

TECHNICAL NOTES

Primary Amino-Terminal Heterobifunctional Poly(ethylene oxide). Facile Synthesis of Poly(ethylene oxide) with a Primary Amino Group at One End and a Hydroxyl Group at the Other End

Yukio Nagasaki, Michihiro Iijima, Masao Kato, and Kazunori Kataoka*

Department of Materials Science and Technology, Science University of Tokyo, Noda 278, Japan.

Received June 16, 1995*

Well-defined poly(ethylene oxide) (PEO) with a cyano group at one end and a hydroxyl group at the other terminus was synthesized by the anionic ring opening polymerization of ethylene oxide (EO) initiated with (cyanomethyl)potassium (CMP) which was prepared by the metalation reaction of acetonitrile with potassium naphthalene in THF. Primary amino-terminal heterotelechelic PEO was obtained by the reduction of the cyano group at the end of the polymer chain by lithium aluminum hydride.

Recently, end-reactive PEOs have become more and more important in a variety of fields such as biology, biomedical science, and surface chemistry, due to their unique properties such as solubility and flexibility of the chains and basicity of the ether oxygens in the main chain (1, 2). For example, surface modifications by the end-reactive PEO prevent protein depositions to provide a biocompatible surfaces (3). Stabilization of proteins has been carried out extensively by a conjugation with the end-reactive PEOs, which induces several other benefits such as a decreased antigenicity and an increased solubility not only in water but also in organic solvents by maintaining their activities (4). Such PEO modification chemistries (sometimes called "PEGylation") have become a key area of interest in bioconjugate chemistry.

In general, PEO is synthesized by the ring opening polymerization of ethylene oxide (EO) initiated with an alkaline initiator such as potassium hydroxide (1). In this case, both chain ends should possess a hydroxyl group, and it is the so-called homotelechelic (5) PEO. PEO possessing a methoxy end group at one end and a hydroxyl group at the other end (semitelechelic PEO; methoxyPEO) can be obtained using potassium 2-methoxyethoxide as the initiator. In the first generation of PEGylation chemistry, the above two PEOs were utilized because of many kinds of commercially available samples with different molecular weights and controlled molecular weight distributions. Abuchowski et al. first reported (6) the activation method of a hydroxyl group using cyanuric chloride, followed by the modification of an enzyme. From this discovery, protein conjugation and also surface modification chemistries were extensively studied utilizing their procedure (2). However, the modifications by the cyanurate activated PEO have several problems since the primary amino groups must be derivatized. Some of the amino groups in the proteins are known to play an important role in its activity. The modification of such amino groups in the active center results in a significant decrease in the activity of the protein (7).

Harris and his co-workers (8) have comprehensively studied the synthesis of end-reactive PEOs possessing several kinds of functional groups such as primary amines, thiols, aldehydes, vinylsulfones, and activated esters. By utilizing such PEOs it has been revealed that a different modification method for the protein PEGylation resulted in activities (9, 10).

Most of the previously mentioned end-functionalized PEOs are semitelechelic or homotelechelic oligomers. To expand the utility of PEO's, a convenient synthesis of heterotelechelic (11) oligomers is needed. If such heterotelechelics can be synthesized easily, then these materials can be utilized as hetero-cross-linkers for different substances with defined spacer lengths and as surface modifiers with remaining reactive moieties at the free end. There are several reports on the synthesis of heterobifunctional PEOs using homotelechelic PEOs as the starting materials (12, 13). The synthetic methods, however, are complicated because they have to use several reaction steps to derivatize the PEO terminus. In addition, the efficiency of the derivatizations are not very high, meaning that the resulting PEO is a mixture of the starting homotelechelics and the resulting heterotelechelics to some extent.

Our strategy for heterotelechelic synthesis is to create a novel polymerization route of EO using new initiators containing defined functionalities. So far, we have synthesized heterotelechelics with a formyl group at one end and a hydroxyl group at the other end using an anionic ring opening polymerization of EO with potassium 3,3-diethoxypropoxide, followed by acid hydrolysis (14). Heterotelechelics with a primary-amino group at one end and a hydroxyl group at the other terminus were also synthesized using a silyl-protected potassium amide (15). In this case, the primary-amino group in *N*-methylethylenediamine was protected by 1,2-bis(dimethylchlorosilyl)ethane, and it was used as the initiator for polymerization of EO after the *sec*-amino group was converted to potassium amide. The silyl-amine at the end of the obtained polymer was easy to hydrolyze by acid to form the primary-amino terminated polymer. However, there were several disadvantages; for example, high skills are required for the synthesis of the silyl-

* Author to whom all correspondence should be addressed.

* Abstract published in *Advance ACS Abstracts*, November 1, 1995.

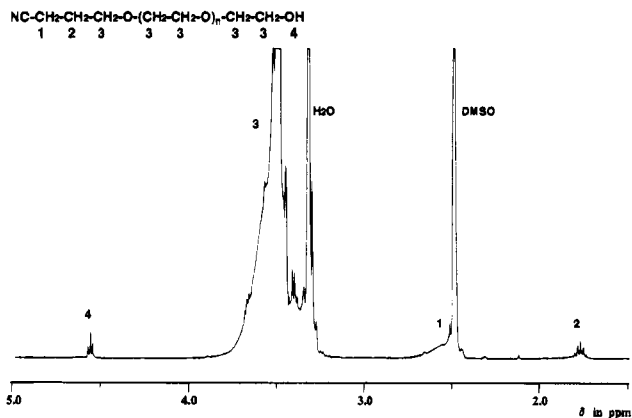


Figure 1. ^1H NMR spectrum of poly(ethylene oxide) initiated with (cyanomethyl)potassium in the presence of 18-crown-6 in THF.

Scheme 1

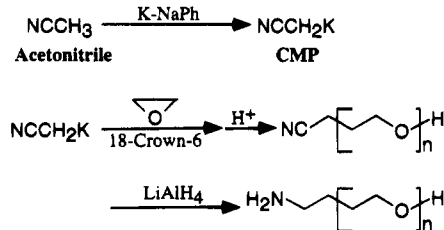
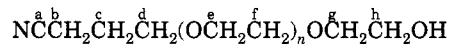


Table 1. ^{13}C NMR Chemical Shift Data of PEO Obtained with CMP as an Initiator (ppm)



carbon	a	b	c	d	e	f	g	h
obsd	119.3	13.8	25.4	68.3	70.3	70.3	72.3	61.4
calcd	120.8	13.7	25.4	68.8	70.6	70.6	72.8	63.7

protected initiator and the resulting polymer possessed one tertiary amine at the α -end group. There are several reports on the EO polymerizations using amine-protected initiators (16, 17). They had the same problems with the protections and the deprotections as our previous study. In this paper, we report the facile synthesis of primary amino-terminal heteroPEO initiated with CMP, which is easily synthesized using acetonitrile and potassium naphthalene, followed by a reduction of the cyano group with lithium aluminum hydride.

Since acetonitrile shows high acidity due to the electron-withdrawing effect of the cyano group ($\text{p}K_{\text{a}} = 25$ (18)), it is easy to metalate using an alkali metal alkyl such as butyllithium and potassium naphthalene (19). If the CMP can be utilized as the initiator for EO polymerization, the cyano-terminal heteroPEO can be formed. For the initiation of the anionic ring opening polymerization of EO, higher nucleophilicity than that of the alkoxylate anion must be provided (20). Since the acidity of the acetonitrile is lower than that of alcohol ($\text{p}K_{\text{a}}$ (methanol) = 15 (16)), it is probable that the CMP has suitable reactivity to act as an initiator for EO polymerization.

To THF (30 mL) containing 18-crown-6 (3 mmol) in a 100 mL flask with a three-way stopcock under an argon atmosphere were added acetonitrile (4 mmol) and a THF solution of potassium naphthalene (21) (2 mmol) to form CMP. The 18-crown-6 was utilized to avoid possible side reactions such as a formation of dianion of acetonitrile. After liquid EO (240 mmol; -20°C) was added via a cooled syringe (22), the mixture was allowed to react for 2 days at room temperature. As the reaction proceeded,

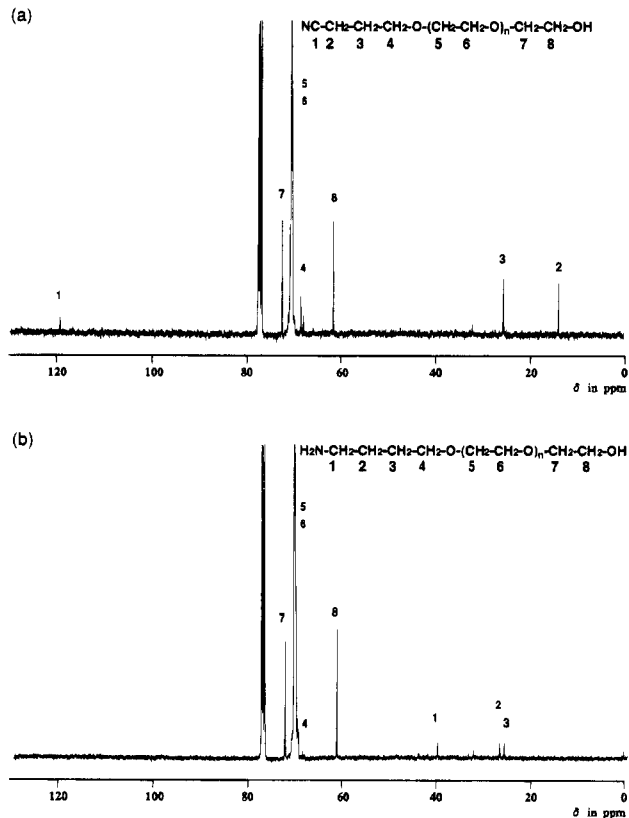


Figure 2. ^{13}C NMR spectra of poly(ethylene oxide) initiated with (cyanomethyl)potassium before (a) and after (b) the reduction by lithium aluminum hydride in THF (the same sample as in Figure 1 was used).

the mixture became a viscous liquid. Polymers thus formed were purified by precipitation with an excess amount of ether and subjected to freeze-drying from benzene solution. A white powder was obtained in 77.5% yield.

From gel permeation chromatographic analysis (GPC), the number average molecular weight (M_n) of the polymer was determined to be 5200, with a molecular weight distribution (M_w/M_n (23)) of 1.17. The M_n of the polymer determined from GPC was slightly lower than that calculated using an initial monomer/initiator ratio ($M_n = \text{MW}(\text{EO})[\text{EO}]_0/[\text{CMP}]_0 + \text{MW}(\text{acetonitrile}) = 44 \times 240/2 + 40 = 5320$), suggesting that a small amount of water participated in the initiation of the polymerization. Figure 1 shows the ^1H NMR spectrum of the polymer after the purification by two fold reprecipitations in ether from THF solution, followed by freeze-drying with benzene. Triad signals appearing at 4.6 ppm are assignable to alcoholic protons (^4H), while the triad signals appearing at 1.8 ppm are the methylene protons adjacent to cyanomethyl groups (^2H) as shown in Figure 1. The ratio of the area of these signals ($^4\text{H}/^2\text{H} = 1.35/2$) was again slightly higher than that expected from the calculated value for cyano-terminal heteroPEO ($\text{OH}/\text{CH}_2\text{CH}_2\text{CN} = 1/2$), indicating that a small amount of hydroxyl-terminal telechelic PEO (both OH end groups) was present as contamination due to the water impurity in the polymerization system. From the ^1H NMR and GPC analyses, it was concluded that ca. 10% of contaminating OH-telechelic PEO was present in the cyano-terminal heterobifunctional PEO (90%). In previous examples (where we have synthesized several types of heterobifunctional PEO (14, 15, 24, 25)) using alkoxide anion (O^-) and amido anion (N^-) as the initiators, no contaminating OH-telechelic PEO was present in the heterobifunctional

PEO formed. Carbanion as the initiator in the present polymerization system may affect the formation of OH-telechelic PEO, *viz.*, the carbanion may react with water, which is always presented the polymerization system. Such a side reaction produces a small amount of KOH which can react with EO to give OH-telechelic PEO. In a large scale polymerization with careful handling, however, the amount of water can be minimized.

Transformation of the terminal cyano group to a primary amino group was carried out by the addition of the THF solution (10 mL) of cyano-terminal-PEO (0.3 mmol) to a suspension of lithium aluminum hydride (6 mmol) in THF (10 mL) over 2 h. From the ^{13}C NMR spectrum (26) of the purified polymer shown in Figure 2, it was found that the signals derived from the cyano moiety completely disappeared and the four signals derived from the amino-methylene moiety appeared at 25.3, 26.9, 40.4, and 67.7 ppm, which are assignable to $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, to $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, to $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, and to $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ at the end of the polymer chain, respectively.

On the basis of the reported results, it is concluded that a heterobifunctional PEO with a primary amino group at one end and a hydroxyl group at the other end was synthesized, though ca. 10% of OH-terminated homotelechelic PEO was obtained due to the water impurities.

LITERATURE CITED

- (1) Bailey, F. E., Jr., and Koleske, J. V., Eds. (1991) *Alkylene Oxide and Their Polymers*, Vol. 35, Marcel Dekker: New York.
- (2) Harris, J. M., Ed. (1993) *Poly(ethylene glycol) Chemistry, Biotechnical and Biomedical Applications*, Plenum Press: New York.
- (3) For reviews see: (a) Merrill, E. W. (1992) Poly(ethylene oxide) and Blood Contact: A Chronicle of One Laboratory. In *PEG Chemistry: Biotechnical and Biomedical Applications* (J. M. Harris, Ed.) pp 199–246, Plenum Press, New York. (b) Gölander, C.-G., Herron, J. N., Lim, K., Claesson, P., Stenius, P., and Andrade, J. D. (1992) Properties of Immobilized PEG Films and the Interaction with Proteins: Experiments and Modeling. In *PEG Chemistry: Biotechnical and Biomedical Applications* (J. M. Harris, Ed.) pp 221–246, Plenum Press, New York.
- (4) For reviews see: (a) Yoshinaga, K., Ishida, H., Sagawa, T., and Ohkubo, K. (1992) PEG-Modified Protein Hybrid Catalyst. In *PEG Chemistry: Biotechnical and Biomedical Applications* (J. M. Harris, Ed.) pp 103–114, Plenum Press, New York. (b) Yomo, T., Urabe, I., and Okada, H. (1992) PEG-Coupled Semisynthetic Oxidases. In *PEG Chemistry: Biotechnical and Biomedical Applications* (J. M. Harris, Ed.) pp 115–126, Plenum Press, New York. (c) Veronese, F. M., Caliceti, P., Schiavon, O., and Sartore, L. (1992) Preparation and Properties of Monomethoxypoly(ethylene Glycol)-Modified Enzymes for Therapeutic Applications. In *PEG Chemistry: Biotechnical and Biomedical Applications* (J. M. Harris, Ed.) pp 127–138, Plenum Press, New York.
- (5) The term telechelic oligomer was defined as an oligomer with reactive groups at the chain ends.
- (6) Abuchowski, A., McCoy, J. R., Palczuk, N. C., van Es, T., and Davis, F. F. (1977) *J. Biol. Chem.* 252, 3582–3586.
- (7) Yoshimoto, T., Nishimura, H., Saito, Y., Sakurai, K., Kamisaki, Y., Wada, H., Sako, M., Tsujino, G., and Inada, Y. (1986) *Jpn. J. Cancer Res.* 77, 1264–1272.
- (8) Harris, J. M., Sedaghat-Herati, M. R., Sather, P. J., Brooks, D. E., and Fyles, T. M. (1992) Synthesis of New Poly(Ethylene Glycol) Derivatives. In *PEG Chemistry: Biotechnical and Biomedical Applications* (J. M. Harris, Ed.) pp 347–370, Plenum Press, New York.
- (9) Chamow, S. M., Kogan, T. p., Venuti, M., Gadek, T., Harris, R. J., Peers, D. H., Mordenti, J., Shak, S., and Ashkennazi, A. (1994) *Bioconjugate Chem.* 5, 133–140.
- (10) Takakura, Y., Kaneko, Y., Fujita, T., Hashida, M., Maeda, H., and Sezaki, H. (1989) *J. Pharm. Sci.* 78, 117–121.
- (11) The term, heterotelechelic, was defined in our previous paper²⁴, which denotes a telechelic oligomer with a functional group at one end and another functional group at the other end.
- (12) Means, G. E., Feeney R. E. (1990) *Bioconjugate Chem.* 1, 2.
- (13) J. M. Harris, and M. Yalpani, Eds. (1985) *Polymer-Ligands Used in Affinity Partitioning and Their Synthesis*, Academic Press: New York.
- (14) Nagasaki, Y., Kutsuna, T., Iijima, M., Kato, M., Kataoka, K., Kitano, S., and Kadoma, Y. (1995) *Bioconjugate Chem.* 6, 231.
- (15) Kim, Y. J., Nagasaki, Y., Kataoka, K., Kato, M., Yokoyama, M., Okano, T., and Sakurai Y. (1994) *Polymer Bull.* 33, 1.
- (16) (a) Furukawa, J., Saegusa, T., Matsushita, H., Kanbara, S., and Tanaka, H. Jpn. Pat. 7,016,468. (b) Huang, Y.-H., Li, Z.-M., and Morawetz, H. (1985) *J. Polym. Sci.* 23, 795.
- (17) Sépulchre, M., Paulus, G., and Jérôme, R. (1983) *Makromol. Chem.* 184, 1849.
- (18) March, J. (1992) *Advanced Organic Chemistry*, 4th ed., John Wiley & Sons, New York.
- (19) Rappoport, Z., Ed. (1970) *The Chemistry of the Cyano Group*, John Wiley & Sons, New York.
- (20) Tsuruta, T., and Kawakami, Y. (1988) Anionic Ring-Opening Polymerization: General Aspects and Initiation. In *Comprehensive Polymer Science: The Synthesis, Characterization, Reactions & Applications of Polymers* (G. C. Eastmond, A. Ledwith, S. Russo, and P. Sigwalt, Eds.) Vol. 3, Chain Polymerization I, pp 457–466, Pergamon Press, Oxford.
- (21) Szwarc, M., Levy, M., and Milkovich, R. (1956) *J. Am. Chem. Soc.* 78, 2656.
- (22) The syringe was cooled by liquid nitrogen for several minutes prior to use.
- (23) M_w and M_n denote weight average and number average molecular weights, respectively.
- (24) Yokoyama, M., Okano, T., Sakurai, Y., Kikuchi, A., Ohsako, N., Nagasaki, Y., and Kataoka, K. (1992) *Bioconjugate Chem.* 3, 275.
- (25) Cammas, S., Nagasaki, Y., and Kataoka, K. (1995) *Bioconjugate Chem.* 6, 223.
- (26) The assignments of these signals were complete in reference to the literature on hydroxyl-terminated PEO²⁷ and are described in Figure 2.
- (27) Kinugasa, S., Takatsu, A., Nakanishi, H., Nakahara, H., and Hattori, S. (1992) *Macromolecules* 25, 4848.

BC950068H