Pillar[5]arene derived covalent organic materials with pre-encoded molecular recognition for targeted and synergistic cancer photo- and chemotherapy

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Supporting Information (23pages)

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.

Fluorescence spectroscopy. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse equipped with a Varian Cary single-cell peltier accessory to control temperature.

UV/Vis spectroscopy. UV/Vis spectra and the optical transmittance were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

TEM microscopy. High-resolution Transmission electron microscopy (TEM) images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 keV. The sample for high-resolution TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried.

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.

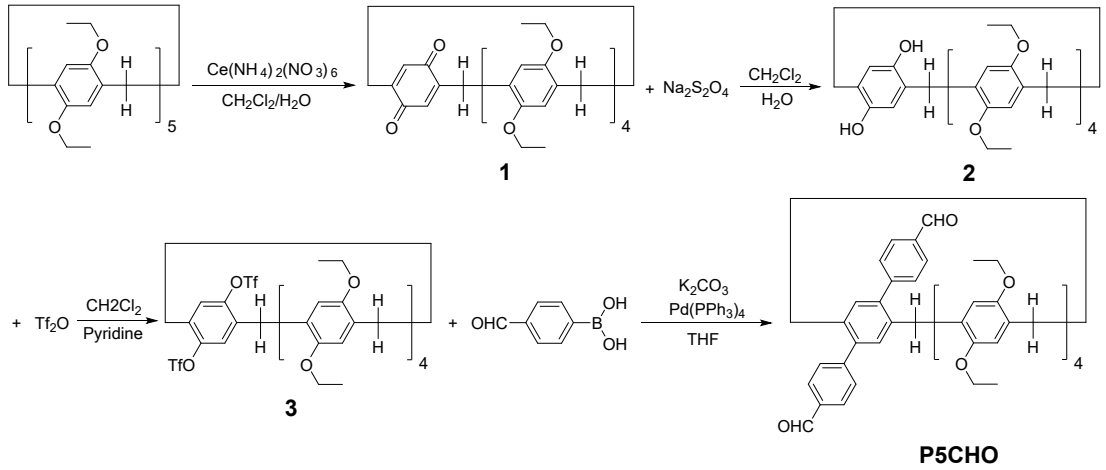
ESI-MS spectroscopy. Electrospray ionization mass spectra (ESI-MS) were measured by Agilent 6520 Q-TOF-MS.

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. 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 the cells were incubated with different groups for 24 h. The relative cellular viability was determined by the MTT assay.

Confocal laser scanning microscopy. HeLa cells were seeded in 6-well plates (5×10^4 cell mL^{-1}, 2 mL per well) for 24 h at 37°C in 5% CO_2. The cells were incubated with the corresponding solution for 4 h. Then the medium was removed, and the cells were washed with phosphate buffer solution for three time. Finally, the cells were subjected to observation by a confocal laser scanning microscope.

2. Synthesis of P5CHO

Scheme S1. Synthetic route of P5CHO



Synthesis of compound **1**: In a 250 mL round bottom flask, (4.45 g, 5 mmol) pillar[5]arene and 100 mL dichloromethane were added, then 20 mL ammonium ceric nitrate aqueous solution (0.25 M) was added drop-wise slowly, and stirred at room temperature for 20 min. After the reaction, saturated $NaHCO_3$ aqueous solution was added for extraction, anhydrous Na_2SO_4 was used to dry the organic layer, the organic solvent was removed by rotating under reduced pressure, and compound **1** was obtained by column chromatography (volume ratio: ethyl acetate: petroleum ether =1: 80).

Synthesis of compound **2**: Compound **1** (1 g, 1.2 mmol), 100 mL CH_2Cl_2 and 20 mL aqueous solution of sodium hydrosulfite (0.15 M) were added into a 250 mL round bottom flask, and stirred at room temperature for 4 hours. After the reaction, saturated NaCl aqueous solution was added for extraction, anhydrous Na_2SO_4 was used to dry the organic layer, and the organic solvent was removed by rotating under reduced pressure to obtain compound **2**.

Synthesis of compound **3**: Add compound **2** (1 g, 1.2 mmol), 100 mL dichloromethane and 4 mL pyridine into a 250 mL round-bottom flask, take an ice bath, then slowly drop 4 mL trifluoromethanesulfonic anhydride, and react overnight at 30°C. After the reaction, the organic solvent was removed by rotating under reduced pressure, and compound **3** was obtained by column chromatography (volume ratio: ethyl acetate: petroleum ether =1: 50).

Synthesis of compound **P5CHO**: Compound **3** (1.10 g, 1 mmol), 4-formylphenylboronic acid (0.89 g, 5.55 mmol), $Pd(PPh_3)_4$ (180 mg, 0.16 mmol), 30

mL K_2CO_3 aqueous solution (0.2 M) and 50mL THF were added into a 250 mL round-bottom flask, and reacted at 80°C for 24 h. After the reaction, the organic layer was washed with water (2 × 100 mL) and brine (100 mL), and dried with anhydrous Na_2SO_4. Rotating under reduced pressure to remove organic solvent, and column chromatography (volume ratio: ethyl acetate: petroleum ether =1: 30) to obtain compound **P5CHO**.

P5CHO: White solid, 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.07 (s, 2H, CHO), 7.76 (d, *J* = 8.1 Hz, 4H, phH), 7.20 (d, *J* = 7.8 Hz, 4H, phH), 7.00 (s, 2H, phH), 6.79 (d, *J* = 14.6 Hz, 4H, phH), 6.59 (s, 2H, phH), 5.82 (s, 2H, phH), 3.99-3.59 (m, 22H, CH_2), 3.52-3.47 (m, 4H, CH_2), 1.46-1.43 (m, 7H, CH_3) , 1.25-1.20 (m, 7H, CH_3), 1.16-1.12 (m, 5H, CH_3), 0.99-0.96 (m, 5H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 192.0, 149.9, 149.7, 149.5, 149.4, 148.8, 139.6, 136.8, 134.8, 132.0, 130.3, 129.4, 129.2, 128.9, 128.5, 127.0, 114.8, 114.7, 114.4, 114.0, 77.4, 77.0, 76.7, 63.9, 63.7, 63.5, 63.2, 58.3, 39.9, 32.8, 29.6, 29.0, 18.3, 15.4, 15.2, 15.0, 14.8; IR (KBr) υ: 2973, 2930, 1702, 1604, 1569, 1503, 1477, 1439, 1407, 1392 1306, 1206, 1111, 1052, 957, 916, 876, 834, 774, 745, 726, 708, 694, 669, 556, 514, 450cm^{-1}; MS (*m/z*): HRMS (ESI) Calcd. for $C_{65}H_{70}O_{10}Na$ ([M+Na]$^+$): 1033.4867, found: 1033.4853.

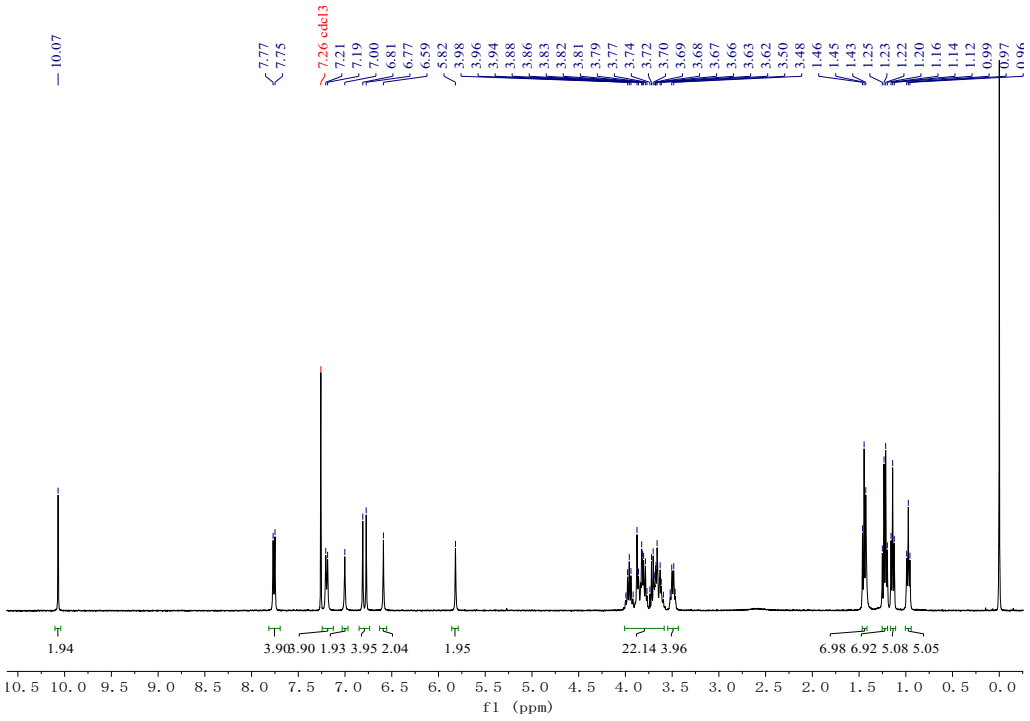


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **P5CHO**.

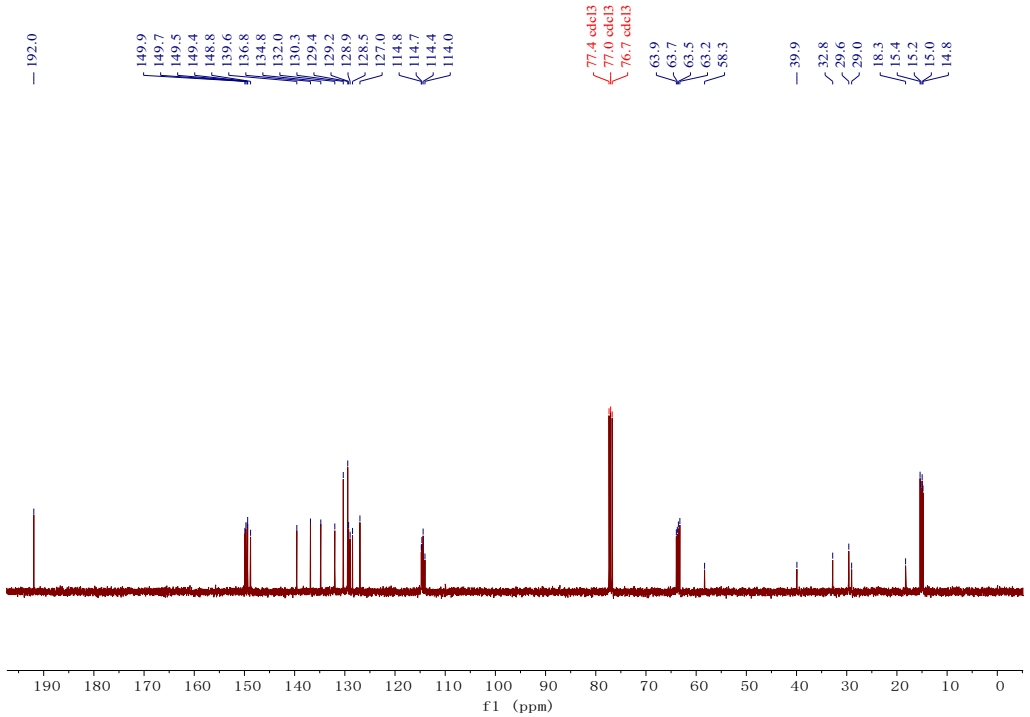


Figure S2. ^{13}C NMR spectrum ($CDCl_3$, room temperature, 100 MHz) of **P5CHO**.

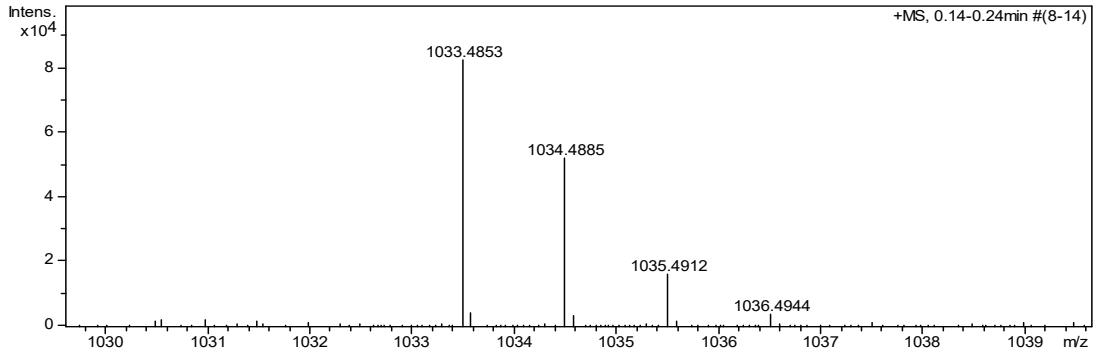


Figure S3. Mass spectra of **P5CHO** Calcd. for $C_{65}H_{70}O_{10}Na$ ($[M+Na]^+$): 1033.4867, found: 1033.4853.

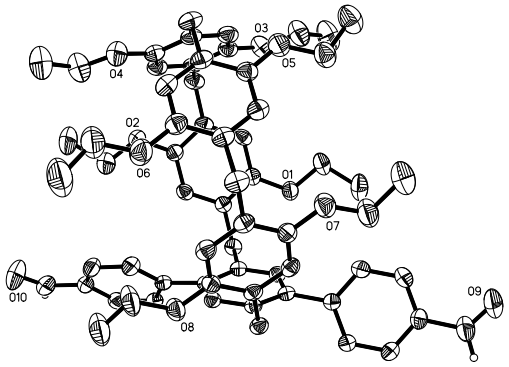


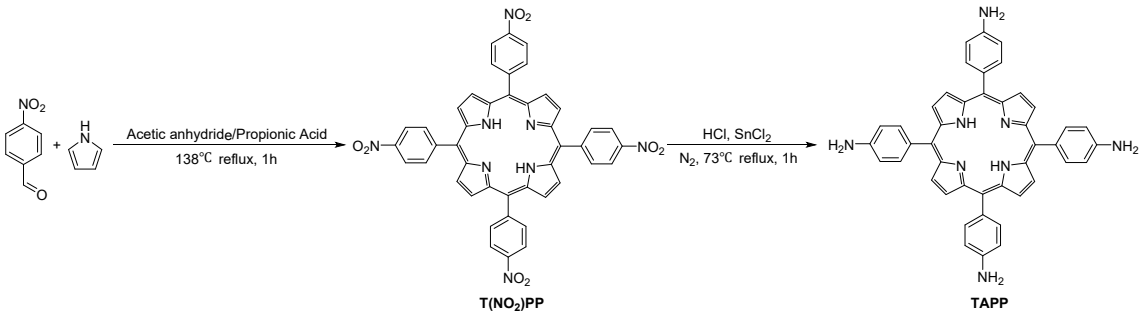
Figure S4. ORTEP-drawing (30% ellipsoid probability) of X-ray structures of **P5CHO** (hydrogen atoms are omitted for clarity). CCDC number: 2076413.

Table S1 Information of crystal data for **P5CHO**

Phase	**P5CHO**
Empirical formula	$C_{65}H_{70}O_{10}$
Formula weight	1011.21
Temperature(K)	273(2)
Wavelength(Å)	0.71073
Crystal system,	Monoclinic,
space group	P2(1)/n
a(Å)	16.7642(10)
b(Å)	20.8125(13)
c(Å)	18.6567(12)
α(°)	90
β(°)	94.942(2)
γ(°)	90
Volume($Å^3$)	6485.2(7)
Z	4
Calculated density($Mg \cdot m^{-3}$)	1.036
Absorption coefficient(mm^{-1})	0.069
F(000)	2160
Crystal size(mm)	0.240 x 0.220 x 0.180
Theta range for data collection(°)	1.469 to 25.999
hkl ranges	-20 to 20, -25 to 22, -23 to 23
Reflections collected	60856 / 12713
unique	R(int)=0.0598
Completeness to theta	99.7%
Absorption correction	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	12713/78/684
Goodness-of-fit on F^2	1.022
Final R indices [I>2σ(I)]	R_1=0.0605, wR_2=0.1644
R indices (all data)	R_1=0.1375, wR_2=0.1918
Largest diff. peak and Hole($e \cdot Å^3$)	0.164 and -0.188

3. Synthesis of TAPP

Scheme S2. Synthetic route of TAPP



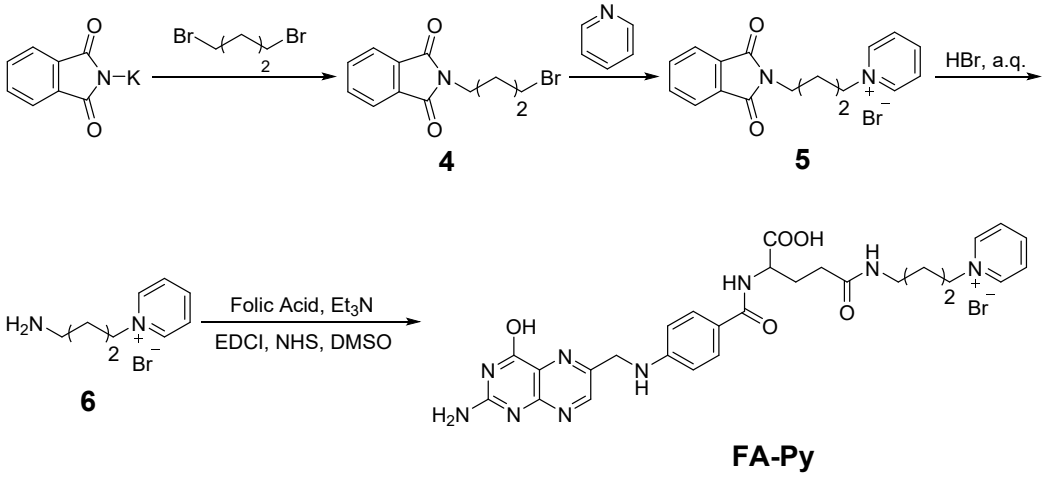
Following a modified procedure from reference S1. To a typical synthesis, a solution of p-nitrobenzaldehyde (11.0 g, 73 mmol), acetic anhydride (12.0 mL, 127 mmol) and propionic acid (300 mL) was stirred at 138 ℃. A mixture of freshly distilled pyrrole (5.0 mL, 73 mmol) and propionic acid was added and the resulting solution was refluxed for 1 h. After cooled to room temperature, the solution was filtered and washed several times with methanol and the dark solid was dried at 100 ℃ in oven. The powdery solid was added into pyridine (80 mL), stirring to reflux for 1h and cooled to room temperature, then methanol (30 mL) was added and stored at -4 ℃ overnight. The mixture was filtered and washed with methanol and acetone until rinsing was clear, then dried to get deep red Tetrakis-5,10,15,20-(4-nitrobenzene) porphyrin [T(NO_2)PP]. A solution of T(NO_2)PP (1.0 g 1.25 mmol) and $SnCl_2 \cdot 2H_2O$ (4.5 g, 20 mmol) in concentrated HCl (130 mL) was bubbled with Ar for 20 min, then stirred at 73 ℃ for 1 h. After cooled to room temperature, concentrated ammonium hydroxide was slowly added at 0 ℃ until the pH was 7, then filtered, washed several times with hot water and dried by oven at 100 ℃. The dark purple solid was Soxhlet extracted with $CHCl_3$ and the purple TAPP was separated from the solution by rotary evaporation.

TAPP: Purple solid, 78%; ^{1}H NMR (400 MHz, DMSO) δ: 8.88 (s, 8H, ArH), 7.85 (d, *J* = 8.2 Hz, 8H, ArH), 7.00 (d, *J* = 8.2 Hz, 8H, ArH), 5.58 (s, 8H, NH), -2.74 (s, 2H,

NH); ^{13}C NMR (100 MHz, DMSO) δ: 149.0, 136.0, 129.2, 121.1, 113.0, 79.7, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.3.

4. Synthesis of FA-Py

Scheme S3. Synthetic route of **FA-Py**



Synthesis of compound **4**: 1,4-dibromobutane (10.80 g, 50 mmol) and potassium phthalimide (1.86 g, 10 mmol) were added to 10 mL DMF and reacted at 90℃ for 24 hours. The organic solvent was removed by rotating under reduced pressure, and compound **4** was obtained by column chromatography (volume ratio: ethyl acetate: petroleum ether =1: 30). White solid, 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.87-7.83 (m, 2H, phH), 7.75-7.70 (m, 2H, phH), 3.73 (t, J = 6.6 Hz, 2H, CH_2), 3.45 (t, J = 6.3 Hz, 2H, CH_2), 1.95-1.82 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 168.3, 134.0, 132.0, 123.2, 77.4, 77.1, 76.8, 36.9, 32.8, 29.8, 27.2.

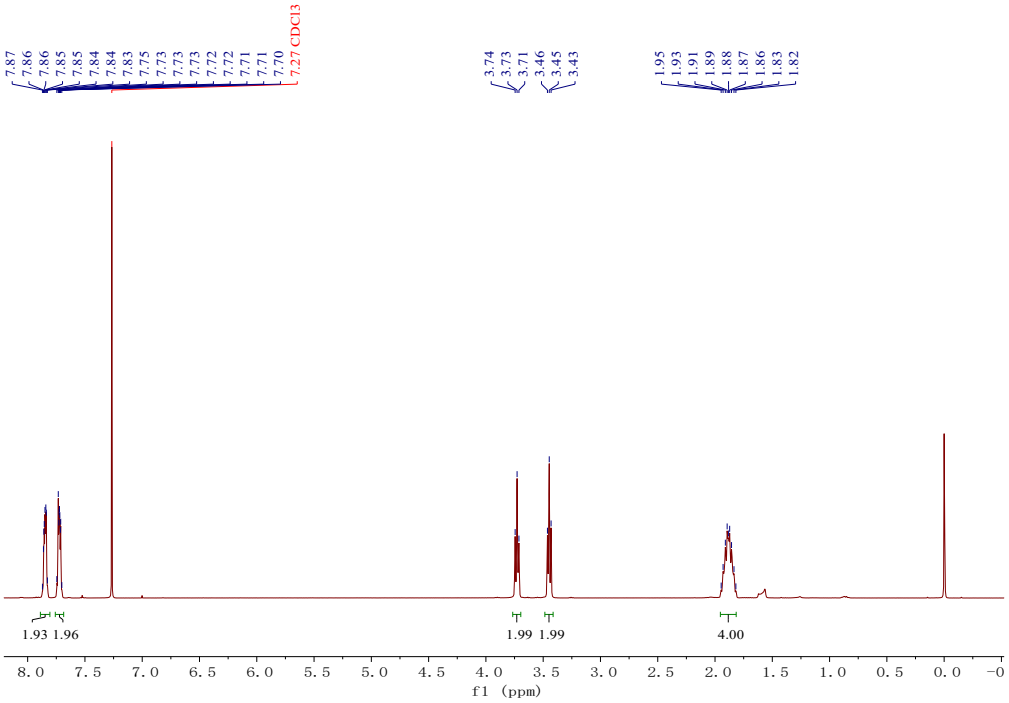


Figure S5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **4**.

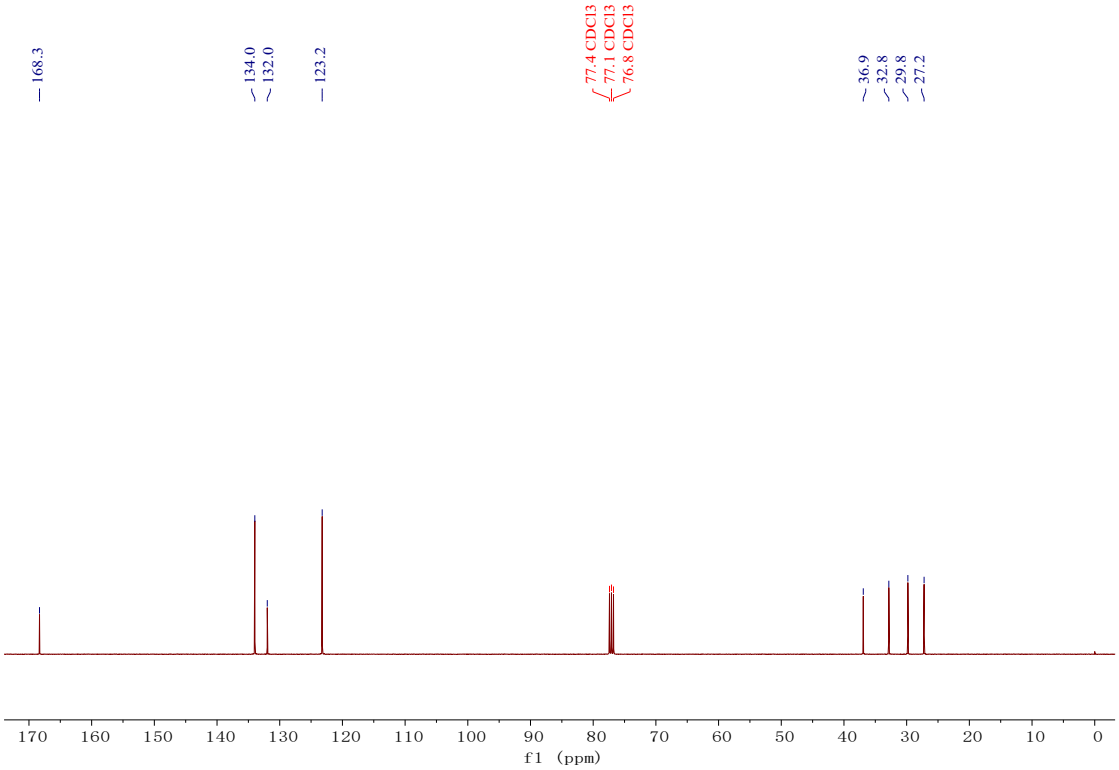


Figure S6. ^{13}C NMR spectrum ($CDCl_3$, room temperature, 100 MHz) of **4**.

Synthesis of compound **5**: Compound **4** (2.81 g, 10 mmol) was dissolved in 10 mL pyridine, and heated and refluxed for 3 hours. After the reaction was cooled, 30 mL of ether was poured, filtered by suction and washed with ether (3 × 30 mL) to obtain white solid **5**. White solid, 90%; ^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.73 (d, J = 5.7 Hz, 2H, ArH), 8.42 (t, J = 7.9 Hz, 1H, ArH), 7.94 (t, J = 7.1 Hz, 2H, ArH), 7.64 (s, 4H, ArH), 4.55(t, J = 7.5 Hz, 2H, CH_2), 3.56 (t, J = 6.9 Hz, 2H, CH_2), 1.98-1.90 (m, 2H, CH_2), 1.64-1.57 (m, 2H, CH_2). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 169.9, 145.7, 144.2, 134.7, 130.7, 128.3, 123.1, 61.0, 36.8, 28.0, 24.5.

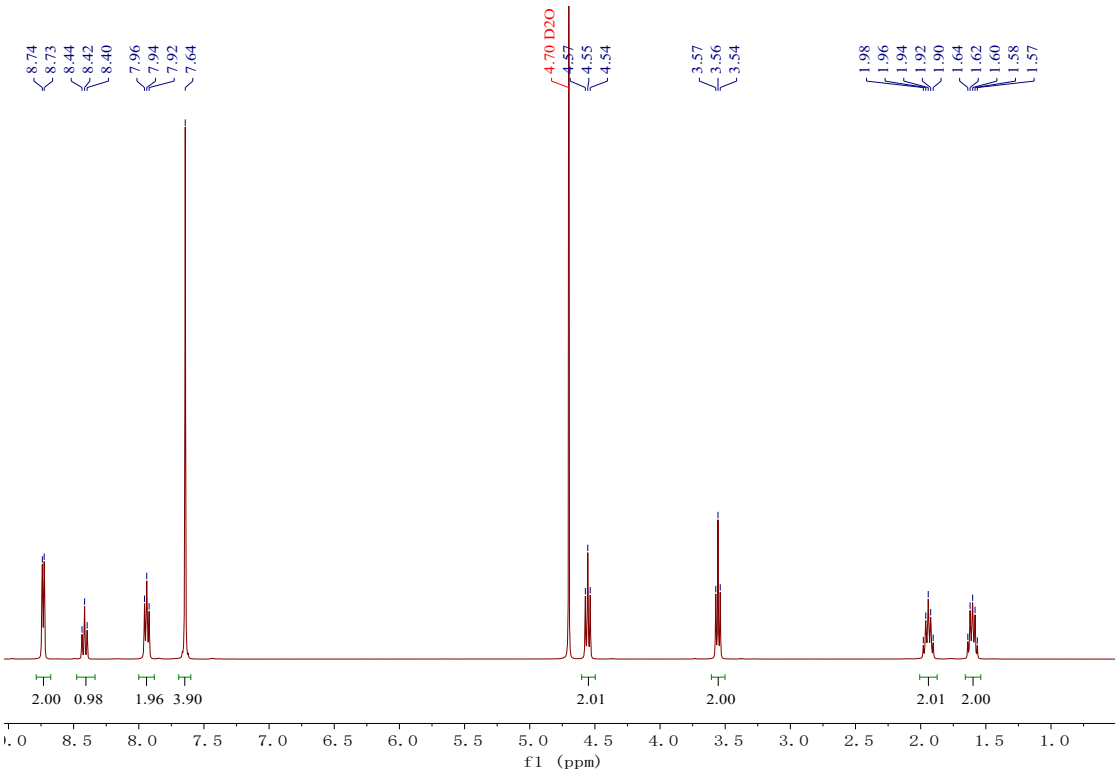


Figure S7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 293 K) of **5**.

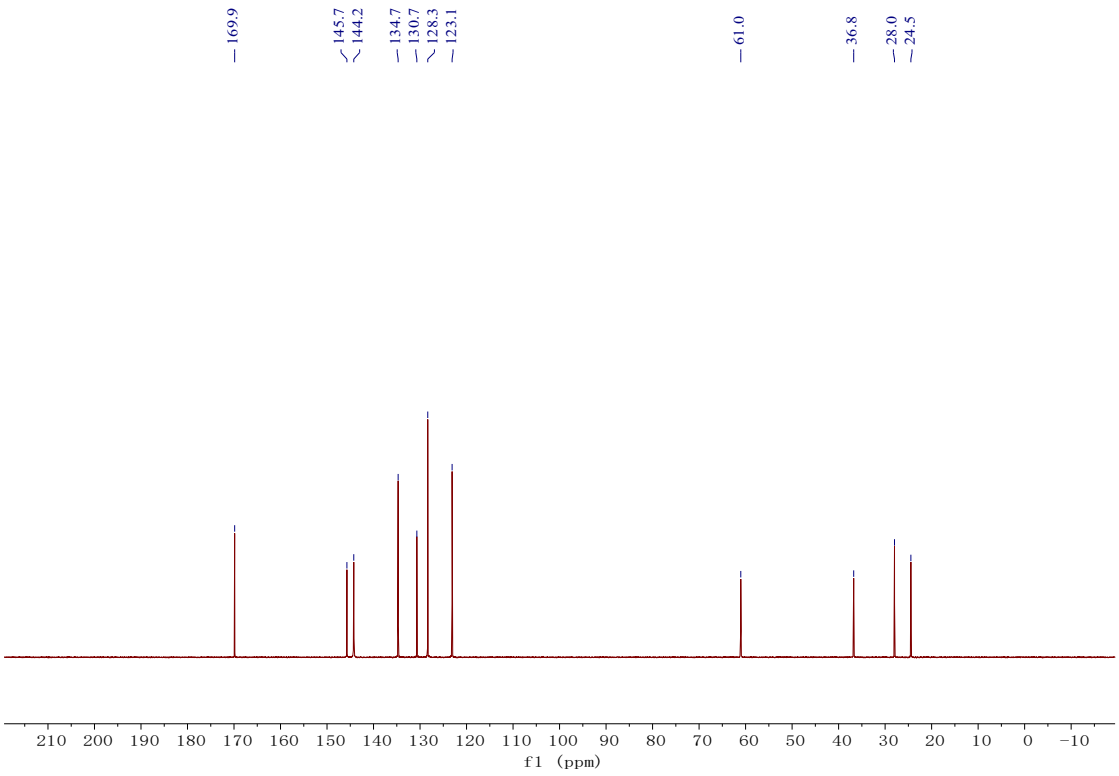


Figure S8. ^{13}C NMR spectrum ($CDCl_3$, room temperature, 100 MHz) of **5**.

Synthesis of compound **6**: Compound **5** (3.46 g, 10 mmol) was dissolved in 30 mL 40% HBr aqueous solution, and heated and refluxed for 3 hours. After the reaction was finished, the reaction was cooled by ice bath, and the precipitated solid was removed by suction filtration. Then, 30 mL isopropanol was added to the filtrate, and the solid was collected by centrifugation and washed with isopropanol (3 × 30 mL) to obtain white solid **6**. Yield: 80%;^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.78 (d, J = 6.3 Hz, 2H, ArH), 8.46 (t, J = 7.9 Hz, 1H, ArH), 7.99 (t, J = 7.0 Hz, 2H, ArH), 4.58 (t, J = 7.5 Hz, 2H, CH_2), 2.98-2.94 (m, 2H, CH_2), 2.06-1.98 (m, 2H, CH_2), 1.70-1.62 (m, 2H, CH_2). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 143.1, 141.5, 125.6, 58.2, 36.0, 24.9, 20.8.

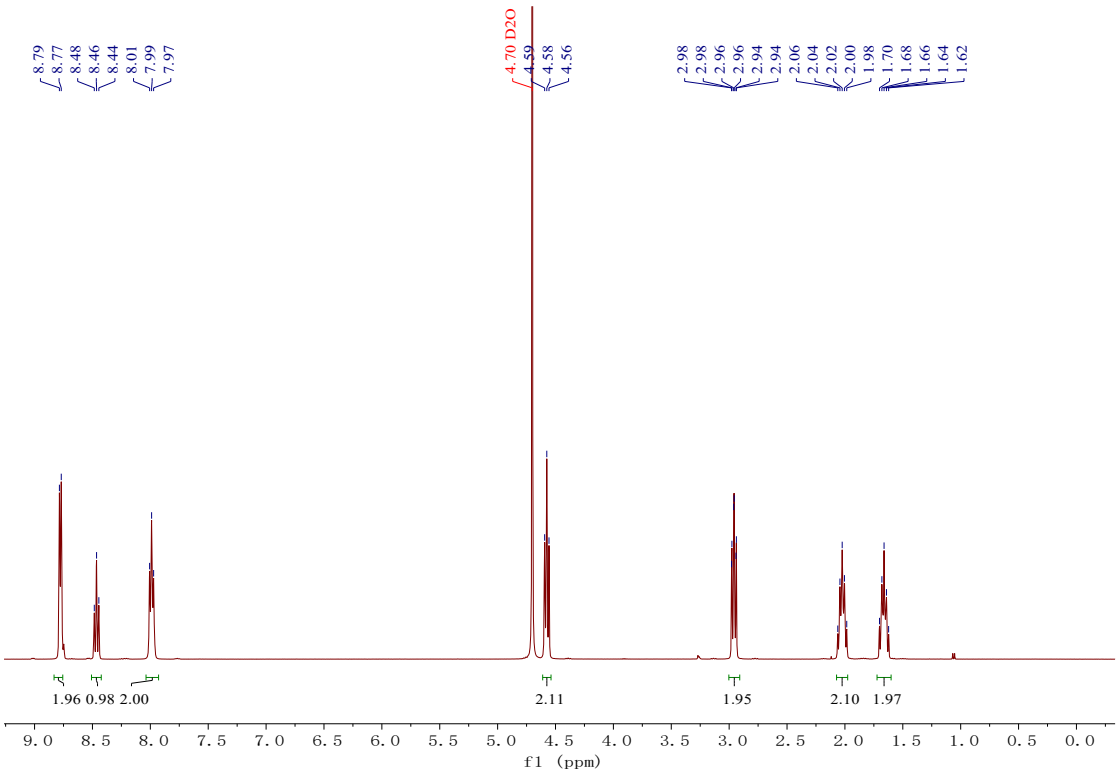


Figure S9. ^{1}H NMR spectrum (400 MHz, D_2O, 293 K) of **6**.

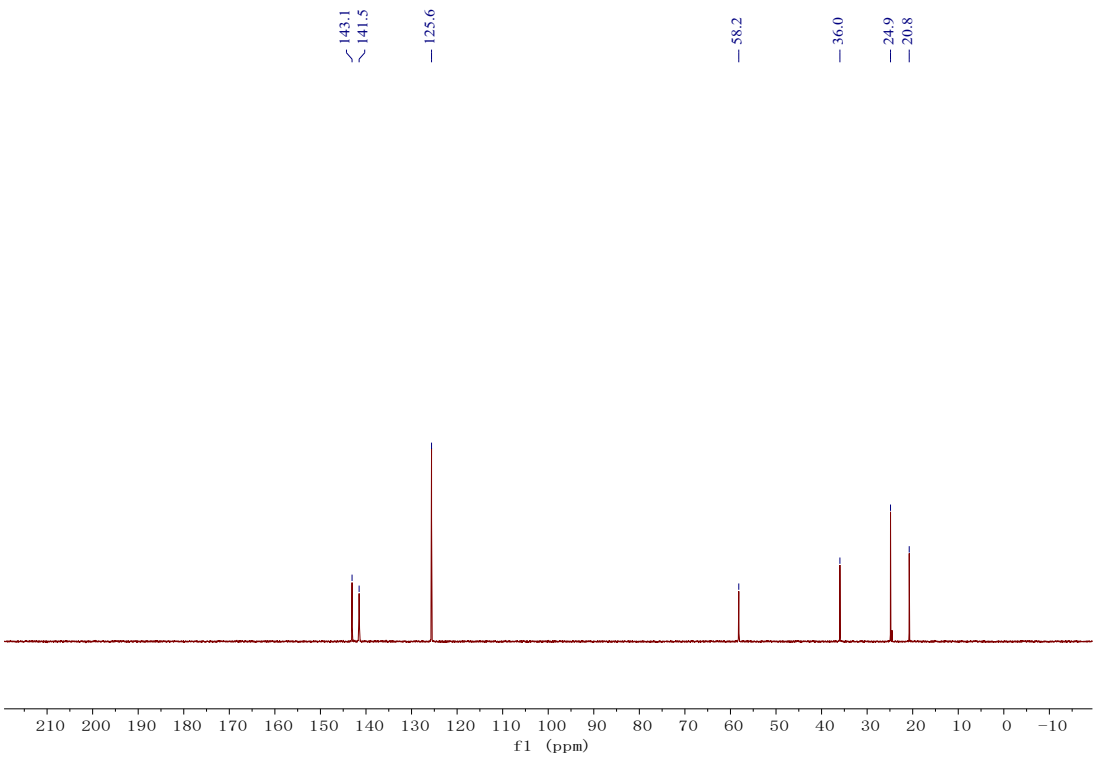


Figure S10. ^{13}C NMR spectrum (100 MHz, D_2O, 293 K) of **6**.

Synthesis of compound **FA-Py**: Folic acid (441 mg, 1 mmol), EDCI (230 mg, 1.2 mmol) and NHS (230 mg, 2 mmol) were added into 20 mL DMSO, reacting at 50℃ for 6 h. Compound **6** (310 mg, 1 mmol) and 200 μL Et_3N were added to react for 6 h at room temperature. After the reaction, the precipitated solid is removed by suction filtration, and 30 mL acetone is added to the filtrate. The solid was collected by centrifugation and washed with acetone (3 × 30 mL) to obtain yellow solid **FA-Py**. Yield: 87%; ^{1}H NMR (400 MHz, DMSO) δ (ppm): 9.13-9.04 (m, 2H, ArH), 8.65-8.62 (m, 1H, ArH), 8.59-8.54 (m, 1H, NH), 8.17-8.08 (m, 2H, ArH), 8.02-7.83 (m, 2H, ArH), 7.66-7.57 (m, 2H, NH), 7.46-7.27 (m, 2H, NH), 7.00-6.90 (m, 2H, ArH), 6.63-6.57 (m, 2H, ArH), 4.64-4.57 (m, 2H, CH_2), 4.45-4.46 (m, 2H, CH_2), 4.27-4.12(m, H, CH), 3.11-3.01 (m, 2H, CH_2), 2.32-2.19 (m, 1H, CH_2), 2.15-2.09 (m, 1H, CH_2), 2.05-1.86 (m, 4H, CH_2), 1.43-1.33 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ (ppm): 175.3, 175.1, 174.9, 172.6, 172.5, 172.3, 166.8, 166.2, 161.9, 156.8, 155.1, 155.0, 154.8, 151.2, 151.1, 148.9, 148.7, 145.9, 145.2, 145.1, 129.5, 129.2, 128.5, 128.5, 128.4, 122.2, 121.7, 111.7, 111.6, 60.9, 60.8, 53.4, 53.0, 46.4, 40.9, 40.6, 40.6, 40.4, 40.2, 40.0, 39.8, 39.5, 39.3, 37.9, 32.6, 31.9, 28.7, 28.5, 28.1, 26.2, 26.1, 26.0. FT-IR (cm^{-1}): 3304, 3060, 1633, 1602, 1484, 1374, 1296, 1174, 1097, 945, 840, 822, 767, 681, 575, 511, 441.

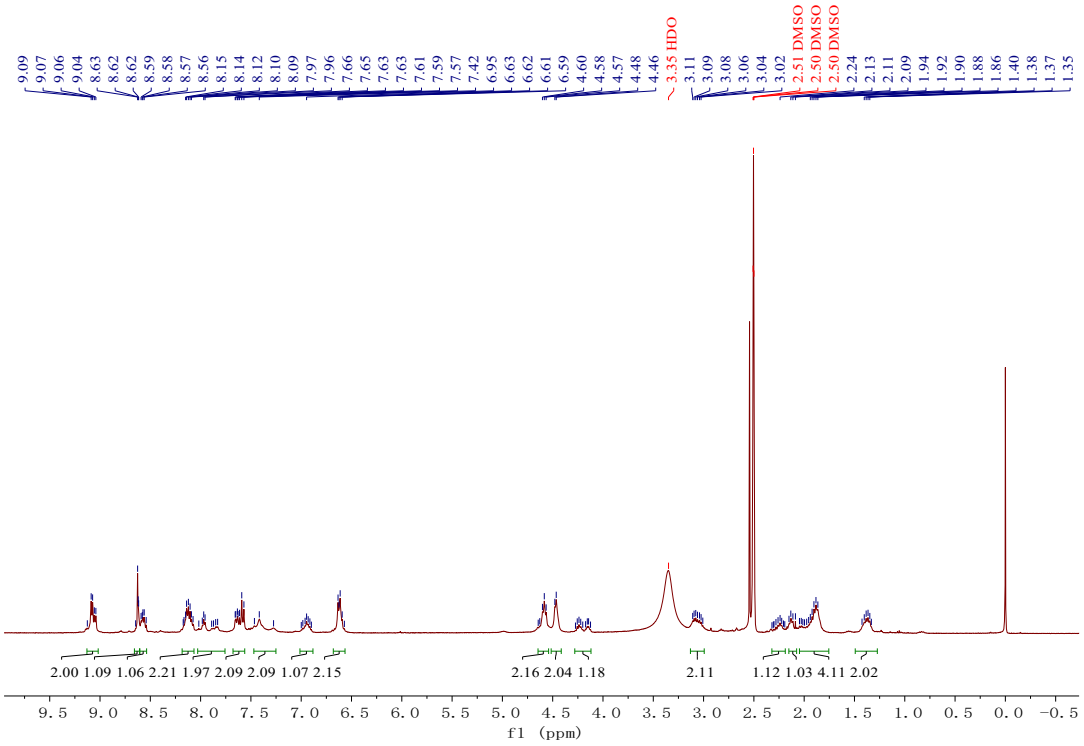


Figure S11. ^{1}H NMR (400 MHz, DMSO) spectrum of **FA-Py**.

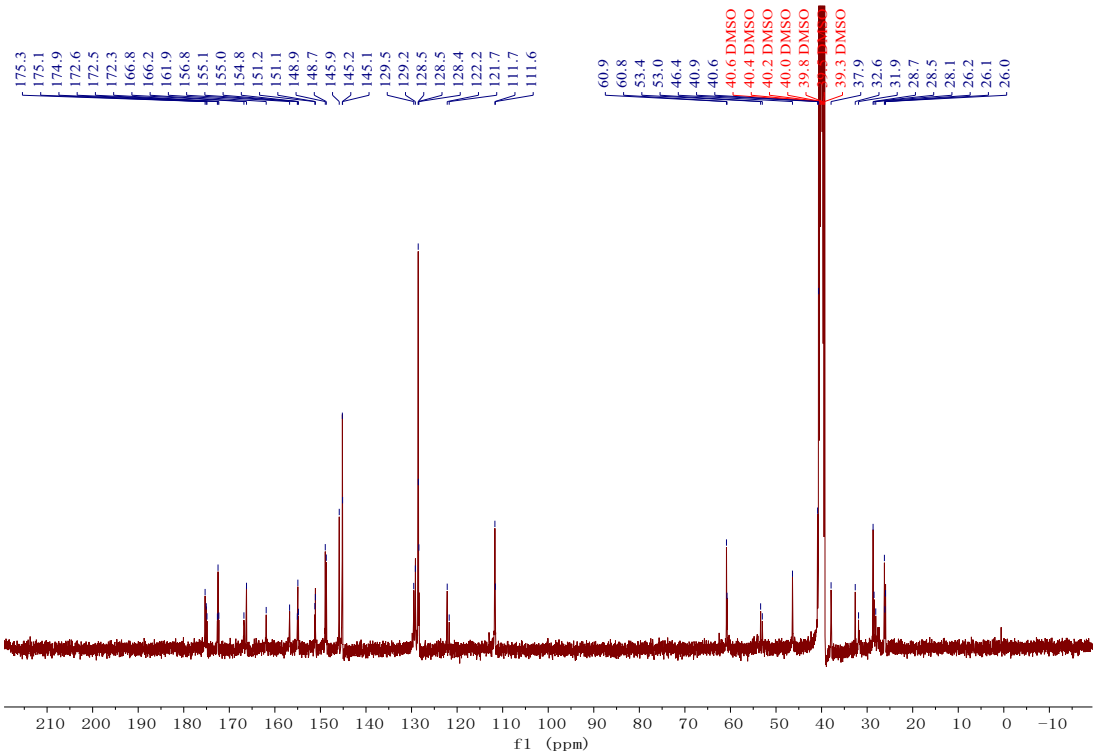


Figure S12. ^{13}C NMR spectra (100 MHz, 298 K, DMSO-d_6) of **FA-Py.**

5. **Host-guest study**

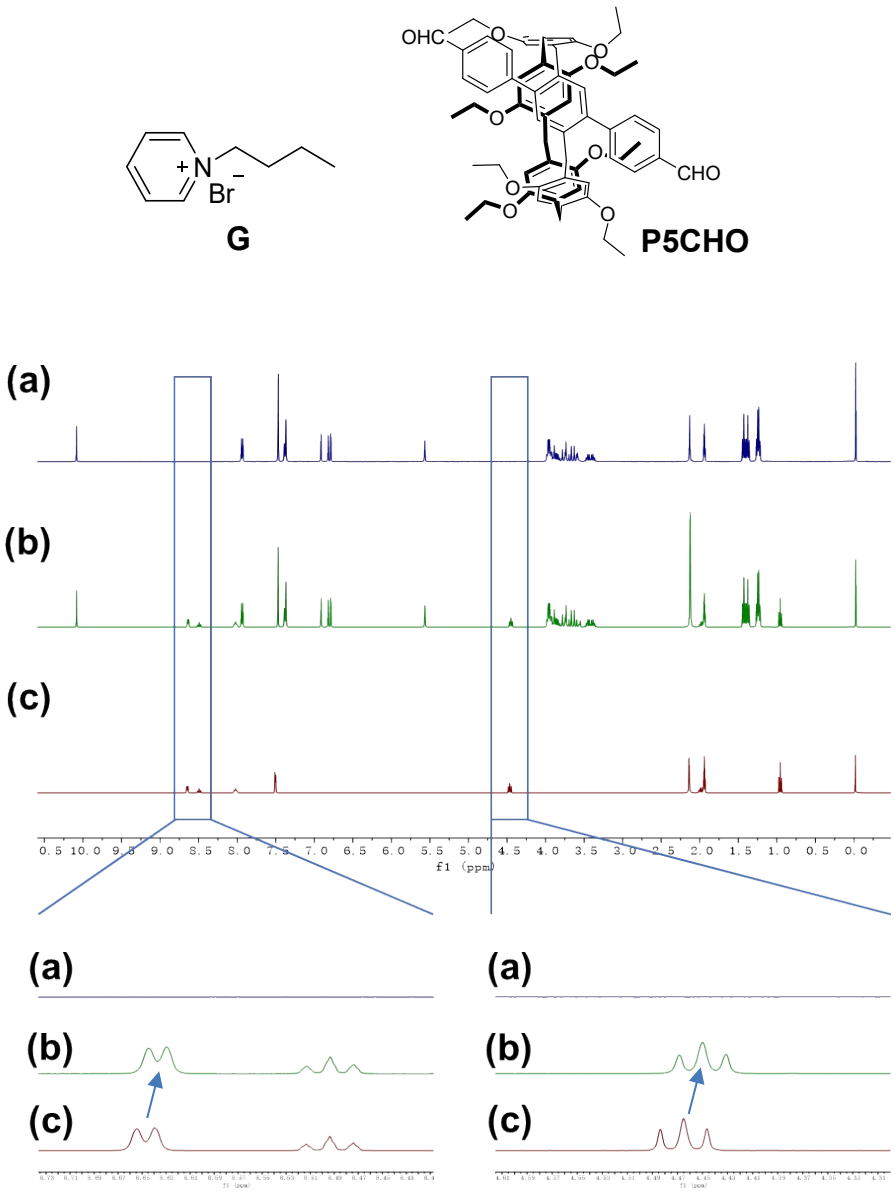
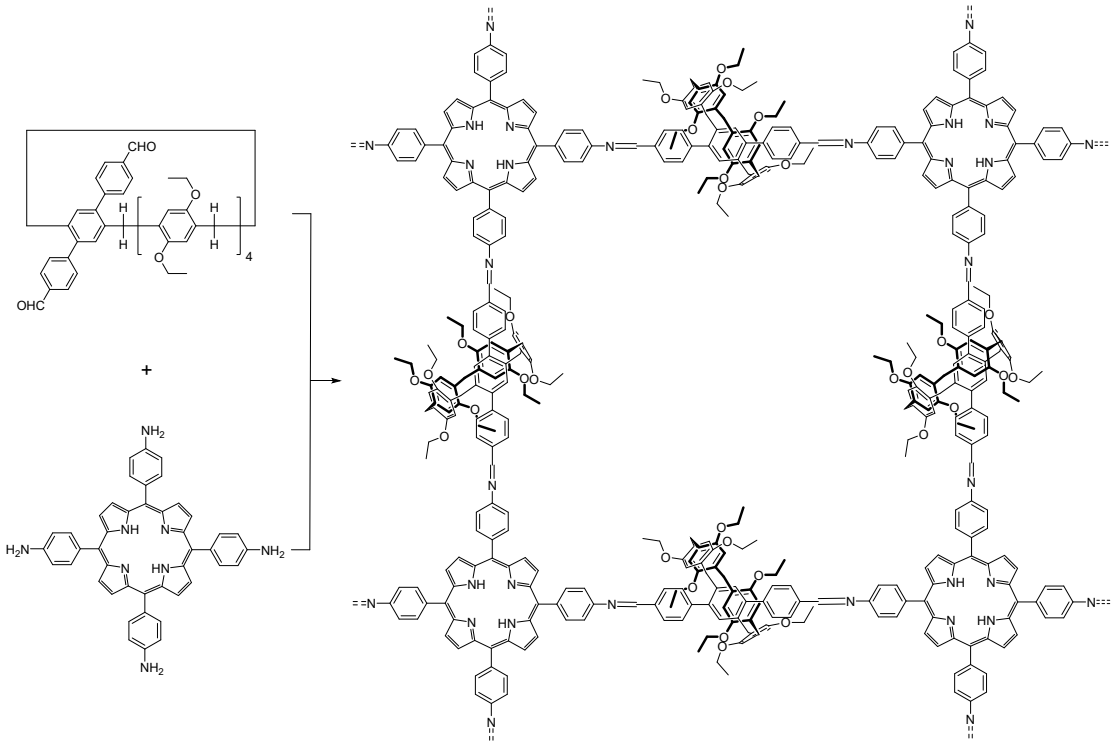


Figure S13. ^{1}H NMR spectra (400 MHz, $CDCl_3$/Acetonitrile-D_3, 298 K) of (a) **P5CHO** (10.0 mM), (b) **P5CHO** + **G** ([**P5CHO**] = 10.0 mM, [**G**]=10.0 mM), and (c) **G** (10.0 mM).

From ^{1}H NMR spectra, we confirmed that strong host-guest interaction exists between P5CHO and the model guest pyridinium (G) with a high association constant (Fig. S13 and S22).

6. **Fabrication and characterization of P5COMs**

Scheme S4. Synthetic route of **P5COMs.**



5,10,15,20- tetra (4-aminophenyl) porphyrin (**TAPP**) (6.75 mg, 0.01 mmol) and **P5CHO** (20.26 mg, 0.02 mmol) were added into a heat-resistant glass tube with a screw cap. Then, 3 mL of n-butanol, 1 mL of mesitylene and 0.1 ml of acetic acid aqueous solution (6.0 M) were added to the mixture. The reaction mixture was ultrasonically treated for five minutes to uniformly disperse all reactants. Seal with nuts and react at 120℃ for five days. After the reaction, the precipitate was collected by centrifugation, washed with anhydrous dichloromethane and acetone for three times, and then dried in vacuum to obtain dark powdery **P5COMs**. FT-IR (transmittance/cm^{-1}): 3368, 2971, 2870, 1698, 1603, 1498, 1474, 1405, 1383, 1286, 1200, 1178, 1104, 1045, 966, 946, 841, 798, 731, 459, 440.

As revealed by FT-IR study (Fig. S14a), the characteristic peak at about 1702 cm^{-1} and 3326 cm^{-1} corresponding to the stretching vibrations of C=O from aldehyde groups and N-H from amino groups decreased obviously, indicating the formation of P5COMs via dynamic covalent bond from amino and aldehyde groups. Then the structure of P5COMs was characterized by solid-state ^{13}C NMR (Fig. S15). Compared with ^{13}C NMR of P5CHO (Fig. S15), the absence of any aldehyde signal at about 190 ppm, indicated virtually quantitative consumption of precursors (Fig. S15). The broad peaks at 15.0 ppm (A) and 30.6 ppm (B) were assigned as the $–CH_3$ and bridged $–CH_2$- moieties, respectively. The peaks at 63.3 ppm (C) were assigned as the $–OCH_3$ unit and the peaks located between 114 to 151 ppm (D, E, F, G) can be attributed to the sp^2 C of phenyl and pyrrole frameworks in the resultant P5COMs.

Meanwhile, TGA analysis confirmed the excellent thermo stability of P5COMs with a decomposition temperature of over 300 °C in air (Fig. S16b).

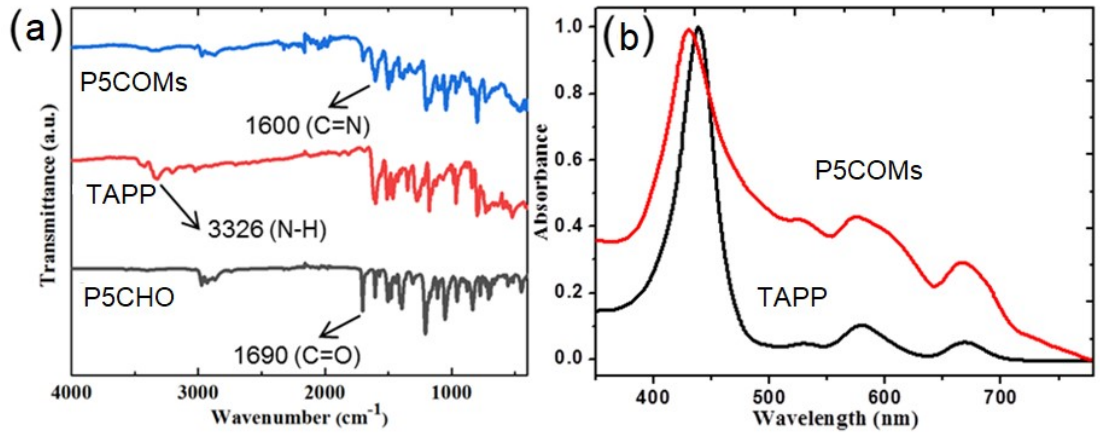


Figure S14. (a) FTIR spectra for **P5COMs** (top) incomparison with model compound **M** and corresponding monomers **P5CHO** and **TAPP**. (b) UV-Vis absorption spectra (DMSO, RT) of **TAPP**, **P5COMs** and **P5CHO**.

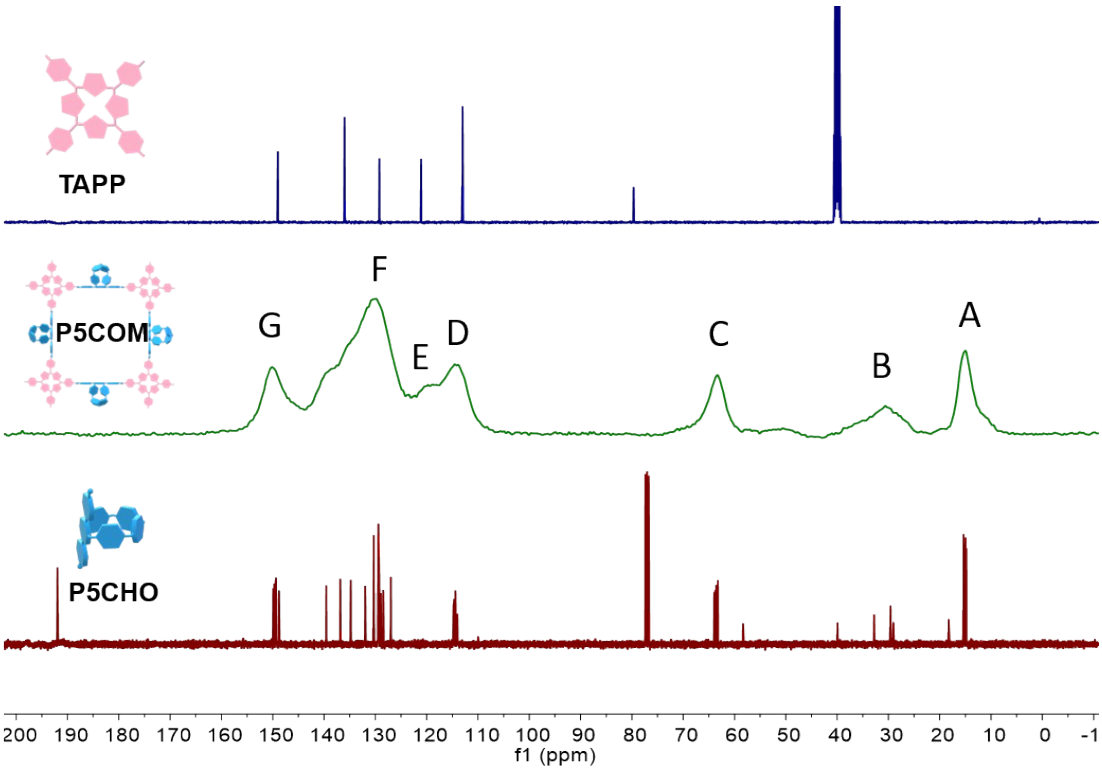


Figure S15. (a) ^{13}C NMR spectrum ($CDCl_3$, 400 MHz, RT) of **TAPP** (b) Solid-state ^{13}C CP MAS NMR spectra of **P5COMs**. (c) ^{13}C NMR spectrum (DMSO-d_6, 400 MHz, RT) of **P5CHO**.

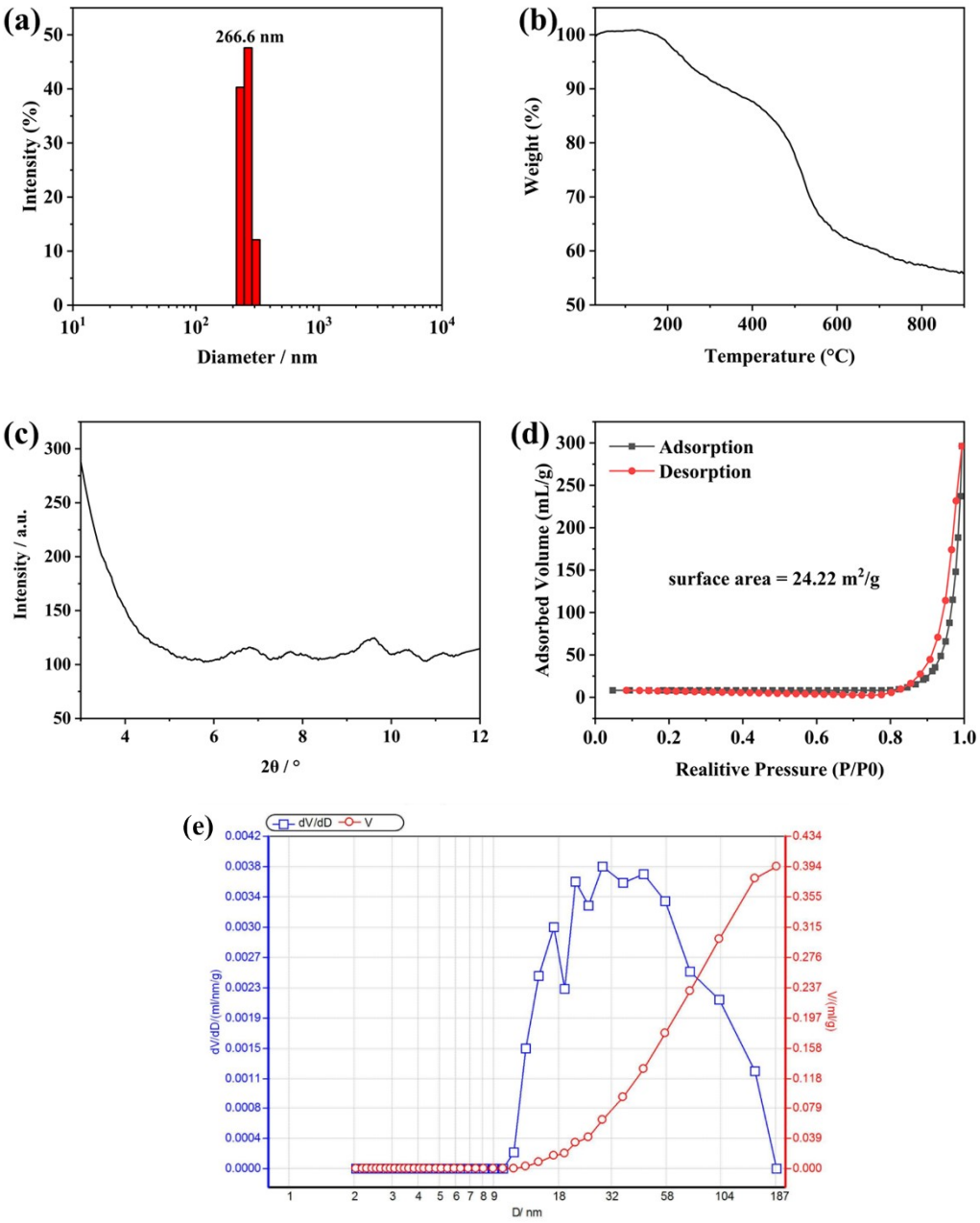


Figure S16. (a) DLS date of **P5COMs** crushed by ultrasonic cell disruptor at 25 ℃. (b) Thermogravimetric (TG) analyses for **P5COMs** under N_2. (c) Powder XRD patterns of experimental data of **P5COMs**. (d) Nitrogen sorption isotherm of **P5COMs**. black symbols: adsorption, red symbols: desorption. (e) Barrett-Joyner-Halenda (BJH) investigation of **P5COMs**.

7. Photothermal performance of P5COMs

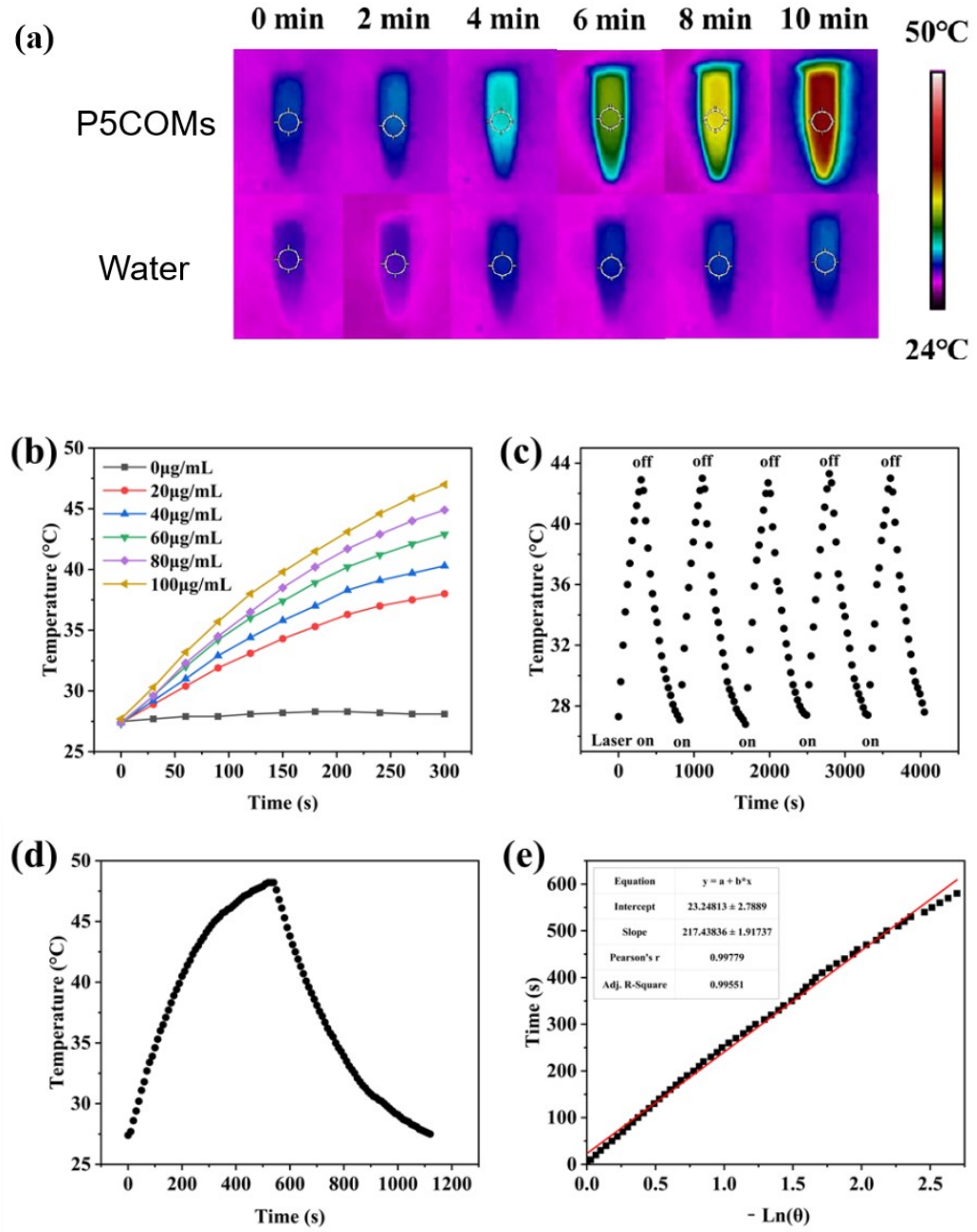


Figure S17. (a) Infrared thermal images of various concentrations and (b) temperature changes of **P5CHO-TAPP-COM** dispersions under 660 nm laser irradiation with the power density of 1 W/cm^2 for 5 min. (c) Photothermal effect curve of **P5CHO-TAPP-COM** dispersion under 660 nm laser irradiation with the power density of 1 W/cm^2 for five cycles. (d)Photothermal effect curve of **P5CHO-TAPP-COM** dispersion under ON/OFF 660 nm laser irradiation with the power density of 1 W/cm^2.

(e) Plot and linear fitting of cooling time versus–ln(θ) received from cooling section of photothermal effect curve in (d).

8. Photodynamic activity of P5COMs

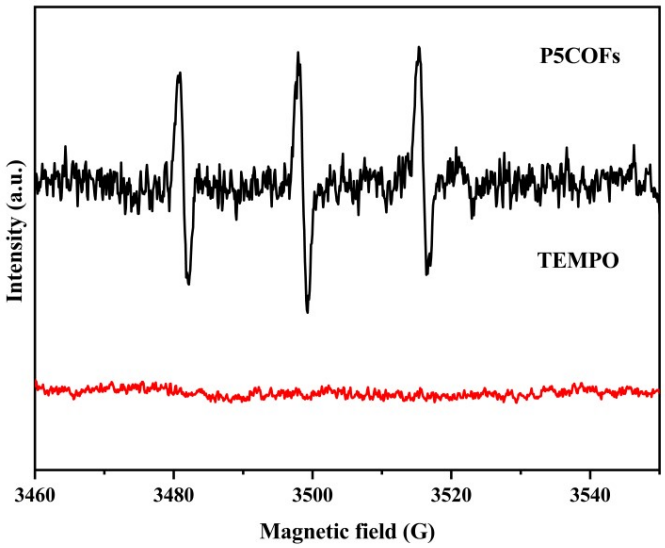


Figure S18. ESR results of singlet oxygen detection for **P5COMs**.

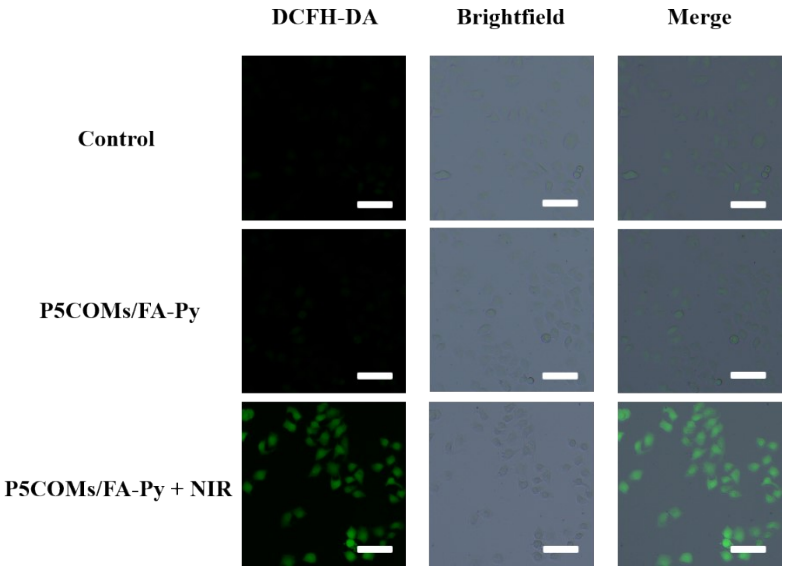


Figure S19. Fluorescence images of ROS generation in HeLa after different treatments: control, only **P5COMs/FA-Py**, and **P5COMs/FA-Py** plus 660 nm laser irradiation for 10 min, as detected with DCFH-DA.

9. Dox loading and releasing activity of P5COMs

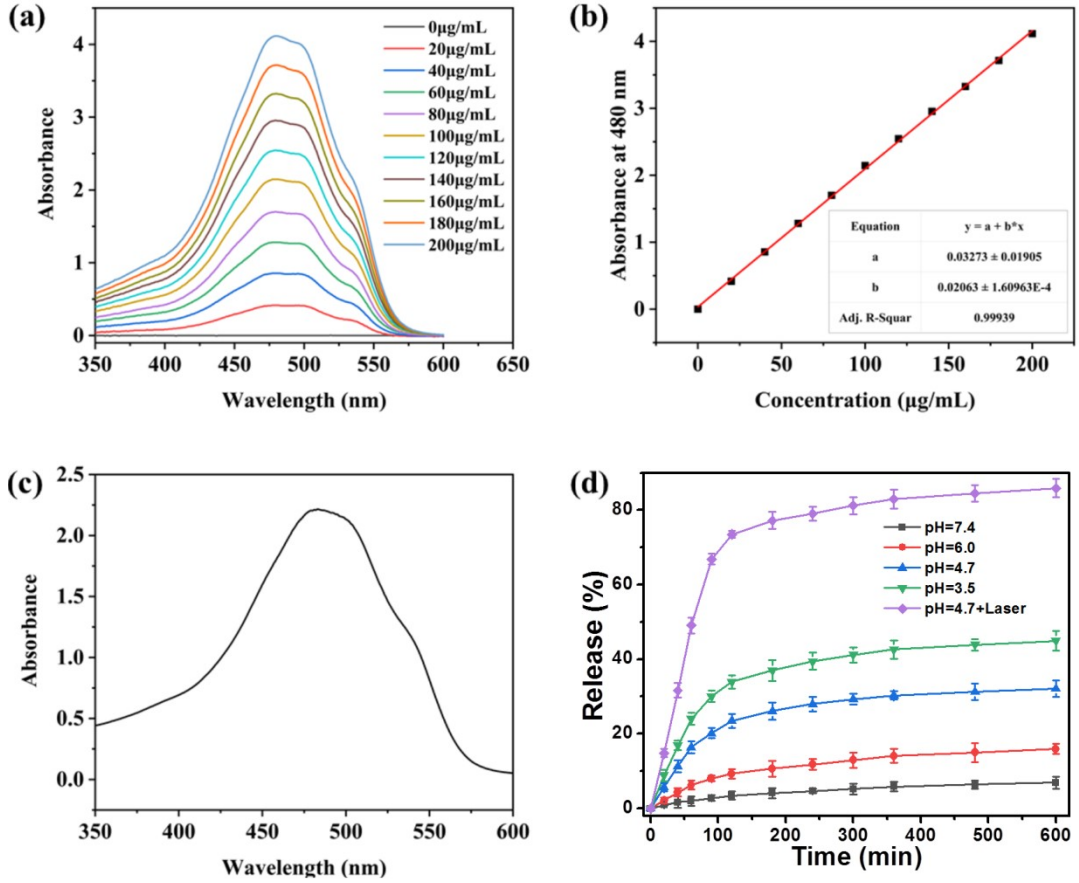


Figure S20. (a) UV-Vis absorption spectra (H_2O, RT) of different concentrations of Dox. (b) Absorbance intensity at 480 nm as a function of the concentration of Dox in solution. (c) The UV-vis absorbance of the supernatant after Dox loading. (d) Drug release curves of **P5COMs/FA-Py/Dox** in PBS solutions of different pH values at 37° C.

10. Cellular uptake and cytotoxicity of P5COMs

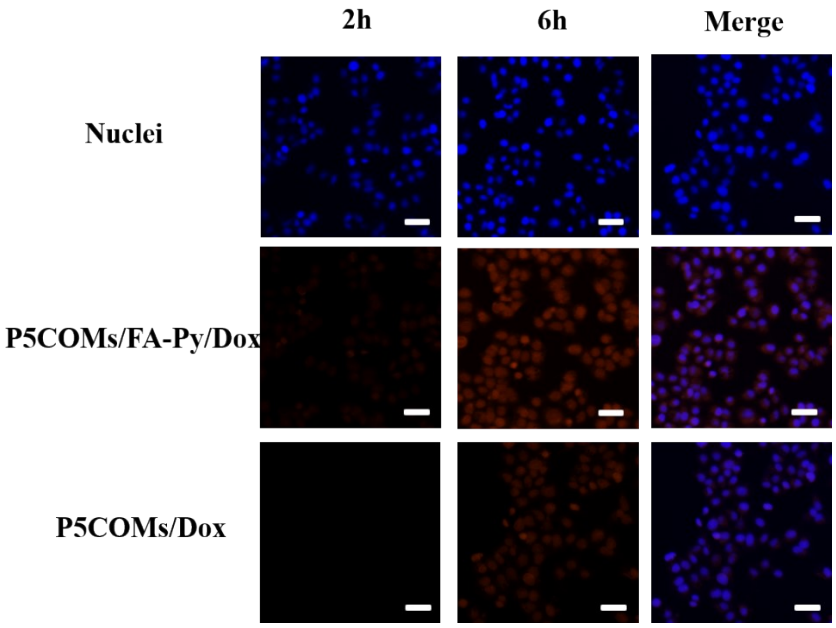


Figure S21. Fluorescence images of HeLa cells incubated with **P5COMs/FA-Py/Dox** or **P5COMs/Dox** for 2 h and 6 h. The cell nuclei were stained as blue by Hoechst 33342, red was the fluorescence of Dox.

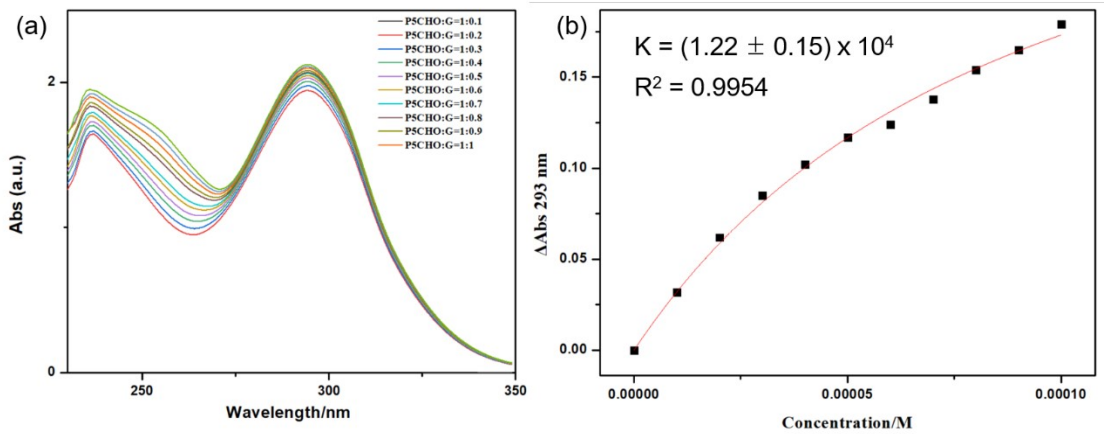


Figure S22. (a) UV-Vis spectra of P5CHO⊃G with different ratio. (b) ΔAbs at 293 nm against the concentration of G.

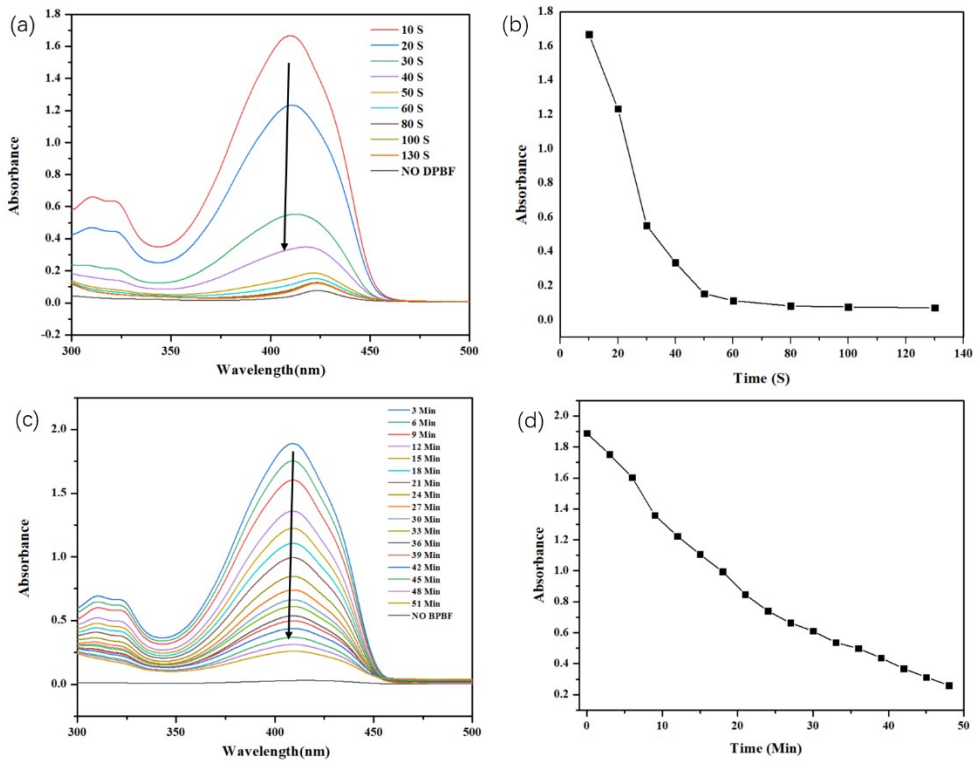


Figure 23. (a) UV-vis spectra of DPBF incubated with P5COMs under light irradiation, (b) The absorbance at 426 nm of (a) with different irradiation time, (c) UV-vis spectra of DPBF incubated with pure TAPP under light irradiation, (d) The absorbance at 426 nm of (c) with different irradiation time. [P5COMs] = [TAPP] = 0.01 mmol, light: 660 nm, 1.0 W cm^{-2}.

References:

S1. A. Bettelheim, B. A. White, S. A. Raybuck, R. W. Murray. *Inorg. Chem.*, **1987**, *26*, 1009-1017.