Supporting Information

Construction of a turn-on probe for fast detection of H₂S in living cells based on a novel H₂S 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

$\text{S1}$

To a solution of $\text{S1}$ (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 $\text{S2}$ was got as colorless oil (14.0 g, 92%), which was used for the next step without further purification.
To a solution of $S_2$ (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 $S_3$ 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 $S_3$ (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 $S_4$ as a white solid. Data for $S_4$: $R_f$ 0.35 (petroleum ether:ethyl acetate = 1:1); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 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$_8$H$_7$N$_3$O$_2$: 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 $S_4$ (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 $S_8$

To a suspension of $S_6$ (6.0 g, 19.1 mmol) and resorcinol $S_7$ (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: Rf 0.35 (petroleum ether:ethyl acetate = 1:1); ¹H 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 C₂₄H₂₁NO₄: 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 ¹H 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 MgSO₄ 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: Rf 0.35 (petroleum ether:ethyl acetate = 4:1); ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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)νmax 3450, 2976, 2928, 2103, 1733, 11419, 1105, 755; HRMS-ESI (m/z) [M + H]+ calcd for C₃₂H₂₇N₄O₅⁺, 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 MgSO₄ 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 CCl₄ (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 NaN₃ (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 MgSO₄, 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$_3$) $\delta$ 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$_3$) $\delta$ 170.57, 150.72, 141.02, 133.38, 132.25, 124.14, 122.83, 52.65; IR/cm$^{-1}$ (thin film) v$_{max}$ 3440, 2928, 2113, 1695, 811, 735; HRMS-ESI (m/z) [M - H]$-$ calcld for C$_9$H$_5$N$_4$O$_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**

![Chemical structure](image)

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$_3$) $\delta$ 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); 13C NMR (100 MHz, CDCl3) 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 C32H26N5O7+, 592.1827, found: 592.1836.

3. In vitro fluorescence spectroscopy measurement

General Procedure for H2S Detection

All UV−vis, fluorescence, and quantum yield measurements were carried out in 10 mM PBS buffer solution containing 5% CH3OH, pH 7.4. In a 5 mL tube, PBS buffer (4 mL) and 250 μL ANR (100 μM in CH3OH) were mixed, and then 4 mM Na2S 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 λex = 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 Na2S to the solution of probe ANR. The quantum yields were calculated using an Eq follows:

Φu = [(AsFAuη2)/(AuFAuη02)]Φs.

Where As and Au 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 η0 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**: $\Phi = 0.0270$

Quantum yield of **3**: $\Phi = 0.3520$

**Detection limit**

To determine the detection limit, the emission intensity of probe **ANR** without Na$_2$S 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 = $3\sigma/k$, where $\sigma$ 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$_2$S was determined as 0.4327 $\mu$M.

Detection limit of **ANR** = $3\sigma$/slope=$3\times0.137799/0.95526=0.4327$

![Fig.S1 Fluorescence response of AR (5 $\mu$M) upon addition of various species (80 eq) in CH$_3$OH/PBS buffer (10 mM, pH = 7.4, 5:95). $\lambda_{ex} = 519$ nm, $\lambda_{em} = 550$ nm.](image-url)
Fig.S2 Bar graph of the fluorescence response of AR (5 μM) upon addition of various species (80 eq) in CH₃OH/PBS buffer (10 mM, pH = 7.4, 5:95). λₑₓ = 519 nm, λₑₘ = 550 nm. Slits: 5/5 nm.

Fig.S3 HRMS chart of AR (5 μM) treated with Na₂S (80 eq).
Fig. S4 Fluorescence response of ANR (5 μM) and ANR (5 μM) treated with 80 eq of Na₂S in CH₃OH/PBS buffer (10 mM, 5:95, pH from 4 to 8). λ_{em} = 519 nm, λ_{ex} = 550 nm. Slits: 5/5 nm.

Fig. S5 HRMS chart of ANR treated with Na₂S (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% CO₂. 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% CO₂ 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: IC₅₀ 69.6 μM.
3T3: IC₅₀ 91.5 μM.

![Graph showing cytotoxicity of the probe ANR](image)

**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% CO₂. 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. References


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