Supplementary Information

A cysteine-triggered fluorogenic donor base on native chemical ligation for tracking H_2S delivery in vivo.

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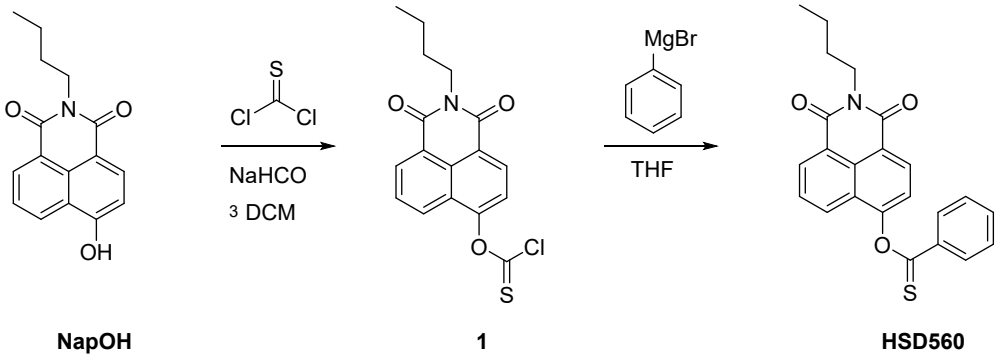
All reagents were purchased from Energy Chemicals, Ltd. Titan Scientific and used directly without further purification. ELISA and Griess reagent kits were purchased from Sangon Biotech. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer. Chemical shifts were exhibited in ppm relative to residual solvent peaks. HRMS was acquired over a Micro-mass GCT spectrometer. UV-Vis absorption spectra were collected on a SHIMADZU UV-2600 plus UV-Vis spectrophotometer. Fluorescence spectra were obtained on a SHIMADZU RF-3000 fluorescence spectrophotometer. The excitation and emission slits were set to 5 nm. The confocal fluorescence images of cells and zebrafish were photographed by Leica SP5 laser confocal microscope.

S1. Synthesis



Scheme S1. Synthetic route of **HSD560**.

Synthetic procedures and characterizations:

O-(2-butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl) carbonochloridothioate (1):

To a dry dichloromethane (DCM) solution (15 mL) of **NapOH** (850 mg, 3.16 mmol) and $NaHCO_3$ (530 mg, 6.31 mmol), thiophosgene (544 mg, 4,73mmol) in dry DCM (15 mL) was added dropwise over 60 min in ice bath. The reaction mixture was stirred at room temperature and monitored by TLC. After removing the solvent, the residue was purified via a flash chromatography with silica gel to give compound **1**. Yield: 750 mg, 68.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.67 (dd, *J*=7.2, 0.8 Hz, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.46 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.86 (t, *J* = 8.0, 1H), 7.65 (d, *J*=8.0, 1H), 4.17 (t, *J*=7.2, 2H), 1.75-1.68 (m, 2H),1.48-1.42 (m, 2H), 0.98 (t, *J*=7.6, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 184.46, 163.72, 163.14, 154.45, 132.07, 131.46, 129.56, 128.08, 127.16, 124.12, 123.32, 122.11, 119.83, 40.42, 30.18, 20.37, 13.83. $[M]^+$ calcd. For C17H14ClNO3S, 347.0383; found 347.0386

O-(2-butyl-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl) benzothioate (HSD560):

Compound **1** (500 mg, 1.44 mmol) was added to an anhydrous 20 mL THF solution under Ar at -78 °C. Then, phenylmagnesium bromide (14.4 mmol, 1.0 M in THF) was added to the mixture THF solution, and stirred for 1 hour at -78 °C. The reaction mixture was stirred at room temperature for another 1 hour. The reaction was quenched by 20 mL deionized water and extracted with 20 mL EtOAc for three times. The organic phase was combined and dried with Na_2SO_4, filtered and evaporated under reduced pressure to afford a crude product, which was purified via a flash chromatography with silica gel to give **HSD560**. Yield: 395 mg, 70.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (d, *J* = 8.0 Hz, 1H), 8.63 (d, *J* = 7.2 Hz, 1H), 8.48 (d, *J* = 7.8 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.76-7.68 (m, 2H), 7.54 (t, *J* = 8.4 Hz, 3H), 4.20 (t, *J* = 7.6 Hz, 2H), 1.77-1.70 (m, 2H), 1.50-1.44 (m, *2*H), 0.99 (t, *J* = 7.6, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.76, 164.00, 163.50, 155.69, 137.14, 134.11, 131.77, 131.63, 129.44, 128.66, 127.92, 127.44, 125.31, 123.29, 121.13, 120.74, 40.35, 30.22, 20.41, 13.86. $[M]^+$ calcd. For C23H19NO3S, 389.1086; found 389.1095.

S2. Spectroscopic Analysis

A stock solution of 10 mM **HSD560** was prepared in DMSO. The fluorescence response of **HSD560** (10 μM) was performance in 3 mL PBS solution with 20% DMSO (10 mM pH = 7.40). Each thiol and reactive oxygen species (ROS) were also prepared in appropriate concentrations. In the selectivity test, the ROS, including H_2O_2, ClO^-, ·OH, $ONOO^-$, NO, were generated according to the previous literature. [1]

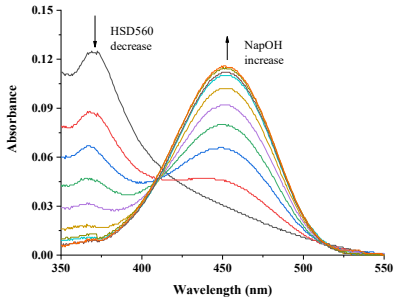


Fig. S1 UV-Vis Absorption titration of **HSD560** by Cys (100 μM)

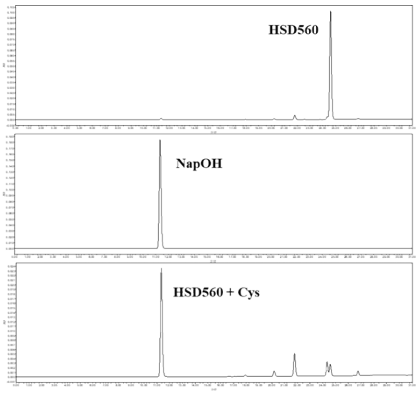


Fig. S2 HPLC and LC-MS analysis of **HSD560** reaction with Cys (100 μM) for 30 min. Eluent solvent: MeCN/H_2O (7/3, v/v), flow rate = 1 mL/min.

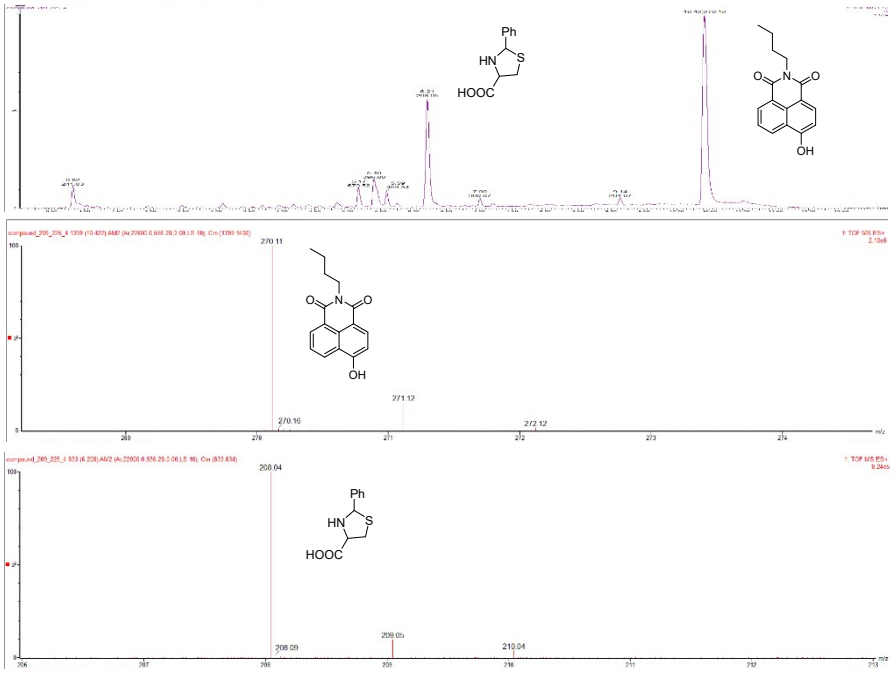


Fig. S3 LC-MS analysis of **HSD560** reaction with Cys (100 μM) for 30 min. Mass of NapOH (m/z =269.1)

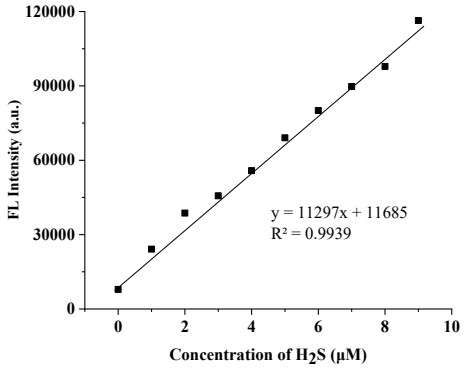


Fig. S4 H_2S (0-10 μM) calibration curve of C7-Az

S3. Cells Assay.

Cell Culture and Cytotoxicity Test.

HepG2 and macro-phage RAW264.7 cells were cultured at 37 °C under a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM), containing10% heat-inactivated fetal bovine serum (FBS).

The cytotoxic effects of **HSD560** were assessed by the CCK8 assays kit. HepG2 and macro-phage RAW264.7 cells were seeded in a 96-well plate at 7 × 103 cells/well and cultured overnight. Then cells were incubated for 24 h upon different concentrations (0,5,10,15, and 20 μM) of **HSD560**. Subsequently, cells in each well were incubated with 10 μL CCK8 reagent for 3 h. The absorbance values of each well were recorded using a microplatereader at 450 nm.

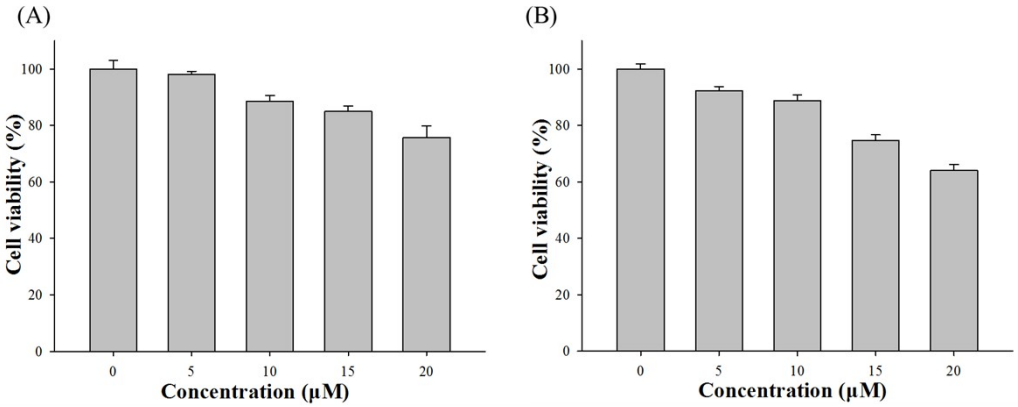


Fig. S5 Cell cytotoxicity of **HSD60** against HepG2 and (B) Raw 264.7 cells evaluated by CCK8 assay. The results are expressed as mean ± SD (n =4).

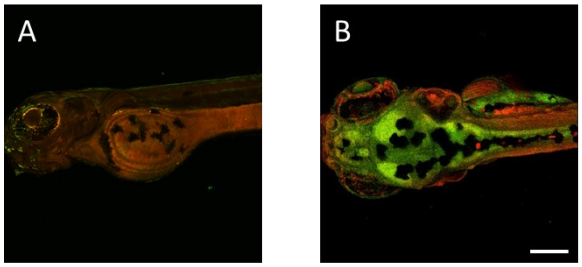
Confocal Microscopy Imaging.

DAPI (4′,6-diamidino-2-phenylindole) was used to stain fixed HepG2 cells for nuclei protocols. HepG2 cells were incubated with 500 μM N-ethylmaleimide (NEM) in FBS-free DMEM at 37 °C for 30 min firstly; the cells were treated with **HSD560** (10 μM) and HS-Az655 (10 μM, H_2S probe) for another 15 min. HS-Az655 is used as a near-infrared fluorescent probe to monitor the release of H_2S from **HSD560** in cells.

Extracellular **HSD560** and HS-Az655 were removed by washing with PBS, cells were treated with or without Cys (200 μM) in FBS-free for 30 min. In the contrast group, cells were cultured with Hcy (200 μM) or GSH (200 μM) for 30 min after washing off the remaining **HSD560** and HS-Az655. Finally, cells were cultured in 2 mL of FBS-free DMEM before imaging. Cell imaging was conducted using a Leica SP5 microscope. **HSD560** was excited at 488 nm, and the fluorescence was collected with wavelength 530-570 nm. HS-Az655 was taken with the excitation/emission wavelength of 488/650 ± 20 nm, and DAPI was set 480 nm as the excitation wavelength, 430 to 470 nm as the emission wavelength range.

Living Zebrafish Imaging.

The AB strain of adult zebrafish was approved by Guangxi Key Laboratory of Efficacy Study on Chinese Materia Medica, Guangxi University of Chinese Medicine (approval number DW20190525-69). Before the experiment, the zebrafishes (72 hpf) were divided into three groups. The first group was treated with **HSD560** (10 μM) and HS-Az655 (10 μM) for 30 min as a control. And in the second group, zebrafishes were pretreated with NEM (500 μM) for 30 min and then co-cultured with **HSD560** (10 μM) and HS-Az655 (10 μM) as a contrast. In the experimental group, after the same operation as the second group, zebrafishes were cultured with Cys (200 μM) for another 30 min. Subsequently, washed three times with PBS buffer to remove the remaining medium, the zebrafish was imaged with the excitation wavelength of 488 nm, and the fluorescence was collected including a green channel (530-570 nm) and red channel (630-670 nm).



Donor	Pearson's coeffcient	Overlap coeffient
HSD560 (A)	0.92	0.93
HSD545 (B)	0.84	0.87

Scheme S2. The contrast of overlapped fluorescence from (a)**HSD560**; (B)HSD545 in zebrafish.[2]

Anti-inflammatory activity of HSD560.

Macrophage RAW 264.7 cells were cultured with 0.5 mL of DMEM in a 24-well plate at 0.5×10^6 cells/well at 37 °C under 5% CO_2 for 24 h.

Detection of the nitrite levels in cells. Briefly, cells were co-cultured with 1 μg/mL lipopolysaccharides (LPS) various concentrations (0,5,10 and 20 μM) of **HSD56** for 24 hour as the experimental group. And the positive group was treated with 1 μg/mL LPS and 5 μg/mL dexamethasone. NO levels were assessed by a Griess reagent kit. Collected cell culture medium supernatant (100 μL/well), and plated into a 96-well plate, then 100 μL the Griess reagent was added, which containing equal volumes of 10

mg/mL sulfanilic acid and 1 mg/mL N-(1-naphthyl) ethylenediamine. The absorbance at 550 nm was collected by a microplatereader. The levels of nitrite were detected based on the standard calibration curve.

Measurement of prostaglandin E2 (PGE2). The LPS (1 μg/mL)-pretreated cells were cultured with **HSD560** (5, 10 and 20 μM), Dex (5 μg/mL) for 24 h. Subsequently, 100 μL supernatant of cell culture medium was collected to calculate the PGE2 levels in each well by using a commercial ELISA kit according to the manufacturer's protocols.

[1] Q. Zhang, Z. Zhu, Y. Zheng, J. Cheng, N. Zhang, Y.-T. Long, J. Zheng, X. Qian, Y. Yang, *J. Am. Chem. Soc*. 134 (2012) 18479-18482.

[2] L. Li, Z. Zhang, *Spectrochim. Acta A*, 264 (2022) 120243-120249.

S4. Original NMR Spectra and HRMS Spectrum.

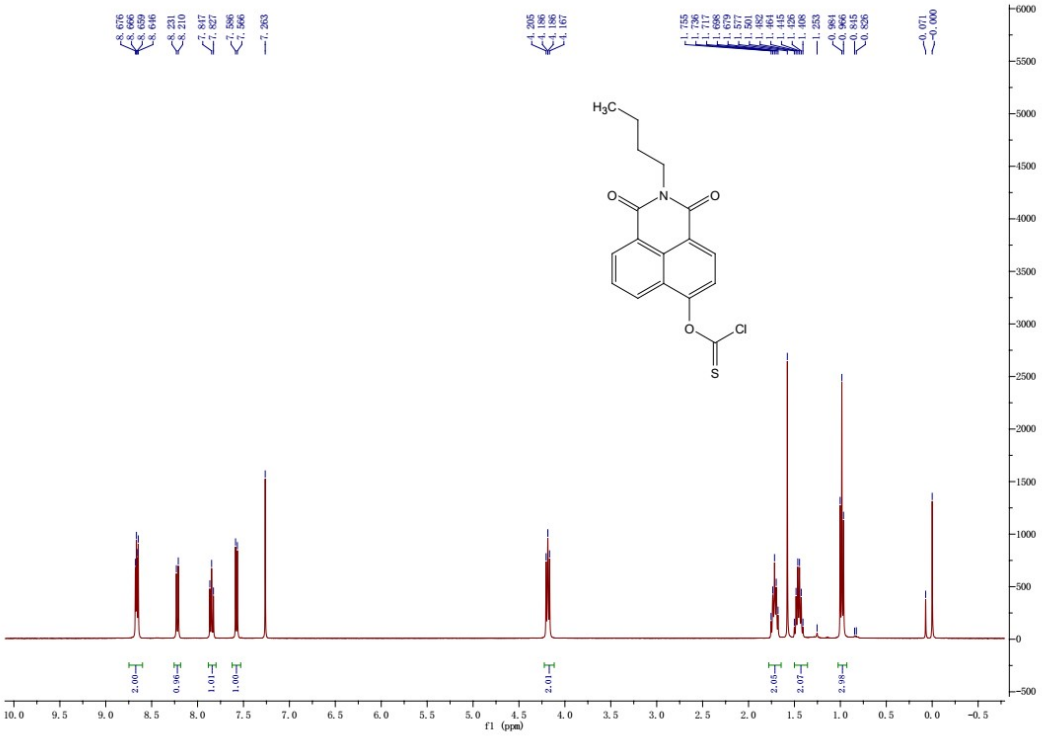


Figure S6. The ^{1}H NMR spectrum of compound **1**.

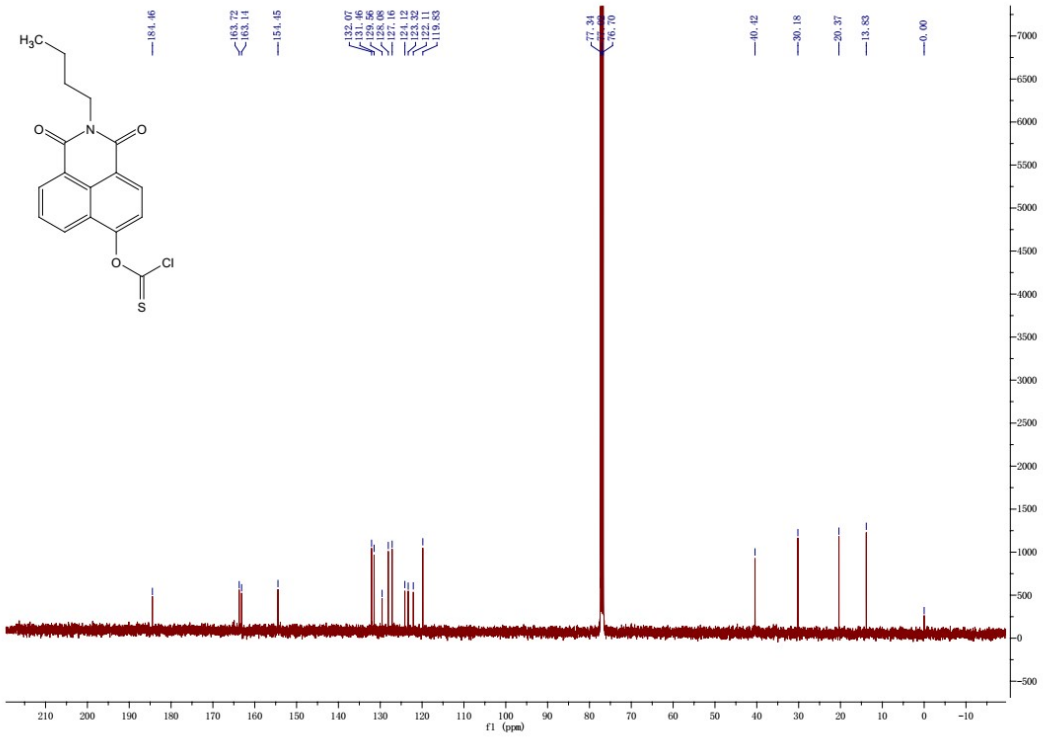


Figure S7. The ^{13}C NMR spectrum of compound **1**.

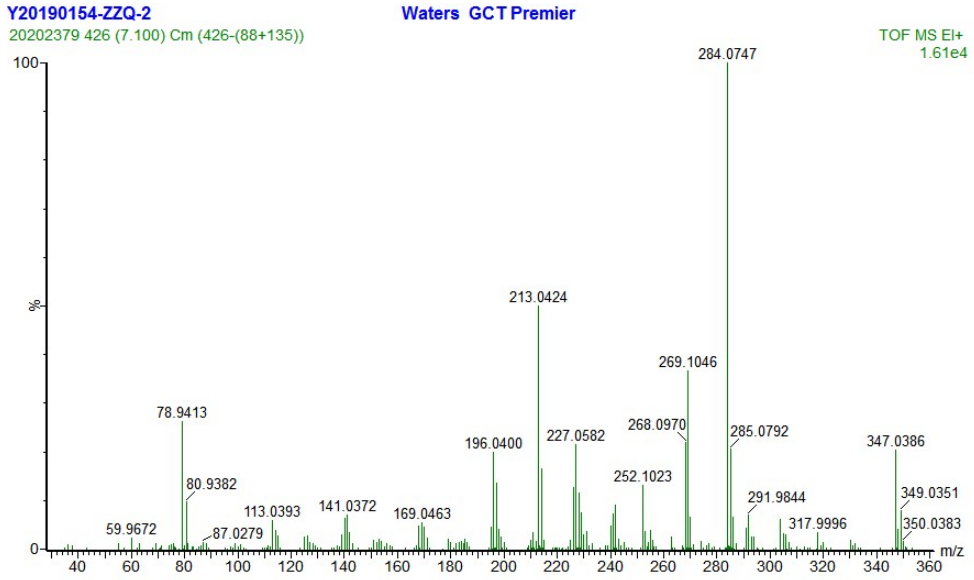


Figure S8. HRMS spectrum of compound **1**.

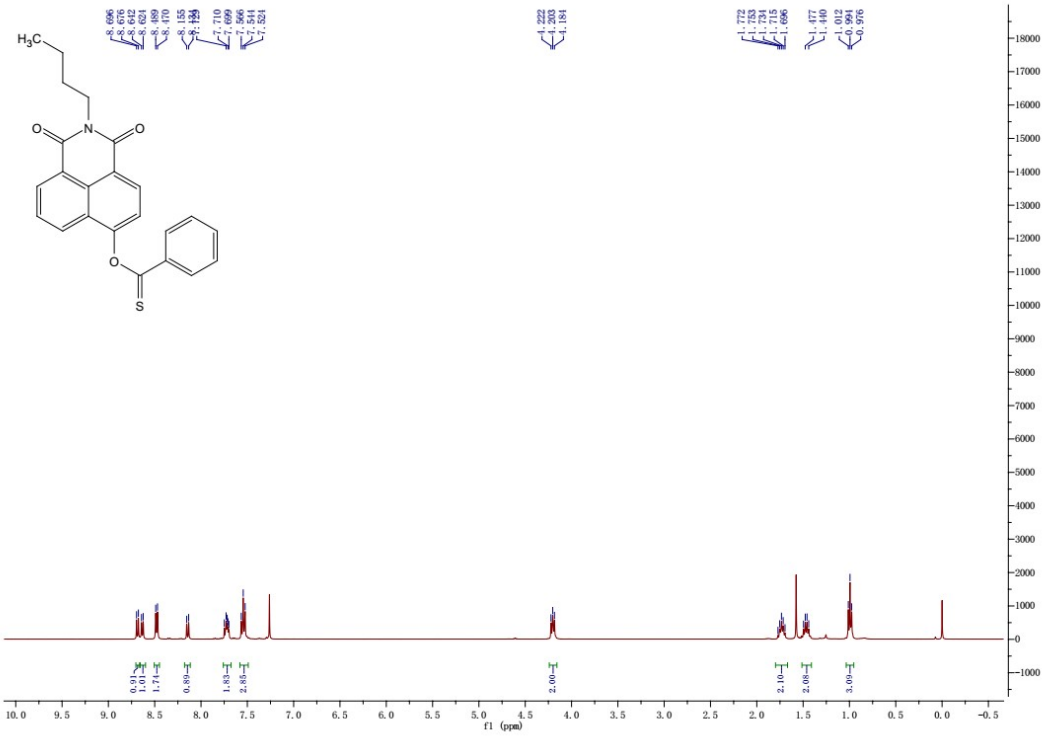


Figure S9. The ^{1}H NMR spectrum of compound **HSD560**.

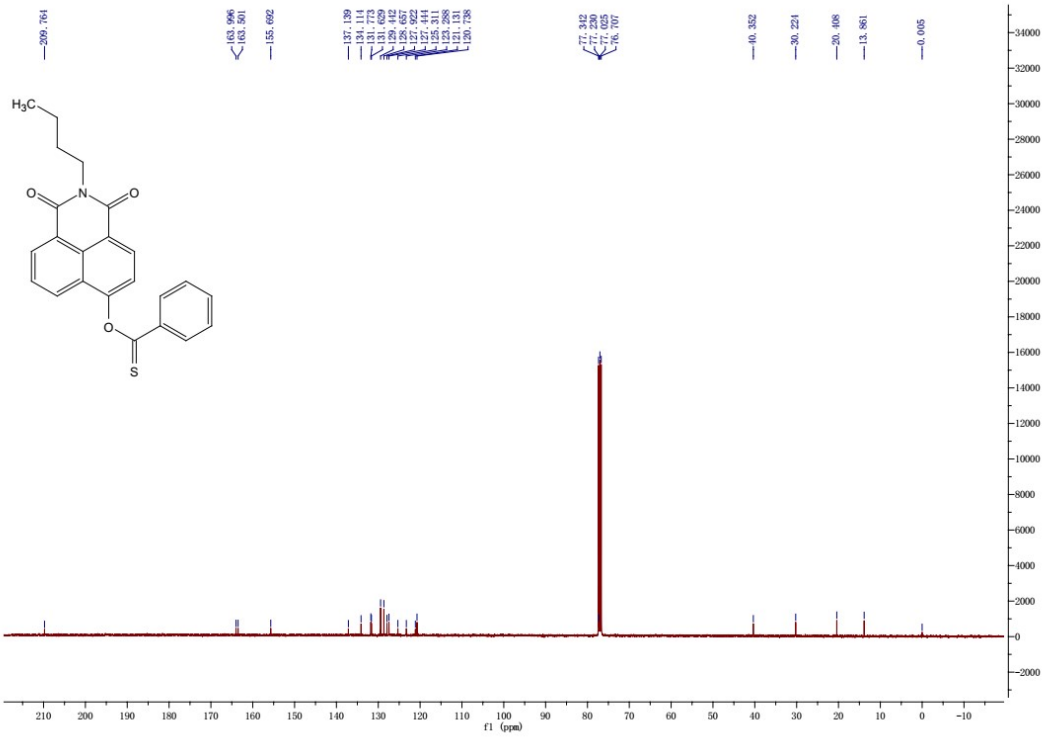


Figure S10. The ^{13}C NMR spectrum of compound **HSD560**.

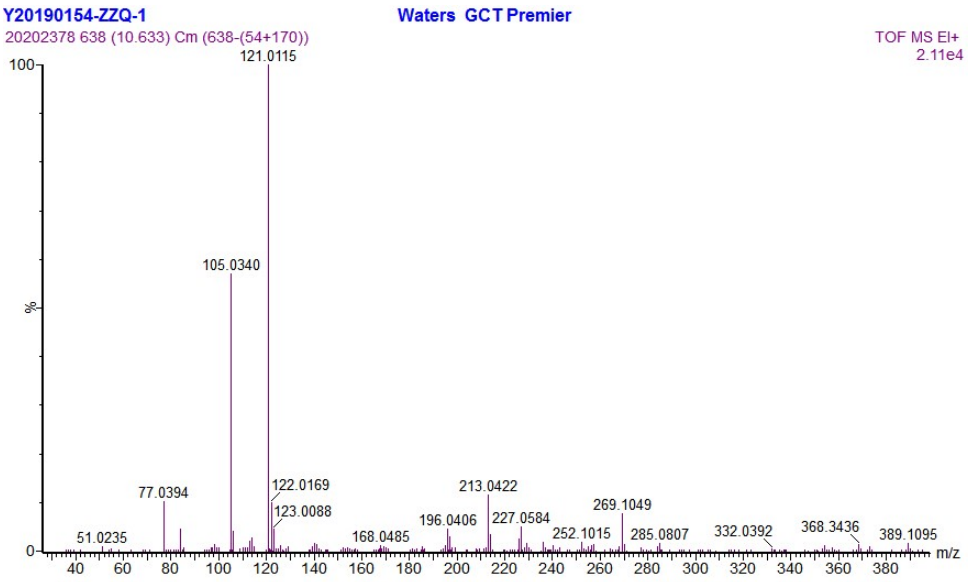


Figure S11. HRMS spectrum of compound **HSD560**.