Supporting Information

Hexaphenylbenzene Based AIEE Active Two Photon Probe for Detection of Hydrogen Sulfide with Tunable Self-assembly in Aqueous Media and Application in Live cell Imaging

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General Experimental Procedures:

Materials and reagents: All reagents were purchased from Aldrich and were used without further purification. THF was dried over sodium and benzophenone as an indicator. UV-vis studies were performed in THF, absolute ethanol, distilled water and HEPES buffer (0.05 M) (pH = 7.05).

Instrumentation: UV-vis spectra were recorded on a SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette (path length, 1 cm). The cell holder was thermostatted at 25°C. The fluorescence spectra were recorded with a SHIMADZU-5301 PC spectrofluorimeter. The FT-IR spectra were recorded with VARIAN 660 IR Spectrometer. The Time resolved fluorescence spectra were recorded with a HORIBA Time Resolved Fluorescence Spectrometer. Elemental analysis was done using a Flash EA 1112 CHNS/O analyzer from Thermo Electron Corporation. The cell holder was thermostatted at 25 °C. The scanning electron microscope (SEM) images were obtained with a field-emission scanning electron microscope (SEM CARL ZEISS SUPRA 55). TEM images were recorded from Transmission Electron Microscope HR-TEM-JEOL 2100. The dynamic light scattering (DLS) data were recorded with MALVERN Instruments (Nano-ZS). ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER-AVANCE-II FT-NMR-AL400 MHz and 500 MHz spectrophotometer using $CDCl_3$ as solvent and tetramethylsilane, $SiMe_4$ as internal standards. Data are reported as follows: chemical shifts in ppm (ı), multiplicity (s = singlet, br = broad signal, d = doublet, t = triplet, m = multiplet), coupling constants *J* (Hz), integration and interpretation. Silica gel 60 (60-120 mesh) was used for column chromatography.

Quantum yield calculations: Fluorescence quantum yield was determined by using optically matching solution of diphenylanthracene (Φ_{fr} = 0.90 in cyclohexane) as standard at an excitation wavelength of 373 nm and quantum yield is calculated using the equation:

$$\Phi_{fs} = \Phi_{fr} \times \frac{1-10^{-ArLr}}{1-10^{-AsLs}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

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

Fluorescence responses of the probes on H_2S:

For each experiment, we have taken 3 mL of a solution that contains a solution of derivative **2** (10^{-3}M) in 15 μL of DMSO diluted with 885 μL DMSO and 2.1 mL of HEPES buffer (0.05 M, pH = 7.05). Typically, the aliquots of freshly prepared standard solutions (10^{-1} to 10^{-3}M) of *tert-butyl ammonium salt* ($^tBu_4N^+X^-$) where X = CN^-, F^-, Cl^-, Br^-, I^-, NO_3^-, AcO^-, ClO_4^- and $H_2PO_4^-$ CO_3^- as K_2CO_3; N_3^- as NaN_3), are used in EtOH; H_2S as aqueous solution of Na_2S; and cysteine and glutathione standard solutions (10^{-1} M to 10^{-3} M) were added to record the UV-vis and fluorescence spectra. Hydrogen peroxide (H_2O_2) and hypochlorite (OCl^-) were delivered from 30% and 5% aqueous solutions, respectively. In titration experiments, each time a 3 ml solution of compound was filled in a quartz cuvette (path length, 1 cm) and spectra were recorded.

Time-dependent experiments: To the solution of HPB-Az, **2** in H_2O/DMSO (7:3, v/v) buffered with HEPES (0.05 M, pH 7.05); Na_2S solution (80 equiv. i.e., 400 μM) were added and incubated for 16 min and the fluorescence spectra were measured in 1 min interval.

Selectivity: The solutions of HPB-Az, **2** in H_2O/DMSO (7:3, v/v) were added Na_2S (10^{-2} M, 400 μM) and other competitive species (10^{-2} M, 500 μM) in water and incubated for 16 min and the fluorescence spectra were measured after 16 minutes.

Blood serum: Real blood sample of medically fit person was used for the experiment and all physiological conditions were maintained. The blood serum was isolated by centrifugation of the fresh blood sample of a healthy volunteer after fasting at 4000 rpm for 20 min at 4°C. The stock solution of blood serum was prepared by dissolving 100 μL of serum in 1 mL solution of HEPES buffer (0.05 M) at pH = 7.05.

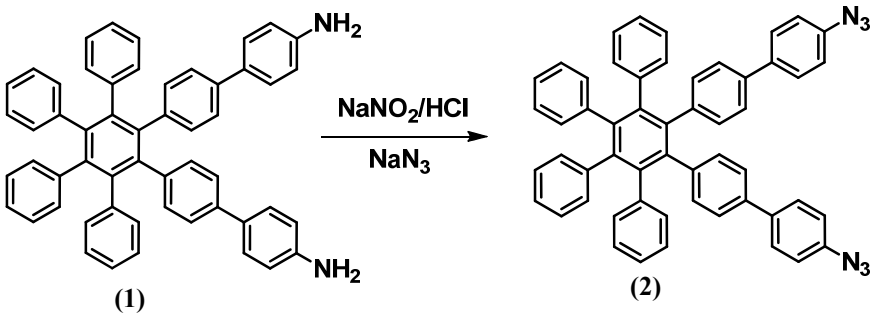
Experimental details of vapour phase sensing of H_2S: For the vapour phase detection of H_2S, the glass vial containing 3 ml of 5 μM solution of derivative **3** in H_2O:DMSO (7:3, v/v) was inserted in other big vial containing 1ml 1M solution of Na_2S in HEPES buffer at the base. The system was sealed so as to obtain saturated vapour pressure at room temperature. The fluorescent spectrum of the sample was then recorded after different time interval (Fig. S19).

Cell Culture: HeLa human cervical carcinoma cells (ATCC, Manassas, VA, USA) were cultured in MEM (WelGene Inc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL). Two days before imaging, the cells were passed and plated on glass-bottomed dishes (NEST). All the cells were maintained in a humidified atmosphere of 5/95 (v/v) of CO_2/air at 37 °C. For labeling, the growth medium was removed and replaced with serum-free MEM (1% DMSO). The cells were treated and incubated with 2.0 μL of 10 mM **2** in DMSO stock solution (20.0 μM **2**) at 37 °C under 5 % CO_2 for 30 min.

Two-Photon Fluorescence Microscopy: Two-photon fluorescence microscopy images of probe-labeled cells and tissues were obtained with spectral confocal and multiphoton microscopes (Leica TCS SP8 MP) with ×10 dry, ×40 oil and ×100 oil objectives, numerical aperture (NA) = 0.30, 1.30 and 1.30, respectively. The two-photon fluorescence microscopy images were obtained with a DMI6000B Microscope (Leica) by exciting the probes with a mode-locked titanium-sapphire laser source (Mai Tai HP; Spectra Physics, 80 MHz pulse frequency, 100 fs pulse width) set at wavelength 710 nm and output power 1690 mW, which corresponded to approximately 7 mW average power in the focal plane. To obtain images at 400-600 nm range, internal PMTs were used to collect the signals in an 8 bit unsigned 512 × 512 and 1024 × 1024 pixels at 400 Hz scan speed, respectively.

Photostability: Photostability of compound **2** in HeLa cells was determined by monitoring the changes in TPEF intensity with time at three designated positions of compound **2**-labeled cells chosen without bias (Fig. 4a-b).

Cell viability: To confirm that compound **2** couldn't affect the viability of HeLa cells in our incubation condition, we used CCK-8 kit (Cell Counting Kit-8, Dojindo, Japan) according to the manufacture's protocol. The results are shown in Fig. 4c.



Scheme S1 Synthesis of hexaphenylbenzene based derivative **2**.

Synthesis of HPB Derivative 2

A mixture of hydrochloric acid (6 mL) and compound **1** (100 mg, 0.14 mmol) were dissolved in ethanol (10 mL). After the solution was cooled to 0°C, sodium nitrite (40 mg, 0.56 mmol) was added and the mixture was stirred for 30 min. Further, sodium azide (37 mg, 0.56 mmol) was added slowly, and the mixture was stirred for another 2 h at room temperature. The suspension was extracted with $CHCl_3$ (3 × 20 mL), and the combined organic layers were concentrated under reduced pressure. The residue was purified by the silica gel chromatography (hexane/$CHCl_3$ = 5:1, v/v) to afford compound **2** as a faint yellow solid recrystallized from methanol as pale yellow solid in 75% yield (80.5 mg); mp: >280°C. The structure of compound **2** was confirmed from its spectroscopic and analytical data (Fig. S29-S32, ESI†); ^{1}H NMR (300 MHz, $CDCl_3$, ppm). δ = 7.40 (d, *J* = 9 Hz, 4H), 7.09 (d, *J* = 9 Hz, 4H), 6.96 (d, *J* = 9 Hz, 4H), 6.91 (d, *J* = 9 Hz, 4H), 6.86-6.85 (m, 20H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm) δ = 158.01, 149.80, 144.89, 144.21, 140.54, 139.62, 137.01, 135.95, 133.53, 131.48, 131.27, 130.26, 129.21, 123.81. ESI-MS mass spectrum of compound **2** showed a parent ion peak, *m/z* = 769.3399 $[M+H]^+$ and 807.2827 $[M+K]^+$. Elemental analysis: Calculated for $C_{54}H_{36}N_6$: C 84.35; H 4.72; N 10.93 Found: C 84.32%; H 4.74%; N 10.94. The FT-IR showed stretching band at 2094 cm^{-1} corresponding to N_3 groups.

Reaction of Compound 2 with H_2S and characterization data of the product:

Compound **2** (50 mg) in THF/Water (1:1) mixture and a solution of aqueous Na_2S was added into it and the mixture was stirred for 60 min at room temperature. The completion of the reaction was confirmed by TLC. The reaction mixture was extracted with EtOAc (5×20 mL), and the combined organic layers were concentrated

under reduced pressure. The residue was purified by the silica gel chromatography (hexane/EtOAc = 4:1, v/v) to afford compound as a light brown solid recrystallized from methanol as yellow solid in 80% yield (37 mg); mp: >280°C. The structure of this compound was confirmed from its spectroscopic and analytical data (Fig. S33-S35, ESI†); ^{1}H NMR (500 MHz, $CDCl_3$, ppm). δ = 7.67 (q, *J* = 5 Hz, 4H), 7.54 (q, *J* = 5 Hz, 4H), 7.47 (q, *J* = 5 Hz, 4H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.05 (d, *J* = 5 Hz, 2H), 6.88-6.84 (m, 12H), 6.69 (d, *J* =10 Hz, $4H_a$), 6.62 (d, *J* =10 Hz, $4H_b$), 3.48 (s, 4H, NH_2); ^{13}C NMR ($CDCl_3$, 125 MHz, ppm) δ = 144.65, 139.29, 133.91, 132.15, 132.07, 131.33, 128.66, 128.48, 126.83, 124.00, 123.47, 115.92, 114.07. ESI-MS mass spectrum of compound showed a parent ion peak, *m/z* = 717.3401 $[M+H]^+$. Elemental analysis: Calculated for $C_{54}H_{40}N_2$: C 90.47; H 5.62; N 3.91, Found: C 90.45%; H 5.65%; N 3.90. The FT-IR showed stretching band at 3385 cm^{-1} corresponding to NH_2 groups. These above experimental data confirmed the formation of compound **1**.

Table S1. Comparison table showing of probe **2** with previous reports of H_2S sensor in the literature.

Sr. No.	Publication	Use of supra-molecular aggregates for the detection of H_2S	Vapour phase detection	Response Time	Detection limit	Fluorescence Response	H_2S induced formation of AIEE active nanoaggregates	Practical application of H_2S sensor by paper strip assay	Sensing ability in blood serum and ground water	TPM imaging in Live cell	Interference with other species
1	**Present Manuscript**	**Yes**	**Yes**	**15 min (Soln.) & 60 min (Vapour)**	**338 nM (0.33 µM)**	**Not-Quenched probe**	**Yes (AIEE active microfibers)**	**Yes**	**Yes**	**Yes**	**No**
2	*Chem. Commun.*, 2015, **51**, 7505	No	No	60 min	0.28 µM	Turn-on	No	No	No	No	Minimal
3	*Chem. Sci.*, 2015, **6**, 2360	No	No	40 min	0.3 µM	Ratiometric	No	No	Yes	Yes	Minimal
4	*Chem. Sci.*, 2015, **6**, 1979	No	No	10 min	5.4 µM	Chemi-luminescent	No	No	No	No	Minimal
5	*Chem. Commun.,* 2015, **51**, 2407	No	No	30 min	0.81 µM	Ratiometric	No	No	No	Yes	Minimal
6	*Chem. Commun.*, 2015, **51**, 1510	No	No	2.5-20 min	91 nM	Ratiometric	No	No	No	No	Yes
7	*Chem. Commun.,* 2014, **50**, 4214	No	No	3-10 min	0.78 nM	Turn-on	No	No	No	No	No
8	*Chem. Commun.*, 2014, **50**, 8892	No	No	5 min	-	Turn-on	No	No	Yes	No	No
9	*J. Am. Chem. Soc.*, 2014, **136**, 9838	No	No	60 min	0.3 µM	Ratiometric	No	No	No	Yes	No
10	*Sci. Rep.*, 2014, **4**, 7053	No	No	9 min	118 µM	Turn-on	No	No	No	No	No
11	*Small*, 2014, **10**, 4874	No	No	30 min	0.13 µM	Ratiometric	No	No	Yes	No	Yes
12	*Chem Biol Drug Des*, 2014, DOI: 10.1111/cbdd.12483	No	No	60 min	1 µM	Turn-on	No	No	Yes	No	No
13	*Analyst*, 2014, **139**, 1945	No	No	30 min	0.7 µM	Turn-on	No	No	No	Yes	Small
14	*Chem. Commun.*, 2013, **49**, 3890	No	No	60 min	3.05 µM	Turn-on	No	No	No	Yes	No
15	*J. Am. Chem. Soc.*, 2013, **135**, 9915	No	No	60 min	0.2 µM	Ratiometric	No	No	No	Yes	Minimum
16	*Analyst*, 2013, **138**, 946	No	No	60 min	0.25 µM	Turn-on	No	No	Yes	Yes	No
17	*Anal. Chem*., 2013, **85**, 7875	No	No	40 min	5-10 µM	Turn-on	No	No	No	Yes	Minimum
18	*Chem. Commun.*, 2012, **48**, 8395	No	No	50 min	5-10 µM	Turn-on	No	No	No	Yes	No
19	*Angew. Chem. Int. Ed.*, 2011, **50**, 9672	No	No	10 min	1 µM	Turn-on	No	No	Yes	No	No
20	*J. Am. Chem. Soc.*, 2011, **133**, 10078	No	No	60 min	5-10 µM	Turn-on	No	No	No	No	No

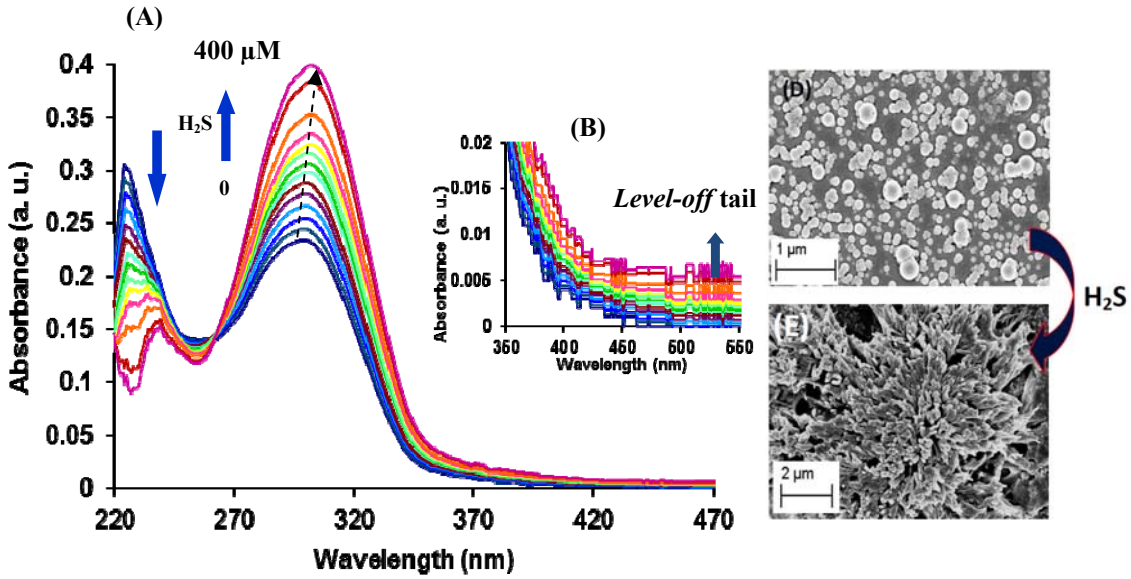


Fig. S1 (A) UV-vis spectrum of **2** (5 µM) in response to 400 µM of H_2S in H_2O/DMSO (7:3, v/v) mixtures buffered with HEPES (pH = 7.05); (B) Showing the presence of level-off tail in the visible region (400-550 nm); SEM images showing (D) before spherical aggregates and (E) after the addition of 400 µM H_2S in H_2O/DMSO (7:3, v/v) buffered with HEPES (pH = 7.05), suggest the formation of flower like supramolecular microfibers.

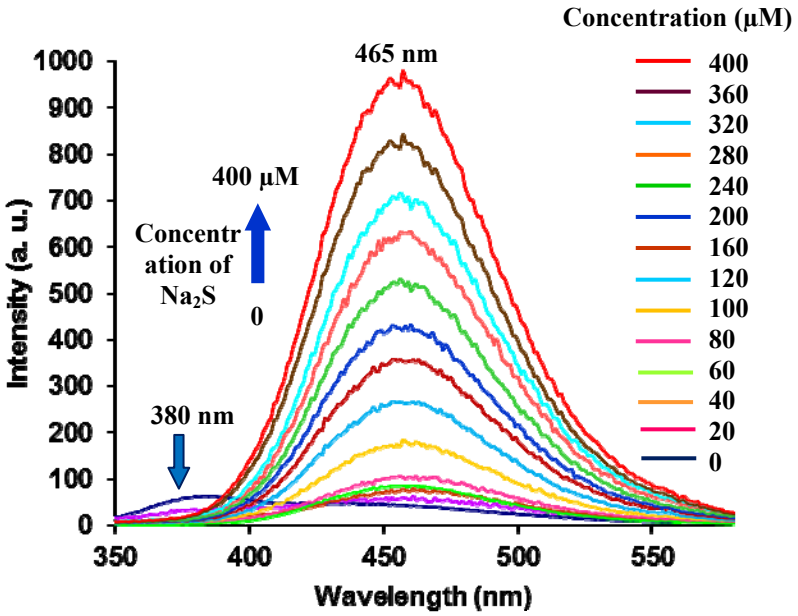


Fig. S2A Fluorescence spectra of **2** (5 μM in H_2O/DMSO (7:3, v/v), in response to various concentration of Na_2S (0-400 μM) in HEPES buffer (pH = 7.05) mixtures in 16 min, λ_{ex} = 300 nm.

Regression Statistics	
Multiple R	0.974105
R Square	0.950316

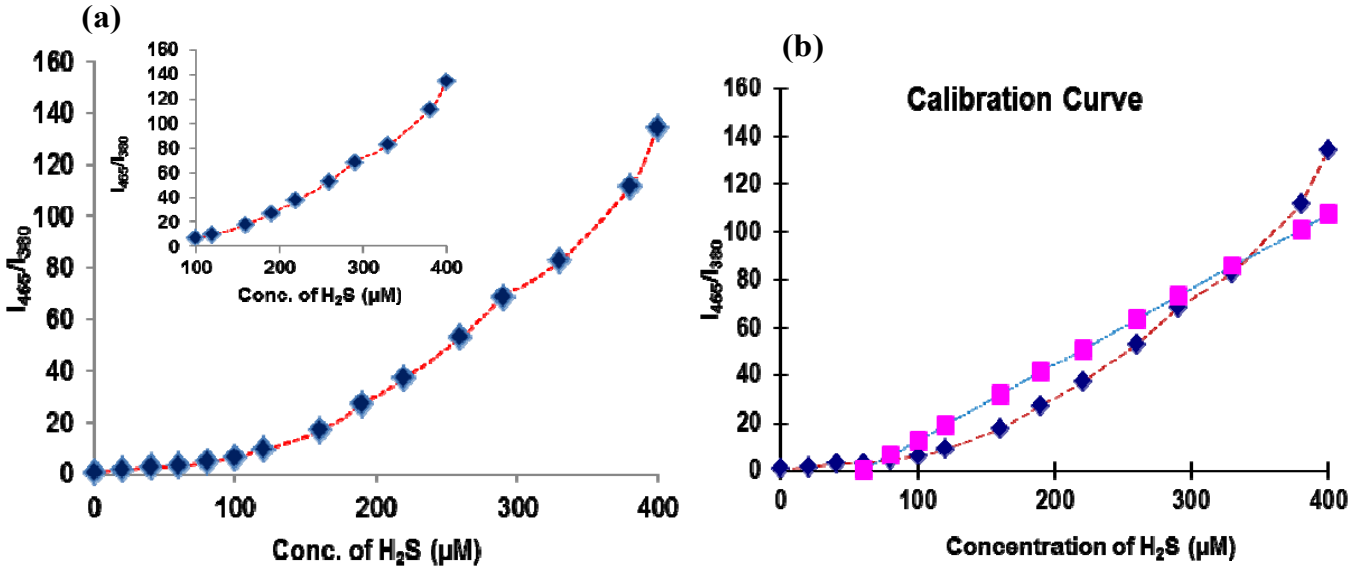
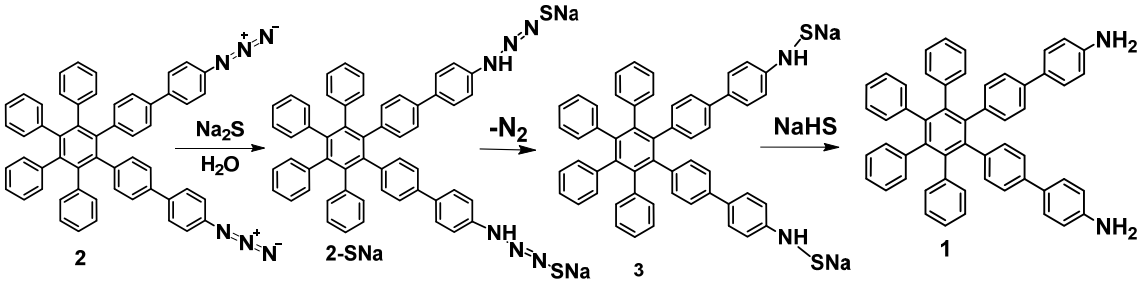


Fig. S2B (a) Change in fluorescence intensity ratio (I_{465}/I_{380}) of **2** (5 μM) in H_2O/DMSO (7:3, v/v) with the increasing concentration of Na_2S (0-400 μM) in HEPES buffer (pH = 7.05) mixtures in 15 min, λ_{ex} = 300 nm; inset of showing the sharp linear increase in intensity above 100 μM; (b) Calibrated curve showing the regression of a.



Scheme S2. Probable mechanistic pathway of reduction of probe **2** in presence of H_2S.

The probable mechanism of the reduction of probe **2** can be examined by varying the amount of Na_2S added and change in optical behaviour. When 50 μM of Na_2S solution was added, all the azido groups should theoretically be reduced to amino groups, which should make the aggregates of **1** highly emissive. But, in present investigation, only quenching of the band at 380 nm along with the appearance of band at 450 nm was observed but not increases even after 16 min, until more than 50 μM of Na_2S was added. Thus, probe **2** could probably be first transformed to a non-emissive intermediate **2-SNa** in presence of only 50 μM of Na_2S, which could be further converted to intermediate **3** through the elimination of N_2 and finally the reduced product **1** was obtained upon addition of >100 μM of Na_2S. Further, in the fluorescence spectrum, the bathochromic shift along with enhancement in the emission intensity at 465 nm is attributed the formation of reduced product **1** which undergo aggregation in aqueous media due to AIEE phenomena. The above results suggest the formation of supramolecular aggregates of **1** in presence of H_2S which restrict the intramolecular rotations of the molecules, induce planarization and makes the molecules more rigid and highly emissive.

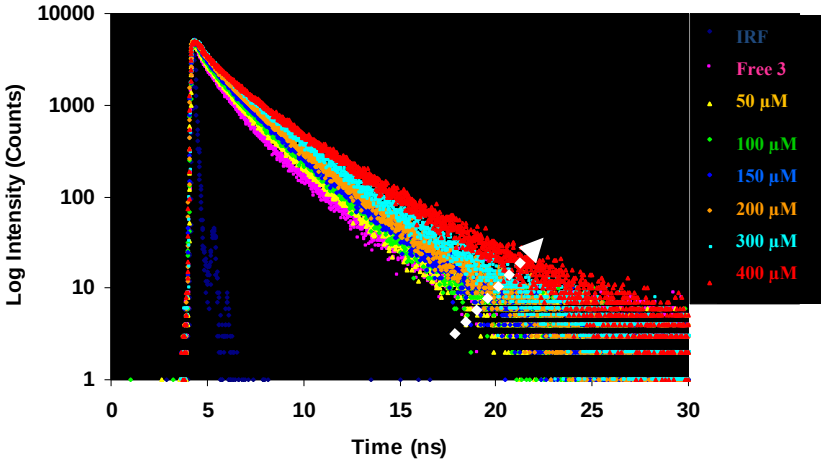


Fig. S3 Exponential fluorescence decays of **2** on addition of Na_2S in HEPES buffer (0-400 µM) measured at 460 nm. Spectra were acquired in H_2O/DMSO (7:3, v/v) mixture buffered with HEPES, pH = 7.05, λ_{ex}= 377 nm.

Na_2S in HEPES (µM)	**Quantum Yield (Φ_f)**	**A_1/A_2**	**τ_1 (ns)**	**τ_2 (ns)**	**τ_{avg} (Average lifetime, ns)**	**k_f (10^9 S^{-1})**	**k_{nr} (10^9 S^{-1})**
0	0.086	90/10	0.72	2.54	1.06	0.081	0.86
400	0.65	20/80	1.03	5.34	4.98	0.13	0.07

Table S2 Fluorescence lifetime of derivative **2** in absence and presence of Na_2S in HEPES buffer (0-400 µM) measured at 465 nm. **A_1**, **A_2:** fractional amount of molecules in each environment. **τ_1, τ_2** and **τ_{avg}:** bi-exponential and average life time of aggregates of **2** in 70 vol% of water in DMSO; **k_f:** radiative rate constant ($k_f = \Phi_f/\tau_{avg}$); **k_{nr}:** non-radiative rate constant ($k_{nr} = (1- \Phi_f)/\tau_{avg}$); λ_{ex}= 377 nm.

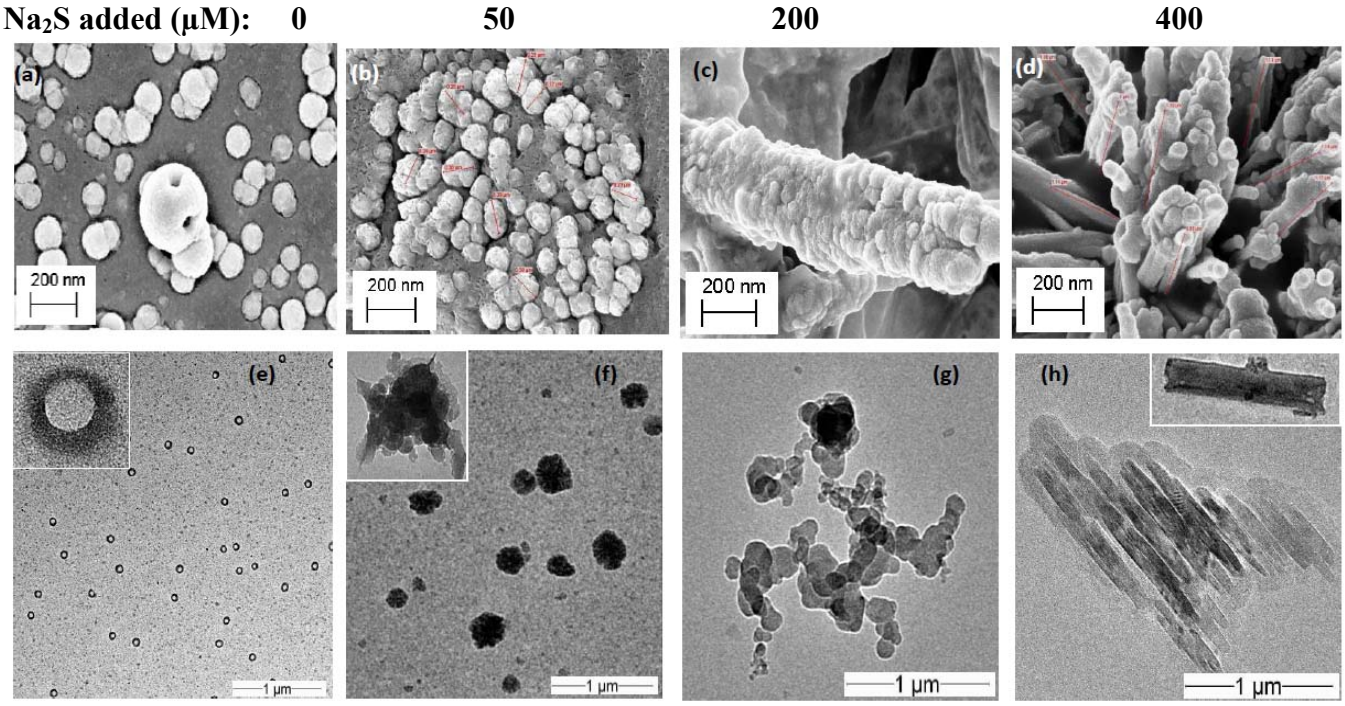
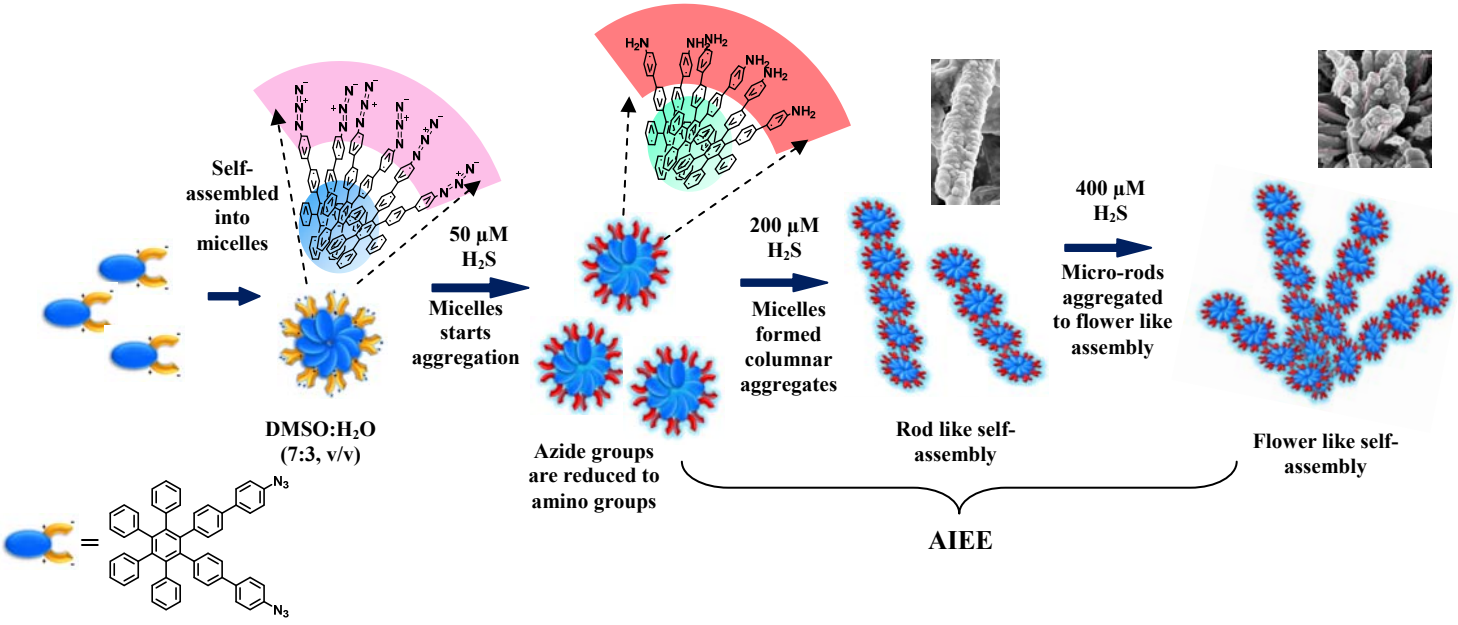
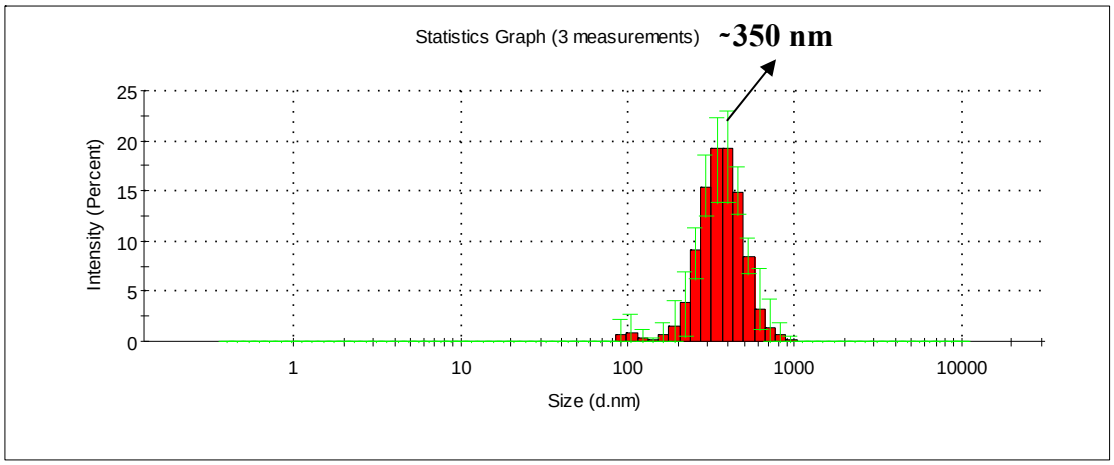


Fig. S4 SEM (a-d) and TEM (e-f) images showing (a & e) spherical aggregates of derivative **2** in H_2O/DMSO (7:3, v/v); After addition of H_2S at various concentration: (b & f) after 50 μM Na_2S addition self-assembled spherical microspheres, (c & g) after 200 μM Na_2S addition rods or chain by self-assembled microspheres and (d & h) self-assembly of micro-rod after the addition of 400 μM Na_2S in the solution of **2** in H_2O/DMSO (7:3, v/v), suggest the formation of more packed supramolecular aggregates; inset of e, f and h showed the expanded tem images.

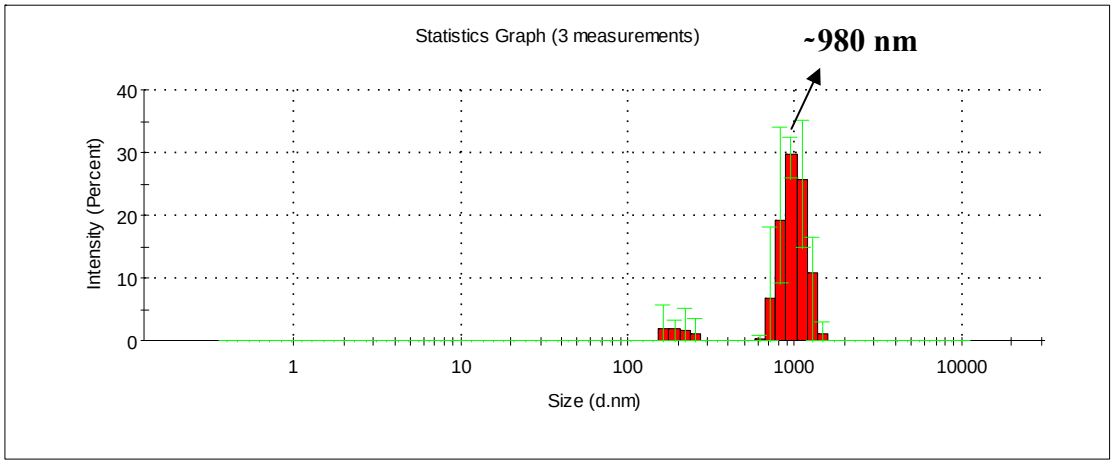


Scheme S3. Probable schematic diagram show H_2S sensing mechanism with tuneable self-assembly and AIEE behaviour.

(A) Aggregates of **2**



(B) Aggregates of **2** after 200 μM Na_2S addition



(C) Aggregates of **2** after 400 μM Na_2S addition

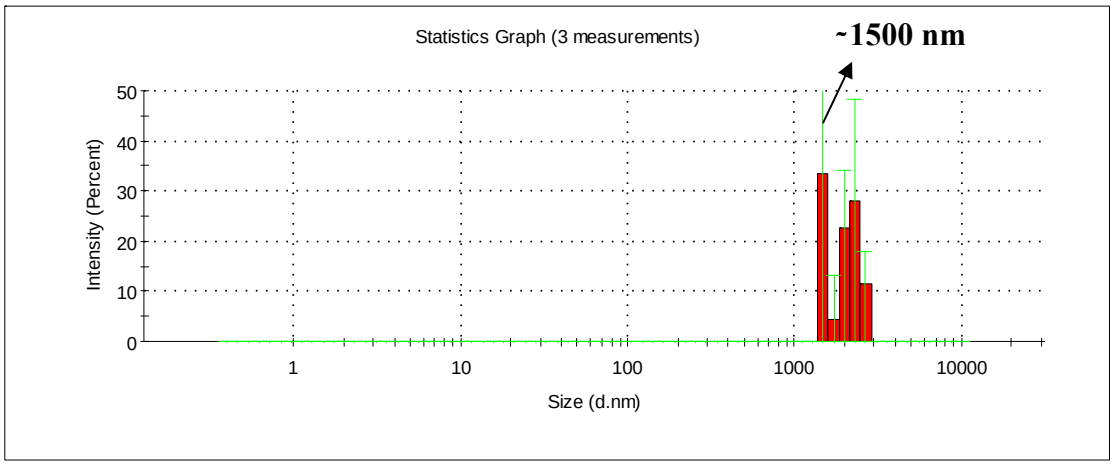


Fig. S5 Dynamic light scattering (DLS) results showing the variation in particle size diameter before (A) and after the addition of 200 μM (B) and 400 μM Na_2S (C) in the aggregates of **2** in H_2O/DMSO (7:3, v/v) mixture.

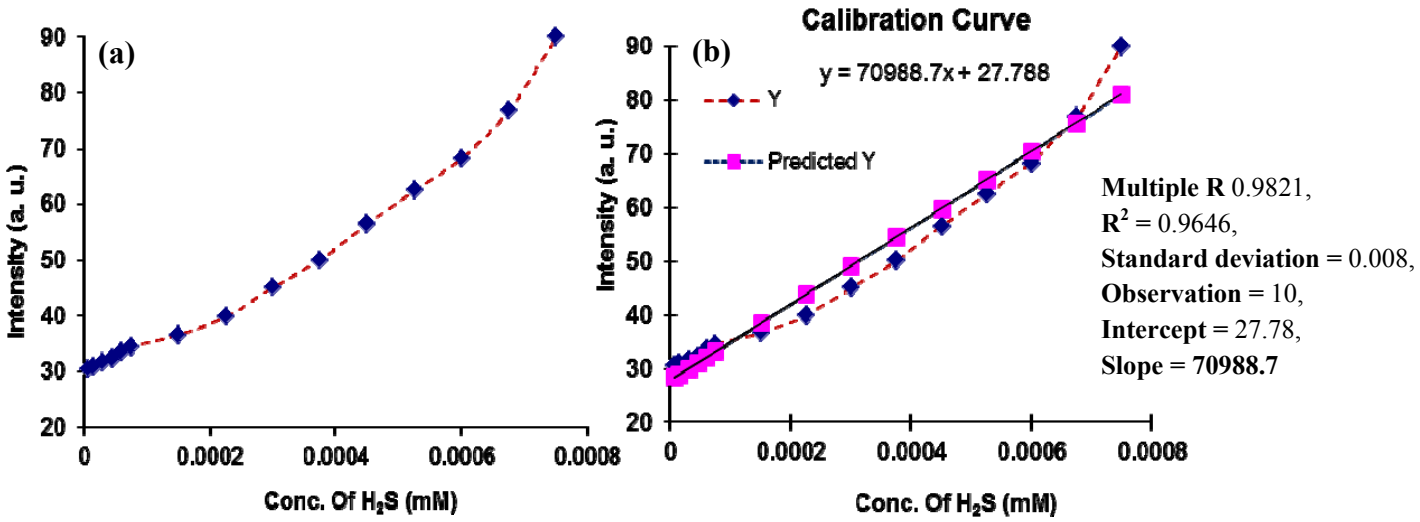


Fig. S6 (a) Showing the fluorescence intensity of compound **2** in H_2O/DMSO (7:3, v/v) buffered with HEPES and (b) Calibrated curve showing the fluorescence intensity of compound **2** at 465 nm as a function of Na_2S concentration (M) in HEPES buffer, pH = 7.05, λ_{ex}= 300 nm.

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of receptor **2** without H_2S was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit is then calculated with the following equation:

DL = 3 × SD/S

Where SD is the standard deviation of the blank solution measured by 10 times; S is the slope of the calibration curve.

From the graph we get slope

S = 261364.3, and SD value is 0.006

Thus using the formula we get the Detection Limit **(DL) = 3 × 0.008/70988.7 = 33.8 × 10^{-8} M = 338 nM = 0.33 µM.**

i.e. probe **2** can detect H_2S in this minimum concentration through fluorescence method.

S.No	Exact concentration of H_2S added (in µM)	Fl Intensity at 465 nm (Y) (in a.u.)	Determination of H_2S concentration (in µM) by using probe 2 (from linear calibration curve and apply equation (X): Conc.= (Y-27.78)/70988.7
1	0.4 (0.0004 mM)	56	3.97×10^{-4} mM = 0.397 µM
2	0.5	63	4.96×10^{-4} mM = 0.496 µM
3	0.6	70	5.94×10^{-4} mM = 0.594 µM
4	0.7	77	6.93×10^{-4} mM = 0.693 µM

Table S3 Results of quantitative determination of H_2S by using aggregates of derivative **2**.

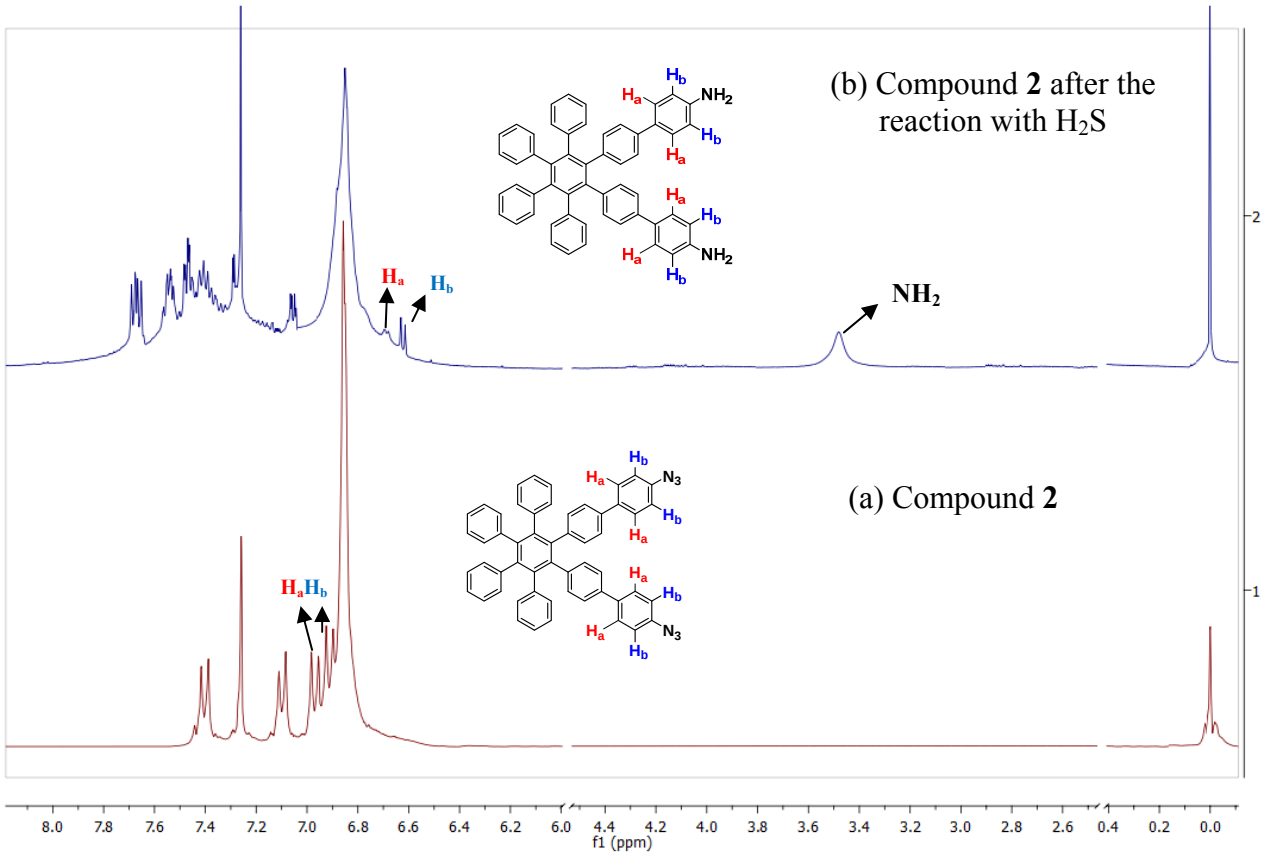


Fig. S7 Overlay of ^{1}H NMR spectrum of compound **2** in $CDCl_3$ (a) and the product after the reaction of **2** with H_2S (b).

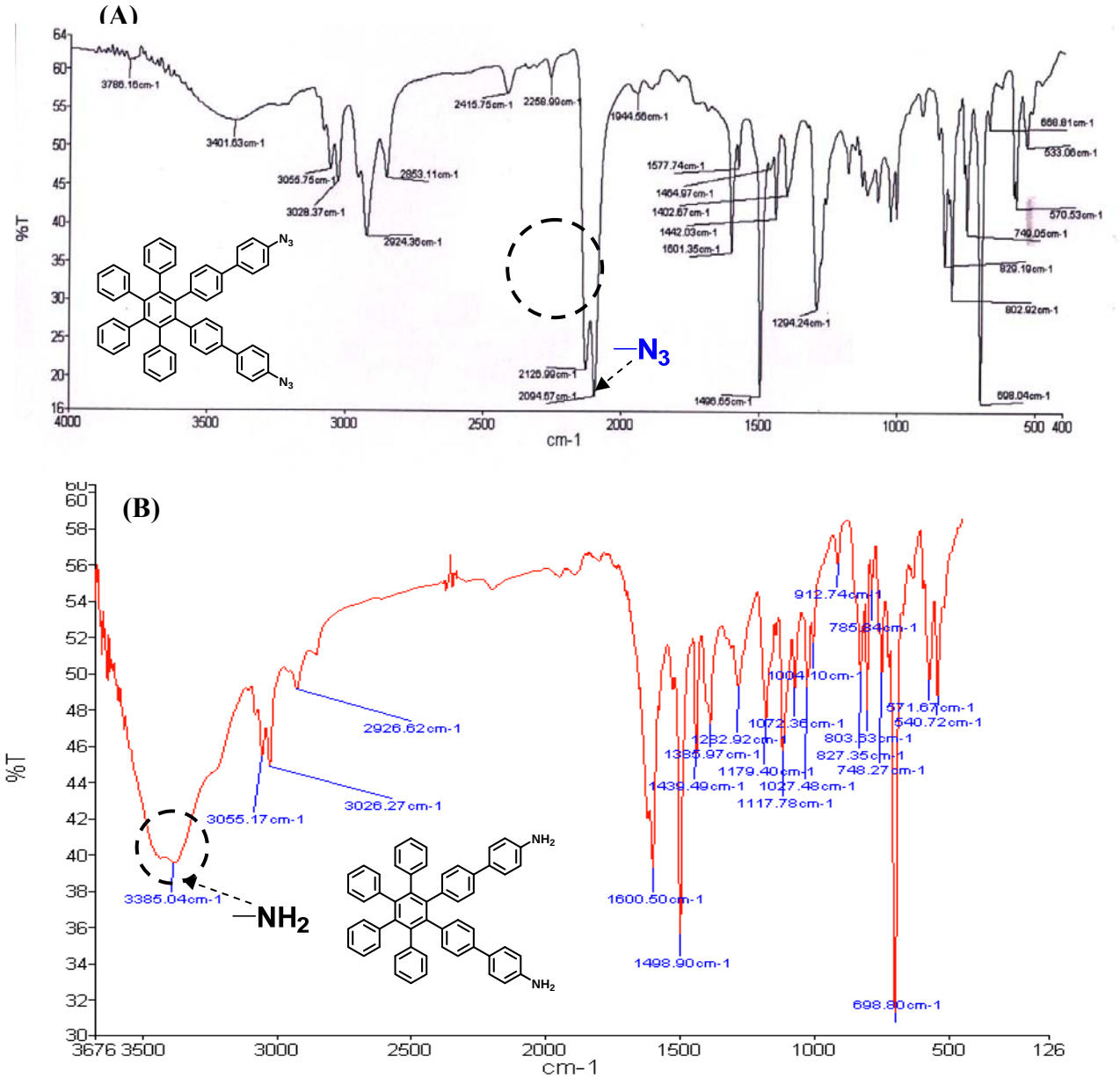


Fig. S8 FT-IR Spectrum of compound **2** (A) before and (B) after the reaction with H_2S.

Regression Statistics	
Multiple R	0.9998
R Square	0.998
Intercept	0.234
Slope	0.0104

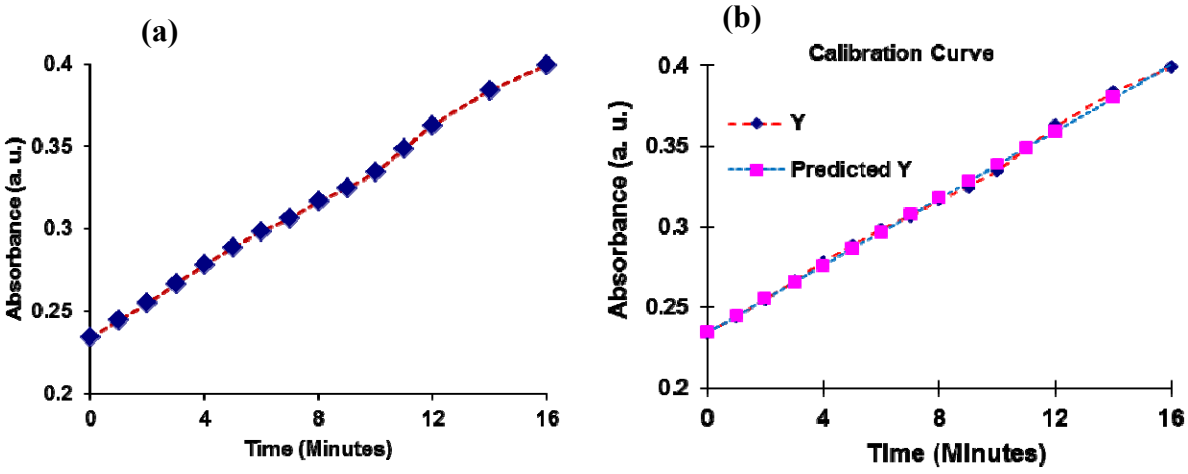


Fig. S9 Graphical representation of the reduction rate of compound **2** to compound **1** in presence of Na_2S. (a) Time (min.) vs. absorbance plot at 340 nm (b) regression plot of a.

The first order rate constant for the formation of compound **1** was calculated from the changes of intensity of absorbance of derivative **2** in response of Na_2S in HEPES buffer at different time interval.

From the time vs. absorbance plot at fixed wavelength 300 nm by using first order rate equation we get the rate constant = k = slope×2.303 = 0.0104×2.303 = **2.39×10^{-2} min^{-1}**.

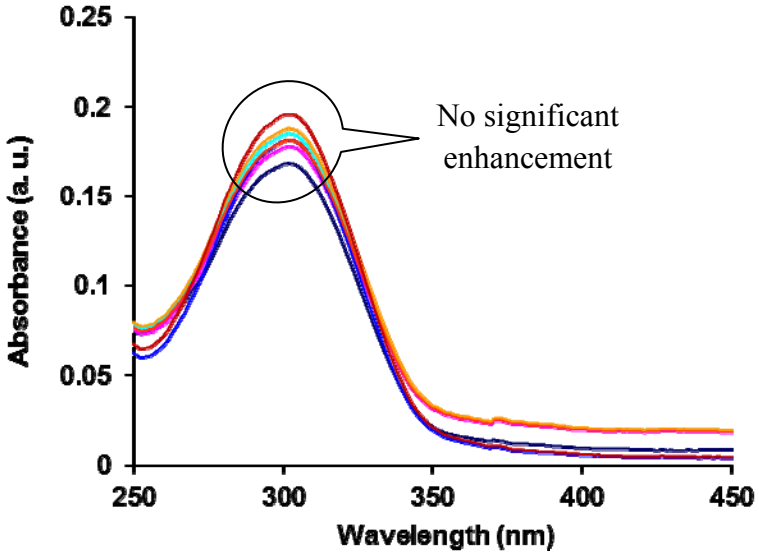


Fig. S10 UV-vis spectrum showing the change in absorbance of compound **2** (5 μM) in Water/ DMSO mixture (0 to 70% volume fraction of water in DMSO).

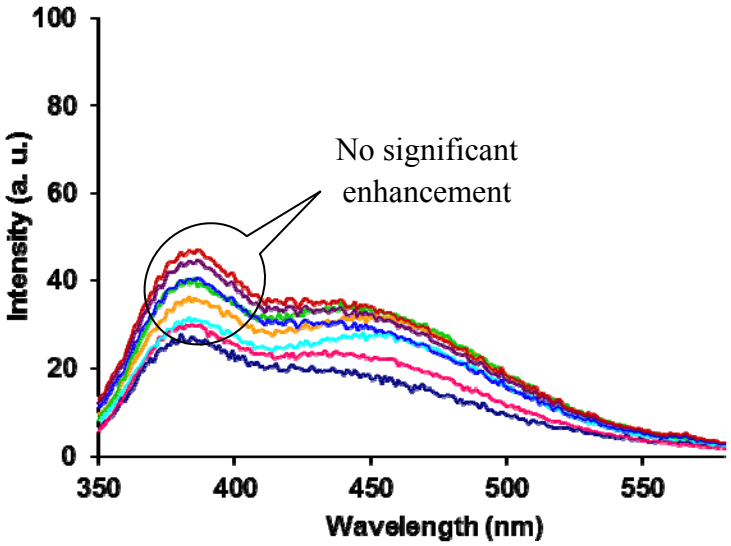


Fig. S11 Fluorescence spectra of compound **2** (5 μM) showing the variation of fluorescence intensity in Water/DMSO mixture (0 to 70% volume fraction of water in DMSO); λ_{ex}= 300 nm.

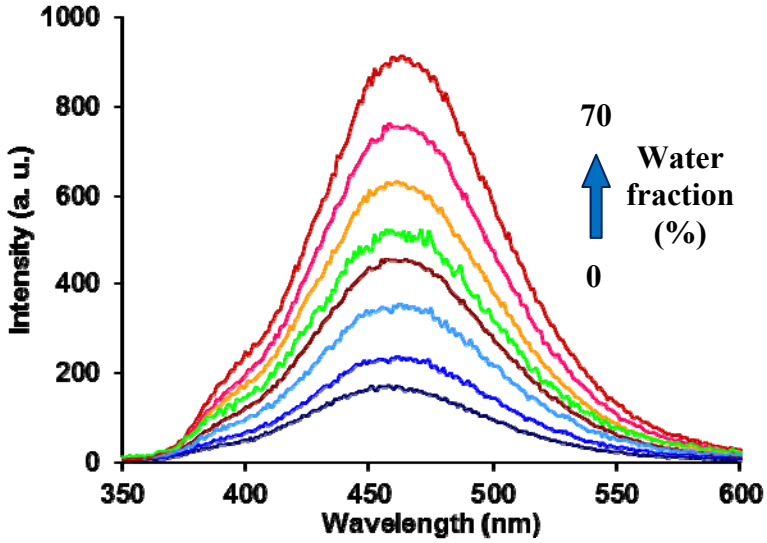


Fig. S12 Fluorescence spectra of compound **1** (5 μM) showing the variation of fluorescence intensity in Water/DMSO mixture (0 to 70% volume fraction of water in DMSO); λ_{ex}= 300 nm.

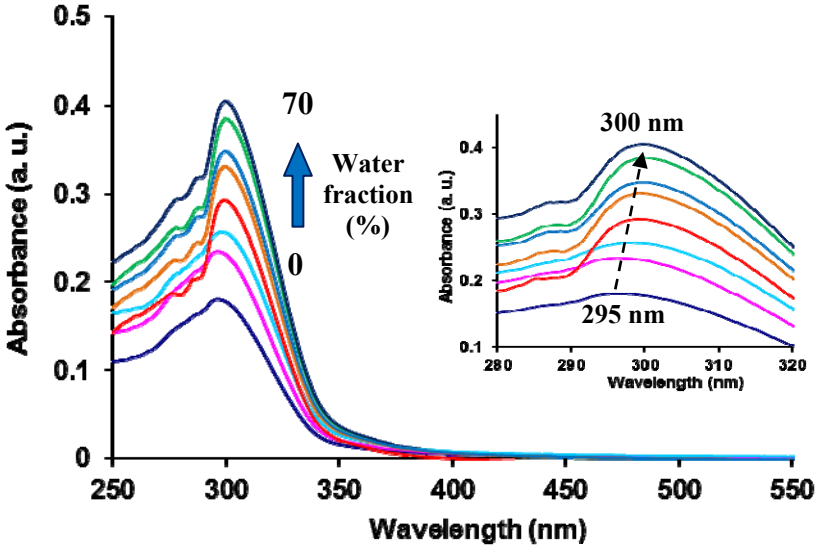


Fig. S13 UV-vis spectrum showing the change in absorbance of compound **1** (5 μM) in Water/ DMSO mixture (0 to 70% volume fraction of water in DMSO); inset showing the 5 nm red shift of absorbance band from 295 to 300 nm

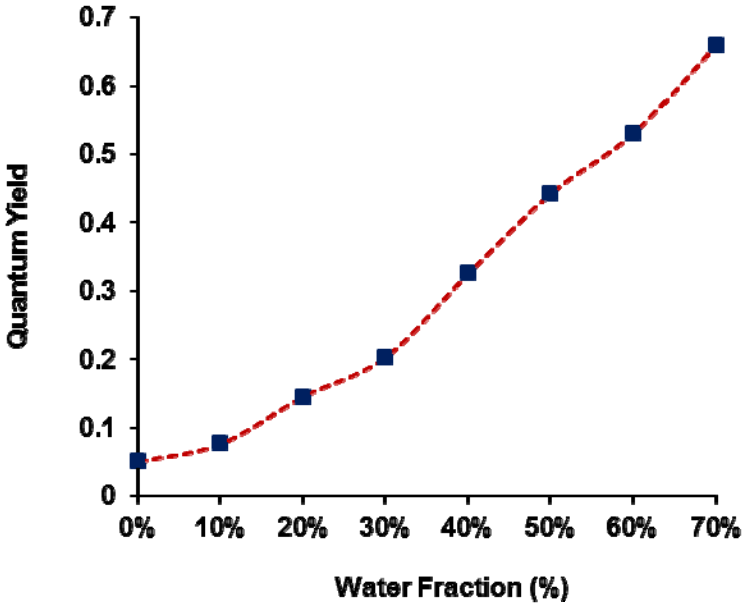


Fig. S14 Variation in quantum yield value of **1** (5 μM) with the variation of water fractions (0 to 70% volume fraction of water in DMSO); λ_{ex}= 300 nm.

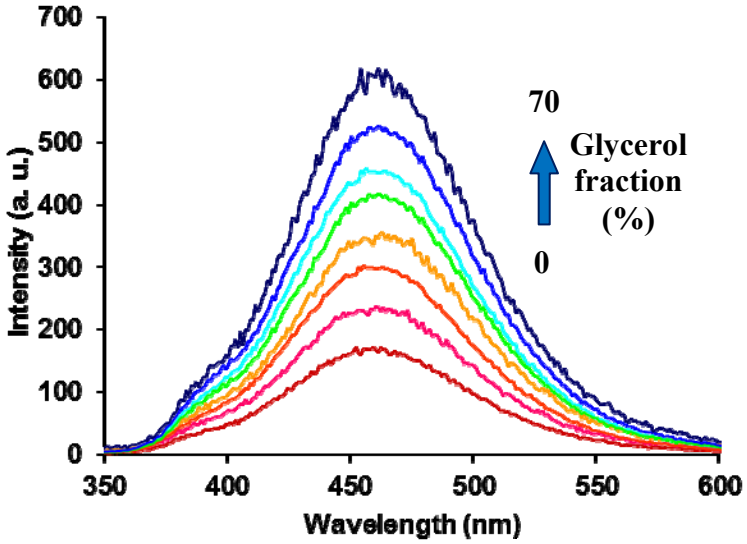


Fig. S15 Fluorescence spectra of compound **1** (5 μM) showing the variation of fluorescence intensity in Glycerol/DMSO mixture (0 to 70% volume fraction of glycerol in DMSO); λ_{ex}= 320 nm.

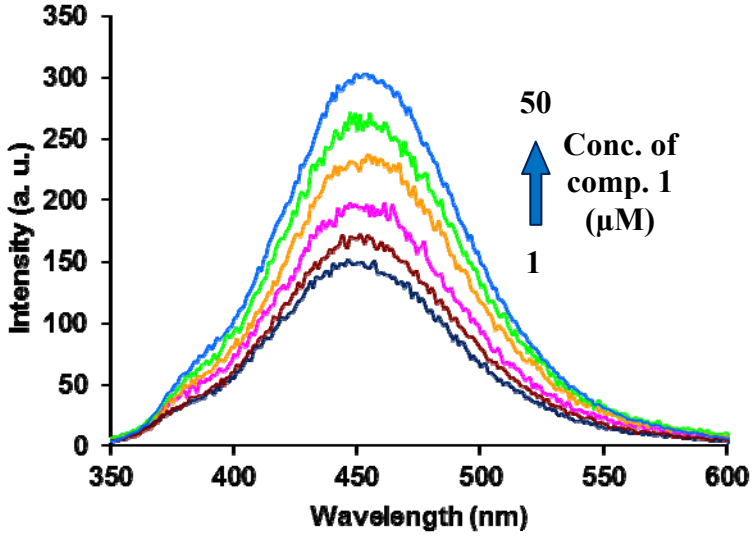


Fig. S16 Fluorescence spectra of compound **1** showing the variation of fluorescence intensity with different concentration of **1** (1μM - 20μM) in DMSO; λ_{ex}= 300 nm.

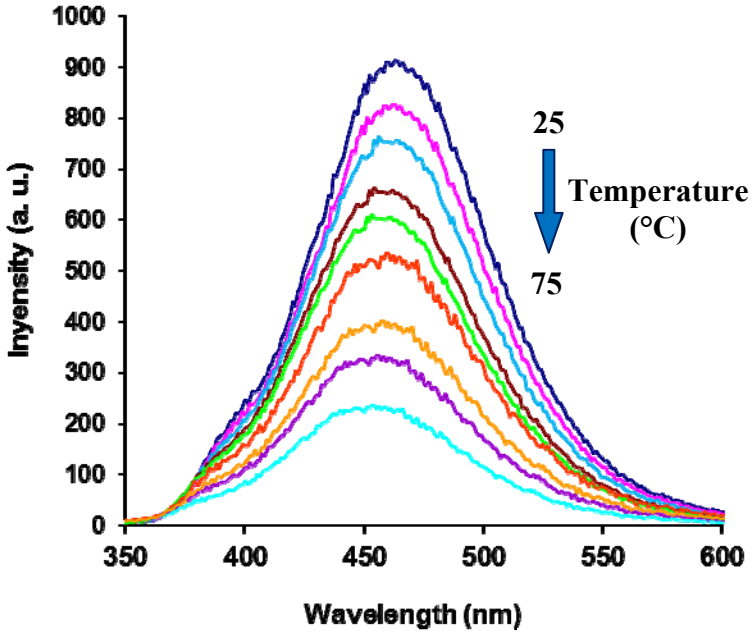


Fig. S17 Fluorescence spectra of derivative **1** (5 μM) showing the variation of fluorescence intensity with increase in temperature (25-75 °C) in H_2O/DMSO mixture (7:3, v/v). λ_{ex}= 300 nm.

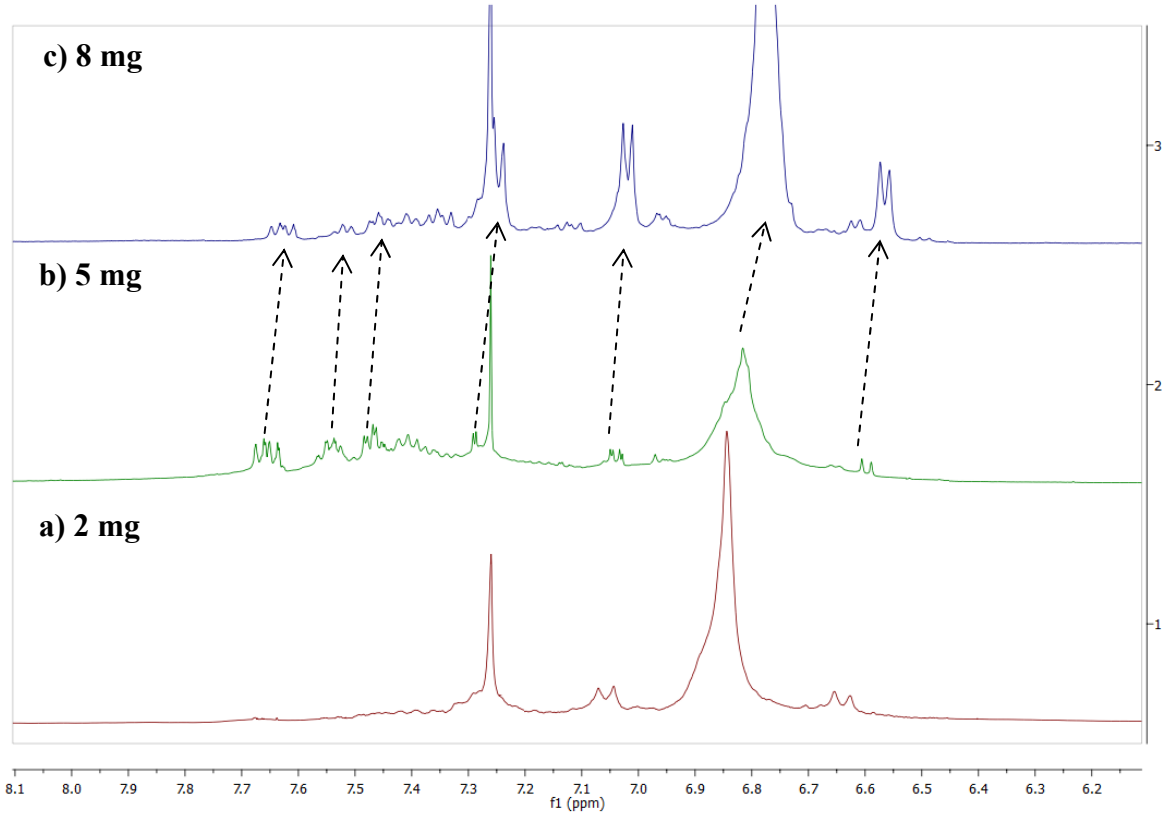


Fig. S18 Concentration dependent ^{1}H NMR spectra of compound **1** in 600µl $CDCl_3$; a) 2 mg; b) 5 mg; c) 8 mg.

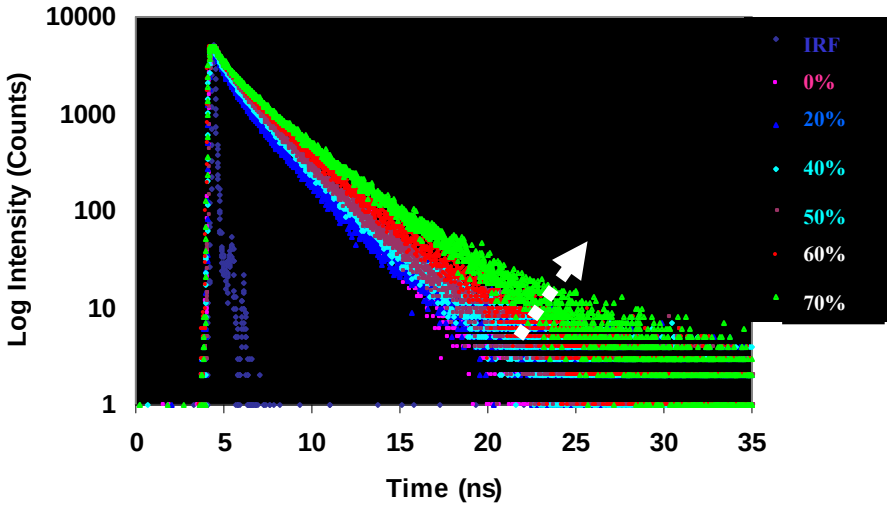


Fig. S19 Exponential fluorescence decays of **1** on addition of water fraction measured at 465 nm. Spectra were acquired in Water/DMSO mixtures (0 to 70% volume fraction of water in Ethanol), λ_{ex}= 377 nm.

Water fraction %	**Quantum Yield (Φ_f)**	**A_1/A_2**	**τ_1 (ns)**	**τ_2 (ns)**	**τ_{avg} (Average lifetime, ns)**	**k_f (10^9 S^{-1})**	**k_{nr} (10^9 S^{-1})**
0	0.08	75/25	0.68	1.2	0.86	0.09	1.07
70	0.66	10/90	1.2	6.24	5.06	0.13	0.067

Table S4 Fluorescence lifetime of derivative **1** in absence and presence of 70% water fraction in DMSO at 465 nm. **A_1, A_2:** fractional amount of molecules in each environment. **τ_1, τ_2** and **τ_{avg}:** bi-exponential and average life time of aggregates in 70 vol% of water in DMSO; **k_f:** radiative rate constant ($k_f = \Phi_f/\tau_{avg}$); **k_{nr}:** non-radiative rate constant ($k_{nr} = (1- \Phi_f)/\tau_{avg}$); λ_{ex}= 377 nm.

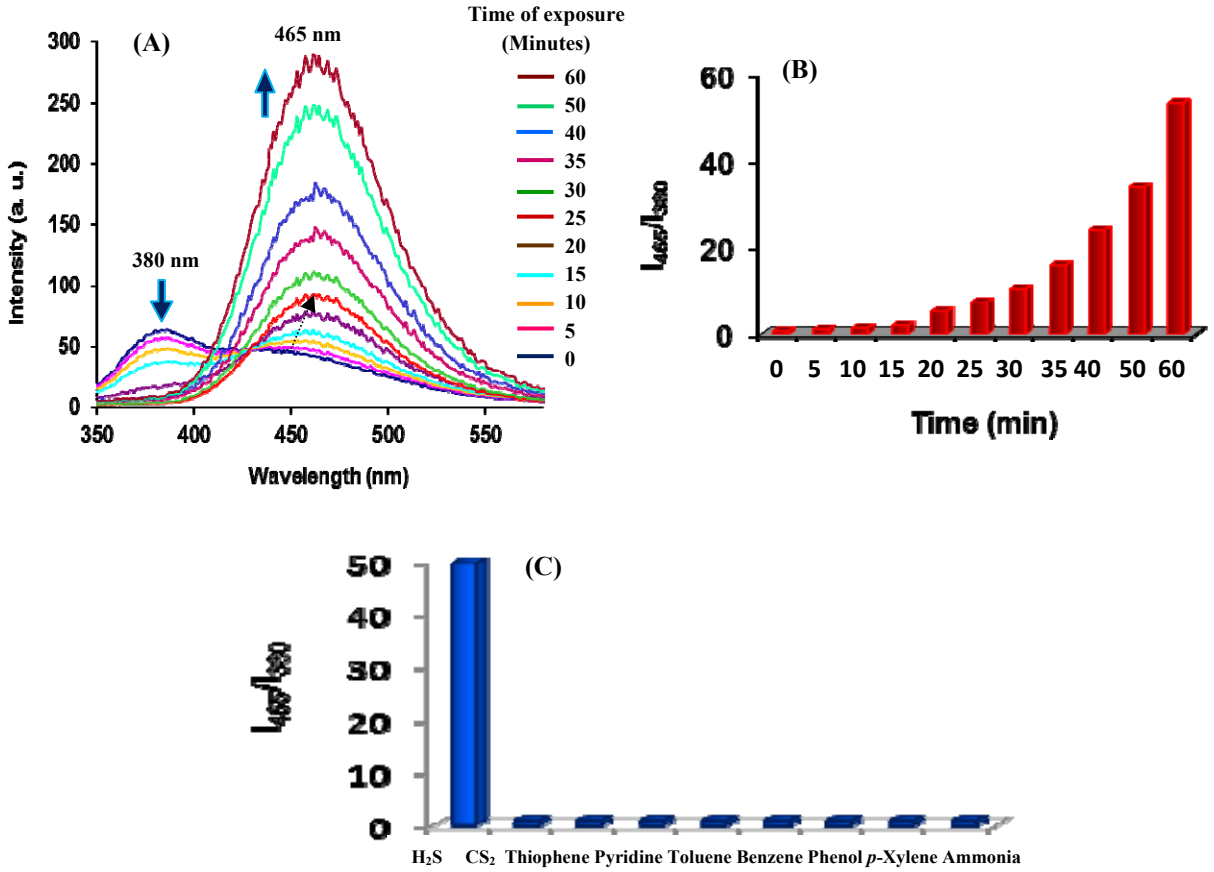


Fig. S20 The change in fluorescence spectra of **2** (5 μM) in H_2O/DMSO (7:3, v/v) on exposing to the vapors of solid Na_2S after 0, 5, 10, 15, 20, 40 and 60 min at λ_{ex} = 300 nm; (B) Showing 6 fold (30%) fluorescence enhancement of **3** in response to vapour of H_2S (0-60 minutes); (C) Showing the selectivity bar diagram in response to vapour of various unpleasant odour of sulphur containing compounds within 60 minutes.

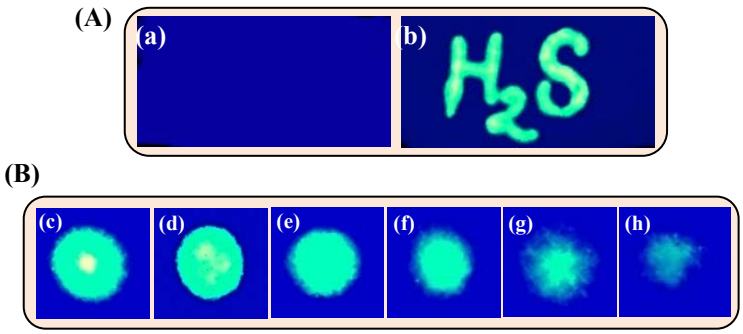


Fig. S21 (A) Fluorescence change under the UV lamp at 365 nm of paper strips coated with chemosensor **2** in response to Na_2S in HEPES buffer (10^{-2} M) after 16 min; (a) blank, (b) after sprayed onto the strip by writing "H_2S" (10^{-2} M) and dried in oven; (B) Change in fluorescence of **2** coated paper strip on addition of different concentration of Na_2S in HEPES buffer (c) 10^{-2} M, (d) 10^{-3} M, (e) 10^{-4} M, (f) 10^{-5} M, (g) 10^{-6} M, (h) 10^{-7} M.

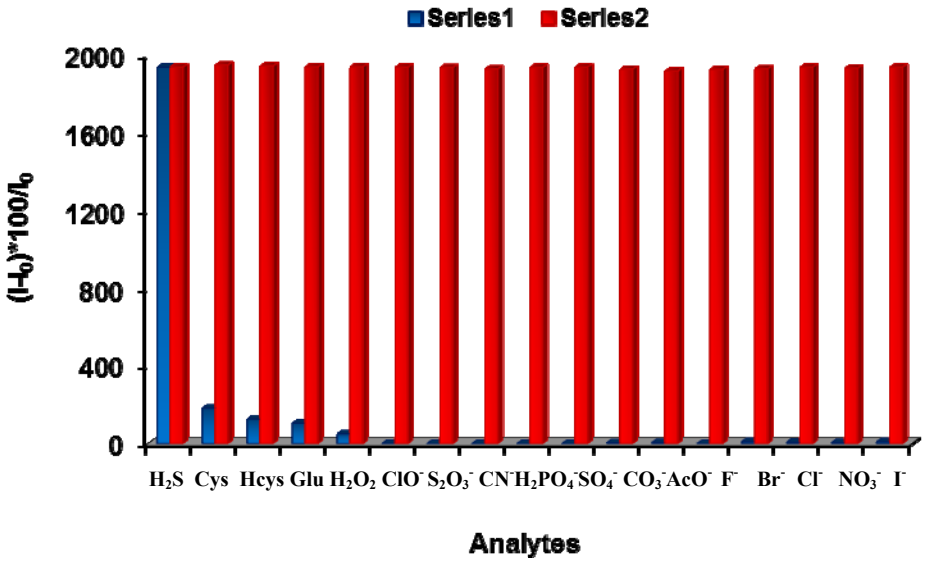


Fig. S22 Fluorescence response of **2** (5.0 μM) to various analytes (400 μM) in H_2O/DMSO (7:3, v/v) buffered with HEPES (pH = 7.05) pH = 7.05; λ_{ex} = 300 nm. Bars represent the increase in percentage of emission intensity at 465 nm. (Series a) Blue bars represent selectivity of **2** upon addition of different analytes (400 μM); (Series b) Red bars represent competitive selectivity of receptor **2** towards of Na_2S in HEPES buffer (400 μM in the presence of other analytes 400 μM).

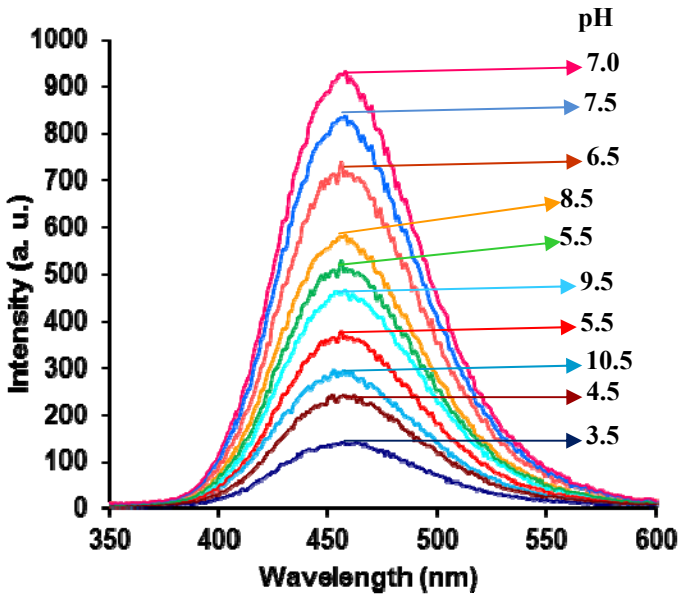


Fig. S23 pH dependent fluorescence spectra of **2** (5 μM), after incubation with 60 μM Na_2S in HEPES buffer for 15 min in H_2O/DMSO (7:3, v/v) buffered with HEPES mixtures, λ_{ex} = 300 nm.

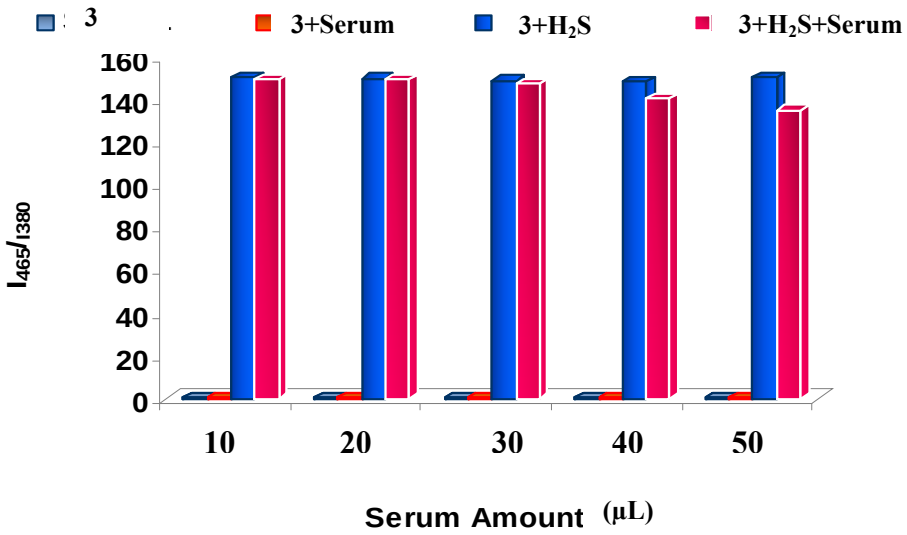


Fig. S24 (A) Histograms showing the fluorescence response of **2** (5 μM) with Na_2S (400 μM) in the presence of blood serum in H_2O/DMSO (7:3, v/v) at pH = 7.05, λ_{ex}= 300nm. The fluorescence change was observed with varying amounts (μL).

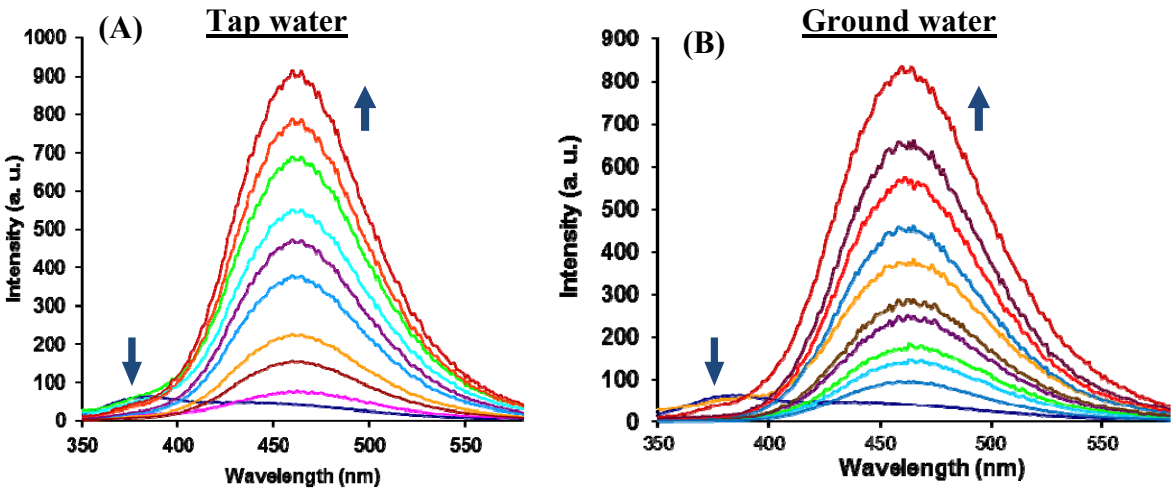


Figure S25 (A) Fluorescence spectra of compound **2** in response to H_2S (400 μM) in tap water, λ_{ex}= 300 nm; (B) Fluorescence spectra of compound **3** (5 μM) in response to H_2S (400 μM) in ground water, λ_{ex}= 300 nm.

Measurement of Two-Photon Cross Section Area of compound 2 before and after H_2S addition:

Compound[a]	$\lambda_{max}^{(1)}$ [b]	λ_{max}^{fl} [c]	Φ[d]	$\lambda_{max}^{(2)}$ [e]	$\delta\Phi$[f]
2	300	nd[g]	0.078	nd[g]	nd[g]
1	300	457	0.66	750	1.31

Table S5 a) All the measurements were performed in HEPES buffer (50 mM, pH 7.05)/DMSO (3:7, v/v). b) λ_{max} of the one-photon absorption spectra in nm. c) λ_{max} of the one-photon emission spectra in nm. d) Fluorescence quantum yield. e) λ_{max} of the two-photon excitation spectra in nm. f) Two-photon action cross-section in 10^{-50} cm^4s/photon (GM units). g) Not determined. The one- and two-photon excited fluorescence signals were too small to determine the values.

Measurement of Two-Photon Cross Section. The two-photon cross section (δ) was determined by using femto second (fs) fluorescence measurement technique as described.[1] **2** (10 μM) was dilluted in HEPES (50 mM, pH 7.05)/DMSO (3:7, v/v) mixture and the two-photon induced fluorescence intensity was measured at 710−880 nm by using rhodamine 6G as the reference, whose two-photon property has been well characterized in the literature.[2] The intensities of the two-photon induced fluorescence spectra of the reference and sample emitted at the same excitation wavelength were determined. The TPA cross section was calculated by using $\delta = \delta_r(S_s\Phi_r\phi_r c_r)/(S_r\Phi_s\phi_s c_s)$: where the subscripts *s* and *r* stand for the sample and reference molecules. The intensity of the signal collected by a CCD detector was denoted as *S*. Φ is the fluorescence quantum yield. ϕ is the overall fluorescence collection efficiency of the experimental apparatus. The number density of the molecules in solution was denoted as *c*. δ_r is the TPA cross section of the reference molecule.

References

[1]. S. K. Lee, W. J. Yang, J. J. Choi, C. H. Kim, S. J. Jeon and B. R. Cho, *Org. Lett.,* 2005, **7**, 323-326.

[2] N. S. Makarov, M. Drobizhev and A. Rebane, *Optics Express*., 2008, **6**, 4029-4047.

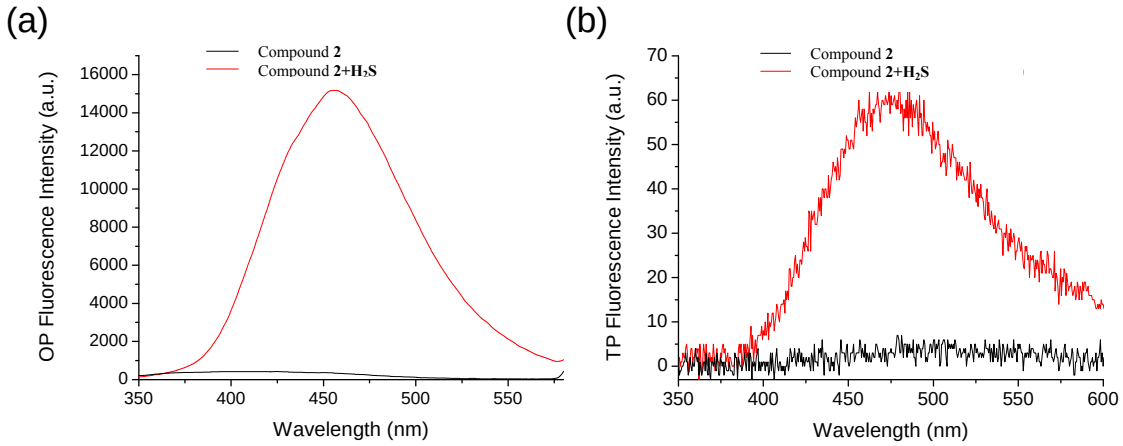


Fig. S26 Fluorescence spectra of **2** (10 μM) before (black lines) and 30 min after (red lines) addition of 1 mM Na_2S in HEPES (50 mM, pH 7.05)/DMSO (3:7, v/v). (a) one-photon fluorescence spectra with excitation at 300 nm. (b) two-photon fluorescence spectra with excitation at 710 nm.

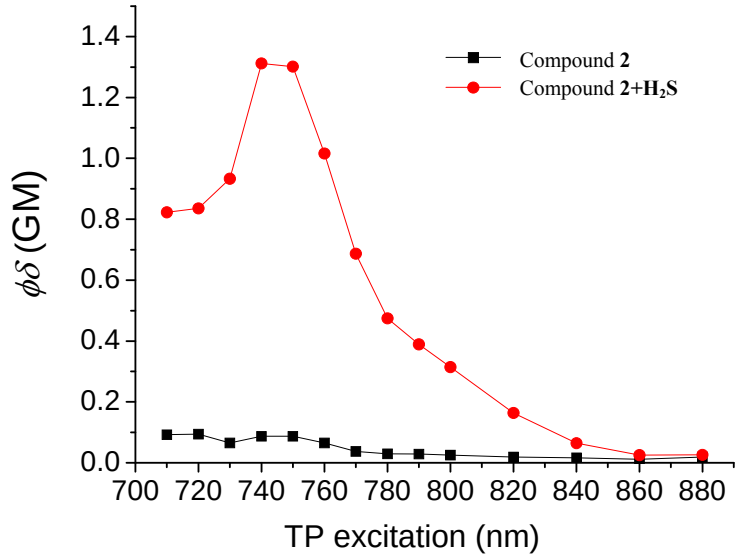


Fig. S27 Two-photon action spectra of 10 μM **2** (black dots) and 10 μM **1** (red dots) in HEPES (50 mM, pH 7.05)/DMSO (3:7, v/v). The estimated uncertainties for the two-photon action cross section values ($\delta\Phi$) are ± 15%.

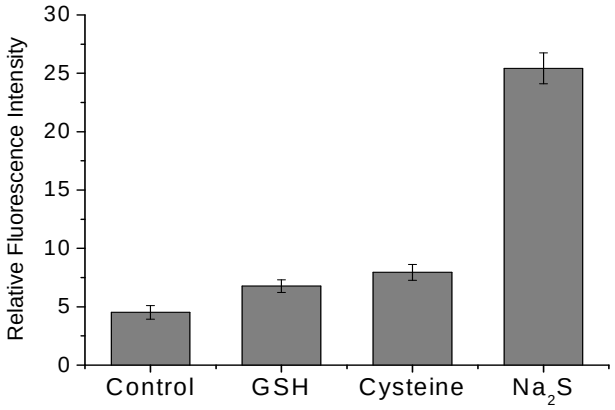


Fig. S28 Average TPEF intensities in (a-d, Fig. 4). The TPM images were obtained by collecting the TPEF at 400–600 nm upon excitation at 710 nm with femtosecond pulses. Cells shown are representative images from replicate experiments (n = 3). Scale bar: 20 μm.

Fig. S29 ^{1}H NMR Spectra ($CDCl_3$, 300 MHz, ppm) of compound 2:

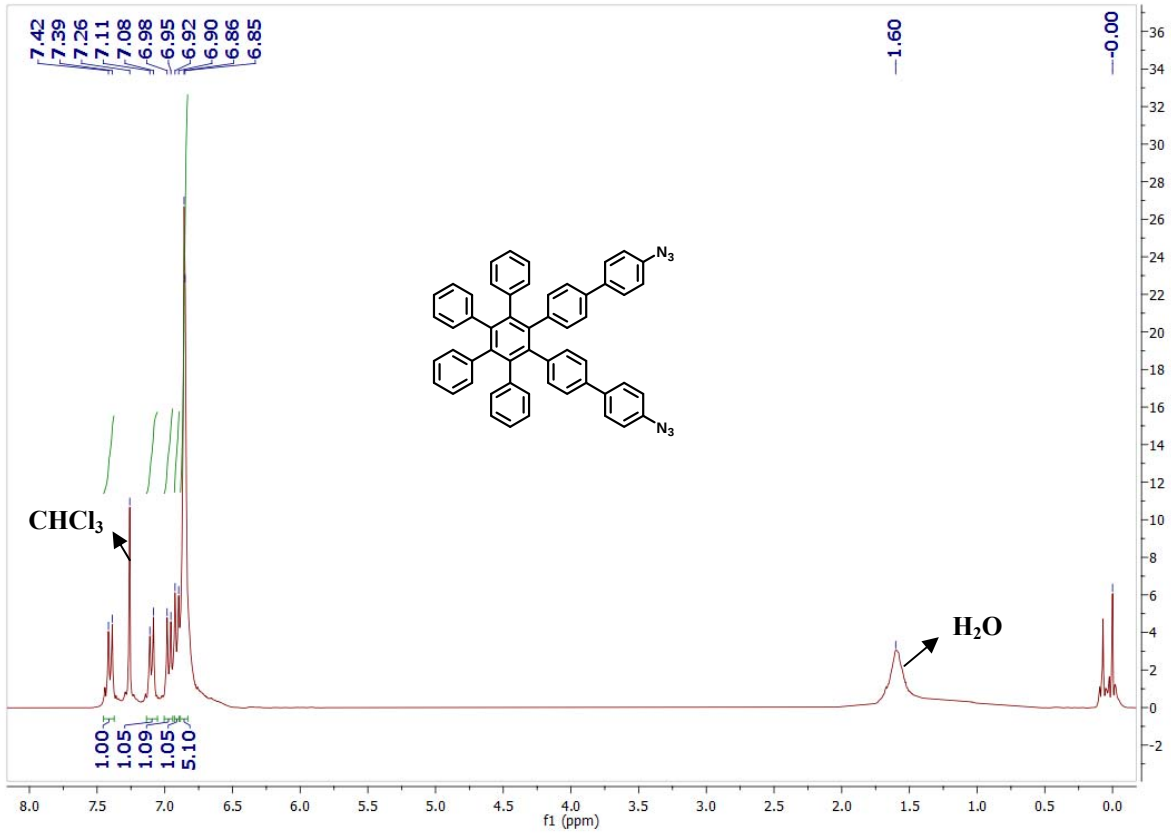


Fig. S30 Expanded ^{1}H NMR Spectra ($CDCl_3$, 300 MHz, ppm) of compound 2:

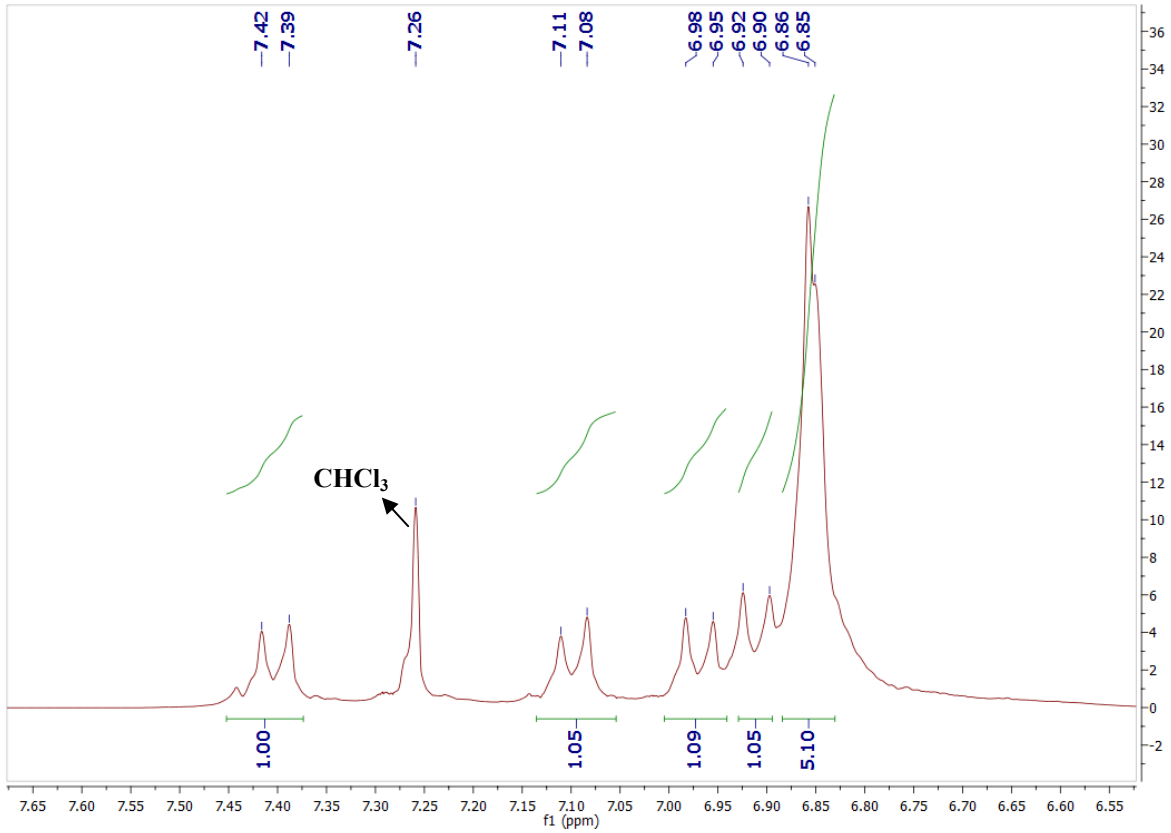


Fig. S31 ^{13}C NMR Spectra ($CDCl_3$, 300 MHz, ppm) of compound 2:

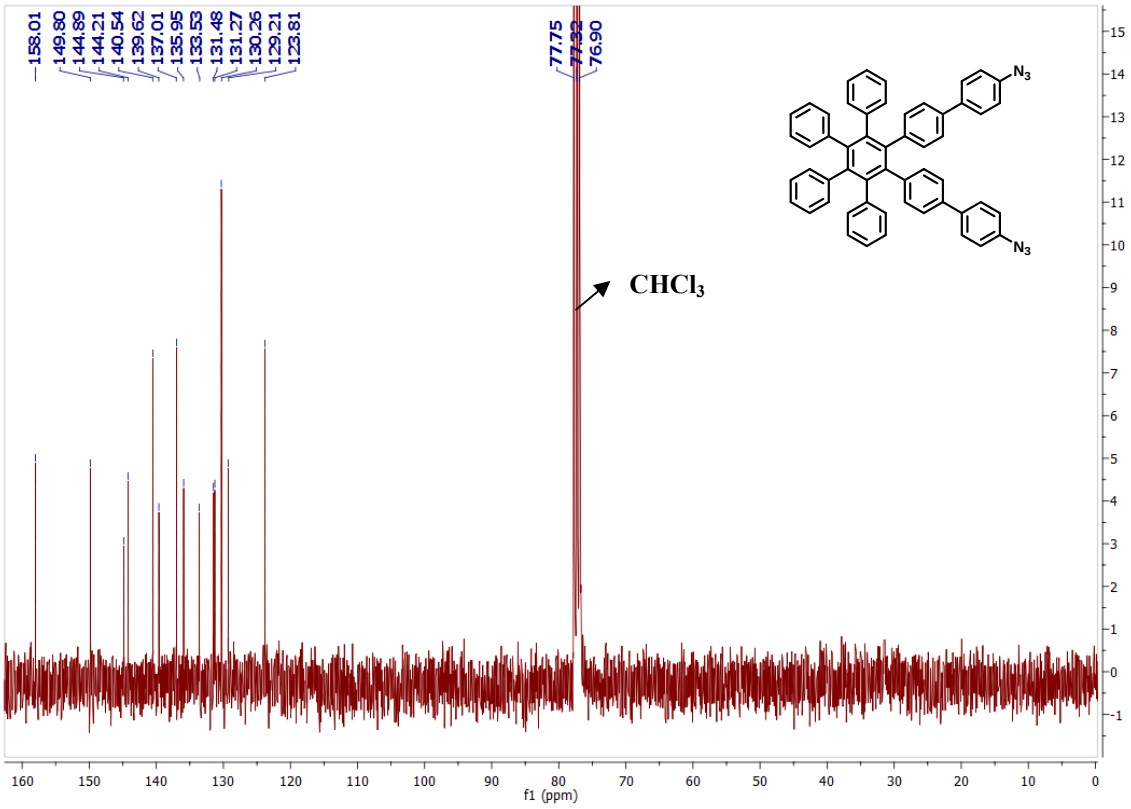


Fig. S32 Mass Spectrum (ESI-MS) of compound 2:

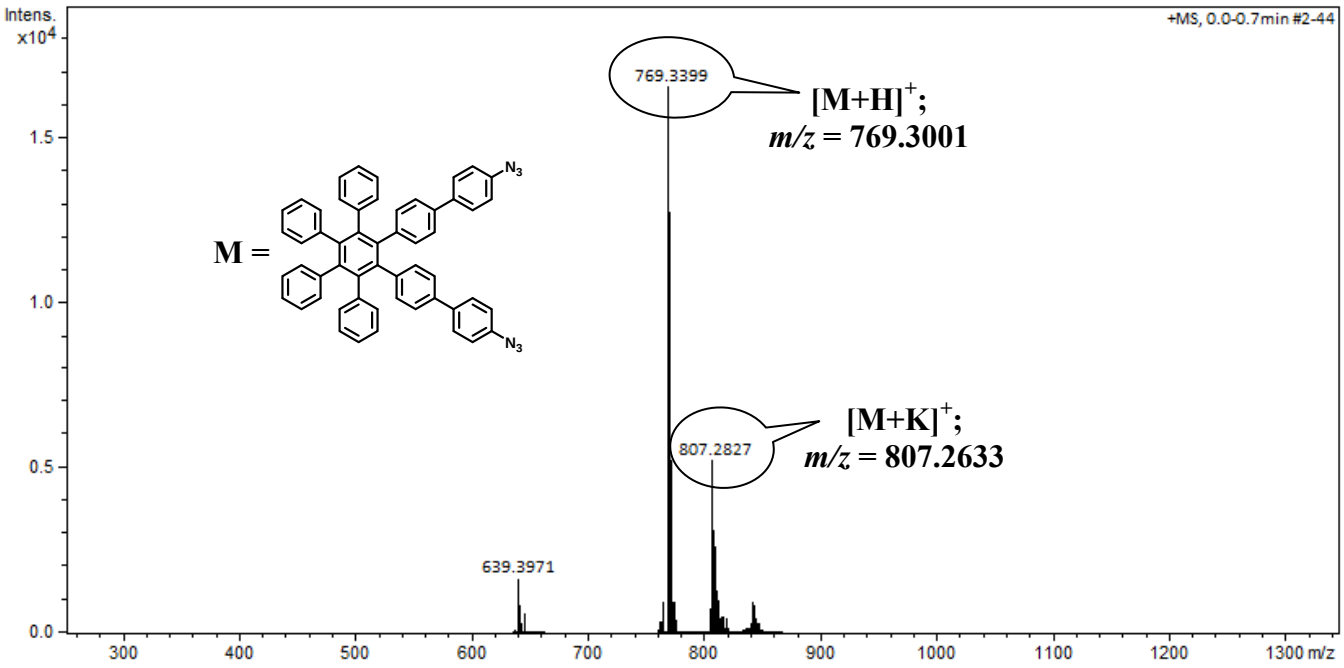


Fig. S33 ^{1}H NMR spectrum of product after the reaction of 2 with H_2S:

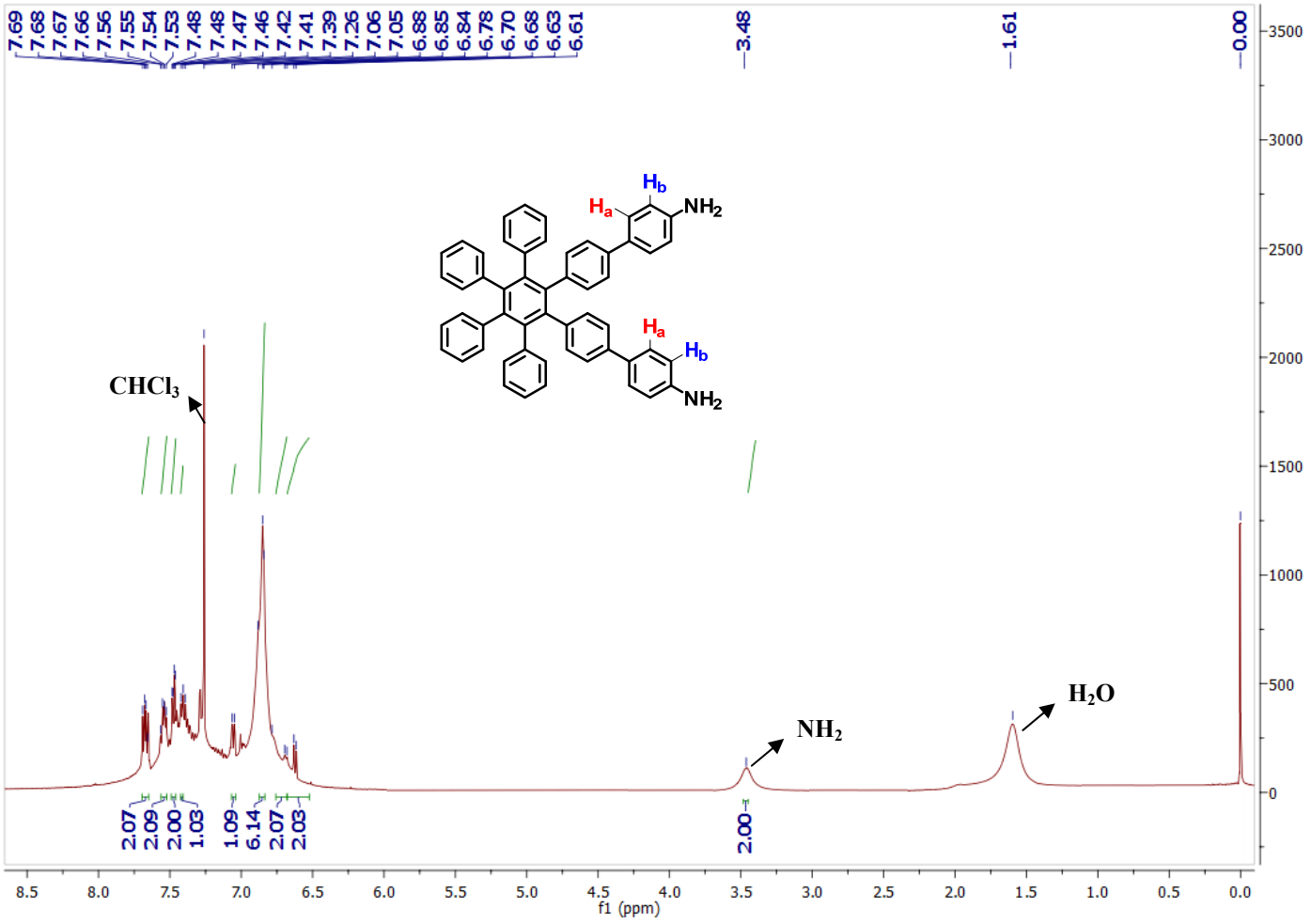


Fig. S34 ^{13}C NMR spectrum of product after the reaction of 2 with H_2S:

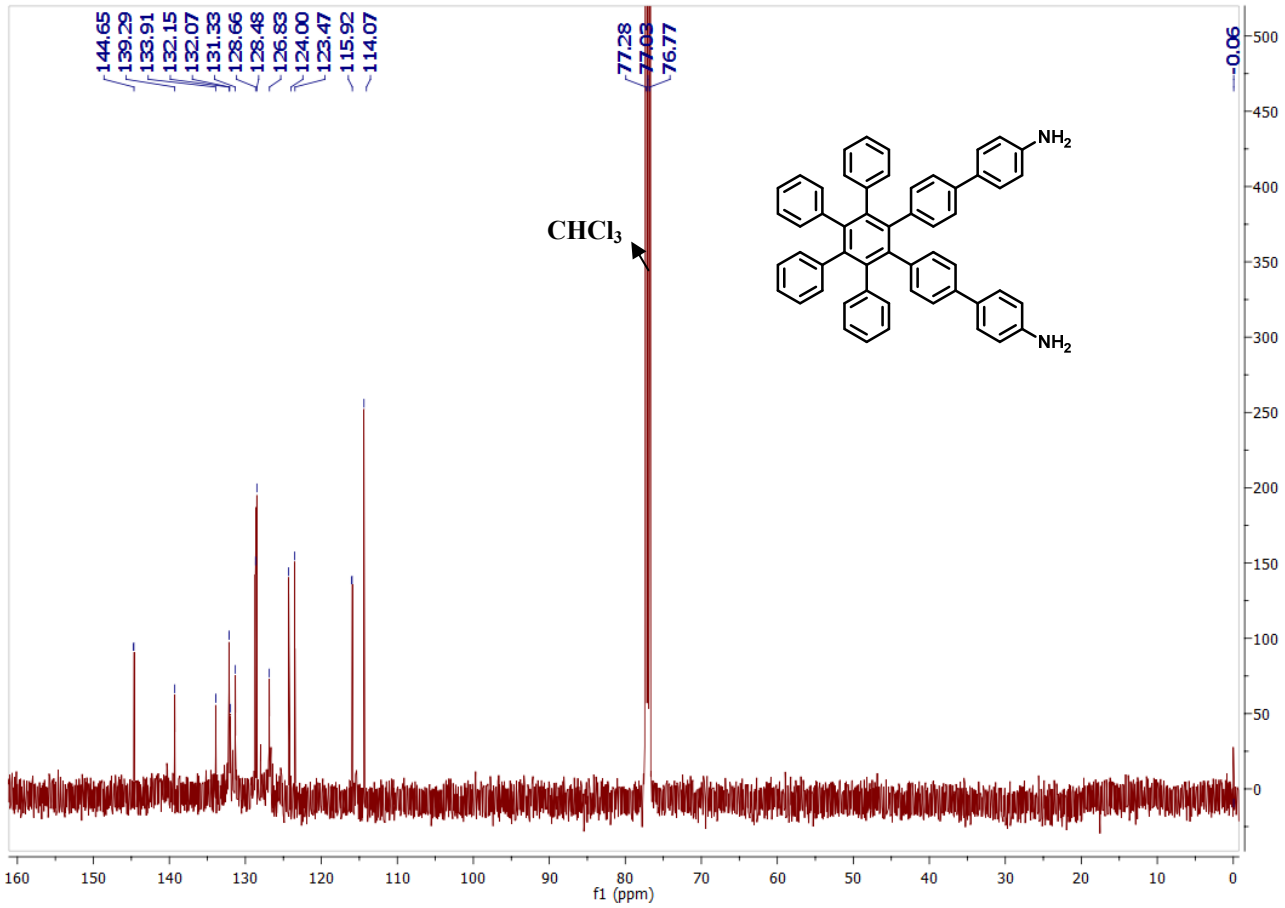


Fig. S35 Mass Spectrum (ESI-MS) of product after the reaction of 2 with H_2S: