Photoresponsive Biodegradable Poly(Carbonate)s with Pendent o-Nitrobenzyl Ester

Huihui Shen, Yingchun Xia, Zhouliang Qin, Juan Wu, Li Zhang, Yanbing Lu, Xinnian Xia, Weijian Xu

Institute of Polymer Science, College of Chemistry & Chemical Engineering, Hunan University, Changsha 410082, China
Correspondence to: Y. Lu (E-mail: yanbinglu@hnu.edu.cn)

Received 27 March 2017; accepted 24 May 2017; published online 00 Month 2017
DOI: 10.1002/pola.28679

ABSTRACT: Photoresponsive amphiphilic diblock poly(carbonate)s mPEG113-b-PMNCn with pendent o-nitrobenzyl ester group were synthesized through ring-opening polymerization (ROP) using 1,8-diazabi-cyclo[5.4.0]undec-7-ene (DBU) as catalyst and monomethoxy poly(ethylene glycol) (mPEG) as macroinitiator. In aqueous solution, the copolymers can self-assemble to spherical micelles with a PC core and a PEG shell. The critical micelle concentration (CMC), size, and morphology of the micelles were demonstrated by means of fluorescence spectroscopy, transmission electron microscopes (TEM), and dynamic light scattering (DLS). Under UV light irradiation, the amphiphilic copolymer micelles disassembled because of the photocleavage of o-NB ester, and the light-controlled release behaviors of payload Nile red were further proved. This study provides a convenient way to construct smart poly(carbonate)s nanocarriers for controlled drug release. © 2017 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. 2017, 00, 000–000

KEYWORDS: polycarbonates; stimuli-sensitive polymers; self-assembly; photoresponsive; amphiphilic block copolymer

INTRODUCTION In the clinical therapy of diseases such as cancer, it is a great challenge to regulate drug delivery and release behaviors which aim to improve the therapeutic efficacy and reduce side effects of drugs. Therefore, the design and preparation of environmentally responsive carriers have attracted significant attention over the past decades because of their potential applications in the biomedical fields.1–3 Amphiphilic copolymers composed of hydrophilic and hydrophobic blocks can self-assemble to different polymorphism of morphologies in water such as micelles, star micelles, vesicles, and complex supramolecular aggregates.4,5 In particular, self-assembled polymeric micelles are designed to improve bioavailability and biodistribution of small drugs in tumor tissues by means of retaining prolonged blood circulation and then extravasated into tumor tissues through enhancing permeability and retention (EPR) effect.6–8 Various stimuli-sensitive polymeric micelles responding to environmental stimuli such as pH, temperature, light, redox potential, and enzymes have been actively studied.9–21 Among these smart polymers, photoresponsive polymers have attracted great attentions due to the accurately adjusted remote, spatiotemporal control, and easy operation.22–24 o-Nitrobenzyl (o-NB) esters are a good candidate for constructing light-responsive materials as they are efficiently cleavable upon UV exposure.25 Historically, the photocleavage compounds containing o-NB groups have gained tremendous attention, which was used as photolabile protecting groups in the area of synthetic organic chemistry.25,26 It is based on the photocleavage of an o-NB ester into a corresponding o-nitrosobenzaldehyde and releasing a free carboxylic acid upon irradiation of UV light.27 Since then, the photocleavage compounds have been employed to kinds of other applications, such as controlled release, photoreconfigurable hydrogels, labels for cell signaling pathway investigations, and microfabrication.3 Early works by Jing et al. explored poly(carbonate)s bearing o-NB esters as photolabile chromophore, which can be cleaved by UV light to the corresponding carboxylic acid.28 Later, Zhao et al. turned their focus to o-NB group as a functional pendent in light responsive amphiphilic block copolymer and proved its light-cleavage nicely. The amphiphilic block copolymer composed of poly (ethylene oxide) and poly(2-nitrobenzyl methacrylate) (PNBM) via atom transfer radical polymerization (ATRP). Upon UV irradiation, 2-nitrobenzyl ester moieties were cleaved from the side chain, and the amphiphilic block copolymer turned into a double hydrophilic block copolymer, finally the micelles disassembled.29 Lee et al. synthesized an amphiphilic block copolymer PEO-b-[2-(1-azidobutryloxy)ethyl methacrylate] (PEO-b-PAzHEMA) via ATRP, and then alkyne-functionalized pyrene with a photolabile 2-nitrobenzyl moiety was added to the hydrophobic block of PEO-b-PAzHEMA via click chemistry to obtained the desired block copolymer. Upon irradiation of UV
light, 2-nitrobenzyl ester moieties were selectively cleaved, leading to the block copolymer released a model drug, 1-pyrenebutyric acid. Then, they encapsulated coumarin 102, another model drug into the core of micelles physically, studying the two drug models released at the same time under UV irradiation.\(^3\) Wang et al. synthesized a dual-stimuli-sensitive amphiphilic block copolymer poly(N-isopropylacrylamide)-block-poly-(2-nitrobenzyl methacrylate) (PNIPAM-b-PNBMM) with a photosensitive PNBM segment and a temperature-sensitive PNIPAM segment.\(^1\) In spite of many different strategies for the construction of photoresponsive polymers with o-NB esters are developed,\(^2\) there have been limited reports to deal with the biocompatible and biodegradable polymers, especially poly(carbonate)s with pendant o-NB group applied to drug delivery system.

As important biodegradable materials, aliphatic poly(ester)s, aliphatic poly(carbonate)s (PC) have received considerable attention for years. These materials are excellent candidates for pharmaceutical applications as a consequence of their low toxicity, biocompatibility, and biodegradability.\(^4\) To construct functional polycarbonates, the introduction of functional pendant groups into the cyclic carbonate monomers before ring opening polymerization (ROP) or further modifications following the polymerization is the common choice. Stimuli-sensitive polycarbonate nanocarriers were designed and showed ideal applications in drug delivery systems.\(^5\)–\(^10\) Hu et al. synthesized light-responsive poly(carbonate)s with a spiropyran chromophore in the side chain via copper-catalyzed azide-alkyne cycloaddition reaction. Under alternative UV and visible light irradiation, the amphiphile carried on a reversible micelle transition in water due to the photoisomerization between SP and MC form.\(^11\) Recently, several stimuli-responsive poly(carbonate)s have been developed. Yang et al. synthesized diblock copolymers of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic biodegradable polycarbonate functionalized with GSH-sensitive disulfide bonds and pH-responsive carboxylic acid groups with PEG as macrominitiator through organocatalytic ring-opening polymerization of functional cyclic carbonates.\(^12\) Xie et al. designed a novel triple-stimuli-sensitive graft copolymer which responds to the changes in temperature, reducing agent, and light. The copolymer consisted of thermoresponsive tetraethylene glycol poly(trimethylene carbonate) (P(MTC-4EG)) as backbone and light-sensitive poly(2-nitrobenzyl methacrylate) (PNBM) as side chain linked by an intervening disulfide bond.\(^13\) However, there are few reports to construct light-responsive pendant of o-NB group in poly(carbonate)s biomaterials used as drug delivery systems.

In this work, we developed a versatile method to prepare photoresponsive amphiphilic diblock poly(carbonate)s mPEG\(_{113}\)-b-PMNC\(_n\) with pendant o-NB ester group. The o-NB ester group was introduced into the cyclic carbonate monomer 5-Methyl-5-(2-nitro-benzoxy-carbonyl)-1,3-dioxan-2-one (MNC) first and then photoresponsive amphiphilic copolymers were obtained through ring-opening polymerization with DBU as catalyst and mPEG\(_{113}\) as macroinitiator (Scheme 2). The amphiphilic copolymer could self-assemble into spherical micelles in aqueous solution because of the photo-cleavage of o-NB ester, the obtained micelles disassembled, leading to the change of micelles size and morphology and the efficiently controlled release of payloads NR. This study provides a convenient way to construct smart poly(carbonate)s nanocarriers for controlled drug delivery.

**EXPERIMENTAL**

**Materials**

Ethyl chloroformate was purchased from Xiya Reagent and used as received. 2,2-Bis(hydroxymethyl)propionic acid, 2-nitrobenzyl bromide, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from Energy Chemical and used as received. mPEG(MWd\(_S\) = 5 kDa, mPEG\(_{113}\)) and Nile red (NR) were purchased from Sigma-Aldrich and used as received. Dimethylformamide (DMF) was dried by commercially available 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled utilizing sodium and benzophenone before used. Dichloromethane (DCM) and triethylamine (TEA) were dried over calcium hydride for 24 h at room temperature and distilled under reduced pressure.

**Measurements**

\(^1\)H NMR spectra were recorded on an INOVA-400 NMR instrument (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane (TMS) as an internal standard in DMSO-\(d_6\).

**SCHEME 1** Illustration of mPEG\(_{113}\)-b-PMNC\(_n\) micelles loading with drugs and photocontrolled release. [Color figure can be viewed at wileyonlinelibrary.com]

**SCHEME 2** Synthesis of o-NB-containing cyclic carbonate and amphiphilic block copolymer with o-NB pendant group.
Fourier-transform infrared (FT-IR) spectra were gained by a WQF-410 spectrophotometer (Beijing Rayleigh Analytical Instrument Co., Beijing, China). Ultraviolet–visible (UV–vis) absorption spectra were obtained on a UV Bluestar Plus UV–Vis spectrophotometer (Beijing LabTech Instruments Co., Ltd., Beijing, China). Fluorescent spectra were performed on a HITACHI F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Minato-ku, Tokyo, Japan) at room temperature, the excitation and emission slit widths were 5.0 and 5.0 nm, respectively. The number-average molecular weight ($M_n$) and molecular distribution ($D$) of the obtained diblock copolymers were determined by a Waters 1515 Gel permeation chromatography (GPC) instrument (Waters Co., Milford, MA, USA) in DMF, calibrated with standard polystyrene with a flow rate of 1 mL min$^{-1}$. Transmission electron microscopy (TEM) images were obtained on an H-7000 NAR transmission electron microscope (Hitachi High-Technologies Co., Minato-ku, Tokyo, Japan) at 100 kV. The mean size of the micelles was determined by dynamic light scattering (DLS) measurements using a Nano-ZS90 zeta-potential and particle analyzer (Malvern Instruments Ltd, Malvern, UK). For the UV-light irradiation, the sample (3 mL of solution) was placed vertically under a high-pressure mercury lamp (wavelength: 365 nm, power: 125 W) with the distance of 10 cm.

**Synthesis of 2-Nitrobenzyl 2,2-Bis(hydroxyethyl)Propionate**

KOH (3.96 g, 71.0 mmol) and 2,2-bis(hydroxyethyl)propionic acid (8.25 g, 61.5 mmol) were dissolved in DMF (75 mL). The solution of 2-nitrobenzyl bromide (12.00 g, 55.5 mmol) in DMF (20 mL) was then added dropwise, and the mixture was stirred at 100 °C for 20 h. After fully reacted, deionized water (200 mL) was added into the solution, and the mixture was extracted with DCM (2 × 100 mL). The resulting mixture was washed with deionized water (4 × 100 mL). The organic phase was then dried over MgSO$_4$ and concentrated in vacuum to yield crude product, which was purified by column chromatography (ethyl acetate/petroleum ether = 1/1, v/v). Yield: 8.36 g (56.1%). $^1$H NMR (400 MHz, d$_6$-DMSO, TMS): δ 8.11 (d, $J = 8.2$ Hz, 1H, aromatic), 8.04–7.69 (m, 2H, aromatic), 7.61 (t, $J = 7.3$ Hz, 1H, aromatic), 5.43 (s, 2H, CH$_2$O), 4.77 (t, $J = 5.3$ Hz, 2H, 2CH$_2$OH), 3.57 (dd, $J = 10.3$, 5.5 Hz, 2H, CH$_2$OH), 3.47 (dd, $J = 10.3$, 5.5 Hz, 2H, CH$_2$OH), 1.11 (s, 3H, CH$_3$).

**Synthesis of 5-Methyl-5-(2-Nitro-Benzoxy-Carbonyl)-1,3-Dioxan-2-One (MNC)**

In a sealed vessel, 2-nitrobenzyl 2,2-bis(hydroxyethyl)-propan-2-one (5.00 g, 18.9 mmol) was dissolved in THF (150mL) at 0 °C and ethyl chloroformate (6.15 g, 56.7 mmol) was added. After stirring for 1 h, triethylamine (5.74 g, 56.7 mmol) was added dropwise, and the whole process was carried out under nitrogen atmosphere. The mixture was allowed to stir for 1 h, after that it was reacted at 30 °C for 6 h. The reaction mixture was then filtered and evaporated in vacuum. The crude product was recrystallized from DCM and diethyl ether, finally obtaining white crystals. Yield: 5.05 g (90.5%). $^1$H NMR (400 MHz, d$_6$-DMSO, TMS): δ 8.14 (d, $J = 8.1$ Hz, 1H, aromatic), 7.80 (t, $J = 7.5$ Hz, 1H, aromatic), 7.76–7.59 (m, 2H, aromatic), 5.54 (s, 2H,CH$_2$O), 4.60 (d, $J = 10.3$ Hz, 2H, CH$_2$O), 4.40 (d, $J = 10.3$ Hz, 2H, CH$_2$O), 1.22 (s, 3H, CH$_3$).

**Typical Procedure for the Synthesis of mPEG$_{113}$-b-PMNC$_{a}$**

The ring-opening polymerization (ROP) method was used to synthesize the mPEG$_{113}$-b-PMNC$_{a}$ block copolymer; the commercially available monomethoxy poly(ethylene glycol) (mPEG$_{15}$) acted as the macronititator. Polymerizations were conducted under an inert atmosphere of nitrogen using standard Schlenk-line techniques. Taking the preparation of mPEG$_{113}$-b-PMNC$_{50}$ for example, MNC (1.0000 g, 3.39 mmol), mPEG$_{113}$ (0.3396 g, 0.07 mmol), DBU (0.0088 g, 0.06 mmol), and dried DCM (10 mL) were placed in a dried Schlenk tube. The mixture solution was further degassed by three vacuum-nitrogen purging cycles to remove trace moisture and oxygen. After stirring at room temperature for 7 h, the resulting mixture was precipitated in cold diethyl ether. After centrifugation, the resulting product was dried in vacuum to yield a white solid. Yield: 1.2284 g (91.7%). $^1$H NMR (400 MHz, d$_6$-DMSO, TMS): δ 8.04 (d, $J = 7.8$ Hz, aromatic), 7.69 (s, aromatic), 7.69–7.33 (m, aromatic), 5.40 (s, CH$_2$O), 4.23 (dd, $J = 19.5$, 11.2 Hz, OCH$_2$CH$_2$OH), 3.51 (s, OCH$_2$CH$_2$OH), 1.14 (d, $J = 24.0$ Hz, CH$_3$).

**Preparation of the Polymeric Nanoparticles and Determination of Critical Micelle Concentration**

Micelles of mPEG$_{113}$-b-PMNC$_{25}$ and mPEG$_{113}$-b-PMNC$_{50}$ were prepared by the solvent exchange method. Ten milligrams of amphiemphic copolymer was first dissolved in 2 mL DMF at room temperature, and then 20 mL deionized water was slowly added dropwise into the solution under vigorous stirring. After vigorous stirring for another 2 h at room temperature, the micelles were obtained and further dialyzed against deionized water in a dialysis membrane (Molecular weight cutoff of 3500 Da) for 48 h to remove DMF. In a 50 mL volumetric flask, the final copolymer concentration was adjusted to 0.2 mg mL$^{-1}$ by adding deionized water.

The critical micelle concentration (CMC) values of the micelles were obtained by using NR as probe molecule. NR in THF (0.1 mg mL$^{-1}$, 30 µL) was added to 10 glass vials through the use of a microsyringe. After THF was completely evaporated, 4 mL micellar solution with different concentrations was added into the glass vials. The concentration of the micellar solution was confected varying from 0.2 to 2 × 10$^{-4}$ mg mL$^{-1}$. Then, the solution was stirred overnight. Finally, fluorescence measurements were recorded at an excitation wavelength of 550 nm and the fluorescence emission spectrum was monitored from 580 to 750 nm.

**Photoresponsiveness Experiments**

To demonstrate the light-induced cleavage of the o-NB groups, the mPEG$_{113}$-b-PMNC$_{25}$ and mPEG$_{113}$-b-PMNC$_{50}$ micelles (0.2 mg mL$^{-1}$, 3mL) were irradiated with UV light at 365 nm. As a control experiment, photochemical cleavage of the o-NB groups of the monomer MNC was carried out so as to evaluate the photolytic cleavage behavior. A solution of monomer MNC (0.2 mg mL$^{-1}$ in DMF, 3mL) was exposed to...
UV light (365 nm). UV-Vis spectroscopy was used to record the whole process.

**Encapsulation and Photo-Controlled Release Experiments**
The amphiphilic copolymers were engineered as nanocarriers for hydrophobic NR payload. NR in THF (0.1 mg mL\(^{-1}\), 30 \(\mu\)L) was added to a glass vial through the use of a microsyringe. After THF was completely evaporated, 4 \(\mu\)L of 0.2 mg mL\(^{-1}\) micellar solution was added into the glass vial. Then, the solution was stirred overnight. Finally, micelles containing NR (3 \(\mu\)L) were exposed to 365 nm UV light. Fluorescence spectroscopy was used to monitor the whole process at an excitation wavelength of 550 nm and the emission monitored from 580 to 750 nm.

**In Vitro Cytotoxicity Assay**
The biocompatibility of blank micelles was estimated by MTT viability assays against the HeLa and HepG2 cells. In the MTT assay, The HeLa or HepG2 cells in 100 \(\mu\)L of medium were incubated for three times and 60 \(\mu\)L of MTT (0.5 mg mL\(^{-1}\)) was added to each well to dissolve intracellular MTT formazan. Finally, the absorbance at 570 nm was measured using a microplate reader (Varioskan Flash, Thermo Scientific). Data were done as average \(\pm\) SD (n = 5).

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of Block Copolymers**
The cyclic carbonate monomer MNC were prepared by a two-step reaction according to the synthetic route showed in Scheme 2. Amphiphilic copolymer mPEG\(_{113}\)-b-PMNC\(_n\) was prepared through the ROP of MNC at room temperature with DBU as catalyst and mPEG\(_{113}\) as macronitiator [Scheme 2(b)]. As shown in Table 1, the degrees of polymerization (DP) of the obtained mPEG\(_{113}\)-b-PMNC\(_n\) with three different monomer-to-initiator ratios were close to the theoretical values, and GPC analysis displayed that the copolymer had a narrow distribution, which demonstrated that very few side reactions occurred during the ROP, probably due to the high efficiency of the catalyst DBU during the ROP of cyclic carbonates.\(^{54}\) The degree of polymerization (DP) was calculated by comparing the peak area of mPEG\(_{113}\). The \(^1\)H NMR and FT-IR spectra provided evidence of the successful synthesis of the monomer (B) and corresponding copolymers (C) showed in Figures 1 and 2. As shown in Figure 1, compared with 2,2-bis(hydroxymethyl)-propionic acid (A), the spectra of monomer (B) appeared the new peaks at 7.5–8.0 and 5.4 ppm representing the characteristic benzyl group peaks, whereas the peaks of hydroxyl at 4.6 ppm and carboxyl at 12.0 ppm disappeared completely which demonstrated the successful introduction of o-NB ester group to the monomer and the formation of cyclic carbonate. The mPEG\(_{113}\)-b-PMNC\(_n\) (C) having just one more peak attributed to the mPEG\(_{113}\) at 3.5 ppm compared with the monomer (B) indicated that the functionalized copolymers synthesized successfully. Similarly, FT-IR spectra shown in Figure 2 provided further evidence. As compared with 2,2-bis(hydroxymethyl)propionic acid in Figure 2(A), the new absorption peaks at \(\approx\)3100 cm\(^{-1}\) assigned to the stretching vibration of the C–H in benzene ring, 1600 cm\(^{-1}\), 1570 cm\(^{-1}\) and 1530 cm\(^{-1}\) assigned to the stretching vibration of the C=C in benzene ring, 798 cm\(^{-1}\) and 739 cm\(^{-1}\) assigned to the out-of-plane bending vibration of the C=H in monosubstituted benzenes appeared in the dashed frame of the spectra monomer MNC (B), and the characteristic alcoholic hydroxyl group and carboxylic hydroxyl group absorption peaks in Figure 2(A), respectively, at 3360 cm\(^{-1}\) and 2850 cm\(^{-1}\) completely disappeared, what is more, compared with monomer MNC (B), a blue shift of the carbanyl group absorption peak at 1640 cm\(^{-1}\) in Figure 2(A) was observed due to the existence of hydrogen bonding, all of which demonstrated that the functionalized copolymers synthesized successfully.

**TABLE 1 Synthesis of mPEG\(_{113}\)-b-PMNC\(_n\) Copolymers**

<table>
<thead>
<tr>
<th>Entry</th>
<th>DP(^a)</th>
<th>(M_n),NMR(^b) (kDa)</th>
<th>(D) (^b)</th>
<th>(M_n),GPC(^b) (kDa)</th>
<th>CMC(^c) (mg/mL)</th>
<th>(D_n)(^d) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPEG(<em>{113})-b-PMNC(</em>{25})</td>
<td>24</td>
<td>12.4</td>
<td>1.31</td>
<td>17.4</td>
<td>0.0163</td>
<td>140.4</td>
</tr>
<tr>
<td>mPEG(<em>{113})-b-PMNC(</em>{50})</td>
<td>45</td>
<td>19.8</td>
<td>1.27</td>
<td>17.7</td>
<td>0.0133</td>
<td>159.5</td>
</tr>
</tbody>
</table>

\(^a\) Determined by \(^1\)H NMR.  
\(^b\) Determined by GPC calibrated with polystyrene standard.  
\(^c\) Obtained by fluorescence spectroscopy using Nile Red as probe.  
\(^d\) Achieved by DLS.

**FIGURE 1** \(^1\)H NMR of 2,2-bis(hydroxymethyl)-propionic acid (A), MNC (B), and mPEG\(_{113}\)-b-PMNC\(_n\) (C). [Color figure can be viewed at wileyonlinelibrary.com]
which illustrated successful synthesis of the monomer (B). The spectra of copolymers (C) was similar to the monomer MNC (B) except for the stronger absorption peak at ~2900 cm\(^{-1}\) resulting from the introduction of mPEG113 which confirmed that the targeted block copolymers were indeed achieved. GPC results in Figure 3 showed that mPEG113-b-PMNC\(_{50}\) had a narrow polydispersity of 1.27 and a number average molecular weight (Mn) of 17.7 kDa.

**Self-Assembly Behaviors and Determination of Critical Micelle Concentration**

As a result of the molecular design of mPEG\(_{113}\)-b-PMNC\(_{n}\) renders it amphiphilic, the photoresponsive copolymer can self-assemble into micelles in aqueous solution with hydrophobic cored and hydrophilic PEG coronae showed in Scheme 1. Self-assemblies of the copolymer were prepared through solvent exchange method and then characterized by means of fluorescence spectroscopy, TEM, and DLS. The micelle formation and calculation of the CMC of mPEG\(_{113}\)-b-PMNC\(_{n}\) were confirmed by fluorescence technique, hydrophobic dye NR was chosen as a fluorescence probe to be loaded into the micelles based on the fact that its fluorescence was ignorable in water but increased crucially and blue shifted in a hydrophobic environment such as the core of micelle. As shown in Figure 4, the curve was almost flat at very low concentration, indicating that the NR was in water and few micelles were formed. With increasing concentration of amphiphile solution, the curve increased dramatically and blue shifted obviously, suggesting more NR were solubilized in hydrophobic micelle core, which proved the self-assembly of micelles. The CMC value of mPEG\(_{113}\)-b-PMNC\(_{n}\) was then identified through plotting the maximum fluorescence emission intensity versus the log of micelle concentration of mPEG\(_{113}\)-b-PMNC\(_{n}\) and the concentration of the inflection point in the plot was CMC, showed in Figure 4. The CMC of mPEG\(_{113}\)-b-PMNC\(_{25}\) and mPEG\(_{113}\)-b-PMNC\(_{50}\) were 0.0163 mg mL\(^{-1}\) and 0.0133 mg mL\(^{-1}\), respectively. mPEG\(_{113}\)-b-PMNC\(_{50}\) showed a smaller CMC value than that of mPEG\(_{113}\)-b-PMNC\(_{25}\) because that its longer hydrophobic chain increased its hydrophobicity.

The size and morphology of the self-assembled mPEG\(_{113}\)-b-PMNC\(_{25}\) and mPEG\(_{113}\)-b-PMNC\(_{50}\) micelles (0.2 mg mL\(^{-1}\)) were further studied by DLS and TEM. The TEM image [Fig. 5(AC)] manifested that mPEG\(_{113}\)-b-PMNC\(_{n}\) spontaneously self-assembled into spherical micelles of a core–shell structure with a diameter of ~130 nm. The DLS results [Fig. 6(A)] showed that the hydrodynamic diameter (D\(_h\)) of mPEG\(_{113}\)-b-PMNC\(_{25}\) micelles in aqueous solution was 140.4 nm and the polydispersity was 0.127, indicating that the micelles had a narrow size distribution. The average size of the spherical micelles determined by TEM was smaller than the results of the DLS analysis, which possibly because of the shrinkage of the PEG shell upon drying. Meanwhile, micelles of mPEG\(_{113}\)-b-PMNC\(_{50}\) had a D\(_h\) of 159.5 nm and a polydispersity of 0.164 [Fig. 6(B)]. The diameter of the micellar nanoparticles of mPEG\(_{113}\)-b-PMNC\(_{50}\) is larger than that of the mPEG\(_{113}\)-b-PMNC\(_{25}\) as mPEG\(_{113}\)-b-PMNC\(_{50}\) had a longer hydrophobic chain. The results demonstrated that the amphiphiles mPEG\(_{113}\)-b-PMNC\(_{n}\) can be self-assembled into spherical micelles in aqueous solution.

**Photocleavage Experiments of o-NB Groups**

As shown in Scheme 1, the pendent o-NB ester group of mPEG\(_{113}\)-b-PMNC\(_{n}\) carried out the photoisomerization into a corresponding o-nitrosobenzaldehyde upon UV irradiation, simultaneously releasing a free carboxylic acid. The mechanism of photoresponsive properties of the copolymer under 365 nm UV irradiation were investigated in details. As a control experiment, the monomer MNC in DMF (0.2 mg mL\(^{-1}\)) was irradiated under 365 nm UV light and the copolymers mPEG\(_{113}\)-b-PMNC\(_{25}\) and mPEG\(_{113}\)-b-PMNC\(_{50}\) in aqueous solution (0.2 mg mL\(^{-1}\)).
spectroscopy shown in Figure 7. Initially, the absorption peak at 270 nm in mPEG113-b-PMNC25 and 265 nm in mPEG113-b-PMNC50 decreased gradually and then leveled off after being irradiated for 100 min. Meanwhile, a following absorption peak at 318 nm in mPEG113-b-PMNC25 and 320 nm in mPEG113-b-PMNC50 appeared and increased relatively slowly, and both of them showed an isobestic point at 301 and 305 nm, respectively, indicating that the o-NB ester groups were cleaved from the copolymers. From Figure S3, we can know that the monomer MNC had the same behavior on UV irradiation.

These results tallyed with the report studied by Kim et al., who found that the photo cleavage of the photosensitive unit had the feature that in UV–Vis spectroscopy existed decreased and increased absorption peaks at 265 and 310 nm, respectively, indicating that the o-NB ester groups were cleaved from the copolymers. From Figure S3, we can know that the monomer MNC had the same behavior on UV irradiation.

In addition, the color of the aqueous solution changed gradually from colourless to slightly brown with UV irradiation, which was caused by o-nitrosobenzaldehyde in the solution.

1H NMR spectra and GPC were further used to verify the photo cleavage of the o-NB ester groups. Figure 8 shows the 1H NMR spectra of mPEG113-b-PMNC50 after irradiation with 365 nm UV light for various times. To prove the photolysis, we used very high concentration solution (15 mg mPEG113-b-PMNC50 dissolved in 0.6 mL DMSO-d6). The peak area of the methylenes in main chain of PMNC at 4.23 ppm was set as 4.00; from Figure 8, we can see that the area of peak at 5.4 ppm assigned to the protons of the methylenes of o-NB groups decreased from 2.01 to 0.53 with the UV irradiation for 20 h, which was a strong proof of the photo cleavage of the o-NB ester groups. Furthermore, the area of the signals from the mPEG113 basically maintained 10, suggesting the chain of PMNC was stable under UV irradiation.59 GPC result of mPEG113-b-PMNC50 after UV irradiation showed that the number average molecular weight decreased to 8.9 kDa with $\bar{D}$ about 1.46 (Fig. 3).

The DLS data of mPEG113-b-PMNC50 micelles after UV irradiation, and adjusting different pH between 11 and 3 also suggested the photo cleavage of the o-NB groups. As shown in Figure 9, after UV irradiation, pH of the solution decreased.
from 5.5 to 3.5, when adjusted its pH to 11, predictably, there was no signal displayed in DLS test. It was because that the free carboxylic acid in the side chain of the copolymer transformed to carboxylate, making the polymer completely soluble in water; when adjusting the pH of the solution back to 3, the micelles reformed.
The photocleavage of \( \alpha \)-NB groups of mPEG113-b-PMNC\(_n\) was also investigated by TEM and DLS. Surprisingly, as displayed in Figure 5(B,D), upon UV light irradiation, spherical micellar nanoparticles disappeared, and some irregularly shaped nanoaggregates were formed. It may result from the fact that hydrophobic 2-nitrobenzyl ester was cleaved from the side chain of the copolymer, transforming to hydrophilic carboxylic acid, leading to the primary hydrophilic–hydrophobic balance of the micelles disrupted. The DLS data of the size changes upon stimulation with UV irradiation were shown in Figure 6. As shown in Figure 6(A), the average diameter of the mPEG113-b-PMNC\(_{25}\) micelles decreased from 140.4 to 118.2 nm, and the polydispersity changed from 0.127 to 0.152 after exposed to 365 nm UV light for 100 min. Meanwhile, the mPEG113-b-PMNC\(_{50}\) micelles obtained the similar tendency of average diameter decreasing from 159.5 to 130.2 nm and polydispersity changing from 0.164 to 0.204 [Fig. 6(B)]. These results also illustrated that the photolysis of the \( \alpha \)-NB esters leaded the photo-triggered disassembly of the nanoparticles.

On the basis of the results of TEM and DLS measurements, it can be concluded that external stimuli such as UV light irradiation could adjust the morphology of the polymeric micellar nanoparticles, which might endow the nanoparticles with great potential to be a new nanocarriers for controlled cargo release.

**Photo-Controlled Release of Micelles**

Stimuli-responsive micelles have been widely used for controlled release because of the ideal nanocarriers for the controlled release of hydrophobic cargoes loaded in the hydrophobic core.\(^{16}\) The controlled release of cargoes NR, from the stimuli-responsive polymeric nanomicelle was investigated by monitoring fluorescence spectroscopy. Supporting
Information, Figure S4 showed the release profile of NR encapsulated in the mPEG 113-b-PMNC25 and mPEG 113-b-PMNC50, from which it can be seen that with the increase of UV irradiating time, fluorescence emission intensity decrease gradually, demonstrating that drug model NR released successfully from the hydrophobic core of micelles, the reason was supposed to be that the primary hydrophilic–hydrophobic balance of the micelles was destroyed because of the conversion from hydrophobic o-NB esters to hydrophilic carboxylic acid due to UV light-induced cleavage and the hydrophobicity of hydrophobic chain segment was weakened. As shown in Figure 10, NR could be hardly released from the mPEG113-b-PMNC25 nanoparticles without UV stimulation, indicating that the micellar assemblies were stable. The NR surplus remained in mPEG113-b-PMNC25 and mPEG113-b-PMNC50 micelles only was 17.0% and 18.4%, respectively, when the irradiation time increased to 75 min, manifesting that the drug model NR was fully released. The remaining NR amount in mPEG113-b-PMNC50 micellar was larger than that of mPEG113-b-PMNC25 micellar, which was probably because of longer hydrophobic chain of mPEG113-b-PMNC50 causing it more hydrophobic. Based on the above results, it can be concluded that the photo-controlled release of guest molecules from the polymeric micellar nanocontainers may vary with the irradiation time of the UV light and hydrophilic or hydrophobic chain length.

Cell Cytotoxicity of mPEG113-b-PMNCn Micelles
For drug delivery system materials, the biocompatibility or cytotoxicity of the drug carrier is a pivotal issue. The biocompatibility of mPEG113-b-PMNCn micelles were evaluated by MTT assay against HeLa cells and HepG2 cells. As shown in Figure 11(A,B), the viabilities of HeLa cells and HepG2 cells after incubated with different concentration of micellar solution for 48 h were above 80%, even at a high concentration of 0.5 mg mL\(^{-1}\). These results indicated that the prepared mPEG113-b-PMNC25 and mPEG113-b-PMNC50 had low cytotoxicity, may be because of the excellent biocompatibility of the PEG and poly(carbonate). Therefore, the light-responsive amphiphilic copolymers mPEG113-b-PMNCn have potential to be an ideal drug delivery system materials.

CONCLUSIONS
In summary, we reported a simple synthetic route for the preparation of photoresponsive amphiphilic diblock copolymers mPEG113-b-PMNCn, which were composed of a hydrophilic mPEG block and a hydrophobic PMNC block with pendent o-NB ester groups. The functional group o-NB ester was first introduced to the six-membered cyclic carbonate monomer 5-methyl-5-(2-nitro-benzoxycarbonyl)-1,3-dioxan-2-one (MNC), and then proceeded ring-opening polymerization at room temperature with DBU as catalyst and mPEG113 as macroinitiator. The obtained amphiphilic copolymer mPEG113-b-PMNC25 and mPEG113-b-PMNC50 can self-assemble to spherical micelles of
REFERENCES AND NOTES

学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具