Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A chemiluminescent sensor for imaging endogenous hydrogen polysulfides imaging in living system

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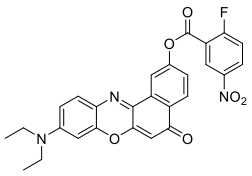
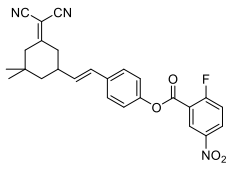
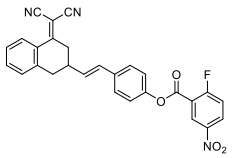
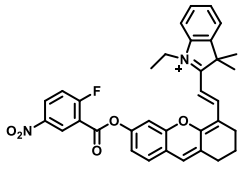
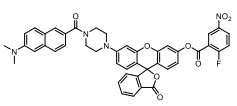
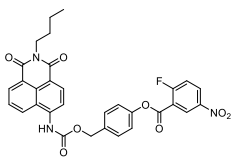
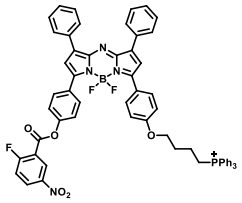
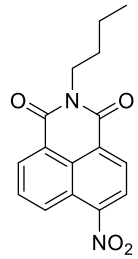
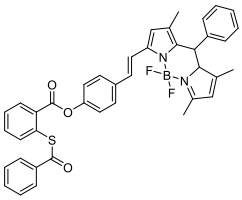
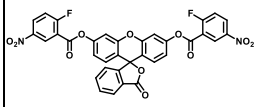
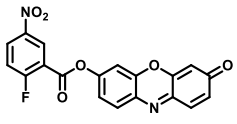
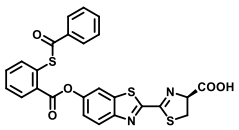
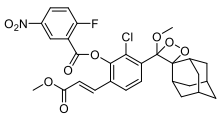
Table S1. Reported H_2S_n probes and CL-HP.

Probe structure	Excitation/ Emission wavelength	Limit of detection	Linear range	Ref.
	550/655 nm	3.9 nM	0-18 μM	Anal. Methods, 2017, 9, 6443–6447
	395/482,655 nm	43 nM	0-50 μM	J. Mater. Chem. B, 2018, 6, 7015--7020
	535/682 nm	8.2 nM	0-20 μM	Sensors and Actuators B 254 (2018) 222–226
	680/720 nm	22 nM	0-10 μM	J. Mater. Chem. B, 2017, 5, 2574--2579
	370/448, 541 nm	700 nM	0-16 μM	Anal. Chem. 2016, 88, 11892−11899
	405/460, 518 nm	100 μM	0-8 μM	Anal. Chem., 2016, 88 (14), 7206–7212

	675/730 nm	25 nM	0-10 μM	Anal. Chem. 2015, 87, 3631−3638
	435/540 nm	26 nM	0.1-100 μM	Sensors & Actuators: B. Chemical 278 (2019) 64–72
	530/584 nm	26 nM	0-10 μM	Chem. Commun., 2018, 54, 3735--3738
	490/515 nm	71 nM	0.5-15 μM	J. Am. Chem. Soc., 2014, 136, 7257-7260.
	550/589 nm	26 nM	0-50 μM	Analyst., 2019, 144, 3221-3225.
	-	100 nM	0.1-2 mM	Chem. Commun., 2019, 55, 4487-4490.
	-	230 nM	0.025-10 mM	This work

Materials and instruments

All chemical solvents and reagents used for synthesis were of analytical grade, and the inhibitors for biological experiments were purchased from commercial suppliers (TCI, Admas, Sigma-Aldrich, and Bide Pharmatech). All materials were applied without further purification. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC). The silica gel chromatographic separation was performed with silica gel (200−300 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 600 MHz instruments at the indicated frequencies. All chemical shifts (δ) recorded in ppm were related to internal standard tetramethylsilane (δ = 0.0 ppm) or signals of residual solvent $CDCl_3$ (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C), and the coupling constants were given in Hz. Mass spectra were recorded on an AB Sciex TripleTOF® 5600+ system. The chemiluminescence signals were measured by Fluoroskan Ascent Fluorescence instrument (Thermo Electron Corporation, USA). The chemiluminescence images of cells and mice was measured by Xenogen IVIS® Spectrum imaging system (Caliper Life Science, USA). UV–Vis absorption spectra were recorded on the Hitachi U-2900 spectrophotometer. The fluorescence spectra were tested on the Hitachi F-7000 fluorescence spectrometer. All solutions of CL-HP1 and CL-HP2 were diluted from the corresponding DMSO stock solution (10 mM). The Na_2S_4 and other analytes' solution is prepared freshly before the experiments.

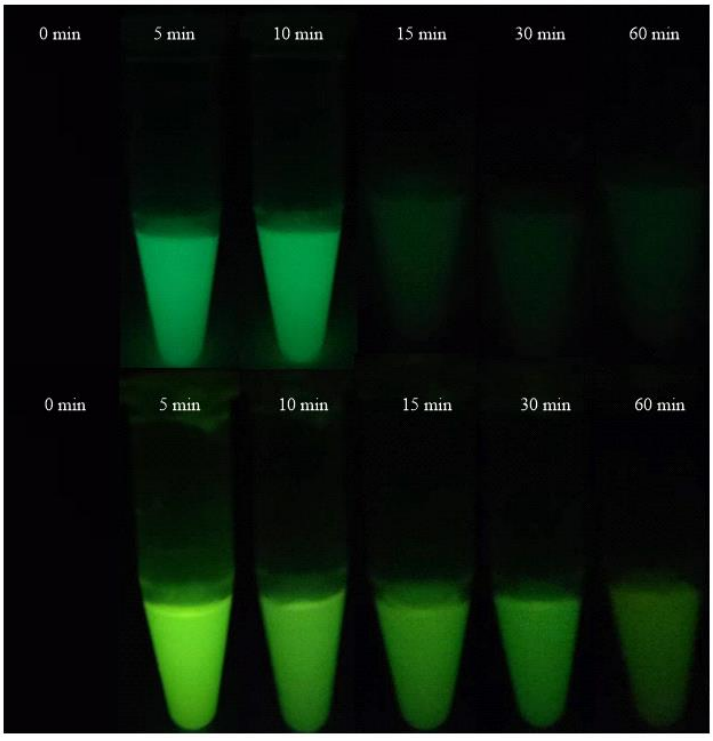


Fig. S1. Time-lapse images of probe CL-HP1 (up) and CL-HP2 (bottom) in 60 min.

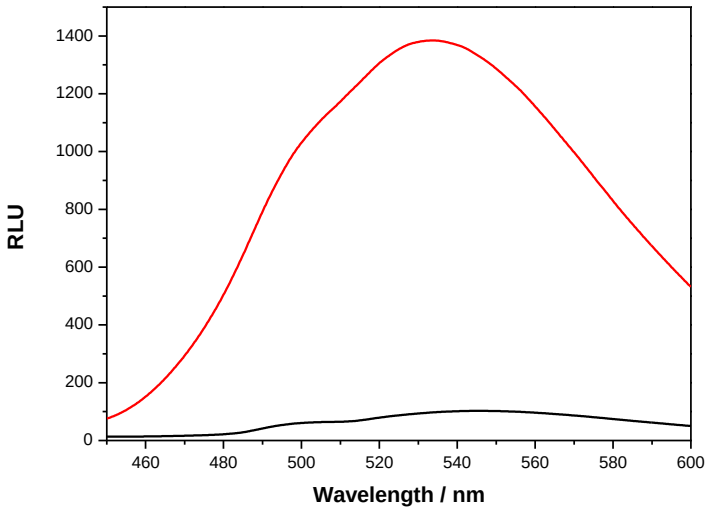


Fig. S2. Fluorescence spectra of probe CL-HP2 (20 μM) before (black line) and after (red line) the incubation with Na_2S_4 in PBS for 30 min (λ_{ex} = 380 nm).

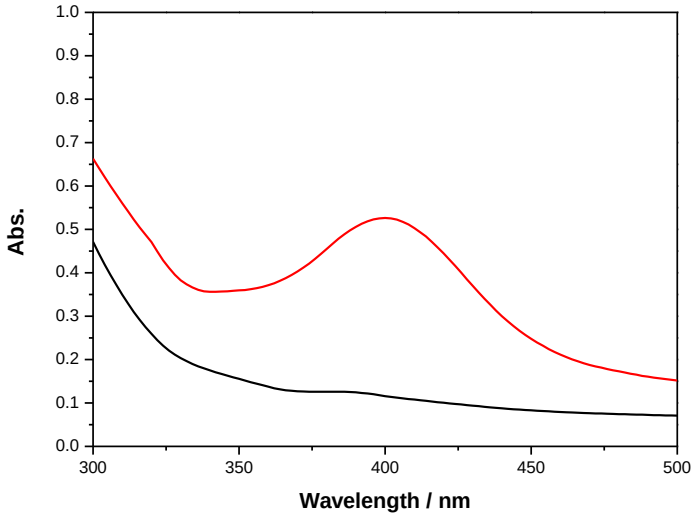


Fig. S3. UV–vis absorption spectra of probe CL-HP2 (20 μM) before (black line) and after (red line) the incubation with Na_2S_4 in PBS for 30 min.

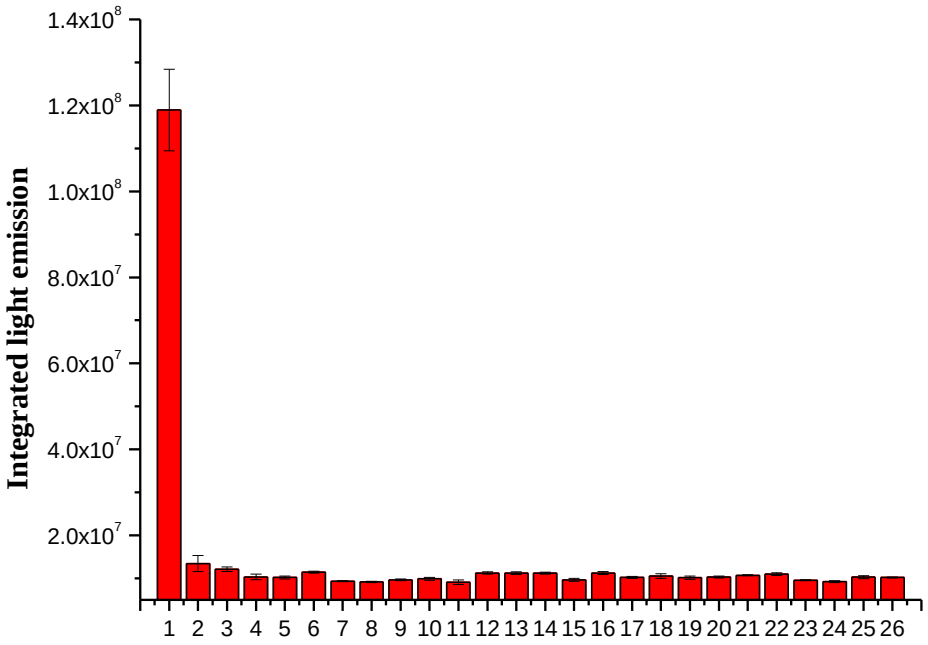


Fig. S4. Chemiluminescence responses of CL-HP2 (20.0 μM) with 100 μM of different analytes. From 1 to 26: Na_2S_4, H_2O_2, ClO^-, His, Asp, Ala, Gly, Glu, Lys, Tyr, Al^{3+}, Ba^{2+}, Ca^{2+}, Cr^{3+}, Li^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, K^+, I^-, Br^-, Cl^-, F^-, NO_3^-, AcO^-.

Cell culture and imaging

MDA-MB-231 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2 incubator for 24 h. In the confocal imaging, MDA-MB-231 cells were plated on a laser confocal dish with a medium of DMEM. Cells then were incubated at 37 °C in a humidified atmosphere of a 5% CO_2 incubator. The cells were washed three times with PBS prior to imaging.

CCK8 assay

MDA-MB-231 cells were seeded into a 96-well microtiter plate at 37 °C in a 5% CO_2/95% air incubator for 12 h. The cells were incubated for an additional 12 h with different concentrations of CL-HP2 (0, 10, 20, 50, 100 μM). Then the cells were washed with PBS three times. Following, the culture media was removed and 100 μL of FBS-free DMEM containing 10% CCK8 was added. After being incubated for 2 h, the absorbance was measured at 450 nm. The results from three individual experiments were averaged. Following formula was used to calculate the viability of cell growth:

Percentage of cell viability (%) = (mean of treatment group – blank)/ (mean of control group – blank) × 100%

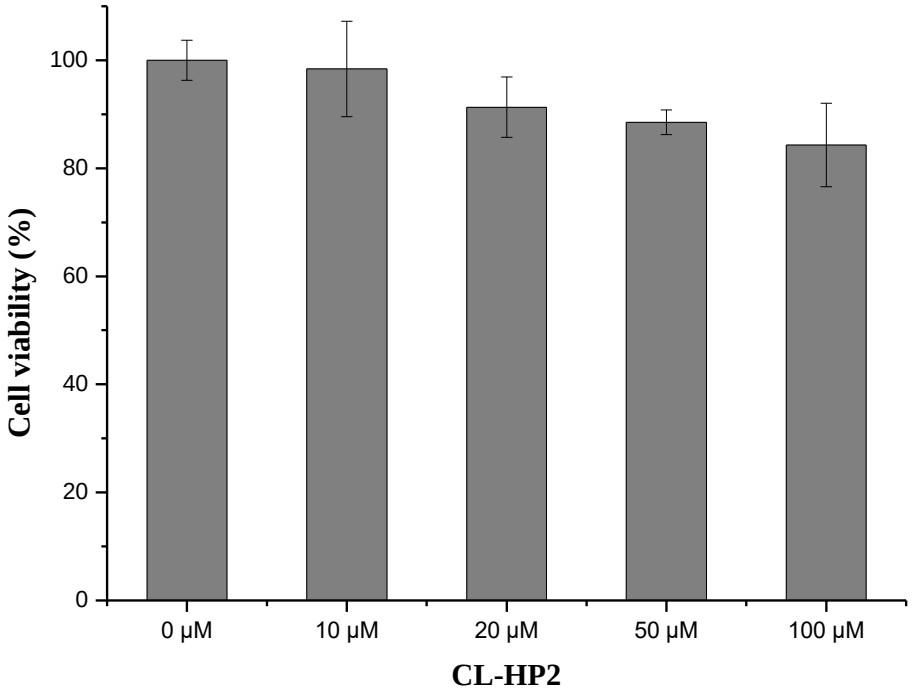


Fig. S6. CCK8 assay for the survival rate of MDA-MB-231 cells treated with probe CL-HP2 (0, 10, 20, 50 and 100 μM) for 12 h.

Real-time chemiluminescence images of H_2S_n in living murine model of inflammation

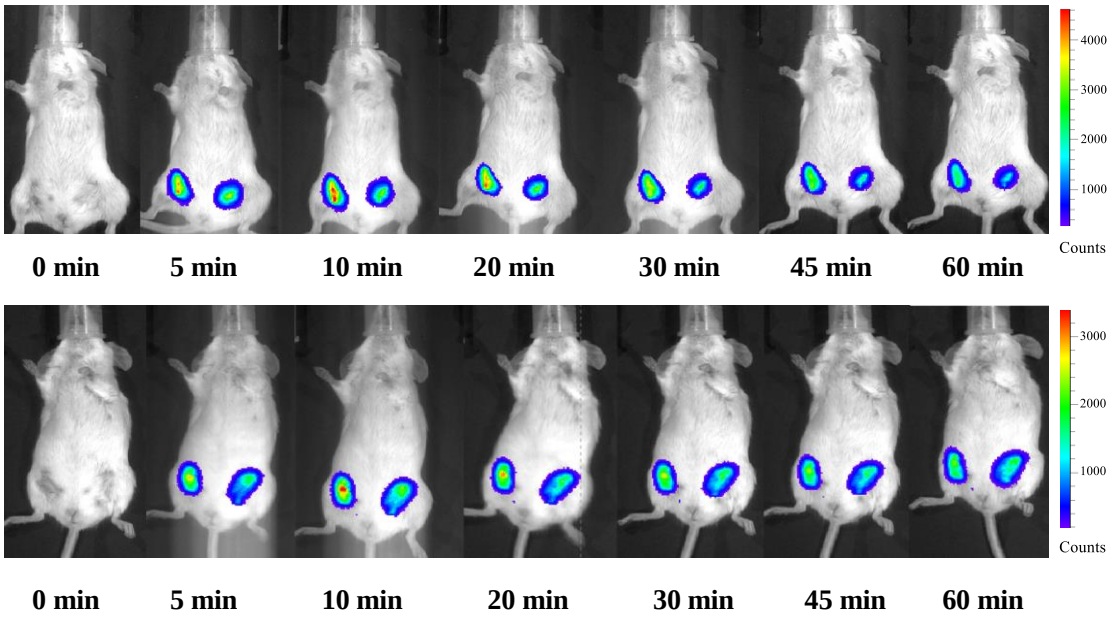
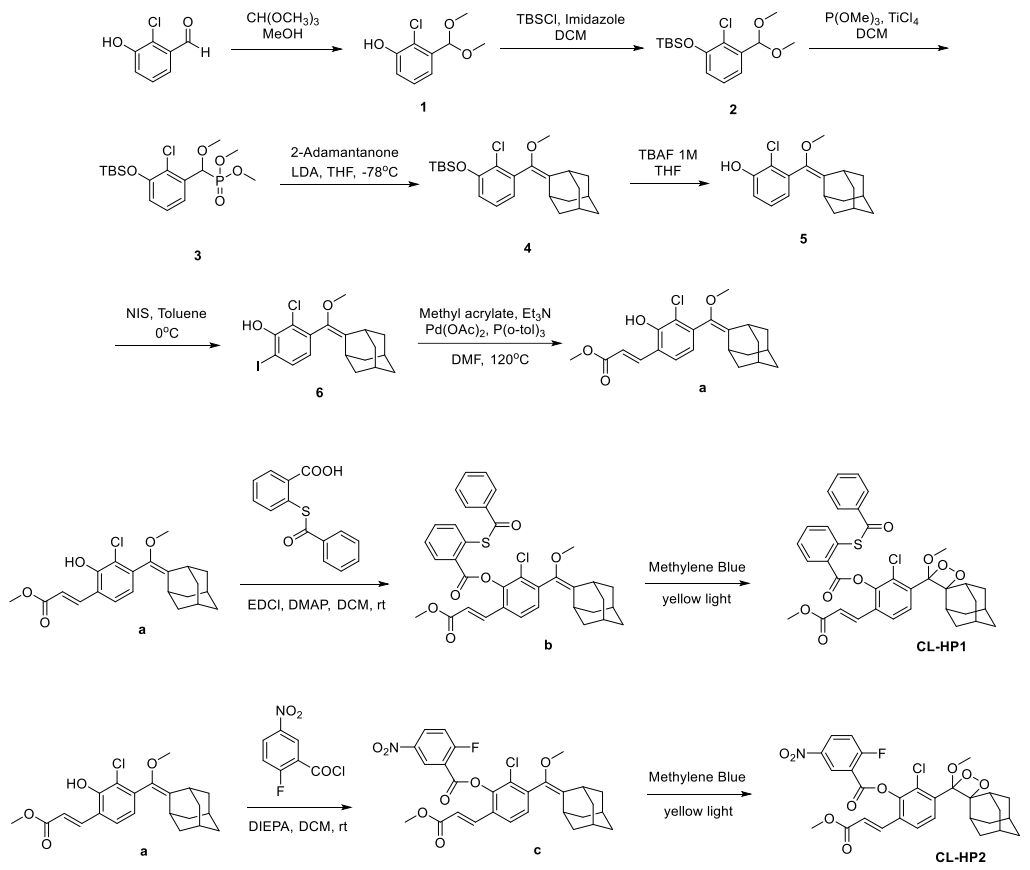


Fig. S7. Real-time chemiluminescence images (exposure time, 40 s) of the LPS injected mice (top) and the *E. coli* infected mice (bottom) after injecting CL-HP2 (20 μM, 100 μL) acquired at 0, 5, 10, 20, 30, 45, and 60 min.



Scheme S1. Synthesis of probe CL-HP1 and CL-HP2.

Compound **a** was synthesized through the reported synthetic route.[1,2]

Synthesis of compound b: A mixture of 2-(benzoylthio)benzoic acid (129 mg, 0.5 mmol), EDCI (115 mg, 0.6 mmol) and DMAP (73 mg, 0.6 mmol) was added in CH_2Cl_2 (25 mL) at RT under Ar atmosphere.. After stirring for 30 min, Compound a (194 mg, 0.5 mmol) was added. The mixture was stirred for 6 h and solution was evaporated. The crude solid product was purified by column chromatography (neutral silica gel, Petroleum Ether /AcOEt = 20:1) to afford compound b as pale white solid (85 mg, yield 27%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (d, J = 7.8 Hz, 2H), 7.74 – 7.53 (m, 9H), 7.2-7.28 (m, 1H), 6.50 (d, J = 16.2 Hz, 1H), 3.74 (s, 3H), 3.35 (s, 3H), 2.16 – 1.65 (m, 14H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 189.3, 167.7, 166.7, 163.9, 146.5, 139.1, 137.9, 137.5, 134.2, 132.3, 130.9, 130.6, 129.5, 129.2, 129.1, 128.8, 128.7, 128.3, 127.6, 124.9, 121.2, 57.4, 51.9, 39.3, 37.0, 36.3, 32.9, 30.6, 29.8, 29.7, 27.5, 19.2. HR-MS: found, m/z 651.1579 $[M + Na]^+$ (calcd, 651.1584).

Synthesis of compound c: A mixture of Compound a (194 mg, 0.5 mmol), 2-fluoro-4-nitrobenzoyl chloride (112 mg, 0.55 mmol), trimethylamine (0.2 mL, 1.5 mmol) was added in CH_2Cl_2 (5 mL) at 0 ℃ under Ar atmosphere. After 30 min, the reaction was stirred for 3 h at room temperature. Subsequently, the solution was diluted with CH_2Cl_2, washed with saturated brine, dried over Na_2SO_4 and concentrated under vacumm. The crude production was purified by column chromatography (neutral silica gel, Petroleum Ether/AcOEt = 15:1) to afford compound c as pale white solid (220 mg, yield 80%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.08 (m, 1H), 8.56 (m, 1H), 7.73 (d, J = 15.6 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.46 (t, J = 9 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 10.2 Hz, 1H), 6.51 (d, J = 15.6 Hz, 1H), 3.77 (s, 3H), 3.34 (s, 3H), 2.14-1.25 (m, 14H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.6, 164.7, 159.4, 159.3, 145.5, 144.1, 138.8, 139.2, 136.9, 130.8, 130.7, 130.1, 128.9, 128.8, 125.0, 121.7, 119.0, 118.8, 57.4, 52.0, 37.0, 32.9, 31.9, 29.8, 29.7, 29.4, 28.3, 28.1, 22.7. HR-MS: found, m/z 554.1422 $[M]^-$ (calcd, 554.1387).

NMR and MS spectra

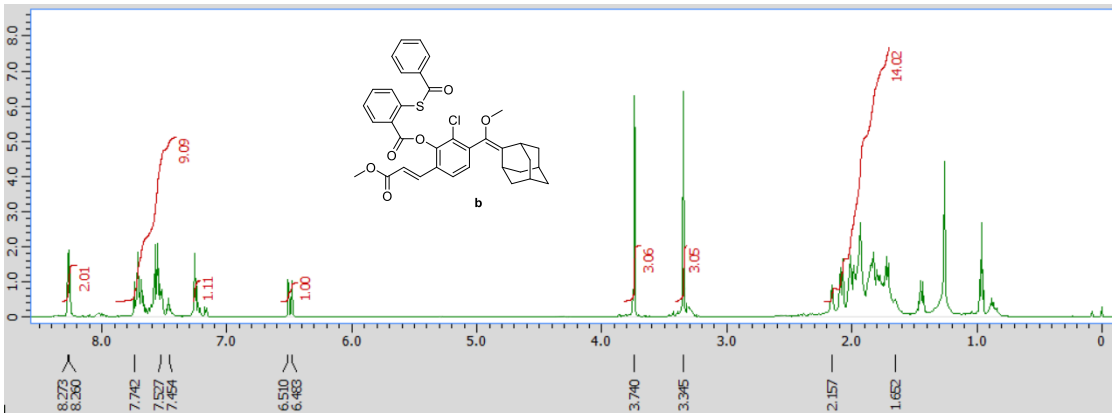


Fig. S8. ^{1}H NMR spectrum of compound **b** in $CDCl_3$.

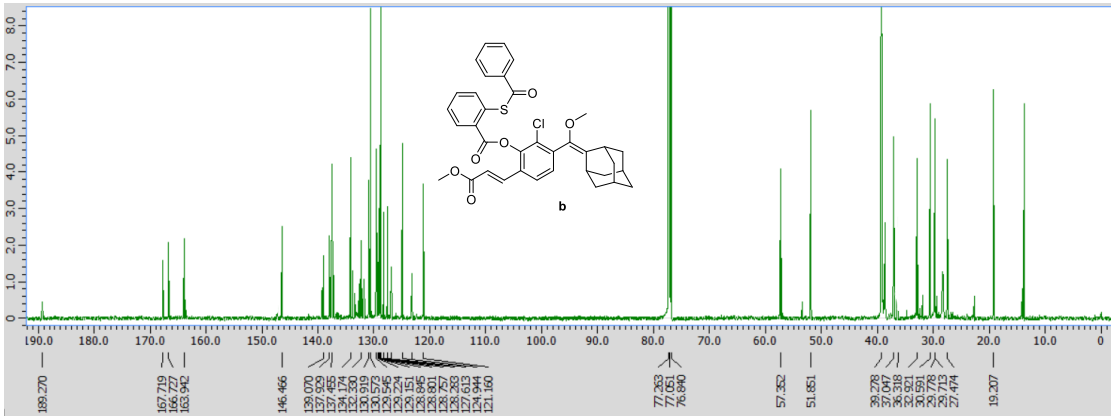


Fig. S9. ^{13}C NMR spectrum of compound **b** in $CDCl_3$.

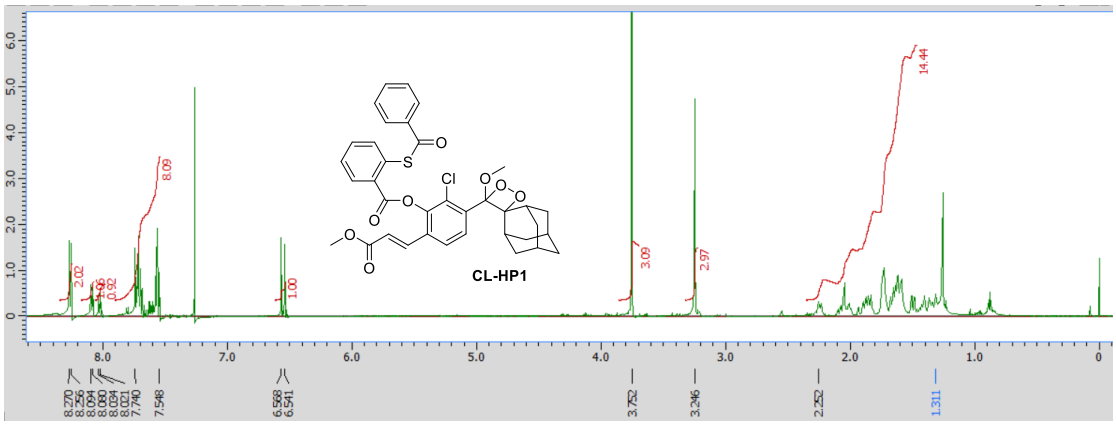


Fig. S10. ^{1}H NMR spectrum of **CL-HP1** in $CDCl_3$.

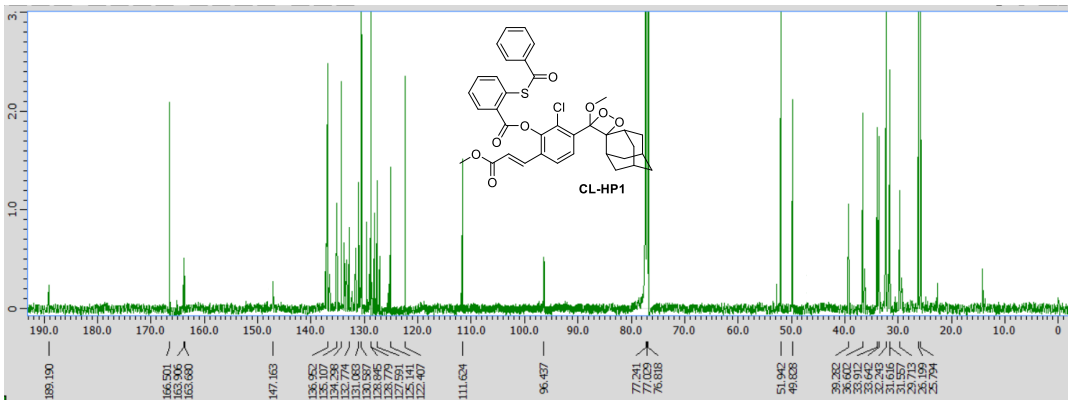


Fig. S11. ^{13}C NMR spectrum of **CL-HP1** in $CDCl_3$.

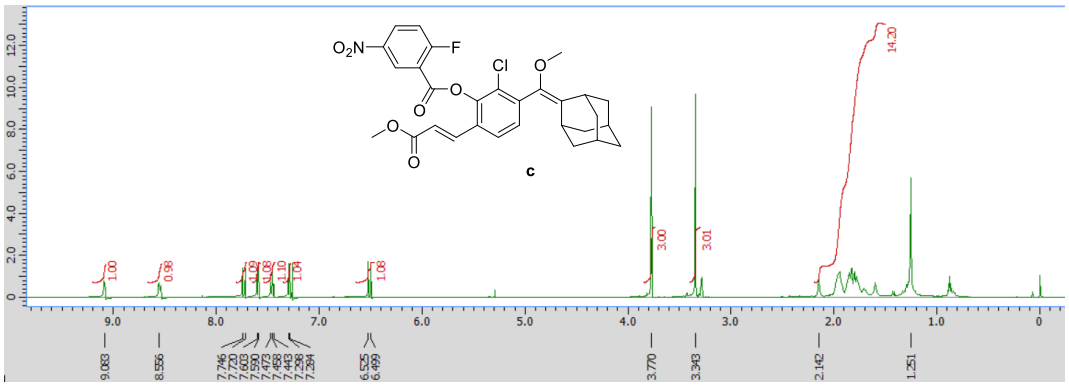


Fig. S12. ^{1}H NMR spectrum of compound **c** in $CDCl_3$.

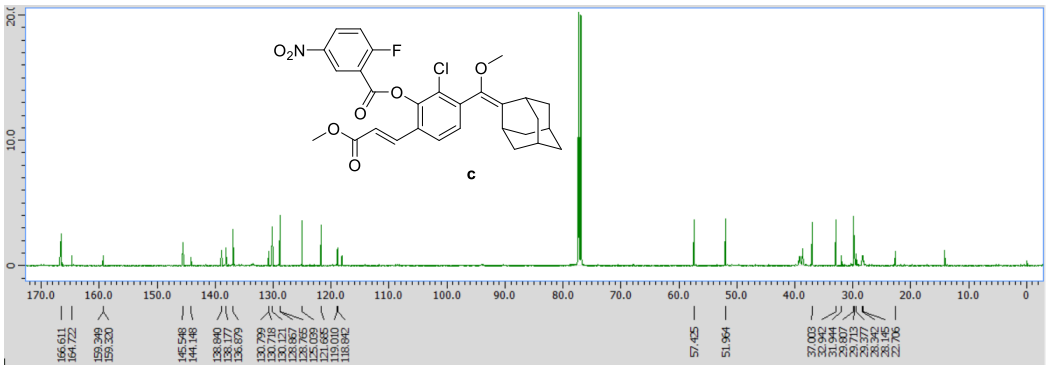


Fig. S13. ^{13}C NMR spectrum of compound **c** in $CDCl_3$.

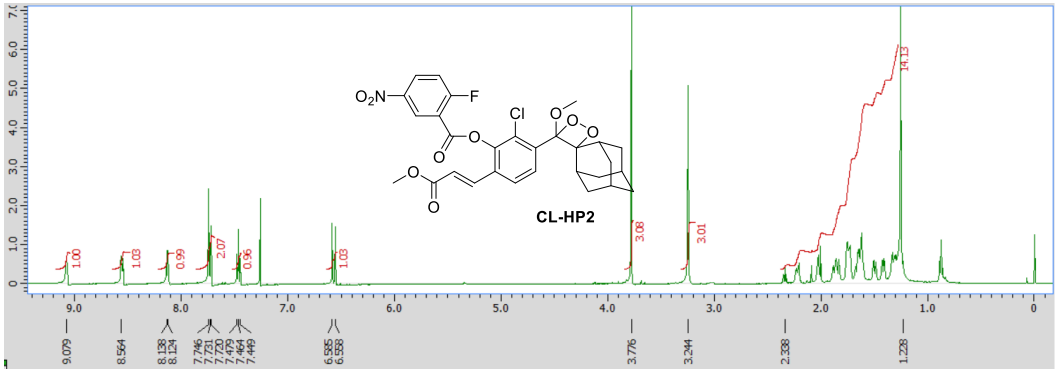


Fig. S14. ^{1}H NMR spectrum of **CL-HP2** in $CDCl_3$.

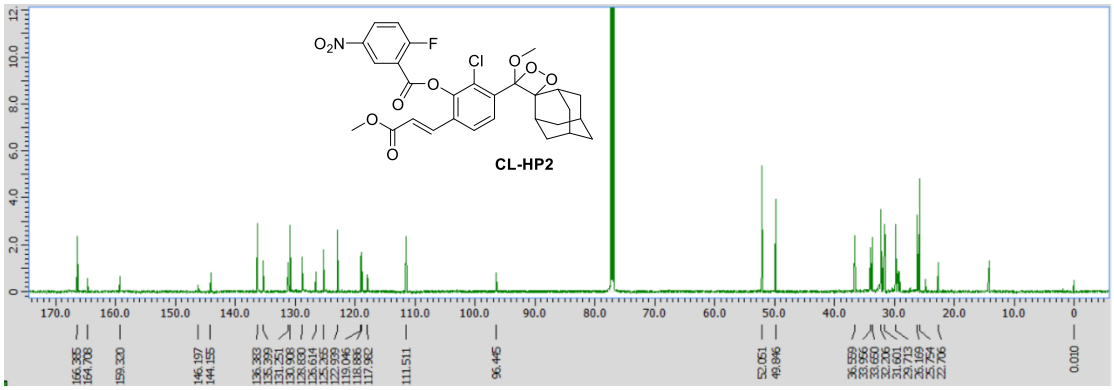


Fig. S15. ^{13}C NMR spectrum of **CL-HP2** in $CDCl_3$.

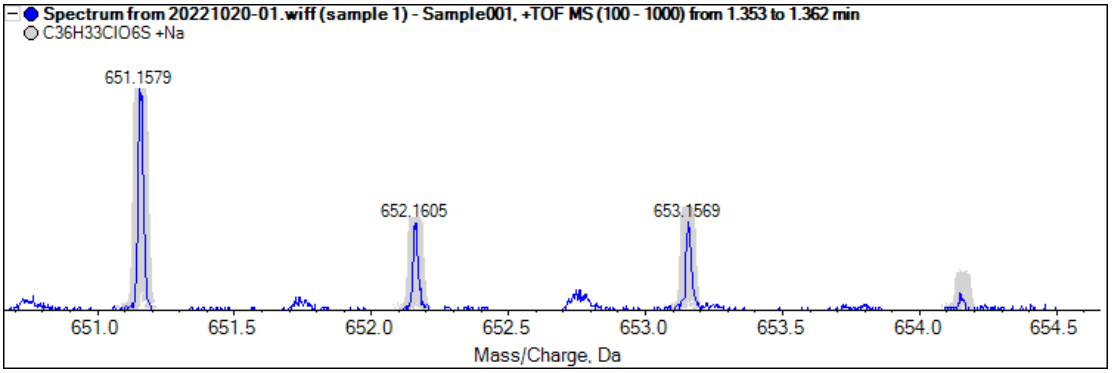


Fig. S16. HRMS spectrum of compound **b**.

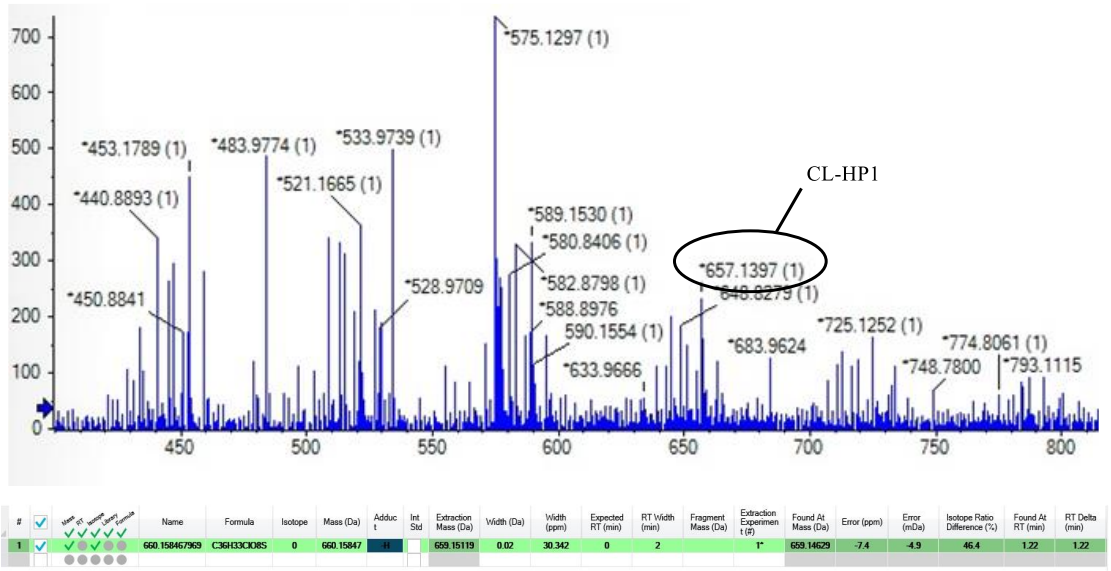


Fig. S17. HRMS spectrum of **CL-HP1**.

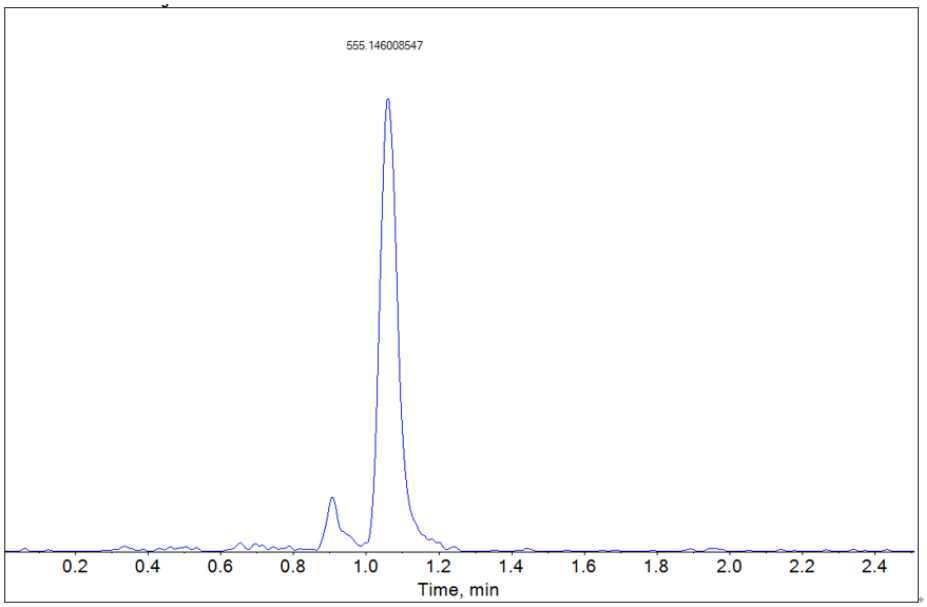


Fig. S18. HRMS spectrum of compound **c**.

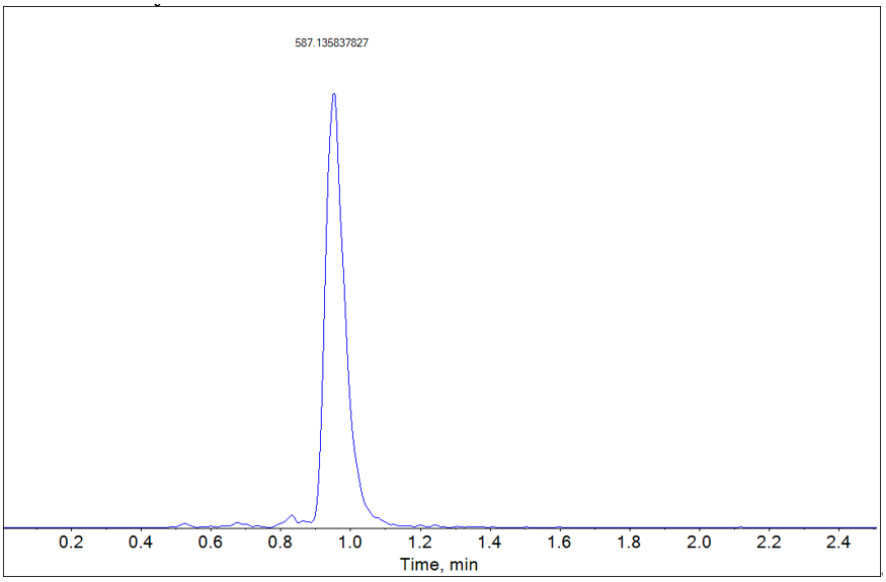


Fig. S19. HRMS spectrum of **CL-HP2**.

References

1 M. Yang, J. Zhang, D. Shabat, J. Fan and X. Peng, *ACS Sens*., 2020, **5**, 3158-3164.

2 O. Green, T. Eilon, N. Hananya, S. Gutkin, C.R. Bauer and D. Shabat, *ACS Cent. Sci*., 2017, **3**, 349-358.