2D amphiphilic organoplatinum(II) metallacycles: syntheses, self-assembly in water and their potential application in photodynamic therapy

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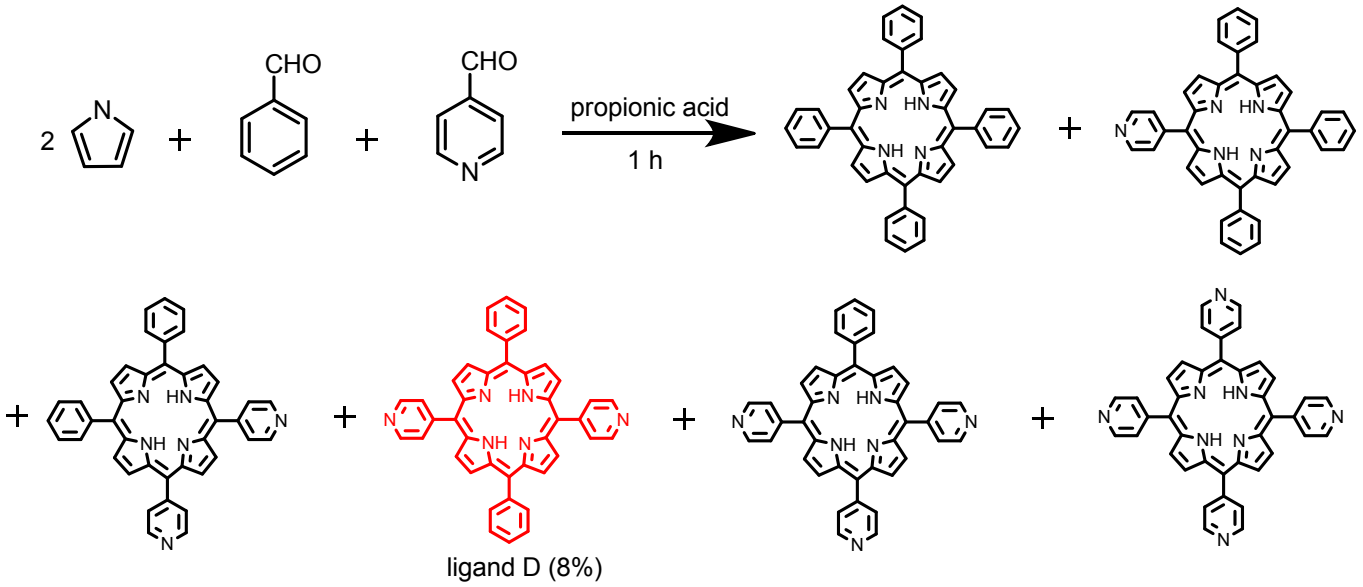
1. Materials/General Methods/Instrumentation

All reagents were commercially available and used as supplied without further purification. Deuterated solvents were purchased from Cambridge Isotope Laboratory (Andover, MA). Ligand D (S1) was prepared according to the published procedures. NMR spectra were recorded on a Varian Unity 400 MHz spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported relative to residual solvent signals, and $^{31}P\{^{1}H\}$ NMR chemical shifts are referenced to an external unlocked sample of 85% H_3PO_4 (δ 0.0). Dynamic light scattering (DLS) was carried out on a Malvern Nanosizer S instrument at room temperature. TEM images were obtained using a Philips TECNAI-12 instrument with an accelerating voltage of 120 kV. SEM images were applied to investigate the morphologies, which was carried out with a Hitachi S-4800 field emission scanning electron microscope. Mass spectra were recorded on a Micromass Quattro II triple-quadrupole mass spectrometer using electrospray ionization with a MassLynx operating system. The melting points were collected on a SHPSIC WRS-2 automatic melting point apparatus.

2. Synthetic Protocols

2.1 Synthesis of metallacycle ***I***

2.1.1 Synthesis of ligand ***D***[S1]



Ligand **D** was synthesized according to a modified procedure[S1]. Pyrrole (7.0 mL, 100 mmol), benzaldehyde (5.0 mL, 50 mmol), and 4-pyridinecarboxaldehyde (5.0 mL, 50 mmol) were refluxed in 250 mL of 99% propionic acid for 1 h. The reaction mixture was then cooled and allowed to stand overnight. Filtration and methanol washing afforded 4.15 g (26% yield) of a purple crystalline product. The product was analyzed by silica gel thin layer chromatography and found to be a mixture of the six possible porphyrin isomers, ligand **D** was the fourth one and has a 8% yield. The ^{1}H NMR spectrum of **D** is shown in Figure S1. ^{1}H NMR ($CDCl_3$, room temperature, 400 MHz) δ (ppm): 9.04 (4 H, d, 2,6-pyridyl), 8.91 (8 H, m, pyrrole β), 8.21 (8 H, m. *o*-phenyl. and 3,5-pyridyl), 7.78 (6 H, m, *m*- and *p*-phenyl).

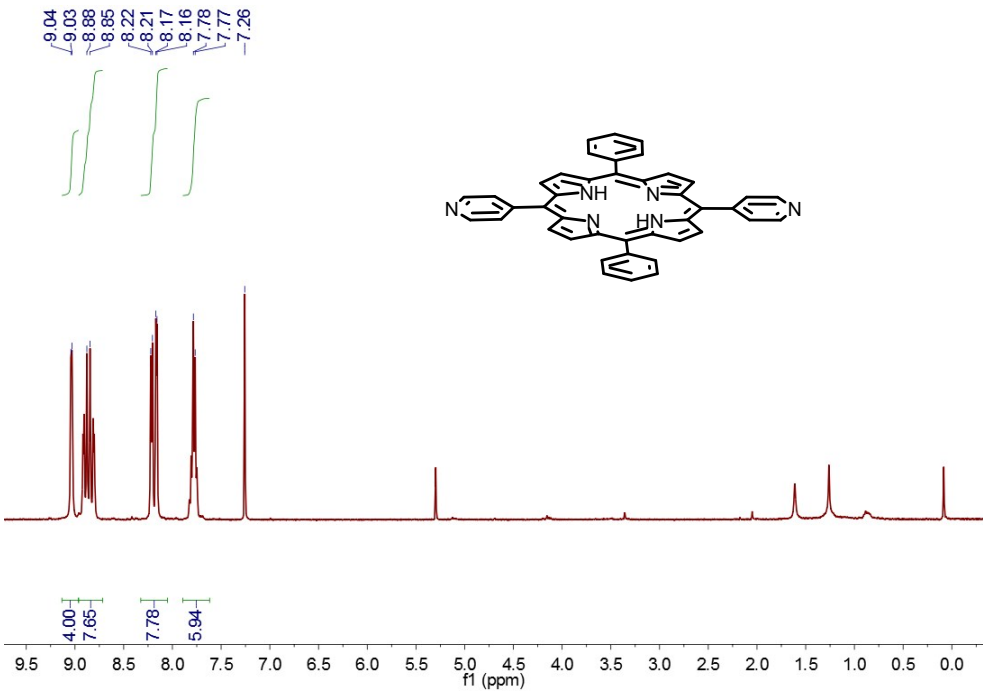
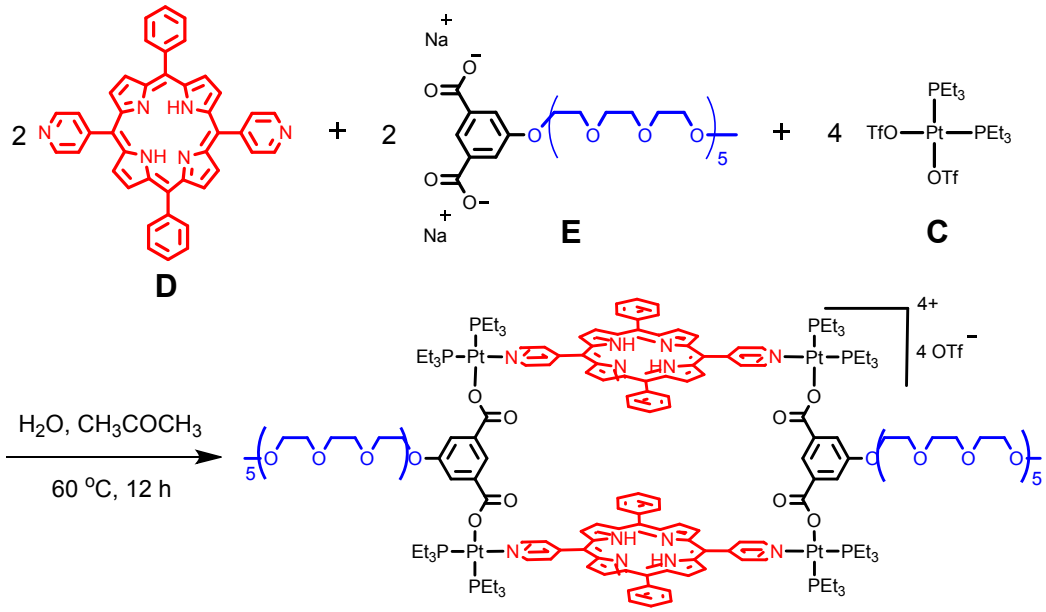


Fig. S1. ^{1}H NMR spectrum ($CDCl_3$, room temperature, 400 MHz) of ligand **D**.

*2.1.2 Synthesis of metallacycle **I***



In a 2:2:4 molar ratio, bipyridylporphyrin **D** (1.23 mg, 2.00 μmol), water soluble carboxylate ligand **E** (1.80 mg, 2.00 μmol), and 90°C Pt (II) acceptor **C** (2.938 mg, 4.00 μmol) were placed in a 2 mL vial, followed by addition of H_2O (0.2 mL) and acetone (0.8 mL). After stirring overnight at 60°C, all solvent was removed by N_2 flow and the solid was dried under vacuum. Acetone (1.0 mL) was then added into the vial, and the solution was stirred for 5 h at room temperature. After storage in a refrigerator for 1 h, the mixture was filtered to remove insoluble materials. The resulting amphiphilic metallacycle **I** was precipitated with diethyl ether, isolated, and dried under reduced pressure (4.48 mg, yield:87%) and then re-dissolved in acetone-d_6 for characterization. The ^{1}H NMR spectrum of metallacycle **I** is shown in Figure S2. ^{1}H NMR (acetone-d_6, room temperature, 400 MHz) δ (ppm): 9.43 (s, 8H),

9.17 (s, 4H), 9.02 (s, 4H), 8.91 (s, 4H), 8.54 (s, 4H), 8.45 (d, J = 4 Hz, 8H), 8.26 (d, J = 4 Hz, 8H), 7.88 (m, 20H), 7.68 (s, 6H), 4.05 (m, 4H), 3.63–3.24 (m, 116H), 1.55–1.11 (m, 120H). The ^{31}P {^{1}H} NMR spectrum of metallacycle **I** is shown in Figure S3. ^{31}P {^{1}H} NMR (acetone, room temperature, 121.4 MHz) δ (ppm): 0.71 ppm ($^{2}J_{p\text{-}p}$ = 21.8 Hz, ^{195}Pt satellites, $^{1}J_{Pt-P}$ = 3374.9 Hz), –5.20 ($^{2}J_{p\text{-}p}$ = 21.8 Hz, ^{195}Pt satellites, $^{1}J_{Pt-P}$ = 3251.1 Hz). ESI-MS is shown in Figure S4: m/z 1604.33 [M – 3OTf]$^{3+}$, 1618.62 [M + 2H_2O – 3OTf]$^{3+}$, 1633.64 [M + H_2O + CH_3COCH_3 – 3OTf]$^{3+}$, 1662.62 [M + Na – 2OTf]$^{3+}$, 1676.23 [M + K + H_2O – 2OTf]$^{3+}$, 1719.87 [M + 2Na – OTf]$^{3+}$, 1777.89 [M + 3Na]$^{3+}$, 1820.05 [M + H_2O + CH_3COCH_3 + 3K]$^{3+}$.

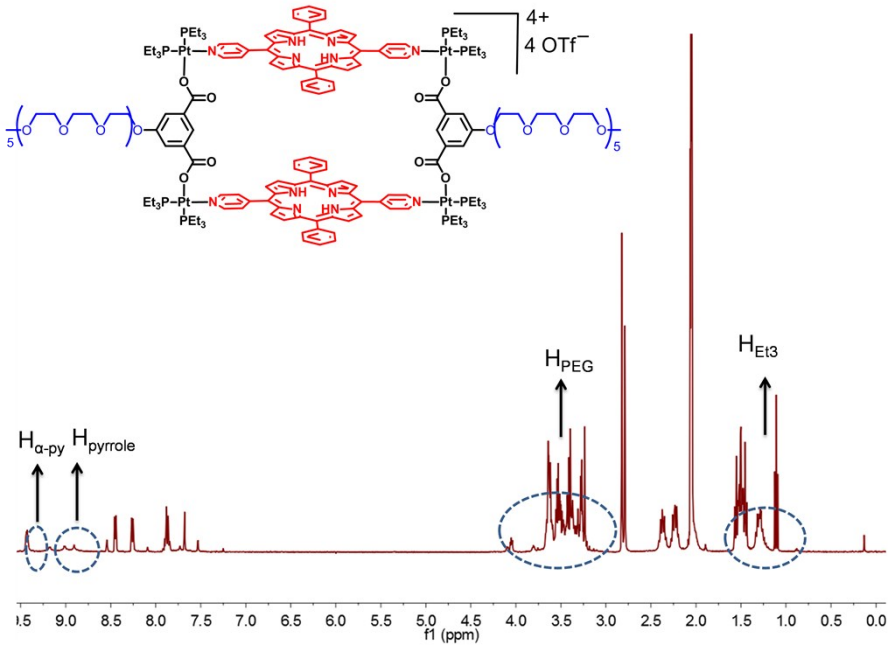


Fig. S2. ^{1}H NMR spectrum (acetone-d_6, room temperature, 400 MHz) of metallacycle **I**.

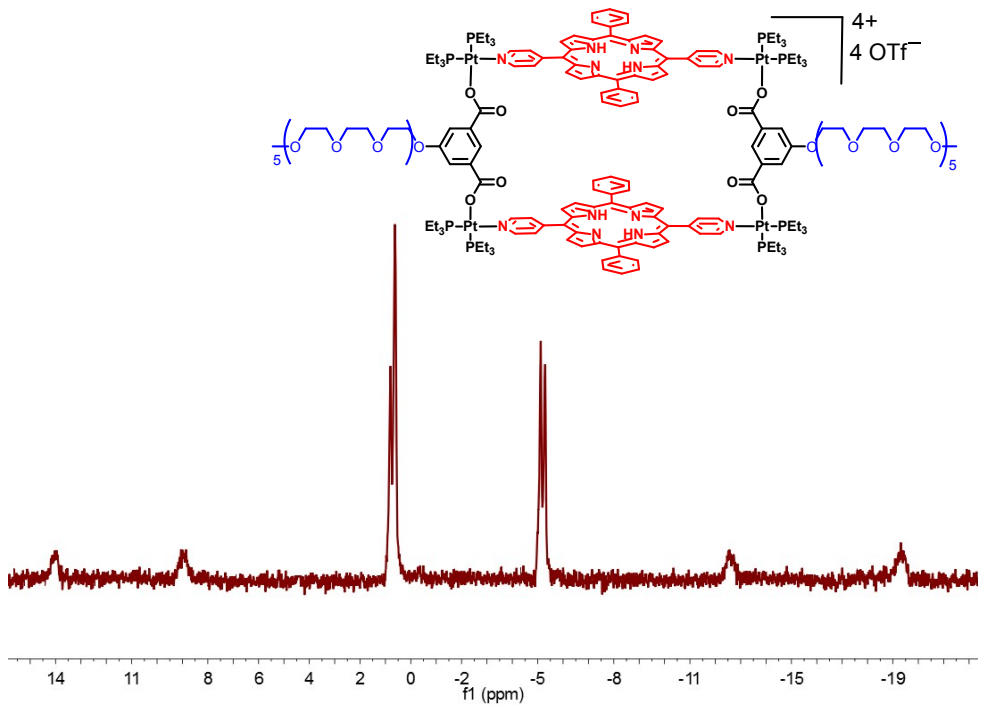
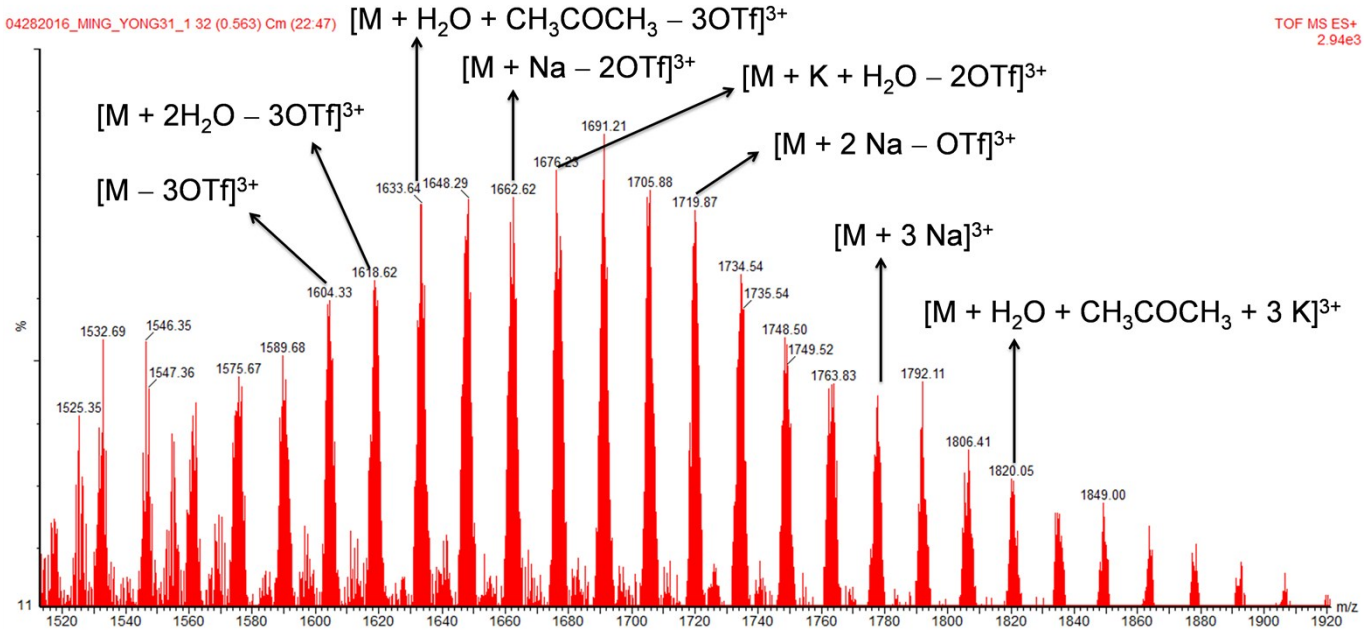


Fig. S3. ^{31}P NMR spectrum (acetone, room temperature, 121.4 MHz) of metallacycle **I**.



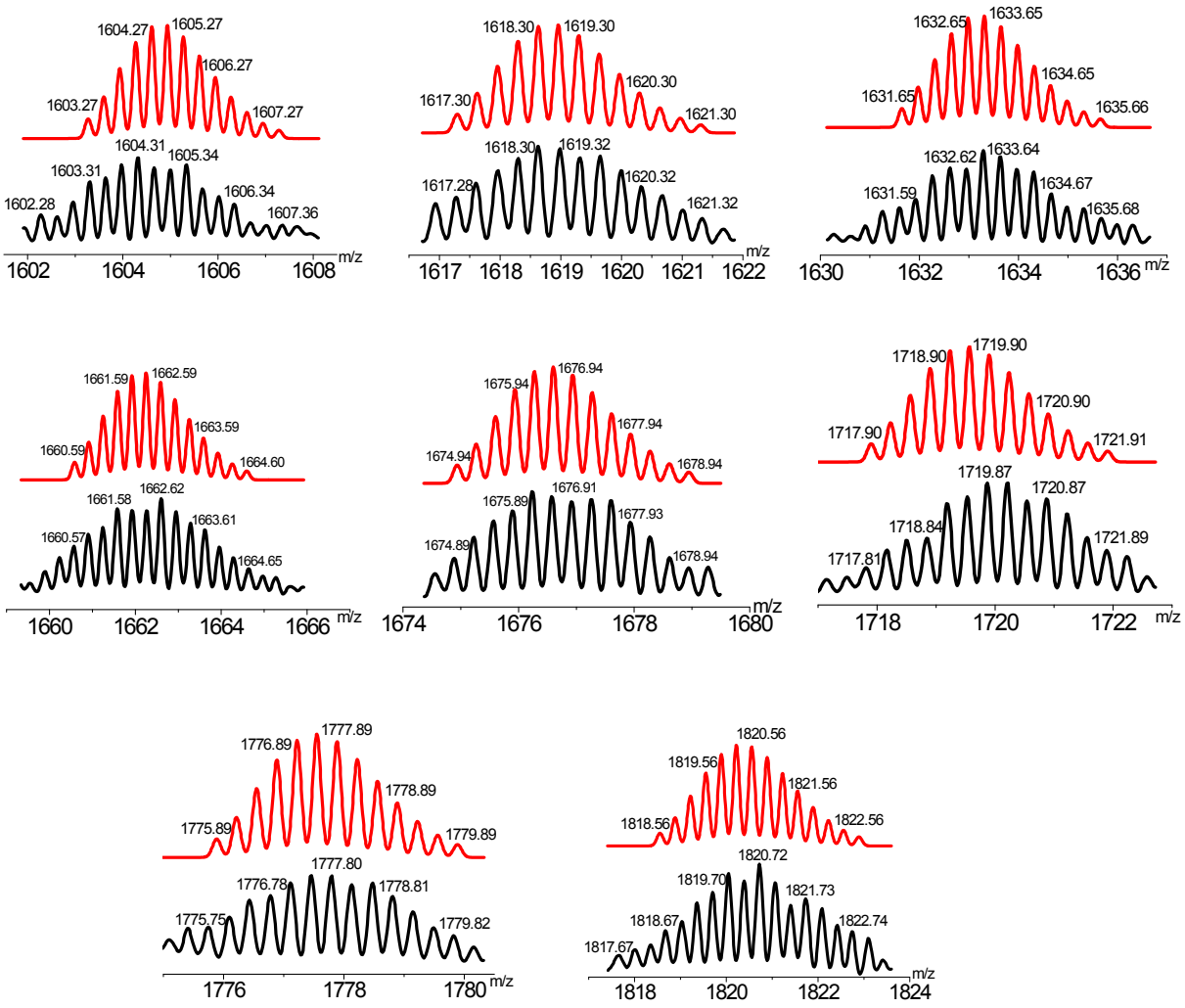
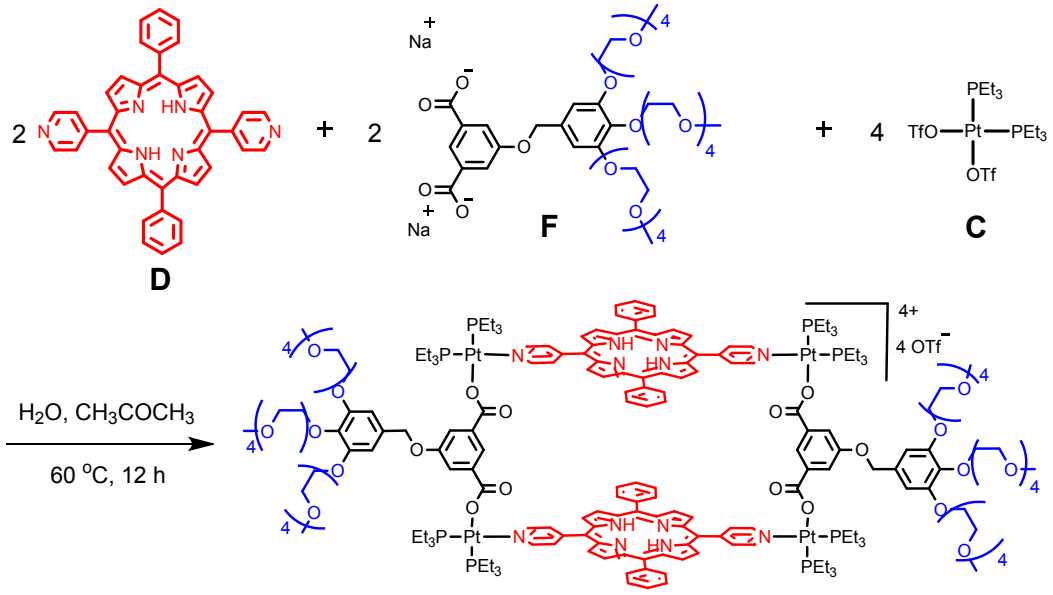


Fig. S4. Experimental (black) and calculated (red) electrospray ionization mass spectrum of metallacycle **I**.

*2.2 Synthesis of metallacycle **II***



In a 2:2:4 molar ratio, bipyridylporphyrin **D** (1.23 mg, 2.00 μmol), water soluble carboxylate ligand **F** (1.87 mg, 2.00 μmol), and 90°C Pt (II) acceptor **C** (2.90 mg, 4.00 μmol) were placed in a 2 mL vial, followed by addition of H_2O (0.2 mL) and acetone (0.8 mL). After stirring overnight at 60°C, all solvent was removed by N_2 flow and the solid was dried under vacuum. Acetone (1.0 mL) was then added into the vial, and the solution was stirred for 5 h at room temperature. After storage in a refrigerator for 1 h, the mixture was filtered to remove insoluble materials. The resulting amphiphilic metallacycle **II** was precipitated with diethyl ether, isolated, and dried under reduced pressure (4.56 mg, yield: 87%) and then re-dissolved in acetone-d_6 for characterization. The ^{1}H NMR spectrum of metallacycle **II** is shown in Figure S5. ^{1}H NMR (acetone-d_6, room temperature, 400 MHz) δ (ppm): 9.31 (s, 8H), 9.21(s, 4H), 9.06 (s, 4H), 8.91 (s, 4H), 8.47 (s, 4H), 8.43 (s, 8H), 8.29 (s, 8H), 7.89 (m, 12H), 7.67 (s, 6H), 6.44 (s, 4H), 5.03 (s, 4H), 3.58–3.31 (m, 114H), 1.57–1.11 (m, 120H). The ^{31}P {^{1}H} NMR spectrum of metallacycle **II** is shown in Figure S6. ^{31}P {^{1}H} NMR (acetone, room temperature, 121.4 MHz) δ (ppm): 0.77 ppm ($^2J_{p\text{-}p}$ = 20.6 Hz, ^{195}Pt satellites, $^1J_{Pt-P}$ = 3425.9 Hz), –5.77 ($^2J_{p\text{-}p}$ = 20.6 Hz, ^{195}Pt satellites, $^1J_{Pt-P}$ = 3425.9 Hz). ESI-MS is shown in Figure S7: *m/z* 1628.66 [M – 3OTf]$^{3+}$, 1685.93 [M + Na – 2OTf]$^{3+}$, 1742.50 [M + 2Na– OTf]$^{3+}$, 1800.08 [M + 3Na]$^{3+}$, 2514.59 [M – 2OTf]$^{2+}$.

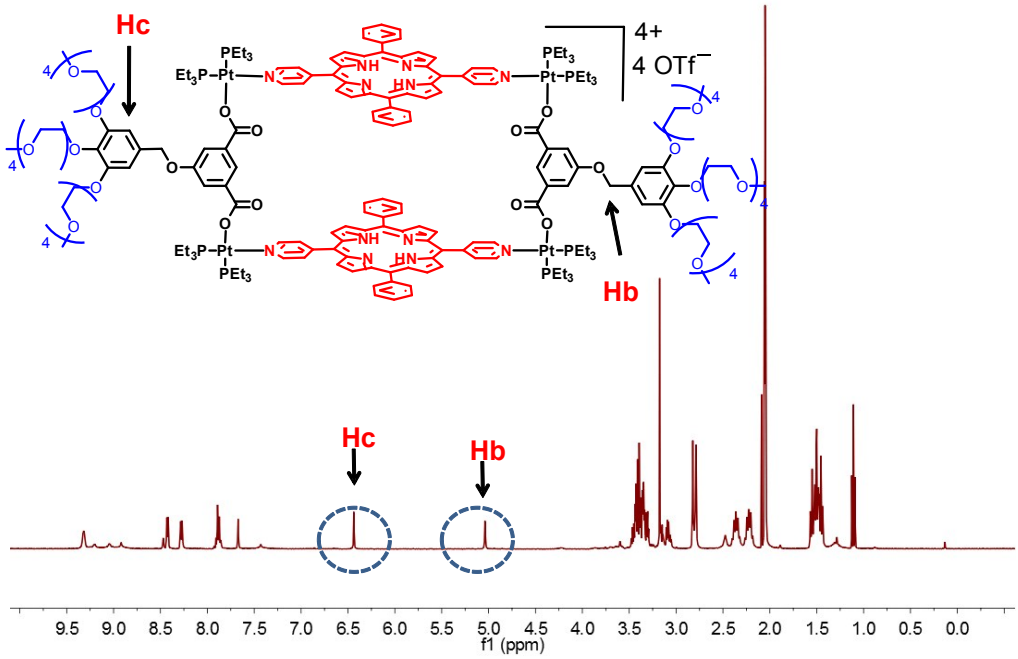


Fig. S5. ^{1}H NMR spectrum (acetone-d_6, room temperature, 400 MHz) of metallacycle **II**.

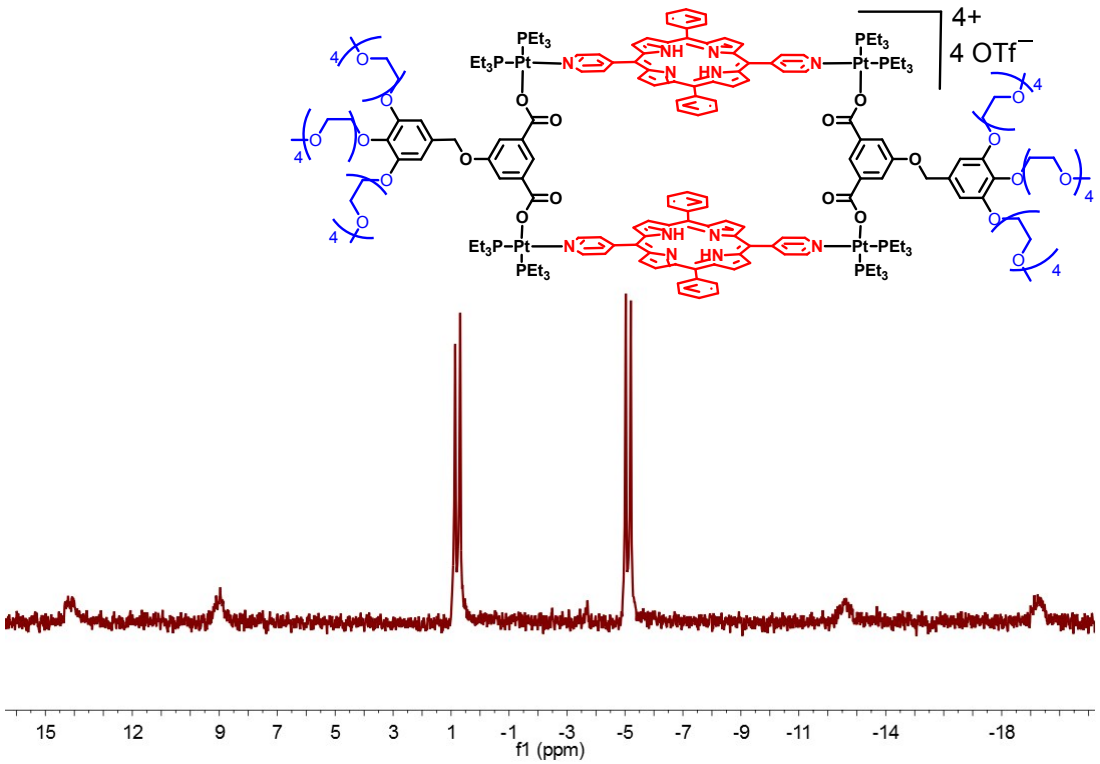
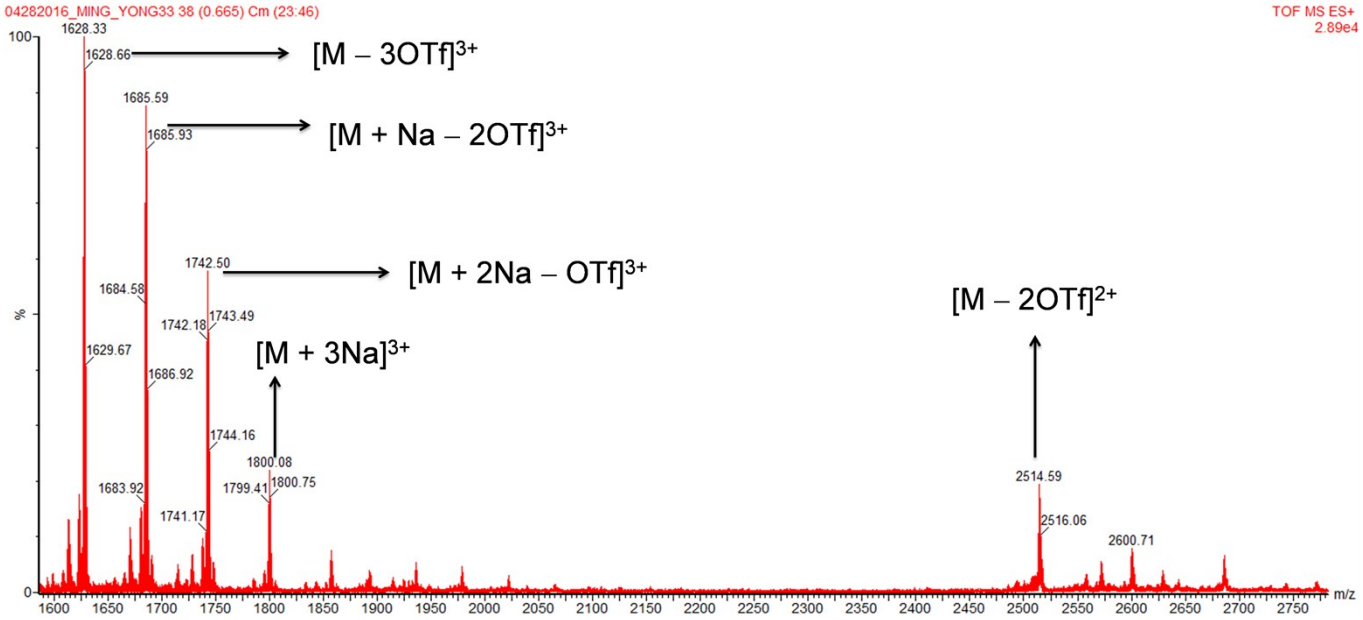


Fig. S6. ^{31}P NMR spectrum (acetone, room temperature, 121.4 MHz) of metallacycle **II**.



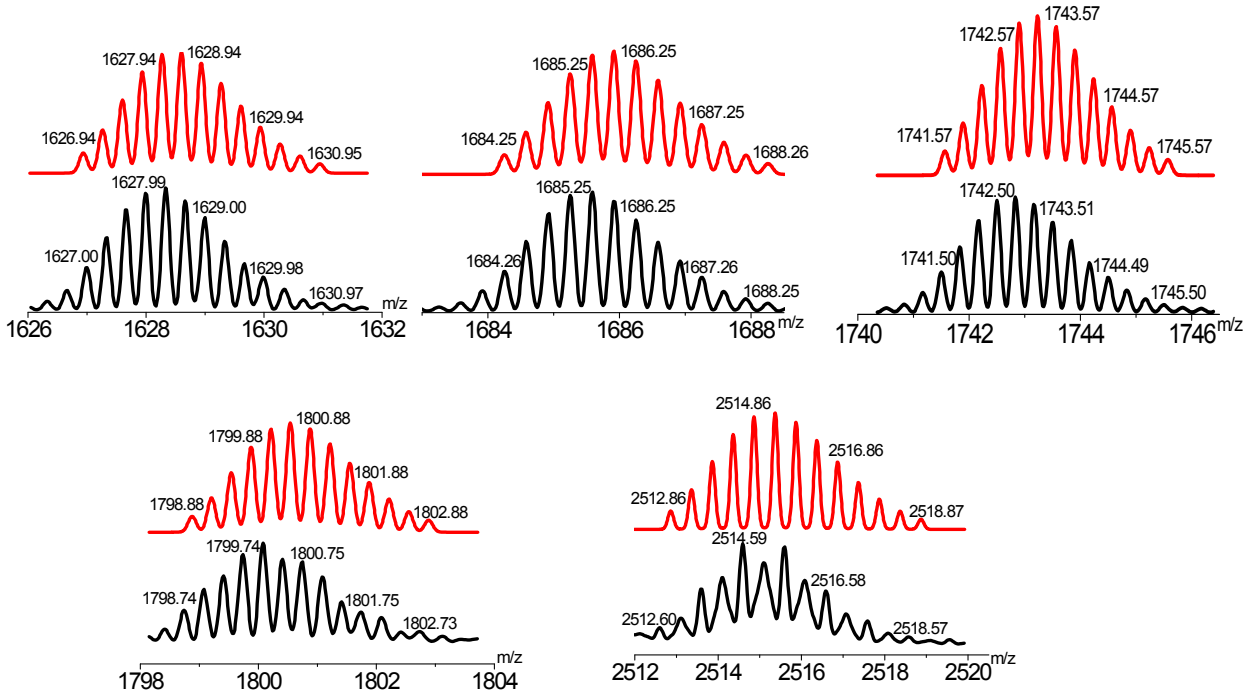
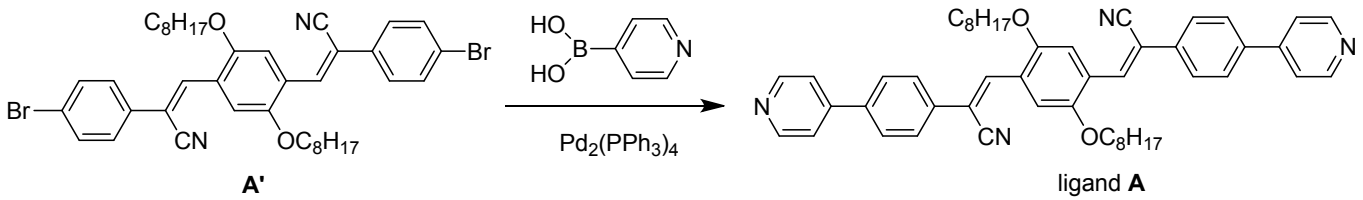


Fig. S7. Experimental (black) and calculated (red) electrospray ionization mass spectrum of metallacycle **II**.

*2.3. Synthesis of metallacycle **III***

*2.3.1 Synthesis of ligand **A***



In a 100 mL round-bottom Schlenk flask, compound **A'** (1.48 g, 2.00 mmol), Pyridine-4-boronic acid (0.600 g, 5.00 mmol), tetrakis(triphenylphosphine)palladium (116 mg, 0.100 mmol), and CTAB (25.0 mg) were added. Toluene (40 mL) and K_2CO_3 aqueous solution (20 mL, 3.00mmol) were added to the flask *via* a syringe under N_2. The mixture was stirred at room temperature for 24h. After removal of the solvent by reduced pressure distillation, the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 100:1 *v*/*v*) to afford **A** as an orange solid (1.37 g, 93%). The ^{1}H NMR spectrum of **A** is shown in Figure S8. ^{1}H NMR ($CDCl_3$, room temperature, 400 MHz) δ (ppm): 8.71 (d, J = 4 Hz, 4H), 8.13 (s, 2H), 7.94 (s, 2H), 7.84–7.74 (m, 8H), 7.55 (d, J = 4 Hz, 4H). 4.16 (t, J = 6 Hz), 1.88 (m, 4H), 1.52–1.28 (m, 24 H), 0.86 (t, J = 8 Hz, 6H). The ^{13}C NMR spectrum of **A** is shown in Figure S9. ^{13}C NMR ($CDCl_3$, room temperature, 100 MHz) δ (ppm): 155.13, 149.04, 148.64, 141.16, 139.95, 131.77, 127.88, 125.42, 121.40, 120.79, 119.82, 119.52, 117.73, 70.37, 31.73, 29.15, 28.80, 26.58, 23.16, and 14.00. LRESIMS is shown in Figure S10: *m*/*z* 765.8 $[M + Na]^+$, 781.4 $[M + K]^+$.

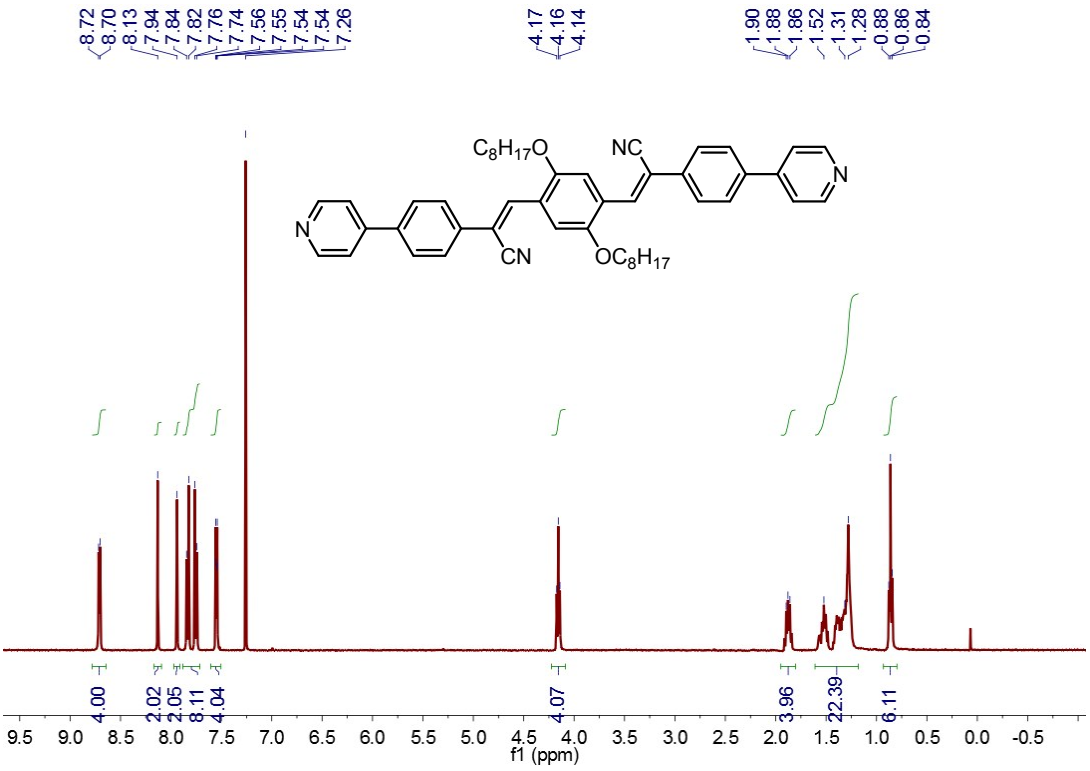


Fig. S8. ^{1}H NMR spectrum ($CDCl_3$, room temperature, 400 MHz) of ligand **A**.

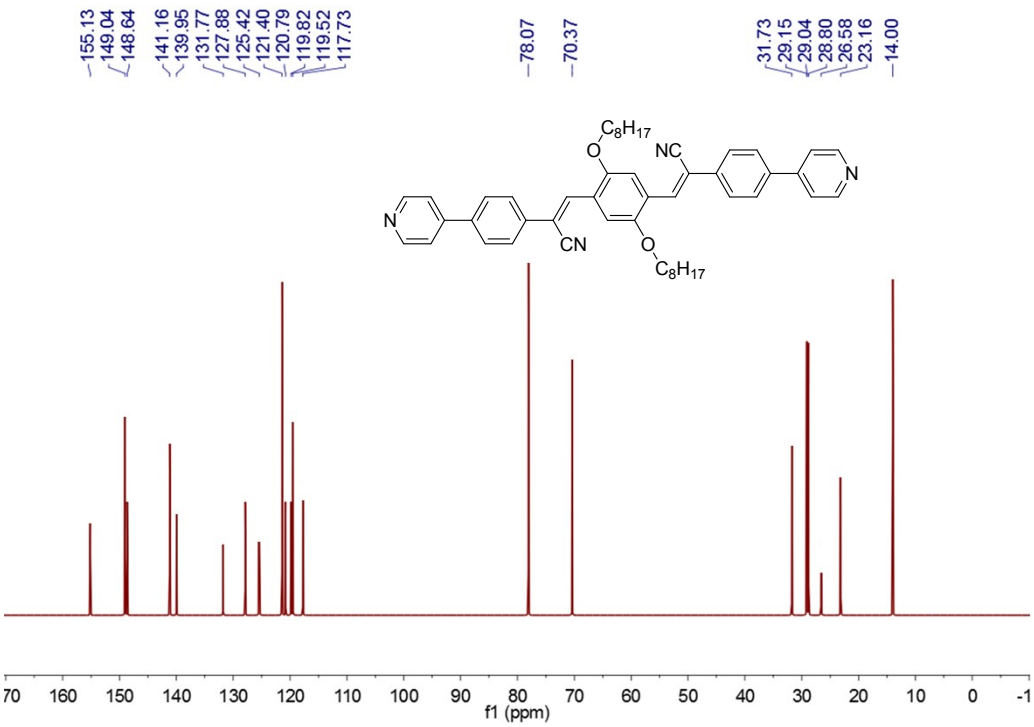


Fig. S9. ^{13}C NMR spectrum ($CDCl_3$, room temperature, 75 MHz) of ligand **A**.

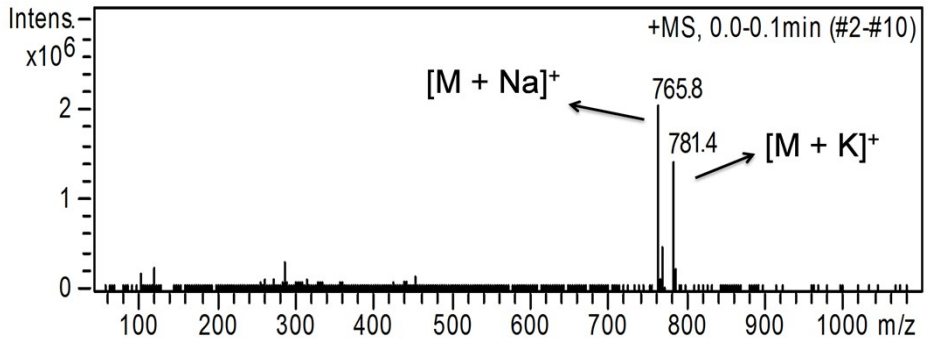
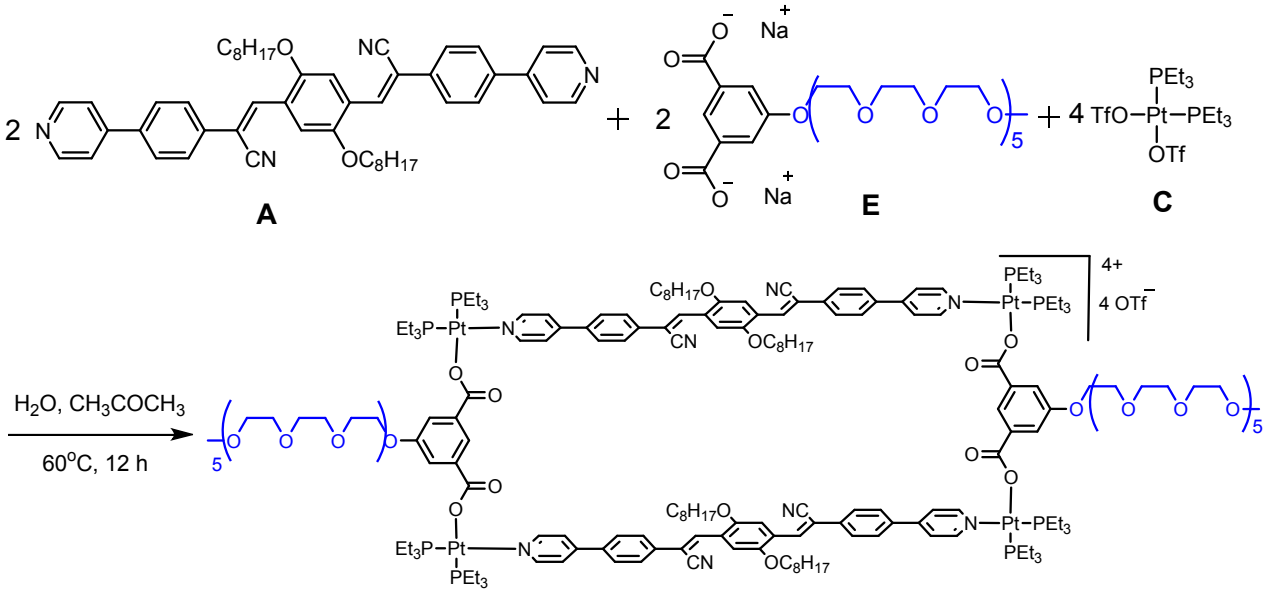


Figure S10. Electrospray ionization mass spectrum of ligand **A**.

*2.3.2 Synthesis of metallacycle **III***



In a 2:2:4 molar ratio, ligand **A** (1.49 mg, 2.00 μmol), water soluble carboxylate ligand **E** (1.80 mg, 2.00 μmol), and 90°C Pt (II) acceptor **C** (2.90 mg, 4.00 μmol) were placed in a 2 mL vial, followed by addition of H_2O (0.2 mL) and acetone (0.8 mL). After stirring overnight at 60°C, all solvent was removed by N_2 flow and the solid was dried under vacuum. Acetone (1.0 mL) was then added into the vial, and the solution was stirred for 5 h at room temperature. After storage in a refrigerator for 1 h, the mixture was filtered to remove insoluble materials. The resulting amphiphilic metallacycle **II** was precipitated with diethyl ether, isolated, and dried under reduced pressure (5.02 mg, yield: 93%) and then re-dissolved in acetone-d_6 for characterization. The ^{1}H NMR spectrum of metallacycle **III** is shown in Figure S11. ^{1}H NMR (acetone-d_6, room temperature, 400 MHz) δ (ppm): 9.05 (s, 8H), 8.06(s, 8H), 7.97 (s, 8H), 7.85 (s, 8H), 7.44 (s, 8H), 4.14–3.39 (m, 130H), 1.41–1.01 (m, 150H). The ^{31}P {^{1}H} NMR spectrum of metallacycle **III** is shown in Figure S12. ^{31}P {^{1}H} NMR (acetone, room temperature, 121.4 MHz) δ (ppm): 1.16 ppm ($^2J_{p\text{-}p}$ = 21.8 Hz, ^{195}Pt satellites, $^1J_{Pt-P}$ = 3365.2 Hz), –6.30 ($^2J_{p\text{-}p}$ = 21.8 Hz, ^{195}Pt satellites, $^1J_{Pt-P}$ = 3195.2 Hz). ESI-MS is shown in Figure S13: *m/z* 1687.44 [M + 2H_2O– 3OTf]$^{3+}$, 1717.36 [M + H_2O + CH_3COCH_3 – 3OTf]$^{3+}$, 1747.01 [M + 3H_2O + 2CH_3COCH_3 – 3OTf]$^{3+}$.

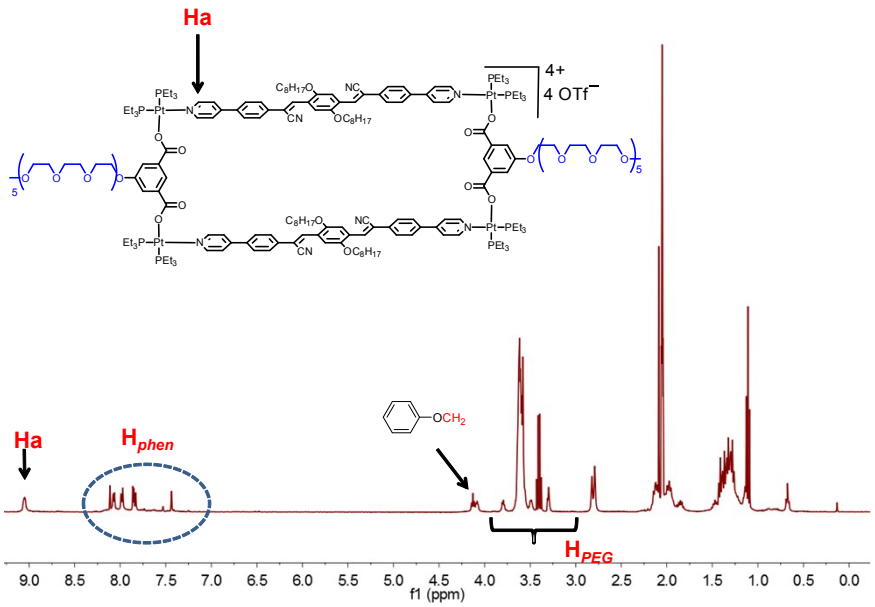


Fig. S11. ^{1}H NMR spectrum (acetone-d_6, room temperature, 400 MHz) of metallacycle **III**.

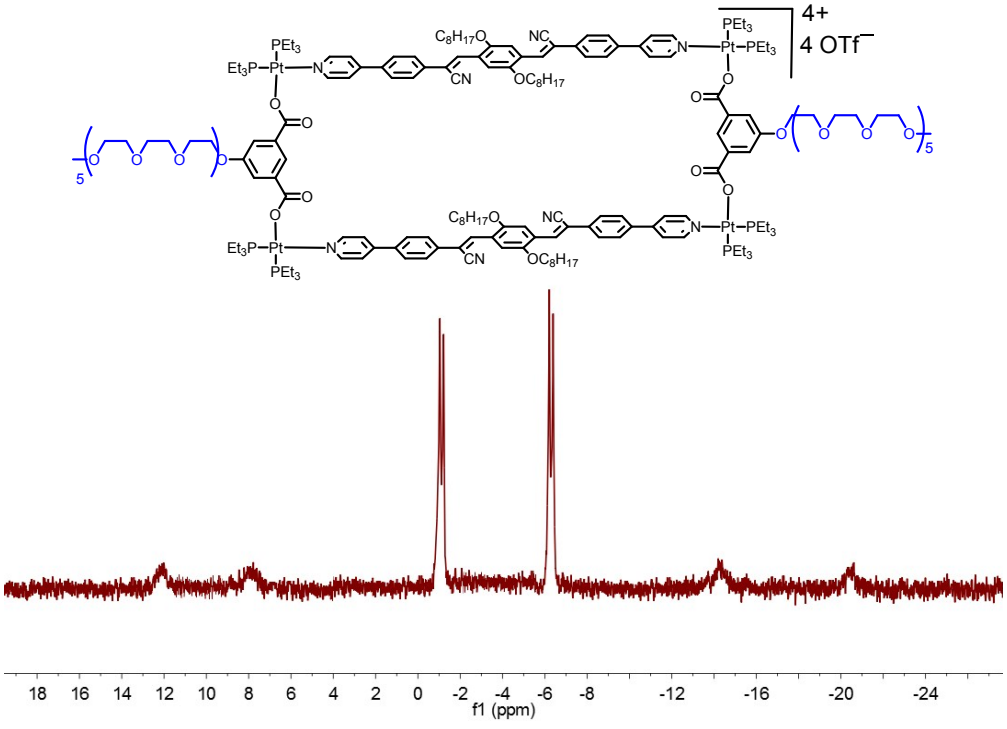


Figure S12. ^{31}P NMR spectrum (acetone, room temperature, 121.4 MHz) of metallacycle **III**.

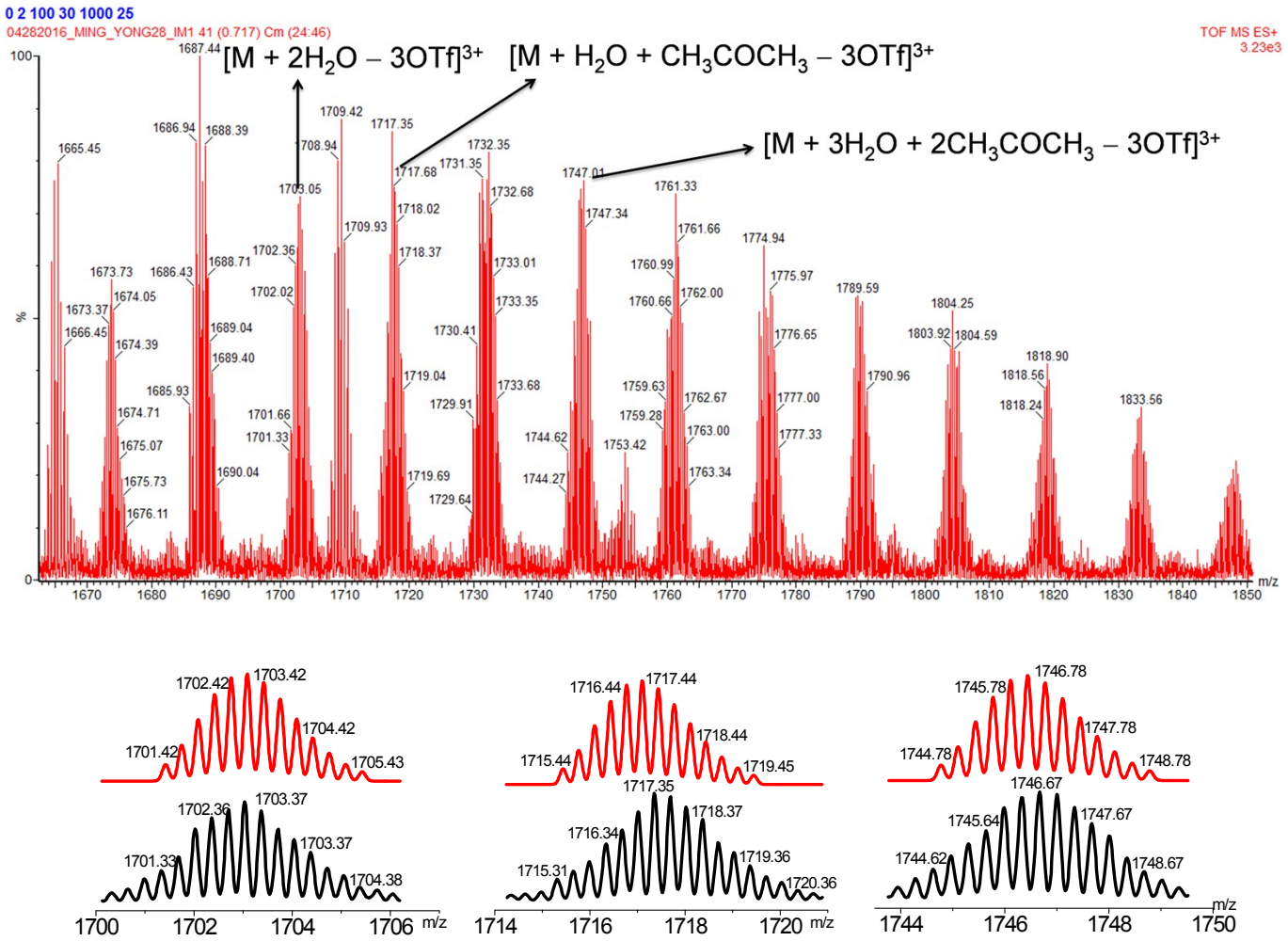
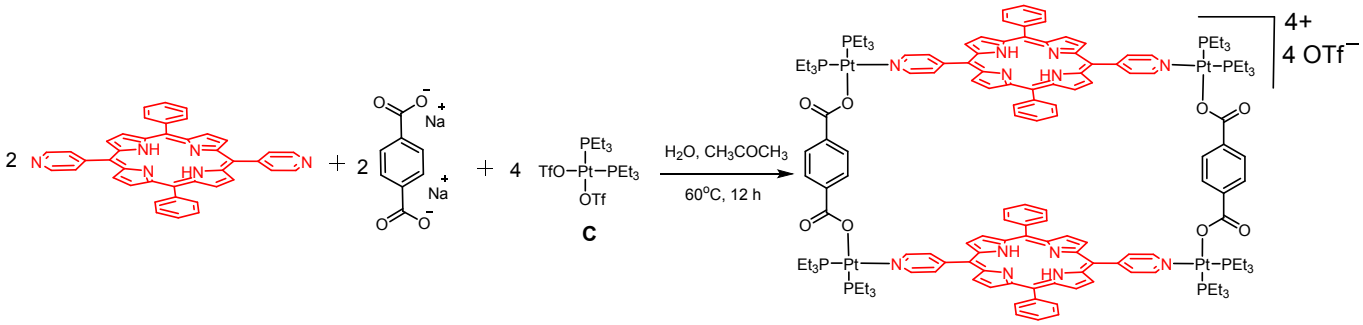


Figure S13. Experimental (black) and calculated (red) electrospray ionization mass spectrum of metallacycle **III**.

*2.4 Synthesis of metallacycle **IV***



In a 2:2:4 molar ratio, bipyridylporphyrin **D** (1.23 mg, 2.00 μmol), water soluble carboxylate ligand **B** (0.42 mg, 2.00 μmol), and 90°C Pt (II) acceptor **C** (2.90 mg, 4.00 μmol) were placed in a 2 mL vial, followed by addition of H_2O (0.2 mL) and acetone (0.8 mL). After stirring overnight at 60°C, all solvent was removed by N_2 flow and the solid was dried under vacuum. Acetone (1.0 mL) was then added into the vial, and the solution was stirred for 5 h at room temperature. After storage in a refrigerator for 1 h, the mixture was filtered to remove insoluble materials. The resulting amphiphilic metallacycle **IV** was precipitated with diethyl ether, isolated, and dried under reduced pressure (3.12 mg, yield: 83%) and then re-dissolved in acetone-d_6 for characterization. The ^{31}P $\{^1H\}$ NMR spectrum of metallacycle **IV** is shown in Figure S14. ^{31}P $\{^1H\}$ NMR (acetone, room temperature, 121.4 MHz) δ (ppm): 0.07 ppm ($^2J_{p\text{-}p}$ = 20.6 Hz, ^{195}Pt satellites, $^1J_{Pt-P}$ = 3408.9 Hz), –5.01 ($^2J_{p\text{-}p}$ = 20.6 Hz, ^{195}Pt satellites, $^1J_{Pt-P}$ = 3299.6 Hz). ESI-MS is shown in Figure S15: *m*/*z* 1792.46 $[M - 2OTf]^{2+}$, 1878.46 $[M + Na - OTf]^{2+}$.

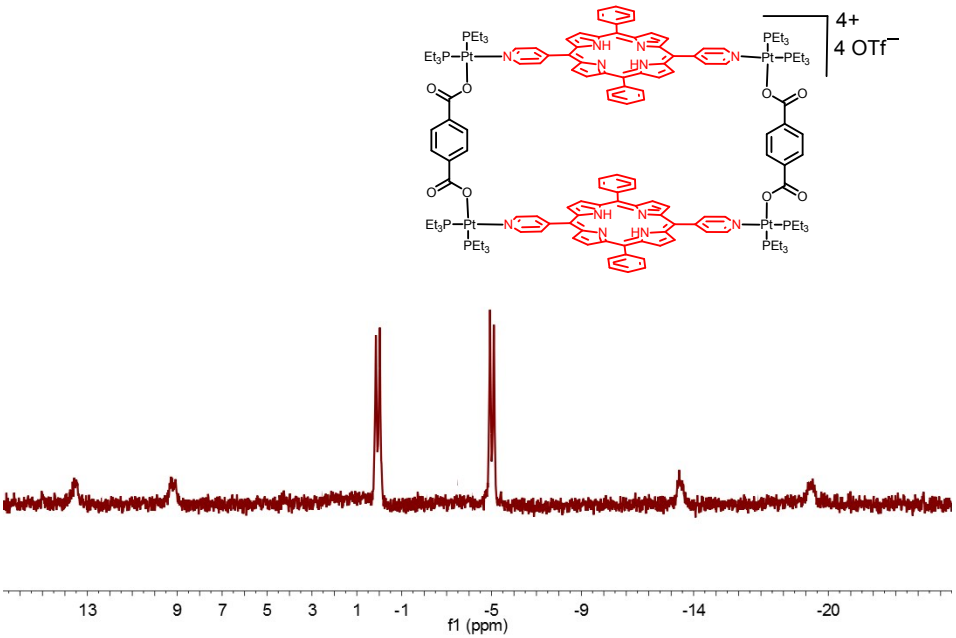


Fig. S14. ^{31}P NMR spectrum (acetone, room temperature, 121.4 MHz) of metallacycle **IV**.

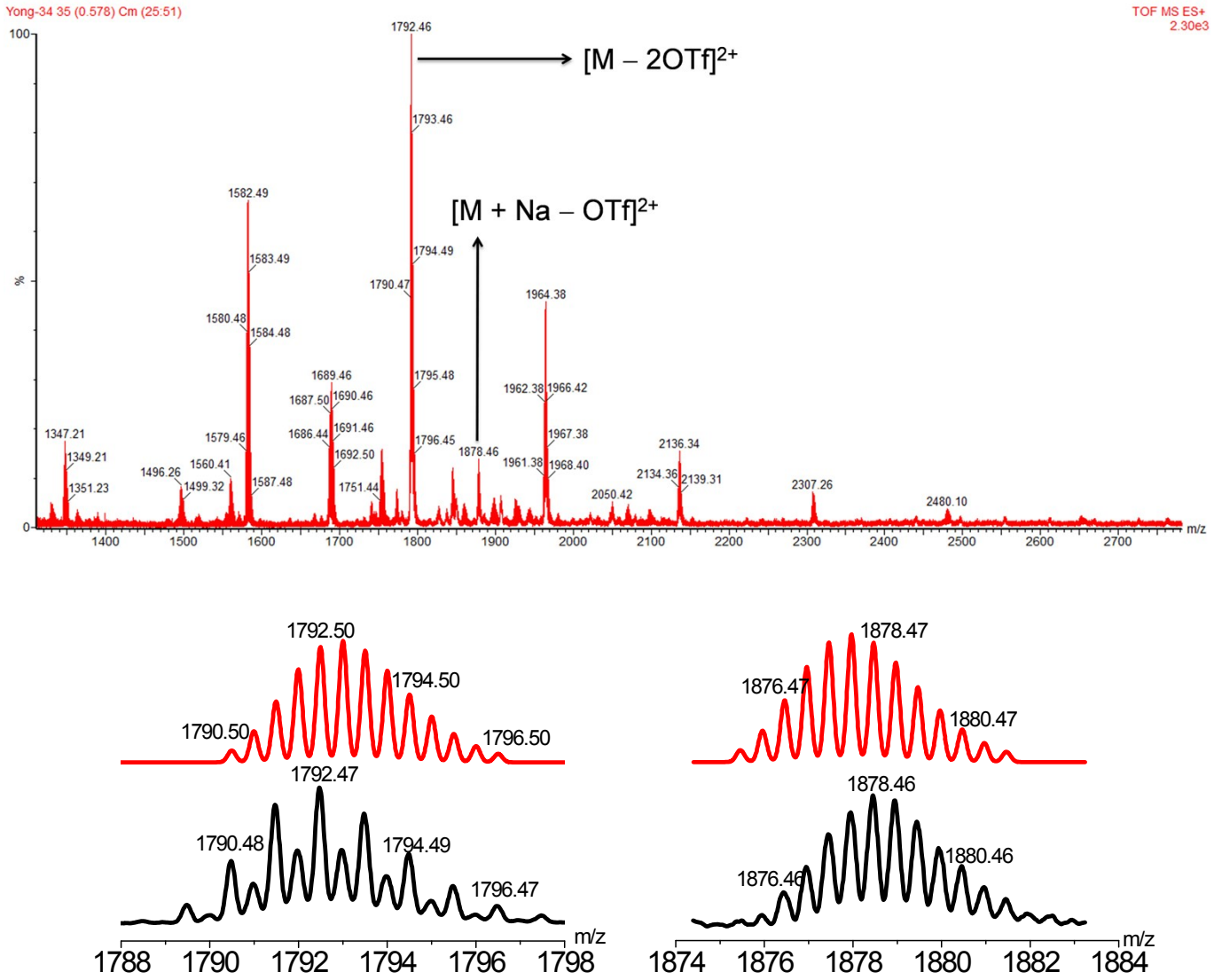


Fig. S15. Experimental (black) and calculated (red) electrospray ionization mass spectrum of metallacycle **IV**.

3. Characterization of Metallosupramolecules

*3.1 Critical aggregate concentration (CAC) values of metallacycles **I**, **II**, **III**, and **IV** in water*

When the metallacycles were dissolved in water, the water surface tension (γ) as a function of the concentration of the metallacycles (C) were measured to determine its critical aggregate concentration (CMC) in water.[S2, S3, S4] There were two linear segments in the γ versus C curve and a sudden reduction in the slope, implying that the CAC values.

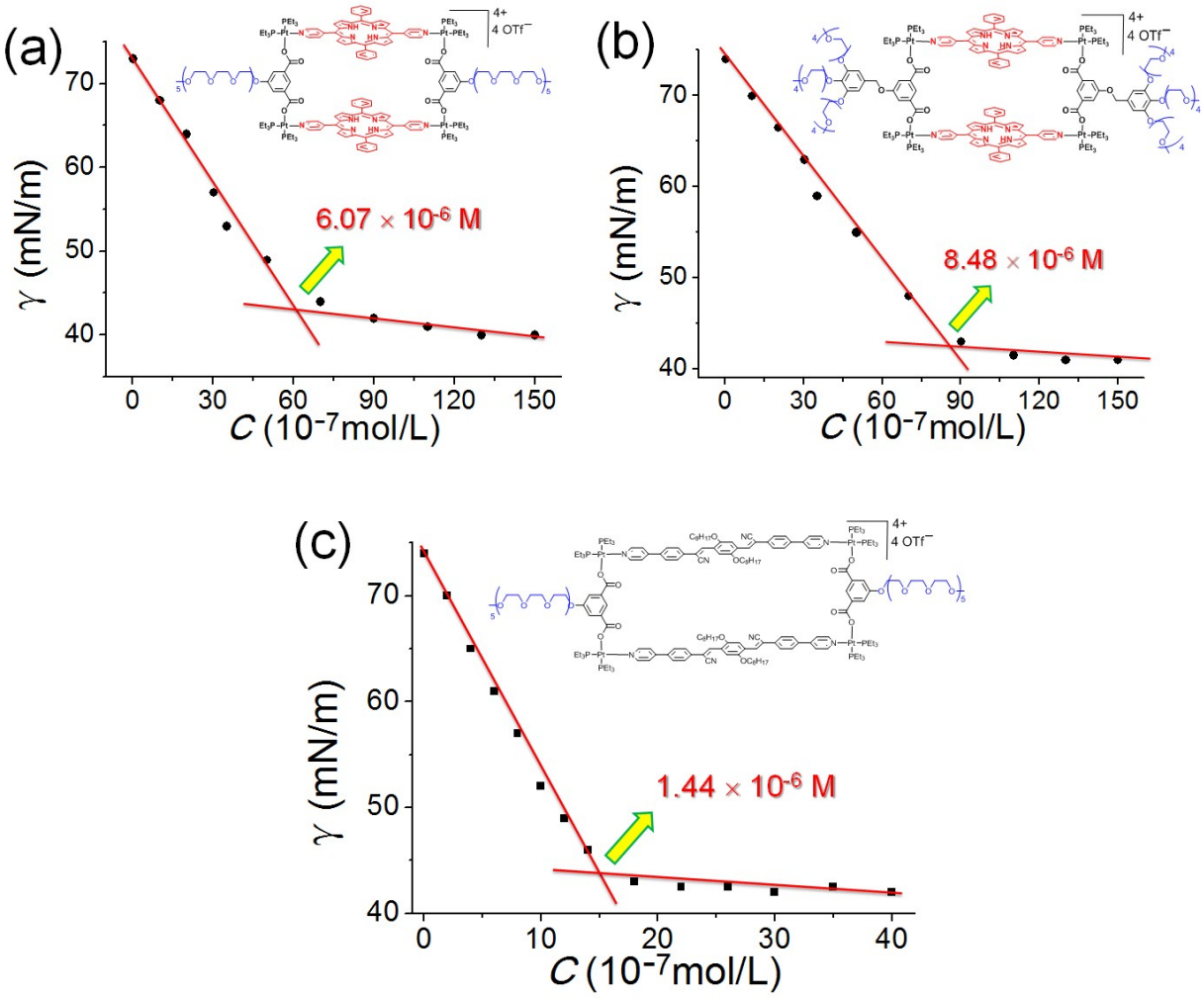


Fig. S16. Surface tension (γ) of water as a function of the concentration of metallacycles **I** (a), **II** (b), and **III** (c). We could not get the CAC value of metallacycle **IV** due to its totally hydrophobic property.

*3.2 DLS studies of metallacycles **I**, **II**, **III**, and **IV** in water*

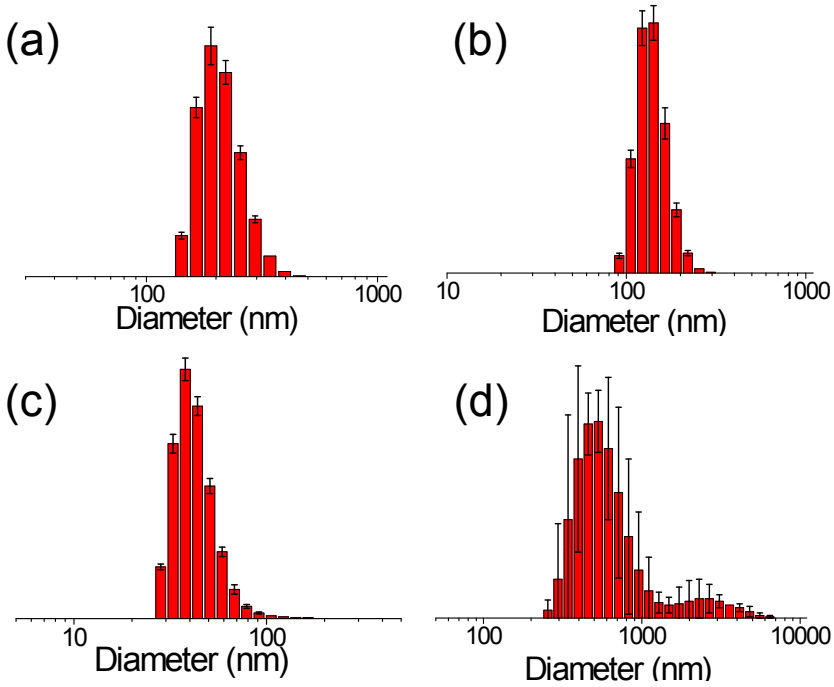


Fig. S17. DLS studies of metallacycles **I** (a), **II** (b), **III** (c), and **IV** (d) self-assembly in water at 1.00 × 10^{-5}M. Metallacycle **IV** was ultrasound for 10 min.

4. Flow cytometry study

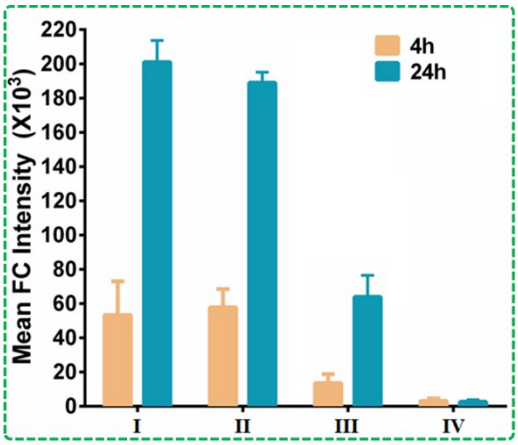


Fig. S18 Flow cytometry of metallacycles **I** (1.00 × 10^{-5} M), **II** (1.00 × 10^{-5} M), **III** (1.00 × 10^{-5} M), and **IV** (1.00 × 10^{-5} M, ultrasound first, not homogeneous solution) were incubated with A549 cells (purchased from American Type Culture Collection -USA) for 4 h and 24 h, respectively.

5. Dark cytotoxicity

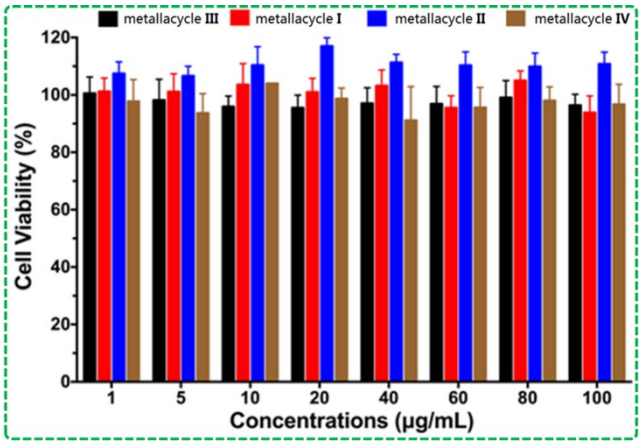


Fig. S19 Viability of A549 cells (purchased from American Type Culture Collection -USA) measured by the MTT assay after treating with metallacycles **I**, **II**, **III**, and **IV** (ultrasound first), without light irradiation.

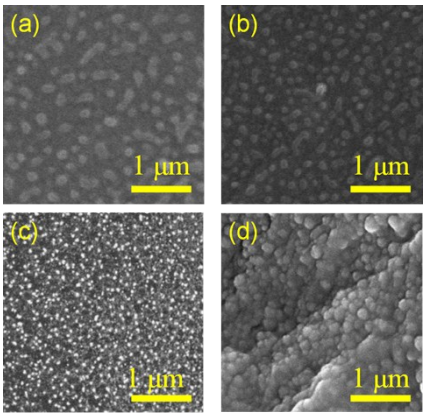


Fig. S20 TEM images of metallacycles **I** (a), **II** (b), **III** (c), and **IV** (d) self-assembly in water at 1.00 10^{-5} M. Metallacycle **IV** was ultrasound for 10 min before preparing sample.

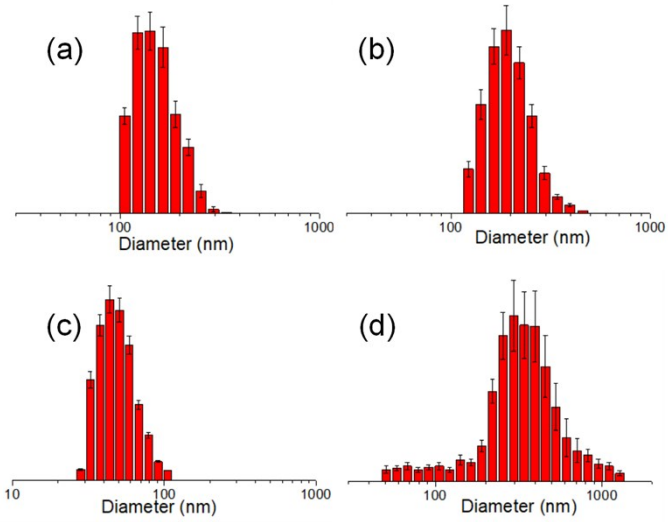


Fig. S21 DLS studies of metallacycles **I** (a), **II** (b), **III** (c), and **IV** (d) self-assembly in PBS aqueous solution at 1.00×10^{-5}M. Metallacycle **IV** was ultrasound for 10 min.

6. References

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