Electronic Supplementary Material (ESI) for Analyst.

Support information

1. Cell imaging

The human breast cancer cells (MCF-7) were cultured in Roswell Park Memorial Institute (RPMI-1640) supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 μg/mL penicillin and 100 μg/mL streptomycin at 37◦C in a humidified atmosphere containing 5% CO_2. 24 h before imaging, cells were seeded in laser scanning confocal microscope (LSCM) culture dishes. The dishes were subsequently incubated at 37◦C in a humidified atmosphere containing 5% CO_2. Then the cells were incubated with Cys (200 μM), Hcy (200 μM), GSH (200 μM), or NEM (1 mM) for 30 min. Subsequently, 10 μM Cz-BDP-NBD was added and incubated at 37◦C for 1 h. The cells were washed three times with Dulbecco's PBS (pH 7.2) to remove free compound before analysis. MCF-7 cells only incubated with NEM (1 mM) for 30 min acted as a control. Confocal luminescence images of MCF-7 cells were carried out on an Olympus FV3000 laser scanning confocal microscope. Green (λem = 510–560 nm) and red (λem = 610–710 nm) channels upon excitations at 488 nm and 592 nm, respectively.

2. Cytotoxic assay

MCF-7 cells were seeded in a 96-well plate (1 × 104 cells/well).After cultivation for 24 h, Cz-BDP-NBD (dissolve in DMSO 10^{-3} M, then added it into the cell culture medium) of different concentrations(0 μM, 2 μM, 5 μM, 10 μM, 15 μM, 20 μM) were added into the wells (n = 6) and incubated for 24 h. Then stock solution of MTT (20 μL; 5 mg/mL) was added into each well. After 4 h incubation at 37◦C, the MTT solution was replaced with 100 μL DMSO in each well. The absorbance in each well was measured at 570 nm with a multi-well plate reader. Cell viability was calculated using the following formula: Cell viability = (Mean absorbance of test wells − Mean

absorbance of medium control wells)/(Mean absorbance of untreated wells − Mean absorbance of medium control well) × 100%.

3. Detection limit [1]:

$$3\delta/k$$

Where δ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity versus Cys, Hcy, and GSH concentration.

4. Fluorescence quantum yields

Dilute solutions of the free probe and probe with Hcy, Cys and GSH (A(λex) ≈0.05) were used. Quantum yields sample were determined according to equation:

$$\Phi_s = \Phi_{ref} \frac{F_{ref} A_{ref}\ n_s^2}{F_s\ \ A_s\ n_{ref}^2}$$

Here, F denotes the integral areas of the corrected fluorescence spectrum, A is the absorbance at the excitation wavelength, n is the refractive index of solvent. "ref" and "s" denote parameters from the reference and unknown experimental samples, respectively. The reference systems at 670 nm used Rhodamine B (Φ_f = 0.65 in EtOH [2]) as reference and fluorescence at 540 nm used Fluorescein dissolved in 0.1 M sodium hydroxide solution as reference [3]

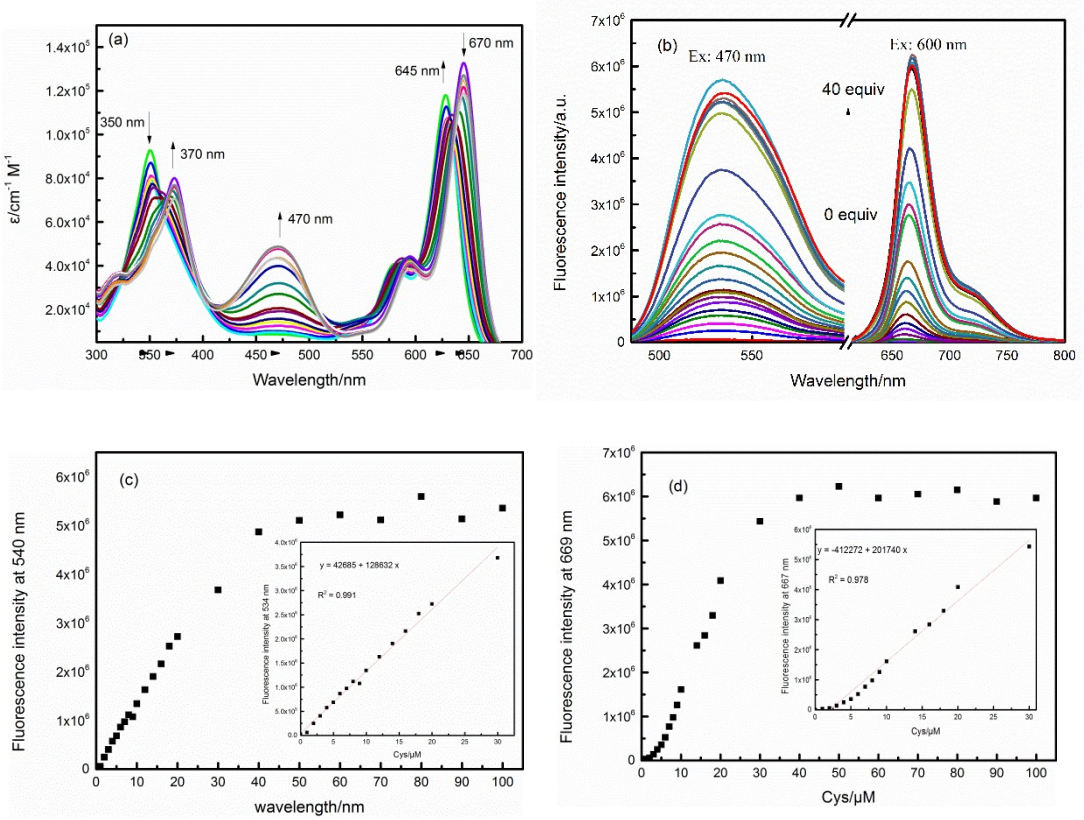

Fig S1. UV-vis absorption and fluorescence titration spectra of Cz-BDP-NBD (10 μM) with Cys, in THF/PBS (v/v = 1:1; pH = 7.4). (a) UV-vis Absorption spectra of probe in the presence of various concentrations of Cys; (b) Fluorescence spectra of Cz-BDP-NBD under different concentrations of Cys; (c) Fluorescence intensity of the probe at 540 nm. Insert photograph: fitted calibration curves of the fluorescence intensities; (d) Fluorescence intensity of the probe at 670 nm.

Insert photograph: fitted calibration curves of the fluorescence intensities.

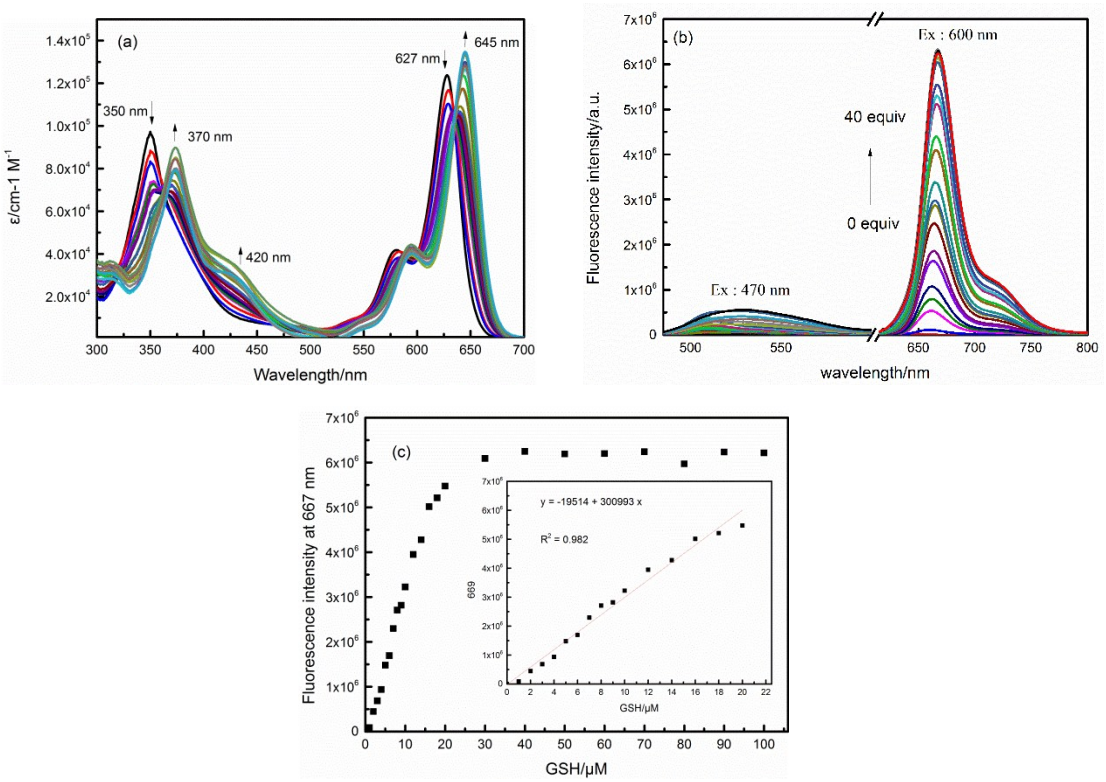

Fig.S2 UV-vis absorption and fluorescence titration spectra of Cz-BDP-NBD (10 μM) with GSH, in THF/PBS (v/v = 1:1; pH = 7.4). (a) UV-vis Absorption spectra of probe in the presence of various concentrations of Cys; (b) Fluorescence spectra of Cz-BDP-NBD under different concentrations of Cys; (c) Fluorescence intensity of the probe at 670 nm. Insert photograph: fitted calibration curves of the fluorescence intensities.

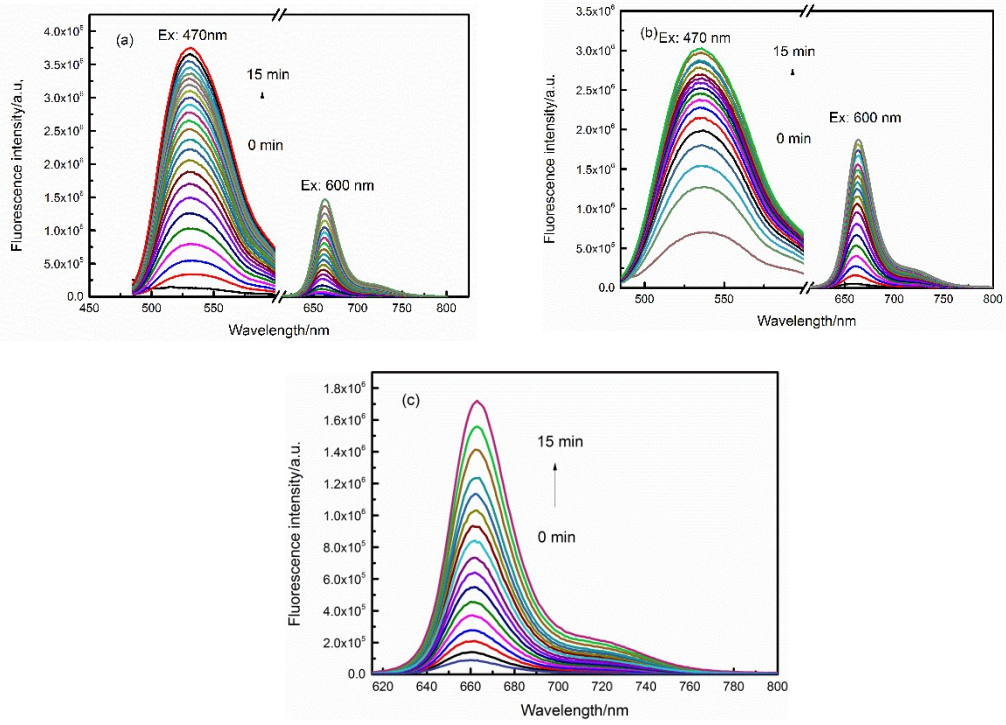

Fig.S3 (a); Responses time of probe Cz-BDP-NBD (10 μM), in present of 200 μM Hcy in a mixture of measured in THF/PBS (v/v = 1:1; pH = 7.4). (b); Responses time of probe Cz-BDP-NBD (10 μM), in present of 200 μM Cys. (c) Responses time of probe Cz-BDP-NBD (10 μM), in present of 200 μM GSH

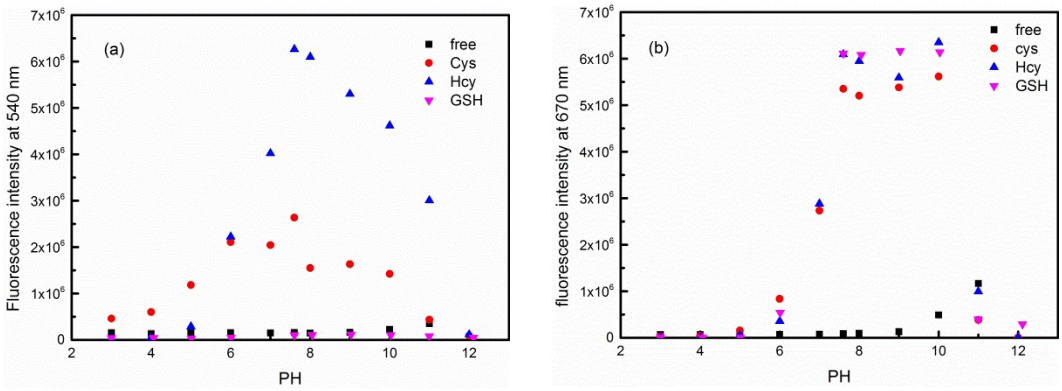

Fig.S4 (a): Fluorescence intensity at 540 nm of probe (10 μM, THF/PBS, v/v = 1:1; pH = 7.4) in the absence or presence of Hcy/Cys and GSH (200 μM) at pH values from 3.0 to 12.0. (b): Fluorescence intensity at 670 nm of probe (10 μM, THF/PBS, v/v = 1:1; pH = 7.4) in the absence or presence of Hcy/Cys and GSH (200 μM) at different pH values. λex = 470 nm and 600 nm, slit 3 nm/3 nm.

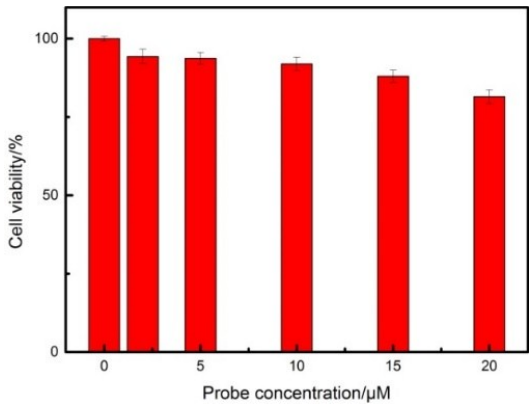

Fig.S5 MTT experiment by the standard method. MCF-7 cells were incubated with different

concentrations of the probe for 24 hours, then the probe was removed and washed, and the cells were subjected to the MTT method.

Table.1 Properties of free probe and the probe (Cz-BDP-NBD) interacted with Hcy, Cys and GSH

	λ_{abs} nm	λ_{em} nm	$\varepsilon(10^5\ cm^{-1}M^{-1})$	Φ/%	LOD /nM	λ_2PET* (nm)	Φδ /GM
probe	350	509	0.76	-			
	627	644	0.96	0.2			
Probe+GSH	420	514	0.21	-			
	645	667	1.33	14.02	26.10	660	196.8
Probe+Hcy	470	530	0.47	27.98	36.90		
	645	667	1.29	14.24		659	180.4
Probe + Cys	470	534	0.48	24.36	99.50		
	645	668	1.33	14.51		660	181.9
Cz-BDP	645	667	1.35	15		661	198.5

*TPEF: two photon excited fluorescence was measured through Fluorescence reference method and serviced the Rhodamine B ($2* 10^{-5}$ M, 150 GM) as reference.

Reference

1. M. Gao, R. Wang, F. Yu, J. You and L. Chen, *Journal of Materials Chemistry B*, 2018, **6**, 2608-2619.

2.D. Kand, T. Saha and P. Talukdar, Sensors and Actuators B: Chemical, 2014, 196, 440-449.

3.J. Lv, Y. Chen, F. Wang, T. Wei, Z. Zhang, J. Qiang and X. Chen, Dyes and Pigments, 2018, 148, 353-358.

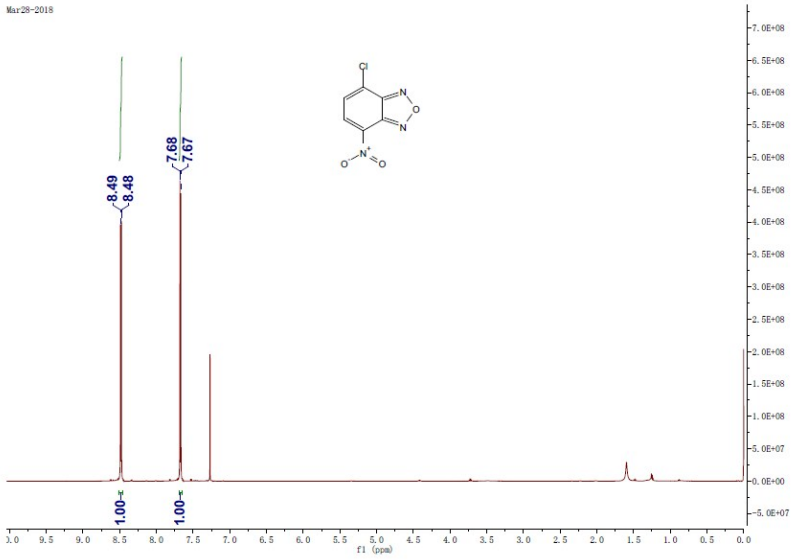

Figure S6.^{1}H-NMR ($CDCl_3$) spectrum of compound NBD-Cl

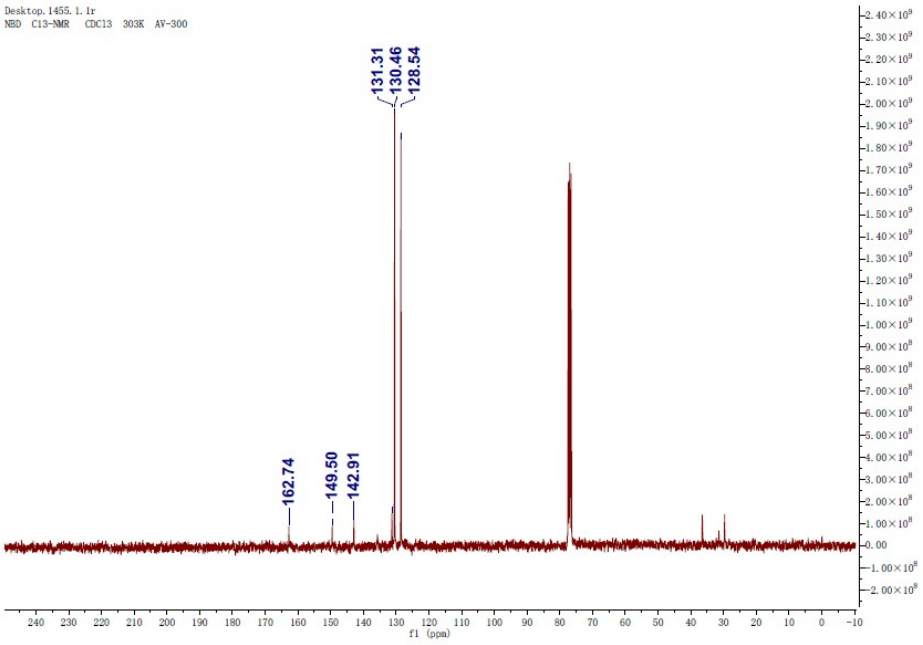

Figure S7.^{13}C-NMR ($CDCl_3$) spectrum of compound NBD-Cl

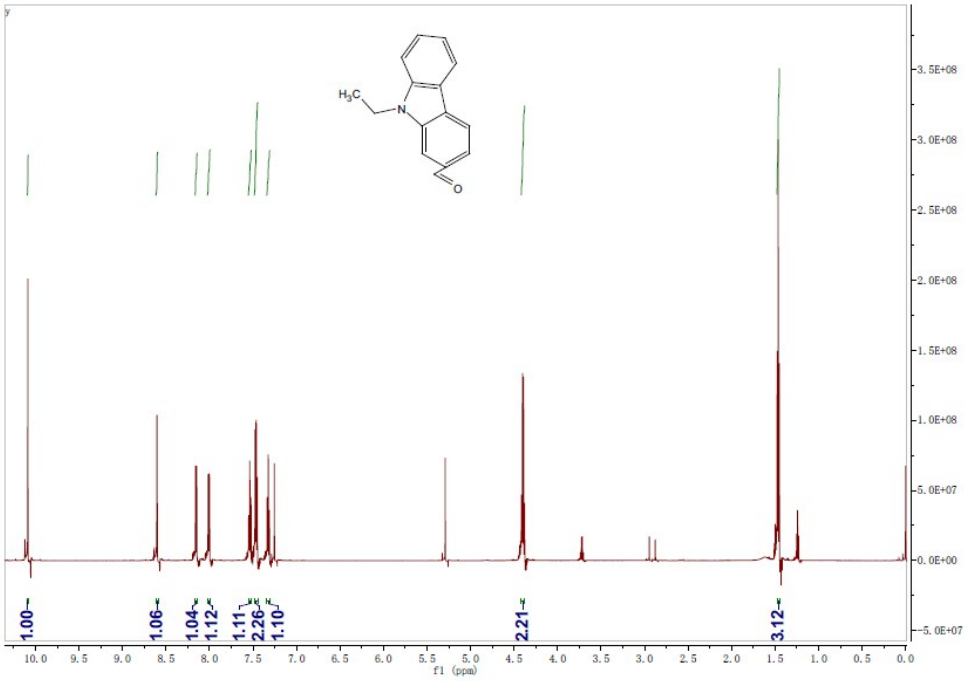

Figure S8.^{1}H-NMR ($CDCl_3$) spectrum of compound KZ-CHO

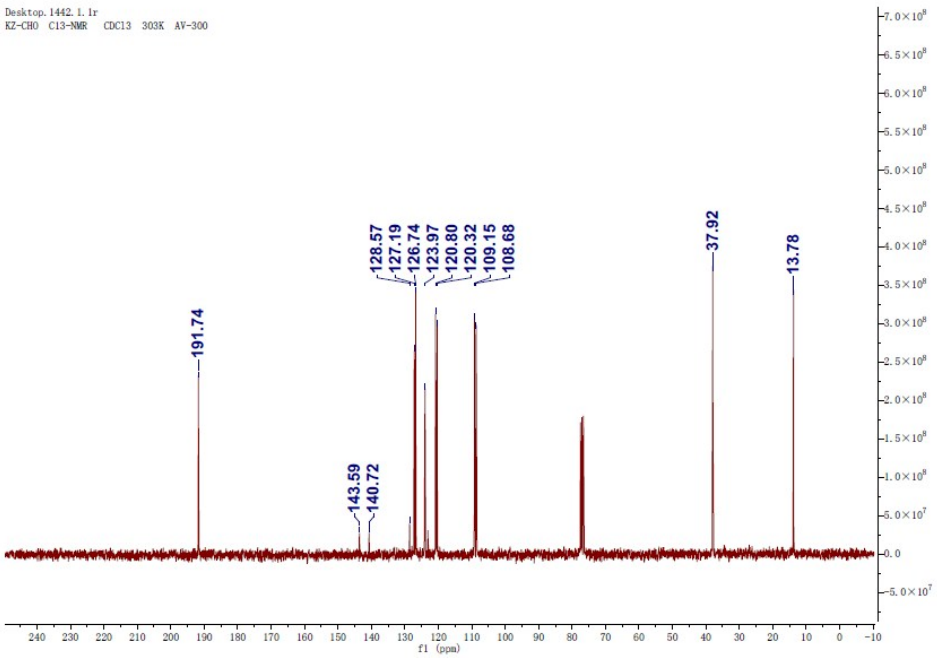

Figure S9.^{13}C-NMR ($CDCl_3$) spectrum of compound KZ-CHO

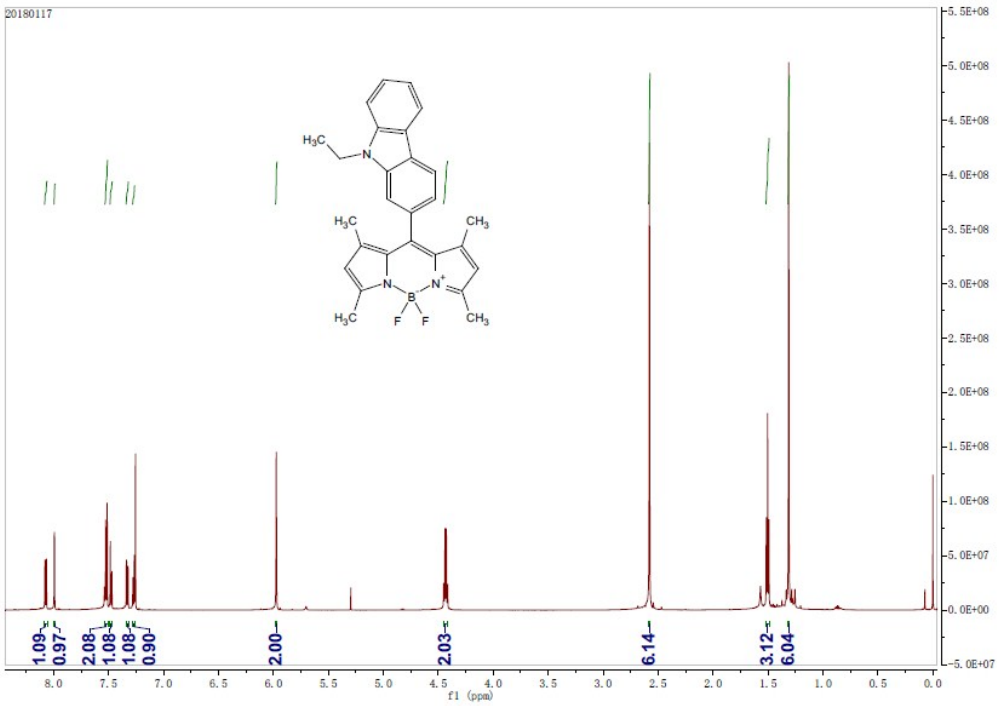

Figure S10.^{1}H-NMR ($CDCl_3$) spectrum of compound M1

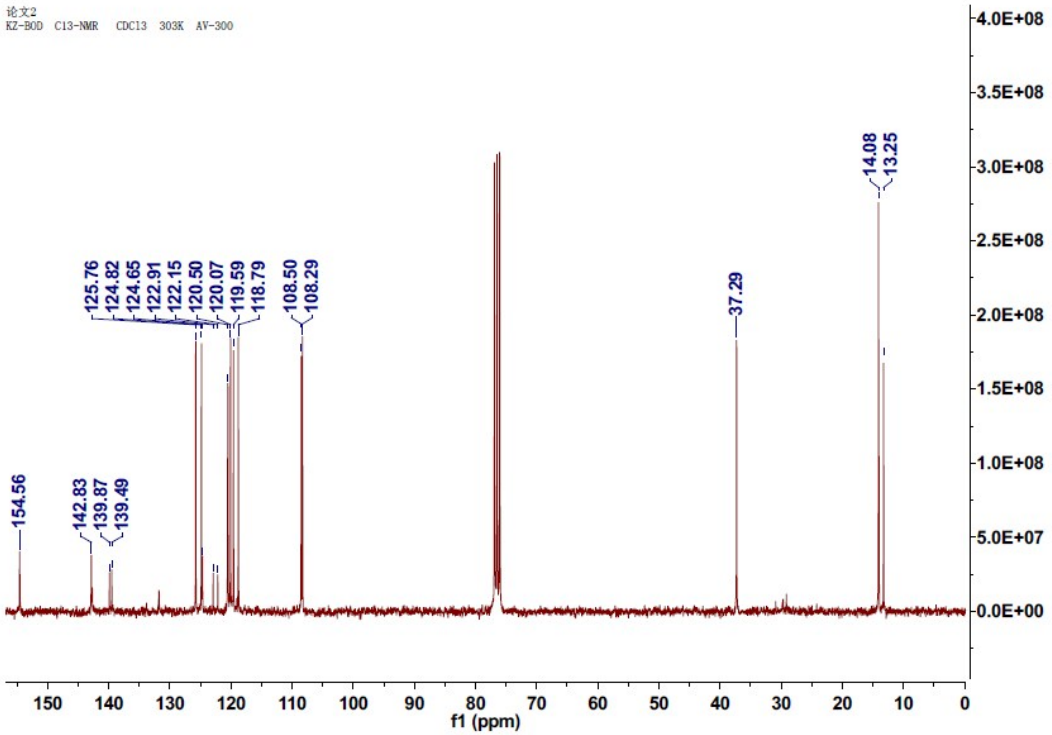

Figure S11. ^{13}C-NMR ($CDCl_3$) spectrum of compound M1

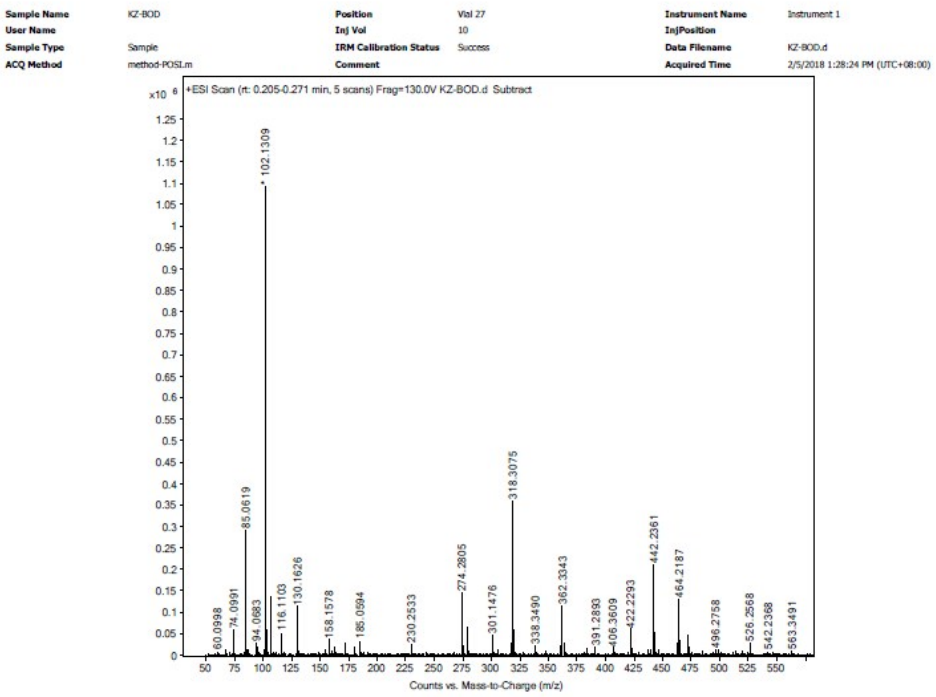

Figure S12. The MS spectrum of compound M1. Calculated for $C_{27}H_{26}BF_2N_3Na$ [M+Na]+: 464.2188, found [M+Na]+ : 464.2187.

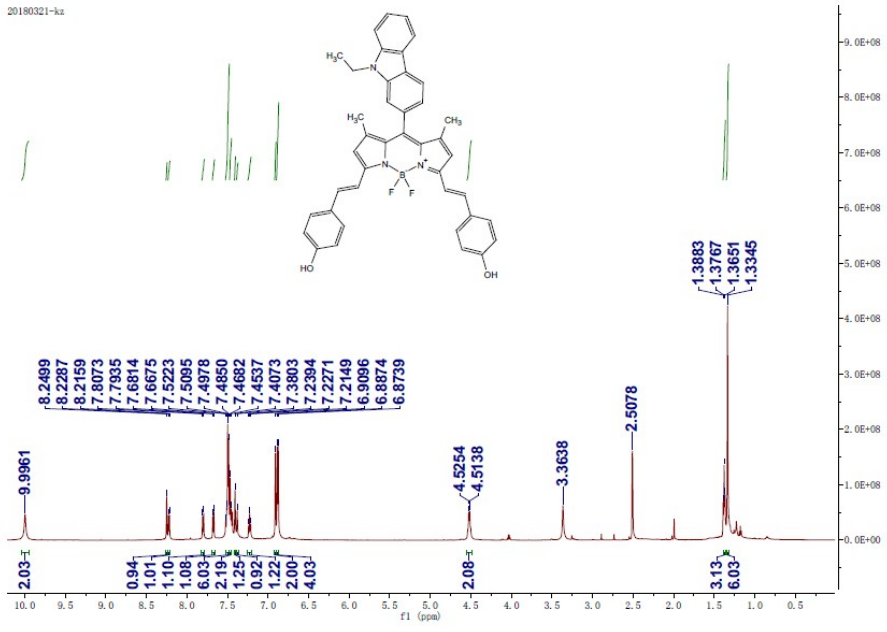

Figure S13.1H-NMR (d_6-DMSO) spectrum of compound M2

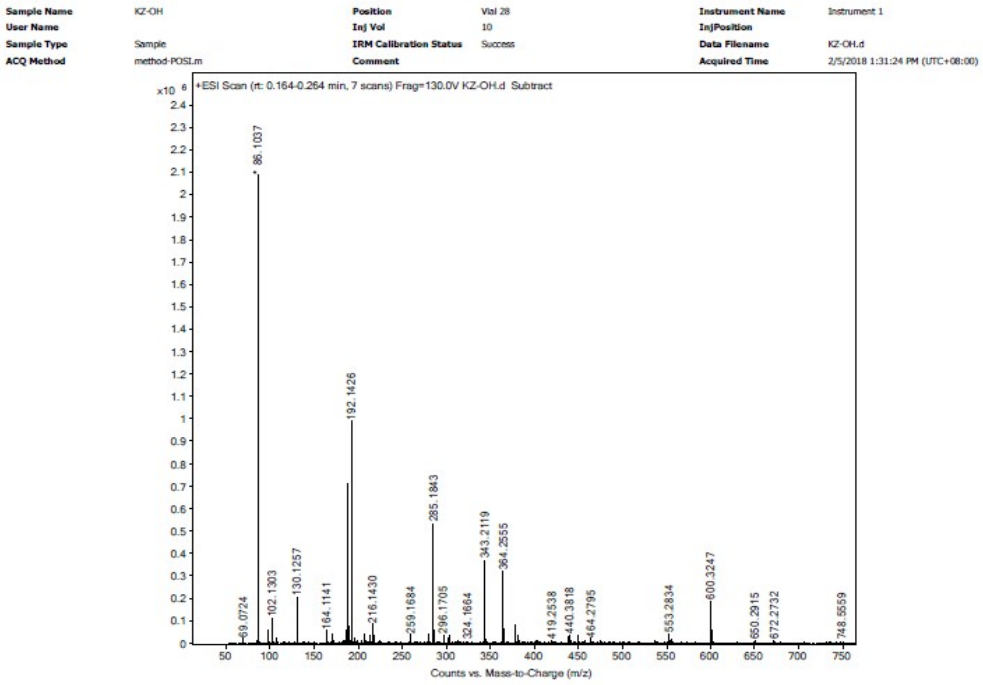

Figure S14. The MS spectrum of compound M2. Calculated for $C_{41}H_{34}BF_2N_3NaO_2$ $[M+Na]^+$: 672.2712, Found $[M+Na]^+$: 672.2732.

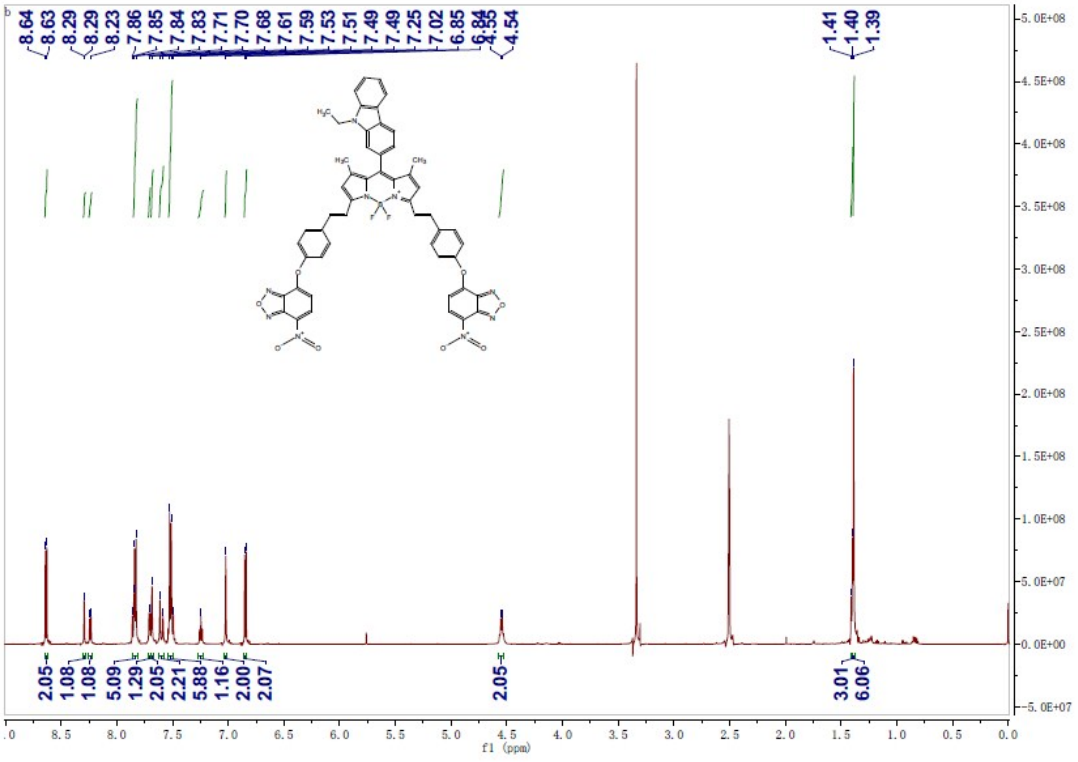

Figure S15.^{1}H-NMR (d_6-DMSO) spectrum of compound Cz-BDP-NBD

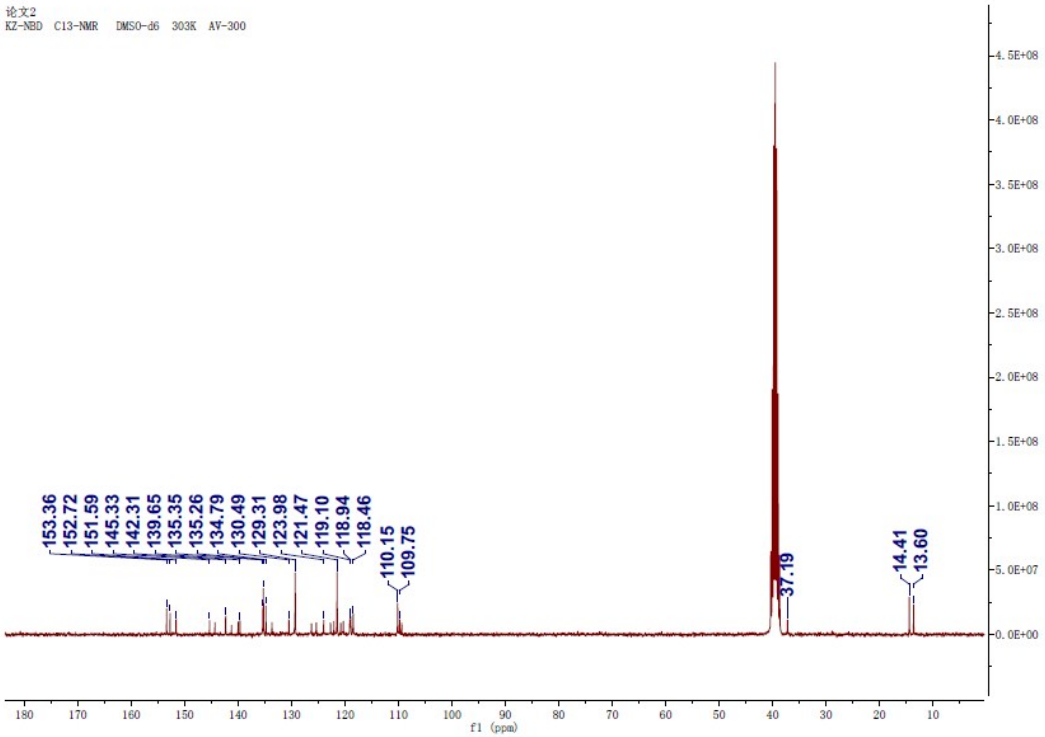

Figure S16.^{13}C-NMR (d6-DMSO) spectrum of compound Cz-BDP-NBD