Supporting Information

Photoswitchable Azobenzene-Rhodamine Tweezers for Biosensing of Al^{3+} Ions

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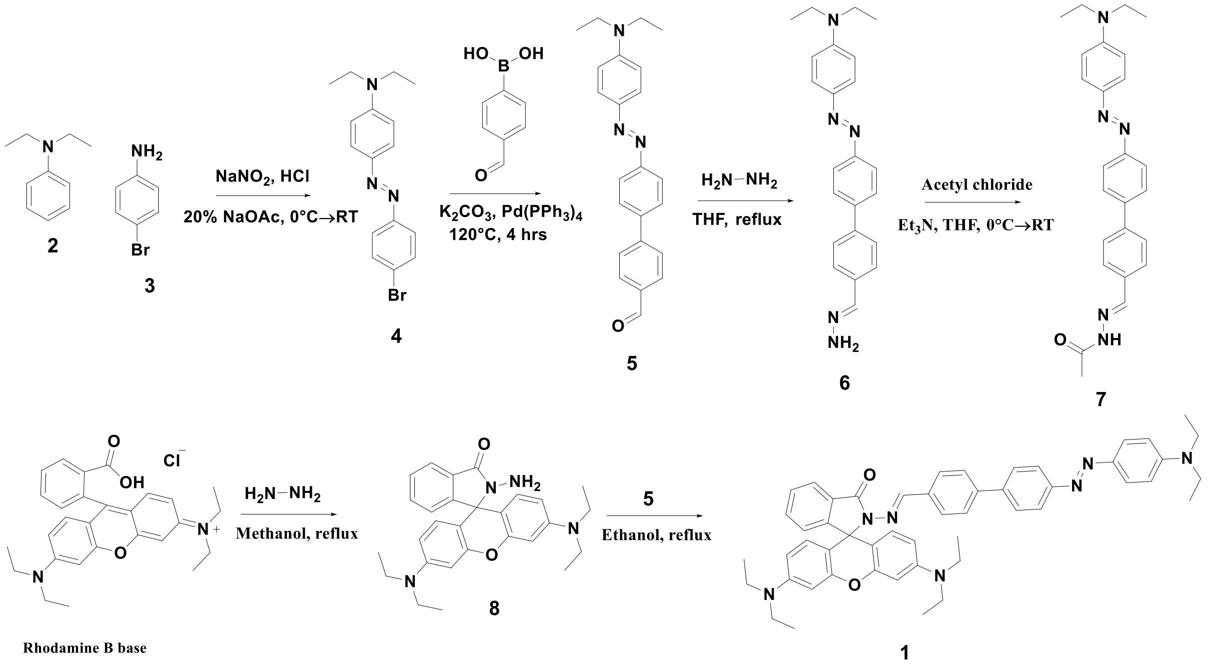
Table of contents **Page No**

Materials and analytical methods:

Solvents and starting materials were purchased from Sigma Aldrich. Other chemicals were of analytical reagent grade and were used without further purification except when specified. Reactions were monitored by thin layer chromatography (TLC) carried out on silica gel plates (Merck 60F-254) using UV light for visualization. Silica gel (Merck 60, particle size 60-120 mesh) was used for column chromatography. Absorption spectra was measured in Shimadzu UV-1800 UV-visible spectrophotometer and fluorescence spectra was recorded on Cary eclipse fluorescence spectrophotometer using quartz cuvettes at room temperature. Photoisomerization studies were performed using UVGL-25 compact 4 watt 365 nm UV lamp. Time-resolved fluorescence life time measurements were performed using a time-correlated single photon counting (TCSPC) spectrometer, Edinburgh Instruments FLS 980 model (UK). The fluorescence decay curves are measured by exciting the molecules at 360 nm, 75 ps pulsed laser. Cell imaging using Fluorescent microscope Leica DMi8, Germany. NMR spectra were recorded on a Bruker 400 MHz (101 MHz for ^{13}C) spectrometer using residual protonated solvent signals as the internal standard. ESI-MS was taken in Finnigan Lcq Advantage Max, Thermofisher. All the metal salts were used as their perchlorates.



Scheme S1. Synthetic route to target **1** and other intermediates **5**, **7** and **8.**

1. Synthesis of (E)-4-((4-bromophenyl)diazenyl)-N,N-diethylaniline (4)

3, 4-Bromoaniline (1 g, 5.81 mmol) was dissolved in an ice water solution of sodium nitrite (0.4 g, 5.81 mmol). After cooling to 0˚C, the solution was added to con.hydrochloric acid (2 mL) and stirred for 30 min. Then the mixture was added dropwise to solution containing **2** i.e, 0.86 g N,N-diethylaniline (5.81 mmol) with NaOAc (0.14 g, 0.3 eq) in MeOH and stirred for 2 h at 0-5˚C. The resulting precipitate was filtered and rinsed with water twice. The crude product was recrystallized from ethanol petroleum ether twice to give yellow crystals with 85% yield (1.6 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.79 (m, 2H), 7.74 – 7.66 (m, 2H), 7.63 – 7.55 (m, 2H), 6.71 (d, J = 9.2 Hz, 2H), 3.45 (q, J = 7.1 Hz, 4H), 1.23 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.13, 150.41, 143.05, 132.05, 125.49, 123.66, 123.00, 111.04, 44.74, 12.66.

2. **Synthesis of (E)-4'-((4-(diethylamino) phenyl)diazenyl)-[1,1'-biphenyl]-4-carbaldehyde (5)**

To a solution of **4** (0.6 g, 1.8 mmol) in toluene is added the 4-formylphenyl boronic acid (0.32 g, 2.16 mmol), tetrakis (triphenylphosphine) palladium (0.104 g, 0.09 mmol) and a 2M aqueous solution of potassium carbonate (1.65 g in 6 ml). The mixture is purged with nitrogen for 10 min. After heating under reflux for 6 h, water is added to the cooled reaction mixture and the product is extracted with ethyl acetate. The organic phase is washed with brine and dried over sodium sulphate. The solvent is evaporated. The crude aldehyde, which is purified by flash chromatography. Yield 75 % (0.48 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.98 (s, 1H), 7.97 – 7.93 (m, 2H), 7.93 – 7.86 (m, 3H), 7.82 (d, J = 8.2 Hz, 2H), 7.78 – 7.70 (m, 2H), 6.74 (d, J = 9.2 Hz, 2H), 3.47 (q, J = 7.1 Hz, 4H), 1.24 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.91, 153.28, 150.39, 146.58, 143.29, 140.03, 135.29, 130.32, 127.96, 127.60, 125.55, 122.80, 111.05, 44.76, 12.68.

3. Synthesis of N,N-diethyl-4-((E)-(4'-((E)-hydrazonomethyl)-[1,1'-biphenyl]-4-yl)diazenyl)aniline (6)

To a 0.2 g of **5** dissolved in 15ml of THF, an excessive hydrazine hydrate (0.15 ml) was added and then the reaction solution was refluxed for 3 hrs. After that, the cooled reaction solution was dried and the solid was washed with hexane and the evaporated. The product yielded 0.16 g (80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (s, 4H), 7.78 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.2 Hz, 3H), 7.57 (d, J = 8.1 Hz, 2H), 6.88 (s, 2H), 6.80 (d, J = 9.0 Hz, 2H), 3.46 (dd, J = 14.0, 6.9 Hz, 4H), 1.15 (t, J = 6.9 Hz, 6H).

4. Synthesis of N'-((E)-(4'-((E)-(4-(diethylamino) phenyl) diazenyl)-[1,1'-biphenyl]-4-yl)methylene)acetohydrazide (7)

To a stirred solution of the **6** (0.26 mmol) in dry THF (5 mL) was added dry triethylamine (0.2 mmol) under N_2. The mixture was cooled to 0°C and the acetyl chloride (0.26 mmol) was added dropwise to the solution. The resulting mixture was stirred for 12 h at room temperature. The crude solution was quenched by a saturated solution of $NaHCO_3$ and extracted with dichloromethane. The combined organic extracts were washed with water, brine, dried over anhydrous sodium sulphate and concentrated in vacuum. The crude material was purified by flash chromatography to give the desired product yielded 0.06 g (55%). ^{1}H NMR (400 MHz, THF-d_8) δ 10.14 (s, 1H), 7.82 – 7.75 (m, 3H), 7.75 – 7.68 (m, 4H), 7.67 (s, 1H), 7.65 (s, 3H), 6.68 (d, J = 9.2 Hz, 2H), 3.39 (q, J = 7.1 Hz, 4H), 2.14 (s, 3H), 1.11 (t, J = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, THF) δ 171.18, 152.64, 150.28, 143.29, 141.22, 141.09, 140.77, 140.66, 134.15, 127.08, 126.86, 125.13, 122.50, 110.79, 44.31, 19.40, 11.98.

5. Synthesis of Rhodamine B hydrazide (8)

To a 1 g of rhodamine B (I) base dissolved in 15ml of methanol, an excessive hydrazine hydrate (1.25 ml) was added and then the reaction solution was refluxed till the pink color disappeared. After that, the cooled reaction solution was poured into distilled water and extracted with ethyl acetate (6×25 ml). The combined extracts were dried with sodium sulfate anhydrous, filtered and then evaporated. The crude product yielded of 0.64 g (68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, J = 5.9, 2.7 Hz, 1H), 7.58 – 7.35 (m, 2H), 7.19 – 6.97 (m, 1H), 6.44 (dd, J = 15.0, 5.6 Hz, 4H), 6.29 (dd, J = 8.8, 2.5 Hz, 2H), 3.61 (s, 2H), 3.34 (q, J = 7.0 Hz, 8H), 1.17 (t, J = 7.0 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.15, 153.88, 151.58, 148.92, 132.50, 130.06, 128.11, 128.09, 123.84, 122.99, 108.07, 104.64, 98.03, 77.36, 77.04, 76.72, 65.92, 44.38, 12.62.

6.**Synthesis of 3',6'-bis(diethylamino)-2-(((E)-(4'-((E)-(4-(diethylamino)phenyl)diazenyl)-[1,1'-biphenyl]-4-yl)methylene)amino)spiro[isoindoline-1,9'-xanthen]-3-one (1)**

A portion of **5** (0.15 g, 0.438 mmol) and **8** (0.2 g, 0.438 mmol) were dissolved in freshly distilled EtOH (10 mL). The reaction solution was refluxed for 24 h and stirred for another 3 h at room temperature to form red solid. Yield 0.27 g (80 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.00 (d, J = 6.5 Hz, 1H), 7.86 (dd, J = 8.8, 3.0 Hz, 4H), 7.64 (dd, J = 8.1, 6.1 Hz, 4H), 7.57 (d, J = 8.3 Hz, 2H), 7.54 – 7.44 (m, 2H), 7.13 (d, J = 6.7 Hz, 1H), 6.72 (d, J = 9.1 Hz, 2H), 6.54 (d, J = 8.8 Hz, 2H), 6.46 (d, J = 2.4 Hz, 2H), 6.25 (dd, J = 8.9, 2.4 Hz, 2H), 3.46 (q, J = 7.0 Hz, 4H), 3.33 (q, J = 7.0 Hz, 8H), 1.23 (t, J = 7.1 Hz, 6H), 1.16 (t, J = 7.0 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.94, 153.23, 152.67, 151.76, 150.19, 149.00, 146.93, 143.42, 141.64, 141.12, 134.71, 133.28, 129.51, 128.27, 128.03, 127.98, 127.47, 126.89, 125.36, 123.89, 123.36, 122.63, 111.05, 108.08, 106.23, 97.98, 66.14, 44.72, 44.33, 12.68, 12.63.

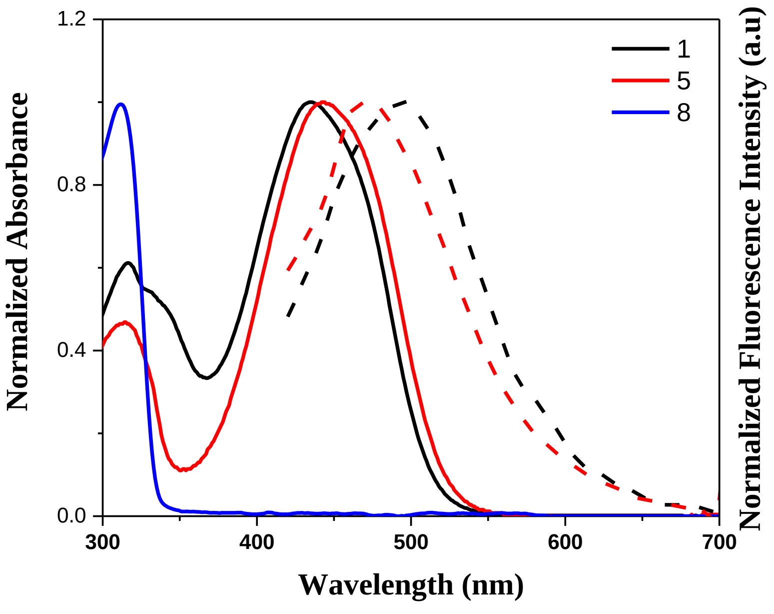


Figure S1 UV-visible absorption (solid line) and emission (dotted line) spectra of the compounds **1, 5,** and **8** in THF

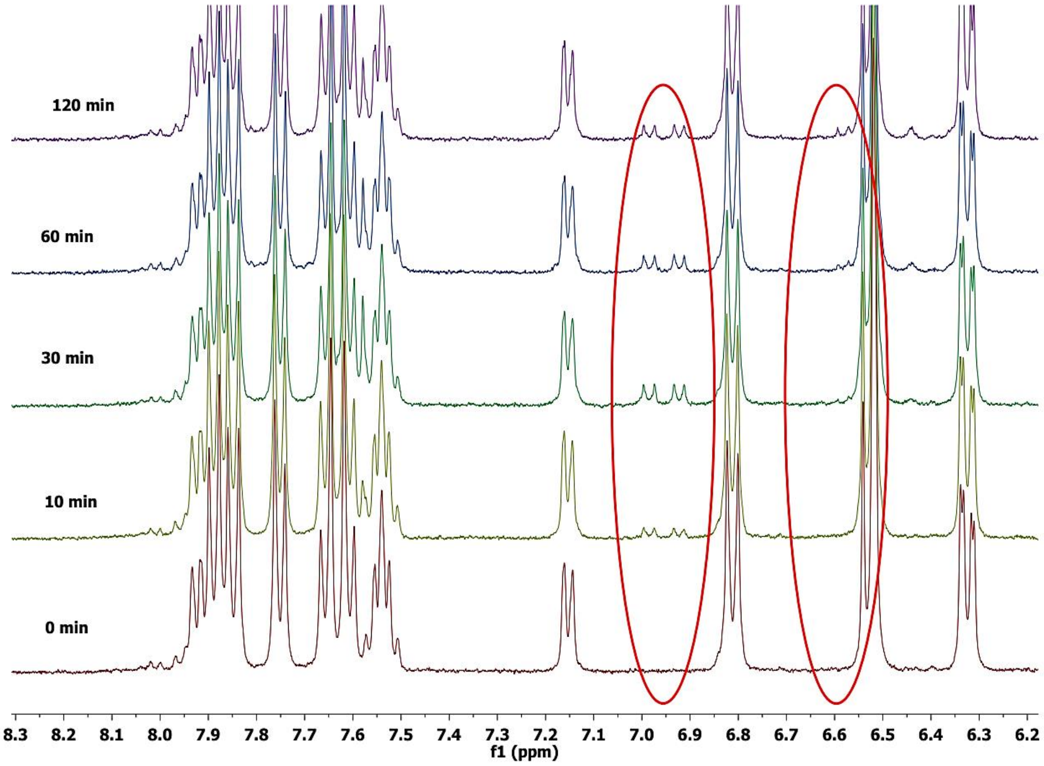


Figure S2 ^{1}H NMR spectra of **1** upon photoirradiation with 365 nm light at different time intervals measured in THF-d_8.

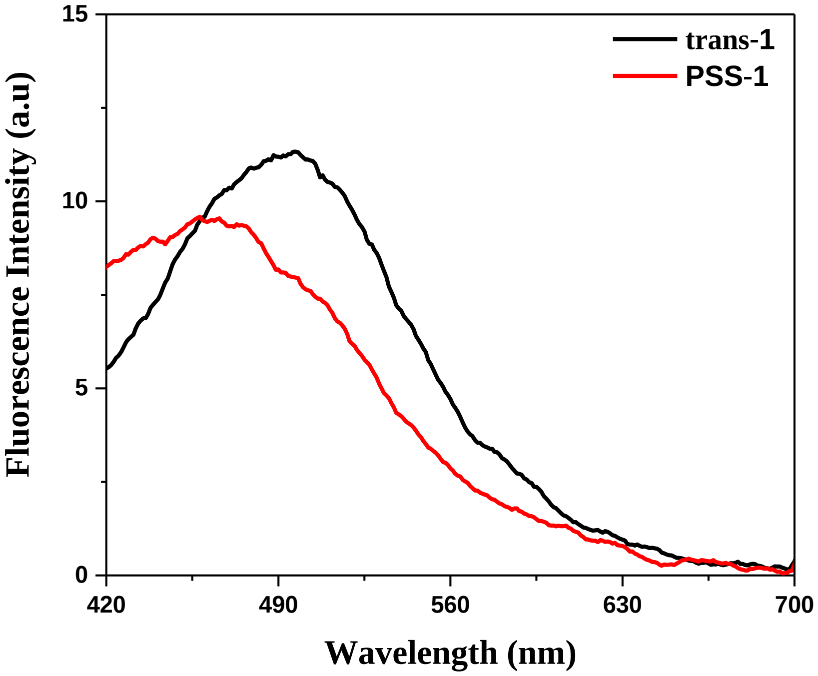


Figure S3 Fluorescence spectra of **1** in trans and PSS state in THF solvent (λ_{ex} = 360 nm).

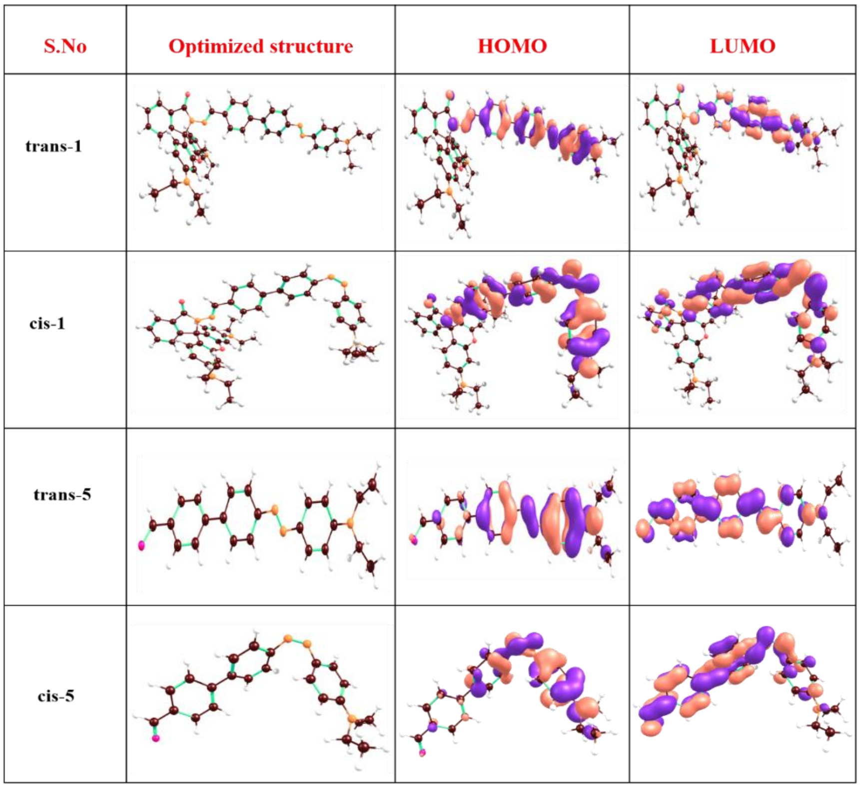


Figure S4 The optimized geometry, Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) of **5** and **1** in trans and cis configuration calculated using Gaussian 03 software at B3LYP/6-31g level of theory.

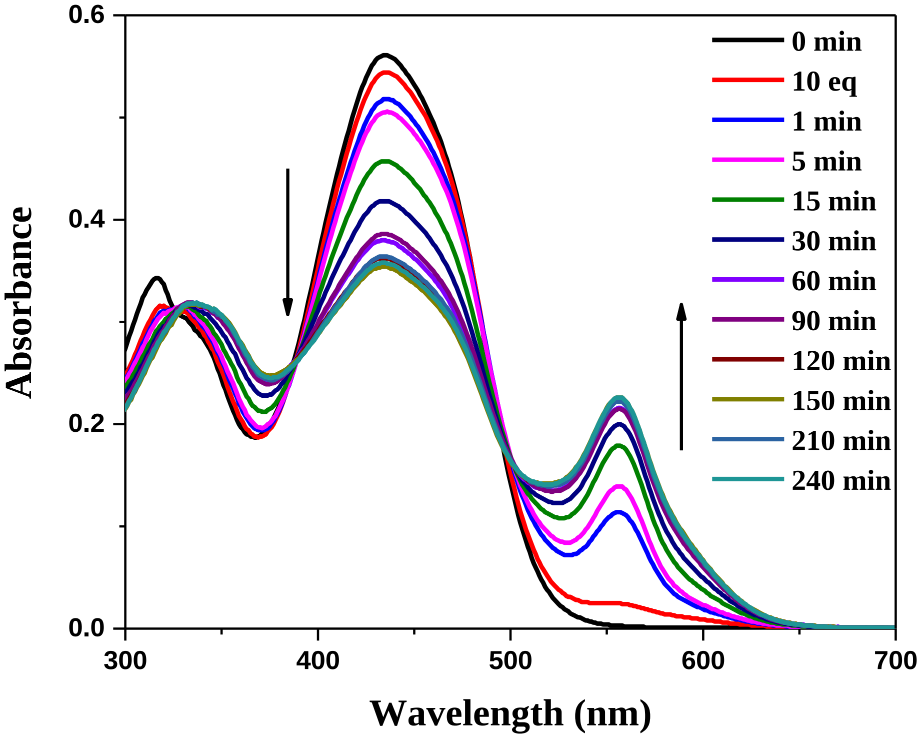


Figure S5 UV-visible absorption spectra of **1** (10 µM) upon photoirradiation with 365 nm light at different time intervals in presence of 10 equivalents of Al^{3+} ions.

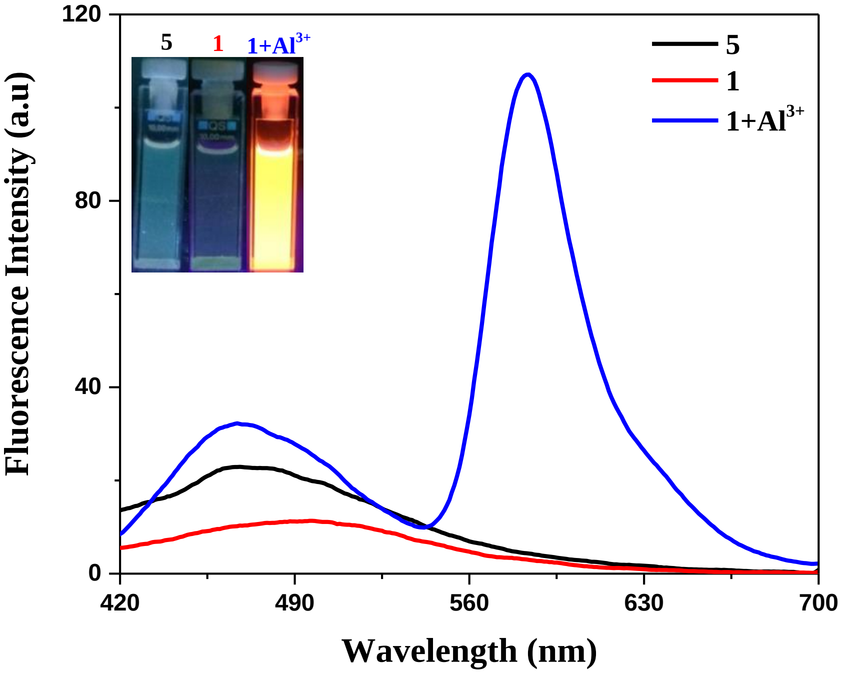


Figure S6 Fluorescence spectra of **5**, **1** and **1**+Al^{3+} complex in 10 μM in THF.

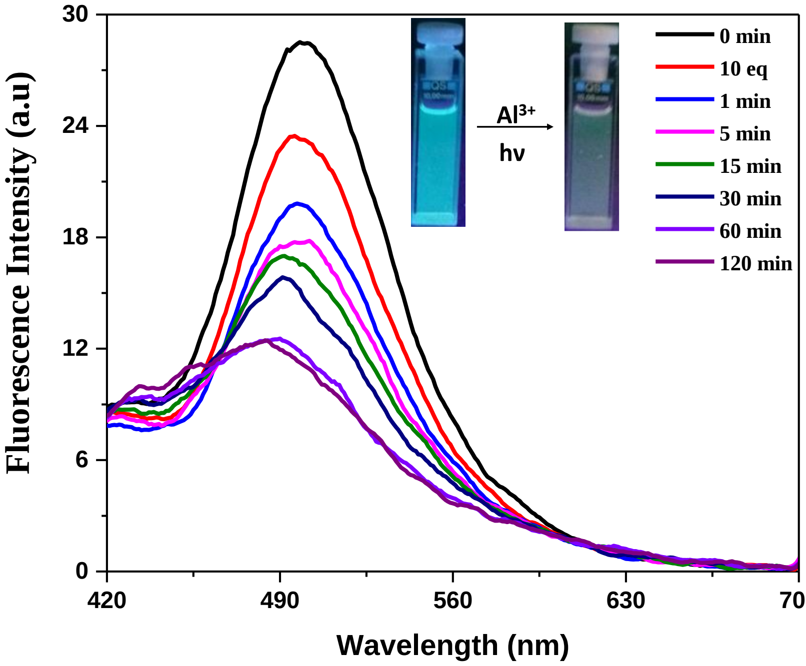


Figure S7 Fluorescence spectra of **7** upon photoirradiation with 365 nm light at different time intervals in presence of 10 equivalents of Al^{3+} in THF.

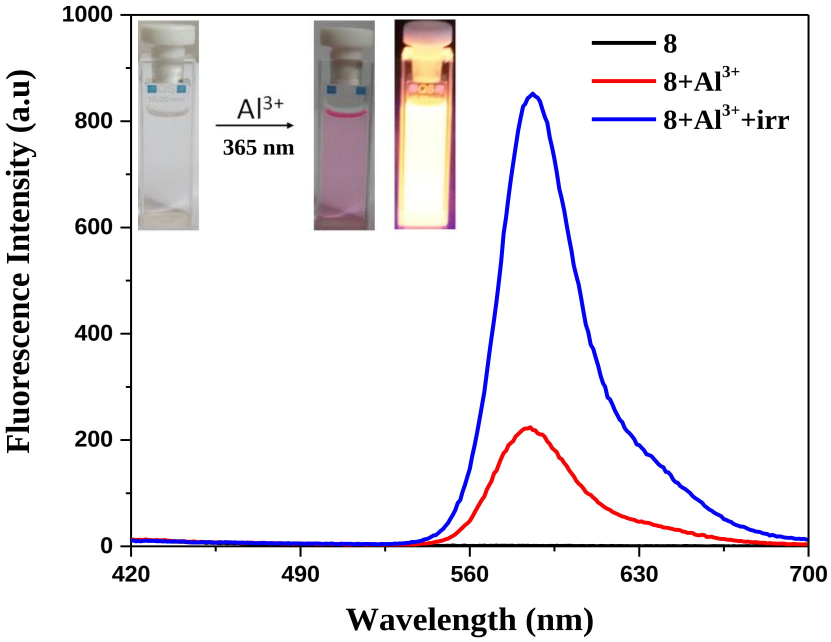


Figure S8 Fluorescence spectral changes of **8** upon photoirradiation with 365 nm light at different time intervals in presence of 10 equivalents of Al^{3+} in THF.

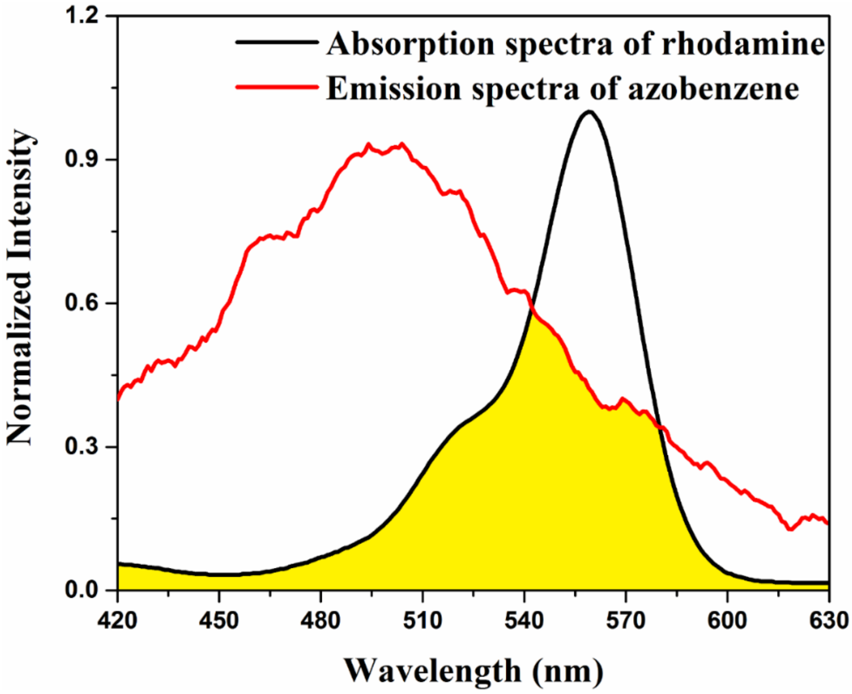


Figure S9 Fluorescence spectra of azobenzene (donor) and absorption spectra of rhodamine (acceptor) in THF solvent. Spectral overlap is indicated in yellow color.

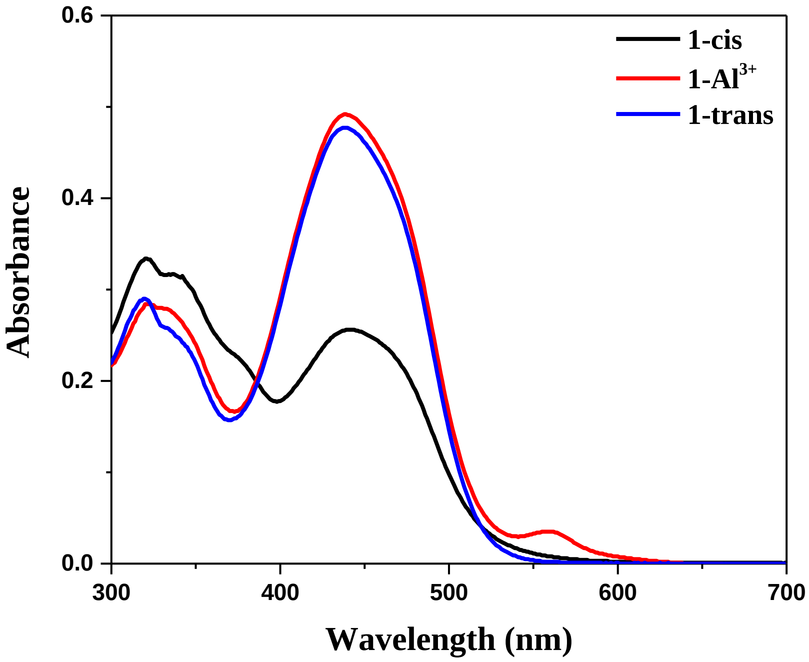


Figure S10 UV-visible absorption spectra of photoirradiated cis rich **1** in presence of Al^{3+} in10 µM in THF under 365 nm light.

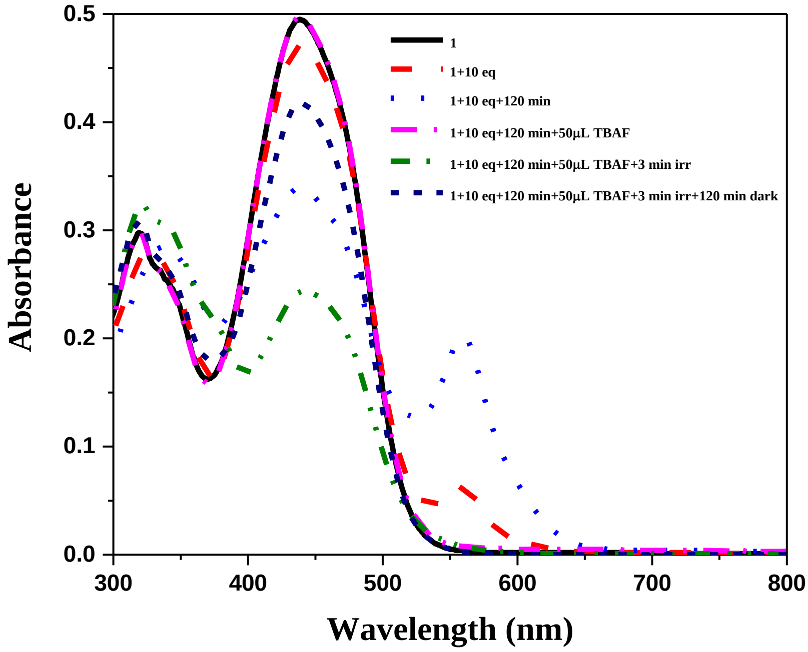


Figure S11 UV-vis absorption spectral changes showing reversibility of photoisomeirzation of **1** with 10 equivalents of Al^{3+} ion by TBAF in THF under 365 nm light.

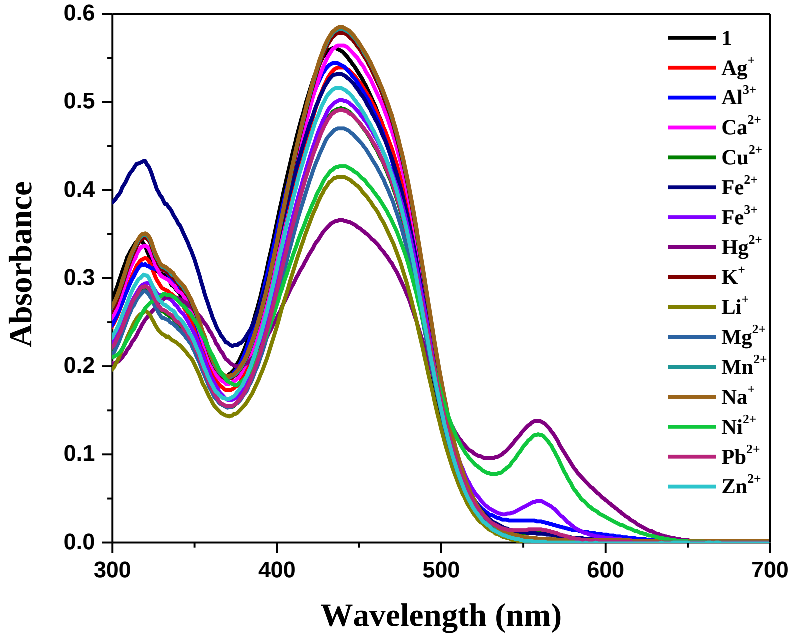


Figure S12 UV-visible absorption spectra of **1** (10μM) in presence of 10 equivalents of different metal ions in THF.

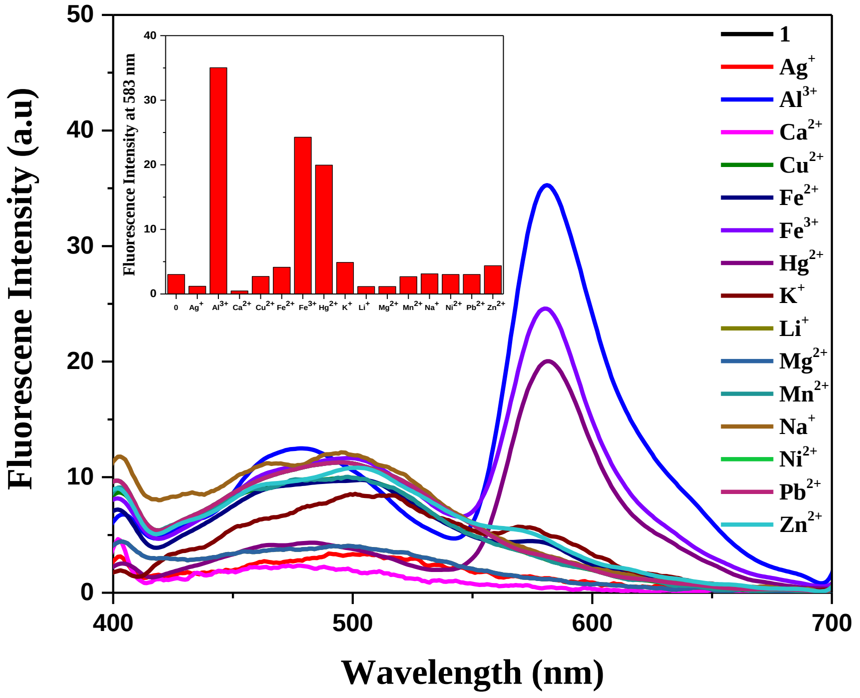


Figure S13 Fluorescence spectra of **1** (10 μM) with 10 eq of different metal ions in THF solution (λ_{exi} = 360 nm). Inset, a plot of fluorescence intensity changes at 583 nm with different metal ions.

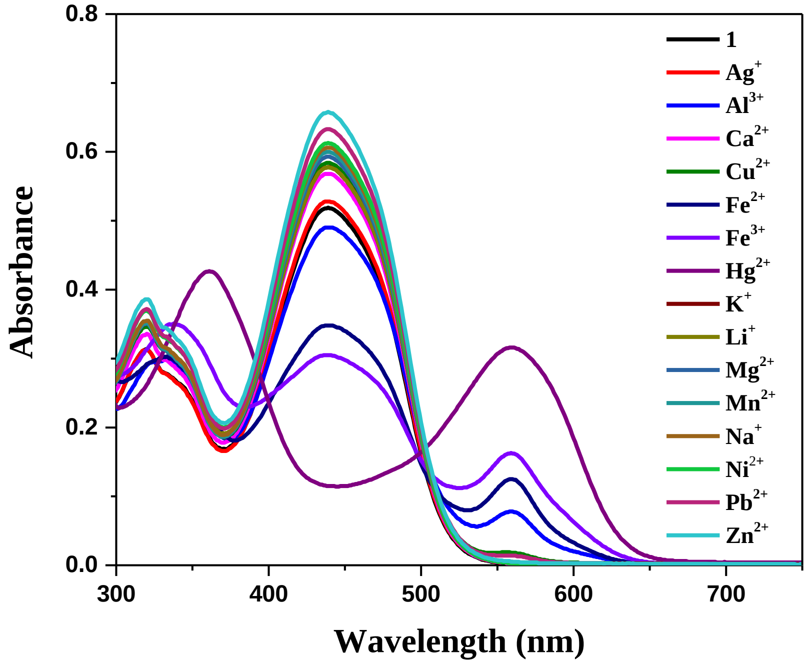


Figure S14 UV-visible absorption spectra of **1** (10 µM) upon photoirradiation with 365 nm light in presence of 10 equivalents different metal ions in THF.

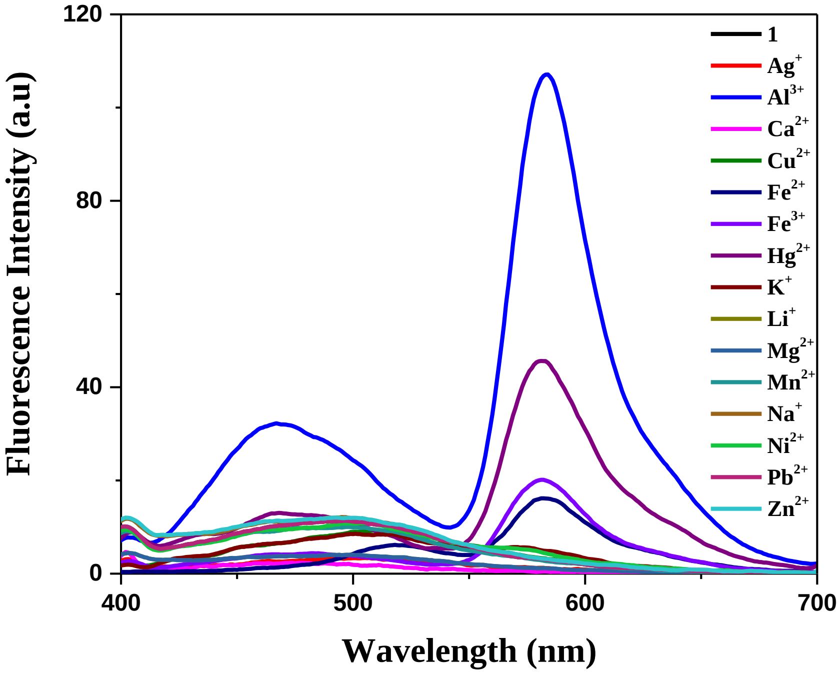


Figure S15 Fluorescence spectra of **1** (10 µM) upon photoirradiation with 365 nm light of in presence of 10 equivalents of different metal ions in THF.

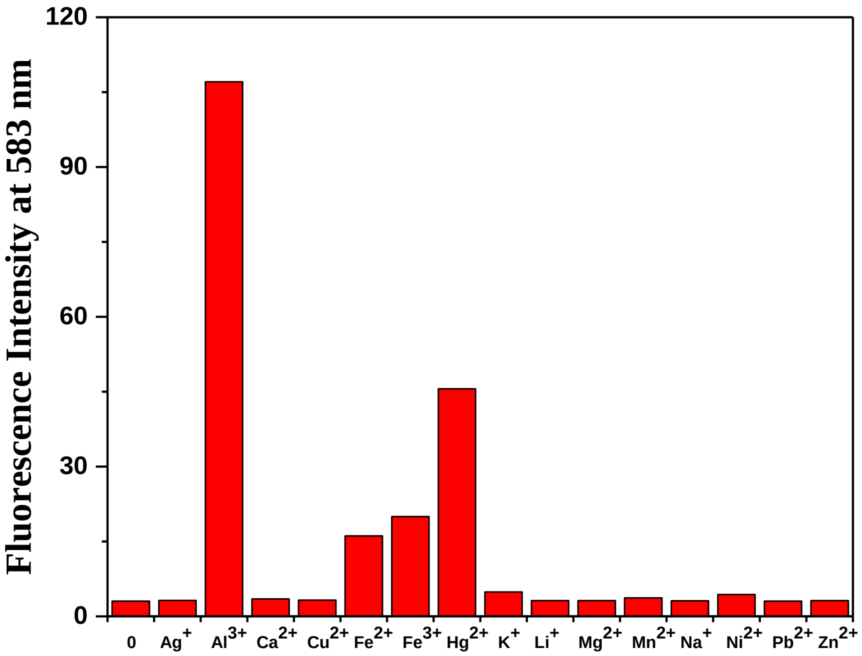


Figure S16 A plot of fluorescence intensity of **1** at 583 nm in presence of different metal ions after photoirradiation with 365 nm light.

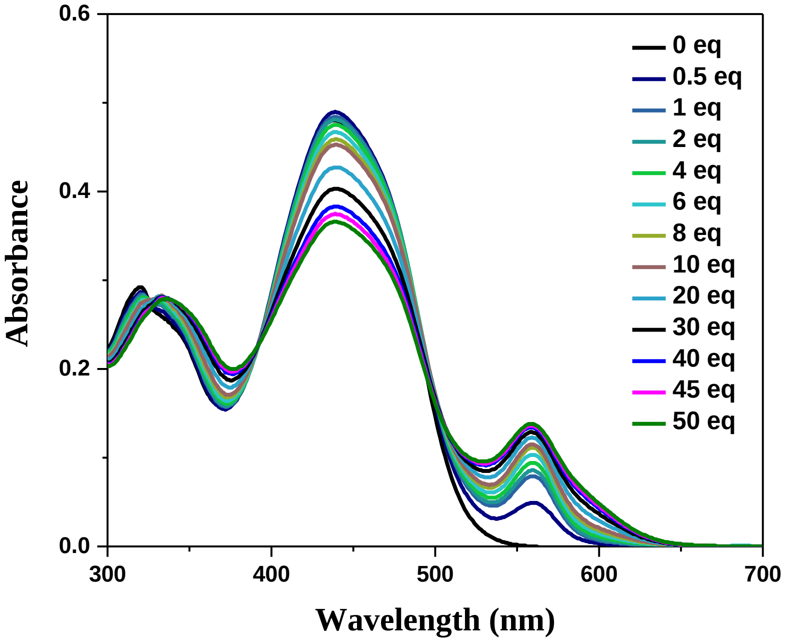


Figure S17 Absorbance changes of titration of **1** (10µM) with different equivalents of Al^{3+} ions in THF.

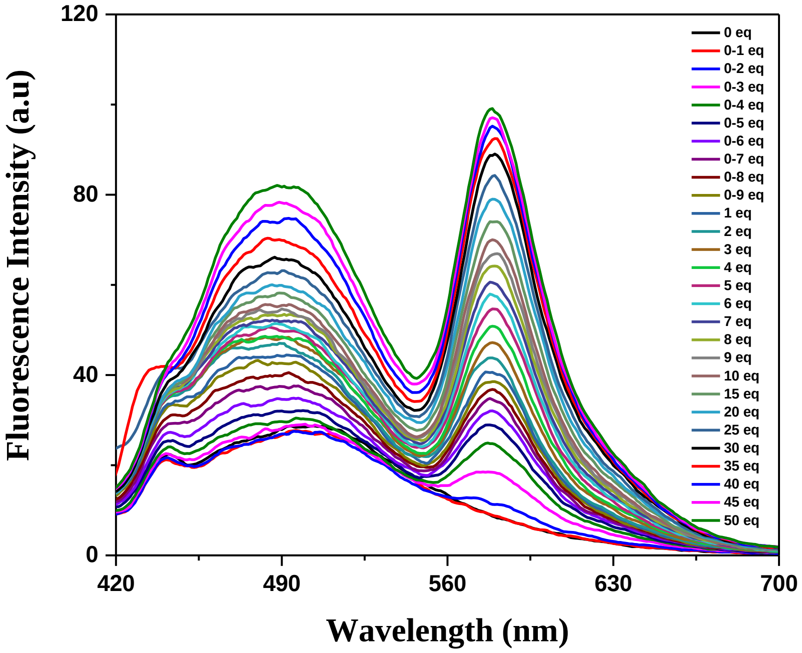


Figure S18 Fluorescence changes of titration of **1** (10µM) with different equivalents of Al^{3+} ions THF.

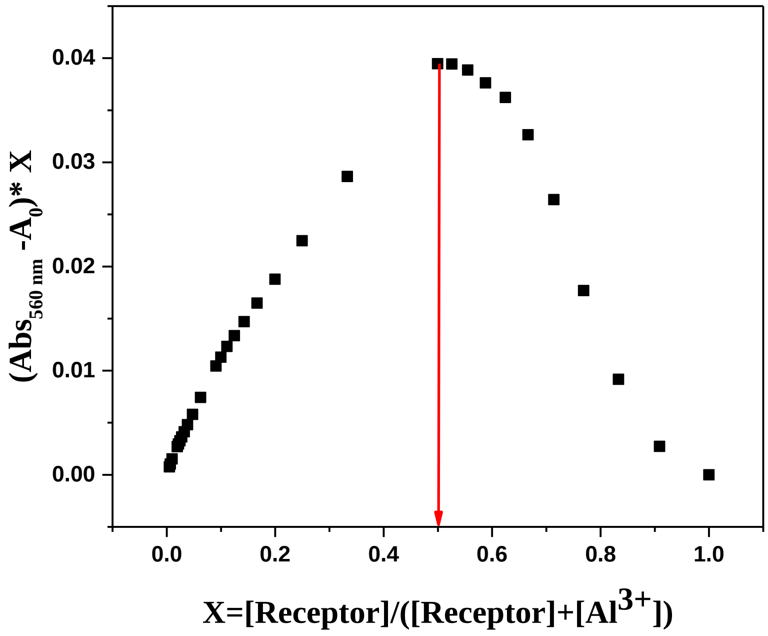


Figure S19 Job's plot of **1** with Al^{3+} ions in THF representing stoichiometry 1:1.

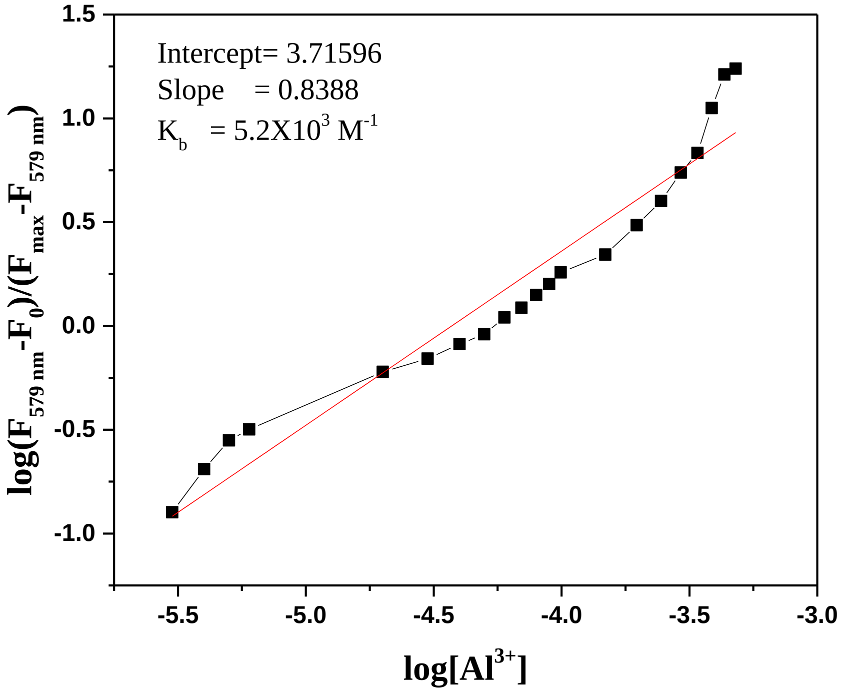


Figure S20 Determination of binding constant (K_b) of **1** for Al^{3+} ($R^2 = 0.986$) determined from the Benesi-Hildebrand method.

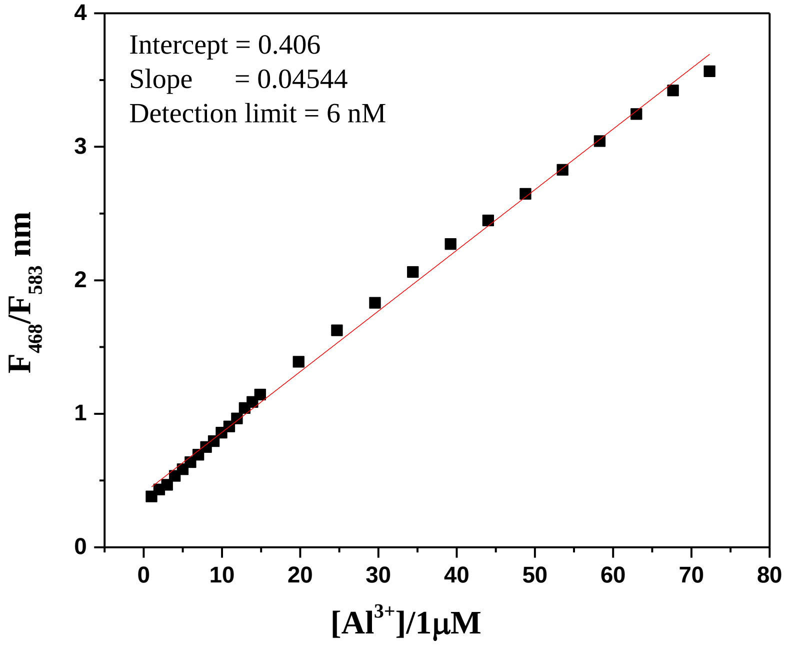


Figure S21 Determination of detection limit of Al^{3+} with **1** in THF.

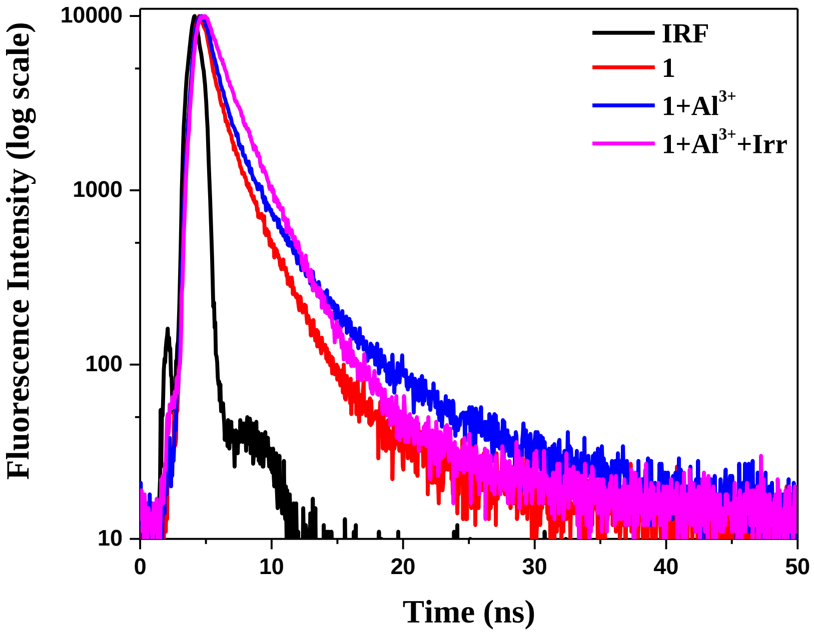


Figure S22 Fluorescence lifetime decay of **1** upon photoirradiation with 365 nm light in presence of 10 equivalents of Al^{3+} in THF (λ_{emi} = 468 nm).

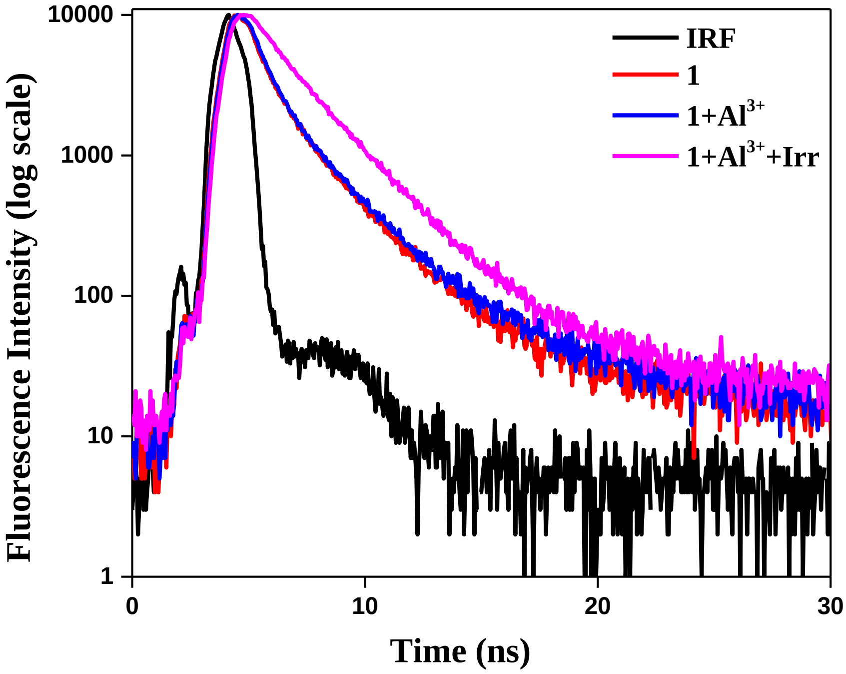


Figure S23 Fluorescence lifetime decay of **1** upon photoirradiation with 365 nm light in presence of 10 equivalents of Al^{3+} in THF (λ_{emi} = 583 nm).

	τ_1 (ns)	**τ_2 (ns)**	**X^2**	**B1**	**B2**
Emi at 468 nm					
1	0.76	2.48	1.5	58.43	41.57
1 + Al^{3+}	0.81	2.75	1.3	62.82	37.18
1 + Al^{3+} +Irr (under 365 nm light)	1.53	2.79	1.16	49.59	50.41
Emi at 583 nm					
1	1.05	3.88	1.24	62.50	37.50
1 + Al^{3+}	1.3	4.1	1.58	60.1	39.9
1 + Al^{3+} + Irr (under 365 nm light)	2.2	4.8	1.15	59.72	40.28

Table 1. Fluorescence life time decay parameters of **1** and its Al^{3+} complex.

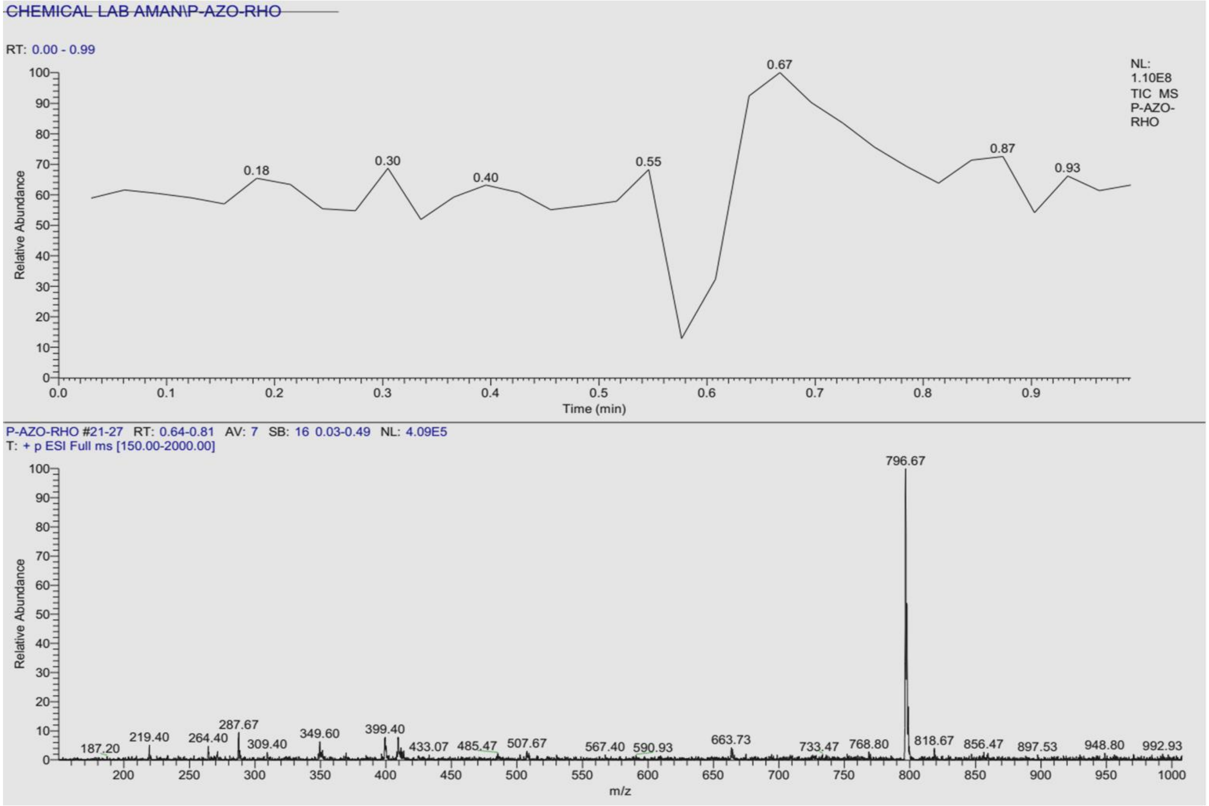


Figure S24 ESI mass spectrum of **1**

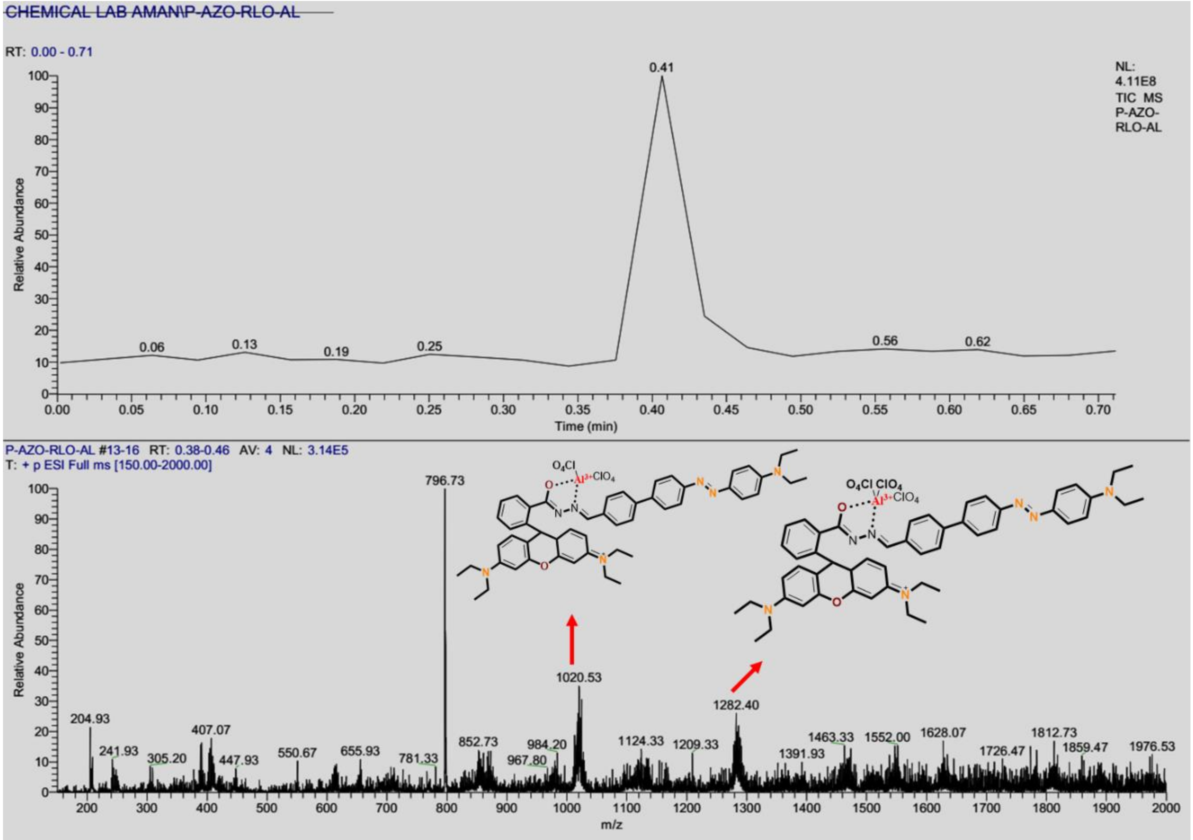


Figure S25 ESI-MS spectrum of **1**+Al^{3+} complex after photoirradiation with 365nm light in THF.

Cell culture maintenance

HeLa cell line obtained from American Type Culture collection (ATCC) were maintained in Dulbecco’s Modified Eagle medium (DMEM) containing high glucose supplemented with 10% Fetal Bovine Serum FBS (v/v), penicillin and streptomycin each (50μ/ml) and L-glutamine (2mM). Cultured flask were kept at 37°C in a humidified 5% CO_2 incubator. Once the cells reached the confluence, culture medium was removed and rinsed twice with 1X Phosphate buffer Saline (PBS) pH8. Subsequently, the confluent cell layers were trypsinized by adding trypsin/EDTA solution and suspended in culture medium. Cell viability and numbers were calculated by with trypan blue (0.4% (w/v) staining using a light microscope.

Cell viability assay (MTT)

Cytotoxicity assay was done in the corning’s cell costar 96 well plate to determine the number of viable cells after adding Al^{3+}, **1** and combination of **1**+Al^{3+} at different concentration. The assay was performed as mentioned in the previously published protocols pertaining to cytotoxicity assay. Different concentrations of above mentioned compounds were added in culture media after seeding 2×10^5 cells/well, incubated at 37°C with 5% CO_2. The assay was performed in a triplicates for each day to study the cell viability at different time intervals like 12 hrs. 24 hrs. At the end of the every exposure period of compound incubated with medium was removed and rinsed with 1X PBS buffer, then the MTT(thiazolyl blue tetrazolium bromide salt) solution (20 µL/ well) was added. After the completion of exposure period, MTT solutions were removed and tiny formazan crystals were solubilized by adding 200µl of DMSO. Two internal controls were set up for each experiment consisting of cells and medium alone. The cell viability was calculated by following formula and expressed in percentage viability.

$$\text{Cell viability (\%)} = \frac{\text{O.D of treated cells}}{\text{O.D of untreated cells}} \times 100$$

Fluorescence microscopic analysis

As described above HeLa cells were cultured overnight at 37°C and supplemented with 5% CO_2, subsequently cells were washed with 1X PBS and individually treated with different concentrations of Al^{3+}, **1** and **1**+Al^{3+} in order to view the cells under fluorescent microscope (Leica DMi8, Germany). Likewise, GFP bearing *E.coli* tyrosine auxotroph (*JW2581*) cells were incubated overnight at 37°C in 180rpm. Next day, 1% of overnight culture was inoculated in 100ml of Luria Bertani (LB) broth and allowed to grow until reaching early mid-log phase (OD_{600}; 0.5-0.6). Then, cells were induced with 1mM of Isopropyl-β-D thioalactopyranoside (IPTG) for 6h followed by cells were harvested and 2×10^6 GFP expressing cells were seeded into 96 well plates. Besides, different concentrations of Al^{3+}, **1** and combination of **1**+Al^{3+} were added and incubated for 30 min to observe the bacterial cells under Fluorescent microscope.

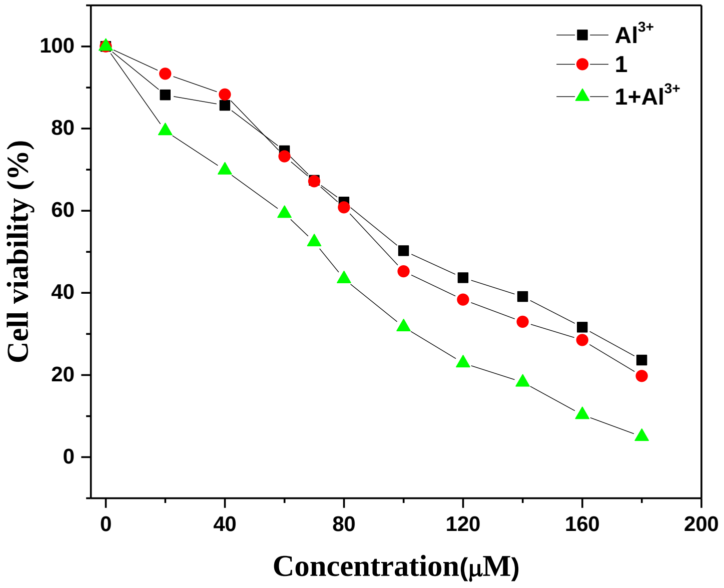


Figure S26 Cytotoxic studies of **1**, Al^{3+} and **1**+Al^{3+}

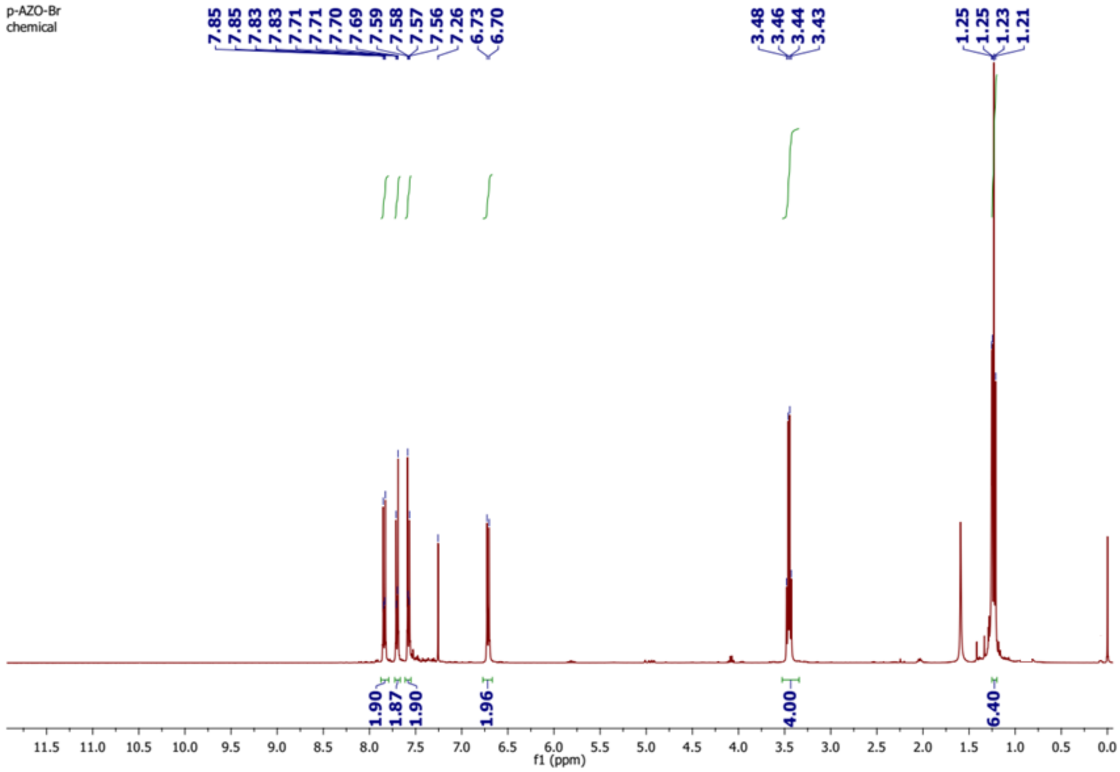


Figure S27. ^{1}H NMR spectrum of **4** in $CDCl_3$

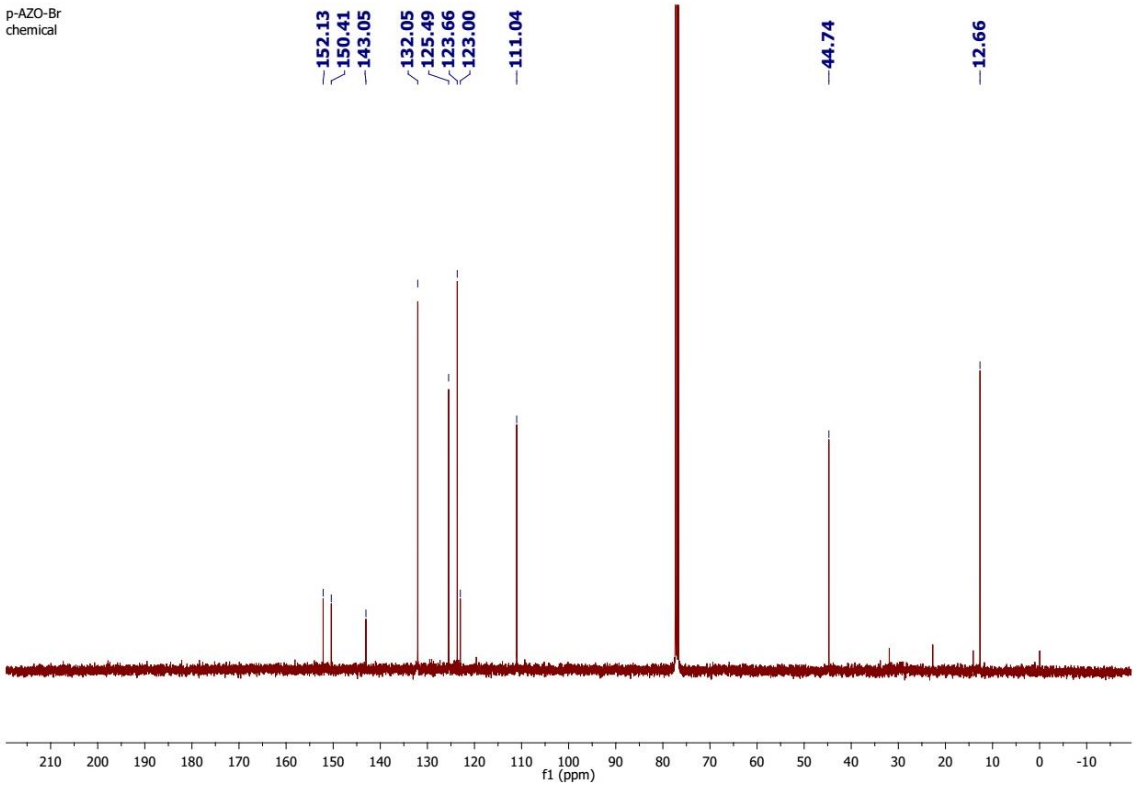


Figure S28 ^{13}C NMR of **4** in $CDCl_3$

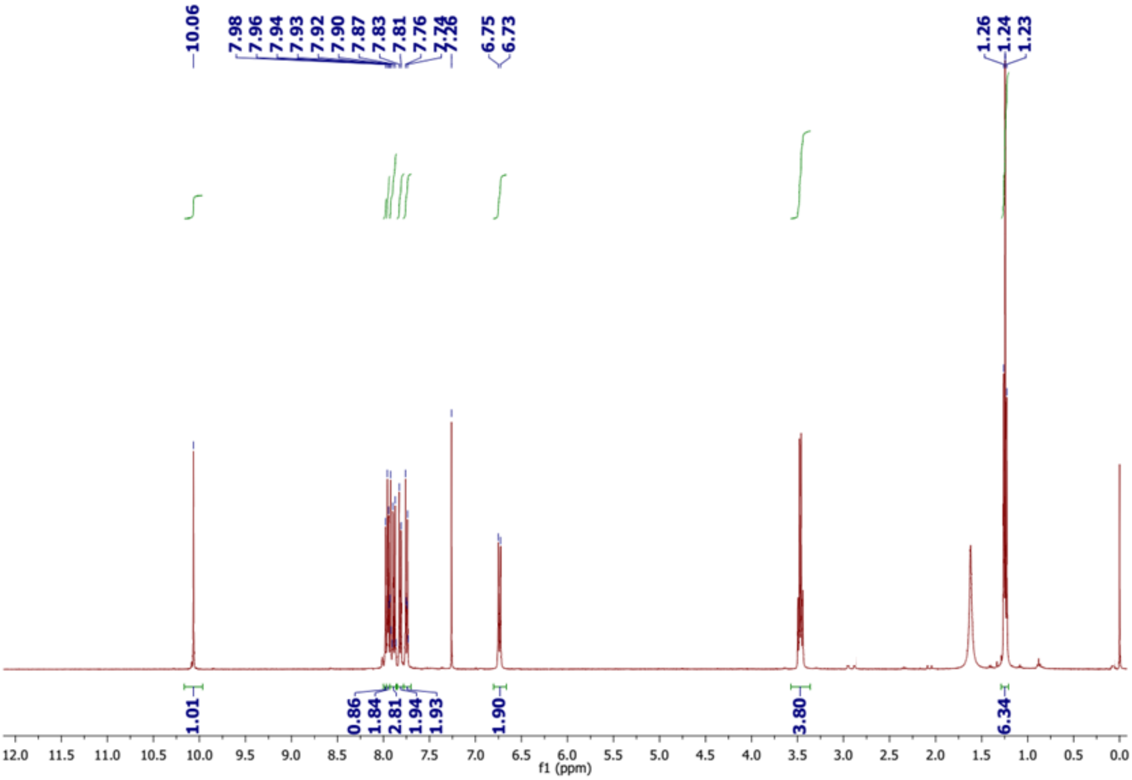


Figure S29 ^{1}H NMR spectrum of **5** in CDCl$_3$

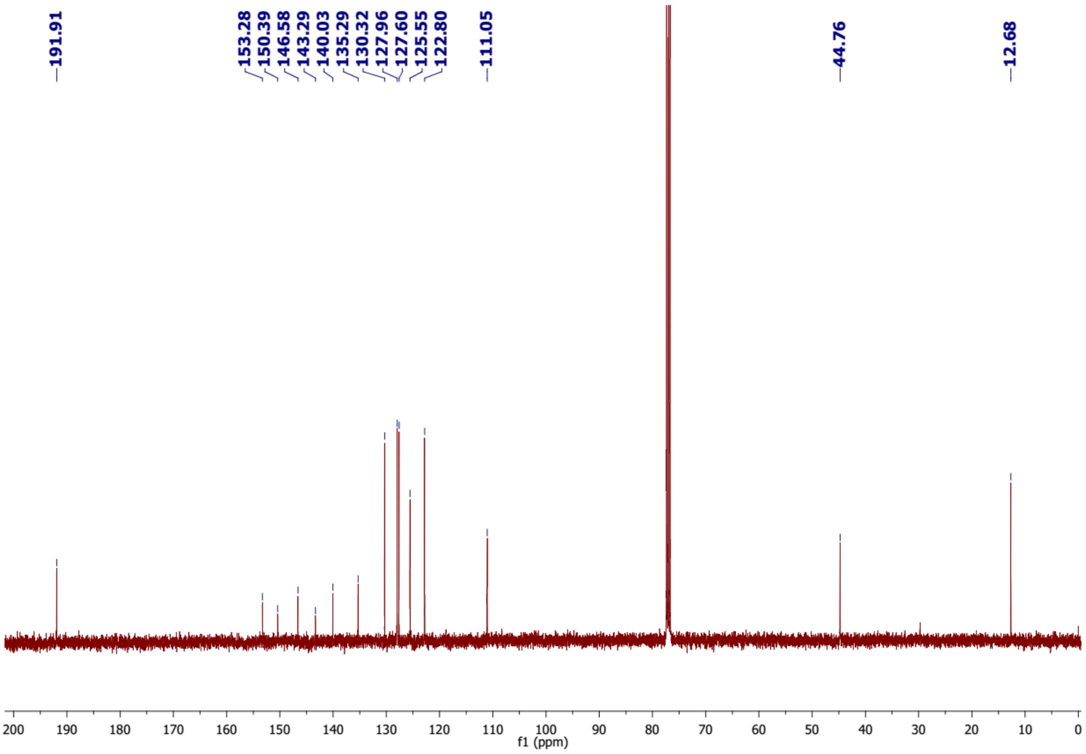


Figure S30 ^{13}C NMR spectrum of **5** in CDCl$_3$

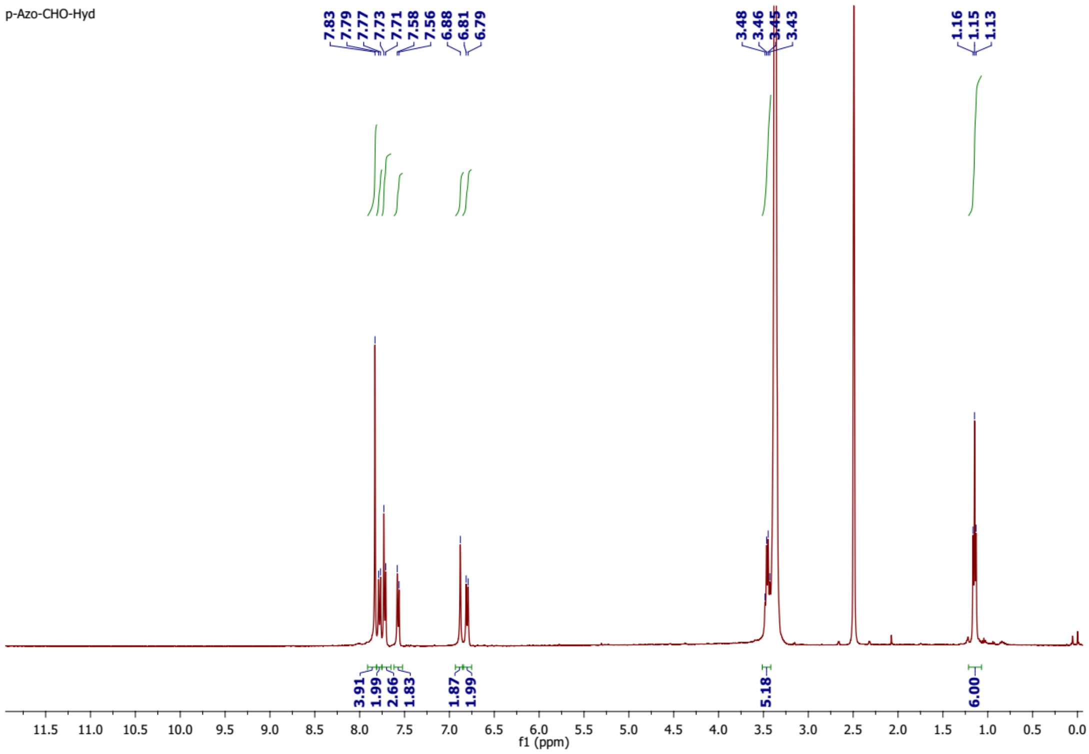


Figure S31 ^{1}H NMR spectrum **6** in DMSO-d_6

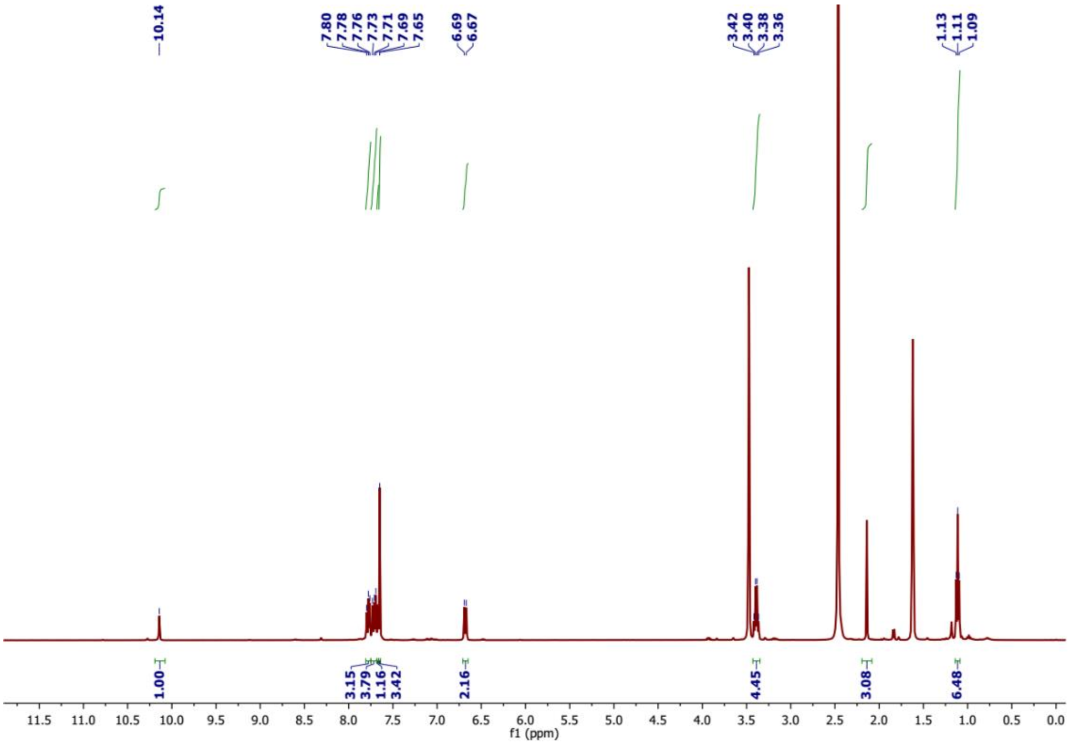


Figure S32 ^{1}H NMR spectrum of **7** in THF-d_8

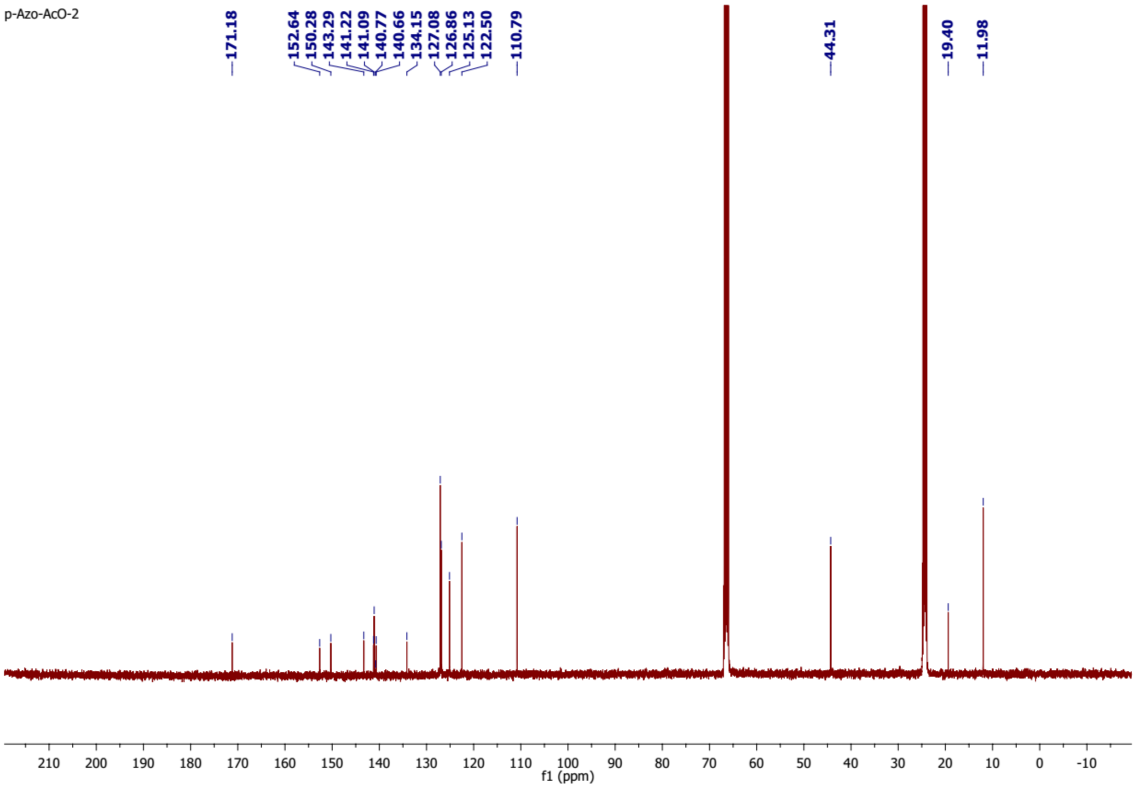


Figure S33 ^{13}C NMR of **7** in THF-d_8

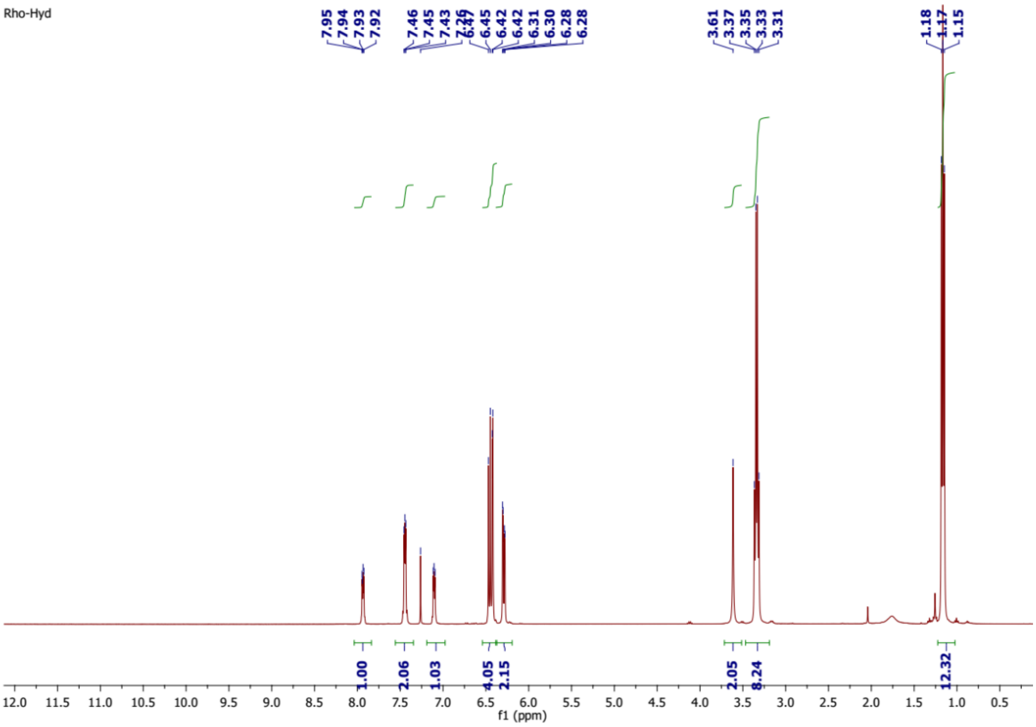


Figure S34 ^{1}H NMR of **8** in $CDCl_3$

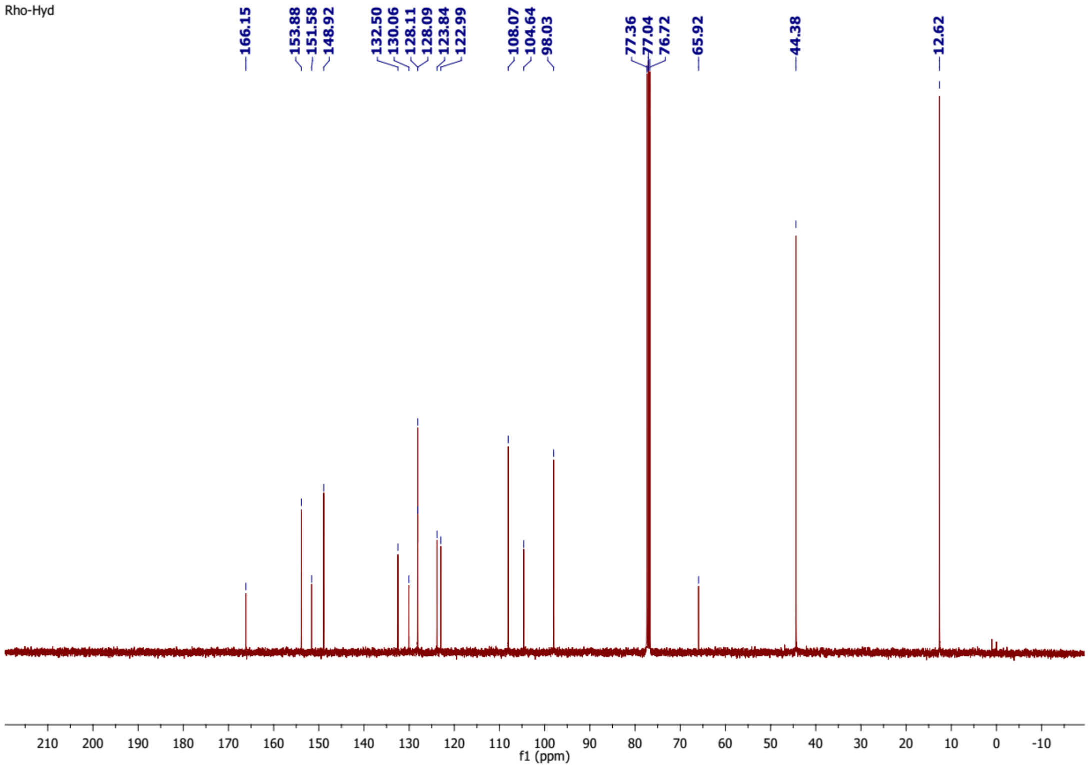


Figure S35 ^{13}C NMR of **8** in CDCl$_3$

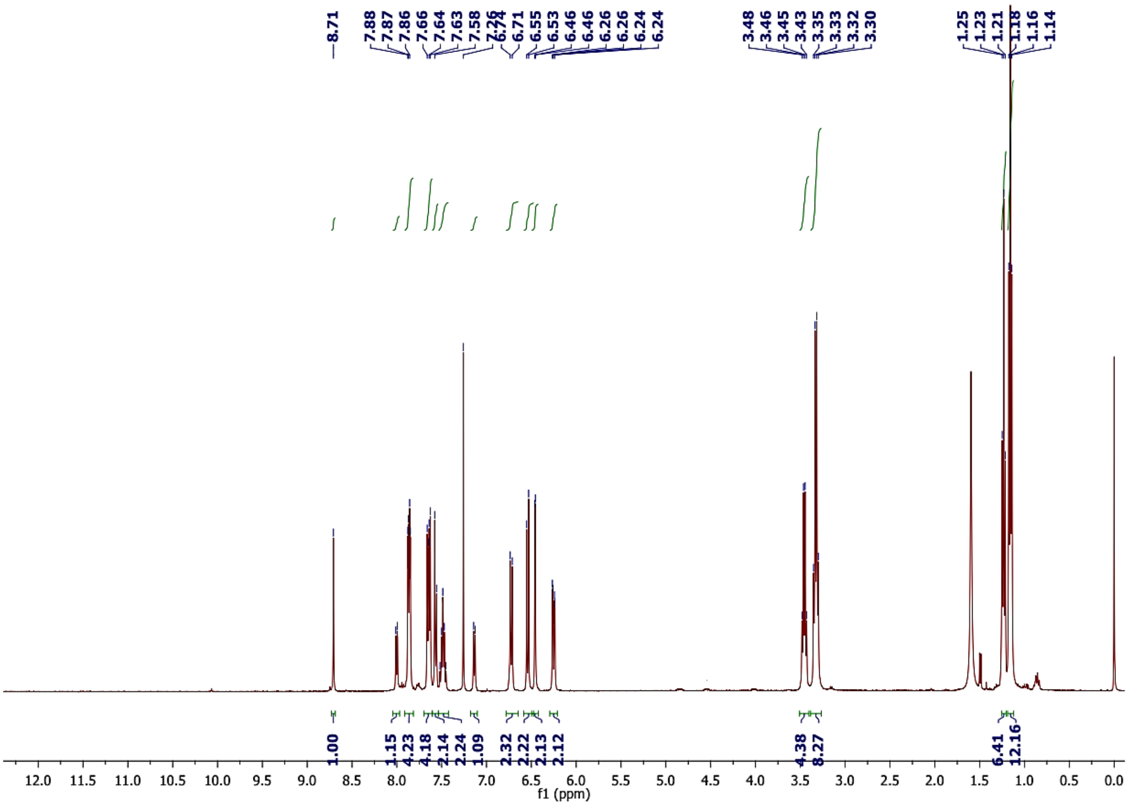


Figure S36 ^{1}H NMR of **1** in CDCl$_3$

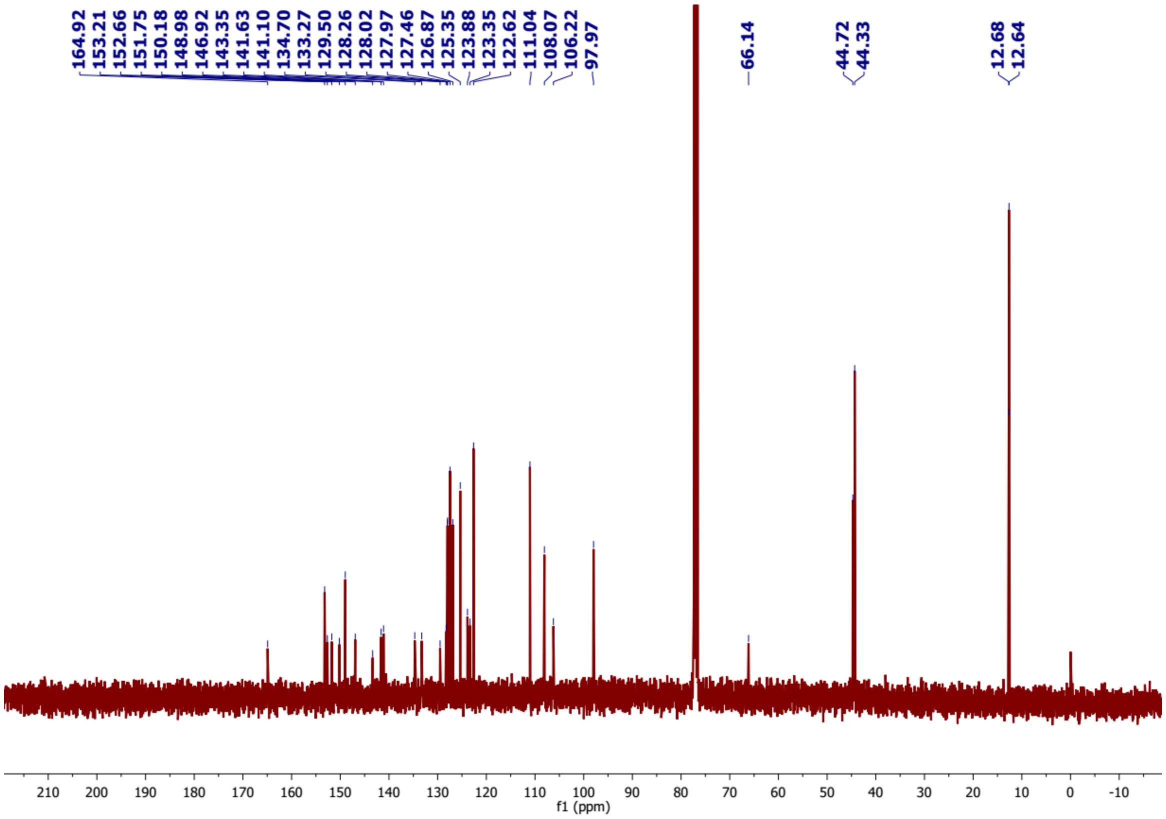


Figure S37 ^{13}C NMR of **1** in CDCl$_3$