Supporting information

Tetraphenylethylene conjugated *p*-Hydroxyphenacyl: Fluorescent Organic Nanoparticles for the Release of Hydrogen Sulfide under Visible light with Real-time Cellular Imaging

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General Information: All commercially available anhydrous solvents dimethylformamide (DMF), dichloromethane (DCM), petroleum ether (PE) and ethyl acetate (EA) and other chemicals were used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. NMR spectra were recorded on a 600 and 400 MHz instrument. ^{1}H NMR chemical shifts were referenced to the tetramethylsilane signal (0 ppm), ^{13}C NMR chemical shifts were referenced to the solvent resonance (77.23 ppm, Chloroform-*d*). Chemical shifts (δ) are reported in ppm, and spin−spin coupling constants (J) are given in Hz. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. UV/vis absorption spectra were recorded on UV/vis spectrophotometer and fluorescence spectra were recorded on fluorescence spectrophotometer. High-resolution mass spectra (HRMS) were recorded on ESI-TOF (electrospray ionization-time-of-flight). Photolysis of H_2S donor was carried out using a 125 W medium pressure mercury lamp. RP-HPLC was taken using mobile phase methanol/water (8:2), at a flow rate of 1mL / min (detection: UV 310 nm). Chromatographic purification was done with 60–120 mesh silica gel. For reaction monitoring, precoated silica gel 60 F254 TLC sheets were used. The morphology of TPE-pHP-H_2S NPs were taken from high-resolution transmission spectroscopy (HR-TEM) JEOL JEM2100, USA at an accelerating voltage at 200 kV. The sample was prepared by dissolving the compound in water and dropping on the surface of copper grid coated with carbon. Dynamic light scattering (DLS) measurement was recorded with Nano ZS ZEN3600.

Experimental Procedure and spectroscopic data:

5-(2-bromoacetyl)-2-hydroxybenzaldehyde (2): To a suspension of aluminum chloride ($AlCl_3$) (1.06 g, 8 mmol) in 60 mL of dichloromethane (DCM), bromoacetyl bromide (0.48 g, 2.4 mmol) was added slowly at 5 °C. The temperature of the mixture was brought to Room Temperature (RT) and the mixture was stirred for an hour. After 1 h in that mixture a solution of salicylaldehyde (**1)** (245 mg, 2 mmol) in DCM was added slowly followed by raising the temperature to RT and the mixture was stirred for 15 h. Then the reaction mixture was quenched with water at 0 °C. The DCM layer was separated and removal of solvent leads to the formation of slurry of product residue. The residue was further purified by column chromatography using 10% ethyl acetate in pet ether to yield 5-(2-bromoacetyl)-2-hydroxybenzaldehyde as white solid (0.316 g, 65%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.52 (s, 1H), 9.99 (s, 1H), 8.30 (s, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 7.10 (d, *J* = 8.8 Hz, 1H), 4.40 (s, 2H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 196.2, 189.0, 165.7, 137.2, 135.8, 126.3, 120.2, 118.5, 29.8. HRMS (ESI^+) calcd for $C_9H_7BrO_3$ $[M+H]^+$, 242.9657, found: 242.9652.

5,5'-(2,2'-thiobis(acetyl))bis(2-hydroxybenzaldehyde) (3): Add a solution of sodium sulfide nonahydrate ($Na_2S.9H_2O$) (0.178 mg, 0.6 mmol) in water (20 ml) to a stir and ice-cooled solution of 5-(2-bromoacetyl)-2-hydroxybenzaldehyde (0.300 g, 1.0 mmol) in an appropriate amount of acetone (10 ml). After completion of the addition, warm the mixture to room temperature for an hour. The completion of reaction was monitored by TLC and it was extracted with EtOAc and washed with water. The collected organic layer dried over Na_2SO_4 and solvent was removed by rotary evaporation under reduced pressure. The crude product was purified by column chromatography using 20% EtOAc in pet ether to give the product as light yellow solid (0.345 g, 78%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.49 (s, 2H), 9.98 (s, 2H), 8.29 (s, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 2H), 3.95 (s, 4H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 196.5, 191.8, 165.8, 137.2, 135.6, 127.9, 120.3, 118.6, 37.4. HRMS (ESI^+) calcd for $C_{18}H_{14}O_6S$ $[M+H]^+$, 359.0589; found:359.0586.

(2-(4-nitrophenyl)ethene-1,1,2-triyl)tribenzene (6): To an oven-dried two-necked flask benzophenone (**4**) (3 g, 16.4 mmol), 4-nitrobenzophenone (**5**) (4.86 g, 21.4 mmol) and zinc dust (6.11 g, 93.5 mmol) were added. The mixture was degassed while purging

nitrogen at least three times, and then freshly distilled fresh THF (80 mL) was injected into the flask. The reaction system was cooled to −78 °C in a dry ice-acetone bath for at least 15 min. $TiCl_4$ (9.02 g, 47.5 mmol) was added dropwise, after which the reaction was continued for about 8 h under refluxing. The excess zinc was filtered after cooling to room temperature. Aqueous potassium carbonate was added, the mixture was extracted with dichloromethane, and the filtrate was dried with anhydrous Na_2SO_4. After removing the solvent with a rotary evaporator, and the residue was purified by column chromatography using 10% EtOAc in pet ether to give the product as yellow solid (2.13 g, 34%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (d, *J* = 8.8 Hz, 2H), 7.18 (m, 11H), 7.03 (m, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 151.04, 146.05, 142.94, 139.08-138.16, 132.39, 127.84, 123.55.

4-(1,2,2-triphenylvinyl)aniline (7): Compound **6** (0.3 g, 0.86 mmol), 10% Pd/C (0. 02g) and hydrazinemonohydrate (80%) (1.2 mL) were added in EtOH (10 mL). The mixture was refluxed for 4 h, then the solid Pd/C was filtered, and the solvent was removed under vacuum to give the product as light yellow solid (0.27 g, 96%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.26 (m, 15H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.43 (d, *J* = 8 Hz, 2H), 3.57 (s, 2H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 147.77, 145.23-144.04, 141.65, 138.32, 132.42-130.48, 126.61, 113.57.

2,2'-thiobis(1-(4-hydroxy-3-((E)-((4-(1,2,2 triphenylvinyl)phenyl)imino)methyl)phenyl)ethan-1-one) (8): Compound **3** (0.05g, 0.14 mmol) is dissolved in 5 mL of methanol. Compound **7** (0.1 g, 0.28 mmol) is added to the mixture and heated at 60 °C for 1 h. The completion of reaction was monitored by TLC and it was extracted with DCM and washed with water. The collected organic layer dried over Na_2SO_4 and solvent was removed by rotary evaporation under reduced pressure. The crude product was purified by column chromatography using 10% EtOAc in pet ether to give the product as yellow solid (0.09 g, 64%).^{1}H NMR (400 MHz, Chloroform-d) δ 14.2 (s, 2H), 8.65 (s, 2H), 8.07 (d, J = 2 Hz, 2H), 8.01 (d, J = 10.8 Hz, 2H), 7.14 (m, 40H), 3.95 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.21, 166.07, 160.91, 143.52-143.41, 139.98, 133.90, 132.58, 132.49, 131.47-131.29, 127.87-127.51, 126.84-126.67, 120.57, 117.89, 37.31. HRMS (ESI+) calcd for $C_{70}H_{53}N_2O_4S$ [M+H]+, 1017.3726; found: 1017.3837.

Characterisation of Photoproduct (E)-2-(4-hydroxy-3-(((4-(1,2,2-triphenylvinyl)phenyl)imino)methyl)phenyl)acetic acid (13): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 14.11 (s, 1H), 11.48 (s, 1H), 8.64 (s, 1H), 8.07 (s, 1H), 8.07(d, J = 2 Hz, 1H), 7.13-7.05 (m, 20H), 3.67 (s, 2H).

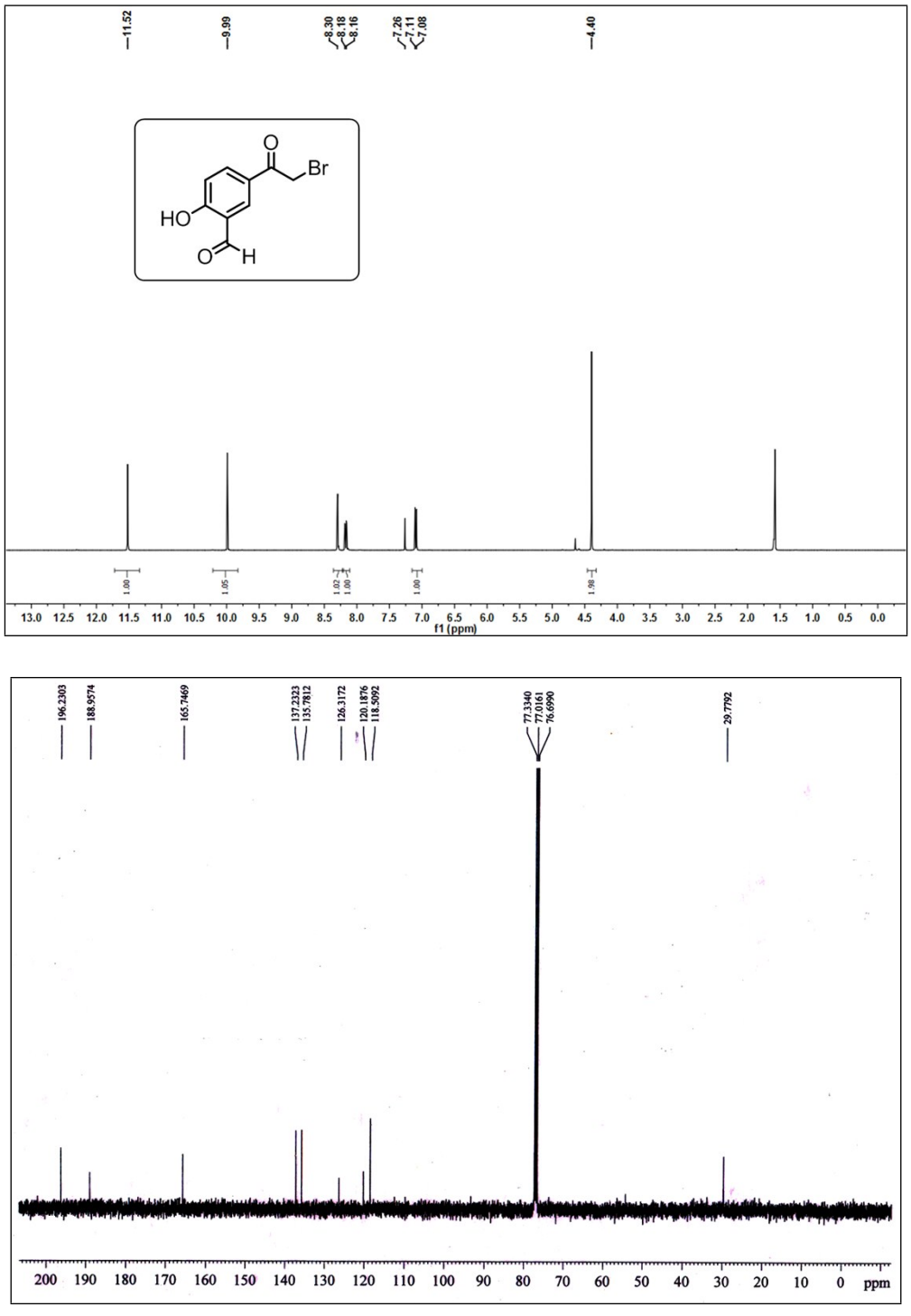


Figure S1. ^{1}H and ^{13}C NMR spectra of **2** in Chloroform-*d*.

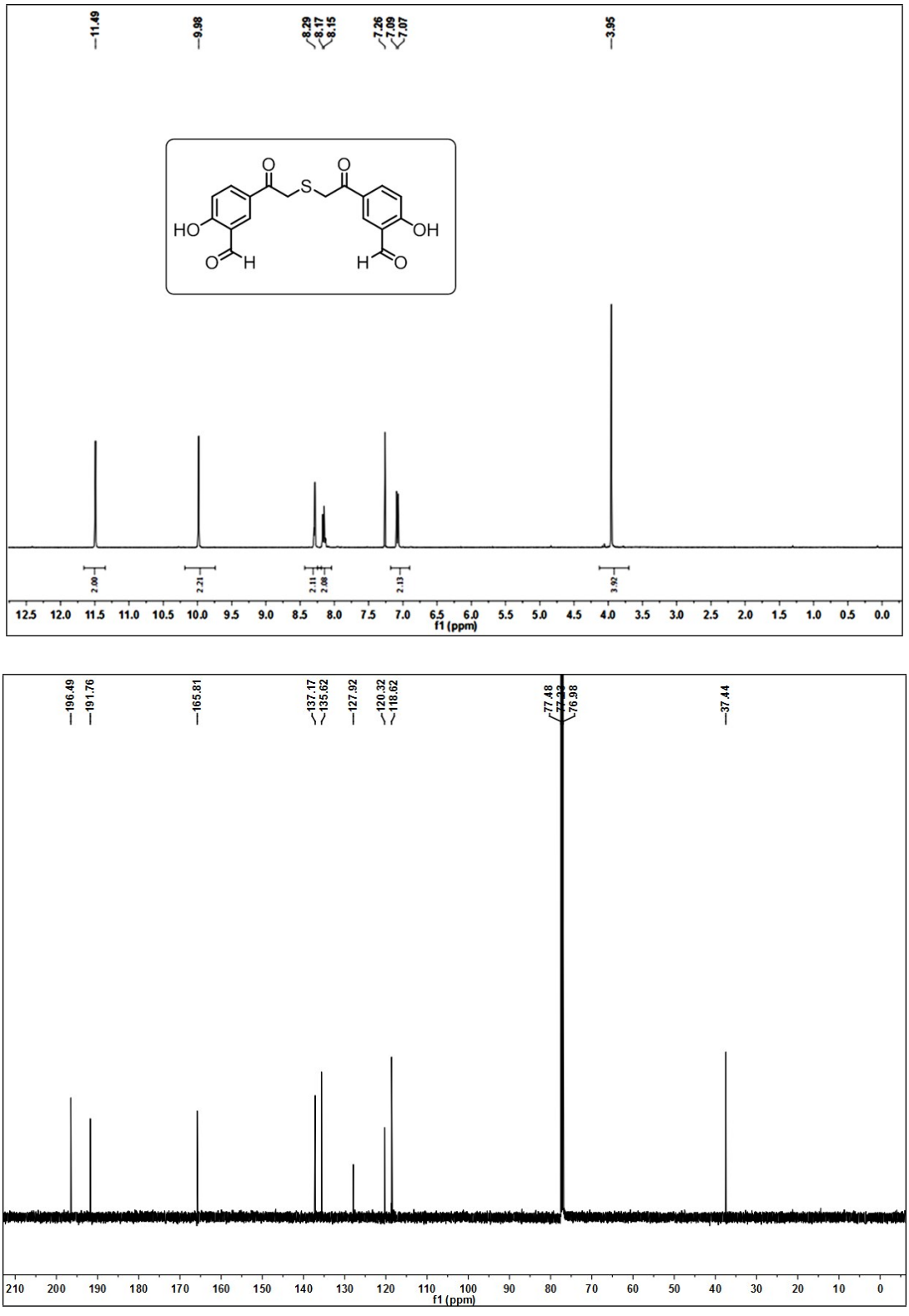


Figure S2. ^{1}H and ^{13}C NMR spectra of **3** in Chloroform-*d*.

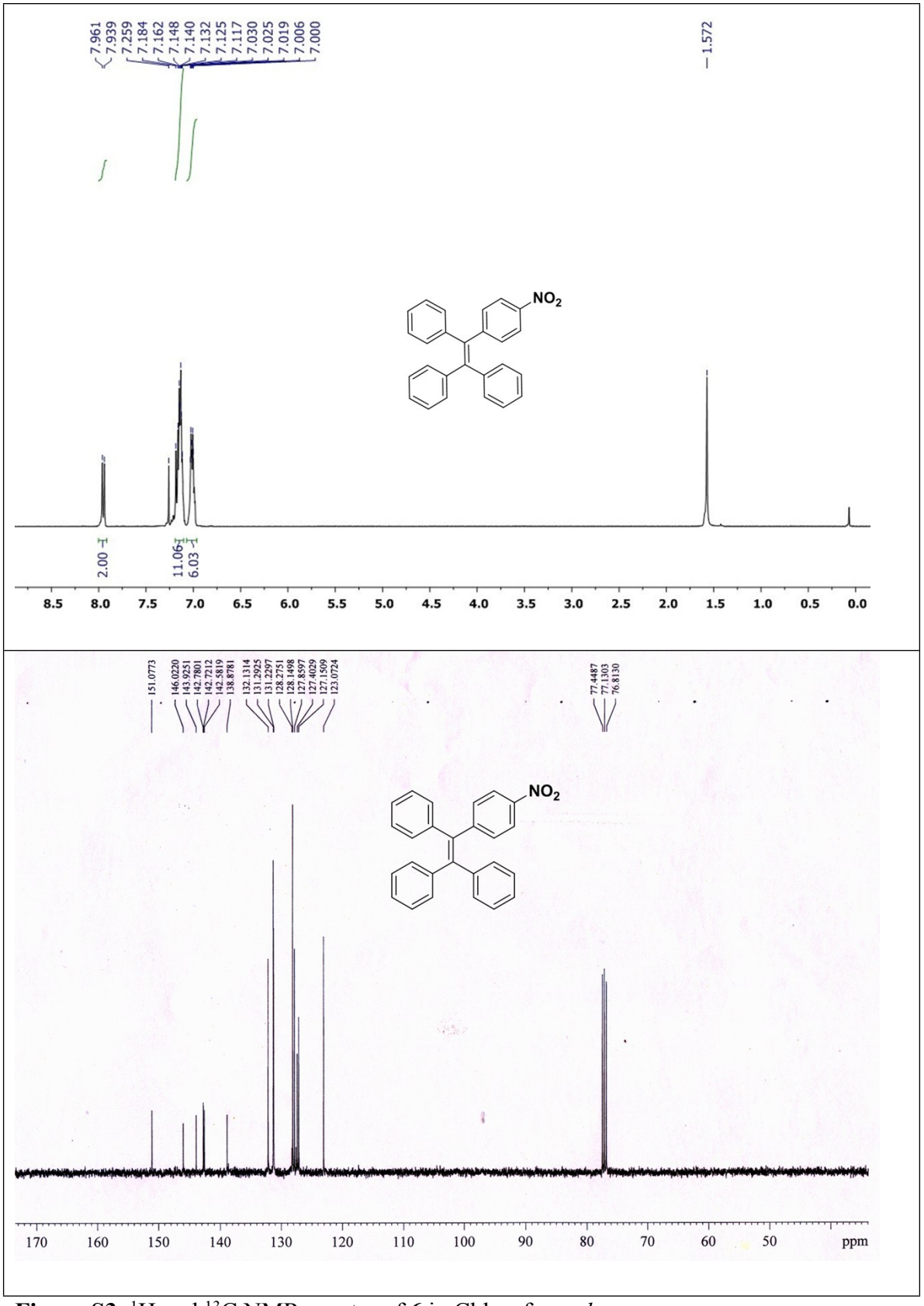


Figure S3. ^{1}H and ^{13}C NMR spectra of **6** in Chloroform-*d*.

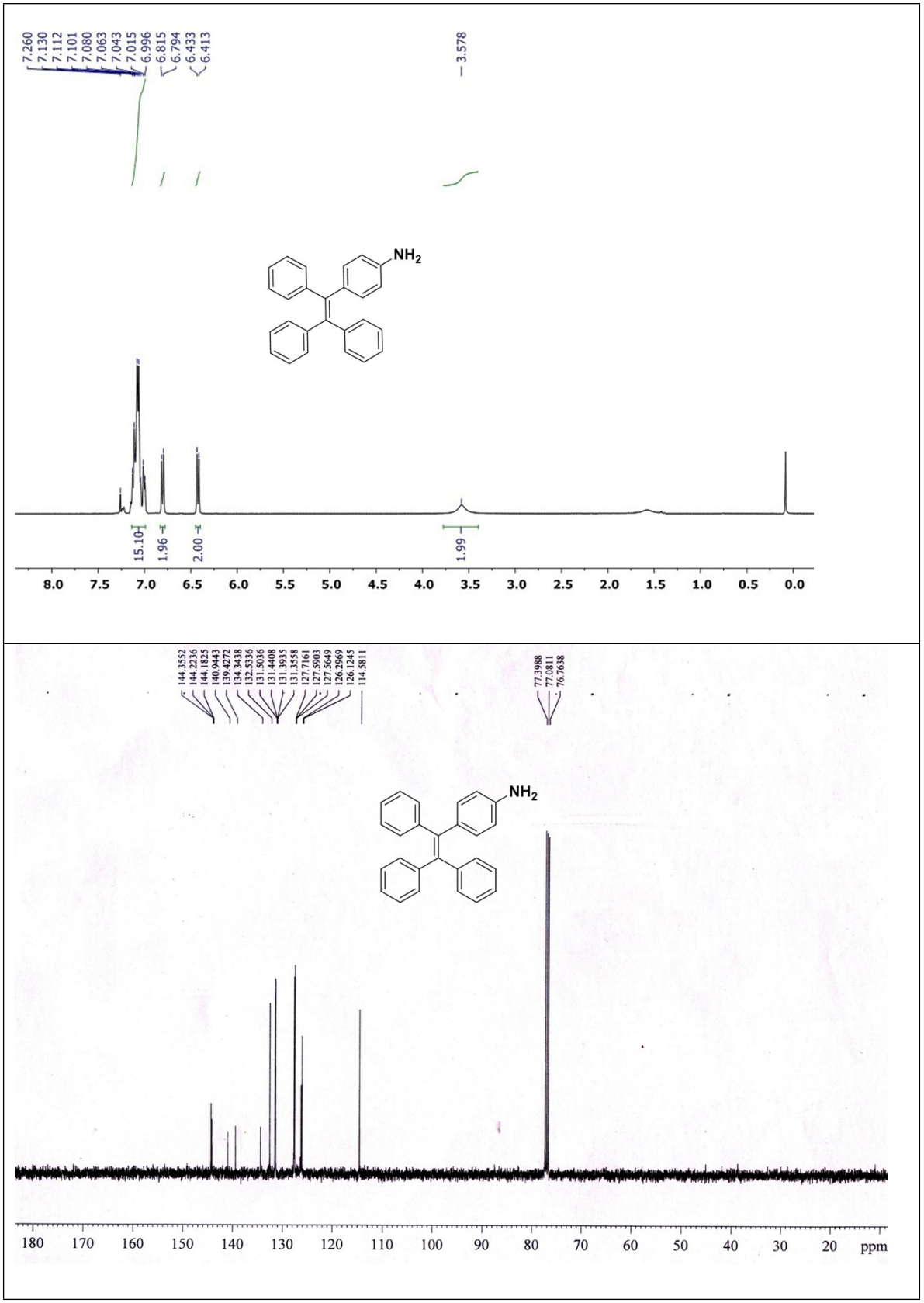


Figure S4. ^{1}H and ^{13}C NMR spectra of **7** in Chloroform-*d*.

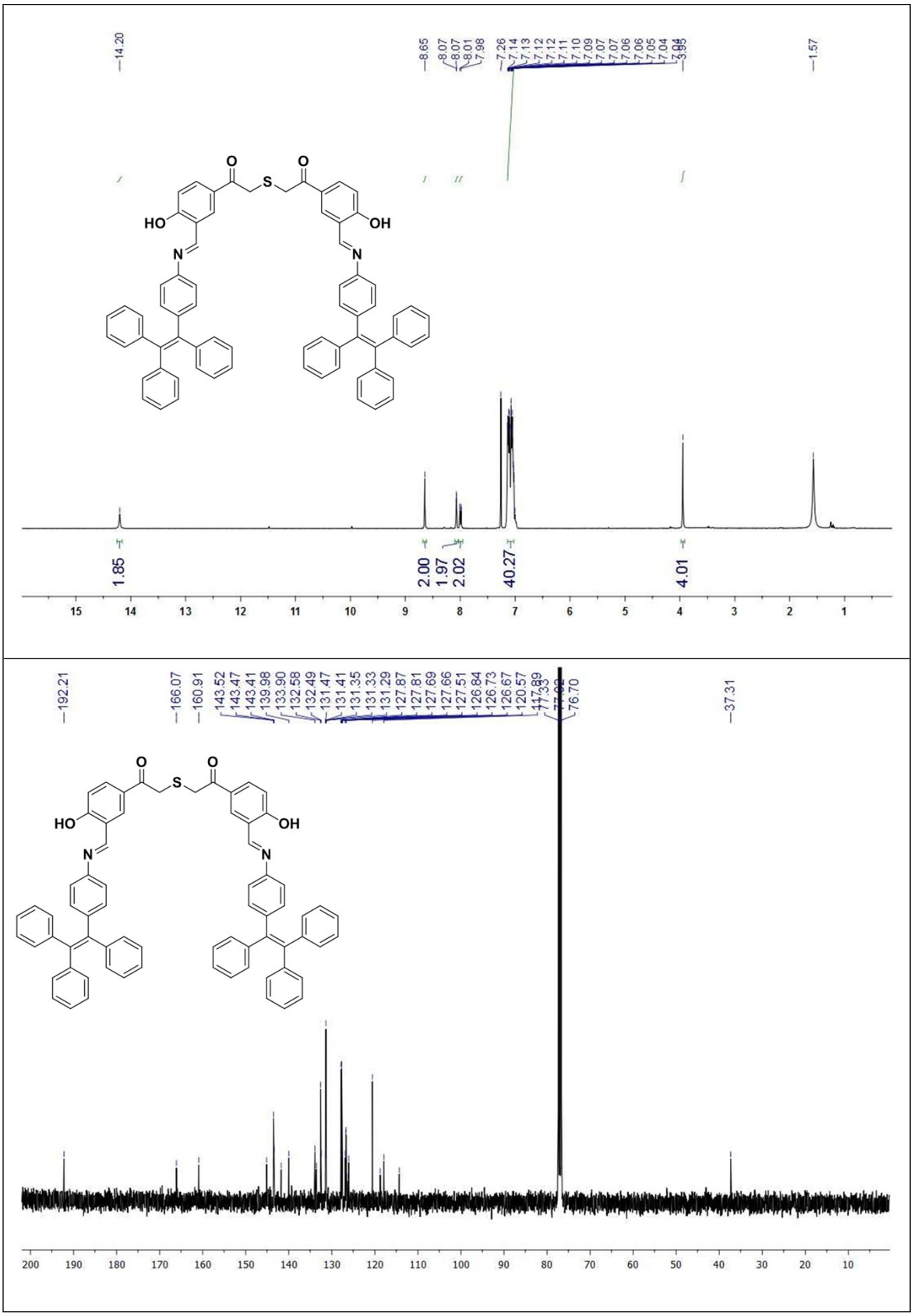


Figure S5. ^{1}H and ^{13}C NMR spectra of **8** in Chloroform-*d*.

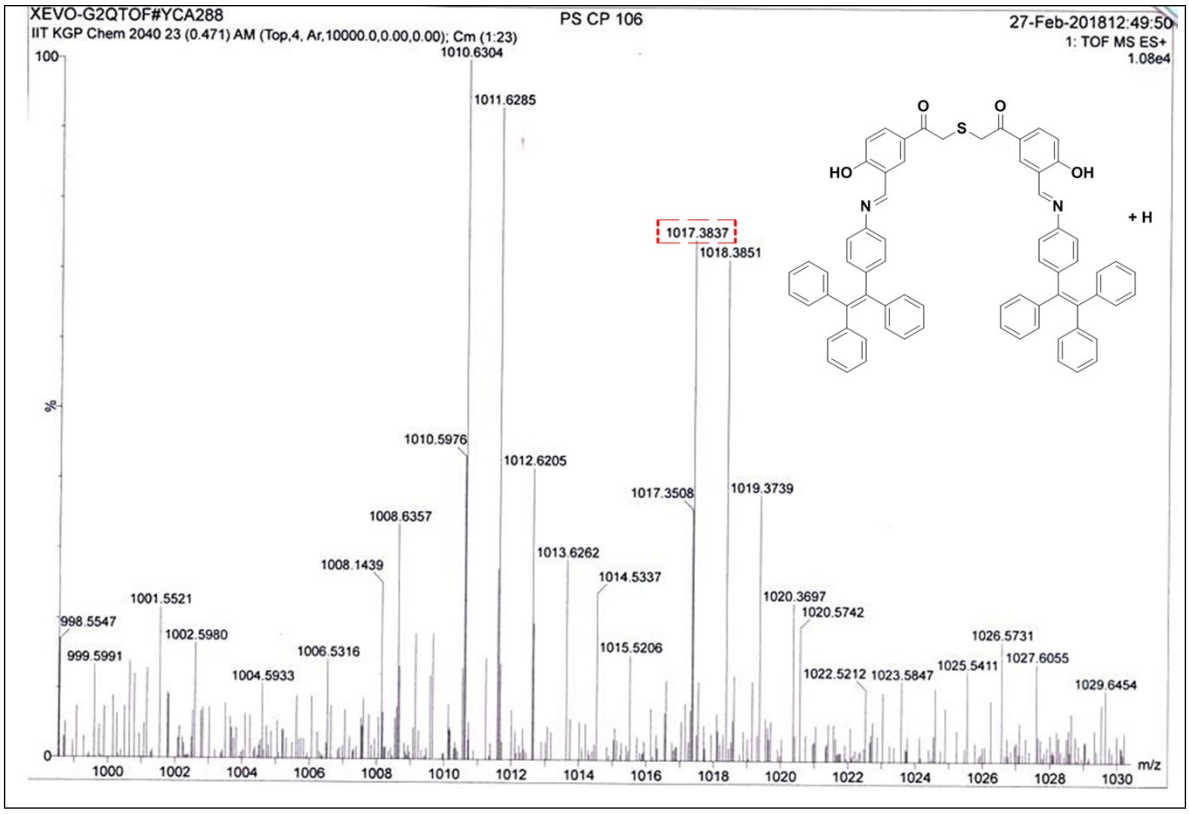
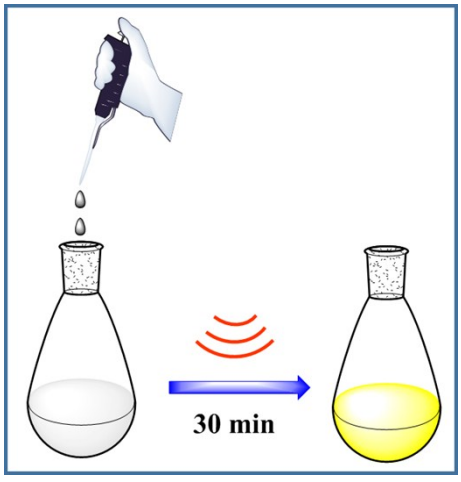


Figure S6. HRMS spectra of **8**

Preparation of TPE-pHP-H_2S nanoparticles:

Photoresponsive TPE-pHP-H_2S nanoparticles were prepared by reprecipitation technique. To 25 mL water, 5 µL ACN solution (1:0.0002 Water:ACN v/v)of TPE-pHP-H_2S (3 mM) was added slowly for 30 min with constant sonication. The size determination of TPE-pHP-H_2S nanoparticles were carried out using TEM and DLS.



Scheme S1: Synthesis of TPE-pHP-H_2S nanoparticles

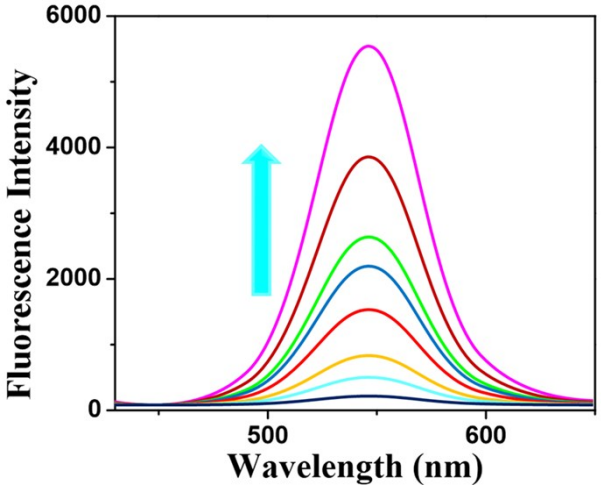


Figure S7. Fluorescence response of TPE-pHP-H_2S NPs donor with increasing the concentration from $1x10^{-5}$ M (0.1:9.9 ACN/PBS buffer at pH 7.4).

Determination of incident photon flux (I_0) of the UV lamp by potassium ferrioxalate actinometry:

The incident photon flux (I_0) was determined by irradiating the Potassium ferrioxalate actinometry using UV lamp. According to literature procedure,[1] solution of potassium ferrioxalate, 1,10-phenanthroline and the buffer solution were prepared. The solution of potassium ferrioxalate (0.006 M) was irradiated using 125 W medium pressure Hg lamp as visible light source (≥410 nm) and $NaNO_2$ (1M) solution as UV cut-off filter. At consistent interval of time (3 min), 1 mL of the aliquots was taken out and to it 3 mL of 1,10-phenanthroline solution and 2 mL of the buffer solution were added and the whole solution was kept in dark for 30 min. The formed phenanthroline-ferrous complex was measured spectrophotometrically at 510 nm. From the calibration graph the amount of Fe^{2+} ion was determined. The calibration graph was plotted by absorbance of phenanthroline-ferrous complex at various known concentration of Fe^{2+} ion in dark. From the slope of the graph the molar absorptivity of the phenanthroline-ferrous complex was calculated to be 1.10×10^{4} M^{-1} cm^{-1} at 510 nm which is found to be similar to reported value. Using the known quantum yield (1.188 ± 0.012) for potassium ferrioxalate actinometer at 406.7 nm, the number of Fe^{2+} ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident intensity (I_0) at 410 nm of the 125W Hg lamp was determined as 2.886×10^{16} quanta s^{-1}.

Deprotection photolysis and measurement of photochemical quantum yield for H_2S donor TPE-pHP-H_2S NPs:

A solution of 1×10^{-4} M of the H_2S donor TPE-pHP-H_2S NPs was prepared in ACN/PBS buffer (0.1:9.9). The solution was passed with nitrogen and irradiated using 125 W medium pressure Hg lamp as a visible light source ($\lambda \geq 410$ nm) using a suitable UV cut-off filter (1M $NaNO_2$ solution) with continuous stirring for 20 min. At a consistent interval of time, 20µl of the aliquots were taken and analyzed by RP-HPLC using mobile phase methanol/water (8:2), at a flow rate of 1mL / min (detection: UV 310 nm). Peak areas were determined by RP-HPLC, which indicated a gradual decrease of the TPE-pHP-H_2S NPs donor with time, and the average of three runs. The reaction was followed until the decomposition of TPE-pHP-H_2S NPs donor is more than 95%. Based on HPLC data, we plotted normalized [A] (HPLC peak area) versus irradiation time. We observed an exponential correlation for the decomposition of TPE-pHP-H_2S NPs donor which suggested a first order reaction. Further, the photochemical quantum yield (Φ_p) was calculated based on the decomposition of TPE-pHP-H_2S NPs donor using below equation (1).

$$(\Phi_p)_S = (\Phi_p)_{act} \frac{(k_p)_S}{(k_p)_{act}} \frac{(F_{act})}{(F_S)} \qquad (1)$$

Where, the subscript 'S' and 'act' denotes sample (TPE-pHP-H_2S NPs donor) and actinometer respectively. Potassium ferrioxalate was used as an actinometer. Φ_p is the photolysis quantum yield, k_p is the photolysis rate constant and F is the fraction of light absorbed.

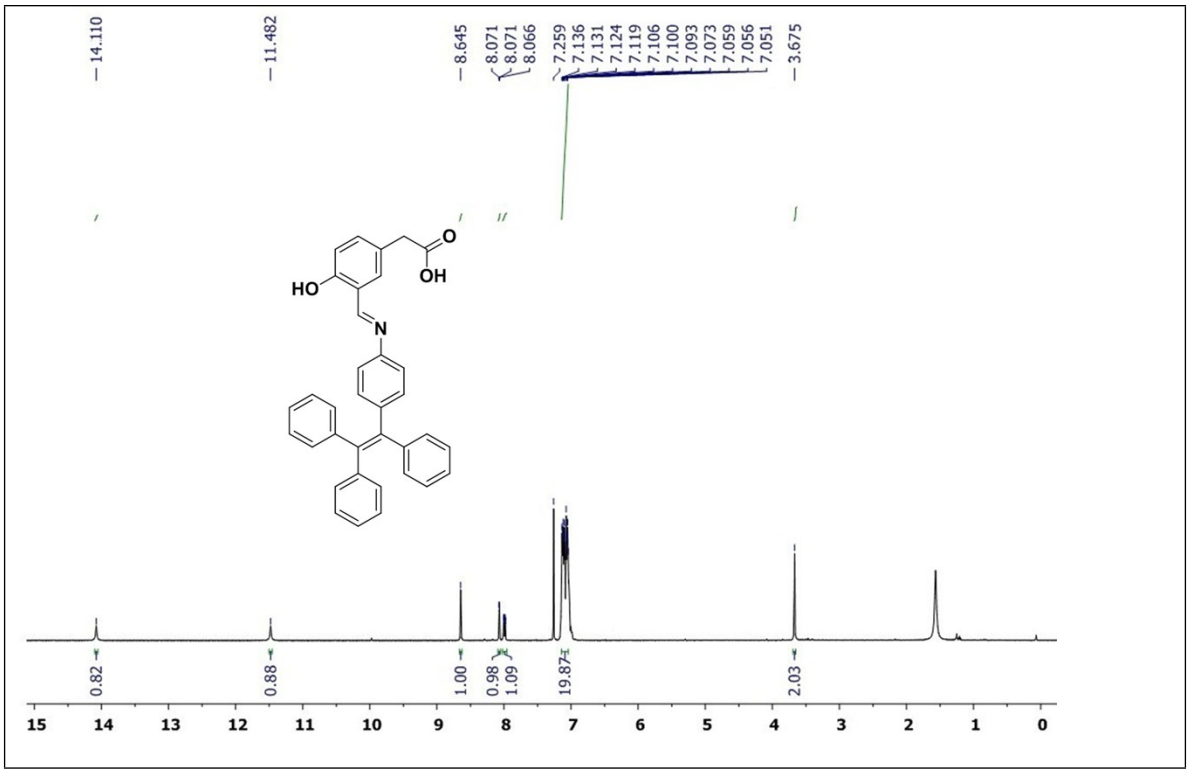


Figure S8. ^{1}H NMR spectra of photoproduct

Methylene Blue assay for H_2S detection:

Methyleneblue assay was carried out as described previously with some modifications.[2]

A 5 mM solution of Na_2S in sodium phosphate buffer (20 mM, pH 7.4)/acetonitrile (HPLC grade) (9.9:0.1) was prepared ($Na_2S.9H_2O$, 120.20 mg in 100 mL volumetric flask) and used as the stock solution. Aliquots of 100, 200, 300, 500, 700, 1000 µL of the Na_2S stock solution were added into a 50 mL volumetric flask and dissolved in a mixture of sodium phosphate buffer/acetonitrile to obtain the standard solutions in 10, 20, 30, 50, 70, 100 µM, respectively.

1 ml aliquot of the respective solution was reacted with the methylene blue (MB+) cocktail: 30 mM $FeCl_3$ (400 µL) in 1.2 M HCl, 20 mM of N,N-dimethyl-1,4-phenylenediamine sulfate (400 µL) in 7.2 mM HCl, 1% w/v of $Zn(OAc)_2$ (100 µL) in H_2O at room temperature for at least 15 min (each reaction was performed in triplicate). The absorbance of methylene blue was measured at λ_{max} = 663 nm. To obtain the molar

absorptivity of (MB+) a linear regression was plotted with the observed absorbance and concentration.

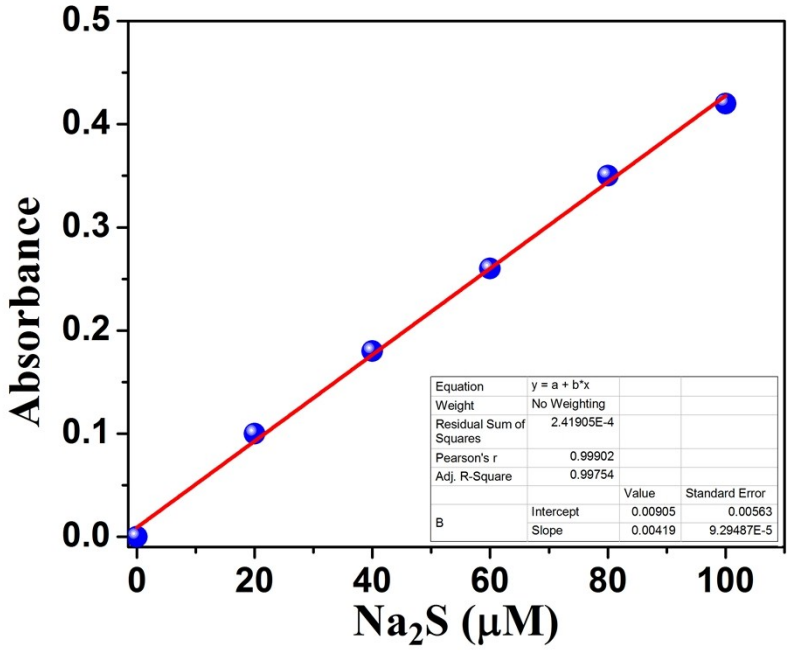


Figure S9. Standard Calibration curve with different concentration of Na_2S.

In this experiment, a 100 μM solution (total volume 20 mL) of the compound TPE-pHP-H_2S NPs donor was prepared in a 9.9:0.1 solution of sodium phosphate buffer (20 mM, pH 7.4)/acetonitrile. This solution was placed in a 24 mL scintillation vial. The resulting reaction vessel was irradiated with a 125 W medium-pressure mercury lamp as the source of visible light ($\lambda \geq$ 410 nm) using a suitable UV cut-off filter (1M $NaNO_2$ solution) with continuous stirring. The aliquot (1 mL) was collected at different time intervals (5, 10, 15, and 20 min) and was mixed immediately with the methylene blue cocktail: 30 mM $FeCl_3$ (200 μL) in 1.2 M HCl, 20 mM of N,N-dimethyl-1,4-phenylenediamine sulfate (200 μL) in 7.2 mM HCl, 1% w/v of $Zn(OAc)_2$ (100 μL) in H_2O at room temperature for at least 20 min. The absorbance of methylene blue was measured at λ_{max}= 663 nm against a blank: 30 mM $FeCl_3$ (400 μL) in 1.2 M HCl, 20 mM of N,N-dimethyl-1,4- phenylenediamine sulfate (400 μL) in 7.2 mM HCl, 1% w/v of $Zn(OAc)_2$ (100 μL) in H_2O, ACN (500 μL), 20 mM sodium phosphate buffer pH 7.4 (500 μL).

Photorelease rate constants at different pHs:

We found that the photorelease rate constants are quite similar in neutral and basic pHs (**Table S1**). This is only possible if ESIPT is helping in the deprotonation process. At acidic pH, the photorelease is much slower which is due to the higher bond order of phenolic –OH in **8**. The below result proves the assistance of ESIPT process in the release of the H_2S.

Table S1: Photorelease rate constants of TPE-pHP-H_2S NPs donor in different pH

pH	Rate Constant
5	**7.87×10^6 s^{-1}**
7	**1.73×10^7 s^{-1}**
9	**1.94×10^7 s^{-1}**

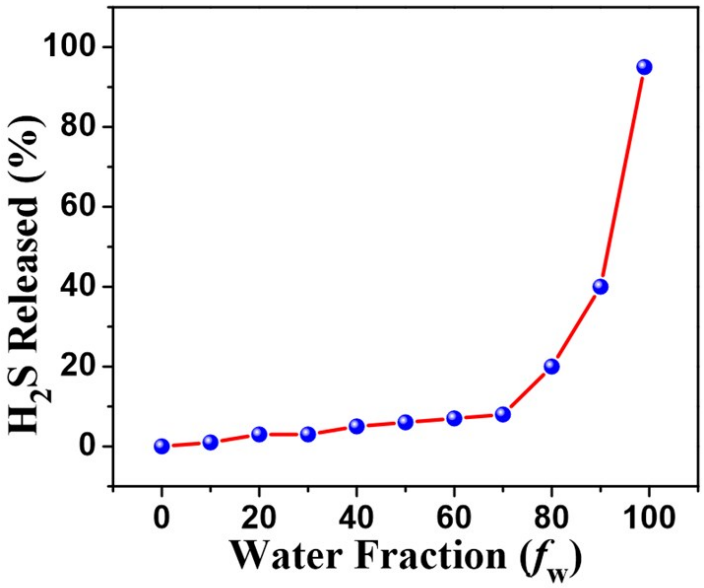


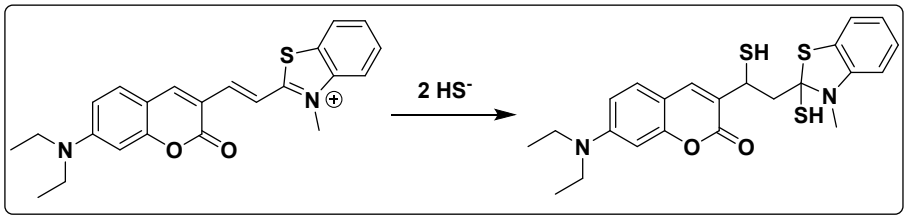
Figure S10 H_2S released upon photolysis against different water fractions (f_w) of acetonitrile. Experiments were repeated in triplicate and the values shown are the mean with s.d

Table S2: Photochemical quantum yields (Φ_p) of TPE-pHP-H_2S NPs donor with different f_w

Water fraction (f_w) Vol (%)	Quantum yield (Φ_p)
70	**0.02**
80	**0.13**
90	**0.18**
99	**0.48**

H_2S detection by using fluorescence sensor (coumarin−hemicyanine dye):

To detect H_2S generation from TPE-pHP-H_2S NPs donor, we have used coumarin-hemicyanine dye as an HS-sensitive probe (Scheme S2). The coumarin-hemicyanine dye (15 μM) in 1:9 v/v ACN: PBS buffer (10 mM, pH 7.4) exhibited a red emission with the maximum at 645 nm (excitation at 545 nm). Then, the dye (15 μM) in the presence of TPE-pHP-H_2S NPs donor (100 μM) in 1:9 v/v ACN: PBS buffer (10 mM, pH 7.4) was irradiated with visible light (λ≥410 nm) with constant stirring. After irradiation for 20 min, we observed the fluorescence signal at 645 nm reduced with a concomitant increase in a new blue emission peak at 476 nm (excitation at 410 nm). However, based on the decrease in the fluorescence intensity at 645 nm, the generation of HS− during the decomposition of TPE-pHP-H_2S NPs donor was studied. There is no visible fluorescence change was observed when the donor was incubated under similar conditions in the absence of light.



Scheme S2. Proposed H_2S sensing mechanism

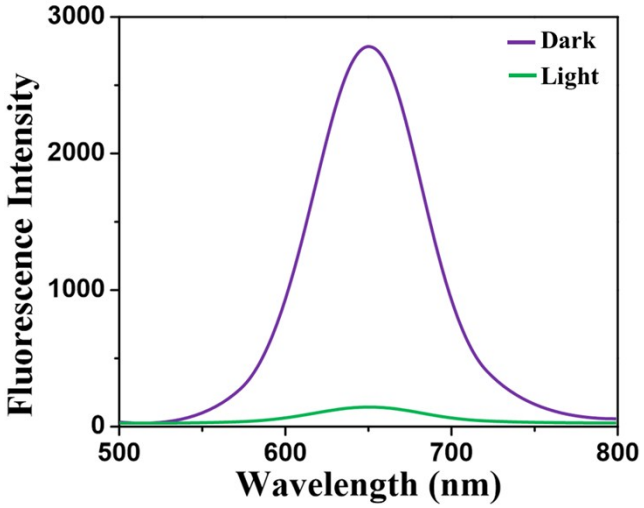


Figure S11. Fluorescence spectra of coumarin−hemicyanine dye (15 μM) in the presence of TPE-pHP-H_2S NPs donor (100 μM) in 1:9 v/v ACN: PBS buffer (10 mM, pH 7.4) under dark and visible light condition for 20 min.

Experimental procedure for biological application study:

***In vitro* real-time cellular uptake and localization studies of TPE-pHP-H_2S NPs donor**

HeLa cells (1×10^5 cells / mL) were seeded on coverslips in MEM medium. After 24 h, one set of cells were treated with 50 μM of TPE-pHP-H_2S NPs donor and incubated for 6 h at 37 °C in a CO_2 incubator and another set was kept as control (no treatment). After incubation, the cells were irradiated by visible light (≥ 410 nm) using UV-visible lamp (Bangalore Genei Pvt. Ltd.) for 0–20 min. Thereafter cells were fixed using 4% paraformaldehyde for 10 min and washed twice with phosphate-buffered saline (PBS). Imaging was done using confocal microscopy (CLSM; Olympus FV 1000 attached to an inverted microscope 1X 81, Japan).

Cytotoxicity assay of TPE-pHP-H_2S NPs donor on the HeLa cell line before and after photolysis:

The cytotoxicity assay of H_2S donor TPE-pHP-H_2S NPs donor *in vitro* was measured using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay on the cervical cancer cells (HeLa). The cells growing in the log phase were seeded into two 96-well cell-culture plate at 1×10^4 cells / mL. Different concentrations of TPE-pHP-H_2S NPs donor (1, 5, 10, 15, 20 μM) were added

into the wells with an equal volume of PBS in the control wells. One 96-well cell-culture plate was irradiated (keeping the cell-culture plate 5 cm apart from the light source) under visible light ($\lambda \geq 410$ nm) by a 125 W medium pressure Hg lamp using a suitable filter (1 M $NaNO_2$ solution) for 20 min. The irradiated and non-irradiated cells were then incubated for 72 h at 37 °C in 5% CO_2. Thereafter, MTT was added to the wells of the 96 well plates at a concentration of 0.4 mg/ml and incubated for an additional 4 h at 37 °C in 5% CO_2 atmosphere. Thereafter, media was removed; formazan crystals were dissolved in appropriate volume of ACN and absorbance readings were taken at 595 nm using a microplate spectrophotometer, Thermo Fisher Scientific, USA. Cell viability was calculated using the formula, Viability (%) = 100 ×A2/A1; [Where A2 = Absorbance of the treated cell; A1 = Absorbance of the control cells].

Real time cellular uptake and localization study:

To study the cellular uptake and localization of TPE-pHP-H_2S NPs donor, HeLa cells were first seeded at a density of 1×10^4 cells/well of a 6-well plate and then incubated with 50 μM of TPE-pHP-H_2S NPs donor for 4 h at 37 °C in a CO_2 incubator. After incubation, the cells were irradiated by UV-visible light (≥ 410 nm) for 0-20 min. Thereafter cells were fixed using 4% paraformaldehyde for 10 min and washed twice with phosphate-buffer saline (PBS). Imaging was done using confocal microscopy (CLSM; Olympus FV 1000 attached to an inverted microscope 1X 81, Japan).

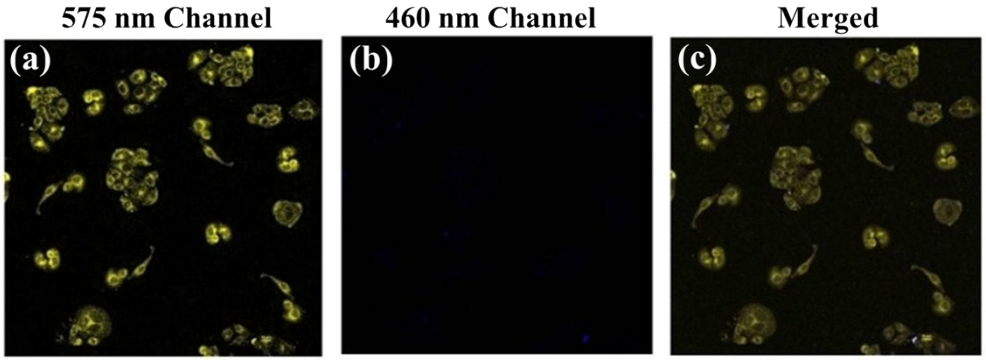


Figure S12. Confocal images of cellular internalization of TPE-pHP-H_2S NPs donor

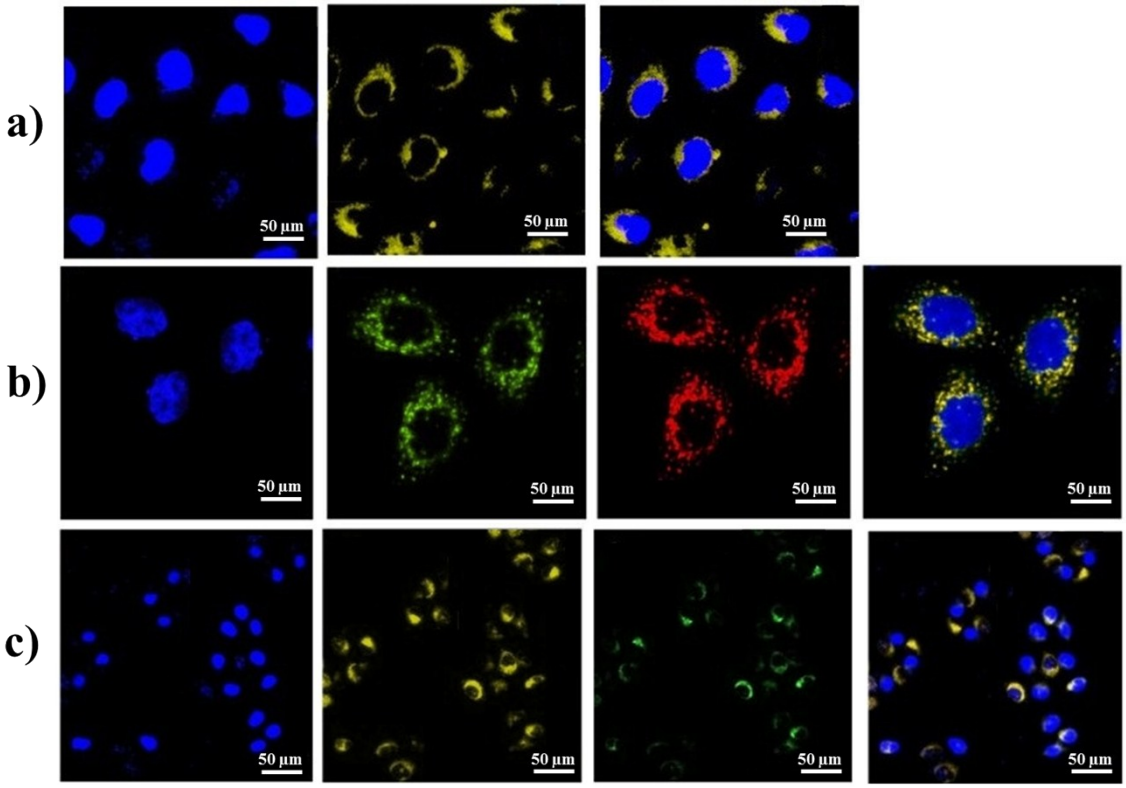


Figure S13. Subcellular localization of TPE-pHP-H_2S NPs donor in HeLa cells, a) 1a, 1b & 1c represent the nuclear staining expermint, b) 2a, 2b, 2c & 2d represent the lyso tracker experiment (Yellow emission were changed to green for better visualization), c) 3a, 3b, 3c & 3d represent the mito tracker experiment.

Fluorescence data for cellular experiment:

In order to study the intracellular fluorescence color change, human cervical cancer HeLa cells were incubated with **8** and coumarin−hemicyanine for 6 h followed by imaging using a fluorescence microscope. The emission signal at 625 nm was observed before photolysis. HeLa cells pretreated with **8** and dye were then irradiated with a light source ($\lambda \geq 410$ nm) for 20 min, and we observed the decrease in the fluorescence signal at 625 nm studied by microscopy. A significant change in fluorescence signal was recorded (Figure S12, light versus dark).

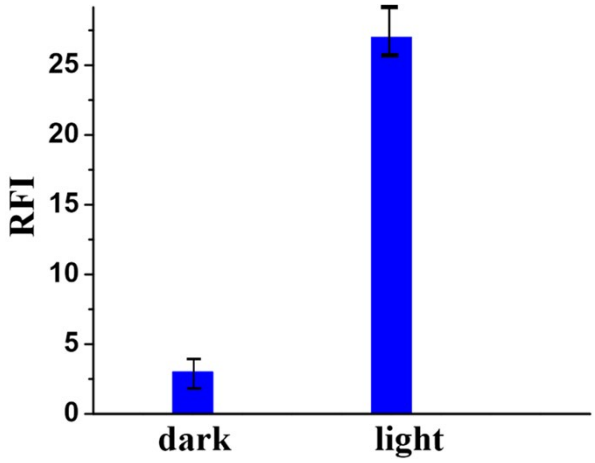


Figure S14. Fluorescence change of coumarin−hemicyanine dye in presence of TPE-pHP-H_2S NPs donor for the cellular experiment under dark and light ($\lambda \geq 410$ for 20 min) conditions.

References:

1) E. T. Ryan, T. Xiang, K. P. Johnston and M. A. Fox, *J. Phys. Chem. A* **1997**, *101*, 1827-1835.

2) Y. Zhao, H. Wang and M. Xian, *J. Am. Chem. Soc.*, **2011**, *133*, 15.