A redox-responsive selenium-containing pillar[5]arene-based macrocyclic amphiphile: synthesis, controllable self-assembly in water, and application in controlled release

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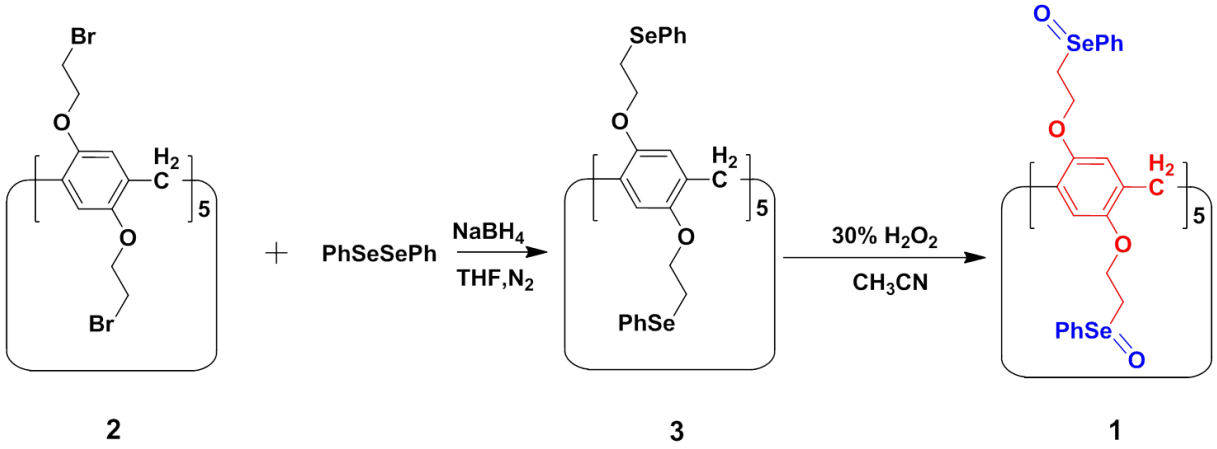
Electronic Supplementary Information (21 pages)

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. pillar[5]arene **3**[S1] was prepared according to a published procedure. ^{1}H NMR, ^{77}Se NMR and ^{13}C NMR spectra were recorded with a Bruker Avance DMX 400, Bruker Avance DMX 500, or Bruker Avance DMX 600 spectrometer 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. UV-vis spectra were taken on a Perkin-Elmer Lambda 35 UV-vis spectrophotometer. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan). The determination of the critical aggregation concentration (CAC) values was carried out on a DDS-307 instrument. 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. MALDI-TOF mass spectrometry was performed with a Bruker UltrafleXtreme instrument. Atomic force microscopy (AFM) experiments were performed on a Multi-Mode Nanoscope-IIIa Scanning Probe Microscope (Veeco Company, USA) in the tapping mode. The small-angle X-ray scattering investigations were carried out on a SAXS/WAXS SYSTEM instrument. Elemental analysis were carried out on a varioMICRO V 1.9.5 instrument.

*2. Synthesis of pillar[5]arene **1***

Scheme S1. Synthetic route to pillar[5]arene **1**.



2 **3** **1**

*2.1 Synthesis of pillar[5]arene **3***

Pillar[5]arene **2** was synthesized according to literature.[S1] To a solution of diphenyldiselane (312 mg, 1.00 mmol) in THF (10.0 mL) under N_2 protection, 2 mL of $NaBH_4$ (152 mg, 4.00 mmol) aqueous solution was injected. Pillar[5]arene **2** (413 mg, 2.20 mmol) was then added. The mixture was stirred for 24 h at 50 °C. Then the solvent was removed and the mixture was purified by column chromatography (silica gel, dichloromethane/petroleum ether = 1:5). Evaporation of the solvent gave **3** as a pale yellow oil (220 mg, 90%). The ^{1}H NMR spectrum of **3** is shown in Fig. S1. ^{1}H NMR (400 MHz, chloroform-*d*, 293 K) δ (ppm): 7.50–7.48 (m, 4 H), 7.21–7.20 (m, 6 H), 6.86 (s, 2 H), 4.26–4.20 (m, 2H), 4.04–4.00 (m, 2H), 3.72 (s, 2H), 3.21–3.14 (m, 4H). The ^{77}Se NMR spectrum of **3** is shown in Fig. S2. ^{77}Se NMR (600 MHz, chloroform-*d*, 293 K) δ (ppm): 269.39. The ^{13}C NMR spectrum of **3** is shown in Fig. S3. ^{13}C NMR (100 MHz, chloroform-*d*, 293 K) δ (ppm): 152.35, 135.30, 132.60, 131.75, 129.63, 118.27, 70.96, 31.99, 29.94. The MALDI-TOF mass spectrum of **3** is shown in Fig. S4: *m*/*z* calcd for [**3** + Na]$^+$ 2472.998; found 2473.261. Anal. Calcd for $C_{115}H_{110}O_{10}Se_{10}$: C, 56.57; H, 4.54. Found: C, 56.65; H, 4.45.

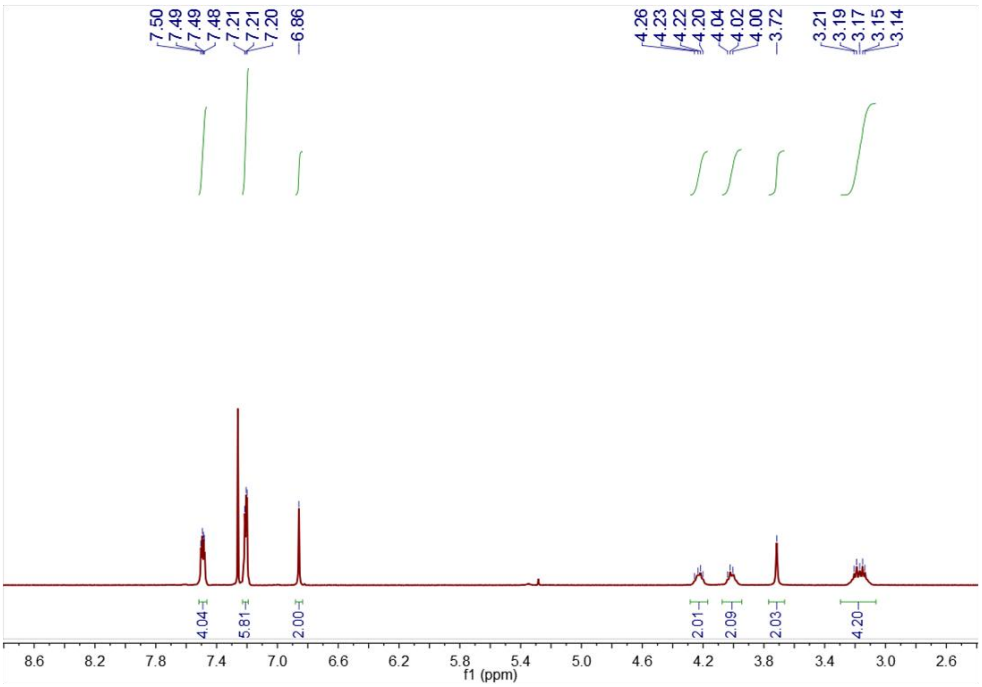


Fig. S1 ^{1}H NMR spectrum (400 MHz, chloroform-*d*, 293 K) of pillar[5]arene **3**.

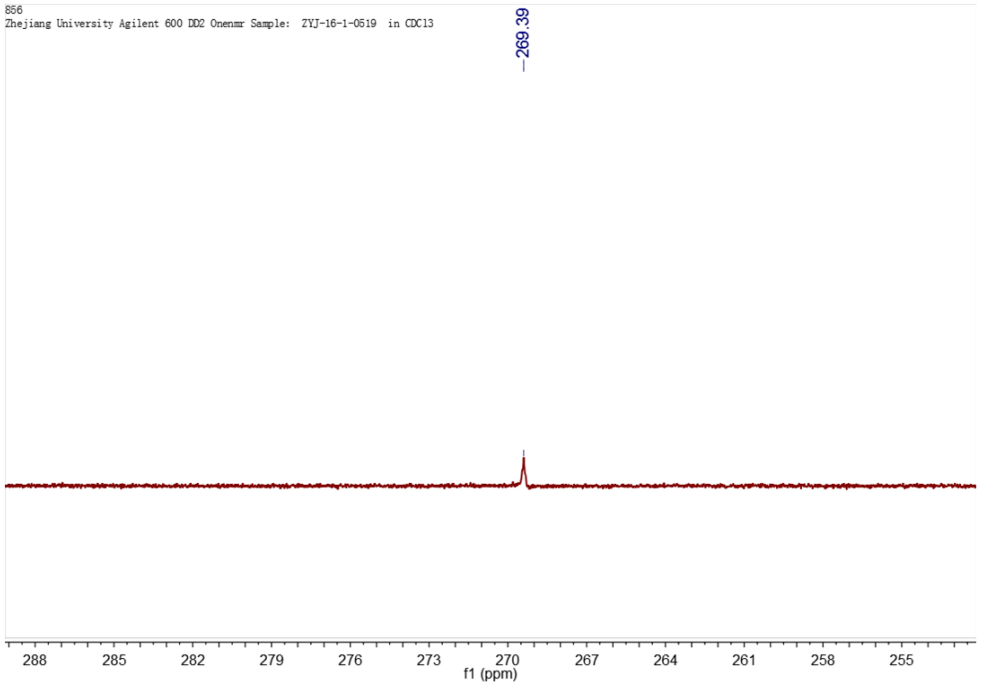


Fig. S2 ^{77}Se NMR spectrum (600 MHz, chloroform-*d*, 293 K) of pillar[5]arene **3**.

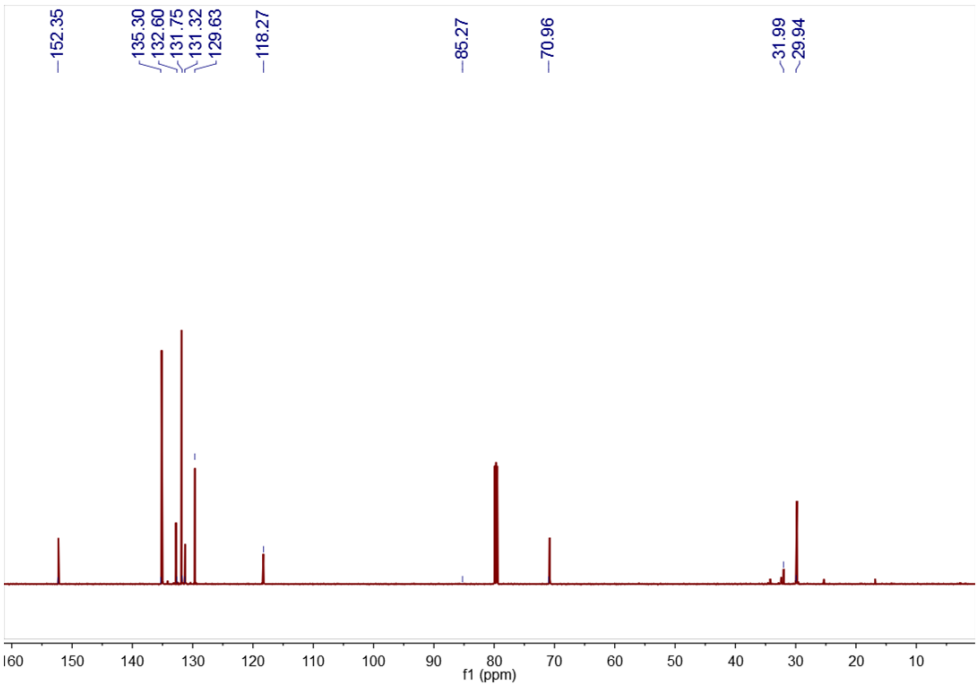


Fig. S3 ^{13}C NMR spectrum (100 MHz, chloroform-*d*, 293 K) of pillar[5]arene **3**.

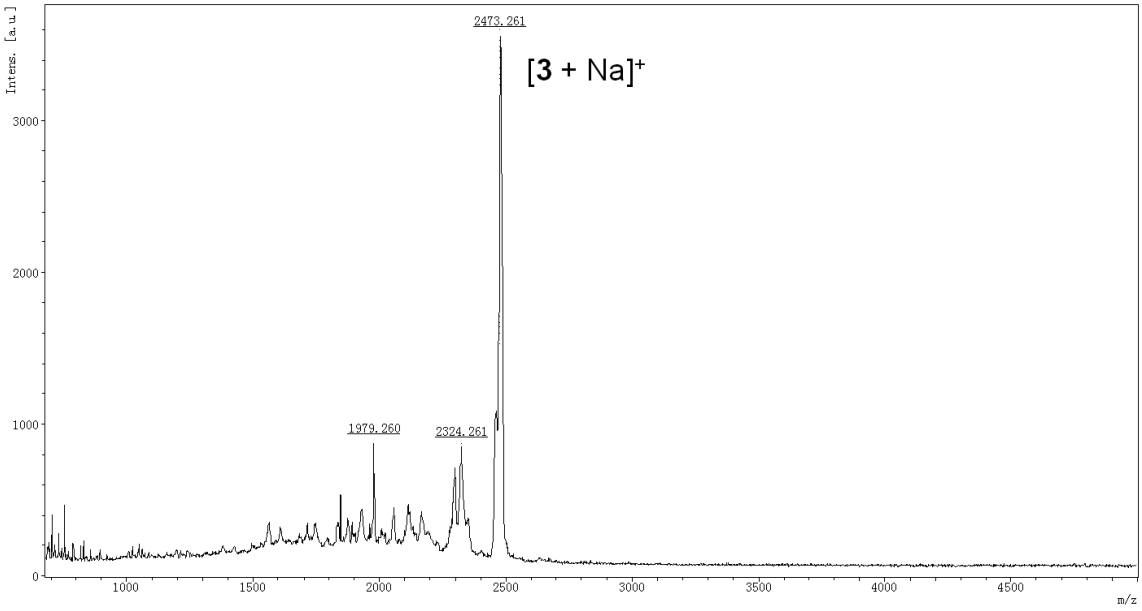


Fig. S4 MALDI-TOF mass spectrum of **3**.

*2.2 Oxidation of pillar[5]arene **3***

To a solution of pillar[5]arene **3** (245 mg, 0.10 mmol) in a mixture of MeCN (5 mL) and H_2O (5 mL), 48 μL of 30% H_2O_2 was added. The mixture was stirred at room temperature for 12 h. MeCN was removed under vacuum and H_2O was removed by freeze-drying to give **1** (248 mg, 95 %) as an oil. ^{1}H NMR spectrum of **1** is shown in Fig. S5. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm): 7.83 (d, J = 4 Hz, 4H), 7.66–7.58 (m, 6H), 6.31 (s, 2H), 4.63–4.60 (m, 2H), 4.52 (s, 2H), 4.10 (s, 2H), 3.56 (s, 4H). The ^{77}Se NMR spectrum of **1** is shown in Fig. S6. ^{77}Se NMR (600 MHz, D_2O, 293 K) δ (ppm): 865.85. The ^{13}C NMR spectrum of **1** is shown in Fig. S7. ^{13}C NMR (100 MHz, acetone-d_6, 293 K) δ (ppm): 139.93, 136.39, 133.06, 128.78, 127.82, 66.83, 62.26, 36.99, 23.56. LRESIMS is shown in Fig. S8: m/z 663.6 [**1** + 2Na + 2H]$^{4+}$; 2610.4 [**1** + H]$^+$. Anal. Calcd for $C_{115}H_{110}O_{20}Se_{10}$: C, 53.09; H, 4.26. Found: C, 53.12; H, 4.18.

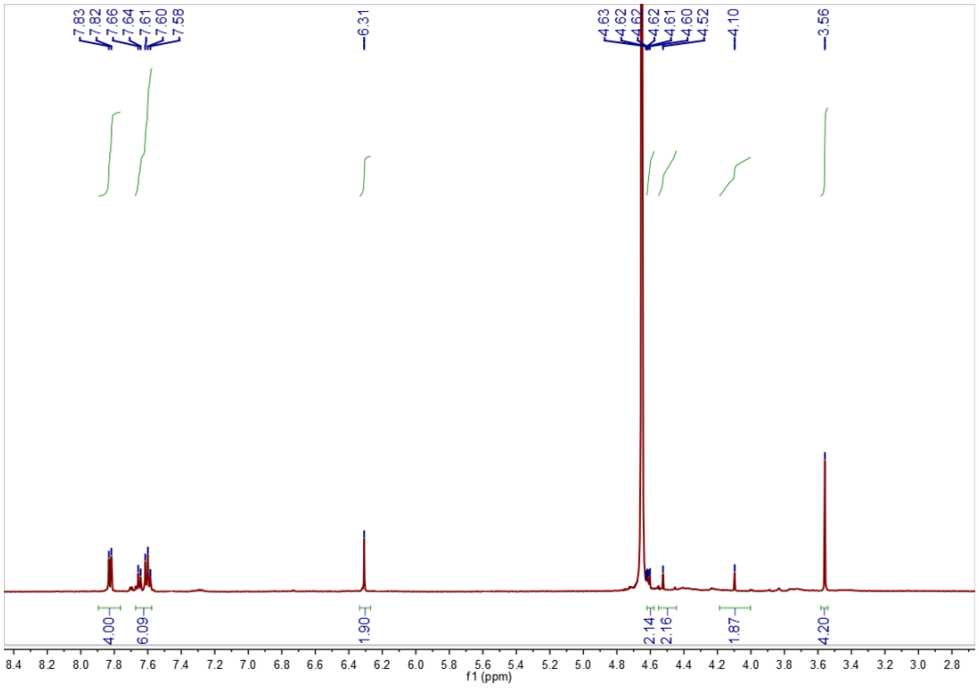


Fig. S5 ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **1**.

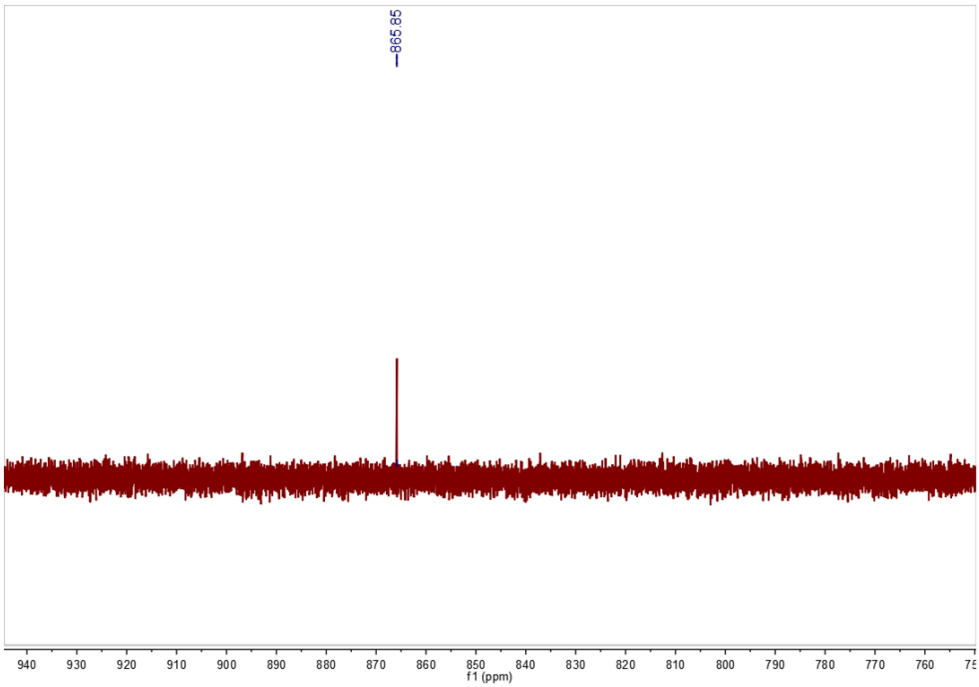


Fig. S6 ^{77}Se NMR spectrum (600 MHz, D_2O, 293 K) of pillar[5]arene **1**.

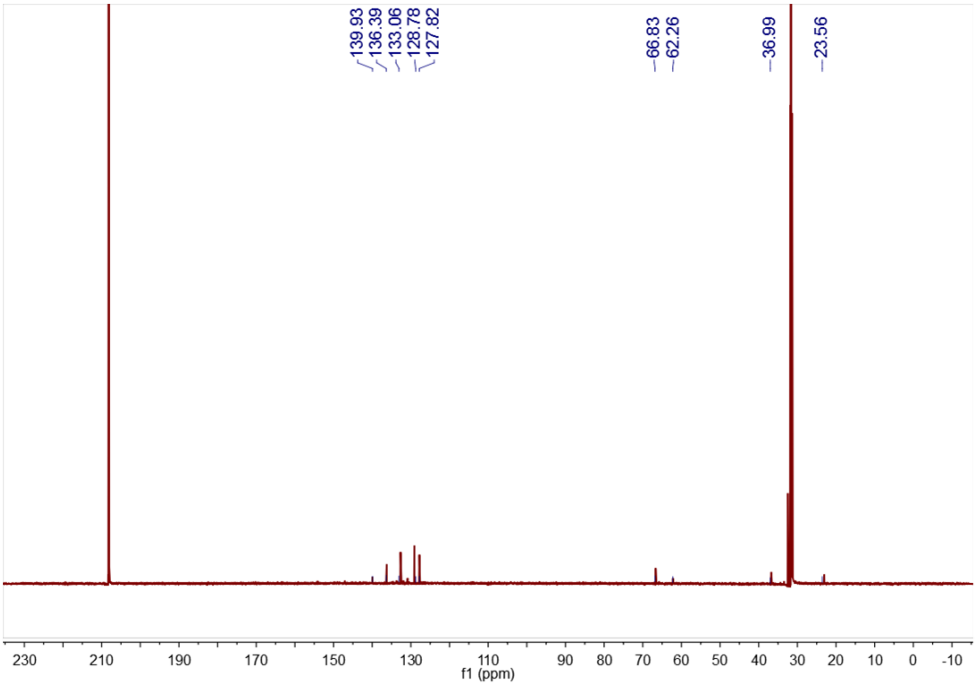


Fig. S7 ^{13}C NMR spectrum (100 MHz, acetone-d_6, 293 K) of pillar[5]arene **1**.

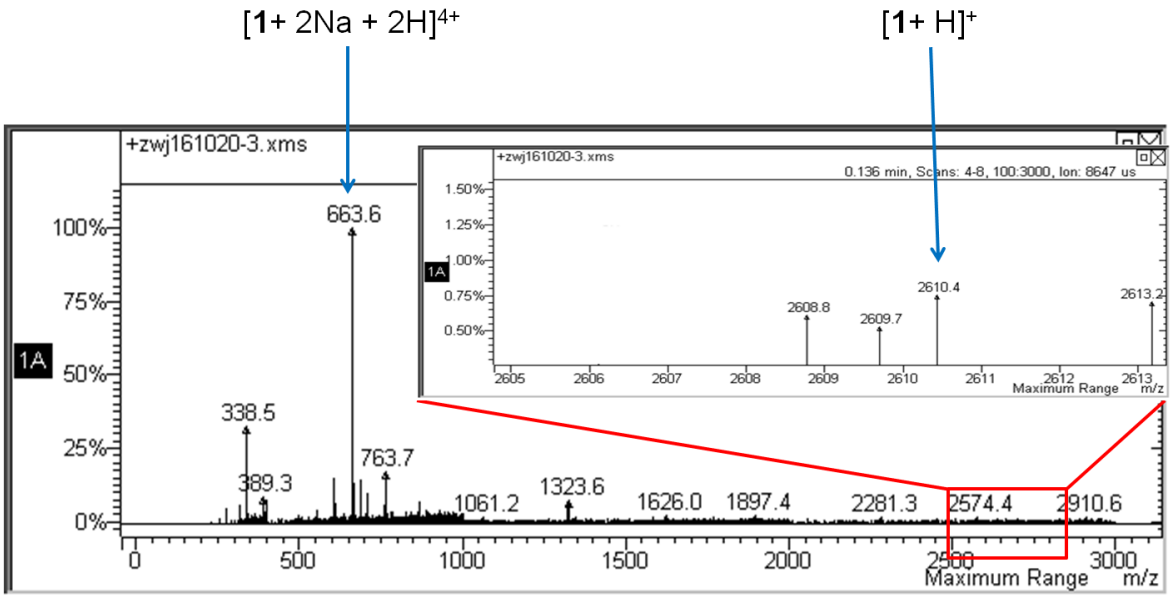
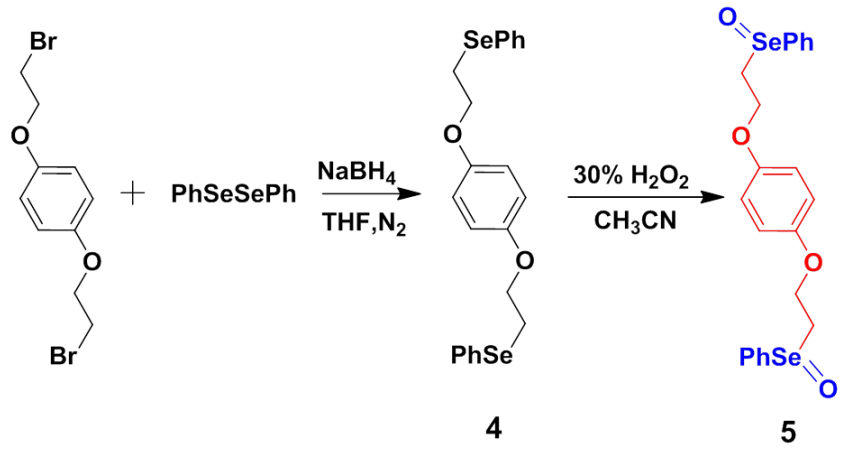


Fig. S8 Electrospray ionization mass spectra of pillar[5]arene **1**. Assignment of the main peak: *m*/*z* 663.6 [**1** + 2Na + 2H]$^{4+}$.

*3. Synthesis of model compound **5***

Scheme S2. Synthetic route to model compound **5**.



*3.1 Synthesis of compound **4***

To a solution of diphenyldiselane (312 mg, 1.00 mmol) in THF (10 mL) under N_2 protection, 2 mL of $NaBH_4$ (152 mg, 4.00 mmol) aqueous solution was injected. 1,4-Bis(2-bromoethoxy)benzene (82.6 mg, 2.20 mmol) was then added. The mixture was stirred for 24 h at 50 °C. The solvent was removed and the mixture was further purified by column chromatography (silica gel, dichloromethane/ petroleum ether = 1:5). Evaporation of the solvent gave **4** as a pale yellow solid (44 mg, 90%), mp 90.5–93.3 °C. The ^{1}H NMR spectrum of **4** is shown in Fig. S9. ^{1}H NMR (400 MHz, chloroform-*d*, 293 K) δ (ppm): 7.55–7.53 (m, 4 H), 7.28–7.26 (m, 6 H), 6.74 (s, 4 H), 4.15–4.12 (m, 4H), 3.21–3.18 (m, 4H). The ^{77}Se NMR spectrum of **4** is shown in Fig. S10. ^{77}Se NMR (600 MHz, chloroform-*d*, 293 K) δ (ppm): 268.13. The ^{13}C NMR spectrum of **4** is shown in Fig. S11. ^{13}C NMR (100 MHz, chloroform-*d*, 293 K) δ (ppm): 152.87,

133.10, 129.49, 129.31, 127.39, 115.84, 68.31, 26.29. The MALDI-TOF mass spectrum of **4** is shown in Fig. S12: *m*/*z* calcd for [**4** + NH_4]$^+$ 494.995; found 494.904. Anal. Calcd for $C_{22}H_{22}O_2Se_2$: C, 55.5; H, 4.66. Found: C, 55.3; H, 4.70.

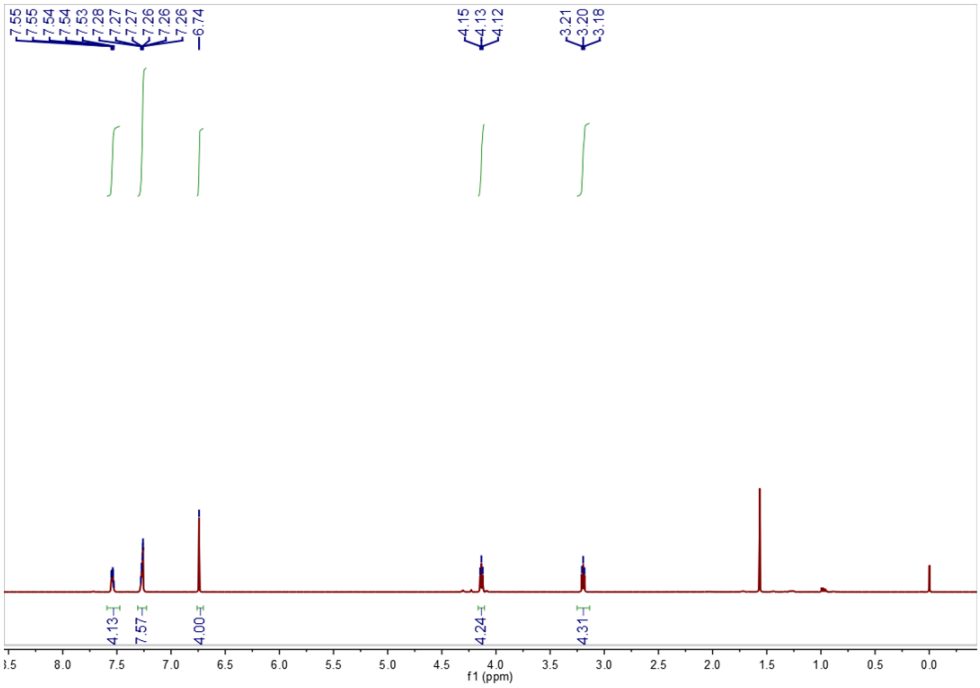


Fig. S9 ^{1}H NMR spectrum (400 MHz, chloroform-*d*, 293 K) of **4**.

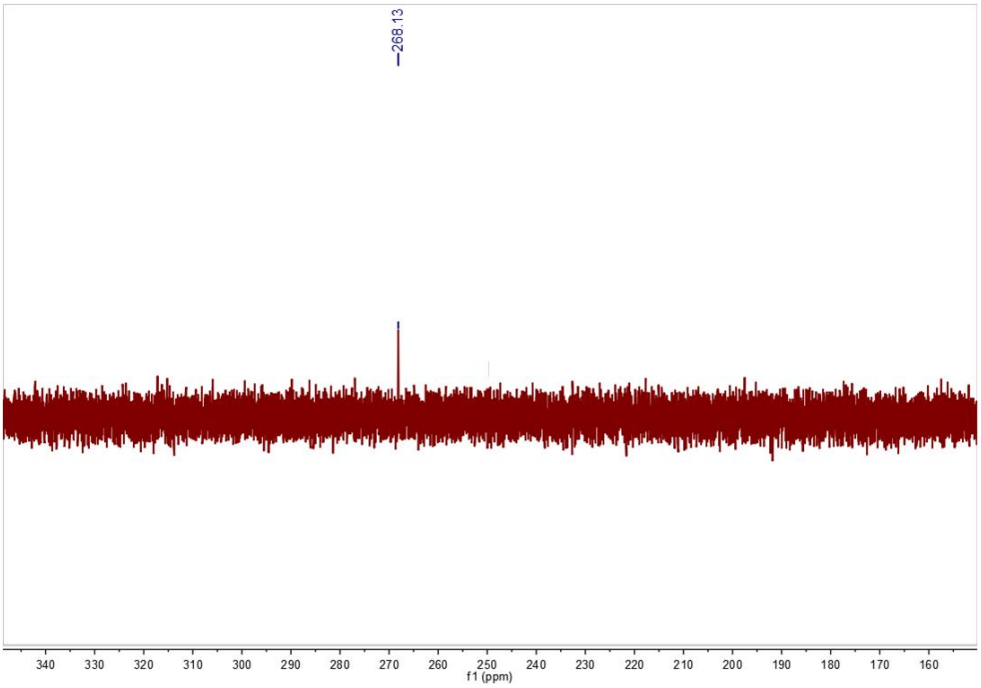


Fig. S10 ^{77}Se NMR spectrum (600 MHz, chloroform-*d*, 293 K) of **4**.

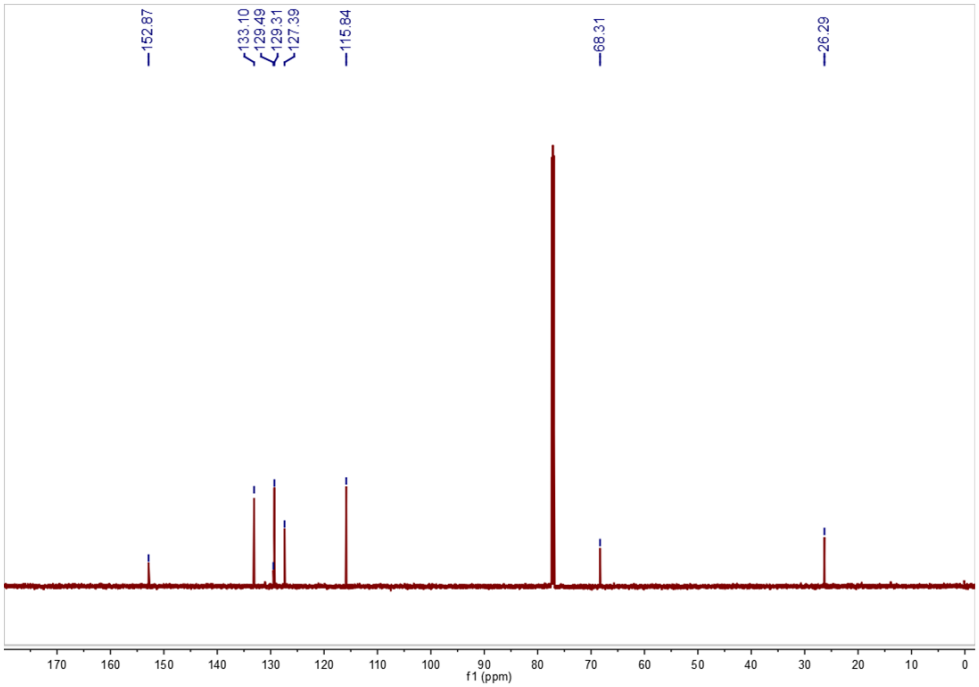


Fig. S11 ^{13}C NMR spectrum (100 MHz, chloroform-*d*, 293 K) of **4**.

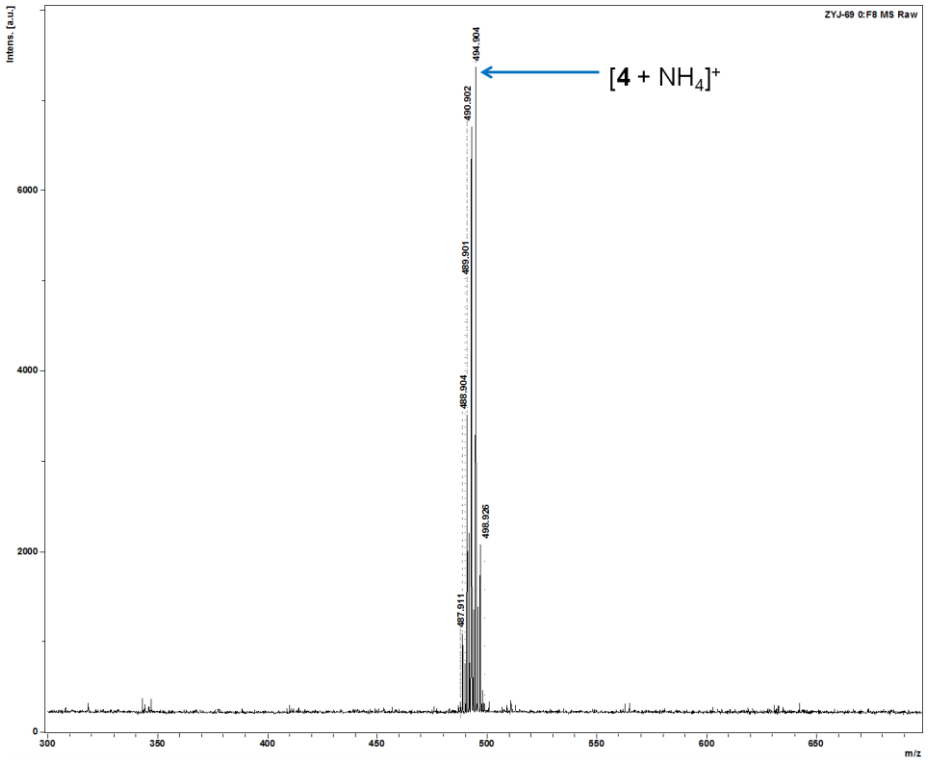


Fig. S12 MALDI-TOF mass spectrum of **4**.

*3.2 Oxidation of **4***

To a solution of **4** (47.7 mg, 0.10 mmol) in a mixture of MeCN (5 mL) and H_2O (5 mL), 13 μL of 30% H_2O_2 was added. The mixture was stirred at room temperature for 12 h. MeCN was removed under vacuum and H_2O was removed by freeze-drying to give **1** (48.3 mg, 95 %) as an oil. ^{1}H NMR spectrum of **5** is shown in Fig. S13. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm): 7.72–7.70 (t, J = 8 Hz, 4H), 7.57–7.48 (m, 6H), 6.23 (s, 4H), 4.40 (s, 2H), 4.00 (s, 1H), 3.76 (s, 1H), 3.45 (s, 4H). The ^{77}Se NMR spectrum of **5** is shown in Fig. S14. ^{77}Se NMR (600 MHz, D_2O, 293 K) δ (ppm): 847.14. The ^{13}C NMR spectrum of **5** is shown in Fig. S15. ^{13}C NMR (100 MHz, D_2O, 293 K) δ (ppm): 169.42, 142.83, 133.95, 130.42, 130.25, 125.66, 62.44, 52.85. The MALDI-TOF mass spectrum of **5** is shown in Fig. S16: m/z calcd for [**5** + $2NH_4$]$^{2+}$ 272.027; found 272.279. Anal. Calcd for $C_{22}H_{22}O_4Se_2$: C, 52.0; H, 4.36. Found: C, 51.9; H, 4.28.

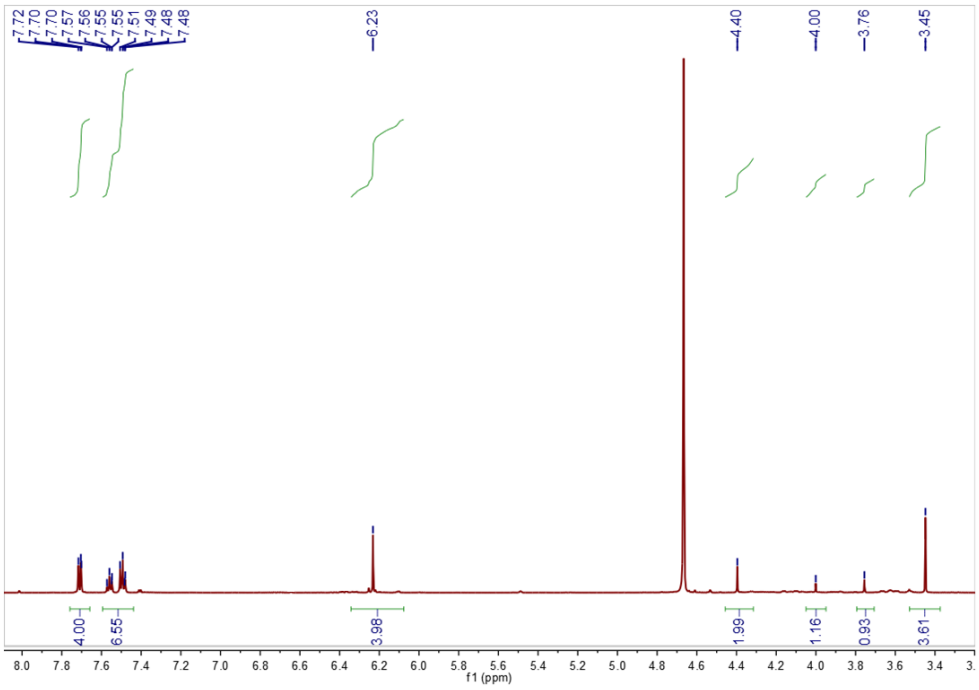


Fig. S13 ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **5**.

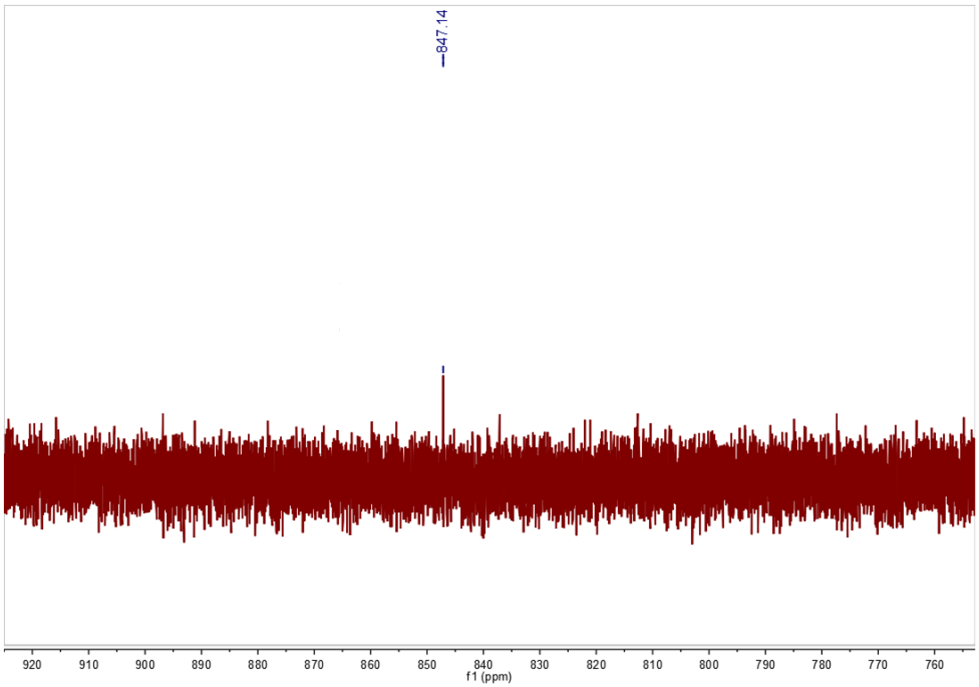


Fig. S14 ^{77}Se NMR spectrum (600 MHz, D_2O, 293 K) of **5**.

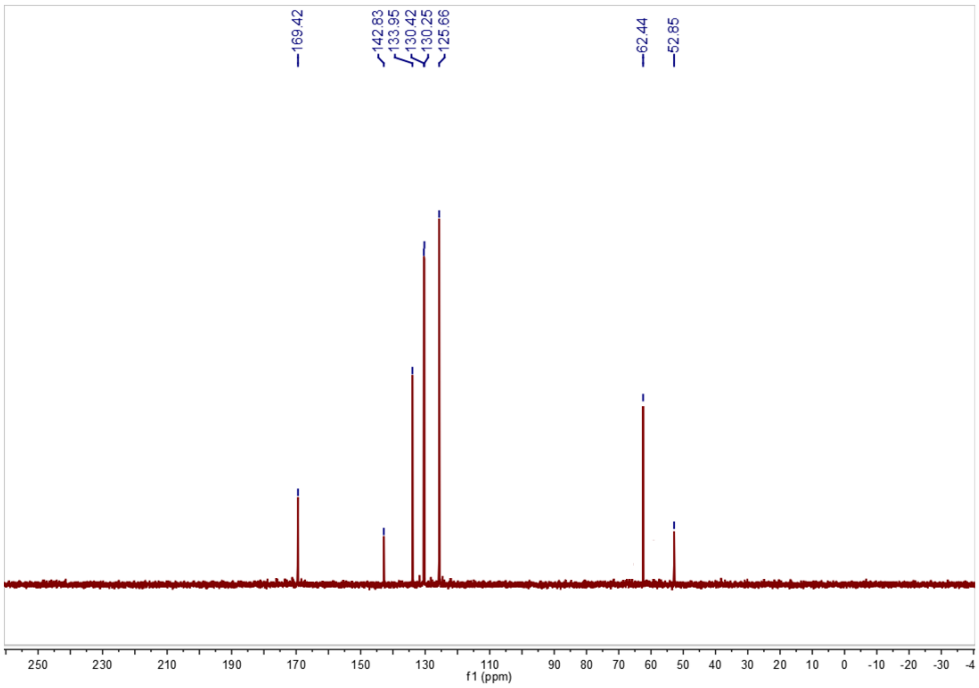


Fig. S15 ^{13}C NMR spectrum (100 MHz, D_2O, 293 K) of **5**.

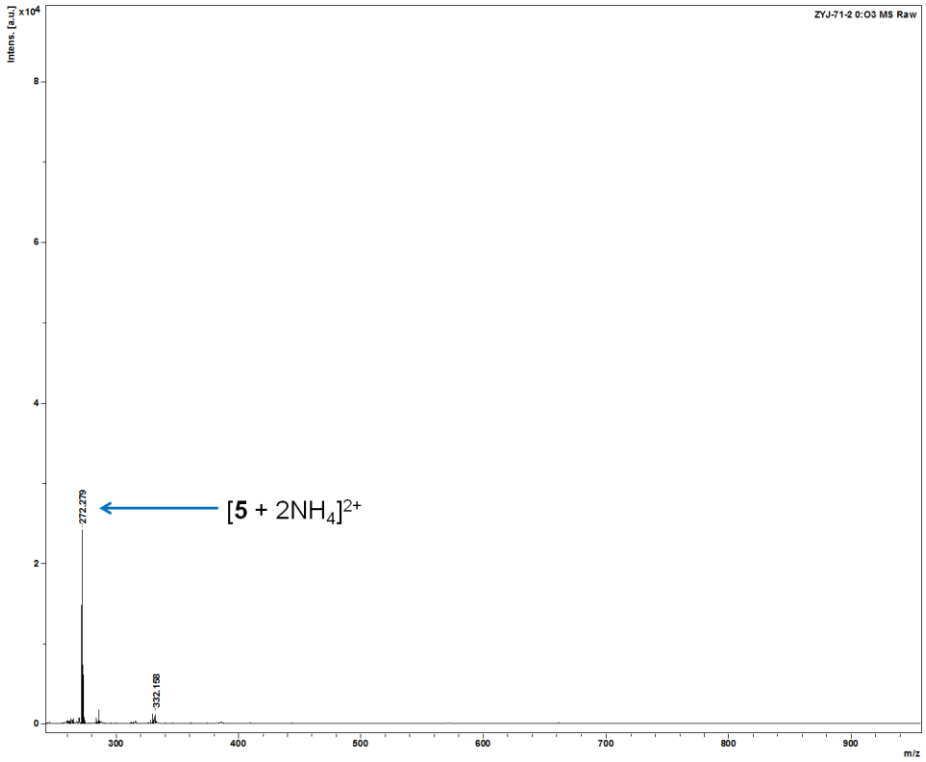


Fig. S16 MALDI-TOF mass spectrum of **5**.

*4. ^{77}Se NMR spectroscopy of redox-responsive behavior of **1***

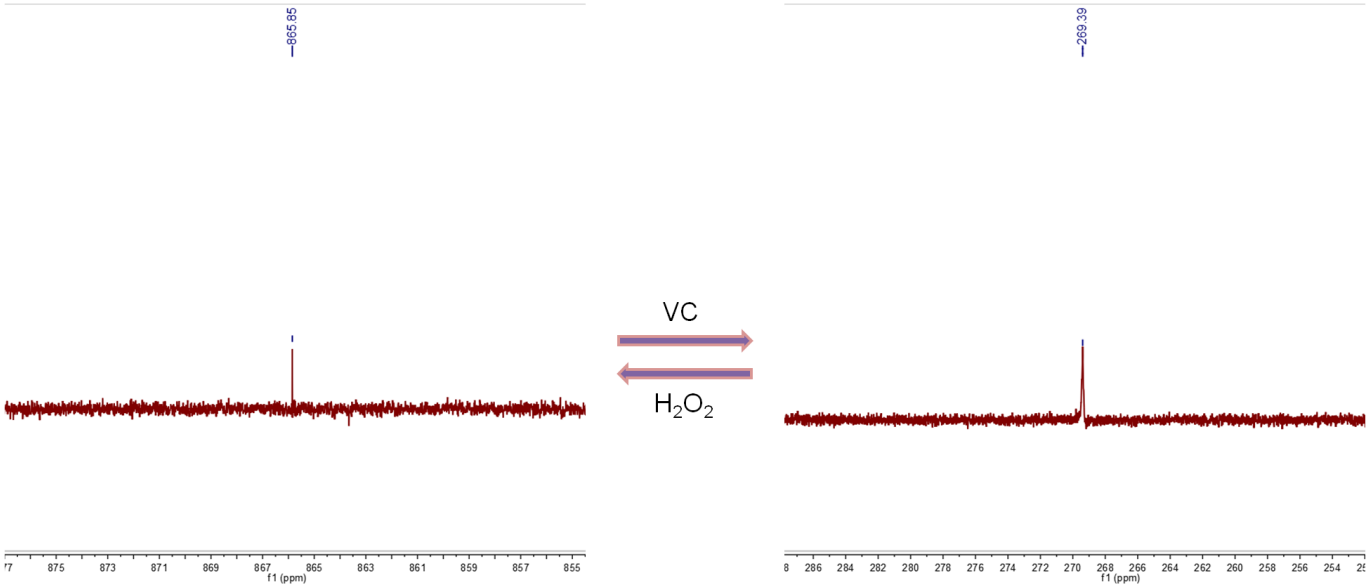


Fig. S17 ^{77}Se NMR spectra (600 MHz, CD_3COCD_3/D_2O (1:4, *v*/*v*), 293 K) of **1** and **3** were switched by redox control.

*5. Photos of an aqueous solution of **1** after adding vitamin C and then H_2O_2*

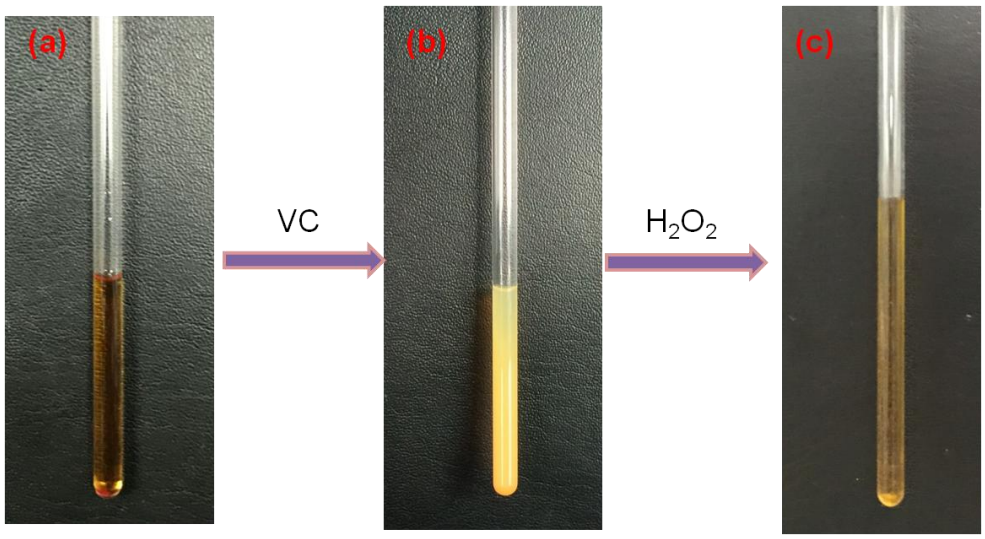


Fig. S18 Photos: (a) an aqueous solution of **1**; (b) a after adding 12 equiv. of vitamin C; (c) b after adding 15 equiv. of H_2O_2.

*6. Fluorescence spectroscopic studies of **1** aggregation after adding vitamin C*

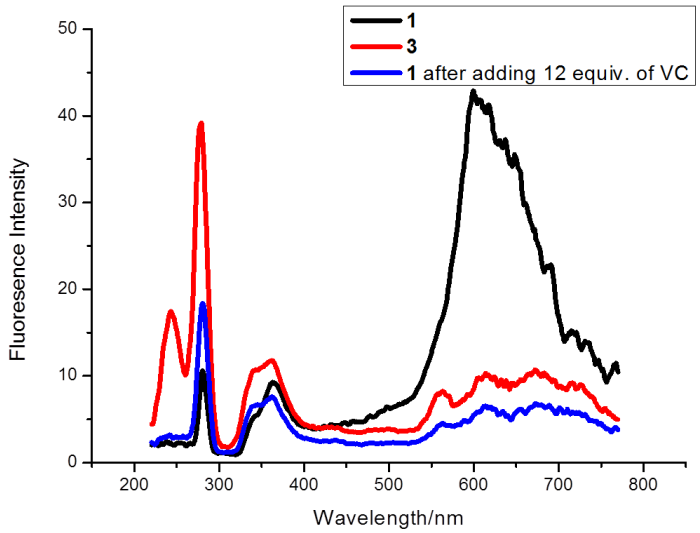


Fig. S19 Fluorescence spectra of **1**, **3**, and **1** after adding vitamin C in CH_3COCH_3/H_2O (1:4, *v*/*v*). The fluorescence spectrum of **1** after adding 12 equiv. of vitamin C is similar to the fluorescence spectrum of **3**.

*7. Determination of critical aggregation concentration of **1** in water*

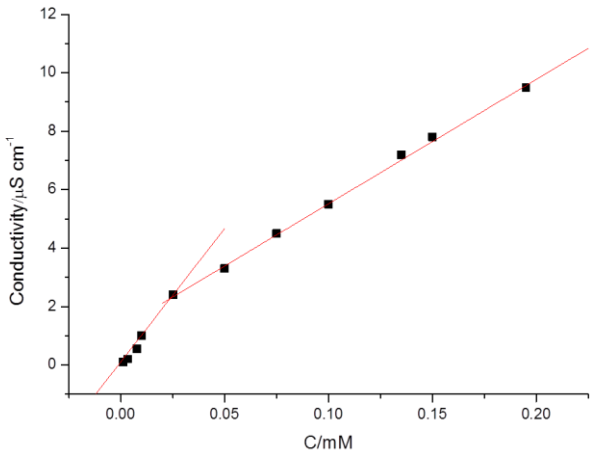


Fig. S20 The concentration-dependent conductivity of **1** in water. The critical aggregation concentration was determined to be 2.38 × 10^{-5} M.

*8. Enlarged TEM image of **1***

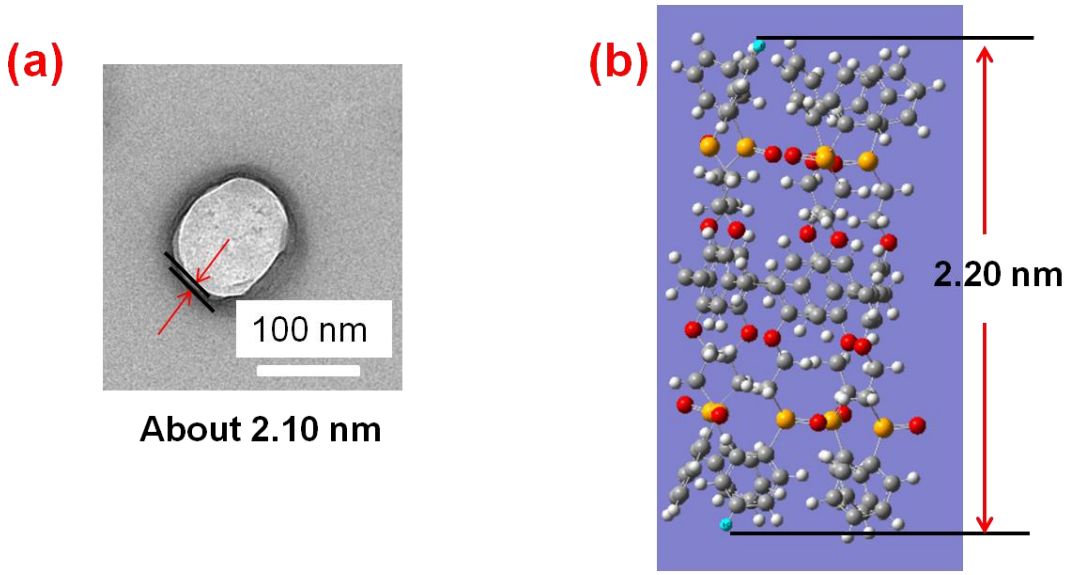


Fig. S21 (a) Enlarged TEM image of **1** aggregates; (b) the energy-minimized structure of **1** (ball and stick mode, side view) obtained from Gaussian (Gaussview V 5.0.8.0). The length of **1** molecule was caculated to be about 2.20 nm.

*9. AFM result of **1***

Pair	Horizontal Distance	Vertical Distance	Surface Distance	Angle	Rmax	Rz	Rz Count	Rms	Ra (Frequency Cutoff)	Frequency Cutoff	Radius	Radius Sigma
1	0.337 (纳)	4.247 (nm)	0.338 (纳)	3.277 (?	21.084 (nm)	0.000 (nm)	0.000	8.480 (nm)	7.608 (nm)	0.000 (纳)	2.172 (纳)	0.582 (nm)
0	0.000 (纳)	0.000 (nm)	0.000 (纳)	0.000 (?	0.000 (nm)	0.000 (nm)	0.000	0.000 (nm)	0.000 (nm)	0.000 (纳)	0.000 (纳)	0.000 (纳)
0	0.000 (纳)	0.000 (nm)	0.000 (纳)	0.000 (?	0.000 (nm)	0.000 (nm)	0.000	0.000 (nm)	0.000 (nm)	0.000 (纳)	0.000 (纳)	0.000 (纳)

Fig. S22 AFM result of the self-assembled vesicles. The height measured from the AFM experiment is the height of two walls of the vesicles. It means that the wall thickness of the vesicles is 2.1 nm, which is equal to half of the vertical distance, 4.247 nm.

*10. Small-angle X-ray scattering scan of **1** in water*

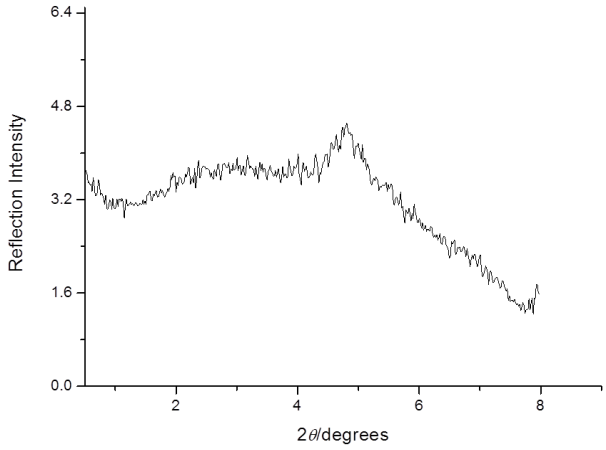


Fig. S23 Small-angle X-ray scattering scan of **1** (5.00×10^{-4} mol/L) in water. $2d\mathrm{Sin}\theta = \lambda$. $\lambda_{\mathrm{CuKa}} = 0.15418$ nm, and $2\theta = 4.4°$. $d = 2.00$ nm.[S2]

*11. Tyndall effect photos of **1** before and after adding vitamin C*

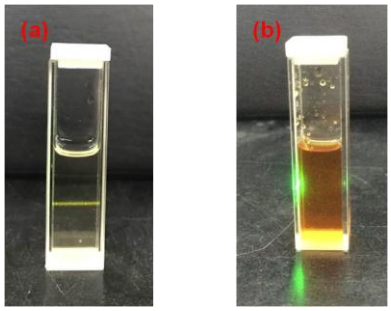


Fig. S24 (a) Tyndall effect of **1** aggregation; (b) a in the presence of excess vitamin C.

*12. ^{1}H NMR spectroscopy of redox-responsive behavior of **5***

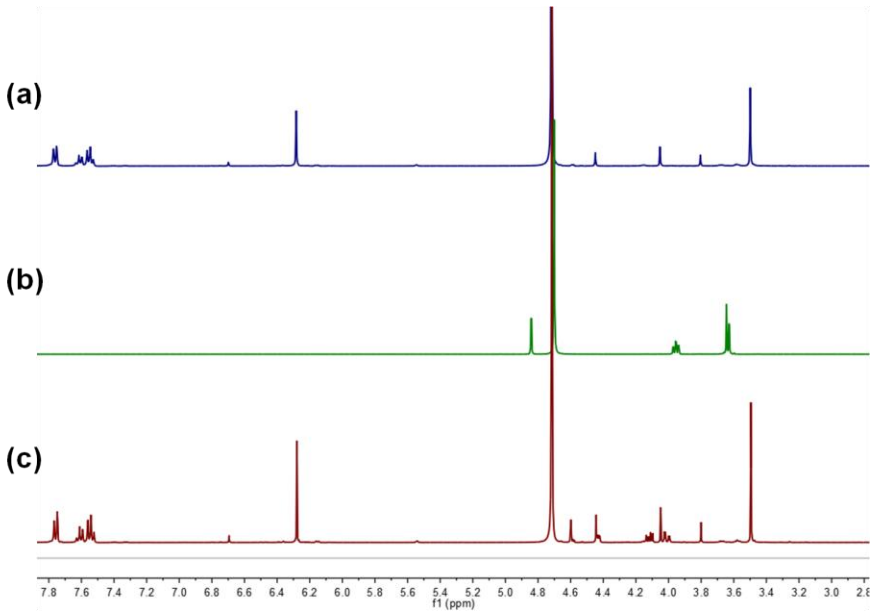


Fig. S25 Partial ^{1}H NMR spectra (400 MHz, D_2O, 25 ℃): (a) **5**; (b) **5** after adding 3 molar equiv. of vitamin C; (c) b after further adding 5 molar equiv. of H_2O_2.

*13. ^{77}Se NMR spectroscopy of redox-responsive behavior of **5***

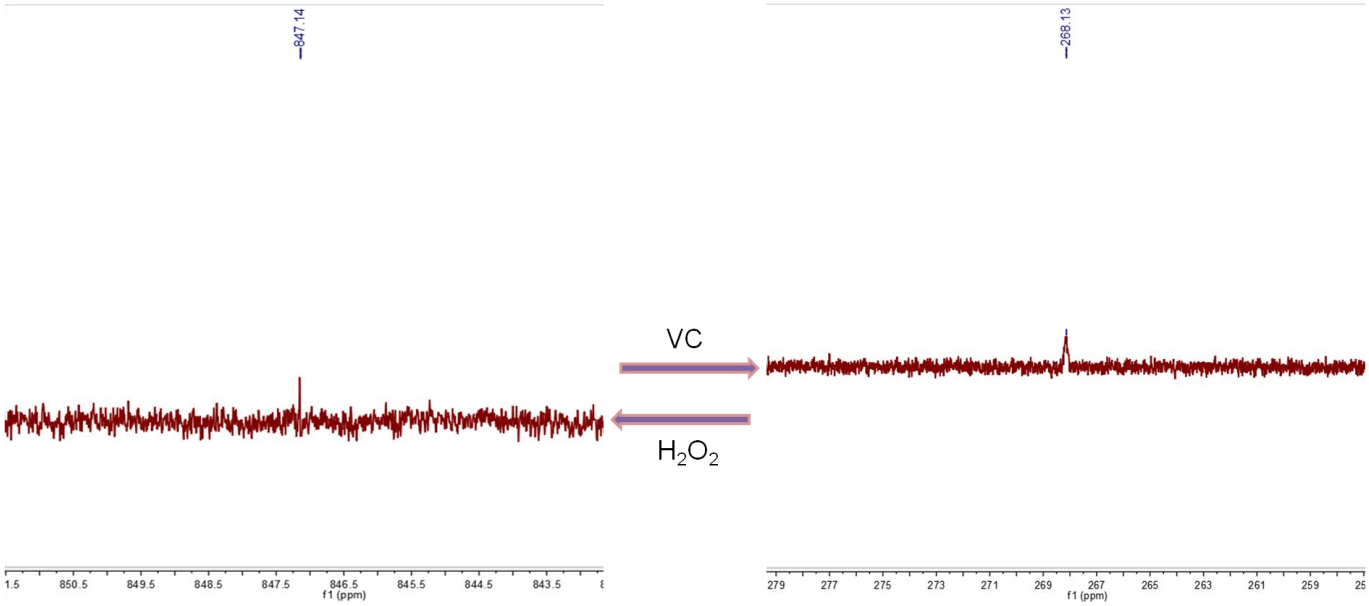


Fig. S26 ^{77}Se NMR spectra (600 MHz, CD_3COCD_3/D_2O (1:4, *v*/*v*), 293 K) of **5** and **4** were switched by redox control.

*14. Photos of an aqueous solution of **5** after adding vitamin C and then H_2O_2*

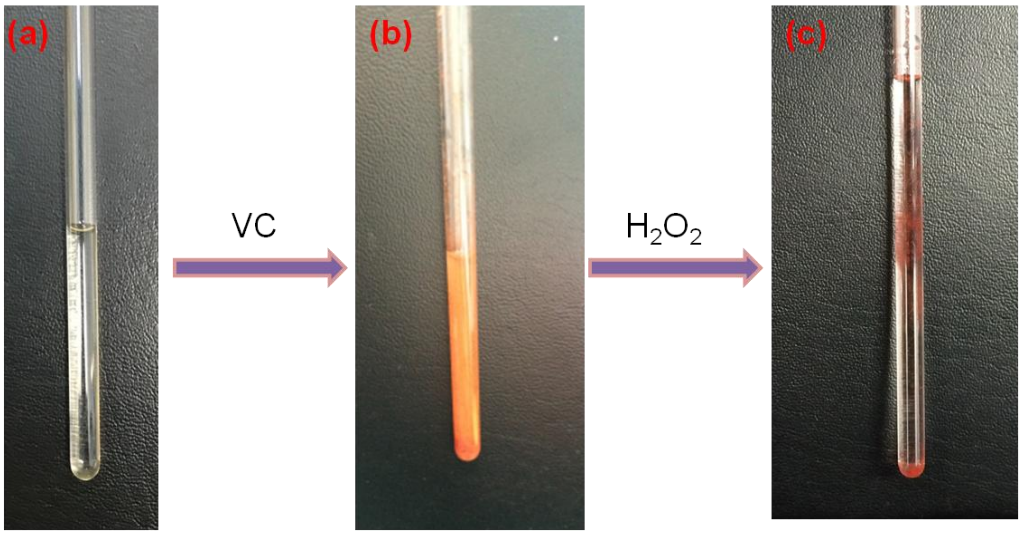


Fig. S27 Photos of aqueous solutions: (a) **5**; (b) **5** after adding 3 molar equiv. of vitamin C; (c) b after further adding 5 molar equiv. of H_2O_2.

*15. Determination of critical aggregation concentration of **5** in water*

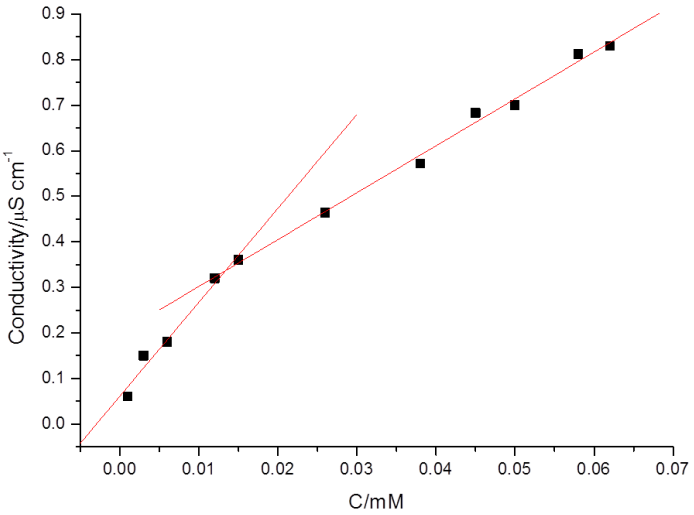


Fig. S28 The concentration-dependent conductivity of **5** in water. The critical aggregation concentration was determined to be 1.35×10^{-5} M.

*16. Enlarged TEM image of **1***

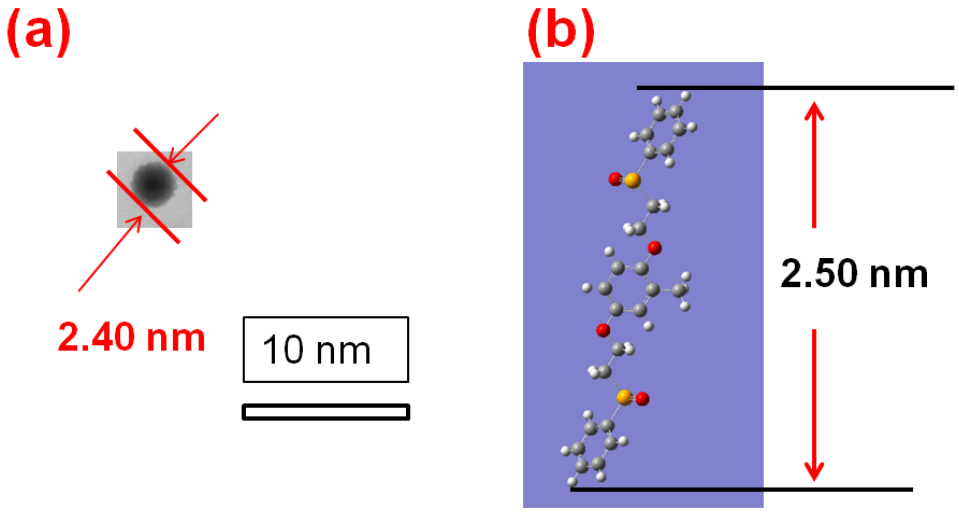


Fig. S29 (a) Enlarged TEM image of **5** aggregation; (b) the energy-minimized structure of **5** (ball and stick mode, side view) obtained from Gaussian (Gaussview V 5.0.8.0). The length of **5** molecule was caculated to be about 2.50 nm.

17. UV−vis absorption spectra of vesicles, DOX, and DOX-loaded vesicles at 25 ℃ in water

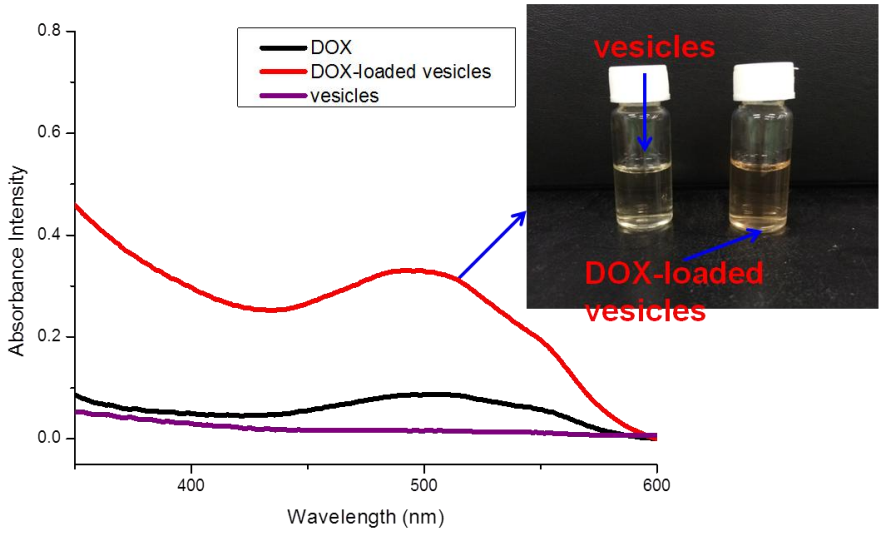


Fig. S30 UV−vis absorption spectra of vesicles, DOX, and DOX-loaded vesicles in water. Inset: colour change of vesicles before (left) and after (right) DOX-loading.

18. Loading and triggered release experiments of DOX

DOX loading experiment: DOX-loaded vesicles were prepared by adding a certain amount of DOX into a freshly prepared aqueous solution of **1** (2.50×10^{-4} M). The ultimate concentrations of DOX and **1** were 0.0500 and 0.250 mM, respectively. The DOX-loaded vesicles were purified by dialysis (molecular weight cutoff = 3500) in distilled water for several times until the water outside the dialysis tube exhibited negligible DOX fluorescence. As a result, DOX was successfully loaded into the vesicles constructed from **1**.

The DOX encapsulation and loading efficiency were calculated by the following equations:[S3]

Encapsulation Efficiency (%) = $(m_{DOX\text{-}loaded} / m_{DOX})100$

Loading EffIciency(%) = $(m_{DOX\text{-}loaded}/m_{vesicles})100$

Here $m_{DOX\text{-}loaded}$, m_{DOX} and $m_{vesicles}$ are mass values of DOX encapsulated in vesicles, DOX added, and DOX-loaded vesicles, respectively. The mass of DOX was measured by a UV spectrophotometer at 490 nm and calculated relative to a standard calibration curve in the concentrations from 5.00×10^{-3} to 2.50×10^{-2} mM in water. 2.00 mL of DOX-loaded assemblies in a dialysis bag were added into 18.0 mL of water. At predetermined time intervals, 2.00 mL of the solution outside the dialysis tube were collected and replaced by 2.00 mL of fresh water. The concentration of DOX was determined by measuring the UV absorbance at 490 nm.

References:

S1. Y. Ma, X. Ji, F. Xiang, X. Chi, C. Han, J. He, Z. Abliz, W. Chen and F. Huang, *Chem. Commun.*, 2011, **47**, 12340.

S2. Q. Zhang, Z. Gao, F. Xu, S. Tai, X. Liu, S. Mo and F. Niu, *Langmuir,* 2012, **28**, 11979.

S3. K. Wang, D.-S. Guo, X. Wang and Y. Liu, *ACS Nano*, 2011, **5**, 2880.