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Feature Article

Synthesis and applications of heterobifunctional poly(ethylene oxide) oligomers

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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. PEO can be chemically modified and reacted with, or adsorbed onto, other molecules and surfaces. 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 applications of heterobifunctional PEO oligomers possessing amine, carboxylate, thiol, and maleimide functional groups.
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1. Introduction

Heterobifunctional PEOs have the structure X–PEO–Y, where X and Y are different functional groups. They can serve as hydrophilic, flexible, biocompatible, and 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 [1–15]. When bounded to other molecules, PEO typically increases their solubility in aqueous media and yields improved circulation times in vivo [16–26]. PEO-modified surfaces also display increased hydrophilicity as well as suppressed protein adsorption, platelet adhesion and macrophage attachment [27–31].

There have been several reviews discussing PEO chemistry, most of which have focused on monofunctional PEO, with only limited treatment of heterobifunctional polymers [32–36]. This review concentrates on synthetic pathways to achieve heterobifunctional oligomers and applications of these materials.

2. 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 repeated unit in the chain. PEO oligomers are most commonly synthesized via anionic ring-opening polymerization of ethylene oxide (EO). 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 [37].

Anionic ring-opening polymerization of EO can be living in nature due to the stability of the propagating species. Such polymerizations of EO are often carried out with a hydroxide or alkoxide initiator (Fig. 1). The reactions take place via nucleophilic attack on an EO methylene to open the ring and form the propagating species.

Epoxide polymerizations can also be conducted with coordination catalysts, usually in conjunction with an alcohol initiator.

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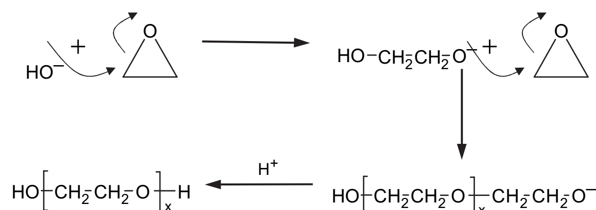


Fig. 1. Anionic ring-opening polymerization of EO initiated by hydroxide.

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 [38–42]. The side reaction that occurs in PO polymerizations caused by hydrogen abstraction from the methyl side chain 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 in other companies including ARCO, Shell, Asahi Glass, and Bayer [39,40,43,44]. One example of a double metal cyanide catalyst, $Zn_3[Co(CN)_6]_2$, was utilized by Huang et al. to synthesize random copolymers of EO and PO [45]. Copolymers with various EO/PO compositions and with unimodal molecular weight distributions in the range of 1.21–1.55 were obtained.

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 [20,46]. When bounded 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 [36]. Another important property of PEO is its solubility in water as well as in many organic solvents. When bounded to a water insoluble compound, the resulting PEO conjugate generally displays increased water solubility or dispersibility [22,47,48].

Since the repeating ether units of PEO are essentially nonreactive, these oligomers must be reacted with, or adsorbed onto, other compounds through terminal or pendant functional groups. Examples include PEO with thiol or carboxylic acid end groups that have been adsorbed onto metal or metal oxide surfaces [11,48–52]. Maleimide functionality on PEO chain ends is one example that has been of great interest in the formation of bioconjugates [53–55].

As an aside, although aliphatic polyethers such as PEO are resistant to hydrolytic degradation or attack by nucleophiles or acids, the relatively labile C–H bonds adjacent to the ether oxygens are susceptible to oxidative degradation through a radical mechanism. This can be especially important in cases where one or more of the PEO end groups has been modified with reactive groups such as methacrylate or acrylate that would subsequently be exposed to a free radical reaction. Other post-reactions such as the deposition of plasma coatings may also lead to PEO degradation due to these aspects.

Two broad methods are commonly employed for synthesizing heterobifunctional PEO oligomers (Fig. 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 partial derivatization of PEO diols, followed by separation of the resultant statistical mixtures to isolate the targeted 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 Fig. 2A [32,34,56–58]. Anionic polymerizations of EO from initiators 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 [59–63]. The hydroxyl terminus can then be derivatized to produce more complex heterobifunctional polymers such as H_2N–PEO–COOH [56]. 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 [58,64–68]. 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 [61]. It is also important to note that the polymerization of ethylene oxide can be dangerous and care must be taken when working with toxic and potentially explosive gases.

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 un-substituted components (Fig. 2B). To obtain heterobifunctional oligomers, only a portion of the hydroxyl end groups are converted to the new functional group,

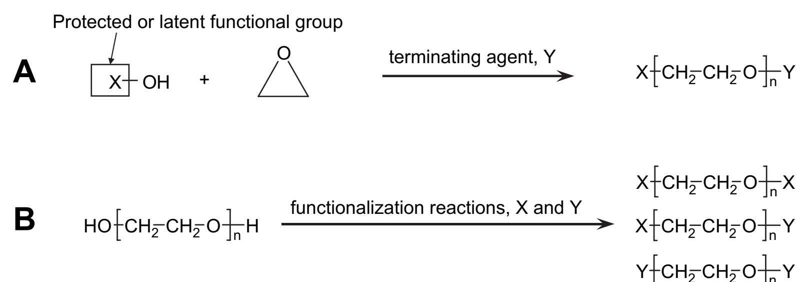


Fig. 2. Synthetic methods to produce heterobifunctional PEO oligomers: direct synthesis of heterobifunctional PEO (A) and end group modification of PEO diols (B).

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 [61,69,70]. As the molecular weights of the starting PEO diols increase, the chemical and physical differences among the mono-, di-, and un-substituted products become increasingly smaller and the heterobifunctional oligomers are therefore harder to isolate [71]. 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.

3. 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 [72–74]. An important example is that a carboxyl terminus of PEO can be activated by forming highly reactive succinimidyl esters. Many biologically relevant ligands have been covalently attached to PEO through amide bonds via these succinimidyl ester intermediates (Fig. 3) [27,75–78]. When using X–PEO–COOH especially in biomedical applications, it is also important to consider the stability of heterobifunctional PEOs linked to substrates via ester bonds. These ester bonds can be hydrolyzed under certain conditions and this phenomenon can be taken advantage to release biological molecules of interest [79].

3.1. Direct synthesis of X–PEO–COOH

3.1.1. Synthesis of $(HOOC)_{1-3}$–PEO–OH

Heterobifunctional PEO with a hydroxyl group on one chain end and 1–3 carboxylic acid groups on the other have been synthesized utilizing vinylsilylpropanol initiators (Fig. 4) [80]. These initiators containing one, two, or three vinyl groups were synthesized from 3-chloropropylchlorodimethylsilane, 3-chloropropyldichloromethylsilane, and 3-chloropropyltri chlorosilane, respectively, by reaction with vinylmagnesium chloride (Fig. 5). The alkyl chlorides were then converted to the corresponding alcohols.

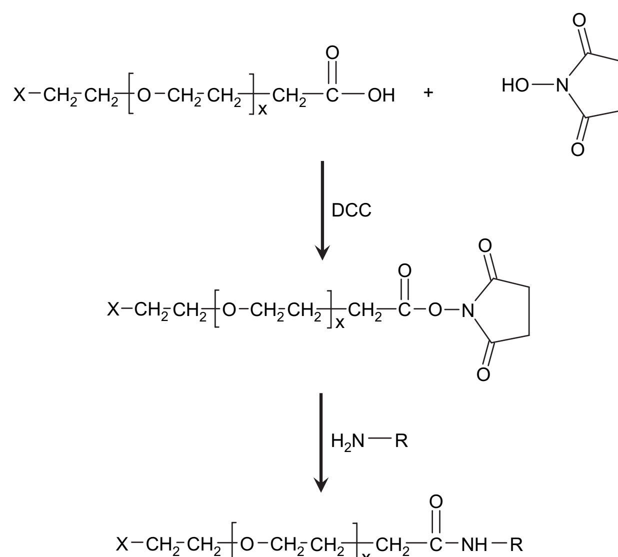


Fig. 3. Formation of an amide linkage via the active succinimidyl ester.

The alkoxide initiators were prepared by reacting the appropriate vinylsilylpropanol with potassium naphthalide in THF (1 mol of –OH:0.95 mol of potassium naphthalide). A slight deficiency of potassium naphthalide ensured that the vinyl groups were preserved during alkoxide formation and polymerization. The anionic initiator was added to EO and reacted at room temperature, and the polymerizations were terminated with acetic acid. The ratio of end group protons observed via ^{1}H 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 ^{1}H NMR and SEC matched well with the targeted values based on the monomer to initiator ratios. Molecular weight distributions were narrow (≤1.13).

Conversions of the terminal vinylsilane moieties into carboxylic acids were achieved via ene–thiol additions utilizing mercaptoacetic acid and AIBN (Fig. 4). The functionalizations were monitored via ^{1}H NMR by following the disappearance of vinyl proton resonances at approximately 6.0 ppm. The NMR resonances of the products corresponded 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 $(H_2N)_{1-3}$–PEO–OH [82].

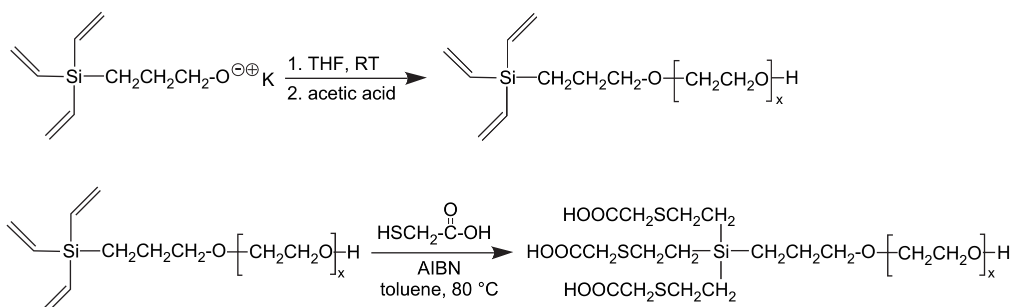


Fig. 4. Synthesis of $(HOOC)_3$–PEO–OH.

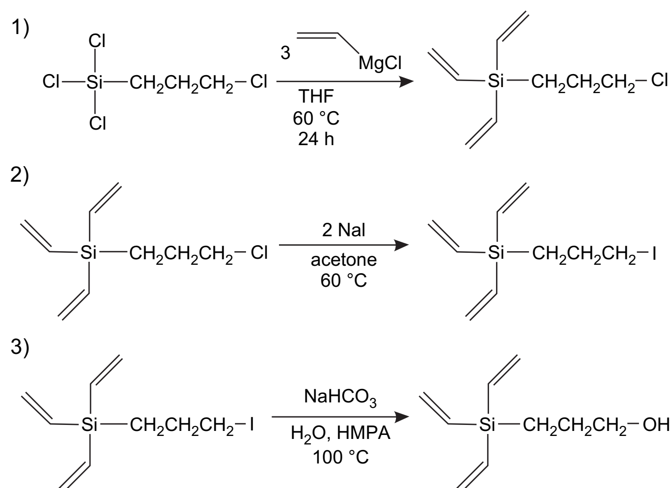


Fig. 5. Preparation of (1) 3-chloropropyltri-vinylsilane, (2) 3-iodopropyltri-vinylsilane, and (3) 3-hydroxypropyltri-vinylsilane.

Heterobifunctional poly(ethylene oxide-*b*-propylene oxide) (PEO-*b*-PPO) block copolymers have also been synthesized utilizing trivinylsilylpropoxide as the initiator [83]. Even under mild reaction conditions, the anionic polymerization of PO leads to some unsaturation at the chain end due to abstraction of a methyl proton on the PO monomer converting it to allyl alkoxide [84–86]. 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 negligible unsaturation and much faster rates of polymerization even with low catalyst concentrations [42,45]. 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 1.84–1.91. Retention of the desired end groups during polymerization was confirmed by ^{1}H NMR. These $(vinyl)_3$–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 have been well documented [42,45,87]. These catalysts are heterogeneous at least in the initial stages of polymerization, and they become somewhat solubilized as the reactions proceed. The broadened molecular weight distributions 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 catalyst 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. 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 observed for these batch reactions.

Carboxylate anions have been widely utilized for adsorbing surfactants or polymers onto the surfaces of magnetite nanoparticles [48,88–90]. 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 [48,52]. Magnetite complexes have been reported with triblock copolymers consisting of a polyurethane anchor block containing carboxylic acid functional groups flanked by PEO and PEO-*b*-PPO tail blocks adsorbed on their surfaces, and the copolymers served as dispersion stabilizers in aqueous media [48,52]. 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.

3.1.2. Synthesis of HOOC–PEO–OH via thiol-initiated anionic polymerization

Zeng and Allen investigated alkoxide and thiolate initiators with 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) [73]. One effective functional initiator was 3-mercaptopropionic acid. Dipotassium-3-mercaptopropionate was prepared by reacting 3-mercaptopropionic acid with 2 equiv 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 (Fig. 6). Molecular weights ranging from 1000 to 25,000 g mol^{-1} were achieved with narrow molecular weight distributions, 1.07–1.15. End group analysis by ^{1}H NMR showed the expected structure of the heterobifunctional PEOs, thus confirming that the carboxylate did not participate in the polymerizations under the conditions utilized.

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 [73]. Poly(ε-caprolactone) blocks were synthesized with molecular weights ranging from 2000

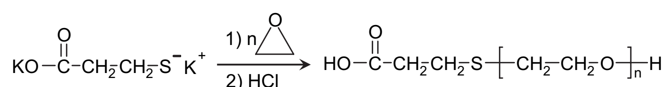


Fig. 6. Thiolate-initiated anionic polymerization of EO in the presence of a weak carboxylate nucleophile.

to 9000 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.

3.1.3. Synthesis of NaOOC–PEO–NH_2 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–NH_2) [56]. 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 molecular weights ranging from 400 to 5000 g mol^{-1} and with molecular weight distributions ≤1.26.

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–NH_2. 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–NH_2 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) [72]. In aqueous media these copolymers self-assembled into micelles with diameters ranging from 30 to 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.

3.1.4. 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 thiols are well known [91]. 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 initiator (Fig. 7) [92]. Allyl alcohol was reacted with potassium naphthalide to afford

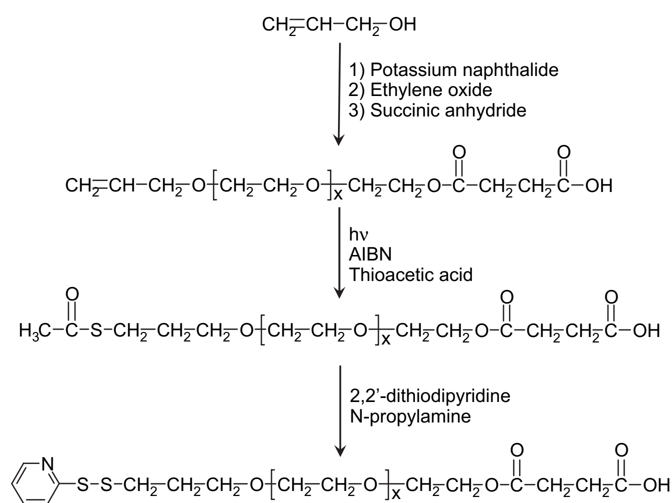
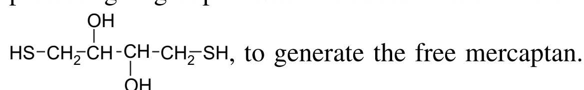


Fig. 7. Synthesis of pyridyl–SS–PEO–COOH.

potassium allyl alkoxide, and this was followed by polymerization of EO. The polymers 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 ^{1}H NMR spectra. The integral ratios of the signals assigned to the methylene protons between the two carboxyl groups and the signal corresponding to the allyl methylene protons was ca. 2:4, indicating that the functionalization of the chain end was quantitative.

Radical additions 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. The thio-ester group was 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 ^{1}H 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 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,



, 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 [92]. Aldehyde–PEO–SH oligomers on the sensors were utilized to immobilize proteins via reductive amination [49]. However, sufficient protein was not always bounded 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 with 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 [78]. 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 was 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. 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 [78]. The SAM was linked to antibodies after activating 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-bounded 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-bounded receptors.

3.2. Synthesis of X–PEO–COOH via end group modification of PEO diols

3.2.1. Synthesis of allyl–PEO–COOH

Völcker et al. synthesized allyl–PEO–COOH from a PEO diol in four steps [57]. First, the PEO diol was tosylated to activate the chain ends toward nucleophilic substitution (TsO–PEO–OTs). End group analysis via ^{1}H 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 un-substituted 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 ^{1}H 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 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 ^{1}H NMR determined that the allyl/carboxymethylthio ratio was 1.0. Relatively narrow molecular weight distributions were obtained via MALDI-TOF MS.

3.2.2. 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 at the other (DSPE–PEO–Hz) was synthesized from a PEO diol (Fig. 8) [77,93]. 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.

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 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

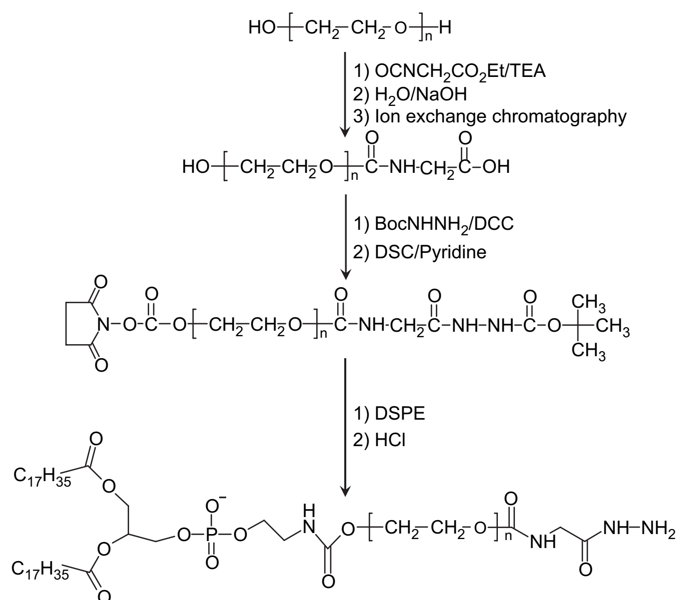


Fig. 8. Synthesis of an amine-terminated lipid–PEO copolymer (DSPE–PEO–Hz).

introducing the succinimidyl carbonate due to the potential reactivities of the boc and hydrazide groups. Quantitative incorporation of succinimidyl carbonate was confirmed by ^{1}H 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 produce 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 and was confirmed by the absence of the ^{1}H NMR signal associated with the boc.

It has been shown that liposomes modified with methoxy–PEO–DSPE exhibited desirable pharmacokinetics and biodistributions [94]. A DSPE–PEO–Hz derivative was utilized to form liposomes bearing hydrazide groups on their surfaces for introducing targeting moieties [77,93]. DSPE–PEO–Hz was 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 [93]. It was determined that covalent attachment of IgG to the PEO termini had no adverse effects on blood circulation times of the liposomes.

3.3. Additional applications of X–PEO–COOH

3.3.1. 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) (Fig. 9) [27]. 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.

A competitive immunosensing approach 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.

3.3.2. 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

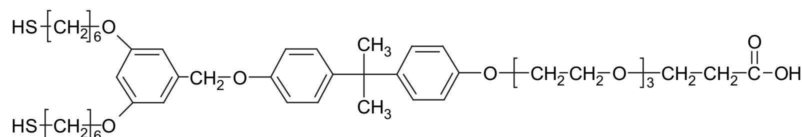


Fig. 9. Structure of $(HS)_2$–PEO–COOH.

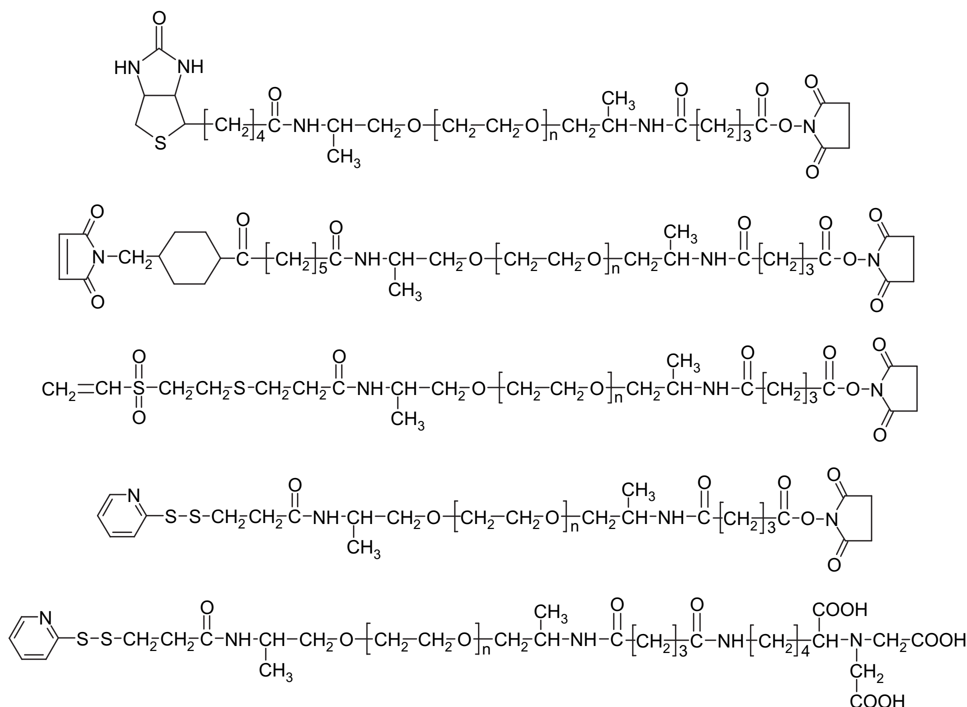


Fig. 10. Heterobifunctional PEOs utilized in SMRFM.

specifically interact with a target molecule. Another key component of SMRFM is a flexible 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 [95]. 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 H_2N−PEO−COOH intermediate for tip-PEO-probe conjugation [76,95]. 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 (Fig. 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 His_6-tagged proteins to AFM tips via noncovalent NTA−Ni^{2+}−His_6 bridges.

3.3.3. Anticoagulant medical devices

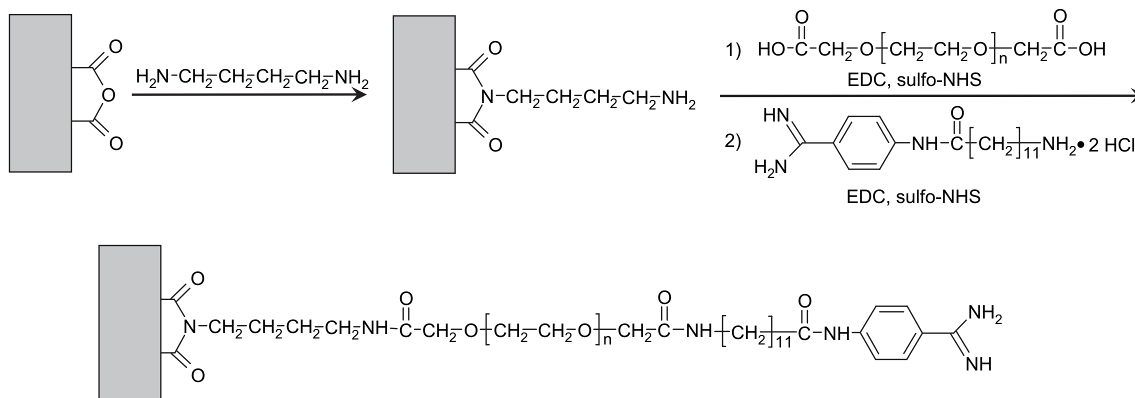
Prevention of blood coagulation on the surfaces of medical devices is important to avoid complications during the clinical use of blood-contacting artificial devices. One method of reducing blood coagulation is by regulating the activity of thrombin, a key enzyme in the coagulation process, through surface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

Salchert et al. utilized H_2N−PEO−COOH and HOOC−PEO−COOH to functionalize polymer coatings with a benzamidine derivative capable of selectively binding thrombin at their free termini (Fig. 11), and thus removing the coagulant from circulation [96]. 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 H_2N−PEO−COOH, the amine terminus was reacted with the maleic anhydride-functional surface to form an imide, and then the benzamidine derivative was bounded.

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 less free carboxyl groups on the surface. Although PEO has the propensity to resist nonspecific protein adsorption, these benzamidine-modified surfaces selectively bound thrombin. The study concluded that the benzamidine−PEO surfaces had significant potential for scavenging thrombin.

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 [3,75]. The siloxane surface was functionalized with Si−H groups using $(MeHSiO)_n$ under acidic conditions. First, the allyl terminus of allyl−PEO−NHS was grafted to the Si−H

Surface modification using HOOC-PEO-COOH



Surface modification using H_2N-PEO-COOH

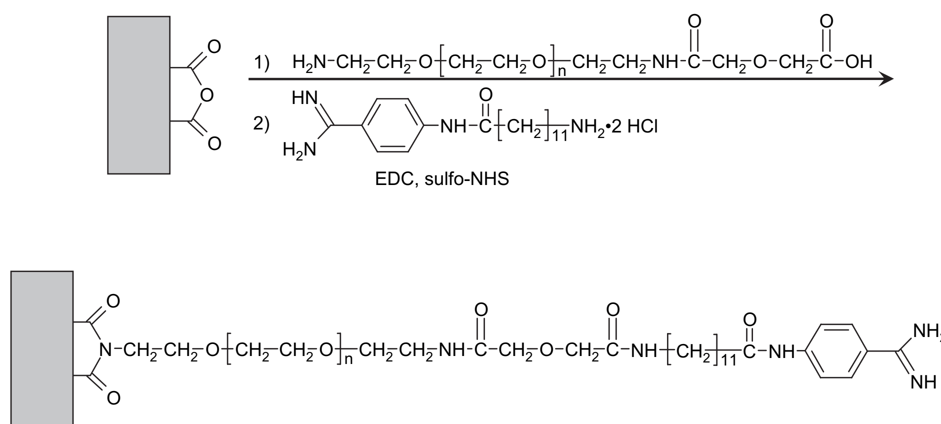


Fig. 11. Surface functionalization with a benzamidine derivative.

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. The heparin modified surfaces demonstrated significantly less thrombin activity as compared to the methoxy–PEO surface, while maintaining resistance to non-specific protein adsorption [3]. 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.

4. Synthesis and applications of H_2N–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 [97–101]. Furthermore, amine-terminated PEO can initiate polymerizations of amino acid *N*-carboxyanhydrides or lactones/lactides for synthesizing biocompatible block copolymers [63,72,102,103].

4.1. Direct synthesis of heterobifunctional H_2N–PEO–X

4.1.1. Schiff base-initiated polymerization of EO

Ethanolamine with a protected amine has been utilized to initiate and polymerize EO to generate α-amino-ω-hydroxy-poly(ethylene oxide) (H_2N–PEO–OH) [59]. 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 protecting group was removed by acidification with hydrochloric acid to yield the H_2N–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 isolated 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 [74]. To avoid this, two steps were 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

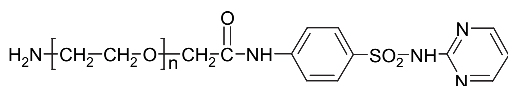


Fig. 12. PEO with a primary amine and sulfadiazine moieties.

synthesized. In the second step, the hydroxyl terminus was deprotonated with a slight molar excess of sodium 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 bounded to the carboxyl end of the PEO (Fig. 12) [74]. The carboxylic acid was transformed to the more reactive acid 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.

4.1.2. 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 (Fig. 13) [60]. Following polymerization, the protecting group was removed by treatment with 0.1 N hydrochloric acid. The resulting polymer was purified by ion exchange chromatography. Molecular weights were in close agreement with predicted values based on the initiator to monomer ratios, and the molecular weight distributions were ~1.1. Titrations confirmed that each mole of polymer contained 1 mol of amine.

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 [104]. PEO was synthesized with potassium bis(trimethylsilyl)-amide to produce the macroinitiator, H_2N–PEO–OH. The block copolymer, H_2N–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 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 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. Methoxy–PEO–PLA and H_2N–PEO–PLA showed no UV signals at low retention times. However, the H_2N–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 H_2N–PEO–PLA diblock copolymer was transformed into an NHS-activated ester by reaction with disuccinimidyl tartrate (DST) or disuccinimidyl succinate (DSS) (Fig. 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.

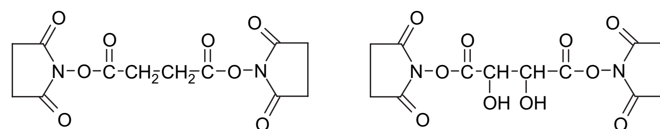


Fig. 14. Structure of DSS and DST.

Potassium bis(trimethylsilyl)amide was also utilized to synthesize H_2N–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 (Figs. 15 and 16) [105]. 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 H_2N–PEO–CBL. The sulfadiazine was covalently bounded to the primary amine terminus by first reacting 2-[N^1-2-pyrimidinyl-(*p*-benzylidene)aminobenzenesulfonamido]ethanol with bis (trichloromethyl)carbonate. Subsequent addition of H_2N–PEO–CBL 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.

To test the activity of chlorambucil bounded 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,

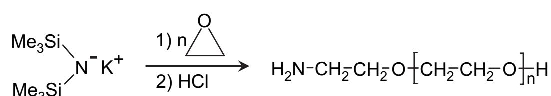


Fig. 13. Potassium bis(trimethylsilyl)amide-initiated polymerization of EO.

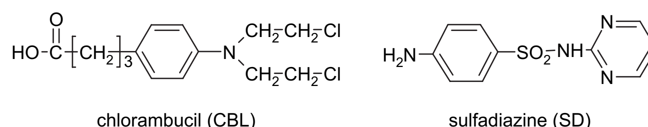


Fig. 15. Structure of chlorambucil and sulfadiazine.

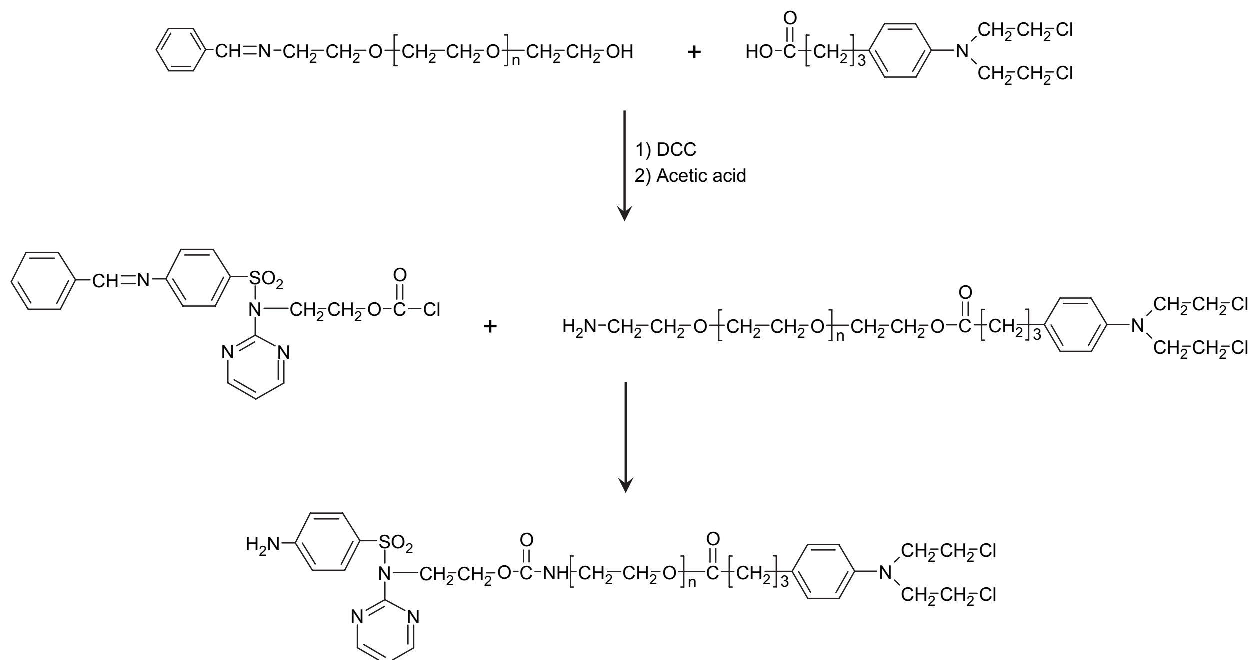


Fig. 16. Synthesis of SD–PEO–CBL.

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-bounded 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.

4.1.3. Potassium TDMA-initiated polymerization of EO

A protected initiator, *N*-2-(2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentyl)-ethylmethylamine (TDMA), was synthesized to prepare a heterobifunctional H_2N–PEO–OH (Fig. 17) [106]. 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. ^{1}H NMR spectra in DMSO-d_6 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. 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 repeated unit plus the molecular weight of the two functional ends, thus providing evidence for purity of the heterobifunctional material.

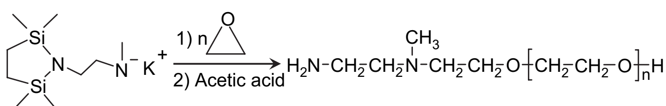


Fig. 17. TDMA-initiated polymerization of EO.

4.1.4. (Cyanomethyl)potassium-initiated PEO

Heterobifunctional PEOs have also been prepared by polymerizing EO initiated with (cyanomethyl)potassium [107]. The synthesis of NC–PEO–OH was carried out in a similar manner as previously described [56]. Following polymerization, the cyano group was reduced with lithium aluminum hydride to afford H_2N–PEO–OH.

A triblock copolymer, poly(γ-benzyl-L-glutamic acid-*b*-ethylene oxide-*b*-ε-caprolactone) (PBLG–PEO–PCL), was synthesized with modifications to the procedure described above [63]. 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 H_2N–PEO–PCL–OH. End group analysis via ^{1}H 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.

4.1.5. Allyl–PEO–OH intermediates for synthesis of H_2N–PEO–OH

Another approach to synthesize 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 narrow molecular weight distribution (1.1) [61]. Molecular weights obtained from SEC and ^{1}H NMR were in good agreement with those targeted.

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. The molecular weights and molecular weight distributions determined by SEC were unaffected, indicating that the radical addition reactions took place without significant side reactions. 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 [91].

4.1.6. Synthesis of vinylbenzyl–PEO–NH_2

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 [108]. 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) was shown to be a suitable initiator for EO to produce an amine-functional PEO macromonomer (Fig. 18) [109]. Initiation with VBA was a preferred method as opposed to obtain the vinylbenzyl group by termination 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. 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 H_2N–PEO–VBA macromonomer. Molecular weights of the macromonomers determined by SEC closely matched the predicted values 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 alkoxide formation. It was reasoned that the coupled products could initiate EO and lead to high molecular weight by-products.

Functionalized nanospheres were prepared in four steps. First, the H_2N–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 nanopsheres 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 (Fig. 19). Lastly, a biological vector, an anti-human α-fetoprotein (AFP) Fab′ fragment, was covalently bounded 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 bounded to the surface, non-specific binding was practically negligible.

4.2. Synthesis of H_2N–PEO–X via end group modification of PEO diols

4.2.1. Synthesis of H_2N–PEO–COOH

Heterobifunctional PEOs were prepared from PEO diols with a carboxylic acid on one chain end and a primary amine on the other [110]. 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

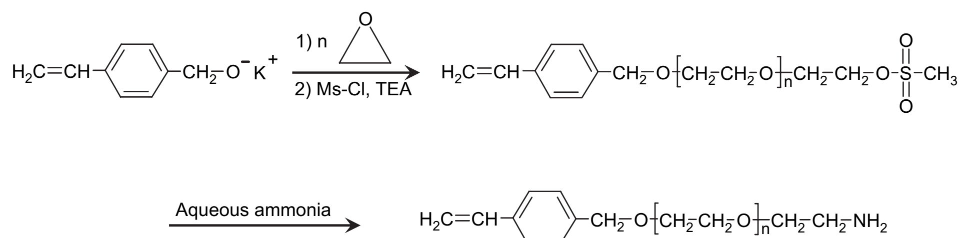


Fig. 18. Synthesis of VBA–PEO–NH_2.

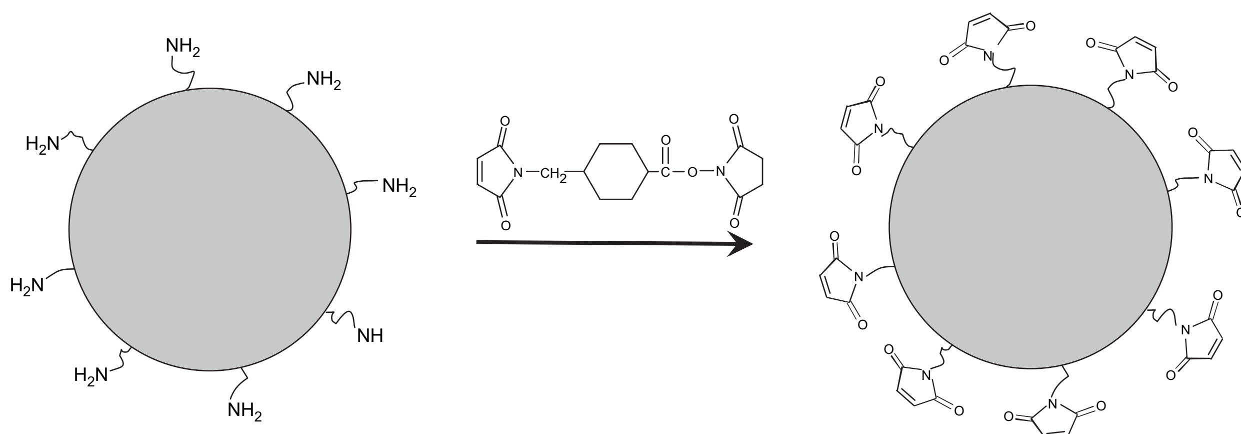


Fig. 19. Synthesis of maleimide-functional nanospheres.

aqueous ammonium hydroxide, and water. The azide was converted to a primary amine by catalytic hydrogenation to afford the target H_2N–PEO–COOH.

4.2.2. Synthesis of aminooxy–PEO–Br

A PEO diol was converted by a series of reactions and separations to yield α-aminooxy-ω-bromo-poly(ethylene oxide) (H_2NO–PEO–Br) spacers for bioconjugates. The ease of oxime ether formation reportedly made this a superior method for targeting aldehydes and ketones compared to reductive amination (Fig. 20) [111]. The oxime ether linkage is stable at physiological pH.

A PEO diol was partially silylated 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), $(H_3C)_2CH–C(=O)–N{=}N–C(=O)–CH(CH_3)_2$, to afford the phthalimido ether. The phthalimide end group was removed via hydrazinolysis to produce the aminooxy-terminated polymer.

Prior to conversion of the silyl ether, the aminooxy terminus was protected by reacting it with di-*tert*-butyldicarbonate (boc_2O) in the presence of triethylamine. Introduction of the bromide was accomplished by desilylation utilizing tetra-*n*-butylammonium 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 H_2NO–PEO–Br.

4.2.3. Synthesis of folate-targeted PEO carboplatin analogues

Carboplatin (Fig. 21) is a chemotherapy drug that is most commonly used to treat ovarian and lung cancers 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 (Fig. 21) on one chain end and a carboplatin analogue on the other [16]. 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.

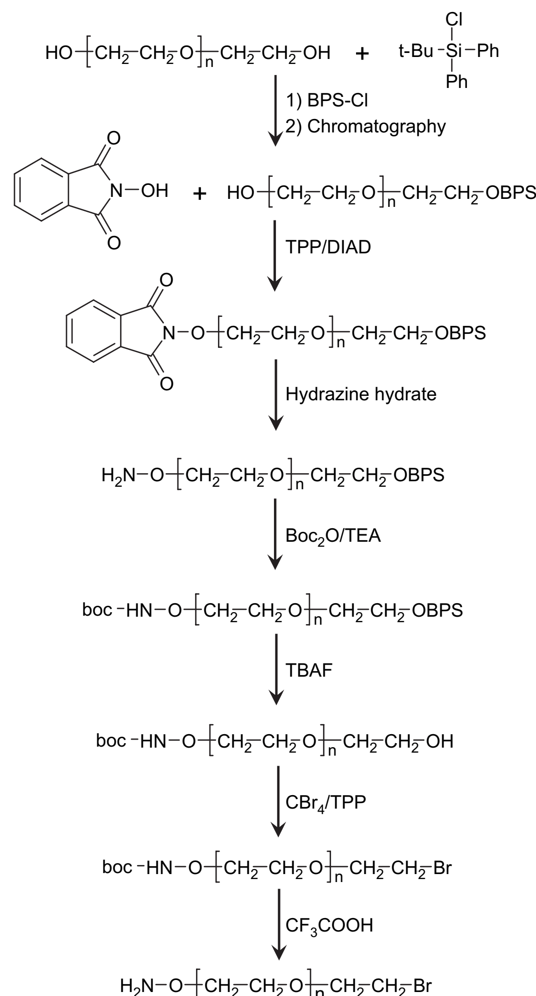


Fig. 20. Synthesis of H_2NO–PEO–Br.

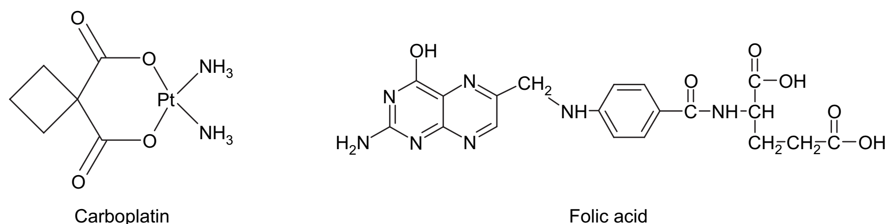


Fig. 21. Structure of carboplatin and folic acid.

The first step toward folate-targeted PEO carboplatin analogues was to synthesize a mono-protected 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, H_2N−PEO−NH_2. An equimolar ratio of fluorenylmethyl chloroformate (fmoc) and PEO diamine was reacted to yield a mixture of un-protected, mono-protected, and bis-protected PEO diamines. The mixture was separated on a Sephadex CM-25 cation exchange column with a yield of ~30% of the mono-protected product. End group analysis by ^{1}H NMR showed that the ratio of signals corresponding to the methylene protons adjacent to the un-protected 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 (Fig. 22). An active ester of folic acid (FA−NHS) was reacted with the fmoc-PEO−NH_2 to yield fmoc-PEO−FA, and this was followed by removal of the fmoc group by reaction with piperidine. The regenerated amine was then reacted with fluorescein isothiocyanate (FITC) to yield 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.

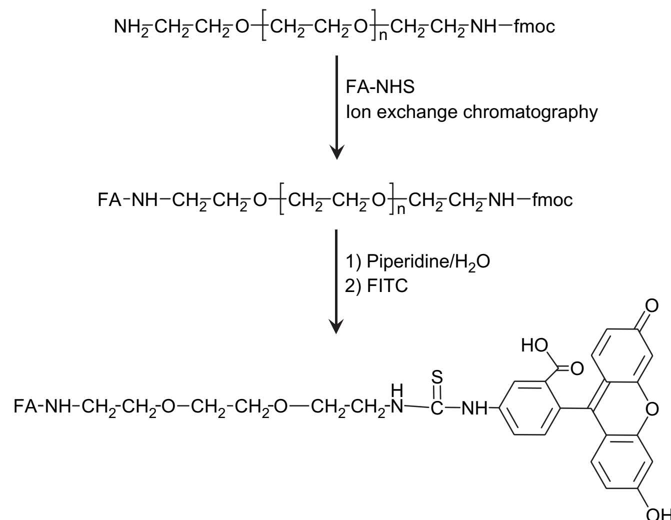


Fig. 22. Synthesis of FITC−PEO−FA.

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−NH_2 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*-$[Pt(NH_3)_2(D_2O)_2]^{2+}(NO_3)_2$ to yield the carboplatin analogue, FA−PEO−Pt (Fig. 23). The platination was monitored by ^{1}H 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.

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.

5. 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 useful for preparing bio-interfaces. Heterobifunctional mercapto or pyridyl disulfide-ended PEOs can be utilized for functionalizing gold and silver surfaces 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 [112]. It is well known that modifying a surface with tethered PEO chains can dramatically decrease non-specific interactions of biopolymers [28,113–115]. A heterobifunctional PEO was required to construct gold nanoparticles possessing both sufficient colloidal stability and biological functionality for bioassays [11,49].

Due to the capacity for pyridyl disulfide moieties to react with thiols, heterobifunctional PEOs bearing a pyridyl disulfide group can serve as linking agents [116]. Another useful property of the pyridyl disulfide moiety is that it releases

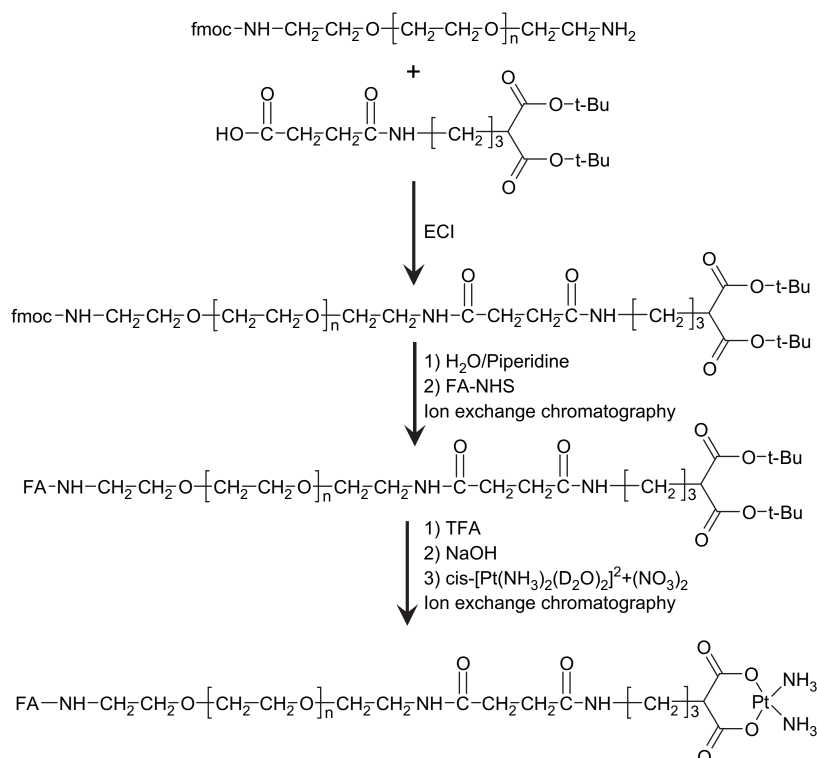


Fig. 23. Synthesis of FA–PEO–Pt.

thiopyridone upon reaction with free mercapto groups (Fig. 24). Thiopyridone can be quantified to determine the number of PEO oligomers bounded to a molecule or surface [116,117].

5.1. Direct synthesis of HS–PEO–X

5.1.1. Synthesis of formyl–PEO–SH and formyl–PEO–SS–pyridyl

A heterobifunctional PEO containing a formyl group and a hydroxyl group was synthesized by polymerizing EO with an initiator that contained an acetal, potassium 3,3-diethoxypropoxide [68]. The initiator was formed by reacting a stoichiometric amount of potassium naphthalide with 3,3-diethoxypropanol in THF, and this was followed by polymerization. The polymers, acetal–PEO–OH, had narrow molecular weight distributions, ~1, and the molecular weights obtained from ^{1}H NMR and SEC were in good agreement with the

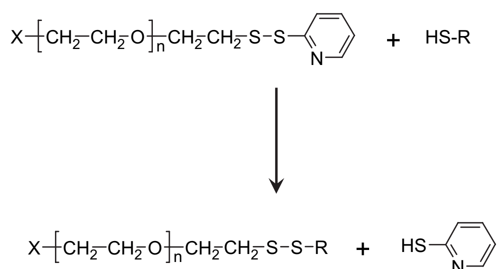


Fig. 24. Release of thiopyridone from PEO bearing a pyridyl disulfide moiety upon reaction with a free mercapto group.

molecular weights calculated from monomer to initiator ratios. The acetal terminus was deprotected to form the aldehyde by reaction with hydrochloric acid.

Modifications to the method described above were employed to synthesize acetal–PEO–SH and acetal–PEO–SS–pyridyl (Fig. 25) [58]. The polymerizations were terminated with methanesulfonyl chloride to produce an activated chain end. Capping with the methanesulfonyl moiety was ~98% based on end group analysis by ^{1}H NMR. Displacement of the methanesulfonyl ester was achieved by reaction with potassium *O*-ethyldithiocarbonate. End group analysis via ^{1}H NMR showed that conversion of the methanesulfonyl ester to the ethyldithiocarbonate 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 ^{1}H NMR end group analysis.

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 [11]. 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 buffers, 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

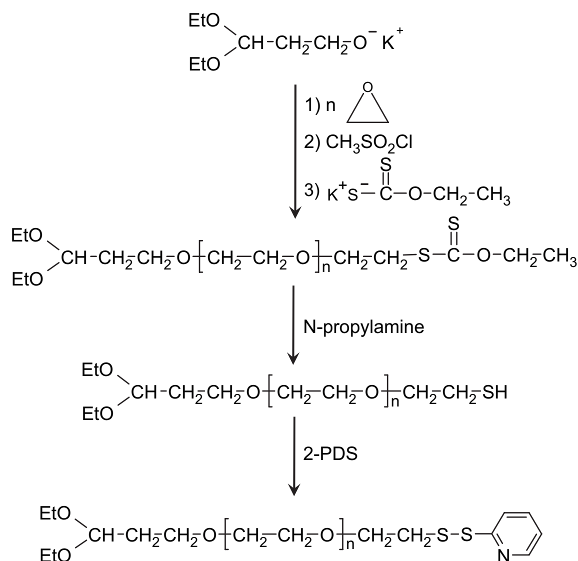


Fig. 25. Synthesis of acetal–PEO–SH and acetal–PEO–SS–pyridyl.

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, *Ricinus communis* agglutinim (RCA_{120}). Additions of RCA_{120} 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 RCA_{120}, 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 RCA_{120}. This process was repeatable over several cycles of aggregation and redispersion. Calibration curves enabled detection of RCA_{120} 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 [118]. 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 (Fig. 26A). The hydroxyl terminus was also converted to a thiol by condensation with an excess of thioglycolic acid to prepare acetal–PEO–SH (Fig. 26B). End group analysis via ^{1}H 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.

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 bounded to the gold electrode by the aldehyde–PEO–SH polymer was demonstrated by cyclic voltammetry. This indicated that the polymer could be utilized to functionalize 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.

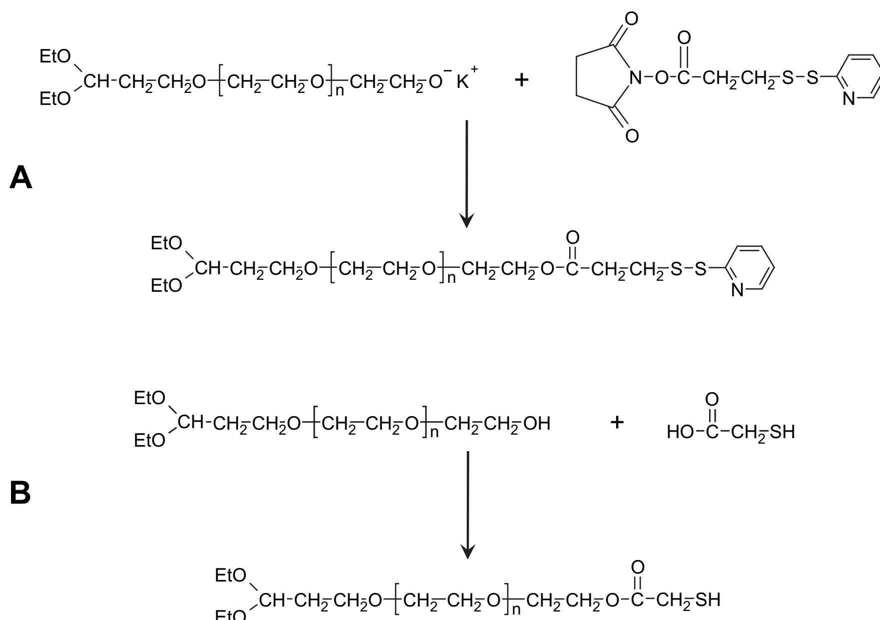


Fig. 26. Synthesis of acetal–PEO–SS–pyridyl and acetal–PEO–SH.

5.1.2. 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 polymerizing EO using potassium 4-(diethoxymethyl)benzylalkoxide (PDA) as an initiator (Fig. 27) [119]. 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 benzaldehyde is that its reaction with an amine produces a sufficiently stable imine that does not require further reduction. 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 ∼1.03 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 ^{1}H 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 ^{1}H 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.

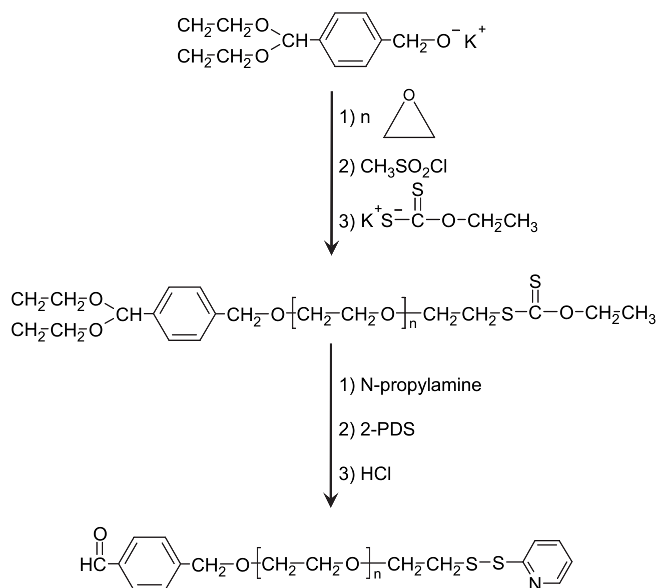


Fig. 27. Synthesis of PEO with both a benzaldehyde and pyridyl disulfide moieties.

5.2. Synthesis of HS–PEO–X via end group modification of homobifunctional PEO

5.2.1. 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 [120]. 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 un-substituted 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.

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–$(CH_2)_{11}$–CH_3 showed significant increase. 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.

5.2.2. Synthesis of pyridyl–SS–PEO–NHS from a PEO diamine

A PEO diamine (H_2N–PEO–NH_2) was utilized for synthesizing PEO oligomers with pyridyl disulfide and NHS functional groups (Fig. 28) [116]. End group asymmetry was introduced by reacting *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) with an excess of the PEO diamine. The monosubstitued PEO (pyridyl–SS–PEO–NH_2) was separated from the statistical mixture of the mono-, di-, and un-substituted PEO oligomers by chromatography on silica gel. The terminal amine was then reacted with glutaric anhydride in the presence of pyridine to introduce a carboxylic acid onto the other chain end. Finally, pyridyl–SS–PEO–NHS was produced by coupling the carboxyl group with NHS using DCC to form the active ester. The signals in the ^{1}H NMR corresponding to the pyridyl disulfide and NHS terminals were present in the expected ratio of 4:4.

Haselgrubler et al. demonstrated that these oligomers could be utilized to form liposomes with antibodies bounded to their surfaces [116]. 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 (Fig. 28).

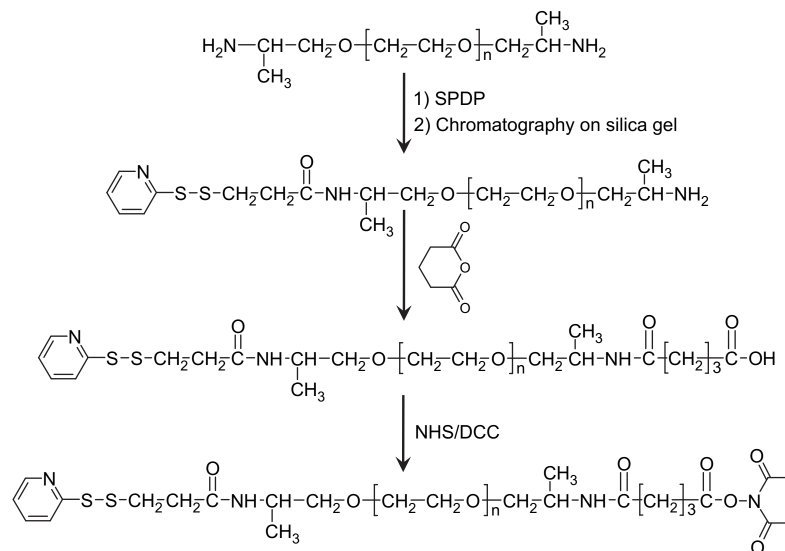


Fig. 28. Synthesis of pyridyl–SS–PEO–NHS.

Pyridyl–SS–PEO–NHS oligomers were also used to prepare liposomes with bovine IgG bounded 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.

5.2.3. 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 end and a pyridyl disulfide group at the other (Fig. 29) [117]. 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.

A stoichiometric amount of a PEO diamine was reacted with di-*tert*-butyldicarbonate (boc$_2$O) 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 avidin binding to biotin–PEO was tested by utilizing biotin–PEO–SS–pyridyl to investigate the stoichiometry and metastability of bounded avidin. The avidin–biotin–PEO conjugates had dissociation kinetics and half-lives similar to those previously reported for spacers with 7–27 atoms [121–124]. These results confirmed that biotin–PEO is a good ligand for avidin.

5.2.4. 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 (Fig. 30) [125]. A PEO diol was activated at one chain end before being bounded to the polymer support. Diglycolic anhydride was reacted with a large excess of PEO diol to obtain mono- and un-substituted 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-bounded PEO.

The hydroxyl terminus of the resin-bounded PEO (resin–PEO–OH) was activated by reacting with an excess of

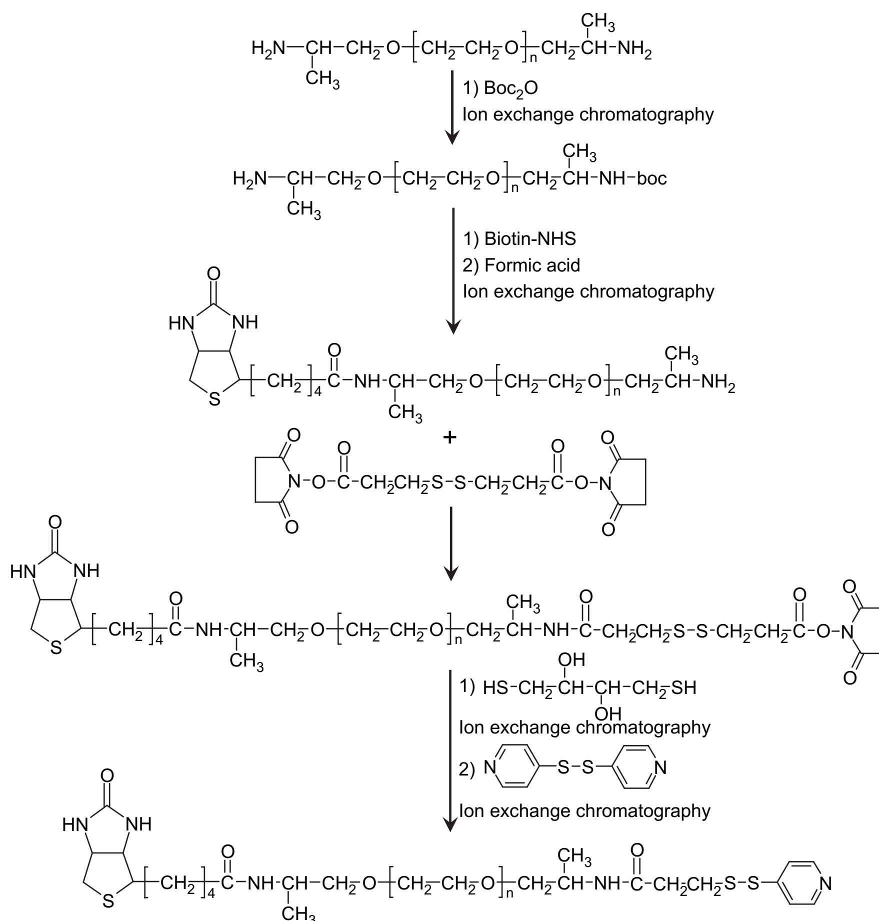


Fig. 29. Synthesis of biotin–PEO–SS–pyridyl.

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 four months at −20 °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*-ethyldithiocarbonate with the resin–PEO–OTs. An excess of potassium *O*-ethyldithiocarbonate lead 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 mild basic polar solvent (tetrahydrofuran/methanol/triethylamine). The pyridyl–SS–PEO–OH was obtained in a global yield of 65%.

5.2.5. 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 end and either a hydroxyl, aldehyde, amine, or azide at the other [70]. The first step in these syntheses was activation of one chain end of a 1500-M_n PEO diol with a equimolar amount of toluene-4-sulfonyl chloride. Tosylation of both chain ends was minimized by tosylation in the presence of silver oxide with a catalytic amount of potassium iodide [126]. 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

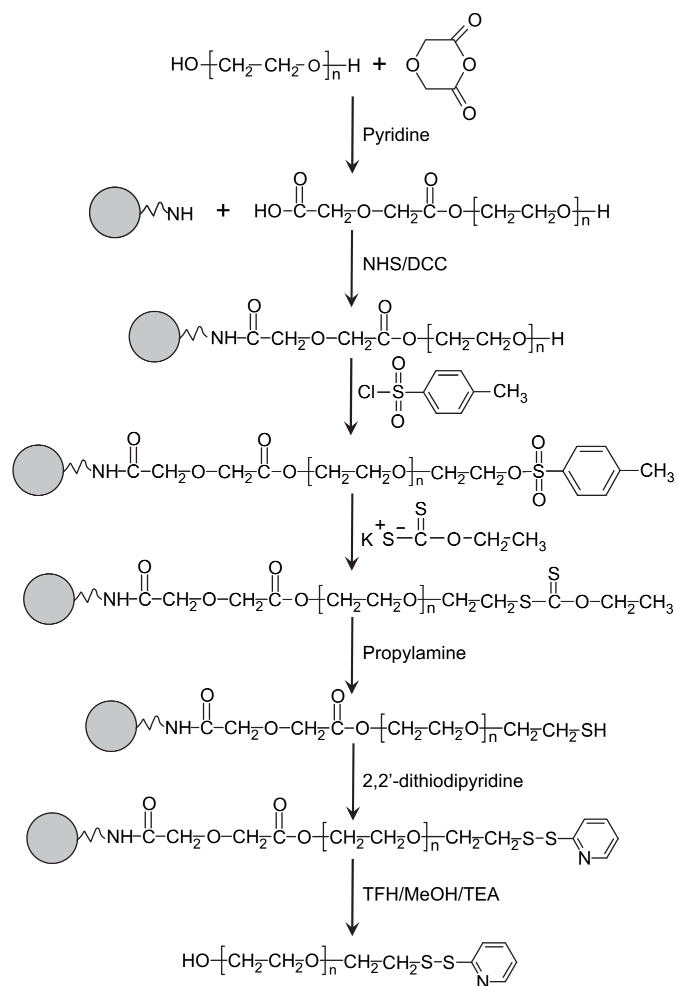


Fig. 30. Polymer supported synthesis of pyridyl–SS–PEO–OH.

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 enabled cellular visualization assays using phase contrast microscopy. The aldehyde–PEO–SAc was covalently bounded 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 [127], the coumarin–PEO–SH functionalized gold nanoparticles displayed significant emission intensity. The reduced fluorescence quenching was attributed to the PEO spacer [128]. Coumarin–PEO–SH functionalized nanoparticles were nontoxic toward MDA-MB-231 human breast adenocarcinoma cells at concentrations up to 200 μg mL^{-1} (higher concentrations were not tested). 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 1 h, the nanoparticles had passed through the cytosol and reached the perinuclear region. Even after 24 h 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.

5.2.6. 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 [49]. 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. 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 unbounded polymer. The protocol was repeated several times to increase brush densities on the sensor chips. The amount of polymer bounded 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 bounded 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.

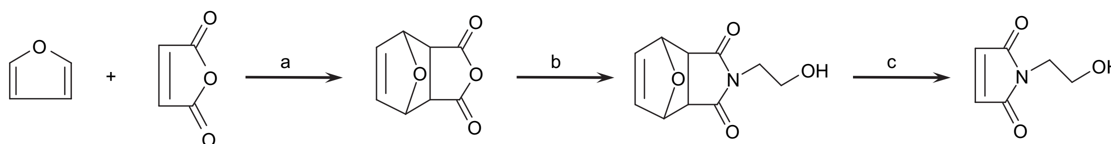


Fig. 31. Synthesis of *N*-(2-hydroxyethyl)maleimide: (a) toluene, RT; (b) ethanolamine, MeOH, reflux; (c) toluene, reflux.

The improved binding of streptavidin and almost negligible non-specific adsorption lead to SPR sensor chips with high signal to noise ratios.

6. 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 [129–133]. The maleimide reacts with mercapto groups under mild conditions to yield stable thioether linkages [134]. PEO oligomers with a maleimide group on one 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 [79,135–137].

6.1. 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 end and a hydroxyl group at the other [83]. Although polymerizations of EO are usually carried out with a basic initiator [138], the sensitivity of *N*-(2-hydroxyethyl)maleimide toward base prohibited this approach [42,45,87]. The zinc hexacyanocobaltate catalyst allowed for polymerizing EO utilizing *N*-(2-hydroxyethyl)maleimide as the initiator with retention of the maleimide functionality.

The heterobifunctional initiator was prepared in three steps (Fig. 31) [139]. 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.

The *N*-(2-hydroxyethyl)maleimide initiator was utilized in a batch polymerization of EO in the presence of Bayer *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 [42,45,87]. As expected from previous studies, the molecular weight distributions obtained from these polymerizations were broad (∼3.3). As determined from ^{1}H 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 ^{1}H NMR confirmed that the maleimide end group was retained during polymerization.

6.1.1. Synthesis of maleimide–PEO–benzophenone (Mal–PEO–BP)

A heterobifunctional PEO with a maleimide on one end and a benzophenone on the other (Mal–PEO–BP) was synthesized for photo-immobilization of biomolecules on

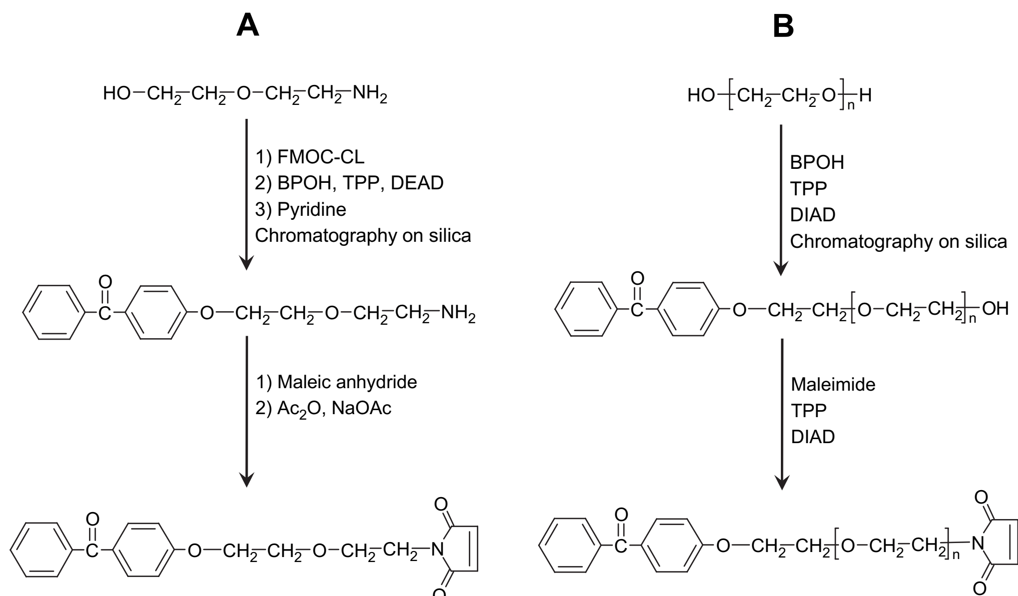


Fig. 32. Synthesis of Mal–PEO–BP.

surfaces [135]. It was reasoned that these materials would be polymeric analogues of a commercially available thiol-reactive, photoactivatable linker, 4-maleimidobenzophenone (BPMI). 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 introducing 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.

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) (Fig. 32A). 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 (Fig. 32B). 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 presence of TPP and DIAD.

6.1.2. 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 [97]. 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) (Fig. 33) and either a maleimide, bromoacetamide, or pyridyl disulfide moiety on the other end for coupling to cysteine residues of peptides.

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

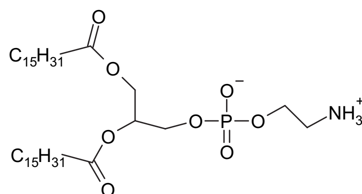


Fig. 33. Structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE).

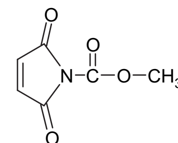


Fig. 34. Structure of 2,5-dioxo-2,5-dihydropyrrole-1-carboxylic acid methyl ester.

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 H_2N–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 (Fig. 34) 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.

Synthesis of the heterobifunctional PEO with 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 (Fig. 35) due to the formation of by-products that were difficult to remove when using DCC.

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 2 h in the pH range of 6–9. 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 min, but at lower pH the reaction was incomplete even after 2 h.

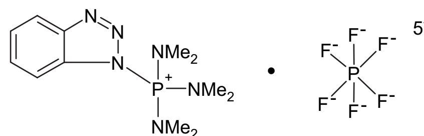


Fig. 35. Structure of (benzotriazol-1-yl-oxy)tris(dimethylamino)phosphonium hexafluorophosphate.

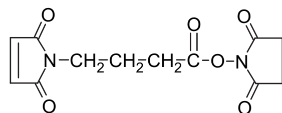


Fig. 36. Structure of *N*-γ-maleimidobutyryloxysuccinimide ester.

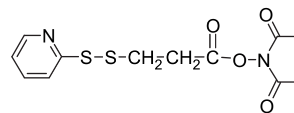


Fig. 37. Structure of *N*-succinimidyl-3-(2-pyridyldithio)propionate.

6.2. Applications of Mal–PEO–X

6.2.1. Improved blood circulation times of rhG-CSF

A heterobifunctional PEO with a carboxylic acid on one 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 [98]. The primary amine terminus of H_2N–PEO–COOH was converted to a maleimide by reaction with *N*-γ-maleimidobutyryloxysuccinimidyl ester (Fig. 36) 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 by reaction of a mercapto group with the maleimide.

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 bounded 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.

6.2.2. Solid-phase enzyme immobilization

To improve the performance of enzymes bounded to a solid support, a heterobifunctional PEO with a maleimide group on one 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 [137]. 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) (Fig. 37). 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 with the heterobifunctional PEO. This decreased activity with the homobifunctional PEO was attributed to the possible crosslinking of the enzymes and steric shielding 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.

6.2.3. Targeted drug delivery

Lipid drug carriers with a targeting moiety tethered to the surface were prepared via a heterobifunctional PEO spacer [79]. The PEO–lipid derivative, DPPE–PEO–Mal, was prepared by reacting DPPE (Fig. 33) 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 group 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 antitumor drug, recombinant human necrosis factor alpha (TNF-α), with a targeting protein, transferrin (Tf) [136]. Approximately five PEO chains were covalently attached to TNF-α through amide bonds via reaction with the NHS active ester of PEO to form PEO_5–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–PEO_5–TNF-α.

The in vivo characteristics of the targeted drug delivery system were investigated using tumor-bearing mice. PEO_5–TNF-α and Tf–PEO_5–TNF-α significantly delayed blood clearance compared to unmodified TNF-α. It was found that Tf–PEO_5–TNF-α showed a 1.8 and 5.3 fold increase in anti-tumor activity over PEO_5–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.

7. Synthesis and applications of other heterobifunctional PEO oligomers

7.1. Heterobifunctional PEO macroinitiators for the synthesis of segmented copolymers

Wagener and coworkers synthesized a heterobifunctional PEO containing an acetal masked hydroxyl group at one chain end and a carboxylic acid group at the other chain end [140,141]. An acetal masked potassium alkoxide was used to polymerize ethylene oxide followed by termination with succinic anhydride. The acetal–PEO–COOH macroinitiator was then employed in the ring-opening polymerization of pivalolactone followed by removal of the acetal group using HCl to yield HO–PEO–PVL–COOH. This heterobifunctional copolymer was then polymerized via step growth polymerization to produce a poly(ethylene oxide-*co*-pivalolactone) segmented copolymer.

7.2. Heterobifunctional PEO macroinitiators for free radical polymerizations

7.2.1. 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 utilized as a macroinitiator for synthesizing poly(ethylene oxide-*b*-styrene) (PEO-*b*-PS) via stable free radical polymerization [142]. 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 (Fig. 38A). 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 (Fig. 38B). End group

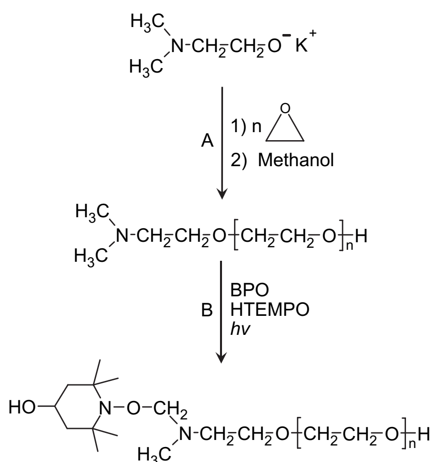


Fig. 38. Synthesis of DME–PEO–OH and TEMPO–PEO–OH.

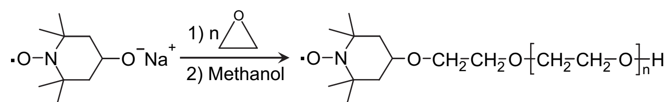


Fig. 39. HTEMPO initiated polymerization of EO.

analysis via ^{1}H 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.

A direct method for synthesizing a heterobifunctional PEO with a 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) moiety at one end and a hydroxyl group at the other has also been developed (Fig. 39) [143]. 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 ^{1}H NMR matched well with the calculated values based on the monomer to initiator ratios, and molecular weight distributions were $\leq$1.11.

The TEMPO–PEO–OH macroinitiator was utilized for stable free radial 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 [143,144]. 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, $<$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 [143].

It has also been shown that TEMPO–PEO–OH can be synthesized from a TEMPO alkoxide (Fig. 40) [145]. 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

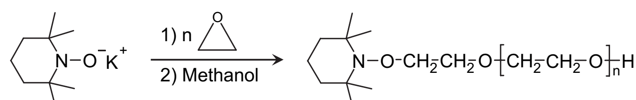


Fig. 40. Synthesis of TEMPO–PEO–OH from TEMPO alcoholate.

ratio of monomer to initiator and with narrow molecular weight distributions (<1.1). Polymer structure was confirmed by ^{1}H NMR, and molecular weights obtained via ^{1}H 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 [143–145].

7.2.2. 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 [142,146]. 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 [147]. The polymers had molecular weight distributions <1.14, and molecular weights determined by ^{1}H NMR matched closely with the predicted values. The ratio of dimethylamino to methacrylate groups was 1:1 as determined via ^{1}H NMR end group analysis. Photoinduced homo- and copolymerization of DME–PEO–methacrylate with methyl methacrylate in the presence of benzophenone produced crosslinked polymers.

7.3. Synthesis of X–PEO–Y via a protected hydroxyl group

7.3.1. 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 [148]. The polymerization of EO utilizing a benzylalkoxide 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 (Fig. 41). Heterobifunctional PPOs have also been produced utilizing benzyl alcohol as an initiator in the presence of an aluminum-porphyrin initiator [149,150].

A HO–PEO–$NH_3^+Cl^-$ 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–$NH_3^+Cl^-$.

HO–PEO–COOH and $Cl^-H_3N^+$–PEO–COOH were also prepared from BzO–PEO–OH. The hydroxyl terminus of BzO–PEO–OH was deprotonated with potassium *tert*-

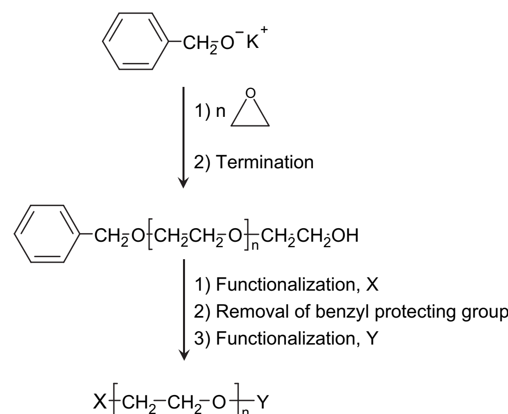


Fig. 41. Synthesis of heterobifunctional PEOs from BzO–PEO–OH.

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, $Cl^-H_3N^+$–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 [148].

7.3.2. Synthesis of HO–PEO–X from TBDMS–PEO–X

Heterobifunctional PEO macromonomers were synthesized having a hydroxyl group at one end and either a methacrylate or vinylbenzyl moiety at the other [151]. 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. [152], 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 ^{1}H NMR and vapor pressure osmometry (VPO) also matched well with the calculated values. 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 Fig. 41.

7.3.3. 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 [71]. 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 PEOs 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. 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 [153].

8. 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 bounded to a targeting moiety through a heterobifunctional PEO spacer. Heterobifunctional PEOs have also been the 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.

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