Supporting Information

Synergistically activated dual-locked fluorescent probes to monitor H_2S-induced DNA damage

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Contents

1. Experimental procedures

All reagents used in the synthesis and purification of probes (**NAN0-N_3** and **NAN6-N_3**) and their intermediates (T) were of analytical grade. Column chromatography using silica gel (200-300 mesh) was used to purify the compounds. **NAN0-N_3** (6.0 mM) and **NAN6-N_3** (6.0 mM) were used as stock solutions for all spectroscopic and cell imaging experiments, respectively. The buffer solution used for the optical experiments of the probes in vitro was tris-HCl (0.05 M, pH = 7.4). Na_2S was selected as the release agent of H_2S for both solution and cell imaging experiments, which was purchased from Aladdin. The DNA used in the in vitro experiments was deoxyribonucleic acid sodium salt from salmon testes (smDNA) purchased from Solarbio. PAG (DL-Propargylglycine, an inhibitor of cystathionine *γ*-lyase during endogenous H_2S synthesis) was purchased from Aladdin as an endogenous H_2S inhibitor. The DNA digestive enzymes used for cell imaging were purchased from Sigma-Aldrich (U.S.A.), and commercial probes Mito-Tracker Deep Red and Hoechst 33258 for co-location imaging experiments were purchased from Thermo Fisher Scientific Company (U.S.A.). The commercial dye PI for dead cell nuclei was purchased from Beyotime Biotechnology for apoptotic experiments. An ultraviolet spectrophotometer (GBC Scientific Equipment Pty LTD, Australia) and fluorescence spectrophotometer FluoroMax-4 (HORIBA, Japan) were used for the spectrometric measurements in vitro. The cell imaging experiments were completed using a FV1200 spectral confocal multiphoton spectrometer (Olympus, Japan). Flow cytometry (BD FACSCanto II, U.S.A.) was used for imaging of big data analysis. Probes and its intermediates were characterized using an Avance 600 MHz spectrometer (Bruker Co., Switzerland).

Spectrometric determination in vitro.

An ultraviolet spectrophotometer (GBC Scientific Equipment Pty LTD, Australia) and fluorescence spectrophotometer FluoroMax-4 (HORIBA, Japan) were used to measure the absorption spectra and fluorescence spectra, respectively. In all spectral experiments, the final solutions contained < 5 ‰ DMSO. Each experiment was carried out in five replicates (n = 5).

Water solubility.

With ultrapure water as the solvent, **NAN0-N_3** and **NAN6-N_3** (0-20.0 μM) were continuously added into 3.0 mL H_2O, respectively. And the absorbance was detected by ultraviolet spectrophotometer, respectively. According to Lambert Beer's law (A= εbc), the solubility of **NAN0-N_3** and **NAN6-N_3** in water were evaluated. All the experimental results obtained were from five parallel experiments.

pH stability.

HCl and NaOH were used as acid and base to prepare test solution with different pH (pH = 3-11) values. Then the fluorescence intensity of **NAN0-N_3** (6.7 μM, λ_{ex} = 370 nm, λ_{em} = 546 nm) and **NAN6-N_3** (6.7 μM, λ_{ex} = 350 nm, λ_{em} = 556 nm) at different pH values were measured, respectively, to determine the influence of different pH values on the detection of the probes to DNA damage induced by H_2S. All the experimental results obtained were from five parallel experiments.

Selectivity.

The biological coexisting substances cation and anion (include $CuSO_4$, $MgSO_4$, $Zn(NO_3)_2 \cdot 6H_2O$, $FeCl_2 \cdot 7H_2O$, $CaCl_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, $CdCl_2$, $CoCl_2$, $HgCl_2$, $MnCl_2$, $AgNO_3$, $K_2HPO_4 \cdot 3H_2O$, Li_2CO_3, $SnCl_2$, Na_2CO_3, $Al(NO_3)_3$) and biomacromolecule (including D-methionine, L-cysteine, Arginine, Hypoxanthine, DL-leucine, Serine, DL-homocysteine, Glutamate, Glycine, Glycine-DL-phenylalanine, L-glutamate, L-lysine, DL-threonine, Valine, Glutamine, Cystine, L-aspartate, Glutathione) were selected as interferences. These were added (20 equivalents of DNA) to the probes (6.7 μM) test solution and the change of fluorescence intensity was measured, respectively. All the experimental results obtained were from five parallel experiments.

Spectral analysis of probes with DNA.

Commercial DNA (smDNA, extracted from salmon testes) was dissolved in ultrapure water and configured into a DNA solution of 3.5 mg/mL for testing. DNA (0-4.5 μg/mL) was added into the **NAN0-N$_3$** (6.7 μM, λ_{ex} = 425 nm), **ND-N$_3$** (6.7 μM, λ_{ex} = 410 nm) and **NAN6-N$_3$** (6.7 μM, λ_{ex} = 410 nm) test solution (tris-HCl, 0.05 M, pH = 7.4), respectively. Then mixed and left for 5.0 min. The responses of probes to DNA were then monitored by changes of the fluorescence spectral signals. All the experimental results obtained were from five parallel experiments.

Spectral analysis of probes with H_2S.

Na_2S was selected as the release agent of H_2S for this research. It was dissolved in ultrapure water and configured into a Na_2S solution of 0.10 M for testing. Na_2S (0-13 mM) was added into the **NAN0-N$_3$** (6.7 μM, λ_{ex} = 425 nm), **ND-N$_3$** (6.7 μM, λ_{ex} = 410 nm) and **NAN6-N$_3$** (6.7 μM, λ_{ex} = 410 nm) test solution (tris-HCl, 0.05 M, pH = 7.4), respectively. Then mixed and left for 5.0 min. The responses of probes to H_2S were then monitored by changes of the fluorescence and absorption spectral signals. All the experimental results obtained were from five parallel experiments.

Spectral analysis of probes with DNA and H_2S.

Na_2S (3.3 mM) was added into the **NAN0-N$_3$** (6.7 μM, λ_{ex} = 425 nm) and **NAN6-N$_3$** (6.7 μM, λ_{ex} = 410 nm) test solution (tris-HCl, 0.05 M, pH = 7.4), respectively. Then, DNA (0-4.5 μg/mL) was successively added, respectively. Mixed and left for 5.0 min. The responses of probes to DNA in the presence of H_2S were then monitored by changes of the fluorescence spectral signals.

DNA (4.5 μg/mL) was added into the **NAN0-N$_3$** (6.7 μM, λ_{ex} = 425 nm) and **NAN6-N$_3$** (6.7 μM, λ_{ex} = 410 nm) test solution (tris-HCl, 0.05 M, pH = 7.4), respectively. Then, Na_2S (0-3.3 mM) was successively added into, respectively. Mixed and left for 5.0 min. The responses of probes to H_2S in the presence of DNA were then monitored by changes of the fluorescence spectral signals. All the experimental results obtained were from five parallel experiments.

Molecular docking of probes with DNA.

Molecular docking was used as a theoretical calculation method to determine the binding pattern of probes to DNA. In this experiment, all the molecular docking experiments were performed on Yinfo Cloud Platform by UCSF DOCK 6.9 software. The crystal structure of the nucleic acid with serial number 6GPI as molecular docking receptor was obtained from the RCSB protein database. Through the process of probes molecular structure drawing, it was converted to a three-dimensional structure with minimal energy, lattice production, semi-flexible docking, etc., then the molecular conformation of **NAN0-N_3** and **NAN6-N_3** which were the best combination with DNA and the combination of probes with DNA were outputted.

Cell culture.

HepG2 cell lines and CHO cell lines were obtained from the Chinese Academy of Medical Sciences. The medium for the cells were made up of phenol red-free Dulbecco's Modified Eagle's Medium (DMEM, WelGene), and 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin. The two kinds of cell lines were all grown in a CO_2 incubator at 37 °C. One day before imaging, the cells mentioned above were seeded into confocal dishes with glass bottoms (MatTek, 1# glass, 0.13-0.16 mm). They were incubated at 37 °C in 5.0 wt %/vol CO_2 for 24.0 h. And then, the cells were incubated with the probes at a certain concentration, respectively.

Cytotoxicity.

HepG2 was prepared for cell viability studies in 96-well plates (1×10^5 cells per well that were incubated in 100 μL). The cells were incubated for an additional 24.0 h with dyes at different concentrations (0, 5.0, 10.0, 15.0 μM). Subsequently, 20 μL 5.0 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co. U.S.A.) was added into each well, followed by further incubation for 4.0 h at 37 °C. The DMEM was removed and DMSO (150 μL/well) added to dissolve the reddish-blue crystals. Optical density (OD) was determined by a microplate reader (Spectra Max M5, Molecular Devices) at 490 nm. The results from the six individual experiments were averaged. The relative cell viability (100%) was calculated using the following equation:

$$\text{Cell viability (\%)} = (OD_{probe}\text{-}OD_{k\text{-}probe})/(OD_{ctrl}\text{-}OD_{k\text{-}ctrl}) \times 100 \quad (2)$$

Fluorescence imaging in cells.

FV1200 spectral Confocal Multiphoton porous (Olympus, Japan) was used for the imaging experiments. An inverted microscope was used in cell imaging. The imaging parameters are as follows. Internal PMTs = 16 bit, pixels = 1600 × 1600. The scan ranges were ascertained according to the fluorescence of the probe.

The HepG2 cells were incubated with **NAN0-N_3** (6.0 μM), **ND-N_3** (6.0 μM) and **NAN6-N_3** (6.0 μM) at 37 °C in 5.0 wt %/vol CO_2 for 30.0 min, respectively. Then washed with PBS (pH = 7.2) three times then imaged under excitation at 405 nm.

Fluorescence imaging of DNA digestive enzymes in cells.

The HepG2 cells incubated with probes (6.0 μM) were quickly washed with iced methanol three times, and 2.0 mL iced methanol was added and stored at 4 °C for 0.5 h, to obtain the fixed cell model. Subsequently, the cells were then washed with PBS (pH = 7.2) three times and incubated with different concentrations of DNA digestibility enzymes (0, 50.0 and 100.0 U/mL) for 1.0 h then imaged under excitation at 405 nm (scan range = 535-575 nm).

Fluorescence imaging of endogenous H_2S in cells.

PAG (DL-Propargylglycine) is an inhibitor of cystathionine γ-lyase during endogenous H_2S synthesis in cells. HepG2 cells were incubated in DMEM medium containing 1.0 mM PAG for 1.0 h to inhibit endogenous H_2S production. Then stained with probes (6.0 μM) for 30.0 min. Washed with PBS (pH = 7.2) three times and imaged under excitation at 405 nm (scan range = 535-575 nm).

Fluorescence imaging of H_2S-induced DNA damage in cells.

HepG2 and CHO cells were pretreated with Na_2S (500 μM) for 4.0 h to establish H_2S-induced DNA damage cell model as experimental groups, respectively. Then stained with probes (6.0 μM) for 30.0 min. Washed with PBS (pH = 7.2) three times and imaged under excitation at 405 nm (scan range = 535-575 nm).

Colocalization imaging.

Commercial mitochondrial dye Mito-Tracker Deep Red and nuclear dye Hoechst 33258 were used as standard localization dyes. The HepG2 cells were incubated with Hoechst 33258 (10.0 μM) and probes (6.0 μM) for 30.0 min under 5.0 wt % /vol CO_2 at 37 °C, respectively. After that, they were washed with PBS (pH = 7.2) three times and imaged under excitation at 405 nm (blue channel = 440-480 nm, green channel = 535-575 nm). The fluorescence imaging was analyzed by means of the colocalization coefficient.

The cells were incubated with Mito-Tracker Deep Red (5.0 μM) and **NAN6-N_3** (6.0 μM) for 30.0 min under 5.0 wt % /vol CO_2 at 37 °C. After that, they were washed with PBS (pH = 7.2) three times and imaged under excitation at 405 nm and 635 nm (green channel = 535-575 nm, red channel = 650-680 nm). The fluorescence imaging was analyzed by means of the colocalization coefficient.

Assessing the health of cells during apoptosis.

The HepG2 and CHO cells were incubated at high temperature for 4.0 h, respectively, to establish apoptotic models as experimental group (Experimental group 1: 56 °C, early apoptosis; Experimental group 2: 65 °C, late apoptosis.). And the untreated cells were healthy cells as control group. Commercial nuclear dye Propidium Iodide (PI) was used as a standardized indicator of the degree of apoptosis. Then, the cells were incubated with **NAN6-N_3** (6.0 μM) and PI (10.0 μM) at 37 °C under 5 wt % /vol CO_2 for 30.0 min. Washed with PBS buffer (pH = 7.2) three times and imaged under excitation at

405 and 559 nm (green channel = 535-575 nm, red channel = 600-630 nm). The experimental data were obtained through five parallel experiments.

HepG2 apoptosis model was dispersed in PBS (pH = 7.2, 10,000 cells / 1.0 mL). Then they were incubated with **NAN6-N_3** (6.0 μM) and PI (10.0 μM) for 30.0 min. Flow cytometry (BD FACSCanto II, USA) was used to collect fluorescence data at 535-575 nm and 600-630 nm under excitation at 488 nm and 515 nm. BD FACSDiva software was used to analyze the average fluorescence intensity. The experimental data were obtained through five parallel experiments.

2. Synthesis of probe NAN0-N_3, ND-N_3, NAN6-N_3

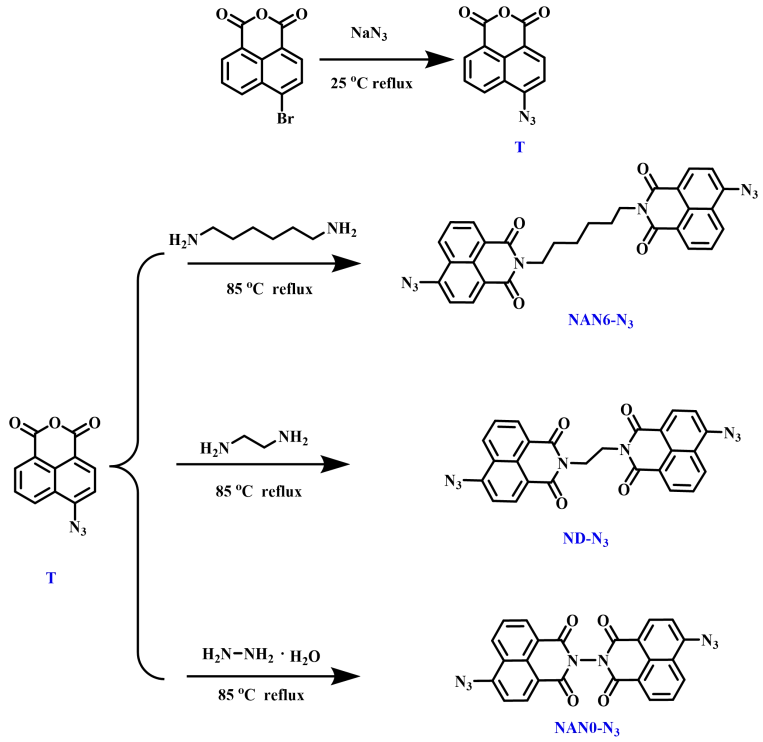


Figure S1. The synthetic route of **NAN0-N_3, ND-N_3, NAN6-N_3**.

Synthesis of intermediate **T**. 4-bromo-1, 8-naphthalene anhydride (0.28 g, 1.0 eq.) was added to a double-mouth round-bottom flask with 25 mL *N*,*N*-dimethylformamide, and stirred at 25 °C till dissolved completely. Then NaN_3 (0.10 g, 1.5 eq.) was dissolved in 2.0 mL of water and dropped into the reaction system within 30.0 min which was then stirred at 25 °C for 4.0 h. After the reaction was complete, it was poured into 20 mL of ice water to stop the reaction and left to stand. The yellow precipitate that formed was filtered and washed with water three times. Then the crude products were purified by column chromatography using petroleum ether/dichloromethane (50:1 to 1:1, v/v) to obtain **T** as yellow solid. Yield 84% (0.20 g). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.57 (d, *J* = 7.2 Hz, 1H), 8.52 (dd, *J* = 8.1, 2.6 Hz, 2H), 7.92 (t, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.20, 160.62, 144.64, 133.80, 133.77, 131.30, 129.99, 128.13, 124.11, 119.77, 116.86, 115.29.

Synthesis of intermediate **NAN0-N$_3$**. **T** (0.35 g, 2.0 eq.) was added to a double-mouth round-bottom flask with 20 mL of anhydrous ethanol then stirred and heated to 85 °C. Hydrazine hydrate (0.038 g, 1.0 eq.) was dissolved in 4.0 mL of anhydrous ethanol, and slowly dropped into the reaction system within 30.0 min which was then stirred at 85 °C for 6.0 h. The reaction was monitored using TLC, cooled to room temperature, then filtered and the filtrate was collected and concentrated to obtain the crude product. The crude product was purified by column chromatography using dichloromethane/ethyl acetate (50:1 to 1:1, v/v) to obtain **NAN0-N$_3$** as yellow solid. Yield 45% (0.16 g). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (d, J = 8.1 Hz, 1H), 8.45 (d, J = 7.1 Hz, 1H), 8.20 (d, J = 8.1 Hz, 1H), 7.67 (t, J = 7.1 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.68, 160.56, 153.62, 134.59, 131.46, 129.84, 128.67, 124.53, 121.78, 119.91, 108.77, 107.35.

Synthesis of intermediate **ND-N$_3$**. **T** (0.35 g, 2.0 eq.) was added to a double-mouth round-bottom flask with 20 mL of anhydrous ethanol then stirred and heated to 85 °C. Ethylenediamine (0.045 g, 1.0 eq.) was dissolved in 4.0 mL of anhydrous ethanol, and slowly dropped into the reaction system within 30.0 min which was then was stirred at 85 °C for 6.0 h. The reaction was monitored using TLC, cooled to room temperature, then filtered and the filtrate was collected and concentrated to obtain the crude product. The crude product was purified by column chromatography using dichloromethane/ethyl acetate (50:1 to 1:1, v/v) to obtain **ND-N$_3$** as yellow solid. Yield 50% (0.18 g). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.57 (d, J = 5.4 Hz, 1H), 8.26 (d, J = 4.5 Hz, 1H), 8.02 (d, J = 5.1 Hz, 1H), 7.58 (t, J = 4.8 Hz, 1H), 6.77 (d, J = 5.4 Hz, 1H), 4.40 (s, J = 6.4 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.65, 163.71, 153.02, 134.24, 131.27, 130.29, 129.63, 124.37, 122.27, 119.83, 108.48, 108.10, 38.69.

Synthesis of intermediate **NAN6-N$_3$**. **T** (0.35 g, 2.0 eq.) was added to a double-mouth round-bottom flask with 20 mL of anhydrous ethanol then stirred and heated to 85 °C. Hexamethylenediamine (0.090g, 1.0 eq.) was dissolved in 4.0 mL of anhydrous ethanol, and slowly dropped into the reaction system within 30.0 min which was then stirred at 85 °C for 6.0 h. The reaction was monitored using TLC, cooled to room temperature, then filtered and the filtrate was collected and concentrated to obtain the crude product. The crude product was purified using column chromatography with dichloromethane/ethyl acetate (50:1 to 1:1, v/v) to obtain **NAN6-N$_3$** as yellow solid. Yield 65% (0.27 g). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60 (d, J = 8.2 Hz, 1H), 8.41 (d, J = 7.2 Hz, 1H), 8.18 (d, J = 8.3 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.42 (s, 2H), 6.84 (d, J = 8.3 Hz, 1H), 3.99 (t, J = 7.0 Hz, 2H), 1.60 (s, 2H), 1.37 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.23, 163.37, 153.14, 134.41, 131.46, 130.15, 129.72, 124.45, 122.28, 119.84, 108.62, 108.07, 28.11, 26.82.

3. Response of NAN0-N_3 and NAN6-N_3 towards H_2S or DNA

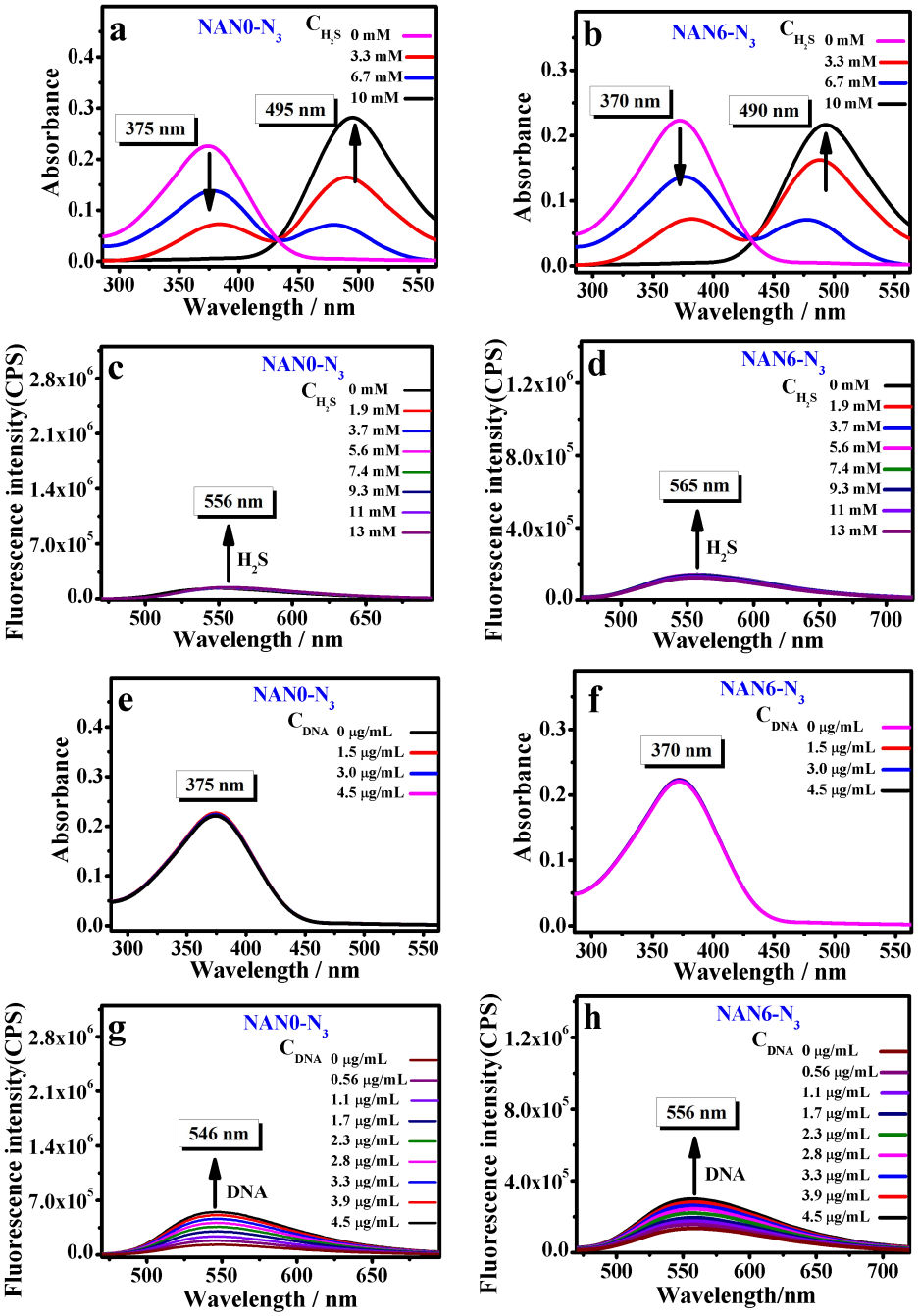


Figure S2. The response of **NAN0-N_3** and **NAN6-N_3** (6.7 μM) towards H_2S or DNA in tris-HCl (0.05 M, pH = 7.4, 37 °C). a, The absorption spectra of **NAN0-N_3** towards Na_2S (0-10 mM). b, The absorption spectra of **NAN6-N_3** towards Na_2S (0-10 mM). e, The absorption spectra of **NAN0-N_3** towards DNA (0-4.5 μg/mL). f, The absorption spectra of **NAN6-N_3** towards DNA (0-4.5 μg/mL). c and g, The emission spectra of **NAN0-N_3** (λ_{ex} = 425 nm) towards Na_2S (c, 0-13 mM) or DNA (g, 0-4.5 μg/mL). d and h, The emission spectra of **NAN6-N_3** (λ_{ex} = 410 nm) towards Na_2S (d, 0-13 mM) or DNA (h, 0-4.5 μg/mL). Na_2S solution (0.1 M) was used as H_2S releasing agent. Commercial smDNA was used for this experiment.

4. Response of ND-N_3 towards H_2S or DNA

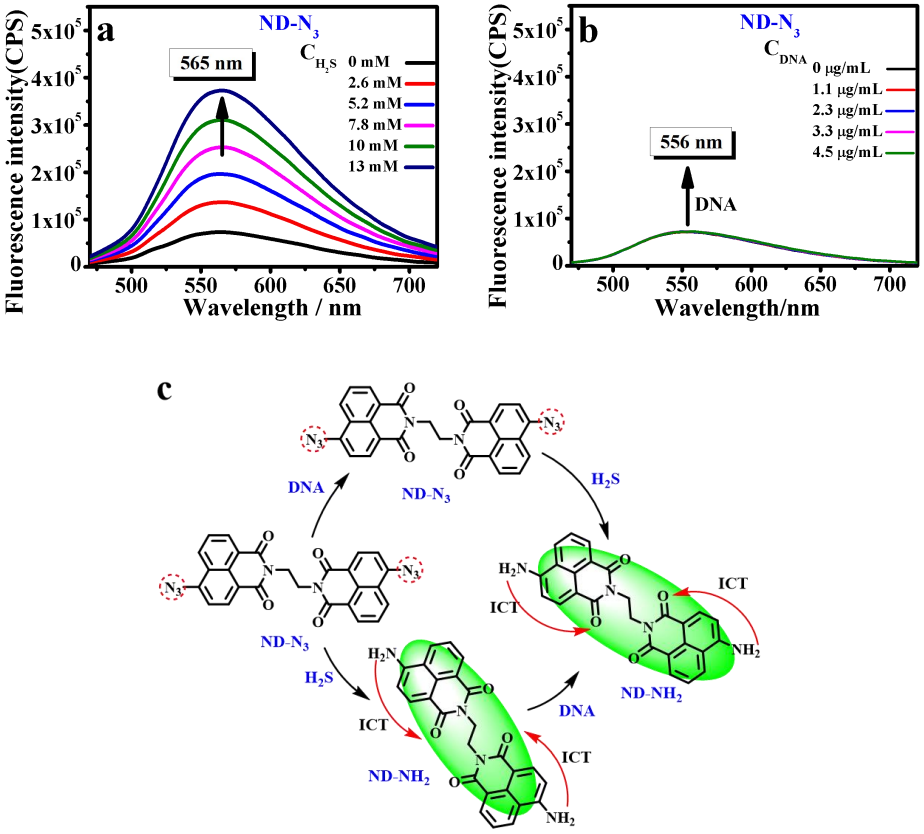


Figure S3. The response of **ND-N_3** towards H_2S or DNA. a-b, The emission spectra of **ND-N_3** (λ_{ex} = 425 nm) towards Na_2S (a, 0-13 mM) or DNA (b, 0-4.5 μg/mL) in tris-HCl (0.05 M, pH = 7.4). c, The response mechanism of **ND-N_3** to H_2S and DNA.

5. Response mechanism of probes to H_2S

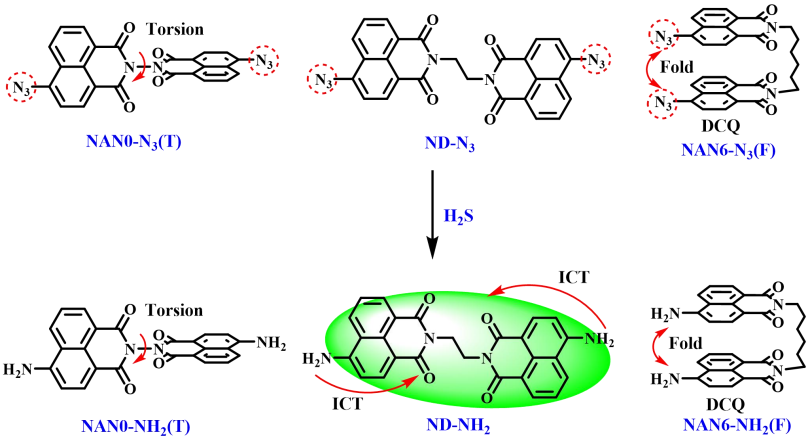


Figure S4. The response mechanism of probes to H_2S.

6. Response mechanism of probes to DNA

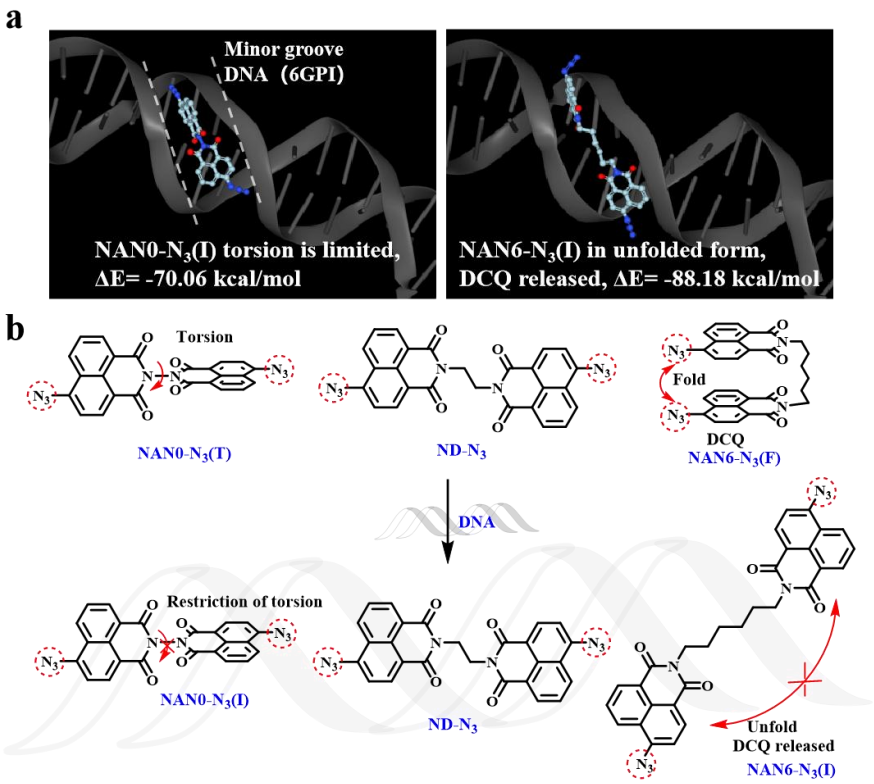


Figure S5. The response mechanism of probes to DNA. a, Molecular docking of probes with DNA (6GPI). b, The response mechanism of probes to DNA.

7. Response of NAN0-N_3 and NAN6-N_3 towards H_2S and DNA

These results are because probes (**NAN0-N_3(T)** and **NAN6-N_3(F)**) underwent conformational changes (**NAN0-N_3(I)** and **NAN0-N_3(I)**) after binding to DNA, which reduced the energy dissipation in the excited state. In addition, H_2S reduces the -N_3 to -NH_2 (**NAN0-NH_2(T)** and **NAN0-NH_2(F)**), and the intramolecular electron cloud density increased. Therefore, under the synergistic action of conformational and structural changes (**NAN0-NH_2(I)**, **NAN6-NH_2(I)**), a highly sensitive fluorescence signal was generated. That is, **NAN0-N_3** and **NAN6-N_3** could be activated by H_2S and DNA in solution.

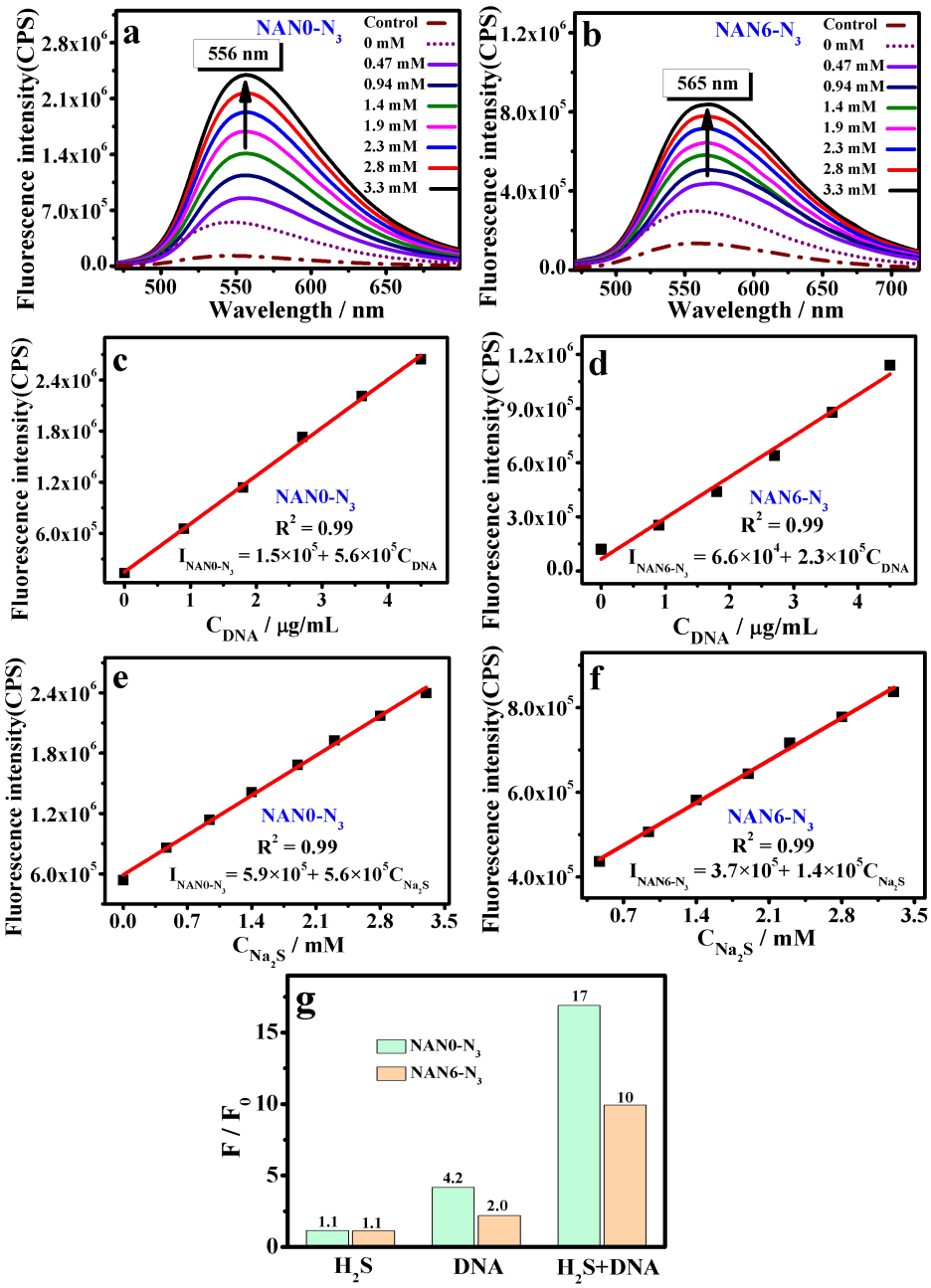


Figure S6. The response of **NAN0-N_3** and **NAN6-N_3** (6.7 μM) towards H_2S and DNA in tris-HCl (0.05 M, pH = 7.4). a and b, The emission spectra of **NAN0-N_3** (a, λ_{ex} = 425 nm) and **NAN6-N_3** (b, λ_{ex} = 410 nm) towards Na_2S (0-3.3 mM) in the presence of DNA (4.5 μg/mL). Dark red dashed line (Control): probe (6.7 μM); Dark purple dotted line (0 mM): probe (6.7 μM) and 4.5 μg/mL DNA. c and d, The linear response of **NAN0-N_3** (c) and **NAN6-N_3** (d) to DNA in the presence of H_2S in the emission spectra, $I_{\mathbf{NAN0\text{-}N3}} = 1.5\times10^5 + 5.6\times10^5 C_{DNA}$ (R^2 = 0.99), $I_{\mathbf{NAN6\text{-}N3}} = 6.6\times10^4 + 2.3\times10^5 C_{DNA}$ (R^2 = 0.99). e and f, The linear response of **NAN0-N_3** (e) and **NAN6-N_3** (f) to H_2S in the presence of DNA (4.5 μg/mL) in the emission spectra (a or b), $I_{\mathbf{NAN0\text{-}N3}} = 5.9\times10^5 + 5.6\times10^5 C_{Na2S}$ (R^2 = 0.99), $I_{\mathbf{NAN6\text{-}N3}} = 3.7\times10^5 + 1.4\times10^5 C_{Na2S}$ (R^2 = 0.99). g, Changes in fluorescence emission intensity before and after the probes responded to H_2S or/and DNA.

8. The selectivity of probes

Fig. S7 showed that the responses of **NAN0-N_3** and **NAN6-N_3** to DNA and H_2S were not affected by biological coexistence such as ions and amino acids.

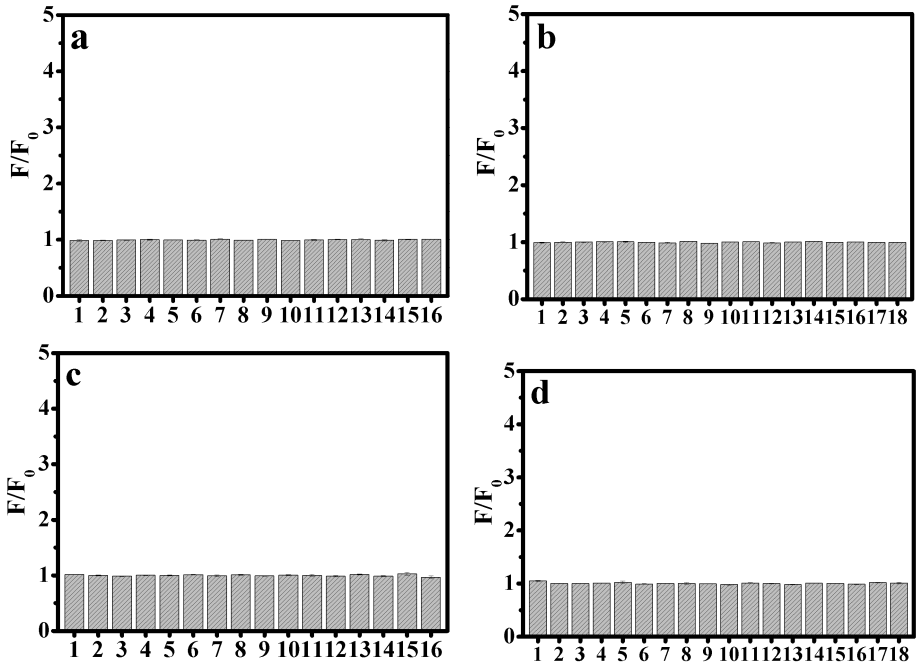


Figure S7. The selectivity of **NAN0-N_3** (6.7 μM, a, b) and **NAN6-N_3** (6.7 μM, c, d) for DNA and H_2S. a and c, ion interference experiment (concentrations for each are 1.0 mM). 1, $CuSO_4$; 2, $MgSO_4$; 3, $Zn(NO_3)_2 \cdot 6H_2O$; 4, $FeCl_2 \cdot 7H_2O$; 5, $CaCl_2 \cdot 6H_2O$; 6, $FeCl_3 \cdot 6H_2O$; 7, $CdCl_2$; 8, $CoCl_2$; 9, $HgCl_2$; 10, $MnCl_2$; 11, $AgNO_3$; 12, $K_2HPO_4 \cdot 3H_2O$; 13, Li_2CO_3; 14, $SnCl_2$; 15, Na_2CO_3; 16, $Al(NO_3)_3$. b and d, macromolecule coexistence interference experiment (concentrations for each are 50 μM). 1, D-methionine; 2, L-cysteine; 3, Arginine; 4, Hypoxanthine; 5, DL-leucine; 6, Serine; 7, DL-homocysteine; 8, Glutamate; 9, Glycine; 10, Glycine-DL-phenylalanine; 11, L-glutamate; 12, L-lysine; 13, DL-threonine; 14, Valine; 15, Glutamine; 16, Cystine; 17, L-aspartate; 18, Glutathione.

9. The photostability and pH-stability of probes

Fig. S8 showed that **NAN0-N_3** and **NAN6-N_3** have good stability (photostability, pH stability), which is beneficial to the fluorescence imaging of probes in cells.

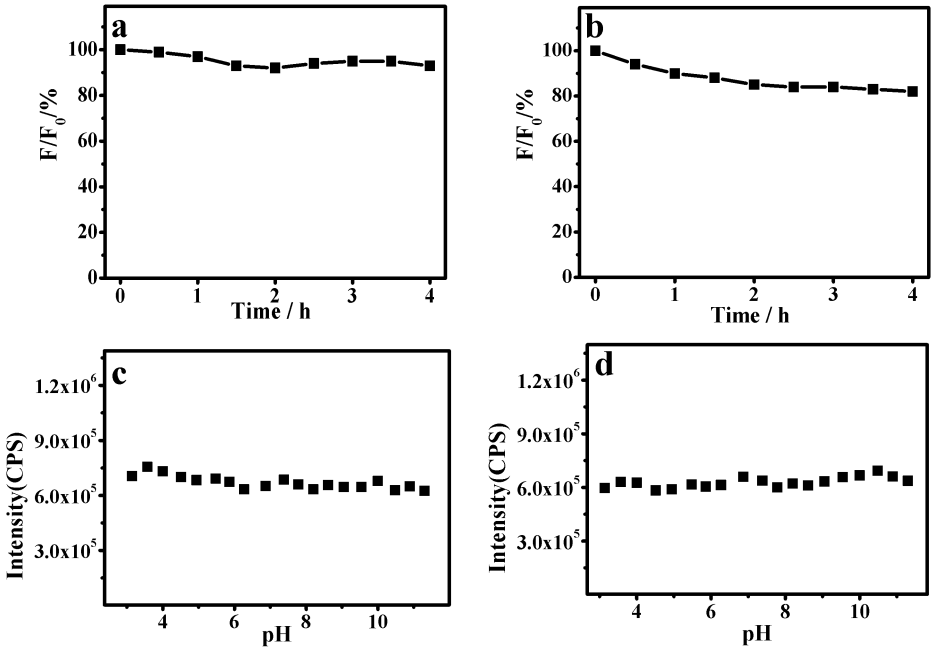


Figure S8. The photostability (a, b) and pH-stability (c,d) of **NAN0-N_3** (a, c) and **NAN6-N_3** (b, d).

10. The solubility of probes

Fig. S9 showed that **NAN0-N_3** and **NAN6-N_3** have good water solubility.

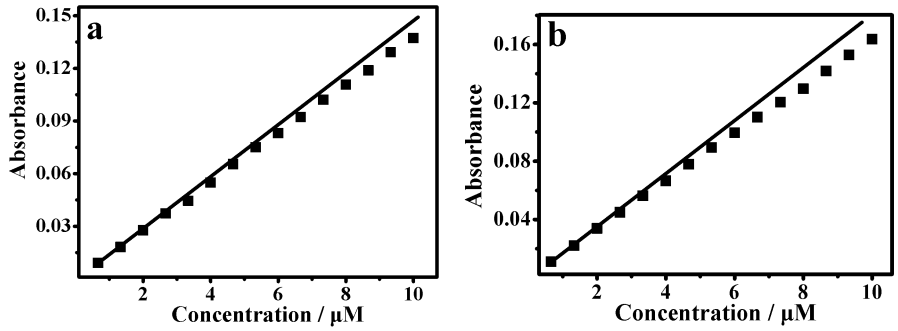


Figure S9. The solubility of **NAN0-N_3** (a) and **NAN6-N_3** (b) in water.

11. The cytotoxicity of probes

Fig. S10 showed that **NAN0-N_3** and **NAN6-N_3** have lower cytotoxicity, which is favorable for biometric detection.

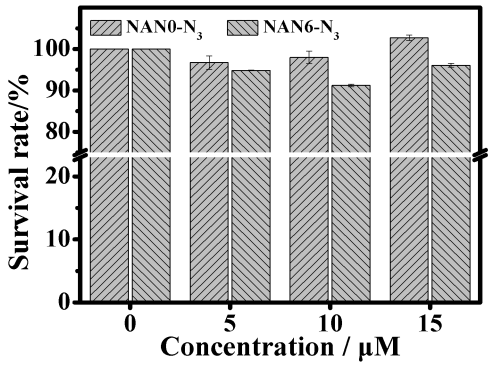


Figure S10. The cytotoxicity of **NAN0-N_3** and **NAN6-N_3** (0 μM, 5.0 μM, 10.0 μM and 15.0 μM).

12. Imaging of DNA digestive enzymes

Fig. S11 indicated that all the probes (6.0 μM) exhibited fluorescence in the green channel (535-575 nm). In addition, as the concentration of DNA digestive enzymes increased, DNA damage was enhanced and the fluorescence intensity of **NAN0-N_3** and **NAN6-N_3** decreased significantly. That is, **NAN0-N_3** and **NAN6-N_3** could only bind with intact DNA in cells, while DNA binding altered the molecular conformation, which reduced the fluorescence quenching caused by intramolecular torsion (**NAN0-N_3**) or DCQ (**NAN6-N_3**), and resulted in the fluorescence being partially turned on. These results are consistent with those from the solution-based experiments (**Fig. S2**). In contrast, the fluorescence of **ND-N_3** did not change after the addition of DNA digestive enzyme, indicating that **ND-N_3** exhibits no response to DNA due to the length of linker.

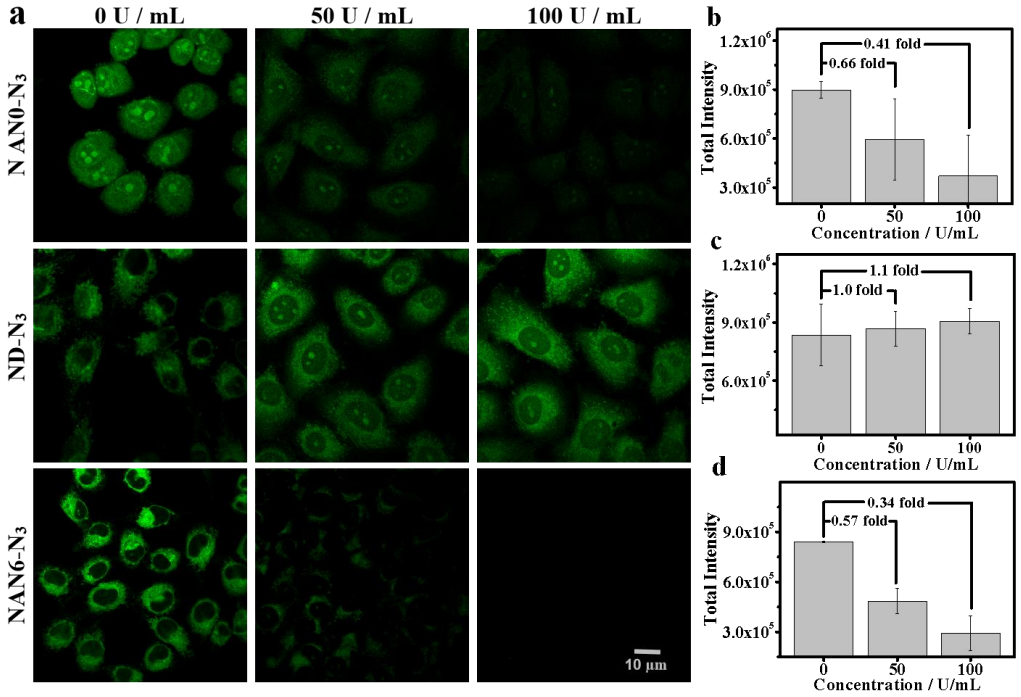


Figure S11. Imaging with DNA digestive enzymes in living HepG2 cells. a, Imaging with DNA digestive enzymes in living HepG2 cells. HepG2 cells were pretreated with different concentrations of DNA digestive enzymes (0, 50.0 and 100.0 U/mL) for 1.0 h. Stained with probes (6.0 μM). Excitation wavelength = 405 nm, scan range = 535-575 nm (green channel). Probes incubation time: 30.0 min. b-d, Histogram of the green channel in (a). Fold: intensity final / intensity at start.

13. Imaging of endogenous H_2S

Fig. S12 showed that when HepG2 cells were pretreated with PAG (DL-Propargylglycine, an inhibitor of cystathionine γ-lyase during endogenous H_2S synthesis), their fluorescence intensity remained constant. That is, normal levels of H_2S expressed in live cells cannot reduce $-N_3$ (one of the locks) to $-NH_2$, and the fluorescence remains in a semi-quenched state. In contrast, the fluorescence of **ND-N_3** decreased significantly with treatment by PAG, indicating that its fluorescence signal was influenced by H_2S.

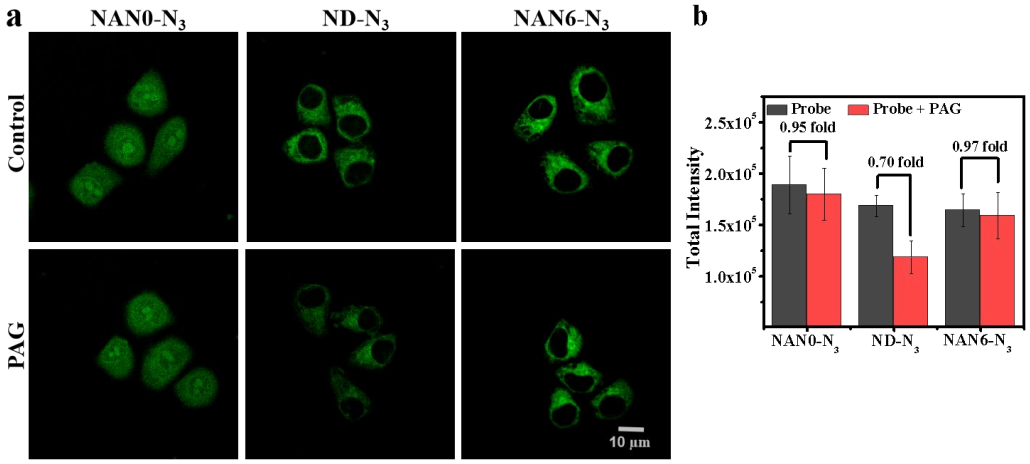


Figure S12. Imaging of endogenous H_2S in living HepG2 cells. a, The imaging of endogenous H_2S in living HepG2 cells. HepG2 cells were pretreated with PAG (1.0 mM) for 1.0 h to inhibit endogenous H_2S production. Stained with **NAN0-N_3**, **ND-N_3** and **NAN6-N_3** (6.0 μM), respectively. Excitation wavelength = 405 nm, scan range = 535-575 nm (green channel). Incubation time: 30.0 min. b, Histogram of the green channel in (a). Fold: intensity final / intensity at start.

14. Colocalization imaging

Fig. S13 showed that the Pearson's correlation coefficient (Rr) for the staining region of **NAN6-N_3**, Hoechst 33258 and Mito-Tracker Deep Red were 0.37, 0.96, respectively, which indicated that **NAN6-N_3** could accumulate in the DNA of the mitochondria, *i.e.* mtDNA. Furthermore, the region stained by **NAN0-N_3** overlapped with that for Hoechst 33258 (Rr = 0.91), suggesting that **NAN0-N_3** could enter the nucleus and specifically detect nDNA. That is, the different length of the linkers could regulate the flexibility, biological activity and lipophilicity of the probes, thus realizing the accumulation in different subcellular regions of the cell. These imaging results confirmed that **NAN0-N_3** and **NAN6-N_3** could achieve specific binding with nDNA and mtDNA, respectively.

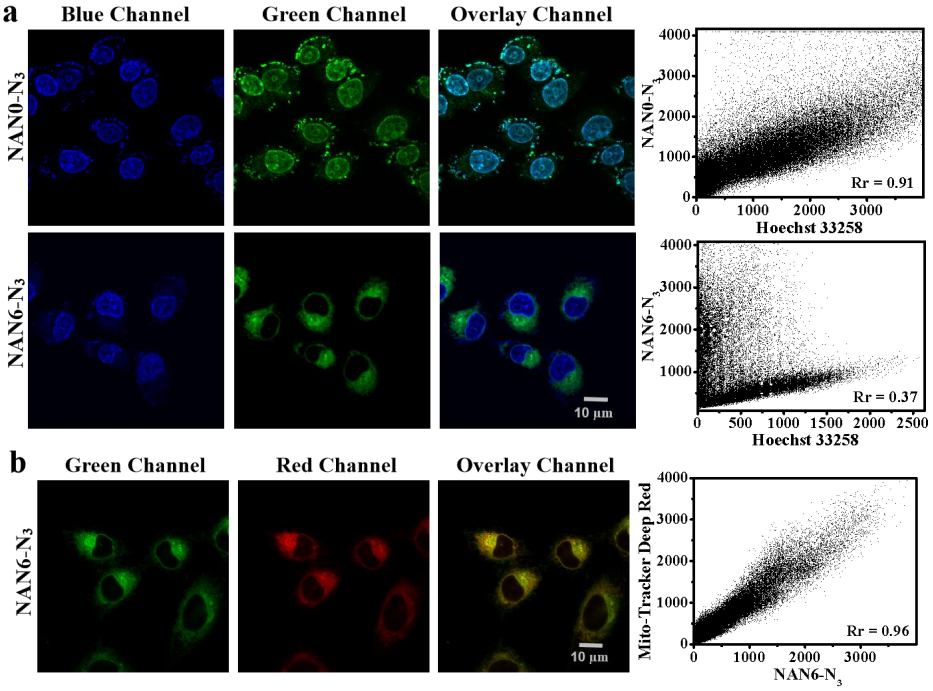


Figure S13. Localization imaging in HepG2 cells. a, Localization images in living HepG2 cells. Stained with Hoechst 33258 (10.0 μM) and probes (6.0 μM). Hoechst 33258: excitation wavelength = 405 nm, scan range = 440-460 nm (blue channel); probes: excitation wavelength = 405 nm, scan range =537-575 nm (green channel). Incubation time: 30.0 min. b, Localization images in living HepG2 cells. Stained with Mito-Tracker Deep Red (5.0 μM) and probes (6.0 μM). probes: excitation wavelength = 405 nm, scan range =537-575 nm (green channel); Mito-Tracker Deep Red: excitation wavelength = 635 nm, scan range = 650-680 nm (red channel). Incubation time: 2.0 h.

15. Imaging of H_2S-induced DNA damage

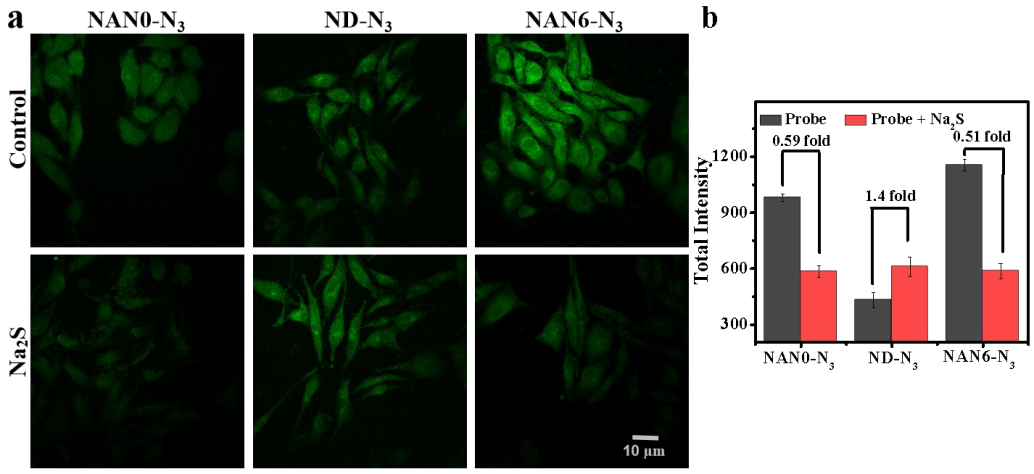


Figure S14. Imaging of H_2S-induced DNA damage in living CHO cells. a, The imaging of H_2S-induced DNA damage in living CHO cells. CHO cells were pretreated with Na_2S (500 μM) for 4.0 h to establish H_2S-induced DNA damage cell model. Stained with **NAN0-N_3**, **ND-N_3** and **NAN6-N_3** (6.0 μM), respectively. Excitation wavelength = 405 nm, scan range = 535-575 nm (green channel). Incubation time: 30.0 min. b, Histogram of the green channel in (a). Fold: intensity final / intensity at start.

16. Assessing the health of cells during apoptosis

Fig. S15 showed that the fluorescence intensity of the green channel decreased significantly with an increase of apoptosis. This is because the damage of mtDNA increases with an increase in the degree of apoptosis. When mtDNA damage increases the amount of binding by **NAN6-N_3** to the minor groove in the non-folded form decreases, and its fluorescence signal intensity decreases. It means that **NAN6-N_3** can be used as a highly sensitive tool to help evaluate the degree of cell apoptosis based on the highly sensitive response to H_2S-induced mtDNA damage.

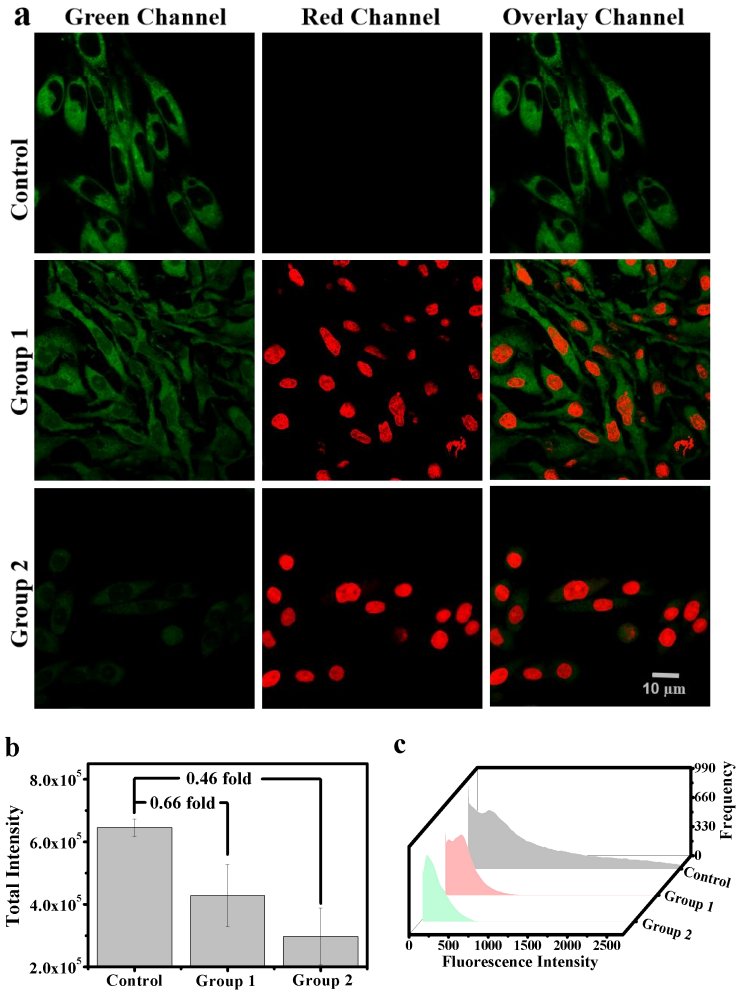


Figure S15. Cell imaging of live CHO cells staining with **NAN6-N_3** (6.0 μM) and PI (10.0 μM) during cell apoptosis. a, Cell images during cell apoptosis. b, Histogram of the green channel in (a). c, Data for the green channel in (a). Control group: untreated cells, healthy cell model. Experimental group 1: the cells were incubated at 56 °C for 4.0 h, early apoptotic cell model. Experimental group 2: the cells were incubated at 65 °C for 4.0 h, late apoptotic cell model. Stained with **NAN6-N_3** (6.0 μM) and PI (10.0 μM). **NAN6-N_3**: excitation wavelength = 405 nm, scan range = 535-575 nm (green channel); PI: excitation wavelength = 559 nm, scan range = 600-630 nm (red channel). Incubation time: 30.0 min. Fold: intensity final / intensity at start.

17. FCM analyses during cell apoptosis

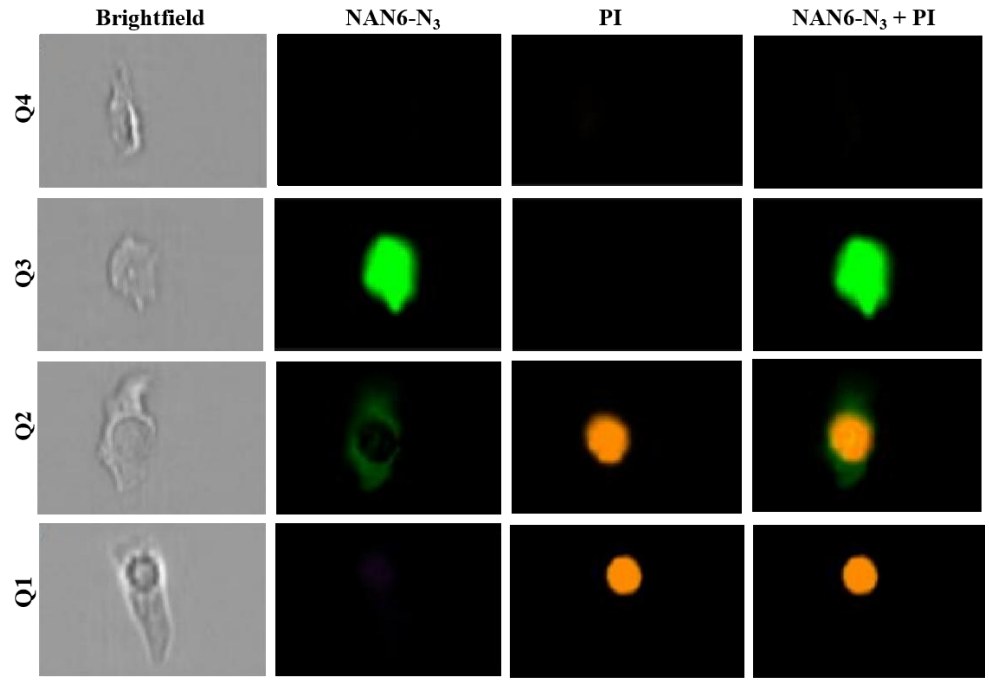


Figure S16. Single cell images of different regions in FCM analyses of HepG2 cells staining with **NAN6-N_3** (6.0 μM) and PI (10.0 μM) during cell apoptosis. Q1: death. Q2: late apoptosis. Q3: early apoptosis. Q4: healthy cells. **NAN6-N_3**: excitation wavelength = 488 nm, scan range = 535-575 nm; PI: excitation wavelength = 515 nm, scan range = 600-630 nm. Incubation time: 30.0 min.

18. HRMS spectra for probes reaction with H_2S

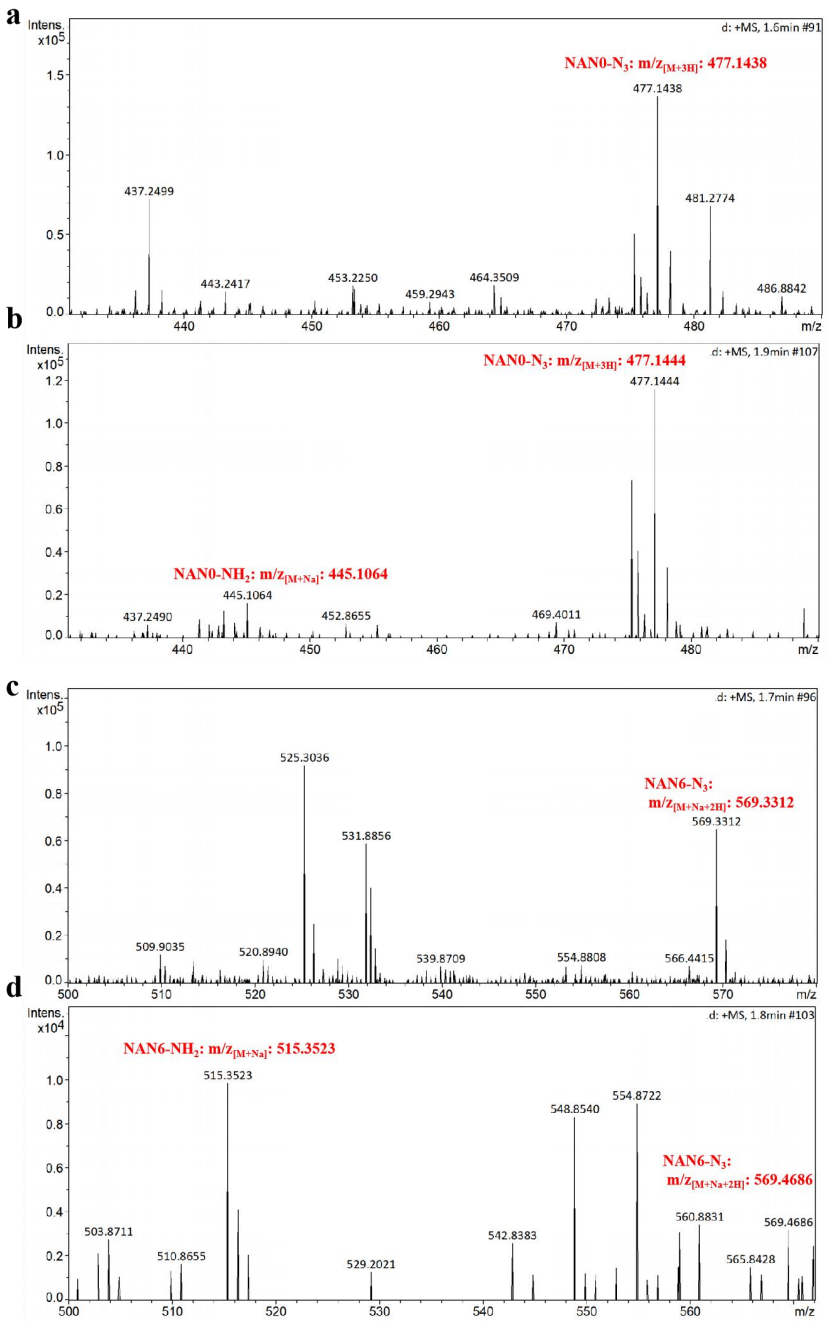
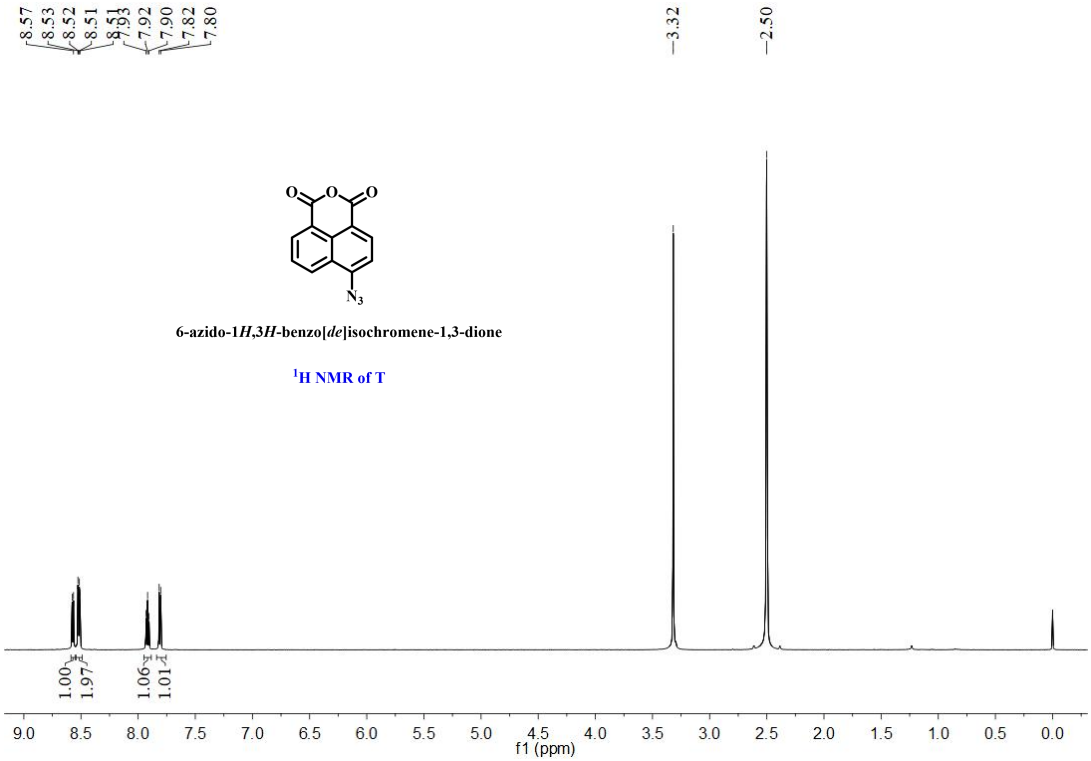


Figure S17. The HRMS spectra of **NAN0-N_3** (a, b) and **NAN6-N_3** (c, d) response to H_2S. a and b, before reaction; c and d, after reaction.

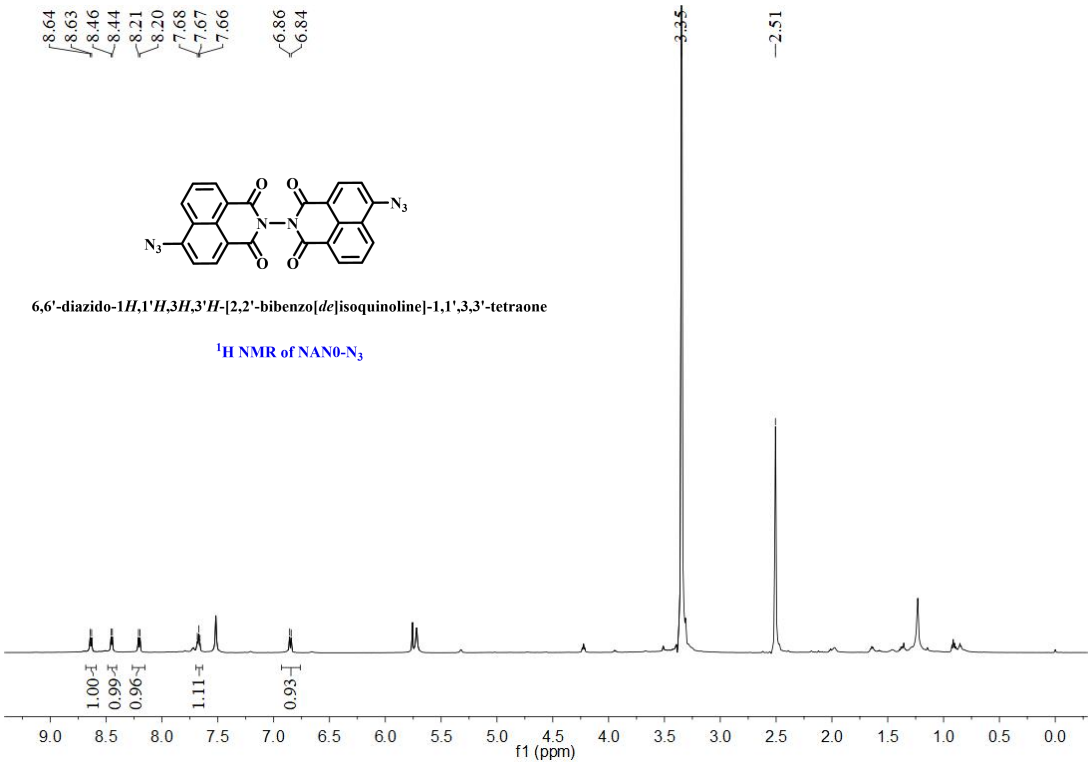
19. NMR Spectra

(1) ^{1}H NMR



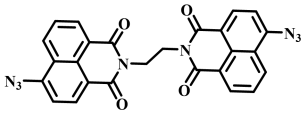
6-azido-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione

^{1}H NMR of T



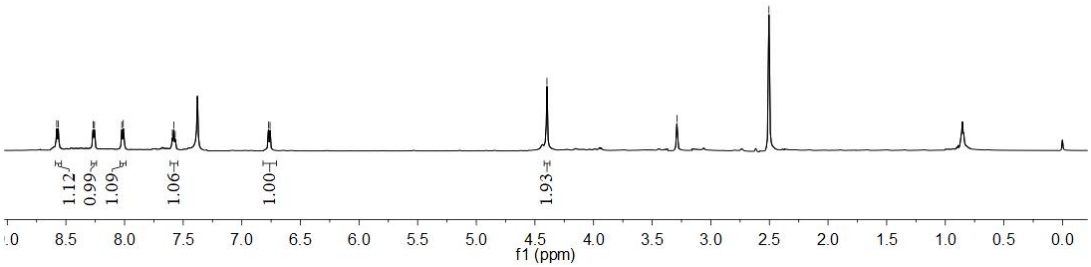
6,6'-diazido-1*H*,1'*H*,3*H*,3'*H*-[2,2'-bibenzo[*de*]isoquinoline]-1,1',3,3'-tetraone

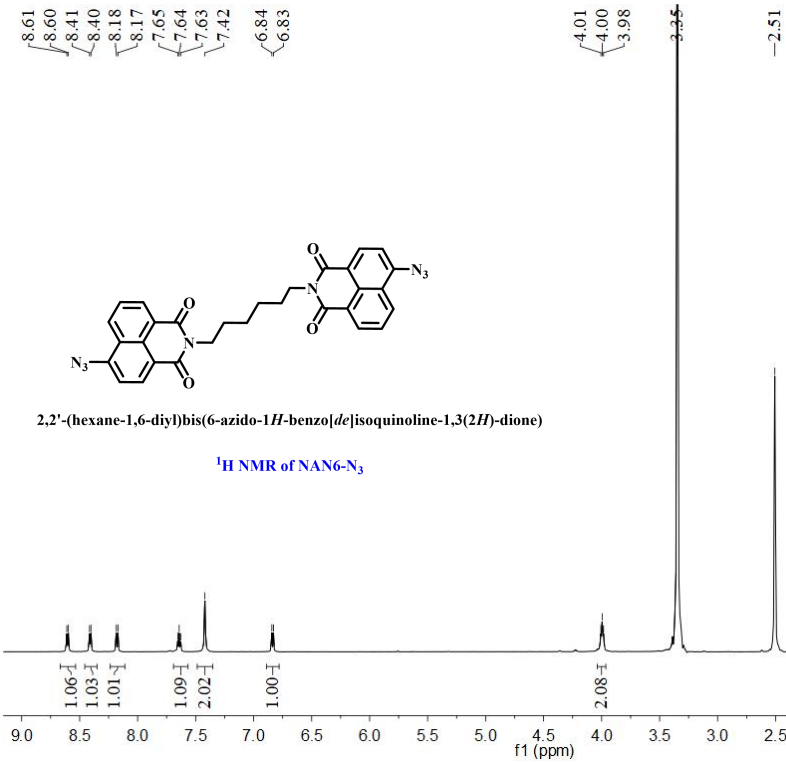
^{1}H NMR of NAN0-N_3



2,2'-(ethane-1,2-diyl)bis(6-azido-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)

^{1}H NMR of ND-N_3

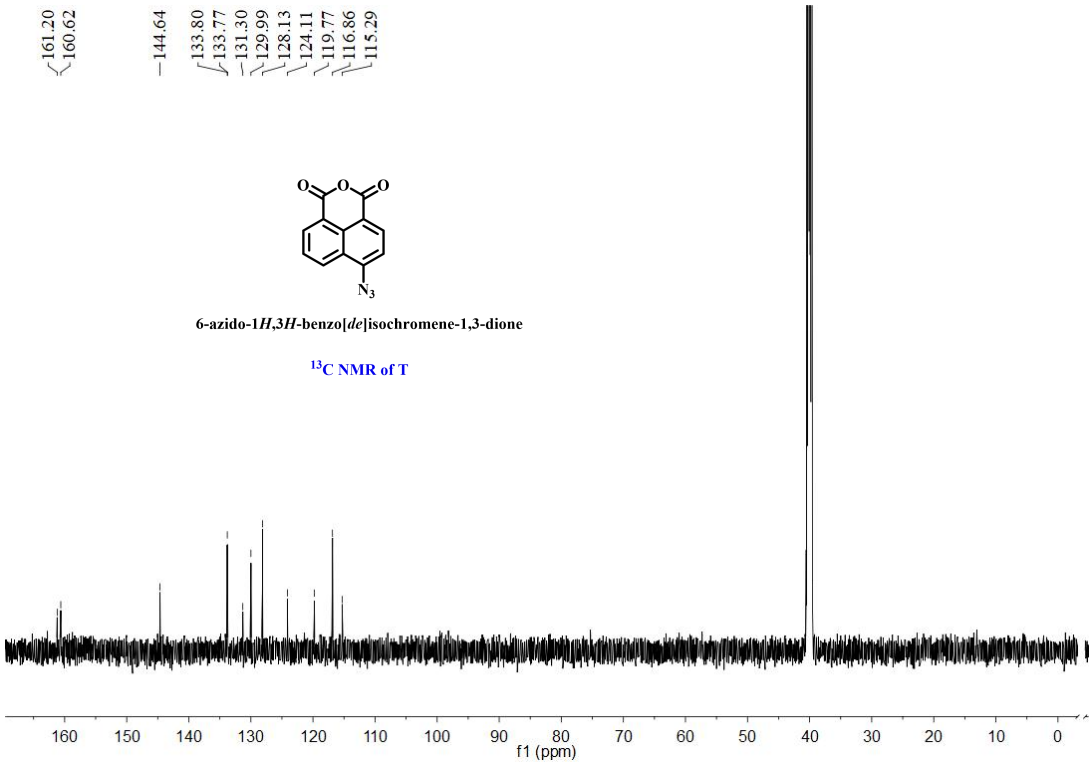




2,2'-(hexane-1,6-diyl)bis(6-azido-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)

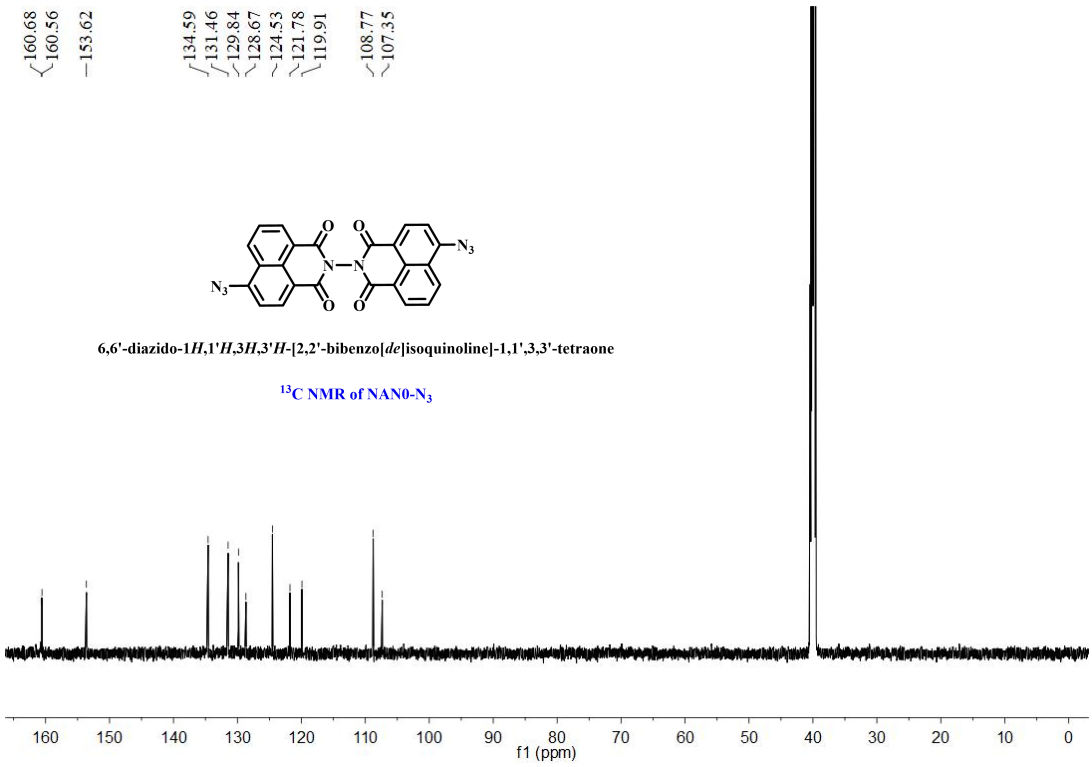
^{1}H NMR of NAN6-N_3

(2) ^{13}C NMR



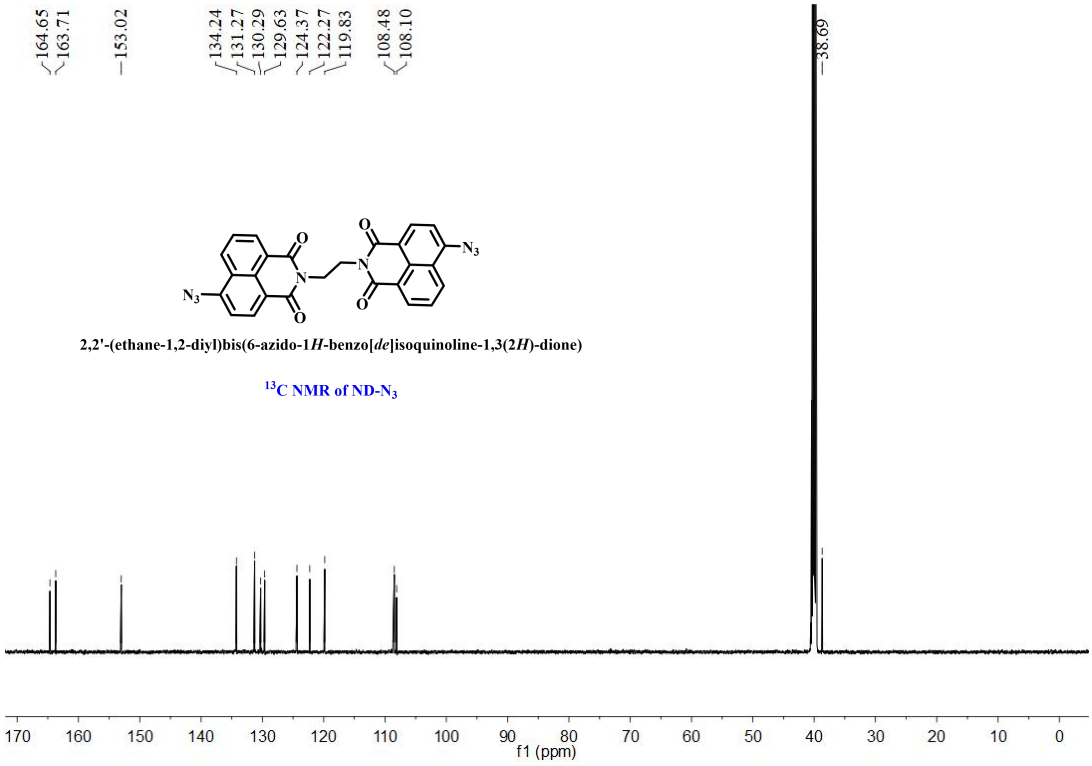
6-azido-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione

^{13}C NMR of T



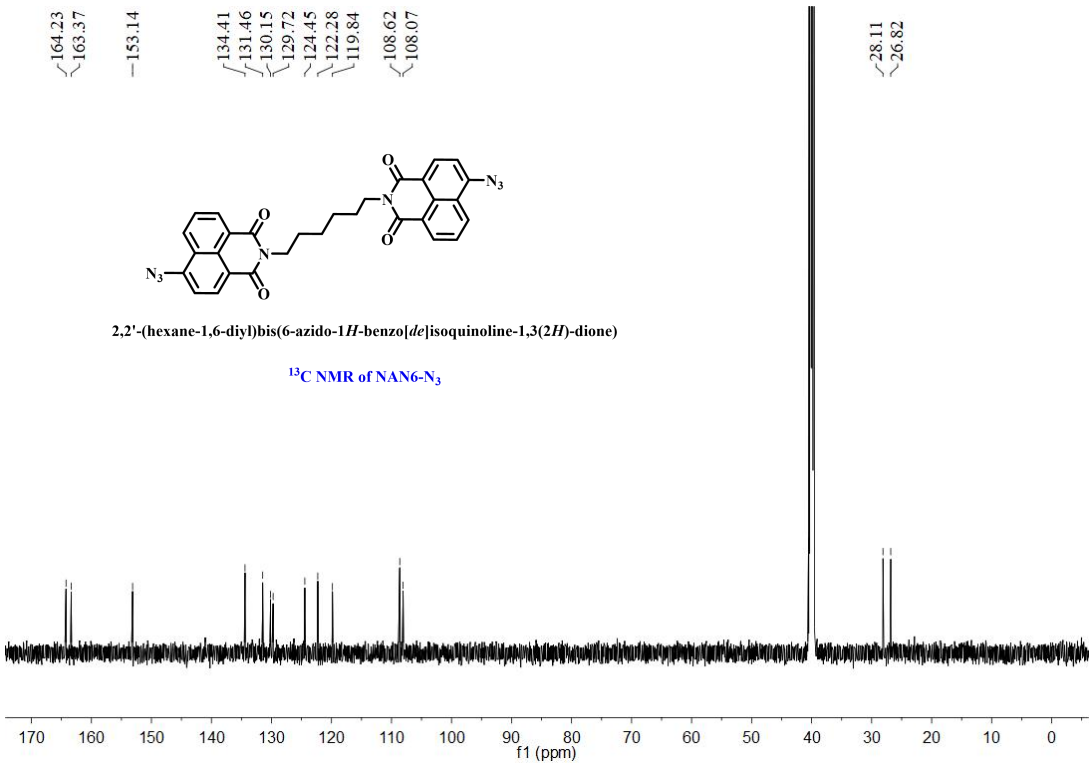
6,6'-diazido-1*H*,1'*H*,3*H*,3'*H*-[2,2'-bibenzo[*de*]isoquinoline]-1,1',3,3'-tetraone

^{13}C NMR of NAN0-N_3



2,2'-(ethane-1,2-diyl)bis(6-azido-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)

^{13}C NMR of ND-N_3



2,2'-(hexane-1,6-diyl)bis(6-azido-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)

^{13}C NMR of NAN6-N_3