Detection of H_2S by a novel fluorescence nanoprobe in plasma and tissue of ASD patients and model mice

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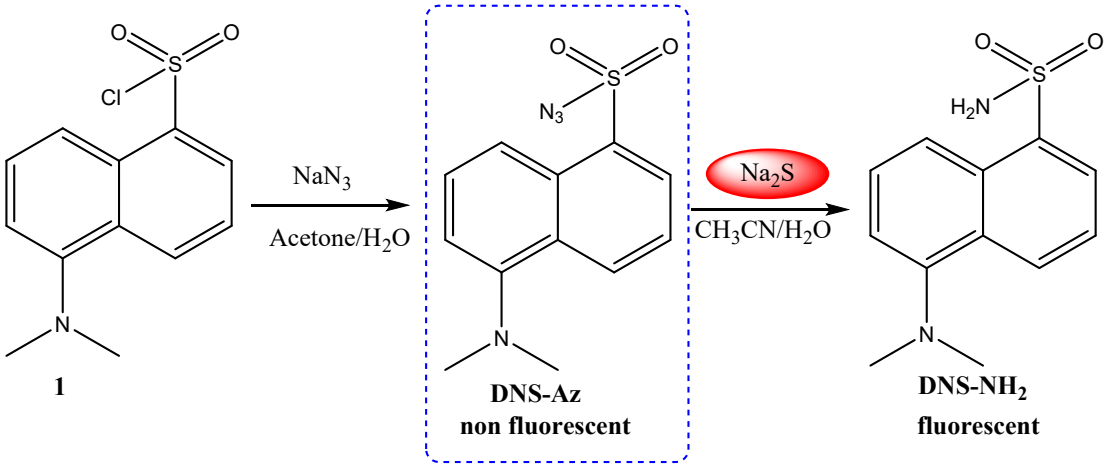
1. Materials

Actone, sodium azide, $MgSO_4$, sodium sulfide nonahydrate, methanol were purchased from Innochem. All other used agents were of the highest commercial grade available.

2. Methods

2.1 Synthesis of H_2S probe (DNS-Az)



Scheme 1. Synthesis of dansyl azide (**DNS-Az**) and **DNS-NH_2**.

A solution of compound **1** (300 mg, 1.12 mmol) in 4 mL of actone was added dropwise into a stirred solution of sodium azide in 12 mL of a mixed solvent (H_2O/Actone, 2:1). Then the reaction mixture was stirred at room temperature for 5 h. The mixture was was evaporated in vacuum to remove the organic solvent, and extracted by DCM. The combined organic layers was washed with brine and then dried over $MgSO_4$. Solvent evaporation gave the crude product, which was purified by flash chromatography to give **DNS-Az** (260 mg, 84.5%) as a light yellow oil. A solution of **DNS-Az** (200 mg, 0.72 mmol) in 20 mL of CH_3CN was added into a solution of sodium sulfide nonahydrate (518 mg, 2.16 mmol) in 1 mL H_2O, Then the reaction mixture was stirred at room temperature for 3 h to finally give **DNS-NH_2** (100 mg, 55.2%) as while solid.

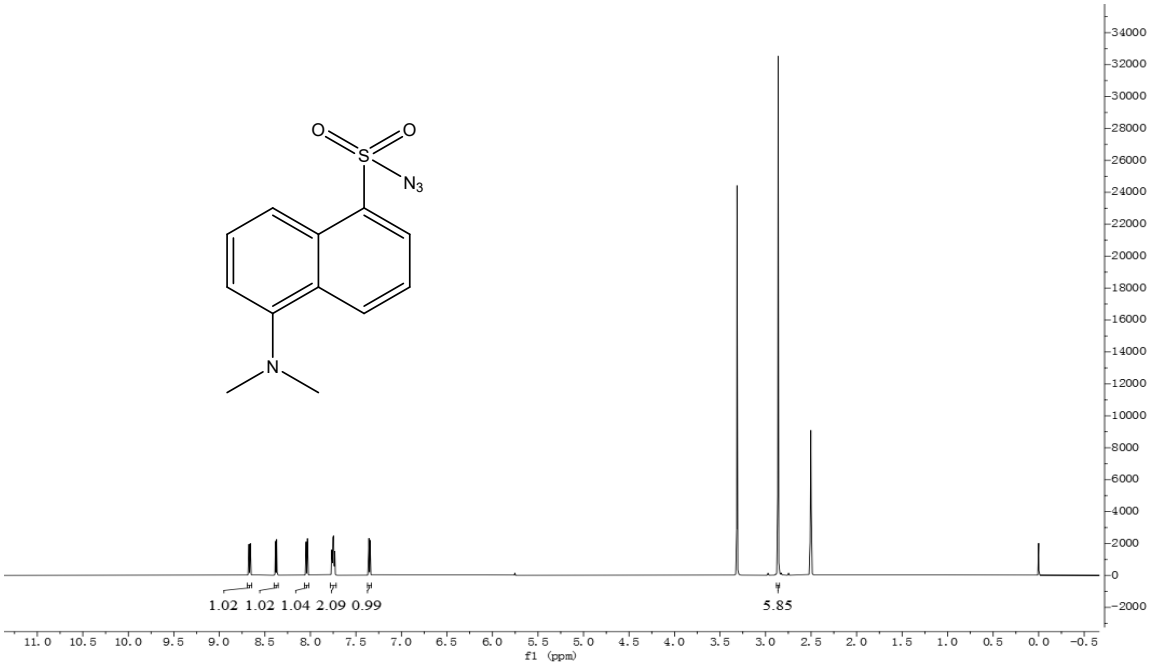


Figure S1. ^{1}H NMR spectrum of **DNS-Az**. 1H NMR (600 MHz, DMSO-d6) δ 8.67 (dd, J = 8.5, 1.2 Hz, 1H), 8.38 (dd, J = 7.4, 1.3 Hz, 1H), 8.04 (dd, J = 8.7, 1.0 Hz, 1H), 7.75 (ddd, J = 8.5, 7.4, 4.2 Hz, 2H), 7.35 (d, J = 7.6 Hz, 1H), 2.86 (d, J = 2.0 Hz, 6H).

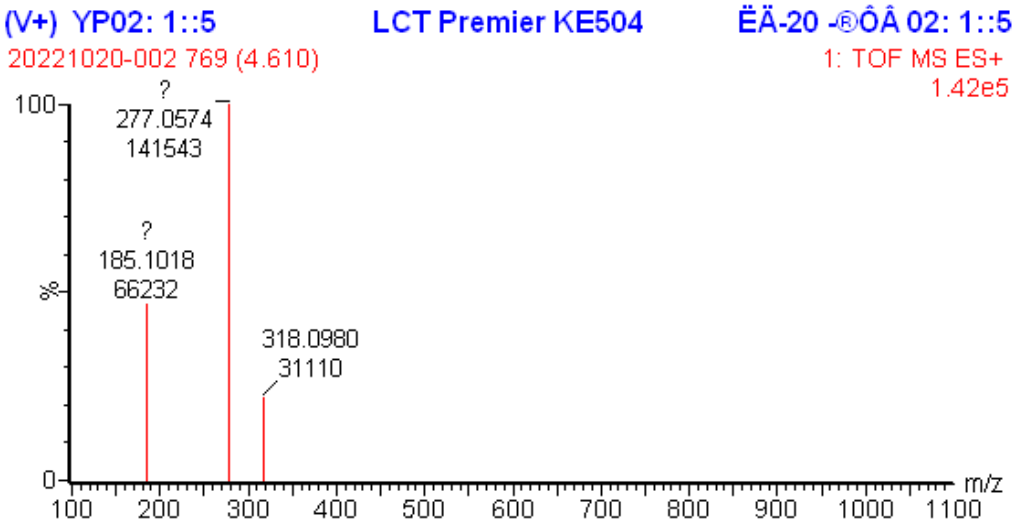


Figure S2. HRMS of **DNS-Az**. HRMS (ESI) Calcd for $C_{12}H_{12}N_4O_2S[M+H]^+$ 277; found 277.

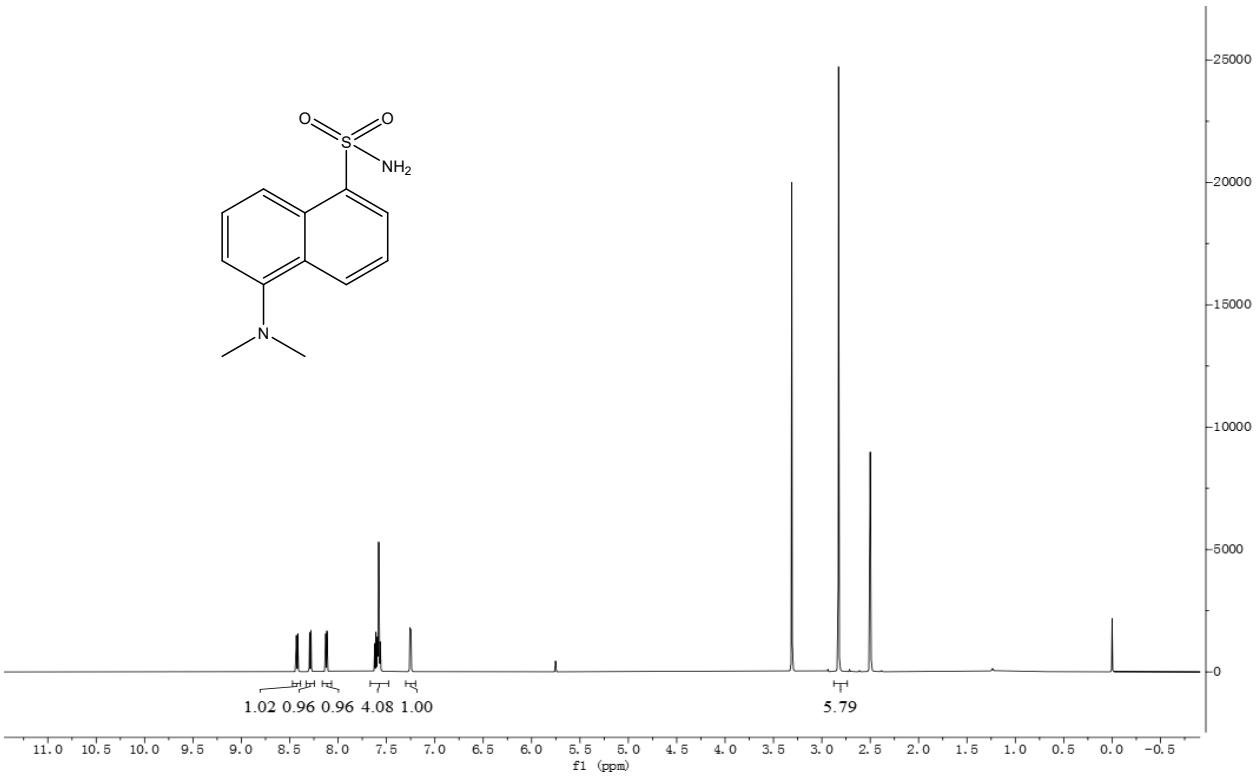


Figure S3. ^{1}H NMR spectrum of **DNS-NH$_2$.** 1H NMR (600 MHz, DMSO-d6) δ 8.42 (dt, J = 8.5, 1.1 Hz, 1H), 8.36 – 8.24 (m, 1H), 8.12 (dd, J = 7.3, 1.3 Hz, 1H), 7.68 – 7.45 (m, 4H), 7.31 – 7.20 (m, 1H), 2.83 (s, 6H).

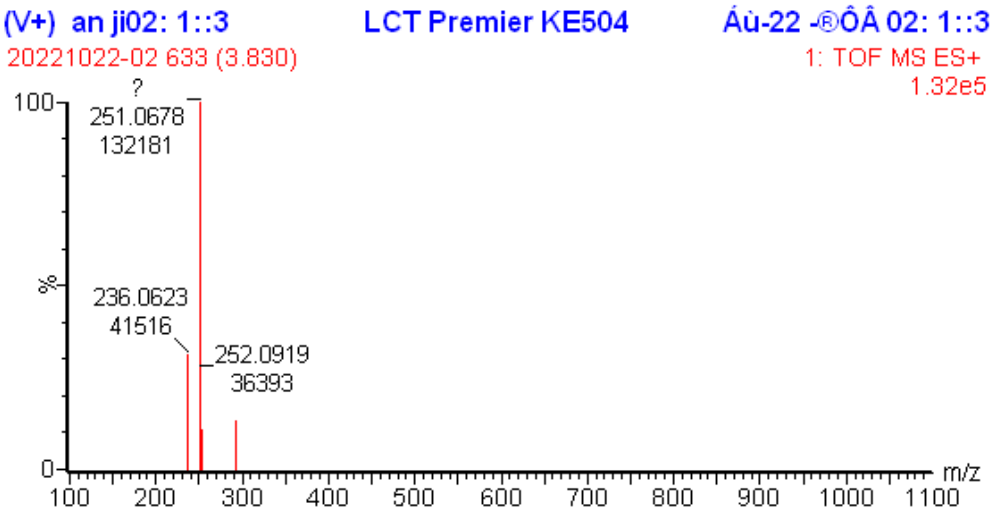


Figure S4. HRMS of **DNS-NH$_2$**. HRMS (ESI) Calcd for $C_{12}H_{14}N_2O_2S[M+H]^+$ 251; found 251.

2.2 DNS-Az-M content detection

DNS-Az is prepared into 1 mg/mL solution with ethanol and diluted to 10, 20, 50, 100, 200, 400 μg/mL, respectively. The different concentrations of **DNS-Az** solution was detected at 350 nm to draw a standard standard curve. **DNS-Az-M** solution 100 μL was added methanol 500 μL, and mixed to obtain demulsified solution, then the OD was detected at 350 nm. **DNS-Az-M** content was calculated according to standard curve of **DNS-Az**.

Figure S5. Standard curve of **DNS-Az**.

2.3 Study on protein removal by different proportion methanol

Plasma 100 μL were added different proportion methanol (volume ratio of 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5), then the mixture was centrifuged at 12000 rpm for 10 minutes at 4℃. Supernatant (100 μL) was gently transferred into 96-well plate, and **DNS-Az-M** (0.4 μL, final concentration 200 μM) was added. Fluorescence intensity was measured every 10 min. λ_{ex}=340 nm，λ_{em}=535 nm.

Figure S6. Fluorescence intensity change in the reaction of **DNS-Az-M** with plasma without methanol treatment.

Figure S7. Fluorescence intensity change of reaction between **DNS-Az-M** and plasma after methanol treatment in different proportions.