

Article

Synthesis of Propargyl-Terminated Heterobifunctional Poly(ethylene glycol)

Changhai Lu ¹ and Wen Zhong ^{1,2,*}

¹ Department of Textile Sciences, Faculty of Human Ecology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada; E-Mail: lcheric2000@yahoo.com.cn

² Department of Medical Microbiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

* Author to whom correspondence should be addressed; E-Mail: zhong@cc.umanitoba.ca; Tel.: +1-204-474-9913; Fax: +1-204-474-7592.

Received: 22 September 2010; in revised form: 4 October 2010 / Accepted: 8 October 2010 /

Published: 13 October 2010

Abstract: Novel propargyl-ended heterobifunctional poly(ethylene glycol) (PEG) derivatives with hydroxyl, carboxyl, mercapto or hydrazide end groups were synthesized with simplicity yet high efficiency. PEG ($M_w = 3500$ Da) with an α -hydroxyl group and an ω -carboxyl was used as the starting polymer. The carboxyl group of the bifunctional PEG was modified into a propargyl, then carboxyl, mercapto or hydrazide groups were introduced to the other end of the bifunctional PEG by modifying the bifunctional PEG's hydroxyl group with succinic anhydride, cysteamide or tert-butyl carbazate, respectively. This method can be useful to the development of PEG-based bioconjugates for a variety of biomedical applications.

Keywords: heterobifunctional; poly(ethylene glycol); “click” chemistry

1. Introduction

Poly(ethylene glycol) (PEG) is a polyether diol that dissolves in organic solvents as well as in water. It can be metabolized by both the kidney and the liver inside the human body, making them ideal to be used in pharmaceutical applications [1]. Food and Drug Administration (FDA) in U.S. has approved PEG and its conjugates for human intravenous, oral and dermal administrations [2]. PEG is

also known for its low level of cell and protein adsorption, so a PEG-grafted surface can deduce the proteinaceous deposition [3]. Because of its low toxicity, good hydrophilicity, excellent biocompatibility and biodegradability, PEG can be used as a promising material in such biomedical applications as protein modification [4], PEG-drug conjugates [1,5], polymer micelles[6,7], and 3-D scaffold materials in tissue engineering [8,9] and regenerative medicine.

However, because of the homobifunctional hydroxyl groups or their derivatives at both ends of the polymer chain, the structure of PEG limits its applications as drug carriers or in other biomedical end uses. If heterogeneous reactive groups can be attached to PEG at its α,ω -terminals, such heterobifunctional PEG will become more “functional” when being linked to biopharmaceutics. To that end, functionalization of the PEG chain is an important method for the development of PEG with heterofunctional groups at each end of the polymer chain.

It has been reported that heterobifunctional PEG can be fabricated through polymer reactions starting from hydroxyl-terminated PEG [10–15]. For example, Kataoka's group [12–14] has reported many kinds of heterofunctional PEG by ring-opening polymerization of ethylene oxide (EO) with the derivative of alcohol such as allyl alcohol, 2-(tetrahydro-2H-pyran-2-yloxy)ethanol and 3,3-diethoxypropanol et al as initiator, followed by several steps of modification to obtain the final functional groups. Owing to its high selectivity, mild reaction conditions, high yields and few by-products, the click reaction has rapidly become one of the most popular reactions to date. For example, Sharpless *et al.* [15] have reported their click reaction in the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and alkynes; Emrick *et al.* [16] have employed the click reaction in the synthesis of PEG- and peptide-grafted aliphatic polyesters; Heinze and Liebert *et al.* [17] in the modification of polysaccharide; Jérôme *et al.* [18] in the functionalization of aliphatic polyester; and Cazalis, *et al.* [19] in the immobilization of carbohydrates and proteins onto the solid surfaces. On the other hand, the azide and alkyne groups can be easily introduced into the polymer backbone. These groups are stable in the common organic reagents and can coexist with other functional groups, such as hydroxyl, amino and carboxyl groups. The triazole, having a five-membered ring of two carbon atoms and three nitrogen atoms, thus formed is quite stable, too. In addition, the copper (I) catalyst system is easily available and insensitive to solvent and pH [19]. For these reasons, azide/alkyne-based click reaction has provided us an appealing approach to the modification of biodegradable polyesters with natural materials such as sugars and proteins, thereby avoiding side reactions caused by the multifunctional and multichiral nature of these molecules [17].

In this paper, we present a novel and highly efficient route to the synthesis of propargyl-capped heterobifunctional PEG with hydroxyl, carboxyl, mercapto and hydrazide groups. The PEGs with new bifunctional groups verified by the ^1H NMR spectrum are useful precursors for click chemistry. This method can be useful to the development of PEG-based bioconjugates for a variety of biomedical applications.

2. Experimental Section

2.1. Materials

Solvents and reagents were purchased from commercial sources and were used as received, unless otherwise noted. Poly(ethylene glycol) ($M_w = 3500$ Da, polydispersity: 1.03) with ω -carboxyl group

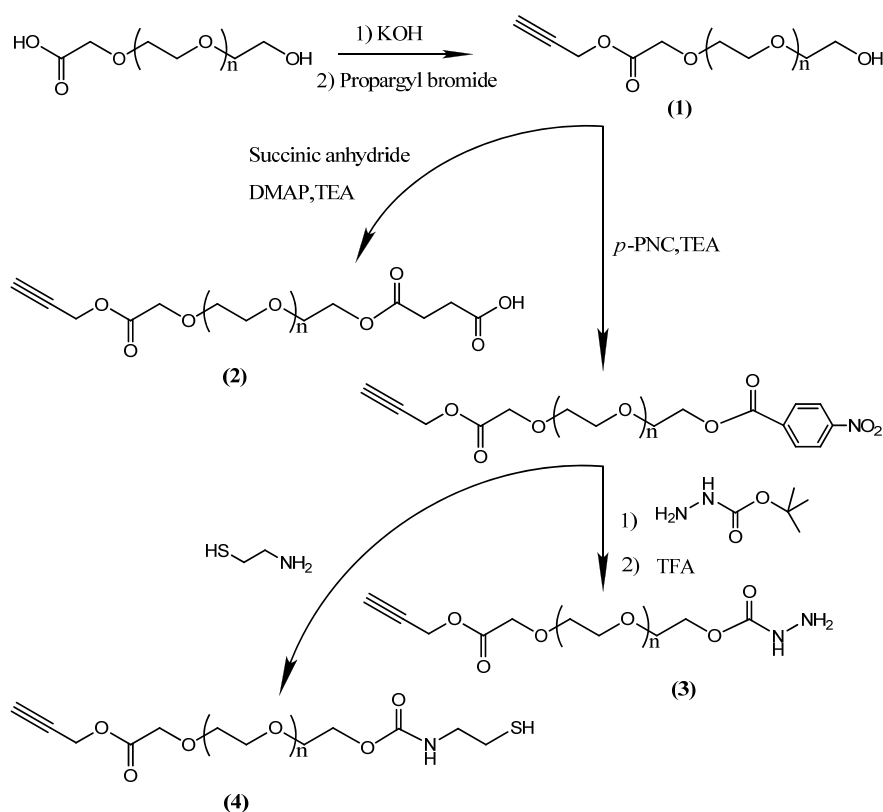
and α -hydroxyl (HOOC-PEG3500-OH, purity 99%) was purchased from JemKem Technology, propargyl bromide from Acros, succinic anhydride, tert-Butyl carbazate, 4-Nitrophenyl chloroformate (*p*-NPC), cysteamide, and 4-(Dimethylamino) pyridine (DMAP) from Sigma-Aldrich.

2.2. Measurements

^1H NMR (300 MHz) spectra were recorded with a Bruker Avance 500 spectrometer in CDCl_3 solution, unless otherwise noted. Chemical shifts for ^1H were reported in parts per million (ppm) downfield from TMS, using residual CHCl_3 (7.27 ppm) as internal standards. FT-IR spectra were recorded on a Bio-Rad Win-IR instrument.

2.3. Synthesis

Scheme 1. Synthetic route to α -hydroxyl- ω -propargyl PEG (**1**), α -carboxyl- ω -propargyl PEG (**2**), α -mercapto- ω -propargyl PEG (**3**) and α -hydrazide- ω -propargyl PEG (**4**).



2.3.1. Synthesis of α -hydroxyl- ω -propargyl PEG (**1**)

1.0 g (0.28 mmol) of HOOC-PEG3500-OH and 16.8 mg (0.30 mmol) of KOH were dissolved in 20 mL DMF, and stirred at 100 °C for 1 h. Propargyl bromide (0.027 mL, 0.30 mmol) was then added dropwise to the solution during 0.5 h, and then the mixture was stirred and allowed to react at 70 °C for 15 h. The reaction was terminated by cooling to room temperature. After filtration and concentration, the residue was dissolved in 10 mL distilled water and extracted with CH_2Cl_2 (3×100 mL). Removal of CH_2Cl_2 in vacuum yielded 0.962 g (rate of yield: 96.2%) α -hydroxyl- ω -propargyl PEG. ^1H

NMR (CDCl_3 , TMS): 2.5 (s, 1H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 3.6 ($-\text{CH}_2$ PEG), 4.1 (m, 2H, $-\text{C}(\text{O})\text{CH}_2-\text{O}-$), 4.7 (m, 2H, $-\text{CH}_2-\text{C}\equiv\text{CH}$).

2.3.2. Synthesis of α -carboxyl- ω -propargyl PEG (2)

6.0 mg succinic anhydride (0.06 mmol) and 7.3 mg DMAP (0.06 mmol) in anhydrous 1,4-Dioxane (10 mL) at 20 °C and triethylamine (TEA, 0.008 mL, 0.06 mmol) were added into the solution of ω -propargyl- α -hydroxyl PEG (0.2 g, 0.057 mmol). The mixture was stirred at room temperature for 24 h. The solution was concentrated in vacuum and then precipitated in diethyl ether. The crude product was purified by crystallization from THF/diethyl ether to give α -carboxyl- ω -propargyl PEG as a white powder, with a rate of yield of 92%. ^1H NMR (CDCl_3 , TMS): 2.5 (s, 1H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 2.7 (t, 4H, $-\text{CH}_2-\text{CH}_2-$ (succinic segment)), 3.6 ($-\text{CH}_2$ PEG), 4.2 (m, 2H, $-\text{C}(\text{O})\text{CH}_2-\text{O}-$), 4.3 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}(\text{O})-$), 4.7 (m, 2H, $-\text{CH}_2-\text{C}\equiv\text{CH}$).

2.3.3. Synthesis of α -hydrazide- ω -propargyl PEG (3)

2.3.3.1. Synthesis of amine-reactive α -hydroxyl- ω -propargyl PEG.

α -hydroxyl- ω -propargyl PEG was activated by using 4-Nitrophenyl chloroformate (*p*-NPC). 0.15 g (0.042 mmol) α -hydroxyl- ω -propargyl PEG and triethylamine (0.017 mL, 0.128 mmol) were dissolved in 15 mL anhydrous dichloromethane at 0 °C. *p*-NPC (0.017 g, 0.084 mmol) dissolved in 5 mL anhydrous dichloromethane was added dropwise into the mixed solution. The reaction proceeded for 2 h at 0 °C, and then at room temperature for 24 h with stirring. After concentration, the product, *Amine-reactive α -hydroxyl- ω -propargyl PEG*, was obtained by precipitation in cold diethyl ether and dried in vacuum with a yield of 0.12 g (80%). ^1H NMR (CDCl_3 , TMS): δ = 2.5 (s, 1H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 3.6 ($-\text{CH}_2$ PEG), 4.2 (m, 2H, $-\text{C}(\text{O})\text{CH}_2-\text{O}-$), 4.5 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}(\text{O})-$), 4.7 (m, 2H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 7.3 (s, 2H, Ar), 8.1 (s, 2H, Ar).

2.3.3.2. Conjugation of hydrazide on α -hydroxyl- ω -propargyl PEG

Amino-reactive α -hydroxyl- ω -propargyl PEG (0.14 g, 0.4 mmol) dissolved in dichloromethane (20 mL) was added into the tert-butyl carbazate (11.3 mg, 0.8 mmol) solution in dichloromethane (5 mL) and reacted for 24 h at room temperature. After the evaporation of dichloromethane, the product was dissolved in 10 mL dichloromethane. 2 mL trifluoroacetic acid (TFA) was added into the solution, which was stirred for 2 h at 0 °C. TFA and CH_2Cl_2 were then removed in vacuum, and the remaining solid was dissolved in a mixture of chloroform (10 mL) and triethylamine (TEA, 1 mL). The reaction was allowed to continue at room temperature for 8 h. The product, *α -hydrazide- ω -propargyl PEG*, was precipitated by the similar procedure described in the previous section, with a yield of 0.12 g (86%). ^1H NMR (CDCl_3 , TMS): δ = 1.80 (t, $-\text{NH}_2$), 2.5 (s, 1H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 4.2 (m, 2H, $-\text{C}(\text{O})\text{CH}_2-\text{O}-$), 3.6 ($-\text{CH}_2$ PEG), 4.3 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}(\text{O})-$), 4.7 (m, 2H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 8.2 (s, $-\text{NH}$).

2.3.4. Synthesis of α -thioglycol- ω -propargyl PEG (4)

Amino-reactive α -hydroxyl- ω -propargyl PEG (0.20 g, 0.57 mmol) and cysteamide (87.9 mg, 1.14 mmol) were dissolved in dichloromethane (20 mL) and were then allowed to react for 24 h at room temperature. After the evaporation of dichloromethane, the product was precipitated by the similar procedure described in the previous section, with a yield of 0.15 g (75%). ^1H NMR (CDCl_3 , TMS): δ = 2.5 (s, 1H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 2.8 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{SH}$), 3.3 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{SH}$), 3.6 ($-\text{CH}_2$ PEG), 4.0 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}(\text{O})-$), 4.2 (m, 2H, $-\text{C}(\text{O})\text{CH}_2-\text{O}-$), 4.7 (m, 2H, $-\text{CH}_2-\text{C}\equiv\text{CH}$), 7.9 (s, $-\text{NH}$).

3. Results and Discussion

In this study, the commercially available HOOC-PEG3500-OH of high purity and confirmed structure was selected as the starting material. α -hydroxyl- ω -propargyl PEG (**1**) was synthesized according to Scheme 1. The propargyl ester α -hydroxyl- ω -propargyl PEG was obtained in a good yield (96.2%) by a procedure described in our previous work [20], *i.e.*, potassium salt of α -hydroxyl- ω -carboxyl PEG was obtained and then allowed to react with propargyl bromide. In contrast with the ^1H NMR spectral for the original HOOC-PEG-OH (Figure 1), the ^1H NMR spectral of the new product (Figure 2) signaled at δ 2.5 and 4.7 ($-\text{C}\equiv\text{C}-\text{H}$ and $-\text{CH}_2-\text{C}\equiv\text{C}-\text{H}$ from the propargyl group), indicating that the ω -propargyl group and α -hydroxyl group of PEG were of the desired structure and high purity. Compared with original α -hydroxyl- ω -carboxyl PEG, the FI-IR spectrum (Figure 3b) shows the characteristic peak of acetylene group at $2130 (\nu_{\text{C}\equiv\text{C}-\text{H}}) \text{ cm}^{-1}$, and the characteristic peak of ester bond is a little stronger at $1760 (\nu_{\text{C}=\text{O}}) \text{ cm}^{-1}$.

Figure 1. ^1H NMR spectrum of poly(ethylene glycol) with ω -carboxyl group and α -hydroxyl group.

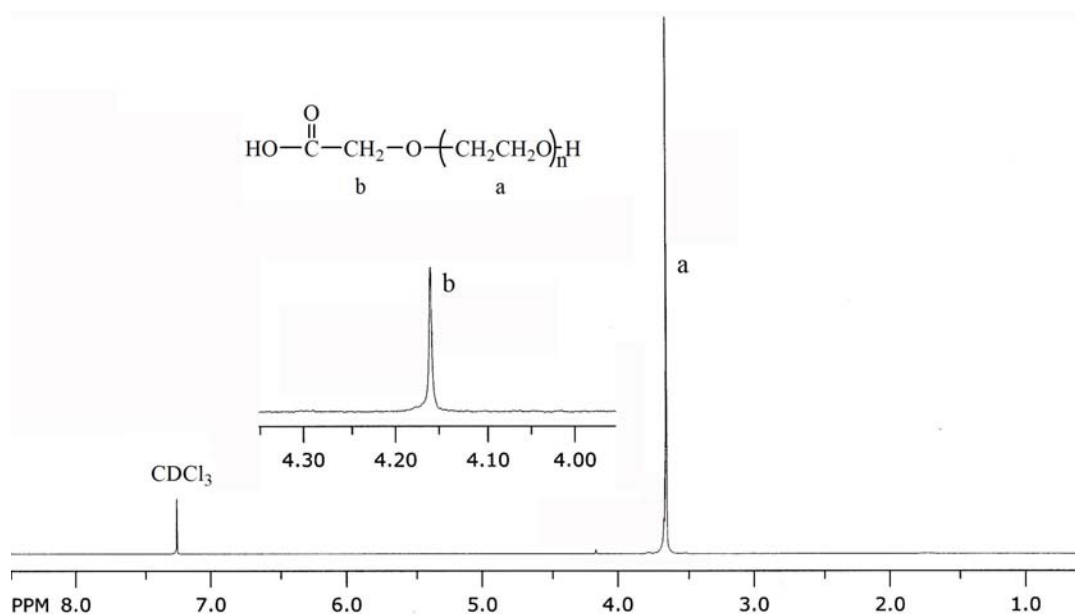


Figure 2. ^1H NMR spectrum of poly(ethylene glycol) with ω -propargyl group and α -hydroxyl group.

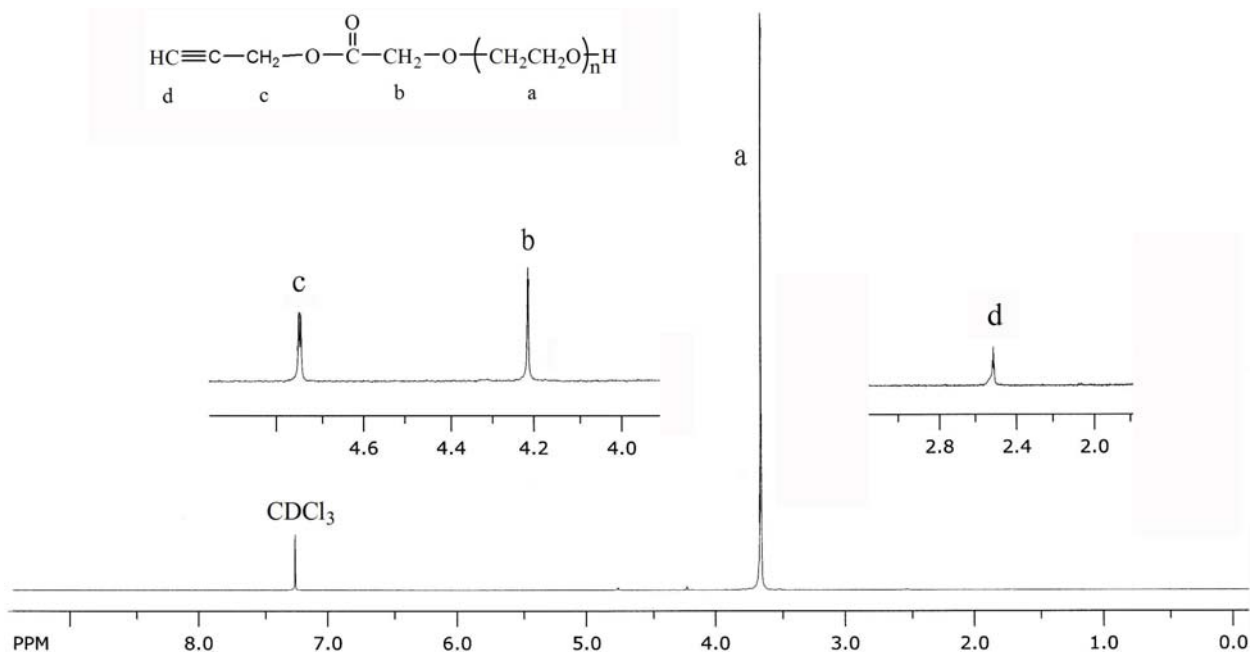
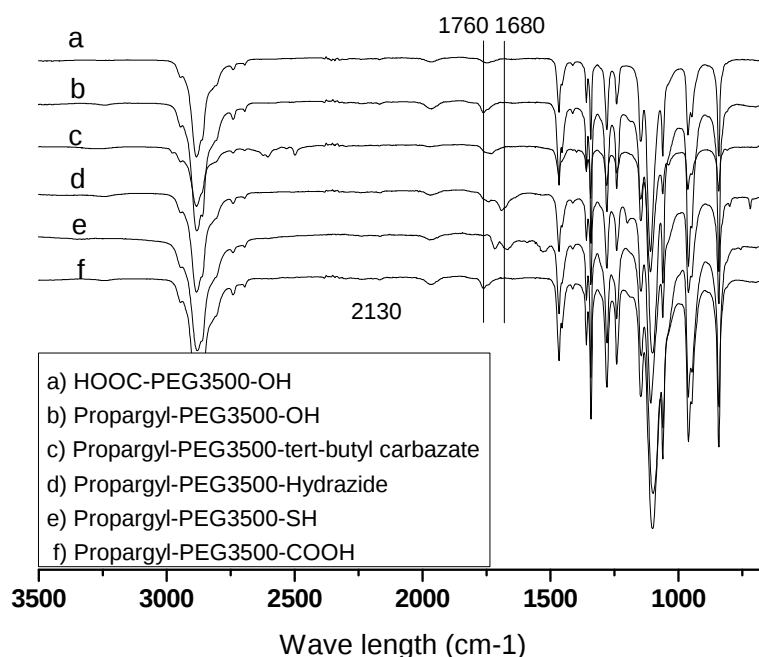


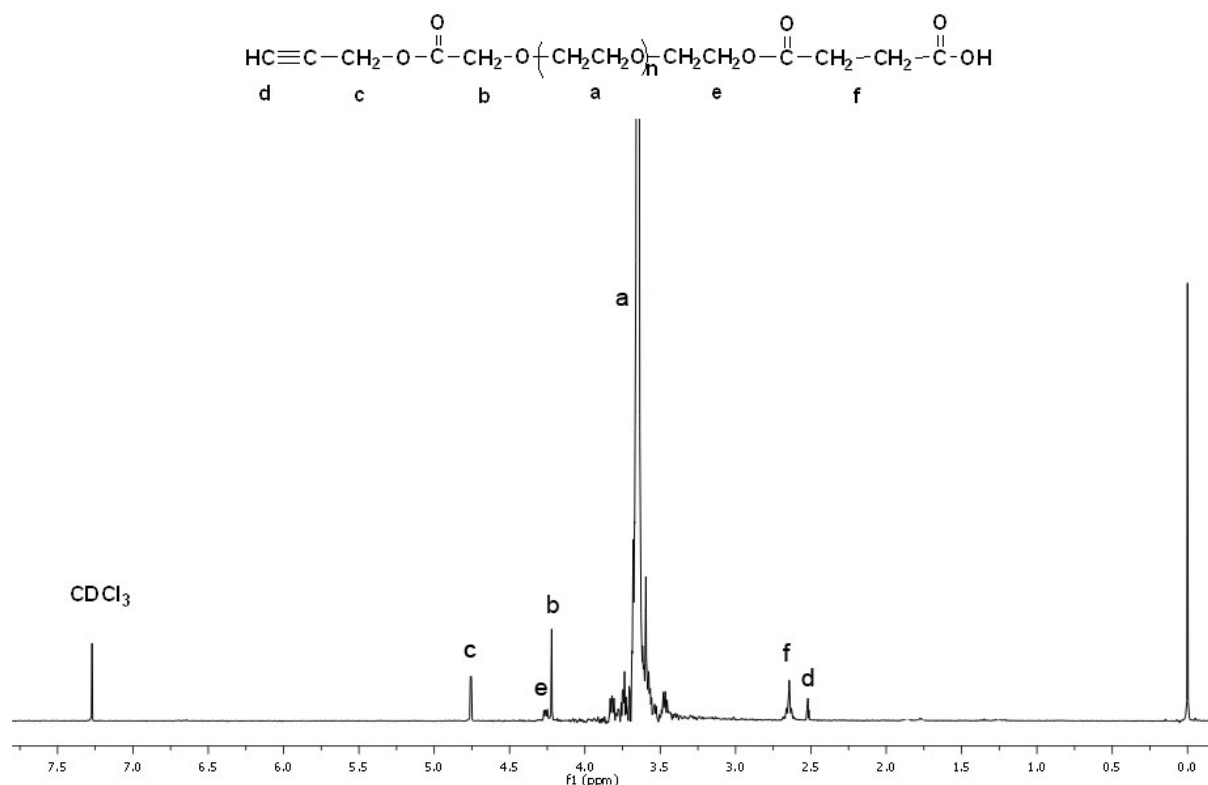
Figure 3. FT-IR spectra of bifunctional PEG.



α -carboxyl- ω -propargyl PEG (**2**) was prepared with succinic anhydride in the presence of DMAP and triethylamine (TEA) according to literatures [21,22]. According to Figure 4, which shows the ^1H NMR spectra of α -carboxyl- ω -propargyl PEG, appearance of the proton signals of CH_2 formed by the reaction with succinic anhydride at 2.75 is obvious, whereas other proton signals almost remain unchanged. The degree of introduction of carboxylation per PEG chain was calculated from the ratio of integration value of 2.75 ppm to that of methylene proton of propargyl signal. From these analyses,

it was found that the ring of succinic anhydride had been quantitatively opened by the terminal hydroxyl groups of PEG. Thus, a terminal carboxyl group was introduced as expected into the PEG.

Figure 4. ^1H NMR spectrum of poly(ethylene glycol) with ω -propargyl group and α -carboxyl group.



α -hydrazide- ω -propargyl PEG (**3**) was obtained through a sequential *p*-NPC-mediated hydroxyl-amine coupling reaction followed by an deprotection of *tert*-butyl group as shown in Scheme 1. As indicated by the ^1H NMR (Figure 5A), the protons of phenyl of *p*-NPC appear at 7.33 and 8.16 ppm in contrast with the spectral in Figure 2. The efficiency of the reaction is 98.2% according to the ratio of integration value of 8.16 ppm to that of methylene proton of propargyl signal (4.35 ppm).

Figure 5B shows the result of the hydroxyl-amine coupling reaction. The methyl proton of *tert*-butyl carbazate signals were detected at 1.44. The substitution percentage is 96% according to Figure 5B. Deprotection of the *tert*-butyl group was carried out in the mixed solvent of trifluoroacetic acid and chloroform at 0 °C. As a result, the methyl ^1H NMR peak of Boc at 1.44 ppm (Figure 5B) disappeared completely in Figure 5C, which demonstrated the successful elimination of the Boc group. After deprotection, the characteristic peak between 1760 and 1680 cm^{-1} in Figure 3d shows the formation of amide bond connection of hydrazide.

α -thioglycol- ω -propargyl PEG (**4**) was synthesized according to the method of polymer **3** with *tert*-butyl carbazate replaced by cysteamide. Figure 6 shows the ^1H NMR spectrum of polymer **4**. The methylene proton of cysteamide appeared at 2.8 and 3.3 ppm, and the proton of NH appeared at 7.9 ppm. Efficiency of the reaction was calculated from the ratio of integration value of 2.8 ppm to that of methylene proton of propargyl signal (4.7 ppm). The ratio was 0.9, which confirmed the high efficiency of this reaction.

Figure 5. ^1H NMR spectra of (A) α -*p*-NPC- ω -propargyl PEG; (B) α -*tert* butyl carbazate- ω -propargyl PEG; (C) α -hydrazide- ω -propargyl PEG.

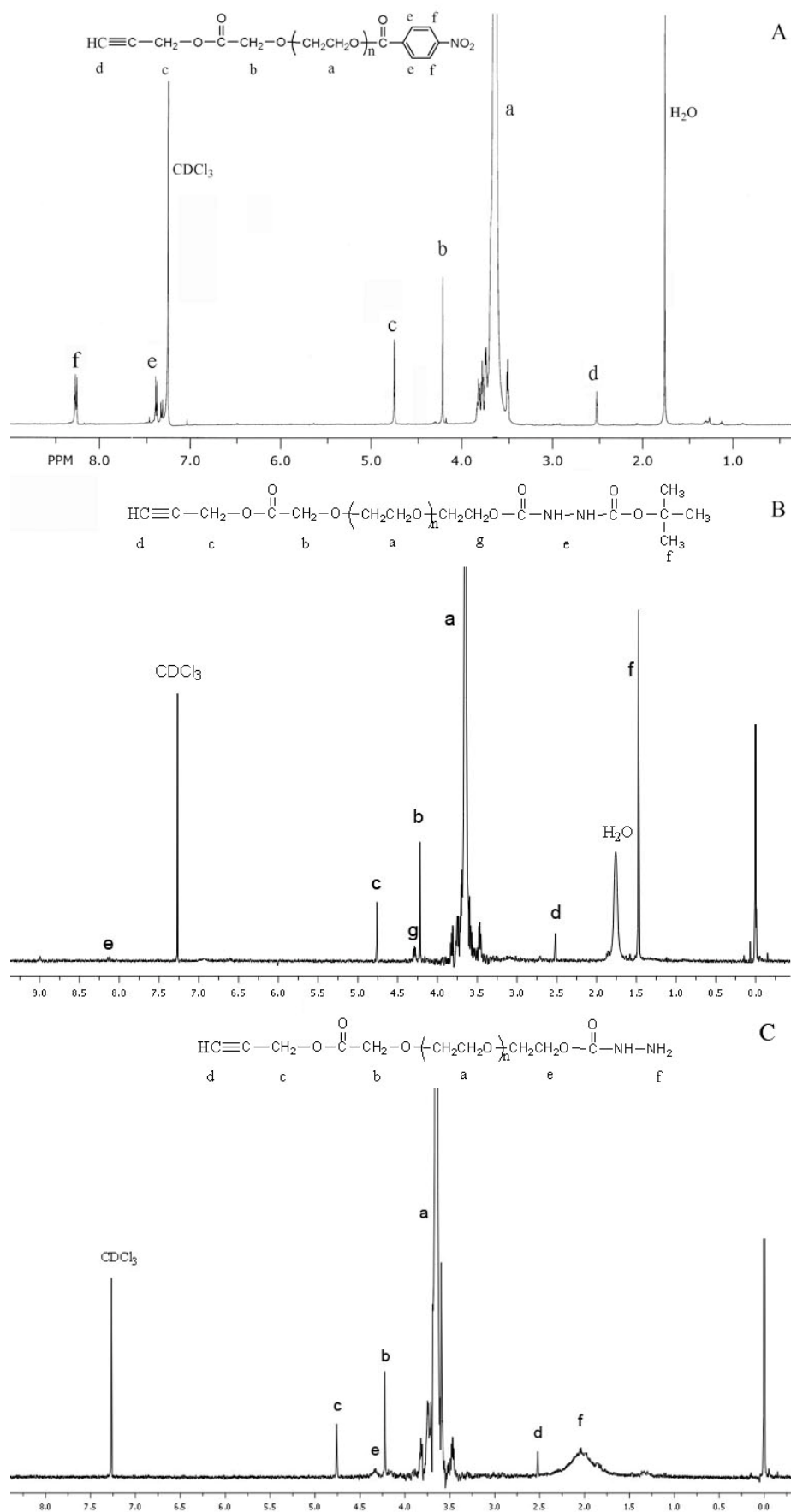
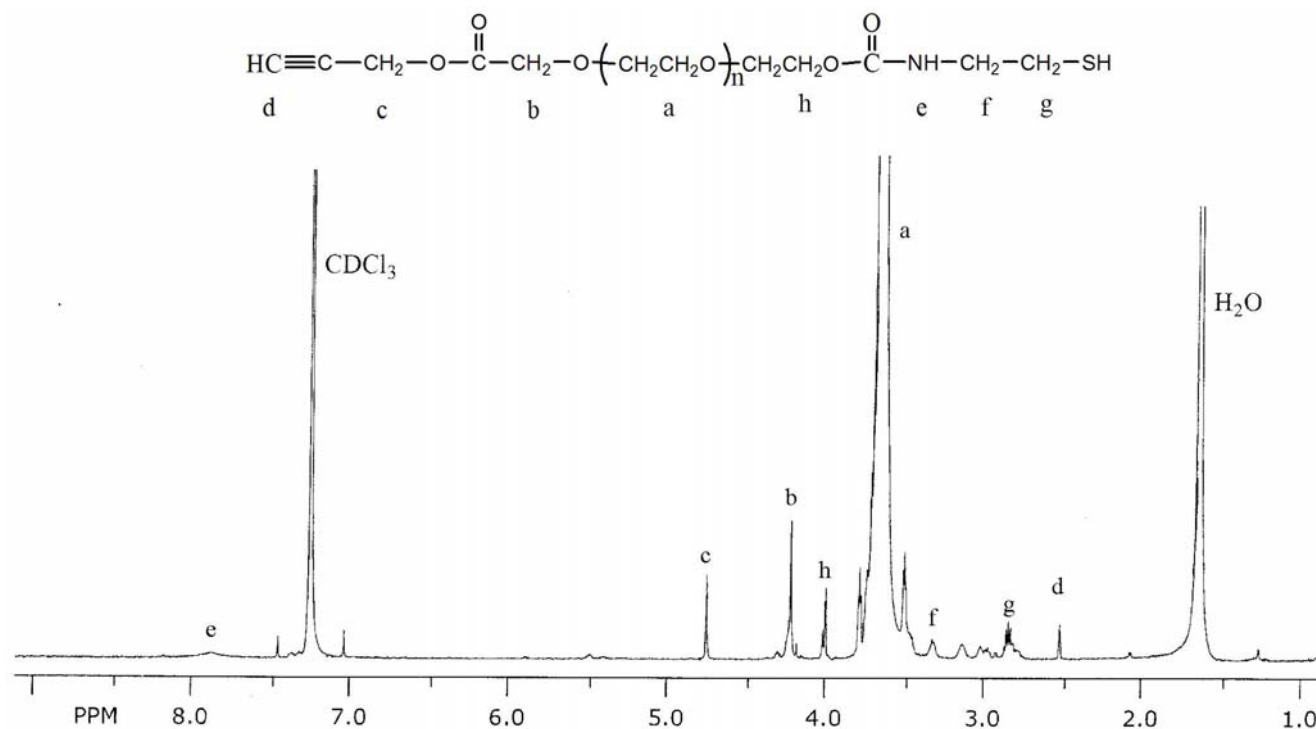


Figure 6. ^1H NMR spectrum of poly(ethylene glycol) with ω -propargyl group and α -thioglycol group.



4. Conclusions

Presented in this paper is a simple, efficient method to obtain the heterobifunctional poly(ethylene glycol) with a ω -propargyl group and a α -hydroxyl (a α -carboxyl, a α -mercapto or a α -hydrazide) group at each terminal by means of the modification of the commercially available α -hydroxyl ω -carboxyl PEG. Structures of these PEGs were confirmed by the ^1H NMR spectrum. Via the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction (click chemistry), it is very convenient to attach azido-containing biomolecules to propargyl-modified PEG, and to further construct the smart vehicles for drug delivery and protein modification. An example of such kind of work is reported in another paper of ours [23].

Acknowledgements

Our work is supported by the NSERC (Natural Sciences and Engineering Research Council of Canada) Discovery Grant and NSERC RTI (Research Tools and Instrument) Grant.

References

1. Greenwald, R.B.; Choe, Y.H.; McGuire, J.; Conover, C.D. Effective drug delivery by PEGylated drug conjugates. *Adv. Drug Deliv. Rev.* **2003**, *55*, 217–250.
2. Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.* **2003**, *2*, 347–360.
3. Hooftman, G.; Herman, S.; Schacht, E. Poly(ethylene glycol)s with reactive endgroups. 2. Practical consideration for the preparation of protein-PEG conjugates. *J. Bioact. Compat. Pol.* **1996**, *11*, 135–159.

4. Pasut, G.; Guiotto, A.; Veronese, F.M. Protein, peptide and non-peptide drug PEGylation for therapeutical application: A review. *Expert Opin. Ther. Patents*. **2004**, *14*, 859–894.
5. Veronese, F.M.; Pasut, G. PEGylation, successful approach to drug delivery. *Drug Discov. Today* **2005**, *10*, 1451–1458.
6. Yoo, H.S.; Park, T.G. Folate receptor targeted biodegradable polymeric doxorubicin micelles. *J. Control. Release* **2004**, *96*, 273–283.
7. Zeng, F.Q.; Lee, H.; Allen, C. Epidermal growth factor-conjugated poly(ethylene glycol)-*block*-poly(delta-valerolactone) copolymer micelles for targeted delivery of chemotherapeutics. *Bioconjugate Chem.* **2006**, *17*, 399–409.
8. Gonen-Wadmany, M.; Oss-Ronen, L.; Seliktar, D. Protein-polymer conjugates for forming photopolymerizable biomimetic hydrogels for tissue engineering. *Biomaterials* **2007**, *28*, 3876–3886.
9. Li, N.; Jie, Q.; Zhu, S.; Wang, R. Preparation and characterization of macroporous sol-gel bioglass. *Ceram. Int.* **2005**, *31*, 641–646.
10. Bettinger, T.; Remy, J.S.; Erbacher, P.; Behr, J.P. Convenient polymer-supported synthetic route to heterobifunctional polyethylene glycols. *Bioconjugate Chem.* **1998**, *9*, 842–846.
11. Kaiser, K.; Marek, M.; Haselgrubler, T.; Schindler, H.; Gruber, H.J. Basic studies on heterobifunctional biotin-PEG conjugates with a 3-(4-pyridyldithio)propionyl marker on the second terminus. *Bioconjugate Chem.* **1997**, *8*, 545–551.
12. Akiyama, Y.; Otsuka, H.; Nagasaki, Y.; Kato, M.; Kataoka, K. Selective synthesis of hetero-bifunctional poly(ethylene glycol) derivatives containing both mercapto and acetal terminals. *Bioconjugate Chem.* **2000**, *11*, 947–950.
13. Hiki, S.; Kataoka, K. A facile synthesis of azido-terminated heterobifunctional poly(ethylene glycol)s for "click" conjugation. *Bioconjugate Chem.* **2007**, *18*, 2191–2196.
14. Hiki, S.; Kataoka, K. Versatile and selective synthesis of "click chemistry" compatible heterobifunctional poly(ethylene glycol)s possessing azide and alkyne functionalities. *Bioconjugate Chem.* **2010**, *21*, 248–254.
15. Kolb, H.C.; Finn, M.G.; Sharpless, K.B. Click chemistry: Diverse chemical function from a few good reactions. *Angew. Chem. Int. Edit.* **2001**, *40*, 2004–2021.
16. Parrish, B.; Breitenkamp, R.B.; Emrick, T. PEG- and peptide-grafted aliphatic polyesters by click chemistry. *J. Am. Chem. Soc.* **2005**, *127*, 7404–7410.
17. Liebert, T.; Hansch, C.; Heinze, T. Click chemistry with polysaccharides. *Macromol. Rapid Comm.* **2006**, *27*, 208–213.
18. Riva, R.; Schmeits, P.; Stoffelbach, F.; Jerome, C.; Jerome, R.; Lecomte, P. Combination of ring-opening polymerization and "click" chemistry towards functionalization of aliphatic polyesters. *Chem. Commun.* **2005**, *42*, 5334–5336.
19. Sun, X.L.; Stabler, C.L.; Cazalis, C.S.; Chaikof, E.L. Carbohydrate and protein immobilization onto solid surfaces by sequential Diels-Alder and azide-alkyne cycloadditions. *Bioconjugate Chem.* **2006**, *17*, 52–57.
20. Lu, C.H.; Shi, Q.; Chen, X.S.; Lu, T.C.; Xie, Z.G.; Hu, X.L.; Ma, J.; Jing, X.B. Sugars-grafted aliphatic biodegradable poly(L-lactide-co-carbonate)s by click reaction and their specific interaction with lectin molecules. *J. Polym. Sci. Pol. Chem.* **2007**, *45*, 3204–3217.

21. Jeon, O.; Lee, S.H.; Kim, S.H.; Lee, Y.M.; Kim, Y.H. Synthesis and characterization of poly(L-lactide)-poly(epsilon-caprolactone) multiblock copolymers. *Macromolecules* **2003**, *36*, 5585–5592.
22. Wang, C.H.; Hsiue, G.H. Polymer-DNA hybrid nanoparticles based on folate-polyethylenimine-*block*- poly(L-lactide). *Bioconjugate Chem.* **2005**, *16*, 391–396.
23. Lu, C.H.; Xing, M.M.Q.; Zhong, W. Shell Cross-linked and Hepatocyte Targeting Nanoparticles Containing Doxorubicin via Acid-Cleavable Linkage. *Nanomed. Nanotechn. Biol. Med.* **2010**, doi:10.1016/j.nano.2010.07.001.

© 2010 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).