Dendron-like Polypeptide/Linear Poly(ethylene oxide) Biohybrids with Both Asymmetrical and Symmetrical Topologies Synthesized via the Combination of Click Chemistry and Ring-Opening Polymerization

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ABSTRACT: Dendron-like poly(γ-benzyl-L-glutamate)/linear poly(ethylene oxide) block copolymers with both *asymmetrical* and *symmetrical* topologies (i.e., *ABn* type D*m*-PBLG-*b*-PEO and *BnABn* type D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG; $n = 2^m$, $m = 0$, 1, 2, and 3; D*m* are the propargyl focal point poly(amido amine) type dendrons having 2^m terminal primary amine groups) were synthesized via the combination of ring-opening polymerization (ROP) of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) and click chemistry according to the "arm-first" and "core-first" strategies. In the arm-first method, the propargyl focal point dendrons D*m* having 2^m terminal primary amine groups were first used for initiating the ROP of BLG-NCA, generating "clickable" *dendron-like* D*m*-PBLG homopolymers having 2^m branches, which were then click conjugated with azide-terminated PEO (PEO-N_3) to produce *asymmetrical* D*m*-PBLG-*b*-PEO. In the core-first strategy, the propargyl focal point D*m* was first click conjugated with PEO-N_3 to generate primary amine-terminated PEO dendrons, which were further used as the macroinitiators for the ROP of BLG-NCA to produce the targeted copolymers with both *asymmetrical* and *symmetrical* topologies. Their molecular structures and physical properties were characterized in detail by FT-IR, NMR, gel permeation chromatography, differential scanning calorimetry, and wide-angle X-ray diffraction. Both spherical and wormlike micelles self-assembled from these D*m*-PBLG-*b*-PEO copolymers in aqueous solution, and mainly the PBLG composition controlled the morphology of nanostructures.

Introduction

Owing to their hierarchical self-assembly, adjustable degradation profiles, and especially 3-dimensional architecture with high surface functional group density, synthetic branched polymers such as star-shaped polymers, hyperbranched polymers, dendrimers, dendrons, and dendronized polymers have attracted much attention in biomedical applications and nanotechnology.[1−6] Reminiscently, naturally branched biomacromolecules (e.g., collagen, glycoprotein, and proteoglycan) and their physically cross-linked networks or gels constitute the connective tissues and the extracellular matrix (ECM) in living systems and human body, having been fabricated into various scaffolds for tissue engineering and matrices for drug delivery.[7−9] To date, a number of artificial peptide mimetics and polypeptides self-assemble into various bioactive nanostructures that provide useful platforms for bionanotechnology.[10−14] Likewise, many researchers focus on the bioconjugates of polypeptides with synthetic biocompatible polymers (e.g., poly(ethylene oxide), PEO or PEG) and the PEG-ylation of protein because they combine the respective advantages of synthetic polymers with biological systems.[15,16] Therefore, from a biomimetic viewpoint, developing new methodology to design polypeptide-based biohybrids with branched topology will be very important for polymer science, molecular therapeutics, and bionanotechnology.

From a synthetic viewpoint, linear polypeptide-based block copolymers are generally prepared by ring-opening polymerization (ROP) of an α-amino acid *N*-carboxyanhydride (NCA) monomer using a primary amine-terminated homopolymer.[17,18] For example, Kataoka et al. thoroughly investigated biocompatible PEG-*b*-polypeptides (e.g., poly(L-aspartic acid), poly(L-glutamic acid), and poly(L-lysine)) and their applications for drug/gene delivery.[16] The hierarchical self-assembly of poly(γ-benzyl-L-glutamate)/PEO block and graft copolymers was investigated in detail.[19−22] As an extension of these efforts, the biodegradable poly(ε-caprolactone)− and/or poly(L-lactide)−polypeptides biohybrids were intensively studied by several research groups.[23−30] In addition, both linear−dendron and dendron−linear−dendron copolymers were synthesized by controlled/"living" radical polymerization using dendron macroinitiator.[31−33] Lee et al. reported the self-assembly of dumbbell-shaped rod amphiphiles.[34,35] However, designing *dendritic* polypeptide-based hybrid copolymers with *asymmetrical* and/or *symmetrical* topologies is very rare.

The copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition between azides and alkynes, termed "click chemistry" by Sharpless and colleagues, is a versatile method for polymers and materials sciences, demonstrating high efficiency and tolerance of functional groups under benign conditions.[36−41] The peptide/protein−polymers bioconjugates have been intensively investigated by click chemistry.[37−42] Very recently, synthetic polypeptide-based block copolymers were also prepared by click chemistry.[43] In this article, according to the "arm-first" and "core-first" synthetic methods, a versatile strategy to prepare *dendron-like* poly(γ-benzyl-L-glutamate)/linear poly(ethylene oxide) block copolymers with both *asymmetrical* and *symmetrical* topologies (i.e., *ABn* type D*m*-PBLG-*b*-PEO and *BnABn* type D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG; $n = 2^m$, $m = 0$, 1, 2, and 3; D*m* are the propargyl focal point poly(amido amine) type dendrons having 2^m terminal primary amine groups) was successfully developed, as shown in Scheme 1. In the arm-first method, the propargyl focal point dendrons D*m* having 2^m terminal primary amines groups were synthesized and then used for initiating the ROP of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA), generating "clickable" *dendron-like* D*m*-PBLG homopolymers having 2^m branches, which were further coupled with azide-terminated PEO (i.e., PEO-N_3) by click chemistry to produce the dendron-like/linear PBLG-*b*-PEO hybrid copolymers with *asymmetrical* topology. In the core-first strategy, the dendrons D*m* were click conjugated with azide-

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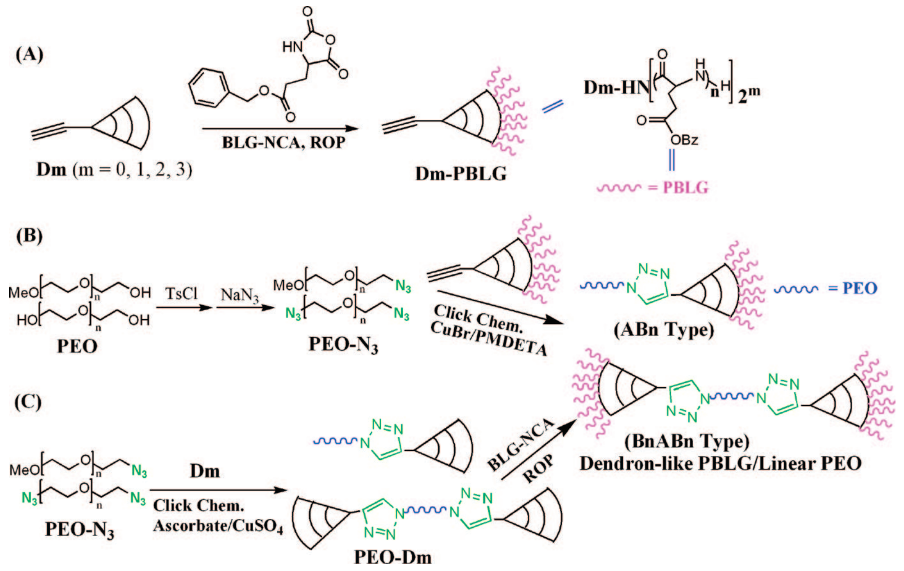
Scheme 1. Synthesis of Dendron-like Poly(γ-benzyl-L-glutamate)/Linear Poly(ethylene oxide) Block Copolymers with Both Asymmetrical and Symmetrical Topologies via the Combination of Ring-Opening Polymerization (ROP) and Click Chemistry



terminated PEO to generate primary amine-terminated PEO-dendrons (i.e., PEO-D*m*), which were then used as the macroinitiators of BLG-NCA monomer to produce the targeted hybrid copolymers with both *asymmetrical* and *symmetrical* topologies. To the best of our knowledge, this is the first synthesis of *dendron-like* polypeptide-based hybrid copolymers with both *asymmetrical* and *symmetrical* topologies via the combination of click chemistry and ROP.

Experimental Section

Materials. Chloroform (99%), ethylenediamine (99%), and methyl acrylate (98%) were purchased from Shanghai Sinopharm Chemical Reagent Corp. and distilled before use. Phosphorus trichloride (Shanghai Sinopharm Chemical Reagent Corp., 95.5%) was distilled and diluted in CH_2Cl_2 (PCl_3:CH_2Cl_2 = 1:2, v/v) before use. Dimethylformamide (DMF, Shanghai Sinopharm Chemical Reagent Corp., 99.5%) was distilled from calcium hydride under reduced pressure and stored over molecular sieves. Poly(ethylene glycol) methyl ether ($M_n = 5000$, Aldrich) was dried at 50 °C in vacuo overnight, and its purity was 100% within the error of ^{1}H NMR measurement. Poly(ethylene glycol) ($M_n = 4000$) was purchased from Shanghai Sinopharm Chemical Reagent Corp. and dried at 50 °C in vacuo overnight before use. Copper(I) bromide (99.999%, Aldrich), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 99%, Aldrich), propargylamine (99%, Acros), and toluene-4-sulfonyl chloride (98.5%, Aldrich) were used as received. 1,6-Diphenyl-1,3,5-hexatriene (DPH, 98%) was purchased from Aldrich and used as a probe molecule. γ-Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) monomer was synthesized from *N-tert*-butyloxycarbonyl-γ-benzyl L-glutamic acid and phosphorus trichloride, as detailed elsewhere.[44] ^{1}H NMR of BLG-NCA (400 MHz, $CDCl_3$): δ (ppm) = 2.05−2.25 (m, 2H, CH_2CH_2COO), 2.58−2.63 (m, 2H, CH_2CH_2COO), 4.34−4.39 (t, 1H, CH), 5.13−5.15 (s, 2H, $PhCH_2O$), 6.17−6.20 (s, 1H, NH), 7.32−7.40 (m, 5H, Ph). The propargyl focal point poly(amido amine) (PAMAM) type dendrons with primary amine groups (i.e., **D*m***) were synthesized using a protocol similar to that described by Lee et al.,[45−48] as detailed in the Supporting Information (Figure S1). According to the previous literatures,[48,49] azide-terminated PEO (i.e., PEO-N_3) was prepared from poly(ethylene glycol) methyl ether and poly(ethylene glycol), respectively. ^{1}H NMR of PEO-N_3 ($CDCl_3$): δ (ppm) = 3.37 (s, CH_3O, 3H), 3.39 (t, $OCH_2CH_2N_3$, 2H), 3.46 (t, $CH_3OCH_2CH_2O$, 2H), 3.54 (t, $CH_3OCH_2CH_2O$, 2H), 3.64 (s, $O(CH_2CH_2O)_n$, 450H), 3.82 (t, $OCH_2CH_2N_3$, 2H).

Methods. FT-IR spectra were recorded on a Perkin-Elmer Paragon 1000 spectrometer at frequencies ranging from 400 to 4000 cm^{-1}. Samples were thoroughly mixed with KBr and pressed into pellet form. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were performed at room temperature on a Varian Mercury-400 spectrometer. D*m*-PBLG homopolymers and D*m*-PBLG-*b*-PEO copolymers were dissolved in mixed solvents of CF_3COOD and $CDCl_3$ (CF_3COOD:$CDCl_3$ = 1:1, v/v). Mass spectra (TOF-MS) were performed on a Waters Acquity UPLC/Premier QTOF MS spectrometer. Molecular weight and polydispersity (M_w/M_n; M_w: weight-average molecular weight; M_n: number-average molecular weight) of polymer was determined on a gel permeation chromatograph (GPC) equipped with two Mixed-B columns (Polymer Laboratory Corp., pore size: 10 μm; column size: 300 × 7.5 mm) and a refractive index detector (Perkin-Elmer Series 200) at 30 °C. The elution phase was DMF (0.01 mol L^{-1} LiBr, elution rate: 1.0 mL/min), and polystyrene was used as the calibration standard. The differential scanning calorimetry (DSC) analysis was carried out using a Perkin-Elmer Pyris 1 instrument under nitrogen flow (10 mL/min). All samples were first heated from 0 to 160 °C at 10 °C/min and held for 3 min to erase the thermal history, then cooled to 20 at 10 °C/min, and finally heated to 160 at 10 °C/min. The indium standard was used for temperature and enthalpy calibrations. The melting temperature (T_m) of PEO and the liquid crystalline phase transition temperature (T_{LC}) of PBLG were taken as the maximum temperature of endothermic peak. Wide-angle X-ray diffraction (WAXD) patterns of powder samples were obtained at room temperature on a Shimadzu XRD-6000 X-ray diffractometer with a Cu Kα radiation source (wavelength = 1.54 Å). The supplied voltage and current were set to 40 kV and 30 mA, respectively. Samples were exposed at a scan rate of $2\theta = 4°$ min^{-1} between $2\theta = 2°$ and 40°. UV−vis spectra of samples were recorded at room temperature using a Spectrumlab54 UV−vis spectrophotometer. The mean size of nanoparticles was determined by dynamic light scattering (DLS) using a Malvern Nano_S instrument (Malvern, UK) at 25 °C. All of the measurements were repeated three times, and the average values reported were the mean diameter ± standard deviation. Transmission electron microscopy (TEM) was performed using a JEM-2010/INCA OXFORD TEM (JEOL/OXFORD) at a 200 kV accelerating voltage. Samples were deposited onto the surface of 300 mesh Formvar-carbon film-coated copper grids. Excess solution was quickly wicked away with a filter paper. The image contrast was enhanced by negative staining with phosphotungstic acid (0.5 wt %).

Preparation of Dendron-like/Linear Dm-PBLG-b-PEO Block Copolymers via the Arm-First Method. D*m*-PBLG with a clickable alkyne focal group (*m* = 0, 1, 2, and 3) was synthesized from the ROP of BLG-NCA using primary amine-terminated D*m* with alkyne focal group in DMF solution at room temperature. In a representative reaction, D1 with two terminal amines (7.9 mg, 0.028 mmol) was dissolved completely in 0.5 mL of DMF under a nitrogen atmosphere, and then a degassed solution of BLG-NCA (734 mg, 2.79 mmol) in 7 mL of DMF was added via a syringe. The resulting solution was stirred vigorously at room temperature for 48 h, diluted with tetrahydrofuran (THF, 2 mL), and then precipitated dropwise into a large excess of diethyl ether (100 mL). The white precipitate was filtered and dried in vacuo at 40 °C to give 520.0 mg of D1-PBLG$_{41}$ {84%, yield = $W_{\text{polymer}}/[(W_{\text{monomer}} - W_{\text{monomer}}/M_{\text{monomer}} \times M_{CO_2}) + W_{\text{initiator}}]$}. Their yields for the synthesis of clickable other D*m*-PBLGs were determined gravimetrically and varied within 77−84%. ^{1}H NMR of D1-PBLG$_{41}$ ($CDCl_3/CF_3COOD$, v:v = 1:1): δ (ppm) = 1.86−2.32 (m, CH_2CH_2CO, 164H), 2.40−2.70 (m, CH_2CH_2CO, 164H), 2.76 (t, $HC{\equiv}CCH_2$, 1H), 2.94 (t, NCH_2CH_2CO, 4H), 3.47 (q, NCH_2CH_2CO + $CONHCH_2CH_2NH$, 8H), 3.66 (q, $CONHCH_2CH_2NH$, 4H), 4.20 (d, $HC{\equiv}CCH_2$, 2H), 4.36−4.76 (m, COC*H*NH, 82H), 5.04−5.26 (m, CH_2Ph, 164H), 7.21−7.42 (m, Ph, 410H). $M_{n,GPC}$ = 4570, M_w/M_n = 1.39. FT-IR (KBr, cm^{-1}): 3293 (ν_{N-H}); 2944 (ν_{C-H}), 1734 ($\nu_{CO\text{-}O}$), 1652 ($\nu_{C=O}$), 1546, 1386 (ν_{CO-NH}).

Thus, the obtained D*m*-PBLG with alkyne focal group was click conjugated with PEO-N_3 to produce the targeted D*m*-PBLG-*b*-PEO block copolymers. A representative procedure for the click conjugation is as follows: D1-PBLG with alkyne focal group (100 mg, 5.6 μmol) and PEO-N_3 (34 mg, 6.7 μmol, 120 mol % per alkyne group) were completely dissolved in DMF (1.5 mL). The solution was bubbled with N_2 for 10 min, and both CuBr (1 mg, 6.7 μmol, 1 fold of PEO-N_3) and PMDETA (1.4 μL, 6.7 μmol, 1 fold of PEO-N_3) were added to the solution. Then the reaction mixture was stirred vigorously under N_2 at 35 °C for 24 h. The solution was precipitated into cold diethyl ether and then washed with cold methanol to remove the excess PEO-N_3. The precipitate was dried in vacuo to give the D$_1$-PBLG$_{41}$-*b*-PEO of 118 mg (yield 92%). ^{1}H NMR of D1-PBLG$_{41}$-*b*-PEO ($CDCl_3/CF_3COOD$, v:v = 1:1): δ (ppm) = 1.86−2.32 (m, CH_2CH_2CO, 164H), 2.40−2.70 (m, CH_2CH_2CO, 164H), 3.54 (s, CH_3O, 3H), 3.83 (s, $O(CH_2CH_2O)_n$, 450H), 4.60−4.70 (m, COC*H*NH, 82H), 5.04−5.22 (m, CH_2Ph, 164H), 7.21−7.42 (m, Ph, 410H), 8.03 (s, 1H, triazole). $M_{n,GPC}$ = 12 800, M_w/M_n = 1.15. The yield for the other click conjugates varied from 90% to 96%.

Synthesis of PEO-Dendron Macroinitiators (PEO-Dm) by Click Chemistry. PEO-N_3 (100 mg, 0.02 mmol) was dissolved in distilled water (1.0 mL) to give a 0.02 M final concentration of terminal azide group (N_3). Then, D*m* (200 mol % per terminal N_3) and aqueous solutions of $CuSO_4$ (30 mol % per N_3) and sodium ascorbate (150 mol % per N_3) were added under stirring. The resulting solution was stirred vigorously at room temperature for 24 h. The reaction solution was removed under reduced pressure, diluted with THF (1 mL), centrifuged to remove the excess D*m*, and then precipitated into diethyl ether (10 mL) to give the final product PEO-D*m* (yield 92−98%). A representative procedure for the synthesis of PEO-D*m* is as follows: PEO-N_3 (100 mg, 0.02 mmol) was dissolved completely in distilled water (1 mL), and then D0 (2.2 mg, 0.04 mmol), 0.05 M sodium ascorbate (0.6 mL), and 0.05 M $CuSO_4$ (0.12 mL) were added to the above solution. After 24 h of reaction time, the reaction solution was removed under reduced pressure, diluted with THF (1 mL), centrifuged to remove the excess D0, and then precipitated into diethyl ether (10 mL) to give the final product PEO-D0 as a pale powder (98 mg, 96%) ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.37 (s, CH_3O, 3H), 3.45 (t, $CH_3OCH_2CH_2O$, 2H), 3.60 (t, $CH_3OCH_2CH_2O$, 2H), 3.64 (s, $O(CH_2CH_2O)_n$, 450H), 3.82 (t, $OCH_2CH_2(CHN_3)CH_2NH_2$, 2H), 4.54 (t, $OCH_2CH_2(CHN_3)CH_2NH_2$, 2H), 4.60 (t, $OCH_2CH_2(CHN_3)CH_2NH_2$, 2H), 7.75 (s, triazole, 1H). $M_{n,NMR}$ = 5080. FT-IR (KBr, cm^{-1}): 3200−3600 (ν_{N-H}), 2883 (ν_{C-H}), 1650 ($\nu_{C=O}$), 1550 (ν_{CO-NH}), 1110 (ν_{C-O}). Similarly, PEO-D1, PEO-D2, and PEO-D3 were successfully synthesized, and they were characterized by ^{1}H NMR and FT-IR. PEO-D1: ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.38 (t, NCH_2CH_2CO, 4H), 2.81 (m, NCH_2CH_2CO + $NHCH_2CH_2NH_2$, 8H), 3.27 (s, $NHCH_2CH_2NH_2$, 4H), 3.37 (s, CH_3O, 3H), 3.46 (t, $CH_3OCH_2CH_2$, 2H), 3.64 (s, $O(CH_2CH_2O)_n$, 450H), 3.82 (t, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 3.86 (s, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 4.53 (m, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 7.70 (s, triazole, 1H). $M_{n,NMR}$ = 5310. PEO-D2: ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.36 (s, NCH_2CH_2CO, 12H), 2.51 (s, $NHCH_2CH_2N$, 4H), 2.72 (s, $NHCH_2CH_2NH_2$, 8H), 2.81 (s, NCH_2CH_2CO, 12H), 3.28 (s, $NHCH_2CH_2N$ + $NHCH_2CH_2NH_2$, 12H), 3.37 (s, CH_3O, 3H), 3.46 (t, $CH_3OCH_2CH_2O$, 2H), 3.54 (t, $CH_3OCH_2CH_2O$, 2H), 3.64 (s, $O(CH_2CH_2O)_n$, 450H), 3.82 (t, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 3.86 (s, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 4.52 (m, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 7.68 (s, triazole, 1H). $M_{n,NMR}$ = 5765. PEO-D3 (D_2O): δ (ppm) = 2.65 (s, NCH_2CH_2CO, 28H), 2.72 (t, NCH_2CH_2CO + $NHCH_2CH_2N$ + $NHCH_2CH_2NH_2$, 56H), 3.02 (t, $NHCH_2CH_2N$, 28H), 3.25 (s, CH_3O, 3H), 3.30 (s, $CH_3OCH_2CH_2O$, 2H), 3.40 (s, $CH_3OCH_2CH_2O$, 2H), 3.53 (s, $O(CH_2CH_2O)_n$, 450H), 3.75 (t, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 3.88 (s, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 4.59 (s, $OCH_2CH_2(CHN_3)CH_2N$, 2H), 7.83 (s, triazole, 1H). $M_{n,NMR}$ = 6700.

Preparation of Dendron-like/Linear PBLG-b-PEO Block Copolymers via the Core-First Method. Dendron-like/linear PBLG-*b*-PEO block copolymers were synthesized by the ROP of BLG-NCA using primary amine-terminated PEO-D*m* as the macroinitiator in DMF solution at room temperature. In a representative reaction, PEO-D1 (21.0 mg, 4.0 μmol) was dissolved completely in 0.5 mL of DMF under a nitrogen atmosphere, and then a degassed solution of BLG-NCA (105.2 mg, 0.4 mmol) in 1 mL of DMF was added via a syringe. The resulting solution was stirred vigorously at room temperature for 48 h, diluted with THF (1 mL), and then precipitated dropwise into a large excess of diethyl ether (20 mL). The white precipitate was filtered and dried in vacuo at 40 °C to give 93.3 mg of D1-PBLG$_{41}$-*b*-PEO (86%, yield = $W_{\text{copolymer}}/[(W_{\text{monomer}} - W_{\text{monomer}}/M_{\text{monomer}} \times M_{CO_2}) + W_{\text{macroinitiator}}]$). ^{1}H NMR of D1-PBLG$_{41}$-*b*-PEO (CDCl3/$CF_3COOD$, v:v = 1:1): δ (ppm) = 1.86−2.32 (m, CH_2CH_2CO, 164H), 2.40−2.70 (m, CH_2CH_2CO, 164H), 3.54 (s, CH_3O, 3H), 3.83 (s, $O(CH_2CH_2O)_n$, 450H), 4.60−4.70 (m, COC*H*NH, 82H), 5.04−5.22 (m, CH_2Ph, 164H), 7.21−7.42 (m, Ph, 410H), 8.03 (s, 1H, triazole). $M_{n,GPC}$ = 13 600, M_w/M_n =1.17. The yield for the polymerization varied from 80% to 90%.

Self-Assembled Nanoparticles from Dm-PBLG-b-PEO Copolymers in Aqueous Solution. A typical procedure for the preparation of D*m*-PBLG-*b*-PEO nanoparticles is as follows. The D*m*-PBLG-*b*-PEO block copolymer (2 mg) was completely dissolved in 2 mL of DMF, and distilled water was then added gradually at a speed of 20 μL/min using a microsyringe. After the appearance of a blue tint indicating the formation of aggregates, another 10−30 wt % of water was added to stabilize the aggregates. After stirring overnight at room temperature, DMF was removed by dialyzing against distilled water for 3 days using a dialysis membrane with a molecular weight cutoff (MWCO) of 7000 Da. The obtained nanoparticles solution was stored at 4 °C before measurement, and both the mean size and morphology of nanoparticles were determined by DLS and TEM, respectively.

Results and Discussion

Synthesis of Dendron-like PBLG/Linear PEO Block Copolymers with Both Asymmetrical and Symmetrical Topologies via the Combination of Click Chemistry and ROP. Polypeptides homopolymers with different macromolecular architectures can be prepared through the ring-opening polymerization (ROP) of α-amino acid NCA monomers using a primary amine-containing compound, where the C-5 carbonyl group of NCA monomer usually undergoes a nucleophilic addition by the amine group.[15−18] In this article, based on the “arm-first” and the “core-first” synthetic strategies, a versatile approach to prepare the *dendron-like* poly(γ-benzyl-L-glutamate)/linear poly(ethylene oxide) block copolymers with both *asymmetrical* and *symmetrical* topologies (i.e., *ABn* type D*m*-PBLG-*b*-PEO and *BnABn* type D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG; $n = 2^m$, m = 0, 1, 2, and 3) was developed by combining click

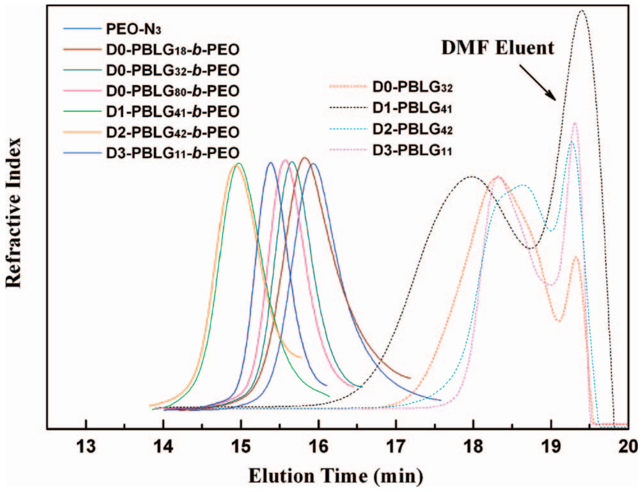


Figure 1. Gel permeation chromatography (GPC) traces of D*m*-poly(γ-benzyl-L-glutamate) (D*m*-PBLG), azide-terminated poly(ethylene oxide) (PEO-N_3) precursors, and the D*m*-PBLG-*b*-PEO copolymers ($m = 0$, 1, 2, and 3; D*m* are the propargyl focal point poly(amido amine) type dendrons having 2^m terminal primary amine groups).

chemistry and ROP, as shown in Scheme 1. In the "arm-first" method (Scheme 1A,B), the propargyl focal point PAMAM type dendrons D*m* with 2^m terminal primary amines groups were synthesized and used for initiating the ROP of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA), generating "clickable" *dendron-like* D*m*-PBLG homopolymers having 2^m branches, which were then click conjugated with azido-terminated PEO (i.e., PEO-N_3) to produce the targeted dendron-like PBLG/linear PEO block copolymers with *asymmetrical topology* (i.e., *ABn* type D*m*-PBLG-*b*-PEO). In the "core-first" strategy (Scheme 1C), the propargyl focal point PAMAM type dendrons D*m* were first click conjugated with PEO-N_3 to generate primary amine-terminated PEO-dendrons (i.e., PEO-D*m*), which were then used as the macroinitiators for the ROP of BLG-NCA monomer to produce the targeted *dendron-like* PBLG/linear PEO biohybrids with both *asymmetrical* and *symmetrical* topologies (i.e., *ABn* type D*m*-PBLG-*b*-PEO and *BnABn* type D*m*-PBLG-*b*-PEO-D*m*-PBLG). These strategies were tested and proved to be successful, as demonstrated in the following parts.

The propargyl focal point PAMAM type dendrons D*m* with 2^m terminal primary amine groups were synthesized using propargylamine as a propargyl focal point according to the divergent synthetic procedure.[45−48] This divergent methodology involves typical stepwise and iterative two-step reaction sequences, consisting of Michael addition of primary amines (e.g., propargylamine, denoted as D0) with methyl acrylate and amidation of methyl ester groups with a large molar excess of ethylenediamine to produce primary amines terminal groups. These dendrons (i.e., D1, D2, and D3) were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and TOF-MS spectroscopy (see Supporting Information Figures S1 and S2), which confirmed their chemical structures and demonstrates that they can be used as initiators for the following ROP. Then, using these dendrons D*m* as initiators, the ROP of BLG-NCA monomer was polymerized in DMF solution at 25 °C to produce the *dendron-like* PBLG homopolymers with a *clickable* alkyne focal group (i.e., D*m*-PBLG having 2^m branches, such as D0-PBLG having one branch, D1-PBLG having two branches, D2-PBLG having four branches, and D3-PBLG having eight branches), as shown in Scheme 1A. The typical GPC curves of the dendron-like D*m*-PBLG homopolymers revealed a partially overlapped elution peak with DMF eluent (Figure 1), and the GPC-determined molecular weight ($M_{n,GPC}$) was much underestimated compared with the ^{1}H NMR-determined molecular weight ($M_{n,NMR}$, Table 1). This is probably attributed to the smaller hydrodynamic volume of dendron-like polymers and the strong molecular interactions within polypeptides. This phenomenon has been often observed for polypeptide-based polymers in the literature,[30,50] and the following as-synthesized D*m*-PBLG-*b*-PEO and D*m*-PBLG-*b*-PEO-D*m*-PBLG copolymers also gave underestimated $M_{n,GPC}$ (Table 2). Note that the dendron-like PBLG polymers need further studies on their absolute molecular weights by multiangle laser light scattering coupled with the GPC technique.

Comparing with the D*m* initiator dissolved in same mixed solvents ($CDCl_3$:CF_3COOH = 1:1, v/v, Figure 2A,B), the representative ^{1}H NMR spectrum of D1-PBLG clearly shows that besides the typical proton signals at 7.42−7.21 and 5.26−5.04 ppm (j) assignable to the benzyl residue, those at 2.70−2.40 and 2.32−1.86 ppm assignable to the methylene groups ($-CH_2CH_2-COOBz$), and those at 4.76−4.36 ppm (g) assignable to the backbone ($-CH-$) of PBLG, there are additional proton signals of its end groups, i.e., the signals assigned to the protons on alkyne group of D*m* residue ($HC'C$, $\delta H^a = 2.76$ ppm, $HC'CCH_2NHCO$, $\delta H^b = 4.20$ ppm). By taking into account the integral ratio of protons "j" or "g" to "b" (Figure 2B), the actual molecular weights of these dendron-like D*m*-PBLG homopolymers can be easily determined by ^{1}H NMR spectroscopy (i.e., $M_{n,NMR}$), and the $M_{n,NMR}$ of D*m*-PBLG is accurately controlled by the molar ratio of BLG-NCA monomer to D*m* initiator (Table 1). ^{13}C NMR spectroscopy further verified the chemical structure of as-synthesized D1-PBLG in comparison with D1 initiator, as shown in the Supporting Information Figure S3. Moreover, the proton signals of methylene connected with primary amine end groups ($NH_2CH_2CH_2-$) at about 3.0 ppm within D1 initiator downshifted to 3.4 ppm within D1-PBLG homopolymer. Furthermore, the carbon signal at 40. 0 ppm ($NH_2CH_2CH_2-$) within D1 upshifted to 31.6 ppm within D1-PBLG. These results indicate that all primary amine end groups of dendron D*m* initiated the ROP of BLG-NCA monomer, which was also confirmed by multifunctional amine-terminated poly(amido amine) initiating ROP of BLG-NCA.[51,52] Note that the PBLG branch within D*m*-PBLG homopolymer cannot be cleaved from the dendron D*m* core by hydrolysis; it is difficult to determine whether each PBLG branch has the same length or not. Meanwhile, the NMR spectra of other D*m*-PBLG homopolymers gave similar results (Supporting Information Figure S4A). Notably, both ^{1}H NMR and ^{13}C NMR analyses indicate that the clickable alkyne focal group retained within D*m*-PBLG during the ROP of BLG-NCA and the branch length of PBLG can be accurately controlled by the molar ratio of BLG-NCA monomer to D*m* initiator. In all, these results demonstrated that the propargyl focal point D*m* with terminal primary amine groups really played the role of initiator for the ROP of BLG-NCA,[17,18] and the "clickable" *dendron-like* D*m*-PBLG having 2^m branches can be easily synthesized according to Scheme 1A. To the best of our knowledge, this is the first example for the synthesis of the "clickable" dendron-like D*m*-PBLG homopolymers having 2^m branches,[43] which can be used for the click conjugation with azide-terminated PEO to produce dendron-like PBLG/linear PEO block copolymers with *asymmetrical topology* (Scheme 1B). Additionaly, the clickable dendron-like D*m*-PBLG homopolymers should provide a straight platform for functionalization of biodegradable polymeric biomaterials such as aliphatic polyesters and natural polysaccharides (e.g., chitin and chitosan) by click chemistry.[37−42]

Commercially available PEO with a mono- and dihydroxyl end group was first tosylated and then converted to azide. The resulting PEO-N_3 was characterized by ^{1}H NMR and FT-IR spectroscopy. The N_3 terminal group within PEO-N_3 was clearly observed at about 2106 cm^{-1} in FT-IR, and the new triplet

Table 1. Synthesis of Dendron-like Poly(γ-benzyl-L-glutamate)/Linear Poly(ethylene oxide) Block Copolymers with *Asymmetrical* Topology (i.e., *ABn* Type D*m*-PBLG-*b*-PEO; $n = 2^m$, $m = 0, 1, 2,$ and 3; D*m* Are the Propargyl Focal Point Poly(amido amine) Type Dendrons Having 2^m Terminal Primary Amine Groups) via the Combination of Click Chemistry and Ring-Opening Polymerization (ROP)

entry[a]	[NCA]/[amine](mol/mol)	$M_{n,GPC}$[b] (×10^3)	M_w/M_n[b]	$M_{n,NMR}$[c] (×10^3)	$M_{n,branch}$[c] PBLG (×10^3)	PEO/PBLG % (wt %)[d]
PEO-N_3		9.99	1.16	5.00		
D0-PBLG$_{32}$	40/1	3.32	1.19	7.06	7.01	
D1-PBLG$_{41}$[e]	50/1	4.57	1.39	18.24	8.98	
D2-PBLG$_{42}$[e]	50/1	2.97	1.12	37.53	9.20	
D3-PBLG$_{11}$[e]	15/1	3.00	1.08	20.92	2.41	
D0-PBLG$_{18}$-*b*-PEO	20/1	12.30	1.14	9.02	39.40	55.9/44.1
D0-PBLG$_{32}$-*b*-PEO	40/1	12.46	1.09	12.09	7.01	41.6/58.4
D0-PBLG$_{80}$-*b*-PEO	100/1	12.65	1.08	22.60	17.52	22.2/77.8
D1-PBLG$_{41}$-*b*-PEO	50/1	12.80	1.15	23.26	8.98	21.8/78.2
D2-PBLG$_{42}$-*b*-PEO	50/1	13.98	1.08	42.56	9.20	12.0/88.0
D3-PBLG$_{11}$-*b*-PEO	15/1	12.74	1.02	25.95	2.41	20.6/79.4

[a] The subscript number represents the degree of polymerization of PBLG branch, which was determined by ^{1}H NMR spectroscopy. [b] The number-average molecular weight ($M_{n,GPC}$) and the polydispersity (M_w/M_n) of polymer was determined by gel permeation chromatography (GPC) using DMF−LiBr (0.01 mmol L^{-1}) as eluent. [c] Both $M_{n,NMR}$ (homopolymer and copolymers) and $M_{n,branch}$ of PBLG are determined by ^{1}H NMR (e.g., Figure 2B and Figure 4). [d] PEO/PBLG % denotes the weight fraction of PEO/PBLG within copolymers. [e] The TOF-MS determined molecular weight of D1, D2, and D3 is 284.2063 [M + H]$^+$, 740.5263 [M + H]$^+$, and 1675.1428 [M + Na]$^+$, respectively.

Table 2. Synthesis of Dendron-like PBLG/Linear PEO Block Copolymers with *Symmetrical* Topology (i.e., *BnABn* Type D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG) via the Combination of Click Chemistry and ROP

entry	[NCA]/[amine] (mol/mol)	$M_{n,GPC}$copolymer (×10^3)	M_w/M_n	$M_{n,NMR}$copolymer (×10^3)	$M_{n,branch}$ PBLG (×10^3)	PEO/PBLG % (wt %)
N_3-PEO-N_3		8.30	1.05	4.00		
D0-PBLG$_{32}$-*b*-PEO-*b*-D0-PBLG$_{32}$	40/1	11.50	1.30	18.18	7.01	22.0/78.0
D1-PBLG$_{28}$-*b*-PEO-*b*-D1-PBLG$_{28}$	35/1	16.47	1.09	32.00	6.13	12.5/87.5
D2-PBLG$_{18}$-*b*-PEO-*b*-D2-PBLG$_{18}$	25/1	10.05	1.15	37.06	3.94	10.8/89.2
D3-PBLG$_{16}$-*b*-PEO-*b*-D3-PBLG$_{16}$	20/1	11.06	1.40	63.42	3.50	6.3/93.7

proton signals of $CH_2{}^{e}CH_2{}^{f}N_3$ occurred at 3.80 and 3.39 ppm in ^{1}H NMR spectrum (Supporting Information Figure S5). Moreover, by taking into account the integral ratio of protons CH_2N_3 (f) or $CH_2CH_2N_3$ (e) to the repeating units protons (CH_2CH_2, d) of PEO-N_3, it can be concluded that the hydroxyl end group of PEO was quantitatively converted into azide within PEO-N_3.

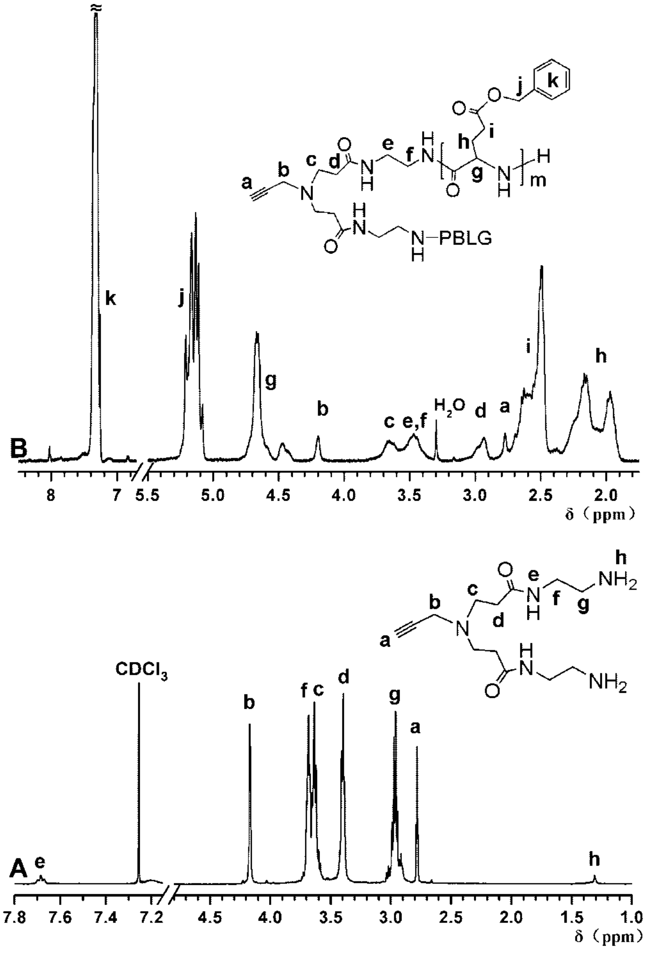


Figure 2. ^{1}H NMR spectra ($CF_3COOD/CDCl_3$ = 1:1, v/v) of dendron D1 initiator (A) and the D1-PBLG homopolymer (B).

The click conjugation between dendron-like D*m*-PBLG and PEO-N_3 was carried out using CuBr/PMDETA as catalyst in DMF solution at 35 °C, and the detailed results for the synthesis of dendron-like PBLG/linear PEO block copolymers are compiled in Table 1. Because the equal molar ratio between D*m*-PBLG (alkyne unit mole) and PEO-N_3 (azide unit mole) cannot be exactly realized in experiments based on the calculation of ^{1}H NMR, and 20% excess PEO-N_3 precursor was used in order to prepare well-defined D*m*-PBLG-*b*-PEO, the yield of the click conjugation was high (90−96 wt %). The unimodal elution peaks of the purified block copolymers apparently shifted toward a lower elution time region with a more narrow polydispersity in comparison with that of the original D*m*-PBLG and PEO-N_3 precursors (Figure 1), which convincingly verified the successful synthesis of the targeted D*m*-PBLG-*b*-PEO block copolymers with *asymmetrical topology*. Moreover, the GPC analysis proved that the excess PEO-N_3 component can be completely removed from the resulting products by simple washing using cold methanol (Supporting Information Figure S6).

FT-IR is a useful tool to verify the click conjugation and the presence of both PBLG and PEO components in these block copolymers (Figure 3). The azide group within PEO-N_3 at about 2106 cm^{-1} completely disappeared within the resulting copolymers, suggesting the occurrence of click reaction. In addition, the D*m*-PBLG-*b*-PEO block copolymers showed the distinct amide I and II bands at 1650 cm^{-1} (CH) and 1550 cm^{-1} for PBLG block, the intense stretching bands at 2883 and 1110 cm^{-1} for PEO block, and the broad band at about 3293 cm^{-1} (NH) for the D*m* initiator and the PBLG backbone. Moreover, comparing with the PEO-N_3 and D*m*-PBLG precursors, the ^{1}H NMR spectra of these copolymers clearly show that, besides the typical proton signals of both PBLG and PEO blocks, new proton signal at 8.03 ppm (h, singlet) typical of methine proton of the triazole ring appeared within the clicked copolymers (Figure 4 and Figure S4B of the Supporting Information).

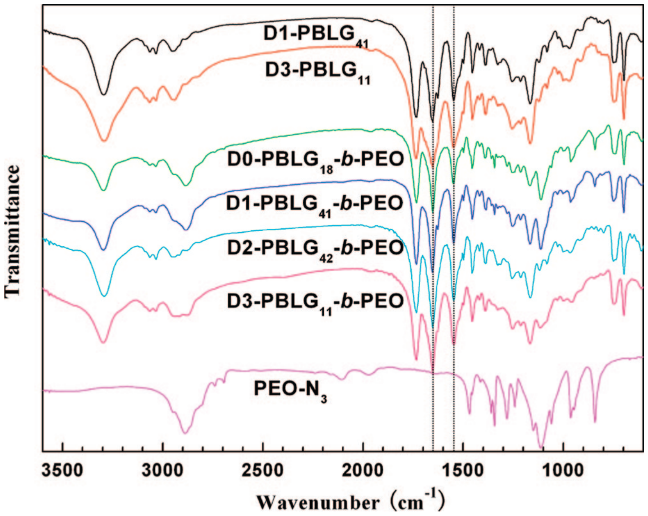


Figure 3. FT-IR spectra of PEO-N_3, D*m*-PBLG, and D*m*-PBLG-*b*-PEO.

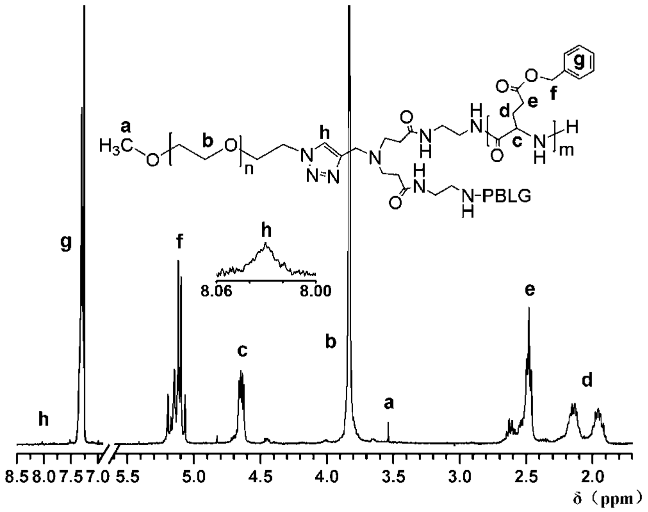


Figure 4. ${}^1H$ NMR spectrum of D1-PBLG-*b*-PEO ($CF_3COOD/CDCl_3$ = 1:1, v/v).

Furthermore, by taking into account the integral ratios of protons "b" or "a" to "c" (or "d", "e", "f"), the click efficiency is about 100% within the error of ${}^1H$ NMR measurement. That is to say, the click conjugation between D*m*-PBLG and PEO-N_3 precursors is quantitative. Meanwhile, the actual molecular weights and the compositions (PEO/PBLG wt %) of these block copolymers can be easily calculated from the integral ratios of the methylene protons of both blocks to methyl protons (Table 1). In all, these results indicate that the click conjugation between azide-terminated PEO and dendron-like D*m*-PBLG with an alkyne focal group provides a versatile strategy for the synthesis of dendron-like PBLG/linear PEO block copolymers with *asymmetrical topology*, as shown in Scheme 1A,B. As a note, the *symmetrical* dendron-like PBLG/linear PEO block copolymers (i.e., *BnABn* type D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG) can be click conjugated between D*m*-PBLG and bifunctional azide-terminated PEO under the excess of D*m*-PBLG homopolymers. However, in our experiments, the excess D*m*-PBLG homopolymer cannot be extracted from the resulting copolymers by solvent. Note that fractional precipitation and/or preparative GPC should be possible for obtaining the purified D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG copolymers via the "arm-first" method, which needs further investigation. On the other hand, the "core-first" strategy was developed for the synthesis of these symmetrical and asymmetrical block copolymers, as shown in Scheme 1C.

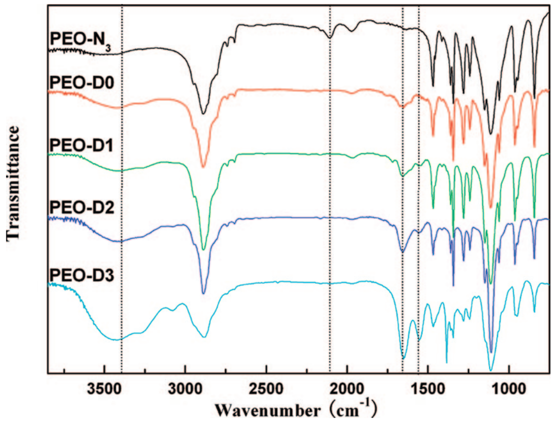


Figure 5. FT-IR spectra of PEO-N_3 and the PEO-D*m* macroinitiators.

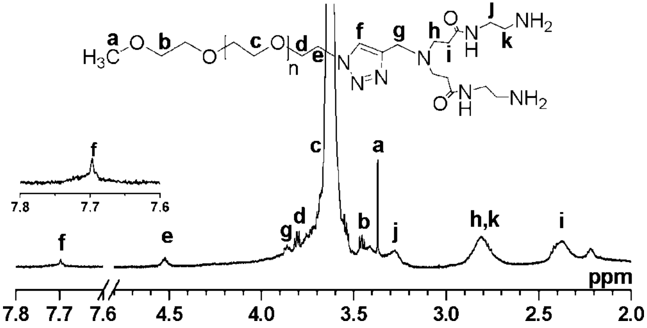


Figure 6. ${}^1H$ NMR spectrum ($CDCl_3$) of the PEO-D1 macroinitiator.

In the "core-first" strategy, the propargyl focal point D*m* with terminal primary amines groups were first click conjugated with PEO-N_3 to generate primary amine-terminated PEO-dendrons (i.e., PEO-D*m*), which were then used as the macroinitiators for the ROP of BLG-NCA to produce the targeted dendron-like PBLG/linear PEO block copolymers. Because both PEO-N_3 and D*m* are easily soluble in water, the PEO-D*m* was click conjugated between PEO-N_3 and D*m* using $CuSO_4 \cdot 5H_2O$/sodium ascorbate as catalyst in aqueous solution at room temperature. All the PEO-D*m* was confirmed by FT-IR and ${}^1H$ NMR spectroscopy. The FT-IR spectra show a complete disappearance of the azide vibrational peak at 2106 cm^{-1}, the amide I and II bands at 1650 and 1550 cm^{-1}, and a broad band (NH and NH_2) at about 3200−3600 cm^{-1} for D*m* (Figure 5). Moreover, the click reaction was confirmed from the appearance of new proton signals at 7.70 ppm (f, singlet) typical of methine proton of the triazole ring and at 4.53 ppm of methylene protons ($-CH_2-$) adjacent to triazole ring (Figure 6 and Figure S7 of the Supporting Information). Note that the methine proton of the triazole ring appeared at 7.70 ppm in $CDCl_3$ solvent in comparison with that at 8.0 ppm in mixed solvents (Figure 4, $CF_3COOD/CDCl_3$ = 1:1, v/v). Furthermore, by taking into account the integral ratios of protons "f" or both "h" and "k" to "c", the click conjugation between PEO-N_3 and dendrons D*m* is quantitative within the error of ${}^1H$ NMR measurement. These results indicate that PEO-N_3 was efficiently and quantitatively converted into PEO-D*m*, which are pure and can be used as the macroinitiators for the ROP of BLG-NCA monomer. The yield of ROP was high (80−90 wt %), and the PBLG branch length can be accurately controlled by the molar ratio of BLG-NCA monomer to PEO-D*m* macroinitiator. The molecular characteristics for the targeted D*m*-PBLG-*b*-PEO block copolymers

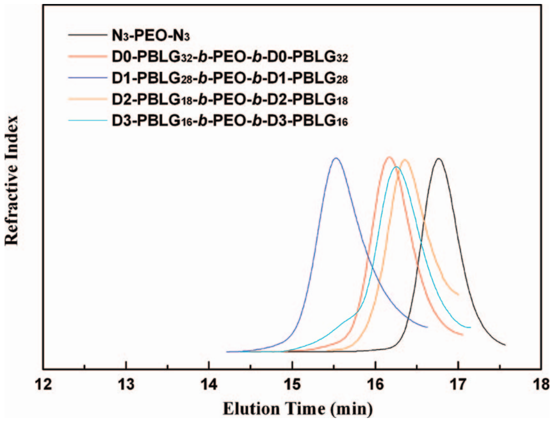


Figure 7. GPC traces of N_3-PEO-N_3 precursor and the D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG copolymers.

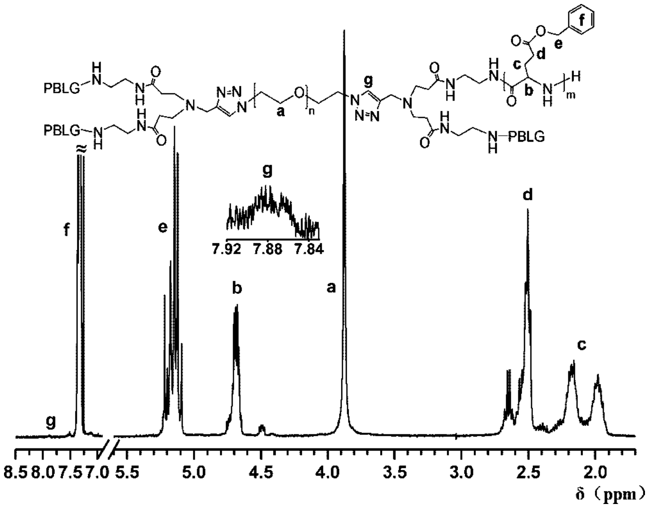


Figure 8. ^{1}H NMR spectrum ($CF_3COOD/CDCl_3$ = 1:1, v/v) of the D1-PBLG-*b*-PEO-*b*-D1-PBLG block copolymer.

synthesized via the "core-first" strategy are basically in agreement with those synthesized via the "arm-first" method. Similar results for *symmetrical* D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG were also obtained (Table 2). As shown in Figure 7, the unimodal elution peaks of the purified D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG block copolymers apparently shifted toward a lower elution time region with a narrow polydispersity compared with that of the original N_3-PEO-N_3 precursor, which convincingly verified the successful synthesis of the targeted block copolymers. As a representative example (Figure 8), ^{1}H NMR spectroscopy also confirmed the chemical structure and composition of these symmetrical block copolymers. In conclusion, these results indicate that both *asymmetrical and symmetrical* dendron-like PBLG/linear PEO biohybrids can be successfully synthesized by the combination of click chemistry and ROP based on the "arm-first" and "core-first" strategies (Scheme 1). Notably, these biohybrids can be further functionalized for the preparation of bioactive biomaterials because the multifunctional amine-terminated dendron-like PBLG segments should be easily conjugated with carboxylic acid, *N*-hydroxysuccinimide activated ester, and/or isothiocyanate derivatives of biological molecules (such as oligopeptides, biotin, and folic acid).[53−55]

FT-IR, DSC, and WAXD Analyses of Dendron-like PBLG/Linear PEO Block Copolymers. FT-IR can also characterize the secondary conformation structures of polypeptides within these D*m*-PBLG-*b*-PEO block copolymers. It is well-known that peaks assignable to the amide I band at ∼1650 cm^{-1} and amide II band at ∼1550 cm^{-1}, together with the bands at ∼1630 and ∼1530 cm^{-1}, are characteristic of the α-helix and *β*-sheet conformations of polypeptides with different chain lengths. Therefore, the PBLG segment within these copolymers mainly presented a α-helix conformation, as verified by the FT-IR analysis in Figure 3. This can be further clarified by the following DSC and WAXD analyses.

DSC was performed to characterize the thermal behavior and supramolecular organization of both PEO and PBLG segments within these D*m*-PBLG-*b*-PEO block copolymers in solid state, as shown in Figure 9 and Table 3. Comparing with the PEO-N_3 and D1-PBLG$_{41}$ precursors, all the D*m*-PBLG-*b*-PEO block copolymers (e.g., D0-PBLG$_{32}$-*b*-PEO, D1-PBLG$_{41}$-*b*-PEO, and D3-PBLG$_{11}$-*b*-PEO) showed the maximal melting temperature (T_m = 52−56 °C) for PEO block and the liquid crystalline phase transition temperature (T_{LC} = 100−105 °C) for PBLG block in the first heating runs. The T_{LC} transition with low enthalpy was induced by two stable helical conformations change from 7/2 α-helix to 18/5 α-helix for PBLG segment,[20,21,24,25,29] where both 7/2 and 18/5 α-helices, being stabilized by intramolecular hydrogen bondings, correspond to 7 amino acid residues in 2 turns and 18 residues in 5 turns, respectively. This also suggests that the PBLG block within these copolymers mainly assumed α-helical conformation, which is consistent with the above FT-IR analysis. Notably, this confomational transition is usually irreversible in homopolypeptides; that is to say, the transition would disappear in the second heating run during DSC experiment. A similar result was obtained for D1-PBLG$_{41}$ homopolymer, which only presented a T_{LC} of 103.5 °C in the first heating run. However, the T_{LC} transition of PBLG block within these D*m*-PBLG-*b*-PEO copolymers again appeared in the second heating runs, suggesting that the PBLG helical conformation transition was reversible. This phenomenon has been observed for linear PBLG-*b*-PEO and PBLG-*b*-poly(*ε*-caprolactone) block copolymers, which is probably attributed to the high mobility of PEO or poly(*ε*-caprolactone) with lower glass transition temperature.[20,29] Furthermore, the degree of crystallinity (X_c) of PEO block within copolymers decreased from 21.6% to 1.0% with increasing PBLG branches and PBLG composition, which demonstrates that the crystallinity of PEO block was progressively suppressed within these copolymers.

WAXD is another useful method to demonstrate the crystalline structure of PEO segment and the secondary conformation of PBLG segment within these block copolymers in solid-state, as shown in Figure 10. Comparing with the PEO-N_3 precursor, both D0-PBLG$_{32}$-*b*-PEO and D1-PBLG$_{41}$-*b*-PEO copolymers showed a similar diffraction peak at about 1.34 and 1.62 Å^{-1} for PEO segment, while the D3-PBLG$_{11}$-*b*-PEO copolymer did not give the crystalline diffraction peaks for PEO. This indicates that the crystallinity of PEO segment was heavily prohibited within D3-PBLG$_{11}$-*b*-PEO having eight PBLG branches and higher PBLG compositions, which is consistent with the above DSC analysis. Meanwhile, all these copolymers presented a diffraction peak at about 0.45 Å^{-1} for the PBLG segment, demonstrating that the PBLG segment within these copolymers mainly assumed a α-helix conformation.[20,21] These results are consistent with the above FT-IR and DSC analyses. Notably, the solid phase behavior and the superstructure of these D*m*-PBLG-*b*-PEO block copolymers, and the effects of both copolymer composition and dendron-like architecture on the hierarchical structures need further investigation, which is on going in our laboratory.

Self-Assembly of Amphiphilic D*m*-PBLG-*b*-PEO in Aqueous Solution. The critical aggregation concentration (cac) of amphiphilic block and graft copolymers was an important parameter for the thermodynamic stability of self-assembled aggregates in aqueous solution, which was measured by the dye

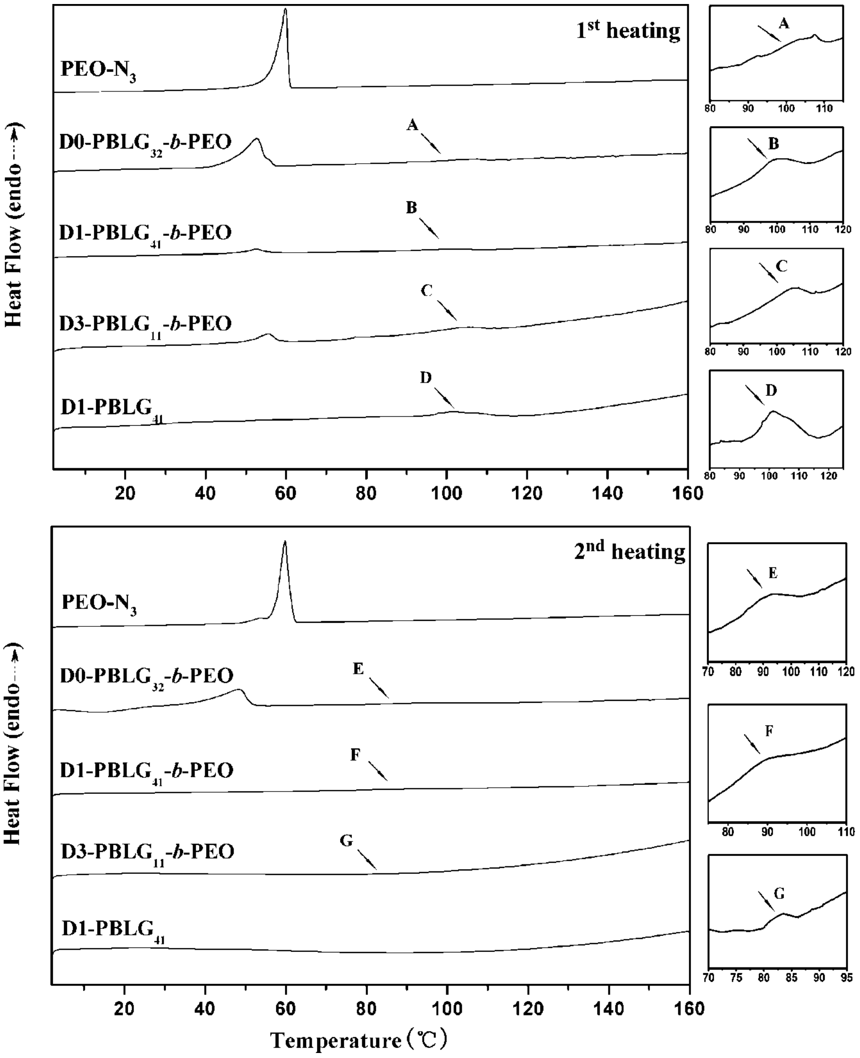


Figure 9. Differential scanning calorimetry (DSC) curves of PEO-N_3, D*m*-PBLG, and D*m*-PBLG-*b*-PEO in the first and the second heating runs, respectively (the letters A−G denote T_{LC}).

Table 3. Melting and Crystallization Properties of the Azide-Terminated PEO (PEO-N_3), D*m*-PBLG Precursors, and D*m*-PBLG-*b*-PEO Copolymers

entry	$T_{\mathrm{m,PEO}}$[a] (°C)	$T_{\mathrm{LC,PBLG}}$[b] (°C)	$\Delta H_{\mathrm{m,PEO}}$[c] (J/g)	$X_{\mathrm{C,PEO}}$[d] (%)
PEO-N_3	59.8		172.8	87.7
D1-$PBLG_{41}$		103.5 (0)		
D0-$PBLG_{32}$-*b*-PEO	52.7	104.8 (92.8)	42.5	21.6
D1-$PBLG_{41}$-*b*-PEO	52.6	100.0 (89.0)	5.5	2.8
D3-$PBLG_{11}$-*b*-PEO	55.4	104.3 (83.0)	2.0	1.0

[a] $T_{\mathrm{m,PEO}}$ denotes the maximal melting temperature of PEO in the first heating run. [b] $T_{\mathrm{LC,PBLG}}$ denotes the liquid crystalline phase transition temperature of PBLG in the first heating run and in the second heating run (i.e., parentheses values), respectively. [c] $\Delta H_{\mathrm{m,PEO}}$ denotes the fusion enthalpy of PEO in the first heating run. [d] $X_{\mathrm{C,PEO}}$ denotes the degree of crystallinity of PEO block within copolymers, where $X_{\mathrm{C}} = \Delta H_{\mathrm{m}}/\Delta H^{0}_{\mathrm{m,PEO}}$, $\Delta H^{0}_{\mathrm{m,PEO}} = 197.0$ J/g.

solubilization method.[56−59] As shown in Figure 11, the absorbance intensity of 1,6-diphenyl-1,3,5-hexatriene dye remained nearly constant below a certain concentration, and then it increased substantially, reflecting the incorporation of dye in the hydrophobic region of aggregates. The cac value was determined by intersecting the two straight lines, and the values of about $(3.4-5.0) \times 10^{-3}$ mg/mL were obtained for these copolymers. This suggests that the self-assembled aggregates are thermodynamically stable in aqueous solution.[56,57]

Both the morphology and the average size of the self-assembled nanostructures from these D*m*-PBLG-*b*-PEO copolymers were investigated by the techniques of TEM and dynamic light scattering (DLS), as shown in Figure 12 and Table S8 of the Supporting Information. To investigate the effect of both *dendron-like* architecture and the weight fraction of hydrophobic PBLG block on the aggregates' morphology, the molecular weight of hydrophilic PEO block was kept equal to 5000. For

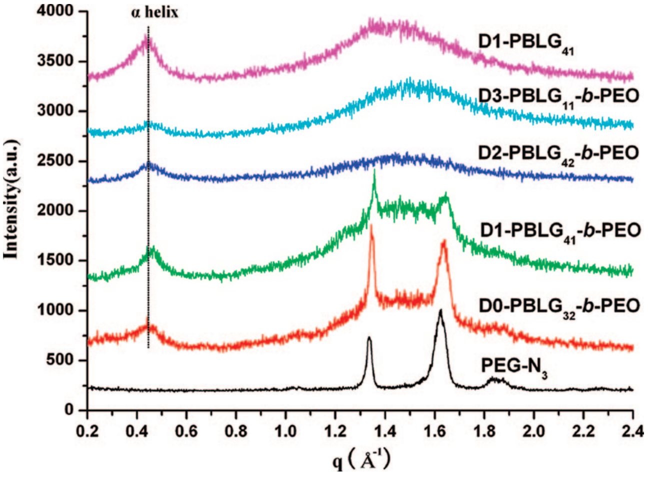


Figure 10. Wide-angle X-ray diffraction (WAXD) patterns of PEO-N_3, D*m*-PBLG, and D*m*-PBLG-*b*-PEO.

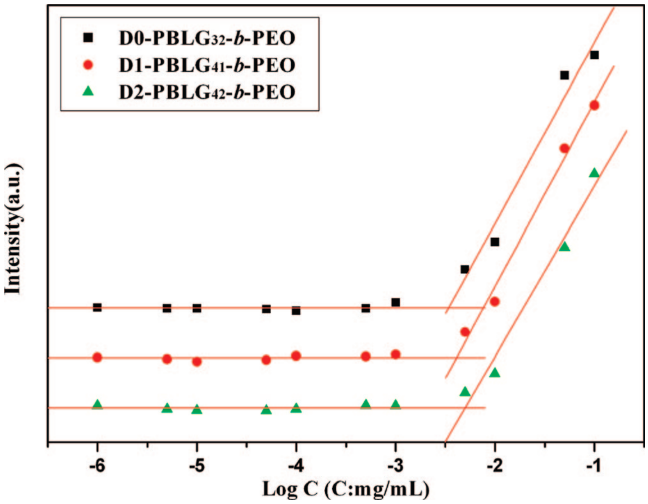


Figure 11. Relationship of the absorbance intensity of 1,6-diphenyl-1,3,5-hexatriene (DPH) as a function of the D*m*-PBLG-*b*-PEO copolymer concentration in aqueous solution at room temperature.

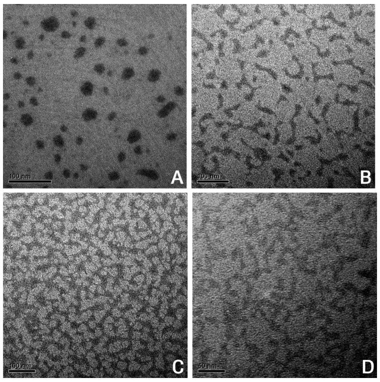


Figure 12. Transmission electron microscopy (TEM) photographs of the self-assembled nanostructures cast from these D*m*-PBLG-*b*-PEO copolymers aqueous solution: (A) D0-PBLG$_{18}$-*b*-PEO; (B) D0-PBLG$_{80}$-*b*-PEO; (C) D1-PBLG$_{41}$-*b*-PEO; (D) D2-PBLG$_{42}$-*b*-PEO.

the D0-PBLG-*b*-PEO copolymer, when the weight fraction of hydrophobic PBLG block was 44.1 wt % within the D0-PBLG$_{18}$-*b*-PEO sample, nearly spherical micelles with an average diameter of 66.7 ± 3.2 nm are shown in Figure 12A. This is similar to the conventional polymeric micelles (10−100 nm), suggesting that they had a helical polypeptide core surrounded by a biocompatible PEO corona. Note that the average size determined by DLS is bigger than that determined from TEM analysis, which can be attributed to the micelle dehydration induced by water evaporation under the high vacuum condition during TEM measurement.[60] However, when the weight fraction of PBLG block increased to 77.8 wt % within D0-PBLG$_{80}$-*b*-PEO, the wormlike micelles with few spherical micelles were obtained (Figure 12B). This result is attributed to both the decreased repulsion among the PEO corona and the increased surface tension resulting from the increased hydrophobicity−hydrophilicity balance.[56,58] As for both D1-PBLG$_{41}$-*b*-PEO and D2-PBLG$_{42}$-*b*-PEO ($f_{\mathrm{PBLG}} = 78-88$ wt %), the wormlike micelles were also generated in aqueous solution compared with D0-PBLG$_{80}$-*b*-PEO having similar PBLG composition (Figure 12C,D). These results demonstrate that the *dendron-like* topology has no apparent effect on the morphology of self-assembled nanostructures in *aqueous solution*, and the PBLG composition within copolymers mainly controlled the final morphology of nanostructures. This fact is rarely reported for the aqueous self-assembly of amphiphilic *dendron-like* copolymers, which provides important parameters for designing branched polypeptides-based copolymers for drug delivery.[56,57]

Conclusion

A versatile strategy to prepare dendron-like PBLG/linear PEO block copolymers with both *asymmetrical* and *symmetrical* topologies (i.e., *ABn* type D*m*-PBLG-*b*-PEO and *BnABn* type D*m*-PBLG-*b*-PEO-*b*-D*m*-PBLG; $n = 2^m$, $m = 0$, 1, 2, and 3) was successfully developed via the combination of ROP and click chemistry based on the “arm-first” and “core-first” strategies. In the arm-first method, the propargyl focal point dendrons D*m* having 2^m terminal primary amine groups were used for initiating the ROP of BLG-NCA monomer, generating “clickable” *dendron-like* D*m*-PBLG homopolymers having 2^m branches, which were then click conjugated with PEO-N$_3$ to produce the D*m*-PBLG-*b*-PEO hybrid copolymers with *asymmetrical* topology. In the core-first strategy, the propargyl focal point D*m* was first click conjugated with PEO-N$_3$ to generate primary amine-terminated PEO-D*m*, which were then used as the macroinitiators for the ROP of BLG-NCA monomer to produce the targeted copolymers with both *asymmetrical* and *symmetrical* topologies. The T_{LC} tranisition at about 100 °C of PBLG segment within these D*m*-PBLG-*b*-PEO copolymers was reversible, and the crystallinity of PEO block was progressively suppressed with the increasing PBLG branches and PBLG composition. Both spherical and wormlike micelles self-assembled from these D*m*-PBLG-*b*-PEO copolymers in aqueous solution, and the PBLG composition mainly controlled the morphology of nanostructures. To the best of our knowledge, this is the first report that describes the synthesis of *dendron-like* polypeptides/linear PEO block copolymers with both *asymmetrical* and *symmetrical* topologies via the combination of ROP and click chemistry.

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Supporting Information Available: Detailed synthesis for D*m*; ^{1}H NMR and ^{13}C NMR for D*m*, D*m*-PBLG, PEO-D*m*, D*m*-PBLG-*b*-PEO, and DLS for nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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