Supporting information

Mitochondria-targeted Fluorescent Probe for Imaging Endogenous Hydrogen Sulfide in Cellular Antioxidant Stress

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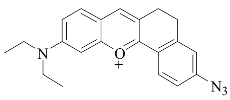
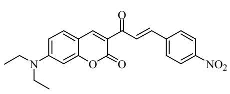
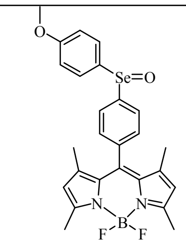
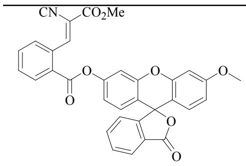
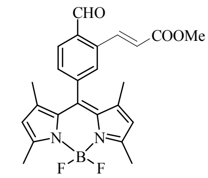
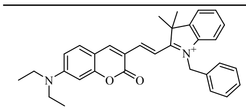
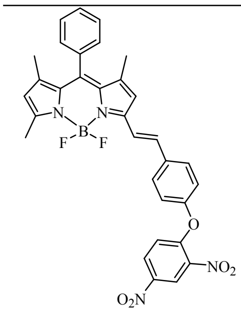
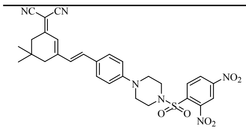
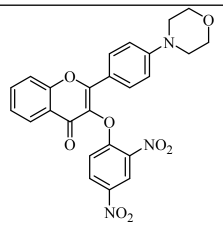
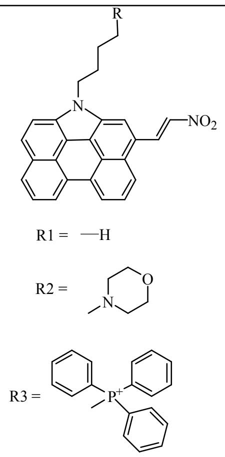
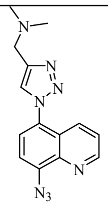
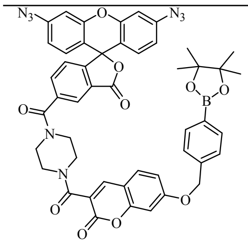
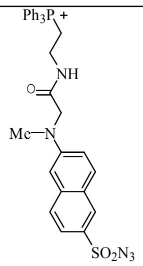
Contents

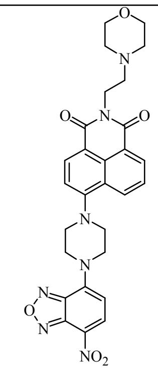
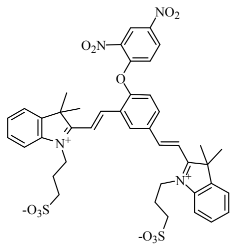
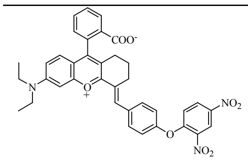
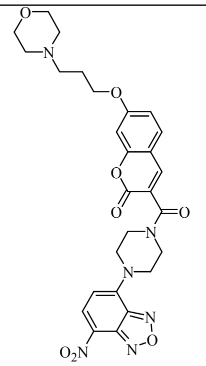
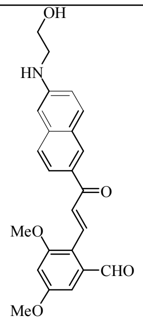
1. Additional data and spectrum

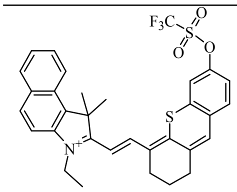
Table S1 Comparison of some reported probes for the detection of H_2S in the literature

Structrure of sensors	Journal	organelle-targeted	Limit of detecton	λ_{em}	Solution
	*Sens Actuators B Chem.*, 2018, **256**, 342-350	Mitochondria -targeted	47 µM	635 nm	PBS/DMSO (v/v, 8/2, 10 mM, pH 7.4)
	*Spectrochim. Acta. A Mol. Biomol. Spectrosc.*,	NO	320 nM	503 nm	PBS/DMSO (v/v, 1/1, 10 mM, pH 7.4)

	2020, **229**, 117987-117995				
	*Chem. Commun.*, 2013, **49**, 1014-1016	NO	--	510 nm	PBS/MeCN (v/v, 7/3, 20 mM, pH 7.4)
	*Org. Lett.*, 2012, **14**, 2184-2187	NO	1 μM	515 nm	PBS (10 mM, pH 7.4)
	*Nat. Commun., 2011, 2, 495-501*	NO		388 nm	PBS buffer (10 mm, pH 7.4, 10% CH_3CN)
	*Angew. Chem. Int. Ed.*, 2013, **52**, 1688-1691	NO	1 μM	652/510 nm	PBS/DMSO (v/v, 98/2, 20 mM, pH 7.4)
	*Talanta*, 2018, **181**, 104-111	NO	1.27 μM	592 nm	HEPES/DMSO (v/v, 1/1, 20 mM, pH 7.4)
	*Sensor. Actuat. B-Chem.*, 2018, **262**, 837-844	NO	6 nM	666 nm	HEPES/DMSO (v/v, 8/2, 10 mM, pH 7.4)

	*Anal. Methods.*, 2018, **10**, 604-610	Lysosome-targeted	69 nM	547 nm	PBS/DMSO (v/v, 4/1, 10 mM, pH 7.4)
	*J. Mater. Chem. B*, 2017, **5**, 2172-2180	R2: Lysosome-targeted R3: Mitochondria-targeted	R1: 139 nM	481 nm	PBS/DMSO (v/v, 7/3, 10 mM, pH 7.4)
	*Org. Biomol. Chem.*, 2018, **16**, 712-716	Lysosome-targeted	214.5 nM	510 nm	PBS/DMSO (v/v, 9/1, 20 mM, pH 7.4)
	*Chem. Eur. J. 2015, **21**, 15167 - 15172*	NO	---	525 nm	PBS
	Dyes Pigments, 2013, **99**, 308-315	Mitochondria-targeted	1.0 µM	440 nm	PBS (pH 7.4, 1% CH_3CN)

	*Sens Actuators B Chem.*, 2018, **260**, 264-273	Lysosome-targeted	330 nM	536 nm	PBS (10 mM, pH 7.4 or 5.0)
	*Spectrochim. Acta. A Mol. Biomol. Spectrosc.*, 2019, **213**, 416-422	Lysosome-targeted	11 nM	680 nm	PBS (10 mM, pH 7.2)
	*Talanta*, 2019, **197**, 326-333	Mitochondria-targeted	89.3 nM	720 nm	PBS/DMSO (v/v, 99/1, 10 mM, pH 7.4)
	*Tetrahedron,* 2015, **71**, 8572-8576	Lysosome-targeted	430 nM	560 nm	PBS/DMSO (v/v, 98/2, 20 mM, pH 7.4 or 6.0)
	*Anal. Chem.***,** 2015, **87**, 1188-1195	NO	50 nM	524 nm	HEPES (pH 7.4, 1% CH_3CN)

	In this paper	Mitochondria -targeted	28.5 nM	455 nm	PBS/EtOH (v/v, 1/1, 10 mM, pH 7.33)

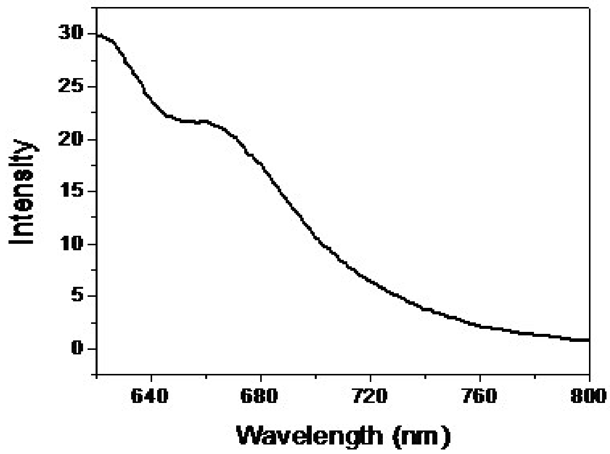


Fig. S1. The fluorescence intensity of L (10 μM) responding to Na_2S (10 eq.) with excitation wavelength at 600 nm.

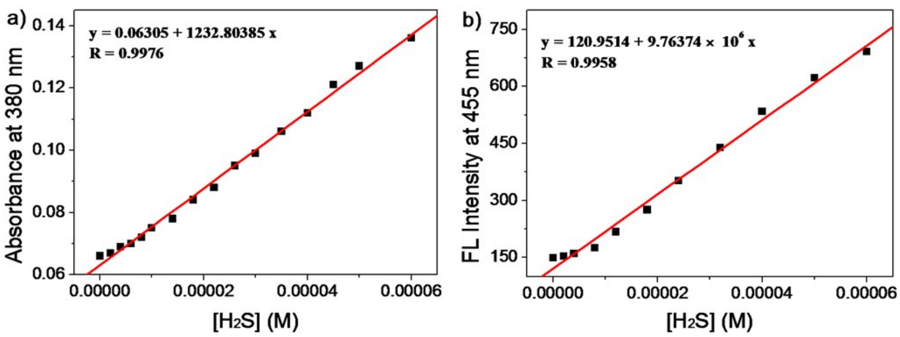


Fig. S2. The absorbance ($A_{380\ nm}$, a) and fluorescence intensity ($F_{455\ nm}$, b) of **L** (10 μM) depending on Na_2S concentration. A linear relationship between the absorbance and the Na_2S concentration (a), and the fluorescence intensity and the Na_2S concentration (b) could be obtained in the 0-60 μM concentration range (R^2 = 0.9976 and 0.9958). The detection limit (DOL) can be calculated with the equation,[1] DOL = $3\sigma/k$, where "k" is the intensity versus [H_2S], and "σ" is the standard deviation of the blank signal obtained without H_2S ($\sigma_L = 1.22 \times 10^{-5}$ and 0.093).

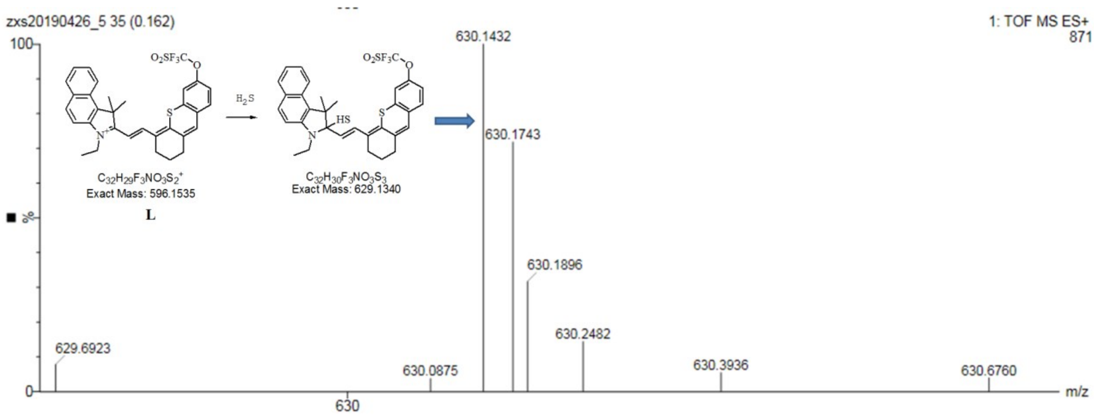


Fig. S3. HRMS spectrum of the solution of **L** interacted with Na_2S.

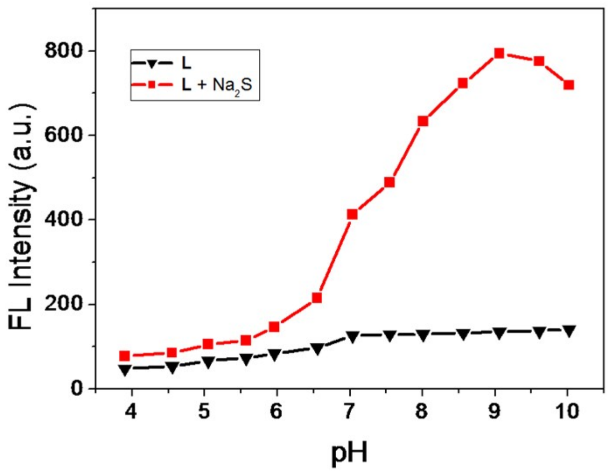


Fig. S4. The pH-dependent fluorescence signals of **L** (10 μM) in the absence and presence of Na_2S (10 eq.). λ_{ex} = 380 nm, λ_{em} = 455 nm.

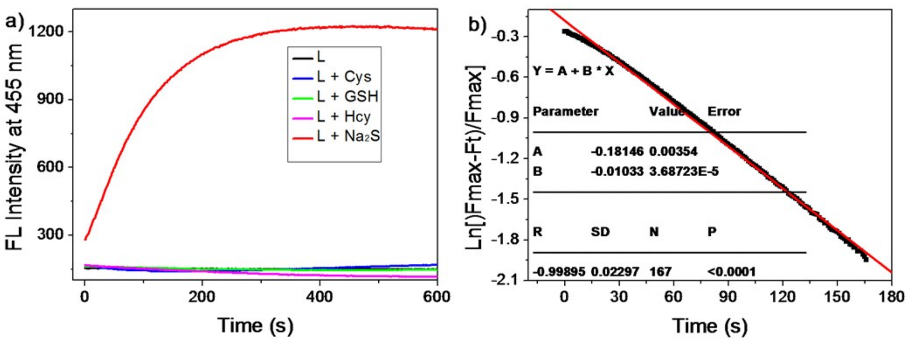


Fig. S5. (a) The time-dependent fluorescence signals of **L** (10 μM) with addition of Na_2S, Cys, Hcy and GSH (100 equiv.), respectively. (b) Kinetic analysis of the reaction of **L** and H_2S. The pseudo-first-order rate constant k can be calculated with the equation,[2] $k = k'C$, where "k'" is $\mathrm{Ln}[(F_{max}-F_t)/F_{max}]$ versus time, "C" is the initial concentration of the probe **L**. λ_{ex} = 380 nm, λ_{em} = 455 nm.

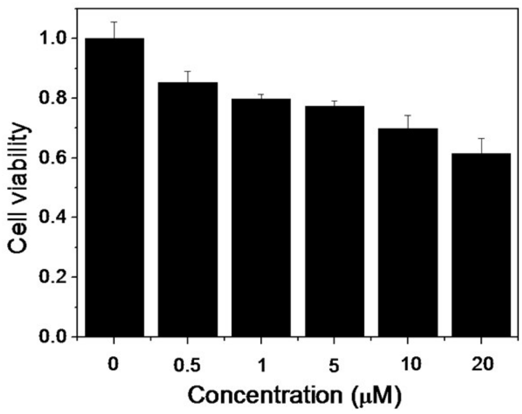


Fig. S6. Cell viability estimated by MTT proliferation tests versus incubation concentrations of **L**.

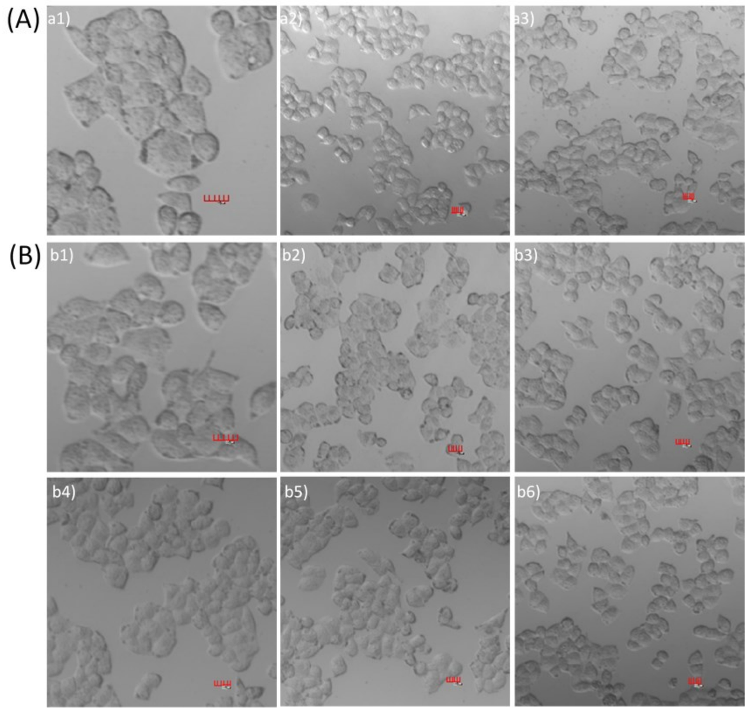


Fig. S7. (A) Bright field of the fluorescence images of Hela cells incubated with **L** (2 μM) (a1), **L** (2 μM) and Na_2S (10 μM) (a2), and **L** (2 μM) and Na_2S (20 μM). (B) Bright field of the fluorescence images of Hela cells incubated with **L** (2 μM), (b1), **L** (2 μM) and Cys (100 μM) (b2), **L** (2 μM) and Cys (200 μM) (b3), **L** (2 μM) and GSH (100 μM) (b4), **L** (2 μM) and GSH (200 μM) (b5), and **L** (2 μM) and Cys/GSH (200 μM) (b6). λ_{ex} = 405 nm, λ_{em} = 420-475 nm. Scale bar: 20 μm.

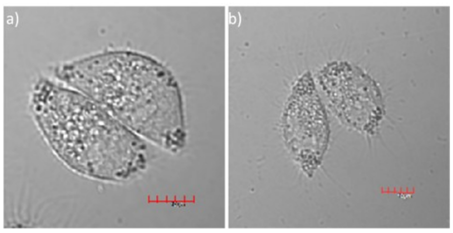


Fig. S8. a) Bright field of the fluorescence images of Hela cells containing **L** and Lyso-tracker incubated with Na_2S (10 μM); b) Bright field of the fluorescence images of Hela cells containing **L** and Mito-tracker incubated with Na_2S (10 μM). Scale bar: 10 μm

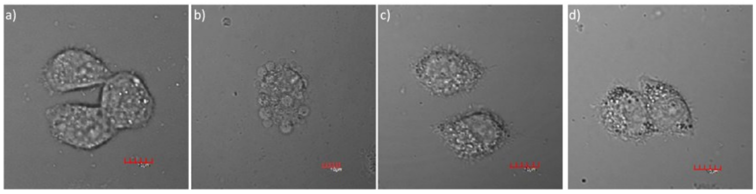


Fig. S9. a) Bright field of the fluorescence images of H_2O_2 pretreated Hela cells containing **L** and Lyso-tracker; b) Bright field of the fluorescence images of H_2O_2 pretreated Hela cells containing **L** and Mito-tracker; c) Bright field of the fluorescence images of PMA pretreated Hela cells containing **L** and Lyso-tracker; d) Bright field of the fluorescence images of PMA pretreated Hela cells containing **L** and Mito-tracker. Scale bar: 10 µm

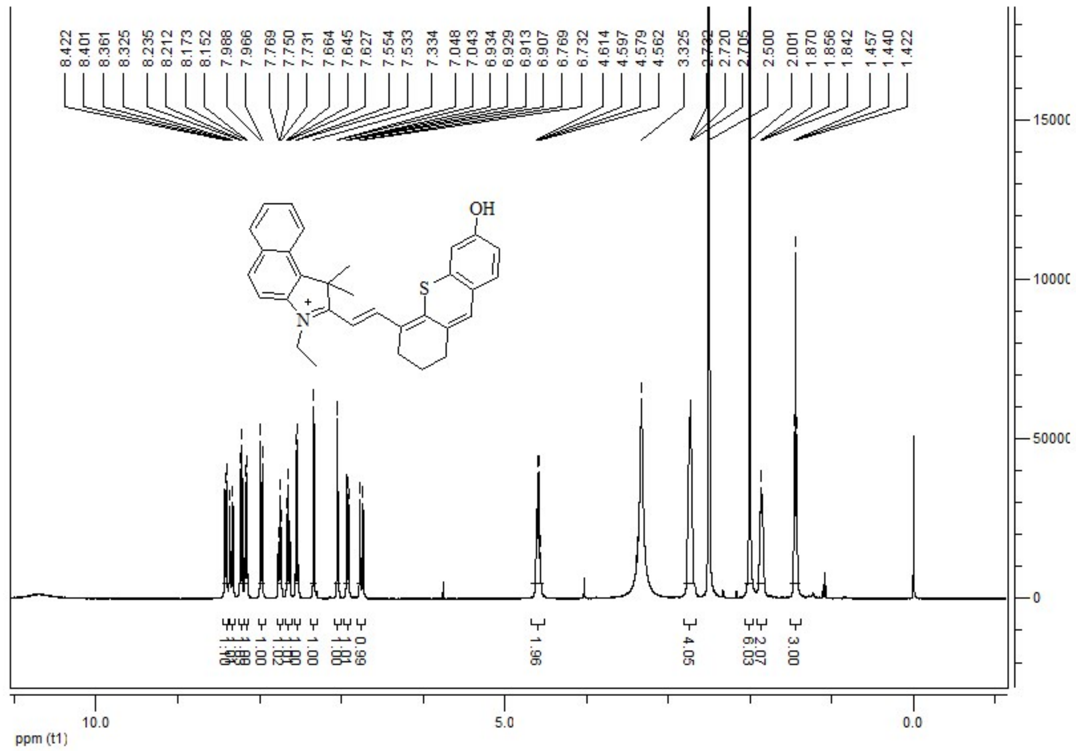


Fig. S10. ^{1}H NMR spectra of **1**

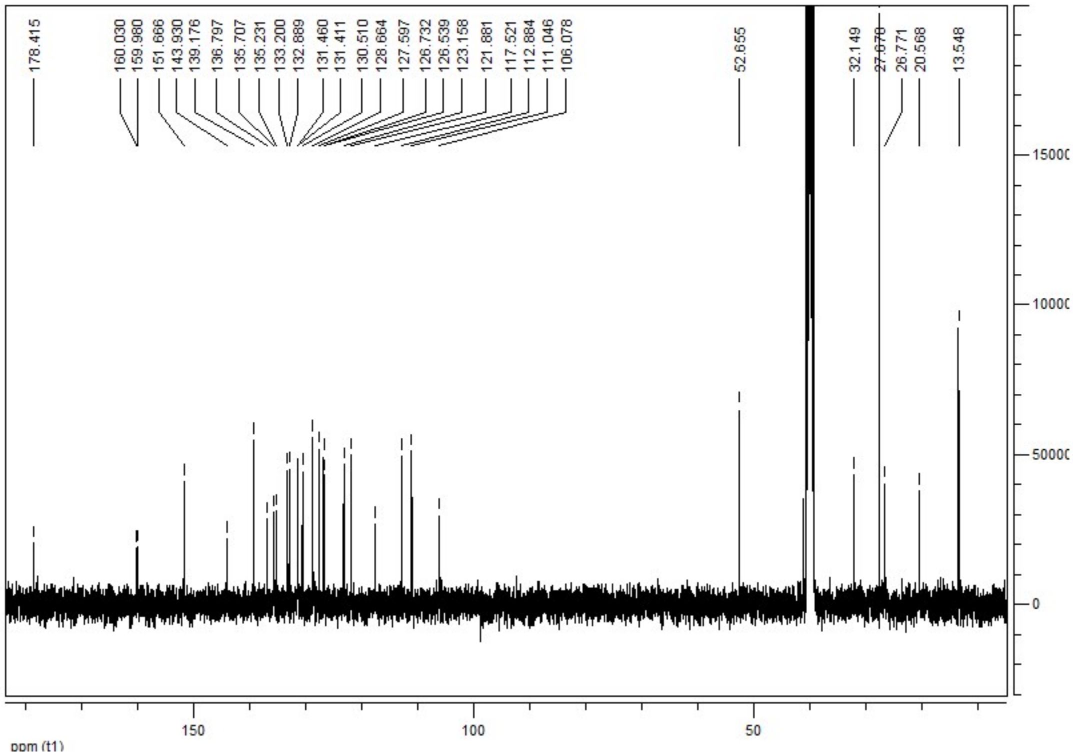


Fig. S11. ^{13}C NMR spectra of **1**

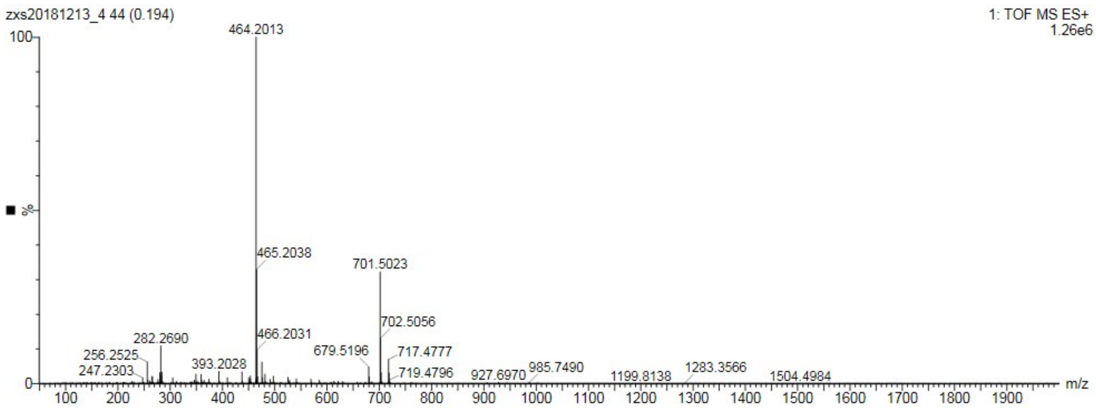


Fig. S12. HRMS spectra of **1**

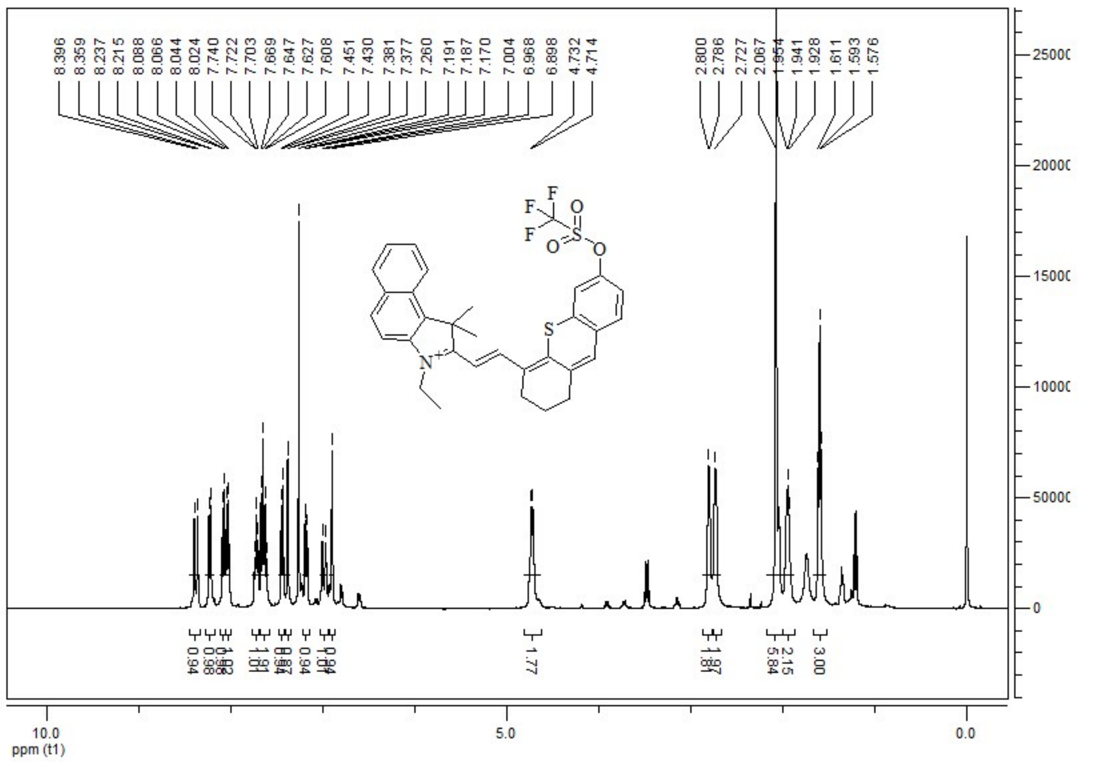


Fig. S13. ^{1}H NMR spectra of **L**

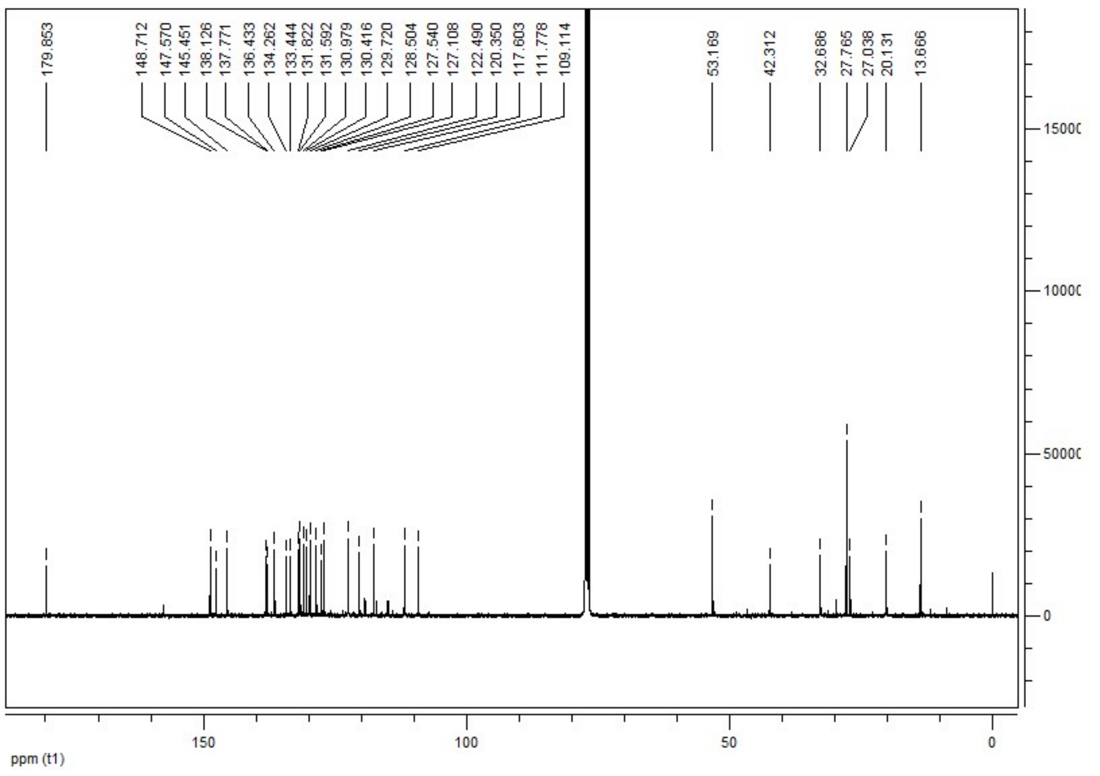


Fig. S14. ^{13}C NMR spectra of **L**

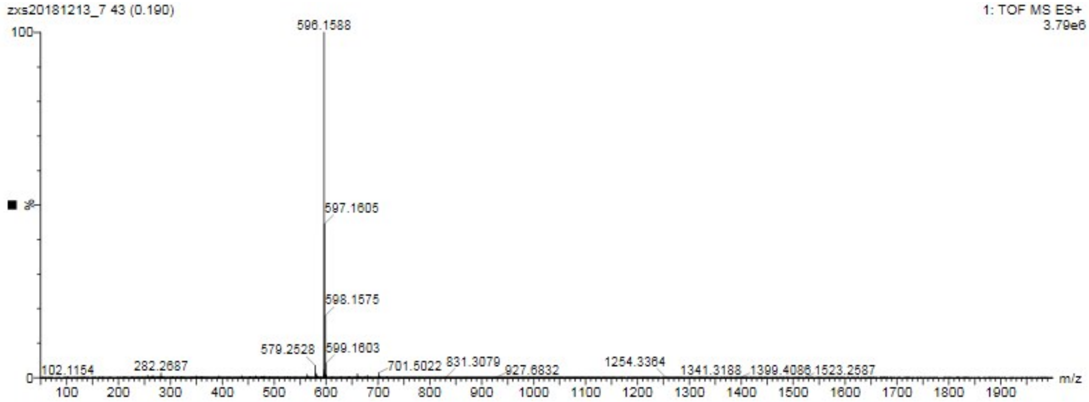


Fig. S15. HRMS spectra of **L**

2. References:

1 G. Long and J. Winefordner, *Anal. Chem.*, 1983, **55**, 712A-24A.

2 Q. X. Han, Z. H. Shi, X. L.Tang, L. Z. Yang, Z. L. Mou, J. Li, J. M. Shi, C. Y. Chen, W. Liu, H. Yang, W. S. Liu, *Org. Biomol. Chem.*, 2014, **12**, 5023−5030.