

# pH-Responsive Biodegradable Micelles Based on Acid-Labile Polycarbonate Hydrophobe: Synthesis and Triggered Drug Release

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pH-responsive biodegradable micelles were prepared from block copolymers comprising of a novel acid-labile polycarbonate hydrophobe and poly(ethylene glycol) (PEG). Two new cyclic aliphatic carbonate monomers, mono-2,4,6-trimethoxybenzylidene-pentaerythritol carbonate (TMBPEC, **2a**) and mono-4-methoxybenzylidene-pentaerythritol carbonate (MBPEC, **2b**) were designed and successfully synthesized via a two-step procedure. The ring-opening polymerization of **2a** or **2b** in the presence of methoxy PEG in dichloromethane at 50 °C using zinc bis[bis(trimethylsilyl)amide] as a catalyst yielded the corresponding block copolymers PEG-PTMBPEC (**3a**) or PEG-PMBPEC (**3b**) with low polydispersities (PDI 1.03–1.04). The copolymerization of D,L-lactide (DLLA) and **2a** under otherwise the same conditions could also proceed smoothly to afford PEG-P(TMBPEC-co-DLLA) (**3c**) block copolymer. These block copolymers readily formed micelles in water with sizes of about 120 nm as determined by dynamic light scattering (DLS). The hydrolysis of the acetals of the polycarbonate was investigated using UV/vis spectroscopy. The results showed that the acetals of micelles **3a**, while stable at pH 7.4 are prone to rapid hydrolysis at mildly acidic pH of 4.0 and 5.0, with a half-life of 1 and 6.5 h, respectively. The acetal hydrolysis resulted in significant swelling of micelles, as a result of change of hydrophobic polycarbonate to hydrophilic polycarbonate. In comparison, the acetals of PMBPEC of micelles **3b** displayed obviously slower hydrolysis at the same pH. Both paclitaxel and doxorubicin could be efficiently encapsulated into micelles **3a** achieving high drug loading content (13.0 and 11.7 wt %, respectively). The in vitro release studies showed clearly a pH dependent release behavior, that is, significantly faster drug release at mildly acidic pH of 4.0 and 5.0 compared to physiological pH. These pH-responsive biodegradable micelles are promising as smart nanovehicles for targeted delivery of anticancer drugs.

## Introduction

Biodegradable micelles that are usually self-assembled from biodegradable amphiphilic block copolymers in water have emerged as one of the most promising nanocarrier systems for various hydrophobic drugs.<sup>1–4</sup> It is remarkable to note that a couple of micellar anticancer drug formulations, for example, NK911 and Genexol-PM, have already advanced to the clinical trials.<sup>5,6</sup> In the recent several years, much research effort has been directed to the development of stimuli-responsive micelles in that release of drug could be readily controlled by exerting an appropriate stimulus (e.g., temperature, pH, glucose, glutathione, etc.) after arrival at the target site.<sup>7–9</sup> Among all applied stimuli, acidic pH as an internal stimulus is particularly appealing due to the mildly acidic pH encountered in tumor and inflammatory tissues as well as in the intracellular compartments such as endosomes and lysosomes of cells.<sup>10</sup> The existing tumoral pH variation has been considered as an ideal trigger for the selective release of anticancer drugs in tumor tissues and within tumor cells, accomplishing tumor-targeted drug delivery.

To obtain acid-responsive micelles, different acid labile bonds such as *ortho*-ester, hydrazone, *cis*-acotynyl, and acetal have been positioned either in the main chain, at the side chain, or at the terminal of the core-forming block.<sup>11</sup> For example, Heller and

co-workers have prepared acid-sensitive micelles based on block copolymers of poly(*ortho* ester) and poly(ethylene glycol) (PEG), in which degradation of the poly(*ortho* ester) block under acidic conditions led to the release of loaded anticancer drugs.<sup>12</sup> Park and co-workers have conjugated doxorubicin (DOX) to the end group of PEG-PLA through an acid sensitive hydrazone or *cis*-aconityl bond,<sup>13</sup> and Kataoka and co-workers prepared environment-sensitive micelles by grafting DOX to a PEG-polyaspartate block copolymer via a hydrazone linkage.<sup>14</sup> These two studies have pointed to the fact that conjugated DOX can be quickly released under endosomal pH, achieving high antitumor activity.

Recently, Frechet and co-workers have exploited acetals as acid-labile linkages for design of pH-responsive polymers, micelles, nanoparticles, and hydrogels.<sup>15–21</sup> In particular, they found that trimethoxybenzylidene acetals attached either to the side chain of poly(aspartic acid) (PAA) segment of PEG-PAA block copolymers or to the periphery of dendron of PEG-dendritic block copolymers showed unprecedented sensitivity toward mildly acidic pH.<sup>15,17</sup> The hydrolysis of acetals effectively disrupted the micellar assembly and quickly released encapsulated Nile Red or DOX under mildly acidic conditions. Bulmus and co-workers employing acetal bisacrylate as a degradable cross-linker prepared acid-labile polymeric core-shell particles<sup>22</sup> as well as core-cross-linked micelles via the reversible addition-fragmentation chain transfer (RAFT) polymerization.<sup>23</sup>

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The encapsulated hydrophobic drugs were released in a pH-dependent manner.

Aliphatic polycarbonates such as poly(trimethylene carbonate) along with aliphatic polyesters such as poly( $\epsilon$ -caprolactone), polylactide, and lactide-glycolide copolymers (PLGA) are among the most important synthetic biodegradable polymers.<sup>24</sup> In the past decade, various functional aliphatic polycarbonates that contain hydroxyl, carboxyl, amine, carboxylate, protected ketone, and vinyl pendant groups have been developed, which may on one hand improve their hydrophilicity, rate of degradation, and mechanical properties and on the other hand provide reactive centers for drug conjugation and further derivatization.<sup>25–36</sup>

In this study, we are interested in developing pH-responsive biodegradable micelles based on polycarbonate hydrophobe. Inspired by Frechet's work on cyclic benzylidene acetals,<sup>15,17,18</sup> we have designed two novel types of cyclic aliphatic carbonate monomers, mono-2,4,6-trimethoxybenzylidene-pentaerythritol carbonate (TMBPEC, **2a**) and mono-4-methoxybenzylidene-pentaerythritol carbonate (MBPEC, **2b**), which provide a direct excess to pH-responsive biodegradable polymers. The ring-opening polymerization of **2a** and **2b** in the presence of methoxy PEG yielded corresponding PEG-polycarbonate block copolymers with low polydispersities. Furthermore, we have shown that cyclic carbonate monomer **2a** can readily copolymerize with D,L-lactide (DLLA) to afford PEG-P(TMBPEC-co-DLLA). The micelle formation, pH-responsive hydrolysis of the micelles, as well as pH-responsive drug release were investigated.

## Experimental Section

**Materials.** *p*-Methoxybenzaldehyde, 2,4,6-trimethoxybenzaldehyde, *p*-toluenesulfonic acid monohydrate, and pentaerythritol were purchased from Alfa Aesar and used as received. Methoxy poly(ethylene glycol) (PEG,  $M_n = 5000$ , PDI = 1.03) was purchased from Fluka and dried by azeotropic distillation from anhydrous toluene. Zinc bis[bis(trimethylsilyl)amide] (97%) was purchased from Aldrich. Toluene and tetrahydrofuran (THF) were dried by refluxing over sodium wire under an argon atmosphere prior to distillation. Triethylamine and dichloromethane were dried by refluxing over CaH<sub>2</sub> under an argon atmosphere. Ethyl chloroformate was freshly distilled before use. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure prior to use.

**Preparation of 2,4,6-Trimethoxybenzylidene-pentaerythritol (1a).** Pentaerythritol (35.0 g, 25.74 mmol) and *p*-toluenesulfonic acid monohydrate (0.65 g, 3.42 mmol) were dissolved in 300 mL of DMF at 70 °C, and then the mixture was allowed to cool undisturbed. When the oil bath temperature cooled to 50 °C, stirring was started and 5.0 g of 2,4,6-trimethoxybenzaldehyde (2.55 mmol) and 40 g of 4 Å molecular sieves were added. The reaction was allowed to proceed with stirring at 50 °C for 4 days. Then, the molecular sieves were filtered off and the filtrate was concentrated under reduced pressure. The residue was washed with 0.1 M phosphate buffer (pH 7.4). The white solid product **1a** was collected by filtration and dried under vacuum for 2 days. Yield: 85.5%. Mp: 163–164 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  3.20 (d, 2H), 3.58 (d, 2H), 3.72 (s, 6H), 3.76 (s, 3H), 3.78 (d, 2H), 3.80 (d, 2H), 4.46 (m, 2H), 5.72 (s, 1H), 6.16 (s, 2H). Anal. Calcd for C<sub>15</sub>H<sub>22</sub>O<sub>7</sub>: C, 57.32; H, 7.01. Found: C, 56.31; H, 6.95.

**Preparation of *p*-Methoxybenzylidene-pentaerythritol (1b).** Compound **1b** was prepared according to a previous report.<sup>37</sup> Typically, pentaerythritol (25.0 g, 183.8 mmol) and water (180 mL) were added into a 250 mL flask, heated to 60 °C with stirring until all the solid was dissolved and then cooled to room temperature undisturbed. Under stirring, catalytic amount of hydrochloric acid and *p*-methoxybenzaldehyde (1 mL, 8.2 mmol) were added. When the precipitate formed, second portion of *p*-methoxybenzaldehyde (22.4 mL, 184 mmol) was

added dropwise. The reaction was allowed to proceed for additional 3 h after completion of addition. The precipitate was collected on a Büchner funnel and washed with ice-cold slightly alkaline water (sodium carbonate solution). The white solid product **1b** was dried over phosphorus pentoxide (42.4 g, 91%). Mp: 164–166 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  3.24 (d, 2H), 3.66 (d, 2H), 3.75 (s, 3H), 3.77 (d, 2H), 3.88 (d, 2H), 4.47 (t, 1H), 4.55 (t, 1H), 5.34 (s, 1H), 6.89 (d, 2H), 7.32 (d, 2H). Anal. Calcd for C<sub>13</sub>H<sub>18</sub>O<sub>5</sub>: C, 61.42; H, 7.09. Found: C, 61.76; H, 7.00.

**Preparation of Mono-2,4,6-trimethoxybenzylidene-pentaerythritol Carbonate (TMBPEC, 2a) and Mono-4-methoxybenzylidene-pentaerythritol Carbonate (MBPEC, 2b).** The two cyclic carbonate monomers were synthesized in practically the same manner. A typical example for the synthesis of **2a** was as follows. In a 250 mL flask were placed **1a** (2.37 g, 7.55 mmol) and dried THF (140 mL). The mixture was heated with stirring until all solid was dissolved and was subsequently cooled to 0 °C. Then ethyl chloroformate (1.6 mL, 15.85 mmol) was charged, followed by dropwise addition of triethylamine (2.4 mL, 17.29 mmol). The reaction mixture was stirred for another 4 h at 0 °C. The precipitated triethylammonium chloride salt was filtered off, and the filtrate was concentrated under reduced pressure. The residue **2a** was recrystallized from anhydrous THF. Yield: 44.7%. Mp: 184–186 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.79 (d, 2H), 3.81 (s, 3H), 3.83 (s, 6H), 4.06 (s, 2H), 4.15 (d, 2H), 4.86 (s, 2H), 6.01 (s, 1H), 6.11 (s, 2H). Anal. Calcd for C<sub>16</sub>H<sub>20</sub>O<sub>8</sub>: C, 56.47; H, 5.88. Found: C, 56.38; H, 6.00. The yield for **2b** after recrystallization from anhydrous THF was 21.5%. Mp: 190–193 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.82 (s, 3H), 3.86 (d, 2H), 4.08 (s, 2H), 4.19 (d, 2H), 4.70 (s, 2H), 5.46 (s, 1H), 6.91 (d, 2H), 7.38 (d, 2H). Anal. Calcd for C<sub>14</sub>H<sub>16</sub>O<sub>6</sub>: C, 60.00; H, 5.71. Found: C, 60.12; H, 5.86.

**Amphiphilic Block Copolymer Synthesis.** The ring-opening polymerization of TMBPEC (**2a**) and MBPEC (**2b**) was carried out in CH<sub>2</sub>Cl<sub>2</sub> at 50 °C using zinc bis[bis(trimethylsilyl)amide] as the catalyst and PEG as an initiator. In a typical example, in the glovebox under a nitrogen atmosphere to a stirring solution of PEG (0.5 g, 0.1 mmol) and TMBPEC (0.4 g, 1.17 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was quickly added zinc bis[bis(trimethylsilyl)amide] (29 mg, 0.08 mmol). The reaction vessel was sealed and placed in an oil-bath thermostatted at 50 °C. The polymerization was allowed to proceed with magnetic stirring for 5 days. The polymer (**3a**) was isolated by twice precipitation from cold diethylether and dried under vacuum at room temperature. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) for polymer **3a**:  $\delta$  3.38 (s, PEG-OCH<sub>3</sub>), 3.64 (s, -CH<sub>2</sub>OCH<sub>2</sub>-), 3.77–3.82 (d, Ar-OCH<sub>3</sub>), 4.10 (d, -OCH<sub>2</sub>CCH<sub>2</sub>O-), 4.74 (s, -COCH<sub>2</sub>C-), 5.98 (s, Ar-CH-), 6.08 (s, aromatic protons). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) for polymer **3b**:  $\delta$  3.38 (s, PEG-OCH<sub>3</sub>), 3.64 (s, -CH<sub>2</sub>OCH<sub>2</sub>-), 3.78 (s, Ar-OCH<sub>3</sub>), 4.12 (s, -OCH<sub>2</sub>CCH<sub>2</sub>O-), 4.56 (s, -COCH<sub>2</sub>C-), 5.39 (s, Ar-CH-), 6.88–7.40 (d, aromatic protons).

**Synthesis of PEG-P(TMBPEC-co-DLLA) (3c).** In the glovebox under a nitrogen atmosphere to a stirring solution of PEG (0.4 g, 0.08 mmol), TMBPEC (0.2 g, 0.59 mmol), and D,L-lactide (DLLA, 0.12 g, 0.83 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was quickly added zinc bis[bis(trimethylsilyl)amide] (29 mg, 0.08 mmol). The reaction vessel was sealed and placed in an oil-bath thermostatted at 50 °C. The polymerization was allowed to proceed with magnetic stirring for 5 days. The polymer was isolated by twice precipitation from cold diethylether and dried under vacuum at room temperature. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) for polymer **3c**:  $\delta$  1.56 (d, -CH<sub>3</sub>), 3.38 (s, PEG-OCH<sub>3</sub>), 3.64 (s, -CH<sub>2</sub>OCH<sub>2</sub>-), 3.78–3.81 (d, Ar-OCH<sub>3</sub>), 4.06 (d, -OCH<sub>2</sub>CCH<sub>2</sub>O-), 4.74 (s, -COCH<sub>2</sub>C-), 5.17 (q, -COCH-), 5.95 (s, Ar-CH-), 6.09 (s, aromatic protons).

**Characterization.** The <sup>1</sup>H NMR spectra were recorded on a Unity Inova 400 spectrometer operating at 400 MHz using deuterated chloroform (CDCl<sub>3</sub>), deuterated dimethylsulfoxide (DMSO-*d*<sub>6</sub>) and deuterated water (D<sub>2</sub>O) as solvents. The chemical shifts were calibrated against residual solvent signals of CDCl<sub>3</sub>, DMSO-*d*<sub>6</sub>, and D<sub>2</sub>O. The micelles **3a** before and after acetal hydrolysis in D<sub>2</sub>O were studied by <sup>1</sup>H NMR. The micelles were prepared by dropwise addition of 2.0 mL

of D<sub>2</sub>O into a THF solution (0.6 mL) of copolymer **3a** (10 mg/mL) followed by stirring overnight to remove THF. The resulting micelles solution (3 mg/mL) was divided into two aliquots. One was used directly for <sup>1</sup>H NMR analysis. The other was treated with one drop of concentrated hydrochloric acid for 4 h, and then measured by <sup>1</sup>H NMR. The molecular weight and polydispersity of the copolymers were determined by a Waters 1515 gel permeation chromatograph (GPC) instrument equipped with two linear PLgel columns (500 Å and Mixed-C) following a guard column and a differential refractive-index detector. The measurements were performed using THF as the eluent at a flow rate of 1.0 mL/min at 30 °C and a series of narrow polystyrene standards for the calibration of the columns. The size of micelles was determined using dynamic light scattering (DLS). The micellar solutions were filtered through a 450 nm syringe filter before measurements. Measurements were carried out at 25 °C using Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He–Ne laser using backscattering detection. Transmission electron microscopy (TEM) was performed using a Tecnai G220 TEM operated at an accelerating voltage of 200 kV. The samples were prepared by dropping 10 µL of 0.1 mg/mL micellar solution on the copper grid followed by staining with phosphotungstic acid.

**Micelle Formation and Critical Micelle Concentration.** Typically, micelles were prepared under stirring by dropwise addition of 10 mL phosphate buffer (10 mM, pH 7.4) to 2.0 mL of block copolymer solution (1 wt %) in THF at room temperature. The resulting solution was stirred overnight to allow complete evaporation of THF.

The critical micelle concentration (CMC) was determined using pyrene as a fluorescence probe. The concentration of block copolymer was varied from  $2.0 \times 10^{-5}$  to 0.2 mg/mL and the concentration of pyrene was fixed at 1.0 µM. The fluorescence spectra were recorded using FLS920 fluorescence spectrometer with the excitation wavelength of 330 nm. The emission fluorescence at 372 and 383 nm were monitored. The CMC was estimated as the cross-point when extrapolating the intensity ratio  $I_{372}/I_{383}$  at low and high concentration regions.

**Determination of the pH-Dependent Hydrolysis Rate of Acetals in the Micelles.** The acetal hydrolysis was followed by UV/vis spectroscopy by measuring the absorbance at 290 nm for micelles **3a/3c** and 279 nm for micelles **3b**, according to the previous reports by Frechet and co-workers.<sup>15,17,18</sup> The micellar solution (2 mg/mL) prepared as above-mentioned was divided into four aliquots and adjusted to pH 4.0, 5.0, 6.0, and 7.4, respectively, by addition of 50 µL of 4.0 M pH 4.0, 5.0, and 6.0 acetate buffer or 7.4 phosphate buffer, while keeping the salt concentration the same. The solutions were shaken at 37 °C. At the desired time points, 80 µL aliquots were removed and diluted with 3.5 mL phosphate buffer (0.1 M, pH 7.4) and the absorbance at 290 nm for micelles **3a/3c** or 279 nm for micelles **3b** was measured. At the end, all the samples were completely hydrolyzed by addition of two drops of concentrated HCl and were measured again to determine the absorbance at 100% hydrolysis, which was used to calculate extent of acetal hydrolysis.

**Confocal Laser Scanning Microscopy (CLSM) Measurement.** A total of 1 mL of micellar solution was prepared at 0.1 mg/mL concentration and brought to pH 4.0 with acetate buffer (final buffer concentration 0.5 M). The samples were kept at 37 °C and change in micelle size was monitored by DLS. When the particle size reached about 1 µm, carboxy fluorescein (in water) and Nile red (in acetone) were added to the micelle solution (each with a final concentration of 5.0 µM) and stirred for 4 h to remove the organic solvent. CLSM analyses were performed with a Nikon Digital Eclipse C1si Confocal Laser Scanning Microscope (Nikon).

**Encapsulation and Release of Nile Red.** Nile red was loaded into micelles **3a**, **3b**, or **3c** by adding 20 µL of 1 mM Nile red in acetone to 0.4 mL of copolymer solution in THF (5 mg/mL), followed by dropwise addition of 10 mL phosphate buffer (10 mM, pH 7.4). To completely evaporate THF and acetone, the solution was stirred overnight and then vacuumed for 1 h. Hence, all micelles have a concentration of 0.2 mg/mL and contain 2.0 µM Nile Red. The solution was divided into three

3 mL samples and the fluorescence intensity of each was measured as 100% intensity. Then the samples were adjusted to pH 4.0, 5.0, and 7.4, respectively, by addition of 75 µL of 4.0 M pH 4.0 and 5.0 acetate buffer or 7.4 phosphate buffer, while keeping the salt concentration the same. The samples were stirred at 37 °C and the fluorescence intensity was measured at the desired time points.

**Encapsulation and Release of Paclitaxel and DOX.** Paclitaxel was loaded into micelles **3a** by dropwise addition of 6 mL of phosphate buffer (10 mM, pH 7.4) to a mixture of 1 mL of copolymer **3a** solution in THF (4 mg/mL) and 40 or 160 µL paclitaxel solution in DMSO (5 mg/mL) under stirring at room temperature, followed by ultrasonication for 1 h and dialysis against phosphate buffer (10 mM, pH 7.4) for 16 h at rt with a molecular weight cutoff (MWCO) of 3500.

The release profiles of paclitaxel from micelles **3a** were studied at 37 °C in three different media, that is, (a) acetate buffer, pH 4.0; (b) acetate buffer, pH 5.0; and (c) phosphate buffer, pH 7.4. The concentrations of the release media were 0.1 M. The above prepared paclitaxel-loaded micelle solution (0.5 mg/mL micelle **3a** and 25 µg/mL paclitaxel) was divided into three aliquots. Each aliquot was adjusted to a certain pH (pH 4.0 or 5.0 using acetate buffer or to pH 7.4 using phosphate buffer), and immediately transferred to a dialysis tube with a MWCO of 12000–14000. The dialysis tube was immersed into 20 mL of corresponding buffer (0.1 M) and the media was stirred at 37 °C. At desired time intervals, 5 mL of release media was taken out for HPLC measurement and replenished with an equal volume of fresh media. The amount of paclitaxel was determined by HPLC (Waters 1525), with UV detection at 227 nm using a 1/1 (v/v) mixture of acetonitrile and water as a mobile phase. The release experiments were conducted in triplicate. The results presented are the average data.

The encapsulation and release of DOX was carried out in a similar way as for paclitaxel, except that the whole procedure was performed in the dark and the amount of DOX was determined using fluorescence (FLS920) measurement (excitation at 600 nm). For determination of drug loading content, DOX loaded micelles were dissolved in DMSO and analyzed with fluorescence spectroscopy, wherein calibration curve was obtained with DOX/DMSO solutions with different DOX concentrations. To determine the amount of DOX released, calibration curves were run with DOX/corresponding buffer solutions with different DOX concentrations at pH 4.0, 5.0 and 7.4, respectively. The emission spectra were recorded from 500 to 680 nm.

Drug loading content and drug loading efficiency were calculated according to the following formula:

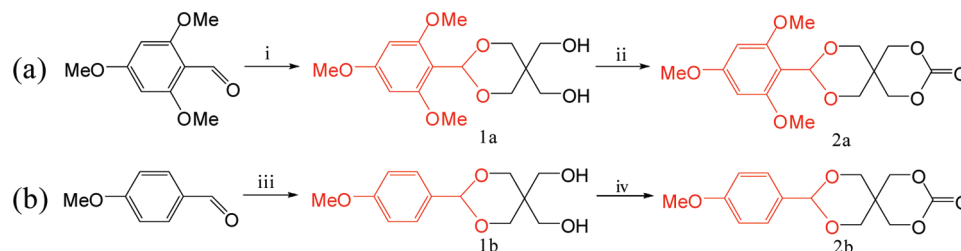
$$\text{Drug loading content (wt\%)} = \frac{\text{weight of loaded drug}}{\text{weight of polymer}} \times 100\%$$

$$\text{Drug loading efficiency (\%)} = \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \times 100\%$$

## Results and Discussion

The aim of the present study was set to develop pH-responsive biodegradable micelles that may be used for targeted delivery of anticancer drugs. In the past years, different micellar systems, of which most are based on biodegradable polyester and polycarbonate hydrophobes due to their unique biocompatibility, biodegradability and nontoxicity, have been investigated for the delivery of a variety of clinically important anticancer drugs in vitro and in vivo.<sup>38–40</sup> However, one significant challenge for the biodegradable micellar drug carriers has been lack of a mechanism to quickly release loaded drugs upon arrival at the tumor site(s). Slow drug release could result in decreased drug efficacy and increase the possibility of developing multidrug resistance (MDR).<sup>41</sup> Rapidly acid-responsive biodegradable micelles may address this issue by taking advantage of the mildly acidic pH encountered in tumor and inflammatory tissues



**Scheme 1.** Synthesis of Mono-2,4,6-trimethoxybenzylidene-pentaerythritol Carbonate (TMBPEC, **2a**) and Mono-4-methoxybenzylidene-pentaerythritol Carbonate (MBPEC, **2b**) Monomers<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i) pentaerythritol, *p*-toluenesulfonic acid, molecular sieves, DMF, 50 °C; (ii, iv) ethyl chloroformate, triethylamine, THF, 0 °C; (iii) pentaerythritol, concentrated HCl, water, rt.

as well as in intracellular compartments such as endosomes and lysosomes of cells, which can elegantly be applied as an internal signal for activated drug release.<sup>10</sup>

To develop rapidly acid-responsive biodegradable micelles, we have designed two novel cyclic carbonate monomers, mono-2,4,6-trimethoxybenzylidene-pentaerythritol carbonate (TMBPEC, **2a**) and mono-4-methoxybenzylidene-pentaerythritol carbonate (MBPEC, **2b**; Scheme 1), which can readily undergo ring-opening (co)polymerization to provide micelle-forming amphiphilic block copolymers.

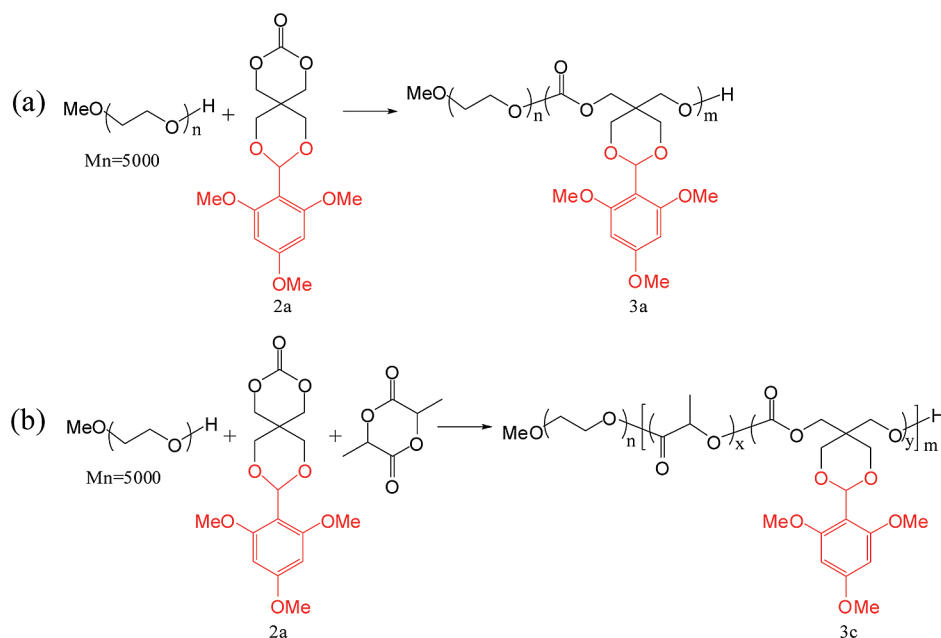
**Preparation of Cyclic Carbonate Monomers.** The two cyclic aliphatic carbonate monomers, **2a** and **2b**, were synthesized in two steps as shown in Scheme 1. The reaction of 2,4,6-trimethoxybenzaldehyde with excess Pentaerythritol in the presence of catalytic amount of *p*-toluenesulfonic acid and excess 4 Å molecular sieves in DMF at 50 °C, followed by washing with 0.1 M pH 7.4 phosphate buffer, produced 2,4,6-trimethoxybenzylidene-pentaerythritol (**1a**) with high yield. The <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>; Figure S1a) revealed signals attributable to the aromatic protons (δ 6.16), acetal proton (δ 5.72), and methoxy protons (δ 3.72, 3.76) of 2,4,6-trimethoxybenzylidene acetal moiety as well as hydroxyl protons (m, δ 4.46), methylene protons neighboring to dioxane (dd, δ 3.58, 3.80), and methylene protons next to hydroxyl group (dd, δ 3.20, 3.78) of pentaerythritol moiety. The integral ratios correlated well with equivalent 2,4,6-trimethoxybenzylidene acetal and pentaerythritol moieties, confirming successful synthesis of **1a**. The synthesis of *p*-methoxybenzylidene-pentaerythritol (**1b**) was slightly different. The reaction was instead carried out in the presence of catalytic amount of HCl in water at room temperature, and the product was washed with cold slightly alkaline water (sodium carbonate solution) and dried over phosphorus pentoxide, as reported previously.<sup>37</sup> The <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) spectrum as well as the signal assignments are given in Figure S1b, which clearly pointed to the successful synthesis of **1b**.

In the following step, cyclic carbonate monomers **2a** and **2b** were synthesized from **1a** and **1b**, respectively. The cyclization was performed in the presence of ethyl chloroformate in dilute anhydrous THF solution at 0 °C via dropwise addition of triethylamine. The products were purified by recrystallization from anhydrous THF to afford **2a** and **2b** with moderate yields (21.5–44.7%). The <sup>1</sup>H NMR spectra (CDCl<sub>3</sub>) of **2a** and **2b** as well as the signal assignments are shown in Figure S2. Taking **2a** as an example, the resonances at δ 6.11, 6.01, and 3.81–3.83 were assignable to the aromatic protons, acetal proton, and methoxy protons, respectively. The two singlets at δ 4.86/4.06 and the double doublets at δ 4.15/3.79 were attributable to the methylene protons next to the carbonate and dioxane, respectively. The integral ratios of signals of the acetal proton and the methylene protons neighboring to the carbonate are close to the theoretical value. Importantly, no peak at δ 3.00–3.75

assignable to the methylene protons adjacent to the hydroxyl group was detected, implying absence of **1a**. The elemental analysis has further confirmed the successful synthesis of **2a**. The <sup>1</sup>H NMR and elemental analysis of **2b** showed similar outcome.

**Synthesis of Amphiphilic Copolymers Comprising PEG and Polycarbonate.** The ring-opening polymerization of **2a** and **2b** was carried out in the presence of methoxy PEG (*M*<sub>n</sub> = 5000) in CH<sub>2</sub>Cl<sub>2</sub> at 50 °C using zinc bis[bis(trimethylsilyl)amide] as a catalyst (Scheme 2). The weight ratio of PEG to monomer in the feed was 1:0.8. The polymerization proceeded slowly, most probably due to the presence of the sterically hindering benzylidene acetal substituent. Nevertheless, polymerization of **2a** and **2b** for 4–5 days yielded corresponding block copolymers, PEG-PTMBPEC (**3a**) and PEG-PMBPEC (**3b**), with notably low polydispersities (PDI 1.03–1.04; Table 1, entries 1, 2). In contrast, an attempt to prepare **3a** using stannous octoate as a catalyst in toluene at 120 °C failed. It has been reported that zinc bis[bis(trimethylsilyl)amide] brings about controlled ring-opening polymerization of lactide and ε-caprolactone under mild conditions.<sup>42,43</sup> The <sup>1</sup>H NMR of **3a** clearly showed peaks corresponding to PTMBPEC besides signals assignable to PEG (Figure S3a). The *M*<sub>n</sub> determined by comparing the integrals at δ 5.98 and 3.38, which were respectively attributed to the acetal proton of PTMBPEC and methoxy protons of PEG end, was 8700. The <sup>1</sup>H NMR of **3b** (Figure S3b) could be analyzed in a similar way and the *M*<sub>n</sub> was determined to be 7000 by comparing the integrals at δ 5.39 and 3.38. The GPC curves for both **3a** and **3b** were narrow and unimodal (not shown), and moreover, the *M*<sub>n</sub> values approximated those determined by <sup>1</sup>H NMR analysis. These results signified successful synthesis of structurally well-defined PEG-polycarbonate block copolymers **3a** and **3b**.

Interestingly, copolymerization of D,L-lactide (DLLA) and **2a** under otherwise the same polymerization conditions could also proceed smoothly. In an initial experiment, the weight ratio of PEG/**2a**/DLLA in the feed was set at 1:0.5:0.3 (Table 1, entry 3). As shown in Figure S3c, the <sup>1</sup>H NMR of the resulting copolymer, PEG-P(TMBPEC-*co*-DLLA) (**3c**), displayed signals attributable to PEG, PTMBPEC, and PDLLA, respectively. Notably, the peak assignable to the methylene protons next to the carbonate group shifted to a lower field and appeared to have two splits compared to polymer **3a**, which is most likely due to random copolymerization with DLLA. The *M*<sub>n</sub> was determined to be 6700 by comparing the integrals at δ 3.38 (methoxy protons of PEG), 5.95 (acetal proton of PTMBPEC), and 5.17 (methine proton of PDLLA). The copolymer had a TMBPEC/DLLA weight ratio of 1.36/1.0, which is close to the ratio in feed (TMBPEC/DLLA = 1.67/1.0). The GPC analysis revealed a narrow and unimodal distribution with a low PDI of 1.17 (Table 1, entry 3). The findings that these new cyclic

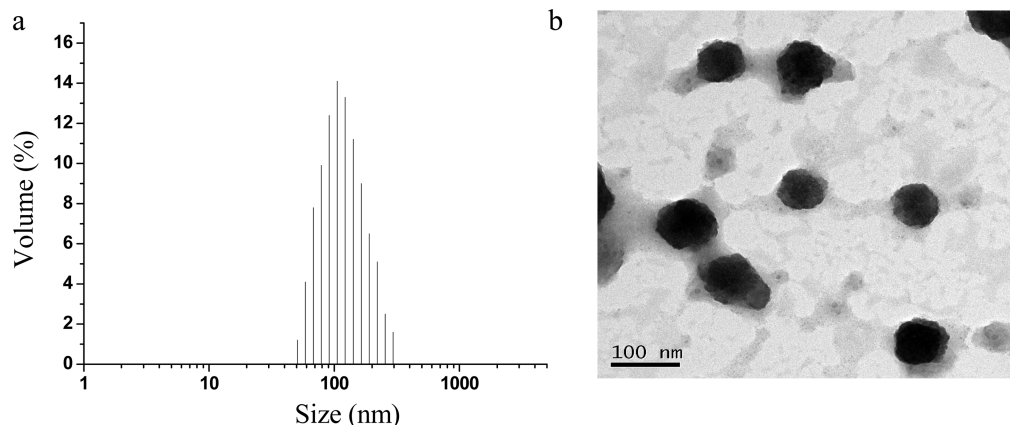
**Scheme 2.** Synthesis of PEG-PTMBPEC (**3a**) and PEG-P(TMBPEC-co-DLLA) (**3c**)<sup>a</sup>

<sup>a</sup> Using zinc bis[bis(trimethylsilyl)amide] as a catalyst in CH<sub>2</sub>Cl<sub>2</sub> at 50 °C.

**Table 1.** Synthesis of Amphiphilic Block Copolymers of PEG and Polycarbonate by Ring-Opening Polymerization<sup>a</sup>

entry	polymer	weight ratio in feed	Conv. (%)	<i>M<sub>n</sub></i> (theory)	<i>M<sub>n</sub></i> ( <sup>1</sup> H NMR)	<i>M<sub>n</sub></i> (GPC)	PDI (GPC)	CMC (mg/L)
1	PEG-PTMBPEC ( <b>3a</b> )	1:0.8	85	8400	8700	10000	1.04	0.43
2	PEG-PMBPEC ( <b>3b</b> )	1:0.8	82	8300	7000	8100	1.03	0.57
3	PEG-P(TMBPEC-co-DLLA) ( <b>3c</b> )	1:0.5:0.3	60	7400	8300	7700	1.17	0.52

<sup>a</sup> Polymerizations were carried out in CH<sub>2</sub>Cl<sub>2</sub> at 50 °C for 4–5 days using zinc bis[bis(trimethylsilyl)amide] as the catalyst and methoxy PEG (*M<sub>n</sub>* = 5000) as an initiator. The monomer concentration is 0.35 M.

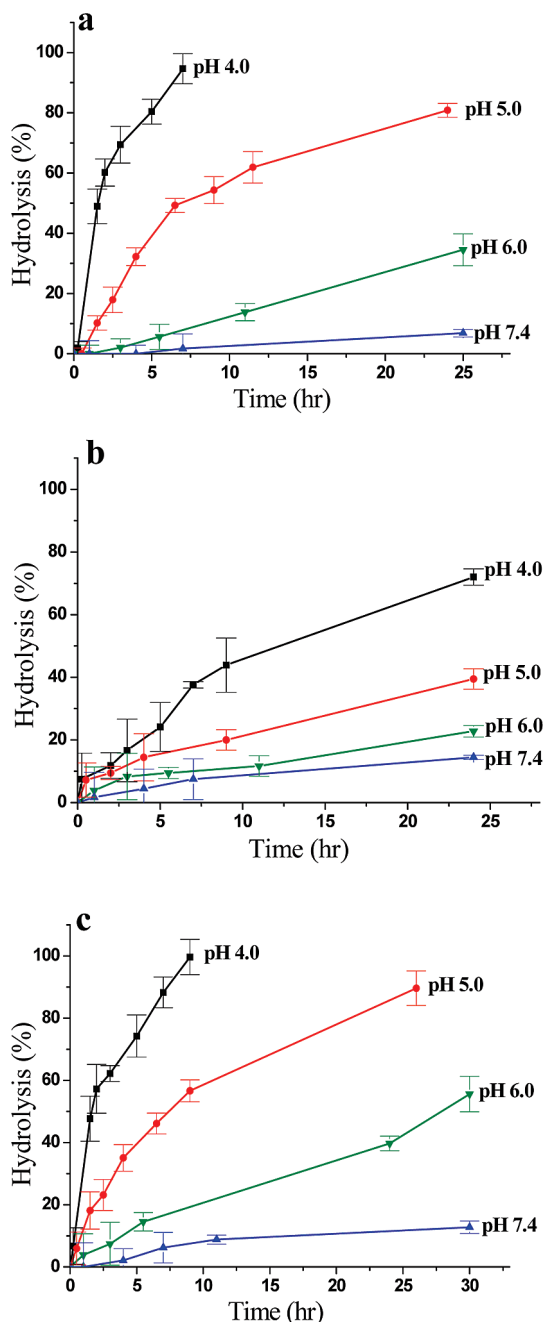
**Figure 1.** Size distribution profile for micelles **3a** determined by DLS (a) and TEM (b).

carbonate monomers are capable of copolymerizing with cyclic esters may greatly expand their potential applications.

**Micelle Formation and pH-Responsive Hydrolysis.** Micelles were prepared by dropwise addition of 10 mL phosphate buffer (10 mM, pH 7.4) to 2.0 mL of 1 wt % block copolymer solution in THF at room temperature, followed by stirring overnight and extensive dialysis to completely remove THF. The size of micelles was determined by DLS and TEM. DLS showed that all three copolymers **3a–c** formed micelles with sizes of approximately 120 nm with narrow size distributions (PDI < 0.10). Figure 1 shows a typical size distribution profile of micelles **3a**. TEM revealed homogeneous distribution of spherical particles with sizes of about 80 nm. The smaller size measured by TEM as compared to DLS was most probably due to shrinkage of the PEG shell upon drying. The CMC was

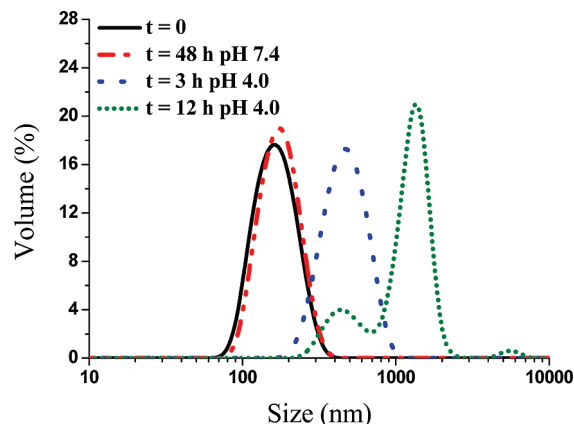
determined by fluorescence study using pyrene as a probe. The results showed that these copolymers had CMC values of 0.43–0.57 mg/L (Table 1).

The hydrolysis of the acetals of the polycarbonate within the micelle cores was investigated for each copolymer micelles at 37 °C and at different pH values (i.e., pH 7.4, 6.0, 5.0, and 4.0). The extent of acetal hydrolysis was conveniently determined as reported by Frechet and co-workers<sup>15,17</sup> using UV/vis spectroscopy by monitoring the absorbance at 290 or 279 nm, which are the characteristic absorbance of the hydrolysis products, 2,4,6-trimethoxybenzaldehyde (**3a** and **3c**) and *p*-methoxybenzaldehyde (**3b**), respectively. The results showed that the hydrolysis rate of the acetals of micelles **3a** is highly pH dependent (Figure 2a). While negligible hydrolysis was observed after 24 h at pH 7.4, rapid hydrolysis took place at



**Figure 2.** pH-Dependent hydrolysis of acetals in micelles (a) **3a**, (b) **3b**, and (c) **3c**.

pH 4.0 and 5.0, with half-lives of 1 and 6.5 h, respectively. The rates of hydrolysis were similar to those reported by Frechet et al. for PEG-PAA and PEG-dendritic block copolymers with trimethoxybenzylidene acetals attached at the side or periphery.<sup>15,17</sup> Interestingly, even at mildly acidic pH of 6.0, over 20% acetals were hydrolyzed after 24 h. It appeared that copolymerization with DLLA did not have much influence on the pH dependent hydrolysis of acetals (Figure 2c). In contrast, the acetals of micelles **3b** displayed obviously slower hydrolysis at the same acidic pH, with less than 40% acetals hydrolyzed after 24 h at pH 5.0 (Figure 2b). It should be noted, nevertheless, that the hydrolysis of acetals of micelles **3b** could be significantly enhanced at a lower pH of 4.0, at which 70% acetals were hydrolyzed after 24 h and an acetal half-life of 12 h was observed. The greater acid-sensitivity of **3a** as compared to **3b** could be attributed to the presence of two additional electron-



**Figure 3.** Time dependence of micelle size for copolymer **3a** at 37 °C at pH 7.4 (0.5 M phosphate buffer) and 4.0 (0.5 M acetate buffer), respectively, monitored by DLS.

donating methoxy groups in the *para*-positions of the aromatic ring relative to the acetal.<sup>15</sup>

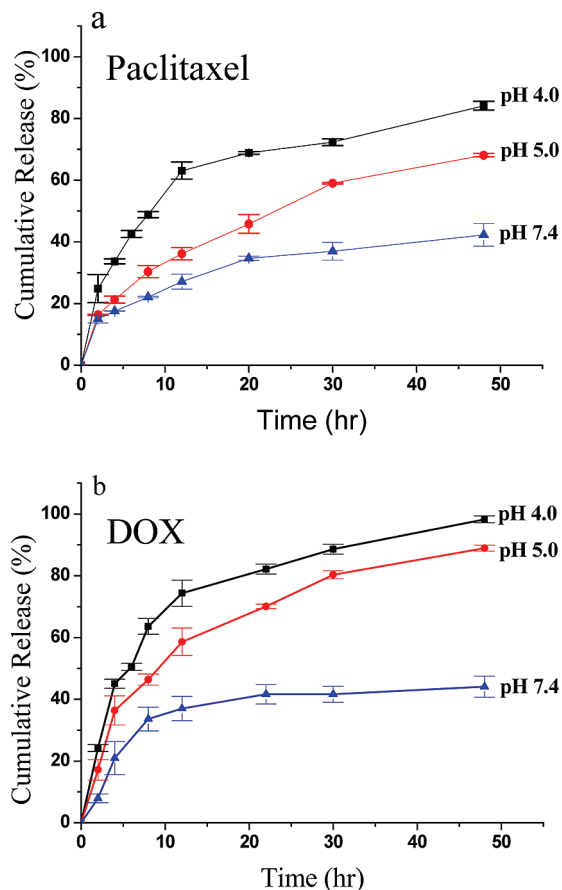
The size change of micelles **3a** in response to acetal hydrolysis was followed by DLS measurement. Interestingly, placement of micelles **3a** into pH 4.0 acetate buffer (0.5 M) resulted in rapid and remarkable swelling of micelles instead of micelle disruption (Figure 3). The micelle size increased from 150 nm to about 400 nm in 3 h, reaching over 1000 nm after 12 h. In contrast, no change of micelle size was observed over 2 days at pH 7.4 at the same buffer concentration. We have also performed a similar study with micelles **3b** as a control and no change of micelle size took place at different pHs over one day. It has been reported previously that hydroxy polycarbonate derived from pentaerythritol is highly hydrophilic but not soluble in water,<sup>27</sup> which could explain the maintenance of micellar structures even after complete acetal hydrolysis.

To confirm the amphiphilic nature of hydroxy polycarbonate, micelles **3a** after acetal hydrolysis were incubated with carboxy fluorescein (hydrophilic) and Nile red (hydrophobic) and then studied using CLSM. The results showed clearly colocalization of carboxy fluorescein and Nile red, supporting swelling of the particles (Figure 4). The <sup>1</sup>H NMR studies on micelles **3a** before and after acetal hydrolysis in D<sub>2</sub>O revealed that the intensity of the backbone peaks increased significantly after acetal hydrolysis (Figure S4), further corroborating swelling of the micelles. Verdenberg and Tian have demonstrated that hydroxy polycarbonate derived from Pentaerythritol is prone to rapid degradation in vitro due to most probably its highly hydrophilic nature.<sup>27</sup> Therefore, it could be hypothesized that micelles **3a** are not only pH-responsive but also subject to fast biodegradation after acetal hydrolysis (Scheme 3).

**Encapsulation and Release of Nile Red, Paclitaxel, and DOX.** The encapsulation and release of Nile red from micelles **3a**, **3b**, and **3c** were investigated at 37 °C at different pHs (i.e., pH 4.0, 5.0, and 7.4). The release of Nile red was followed by measurement of fluorescence and the results showed clearly a pH-dependent release behavior from micelles **3a**, in which the fluorescence of Nile red decreased more than 20 and 30% at pH 5.0 and 4.0, respectively, within 5 h, while little change was observed for the sample at pH 7.4 (Figure 5a). In comparison, the release rates from micelles **3b** and **3c** revealed much smaller dependence on the pH than micelles **3a** (Figure 5b,c). Given the fact that the acetal hydrolysis rates are similar for micelles **3a** and **3c** (Figure 2), the slow release of Nile red from micelles **3c** is most probably due to the presence of the hydrophobic DLLA units. In contrast, the slow release from







**Figure 6.** Cumulative release of paclitaxel (a) and DOX (b) using micelles **3a** at 37 °C and different pHs.

5.0, whereas at pH 7.4 no precipitation was observed after 1 day. We were unable to determine the exact release profile due to drug precipitation. However, drug precipitation occurred in a time scale similar to that of the acetal hydrolysis, indicating that fast drug release at mildly acidic conditions was triggered by acetal hydrolysis. The combination of high drug loading capacity and rapid drug release at endosomal pHs renders these pH-responsive biodegradable micelles promising as smart nanovehicles for targeted delivery of anticancer drugs.

### Conclusions

We have successfully developed pH-responsive biodegradable micelles based on block copolymers comprising of a novel acid-labile polycarbonate hydrophobe. The rational design of pH-sensitive cyclic ester or carbonate monomers has allowed facile access to pH-responsive biodegradable polymers via ring-opening (co)polymerization. The present work is interesting in that (1) it allows easy access to predesigned amphiphilic block copolymers with desired PEG and acid-sensitive polycarbonate block lengths; (2) the cyclic carbonate monomers **2a** and **2b** are capable of copolymerizing with cyclic ester and/or other cyclic carbonate monomers to afford micelle cores with varying properties; (3) the acetal hydrolysis while negligible at neutral pH is rapid under mildly acidic conditions; and (4) these micelles show a high drug loading capacity and a significantly faster drug release at endosomal pHs than at physiological pH. We are convinced that these pH-responsive biodegradable micelles will have potentials as intelligent nanovehicles for targeted delivery of various types of anticancer drugs.

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**Supporting Information Available.**  $^1\text{H}$  NMR spectra of monomers, polymers, and micelles **3a** before and after acetal hydrolysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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