ELECTRONIC SUPPLEMENTARY INFORMATION

Design strategy for analyte-compensated fluorescent probe to reduce the toxicity

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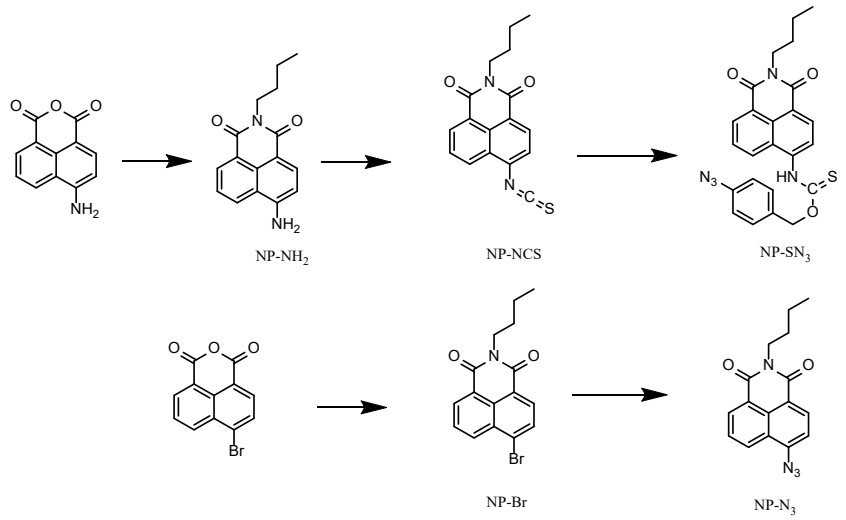
1. Materials and General Methods

1.1. Reagents and instruments

All reagents used in the experiment are of analytical grade unless otherwise stated and no further purification is required. All reagents were purchased from legal commercial sources. The NMR spectra were measured using Liquid Nuclear Magnetic Resonance Spectrometer JNM-ECS 400 M (Japan). The fluorescence emission spectra were recorded on a fluorescence spectrometer RF-5301pc (Japan). The absorption spectra were measured on an ultraviolet–visible spectrophotometer TU-1810 (China). A Bruker Daltonics APEX II FT-ICR spectrometer (USA) with ESI mode was used to measure high-resolution mass spectra. The digital pH meter PHSJ-3F (China) was used to measure the pH value. A confocal laser scanning microscope Axio Imager.Z2 (Germany) with objective lens (× 20) was used to take fluorescence images of cells. Cell viability and cytokines were recorded by using a microplate reader Flash3001 (USA). BCA protein quantification kit, α-SMA enzyme-linked immunoassay kit were purchased from J&L Biological (China). HepG2, HSC-T6 were provided by the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Zebrafish embryos were purchased from Nanjing Yishulihua Biotechnology Co. Ltd.

1.2. Synthesis



Scheme S1 Synthetic Route to Probes

1.2.1. Synthesis of NP-NH_2

4-Amino-1,8-naphthalenedicarboxylic anhydride (400 mg, 1.88 mmol) and 1-butylamine (411.7 mg, 3.01 mmol) were dissolved in ethanol (25 mL) in a round bottom flask under argon, heated to reflux for 4 hours, cooled to room temperature, evaporated under reduced pressure to remove the solvent, and purified by column chromatography to obtain the product. (hexane:ethyl acetate=7:3) ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.61 – 8.52 (m, 1H), 8.38 (d, *J* = 7.2 Hz, 1H), 8.18 – 8.10 (m, 1H), 7.61

(d, J = 8.8 Hz, 1H), 7.40 (s, 2H), 6.82 – 6.76 (m, 1H), 3.99 – 3.92 (m, 2H), 1.60 – 1.46 (m, 2H), 1.28 (s, 2H), 0.87 (d, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-D_6) δ 164.28, 163.42, 153.21, 134.46, 131.50, 130.19, 129.79, 124.49, 122.32, 119.88, 108.67, 108.08, 40.24, 30.37, 20.37, 19.18, 14.29. HRMS (ESI) $[M+H]^+$ calcd for $C_{16}H_{17}N_2O_2$, 269.1290, found, 269.1286.

1.2.2. Synthesis of NP-NCS

Thiophosgene dissolved in acetone (25 mL) was added dropwise to **NP-NH$_2$** (286 mg, 1.02 mmol) in acetone (70 mL) over 30 min at 0 °C. The reaction mixture was stirred at room temperature overnight, then the solvent was evaporated under reduced pressure, and the product was purified by column chromatography. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.58 (s, 1H), 8.46 (d, J = 8.0 Hz, 1H), 8.36 (d, J = 8.8 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.57 (d, J = 9.7 Hz, 1H), 4.12 (d, J = 2.2 Hz, 2H), 1.65 (s, 2H), 1.47 – 1.36 (m, 2H), 0.94 (d, J = 8.3 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*)) δ 164.02, 163.60, 143.38, 132.21, 131.69, 129.16, 128.73, 126.89, 124.36, 122.71, 119.01, 114.70, 65.65, 40.34, 30.29, 20.47, 13.94. HRMS (ESI) $[M+H]^+$ calcd for $C_{17}H_{15}N_2O_2S$, 311.0854, found, 311.0863.

1.2.3. Synthesis of NP-Br

4-Bromo-1,8-naphthalenedicarboxylic anhydride (4.0 g, 14.43 mmol) was dissolved in ethanol (80 mL) and butylamine (1.005 g, 14.43 mmol) was added. The reaction mixture was stirred in refluxing ethanol for 24 hours. After cooling to room temperature, the mixture was filtered and dried under reduced pressure to give a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.64 (d, J = 7.3 Hz, 1H), 8.54 (d, J = 8.5 Hz, 1H), 8.39 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.85 – 7.80 (m, 1H), 4.19 – 4.12 (m, 2H), 1.70 (p, J = 7.6 Hz, 2H), 1.43 (h, J = 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.72, 163.70, 133.30, 132.09, 131.29, 131.17, 130.71, 130.27, 129.09, 128.16, 123.24, 122.38, 40.47, 30.25, 20.45, 13.93. HRMS (ESI) $[M+H]^+$ calcd for $C_{16}H_{15}BrNO_2$, 332.0286, found, 332.0280.

1.2.4. Synthesis NP-SN$_3$

NaH (40 mg, 60% in oil) was dissolved in 10 mL anhydrous THF, p-azidobenzyl alcohol (149 mg) was added to this solvent at 0 °C. The compound **NP-NCS** (310 mg) was dissolved in 10 mL THF and added dropwise to the mixture. The resultant mixture was stirred at 0 °C for 30 min, then stirred at r.t. until the completion of the reaction indicated by TLC. The reaction was quenched by adding brine and the reactant was extracted with ethyl acetate. The organic layers were combined, dried over $MgSO_4$, and evaporated under vacuum. The crude product was purified by column chromatography to give a pale-yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 – 8.50 (m, 2H), 8.24 (d, J = 9.5 Hz, 1H), 7.73 (s, 1H), 7.36 – 7.30 (m, 2H), 7.01 – 6.94 (m, 2H), 5.56 (s, 2H), 4.13 (d, J = 3.3 Hz, 2H), 1.66 (d, J = 12.4 Hz, 2H), 1.46 – 1.34 (m, 2H), 1.24 (s, 2H), 0.94 (d, J = 10.6 Hz, 3H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 189.34, 163.99, 163.51, 140.73, 131.64, 131.39, 131.33, 130.37, 129.01, 127.77, 127.38, 126.21, 123.35, 120.98, 119.32, 99.99, 77.32, 73.19,

53.53, 40.40, 30.25, 29.78, 20.44, 13.90. HRMS (ESI) $[M-H]^-$ calcd for $C_{24}H_{20}N_5O_3S$, 458.1281, found, 458.1305.

1.2.5. Synthesis of NP-N_3

NP-Br (3.31 g, 10 mmol) and NaN3 (0.65 g, 10 mmol) were dissolved in 50 mL of anhydrous DMF. After stirring the mixture at 80°C for 6 h, the solution was diluted with H_2O and extracted with EtOAc. The organic phase was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography gave a yellow solid. ^1H NMR (400 MHz, Chloroform-*d*) δ 8.61 – 8.56 (m, 1H), 8.52 (d, *J* = 9.6 Hz, 1H), 8.37 (d, *J* = 9.9 Hz, 1H), 7.69 (s, 1H), 7.41 (d, *J* = 10.8 Hz, 1H), 4.17 – 4.10 (m, 2H), 1.74 – 1.63 (m, 2H), 1.41 (d, *J* = 13.2 Hz, 2H), 0.95 (dd, *J* = 10.1, 6.2 Hz, 3H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.02, 163.60, 143.38, 132.21, 131.69, 129.16, 128.73, 126.89, 124.36, 122.71, 119.01, 114.70, 40.34, 30.29, 20.47, 13.94. HRMS (ESI) $[M+Na]^+$ calcd for $C_{16}H_{14}N_4NaO_2$, 317.1042, found, 317.1065.

1.3. Spectrometric Study

The probe **NP-SN_3** (or **NP-N_3**) was dissolved in DMSO to obtain a 1.0 mM stock solution. Stock solutions (10.0 mM) of analytes, including metal ions (K^+, Na^+, Mg^{2+}), anions (F^-, Cl^-, Br^-, I^-, NO_3^-, SO_3^{2-}, HSO_3^-, SO_4^{2-}, AcO^-), amino acids (GSH (1.0 mM), Hcy, Cys,Thr, Gly), and active oxygen (ClO^-, H_2O_2) were prepared by dissolving in phosphate-buffered saline (PBS). All spectrometric measurements were performed in DMSO/PBS (v/v = 4:1, pH = 7.4, 10 mM) at 37 °C with 10.0 μM of probe and 200 μM of analyte, unless otherwise specified. The fluorescence experiment was performed with the excitation and emission wavelengths 450/520 nm (slit width: 3 and 3 nm, respectively) and 1.0 cm Quartz cuvette.

1.4. Imaging experiments

1.4.1. Fluorescence Imaging of H_2S in Cells

HepG2 and HSC-T6 cells were grown in Dulbecco's modified Eagle's medium (DMEM) including 10% fetal bovine serum (FBS) and 1% antibiotics (streptomycin, Hyclone) at 37 °C in a humidified incubator which contained 5% CO_2 and 95% air.

The cytotoxicity of **NP-SN_3** (or **NP-N_3**) were investigated with Cell Counting Kit-8 (CCK-8). The cells were incubated for 12 h upon different concentrations (0, 2.5, 5.0, 10.0, 20.0, 25.0, and 50.0 μM) of the probes and CCK-8 (10%) solution was added into each well. Then, the plate was incubated further for 2 h before the measurement of the absorbance values of each well using a microplate reader at 450 nm with six sets for each concentration.

HepG2 cells were inoculated in confocal plates for 24 h, and washed with PBS three times after adherence. For the blank group, HepG2 cells were incubated with the probes at 37 °C for 0.5 h before imaging. For the exogenous H_2S group, the cells were pretreated with 100 μM NaHS at 37 °C for 0.5 h and then operated as that as the blank group. For the endogenous H_2S group, the cells were pretreated with 200 μM L-Cys at 37 °C for 1 h and then treated as that as the blank group. In the control group, cells

were pretreated with 1 mM DL-propargylglycine (PAG) for 1 h and with L-Cys (200 μM) for 1 h at 37 °C in sequence, then treated as the way of blank group. The concentration of **probes** used for imaging of cell was 10 μM.

1.4.2. Fluorescence Imaging of H_2S in Zebrafish

Embryos were examined under a dissecting microscope and normally developing specimens were selected for further experiments as described in the literature (Kimmel, Ballard et al. 1995). Eligible zebrafish embryos were divided into three groups for bioimaging. In the first group, the probes were added to zebrafish larvae to incubate for 0.5 h. In the second group, the zebrafish larvae was pretreated with NaHS (100 μM) for 0.5 h and then treated as the way for the first group. In the third group, zebrafish embryos were incubated with the probes and hatched into larvae at 28°C. The zebrafish embryos and the larvae from the three groups were imaged separately after washing with PBS. The concentration of two probes used for imaging of larvae and embryos was 10 μM.

1.5. Detection of H_2S Released

1.5.1. Detection of H_2S Released by Probes *in vitro*

We chose the probe $CySO_3N_3$ as another probe to detect hydrogen sulfide. In DMSO: PBS (v/v=4:1) solution, the probe NP-SN_3 (50 μM) or NP-N_3 (50 μM) was reacted with NaHS (50 μM) for 1 h, and then the probe $CySO_3N_3$ was added. The fluorescence emission intensities were recorded separately at different excitation wavelengths (450 nm and 675 nm).

1.5.2. Detection of H_2S Released by Probes in Cells

HePG2 cells were divided into three groups. The first group only added probe $CySO_3N_3$ as blank control. The second group added probe NP-N_3 and incubated for 1 h before adding probe $CySO_3N_3$. The third group added probe NP-SN_3 and incubated for 1 h before adding probe $CySO_3N_3$. All probe concentrations in imaging experiments were 10 μM.

1.5.3. Detection of H_2S Released by Probes in Zebrafish

Zebrafish larvae were divided into two groups. The first group added probe NP-N_3 and incubated for 1 h before adding probe $CySO_3N_3$. The second group added probe NP-SN_3 and incubated for 1 h before adding probe $CySO_3N_3$. All probe concentrations in imaging experiments were 10 μM.

1.6. Assessment of Liver Fibrosis

HSC-T6 cells were seeded in a twelve-well plate at a density of 2.5×10^4 /mL and cultured for 24 h. The cells were divided into two groups, blank group and LPS group. For blank group, HSC-T6 cells were incubated with **NP-SN_3** or **NP-N_3** for 0.5 h at 37°C for imaging. For LPS group, HSC-T6 cells were incubated with LPS (10 μg/L) for 24 h, followed by incubation with **NP-SN_3** or **NP-N_3** at 37 °C for 0.5 h for imaging.

The levels of cytokine α-SMA were assessed with the above cells using a commercially available α-SMA kit according to the manufacturer's instruction. After lysis with RIPA Lysis buffer, HSC-T6 cells were collected and centrifuged at 12,000 g for 10 min to remove any particle. After the protein concentration was measured by the BCA protein assay kit, a protein solution at a concentration of 0.1 mg/mL was added to a 96-well plate pre-coated with mouse affinity-purified polyclonal antibodies against α-SMA cytokines. The cytokine level in each sample was calculated from the standard curve according to the kit operation instruction.

1.7. Toxicity

1.7.1. Developmental Toxicity in Zebrafish

We selected a certain number of normal zebrafish embryos and divided the embryos into 3 groups: blank group, solvent group and experimental group. It consisted of 100 embryos in each group, and each group consisted of 3 parallel groups. The experimental group were added with different concentrations (1, 5, 10, 20 μM) of **NP-N_3** or **NP-SN_3**. DMSO (10 μL) was added to the solvent group. All the treated zebrafish embryos were stored in sterile 96-well plates containing 200 μL of solution per well, covered with sealing film. The numbers of dead zebrafish embryos were recorded separately after 24 h and the numbers of hatching zebrafish embryos were recorded separately after 48 h in each group. Ten zebrafish larvae were randomly selected in each group after 72 h to observe under a microscope. The number of malforms was recorded.

1.7.2. Continuous Administration Toxicity

WISTAR rats (GLP laboratory of the Laboratory Animal Center of Lanzhou University, 8 weeks old, weighing 200 ± 25 g, 1 animal per cage, reared in separate cages, free to eat and drink, and experiment after 7 days of rearing) were chosen to study the toxicity of probes after continuous administration. The **NP-N_3** or **NP-SN_3** was dissolved in DMSO. In the experimental group, normal WISTAR rats were intraperitoneally injected with the probe at a dose of 0.01 mol/kg, and the control group was treated with the same dose of DMSO. The administration was continued for 14 days, then the liver, kidney and spleen were collected. The collected organs were immediately fixed with 4% glutaraldehyde solution, sliced, stained and observed under a microscope. Collect the urine and blood from rats for testing.

The zebrafish and rats use and handling protocol conformed to *the Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Use and Care Committee (IAUCC) of the Lanzhou University.

2. Structure Characterizations

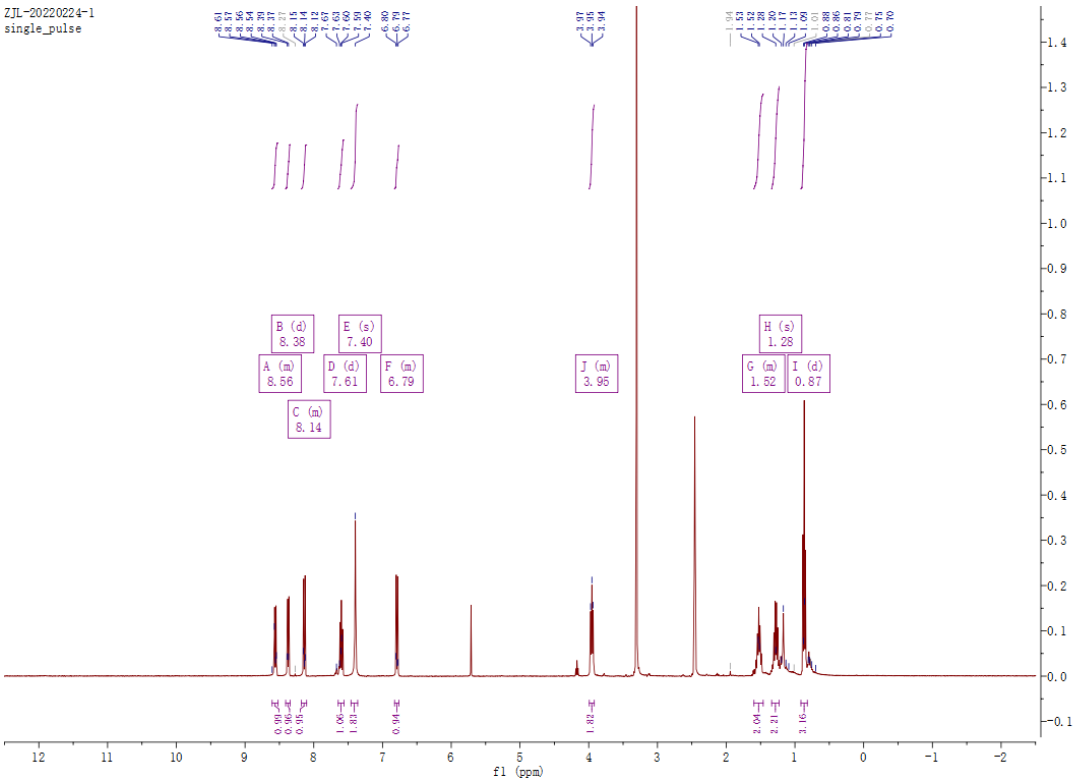


Fig. S1 ^{1}H NMR of **NP-NH$_2$**

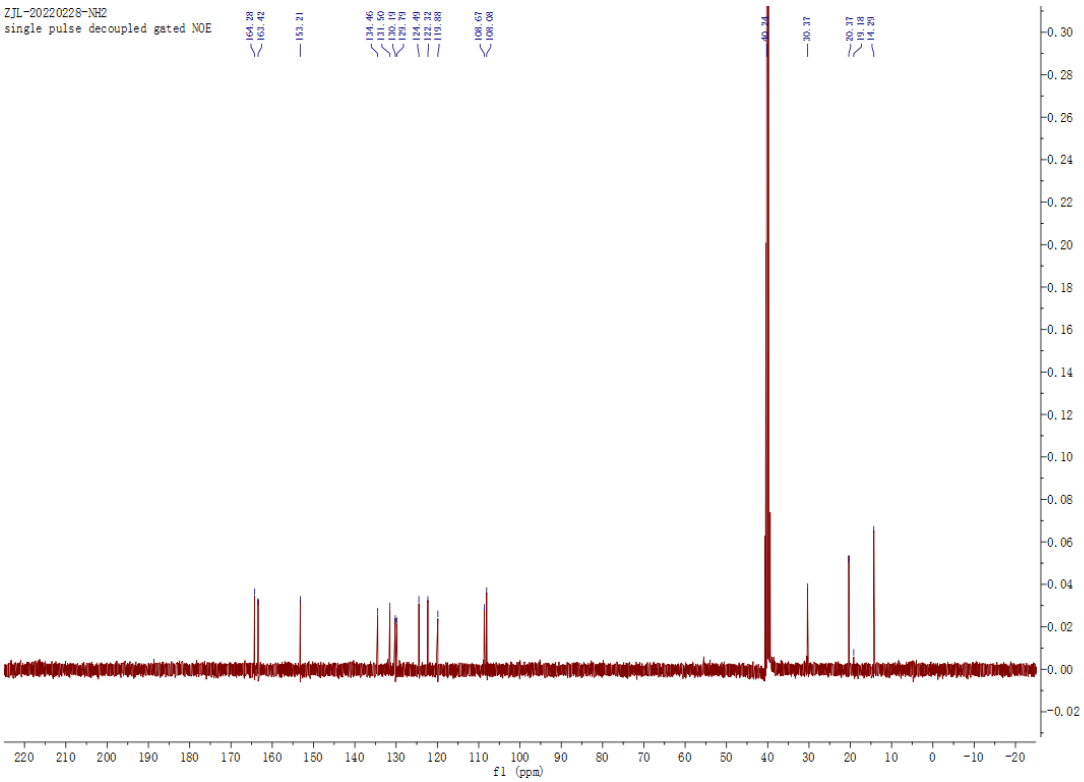


Fig. S2 ^{13}C NMR of **NP-NH$_2$**

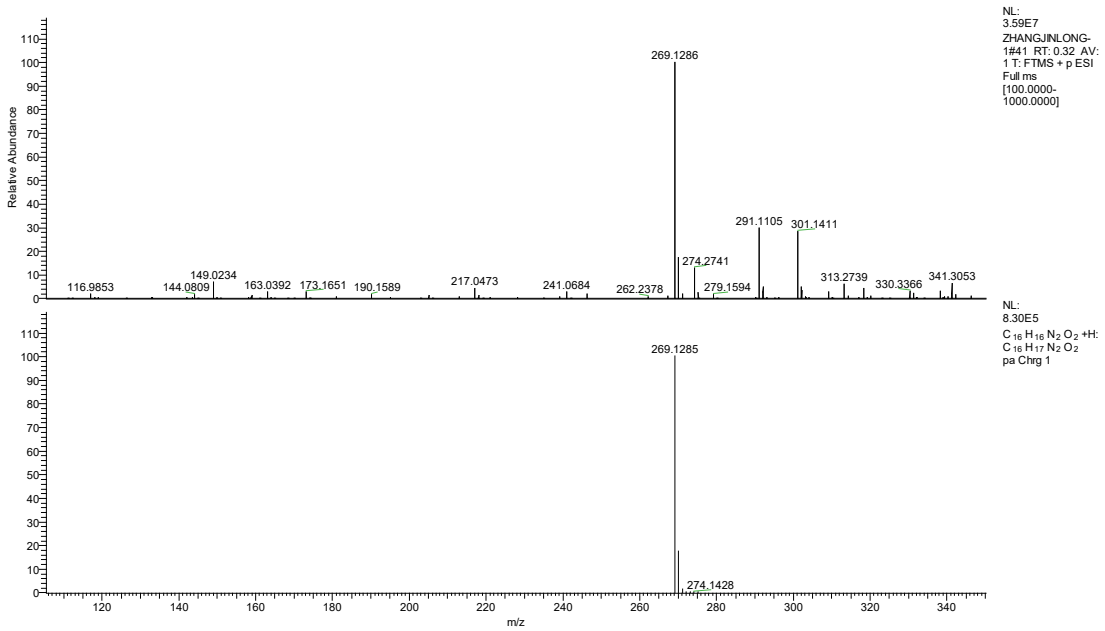


Fig. S3 Mass spectrum of **NP-NH$_2$**

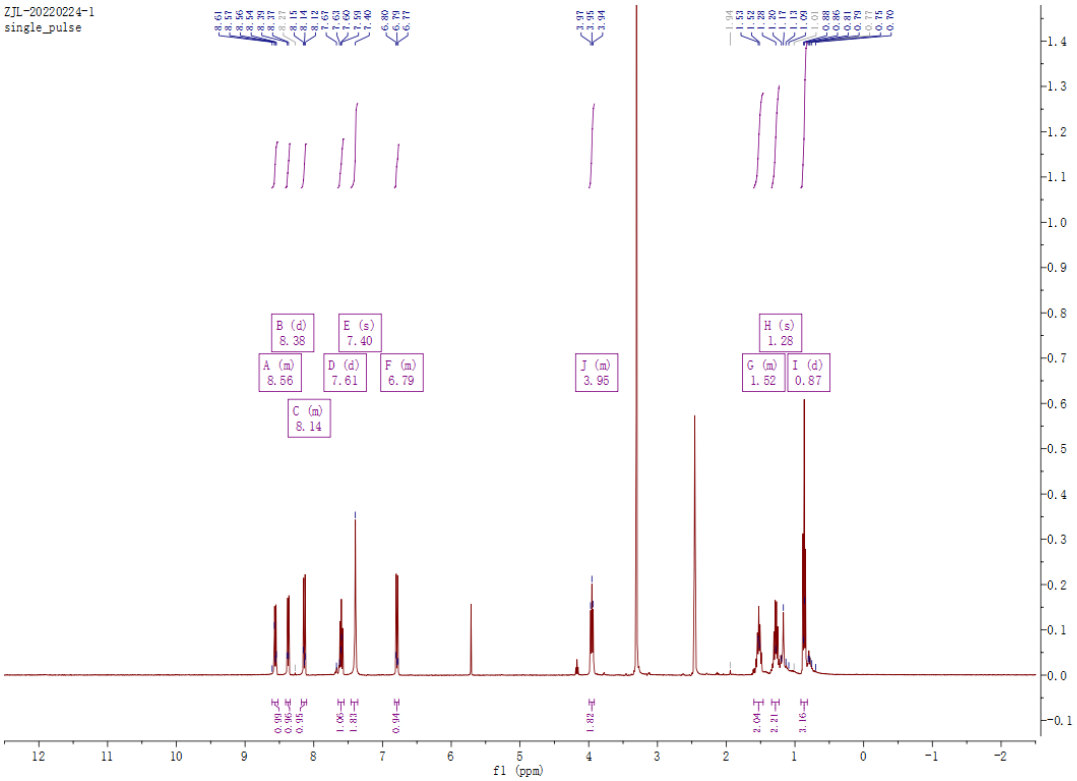


Fig. S4 ^{1}H NMR of **NP-NCS**

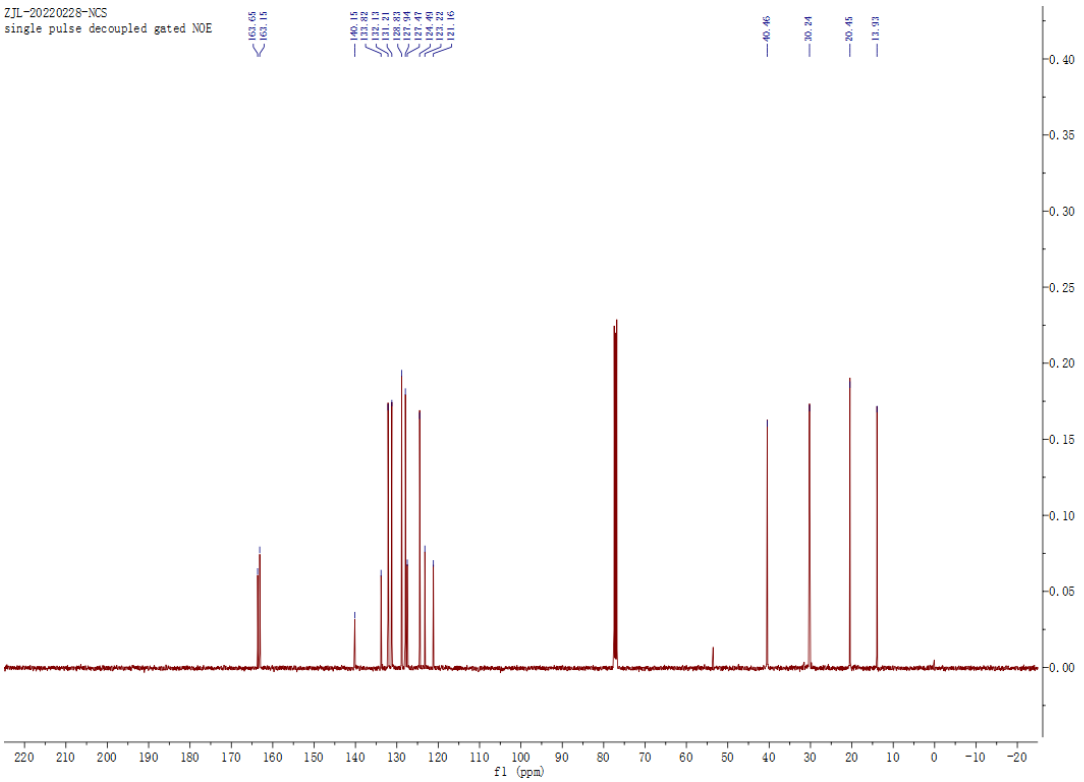


Fig. S5 ^{13}C NMR of **NP-NCS**

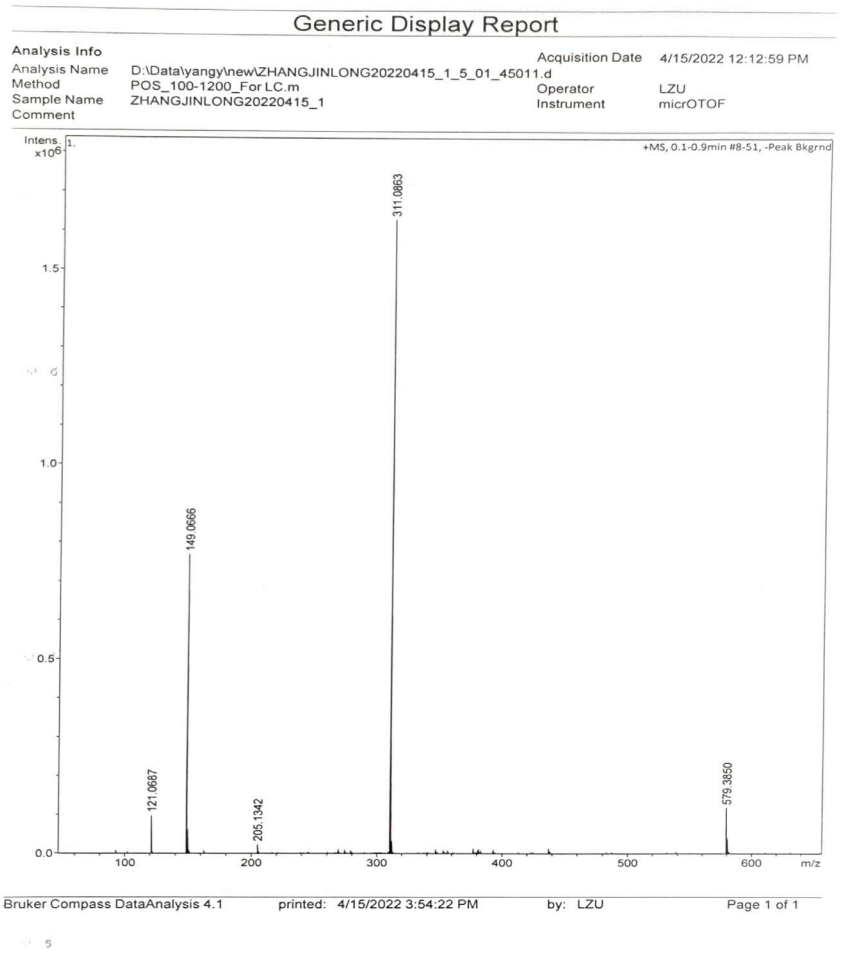


Fig. S6 Mass spectrum of **NP-NCS**

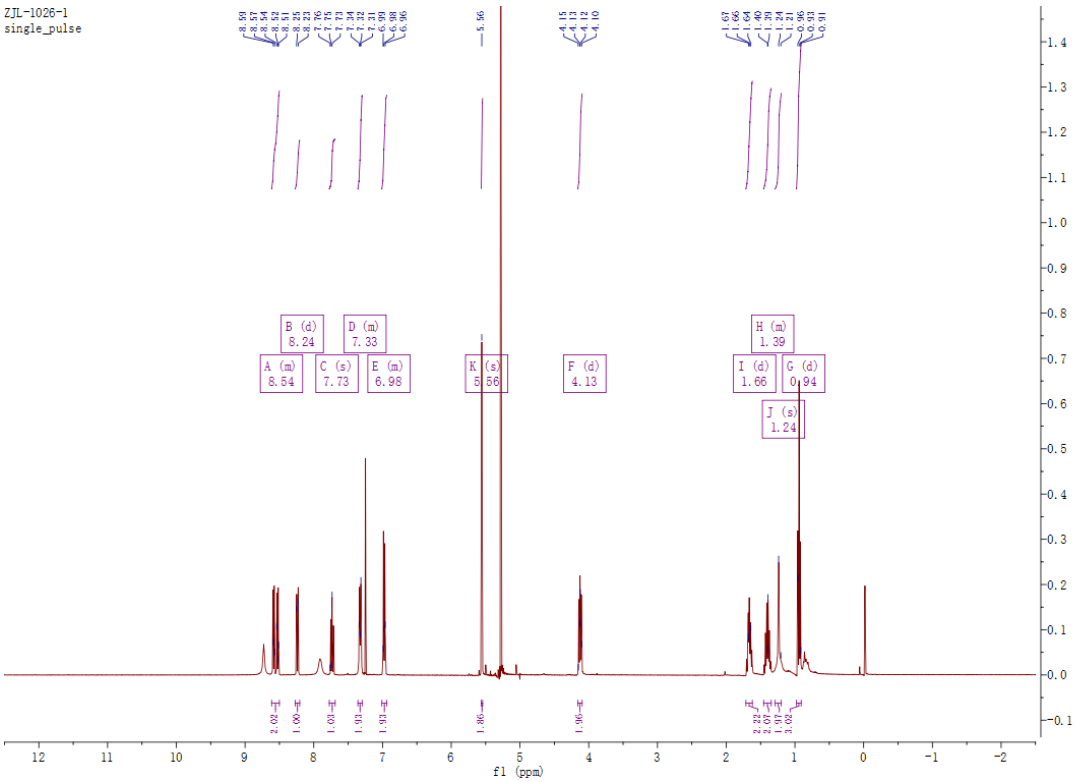


Fig. S7 ^{1}H NMR of **NP-SN$_3$**

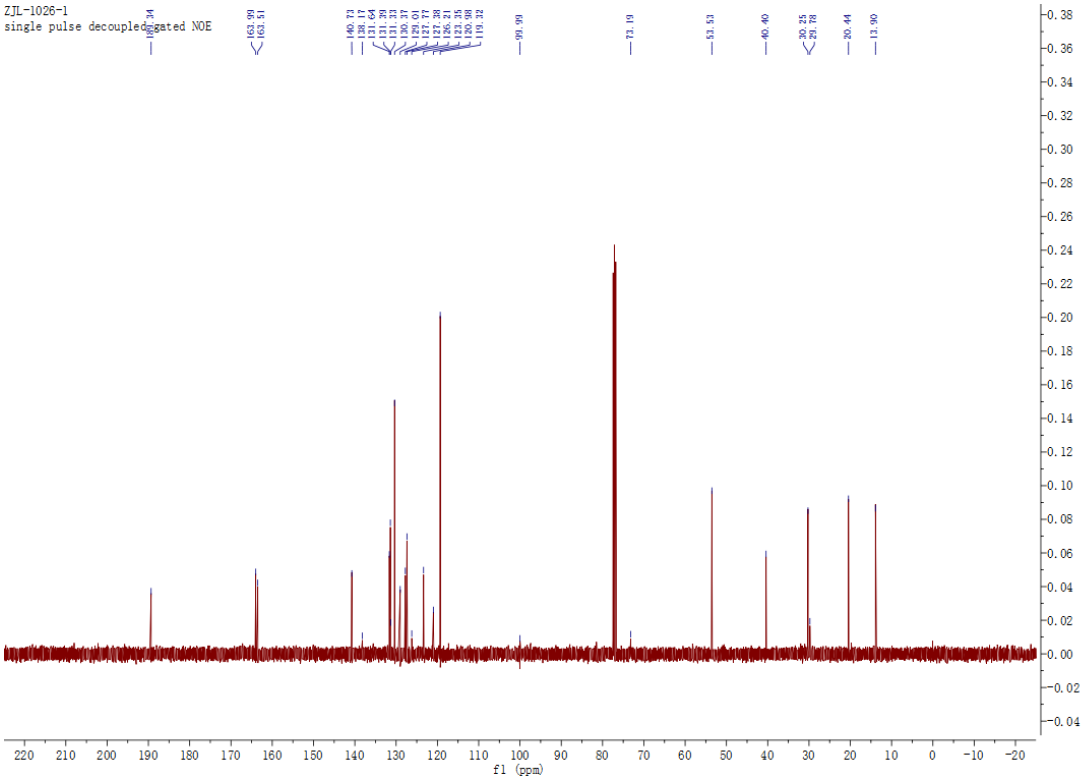


Fig. S8 ^{13}C NMR of **NP-SN$_3$**

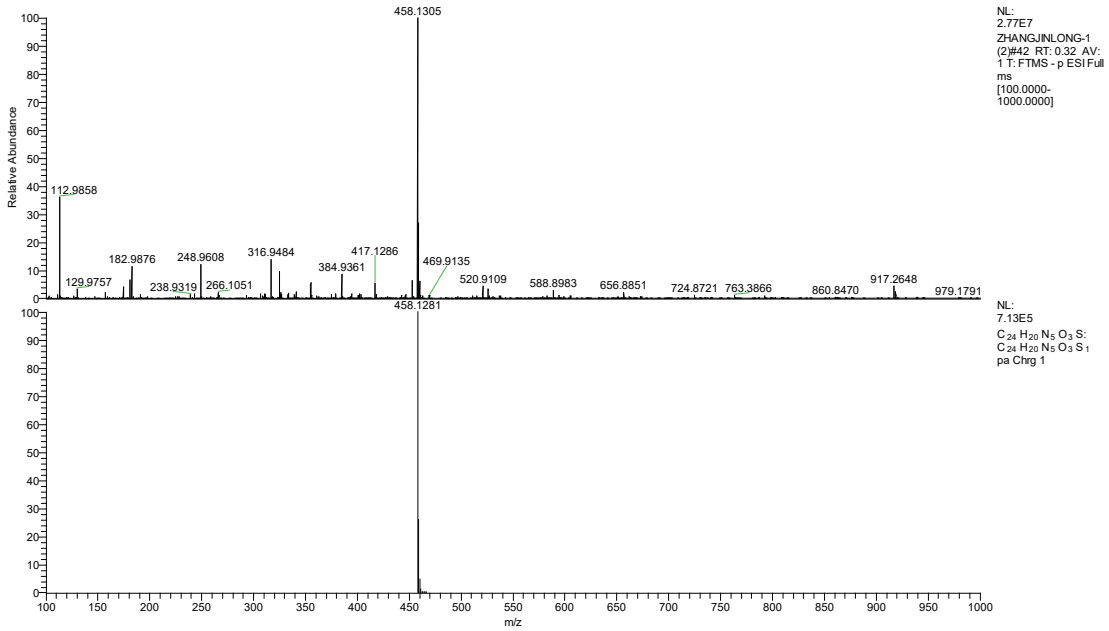


Fig. S9 Mass spectrum of **NP-SN$_3$**

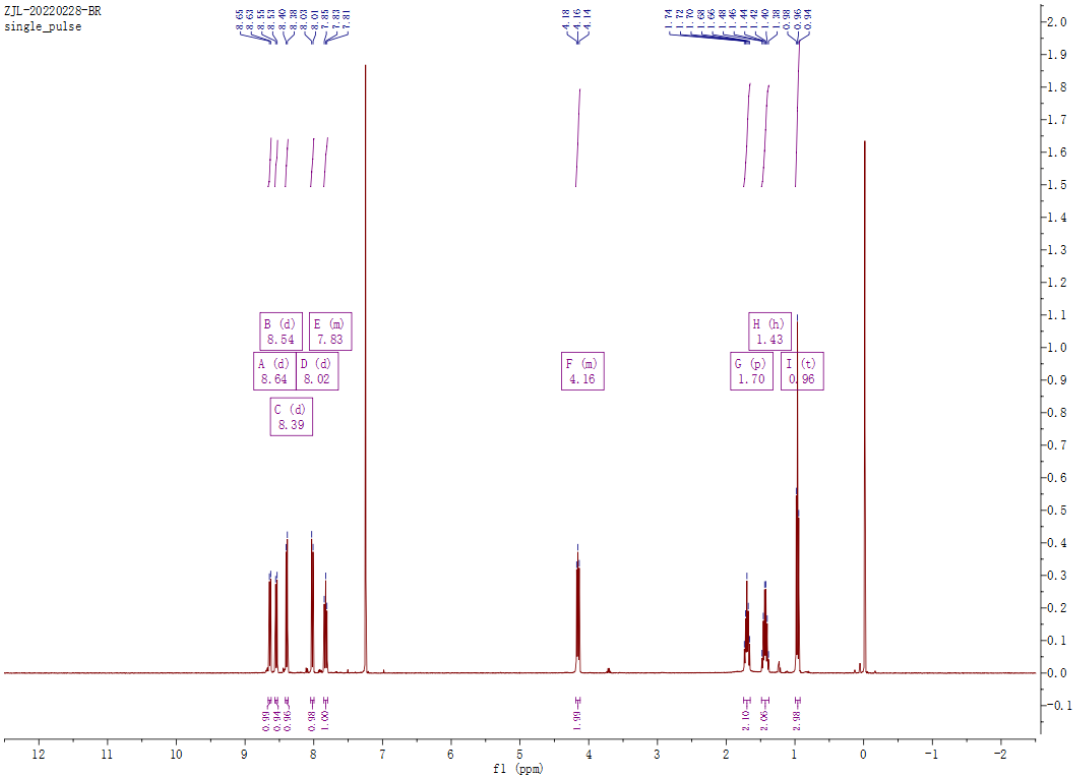


Fig. S10 ^{1}H NMR of **NP-Br**

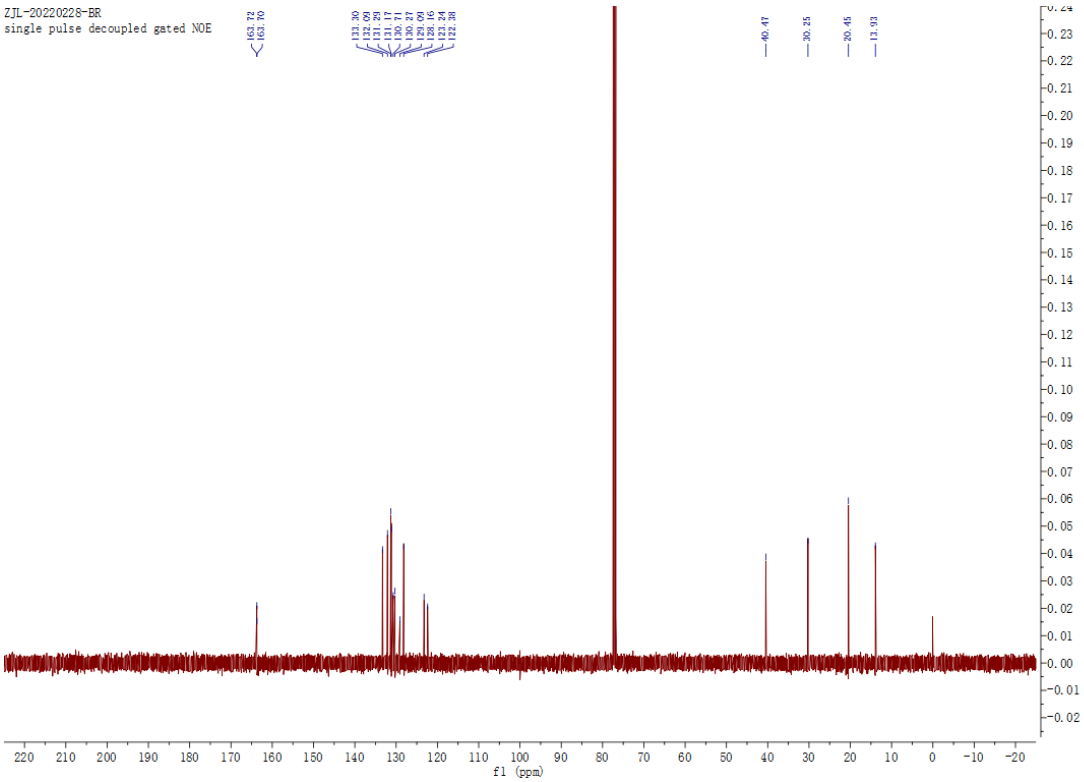


Fig. S11 ^{13}C NMR of **NP-Br**

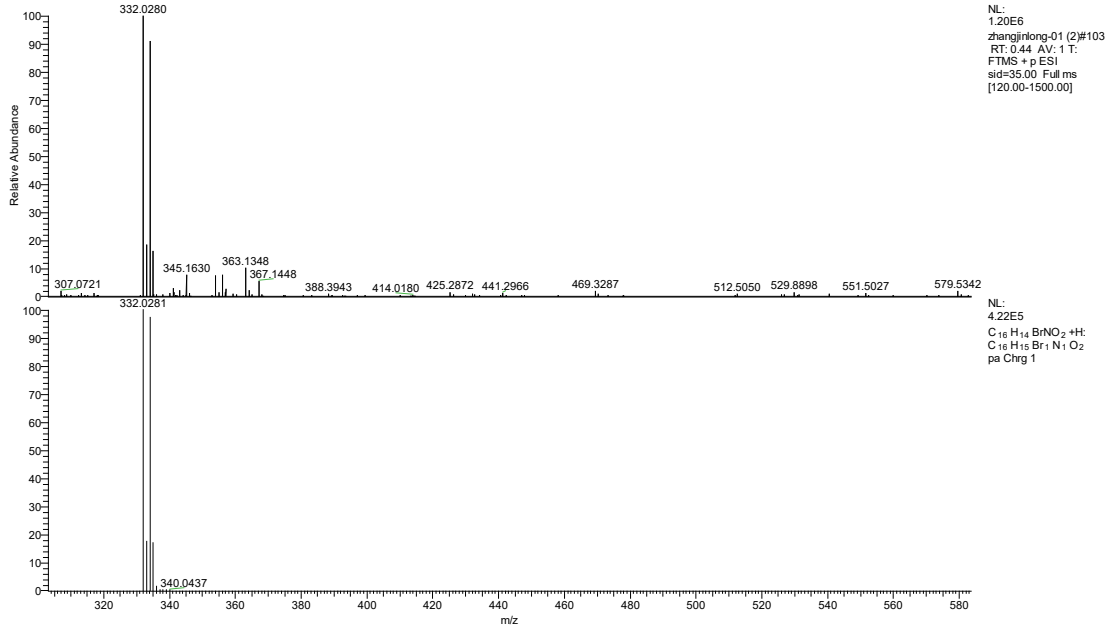


Fig. S12 Mass spectrum of **NP-Br**

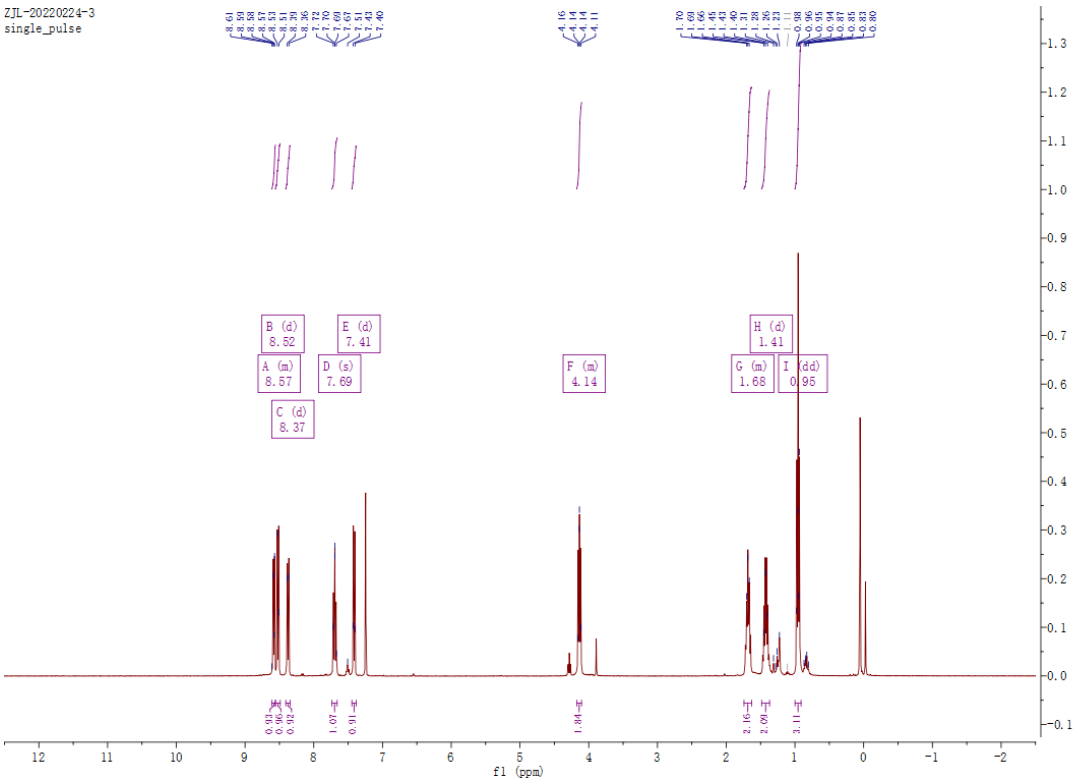


Fig. S13 ^{1}H NMR of **NP-N$_3$**

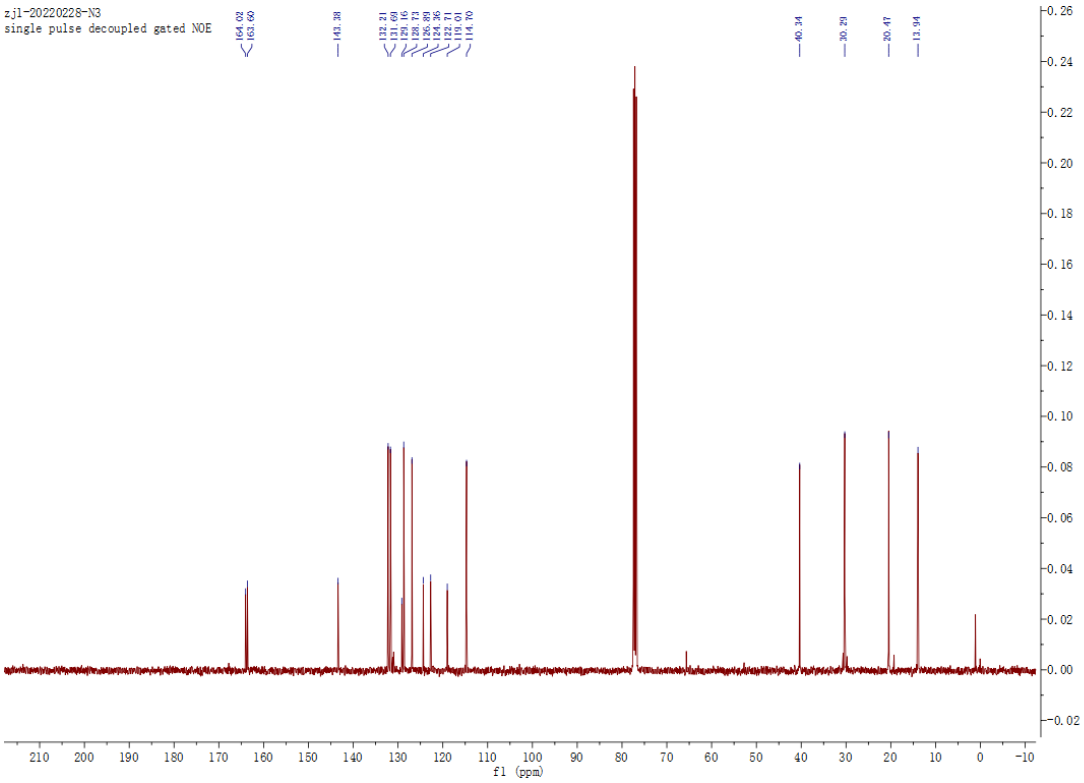


Fig. S14 ^{13}C NMR of **NP-N$_3$**

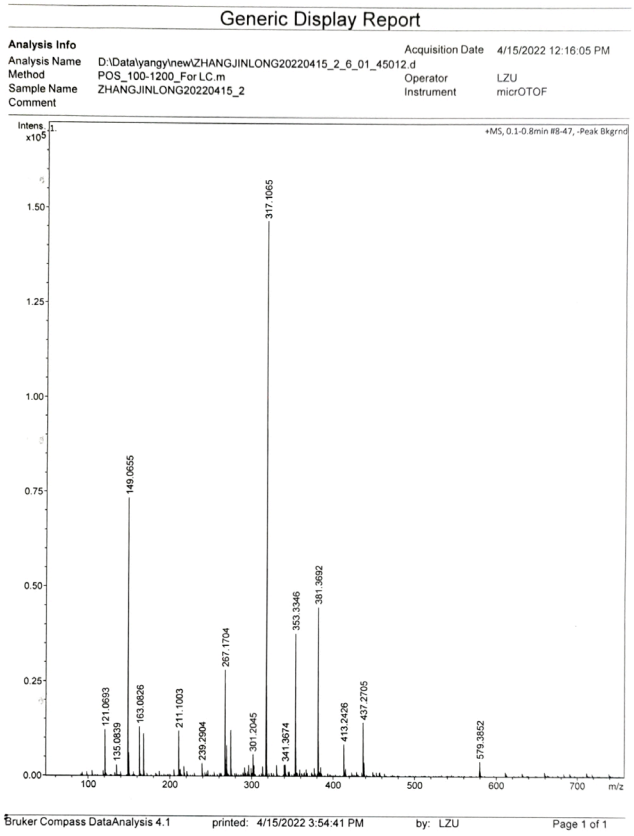


Fig. S15 Mass spectrum of **NP-N_3**

3. Spectral Supplementary Data of NP-SN_3 and NP-N_3

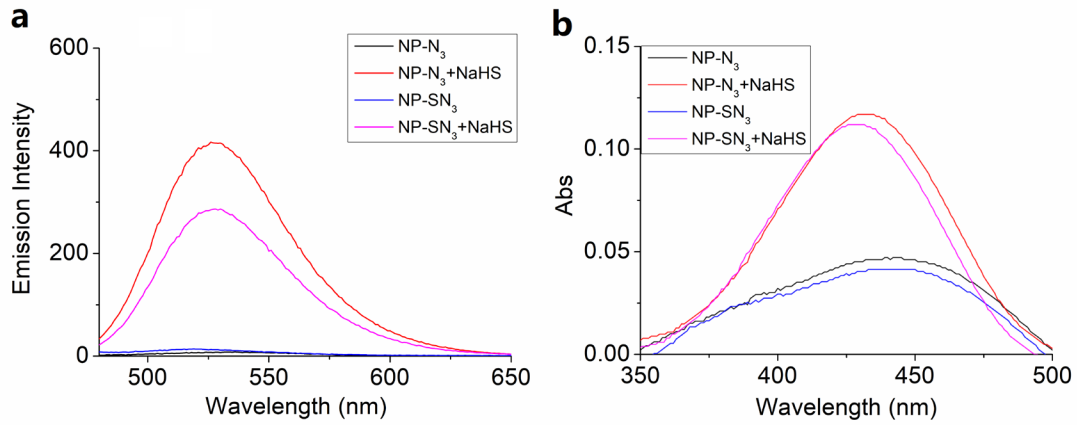


Fig. S16 Emission changes (a) and absorption changes (b) of probes after addition of NaHS.

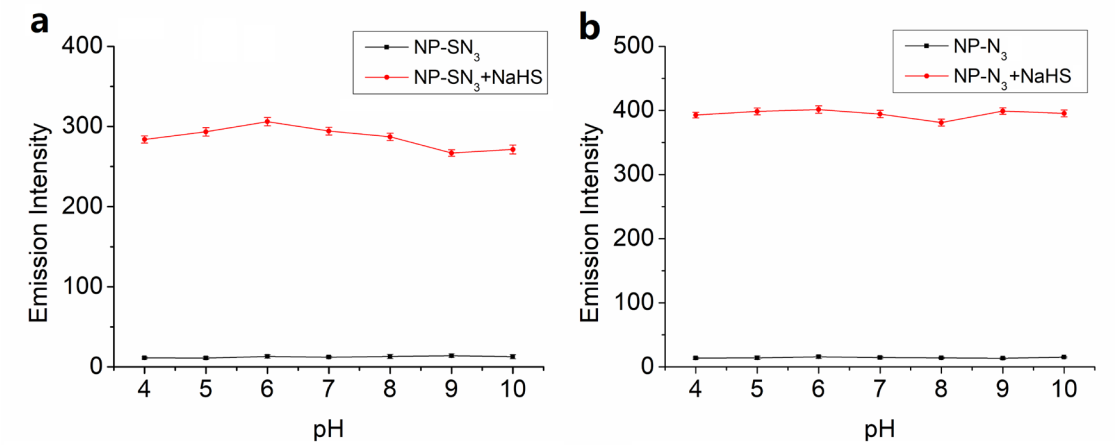


Fig. S17 Influence of pH on the fluorescence response of **NP-SN$_3$** (a) and **NP-N$_3$** (b) at 520 nm in the presence and absence of NaHS. λex = 450 nm

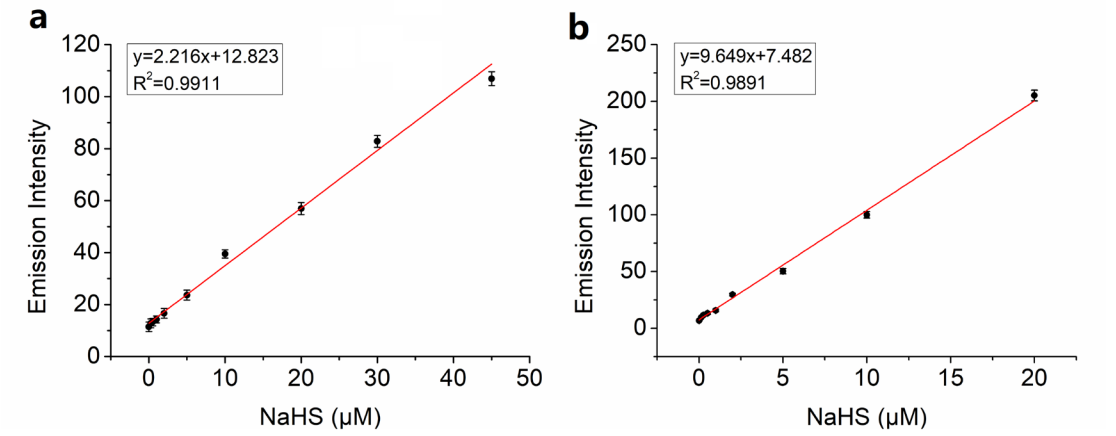


Fig. S18 Linear relationship between H_2S concentration and fluorescence intensity of probes **NP-SN$_3$** (a) or **NP-N$_3$** (b) at 520 nm. λex = 450 nm

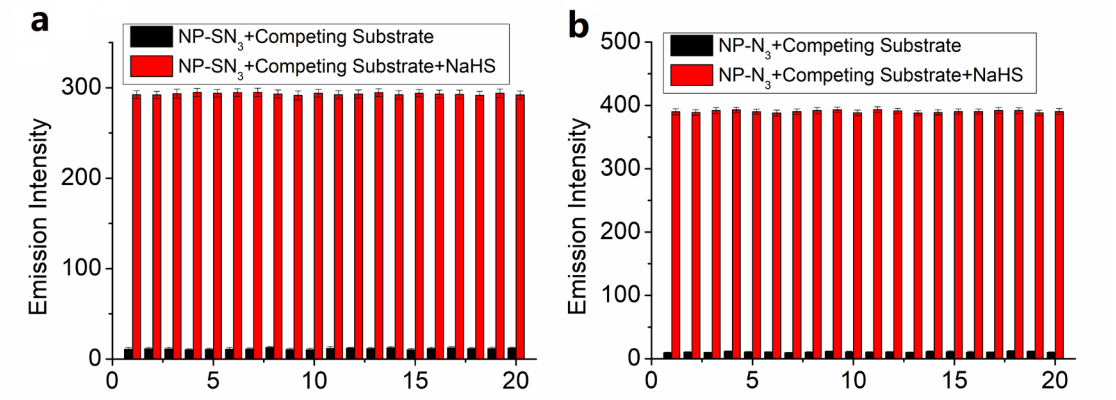


Fig. S19 Competition experiments on NaHS with **NP-SN$_3$** (a) and **NP-N$_3$** (b) in the presence of various analysts at 37 ℃. Analytes 1–20: blank, GSH (1 mM), Hcy, Cys,Thr, Gly, K^+, Na^+, Mg^{2+}, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_3^{2-}, HSO_3^-, SO_4^{2-}, AcO^-, ClO^-, H_2O_2. λex = 450 nm

4. In Vitro, Cellular and Zebrafish Imaging Data of NP-N$_3$

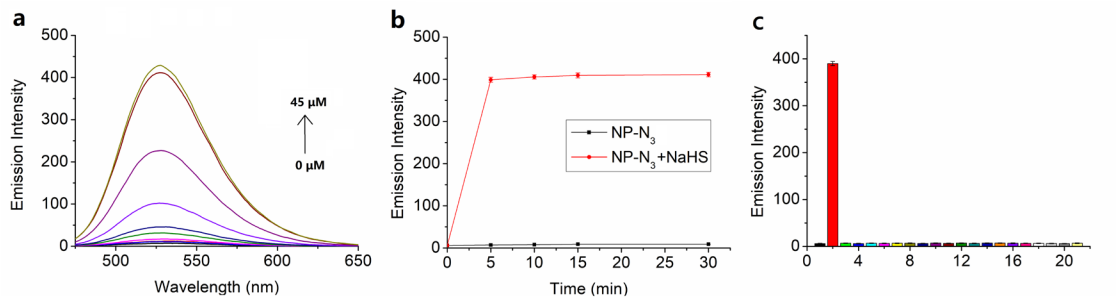


Fig. S20 (a) Concentration-dependent fluorescence spectra of **NP-N$_3$** towards H_2S (0–45 μM). (b) Fluorescence intensity of **NP-N$_3$** (10 μM) as a function of time in the presence or absence of NaHS (100 μM) at 520 nm; (c) Fluorescence intensity changes of **NP-N$_3$** for various analytes (200 μM unless stated otherwise) in PBS at 37 °C. Analytes 1–21: blank, NaHS (100 μM), GSH (1 mM), Hcy, Cys,Thr, Gly, K^+, Na^+, Mg^{2+}, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_3^{2-}, HSO_3^-, SO_4^{2-}, AcO^-, ClO^-, H_2O_2. λex = 450 nm.

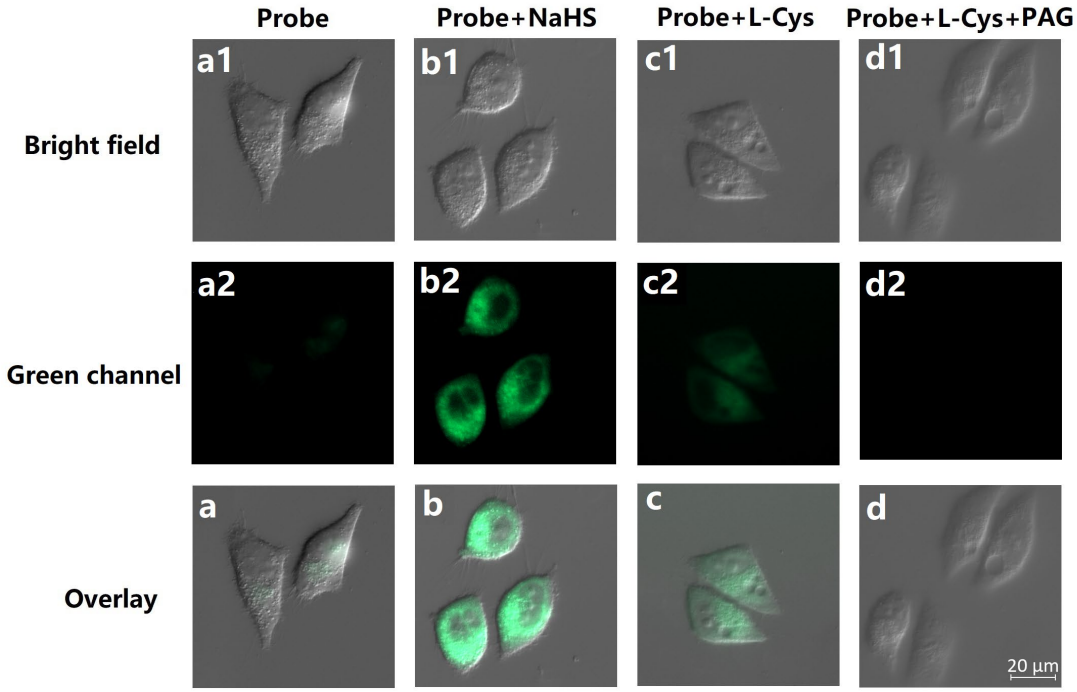


Fig. S21 Bioimaging of exogenous and endogenously produced H_2S with **NP-SN$_3$** in HepG2 cells. (a) Cells incubated with **NP-SN$_3$**. (b) Cells incubated with NaHS (100 μM) for 0.5 h and then stained by **NP-SN$_3$**. (c) Cells incubated with Cys (200 μM) for 1 h and then by **NP-SN$_3$**. (d) Cells incubated for 1 h with 1 mM PAG and 200 μM Cys, and then by **NP-SN$_3$**. (a1−d1) Bright field images. (a2−d2) Green channel images. (a–d) Merged images. λem =500-550 nm.

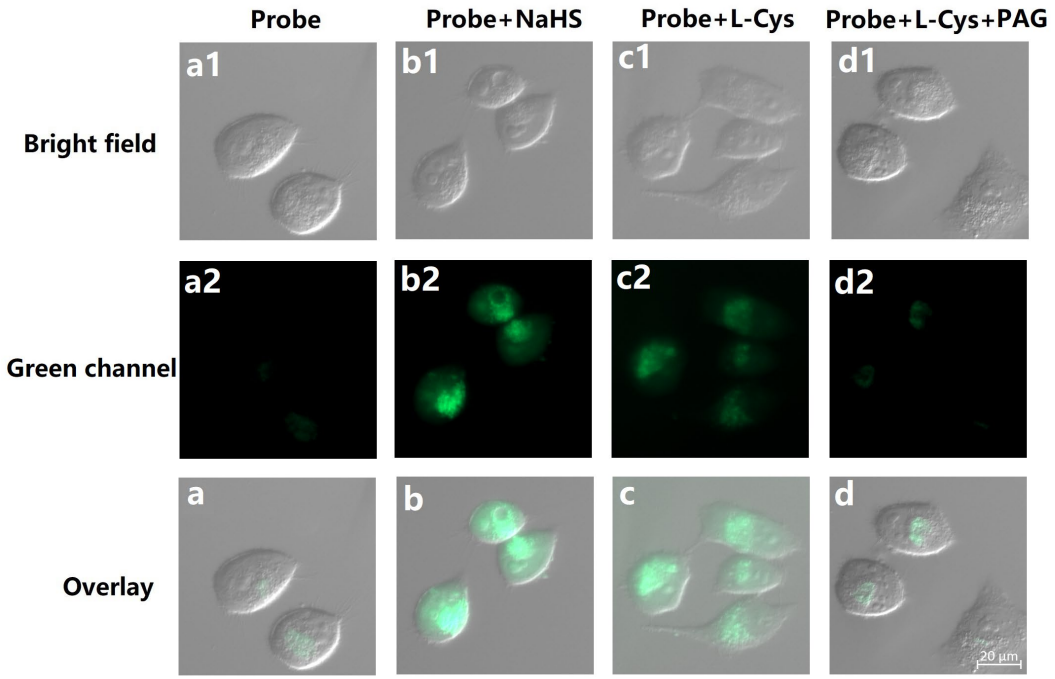


Fig. S22 Bioimaging of exogenous and endogenously produced H_2S with **NP-N_3** in HepG2 cells. (a) Cells incubated with **NP-N_3**. (b) Cells incubated with NaHS (100 μM) for 0.5 h and then stained by **NP-N_3**. (c) Cells incubated with Cys (200 μM) for 1 h and then by **NP-N_3**. (d) Cells incubated for 1 h with 1 mM PAG and 200 μM Cys, and then by **NP-N_3**. (a1−d1) Bright field images. (a2−d2) Green channel images. (a–d) Merged images. λem =500-550 nm.

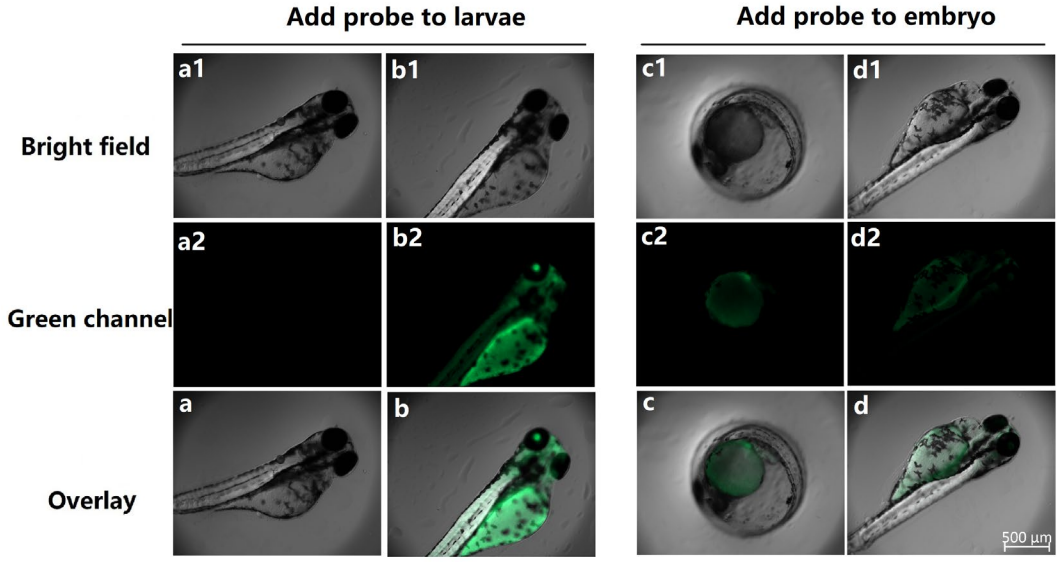


Fig. S23 Bioimaging of exogenous and endogenously produced H_2S with **NP-N_3** in zebrafish. (a) Zebrafish larvae incubated with **NP-N_3** for 0.5 h; (b) Zebrafish larvae were incubated with NaHS (100 μM) for 0.5 h and then stained with **NP-N_3**. Imaging of zebrafish embryo (c) and zebrafish larvae (d) after adding **NP-N_3** during zebrafish

embryonic growth. (a1−d1) Brightfield image. (a2−d2) Green channel image. (a-d) Merged images. λem =500-550 nm.

5. Detection of H_2S Released by Probes in Vitro, Cells and Zebrafish

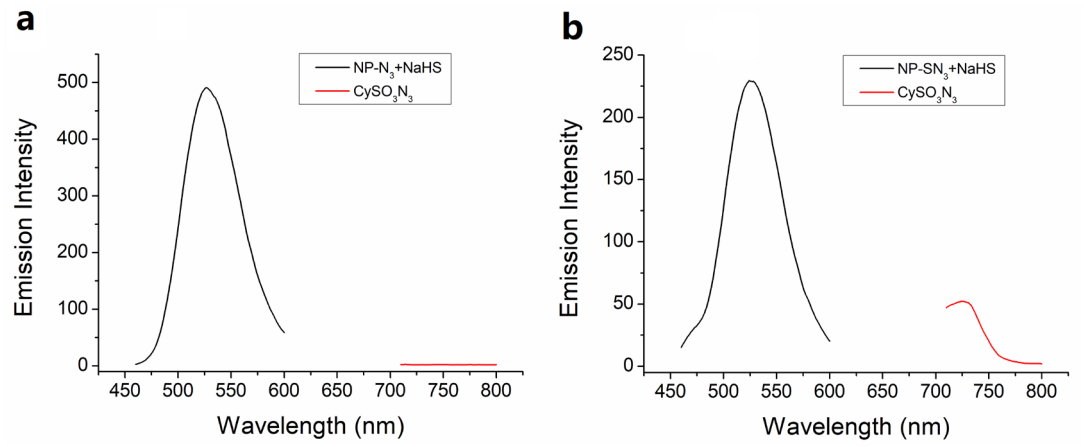


Fig. S24 Fluorescence intensities of NP-N_3 (a) and NP-SN_3 (b) with $CySO_3N_3$ added after NaHS addition. λem=450 nm and 675 nm. slit width: 3,3 and 10,5.

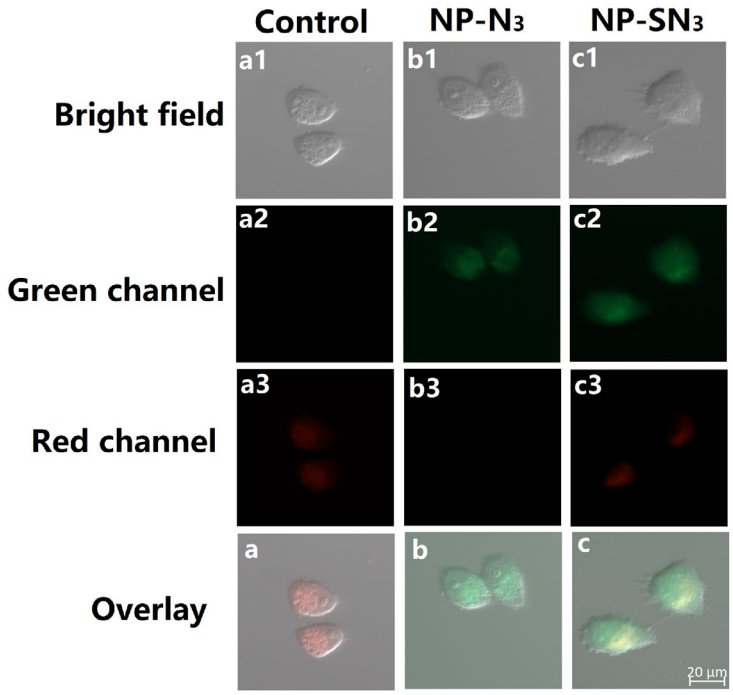


Fig. S25 Bioimaging of HePG2 cells with $CySO_3N_3$ added 1 h after adding probes. (a) Cells were incubated $CySO_3N_3$. (b) Cells were incubated with NP-N_3 for 1 h before adding $CySO_3N_3$. (c) Cells were incubated with NP-SN_3 for 1 h before adding $CySO_3N_3$. (a1−c1) Bright field images. (a2−c2) Green channel images. (a3−c3) Red channel images. (a−c) Merged images. λem=500-550 nm and 650-710 nm.

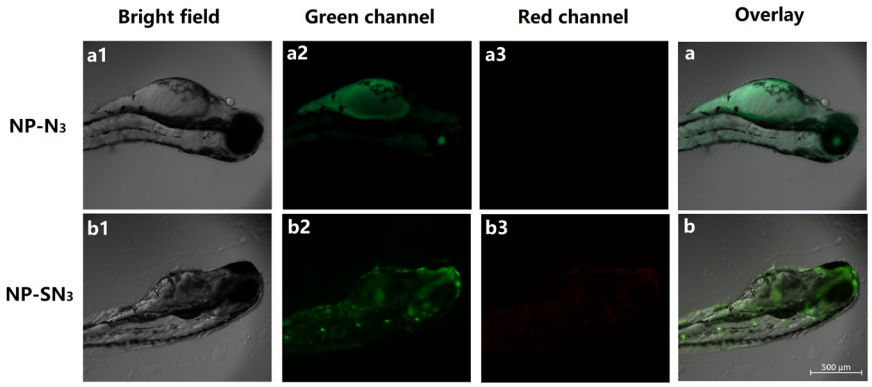


Fig. S26 Bioimaging of zebrafish with $CySO_3N_3$ added 1 h after adding probes. (a) Zebrafish larvae were incubated with NP-N_3 for 1 h before adding $CySO_3N_3$. (b) Zebrafish larvae were incubated with NP-SN_3 for 1 h before adding $CySO_3N_3$. (a1−b1) Bright field images. (a2−b2) Green channel images. (a3−b3) Red channel images. (a−b) Merged images. λem=500-550 nm and 650-710 nm.

6. Cytotoxicity Assay

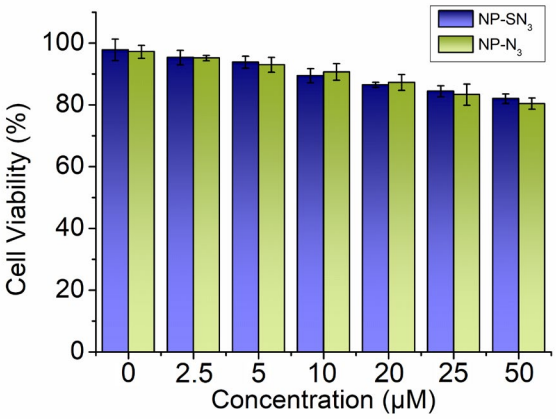


Fig. S27 Viability of HepG2 cell in the presence of different concentrations of **NP-SN_3** and **NP-N_3**.

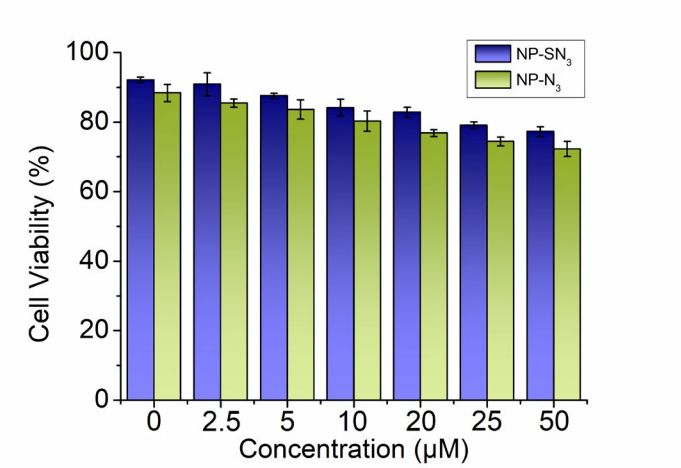


Fig. S28 Viability of HTS-T6 cell in the presence of different concentrations of **NP-SN$_3$** and **NP-N$_3$**.

7. Quantitative Standard Curve

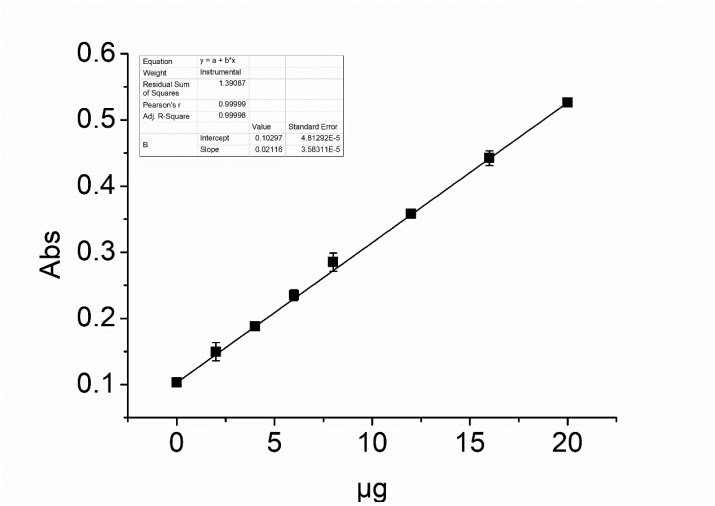


Fig. S29 BCA protein quantitative standard curve

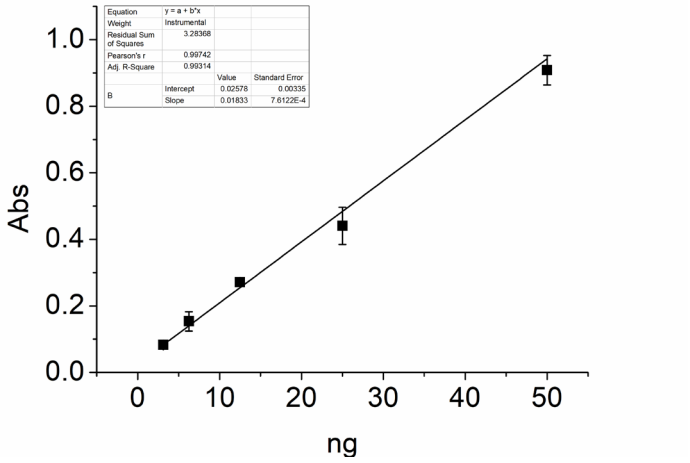
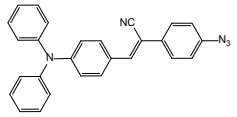
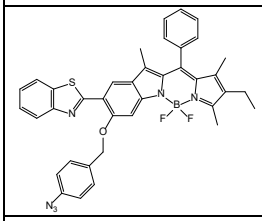
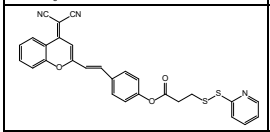
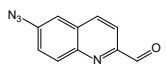
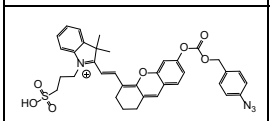
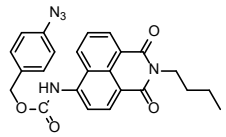


Fig. S30 α-SMA quantitative standard curve

8. Comparison with the Existing H_2S Sensor

Table S1 Comparison with the existing H_2S sensor

Probe for H_2S	Test system(v/v)	λex/λem	Response time	LOD	Toxicity evaluation	Ref
	PBS:DMF=7:3	*420/590 nm*	5 min	520 nM	cytotoxicity	14
	PBS:CH_3CN=1:1	*620/700 nm*	30 min	46 nM	cytotoxicity	15
	PBS:CH_3CN=1:1	*450/650 nm*	30 min	25 nM	cytotoxicity	16
	PBS	*335/533 nm*	3.4 min	46 nM	cytotoxicity	17
	PBS	*675/695 nm*	30 min	38 nM	cytotoxicity	30
	PBS:DMSO=1:4	*450/520 nm*	60 min	97 nM	1) cytotoxicity 2) continuous administration toxicity 3) developmental toxicity 4) detection-induced toxicity	This work

9. The examinations of blood and urine routine in rats

Table S2 The changes of blood routine examinations in rats after continuous injection of probes

Test items	Control	NP-N_3	NP-SN_3
Leukocyte (10^9/L)	5.23	1.65*	4.71
Red blood cells (10^{12}/L)	6.61	6.79	6.37
Hemoglobin (g/L)	173	146	151
Platelets (10^9/L)	791	245*	518
Neutrophils (%)	40.9	45.6	44.5
Lymphocytes (%)	57.5	56.4	51.5

*$p < 0.05$

Table S3 The changes of urine routine examinations in rats after continuous injection of

probes

Test items	Control	NP-N_3	NP-SN_3
pH	7.3	7.3	7.3
proportion	1.020	1.020	1.020
protein(g/L)	2.8	2.9	2.9
glucose	0	0	0
ketone bodies (mmol/L)	2.4	1.6	1.9
Bilirubin	Negative	Negative	Negative
Urobilinogen	Negative	Negative	Negative
Urinary white blood cells	Negative	Negative	Negative
VC	Negative	Negative	Negative