Macromolecules
ARTICLE

Macromolecules **2010**, *43*, 6671–6677 **6671**
DOI: 10.1021/ma100653u

Miktoarm Copolymers Bearing One Poly(ethylene glycol) Chain and Several Poly(ε-caprolactone) Chains on a Hyperbranched Polyglycerol Core

Xiaojin Zhang, Juan Cheng, Qingrong Wang, Zhenlin Zhong,* and Renxi Zhuo*

Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, Wuhan 430072, P. R. China

Received March 26, 2010; Revised Manuscript Received July 11, 2010

ABSTRACT: Amphiphilic miktoarm copolymers mPEG-(*hb*-PG)-*g*-PCL bearing one monomethoxy poly(ethylene glycol) (mPEG) chain and several linear poly(ε-caprolactone) (PCL) chains on a hyperbranched polyglycerol (*hb*-PG) core were designed and synthesized via a combination of anionic polymerization of glycidol and ring-opening polymerization of ε-caprolactone (CL). The polymers were characterized by ^{1}H NMR and gel permeation chromatograph (GPC). The amphiphilic miktoarm copolymers could form stable micelle solutions by adding water to a THF solution of the polymer followed by removal of the organic solvent by dialysis. Dynamic light scattering (DLS) measurements showed that the micelles had a narrow unimodal size distribution. Transmission electron microscopy (TEM) images displayed that the micelles were in regular spherical shape with narrow size distribution. The properties of the copolymers as drug carriers were investigated with prednisone acetate as a model drug. Linear mPEG-*b*-PCL block copolymers with similar composition were synthesized and used for comparison to investigate the effects of the hyperbranched architecture. The drug loading capacity (DLC) and entrapment efficiency (EE) of amphiphilic hyperbranched miktoarm copolymers mPEG-(*hb*-PG)-*g*-PCL were higher than those of amphiphilic linear block copolymers mPEG-*b*-PCL. Further, the amphiphilic hyperbranched miktoarm copolymers had more sustained drug release behavior.

Introduction

Amphiphilic block copolymers composed of hydrophilic and hydrophobic blocks can form micelles with a core−shell structure by self-assembly in solvents that are selective for only one of the blocks.[1−5] Depending on the nature of the block copolymer, the length of the blocks, the temperature, and the solvent quality,[6−12] the micelles might be spherical, cylindrical, toroidal, helical, worm-like, or vesicle-like.[13−17] Block copolymers could be used as stabilizing agents of emulsion, surfactants, and drug carriers.[18]

Linear−dendritic block copolymers with a hybrid structure can increase capacity and chemical flexibility of copolymer micelles due to the advantages conferred from the dendritic architecture that has inner porosity and dense surface functionality.[19] The micellar characteristics are highly dependent on the generation of the dendritic units.[20] The rate of degradation increases with the increase of arm numbers or the decrease of arm lengths[21] and the generation order can affect self-assembled morphology.[22] However, the synthetic route based on multistep approaches is tedious, and it is hard to afford very high purity materials, especially for the preparation of high-generation dendrimers.[23] Therefore, a facile method to prepare linear−dendritic-like block copolymers should be attractive. Although the structure of hyperbranched polymers is not as perfect as dendrimers, the preparation is much more facile and their properties are similar. Linear−hyperbranched block copolymers with novel structures such as double-hydrophobic,[24−26] double-hydrophilic,[27−29] and amphiphilic,[30−34] have received great attention in recent years for their unique properties. Linear−hyperbranched polymers are capable of forming highly ordered nanophase-segregated morphologies, despite of structural isomerism of the branched block in addition to the polydispersity of molecular weight.[24] Amphiphilic linear−hyperbranched block copolymers can form micelles in aqueous solution by self-assembly. The micelle size and critical micelle concentration obviously depended on the hydrophobic hyperbranched block content in the copolymer.[30] The multiply branched structure should impose a steric barrier for embedded guest molecules, potentially allowing good release control.[32]

In this paper, we report amphiphilic miktoarm copolymers mPEG-(*hb*-PG)-*g*-PCL with a linear−hyperbranched architecture bearing one monomethoxy poly(ethylene glycol) (mPEG) chain and several poly(ε-caprolactone) (PCL) chains on a hyperbranched polyglycerol (*hb*-PG) core. Linear−hyperbranched block copolymer mPEG-(*hb*-PG) similar to that reported by Wurm et al[27] was used as a macroinitiator for the ring-opening polymerization (ROP) of ε-caprolactone (CL). The ROP grafted PCL chains on the *hb*-PG core and converted the double-hydrophilic mPEG-(*hb*-PG) to amphiphilic miktoarm mPEG-(*hb*-PG)-*g*-PCL copolymers. Linear mPEG-*b*-PCL block copolymers were prepared and used for comparison. The polymers were characterized by ^{1}H NMR and GPC. Micelle preparation, properties (size, morphology, drug loading content, and entrapment efficiency), and in vitro drug release behavior were investigated.

Experimental Section

Materials. Tetrahydrofuran (THF) was distilled over Na−K alloy in the presence of benzophenone before use. Diglyme was purchased from Sinopharm Chemical Reagent Co., Ltd., in China and purified by distillation over CaH_2 directly prior to

*To whom correspondence should be addressed. Telephone: (+86) 27 6875 4061. Fax: (+86) 27 6875 4509. E-mail: zlzhong@whu.edu.cn (Z.Z.); bmp@whu.edu.cn (R.Z.).

Published on Web 07/28/2010

use. Monomethoxy poly(ethylene glycol) ($mPEG_{113}$) with average molecular weight of 5000 and degree of polymerization (DP) of 113 was purchased from Fluka and used as received. Glycidol (96%) and ε-caprolactone (ε-CL) (99%) were purchased from Acros and purified by distillation under reduced pressure prior to use. $Sn(Oct)_2$ (95%) was purchased from Aldrich and purified by distillation under reduced pressure and then dissolved in dry toluene prior to use. Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., in China and used as received.

Characterization. ^{1}H NMR spectra were recorded on a Mercury VX-300 spectrometer using tetramethylsilane (TMS) as an internal reference and deuterated chloroform ($CDCl_3$), deuterium oxide (D_2O), and deuterated dimethyl sulfoxide (DMSO-d_6) as a solvent. FT-IR spectra were recorded on a Perkin-Elmer-2 spectrometer. The molecular weight and polydispersity of copolymers were determined by gel permeation chromatograph (GPC) using a Waters high-pressure liquid chromatographic system equipped with a model 2690D separation module, a model 2410 refractive index detector, and Styragel HR1 THF and HR4 THF columns. The measurements were performed using DMF as an eluent at a flow rate of 0.3 mL min^{-1} at 35 °C and a series of narrow polystyrene standards for the calibration of the columns.

Preparation of Linear-Hyperbranched Copolymer mPEG-(*hb*-PG). A 5.0 g sample of linear macroinitiator $mPEG_{113}$ (1.00 mmol) was placed in a flask and heated at 100 °C under reduced pressure (oil pump) for 6 h. Then, 10 mg of potassium (0.26 mmol) was added to achieve about 25% deprotonation of the hydroxyl groups of $mPEG_{113}$. After heating and evacuation (oil pump) at 100 °C for 6 h, 25 mL of dry diglyme was added. Subsequently a solution of glycidol (2.0 g, 0.027 mol) in dry diglyme (10 mL) was added slowly over a period of approximately 12 h. After cooling, excess methanol (50 mL) and acidic cation exchange resin were added for terminating the reaction. The mixture was filtered and the filtrate was precipitated in cold diethyl ether thrice. The precipitate was collected and dried under vacuum at 40 °C for 2 days to give a pale yellow solid in 83% yield. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.88−4.41 (m, O*H*, disappeared after D_2O exchange), 3.84−3.41 (m, CH_2 and C*H*), 3.24 (s, OCH_3).

Total Acetylation of mPEG-(*hb*-PG) for Determination of Hydroxyl Number. A 60 mg sample of mPEG-*b*-(*hb*-PG), 0.622 g of potassium carbonate anhydrous (4.50 mmol) and 1.68 mg of 4-dimethyaminopyridine (0.015 mmol) were placed in flask. After thorough grinding of the mixture, 2 mL of anhydrous THF and 0.118 g of freshly distilled acetyl chloride (1.50 mmol) were added into the flask. The mixture was stirred under argon atmosphere at 50 °C for 24 h. The mixture was filtered and the filtrate was concentrated under reduced pressure and dried under vacuum at 40 °C for 24 h to give 63 mg of yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 3.92−3.50 (m, CH_2 and C*H*), 3.39 (s, OCH_3), 2.08 (s, OCOCH_3).

Preparation of Amphiphilic Miktoarm Copolymers mPEG-(*hb*-PG)-*g*-PCL. A round-bottom flask pretreated with trimethylchlorosilane was charged with predetermined amounts of mPEG-(*hb*-PG), ε-CL, and 0.1 M $Sn(Oct)_2$ solution in anhydrous toluene ([monomer]:[catalyst] = 500:1). The total mass was about 1 g. The flask was evacuated and charged with argon three times, and then sealed under vacuum with a magnetic stirring bar inside. After the mixture was stirred at 140 °C for 48 h, the polymerization was quenched by immersing the flask in a cool water bath. The product was purified by precipitation from chloroform with cold methanol-diethyl ether mixture (1:1 by volume) thrice and dried under vacuum at 40 °C for 24 h to give a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 4.13−4.01 (t, $OCOCH_2CH_2CH_2CH_2CH_2$), 3.96−3.84 (m, C*H* of glycidol), 3.74−3.58 (m, CH_2 of mPEG and CH_2 of glycidol), 3.39 (s, OCH_3), 2.40−2.24 (t, $OCOCH_2CH_2CH_2CH_2CH_2$), 1.82−1.54 (m, $OCOCH_2CH_2CH_2CH_2CH_2$), 1.47−1.30 (t, $OCOCH_2CH_2CH_2CH_2CH_2$).

Preparation of Amphiphilic Linear Block Copolymers mPEG-*b*-PCL. A round-bottom flask pretreated with trimethylchlorosilane was charged with predetermined amounts of $mPEG_{113}$, ε-CL, and 0.1 M $Sn(Oct)_2$ solution in anhydrous toluene ([monomer]:[catalyst] = 1000:1). The total mass was about 1 g. The flask was evacuated and charged with argon three times, and then sealed under vacuum with a magnetic stirring bar inside. After the mixture was stirred at 130 °C for 24 h, the polymerization was quenched by immersing the flask in a cool water bath. The product was purified by precipitation from chloroform with cold methanol-diethyl ether mixture (1:1 by volume) thrice and dried under vacuum at 40 °C for 24 h to give a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz, TMS): δ 4.14−4.00 (t, $OCOCH_2CH_2CH_2CH_2CH_2$), 3.75−3.56 (m, CH_2 of mPEG), 3.40 (s, OCH_3), 2.38−2.24 (t, $OCOCH_2CH_2CH_2CH_2CH_2$), 1.75−1.54 (m, $OCOCH_2CH_2CH_2CH_2CH_2$), 1.48−1.29 (t, $OCOCH_2CH_2CH_2CH_2CH_2$).

Micelle Preparation and Characterization. To a stirred solution of amphiphilic linear or miktoarm copolymer (20 mg), with or without prednisone acetate (2.0 mg) in 4 mL of THF, was added 10 mL of deionized water dropwise. The resulting solution was stirred continuously overnight and then dialyzed against deionized water for 2 days, during which the water was renewed each 8 h, to remove THF and residual drug. The micelle solution was obtained after filtration through a membrane filter with a pore size of 0.45 μm.

The drug-free micelle solution was used directly for micelle size analysis. The drug-loaded micelle solution was divided into three portions. The first portion (1.0 mL) was used directly for micelle size analysis. The second portion (2.0 mL) was concentrated and dried by rotary evaporation. The resulting polymer membrane was dissolved in 3 mL of acetonitrile (chromatographic grade) for micelle drug loading determination. The last portion was placed in a dialysis bag (MWCO 3500 Da) for in vitro drug release study.

The micelle size and size distribution were determined by dynamic light scattering (DLS). Measurements were carried out and repeated three times at 25 °C with a scattering angle (θ) of 90° in optically homogeneous quartz cylinder cuvette by using a Beckman Coulter N4 Plus submicrometer particle sizer. JEOL JEM-100CXII and JEM-2010HT transmission electron microscopes (TEM) were used to characterize the size and morphology of micelles. The samples for TEM analysis were prepared as follows: One drop of micellar solution was added onto a carbon-coated copper grid. After 3−5 min, most of the solution was removed by touching edge of filter paper until the grid surface is nearly dry. A drop of 1% phosphotungstic acid solution (pH 7.2 adjusted with NaOH) was added onto the copper grid for negative staining. 1−2 min later, the staining solution was removed by touching on a piece of filter paper. The grid was allowed to dry under ambient conditions.

Determination of Drug Loading Content (DLC) and Entrapment Efficiency (EE). To determine the DLC and EE, the drug-loaded micelle solution was dried by rotary evaporation and then dissolved in acetonitrile (chromatographic grade). The UV absorbance at 235 nm was measured to determine the drug concentration with a Perkin-Elmer Lambda Bio 40 UV−vis spectrophotometer.

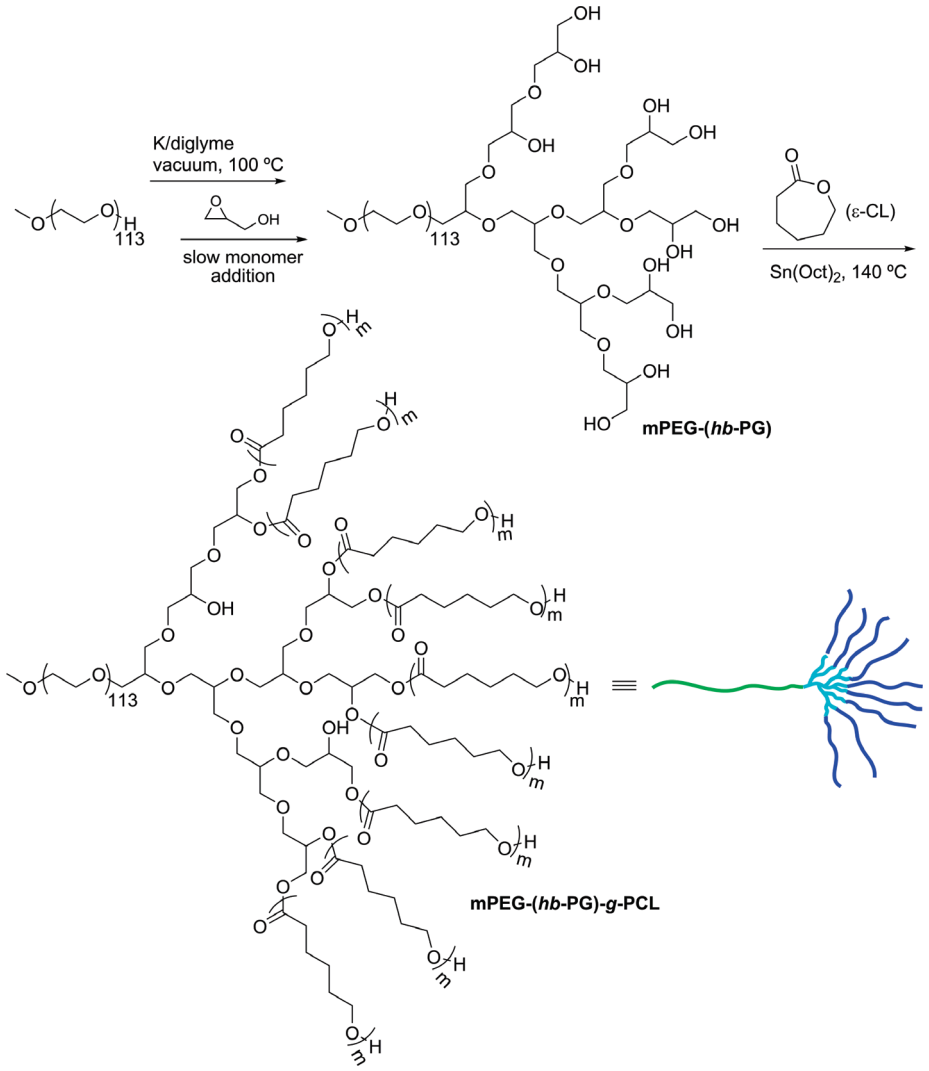
DLC and EE were calculated as follows:

$$\text{DLC}\ (\%) = \text{weight of loaded drug/weight of polymer and loaded drug} \times 100$$

$$\text{EE}\ (\%) = \text{weight of loaded drug/weight of drug in feed} \times 100$$

In Vitro Drug Release Behavior. The drug-loaded micelle solution was placed in a dialysis bag (MWCO 3500 Da). The dialysis bag was sealed and immersed in 40 mL of phosphate-buffered

Scheme 1. Synthesis of Amphiphilic Linear-Hyperbranched mPEG-(*hb*-PG)-*g*-PCL Block Copolymers



saline (PBS) (pH 7.4). In vitro drug release study of drug-loaded micelle was carried out in a shaking water bath at 37 °C. A 3 mL aliquot of solution was taken out, and the same volume of PBS solution was added after each sampling at predetermined time intervals. The drug concentration was determined by measuring the absorbance of prednisone acetate at 242 nm. The rate of drug release was measured by the released concentration of prednisone acetate at predetermined time intervals according to the calibration curve of prednisone acetate.

Results and Discussion

Amphiphilic block copolymer could form micelle with core−shell structure by self-assembly in water. Biocompatibility micelles have been extensively studied on drug controlled release in the past decades. In this study, amphiphilic miktoarm copolymers with a hyperbranched core were synthesized and characterized. Linear block copolymers were used for comparison. The properties of micelles of the amphiphilic hyperbranched miktoarm copolymers and linear block copolymers in water were investigated and compared.

The synthesis route of amphiphilic hyperbranched miktoarm copolymers mPEG-(*hb*-PG)-*g*-PCL is shown in Scheme 1. First, linear−hyperbranched block copolymer mPEG-(*hb*-PG) of ethylene glycol and glycerol was synthesized via anionic ring-opening polymerization of glycidol with partially deprotonated mPEG as an initiator, using a slow monomer addition technique.[27,35] Then, mPEG-(*hb*-PG) was used as a macroinitiator for the ring-opening polymerization of ε-CL that grafts PCL chains on the hyperbranched core converting the hydrophilic linear−hyperbranched block copolymer to amphiphilic miktoarm mPEG-(*hb*-PG)-*g*-PCL.

The synthesis and structure of the linear−hyperbranched mPEG-(*hb*-PG) block copolymer are similar to but different from those reported by Wurm et al.[27] In the literature, linear−hyperbranched mPEG-(*hb*-PG) block copolymers were synthesized by a four-step two-pot procedure: First, linear mPEG-*b*-PG copolymer was prepared by removal of the ethoxyethyl protection groups of its protected form, which was synthesized by ROP of ethoxyethyl glycidyl ether with deprotonated mPEG as an initiator. Then, the linear mPEG-*b*-PG was used as a macroinitiator for the ROP of glycidol to achieve the synthesis of linear−hyperbranched mPEG-(*hb*-PG) block copolymers.[27] In the current work, the linear−hyperbranched mPEG-(*hb*-PG) block copolymer was synthesized directly using partially deprotonated mPEG as an initiator. Partial deprotonation other than full deprotonation condition was adopted because of two considerations. First, the proton exchanging reaction between deprotonated and undeprotonated hydroxyl groups is much faster than the ring-opening polymerization of glycidol. That means partially deprotonation would not affect the structure and molecular weight of the polymer product.[35] Second, partially deprotonation of mPEG could avoid phase separation at the beginning of the polymerization reaction. Homopolymerization

Table 1. Synthetic Results of Amphiphilic Block Copolymers mPEG-*b*-PCL[a] and mPEG-(*hb*-PG)-*g*-PCL[b]

				^{1}H NMR		GPC	
sample	w_{mPEG}[c] (predicted)	yield (%)	M_n (predicted)	W_{mPEG}[d]	M_n	M_n	PDI
$mPEG_{113}$-*b*-PCL_{25}	0.63	93	8000	0.64	7800	6900	1.16
$mPEG_{113}$-*b*-PCL_{48}	0.45	96	11 000	0.48	10 500	10 300	1.44
$mPEG_{113}$-*b*-PCL_{105}	0.29	92	17 000	0.29	17 000	15 400	1.55
$mPEG_{113}$-(*hb*-PG)$_{10}$	0.71	83	7000	0.88	5800	5100	1.11
$mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{25}	0.56	87	9000	0.58	8600	7900	1.31
$mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54}	0.42	92	12 000	0.42	11 900	10 600	1.67
$mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{98}	0.28	88	18 000	0.30	16 900	16 100	1.95

[a] Polymerization conditions: [monomer]/[Sn(Oct)$_2$] = 1000, 130 °C, 24 h, in bulk. [b] Polymerization conditions: [monomer]/[Sn(Oct)$_2$] = 500, 140 °C, 48 h, in bulk. [c] Weight fraction of mPEG chain in the initiator/monomer mixtures. [d] Weight fraction of mPEG chain in the copolymers.

of glycidol is an inevitable by-reaction, but it could be minimized by slow monomer addition.[35] Initial oligoglycidols with an active epoxy head may graft onto mPEG-containing copolymers by reacting with the hydroxyl groups of the copolymers. If the epoxy group is consumed by tail-biting reaction with an intramolecular hydroxyl group, however, the homopolymer becomes a "dead" byproduct. Therefore, increasing the number of hydroxyl groups on the initiator or copolymer could reduce byproduct homopolymers. From this point of view, though more complicated, the multistep route by Wurm et al.[27] should be superior than our one-step route in minimizing homopolymerization. The relative low yield of 83% and lower NMR-calculated M_n of 5800 in comparison with predicted M_n of 7000 suggest that a portion of glycidol was consumed by homopolymerization during the synthesis of mPEG-(*hb*-PG). GPC analysis showed the mPEG-(*hb*-PG) copolymer had a unimodel and narrow molecular weight distribution (PDI = 1.11), implying that the oligomer byproduct of homopolymerization was removed by the precipitation.

Amphiphilic miktoarm copolymers mPEG-(*hb*-PG)-*g*-PCL were synthesized via ROP of ε-CL with mPEG-(*hb*-PG) as a macroinitiator and Sn(Oct)$_2$ as a catalyst. Analogously, amphiphilic linear block copolymers mPEG-*b*-PCL were synthesized via bulk polymerization of ε-CL with mPEG as an initiator and Sn(Oct)$_2$ as a catalyst. For comparison, three kinds of mPEG-*b*-PCL and mPEG-(*hb*-PG)-*g*-PCL with different hydrophobic block lengths and same hydrophilic block length were designed and synthesized. Synthetic results of amphiphilic block copolymers mPEG-*b*-PCL and mPEG-(*hb*-PG)-*g*-PCL are listed in Table 1. The M_n of hydrophilic block mPEG-(*hb*-PG) was 7000 predicted and 5800 (PDI=1.11) determined by GPC. The M_n of hydrophobic PCL blocks of mPEG-(*hb*-PG)-*g*-PCL were 3000, 6000, and 12000 predicted on the basis of feed ratio, and 2600, 5900, and 11900 calculated from ^{1}H NMR spectra, respectively. The composition of copolymers calculated from ^{1}H NMR spectra was similar to the feed ratios. Therefore, the molecular weights could be controlled by initiator/monomer feed ratios. The M_n of all the copolymers from GPC was slightly lower than the M_n values predicted from feed ratio or calculated from ^{1}H NMR spectra.

The hydroxyl number and degree of polymerization (DP) of the polyglycidol (PG) units in mPEG-(*hb*-PG) are very important parameters for the preparation and characterization of mPEG-(*hb*-PG)-*g*-PCL. The DP was calculated to be 10.5 on the basis of the ^{1}H NMR spectra of mPEG and mPEG-(*hb*-PG) in D_2O by comparing the integration of signals at 4.10−3.41 ppm for CH_2 of mPEG and CH and CH_2 of mPEG-(*hb*-PG) using the singlet at 3.38 ppm for methoxy-end group of mPEG as an internal standard. This result was further confirmed by total acetylation of mPEG-(*hb*-PG) for determination of hydroxyl number. First, the hydroxyl groups of mPEG-(*hb*-PG) were acetylated by excessive acetyl chloride. Complete acetylation was verified by complete disappearance of the broad absorbance bond at 3436 cm^{-1} for OH groups and emergence of strong absorbance at 1743 cm^{-1} for the acetate carbonyl group in the FT-IR spectrum of mPEG-(*hb*-PG)-Ac shown in Figure 1. Then, the acetate number in the acetylated derivative was determined by comparing the integration of strong and sharp signals at 3.92−3.50 ppm for PEG and PG units and 2.08 ppm for the acetate groups (Figure 2). The number was 11, which means that on average 10 glycidol units were grafted to each $mPEG_{113}$ chain.

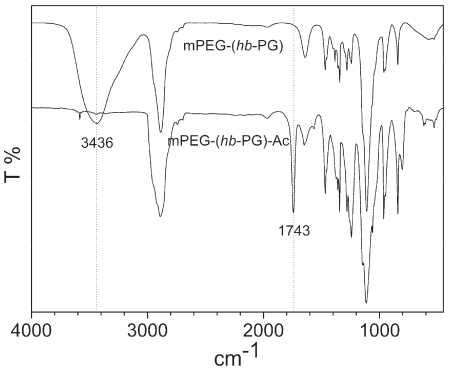


Figure 1. FT-IR spectra of (upper) mPEG-(*hb*-PG) and (lower) the acetylated derivative mPEG-(*hb*-PG)-Ac.

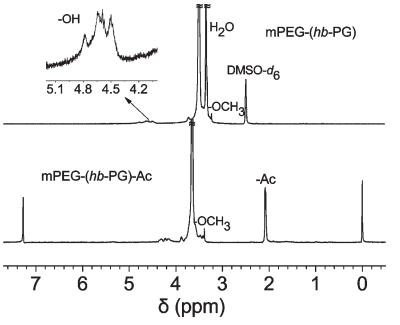


Figure 2. ^{1}H NMR spectra of (upper) linear−hyperbranched block copolymer mPEG-(*hb*-PG) in DMSO-d_6 and (lower) acetylated derivative mPEG-(*hb*-PG)-Ac in $CDCl_3$.

The ^{1}H NMR spectra of amphiphilic block copolymers mPEG-*b*-PCL and mPEG-(*hb*-PG)-*g*-PCL in $CDCl_3$ are shown in Figure 3. Signals at 3.96−3.84 ppm for C*H* of glycidol unit on the ^{1}H NMR spectrum of mPEG-(*hb*-PG)-*g*-PCL were clearly viewable, while no such signals presented on the ^{1}H NMR spectrum of mPEG-*b*-PCL. The DP and M_n of the two types of amphiphilic block copolymers were calculated from the ^{1}H NMR spectra by comparing the integration of signals at 3.65 ppm for mPEG units and 2.35 ppm for ε-CL units. The results are shown in Table 1. The compositions of all the copolymers were close to the feed ratios. Therefore, the hydrophobic block lengths in the copolymer chains could be adjusted through changing the weight ratios of the initiator and monomer.

The molecular weight and polydispersity of polymers determined by GPC further verified the successful synthesis of the

block copolymers. The measurements were performed using DMF as an eluent at a flow rate of 0.3 mL min^{-1} at 35 °C and a series of narrow dispersity polystyrene standards for the calibration of the columns. The data are listed in Table 1. For amphiphilic block copolymers mPEG-*b*-PCL and mPEG-(*hb*-PG)-*g*-PCL, the polydispersities increased with increasing hydrophobic block lengths (molecular weights). Normalized GPC curves of mPEG, $mPEG_{113}$-(*hb*-PG)$_{10}$, $mPEG_{113}$-*b*-PCL_{48}, and $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} are shown in Figure 4. All the polymers were in unimodal molecular weight distributions with moderate polydispersities. This proved the successful synthesis of mPEG-(*hb*-PG), mPEG-*b*-PCL and mPEG-(*hb*-PG)-*g*-PCL.

Micelles were prepared by adding deionized water dropwise to a THF solution of amphiphilic block copolymer under vigorous stirring, followed by removal of the organic solvent by dialysis against deionized water for 2 days. The micelle solutions were filtered through a syringe filter (pore size: 0.45 μm). As shown by the TEM images in Figure 5, the micelle particles are nanosized in spherical shape with narrow size distribution. The particle size and size distribution of the micelles were determined by dynamic light scattering (DLS) with the distribution profiles shown in Figure 5D−F and the results were summarized in Table 2. The results showed that the size of the micelles increased with the increasing content of the hydrophobic PCL blocks while the length of hydrophilic PEG block was kept constant. At similar composition ratios of hydrophobic/hydrophilic blocks, the hyperbranched miktoarm mPEG-(*hb*-PG)-*g*-PCL copolymers formed larger micelles than the linear mPEG-*b*-PCL analogues. All the micelles had a narrow unimodal size distribution. The size and size distribution had no significant change after the micelle solutions were placed at room temperature for 1 week or exposed to ultrasonic for 5 min, showing a good stability of the micelle in water. Depending on the hydrophilic−lipophilic balance, amphiphilic copolymers may form micelles or vesicles of various morphologies such as spherical, worm-like, and tubular assemblies.[36] It was reported that PEG-*b*-PCL with high PEG/PCL ratio formed micrometer-sized vesicles when it was dissolved directly in water.[37] In our case, though the copolymers have a wide range of hydrophilic−lipophilic balance volume (weight fraction of PEG chain from 0.29 to 0.64), only nanosized spherical particles were observed in the micelle solutions prepared by adding water in THF solution of the copolymers. This could be understood that an amphiphilic polymer of certain structure can form different assembly morphologies under different preparation conditions.[38]

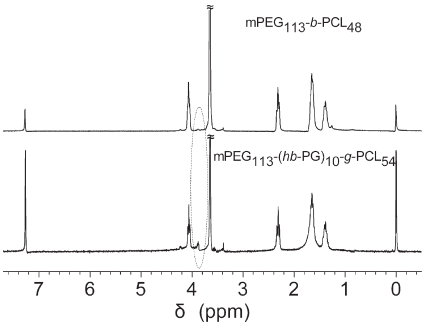


Figure 3. ^{1}H NMR spectra of amphiphilic block copolymers $mPEG_{113}$-*b*-PCL_{48} and $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} (300 MHz, $CDCl_3$).

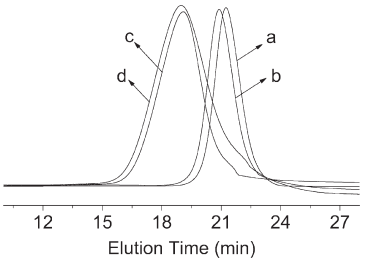


Figure 4. Normalized GPC curves of (a) mPEG, (b) $mPEG_{113}$-(*hb*-PG)$_{10}$, (c) $mPEG_{113}$-*b*-PCL_{48}, and (d) $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} (DMF as an eluent).

Prednisone acetate, an adrenocortical hormone drug, was used as a model drug to test the properties of the polymer micelles as drug carriers. The drug-loaded micelles were prepared from solutions of 10:1 (w/w) copolymer/drug mixtures by a procedure similar to that for drug-free micelles. Unenclosed residual drug was removed by filtration through a 0.45 μm micropore membrane and dialysis the micelle solution against water. To determine the drug loading capacity (DLC) and entrapment efficiency (EE), a portion of the drug-loaded micelle solution in water was dried by rota-evaporation, and the residue was dissolved in acetonitrile to determine the drug concentration through measuring the UV absorbance at 235 nm. As revealed by the DLS results in Table 2, the drug-loaded micelles were a little bigger than the corresponding drug-free ones. When the length of hydrophilic PEG block was kept constant, DLC and EE were dependent to the length of hydrophobic PCL blocks. Increasing

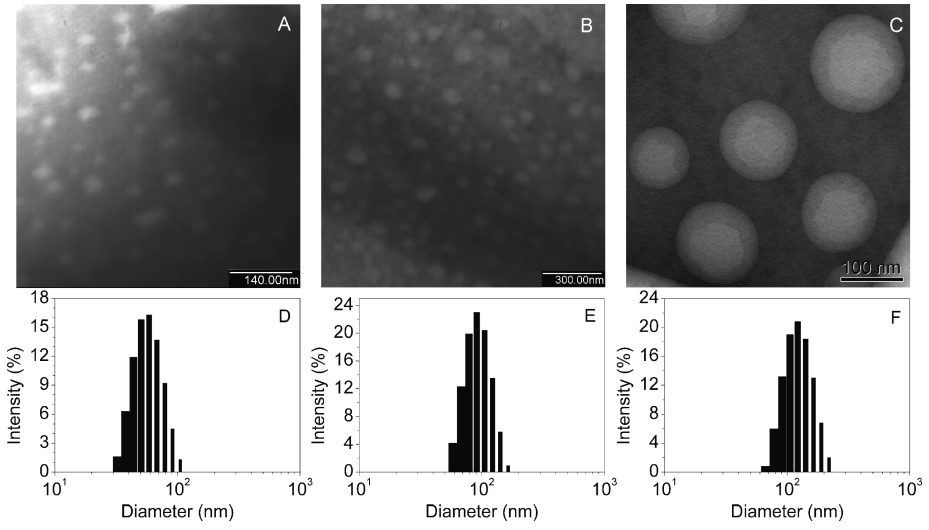


Figure 5. TEM images of $mPEG_{113}$-*b*-PCL_{48} (A), $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} (B), and $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{98} (C) micelles; size distribution profiles of $mPEG_{113}$-*b*-PCL_{48} (D), $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} (E), and $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{98} (F) micelles determined by DLS.

Table 2. Influence of Copolymer Composition on Micellar Properties

		drug-free micelle[b]		drug-loaded micelle[b,c]			
copolymers	W_{mPEG}[a]	diameter (nm)	PDI	diameter (nm)	PDI	DLC[d] (%)	EE[e] (%)
$mPEG_{113}$-*b*-PCL_{25}	0.64	41	0.132	60	0.153	1.41	14.3
$mPEG_{113}$-*b*-PCL_{48}	0.48	58	0.129	73	0.150	2.01	20.5
$mPEG_{113}$-*b*-PCL_{105}	0.29	76	0.134	85	0.162	2.80	28.8
$mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{25}	0.58	64	0.125	96	0.156	2.07	21.1
$mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54}	0.42	88	0.124	111	0.147	3.52	36.5
$mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{98}	0.30	117	0.136	127	0.165	4.26	44.5

[a] Weight fraction of mPEG chain in the copolymers. [b] Measured at a concentration of 0.5 mg/mL. [c] Prepared with 20 mg of copolymers and 2.0 mg of prednisone acetate as a model drug. [d] Drug loading content (DLC). [e] Entrapment efficiency (EE).

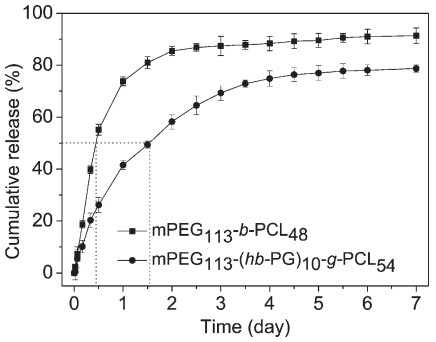


Figure 6. Release profile of prednisolone acetate from amphiphilic block copolymers $mPEG_{113}$-*b*-PCL_{48} and $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} (PBS, 0.1 M, pH 7.4; 37 °C).

the hydrophobic block length was beneficial for improving DLC and EE probably because of increased size of the hydrophobic core. The DLC and EE of miktoarm mPEG-(*hb*-PG)-*g*-PCL were higher than those of linear mPEG-*b*-PCL with similar copolymer composition. For example, $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{98} had an EE of 44.5%, which was about 55% higher than the 28.8% of $mPEG_{113}$-*b*-PCL_{105}. The multibranched structure of mPEG-(*hb*-PG)-*g*-PCL results in steric barrier for ordered stacking of PCL blocks. Also, at the same PCL/PEG ratio, the average PCL chain length in the branched miktoarm polymers is much shorter than that in the linear ones. Shorter chain length might weaken chain−chain interactions. Overall, the hyperbranched structure would increase the disorder and space between PCL chains, decrease the crystalline degree, and thus enhance the interaction of polymer chains with drug molecules.

The release rate was monitored by determining the concentration of released drug at predetermined time intervals. The in vitro drug release profile of prednisone acetate from amphiphilic block copolymers $mPEG_{113}$-*b*-PCL_{48} and $mPEG_{113}$-(*hb*-PG)$_{10}$-*g*-PCL_{54} is shown in Figure 6. For linear block copolymer micelle, the release was quite quick at the beginning and 85% of drug enwrapped in the micelle was released after 2 days. For linear−hyperbranched block copolymer micelle, the release was slow and continuous in a week and only 58% of drug enwrapped in the micelle was released after 2 days. Most of the drug was released after 3 days for linear block copolymer micelle and 7 days for linear−hyperbranched block copolymer micelle. The time to release half of the drug was about 10.5 h for linear block copolymer micelle and 37 h for linear−hyperbranched block copolymer micelle. Amphiphilic linear−hyperbranched block copolymers mPEG-(*hb*-PG)-*g*-PCL with a lot of arms had more sustained drug release behavior that is favorable for drug controlled release.

Conclusions

Amphiphilic linear−hyperbranched block copolymers mPEG-(*hb*-PG)-*g*-PCL with a lot of arms were successfully synthesized via a facile strategy of two steps of ring-opening polymerization. The copolymer composition and molecular weight were in good accordance to the predicted from the feed ratio. All the polymers were in unimodal molecular weight distributions with moderate polydispersities. The amphiphilic block copolymers formed stable micelle solutions via adding water to a THF solution of the polymer followed by removal of the organic solvent by dialysis. All the micelles had a narrow unimodal size distribution. The micelle sizes depended much on the copolymer composition. Comparative studies revealed the advantages of the multibranched copolymers over linear mPEG-*b*-PCL analogues for use as drug carriers. The DLC and EE of amphiphilic linear−hyperbranched block copolymers mPEG-(*hb*-PG)-*g*-PCL were higher than those of amphiphilic linear block copolymers mPEG-*b*-PCL with similar copolymer composition. Furthermore, the amphiphilic linear−hyperbranched block copolymers had more sustained drug release behavior. Therefore, the amphiphilic linear−hyperbranched block copolymers mPEG-(*hb*-PG)-*g*-PCL are potentially a type of favorable biomaterials for drug controlled release.

Acknowledgment. This research is supported by National Natural Science Foundation of China (20774067 and 20974082) and National Basic Research Program of China (2005CB623903 and 2009CB930300).

References and Notes

(1) Timo, R.; Meyer, W. H.; Wegner, G. *Macromol. Chem. Phys.* **1999**, *200*, 1672–1680.
(2) Tan, B. H.; Hussain, H.; Liu, Y.; He, C. B.; Davis, T. P. *Langmuir* **2010**, *26*, 2361–2368.
(3) Zehm, D.; Laschewsky, A.; Gradzielski, M.; Prevost, S.; Liang, H.; Rabe, J. P.; Schweins, R.; Gummel, J. *Langmuir* **2010**, *26*, 3145–3155.
(4) Shvartzman−Cohen, R.; Monje, I.; Florent, M.; Frydman, V.; Goldfarb, D.; Yerushalmi−Rozen, R. *Macromolecules* **2010**, *43*, 606–614.
(5) Danquah, M.; Fujiwara, T.; Mahato, R. I. *Biomaterial* **2010**, *31*, 2358–2370.
(6) Jain, S.; Bates, F. S. *Science* **2003**, *300*, 460–464.
(7) Bhargava, P.; Tu, Y. F.; Zheng, J. X.; Xiong, H. M.; Quirk, R. P.; Cheng, S. Z. D. *J. Am. Chem. Soc.* **2007**, *129*, 1113–1121.
(8) Yu, Y. S.; Eisenberg, A. *J. Am. Chem. Soc.* **1997**, *119*, 8383–8384.
(9) Bhargava, P.; Zheng, J. X.; Li, P.; Quirk, R. P.; Harris, F. W.; Cheng, S. Z. D. *Macromolecules* **2006**, *39*, 4880–4888.
(10) Tang, R. P.; Ji, W. H.; Wang, C. *Macromol. Biosci.* **2010**, *10*, 192–201.
(11) Smith, A. E.; Xu, X. W.; Kirkland−York, S. E.; Savin, D. A.; Mccormick, C. L. *Macromolecules* **2010**, *43*, 1210–1217.
(12) Nese, A.; Mosnacek, J.; Juhari, A.; Yoon, J. A.; Koynov, K.; Kowalewski, T.; Matyjaszewski, K. *Macromolecules* **2010**, *43*, 1227–1235.
(13) Cornelissen, J. J. L. M.; Fischer, M.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Science* **1998**, *280*, 1427–1430.
(14) Borsali, R.; Minatti, E.; Putaux, J. L.; Schappacher, M.; Deffieux, A.; Viville, P.; Lazzaroni, R.; Narayanan, T. *Langmuir* **2003**, *19*, 6–9.
(15) Ravenelle, F.; Marchessault, R. H. *Biomacromolecules* **2003**, *4*, 856–858.
(16) Pochan, D. J.; Chen, Z. Y.; Cui, H. G.; Hales, K.; Qi, K.; Wooley, K. L. *Science* **2004**, *306*, 94–97.

(17) Liu, X. Y.; Kim, J. S.; Wu, J.; Eisenberg, A. *Macromolecules* **2005**, *38*, 6749–6751.
(18) Harada, A.; Kataoka, K. *Prog. Polym. Sci.* **2006**, *31*, 949–982.
(19) Suek, N. W.; Lamm, M. H. *Langmuir* **2008**, *24*, 3030–3036.
(20) Chang, Y.; Kwon, Y. C.; Lee, S. C.; Kim, C. *Macromolecules* **2000**, *33*, 4496–4500.
(21) Lu, D. D.; Yuan, J. C.; Li, H. G.; Lei, Z. Q. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 3802–3812.
(22) Chen, T.; Wang, L.; Jiang, G. H.; Wang, J. J.; Wang, X. J.; Zhou, J. F.; Wang, W. *Eur. Polym. J.* **2006**, *42*, 687–693.
(23) Ihre, H.; Jesús, O. L. P. D.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2001**, *123*, 5908–5917.
(24) Marcos, A. G.; Pusel, T. M.; Thomann, R.; Pakula, T.; Okrasa, L.; Geppert, S.; Gronski, W.; Frey, H. *Macromolecules* **2006**, *39*, 971–977.
(25) Zou, P.; Yang, L. P.; Pan, C. Y. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 7628–7636.
(26) Xiao, A. G.; Wang, L.; Liu, Q. Q.; Yu, H. J.; Wang, J. J.; Huo, J.; Tan, Q. H.; Ding, J. H.; Ding, W. B.; Amin, A. M. *Macromolecules* **2009**, *42*, 1834–1837.
(27) Wurm, F.; Nieberle, J.; Frey, H. *Macromolecules* **2008**, *41*, 1184–1188.
(28) Wurm, F.; Klos, J.; Räder, H. J.; Frey, H. *J. Am. Chem. Soc.* **2009**, *131*, 7954–7955.
(29) Ge, Z. S.; Liu, S. Y. *Macromol. Rapid Commun.* **2009**, *30*, 1523–1532.
(30) Tian, H. Y.; Deng, C.; Lin, H.; Sun, J. R.; Deng, M. X.; Chen, X. S.; Jing, X. B. *Biomaterials* **2005**, *26*, 4209–4217.
(31) Barriau, E.; Marcos, A. G.; Kautz, H.; Frey, H. *Macromol. Rapid Commun.* **2005**, *26*, 862–867.
(32) Istratov, V.; Kautz, H.; Kim, Y. K.; Schubert, R.; Frey, H. *Tetrahedron* **2003**, *59*, 4017–4024.
(33) Wurm, F.; Schüle, H.; Frey, H. *Macromolecules* **2008**, *41*, 9602–9611.
(34) del Barrio, J.; Oriol, L.; Alcala, R.; Sanchez, C. *Macromolecules* **2009**, *42*, 5752–5760.
(35) Sunder, A.; Hanselmann, R.; Frey, H.; Mülhaupt, R. *Macromolecules* **1999**, *32*, 4240–4246.
(36) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967–973.
(37) Ahmed, F.; Discher, D. E. *J. Controlled Release* **2004**, *96*, 37–53.
(38) Guo, Z. G.; Li, Y. H.; Tian, H. Y.; Zhuang, X. L.; Chen, X. S.; Jing, X. B. *Langmuir* **2009**, *25*, 9690–9696.