Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Construction of a turn-on probe for fast detection of H_2S in living cells based on a novel H_2S trap group with an electron rich dye

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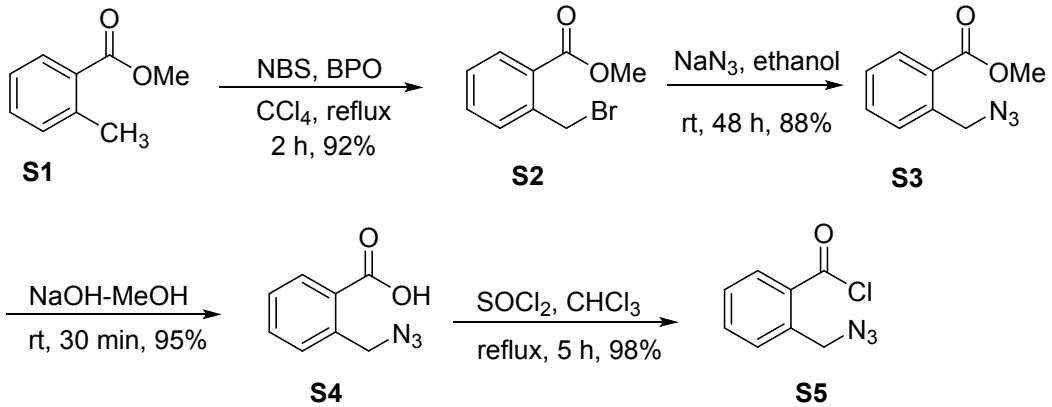
1. General information

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Deionized water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). Column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factor. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE AV400 at 400 and 100 MHz, respectively. All NMR chemical shifts were referenced to residual solvent peaks or to $Si(CH_3)_4$ as an internal standard, spectra recorded in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm for ^{1}H or 77.0 ppm for ^{13}C. All coupling constants *J* are quoted in Hz. FTIR spectra were obtained with a Bruker Vertex 70 FT-IR spectrometer with KBr pellets. All IR samples were prepared as thin film and reported in wave numbers (cm^{-1}). High resolution mass spectra were obtained on a Q-TOF6510 instrument mass spectrometer. Fluorescence spectra were carried out on an Edinburgh Instruments Ltd-FLS920 fluorescence spectrophotometer.

2. Synthesis and Characterization of Compounds

Synthesis of **S5**



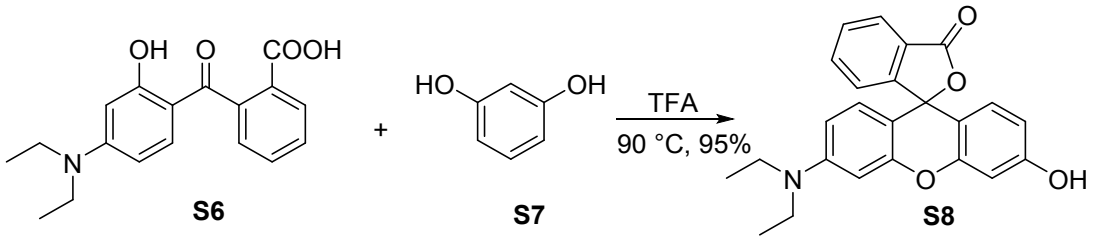
To a solution of **S1** (10.0 g, 66.5 mmol) in CCl_4, was added NBS (13.0 g, 73.2 mmol) and BPO (129 mg, 0.5 mmol). The resulting solution was heated to reflux for 2 h. The solution was cooled to room temperature, and then filtered and concentrated. The crude product **S2** was got as colorless oil (14.0 g, 92%), which was used for the next step without further purification.

To a solution of **S2** (14.0 g, 61.2 mmol) in absolute ethanol, was added NaN_3 (6.5 g, 99.8 mmol). The resulting solution was warmed to room temperature and stirred for 48 h. The mixture was quenched by addition of brine and extracted with ethyl acetate (3×10 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated to yield 10.3 g (88%) of **S3** as light yellow oil, which was used for the next step without further purification.

To a solution of aq NaOH–MeOH (aq NaOH, 2 M, 1:1, v:v), was added **S3** (10.2 g, 53.8 mmol). The resulting solution was stirred for 30 min. Then the mixture was extracted with DCM (3×10 mL). The aqueous layer was acidified to pH = 3 and then extracted with DCM (3×10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to yield 9 g (95%) of **S4** as a white solid. Data for **S4**: R_f 0.35 (petroleum ether:ethyl acetate = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, *J* = 7.8, 1.3, 1H), 7.63 (td, *J* = 7.6, 1.4, 1H), 7.58–7.52 (m, 1H), 7.46 (td, *J* = 7.7, 1.3, 1H), 4.89 (s, 2H); Anal. Calcd for $C_8H_7N_3O_2$: C, 54.23; H, 3.98; N, 23.72. Found: C, 54.43; H, 4.10; N, 23.60; mp 65 °C. The ^{1}H spectra matched those reported by Zhen Xi.[1]

To a solution of **S4** (354 mg, 2.0 mmol) in DCM, was added $SOCl_2$ (0.43 mL, 6.0 mmol) at room temperature. The resulting solution was heated to reflux for 5 h. The mixture was cooled to room temperature and concentrated to yield 384 mg (98%) of S**5** as light yellow oil, which was used for the next step without further purification.

Synthesis of **S8**

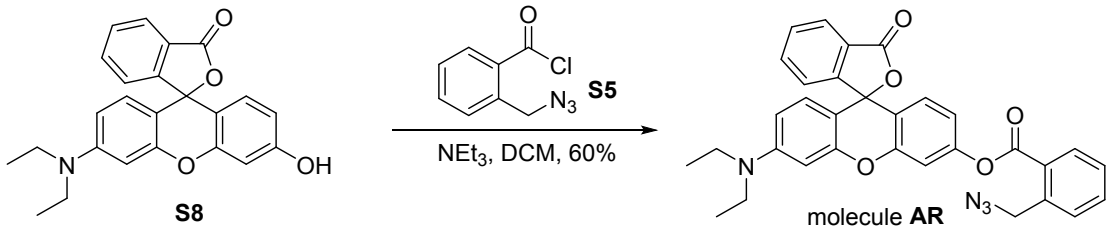


S6 **S7** **S8**

To a suspension of **S6** (6.0 g, 19.1 mmol) and resorcinol **S7** (2.1 g, 19.1 mmol) in TFA (20 mL) in a sealing tube, was added molecular sieve (2.0 g). The resulting mixture was heated to 90 °C and stirred for 6 h. Then the mixture was cooled to room temperature and concentrated to yield a crude product, which was recrystallized in

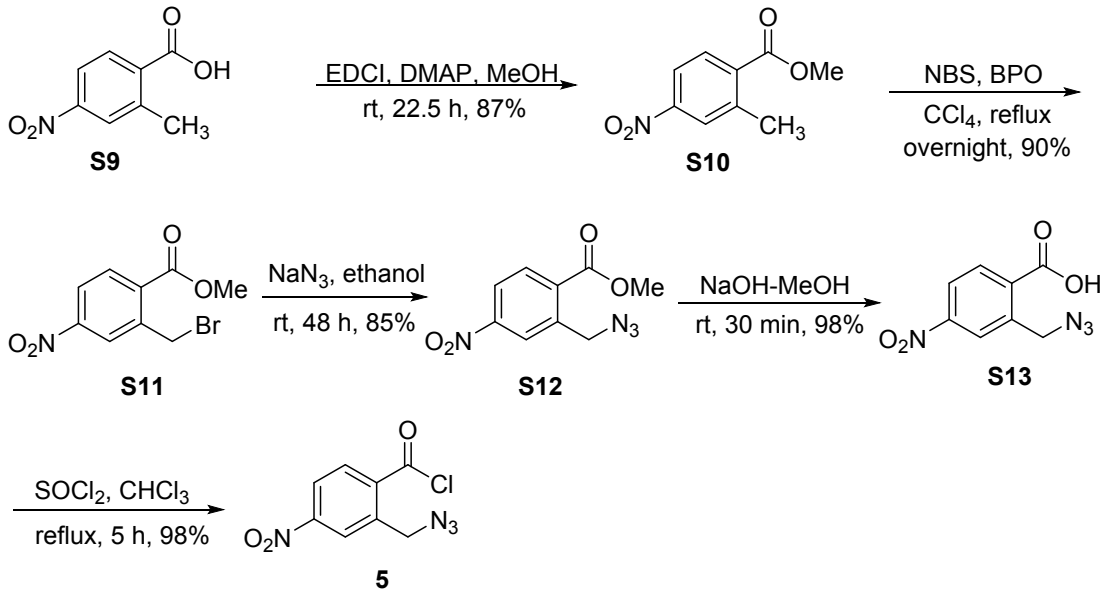
ethyl 1:1 petroleum ether:ethyl acetate affording **S8** as a red solid (7.0 g, 95% yield). Data for **S8**: R_f 0.35 (petroleum ether:ethyl acetate = 1:1); ^{1}H NMR (400 MHz, DMSO-$_{d6}$) δ 7.96 (d, *J* = 7.6, 1H), 7.80 (dt, *J* = 27.8, 7.5, 1H), 7.64 (dt, *J* = 30.2, 7.5, 2H), 7.38 (d, *J* = 7.4, 2H), 6.79 (d, *J* = 9.1, 2H), 6.18 (d, *J* = 9.2, 1H), 6.07 (d, *J* = 2.4, 1H), 3.38 (q, *J* = 6.9, 4H), 1.09 (t, *J* = 7.0, 6H); Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.31; H, 5.30; N, 3.55; mp 184.5-185.5 °C. The ^{1}H spectra matched those reported by Xiao-Feng Yang.[2]

Synthesis of molecule **AR**



To a suspension of **S8** (379 mg, 1.9 mmol) and DMAP (315 mg, 2.6 mmol) in DCM (5 mL) at 0 °C, was added **S5** (500 mg, 1.3 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The resulting mixture was extracted with DCM (3×20 mL). Combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated to afford a red oil. Flash chromatography of the crude product (4:1 petroleum ether:ethyl acetate) provided the desired product as colorless oil (423 mg, 60%). Data for **AR**: R_f 0.35 (petroleum ether:ethyl acetate = 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.8, 1.0, 1H), 8.03 (d, *J* = 7.5, 1H), 7.77–7.61 (m, 2H), 7.63–7.54 (m, 2H), 7.54–7.44 (m, 1H), 7.23 (d, *J* = 7.5, 1H), 7.17 (d, *J* = 2.1, 1H), 6.95–6.78 (m, 2H), 6.59 (d, *J* = 8.9, 1H), 6.46 (d, *J* = 2.5, 1H), 6.38 (dd, *J* = 8.9, 2.6, 1H), 4.87 (s, 2H), 3.37 (q, *J* = 7.0, 4H), 1.18 (t, *J* = 7.0, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.56, 164.67, 153.02, 152.74, 152.48, 151.70, 149.69, 138.42, 134.92, 133.74, 131.78, 130.00, 129.65, 129.27, 128.88, 128.39, 127.44, 127.06, 124.95, 124.19, 117.52, 117.00, 110.42, 104.78, 97.58, 83.63, 53.12, 44.51, 12.51; IR (thin film)vmax 3450, 2976, 2928, 2103, 1733, 11419, 1105, 755; HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{32}H_{27}N_4O_5^+$, 547.1976, found: 547.1968.

Synthesis of **5**



To a solution of **S9** (10.0 g, 55.2 mmol) in MeOH (100 mL), was added EDCI (31.7 g, 165.6 mmol) and DMAP (0.7 g, 5.2 mmol). The resulting solution was stirred for 20 h. The resulting solution was poured into HCl (30 mL, 1 M), and then extracted with DCM (3×100 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated to afford **S10** as colorless oil (9.4 g, 87%), which was used for the next step without further purification.

To a solution of **S10** (6.3 g, 32.3 mmol) in CCl_4 (120 mL), was added NBS (6.3 g, 35.5 mmol) and BPO (62.6 mg, 0.3 mmol). The resulting solution was heated to reflux for 2 h. The solution was cooled to room temperature, and then filtered and concentrated. The crude product **S11** was got as colorless oil (8.0 g, 90%), which was used for the next step without further purification.

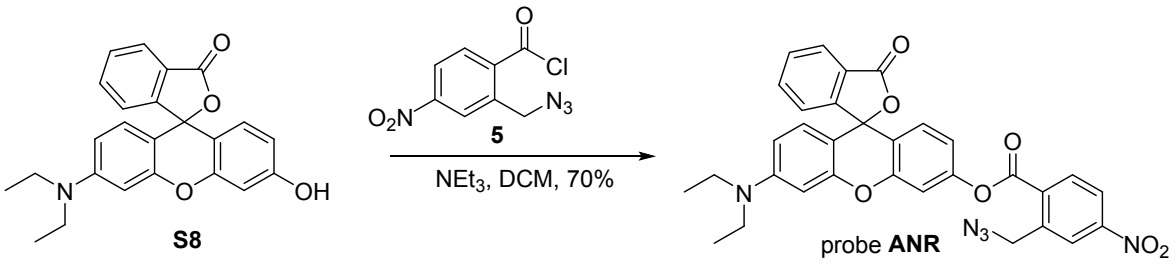
To a solution of **S11** (8.0 g, 29.1 mmol) in absolute ethanol (50 mL), was added NaN_3 (2.9 g, 43.6 mmol). The resulting solution was warmed to room temperature and stirred for 48 h. The mixture was quenched by addition of brine and extracted with ethyl acetate (3×50 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated to yield 5.8 g (85%) of **S12** as light yellow solid, which was used for the next step without further purification.

To a solution of aq NaOH–MeOH (60 mL, aq NaOH, 2 M, 1:1, v:v), was added **S12** (5.8

g, 24.7 mmol). The resulting solution was stirred for 30 min. Then the mixture was extracted with DCM (3×50 mL). The aqueous layer was acidified to pH = 3 and then extracted with DCM (3×50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to yield 4.3 g (98%) of **S13** as a light yellow solid. Data for **S13**: R_f 0.35 (petroleum ether:ethyl acetate = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 2.0, 1H), 8.07 (d, J = 8.6, 1H), 8.01 (dd, J = 8.6, 2.2, 1H), 4.74 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.57, 150.72, 141.02, 133.38, 132.25, 124.14, 122.83, 52.65; IR/cm^{-1} (thin film) vmax 3440, 2928, 2113, 1695, 811, 735; HRMS-ESI (m/z) $[M - H]^-$ calcd for $C_8H_5N_4O_4^-$, 221.0305, found: 221.0312; mp 112.5-113.9 °C.

To a solution of **S13** (2.0 g, 9.0 mmol) in DCM (30 mL), was added $SOCl_2$ (3.2 mL, 27.0 mmol) at room temperature. The resulting solution was heated to reflux for 5 h. The mixture was cooled to room temperature and concentrated to yield 2.1 g (98%) of **5** as light yellow oil, which was used for the next step without further purification.

Synthesis of probe **ANR**



To a suspension of **S8** (1.9 g, 7.7 mmol) and DMAP (1.3 g, 10.3 mmol) in DCM (20 mL) at 0 °C, was added **5** (2.0 g, 5.2 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The resulting mixture was extracted with DCM (3×30 mL). Combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated to afford colorless oil. Flash chromatography of the crude product (4:1 petroleum ether:ethyl acetate) provided the desired product as colorless oil (2.2 g, 70%). Data for **ANR**: R_f 0.35 (petroleum ether:ethyl acetate = 4:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (dd, J = 34.2, 5.4, 1H), 8.34–8.26 (m, 1H), 8.22–8.12 (m, 1H), 8.08–7.97 (m, 1H), 7.77–7.54 (m, 2H), 7.23 (d, J = 7.5, 1H), 7.17 (d, J = 1.2,

1H), 6.93–6.81 (m, 2H), 6.59 (d, J = 8.9, 1H), 6.45 (d, J = 2.4, 1H), 6.38 (dd, *J* = 9.0, 2.5, 1H), 4.99 (s, 2H), 3.37 (q, *J* = 7.0, 4H), 1.18 (t, *J* = 7.1, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.51, 163.18, 152.51, 151.42, 151.15, 150.46, 149.73, 141.00, 134.98, 132.86, 129.46, 128.90, 127.03, 126.49, 125.02, 124.14, 122.87, 120.88, 118.08, 117.79, 116.76, 116.61, 110.20, 108.73, 104.66, 97.51, 83.47, 52.57, 44.52, 12.51. IR/cm^{-1}(thin film) νmax 3450, 2928, 2113, 1761, 1515, 1419, 1345, 1105, 821, 755; HRMS-ESI (m/z) $[M + H]^+$ calcd for $C_{32}H_{26}N_5O_7^+$, 592.1827, found: 592.1836.

3. In *vitro* fluorescence spectroscopy measurement

General Procedure for H_2S Detection

All UV−vis, fluorescence, and quantum yield measurements were carried out in 10 mM PBS buffer solution containing 5% CH_3OH, pH 7.4. In a 5 mL tube, PBS buffer (4 mL) and 250 μL **ANR** (100 μM in CH_3OH) were mixed, and then 4 mM Na_2S solution (50 μL, 80 eq) was added. The final solution volume was adjusted to 5 mL with PBS buffer to obtain a final concentration of 5 μM. After rapid mixing of the solution, it was placed for 4 min then transferred to a 10 × 10 mm quartz cell and incubated at 37 °C for in vitro detection. Fluorescence spectra were recorded in the range from 537 to 700 nm with λ_{ex} = 519 nm, and absolute emission quantum yields were determined accordingly.

Quantum Yields

Fluorescence quantum yields of **ANR** was determined in PBS buffer (10 mM, pH 7.4) with rhodamine B (Φ = 0.89, in ethanol) as a reference. *N,N*-diethylrhodol **3** was obtained in the experiment by addition of 80 eq of Na_2S to the solution of probe **ANR**. The quantum yields were calculated using an Eq follows:

$$\Phi_u = [(A_s F A_u \eta^2)/ (A_u F A_s \eta_0^2)]\Phi_s.$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and

reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

Quantum yield of **ANR**: Φ = 0.0270

Quantum yield of **3**: Φ = 0. 3520

Detection limit

To determine the detection limit, the emission intensity of probe **ANR** without Na_2S was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit was then calculated with the equation: detection limit = 3σ/k, where σ was the standard deviation of blank measurements, k was the slope between intensity difference versus sample concentration. According to fluorometric method, the detection limit of probe **ANR** for H_2S was determined as 0.4327 μM.

Detection limit of **ANR** = 3σ/slope=3*0.137799/0.95526=0.4327

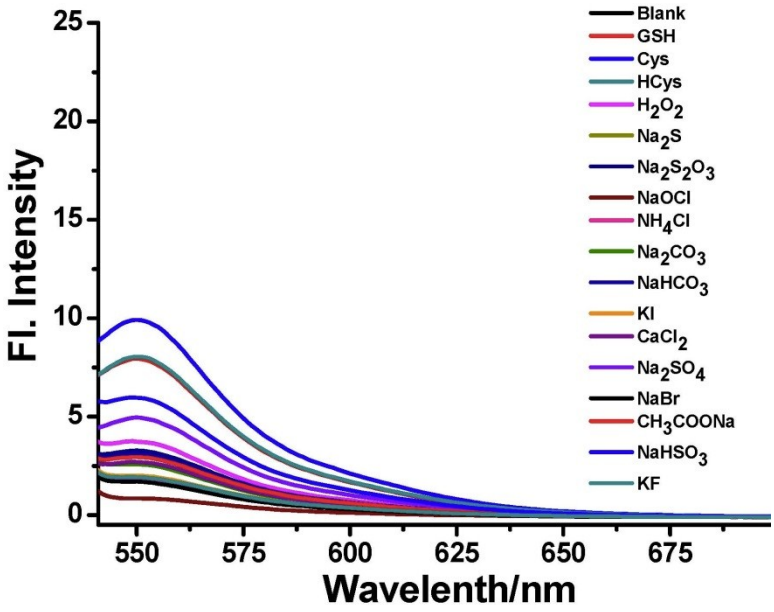


Fig.S1 Fluorescence response of **AR** (5 μM) upon addition of various species (80 eq) in CH_3OH/PBS buffer (10 mM, pH = 7.4, 5:95). λ_{ex} = 519 nm, λ_{em} = 550 nm.

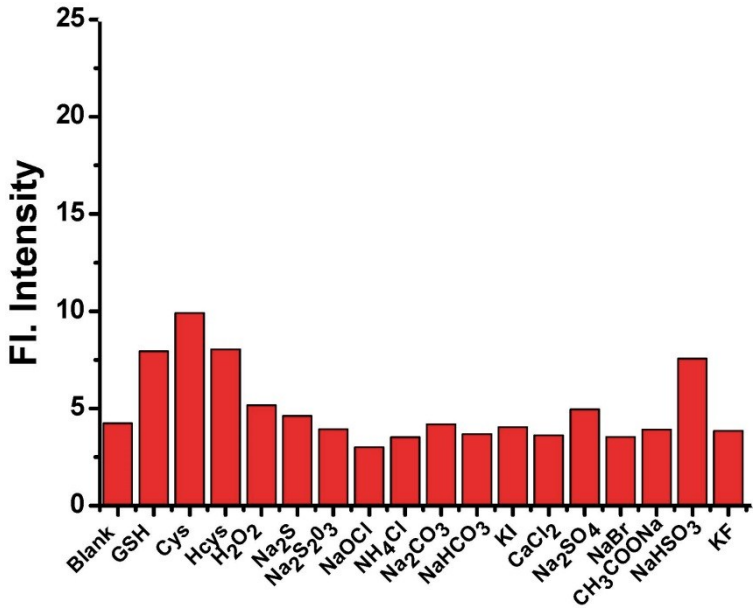


Fig.S2 Bar graph of the fluorescence response of **AR** (5 μM) upon addition of various species (80 eq) in CH_3OH/PBS buffer (10 mM, pH = 7.4, 5:95). λ_{ex} = 519 nm, λ_{em} = 550 nm. Slits: 5/5 nm.

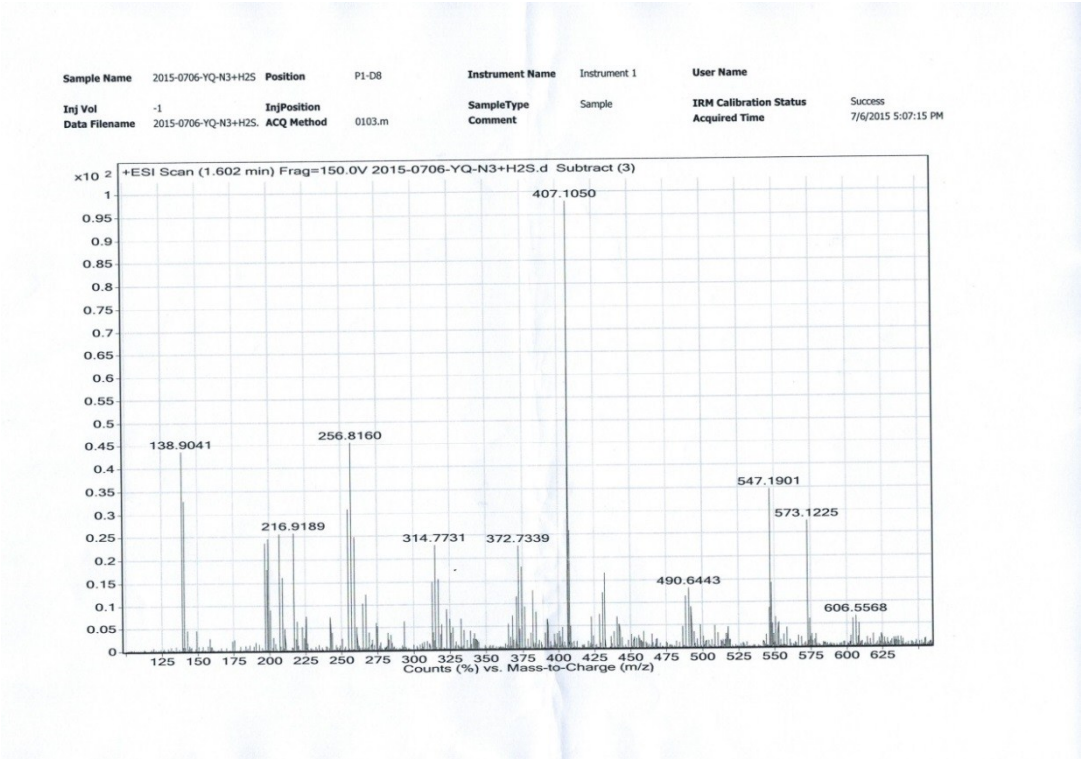


Fig.S3 HRMS chart of **AR** (5 μM) treated with Na_2S (80 eq).

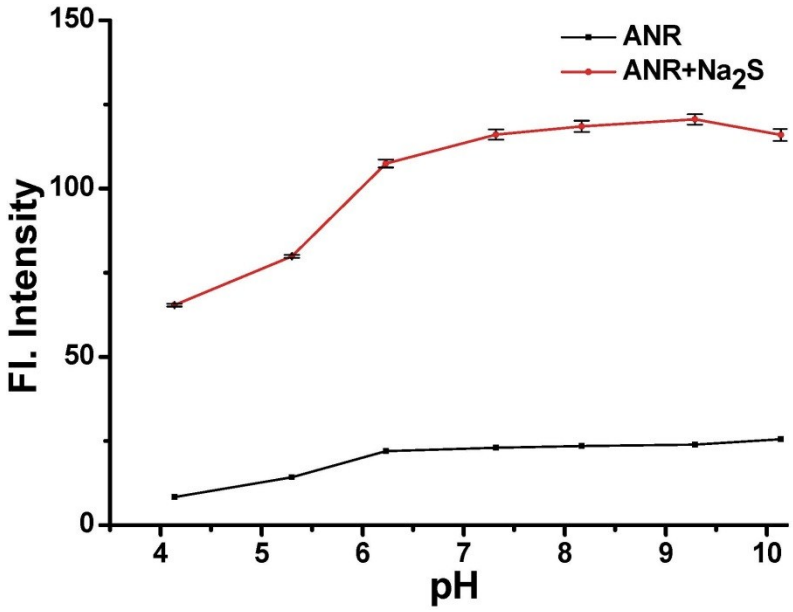


Fig.S4 Fluorescence response of **ANR** (5 μM) and **ANR** (5 μM) treated with 80 eq of Na_2S in CH_3OH/PBS buffer (10 mM, 5:95, pH from 4 to 8). λ_{ex} = 519 nm, λ_{em} = 550 nm. Slits: 5/5 nm.

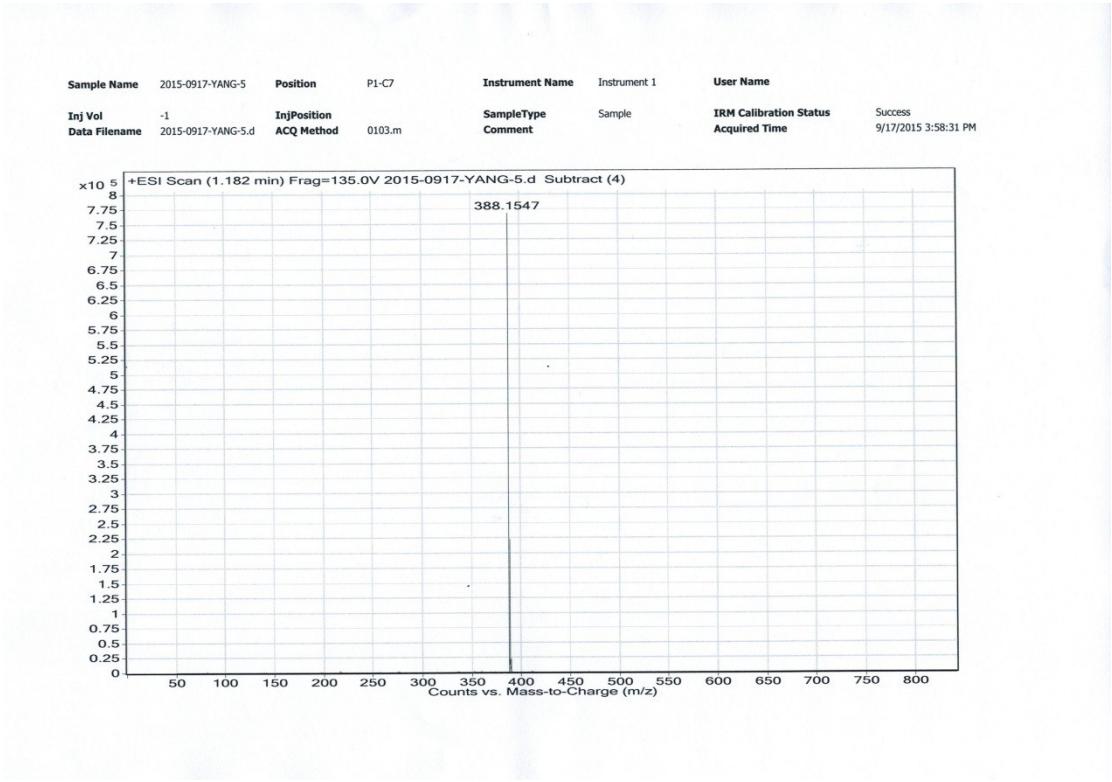


Fig.S5 HRMS chart of **ANR** treated with Na_2S (80 eq).

4. MTT assay

The MTT assay was used to evaluate the cytotoxicity of probe. MCF-7 cells and 3T3 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere containing 5% CO_2. MCF-7 cells and 3T3 cells were

seeded onto 96-well plates at a density of 1×104 cells/well and incubated for 24 h. The medium was replaced by various probe over a range of concentrations (0.5 μM to 30 μM) dissolved in culture medium. After incubation at 37 °C, 5% CO_2 for 24 h, each well of cells were treated with 20 μl MTT solution (5 mg/mL), and incubated for another 4 h. After that, the medium was removed, and 100 μL of DMSO were added to dissolve the formazan crystals. The plate was agitated for 10 min, and each well was finally analyzed by the microplate reader (Thermo Scientific, Multiskan FC) and detected by the absorbance at 570 nm.

MCF-7: IC_{50} 69.6 μM.

3T3: IC_{50} 91.5 μM.

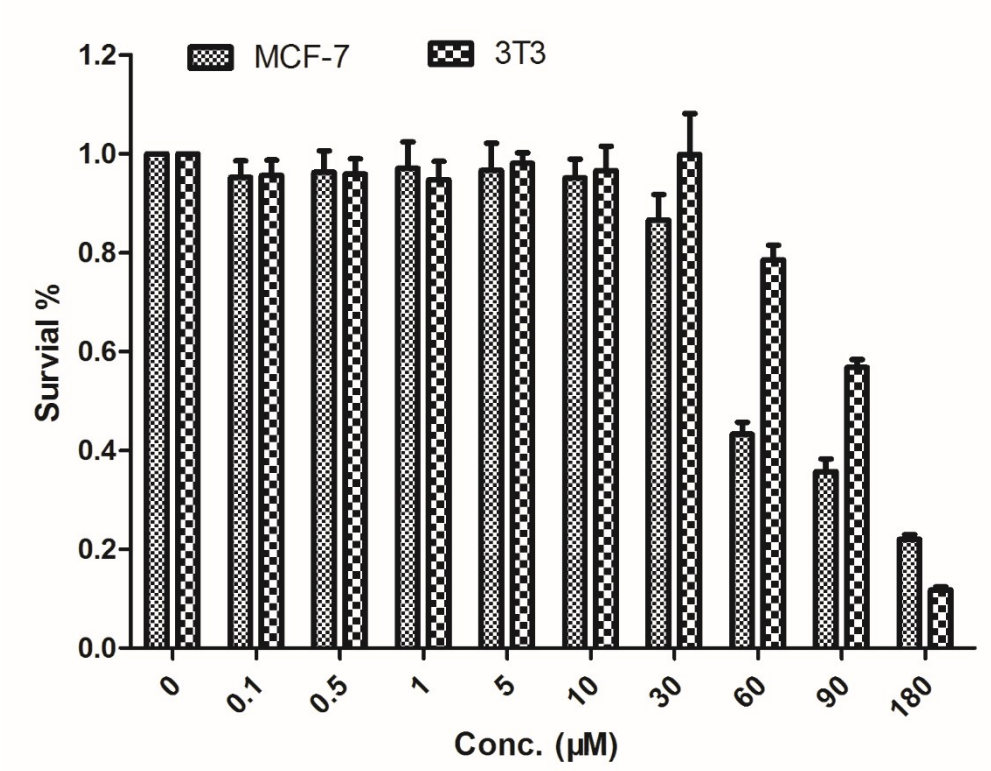


Fig.S6 The cytotoxicity of the probe **ANR** evaluated by the MTT assay

5. Cell culture and fluorescence imaging

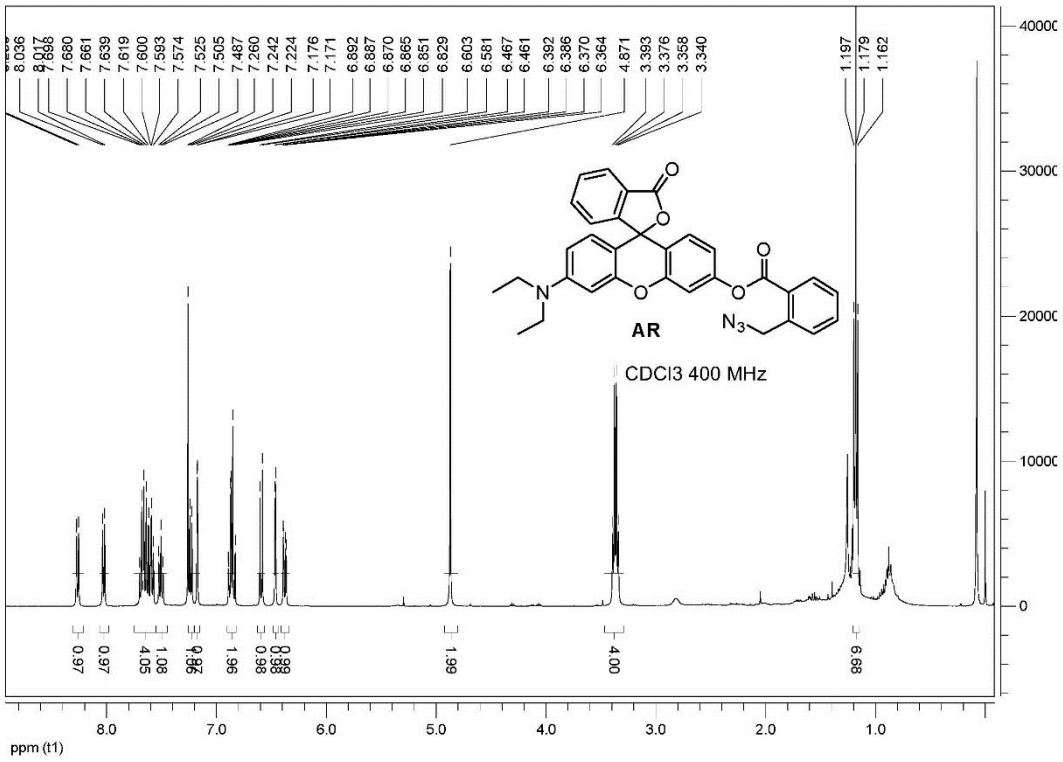
The mammalian cells MCF-7 were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere containing 5% CO_2. For live cell imaging, probe **ANR** was added to the cells and incubated for 30 min and washed with PBS (phosphate-buffered saline) three times. After replacement of the

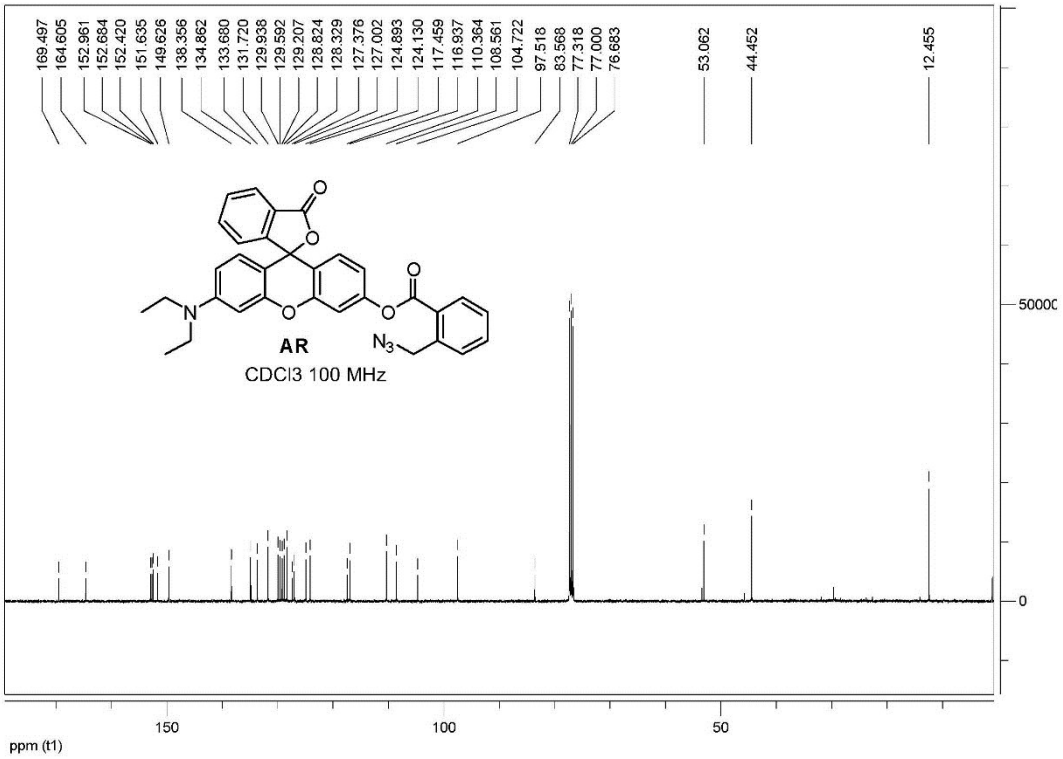
medium, cells were imaged using an Olympus (FV1000) confocal laser scanning microscope with a 200×objective lens.

6. R references

1. J. Huang; Z. Xi, Tetrahedron Lett., 2012, **53**, 3654.
2. W. Dong; H. Wen; X. -F. Yang; H. Li, Dyes Pigments, 2013, **96**, 653.

7. ^{1}H NMR, ^{13}C NMR chart of compounds AR and ANR

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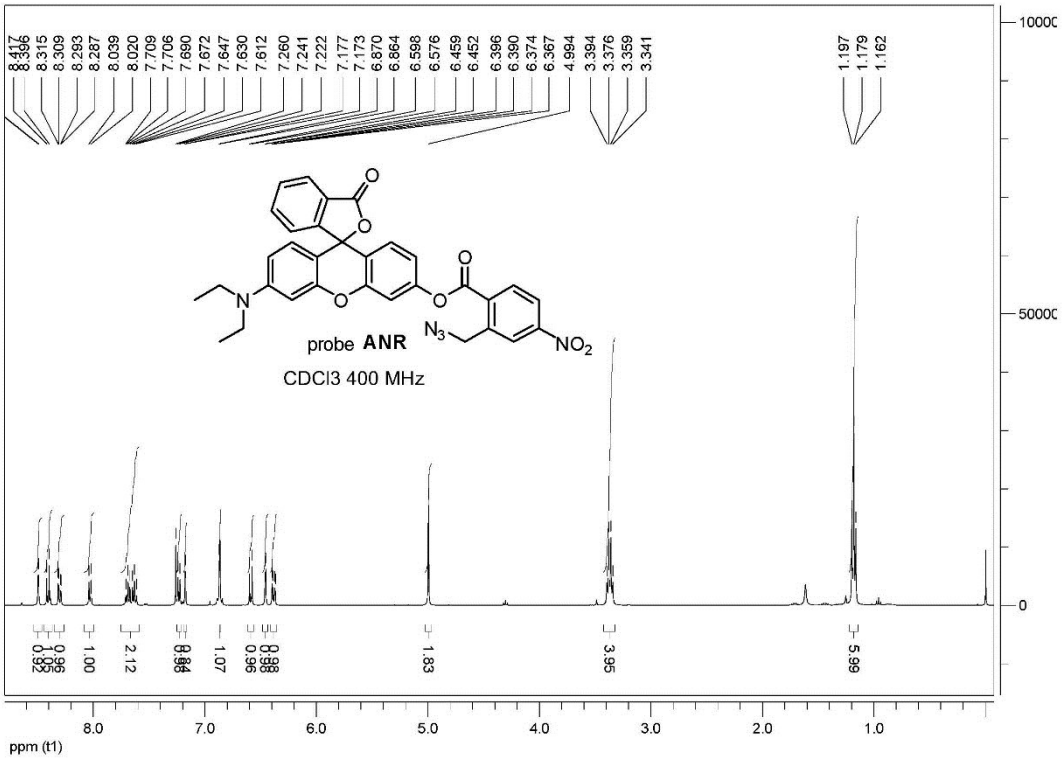
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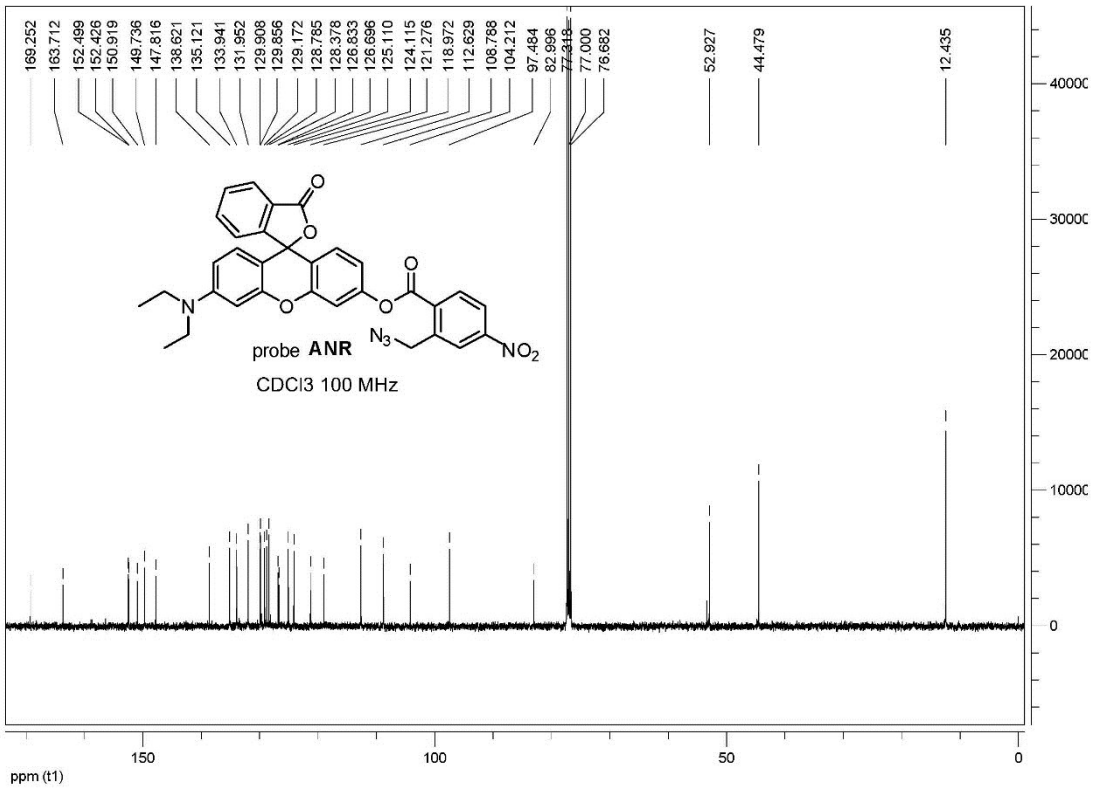
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100
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ppm (t1)