Electronic Supplementary Information

Through Bond Energy Transfer Based Ratiometric Probe for Fluorescent Imaging of Sn^{2+} Ions in Living Cells

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General information:

Chemicals and solvents were purchased from commercial suppliers and used as received. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD (300 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.16) or tetramethylsilane (TMS δ 0.00) was used as a reference. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), bs (broad singlet). Coupling constants were reported in Hertz (Hz). High resolution mass spectra were obtained on a XeVO TQ-S: Waters spectrometer. IR spectra were measured on Thermo Scientific Nicolet 380 instrument. For thin layer chromatography (TLC), Merck precoated TLC plates (Merck 60 F254) were used, and compounds were visualized with a UV light at 254 nm. Further visualization was achieved by staining with iodine. Flash chromatography separations were performed on SRL 230-400 mesh silica gel. Milli-Q Milipore 18.2 MΩ cm^{-1} water was used throughout all experiments. A JASCO (model V-570) UV-vis spectrophotometer was used for recording UV-vis spectra. FTIR spectra were recorded on a JASCO FTIR spectrophotometer (model FTIR-H2O). Melting point was measured with a VEEGO digital melting point apparatus. Steady state fluorescence emission and excitation spectra were recorded with a Hitachi-Hitachi F-4500 spectrofluorometer. A Systronics digital pH meter (model 335) was used to measure the solution pH. Either 50 mM HCl or KOH was used for pH adjustment.

Synthesis of compound **1**:

Compound 1 was synthesized according to literature procedure.[1]

Synthesis of compound **2**:

To the mixture of 1-iodo naphthalene (762.21 mg, 3 mmol), CuI (5.7 mg, 0.03 mmol), and $Pd(PPh_3)_4$ (69.3 mg, 0.06 mmol) were added anhydrous THF (40 mL) and TEA (20 mL) under argon. While stirring, 2-methyl-3-butyn-2-ol (0.50 g, 6 mmol) was injected through a syringe. The reaction mixture was stirred at 75 °C overnight under argon atmosphere and was monitored by TLC. Upon completion, the solution was evaporated in vacuo to dryness. The crude product was purified by silica gel flash column chromatography (CH_2Cl_2/EtOAc, 4/1) to give compound 2 (0.45 g, 17.7 mmol, 71%) as a yellow liquid.

^1H NMR (300 MHz, $CDCl_3$): δ 8.460-8.432 (d, 1H), 7.851-7.780 (m, 2H), 7.730-7.702 (m, 1H), 7.638-7.584 (m, 1H), 7.548-7.499 (t, 1H), 7.414-7.363 (t, 1H), 1.844 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 133.32, 130.42, 128.52, 126.89, 126.32, 125.23, 120.61, 99.42, 80.21, 65.84, 31.34. QTOF MASS (Fig. S) m/z $(M+H)^+$ calculated. For $C_{15}H_{15}O^+$: 211.1122 found:

Synthesis of compound **3**:

To a toluene (30 mL) solution compound of 3 (490 g, 3.6 mmol) was added NaH (0.44 g, 18 mmol), and the mixture was stirred at 80 °C for over 20 min and monitored by TLC. Upon completion, the solution was evaporated in vacuo to dryness. The crude product was purified by silica gel flash column chromatography (petroleum ether) to give compound 3 (0.49 g, 3.21 mmol, 89%) as a light yellow liquid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.361-8.333 (d, 1H), 7.787-7.761 (d, 2H), 7.713-7.650 (m, 1H), 7.531-7.421 (m, 2H), 7.372-7.324 (t, 1H), 3.420-3.382 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 133.61, 133.17, 131.22, 129.29, 128.32, 126.96, 126.46, 126.13, 125.10, 119.90, 81.93.

QTOF MASS (Fig. S) m/z $(M+H)^+$ calculated. For $C_{12}H_9^+$:153.0704 found:

Synthesis of compound **4**:

To the mixture of 4-iodo aniline (657.60 mg, 3 mmol), CuI (5.7 mg, 0.03 mmol), and $Pd(PPh_3)_4$ (69.3 mg, 0.06 mmol) were added anhydrous THF (40 mL) and TEA (20 mL) under argon. While stirring, compound 3 (532.70 g, 3.5 mmol) in THF was injected through a syringe. The reaction mixture was stirred at 75 °C overnight under argon atmosphere and was monitored by TLC. Upon completion, the solution was evaporated in vacuo to dryness. The crude product was purified by silica gel flash column chromatography (petroleum ether /EtOAc, 7/3) to give compound 3 (0.470 g, 17.7 mmol, 64%) as a yellow liquid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.454-8.427 (d, 1H), 7.850-7.722 (m, 2H), 7.722-7.699 (d, 1H), 7.597-7.398 (m, 5H), 6.786-6.639 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 146.84, 133.12, 129.77, 128.09, 126.45, 125.32, 121.59, 114.84, 113.96, 112.70, 95.23. QTOF MASS (Fig. S-9) m/z $(M+H)^+$ calculated. For $C_{18}H_{14}N^+$:244.1121 found: 244.1124.

Synthesis of compound **5**:

To the mixture of compound 4 (0.400g, 1.64 mmol) in 7 ml acetic acid added 2ml of distilled acetic anhydride. The mixture was stirred for 2h. Then the reaction mixture was poured into ice and stirred for 10 min. The solid product obtained was further purified by silica gel flash column chromatography (petroleum ether /EtOAc, 7/3) to give compound 3 (0.390 g, 1.36 mmol, 84%) as a yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36-8.33 (d, 1H), 7.80-7.77 (m, 2H), 7.68-7.65 (d, 1H), 7.52-7.35 (m, 6H), 7.19 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 168.30, 138.00, 132.91, 132.08, 129.93, 128.31, 129.98, 126.10, 126.10, 125.89, 124.96, 120.66, 119.18, 118.53, 93.83, 24.26. QTOF MASS (Fig. S) m/z (M+ Na)$^+$ calculated. For $C_{20}H_{15}NO^+Na$: 308.1051 found: 308.1052

Synthesis of **NAP-RD**:

To the mixture of compound 1 (0.600 g, 1.20 mmol) in dichloromethane added few drops of triethylamine. Then compound 4 (0.270 g, 1.10 mmol) in dichloromethane was drop wise. The reaction mixture was stirred for additional 6 h. Upon completion of the reaction monitored by TLC, the solution was evaporated in vacuo to dryness. The crude product was purified by silica gel flash column chromatography (petroleum ether /EtOAc, 7/3) to give **NAP-RD** (0.500 g, 0.74 mmol, 68%) as a light yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.45-8.42 (m, 1H), 7.96-7.92 (m, 1H), 7.82-7.79 (m, 2H), 7.64-7.62 (m, 1H), 7.68-7.42 (m, 9H), 7.18-7.14 (m, 1H), 6.95-6.88 (m, 2H), 6.67-7.62 (m, 2H), 6.29-6.23 (m, 2H), 7.19 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 168.30, 138.00, 132.91, 132.08, 129.93, 128.31, 129.98, 126.10, 126.10, 125.89, 124.96, 120.66, 119.18, 118.53, 93.83, 24.26. QTOF MASS (Fig. S-15) m/z (M+H)$^+$ calculated. For $C_{46}H_{42}N_3O_2^+$: 668.3272 found: 668.3272.

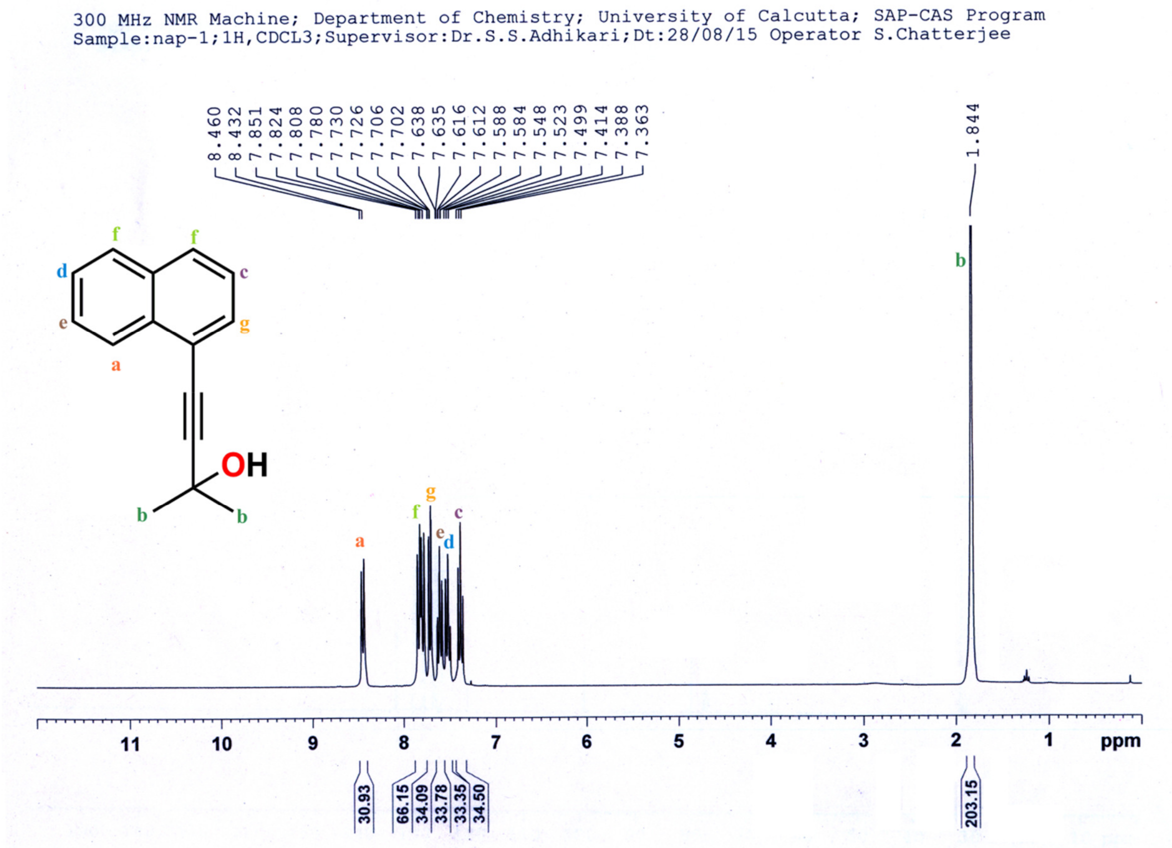


Fig. S-1[1]H NMR spectra of compound **2**

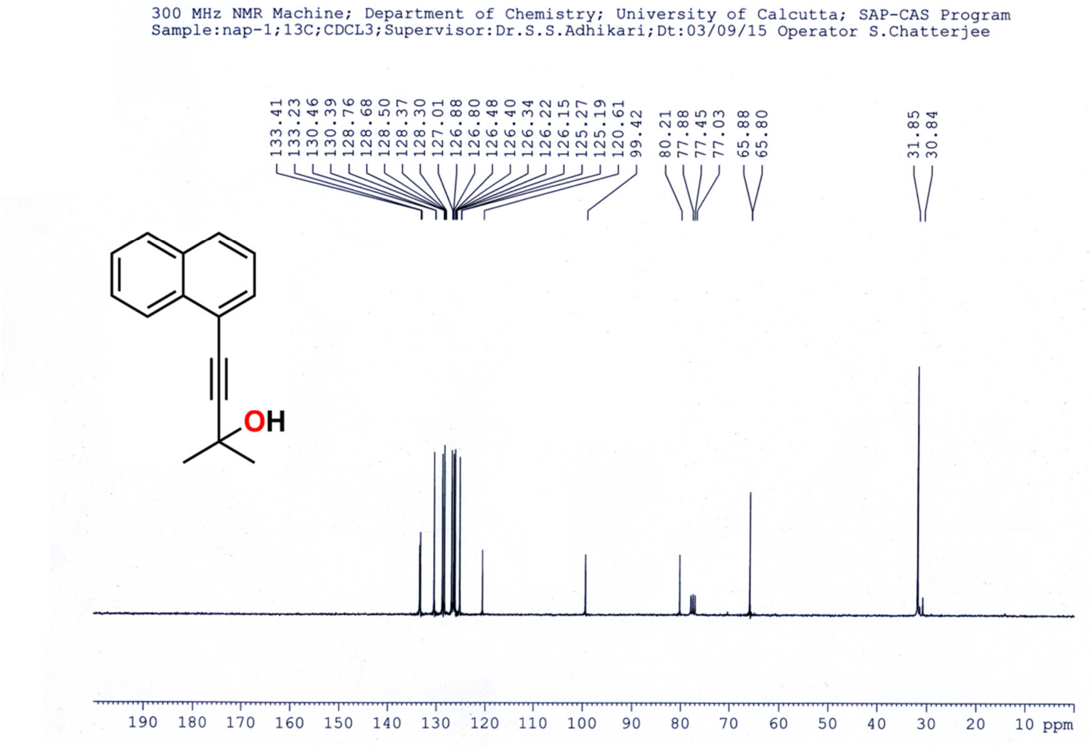


Fig. S-2 ^{13}C spectra of compound **2**

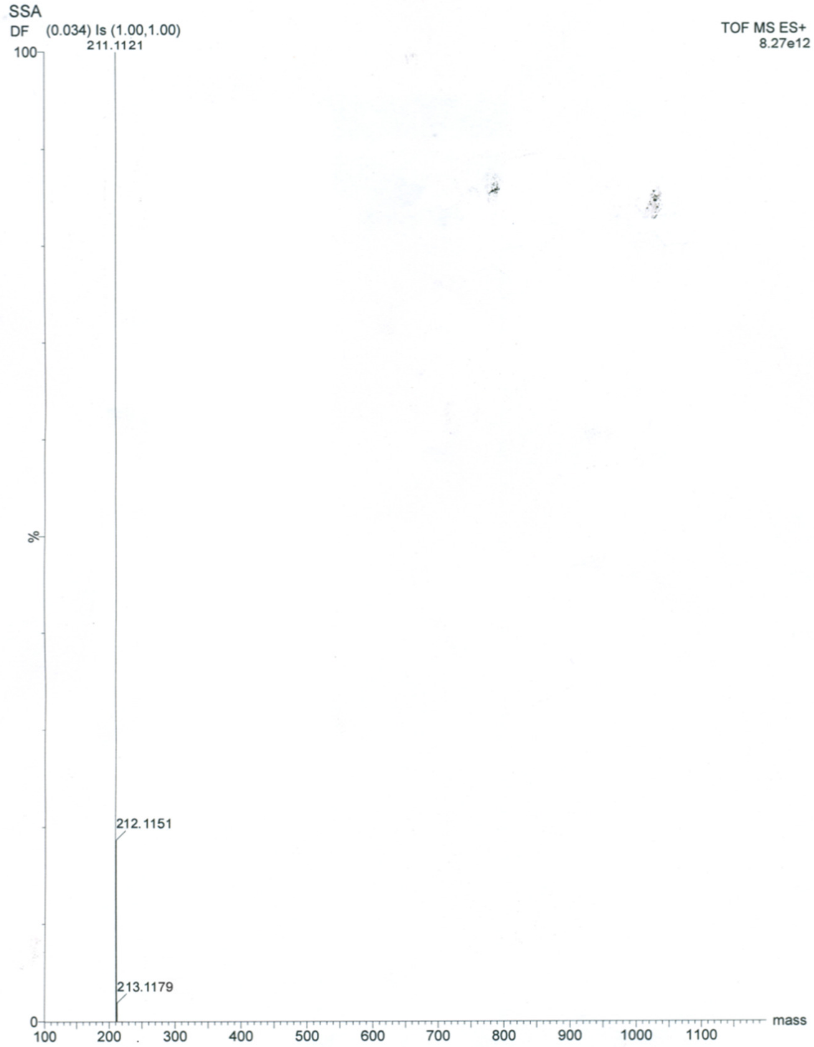


Fig. S-3 Q-tof MS spectra of compound **2**

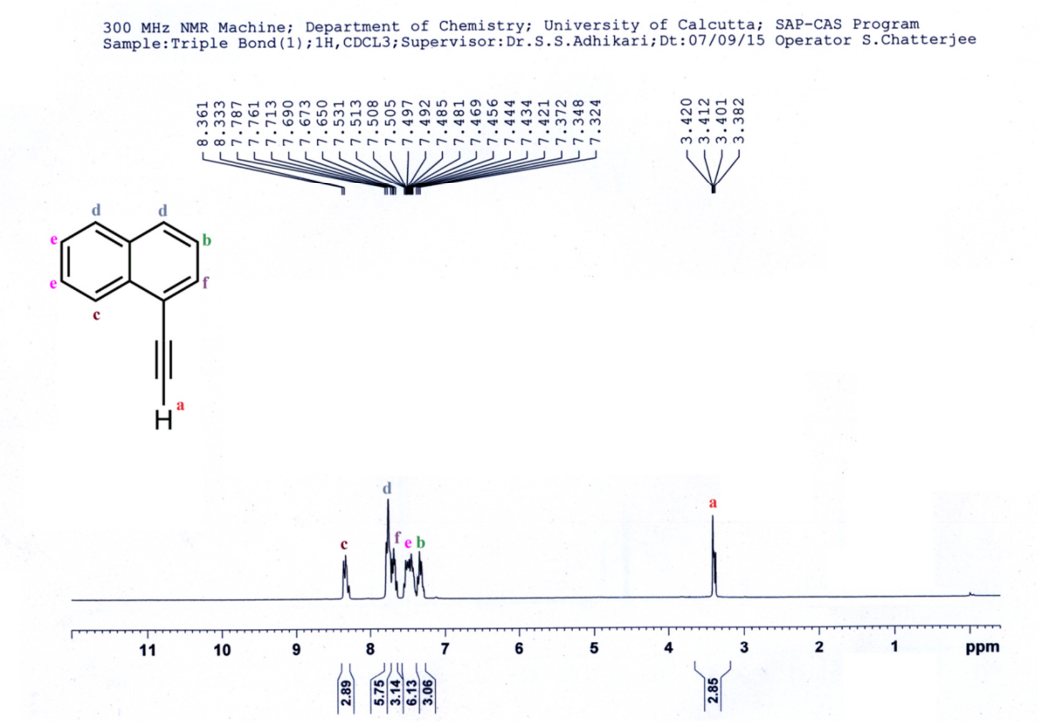


Fig. S-4 ^{1}H NMR spectra of compound **3**

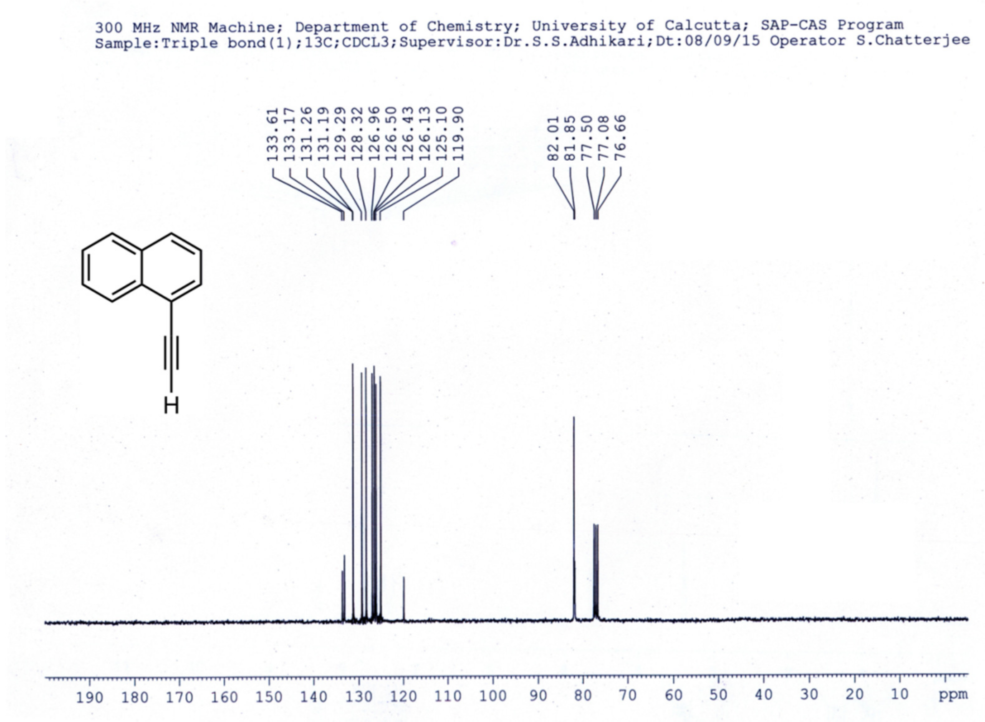


Fig. S-5 ^{13}C spectra of compound **3**

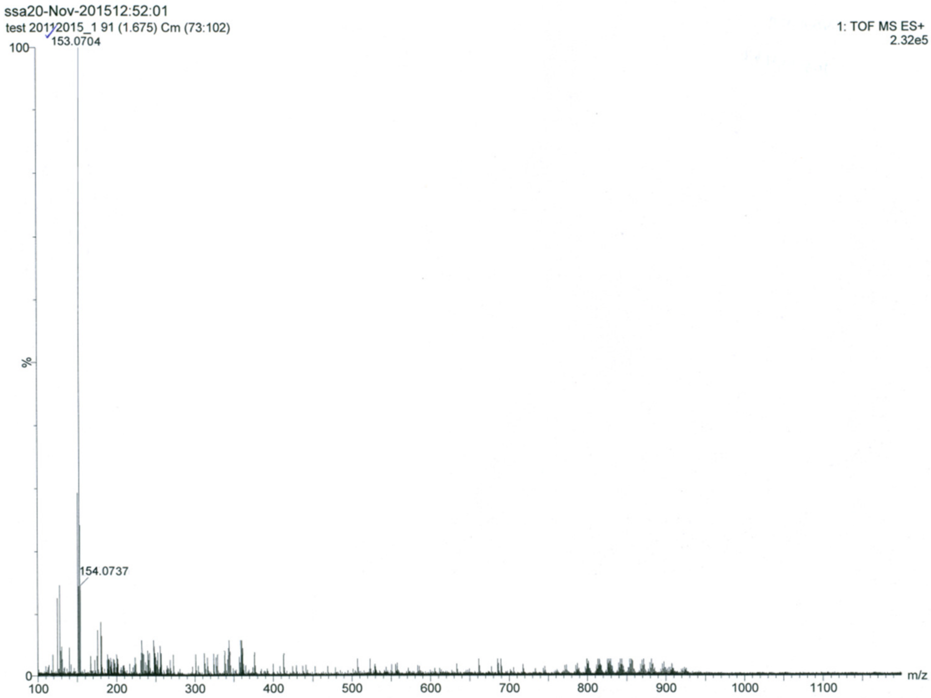


Fig. S-6 Q-tof MS spectra of compound **3**

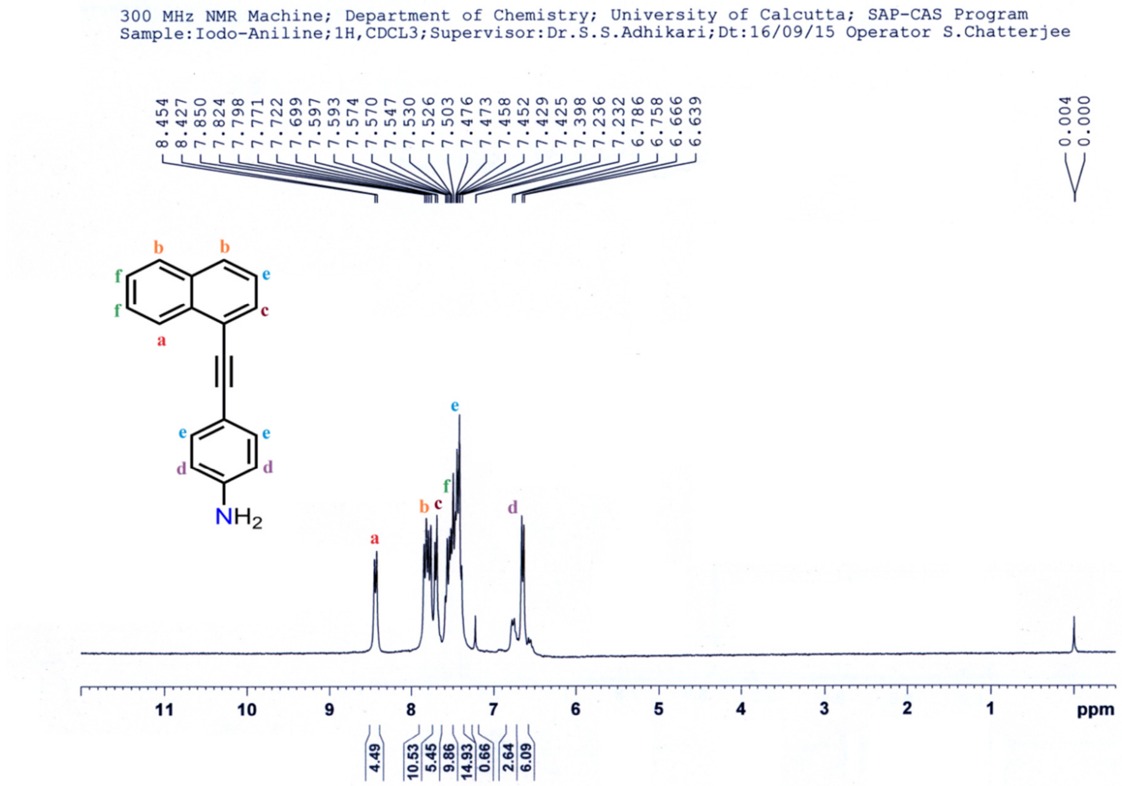


Fig. S-7 [1]H NMR spectra of compound **4**

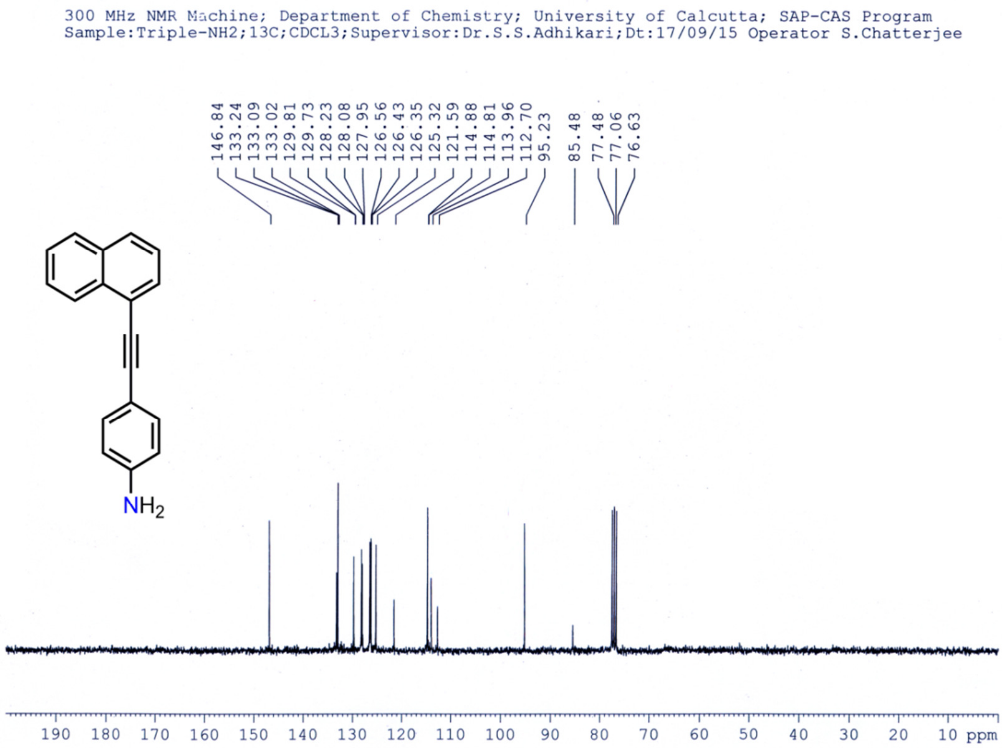


Fig. S-8 ^{13}C spectra of compound **4**

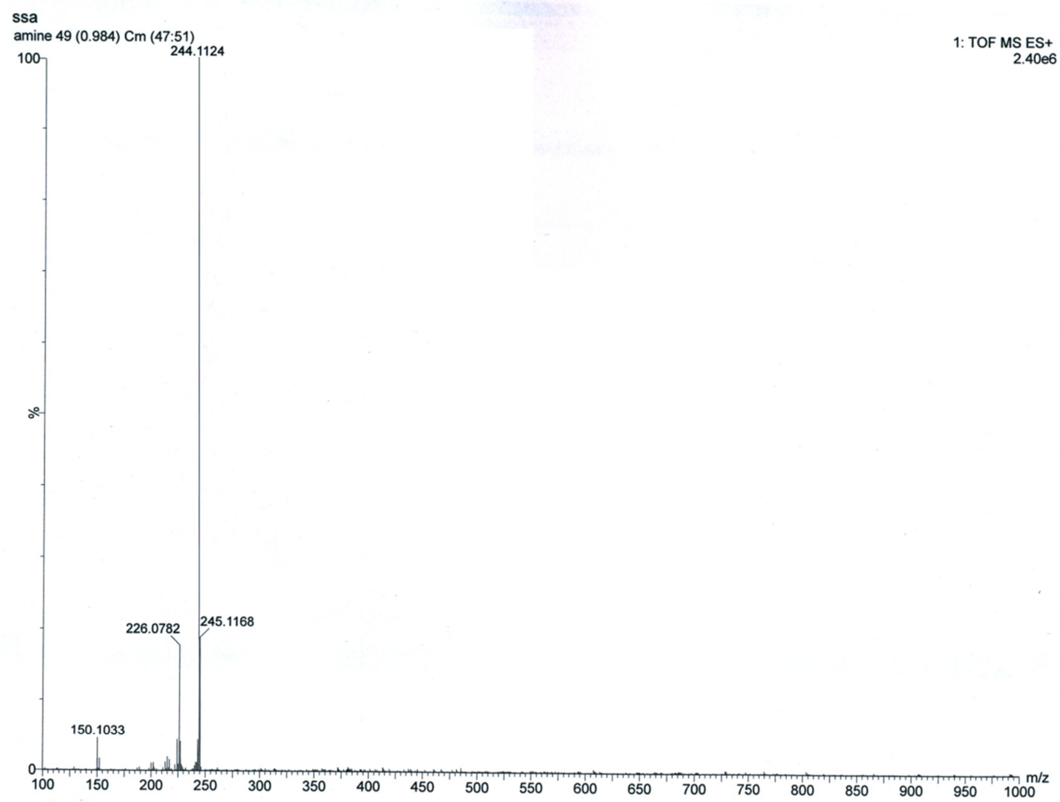


Fig. S-9 Q-tof MS spectra of compound **4**

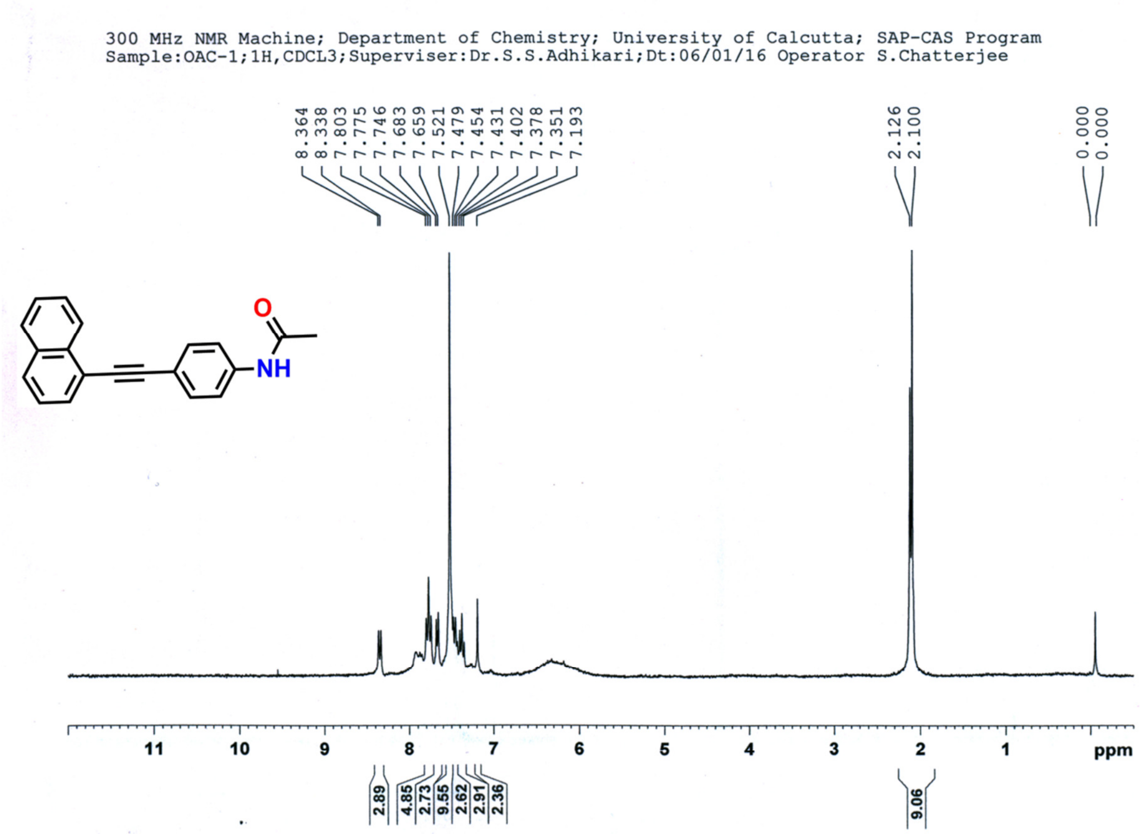


Fig. S-10 ^{1}H NMR spectra of compound **5**

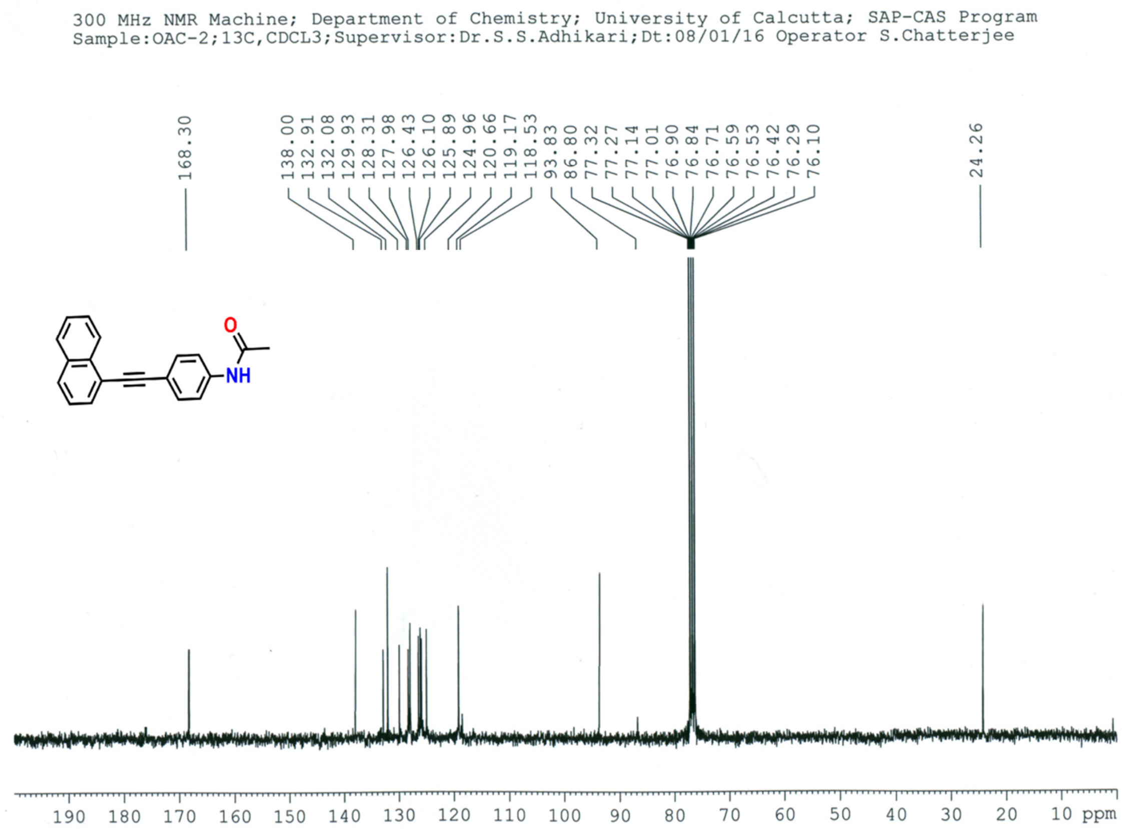


Fig. S-11 ^{13}C spectra of compound **5**

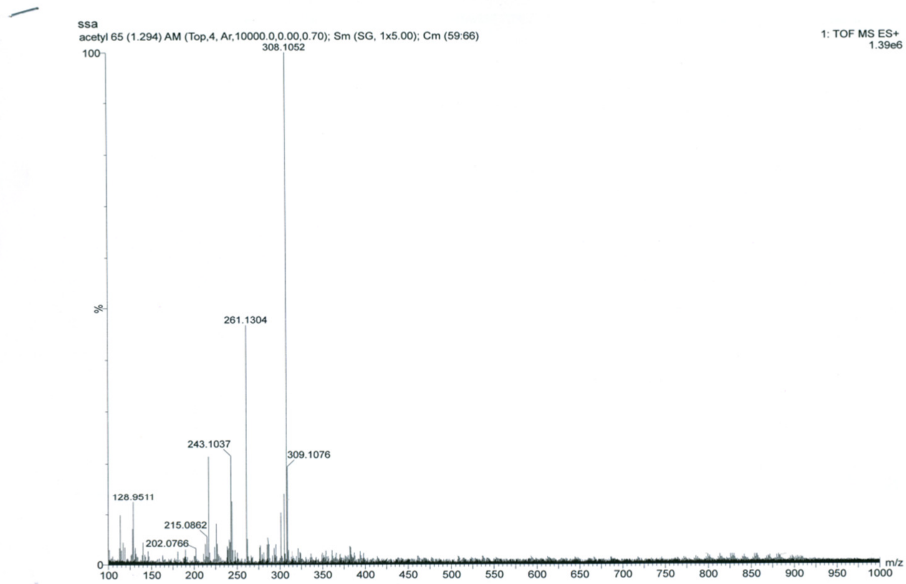


Fig. S-12 Q-tof MS spectra of compound **5**

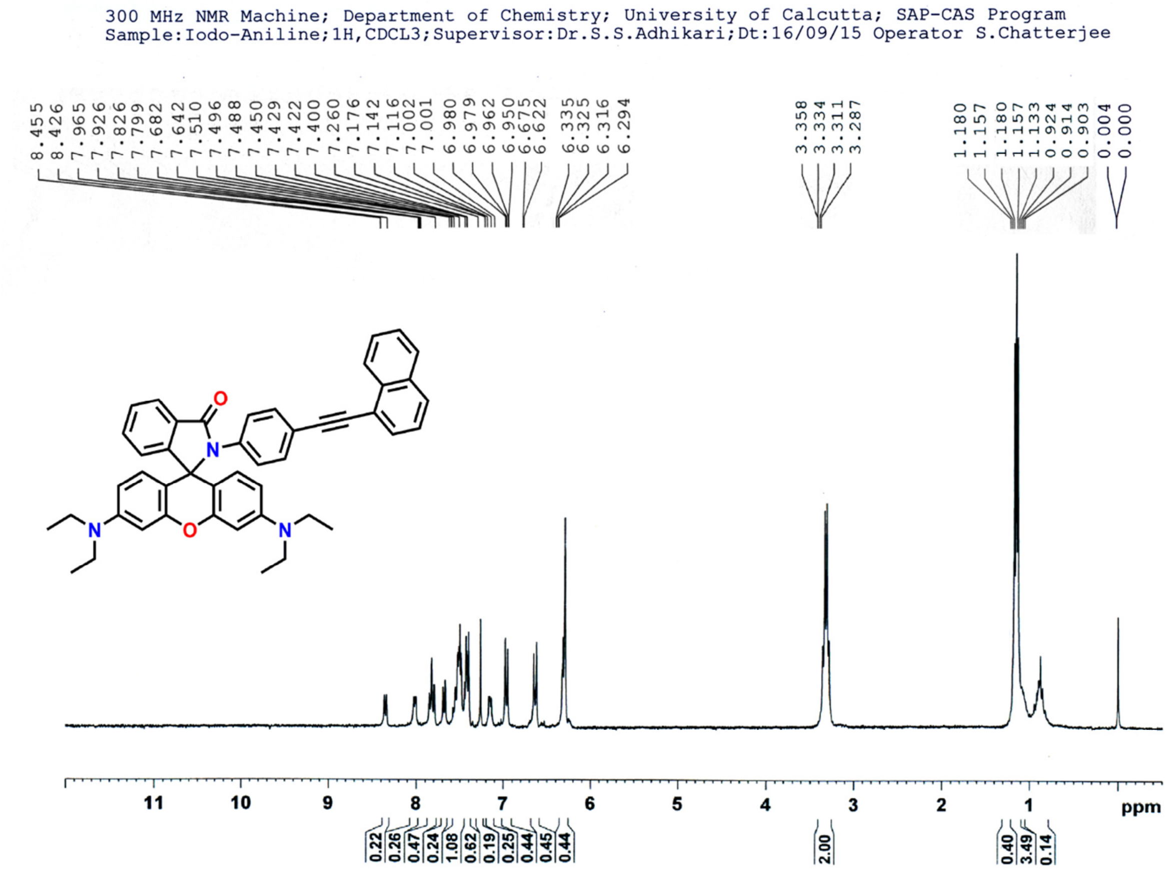


Fig. S-13 ^{1}H NMR spectra of compound **NAP-RD**

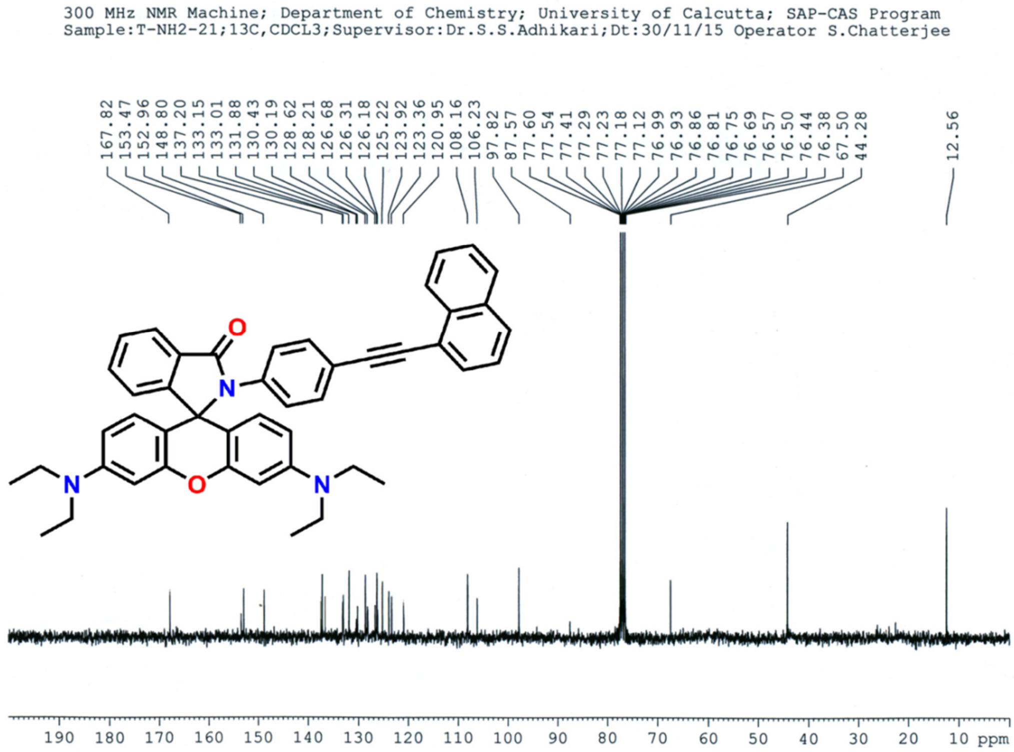


Fig. S-14 ^{13}C spectra of compound **NAP-RD**

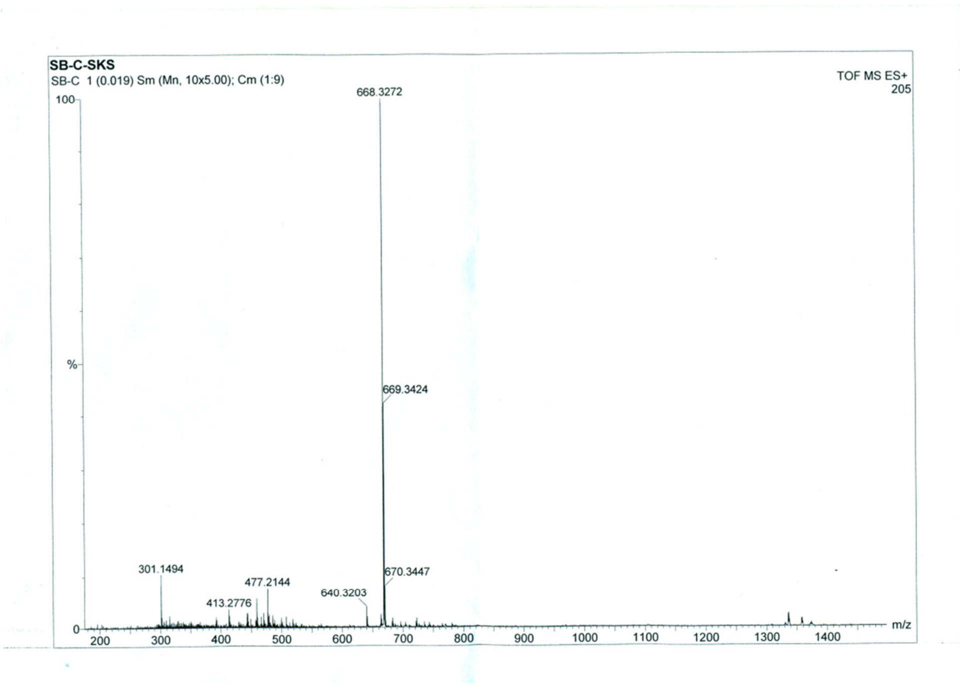


Fig. S-15 Q-tof MS spectra of compound **NAP-RD**

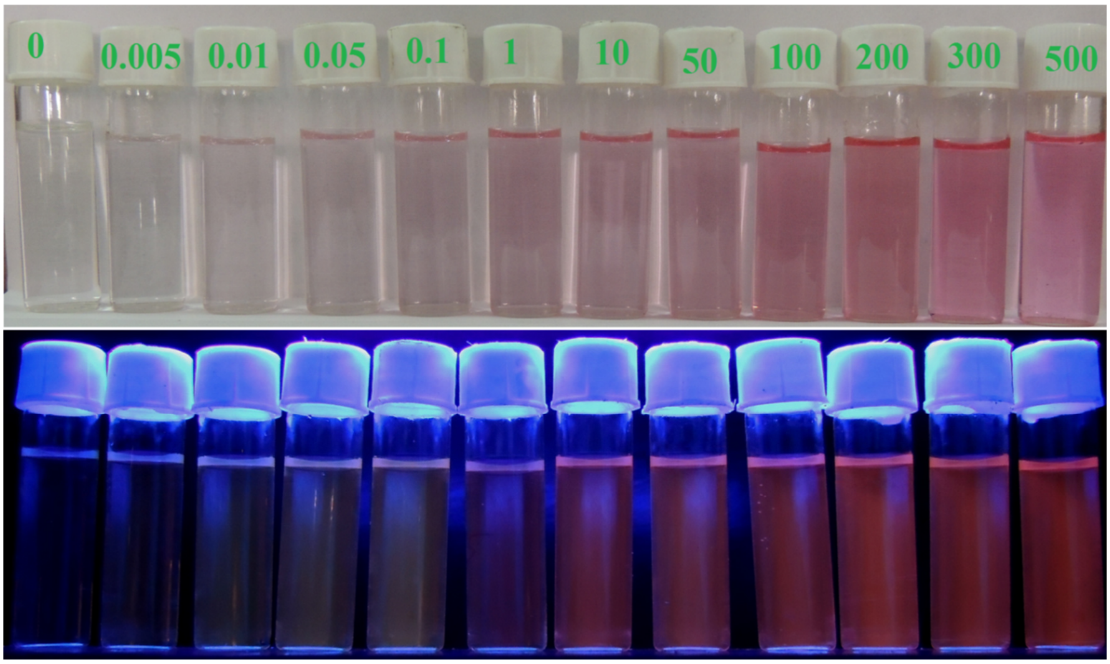


Fig. S-16 Colour changes of **NAP-RD** (10 µM) in 1:4 acetonitrile: HEPES buffer (10 mM, pH 7.4) media upon gradual addition of Sn^{2+} (0.0 to 500.0 µM) in visible light (top) and under a hand held UV lamp (bottom).

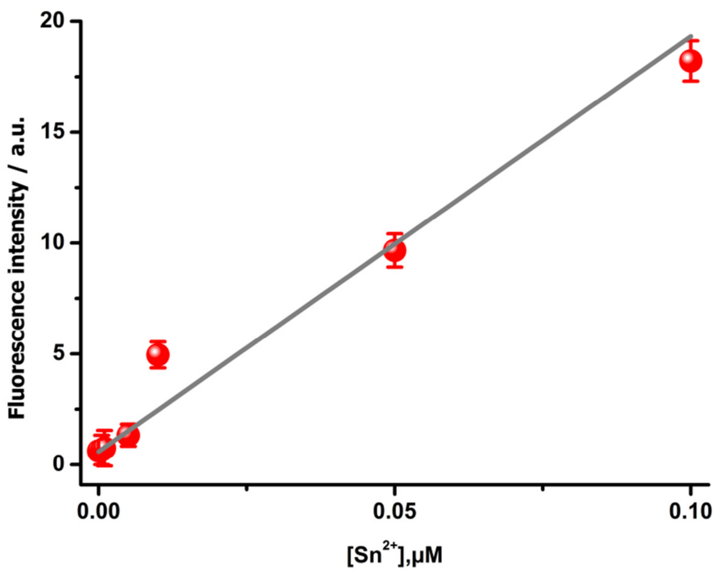


Fig. S-17 The expanded linear region of the plot (emission intensity *vs.* Sn^{2+} concentration) up to 0.10 µM of Sn^{2+}.

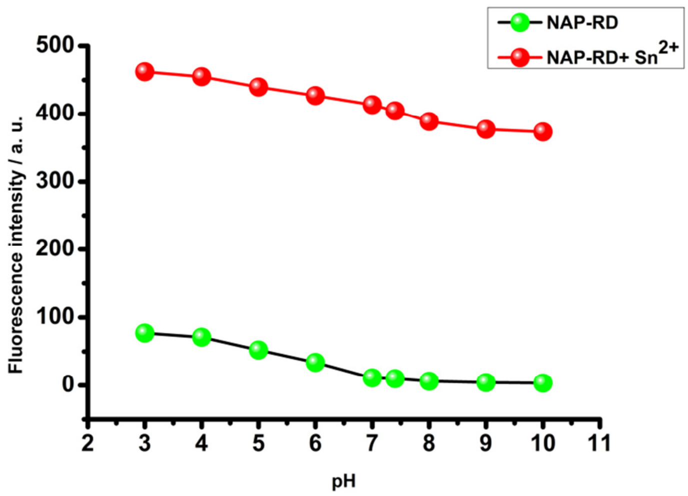


Fig. S-18 Effect of pH on the emission intensity of **NAP-RD** (10 µM) and **[NAP-RD –Sn^{2+}]** systems in 1:4 acetonitrile: HEPES buffer (10 mM, pH 7.4).

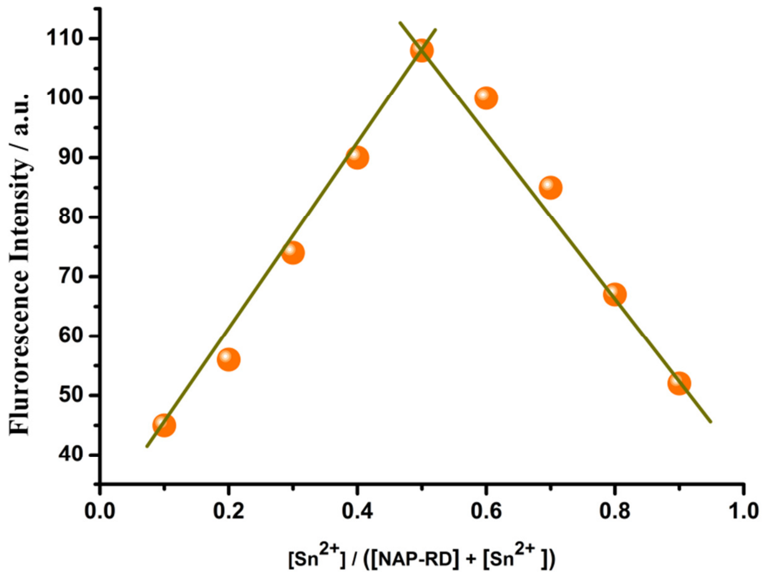


Fig. S-19 Job's plot (stoichiometry determination of the [**NAP-RD –Sn^{2+}**] adduct) in 1:4 acetonitrile: HEPES buffer (10 mM, pH 7.4).

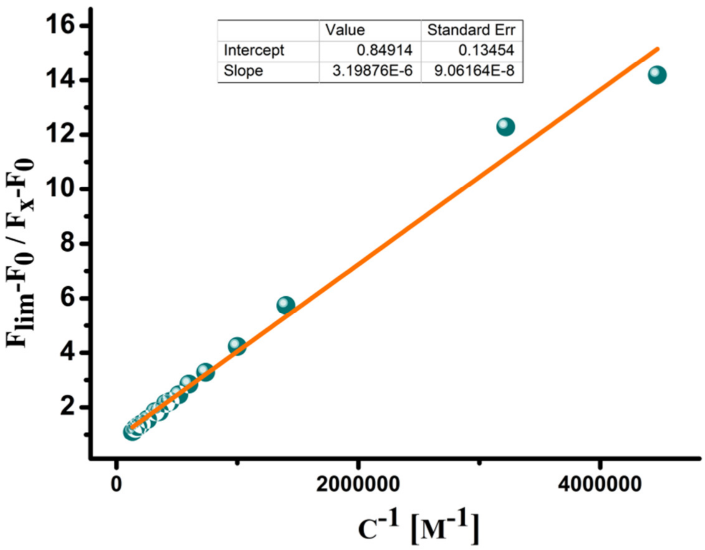


Fig. S-20 Determination of the binding constant of **NAP-RD** with Sn^{2+}using the fluorescence technique.

Table S-1 Comparison of the present probe with existing Sn^{2+} probes

Probe type	**Solvent System**	**LOD**	**Reference**
Turn on	acetonitrile	25.7 nM	*Dalton Trans*. **2015**, *44*, 14388–14393.
Turn on	MeOH/H_2O (2:3, v/v, pH 5.95)	0.044 µM	.*Molecules* **2014**, *19*, 7817-7831.
Turn on	ethanol–water (1 : 1, v/v), pH 7.04.	4.6×10^{-7} M	*Analyst*, **2014**, *139*, 5223–5229.
TBET, ratiometric turn on	1:4 acetonitrile: HEPES buffer (10 mM, pH 7.4)	5×10^{-9} M	***Present work***

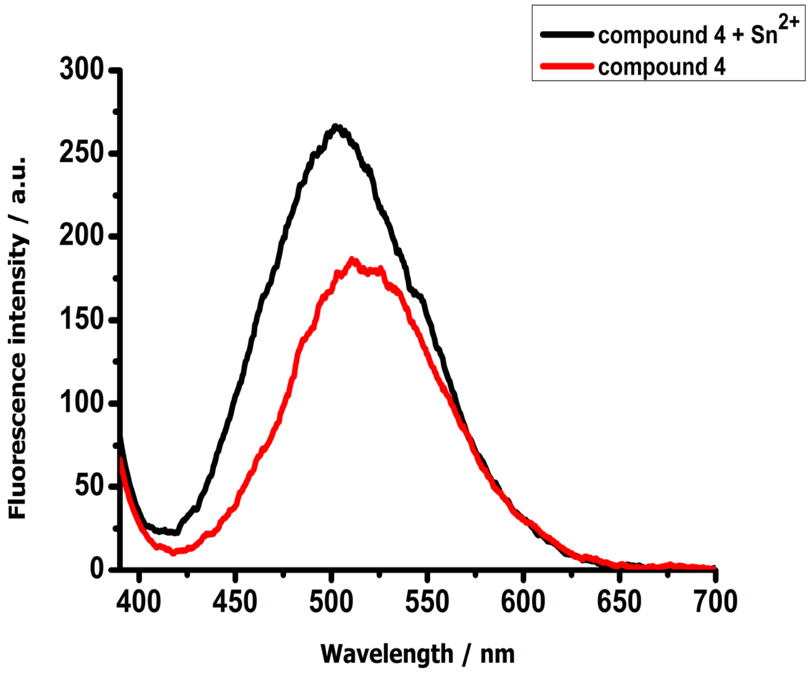


Fig. S-21 Changes in the emission spectra of compound **4** (10 µM), upon gradual addition of Sn^{2+} (10 µM).

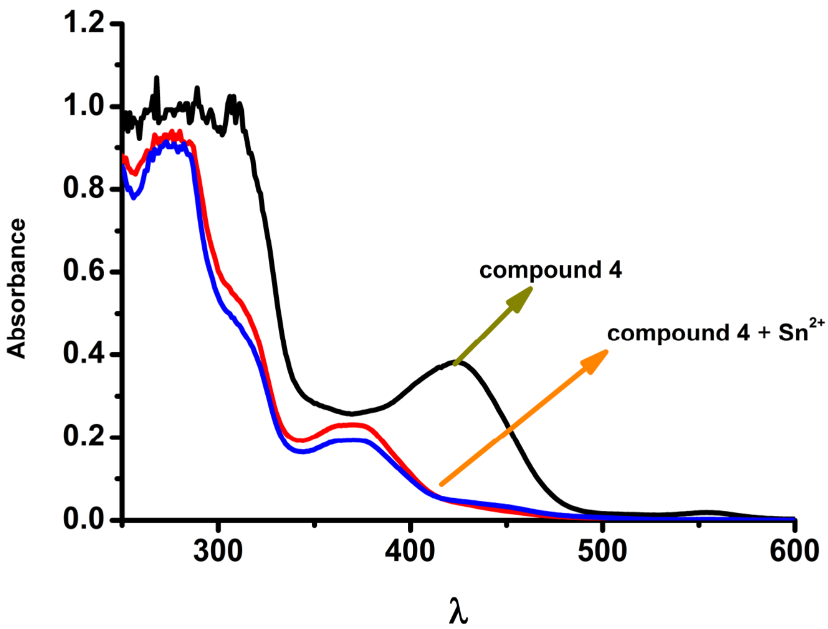


Fig. S-22 Changes in the absorbance spectra of compound **4** (10 μM), upon gradual addition of Sn^{2+}(10 μM).

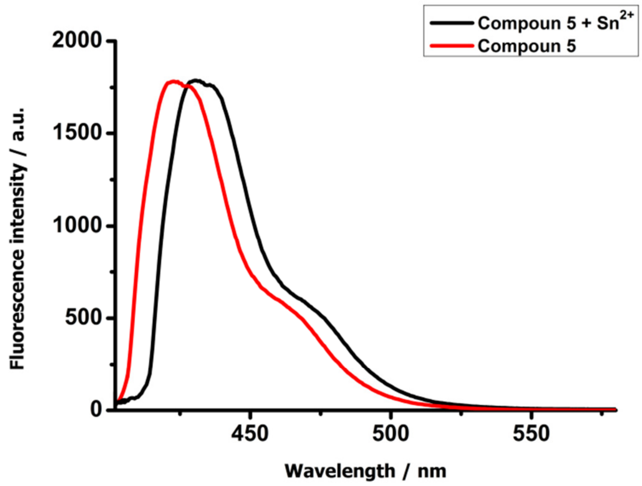


Fig. S-23 Changes in the emission spectra of compound **5** (10 μM), upon gradual addition of Sn^{2+} (10 μM).

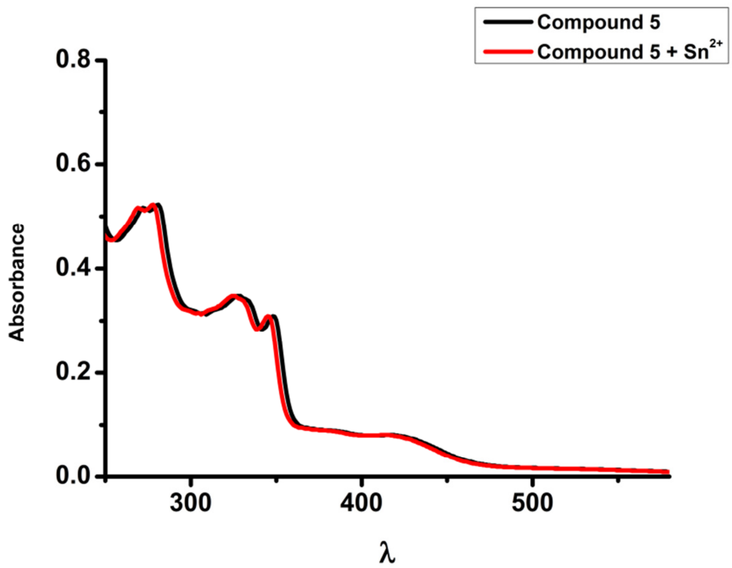


Fig. S-24 Changes in the absorbance spectra of compound **5** (10 μM), upon gradual addition of Sn^{2+}(10 μM).

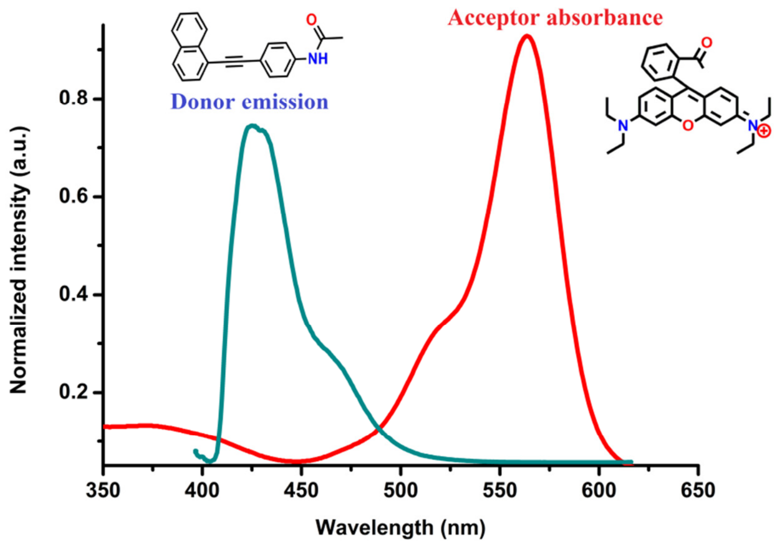


Fig. S-25 Normalized spectra of donor emission (blue) and acceptor absorbance (open ring rhodamine B) and the Spectral overlap between them.

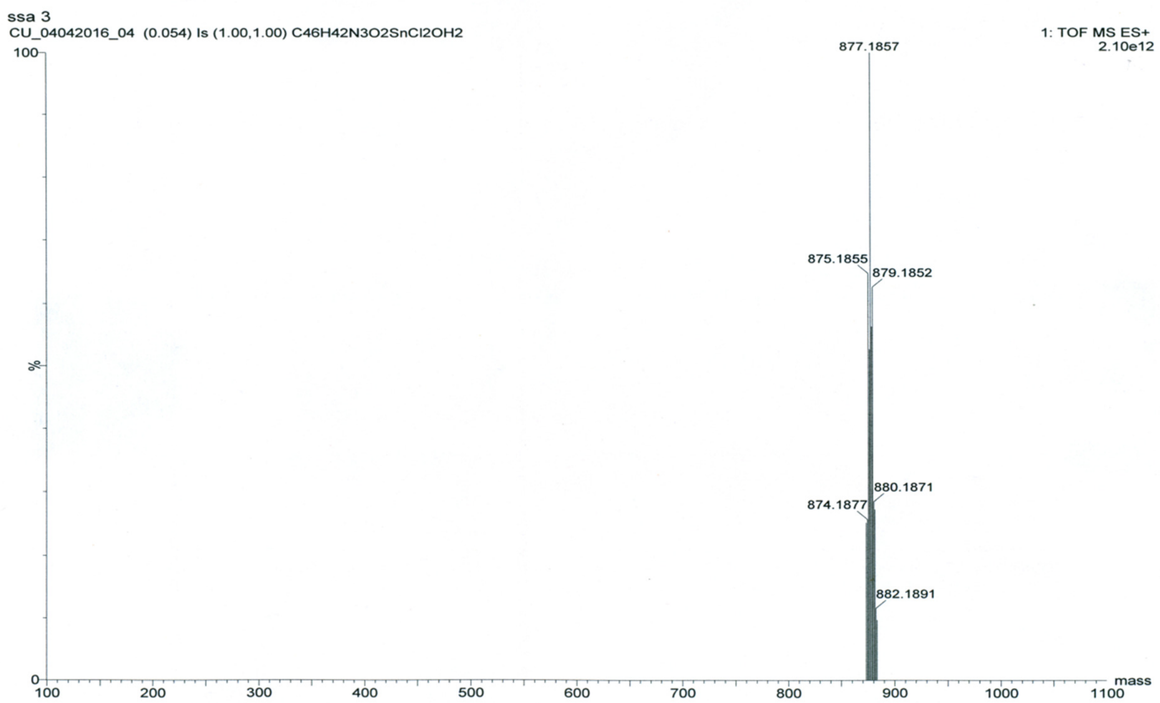


Fig. S-26 The QTOF-MS spectra of the resulting complex of [NAP-RD-Sn^{2+}]

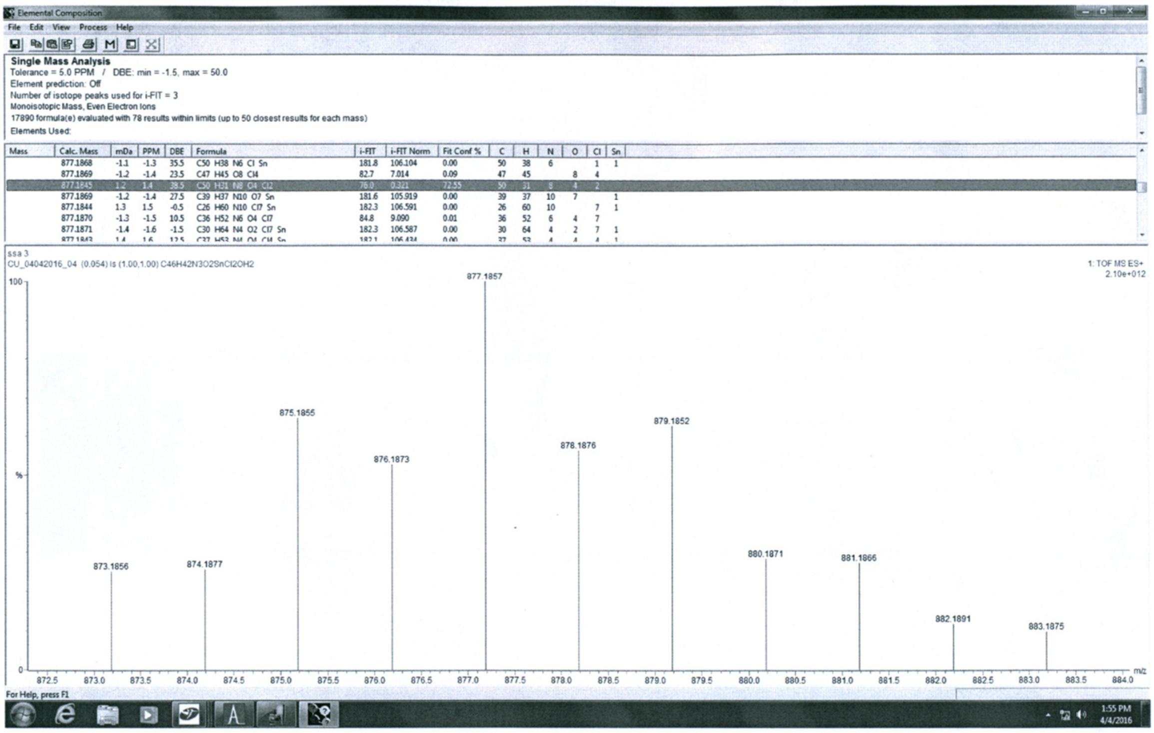


Fig. S-27 Expanded QTOF-MS spectra of the resulting complex of [NAP-RD-Sn^{2+}]

Energy transfer efficiency calculations: [2]

Energy transfer efficiency (ETE) = [(fluorescence of donor) - (fluorescence of donor in cassette)/ (fluorescence of donor)] ×100%.

For **NAP-RD**, ETE = [1239.402-71.638]/1239.638 ×100% = 94.22%.

Calculation for detection limit

To determine the detection limit, fluorescence titration of **NAP-RD** with Sn^{2+} was carried out by adding aliquots of micro molar concentration of Sn^{2+}. From the concentration at which there was a sharp change in the fluorescence intensity multiplied with the concentration of **NAP-RD** gave the detection limit.

Equation used for calculating detection limit (DL)

DL = CL × CT

CL = Conc. of ligand; CT = Conc. of Sn^{2+} at which fluorescence enhanced.

Thus;

$DL = 1 \times 10^{-6} \times 0.005 \times 10^{-6} = 0.005 \times 10^{-6}$

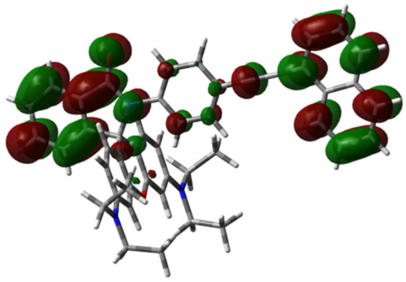
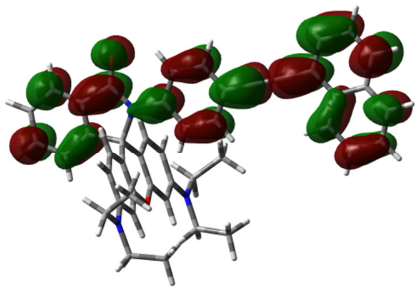
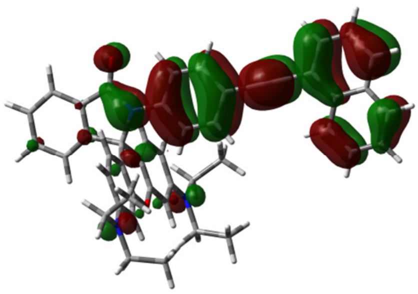
The fluorescence quantum yield

The fluorescence quantum yield was determined using optically matching solutions of rhodamine B (Φ_{fr} = 0.65 in ethanol) as standards at an excitation wavelength of 540 nm, and the quantum yield is calculated using the equation

$\Phi_{fs} = \Phi_{fr} \times 1 - 10^{-A_s L_s} / 1 - 10^{-A_r L_r} \times N_S^2 / N_r^2 \times D_s / D_r$

Φ_{fs} and Φ_{fr} are the radiative quantum yields of the sample and reference, respectively, A_s and A_r are the absorbances of the sample and reference, respectively, D_s and D_r are the respective areas of emission for the sample and reference, respectively, L_s and L_r are the lengths of the absorption cells of the sample and reference, respectively, and N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed), respectively.

Table S-2 Frontier molecular orbitals (MOs) of **NAP-RD** and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/6-31G/level using Gaussian 09.

Frontier orbital	Energy (a.u.)	Energy optimised geometry
LUMO+1	-0.03705	
LUMO	-0.05266	
HOMO	-0.18572	

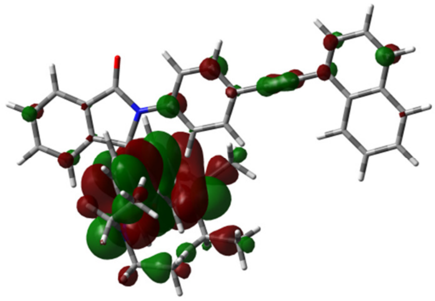
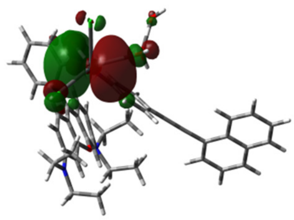
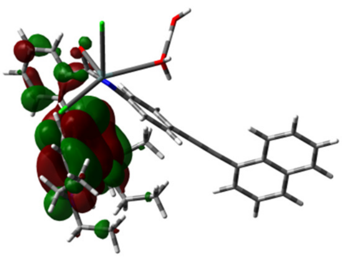
HOMO-1	-0.18968	

Table S-3 Frontier molecular orbitals (MOs) of [**NAP-RD-Sn^{2+}**] and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/3-21G/level using Gaussian 09.

Frontier orbital	**Energy (a.u.)**	**Energy optimised geometry**
LUMO+1	-0.09297	
LUMO	-0.12871	

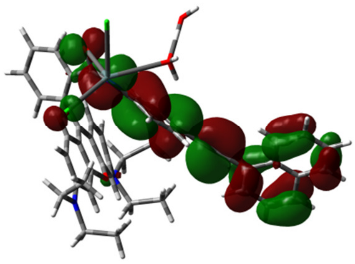
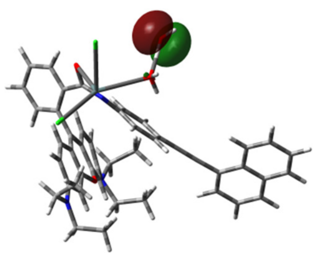
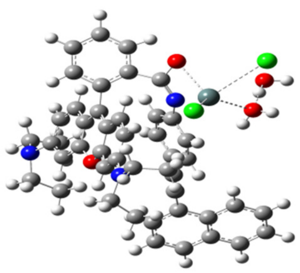
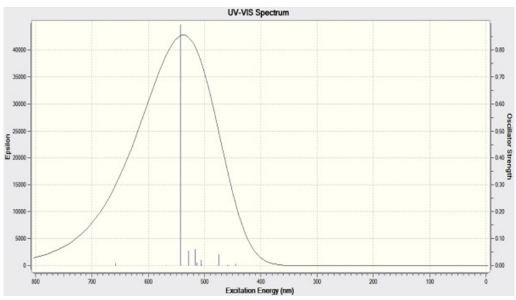
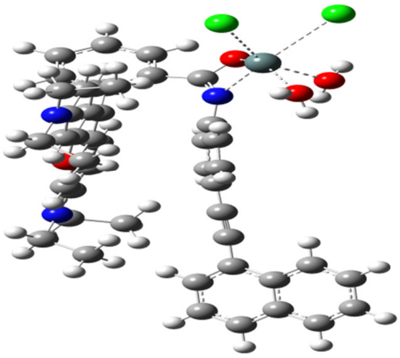
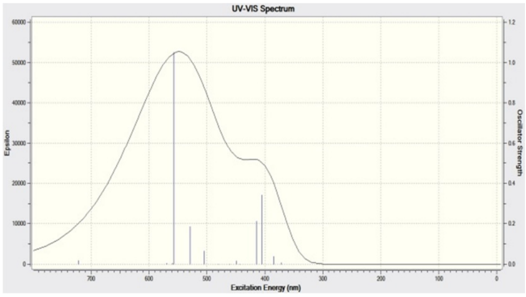
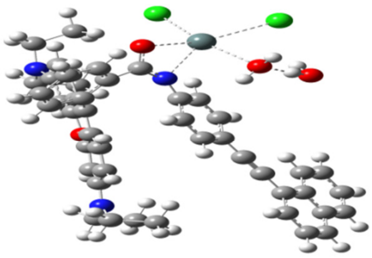
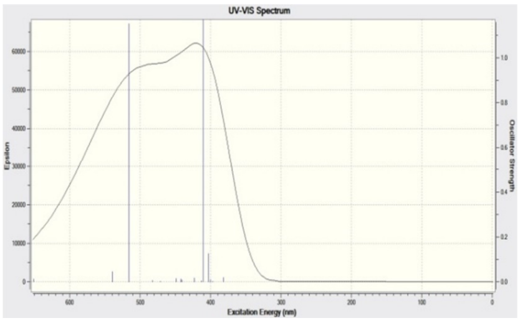
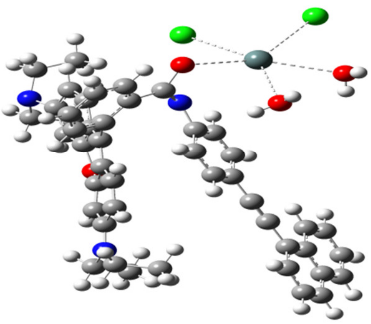
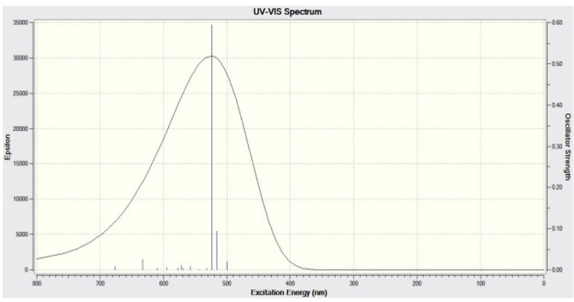
HOMO	-0.21177	
HOMO-1	-0.21360	

Table S-4 Mode of probable binding of NAP-RD with Sn^{2+}

Mode of probable binding of NAP-RD with Sn^{2+}	**Theoritical Uv calculated from TDDFT calculation in different modes**
	

	 UV-VIS Spectrum Epsilon Oscillator Strength Excitation Energy (nm)
	 UV-VIS Spectrum Epsilon Oscillator Strength Excitation Energy (nm)
	 UV-VIS Spectrum Epsilon Oscillator Strength Excitation Energy (nm)

In vitro cell imaging

RAW264.7 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C and 5% CO_2. For *in vitro* imaging studies, the cells are seeded in 6-well tissue culture plates with a seeding density of 10^5 cells per well. After reaching 60%–70% confluence, the previous DMEM medium was replaced with serum free DMEM medium, supplemented with 10 µM of **NAP-RD** and incubated for 2 h. Then cells were washed three times with PBS buffer to remove extracellular **NAP-RD**. Then Sn^{2+} (20 µM) was added into the medium and then further incubated for 3h to facilitate metal ion uptake by cells. After washing with PBS buffer, images of live cells were taken by Olympus IX81 microscope. Differential interference contrast (DIC) and fluorescence images of live cells were obtained by Olympus IX81 microscope using image-pro plus version 7.0 software.

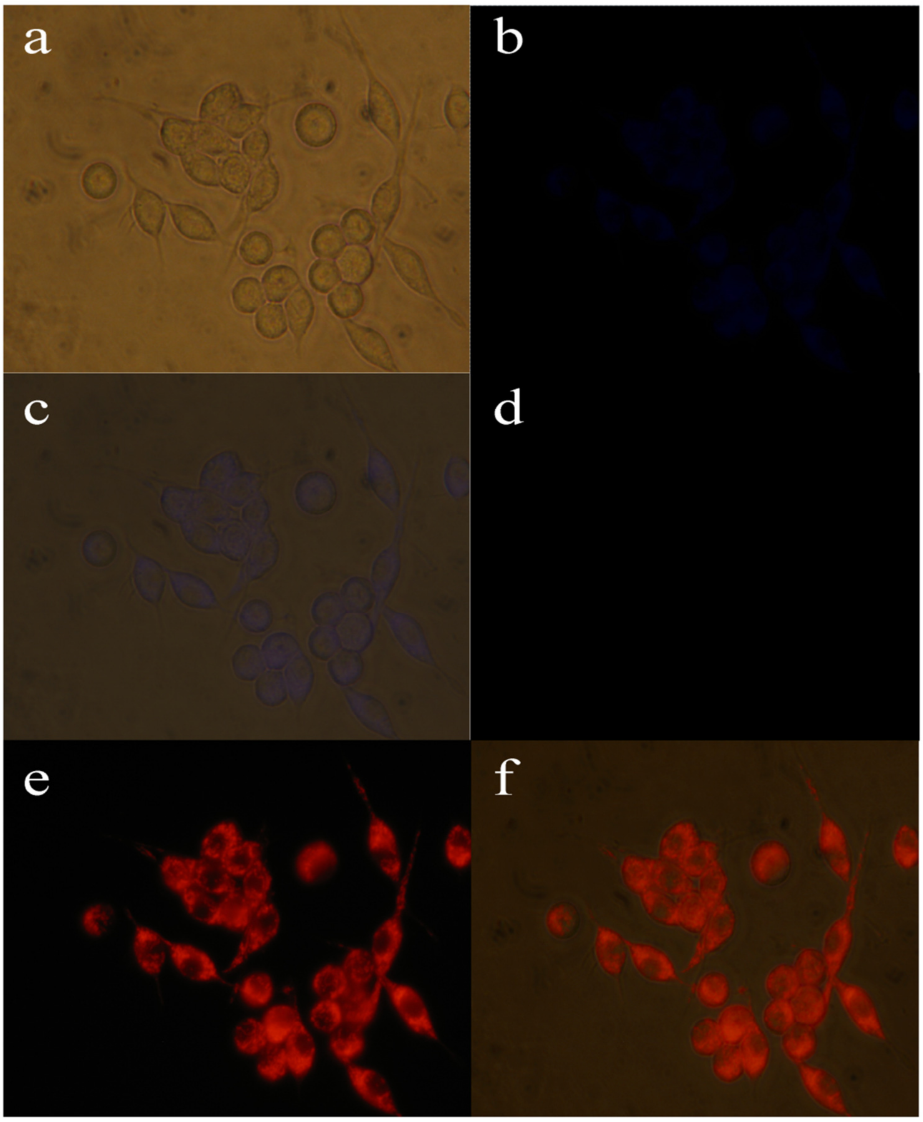


Fig. S-28 Fluorescence imaging of Sn^{2+} in RAW264.7 cells: (a) bright field images of cells after incubation with 10 μM NAP-RD; (b) fluorescence image of those cells in blue channel; (c) overlay image of a and b; (d) fluorescence image of those cells in b in red channel; (e) fluorescence image of those cells in b after incubation with 20 μM of Sn^{2+}; (f) overlay image of a and e; **NAP-RD** was prepared in ~ 0.3 % DMSO in water.

Cytotoxicity assay

In vitro cytotoxicity was measured by using the colorimetric methyl thiazolyl tetrazolium (MTT) assay against RAW264.7cells. Cells were seeded into 24-well tissue culture plate in presence of 500 µL Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ºC temperature and 5 % CO_2 atmosphere for overnight and then incubated for 12 hours in presence of **NAP-RD** at different concentrations (10-100 µM). Then cells were washed with PBS buffer and 500 µL supplemented DMEM medium was added. Subsequently, 50 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT (5 mg/mL) was added to each well and incubated for 4 hours. Next, violet formazan was dissolved in 500 µL of sodium dodecyl sulfate solution in water/DMF mixture. The absorbance of solution was measured at 570 nm using microplate reader. The cell viability was determined by assuming 100 % cell viability for cells without **NAP-RD**.

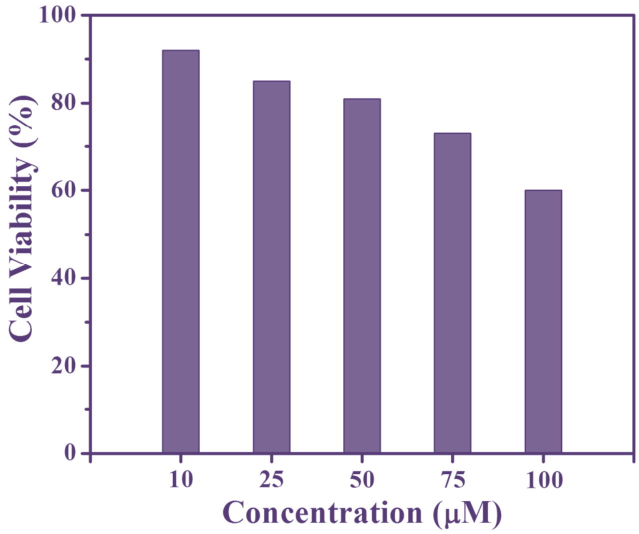


Fig. S-29 Cell viability of **NAP-RD** at different concentration against RAW264.7 cells after 12 hours incubation.

(1) V. Dujols, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.*, 1997, **119**, 7386.

(2) V. Bhalla, Roopa, M. Kumar, P. R. Sharma and T. Kaur, *Inorg. Chem.*, 2012, **51**, 2150.