Polypeptide End-Capping Using Functionalized Isocyanates: Preparation of Pentablock Copolymers

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ABSTRACT: The use of electrophiles (isocyanates, isothiocyanates, acid chlorides) to cap the N-terminal ends of polypeptides and the use of isocyanates to prepare poly(γ-benzyl-L-glutamate)-*b*-(nonpeptide polymer) block copolymers are described. This chemistry was also used to prepare poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate)-*b*-(**polymer**)-*b*-poly(γ-benzyl-L-glutamate)-*b*-poly(ethylene glycol) pentablock copolymers, where **polymer** = polyoctenamer, poly(ethylene glycol), or poly(dimethylsiloxane). These α,ω-diamino-terminated polymers (**polymer**) were used to prepare difunctional macroinitiators for the living polymerization of γ-benzyl-L-glutamic acid-*N*-carboxyanhydride (Glu NCA) to form triblock copolymers that were subsequently capped with isocyanate terminated poly(ethylene glycol) to give the pentablock copolymers. These methods allow the facile functionalization of the N-terminal ends of polypeptides from NCA polymerizations. They also were shown to allow the controlled preparation of “rod−coil” polypeptide−(nonpeptide polymer) multiblock architectures with good control over the chain lengths of the domains and without formation of homopolypeptide contaminants.

Introduction

Much work has been done on the synthesis of polypeptide-*b*-nonpeptide block copolymers where the polypeptide domain is typically grown from an amino-terminated polymer via addition of α-amino acid-*N*-carboxyanhydride (NCA) monomers.[1] A major limitation in these syntheses has been side reactions that occur during formation of the polypeptide segments, where the presence of chain termination and transfer reactions result in block copolymers containing significant homopolypeptide and oligopeptide contamination.[2] Pure block copolymers are thus only obtained after time-consuming fractionation and extraction steps that decrease yields.[2,3] These side reactions also make it difficult to accurately control block copolymer composition.[2] Another subtle feature of this methodology is that it only allows for coupling of polymers to the carboxy-terminal (C-terminal) ends of polypeptides. The mode of attachment is important since polypeptide chains are directional and can possess considerable dipole moments along the chain axis.[4] Thus, the connectivity not only affects the nature of the free end group (amine vs carboxylate) but also may affect overall copolymer properties if packing of helical dipoles is involved. For these reasons, the ability to functionalize amino-terminal (N-terminal) ends of polypeptide segments is desirable.

In previous work we demonstrated that amido−amidate nickelacycle end groups can be incorporated onto amine-terminated polymers, and the resulting materials can be used as macroinitiators for addition of polypeptide segments.[5] The nickel complexes are known to initiate controlled NCA polymerizations with few side reactions, as shown in previous work.[6] These methods allowed the controlled preparation of PBLG-*b*-(**polymer**)-*b*-PBLG triblock copolymers (PBLG = poly(γ-benzyl-L-glutamate); **polymer** = polyoctenamer (POCT), polyethylene) with superior control over polypeptide chain lengths and no formation of homopolypeptide contaminants. Similar to other work, the polypeptide segments were attached to the hydrocarbon polymers via their C-terminal ends. In this report, we have extended this chemistry to incorporate inorganic and hydrophilic domains (poly(dimethylsiloxane), PDMS, and poly(ethylene glycol), PEG) as the central segments of analogous triblock copolymers. In addition, the N-terminal functionalization of polypeptides using electrophiles (isocyanates, isothiocyanates, acid chlorides) has been developed. This chemistry was used to terminate metal-initiated NCA polymerizations, resulting in quantitative capping of the N-terminal ends. The end-capping reagents can either be small molecules or readily prepared isocyanate-terminated polymers, the latter resulting in formation of polypeptide-*b*-(nonpeptide) block copolymers. Using this chemistry, PEG-*b*-PBLG diblock copolymers were prepared, where the PEG segments were attached to the N-terminal ends of the polypeptide chains. This methodology, in conjunction with the amido−amidate nickelacycle chemistry, also allowed preparation of highly complex copolymer sequences. Specifically, pentablock copolymers of the sequences PEG-*b*-PBLG-*b*-POCT-*b*-PBLG-*b*-PEG, PEG-*b*-PBLG-*b*-PEG-*b*-PBLG-*b*-PEG, and PEG-*b*-PBLG-*b*-PDMS-*b*-PBLG-*b*-PEG were synthesized and characterized. Such control over copolymer architecture is expected to lead to expanded opportunities for these types of copolymers in biomedical applications.

Experimental Section

Instrumentation. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer using NaCl plates. GPC data were obtained using an SSI Acuflow series II pump equipped with a SSI refractive index detector, HPLC grade THF as the mobile phase, and a column bank consisting of four Phenomenex 5μ columns (10^5, 10^4, 10^3, and 500 Å) at 25 °C as the stationary phase. A constant flow rate of 1 mL/min was maintained, and the instrument was calibrated using polystyrene standards. Circular dichroism spectra were recorded on an OLIS RSM CD spectrophotometer running in conventional scanning mode. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on an SSI Acuflow series III liquid chromatography pump equipped

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with a Wyatt DAWN DSP light scattering detector (633 nm HeNe laser) and Wyatt Optilab DSP RI detector (633 nm). d*n*/d*c* values were determined using guidelines of the manufacturer at 633 nm. Separations were effected by 10^5, 10^4, 10^3, and 500 Å Phenomenex 5μ columns using 0.1 M LiBr in DMF eluent at 60 °C. NMR spectra were measured on Bruker AVANCE 200 MHz spectrometer using chloroform-*d* as solvent, with 0.5% v/v TMS added as an internal reference. Differential scanning calorimetry (DSC) analyses were perormed on a TA Instruments DSC 2920 differential scanning calorimeter. DSC samples were scanned at a heating rate of 10 °C/min from 0 to 100 °C with data collected during second cycle in the selected temperature ranges. Calibrations were made using indium as the standard for both temperature transitions and the heats of fusion. Melting transition temperatures (T_m) were determined as the peak maxima of the transition.

Materials. 1,9-Decadiene (from Aldrich) was purified by fractional distillation from CaH_2, degassed under high vacuum by several freeze−pump−thaw cycles, and vacuum transferred successively into sodium-mirrored flasks until no reaction was observed. Alloc-L-aminoamides[7] and γ-benzyl-L-glutamate-*N*-carboxyanhydride, Glu NCA,[8] were prepared according to literature procedures. Hexane, THF, and THF-d_8 were purified by first purging with dry nitrogen, followed by passage through columns of activated alumina.[9] DMF, DMF-d_7, chloroform, and methylene chloride (from Aldrich) were purified by drying over 4 Å molecular sieves and then degassed by three freeze−pump−thaw cycles before being transferred into drybox. 11-Phthalimide-1-undecene, bis(11-undecenylphthalimide) polyoctenamer, α,*ω*-bis(amino-terminated polyoctenamer), alloc-L-leucine, alloc-L-leucine-*N*-hydroxysuccinimidyl ester, (*S*)-$\mathrm{depe}\overline{\mathrm{NiNHC(H)R^1C(O)N}}\mathrm{R^2}$ ($R^1 = -CH_3$, $R^2 = CH_2C(CH_3)_3$ **1**), bis-alloc-L-leucine-terminated polyoctenamer, $\mathrm{(depe)}\overline{\mathrm{Ni{-}NH\text{-}CH(R')C(O)N}}\mathrm{(CH_2)_9{-}[CH{=}CH(CH_2)_6]_{\mathit{n}}{-}(CH_2)_9}\overline{\mathrm{NC(O){-}CH(R')\text{-}NH{-}Ni}}\mathrm{(depe)}$ (**2**), and triblock (γ-benzyl-L-glutamate)-*b*-(polyoctenamer)-*b*-(γ-benzyl-L-glutamate) PBLG-*b*-POCT-*b*-PBLG copolymers (**3**) were synthesized according to our previous paper.[5] 2,2′-BipyridylNi(1,5-cyclooctadiene) was prepared as previously described.[10] 2,2′-Bipyridyl (from Aldrich), 1,3-bis(diethylphosphino)ethane (depe, from Strem), O_α-(2-aminoethyl)-O_ω-(methyl)-poly(ethylene glycol) ($M_n = 5000$, from Fluka), O_α,O_ω-bis(2-aminoethyl)poly(ethylene glycol) ($M_n = 2000$ from Fluka), α,*ω*-bis(3-aminopropyl)poly(dimethylsiloxane) ($M_n = 2500$ from Fluka), and phosgene solution (1.96 M in toluene, from Fluka) were used without purification.

Isocyanate N-Terminal End-Capping and Quenching of PBLG Initiated with 1. In the drybox, Glu NCA (50 mg, 0.20 mmol) was dissolved in DMF (1.0 mL) and placed in a 25 mL reaction tube which could be sealed with a Teflon stopcock. An aliquot of **1** (100 μL of a 16 mM solution in DMF) was added via syringe to the flask. A stir bar was added, and the flask was sealed. The polymerization was stirred at 25 °C for 4 h after which 4 equiv of *p*-tolyl isocyanate (2 μL, 16 μmol) was added to the solution. After 4 h a small aliquot was removed for molecular weight determination. A second quantity of Glu NCA (50 mg, 0.20 mmol) was then added to the polymerization solution which was stirred for an additional 12 h. The FTIR spectrum of the resulting solution contained unreacted Glu NCA, and the molecular weight of the polymer component remained essentially unchanged from before. Polymer was isolated by addition of the reaction mixture to methanol containing HCl (1 mM), causing precipitation of the polymer. The polymer was dried in vacuo to give a white solid, PBLG (19 mg, 45% yield based on total amount of Glu NCA added). ^{13}C NMR, ^{1}H NMR, and FTIR spectra of this material were identical to data found for authentic samples of PBLG.[4] GPC of the polymer in 0.1 M LiBr in DMF at 60 °C: first aliquot: $M_n = 38\,810$; $M_w/M_n = 1.31$; final sample: $M_n = 40\,180$; $M_w/M_n = 1.28$.

Isocyanate N-Terminal End-Capping and Quenching of PBLG Initiated with (2,2′-bipyridyl)Ni(COD). In the drybox, two samples of Glu NCA (0.10 g, 0.38 mmol each) were dissolved in DMF (2.0 mL) and placed in 25 mL reaction tubes which could be sealed with Teflon stopcocks. An aliquot of (2,2′-bipyridyl)Ni(COD) (50 μL, 3.6 μmol of a solution in THF) was added via syringe to each flask. Stir bars were added and the flasks were sealed. The polymerizations were stirred at 25 °C for 4 h to consume all the NCA monomers, after which *p*-tolyl isocyanate [either 20 equiv/Ni (**a**, 10 μL, 80 μmol) or 4 equiv (**b**, 2.0 μL, 16 μmol)] was added to separate flasks. After letting react for either 1 h (**a**) or 30 min (**b**) a small aliquot was removed from each for molecular weight determination. A second quantity of Glu NCA (0.10 g, 0.38 mmol each) was then added to each polymerization solution, which were stirred for an additional 24 h. The FTIR spectrum of solution (**a**) showed the presence of unreacted Glu NCA, while that of solution (**b**) showed that all the NCA had been consumed. Polymers were isolated by addition of the reaction mixtures to methanol containing HCl (1 mM) causing precipitation of the polymers. The polymers were dried in vacuo to give white solids, PBLG (**a**, 0.09 g, 45% yield; **b**, 0.13 g, 63% yield, based on total amount 0.20 g of Glu NCA added). ^{13}C NMR, ^{1}H NMR, and FTIR spectra of this material were identical to data found for authentic samples of PBLG.[4] GPC of the polymers in 0.1 M LiBr in DMF at 60 °C: (**a**) first aliquot: $M_n = 16\,900$; $M_w/M_n = 1.4$; final sample: $M_n = 18\,700$; $M_w/M_n = 1.4$; (**b**) first aliquot: $M_n = 15\,200$; $M_w/M_n = 1.3$; final sample: $M_n = 28\,600$; $M_w/M_n = 1.9$.

Isothiocyanate N-Terminal End-Capping and Quenching of PBLG Initiated with (2,2′-bipyridyl)Ni(COD). In the drybox, two samples of Glu NCA (0.10 g, 0.38 mmol each) were dissolved in DMF (2.0 mL) and placed in 25 mL reaction tubes which could be sealed with Teflon stopcocks. An aliquot of (2,2′-bipyridyl)Ni(COD) (50 μL, 3.6 μmol of a solution in THF) was added via syringe to each flask. Stir bars were added, and the flasks were sealed. The polymerizations were stirred at 25 °C for 4 h to consume all the NCA monomers, after which *p*-tolyl isothiocyanate [either 12 equiv/Ni (**a**, 6.0 μL, 48 μmol) or 4 equiv (**b**, 2.0 μL, 16 μmol)] was added to separate flasks. After letting react for either 1 h (**a**) or 30 min (**b**) a small aliquot was removed from each for molecular weight determination. A second quantity of Glu NCA (0.10 g, 0.38 mmol each) was then added to each polymerization solution, which were stirred for an additional 24 h. The FTIR spectra of both solutions showed the presence of unreacted Glu NCA. Polymers were isolated by addition of the reaction mixtures to methanol containing HCl (1 mM) causing precipitation of the polymers. The polymers were dried in vacuo to give white solids, PBLG (**a**, 0.09 g, 45% yield; **b**, 0.085 g, 43% yield, based on total amount 0.20 g of Glu NCA added). ^{13}C NMR, ^{1}H NMR, and FTIR spectra of this material were identical to data found for authentic samples of PBLG.[4] GPC of the polymers in 0.1 M LiBr in DMF at 60 °C: (**a**) first aliquot: $M_n = 11\,900$; $M_w/M_n = 1.4$; final sample: $M_n = 12\,600$; $M_w/M_n = 1.2$; (**b**) first aliquot: $M_n = 19\,300$; $M_w/M_n = 1.2$; final sample: $M_n = 14\,200$; $M_w/M_n = 1.4$

Acid Chloride N-Terminal End-Capping and Quenching of PBLG Initiated with (2,2′-bipyridyl)Ni(COD). In the drybox, two samples of Glu NCA (**a**, 0.10 g, 0.38 mmol or **b**, 0.14 g, 0.53 mmol) were dissolved in DMF (2.0 mL) and placed in 25 mL reaction tubes which could be sealed with Teflon stopcocks. An aliquot of (2,2′-bipyridyl)Ni(COD) (50 μL, 3.6 μmol of a solution in THF) was added via syringe to each flask. Stir bars were added, and the flasks were sealed. The polymerizations were stirred at 25 °C for 4 h to consume all the NCA monomers, after which *p*-toluoyl chloride [either 20 equiv/Ni (**a**, 10 μL, 80 μmol) or 4 equiv (**b**, 2.0 μL, 16 μmol)] was added to separate flasks. After letting react for either 1 h (**a**) or 30 min (**b**) a small aliquot was removed from each for molecular weight determination. A second quantity of Glu NCA (0.10 g, 0.38 mmol each) was then added to each polymerization solution, which were stirred for an additional 24 h. The FTIR spectra of both solutions showed the presence of unreacted Glu NCA. Polymers were isolated by addition of the reaction mixtures to methanol containing HCl (1 mM) causing precipitation of the polymers. The polymers were dried in vacuo to give white solids, PBLG (**a**, 90 mg, 45% yield; **b**, 0.10 g, 42% yield, based on total amounts 0.20 g (**a**) and 0.24 g (**b**) of Glu NCA added). ^{13}C NMR, ^{1}H NMR, and FTIR spectra of this material were identical to data found for authentic samples of PBLG.[4] GPC of the polymers in 0.1 M LiBr in DMF at 60 °C: (**a**) first aliquot: $M_n = 15\,700$; $M_w/M_n = 1.3$; final sample: $M_n = 18\,500$; $M_w/M_n = 1.3$; (**b**) first aliquot: $M_n = 29\,900$; $M_w/M_n = 1.4$; final sample: $M_n = 27\,100$; $M_w/M_n = 1.4$.

Synthesis of Isocyanate-Terminated Poly(ethylene glycol), 4. A solution of phosgene (10 mL, 1.96 M in toluene) was added to a solution of α-(2-aminoethyl)-*ω*-(methyl)-poly(ethylene glycol) ($M_n = 5000$, 2.3 g, 0.40 mmol) in dry CH_2Cl_2 (5 mL) and stirred at 40 °C overnight. The solvent was then removed leaving the product as a white solid. The product was washed with hexanes (3 × 10 mL) and dried in vacuo (2.2 g, 94% yield). The following spectral properties were observed: ^{1}H NMR: δ 3.65 ($-CH_2CH_2O-$), 3.64 (CH_3O-). ^{13}C NMR: δ 122.2 (N=C=O), 70.6 ($-CH_2CH_2O-$), 59.7 (CH_3O-). IR (NaCl, THF): 2266 cm^{-1}.

Synthesis of Diblock PBLG-*b*-PEG Using (2,2′-bipyridyl)Ni(COD) Initiator and End-Capping Agent 4. In the drybox, Glu NCA (0.19 g, 0.72 mmol) was dissolved in 3 mL of DMF and placed in a 15 mL reaction tube that could be sealed with Teflon stopcock. An aliquot of (2,2′-bipyridyl)Ni(COD) initiator (0.10 mL, 0.0073 mmol) in THF was added via syringe to the flask. A stir bar was added, and the flask was sealed. Polymerization was allowed to proceed for 24 h at 25 °C, followed by termination with 0.20 g (0.040 mmol) **4** dissolved in 2 mL of THF. After 48 h at room temperature the copolymer was isolated by addition of the reaction mixture to methanol containing HCl (1 mM) causing precipitation. To remove any unreacted **4**, the copolymer was precipitated multiple times into methanol, a nonsolvent for the copolymer but a good solvent for PEG (0.26 g, 79% yield). GPC of the polymer in 0.1 M LiBr in DMF at 60 °C: $M_n = 19\,200$ and $M_w/M_n = 1.17$. The following spectral properties were observed: ^{1}H NMR: δ 8.01 (−NH−, PBLG), 7.27 ($-C_6H_5$, PBLG), 5.05, 4.0 (PBLG), 3.65 ($-CH_2CH_2O-$), 2.25 (PBLG). ^{13}C NMR: δ 175.4 (C=O, PBLG), 136.1, 130.3, 128.5 ($-C_6H_5$, PBLG), 70.6 ($-CH_2CH_2O-$). IR (NaCl, THF): 3565.2, 3291.4, 2981.5, 2681.4, 2358.0, 1955.9, 1732.9, 1651.0, 1540.3, 1456.8, 1363.8, 1288.7, 1244.4, 1174.9, 1077.5, 913.6, 659.6 cm^{-1}.

Synthesis of Pentablock PEG-*b*-PBLG-*b*-POCT-*b*-PBLG-*b*-PEG, 5, Using Macroinitiator 2 and End-Capping Agent 4. In the drybox, Glu NCA (0.20 g, 0.76 mmol) was dissolved in either 3 mL of DMF (**5a**) or in 3 mL of THF (**5b**) and each placed in separate 15 mL reaction tubes that could be sealed with Teflon stopcocks. An aliquot of macroinitiator **2** (0.25 mL, 0.0091 mmol,) in THF was added via syringe to each flask. Stir bars were added, and the flasks were sealed. Precipitation was observed for **5a** since the polyoctenamer (POCT) component of **2** is insoluble in DMF. After a few hours this reaction mixture clarified as the growing PBLG blocks solubilized the copolymer. These polymerizations were allowed to proceed for 24 h at 25 °C, followed by termination with 0.41 g (0.083 mmol) of **4** dissolved in 2 mL of THF. After 36 h at room temperature the copolymers **5a** and **5b** were isolated by addition of the reaction mixtures to methanol containing HCl (1 mM) causing precipitation. The copolymers were repeatedly dissolved in THF and precipitated into methanol, a nonsolvent for the PBLG and POCT domains, but a solvent for any uncoupled PEG. **5a** (0.26 g, 81% yield). GPC of the polymer in 0.1 M LiBr in DMF at 60 °C: $M_n = 33\,700$ and $M_w/M_n = 1.1$, DSC: $T_m = 57$ °C. **5b**: 0.3 g, 88.7% yield, GPC (DMF): $M_n = 77\,000$ and $M_w/M_n = 1.4$, DSC: $T_m = 59$ °C. The following spectral properties were observed: ^{1}H NMR: δ 8.01 (−NH−, PBLG), 7.27 ($-C_6H_5$, PBLG), 5.40 (−CH=CH−, POCT), 5.05, 4.0 (PBLG), 3.65 ($-CH_2CH_2O-$), 2.25 (PBLG), 1.99 (POCT). ^{13}C NMR: δ 175.4 (C=O, PBLG), 136.1, 130.3 (CH=CH, trans, POCT), 129.9 (CH=CH, cis, POCT), 128.5 ($-C_6H_5$, PBLG), 70.6 ($-CH_2CH_2O-$), 36.5, 32.6, 31.5, 29.6, 29.0, 27.2. IR (NaCl, THF): 3555.6, 3500.0, 3288.9, 2966.7, 2677.8, 2366.7, 1966.7, 1733.3, 1650.0, 1544.4, 1461.1, 1366.7, 1288.9, 1244.4, 1177.8, 1066.7, 906.6, 656.0 cm^{-1}.

(S,S)-AllocNHCH(R)C(O)NHCH$_2$CH$_2$−(OCH$_2$CH$_2$)$_n$−NH-C(O)CH(R′)−NHAlloc; R = −CH$_2$CH(CH$_3$)$_2$, 6. A solution of alloc-L-leucine succinimidyl ester (0.80 g, 2.6 mmol) in chloroform was added to a solution of α,*ω*-bis(2-aminoethyl)-poly(ethylene glycol), $M_n = 2000$ (1.6 g, 1.1 mmol) in chloroform (3 mL). The solution was stirred for 24 h and the chloroform then removed in vacuo. The solid was washed in dry THF to remove the excess alloc-L-leucine succinimidyl ester and free *N*-hydroxy succinimide. The product was then dried in vacuo (1.1 g, 70% yield). ^{1}H NMR: δ 6.9 ($-NH-$), 5.88 ($CH_2{=}CH-$), 5.26 ($CH_2{=}CH-$), 4.57 ($CH_2{=}CH-CH_2-O-$), 3.77 ($-CH-CH_2-(CH_3)_2$), 3.65 ($-OCH_2CH_2-O-$), 3.39 ($-CH_2-NH-$), 2.64 ($-CH_2-O-CH_2CH_2CH_2-$), 1.8 [$-CH_2-$, $-CH_2-CH(CH_3)_2$], 1.5 [$-CH-(CH_3)_2$], 0.96 [$-CH-(CH_3)_2$]. ^{13}C NMR: δ 170.5 ($-C{=}O$), 133.0 ($CH_2{=}CH-$), 118.1 ($CH_2{=}CH-$), 70.6 ($-OCH_2CH_2-O-$), 67.2 ($CH_2{=}CH-CH_2-O-$), 58.9 [$-CH-CH_2-(CH_3)_2$], 36.9 [$-CH_2-(CH_3)_2$], 29.1, 28.5, 26.7, 26.5, 25.9, 24.2 [$-CH-(CH_3)_2$]. IR (NaCl, THF): 3566.6, 3488.8 (ν, NH), 2855.5, 2666.6, 2355.5, 1961.1, 1722.2 (ν, CO), 1644.0, 1455.5, 1361.1, 1283.3, 1244.4, 1177.8, 1076.6, 911.1, 655.5 cm^{-1}.

Synthesis of (S,S)-depeNiNHCH(R)C(O)NCH$_2$CH$_2$−(OCH$_2$CH$_2$)$_n$−NC(O)−CH(R′)NHNidepe; R = −CH$_2$CH-(CH$_3$)$_2$, 7. Depe (34 μL, 0.15 mmol) in 1 mL of THF was added to a solution of $Ni(COD)_2$ (40 mg, 0.15 mmol) in THF (1 mL) and let stand at room temperature for 10 min after which a solution of (depe)Ni(COD) had formed. **6** (0.15 g, 0.073 mmol) in THF (2 mL) was then added to the yellow solution, which subsequently became orange-yellow in color. The solution was heated at 80 °C for 24 h to yield the product as an orange solution in THF. A ^{1}H NMR spectrum could not be obtained in THF-d_8, most likely due to paramagnetism of the metal centers. (Only broad lines for the poly(ethylene glycol) repeats were observed.)

Preparation of Triblock PBLG-*b*-PEG-*b*-PBLG, 8, Using 7. In the drybox, Glu NCA (0.23 g, 0.87 mmol) was dissolved in THF (2.0 mL) and placed in a 25 mL reaction tube which could be sealed with a Teflon stopcock. A solution of **7** (1.0 mL, 0.036 mmol) in THF (1 mL) was added via syringe to the flask. A stir bar was added and the flask was sealed, removed from the drybox, and stirred at 25 °C in a thermostated bath for 24 h. Polymer was isolated by addition of the reaction mixture to methanol containing HCl (1 mM) causing precipitation of the polymer. The polymer was repeatedly dissolved in THF and precipitated into methanol, a nonsolvent for the PBLG, but solvent for any uncoupled PEG. The polymer was dried in vacuo to give **8** as a white solid (0.19 g, 82% yield). GPC of the polymer in 0.1 M LiBr in DMF at 60 °C: $M_n = 12\,000$; $M_w/M_n = 1.2$. The following spectral properties were observed: ^{1}H NMR: δ 8.01 (−NH−, PBLG), 7.25 ($-C_6H_5$, PBLG), 5.03, 4.0 (PBLG), 3.65 ($-CH_2CH_2O-$), 2.25 (PBLG). ^{13}C NMR: δ 175.4 (C=O, PBLG), 136.1, 130.3, 128.5 ($-C_6H_5$, PBLG), 70.6 ($-CH_2CH_2O-$). IR (NaCl, THF): 3565.2, 3291.4, 2981.5, 2681.4, 2358.0, 1955.9, 1732.9, 1651.0, 1540.3, 1456.8, 1363.8, 1288.7, 1244.4, 1174.9, 1077.5, 913.6, 659.6 cm^{-1}.

Synthesis of Pentablock PEG-*b*-PBLG-*b*-PEG-*b*-PBLG-*b*-PEG, 9, Using Macroinitiator 7 and End-Capping Agent 4. In the drybox, Glu NCA (0.23 g, 0.87 mmol) was dissolved in 2 mL of THF and placed in 15 mL reaction tube which could be sealed with Teflon stopcock. An aliquot of macroinitiator **7** (1.0 mL of a solution in THF, 0.037 mmol) was added via syringe to the flask. A stir bar was added, and the flask was sealed. This polymerization was allowed to proceed for 48 h at 25 °C, followed by termination with 0.72 g (0.14 mmol, 4.0 equiv) of **4** dissolved in 2 mL of THF. After 48 h at room temperature the copolymer **9** was isolated by addition of the reaction mixture to ethyl ether causing precipitation. The copolymer was repeatedly dissolved in THF and precipitated into methanol, a nonsolvent for the copolymer, but a good solvent for unreacted **4**, for 5 h (0.46 g, 78% yield). GPC (DMF): $M_n = 24\,200$ and $M_w/M_n = 1.2$, DSC: $T_m = 48$ °C. The normalized integral ratio for PEG signals at 3.7 ppm ($-CH_2OCH_2-$, 4H), [$I_{3.7}$] and for PBLG signals at 7.3 ppm ($-C_6H_5$, 5H) [$I_{7.3}$] was found to be $[I_{3.6}]/4{:}[I_{7.3}]/5 = 1.6{:}1$. The calculated ratio for the expected copolymer was 1.5:1. The following spectral properties were observed: ^{1}H NMR: δ 8.01 (−NH−, PBLG), 7.27 (−C$_6$H$_5$, PBLG), 5.05, 4.0 (PBLG), 3.67 (PEG), 2.25 (PBLG). ^{13}C NMR: δ 175.4 (C=O, PBLG), 128.5 (−C$_6$H$_5$, PBLG), 70.6 (PEG).

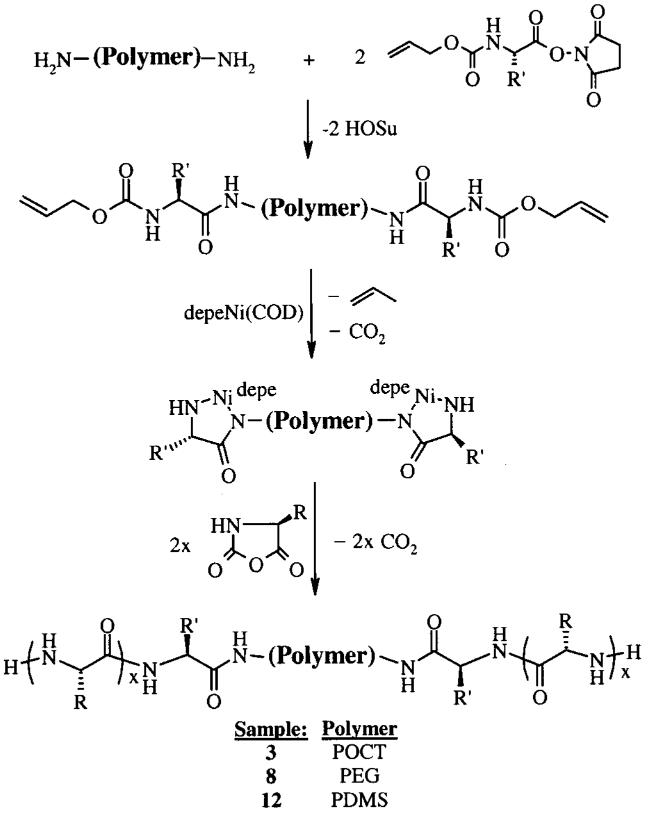
(S,S)-AllocNHCH(R)C(O)NHCH$_2$CH$_2$CH$_2$−(Si(CH$_3$)$_2$O)$_n$−Si(CH$_3$)$_2$CH$_2$CH$_2$−CH$_2$NHC(O)CH(R)NHAlloc, 10. A solution of 2500 molecular weight α,*ω*-bis(3-aminopropyl)poly(dimethylsiloxane) (5.0 g, 2.0 mmol) in THF (10 mL) was added to a solution of alloc-L-leucine succinimidyl ester (2.4 g, 7.6 mmol) in THF (5 mL). The reaction was stirred for 18 h after which the product was precipitated into methanol (35 mL). The product was washed twice more with methanol (25 mL) and dried in vacuo (3.8 g, 73% yield). ^{1}H NMR: δ 6.3 (−N*H*−), 5.90 (CH$_2$=C*H*−), 5.26 (C*H*$_2$=CH−), 4.59 (CH$_2$=CH−C*H*$_2$−O−), 3.77 [-C*H*-CH$_2$−(CH$_3$)$_2$], 3.64 [−C*H*$_2$−O−Si(CH$_3$)$_2$−], 3.30 (−C*H*$_2$−NH−), 1.7 [−C*H*$_2$−, −C*H*$_2$−CH(CH$_3$)$_2$], 1.5 [−C*H*−(CH$_3$)$_2$], 0.97 (−CH−(C*H*$_3$)$_2$], 0.1 (−O−Si(C*H*$_3$)$_2$−). ^{13}C NMR: δ 170.5 (−*C*=O), 133.0 (CH$_2$=*C*H−), 118.1 (*C*H$_2$=CH−), 67.2 (CH$_2$=CH−*C*H$_2$−O−), 58.9 [−*C*H−CH$_2$−(CH$_3$)$_2$], 56.5 (−*C*H$_2$−O−Si(CH$_3$)$_2$−), 41.1 (−*C*H$_2$−NH−), 36.9 [−*C*H$_2$−(CH$_3$)$_2$], 29.1, 28.5, 26.7, 25.5, 24.7 [−CH−(*C*H$_3$)$_2$], 1.9 [−Si(*C*H$_3$)$_2$]. IR (NaCl, THF): 3511.1, 3311.1 (ν, NH), 2966.6, 2855.5, 2677.7, 2355.5, 1966.6, 1722.2 (ν, CO), 1677.8, 1533.3, 1455.5, 1411.1, 1361.1, 1255.5, 1077.8, 1022.2, 911.1, 861.1, 800.0, 700.0, 655.5 cm^{-1}.

Synthesis of (S,S)-depeNiNHCH(R)C(O)NCH$_2$CH$_2$CH$_2$−(Si(CH$_3$)$_2$O)$_n$−Si(CH$_3$)$_2$CH$_2$CH$_2$CH$_2$NC(O)CH(R)NHNi-depe; R = −CH$_2$CH(CH$_3$)$_2$, 11. Depe (34 μL, 0.15 mmol) in 1 mL of THF was added to a solution of Ni(COD)$_2$ (40 mg, 0.15 mmol) in THF (1 mL) and let stand at room temperature for 10 min after which a solution of (depe)Ni(COD) had formed. **10** (0.18 g, 0.073 mmol) in THF (2 mL) was then added to the yellow solution, which subsequently became orange-yellow in color. The solution was heated at 80 °C for 24 h to yield the product as an orange solution in THF. A ^{1}H NMR spectrum could not be obtained in THF-d_8, most likely due to paramagnetism of the metal centers. (Only broad lines for the poly(dimethylsiloxane) repeats were observed.)

Preparation of Triblock PBLG-*b*-PDMS-*b*-PBLG, 12, Using 11. In the drybox, Glu NCA (0.23 g, 0.87 mmol) was dissolved in THF (2.0 mL) and placed in a 25 mL reaction tube which could be sealed with a Teflon stopcock. A solution of **11** (1.0 mL, 0.037 mmol) in THF (1 mL) was added via syringe to the flask. A stir bar was added, and the flask was sealed, removed from the drybox, and stirred at 25 °C in a thermostated bath for 24 h. Polymer was isolated by addition of the reaction mixture to methanol containing HCl (1 mM) causing precipitation of the polymer. The polymer was dried in vacuo to give **12** as a white solid (0.18 g, 81% yield). The following spectral properties were observed: ^{1}H NMR: δ 8.01 (−NH−, PBLG), 7.27 (−C$_6$H$_5$, PBLG), 5.06, 4.0 (PBLG), 2.25 (PBLG), 0.20 (PDMS). ^{13}C NMR: δ 175.4 (C=O, PBLG), 128.5 (−C$_6$H$_5$, PBLG), 1.31 (PDMS).

Synthesis of Pentablock PEG-*b*-PBLG-*b*-PDMS-*b*-PBLG-*b*-PEG, 13, Using Macroinitiator 11 and End-Capping Agent 4. Since PDMS is insoluble in DMF, the Glu NCA additions were carried out in THF. In the drybox, Glu NCA (0.23 g, 0.87 mmol) was dissolved in 3 mL of THF and placed in a 15 mL reaction tube which could be sealed with Teflon stopcock. An aliquot of macroinitiator **11** (1 mL of a solution in THF, 0.037 mmol) was added via syringe to the flask. A stir bar was added, and the flask was sealed. This polymerization was allowed to proceed for 48 h at 25 °C, followed by termination with 0.72 g (0.15 mmol, 4.0 equiv) of **4** dissolved in 2 mL of THF. After 48 h at room temperature the copolymer **13** was isolated by addition of the reaction mixture to ethyl ether causing precipitation. The copolymer was repeatedly dissolved in THF and precipitated into methanol, a nonsolvent for the copolymer, but a good solvent for unreacted **4**, for 5 h (0.46 g, 80% yield). DSC: T_m = 60 °C. The following spectral properties were observed: ^{1}H NMR: δ 8.01 (−NH−, PBLG), 7.27 (−C$_6$H$_5$, PBLG), 5.06, 4.0 (PBLG), 3.65 (PEG), 2.25 (PBLG), 0.20 (PDMS). ^{13}C NMR: δ 175.4 (C=O, PBLG), 128.5 (−C$_6$H$_5$, PBLG), 70.6 (PEG), 1.31 (PDMS).

Scheme 1. Synthesis of C-Terminal Linked Polypeptide Triblock Copolymers Using Bis(amino)-Terminated Macroinitiators (R′ = −CH$_2$CH(CH$_3$)$_2$, R = −CH$_2$CH$_2$CO$_2$CH$_2$C$_6$H$_5$, depe = 1,2-Bis(diethylphosphino)ethane; COD = 1,5-Cyclooctadiene)



Results and Discussion

Triblock Copolymers. Previously, we reported the conversion of α,*ω*-bis(amino)-terminated polyoctenamers to bifunctional initiators that were used to grow PBLG segments onto both ends of the hydrocarbon polymer chains.[5] We have now used similar chemistry to prepare PBLG-*b*-PEG-*b*-PBLG and PBLG-*b*-PDMS-*b*-PBLG triblock copolymers from corresponding α,*ω*-bis(amino)-terminated PEG and PDMS chains (Scheme 1). GPC data on growth of the PBLG segments (Table 1) showed that the macroinitiators gave controlled polymerization, and block copolymer formation was verified by selective solvent extractions and ^{1}H NMR compositional analysis. These results show that this methodology for preparation of block copolymers is general and can be used with a wide range of amino-terminated polymers.

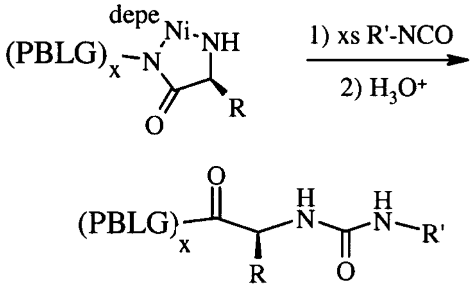
Polypeptide N-Terminal Capping with Electrophiles. The above-mentioned strategy for synthesizing triblock copolymers is versatile for preparation of C-terminal functionalized polypeptides and polypeptide-*b*-(nonpeptide) block copolymers. However, since peptide bonds are directional, the ability to selectively functionalize the N-terminal ends of polypeptides was also desired. Since polypeptides produced through metal-mediated NCA polymerizations grow from the C-terminus to N-terminus, amino end-capping would be accomplished by terminating the growing chain with some reagent that could couple to the propagating amido end group. Isocyanates, common impurities in NCA mono-

Table 1. Characterization Data on Triblock and Pentablock Copolymers Containing Polypeptide Segments

sample	solvent	M_n center[a]	M_n (calcd)[b]	M_n (GPC)[c]	PDI (M_w/M_n)	yield (%)[d]	T_m (°C)
3a	DMF	3300	11 200	11 900	1.1	90	49
3b	THF	3300	9 100	46 000	2.0	86	50
8a	THF	2000	8 600	12 000	1.2	82	NA[f]
8b	DMF	2000	12 900	16 900	1.3	81	NA
8c	THF	2000	12 900	53 400	1.2	85	NA
8d	THF	2000	23 900	109 700	1.1	87	NA
12a	THF	2500	7 700	ND[e]	ND	81	NA
12b	THF	2500	13 900	ND	ND	75	NA
12c	THF	2500	24 500	48 900	1.2	77	NA
12d	THF	2500	46 500	80 130	1.2	78	NA
5a	DMF	3300	31 500	33 700	1.1	81	57
5b	THF	3300	31 500	77 000	1.4	88	59
9a	THF	2000	17 200	21 000	1.2	78	48
9b	DMF	2000	22 400	27 700	1.2	80	NA
9c	DMF	2000	28 000	30 000	1.2	80	NA
13a	THF	2500	17 700	ND	ND	80	60
13b	THF	2500	22 000	85 000	1.2	79	NA
13c	THF	2500	28 200	117 000	1.3	85	NA

[a] Molecular weight of macroinitiator central domain. [b] Determined from ratio of Glu NCA to macroinitiator assuming 100% conversion. For pentablocks, the mass of two PEG_{5000} segments was also added. [c] Determined by GPC (0.1 M LiBr in DMF). [d] Total isolated yield of block copolymer. [e] Not determined due to solubility limitations. [f] Not applicable.

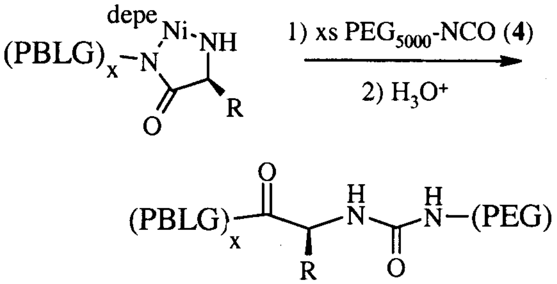
mers and polymerizations, are known to cause termination of growing chains in anionic NCA polymerizations.[2] Thus, it seemed possible that isocyanates, or other electrophiles, added to polymerizations, might be able to react with the amido−metallacycle propagating species resulting in N-terminal capping of the chains through formation of stable urea linkages (eq 1).



(1)

To test this idea, attempts were made to quench nickel-initiated polymerizations of Glu NCA with small molecule isocyanates, isothiocyanates, and acid chlorides. After polymerization of a first aliquot of monomer, 4 equiv of *p*-tolyl isocyanate per metal center was added to the polymerization mixture, which was then stirred for 4 h. A second aliquot of Glu NCA was then added, and the resulting mixture was stirred for 12 h. The second aliquot of monomer was not consumed, and polypeptide chain length did not significantly increase from the value determined after polymerization of the first segment. From these data, termination of the growing chains with both alkyl and aromatic isocyanates appeared to be near quantitative. Isothiocyanates and acid chlorides also showed good chain quenching ability (Table 2).

This termination reaction showed potential broad synthetic use since practically any primary amine can be readily converted into an isocyanate using phosgene. To verify that isocyanates not only terminated NCA polymerizations, but were also attached to the N-terminal ends of the polypeptides, a diblock copolymer was synthesized by capping a Glu NCA polymerization with excess isocyanate functionalized PEG (M_n = 5000) (eq 2). After repetitive selective-solvent precipitations of the product, the formation of the diblock copolymer was confirmed by both GPC and NMR spectroscopy. Compositional analysis by ^1H NMR verified that the amount of PEG coupling was near quantitative. These results demonstrate a straightforward methodology for preparation of N-terminal functionalized polypeptide block copolymers that complements the established technique of using amino-functionalized polymers to prepare C-terminal functionalized polypeptides.



(2)

Synthesis of Pentablock Copolymers. Recognizing that the methods we have used to prepare both N-end and C-end functionalized polypeptides should be compatible, their combination for synthesis of complex, multiblock copolymers was pursued. More specifically, use of amino-terminated polymer segments as macroinitiators was envisioned to produce C-terminal functionalized polypeptide copolymers that could subsequently be derivatized on their N-terminal ends with other arbitrary polymers (Scheme 2). This strategy would produce ABC triblock sequences, assuming a monofunctional macroinitiator and a simple homopolypeptide domain. With bifunctional macroinitiators, ABCBA pentablock architectures should be possible (Scheme 2). The ability to readily prepare such materials, with control over both the nature and sizes of different domains, would greatly enhance the utility of these materials in applications where engineering of their multifunctionality is crucial.

The methods described above for preparation of PBLG-*b*-(**polymer**)-*b*-PBLG triblock copolymers (**polymer** = POCT, PEG, PDMS) were modified only slightly for preparation of pentablock copolymers. Rather than quenching the polymerizations with MeOH/HCl after synthesis of the polypeptide domains, the active chains were instead terminated with excess isocyanate functionalized PEG (**4**) (Scheme 2). The product copolymers were purified by repeatedly dissolving in THF followed by precipitation into methanol, a nonsolvent for the copolymers but a good solvent for any uncoupled PEG. The ^1H and ^{13}C NMR spectra of the purified copolymers confirmed the expected compositions and verified coupling of the PEG segments. Molecular weight and physical data for these copolymers are given in Table 1.

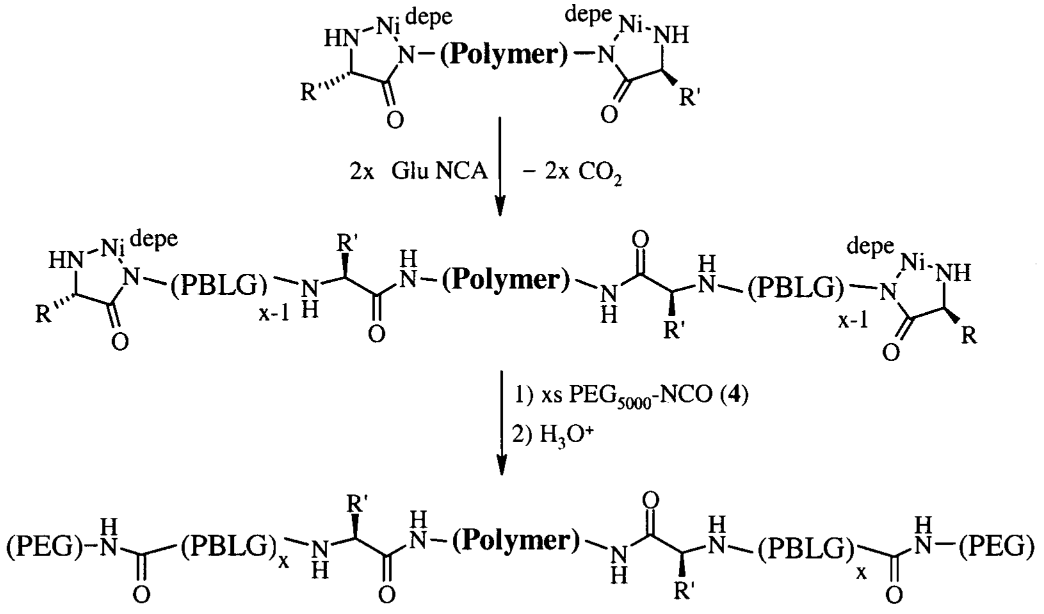
The GPC traces for these pentablock copolymers were unimodal, with no detectable residual macroinitiator segments or poly(ethylene glycol) homopolymer (Figure 1). Thermal analysis of these copolymers using DSC showed the expected melting transitions for the nonpeptide polymer segments. For copolymer **5**, the POCT and PEG segments have overlapping transitions at 57

Table 2. Quenching of Polypeptide Chain Growth with Electrophiles[a]

initiator	first monomer[a]	M_n (GPC)	M_w/M_n	capping agent	second monomer[a]	M_n (GPC)	M_w/M_n	yield (%)[b]
1	125 Glu NCA	38 810	1.31	4 *p*-tolyl isocyanate	125 Glu NCA	40 180	1.28	45
1	125 Glu NCA	38 810	1.20	none	125 Glu NCA	78 130	1.20	90
bpyNi(COD)	125 Glu NCA	28 140	1.20	4 *n*-propyl isocyanate	125 Glu NCA	31 220	1.27	43
bpyNi(COD)	125 Glu NCA	28 140	1.18	none	125 Glu NCA	88 110	1.23	94
bpyNi(COD)	100 Glu NCA	16 900	1.40	20 *p*-tolyl isocyanate	100 Glu NCA	18 700	1.40	45
bpyNi(COD)	100 Glu NCA	11 900	1.20	12 *p*-tolyl isothiocyanate	100 Glu NCA	12 600	1.20	45
bpyNi(COD)	100 Glu NCA	15 700	1.30	20 *p*-toluoyl chloride	100 Glu NCA	18 500	1.30	45

[a] Aliquots of monomer added to initiator before (first) and after quenching (second) with electrophile. Number indicates the number of equivalents of monomer per initiator. [b] Total isolated yield of polymer after both monomer additions. Molecular weights were determined by GPC (0.1 M LiBr in DMF).

Scheme 2. Synthesis of Polypeptide Containing Pentablock Copolymers Using Bis(amino)-terminated Macroinitiators and Isocyanate End-Capping ($R' = -CH_2CH(CH_3)_2$, $R = -CH_2CH_2CO_2CH_2C_6H_5$)



Sample:	Polymer
5	POCT
9	PEG
13	PDMS

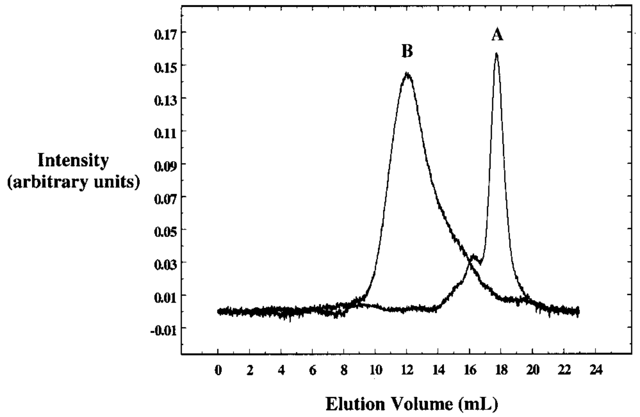


Figure 1. GPC chromatograms of (A) end-capping reagent **4** (M_n = 5000 and PDI = 1.2) and (B) pentablock copolymer **5** (M_n = 77 000 and PDI (M_w/M_n) = 1.4).

and 60 °C, respectively. The presence of stable α-helical conformations in the PBLG domains was expected based on circular dichroism analysis of triblock copolymers.[5] Thus, these copolymers can be viewed as "coil−rod−coil−rod−coil" architectures where the coil segments can be either hydrophilic (PEG) or hydrophobic (PDMS) and either crystalline (POCT) or amorphous (PDMS). It is also possible to remove the benzyl groups from the PBLG segments to produce free carboxylate groups within these segments. Polypeptide-*b*-PEG diblock copolymers are currently being studied for use in many biomedical applications such drug and gene delivery, where the functionality and potential degradability of the polypeptide segments provide advantages.[11] The pentablock copolymers described here are expected to possess added capabilities for such applications since extra functionality (e.g., a hydrophobic center domain for drug loading) and potential for more complex self-assembled structures (e.g., multilayered micelles) can be readily engineered into the materials.

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