Supplementary data

PEGylated alternating copolymer with oxidation-sensitive phenylboronic ester pedants for anticancer drug delivery

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Cell and animal experiments

1. Cell culture and *in vitro* cytotoxicity

The 4T1 mice breast cancer cells were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). All cells were grown in DMEM (supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin) at 37 °C in an incubator with 5% CO_2 and humidified atmosphere.

The cytotoxicities of PAPBN, free DOX, and PAPBN/DOX were evaluated by MTT assay. The cells were seeded into 96-well plates at a density of 8000 cells per well in 200 μL of DMEM and incubated for 24 h. Then, the media was completely removed and 200 μL of fresh media containing PAPBN, free DOX, and PAPBN/DOX was added. After incubation for another 48 or 72 h, the cells were subjected to MTT assay. The absorbance at 570 nm was determined by a Bio-Rad 680 microplate reader (USA). Cell viability (%) was calculated using the following equation: Cell viability (%) = ($A_{sample}/A_{control}$) × 100%, where A_{sample} and $A_{control}$ are the absorbance of the sample and control wells, respectively.

2. Cellular uptake and intracellular drug release

The cellular internalization and intracellular release of DOX were studied in 4T1 cells by using confocal laser scanning microscopy (CLSM) and Flow cytometry (FCM). 4T1 cells were seeded on coverslips in 6-well plates (1×10^5 cells per well) in 2.0 mL of DMEM and incubated for 24 h. Then fresh DMEM containing PAPBN, free DOX or PAPBN/DOX was added to replace the original medium. The DOX concentration for free DOX or PAPBN/DOX in DMEM was set at 4.0 μg mL^{-1}. After incubation for 1 h or 3 h, the cells were washed with PBS for five times and fixed with 4% formaldehyde for 20 min. The cell nuclei were stained with DAPI before observation with confocal microscope (Carl Zeiss, LSM 780).

The process of FCM is basically the same as above. The 4T1 cells were seeded in 6-well plates (2×10^5 cells per well) in 2.0 mL of DMEM and incubated for 24 h. Then fresh DMEM containing PAPBN, free DOX or PAPBN/DOX was added to replace the original medium, and the cells without drug treatment were set as control. The DOX concentration for free DOX or PAPBN/DOX in DMEM was set at 4.0 μg mL^{-1}. After incubation for 6 h, the cells were washed

with PBS and detached with trypsin. The harvested cells suspended in 1.0 mL of PBS were centrifuged at 1000 rpm for 5 min at 4 °C. After the supernatants were removed, the cells were washed with 1.0 mL of PBS twice. Finally, the cells were resuspended in 0.5 mL of PBS and subjected to measurements using a Guava Easy Cyte™ 12 Flow Cytometer (Millipore, Billerica, MA, USA). Analysis was performed on 5000-gated events per sample (λ_{ex}=488 nm).

3. In vivo antitumor efficiency

Female Balb/C mice (6–8 weeks old) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Jilin University and approved by the Animal Ethics Committee of Jilin University. The tumor-bearing mice were prepared by subcutaneously injecting 1.5×10^6 4T1 cells suspended in 0.1 mL medium (PBS: matrigel =1: 1, *V/V*) into the mammary gland of mice. When the tumors grew to about 35 mm^3, the mice were randomly divided into 4 groups and received treatment of PBS, PAPBN (25 mg kg^{-1}), free DOX (4 mg kg^{-1}) and PAPBN/DOX (2 and 4 mg kg^{-1} DOX) respectively. Treatments were performed on day 0, 4, 8. The body weights and tumor sizes were measured every two days, and the tumor volume (V; mm^3) and tumor inhibition rate (%) was calculated by the following equations:

$$V\,(\mathrm{mm}^3) = \frac{a \times b^2}{2} \qquad \text{(I)}$$

in this equation, a and b (mm) are the longest and shortest diameter of tumors, respectively.

$$\text{Tumor inhibition rate (\%)} = \frac{V_{control} - V_{sample}}{V_{control}} \times 100\% \qquad \text{(II)}$$

in this equation, $V_{control}$ and V_{sample} represent the tumor volumes in control and sample groups, respectively.

4. Ex vivo DOX fluorescence imaging

Biodistribution of free DOX and PAPBN/DOX after systemic administration was evaluated with ex vivo imaging studies in 4T1 tumor-bearing mice. At 3 and 6 h post-injection, imaging of the isolated visceral organs and tumors was carried out in a Maestro Imaging System (Cambridge Research & Instrumentation, Inc.).

5. Histological and immunohistochemical analyses

One day after the last intravenous injection, the mice were sacrificed. The tumor and major organs (heart, liver, spleen, lung, and kidney) were collected and fixed in 4% paraformaldehyde overnight, and then embedded in paraffin. The paraffin-embedded tumors and organs were sliced at a thickness of 5 mm for the following hematoxylin and eosin (H&E) staining and in situ cell apoptosis analyses (TUNEL). The evaluation of histological alterations was performed using a microscope (Nikon Eclipse Ti, Optical Apparatus Co., Ardmore, USA).

6. Statistical analysis

All experiments were performed for at least three times and the data were shown as mean ± standard deviation (SD). *$p < 0.05$ was considered statistically significant. **$p < 0.01$ and ***$p < 0.001$ were considered highly significant.

Characterization of synthetic compounds and micelles

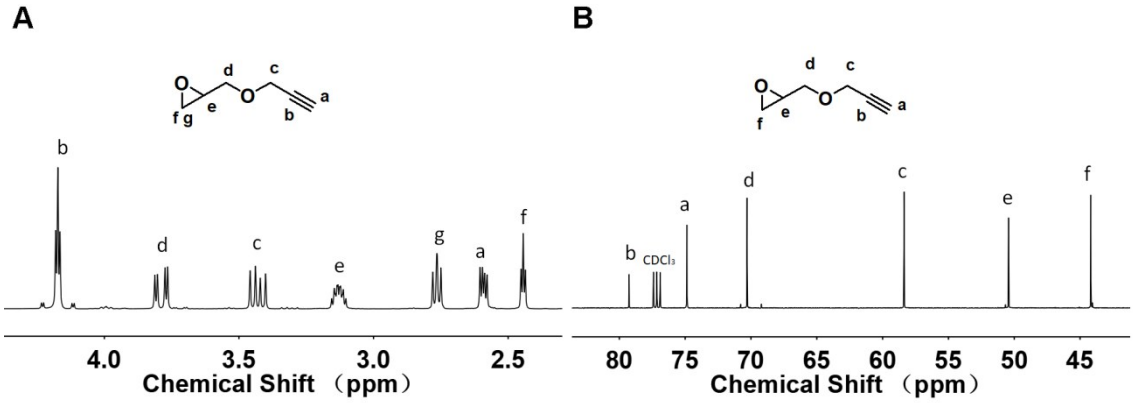


Fig. S1. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of glycidyl propargyl ether (GPE) monomer in $CDCl_3$.

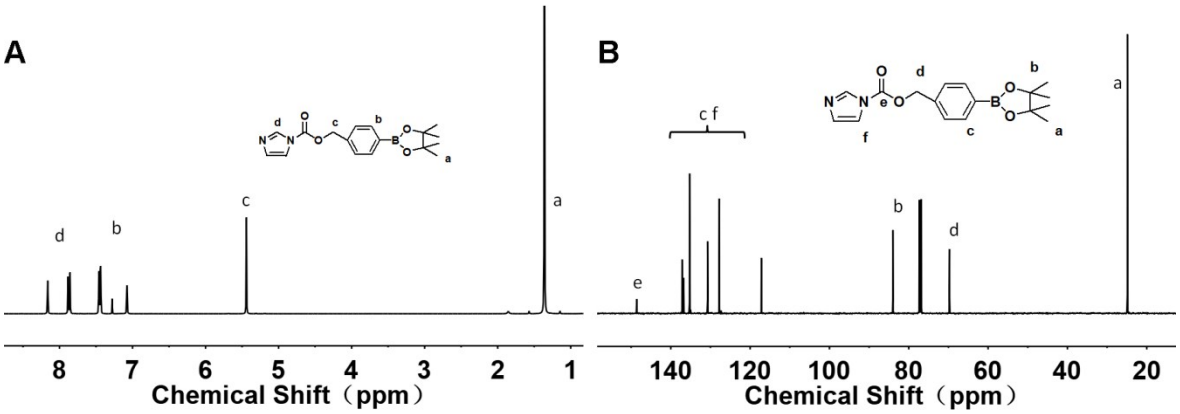


Fig. S2. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of HPBAe-CDI in $CDCl_3$.

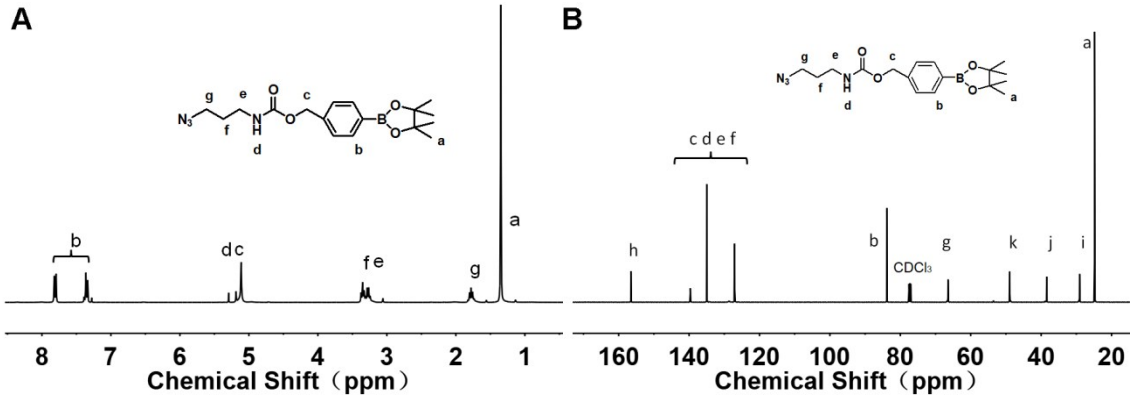


Fig. S3. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of N_3-PBAe in $CDCl_3$

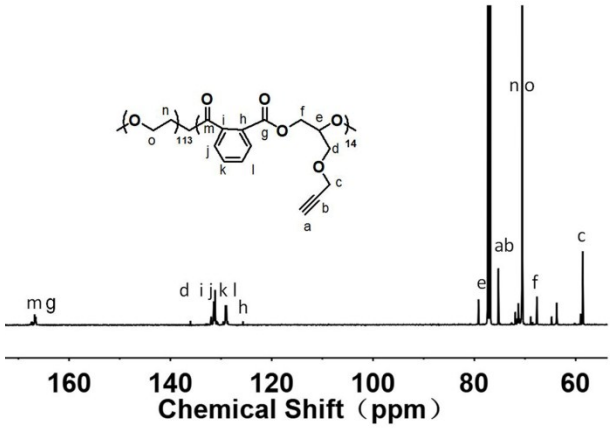
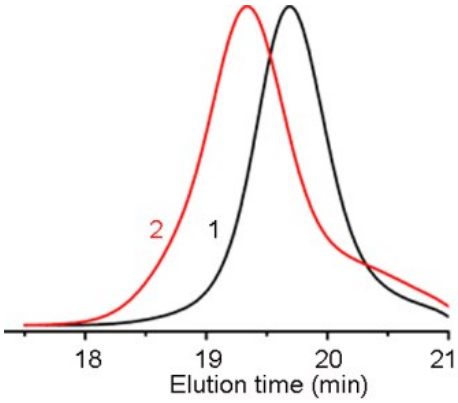


Fig. S4. ^{13}C NMR spectrum of mPEG-*b*-P(PA-*alt*-GPE) in $CDCl_3$.



		***M*n**	***Đ***
1	**mPEG-*b*-P(PA-*alt*-GPE)**	**15800**	**1.30**
2	**mPEG-*b*-P(PA-*alt*-GPBAe)**	**18100**	**1.22**

Fig. S5. GPC analyses of mPEG-*b*-P(PA-*alt*-GPBAe) and its precursor polymer mPEG-*b*-P(PA-*alt*-GPE)**.**

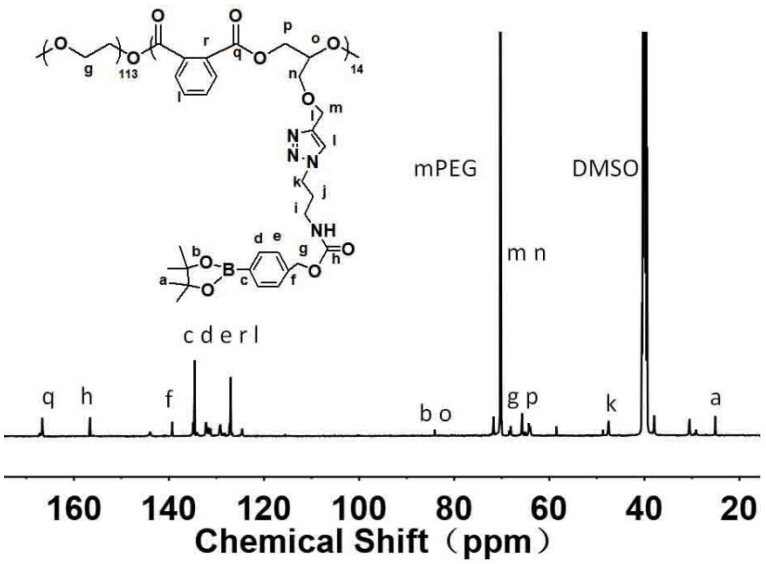


Fig. S6. ^{13}C NMR spectrum of mPEG-*b*-P(PA-*alt*-GPBAe) in DMSO-*d*6.

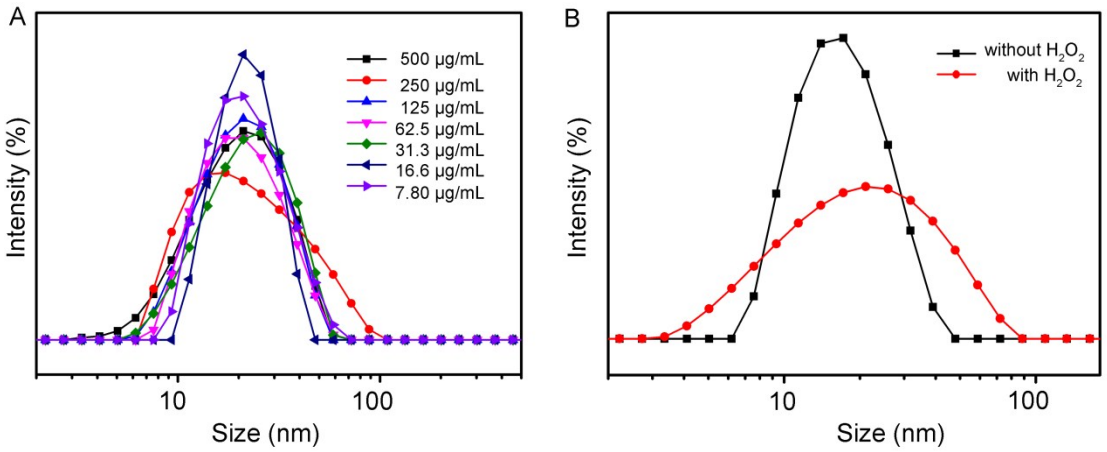


Fig. S7. The hydrodynamic radius of the mPEG-*b*-P(PA-*alt*-GPBAe) micelles (PAPBN) at the concentrations of 7.8–500 μg mL^{-1} (A) and PAPBN before and after treatment with 200 mM H_2O_2 for 24 h (B) .

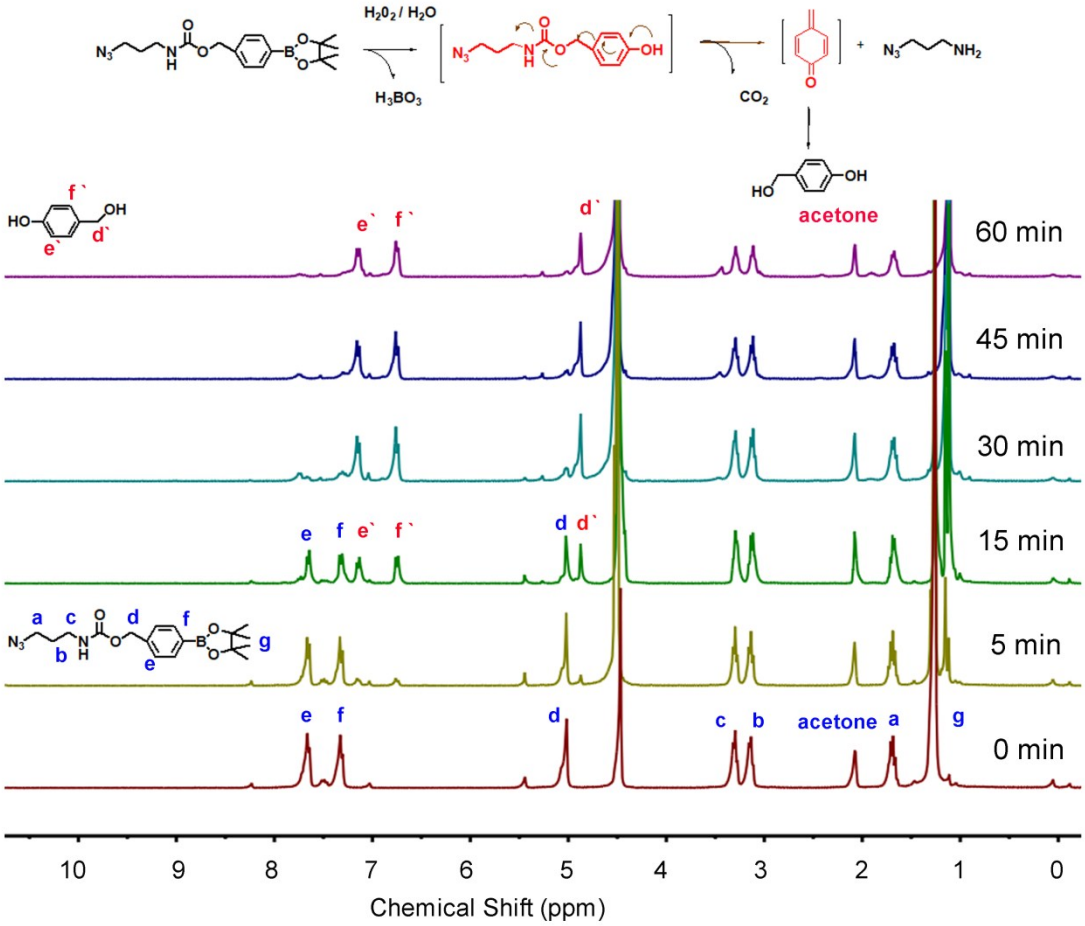


Fig. S8. Time-dependent ^1H NMR spectra of N_3-PBAe oxidized by H_2O_2 in acetone/D_2O(*V*/*V*=3:2*)* with the H_2O_2/ N_3-PBAe molar ratio of 1:1 at room temperature.

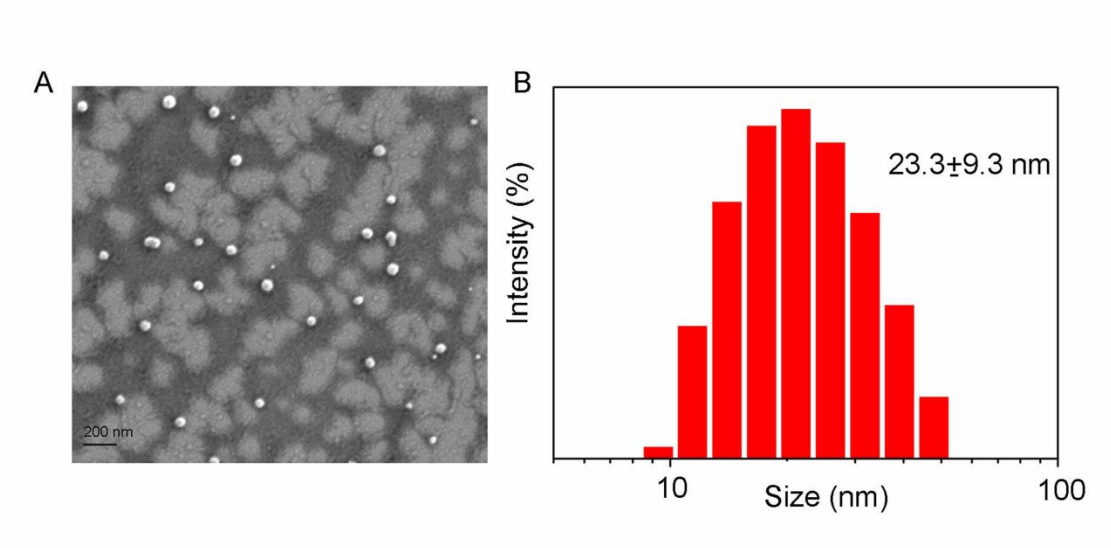


Fig. S9. The SEM image (A) and hydrodynamic radius (B) of PAPBN/DOX.