New Synthetic Methodologies for Amphiphilic Multiblock Copolymers of Ethylene Glycol and Propylene Sulfide

A. Napoli, N. Tirelli,* G. Kilcher, and J. A. Hubbell

Institute for Biomedical Engineering and Department of Materials, Swiss Federal Institute of Technology (ETH) and University of Zurich, Moussonstrasse 18, CH-8044 Zurich, Switzerland

Received May 10, 2001; Revised Manuscript Received September 25, 2001

ABSTRACT: A new *one pot* synthetic method has been developed for preparing amphiphilic block copolymers. The method makes use of episulfide anionic polymerization for generating symmetric or asymmetric tri- or multiblock copolymers with living mechanism. Important features of the method are (a) the use of in situ generated thiolates, which ensures reproducibility in the initiator concentration; (b) the mild character of the reactive species; therefore, also sensitive bioactive groups could be easily incorporated into the polymer structure; (c) the tolerance to impurities in the reagents and the one pot character, which allow the use of the method also by operators with limited skills in organic synthesis.

Introduction

There exists extensive interest in amphiphilic block copolymers that self-assemble in aqueous environments, for example, in the field of controlled drug delivery. The aqueous phase behavior of these polymers can show a high degree of richness and complexity. A variety of supermolecular structures can be generated, such as micellar and vesicular assemblies, both of which are potentially interesting for a variety of pharmacological applications. Extensive investigations have been conducted on poly(ethylene glycol) (PEG)-containing block copolymers, such as the Poloxamer (also known as Pluronic) series of PEG-*bl*-poly(propylene glycol) di- and triblock copolymers,[1−3] and more recently on poly(ethylethylene)-*bl*-PEG,[4] poly(styrene)-*bl*-PEG,[5] and others,[6,7] as well as on poly(oxazoline)-containing block terpolymers.[8,9] Such block copolymers are generally prepared via ionic polymerization under strictly anhydrous conditions; the synthetic conditions make it generally difficult to obtain asymmetric multiblock structures (such as ABC block copolymers). Furthermore, only a limited number of functional groups can be tolerated, making the incorporation of biological species sometimes difficult.

In this paper we report a versatile and mild synthetic method for the preparation of amphiphilic multiblock copolymers in which the hydrophobic block is poly(propylene sulfide) (PPS). The method allows the preparation of AB, ABA, ABC, and ABA′ structures (as well as potentially higher multiblocks), where A represents a hydrophilic block, B the PPS block, and C and A′ an additional hydrophilic block, chemically different and chemically identical but of different MW from A. Interesting lyotropic behavior of PEG-*bl*-PPS-*bl*-PEG′ copolymers has been observed.

Experimental Section

Materials and Methods. Solvents and reagents were purchased from Fluka (Buchs, Switzerland) and used without further purification unless otherwise specified. THF was refluxed over sodium/potassium alloy and benzophenone for 3 h before being distilled under an inert atmosphere. ^{1}H NMR spectra were recorded on a 300 MHz Bruker spectrometer. FT-IR spectra were recorded in ATR mode on a Spectrum One Perkin-Elmer spectrometer. GPC was performed in THF on a Polymer Laboratories GPC equipped with refractive index and viscosity detectors; the M_n data were obtained using a universal calibration with poly(styrene) standards.

Yields are reported as the ratio between the weight of recovered polymeric material and the total weight of polymeric material. Conversions are reported as ratio between number of reacted groups and number of potentially reactive groups in the recovered material.

PEG Tosylate. PEG tosylate was synthesized from PEG750 monomethyl ether according to a literature procedure.[18] Yield 74%, conversion 100% after double precipitation in diethyl ether. ^{1}H NMR ($CDCl_3$): δ = 2.48 (s, 3H, C**H$_3$**-tosyl), 3.37 (s, 3H, C**H$_3$**−O−PEG), 3.67−3.70 (t, 2H, O−C**H$_2$**CH_2OSO_2−), 4.14−4.17 (t, 2H, O−CH_2C**H$_2$**OSO_2−), 7.33−7.36 (d, 2H, aromatic CH, *ortho* to the methyl group), 7.78−7.81 ppm (d, 2H, aromatic CH, *meta* to the methyl group). FT-IR (thin film): 3055 (ν aromatic C−H), 2990−2790 (ν aliphatic C−H), 1595 (aromatic ring stretching), 1452 (δ_s CH_2), 1373 (ν_{as} SO_3), 1350, 1295, 1250, 1174 (ν_s SO_3), 1097 (ν_{as} C−O−C), 947, 923, 842 (ν_s C−O−C), 816 cm^{-1} (δ_{oop} *para*-substituted aromatic CH).

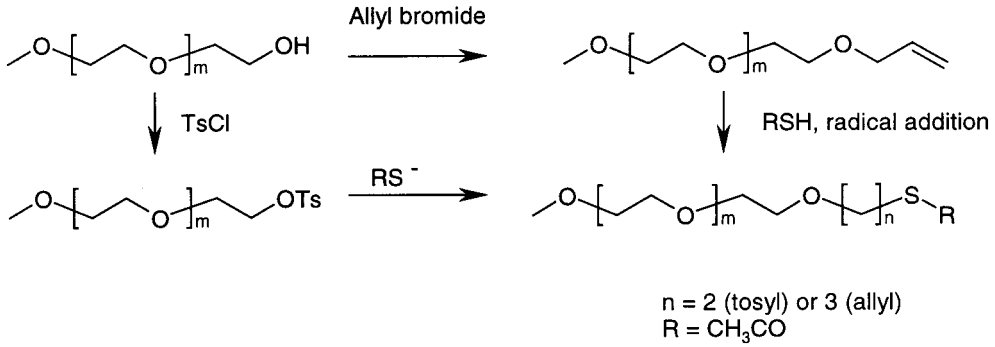
PEG Thioacetate (1). PEG thioacetate was synthesized from PEG tosylate according to a literature procedure.[18] After dissolution in CH_2Cl_2, extraction with water, treatment with activated charcoal, and double precipitation in cold hexane, a brownish viscous material was obtained. Yield 50%, conversion 100%. ^{1}H NMR ($CDCl_3$): δ = 2.33 (s, 3H, −SCOC**H$_3$**), 3.06−3.11 (t, 2H, −C**H$_2$**$SCOCH_3$), 3.37 (s, 3H, −OC**H$_3$**), 3.54−3.58 (t, 2H, −OC**H$_2$**CH_2S−), 3.5−3.7 (broad, PEG chain protons) ppm. FT-IR (thin film): 2990−2790 (ν C−H), 1691 (ν C═O), 1460 (δ_s CH_2), 1344, 1281, 1242, 1103 (ν_{as} C−O−C), 948, 842 cm^{-1} (ν_s C−O−C).

PEG Allyl Ether. PEG750 monomethyl ether (23.14 g, 31 mmol) was dissolved in toluene (250 mL) under an inert atmosphere and heated to reflux for 3−4 h in a Soxhlet apparatus filled with molecular sieves. The solution was then cooled to 0 °C, and 3 equiv of NaH (2.22 g, 93 mmol) was added under stirring. Gas evolved for about 15 min, and then 5 equiv of allyl bromide (13.34 mL, 155 mmol) was added dropwise; the reaction mixture was stirred at room temperature overnight. After filtration and evaporation of the solvent, the obtained solid was dissolved in CH_2Cl_2 and extracted twice with water. The organic phase was dried over Na_2SO_4, treated with activated charcoal, and precipitated twice in hexane. A white solid polymer (16.71 g, yield 82%, conversion 100%) was finally collected. ^{1}H NMR ($CDCl_3$): δ = 3.37 (s, 3H, −OC**H$_3$**), 3.5−3.7 (broad, PEG chain protons), 4.01−4.04 (dd, 2H,

* To whom correspondence should be addressed. Tel +41 1 632 45 85; FAX +41 1 632 12 14; e-mail tirelli@biomed.mat.ethz.ch.

10.1021/ma0108057 CCC: $20.00
Published on Web 11/21/2001

Scheme 1



$-CH_2OC\mathbf{H_2}CH{=}CH_2$), 5.15−5.30 (m, 2H, $-OCH_2CHC\mathbf{H_2}$), 5.85−5.98 ppm (m, 1H, $-OCH_2C\mathbf{H}CH_2$). FT-IR (thin film): 2990−2790 (ν C−H), 1456 (δ_s CH_2), 1349, 1296, 1249, 1097 (ν_{as} C−O−C), 945, 849, 842 cm^{-1} (ν_s C−O−C).

PEG Thioacetate (II). PEG allyl ether (15.0 g, 18.97 mmol) was introduced into a Schlenck tube and dissolved in THF (90 mL). 0.1 equiv of AIBN (0.312 g, 1.87 mmol) and 4 equiv of thioacetic acid (5.41 mL, 75.97 mmol) were added under stirring. The reaction mixture was degassed by freezing the mixture under liquid nitrogen, evacuating under high vacuum, and filling the Schlenck tube with argon, while bringing it back to room temperature. The degassing procedure was repeated three times. The reaction mixture was then stirred for 18−20 h at 60−65 °C. After cooling at room temperature, 5.0 g of Dowex resin 1 × 8 was introduced, and the mixture was stirred for 1 h. The resin was filtered away, the solvent evaporated, and the polymer redissolved in CH_2Cl_2. After extraction with water 5% $NaHCO_3$ and then water, the organic phase was dried over Na_2SO_4. The solution was filtered, concentrated, and precipitated twice in cold diethyl ether. A white solid polymer (10.7 g, yield 65%, conversion 100%) was finally collected. ^{1}H NMR ($CDCl_3$): δ = 1.81−1.90 (broad q, 2H, $-OCH_2C\mathbf{H_2}CH_2S-$), 2.32 (s, 3H, $-SCOC\mathbf{H_3}$), 2.92−2.97 (t, 2H, $-C\mathbf{H_2}SCOCH_3$), 3.38 (s, 3H, $-OC\mathbf{H_3}$), 3.49−3.52 (t, 2H, $-OC\mathbf{H_2}CH_2CH_2S$), 3.5−3.7 ppm (broad, PEG chain protons). FT-IR (thin film): 2990−2790 (ν C−H), 1691 (ν C=O), 1460 (δ_s CH_2), 1344, 1281, 1242, (ν_{as} C−O−C), 952, 842 (ν_s C−O−C) cm^{-1}. Elemental analysis: Calculated (considering 16.5 EG repeating units) [C]: 53.54%, [H]: 8.93%, [O]: 33.86%, [S]: 3.67%. Experimental [C]: 53.69%, [H]: 8.96%, [O]: 33.82%, [S]: 3.53%.

One-Pot Process. PEG thioacetate was introduced in a Schlenk tube under an inert atmosphere and was dissolved in THF (1 mL/40 mg of PEG). 1.05 equiv of sodium methylate (0.5 M in methanol) was then added via syringe, and the mixture was stirred at room temperature for 30 min. To the mixture a variable quantity (e.g., 25 or 50 equiv) of propylene sulfide was added, and the reagents were allowed to react for 45 min. An excess of end-capping agent (3-fold for iodoacetamide, 10-fold for PEG monoacrylate) was finally added, and the mixture was stirred overnight at room temperature. The solvent was removed, and the resulting viscous liquid was twice extracted with methanol. Yields > 90%. ^{1}H NMR ($CDCl_3$): δ = 1.35−1.45 (d, $C\mathbf{H_3}$ in PPS chain), 1.81−1.90 (broad q, 2H, $-OCH_2C\mathbf{H_2}CH_2S-$), 2.6−2.7 (m, C**H** in PPS chain), 2.85−3.0 (m, $C\mathbf{H_2}$ in PPS chain), 3.38 (s, 3H, $-O\mathbf{CH_3}$), 3.52−3.58 (t, 2H, $-O\mathbf{CH_2}CH_2CH_2S$), 3.5−3.7 ppm (broad, PEG chain protons) (Figure 1). ^{13}C NMR ($CDCl_3$): δ = 172 or 173 (C=O, amide or ester), 70.6 (PEG chain), 41.2 (**C**H in PPS chain), 38.3 ($\mathbf{C}H_2$ in PPS chain), 20.7 ppm ($\mathbf{C}H_3$ in PPS chain). FT-IR (thin film): 3427 and 3310 (ν NH_2), **2958** (ν_s CH_3), **2918** (ν_{as} CH_2), ***2867*** (ν_{as} CH_3 and ν_s CH_2), 1735 or 1683 (ν C=O for ester or amide, respectively), ***1450*** (δ_s CH_2) **1372**, 1281, *1250*, **1225 1173**, *1102* (ν_{as} C−O−C), 949, *849* cm^{-1} (ν_s C−O−C) (the characteristic absorptions of the PEG chain are underlined, while the ones of PPS chain are in bold). Elemental analysis (amide end-capped polymer): Calculated (considering 16.5 EG and 25 PS repeating units) [C]: 49.9%, [H]: 8.4%, [O]: 10.8%, [S]: 30.4%, [N]: 0.5%. Experimental [C]: 49.4%, [H]: 8.4%, [O]: 9.8%, [S]: 31.9%, [N]: 0.35%. This agreement is more than satisfactory, considering the polydispersity of the sample. The discrepancy on the nitrogen content is likely due to the usual high errors of elemental analysis applied onto polymeric samples. (In this case FT-IR amide absorption gave more reliable results.)

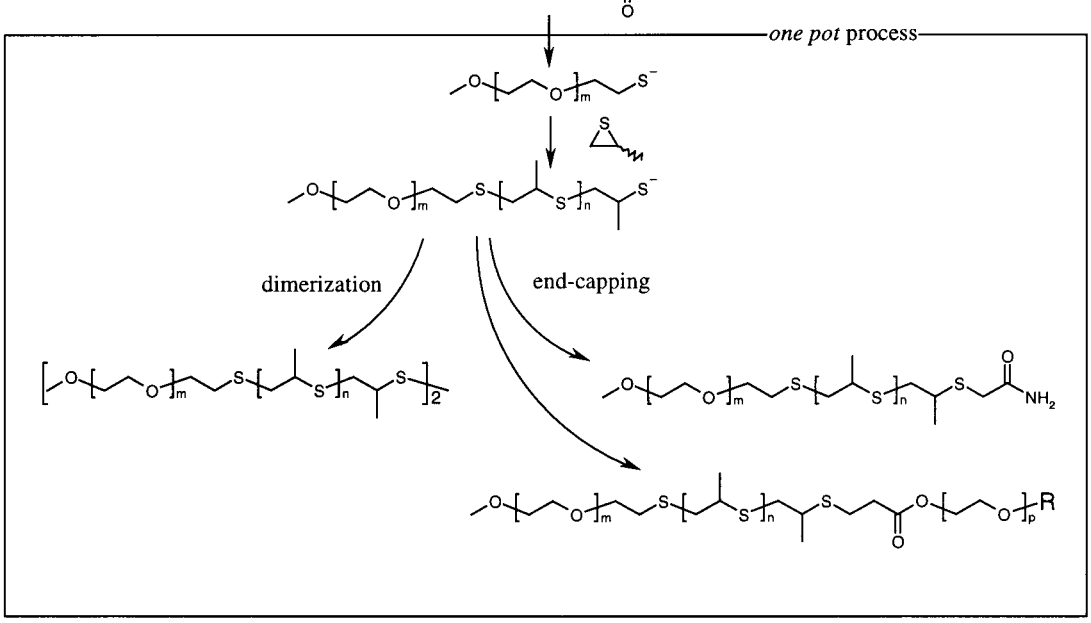
Results and Discussion

Strategy. The core of the synthetic procedure is the preparation of the B block, obtained by anionic ring-opening polymerization of episulfides, using polymeric initiators (the A block) and end-capping agents (the A, A′, or C blocks). Key features (which differentiate our method from the literature syntheses of PEG−polysulfide linear block copolymers[10,11]) are the proper choice of the initiator groups and of the end-capping reaction, which determine the symmetric or asymmetric nature of the final block terpolymer. Because of the polysulfide central block and the conceptual similarity to the poloxamers (with an oxygen atom rather than a sulfur atom in the central hydrophobic block), we refer to this class of block copolymers as "sulfamers".

Polymerization Technique. The anionic polymerization of episulfides has been the object of a number of papers, which, for example, reported the influence of different initiators,[12−15] demonstrated the negligible influence of protic groups such as alcohols[14,16] during the polymerization, and showed the amphiphilic character of comblike polymers with PEG (obtained from the corresponding functional monomers).[17] This polymerization technique, due to the mild character of the propagating species (thiolates), does not require extremely anhydrous conditions, makes use of a living polymerization, and tolerates a number of functional groups, thus allowing the presence of bioactive groups (such as peptides) in at least one block. Initiation can be accomplished by a variety of nucleophiles, but sulfur-based moieties (thiolates and the salts of thio- or dithio acids[13]) have shown by far the best results. PEG alcoholates, for example, are not effective initiators, having been demonstrated to be capable of conversion of episulfides to allyl thiolates by proton abstraction, thus generating another initiator species.[11]

Initiators. In the present case, thiolates were used as initiators; thio- or dithio acid derivatives were avoided, because of the well-known hydrolytic instability of the corresponding thio- or dithioesters formed in the initiation step. The direct use of thiols for thiolate generation is often disadvantageous: their easy oxidation to disulfides makes it difficult to estimate the actual concentration of the active thiolate group. In particular, commercial PEG thiols contain high quantities of the corresponding disulfides and have to be reduced and

Scheme 2



isolated in oxygen-free environments immediately before their use.[18] To avoid the likelihood of dimerization, enhance the shelf life of the initiators, and know in a precise and reproducible way their concentration, we adopted the approach of preparation of protected thiols as the initiating A block, deprotecting them in situ to form the active initiator. As protecting groups, thioacetates were well employed;[19] however, it is noted that dithioacetates, trithiocarbonates, xantates, and related groups could fit well in this scheme.

Two different synthetic schemes were explored for the preparation of PEG thioacetate: from PEG tosylate by direct substitution with potassium thioacetate or from PEG allyl ether by radical addition of thioacetic acid (Scheme 1). The second procedure gave an almost colorless material of very high purity, while the product of the substitution reaction generally exhibited a dark brown tone (from oxidation products of the thioacetate). This inconvenience has been noted previously, where for example PEG thioacetate was reported to have a red color.[18]

One-Pot Copolymer Synthesis. The multiblock copolymers were prepared by the means of a series of successive reactions: the PEG thioacetate was first

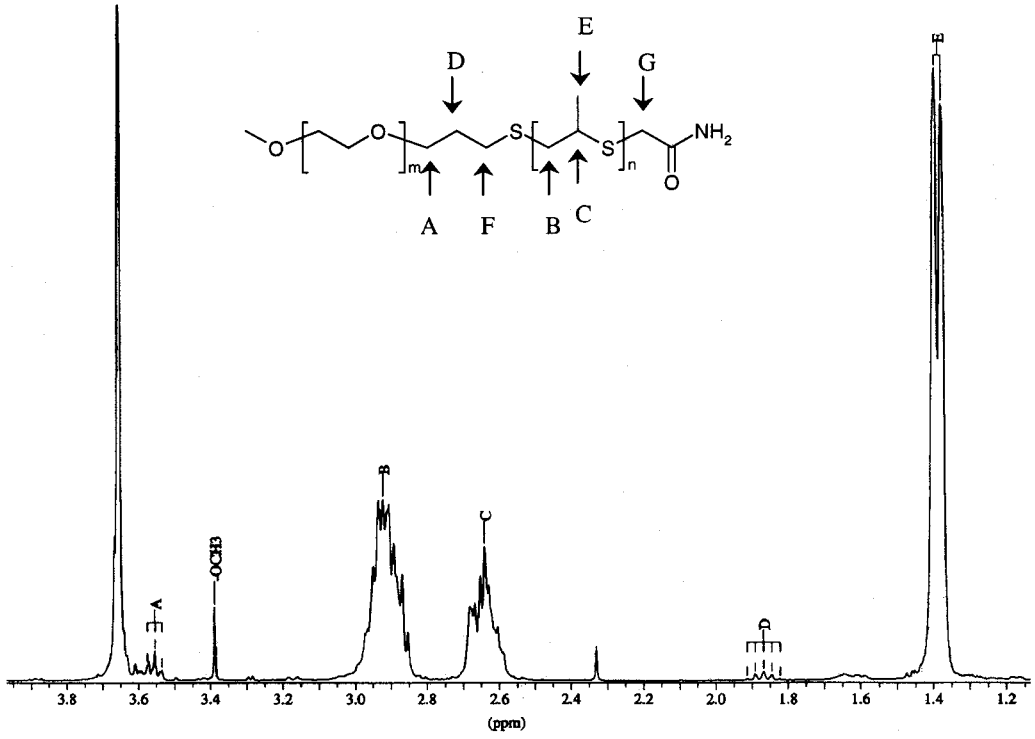


Figure 1. ^{1}H NMR spectrum of a diblock copolymer. Protons F are covered by the C ones, and protons G are covered by the PEG absorption (3.65−3.7 ppm).

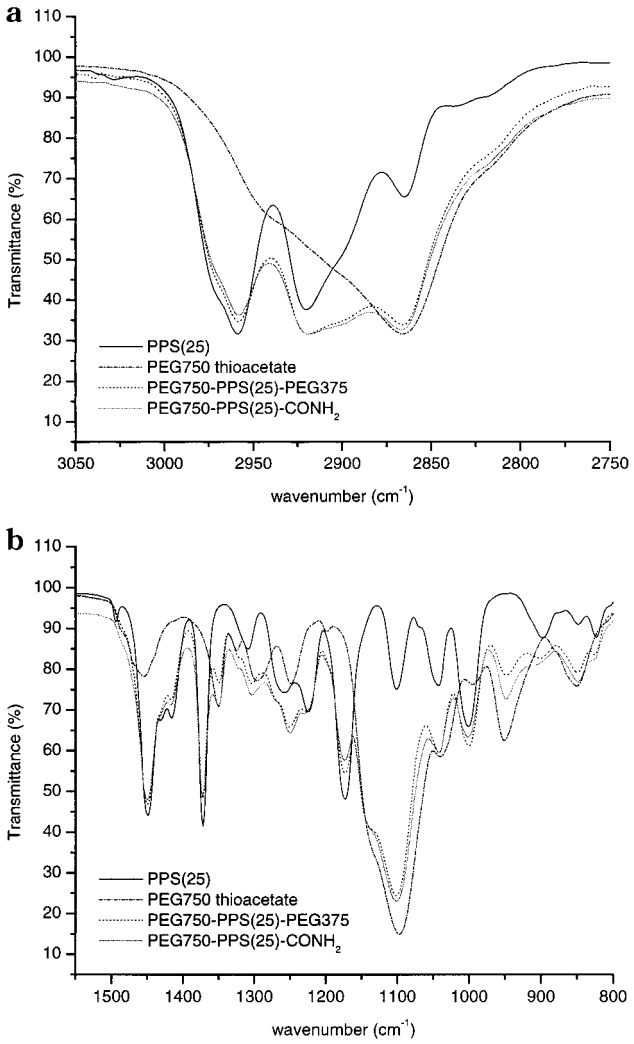


Figure 2. IR absorptions in the C−H stretching region (a) and in the fingerprints region (b) of poly(propylene sulfide) homopolymer, d.p. 25 PS units, PEG750 monomethyl ether thioacetate, PEG750-PPS-CH_2CONH_2, d.p. 25 PS units, PEG750-PPS−PEG375, d.p. 25 PS units. The two block copolymers show distinctly the absorptions of the two homopolymers, and no additional peak is detected outside of the carbonyl region (not shown).

deprotected to reveal the initiating thiolate, the monomer (propylene sulfide, PS) was then added and promptly polymerized, and finally the polymerization was stopped by the introduction of an end-capping agent (Scheme 2). After precipitation in methanol, the block copolymers were isolated in almost quantitative yields; possible traces of the PEG initiator and the unreacted excess of end-capping agent were eliminated in this stage. IR analysis after the workup showed the presence of characteristic absorption of both PEG and PPS blocks, proving their contemporary presence in a blocky structure (Figure 2).

Diblock Copolymers. The quantitative deprotection of the thioacetate groups and the living character of the episulfide polymerization were demonstrated by using iodoacetamide as an end-capping agent (a well-known method for thiol quantitative derivatization[20]). By definition, in a living polymerization the number of active groups does not change during the reaction; thus, the number of end-capping sites should be equal to the number of initiator molecules. The iodoacetamide was added, varying the iodoacetamide/thioacetate ratio (φ) between 0.2 and 2, and the polymers were analyzed by

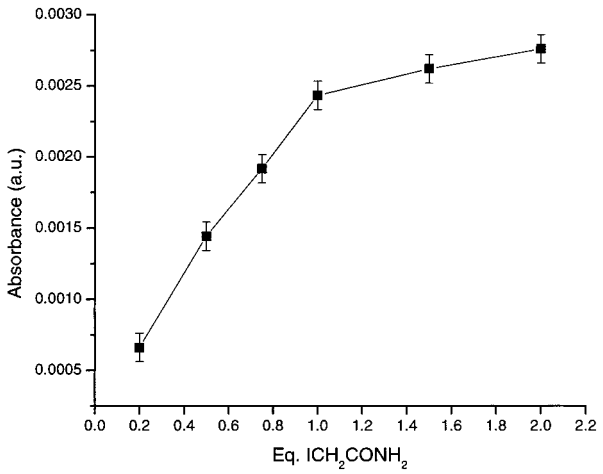


Figure 3. Amide absorbance vs equivalents of end-capping agent for the termination reaction with iodoacetamide, demonstrating the living nature of the PS polymerization.

Table 1. Molecular Weight Determination on Multiblock Sulfamers

polymer	PS units[a]	theor MW	M_n[b]	M_w/M_n
PEG750-PPS-CH_2CONH_2	25	2730	2900 (±200)	1.23 (±0.04)
$(PEG750\text{-}PPS)_2$	25	5350	5500 (±350)	1.29 (±0.03)
PEG750-PPS-PEG375	25	3050	3180 (±220)	1.24 (±0.02)

[a] In the feed. [b] From GPC data, averaged on five samples, mean and SD.

monitoring the absorbance of the amide group at 1683 cm^{-1} (amide I band). The absorbance linearly increased with the iodoacetamide quantity until $\varphi = 1$; this value remained then almost constant with $\varphi \geq 1$, showing in this way the 1:1 relationship between the number of reactive end groups and that of original thioacetic esters (Figure 3). The unreacted diblock copolymer readily dimerized when exposed to air: therefore, a mixture of "monomeric" diblock PEG-*bl*-PPS-amide and disulfide-linked "dimeric" triblock $(PEG\text{-}bl\text{-}PPS)_2$ was detected in samples end-capped with iodoacetamide in stoichiometric deficit (Figure 4). The low polydispersity of both these block copolymers (Table 1) proved that the PS polymerization was not only living but also controlled; moreover, no trace of unreacted PEG initiator was observed in the GPC trace.

Triblock Copolymers. Symmetric ABA triblock copolymers were readily obtained by exposing the thiolate-terminated diblock copolymers to air, to achieve disulfide linking as mentioned above. Asymmetric ABA′ triblock copolymers were generated by Michael-type addition of the thiolate terminal groups onto PEG monoacrylates. Quantitative yields were observed in the coupling reaction accomplished in an excess (e.g., 10-fold) of PEG monoacrylate (from comparison of PEG protons in the ^{1}H NMR of diblock and triblock copolymers). The Michael-type addition reaction employed for attachment of the A′ or C block tolerates a variety of bioactive functional groups,[21,22] thus permitting inclusion of a variety of biofunctional groups, such as peptides, in the PEG acrylate structure. Moreover, a variety of other useful features, such as hydrolyzable oligoesters, could be included.

Lyotropic Behavior. Both symmetric (e.g., PEG750-PPS-PEG750, d.p. 25 PS units) and asymmetric (e.g., PEG750-PPS-PEG375, d.p. 25 PS units) triblock copolymers have shown a distinct lyotropic behavior, building

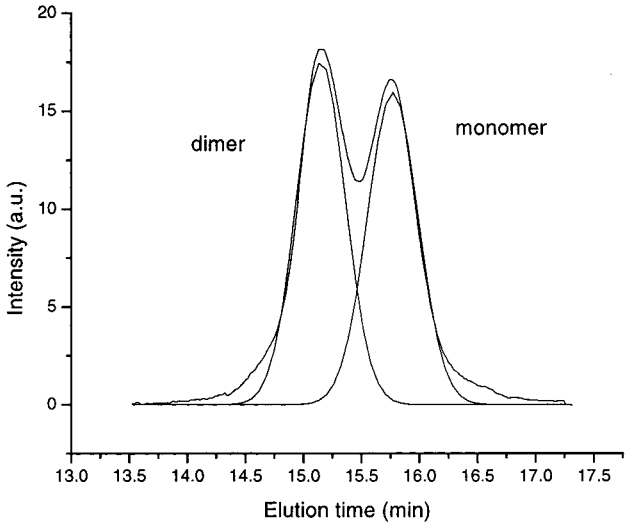


Figure 4. GPC trace in THF for a polymer mixture constituted by 55/45 dimeric disulfide-linked triblock copolymer and monomeric amide-terminated diblock copolymer.

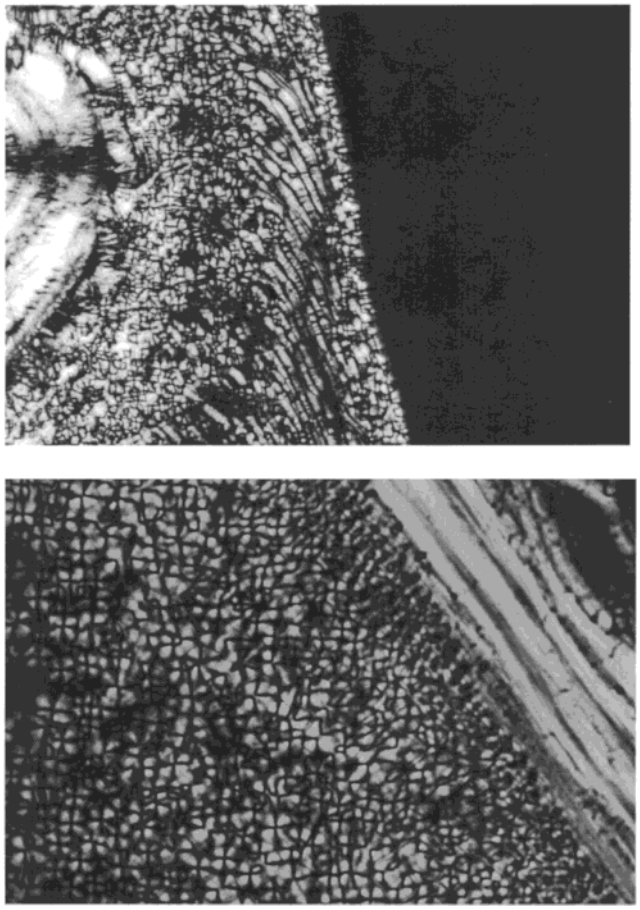


Figure 5. Cross-polarizers optical microscope images of 100 μm thick films of an asymmetric (PEG750-PPS-PEG375, d.p. 25 PS units) (a, top) and a symmetric (PEG750-PPS-PEG750, d.p. 25 PS units) (b, bottom) triblock copolymer, when put in contact with water under the microscope light. In (a) the black zone is the dry polymer, which does not show any birefringence pattern.

anisotropic structures when put in contact with water (Figure 5a,b); the correlation of the recorded birefringence patterns with a precise mesoscopic structure is difficult, but a certain similarity to nematic patterns can be seen.

Conclusions. Di- and triblock copolymeric amphiphiles with alternating hydrophilic and hydrophobic blocks were produced with low polydispersity under very mild conditions with a one-pot process. To ensure the reproducibility of the synthetic conditions, the only necessary condition is in the use of a dry solvent; the process tolerated the impurities present in commercial grade reagents. The synthetic procedure reported here is particularly promising for generating polymeric amphiphiles for potential in generating supermolecular assemblies, especially as drug carriers, because the scheme does not require complicated purifications or workups (the process tolerated the impurities present in commercial grade reagents; only the solvent was to be dried in order to improve the reproducibility of the PPS molecular weight), is not sensitive to the presence of most common polar groups (such as alcohols, esters, amides, carboxylic acids, which are found in a variety of biomolecules that might be used as potential targeting agents), and allows the synthesis of well-defined block copolymers.

The sulfamers, due to their amphiphilic nature and to the tailor-made chemical structure, are potentially able to form a variety of supermolecular structures in water. A detailed investigation of their lyotropic properties will be the subject of a forthcoming paper.

References and Notes

(1) Alexandridis, P. *Curr. Opin. Colloid Interface Sci.* **1997**, *2*, 478.
(2) Schillén, K.; Bryskhe, K.; Mel'nikova, Y. S. *Macromolecules* **1999**, *32*, 6885−6888.
(3) Booth, C.; Attwood, D. *Macromol. Chem., Rapid Commun.* **2000**, *21*, 501−527.
(4) Discher, B. M.; Won, Y.-Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143−1146.
(5) Eisenberg, A.; Yu, K. *Macromolecules* **1998**, *31*, 3509.
(6) Cornelissen, J. L. M.; Fischer, M.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Science* **1998**, *280*, 1427−1430.
(7) Sommerdijk, N. A. J. M.; Holder, S. J.; Hiorns, R. C.; Jones, R. G.; Nolte, R. J. M. *Macromolecules* **2000**, *33*, 8289−8294.
(8) Nardin, C.; Hirt, T.; Leukel, J.; Meier, W. *Langmuir* **2000**, *16*, 1035−1041.
(9) Nardin, C.; Winterhalter, M.; Meier, W. *Langmuir* **2000**, *16*, 7708−7712.
(10) Watanabe, Y.; Aida, T.; Inoue, S. *Macromolecules* **1990**, *23*, 2612−2617.
(11) Boileau, S.; Sigwalt, P. *Makromol. Chem.* **1973**, *171*, 11−18.
(12) Oswald, A. A. US Patent 4,163,832, Exxon Research & Engineering Co., 1979.
(13) Bonnans-Plaisance, C.; Levesque, G.; Midrak, A. *Eur. Polym. J.* **1994**, *30*, 239−244.
(14) Bonnans-Plaisance, C.; Levesque, G. *Macromolecules* **1989**, *22*, 2020−2023.
(15) Nicol, E.; Bonnans-Plaisance, C.; Levesque, G. *Macromolecules* **1999**, *32*, 4485−4487.
(16) Bonnans-plaisance, C.; Retif, P.; Levesque, G. *Polym. Bull.* **1995**, *34*, 141−147.
(17) Bonnans-Plaisance, C.; Nakache, E.; Rétif, Ph. *Colloid Polym. Sci.* **1998**, *276*, 297−304.
(18) Woghiren, C.; Sharma, B.; Stein, S. *Bioconjugate Chem.* **1993**, *4*, 314−318.
(19) Zalipsky, S. *Bioconjugate Chem.* **1995**, *6*, 150−165.
(20) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: New York, 1996.
(21) Romanowska, A.; Meunier, S. J.; Tropper, F. D.; Laferrière, C. A.; Roy, R. *Methods Enzymol.* **1994**, *242*, 90−101.
(22) Luetolf, M.; Tirelli, N.; Cavalli, L.; Hubbell, J. A. *Bioconjugate Chem.*, in press.

MA0108057