Supporting Information for

Recognition of *myo*-Inositol 1,4,5-Trisphosphate using Fluorescent Imidazolium Receptor †

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Experimental

1. General methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with the thickness of 1 mm.

Melting points were measured using a Büchi 530 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 MHz, 300 MHz or Varian 500 MHz. Chemical shifts were given in ppm and coupling constants (*J*) in Hz. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

2. Synthesis

Synthesis of 1-((pyren-3-yl)methyl)-1H-imidazole 3 ***(Tetrahedron Letter, 46(39), 6617-20, 2005)***

NaH (36.7mg, 0.92mmol, 60% in mineral oil) was added to a mixture of imidazole (57mg, 0.84mmol) in THF (20mL) at 0℃. After the reaction mixture had been stirred for 20min at 0℃, 1-bromomethylpyrene (200mg, 0.68mmol) was added. After additional stirring for 1h at room temperature, water (50mL) was added to the reaction mixture and the mixture extracted with $CHCl_3$. The organic layer was separated, dried with anhydrous magnesium sulfate, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (Hexane:EA=1:2) afforded **2** (142mg, 74.3%) as a pale-yellow solid. ^{1}H NMR (CD_3CN, 250 MHz) : δ 8.34 (d, *J* = 3.35 Hz, 2H), 8.30-8.24 (m, 3H), 8.17-8.10 (m, 3H), 7.85 (d, *J* = 7.82 Hz,

1H), 7.68 (s, 1H), 7.08 (s, 1H), 6.96 (s, 1H), 5.93 (s, 2H). ^{13}C NMR (CD_3CN, 62.5 MHz) : δ 137.54, 131.36, 131.24, 130.62, 128.70, 128.32, 127.70, 127.31, 126.78, 126.45, 125.69, 125.54, 125.09, 122.25, 119.64, 48.13. HRMS (FAB) calcd for $C_{20}H_{15}N_2$ $[M+H]^+$ 283.1157; found 283.1232.

Synthesis of 1

A mixture of **3** (263mg, 0.93mmol) and 1,2,4,5-tetrakis(bromomethyl)benzene (100mg, 0.22mmol) in acetonitrile (10mL) was heated at reflux for 24h under N_2. After cooling to room temperature, the precipitate was filtered and washed with cold CH_2Cl_2 to give **1** as a white solid (320mg, 91%). m.p. decompose. ^{1}H NMR (D_2O, 250 MHz) : δ 9.45 (s, 4H), 8.43 (d, *J* = 9.29 Hz, 4H), 8.33-8.19 (m, 20H), 8.14 (s, 4H), 8.10-8.03 (m, 8H), 7.75 (s, 4H), 7.66 (s, 4H), 7.44 (s, 2H), 6.21 (s, 8H), 5.54 (s, 8H). ^{13}C NMR (D_2O, 62.5 MHz) : δ 137.47, 134.95, 133.18, 132.14, 131.32, 130.72, 129.47, 129.22, 128.88, 128.54, 127.86, 127.74, 127.35, 126.72, 126.45, 125.83, 124.71, 124.27, 123.51, 123.05, 50.83, 49.53. HRMS (FAB) calcd for $C_{58}H_{50}Br_3N_8$ $[M-Br]^+$ 1495.2955; found 1495.2957.

Synthesis of 2

A mixture of **3** (200mg, 0.71mmol) and 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (89.3mg, 0.20mmol) in acetonitrile (10mL) was heated at reflux for 24h under N_2. After cooling to room temperature, the precipitate was filtered and washed with cold CH_2Cl_2 to give **2** as a yellow solid (225mg, 88%). ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) : δ 9.83 (s, 3H), 8.49-8.11 (m, 27H), 7.93 (m, 6H), 6.35 (s, 6H), 5.56 (s, 6H), 2.67 (d, *J* = 7.2 Hz, 6H), 0.85 (t, 9H). ^{13}C NMR (DMSO-*d*$_6$, 500 MHz) : δ 148.49, 136.51, 131.96, 131.30, 130.66, 129.43, 129.11, 129.04, 128.74, 128.47, 128.05, 127.83, 127.22, 126.60, 126.38, 125.73, 124.67, 124.25, 124.20, 123.55, 123.07, 50.53, 47.75, 23.67, 16.16. HRMS (FAB) calcd for $C_{75}H_{63}Br_2N_6$ $[M-Br]^+$ 1205.3475; found 1205.3477.

3. Fluorescent study

Stock solutions (1 mM) of IP_1, IP_2, IP_3, IP_4, IP_5, IP_6, *scyllo*-IP_3, PPi, and ATP in doubly distilled water were prepared. Stock solution of host **1** (0.1 mM) was also prepared in DMSO. Test solutions were prepared by placing 300 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each stock, and diluting the solution to 3 mL with DMSO-HEPES buffer (0.02 M, pH 7.4) (1:9, v/v).

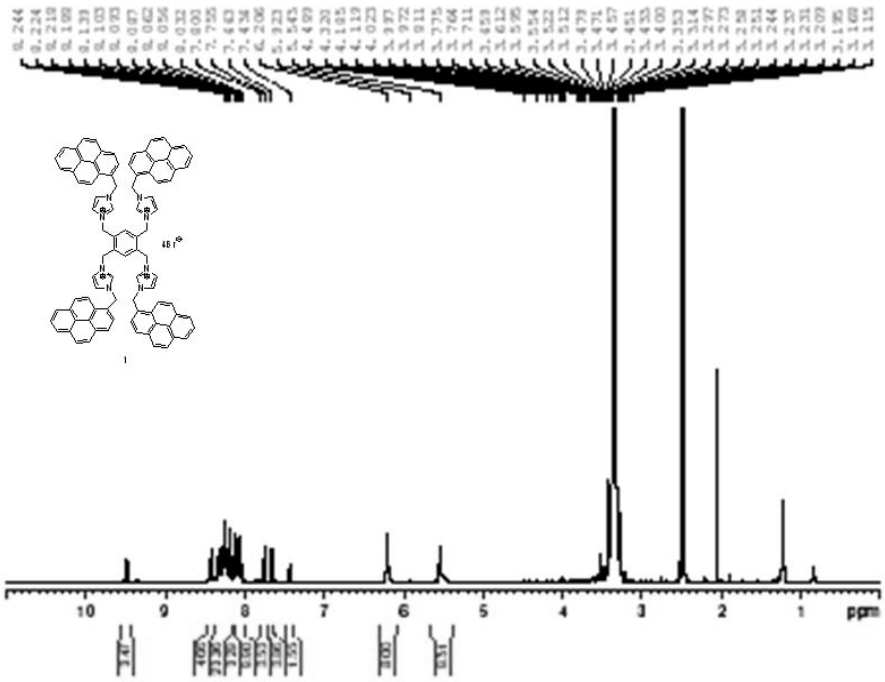

Fig. S1. ^{1}H NMR (250 MHz) of compound **1** in D_2O.

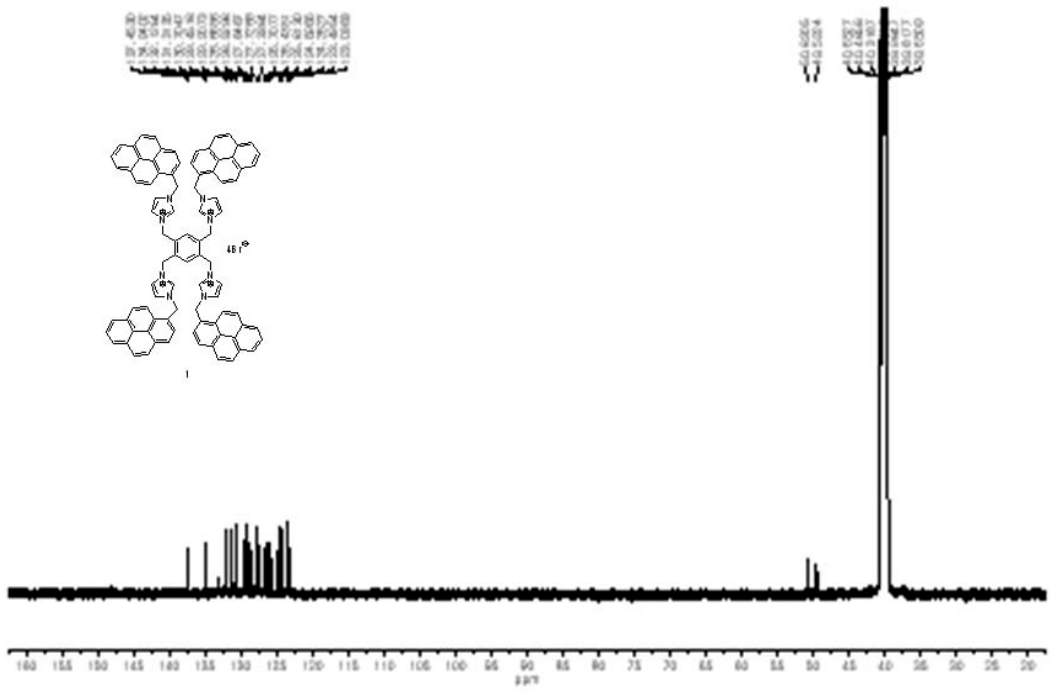

Fig. S2. ^{13}C NMR (62.5 MHz) of compound **1** in D_2O.

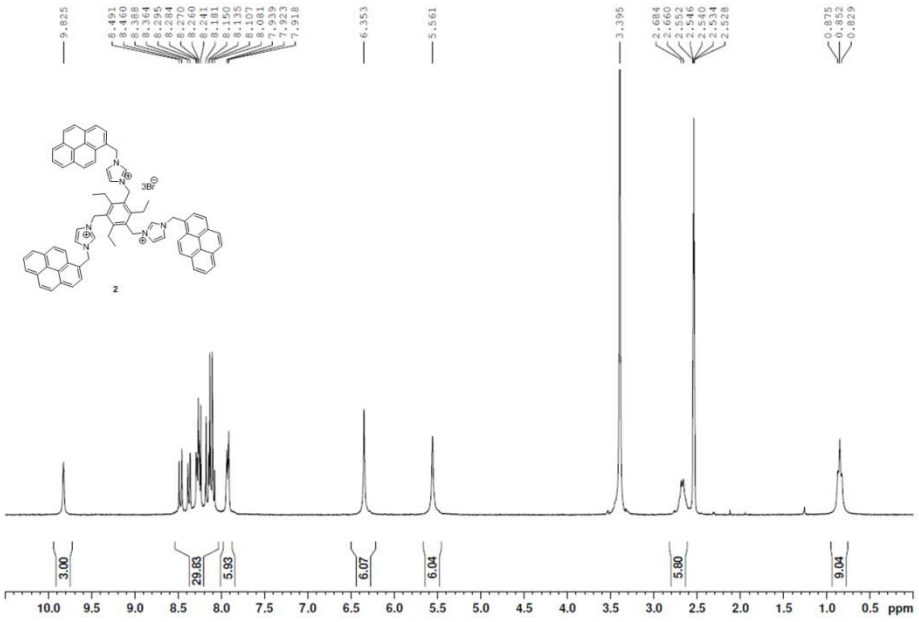

Fig. S3. ^{1}H NMR (300 MHz) of compound **2** in DMSO-d_6.

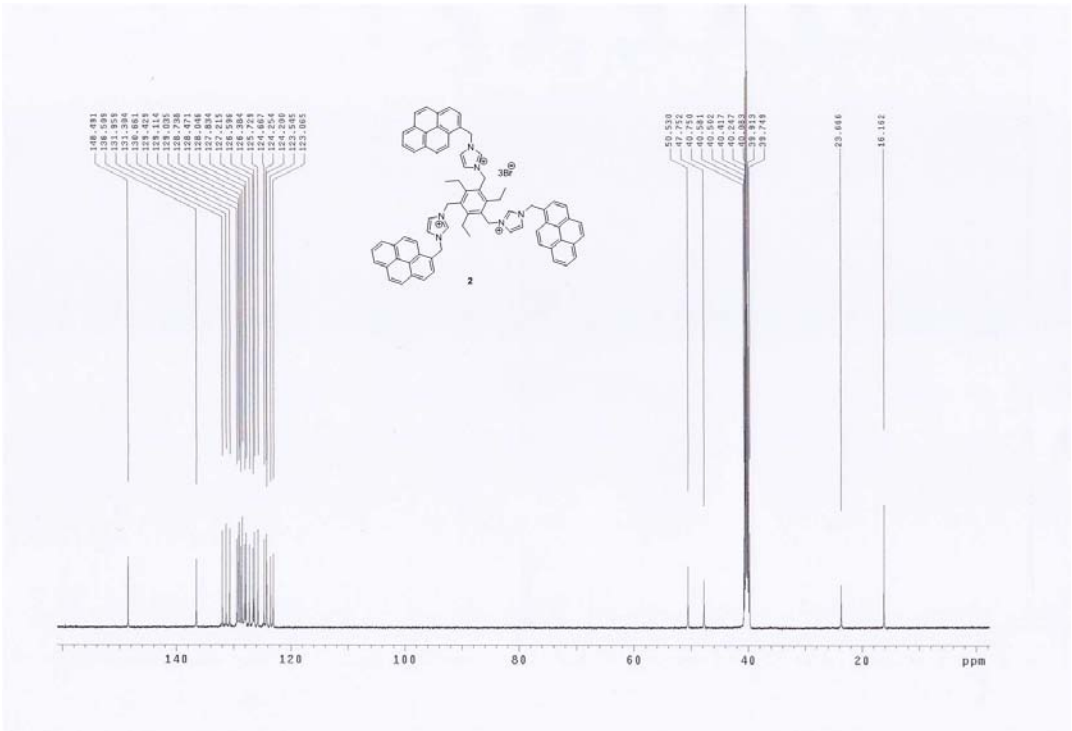

Fig. S4. ^{13}C NMR (300 MHz) of compound **2** in DMSO-d_6.

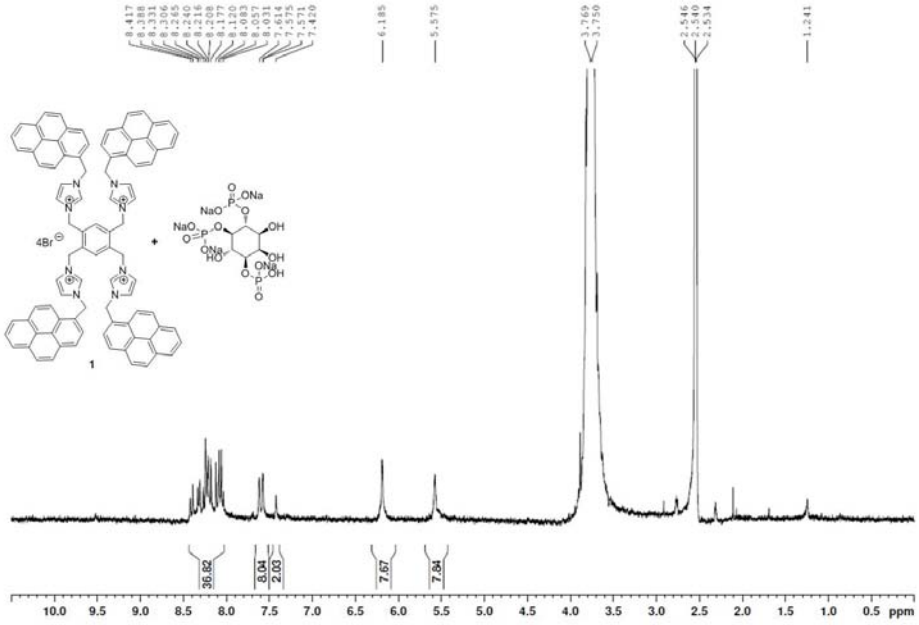

Fig. S5. ^{1}H NMR (300 MHz) of compound **1** with IP_3 in DMSO-d_6-D_2O (9:1, v/v).

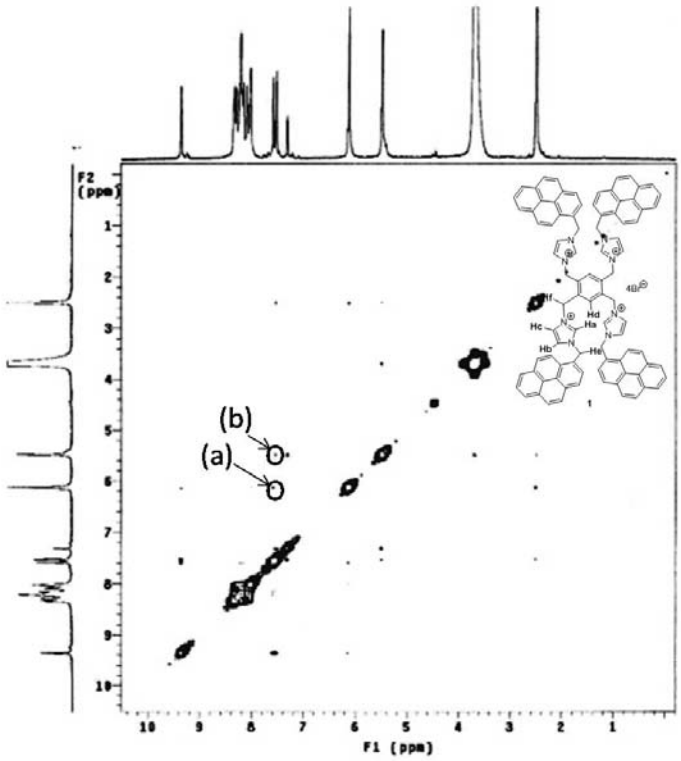

Fig. S6. Partial 2D-COSY NMR spectra of **1** in DMSO-d_6-D_2O (9:1, v/v) cross peak **A** between Hb and He(a), cross peak **B** between Hc and Hf(b).

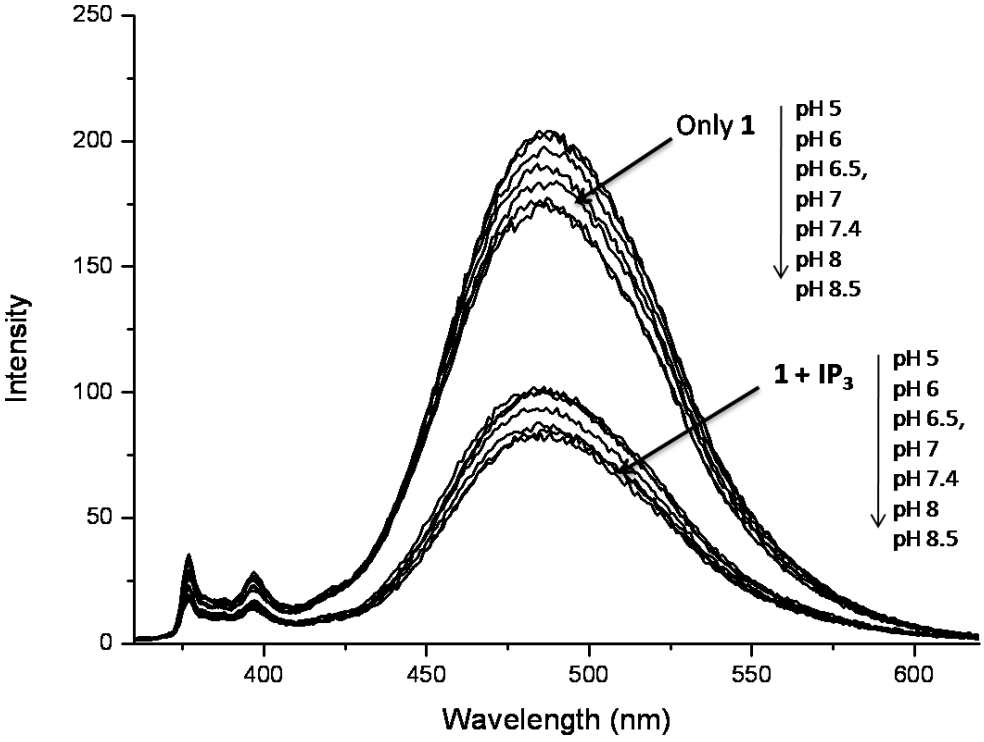

Fig. S7. Fluorescent changes of **1** (1×10^{-5} M, DMSO-HEPES buffer (0.02 M, pH 7.4) (1: 9, v/v) and **1**+IP_3(2.0 equiv.) at different pHs (excitation at 340 nm).

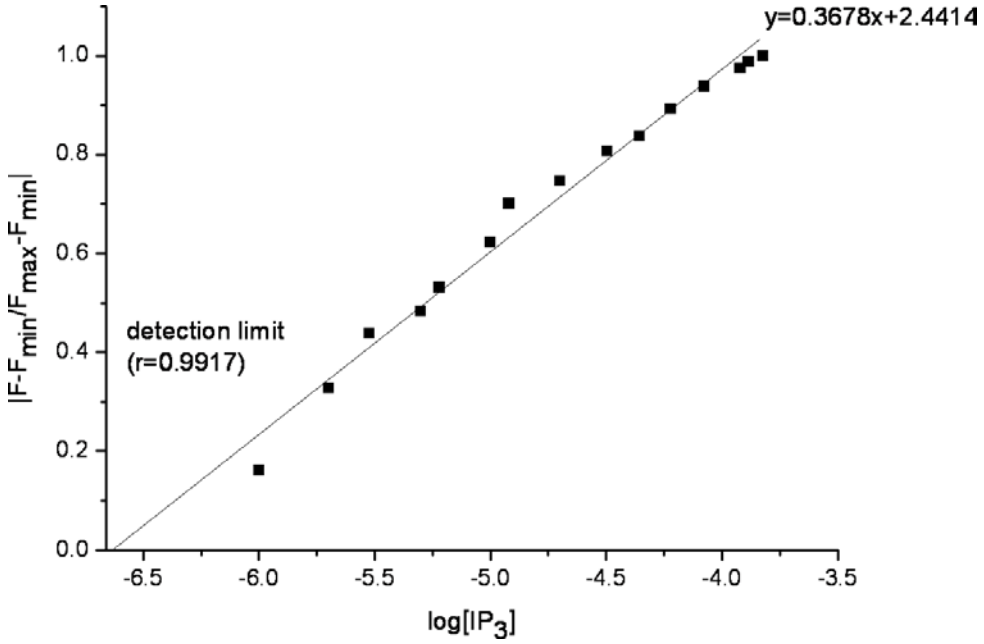

Fig. S8. Normalized fluorescence responses of **1** (1×10^{-5} M) to changing IP_3 concentrations in DMSO-HEPES buffer (0.02 M, pH = 7.4) (1:9, v/v).