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Electronic Supplementary Information

A highly selective and efficient single molecular FRET based sensor for ratiometric detection of Fe^{3+} ions

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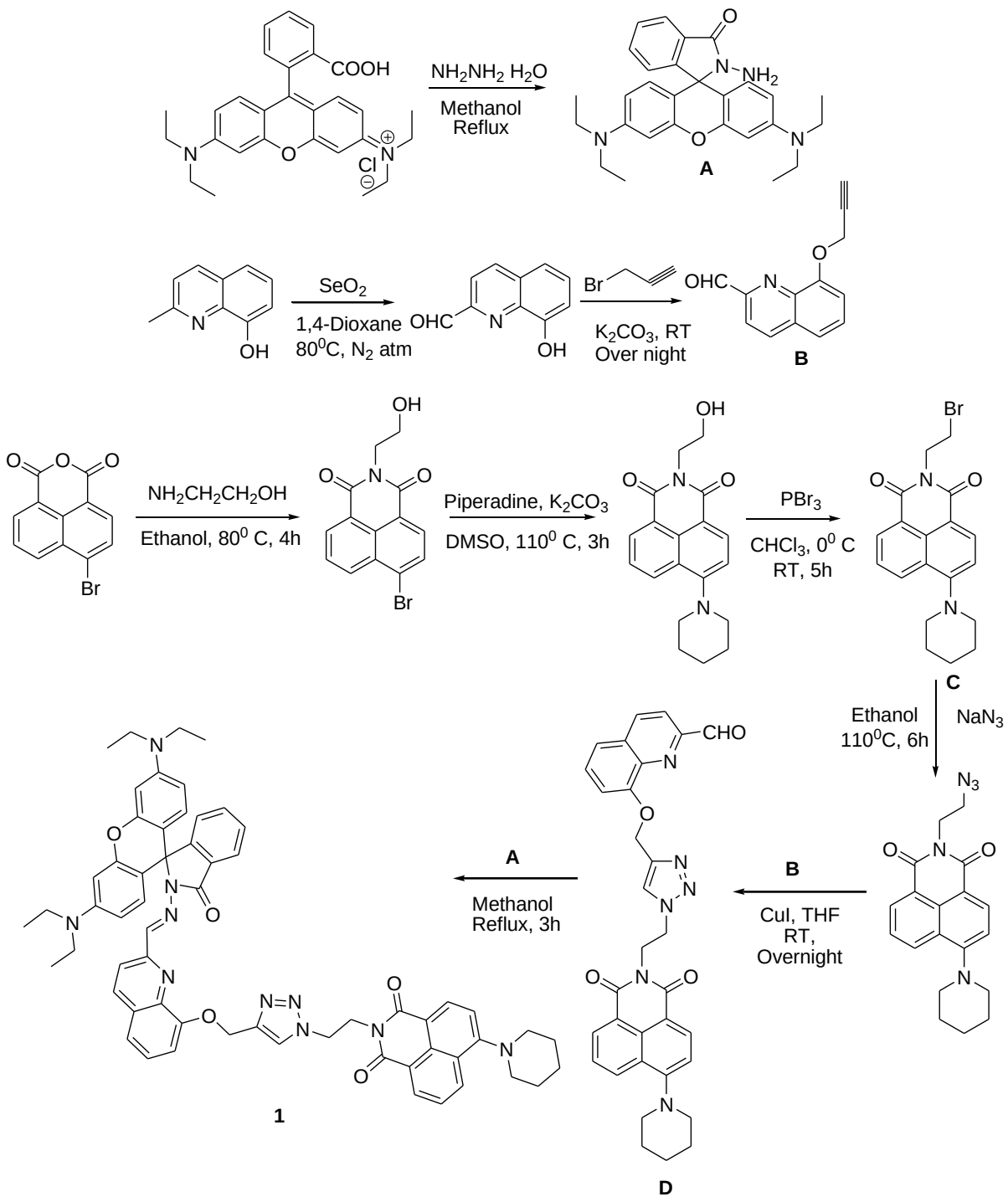

Scheme S1. Synthesis of sensor **1**

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General Information

Dry acetonitrile and double distilled water were used in all experiments. All the materials for synthesis were purchased from commercial suppliers and used without further purification. The solutions of metal ions were prepared from the corresponding chloride salts. Absorption spectra were recorded on a SPECORD 200 PLUS UV-visible spectrophotometer. Fluorescence measurements were performed on a HITACHI F-4500 fluorescence spectrophotometer (Excitation wavelength 420 nm; Slit width 5 nm). All pH measurements were made with a Systronics µpH System Model 361. NMR spectra were recorded using a JEOL–ECP500 spectrometer operating at 500 MHz. ESI-MS spectra were obtained on a PE Sciex API3000 mass spectrometer. Fluorescence imaging experiments were performed using a Leica DM IRB microscope equipped with EBQ-100 UV-lamp. All measurements were carried out at room temperature. Stock solutions of rhodamine derivative (probe) were prepared by dissolving 1.0 mmol of probe (9.98 mg) in 1:1 v/v 0.01M Tris HCl-CH_3CN (pH 7·4) separately, and making up to the mark in a 10 mL volumetric flask. Further dilutions were made to prepare 100 µM solutions for the experiments. Stock solutions of metal ions (1 M) were prepared in de-ionised water.

Synthesis of rhodamine hydrazide (A)

Rhodamine hydrazide was synthesized according to the reported procedure. [1]

Synthesis of 8-(prop-2-ynyloxy)quinoline-2-carbaldehyde (B)

The 8-(prop-2-ynyloxy)quinoline-2-carbaldehyde **B** was synthesized in a two-step procedure. To a solution of SeO_2 (1.66 g, 15 mmol) in 1,4-dixane (20 mL) under N_2 atmosphere, 2-methyl 8-quinolinol (1.59 g, 10 mmol) in 1,4-dixane (20 mL) was added dropwise, the resulted mixture was allowed to stir for 8h under N_2 atmosphere at 70^0 C and progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was allowed to cool to room temperature, filtered and subjected silica gel 100-200 mesh column chromatography using 95:5 hexane-ethtylacetate as eluent to get 0.87 g (50%) of 8-hydroxyquinoline 2-aldehyde in pure form as yellow colour solid. To a solution of 8-hydroxyquinoline 2-aldehyde (0.87 g, 5 mmol) in DMF (20 mL) was added potassium

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carbonate (1.04 g, 7.5 mmol) and the solution was stirred at room temperature. Propargyl bromide (0.7 mL, 7.5 mmol) was added drop wise and the resulting mixture was allowed to stir overnight. After completion of reaction, the reaction mixture was partitioned between DCM and water, and the DCM layer was collected. The aqueous layer was extracted three times with DCM, and the combined organic extracts was dried over anhydrous Na_2SO_4, and subjected to silicagel column chromatogaphy to obtain the desired propargylated aldehyde (0.85 g, 4.0 mmol, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.60 (t, *J* = 7.0 Hz, 1H), 5.10 (d, *J* = 2.5 Hz, 2H), 7.36 (d, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 8.5 Hz, 1H), 7.64 (t, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 10.31 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 56.94, 76.75,77.90, 111.04, 118.07,120.76, 129.58, 131.49, 137.49, 140.14, 153.83, 193.77.

Synthesis of 2-(2-bromoethyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (C)

The intermediate **C** was synthesized in a three step procudure. Ethanolamine (3mL) was added dropwise to a solution of 4-bromo1,8-naphthanoic anhydride (5.0 mmol, 1.38g) in ethanol at 80^o C. the resulting solution was allowed to stir for 4h and the solid was filterd, washed three times with cold methanol, dried under vacuum. To this solid dissolved in DMSO, K_2CO_3 (5 mmol, 0.69 g) and piperadine (4 mL) were added and stirred under heating at 110 ^{o} C for 3h. After completion of the reaction moniterd by TLC, the solution was cooled and partitioned between water and DCM. The DCM layer was collected and the aqueous layer was further extrated with DCM (3x30 mL). The combined organic extracts was washed with brine and dried under vacuum to yield yellow colour solid. The obtained yellow colour solid was dissolved in dry $CHCl_3$, cooled to 0^o C using ice bath. PBr_3 (1.2 mL) was added dropwise and the solution was allowed to heat to room temparature and stirred for 5h. After

completion of the reaction moniterd by TLC, saturated NaOAc (50 mL) was added slowly, extracted with DCM, dried and subjected to silica gel column chromatography to efford C as yellow solid (0.97 g, 2.5 mmol, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 1.73 (m, 2H), 1.88 (m, 4H), 3.23(s, 4H), 3.64 (t, *J* = 7.5 Hz, 2H), 4.56 (t, *J* = 7.5 Hz, 2H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.46 (d, *J* = 8.5 Hz, 1H), 8.53 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 24.41, 26.29, 28.09, 41.11, 54.60, 114.79, 115.32, 122.71, 125.43, 130.05, 131.07, 131.36, 133.08, 157.68, 163.82, 164.38.

Synthesis of 8-((1-(2-(1,3-dioxo-6-(piperidin-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)quinoline-2-carbaldehyde (D)

To the solution of **C** (0.77 g, 2 mmol) in ethanol, NaN_3 (0.69 g, 6 mmol) was added and refulxed at 110^{o} C for 6h. The resulted solution was colled to room temparature, dried under vacuum, suspended in DCM. The DCM solution was washed with water for 3 times and drieed under vacuum to yield pale yellow solid. The solid was dissolved in THF (20 mL), propargylated hydroxyquinoline aldehyde (**B**) (0.46 g, 2.2 mmol) and CuI (c.a) were added. The resulted solution was sturred over night, filterd, dried under vacuum and subjected to silica gel column chromato graphy to yield **D** as yellow solid (0.62 g, 1.1 mmol, 55% yield). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 1.73 (m, 2H), 1.88 (m, 4H), 3.21(s, 4H), 4.64 (t, *J* = 6.0 Hz, 2H), 4.76 (t, *J* = 6.0 Hz, 2H), 5.59 (s, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.57 (m, 2H), 7.85(s, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 8.27-8.34 (m, 3H), 8.39 (d, *J* = 7.0 Hz, 1H), 10.26 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 24.39, 26.26, 39.60, 48.21, 54.58, 63.41, 111.30, 114.73, 114.95, 117.88, 120.17, 122.39, 123.87, 125.36, 126.24, 129.99, 130.11, 131.21, 131.40, 133.14, 137.40, 140.19, 143.90, 151.49, 154.69, 157.82, 163.82, 164.40, 193.91. ESI-MS (+ve mode, m/z): 561.20 ($M+H^{+}$), Calc. for $C_{32}H_{28}N_6O_4$ is 560.22.

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Synthesis of sensor 1

To the solution of **D** (0.28 g, 0.5 mmol) in methanol (20 mL), rhodamine hydrazide **A** (0.25g, 0.55 mmol) was added and refluxed for 3h. After completion of the reaction moniterd by TLC, methanol was evoparated under vacuum, the resultant solid was dissolved in DCM and subjected to silica gel column chromatography to obtain **1** as yellow solid (0.33 g, 0.3 mmol, 60% yield). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 1.14 (t, *J* = 7.0 Hz, 12H), 1.71 (m, 2H), 1.86 (m, 4H), 3.20(s, 4H), 3.31 (q, *J* = 7.0 Hz, 8H), 4.65 (t, *J* = 6.0 Hz, 2H), 4.75 (t, *J* = 6.0 Hz, 2H), 5.49 (s, 2H), 6.23 (d, *J* = 8.5 Hz, 2H), 6.48 (s, 2H), 6.55 (d, *J* = 9.0 Hz, 2H), 7.07 (d, *J* = 8.5 Hz, 1H), 7.15 (d, *J* = 7.0 Hz, 1H), 7.20 (d, *J* = 7.0 Hz, 1H), 7.31 (m, 2H), 7.44-7.52 (m, 2H), 7.59 (t, *J* = 8.5 Hz, 1H), 7.89(s, 1H), 7.96-8.05 (m, 3H), 8.34 (d, *J* = 8.0 Hz, 2H), 8.42 (d, *J* = 6.5 Hz, 1H), 9.01 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 12.71, 24.42, 26.27, 29.79, 39.60,44.41, 48.12, 54.59, 63.62, 66.44, 98.23, 106.12, 108.04, 111.28, 114.75, 115.14, 118.96, 120.28, 122.50, 123.98, 125.38, 126.29, 127.21, 127.86, 128.40, 128.88, 129.52, 130.13, 131.10, 131.40, 133.13, 133.72, 135.94, 139.95, 144.39, 148.23,149.02, 152.02, 153.35,153.95, 154.12, 157.73, 163.80, 164.38, 165.23. ESI-HRMS (+ve mode, m/z): 999.4677 ($M+H^+$), Calc. for $C_{60}H_{58}N_{10}O_5$ is 999.4670.

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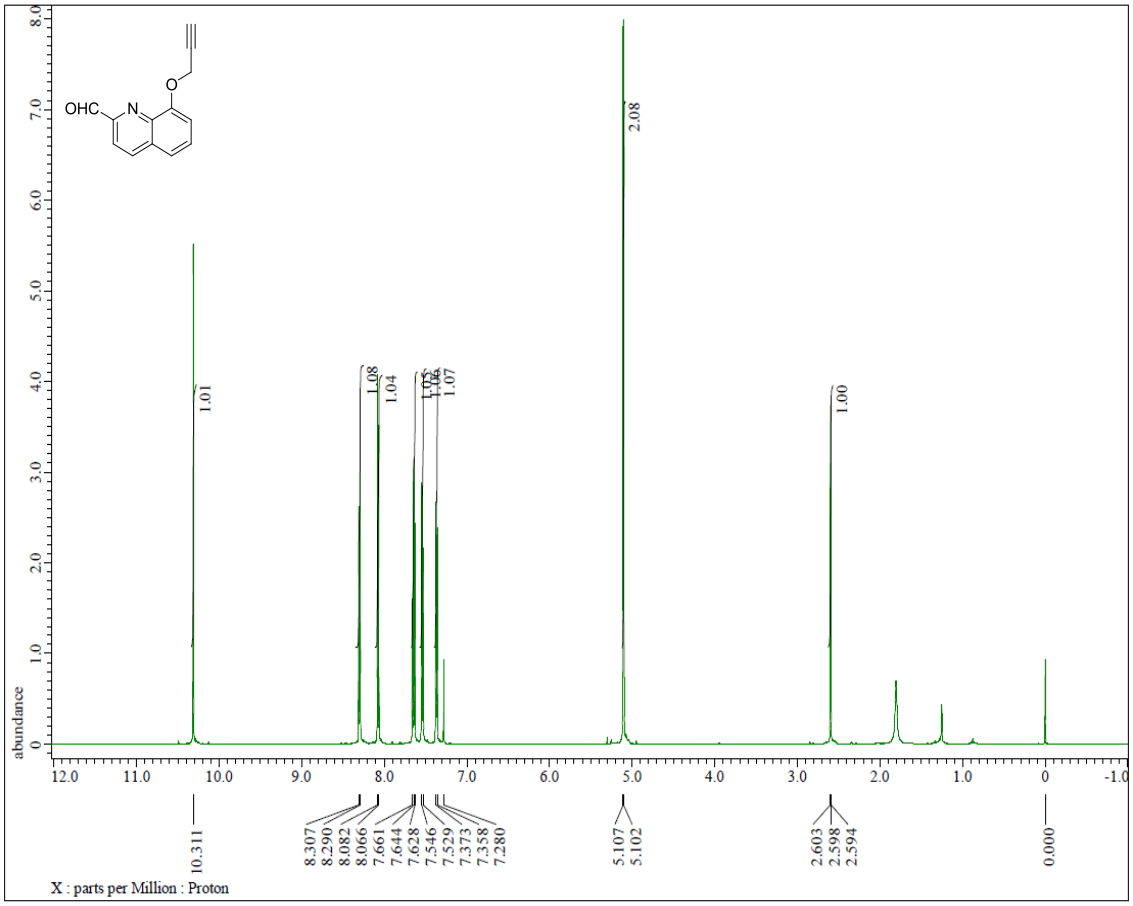

Fig. S1. ^{1}H NMR spectrum of **B** in $CDCl_3$

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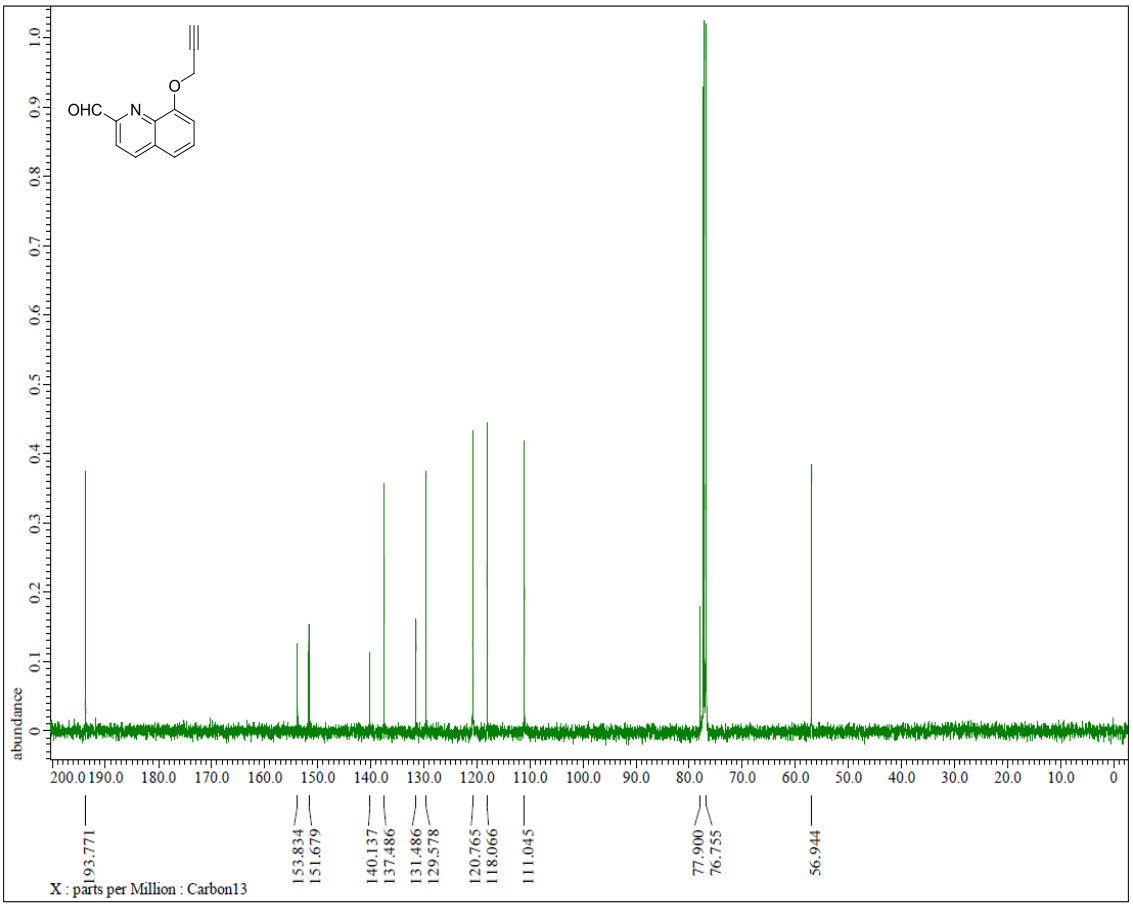

Fig. S2. ^{13}C NMR spectrum of **B** in $CDCl_3$

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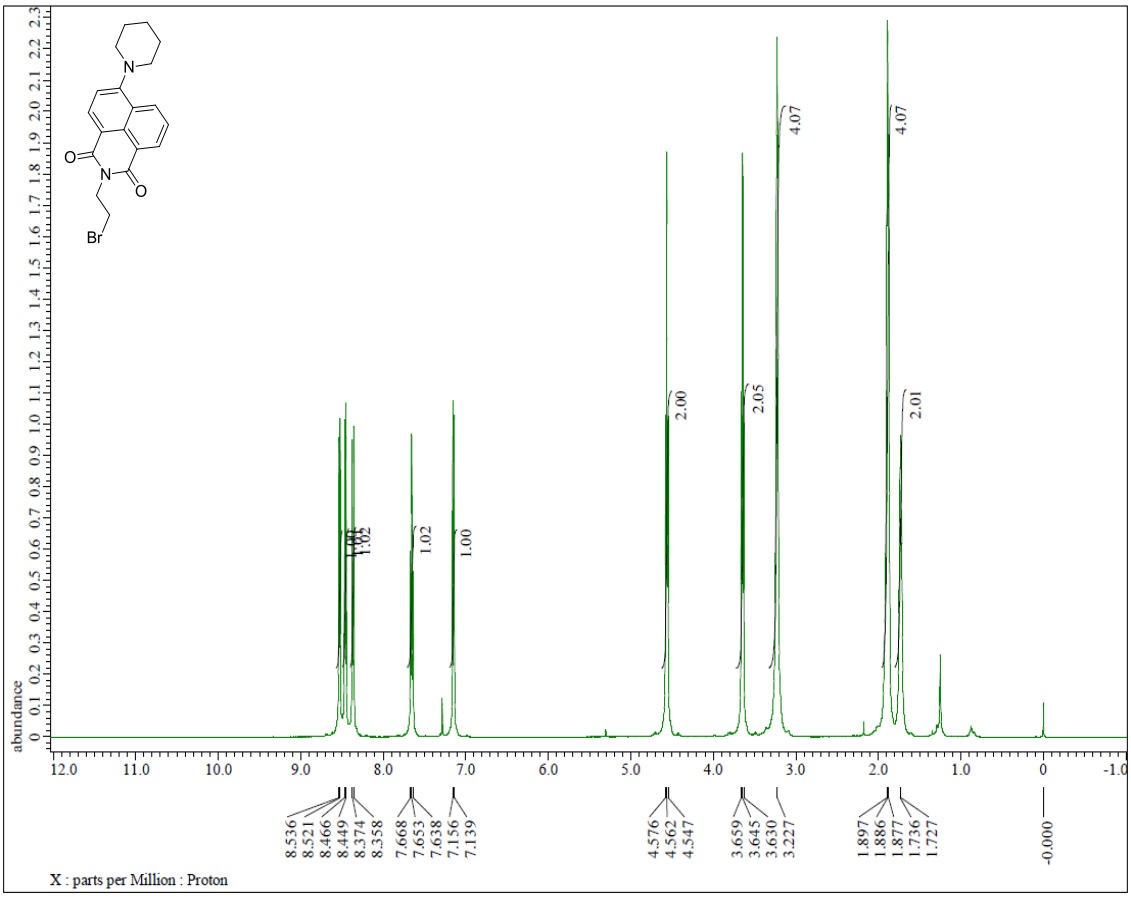

Fig. S3. ^{1}H NMR spectrum of **C** in $CDCl_3$

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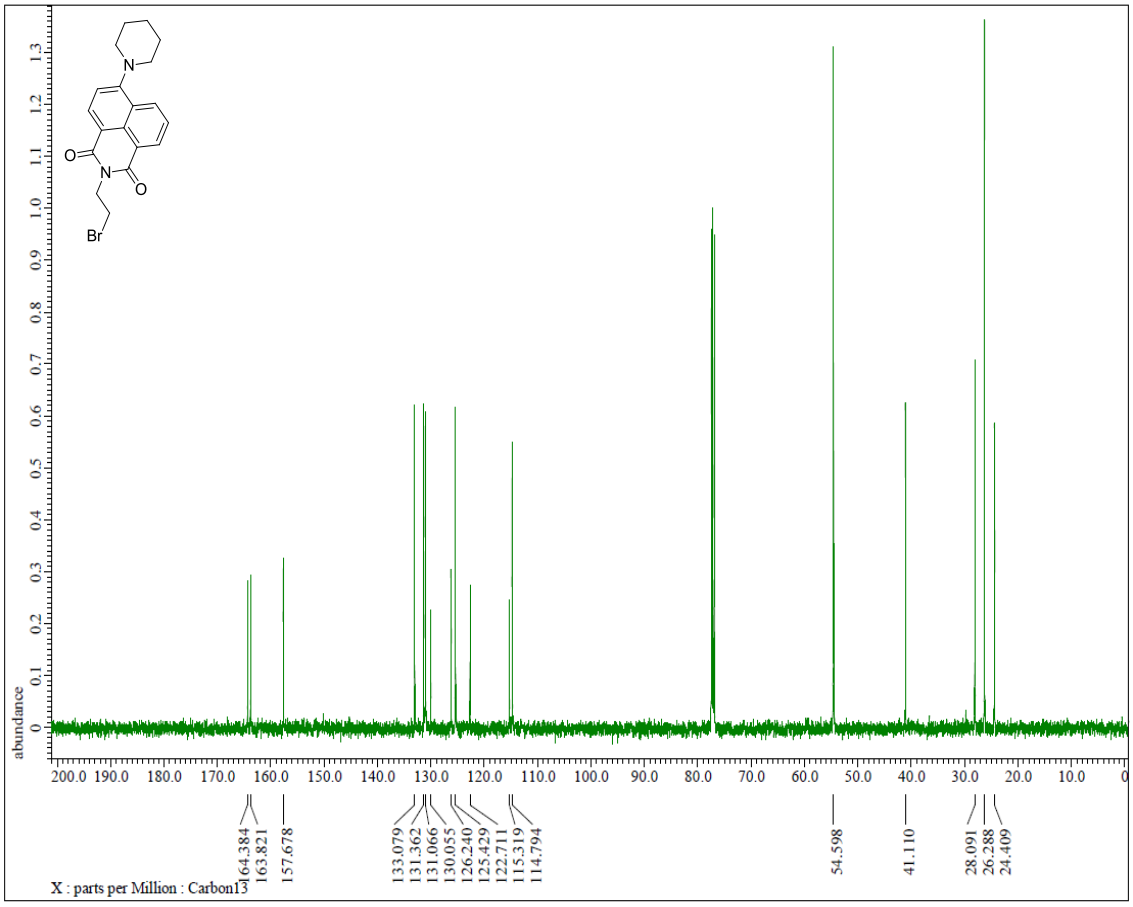

Fig. S4. ^{13}C NMR spectrum of **C** in $CDCl_3$

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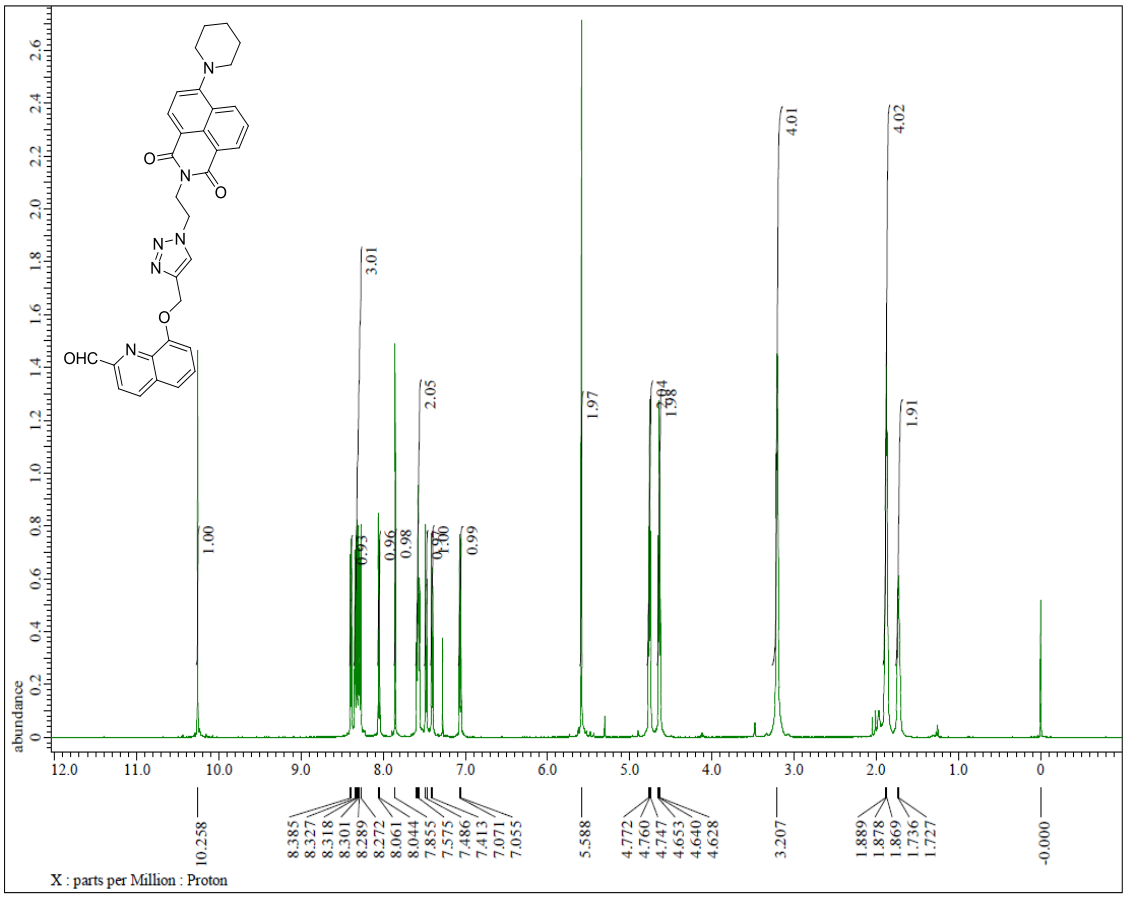

Fig. S5. ^{1}H NMR spectrum of **D** in $CDCl_3$

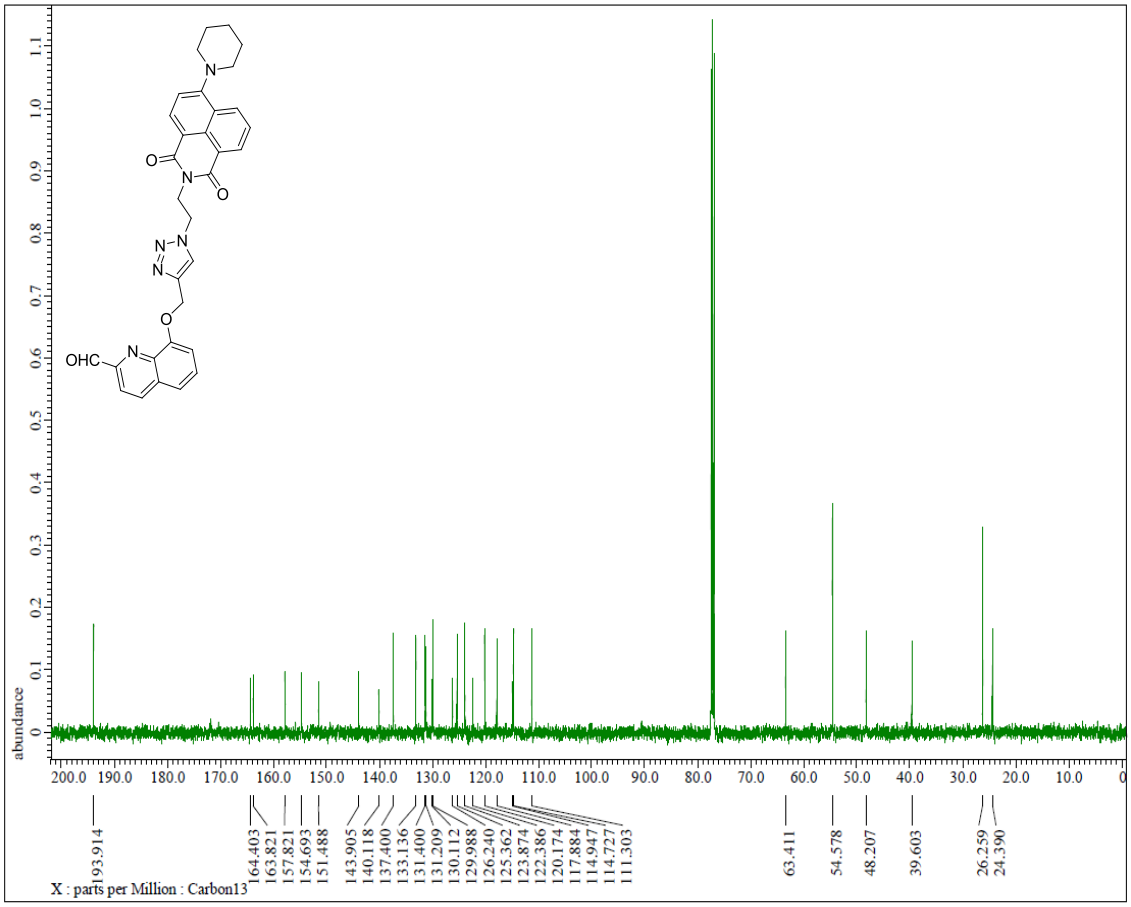

Fig. S6. ^{13}C NMR spectrum of **D** in $CDCl_3$

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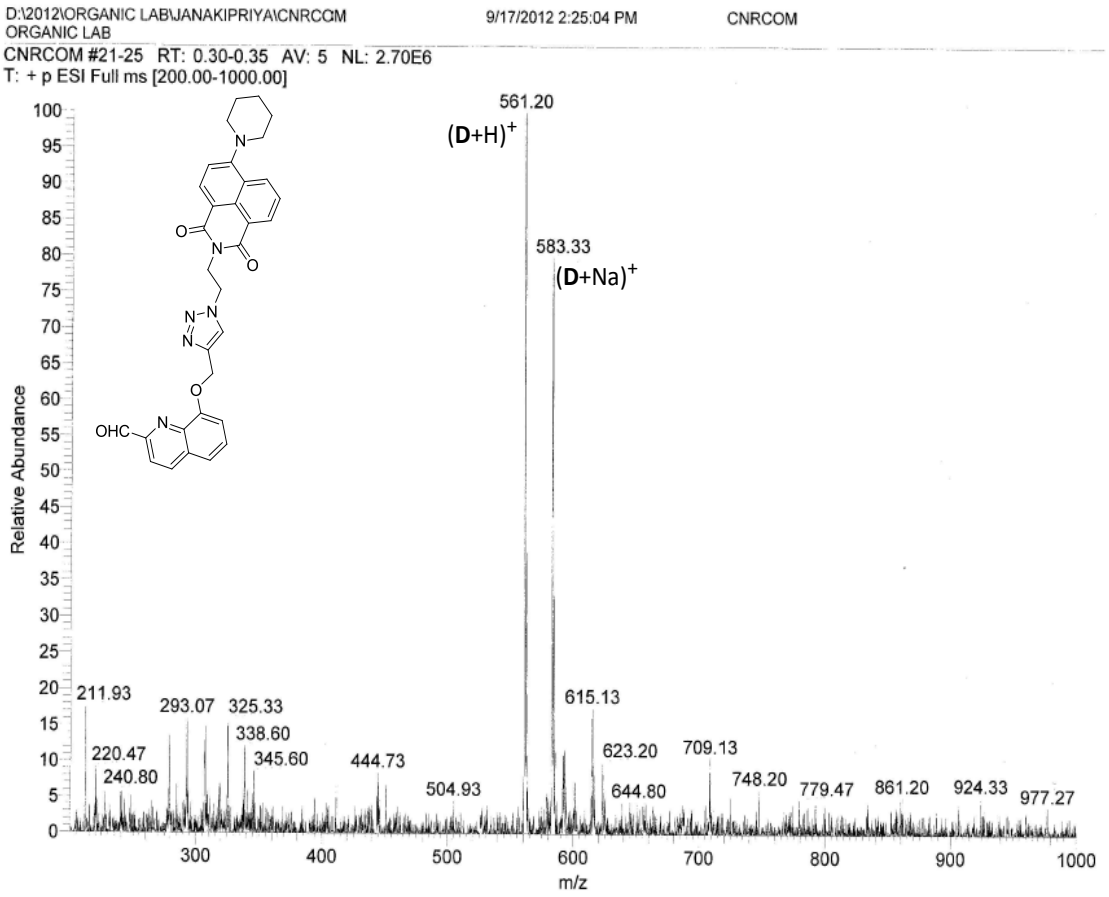

Fig. S7. ESI Mass spectrum of **D**

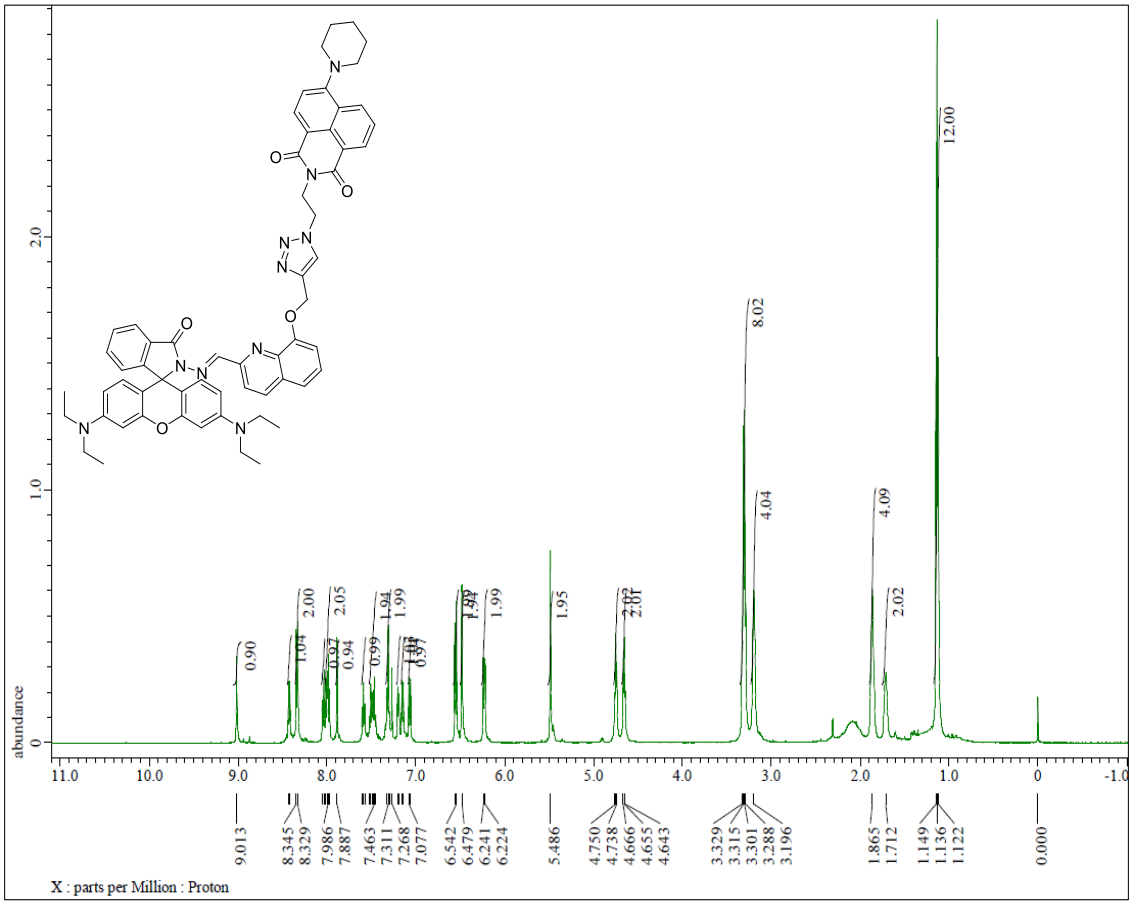

Fig. S8. ^{1}H NMR spectrum of **1** in $CDCl_3$

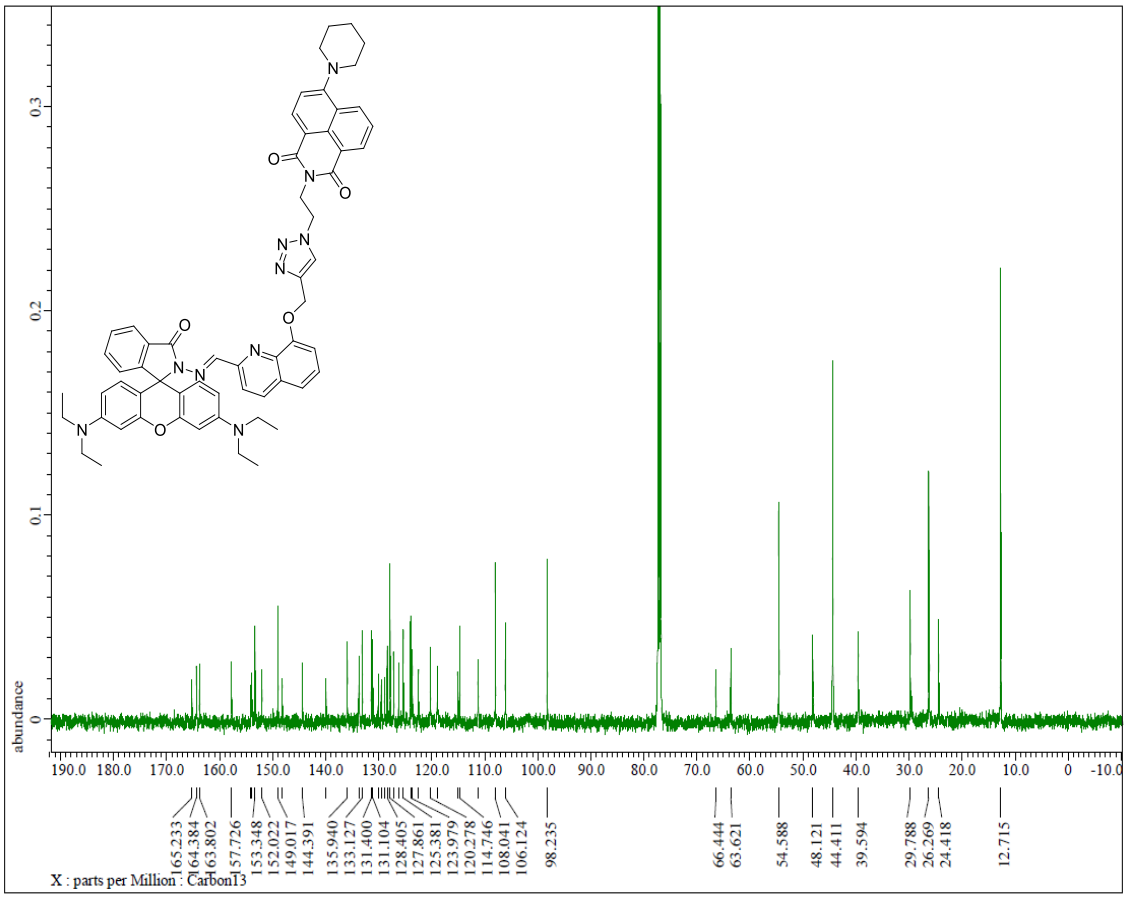

Fig. S9. ^{13}C NMR spectrum of **1** in $CDCl_3$

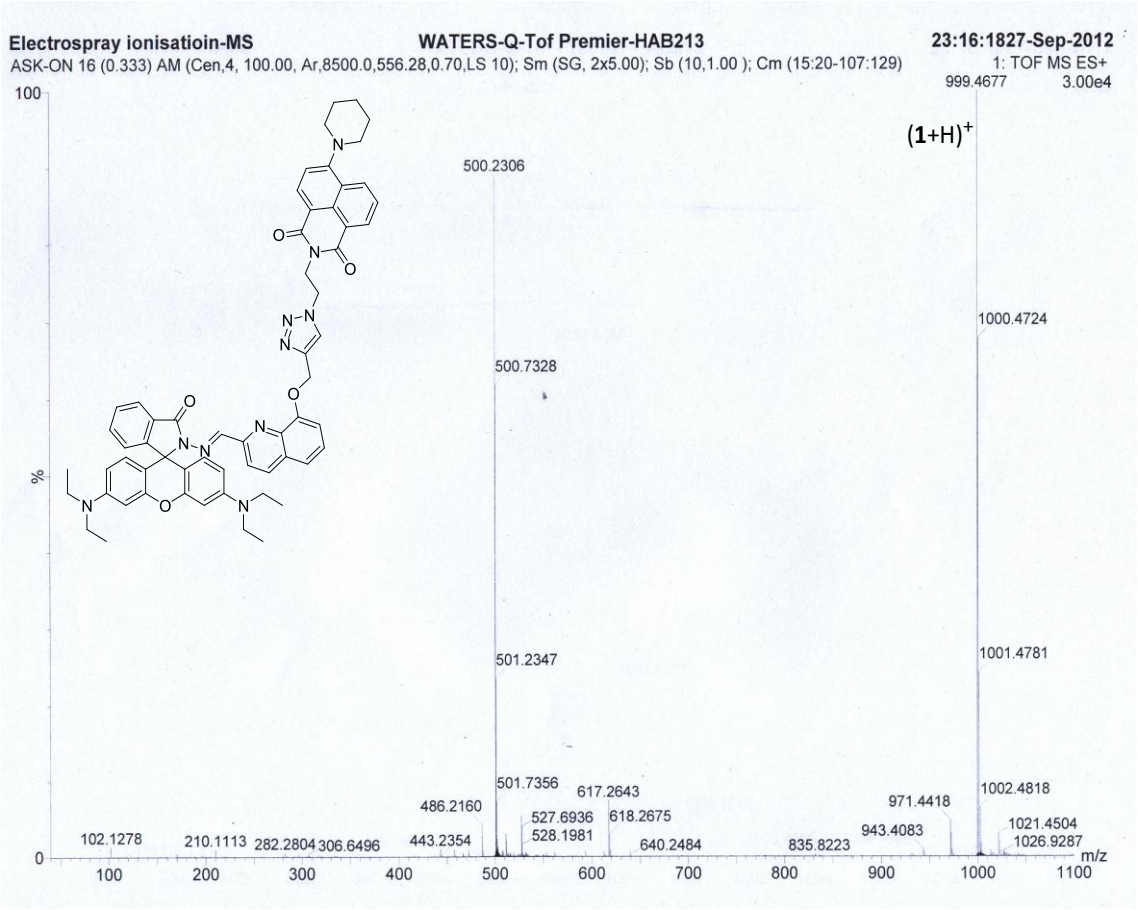

Fig. S10. ESI Mass spectrum of **1**

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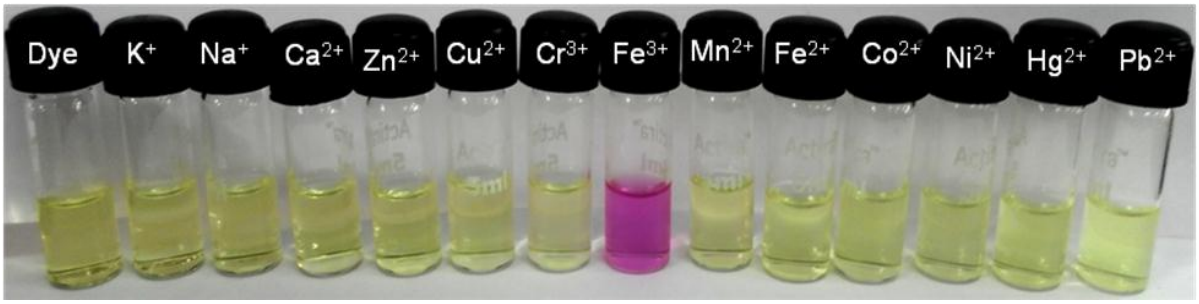

Fig. S11. Effect of addition of various metal ions (20 µM) to 20 µM solutions of fluorescent sensor **1** in 1:1 v/v 0.01M Tris HCl-CH_3CN, pH 7.4.

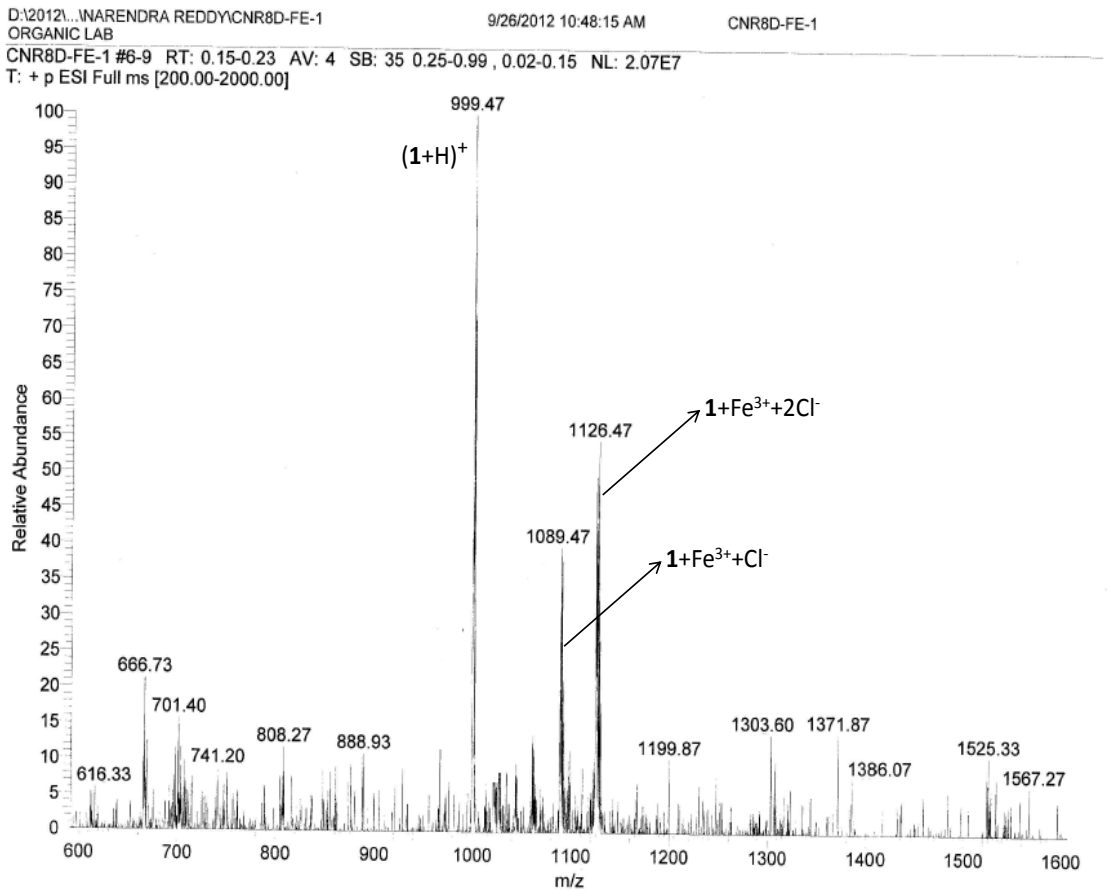

Fig. S12. ESI Mass spectrum of **1**-Fe^{3+} complex

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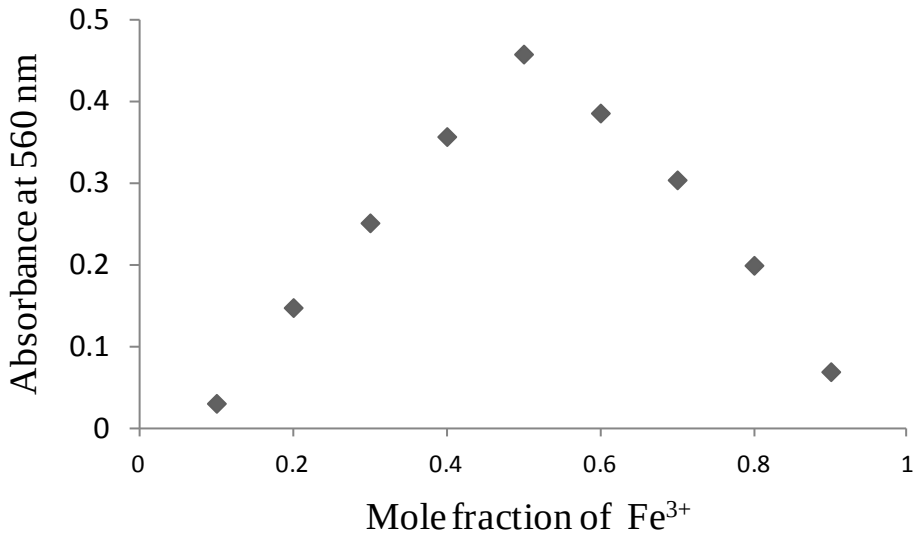

Fig. S13. Job plot of 1:1 complex of **1** and Fe^{3+} in 1:1 v/v 0.01 M Tris HCl-CH_3CN, pH 7.4, the absorption at 560 nm was plotted against the mole fraction of Fe^{3+} ions. The total concentration of Fe^{3+} ions with **1** was 20 µM.

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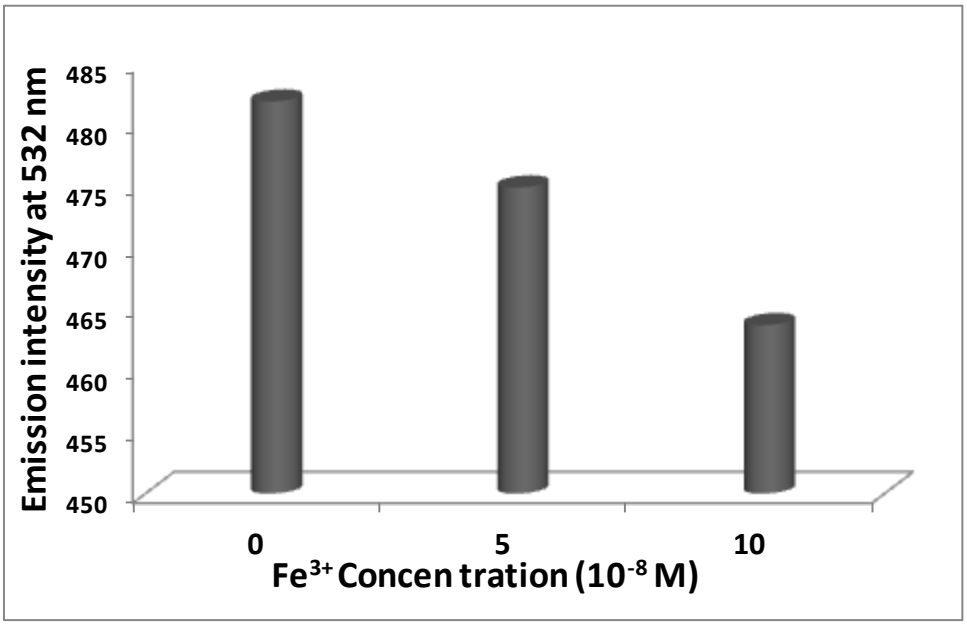

Fig. S14. Detection limit of sensor **1**. The variation in the fluorescence intensity of **1** (10 μM) at 532 nm in 1:1 v/v 0.01 M Tris HCl-CH_3CN, pH 7.4, upon addition of Fe^{3+} ions. The fluorescence intensity at 532 nm was decreased due to the Fe^{3+} induced FRET from napthalimide donor (532 nm) to rhodamine acceptor (580 nm).

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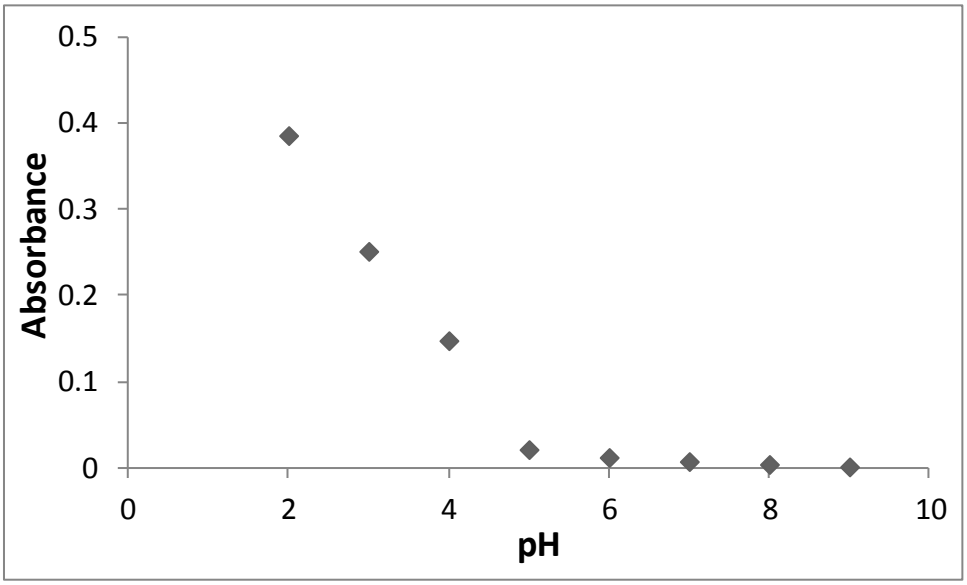

Fig. S15. pH dependant variation in absorption intensity of **1** (10 μM) at 560 nm.

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Cell viability assay

The cell viability assay of sensor **1** on NIH3T3 cells was determined by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide) assay (*J Immunol Methods*, 1983, **65**, 55.). The NIH3T3 cells were trypsinised and seeded in 48-well flat-bottom culture plates at a density of 4 x 10^3 cells per well in 250 µL DMEM supplemented with 10% FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin, 30 µg/mL gentamicin. The cells were allowed for 24 hours to adhere and grow at 37 ºC in CO_2 incubator. Then the medium was replaced with 250 µL fresh medium containing various concentrations of sensor **1** (0 to 6 µM) and incubated for 12 hours in a humidified chamber with 5% CO_2 after which the medium was removed. The cells were further incubated for 3 hours with 250 µL of fresh medium containing 1 mg/mL MTT reagent. The medium was then removed to eliminate unreacted MTT reagent. DMSO (100 µL) was added into each well to dissolve the formazan precipitate formed and was measured spectrophotometrically using a microplate reader at 570 nm. The assay was performed in quadruplet for each concentration. The cytotoxicity of the sensor **1** was expressed in terms of percentage of cell viability relative to the untreated control cells which was taken as 100 percent viable.

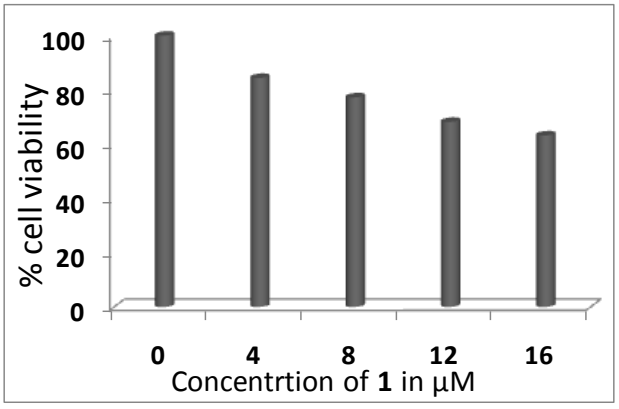

Fig. S16. The cell viability Percentage of NIH 3T3 cells after treatment with different concentrations of sensor **1**.

1. N. R. Chereddy and S. Thennarasu, *Dyes Pigm*. 2011, **91**, 378