Selective Recognition of Tryptophan Through Inhibition of Intramolecular Charge-Transfer Interactions in Aqueous Medium

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SUPPLEMENTARY SUPPORTING INFORMATION

Details of synthesis, characterization and calculations, Figures S1 to S13 showing fluorescence and ^{1}H NMR spectra of the conjugate **1** under various conditions.

1. General experimental techniques. ^{1}H and ^{13}C NMR were measured on a 300 MHz Bruker advanced DPX spectrometer. The electronic absorption spectra were recorded on a Shimadzu UV-VIS-NIR spectrophotometer. Fluorescence spectra were recorded on a SPEX-Fluorolog F112X spectrofluorimeter. Quinine sulphate (Φ_f = 0.54) in 0.1 N H_2SO_4 was used as the standard. The quantum yields of fluorescence were calculated using the equation 1,

$$\Phi_u = \frac{A_s F_u n_u^2}{A_u F_s n_s^2} \Phi_s \qquad (1)$$

where, A_s and A_u are the absorbance of standard and unknown, respectively. F_s and F_u are the areas of fluorescence peaks of the standard and unknown and n_s and n_u are the refractive indices of the solvents used for the standard and unknown, respectively. Φ_s and Φ_u are the fluorescence quantum yields of the standard and unknown compound.

Fluorescence lifetimes were measured using a IBH Picosecond single photon counting system. The fluorescence decay profiles were deconvoluted using IBH data station software V2.1, fitted with monoexponential decay and minimizing the χ^2 values of the fit to 1 ± 0.1. Cyclic voltammograms were recorded in Bioanalytical Systems Inc., BAS-CV50W cyclic voltammeter. Doubly distilled water was used in all the studies. Petroleum ether used was the fraction with boiling range 60-80 ºC. All experiments were carried out at room temperature (25 ± 1 °C), unless otherwise mentioned.

2. Calculation of association constants (K_{ass}). Amino acids (Sigma Aldrich) solution were prepared in distilled water. The binding affinities were calculated using Benesi-Hildebrand equation 2, where, K is the association constant, I_f is the fluorescence intensity at

$$1/(I_f - I_{ob}) = 1/(I_f - I_{fc}) + 1/K_{ass}(I_f - I_{fc})[\text{Amino acids}] \qquad (2)$$

475 nm of free viologen linked pyrene conjugate, I_{ob} is the observed fluorescence intensity at 475 nm in the presence of various amino acids and I_{fc} is the fluorescence intensity at saturation. The linear dependence of $1/(I_f - I_{ob})$ on the reciprocal of the ligand concentration indicates the formation of a 1:1 molecular complex between amino acid and the viologen linked pyrene conjugate.

3. Synthesis of Starting Materials

3.1. Synthesis of the conjugate 1. *Preparation of 1-(bromomethyl)pyrene:* To an ice cold solution of 1-(hydroxymethyl)pyrene (0.65 mmol) in 35 mL of dry chloroform, phosphorus

tribromide (0.21 mmol) was added and the resulting solution was stirred for 12 h. Reaction mixture was neutralized with saturated sodium bicarbonate solution. The organic layer was separated and evaporated under reduced pressure to give 82% of 1-(bromomethyl)pyrene. The product was recrystallized from chloroform, mp 136-137 ^{0}C: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.25 (2H, s), 8.06-8.13 (5H, m), 8.20-8.26 (3H, m), 8.39 (1H, d, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 32.2, 122.8, 124.8, 125.6, 126.2, 127.3, 127.7, 127.9, 128.2. Anal. Calcd for $C_{17}H_{11}Br$: C, 69.17; H, 3.76. Found: C, 69.31; H, 3.57.

*Preparation of **1***: To a solution of 1-(bromomethyl)pyrene (0.6 mmol) in dry acetonitrile (50 mL), 1-butyl-4,4'-bipyridinium bromide (0.6 mmol) was added and stirred at room temperature for 12 h. Precipitated product was filtered and dried to give 29% of 1-[(pyren-1-yl)methyl]-1'-butyl-4,4'-bipyridinium dibromide (**1**), which was recrystallized from a mixture (7:3) of methanol and ethyl acetate, mp 286-290 °C: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.93 (3H, t, J = 7.4Hz), 1.29-133 (2H, m), 1.93-1.97 (2H, m), 4.69 (2H, t, J = 7.1Hz), 6.82 (2H, s), 8.17-8.75 (9H, m), 8.58-9.51 (8H, m); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ 13.3, 18.8, 25.4, 25.7, 28.3, 28.6, 28.7, 28.9, 29.2, 30.7, 32.7, 38.7, 38.9, 39.2, 39.5, 39.8, 40.1, 40.3, 60.6, 60.8, 69.6, 70.4, 109.0, 112.7, 123.6, 123.9, 124.5, 125.2, 125.3, 126.3, 126.6, 127.0, 127.2, 127.3, 127.4, 128.7, 130.3, 130.5, 130.7, 132.2, 145.7, 148.5; HRMS (ESI) Calcd for $C_{31}H_{28}BrN_2$: 508.4715. Found: 508.4711. Anal. Calcd for $C_{31}H_{28}Br_2N_2$: C, 63.28; H, 4.80; N, 4.76. Found: C, 63.14; H, 4.69; N, 4.86.

3.2. Synthesis of the model compound 2. To a solution of 1-(bromomethyl)pyrene (0.65 mmol) in dry acetonitrile (30 mL), triethylamine (0.65 mmol) was added and stirred for 12 h at room temperature. Precipitated product was filtered and dried under vacuum oven, to give 45% of 1-[(pyren-1-yl)methyl]-1'-*N*,*N'*,*N''*-triethylammonium bromide (**2**), which was recrystallized from a mixture (6:4) of methanol and ethyl acetate, mp 184-185 °C: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.25-1.29 (9H, m), 3.35-3.42 (6H, m), 5.31 (2H, s), 8.17-8.73 (9H, m); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ 8.1, 53.1, 58.7, 121.7, 123.0, 123.5, 124.0, 124.8, 125.9, 126.3, 126.7, 127.1, 128.9, 129.0, 129.9, 130.7, 131.6, 132.2; HRMS (ESI) Calcd for $C_{23}H_{26}BrN$: 396.3633. Found: 396.3639. Anal. Calcd for $C_{23}H_{26}BrN$: C. 69.70; H, 6.61; N, 3.53. Found: C, 69.59; H, 6.83; N, 3.71.

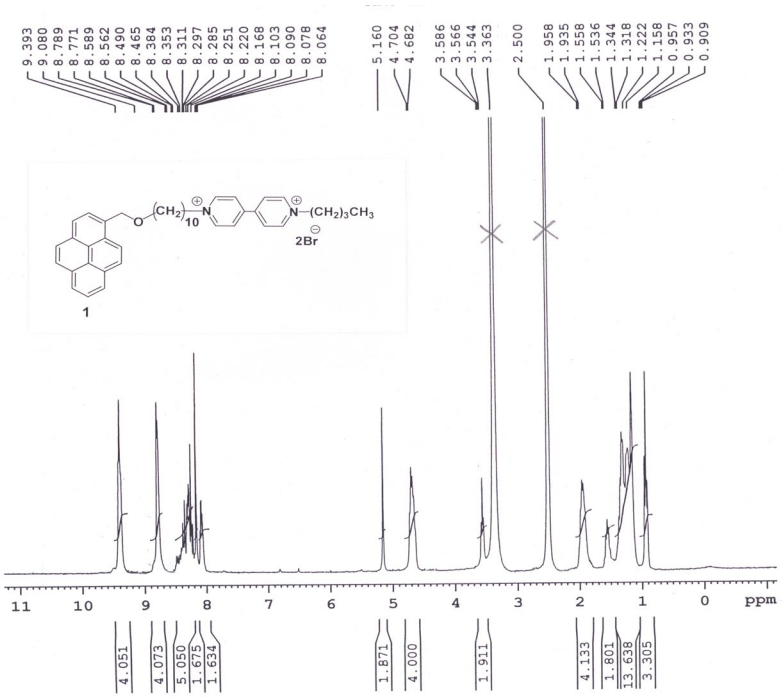

Figure S1. ^{1}H NMR (300 MHz) spectrum of the conjugate **1** in DMSO-d_6.

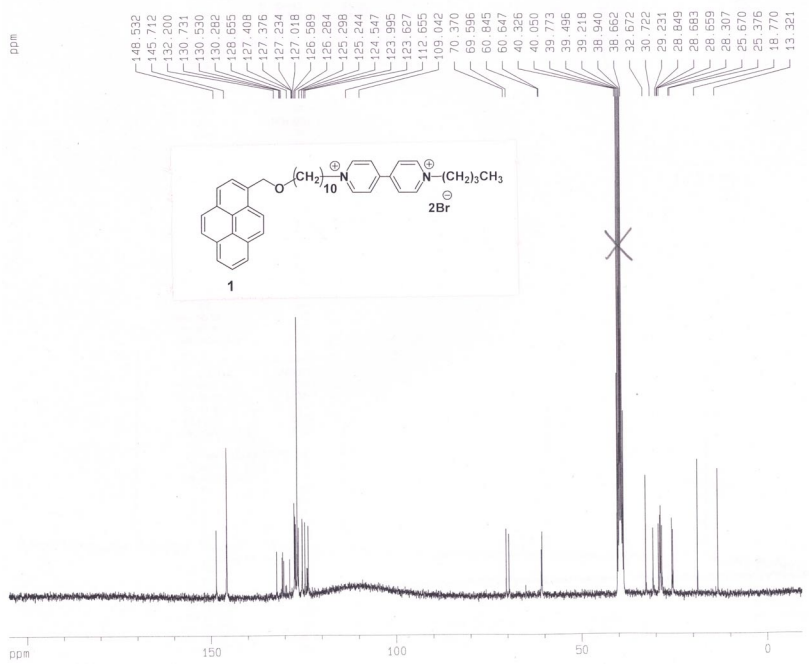

Figure S2. ^{13}C NMR (75.47 MHz) spectrum of the conjugate **1** in DMSO-d_6.

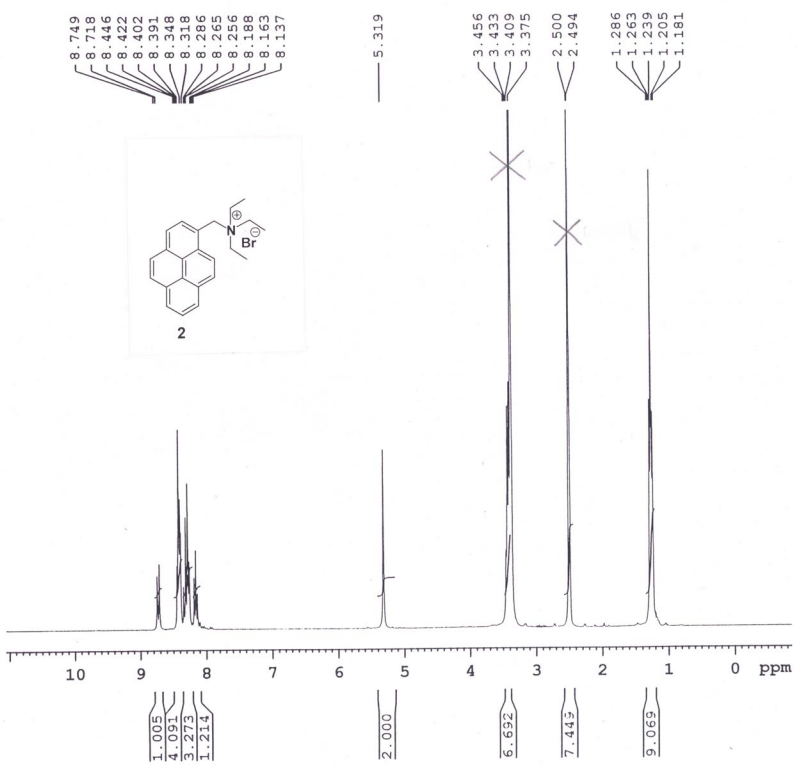

Figure S3. ^{1}H NMR (300 MHz) spectrum of the model compound **2** in DMSO-d_6.

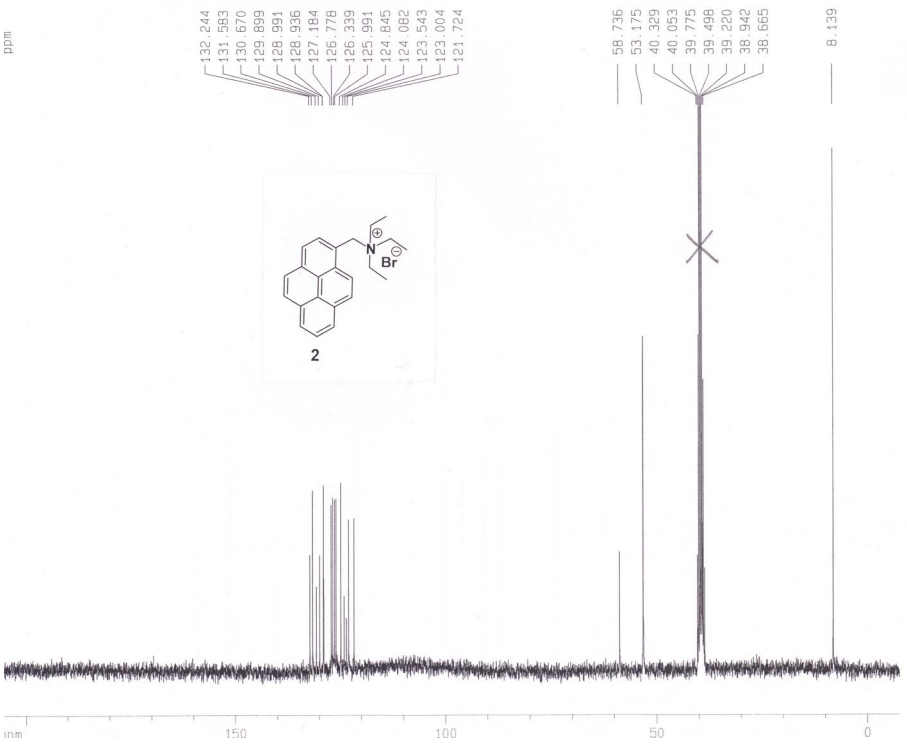

Figure S4. ^{13}C NMR (75.47 MHz) spectrum of the model compound **2** in DMSO-d_6.

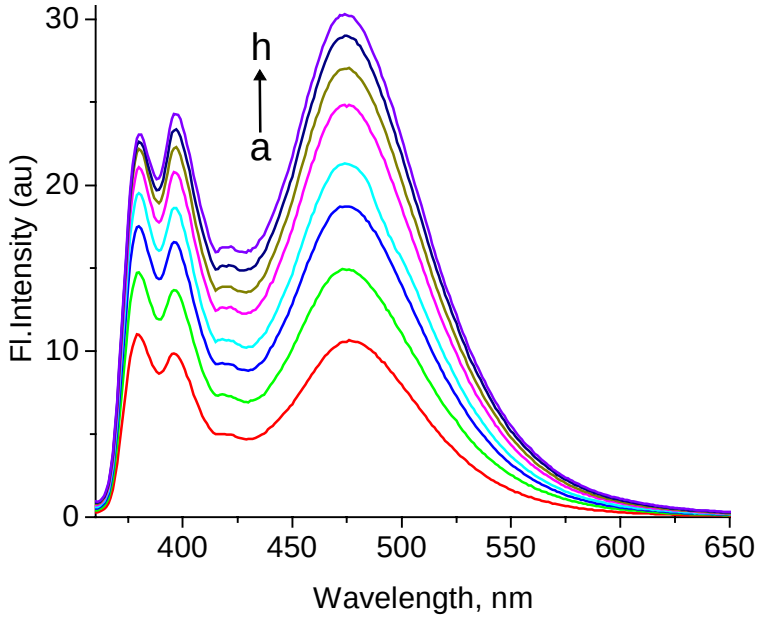

Figure S5. Concentration dependent fluorescence spectra of **1** in aqueous medium. (a) 1.7 and (h) 11.7 μM. Excitation wavelength, 340 nm.

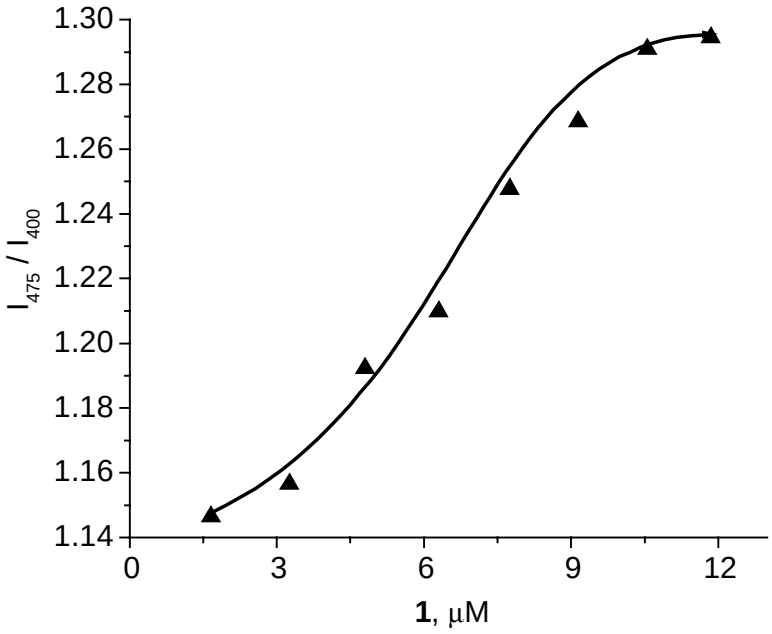

Figure S6. Increase in the relative fluorescence intensity at 475 nm (I_{475} / I_{400}) with increase in concentration of **1**. Excitation wavelength, 340 nm.

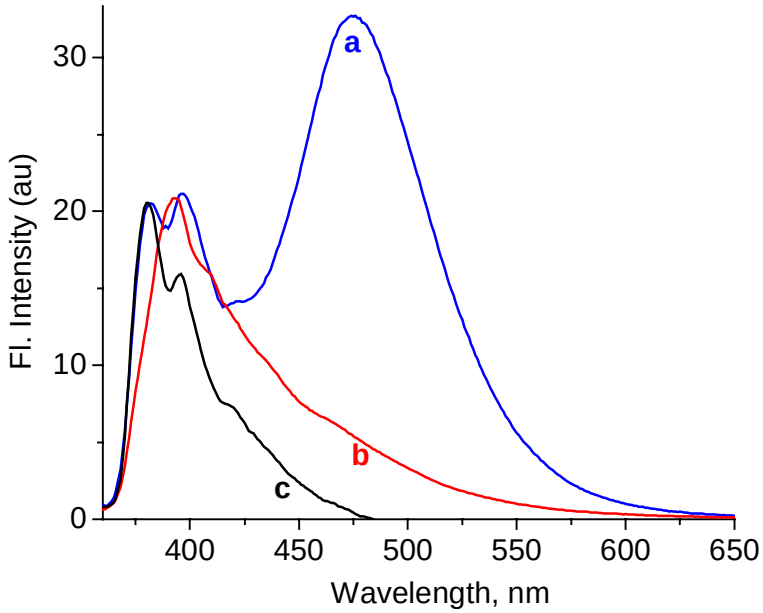

Figure S7. Solvent dependent fluorescence spectra of **1** (13 μM) in (a) aqueous medium, (b) methanol and (c) 10 mM phosphate buffer.

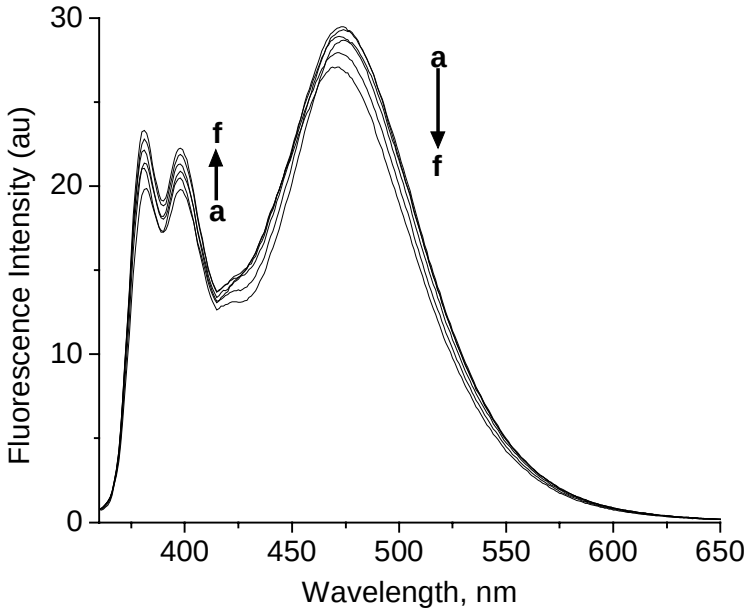

Figure S8. Change in fluorescence spectra of compound **1** with increase in temperature; (a) 25, (b) 35, (c) 45, (d) 55, (e) 65 and (f) 75 °C. Excitation wavelength, 340 nm.

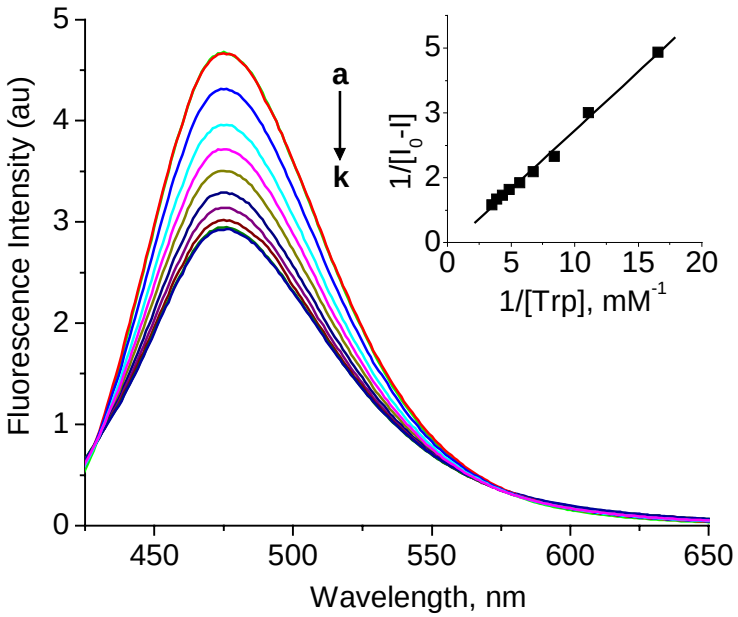

Figure S9. Fluorescence spectra of **1** (13 μM) in aqueous medium with increase in addition of tryptophan. [Trp] (a) 2.9 and (k) 27.0 x 10^{-4} M. Excitation wavelength, 400 nm.

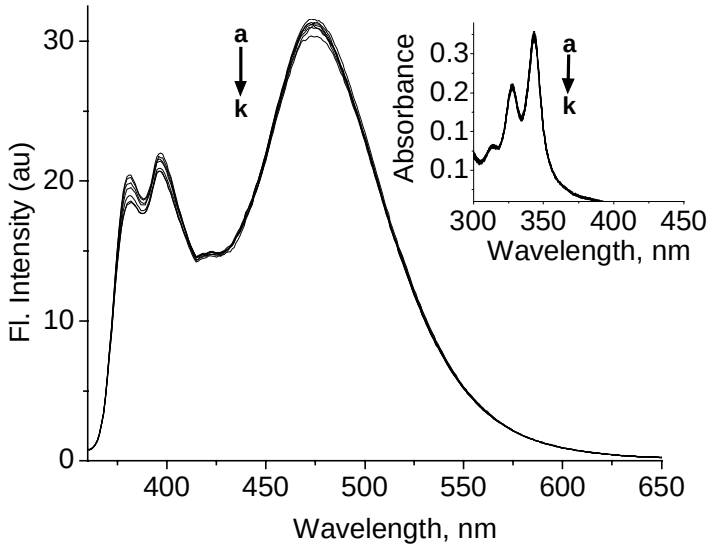

Figure S10. Fluorescence spectra of **1** (13 μM) in aqueous medium with increase in addition of phenylalanine. [Phe] (a) 2.9 and (k) 27.0 x 10^{-4} M. Inset shows the corresponding changes in the absorption spectra. Excitation wavelength, 340 nm.

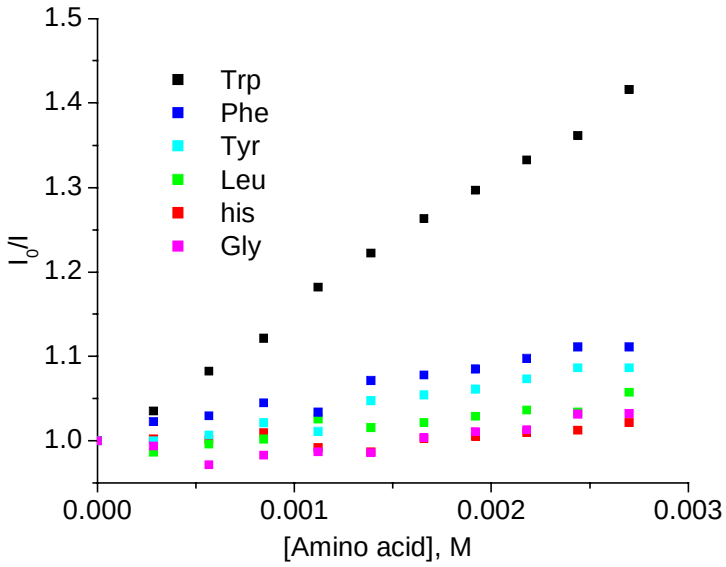

Figure S11. Relative change in fluorescence intensity of the conjugate **1** (13 μM) in aqueous medium in the presence of various amino acids.

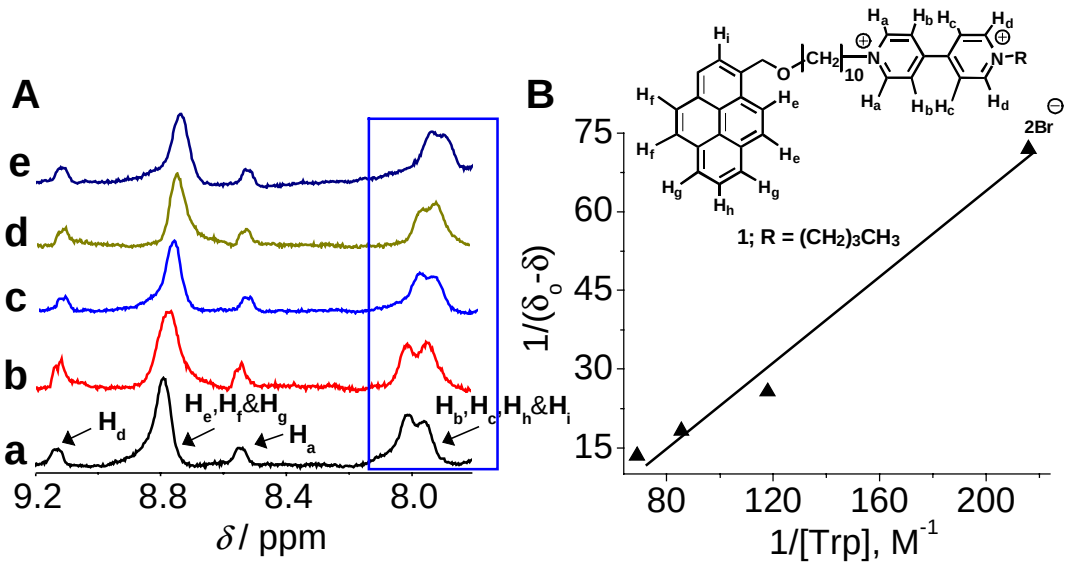

Figure S12. (A) Changes in ^{1}H NMR spectra of **1** (13.4 mM) in D_2O with increase in concentration of tryptophan. [Trp] (a) 0, (b) 4.6, (c) 8.5, (d) 11.7 and (e) 14.5 mM. (B) Benesi-Hildebrand plot for the corresponding changes in chemical shift of the protons (H_b) corresponding to the viologen moiety of the conjugate **1**.

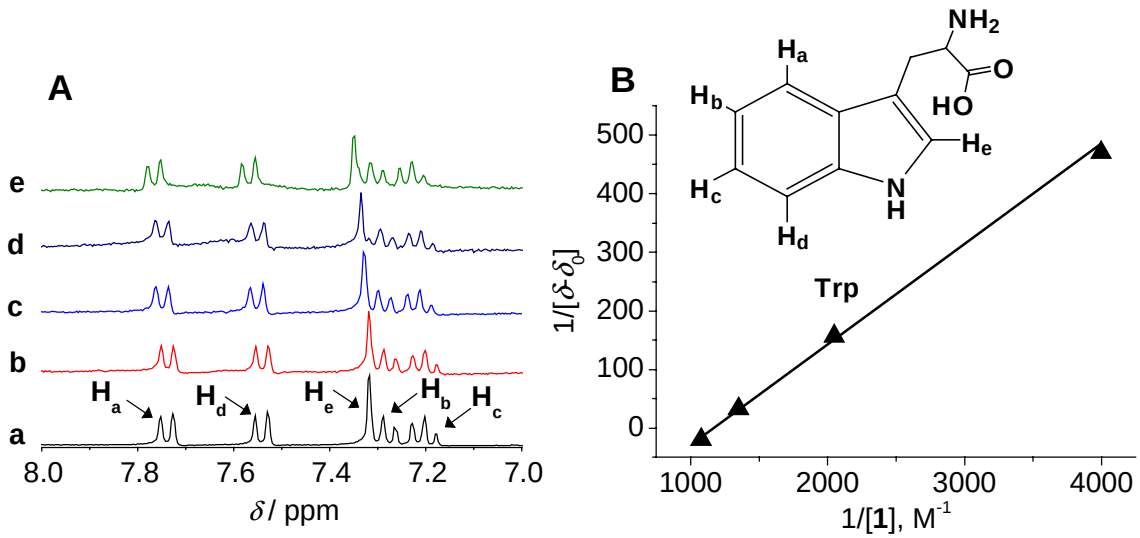

Figure S13. (A) Changes in ^{1}H NMR spectra of tryptophan (3.69 mM) in D_2O with increase in concentration of the conjugate **1**. [**1**] (a) 0, (b) 0.3, (c) 0.5, (d) 0.74 and (e) 0.93 mM. (B) Benesi-Hildebrand plot for the corresponding changes in chemical shift of the proton corresponding to the indole moiety of tryptophan.