

Supporting Information for

**A dual-site two-photon fluorescent probe for visualizing lysosomes
and tracking lysosomal hydrogen sulfide with two different sets of
fluorescence signals in the living cells and mouse liver tissues**

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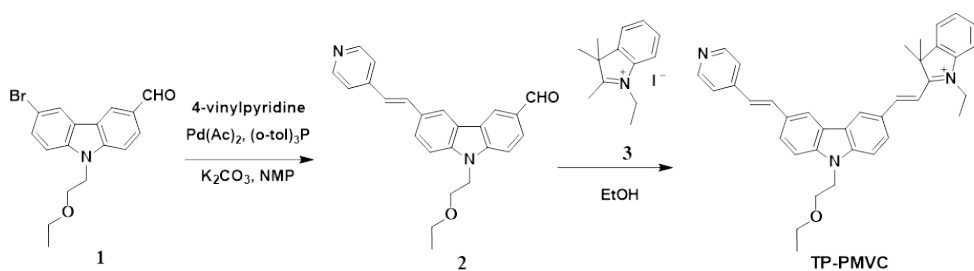
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Material

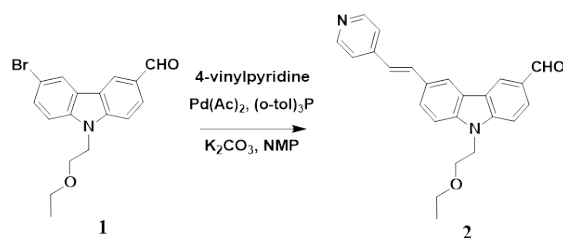
Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR spectrometer. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. Fluorescence imaging of the cells and tissues slices was obtained with Nikon A1MP two-photon confocal microscopy. Two-photon imaging was conducted on a SpectroPro300i and the pump laser beam came from a mode-locked Ti: sapphire laser system at the pulse duration of 200 fs, a repetition rate of 76 MHz (Coherent Mira900-D).

Synthesis



Scheme. S1. The synthetic route to the probe TP-PMVC.

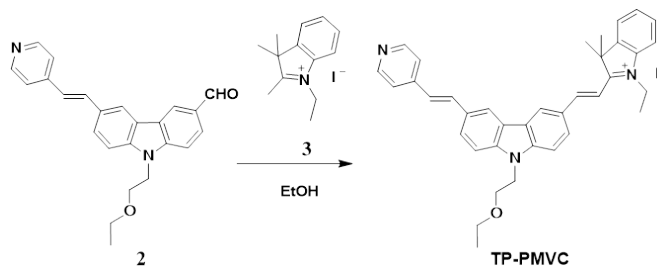
Synthesis of 6-bromo-9-(2-ethoxyethyl)-9H-carbazole-3-carbaldehyde (**2**):



Compound **1** ⁽¹⁾ (0.69 g, 2.0 mmol) was added into a flask containing a mixture of palladium(II) acetate (44.9 mg, 0.2 mmol), tri-(*o*-tolyl) phosphine (182.62 mg, 0.6 mmol), K₂CO₃ (2.2 g, 16.0 mmol), and 35 mL of *N*-methyl-2-pyrrolidone (NMP). 4-vinylpyridine (0.43 mL, 4.0 mmol) was then added after the above mixture was bubbled with nitrogen for 30 min. The system was heated at 130 °C for 36 h under the protection of nitrogen and a dark-red suspension was obtained, which was cooled to room temperature and then poured into H₂O (500 mL) and extracted with CH₂Cl₂. The organic phase was separated, dried with MgSO₄, and removed by vacuum distillation. The product was obtained as a yellow solid with a yield of 60% after the residue was purified by column chromatography with ethyl acetate/petroleum ether (2:1, v/v) as eluent. ¹H NMR (400 MHz, CDCl₃) δ 10.12 (s, 1H), 8.64 (s, 1H), 8.60 (d, *J* = 6.0 Hz, 2H), 8.31 (s, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.75 (d, *J* = 8.5 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 1H), 7.54 (s, 1H), 7.51 (d, *J* = 7.2 Hz, 1H), 7.43 (d, *J* = 6.0 Hz, 2H), 7.09 (d, *J* = 16.3 Hz, 1H), 4.53 (t, *J* = 5.7 Hz, 2H), 3.84 (t, *J* = 5.7 Hz, 2H), 3.42 (q, *J* = 7.0 Hz, 2H), 1.09 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 191.66, 149.98, 145.18, 144.88, 141.77, 133.75, 129.01, 127.57, 125.85, 124.17, 123.81, 123.55, 123.10, 120.73, 119.56, 110.10, 109.74, 68.53, 66.98, 43.95, 15.08.

1. J. Wang, Y. Sun, W. Zhang, Y. Liu and X. Yu, *Talanta*, 2014, **129**, 241.

Synthesis of 2-((E)-2-(9-(2-ethoxyethyl)-6-((E)-2-(pyridin-4-yl)vinyl)-9H-carbazol-3-yl)vinyl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium iodide (**TP-PMVC**):



Under the protection of nitrogen, compounds **2** (0.37 g, 1.00 mmol) and **3** (0.34 g, 1.1 mmol) were dissolved in 15 mL of EtOH and the resulting solution was allowed to react for 24 h at 80 °C. Ethanol in the mixture was evaporated, and then ether (30 mL) was poured into to form a red solid, which was washed by ether for several times, and the resulting solid was dried to afford the product **TP-PMVC** as a red solid with a yield of 85%. ¹H NMR (400 MHz, CDCl₃) δ 9.76 (s, 1H), 9.40 (s, 1H), 8.35 (dd, *J* = 10.1, 5.4 Hz, 3H), 8.11 (d, *J* = 15.6 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.78 – 7.61 (m, 4H), 7.60 – 7.51 (m, 4H), 7.49 (d, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 5.13 (d, *J* = 7.1 Hz, 2H), 4.51 (t, *J* = 5.3 Hz, 2H), 3.85 (t, *J* = 5.4 Hz, 2H), 3.42 (q, *J* = 7.0 Hz, 2H), 1.88 (s, 5H), 1.70 (t, *J* = 7.1 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (100MHz, CDCl₃) δ 180.13, 156.93, 148.29, 146.60, 145.45, 142.97, 141.69, 140.40, 132.15, 129.56, 128.83, 127.27, 125.60, 124.87, 124.36, 123.86, 122.61, 121.95, 121.14, 113.62, 110.21, 109.86, 108.58, 68.52, 66.89, 51.72, 44.21, 43.53, 15.12, 14.41. HRMS (*m/z*): M⁺calcd for C₃₆H₃₆N₃O⁺: 540.30; found, 540.30.

General procedure for the spectral measurement

The stock solution of the probe **TP-PMVC** was prepared at 1 mM in DMSO. The different pH (4.0-8.0) PBS solutions were prepared. The solutions of various testing species were prepared from NaCl, KCl, CaCl₂, MgCl₂, glutathione (GSH), H₂O₂, homocysteine (Hcy), NaHSO₃, KI, cysteine (Cys), NaNO₂, Na₂S₂O₃, Na₂SO₃, Na₂SO₄, ZnCl₂, and NaHS in the twice-distilled water. The test solution of the probe **TP-PMVC** (10 μM) in 3 mL PBS buffer (pH 4.4) with 5% DMSO was prepared by

placing 0.03 mL of the probe **TP-PMVC** stock solution and 0.15 mL DMSO in 2.82 mL of the aqueous buffer. The resulting solution was shaken well and incubated with appropriate testing species for 5 min at ambient temperature before recording the spectra. For the pH response experiments, the excitation wavelength was 488 nm, and the excitation and emission slit widths were 5 and 10 nm, respectively. Unless otherwise noted, for the titration and selectivity experiments, the excitation wavelength was 405 nm, and the excitation and emission slit widths were 5 and 10 nm, respectively.

Quantum yields

The fluorescence quantum yields can be calculated by means of equation (1):

$$\Phi_s = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{n_s^2}{n_r^2} \right) \frac{F_s}{F_r} \quad (1)$$

Where the subscripts *s* and *r* refer to the sample and the reference, respectively. Φ is quantum yield, *F* is the integrated emission intensity, *A* stands for the absorbance, and *n* is refractive index.

Two-photon absorption (TPA) cross sections

Two-photon absorption (TPA) cross sections were measured using the two-photon induced fluorescence method, and the cross section can be calculated by means of equation (1):²⁻⁴

$$\delta_s = \delta_r \frac{\Phi_r c_r n_r F_s}{\Phi_s c_s n_s F_r} \quad (1)$$

Where the subscripts *s* and *r* refer to the sample and the reference, respectively. The terms *c* and *n* are the concentration and refractive index of the solution, respectively. *F* is two-photon excited fluorescence integral intensity. Φ is the fluorescence quantum yield. *r* is the TPA cross-section of coumarin 307 in methanol ($\delta = 27.7 \text{ GM}$) at 800 nm.⁵

2. X. Zhang, X. Q. Yu, Y. M. Sun, H. Y. Xu, Y. G. Feng, B. B. Huang, X. T. Tao and M. H. Jiang, *Chem. Phys.*, 2006, **328**, 103.
3. X. Zhang, X. Q. Yu, J. S. Yao and M. H. Jiang, *Synth. Met.*, 2008, **158**, 964.
4. X. Zhang, Y. M. Sun, X. Q. Yu, B. Q. Zhang, B. B. Huang and M. H. Jiang, *Synth. Met.*, 2009, **159**, 2491.
5. C. Xu, *J. Opt. Soc. Am. B*, 1996, **13**, 481.

Cell culture and Imaging

A549 cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO₂ incubator at 37 °C. Before the imaging experiments, A549 cells were subcultured and seeded in the glass bottom culture dishes (Nest) at a density of 1×10⁵/ mL. The cells were placed on glass coverslips and allowed to adhere for 24 h. When the cells reached about 70 % confluence, they were then subjected to the imaging experiments.

The co-localization experiment: The A549 cells were incubated with 5 μM **TP-PMVC** in the culture medium for 20 min, and then the medium was removed and washed three times with PBS to remove the excess probe. Subsequently, 1mL of the culture medium containing 2 μM LysoTracker Deep Red was added to the cells and incubated for 15 min. The residual LysoTracker Deep Red was removed by washing three times using PBS before the imaging by Nikon fluorescence microscope equipped with 40×objective lens. Finally, confocal fluorescence imaging was carried out. Fluorescence images of LysoTracker Deep Red were collected between 660 and 700 nm upon excitation at 647 nm; Fluorescence images of **TP-PMVC** were collected between 650 and 675 nm upon excitation at 488 nm.

The PMA control experiment: Firstly, for the experimental group, the culture medium of the cells was changed to a fresh medium containing 5 μM **TP-PMVC**, and then incubated for 20 min. Subsequently, the medium was removed and washed three times with PBS to remove the excess probe. Secondly, we carried out the negative control experiment, in which the culture medium of the cells was changed to a fresh medium containing 50.0 μL (1 μg/mL) PMA and incubated for 1 h. Then, the medium

was removed and washed three times with PBS to remove the excess PMA. After that, 1mL of the medium containing 5 μM **TP-PMVC** was added and then incubated for 20 min. Finally, the confocal imaging was carried out in the red ($\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 650\text{-}675 \text{ nm}$) and green channels ($\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 475\text{-}550 \text{ nm}$).

Dual-color fluorescence imaging of lysosomes and lysosomal H_2S in the living cells: First, the A549 cells were incubated with 5 μM **TP-PMVC** in the culture medium for 20 min. Subsequently, the medium was removed and washed three times with PBS to remove the excess probe. Finally, one- and two-photon fluorescence imaging was carried out. The cells exhibited lysosomes and lysosomal H_2S fluorescence in the red ($\lambda_{\text{ex}} = 488 \text{ nm} / 960 \text{ nm}$, $\lambda_{\text{em}} = 650\text{-}675 \text{ nm}$) and green channels ($\lambda_{\text{ex}} = 405 \text{ nm} / 810 \text{ nm}$, $\lambda_{\text{em}} = 475\text{-}550 \text{ nm}$).

Real-time imaging of lysosomes and lysosomal H_2S : First, the A549 cells were incubated with 5 μM **TP-PMVC** in the culture medium for 20 min. After that, the medium was removed and washed three times with PBS to remove the excess probe. Finally, lysosomes and lysosomal H_2S fluorescence imaging was acquired under successive irradiation. Red channel : $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 650\text{-}675 \text{ nm}$; Green channel: $\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 475\text{-}550 \text{ nm}$.

Two-photon fluorescence spectra of A549 cells

The live cells were incubated with 5 μM **TP-PMVC** for 20 min. Subsequently, the cells were washed three times with PBS, transferred to glass-bottomed dishes, and observed by the infrared spectra detector (IR SD) of Nikon A1MP two-photon confocal microscope. The cells were excited at 810 and 960 nm, and the fluorescence spectral data of the A549 cells were analyzed by Nikon analysis software NIS-Elements AR.

Cell viability evaluated by MTT assays

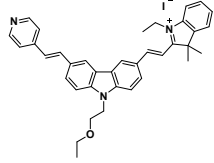
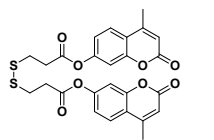
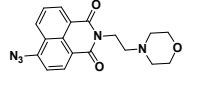
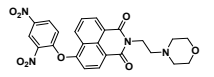
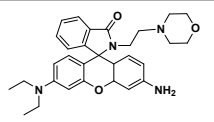
A549 cells were seeded per well in a 96-well plate. The next day the medium was changed into a medium containing 10 μM **TP-PMVC**. After different incubation times (0.5, 1, 4, 8, 12 h), the medium and the excess probe were removed, and then 10

μL MTT (5 mg/mL in PBS) was added. Subsequently, the culture medium was removed, and 100 μL DMSO was added into the dishes to dissolve the formazan crystal product. The plate was shaken for 10 min, and then the absorbance at 490 nm was measured by the microplate reader. The cell viability (%) = $(\text{OD}_{490 \text{ sample}} - \text{OD}_{490 \text{ blank}})/(\text{OD}_{490 \text{ control}} - \text{OD}_{490 \text{ blank}}) \times 100\%$. $\text{OD}_{490 \text{ sample}}$ denotes the cells incubated with the probe for different incubation time, $\text{OD}_{490 \text{ control}}$ denotes the cells without the probe, $\text{OD}_{490 \text{ blank}}$ denotes the wells containing only the culture medium.

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

The slices were prepared from the liver of 14 day-old mice, and they were cut to 400 μm thickness by using a vibrating-blade microtome in 25 mM PBS (pH 7.4). The slices were incubated with 10 μM TP-PMVC in PBS buffer bubbled with 95% O_2 and 5% CO_2 for 1 h at 37 $^\circ\text{C}$, and then were washed three times with PBS, transferred to glass-bottomed dishes, and observed under a two-photon confocal microscope (Nikon A1MP). The 3D fluorescence images of the slices were acquired using 960 and 810 nm excitation and fluorescence emission windows of 650–675 nm and 475–550 nm.

Table S1. Properties of the new probe **TP-PMVC** and the reported lysosome-targeted H₂S fluorescent probes.

Probes	Lysosome-targetable	Two-photon imaging of lysosomal H ₂ S	Dual-channel imaging of lysosomes and lysosomal H ₂ S	Reference
	Yes	Yes	Yes	This work
	Yes	No	No	<i>Chem. Commun.</i> , 2014, 50 , 13833.
	Yes	No	No	<i>RSC. Adv.</i> , 2014, 42 , 5790.
	Yes	No	No	<i>Org. Lett.</i> , 2013, 15 , 2310.
	Yes	No	No	<i>Anal. Chem.</i> , 2014, 86 , 7508.

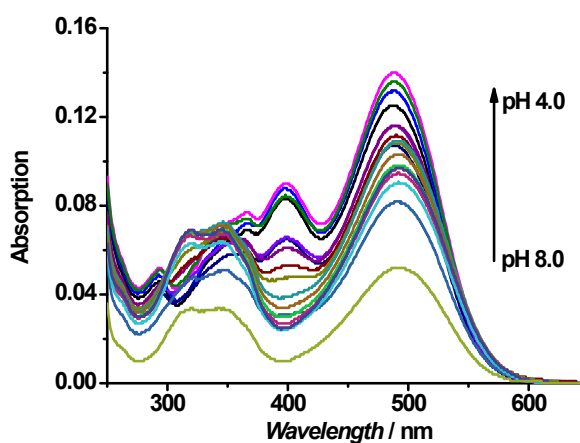


Fig. S1. Absorption spectra of **TP-PMVC** (10 μ M) in distinct pH 4.0-8.0 PBS buffer solutions.

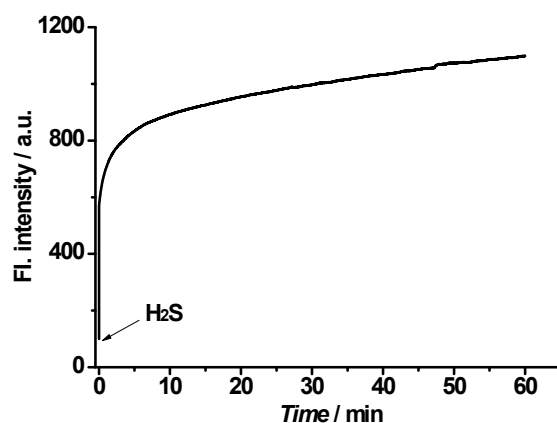


Fig. S2. Time course of the fluorescence intensity of TP-PMVC (10 μM) at 550 nm after adding 220 equiv. H_2S . $\lambda_{\text{ex}} = 405 \text{ nm}$.

Detection limit: The detection limit was determined from the fluorescence titration data based on a reported method.⁶ According to the result of the titration experiment, the fluorescent intensity data at 550 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescent intensity data (Fig. S3), and the point at which this line crossed the axis was considered as the detection limit (3.2 μM).

6. A. Caballero, R. Martinez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tarraga, P. Molina and J. Veciana, *J. Am. Chem. Soc.*, 2005, **127**, 15666.

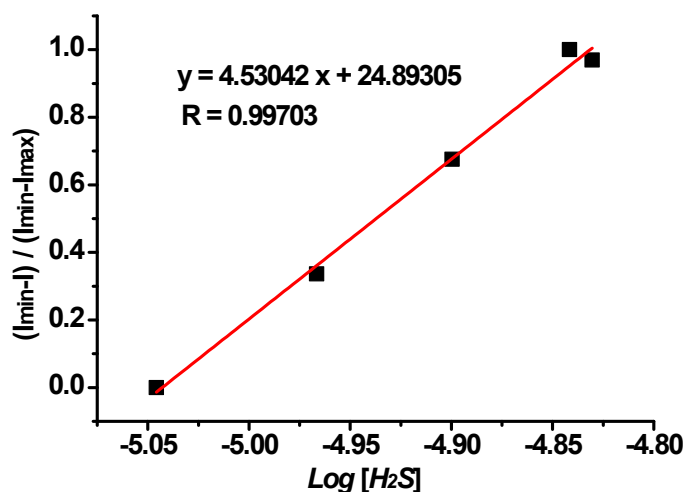


Fig. S3. Normalized response of the fluorescence signal by changing the concentration of H_2S .

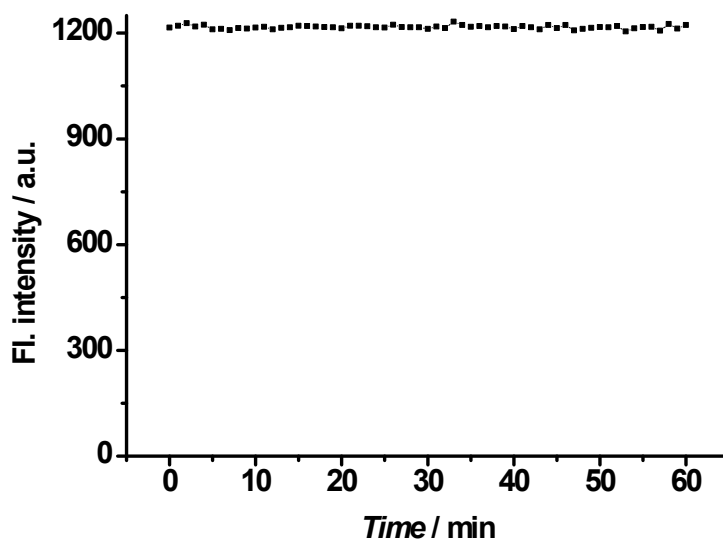


Fig. S4. Fluorescence intensities of **TP-PMVC** (10 μM) to H_2S (150 equiv.) in pH 4.4 PBS buffer solution (containing 5% DMSO) under successive irradiation ($\lambda_{\text{ex}} = 405$ nm) for different times. The intensities were recorded after incubation of the probe with H_2S for 5 min.

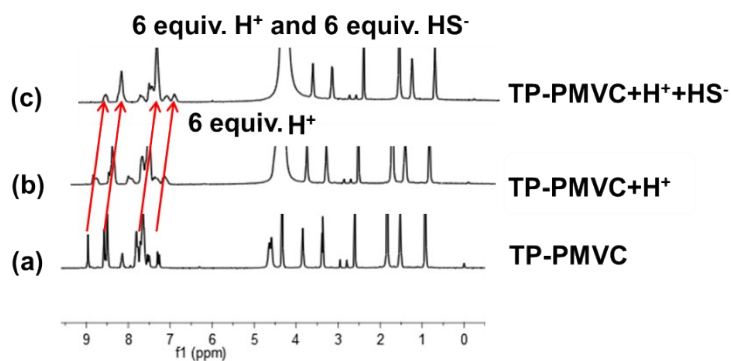


Fig. S5. (a) The ^1H NMR spectrum of **TP-PMVC** in d_6 - DMSO/ D_2O (4/1); (b) The ^1H NMR spectrum of addition of 6 equiv. H^+ ions to **TP-PMVC** in d_6 - DMSO/ D_2O (4/1); (c) The ^1H NMR spectrum of addition of 6 equiv. H^+ ions and 6 equiv. HS^- to **TP-PMVC** in d_6 - DMSO/ D_2O (4/1).

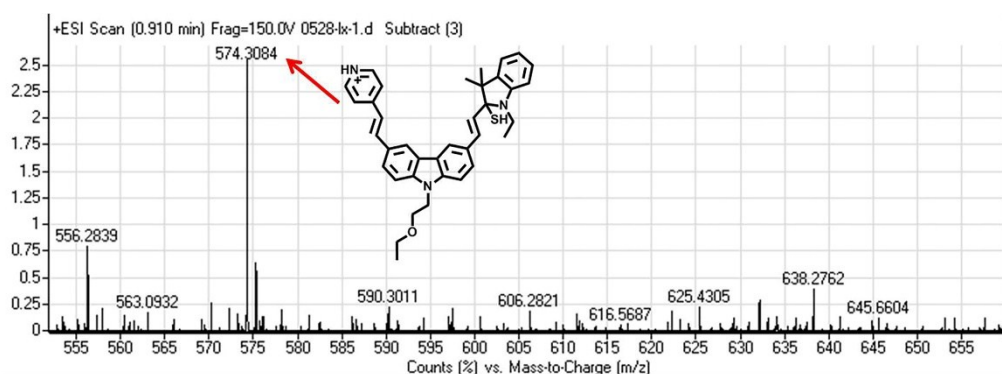


Fig. S6. The formation of TP-PMVC-H⁺-SH from the reaction of the probe with H₂S in the acidic conditions was confirmed by HRMS. The intense peak at m/z 574.3084 in the HRMS spectrum corresponds to (TP-PMVC-H⁺-SH)⁺.

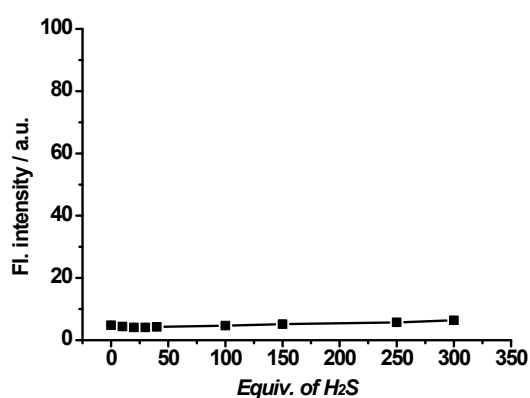


Fig. S7. Fluorescence intensity changes of TP-PMVC (10 μM) at 550 nm with the addition of H₂S in the neutral environment (pH 7.4). λ_{ex}: 405 nm.

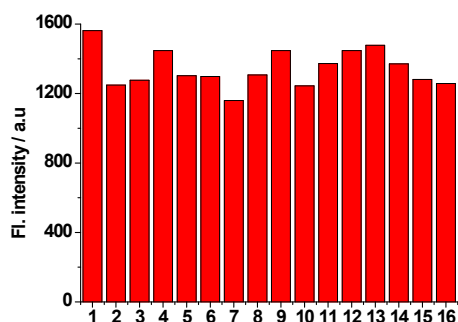


Fig. S8. Fluorescence responses of TP-PMVC (10 μM) in pH 4.4 PBS buffer (containing 5% DMSO) to HS⁻ in the presence of various analytes at room temperature. From left to right: 1. TP-PMVC+HS⁻; 2. TP-PMVC+HS⁻+Cl⁻; 3. TP-PMVC+HS⁻+K⁺; 4. TP-PMVC+HS⁻+Ca²⁺; 5. TP-PMVC+HS⁻+Mg²⁺; 6. TP-PMVC+HS⁻+GSH; 7. TP-PMVC+HS⁻+H₂O₂; 8. TP-PMVC+HS⁻+Hcy; 9. TP-PMVC+HS⁻+HSO₃⁻; 10. TP-PMVC+HS⁻+I⁻; 11. TP-PMVC+HS⁻+Cys; 12. TP-PMVC+HS⁻+NO₂⁻; 13. TP-PMVC+HS⁻+Mg²⁺; 14. TP-PMVC+HS⁻+S₂O₃²⁻; 15. TP-PMVC+HS⁻+SO₃²⁻; 16. TP-PMVC+HS⁻+SO₄²⁻. λ_{ex}=405nm.

Table S2. Cytotoxicity Data of TP-PMVC (5 μ M) in A549 cells^a.

Incubation time	0.5 h	1 h	4 h	8 h	12 h
Survival (%)	98 \pm 3	97 \pm 3	97 \pm 3	92 \pm 1	88 \pm 1

^a Cell viability was quantified by the MTT assays (mean \pm SD).

Positive control experiment: Positive control experiment was determined from a reported method.⁷ As shown in Fig. S9, imaging results of (a-c) and (d-f) were carried out at the same conditions using Nikon confocal fluorescence microscope.

Imaging conditions: HV: 98; Offset: -67; laser: 16.8; Pinhole: 1.1. $\lambda_{\text{ex}} = 405$ nm; $\lambda_{\text{em}} = 475$ -550 nm. Other conditions see caption of Fig. S10.

7. (a) B. W. Michel, A. R. Lippert and C. J. Chang, *J. Am. Chem. Soc.*, 2012, **134**, 15668. (b) T. Liu, Z. Xu, D. R. Spring and J. Cui, *Org. Lett.*, 2013, **15**, 2310.

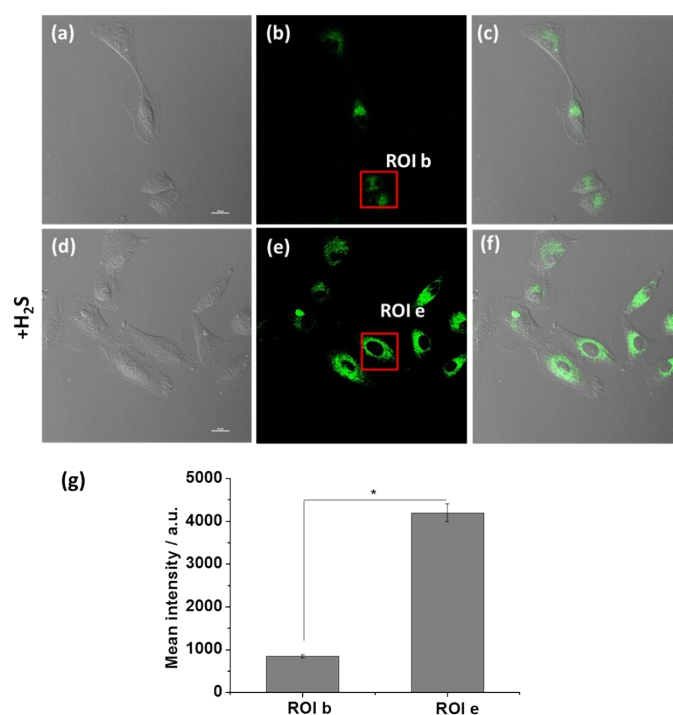


Fig. S9. Fluorescence imaging of H₂S in the lysosomes of A549 cells using TP-PMVC in the absence or presence of added NaHS: (a-c) Confocal images of A549 incubated with 5 μ M TP-PMVC for 20 min followed by incubation in 0.5 % trypan blue solution for 4 min. (d-f) Images of A549 treated with NaHS for 0.5 h and then incubated with 5 μ M TP-PMVC for 40 min followed by incubation in 0.5 % trypan blue solution for 4 min. (g) The comparison of the mean fluorescence intensities of ROI b and ROI e. (a and d): Bright-field images; (b and e): Green channel fluorescence images ($\lambda_{\text{ex}} = 405$ nm; $\lambda_{\text{em}} = 475$ -550 nm); (c and f): Merge pictures of (a and b) and (d and e); Scale bar = 20 μ m. Statistical analyses were performed with Student's *t*-test (n = 4). * *P* < 0.05. Area of ROI b and ROI e are the same.

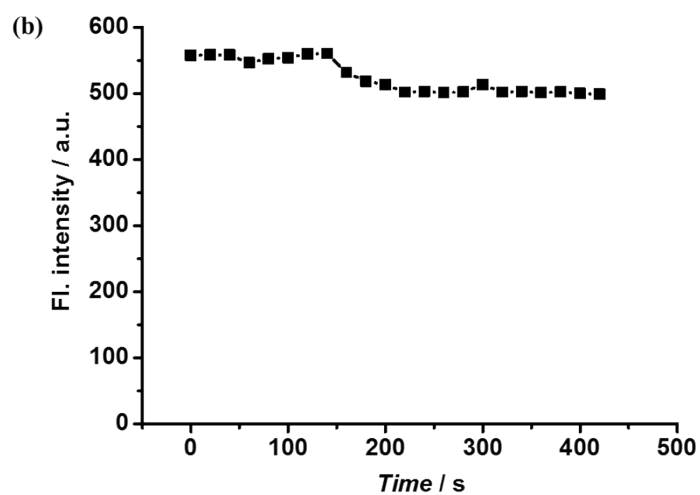
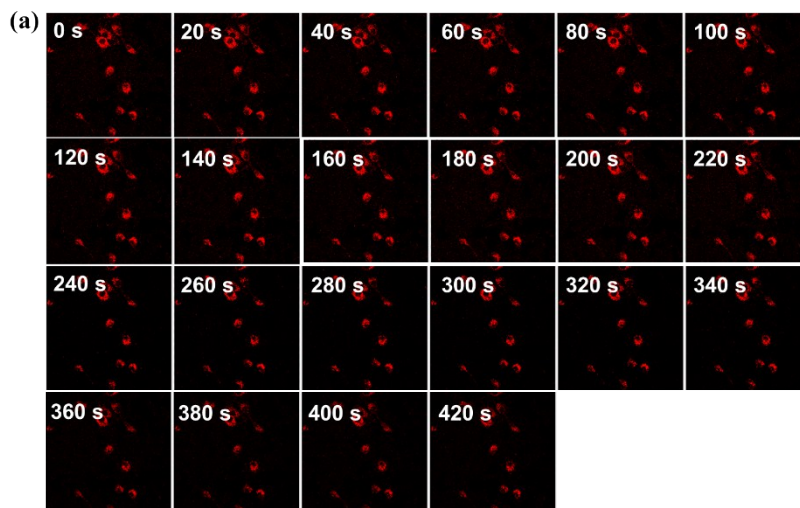


Fig. S10. (a) Fluorescence images (the red channel) of A549 cells incubated with TP-PMVC (5 μ M) acquired at different times under successive excitation. (b) Mean intensities of the cells incubated with probe in the red channel under successive excitation at different times. λ_{ex} : 488 nm, λ_{em} : 650-675 nm.

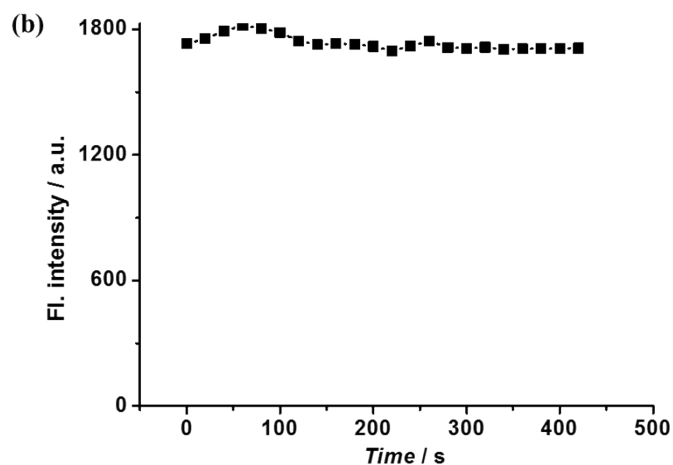
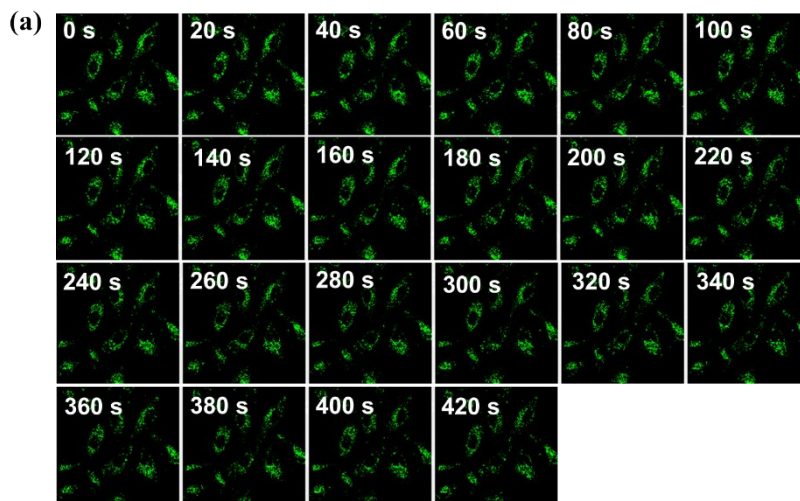


Fig. S11. (a) Fluorescence images (the red channel) of A549 cells incubated with **TP-PMVC** (5 μ M) acquired at different times under successive excitation. (b) Mean intensities of the cells incubated with probe in the green channel under successive excitation at different times. λ_{ex} : 405 nm, λ_{em} : 475-550 nm.

Movie S1: Confocal imaging of the lysosomes of the living A549 cells treated with **TP-PMVC** (5 μ M) at different scanning time (0 - 420 s). $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 650$ -675 nm. Scanning speed: 20 s/scan.

Movie S2: Confocal imaging of the lysosomal H_2S of the living A549 cells stained with **TP-PMVC** (5 μ M) at different scanning time (0 - 420 s). $\lambda_{\text{ex}} = 405$ nm, $\lambda_{\text{em}} = 475$ -550 nm. Scanning speed: 20 s/scan.

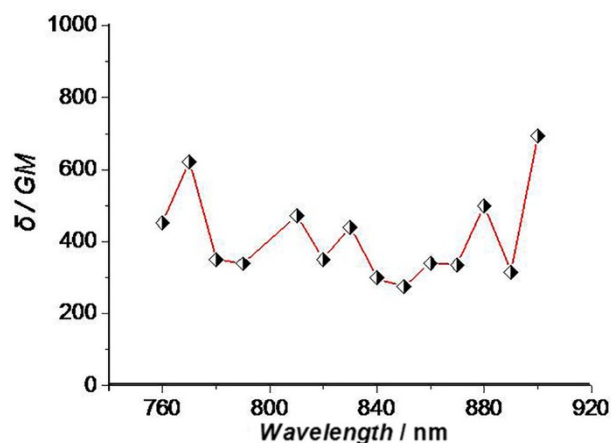


Fig. S12. Two-photon absorption cross sections (δ) of the probe **TP-PMVC** (10 μ M) at 760-900 nm in pH 4.4 PBS buffer (containing 5% DMSO).

Table S3. Photophysical properties of the compound **TP-PMVC** in acidic and neutral pH PBS buffer solution.

	<i>Solvent</i>	$\Phi^a/\%$	δ^b/GM	$\delta\Phi^c/GM$
TP-PMVC	Buffer (pH 4.4)	3.00	750	22.5
	Buffer (pH 7.4)	0.038	—	—

^a refers to fluorescence quantum yield determined using fluorescein ($\Phi = 0.95$) as the standard. ^b refers to two-photon absorption cross sections, and ^c refers to two-photon action cross sections at 900 nm, determined using fluorescein ($\delta = 16$ GM) as the standard at 900 nm. $1GM = 10^{-50} \text{ cm}^4 \text{ s photon}^{-1}$; $[\text{TP-PMVC}] = 10 \mu\text{M}$. The δ and $\delta\Phi$ of the probe in pH 7.4 PBS are negligible.

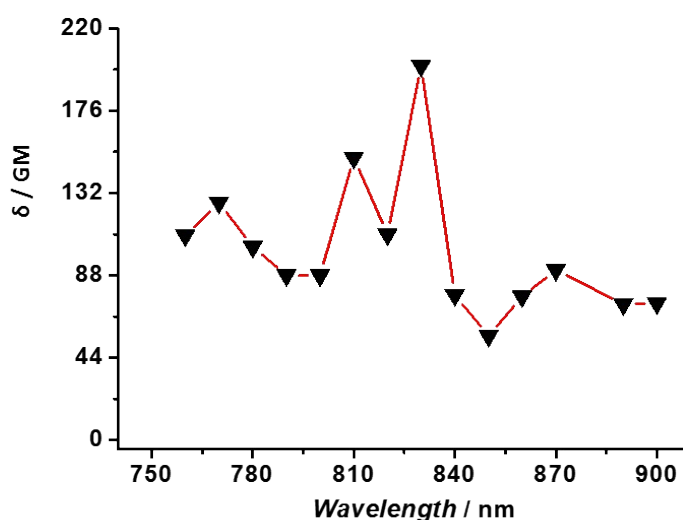


Fig. S13. Two-photon absorption cross sections (δ) of the probe **TP-PMVC** (10 μ M) to H_2S (220 equiv.) at 760-900 nm in pH 4.4 PBS buffer (containing 5% DMSO).

Table S4. Photophysical properties of the compound **TP-PMVC** in the presence of H₂S in pH 4.4 PBS buffer solution (containing 5% DMSO).

	Solvent	λ^a/λ^b (nm)	ε^c ($\times 10^4$)	Φ^d (%)	δ^e (GM)	$\delta\Phi^f$ (GM)
TP-PMVC+H₂S	pH 4.4 buffer	405/550	3.9	11	203	22.3

^a and ^b are linear absorption and fluorescent maximum peaks in nm respectively; Abs is absorbance, ^c ($\times 10^4$) is molar absorptivity in $M^{-1}\cdot cm^{-1}$; ^d is fluorescence quantum yield determined using fluorescein ($\Phi = 0.95$) as the standard. ^e refers to two-photon absorption cross sections, and ^f refers to two-photon action cross sections at 830 nm, determined using fluorescein ($\delta = 29$ GM) as the standard at 830 nm. $1GM = 10^{-50} cm^4 s photon^{-1}$; [TP-PMVC] = 10 μM . 220 equiv. HS⁻

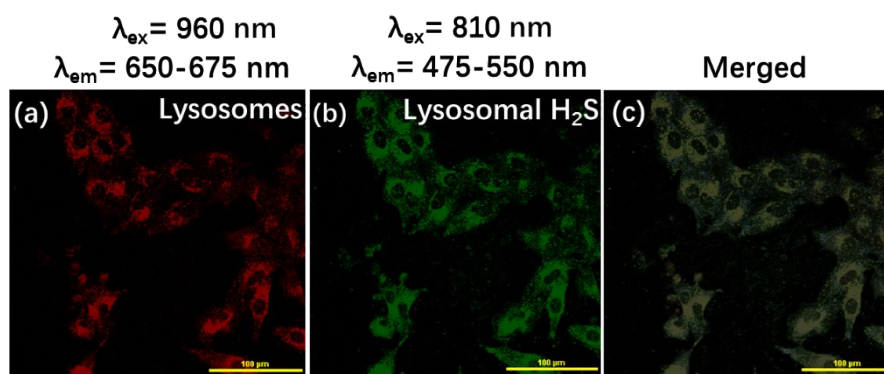


Fig. S14. TP fluorescence images of A549 cells incubated with **TP-PMVC** (5 μM , 20 min). (a) Lysosomes TP fluorescence image ($\lambda_{ex} = 960$ nm, $\lambda_{em} = 650-675$ nm); (b) Lysosomal H₂S TP fluorescence image ($\lambda_{ex} = 810$ nm, $\lambda_{em} = 475-550$ nm); (c) Merged image of a and b. Scale bar = 100 μm .

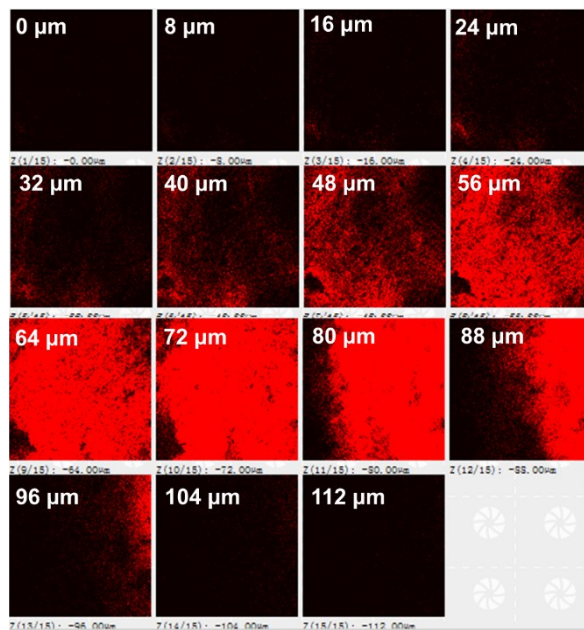


Fig. S15. Two-photon fluorescence images (λ_{ex} : 960 nm. λ_{em} : 650-675 nm) of the mouse liver slice incubated with 10 μM TP-PMVC.

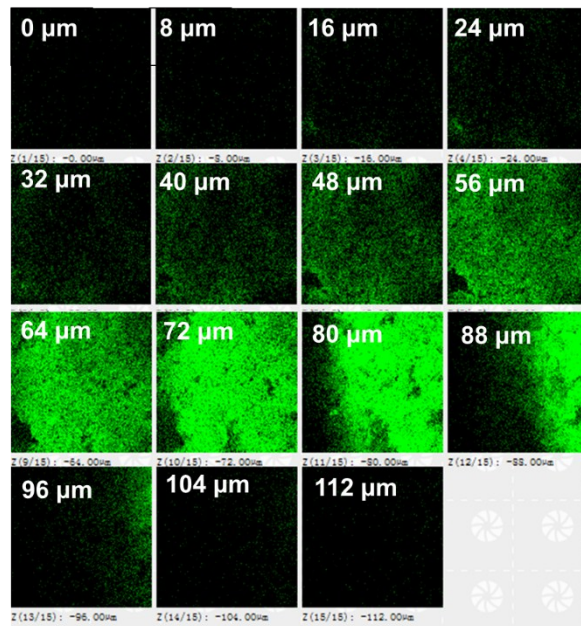


Fig. S16. Two-photon fluorescence images (λ_{ex} : 810 nm. λ_{em} : 475-550 nm) of the mouse liver slice incubated with 10 μM TP-PMVC.

Spectral characterization

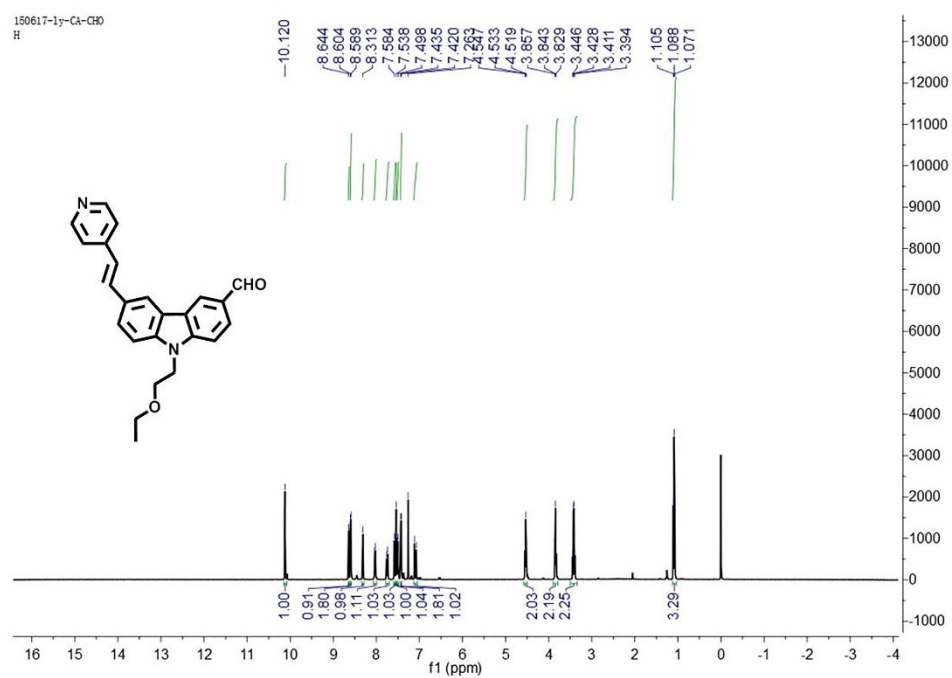


Fig. S17. ¹H NMR spectrum of the compound 2

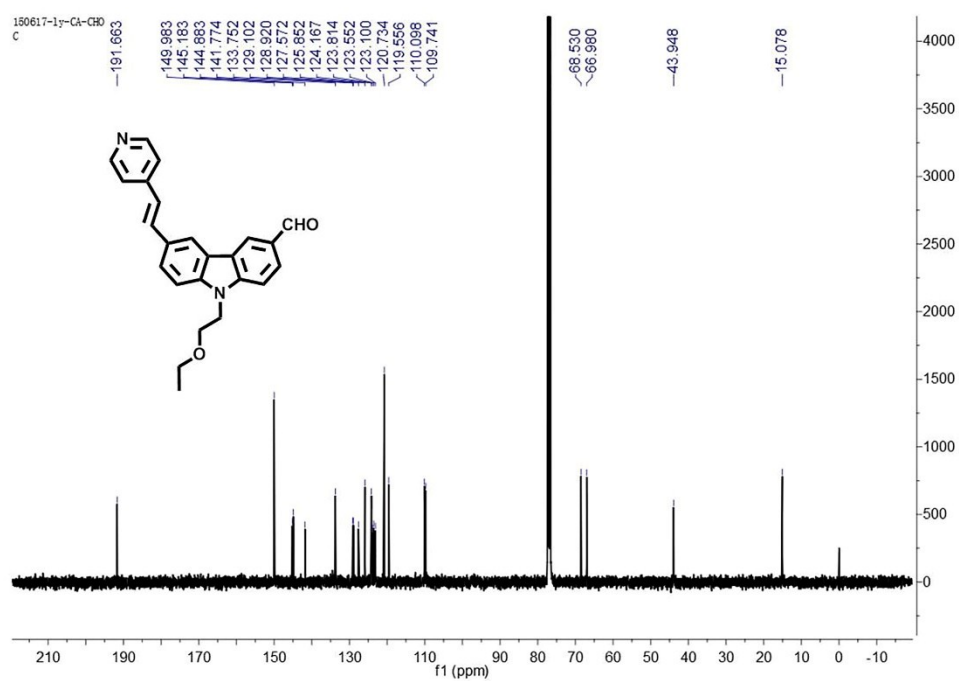


Fig. S18. ¹³C NMR spectrum of the compound 2

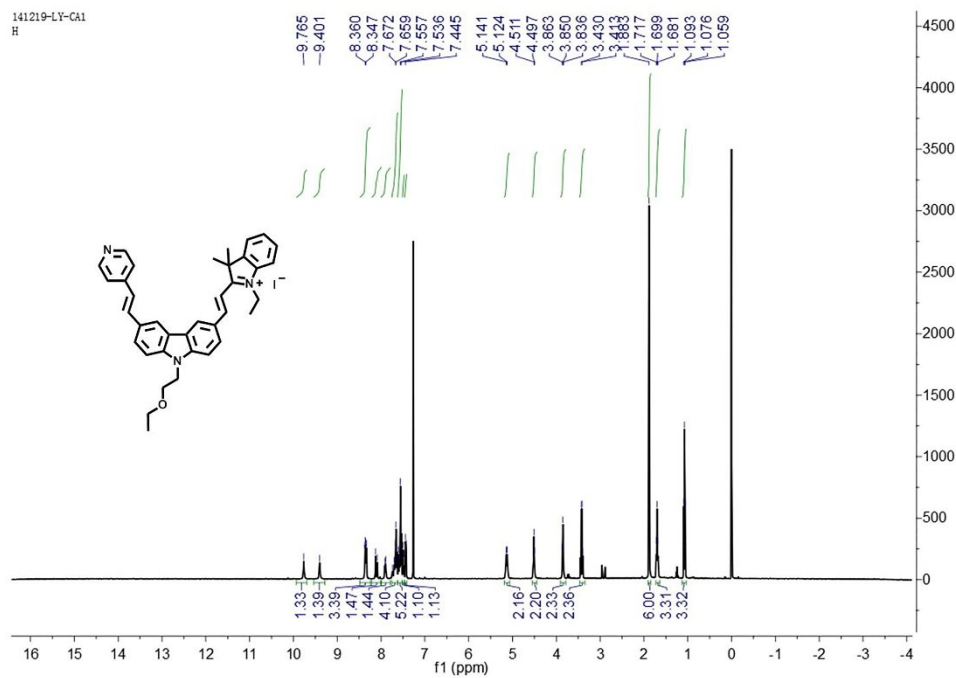


Fig. S19. ^1H NMR spectrum of the compound TP-PMVC

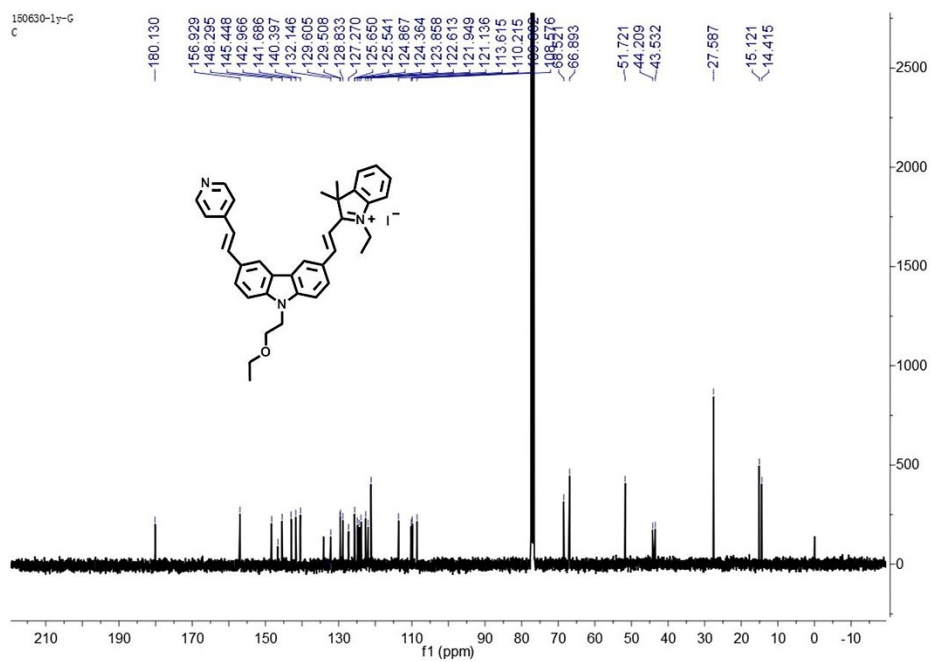


Fig. S20. ^{13}C NMR spectrum of the compound TP-PMVC

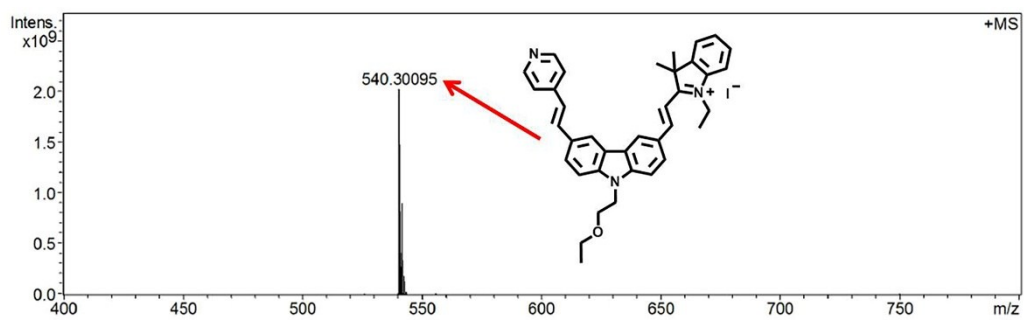


Fig. S21. HRMS spectrum of the compound **TP-PMVC**.