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-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 homopolymer 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.4 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 amine-terminal (N-terminal) ends of polypeptide segments is desirable.

In previous work we demonstrated that amido–amine 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 homopolymer 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 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–amide 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-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, 10, 10, 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
with a Wyatt DAWN DSP light scattering detector (633 nm HeNe laser) and Wyatt Optilab DSP RI detector (633 nm). dn/dc values were determined using guidelines of the manufacturer at 633 nm. Separations were effected by 10, 10, 10, and 50 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 performed 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 (Tm) were determined as the peak maxima of the transition.

Materials. 1,9-Decadiene (from Aldrich) was purified by fractional distillation from CaH₂ degassed under high vacuum by several freeze–pump–thaw cycles, and vacuum transferred successively into sodium-mirrored flasks until no reaction was observed. Alloc-ω-aminoamides and ω-benzyl-ε-glutamate-N-carboxyanhydride, Glu NCA, were prepared according to literature procedures. Hexane, THF, and THF-d₄ were purified by first purging with dry nitrogen, followed by passage through columns of activated alumina. DMF, DMF-d₇, and methylene chloride (from Aldrich) were purified by drying over columns using 0.1 M LiBr in DMF at 60 °C. The polymers were dried in vacuo to give white solids, PBLG (0.10 g, 0.38 mmol each) was then 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) 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 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). 13C NMR, 1H NMR, and FTIR spectra of this material were identical to data found for authentic samples of PBLG. GPC of the polymers in 0.1 M LiBr in DMF at 60 °C: (a) first aliquot: Mₙ = 13 900; Mₚ/Mₙ = 1.4; final sample: Mₚ = 18 700; Mₚ/Mₙ = 1.4; (b) first aliquot: Mₙ = 15 200; Mₚ/Mₙ = 1.3; final sample: Mₚ = 28 600; Mₚ/Mₙ = 1.9.

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 spectra of both solutions 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 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). 13C NMR, 1H NMR, and FTIR spectra of this material were identical to data found for authentic samples of PBLG. GPC of the polymers in 0.1 M LiBr in DMF at 60 °C:

- Isocyanate N-Terminal End-Capping and Quenching of PBLG Initiated with (2,2′-bipyridyl)(Ni(COD)). The 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.
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). 13C NMR, 1H 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: Mw = 15 700; Mw/Mn = 1.3; final sample: Mw = 18 500; Mw/Mn = 1.3; (b) first aliquot: Mw = 29 500; Mw/Mn = 1.4; final sample: Mw = 27 100; Mw/Mn = 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 a (2-amino-ethyl)mono-(methyl)-poly(ethylene glycol) (Mn = 5000, 2.3 g, 0.40 mmol) in dry CH2Cl2 (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 × 5 mL) and dried in vacuo (2.2 g, 94% yield). The following spectral properties were observed: 1H NMR: δ 4.05 (CH2O CH2O m), 3.73 (CH3O CH3O n), 3.97 (CH2 CH2 O), 5.68 (CH2 NH Alloc). IR (NaCl, THF): 3565.2, 3291.4, 3062.0, 2953.8, 2845.8, 1743.3, 1650.0, 1544.4, 1461.1, 1366.7, 1288.9, 1244.4, 1177.8, 1066.7, 906.6, 656.0 cm⁻¹.

Synthesis of Diblock PBLG-b-PPEG Using (2,2'-bipyridyl)-(S,S)-depennNHCHR(OCH2CH2O)2-NH-(OC(O)CH2CH2O)-NHnidepe; R = -CH2-CH2(CH2)n. 7. Depe (34 mL, 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)2 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 in a thermostated bath for 24 h. Polymer 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 unreacted PEG (0.26 g, 79% yield). GPC of the polymer in 0.1 M LiBr in DMF at 60 °C, Mw = 19 200 and Mw/Mn = 1.17. The following spectral properties were observed: 1H NMR: δ 8.01 (NH—, PBLG), 7.27 (–CH2H, PBLG), 5.05, 4.0 (PBLG), 3.65 (–CH2CH2O–), 2.25 (PBLG). 13C NMR: δ 175.4 (C=O, PBLG), 136.1, 130.3, 128.5 (–CH2CH2O–), 70.6 (–CH2CH2O–), IR (NaCl, THF): 3565.2, 3291.4, 2981.5, 2681.4, 2358.0, 1955.9, 1732.9, 1651.0, 1540.4, 1456.8, 1363.8, 1288.7, 1244.4, 1174.9, 1077.5, 913.6, 659.6 cm⁻¹.

Synthesis of Pentablock PBLG-b-PPEG-b-PBLG-b-PPEG, 5. Using Macroinitiator 2 and End-Capping Agent 4. In the drybox, Glu NCA (0.23 g, 0.87 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)2 (200 mg, 0.76 mmol) in THF was added via syringe to the flask. A stir bar was added, and the flask was sealed with Teflon stopcock. An aliquot of (2,2'-bipyridyl)Ni(COD)2 (200 mg, 0.76 mmol) in THF was added via syringe to the flask. A stir bar was added, and the flask was sealed with Teflon stopcock. An aliquot of (2,2'-bipyridyl)Ni(COD)2 (200 mg, 0.76 mmol) in THF was added via syringe to the flask. A stir bar was added, and the flask was sealed with Teflon stopcock. An aliquot of (2,2'-bipyridyl)Ni(COD)2 (200 mg, 0.76 mmol) in THF was added via syringe to the flask. A stir bar was added, and the flask was sealed with Teflon stopcock. An aliquot of (2,2'-bipyridyl)Ni(COD)2 (200 mg, 0.76 mmol) in THF was added via syringe to the flask. A stir bar was added, and the flask was sealed with Teflon stopcock.
calculated ratio for the expected copolymer was 1.5:1. The following spectral properties were observed: 1H NMR: δ 8.01 (−NH−, PBLG), 7.27 (−CH₃, PBLG), 5.05, 4.0 (PBLG), 3.67 (PEG), 2.25 (PBLG). 13C NMR: δ 175.4 (C=O, PBLG), 128.5 (−CH₃, PBLG), 70.6 (PEG).

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**Polypeptide End-Capping Using Isocyanates**

Results and Discussion

**Triblock Copolymers.** Previously, we reported the conversion of α,ω-bis(aminoo)minated polycateners to bifunctional initiators that were used to grow PBLG segments onto both ends of the hydrocarbon polymer chains. We now have used similar chemistry to prepare PBLG-PEG-PBLG triblock copolymers from corresponding α,ω-bis(aminoo)minated 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 1H 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-

**Scheme 1. Synthesis of C-Terminal Linked Polypeptide Triblock Copolymers Using Bis(aminoo)minated Macropolymer (R = −CH₂CH₂CO₂CH₂C₆H₅, depe = 1,2-Bis(diethylphosphino)ethane; COD = 1,5-Cyclooctadiene)**

H₂N−(Polymer)NH₂ + 2 depeNi(COD) → 2 CO₂−

**Synthesis of (S,S)-depeNiNHCH(R)C(O)NCH₂CH₂CH₂, 11.** Depe (34 mL, 0.15 mmol) in 1 mL of THF was added to a solution of Ni(COD)₂ (40 mg, 0.15 mmol) and let stand at room temperature for 10 min after which a solution of (depe)Ni(COD) had formed. The solution was heated at 80 °C for 24 h to yield the product as an orange solution in THF. A 1H NMR spectroscopic analysis of the reaction mixture revealed the presence of (S,S)-depeNiNHCH(R)C(O)NCH₂CH₂CH₂, 11. The following spectral properties were observed: 1H NMR: δ 8.01 (−NH−, PBLG), 7.27 (−CH₃, PBLG), 5.06, 4.0 (PBLG), 3.65 (PEG), 2.25 (PEG).
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. Composition analysis via \(^1\)H 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.

The methods described above for preparation of PBLG-b-(polymer)-b-PBLG diblock copolymers \((\text{polymer} = \text{POCT}, \text{PEG}, \text{PDMS})\) 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 \(^1\)H 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 non-peptide polymer segments. For copolymer \(5\), the POCT and PEG segments have overlapping transitions at 57...
and 60 °C, respectively. The presence of stable R-helical conformations in the PBLG domains was expected based on circular dichroism analysis of triblock copolymers. 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 as drug and gene delivery, where the functionality and potential degradability of the polypeptide segments provide advantages. 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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**References and Notes**


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