A water-soluble cationic [2]biphenyl-extended pillar[6]arene: synthesis, host-guest interaction with hemin and application in chemodynamic/photodynamic cancer therapy

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Supporting Information (14 pages)

1. Materials and methods

Materials

All reagents were commercially available and used as supplied without further purification. Solvents were either employed as purchased or dried according to procedures described in the literature.

Measurements

NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AV400 spectrometer.

UV/Vis spectroscopy. UV/Vis spectra and the optical transmittance were recorded in a quartz cell on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

SEM microscopy. (SEM) was scanned by Gemini SEM 3600 (Britain).

DLS spectroscopy. Solution samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at a scattering angle of 90°. The hydrodynamic diameter (Dh) was determined by DLS experiments at 25°C.

Cytotoxicity experiments. Human cervical cancer cells (HeLa cells) were incubated in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin (Control group). HeLa cells were seeded in 96-well plates (5×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37°C in 5% CO_2. Then DMEM containing different concentrations of hemin or $CBpExP_6 \supset$Hemin was introduced to replace the original medium. Four hours later, the cells were treated with or without a 660 nm laser (1 W cm^{-2}). Then the cells were incubated with different groups for 24 h. The relative cellular viability was determined by the MTT assay.

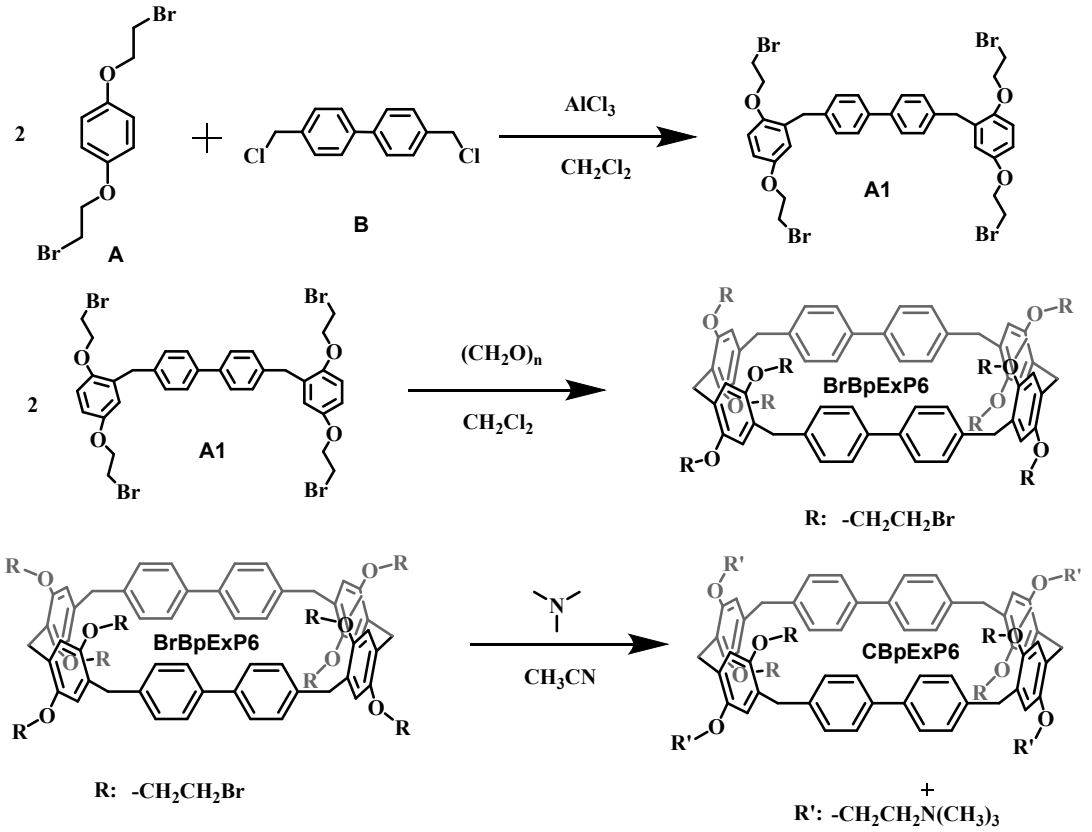
Cell viability assay. The live/dead cell assay was performed to verify the cytotoxicity of the material. HeLa cells were seeded into 6-well plates and cultured in DMEM with various concentrations of different formulations for 4 h. The used medium was then discarded, and a fresh medium containing Calcein-AM (10 μg/mL) and PI (5

μg/mL) was added. After incubation for 20 min, the cells were observed by a confocal laser scanning microscope after washing with PBS.

Detection of intracellular reactive oxygen species by fluorescence. 1. Detection of ROS： The detection of ROS was performed by diluting DCFH - DA with serum-free culture medium at a ratio of 1:1000 to a final concentration of 10 μmol/L. The cell culture medium was removed, and an appropriate volume of the diluted DCFH-DA was added. The volume added should be sufficient to cover the cells completely. Typically, for one well of a six-well plate, not less than 1 mL of diluted DCFH-DA should be added. The cells were incubated in a 37 ℃ cell culture incubator for 20 min. The cells were washed three times with serum-free cell culture medium to fully remove the DCFH-DA that did not enter the cells. **2. Detection of singlet oxygen:** Cells were seeded in 6-well plates at a density of 20×10^4 cells/mL. After seeding, the cells were incubated in a 5% CO_2 atmosphere and 37 ^oC for 12 h to allow the suspended cells to adhere to the wall. Once the cells adhered to the wall, the nano-drug at a specific concentration was added to the DMEM medium and continued to culture for 4 h. Then, the cells were irradiated with a near-infrared laser with a specific wavelength and power for 10 min. Subsequently, the cells were stained with SOSG (2 μM) for 30 min. After staining, the cells were washed three times with PBS buffer, and then the cellular fluorescence was observed by a confocal fluorescence microscope, and the confocal images were captured. (λex: 460–495 nm, λem: > 510 nm). **3. Detection of hydroxyl radical:** Cells were seeded in 6-well plates at a density of 20×10^4 cells/mL. After seeding, the cells were incubated in a 5% CO_2 atmosphere and 37 ^oC for 12 h to allow the suspended cells to adhere to the wall. Once the cells were adherent, specific concentrations of NPs were added to the DMEM medium and the cells were further cultured for 4 h. Then, the cells were irradiated with a near-infrared laser with a specific wavelength and power for 10 min. Subsequently, the cells were stained with APF (2 μM) for 30 min. After staining, the cells were washed three times with PBS buffer, and then the cellular fluorescence was observed by a confocal fluorescence microscope, and the confocal images were captured. (λex: 460–495 nm, λem: > 510 nm)**.**

2. Synthesis of $CBpExP_6$

Scheme S1. Synthetic route of $CBpExP_6$



Synthesis of compound A1: Refer to the existing reports,[S1] in a 500 mL round bottom flask, compound A (6.48 g, 20 mmol), $AlCl_3$ (0.53 g, 4 mmol) were dissolved in CH_2Cl_2 (180 mL). Compound B was dissolved CH_2Cl_2 (20 mL) and added dropwise to the round bottom flask during 30 minutes and stirred for about 30 minutes. After the reaction, the reaction solution was slowly poured into the prepared ice water, the organic solvent was removed by rotating under reduced pressure, and compound **A1** was obtained by column chromatography (volume ratio: dichloromethane: petroleum ether = 1: 2).

A1: White solid, 62%; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.47 (d, 4H, ArH), 7.28 (d, 4H, ArH), 6.75 (m, 6H, ArH), 4.22 (q, 8H, OCH_2), 4.01 (s, 4H, CH_2), 3.59 (m, 8H, CH_2).

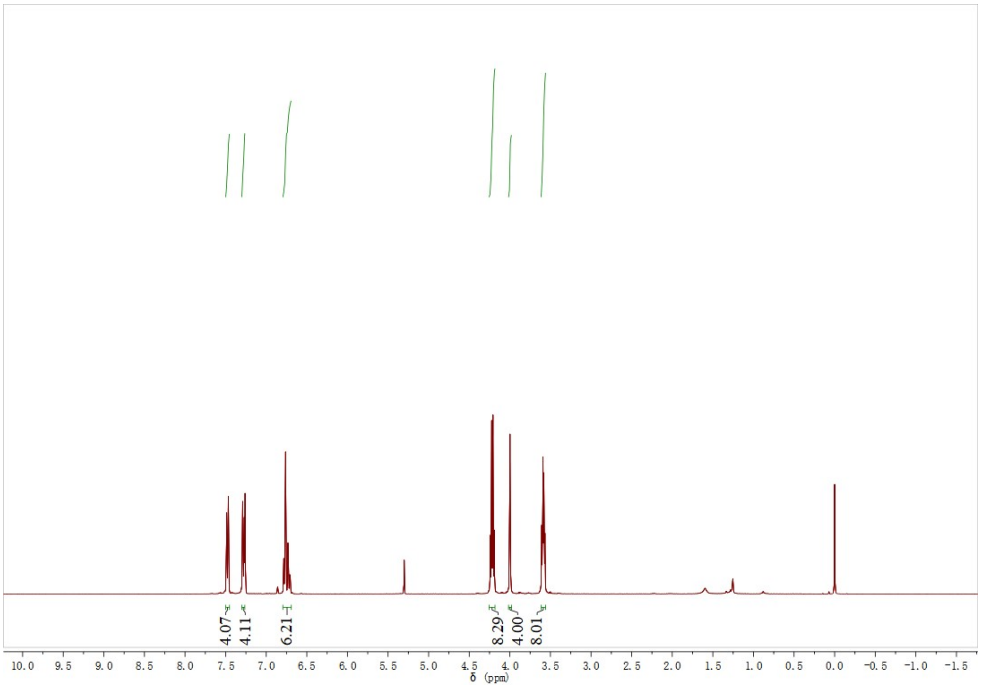


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **A1**.

Synthesis of compound $BrBpExP_6$: Compound **A1** (1 g, 1.2 mmol), $(CH_2O)_n$ (0.11 g, 1.2 mmol), 100 mL CH_2Cl_2 were added into a 250 mL round bottom flask. The $BF_3{\cdot}(C_2H_5)_2O$ (2 mL) solution was slowly added into flask through a constant-pressure dropping funnel and stirred at room temperature for 1.5 hours. After the reaction, saturated $NaHCO_3$ aqueous solution was added for extraction, and the organic solvent was removed by rotating under reduced pressure. Compound **$BrBpExP_6$** was obtained by column chromatography (volume ratio: dichloromethane: petroleum ether = 1: 1).

$BrBpExP_6$: White solid, 46%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37 (d, 8H, ArH), 7.21 (d, 8H, ArH), 6.98 (s, 4H, ArH), 6.67 (s, 4H, ArH), 4.24 (t, 8H, OCH_2), 4.19 (t, 8H, OCH_2), 3.94 (d, 12H, CH_2), 3.65 (t, 8H, CH_2), 3.51 (t, 8H, CH_2).

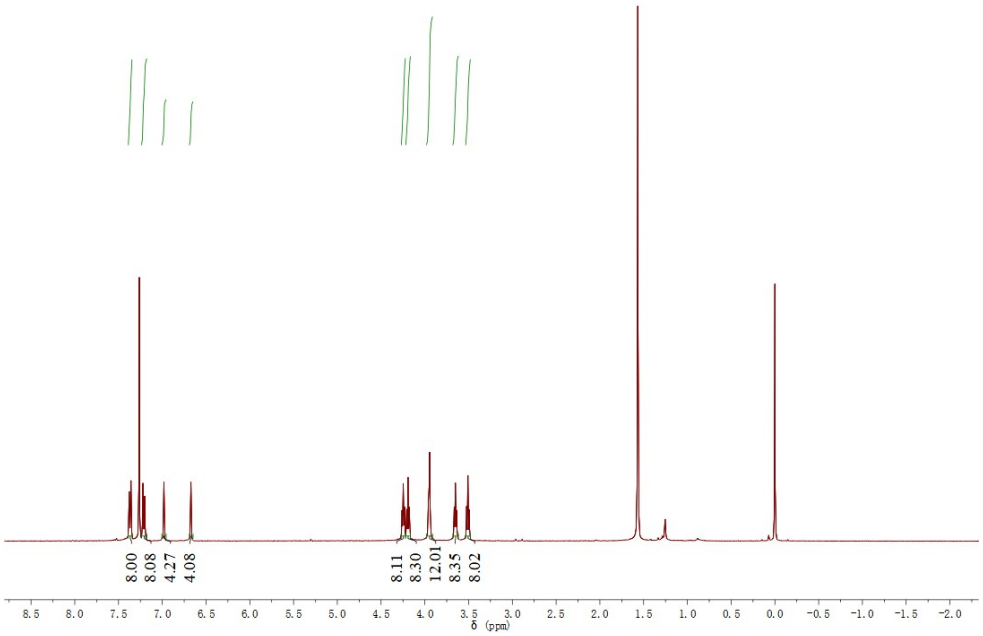


Figure S2. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **$BrBpExP_6$**.

Synthesis of compound $CBpExP_6$: Compound **$BrBpExP_6$** (500 mg, 0.3mmol), Trimethylamine solution (1.2 mL, 1.2 mmol), 100 mL CH_3CN were added into a 250 mL round bottom flask. The mixture was refluxed at 80 ℃ for 12 h, and then the solvent was removed by rotating under reduced pressure. After dissolving in water and filtering to remove impurities, the filtrate was removed by rotating under reduced pressure **$CBpExP_6$**.

$CBpExP_6$: White solid, 76%; ^{1}H NMR (400 MHz, D_2O): 7.43 (d, 8H, ArH), 7.16 (d, 8H, ArH), 6.84 (s, 4H, ArH), 6.66 (s, 4H, ArH), 4.22 (d, 16H, OCH_2), 3.91 (s, 8H, CH_2), 3.86 (s, 4H, CH_2), 3.52 (d, 16H, CH_2), 2.94 (d, 72H, CH_3). ^{13}C NMR (101 MHz, D_2O): 149.9, 149.5, 140.5, 137.8, 129.2, 128.7, 128.4, 126.8, 115.3, 114.8, 65.3, 65.1, 62.7, 62.5, 53.7, 35.2.

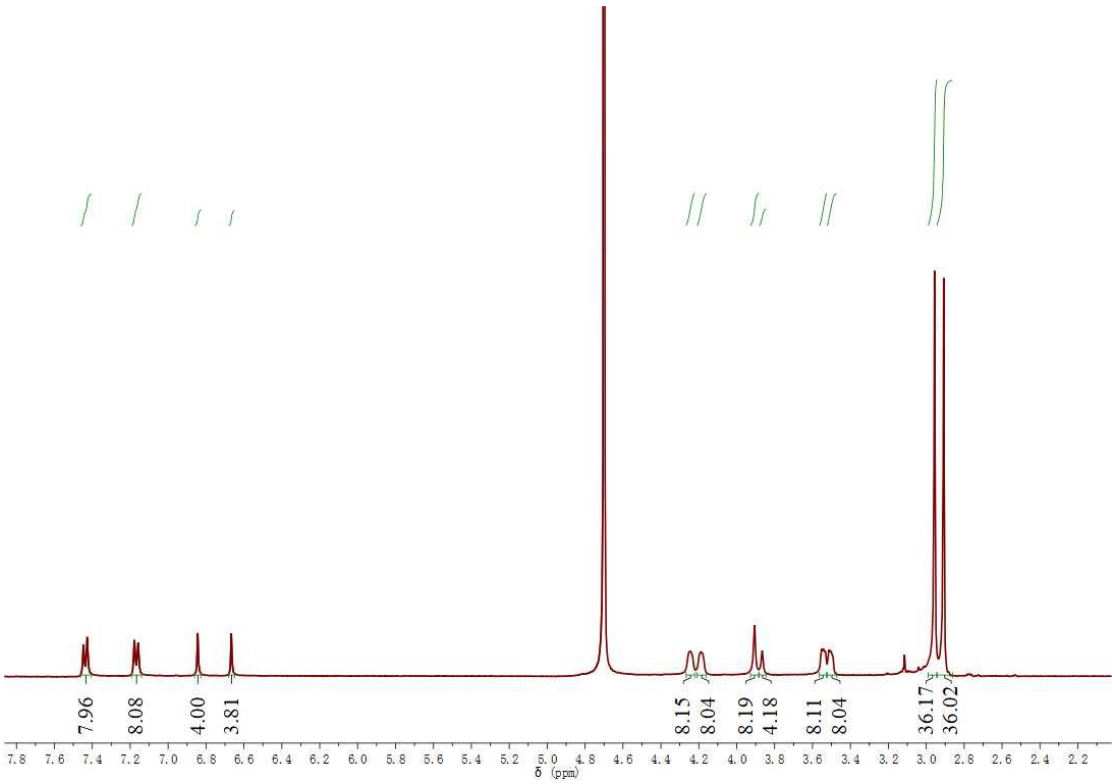


Figure S3. ^{1}H NMR spectrum (400 MHz, D_2O, 293 K) of **CBpExP$_6$**.

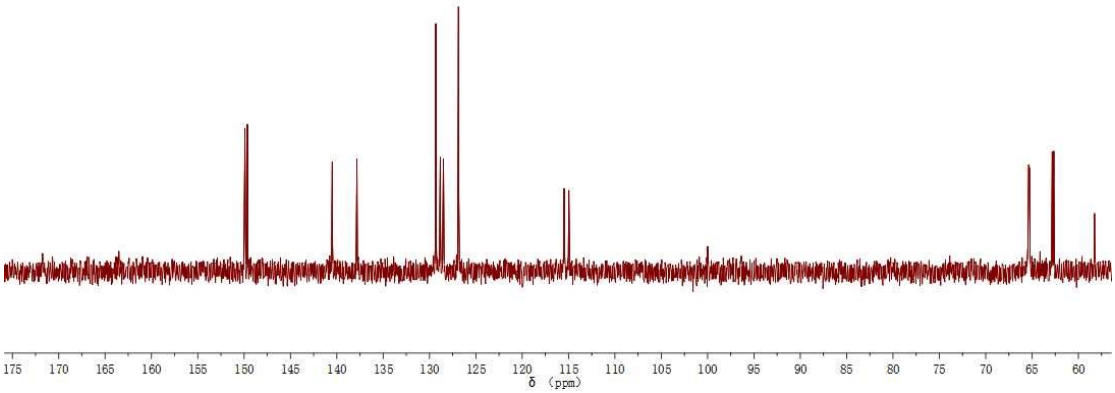


Figure S4. ^{13}C NMR spectrum (101 MHz, D_2O, 293 K) of **CBpExP$_6$**.

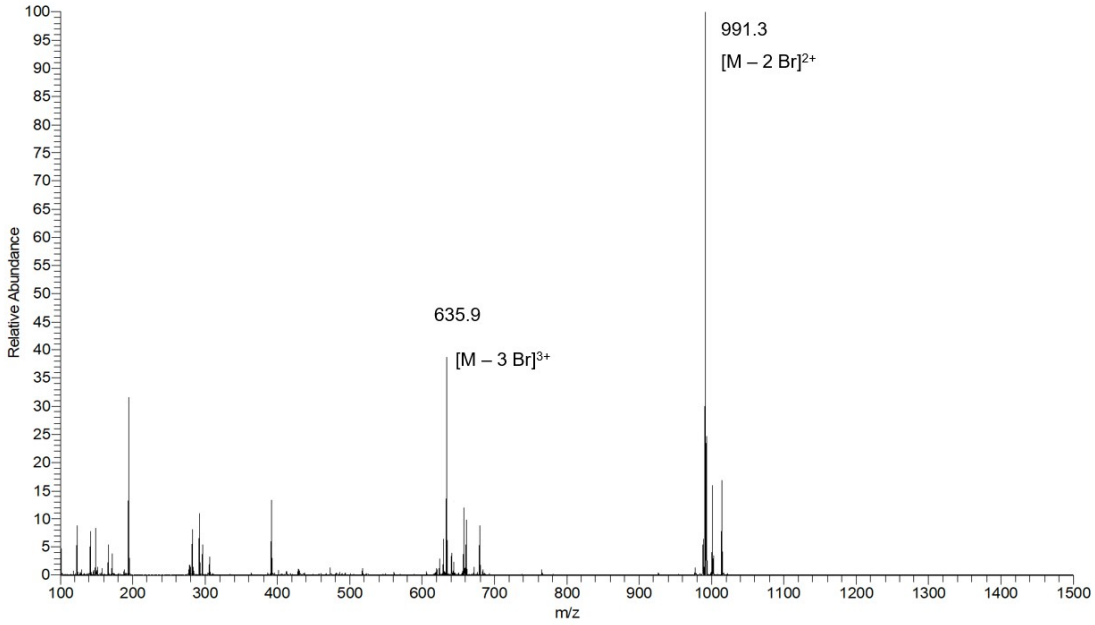


Figure S5. MS spectrum of **CBpExP$_6$**. $[M - 2Br]^{2+}$: C94H140Br6N8O8, found: 991.3; $[M - 3Br]^{3+}$: C94H140Br5N8O8, found: 635.9.

3. Host-guest interaction

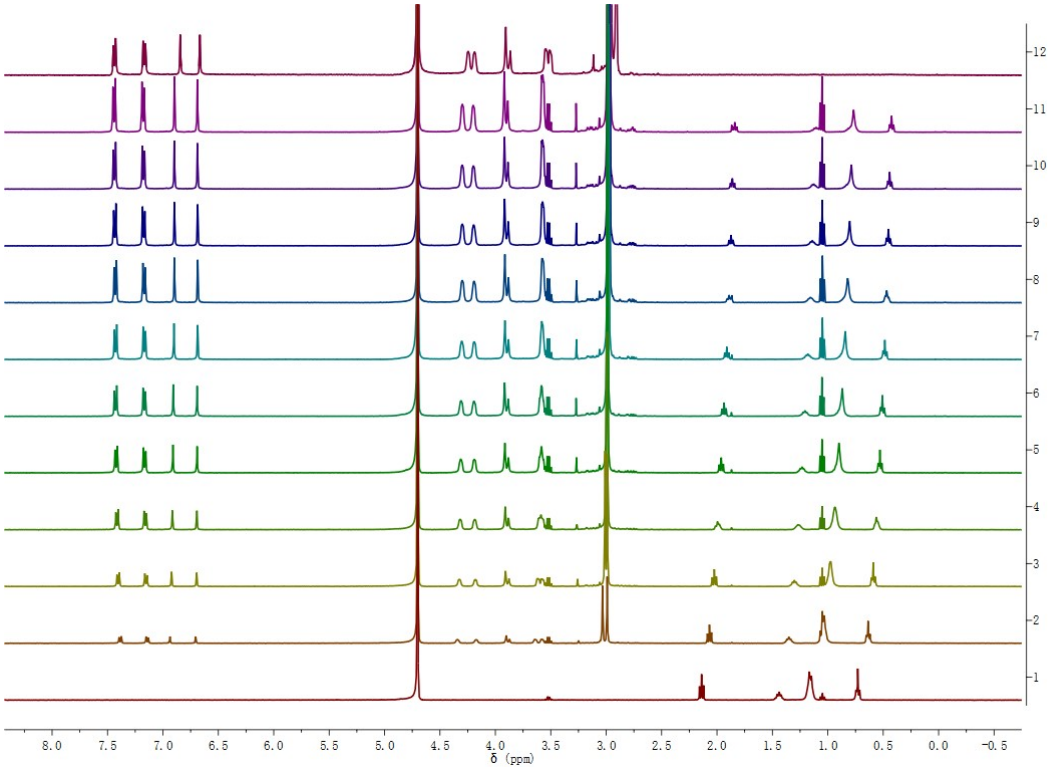


Figure S6. Host-guest interaction between **CBpExP$_6$** and model guest ***n*-Octanoic acid (G_M).**

(1-11) The ^{1}H-NMR spectra of the **CBpExP$_6$** and ***n*-Octanoic acid** (G_M) (D_2O， 293 K， 400 MHz), G_M (3 mM) and different concentrations of **CBpExP$_6$**: (1) 0.0 mM, (2) 0.6 mM, (3) 1.2 mM, (4) 1.8 mM, (5) 2.4 mM, (6) 3.0 mM; (7) 3.6 mM; (8) 4.2 mM; (9) 4.8 mM; (10) 5.4 mM; (11) 6 mM. (12) The ^{1}H-NMR spectra of **CBpExP$_6$**.

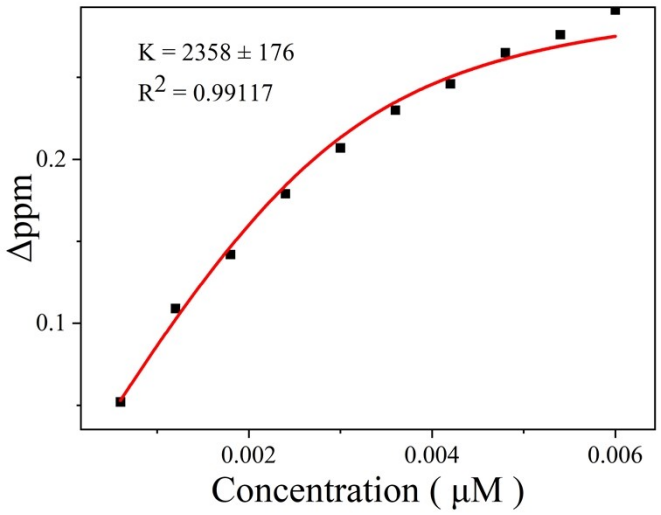


Figure S7. Chemical shift changes of H_d on $\mathbf{G_M}$ with the addition of **CBpExP$_6$**.

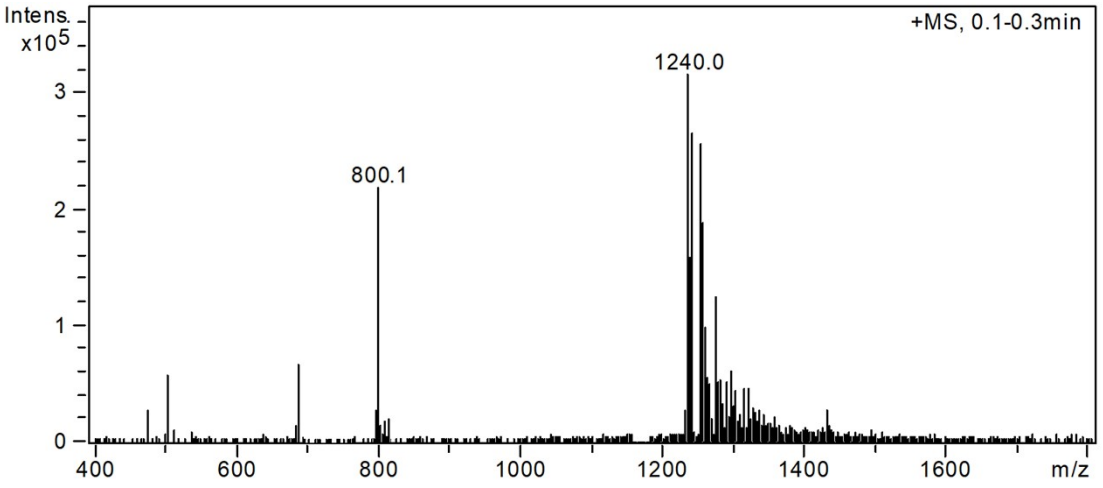


Figure S8. MS spectra of **CBpExP$_6$**⊃Hemin. Peaks at m/z 1240.0 and 800.1, corresponding to [CBpExP$_6$⊃Hemin – $2H^+$ – $4Br^-$]$^{2+}$ and [CBpExP$_6$⊃Hemin – $2H^+$ – $5Br^-$]$^{3+}$, respectively

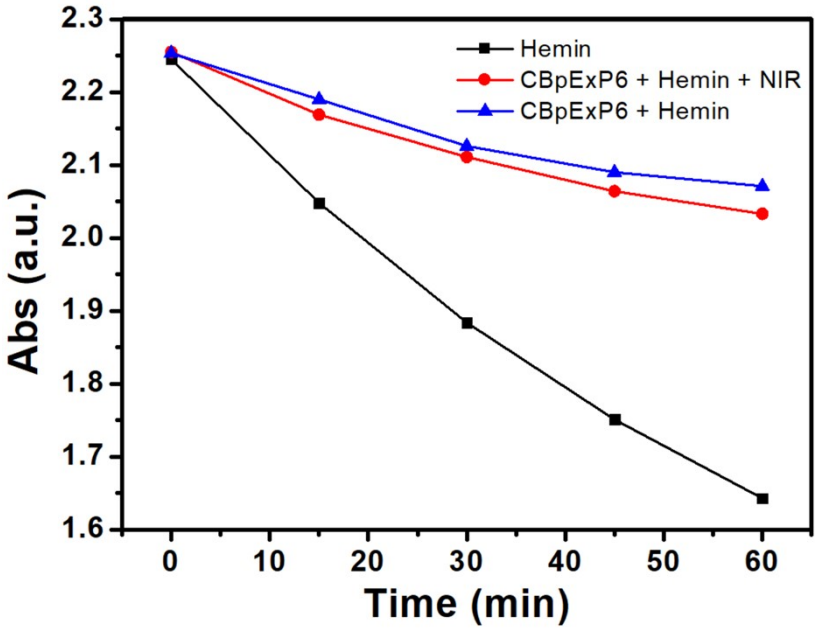


Figure S9. Time-dependent UV absorbance intensity at 390 nm of Hemin, **CBpExP$_6$**⊃Hemin and **CBpExP$_6$**⊃Hemin + NIR.

4. Self-assembly of **CBpExP$_6$**⊃Hemin in water

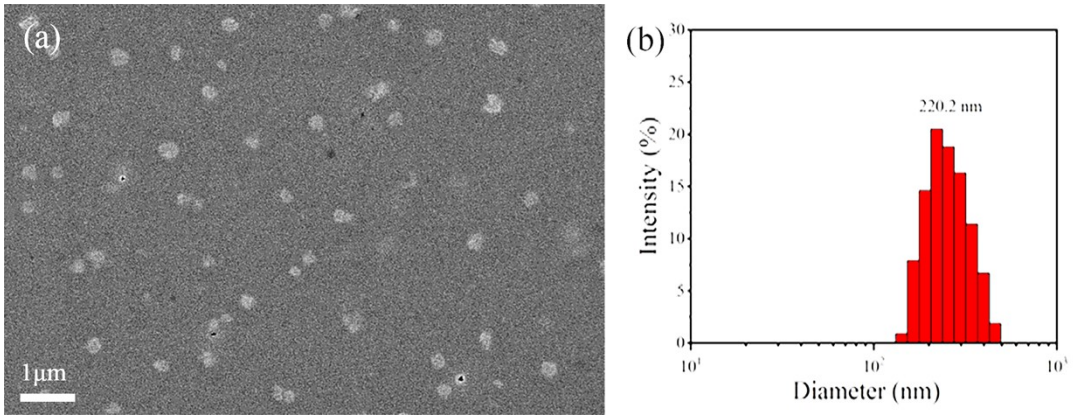


Figure S10. (a) SEM image of **CBpExP$_6$**⊃Hemin NPs; (b) DLS study of **CBpExP$_6$**⊃Hemin NPs at room temperature.

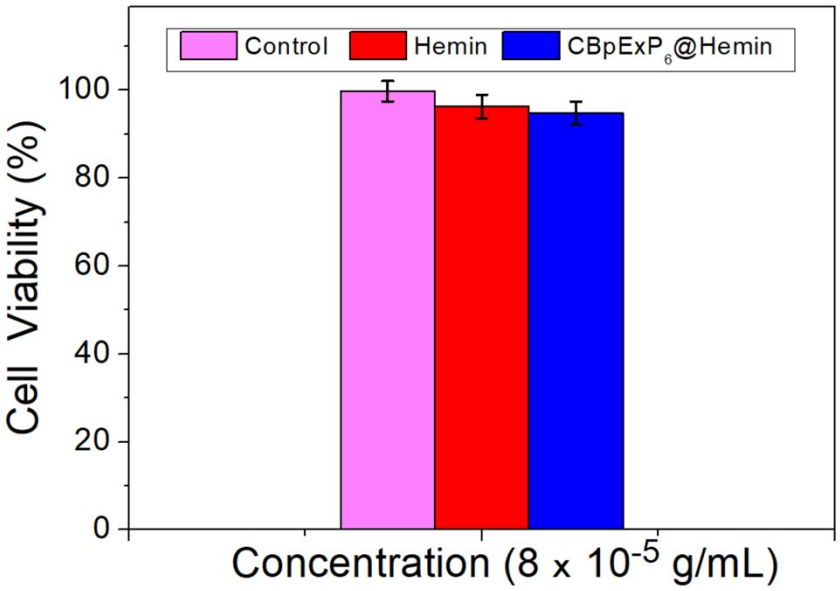


Fig. S11 Cell viability of Control, Hemin and $CBpExP_6$⊃Hemin to the normal cells HEK293.

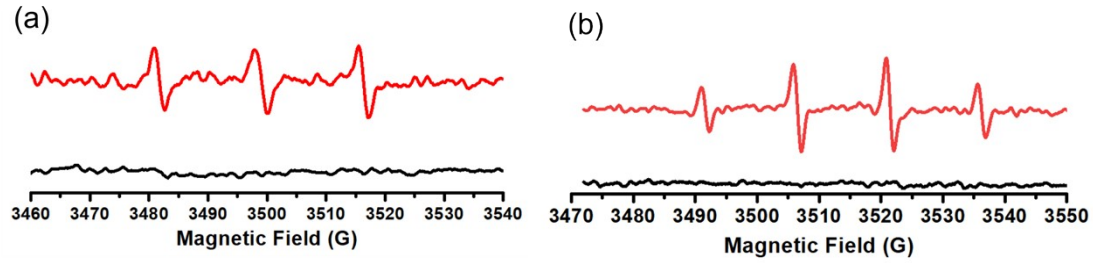


Fig. S12 (a) Electron spin resonance spectra of $^1O_2$ trapped by DMPO in $CBpExP_6$⊃Hemin NPs + NIR (red line), and PBS (black line). (b) Electron spin resonance spectra of $^1O_2$ trapped by DMPO in $CBpExP_6$⊃Hemin NPs + H_2O_2 (red line), and PBS (black line).

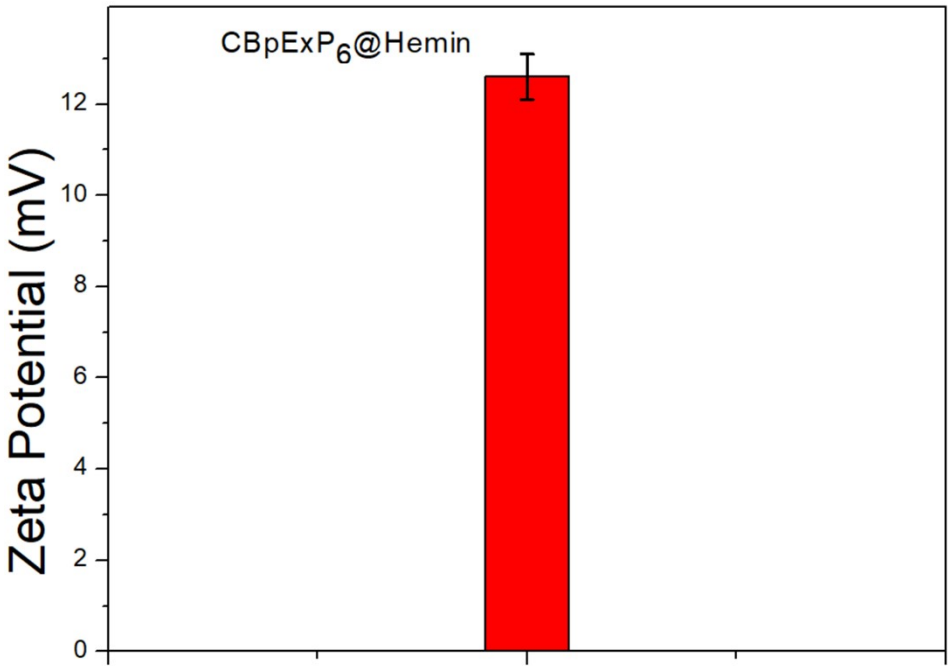


Fig. S13 Zeta potential of $CBpExP_6$⊃Hemin NPs.

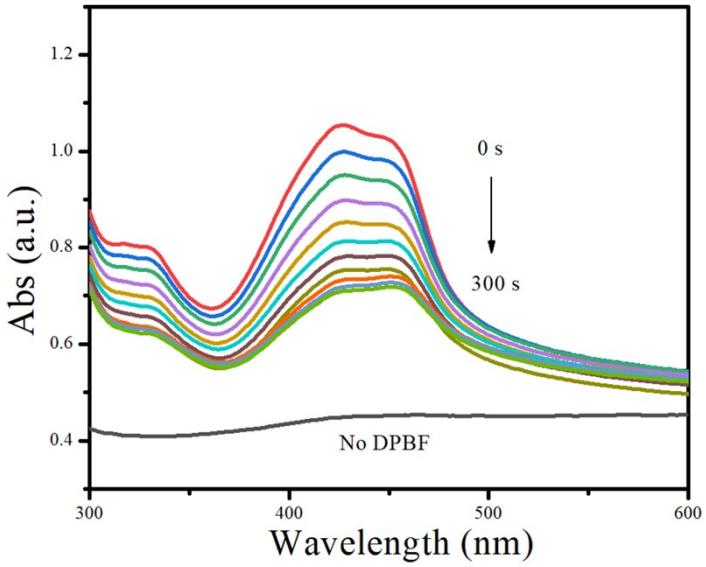


Fig. S14 Time-dependent UV-vis spectra of Hemin with DPBF after irradiation (660 nm, 1.0 W/cm^2).

The max. Abs in $CBpExP_6$⊃Hemin group decrease from 1.21 to 0.62, while in Hemin group, it decreases from 1.05 to 0.75 in 300 s.

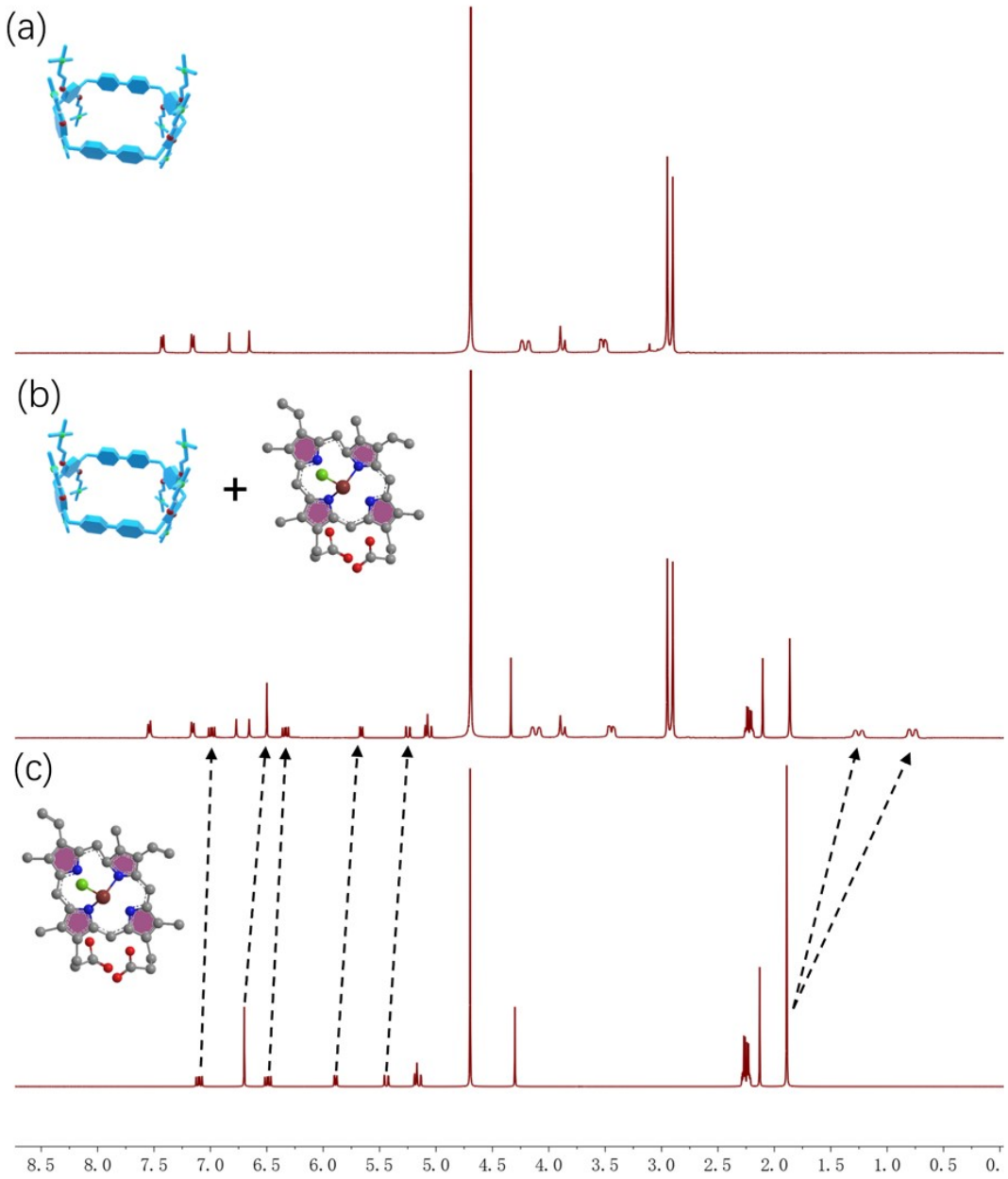


Fig. S15. ^{1}H NMR (400 MHz, D_2O, r.t.) spectra of (a) $CBpExP_6$ (1 mmol), (b) $CBpExP_6$ (1 mmol) and hemin (1 mmol), (c) hemin (1 mmol).

Reference

S1. (a) D. Dai, Z. Li, J. Yang, C. Wang, J.-R. Wu, Y. Wang, D. Zhang and Y.-W. Yang, *J. Am. Chem. Soc.*, **2019**, *141*, 4756–4763; (b) J.-R. Wu and Y.-W. Yang, *J. Am. Chem. Soc.*, **2019**, *141*, 12280–12287.