Supporting Information

Axial modification inhibited H-aggregation of phthalocyanine in polymeric micelles for enhanced PDT efficacy

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1. Materials

Monomethoxy poly(ethylene glycol) (CH_3O-PEG_{114}-OH; M_w = 5000 g/mol; M_w/M_n = 1.05; 99%) was purchased from Sigma-Aldrich. ε-Caprolactone (ε-CL, 99%) from Alfa was dried over calcium hydride and purified by distillation under reduced pressure before use. 4-Tert-butylphthalonitrile (98%) was purchased from TCI and used directly. Triphenylchlorosilane (98%) was purchased from Energy Chemical. Stannous octoate ($Sn(Oct)_2$, 95%), trichlorosilane ($HSiCl_3$, 98%) and *N, N*-diisopropylethylamine (DIEA, 99.5%) were purchased from J&K. 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA, 90%) and lithum (98.5%) were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. CCK-8 (Dojindo) was purchased from Tianjin BaiBei bio-technology co. LTD. Calcein-AM/PI Double Stain Kit was purchased from Tianjin Solomon biotechnology co. LTD.

2. Synthesis of polymer and photosensitizers

2.1 Synthesis of mPCL-*b*-PEG

mPEG-*b*-PCL was synthesized by ring-opening polymerization (ROP) of ε-CL using CH_3O-PEG_{114}-OH as macroinitiator and $Sn(Oct)_2$ as catalyst. In detail, CH_3O-PEG_{114}-OH (1.0 g, 0.2 mmol), ε-CL (5.0 g, 43.9 mmol) and one drop of $Sn(Oct)_2$ were dissolved into 15 mL of toluene in a Schlenk flask. After freeze-pump-thaw for three times, polymerization was carried out at 110 °C for 12 h. Then, the reaction

mixture was precipitated into excess diethyl ether. The precipitate was collected and dried under vacuum to obtain a white powder. According to the ^{1}H NMR result (Fig. S2), the composition of polymer was determined as $mPEG_{114}$-*b*-PCL_{87} by comparing the integration of H^a (3.63 ppm) and H^b (2.30 ppm).

2.2 Synthesis of Tetra(4-tert-butyl)-phthalocyanine (TbPc)

A mixture of 4-tert-butylphthalonitrile (2 g, 10.9 mmol) and lithum (160 mg, 22.8 mmol) in 1-pentanol (50 mL) was refluxed for 4 h. After being cooled to room temperature and condensed to around 10 mL by rotavapor, the resulting solution was precipitated into methanol (100 mL) containing a few drops of concentrated HCl. The precipitate was collected by filtration and chromatographed on a silica gel column using CH_2Cl_2/hexane (1:1) as eluent. The blue band was collected and concentrated by rotavapor. ^{1}H NMR (400 MHz, $CDCl_3$): 9.27-8.51 ppm (m, 8H), 8.1 ppm (m, 4H), 1.87 ppm (s, 36H), -2.71 ppm (s, 2H). MALDI-TOF m/z (M+): TbPc: calcd for $C_{48}H_{50}N_8$ 738.42, found 738.43.

2.3 Synthesis of Bis-triphenylsilyloxy-silicon-tetra(4-tert-butyl)-phthalocyanine (BtPc)

Tetra(4-tert-butyl)-phthalocyanine (200 mg, 0.27 mmol) was dissolved in anhydrous DCM (100 mL). The mixture was stirred for 10 min at room temperature. $HSiCl_3$ (100 µL, 1 mmol) was added and the mixture was stirred for another 10 minutes. Subsequently, DIEA (0.5 mL, 3 mmol) was added and the reaction mixture was stirred at room temperature. After 6 h, the mixture was slowly poured into water (20 mL) and stirred until gas evolution had ceased. The organic layer was collected, filtered and evaporated under reduced pressure. The intermediate was purified by silica gel column using CH_2Cl_2/MeOH (v:v = 100:1) as eluent to obtain a blue solid. The blue solid, imidazole (41 mg, 0.60 mmol) and triphenylchlorosilane (148 mg, 0.50 mmol) were dissolved in anhydrous 50 mL of DMF and refluxed for 6 h. After being cooled to room temperature, the solvents were evaporated under reduced pressure. BtPc was purified by silica column chromatography using gradient hexane/EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$): 9.57 ppm (m, 4H), 9.47 ppm (m, 4H), 8.55 ppm (m, 4H), 6.76 ppm (t, 6H), 6.34 ppm (t,12H), 4.83 ppm (d, 12H), 1.87 ppm (d, 36H). MALDI-TOF m/z (M+): BtPc: calcd for $C_{84}H_{78}N_8O_2Si_3$ 1314.56, found 1314.57. Uv-vis maximum absorption in DMF is 682.5nm.

3. Preparation of micelles

mPEG-*b*-PCL was dissolved in THF at 10 mg/mL and photosensitizer (TbPc or BtPc) was dissolved in THF at 2 mM. The polymer solution and photosensitizer solution were mixed at the ratio in Table S1:

Table S1. Polymer and photosensitizer amount used in preparation of micelles.

Micelles	THF / mL	mPEG-*b*-PCL (10 mg / mL) / mL	TbPc (2 mM) / mL	BtPc (2 mM) / mL
TbPcM-10	0.330	0.100	0.320	--
TbPcM-20	0.230	0.200	0.320	--
TbPcM-40	0.030	0.400	0.320	--
BtPcM-10	0.350	0.100	--	0.300
BtPcM-20	0.250	0.200	--	0.300
BtPcM-40	0.050	0.400	--	0.300

The mixed solution was rapidly added into 8 mL water, sonicated for 15 min and dialyzed against water for 2 days. The micelles solution was filtrated using 0.8 μm filter membrane and diluted to 10 mL.

4. Photosensitizer concentration measurement

Twenty microliter of micelle solution was added into 2 mL DMF, sonicated for 1 min and scanned by UV-vis. The concentration of photosensitizer (shown in Table S2) was calculated according to the standard curve. The loading efficiency of photosensitizer in micelles was calculated according to Formula 1.

Table S2. Concentration and loading efficiency of photosensitizers in micelles.

	TbPcM-10	TbPcM-20	TbPcM-40	BtPcM-10	BtPcM-20	BtPcM-40
Abs.	0.084	0.085	0.086	0.144	0.143	0.146
c (μM)	53.0	53.6	54.3	55.0	54.6	55.8
Loading Efficiency (%)	82.8	83.8	84.8	91.7	91.0	93.0

$$\text{Loading Efficiency} = \frac{\text{photosensitizer amount loaded in the micelle}}{\text{photosensitizer amount added initially}} \quad \text{(Formula 1)}$$

5. Spectral analysis

In this experiment, BtPcM-R and TbPcM-R were diluted using water to a concentration of photosensitizer at 2.0 μM. BtPc and TbPc were also dissolved in DMF (2 μM) for comparison. Uv-vis and fluorescence spectra were measured. Fluorescence excitation wavelength was 620 nm.

6. Singlet oxygen measurement

BtPcM-R and TbPcM-R were diluted using water to a concentration of photosensitizer at 0.5 μM with a final volume of 3.5 mL, containing 0.028 mg/mL ABDA for singlet oxygen measurement. The solution was illuminated by 684 nm 96-diodes array (0.15 W/cm^2). The UV-vis absorption spectra at 380 nm were measured at 1 min intervals for 10 min and plotted as a function of the irradiation time. Experiments were performed in triplicate.

7. *In vitro* PDT effect

HeLa cells were provided by Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Science & Peking Union Medical College. HeLa cells were grown in DMEM medium containing 10% FCS and supplemented with penicillin (172000 units/L) and streptomycin (172 mg/mL). HeLa cells (1×10^4 cells per well) were seeded in 96-well plates and incubated at 37 °C in a humidified 5% CO_2 atmosphere. One day later, the medium was exchanged with 80 μL fresh medium together with 20 μL of diluted TbPcM-40 or BtPcM-40 solution, the final photosensitizer concentration were 0 μM, 2 μM, 4 μM, 6 μM, 8 μM and 10 μM. After incubation for 12 h, the cells were illuminated by 684 nm 96-diodes array (0.15 W/cm^2) for 30 min, and a parallel experiment was conducted without illumination. After another 4 h incubation, the standard CCK-8 assay was carried out to evaluate the cell viability. Finally, the medium was removed and cells were rinsed with PBS (pH 7.4) and stained with Calcein-AM/PI for fluorescence microscope. Experiments were performed in triplicate.

8. Cell uptake of micelles

The cellular uptake experiment was performed as follow: HeLa cells were seeded into 20 mm

confocal dishes (glass bottom dish) at a density of 10^5 cells per dish. After incubation for 24 h, the culture medium was replaced with 1 mL of fresh medium containing 200 μL TbPcM-40 and BtPcM-40 respectively (10 μM photosensitizer). After further incubation for 6 h, the cells were washed three times with 1 mL PBS buffer. The cellular uptake of micelles was observed using confocal laser scanning microscopy (CLSM, TCS SP8, Leica, Germany, excitation at 633 nm, emission at 640-700 nm).

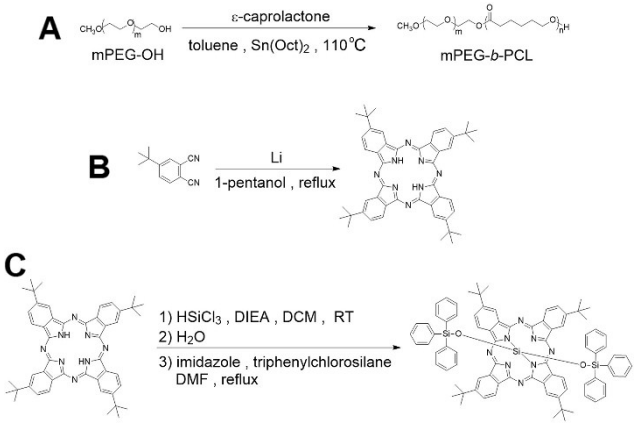

Fig. S1. Synthesis route of mPEG-*b*-PCL (A), TbPc (B) and BtPc (C).

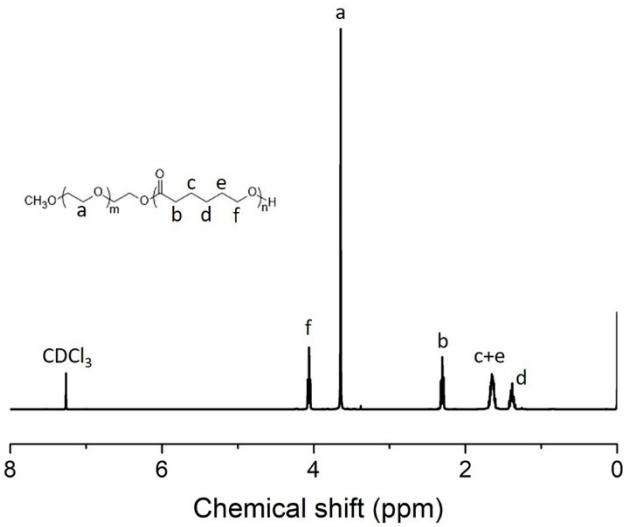

Fig. S2. ^{1}H NMR spectra of mPEG$_{114}$-*b*-PCL$_{87}$ in $CDCl_3$.

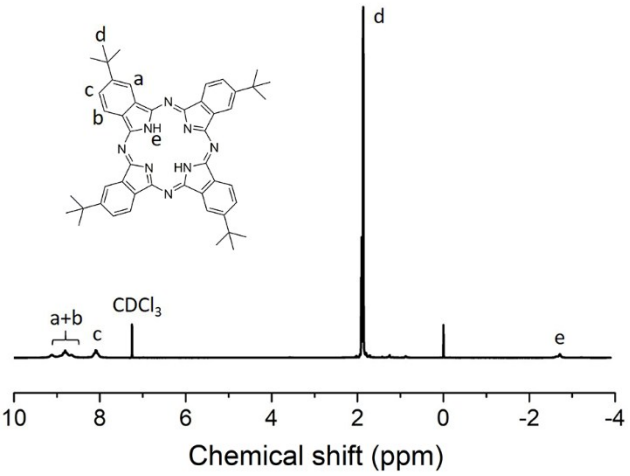

Fig. S3. ^{1}H NMR spectra of TbPc in $CDCl_3$.

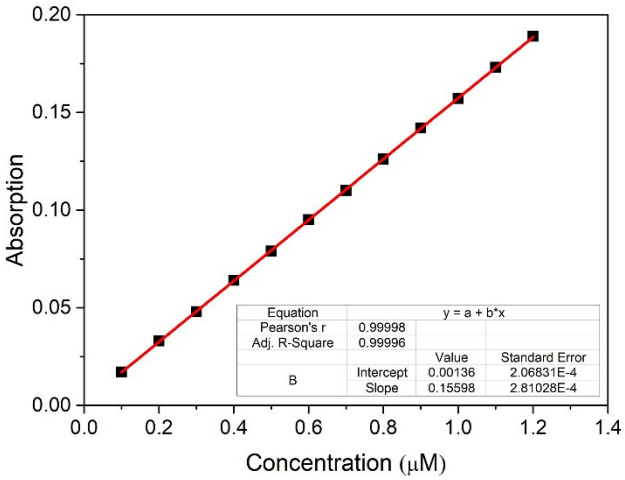

Fig. S4. Standard curve of TbPc in DMF measured by Uv-vis absorbance at 697 nm for calculating the concentration of TbPc in micelle.

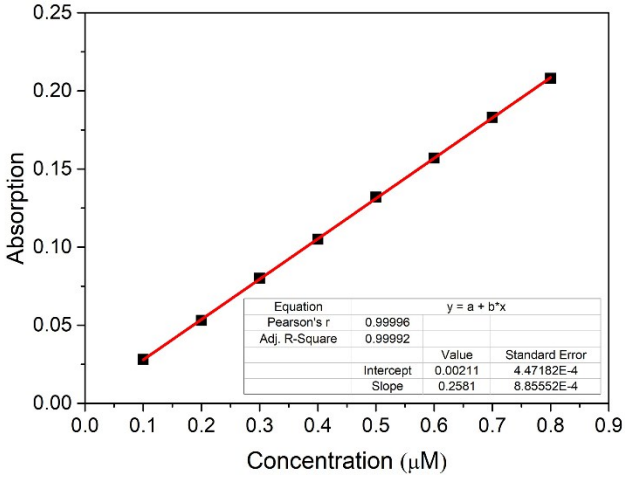

Fig. S5. Standard curve of BtPc in DMF measured by Uv-vis absorbance at 682.5 nm for calculating the concentration of BtPc in micelle.

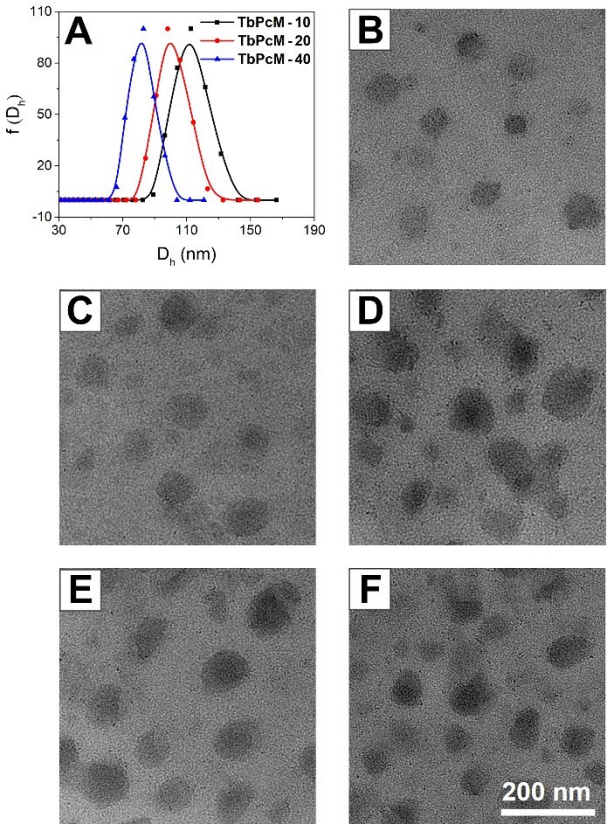

Fig. S6. Hydrodynamic diameter distribution of TbPcM-R (A). TEM images of TbPcM-10 (B), TbPcM-20 (C), TbPcM-40 (D), BtPcM-20 (E) and BtPcM-40 (F).