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Supporting Information

Water-Soluble Pillar[6]arene Bearing Pyrene on Alternating Methylene Bridges for Direct Spermine Sensing

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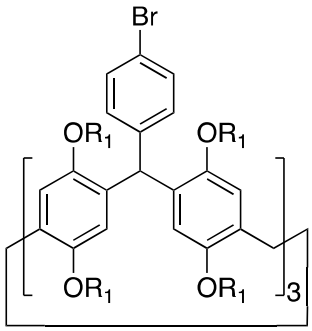
1. General Experimental

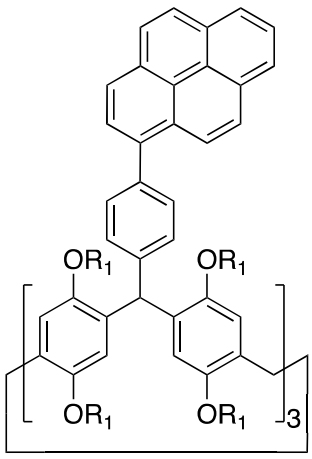
All starting materials, reagents and solvents were purchased from commercial vendors and used without further purification. ^{1}H NMR spectra were recorded on Bruker Avance III 400MHz Spectrometer at 298K. UV-Vis absorbance spectroscopy was measured on an Agilent Cary 100 UV. Fluorescence spectroscopy was measured on Shimadzu RF-6000 (slit width: 5 nm). Confocal laser scanning microscopy (CLSM) images were performed on a LSM880. Cell culture was carried out in an incubator with a humidified atmosphere of 5% CO_2 at 37 ℃.

2. Synthetic procedures



2 $R_1 = CH_2COOEt$

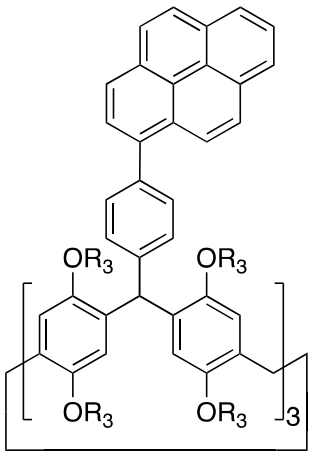
Compound **1** was dissolved in anhydrous CH_2Cl_2. BBr_3 (227 μL, 2.4 mmol) was slowly added into a solution of compound **1** (136.6 mg, 0.1 mmol). The mixture was stirred at room temperature for 12 h, and subsequently quenched by the addition of water (20 mL) to form precipitate. The precipitate was collected by centrifugation, and washed by diluted aqueous HCl (1 M) by five times and water by three times. The resulting per-phenol compound was a white powder, and instaneously used for the subsequent reaction. Under the protection of N_2, the per-phenol compound was mixed with anhydrous K_2CO_3 (663.3 mg, 4.8 mmol), and added into anhydrous CH_3CN (8 mL). To the suspension, $BrCH_2COOEt$ (266 μL, 2.4 mmol) was slowly added. The mixture was refluxed at 80 ℃ for 48 h. The suspension was filtered, and the filtrate was dried by rotary evaporation to yield a yellow oil. The yellow oil was dissolved in ethyl acetate, and washed by saturated NH_4Cl for three times. The organic phase was dried by anhydrous Na_2SO_4, and the solvent was removed by rotary evaporation. The crude product was purified by column chromotagraphy (*n*-hexane:ethyl acetate = 2:1, v/v) to yield compound **2** as an orange solid (126.3 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.0 Hz, 6H), 6.96 (d, *J* = 8.0 Hz, 4H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.92 (s, 4H), 6.89 (s, 2H), 6.54 (s, 2H), 6.46 (s, 2H), 6.28 (s, 1H), 6.26 (s, 2H), 6.06 (s, 2H), 4.47−4.34 (m, 24H), 4.17 (s, *J* = 14.0 Hz, 1H), 4.17−4.03 (m, 24H), 3.93 (s, 4H), 3.82 (d, *J* = 14.0 Hz, 1H), 1.21−1.11 (m, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 169.1, 169.1, 150.5, 150.3, 150.2, 150.2, 142.9, 142.2, 131.0, 131.0, 130.9, 130.8, 130.7, 129.6, 128.8, 128.6, 128.4, 119.8, 119.6, 116.8. 116.7, 116.0, 115.2, 114.9, 114.1, 66.8, 66.5, 66.4, 66.2, 66.1, 65.9, 61.1, 61.0, 60.8, 60.8, 44.6, 42.4, 31.4, 30.4, 14.2, 14.1. HR-MS (m/z): $[M+NH_4]^+$ calcd for $C_{108}H_{121}Br_3NO_{36}$ 2244.5213; found 2244.5213.



3 $R_1 = CH_2COOEt$

Compound **3**: Compound **2** (150.3 mg, 0.067 mmol), 1-pyreneboronic acid (90.5mg, 0.41 mmol), $Pd(dppf)Cl_2$ (15.6 mg, 0.02 mmol) and anhydrous K_3PO_4 (173.6 mg, 0.81 mmol) was added into a mixed solvent system containing 2.5 mL 1,4-dioxane and 0.3 mL H_2O. The mixture was refluxed at 80 ℃ for 18 h. The reaction mixture was filtered, and the filtrate was added into ethyl acetate (20 mL). The organic phase was washed with saturated NH_4Cl aqueous solution for three times. After dried by anhydrous Na_2SO_4, the solvent was removed by rotary evaporation to yield an oil. The crude product was purified by silica gel column chromotography (*n*-hexane:ethyl acetate = 5:4, v/v) to yield compound **3** as a yellow solid (58.7 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26−8.16 (m, 12H), 8.12−8.08 (m, 6H), 8.04−7.99 (m, 9H), 7.53 (d, *J* = 8.0 Hz, 6H), 7.34 (d, *J* = 8.0 Hz, 4H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.07 (s, 2H), 7.04 (s, 2H), 6.98 (s, 2H), 6.73 (s, 2H), 6.70 (s, 2H), 6.56 (s, 1H), 6.52 (s, 2H), 6.35 (s, 2H), 4.58−4.46 (m, 24 H), 4.21 (d, *J* = 14.0 Hz, 1H), 4.21−4.08 (m , 24 H), 4.03 (s, 4H), 3.87 (d, *J* = 14.0 Hz, 1H), 1.24−1.13 (m, 36H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 169.4, 169.3, 150.7, 150.7, 150.6, 150.5, 150.4, 142.9, 142.3, 138.7, 138.6,

138.1, 138.0, 131.7, 131.6, 131.2, 130.6, 130.3, 130.2, 129.3, 129.2, 128.9, 128.8, 128.7, 128.5, 127.8, 127.6, 127.4, 126.1, 125.2, 125.1, 124.9, 124.8, 117.3, 117.1, 116.6, 115.5, 115.2, 114.4, 67.2, 67.0, 66.8, 66.5, 66.4, 66.2, 61.2, 61.1, 60.9, 44.8, 42.6, 31.3, 30.6, 14.4, 14.3. HR-MS (m/z): $[M+NH_4]^+$ calcd for $C_{156}H_{148}NO_{36}$ 2610.9776; found 2610.9984.



4 $R_3 = CH_2COOH$

Compound **4**: Compound **3** (58.7 mg, 0.023 mmol) and KOH (313.1 mg, 5.52 mmol) were dissolved in a mixed solvent system of THF and water (1:1, v:v, 10 mL), and heated at 80 ℃ for 48 h. The reaction mixture was filtered. The filtrate was adjusted to pH 7 by adding aqueous HCl (12 M). The precipitate was collected by centrifugation and dried under vacuum to afford compound **4** as a yellow solid (24.0 mg, 46%). ^{1}H NMR (400 MHz, D_2O) δ 8.41−8.33 (m, 12H), 8.27−8.24 (m, 6H),8.22−8.11 (m, 9H), 7.70 (d, *J* = 8.0 Hz, 4H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 4H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.07 (s, 2H), 7.05 (s, 2H), 6.70 (s, 2H), 6.62 (s, 2H), 6.58 (s, 2H), 6.55 (s, 2H), 6.53 (s, 3H), 4.51 (d, *J* = 16.0 Hz, 1H), 4.49−4.20 (m, 24 H), 4.02 (s, 4H), 3.58 (d, *J* = 16.0 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 177.4, 177.2, 176.9, 176.7, 151.0, 150.7, 150.1, 149.8, 142.4, 142.1, 138.6, 137.2, 132.2, 131.8, 131.7, 131.1, 130.6, 130.5, 130.2, 129.4, 128.8, 128.3, 127.9, 127.8, 127.7, 127.4, 126.4, 125.0, 124.9, 124.1, 124.0, 118.3, 118.0, 114.8, 70.3, 70.1, 69.5, 68.2, 68.0, 67.9, 40.1, 41.5, 31.2, 29.8. HR-MS (m/z): $[M-H]^-$ calcd for $C_{132}H_{95}O_{36}$ 2255.5609; found 2255.5598.

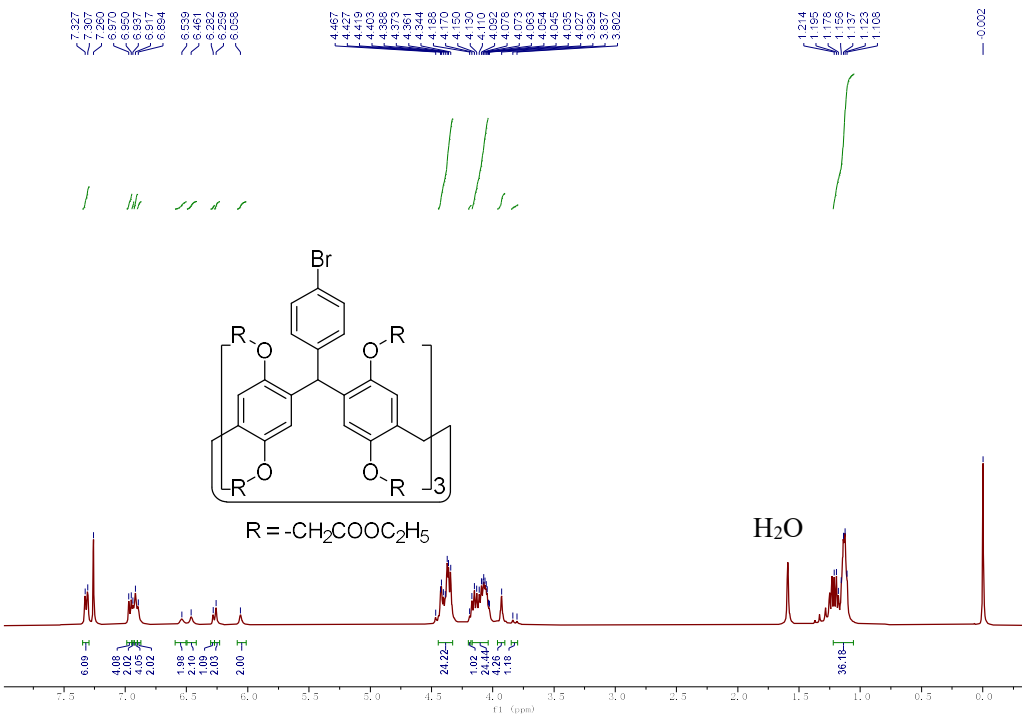


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) recorded for compound **2**.

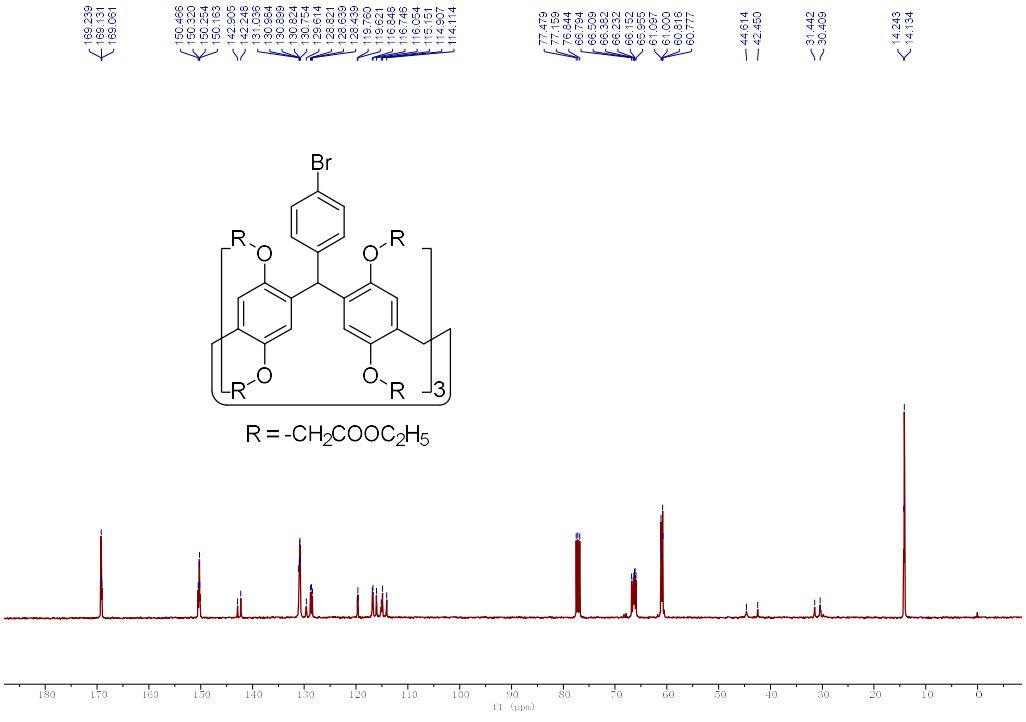


Figure S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) recorded for compound **2**.

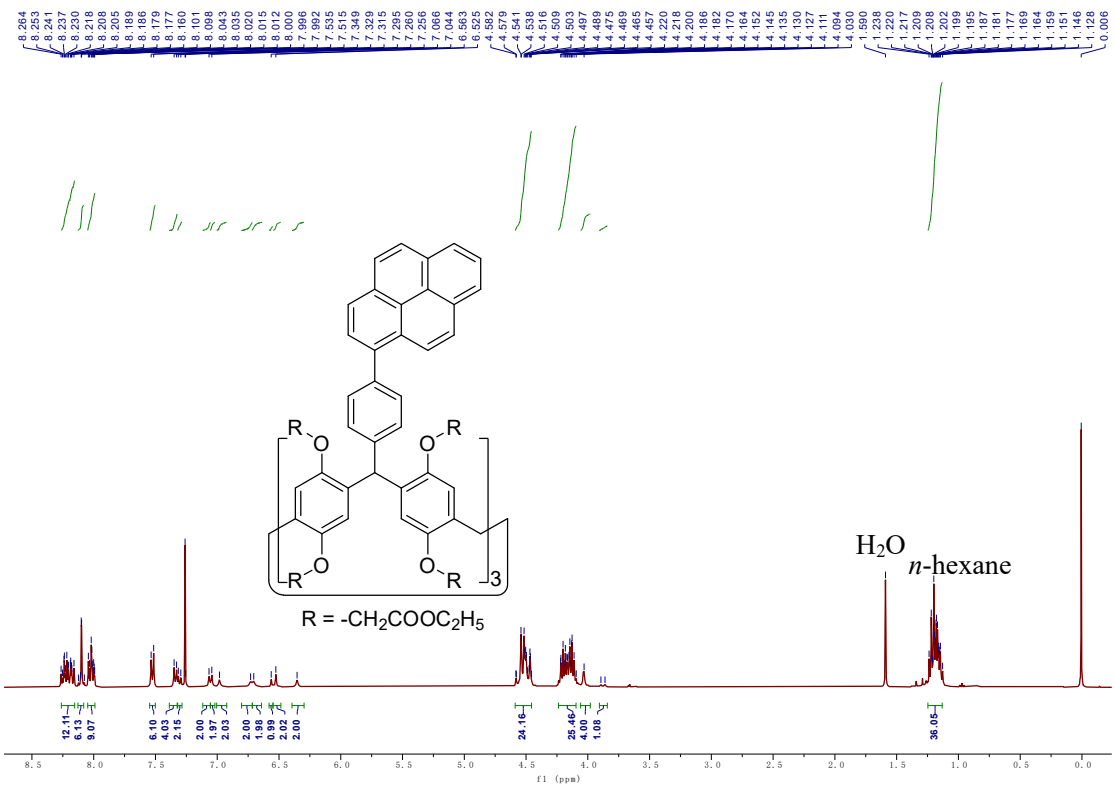


Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) recorded for compound **3**.

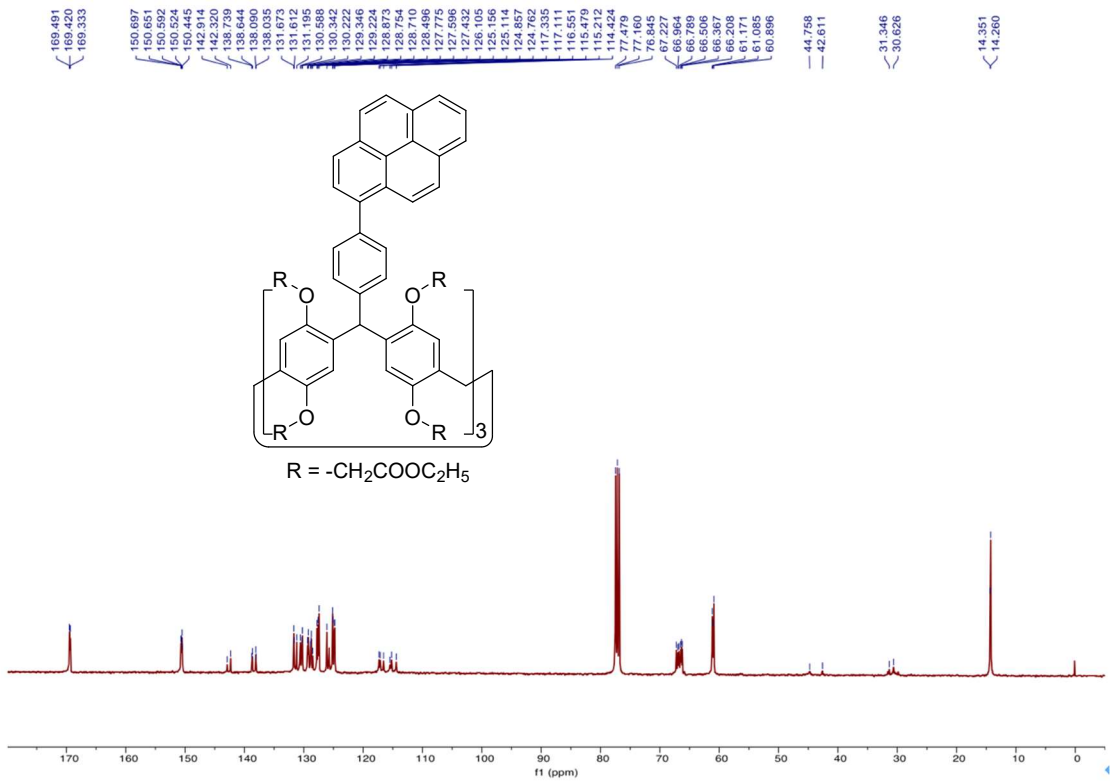


Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) recorded for compound **3**.

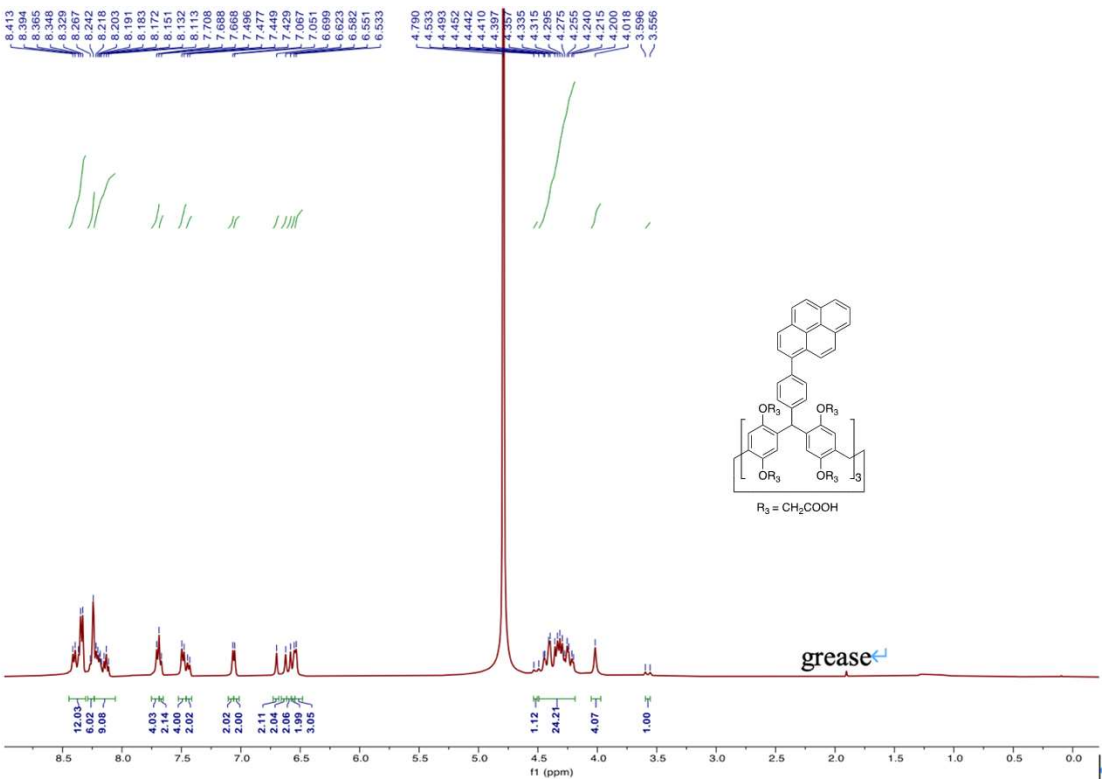


Figure S5. ^{1}H NMR spectrum (400 MHz, D_2O) recorded for compound **4**.

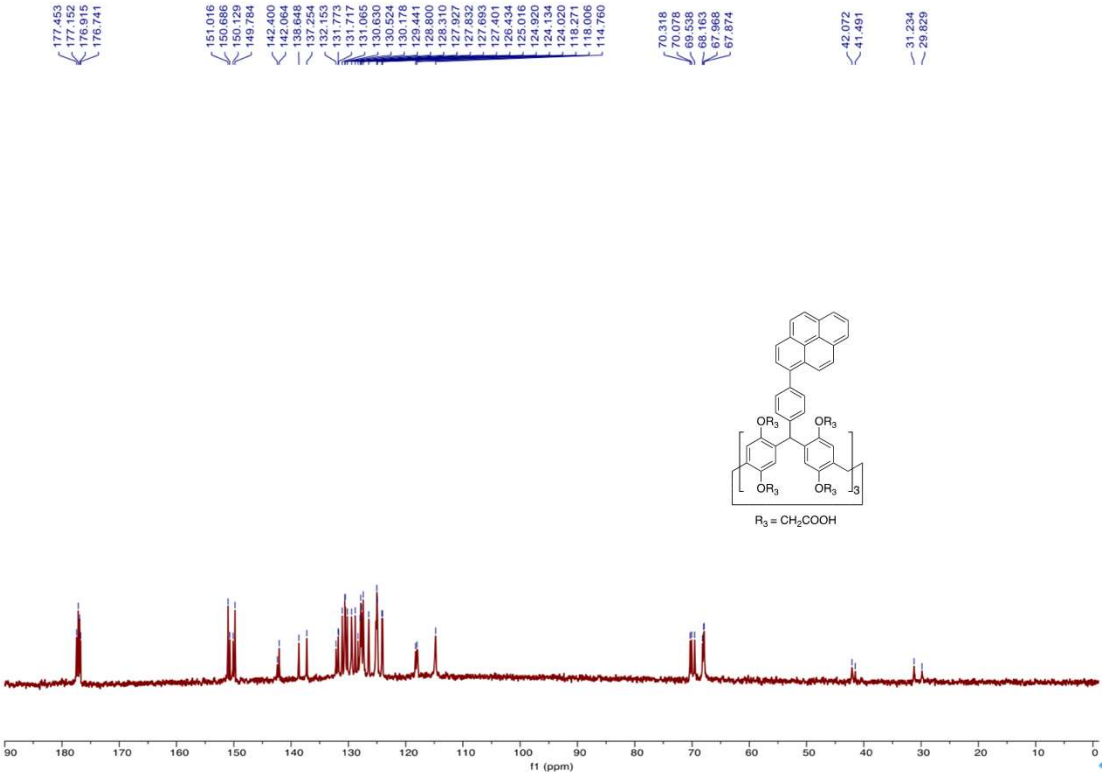


Figure S6. ^{13}C NMR spectrum (100 MHz, D_2O) recorded for compound **4**.

3. Fluorescence analysis

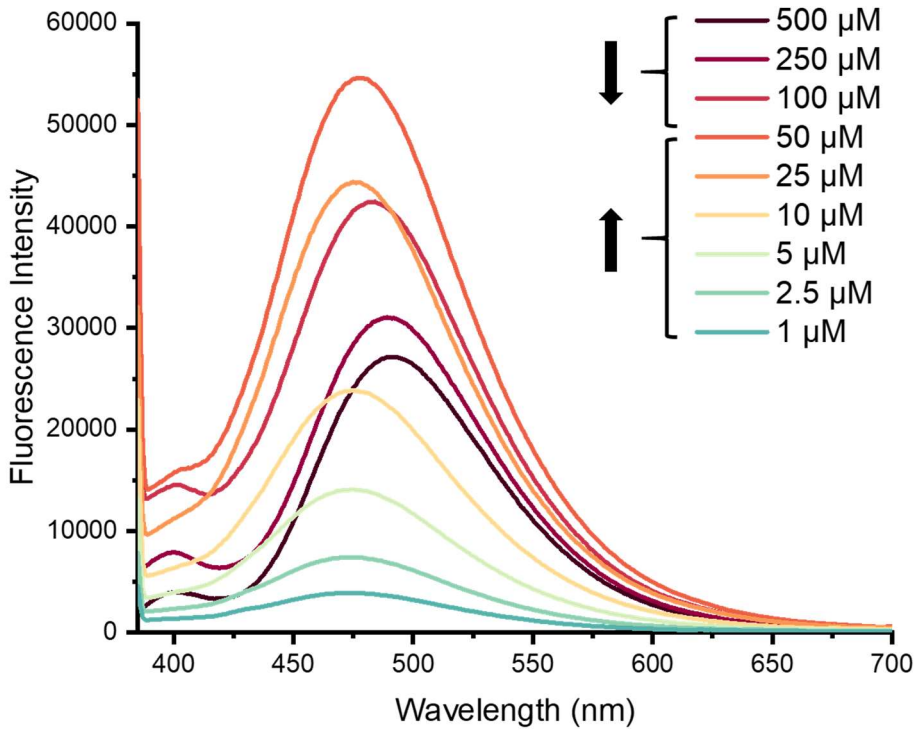


Figure S7. Fluorescence spectra recorded for host **4** at different concentrations (ex 375 nm).

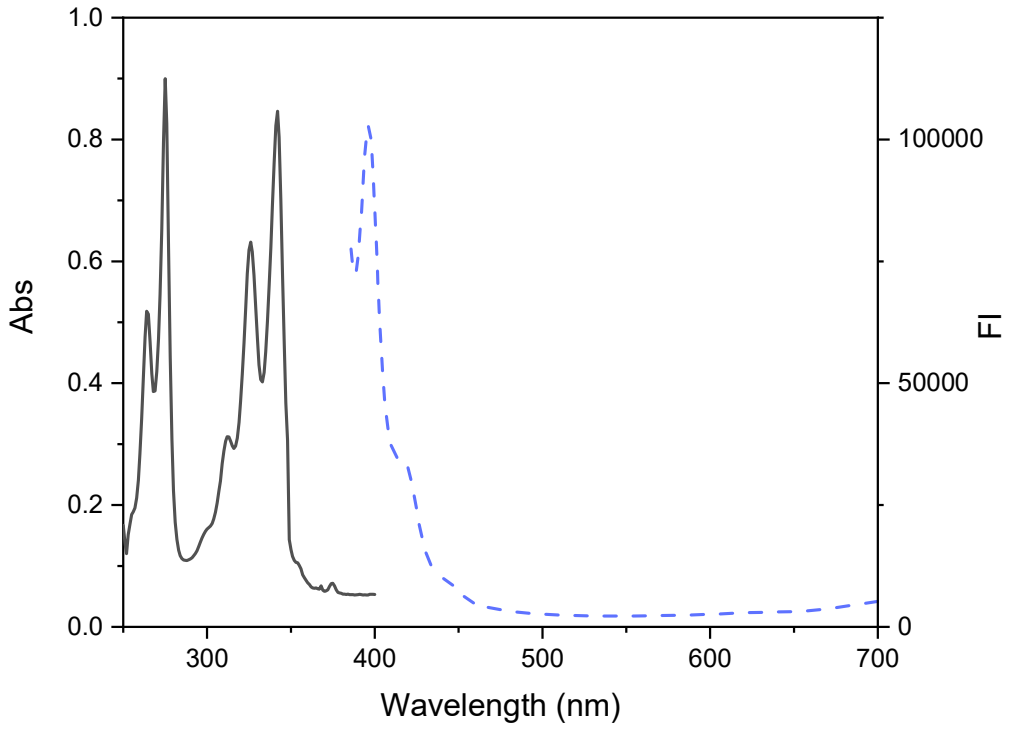


Figure S8. UV-Vis absorption (solid line, left axis) and fluorescence emission (dashed line, right axis) spectra recorded for compound **5**.

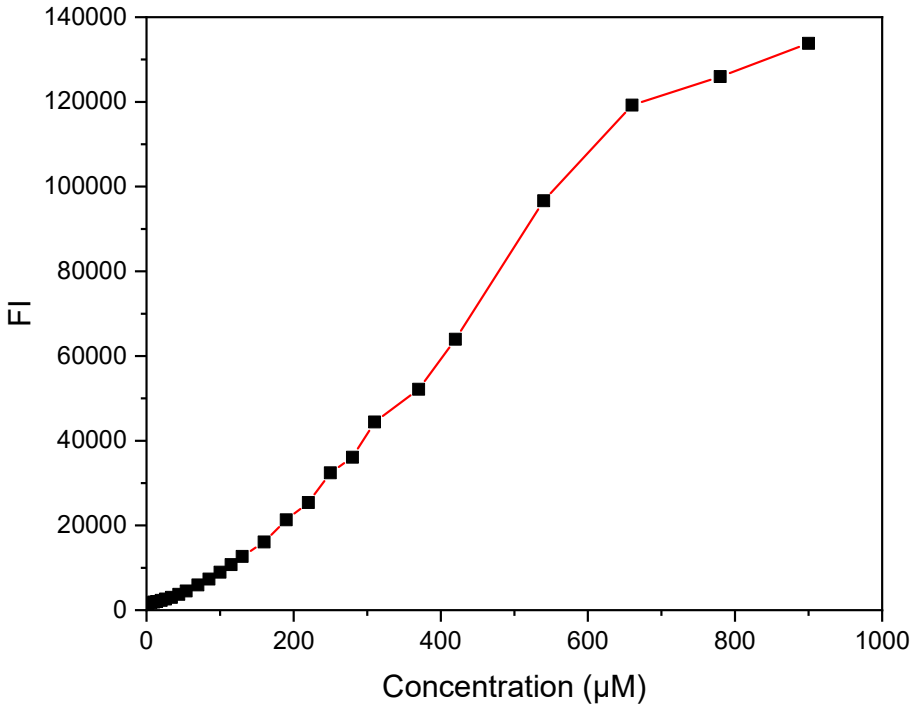


Figure S9. The concentration-dependent change of fluorescence emission intensity for compound **5**. Ex 375 nm, em 500 nm.

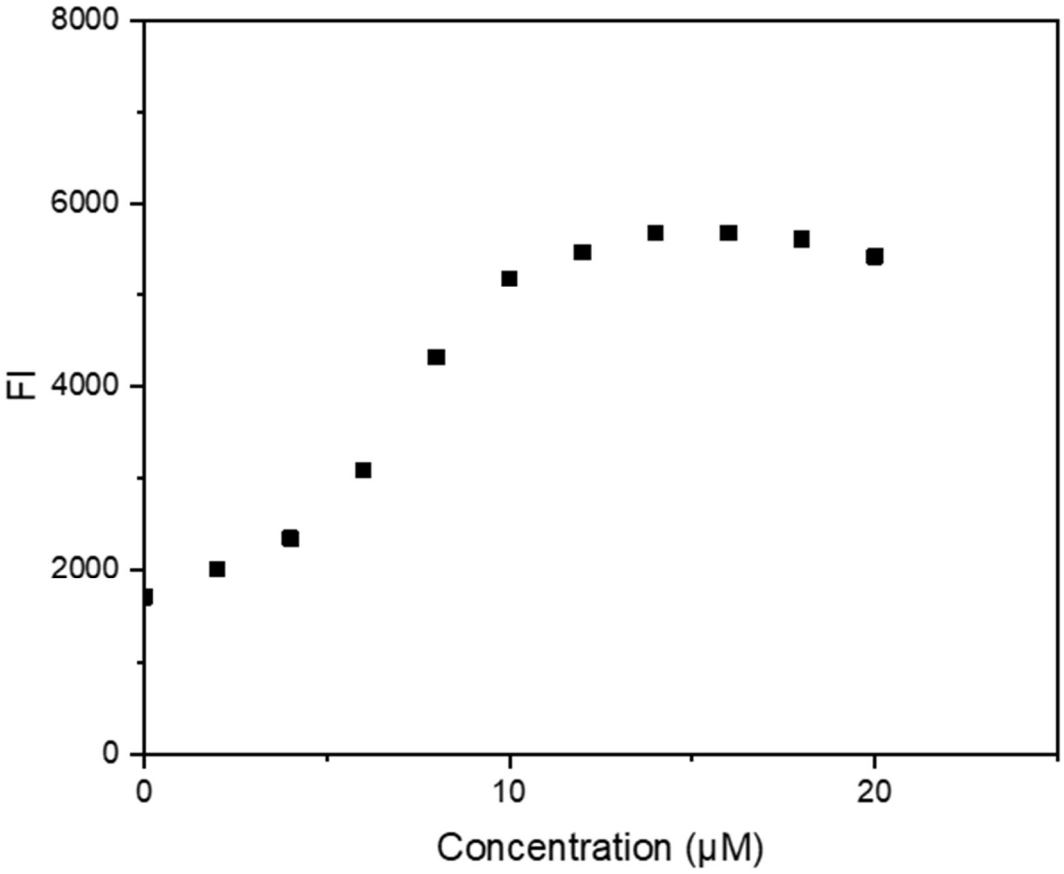


Figure S10. Fluorescence intensity-concentration correlation for host **4** (2 μM) and added spermine. Ex 375 nm, em 475 nm.

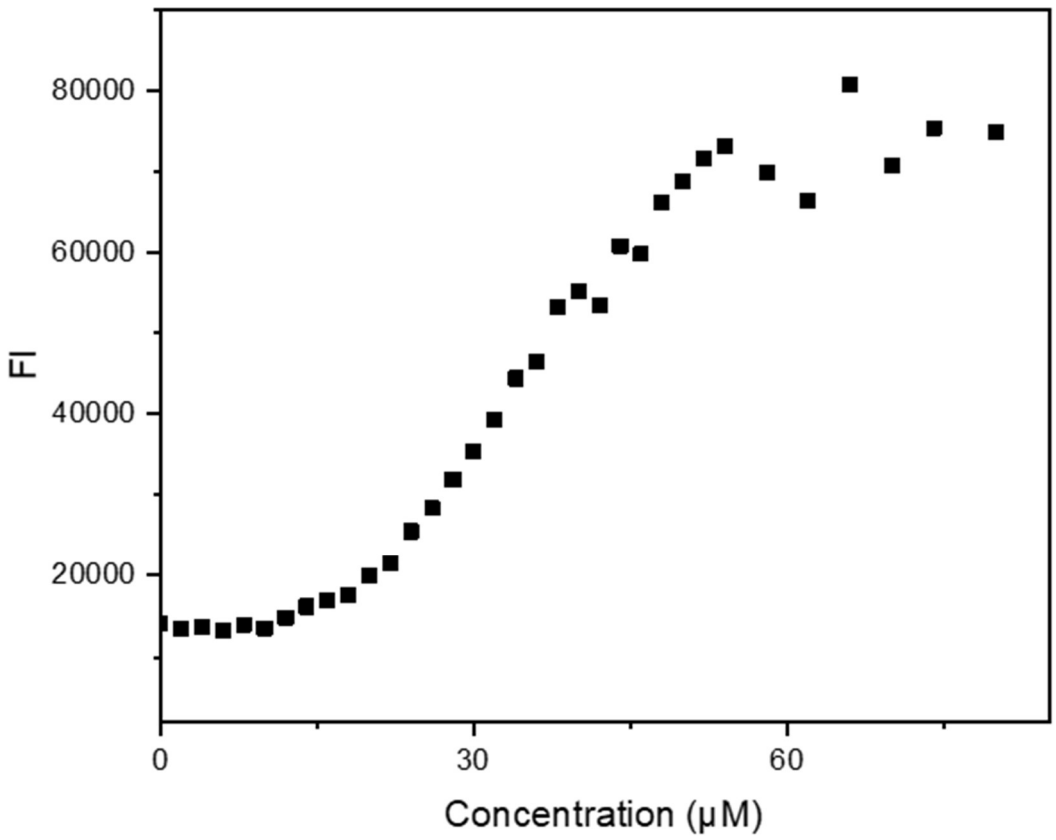


Figure S11. Fluorescence intensity-concentration correlation for host **4** (20 μM) and added spermine. Ex 375 nm, em 475 nm.

4. Host-guest chemistry

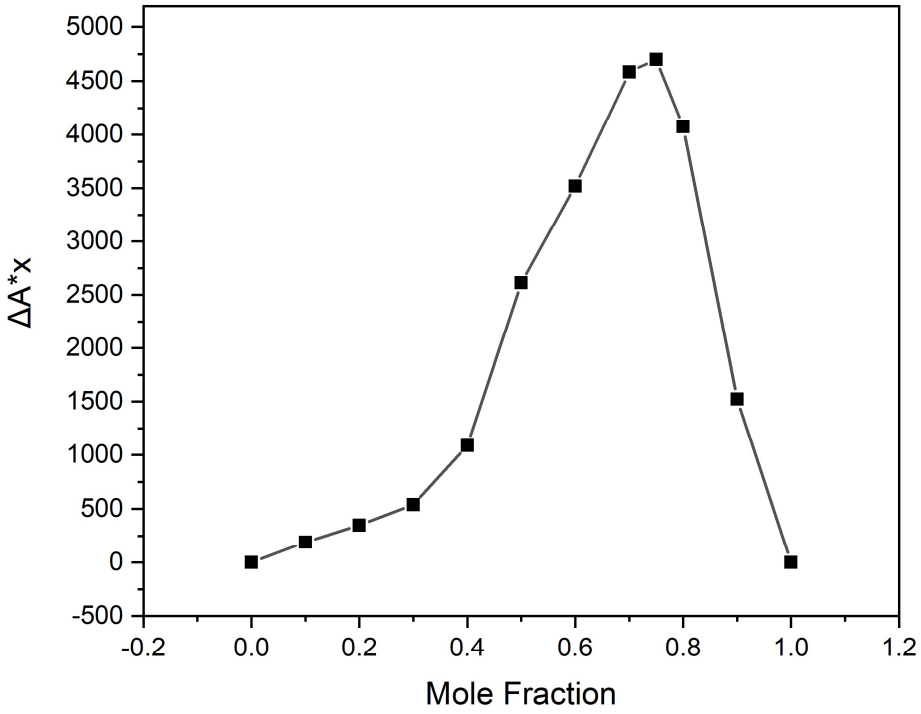


Figure S12. Job plot for the complex of host **4** and spermine by fluorescence spectroscopy in 20 mM sodium phosphate buffer, pH 7.4. ex 375 nm, em 475 nm. [**4**] + [spermine] = 10 μM.

5. Confocal laser scanning microscopy.

B16-F10 cells were seeded in a glass bottom cell culture dish (2×10^5 cells per dish) for 24 hours. The cells were rinsed with Hanks' balanced buffer for three times, and incubated with host **4** (100 μM) in Hanks' balanced buffer for 2 h. Next, cells were washed, and a solution of spermine (200 μM) in Hanks' balanced buffer was added. In the control group, Hanks' balanced buffer without spermine was added. The cells were further incubated for another 1 h, washed twice with Hanks' balanced buffer, and imaged by LSCM.

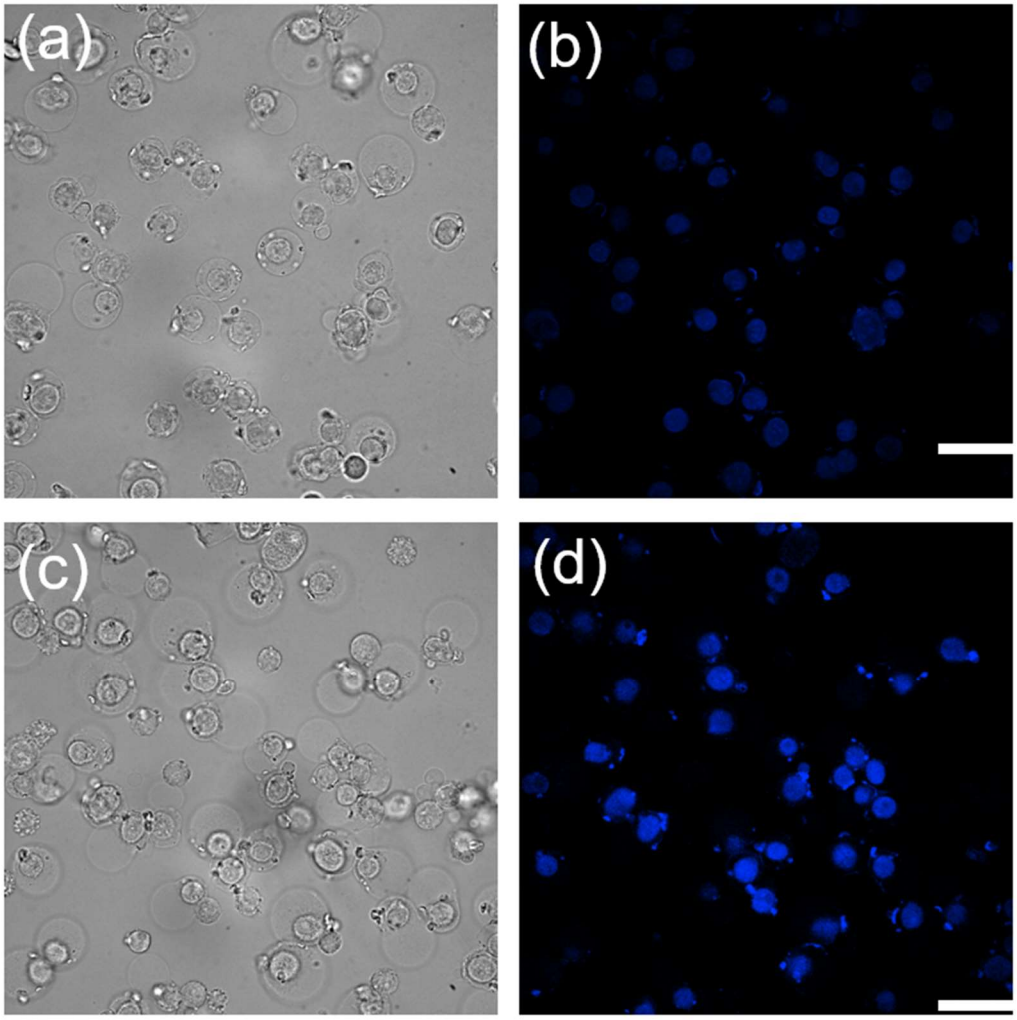


Figure S13. CLSM images recorded for HeLa cells treated with host **4** on the bright field channel (a), or on the fluorescent channel (b). CLSM images recorded for HeLa cells treated with host **4** and spermine on the bright field channel (c), or on the fluorescent channel (d). Scale bar: 50 μm. Ex 405 nm. [**4**] = 100 μM, [spermine] = 200 μM.