Supporting Information for

A fast-response, highly sensitive and selective fluorescent probe for the ratiometric imaging of nitroxyl in living cells

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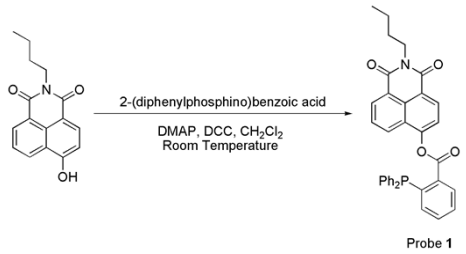
Contents

1. Materials and general methods

All of analytical reagents in this paper were obtained from commercial suppliers and used without further purification. Ultrapure water was prepared through Sartorious Arium 611DI system and used throughout the experiment. Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$), using tetramethylsilane (TMS) as the internal standard. High-resolution mass data was measured with fourier transform ion cyclotron resonance mass spectrometer (APEX IV). Absorption spectra were measured with UV-3101PC spectrophotometer. Fluorescence emission spectra were carried out on Perkin-Elmer Model LS-55 with excitation wavelength of 375 nm. All the fluorescence spectra were uncorrected. All pH measurements were made with a Sartorius basic pH-meter PB-10.

2. Synthesis of probe 1



Probe **1**

A mixture of *N*-butyl-4-hydroxy-1,8-naphthalimide (269 mg, 1 mmol), 2-(diphenylphosphino)benzoic acid (367 mg, 1.2 mmol), 4-dimethylaminopyridine (DMAP, 83 mg, 0.68 mmol) and dicyclohexylcarbodiimide (DCC, 265.8 mg, 1.288 mmol) in CH_2Cl_2 (20 mL) was stirred at 45 °C for 4 hours. After cooled to room temperature, the reaction mixture was purified by silica column chromatography (CH_2Cl_2 as eluent) in order to get pure probe **1** (352 mg, yield 63%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (*10^{-6}): 0.975(t, *J* = 7.6 Hz, 3H), 1.413-1.470(m, 2H), 1.666-1.742(m,

2H), 4.171(t, *J* = 7.6 Hz, 2H), 7.053-7.084(m, 1H), 7.296-7.380(m, 11H), 7.551(t, *J* = 7.6 Hz, 2H), 7.644(t, *J* = 8.0 Hz, 1H), 8.035(d, *J* = 8.4 Hz, 1H), 8.416-8.439(m, 1H), 8.540(d, *J* = 8.0 Hz, 1H), 8.586(d, *J* = 7.2 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ (*10^{-6}): 13.98, 20.50, 30.32, 40.39, 119.63, 120.55, 122.94, 127.24, 128.07, 128.65, 128.78, 128.85, 129.13, 129.40, 131.66, 131.82, 132.13, 132.31, 133.38, 134.10, 134.31, 134.79, 137.24, 137.35, 142.47, 142.76, 151.59, 163.65, 164.21, 164.57. HRMS (ESI positive) calcd for $C_{35}H_{29}NO_4P$ $[M+H]^+$ 558.18287, found 558.18271.

3. General procedure for analysis

Stock solution of probe **1** (1 mM) were prepared in ethanol. Test solutions were prepared by placing 25 μL of the probe stock solutions into a test tube, adding an appropriate aliquot of each stock solution, and then diluting the solution to 10 mL with the mixture of ethanol and ultrapure water (3:7, v/v) containing PBS (5 mM, pH 7.4). Stock solution of AS (10 mM) was prepared in PBS (60 mM, pH 7.4). Superoxide (O_2^-) was added as solid KO_2. Hydrogen peroxide and *tert*-butylhydroperoxide (TBHP) were delivered from 30% and 70% aqueous solutions, respectively. Hydroxyl radical ($^{\cdot}OH$) and *tert*-butoxy radical ($^{\cdot}O^tBu$) were generated by reaction of 1 mM Fe^{2+} with 1 mM H_2O_2 or 1 mM TBHP, respectively. Stock solution of other analytes (10 mM) was prepared in ultrapure water. All the spectra were obtained in a quartz cuvette (path length = 1 cm). All measurements were made at room temperature (25 °C).

4. Absorption responses of 1 toward HNO

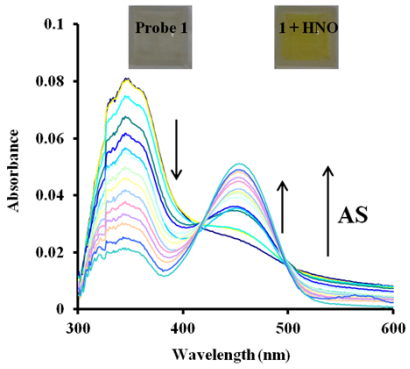


Fig. S1. Absorption spectra of **1** (5 μM) in the presence of different concentrations of AS (from 0 to 50 μM) in PBS (5 mM, pH 7.4) solution (ethanol/water = 3:7, v/v).

5. Investigations of kinetic profiles of the reaction of probe 1 and HNO

The calculate method of *pseudo*-first-order and second-order rate constant referred to the previous report (Chem. Commun., 2011, **47**, 6275-6277).

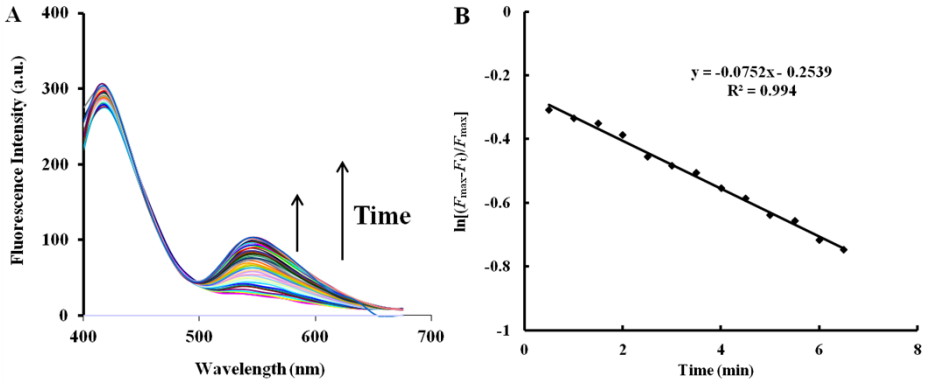


Fig. S2. (A) The fluorescence spectra of probe **1** (5 μM) incubated with AS (40 μM) for 0-30 min; (B) *Pseudo* first-order kinetic plot of the reaction of probe **1** (5 μM) with AS (40 μM). Slope = 0.0752 min^{-1}.

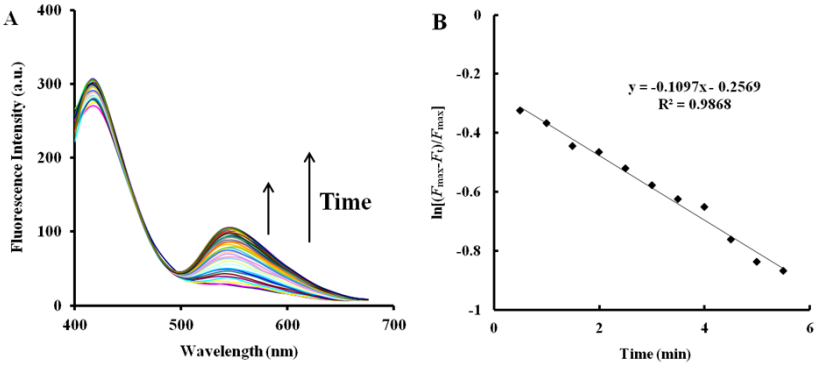


Fig. S3. (A) The fluorescence spectra of probe **1** (5 μM) incubated with AS (50 μM) for 0-30 min; (B) *Pseudo* first-order kinetic plot of the reaction of probe **1** (5 μM) with AS (50 μM). Slope = 0.1097 min^{-1}.

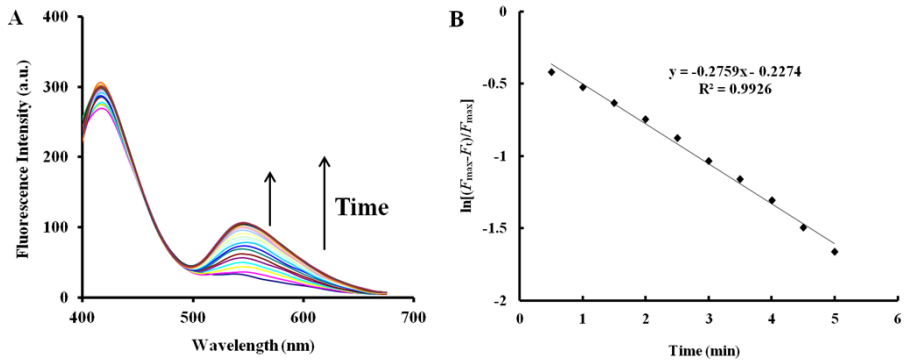


Fig. S4. (A) The fluorescence spectra of probe **1** (5 μM) incubated with AS (100 μM) for 0-30 min; (B) *Pseudo* first-order kinetic plot of the reaction of probe **1** (5 μM) with AS (100 μM). Slope = 0.2759 min^{-1}.

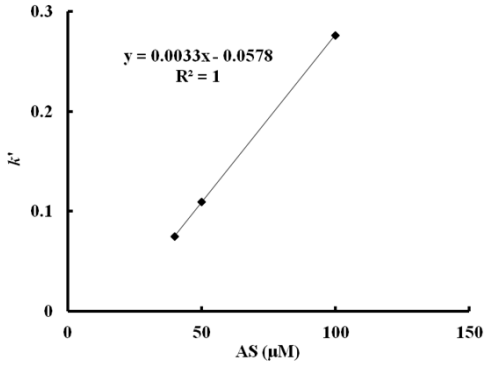


Fig. S5. Plot of the apparent rate constant *k'* versus the concentrations of AS. Slope = 3.3 × 10-3 μM-1 min-1. Thus, second-order rate constant, k' = 3.3 × 10-3 μM-1 min-1.

6. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. In the absence of AS, the fluorescence emission spectrum of probe **1** was measured by five times and the standard deviation of blank measurement was achieved. To gain the slope, the ratio of the fluorescence intensity at 546 nm to the fluorescence intensity at 418 nm (F_{546}/F_{418}) was plotted as a concentration of AS. So the detection limit was calculated with the following equation:

Detection limit = 3σ/k

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity ratio versus AS concentration.

7. The effects of interference of various analytes on monitoring HNO

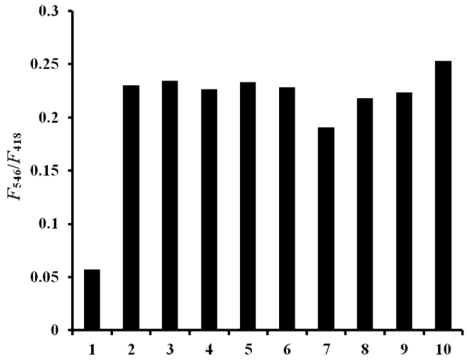


Fig. S6. Fluorescence responses of probe **1** (5 μM) toward various analytes (50 μM) in the presence of AS (50 μM) under PBS (5 mM, pH 7.4) solution (ethanol/water = 3:7, v/v). 1, only probe **1**; 2, NO_3^-; 3, NO_2^-; 4, O_2^-; 5, ascorbate; 6, TBHP; 7, H_2O_2; 8, $^{\cdot}O^tBu$; 9, $^{\cdot}OH$; 10, AS. All data represent the fluorescence intensity ratio F_{546}/F_{418}. Each spectrum was obtained after various analytes addition at 25 °C for 20 min.

8. Photostability study

The photostability of probe **1** in the above-mentioned analytical conditions was measured by continuous irradiation with a Xe lamp at 15 nm slit width at excitation wavelength 375 nm.

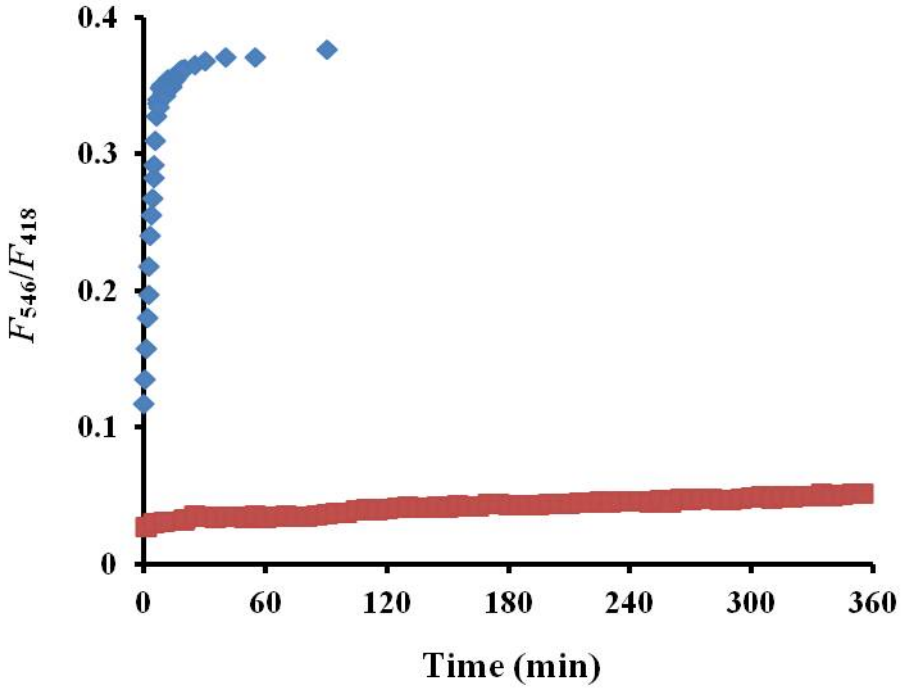


Fig. S7. The photostability study of probe **1**. Blue data represent the fluorescence responses of probe **1** (5 μM) toward AS (100 μM) for 1-90 min; red data represent the fluorescence changes of probe **1** (5 μM) in the absence of AS for 0-360 min;

9. Cell culture and fluorescence imaging

RAW264.7 macrophage cells (gifted from the center of cells, Peking Union Medical College) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 U/mL of penicillin and 100 μg/mL streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2. RAW264.7 macrophage cells were seeded in a 96-well plate at a density of 10^4 cells per well in culture media. After 24 h, they were incubated with 5 μM probe **1** in culture media for 20 min at 37 °C. In additionally, RAW264.7 macrophage cells were incubated with 5 μM probe **1** in culture media for 20 min, and then they were treated with 100 μM AS for another 10 min. After the medium was removed, the cells were carefully washed with PBS for twice. The fluorescence imaging of cells was carried out, respectively. The fluorescence imaging of RAW264.7 macrophage cells was observed under Nikon Cl Si confocal and multi-photo system.

10. Cytotoxicity assays

RAW264.7 macrophage cells were cultured in culture media (DMEM) in an atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were seeded into 96-well plates at a density of 3×10^3 cells per well in culture media, then 0, 5, 10, 20 and 30 μM (final concentration) probe **1** were added respectively. Next, the cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h. Finally, 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) was added and were cultured for another 4 h, respectively.

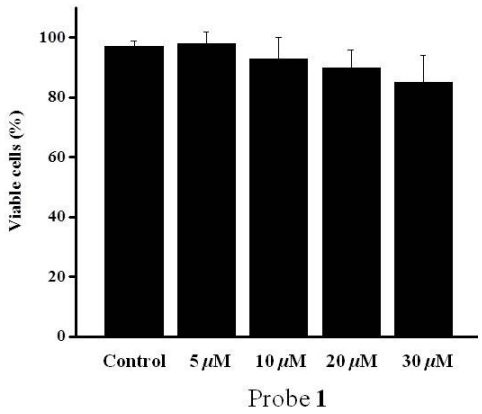


Fig. S8. Cytotoxicity assays of probe **1** at different concentrations for RAW 264.7 macrophage cells.

11. Determination of quantum yield

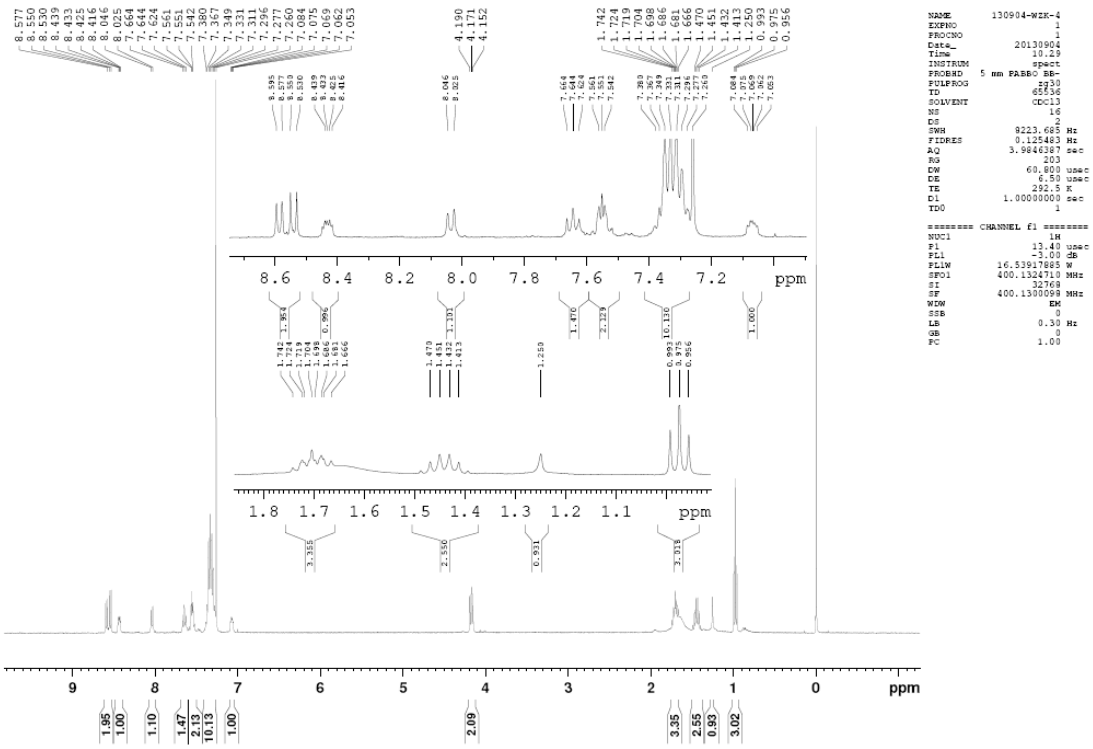
The quantum yield of probe **1** was determined according to the following equation:

$$\phi_1 = \frac{\phi_B I_1 A_B \lambda_{exB} \eta_1}{I_B A_1 \lambda_{ex1} \eta_B}$$

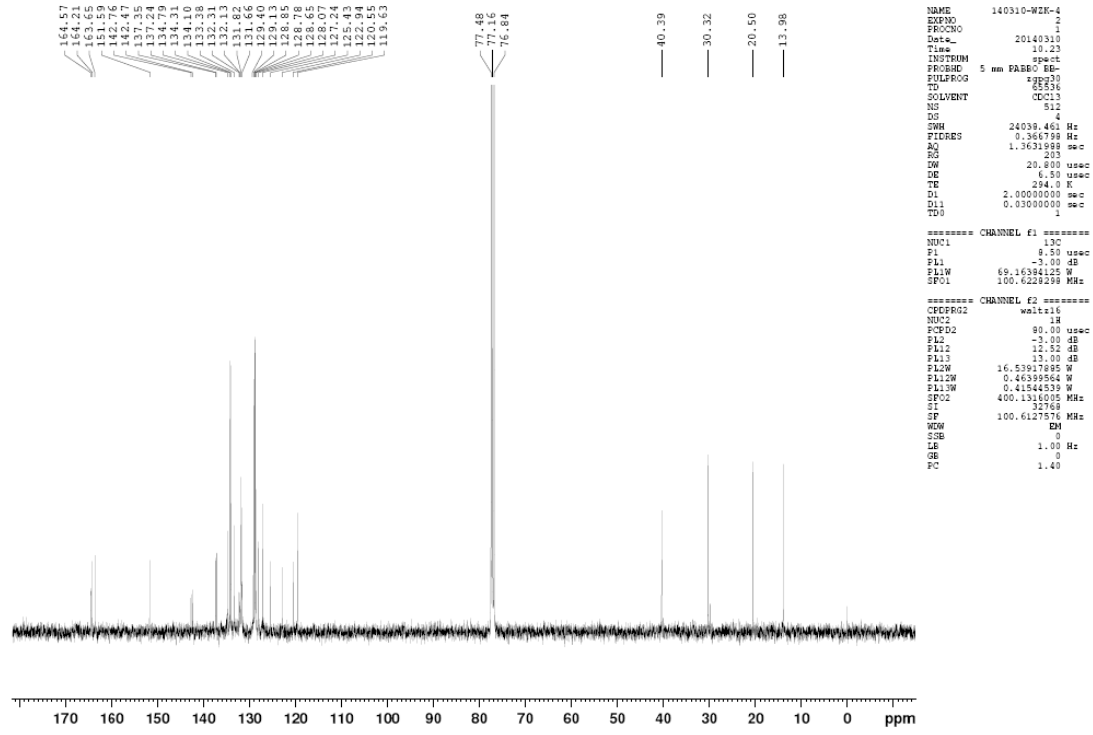
Where Φ is quantum yield; I is integrated area under the uncorrected emission spectra; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts 1 and B refer to probe **1** and the standard, respectively. *N*-butyl-4-butylamino-1,8-naphthalimide in absolute ethanol was used as the standard, which has a quantum yield of 0.810.[1]

12. The characterization data of probe 1

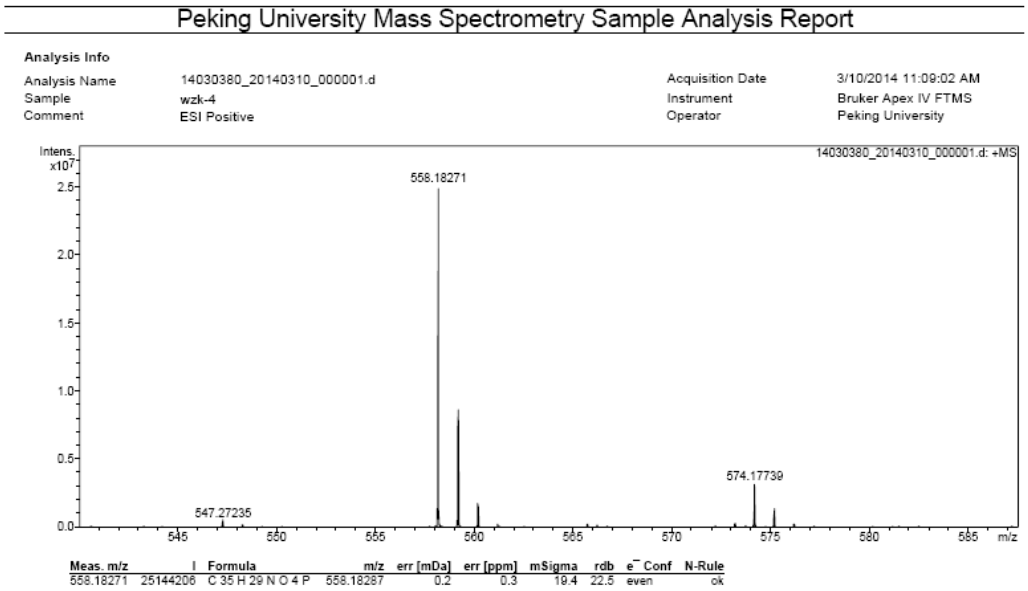
[1]H-NMR of probe 1



[13]C-NMR of probe 1



HRMS of probe 1



13. References

1. B. Zhu, X. Zhang, H. Jia, Y. Li, S. Chen and S. Zhang, *Dyes Pigm.*, 2010, **86**, 87-92.