Electronic Supplementary Material (ESI) for Analyst.

Supporting Information For

An ultrafast turn-on thiol probe for protein labeling and bioimaging

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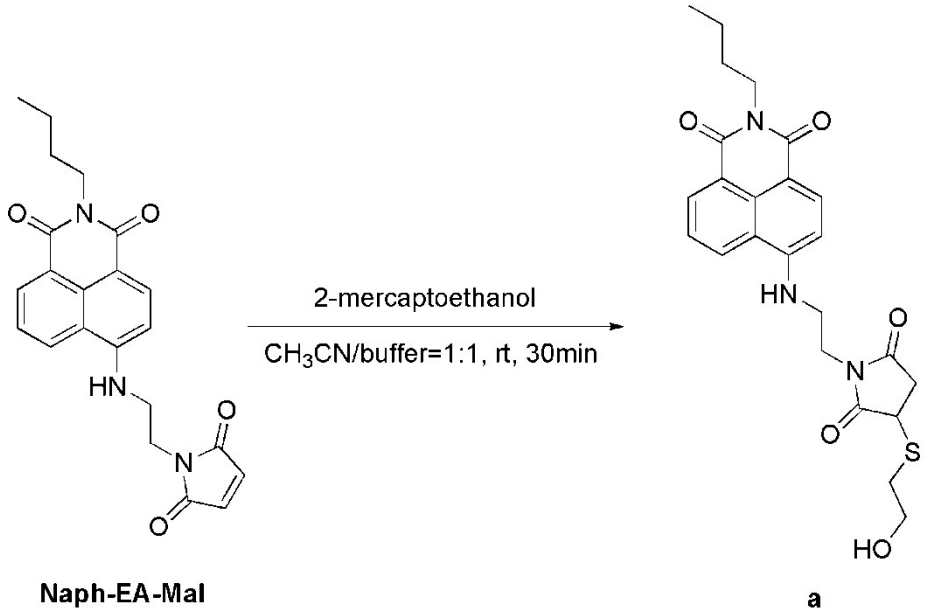
Mechnism Studies:



Scheme S1. The reaction of **Naph-EA-mal** with 2-mercaptoethanol.

Naph-EA-mal (187 mg, 1 mmol) and 2-mercaptoethanol (160 mg, 2 mmol) was dissolved in CH_3CN/Buffer (Tris-HCl, 50 mM, pH=7.4) = 1:1, and then the mixture was stirred vigorously for another 30 min at room temperature. And the solvent was removed under reduced pressure, the residual sample was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate = 5:1) to give **a**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (dd, *J* = 7.3, 0.7 Hz, 1H), 8.38 (d, *J* = 8.3 Hz, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.64 (dd, *J* = 8.3, 7.5 Hz, 1H), 6.58 (d, *J* = 8.4 Hz, 1H), 6.09 (s, 1H), 4.17-4.10 (m, 2H), 4.07 (q, *J* = 5.2 Hz, 2H), 3.97 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.88 (s, 2H), 3.58 (dd, *J* = 9.9, 5.2 Hz, 2H), 3.28 (dd, *J* = 18.9, 9.2 Hz, 1H), 3.13 (m, 1H), 2.88 (m, 1H), 2.66 (dd, *J* = 18.9, 4.0 Hz, 1H), 2.62-2.55 (m, 1H), 1.69 (m, 2H), 1.44 (dd, *J* = 14.7, 7.4 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.60 (s), 175.68 (s), 164.53 (s), 163.96 (s), 148.86 (s), 134.10 (s), 131.03 (s), 129.42 (s), 126.18 (s), 125.04 (s), 122.93 (s), 120.13 (s), 110.85 (s), 103.72 (s), 61.88 (s), 43.29 (s), 39.96 (s), 39.73 (s), 38.19 (s), 36.45 (s), 35.50 (s), 30.27 (s), 20.42 (s),

13.88 (s). HRMS (ESI) m/z calculates for $C_{22}H_{21}N_3O_4$, 469.1671, found 470.1744 $[C_{22}H_{21}N_3O_4 + H]^+$.

Limit of detection (LOD) Studies:

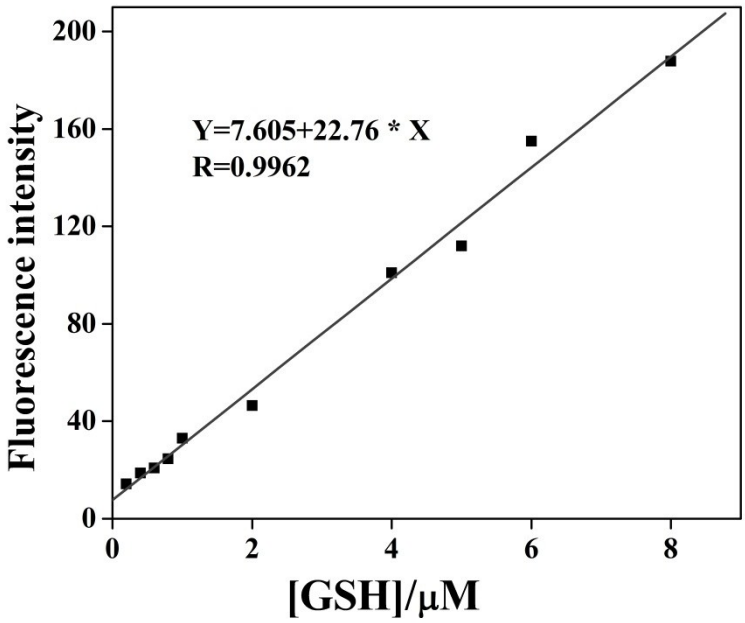


Fig. S1. Plot of the fluorescence intensity at 540 nm as a function of the GSH concentration.

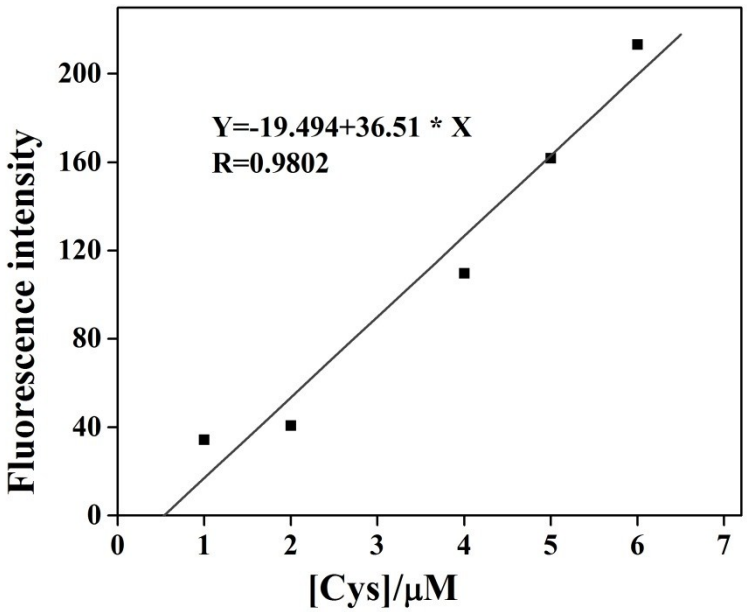


Fig. S2. Plot of the fluorescence intensity at 540 nm as a function of the Cys concentration.

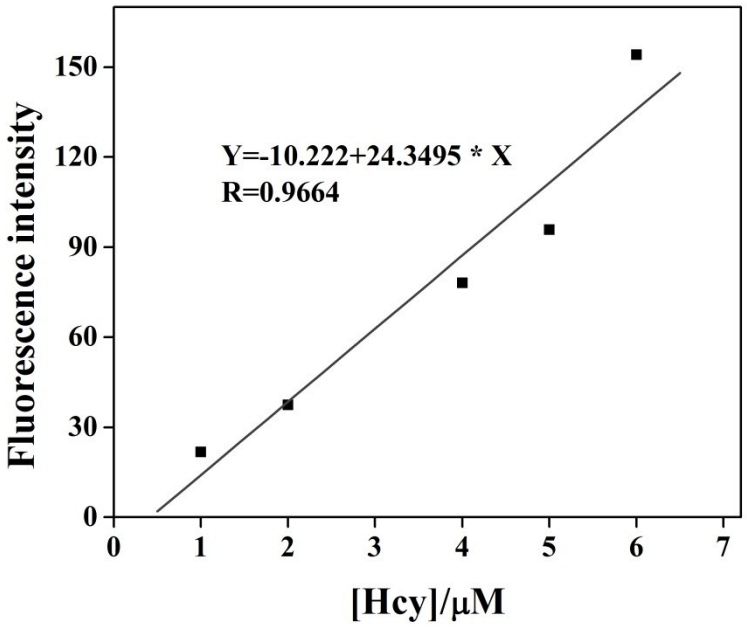


Fig. S3. Plot of the fluorescence intensity at 540 nm as a function of the Hcy concentration.

Kinetic Studies:

The reaction rate constant of the probe **Naph-EA-mal** with Cys, Hcy, and GSH was evaluated under pseudo-first-order kinetics conditions (10 μM **Naph-EA-mal** and 500 μM thiol). The reaction of **Naph-EA-mal** with thiols in aqueous conditions (Tris-HCl, pH=7.4) was monitored using the fluorescence intensity at 540 nm. The pseudo-first-order rate constant was determined by fitting the fluorescence intensities of **Naph-EA-mal** to the pseudo-first-order equation:

$$\ln [(F_{max}-F_t) / F_{max}] = -k't$$

Where F_t and F_{max} are the fluorescence intensities at 540 nm at time t and the maximum value obtained after the reaction was completed. k′ is the pseudo-first-order rate constant. Figure S4-S6 are the pseudo-first-order plot for the reaction of **Naph-EA-mal** with Cys, Hcy, and GSH, respectively. The negative slope of the line provides pseudo-first-order rate constant: k′.

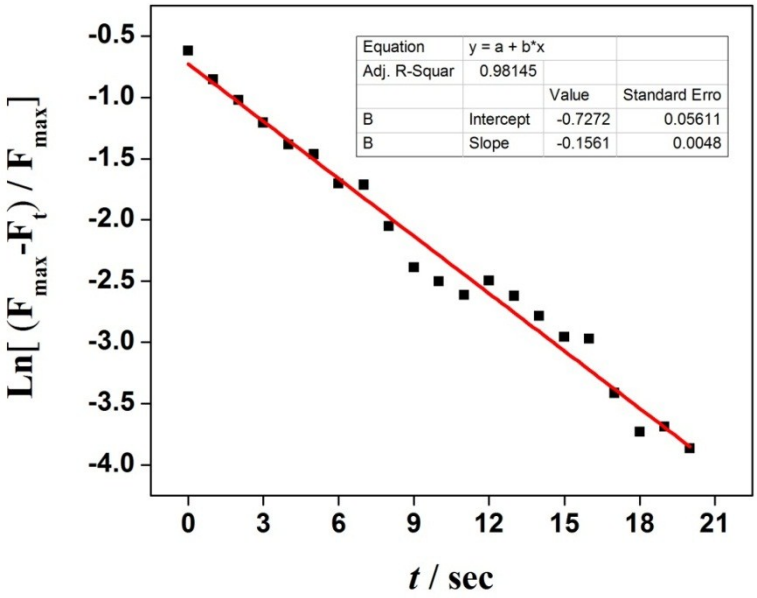


Figure S4. Peudo-first-order kinetic plot of the reaction of probe incubated with Cys in aqueous conditions (50 mM Tris-HCl, pH=7.4). Slope = -0.1561 S^{-1}.

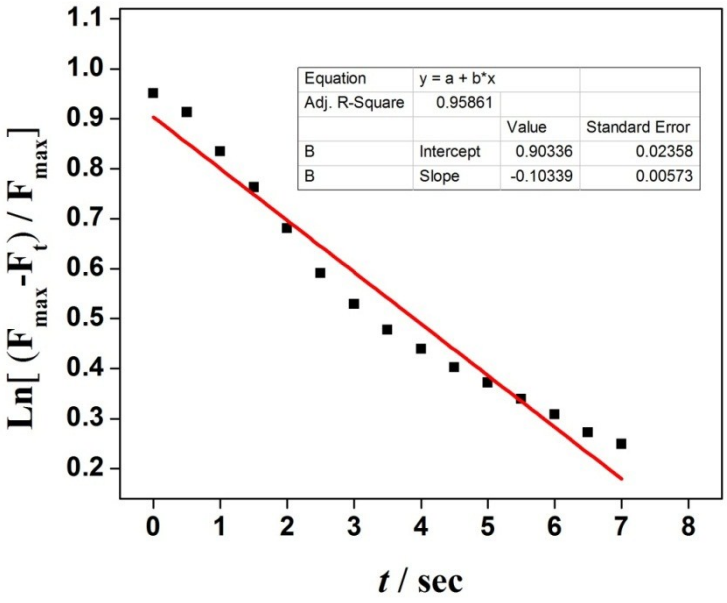


Figure S5. Peudo-first-order kinetic plot of the reaction of probe incubated with Hcy in aqueous conditions (50 mM Tris-HCl, pH=7.4). Slope = -0.10339 S^{-1}.

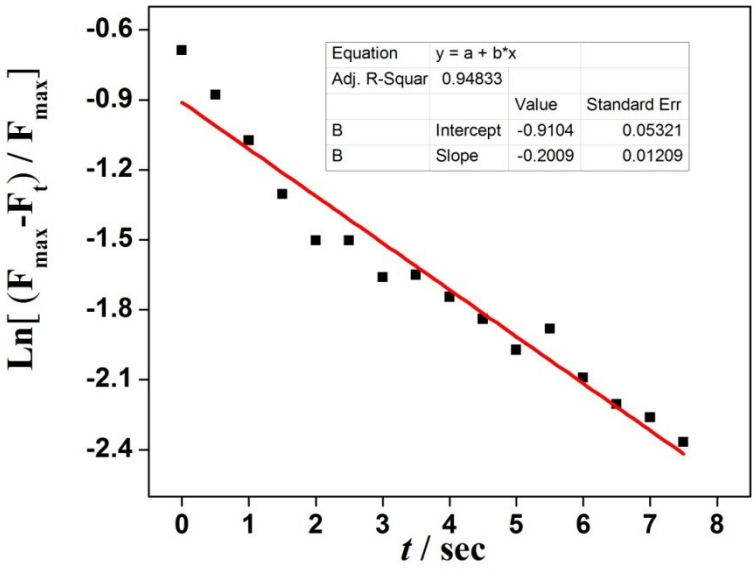


Figure S6. Peudo-first-order kinetic plot of the reaction of probe incubated with GSH in aqueous conditions (50 mM Tris-HCl, pH=7.4). Slope = -0.2009 S^{-1}.

^{1}H NMR, ^{13}C NMR, HRMS spectra:

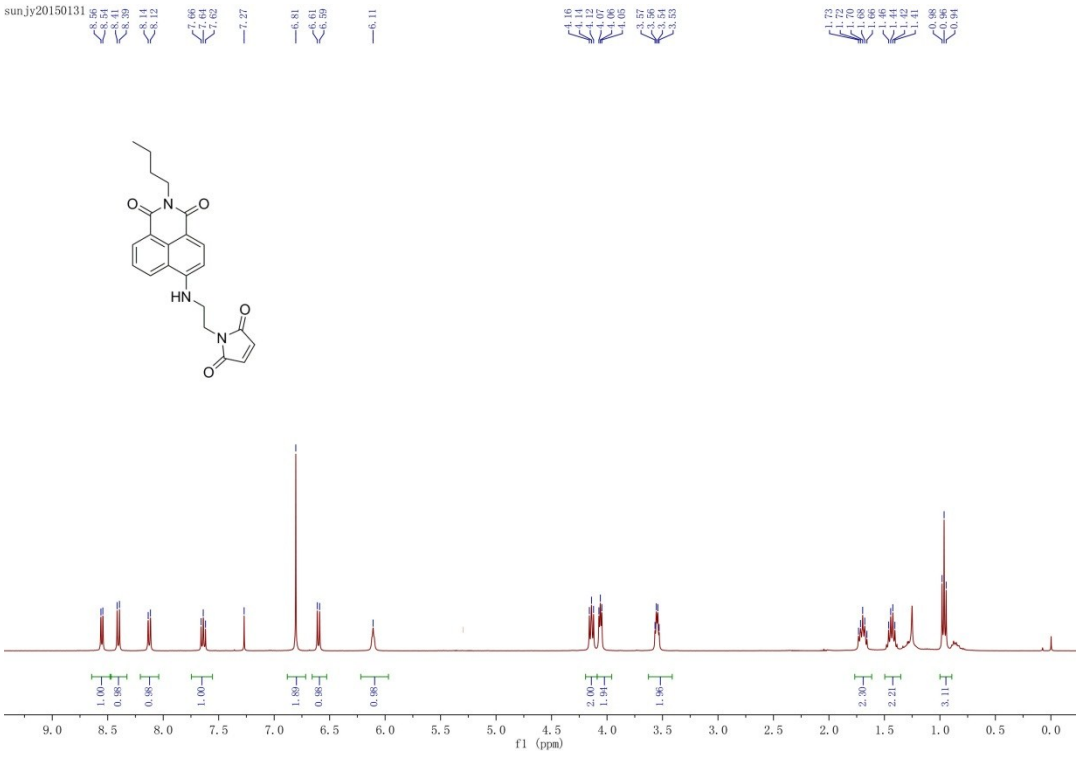


Figure S7. ^{1}H NMR (400 MHz) spectrum of **Naph-EA-mal** in $CDCl_3$.

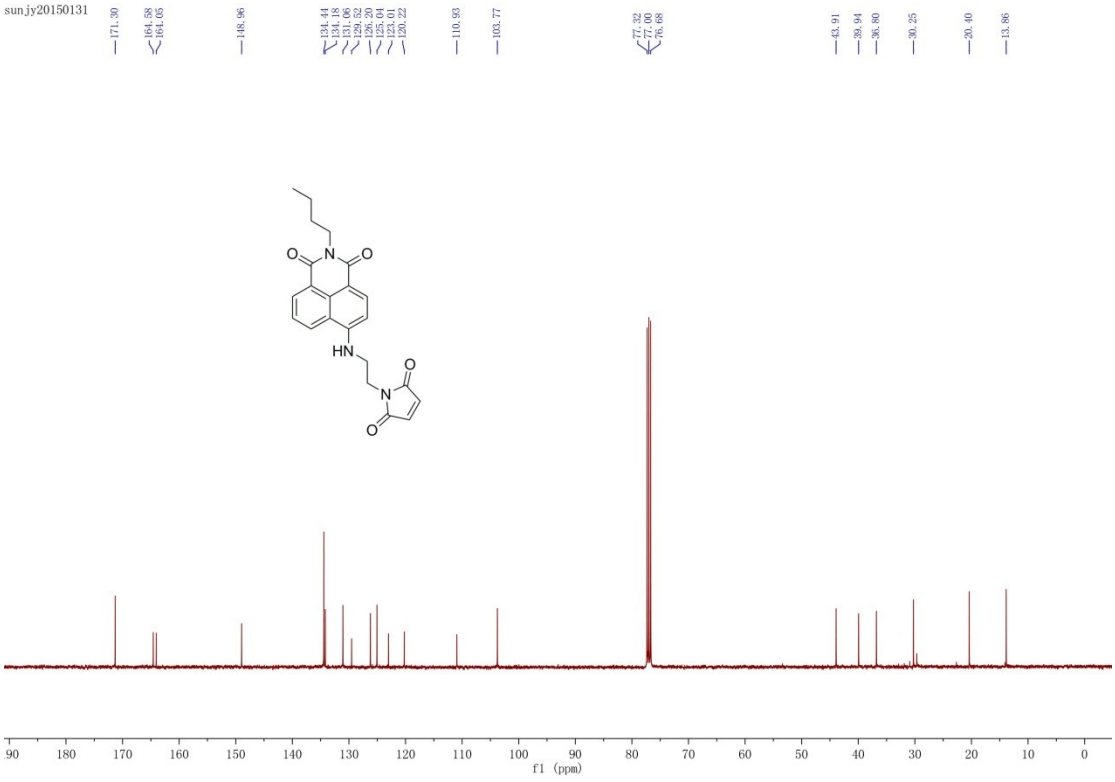


Figure S8. ^{13}C NMR (100 MHz) spectrum of **Naph-EA-mal** in $CDCl_3$.

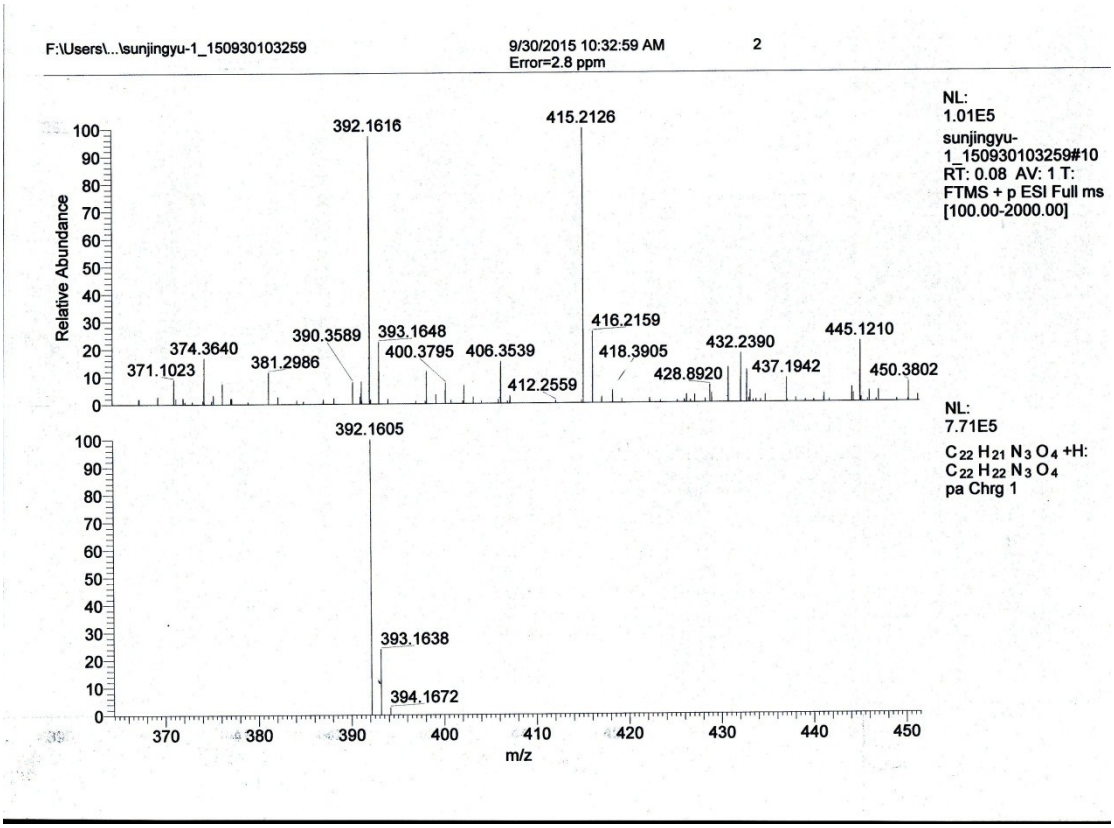


Figure S9. High resolution mass spectrum of **Naph-EA-mal.**

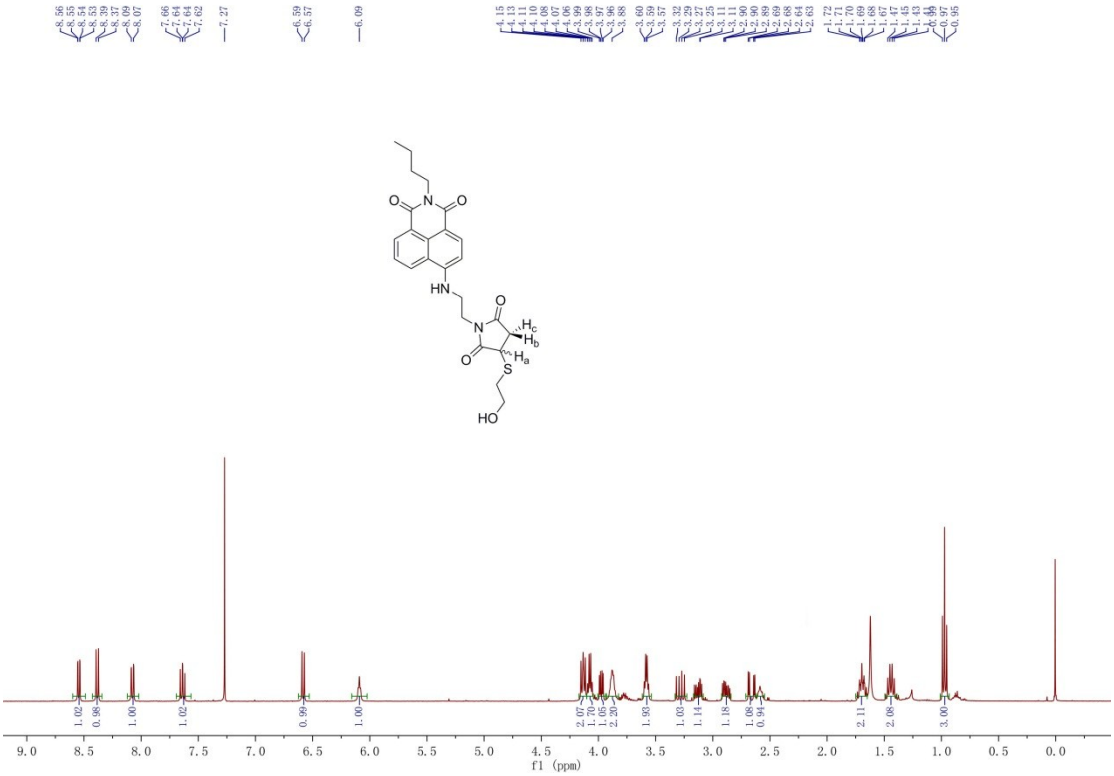


Figure S10. ^{1}H NMR (400 MHz) spectrum of **a** in $CDCl_3$.

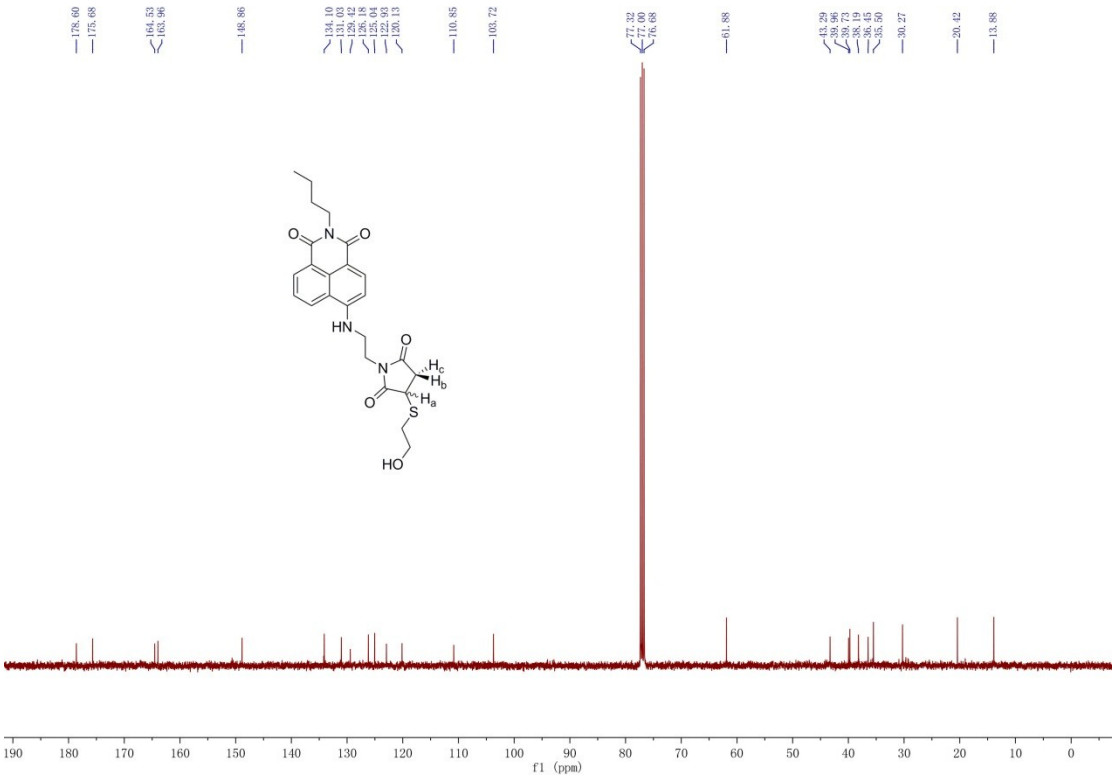


Figure S11. ^{13}C NMR (100 MHz) spectrum of **a** in $CDCl_3$.

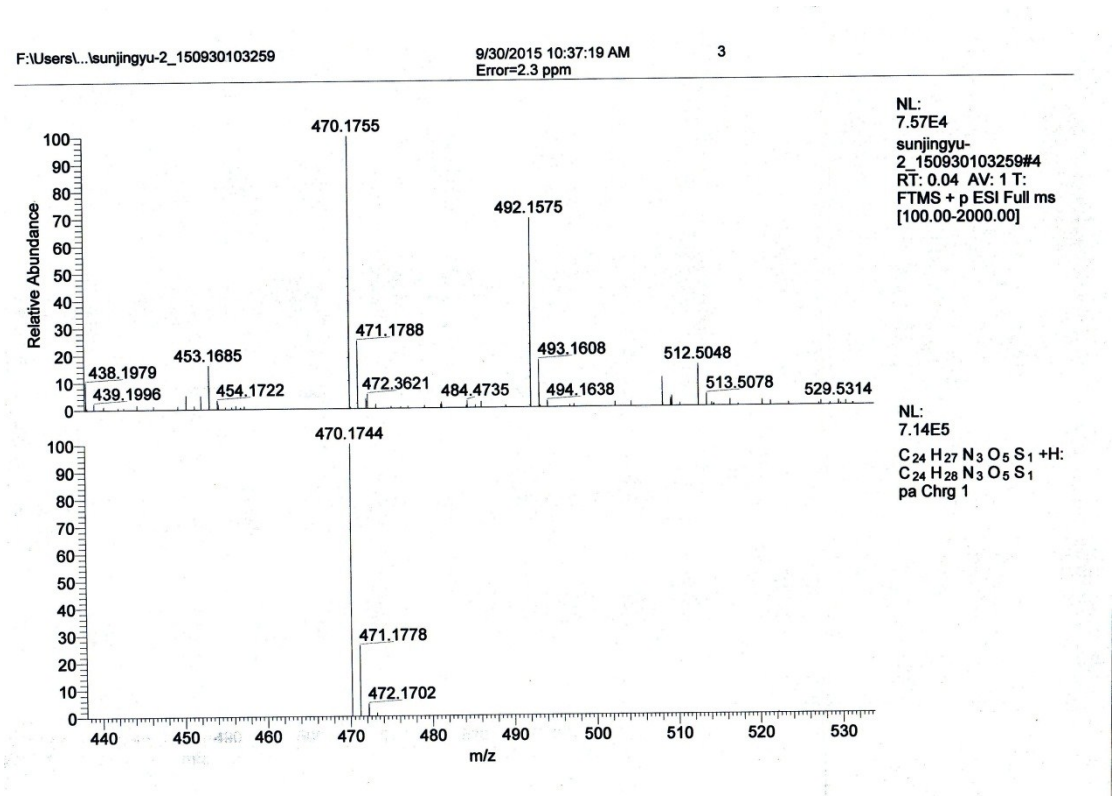


Figure S12. High resolution mass spectrum of **a**.