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One-Pot Syntheses of Amphiphilic Centipede-like Brush Copolymers via Combination of Ring-Opening Polymerization and "Click" Chemistry

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ABSTRACT: Amphiphilic centipede-like brush copolymers with biodegradable poly(ε-caprolactone) and poly(ethyl ethylene phosphate) side segments were prepared by a one-pot syntheses strategy. The syntheses combined ring-opening polymerization of 2-ethoxy-2-oxo-1,3,2-dioxaphospholane through a "grafting from" strategy and "click" reaction with α-propargyl-ω-acetyl-poly(ε-caprolactone) through a "grafting to" strategy, using multifunctional poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate) that bears hydroxyl and azide groups from junction points. The reactions are controllable, and the structure of obtained centipede-like brush copolymer is well characterized. These brush copolymers are amphiphilic and self-assemble into spherical micellar structure in aqueous solution with critical aggregation concentration around 10^{-3} mg mL^{-1} and average diameters of 50−90 nm. Such micelles formed from centipede-like brush copolymers can be used as drug carriers for biomedical applications.

Introduction

The preparation of structurally well-defined macromolecules such as star, block, miktoarm, and brush copolymers has gained increased attention in view of the ability of such polymers that could self-assemble into well-defined nano-objects,[1−3] which are potential for applications in the fields including the solubilizer,[4] drug delivery,[5−8] and microelectronics.[9] Brush copolymers are a special class of graft copolymers in which side chains are distributed densely on a polymer backbone, and because of their crowding arrangement, those side chains are stretched away from the backbone to form a brushlike or a wormlike cylindrical conformation.[2,10−12] Thus, they offer unique possibility of tailoring material properties through facile selection of the polymer backbone or the graft chains.[2,13−17] Brush copolymers are usually prepared by grafting-to, grafting-through, and grafting-from strategies.[2,3] Interest in brush copolymers with hydrophobic and hydrophilic grafts distributed on the same unit of the backbone, named amphiphilic centipede-like brush copolymers, is steadily growing.[18−20] Those polymers could self-assemble into a unimolecular micelle, first reported by Newkome and co-workers.[21] In other typical examples, Malmström et al. prepared unimolecular nanocontainers by ring-opening polymerization (ROP) and subsequent atom transfer radical polymerization (ATRP) from hydroxypropylcellulose with hydrophobic poly(ε-caprolactone) (PCL) and hydrophilic poly(acrylic acid) as the side chains.[22] Huang et al. prepared amphiphilic centipede-like brush copolymers with poly(ethylene glycol) and polystyrene as the side chains by two consecutive ATRP.[19] Xie et al. prepared well-defined centipede-like brush copolymers with amphiphilic PCL and poly(2-(dimethylamino)ethyl methacrylate) as the side chains by combination of ROP, ring-opening metathesis polymerization, and ATRP.[20] However, the studies of such grafted copolymers are still restrained due to the synthetic difficulties.

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One-pot reaction strategy has been widely applied in the synthesis of different polymer architectures,[23−28] which accelerates synthetic procedure and reduces the number of reactions as well as purification steps, therefore leading to more environment-friendly products. Recently, "click" chemistry has been utilized extensively in polymer and material science to construct various polymer architectures because of its high selectivity, near-perfect reliability, and high yield. Most importantly, it is exceptionally tolerant toward a wide range of functional groups and reaction conditions.[29,30] Such advantages have been applied for the synthesis of brush copolymers by combination of "click" chemistry with controlled/living polymerization previously.[18,31−35] In the present work, by combination of ROP and "click" chemistry, we report a facile and useful one pot reaction strategy for preparing centipede-like brush copolymers composed of PCL, poly(ethyl ethylene phosphate) (PEEP) side chains, using poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate) as the backbone. We selected PCL and PEEP as the side chains since PCL is a biocompatible and hydrophobic material being widely investigated in biomedical applications,[36] while PEEP is a typical polyphosphoester, which has received considerable attention in biomedical applications due to its biodegradability and good biocompatibility.[37,38] Combination of biocompatible and biodegradable PCL and PEEP segments into the unique architecture of centipede-like molecular polymer brushes will introduce versatile vehicles for drug delivery applications.

Experimental Section

Materials. 2-Ethoxy-2-oxo-1,3,2-dioxaphospholane (EEP) was synthesized by a method described previously and distilled under reduced pressure just before use.[39] Stannous octoate ($Sn(Oct)_2$) (Sinopharm Chemical Reagent Co., China) was purified according to a method described in the literature.[40] α-Propargyl-ω-acetyl-poly(ε-caprolactone) was prepared by ROP of ε-caprolactone under the co-initiation of propargyl alcohol and $Sn(Oct)_2$ followed by blocking of the hydroxyl end groups

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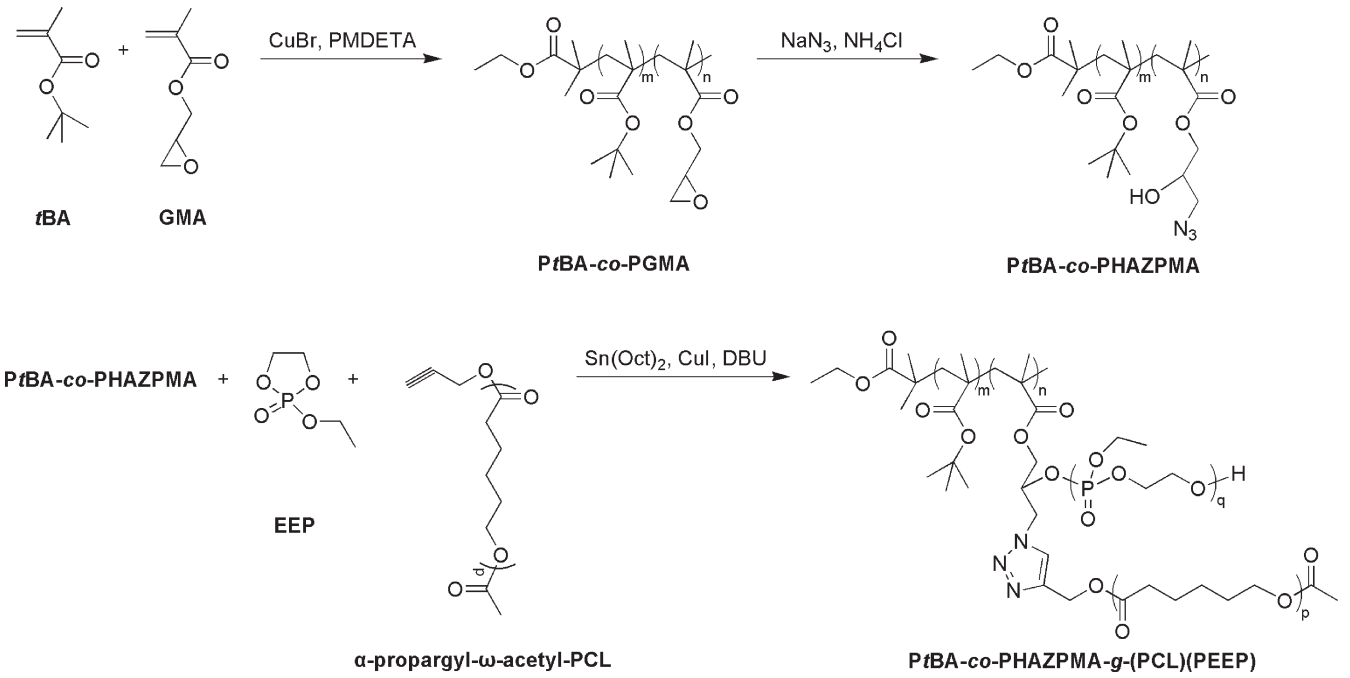
Scheme 1. Synthesis Route of the Amphiphilic Centipede-like Brush Copolymer P*t*BA-*co*-PHAZPMA-*g*-(PCL)(PEEP) by Combination of Ring-Opening Polymerization and "Click" Chemistry



of propargyl-terminated poly(ε-caprolactone) with acetyl chloride according to our previous report.[41] CuBr was purified by stirring in acetic acid and washing with methanol and then dried under vacuum. Ethyl 2-bromoisobutyrate (EBiB, 99%), *tert*-butyl methacrylate (*t*BA), and glycidyl methacrylate (GMA) were distilled under reduced pressure just before use. Pentamethyldiethylenetriamine (PMDETA) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) were from Alfa Aesar and used as received. Tetrahydrofuran (THF) and toluene were refluxed over potassium−sodium alloy under a N_2 atmosphere and distilled just before use. All other reagents and solvents were of analytical grade and used as received.

Synthesis of Poly(*tert*-butyl methacrylate)-*co*-poly(glycidyl methacrylate) (P*t*BA-*co*-PGMA) by ATRP. In a typical procedure to synthesize P*t*BA_{63}-*co*-$PGMA_{35}$, GMA (1.00 g, 7.04 mmol), *t*BA (3.00 g, 21.12 mmol), EBiB initiator (27.60 mg, 0.14 mmol), and diphenyl ether (2.00 mL) as the solvent were charged into a dry 25 mL Schlenk flask. The mixture was subjected to four freeze−pump−thaw cycles and then transferred into a glovebox (H_2O and O_2 contents <0.1 ppm), and CuBr (20.00 mg, 0.14 mmol) and PMDETA (24.20 mg, 0.14 mmol) were added. The solution was then immediately immersed into an oil bath set at 30 °C to start the polymerization under stirring. After 3 h, the mixture was diluted with THF and passed through a short neutral alumina column to remove the copper catalyst. The collected eluent was concentrated and precipitated into excess of diethyl ether twice. The product was obtained by filtration and dried under vacuum overnight with a yield of ca. 41%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 4.31 and 3.82 ($COOCH_2CH$), 4.13 (CH_3CH_2COO), 3.25 ($COOCH_2CH$), 2.65 and 2.84 ($COOCH_2CHCH_2O$), 2.00−2.38 ($-CH_2C(CH_3)$-$COO-$), 1.47 ($-C(CH_3)_3$), 0.81−1.22 (CH_3CH_2COO, $COOC$-$(CH_3)_2-$, $-CH_2CH(CH_3)COO-$).

Reaction of P*t*BA-*co*-PGMA with Sodium Azide in the Presence of Ammonium Chloride. P*t*BA_{63}-*co*-$PGMA_{35}$ (1.00 g, 2.52 mmol epoxide groups) was dissolved in *N,N*-dimethylformamide (DMF, 20 mL) and then sodium azide (0.49 g, 7.56 mmol), ammonium chloride (0.39 g, 7.56 mmol) were added to this solution. The mixture was stirred at 50 °C for 24 h. After the reaction, insoluble impurities were removed by filtration. Then the solution was concentrated and precipitate into diethyl ether. The desired polymer poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate) (P*t*BA-*co*-PHAZPMA) was obtained by filtration and dried overnight under vacuum, affording a yield of ca. 76%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 4.35−3.95 ($COOCH_2CH(OH)$, CH_3CH_2COO, $COOCH_2CH$-(OH)), 3.65 and 3.40 ($-CH_2N_3$), 2.00−2.38 ($-CH_2C(CH_3)$-$COO-$), 1.47 ($-C(CH_3)_3$), 0.81−1.22 (CH_3CH_2COO, $COOC$-$(CH_3)_2-$, $-CH_2CH(CH_3)COO-$).

One-Pot Syntheses of Centipede-like Brush Copolymers P*t*BA-*co*-PHAZPMA-*g*-(PCL)(PEEP) (Scheme 1). Typically, P*t*BA_{63}-*co*-$PHAZPMA_{35}$ (25 mg, 0.057 mmol of azide/hydroxyl groups) and α-propargyl-ω-acetyl-PCL_{41} (0.27 g, 0.057 mmol) were azeotropically distilled from toluene and dried under vacuum overnight. They were dissolved in anhydrous THF (3 mL), and the predetermined amount of EEP (0.43 mg, 2.85 mmol) was added. The mixture was degassed by five freeze−pump−thaw cycles, and then $Sn(Oct)_2$ (11.5 mg, 0.028 mmol), CuI (10.8 mg, 0.057 mmol), and DBU (8.6 mg, 0.057 mmol) were added in a glovebox (H_2O and O_2 contents <0.1 ppm). The reaction was carried out at 35 °C for 3 h. After the reaction, the solution was passed through a short neutral alumina column to remove the copper catalyst using THF as the eluent. The solution was then concentrated and poured into cold diethyl ether/methanol (v/v = 5:1), and the crude product was obtained by filtration. The crude product was loaded to a chromatography column with silica gel (200−300 mesh), and toluene was used to wash off the unreacted α-propargyl-ω-acetyl-PCL_{41}. The product was obtained by further washing with THF, following precipitation in cold diethyl ether and a drying process under vacuum, with a yield of ca. 37%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 7.62 (1H in triazole ring) 5.55 ($-CH_2-$triazole ring), 4.52 ($COOCH_2CH$), 4.40−4.20 (CH_3CH_2COO, $COOCH_2$-$CHCH_2-$, $-POCH_2CH_2O-$), 4.18 ($-OCH_2CH_3$), 4.05 ($-CO$-$CH_2CH_2CH_2CH_2CH_2O-$), 2.25−2.35 ($-CH_2C(CH_3)COO-$, $-COCH_2CH_2CH_2CH_2CH_2O-$), 2.03 ($OCOCH_3$), 1.64 ($-CO$-$CH_2CH_2CH_2CH_2CH_2O-$), 1.47−1.23 ($-COCH_2CH_2CH_2CH_2$-$CH_2O-$, $-OCHH_2CH_3$, $-C(CH_3)_3$), CH_3CH_2COO, $COOC$-$(CH_3)_2-$, $-CH_2CH(CH_3)COO-$).

Characterization. Number- and weight-average molecular weights (M_n and M_w) and molecular weight distributions (polydispersity index, PDI = M_w/M_n) were determined by gel permeation chromatography (GPC) measurements on a Waters

Table 1. Preparation and Characterization of Poly(*tert*-butyl methacrylate)-*co*-poly(glycidyl methacrylate) (P*t*BA-*co*-PGMA) and Poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate) (P*t*BA-*co*-PHAZPMA)

	P*t*BA-*co*-PGMA					P*t*BA-*co*-PHAZPMA		
no.	feed ratio of *t*BA/GMA/EBiB	DP_n of *t*BA/GMA[a]	M_n[a]	M_n[b]	PDI[b]	M_n[a]	M_n[b]	PDI[b]
1	150/50/1	63/35	13920	7780	1.22	15390	8830	1.25
2	75/25/1	38/15	7530	4040	1.19	8160	4310	1.26

[a] Calculated based on ^{1}H NMR analyses. DP_n is the degree of polymerization. [b] Determined by GPC analyses. *t*BA, GMA, and EBiB represent *tert*-butyl methacrylate, glycidyl methacrylate, and ethyl 2-bromoisobutyrate, respectively.

1515 GPC system, which was equipped with a Waters 2414 refractive index detector and three Waters Styragel high-resolution columns (HR4, HR2, and HR1, effective molecular weight range 5000−500 000, 500−20 000, and 100−5000, respectively). HPLC grade chloroform was purchased from J.T. Baker and used as the eluent at 40 °C, delivered at a flow rate of 1.0 mL min^{-1}. Monodispersed polystyrene standards obtained from Waters Co. were used to generate the calibration curve.

A Bruker AV300 NMR spectrometer was used for ^{1}H NMR analyses to determine the structure and composition of the polymers. FT-IR spectra were measured on a Bruker Vector 22 Fourier transform infrared spectrometer at wavenumbers 400−4000 cm^{-1} with a resolution of 2 cm^{-1} using the KBr disk method.

For cryo-TEM measurements, 10 μL of sample (1.0 mg mL^{-1}) was applied to a holey carbon film grid (R1.2/1.3 Quantifoil Micro Tools GmbH, Jena, Germany), and then the excess solution was absorbed by filter paper from the other side of the grid. Afterward, the grid was immediately plunged into precooled liquid ethane for flash freeze at −172 °C. The cryo-grid was held in a Gatan 626 cryo-holder and transferred into JEOL JEM-2010 transmission electron microscope with 200 kV LaB6 filament) at −172 °C. The micrographs were recorded by a Gatan 832 CCD camera at a magnification of 6000−50 000× and at the defocus of 3−5.46 μm.

The size and size distribution of micelles in aqueous solution were measured by dynamic light scattering carried out on a Malvern Zetasizer Nano ZS90 with a He−Ne laser (633 nm) and 90° collecting optics. All samples were prepared in aqueous solution at a concentration of 1.0 g L^{-1} and filtered through Millipore 0.45 μm filter prior to measurements. All measurements were carried out at 25 °C, and data were analyzed by Malvern Dispersion Technology Software 4.20.

Laser light scattering was conducted on a commercial spectrometer (ALV/DLS/SLS-5022F) equipped with an ALV5000 multi-τ digital time correlator and a cylindrical 22 mW UNIPHASE He−Ne laser (λ_0 = 632 nm) as the light source to characterize the polymeric micelles (1.0 mg mL^{-1}) at a scattering angle of 90° and at a constant temperature of 25 °C. Static light scattering (SLS) studies were conducted at 25 °C using the same instrument at scattering angles ranging from 0° to 150°. The micelle solution was filtered using a 0.45 μm membrane filter prior to measurement for all of the experiments.

Preparation of Micelles of Centipede-like Brush Copolymers. Micelles were prepared by the dialysis method. Briefly, 10 mg of centipede-like brush copolymer P*t*BA-*co*-PHAZPMA-*g*-(PCL)-(PEEP) was dissolved in 1 mL of DMF. A predetermined volume of Milli-Q water (Millipore Milli-Q Synthesis, 18.2 MΩ) was added dropwise under gentle stirring. After the mixture was stirred for another 2 h, DMF was removed by dialysis (MWCO 2000) against water at ambient temperature overnight. The final volume of the aqueous solution was adjusted to 10 mL using Milli-Q water to obtain the desired concentration of polymer micelles.

Determination of Critical Aggregation Concentration (CAC) of Centipede-like Brush Copolymers. Critical aggregation concentration of the centipede-like brush copolymers were estimated by the fluorescence spectroscopy method using pyrene as the fluorescence probe. A predetermined amount of pyrene solution in acetone was added into a series of volumetric flasks, and the acetone was then evaporated completely overnight at ambient temperature. A series of micelle solutions at different concentrations ranging from 1.0×10^{-5} to 0.5 mg mL^{-1} were added to the flasks, while the concentration of pyrene in each flask was fixed at a constant value (6.0×10^{-7} mol L^{-1}). The excitation spectra were recorded at 25 °C on a Shimadzu RF-5301PC spectrofluorophotometer with the maximum emission wavelength at 390 nm and a slit width of 5 nm.

Results and Discussion

Synthesis of P*t*BA-*co*-PHAZPMA (Scheme 1). "Click" chemistry, mainly denoted as the 1,3-dipolar cycloaddition reaction between azide and alkynes, serves as a powerful strategy in preparation of polymers with various architectures.[29] The synthesis of polymer containing azide groups for further modification is still challenging. Reaction of polymers containing epoxide groups with sodium azide at relatively moderate reaction conditions is one of the most convenient routes for preparation of polymers with azides group.[42,43] Herein, to get well-defined multifunctional macroinitiator for amphiphilic centipede-like brush copolymer syntheses by combination of ring-opening polymerization and "click" chemistry, we prepared random precursor copolymer P*t*BA-*co*-PGMA via ATRP. Following ring-opening reaction of epoxide of P*t*BA-*co*-PGMA with sodium azide in the presence of ammonium chloride according to literatures,[41,42] we obtained P*t*BA-*co*-PHAZPMA copolymers, which contain both hydroxyl and azide groups from junction points.

GMA is a functionalizable vinyl monomer that contains epoxy group. In this contribution, we used CuBr/PMDETA as the catalyst and EBiB as the initiator in the polar solvent of diphenyl ether to synthesize P*t*BA-*co*-PGMA via ATRP. PMDETA is a triamine which will not react with GMA monomer, thus avoid branching or even cross-linking during the polymerization. The mixture was lightly green and homogeneous during the polymerization. The reaction was terminated by exposing the mixture to the air. After passing the mixture through a short neutral alumina column, the eluent was concentrated and precipitated into diethyl ether to obtain P*t*BA-*co*-PGMA.

As summarized in Table 1, two P*t*BA-*co*-PGMA copolymers with different compositions were synthesized. Figure 1 shows the comparison of GPC curves of $PtBA_{63}$-*co*-$PGMA_{35}$ (A) and $PtBA_{38}$-*co*-$PGMA_{15}$ (B), where the subscripts represent the degree of polymerization (DP) of each monomer. Both show a single peak with narrow PDIs around 1.20, indicating the well-control of polymerization. A typical ^{1}H NMR spectrum of $PtBA_{63}$-*co*-$PGMA_{35}$ in $CDCl_3$ is given in Figure 2A. The DPs were calculated according to the relative integration ratio of proton signals at 4.13 (*b*), 3.25 (*m*), and 1.47 (*h*) ppm, assigned to the methylene protons of the initiator (CH_3CH_2O−), methine protons of PGMA (C*H*−O from the epoxide ring), and methyl protons of P*t*BA (−C(CH_3)$_3$). The molecule weights

determined by GPC in chloroform using polystyrene as the standard were smaller than those calculated based on ^{1}H NMR analyses, which were also observed by other study.[44]

The epoxide groups of P*t*BA-*co*-PGMA were then opened with sodium azide. Figure 1C,D shows the GPC curves of obtained P*t*BA-*co*-PHAZPMA copolymers, which exhibits similar pattern with that of precursor P*t*BA-*co*-PGMA copolymers. The PDIs only increased very slightly to 1.25 and 1.26 as summarized in Table 1, which were in accordance with the previous report.[42] In addition, the singlet peak indicates no coupling or cross-linking happened during the opening of the epoxide groups. The opening of epoxide groups by sodium azide led to multifunctional structure with reactive azide and hydroxyl groups, which can be used for "click" chemistry and ring-opening polymerization of EEP, respectively. As shown in Figure 2B, proton signals from epoxide ring (*m*, *n*) disappeared completely. Instead, newly formed resonances at 3.40, 3.65 ppm (*t*, $-CH_2N_3$) and enhanced resonance at 4.12 ppm (*r* and *s*, $-O-CH_2-CH(OH)-CH_2-$) demonstrated the successful transformation of epoxide to azide and hydroxyl groups. On the other hand, from the FT-IR spectra shown in Figure 3, P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$ exhibited a typical absorbance at 2106 cm^{-1}, which is the characteristic absorbance of azide groups. It is not observed in the spectrum of P*t*BA$_{63}$-*co*-PGMA$_{35}$, further demonstrating the efficient epoxide ring-opening.

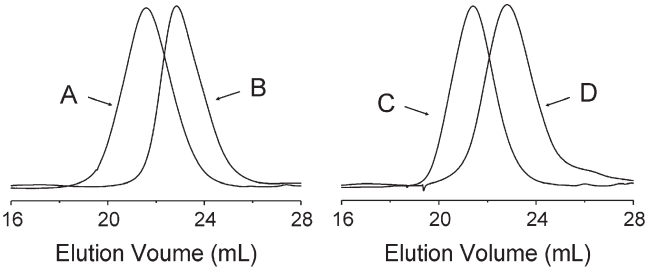


Figure 1. Gel permeation chromatography measurements of poly(*tert*-butyl methacrylate)-*co*-poly(glycidyl methacrylate) and poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate): (A) P*t*BA$_{63}$-*co*-PGMA$_{35}$, (B) P*t*BA$_{38}$-*co*-PGMA$_{15}$, (C) and P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$, and (D) P*t*BA$_{38}$-*co*-PHAZPMA$_{15}$.

One-Pot Preparation of Centipede-like Brush Copolymer P*t*BA-*co*-PHAZPMA-*g*-(PCL)(PEEP) (Scheme 1). One-pot reaction strategy has been widely applied in the synthesis of varies architectures of polymers,[23−28] which accelerates synthetic procedure and reduces the number of reactions as well as purification steps, therefore leading to more environment-friendly products. As an example, triblock copolymers have been synthesized via in situ "click" [3 + 2] and Diels−Alder [4 + 2] reactions via a one-pot reaction.[23] Graft copolymers have also been synthesized in one pot by combination of free radical polymerization and polyaddition.[28]

In this study, we used two α-propargyl-ω-acetyl-PCL polymers with different molecule weights, which were prepared as previously reported.[41] The DPs of PCL are 41 and 21, respectively, while the polydispersities are 1.11 and 1.14 as determined by GPC analyses. We then performed the one-pot reactions to synthesize the centipede-like brush copolymers using multifunctional macroinitiator P*t*BA-*co*-PHAZPMA for EEP ring-opening polymerization through a "grafting from" strategy and "click" reaction of α-propargyl-ω-acetyl-PCL through a "grafting to" strategy. The reactions were catalyzed by Sn(Oct)$_2$ for ROP and CuI/DBU for "click" reaction in THF. After the reaction, centipede-like brush copolymers were recovered by precipitation of the mixture into diethyl ether/methanol (v/v = 5:1), following the removal of unreacted α-propargyl-ω-acetyl-PCL via column chromatography using toluene as the eluent.

The successful syntheses of centipede-like brush copolymers were demonstrated by GPC and ^{1}H NMR analyses. As shown in Figure 4, GPC curves of centipede-like brush copolymer shifts to higher molecular weight direction compared to that of precursor polymer, namely, α-propargyl-ω-acetyl-PCL$_{41}$ or P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$ with slightly increased polydispersity. The molecule weights and PDI are summarized in Table 2. On the other hand, the ^{1}H NMR spectrum of P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$)(PEEP) (1) is given in Figure 5 as an example to demonstrate the chemical structure. Characteristic proton signals of PCL and PEEP segments were assigned. More importantly, resonances at 7.62 (*s*) and 5.55 ppm (*n*) were clearly observed, which should be assigned to the protons of the 1,2,3-trizole

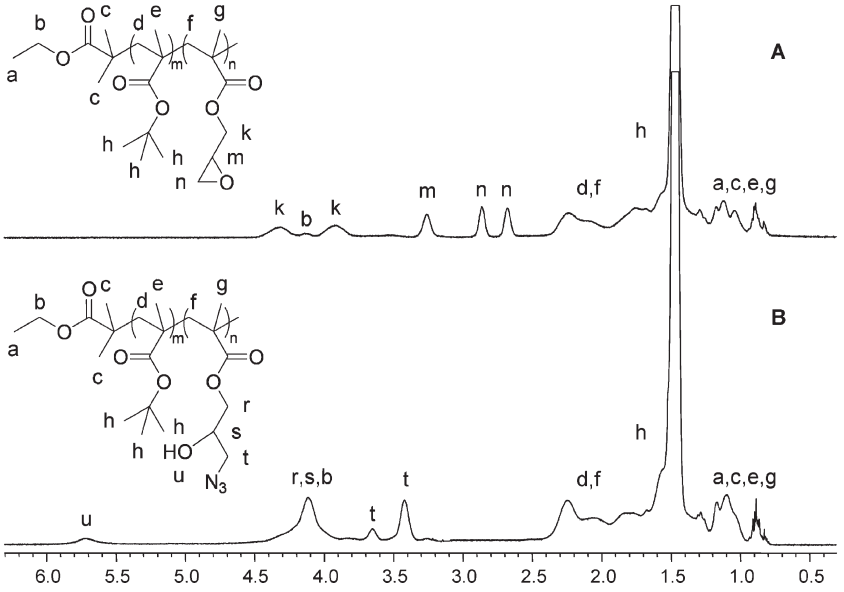


Figure 2. Representative ^{1}H NMR spectra of poly(*tert*-butyl methacrylate)-*co*-poly(glycidyl methacrylate) (A: P*t*BA$_{63}$-*co*-PGMA$_{35}$) and poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate) (B: P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$) in $CDCl_3$ (ppm).

ring and the methylene protons of PCL conjoint to the 1,2,3-trizole ring, demonstrating the successful performance of "click" reaction. Besides, characteristic signals of PEEP segments were also observed at 1.37 (*q*), 4.18 (*p*), and 4.26 ppm (*y*), which should be assigned to the pendent methyl ($-CH_2CH_3$), methylene ($-OCH_2CH_3$) protons, and methylene protons ($-POCH_2CH_2O-$) of PEEP, respectively. Because of the initiation of the EEP monomer, the methine protons at the junction point ($CH-O-$) should shift to the low field, which could be assigned to the signals at 4.52 ppm (*k*).

The average graft efficiency of α-propargyl-ω-acetyl-PCL to the backbone of centipede-like brush copolymers was calculated based on the resonance intensities of methylene protons of α-propargyl-ω-acetyl-PCL (*r*) and methine protons at the junction point (*k*, $CH-O-$) according to the following equation:

$$EF_{(click\ \%)} = (I_{r,d,f} - 4I_k)/2DP_{NMR}(PCL)(I_k)$$

where $DP_{NMR}(PCL)$ is the degree of polymerization of PCL in α-propargyl-ω-acetyl-PCL polymer. $I_{r,d,f}$ and I_k are the relative integrations of resonances at 2.25−2.35 and 4.52 ppm, respectively. The graft efficiency was in the range of 47−60% as calculated based on the above equation, while previous "click" reaction between azide and alkyne with similar structures showed comparable graft efficiency.[42,45,46] The inability of all the azide groups in P*t*BA-*co*-PHAZPMA copolymers to participate in the 1,3-dipolar cycloaddition reaction might be attributed to several factors such as the spatial separation between reactive groups on the backbone and the dimensions of the grafted polymer.

Moeller et al. compared the chemical and enzymatic catalyzed ring-opening polymerization of ε-caprolactone using polyglycidols as the macroinitiators and found that for the enzymatically catalyzed polymerization only 15−20% of the hydroxyl groups could initiate the polymerization, while with the chemically catalyzed polymerization, all the hydroxyl groups of the polyglycidols could initiated polymerization.[47] So herein, we assumed that all of the hydroxyl groups of P*t*BA-*co*-PHAZPMA could initiate the polymerization of EEP in the presence of stannous octoate; therefore, the DP of PEEP is calculated by the intensity of the pendent methylene ($-OCH_2CH_3$) protons and methylene protons ($-POCH_2CH_2O-$) of PEEP backbone by comparison with that of methine protons at the junction point ($CH-O-$) according to the

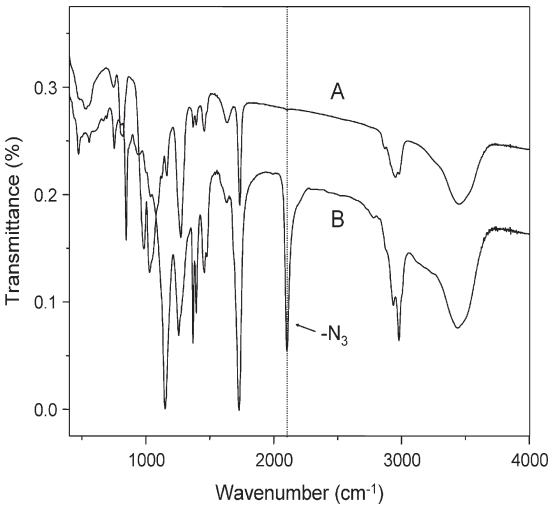


Figure 3. Comparison of FT-IR spectra of poly(*tert*-butyl methacrylate)-*co*-poly(glycidyl methacrylate) and poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate): (A) P*t*BA_{63}-*co*-$PGMA_{35}$; (B) P*t*BA_{63}-*co*-$PHAZPMA_{35}$.

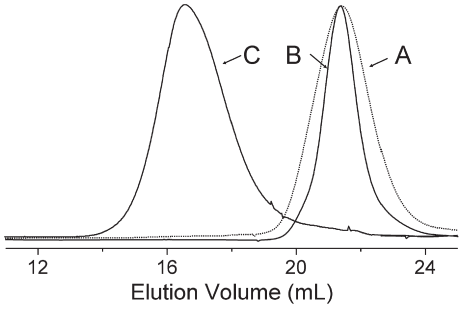


Figure 4. Gel permeation chromatography measurements of the precursor polymers and the centipede-like brush copolymer: (A) P*t*BA_{63}-*co*-$PHAZPMA_{35}$, (B) α-propargyl-ω-acetyl-PCL_{41}, and (C) P*t*BA_{63}-*co*-$PHAZPMA_{35}$-*g*-(PCL_{41})(PEEP) (1).

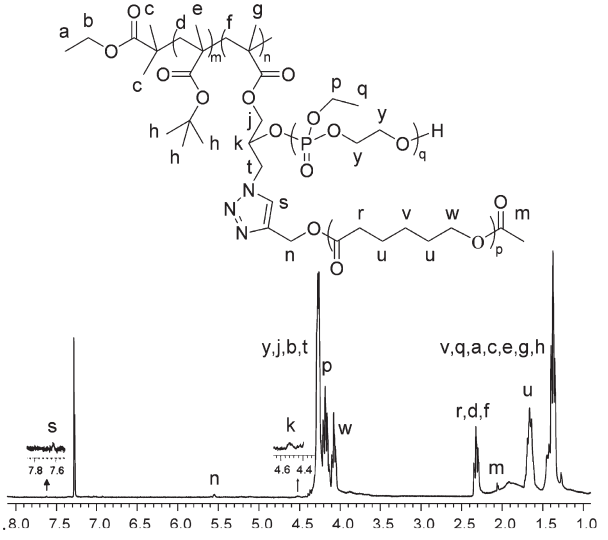


Figure 5. ^1H NMR spectrum of the centipede-like brush copolymer P*t*BA_{63}-*co*-$PHAZPMA_{35}$-*g*-(PCL_{41}) (PEEP) (1) in $CDCl_3$ (ppm).

Table 2. Characterization of P*t*BA-*co*-PHAZPMA-*g*-(PCL)(PEEP) Centipede-like Brush Copolymers

code	$[M]_0/[I]_0$[a]	M_n[b]	M_n[c]	PDI[c]	DP[d]	EF[e] (%)
P*t*BA_{63}-*co*-$PHAZPMA_{35}$-*g*-(PCL_{41})(PEEP) (1)	50	194 800	106 600	1.45	18	52
P*t*BA_{63}-*co*-$PHAZPMA_{35}$-*g*-(PCL_{41})(PEEP) (2)	100	223 800	121 100	1.64	25	47
P*t*BA_{63}-*co*-$PHAZPMA_{35}$-*g*-(PCL_{21})(PEEP)	50	149 300	63 400	1.52	16	60
P*t*BA_{38}-*co*-$PHAZPMA_{15}$-*g*-(PCL_{41})(PEEP)	50	80 300	41 200	1.42	15	55

[a] $[M]_0$ and $[I]_0$ are the initial concentrations of 2-ethoxy-2-oxo-1,3,2-dioxaphospholane (EEP) and hydroxyl groups of poly(*tert*-butyl methacrylate)-*co*-poly(2-hydroxy-3-azidopropyl methacrylate), respectively. [b] Calculated based on ^1H NMR analyses. [c] Determined by GPC analyses. [d] DP is the average degree of polymerization per poly(ethyl ethylene phosphate) (PEEP) block, calculated by ^1H NMR analyses. [e] Average graft efficiency of α-propargyl-ω-acetyl-poly(ε-caprolactone), calculated based on ^1H NMR analyses.

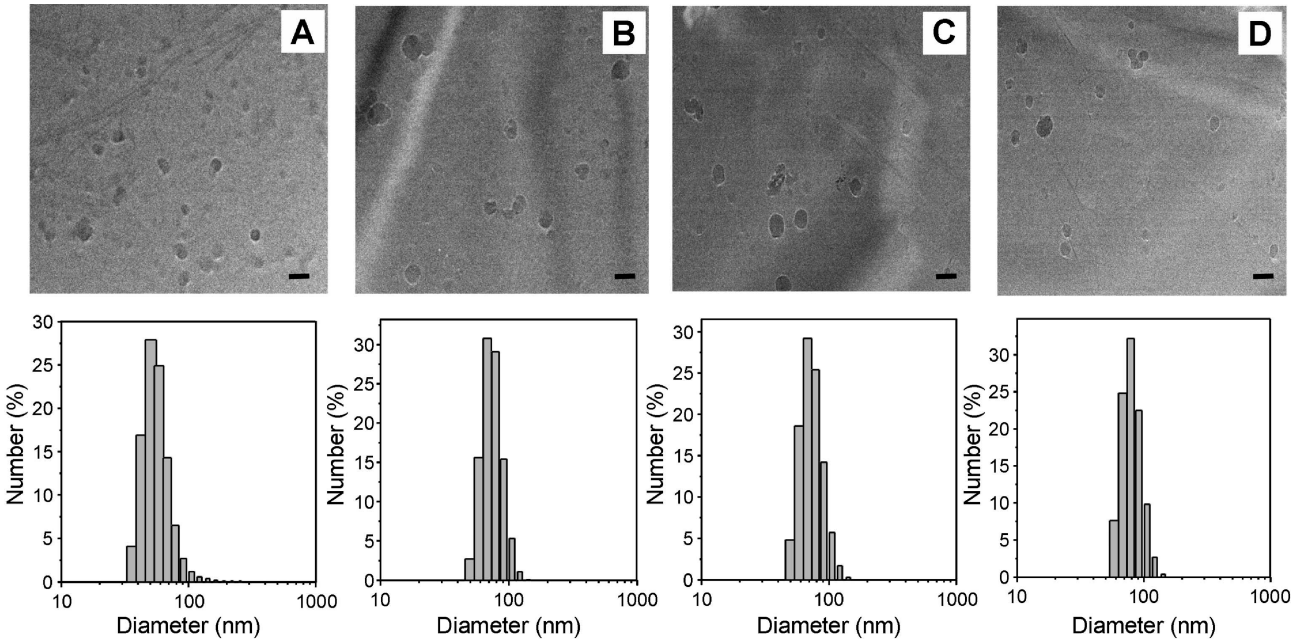


Figure 6. Cryo-TEM images and size distributions of centipede-like brush copolymer micelles: (A) P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$)(PEEP) (1); (B) P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$)(PEEP) (2); (C) P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{21}$)(PEEP); (D) P*t*BA$_{38}$-*co*-PHAZPMA$_{15}$-*g*-(PCL$_{41}$)-(PEEP). Scale bar = 100 nm.

Table 3. Properties of Micelles Formed from P*t*BA-*co*-PHAZPMA-*g*-(PCL)(PEEP) Centipede-like Brush Copolymers in Aqueous Solution

code	CAC[a] (mg L^{-1})	D_h[b] (nm)	polydispersity[b]	N_{agg}[c]
P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$)(PEEP) (1)	3.7	66.3	0.281	14
P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$)(PEEP) (2)	6.1	74.9	0.248	17
P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{21}$)(PEEP)	4.2	71.2	0.252	24
P*t*BA$_{38}$-*co*-PHAZPMA$_{15}$-*g*-(PCL$_{41}$)(PEEP)	3.1	76.5	0.221	44

[a] The critical aggregation concentration (CAC) is determined by fluorescence technique using pyrene as a fluorescence probe. [b] Hydrodynamic diameter (D_h) and polydispersity of micelles determined by dynamic light scattering. [c] N_{agg}: the average aggregation number of brush copolymer molecules in the micellar structure.

following equation:

$$DP_{NMR}(PEEP) = (I_{y,j,b,t} + I_p - 4I_k)/(6I_k)$$

where I_k and $I_{y,j,b,t}$, I_p are the integrals of the resonances at 4.52 and 4.10−4.40 ppm, respectively. Lower conversion of EEP monomer (around 30%) with such multifunctional macroinitiator compared to our previous linear initiator is mostly due to the higher steric hindrance.[48] The molecule weights of obtained brush copolymers were also calculated based on ^{1}H NMR analyses and given in Table 2. The values were larger than that determined by GPC analyses, likely due to its brush structure as reported by other groups.[18,46]

Self-Assembly of Centipede-like Brush Copolymers in Aqueous Solution. Similar to many other amphiphilic copolymers, centipede-like brush copolymer with two amphiphilic side chains could also self-assemble into micellar-like structure in aqueous solution.[19] With hydrophilic PEEP and hydrophobic PCL segments, P*t*BA-*co*-PHAZPMA-*g*-(PCL)-(PEEP) brush copolymers self-assembled into micellar structure in aqueous solution, demonstrated by cryo-TEM measurements. It is believed that hydrophobic PCL segments are locked in the inner core, while PEEP chains form the corona shell due to the more hydrophilic nature. The micellar structures formed by these brush copolymers with spherical morphologies and the size distributions are shown in Figure 6. From the cryo-TEM images, we can find that the micelles show a diameter ranged from 50 to 90 nm in average, while DLS analyses showed relative narrow size distributions. The average aggregation number of the brush copolymer molecules in the micellar structure was determined by SLS and calculated using equation $N_{agg} = M_{w,micelle}/M_{w,unimers}$, which are listed in Table 3, where $M_{w,micelle}$ and $M_{w,unimers}$ are the micelle molar mass determined by SLS and weight-average molecular weight of the individual brush copolymer determined by GPC, respectively.[49] Hadjichristidis et al. reported that the aggregation number of micelles formed by brush copolymers decreases as the number of branches increases.[50] Similar results were observed in this study, which should be due to the complex structure of brush polymer compared with linear block copolymer.

The CAC value is a strong evidence for self-assembly of an amphiphilic copolymer into micellar structures. It is also an important parameter for biomedical applications in drug delivery to estimate the *in vivo* performance while injected into the body when diluted with the large volume of blood (5−6 L per 50−60 kg human body). Polymeric micelles with lower CAC values are usually more suitable in drug delivery application because they will remain stable after being administrated with the dilution of the blood. CAC of these centipede-like brush polymers in aqueous solution were determined by fluorescence technique using pyrene as the probe according to a reported method.[51] The fluorescence spectrum of pyrene is sensitive to the environment and the polarity of its surrounding. As shown in Figure 7, as the concentration of the copolymer increased, the intensity ratio started to increase dramatically, reaching the characteristic

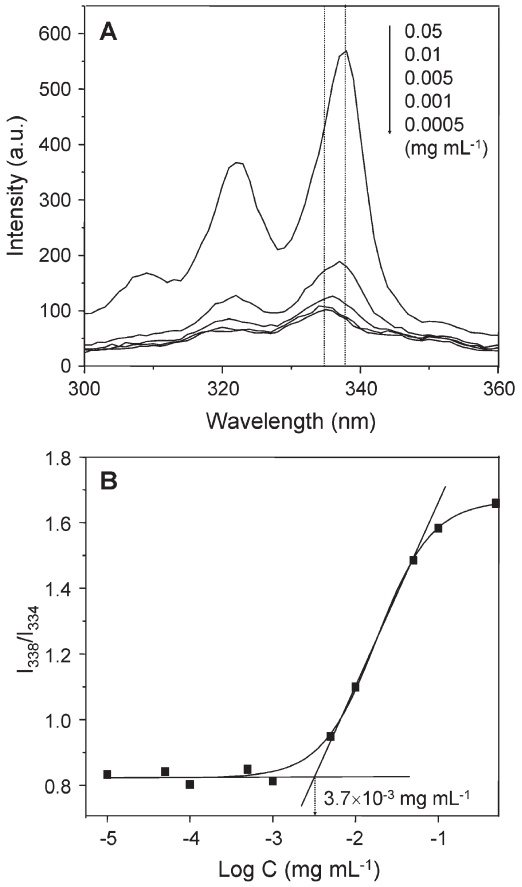


Figure 7. Excitation spectra of pyrene at 390 nm with P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$) (PEEP) (1) at various concentrations (A) and plot of the I_{338}/I_{334} ratio from pyrene excitation spectra versus log *C* of P*t*BA$_{63}$-*co*-PHAZPMA$_{35}$-*g*-(PCL$_{41}$) (PEEP) (1) (B).

of pyrene entirely in a hydrophobic environment at certain copolymer concentrations. The red-shift results from the transfer of pyrene molecules from a water environment to the hydrophobic micellar core and thus provides information on the location of the pyrene probe in the system, in fact, also indicating the formation of micelles. From the sigmoidal shape curve shown in Figure 7, CAC of the brush copolymers were determined and summarized in Table 3.

The CAC values of the brush copolymers are around 10^{-3} mg mL^{-1}. The lower CAC values of P*t*BA-*co*-PHAZPMA-*g*-(PCL)(PEEP) are related with the branched structure of graft copolymers and the compositions of the hydrophobic and hydrophilic blocks. It is reasonable that the higher content of the hydrophobic segments will result in stronger interactions between each other, leading to a more stable micellar structure and, therefore, to lower CAC value. On the other hand, the intramolecular interaction of PCL side chains may also decrease the CAC value. With such low CAC values, amphiphilic centipede-like copolymers can form highly stable micellar aggregates with low rates of dissociation *in vivo*.

Conclusions

Amphiphilic centipede-like brush copolymers with P*t*BA-*co*-PHAZPMA as the backbone and hydrophobic PCL and hydrophilic PEEP as the side chains have been prepared by a one-pot syntheses method by combination of ROP and "click" chemistry methods. Azide and hydroxyl groups on each repeating unit of PGMA are used for "click" chemistry with α-propargyl-ω-acetyl-PCL and ring-opening polymerization of EEP, respectively. The polymerization is controllable, and the structures of brush polymers have been well characterized. Such centipede-like brush copolymers containing both hydrophobic and hydrophilic side segments form spherical micelles in aqueous solution, with average diameters in the range of 50−90 nm, demonstrated by cryo-TEM and DLS measurements. With biocompatible and biodegradable PCL and PEEP side segments and self-assembly ability in aqueous solution at lower CAC ($\sim 10^{-3}$ mg mL^{-1}), these amphiphilic centipede-like brush copolymers can be used for biomedical applications particularly in drug delivery.

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