Controlled Synthesis of Peptide-Based Amphiphilic Copolymers

Ivaylo V. Dimitrov,*,† Iliyana V. Berlinova,† Plamen V. Iliev,† and Nikolay G. Vladimirov‡

Institute of Polymers, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria, and Hercules Incorporated, Research Center, Wilmington, Delaware 19808-1599

Received August 9, 2007
Revised Manuscript Received October 25, 2007

Introduction

Peptide-based amphiphiles are an attractive class of self-assembling systems suitable for numerous biomedical applications.[1] They can be constructed only of amino acids, long alkyl chains attached to a hydrophilic peptide sequence, or hybrid copolymer systems combining polypeptide segments with synthetic polymers.[2] Peptides modified with one or more alkyl tails form various supramolecular organization exhibiting α-helices,[3] β-sheets,[4] and fibers.[5] Among the hybrid block copolymers, those containing poly(oxyethylene) (PEO) as a synthetic segment are of special interest because of the hydrophilic, nontoxic, and nonimmunogenic properties of PEO.[6]

The precise molecular design allows control over the peptide conformation and intermolecular interactions. Polymers containing polypeptide motifs can be prepared by either solid-phase synthesis (Merrifield synthesis),[7,8] ring-opening polymerization of α-amino acid *N*-carboxyanhydride (NCA),[9,10] or linking polymers with appropriate functionalities to the active sites of peptides.[11] Recent advances in NCA polymerization enabled the controlled synthesis of a variety of polypeptide hybrid copolymers.[12] The ammonium-mediated ring-opening polymerization of NCA has attracted attention as an improved synthetic route to well-defined hybrid copolymers of various architectures.[13−17]

Herein, we present a synthetic route to the preparation of a lipophilic initiator and modified PEO-macroinitiators for ammonium-mediated polymerization of α-amino acid *N*-carboxyanhydrides. It is a multistep procedure involving the preparation of derivatives of maleic acid followed by a Michael addition of ammonium-containing thiols to the double bond. This method enables the preparation of initiators modified at the junction point with various low molar mass compounds or polymer chains. The attached ammonium groups were then used to initiate the ring-opening polymerization of Z-L-lysine NCA. When the lipophilic initiator was used, lipopolypeptides were obtained after cleavage of Z-protecting groups. Amphiphilic hybrid block copolymers modified with dodecylamine or α-naphthylamine at the junction point were synthesized when the PEO-based macroinitiators were used.

Experimental Section

Materials. Reagent grade chemicals were purchased from Fluka unless otherwise indicated. Maleic anhydride (≥99%) was recrystallized from benzene. Dichloromethane (CH_2Cl_2, ≥99.5%) and *N*,*N*-dimethylformamide (DMF, >99.5%) were dried over phosphorus pentoxide. DMF was distilled under reduced pressure prior to use. Ethyl acetate (>99%) was distilled from CaH_2. Tetrahydrofuran (THF, ≥99%) and toluene (≥99%) were refluxed over sodium−potassium alloy and distilled. α-Naphthylamine was sublimed. α-Methoxy-ω-hydroxypoly(oxyethylene) (PEO) with M_n = 2000 was dried azeotropically with toluene. *N*,*N*′-Dicyclohexylcarbodiimide (DCC, ≥99%), 1-hydroxybenzotriazole (HOBT, >95%, Aldrich,), dodecylamine (≥99%), 2-aminoethanethiol hydrochloride (AET.HCl, ≥98%, Aldrich), L-cysteine ethyl ester hydrochloride (Cys-OEt.HCl, 98%, Aldrich), ϵ-benzyloxycarbonyl-L-lysine (ZLLys, 98%), triphosgene (>99%), and *N*,*N*-dimethylacetamide (DMA, ≥99.5%) were used as received.

*N*ϵ-(Benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride (ZLLys-NCA) was prepared from ZLLys and triphosgene in ethyl acetate applying the advanced purification procedure described by Poché et al.[18] Yield: 88%. IR (KBr pellets): N−H (NCA, Z), ν 3342 cm^{-1}; CH_2, ν_{as} 2936 cm^{-1}; CH_2, ν_s 2863 cm^{-1}; C═O (NCA), ν 1814, 1774 cm^{-1}; C═O(Z), ν 1687 cm^{-1}; N−H(Z), δ 1533 cm^{-1}; C(═O)−O, ν 1258 cm^{-1}.

Synthesis of Lipophilic Initiator. *1. Preparation of Dodecylmaleamide (**I**).* Dodecylamine (9.27 g, 50 mmol) was dissolved in 40 mL of CH_2Cl_2. A solution of maleic anhydride (4.91 g, 50 mmol) in 60 mL of CH_2Cl_2 was added dropwise. The reaction mixture was stirred overnight at room temperature. The product precipitated from the solution. The solvent was filtered out, and the residue was washed with diethyl ether. Yield: 13.4 g, 94%. ^{1}H NMR ($CDCl_3$): δ 0.88 (CH_3); δ 1.25 (CH_3-$(CH_2)_9$); δ 1.55 ($(CH_2)_9$−CH_2); δ 3.28 (CH_2−NH); δ 6.35−6.52 (CH═CH); δ 8.24 (HN−C═O).

*2. Synthesis of Didodecylmaleamide (**II**).* In a typical reaction **I** (3.39 g, 12 mmol) was dissolved in 100 mL of THF, and the solution was chilled with ice/water. Then a solution of dodecylamine (2.22 g, 12 mmol) in 10 mL of THF was added followed by the addition of HOBT (1.62 g, 12 mmol) and DCC (2.47 g, 12 mmol). The reaction mixture was stirred overnight at 0 °C. The product precipitated from the chilled solution. It was recrystallized from ethanol. Yield: 5.6 g, 82%. ^{1}H NMR ($CDCl_3$): δ 0.87 (CH_3 + CH_3); δ 1.25 (CH_3−$(CH_2)_9$ + CH_3−$(CH_2)_9$); δ 1.55 ($(CH_2)_9$−CH_2 + $(CH_2)_9$−CH_2); δ 3.28 (CH_2−NH + CH_2−NH); δ 6.10 (CH═CH); δ 8.24 (HN−C═O + HN−C═O).

*3. Michael Addition of 2-Aminoethanthiol Hydrochloride to Didodecylmaleamide (**III**).* AET·HCl (6.02 g, 53 mmol) and **II** (2.54 g, 6 mmol) were mixed in DMF (5 mL). The reaction was completed in 36 h at 70 °C under an argon atmosphere. The solvent was evaporated, and the residue was dispersed in CH_2Cl_2. The solvent was filtered out, and the residue was washed extensively with water to remove the excess of AET·HCl. Yield: 2.35 g, 75%. ^{1}H NMR (DMSO-d_6): δ 0.88 (CH_3 + CH_3); δ 1.17 (CH_3−$(CH_2)_9$ + CH_3−$(CH_2)_9$); δ 1.24−1.36 ($(CH_2)_9$−CH_2 + $(CH_2)_9$−CH_2); δ 2.80 (S−CH_2); δ 2.95−3.05 (O═C−CH_2−CH + CH_2−NH + CH_2−NH + CH_2−N^+); δ 3.65 (O═C−CH−S); δ 7.82 (CH_2−NH−(C═O)−CH_2); δ 7.99 (CH_2−NH−(C═O)−CH−S).

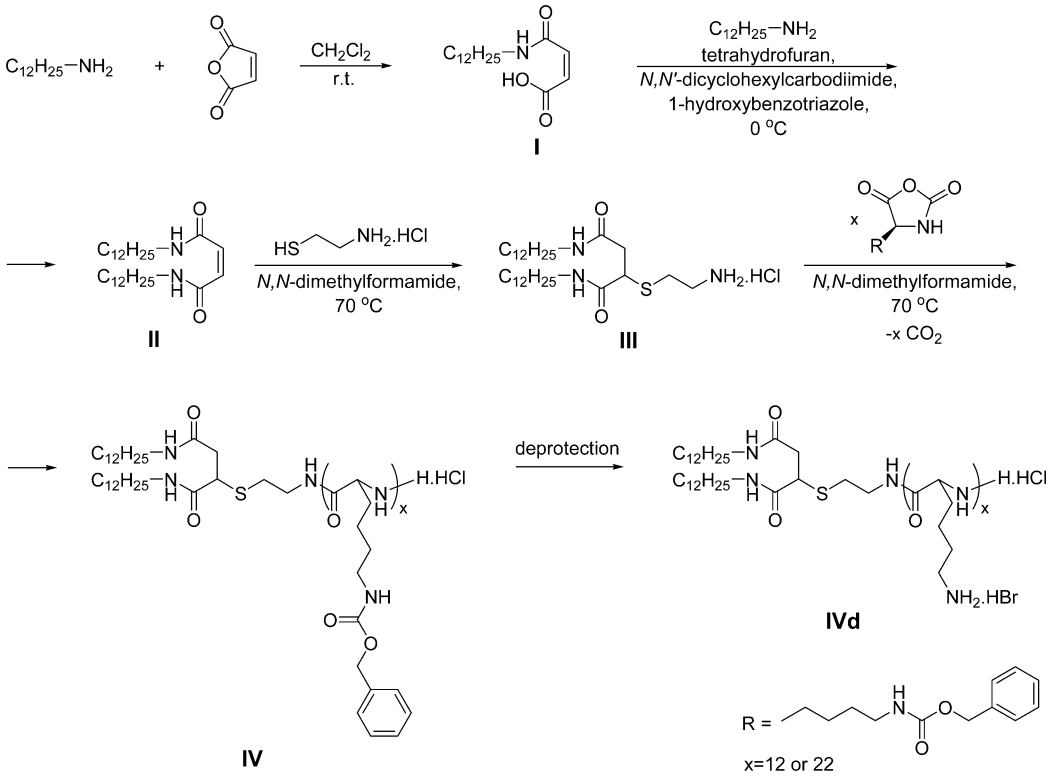
Synthesis of PEO-Macroinitiators. *1. Preparation of Poly(oxyethylene) Monoesters of Maleic Acid (**Ia**).* Maleic anhydride (2.94 g, 30 mmol) and PEO (10.0 g, 5 mmol of OH groups) were dissolved in dry toluene (40 mL) and heated at 80 °C for 30 h. Toluene was distilled off, and the residue was taken in CH_2Cl_2, filtered, and precipitated into diethyl ether. Yield: 12.5 g, 96%. ^{1}H NMR ($CDCl_3$): δ 3.38 (CH_3−O); δ 3.65 (O−CH_2−CH_2−O); δ 4.37 (CH_2−O−C═O); δ 6.19−6.44 (CH═CH).

*2. Preparation of Maleamates (**IIa**).* It was performed by amidation of **Ia** with dodecylamine or α-naphthylamine to obtain: methoxypoly(oxyethylene) dodecylmaleamate (**IIa-1**) and methoxypoly(oxyethylene) α-naphthylmaleamate (**IIa-2**). In a typical procedure, **Ia** (3.991 g, 1.9 mmol) and HOBT (0.284 g, 2.1 mmol) were dissolved in CH_2Cl_2 (55 mL). The solution was cooled with ice/water, and dodecylamine (0.389 g, 2.1 mmol) was added under stirring. Then DCC (0.433 g, 2.1 mmol) dissolved in a small amount of CH_2Cl_2 was added, and the reaction mixture was left at 0 °C for

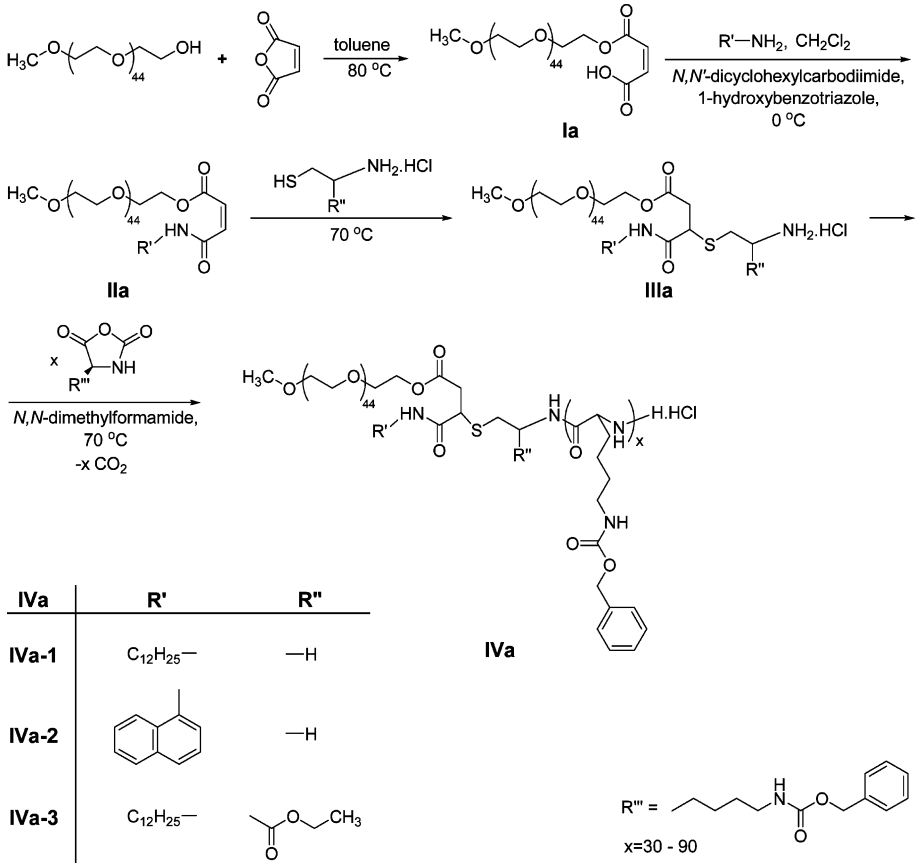
* To whom correspondence should be addressed. E-mail: dimitrov@polymer.bas.bg.
† Institute of Polymers, Bulgarian Academy of Sciences.
‡ Hercules Incorporated, Research Center.

10.1021/ma071798a CCC: $40.75
Published on Web 01/16/2008

Scheme 1. Synthetic Route to Lipopolypeptides



Scheme 2. Synthesis of Amphiphilic Hybrid Block Copolymers



IVa	R'	R"
IVa-1	$C_{12}H_{25}$—	—H
IVa-2		—H
IVa-3	$C_{12}H_{25}$—	

20 h. The mixture was filtered, the solution was concentrated, and the product (**IIa-1**) was precipitated into diethyl ether. Yield: 3.7 g, 85%. ^{1}H NMR ($CDCl_3$): δ 0.87 (CH_3); δ 1.25 ($CH_3-(CH_2)_9$); δ 1.55 ($(CH_2)_9-CH_2$); δ 3.29 (CH_2-NH); δ 3.37 (CH_3-O); δ 3.65 ($O-CH_2-CH_2-O$); δ 4.32 ($CH_2-O-C=O$); δ 6.12−6.43 ($CH=CH$); δ 8.23 ($HN-C=O$).

*3. Preparation of PEO-Conjugates Containing an Ammonium Group (**IIIa**).* The thiol group in AET·HCl or Cys-OEt·HCl was added to the double bond of **IIa**. Typically, 3.193 g (1.4 mmol) of methoxypoly(oxyethylene) dodecylmaleamate (**IIa-1**) and AET·HCl (2.431 g, 21.4 mmol) were stirred at 70 °C for 24 h. The mixture was taken in CH_2Cl_2 and passed through a 0.45 μm filter. The

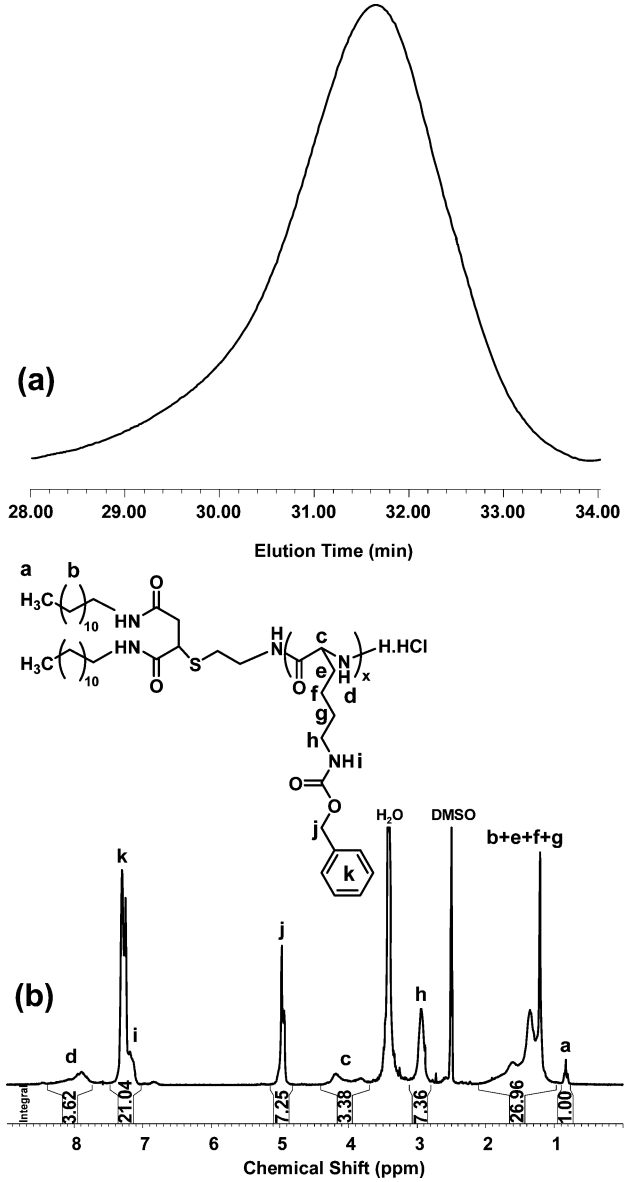


Figure 1. Experimental evidence for the lipopolypeptide formation by ammonium-mediated ring-opening polymerization of Z-L-lysine *N*-carboxyanhydride: (a) gel permeation chromatogram in dimethyl sulfoxide (+0.5 wt % LiCl) at 50 °C of the protected lipopolypeptide **IV-2** ($M_w/M_n = 1.18$); (b) ^{1}H NMR spectrum of the same lipopolypeptide in dimethyl-d_6 sulfoxide at 50 °C.

solution was concentrated, and the macroinitiator (**IIIa-1)** was precipitated into diethyl ether. The same procedure was repeated by dissolving the product into dry benzene. Yield: 2.3 g, 73%. GPC in *N*,*N*-dimethylacetamide (vs PEO standards): $M_w/M_n = 1.29$. ^{1}H NMR ($CDCl_3$): δ 0.87 (CH_3); δ 1.23 ($CH_3-(CH_2)_9$); δ 1.55 ($(CH_2)_9-CH_2$); δ 2.85 ($S-CH_2$); δ 3.05−3.29 ($CH_2-N^+ + CH_2-NH + O{=}C-CH_2-CH$); δ 3.37 (CH_3-O); δ 3.65 ($O-CH_2-CH_2-O$); δ 3.95 ($O{=}C-CH-S$); δ 4.30 ($CH_2-O-C{=}O$); δ 8.32 ($HN-C{=}O$). For the characteristics of the other two PEO-based macroinitiators (**IIIa-2** and **IIIa-3**), see the Supporting Information.

Synthesis of Peptide-Based Amphiphilic (Co)polymers. The peptide-based (co)polymers were prepared by ring-opening polymerization of ZLLys-NCA initiated by the ammonium group of **III** or **IIIa**. In a typical procedure, **III** (0.0846 g, 0.15 mmol) was dissolved in 6 mL of DMF and degassed. Separately, 0.918 g (3.0 mmol) of ZLLys-NCA was dissolved in 4 mL of DMF and degassed. The two solutions were combined via transfer needle and stirred under a dry argon atmosphere. The polymerization was performed at 70 °C for 5 days. The reaction mixture was diluted with DMF and passed through a 0.45 μm filter. The solvent was evaporated, and the residue was extracted with methanol to give lipopolypeptide **IV-2**. Yield: 0.62 g, 75%. GPC in dimethyl sulfoxide (vs pullulane standards): $M_w/M_n = 1.18$. ^{1}H NMR (DMSO-d_6): δ 0.82 ($CH_3 + CH_3$); δ 1.10−2.00 ($CH_3-(CH_2)_{10} + CH_3-(CH_2)_{10} + \alpha CH-(CH_2)_3$); δ 2.80−3.10 ($S-CH_2 + \alpha CH-(CH_2)_3CH_2 + O{=}C-CH_2-CH + (CH_2)_{10}-CH_2-NH + (CH_2)_{10}-CH_2-NH + S-CH_2-CH_2-NH$); δ 3.65−4.35 ($O{=}C-CH-S + \alpha CH$-NH); δ 4.97 ($Z-CH_2$); δ 7.15−7.35 ($C_6H_5 + \alpha CH-(CH_2)_4-NH + S-(CH_2)_2-NH$); δ 7.72−8.51 ($\alpha CH-NH + CH_2-NH-(C{=}O)-CH_2 + CH_2-NH-(C{=}O)-CH-S$). An exemplary polymerization procedure for the synthesis of peptide-based amphiphlic block copolymers and their characterization are given in the Supporting Information.

The Z-protecting groups were removed from the lipopolypetides containing didodecyl tail following the general procedure: 0.457 g of the polymer (1.6 mmol of protecting groups) was dissolved in 3.5 mL of trifluoroacetic acid. Then 1.6 mL of 4 N HBr in glacial acetic acid was added. The reaction mixture was stirred for an hour at room temperature followed by the addition of 10 mL of distilled water. The mixture was extracted several times with diethyl ether. The aqueous layer was neutralized with 10 N NaOH and dialyzed against distilled water. The product was recovered through liophilization. Yield: 0.62 g, 75%. ^{1}H NMR (D_2O): δ 0.72 ($CH_3 + CH_3$); δ 1.14−1.60 ($CH_3-(CH_2)_{10} + CH_3-(CH_2)_{10} + \alpha CH-(CH_2)_3$); δ 2.88 ($\alpha CH-(CH_2)_3CH_2$); δ 4.19 ($\alpha CH-NH$).

Characterization. ^{1}H NMR spectra were recorded on a Bruker 250 MHz instrument. Gel permeation chromatography (GPC) for lipopolypeptides was performed in dimethyl sulfoxide (DMSO) + 0.5 wt % LiCl at a flow rate of 0.5 mL/min on a PL Gel Linear B + 500 Å column, calibrated versus pullulane narrow molar mass standards; column and DRI detector temperature was 50 °C. GPC analyses for PEO−PZLys hybrid block copolymers were performed in *N*,*N*-dimethylacetamide + 0.5 wt % LiCl at a flow rate of 1.0 mL/min on a set of GRAM Linear and GRAM 100 Å, 300 × 8 mm, 10 μm columns (Polymer Standards Service GmbH, Mainz, Germany), calibrated versus PEO narrow molar mass standards; the column and DRI detector temperature was 50 °C. Scanning electron microscopy (SEM) measurements were performed on a Jeol JSM-5510 at 10 kV. The samples were prepared by spin-casting of polymer aqueous solutions on clean glass slides and sputter-coated with gold.

Results and Discussion

1. Lipopolypeptides. A three-step procedure was applied to obtain the lipophilic initiator bearing an ammonium group. The first step is the amidation of maleic anhydride with dodecylamine at room temperature in dichloromethane followed by the preparation of didodecylmaleamide. Both reactions proceeded quantitatively as evidenced from the ^{1}H NMR analyses. The third reaction step is the Michael addition of AET·HCl to the didodecylmaleamide double bond (Scheme 1). The reaction proceeded in DMF at 70 °C and using a 10-fold excess of AET·HCl. The product was washed extensively with water to remove any traces from the thiol-compound which, if present, can also initiate the NCA polymerization.

The ammonium-mediated ring-opening polymerization of ZLLys was initiated by the amine hydrochloride group of the lipophilic initiator. Polymerizations were conducted in DMF as a solvent at 70 °C. Upon completion, the reaction mixtures were filtered and the crude products were extracted with methanol. The purified polymers were characterized by GPC and ^{1}H NMR analyses (Figure 1). The molar mass distributions of lipopolypeptides were monomodal as evidenced from GPC analyses in DMSO with polydispersity indices in the 1.18−1.26 range (Figure 1a).

The polymer compositions were estimated from the ^{1}H NMR analyses in DMSO-d_6 performed at 50 °C (Figure 1b). The average degree of Z-L-lysine polymerization (DP_{Lys}) was

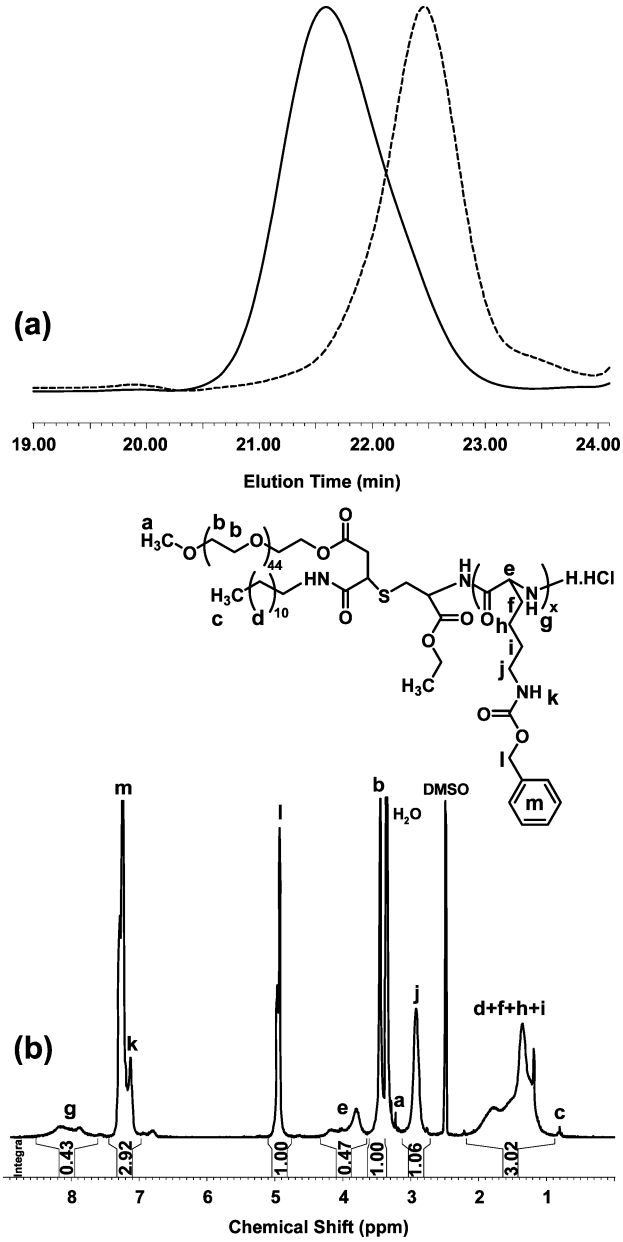


Figure 2. Experimental evidence for the block copolymer formation by ammonium-mediated ring-opening polymerization of Z-L-lysine *N*-carboxyanhydride: (a) gel permeation chromatograms in dimethyl sulfoxide (+0.5 wt % LiCl) at 50 °C of the macroinitiator **IIIa-3** (---), $M_w/M_n = 1.34$, and the corresponding block copolymer **IVa-3.2** (—), $M_w/M_n = 1.41$; (b) ^{1}H NMR spectrum of the block copolymer **IVa-3.2** in dimethyl-d_6 sulfoxide at 50 °C.

calculated from the ratio of the integral areas of methylene signal of the Z-protecting group from the peptide repeating units at 5.0 ppm to the methyl protons signals from the initiator at 0.82 ppm. The initiator efficiency was calculated from the ratio of the targeted DP_{Lys} and the experimental one as estimated from the ^{1}H NMR spectra and was in the 0.83−0.91 range.

The final reaction step was the removal of the Z-protecting groups from the peptide chain using an acidic conditions and HBr (Scheme 1). The resulting product was soluble in water, and the complete removal of Z-protecting groups was confirmed by ^{1}H NMR analysis in D_2O. The signals characteristic for the aromatic protons at 7.36 ppm and O−CH_2 at 4.8 ppm from the Z-protecting groups completely disappeared from the ^{1}H NMR spectrum of the product.

Preliminary studies on the self-assembly of deprotected lipopolypeptide by SEM indicate the formation of fibrous aggregates (Figure S1, Supporting Information). This is consistent with the self-assembling behavior of peptide amphiphiles.[19,20] However, more detailed work is needed to confirm this observation.

Table 1. Characteristics of the Peptide-Based Amphiphilic Copolymers

entry	code	target DP_{Lys}[a]	DP_{Lys}[b]	M_w/M_n[c]	In_{eff}[d]
1	IV-1	10	12	1.26[e]	0.83
2	IV-2	20	22	1.18[e]	0.91
3	IVa-1.1	15	29	1.38	0.52
4	IVa-1.2	30	54	1.35	0.55
5	IVa-2.1	15	28	1.33	0.54
6	IVa-2.2	30	59	1.30	0.51
7	IVa-3.1	15	48	1.36	0.31
8	IVa-3.2	30	90	1.41	0.33

[a] The target degree of polymerization of peptide chain, target $DP_{Lys} = [ZLLys-NCA]_0/[NH_2 \cdot HCl]_0$, where $[NH_2 \cdot HCl]_0$ is the active centers concentration. [b] Number-average degree of ZLLys polymerization, as determined by ^{1}H NMR analysis (see Results and Discussion). [c] Determined by GPC in *N*,*N*-dimethylacetamide (vs PEO standards). [d] The initiator efficiency, In_{eff} = target DP_{Lys}/DP_{Lys}. [e] Determined by GPC in dimethyl sulfoxide (vs pullulane standards).

2. PEO−PZLys Hybrid Block Copolymers Modified at the Junction Point. The synthesis involved the preparation of PEO-based macroinitiators, followed by ring-opening polymerization of ZLLys−NCA (Scheme 2). The first step was the synthesis of monoester of maleic acid, followed by amidation with amine containing compounds (dodecylamine or α-naphthylamine). The intermediates were characterized by ^{1}H NMR spectroscopy in $CDCl_3$. Then two different thiols containing an ammonium group (AET·HCl or Cys−OEt·HCl) were added to the double bond, and thus PEO-macroinitiators for NCA polymerization were prepared. Different monomer/macroinitiator molar ratios were used to obtain block copolymers with various lengths of polypeptide blocks. The hybrid block copolymers modified at the junction point were characterized by UV, GPC, and ^{1}H NMR analyses. GPC analyses of the macromonomers and the hybrid block copolymers were performed in DMA at 50 °C. They showed that the products elute as monomodal species (M_w/M_n = 1.3−1.4, vs PEO standards), and the copolymers elution times are shifted toward higher molar masses (Figure 2a). There were no traces of macroinitiator left in the purified samples.

Since the molar mass of the PEO-macroinitiator is known, the experimental degree of peptide polymerization was calculated from the ^{1}H NMR spectra in DMSO-d_6 from the ratio of the intensities of methylene protons at 5.0 ppm (Z-protecting group) and oxyethylene protons from the PEO chain at 3.5 ppm (Figure 2b). The calculated values for DP_{Lys} were in the 30−90 range. The macroinitiator efficiency was significantly lower than that of the lipophilic initiator (0.3−0.58). The presence of an optical label in the block copolymer containing naphthyl residue at the junction point enabled molar mass determination via UV analyses of copolymer solutions in DMA. The results are in good agreement with those obtained from ^{1}H NMR (^{1}H NMR: DP_{Lys} = 59; UV: DP_{Lys} = 62).

The molar mass characteristics of the peptide-based amphiphilic (co)polymers obtained and the (macro)initiator efficiencies are summarized in Table 1.

The macro(initiator) efficiency decreased in the order lipophilic initiator > PEO−AET·HCl > PEO−Cys−OEt·HCl (Table 1). Most likely, the steric hindrance in PEO−Cys−OEt·HCl macroinitiator reduces the efficiency of initiation.

Conclusions. A lipophilic initiator comprised of two aliphatic groups and an ammonium group as well as PEO-based macroinitiators were synthesized and used to initiate the ammonium-

mediated ring-opening polymerization of N^{ϵ}-(benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride. Well-defined lipopolypeptides and amphiphilic hybrid PEO−poly(Z-L-lysine) block copolymers were obtained. The lipopolypeptides were deprotected, and the initial studies on their self-assembly indicate the formation of fibrous structures. The PEO−PZLLys diblock copolymers were modified at the junction point either with an alkyl group or with an optical label. The (co)polymers were characterized by GPC, ^{1}H NMR, and UV spectroscopic analyses. The peptide-based amphiphiles obtained have a potential application as surfactants or in encapsulation and delivery of drugs and other biological molecules.

Acknowledgment. Support of this work by the National Science Fund, Bulgarian Ministry of Education and Science, through Project X-1303 is gratefully acknowledged.

Supporting Information Available: Characterization of PEO-based macroinitiators; typical reaction procedure for the synthesis of peptide-based block copolymers and their characterization; SEM image of deprotected lipopolypeptide. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Luk, Y.-Y.; Abbott, N. *Curr. Opin. Colloid Interface Sci.* **2002**, *7*, 267−275.
(2) Löwik, D.; van Hest, J. *Chem. Soc. Rev.* **2004**, *33*, 234−245.
(3) Yu, Y.-C.; Berndt, P.; Tirrell, M.; Fields, G. *J. Am. Chem. Soc.* **1996**, *118*, 12515−12520.
(4) Löwik, D.; Garcia-Hartjes, J.; Meijer, J.; van Hest, J. *Langmuir* **2005**, *21*, 524−526.
(5) Yuwono, V.; Hartgerink, J. *Langmuir* **2007**, *23*, 5033−5038.
(6) Veronese, F. *Biomaterials* **2001**, *22*, 405−417.
(7) Merrifield, R. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.
(8) Vandermeulen, G.; Tziatzios, C.; Klok, H.-A. *Macromolecules* **2003**, *36*, 4107−4114.
(9) Kricheldorf, H. *α-Aminoacid-N-Carboxyanhydrides and Related Heterocycles*; Springer: Berlin, 1987.
(10) Kricheldorf, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 5752−5784.
(11) Börner, H.; Schlaad, H. *Soft Matter* **2007**, *3*, 394−408.
(12) Deming, T. *Adv. Polym. Sci.* **2006**, *202*, 1−18.
(13) Dimitrov, I.; Schlaad, H. *Chem. Commun.* **2003**, 2944−2945.
(14) Lutz, J.-F.; Schütt, D.; Kubowitz, S. *Macromol. Rapid Commun.* **2005**, *26*, 23−28.
(15) Lee, C.; Fréchet, J. M. J. *Macromolecules* **2006**, *39*, 476.
(16) Dimitrov, I.; Berlinova, I.; Vladimirov, N. *Macromolecules* **2006**, *39*, 2423−2426.
(17) Meyer, M.; Schlaad, H. *Macromolecules* **2006**, *39*, 3967−3970.
(18) Poché, D.; Moore, M.; Bowles, J. *Synth. Commun.* **1999**, *29*, 843−854.
(19) Hartgerink, J.; Beniash, E.; Stupp, S. *Science* **2001**, *294*, 1684−1688.
(20) Paramonov, S.; Jun, H.-W.; Hartgerink, J. *J. Am. Chem. Soc.* **2006**, *128*, 7291−7298.

MA071798A