Supporting Information to:

A Colorimetric and Fluorescent Dual-Modal Displacement Probe Based on Host-Assisted Modulation of Intramolecular Charge Transfer and Deaggregation

Gyan H. Aryal,[a] Kunchao Lu,[b] Guosong Chen,[b] Kenneth W. Hunter,*[a] and Liming Huang*[a]

[a.] *Department of Microbiology and Immunology, School of Medicine, University of Nevada, Reno, NV 89557, USA.*
[b.] *College of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing 210009, China*
E-mails: huang@med.unr.edu

Materials and Methods

Unless otherwise stated, all solvents and chemicals were purchased from Sigma-Aldrich and used without further purification. Cucurbit[8]uril (CB8) was purchased from Strem Chemicals Inc. (Newburyport, MA) and dried at 110 ºC for 24 hours before use.

Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy

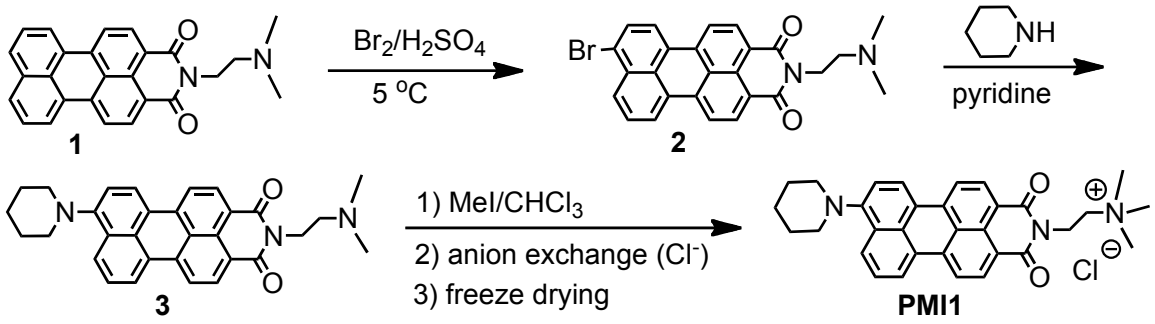
^{1}H- and ^{13}C-NMR spectra were recorded on either a Varian 400 or 500 MHz spectrometer in $CDCl_3$, DMSO-d_6, CF_3COOD, or D_2O. Mass spectra were recorded on a Bruker Autoflex3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS).

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 polystyrene (PS) plastic cuvettes were used for UV-vis and fluorescence studies. Quantum yields were determined using another a previous reported water-soluble perylenemonoimide as a standard (QY=0.50 in methanol).[1,2] The binding constants of CB8•**PMI1** complexes were determined by fluorescence titration and calculated with a 1:1 binding model using the Origin program and the equation

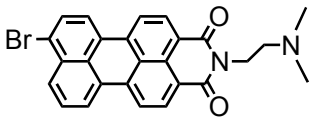
(Int=Igh+(Ig-Igh)*(((Go-Ho-1/Kg)/2+sqrt(((Ho+Go+1/Kg)^2)/4-Ho*Go))/Go), where Ig is the fluorescence intensity of guest, Igh is the intensity of host-guest complexes, Go is the concentration of the guest, and Kg is the binding constant. The binding constant of CB8 with different guests (e.g. MV) was 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 FGL550 color filter (550 nm cut-off, Thorlabs) when samples were excited using a blue LED flashlight (SHLEIS, purchased from Amazon.com).

Synthesis of PMI1



Scheme S1: Synthesis of **PMI1**.

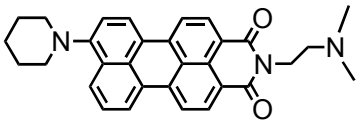
Synthesis of 2



Compound **1** (0.85 g, 2.16 mmol, **1** was synthesized according to previous reported procedure[3,4]) was added into concentrated sulfuric acid (65 mL) in a 250-mL round bottom flask. The mixture was cooled to 5 °C in an ice bath and 0.17 mL of bromine was added slowly. The reaction mixture was stirred at 5 °C

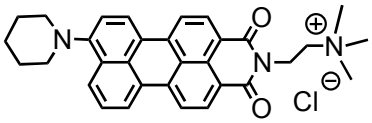
for 5 hours. The reaction mixture was poured into 300 mL of ultrapure water in a 1000 mL flask with an ice bath and the solution was precipitated by adding 30 % NH_4OH slowly until the pH reached 8-9. The precipitate was collected by suction filtration and washed with 5 % NH_4OH and ultrapure water. The solid was dried under vacuum oven at 110 °C. The red crude product was purified by recrystallization with DMF containing 1% of triethylamine. The solid was collected by filtration and washed with ethyl ether. The solid was dried under a vacuum oven overnight to afford compound **2** (0.55 g, 0.1.17 mmol, 54 %). ^{1}H NMR (Varian 400 MHz, CF_3COOD) δ 8.58-8.48 (m, 3H), 8.42 (d, *J* = 8 Hz, 1H), 8.37-8.31 (dd, *J* = 8 Hz, 2H), 8.14 (d, *J* = 8 Hz, 1H), 7.84 (d, *J* = 8 Hz, 1H), 7.74 (t, *J* = 8 Hz, 1H) 4.91 (br, 2H), 3.86 (br, 2H), 3.32 (s, 6H) ppm; ^{13}C NMR (Varian 100 MHz, CF_3COOD): δ = 163.8, 1.38.3, 132.8, 131.5, 130.7, 128.8, 127.5, 126.6, 125.5, 125.4, 124.5, 120.3, 119.9, 57.8, 43.8, 36.2 ppm; MALDI-TOF: m/z = 472.45 $[M+2H]^{2+}$ (calc'd. 472.08 for $C_{26}H_{21}BrN_2O_2$).

Synthesis of 3



Compound **2** (200 mg, 0.42 mmol) was added to pyridine (5 mL) in a round bottom flask. The reaction mixture was stirred at 120 °C for 48 hours under nitrogen. Most of the solvent was removed by vacuum distillation and the resulting solid was suspended in water. A solid was collected by suction filtration and washed with excess water. Then it was dried under vacuum oven at 110 °C to yield compound **3** (160 mg, 0.34 mmol, 80%). ^{1}H NMR (Varian 400 MHz, $CDCl_3$) δ 8.45 (t, *J* = 8 Hz, 1H), 8.35-8.30 (m, 2H), 8.28-8.24 (m, 2H), 8.17 (t, *J* = 8 Hz, 1H)), 7.84 (d, *J* = 8 Hz, 1H), 7.57 (t, *J* = 8 Hz, 1H) 7.12 (d, *J* = 8 Hz, 1H), 4.39-4.36 (br, 2H), 3.16 (br, 2H), 2.80 (br, 4H), 2.47 (s, 6H), 1.91-1.85 (br, 4H), 1.71 (br, 2H) ppm; ^{13}C NMR (Varian 100 MHz, CF_3COOD): δ = 166.6, 138.1,137.9, 137.7, 133.6, 133.3, 131.6, 130.2, 129.5, 128.7, 126.3, 125.2, 123.9, 122.1, 121.9, 120.8, 119.1, 58.8, 58.3, 43.8, 36.1, 23.5, 20.5ppm; MALDI-TOF: m/z = 476.98 $[M+H]^+$(calc'd. 476.23 for $C_{31}H_{30}N_3O_2$).

Synthesis of PMI1



Compound **3** (0.05 g, 0.11 mmol) was added into 15 mL of $CHCl_3$ in a round-bottom flask. Then methyl iodide (0.5 g, 3.52 mmol) was added. The resulting mixture was stirred at 70 °C for 24 hours. The solvent was removed by vacuum distillation and the solid was precipitated with ethyl acetate. The precipitate was collected by suction filtration and washed with ethyl acetate. Then it was dried under a vacuum oven at 110 °C overnight. After that, the solid was dissolved in 10 mL of ultrapure water for anion exchange. 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 flack and treated for one hour. The resin solution was placed in a 1-cm diameter glass column and washed with ultrapure water to remove excess NaCl. The purple dye solution was poured into the anion-exchange column and eluted with ultrapure water. The collected purple solution (30 mL) was dried using a Labconco freeze dryer (Free Zone 2.5 Liter -84C Benchtop) for 24 hours. A total of 44 mg (0.08 mmol, 76 % yield) of purple solid product **PMI1** was collected. ^{1}H NMR (Varian 400 MHz, DMSO-d_6) δ 8.70 (d, J = 8 Hz, 1H), 8.66 (d, J = 8 Hz, 2H), 8.61 (d, J = 8 Hz, 1H), 8.55 (d, J = 8 Hz, 1H), 8.47-8.42 (m, 2H), 8.17 (d, J = 8 Hz, 1H), 7.72 (t, J = 8 Hz, 1H), 7.26 (d, J = 8 Hz, 1H), 4.91 (br, 2H), 4.45 (t, J = 8 Hz, 1H), 3.62 (d, J = 8 Hz, 1H), 3.19 (s, 6H), 3.12 (br, 4H), 1.81 (br, 4H), 1.65 (br, 2H) ppm; ^{13}H NMR (Varian 100 MHz, CF_3COOD) δ = 166.2, 138.8, 138.1, 137.8, 133.6, 133.4, 131.6, 130.2, 129.6, 129.4, 128.8, 126.4, 126.3, 125.4, 124.0, 122.2, 121.9, 121.5, 119.6, 119.0, 62.5, 58.9, 53.3, 34.4, 23.6, 20.6 ppm; MALDI-TOF: m/z = 490.82 $[M]^+$ (calc'd. 490.25 for $C_{32}H_{32}N_3O_2$).

Supplementary Figures

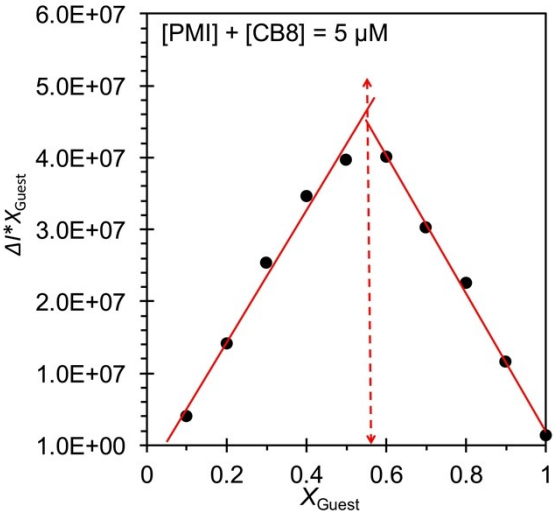


Figure S1. a) A Job plot for CB8•**PMI1** (the total concentration of the guest **PMI1** and the host CB8 was fixed at 5 μM).

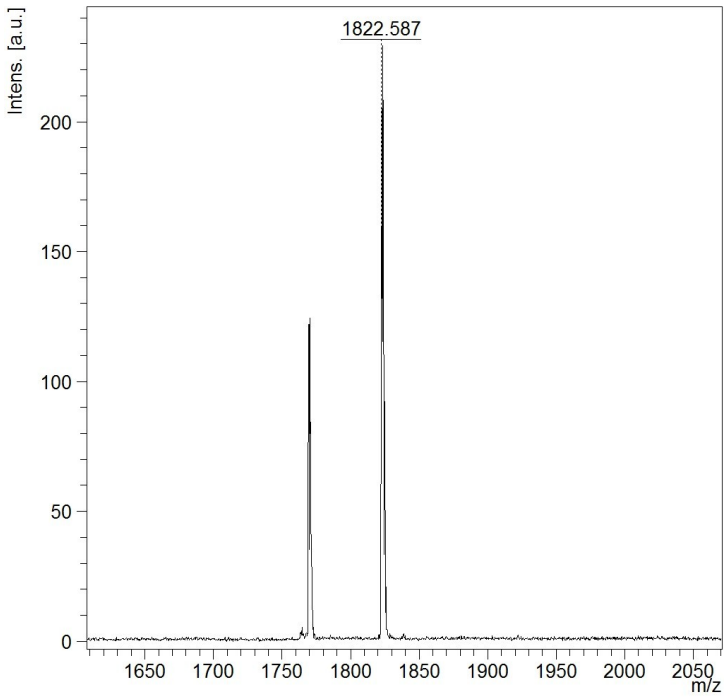


Figure S2. MALDI mass of CB8•**PMI1** (1:1) in H_2O using *α*-Cyano-4-hydroxycinnamic acid as a matrix. ([CB8•**PMI**+3H]$^{4+}$: observed mass 1822.58 Da, calculated mass 1822.73 Da).

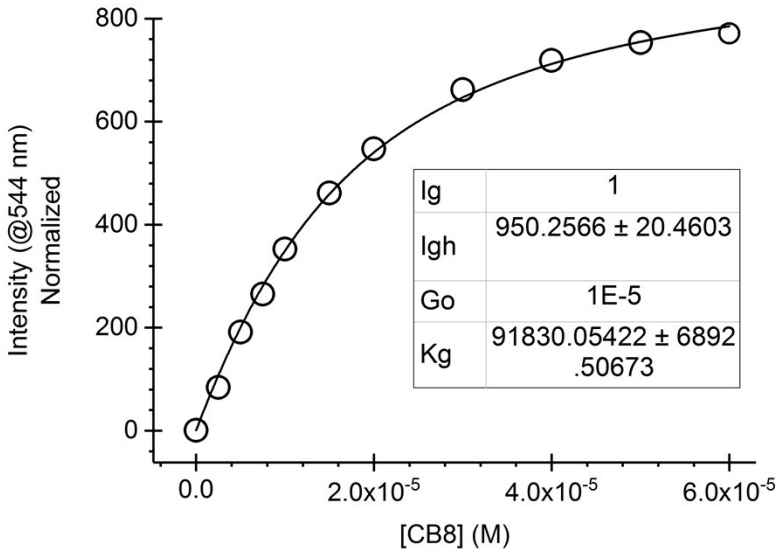


Figure S3. The binding curve for CB8•**PMI1** (The fluorescence titration experiment of **PMI1** (10 μM) in ultrapure water in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding.

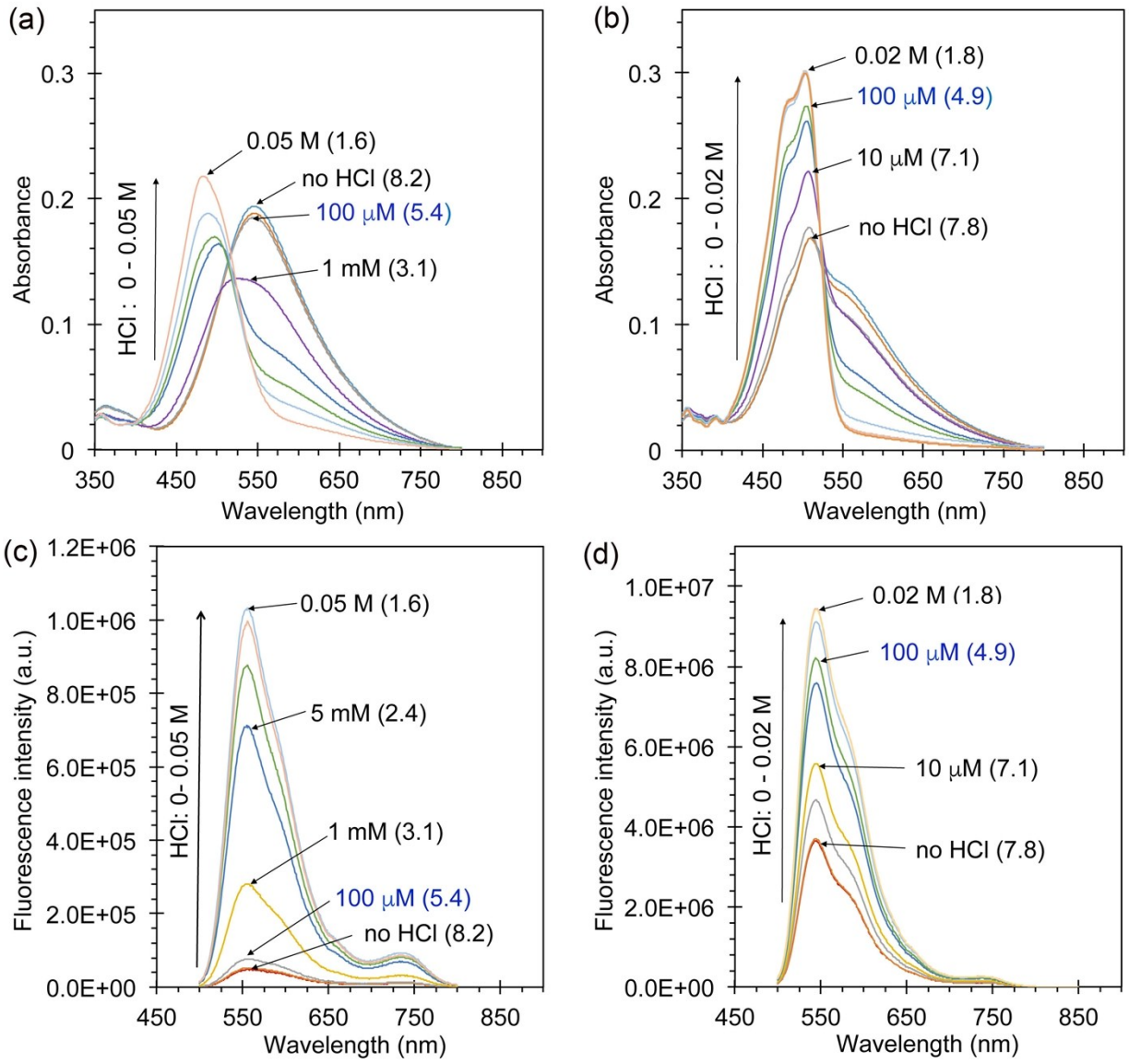


Figure S4. (a) UV-vis absorbance and (c) fluorescence emission spectra of **PMI1** (10 μM) in the absence (pH=8.2) and presence of 10 μM (pH=7.7), 100 μM (pH=5.4), 1 mM (pH=3.1), 5 mM (pH=2.4), 0.01 M (pH=2.1), 0.02 M (pH=1.8), and 0.05 M (pH=1.6) HCl in water. (b) UV-vis absorbance and (d) fluorescence emission spectra of CB8•**PMI1** (1:1, 10 μM) in the absence (pH=7.8) and presence of 1 μM (pH=7.4), 5 μM (pH=7.3), 10 μM (pH=7.1), 50 μM (pH=6.3), 100 μM (pH=4.9), 1 mM (pH=3.0), 5 mM (pH=2.4), 0.01 M (pH=2.1), and 0.02 M (pH=1.8) HCl in water. The fluorescence spectra were collected when samples were excited at 490 nm.

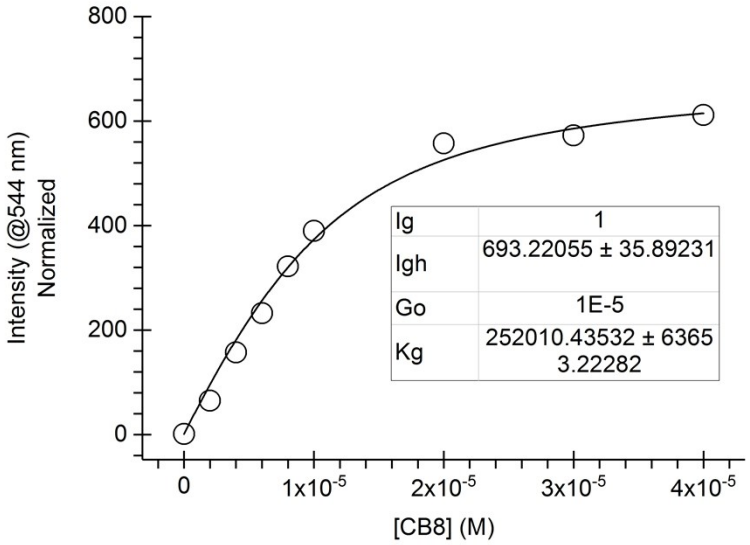


Figure S5. The binding curve for CB8•**PMI1** (The fluorescence titration experiment of **PMI1** (1.0 μM, 100 μM HCl) in ultrapure water in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding.

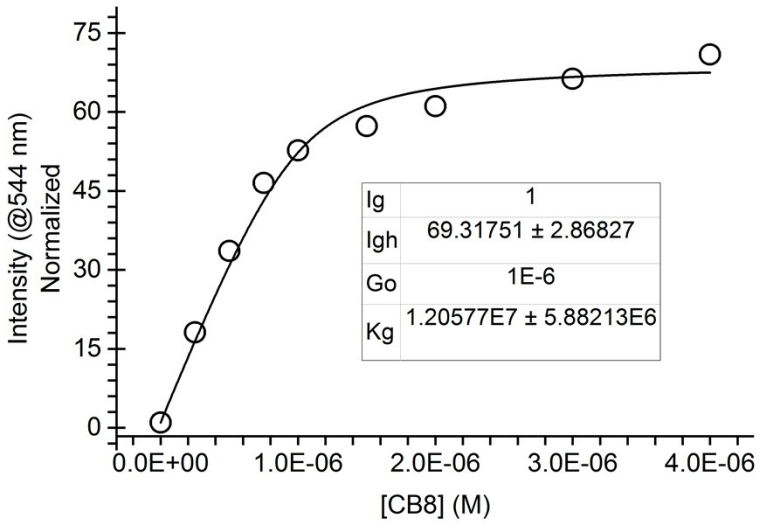


Figure S6. The binding curve for CB8•**PMI1** (The fluorescence titration experiment of **PMI1** (1.0 μM, 100 μM HCl) in ultrapure water in the presence of CB8 and the fluorescence intensity was plotted against the concentration of CB8). The solid line represents the best fitting of the data according to a 1:1 binding.

Detection Limit:

The limit of detection (LOD) was calculated from the fluorescence titration using following equation.[5]

$$\mathrm{LOD} = 3\,\boldsymbol{\sigma}/\mathrm{K}$$

Where $\boldsymbol{\sigma}$ is the standard deviation (the standard deviation of the fluorescence changes of the CB8•**PMI1** (1:1, 10 μM, 100 μM HCl) in water in the presence of 1.0 μM MV with four duplicated solutions) and K is the slope between the fluorescence intensity of CB8•**PMI1** versus MV concentration.

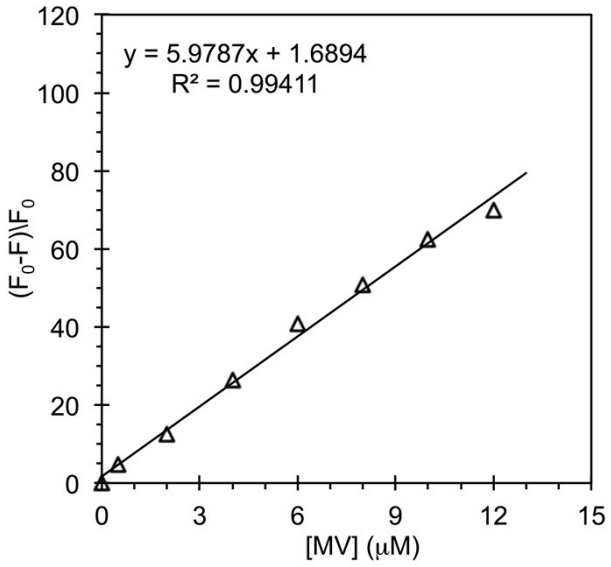


Figure S7. A linear plot of $(F_0\text{-}F)/F_0$ vs. MV in the concentration range of 0 to 15 μM. Where F_0 and F are the fluorescence intensity of CB8•**PMI1** (1:1, 10 μM) in ultrapure water with 100 μM HCl in the absence and presence of MV, respectively.

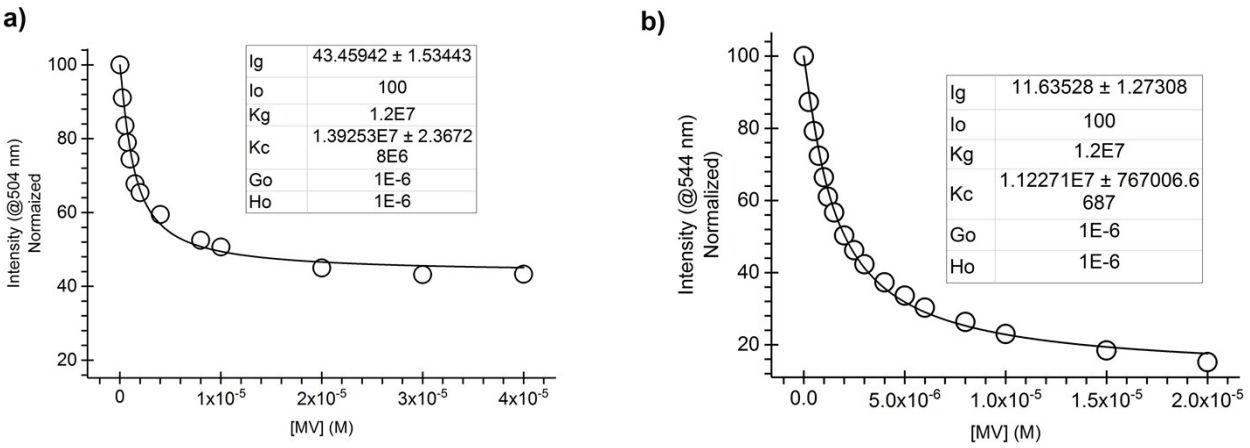


Figure S8. The binding curve for the displacement of CB8•**PMI1** with MV (a: The UV-vis titration experiment of CB8•**PMI1** (10 μM, 1:1, 100 μM HCl) and b: The fluorescence titration experiment of CB8•**PMI1** (10 μM, 1:1, 100 μM HCl) in the presence of MV and the UV-vis absorbance at 503 nm and the fluorescence intensity at 544 nm were plotted against the concentration of MV, respectively).

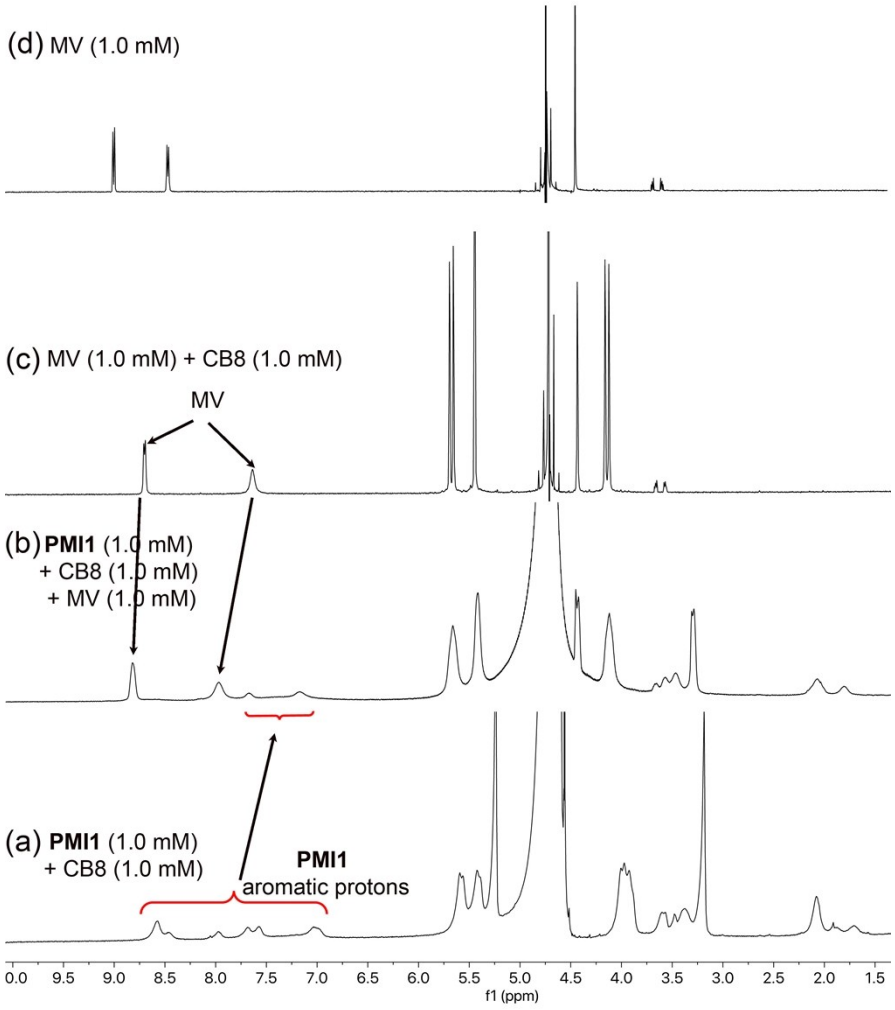


Figure S9. [1]H-NMR spectrum of (a) the CB8•**PMI1** complex (1:1, 1.0 mM), (b) the CB8•**PMI1** complex (1:1, 1.0 mM) in the presence of 1.0 equivalent of MV, (c) the CB8•MV complex (1:1, 1.0 mM), and (d) MV (1.0 mM) in D2O with 10 mM DCl.

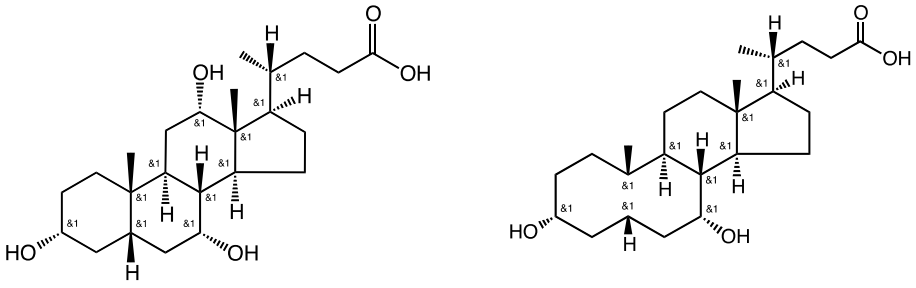


Figure S10. The structures of cholic acid (CA) (left) and deoxycholic acid (DCA) (right).

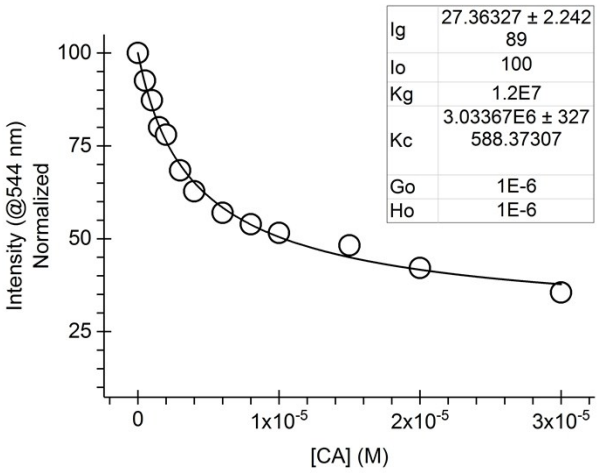


Figure S11. The binding curve for the displacement of CB8•**PMI1** with cholic acid (CA) (The fluorescence titration experiment of CB8•**PMI1** (1 μM, 1:1, 100 μM HCl) in the presence of CA and the fluorescence intensity at 544 nm were plotted against the concentration of CA).

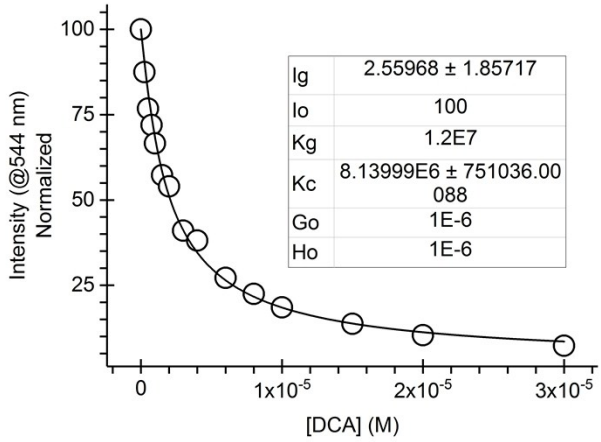


Figure S12. The binding curve for the displacement of CB8•**PMI1** with deoxycholic acid (DCA) (The fluorescence titration experiment of CB8•**PMI1** (1 μM, 1:1, 100 μM HCl) in the presence of DCA and the fluorescence intensity at 544 nm were plotted against the concentration of DCA).

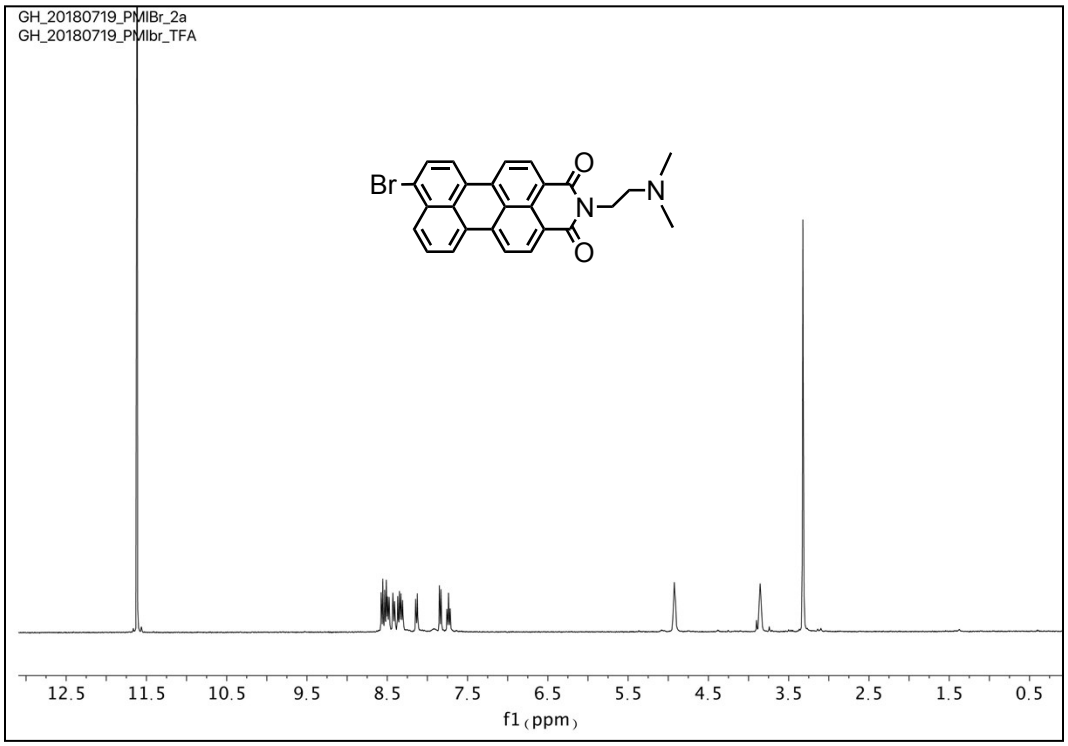


Figure 13. ^{1}H-NMR of **2** in CF_3COOD.

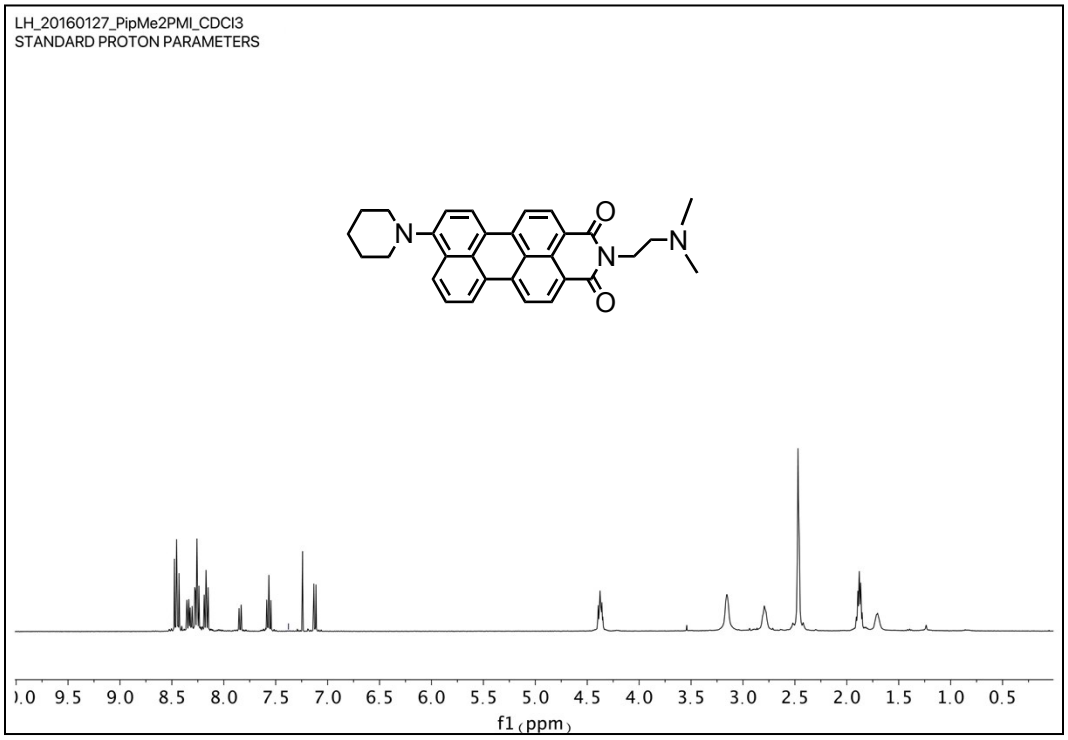


Figure 14. ^{1}H-NMR of **3** in $CDCl_3$.

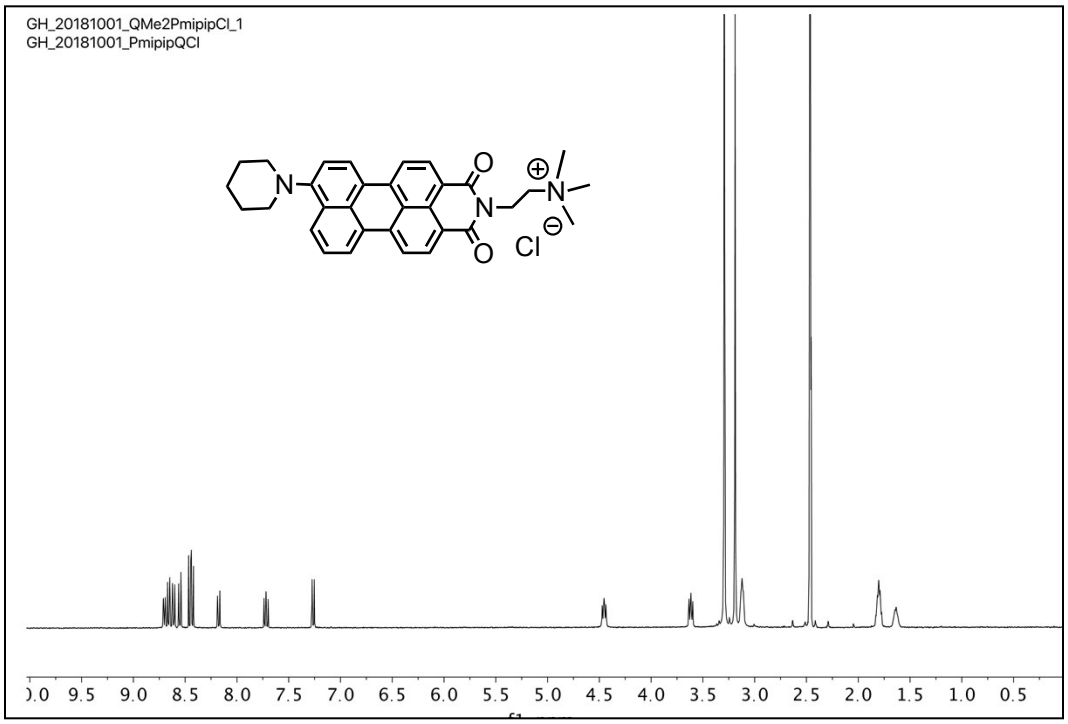


Figure 15. ^{1}H-NMR of **PMI1** in DMSO-d_6.

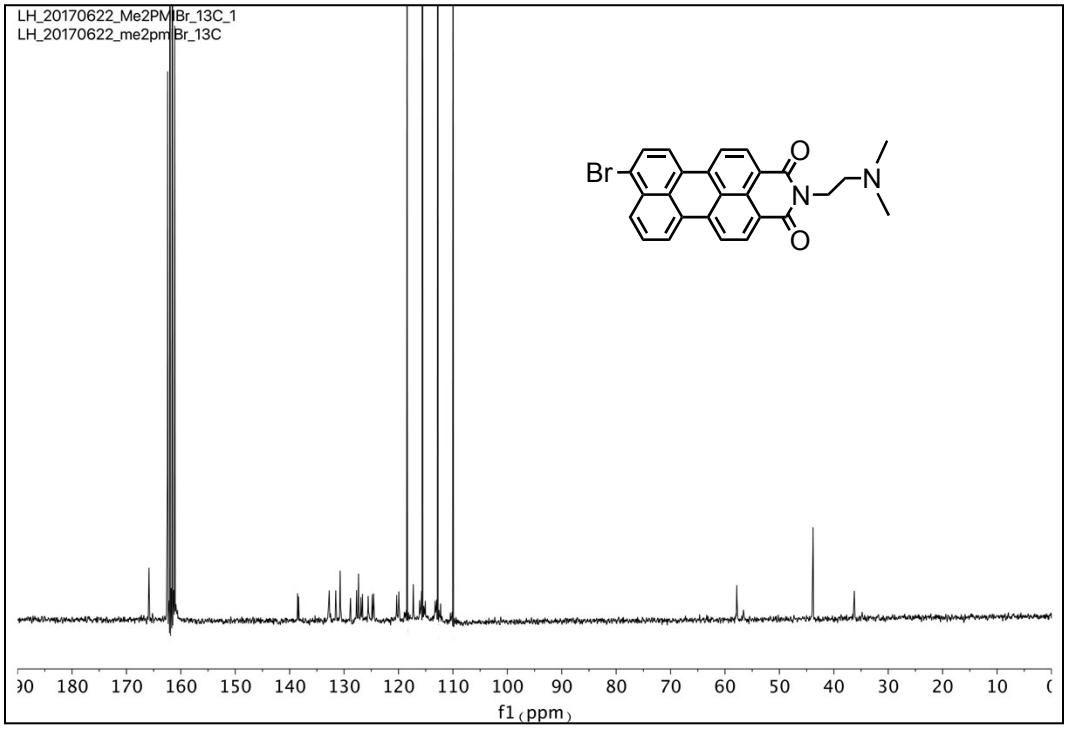


Figure 16. ^{13}C-NMR of **2** in CF_3COOD.

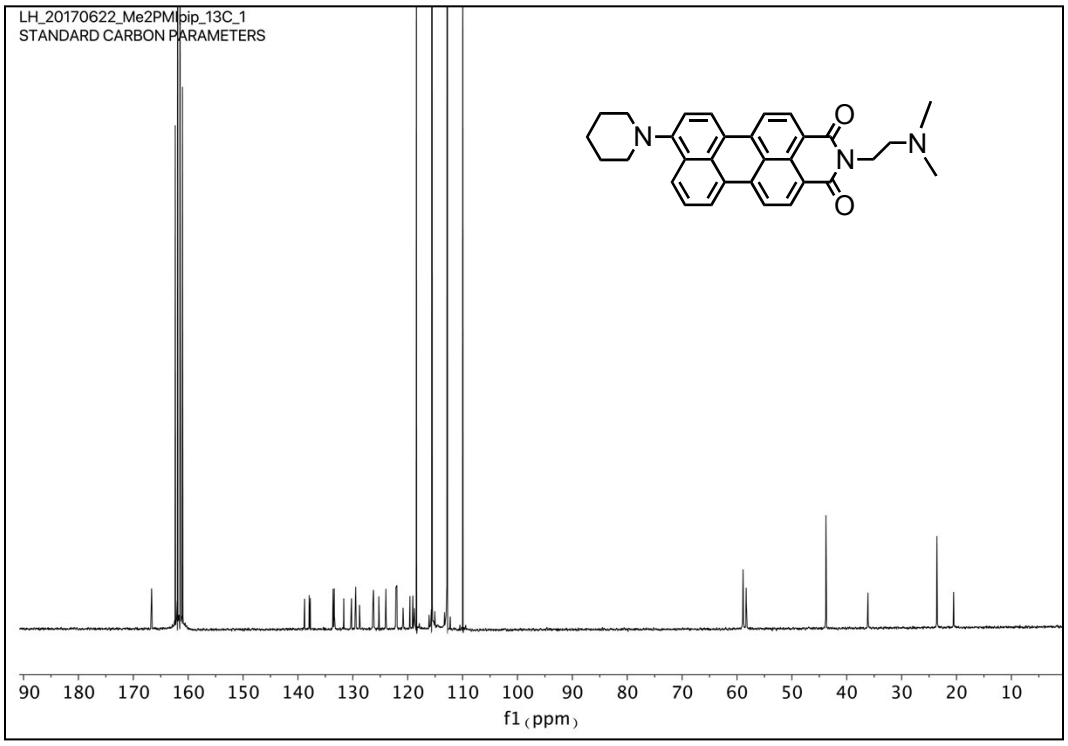


Figure 17. ^{13}C-NMR of **3** in CF_3COOD.

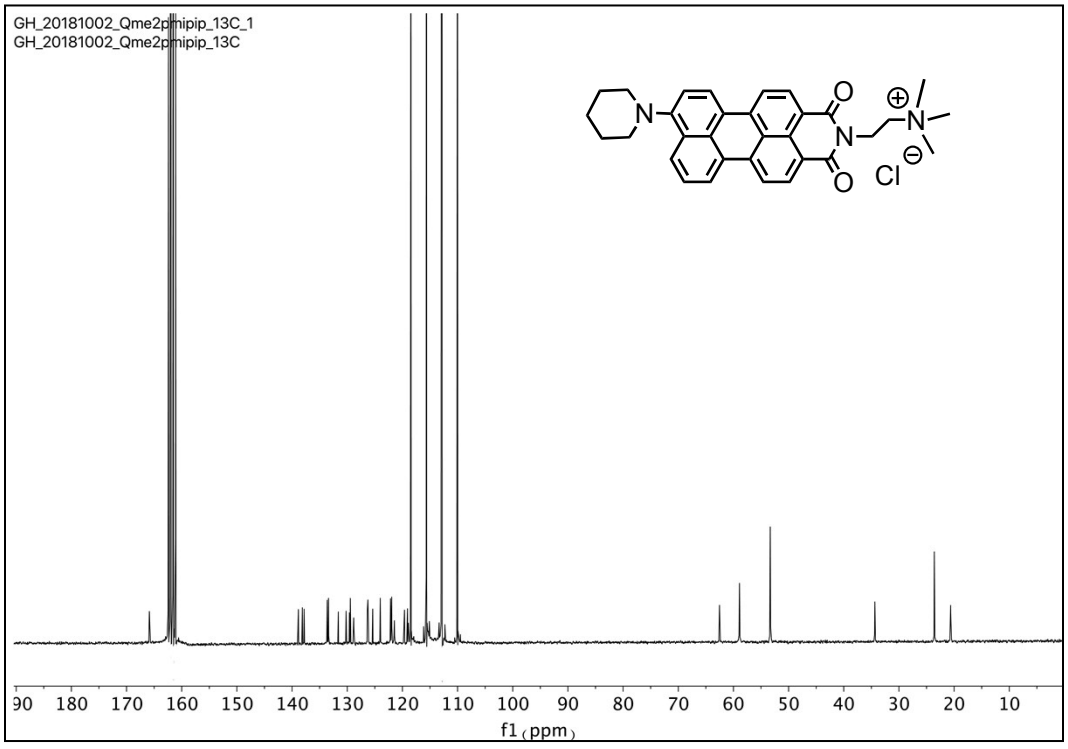


Figure 18. ^{13}C-NMR of **PMI1** in CF_3COOD.

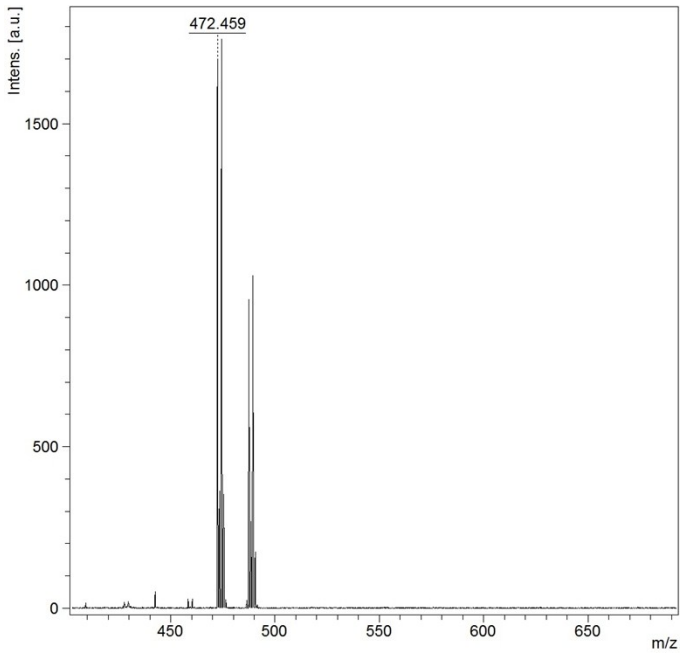


Figure S19. MALDI mass of **2** in methanol using 2-5-dihydroxy benzoic acid acid as a matrix. ($[2+3H]^{3+}$: observed mass 472.45 Da, calculated mass 472.08 Da).

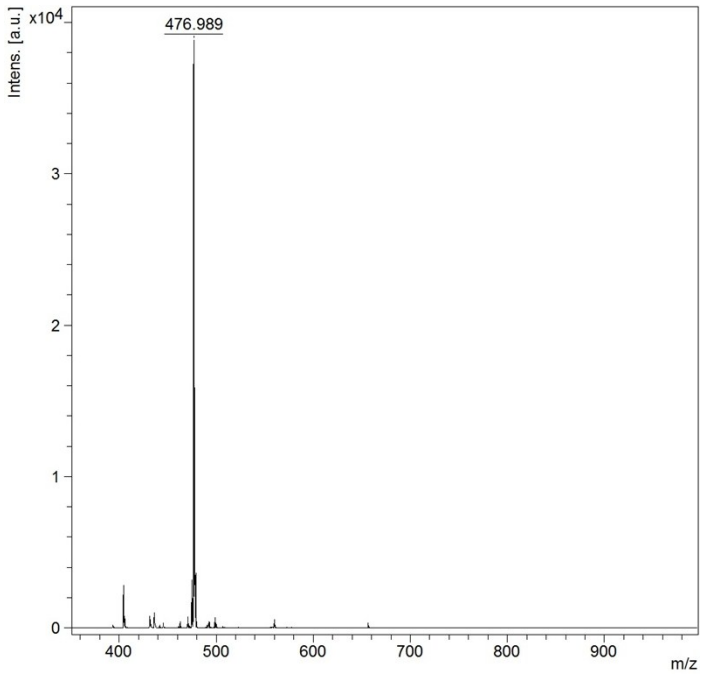


Figure S20. MALDI mass of **3** in methanol using *α*-Cyano-4-hydroxycinnamic acid as a matrix. ($[3+H]^{+}$: observed mass 476.98 Da, calculated mass 476.23 Da).

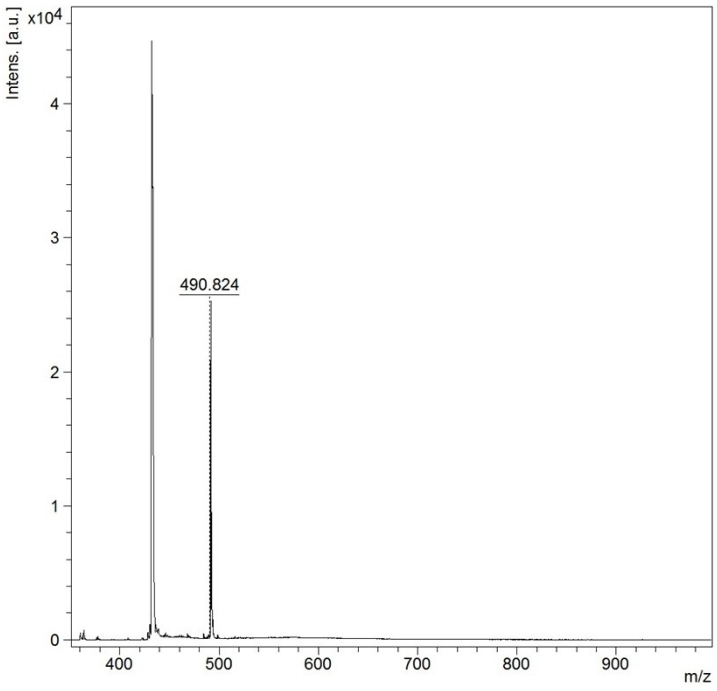


Figure S21. MALDI mass of **PMI1** in methanol using α-Cyano-4-hydroxycinnamic acid as a matrix. ([**PMI**]$^+$: observed mass 490.82 Da, calculated mass 490.63 Da)

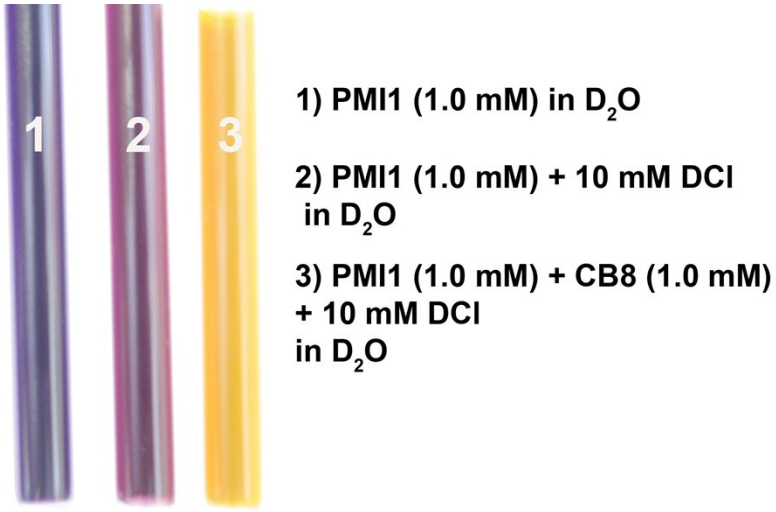


Figure S22. Images of solutions for ^{1}H-NMR studies.

References

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