these two phenomena that microspheres can be formed that will possess a core of biodegradable poly(D,L-lactide) and a hydrophilic corona of poly(ethylene oxide) with a terminal functionality that would allow the incorporation of ligands at the surface with controllable densities. The utilization of flexible poly(ethylene oxide) spacers for ligand coupling could also be advantageous by allowing maximum accessibility for the ligand to interact with its target.

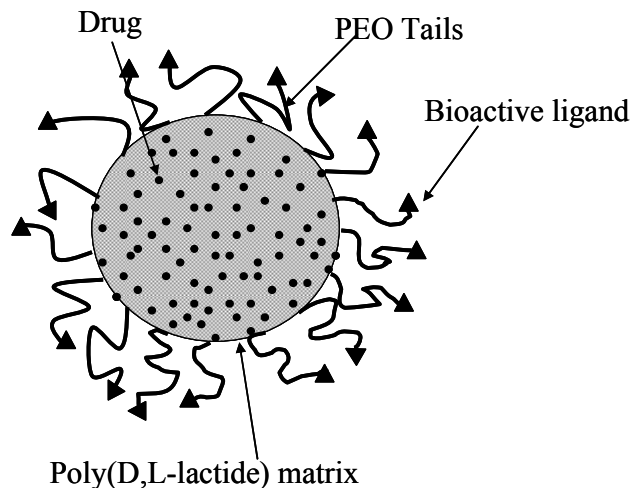


Figure 5.1. Surface functionalized microspheres that can be utilized for site-specific drug delivery

The motivation for the proposed research is to create surface functionalized microspheres that can be utilized for site-specific drug delivery. Biologically active materials could be entrapped in the microspheres and delivered to a specific site in the body by tailoring the ligand on the surface of the microspheres. Another important potential application of the surface functionalized microspheres is the separation of target molecules from complex biological media. For example, the surfaces of the microspheres could be utilized to bind and remove target molecules from the blood stream. Once separated from the matrix, the target molecules could possibly be analyzed or discarded depending on the specific application.

Questions that will need to be answered by the proposed research will be (1) whether surface functionalized microspheres can be prepared from poly(ethylene oxide-*b*-D,L-lactide) block copolymers possessing terminal biologically active molecules, (2) whether or not the density of the ligands on the surface of the microsphere can be controlled, and (3) if the functional microspheres can bind to target molecules. The length of the poly(ethylene oxide) spacer and its effects on the efficiency of the functionalized microspheres to bind with target

molecules will also need to be studied. Another interesting aspect that should be investigated is the ability to form microspheres with a combination of ligands bound to the surface of the microspheres, and to understand what advantages this might offer for the intended biotechnological applications.

The previous chapters have discussed various methods for producing amphiphilic dispersion stabilizers that can adsorb to the surfaces of magnetite nanoparticles. The design and synthesis of these dispersion stabilizers is only the first step in preparing surface functional magnetic nanoparticles. The lengths of the dispersion stabilizers as well as the percentage of the hydrophilic and hydrophobic components need to be investigated to better understand how the hydrophilic/hydrophobic surface properties interact with cell walls. It is also yet to be seen how varying these characteristics can affect the ability of these surface coated magnetic particles to act as MRI contrast agents.

Chapter 4 discussed the synthesis of heterobifunctional polyethers containing a hydroxyl group at one chain end and either vinylsilane or carboxylic acid groups at the other chain end. These polymers lend themselves to preparing more complex surface functionalized magnetic particles where a bioactive molecule could be tethered to the magnetic particle via a polyether spacer (Figure 5.2). These materials could open up a wide range of applications for these nanoparticles. The functional groups tethered to the surfaces of the magnetic particles could be utilized to bind molecules of interest in complex solutions for magnetic assay devices or for improved separations. The functional groups could serve as targeting vectors to accumulate the particles in a specific area of the body to improve MRI contrast. The incorporation of more than one type of bioactive molecule tethered to the surface may also lead to further applications of these materials.

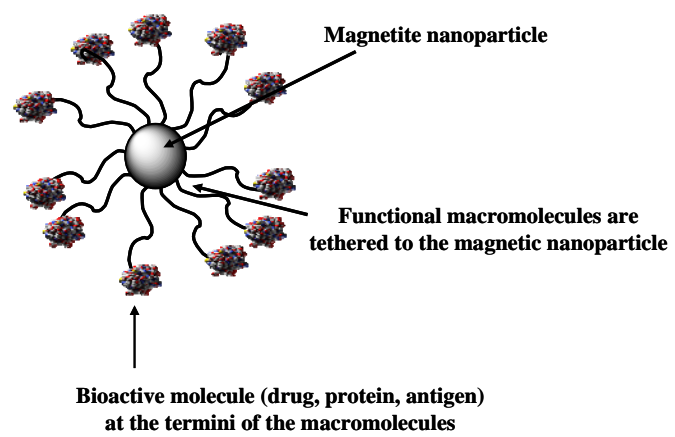


Figure 5.2. Biologically active ligands tethered to the surface of a magnetic nanoparticle

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