Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting information

Lighting up Cysteine and Homocysteine in Sequence Based on Kinetic Difference of Cyclization/Addition Reaction

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Materials

All chemicals used are of analytical grade, carbazole and NBS were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Palladium (II) acetate and tri-*o*-tolylphosphine were purchased from J&K Chemical (Beijing, China). GSH, DTT, amino acids and PBS were purchased from Seikagaku Corporation (Japan). The solvents used in the spectral measurement are of chromatographic grade. All spectroscopic measurements of **CB1** were performed in ethanol/phosphate buffer solution with pH = 7.4 and v/v of 4:1. TLC analyses were performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from Qingdao Ocean Chemicals.

Methods

Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were obtained on a Bruker Avanace 400 spectrometer. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. The HRMS spectra were recorded on Agilent Technologies 6510 Q-TOF LC/MS or ThermoFisher LCQ FLEET. The elemental analyses were performed on a Perkin-Elmer 2400 instrument.

Spectroscopic Measurements

The UV-visible to near-IR absorption spectra of dilute solutions were recorded on a U2910 spectrophotometer using a quartz cuvette having 1 cm path length. IR spectra were measured on Nexus 670. One-photon fluorescence spectra were obtained on a HITACH F-2700 spectrofluorimeter equipped with a 450-W Xe lamp. Two-photon ones were measured 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).

Cell Culture and Staining

SiHa cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO_2 incubator at 37 ℃. Cells (1×10^5 / mL) were placed on glass coverslips and allowed to adhere for 24 h. For living cells imaging experiment of **CB1**, cells were incubated with 1 μM **CB1** in the culture medium for 40 min and 24 h at 37 ℃, respectively. After rinsing with PBS three times, cells were imaged immediately. For *N*-ethylmaleimide (NEM) treated experiment, SiHa cells were pretreated with a DMSO-PBS (1:49, v/v, pH = 7) solution of 10 mM NEM for 40 min in a 5% CO_2 incubator at 37 ℃, and then incubated with 1 μM **CB1** in the culture medium for 40 min and 24 h at 37 ℃. Fluorescence imaging was then carried out after washing cells with the PBS buffer.

Fluorescence Imaging

Wide-field fluorescence microscopy images were acquired with an Olympus IX71 inverted microscope coupling with CCD and display controller software. The fluorescence of **CB1** was excited and collected through U-MNIBA3 and U-MWU2, respectively.

Two-photon fluorescence imaging were obtained with Olympus FV 300 Laser Confocal Microscope with a 60× water-immersion objective lens (N.A. = 1.25). Emission was collected with a beam splitter IF565 nm, BA510–540 nm or BA430–460 nm band pass filter combination. In two-photon experiment, the excitation wavelength used was 760 nm from a Ti:sapphire femtosecond laser source (Coherent Chamelon Ultra), and the incident power on samples was modified by means of an attenuator and examined with Power Monitor (Coherent).

Cell-Viability Assay

The study of the effect of CB1 on viability of cells was carried out using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay.[S1] SiHa cell cultures were grown in 96-well plates (ca. 1 × 104 cells/well) in DMEM containing 10% bovine calf serum in a 5% CO2 incubator at 37 ℃. For examining the short-term cytotoxic effect, cells were then incubated with CB1 at 1 μM for 1 and 24 h, and then MTT assay was used as described. Non-treated cells were used as the control. Each individual cytotoxic experiment was repeated for three times.

Photostability Experiments. [S2]

The photostability test was carried out in square cross-section quartz cells (l × 1 cm) and solutions of the samples were irradiated under full Xe arc light at room temperature. To eliminate the heat and absorb short wavelength light, a cold trap (3 L solution of 60 g/L $NaNO_2$ in 8 cm (width) × 25 cm (length) × 20 cm (height) was set up between the cells and the lamp. The distance between the cells and the lamp was 15 cm. Fluorescent intensities were captured every 5 min.

References:

[1] J. M. Edmondson, L. S. Armstrong and A. O. Martinez, *J. Tissue Cult. Methods.* 1988, **11**, 15–17.

[2] S. Zhang, T. Wu, J. L. Fan, Z.Y. Li, N. Jiang, J. Y. Wang, B. R. Dou, S. G. Sun, F. L. Song and X. J. Peng, *Org. Biomol. Chem.* 2013, **11**, 555–558.

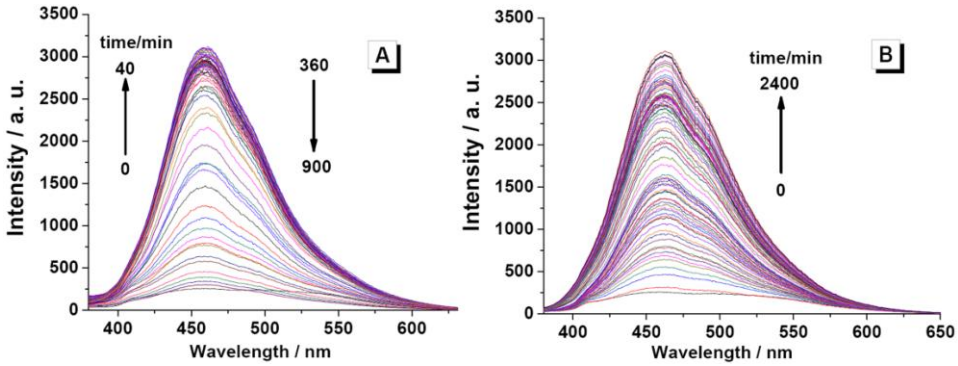


Figure S1. Fluorescence spectra of **CB1**+Cys (A) and **CB1**+Hcy (B) at different time in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4) at room temperature. (A: 0~900 min; B: 0~2400 min)

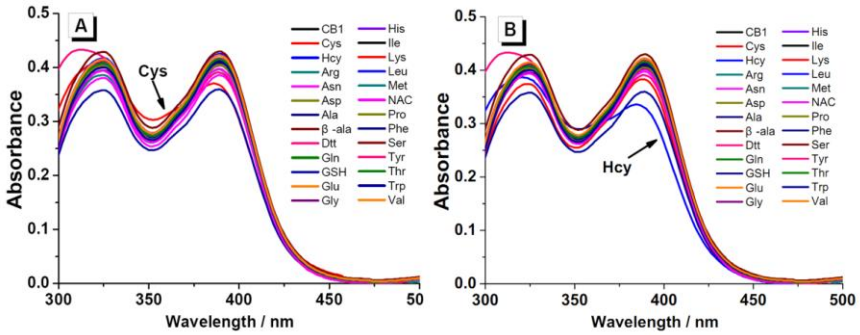
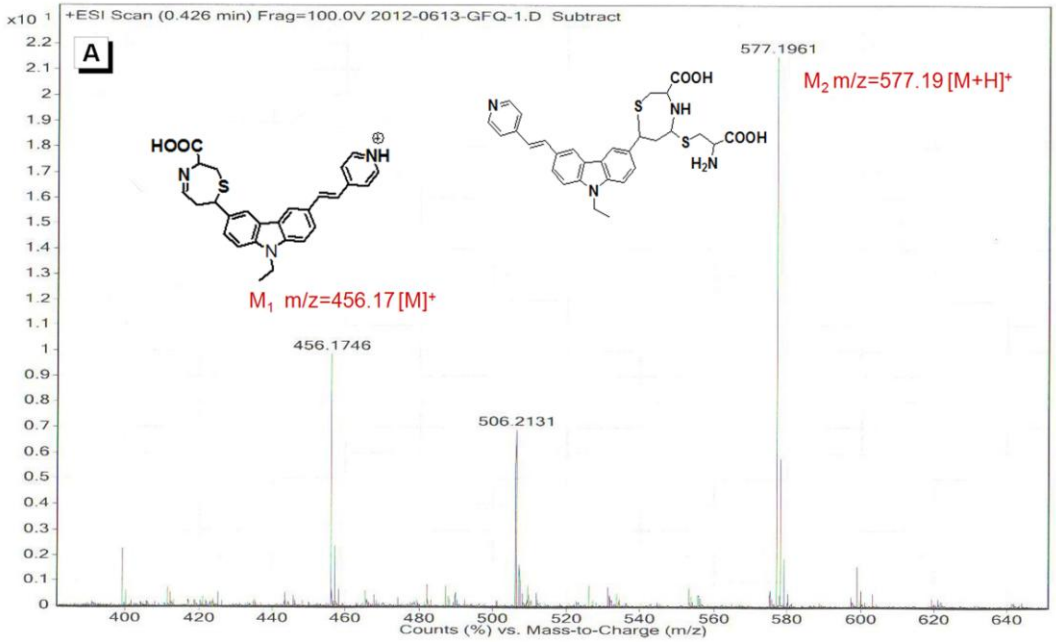


Figure S2. Absorption spectra for **CB1** (10 μM) in the absence and presence of various bioanalytes (600 μM) in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4) at room temperature. A: at 40 min and B: at 24 h.



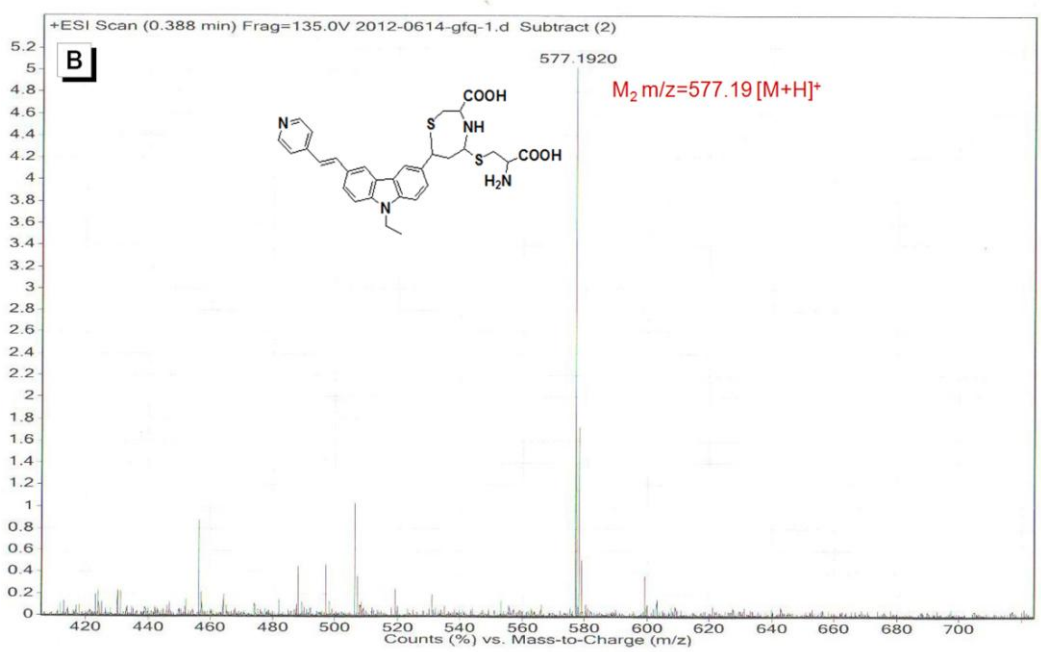


Figure S3. HRMS of **CB1** (10 μM) in the presence of Cys (600 μM) in EtOH at different time. A: 2 h, B: 24 h.

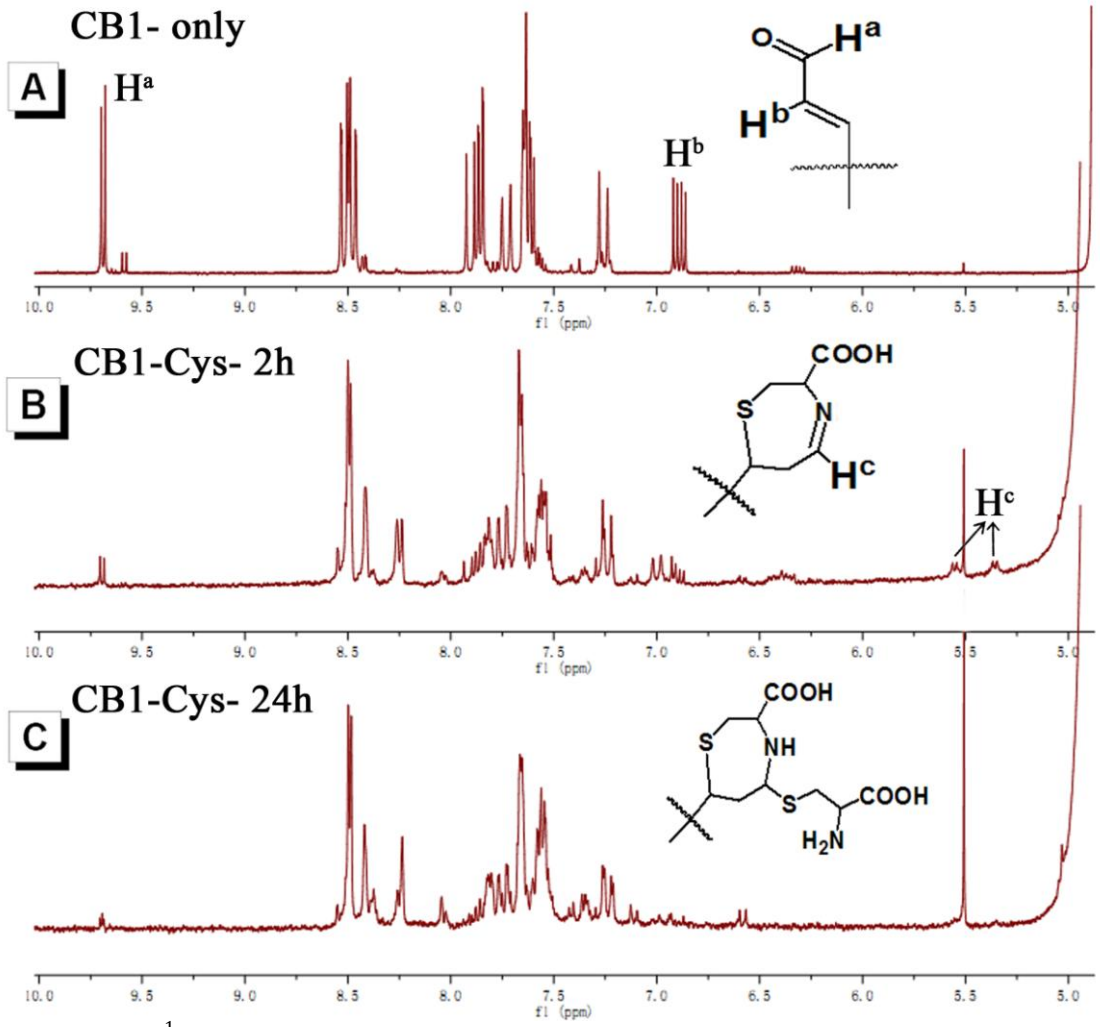


Figure S4. ^{1}H NMR spectra of **CB1** with Cys (n/n 1:2) in CD_3OD at different time.

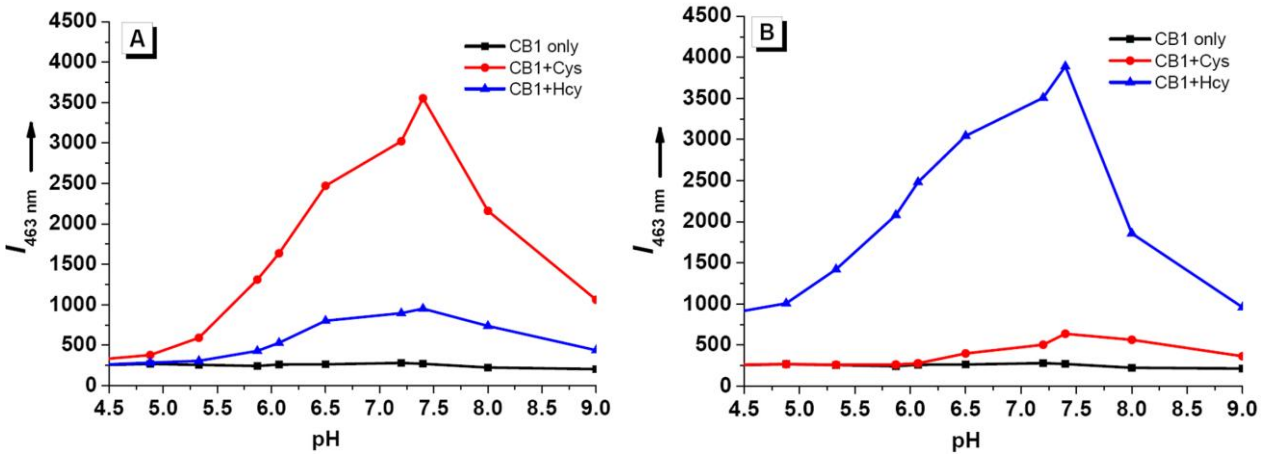
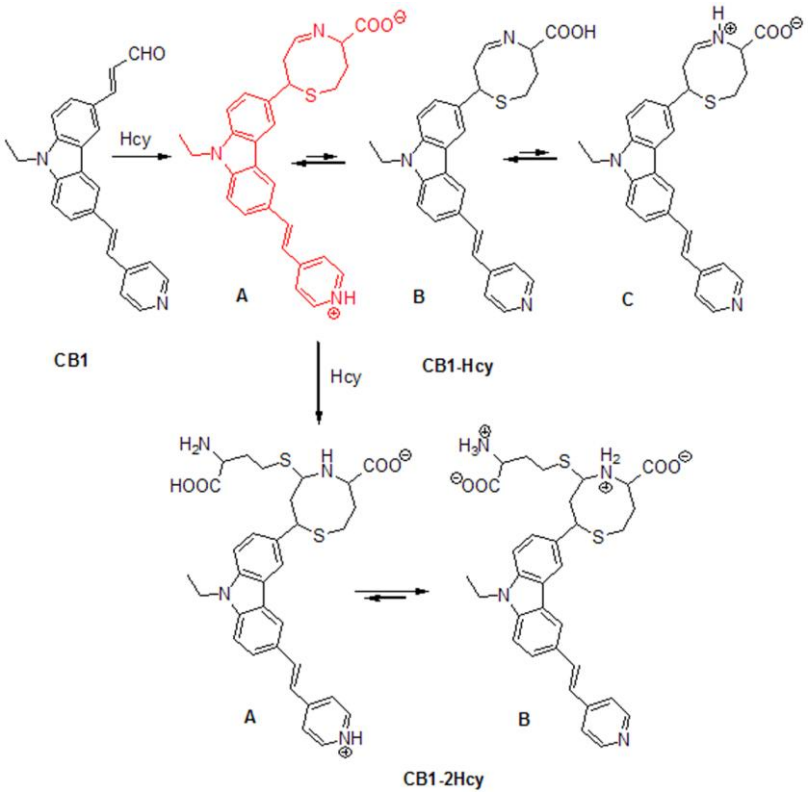


Figure S5. Fluorescence intensity at 463 nm of CB1 (10 μM), CB1 (10 μM) + Cys (600 μM) and CB1 (10 μM) + Hcy (600 μM) in EtOH/PBS buffer solution (v:v, 4:1) at different pH values. λex = 365 nm. A: 40 min, B: 24 h.



Scheme S1. Proposed mechanism for the reaction between **CB1** and Hcy.

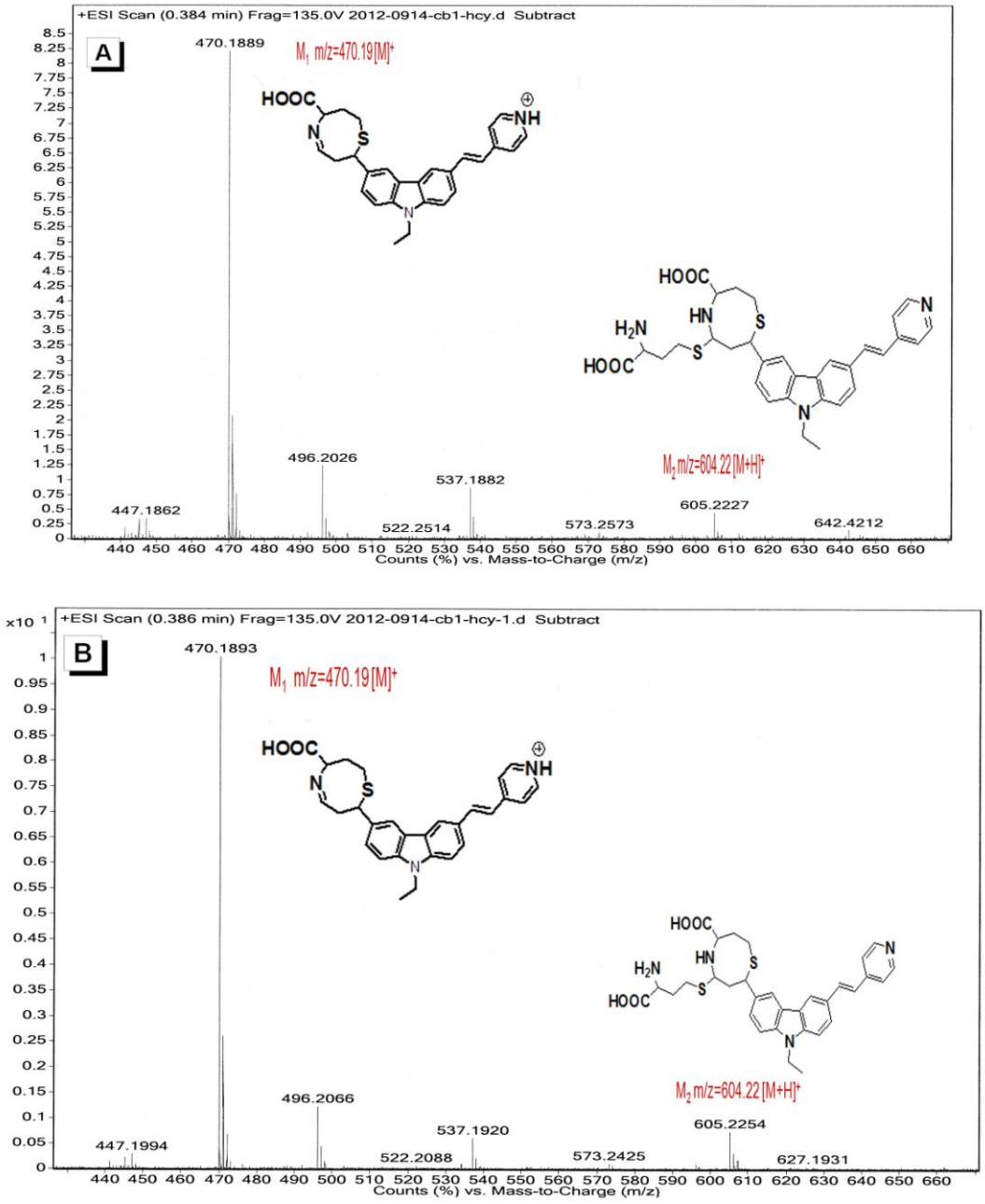


Figure S6. HRMS of **CB1** (10 μM) in the presence of Hcy (600 μM) in EtOH at different time. A: 24 h, B: 36 h.

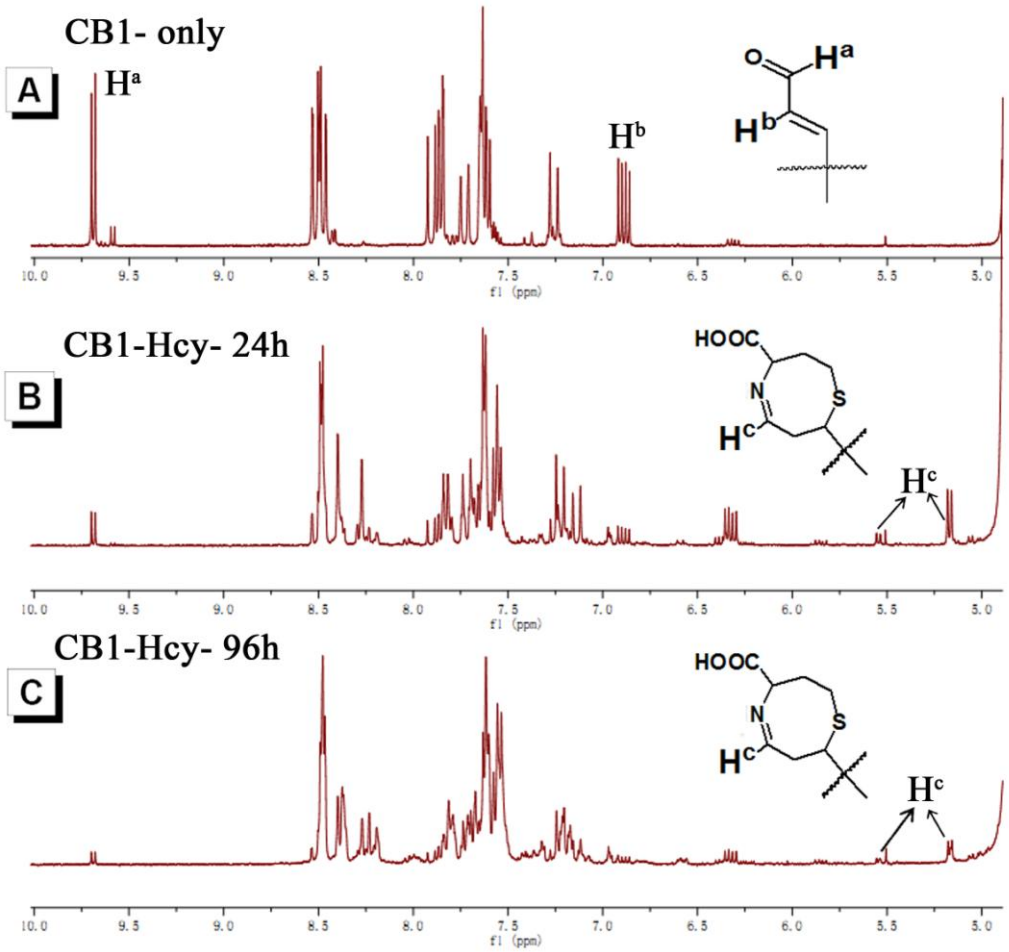


Figure S7. ^{1}H NMR spectra of **CB1** with Hcy (n/n 1:2) in CD_3OD at different time.

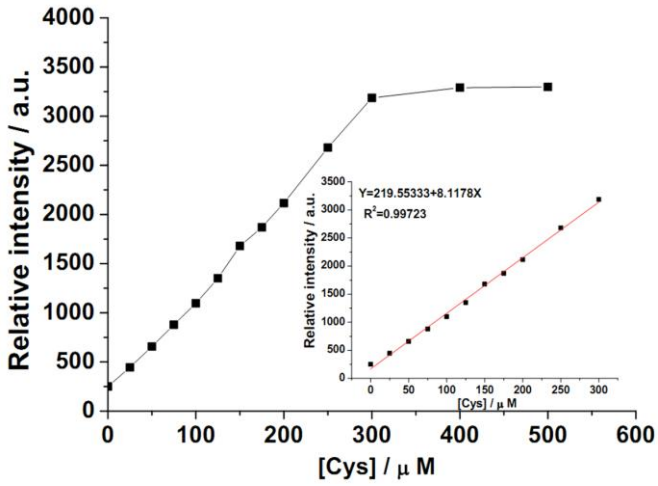


Figure S8. SPEF intensity change spectra of **CB1** (10 μM) at 463 nm in various concentrations of Cys (0–500 μM) at 40 min in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4). Inset: Plot of fluorescence intensity as a function of Cys concentration (0–300 μM). The detection limit of Cys is 59.8 μM. λ_{ex} = 365 nm.

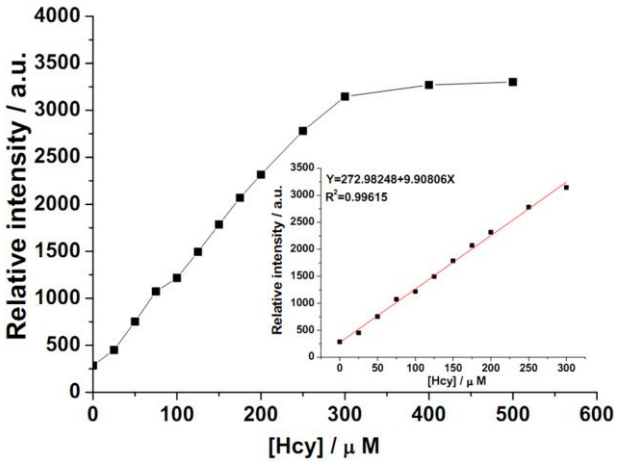


Figure S9. SPEF intensity change spectra of **CB1** (10 μM) at 463 nm in various concentrations of Hcy (0–500 μM) at 24 h in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4). Inset: Plot of fluorescence intensity as a function of Hcy concentration (0–300 μM). The detection limit of Hcy is 58.6 μM. λ_{ex} = 365 nm.

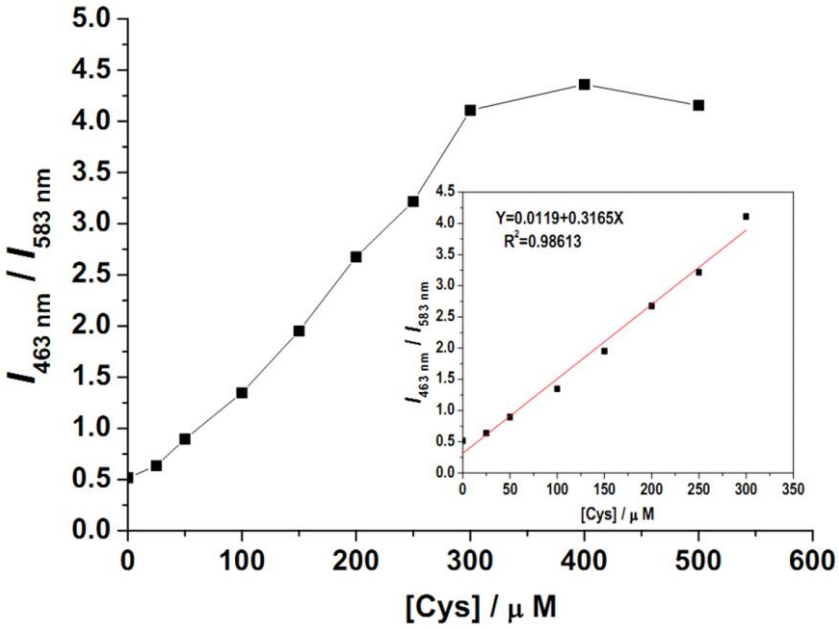


Figure S10. TPEF intensity ratio change of **CB1** (10 μM) in various concentrations of Cys (0–500 μM) at 40 min in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4). Inset: Plot of fluorescence intensity as a function of Cys concentration (0–300 μM). The detection limit of Cys is 3.2 μM. λ_{ex} = 365 nm.

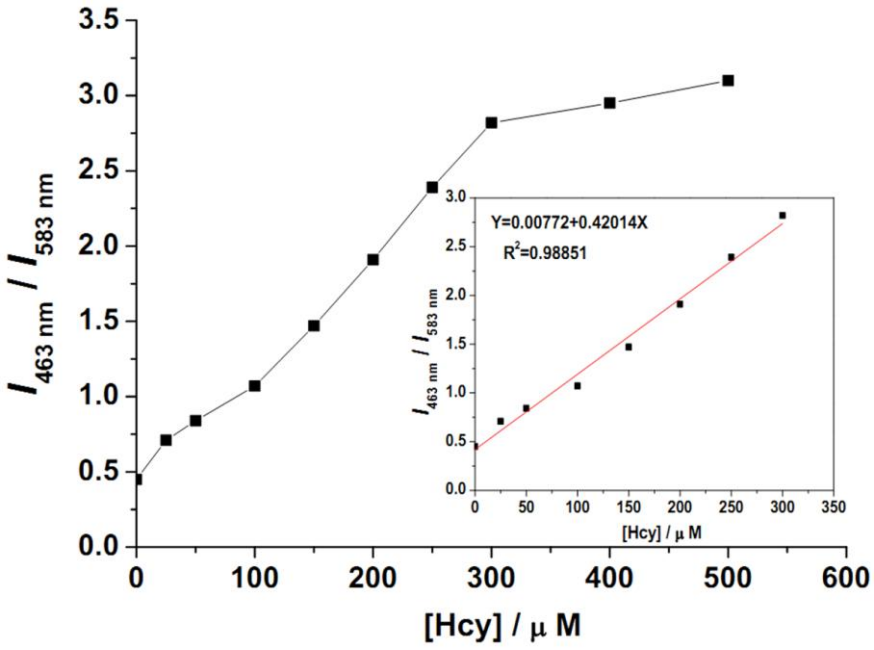


Figure S11. TPEF intensity ratio change of **CB1** (10 μM) in various concentrations of Hcy (0–500 μM) at 24 h in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4). Inset: Plot of fluorescence intensity as a function of Hcy concentration (0–300 μM). The detection limit of Hcy is 4.8 μM. λ_{ex} = 365 nm.

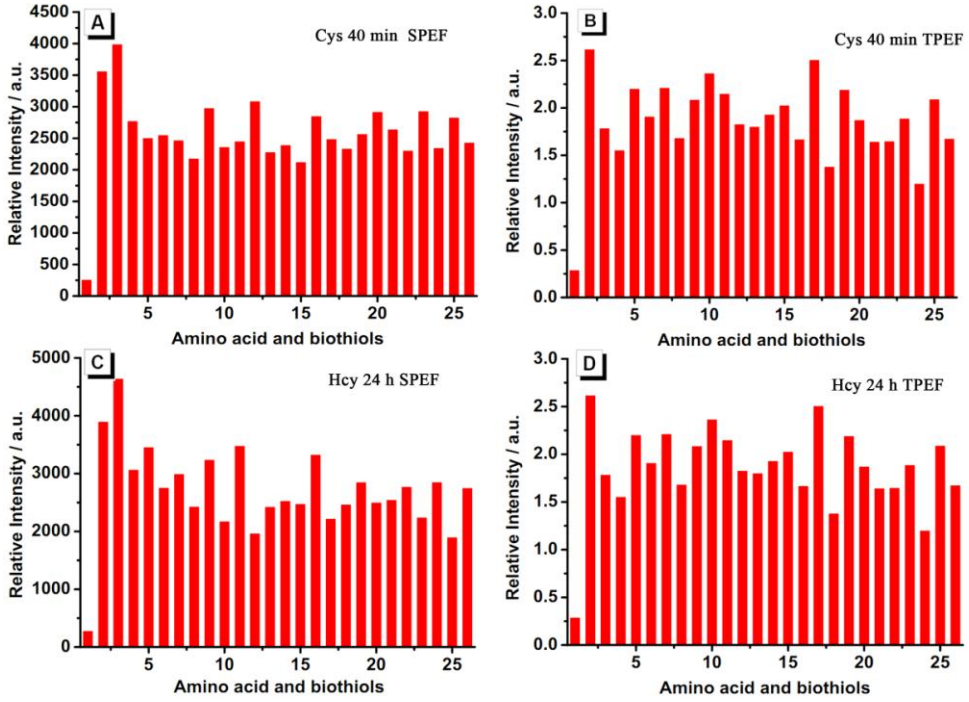


Figure S12. One- (A, C)and two-photon (B, D) excited fluorescence intensity at 463 nm of **CB1** (10 μM) in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4) with various bioanalytes (600 μM) in the presence of Cys (A, B) or Hcy (C, D). From left to right: 1: **CB1** without Hcy/Cys, 2: **CB1**+Cys/Hcy, 3: 2+Cys (C, D) or Hcy (A, B), 4: 2+Arg, 5: 2+Asn, 6: 2+Asp, 7: 2+Ala, 8: 2+*β*-ala, 9: 2+DTT, 10: 2+Gln, 11: 2+GSH, 12: 2+Glu, 13: 2+Gly, 14: 2+His, 15: 2+Ile, 16: 2+Lys, 17: 2+Leu, 18: 2+Met, 19: 2+NAC, 20: 2+Pro, 21: 2+Phe, 22: 2+Ser, 23: 2+Tyr, 24: 2+Thr, 25: 2+Trp, 26: 2+Val. Excitation wavelength: SPEF, 365 nm; TPEF, 760 nm.

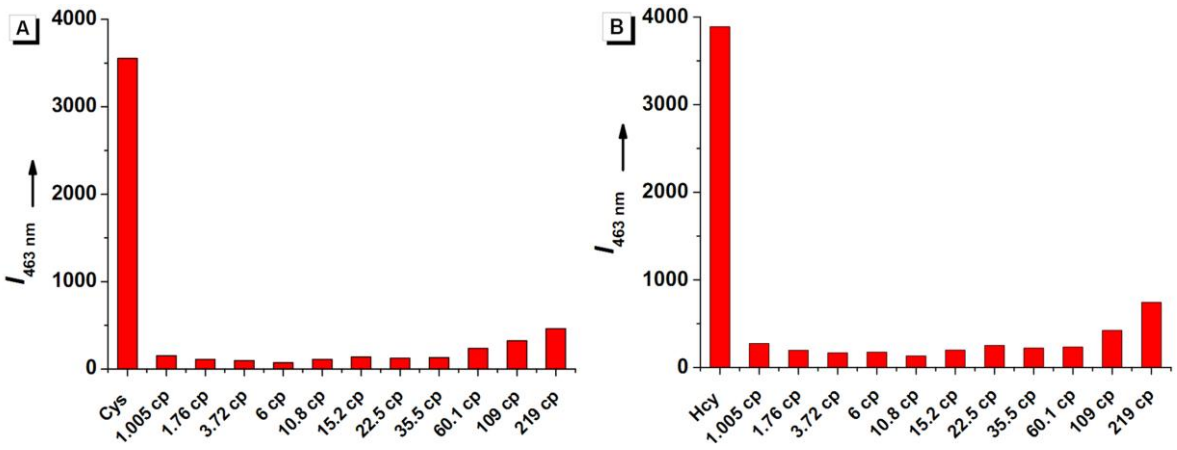


Figure S13. Fluorescence intensity at 463 nm of **CB1** (10 μM) with Cys/Hcy (600 μM) in EtOH/PBS buffer solution (v/v 4:1, pH 7.4, η 2.037 cp) and free probe in different viscosity values (glycerol/aqueous solution, 1.005~219 cp, λ_{ex} = 365 nm) at different time. A: 40 min, B: 24 h.

Table S1. Cytotoxicity Data (SiHa, 1μM) [a]

incubate time	1 h	24 h	36 h
% cell survival	100 %	93 ± 3%	83 ± 3%

[a] Cell viability was quantified by the MTT assay (mean ± SD).

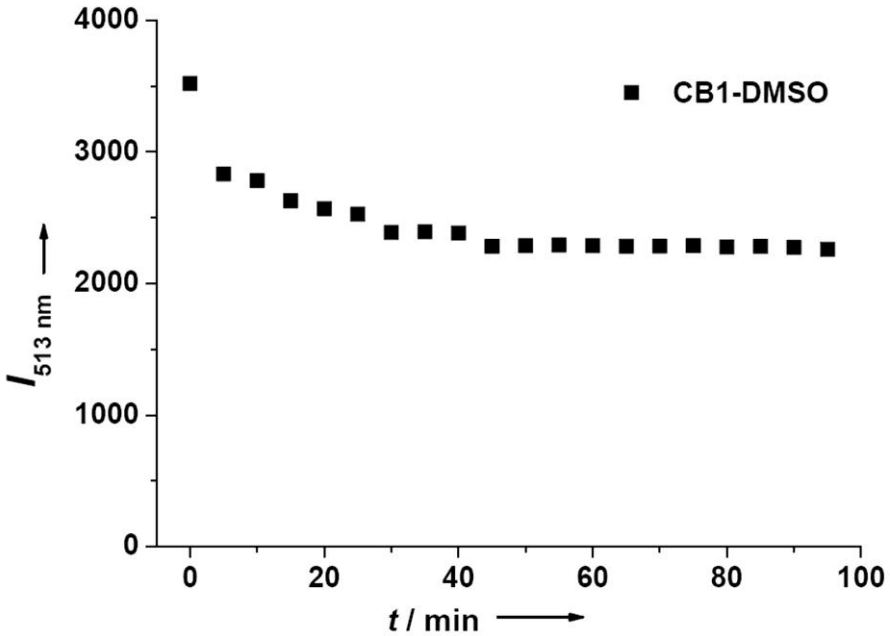


Figure S14. Photostability of **CB1**. **CB1** (10μM) was dissolved in DMSO, and fluorescent intensities were captured every 5 minutes with λ_{ex} = 365 nm.

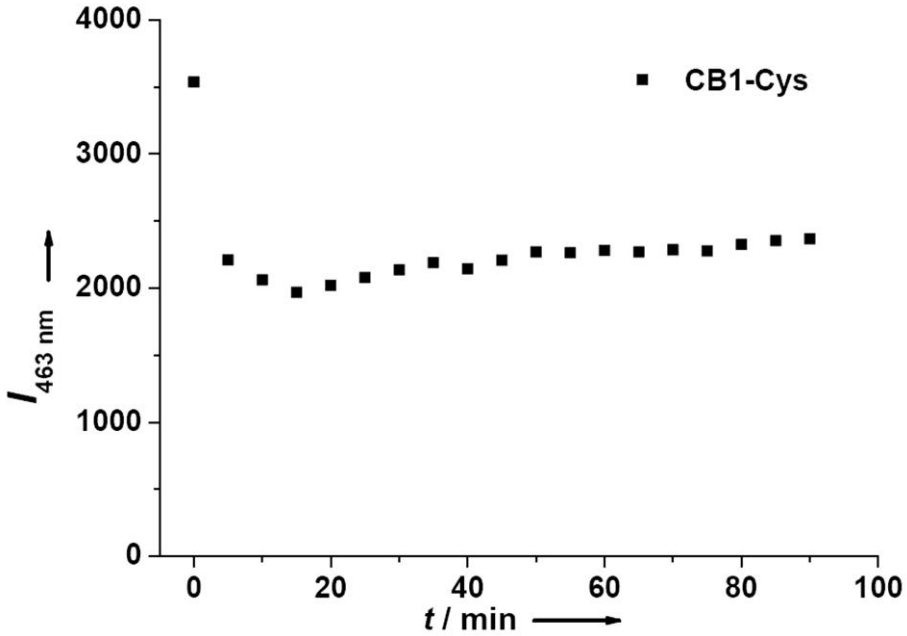


Figure S15. Photostability of **CB1**+Cys after 40 min. **CB1** (10 μM) and Cys (600 μM) were in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4), and after 40 min fluorescent intensities were captured every 5 minutes with λ_{ex} = 365 nm.

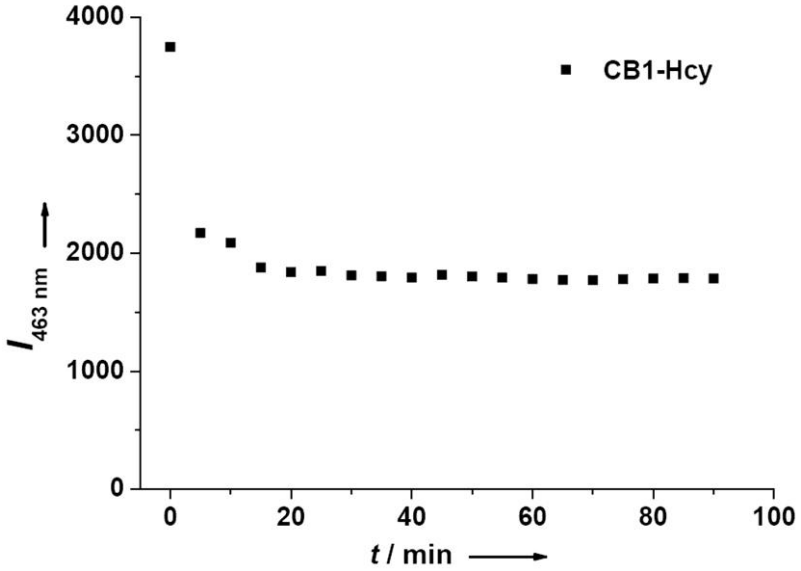


Figure S16. Photostability of **CB1**+Cys after 24 h. **CB1** (10 μM) and Hcy (600 μM) were in EtOH/PBS buffer solution (v/v 4:1, 20 mM, pH 7.4), and after 24 h fluorescent intensities were captured every 5 minutes with λ_{ex} = 365 nm.

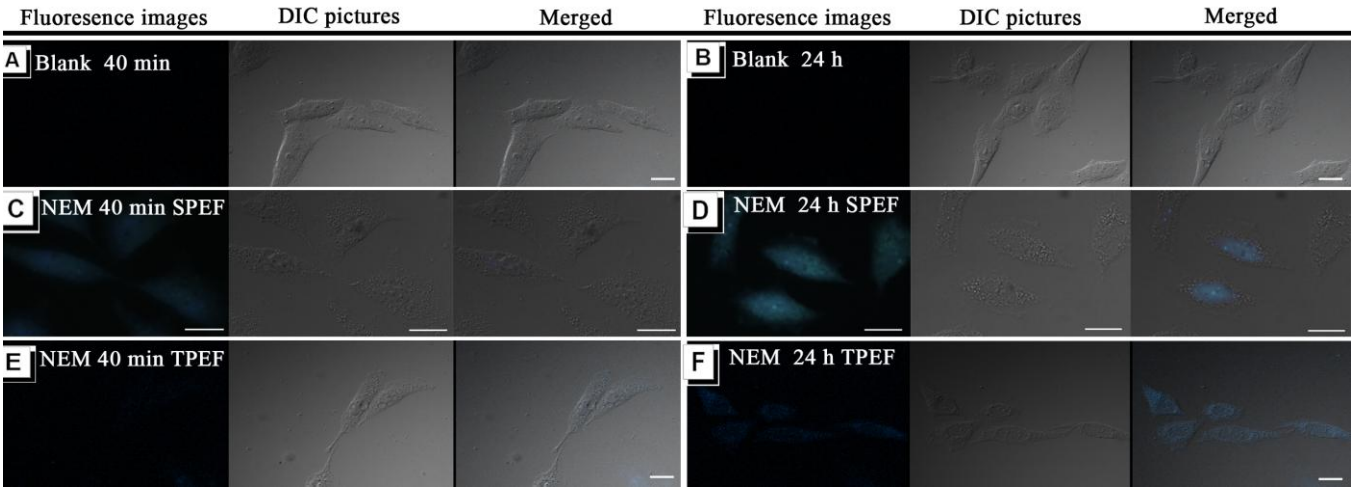
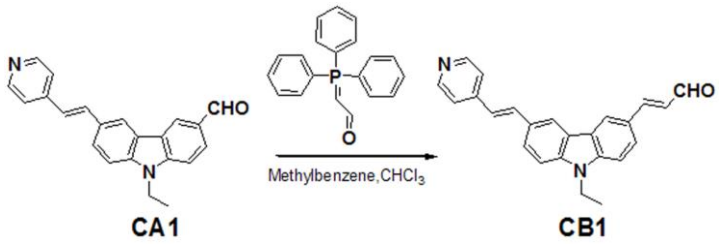


Figure S17. Fluorescence images of SiHa cells treated with 5 mM *N*-ethylmaleimide for 0.5 h at 37 ℃ and then incubated with **CB1** (1 μM) for 40 min and 24 h at 37 ℃. (SPEF: λ_{ex} = 330–385 nm, λ_{em} > 410 nm; TPEF: λ_{ex} = 760 nm).

Synthesis of CB1



Synthesis of hexenal -6-[2′-*para*-pyridylethenyl-9-ethylcarbazole] (CB1):

Compound **CA1**[S3] (0.61 g, 2.0 mmol) was dissolved in 40 mL of methylbenzene, then added into a flask and bubbled with argon for 30 min. A solution of triphenylphosphoranylidene (182.62 mg, 0.6 mmol) in chloroform (10 ml) was added into the system. The mixture was then bubbled with argon for 30 min and heated at 110 ℃ for 28 h under the protection of argon and a brownish red suspension was obtained. The mixture was distilled to remove solvent, then poured into H_2O (500 mL) and extracted with CH_2Cl_2 after the resulting mixture was cooled to room temperature. The organic phase was separated, dried with $MgSO_4$ and removed by vacuum distillation. The title product was obtained after the residue was purified by column chromatography with ethyl acetate/petroleum ether (1:10, v/v) as eluent with a yield of 16.2%. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 9.69 (s, 1H), 8.54–8.46 (m, 4H), 7.92–7.84 (m, 3H), 7.73 (d, J = 16.32 Hz, 1H), 7.64–7.59 (m, 4H), 7.26 (d, J = 16.32, 1H), 6.89 (dd, J_1=J_2 = 15.72 Hz, 1H), 4.49 (t, J = 7.23 Hz, 2H), 1.46 (q, J = 4.79 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): δ = 193.7, 154.2, 150.1, 145.1, 142.2, 140.8, 133.8, 128.4, 126.6, 126.2, 125.7, 125.6, 123.8, 123.4, 123.2, 121.8, 120.7, 119.6, 109.4, 109.3, 38.0, 13.9. MS (m/z): M^+ calcd for $C_{24}H_{20}N_2O$: 353.16; found, 353.16. Anal. calcd for $C_{24}H_{20}N_2O$: C 81.78, H 5.67, N 7.95; found: C 81.65, H 5.46, N 7.84. IR (cm^{-1}): 1669 ($\upsilon_{C=O}$). (s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad.)

References:

[S3] Z. G. Yang, N. Zhao, Y. M Sun, F. Miao, Y. Liu, X. Liu, Y. H. Zhang, W. T. Ai, G.F. Song, X. Y. Shen, X. Q. Yu, J. Z. Sun, W.-Y. Wong, *Chem. Commun.* **2012**, *48*, 3442–3444.

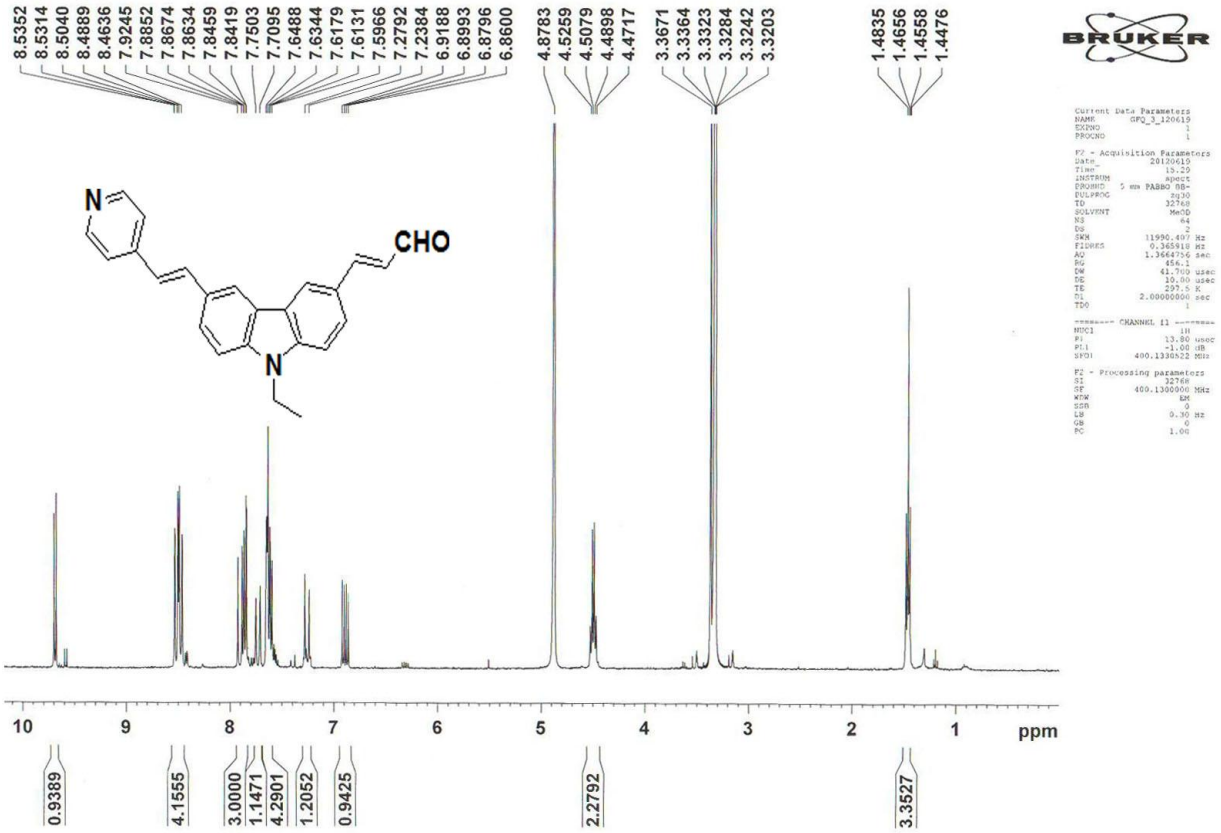


Figure S18. ^{1}H NMR spectrum of **CB1** in CD_3OD.

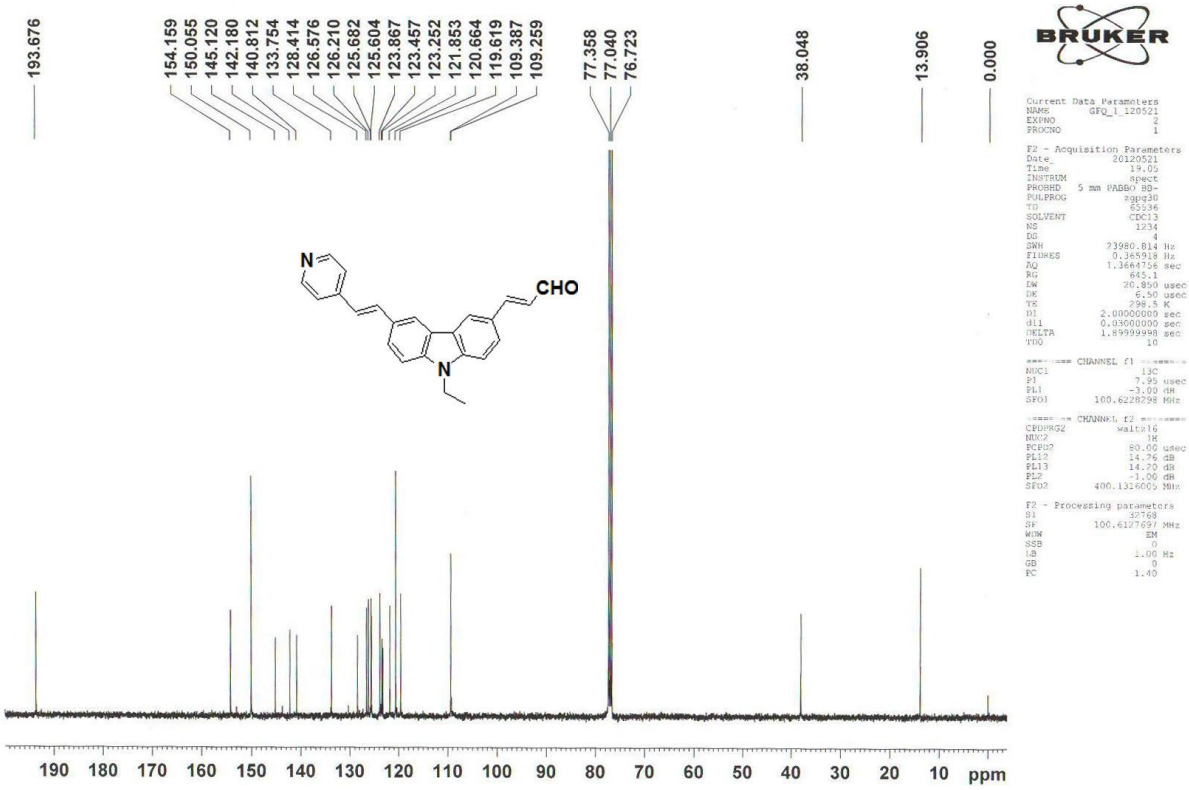


Figure S19. ^{13}C NMR spectrum of **CB1** in $CDCl_3$.

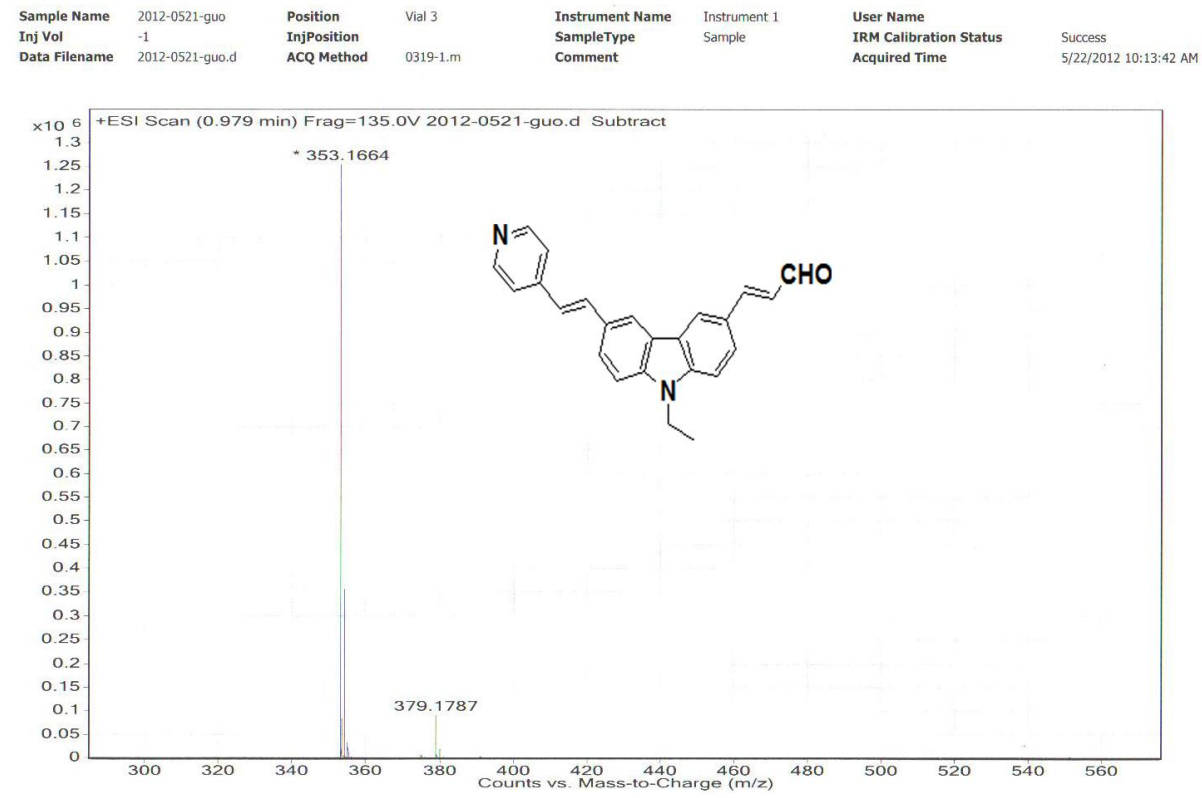


Figure S20. HRMS of **CB1** in EtOH.

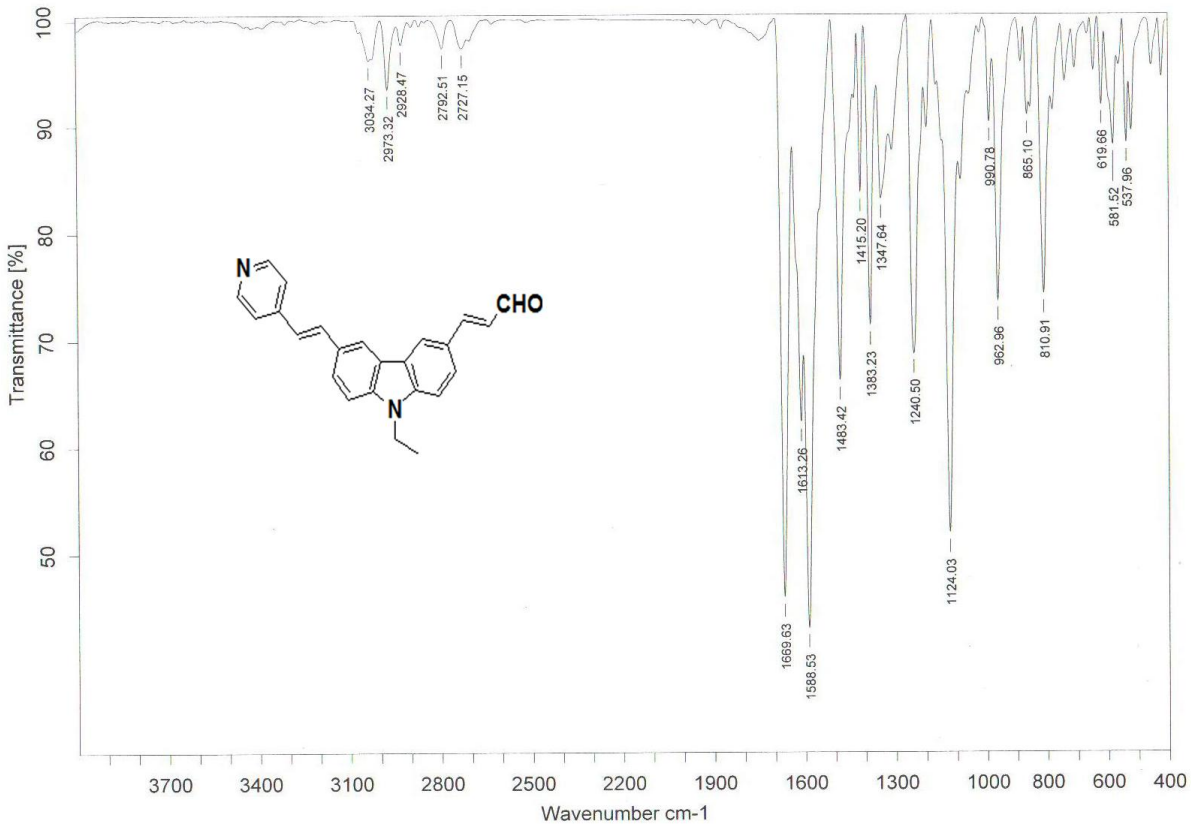


Figure S21. IR spectrum of **CB1**.