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Supporting information for

Development of green to near-infrared turn-on fluorescent probes for multicolour imaging of nitroxyl in living system

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General procedure for the spectral measurement

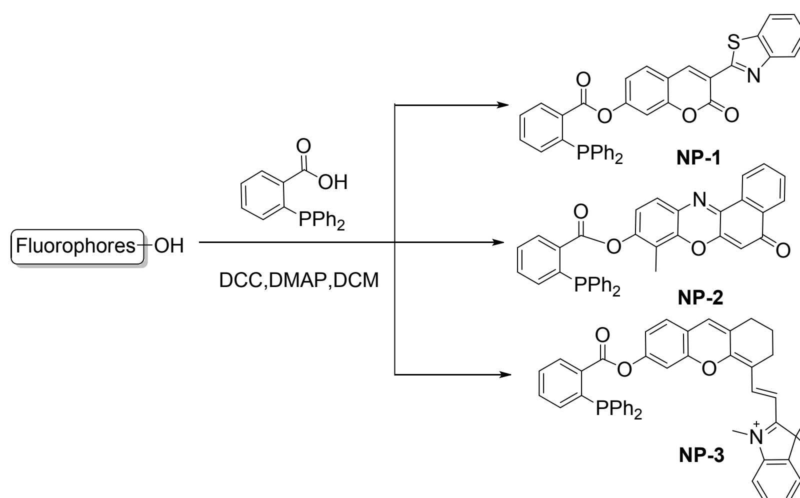
The stock solution of the probe **NP-1** was prepared at 0.5 mM in EtOH. The solutions of various testing species were prepared from, Na_2S, $FeCl_3$, NaN_3, $NaNO_2$, $NaNO_3$, ascorbic acid, GSH, cysteine, H_2O_2, NaClO in the twice-distilled water, and Superoxide ($O_2^{\cdot -}$) and CORM-2 (CO) in the DMSO. AS (HNO) and DEA/NONOate (NO) in 0.01 M NaOH. The test solution of the probe **NP-1** (5.0 μM) in 3 mL 25 mM PBS buffer (pH 7.4) with 10% ethanol was prepared by placing 0.03 mL of the probe **NP-1** stock solution and 0.3 mL ethanol in 2.7 mL of the aqueous buffer. The probe **NP-2** (10 μM) in 3 mL 25 mM PBS buffer (pH 7.4) with 30% ethanol was prepared by placing 0.03 mL of the probe **NP-1** stock solution and 0.9 mL ethanol in 2.1 mL of the aqueous buffer. The probes **NP-3** (5.0 μM) in 3 mL 25 mM PBS buffer (pH 7.4) with 30% ethanol was prepared by placing 0.03 mL of the probe **NP-3** stock solution and 0.9 mL ethanol in 2.1 mL of the aqueous buffer. The resulting solution was shaken well and incubated with appropriate testing species for 45 min at ambient temperature before recording the spectra. Unless otherwise noted, for all measurements, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

Determination of the fluorescence quantum yields

Fluorescence quantum yields for **NP-1~3** were determined by using Rhodamine 6G (Φ_f = 0.95 in H_2O) or ICG (Φ_f = 0.13 in DMSO) as a fluorescence standard.[1] The quantum yield was calculated using the following equation:

$$\Phi_{F(X)} = \Phi_{F(S)} \, (A_S F_X / A_X F_S) \, (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and the unknown, respectively.



Scheme S1. Synthetic routes to the probes **NP-1~3**

Table S1. Fluorescent properties of probes **NP1-3**.

probes	$\lambda_{ex/}$ (nm)	$\lambda_{em/}$ (nm)	Φ_f	Solvent
NP-1	450	490	0.015	PBS: EtOH = 9:1
NP-2	570	635	0.009	PBS: EtOH = 7:3
NP-3	670	710	0.011	PBS: EtOH = 7:3

Detection limit: The detection limit was determined from the fluorescence titration data based on a reported method.[2] According to the result of titration experiment, the fluorescent intensity data at 490 nm (635 nm, 710 nm) were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescent intensity data and the point at which this line crossed the axis was considered as the detection limit (**NP-1**: 6.48×10^{-7} M; **NP-2**: 6.08×10^{-7} M; **NP-3**: 6.22×10^{-7} M.).

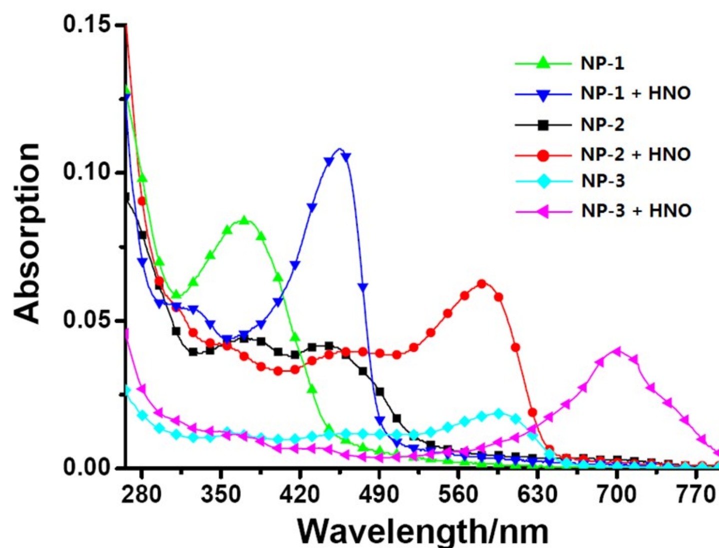


Fig. S1 Absorption spectra of **NP-1~3** (5.0 μM) in pH 7.4 PBS buffer (10% ethanol for **NP-1**, 30% ethanol for **NP-2~3**) in the absence or presence of AS (100 μM). The spectra were recorded after incubation of the probe with AS for 45 min.

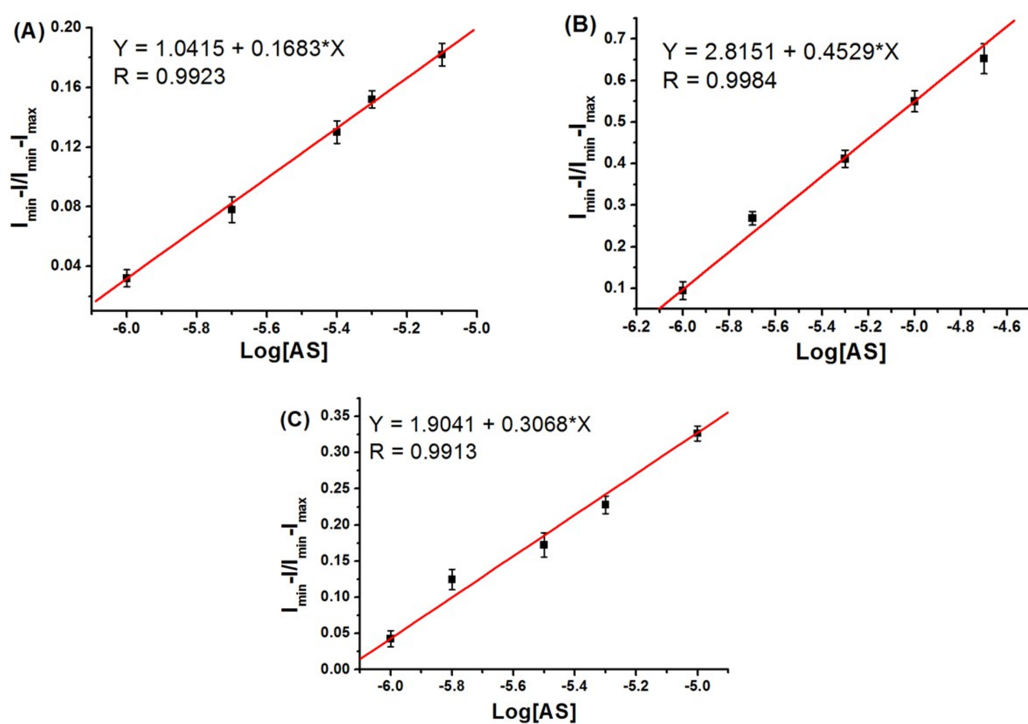


Fig. S2. Normalized response of fluorescence signal by changing the concentrations of AS.

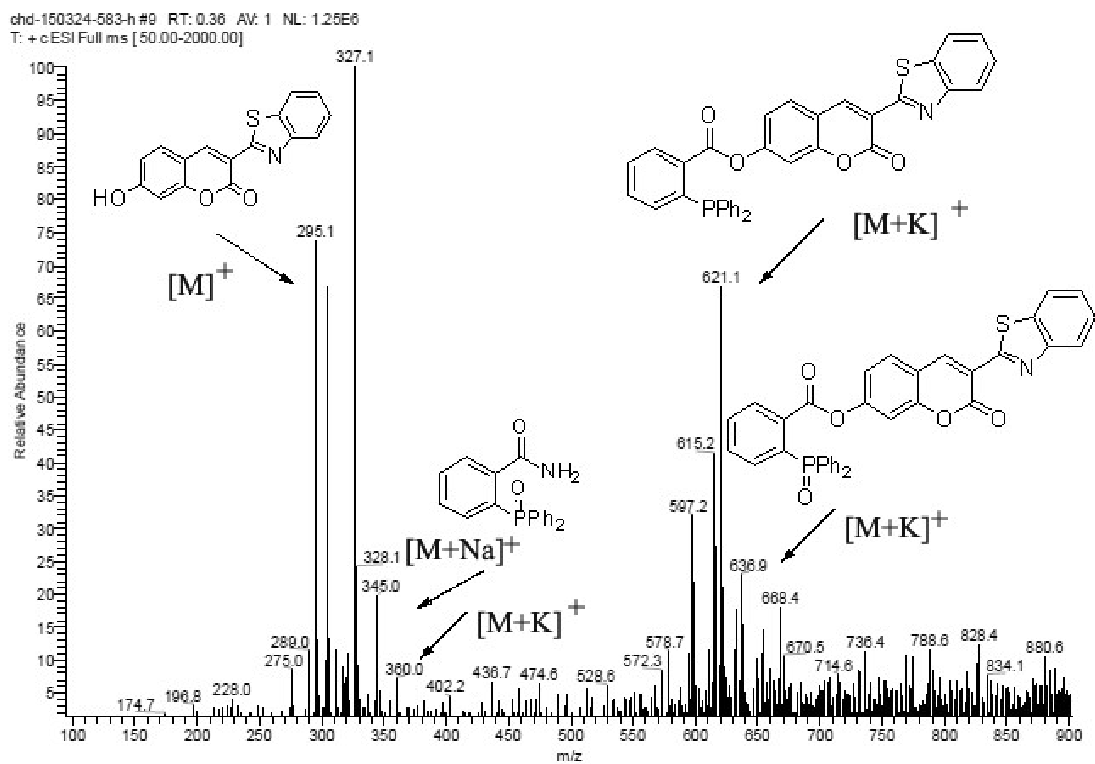
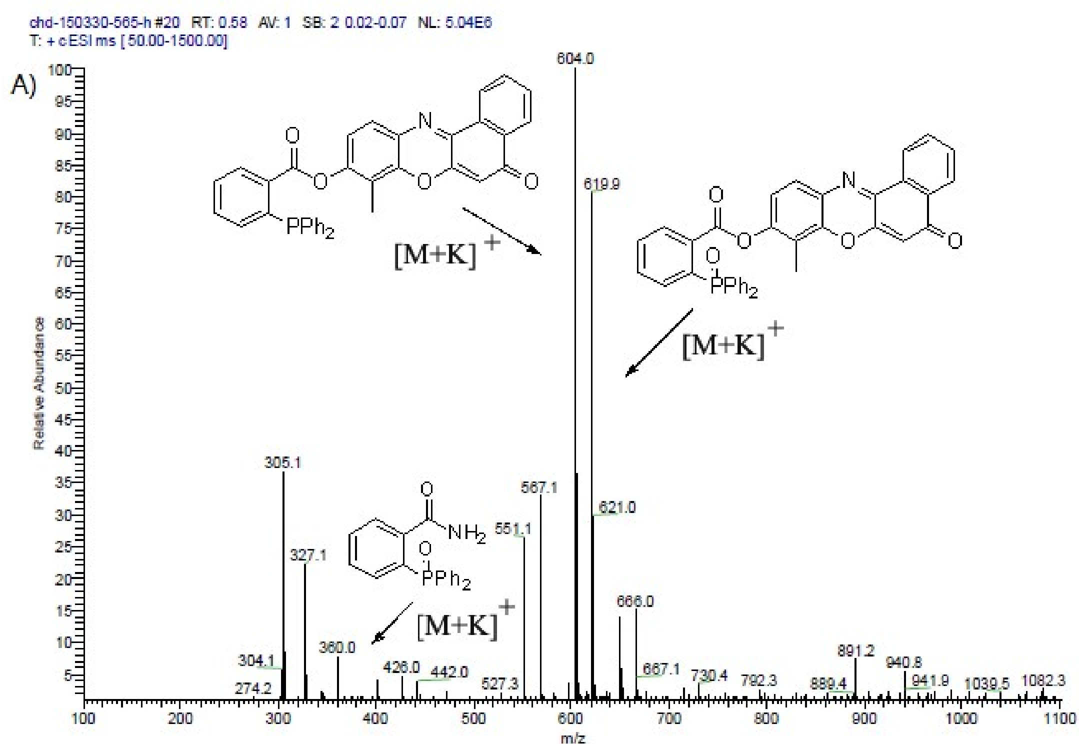


Fig. S3. ESI-MS spectrum of **NP-1** (1 mL **NP-1** at 250 μM, EtOH/PBS=1:1) with AS (0.5 mL AS at 2 mM, 0.01 M NaOH).



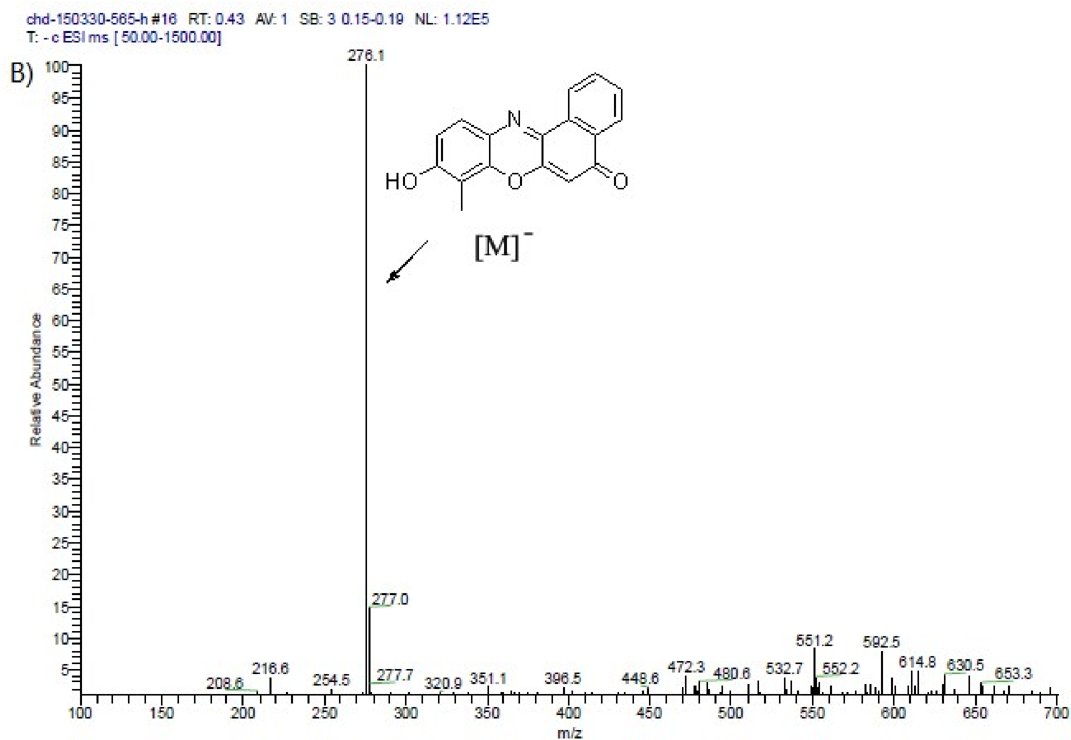
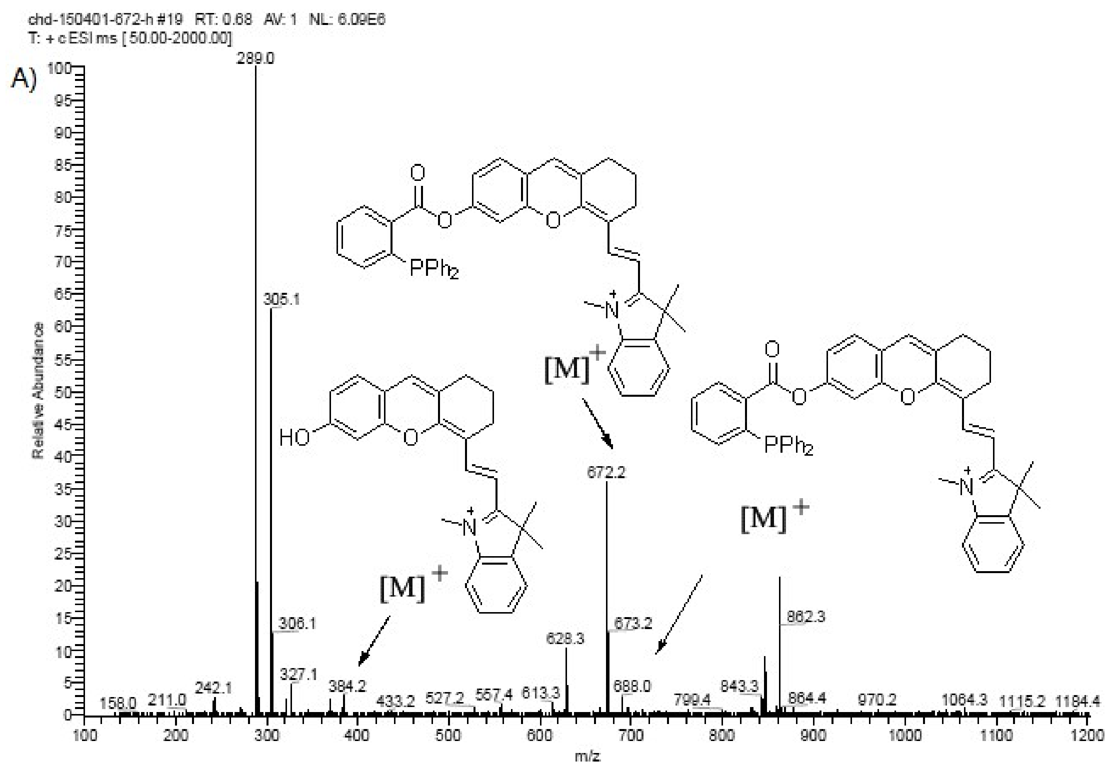


Fig. S4. ESI-MS spectrum of **NP-2** (1 mL **NP-2** at 250 μM, EtOH/PBS=1:1) with AS (0.5 mL AS at 2 mM, 0.01 M NaOH). A (the positive mode of mass spectrometry), B (the negative mode of mass spectrometry).



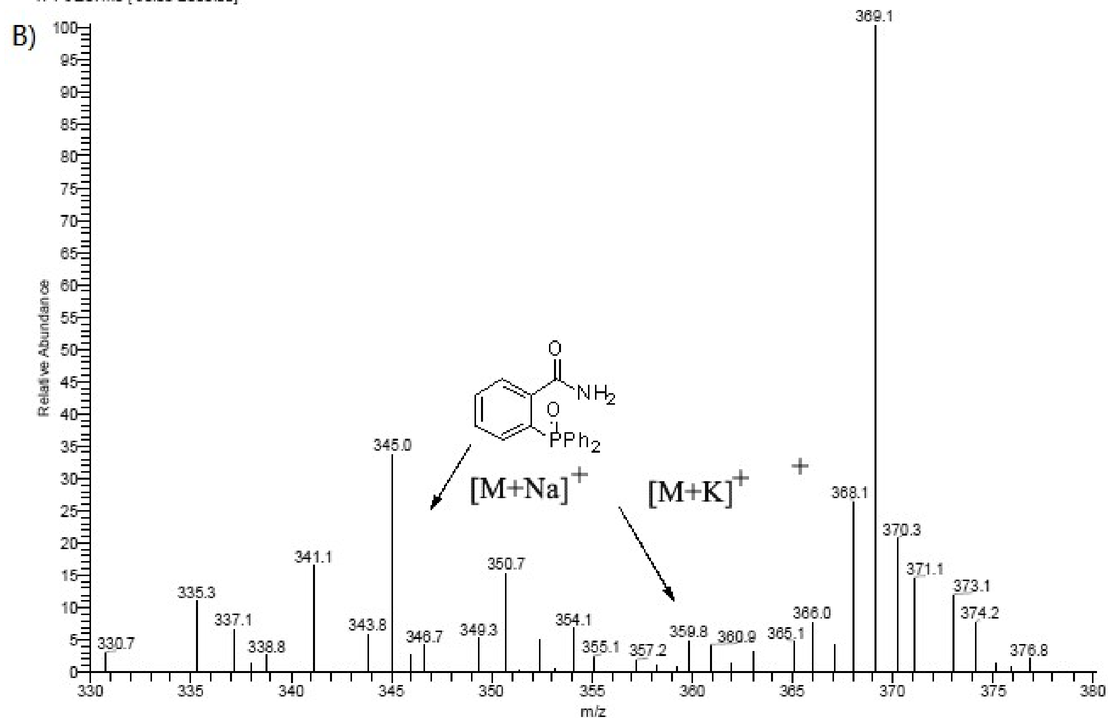


Fig. S5. ESI-MS spectrum of **NP-3** (1 mL **NP-3** at 250 μM, EtOH/PBS=1:1) with AS (0.5 mL AS at 2 mM, 0.01 M NaOH). B (the magnification spectrum of M/Z from 330 to 380).

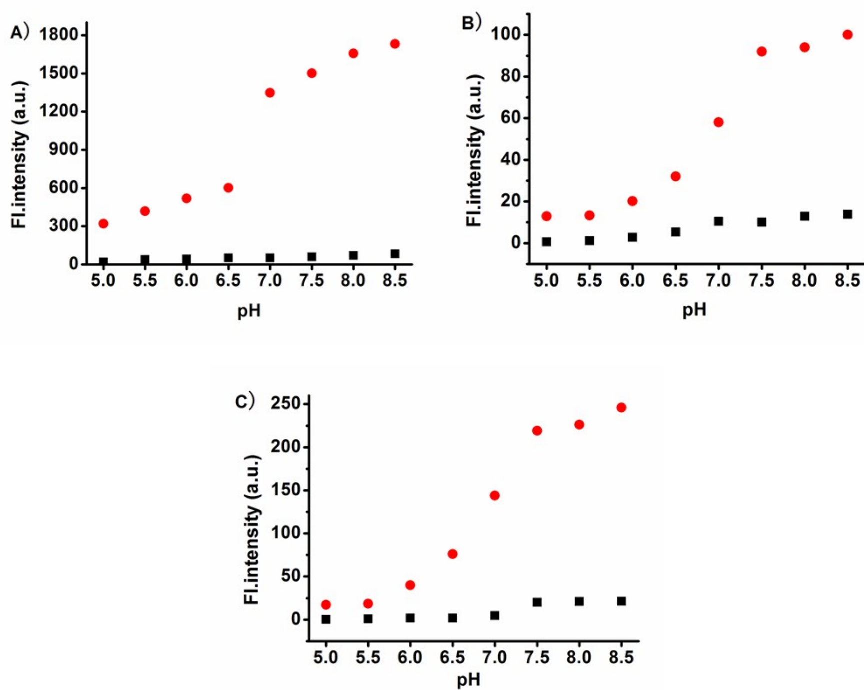


Fig. S6. Fluorescence intensity changes of the probes **NP-1~3** at different pH values in the absence (■) or presence (●) of AS.

Cytotoxicity assays:

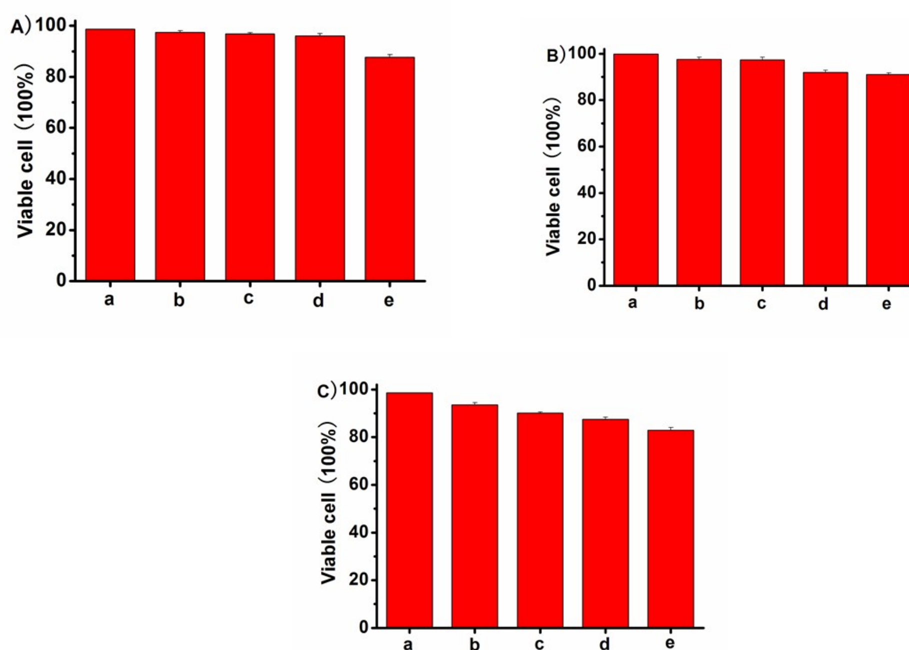


Fig. S7. Cytotoxicity assay of the probes **NP-1~3** at different concentrations (a: 0 μM; b: 5 μM; c: 10 μM; d: 20 μM; e: 30 μM) for A549 cells.

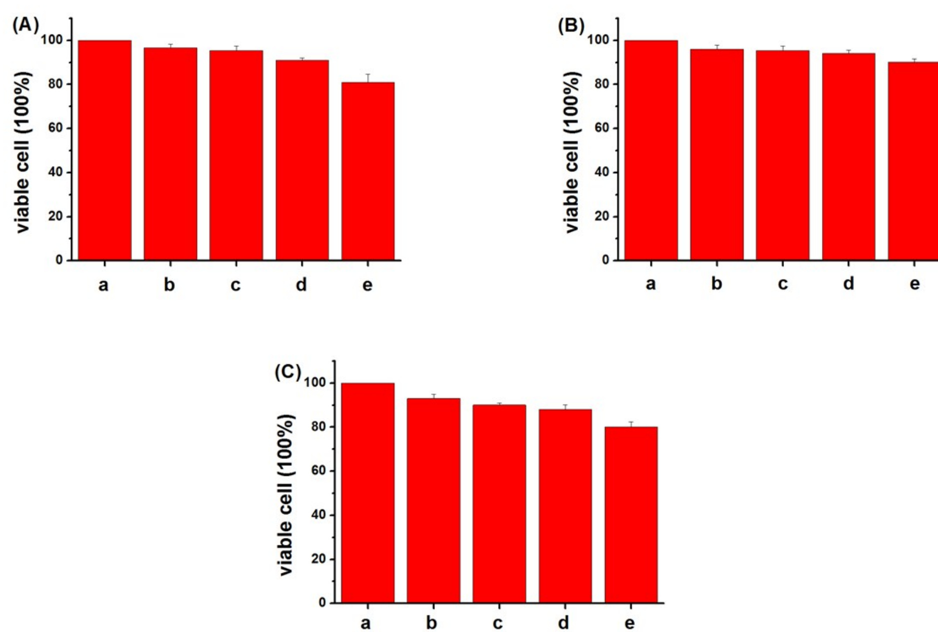


Fig. S8. Cytotoxicity assay of the probes **NP-1~3** at different concentrations (a: 0 μM; b: 5 μM; c: 10 μM; d: 20 μM; e: 30 μM) for HeLa cells.

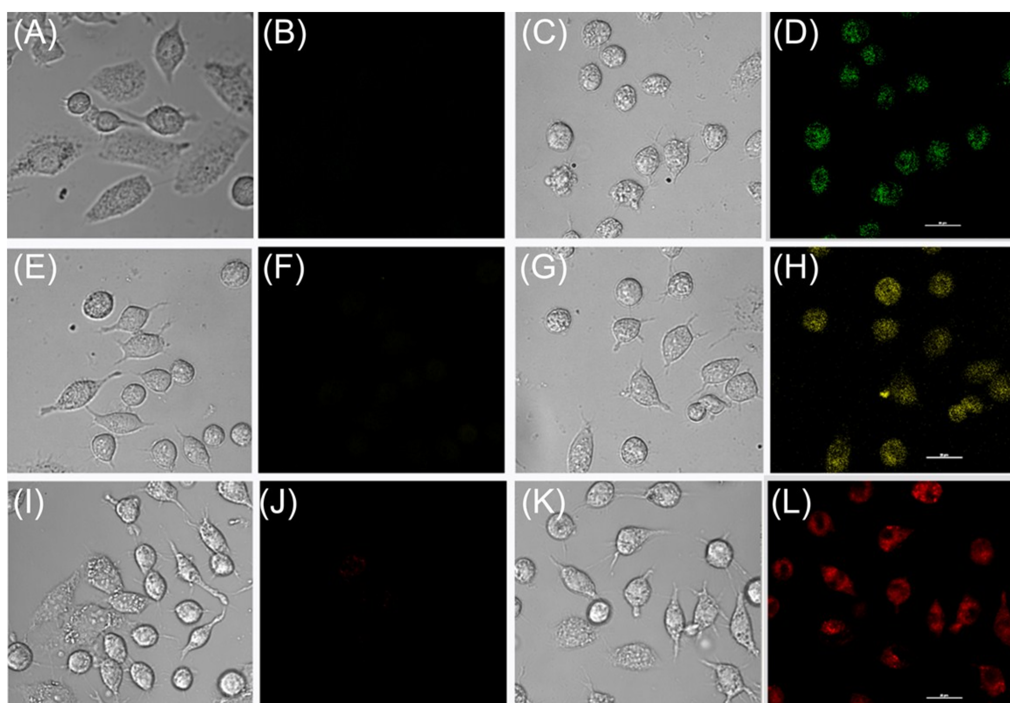


Fig. S9 Confocal fluorescence images of A549 cells using Nikon A1R confocal microscope: (a) Bright-field image of live A549 cells incubated with only **NP-1** (5.0 μM) for 20 min; (b) Fluorescence image of (a); (c) Bright-field image of live HeLa cells incubated with **NP-1** (5.0 μM) for 20 min, then with AS (75 μM) for 45 min; (d) Fluorescence image of (c). Excitation at 488 nm, emission window of 500-550 nm; (e) Bright-field image of live A549 cells incubated with only **NP-2** (10 μM) for 20 min; (f) Fluorescence image of (e); (g) Bright-field image of live A549 cells incubated with **NP-2** (10 μM) for 20 min, then with AS (100 μM) for 45 min; (h) Fluorescence image of (g). Excitation at 561 nm, emission window of 570-620 nm. (i) Bright-field image of live A549 cells incubated with only **NP-3** (5.0 μM) for 20 min, (j) Fluorescence image of (i); (k) Bright-field image of live A549 cells incubated with **NP-3** (5.0 μM) for 20 min, then with AS (75 μM) for 45 min; (k) Fluorescence image of (l). Excitation at 647 nm, emission window of 663-738 nm. Scale bar = 20 μm.

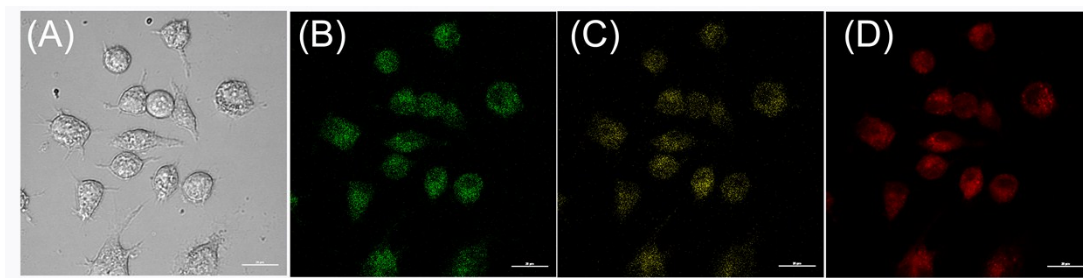


Fig. S10. Confocal fluorescence images of living A549 cells using Nikon A1R confocal microscope: (a) Bright-field image of live A549 cells incubated with **NP-1**(5.0 μM), **NP-2** (10 μM) and **NP-3** (5.0 μM) for 20 min, then with AS (200 μM) for 45 min; (b) Fluorescence image of (a), excitation at 488 nm, emission window of 500-550 nm; (c) Fluorescence image of (a), excitation at 561 nm, emission window of 570-620 nm.; (d) Fluorescence image of (a), excitation at 647 nm, emission window of 663-738 nm.. Scale bar = 20 μm.

References

1. R. C. Benson, H. A. Kues.. *J. Chem. Eng. Data.* **1977**, *22*, 379-383.
2. (a) M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, Anal. Chem., 1996, 68, 1414; (b) A. Caballero, R. Martınez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tarraga, P. Molina, J. Veciana, J. Am. Chem. Soc., 2005, 127, 15666. (c) W. Lin, L. Yuan, Z. Cao, Y. Feng, L. Long, Chem. Eur. J., 2009, 15, 5096.

Fig. S11. ^{1}H NMR spectrum of the probe **NP-1**.

Fig. S12. ^{13}C NMR spectrum of the probe **NP-1.**

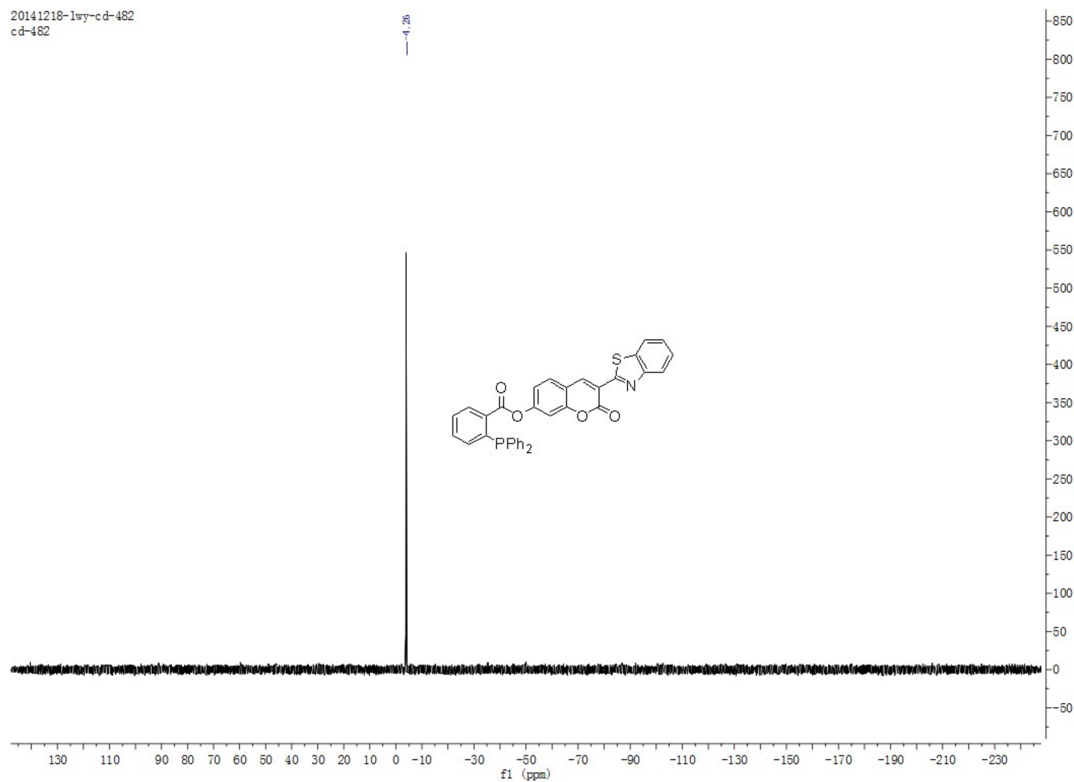


Fig. S13. ^{31}P NMR spectrum of the probe **NP-1.**

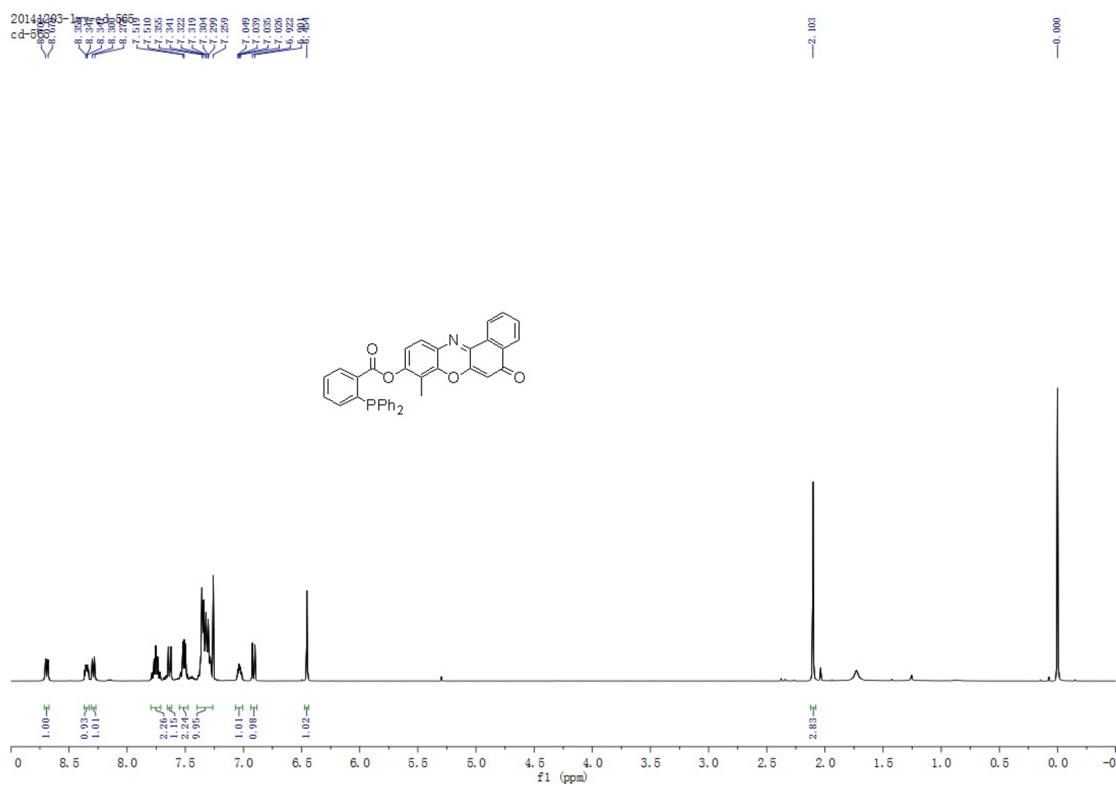


Fig. S14. ^{1}H NMR spectrum of the probe **NP-2**.

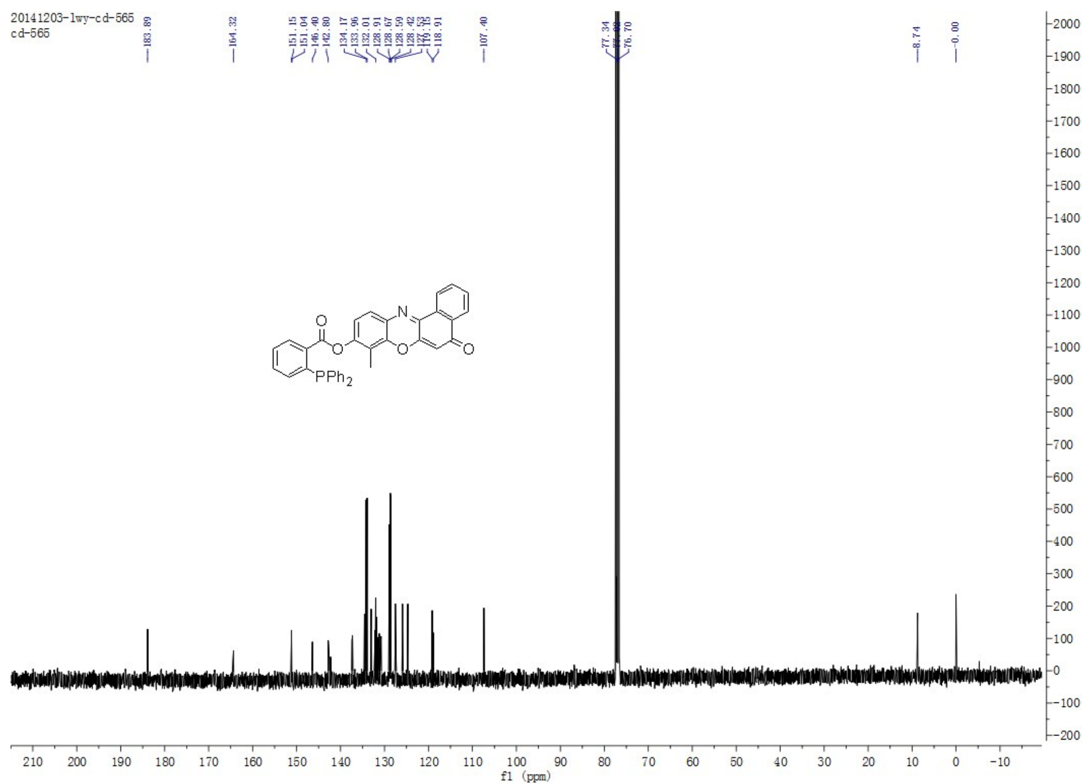


Fig. S15. ^{13}C NMR spectrum of the probe **NP-2.**

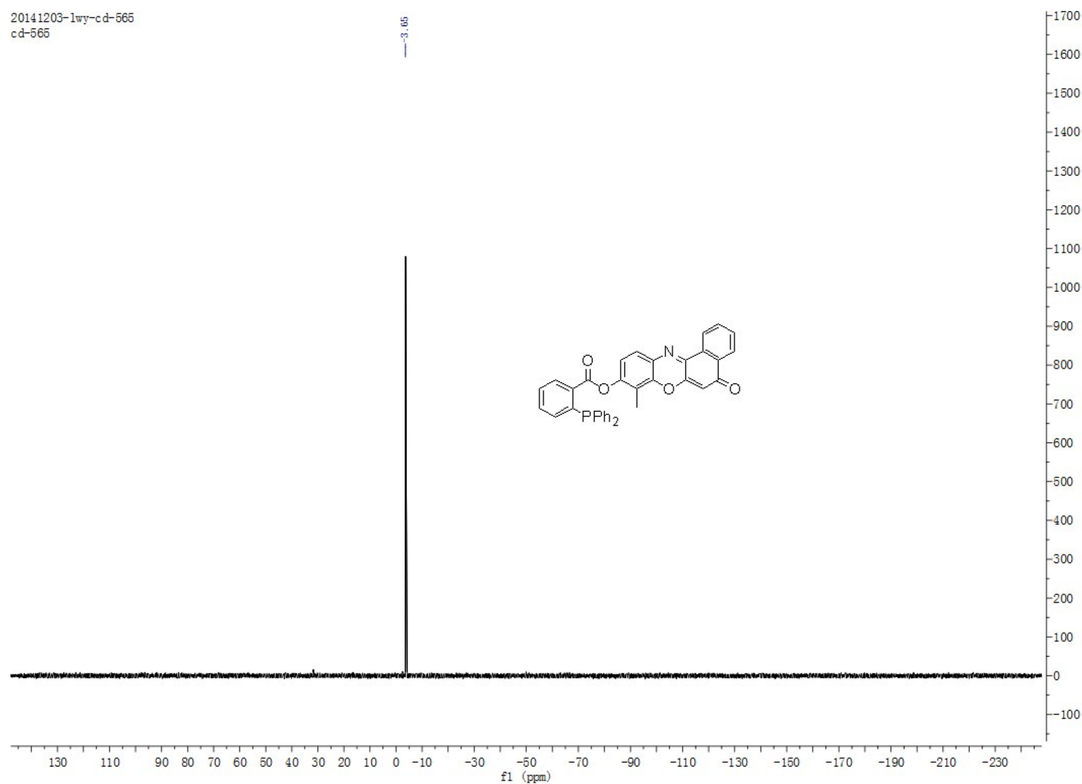


Fig. S16. ^{31}P NMR spectrum of the probe **NP-2.**

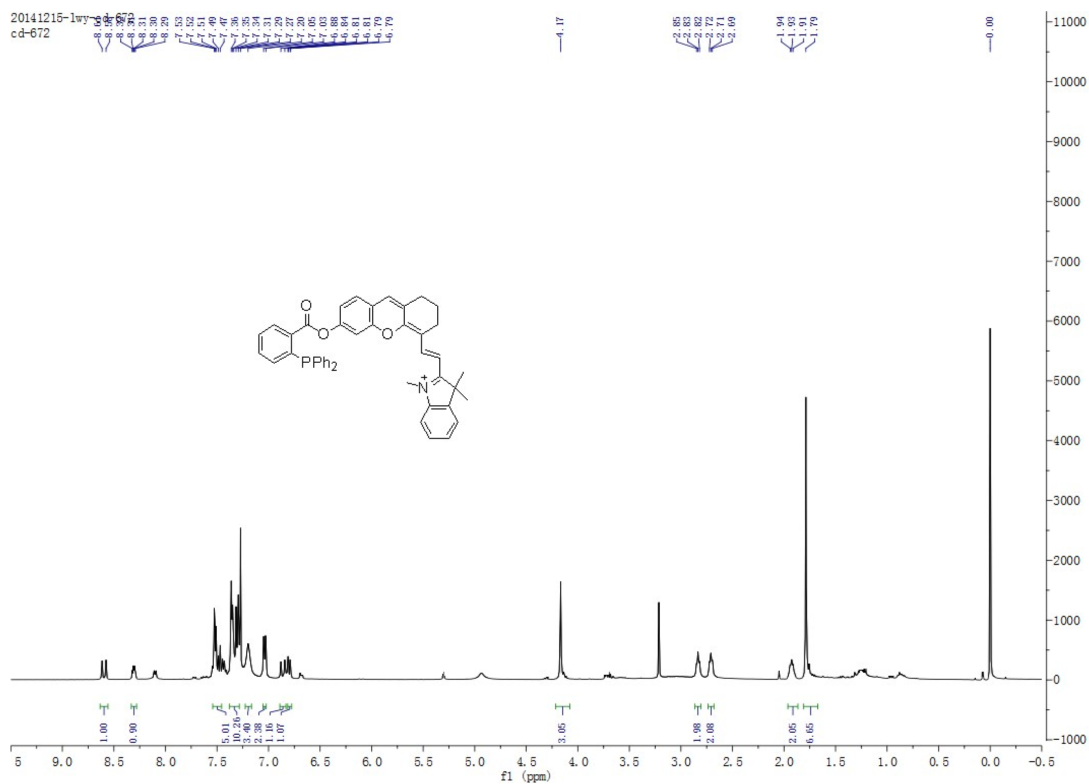


Fig. S17. ^{1}H NMR spectrum of the probe **NP-3**.

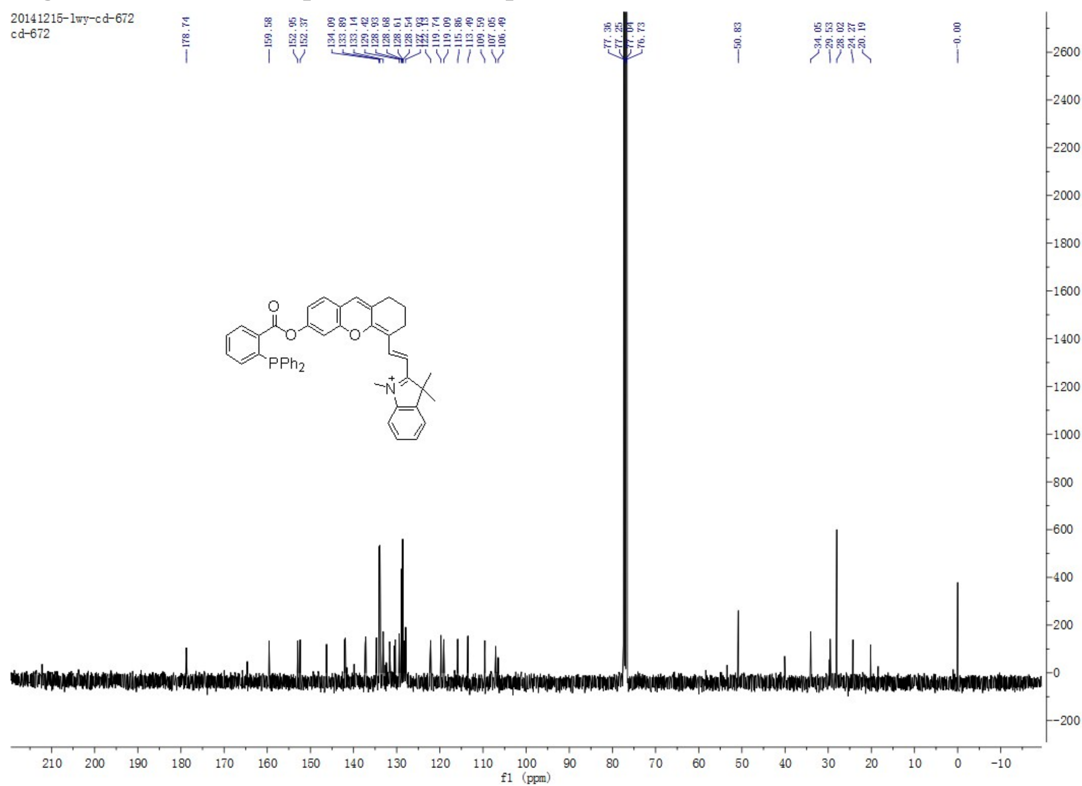


Fig. S18. ^{13}C NMR spectrum of the probe **NP-3.**

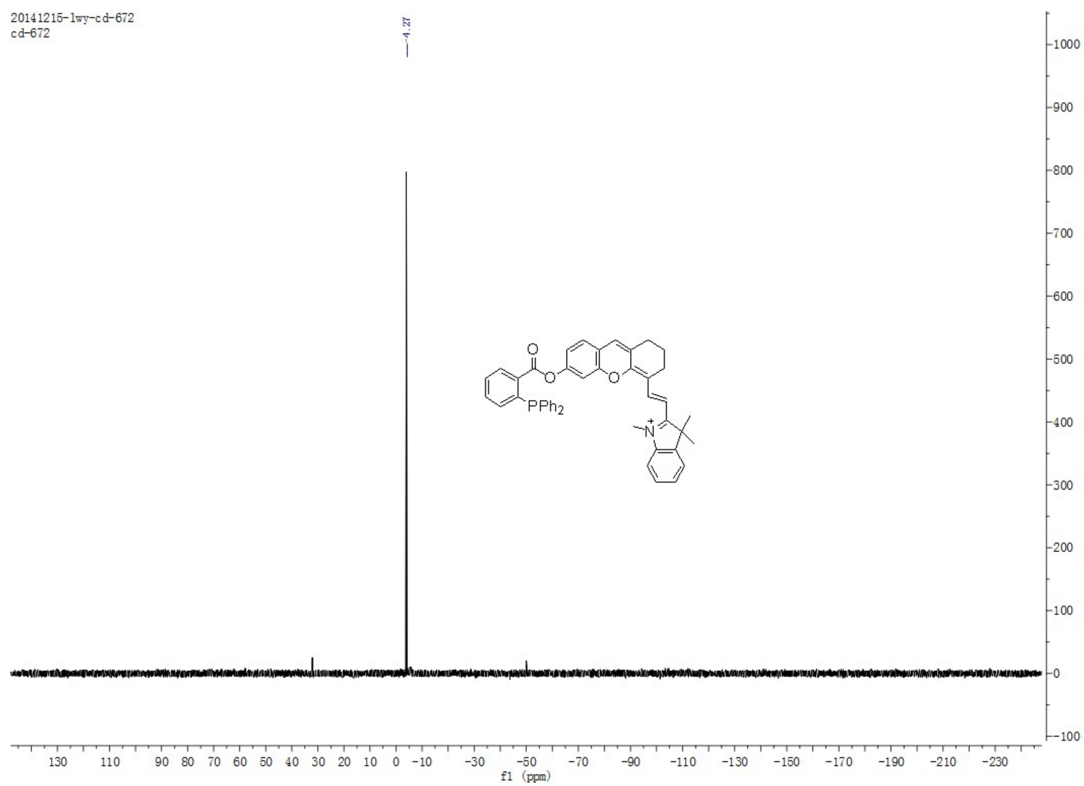


Fig. S19. ³¹P NMR spectrum of the probe **NP-3.**