Synthesis of the first amphiphilic pillar[6]arene and its enzyme-responsive self-assembly in water

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Electronic Supplementary Information (16 pages)

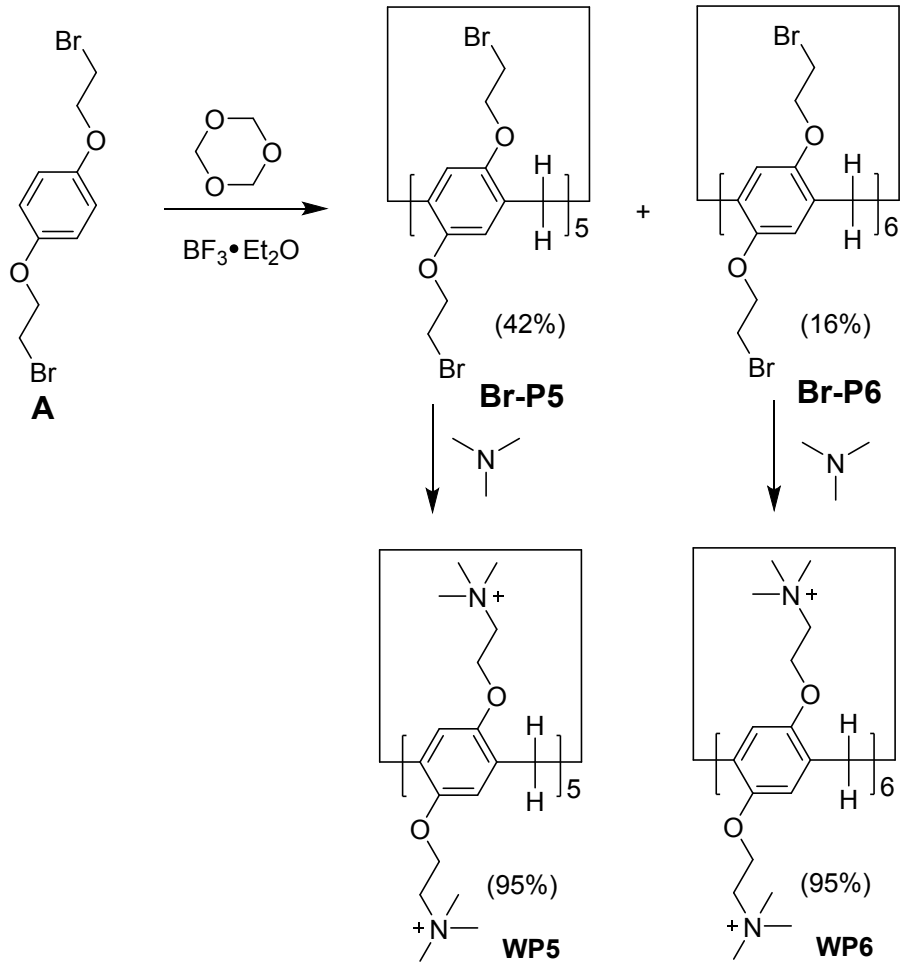
1. Materials and methods

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. ^{1}H NMR and ^{13}C HMR spectra were recorded with a Bruker Avance DMX 400 spectrophotometer using the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. Low-resolution electrospray ionization mass spectra were recorded with a Bruker Esquire 3000 Plus spectrometer. High-resolution mass spectrometry experiments were performed with IonSpec 4.7 Tesla FTMS. Transmission electron microscopy investigations were carried out on a JEM-1200EX instrument. Dynamic light scattering was carried out on a Malvern Nanosizer S instrument at room temperature. UV-Vis spectra were taken on a PerkinElmer Lambda 35 UV-vis spectrophotometer.

*2. Syntheses of **WP5**, **WP6**, and **AP6***

*2.1 Syntheses of **WP5** and **WP6***

*Scheme S1. Syntheses route to **WP5** and **WP6***



WP5 and **WP6** were synthsized according previous report.[S1,S2] By condensation of **A** with boron trifluoride etherate as the catalyst in $ClCH_2CH_2Cl$, bromoethyl substituted pillar[n]arene **Br-P5** and **Br-P6** were synthesized. Then **WP5** and **WP6** were obtained by refluxing a solution of **Br-P5** or **Br-P6** with trimethylamine in toluene. The ^1H NMR spectra of **Br-P5**, **Br-P6**, **WP5**, and **WP6** were shown in Fig. S1–Fig.S4, which were the same as the previous reports.

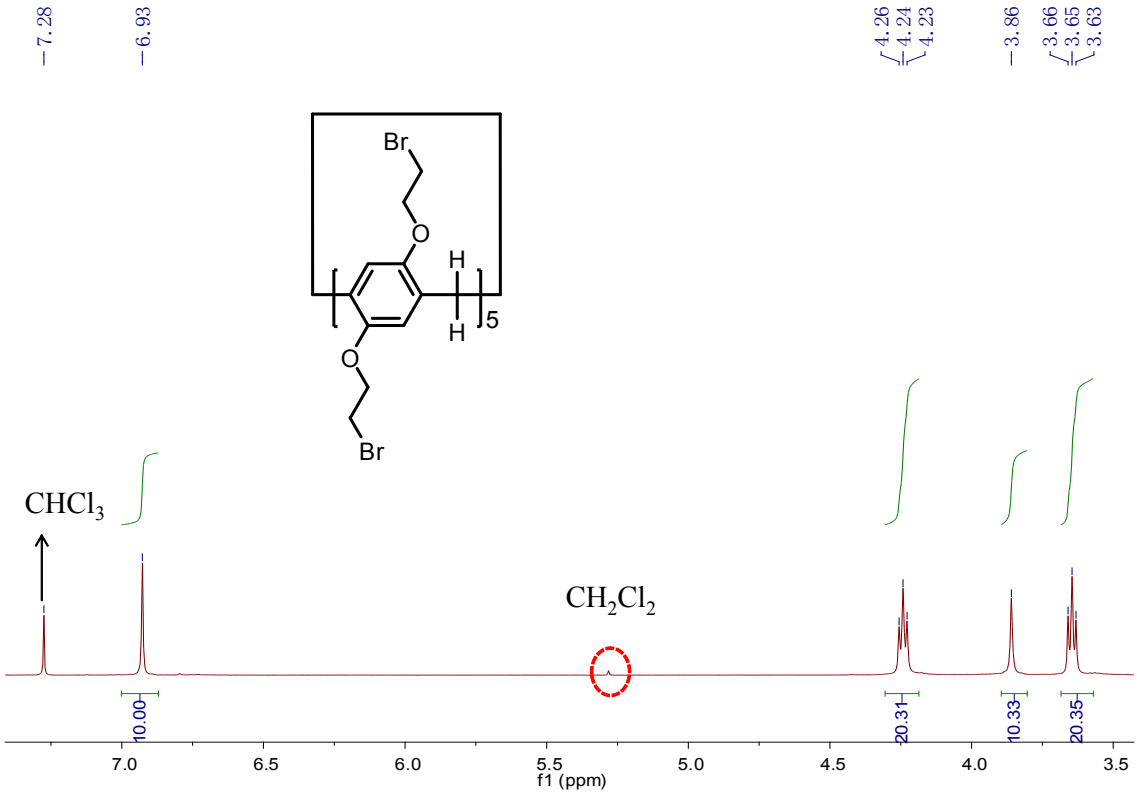


Fig. S1 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, rt) of **Br-P5**.

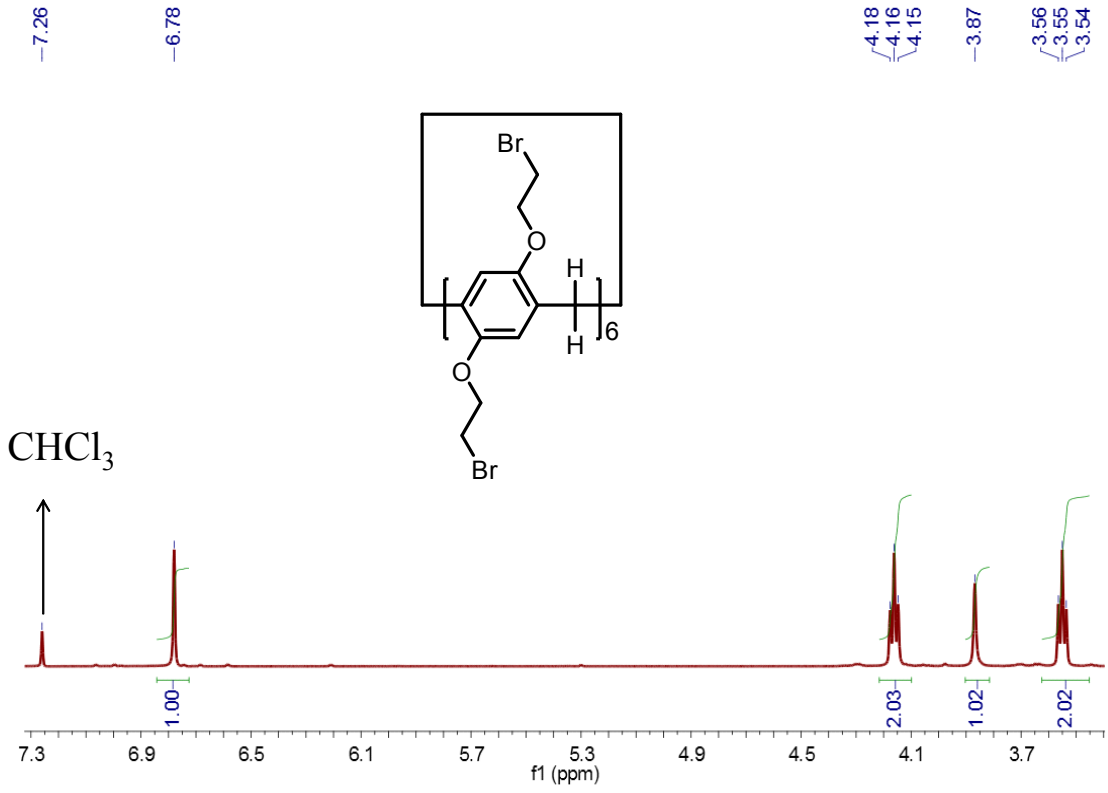


Fig. S2 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, rt) of **Br-P6**.

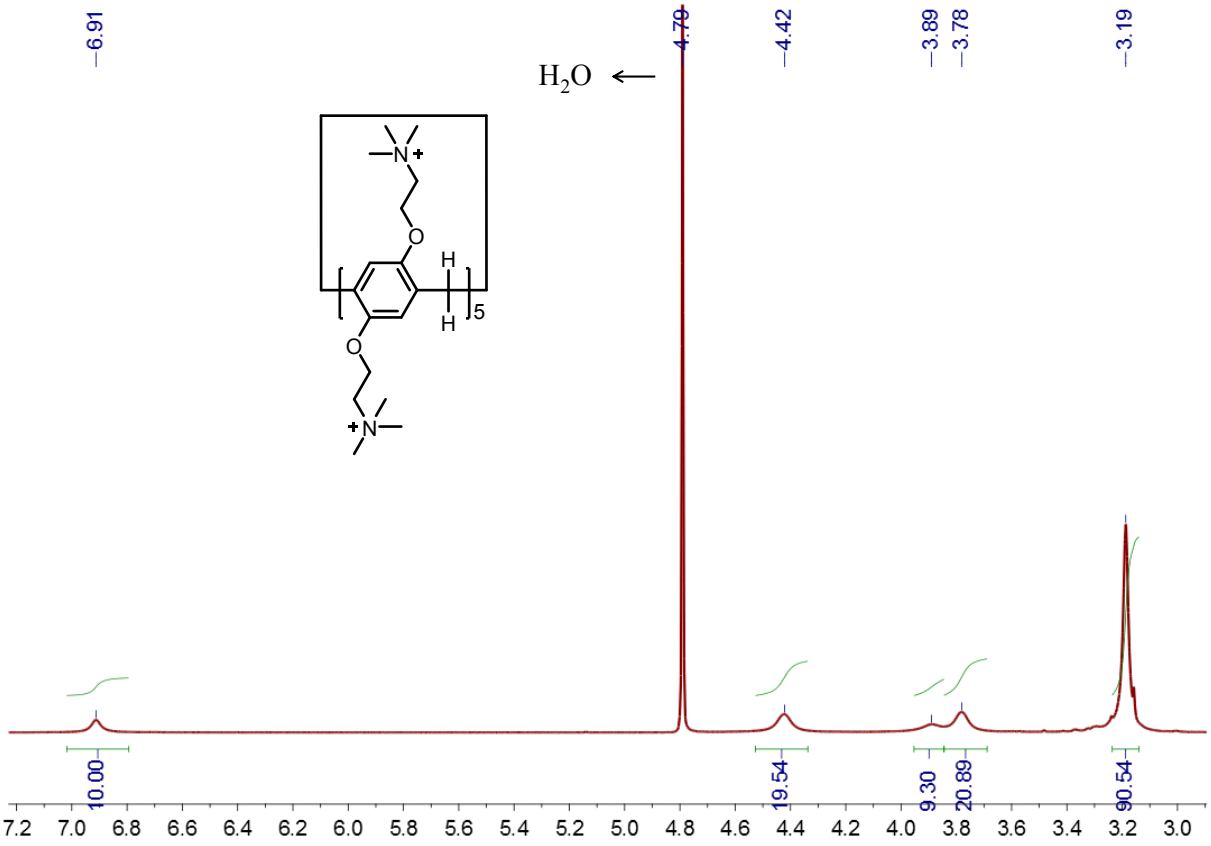


Fig. S3 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, rt) of **WP5**.

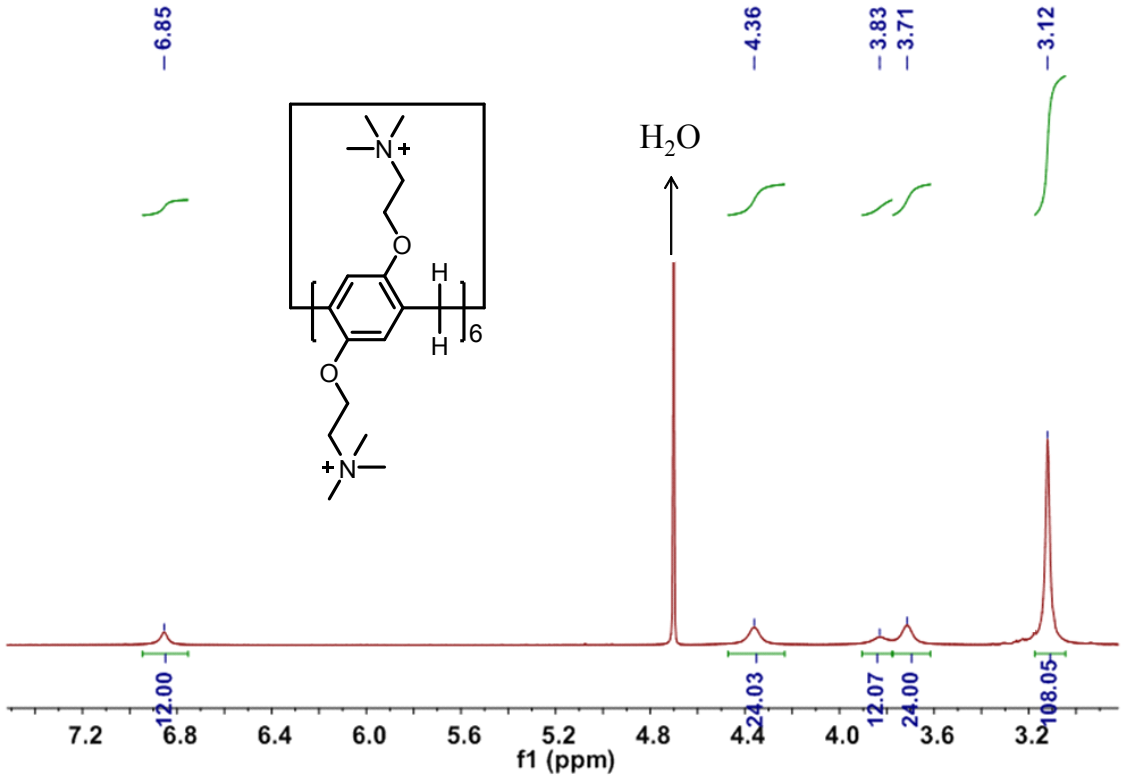
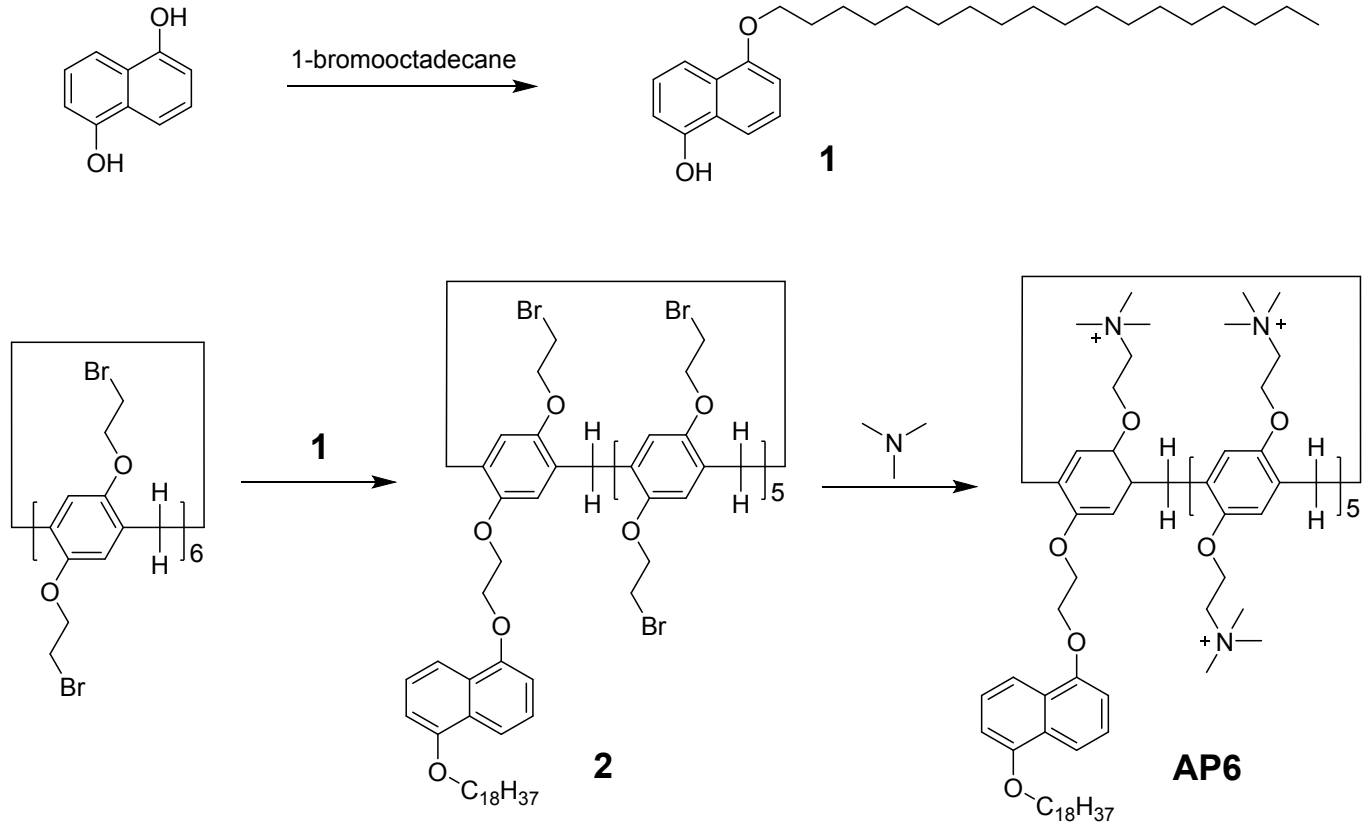


Fig. S4 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, rt) of **WP6**.

*2.2 Synthesis of **AP6***

*Scheme S1. Synthesis route to **AP6***



Anhydrous potassium carbonate (27.6 g, 200 mmol) was added to a solution of 1,5-dihydroxy-naphthalene (16.0 g, 100 mmol) and 1-bromooctadecane (33.3 g, 100 mmol) in dry acetonitrile (500 mL) under vigorous stirring. The mixture was stirred at 80 °C for 24 hours under nitrogen. After removal of the inorganic salt, the solvent was evaporated and the residue was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 100:1) to give **1** as a white solid. The yield of **1** was 75%. The melting point of **1** is 61.0 °C. The ^{1}H NMR spectrum of **1** is shown in Fig. S5. ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) δ (ppm): 7.76 (d, J = 4.0 Hz, 1H), 7.62 (d, J = 4 Hz, 1H), 7.26 (t, J = 6 Hz, 1H), 7.21 (d, J = 6 Hz, 1H), 6.38−6.22 (m, 2H), 5.17 (s, 1H), 4.14 (t, J = 4 Hz, 2H), 1.86−1.84 (m, 2H), 1.56−1.32 (m, 30H), 0.95 (t, J = 4 Hz, 3H).[S3]

Anhydrous potassium carbonate (5.52 g, 40 mmol) was added to a solution of **1** (4.12 g, 10.0 mmol) and **Br-P6** (16.8 g, 8.33 mmol) in dry acetonitrile (250 mL) under vigorous stirring. The mixture was stirred at 80 °C for 24 hours. After removal of the inorganic salt, the solvent was evaporated and the residue was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 10:1) to give the crude product as a white solid. A mixture of the crude product with excess trimethylamine (30 equiv) were dissolved in ethanol and refluxed for 24 h. The solvent was evaporated, and the residue was poured into $CHCl_3$ and stirred. The solution was extracted with water (3 × 100 mL), and the aqueous phase was obtained. The white solid **AP6** was isolated after evaporation of the solution. The melting point of 1 is 135.2 °C. The ^{1}H NMR spectrum of **AP6** is shown in Fig. S6. ^{1}H NMR (400 MHz, DMSO-d_6, 293 K) δ (ppm): 7.94 (t, J = 8 Hz, 2H), 7.40−7.35 (m, 2H), 6.99 (s, 1H), 6.94−6.86 (m, 12H), 6.78 (s, 1H), 4.52 (s, 2H), 4.27 (s, 2H), 4.24−4.13 (m, 22H), 4.12−3.84 (m, 12H), 3.66−3.62 (m, 22H), 3.07 (t, J = 4 Hz, 2H), 2.27 (s, 99H), 1.92−1.91 (m, 2H), 1.26 (s, 32H), 0.89 (t, J = 8 Hz, 3H). The ^{13}C NMR spectrum of **AP6** is shown in Fig. S7. ^{13}C NMR (100 MHz, DMSO, 293 K) δ (ppm): 155.44, 154.62, 153.08, 150.27, 129.95, 128.55, 126.47, 125.47, 124.25, 117.31, 116.98, 115.66, 113.75, 111.40, 109.81, 71.79, 70.10, 69.11, 68.53, 68.12, 68.03, 67.84, 66.44, 64.69, 54.69, 40.70, 31.62, 28.93, 26.46, 22.91, 13.99. LRESIMS is shown in Fig. S8: m/z 1418.3 $[M - 2Br]2^+$. HRESIMS m/z calcd for $[M - 2Br]^{2+}$ $C_{127}H_{214}O_{14}N_{11}Br_9^{2+}$, 1418.4481; found 1418.4479; error –0.14 ppm.

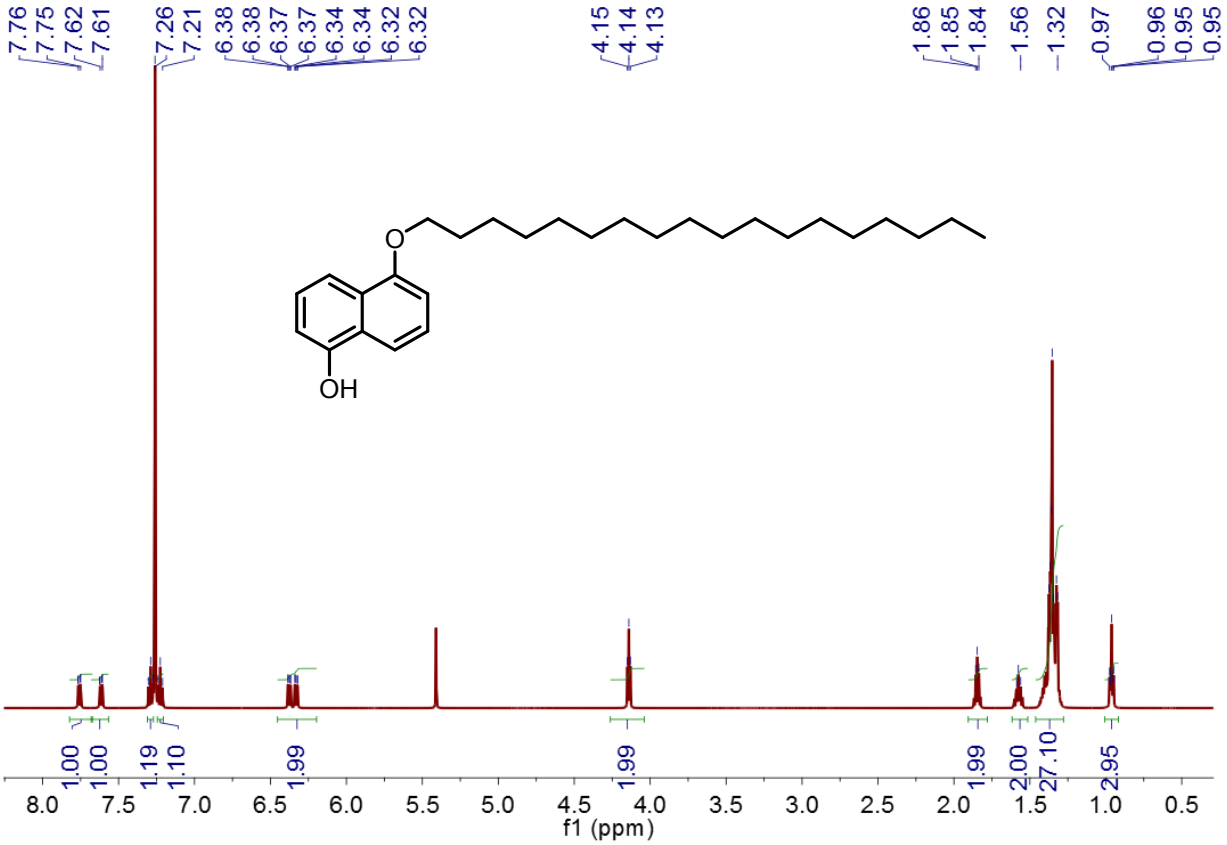


Fig. S5 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, rt) of **1**

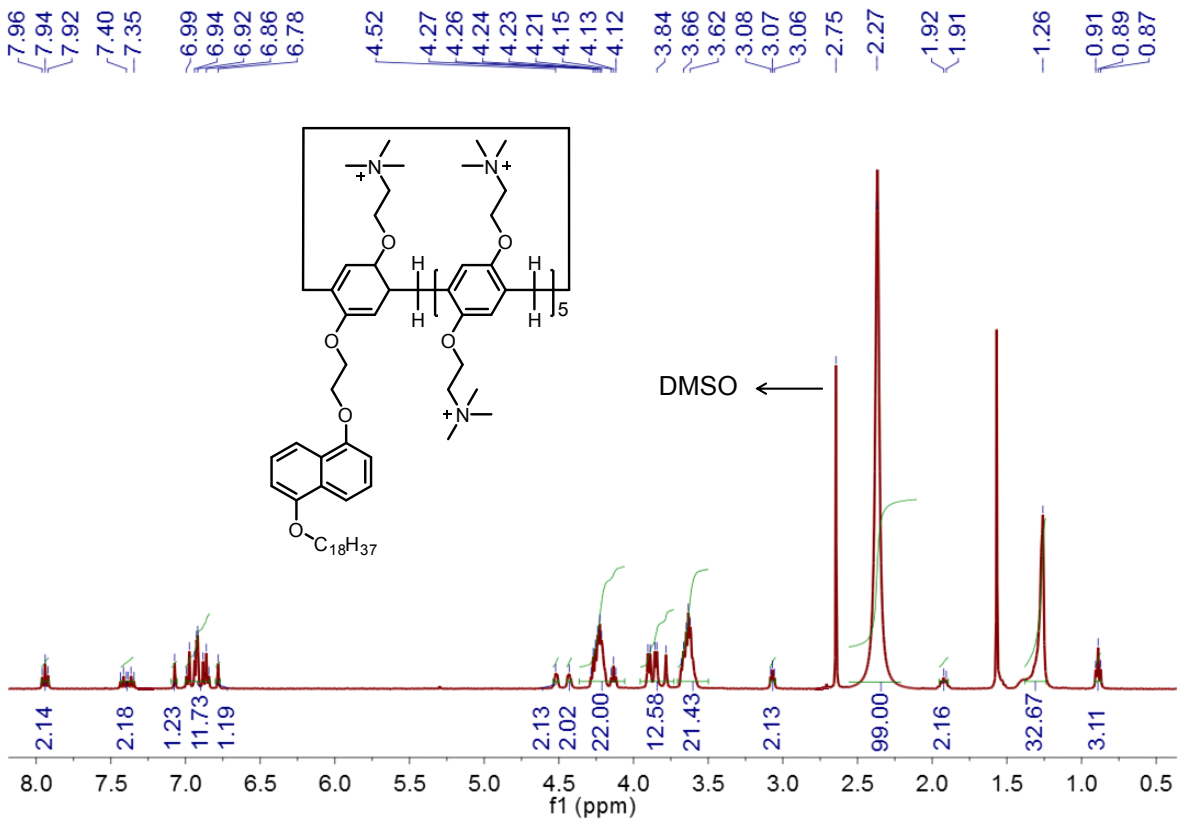


Fig. S6 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, rt) of **AP6**

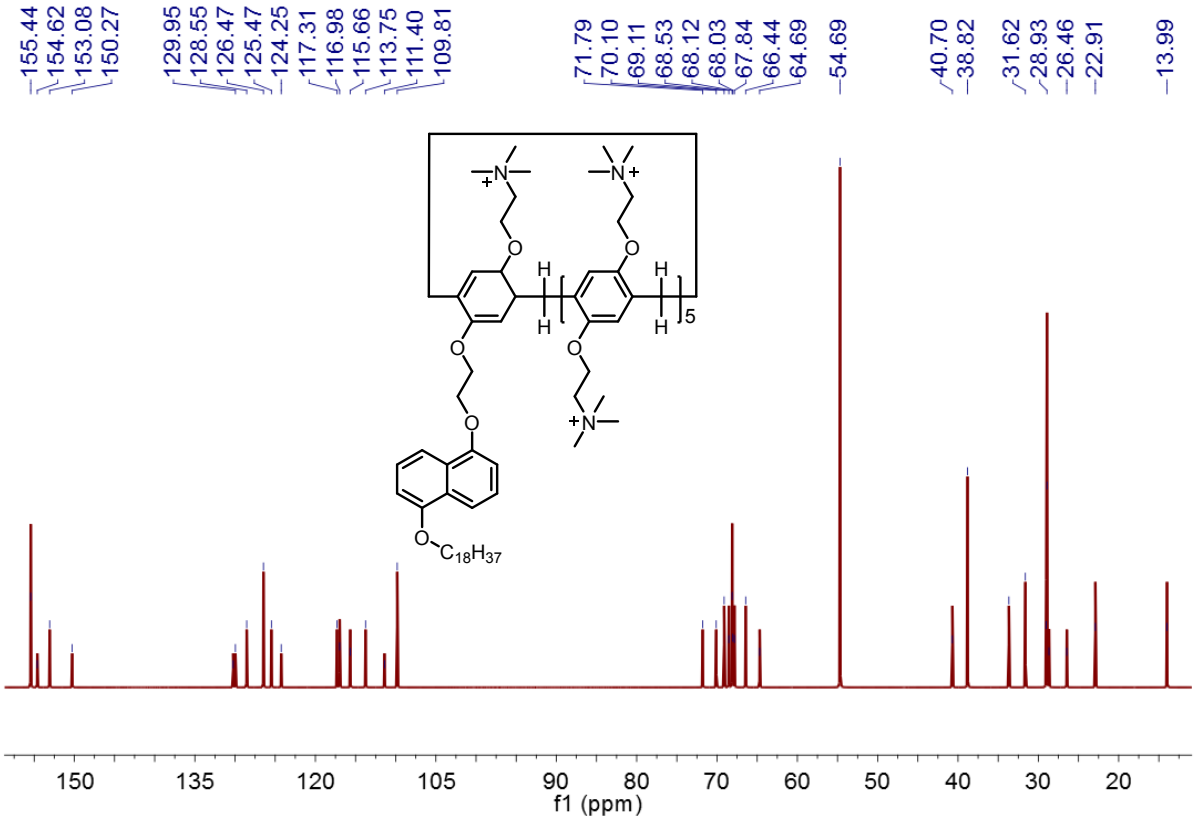


Fig. S7 ^{13}C NMR spectrum (100 MHz, DMSO, rt) of **AP6**

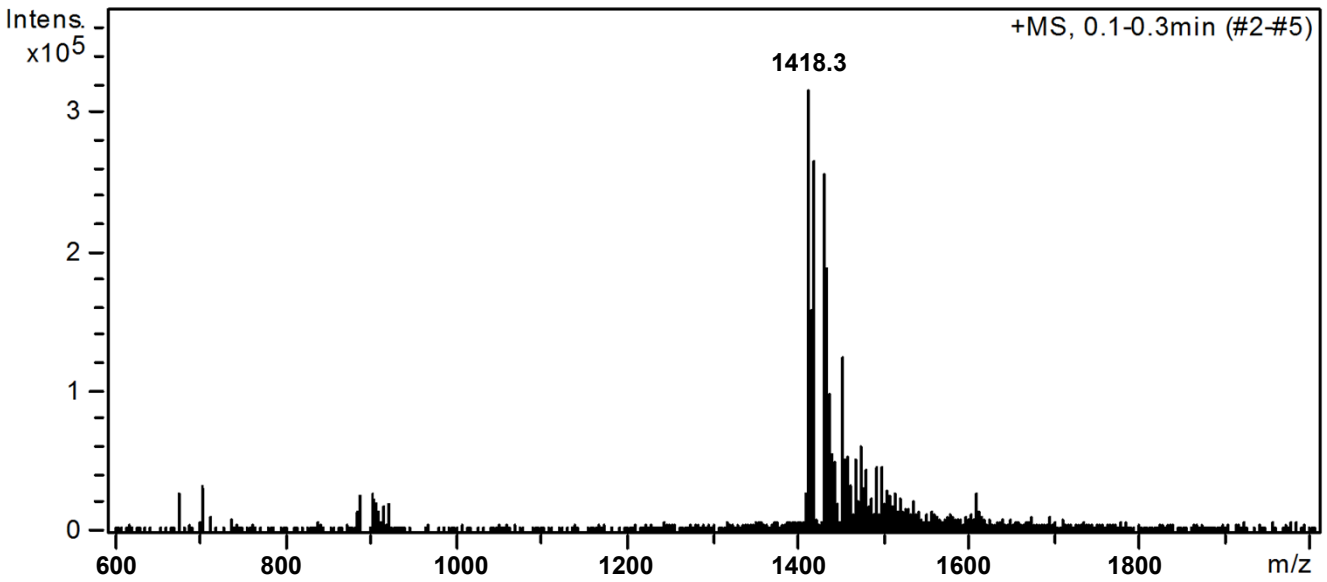


Fig. S8 Electrospray ionization mass spectrum of **AP6**. Assignment of the main peak: *m*/*z* 1418.3 $[M - 2Br]^{2+}$.

*3. Self-assembly of **AP6** in water*

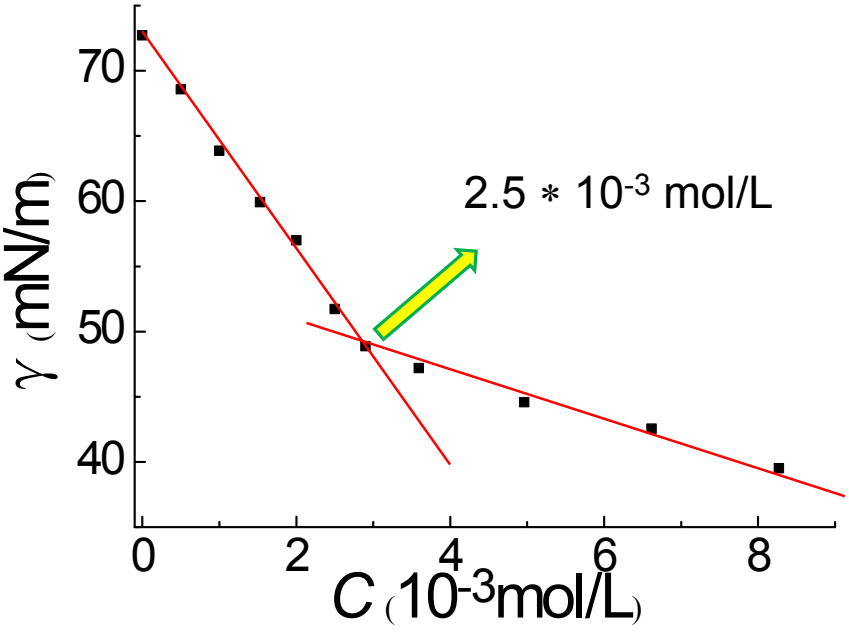


Fig. S9 CAC value of the **AP6** in water

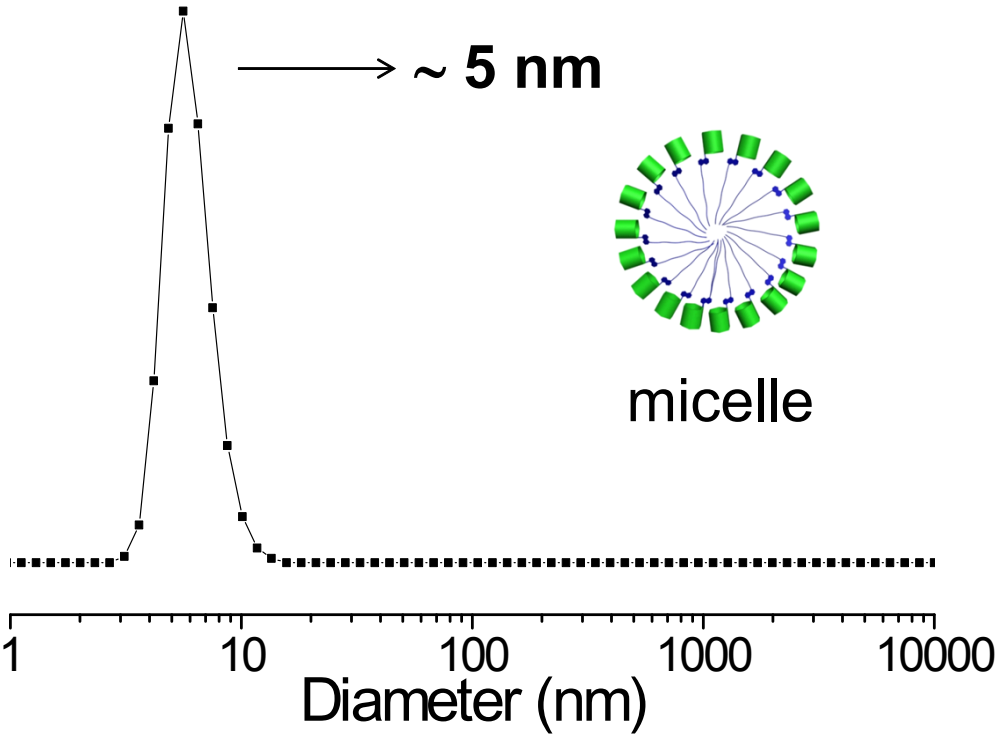


Fig. S10 DLS study of **AP6** in water. (The concentration of **AP6** was 3.00×10^{-3} mol/L)

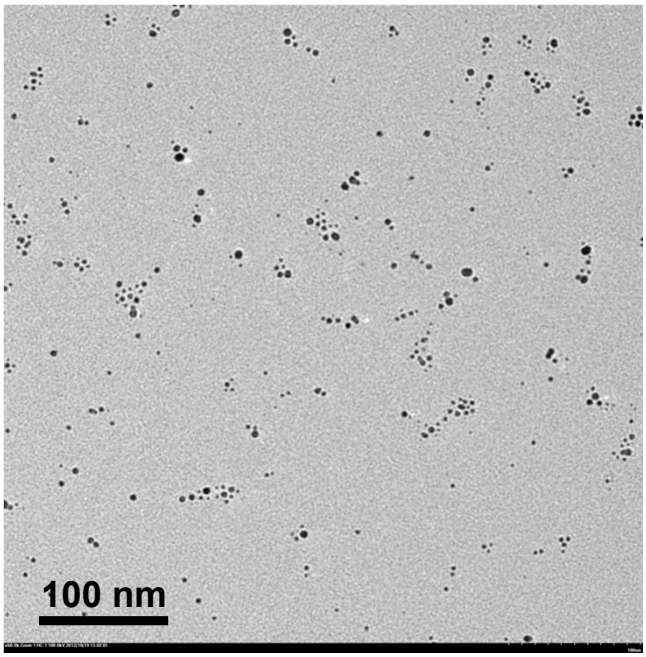


Fig. S11 TEM image of **AP6** self-assembly in water.

*4. Host-guest interaction between **ATP** and **WP6***

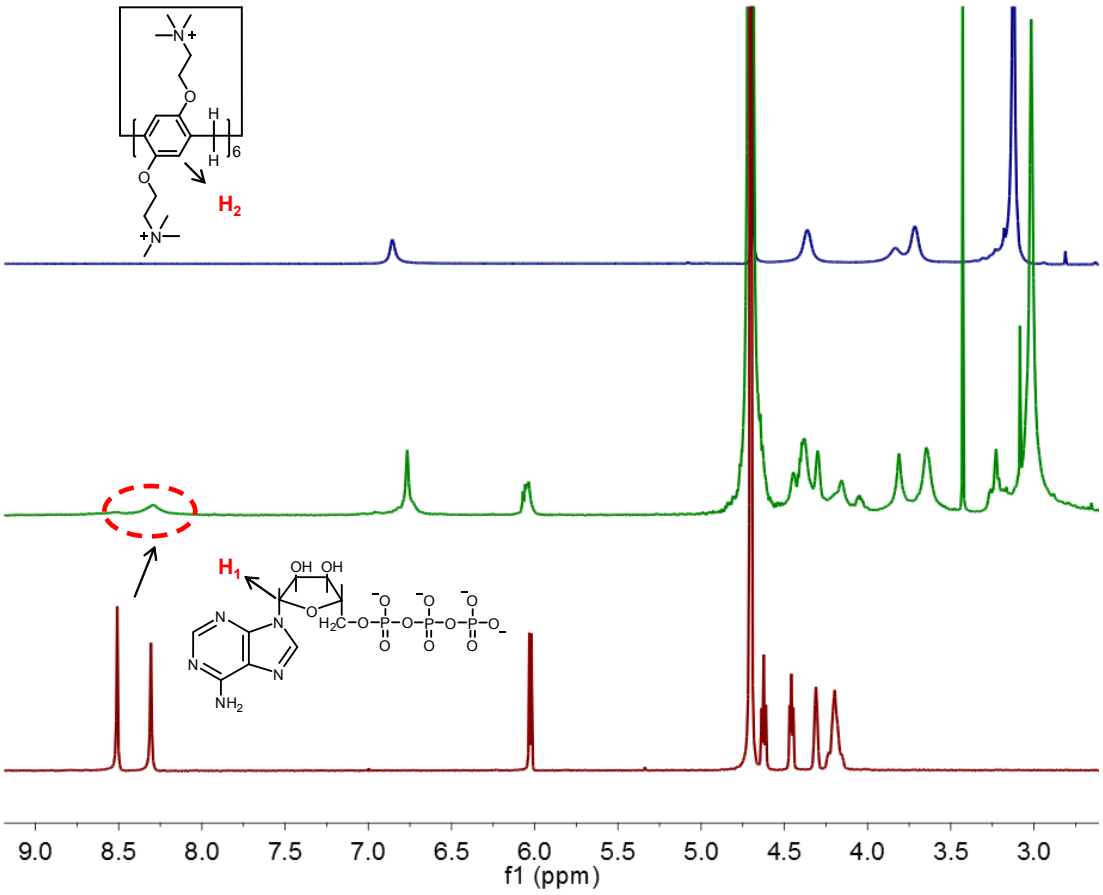


Fig. S12 ^{1}H NMR spectra (400 MHz, D_2O) of **WP6**, **WP6⊃ATP**, and **ATP**.

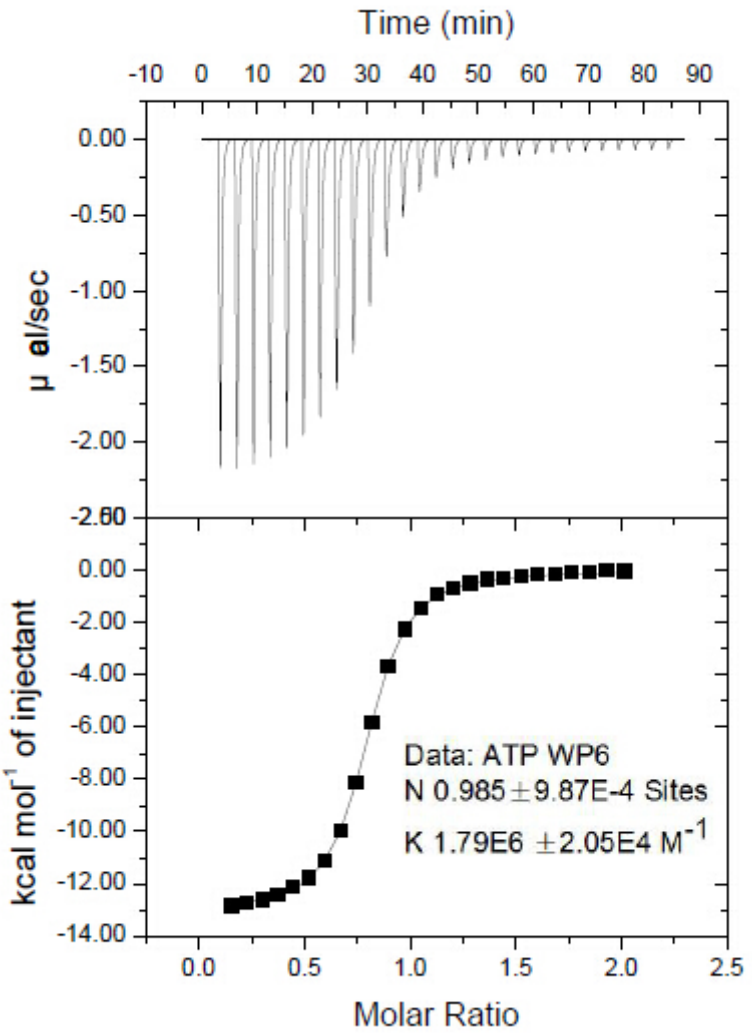


Fig. S13 ITC studies of **WP6⊃ATP**.

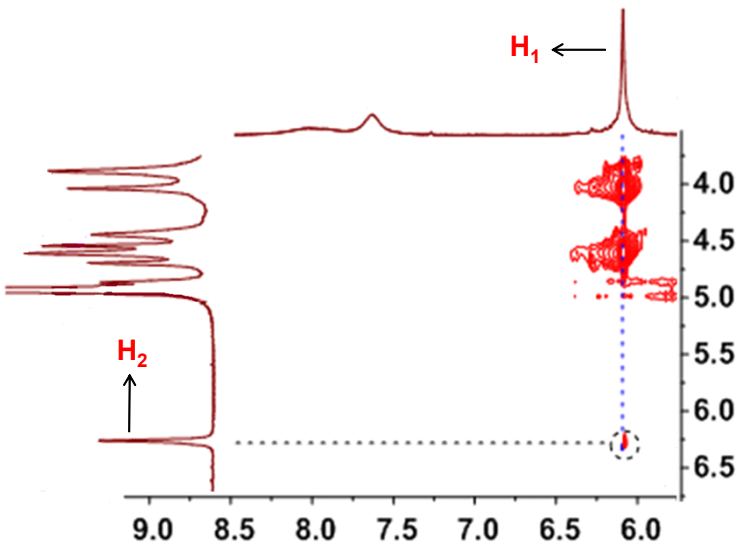


Fig. S14 2D NOESY NMR spectrum of **WP6⊃ATP**.

*5. Self-assembly of **AP6⊃ATP** in water*

Fig. S15 DLS study of **AP6** in water. (The concentration of **AP6** was 5.00×10^{-5} mol/L)

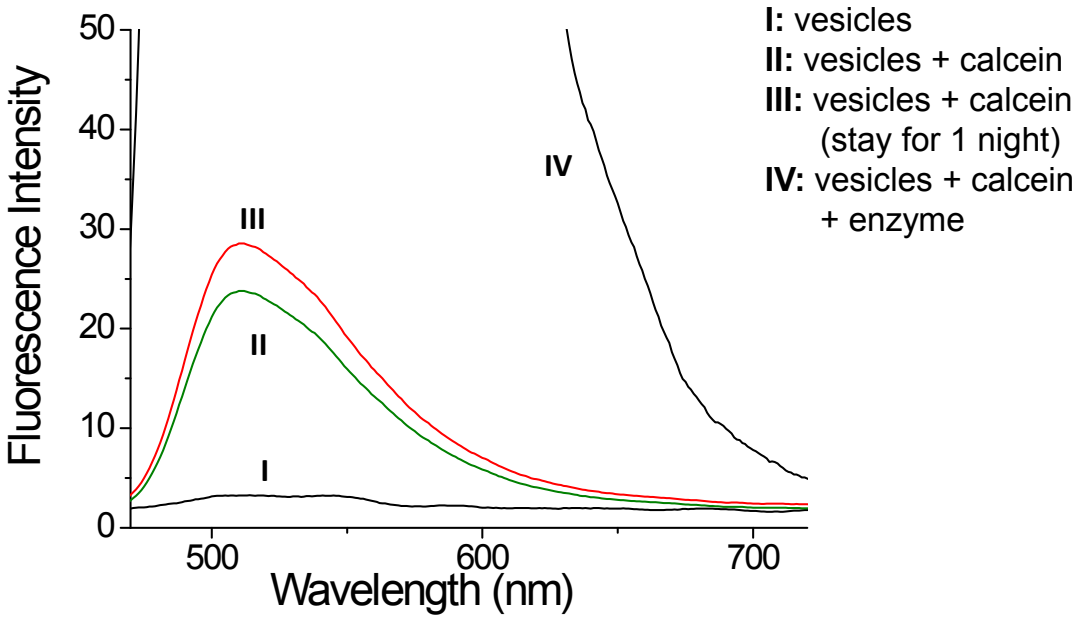


Fig. S16 Enlarged picture of Fig. 3b. (The fluorescence intensity of **AP6** vesicles was very week, when calcein was encapsulated into the vesicles, the fluorescence intensity become a litter strong, after 1 night, the fluorescence intensity was similar to 1 night before, which indicate the vesicles are stable, however, after adding anzyme, the fluorescence intensity become very strong, which indicate that calcein released from the vesicles.)

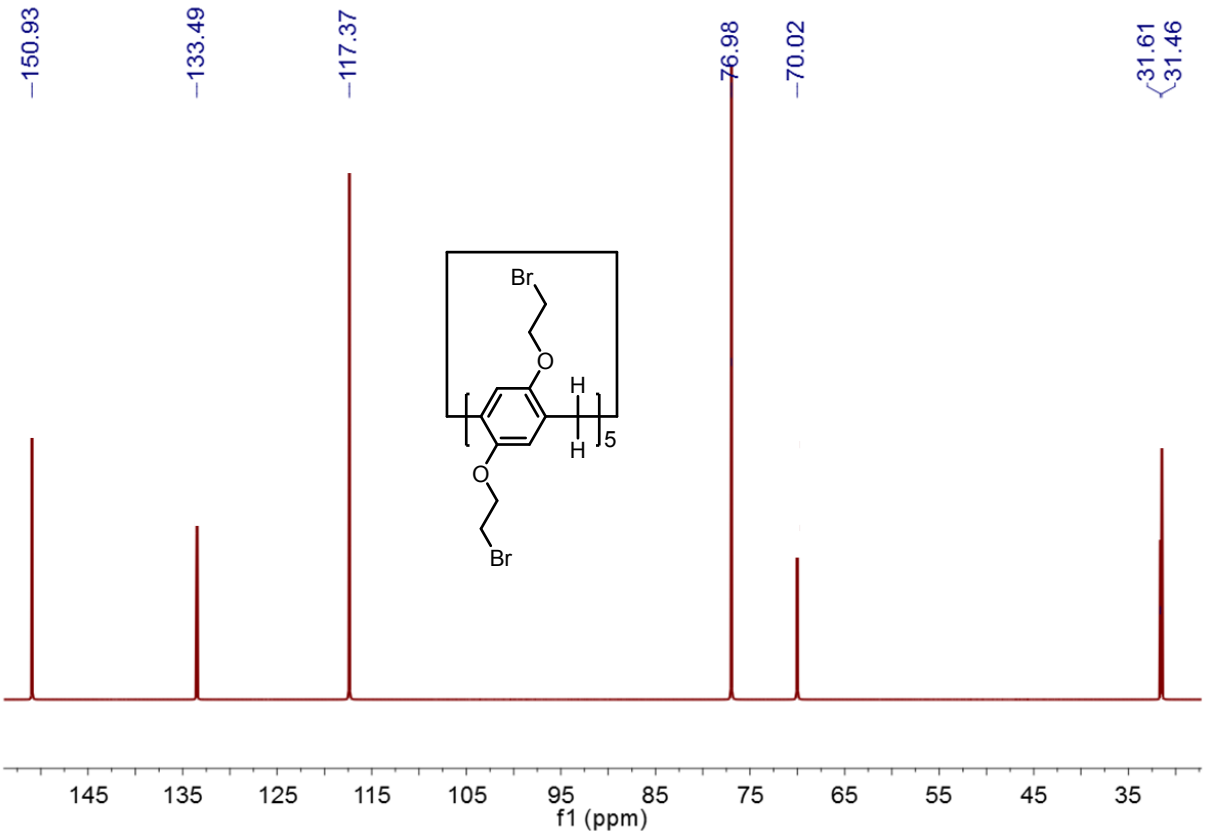


Fig. S17 ^{13}C NMR spectrum (100 MHz, DMSO, rt) of **Br-P5**.

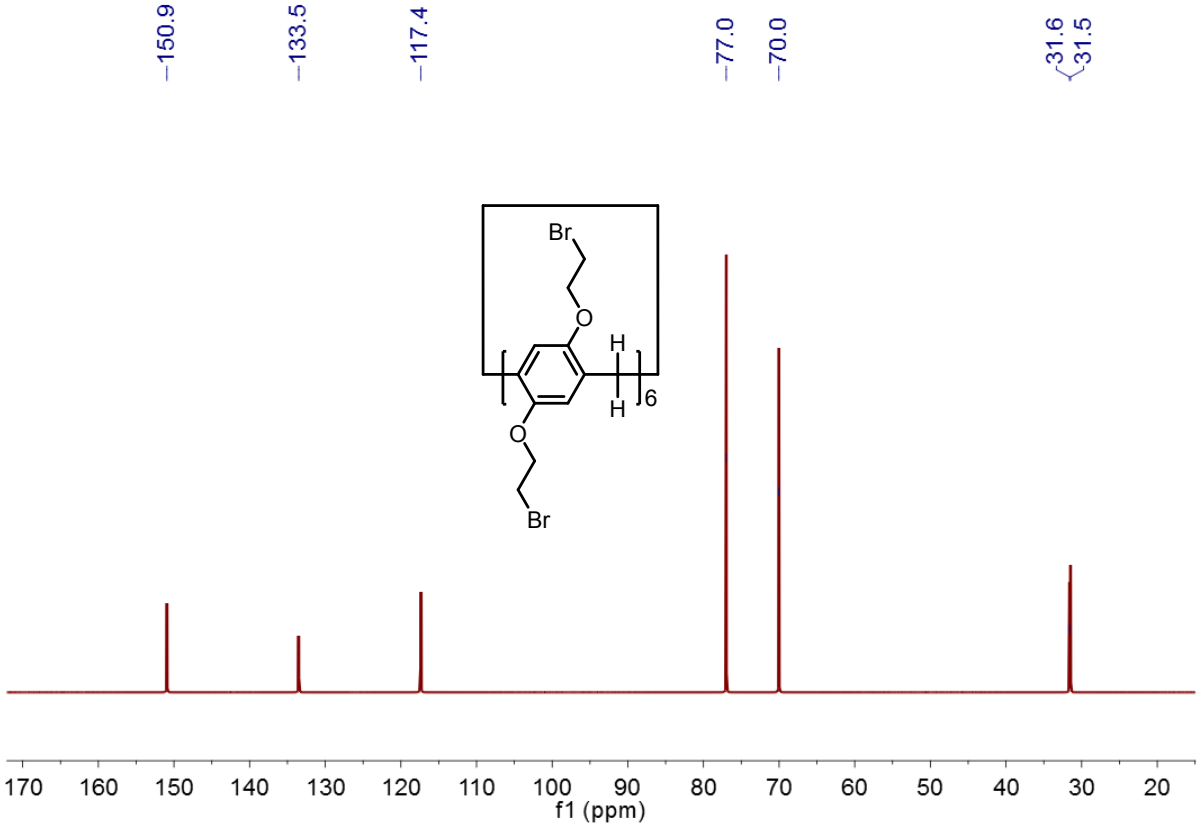


Fig. S18 ^{13}C NMR spectrum (100 MHz, DMSO, rt) of **Br-P6**.

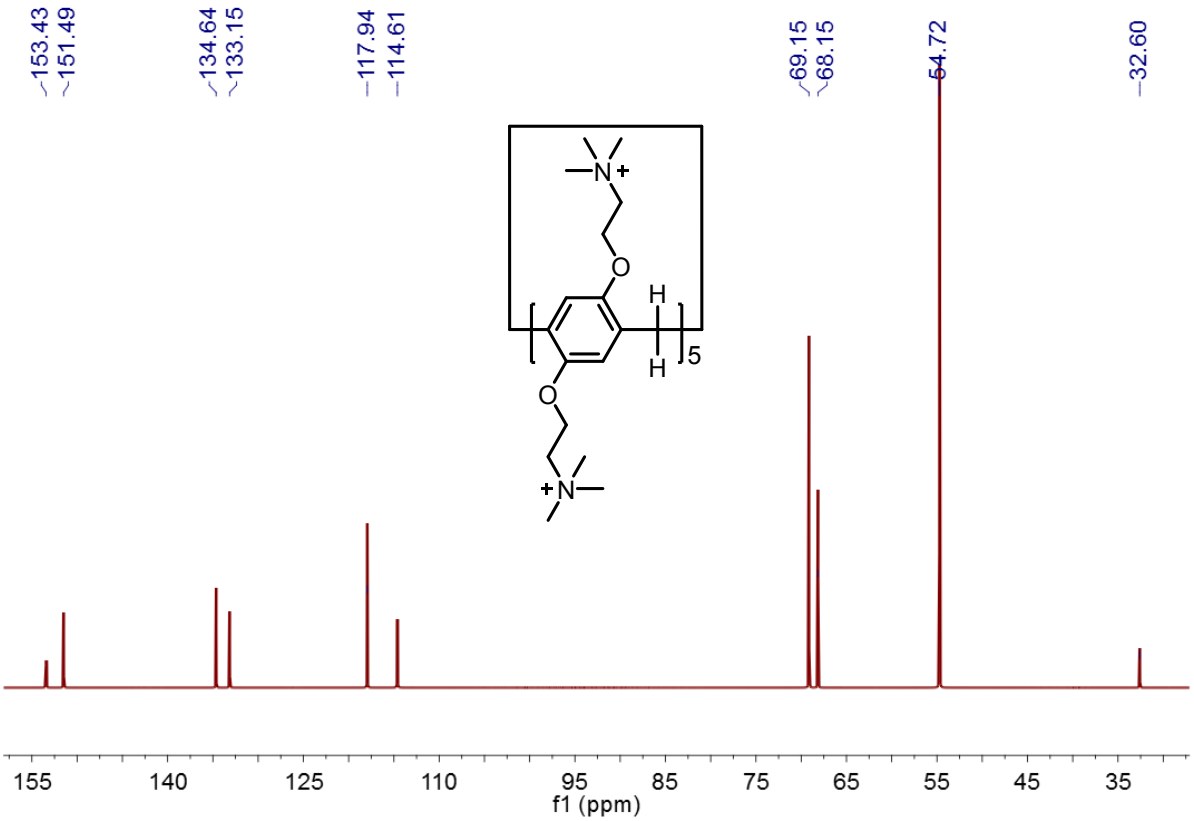


Fig. S19 ^{13}C NMR spectrum (100 MHz, DMSO, rt) of **WP5**.

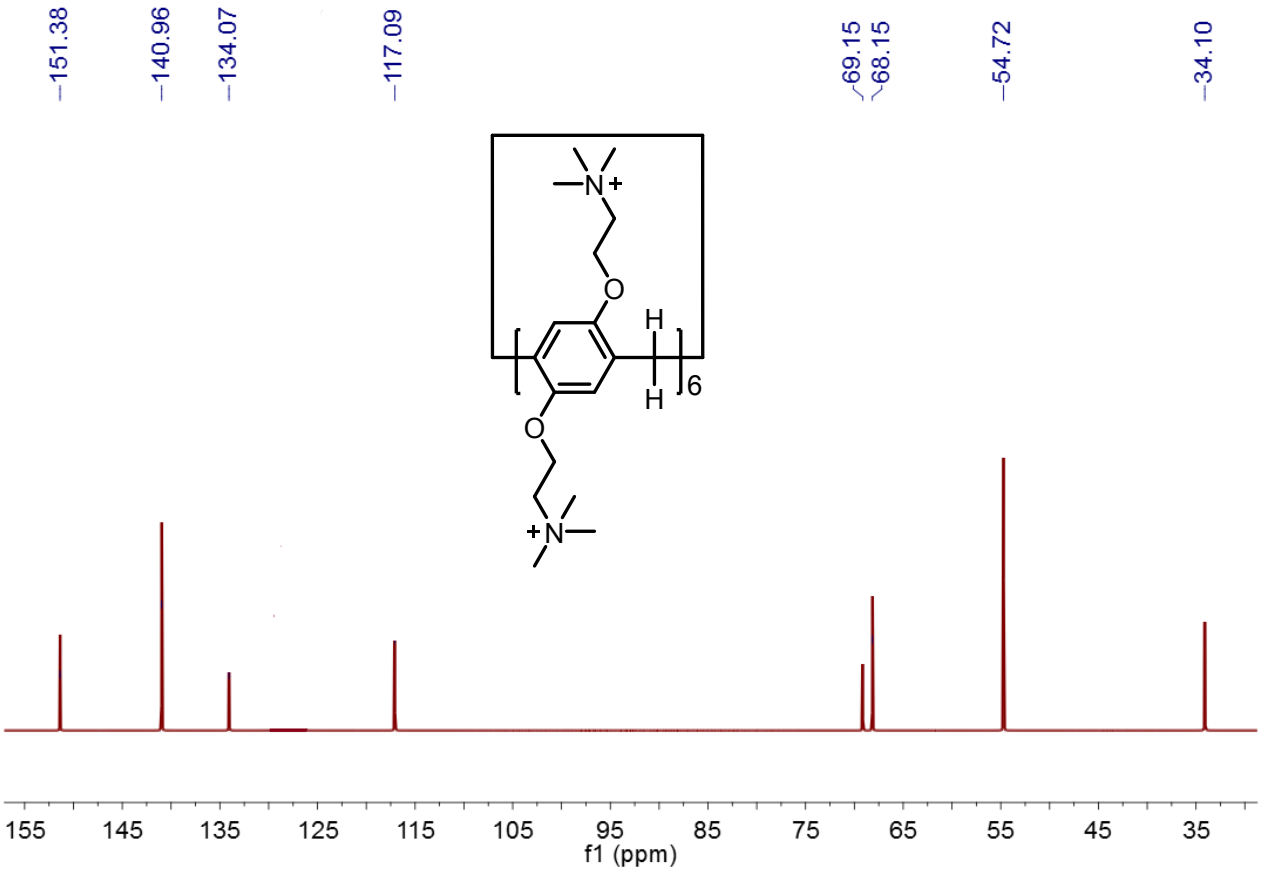


Fig. S20 ^{13}C NMR spectrum (100 MHz, DMSO, rt) of **WP6**.

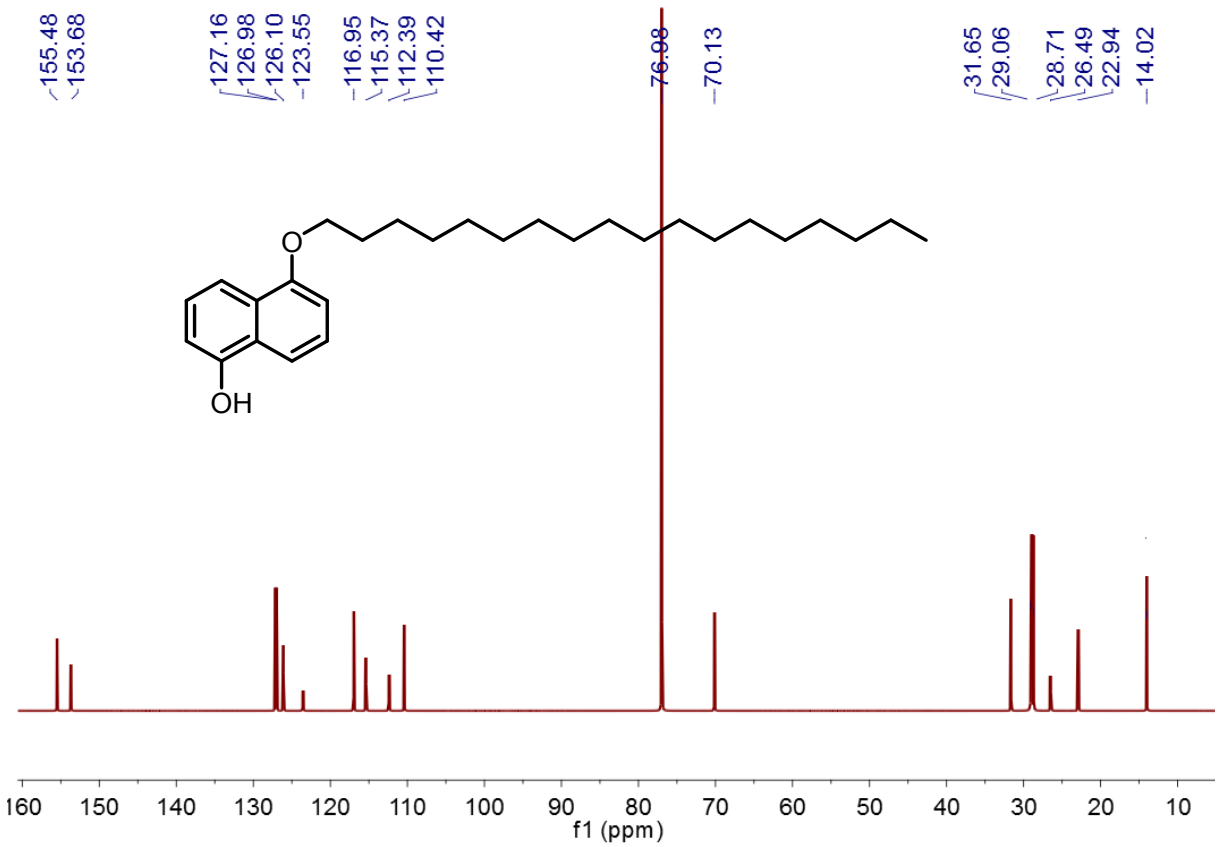


Fig. S21 ^{13}C NMR spectrum (100 MHz, DMSO, rt) of **1**.

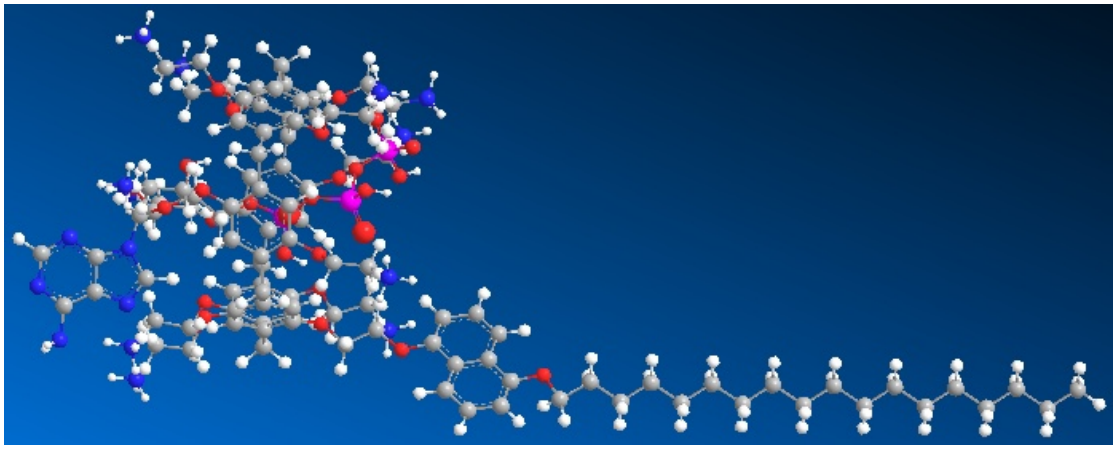


Fig. S22 Energy-minimized structure of **AP6⊃ATP**

6. References

S1. Y. Yao, J. Li, J. Dai, X. Chi and M. Xue, *RSC Adv.*, 2014, **4**, 9039.

S2. Y. Yao, K. Jie, Y. Zhou and M. Xue, *Tetrahedron Lett.*, 2014, **55**, 3195.

S3. K. Jie, Y. Yao, X. Chi and F. Huang, *Chem. Commun.*, 2014, **50**, 5503.