Supporting information for

A dual-site two-photon fluorescent probe for visualizing mitochondrial aminothiols in living cells and mouse liver tissues

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Preparation of fresh mouse liver slices and two-photon fluorescence imaging

All of these experiments are performed in compliance with the relevant laws and institutional guidelines, and are approved by the Animal Ethical Experimentation Committee of Shandong University. The slices were prepared from the liver of 14 day-old mice, and they were cut to 400 mm thickness by using a vibrating-blade microtome in 25 mM PBS (pH 7.4). The slices were incubated with 30 μM **CA-TPP** in PBS buffer bubbled with 5% CO_2 for 1 h at 37 ^oC, and then were washed three times with PBS buffer solution, transferred to glass-bottomed dishes, and observed under a two-photon confocal microscope (Nikon A1MP). The fluorescence images of the slices were acquired using 770 nm excitation and fluorescence emission windows of 475-500 nm.

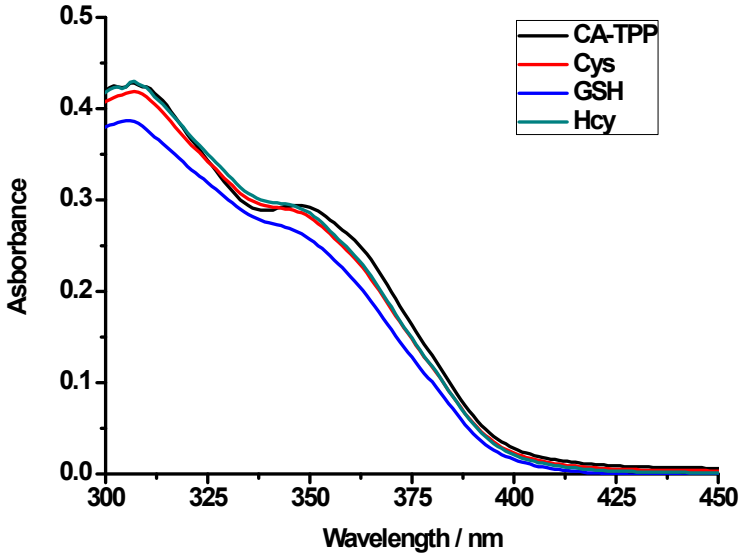

Fig. S1. UV-vis absorption spectra of **CA-TPP** in solutions containing different RSH.

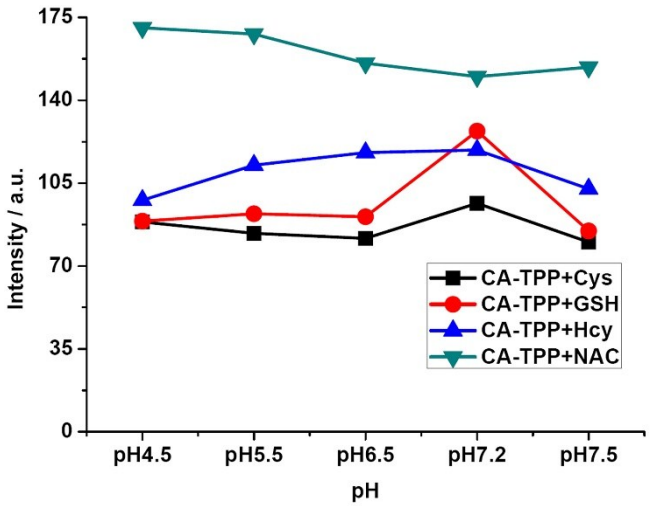

Fig. S2. Relative fluorescence intensity of **CA-TPP** + Cys, GSH, Hcy, NAC in CH_3OH : tris-HCl buffer solution at different pH value. Excitation wavelength: 350 nm.

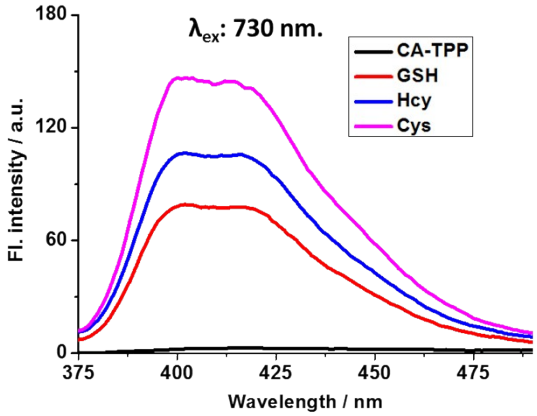

Fig. S3. Selectivity of **CA-TPP** in mixtures (**CA-TPP**, **CA-TPP**+Hcy**, CA-TPP**+Cys**, CA-TPP**+GSH). [Hcy] = [GSH] = [Cys] = 1×10^{-3} M. λ_{ex}: 730 nm.

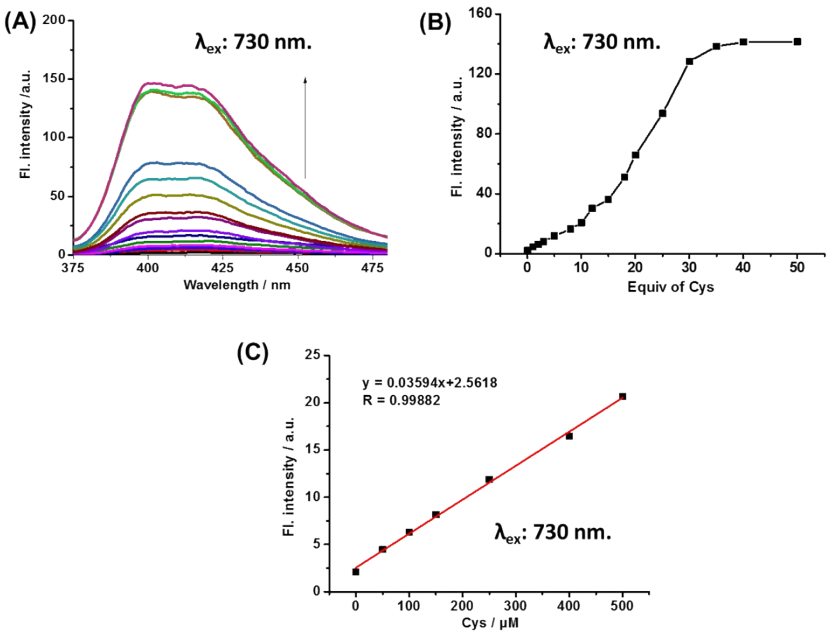

Fig. S4. (A) Fluorescence intensity change at 410 nm in varied equiv of Cys. (B) Change curve of the fluorescence intensity. (C) Plot of fluorescence intensity as a function of concentration of Cys. [**CA-TPP**]: 50 μM. λ_{ex}: 730 nm.

Table S1. Photophysical properties of the compound **CA-TPP** at 760 nm/770 nm/780 nm excitations.

Sample	λ^a /λ^b (nm)	ε [c] ($\times 10^4$)	Φ [d] (%)	δ [e] (GM) 760/770/780 nm
CA-TPP	365/415	2.62	0.067	—/—
CYS	362/414	1.52	2.86	1.7/1.0/0.6
HCY	362/415	1.74	1.93	2.7/1.6/1.0
GSH	365/414	2.52	0.66	10.5/7.2/5.3

[a] and [b] are linear absorption and fluorescent maximum peaks in nm respectively; [c] ($\times 10^4$) is molar absorptivity in $M^{-1} \cdot cm^{-1}$; [d] is fluorescence quantum yield determined using fluorescein (Φ = 0.95) as the standard. [e] refers to two-photon absorption cross sections at 760 and 770 nm, determined using fluorescein (δ = 28.5 GM/29 GM/29.5GM) as the standard at 760 nm/770 nm/780 nm. 1GM = $10^{-50} cm^4$ s photon $^{-1}$; [**CA-TPP**] = 50 μM. 30 equiv. RSH. The δ of the probe is negligible.

Table S2. Representative C-C (N or O) bond lengths of **CA-TPP**

C-C Bond	Bond Lengths (in pm)	C-C Bond	Bond Lengths (in pm)	C-N/O Bond	Bond Lengths (in pm)
C5-C6	138.9	C16-C17	135.5	C14-N17	146.7
C10-C11	139.5	C17-C18	148.0	C4-N7	138.5
C11-C12	142.3	C18-C19	141.2	C48-O49	125.8
C12-C16	147.5	C20-C21	140.1	C21-O24	139.7

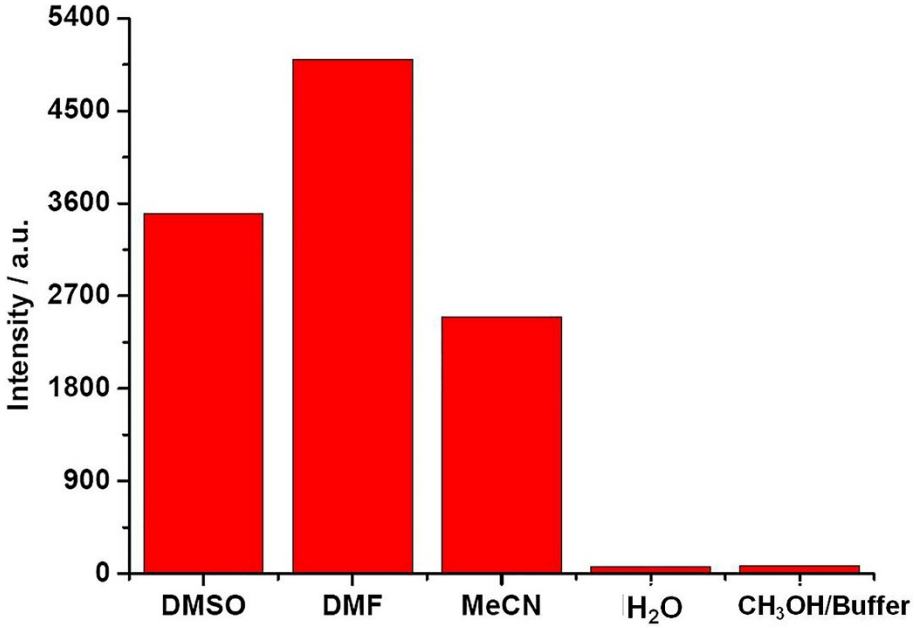

Fig. S5. One-photon fluorescent intensity of **CA-TPP** in four solvents (DMSO, DMF, MeOH, H_2O and CH_3OH/Tris-HCl = 40:1). Concentration: 50 μM.

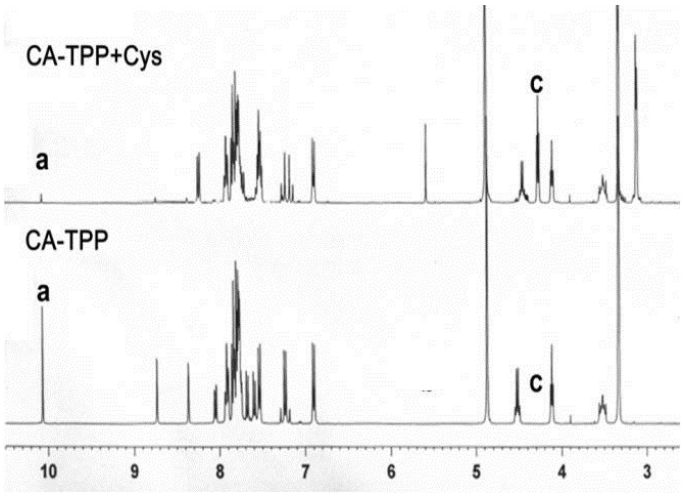

Fig. S6. ^{1}H NMR spectrum of **CA-TPP** in the presence (top) and absence (bottom) of Cys in deuterated methanol.

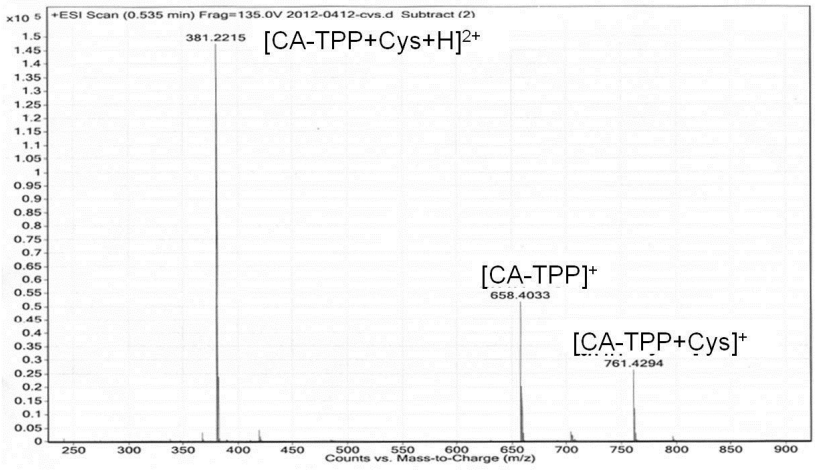

Fig. S7. HRMS spectrum of **CA-TPP+Cys**

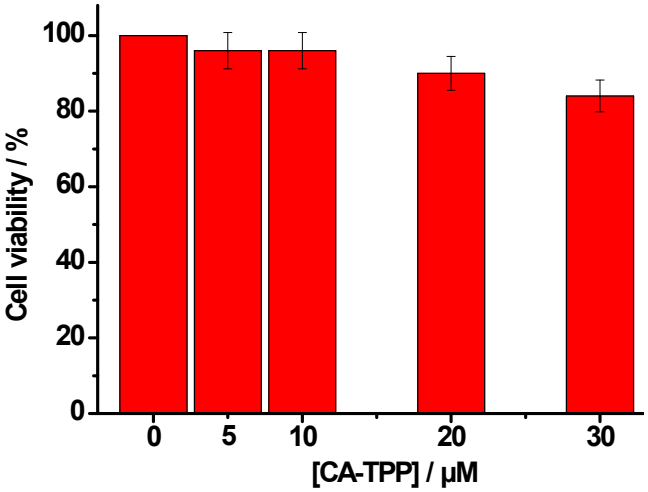

Fig. S8. Cytotoxicity experiment of **CA-TPP**

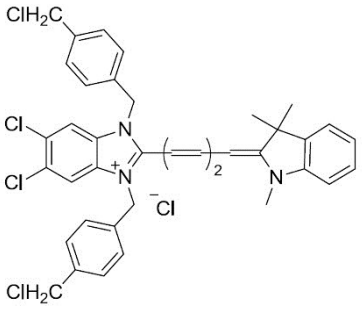

Fig. S9. Molecular structure of MitoTracker® Red FM.

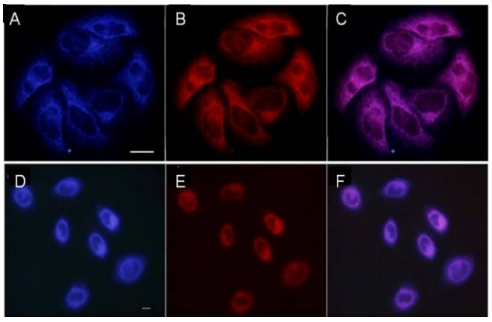

Fig. S10. Wide-field fluorescence imaging of living HeLa (A-C) and SiHa (D-E) cells with **CA-TPP** (A, D) and MTR (B, E). (C, F) Merged image. Excitation wavelength: 330-385 nm , emission windows : > 410 nm. Scale bar = 20 μm

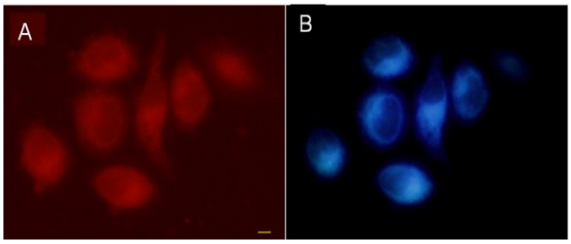

Fig. S11. Wide-field images of CCCP (10 μM) treated SiHa cells stained with (A) MTR (250 nM) for 20 min and (B) **CA-TPP** (5 μM) for 30 min. Excitation wavelength: 561 nm (for MTR) and 330-385 nm (for **CA-TPP**). Scale bar = 20 μm.

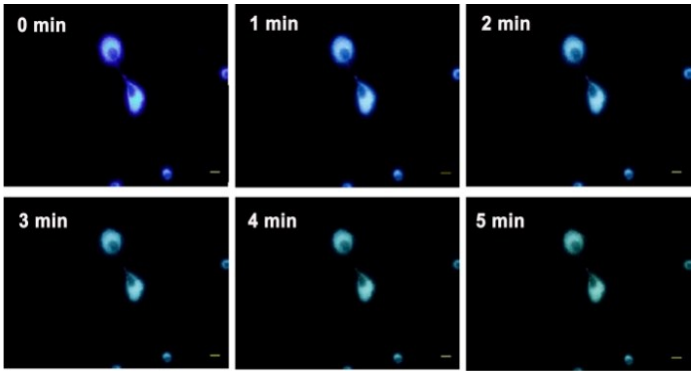

Fig. S12. Fluorescent images of living SiHa cells incubated with **CA-TPP**. Excitation power from 330-385 nm (mercury lamp) channels and 70 μW power of the olympus wide field microscope was unified. Compound concentration: 5 μM, **CA-TPP**; Detection wavelength: > 410 nm. Scale bar = 20 μm.

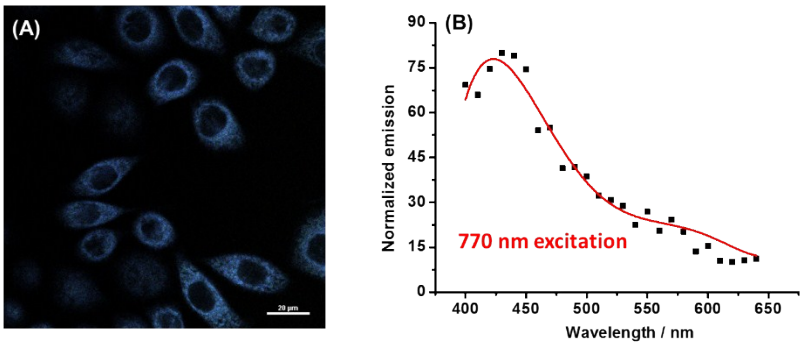

Fig. S13. TP fluorescence images and spectra of the HeLa cells incubated with **CA-TPP** (5 μM) by the Nikon infrared spectra detector (IR SD). (A) TP fluorescence images of cells with excitation at 770 nm. (B) TP fluorescence spectra of the cells with excitation at 770 nm, Scale bar = 20 μm.

Characterization

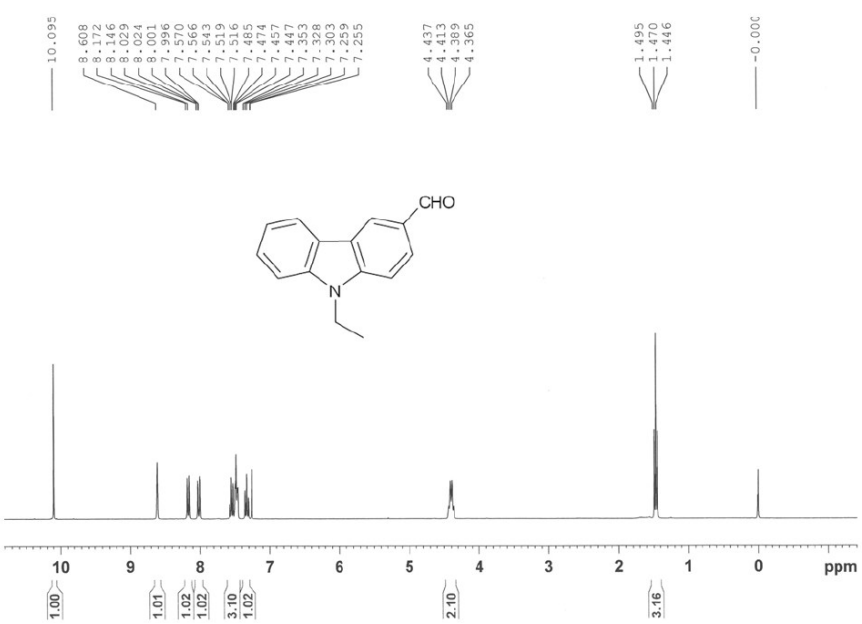

Fig. S14. ^{1}H NMR spectrum of compound **1** in $CDCl_3$

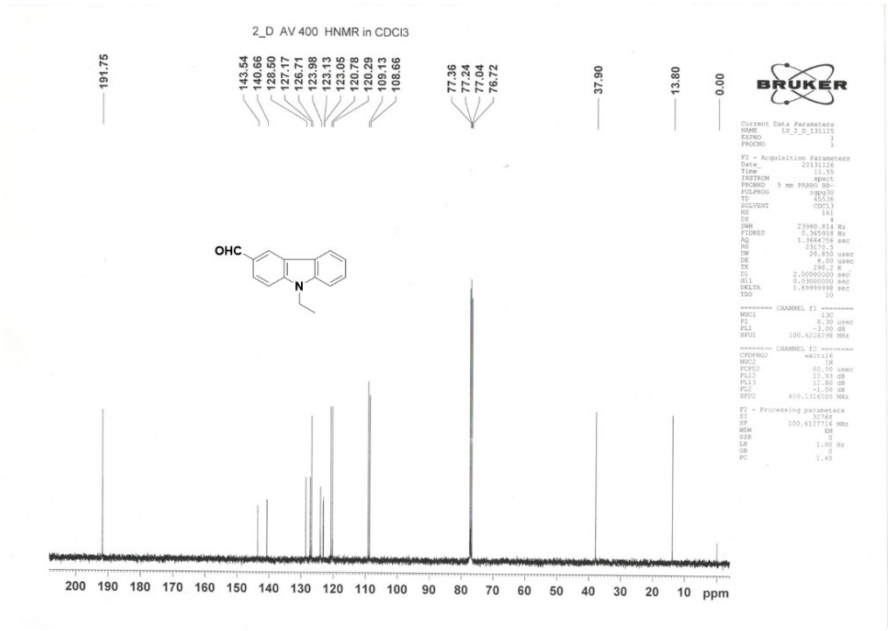

Fig. S15. ^{13}C NMR spectrum of **1** in $CDCl_3$

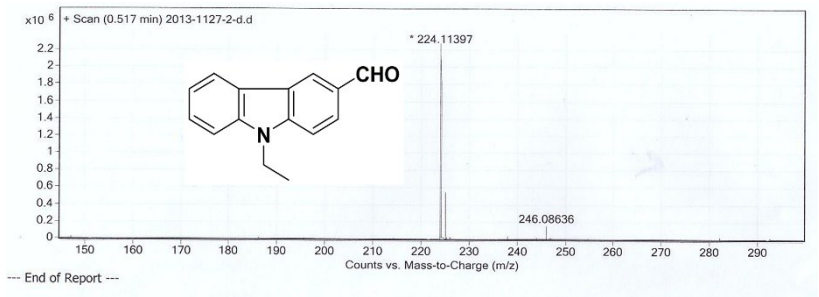

Fig. S16. High resolution mass spectrum (HRMS) of **1**

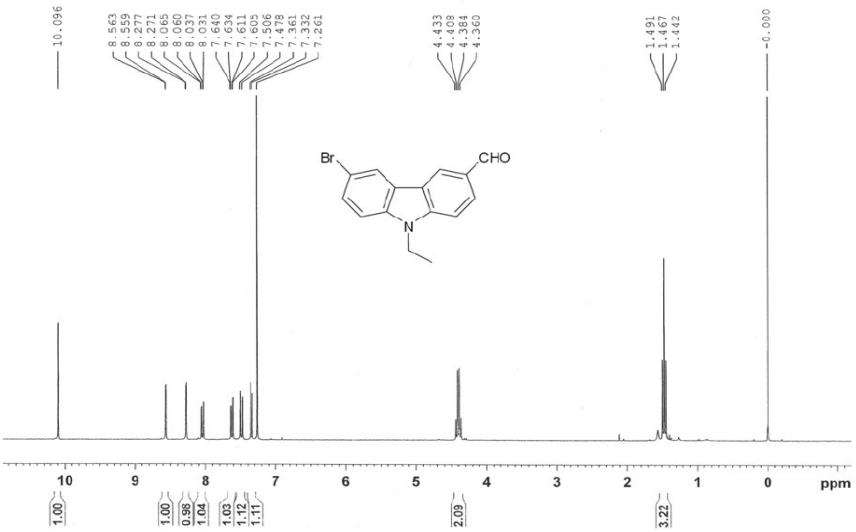

Fig. S17. ^{1}H NMR spectrum of compound **2** in $CDCl_3$

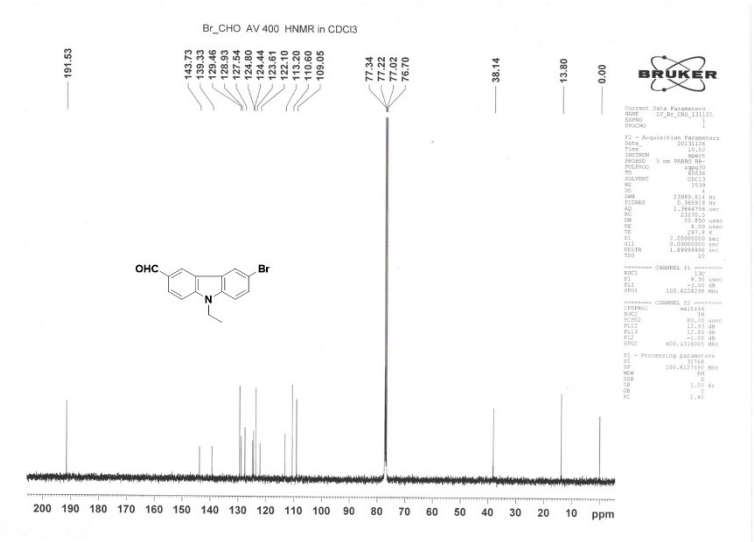

Fig. S18. ^{13}C NMR spectrum of **2** in $CDCl_3$

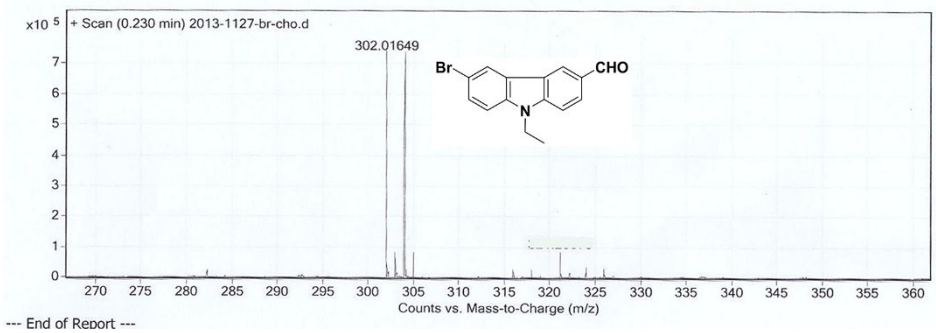

Fig. S19. High resolution mass spectrum (HRMS) of **2**

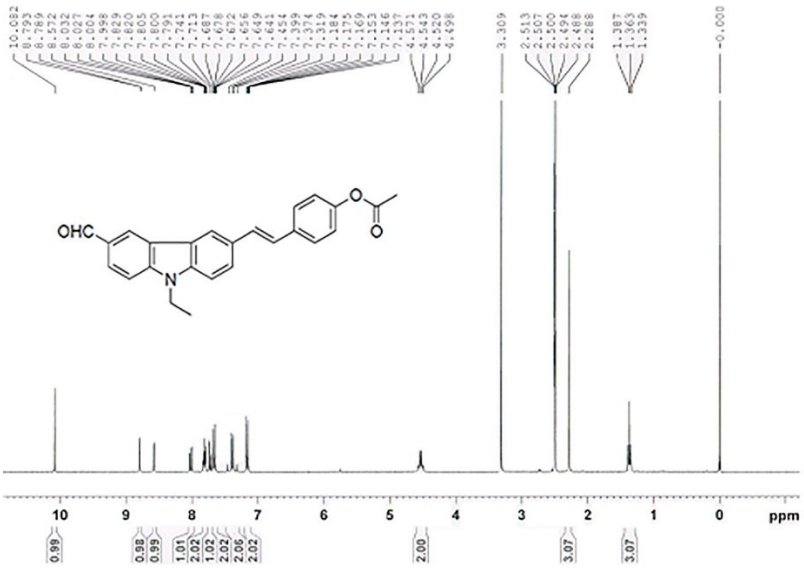

Fig. S20. ^{1}H NMR spectrum of **3** in DMSO-d_6

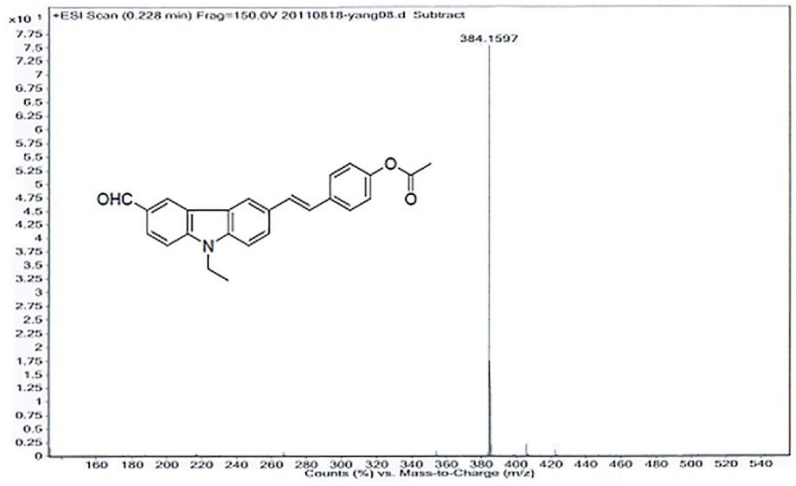

Fig. S21. High resolution mass spectrum (HRMS) of **3**

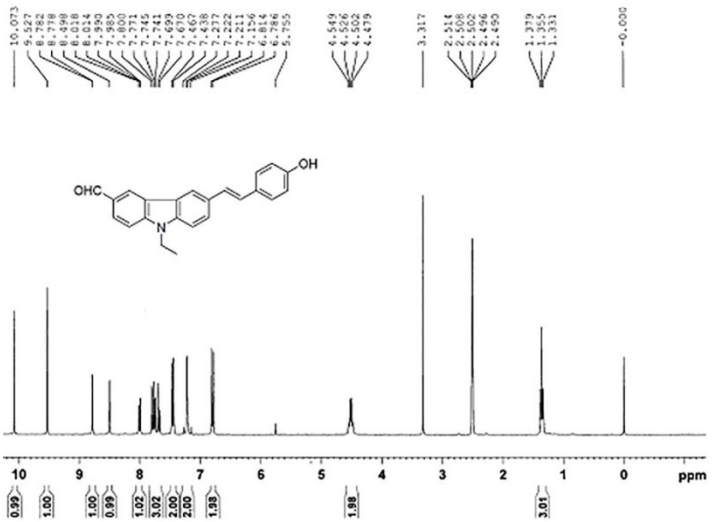

Fig. S22. ^{1}H NMR spectrum of **4** in DMSO-d_6

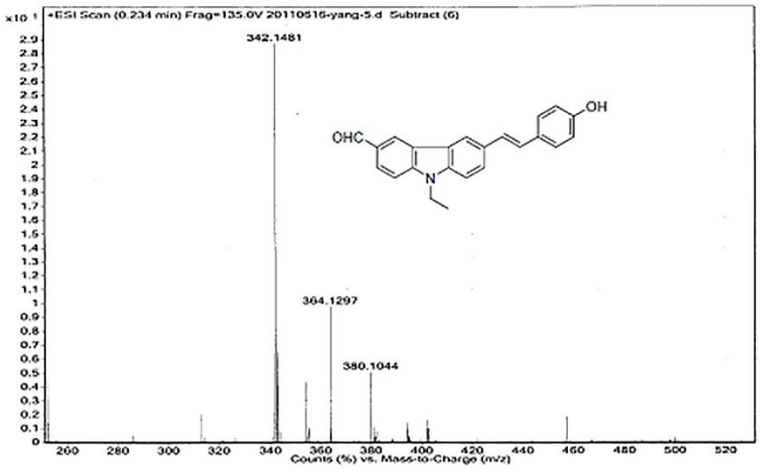

Fig. S23. [1]H NMR spectrum of **4** in DMSO-d_6

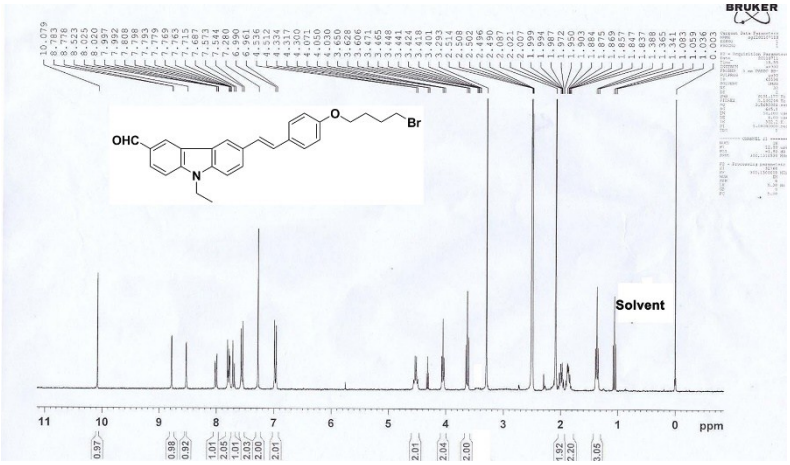

Fig. S24. [1]H NMR spectrum of **5** in DMSO-d_6

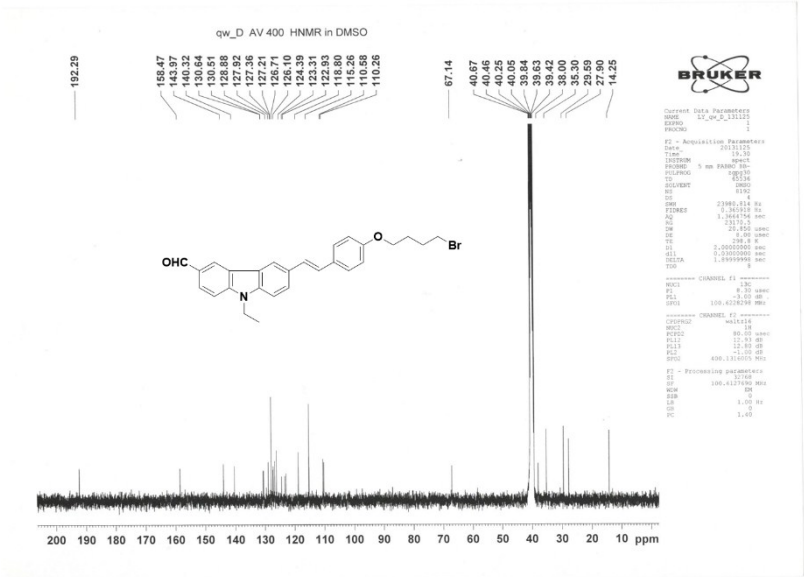

Fig. S25. [13]C NMR spectrum of **5** in DMSO-d_6

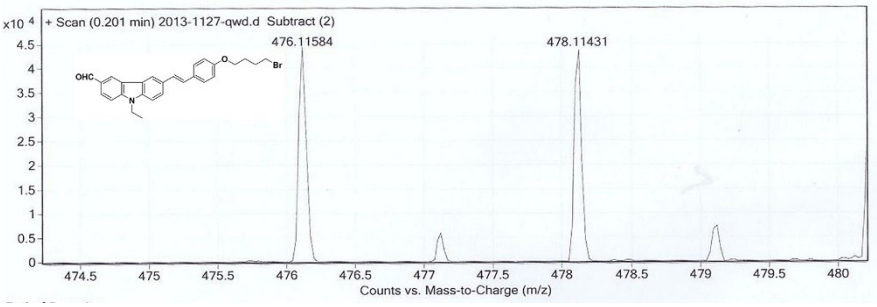

Fig. S26. HRMS spectrum of **5**

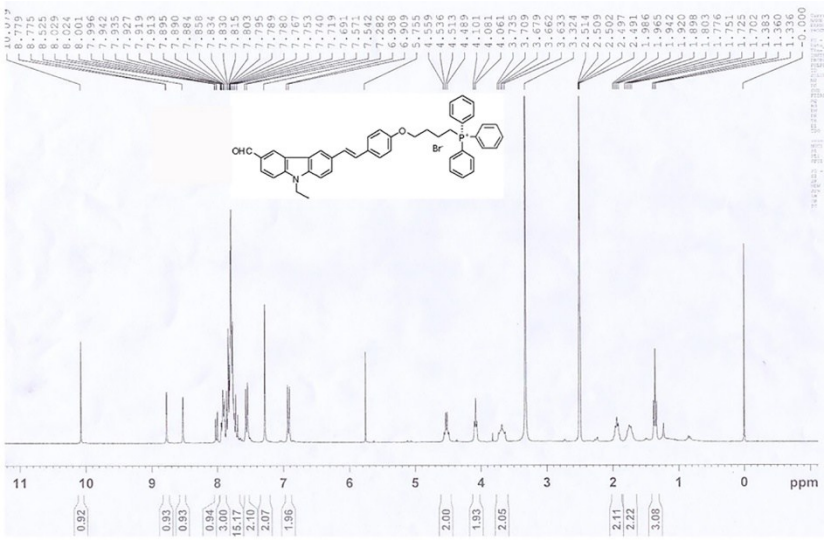

Fig. S27. ^{1}H NMR spectrum of **CA-TPP** in DMSO-d_6

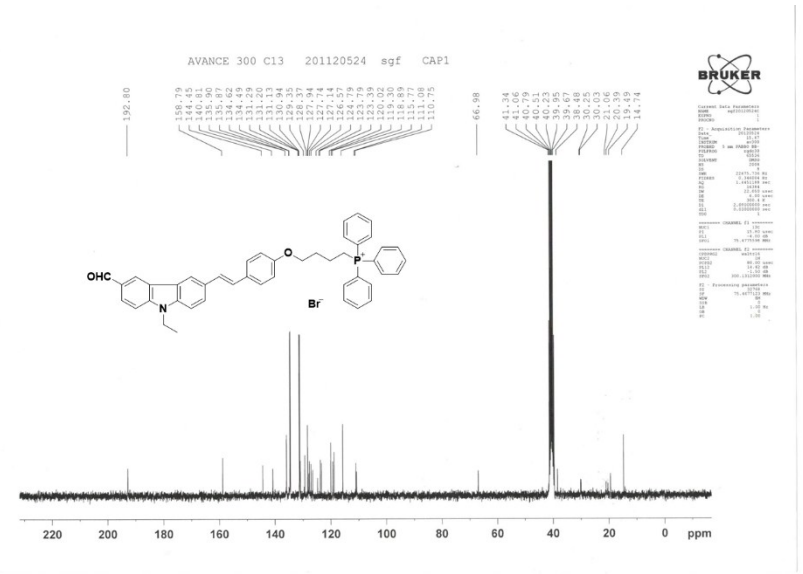

Fig. S28. ^{13}C NMR spectrum of **CA-TPP** in DMSO-d_6

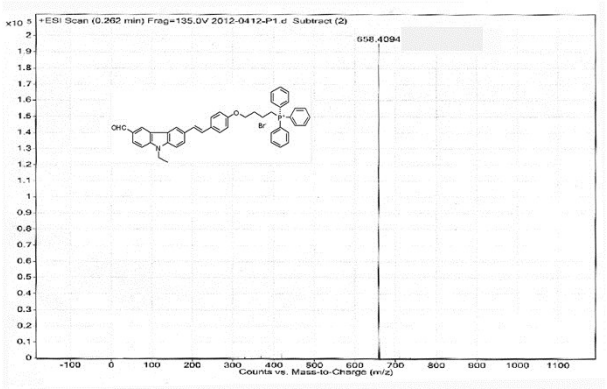

Fig. S29. HRMS spectrum of **CA-TPP**