Supporting Information

Spatiotemporal Photorelease of Hydrogen Sulphide from β-Carboline Derived Nanoparticles for Therapeutic Applications

Antara Sikder[a], Rakesh Mengji,[b,c] SaugatMondal,[a] AvijitJana[b,c] and N. D. Pradeep Singh[a]**

[a]*Department of Chemistry, Indian Institute of Technology Kharagpur*

[b]*Department of Natural Products and Medicinal Chemistry, CSIR-Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad-500007, India*

[c]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India-201002*

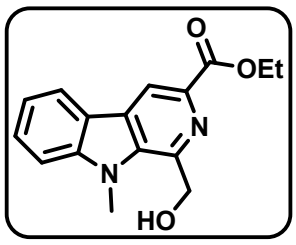
Contents

1. Materials and methods

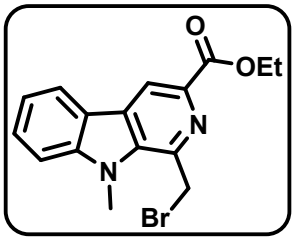
All starting materials were obtained from commercial suppliers (Sigma-Aldrich, Merck, Alfa Aesar, BLD Pharm) and used without further purification. Analytical thin-layer chromatography (TLC) was performed on Merck's silica gel 60 F254 precoated aluminum TLC plates. Preparative column chromatography was performed on silica gel mesh size 60-120. ^{1}H (400 MHz and 500 MHz) spectra were recorded on a BRUKER-AC 400 and 500 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane with the solvent resonance as the internal standard ($CDCl_3$: 7.26 ppm, DMSO-d_6: 2.5 ppm). Coupling constants (J) are reported in Hertz (Hz). ^{13}C NMR (125 MHz) spectra were recorded on a 500 MHz Spectrometer. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance the internal standard (deuterochloroform: 77.0 ppm DMSO-d6: 39.5 ppm). The exact masses were recorded on an Agilent mass spectrometer. The photolysis of the H_2S donor was carried out using a 125W medium-pressure Hg lamp supplied by SAIC (India). Analytical RP-HPLC measurements were employed using an Agilent ZORBAX SB-C18 column (4.6 × 250 mm) in which the stationary phase is 5 μm silica with a pore size of 80 Å. The chromatograms were detected by a UV-Vis diode array (254 and 300 nm). The samples were dissolved in HPLC grade MeCN-H_2O mixtures or in THF water in the case of nanoparticles. Spectroscopic measurements were performed on a Shimadzu UV-2450 UV/vis spectrophotometer. Fluorescence emission spectra were recorded on a Shimadzu RF-6000 fluorescence spectrophotometer. Quartz cuvettes with a path length of 1 cm were used. Quinine sulphate in 0.1 M H_2SO_4 was used for the fluorescence quantum yield determination with a Φ_{fl} value of 0.54.

2. Synthesis of the compounds 2, 3, and BCS

Synthesis of compound 2:



To a solution of compound **1**[1] (1 g, 3.54 mmol) in dry THF, $LiAlH_4$ (157 mg, 1.2 eq.) was added at 0 ◦C and stirred for 1 hour. Reaction progress was monitored by TLC and quenched with sat. Na_2SO_4. The organic part was extracted with ethyl acetate and concentrated in vacuo. The product was purified by column chromatography with an eluent of ethyl acetate: Hexane 1:1 and separated as a yellow solid. Yield: 96 %, 967 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.65 (t, J = 7.7 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.36 (t, J = 7.5 Hz, 1H), 7.25 (s, 1H), 5.34 (s, 2H), 4.48 (q, J = 7.1 Hz, 2H),

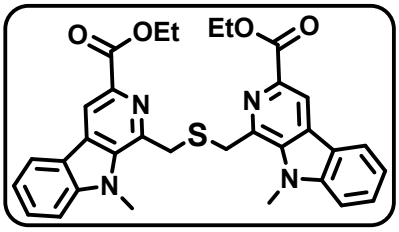


4.13 (s, 3H), 1.47 (t, J = 7.1 Hz, 3H).. ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.12, 142.86, 142.40, 136.19, 129.74, 129.33, 122.12, 121.89, 121.30, 117.54, 110.29, 63.03, 61.83, 32.50, 14.85. HRMS (ESI): calcd. for $C_{16}H_{17}N_2O_3^+$ $[M+H]^+$ 285.1234, found 285.1234.

Synthesis of compound 3:

To 20 ml of dry DCM solution of compound **2** (500 mg, 1.75 mmol) in ice-cold condition, 200 µL PBr_3 was added dropwise to the solution and stirred for 2 hours in rt. The reaction was monitored by TLC and quenched with water. The organic layer was separated by ethyl acetate and concentrated in vacuo. The pure product was obtained as a white powder by column chromatography (Eluent EtoAc: H_2O = 30:70). Yield 86 %, 524 mg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.66 (t, J = 7.7 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.36 (t, J = 7.5 Hz, 1H), 5.15 (s, 2H), 4.52 (q, J = 7.1 Hz, 2H), 4.30 (s, 3H), 1.48 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.72, 142.82, 139.37, 137.37, 136.43, 130.82, 129.30, 121.72, 121.46, 121.14, 117.99, 110.08, 61.71, 33.26, 32.10, 14.61. HRMS (ESI): calcd. for $C_{16}H_{16}BrN_2O_2$ $[M+H]^+$ 347.0390, obtained 347.0397.

Synthesis of 1,1'-(thiobis(methylene))bis(N-methyl-β-carboline-3-carboxylate (BCS):



To compound **3** (200 mg, 0.57 mmol) in 20 ml of acetone-water (8:2 v/v), 0.6 eq., 26 mg of $Na_2S•9H_2O$ in water was added dropwise in ice-cold condition and slowly warmed to rt. Thereafter reaction was stirred for 20 min. Reaction progress was monitored by TLC and quenched with water. The product was purified by column chromatography. Yield: 48 %, 157 mg as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 8.37 (d, J = 7.7 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.65 (t, J = 7.7 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 4.67 (s, 2H), 4.29 (q, J = 7.0 Hz, 2H), 4.15 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 166.8, 164.6, 147.5, 141.8, 140.6, 135.6, 135.4, 128.5, 121.4, 120.2, 116.1, 110.3, 60.2, 35.9, 31.6, 14.0. HRMS (ESI): calcd. for $C_{32}H_{31}N_4O_4S^+$ $[M+H]^+$ 567.2061, found 567.2074.

3. Spectrum of the synthesized compounds

3.1 NMR of compounds 2, 3, and BCS

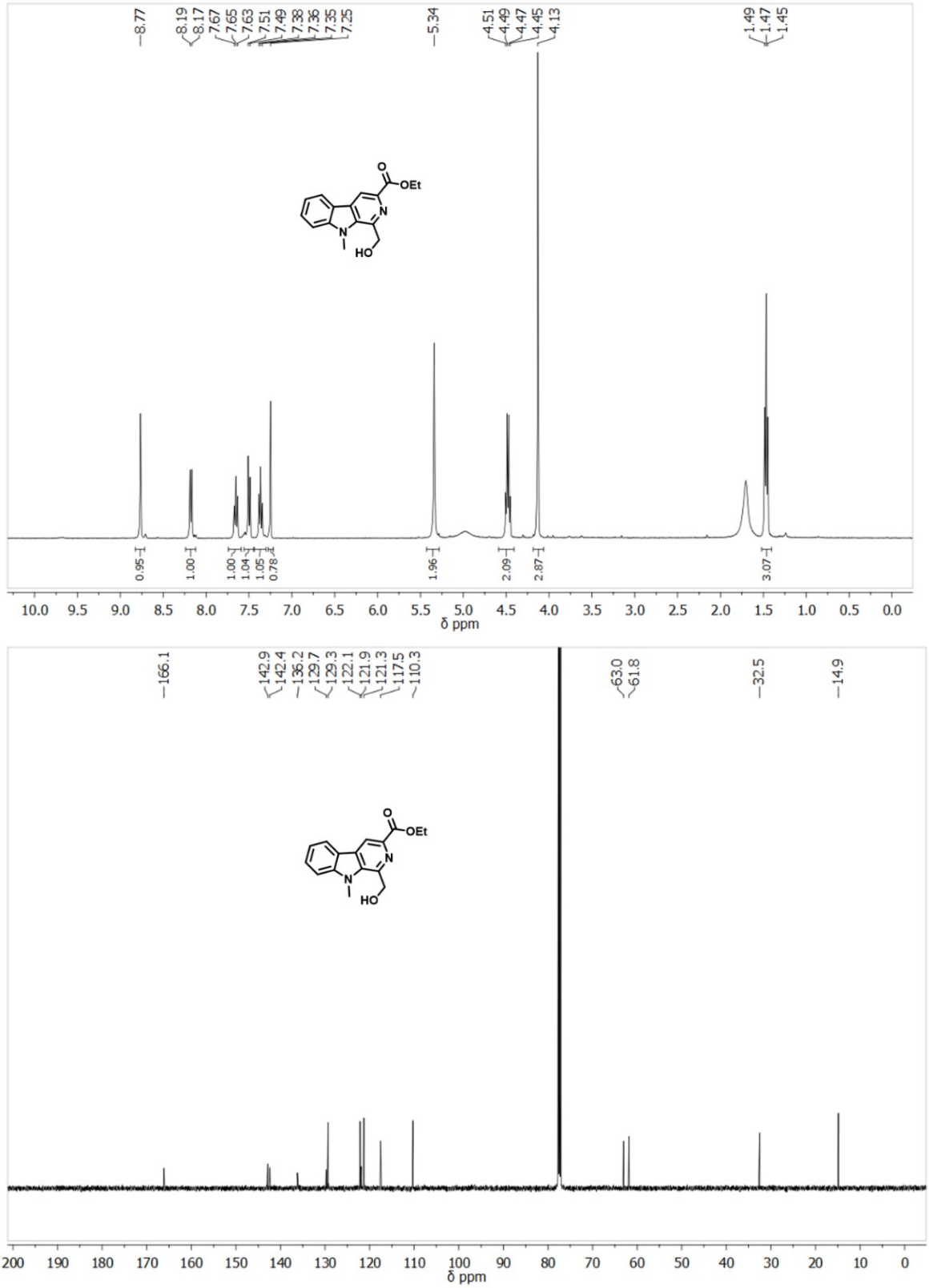


Figure S1: ^{1}H and ^{13}C- NMR spectrum of compound **2**.

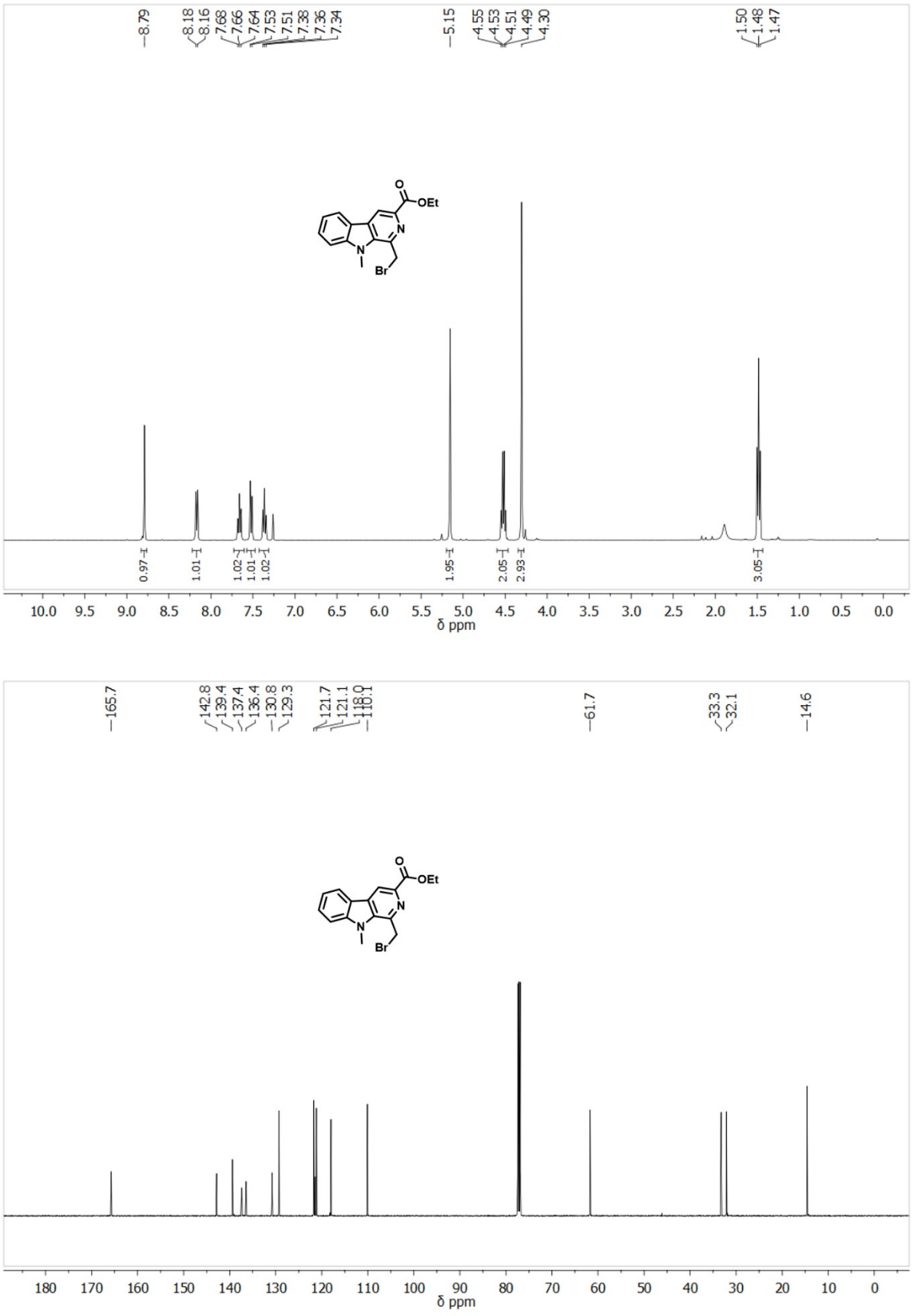


Figure S2: ^{1}H and ^{13}C-NMR spectrum of compound **3**.

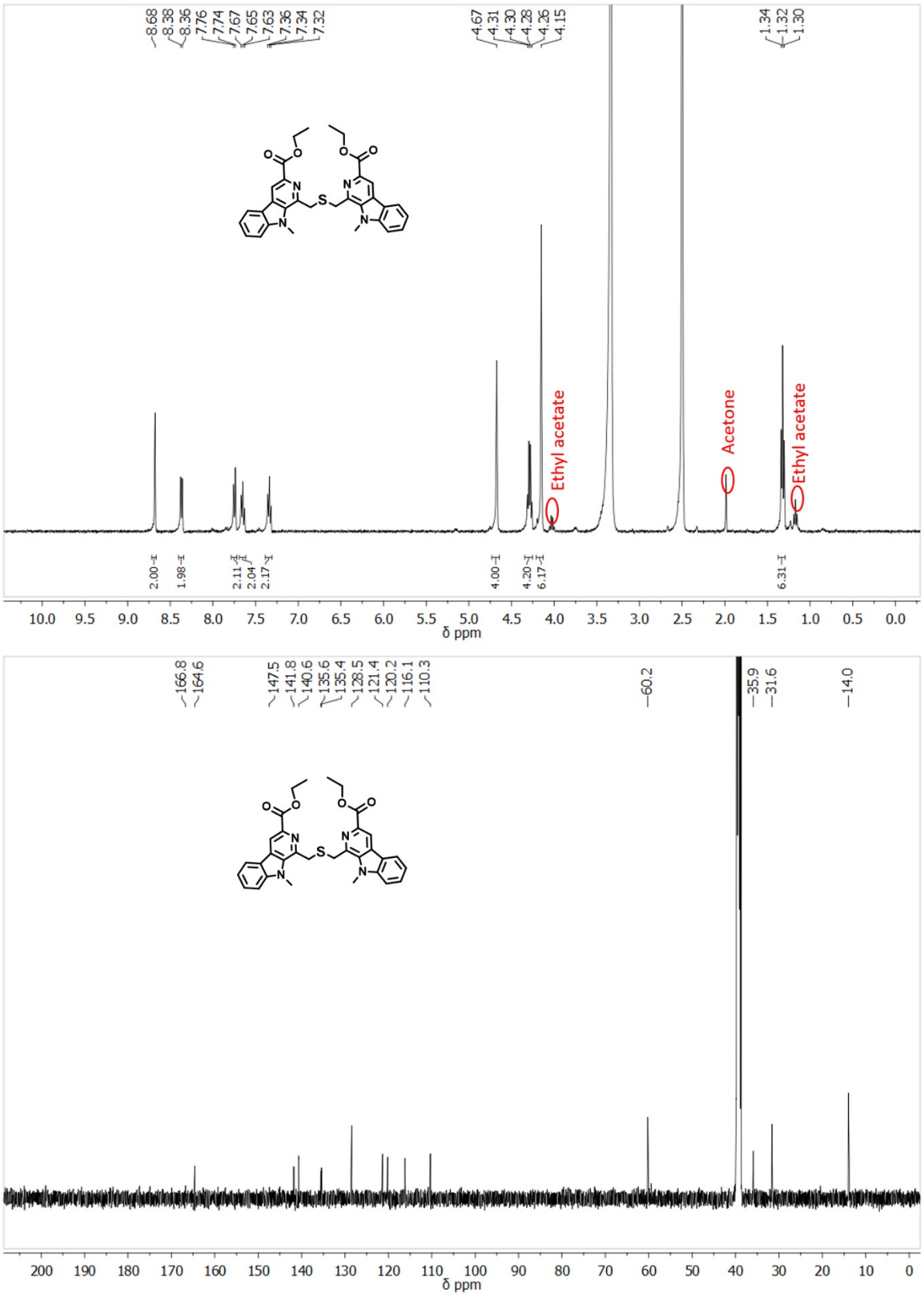


Figure S3: ^{1}H and ^{13}C-NMR spectrum of **BCS**.

3.2 HRMS spectra of the compounds

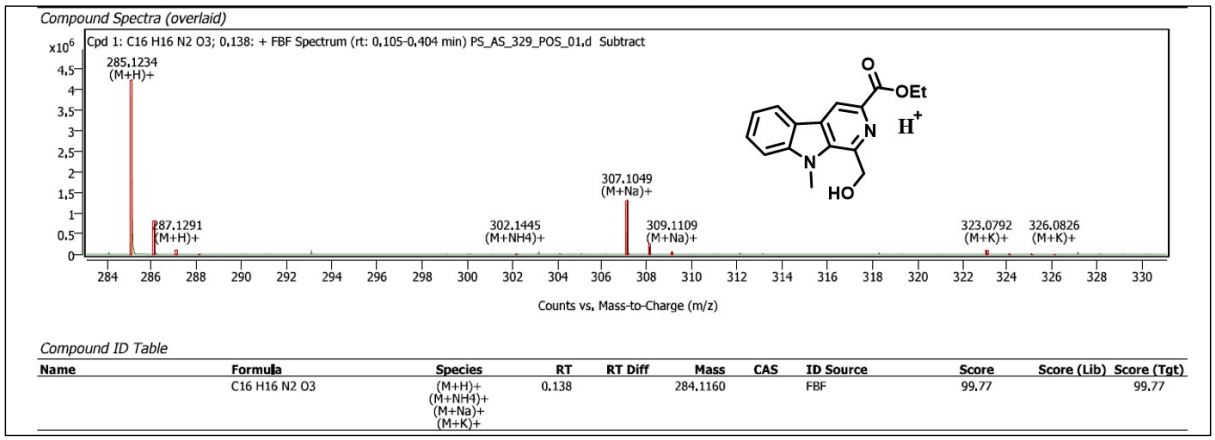


Figure S4: HRMS spectrum of compound **2**.

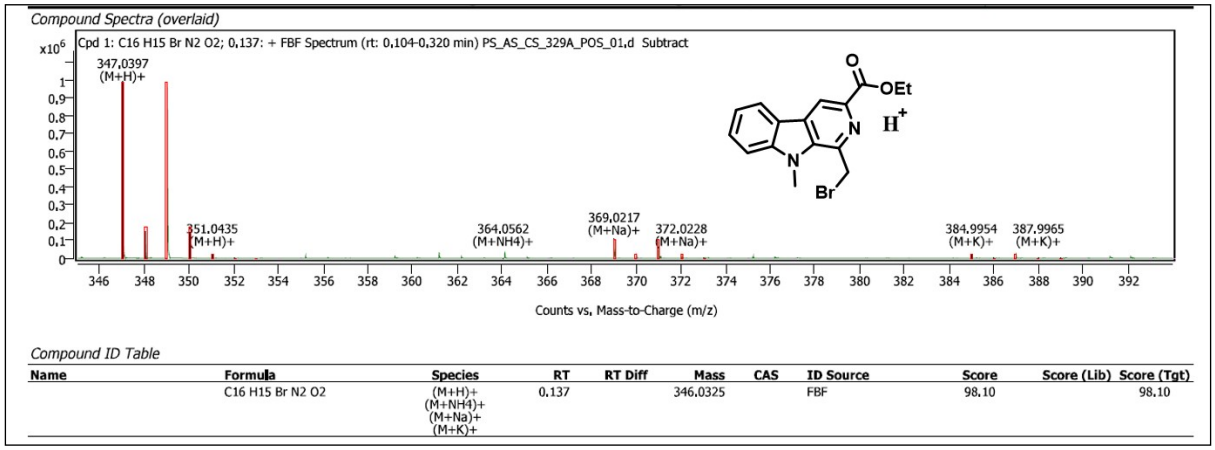


Figure S5: HRMS spectrum of compound **3**.

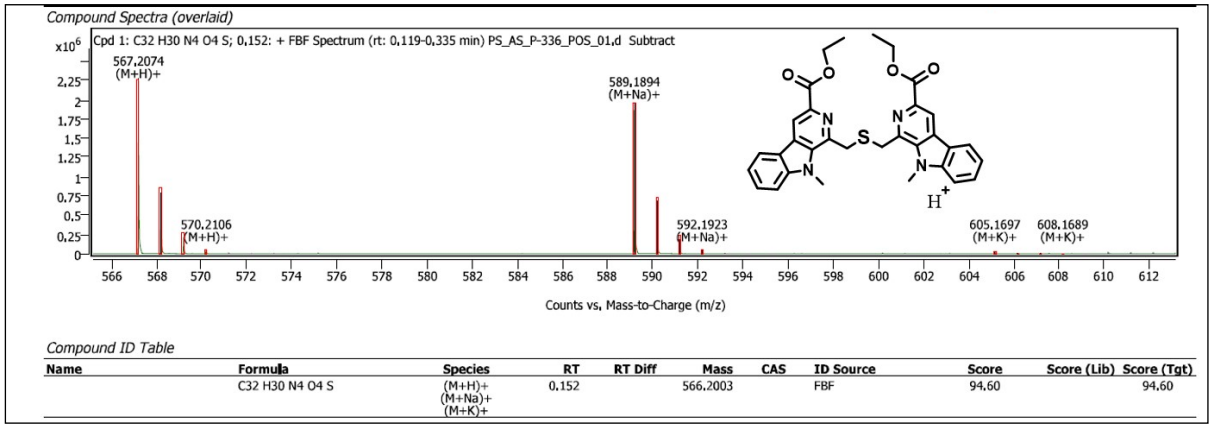


Figure S6: HRMS spectrum of **BCS**.

4. Characterisation of BCS nano, Plu@BCS nano, and Plu@BCS hydrogel

4.1 Zeta potential of BCS nanoparticles Plu@BCS nanoparticles

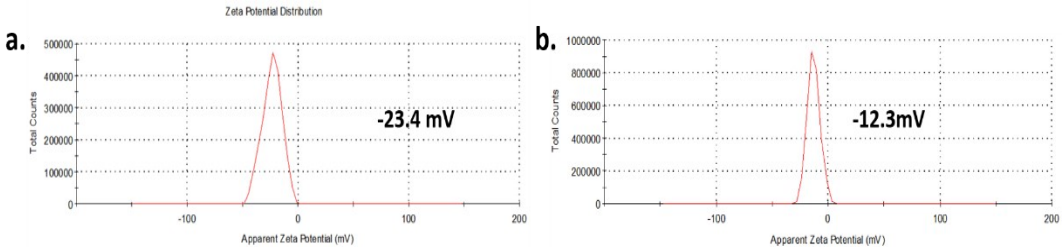


Figure S7. Zeta potential of (a) BCS nanoparticles and (b) Plu@BCS nanoparticles.

4.2 SEM images and EDAX spectrum of Plu@BCS hydrogel

The Plu@BCS hydrogel and pluronic hydrogels were freeze-dried to obtain a flaky white solid and stored in freeze. The samples were gold coated before taking the SEM images.

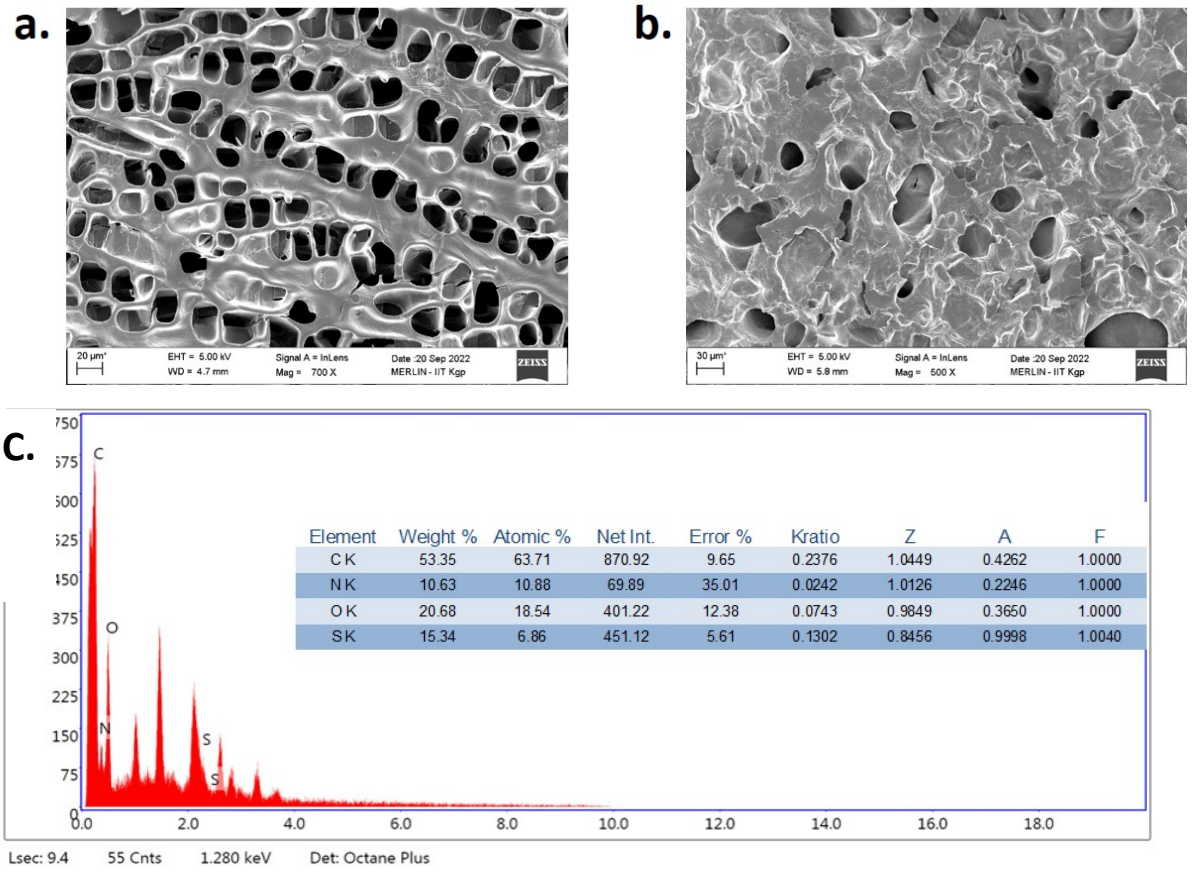


Figure S8. SEM images of a) Plu@BCS hydrogel and b) Pluronic hydrogel, and c) EDAX spectrum of Plu@BCS hydrogel.

5. Hydrolytic decomposition of BCS nano and Plu@BCS nano

The hydrolytic decomposition of the sample solutions (100μM in PBS buffer) was kept in the dark, and HPLC was recorded for upto 7 days.

Table S1: Hydrolytic decomposition of BCS nano and Plu@BCS nano.

	% of decomposition			
	1 Day	**3 Days**	**5 Days**	**7 Days**
BCS nano	<1	~7	~16	~25
Plu@BCS nano	<1	~5	~8	~15

6. Spectroscopic characterization

6.1 Measurement of fluorescence quantum yield

The fluorescence quantum yield (QY) of the compound BCS and BCS nano were determined by the reference point method. A solution of quinine sulfate ($\Phi_f = 0.54$ in 0.1 M H_2SO_4) was used as a standard for the fluorescence quantum yield determinations. An error of 10% is estimated for the fluorescence quantum yields. The absorbance values of the solutions at the excitation wavelength were measured with Shimazu 2600 UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra of all the sample solutions were recorded by Hitachi F-7000 fluorescence spectrophotometer at the respective excitation wavelength of compounds.

$$\frac{\Phi_S}{\Phi_R} = \frac{A_S}{A^R} \times \frac{(Abs)_R}{(Abs)_S} \times \frac{\eta_S^2}{\eta_R^2}$$

Where Φ represents quantum yield, Abs represents absorbance, A represents the area under the fluorescence curve, and η is the refractive index of the medium. The subscripts S and R denote the corresponding parameters for the sample and reference, respectively.

7. Uncaging studies

7.1 Experimental details

BCS solution 50 μM in ACN-H_2O (1:1 v/v) was prepared and placed in front of medium pressure Hg lamp with a suitable solution filter at 5 cm distance. We used 1M $CuSO_4$ solution filter to obtain $\lambda \geq 365$ nm and 10 wt% $NaNO_2$ solution filter to obtain $\lambda \geq 410$ nm wavelength

from the 125-watt Hg lamp. The solution was irradiated for a certain time interval and monitored by UV, fluorescence spectroscopy, and HPLC chromatography.

7.2 Photochemical quantum yield determination

Uncaging quantum yield Φ_u of a photocage is usually defined as $\Phi_u = (I \cdot 1000 \cdot \varepsilon \cdot t_{90})^{-1}$ where I is the irradiation intensity (in einstein.cm^{-2}s^{-1}), ε the molar extinction coefficient (in M^{-1}cm^{-1}), and t_{90} the irradiation time necessary to reach 90% conversion (in s).

The uncaging sensitivity of our H_2S donor was determined in a relative way by comparing their conversion kinetics with respect to a reference [β-carboline] (Φ_u = 0.6 in ACN/water)[3] for photolysis at 365 nm). According to the previous equation, the ratio of the rate constants is directly proportional to the uncaging sensitivities of the new compound ($\varepsilon^{com}Q^{com}{}_u$) and the reference compound ($\varepsilon^{ref}\Phi^{ref}{}_u$):

$$\frac{K^{com}}{K^{ref}} = \frac{\varepsilon^{com} Q^{com}_u}{\varepsilon^{ref} \Phi^{ref}_u}$$

Determination of the ε values at the excitation wavelength, measured in the solvent of the photolysis, then allowed the determination of the ratio Q^{com} / Φ^{ref}_u .The conversion rate (K) was monitored over time by RP-HPLC.

7.3 Full ^{1}H-NMR spectra of BCS photocage during photolysis

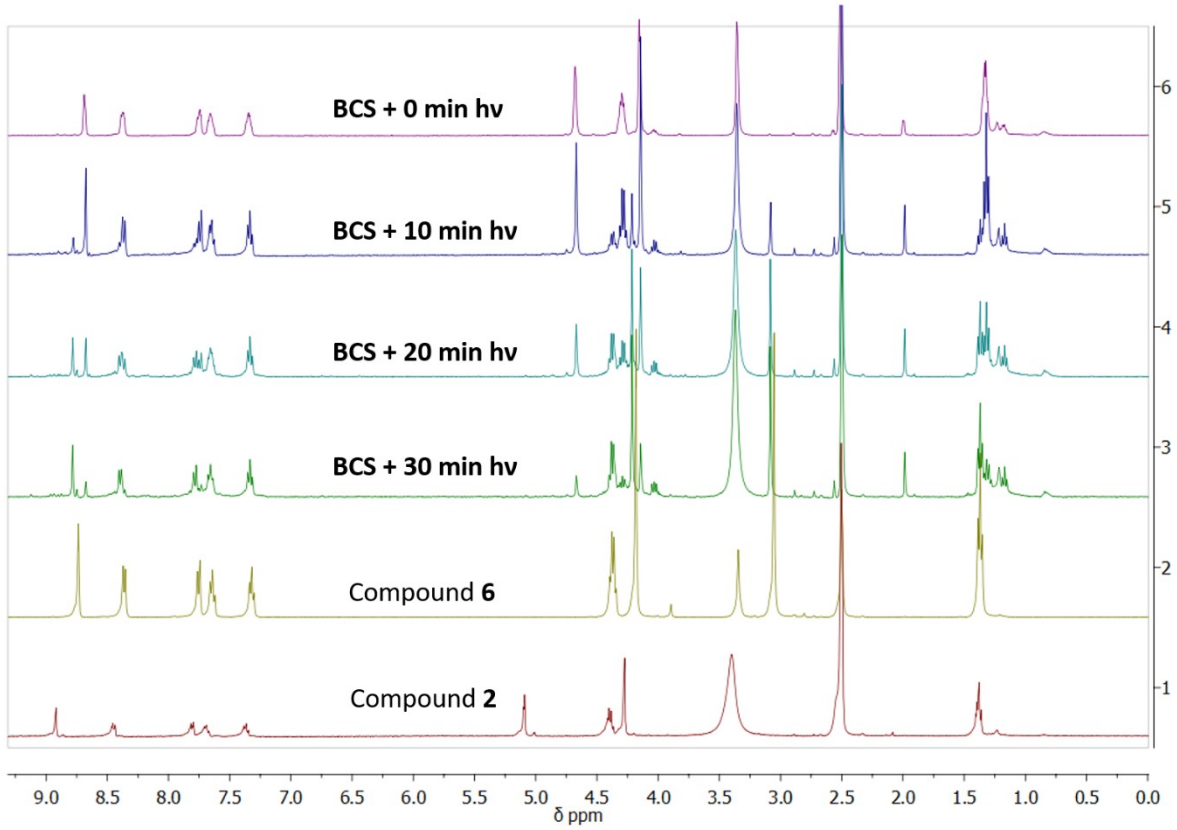


Fig S9. Full ^{1}H-NMR spectra of BCS photocage in DMSO-d_6 during photolysis with ≥365 nm for 30 min

7.4 HRMS of photoproducts

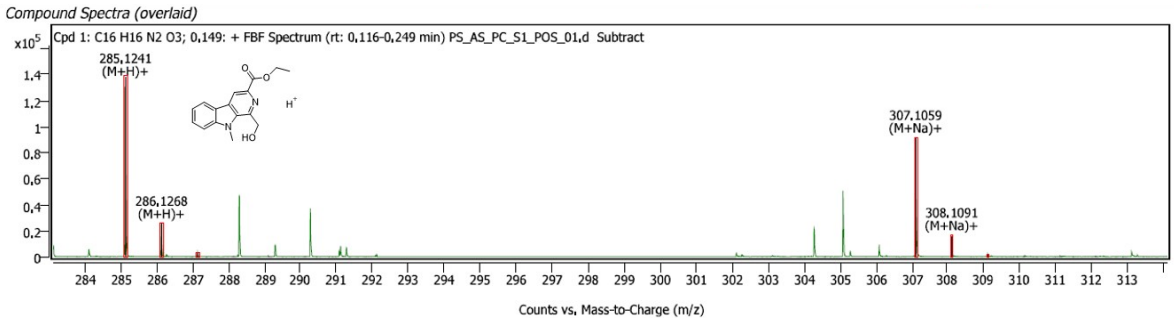


Fig S10. HRMS spectrum of BCS photoproduct.

7.5 Fluorescence change of BCS photocage upon light irradiation

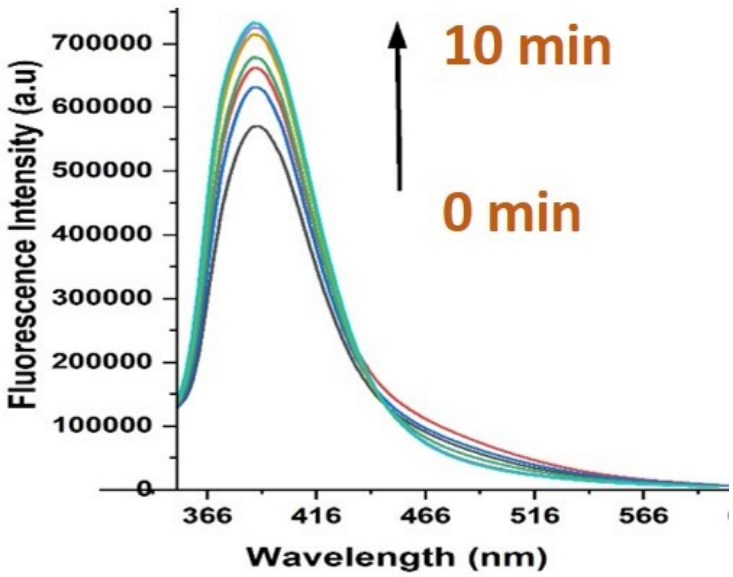


Fig S11. Change in emission intensity of BCS photocage upon UV light irradiation.

7.6 Rate constant for photolysis of BCS photocage

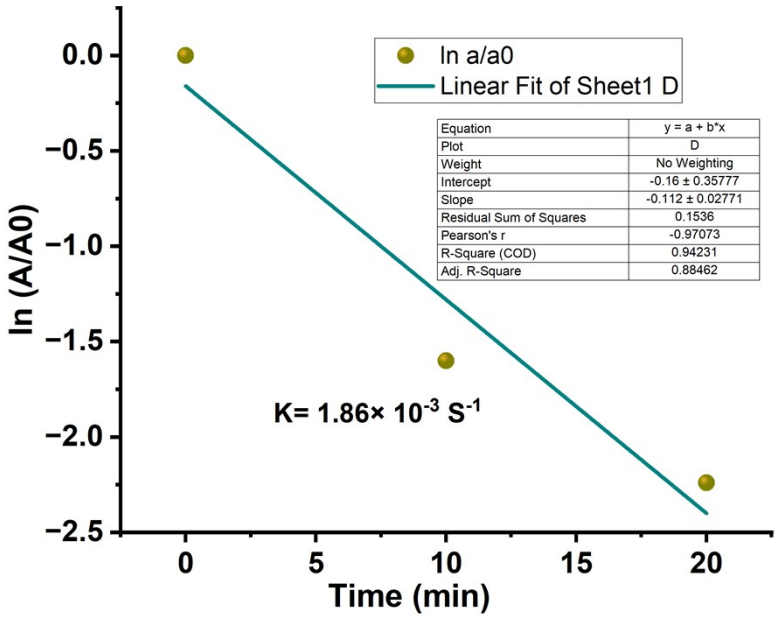


Figure S12: First order rate constant for the photolysis of BCS photocage.

7.7 Generation of H_2S from Plu@BCS nano with visible light irradiation

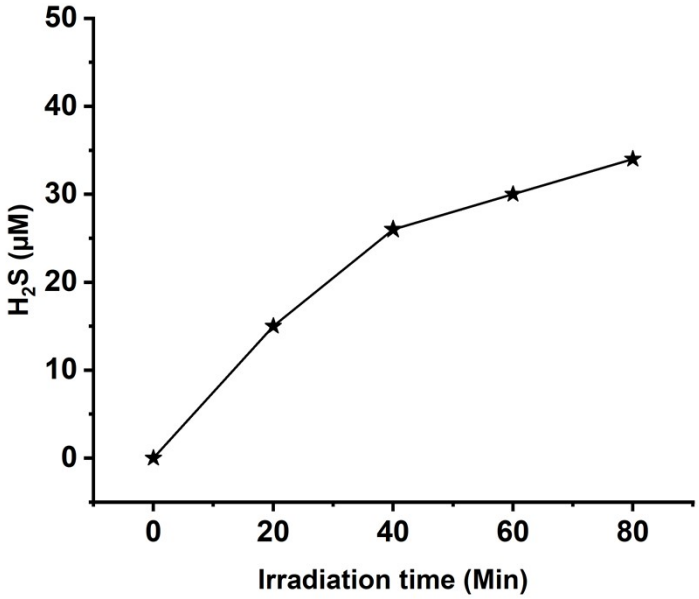


Figure S13. Generation of H_2S from Plu@BCS nano with visible light irradiation ≥ 410 nm.

8. H_2S detection and quantification from BCS by H_2S selective fluorescent probe (D1):

8.1 Physical appearance of the cuvette during photolysis of BCS solution

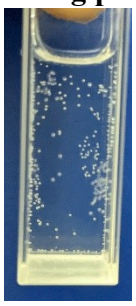
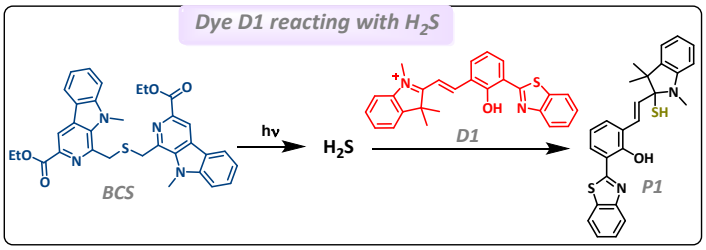


Fig S14: Tiny H_2S bubbles formation during photolysis of BCS solution

8.2. Reaction scheme of H_2S detection from BCS with specific dye D1



Scheme S1: Schematic presentation of detection of H_2S generated from BCS upon light irradiation with H_2S-specific dye D1

8.3 Control experiment of dye D1 with Light

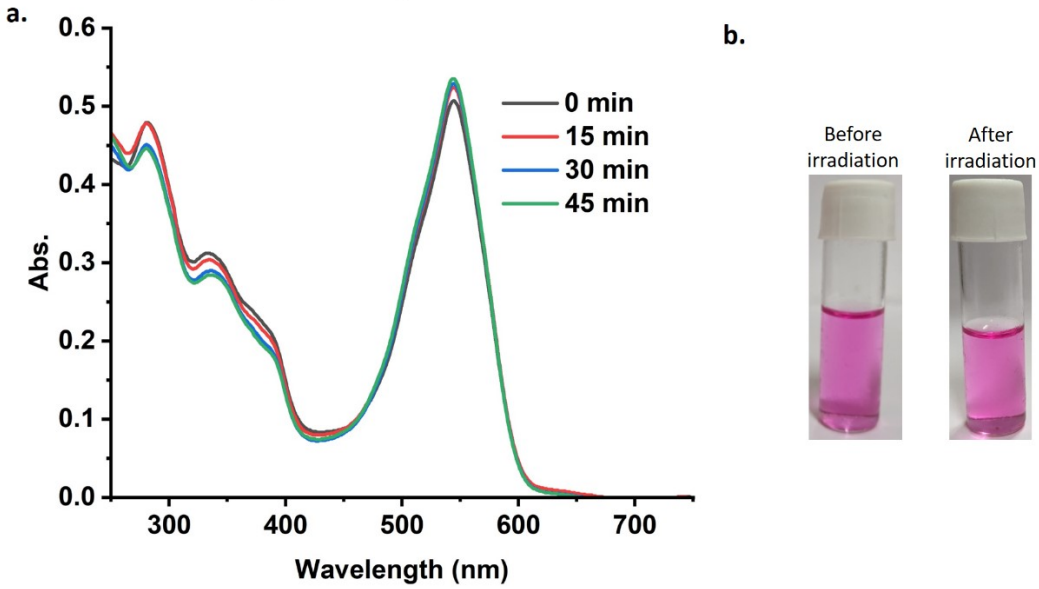


Fig S15: a) Change of UV absorption spectrum of Dye D1 with light irradiation b) colour of D1 solution before and after light irradiation.

8.4. Calibration curve for H_2S quantification

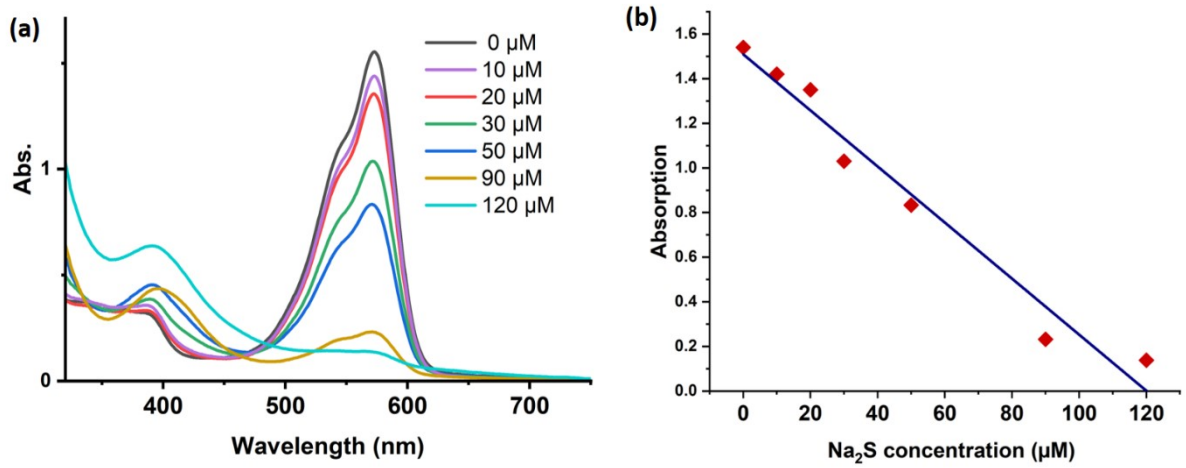


Figure S16. a) Change in the UV spectrum of dye D1 with reaction with different concentrations of Na_2S (0-120 µM) b) Calibration curve for determining H_2S concentration generated from BCS photocage.

To determine the amount of H_2S generated from BCS, we only take account of the change in absorption at 573 nm and not the ratio of A390/570 because our compound BCS absorbs at 390 nm, which will interfere with the calculation. The dye absorption at 573 nm changes proportionally with H_2S concentration, and our molecule has no absorption at 573 nm.

9. Mechanism of photorelease from BCS photocage

9.1 Formation of the carboline-TEMPO adduct during photolysis

BCS and TEMPO 1:1 w/w were taken in an NMR tube in acetonitrile-d_3 and irradiated for 30 min at 365 nm. NMR is obtained after irradiation.

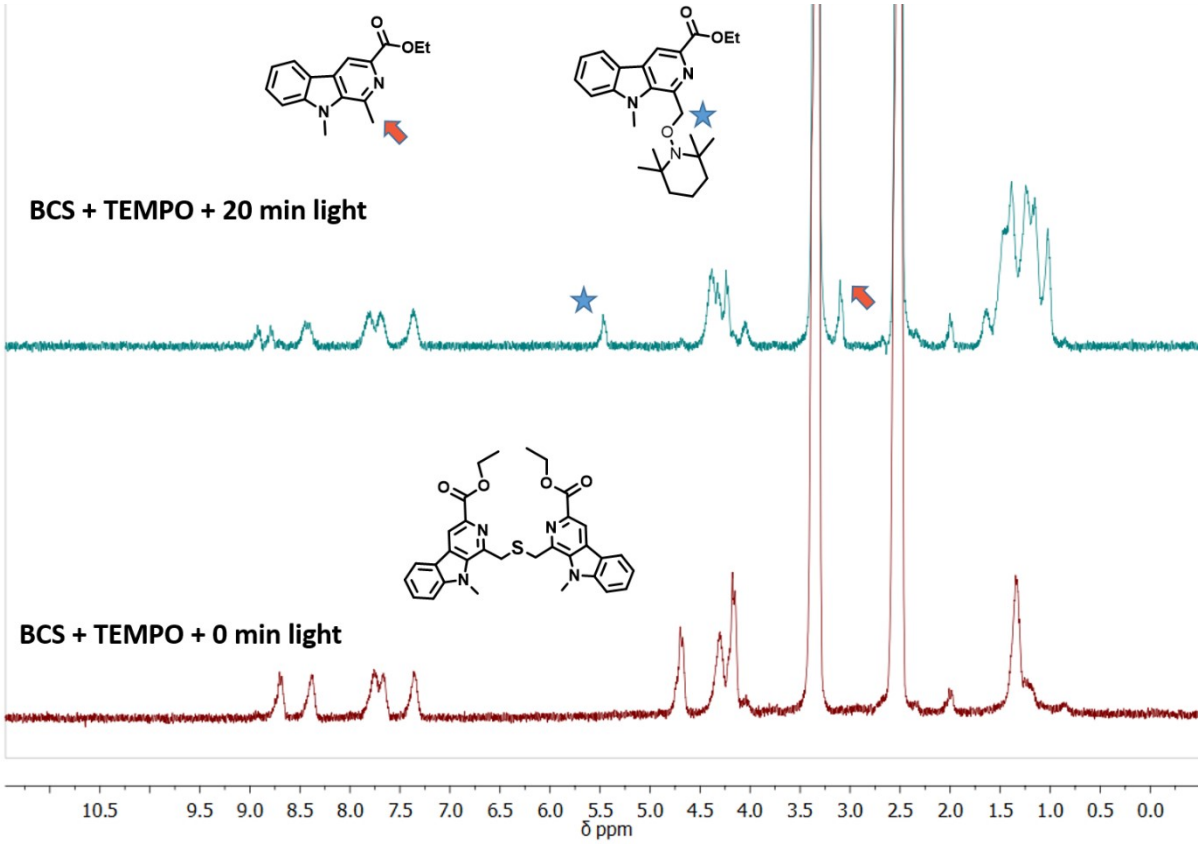
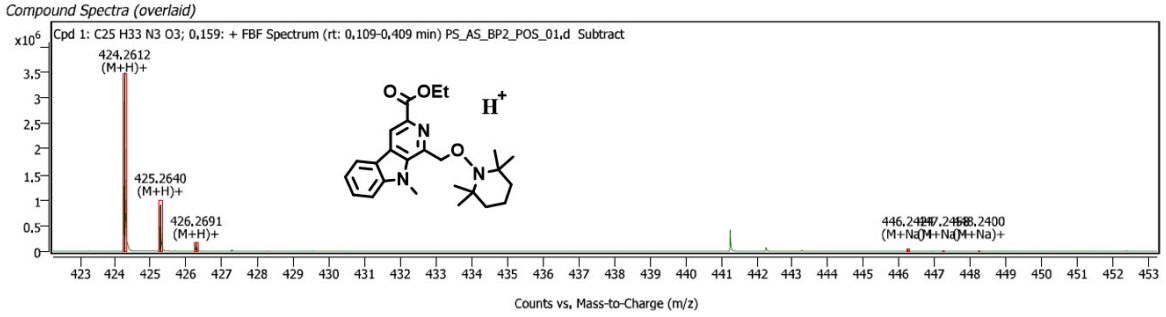


Figure S17. Stacked spectra of (BCS + TEMPO) at different time intervals of photolysis.

9.2 HRMS of the β-carboline-TEMPO adduct



Compound ID Table

Name	Formula	Species	RT	RT Diff	Mass	CAS	ID Source	Score	Score (Lib)	Score (Tgt)
	C25 H33 N3 O3	(M+H)+ (M+Na)+	0.159		423.2539		FBF	91.70		91.70

Figure S18. HRMS of the TEMPO adduct of BCS during photolysis.

10. Cell viability assay

HEK 293 and MDA-MB-231 cells were used to evaluate the cytotoxicity of BCS and Plu@BCS nano by MTT assay according to ISO 10993-5.

10.1 Cell imaging

For the co-localization imaging, the cells were stained firstly with the fresh cell growth medium supplemented with Plu@BCS nano and dye D2 (final concentration: 27 μgmL^{-1}) with a light treatment of 0 min, 5 min, and 15 min for 3 h, then nucleus staining dye DAPI (500 nM) in cell culture medium was added to pre-washed cells and incubated at 37 °C for 30 min, rinsed with PBS three times, and then the fluorescence images were acquired through a Nikon A1MP confocal microscope equipped with a cooled CCD camera.

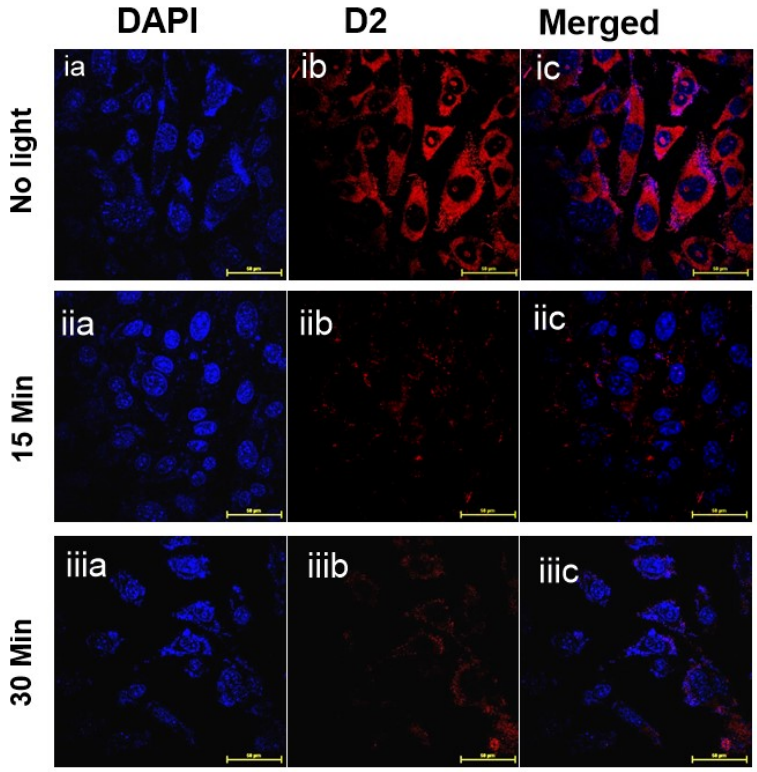


Figure S19. Confocal microscopy images of H_2S release from Plu@BCS NPs inside MDA-MB-231 cells. Gradual release of H_2S from Plu@BCS NPs monitored using H_2S sensitive fluorescent probe (D2) in non-irradiated cells (i) and in light irradiated cells (λ ≥ 365 nm) (ii) 15 min and (iii) 30 min. (a) Images of cells stained with DAPI (1.0 μg mL^{-1}) for 10 min (blue channel: λ_{ex} = 405 nm, λ_{em} = 425–475 nm); (b) image of cells preincubated with Plu@BCS

NPs (5 μM) followed by the dye D1 (5 μM) for 30 min (red channel: λ_{ex} = 635 nm; λ_{em} = 655–755 nm); (c), overlay of (a) and (b); Scar bar: 30 μm

References:

1. Chen, X., Guo, L., Ma, Q., Chen, W., Fan, W. and Zhang, J. Design, synthesis, and biological evaluation of novel N-acylhydrazone bond linked heterobivalent β-carbolines as potential anticancer agents. **2019**. *Molecules*, *24*(16), 2950.

2. Sikder, A., Banerjee, M., Singha, T., Mondal, S., Datta, P. K., Anoop, A. and Singh, N. P. A natural alkaloid, β-carboline, as a one-and two-photon responsive fluorescent photoremovable protecting group: sequential release of the same or different carboxylic acids. *Organic Letters*, **2020**, *22*(17), 6998-7002.