Synthesis and Characterization of Thiol-Terminated Poly(ethylene oxide) for Chemisorption to Gold Surface

Ying Jun Du, John L. Brash

Department of Chemical Engineering, McMaster University, Hamilton, Ontario, Canada L8S 4L7

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ABSTRACT: Thiol-terminated poly(ethylene oxide) (PEO) was synthesized using two different approaches: esterification of terminal hydroxyl groups with mercaptoacetic acid and amidation using *N*-hydroxysuccinimidyl PEO (NHS–PEO) and cysteine. The reaction of hydroxyl-terminated PEO with mercaptoacetic acid was carried out in boiling toluene. Different thiolated PEOs, including linear PEOs of varying molecular weights and end-group types, and star-type PEOs were synthesized. Nuclear magnetic resonance and infrared spectroscopy were used to characterize the products. The reaction kinetics were also briefly investigated. Gel permeation chromatography was used to investigate the relative amounts of the mono- and disubstituted products in the α,ω-dihydroxy PEOs. NHS–PEO was used both to attach terminal thiol groups via reaction with cysteine and to conjugate other amino acids (and potentially any amino-containing molecule) to PEO. Reactions using NHS–PEO were carried out at room temperature in water. The chemisorption of these thiolated PEOs to gold was expected to yield surfaces resistant to biofouling, in particular to unwanted protein adsorption. Chemisorption of amino acid–, peptide-, or protein-terminated PEOs in addition may yield surfaces having specific biological activity. Work on these aspects will be reported elsewhere. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 594–607, 2003

Key words: poly(ethylene oxide); thiolation; amino acids; NMR; gel permeation chromatography

INTRODUCTION

Passivation to minimize nonspecific protein adsorption and introduction of bioactive ligands to promote adsorption of specific targeted proteins are believed to be useful approaches to improve the biocompatibility of biomaterials.[1–4] Surface attachment of hydrophilic polymers by their chain ends has been found to be effective for passivation. The steric repulsion effects believed to be associated with such polymers may exclude proteins and other macromolecules from the surface.[5–9] Poly(ethylene oxide) (PEO) has been extensively studied for this purpose,[10] and has been grafted to different types of surfaces using a variety of techniques.[11–31]

Surfaces consisting of self-assembled monolayers (SAMs) containing PEO on gold[32–35] may be useful for biomaterial applications because of the bioinertness of gold and the potentially high surface density of the SAMs.[36–38] Gold surfaces can readily be modified via reaction with thiol-containing compounds at room temperature under generally mild conditions.[38] On the (1,1,1) surface of crystalline gold the modification sites are in the threefold hollows between gold atoms spaced about 5 Å apart, corresponding to a surface density of 4.6×10^{14} molecules/cm^2 if all sites are occupied.[39] When an alkyl thiol compound is used, it can form densely packed, crystal-like assemblies with fully extended zigzag carbon chains tilted at about 30° to the normal in order to maximize interactions between adjacent chains when the chain length is of an optimum value.[40] A number of factors, such as the method of preparing the gold substrate[32,33,41–46] and the solvent from which the thiol is chemisorbed,[47,48] have been shown to influence the structure and quality of alkane thiol monolayers on gold. Surfaces consisting of layers of amphiphilic diglycol-alkyl chains on gold were reported to have significant protein-repelling properties.[17] It has also been shown[49] that PEO-modified gold exhibits excellent passivation toward protein adsorption and platelet adhesion. Although it is not clear whether chain-end thiolated PEO chemisorbed on gold will form high-density self-assembled monolayers, it is expected that such surfaces should be able to inhibit nonspecific protein interactions to some degree.

A few reports have appeared on the thiolation of PEO. Harris et al.[50] synthesized PEO thiols using tosylated PEO and the thiolation agent sodium hydrosulfide. Herron et al.[51] have used *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) to thiolate PEO using an amino-functionalized PEO. However, neither method allowed one-step direct thiolation, and the formation of disulfide appeared to be a complication.

In this article we present a method for the direct thiolation of hydroxy-terminated PEO by reaction

Correspondence to: Dr. J. L. Brash (brashjl@mcmaster.ca).

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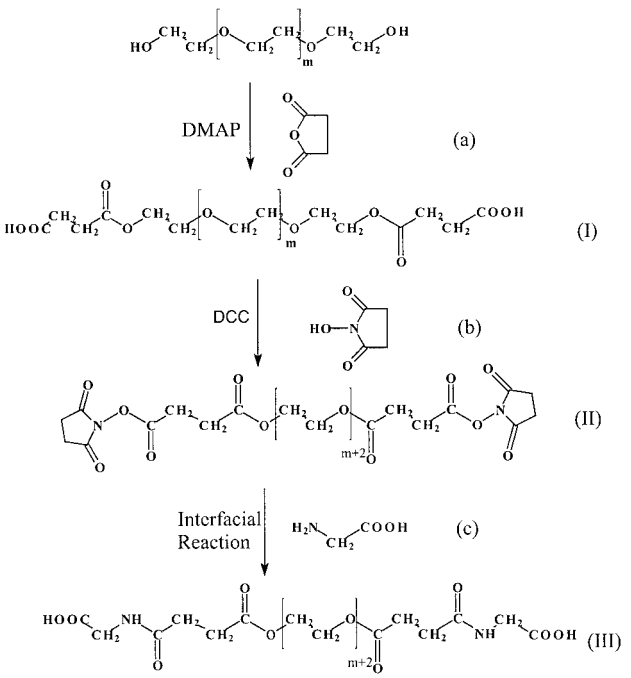

Scheme 1 Conjugation of amino acids to PEO: (a) carboxylation of chain ends, (b) conversion of carboxyls to NHS esters, (c) conjugation of amino acid (glycine example).

with mercaptoacetic acid. A reaction scheme to derivatize PEO with a thiol group at one chain end and an amino acid residue at the other was also investigated. In this protocol (by which peptides and proteins, as well as most amino acids may be attached to PEO), the PEO is first activated by derivatization at the chain ends with *N*-hydroxysuccinimide (NHS).[52] The NHS moiety can then react with an amino group of the amino acid, peptide or protein. Thiolation is accomplished by reacting the NHS–PEO with cysteine.

EXPERIMENTAL

Materials

Linear PEOs with a hydroxyl group at one end of the chain and a methoxyl group at the other (molecular weights of 100–5000), linear PEOs with hydroxyl groups at both chain ends (molecular weights of 100–5000), and star-type PEOs with hydroxyl groups at all chain ends (total molecular weights of 2000 and 8000) were from Shearwater Polymers, Inc. (Huntsville, AL).

Tetrahydrofuran, toluene, succinic anhydride, glycine methyl ester, aminoethane thiol, ether, potassium carbonate, isopropyl ether, *N*,*N*-dimethylaminopyridine (DMAP), *N*-hydroxysuccinimide (NHS), dicyclohexyl carbodiimide (DCC), mercaptoacetic acid, isopropyl ether, dichloromethane, and dimethyl formamide were from Aldrich (Oakville, Ontario, Canada). Cysteine and lysine were from Sigma (Oakville, Ontario, Canada).

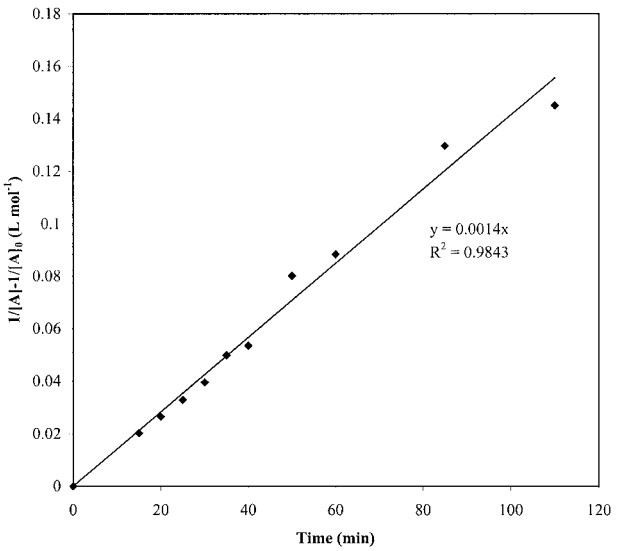

Figure 1 Second-order plot of kinetic data for the self-condensation of mercaptoacetic acid catalyzed by sulfuric acid (temperature: 113°C). The solid line is a linear regression of the data over the first 110 min [*A*] = molar concentration of mercaptoacetic acid derived from ($[A]_0 - [D]$), in which $[A]_0$ is the concentration of *A* at time zero and [*D*] is the concentration of water at time *t*.

Thiolation of PEO using mercaptoacetic acid

A typical thiolation reaction was carried out as follows. Monomethoxy PEO with a molecular weight of 350 [MPEO (350)–OH; 10.00 g, 10 mmol] was introduced into a 50-mL three-necked flask equipped with a stirrer and a graded distillation trap prefilled with toluene. (For kinetic studies, water condensing in the

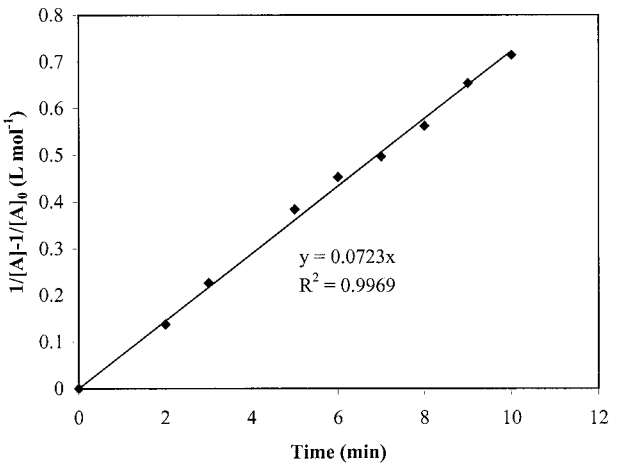

Figure 2 Second-order plot of kinetic data for the reaction of mercaptoacetic acid with MPEO (350)–OH catalyzed by sulfuric acid (temperature: 113°C). The solid line is a linear regression of the data over the first 10 min [*A*] = molar concentration of mercaptoacetic acid derived from ($[A]_0 - [D]$), in which $[A]_0$ is the concentration of *A* at time zero and [*D*] is the concentration of water at time *t*.

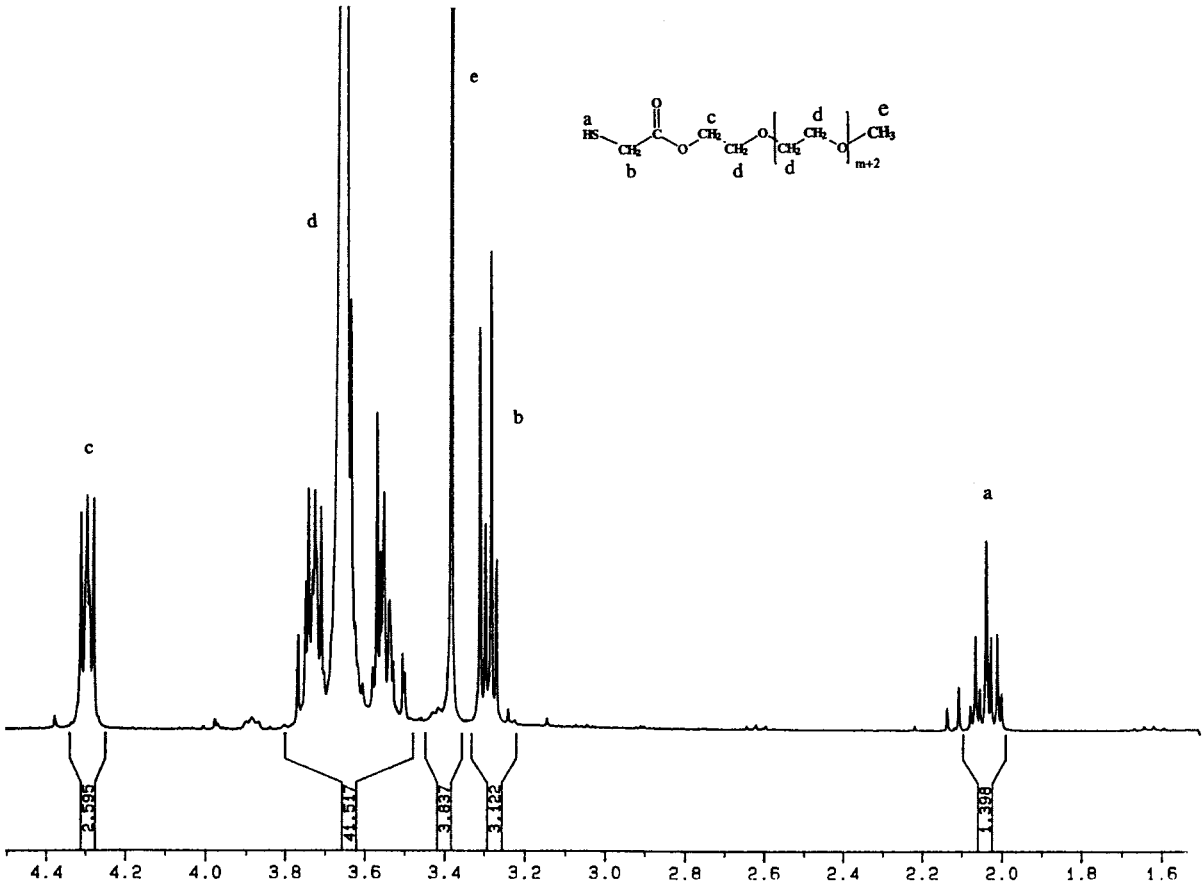

Figure 3 Proton NMR spectrum of the product of reaction between MPEO (350)–OH and mercaptoacetic acid. Peak (c) at 4.3 ppm is assigned to the methylene protons of PEO directly bonded to the carboxyl group of mercaptoacetic acid. The presence of peak (a) at about 2.1 ppm indicates that the thiol group did not react further to give a disulfide-linked dimer.

trap displaced the toluene, which flowed back to the flask to maintain a constant volume.) The system was evacuated over a 30-min period at 80°C. Toluene (40 mL) was then introduced to dissolve the PEO. The flask was heated in an oil bath to 110°C. Mercaptoacetic acid (2.76 g, 30 mmol) and concentrated sulfuric acid (2 drops) were then added. The reaction was allowed to proceed for 2 h. The product [MPEO (350)–

TABLE I
Thiolation of Linear PEOs of Different Molecular Weights and End Groups by Reaction with Mercaptoacetic Acid (Data are Percent Hydroxyl Groups Converted To—$OCOCH_2$—SH, as Determined by NMR)

MW of PEO or PPO	114	300	600	750	1025	1500	3400	4600	5000
HO—PEO—OH ↓ HS—PEO—OH	nd	53	50	nd	nd	47	nd	47	nd
HO—PEO—OCH_3 ↓ HS—PEO—OCH_3	~100	nd	nd	~100	nd	nd	~100	nd	~100
HO—PEO—OH ↓ HS—PEO—SH	nd	~100	~100	nd	nd	~100	nd	~100	nd
HO—PPO—OH ↓ HS—PPO—OH	nd	nd	nd	nd	~50	nd	nd	nd	nd

[a] nd = not done. Data precision is on the order of ±5%.

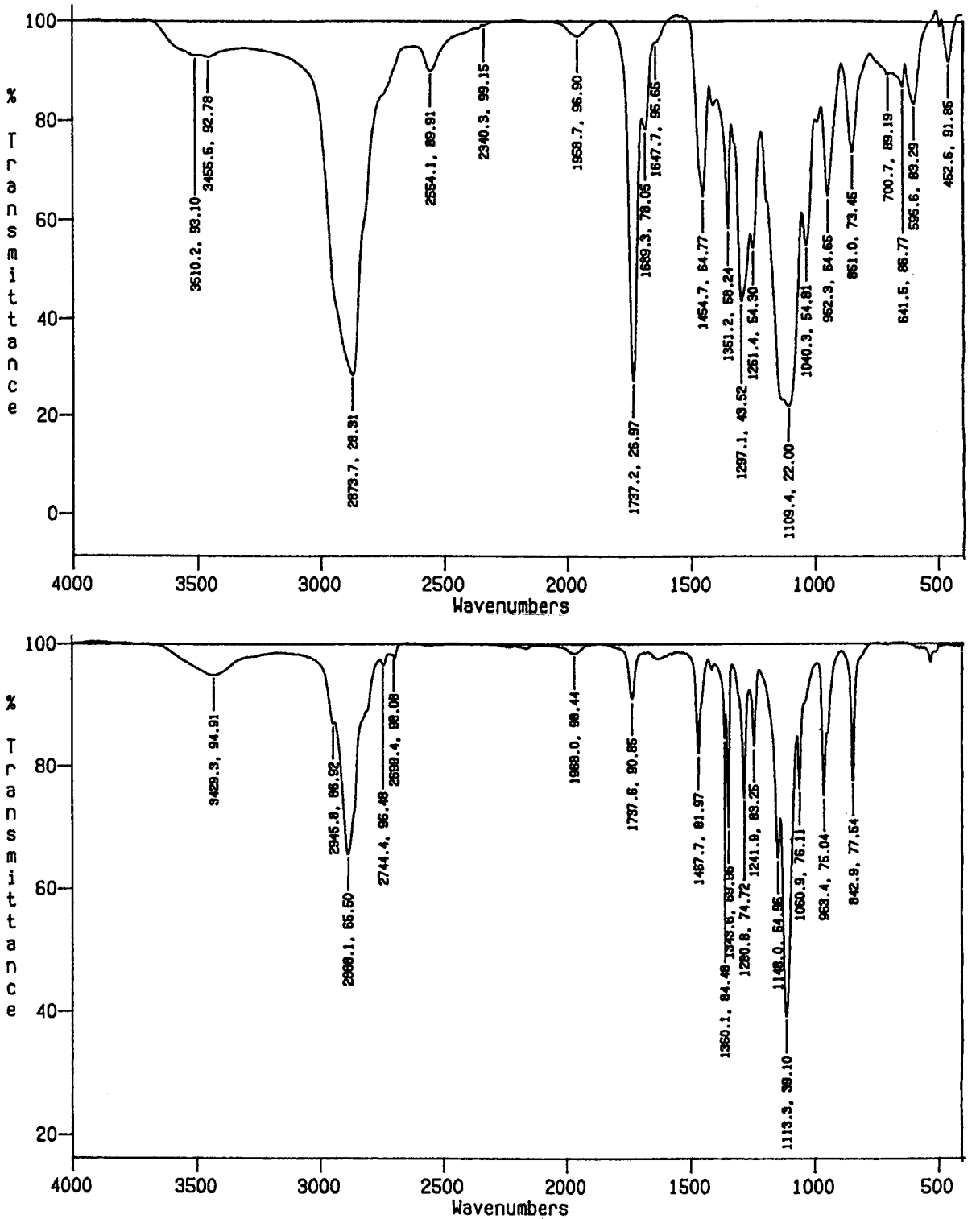

Figure 4 FTIR spectra of thiolated PEO. The upper and lower panels show the spectra of thiolated PEO of molecular weights 350 and 3400, respectively. Both spectra show a peak at 1737 cm^{-1}, indicating the presence of an ester group. The intensity of this peak in the two spectra relative to an internal reference, for example, the C—O—C vibration at about 1110 cm^{-1}, indicates the relative numbers of ester bonds.

SH] was obtained by precipitating three times in ether and then drying under vacuum at 40°C overnight. The volume of water produced was measured as a function of reaction time by collection in the distillation trap, and the data were used to investigate the reaction kinetics.

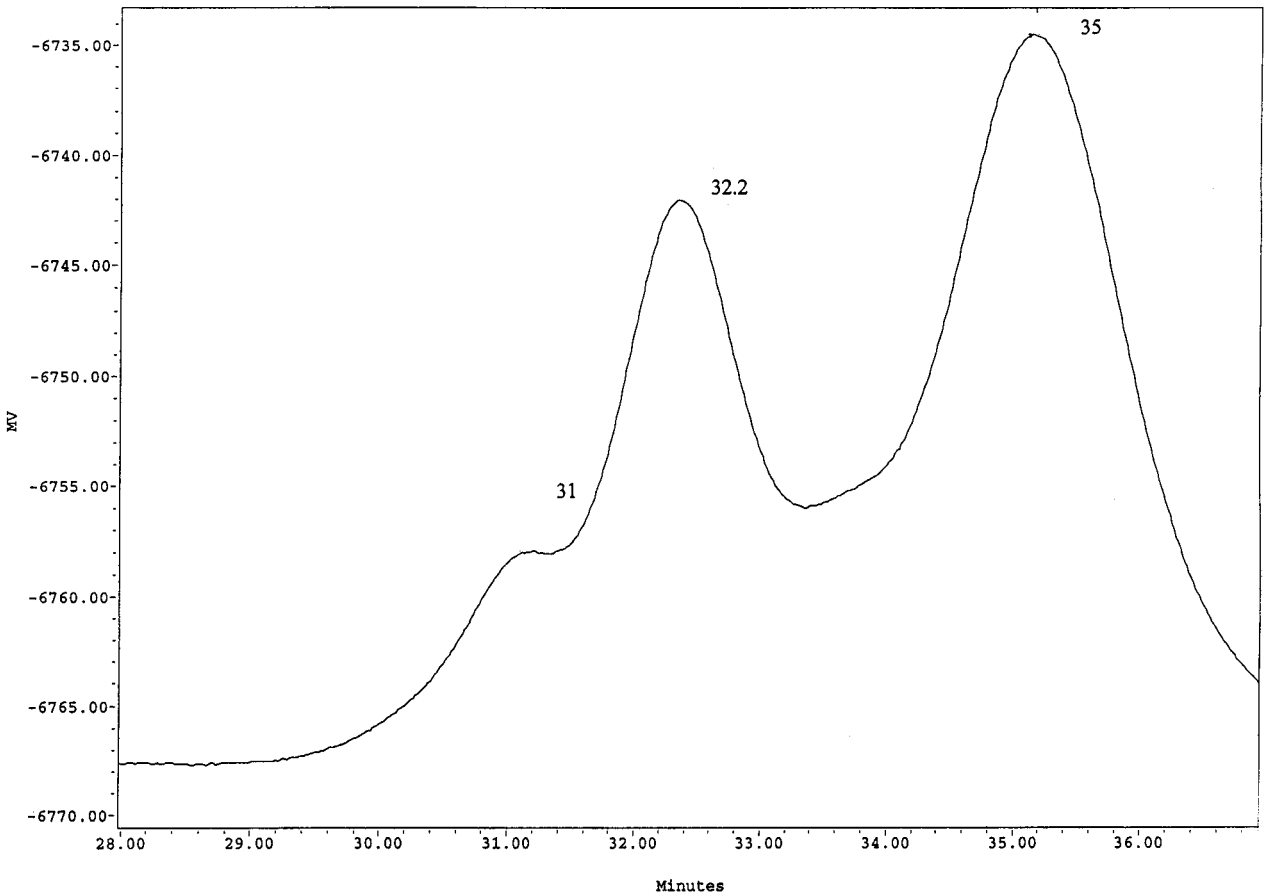

Figure 5 GPC chromatogram of the product of reaction between dihydroxy PEO (*MW* 2000) and carboxy-methoxy PEO (*MW* 1000) with a molar ratio 1:0.5. Three peaks are resolved and may be assigned to the disubstituted product (31 min), the monosubstituted product (32 min), and the unreacted PEO (dihydroxy PEO of *MW* 2000, 35 min). The assignments of peaks are based on the calibration curve constructed from a series of monodispersed polystyrenes.

Conjugation of amino acids to PEO

Conjugation of amino acids to PEO was carried out in three steps. In the first step, PEO–OH was converted to PEO–COOH by reaction with succinic anhydride (reaction a, Scheme 1). For example, MPEO (350)–OH (3.50 g, 10 mmol) was vacuum-dried at 80°C for 30 min in a round flask and dissolved in tetrahydrofuran (THF, 40 mL). Succinic anhydride (SA; 2.00 g, 20 mmol) and *N*,*N*-dimethylaminopyridine (DMAP; 0.12 g, 1.0 mmol) were then added. The solution was held at room temperature for 8 h. The product was precipitated using isopropyl ether. The workup procedure was repeated twice more by dissolving the product in dichloromethane and precipitating in isopropyl ether (volume ratio 1:5). The final product was dried under vacuum at room temperature overnight.

In the second step PEO–COOH was converted to PEO–NHS (reaction b, Scheme 1). For example, MPEO (350)–COOH obtained from reaction a (1.73 g, 4.0 mmol) was dissolved in THF (20 mL). *N*-Hydroxysuccinimide (NHS; 0.42 g, 4.0 mmol), and dicyclohexylcarbodiimide (DCC; 1.04 g, 5.0 mmol) were then added. The solution was held at room temperature for 4 h. After filtration to remove impurities, the solid product, NHS–PEO, was obtained by evaporating the solvent. It was then extracted with methylene chloride.

In the third step the amino acid was conjugated to NHS–PEO via an amide linkage (reaction c, Scheme 1). For example, MPEO (350)–NHS (1.04 g, 2.0 mmol) from reaction b was dissolved in THF (20 mL). Glycine methyl ester (0.25 g, 2.0 mmol) and potassium carbonate (0.15 g, 1.0 mmol) in 20 mL of distilled water were added to the solution, and the mixture was allowed to react at room temperature for 1 h. The product, MPEO (350)–amino acid, was precipitated using isopropyl ether. The workup procedure was repeated twice more by dissolving the product in dichloromethane and precipitating in isopropyl ether (volume ratio 1:5). The product was dried under vacuum at room temperature overnight.

To obtain conjugates that had a thiol group at one end of the PEO and an amino acid at the other, NHS–PEO–NHS was used as the starting material, and a

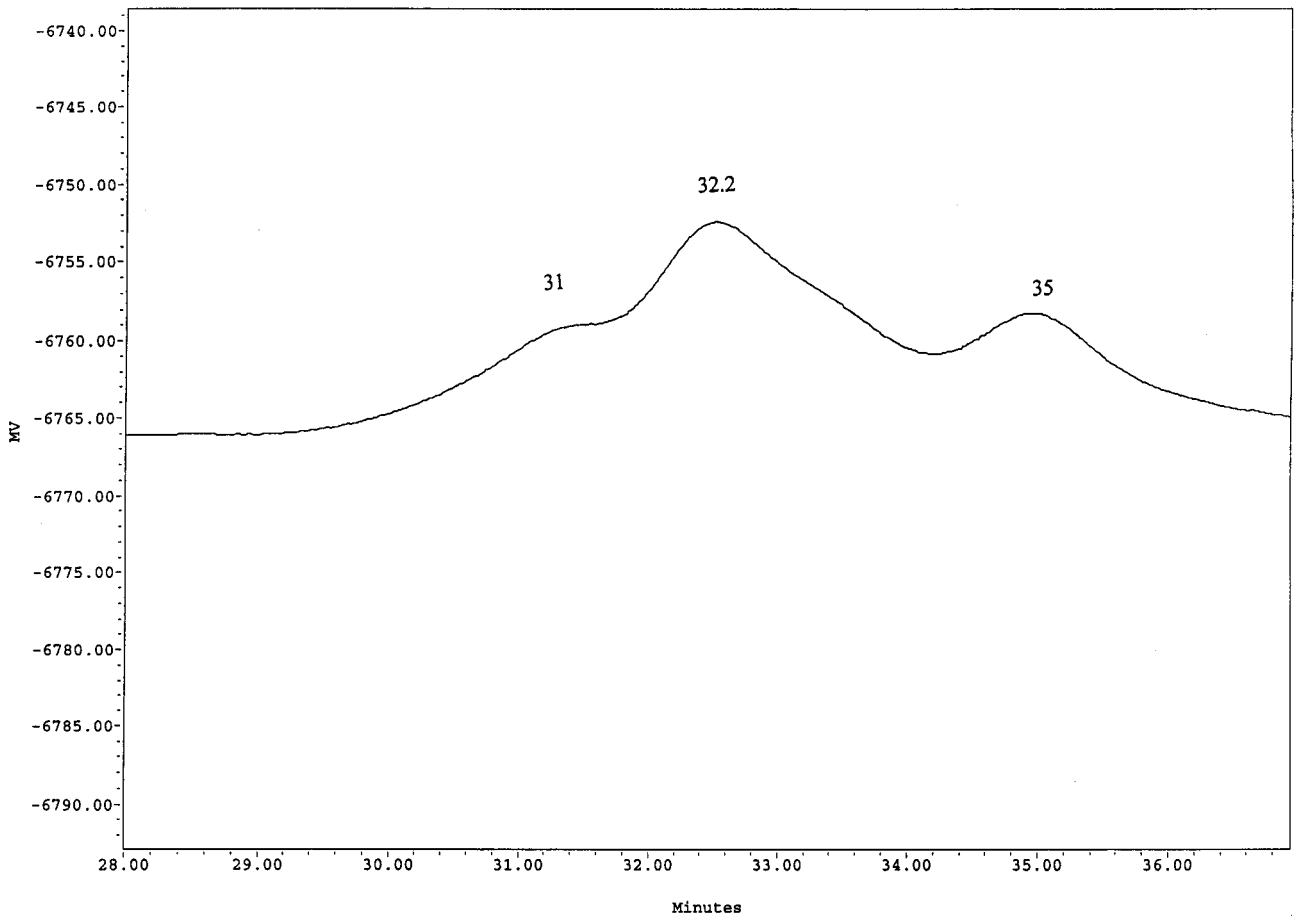

Figure 6 GPC chromatogram of the product of reaction between dihydroxy PEO (*MW* 2000) and carboxy-methoxy PEO (*MW* 1000) with a molar ratio of 1:1. Three peaks are resolved and may be assigned to the disubstituted product (31 min), the monosubstituted product (32 min), and the unreacted PEO (dihydroxy PEO of *MW* 2000, 35 min). The assignments of peaks are based on the calibration curve constructed from a series of monodispersed polystyrenes.

mixture of aminoethane thiol and amino acid was used in the final step. Because it was not intended for all of the product molecules to have an amino acid residue, ratios of amino acid to aminoethane thiol of less than 1 (generally 1:5) were used. To minimize the probability that both chain ends would be the same, the amino acid was added to the reaction 5 min prior to the aminoethane thiol.

Polymer characterization

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-300 spectrometer (Karlsruhe, Germany). Yields of the modified PEO products were calculated from the relative intensities of the methylene protons at 4.3 and 3.7 ppm. Infrared spectra were taken with a Mattson Polaris Fourier transform infrared (FTIR) spectrometer, with samples in the form of KBr pellets. Gel permeation chromatography analysis was carried out using a Waters 600 system (Waters Associates, USA) with a Waters 410 differential refractometer detector. The eluent, dimethyl formamide, flowed at 1 mL/min through a Waters styragel column combination of HR-4, HR-2, HR-1, and HR-0.5 maintained at 80°C. The columns were calibrated with narrowly dispersed (M_w/M_n of 1.01–1.03) polystyrene standards purchased from Toyosoda Corporation (Tokyo, Japan).

RESULTS AND DISCUSSION

Thiolation of PEO using mercaptoacetic acid

Thiolation of linear monomethoxy PEO and dihydroxy PEO of various molecular weights was carried out by reaction with mercaptoacetic acid using sulfuric acid as catalyst (Reaction 1).

$$\mathrm{HS{-}CH_2{-}COOH + HO{-}PEO{-}R} \underset{\text{Toluene}}{\overset{\mathrm{H_2SO_4}}{\rightleftharpoons}} \mathrm{HS{-}CH_2{-}COO{-}PEO{-}R + H_2O} \quad (1)$$

To force the equilibrium to the product side, the water-toluene azeotrope formed was distilled as the reaction proceeded.

TABLE II
GPC Peak Areas (Given as Ratios) of Products from Esterification of HO—PEO—OH (D) by MPEO—COOH (M) using two M:D Molar Ratios

MW	Peak evaluation		
	2000	3000	4000
Elution time (min)	35	32.2	31
Peak areas (M:D = 0.5, experiment)	1.67	1.0	0.33
Peak areas (M:D = 0.5, theory)	1.5	1.0	0.17
Peak areas (M:D = 1.0, experiment)	0.4	1.0	0.32
Peak areas (M:D = 1.0, theory)	0.5	1.0	0.5

It should be noted that mercaptoacetic acid can undergo self-condensation, as indicated in Reaction 2.

$$2\ HS{-}CH_2{-}COOH \underset{\text{Toluene}}{\overset{H_2SO_4}{\rightleftharpoons}} HS{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}S{-}CH_2{-}COOH + H_2O \quad (2)$$

To investigate the extent to which this side reaction might be occurring, kinetic studies were carried out on the self-condensation and conjugation reactions by measuring the production of water, using a volumetric method. Figures 1 and 2 show plots of the kinetic data for, respectively, the self-condensation and conjugation reactions. The expectation from the simple mechanisms $A + B \rightarrow C + D$ and $2B \rightarrow E + D$ (where *A*, *B*, *C*, *D*, and *E* are PEO, mercaptoacetic acid, ester, water, and mercaptoacetic acid dimer, respectively) is that the reactions should follow second-order kinetics. Accordingly, plots of $1/[A]$, in which $[A]$ was derived from $([A]_0 - [D])$, were constructed to test the data. As can be seen, the plots are reasonably linear, over the first 100 min for the self-condensation reaction(Fig. 1) and over the first 10 min for the esterification reaction (Fig. 2). The rate constants were estimated from the slopes of the regression lines. The estimated rate constant for the self-condensation reaction (1.4×10^{-3} L mol^{-1} min^{-1}) was found to be considerably smaller than that for the esterification reaction (7.2×10^{-2} L mol^{-1} min^{-1}), indicating that the latter reaction was favored, making it unlikely that the self-condensation polymerization of mercaptoacetic acid occurred to any significant extent under these conditions.

As indicated previously, linear PEOs of different molecular weights and having different groups at the chain ends were used in this work. NMR and infrared spectroscopy were used to characterize the reaction products. Figure 3 shows a proton NMR spectrum of the product from MPEO (350)–OH. Peak (c), at 4.3 ppm, is assigned to the methylene protons of PEO bonded directly to the carboxyl group of mercaptoacetic acid. The presence of peak (a) at about 2.1 ppm indicates that the SH group did not react further to form a disulfide-linked dimer. The yield of this reaction calculated from integration of the NMR peaks was essentially quantitative, as shown below.

Integration of the NMR spectral peaks was used to estimate the yields of the reactions. The intensity of peak (d), at 3.67 ppm ($I_{3.67}$), indicates the number of methylene protons in the PEO repeat units excluding the methylene group adjacent to the ester bond. The latter are represented by peak (c), at 4.3 ppm ($I_{4.3}$). The esterification yield may be defined as:

$$\text{yield} = \frac{\text{Number of end group protons reacted}}{\text{Number of end group protons initially}}$$

For the NMR data this can be written as

$$\text{Yield} = \frac{I_{4.3}}{\dfrac{I_{3.67} + I_{4.3}}{N}} \quad (1)$$

where N is the chain length of the PEO (estimated from the molecular weight data provided by the supplier). Table I lists typical data, which indicate that the esterification yields were in general greater than 90% of the expected values.

Infrared spectroscopic data confirmed that esterification occurred. Figure 4 shows the FTIR spectra of MPEO (350)–SH and MPEO (3400)–SH. The presence of an ester group was expected to give rise to a peak around 1750 cm^{-1} (C═O stretch). Thus, the peak at 1737 cm^{-1} in the spectra of Figure 4 suggests that esterification has occurred. Also, as expected, the intensity of this peak in MPEO (350)–SH is much higher than that in MPEO (3400)–SH, in accordance with the expected numbers of ester groups in these molecules.

Investigation of mono- and disubstitution of HO–PEO–OH in thiolation reactions

In the thiolation of PEO when both chain ends are hydroxyl groups, it was expected that a mixture of products would be obtained with one or both chain ends having reacted (Reaction 3).

TABLE III
Calculation of Degree of Thiolation of 8-Arm PEO with Mercaptoacetic Acid According To eq. (2)

M	0	1	2	3	4	5	6	7	8
n-m	8	7	6	5	4	3	2	1	0
Pm	0.344	0.393	0.196	0.056	0.011	0.001	0	0	0
Pm*	0.875	1.0	0.499	0.143	0.028	0.003	0	0	0

Pm*: Pm normalized to monosubstituted (m = 1) PEO.

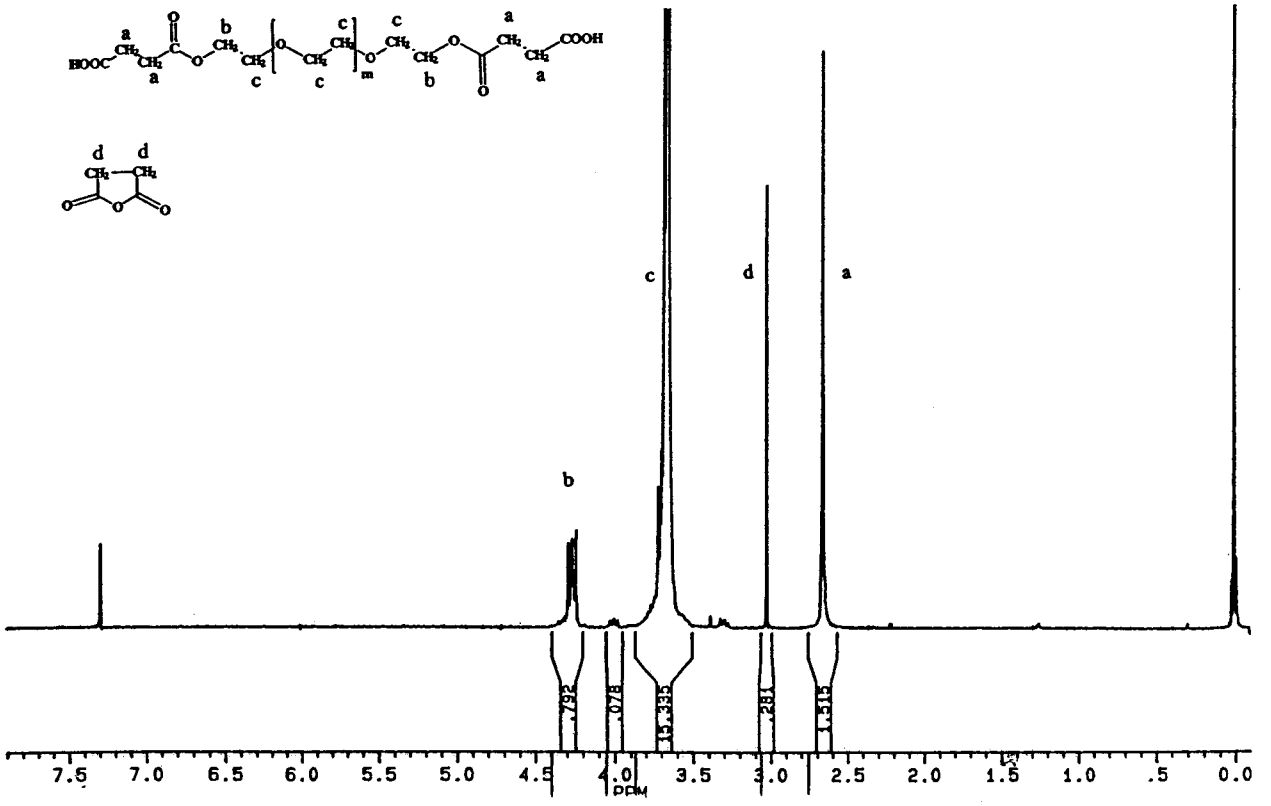

Figure 7 The proton NMR spectrum of the product of reaction between HO–PEO(1000)–OH and succinic anhydride using a 1:1 molar ratio. *p*-Dimethylaminopyridine was used as catalyst. The reaction was carried out at room temperature for 8 h. The peaks (a) at 2.64 ppm and (b) at 4.3 ppm verify the ring opening of succinic anhydride and the esterification reaction.

$$\text{HO—PEO—OH} + \text{HS—CH}_2\text{—COOH} \underset{\text{Toluene 110 °C}}{\overset{\text{H}_2\text{SO}_4}{\rightleftharpoons}} \begin{matrix} \text{HS—CH}_2\text{—COO—PEO—OH} \\ \text{HS—CH}_2\text{—COO—PEO—OOC—CH}_2\text{—SH} \end{matrix} \quad (3)$$

Theoretically, the product distribution should be dependent on the ratio of the reactants. For example, in reaction 3, if p is the ratio of carboxyl to hydroxyl groups, and if the reaction of the hydroxyl at one end of the PEO chain has no effect on the hydroxyl at the other end, then the probabilities that reaction will occur at both chain ends (product A), one end only (B), and not at all (C) will be p^2, $2p(1-p)$ and $(1-p)^2$, respectively. If p is 0.5, as is the case for most of the reactions studied here, these probabilities are then 0.25, 0.5, and 0.25, respectively.

A model system was used to investigate the distribution of the three products, *A*, *B*, and *C*. The reaction between dihydroxy PEO with a MW of 2000 (D) and monomethoxy, monocarboxy PEO with a MW of 1000 (M) was carried out (Reaction 4)

$$\text{HO—PEO—OH} + \text{M—PEO—COOH} \underset{\text{Toluene 110 °C}}{\overset{\text{H}_2\text{SO}_4}{\rightleftharpoons}} \begin{matrix} \text{M—PEO—COO—PEO—OH} \\ \text{M—PEO—COO—PEO—OOC—PEO—M} \end{matrix} \quad (4)$$

using different stoichiometries. The products were expected to have different molecular weights and should therefore have been amenable to analysis by gel permeation chromatography (GPC). The molecular weights of the PEOs were chosen such that the different products should be easily separated by the GPC system used.

Figures 5 and 6 show typical chromatograms. Figure 5 shows data for a molar ratio of 0.5, M:D that is,

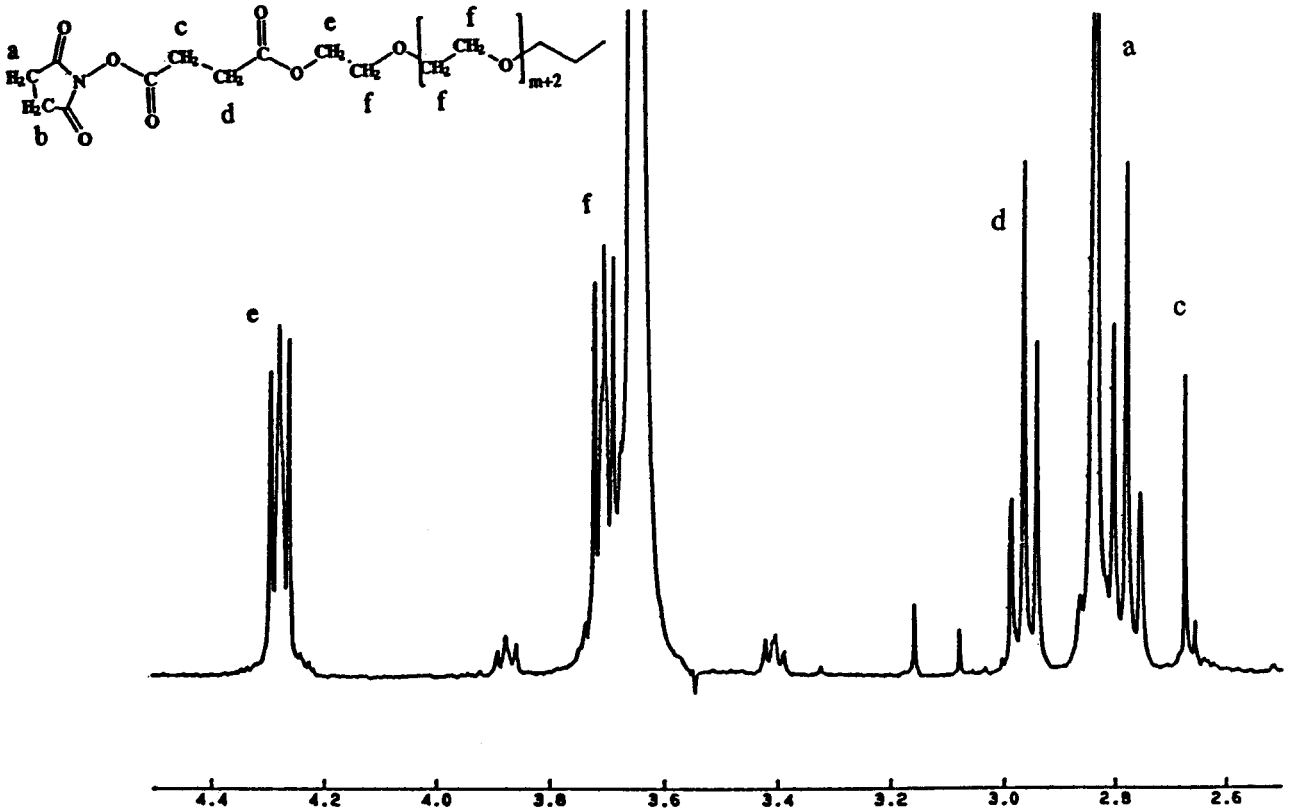

Figure 8 Proton NMR spectrum of the product of reaction between PEO-COOH and NHS (II in Scheme 1) at a 1:1 molar ratio. The reaction was carried out at room temperature. The peak at 2.83 ppm (a) is assigned to the protons of NHS after reaction (see text). The details at the right end of the inset structure are omitted.

a functional group ratio, COOH:OH, of 0.25. Three peaks are resolved and may be assigned to the disubstituted product (31 min), the monosubstituted product (32 min), and unreacted PEO (35 min). It can be seen that considerable unreacted HO–PEO–OH remained, that a significant amount of monosubstituted PEO was present, and that the amount of disubstituted product was small. Unreacted M–PEO–COOH was not detected, possibly because the lower separation bound of the column ($MW = 600$) was too close to the molecular weight of this species. In effect, material with a MW of about 1000 would elute with the solvent. With a molar ratio, M:D, of 1.0 (functional group ratio of 0.5) the amounts of unreacted PEO and disubstituted products appear to have been less than the amount of monosubstituted product (Fig. 6).

All these observations are in accordance with the theoretical prediction discussed above. Quantitative data on the peak areas (normalized to the peak for monosubstituted product) are listed in Table II and are compared to the calculated values. It can be seen from Table II that the experimental and calculated values are in reasonably good agreement.

It should be mentioned that when mixtures of products, as indicated in reaction 3, are used as reagents for chemisorption to gold, the mono- and disubstituted PEO are expected to adsorb, whereas the unreacted PEO is not. Clearly, the disubstituted PEO may chemisorb using either one or two chain ends. In the latter case PEO in a loop configuration would be present on the surface.

Thiolation of star-type PEO

The star-type PEOs used had hydroxyl groups at the chain ends. Thiolation was again carried out by reaction with mercaptoacetic acid. As for the linear PEOs, the self-esterification of mercaptoacetic acid can occur in these reactions. Also, a distribution of products having different numbers of hydroxyl groups converted to thiol is possible. However, for the same molar ratio of PEO to mercaptoacetic acid as in the linear PEO reaction, the ratio OH:SH becomes much

Figure 9 Proton NMR spectrum of the product of reaction between NHS–PEO and glycine methyl ester: (a) 0–8 ppm, (b) expanded scale 2.4–4.4 ppm. The new peaks at 3.78 ppm and 4.04 ppm indicate, respectively, the presence of methyl and methylene protons similar to those of glycine methyl ester. The disappearance of the peaks in the vicinity of 2.8 ppm indicates the elimination of NHS groups. The details at the right end of the inset structure in (a) are omitted.

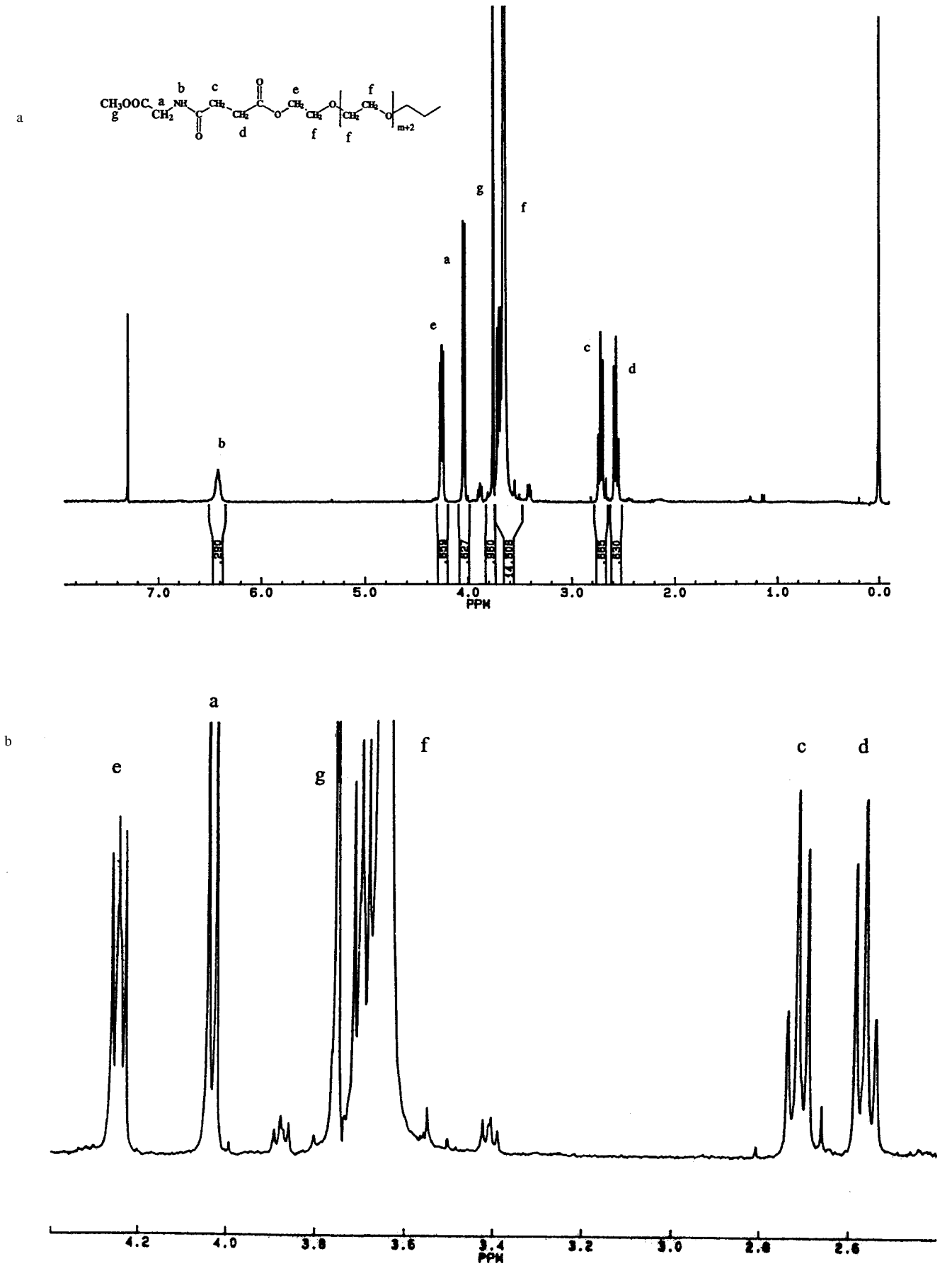
a
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Figure 9

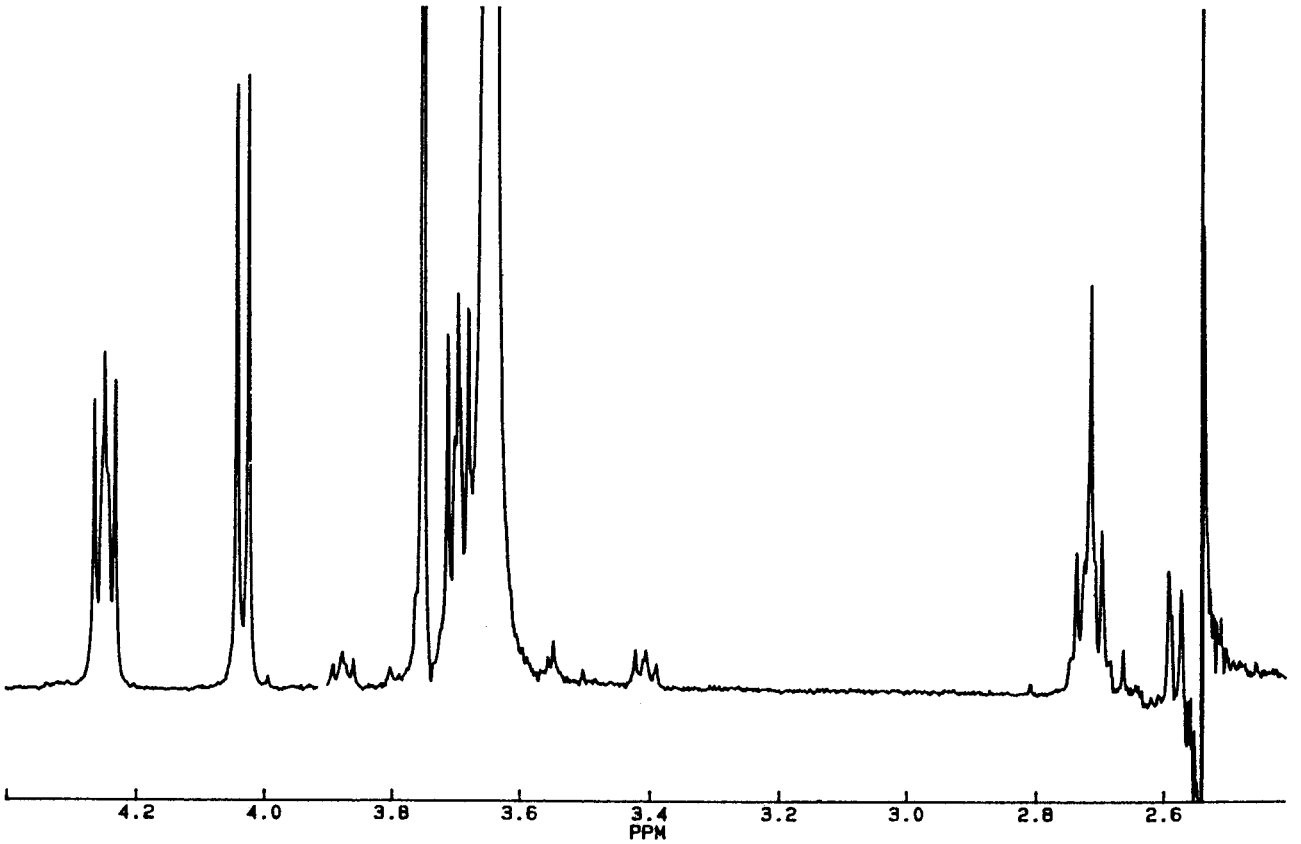

Figure 10 Spin-decoupled proton NMR spectrum of the product of reaction between NHS-PEO and glycine methyl ester. The peak at 2.71 ppm is partly collapsed to a singlet, in contrast to the triplet, which is seen before decoupling [(c) in Fig. 9].

higher. Therefore, the effect of self-esterification and the probability of di-, tri-, and higher-order substitution would be even smaller and probably negligible. As an example, estimates of the distribution of products for a star-type PEO can be made in the same way as for linear PEO. If p is the ratio COOH:OH, n is the number of arms on the PEO, and m is the number of hydroxyls esterified, the probability of a PEO having n arms with m arms reacted is

$$P_m = \frac{n!}{m!(n-m)!}\, p^m\, (1-p)^{n-m} \qquad (2)$$

To compare with linear PEO, suppose the molar ratio of eight-arm PEO to mercaptoacetic acid is 1:1, then p, the ratio COOH:OH, becomes 1:8. The substitution yields calculated according to eq. (2) are shown in Table III. It can be seen that most of the products are nonsubstituted ($m = 0$), monosubstituted ($m = 1$), and disubstituted ($m = 2$) PEOs, leaving most of the hydroxyls in the multiarmed PEOs unreacted. The yield of fully substituted PEO is essentially zero. In contrast, for linear PEO it has been calculated that the ratio of unreacted, monosubstituted, and disubstituted PEO is 1.5:1.0:0.17. In this work star PEOs having four arms (*MW* 2000, 10000) and eight arms (*MW* 2000) were thiolated using a molar ratio of PEO to mercaptoacetic acid of 1:1. The degree of thiolation is very close to the stoichiometric ratio. Although the distribution of the different degrees of substitution was not studied, X-ray photoelectron spectroscopy data (not shown) indicated that most products had a low degree of substitution.

Thiolation of poly(propylene oxide)

Thiolation of linear poly(propylene oxide) (PPO) of MW 1025 was also carried out by reaction with mercaptoacetic acid, using sulfuric acid as catalyst (Reaction 5).

$$\mathrm{HO{-}PPO{-}OH + HS{-}CH_2{-}COOH} \underset{\text{Toluene}}{\overset{\mathrm{H_2SO_4}}{\rightleftharpoons}} \mathrm{HS{-}CH_2{-}COO{-}PPO{-}OH + H_2O} \qquad (5)$$

The reactants were present in a 1:1 molar ratio, thus favoring the monosubstituted product as discussed for PEO.

Conjugation of PEO to amino acids

As detailed in the experimental section (Scheme 1), amino acids were coupled to the chain ends of PEO

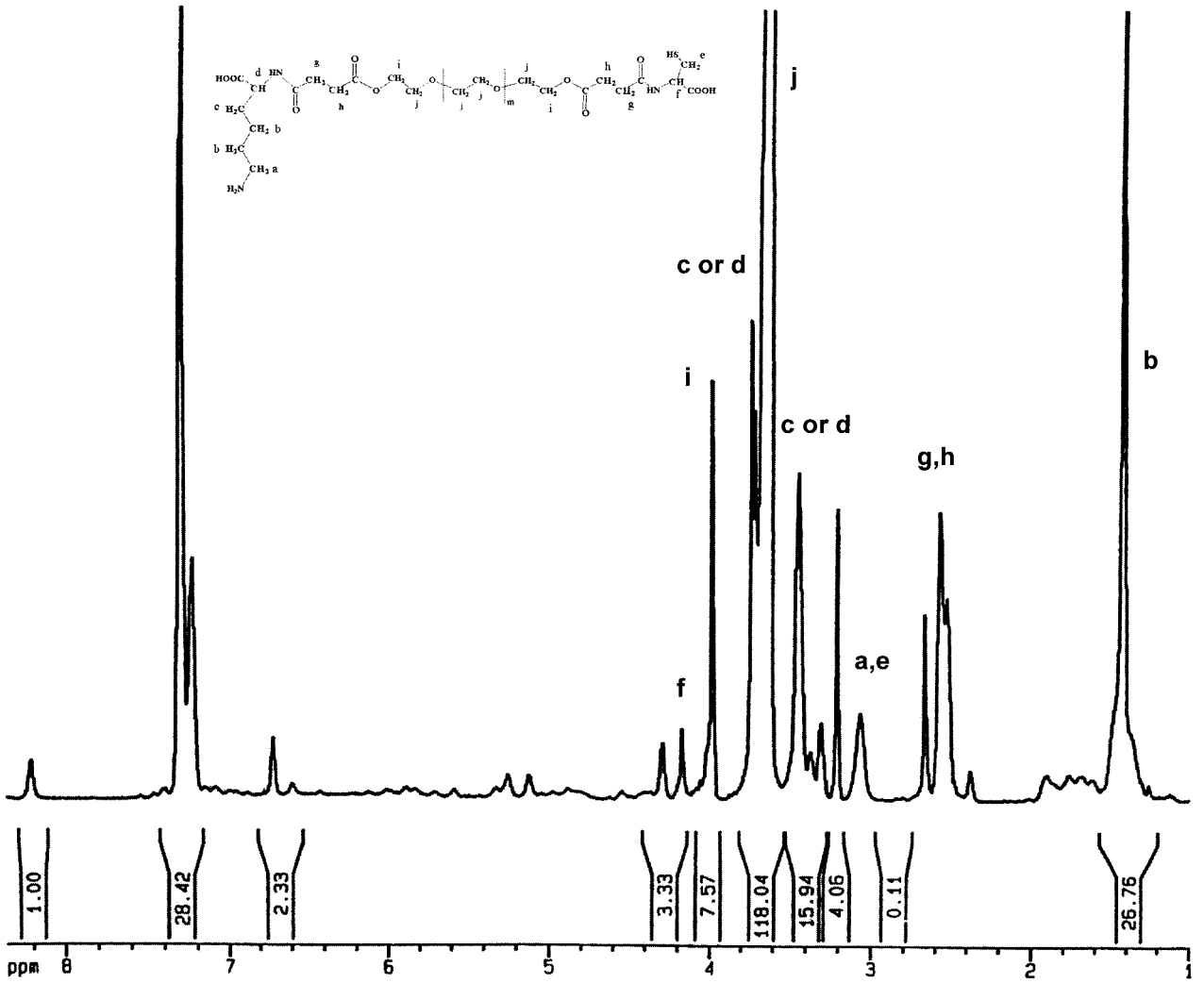

Figure 11 Proton NMR spectrum of the product of reaction between lysine, cysteine, and NHS–PEO–NHS. All peaks expected for cysteine (3.10 and 4.03 ppm) and lysine (1.40, 3.02, and 3.88 ppm) are present, indicating the reaction was successful.

via their amino groups. Data relating to each reaction step in this process are discussed in turn.

Conversion of PEO–OH to PEO–COOH by reaction with succinic anhydride

This reaction (reaction a, Scheme 1) was verified by proton NMR analysis of the products. Figure 7 shows the spectrum of the product of the reaction of succinic anhydride (SA) with HO–PEO (1000)–OH. The singlet peak at 2.64 ppm (peak a) is assigned to the protons of SA after ring opening although it consists of signals from hydrogen bonded to different carbons. This assignment is consistent with the spectrum of the monomethyl ester of succinic acid found in the literature.[53] The peaks in the vicinity of 4.3 ppm (b) are assigned to the methylene protons adjacent to the hydroxyl group of PEO after esterification by SA. Estimation of the product yield based on integration of the spectral peaks indicated that the yield was essentially quantitative.

Conversion of PEO–COOH to the *N*-hydroxysuccinimide derivative

Figure 8 shows the NMR spectrum of the product of the reaction of PEO–COOH with NHS (see Scheme 1). To ensure that unreacted *N*-hydroxysuccinimide (NHS) was removed, extensive washing with dichloromethane, a good solvent for PEO and a nonsolvent for NHS, was carried out. The peaks at 2.83 ppm (a) indicate the presence of protons in NHS that has reacted. Also, the singlet peak that appears at 2.64 ppm in Figure 7 (peak a) is now split into two triplets centered at 2.78 (c) and 2.92 ppm (d), respectively. This is because the chemical and magnetic environments of the protons of the two methylene groups in succinic acid are different after reaction [one is adjacent to PEO

(d) and the other to NHS (c)]. Based on peak integration, the yields of this reaction were estimated to be greater than 90%.

Conjugation of amino acids to NHS-activated PEO

This reaction was performed using an interfacial reaction technique with water as one phase and methylene dichloride as the other. The advantage of this approach is its suitability for the many amino acids which are soluble in water at room temperature. The reactions of NHS–PEO–NHS with glycine, glycine methyl ester, *N*(ε)-*t*-BOC-lysine, lysine methyl ester, and cysteine were carried out using this method. Also, the reaction of NHS–PEO–NHS with both cysteine and lysine was attempted with the goal of synthesizing PEO derivatized with cysteine at one end, and lysine at the other. The ε-amino group of lysine was protected so that the α amino group would be used in the conjugation reaction, leaving the ε-amino group (after deprotection) free, as in proteins.

Figure 9 shows the NMR spectrum of the product of the reaction of glycine methyl ester with NHS–PEO. The new peaks at 3.78 ppm (g) and 4.04 ppm (a) indicate the presence, respectively, of methyl and methylene protons having chemical shifts in accordance with the structure of glycine methyl ester. Further, compared to what is shown in Figure 8, the disappearance of the peaks at 2.83 ppm also indicates that the NHS end groups have been eliminated, thus confirming that the reaction has taken place. Because the methylene proton signal at 2.64 ppm in the product of reaction (a) in Scheme 1 is a singlet [(a) in Fig. 7], which indicates the presence of succinic acid, there was some doubt whether the triplets at 2.57 ppm and 2.71 ppm [(d) and (c) in Fig. 9] are due to the protons of succinic acid. Therefore, spin decoupling at 4214.26 Hz (2.57 ppm in the 300 MHz machine) under a decoupling power of 12 L was performed. Figure 10 shows the spectrum obtained from the decoupling experiment. From a comparison with what is shown in Figure 9, it can be seen that the peak centered at 2.71 ppm (c) has partly collapsed to a singlet. This provides additional evidence that the glycine methyl ester was linked to the succinic acid group of PEO, because otherwise the methylene protons in succinic acid should appear as in Figure 7.

The NMR data indicate that the yield of this reaction was higher than 90%, in fact, somewhat higher than expected because interfacial reactions usually have low yields. One reason for such high yields in this case may be the high reactivity of the NHS ester with amino groups. It should also be noted that interfacial reactions usually do not occur without surfactant. However, PEO is itself a powerful surfactant, and this may also contribute to the high yield of this reaction.

Lysine was also investigated for conjugation to PEO. Figure 11 shows the proton NMR spectrum for the product of the reaction of NHS–PEO–NHS with lysine and cysteine, the latter to provide a thiol group for chemisorption to gold. The molar ratio of lysine: cysteine:PEO was 0.2:1:1. To increase the likelihood that the PEO would be thiolated, cysteine was added to the reactor first, followed by lysine. Figure 11 shows the NMR spectrum of the reaction product. It can be seen that the expected signals for lysine (1.40, 3.02, and 3.88 ppm) and for cysteine (3.10 and 4.03 ppm) are present.[53] Because the purification process (dissolution in dichloromethane and reprecipitation in isopropylether) removed the unreacted lysine and cysteine efficiently, it is evident that both lysine and cysteine were bound to PEO.

CONCLUSIONS

The synthesis of thiol-terminated PEO and PPO starting with linear PEOs of variable molecular weight, linear PPO, and star-type PEOs was demonstrated. Mercaptoacetic acid reacted with hydroxy-terminated PEO to form an ester, as shown by NMR spectroscopy. Kinetic studies suggested that the self-condensation of mercaptoacetic acid, a possible side reaction, did not occur to any significant extent. The distribution of products agreed with statistical predictions.

Methods for synthesizing linear PEO, one end bearing a thiol group and the other an amino acid or other potentially bioactive group, were also investigated. For this purpose, *N*-hydroxysuccinimide activation was used to conjugate the amino acids cysteine (to provide a thiol), glycine methyl ester, and lysine to PEO. These reactions were carried out successfully as demonstrated by NMR spectra of the products.

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