Supporting Information

Multi-Responsive Supramolecular Heparin-Based Biohybrid Metallogel Constructed by Controlled Self-Assembly Based on Metal–Ligand, Host-Guest and Electrostatic Interactions

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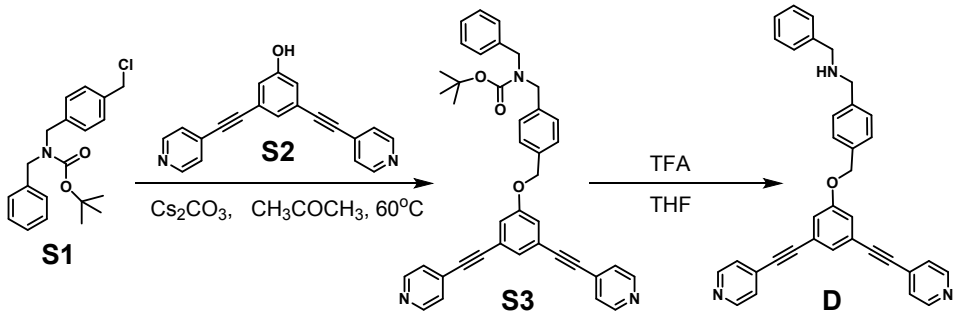
1. Materials and methods

All solvents were dried according to standard procedures and all of them were degassed under N2 for 30 minutes before use. All air-sensitive reactions were carried out under inert N_2 atmosphere. ^{1}H, ^{13}C NMR and ^{31}P NMR spectra were recorded on Bruker 400MHz Spectrometer (^{1}H: 400 MHz; ^{31}P: 161.9 MHz) and 500MHz Spectrometer (^{1}H: 500MHz) at 298 K. The ^{1}H and ^{13}C 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 SEM samples were prepared on clean Si substrates. To minimize sample charging, a thin layer of Au was deposited onto the samples before SEM examination. All the SEM images were obtained using a S-4800 (Hitachi Ltd.) with an accelerating voltage of 3.0-10.0 kV.

Synthetic experimental details and characterizations of new compounds

Scheme S1. The synthetic procedure for the donor **D**.

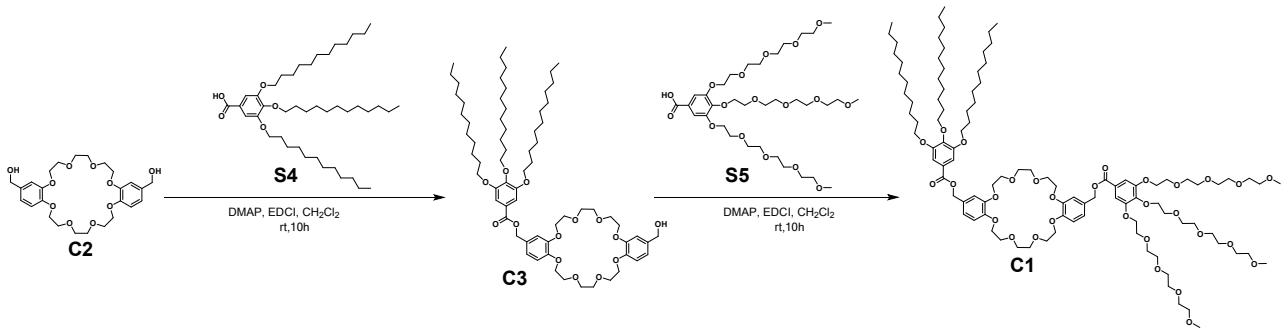


Synthesis of the donor D. A 100 mL Schlenk flask was charged with **S1** (370 mg, 1.07mmol),[1] Cs_2CO_3 (929.09 mg, 4.81 mmol), **S2** (475.5 mg, 1.61 mmol), degassed, and back-filled three times with N_2. Anhydrous CH_3COCH_3 (40mL) were introduced into the reaction flask by syringe. The reaction was stirred under an inert atmosphere at 60℃ for a night. The solvent was extracted in CH_2Cl_2/H_2O mixture (100/50 mL). The organic phase was washed with H_2O (3×100 mL). The organic phases were collected and dried over anhydrous Na_2SO_4, and the solution was evaporated in vacuo. After column chromatography on SiO_2 (CH_2Cl_2-CH_3OH 0% to 2%), **S3** was obtained in 81% yield (524.88 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.64 (dd, J = 4.5, 1.5 Hz, 4H), 7.50 (dd, J = 4.5, 1.5 Hz, 6H), 7.44 – 7.20 (m, 10H), 5.25 (s, 2H), 4.43 (d, J = 24.0 Hz, 4H), 1.46 (s, 9H).

S3 (400 mg, 0.66 mmol) was added to THF at room temperature. Trifluoromethanesulfonic acid (150.51mg, 1.32 mmol) was added to the stirred solution for an hour. Neutralize the solution to neutral by adding 10% sodium hydroxide solution, the solvent was extracted in CH_2Cl_2/H_2O mixture (100/50 mL). The organic phase was washed with H_2O (3×100 mL). The organic phases were collected and dried over anhydrous Na_2SO_4, and the solution was evaporated in vacuo. Donor **D** was obtained in 92% yield (307.01 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.70-8.57 (m, 4H), 7.53-7.17 (m, 16H), 5.24 (s, 2H), 3.79 (d, J = 6.7 Hz, 4H). ^{13}C NMR (126 MHz, acetone-d_6) δ 159.02 (s), 150.09 (s), 141.11 (d, J = 13.6 Hz), 134.99 (s), 130.34 (s), 128.32-127.92 (m), 127.65 (s), 127.48 (s), 126.57 (s), 125.34 (s),

123.76 (s), 119.10 (s), 91.92 (s), 87.15 (s), 70.02 (s), 52.77 (s), 52.46 (s). MALDI-TOF-MS of **D**: m/z calcd for $C_{35}H_{27}N_3O$: 505.62, Found: 506.1.

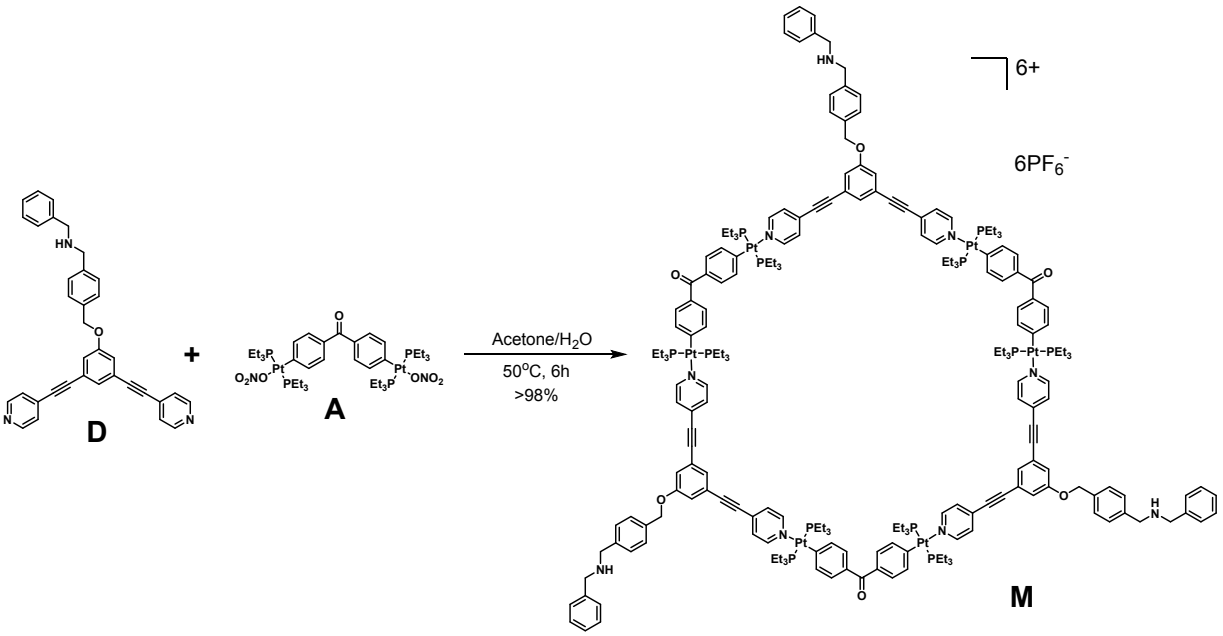
Scheme S2. The synthetic procedure for the crown ether containing **C1**.



To the mixture of crown ether **C2** (580 mg, 1.14 mmol) and compound **S4** (702.1 mg, 1.04 mmol) in dry CH_2Cl_2 (30 mL) was added DMAP (139.3 mg, 1.14 mmol) and EDCI (218.5 mg, 1.14 mmol) under stirring. The mixture was stirred overnight under Ar atmosphere. Next, the mixture was stirred for two hours and poured into 50 mL deionized water then the aqueous phase was extracted with CH_2Cl_2 (3 × 50 ml). The solvent was removed in vacuo to give a colorless liquid, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH = 100/1) to give compound **C3** (606.1 mg, 50 %) as a colorless viscous liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (s, 2H), 7.01-6.79 (m, 6H), 5.21 (s, 2H), 4.58 (d, J = 7.6 Hz, 2H), 4.19-4.08 (m, 6H), 4.04-3.94 (m, 6H), 3.91 (s, 6H), 3.83 (t, J = 7.2 Hz, 6H), 1.84-1.68 (m, 6H), 1.44 (dt, J = 14.6, 7.1 Hz, 6H), 1.38-1.18 (m, 30H), 0.88 (t, J = 6.9 Hz, 9H). To the mixture of crown ether **C3** (300 mg, 0.26 mmol) and compound **S5** (247.9 mg，0.33 mmol) in dry CH_2Cl_2 (30 mL) was added DMAP (31.5 mg, 0.26 mmol) and EDCI (197.4 mg, 0.99 mmol) under stirring. The mixture was stirred overnight under Ar atmosphere. Next, the mixture was stirred for two hours and poured into 50 mL deionized water then the aqueous phase was extracted with CH_2Cl_2 (3 × 50 ml). The solvent was removed in vacuo to give a colorless liquid, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH = 100/1) to give compound **C1** (392.8 mg, 80 %) as a colorless waxy solid. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.34 (s, 2H), 7.29 (s, 2H), 7.10 (s, 2H), 7.01 (d, J = 8.2 Hz, 2H), 6.95 (d, J = 8.1 Hz, 2H), 5.24 (s, 4H), 4.25-4.10 (m, 14H), 4.02 (q, J = 6.4 Hz, 6H), 3.88-3.80 (m, 12H), 3.80-3.71 (m, 10H), 3.66 (dt, J = 8.1, 4.7 Hz, 6H), 3.63-3.51 (m, 24H), 3.48-3.41 (m, 6H), 3.27 (d, J = 3.2 Hz, 8H), 1.86-1.67 (m, 6H), 1.52 (dd, J = 14.9, 7.2 Hz, 6H), 1.29 (s, 47H), 0.88 (dd, J = 7.4, 6.0 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.66, 158.17, 157.79, 154.70- 154.12, 148.20, 147.66, 134.68, 130.20, 126.64, 119.92, 119.26, 114.11, 113.02, 78.07, 77.60,

76.99, 76.15, 75.95-75.22, 74.97-74.59, 74.53, 74.27, 74.04, 71.46, 63.15, 37.01, 35.53, 35.00-34.56, 31.26, 27.69, 18.74.

Scheme S3. The synthetic procedure for the supramolecular [3+3] hexagon **M**.



Synthesis of metallacycles 1. Self-assembly of supramolecular [3+3] hexagon **M** from ligand **D** and diplatinum acceptor **A**. The dipyridyl donor ligand **D** (6 mg, 11.87 μmol) and 120° organoplatinum acceptor **A** (13.85 mg, 11.87 μmol) were weighed accurately into a glass vial. To the vial was added 2.0 mL acetone and 0.4 mL H_2O, and the reaction solution was stirred at 50°C for 6 hours. The PF_6^- salt of **M** was synthesized by dissolving the NO_3^- salt of **M** in acetone/H_2O and adding a saturated aqueous solution of KPF_6 to precipitate the product, which was collected by vacuum filtration. Yield: 18.38 mg, 99%. ^{1}H NMR (500 MHz, d_6-acetone) δ 9.53 (d, J = 5.9 Hz, 4H), 8.38 (d, J = 6.2 Hz, 4H), 8.15 (d, J = 7.8 Hz, 4H), 8.03 (dd, J = 17.3, 9.6 Hz, 6H), 7.98-7.88 (m, 7H), 7.84 (d, J = 7.5 Hz, 2H), 7.77 (t, J = 7.5 Hz, 2H), 7.69 (t, J = 7.3 Hz, 1H), 5.72 (s, 2H), 4.25 (t, J = 21.6 Hz, 4H), 2.11-1.84 (m, 24H), 1.84-1.52 (m, 36H). ^{13}C NMR (126 MHz, d_6-acetone) δ 159.04, 150.10, 141.11, 135.00, 130.34, 128.67-127.74, 127.65, 127.46, 126.56, 125.33, 123.77, 119.11, 91.87, 87.12, 70.03, 52.74, 52.42. ^{31}P NMR (acetone-d_6, 161.9 MHz): δ 14.18 (s). ESI-TOF-MS of **M**: calcd for $[M–4PF_6]^{4+}$: 1233.65,

found: 1233.65; calcd for $[M–5PF_6]^{5+}$: 958.14, found: 958.09.

3. The construction and characterization of metallacycle M.

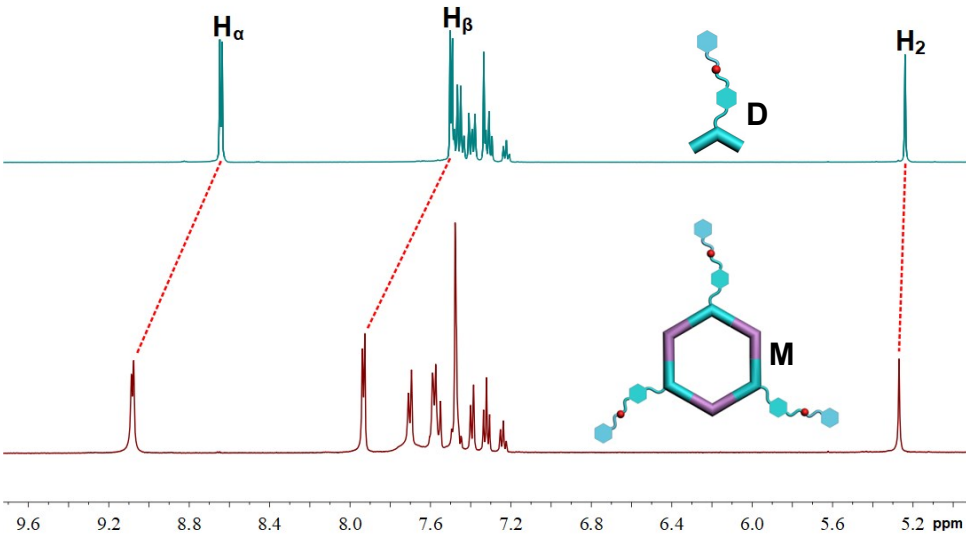


Figure S1. Partial ^{1}H NMR spectra of 120°donor **D** and hexagon metallacycle **M** (500 MHz, 298K) in acetone-d_6.

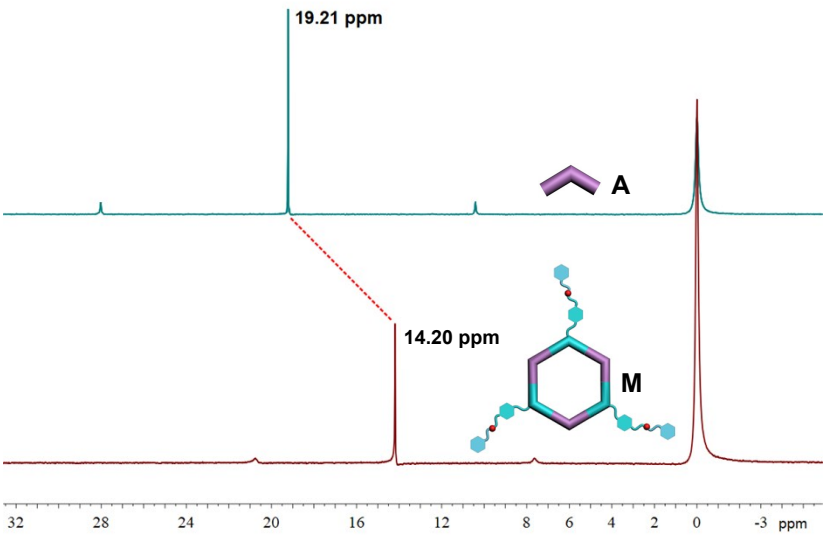
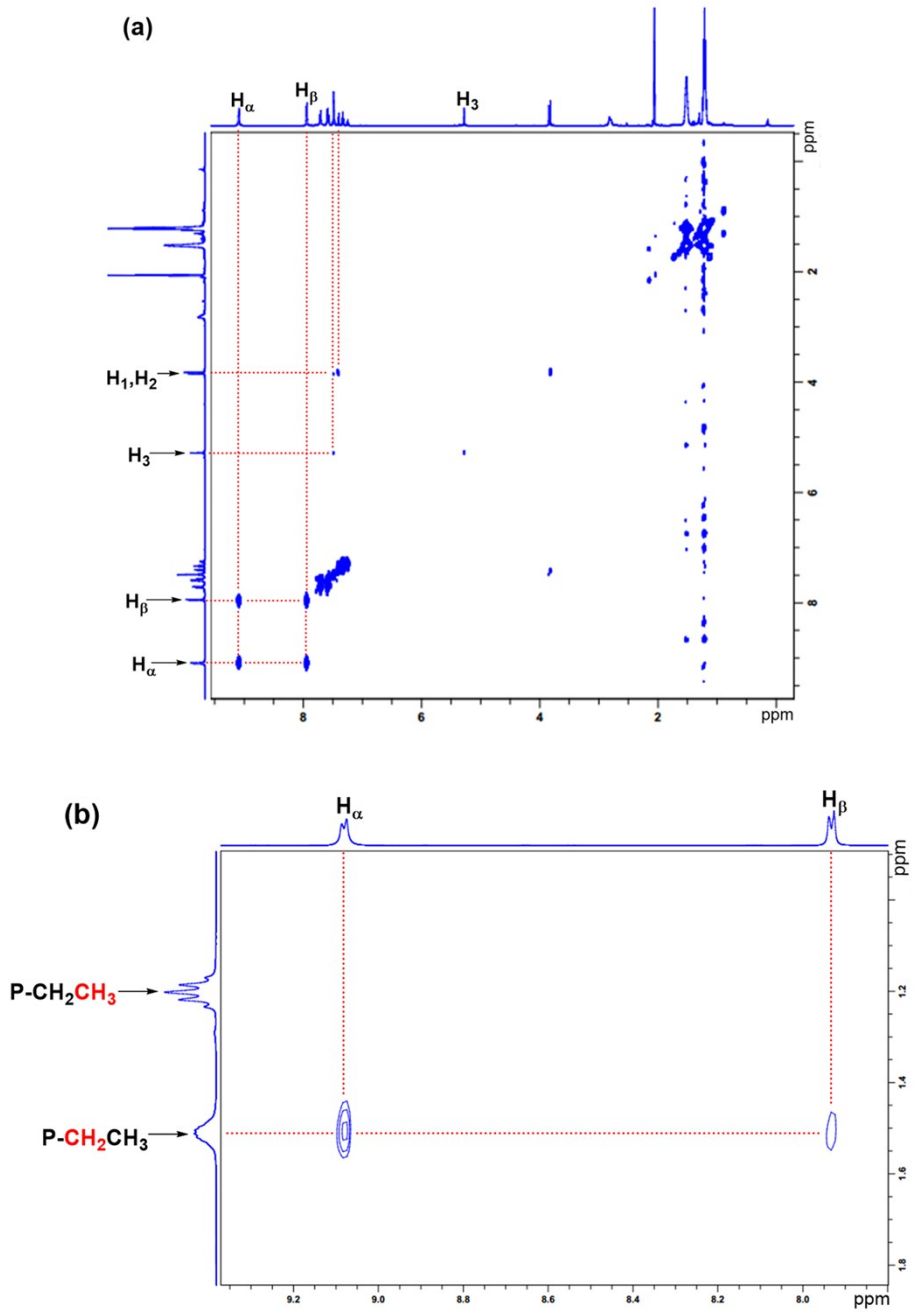


Figure S2. Partial ^{31}P NMR spectra of 120°donor **A** and hexagon metallacycle **M** (500 MHz, 298K) in acetone-d_6.


(a)
Hα
Hβ
H3
H1,H2
H3
Hβ
Hα
ppm
8
6
4
2
(b)
Hα
Hβ
P-CH2CH3
P-CH2CH3
ppm
9.2
9.0
8.8
8.6
8.4
8.2
8.0

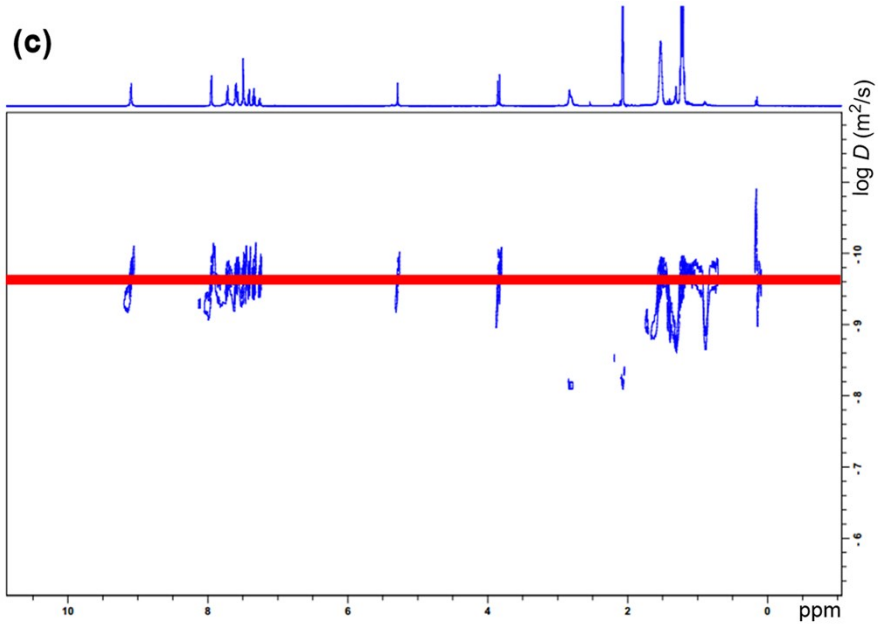
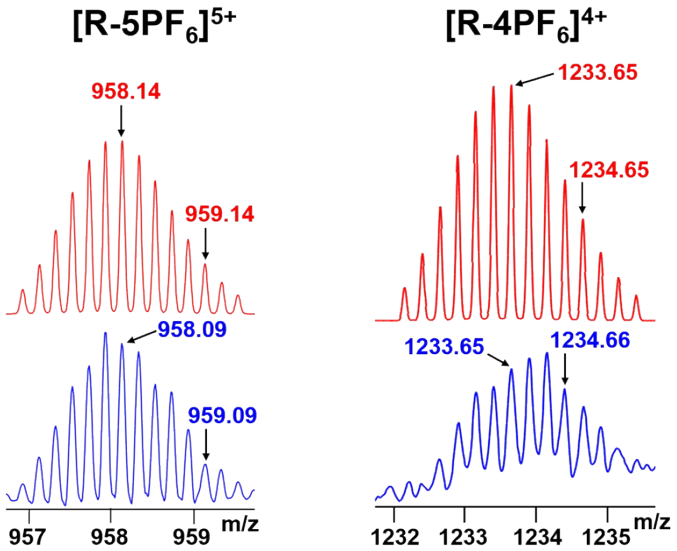


Figure S3. ^{1}H-^{1}H COSY NMR (a), ^{1}H-^{1}H NOESY NMR (b) and 2D DOSY NMR (c) spectra of 1.5 mM hexagonal metallacycles **M** in acetone-d_6 (500 MHz, 298 K).



5 **Figure S4**. Theoretical (top) and experimental (bottom) ESI-TOF-MS spectra for the different charge states (5+ to 4+) observed from of hexagonal metallacycle **M** (PF_6^- as counterion).

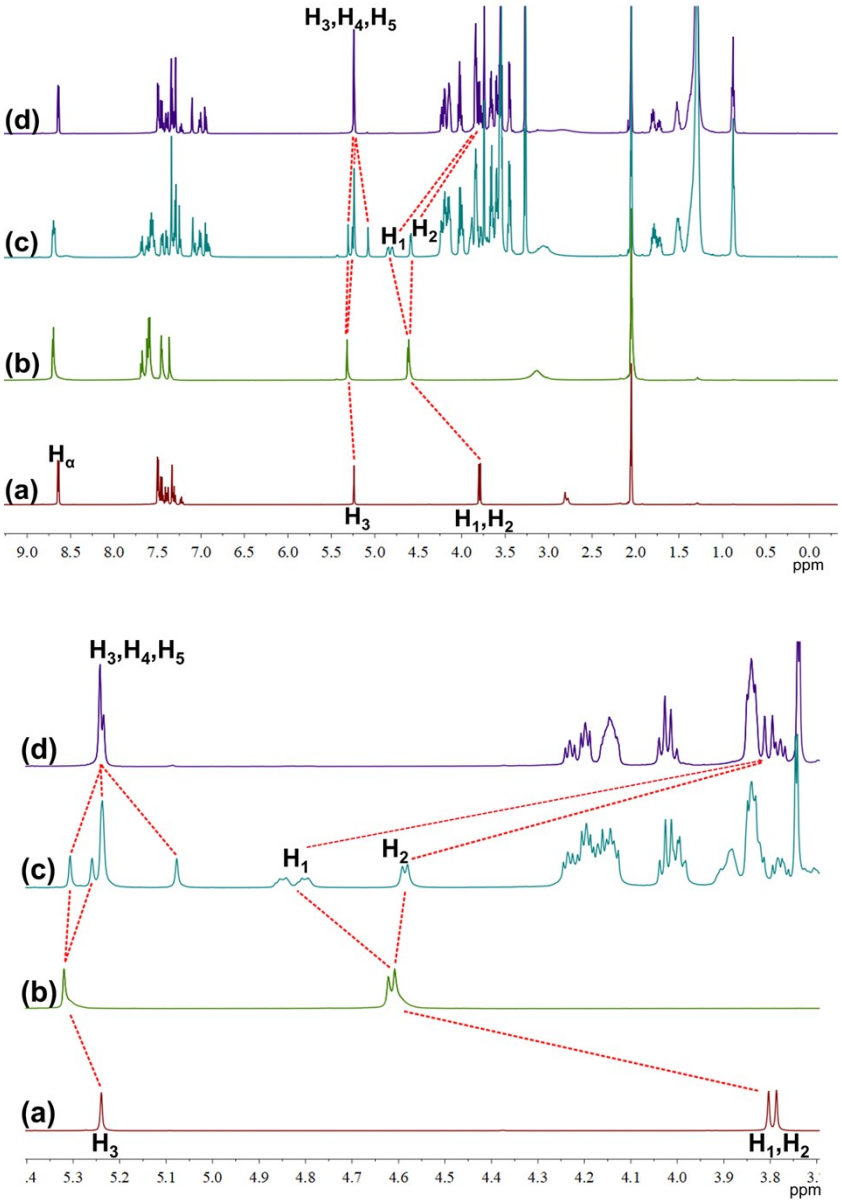


Figure S5. ^{1}H NMR spectra (500 MHz, acetone-d_6, 298K) of (a) **D** (16.0 mM) (b) **D** + 1.0 equiv. TfOH, (c) **D** + 1.0 equiv. TfOH + 1.0 equiv. **C1**, (d) **D** + 1.0 equiv. TfOH +1.0 equiv. **C1** + 2.0 DIEA.

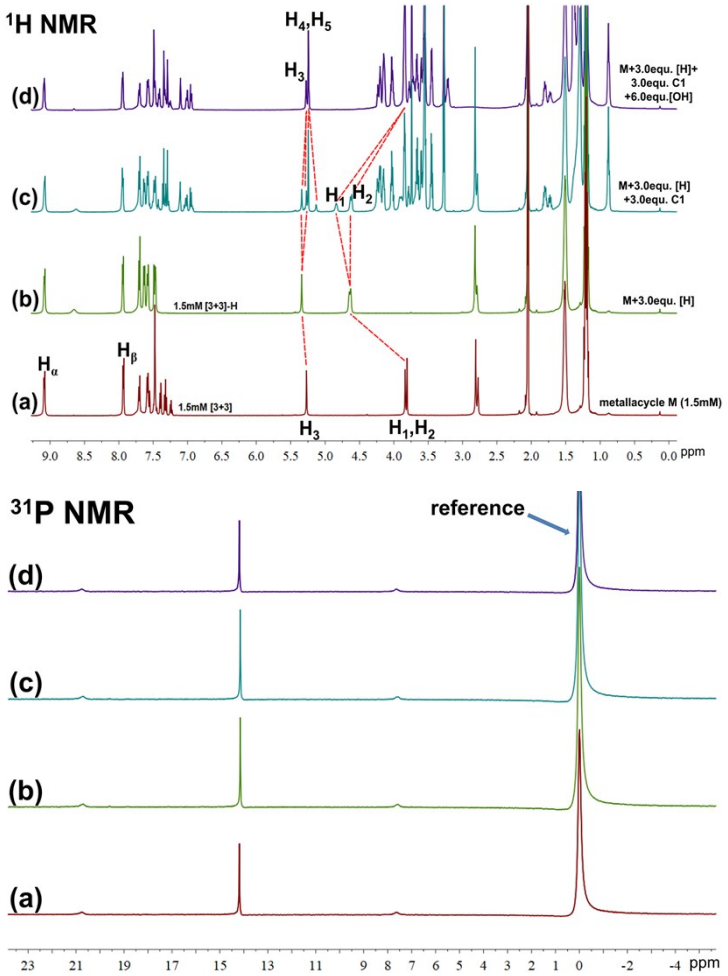


Figure S6. ^{1}H NMR spectra (500 MHz, acetone-d_6, 298K) and ^{31}P NMR spectra of (a) **M** (1.5 mM) (b) **M** + 3.0 equiv. TfOH, (c) **M** + 3.0 equiv. TfOH +3.0equiv. **C1**, (d) **M**+ 3.0 equiv. TfOH +3.0 equiv. **C1**+6.0 DIEA.

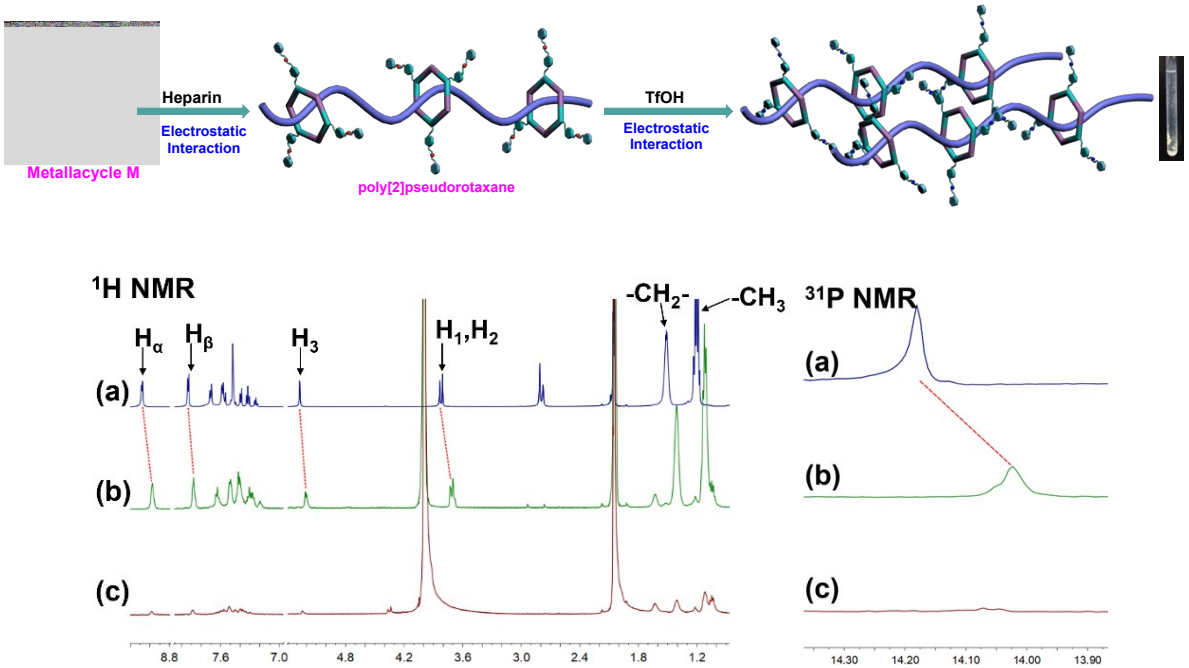


Figure S7. Partial $^{1}H\{^{31}P\}$ NMR spectra of dibenzylamine containing metallacycle **M** (a), **M**+heparin (b), and self-assembly **M** + heparin + 3.0 equiv. TfOH (c) (500 MHz, acetone-d_6 and deuterium oxide 5 (v/v, 5/1), 298 K).

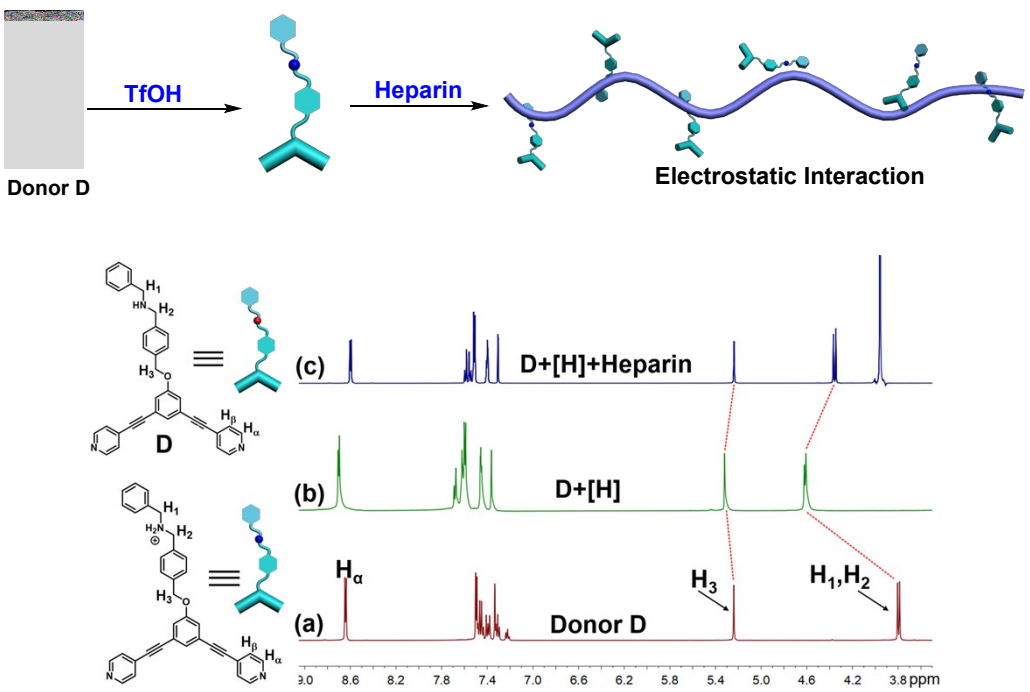


Figure S8. The partial ^{1}H NMR spectra (500 MHz, acetone-d_6 and deuterium oxide (v/v, 3/1), 298 K)

of (a) **D** (16 mM) (b) **D** + 1.0 equiv. TfOH, (c) **D** + 1.0 equiv. TfOH + 0.1 mg heparin.

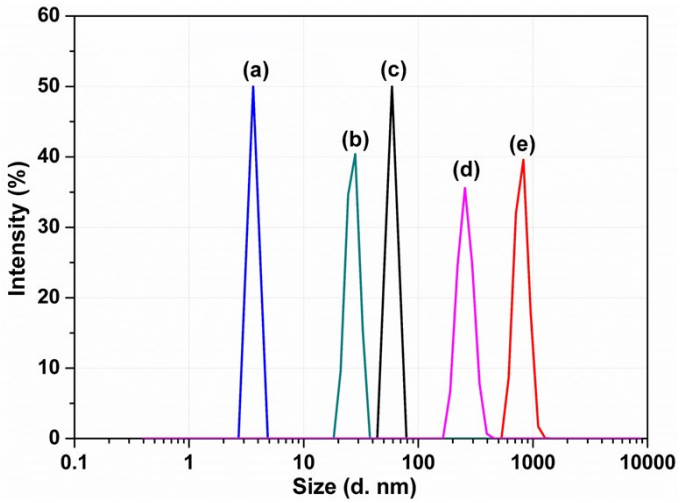


Figure S9. Dynamic Light Scattering: size distributions of 0.03 mM metallacycle **M** (a), heparin (b), 0.03 mM **M** + 3.0 qeuiv. TfOH + 3.0 equiv. **C1** (c), 0.03 mM **M** + heparin (d), 0.03 mM **M**□(heparin)□(**C1**)$_3$ (e).

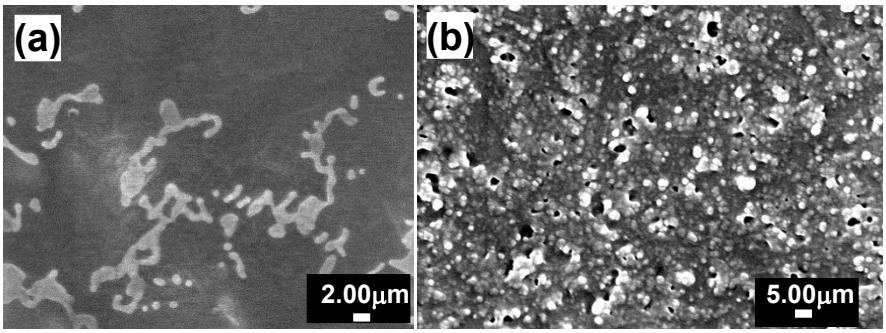


Figure S10. SEM images of building block **D** (0.2 mM) (a) and metallacycle **M** (1.0 mM) (b) prepared in acetone.

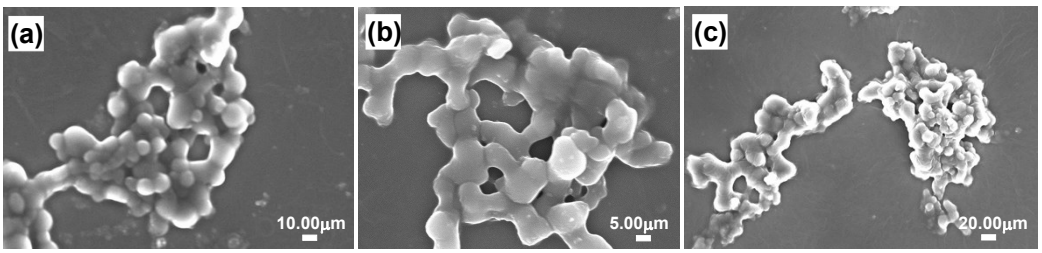
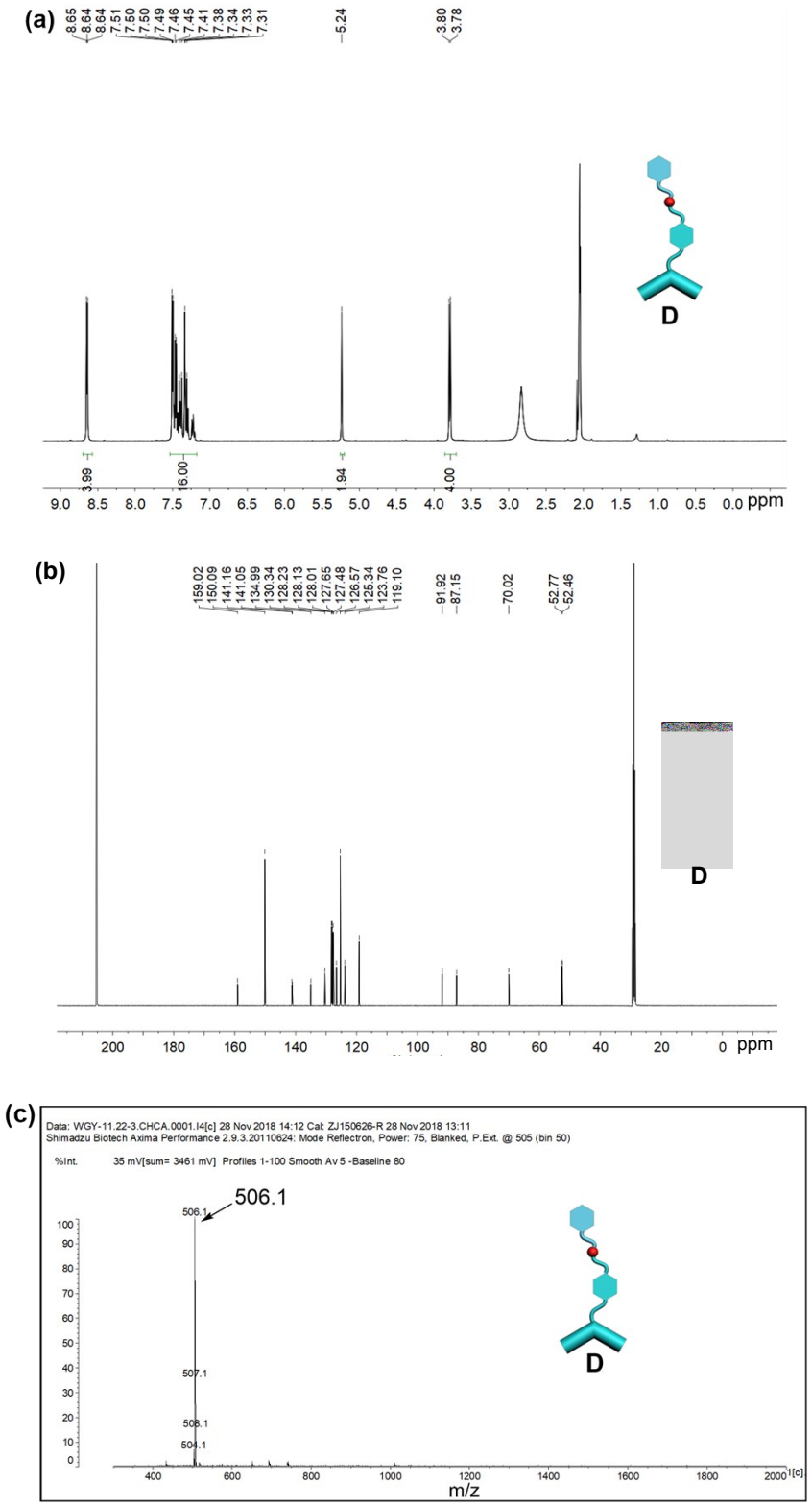


Figure S11. Concentration-dependent SEM images of metallacycle **M** + 3.0 equiv. TfOH + 3.0 equiv. **C1** + Heparin + 6.0 equiv. DIEA + 9.0equ. TfOH: (a) ca. 0.01 mM; (b) ca. 0.02 mM; (c) ca. 0.1 mM.

5. Multiple nuclear NMR (^{1}H and ^{13}C NMR) spectra and MS of new compounds.



5 **Figure S12**. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of the donor **D** in CD_3COCD_3; (c) ESI-TOF-MS of

D: Exact mass calcd. For $C_{35}H_{27}N_3O$: 505.62, Found: 506.1.

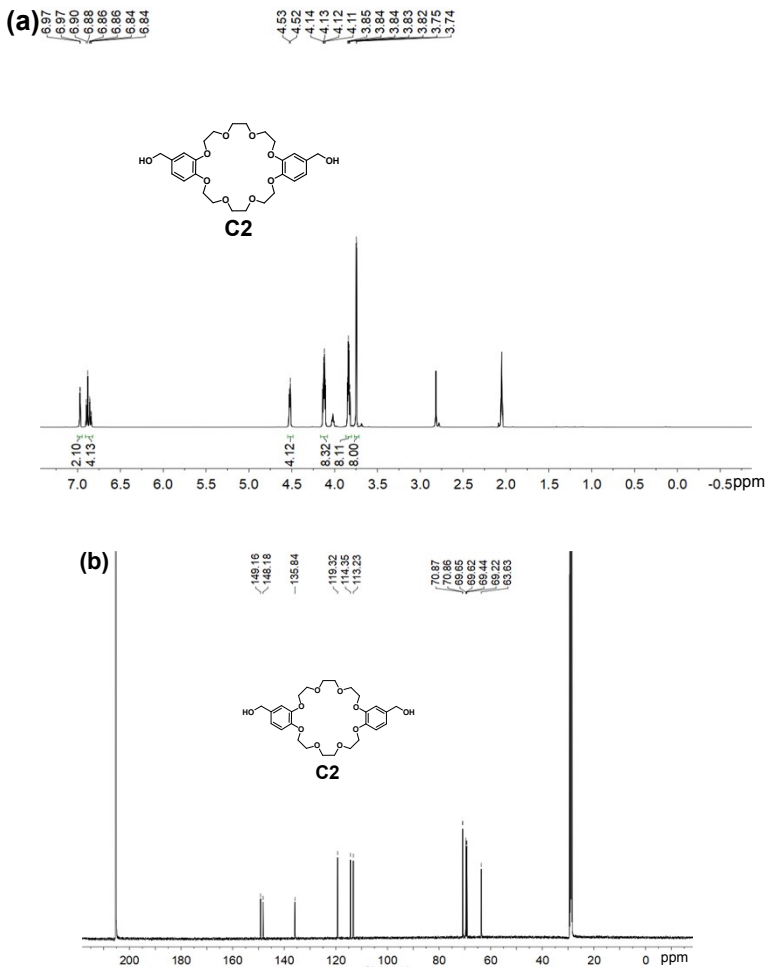
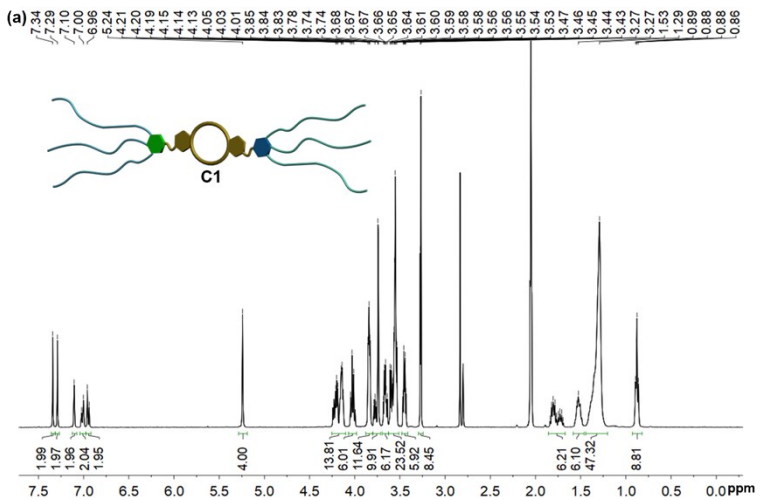


Figure S13. (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of **C2** (500 MHz, acetone-d_6, 298K).



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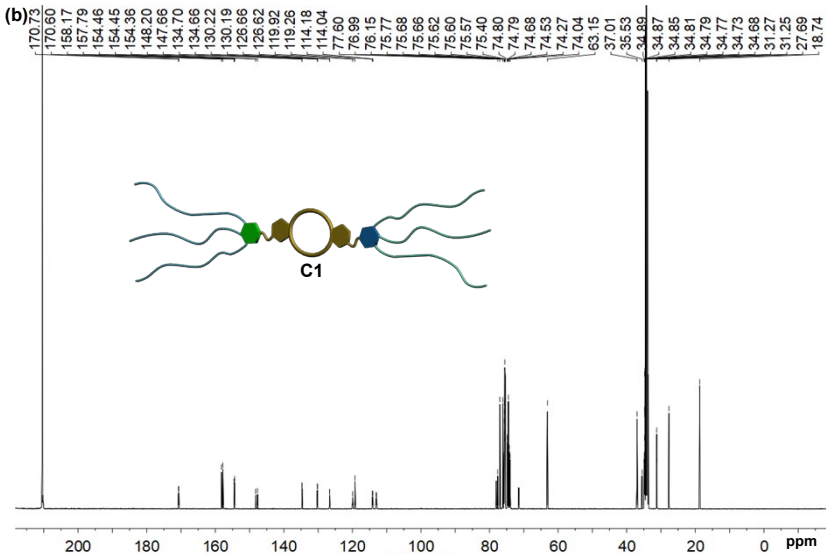
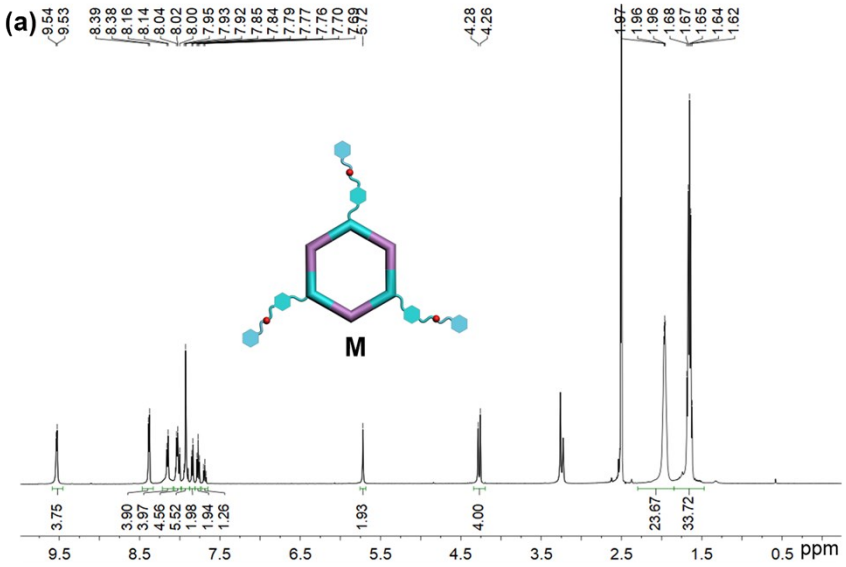


Figure S14. (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of **C1** (500 MHz, acetone-d_6, 298K).



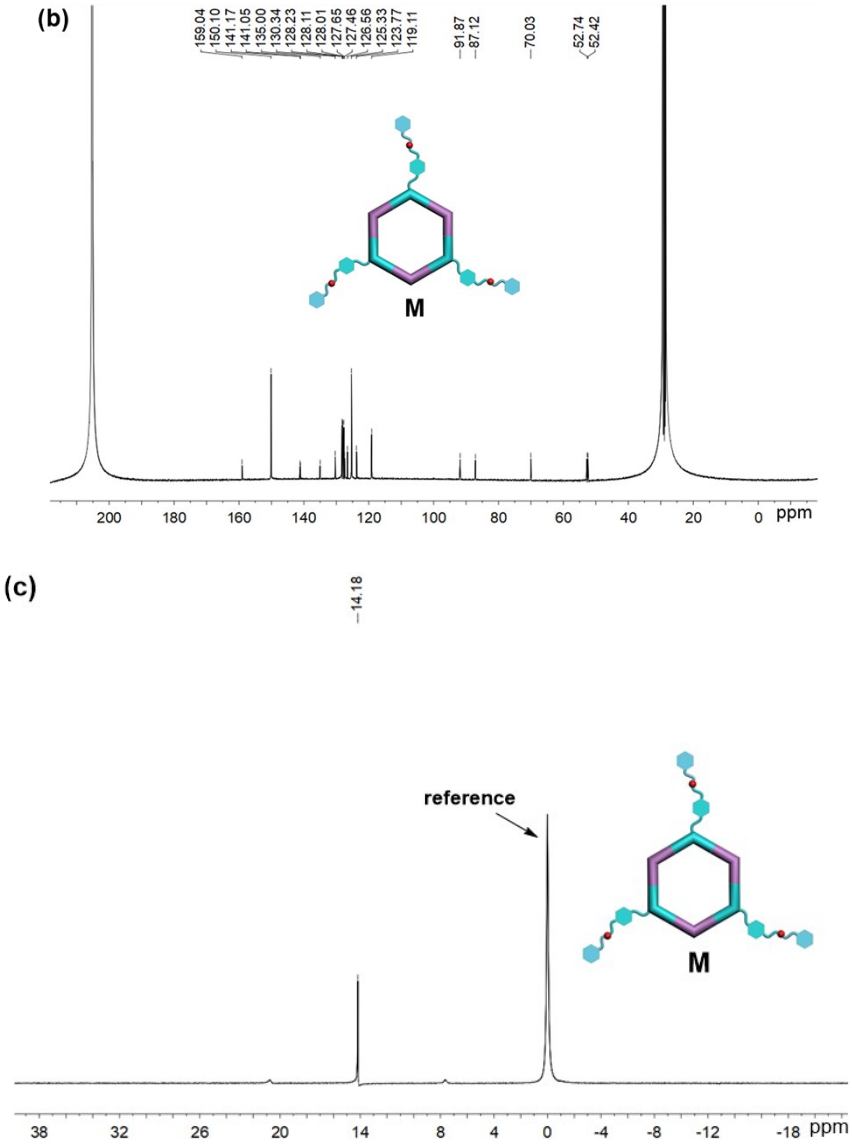


Figure S15. (a) ^{1}H NMR spectrum, (b) ^{13}C NMR spectrum of metallacycle **M** and (c) ^{31}P NMR spectrum (500 MHz, acetone-d_6, 298K).