Supporting information for

A long-wavelength fluorescent turn-on probe for video detection of biological thiols in living cells

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Materials and instruments.Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at 37˚C with a HITACHI F4600 fluorescence spectrophotometer. Cells imaging was performed with a Nikon Eclipse TE300 equipped with a CCD camera. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the fluorescence quantum yield[1-3]: Fluorescence quantum yields for **FR-OH, FR-OCH3** were determined by using 6G (Φ_f = 0.95 in H_2O) 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 to the unknown, respectively.

Calculation of pK_a Values. pK_a values of **FR-OH** dye at acidic to near-neutral pH regions were calculated by regression analysis of the fluorescence data to fit equation (1)

$$pH - pK_a = \log (F_{max} - F)/(F - F_{min}) \qquad (1)$$

Where F is the area under the corrected emission curve, F_{max} and F_{min} are maximum and minimum limiting values of F, respectively.

HeLa cell Culture and Imaging Using FR-thiol. HeLa cells were seeded in a 12-well plate in Dulbecco's modified Eagle'smedium (DMEM) supplemented with 10% fetal bovine serum for 24 h. HeLa cells were then incubated with or without N-ethylmaleimide (as a thiol blocking agent) in the culture medium for 30 min at 37 °C. After washing with PBS three times to remove the remaining N-ethylmaleimide, the cells were further incubated with the probe **FR-thiol** (5 μM) for 30 min at 37 °C. After washing the cells with PBS three times, the cells were imaged using OLYMPUS FV1000 (TY1318) confocal microscope with an excitation filter of 546 nm.

Cytotoxicity assays. HeLa cells were grown in the modified Eagle's medium (MEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ºC. Immediately before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of probe **FR-thiol** (99% MEM and 1% DMSO). The final concentrations of **FR-thiol** was 5, 10, 20, 30 μM (n = 5), respectively. The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air at 37 ºC for 24 h, followed by MTT assays. Untreated assay with MEM (n = 5) was also conducted under the same conditions.

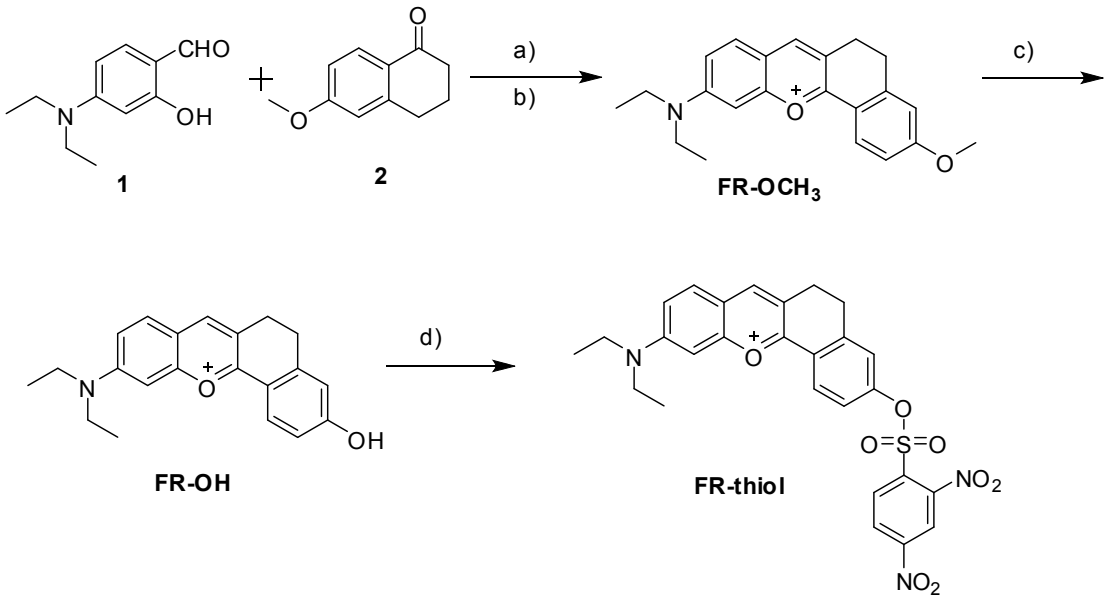
Synthesis of compounds FR-OH, FR-OCH3 and FR-thiol:

The synthesis of **FR-OCH3**, a mixture of salicylaldehyde derivatives (0.01 M, 1.93 g) and benzocyclohexanone derivatives (0.01M, 1.76 g) in 70%concentrated sulfuric acid was stirred at 90℃ for 5h at N_2 atmosphere. After 5 h, it was poured onto 100 g of crushed iced and mixed carefully, and then the aqueous phase is extracted with dichloromethane/MeOH (5 × 100 mL, V/V 20: 1). The organic layers were collected, dried over Na_2SO_4, and evaporated under reduced pressure. The red solid is purified by column chromatography on silica gel flash chromatography using CH_2Cl_2/EtOH (50: 1 to 20: 1). The desired product is obtained as a red solid.^{1}H NMR (400 MHz, DMSO) δ 8.64 (s, 1 H), 8.20 (d, *J*=8.4 Hz, 1 H), 7.92(d, *J*=9.6 Hz, 1 H), 7.43 (dd, *J*=9.6, 2.4 Hz, 1 H), 7.30 (d, *J*=2 Hz, 1 H), 7.13-7.09 (m, 2 H), 3.92 (s, 3 H), 3.68 (q, *J*=7.2Hz, 4 H), 3.36 (s, 4 H), 1.25 (t, *J*=7.2 Hz, 6 H). ^{13}C NMR (100 MHz, DMSO) δ 164.93, 163.37, 158.23, 155.33, 148.44, 145.46, 131.98, 128.55, 120.62, 119.21, 118.03, 117.89, 114.63, 114.37, 95.95, 56.32, 45.61, 26.82, 25.00, 12.71. MS (ESI)

m/z = 334.2 $[M]^+$; HRMS (ESI) Calcd for $C_{22}H_{24}NO_2^+$ ($[M]^+$): 334.1799, Found, 334.1802.

The synthesis of **FR-OH**, to a solution of compounds **FR-OCH3** (200mg, 0.6mmol) and BBr_3 (148 mg) in dry Dichloromethane (5 ml) was stirred under ice bath for 6 h at N_2 atmosphere. After 6 h, it was poured onto 100 g of crushed iced and mixed carefully, and then the aqueous phase is extracted with dichloromethane/MeOH (5 × 100 mL, V/V 10: 1). The organic layers were collected, dried over Na_2SO_4, and evaporated under reduced pressure. The red solid is purified by column chromatography on silica gel flash chromatography using CH_2Cl_2/EtOH (20:1). The desired product is obtained as a red solid.^{1}H NMR (400 MHz, DMSO) δ 8.61 (s, 1 H), 8.10 (d, *J*=8.8 Hz, 1 H), 7.88(d, *J*=9.2 Hz, 1 H), 7.36 (dd, *J*=9.2, 1.2 Hz, 1 H), 7.24 (s, 1 H), 6.91 (dd, *J*=8.8, 2 Hz, 1 H), 6.84 (s, 1 H), 5.75 (s, 1 H), 3.64 (m, 4 H), 2.98 (s, 3 H), 1.21 (t, *J*=6.8 Hz, 6 H). ^{13}C NMR (100 MHz, DMSO) δ 169.48, 168.85, 162.82, 159.89, 152.93, 150.77, 136.68, 134.05, 125.25, 122.56, 122.31, 122.78, 120.86, 100.86, 50.44, 31.68, 29.78, 17.62. MS (ESI) m/z = 320.2$[M]^+$; HRMS (ESI) Calcd for $C_{21}H_{22}NO_2^+$ ($[M]^+$): 320.1642, Found, 320.1645.

The synthesis of probe **FR-thiol**, to mixture of **FR-OH** (0.001M, 419 mg) and 2,4-dinitrobenzene-1-sulfonyl chloride (0.001M, 265mg) in dry dichloromethane was stirred at room temperature for 4h at N_2 atmosphere. The red solid is purified by column chromatography on silica gel flash chromatography using CH_2Cl_2/EtOH (30:1). The desired product is obtained as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 9.15 (d, *J*=2 Hz, 1 H), 8.73 (s, 1 H), 8.66 (dd, *J*=8.4, 2.0 Hz, 1 H), 8.36 (d, *J*=8.4 Hz, 1 H), 8.25(d, *J*=8.4 Hz, 1 H), 8.03 (d, *J*=9.6 Hz, 1 H), 7.55 (dd, *J*=9.6, 2.0 Hz, 1 H), 7.42 (d, *J*=2 Hz, 1 H), 7.37-7.34 (m, 2 H), 3.73 (d, *J*=9.2 Hz, 4 H), 3.34 (s, 4 H), 1.26 (t, *J*=7.2 Hz, 6 H). ^{13}C NMR (100 MHz, DMSO) δ 160.08, 158.87, 156.34, 151.89, 149.06, 148.37, 144.52, 133.84, 132.55, 131.06, 127.93, 126.54, 122.66, 121.65, 121.52, 120.51, 119.45, 95.91, 46.02, 40.22, 26.20, 24.31. MS (ESI) m/z =550.1 $[M]^+$; HRMS (ESI) Calcd for $C_{27}H_{24}N_3O_8S^+$ ($[M]^+$): 550.1279, Found, 550.1274.



Scheme S1. Synthesis of Compounds probe **FR-thiol**. Conditions: (a) 70%concentrated sulfuric acid, 90℃; (b) 70% $HClO_4$, room temperature; (c) BBr_3, CH_2Cl_2, room temperature; (d) CH_2Cl_2, room temperature.

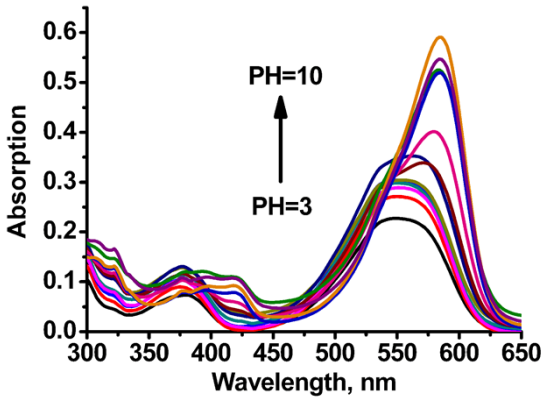


Figure. S1. pH-dependence of the absorption spectra of compound **FR-OH** (5 μM) with the arrows indicating the change of the absorption intensities with pH enhancement from 3 to 10.

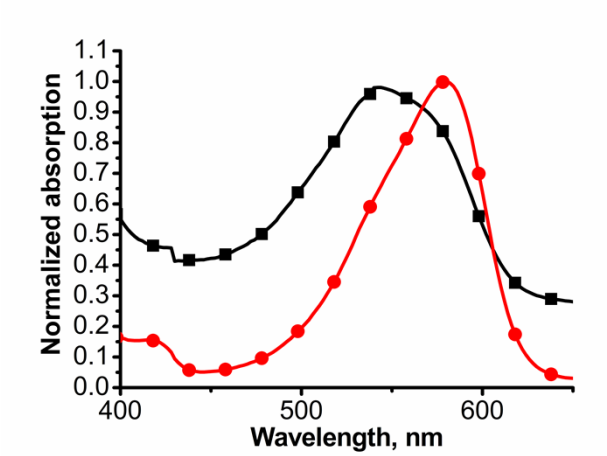


Figure S2. (a) Normalized absorption of 5 μM probe **FR-thiol** before (■) and after (●) treating with 100 eq. cysteine.

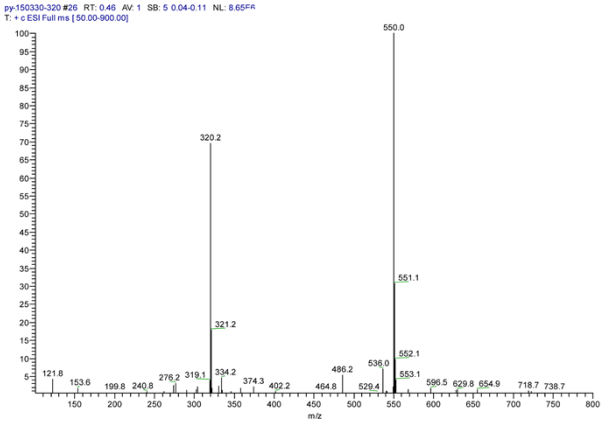


Figure S3. Mass spectrum (ESI) of the reaction mixture of the probe **FR-thiol** with cysteine.

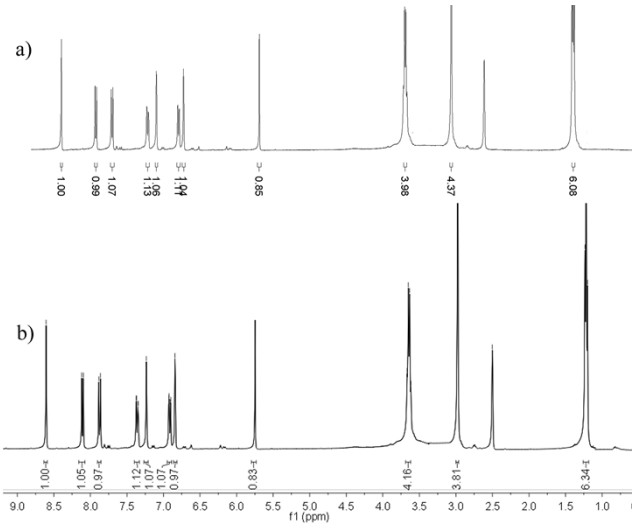


Figure S4.[1]H NMR spectra of the isolated product of the probe **FR-thiol** with cysteine (a) and the compound **FR-OH** (b).

Detection limit: The detection limit was determined from the fluorescence titration data. According to the result of titration experiment, the fluorescent intensity data at 624 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescent intensity data (Figure. S4), and the point at which this line crossed the axis was considered as the detection limit (1.87×10^{-6} M).

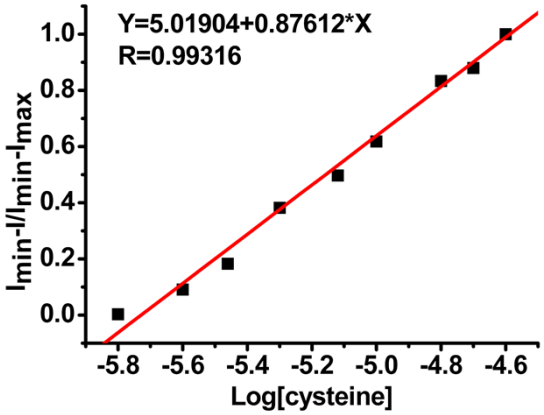


Figure S5. Normalized response of fluorescence signal to changing cysteine concentrations.

Kinetic Studies:

The reaction of the probe **FR-thiol** (5 μM) with cysteine or GSH (100 μM) in pH 7.4, 25 mM PBS was monitored using the fluorescence intensity at 624 nm. The reaction was carried out at 25 ˚C. The *pseudo*-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the *pseudo* first-order equation:

$$\text{Ln}\,[(F_{max} - F_t) / \text{F}_{\text{max}}] = - k't$$

Where F_t and F_{max} are the fluorescence intensities at 624 nm at time t and the maximum value obtained after the reaction was complete. k' is the *pseudo*-first-order rate constant.

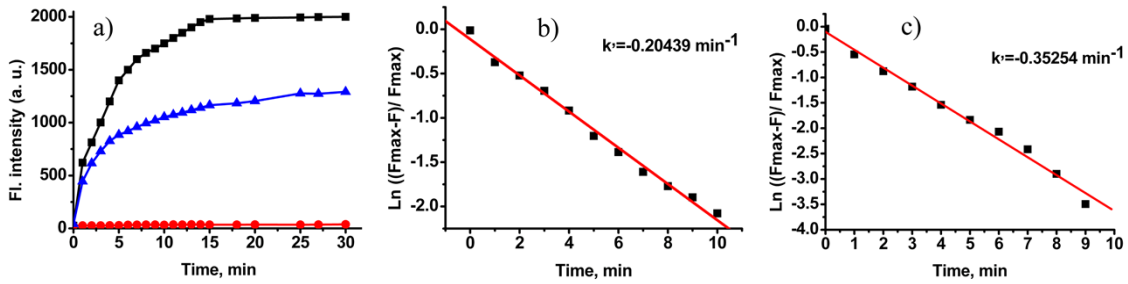


Figure S6. a) Reaction-time profiles of **FR-thiol** (5.0 μM) in the absence (●) or presence of 100 μM cysteine(■) or GSH (▲); b) *Pseudo* first-order kinetic plot of the reaction of the probe **FR-thiol** (5 μM) with cysteine (100 μM) in pH 7.4, 25 mM PBS. Slope = 0.2044 min^{-1}; b) *Pseudo* first-order kinetic plot of the reaction of the probe

FR-thiol (5 μM) with cysteine (100 μM) in pH 7.4, 25 mM PBS. Slope = 0.3525 min^{-1}.

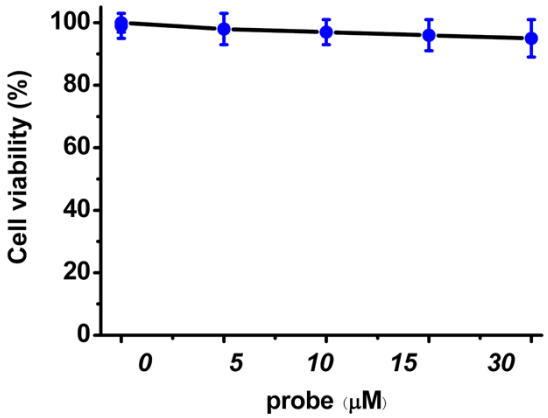


Figure S7. Cytotoxicity of the probe **FR-thiol** on HeLa cells determined by MTT.

References

1. B. Valeur, Molecular Fluorescence: Principles and Applications, Wiley-VCH, 2001.
2. D. Magde, G. E. Rojas and P. Seybold, *Photochem. Photobiol.*, 1999, **70**, 737.
3. D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano and T. Nagano, *J. Am. Chem. Soc*., 2010, **132**, 2795.

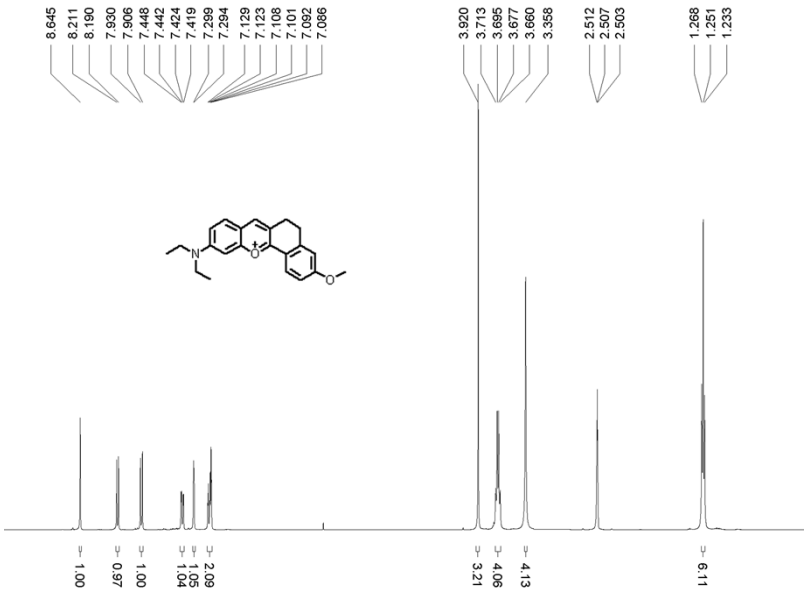


Figure S8. The ^{1}H NMR of **FR-OCH3.**

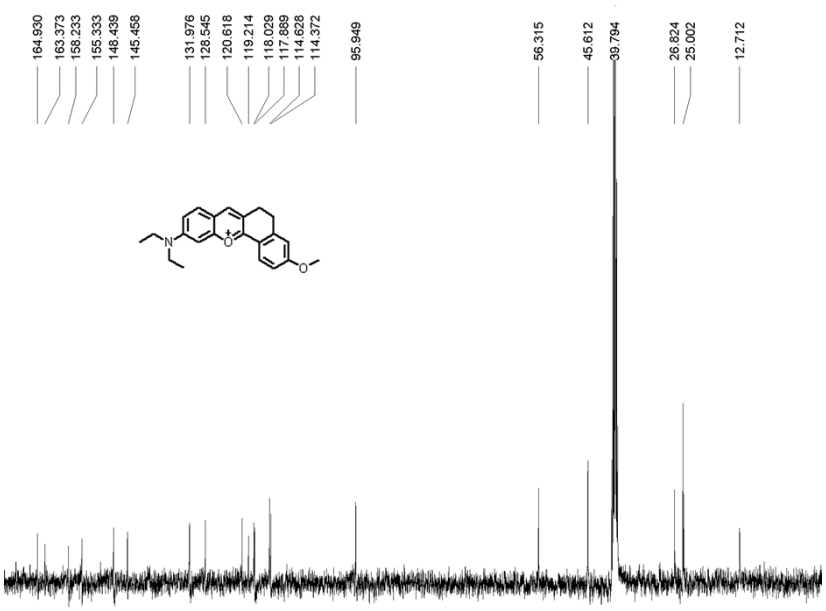


Figure S9. The ^{13}C NMR of **FR-OCH3.**

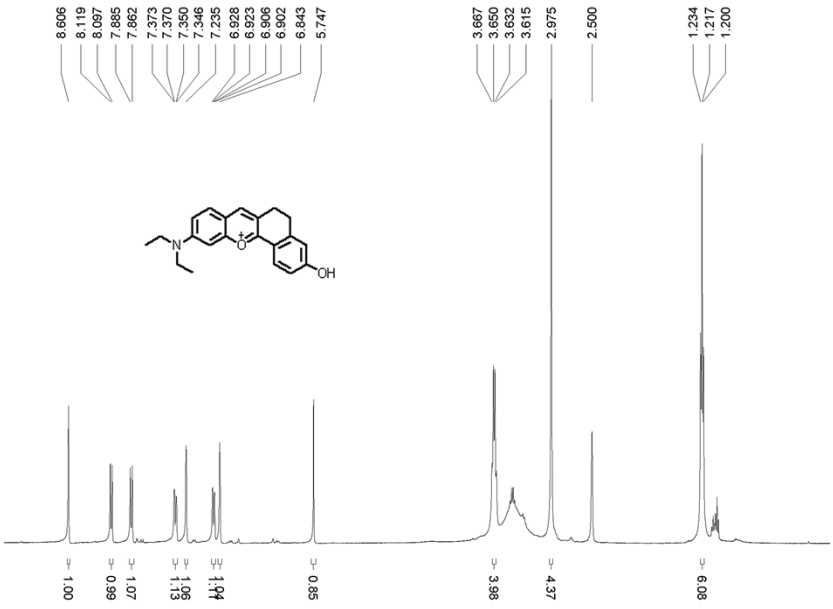


Figure S10. The ^{1}H NMR of **FR-OH.**

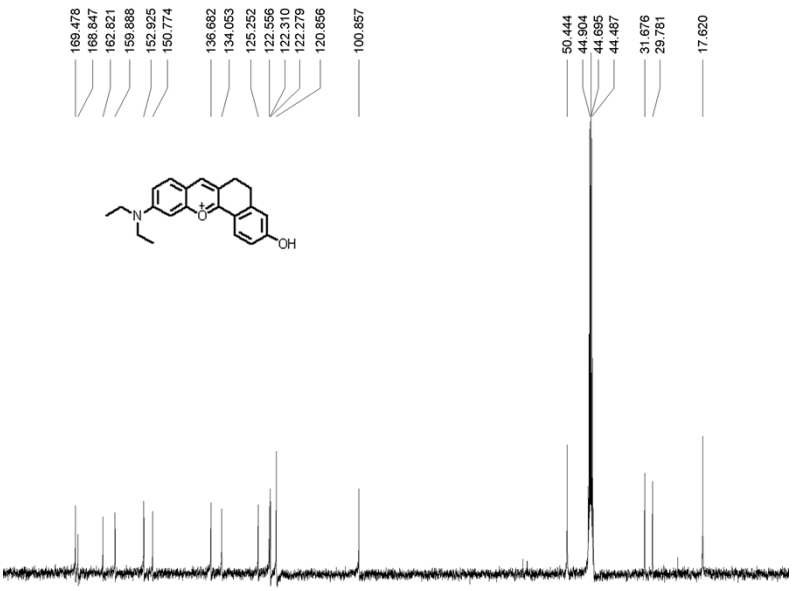


Figure S11. The ^{13}C NMR of **FR-OH.**

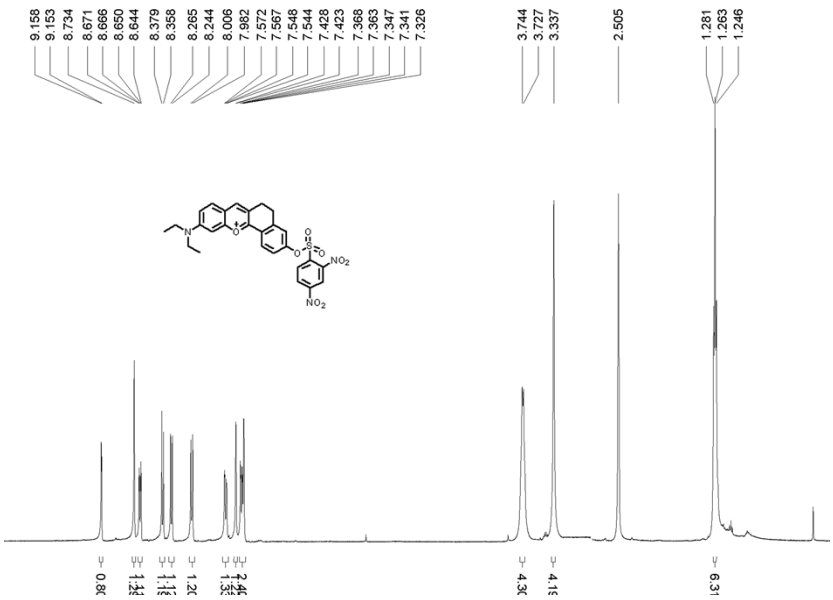


Figure S12. The ^{1}H NMR of **FR-thiol.**

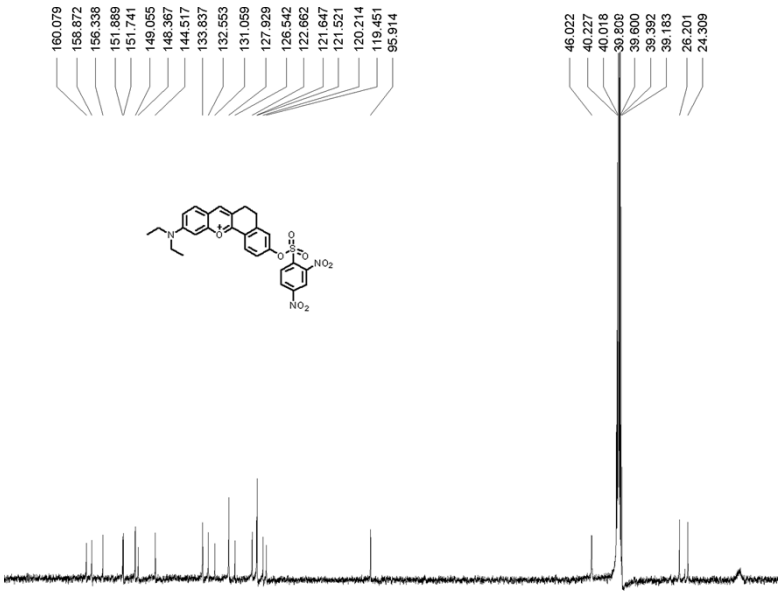


Figure S13. The ^{13}C NMR of **FR-thiol.**