Electronic Supplementary Information

Porphyrin-functionalized coordination star polymers and their potential applications in photodynamic therapy

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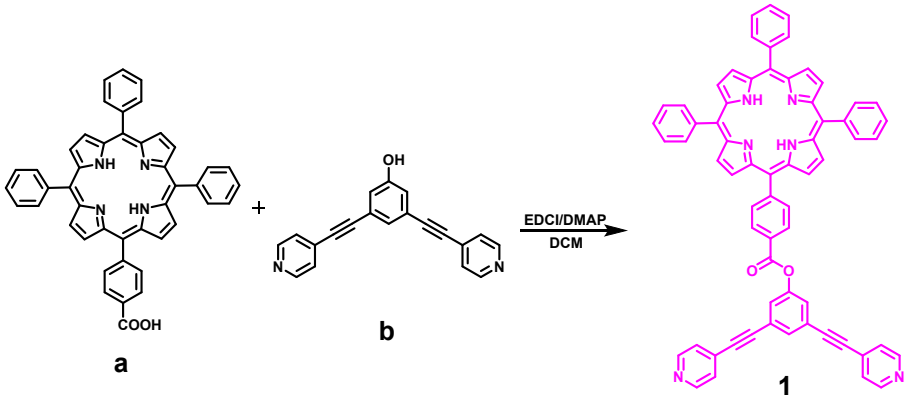
Contents

1. General Information

All reagents were of analytical purity and used without further treatment. Poly(ethylene glycol) methacrylate (OEGMA, M_n = 300 g/mol) and azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich. Deuterated solvents were purchased from Cambridge Isotope Laboratory (Andover, MA). All air-sensitive reactions were carried out under argon atmosphere. The ^{1}H NMR chemical shifts are reported relative to the residual solvent signals, and ^{31}P NMR resonances are referenced to an internal standard sample of 85% H_3PO_4 (δ 0.0). The DLS samples were prepared in water at a concentration of 0.5 mg/mL. DLS measurements were performed under a Malvern Zetasizer Nano-ZS light scattering apparatus (Malvern Instruments, U.K.) with a He-Ne laser (633 nm, 4 mW). TEM images were recorded on a Tecnai G^2 F30 (FEI Ltd.). The sample for TEM measurements was prepared by dropping the solution onto a carbon-coated copper grid. Molecular weights and distributions were determined by Gel permeation chromatography (GPC) analysis with Shimadzu RID-20A refractive index detector and KD-804 column. DMF with 10 mM $LiPF_6$ was used as eluent at the flow rate of 1 mL/min at 40 ^{o}C. Polystyrene was used as the standard to calibrate the GPC system. UV-vis spectra were recorded in a quartz cell (light path 10 mm) on a Cary 50 Bio UV-Visible spectrophotometer. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Cary Eclipse fluorescence spectrophotometer. Infrared (IR) spectra were obtained by using a Bruker tensor 27 infrared spectrometer. Confocal laser scanning microscopy (CLSM) imaging was performed with Nikon AIR confocal microscope. A 420 nm laser was selected as the excitation source. A 500 W xenon lamp (BL Ltd.) with 420 nm pass filter was used to generate singlet oxygen (Power density ~100 mW/cm^2). 9,10-dimethylanthracene (DMA) was used as singlet oxygen generation scavenger. Mass spectra were recorded with a Waters Synapt G2 mass spectrometer.

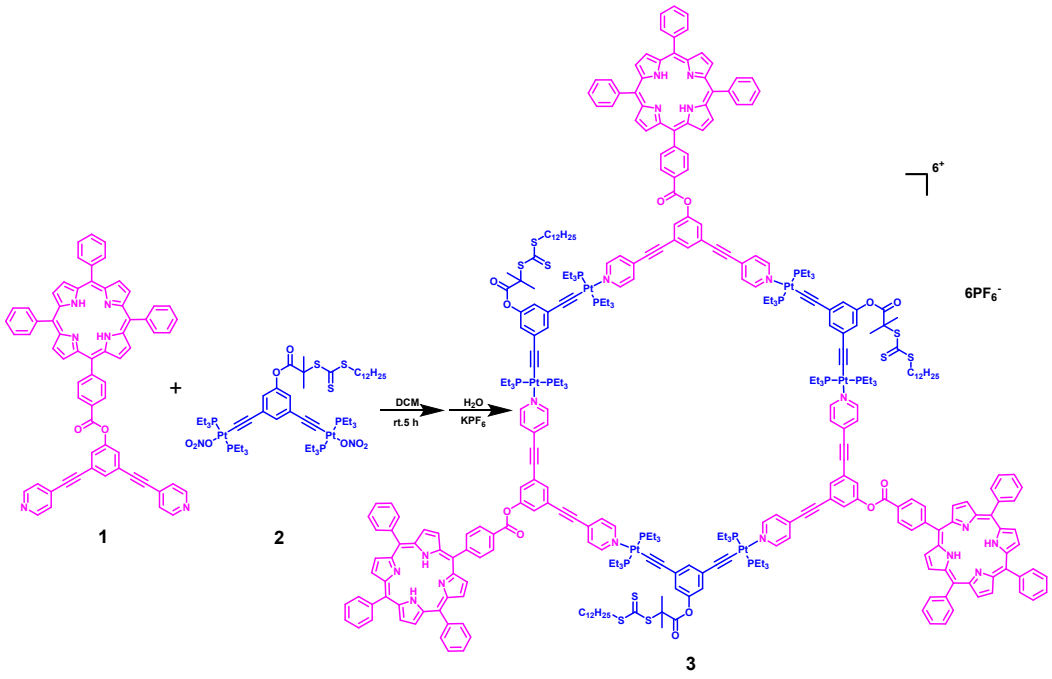
2. Synthesis and Characterization of the Compounds

Scheme S1. Synthesis of 120° Porphyrin Containing dipyridyl ligand **1**.



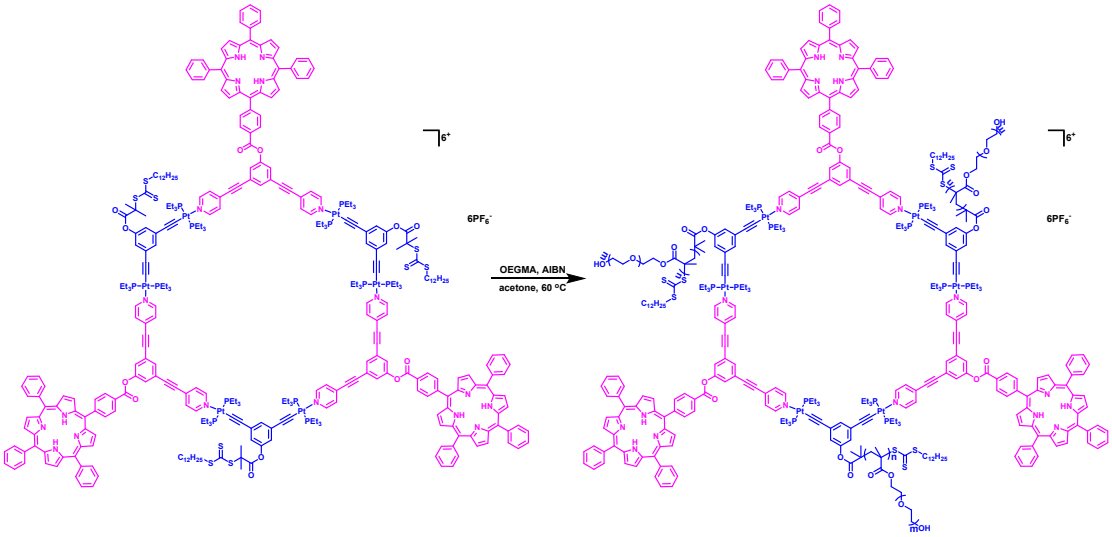
Synthesis of 120° porphyrin containing dipyridyl ligand **1**: compounds **a** and **b** was synthesized according to the literature.[S1] Compounds **a** (150 mg, 0.23 mmol, 1 eq.), **b** (101 mg, 0.34 mmol, 1.5 eq.), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (106 mg, 0.68 mmol, 3.0 eq.), and 4-dimethylaminopyridine (2.8 mg, 0.023 mmol, 0.1 eq.) were added to a 100 mL Schlenk flask and the flask was evacuated under vacuum and bsckfilled with N_2 for three times. 25 mL anhydrous dichloromethane was syringed into the flask, then the mixture was stirred at room temperature overnight. Purification by column chromatography (dichloromethane:acetone = 4:1) afforded ligand **1** (160 mg, 75%) as a purple solid. R_f = 0.3 (dichloromethane:acetone = 4:1). ^{1}H NMR (acetone, 500 MHz): δ 8.84-8.97 (m, 8H, H_{5-8}), 8.67 (dd, 4H, J_1 = 6 Hz, J_2 = 2.5 Hz, H_α-Py), 8.62 (d, 2H, J = 8 Hz, H_3), 8.47 (d, 2H, J = 8 Hz, H_4), 8.26(d, 6H, J = 8 Hz, H_{9-11}), 7.81-7.88 (m, 12H, H_{1-2}+H_{12-16}), 7.44 (dd, 4H, J_1 = 6 Hz, J_2 = 2.5 Hz, H_β-Py), -2.71 (s, 2H, *NH*). ^{13}C NMR (acetone-d_6, 125 MHz): δ 165.56, 152.57, 151.16, 148.87, 142.87, 135.81, 135.36, 133.44, 131.19, 129.60, 129.50, 129.06, 127.93, 127.40, 126.43, 125.09, 121.53, 119.41, 92.06, 89.15. ESI-FTMS: m/z calcd for $[M + H]^+$: 937.32, found: 937.33.

Scheme S2. Self-assembly of supramolecular metallacycle **3** from ligand **1** and the 120° CTA-based diplatinum acceptor **2**.



Self-assembly of supramolecular metallacycle 3 from ligand 1 and the 120° CTA-based diplatinum acceptor 2: The dipyridyl donor ligand **1** (16.45 mg, 17.55 μmol) and the 120° organoplatinum acceptor **2**[S2] (25.87 mg, 17.55 μmol) were weighed into a glass vial. 2.5 mL acetone was added into the vial, and the solution was then stirred at room temperature for 6 h to yield a purple solution. Then the addition of a saturated aqueous solution of KPF_6 into the vial to precipitated the product. The reaction mixture was centrifuged, washed three times with water. Finally, the purple solid product **3** was obtained by removing the solvent under vacuum. Yield: 38.09 mg, 90%. ^{1}H NMR (acetone-d_6, 500 MHz): δ 9.04-9.14 (s, 4H, H_α-Py), δ 8.84-8.97 (s, 8H, $H_{5\text{-}8}$), 8.65-8.71 (m, 2H, J = 6.0 Hz, H_3), 8.50-8.56 (d, 2H, J = 5.0 Hz, H_4), 8.25-8.30 (m, 6H, $H_{9\text{-}11}$), 7.6-8.03 (m, 4H, $H\beta$-Py), 7.81-7.94 (m, 12H, $H_{1\text{-}2}$+$H_{12\text{-}16}$), 7.07 (s, 1H, H_a), 6.94 (s, 2H, H_b), 3.40-3.46 (t, 2H, J = 7.5 Hz), 1.70-2.04 (m, 32H), 1.22-1.37 (m, 51H), 0.84-0.90 (t, 6H, J = 7.0 Hz); ^{31}P NMR (acetone-d_6, 202 MHz): δ 17.40 (s, $J_{Pt\text{-}P}$ = 2308.8 Hz); IR (neat): 3317, 2926, 1741, 1608, 1456, 1415, 1259, 1199, 1130, 1066, 837, 798, 731 cm^{-1}; ESI-TOF-MS of **3**: calcd for $[M\text{-}6PF_6^-]^{6+}$: 1143.40, found: 1143.32; calcd for $[M\text{-}5PF_6^-]^{5+}$: 1401.06, found: 1401.02, calcd for $[M\text{-}4PF_6^-]^{4+}$: 1787.57, found: 1787.57.

Scheme S3. Synthesis route of supramolecular star polymer **4**.



Synthesis of star supramolecular polymer 4: Supramolecular [3+3] metallacycle **3** (20.8 mg, 2.69 μmol), AIBN (0.22 mg, 1.35 μmol), poly(ethylene glycol) methacrylate (OEGMA)(290 mg, 0.805 mmol), and 1.5 mL of acetone were added in a 10.0 mL flask . The flask was then degassed via three freeze-pump-thaw cycles. The mixed solution was then transferred to the preheated oil bath at 60 °C to start the polymerization. After 3 h, the polymerization was quenched by liquid N_2, and the resulting mixture was precipitated into excess diether ether. The precipitate was dissolved in acetone and then precipitated again in the presence of diether ether, then cycled this process for three times. The final product was dried in vacuum, as a purple solid ($M_{n,\ NMR}$ = 53 kDa, $M_{n,\ GPC}$ = 44 kDa, PDI = 1.29). Yield: 200 mg, 64.35%. ^{1}H NMR (acetone-d_6, 500 MHz): δ 4.05-4.30 (s, H_c), 3.51-3.87 (m, H_{e+f+g}), 0.91-1.07(s, H_d), ^{31}P NMR (acetone-d_6, 202 MHz): δ 17.38 (s, $J_{Pt\text{-}P}$ = 2308.6 Hz); IR (neat): 3441, 2878, 1703, 1622, 1423, 1357, 1240, 1034, 833, 733, 679, 557 cm^{-1}.

3. GPC Traces of Metallacycle 3 and Star Supramolecular Polymer 4

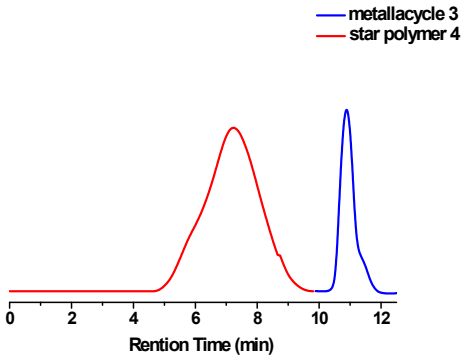


Figure S1. GPC curves of hexagonal metallacycle **3** (blue) and star supramolecular polymer **4** (red).

4. Self-assembly of Star Supramolecular Polymer 4

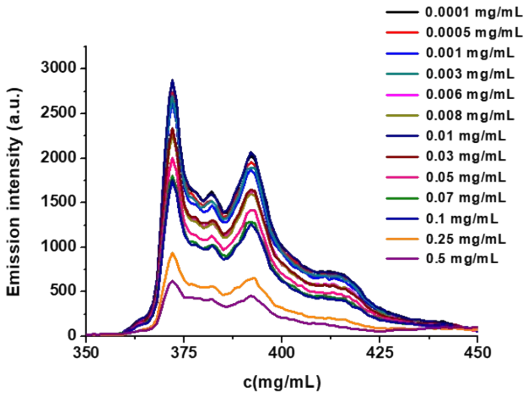


Figure S2. Determination of CAC for the star polymer **4** by using the fluorescent method with pyrene as a probe.

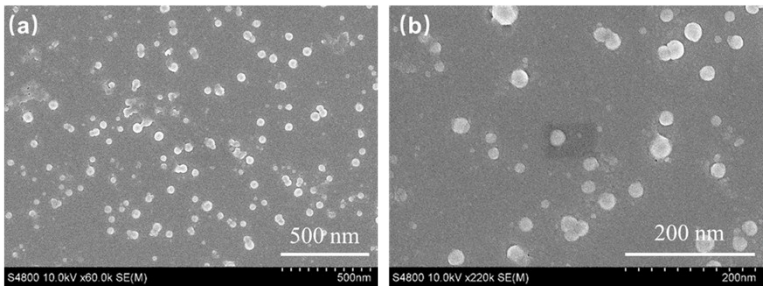


Figure S3. SEM images of porphyrin-based star polymer **4** (0.5 mg/mL, aqueous solution at 25 ^{o}C.)

5. The Generation of Singlet Oxygen

The singlet oxygen yield of the compound is calculated by measuring the change in absorbance in the fluorescence emission spectrum at 420 nm, determination of tetraphenylporphyrin (TPP) as a reference[S3] calculated according to equation (1):

$$\Phi_{\Delta}{}^{S} = \Phi_{\Delta}{}^{R}\frac{K^{S}F^{R}}{K^{R}F^{S}} \quad \textbf{(1)}$$

Where $\Phi_{\Delta}{}^{R}$ is the singlet oxygen generation yield of the standard TPP, $\Phi_{\Delta}{}^{S}$ is the singlet oxygen generation yield of polymer **4** ; K^{S} and K^{R} are the DMA fluorescence intensity change rates (K = $\ln[DMA]_0/[DMA]_t$); F^{R} and F^{S} are the DMA photobleaching in the presence of the analyte and the standard, respectively. The concentration of the DMA was chosen as 3×10^{-5} M. The experiments were carried out in DMF.

The summary of all fitting results is included in Table S1.

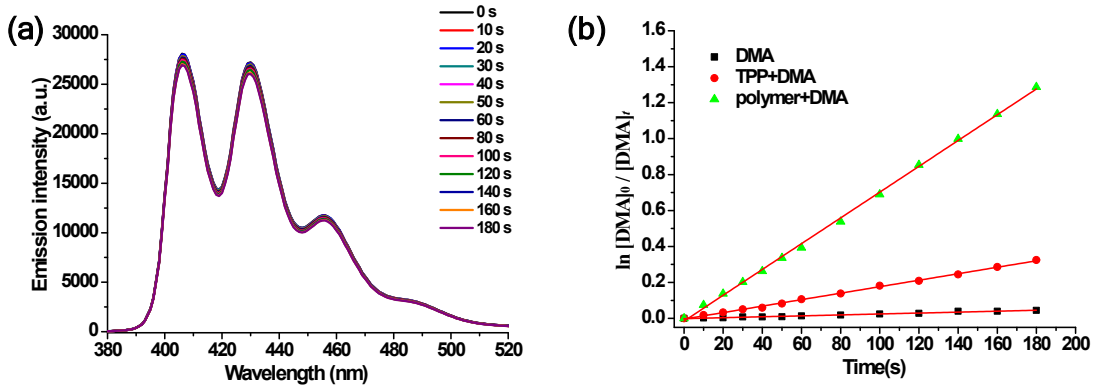


Figure S4. (a) The fluorescence emission spectra of DMA. (b) The decomposition of DMA by TPP and star polymer **4** in DMF. $[DMA]_0$ and $[DMA]_t$ are the fluorescence intensity of DMA at 430 nm before and after irradiation, respectively.

Table S1. Fitting parameters of singlet oxygen generation rate of DMA, TPP and star polymer **4.**

	r^2	K	normalized yield
DMA	0.98574	0.00026	none
TPP	0.99830	0.00180	1
Star polymer **4**	0.99893	0.00717	3.9833

6. The Cell Uptake and Cytotoxicity of Coordination Star Polymer 4

Cell culture

The human cervical cancer cells line Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C under 5% CO_2.

Cell uptake

The cellular uptake was characterized by flow cytometry, and the localization was performed by confocal laser scanning microscope (CLSM, Nikon AIR). In a simple process, Hela cells were fostered with 2 mL of culture medium at a density of 1×10^5 cells/well in a 6-well plate for 24 h at 37 °C. Then the culture medium was replaced with a fresh culture medium containing the star polymer **4** (final porphyrin concentration: 20 μg/mL) and incubated for another 24 h at 37 °C. The cells were trypsinized and washed thrice with PBS, followed by suspension using centrifuge. Furthermore, the fluorescence intensity was measured on a BD FACS Calibur flow cytometer and the flow cytometry was analyzed by Qdot 605-A (Quantum dots nanocrystals, 605 nm) to study the cellular uptake. In addition, 4% paraformaldehyde was added in the wells. After 20 min, the cells were washed with PBS thrice, and then DAPI was used to stain the cell nucleus for 3 min. After the cells were washed with PBS thrice, the images were obtained using CLSM.

In *vitro* dark cytotoxicity and phototoxicity

The cytotoxicity of Coordination Star Polymer **4** in Hela cells was determined *via* MTT assay. Hela cells were seeded in 96-well plates at 5000 cells per well in 200 μL DMEM supplemented with 10% FBS for 24 h in normoxia conditions. Subsequently, the cells were incubated with DMEM containing free Star Polymer 4 micelles with different concentrations for 24 h. Then, the culture medium was replaced with RPMI

1640 medium containing 5 mg mL^{-1} MTT to incubate cells for 4 h. The dark cytotoxicity was assessed by a spectrophotometric microplate reader (Thermo Multiskan MK3 spectrometer).

Cell viability (%) = $(OD_{test})/(OD_{control}) \times 100$, where OD_{test} is the absorbance at 492 nm in the presence of sample solutions and $OD_{control}$ is the absorbance without treatment.

The phototoxicity of star polymer **4** was evaluated through similar procedure. After 24 h incubation with polymer micelles, the cells were exposed to laser irradiation with a power density of 0.6 W/cm^2 for 20 min. After irradiation, the cells were further incubated at 37 °C for 20 h, and the cell killing efficiencies were analyzed by a standard cell viability MTT assay.

7. Characterization data and spectra

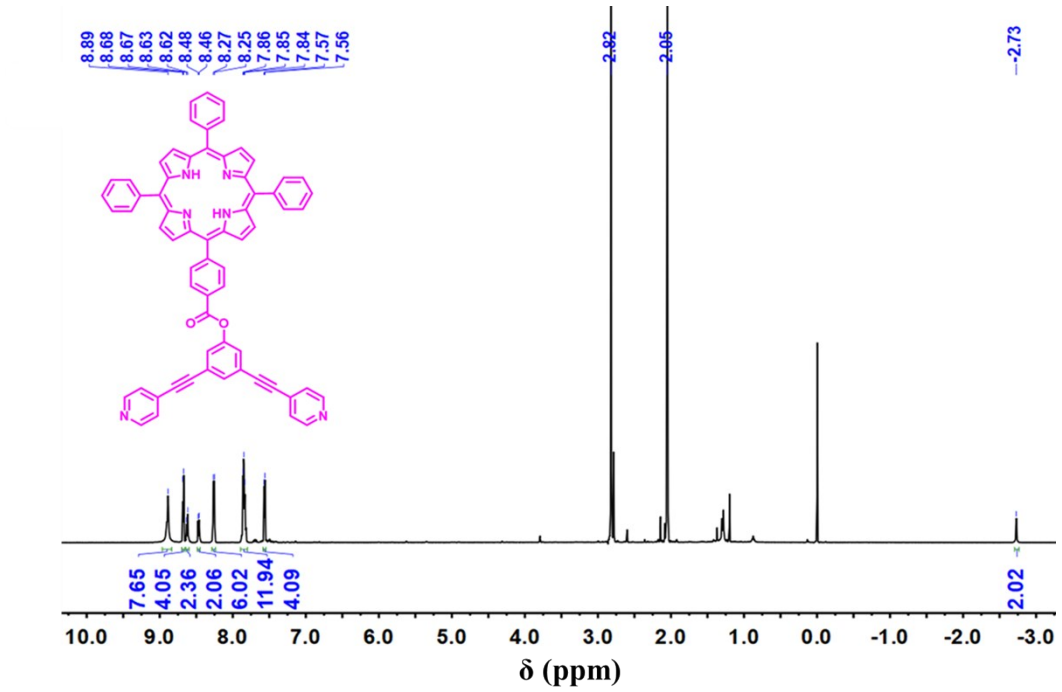


Figure S5. ^{1}H NMR (500 MHz, 298 K) spectra of 120° porphyrin containing dipyridyl ligand **1** in acetone-d_6.

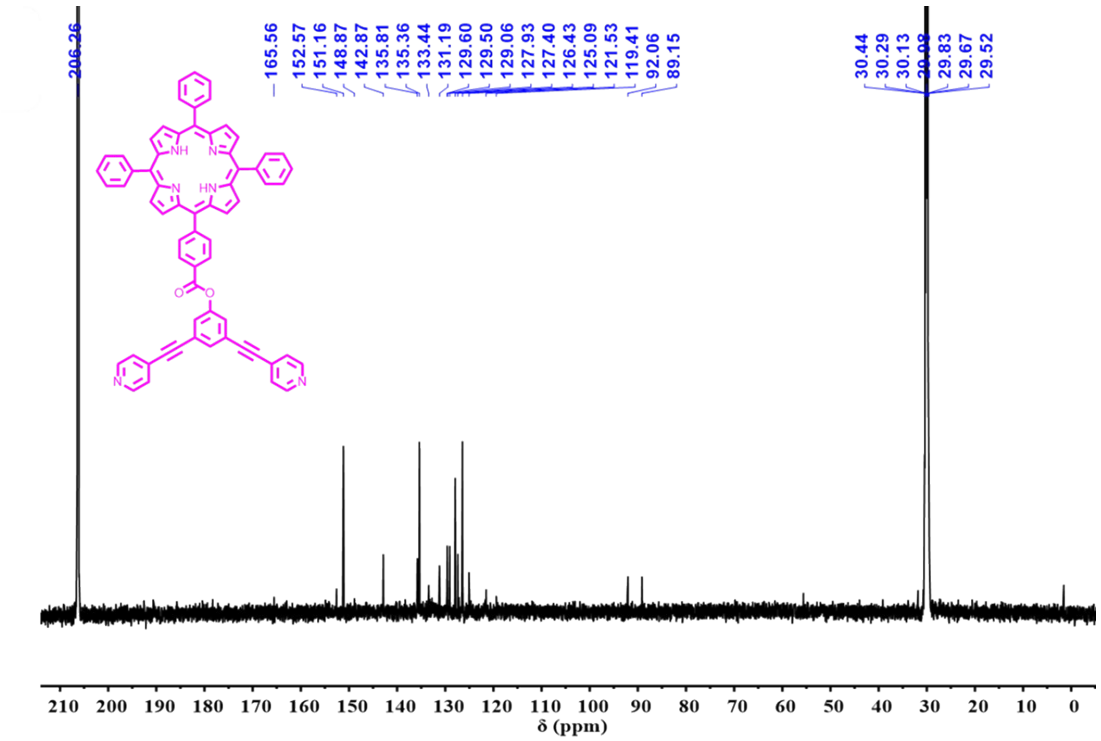


Figure S6. ^{13}C NMR (125 MHz, 298 K) spectra of 120° porphyrin containing dipyridyl ligand **1** in acetone-d_6.

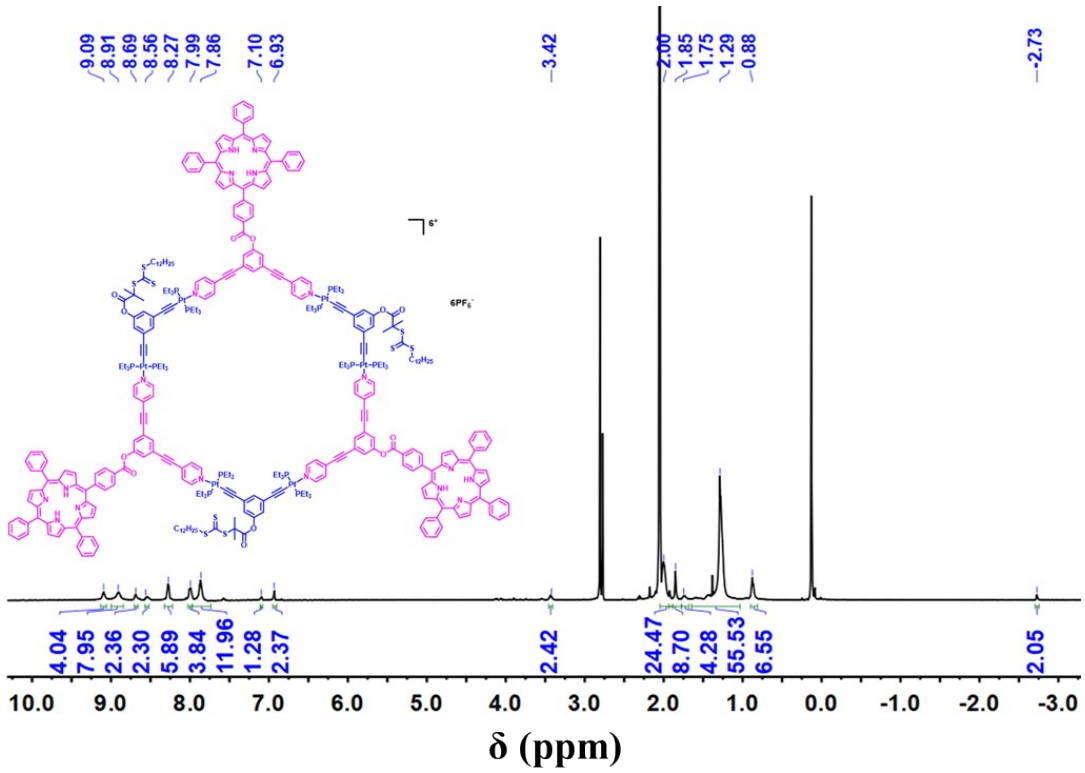


Figure S7. ^{1}H NMR (500 MHz, 298 K) spectra of metallacycle **3** in acetone-d_6.

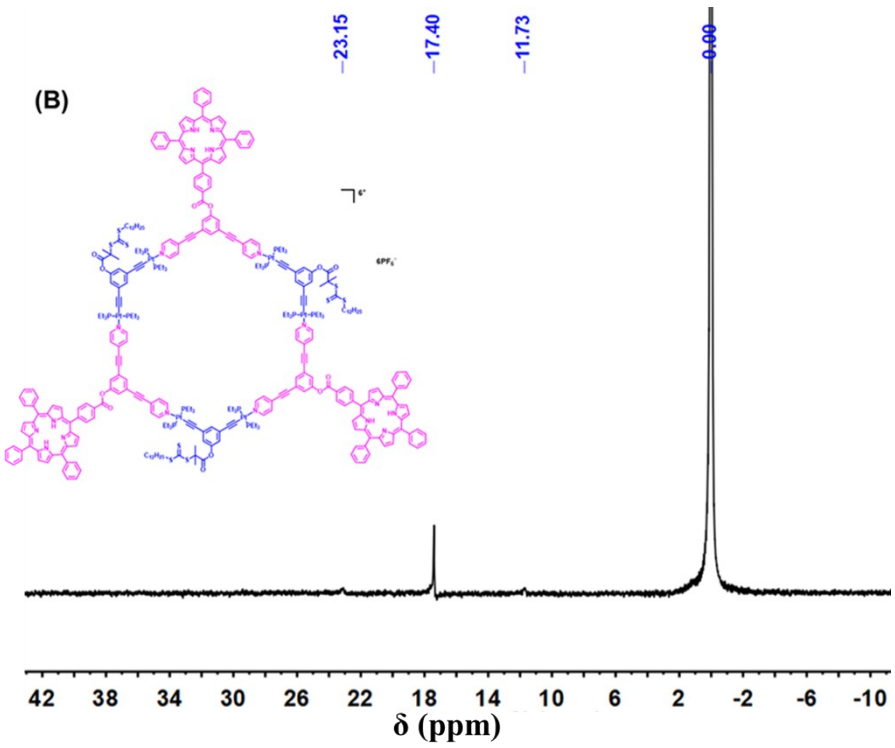


Figure S8. ^{31}P NMR (202 MHz, 298 K) spectra of metallacycle **3** in acetone-d_6.

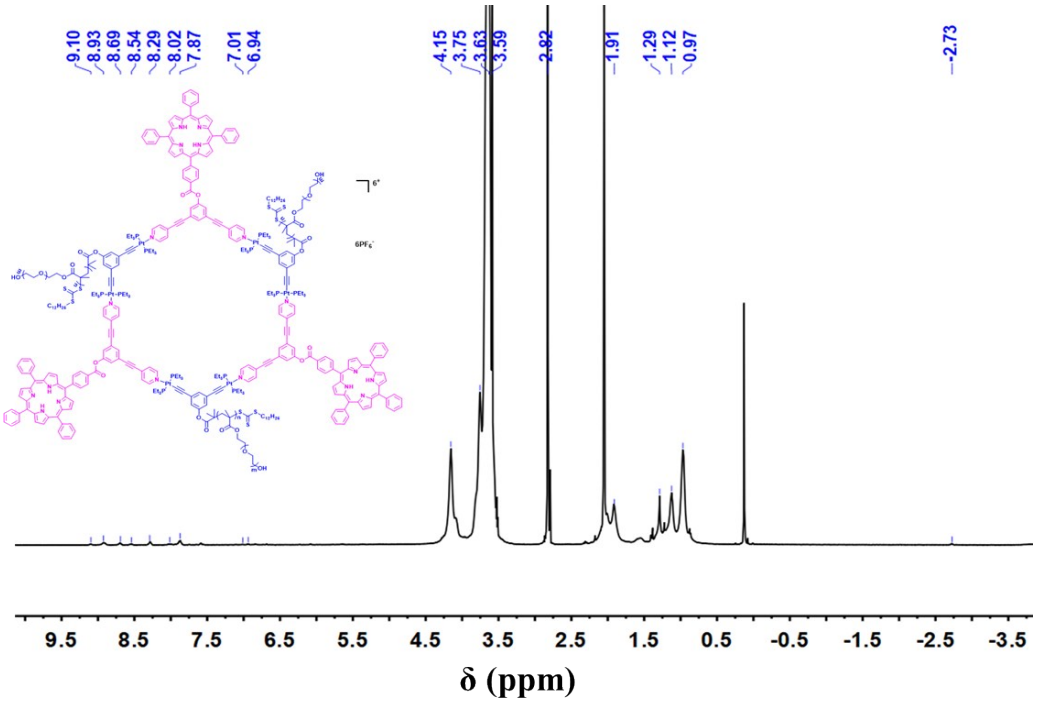


Figure S9. ^{1}H NMR (500 MHz, 298 K) spectra of star polymer **4** in acetone-d_6.

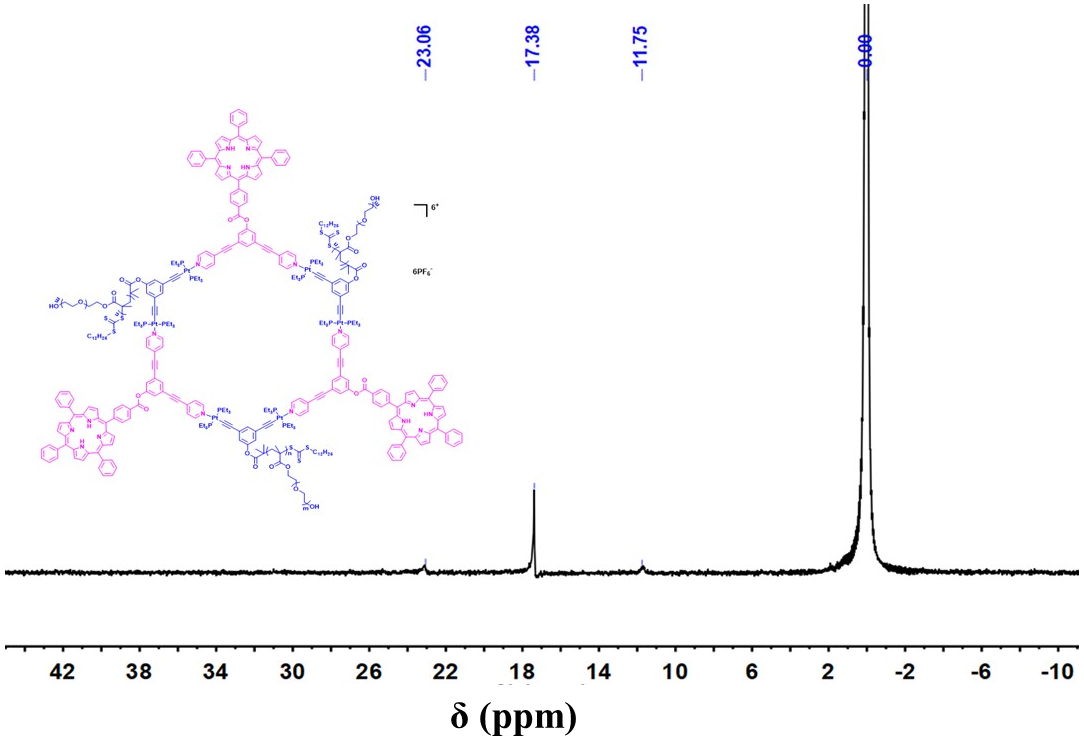


Figure S10. ^{31}P NMR (202 MHz, 298 K) spectra of star polymer **4** in acetone-d_6.

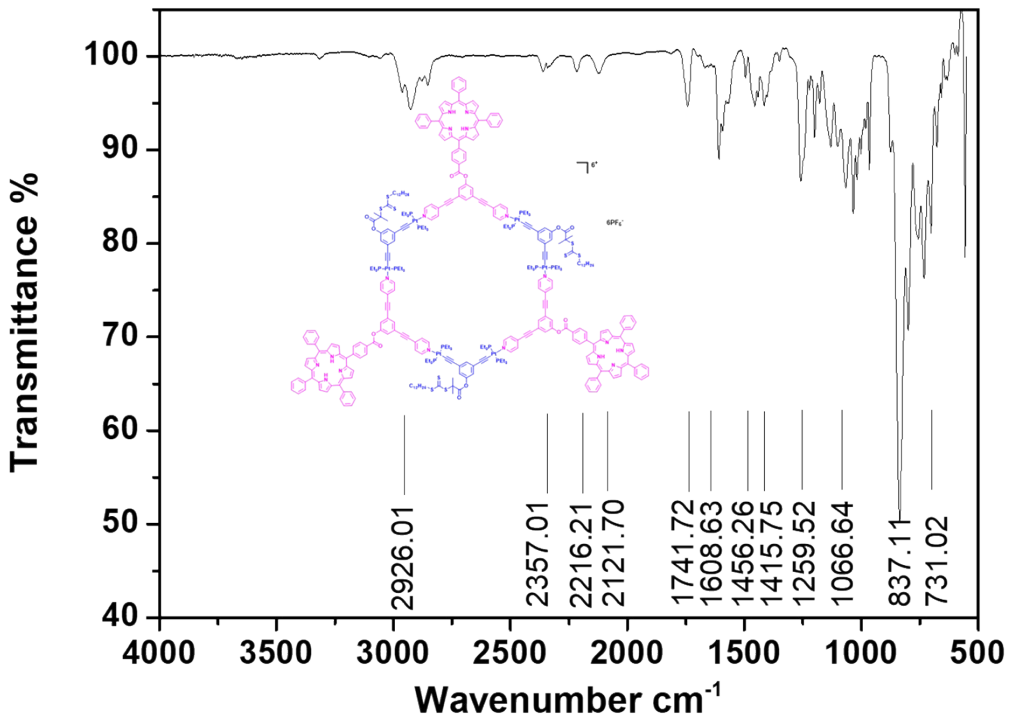


Figure S11. FTIR spectrum of supramolecular metallacycle **3**.

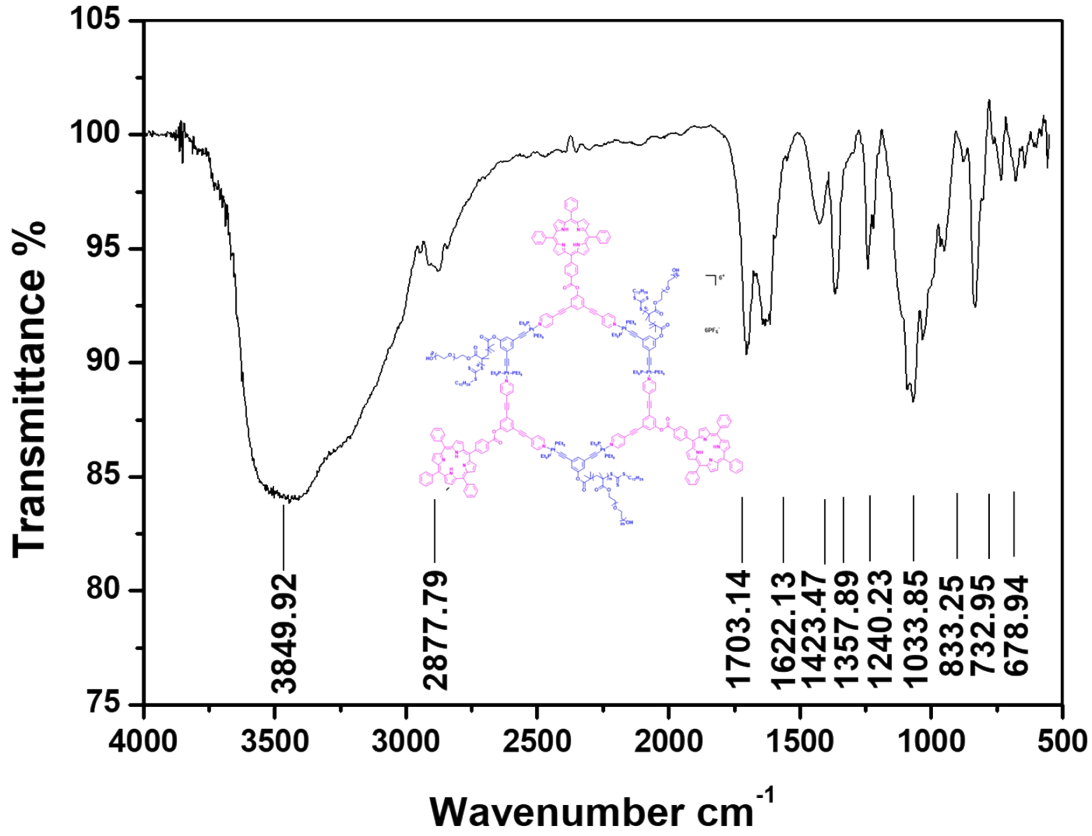


Figure S12. FTIR spectrum of star polymer **4**.

8 References

[S1] L.-J. Chen, S. Chen, Y. Qin, L. Xu, G.-Q. Yin, J.-L. Zhu, F.-F. Zhu, W. Zheng, X. Li and H.-B. Yang, *J. Am. Chem. Soc.*, 2018, **140**, 5049-5052.

[S2] W. Zheng, G. Yang, S. T. Jiang, N. N. Shao, G. Q. Yin, L. Xu, X. P. Li, G. S. Chen and H.-B. Yang, *Mater. Chem. Front.*, 2017, **1**, 1823-1828.

[S3] P. Kluson, M. Drobek, A. Kalaji, S. Zarubova, J. Krysa and J. Rakusan, *J. Photochem. Photobiol. A*, 2008, **199**, 267-273.