TECHNICAL NOTES

Heterobifunctional Poly(ethylene oxide): Synthesis of α-Methoxy-ω-amino and α-Hydroxy-ω-amino PEOs with the Same Molecular Weights

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Well-defined α-methoxy-ω-amino and α-hydroxy-ω-amino poly(ethylene oxide)s (PEOs) were obtained after chemical modifications of α-hydroxy-ω-allyl PEO which was synthesized by anionic polymerization of ethylene oxide (EO) with allyl alcoholate as initiator; molecular weights of the prepolymer were controlled by the monomer/initiator ratio. Addition of methyl iodide on the hydroxy function of this prepolymer led to an α-methoxy-ω-allyl PEO; completion of the reaction and purity of the resulting polymer were demonstrated by ^{1}H, ^{13}C NMR and GPC studies. Addition reactions of 2-aminoethanethiol hydrochloride on α-hydroxy-ω-allyl PEO and α-methoxy-ω-allyl PEO in the presence of azobisisobutyronitrile (AIBN) led to the expected homopolymers without any side reactions as shown by ^{1}H and ^{13}C NMR spectra.

INTRODUCTION

Owing to its nontoxicity and water solubility, poly(ethylene oxide) (PEO) has numerous applications in biochemical and biomedical fields (*1*). For example, this synthetic polymer is used as a promotor for cell fusion and hybridization (*2*) and as a chemical modification reagent for reducing or controlling the antigenicity of immunogenic proteins (*3*, *4*). Nevertheless, PEO polymers present an important disadvantage: lack of reactive groups in ethylene oxide units. For this reason, synthesis of polymers having reactive end groups is of great interest.

In the last few years, numerous studies have been focused on the synthesis of well-defined homotelechelic (*5*) and heterotelechelic (*6*) PEOs (*7*–*9*). End groups allow us to control and adjust physicochemical properties of the resulting materials. They also permit copolymerization between PEOs oligomers and defined comonomers in order to obtain hydrophobic/hydrophilic block-copolymers, for example. Furthermore, the preparation of well-defined heterotelechelic PEOs is of great interest for bioconjugation of these polymers with molecules such as proteins (*10*–*13*) or with liposomes (*14*–*16*).

Uses of proteins or liposomes as therapeutic agents are limited because of their degradation by proteolytic enzymes, thermal instability, or immunogenicity. This problem can be reduced by the formation of a conjugate between the protein or liposome and a poly(ethylene oxide). Moreover, if heterotelechelic PEOs are used, a functional group is available at the free end of PEO chains. This group allows the introduction of a homing device, for example. Low and co-workers had described the preparation of PEO-conjugated liposomes having folic acid bound to the free end of PEO. These liposomes can be targeted to cancer cells having a folic acid receptor (*15*). These "functionalized" liposomes have been also described by Crommelin and co-workers in their review on liposomes (*16*).

Ito and co-workers had prepared amphiphilic PEO macromonomers having their hydrophilic/hydrophobic balance influenced by the terminal alkyloxy group and/or the PEO chain length (*17*, *18*). Moreover, they had shown that reactivities of such PEO macromonomers for copolymerization reactions with styrene or benzylmethacrylate depended on the nature of α and ω end groups (*17*, *18*).

Recently, one of us (K.K.) and his co-workers have been studying a polymeric micelle system which can be used as high-performance vehicles for drug delivery (*19*, *20*). These polymeric micelles were prepared from PEO-poly(β-benzyl-L-aspartate) block copolymer which was synthesized by ring-opening polymerization of β-benzyl-L-aspartate *N*-carboxyanhydride (BLA-NCA) initiated with primary amino ended PEO (*21*, *22*). These polymeric micelles can entrap, chemically or physically, drugs such as adriamycin; the drug entrapped in the micelles core can be stabilized in the body and especially in the blood. Actually, a 1000-fill elevated adriamycin concentration can be maintained in the blood without any trouble as compared with adriamycin itself. Such a high concentration may be one of the reasons for the extremely high antitumor activity of this preparation (*19*).

The surface of the polymeric micelles thus prepared should be surrounded by methoxy groups. If another group, such as hydroxy, carboxyl, or thiol functions, can be introduced on the surface of the micelles, instead of methoxy groups, the resulting micelles would exhibit two interesting features: (i) characteristics of the micelle itself

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and (ii) targeting by introduction of functional substances such as sugar or antibody on the surface of the polymeric micelles.

In this paper, we report on the synthesis of heterotelechelic PEOs having the same molecular weight and distributions. These heterotelechelic PEOs are synthesized with the aim to be used for further preparation of functional polymeric micelles as described above.

EXPERIMENTAL PROCEDURES

THF, DMF, EO, allyl alcohol, and methyl iodide were purified using conventional methods (*33*). Potassium, naphthalene, AIBN, and 2-aminoethanethiol hydrochloride were used as received.

Potassium naphthalide solution was prepared by addition of potassium over naphthalene solution in THF. The mixture was stirred for 24 h under argon (Ar) atmosphere at 15 °C, and the concentration of the solution was measured by titration with 0.1 N HCl solution.

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using JEOL EX400 and EX90Q spectrometers. GPC measurements were done by using a Tosoh gel permeation chromatograph HLC-8020 equipped with a Tosoh degasser, a TSK-Gel G2000HxL precolumn, TSK-Gel G4000HxL, G3000HxL, and G2000HxL columns, an internal RI detector, and a UV-8010 detector. THF was used as solvent, and standards were poly(ethylene glycol).

Synthesis of α-hydroxy-ω-allyl PEO (1). In the polymerization flask, 160 mL of anhydrous THF, 0.54 mL (7.98 mmol) of anhydrous allyl alcohol, and 20.3 mL (7.98 mmol) of potassium naphthalide solution were added under Ar stream. This mixture was cooled into a water bath, and 50 mL (0.91 mol) of distilled EO was added via a cooled syringe under Ar stream. The solution was stirred for 48 h in the water bath under Ar atmosphere. Distilled water was added, and the polymer was extracted with chloroform. The organic phase was dried over Na_2SO_4, filtrated, and concentrated. Polymer was recovered by precipitation in a large excess of ether and freeze-dried into benzene to lead to a white powder (41.96 g, yield = 96%). GPC (THF, PEG standards): number-average molecular weight ($\bar{M}_n$) = 4900; weight-average molecular weight ($\bar{M}_w$) = 5180; polydispersity index (Ip) = 1.05. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 2.92 (s, OH, 1H), 3.63 (m, CH_2 of PEO, 480H), 4.02 (d, CH_2 of allyl group, 2H), 5.17−5.30 (dd, CH_2 of allyl group, 2H), 5.87−5.96 (m, CH of allyl group, 1H).

Synthesis of α-Methoxy-ω-allyl PEO (2). The polymer **1** (21.06 g) was put into the reaction flask and dried under vacuum at room temperature overnight. Anhydrous THF (210 mL) was added under an Ar stream, and potassium naphthalide solution was added until a green color remained (2 equiv, 7.86 mmol). Then, 3.7 mL (15 equiv, 59 mmol) of distilled methyl iodide was added under Ar stream. The mixture was stirred for 72 h at 50 °C. Water was added, and polymer was extracted by chloroform. The organic phase was dried over Na_2SO_4. After filtration and concentration, the polymer was precipitated into ether and freeze-dried into benzene to lead to polymer **2** as a white powder (16.68 g, yield = 79%). GPC (THF, PEG standards): $\bar{M}_n$ = 4870; $\bar{M}_w$ = 5110; Ip = 1.05. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 3.37 (s, CH_3, 3H), 3.63 (m, CH_2 of PEO, 568H), 4.02 (d, CH_2 of allyl group, 2H), 5.17−5.30 (dd, CH_2 of allyl group, 2H), 5.87−5.96 (m, CH of allyl group, 1H).

Synthesis of α-Hydroxy-ω-amino PEO (3). The polymer **1** (20.90 g) was put in the reaction flask and dried under vacuum at room temperature. Anhydrous DMF (150 mL) was added under an Ar stream. Separately, 6.6 g (15 equiv, 58.4 mmol) of 2-aminoethanethiol hydrochloride and 643 mg (7.5×10^{-1} equiv, 2.3 mmol) of AIBN were weighed and dried under vacuum at room temperature. Thirty mL of anhydrous DMF was added under an Ar stream. This solution was transferred into the previous one under an Ar stream, and the mixture was stirred at 70 °C for 24 h under Ar atmosphere. The polymer was precipitated two times in a large excess of ether. After filtration, the white powder was dissolved into methanol, and 0.22 g (1 equiv, 3.8 mmol) of KOH dissolved in water was added. The mixture was stirred for about 30−60 min. Then, water was added and polymer was extracted by chloroform. After drying over Na_2SO_4, filtration, and concentration, the polymer was precipitated into ether and freeze-dried into benzene to lead to a white powder (17.37 g, yield = 83%). GPC (THF, PEG standards): $\bar{M}_n$ = 5190; $\bar{M}_w$ = 5560; Ip = 1.07.

Synthesis of α-Methoxy-ω-amino PEO (4). Experimentals details are the same as described above for the preparation of polymer **3** with 16.5 g of polymer *2*, 120 mL of anhydrous THF, 5.2 g of 2-aminoethanethiol hydrochloride, and 378 mg of AIBN. The polymer **4** was recovered as a white powder (15 g, yield = 91%). GPC (THF, PEG standards): $\bar{M}_n$ = 4800; $\bar{M}_w$ = 5150; Ip = 1.07.

RESULTS AND DISCUSSION

The synthesis of heterotelechelic PEO, having (primary amino/hydroxy) and (primary amino/methoxy) end groups with the same molecular weight and distributions, contains the three following steps: (i) synthesis of heterotelechelic PEO with (allyl/hydroxy) end groups; (ii) methylation of hydroxy terminal group; and (iii) amination of allyl end groups.

Use of allyl alcoholate as initiator for anionic polymerization of EO allowed the introduction of an unsaturated double bond at one end and a hydroxy function at the other end of the PEO chain. These two functional groups allow several kinds of chemical modifications such as radical addition reactions (*23*) for allyl group and introduction of an active group via ether or ester bonds for the hydroxy function (*24*).

The initiator, allyl alcoholate, was prepared *in situ* by reaction between allyl alcohol and potassium naphthalide solution which was prepared by addition of potassium on naphthalene solution in THF (Scheme 1). EO was added to the allyl alcoholate solution and reacted for 2 days at room temperature (disappearence of monomer and oligomer peaks on GPC chromatogram). Hydroxy end groups were recovered by addition of an excess of acetic acid (Scheme 1). After chloroform was added to the reaction mixture, the solution was washed with water several times in order to remove impurities. The PEO thus obtained was further purified by precipitation into ethyl ether and then freeze-dried from benzene solution. The ^{1}H NMR spectrum of this α-hydroxy-ω-allyl PEO (**1**) showed a high degree of purity; this result was confirmed by ^{13}C NMR. Moreover, molecular weights determined by GPC (THF, PEG standards) and calculated from ^{1}H NMR were in good agreement with molecular weights obtained from the ratio monomer/initiator (Table 1). These results demonstrated that anionic polymerization of EO by allyl alcoholate led to the expected PEO with an allyl group at one end, hydroxy function at the other end, and well-defined molecular weights and narrow dispersity.

Addition of methyl iodide on hydroxy end groups of polymer was described by Kobayashi and his coworkers (*25*). However, their experimental conditions were not adapted for our case. Indeed, after 24 h at room temperature with 5 equiv of CH_3I, ^{1}H and ^{13}C NMR spectra

Scheme 1. Synthetic Route to α-Hydroxy-ω-amino PEO (3) and α-Methoxy-ω-amino PEO (4)

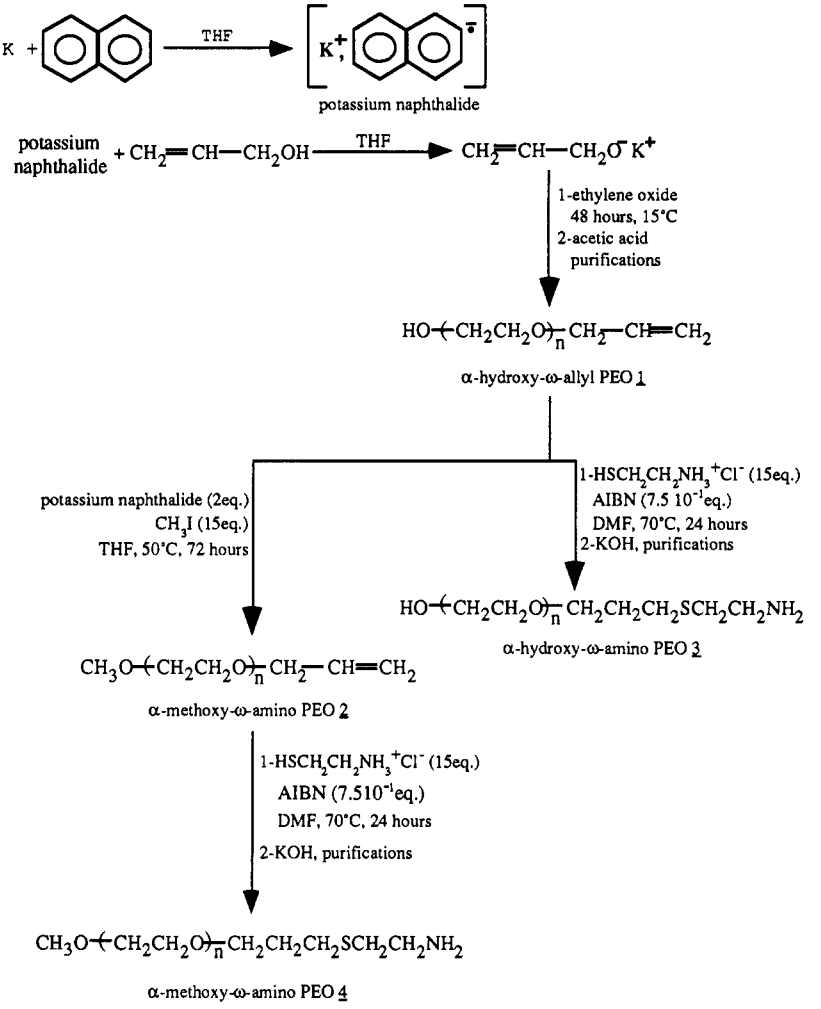

Table 1. Data for Molecular Weights of Polymers 1–4 and Their Distributions

	$\bar{M}_n$[a]	$\bar{M}_w$[a]	Ip	M_s[a]	M_{NMR}[b]	M_{cal}[c]
1	4900	5180	1.05	5100	5280	5000
2	4870	5110	1.05	5100	5630	5000
3	5190	5560	1.07	5100	5060	5000
4	4800	5150	1.07	5100	5320	5000

[a] Determined by GPC in THF with PEG standards. [b] Calculated from ^{1}H NMR spectra in $CDCl_3$. [c] Calculated from the ratio ethylene oxide/allyl alcoholate.

of the resulting polymer showed the presence of unreacted hydroxy end groups (about 30%). In order to determine if some side reactions could take place when the temperature was increased, we studied the model reaction between allyl alcohol, methyl iodide, and potassium naphthalide in THF at 50 °C. After 12 h at 50 °C, the reaction milieu was examined by GC/MS: absence of any kind of adduct on the double bond of allyl alcohol and formation of addition compound between allyl alcohol and methyl iodide via the hydroxy function were demonstrated. In view of these results, methyl iodide addition on α-hydroxy-ω-allyl PEO (**1**) was carried out as described in the Experimental Procedures. The ^{1}H NMR spectrum of the purified polymer demonstrated completion of this addition reaction: the peak corresponding to the hydroxy function at 2.9 ppm disappeared and a new peak appeared at 3.37 ppm, corresponding to methoxy group. These results were confirmed by the ^{13}C NMR spectrum: peaks corresponding to methylene adjacent to hydroxy function (CH_2CH_2OH: δ = 61.5 ppm, CH_2CH_2-OH: δ = 72.33 ppm) disappeared in favor of the appearance of new peaks at 59.05 ppm (OCH_3) and at 72 ppm ($CH_2CH_2OCH_3$). Moreover, molecular weights determined by GPC (THF, PEG standards) and by ^{1}H NMR were very similar to those of PEO **1**; dispersity was also very narrow (Ip = 1.05, Table 1). These data demonstrated that α-hydroxy-ω-allyl PEO (**1**) was successfully transformed in α-methoxy-ω-allyl PEO (**2**) via addition of methyl iodide on hydroxy function, without any side reactions and detectable chain degradation.

Thiol compounds are known to control molecular weights of elastomers prepared by emulsion polymerization (*26*). These transfer reagents are also used to prepare specific macromonomers (*27*). Moreover, functional groups can be introduced efficiently to the end of growing polymeric chains using thiol compounds having functional groups. In addition, molecular weights of corresponding polymers can be regulated by radical telomerization via chain-transfer reactions (*23*). Takei and co-workers (*28*) have described the telomerization reaction of *N*-isopropylacrylamide using 3-mercaptopropionic acid as a chain transfer agent in the presence of AIBN at 70 °C in DMF.

On the basis of the above reports, we adopted radical addition reaction of 2-aminoethanethiol hydrochloride with an allyl end group of PEO in order to get primary amino-ended PEO at one end. The reactions were carried out under the conditions of [PEO(**1** or **2**)]$_0$/[$HSCH_2CH_2NH_3^+Cl^-$]$_0$/[AIBN]$_0$ = 1/15/7.5 × 10^{-1} mol equiv in DMF at 70 °C. Crude polymers (**4** and **5**), thus obtained, were precipitated two times in ether in order to remove excess

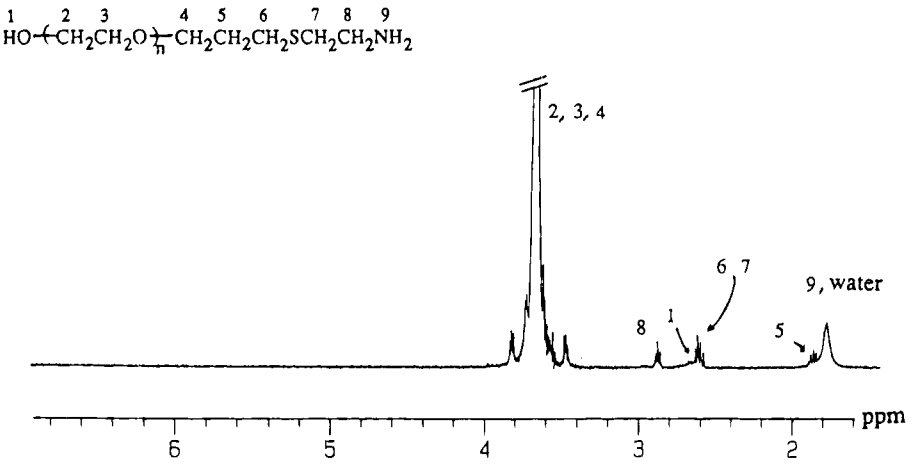

Figure 1. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of α-hydroxy-ω-amino PEO (**3**).

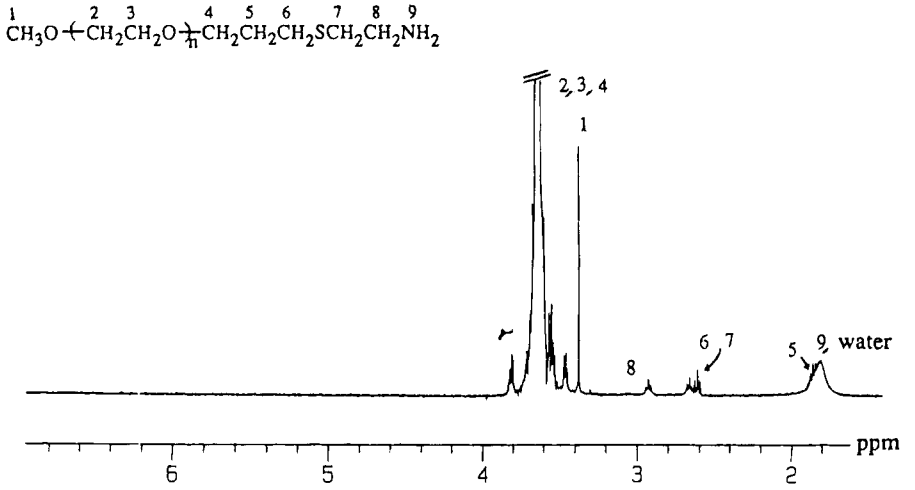

Figure 2. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of α-methoxy-ω-amino PEO (**4**).

of reagents. Primary amine end groups were recovered by addition of potassium hydroxide solution (Scheme 1). ^{1}H NMR spectra of both α-hydroxy-ω-amino and α-methoxy-ω-amino PEOs showed completion of reaction without formation of any byproducts (Figures 1 and 2), results confirmed by ^{13}C NMR. Molecular weights determined by GPC (THF, PEG standards) were in good agreement with molecular weights calculated from ^{1}H NMR spectra and with those of starting polymer (Table 1), moreover, dispersity of both modified homopolymers stayed very narrow (Ip = 1.07). These results demonstrated that the addition reactions of 2-aminoethanethiol hydrochloride on α-hydroxy-ω-allyl and on α-methoxy-ω-allyl PEOs (**1**) and (**2**) gave access to the expected heterobifunctional PEOs without any side reactions and significant polymer alteration.

This new synthetic route is very interesting. Indeed, amino-ended PEOs are important as intermediate in the synthesis of other derivatives or in direct applications, and several routes for their preparation have been explorated (*9*). In 1979, Kern and co-workers have described a direct route to α,ω-diamine oligo(oxyethylene) by reaction of ditosyl esters of α,ω-dihydroxy oligo(oxyethylenes) with potassium 2-aminoethanolate: percentage of modification decreased when molecular weight of the polymers increased (*29*). Ziegast and co-workers brought some modifications to this synthesis route in order to modify higher molecular weight PEOs; percentage of modification is higher but not quantitative (up to 95%) (*30*). In 1981, Bückmann and co-workers as well as Johansson and co-workers have described two direct syntheses for primary amino-ended PEOs. The first group used gaseous ammonium, high temperature, and high pressure (glass autoclave is required): although this method gives 100% substitution, difficulty to set up limited its use (*31*). The second group described a method which is easily applied but primary and secondary amine are produced (*32*). In comparison, addition reaction of 2-aminoethanethiol hydrochloride on α-hydroxy (or methoxy)-ω-allyl PEOs provides a simple and reproducible way to introduce primary amino end group at one end of PEO chains. Moreover, this one-step synthesis allows us to obtain 100% modification.

CONCLUSION

Heterobifunctional PEOs having a hydroxy or methoxy group at one end and primary amine function at the other end were synthesized with well-controlled molecular weights and in high yield (more than 80%). Moreover, nonmodified and modified homopolymers had a very narrow dispersity (≤1.07) probe of the efficiency of anionic polymerization of EO with allyl alcoholate and absence of any side reactions and chain degradation during chemical modifications of end-groups.

The possibility to introduce an allyl group at one end and alkyl or functional group at the other end of the PEO chain is of great interest. Indeed, physicochemical properties (solubility, copolymerization, biocompatibility) can be adjusted through the chemical modifications of these end-groups. As a result, a large family of derivatives having various applications, e.g., as surface coating

or drug delivery systems, can be synthesized. The allyl end group also allows quantitatively conversion into a primary amino moiety via radical addition reactions of amino-thiol compound.

Synthesis of poly(ethylene oxide)-*co*-poly(β-benzyl-L-aspartate), PEO/PBLA block-copolymers, with a hydroxy and/or methoxy end group with the same molecular weight and their characteristics as micelles will be published elsewhere.

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