Synthesis and Characterization of Heterotelechelic Poly(ethylene glycol)s with Amino Acid at One End and Hydroxyl Group at Another End

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ABSTRACT: Heterotelechelic poly(ethylene glycol)s are widely used in the modification, conjugation, and crosslinking of biomacromolecules. A series of heterotelechelic PEGs with amino acid at one end and hydroxyl group at another end, including α-glycine-ω-hydroxyl-PEG, α-proline-ω-hydroxyl-PEG, and α-phenylalanine-ω-hydroxyl-PEG, were first synthesized in this study. The reaction proceeded at ambient temperature under alkaline conditions via an aqueous solution polymerization of ethylene oxide. Amino group of glycine, proline, and phenylalanine was the initiating center in the polymerizations, and carboxyl group of these amino acids was reserved as one of the active end groups of the obtained heterotelechelic PEG. Purification of the desired products was accomplished by silica gel column chromatography. The obtained heterotelechelic PEGs were characterized by means of FT-IR, ^{1}H NMR, ^{13}C NMR, MS, and RP-HPLC. They were in different forms depending on the type of initiating amino acid, e.g. α-glycine-ω-hydroxyl-PEG and α-phenylalanine-ω-hydroxyl-PEG are in branched form, and α-proline-ω-hydroxyl-PEG is linear. Amino acids were conjugated to PEG chains through the stable carbon–nitrogen bond. Compared with the traditional critical polymerization conditions, the advantage of this method is that various amino acid ended heterotelechelic PEGs can be designed and obtained by using different amino acid as the initiator through a much more convenient route, which proceeded in aqueous solution at ambient temperature. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 2432–2439, 2008

Key words: polyethers; ring-opening polymerization; crosslinking

INTRODUCTION

PEG derivatives, possessing of useful properties such as nontoxicity, water solubility, low protein adsorption, and high resistance to bacterial and animal cell adhesion, have been utilized for a variety of applications including synthesis of peptides, phase transfer catalysis, solid surface modification, drug modification, and so on.[1,2] In recent research, heterotelechelic PEGs with different functional groups at each chain end were used as crosslinkers to conjugate the enzymes or other biomolecules to hydrophobic carriers, while these biomolecules can retain their activities because of the biocompatibility of PEG. For example, OPSS-PEG-NHS (*o*-pyridyldisulfide-PEG-*n*-hydroxylsuccinimide) was used to tether antibodies, such as anti-HER2 or anti-IgG, onto gold shells of nanoparticles to produce immunotargeted nanoparticles, with optical properties suitable for combined imaging and therapy. The covalently bound heterotelechelic PEG in the complex could reduce unwanted protein adhesion and assist in maintaining antibodies activity in targeting delivery.[3] The parallel application of *p*-mercaptoaniline-PEG-fluorescein in Halas's research was also reported.[4]

The ideal heterotelechelic PEG spacers as reported should provide distinct reactivity on both termini: conjugating with specific functional groups and preventing undesired coupling. These requirements give challenges to synthesis work. Two general approaches have been investigated to produce heterotelechelic PEGs: derivation of diol PEG and polymerization of ethylene oxide by using specific initiator or terminator. The first one is to derive one of hydroxyl end groups of diol PEG by reacting with some appropriate reagents. For example, α-pyridyldithio-ω-hydroxy-PEG was prepared from diol PEG through six steps,[5] and DMT-PEG-Fmoc (α-dimethoxytrityl-ω-fluorenylmethyloxycarbonyl-PEG) was produced from diol PEG with two synthetic steps followed by chromatographic purification.[6] Since several reaction steps were needed and the efficiency of these derivations was not high, the yield of the product usually was low after several reactions and purification steps. The second way developed in recent years is the direct polymerization of ethylene

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oxide with a certain reagent as catalyst and a specially derived compound as the starting material. In 1990s, Kataoka and Nagasaki have synthesized heterotelechelic PEG with a primary amino group at one end and a hydroxyl group at another end by anionic polymerization of ethylene oxide with cyanomethyl potassium as an initiator followed by the reduction of cynao end group of the polymer chain to amino group.[7] The α-methacryloyl-ω-formyl PEG was also successfully synthesized by using potassium (3,3-diethoxypropyl) alkoxide as an initiator, which has an acetal moiety derived from aldehyde group.[8] However, besides the polymerization, the whole synthetic route also includes the protection of starting material and the deprotection of the terminal group of the synthetic polymer.[7–12]

In this article, we developed a novel strategy for the synthesis of new heterotelechelic PEGs using special agents as starting materials, which contain both amino group as initiating center and other defined functionalities as designed end groups of PEG chain, in the polymerization of ethylene oxide. Several amino acids, which have never been used as initiators in the preparation of PEG and its derivatives, have been successfully introduced as initiators in the polymerization of ethylene oxide. They effectively initiated the ring opening of ethylene oxide by using their amino group and produced desired heterotelechelic PEGs. In most of the previous reports,[7–12] the active initiating center of the direct polymerization of ethylene oxide to produce heterotelechelic PEGs was hydroxyl group, and amino group was scarcely used as initiator directly in the preparation of heterotelechelic PEGs. However, amino group showed great nucleophilic attack ability in the ring-opening reaction of epoxide compounds.[13–20] Micheloni et al. synthesized bis(2-chloroethyl) pentylamine by the nucleophilic attack of pentylamine on ethylene oxide with ethanol as solvent.[14] Chakraborti et al. have used montmorillonite K 10 to catalyze the ring opening of epoxides by amines under solvent-free conditions at room temperature and produced 2-amino alcohols.[17]

The polymerizations proceeded under a mild condition in aqueous solution, with alkali as catalyst. The newly synthesized heterotelechelic PEGs have amino acid at one end of the polymers and hydroxyl group at another end, in which amino acid was conjugated to PEG chain through carbon–nitrogen bond.

EXPERIMENTAL PROCEDURES

Materials and reagents

Ethylene oxide (Changzhou, China) was condensed onto CaH_2 (Tianjin, China) and stayed for more than 24 h, then was distilled, and kept under inert atmosphere. Sodium hydroxide, hydrochloric acid, ethanol, glycine, proline, and phenylalanine were commercially available (Beijing, China) and used in analytical grade as received.

Analytic techniques

The desired heterotelechelic PEGs were purified by column chromatography using silica gel (200–300 mesh) (Qingdao, China) as matrix and ethanol (C_2H_5OH)/water (H_2O) mixture as eluent. Unless specified, the ratios describing the composition of solvent mixtures represent relative volume fractions. FT-IR transmission spectra were recorded on a JASCO FT-IR 660 spectrophotometer using 4 cm^{-1} resolution and 128 scans. ^1H NMR spectra and ^{13}C NMR spectra were recorded on an AVANCE Bruker spectrometer operating at 400.13, 100.61 MHz, respectively. Deuterated DMSO was used as the solvent for ^1H NMR spectroscopy and tetramethylsilane was used as an internal standard. Deuterated water was used as the solvent for ^{13}C NMR. MS analyses were carried out on a LCQ Deca XP Thermo Finngian (San Jose, CA) ion trap MS equipped with an electrospray ionization source. Data acquisition and process was performed with Xcalibur 1.2 software. The mass spectrometer was operated in positive ion mode. Analytical reversed-phase HPLC was performed on a Waters 2695 chromatograph equipped with a SEDEX 75 evaporative light scattering detector, and a Vydac 214MS54 C4 column (46 mm × 250 mm, 50 μm, 300 Å) was used. The flow rate was 0.5 mL min^{-1}. Eluent was consist of solvent A (0.1% trifluoroacetic acid (TFA)/H_2O) and solvent B (0.1% TFA/CH_3CN). The gradient was sequential linear from 0 to 40% solvent B in 40 min.

General procedure for the synthesis of heterotelechelic PEG with amino acid at one end and hydroxyl group at another end

Five milliliters of H_2O, 2 mmol amino acid, and 2 mmol NaOH were added to a round-bottomed glass vessel, and then the reaction system was sealed and stirred for about 30 min until all of the substances were dissolved. After the system was cooled down to about 0°C by salt-ice bath, 3 mL ethylene oxide (2.6 g, 60 mmol) was added via a cooled syringe. After the reaction proceeded for about 40 h at ambient temperature, 1.0 mL of diluted hydrochloric acid (4 mol L^{-1}, 4 mmol) was added, and the solution was stirred for a further 0.5 h. The obtained mixture was evaporated to dryness, and then was dissolved in methylene dichloride. After the solution was filtered to clear away the unreacted amino acid, the filtrate was evaporated to obtain viscous colorless oil. The raw product was purified by silica gel column chromatography, with ethanol solution (90%) as the eluent.

Heterotelechelic PEG with glycine at one end and hydroxyl group at another end (a)

TLC (EtOH/H_2O 85 : 15): R_f = 0.40. FT-IR (KBr, cm^{-1}): 3388 (νO—H), 2874 (νCH_2), 1397 (νC—N), 1115 (ν_aC—O—C). ^{1}H NMR (DMSO—d_6), δ (ppm): 3.72 (HOOCCH_2); 3.89 (NCH_2CH_2O); 3.40–3.60 (OCH_2CH_2O); 3.78 (CH_2OH); 4.68 (OH). ^{13}C NMR (H_2O-d_2), δ (ppm): 168.08 (COOH); 63.86 (HOOC—CH_2—N); 59.59 (NCH_2CH_2O); 69.20–69.49 (OCH_2CH_2O); 71.49 (CH_2CH_2OH); 60.19 (CH_2CH_2 OH).

Heterotelechelic PEG with proline at one end and hydroxyl group at another end (b)

TLC (EtOH/H_2O 85 : 15): R_f = 0.45. FT-IR (KBr, cm^{-1}): 3395 (νO—H), 2874 (νCH_2), 1393 (νC—N), 1100 (ν_aC—O—C). ^{1}H NMR (DMSO-d_6), δ (ppm): 3.42 (NCH_2CH_2); 1.96 (NCH$_2$CH_2); 2.11, 2.23 (HOOCCHCH_2); 4.01 (HOOCCH); 3.85 (NCH_2CH_2O); 3.40–3.60 (OCH_2CH_2O); 3.78 (CH_2OH); 4.68 (OH). ^{13}C NMR (H_2O-d_2), δ (ppm): 171.52 (COOH); 18.71 (HOOCCHCH$_2$CH$_2$CH$_2$N); 25.35 (HOOCCHCH$_2$CH$_2$ CH$_2$N); 64.46 (HOOCCHCH$_2$CH$_2$CH$_2$N); 75.13 (HOO CCHCH$_2$CH$_2$CH$_2$N); 55.61 (NCH$_2$CH$_2$O); 69.2069.40 (OCH$_2$CH$_2$O); 71.46 (CH$_2$CH$_2$OH); 60.16 (CH$_2$CH$_2$OH).

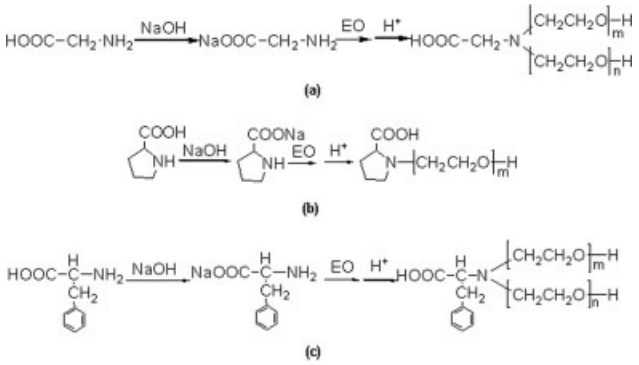
Heterotelechelic PEG with phenylalanine at one end and hydroxyl group at another end (c)

TLC (EtOH/H_2O 85 : 15): R_f = 0.50. FT-IR (KBr, cm^{-1}): 3382 (νO—H), 2875 (νCH_2), 1389 (νC—N), 1117 (ν_aC—O—C). ^{1}H NMR (DMSO-d_6):7.15–7.29 (Ph); 3.21, 3.07 (HOOCCHCH_2); 3.87 (HOOCCH); 3.83 (NCH_2CH_2O); 3.40–3.68 (OCH_2CH_2O); 3.83 (CH_2OH); 4.76 (OH). ^{13}C NMR (H_2O-d_2): 170.36 (COOH); 127.26–134.77 (Ph); 32.61 (PhCH$_2$); 78.25 (HOOC(Ph)CHN); 59.77 (NCH$_2$CH$_2$O); 69.40 (NCH$_2$ CH$_2$O); 69.51 (OCH$_2$CH$_2$O); 71.45 (CH$_2$CH$_2$OH); 64.34 (CH$_2$CH$_2$OH).

RESULTS AND DISCUSSION

In this article, various new heterotelechelic PEGs with amino acid at one end and hydroxyl group at another end were synthesized via the direct polymerization of ethylene oxide initiated by the amino group of the corresponding amino acid following the path of Scheme 1.

Glycine, proline, and phenylalanine were selected as the initiators to produce amino acid ended heterotelechelic PEGs because these α-amino acids did not contain other active groups which could serve as initiating center in the polymerization of ethylene oxide, such as hydroxyl group, mercapto group, amide, and so on.



Scheme 1 Polymerization of ethylene oxide initiated by amino acid. (a) glycine, (b) proline, and (c) phenylalanine.

In the reaction systems, amino group of amino acid could sucessfully lead to the formation of amino acid ended heterotelechelic PEG in aqueous solution. As we know, amino group and hydroxyl groups both are nucleophilic reagents and can open the ethylene oxide ring in the same reaction system. Nucleophilicity of them is greatly influenced by the species of solvent. In the traditional anionic polymerization of ethylene oxide, hydroxyl group was generated to alcoholate as the initiating center.[20] Moreover, the reaction must proceed in absolute dry nonprotonic solvent, which was used as reaction medium to enhance the activity of alcoholate without increasing that of amino group.[20] On the other hand, the presence of protonic solvents such as ethanol, methanol, and water could enhance the reactivity of amino group but not that of hydroxyl group.[13,15,21] In our reaction system, the selected amino acid was derived to alkaline metal carboxylate by the addition of equal mole amount of inorganic alkali such as sodium hydroxide, and then the amino group of amino acid in free form in the alkaline reaction system could initiate the ring opening of ethylene oxide more effectively compared to the usual protonated form in neutral or acidic solution.

Diol PEG was inevitably produced accompanied with the formation of the desired heterotelechelic PEGs in the aforementioned reaction system, for the polymerization of ethylene oxide initiated by the hydroxyl group of water under the catalysis of alkaline conditions was the major side reaction in these reaction systems. The amino acid ended heterotelechelic PEGs were then obtained via silica gel column chromatography of the raw products, with the mixture of ethanol and water (EtOH/H_2O, 9/1) as eluent. The isolated yields of amino acid ended heterotelechelic PEG were around 40%, because a considerable amount of heterotelechelic PEG was adsorbed on the silica during the elution.

FT-IR analyses were carried out to characterize the backbone structure of PEG chain and the conjugations of amino acid terminal groups to polymers.

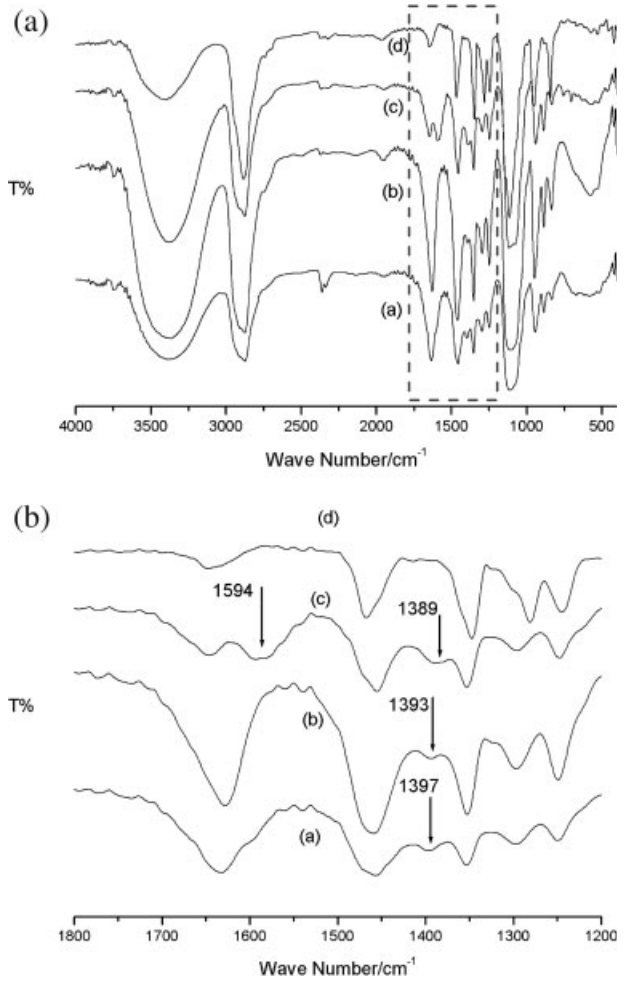


Figure 1 FT-IR spectra of the amino acid ended heterotelechelic PEGs and diol PEG. (a) Gly-PEG, (b) Pro-PEG, (c) Phe-PEG, and (d) Diol PEG.

The comparison of FT-IR spectra of the obtained heterotelechelic PEGs to that of diol PEG is shown in Figure 1. The obtained heterotelechelic PEGs have amino acid at one end and hydroxyl group at another end, while diol PEG has hydroxyl groups at both ends. The main difference between amino acid ended heterotelechelic PEGs and diol PEG is the conjugation of end group with PEG chain, that is, amino acid was conjugated to PEG chain through carbon–nitrogen bond. The absorption bands around 1390 cm^{-1} in Figure 1(a–c) are the characteristic peak of the C—N bond. The appearances of these peaks suggested that the amino group of amino acid (glycine/proline/phenylalanine) had nucleophilicity that attacked the ring of the ethylene oxide effectively and formed steady conjugation of amino group to PEG chain. The absorption band that appeared at around 1594 cm^{-1} in Figure 1(c) is the characteristic peak of phenyl group of phenylalanine conjugated to PEG chain. The strong absorptions at 1100 cm^{-1} were attributed to C—O asymmetric stretching of the ether backbond. The other peaks of the obtained amino acid ended heterotelechelic PEG were in accordance with those of diol PEG, which implied that the basic structures of the obtained polymers were almost the same as the diol PEG, except the terminal groups.

Informations concerning the chain length and end groups of the polymer can be derived directly and accurately from NMR analysis. The ^{13}C NMR analyses of the obtained polymers were carried out with deuterated water as solvent. Figure 2(a–c) show the ^{13}C NMR spectra of the purified heterotelechelic PEGs ended with glycine, proline, and phenylala-

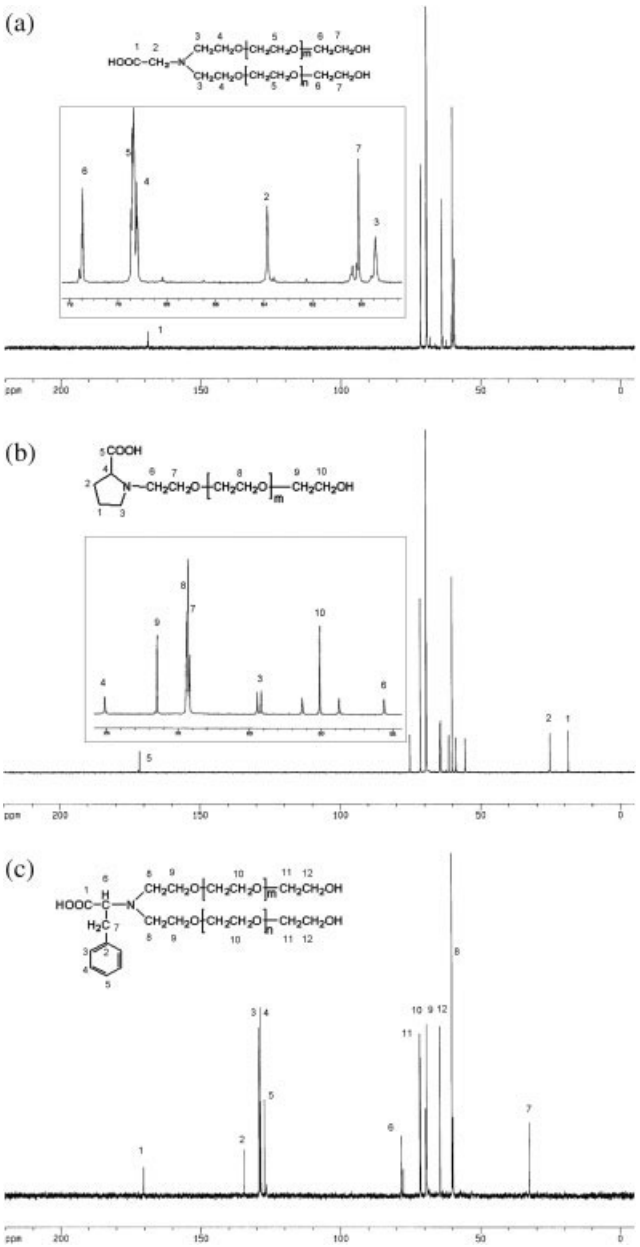


Figure 2 ^{13}C NMR spectra of the amino acid ended heterotelechelic PEGs (Table I). (a) Gly-PEG, (b) Pro-PEG, and (c) Phe-PEG.

TABLE I
^{13}C NMR Chemical Shift Data of the Amino Acid Ended Heterotelechelic PEGs

	δ (ppm)					
	Gly-PEG (a)		Pro-PEG (b)		Phe-PEG (c)	
C	Obsd.	Calcd	Obsd.	Calcd	Obsd.	Calcd
1	168.08	171.02	18.71	21.7	170.36	174.2
2	63.86	64.5	25.35	26.7	134.57	136.3
3	59.59	55.7	64.46, 64.19	64.7	129.10	130.2
4	69.20	69.7	75.13	77.1	128.60	129.9
5	69.23–69.46	70.6	171.52	175.46	127.26	128.4
6	71.49	72.5	55.61	55.7	77.53	77.7
7	60.19	61.5	69.19	69.7	32.61	37.7
8			69.23–69.40	70.6	59.77	55.7
9			71.46	72.5	69.40	69.7
10			60.16	61.5	69.40	70.6
11					71.45	72.5
12					64.34	61.5

nine, respectively. By referring to the literature on hydroxyl-terminated PEG and using glycine, proline, and phenylalanine as reference compounds,[23] the assignments of these signals were carried out, and the results were shown in Table I. It can be seen that the determined chemical shifts are in good accordance with the estimated data, which were calculated based on the empirical equations.[23] For example, the estimated chemical shift of 6# C atom in Figure 2(b) was 55.7 ppm, which is close to the measured data, 55.61 ppm. In Figure 2(a), it should be noted that the signal appeared at 63.86 ppm is the characteristic signal of methylene carbon of glycine terminal group of the polymer. It demonstrated that the amino group of glycine was dialkylated, that is to say, there were two PEG chains conjugated to the nitrogen atom and the obtained polymer was in a branched form, because the estimated shift of 2# C would be around 59.5 ppm if the amino group were monoalkylated. The signal at 77.5 ppm shown in Figure 2(c) also indicated that the primary amino group of phenylalanine initiated the ring opening of ethylene oxide and produced a branched PEG derivative. For proline, its secondary amino group has only one proton that could be subsitituted after its nucleophilic attack on the ethylene oxide ring, and it determines that the resulted polymer should be linear if the polymerization proceeds successfully. In Figure 2(b), the signal shown at 75.1 ppm was the characteristic of methine carbon (4#) which indicated that the PEG chain had been successfully connected to the amino group of proline through the carbon–nitrogen bond. Therefore, the resulted proline ended heterotelechelic PEG was in linear form.

Figure 3(a–c) show the ^{1}H NMR spectra of the obtained amino acid ended heterotelechelic PEGs, respectively. It has been reported that[24] ^{1}H NMR spectra of PEG derivatives in deuterated dimethyl sulfoxide (DMSO-d_6) showed a clear peak at 4.56 ppm for the hydroxyl group at polymer terminus, which did not shift or broaden in other solvents. It was well separated from the large peak of methylene protons of PEG backbone chain at around 3.0–4.0 ppm. According to Harris's research,[24] the peaks appeared at around 4.80 in Figure 3(a–c) were the characteristic peaks of the hydroxyl group of the obtained polymers. The broad peaks shown around 3.4–3.6 ppm are assigned to the methylene protons of the PEG chain. The signals (3.7–3.9 ppm) appeared in front of the broad peak are the characteristic of protons that are attributed to the methylene groups connected to the terminal hydroxyl group and amino group, such as peak 2, 6 in Figure 3(a), 5, 9 in Figure 3(b), and 6, 10 in Figure 3(c). The signals (3.3–3.4 ppm) appeared behind the broad peak are the shifts of the protons which belong to the methylene groups next to the aforementioned methylene groups, such as peak 3, 5 in Figure 3(a), 6, 8 in Figure 3(b), and 7, 9 in Figure 3(c).

The MS measurement can precisely analyze the end functionalization of oligomers as well as the determination of absolute molar masses. The MS results of the obtained polymers are shown in Figure 4(a–c). Typical ''Gaussian distribution'' mass spectra of the obtained heterotelechelic polymers were observed. The number at each peak corresponds to the absolute molar mass of a single polymer chain and is generally within 1 g/mol or 23g/mol of the theoretically expected value [m+44.032n] [eqs. (1) and (2)], because the m/z show the Na+ adducts.

Amino acid ended PEG:

$$m/z[\mathrm{H} + \mathrm{adduct}] = \mathrm{m} + 44.032n + 1.008 \quad (1)$$

$$m/z[\mathrm{Na} + \mathrm{adduct}] = m + 44.032n + 22.99 \quad (2)$$

$$m = 75.01\ (\text{glycine}), 115.13\ (\text{proline}), 165.19\ (\text{phenylalanine})$$

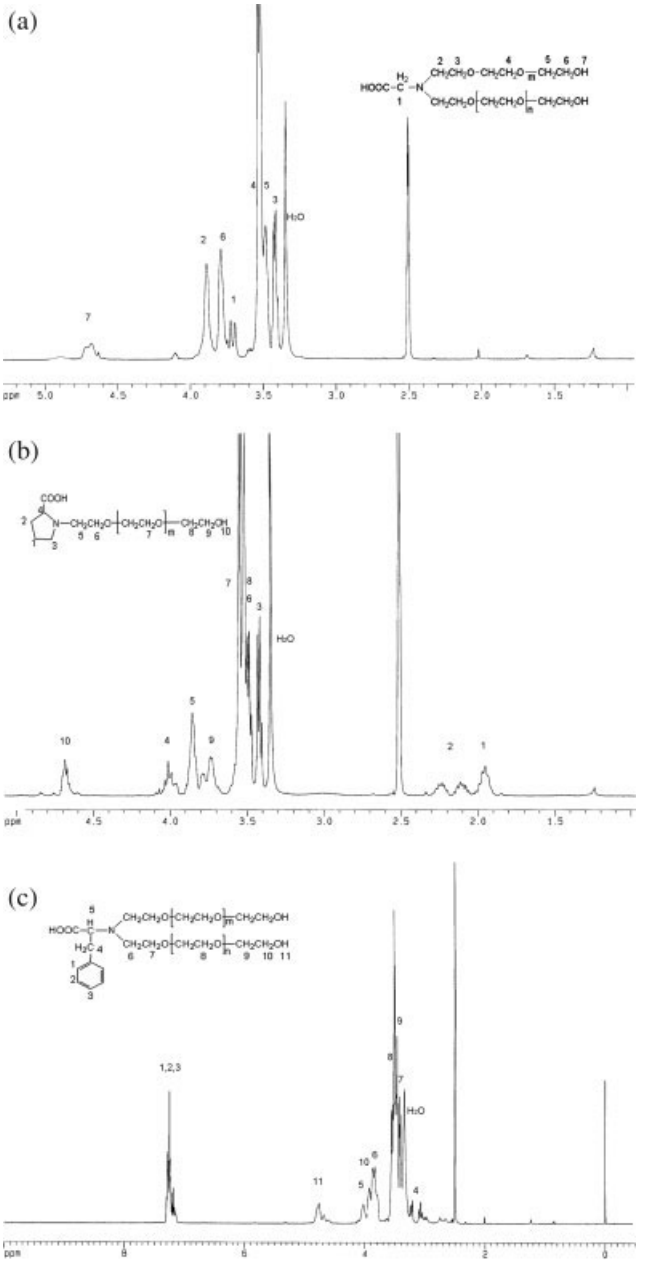


Figure 3 ^{1}H NMR spectra of amino acid ended heterotelechelic PEGs. Gly-PEG, (b) Pro-PEG, and (c) Phe-PEG.

where m is the molecular weight of the desired amino acid; n is the degree of polymerization; 44.032, 1.008, and 22.99 are the molar masses of ethylene oxide monomer, proton, and sodium ion, respectively.

For example, the peak molar mass in Figure 4(b), m/z = 820.7, is assigned to the heterotelechelic PEG ended with proline, whose degree of polymerization is 16. Meanwhile, the peak m/z = 842.7 is the sodium adduct of the corresponding polymer.

Therefore, the present MS results provide the proof for the formation of heterotelechelic PEGs, with amino acid at one end and hydroxyl groups at another end by the ring-opening polymerization of ethylene oxide initiated with the amino group of amino acid.

Furthermore, the HPLC spikings of the obtained amino acid ended heterotelechelic PEGs shown in Figure 5(a–c) were in good accord with their MS signals appeared in Figure 4(a–c). It revealed that the RP-HPLC system was capable of separating the whole entity of oligomeric PEG derivatives. Approximately, baseline separations of amino ended heterotelechelic PEGs were achieved in RP-HPLC analyses by using a binary solvent gradient of acetonitrile and water. The chromatograms provided the evidence that almost no pronounced peak broadening of late-eluting oligomers with respect to their early-eluting homologues was observed.

The degree of polymerization of polymer was around 10–15, and it could hardly increase with the increasing of the ratio of ethylene oxide to amino acid (Table II). The limitation of degree of polymer-

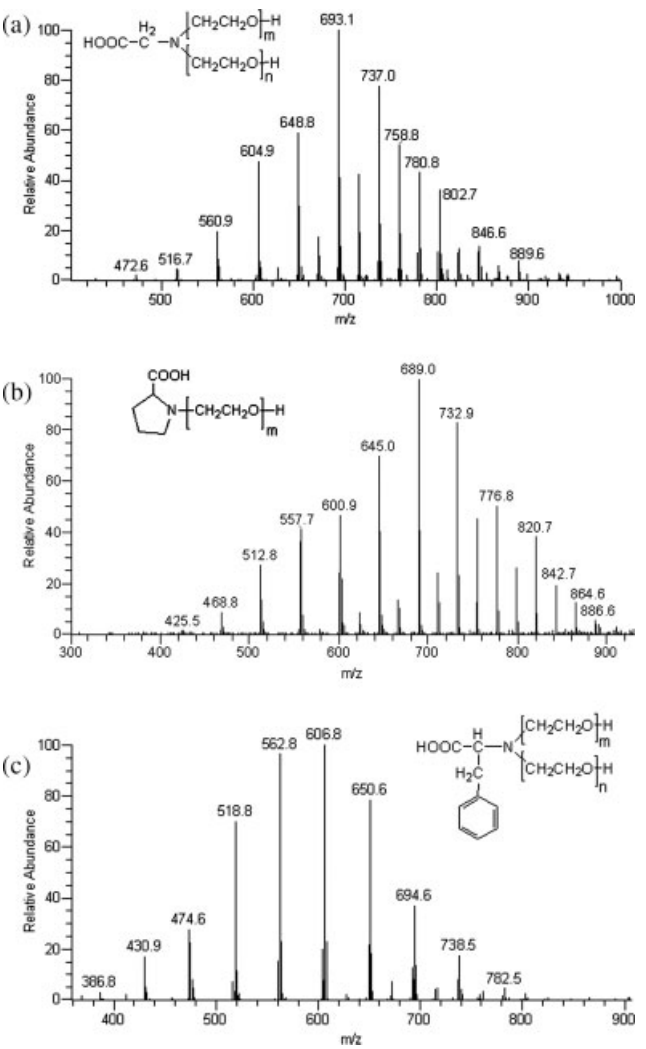


Figure 4 MS spectra of amino acid ended heterotelechelic PEGS. (a) Gly-PEG, (b) Pro-PEG, and (c) Phe-PEG.

ization probably resulted from two reasons. First, polymerization condition was so mild (aqueous solution and room temperature) that the reactivity of amino group was not high enough to initiate ethylene oxide ring thoroughly, and the superiority of amino group over hydroxyl group is not high as we expected. The unreacted amino acid was finally cleared away through the followed filtration. Second, diol PEG, which originated from water and was inevitably produced accompanied with amino acid ended PEG, consumed a fair part of ethylene oxide for the mole amount of water that far exceeded the amino acid. Therefore, they led to the resulted low degree of polymerization of amino acid ended heterotelechelic PEG.

TABLE II
Effects of the Ratio of EO to Glycine on the Degree of Polymerization

No.	EO : glycine (mole ratio)	Average degree of polymerization
1	13 : 1	15
2	30 : 1	15
3	40 : 1	12

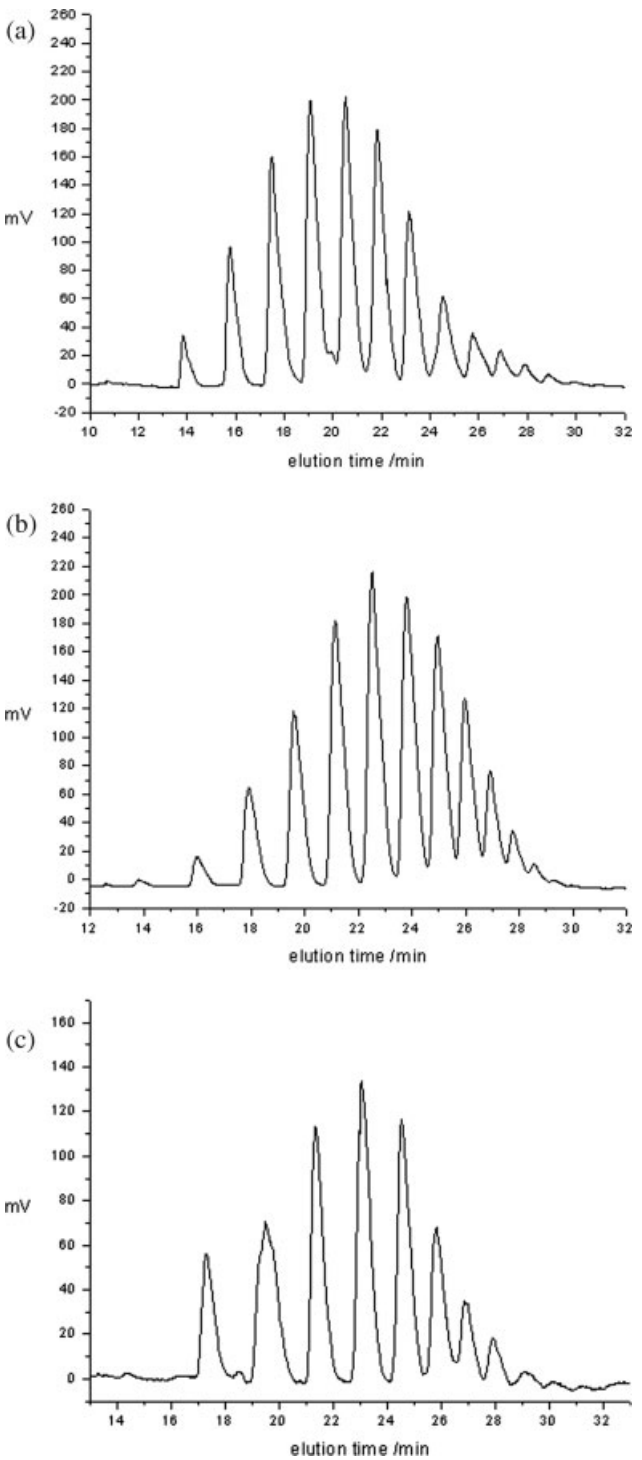


Figure 5 RP-HPLC of amino acid ended heterotelechelic PEGs. (a) Gly-PEG, (b) Pro-PEG, and (c) Phe-PEG.

As we can see, the MW of heterotelechelic PEGs was often around several hundreds to 3K.[25–27] It was not as high as that of monomethoxyl polyethylene glycol. Because high molecular weight mPEG was mainly used for protein modifications to modulate physicochemical or biochemical characterizations of those biomolecules, while heterotelchelic PEGs were mostly used as crosslinkers between biomacromolecules and carriers or biomolecules and solid surfaces. For their different end groups could react with different reagents, respectively, without unwanted crosslinkings, such as crosslinking between two same biomolecules, the newly synthesized amino acid ended heterotelechelic PEGs could play the same role as those spacers to conjugate biomolecules and carriers or solid surfaces. For example, it could be covalently bound to the silicone surface through carboxyl end group, and then hydroxyl group was activated to immobilize proteins or other biomolecules.[22]

CONCLUSIONS

In this study, new heterotelechelic PEGs with amino acid (glycine, proline, and phenylalanine) at one end and hydroxyl group at another end were synthesized successfully by aqueous solution polymerization of ethylene oxide initiated by the amino group of amino acid. The reactions proceeded at ambient temperature, and the polymerization time was about 40 h. After the crude products were purified by the silica gel column chromatography with the mixture of ethanol and water as eluent, the desired heterotelechelic polymers were obtained. They were characterized by means of FT-IR, ^{1}H NMR, ^{13}C NMR, MS, and RP-HPLC, which provided satisfied information on chemical structure as well as molecular weight. Based on the earlier measurement, amino acid was conjugated with PEG chains through the stable

carbon–nitrogen bond, and the obtained heterotelechelic PEGs were in linear or branched forms which varied with the species of amino acid.

The possibility of introducing an amino acid to the end of the PEG chains is of great interest. This reaction system can be applied to the syntheses of other amino acid ended heterotelechelic PEGs. As a result, a large family of heterotelechelic PEG derivatives can be synthesized through the convenient solution polymerization of ethylene oxide proceeded under mild conditions. They have the potential to be used as the crosslinkers to tether two different proteins or protein and carrier, because they can reduce nonspecific adsorption of proteins on the carrier surfaces and retain the activity of covalently bound proteins simultaneously. Above all, various applications of newly synthetic amino acid ended heterotelechelic PEGs may be expected in surface coating, drug delivery system, and other biomedical science.

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