Supporting Information to:

A supramolecular red to near-infrared fluorescent probe for detection of drugs in urine

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Materials and Methods

Unless otherwise stated, all solvents and chemicals were purchased from Sigma-Aldrich and used without further purification. Cucurbit[7]uril and cucurbit[8]uril were purchased from Strem Chemicals Inc. (Newburyport, MA) and dried at 110 ºC for 24 hours before use. The synthetic urine was purchased from Clear Test (www.cleartest.com).

Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy

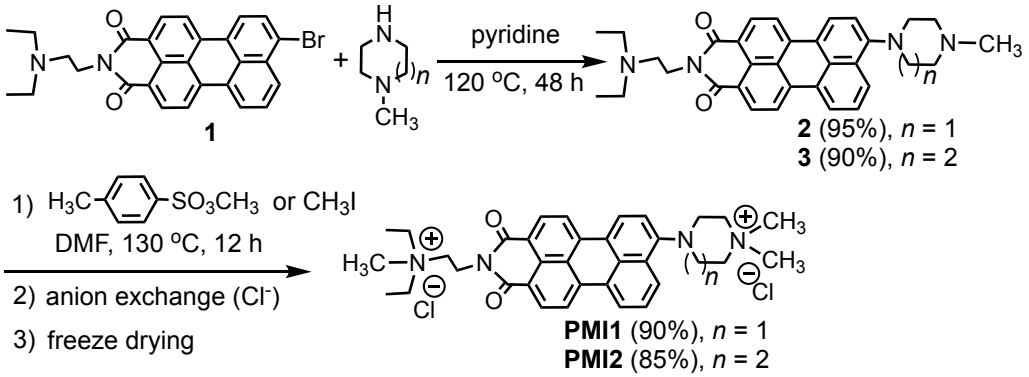
^{1}H- and ^{13}C-NMR spectra were recorded on either a Varian 400 or 500 MHz spectrometer in $CDCl_3$, DMSO-d_6, CF_3COOD, or D_2O. Mass spectra were recorded on a Bruker Autoflex3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS), Waters Micromass XQ detector using ESI^+ and ESI^-, or ESI-TOF MS: Agilent G6230A instrument with purine and HP-Ø921 as internal calibrants internal calibrants.

UV-Vis Spectroscopy, Fluorescence Spectroscopy, and Optical Images

UV-vis spectra were recorded with a dual-beam Perkin Elmer Lambda 950 Spectrophotometer using UV-WIN Lab version 5.1.5 software. Fluorescence spectra were acquired using a Jobin-Yvon Horiba Fluorolog 3-222 Fluorescence Spectrophometer. 1-cm quartz cuvettes or methacrylate plastic cuvettes were used for UV-vis and fluorescence studies. The binding constants of CB8•**PMI1** and CB8•**PMI2** complexes were determined by fluorescence titration and calculated with a 1:1 binding model using the Origin program and the equation (Int=Igh+(Ig-Igh)*(((Go-Ho-1/Kg)/2+sqrt(((Ho+Go+1/Kg)^2)/4-Ho*Go))/Go), where Ig is the fluorescence intensity of guest, Igh is the intensity of host-guest complexes, Go is the concentration of the guest, and Kg is the binding constant. The binding constant of CB8 with different guests (e.g. drugs) was determined by fluorescence displacement titration and calculated with a 1:1:1 host-guest-competitor model using the Origin program and the equation (y=Ig+(Igh(Io,Ig,Kg,Go,Ho)-

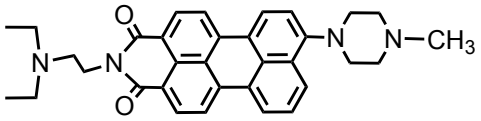
Ig)*Kg*rac(Kg,Kc,x,Go,Ho)/(1+Kg*rac(Kg,Kc,x,Go,Ho)), where Ig is the fluorescence intensity of guest, Io is the fluorescence intensity of host-guest complexes, Kg is the binding constant of host-guest complexes, Kc is the binding constant of host-competitor complexes, Go is the concentration of the guest, Ho is the concentration of the host). The optical images of samples were acquired using a Canon D60 digital camera under room light. The fluorescence images were acquired using a Canon D60 digital camera through a 2" square RG665 color filter (655 nm cut-off, Thorlabs) when samples were excited using an Ulako green LED flashlight (purchased from Amazon.com).

Synthesis of PMI1 and PMI2



Scheme S1: Synthesis of **PMI** and **PMI2**.

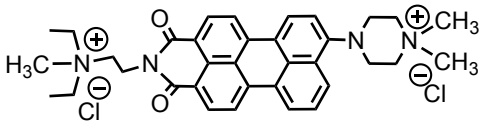
S**ynthesis of 2**



1 (0.5 g, 1.0 mmol, **1** was synthesized according to previous reported procedure[1]) and 1-methylpiperazine (3.0 g, 30.0 mmol) were mixed in pyridine (15 mL) in a round bottom flask. The mixture was stirred at 120 °C for 48 hours under N_2, then most of solvent was removed by vacuum distillation followed by precipitation with excess acetone. The precipitate was collected by suction filtration, washed with acetone, and dried under vacuum at 110 °C for overnight to afford **2** (0.49 g, 0.95 mmol, 95%). ^{1}H NMR (Varian 400 MHz, $CDCl_3$) δ 8.51-8.46 (m, 2H), 8.39 (d, J = 8 Hz, 1H), 8.34-8.30 (m, 2H), 8.24-8.8.20 (m, 2H), 7.60 (t, J = 8 Hz, 1H)), 7.19 (d, J = 8 Hz, 1H), 4.38 (br, 2H), 3.27 (br), 2.98-2.80 (br, 10H), 2.47 (s, 3H),

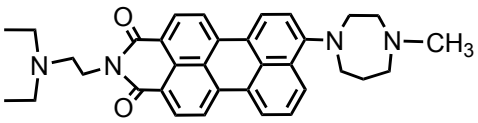
1.25 (br, 6H) ppm; ^{13}C NMR (Varian 100 MHz, $CDCl_3$): δ = 168.2, 152.6, 137.3, 137.2, 131.2, 129.5, 128.7, 126.7, 126.0, 124.5, 123.8, 120.2, 119.4, 118.7, 115.7, 55.4, 52.9, 49.6, 47.7, 46.2, 37.5, 12.1 ppm; ESI$^+$: m/z = 519.26 $[M+H]^+$(calc'd. 519.28 for $C_{33}H_{35}N_4O_2$).

Synthesis of PMI1



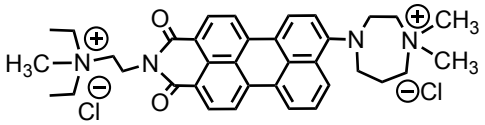
Compound **2** (0.12 g, 0.23 mmol) was added into 4 mL of DMF in a round-bottom flask. Then Methyl p-toluenesulfonate (1.0 g, 5.4 mmol) was added. The resulting mixture was stirred at 130 °C for 12 hours and most of solvent was removed by vacuum distillation. A mixture of acetone and TEA (9:1 in ratio) was added to form a precipitate and it was collected by suction filtration, washed with acetone, and dried under vacuum at 110 °C overnight. The solid was dissolved in 10 mL of double distilled water for anion exchanged. A total of 3.5 g of anion exchange resin (Dowex 1-X8, 100-200 mesh, J.T. BAKER Chemical CO.) was added to a 17% NaCl(aq) solution in a 125-mL boiling flack and treated for one hour. The resin solution was placed in a 1-cm diameter glass column and washed with double distilled water to remove excess NaCl. The purple dye solution was poured into the anion exchange column and eluted with double distilled water. The collected purple solution was dried using a Labconco freeze dryer (FreeZone 2.5 Liter -84C Benchtop) for 24 hours. A total of 0.127 g (0.207 mmol, 90% yield) of purple solid of **PMI1** was collected. ^{1}H NMR (Varian 400 MHz, DMSO-d_6) δ 8.77-8.66 (m, 4H), 8.51-8.47 (m, 2H), 8.25 (d, J = 8 Hz, 1H), 7.75 (t, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 4.40 (t, J = 8 Hz, 2H), 3.72 (br, 2H), 3.49-3.44 (br, 10H), 3.07 (s, 3H), 1.31 (t, J = 8 Hz, 6H) ppm; ^{13}H NMR (Varian 125 MHz, DMSO-d_6) δ = 163.4, 151.2, 137.6, 131.8, 129.7, 129.3, 128.5, 127.5, 126.2, 125.6, 124.5, 121.3, 120.6, 119.6, 117.9, 61.4, 56.7, 55.5, 47.4, 46.5, 33.4, 7.9 ppm; ESI$^+$: m/z = 274.06 $[M]^{2+}$ (calc'd. 274.16 for $C_{35}H_{40}N_4O_2$).

Synthesis of 3



A mixture of **1** (0.2 g, 0.40 mmol) and 1-Methylhomopiperazine (0.5 g, 4.37 mmol) in pyridine (5 mL) was stirred at 120°C for 48 hrs under N_2. The solvent was removed by vacuum distillation and the resulting mixture was suspended in water. The precipitate was collected by suction filtration and washed with water. The solid residue was dried under vacuum at 110 °C for overnight to afford **3** (0.191 g, 0.36 mmol, 90%). ^{1}H NMR (Varian 400 MHz, $CDCl_3$) δ 8.49-8.45 (m, 2H), 8.39 (d, *J* = 5 Hz, 1H), 8.30-8.28 (m, 2H), 8.23 (d, *J* = 10 Hz, 1H), 8.19 (d, *J* = 10 Hz, 1H)), 7.60 (t, *J* = 5 Hz, 1H), 7.21 (d, *J* = 10 Hz, 1H), 4.35 (t, *J* = 10 Hz, 2H), 3.69 (br, 2H), 2.97-2.90 (br, 4H), 2.81-2.80 (br, 4H), 2.57 (s, 3H), 2.16 (br, 2H), 1.21 (t, *J* = 5 Hz, 6H) ppm; ^{13}H NMR (Varian 125 MHz, DMSO-d_6) δ 165.9, 154.4, 137.7, 131.5, 131.2, 129.9, 129.2, 128.7, 127.7, 125.6, 124.9, 124.0, 119.4, 118.5, 115.9, 57.9, 55.0, 49.7, 47.8, 47.1, 28.0, 12.2 ppm; ESI$^+$: m/z = 533.30 $[M+H]^+$ (calc'd. 533.28 for $C_{34}H_{37}N_4O_2$).

Synthesis of PMI2



3 (50 mg, 0.094 mmol) and methyl iodide (0.5 g, 3.52 mmol) were mixed in DMF (5 mL) in a round bottom flask. The resulting mixture was stirred at 100 °C for 24 hrs. The solvent was removed by vacuum distillation and precipitated with ethyl acetate. The precipitate was collected by suction filtration and dried under vacuum at 110 °C overnight. The product was dissolved in 5 mL of double distilled water for anion exchange. The purple solution was poured into an anion exchange column (Dowex 1-X8, 100-200 mesh, J.T. BAKER Chemical CO.) and eluted with excess ultra-pure water. The collected purple solution was dried using a Labconco freeze dryer (FreeZone 2.5 Liter -84C Benchtop) for 24 hours. A total of 50 mg (0.080 mmol, 85% yield) of **PMI2** was collected. ^{1}H NMR (Varian 400 MHz, DMSO-d_6) δ 8.75-8.61 (m, 4H), 8.50-8.46 (d,d, *J* = 8 Hz, 2H), 8.24 (d, *J* = 8 Hz, 1H), 7.75 (t, *J* = 4 Hz, 1H), 7.38 (d, *J* = 8 Hz, 1H), 4.40 (br, 2H), 3.86 (br, 2H), 3.75-3.69 (br, 4H) 3.49-3.42 (m, 8H), 3.21 (s, 3H), 3.06 (s, 6H), 2.29 (br, 2H), 1.32 (t, *J* = 4 Hz, 6H) ppm; ^{13}C NMR (Varian 100 MHz, DMSO-d_6) δ 165.9, 138.9, 137.7, 133.7, 132.2, 130.3, 129.8, 130.0, 129.0, 126.5, 124.2, 122.5, 122.2, 120.0, 119.3, 65.1, 61.0, 57.9, 56.2, 55.4, 54.7, 53.0, 47.3, 33.0, 20.8, 6.5 ppm; HRMS (ESI-TOF) m/z = 281.16 $[M]^{2+}$ (calc'd. 281.16 for $C_{36}H_{42}N_4O_2$).

Supplementary Figures

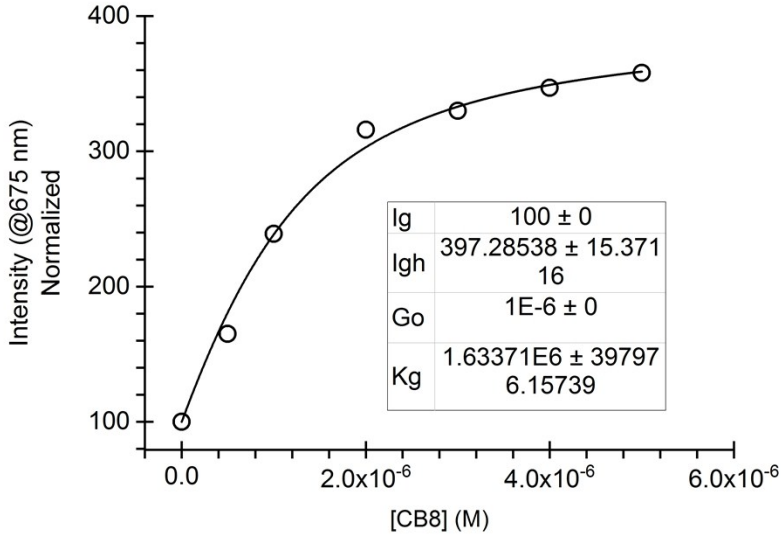


Figure S1. The binding curve for CB8•**PMI1** (fluorescence titration experiment of **PM1** (1.0 μM) in ultra pure water in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding. The fluorescence intensity of **PMI1** at 675 nm was normalized to 100.

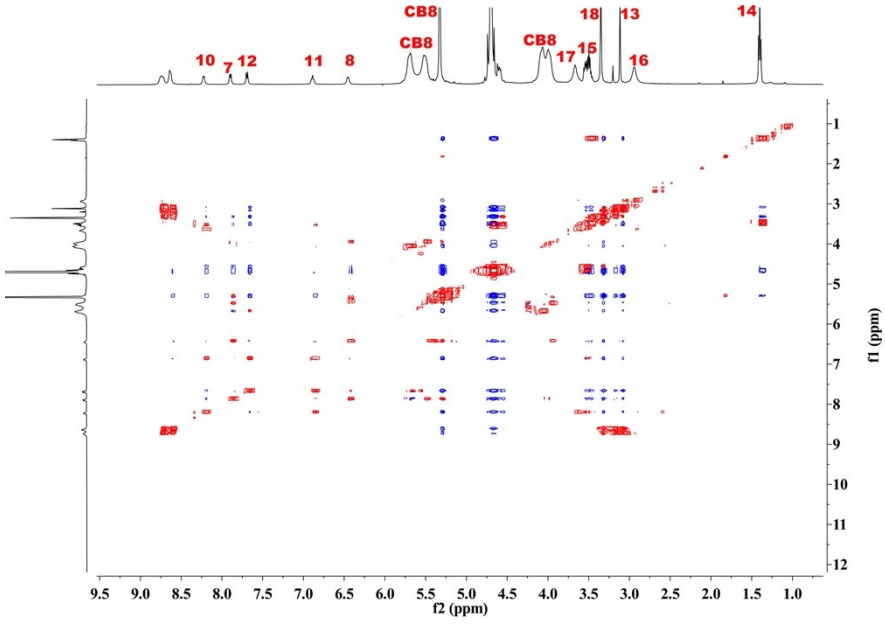


Figure S2. 2D NMR spectrum (COSY) of CB8•**PMI1** complex (1:1, 1.0 mM) in D_2O.

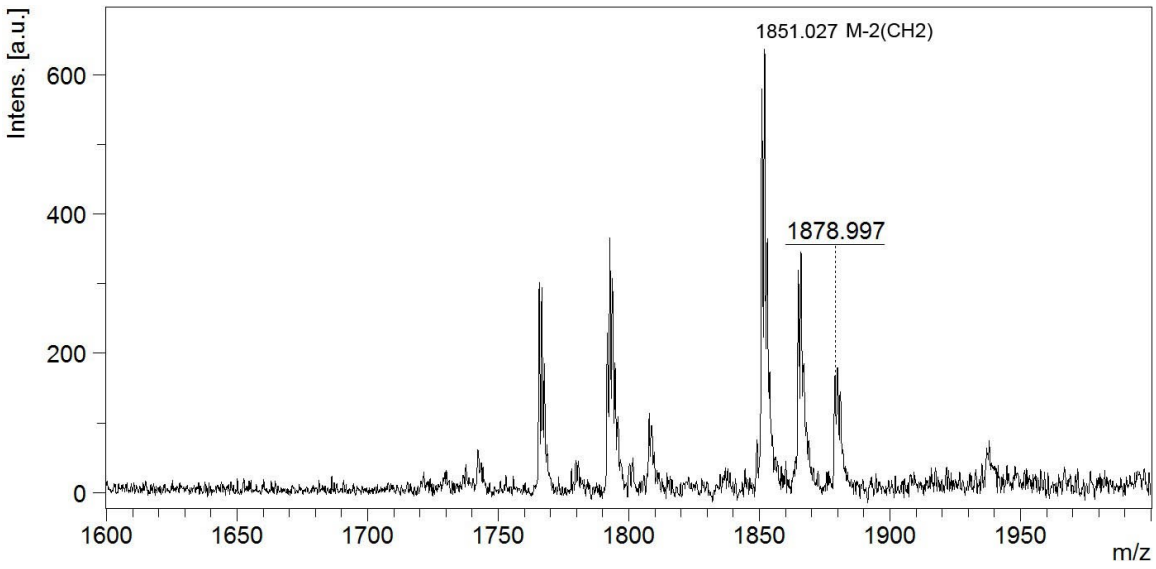


Figure S3. MALDI mass of CB8•**PMI1** (1:1) in H_2O using *α*-Cyano-4-hydroxycinnamic acid as a matrix. ([CB8•**PMI1**+H]$^{2+}$: observed mass 1878.997 Da, calculated mass 1878.41Da)

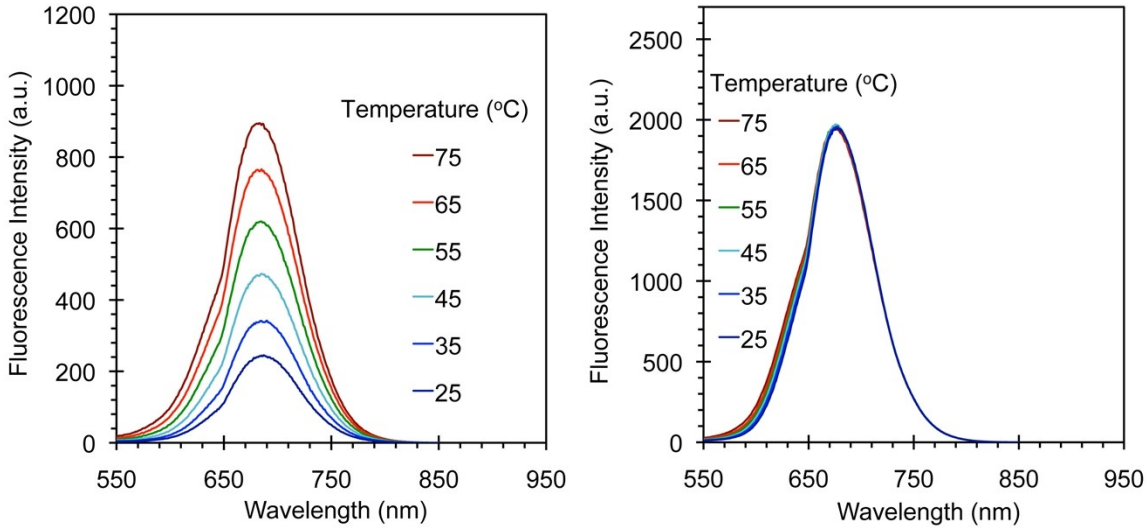


Figure S4. Fluorescence emission spectra of **PMI1** (10 μM) (left) and the CB8•**PMI1** complex (1:1, 10 μM) (right) in water at various temperatures.

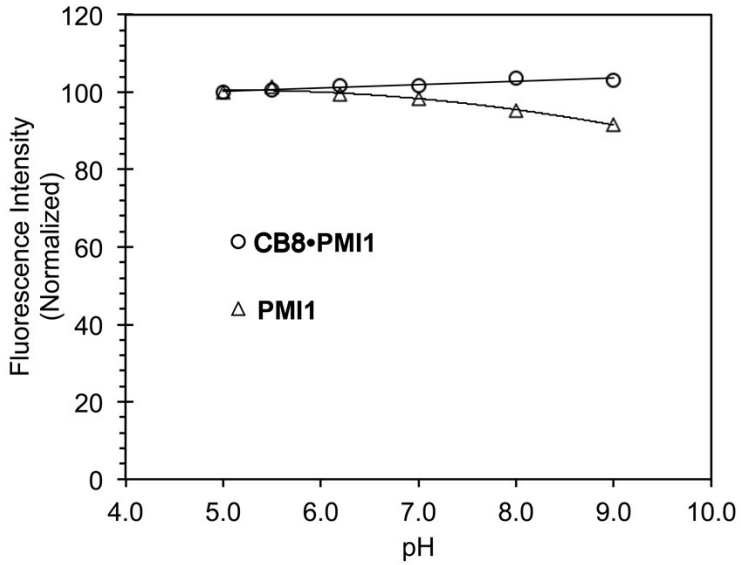


Figure S5. Fluorescence intensity of **PMI1** (10 μM) and the CB8•**PMI1** complex (1:1, 10 μM) in ammonium-acetate buffers with different pH from 5 to 9. The fluorescence intensity of **PMI1** and CB8•**PMI1** at λ_{max} was normalized to 100.

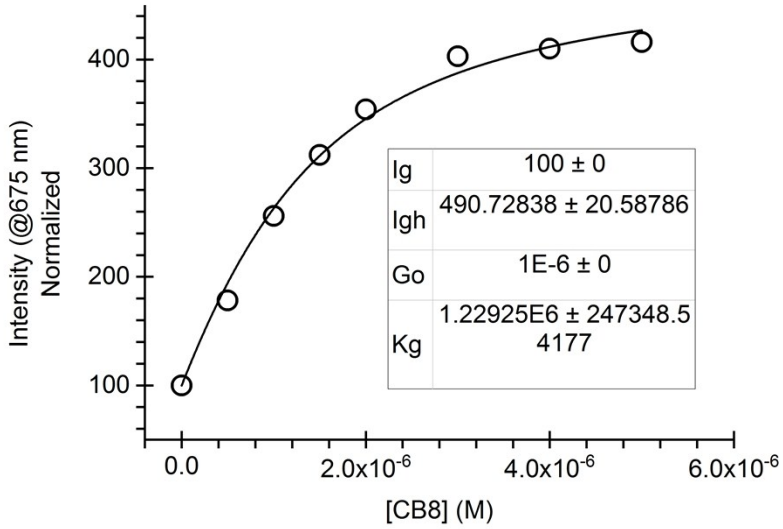


Figure S6. The binding curve for CB8•**PMI1** (fluorescence titration experiment of **PMI1** (1.0 μM) in ammonium-acetate buffer at pH 5.0 in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding. The fluorescence intensity of **PMI1** at 675 nm was normalized to 100.

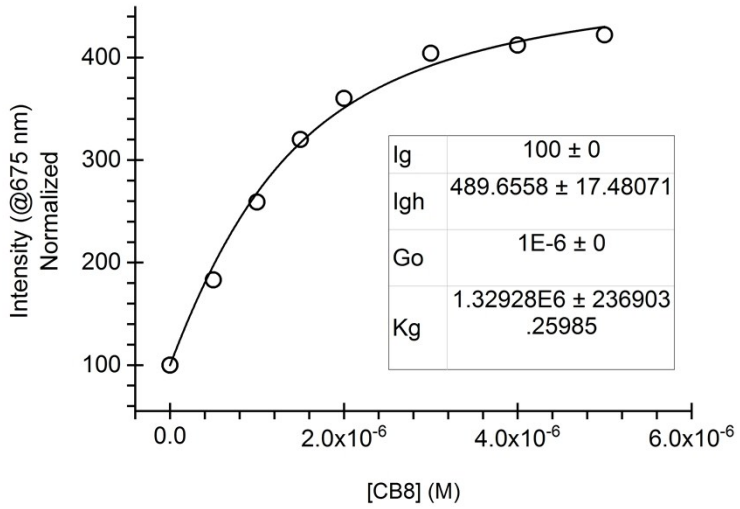


Figure S7. The binding curve for CB8•**PMI1** (fluorescence titration experiment of **PMI1** (1.0 μM) in ammonium-acetate buffer at pH 7.0 in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding. The fluorescence intensity of **PMI1** at 675 nm was normalized to 100.

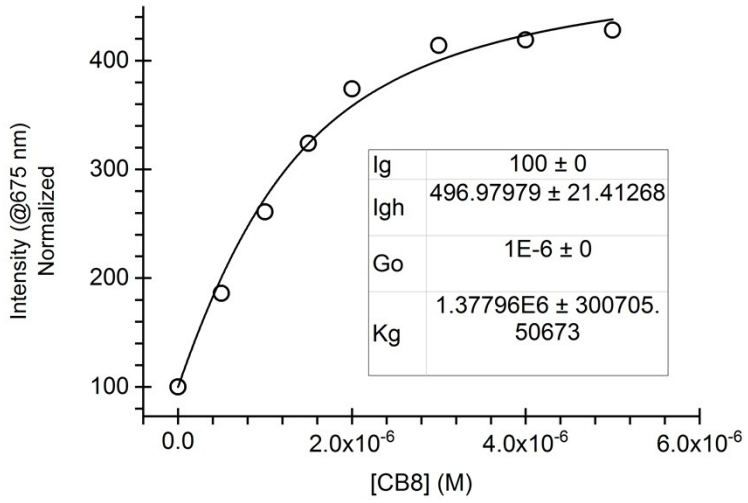


Figure S8. The binding curve for CB8•**PMI1** (fluorescence titration experiment of **PMI1** (1.0 μM) in ammonium-acetate buffer at pH 9.0. in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding. The fluorescence intensity of **PMI1** at 675 nm was normalized to 100.

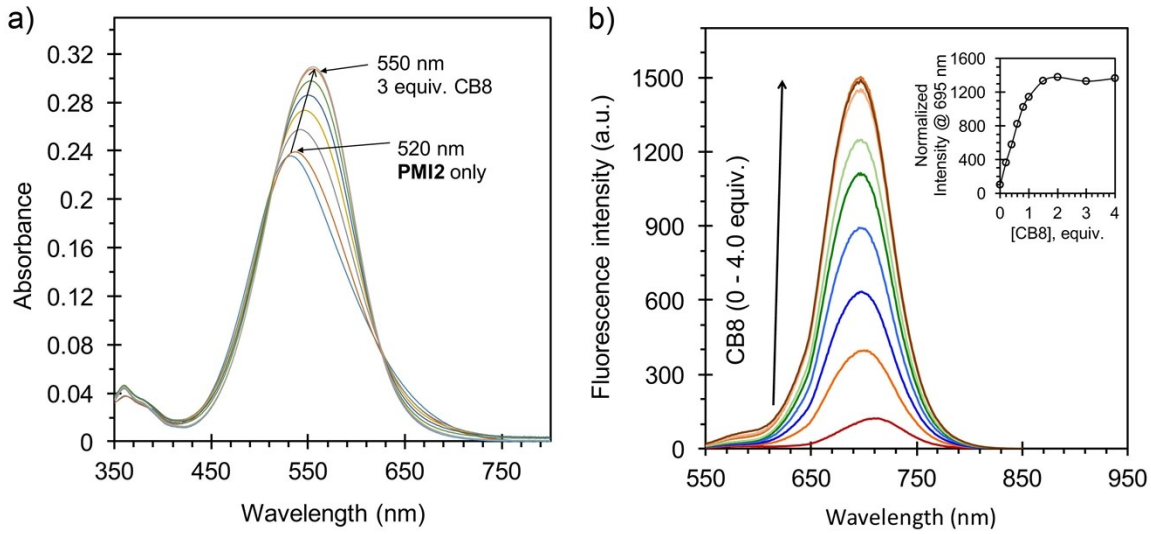


Figure S9. a) UV-vis absorbance and b) fluorescence emission spectra of **PMI2** (10 μM) in the presence of CB8.

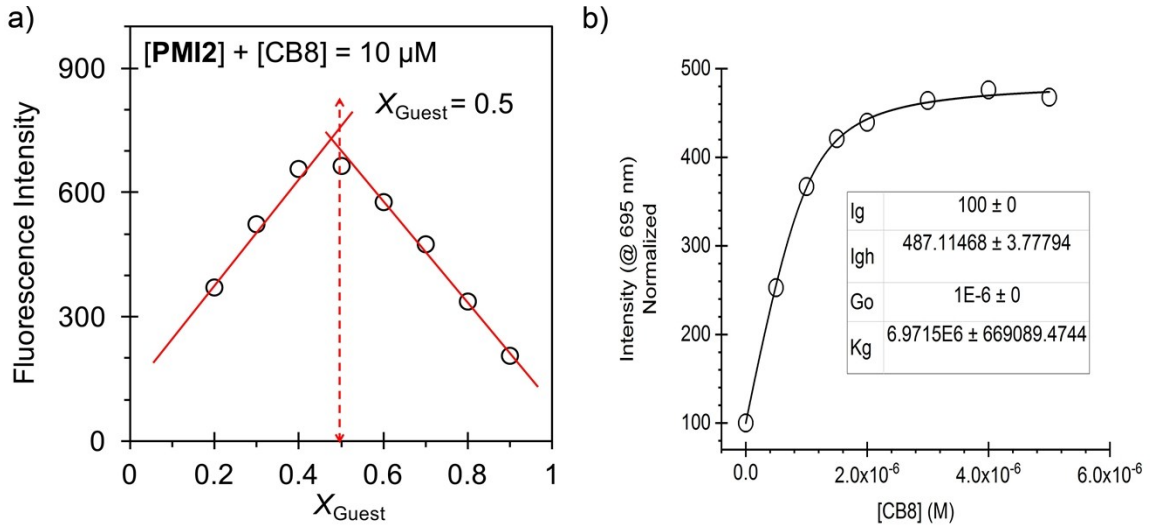


Figure S10. a) A Job plot for CB8•**PMI2** (the total concentration of guest **PMI2** and CB8 was fixed at 10 μM). b) The binding curve for CB8•**PMI2** (fluorescence titration of 1.0 μM **PMI2** in the presence of 0 to 4.0 equivalents CB8. Fluorescence intensity at 695 nm was plotted against the concentration of CB8. The solid line represents the best fitting of the data to a 1:1 binding. The fluorescence intensity of **PMI2** at 695 nm was normalized to 100.

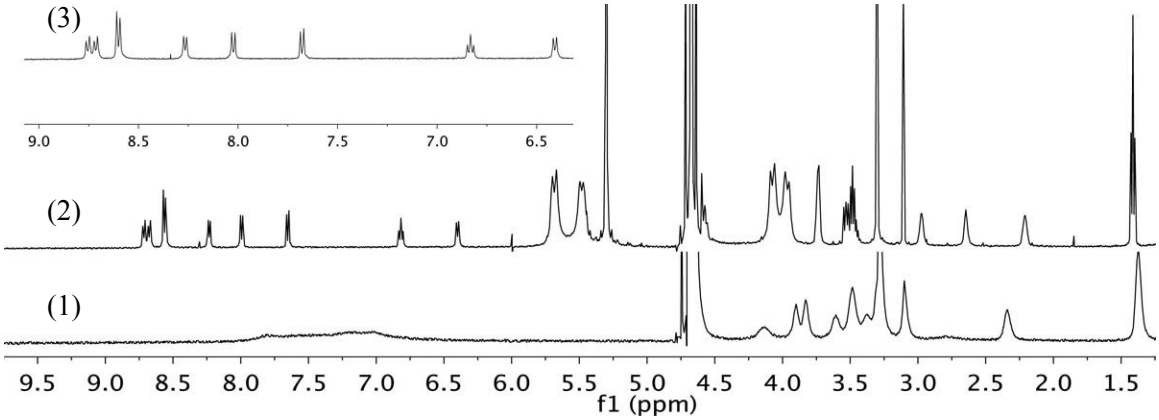


Figure S11. (1) ^{1}H-NMR of **PMI2** (1.0 mM), (2) CB8•**PMI2** (1:1, 1.0 mM) in D_2O, and (3) the highlighted area of aromatic protons of CB8•**PMI2.**

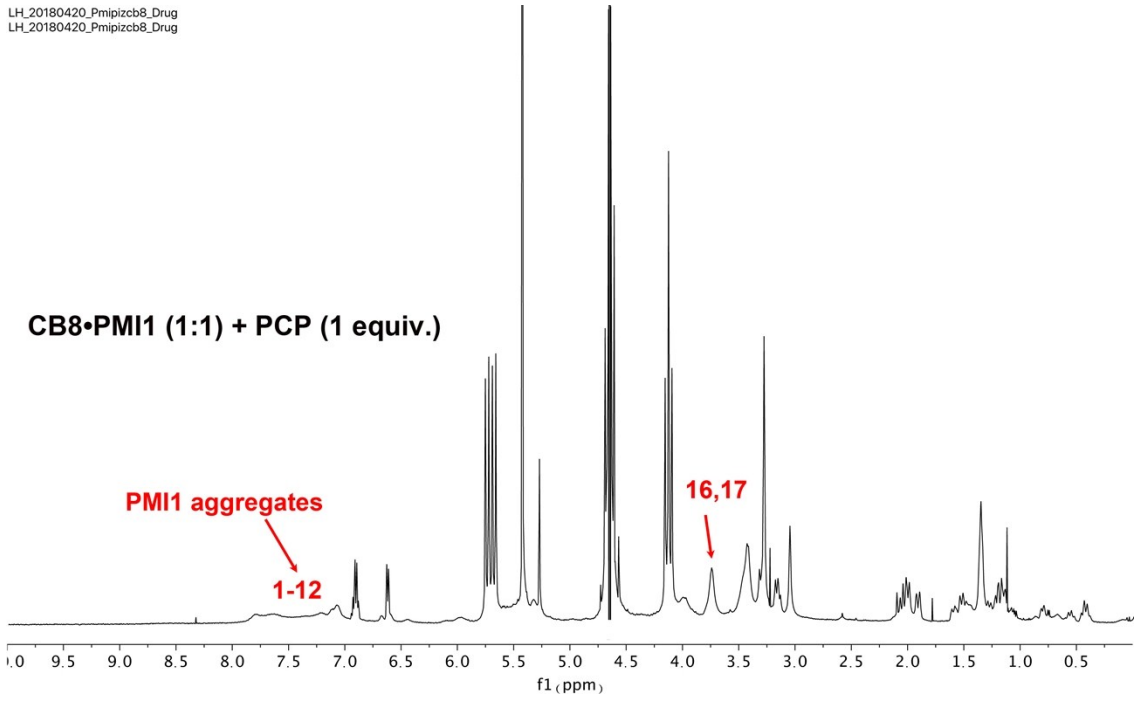


Figure S12. ^{1}H-NMR of CB8•**PMI1** (1:1, 1.0 mM) in the presence of 1.0 equiv. of PCP in D_2O.

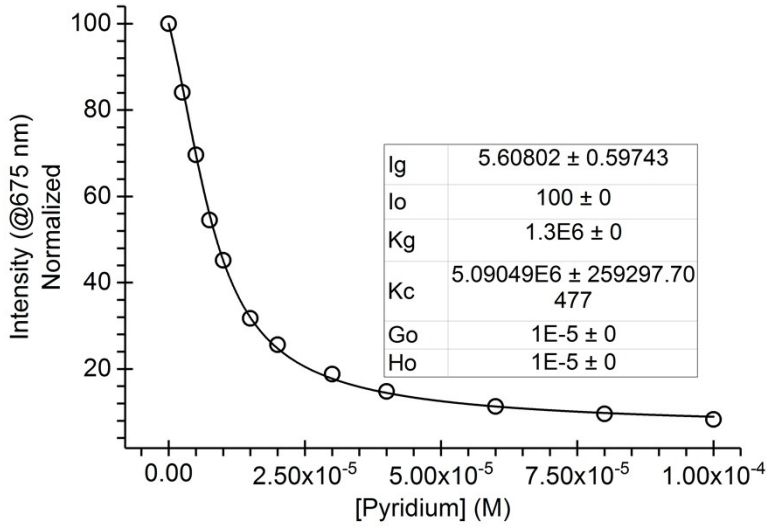


Figure S13. The binding curve for the displacement of CB8•**PMI1** with Pyridium (fluorescence titration experiment of CB8•**PMI1** (10 μM, 1:1) in Tris-HCl buffer (pH 7.4) in the presence of Pyridium and the fluorescence intensity at 675 nm was plotted against the concentration of Pyridium). The fluorescence intensity of CB8•**PMI1** at 675 nm was normalized to 100.

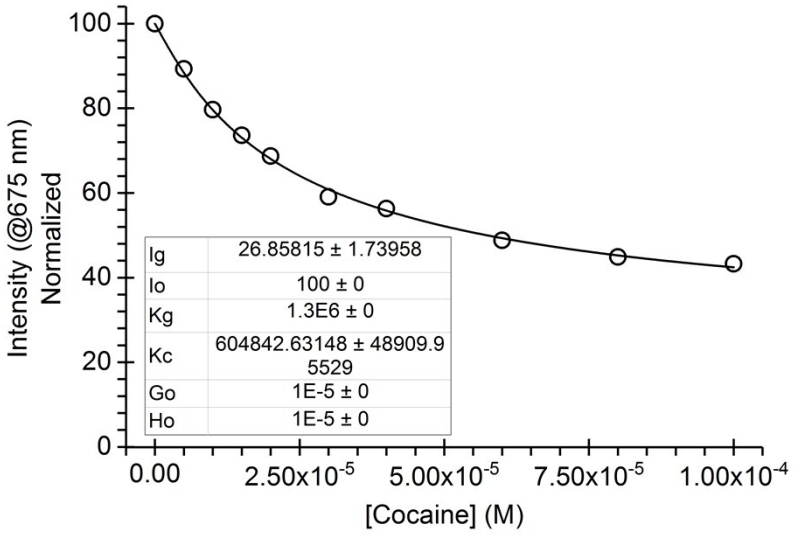


Figure S14. The binding curve for the displacement of CB8•**PMI1** with Cocaine (fluorescence titration experiment of CB8•**PMI1** (10 μM, 1:1) in Tris-HCl buffer (pH 7.4) in the presence of Cocaine and the fluorescence intensity at 675 nm was plotted against the concentration of Cocaine). The fluorescence intensity of CB8•**PMI1** at 675 nm was normalized to 100.

Detection Limit:

The limit of detection (LOD) was calculated from the fluorescence titration using following equation.[2]

$$LOD = 3\ \sigma/K$$

Where σ is the standard deviation and K is the slope between the fluorescence intensity of CB8•**PMI1** versus drug concentration.

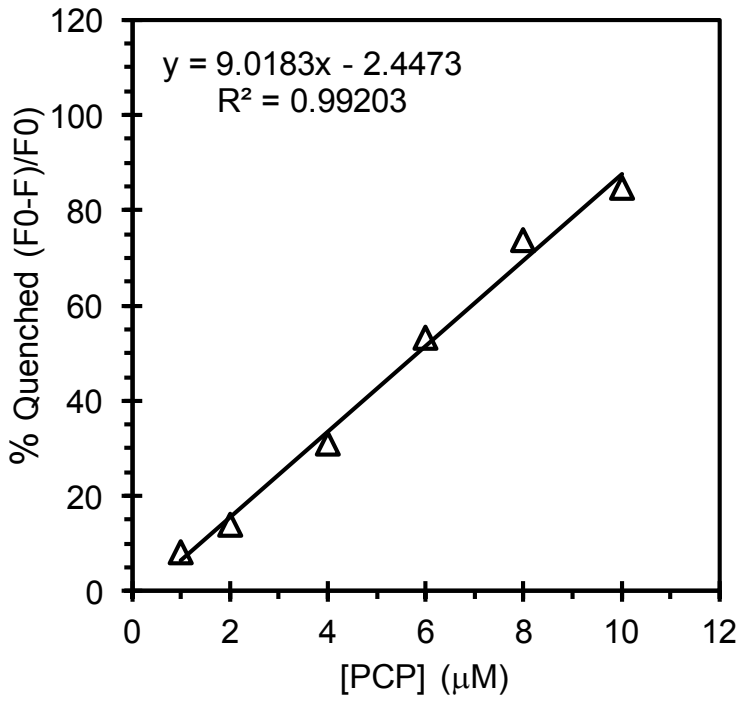


Figure S15. Linear plots of (F0-F) in the presence of increasing concentration of PCP. Where F0 and F are the fluorescence intensity of CB8•**PMI1** (1:1, 10 μM) in Tris-HCl buffer (pH 7.4) in the absence and presence of PCP, respectively.

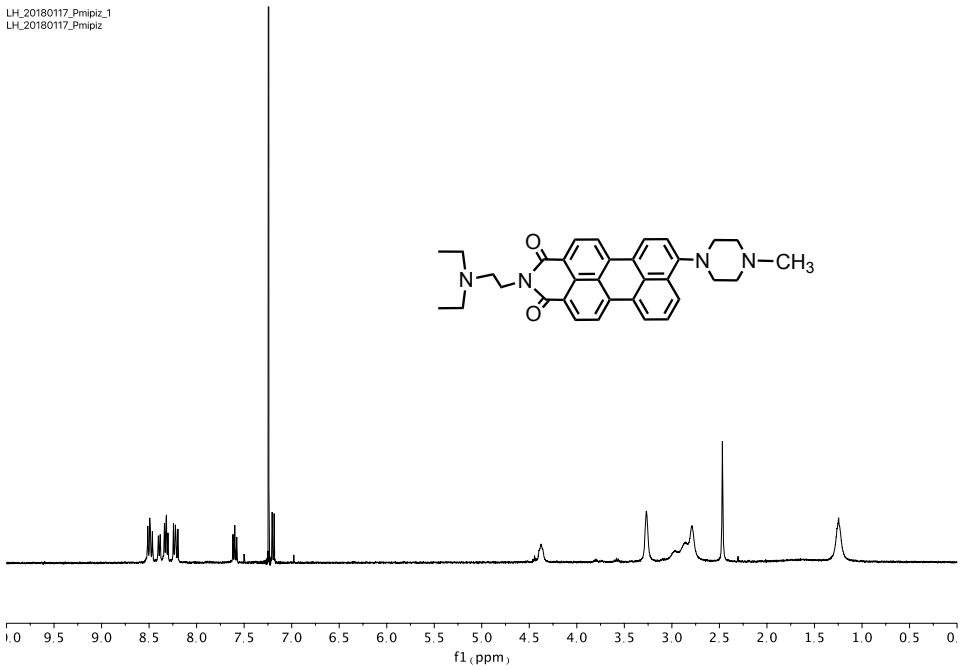


Figure S16. ^{1}H-NMR of **2** in $CDCl_3$.

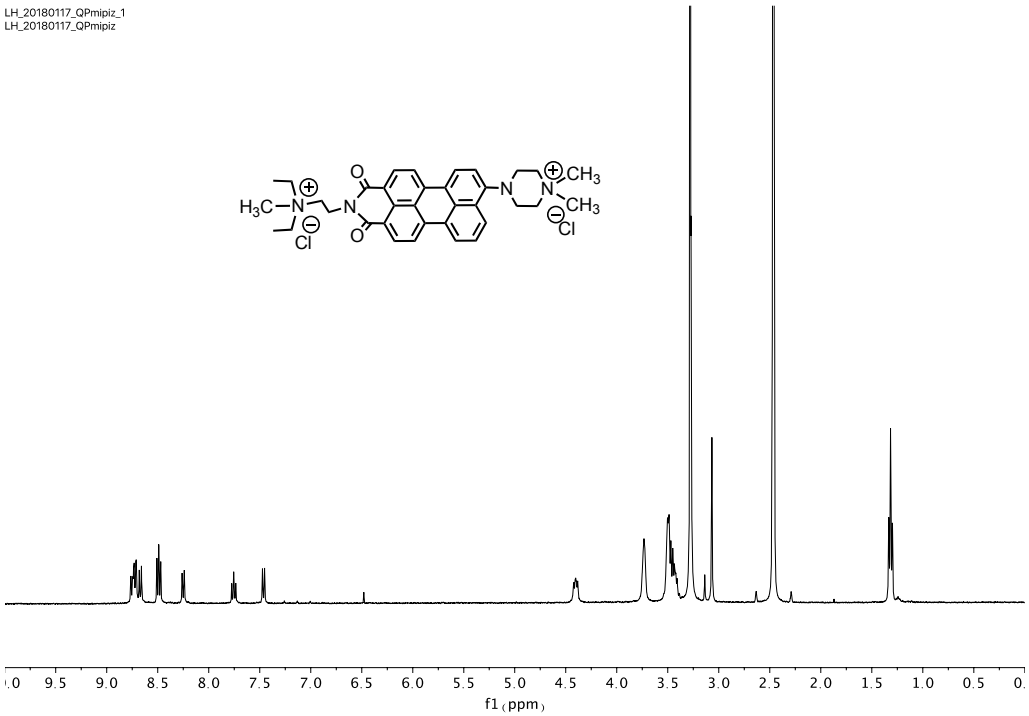


Figure S17. ^{1}H-NMR of **PMI1** in DMSO-d_6.

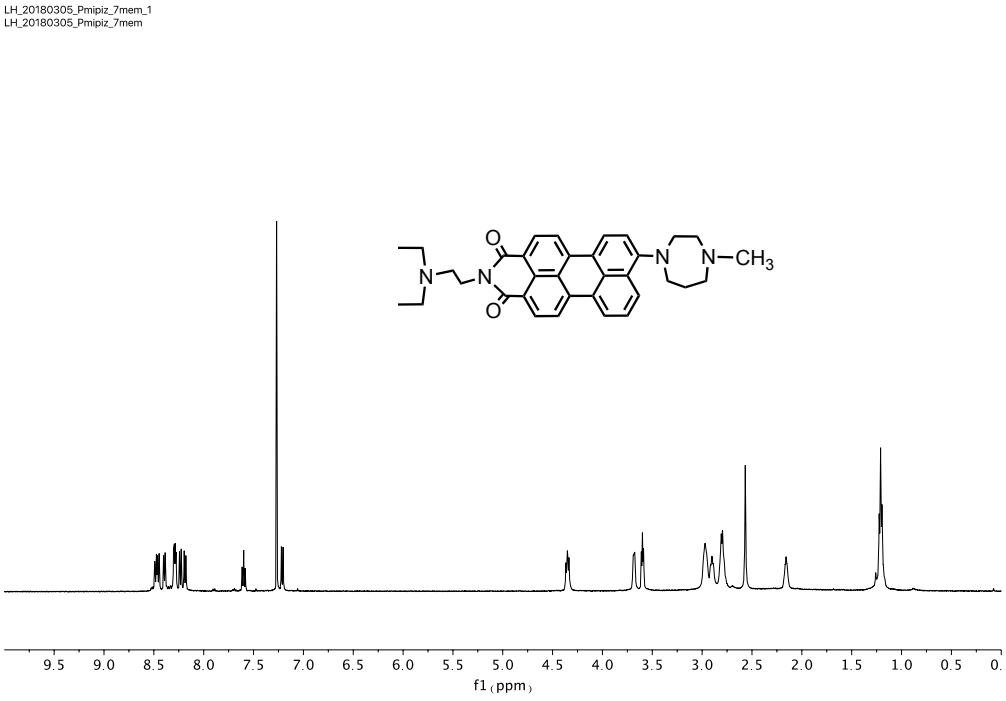


Figure S18. ^{1}H-NMR of **3** in $CDCl_3$.

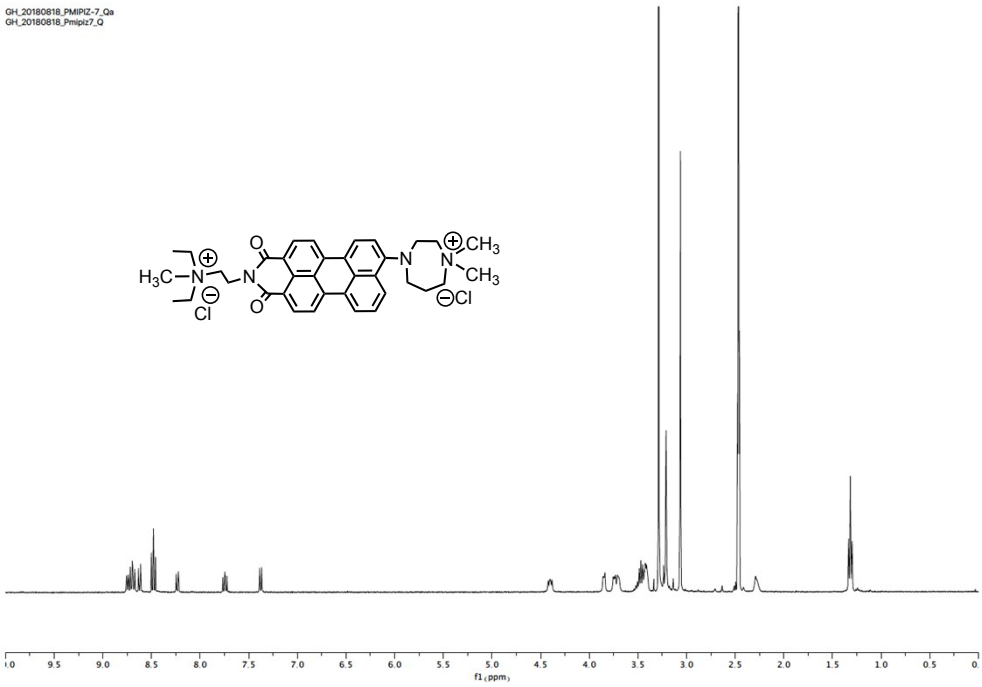


Figure S19. ^{1}H-NMR of **PMI2** in DMSO-d_6.

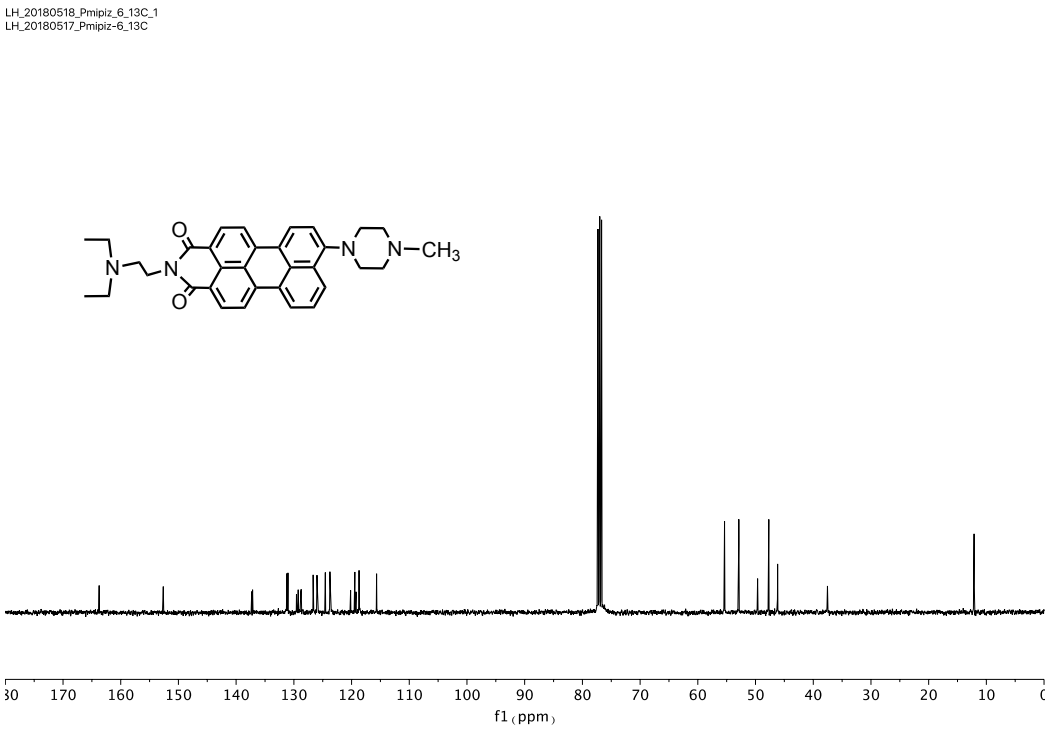


Figure S20. ^{13}C-NMR of **2** in $CDCl_3$

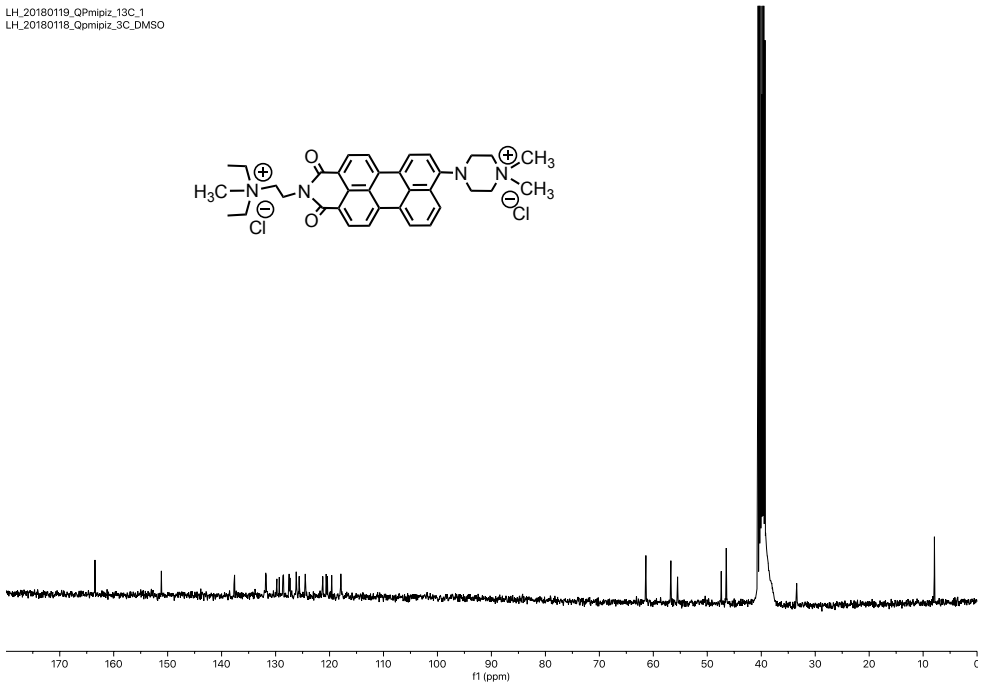


Figure 21. ^{13}C-NMR of **PMI1** in DMSO-d_6.

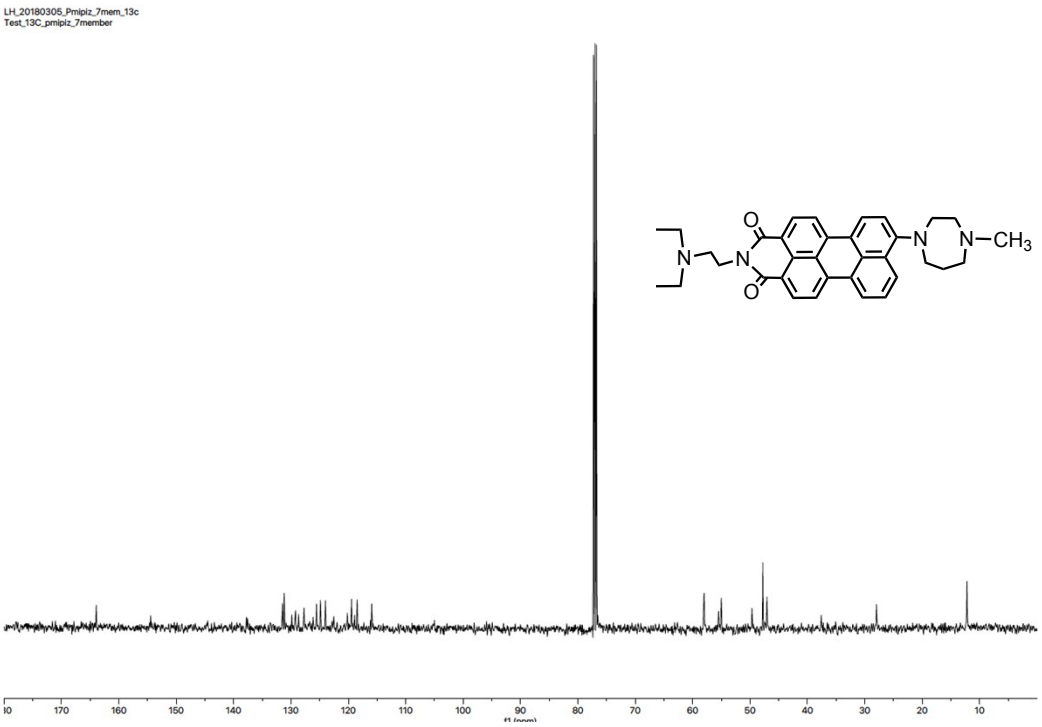


Figure S22. ^{13}C-NMR of **3** in $CDCl_3$

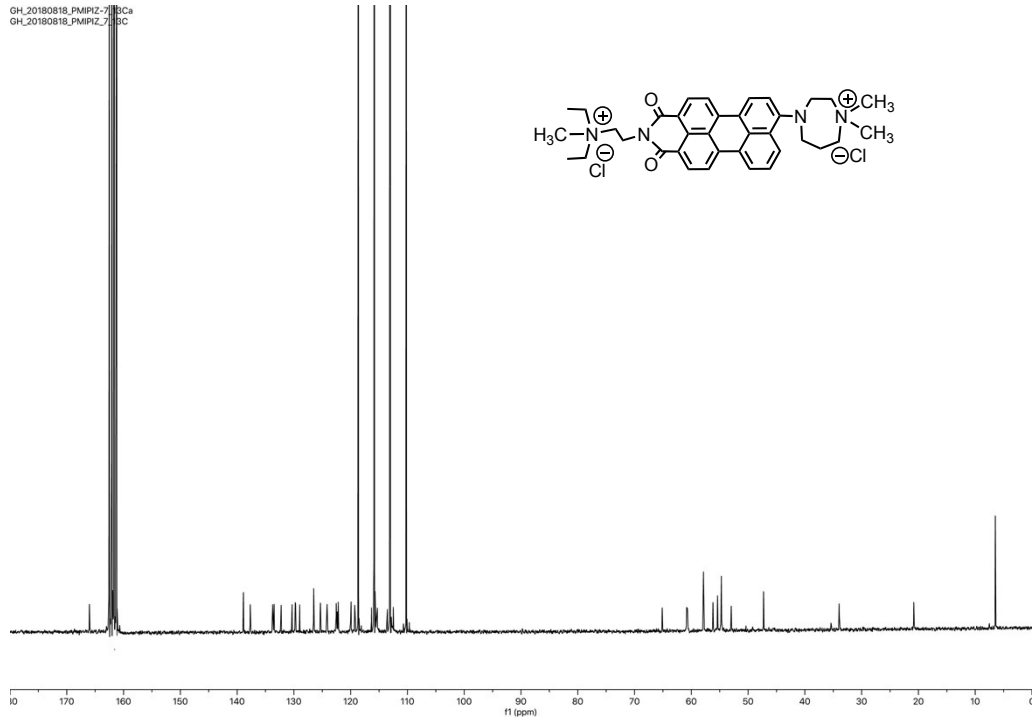


Figure S23. ^{13}C-NMR of **PMI2** in DMSO-d_6

Host-Guest Complex	Solvent/pH	Binding Constant
CB8•**PMI1**	ultra pure water	$K_a = 1.63 \times 10^6\ M^{-1}$
CB8•**PMI1**	Tris-HCl/pH 7.4	$K_a = 1.32 \times 10^6\ M^{-1}$
CB8•**PMI1**	ammonium acetate/pH 5	$K_a = 1.23\times 10^6\ M^{-1}$
CB8•**PMI1**	ammonium acetate/ pH 7	$K_a = 1.33 \times 10^6\ M^{-1}$
CB8•**PMI1**	ammonium acetate/ pH 9	$K_a = 1.38 \times 10^6\ M^{-1}$
CB8•**PMI2**	Tris-HCl/ pH 7.4	$K_a = 6.97 \times 10^6\ M^{-1}$
CB8•Pyridium	Tris-HCl/ pH 7.4	$K_a = 5.09 \times 10^6\ M^{-1}$
CB8•COC	Tris-HCl/ pH 7.4	$K_a = 6.05 \times 10^5\ M^{-1}$

Table S1. Binding constant of CB8•**PMI1**, CB8•**PMI2**, CB8•pyridium and CB8•COC.

1.8 References

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2. X.-F. Yang, L. Wang, H. Xu, M. Zhao, *Analytical Chemical Acta*, 2009, **631**, 91-95.