ARTICLE

Synthesis of an Amphiphilic Dendrimer-Like Block Copolymer and Its Application on Drug Delivery

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Dendrimer-like amphiphilic copolymer is a kind of three-dimensional spherical structure polymer. An amphiphilic dendrimer-like diblock copolymer, PEEGE-G2-b-PEO$(OH)_{12}$, constituted of a hydrophobic poly(ethoxyethyl glycidol ether) inner core and a hydrophilic poly(ethylene oxide) outer layer, has been successfully synthesized by the living anionic ring-opening polymerization method. The intermediates and targeted products were characterized with ^{1}H NMR spectroscopy and gel permeation chromatography. The application on drug delivery of dendrimer-like diblock copolymer PEEGE-G2-b-PEO$(OH)_{12}$ using DOX as a model drug was also studied. The drug loading content and encapsulation efficiency were found at 13.07% and 45.75%, respectively. *In vitro* release experiment results indicated that the drug-loaded micelles exhibited a sustained release behavior under acidic media.

Key words: Anionic ring opening polymerization, Living polymerization, Dendrimer-like copolymer, Amphiphilic diblock copolymer, Drug delivery

I. INTRODUCTION

Poly(ethylene oxide) (PEO), often referred as PEG for poly(ethylene glycol), has many potential applications in biomedical and pharmaceutical areas owing to its water solubility, nontoxicity, chemical stability, and non-recognition by the immune system [1]. Different structures such as star-like PEO's [2], hyperbranched PEO's [3, 4], arborescent PEO's [5, 6], or dendrimer-like PEO's [7−9] were proposed and synthesized.

Dendrimer-like polymers exhibit molecular features similar to those regular dendrimers, such as the presence of a central core, a precise number of branching points and terminal functions, but comprise of generations of macromolecular size between their branching junctions [10]. Recently, Feng and co-workers have developed an easy access to well-defined dendrimer-like PEOs based on an iterative divergent method combining the "living anionic ring-opening polymerization of ethylene oxide and chain end functionalization/branching reaction" [11]. In this way, dendrimer-like PEOs was obtained up to eighth generation, carrying 384 hydroxyl end groups. It was a promising drug carrier because of their special structures.

Hydrophobic drugs, taking most percentage of existing ones, can be loaded into the hydrophobic cores of the micelles and solubilized in an aqueous media. Such encapsulation could keep drugs' original properties without affecting their therapeutic performances. However, the stabilities of micelles are dependent on many exterior environmental parameters. For instance, in the case of micelles formed from linear amphiphilic copolymer [12−14], they could be dissociated under diluted conditions (below CMC), resulting low therapeutic efficiency. On the other hand, dendrimer-like copolymers with multiple generations avoid such concerns. The micelles formed by dendrimer-like copolymer are unimolecular, which are stable under extremely diluted conditions [15, 16].

In this work, a well-defined amphiphilic dendrimer-like diblock copolymer PEEGE-G2-b-PEO$(OH)_{12}$ carrying hydroxyl end groups was successfully synthesized by anionic ring-opening polymerization using "core-first" divergent methodology (Fig.1). An acetal-protected ethoxy ethyl glycidol ether (EEGE) can be living polymerized [17−20]. The obtained dendrimer-like polymer possessed a hydrophobic PEEGE core and a hydrophilic PEO outer layer through sequential polymerization of EEGE and EO after branching reaction. The encapsulated drugs could be released with the inversion of philicity of core from hydrophobicity to hydrophilicity under acidic conditions due to the deprotection of acetal group.

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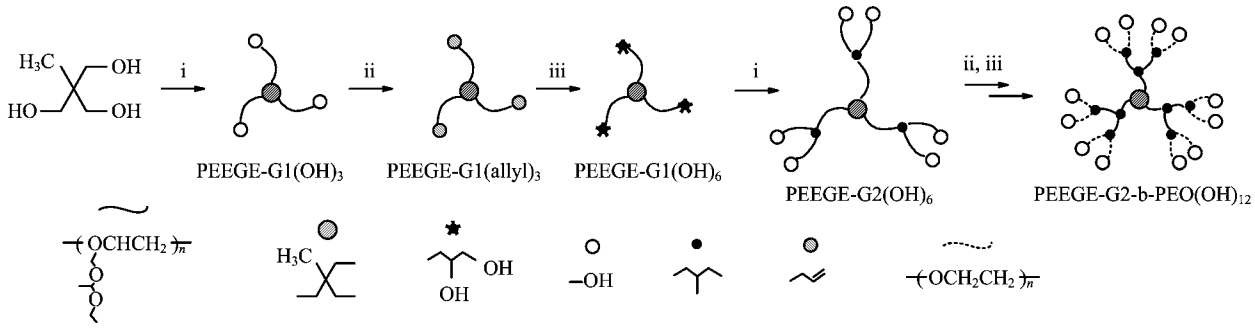

FIG. 1 Synthetic routes amphiphilic dendrimer-like polymer, PEEGE-G2-b-PEO$(OH)_{12}$. (i) DPMK, THF/DMSO, and EEGE (the third generation was EO), 2 day, r.t. (ii) NaH, THF, and allyl chloride, 50 °C, 48 h. (iii) *N*-methylmorpholine-*N*-oxide, OsO_4, THF/H_2O/*t*-BuOH, r.t., 24 h.

II. EXPERIMENTS

A. Materials and measurements

Ethylene oxide (EO) (Aladdin, 99.5%) and tetrahydrofuran (THF) were distilled over sodium into a buret. Diphenylmethylpotassium (DPMK) was prepared according to a known procedure and titrated with acetanilide [21, 22]. EEGE was synthesized according to established procedures [17, 22, 23]. Dimethyl sulfoxide (DMSO) and dioxane were distilled over CaH_2. All hydroxylated precursors used for the polymerization of EEGE or EO were dried by freeze-drying from a dried dioxane solution. All other chemicals were purchased from Aladdin and used without further purification.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV 300 NMR (400 MHz) spectrometer operated in the Fourier transform mode with $CDCl_3$ as solvent other than stated. Molecular weights and molecular distributions were determined by gel permeation chromatography (GPC) equipped with one guard column PLGel 5 μm (50 mm×7.5 mm) and two PLGel 5 with THF (1 g/L LiBr) as eluent (1 mL/min) at 40 °C. The apparent molar masses were calculated using linear poly(styrene) (PS) standards. UV-Vis absorption measurement was performed on a UV-3100 spectrophotometer. The calibration curve of absorbance at 480 nm versus the concentration of DOX was used to calculate the loading of DOX in micelles.

B. Synthesis of three-arm PEEGE-G1$(OH)_3$

The synthesis procedure of three-arm PEEGE-G1$(OH)_3$ was similar to that of PEO-G1$(OH)_3$ [11]. Typically, to a two-neck 250 mL of flask equipped with a magnetic stirrer, the anhydrous trifunctional precursor 1,1,1-tris(hydroxymethyl) ethane (0.36 g, 3.0 mmol) was added. After it was freeze-dried with a dioxane solution under vacuum, THF and DMSO were added successively. A solution of DPMK in THF (2.23 mL, 0.6 mmol) was slowly added to get a homogeneous solution with yellowish color. EEGE (29.2 mL, 0.18 mol) was then added. The solution was stirred at 80 °C for 48 h. The alkoxides were deactivated by adding a few drops of methanol. After removal of the solvent under reduced pressure, the crude product was precipitated with an excess of deionized water. Then, the precipitates were extracted with CH_2Cl_2. The solution was dried and concentrated. The product was obtained by precipitating into excess cold petroleum ether (28.63 g, 97.1%).

C. Synthesis of PEEGE-G1$(allyl)_3$

To a solution of PEEGE-G1$(OH)_3$ (6.43 g, 2.17 meq. OH) and THF (50 mL), NaH (500 mg) was added. After stirring for 30 min at 50 °C, allyl chloride (1.72 mL, 21.70 mmol) was added under N_2. The solution was kept for 48 h at 50 °C under vigorous stirring. The solvents were removed and the residues were extracted with CH_2Cl_2. The solution was dried and concentrated. The product was obtained by precipitating into excess cold petroleum ether (5.25 g, 80.5%).

D. Synthesis of PEEGE-G1$(OH)_6$

To a solution of PEEGE-G1$(allyl)_3$(5.25 g, 1.67 meq. C=C), *N*-methylmorpholine-*N*-oxide (5.01 mmol, 0.59 g) in THF (10 mL), distilled water (2 mL) and *t*-butanol (10 mL), 50 μL of a 4wt% OsO_4 solution in water were added under N_2. The mixture was stirred for 24 h at room temperature. After removing the organic solvents, the residue was extracted with CH_2Cl_2 and concentrated. The solution was precipitated into cold petroleum ether and the product (4.43 g, 83.4%) was vacuum dried at room temperature.

E. Synthesis of PEEGE-G2$(OH)_6$

A similar synthesis procedure of PEEGE-G2$(OH)_6$ to that of amphiphilic three-arm star, PEEGE-G1$(OH)_3$,

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was employed. To a two-neck 250 mL schlenk flask charged with lyophilized dry precursor PEEGE-G1$(OH)_6$ (4.43 g, 2.78 meq. OH), dried THF and DMSO (50 mL, volume ratio of 2/3) was added under vacuum. DPMK (1.27 mL) in THF was slowly introduced and stirred until the red color of DPMK disappeared and a homogenous solution was formed. Then about 10% of the EEGE (8.71 mL, 55.6 mmol) was added. The solution was stirred for 12 h at 80 °C and then the rest of the monomer was added. The polymerization was carried out at 80 °C for another 48 h. The alkoxides were neutralized with methanol. The solution was concentrated under vacuum and the polymer (11.84 g, 94.3%) was obtained by precipitation twice in distilled water and cold petroleum ether respectively.

F. Synthesis of PEEGE-G2-b-PEO$(OH)_{12}$

To a two-neck 250 mL schlenk flask charged with lyophilized dry precursor PEEGE-G2$(OH)_{12}$ (6.97 g, 3.09 meq. OH), dried THF and DMSO (50 mL, volume ratio of 2/3) was added under vacuum. DPMK (1.15 mL) in THF was slowly introduced. Then about 10% of the EO (6.24 mL, 123.6 mmol) was added. After this addition, the system was stirred for 12 h at 30 °C and the rest of the monomer was added. The polymerization was carried out at 30 °C for another 48 h. The alkoxides were neutralized with methanol. Then all the solvents were removed under reduced pressure. The crude product was dissolved in CH_2Cl_2, filtered, dried over anhydrous $MgSO_4$ overnight and then filtered again to remove $MgSO_4$. The polymer (11.29 g, 91.1%) was obtained by precipitation twice in cold petroleum ether.

G. Preparation of micelles in aqueous solutions

The micelles in aqueous solutions were prepared via a solvent evaporation method. Doxorubicin hydrochloride salt (5 mg) was dissolved in 2 mL of THF. Triethylamine was added and the solution was stirred for 1 h. Dendrimer (25 mg) was then added to the solution and stirred for 30 min. The mixture obtained was dropped in 10 mL of DI water and stirred at room temperature for 1 h. THF was removed by evaporation. DOX-loaded unimolecular micelles solution was obtained after centrifugation. The drug loading content was determined by UV-Vis spectrophotometer at a wavelength of 480 nm.

H. *In vitro* drug release

10 mL of micelles solution loaded with 13.07wt% DOX was charged into a dialysis bag (molecular weight cutoff 3000), and the dialysis bag was incubated in 100 mL phosphate buffer saline (PBS, pH=5.0, 7.4) at 37 °C in a water bath with shaking. At time intervals, 2 mL of the solution outside the dialysis bag was taken and 2 mL of fresh PBS was then added. The DOX concentration in the solution was determined by measuring its UV-Vis absorbance at 480 nm and the percentage of the DOX released was then calculated.

III. RESULTS AND DISCUSSION

A. Synthesis and characterization of amphiphilic dendrimer-like copolymers

Well-defined amphiphilic dendrimer-like diblock copolymer, PEEGE-G2-b-PEO$(OH)_{12}$, was synthesized based on an iterative divergent method combining AROP and chain-end functionalization/branching reactions. As discussed in detail in a previous work [11], the best conduction suited to polymerize EEGE from multifunctional hydroxylated precursors required that the latter was partially deprotonated (below 30%) by a solution of DPMK and carried out in the mixed solvent of THF and DMSO (volume ratio of 2/3).

The three-arm PEEGE star was first prepared by AROP of EEGE from 1,1,1-tri(hydroxymethyl)ethane [9−11]. PEEGE-G1$(OH)_3$ had a low polydispersity index and predefined molar masses because of the living character of AROP of EEGE. A typical ^{1}H-NMR spectrum was shown in Fig.2(A). The peaks at δ=4.75−4.63 ppm are ascribed to the methene protons of the EEGE moiety, the doublets at δ=1.30 ppm and the triplet at δ=1.19 ppm are ascribed to the methyl protons of EEGE moiety and the chemical shift at δ=4.0−3.2 ppm is assigned to the protons of the main chain and the protons of the lateral chains. The methyl protons of the core are clearly detected at 0.9 ppm.

The integration ratio of these signals provided us with an estimation of the molar mass of PEEGE-G1$(OH)_3$ sample (see Table I), using the following equation:

$$\overline{M}_n = 120 + 146.18\frac{3I_a}{7I_b} \tag{1}$$

$$M_{n\mathrm{NMR}} = 3(2^{n-1}-1)\times 75 + 146.18\frac{3I_a}{7I_b} + 120, \quad n \le 2 \tag{2}$$

$$M_{n\mathrm{NMR}} = 3(2^{n-1}-1)\times 75 + 146.18\frac{3I_{a(n-1)}}{7I_{b(n-1)}} + 44.04\times \frac{I_{a(n)} - I_{\mathrm{a}(n-1)}}{4} + 120, \quad n = 3 \tag{3}$$

where I_a is the integration of the peak due to the methene protons of the EEGE main chain at 4.0−3.2 ppm, I_b is the integration of the peak due to the methyl protons of the core at 0.9 ppm, 146.18 is the molar mass of one EEGE unit, 120 is the molar mass of

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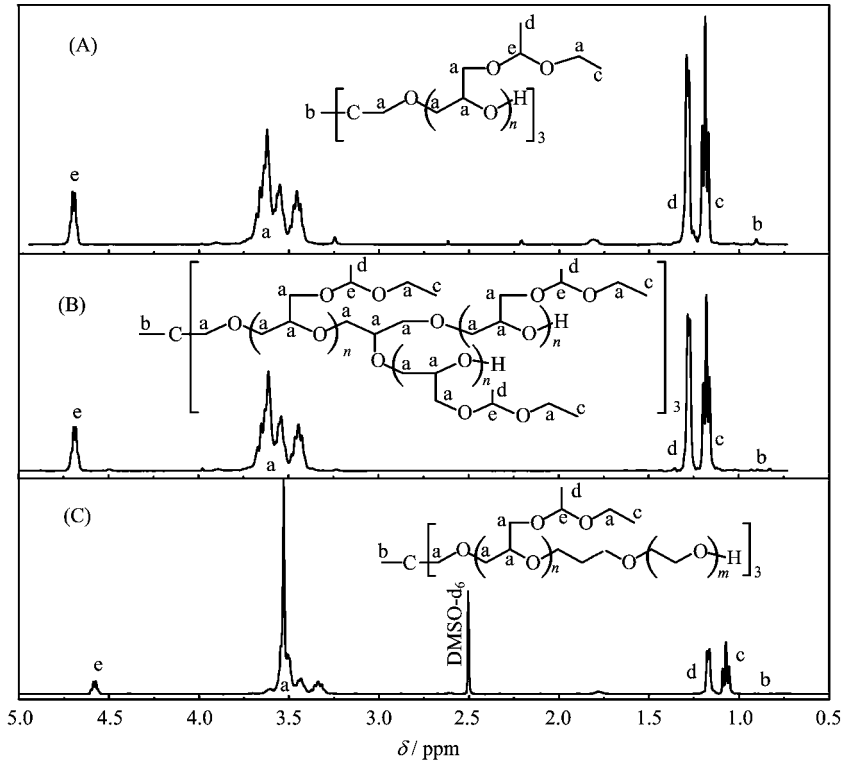

FIG. 2 ^{1}H-NMR spectra of different generation polymers. (A) PEEGE-G1(OH)$_3$, (B) PEEGE-G2(OH)$_6$, and (C) PEEGE-G2-b-PEO(OH)$_{12}$.

TABLE I Characteristic data of amphiphilic dendrimer-like diblock copolymer.

Sample	$\overline{M}_{\text{theor}}$	$\overline{M}_{n\text{NMR}}$[a]	$\overline{M}_{n\text{GPC}}$	PDI	N_{OH}[b]
PEEGE-G1(OH)$_3$	8880	9337	8198	1.15	3
PEEGE-G2(OH)$_6$	26656	26994	24917	1.32	6
PEEGE-G2-b-PEO(OH)$_{12}$	48235	46182	41592	1.22	12

[a] The total molar masses were calculated by Eqs. (1)−(3).
[b] Theoretical values of peripheral hydroxyl groups.

the core, n is the number of generation, 75 is the molar mass of branching points, 44.04 is the molar mass of the EO unit. For the sake of simplicity, we omitted the contribution from the five protons arising from each branching points and did not subtract the corresponding intensity from the peak appearing at around 4.0−3.2 ppm. The average molar mass calculated by the equation above from ^{1}H NMR spectra was 9.337 kg/mol corresponding to the GPC result (Fig.3(a)).

Each hydroxyls of PEEGE-G1(OH)$_3$ was derivatized twice to get PEEGE-G1(OH)$_6$. A two-step reaction was employed, which is shown in Fig.4. First, the branching agent reacted with PEEGE-G1(OH)$_3$ in a dried THF, affording a three-arm PEEGE star, denoted as PEEGE-G1(allyl)$_3$, carrying three vinyl groups. Secondly, the later star compound was in turn submitted to a bis-hydroxylation reaction using OsO_4 and N-methylmorpholine-N-oxide in a mixture of THF, water, and tert-butyl alcohol. This allowed us to obtain PEEGE-G1(OH)$_6$, which was end-functionalized with primary and secondary hydroxyl groups at each arm.

The branching reaction was monitored by ^{1}H NMR spectroscopy. Figure 5 shows ^{1}H NMR spectra of the PEEGE derivatives, PEEGE-G1(allyl)$_3$ and PEEGE-G1(OH)$_6$, respectively. After the treatment with the branching agent, the protons characteristic of the double bonds of PEEGE-G1(allyl)$_3$ are detected between 5 and 6 ppm. After the treatment with OsO_4 and N-methylmorpholine-N-oxide, the vinyl protons completely vanish.

Using PEEGE-G1(OH)$_6$ as a precursor, a sequence of steps (i), (ii), and (iii) shown in Fig.1 was applied to produce the second-generation dendrimer-like PEEGE, denoted as PEEGE-G2(OH)$_6$. The degree of polymerization (DP) of each branching arms of the second generation (PEEGE-G2(OH)$_6$) was set to be 20. The same

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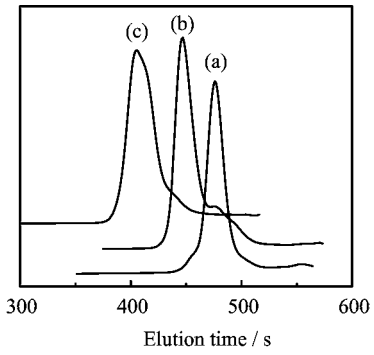

FIG. 3 Gel permeation chromatography (GPC) traces of intermediates and product. (a) PEEGE-G1$(OH)_3$, (b) PEEGE-G2$(OH)_6$, and (c) PEEGE-G2-b-PEO$(OH)_{12}$.

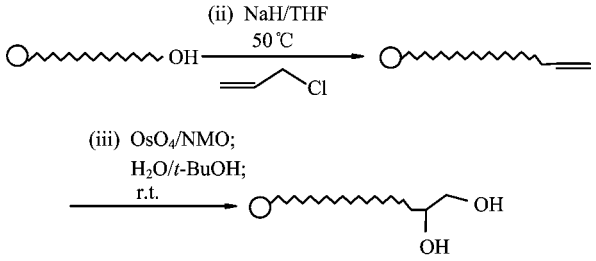

FIG. 4 Branching reaction onto PEEGE arm ends involving steps (ii) and (iii).

as before, only 30% of the end hydroxyls were deprotonated using DPMK in the solvent of DMSO/THF in step (i) of Fig.1.

The dendrimer-like PEEGE sample of the second generation was obtained after 48 h of reaction. Figure 2(B) gives the ^{1}H NMR spectra of PEEGE-G2$(OH)_6$ and shows an average molar mass of 26.994 kg/mL closed to the theoretical value. Simultaneously, the GPC trace of PEEGE-G2$(OH)_6$ indicates a symmetrical and unimodal shape with a marked shift to the higher molar mass region with regard to that of the PEEGE-G1$(OH)_3$ precursor (Fig.3(b)).

To get an amphiphilic dendrimer-like diblock copolymer, EO was extended to the outer layer of polymer instead of EEGE, and the degree of polymerization was set to be 40. The same procedure as the synthesis of PEEGE-G2$(OH)_6$ was applied for the PEEGE-G2-b-PEO$(OH)_{12}$. The sample thus obtained was characterized by ^{1}H NMR and GPC spectroscopy, as shown in Fig.2(C) and Fig.3(c). Figure 2(C) shows an intensity increase at 4.0−3.3 ppm due to the PEO linked to outer layer of dendrimer.

B. *In vitro* drug release property

We prepared DOX-loaded micelles and investigated their potential application in controlled drug release. In this case, DOX was physically entrapped and stabilized

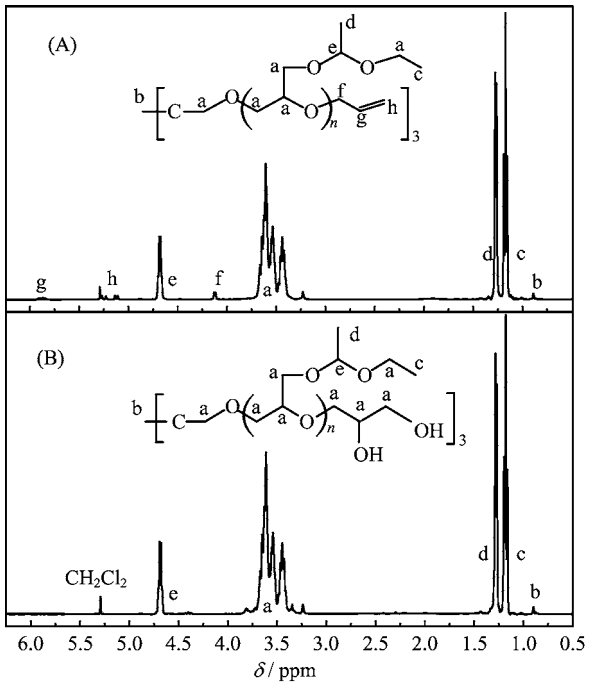

FIG. 5 ^{1}H NMR spectra of the PEEGE derivatives. (A) PEEGE-G1(allyl)$_3$, (B) PEEGE-G1$(OH)_6$.

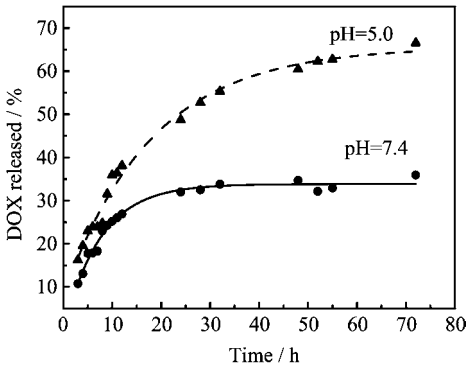

FIG. 6 Cumulative release profiles of DOX from the DOX-loaded micelles at pH=7.4 and 5.0 respectively at 37 °C.

in the hydrophobic cores of the micelles by hydrophobic interactions against water. It was found that DOX was successfully incorporated into polymeric micelles. The drug loading content and encapsulation efficiency were around 13.07% and 45.75%, respectively.

In order to study the release behaviors of drug under acidic conditions, the release experiment of DOX was performed respectively in the PBS buffer solutions of pH=5.0 and 7.4. As shown in Fig.6, the release profile was characterized by a burst release in initial stage (0−12 h), followed by a level off up to 72 h for pH=7.4. On the contrary, the DOX release rate at pH=5.0 was much faster and more sustained than that at pH=7.4. For example, 67% of the DOX was released from the micelles at pH=5.0 at 72 h, whereas only 36% of the

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drug was released at pH=7.4 during the same period of time. The fast and sustained release of DOX at pH=5.0 was mainly attributed to the hydrolysis of PEEGE inner core. The ethoxyethyl protecting group can be removed in acid condition [24−26], yielding a hydrophilic PG. This pH-dependent releasing behavior is considered to be beneficial to the application of tumor-targeted DOX delivery. It is well known that the pH is about 5.0−6.5 in endosome, but the pH is about 7.4 in cytosol and blood [27]. Further improvement of the structure of dendrimer-like polymer for drug carrier and detailed release investigation are in progress.

IV. CONCLUSION

In conclusion, a well-defined dendrimer-like PEEGE-G2-b-PEO$(OH)_{12}$ with 12 hydroxyl groups at the periphery was successfully prepared through an iterative divergent approach based on AROP of the acetal-protected ethoxy ethyl glycidol ether and ethylene oxide. NMR and GPC characterizations confirmed the structures of reaction intermediates and final product (PEEGE-G2-b-PEO$(OH)_{12}$). The dendrimer-like copolymers can form micelles in aqueous solution. DOX was loaded into the micelles and a sustained release was observed *in vitro* experiments at pH=7.4, 37 °C. It is found that the sustained release rate can be accelerated at pH=5.0 due to the hydrolysis of PEEGE under these conditions. A detailed study on drug delivery is under considering.

V. ACKNOWLEDGMENTS

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