Supplementary Material

A two-photon off-on fluorescence probe for imaging thiols in live cells and tissues

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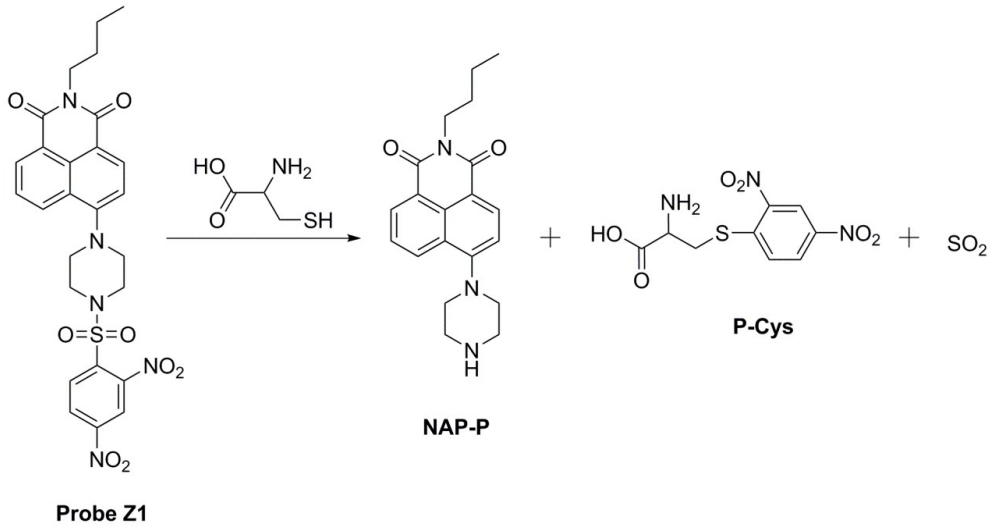
List

1. Sensing Mechanism of Z1 with Thiols



Scheme S1. Proposed mechanism of **Z1** responding to Cys

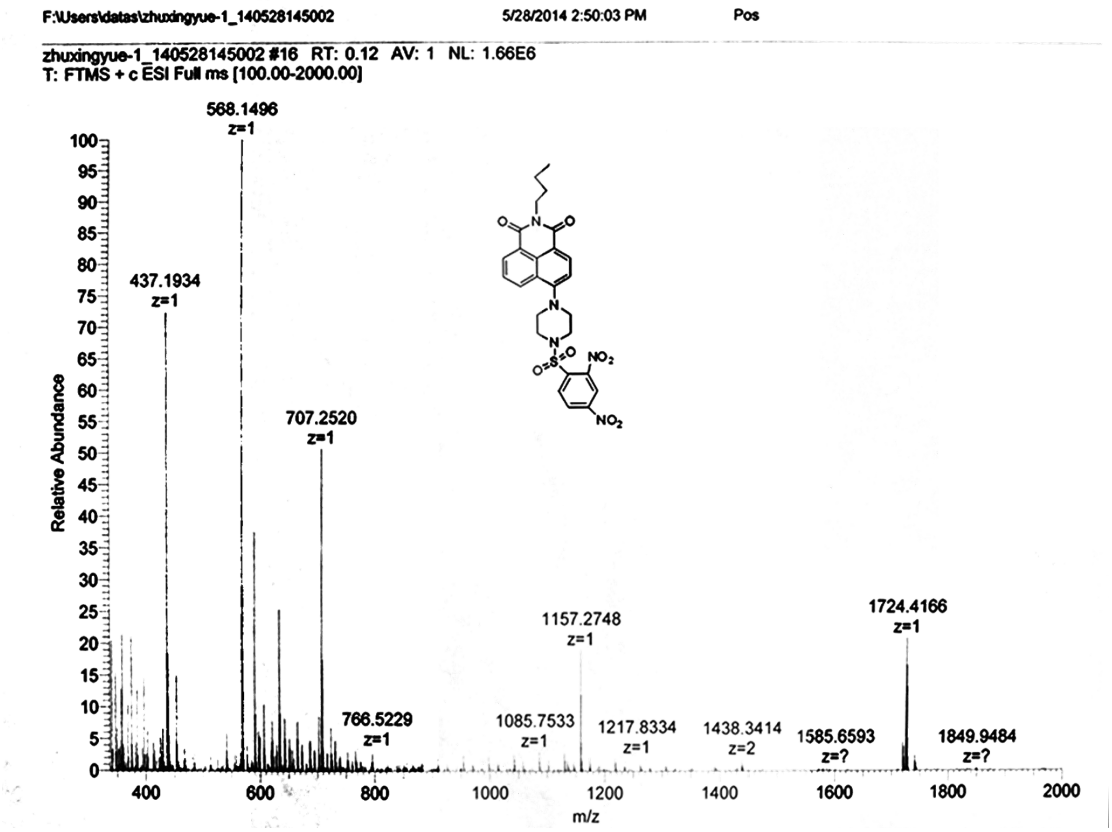


Figure S1. HRMS of probe **Z1**.

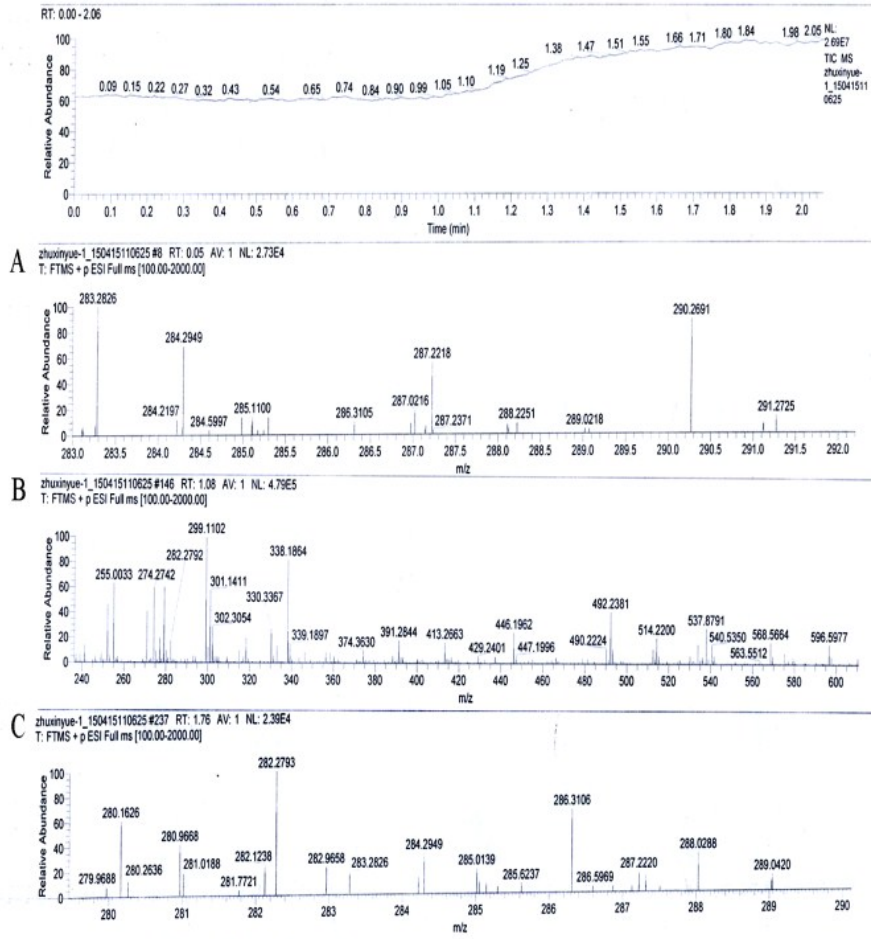


Figure S2. HRMS of probe **Z1** adding with excess of Cys.

2. Spectroscopic Measurement

UV-Vis absorption spectra

The UV-Vis absorption spectra of probe **Z1** toward Cys/Hcy/GSH were examined. as displayed in Figure S2, the UV-Vis absorption of **Z1** increased with the increasing concentration of three kinds of analytes at around 409 nm.

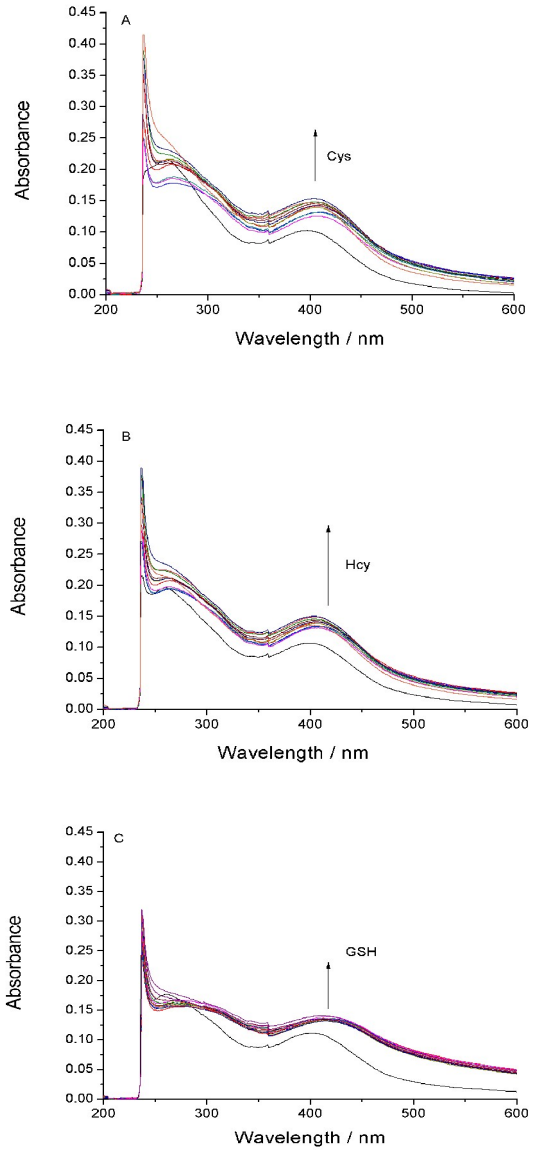


Figure S3. UV-Vis absorption spectra change of probe **Z1** (10 μM) toward Cys (A)/Hcy (B)/GSH (C) with different concentrations (2.0, 4.0, 6.0, 8.0, 10, 20, 40, 60, 80, 100, 150, 200 μM, bottom to up).(20 mM PBS, pH 7.4, 5% DMSO, λex = 409 nm)

Fluorescence spectra

The fluorescence change of probe **Z1** toward different reaction times.

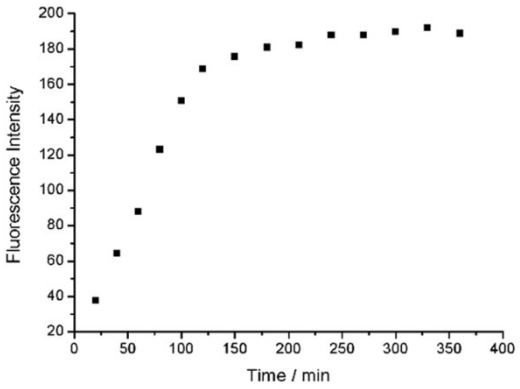


Figure S4. Fluorescence change of **Z1** toward different reaction times (probe **Z1**: 10μM, Cys: 100 μM).

The Fluorescence spectra of probe **Z1** toward Hcy/GSH were obtained using the method described in text. The fluorescence intensities increased with the increasing concentration of Hcy/GSH at around 530 nm.

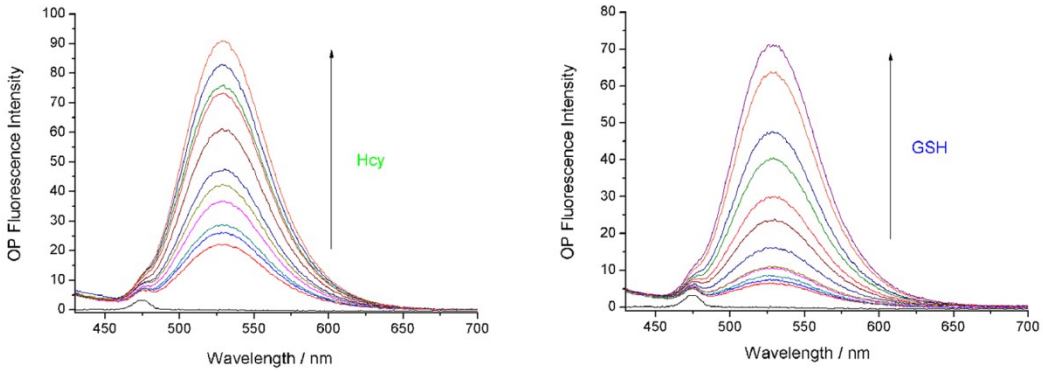


Figure S5. OP fluorescence spectra of probe **Z1** (10 μM) toward Hcy (A)/GSH (B) with different concentrations (2.0, 4.0, 6.0, 8.0, 10, 20, 40, 60, 80, 100, 150, 200μM, bottom to up). (20 mM PBS, pH 7.4, 5% DMSO, λex = 409 nm).

4. Measurement of Two-Photon TPA Value

The two-photon absorption cross section (δ) was determined by using femto second (fs) fluorescence measurement technique as described.[1] To measure the two-photon absorption cross section (δ) of the reaction product of probe **Z1** (5.0×10^{-6} M) and Cys (1.0×10^{-4} M), the reaction mixture dissolved in 20 mM PBS (5% DMSO, pH 7.4) was kept at 37°C for 2 h before the measurement was conducted (**NAP-P**, QY%: 11.11). The two-photon induced fluorescence intensity was measured at 700-880 nm by using Rhodamine 6G (1.3×10^{-6} M, QY%: 95, ethyl alcohol) as the reference, whose two-photon property has been well characterized in the literature.[2] The intensities of the two-photon induced fluorescence spectra of the reference and sample emitted at the same excitation wavelength were determined. The TPA cross section was calculated according to Equation S1.

$$\delta_s = \delta_r \frac{\phi_r}{\phi_s} \frac{C_r}{C_s} \frac{n_r}{n_s} \frac{S_s}{S_r} \qquad \text{(Equation S1)}$$

Where the subscript s and r standed for **Z1** and reference (Rhodamine 6G), respectively. δ was the TPA value, ϕ was the fluorescence quantum yield, *n* was the refractive index of the solvents, *C* was the concentration and *S* represented the intensity of TPE fluorescence emission.

References

1. M. A. Albota, C. Xu, W. W. Webb, Applied Optics, 1998, **37**, 7352-7356.

2. N. S. Makarov, M. Drobizhev, A. Rebane, Opt. Express, 2008, **16**, 4029-4047.

1. Characteristic of Z1

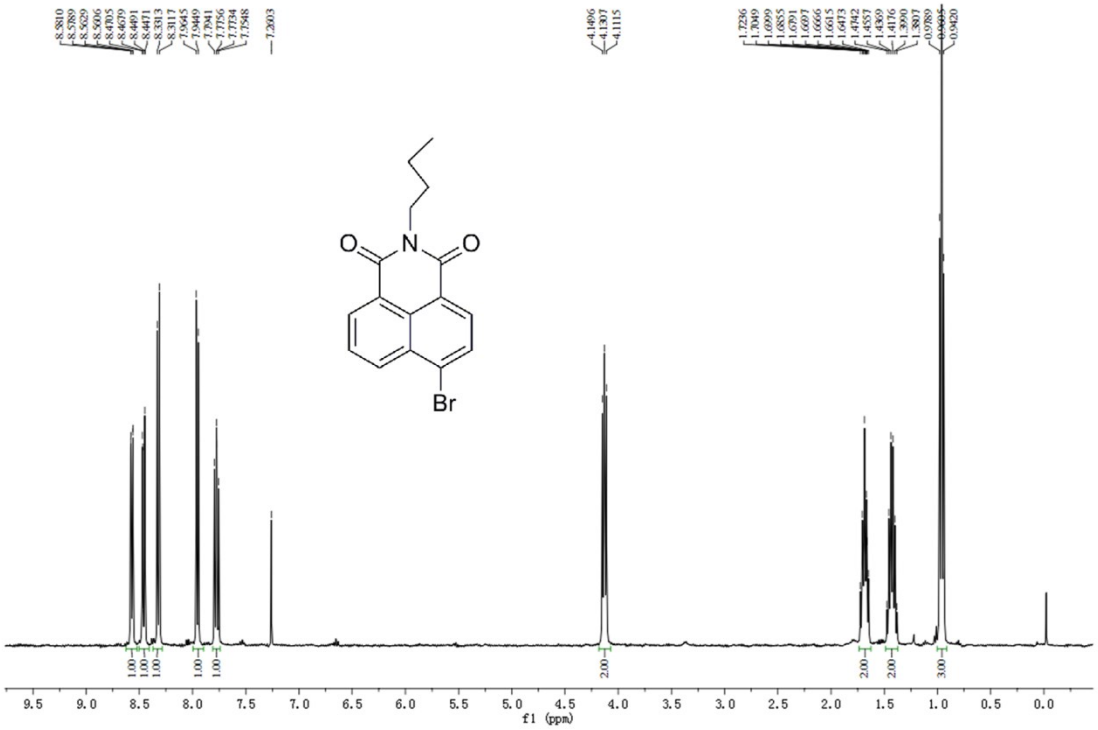


Figure S6. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

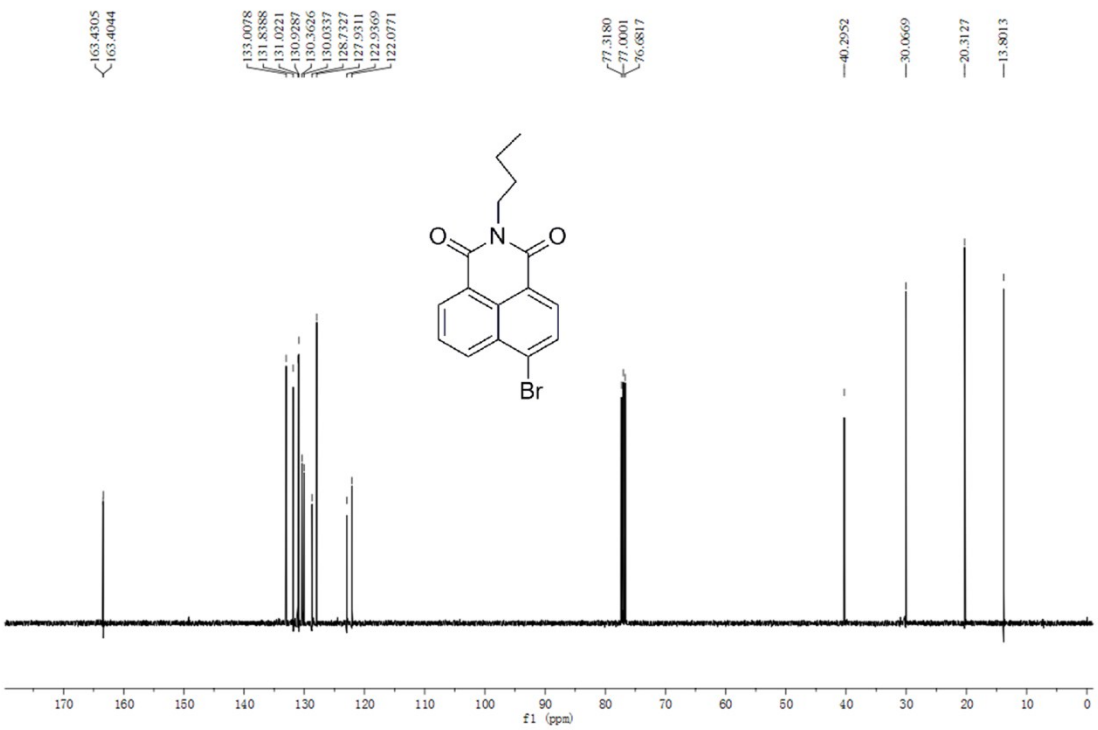


Figure S7. ^{13}C NMR spectrum of compound **1** in $CDCl_3$.

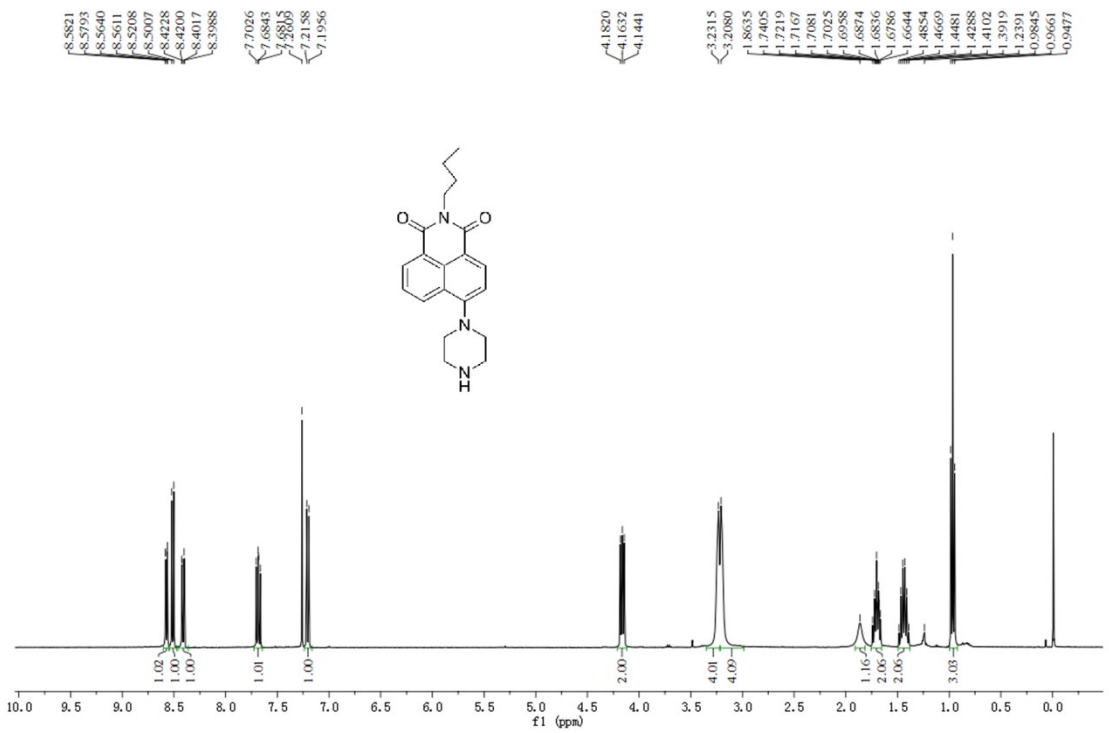


Figure S8. ^{1}H NMR spectrum of **NAP-P** in $CDCl_3$.

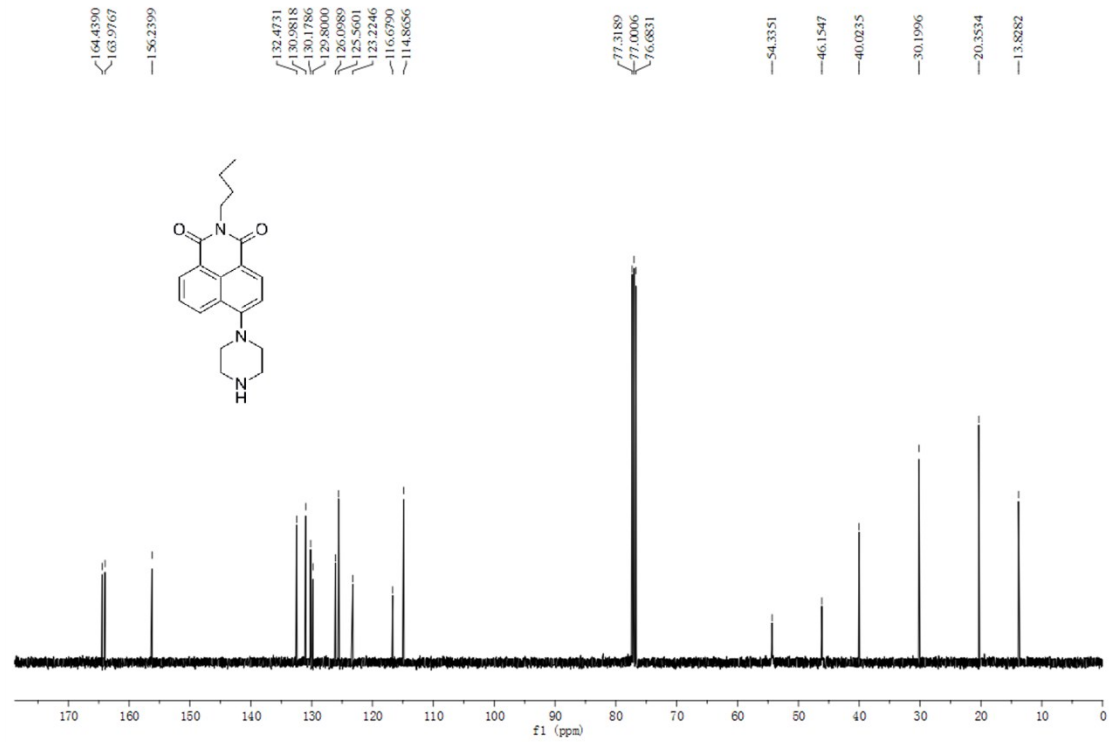


Figure S9. ^{13}C NMR spectrum of **NAP-P** in $CDCl_3$.

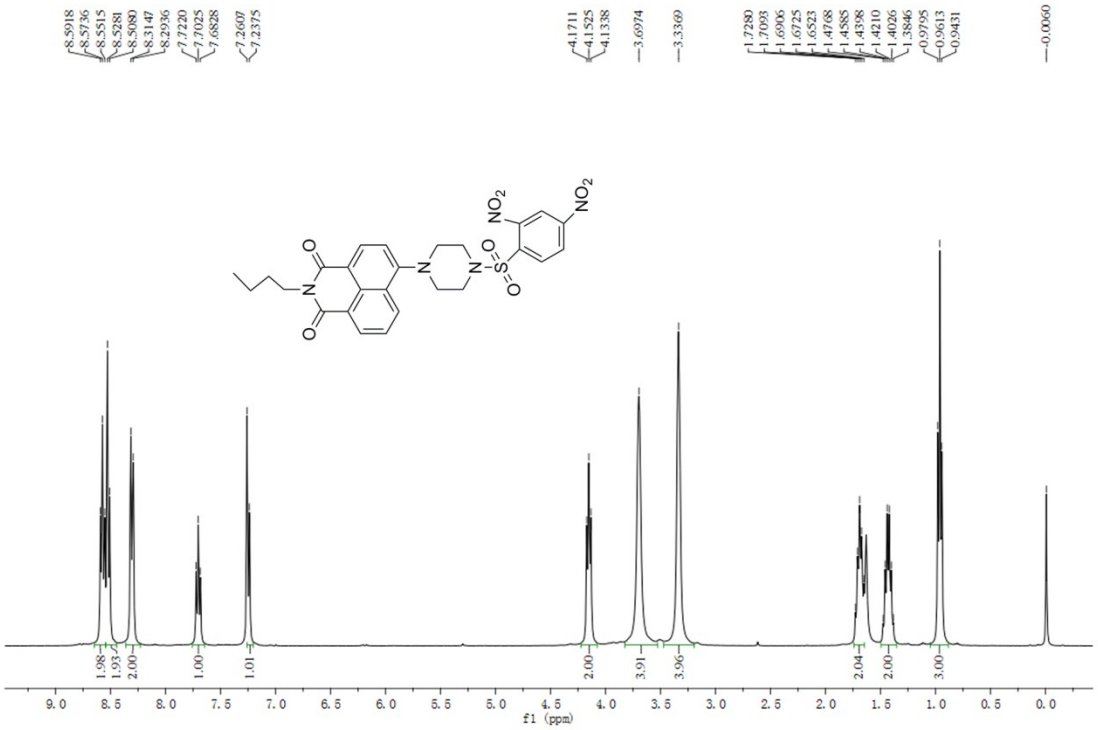


Figure S10. ^{1}H NMR spectrum of probe **Z1** in $CDCl_3$.

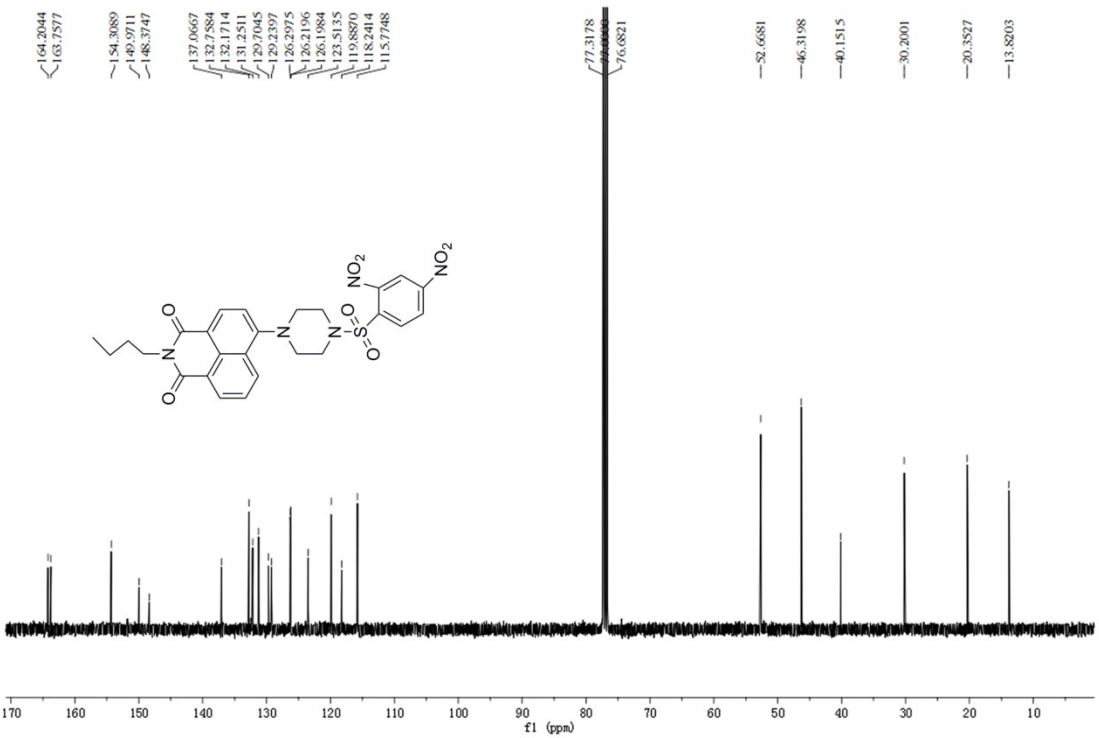


Figure S11. ^{13}C NMR spectrum of probe **Z1** in $CDCl_3$.