Supporting information

Water Soluble Light Activated Hydrogen Sulfide Donor Induced by Excited State Meta Effect

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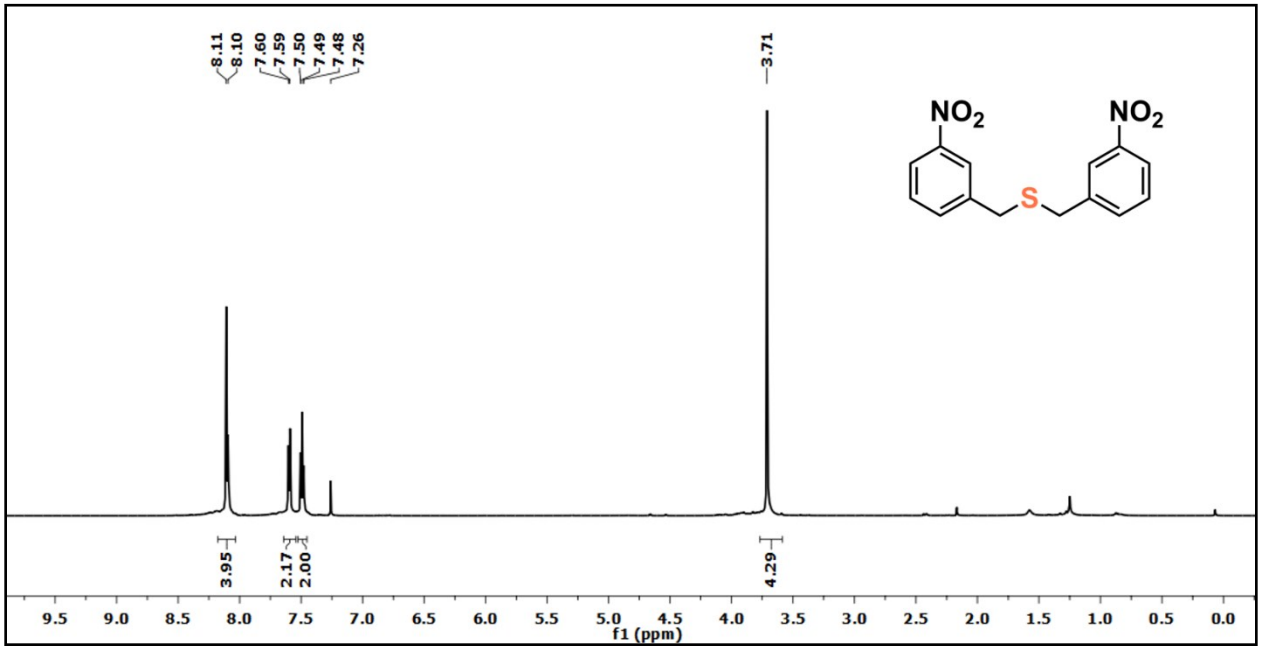
1. Characterization of synthesized molecules and photoproduct by NMR Spectroscopy:



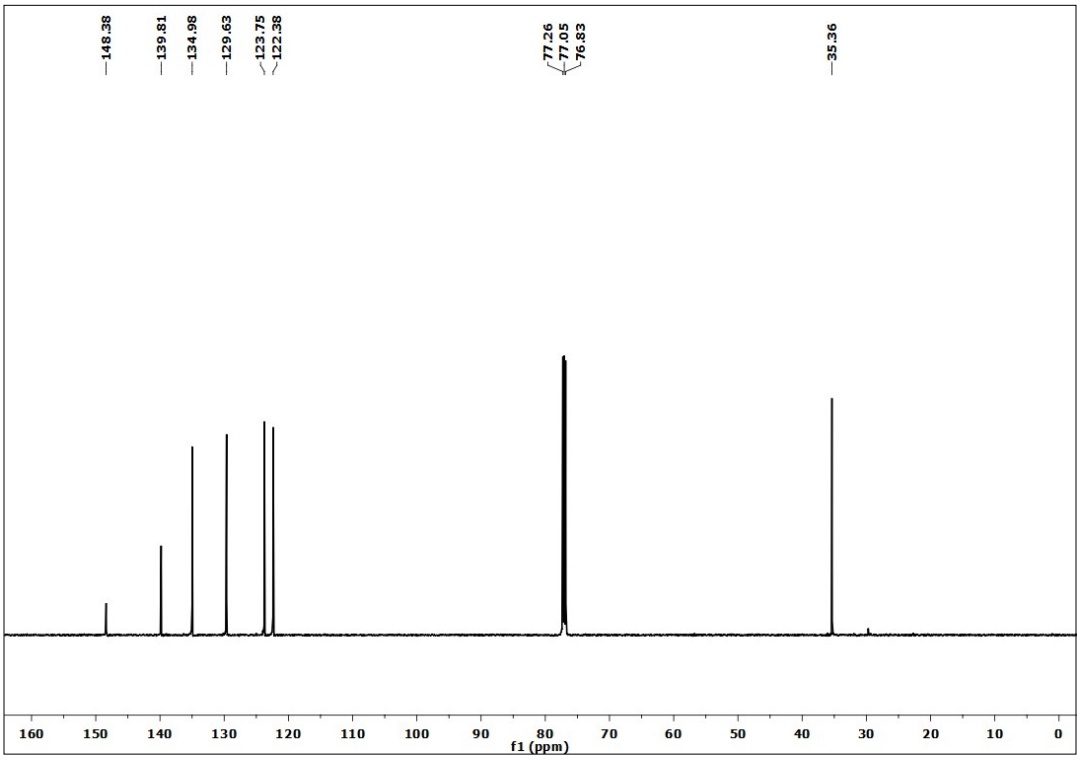


Fig. S1. ^{1}H and ^{13}C NMR spectra of **2** in $CDCl_3$.

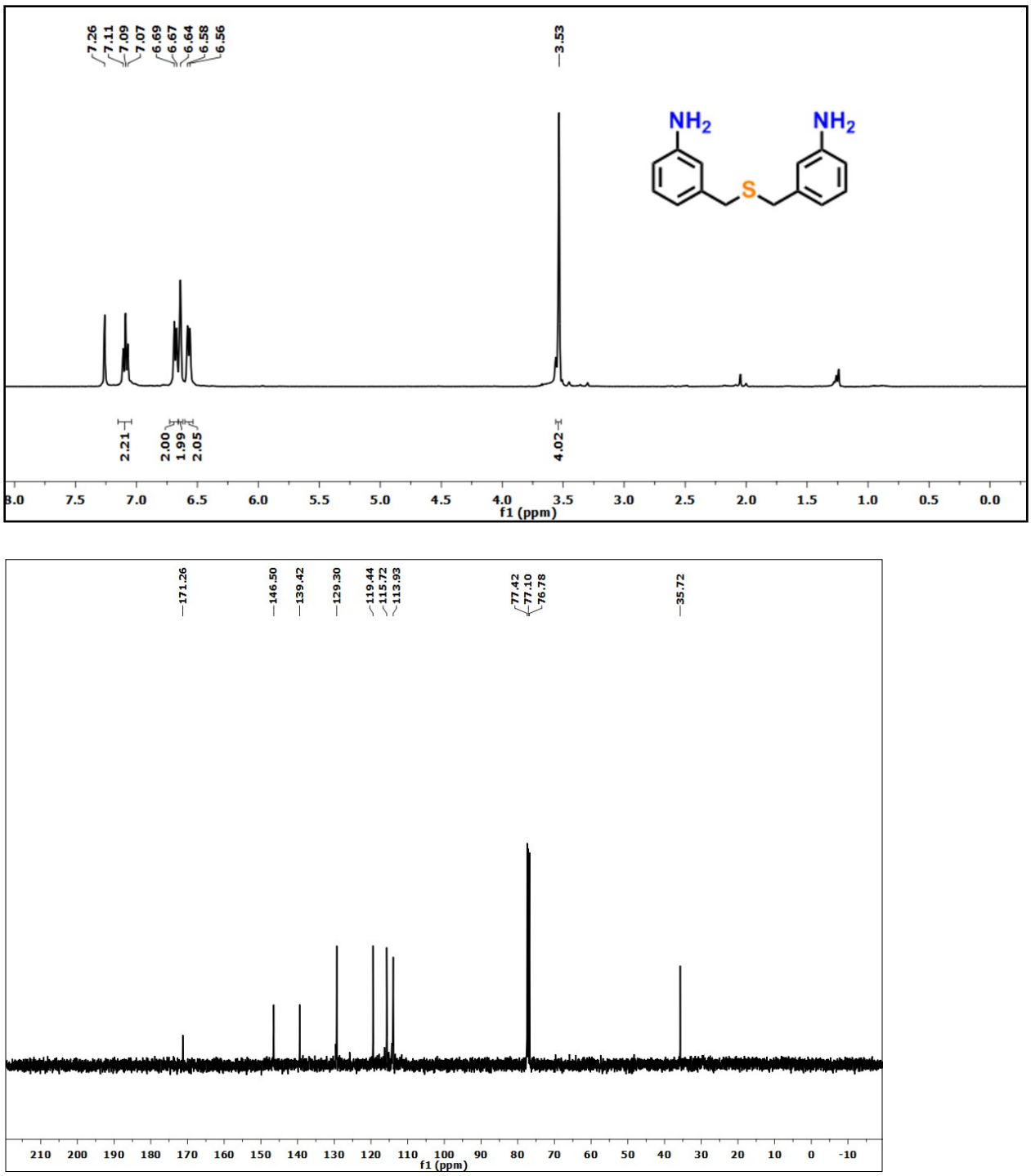


Fig. S2. ^{1}H and ^{13}C NMR spectra of **3** in $CDCl_3$.

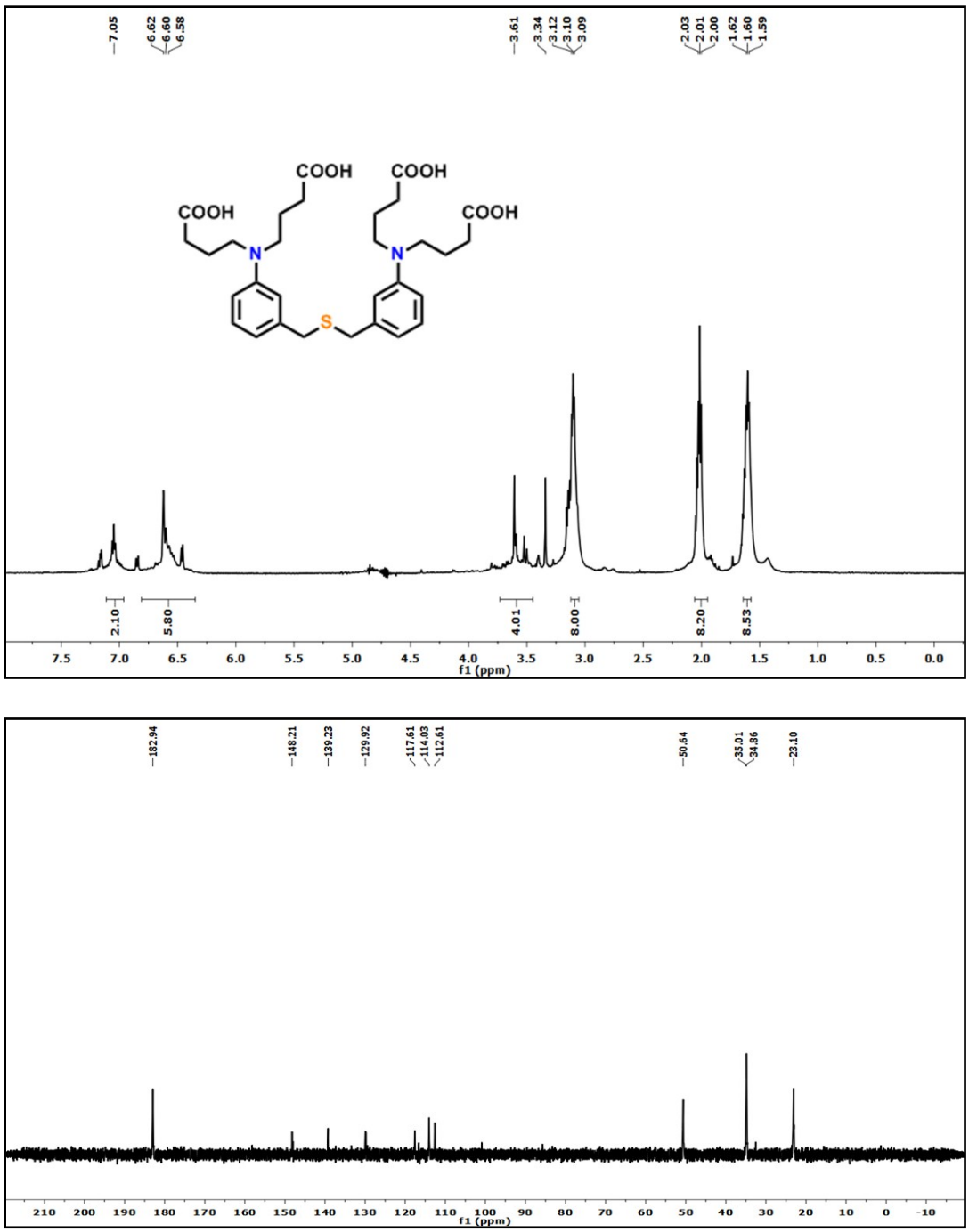


Fig. S3. ^{1}H NMR (in D_2O – CD_3OD) and ^{13}C NMR (in D_2O) spectra of **4**.

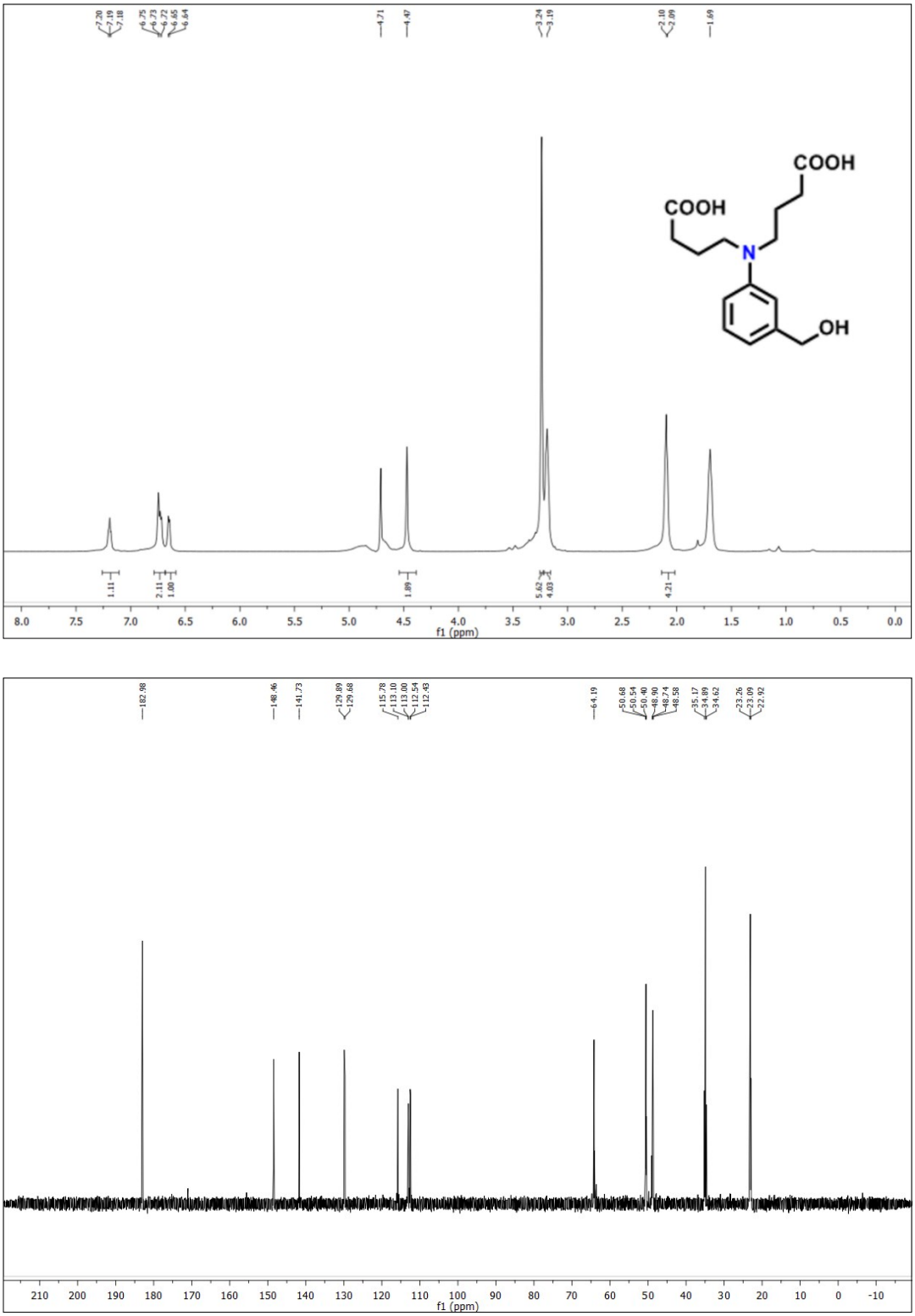


Fig. S4. ^{1}H and ^{13}C NMR spectra of the photoproduct, 4,4'-((3-(hydroxymethyl)phenyl)azanediyl)dibutanoic acid (**6**) in D_2O and few drops of CD_3OD.

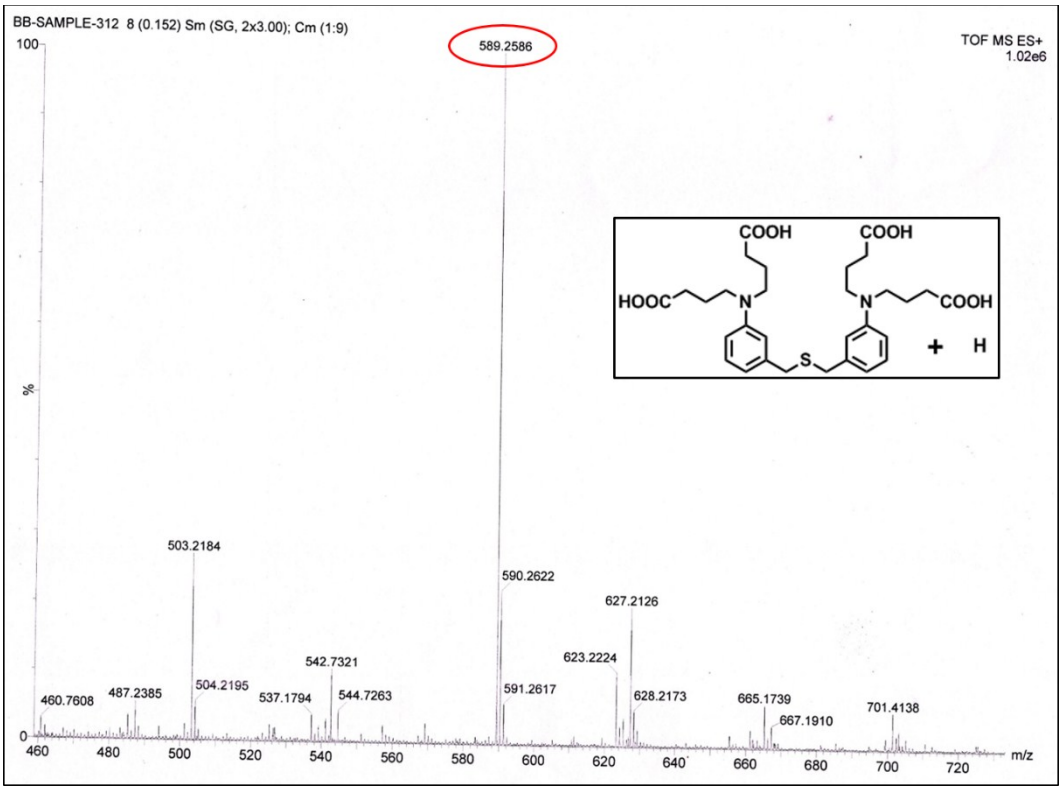


Fig. S5. HRMS of H_2S donor **4.**

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

Potassium ferrioxalate actinometry was used for the determination of incident photon flux (I_0) of the UV lamp used for irradiation. Solution of potassium ferrioxalate, 1, 10-phenanthroline and the buffer solution were prepared following the literature procedure.[1] Solution (0.006 M) of potassium ferrioxalate was irradiated using 125 W medium pressure Hg lamp as UV light source ($\geq$ 365 nm) and 1 M $CuSO_4$ solution as UV cut-off filter. At regular 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 absorbance of red phenanthroline-ferrous complex formed was then measured spectrophotometrically at 510 nm. The amount of Fe^{2+} ion was determined from the calibration graph. The calibration graph was plotted by measuring the absorbance of phenanthroline-ferrous complex at several 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 30 reported value. Using the known quantum yield (1.283 ± 0.023) for

potassium ferrioxalate actinometer at 363.8 nm,[2] the number of Fe^{2+} ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident photon flux (I_0) at 350 nm of the 125 W Hg lamp was determined as $1.55x10^{17}$ photons s^{-1} cm^{-2}.

3. Deprotection photolysis and measurement of photochemical quantum yield for H_2S donor 4:

A solution of 1×10^{-4} M of the H_2S donor **4** was prepared in ACN/PBS buffer (3:7). Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated (keeping the quartz cuvette 5 cm apart from the light source) using 125 W medium pressure Hg lamp as UV light source (≥ 365 nm) and 1 M $CuSO_4$ solution as UV cut-off filter with continuous stirring for 20 min. At a regular interval of time, 20 µL of the aliquots were taken and analyzed by RP-HPLC using mobile phase acetonitrile/water (8:2), at a flow rate of 1 mL / min (detection: UV 310 nm). Peak areas were determined by RP-HPLC, which indicated a gradual decrease of the **4** with time, and the average of three runs. The reaction was followed until the decomposition of **4** 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 **4** which suggested a first order reaction. Further, the photochemical quantum yield (Φ_p) was calculated based on the decomposition of **4** using below equation (1).

$$(\Phi)_{CG} = (\Phi)_{act} \times [(k_p)_{CG}/(k_p)_{act}]\times[F_{act}/F_{CG}] \text{ ---------- (1)}$$

Where the subscript 'CS' and 'act' denotes caged substrate and actinometer respectively. 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.

4. Methylene Blue assay for H_2S detection:

Methyleneblue assay was carried out as described previously with some modifications.[3] A 5 mM solution of Na_2S in sodium phosphate buffer (20 mM, pH 7.4)/acetonitrile (HPLC grade) (7:3) 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. Fig. S5. Standard Calibration curve with different concentration of Na_2S.

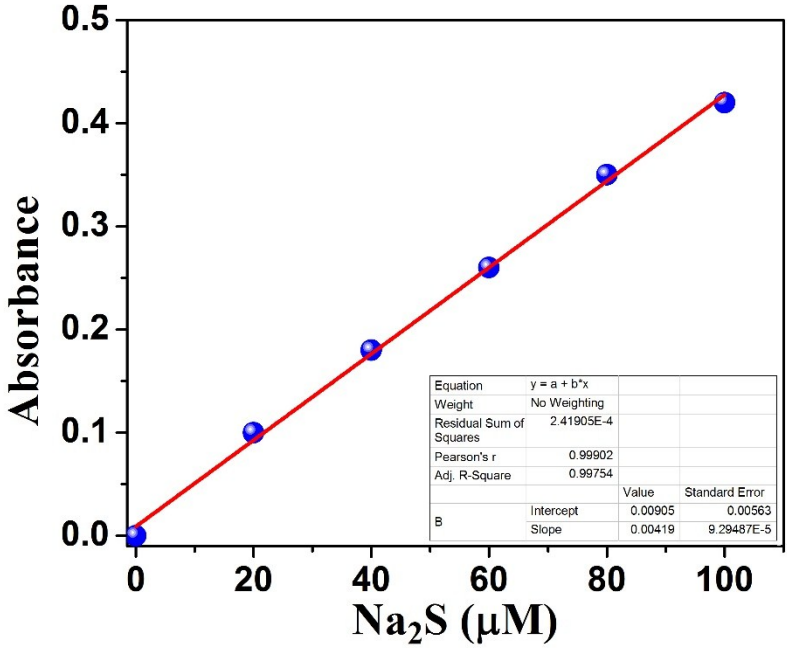


Fig. S6. Standard Calibration curve with different concentration of Na_2S.

In this experiment, a 100 µM solution (total volume 20 mL) of the compound **4** was prepared in a 7:3 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 UV-Vis light ($\lambda \geq 365$ nm) using a suitable UV cut-off filter (1M $CuSO_4$ 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- 11 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,Ndimethyl-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).

5. Spatio Control Study:

To shows the spatio control of our H_2S donor 4 by UV light we carried out the experiment as follows. We prepared six solutions in six different test tubes containing similar concentration of the dye (D1), H_2S donor (4) in water (1 x 10^{-4} M). Then a homemade photomask (a cardboard covered with aluminium foil with a hole middle of it of such a size that only two test tubes can exposed by UV light) placed over the test tubes such a way that only two of them can exposed by the UV light. After that we irradiated the system for 30 mins by a medium-pressure mercury lamp (125 W) as the source of light ($\lambda \geq 365$ nm) using 1 M $CuSO_4$ solution as a UV cut-off filter. After removing the photomask we found that the color of the two test tubes those were exposed to UV light changed color while others are remain same. Hence, we can conclude that our synthesized H_2S donor is capable of spatio control to release H_2S on demand.

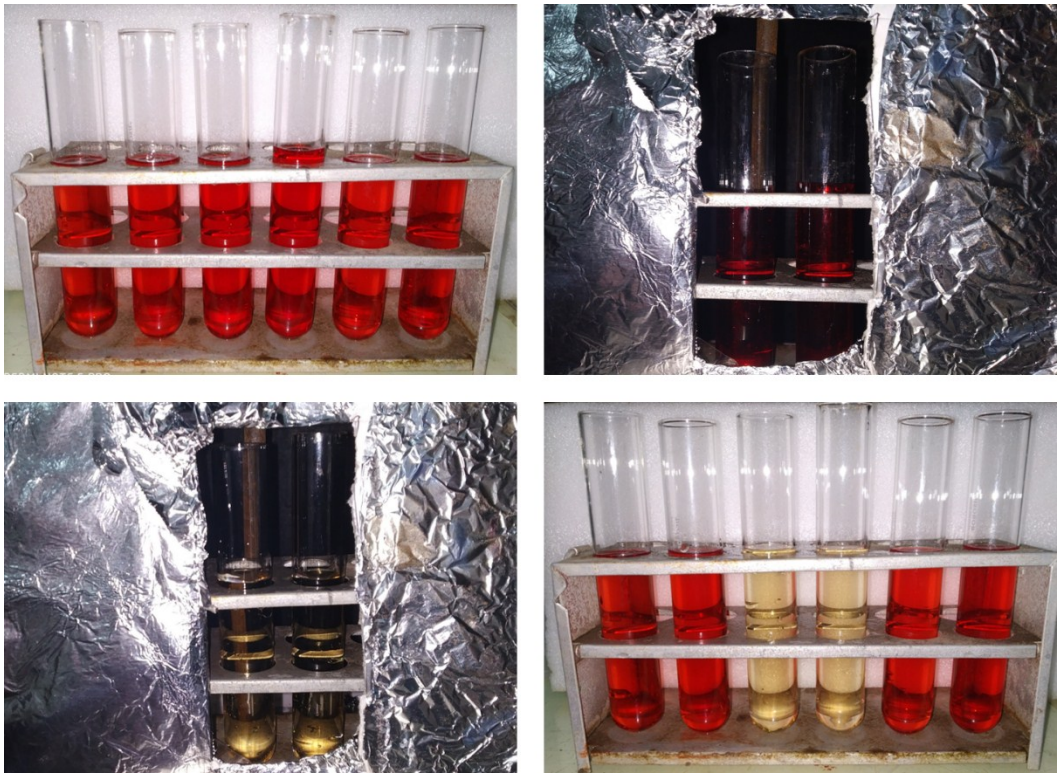


Fig. S7. Spatio control studies for H_2S donor **4** in presence of dye (D1).

Furthermore, we carried out two different experiments on parallel (i) irradiated dye (10 μM) by UV-Vis light ($\lambda \geq 365$ nm) in the presence of H_2S donor **4** (100 μM) in PBS buffer (10 mM) pH~7.4) with continuous stirring (ii) dye (10 μM) in PBS buffer (10 mM, pH~7.4) was mixed with Na_2S (100 μM) in the absence of an external light. We recorded the absorption spectrum (**Fig. S8**) of both the experiments and we noted the absorption spectrum was similar except in the case of light the release was in a controlled fashion. Hence from the above experiments, we can infer that the photoproduct has no role in the quenching the fluorescence of the dye.

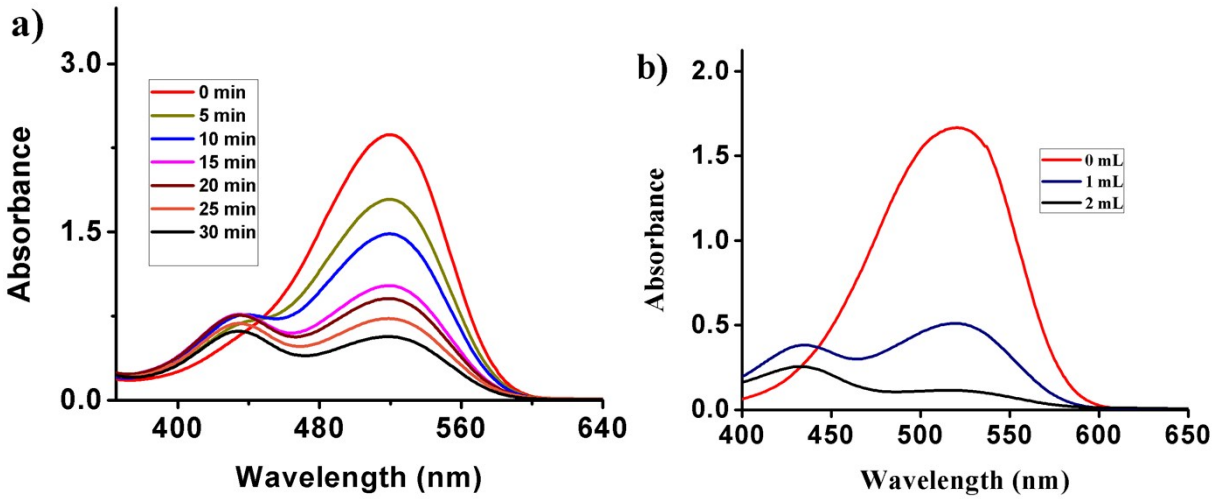


Fig. S8. Absorption spectra (a) dye (D1) and compound **4** at different irradiation time using UV light. (b) dye (D1) and Na_2S without irradiation by UV light.

6. Photostability of N,N-dimethylaniline−hemicyanine dye (D1):

The N,N-dimethylaniline−hemicyanine dye known to have good photostability.[1] We took 3 ml of 10^{-4} M dye (D1) in water and irradiated under a medium-pressure mercury lamp (125 W) as the source of light ($\lambda \geq 365$ nm) using 1 M $CuSO_4$ solution as a UV cut-off filter. After 30 mins of irradiation, we observed the emission intensity using fluorescence spectrophotometer. We found a negligible change in fluorescence intensity and almost same color of the solution (naked eye) before and after the experiment.

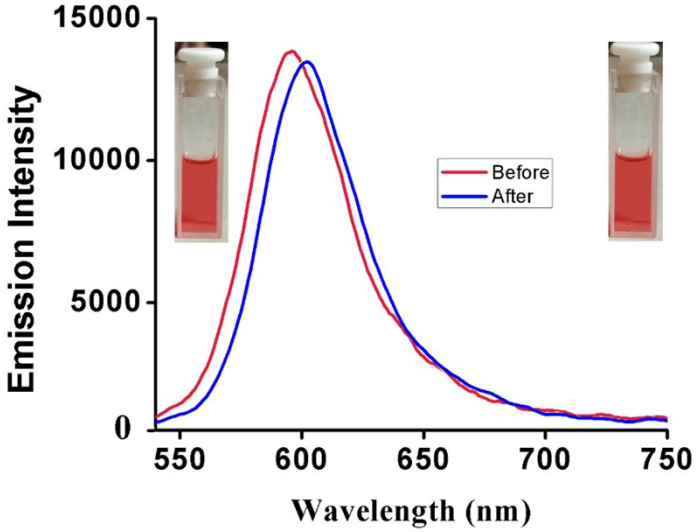


Fig. S9. Emission Spectrum of N,N-dimethylaniline−hemicyanine dye (D1) before and after irradiation of 30 mins .

7. Experimental procedure for biological application study: In vitro real-time cellular uptake and localization studies of H_2S donor **4** 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 **4** 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 with light (≥ 365 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 **4** on the HeLa cell line before and after photolysis: The cytotoxicity assay of H_2S donor **4** 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 plates at 1×10^4 cells / mL. Different concentrations of H_2S donor **4** (5, 10, 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 UV-Vis light (λ ≥ 365 nm) by a 125 W medium pressure Hg lamp using a suitable filter (1 M $CuSO_4$ 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 an appropriate volume of DMSO and absorbance readings were taken at 595 nm using a 16 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].

8. Cellular Internalization and Cell Viability study:

H_2S releasing capability of **4** was measured in the live cells using H_2S−sensitive probe D1 by confocal microscopy imaging. Cervical cancer cells (HeLa) were incubated with **4** and D1 for 6h followed by irradiation with light (λ ≥ 365 nm) for 30 min. Initially the cells emitted bright red fluorescence from the incubated dye (D1) (**Fig. 10**). With the increase in irradiation time from 0-30 min a gradual decrease in the fluorescence intensity of the cells was observed, indicating a steady time dependent release of H_2S from **4 (Fig. S10a-c**). The cells incubated with dye D1 that were either treated only with compound **4** or exposed only to light without compound **4** showed no significant decrease in fluorescence intensity of D1 (**Fig. S10 e,f**). On the contrary, when the cells incubated with dye D1 were exposed

light for 30 min in presence of compound **4,** showed a significant decrease in fluorescence intensity of D1, indicating the intracellular release of H_2S from compound **4** in presence of light (**Fig. S10g**).

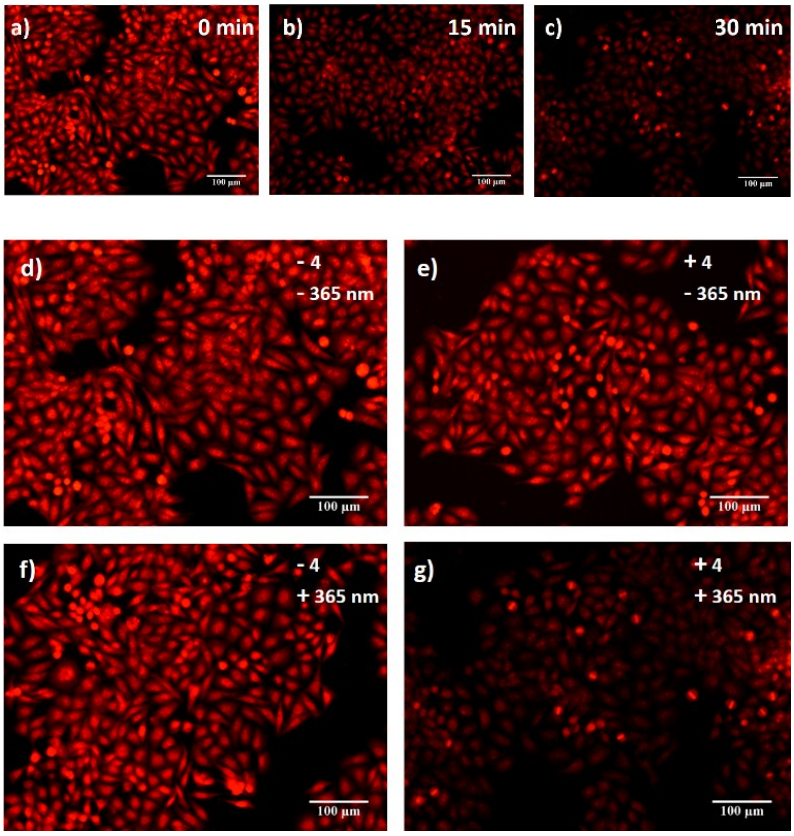


Fig. S10 Confocal microscopy images of H_2S release from **4**. Gradual release of H_2S from **4** monitored using H_2S sensitive fluorescent probe (D1) at different time intervals during irradiation with light ($\lambda \geq 365$ nm), (a) 0 min; (b) 15 min; (c) 30 min. HeLa cells were incubated with D1 for 6 h, washed and then (d) incubated for 30 min in the dark; (e) treated with 60 µM **4** for 30 min in the dark (f); exposed to light ($\lambda \geq 365$ nm) for 30 min; (f) treated with **4** and exposed to red light ($\lambda \geq 365$ nm) for 30 min.

Also we calculated the fluorescence intensities of images in **Fig. 7** provided in manuscript by using ImageJ software. We found the fluorescence intensity decreased 5 times after 30 mins of irradiation by UV light in HeLa cells.

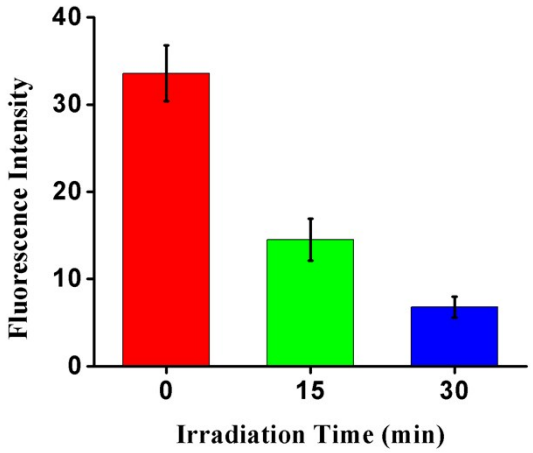


Fig. S11 Changes in fluorescence intensities with different irradiation time (min) from the confocal microscopy images.

9. References:

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