Electronic Supplementary Material (ESI) for Analytical Methods
This journal is © The Royal Society of Chemistry 2013

Supporting Information for

Development of a near-infrared fluorescent probe for monitoring hydrazine in serum and living cells

Sasa Zhu, Weiying Lin,* Lin Yuan

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, P. R. China.

E-mail: weiyinglin@hnu.edu.cn

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. Low resolution mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP 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 spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; TLC analyses were 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.

Cell culture and imaging: HeLa cells 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 under an atmosphere of 5% CO_2 and 95% air at 37 ℃. HeLa cells were seeded in a 12-well plate in culture media. After 24 h, they were incubated with the probe **NIR-N_2H_4** (5 μM, final concentration) in culture media (1% CH_3CN) for 30 min at 37 ℃, and washed with phosphate buffered saline (PBS) three times. After incubating with hydrazine (30 μM) for another 30 min at 37 ºC, the HeLa cells were rinsed with PBS three times, and the fluorescence images were acquired through fluorescence microscope.

Cytotoxicity assays: HeLa cells were cultured in DMEM containing 10% FBS supplemented with 100 U/mL of penicillin and 100 μg/mL streptomycin in an atmosphere of 5% CO_2 and 95% air at 37 ℃. The cells were seeded into 96-well plates, and then 2.5, 5.0, 7.5, 10.0, or 15.0 μM (final concentration) **NIR-N_2H_4** (99% DMEM and 1% DMSO) was added, respectively (n = 6). Subsequently, the cells were incubated at 37℃ in an atmosphere of 5% CO_2 and 95% air for 1 h. Untreated assay with DMEM (n = 6) was also conducted under the same conditions.

Synthesis of Compound 4. 4-chloro resorcin **3** (134.1 mg, 0.93 mmol) and triethylamine (0.3 mL) were placed in a flask containing DMF (2.0 mL), and the mixture was stirred at room temperature under nitrogen atmosphere for 10 min. Compound **2** (200.0 mg, 0.31 mmol) in DMF (1.0 mL) was introduced to the mixture via a syringe, and the reaction mixture was heated at 75 °C for 4 h. The solution was then removed under reduced pressure. The crude product was purified by silica gel flash chromatography using CH_2Cl_2/EtOH (50:1) as eluent to give compound **4** as a blue-green solid (60.7 mg, yield 35.0%). ^{1}HNMR (400 MHz, $CDCl_3$): δ = 1.67 (s, 6H), 1.89 (t, *J* = 5.6 Hz, 2H), 2.61 (t, *J*= 5.6 Hz, 2H), 2.68 (t, *J* = 5.8 Hz, 2H), 3.37 (s, 3H)，5.61 (d, *J* = 13.6 Hz, 1H)，6.68 (s, 1H), 6.86 (d, *J* =7.6 Hz,1H), 7.07 (t, *J* = 7.2Hz, 1H), 7.28-7.31 (3H), 7.40 (s, 1H), 8.09 (d, *J* = 13.6 Hz，1H). ^{13}CNMR (100MHz, $CDCl_3$): δ=21.2, 24.4, 28.0, 28.6, 30.1, 47.5, 103.8, 108.0, 115.3, 116.1, 117.0, 122.1, 122.5, 127.3, 128.2, 131.8, 133.4, 139. 7, 143.7, 157.7, 160.4, 166.7, 174.8. HRMS (EI) m/z calcd for $C_{26}H_{24}NO_2Cl$ (M-H): 417.1490. Found: 417.1485.

Synthesis of Compound NIR-N_2H_4. Levulinic acid (90 mg, 0.776 mmol) and EDC (198 mg,1.036 mmol), DMAP (12.2 mg, 0.103 mmol) were dissolved in CH_2Cl_2. The mixture was stirred at room temperature for 10 min, and then compound **4** (108 mg, 0.259 mmol) was added. After 1h reaction, the solvent was removed under reduced pressure. The resulting residue was purified by a silica gel column (CH_2Cl_2/EtOH =30:1) to afford compound **NIR-N_2H_4** as a blue solid (106 mg, isolated yield: 79.6%): ^{1}H NMR (400 Hz, $CDCl_3$): δ =1.81 (s, 6H), 1.93 (t, *J* = 5.4 Hz, 2H), 2.27 (s, 3H), 2.71-2.72 (d, 2H), 2.84 (t, J = 5.4Hz, 2H), 2.95 (s, 4H), 4.21 (s, 3H), 6.90 (d, *J* = 15.6 Hz, 1H), 6.94 (s, 1H), 7.14 (s, 1H), 7.39 (s, 1H), 7.46 (d, *J* = 7.0 Hz, 1H), 7.51-7.53 (2H), 7.57 (t, *J* = 7.6 Hz, 1H), 8.59 (d, *J* = 15.2 Hz, 1H). ^{13}C NMR (400Hz, CDCl3): 20.1, 24.3, 27.9, 29.6, 29.9, 34.4, 37.7, 51.1, 108.0, 111.6, 113.8, 116.1, 120.9, 122.3, 123.4, 127.6, 128.3, 128.4, 129.5, 131.6, 142.1, 146.2, 148.2, 151.2, 158.4, 170.3, 179.3, 206.4. HRMS (ESI) m/z calcd for MS (ESI) m/z calcd for $^+C_{31}H_{31}NO_4Cl$ (M^+): 516.1936. Found: 516.1928.

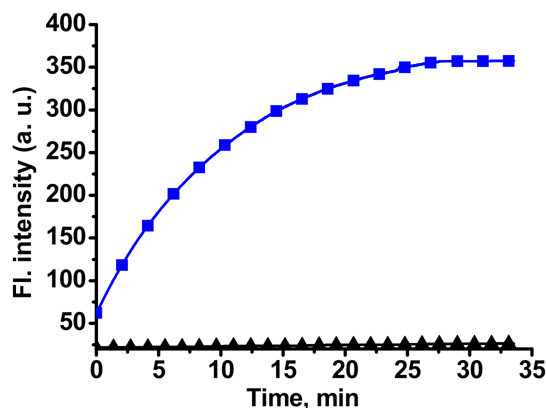


Fig. S1 Reaction-time profile of **NIR-N_2H_4** (5 μM) in HEPES/CH_3CN (pH =7.0, 7: 3) in the absence (▲) and presence (■) of hydrazine (200 equiv.) in pH 7.0, HEPES/CH_3CN (7: 3). Kinetic studies were performed at room temperature. The emission intensity at 725 nm was continuously monitored at time intervals. Excitation wavelength was 670 nm.

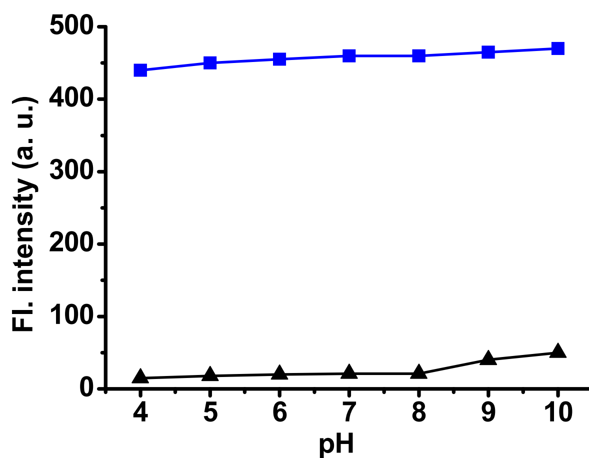
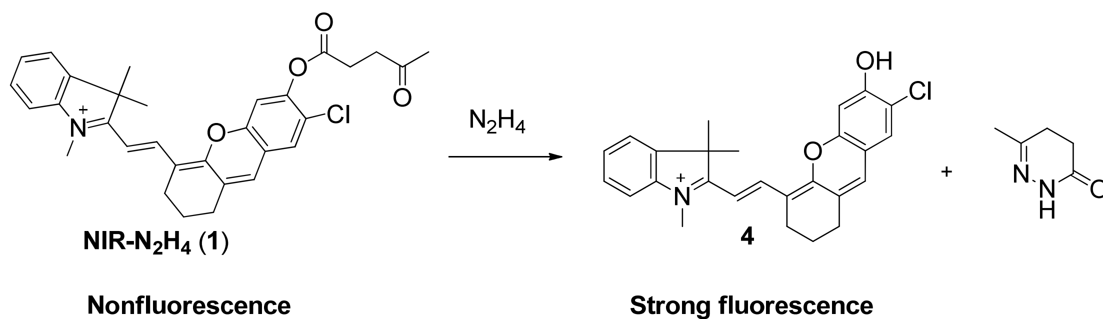


Fig. S2 Fluorescent emission intensities at 725 nm of probe **NIR-N_2H_4** (5 μM) in the absence (▲) or presence (■) of hydrazine (100 equiv.) at various pH values. Excitation wavelength was 670 nm.



Scheme S1. The proposed sensing mechanism of the probe **NIR-N_2H_4**.

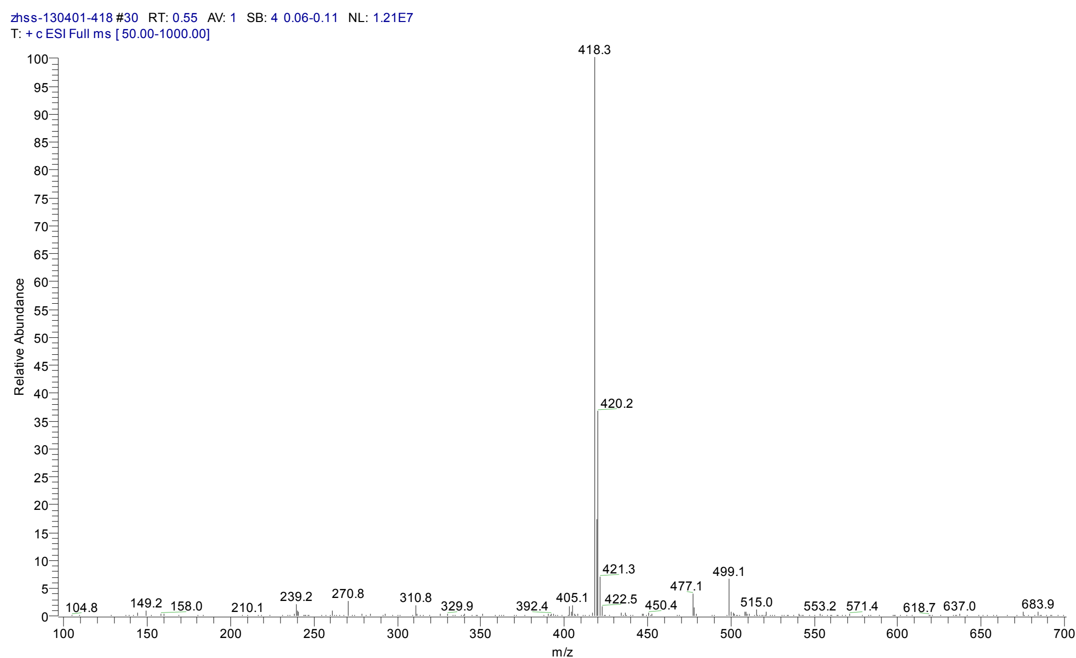


Fig. S3 MS (ESI) of the reaction product of the probe **NIR-N_2H_4** with hydrazine.

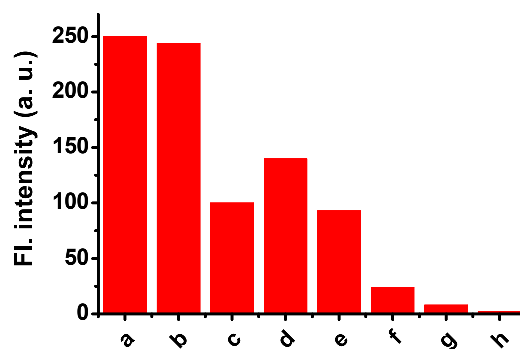


Fig. S4 The autofluorescence of newborn calf serum samples: a ($\lambda_{ex}/\lambda_{em}$ = 370/465 nm); b ($\lambda_{ex}/\lambda_{em}$ = 370/503 nm); c ($\lambda_{ex}/\lambda_{em}$ = 400/513 nm); d ($\lambda_{ex}/\lambda_{em}$ = 450/527 nm); e ($\lambda_{ex}/\lambda_{em}$= 510/578 nm); f ($\lambda_{ex}/\lambda_{em}$ = 550/600 nm); g ($\lambda_{ex}/\lambda_{em}$ = 650/680 nm); and h ($\lambda_{ex}/\lambda_{em}$ = 670/720 nm).

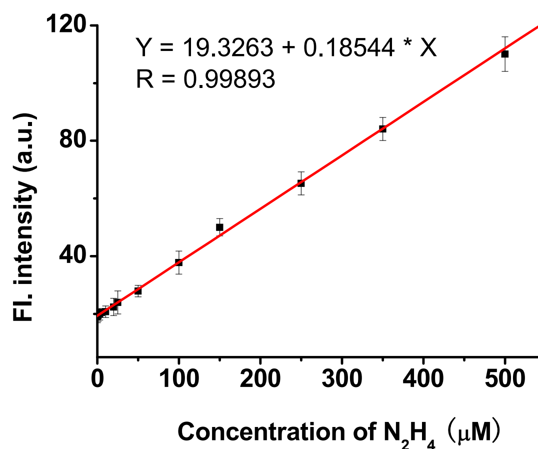


Fig. S5 Plot of the fluorescent intensity at 725 nm of probe **NIR-N_2H_4** as a function of the hydrazine concentration. Each spectrum was acquired 1 min after hydrazine addition at 25 ^{o}C.

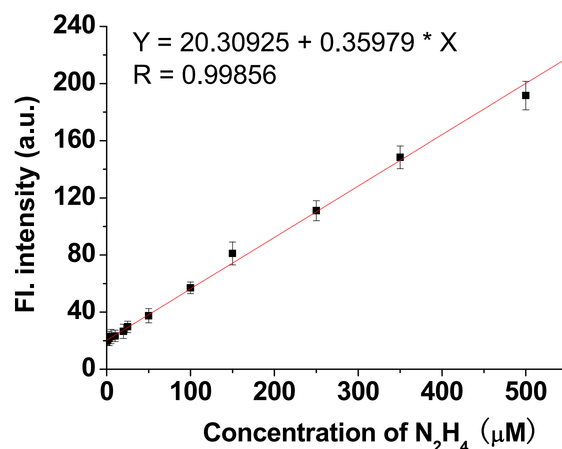


Fig. S6 Plot of the fluorescent intensity at 725 nm of probe **NIR-N_2H_4** as a function of the hydrazine concentration. Each spectrum was acquired 5 min after hydrazine addition at 25 °C.

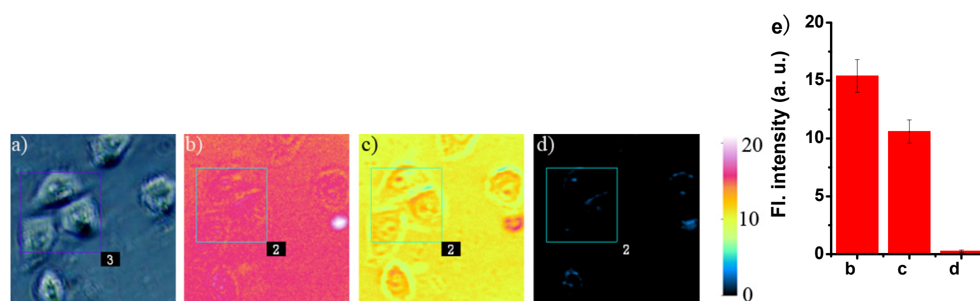


Fig. S7 Bright-field (a) and the autofluorescence of HeLa cells excited at 405 nm (b), 488 nm (c), and 633 nm (d). (e) Quantification of fluorescence emission intensity from the panels b-d.

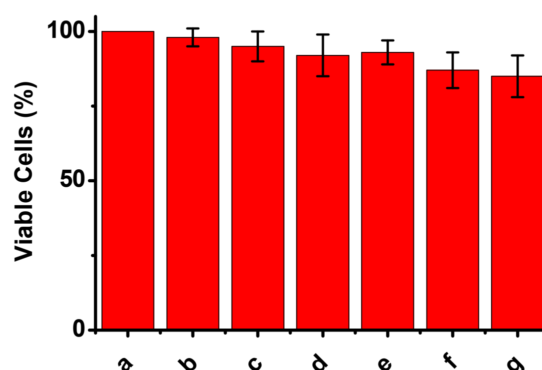
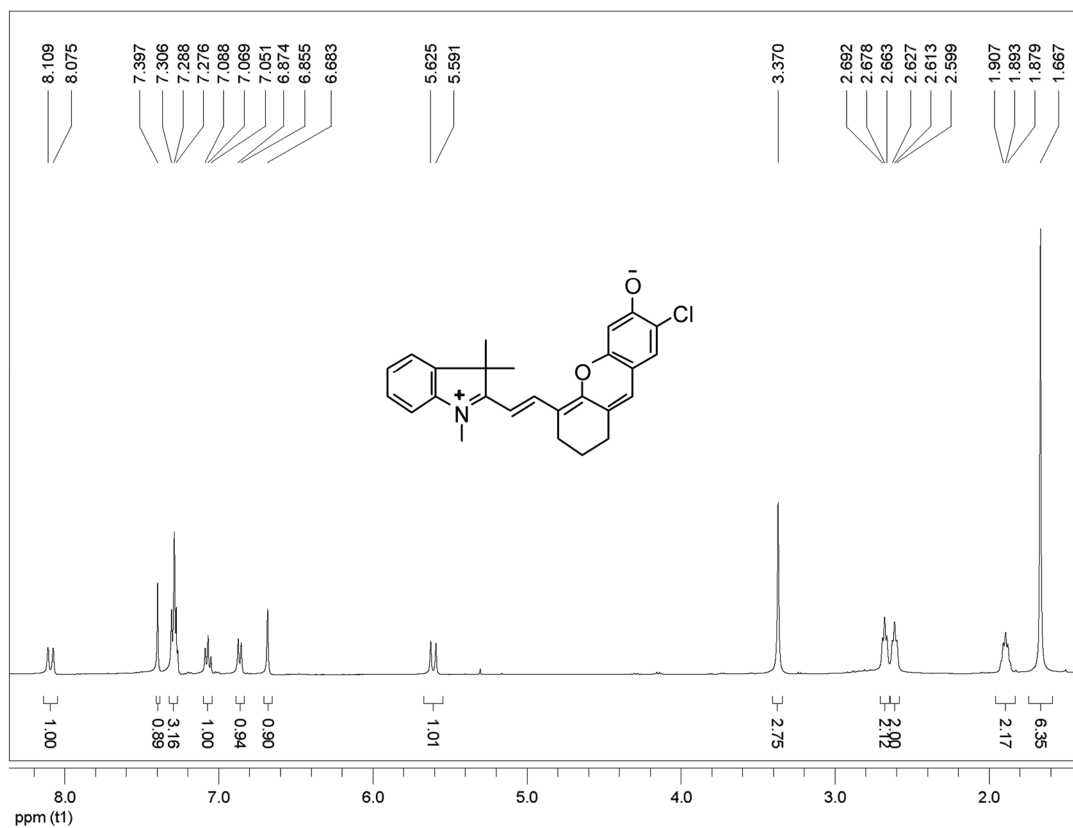
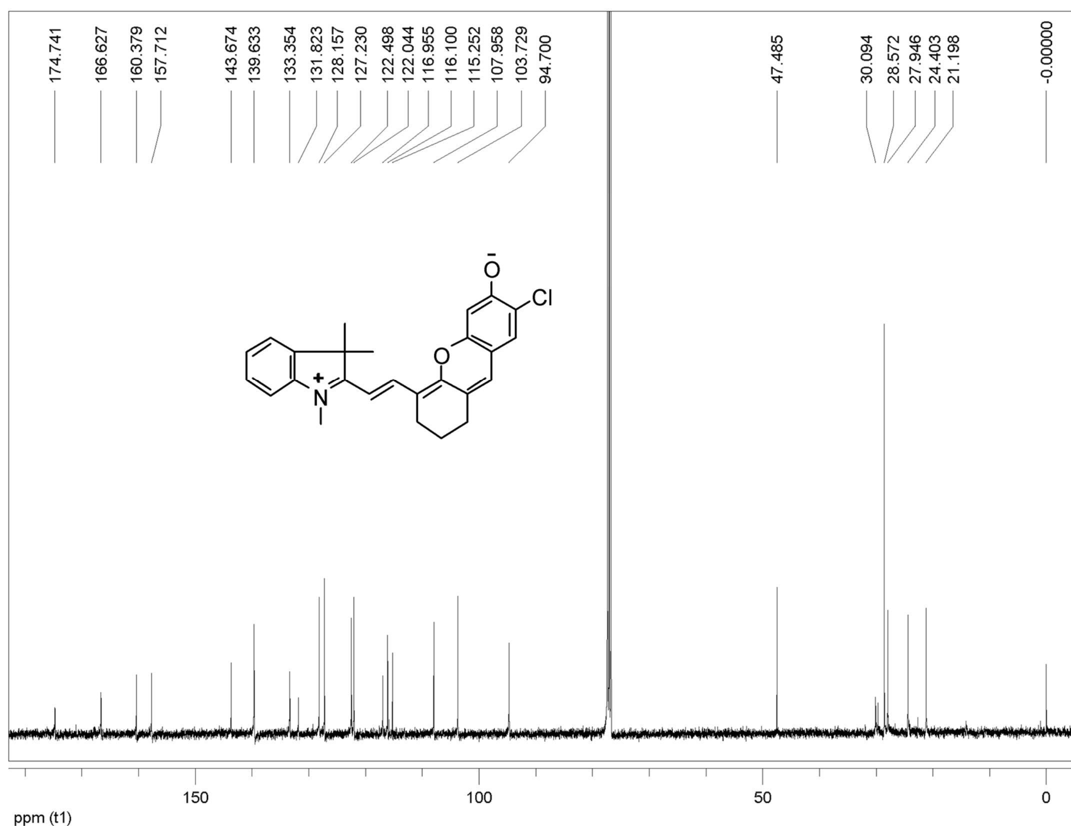
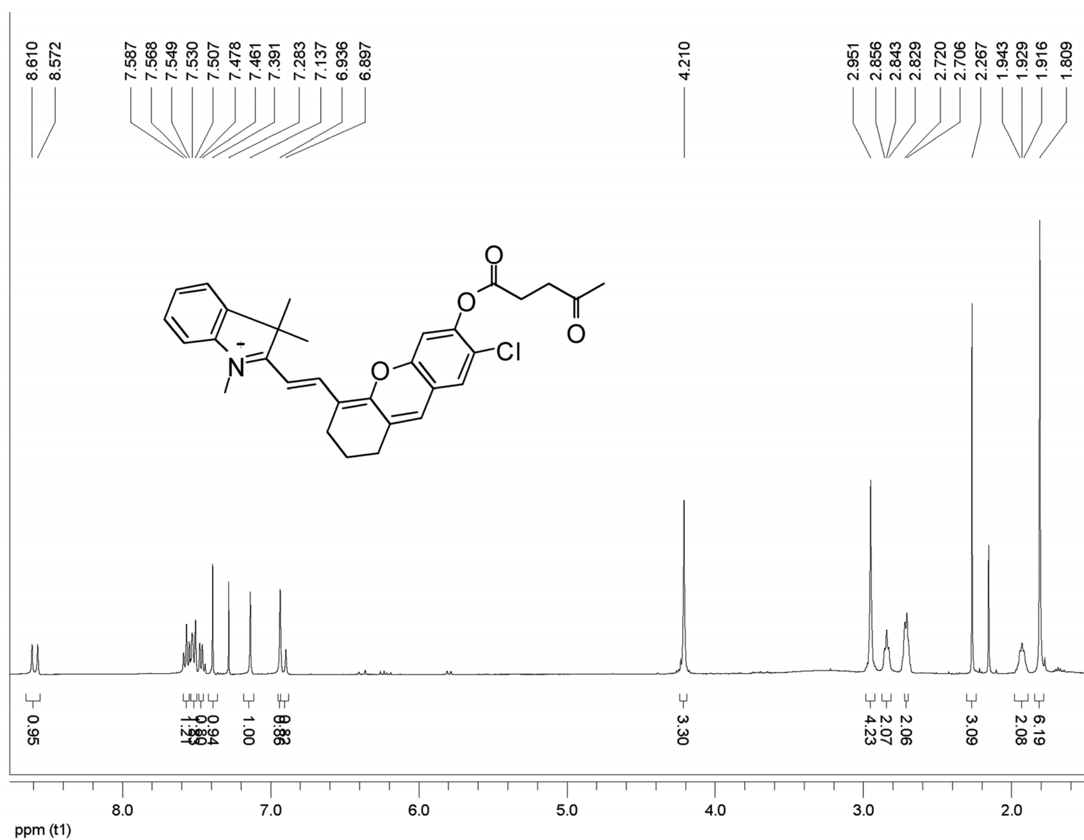
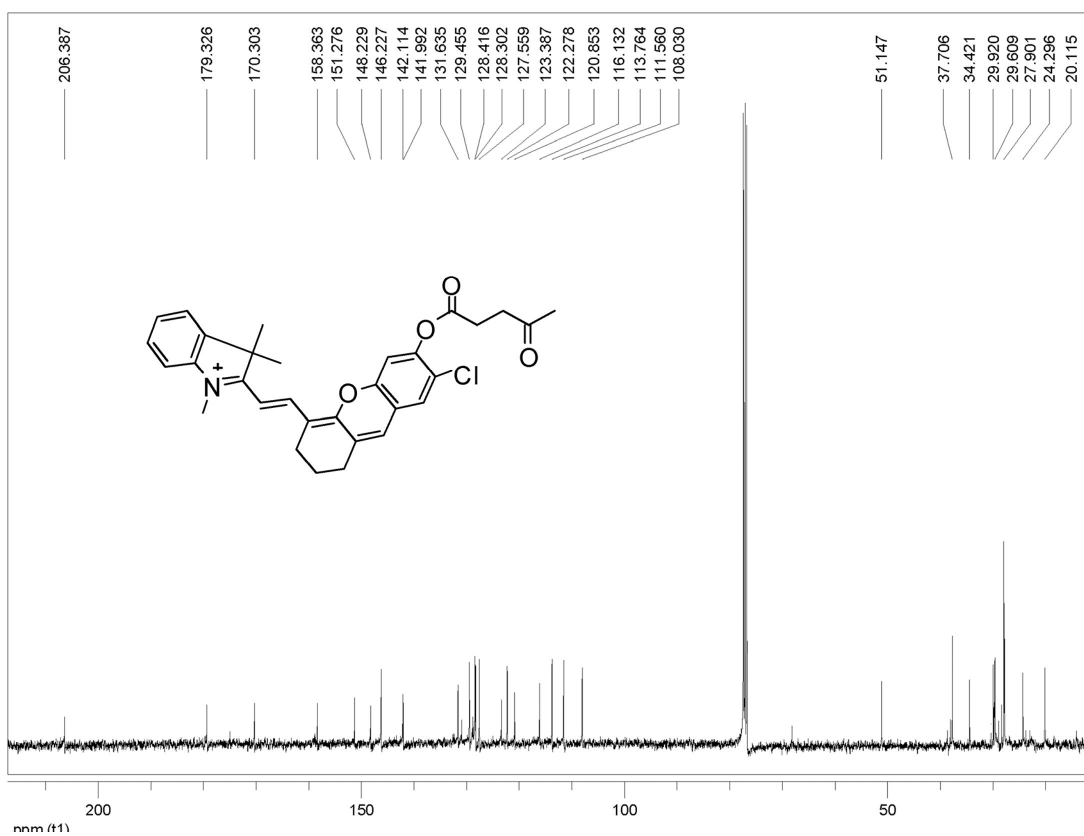


Fig. S8 Cytotoxicity assay of the probe **NIR-N_2H_4** at different concentrations (a: 0 μM; b: 1.0 μM; c: 2.5 μM; d: 5.0 μM; e: 7.5 μM; f: 10.0 μM; g: 15.0 μM;) for HeLa cells.






8.610
8.572
7.587
7.568
7.549
7.530
7.507
7.478
7.461
7.391
7.283
7.137
6.936
6.897
4.210
2.951
2.856
2.843
2.829
2.720
2.706
2.267
1.943
1.929
1.916
1.809
N
O
O
O
O
Cl
0.95
1.00
3.30
4.23
2.07
2.06
3.09
2.08
6.19
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)


206.387
179.326
170.303
158.363
151.276
148.229
146.227
142.114
141.992
131.635
129.455
128.416
128.302
127.559
123.387
122.278
120.853
116.132
113.764
111.560
108.030
51.147
37.706
34.421
29.920
29.609
27.901
24.296
20.115
N
O
O
O
O
Cl
200
150
100
50