Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

ESIPT-Induced Fluorescent o-Hydroxycinnamate: Self-Monitoring Phototrigger for Prompt Image-Guided Uncaging of Alcohols

Amrita Paul, Rakesh Mengji, Olive Abraham Chandy, Surajit Nandi, Manoranjan Bera, Avijit Jana*, Anakuthil Anoop,* and N. D. Pradeep Singh*

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1. ^{1}H and ^{13}C NMR of the caged compounds

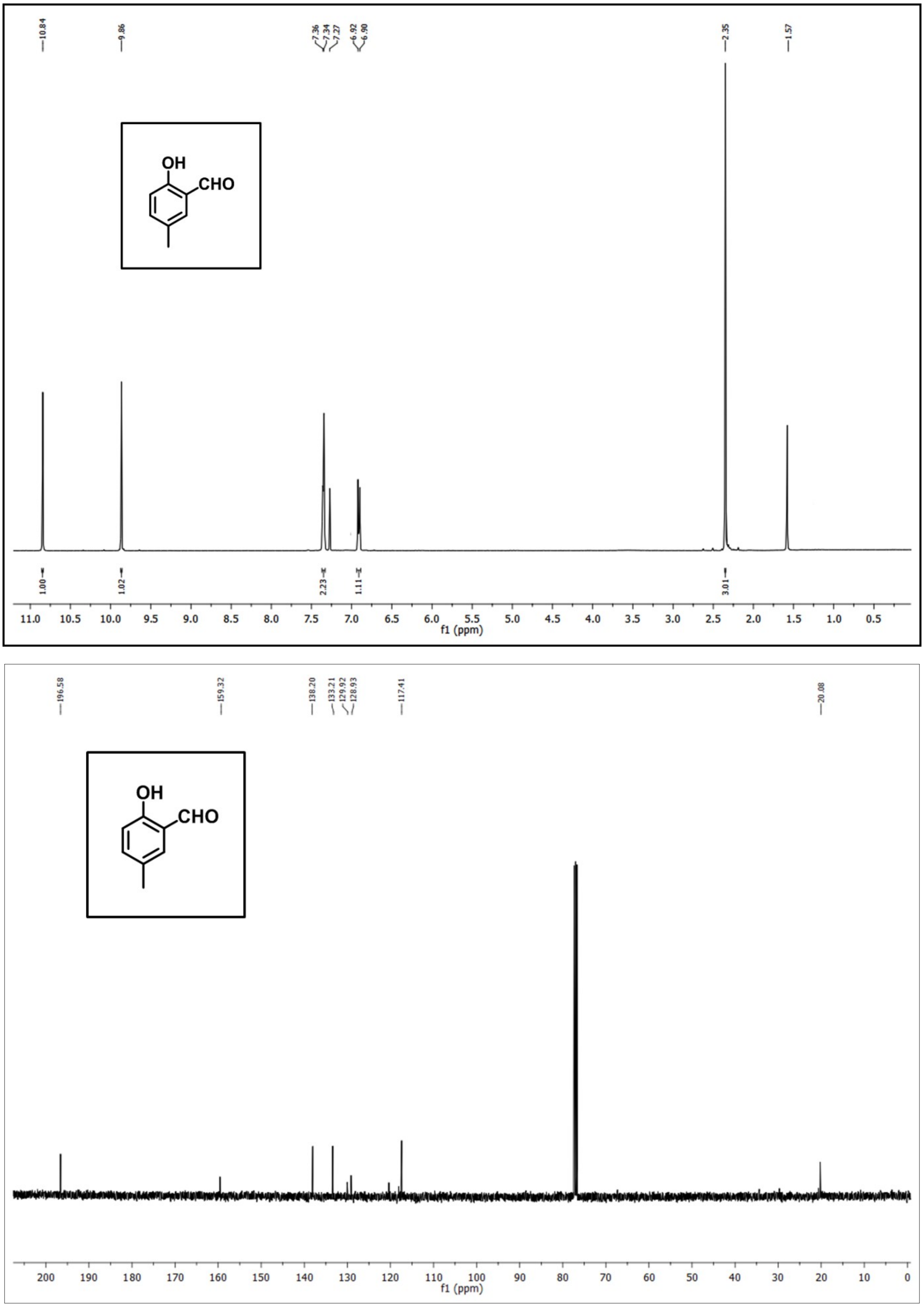

Figure S1. ^{1}H and ^{13}C NMR spectra of **2** in $CDCl_3$.

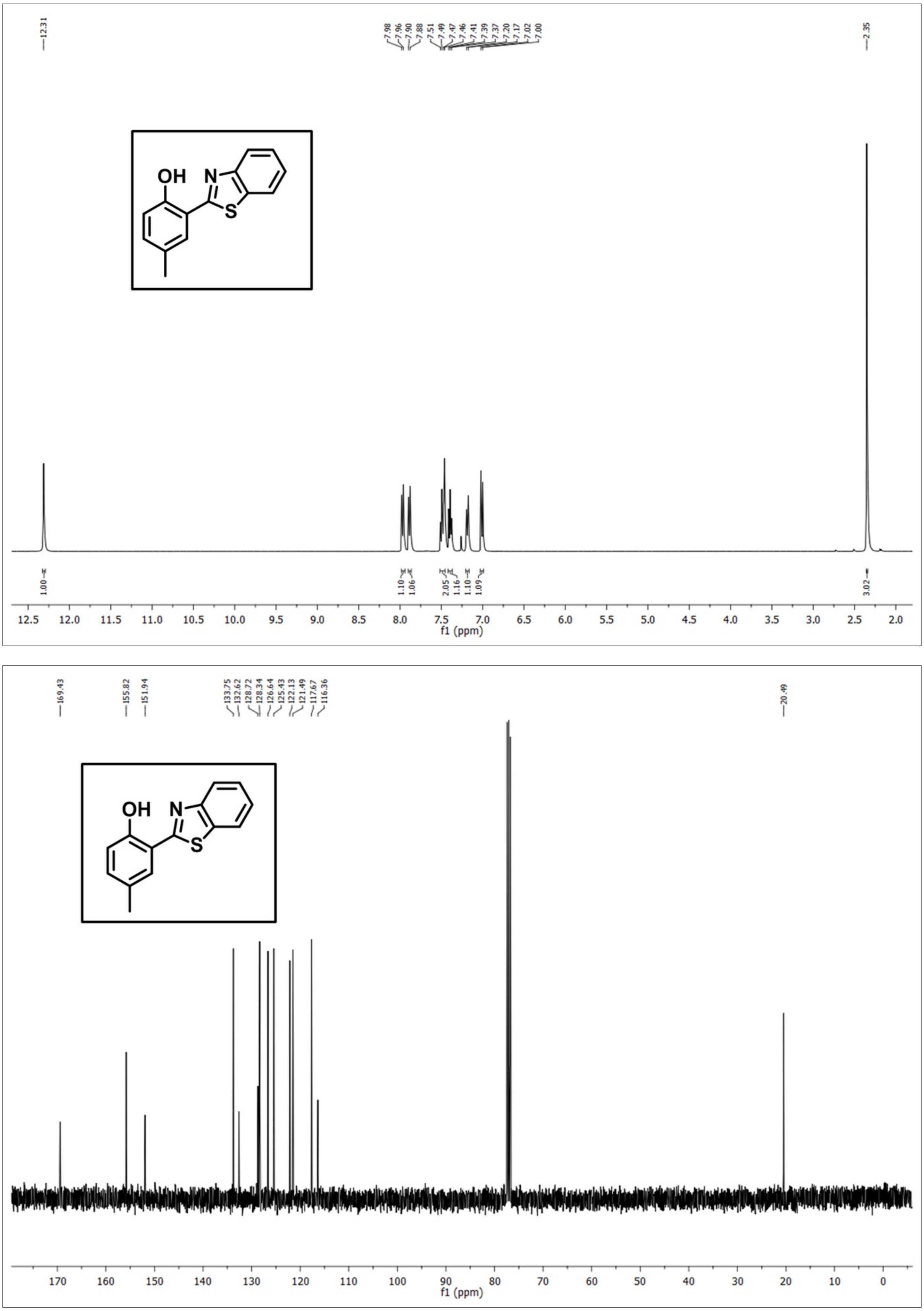

Figure S2. ^1H and ^{13}C NMR spectra of **3** in $CDCl_3$.

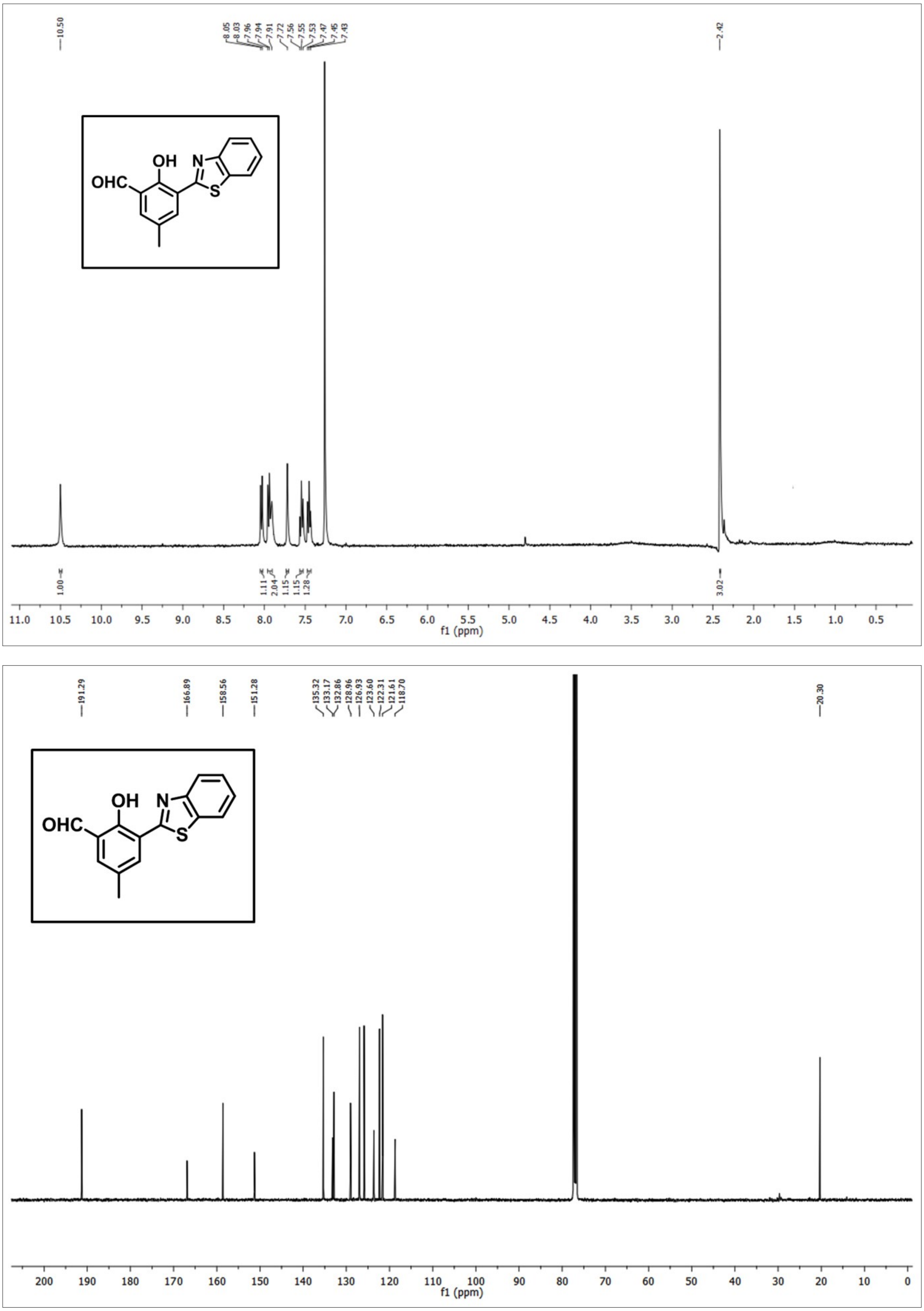

Figure S3. ^{1}H and ^{13}C NMR spectra of **4** in $CDCl_3$.

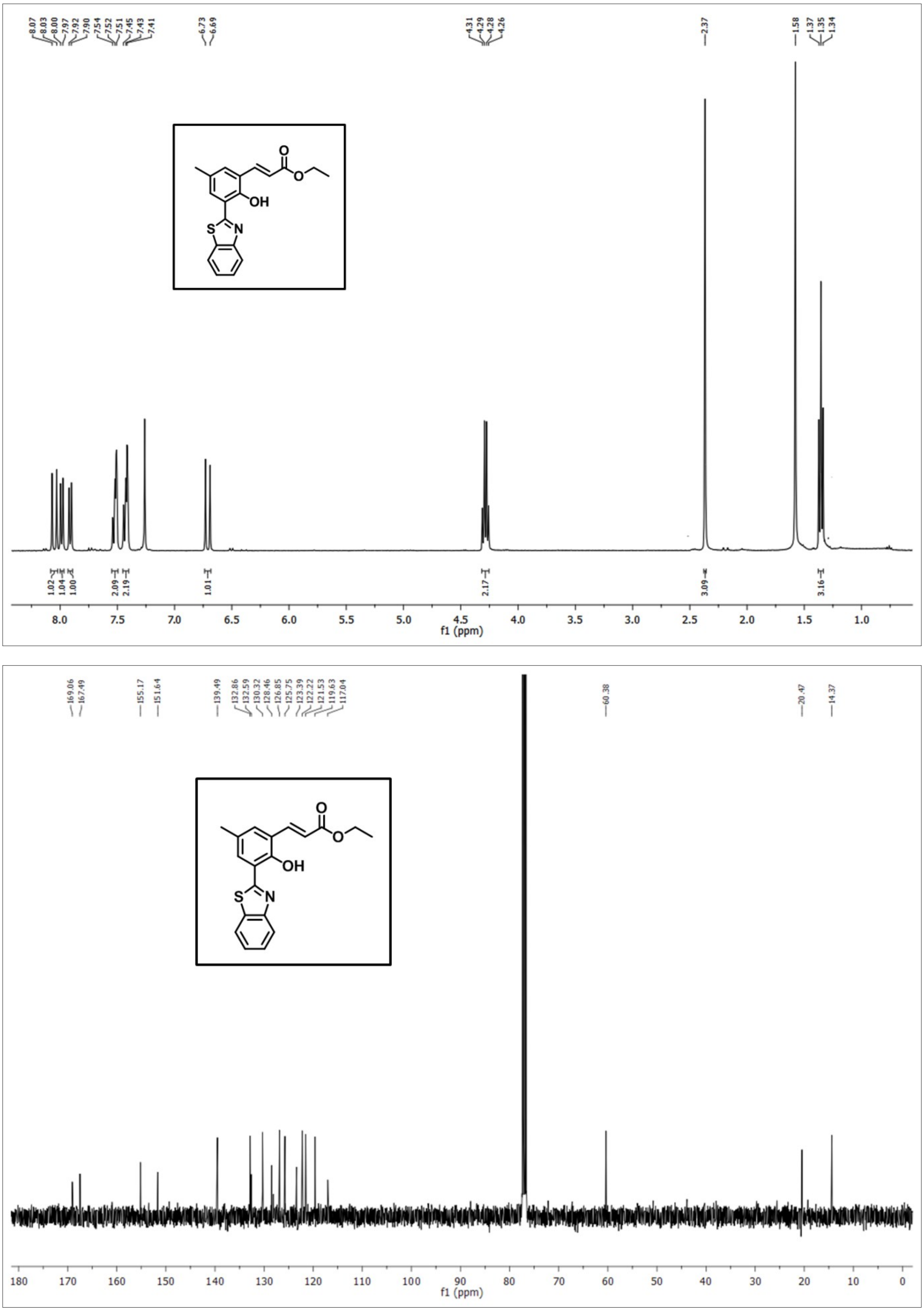

Figure S4. ^{1}H and ^{13}C NMR spectra of **5** in CDCl$_3$.

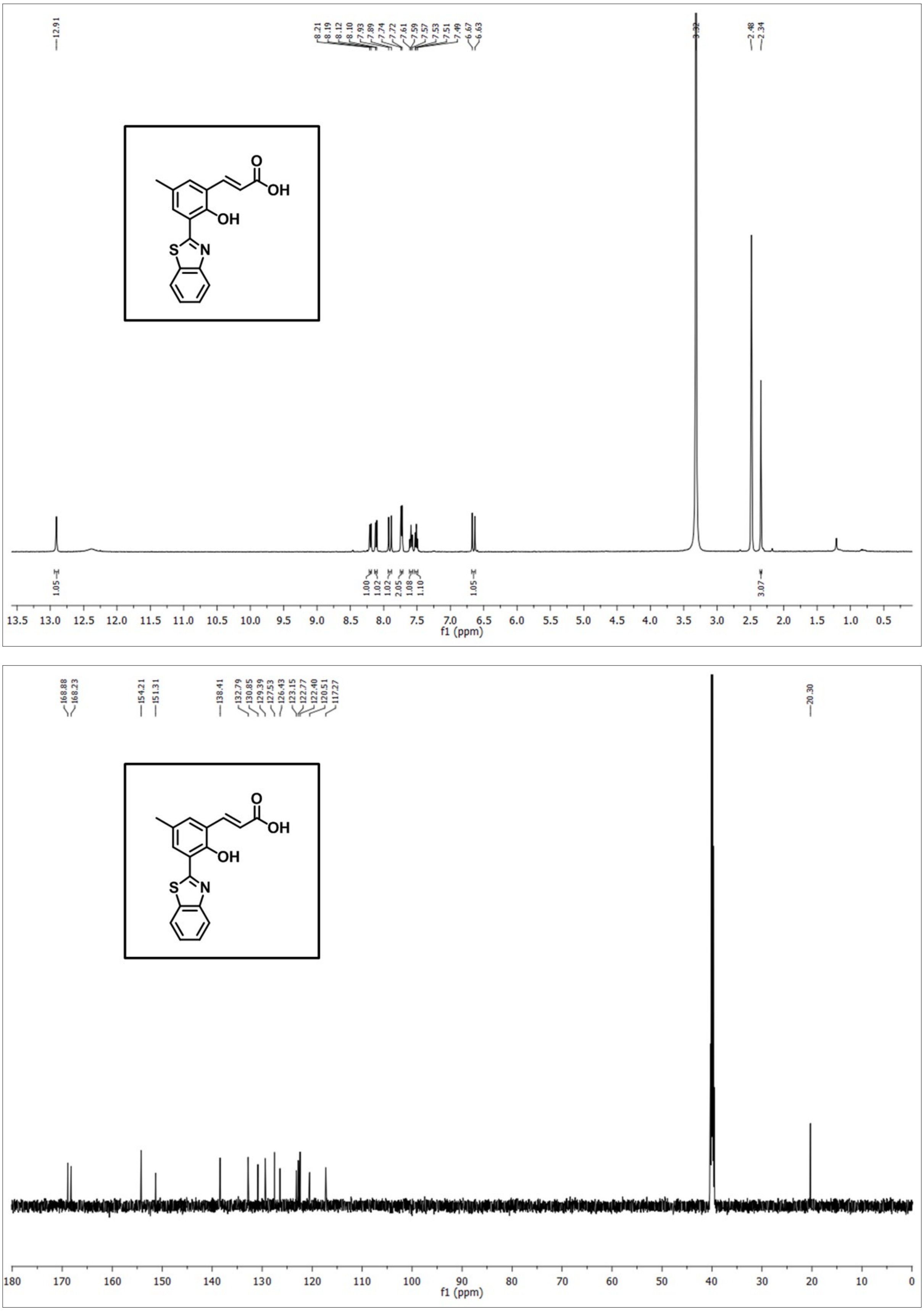

Figure S5. ^{1}H and ^{13}C NMR spectra of **6** in $CDCl_3$.

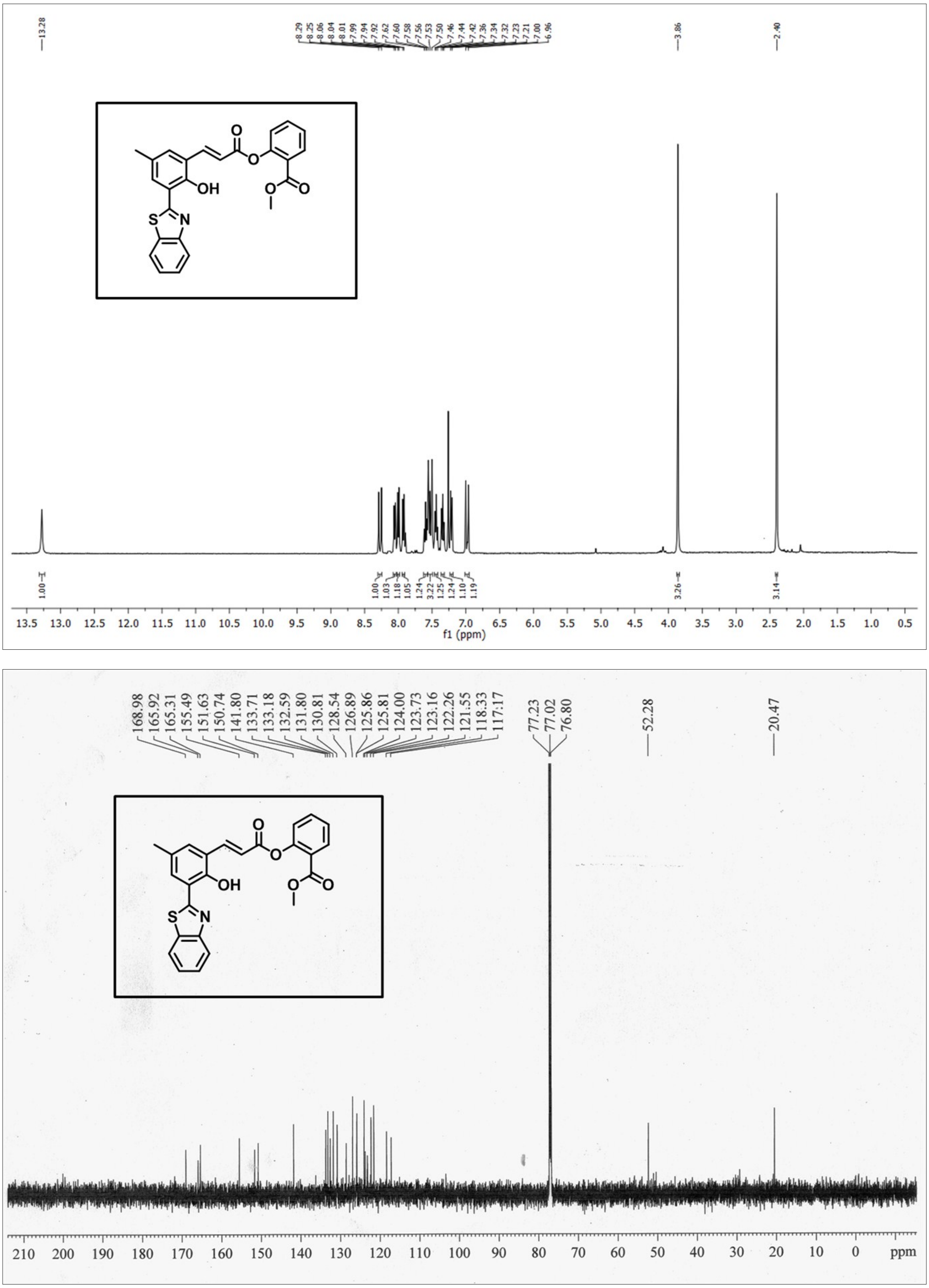

Figure S6. ^{1}H and ^{13}C NMR spectra of **7** in $CDCl_3$.

2. HRMS spectrum of caged compounds

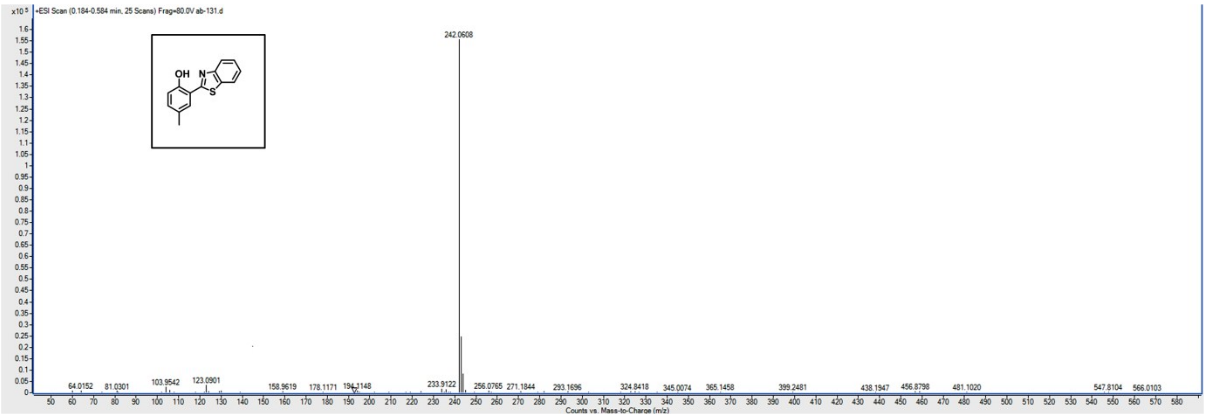

Figure S7. HRMS spectrum of **3.**

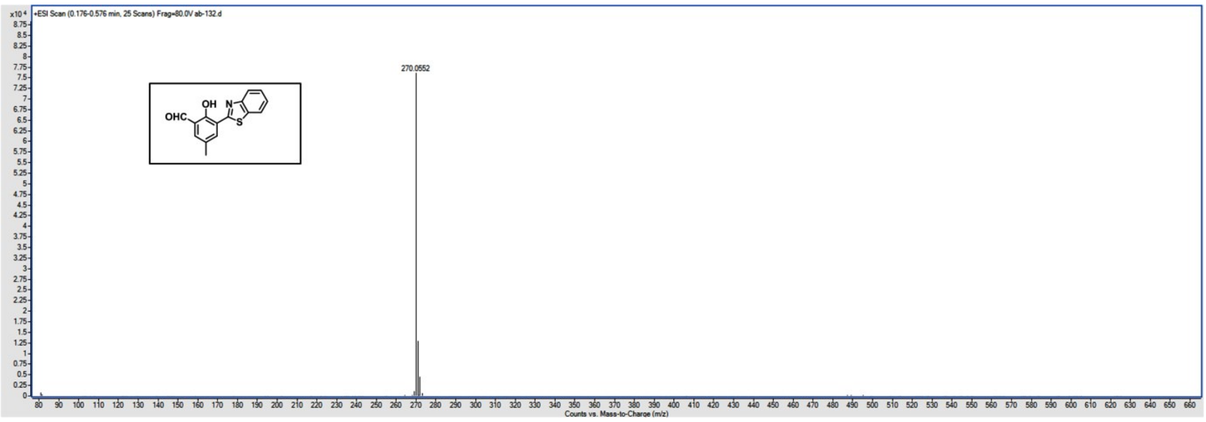

Figure S8. HRMS spectrum of **4.**

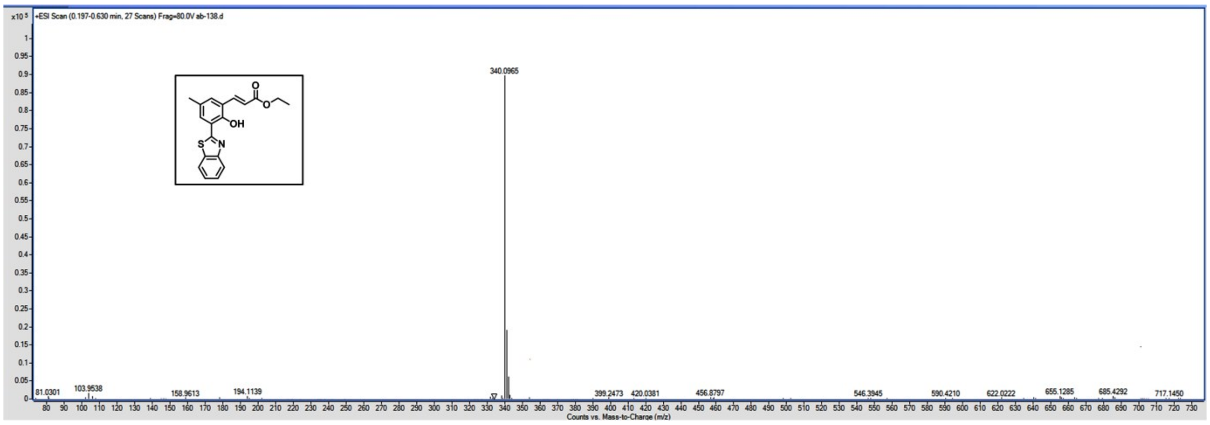

Figure S9. HRMS spectrum of **5.**

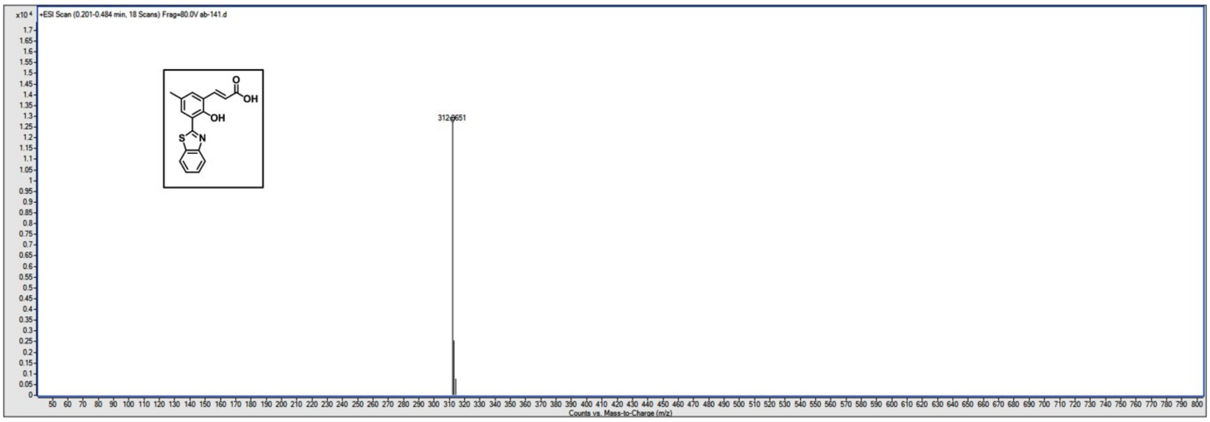

Figure S10. HRMS spectrum of **6.**

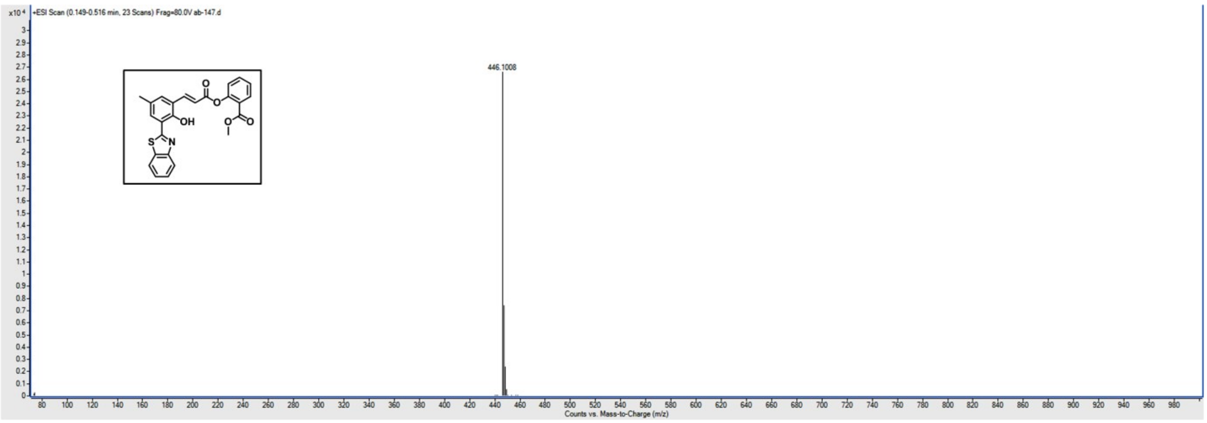

Figure S11. HRMS spectrum of **7.**

3. Photophysical properties of 5 and 7:

The absorption and emission spectra of a degassed solution of **5** and **7** (1×10^{-5} M) in different solvents were recorded on a UV-vis spectrophotometer and fluorescence spectrophotometer respectively.

Measurement of fluorescence quantum yields[1]

The fluorescence quantum yield (QY) of the compound **5** and **7** was determined by reference point method. [1] Quinine sulphate in 0.1 M H_2SO_4 (literature quantum yield: 54 %) was used as a standard sample to calculate the QY. Compound **5** was dissolved in acetonitrile. The absorbance values of the solutions at the excitation wavelength were measured with UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra of all the sample solutions were recorded by Hitachi F-7000 fluorescence spectrophotometer at an excitation wavelength of 370 nm.

$$\frac{\Phi_S}{\Phi_R} = \frac{A_S}{A_R}\,\frac{(Abs)_R}{(Abs)_S}\,\frac{\eta_S^2}{\eta_R^2}$$

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

Table S1: Quantum yield of the fluorescent compound **5** and **7**

Samples	**Quantum yield at 370nm (Q)**
Quinine sulphate	54 % (known)
Compound **5** in acetonitrile	14.6 %
Compound **7** in acetonitrile/HEPES buffer (1:9) solution	16.3 %

Fluorescence quantum yield (excitation wavelength 370 nm, error limit within ± 5 %).

4. 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.[2] Solution (0.006 M) of potassium ferrioxalate was irradiated using 125W medium pressure Hg lamp as UV light source (≥ 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 reported value. Using the known quantum yield (1.283 ± 0.023) for potassium ferrioxalate actinometer at

363.8 nm, the number of Fe2+ ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident photon flux (I_0) at 350 nm of the 125W Hg lamp was determined as $1.55x10^{17}$ photons $s^{-1}cm^{-2}$.

5. Photochemical rate constant determination for the photodegradation of compound 5 and 7:

A 20 mL solution of **5** (1×10^{-4} M) was prepared in acetonitrile / water (1:1). Nitrogen was passed throughout the solution for 30 min and irradiated using 125 W medium pressure Hg lamp as the UV light source ($\lambda \geq 365$ nm) and 1 M $CuSO_4$ solution as the UV cut−off filter. At regular interval of time, 20 µl of the aliquots was taken and analyzed by RP-HPLC using mobile phase acetonitrile , at a flow rate of 1 mL/min (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated gradual decrease of the caged compound with time, and the average of three runs. The reaction was followed until the consumption of the ester is less than 5 % of the initial area. Based on HPLC data for caged compound, the natural logarithm of the concentration of caged compound (lnC) (from HPLC peak area) versus irradiation time was plotted. We observed an exponential correlation for the disappearance of the caged compounds which suggested a first order reaction. Further, the quantum yield for the photolysis of caged compound was calculated using equation (1)

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

Where, the subscript ‘CG’ and ‘act’ denote caged compound and actinometer, respectively. Φ is the relative photolysis quantum yield, k_p is the photolysis rate constant and F is the fraction of light absorbed. Potassium ferrioxalate was used as an actinometer.

6. Time-correlated single photon counting (TCSPC) study:

Fluorescence lifetimes were obtained from a time-correlated single photon counting (TCSPC) spectrometer using nano-LED (IBH, U.K.) as the light source at 375 nm. The experimental setup for picosecond TCSPC has been described elsewhere.[3] Briefly, the samples were excited at 375 nm using a picosecond laser diode (IBH, Nanoled), and the signals were collected at the magic angle (54.7°) using a Hamamatsu microchannel plate photomultiplier tube (3809U). The instrument response function of our setup was 90 ps. The average fluorescence lifetimes for the decay curves were calculated from the decay times and the relative contributions of the components using the following equation (2).

$$\boxed{\langle\tau\rangle = a_1\tau_1 + a_2\tau_2} \text{ --------------- } (2)$$

where τ_1 and τ_2 are the first and second components of the decay time of our caged compound (5) and a_1 and a_2 are the corresponding relative amplitudes of these components.

A 5 mL (1×10^{-4} M) solution of caged compound (**5**) was prepared in acetonitrile. and the time-resolved fluorescence decays were recorded at emission wavelength of 590 nm with an excitation wavelength of 375 nm. The representative time resolved fluorescence decay profiles of caged compound (**5**) at 590 nm is depicted in **Figure S14**, and the lifetime value is obtained from the biexponential fitting of the decay.

7. Computational Details, Coordinates, and Energies:

All the optimizations and relaxed surface scan were done using CAM-B3LYP/cc-pVDZ level of theory.[4,5] The stationary points in the ground state were characterized by frequency analysis. The ground states were confirmed by all real frequencies. The calculations were carried out in Gaussian 09 program package. Single point calculations of all the points on the PES were done using CAM-B3LYP/cc-pVTZ level of theory. The conical intersection for the cis-trans isomerization was confirmed by single point calculation and excitation at CASSCF(4,4)/cc-pVDZ[6] level of theory.

Cartesian coordinates of compound **5** (as depicted in **Fig. 5**)

i) E (S_0) - CAM-B3LYP/cc-pVDZ = -1412.50749919 Hatrees
ii) E(S_0) - CAM-B3LYP/cc-pVTZ = -1412.80698891 Hatrees

C	1.00422700	1.26589800	-0.00000100
C	1.15873200	2.66156500	0.00000100
C	0.07295700	3.52006500	0.00000100
C	-1.20104300	2.94568300	-0.00000200
C	-1.42158200	1.56799500	-0.00000300
C	-0.29671600	0.70489100	-0.00000300
H	2.16441200	3.08820800	0.00000300
C	0.24331100	5.01520500	0.00000100
H	-2.07702100	3.59974900	-0.00000200
C	-2.80967400	1.10987300	-0.00000600
O	-0.49838200	-0.61295700	-0.00000600
C	2.16506100	0.37984700	-0.00000200
N	2.06285200	-0.91959600	-0.00000700
C	3.29394300	-1.54639900	-0.00000500
C	4.39530400	-0.67215900	0.00000300
S	3.82715000	0.98061900	0.00000400
C	3.50120200	-2.92977400	-0.00000800
C	4.80194000	-3.40884700	-0.00000400
C	5.89529600	-2.52801000	0.00000400
C	5.70520200	-1.15245100	0.00000800
H	0.39268400	-1.06113500	-0.00000800
H	-0.22738700	5.46825500	-0.88765800
H	-0.22740200	5.46825700	0.88765200
H	1.30765200	5.29299800	0.00001000
H	2.64189500	-3.60110500	-0.00001400

H	4.98015800	-4.48518900	-0.00000600
H	6.91021900	-2.92841900	0.00000700
H	6.55478100	-0.46877600	0.00001300
C	-3.30491300	-0.13604200	0.00000500
H	-3.55339400	1.91418100	-0.00001700
C	-4.77210500	-0.31283300	0.00000200
H	-2.68312000	-1.02601900	0.00001700
O	-5.09605800	-1.61966200	0.00001800
O	-5.59966100	0.57171800	-0.00001300
C	-6.50047500	-1.90535500	0.00001200
C	-6.65016800	-3.40864600	-0.00000700
H	-6.96048500	-1.44056000	0.88655500
H	-6.96048400	-1.44053500	-0.88651800
H	-7.71691100	-3.68135400	-0.00000900
H	-6.17508300	-3.84354200	-0.89290900
H	-6.17508000	-3.84356500	0.89288200

Cartesian coordinates of compound **9** (as depicted in **Fig. 5**)

i) E(S_0) - CAM-B3LYP/cc-pVDZ = -1412.50749919 Hatrees
ii) E(S_0) - CAM-B3LYP/cc-pVTZ = -1412.80698891 Hatrees

C	1.01331000	1.31805100	-0.00004600
C	1.13681300	2.69605800	-0.00008800
C	0.00734600	3.56746000	-0.00011600
C	-1.25718400	3.01569100	-0.00010200
C	-1.47008500	1.60869400	-0.00006000
C	-0.33540000	0.70943200	-0.00003200
H	2.13006300	3.15240300	-0.00009900
C	0.20872500	5.05421900	-0.00016100
H	-2.13305700	3.66806800	-0.00012200
C	-2.82738700	1.14018300	-0.00004400
O	-0.46355900	-0.54240300	0.00000300
C	2.15821100	0.45934200	-0.00001600
N	2.08273500	-0.89519400	0.00002300
C	3.27209300	-1.56754600	0.00004900
C	4.37638400	-0.69374400	0.00003100
S	3.83364500	0.98322300	-0.00002100
C	3.46501600	-2.95348800	0.00008900
C	4.76847200	-3.43423100	0.00011200
C	5.86571100	-2.56437000	0.00009400
C	5.67689900	-1.18241700	0.00005400
H	1.10943200	-1.26053400	0.00002900
H	-0.25997600	5.51380300	-0.88636400
H	-0.26000500	5.51386100	0.88599500
H	1.27646900	5.31764600	-0.00015300
H	2.60743600	-3.62719300	0.00010300
H	4.93808300	-4.51210500	0.00014300
H	6.87854500	-2.96863600	0.00011300
H	6.52848800	-0.50104500	0.00004000
C	-3.29836600	-0.12980800	-0.00000200
H	-3.59233800	1.92487000	-0.00006800
C	-4.75359400	-0.34408100	0.00000900
H	-2.64074400	-0.99423000	0.00002500
O	-5.04675600	-1.66071500	0.00005300
O	-5.60634800	0.51943100	-0.00002100
C	-6.44306400	-1.97789100	0.00006700

C	-6.55894900	-3.48434500	0.00012000
H	-6.91472100	-1.52383600	0.88634600
H	-6.91472600	-1.52389800	-0.88624100
H	-7.61919600	-3.78158600	0.00013300
H	-6.07381300	-3.90831500	-0.89268900
H	-6.07380800	-3.90825200	0.89295700

Cartesian coordinates of compound **15** (as depicted in **Fig. 5**)

i) $E(S_0)$ - CAM-B3LYP/cc-pVDZ = -1412.34476242 Hatrees
ii) $E(S_0)$ - CAM-B3LYP/cc-pVTZ = -1412.6679481 Hatrees

C	0.74226400	1.46065300	-0.07734800
C	0.90928700	2.81608600	0.14415000
C	-0.17609300	3.74060000	0.09661900
C	-1.44138000	3.26567700	-0.18083800
C	-1.69752600	1.88657300	-0.42048200
C	-0.60791300	0.93422400	-0.37623100
H	1.90388400	3.21180900	0.36545500
C	0.07130600	5.19920100	0.34618300
H	-2.28383200	3.95950700	-0.22112800
C	-3.05158100	1.49913700	-0.70012400
O	-0.77538700	-0.29621200	-0.57993200
C	1.84328300	0.54782500	-0.02555300
N	1.72439900	-0.78708300	-0.23614700
C	2.87537900	-1.51815600	-0.15008400
C	3.99319100	-0.71592300	0.14976500
S	3.51292100	0.97224600	0.31165200
C	3.02018200	-2.89909100	-0.32359300
C	4.28998100	-3.44710200	-0.19167100
C	5.40070800	-2.64837200	0.10650400
C	5.25983000	-1.27161000	0.28006500
H	0.75246100	-1.09395100	-0.44099400
H	-0.23252100	5.80354100	-0.52486100
H	-0.51927500	5.55594200	1.20653200
H	1.13304300	5.40004300	0.55074900
H	2.15226200	-3.51727100	-0.55573500
H	4.42189900	-4.52217500	-0.32357100
H	6.38638200	-3.10455900	0.20450400
H	6.12209200	-0.64551600	0.51240300
C	-3.55776900	0.26988100	-0.95974200
H	-3.78029100	2.31758100	-0.70375700
C	-4.03062100	-0.55185600	0.16487400
H	-3.62682400	-0.13001000	-1.96721900
O	-4.50045100	-1.73913200	-0.26998700
O	-4.01679100	-0.23982900	1.33760200
C	-4.98697700	-2.61712100	0.75114300
C	-5.46271100	-3.87753400	0.06713400
H	-4.17678400	-2.81450000	1.47138300
H	-5.79574300	-2.11242300	1.30367800
H	-5.84873000	-4.58946200	0.81320500
H	-6.26622100	-3.64741100	-0.64954600
H	-4.63533500	-4.35466000	-0.48060500

Cartesian coordinates of compound **12** (as depicted in **Fig.5**)

i) $E(S_0)$ - CAM-B3LYP/cc-pVDZ = -1412.49676122 Hatrees
ii) $E(S_0)$ - CAM-B3LYP/cc-pVTZ = -1412.74716204 Hatrees

C	0.38780400	1.46158000	-0.12959500
C	0.41764600	2.79550300	0.31259800
C	-0.69717300	3.61147700	0.25857000
C	-1.88063900	3.05576700	-0.24494800
C	-1.97463900	1.73279500	-0.65504900
C	-0.81833300	0.91934300	-0.63288700
H	1.35074400	3.20864800	0.70276800
C	-0.65786900	5.04211700	0.72329000
H	-2.77671900	3.68012500	-0.30084100
C	-3.26805800	1.23768800	-1.16297000
O	-0.90347800	-0.31881700	-1.11578400
C	1.58044100	0.62256300	-0.07829900
N	1.60033600	-0.61011200	-0.50126300
C	2.83837500	-1.20158000	-0.34399800
C	3.81737800	-0.37085500	0.23007800
S	3.12274900	1.19573200	0.57194900
C	3.16270600	-2.51367000	-0.70454300
C	4.45426600	-2.96695000	-0.48461100
C	5.42447000	-2.13000100	0.08901000
C	5.11763200	-0.82505100	0.45205900
H	-0.01660200	-0.75250900	-1.00204700
H	-0.97031100	5.73032700	-0.07912200
H	-1.33846700	5.20214200	1.57573300
H	0.35596000	5.32751800	1.04096100
H	2.39797700	-3.15147900	-1.14900400
H	4.72186600	-3.98808900	-0.76050700
H	6.43463900	-2.50840800	0.25294000
H	5.87138500	-0.17515600	0.89796000
C	-3.90215900	0.10392100	-0.84556500
H	-3.76713000	1.90006200	-1.87827000
C	-3.45123200	-0.85593700	0.18755300
H	-4.84101600	-0.14558000	-1.34280400
O	-4.09418400	-2.03160800	0.04067900
O	-2.65776400	-0.64462000	1.07202400
C	-3.74440700	-3.04127300	0.99587000
C	-4.55022300	-4.27382000	0.65886700
H	-2.65950800	-3.22358500	0.94011200
H	-3.95962700	-2.66587300	2.00899700
H	-4.31496200	-5.08329100	1.36718300
H	-5.62836500	-4.05813200	0.71628000
H	-4.31871700	-4.61900700	-0.36061000

8. *In Vitro* Cytotoxicity assay

The cytotoxicity of compound **7** was determined before and after irradiation on NIH 3T3 cells using MTT assay. NIH 3T3 cells (1 × 10^5 cells/well of a 96-well plate) were treated for 48 h with different concentrations (0.1–50 µg mL^{-1}) and cell viability was determined by MTT assay (Mossman, 1983), measuring absorbance at 595 nm using a micro plate reader (Bio-Rad 550). Cell viability was calculated using the formula, Viability (%) = 100 × A2/A1; [Where A2 = Absorbance of the treated cell; A1 = Absorbance of the control cells].

Briefly, cells in exponential growth phase were trypsinised and seeded in 96-well culture plates (1 × 10^5 cells/well). After 12 h of cell seeding, the medium was replaced with a fresh complete medium (DMEM) containing 50 µg mL^{-1} of compound **7** and was kept for 4 h at 37 °C in a CO_2 incubator. Treated cells were irradiated with UV-light (≥ 365 nm) for 0–60 min.

9. Overlay of HPLC chromatograms of the 5 at regular time intervals of irradiation with light

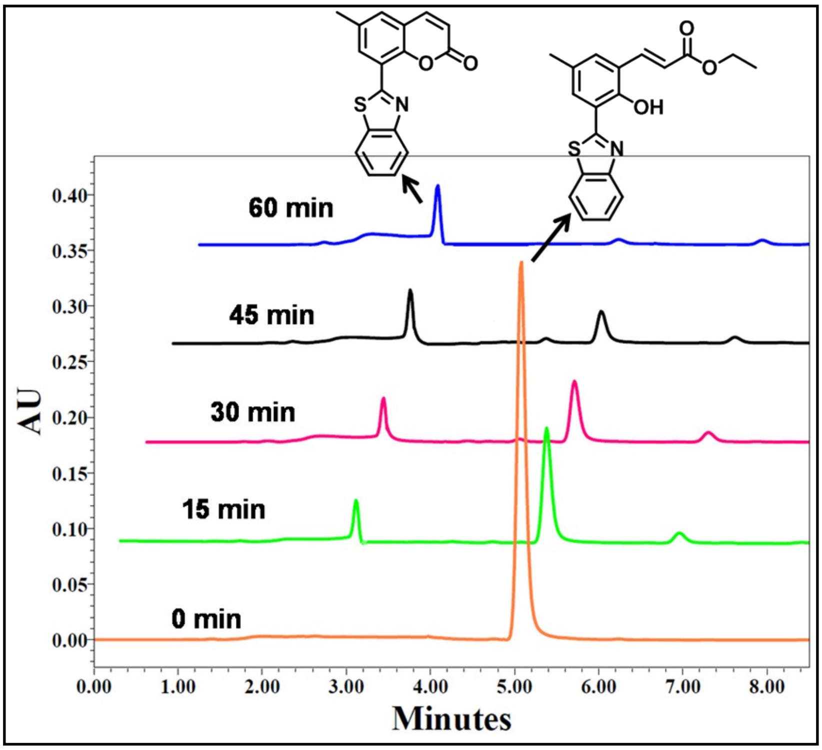

Figure S12. Overlay of HPLC chromatograms of the caged compound **5** at regular time intervals of irradiation with light (≥ 365nm). The y axes are offset by 10 mAU and the x axes are offset by 15 s to facilitate visualization. AU= arbitrary units.

Table S2: Photochemical quantum yield of caged compound **5** and **7**

Compound name	Quantum Yield $(\Phi)_{CG}$
Caged compound **5**	0.10
Caged compound **7**	0.10

10. Fluorescence quenching study of compound 5

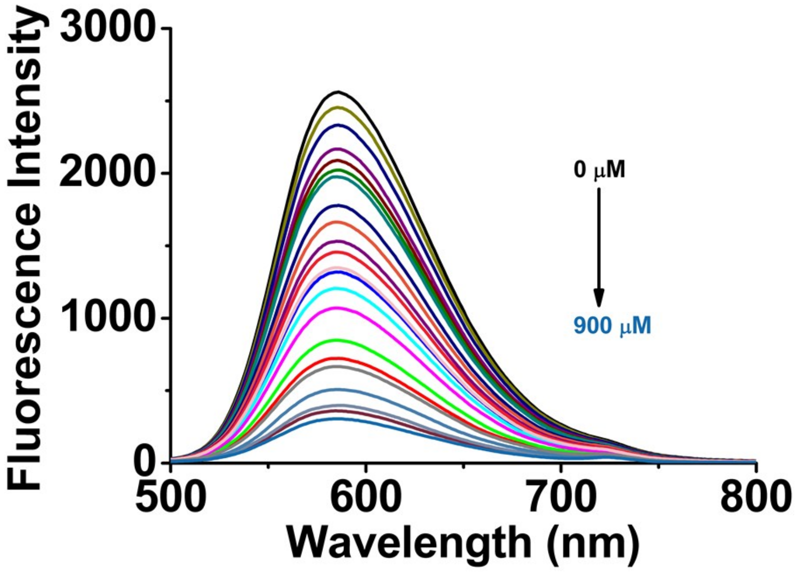

Figure S13: Fluorescence quenching study of caged compound **5** in presence of different concentration of triplet sensitizer (Benzophenone).

11. Time resolved florescence decay curve of caged compound 5

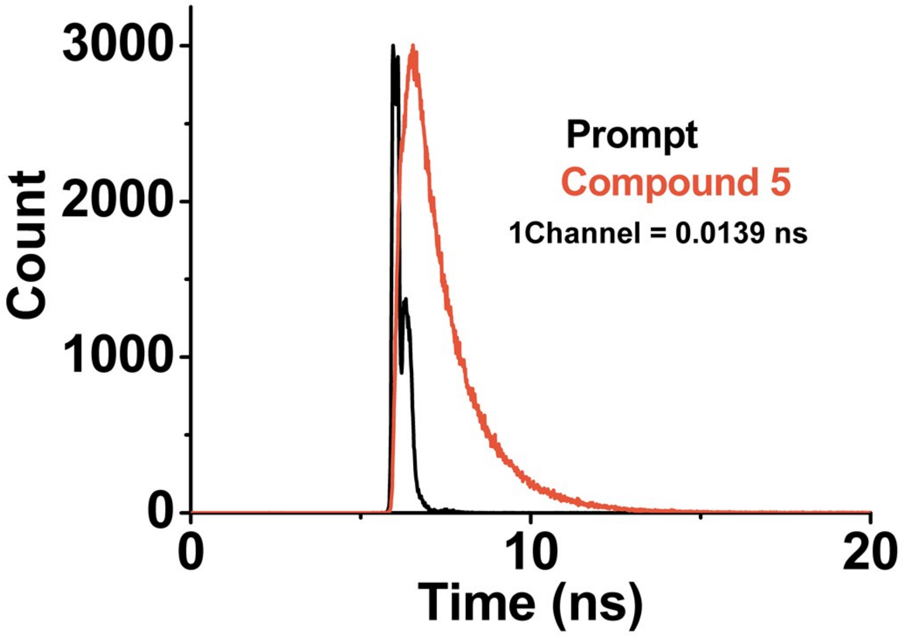

Figure S14: Time resolved florescence decay curve of caged compound **5** at 590 nm.

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