Supporting Information

Tandem Reaction-Powered Near-Infrared Fluorescent Molecular Reporter for Real-Time Imaging of Lung Diseases

Yan Hu,[‡ a] Hongshuai Zhang,[‡ a] Yiteng Ding,[‡ a] Weirui Chen,[a] Changqie Pan,[* e] Longwei He,[* b, c] Dan Cheng,[* a, c] Lin Yuan [d]

[a] *Department of Gastroenterology, the Affiliated Nanhua Hospital, Hengyang Medical School, University of South China, Hengyang 421002, Hunan, China.*

[b] *MOE Key Lab of Rare Pediatric Diseases, Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, Department of Pharmacy and Pharmacology, Hengyang Medical School, University of South China, Hengyang, China.*

[c] *School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang, 453007, P. R. China.*

[d] *State Key Laboratory of Chemo and Biosensing, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China.*

[e] *Department of Thoracic Oncology, Hunan Cancer Hospital/The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, China, 410013.*

E-mail: flychand0110@163.com, helongwei0110@163.com, panchangqie@hnca.org.cn

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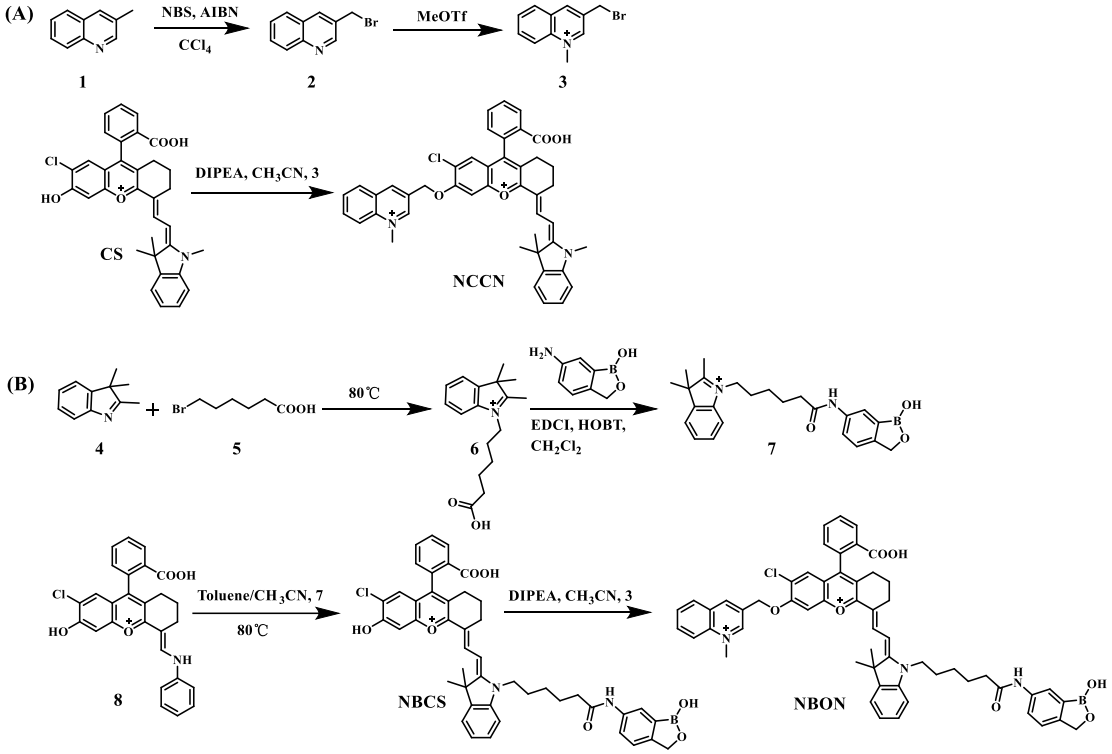
1. Materials and General Experimental Methods

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. NMR spectra were recorded on a Bruker-500 spectrometer, using TMS as an internal standard. Photoluminescent spectra were recorded at room temperature with a HITACHI F7100 fluorescence spectrophotometer (1 cm standard quartz cell). The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; the fluorescence images were acquired with a confocal laser scanning microscope (Nikon); The in vivo (living mice) imaging was carried out using the FUSION FX imaging system (Vilber Lourmat); TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300).

2. Synthesis and Characterization



Scheme S1 Synthetic routes of NADH probe **NBON** and control probe **NCCN**.

Synthesis of compound 2. A mixture of compound 3-methylquinoline (1, 429 mg, 3 mmol), 1-Bromopyrrolidine-2,5-dione (NBS, 561 mg, 3.15 mmol) and 2,2-Azobis(2-methylpropionitrile) (AIBN, 492 mg, 3 mmol) were added to carbon tetrachloride (6 mL) and the mixture was heated to 80 ℃ with stirring. The reaction was monitored by TLC. After 2 h, the solvent was removed under reduced pressure to give

the crude product, which was purified by silica gel flash chromatography to afford compound 2 (298 mg, yield: 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.06 – 7.99 (m, 2H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 1H), 4.56 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.2, 147.7, 135.7, 130.7, 130.1, 129.3, 127.8, 127.5, 127.2, 30.1.

Synthesis of compound 3. A mixture of compound 2 (44 mg, 0.2 mmol) and methyl trifluoromethanesulfonate (0.08 mL) in 5 mL dichloromethane was stirred at room temperature for 24 h under nitrogen atmosphere. Ether was added after the solvent was removed in vacuo, and then the precipitate was filtered and purified by column chromatography on silica gel to give compound 3 (54 mg, yield 70%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 9.72 (s, 1H), 9.36 (s, 1H), 8.52 (d, *J* = 8.9 Hz, 1H), 8.47 (d, *J* = 8.1 Hz, 1H), 8.30 (t, *J* = 7.4 Hz, 1H), 8.08 (t, *J* = 7.6 Hz, 1H), 5.04 (s, 2H), 4.65 (s, 3H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 151.4, 146.8, 138.1, 136.3, 133.0, 131.0, 130.8, 129.3, 119.7, 46.1, 28.9.

Synthesis of compound NCCN. Compound CS was synthesized according to a previous literature.[1]

A mixture of compound CS (4, 64 mg, 0.1 mmol), compound 3 (38 mg, 0.1 mmol) and ethyldiisopropylamine (DIPEA, 0.02 mL) were mixed in acetonitrile (15 mL) and stirred for 2 h at 50 ℃. Then the solvent was removed under vacuum condition, and the mixture was purified by column chromatography on silica gel to offer compound **NCCN** as a solid (30 mg, yield 35%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 9.72 (s, 1H), 9.44 (s, 1H), 8.64 (d, *J* = 15.1 Hz, 1H), 8.57 (d, *J* = 9.6 Hz, 2H), 8.35 (t, *J* = 7.7 Hz, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 8.13 (t, *J* = 7.6 Hz, 1H), 7.87 (t, *J* = 7.6 Hz, 1H), 7.76 (dd, *J* = 17.3, 7.8 Hz, 3H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.57 – 7.50 (m, 2H), 7.40 (d, *J* = 7.5 Hz, 1H), 6.69 – 6.61 (m, 2H), 5.76 (s, 2H), 4.71 (s, 3H), 3.69 (s, 3H), 2.68 (t, *J* = 5.7 Hz, 2H), 2.22 (m, 2H), 1.83 – 1.68 (m, 8H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 178.9, 166.0, 158.9, 155.5, 152.1, 150.4, 146.2, 145.1, 142.8, 142.7, 142.3, 138.6, 136.3, 135.2, 134.2, 131.3, 131.0, 130.6, 130.5, 130.2, 129.6, 129.4, 129.3, 128.1, 126.2, 125.6, 123.1, 122.4, 119.8, 118.9, 117.9, 115.1, 114.1, 107.0, 103.0, 68.2, 55.4, 51.1, 46.2, 40.9, 33.5, 27.5. HRMS (ESI) $C_{44}H_{38}ClN_2O_4^+$ $[M]^+$, calculated 693.2520, found 693.2523.

Synthesis of compound 6. A mixture of compound 2,3,3-trimethylindolenine (4, 159 mg, 1.0 mmol) and 6-bromohexanoic acid (5, 194 mg, 1.0 mmol) were mixed and stirred for 6 h at 80 ℃. Then the solvent was added acetone to get solid precipitation, and the precipitation was filtered and purified by column chromatography on silica gel to offer compound 6 as a solid (265 mg, yield 75%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.07 – 8.02 (m, 1H), 7.91 (m, 1H), 7.71 – 7.66 (m, 2H), 4.53 (t, *J* = 7.7 Hz, 2H), 2.92 (s, 3H), 2.29 (t, *J* = 7.2 Hz, 2H), 1.96 – 1.87 (m, 2H), 1.60 (s, 8H), 1.50 (m, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 197.0, 174.8, 142.4, 141.5, 129.9, 129.4, 124.0, 116.0, 54.7, 48.0, 33.9, 27.4, 25.9, 24.5, 22.5, 14.5.

Synthesis of compound 7. A mixture of compound 6 (177 mg, 0.5 mmol), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 115 mg, 0.6 mmol) and Hydroxybenzotriazole (HOBT, 81 mg,

0.6 mmol) were mixed and dissolved in 8 mL dichloromethane (CH_2Cl_2). After stirring for 10 min, 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (74 mg, 0.5 mmol) was added and stirred for 6 h. Then the solvent was removed under vacuum condition, and the mixture was purified by column chromatography on silica gel to offer compound 7 as a solid (123 mg, yield 51%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (s, 1H), 7.62 (d, *J* = 6.1 Hz, 2H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.06 (d, *J* = 7.4 Hz, 1H), 6.69 (t, *J* = 7.3 Hz, 1H), 6.56 (d, *J* = 7.8 Hz, 1H), 5.02 (s, 2H), 3.54 (t, *J* = 7.2 Hz, 2H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.77 – 1.74 (m, 2H), 1.72 – 1.68 (m, 2H), 1.58 (s, 3H), 1.48 – 1.44 (m, 2H), 1.28 (s, 6H). HRMS (ESI) $C_{24}H_{30}BN_2O_3$ $[M]^+$, calculated 405.2349, found 405.2354.

Synthesis of compound NBCS. Compound 8 (67 mg, 0.12 mmol), potassium acetate (AcOK, 14 mg, 0.14 mmol) and compound 7 (87 mg, 0.18 mmol) were dissolved in acetonitrile/toluene (v/v = 3/1, 10 mL), and the reaction mixture was heated to 80 °C for 8 h. Then the solvent was removed under vacuum condition and the mixture was purified by silica gel flash chromatography to offer compound NBCS as a solid (31 mg, yield 30%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.06 (d, *J* = 4.3 Hz, 1H), 7.98 (d, *J* = 14.4 Hz, 2H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.41 (d, *J* = 7.2 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 2H), 7.08 – 6.99 (m, 3H), 6.71 (s, 1H), 6.32 (s, 1H), 5.77 (d, *J* = 13.0 Hz, 1H), 4.86 (s, 2H), 3.90 (s, 2H), 2.34 (t, *J* = 6.4 Hz, 2H), 2.23 (m, 1H), 2.04 (m, 1H), 1.64 (m, 13H), 1.41 (m, 3H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 173.1, 171.6, 170.1, 164.8, 158.6, 157.6, 148.7, 143.7, 139.8, 138.7, 134.7, 131.9 130.7, 130.3, 129.5, 129.11, 128.8, 128.6, 128.2, 126.8, 122.7, 122.2, 121.7, 121.4, 116.7, 116.5, 113.2, 109.0, 102.4, 94.9, 70.1, 47.2, 42.4, 36.6, 28.9, 26.5, 26.4, 26.0, 25.4, 24.2, 21.1. HRMS (ESI) $C_{45}H_{43}BClN_2O_7$ $[M]^+$, calculated 769.2852, found 769.2848.

Synthesis of compound NBON. A mixture of compound NBCS (43 mg, 0.05 mmol), compound 3 (19 mg, 0.05 mmol) and ethyldiisopropylamine (DIPEA, 0.01 mL) were mixed in acetonitrile (10 mL) and stirred for 4 h at 50 ℃. Then the solvent was removed under vacuum condition, and the mixture was purified by column chromatography on silica gel to offer probe **NBON** as a solid (13 mg, yield 25%). ^{1}H NMR (500 MHz, CD_3OD) δ 9.60 (s, 1H), 9.38 (s, 1H), 9.12 (s, 1H), 8.89 (d, *J* = 15.0 Hz, 1H), 8.54 (d, *J* = 8.9 Hz, 1H), 8.48 (d, *J* = 9.2 Hz, 1H), 8.39 (d, *J* = 8.2 Hz, 1H), 8.32 (t, *J* = 7.5 Hz, 1H), 8.25 (d, *J* = 8.5 Hz, 1H), 8.10 (t, *J* = 7.6 Hz, 1H), 8.04 (t, *J* = 7.7 Hz, 1H), 7.83 (t, *J* = 7.1 Hz, 1H), 7.69 (d, *J* = 7.3 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.54 (s, 1H), 7.50 – 7.43 (m, 2H), 7.34 (d, *J* = 7.4 Hz, 1H), 6.95 (s, 1H), 6.73 (s, 1H), 6.60 (d, *J* = 15.1 Hz, 1H), 5.76 (s, 2H), 4.77 (s, 2H), 4.70 (s, 3H), 3.74 (s, 2H), 2.80 (t, *J* = 7.4 Hz, 1H), 2.72 (d, *J* = 6.5 Hz, 1H), 2.55 (t, *J* = 7.4 Hz, 1H), 2.35 (t, *J* = 7.3 Hz, 2H), 2.26 (t, *J* = 7.4 Hz, 3H), 2.03 (m, 4H), 1.97 – 1.93 (m, 2H), 1.91 (s, 6H). HRMS (ESI) $C_{56}H_{52}BClN_3O_7$ $[M]^+$, calculated 924.3587, found 924.3566.

3. Spectrometric Studies

Measurement of photophysical properties. For photophysical characterization, the compound NCCN and probe **NBON** were dissolved in EtOH to make the stock solutions (500 μM), which were diluted to 5 μM as the testing solutions with PBS buffer solution (25 mM, 30% EtOH, pH 7.4). Absorption and fluorescence spectroscopic studies were performed on a UV2600 spectrophometer and a Hitachi F-7100 fluorescence spectrophotometer.

4. Fluorescence Microscopic Studies

Cell culture. A549 and WI-38 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, BI), and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) at 37 °C and 5% CO_2. Cells were carefully harvested and split when they reached 80% confluence to maintain exponential growth.

Fluorescence microscopic imaging. All the experiments were conducted in live cells. Microscopic imaging uses Nikon confocal microscope with an excitation filter of 640 nm and a collection wavelength range of 662-737 nm.

For endogenous NADH imaging experiments in live A549 cells, A549 cells were incubated with 15 μM **NBON** within 60 min, then image.

For exogenous NADH imaging experiments, A549 cells were incubated with different concentration of NADH (0-3000 μM) for 2 h, then incubated with **NBON** (15 μM) for 40 min. Cell imaging was performed after washing the cells with PBS for three times.

For LA/Py-stimulated cell imaging using **NBON**, A549 cells were pretreated with different concentrations of LA/Py (20/0, 0/10 and 0/20 mM) for 30 min, then washed, and incubated with 15 μM **NBON** for 40 min prior to imaging.

For rotenone-stimulated cell imaging using **NBON**, A549 cells were pretreated with different concentrations of rotenone (5, 20 and 30 μM) for 60 min, then washed, and incubated with 15 μM **NBON** for 40 min prior to imaging.

For CCCP-stimulated cell imaging using **NBON**, A549 cells were pretreated with different concentrations of CCCP (10, 20 and 30 μM) for 60 min, then washed, and incubated with 15 μM **NBON** for 40 min prior to imaging.

For GSH-stimulated cell imaging using **NBON**, A549 cells were pretreated with different concentrations of GSH (5, 10 and 20 mM) for 60 min, then washed, and incubated with 15 μM **NBON** for 40 min prior to imaging.

For glucose-stimulated cell imaging using **NBON** in live A549 and WI-38 cells, cells were pretreated with different concentrations of glucose (5, 20 and 30 mM) for 12 h, then washed, and incubated with 15 μM **NBON** for 40 min prior to imaging.

For the protection of lung diseases in living cells, A549 and WI-38 cells were preincubated with EPS (0-50 μM) for 24 h before glucose treatment for 12 h. Then, the cells were treated with 15 μM **NBON** for 40 min. Cell imaging was carried out after washing the cells three times with PBS.

5. Cell cytotoxicity in MTT assay

Cells were plated in 96-well flat-bottomed plates at 1×10^5 cells per well and allowed to grow overnight prior to exposure to **NBON**. Then the MTT (0.5 mg/mL) reagent was added for 4 hours at 37 °C and DMSO (100 μL/well) was further incubated with cells to dissolve the precipitated formazan violet crystals at 37 °C for 15 min. The absorbance was measured at 490 nm by a multidetection microplate reader. The following formula was used to calculate the viability of cell growth: Cell viability = mean of A value of treatment group / mean of A value of control.

6. Determining the subcellular location of probe

Live A549 cells cultured in 35-mm glass bottom culture dishes. Cells were incubated with **NBON** (15 μM) for 40 min at 37 °C. Then, MitoTracker Green (1.0 μM) or LysoTracker Green (1.0 μM) was added and incubated for another 10 min. Cells were washed with PBS prior to imaging.

7. Flow Cytometry Analysis

Flow cytometry (BD FACS Calibur) was employed to determine NADH in the drug-stimulated situation with developed probe. A549 and WI-38 cells in 6-well plate were precultured for 24 h and treated with a range of concentrations of EPS (0-50 μM) for 24 h before glucose (40 mM) treatment for 12 h. Then, the cells were then treated with 15 μM probe **NBON** for 40 min. After incubation, the cells were treated with trypsin, washed twice with medium and subjected to flow cytometry analysis. The fluorescence signal was determined in FL-7 detector by flow cytometry.

8. Measurement of NADH and GSH/GSSG

A549 cells in 6-well plate were precultured for 24 h and pretreated with EPS (0 and 50 μM, 24 h) in the presence of glucose (0 and 40 mM, 12 h), respectively. Then, the NADH was determined using a

NAD^+/NADH Assay Kit with WST-8 (S0175, Beyotime) and GSH and GSSG Assay Kit (S0053, Beyotime) according to the manufacturer's instructions.

9. Measurement of Total Antioxidant Capacity

A549 cells in 6-well plate were precultured for 24 h and pretreated with EPS (0 and 50 μM, 24 h) in the presence of glucose (0 and 40 mM, 12 h), respectively. Then, the total antioxidant capacity was determined using a Total Antioxidant Capacity Assay Kit with FRAP method (T-AOC Assay Kit) (S0116, Beyotime) according to the manufacturer's instructions.

10. *In vivo* Imaging Studies

All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved (approval number: No. SYXK (Xiang) 2020-0002) by University of South China. All Kunming mice (18-20 g) and BALB/c mice (18-20 g) were obtained from Hunan SJA Laboratory Animal Co. Ltd. and operated in accordance with University of South China guidelines on the care and use of animals for scientific purposes.

Kunming mice were with different treatment by receiving injection of PBS, STZ (150 mg kg^{-1}, intraperitoneal) for 7 days, STZ/MET (150/200 mg kg^{-1}, intraperitoneal) for 7/28 days and STZ/EPS (150/100 mg kg^{-1}, intraperitoneal/gavage) for 7/49 days. All mice were then injected intravenously with 60 μL PBS containing 200 μM **NBON** for *in vivo* imaging. The *in vivo* (living mice) imaging was carried out using the FUSION FX imaging system (Vilber Lourmat). Excitation wavelength was 680 nm. The emission band was at 735–765 nm.

Imaging of NADH *in vivo.* Kunming mice were preinjected with PBS and NADH (50 μL, 500 μM) for 3 h on the left and right leg of mice, respectively, followed by **NBON** (50 μL, 200 μM) for 2 h. All the mice were anaesthetized and performed *in vivo* imaging under a FUSION FX imaging system. Excitation wavelength was 680 nm. The emission band was at 735–765 nm.

Imaging of lung metastasis *in vivo*. For establishing a mouse lung metastasis model, the 4T1 cells (1×10^6 cells) were chose to intravenously inject into approximately 18-20 g BALB/c mice. After 21 days, the mice were injected intravenously with 100 μL PBS containing 200 μM **NBON** for *in vivo* imaging. The *in vivo* (living mice) imaging was carried out using the FUSION FX imaging system (Vilber Lourmat). Excitation wavelength was 680 nm. The emission band was at 735–765 nm.

Image-guided tumor resection *in vivo*. For establishing a mouse tumor model, the A549 cells (1×10^6 cells) were chose to transplant beside the thighs of approximately 18-20 g BALB/c mice. After 20 days

inoculation, the xenograft tumor mice were sprayed with 30 μL PBS containing 100 μM **NBON** for *in vivo* imaging within the period of mice anesthesia. The *in vivo* (living mice) imaging was carried out using the FUSION FX imaging system (Vilber Lourmat). Excitation wavelength was 680 nm. The emission band was at 735–765 nm.

11. Toxicity and biodistribution studies

All studies were completed in immunocompetent 18-20 g female Kunming mice. After administration of **NBON**, animals were killed at the indicated timepoints and their organs (heart, liver, spleen, lung, kidney) for hematoxylin and eosin (H&E) staining and fluorescence measurements. The imaging was carried out using the FUSION FX imaging system (Vilber Lourmat). Excitation wavelength was 680 nm. The emission band was at 735–765 nm.

12. Histology

All tissues of Kunming and BALB/c mice were fixed in 10% formaldehyde immediately after sacrifice. Histological examination was according to a conventional method, and stained with hematoxylin and eosin (H&E). Classify and record the morphology of any observed lesions was according to classification criteria.[2]

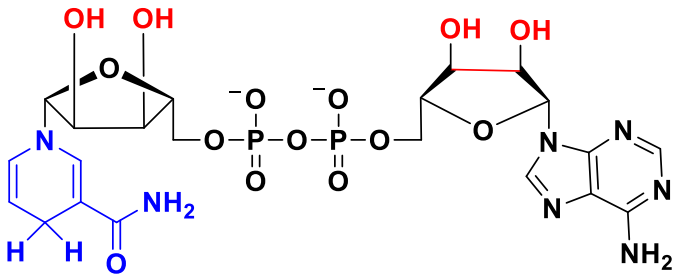
13. Measurement of NAD^+/NADH Ratio

Kunming mice were pretreated with PBS, STZ (150 mg kg^{-1}, intraperitoneal) for 7 days, STZ/MET (150/200 mg kg^{-1}, intraperitoneal) for 7/28 days and STZ/EPS (150/100 mg kg^{-1}, intraperitoneal/gavage) for 7/49 days, respectively. Then, the mice were sacrificed and lung tissue was obtained for the determination of NAD^+/NADH ratio using a NAD^+/NADH Assay Kit with WST-8 (S0175, Beyotime, China) according to the manufacturer's instructions.

14. Measurement of Total Antioxidant Capacity

Kunming mice were pretreated with PBS, STZ (150 mg kg^{-1}, intraperitoneal) for 7 days, STZ/MET (150/200 mg kg^{-1}, intraperitoneal) for 7/28 days and STZ/EPS (150/100 mg kg^{-1}, intraperitoneal/gavage) for 7/49 days, respectively. Then, the mice were sacrificed and lung tissue was obtained for the determination of Total Antioxidant Capacity Assay Kit with FRAP method (T-AOC Assay Kit) (S0116, Beyotime) according to the manufacturer's instructions.

15. Supplemental Figures



NADH

Fig. S1 The structure of NADH.

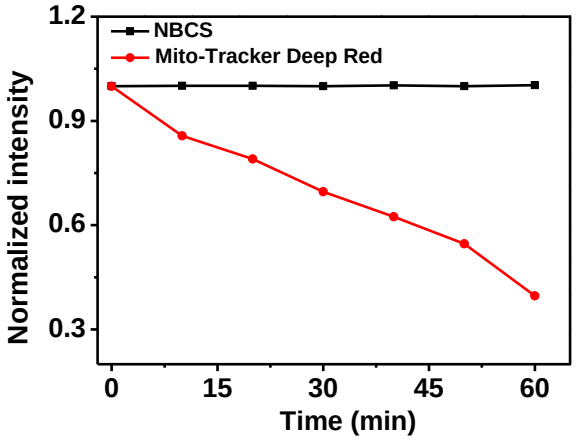


Fig. S2 Photostability of **NBCS** and Mito-Tracker Deep Red in PBS buffer solution (pH 7.4). The samples were continuously irradiated by white light (50 W) in ice-bath condition.

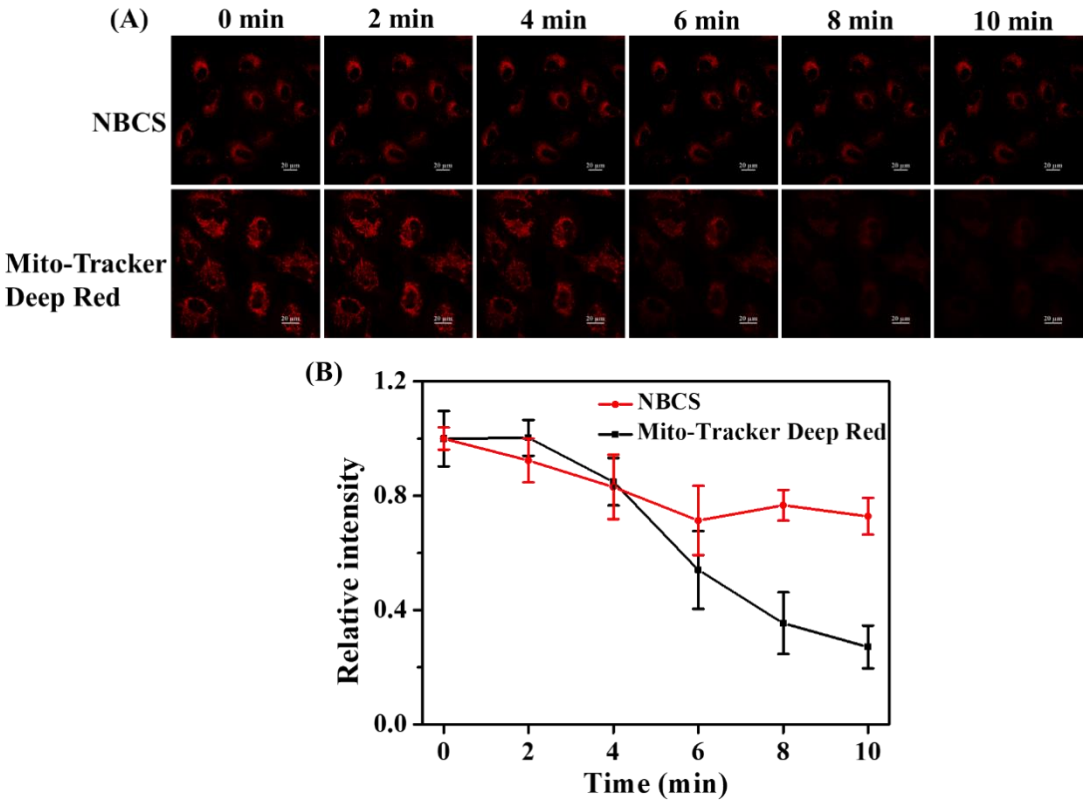


Fig. S3 (A) Confocal fluorescence images of living A549 cells cultured with **NBCS** and Mito-Tracker Deep Red with continuous irradiation using confocal microscope with the same parameters. (B) Quantification of the relative mean fluorescence levels of cells from the images of **NBCS** and Mito-Tracker Deep Red. λ_{ex} = 640 nm and λ_{em} = 662–737 nm. Scale bar = 20 μm.

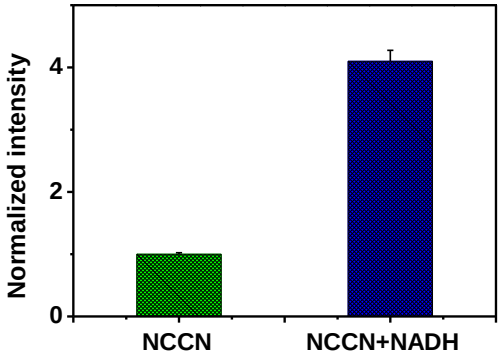
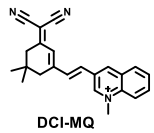
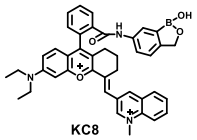
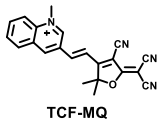
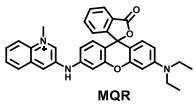
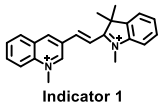
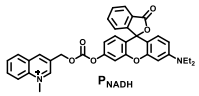
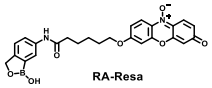
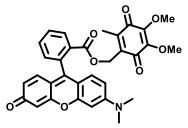
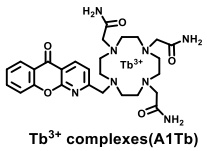
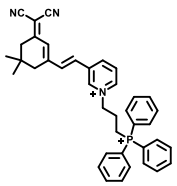
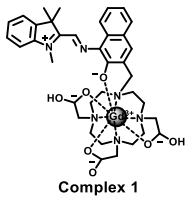
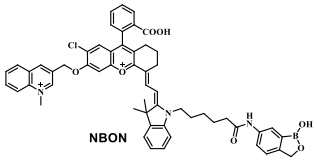
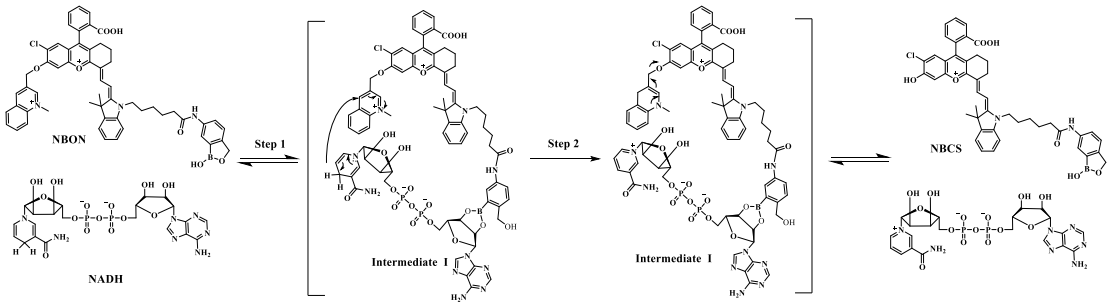


Fig. S4 Normalized fluorescence intensity of **NCCN** for NADH (100 μM) in PBS buffer solution (25 mM, 30% EtOH, pH 7.4).

Table S1. Representative NADH fluorescent probes

Probe	λ_{ex} (nm)	λ_{em} (nm)	LOD	Application	Reference

 DCI-MQ	568	660	12 nM	*In vivo* and cells	3
 KC8	680	745	0.4 μM	*In vivo* and cells	4
 TCF-MQ	576/582	604/610	6 nM	*In vivo* and cells	5
 MQR	548	560	-	In cells	6
 Indicator 1	537	561	0.1 μM	In cells	7
 P_{NADH}	510	552	-	In cells	8
 RA-Resa	480	575	87 nM	In cells	9
 UQ-Rh	492	518	-	In cells	10
 Tb^{3+} complexes(A1Tb)	340/488	545	-	-	11
 Probe 1	570	615	1.2 μM	In cells	12
 Complex 1	460	539	-	In cells	13
 NBON	660	721	45 nM	*In vivo* and cells	This work



Scheme S2 The proposed reaction mechanism of probe **NBON** and NADH.

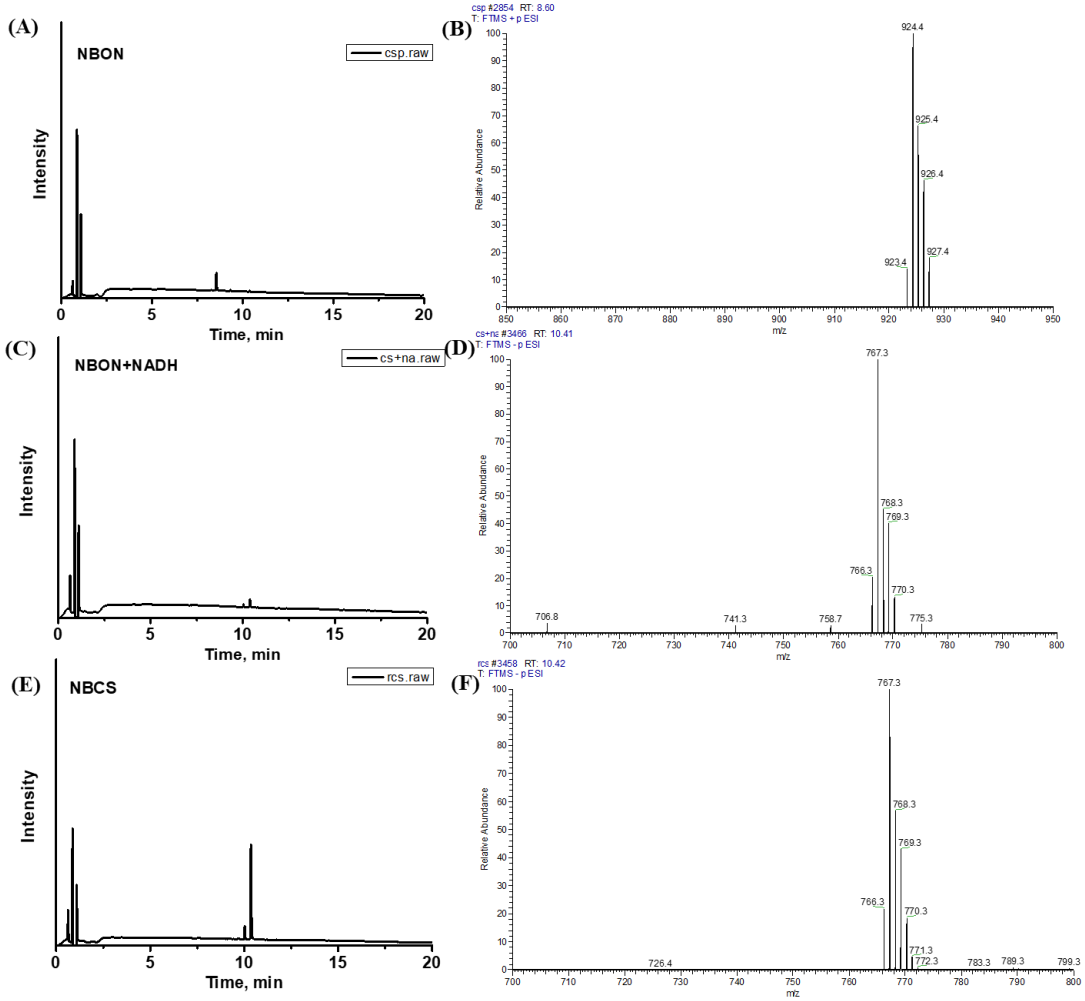


Fig. S5 The proposed reaction mechanism of probe **NBON** toward NADH by HPLC-MS analysis using a Dionex Ultimate 3000 RS UHPLC equipped with a quaternary pump and LPG-3400SD vacuum degasser unit (Thermo Fisher Scientific, California, CA, USA) and a Q-Exactive Orbitrap MS mass spectrometer with an electrospray ionization (ESI) source.

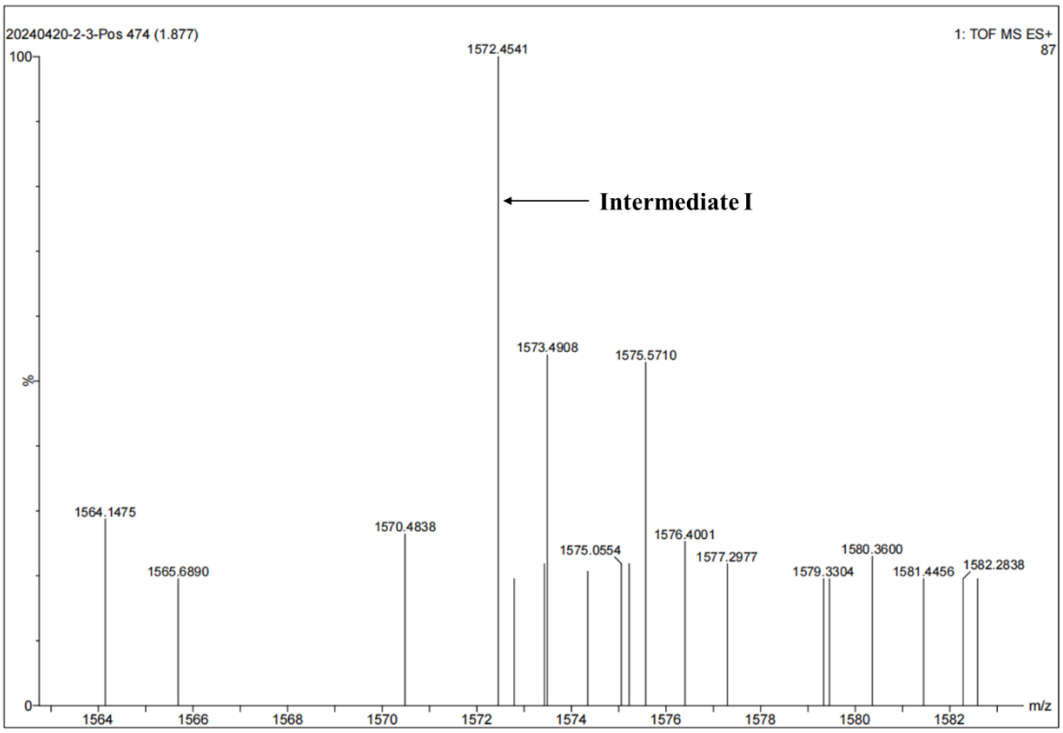


Fig. S6 The MS-ESI mass spectrum of probe **NBON** in the presence of NADH to obtain intermediate.

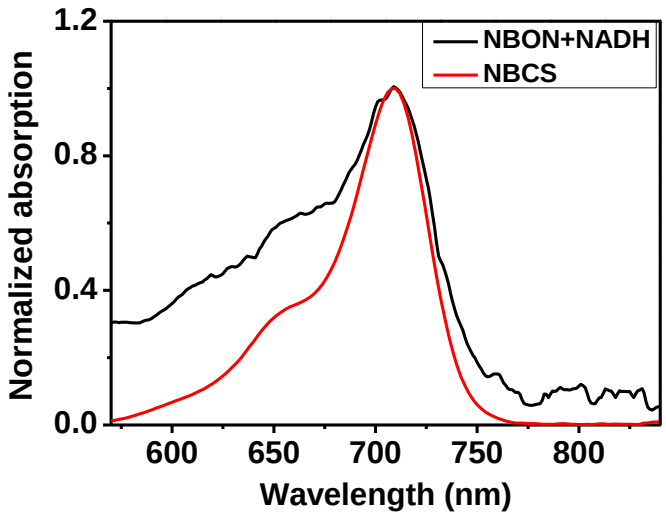


Fig. S7 Normalized absorption of **NBON** (5 μM) in the presence of NADH (70 μM) and **NBCS** (5 μM).

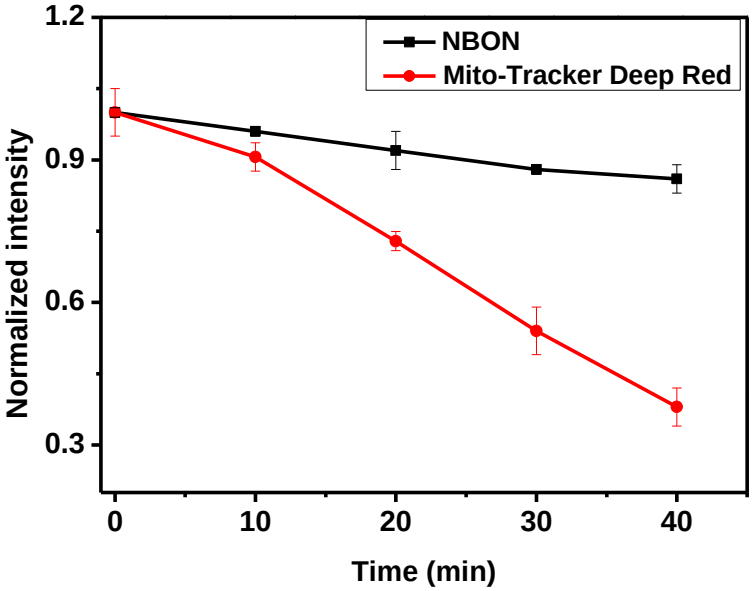


Fig. S8 Photostability of **NBON** and Mito-Tracker Deep Red. The samples were continuously irradiated by white light (50 W) in ice-bath condition.

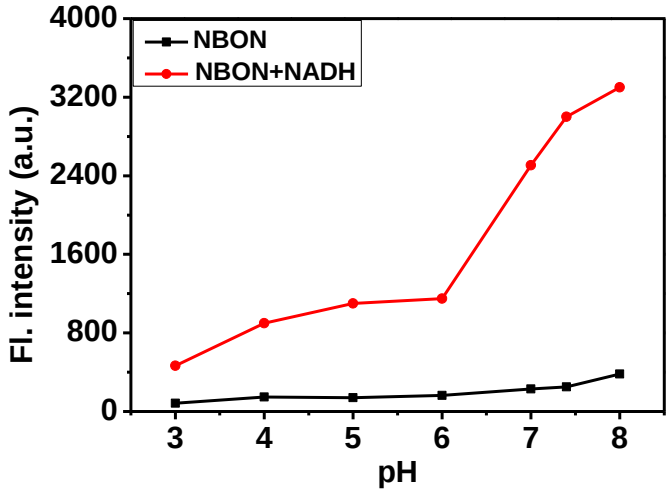


Fig. S9 Effects of pH on the fluorescence of probe **NBON** (5 μM) reacting with NADH (70 μM). $\lambda_{ex}/\lambda_{em}$ = 660/721 nm.

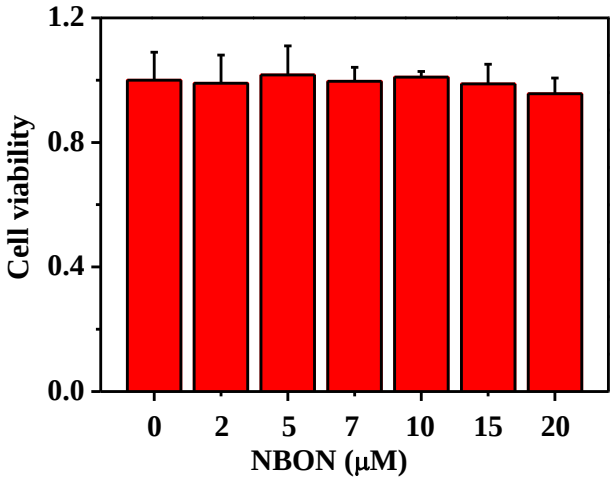


Fig. S10 Cytotoxicity of **NBON** for A549 cells. Cells were incubated with the probe at corresponding concentrations for 24 hours. Cell viability was measured by MTT assay and the results are reported as percentage relative to untreated cells (mean ± SD).

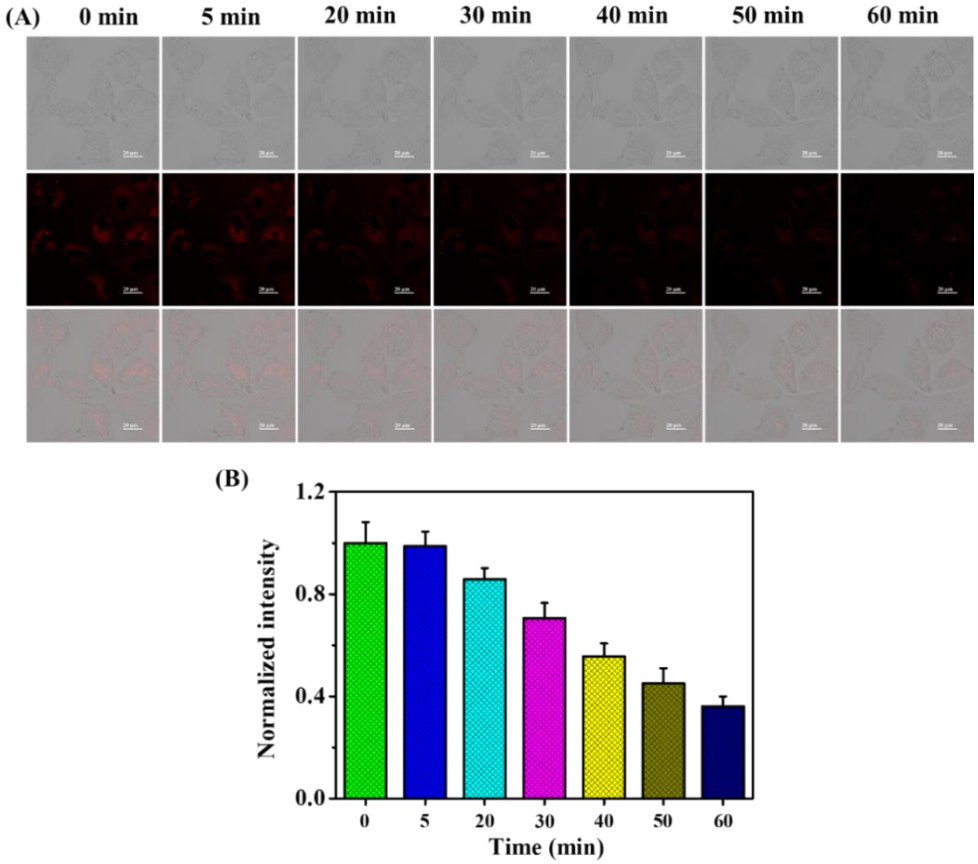


Fig. S11 (A) Real-time images of Py-stimulated live cells. HepG2 cells were pretreated with **NBON** (15 μM) for 40 min, and then with Py (20 mM). λ_{ex}= 640 nm, λ_{em} = 662-737 nm. Scale bar = 20 μm. (B) Normalized intensity in A. Data are expressed as mean ± SD of three experiments.

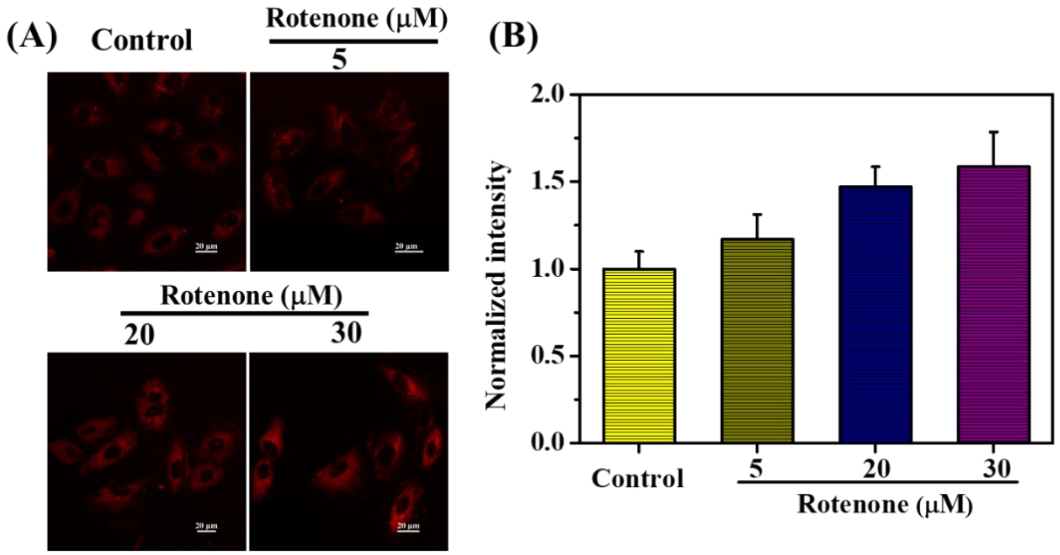


Fig. S12 (A) Fluorescence images of NADH by confocal fluorescence images based on the different concentration of rotenone (5-30 μM). λ_{ex}= 640 nm, λ_{em} = 662-737 nm. Scale bar = 20 μm. (B) Normalized intensity in A. Data are expressed as mean ± SD of five experiments.

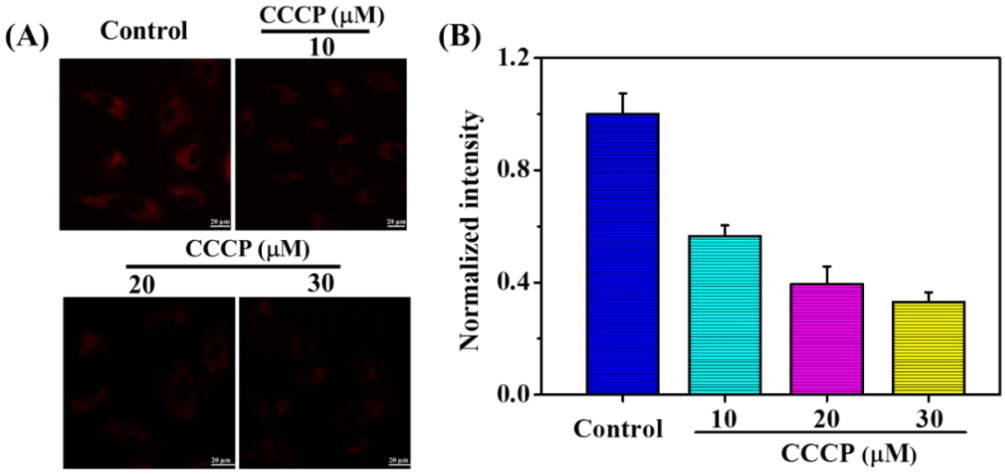


Fig. S13 (A) Fluorescence images of NADH by confocal fluorescence images based on the different concentration of CCCP (10-30 μM). λ_{ex}= 640 nm, λ_{em} = 662-737 nm. Scale bar = 20 μm. (B) Normalized intensity in A. Data are expressed as mean ± SD of five experiments.

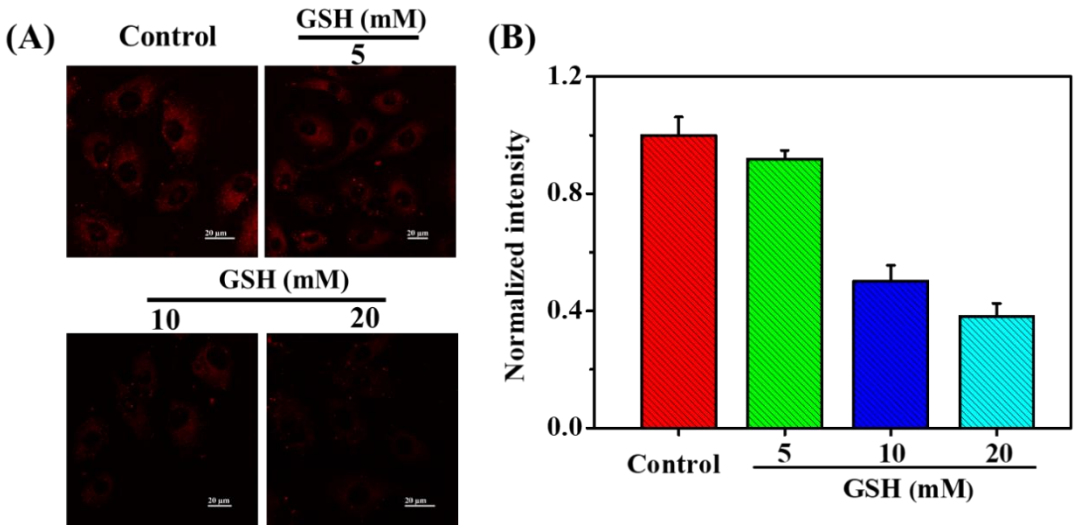


Fig. S14 (A) Fluorescence images of NADH by confocal fluorescence images based on the different concentration of GSH (5-20 mM). λ_{ex}= 640 nm, λ_{em} = 662-737 nm. Scale bar = 20 μm. (B) Normalized intensity in A. Data are expressed as mean ± SD of five experiments.

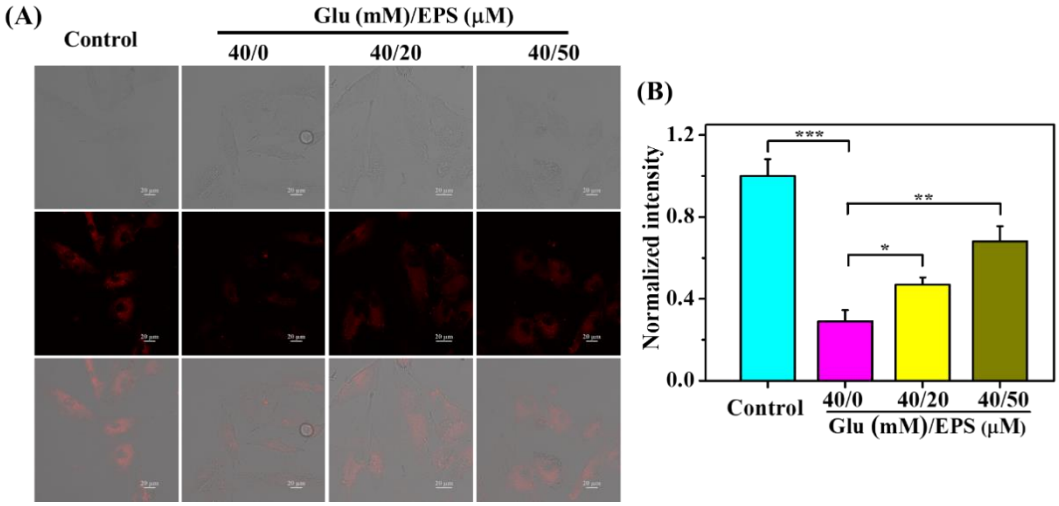


Fig. S15 (A) Fluorescence images of GSH in A549 treated with only reported probe **TCF-GSH** (10 μM) or incubated with EPS (0, 20 and 50 μM) for 24 h before glucose stimulation (40 mM) for 12 h (λ_{ex} = 640 nm, λ_{em} = 662-737 nm; Scale bar: 20 μm). (B) Relative fluorescence intensity in A (n = 3, data expressed as mean ± SD, *P < 0.05, **P < 0.01, and ***P < 0.001, Student's t-test). λ_{ex}= 640 nm, λ_{em} = 662-737 nm. Scale bar = 20 μm.

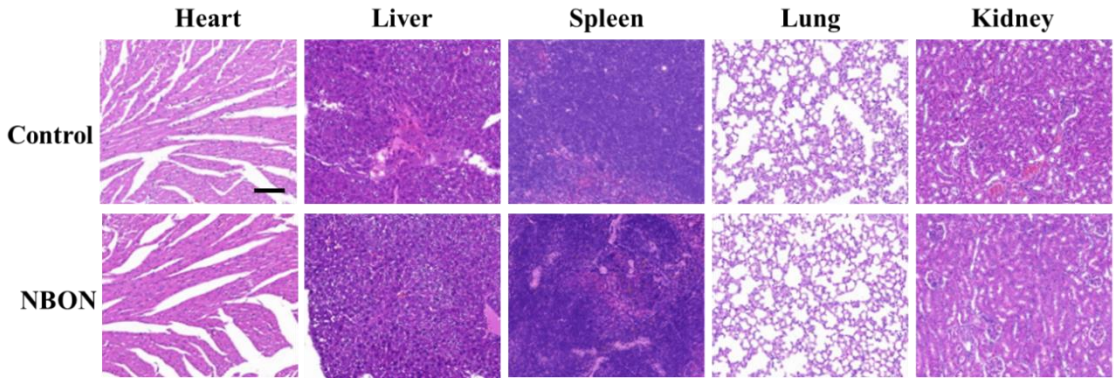


Fig. S16 Representative histological sections (H&E staining) for main organs of the mice after intravenous injection of saline (control) or **NBON** for 24 h. Scale bar = 100 mm.

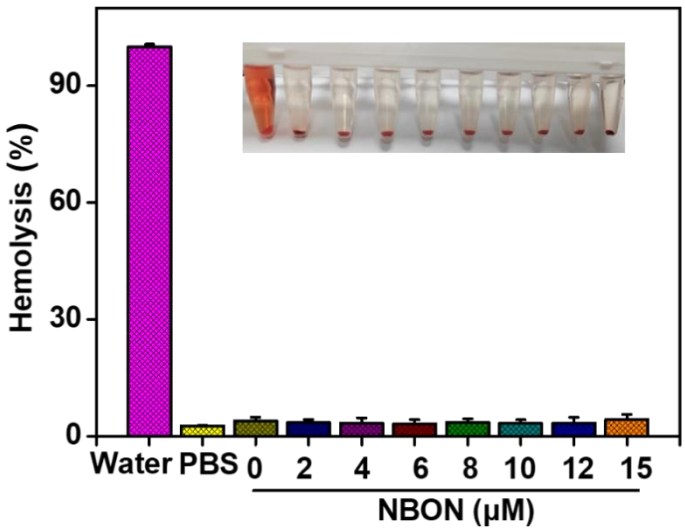


Fig. S17 The hemolysis of probe **NBON**.

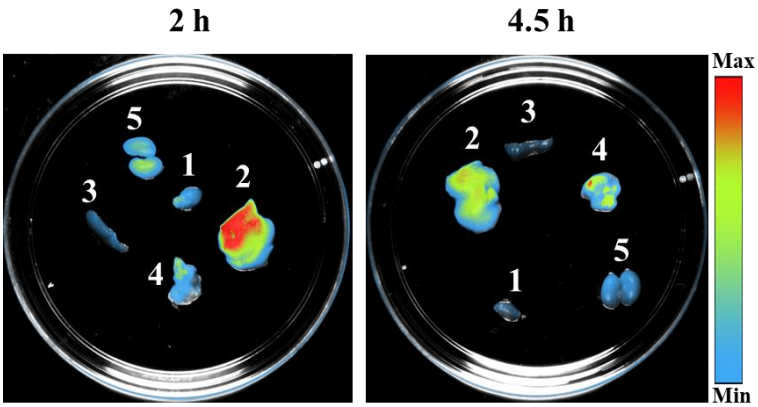


Fig. S18 Fluorescence images of representative organs of mice after intravenously injected with **NBON** (60 μL, 200 μM) for 2 h and 4.5 h. 1, Heart; 2, Liver; 3, Spleen; 4, Lung; 5, Kidney.

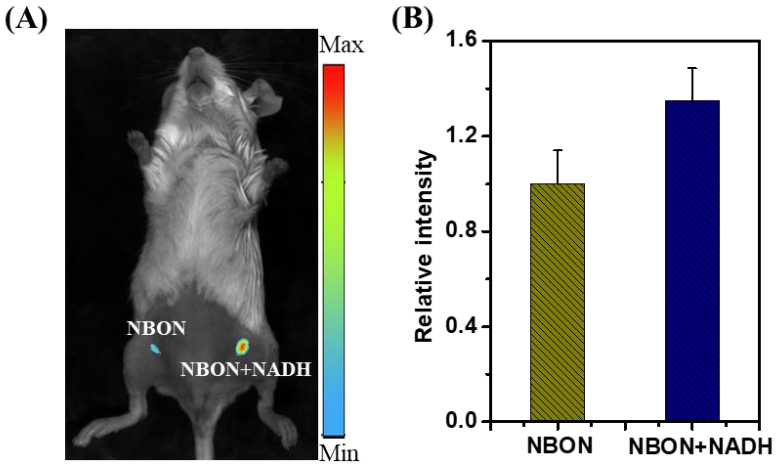


Fig. S19 Fluorescence images of BALB/c mice receiving NADH (50 μL, 500 μM, 3 h). Left: PBS, then **NBON** (50 μL, 200 μM, 1 h). Right: NADH (50 μL, 500 μM, 3 h), then **NBON** (50 μL, 200 μM, 1 h) (λ_{ex} = 680 nm, λ_{em} = 735–765 nm). (B) Relative fluorescence intensity in A. Data are expressed as mean ± SD of three experiments.

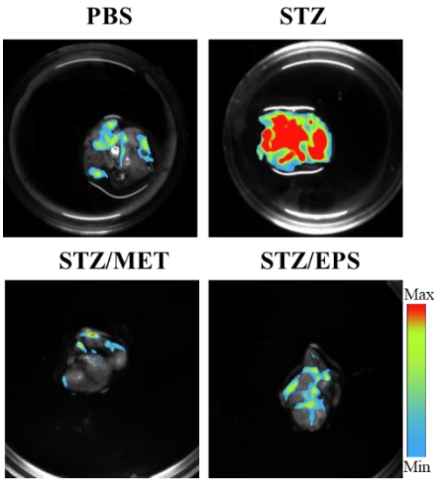


Fig. S20 Fluorescence imaging of lung tissues from Kunming mice with different treatment by receiving injection of PBS, STZ (150 mg kg^{-1}, intraperitoneal), STZ/MET (150/200 mg kg^{-1}, intraperitoneal) and STZ/EPS (150/100 mg kg^{-1}, intraperitoneal/gavage), then the lung tissues were dissected, followed by incubated with **NBON**. λ_{ex} = 680 nm, λ_{em} = 735–765 nm.

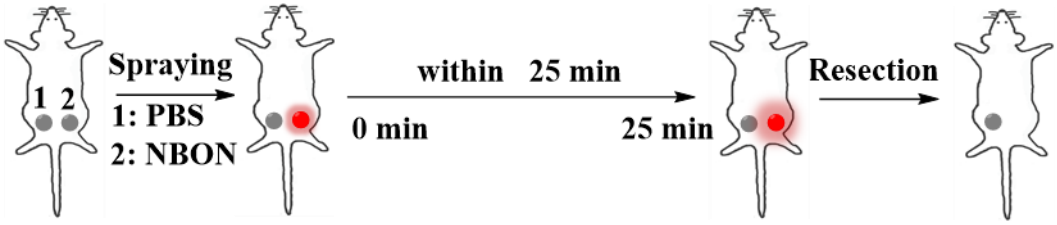


Fig. S21 Schematic representation for lung tumor model and imaging.

16. Data

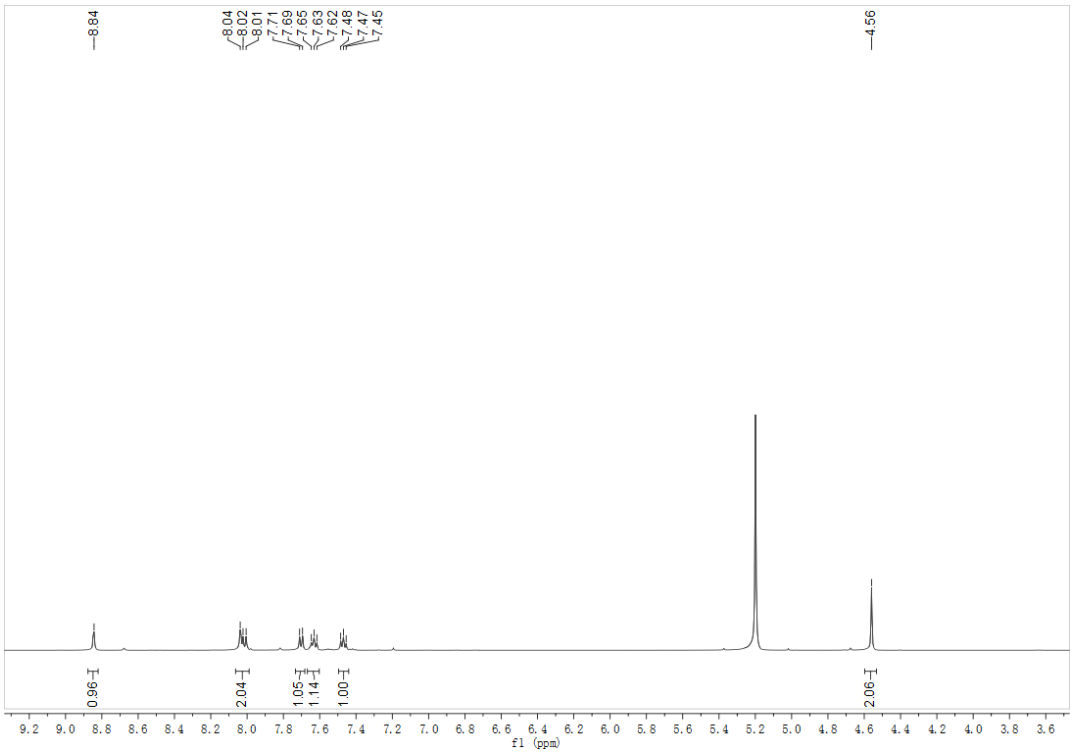


Fig. S22 The ^{1}HNMR spectrum of compound **2**.

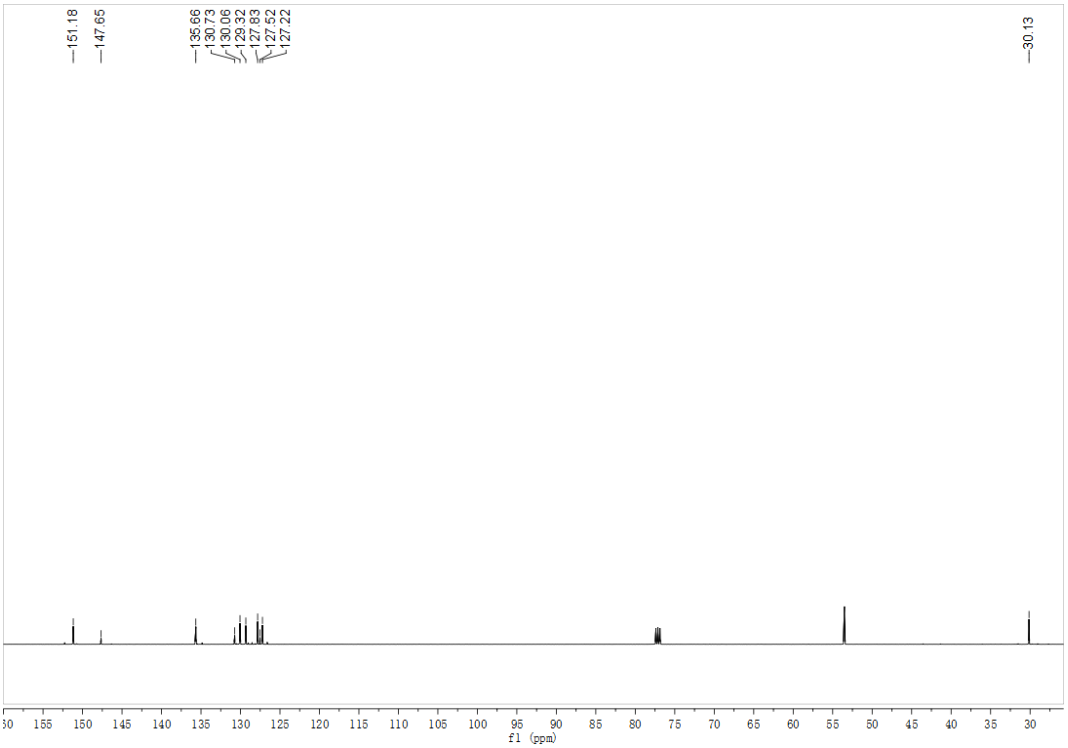


Fig. S23 The ^{13}CNMR spectrum of compound **2**.

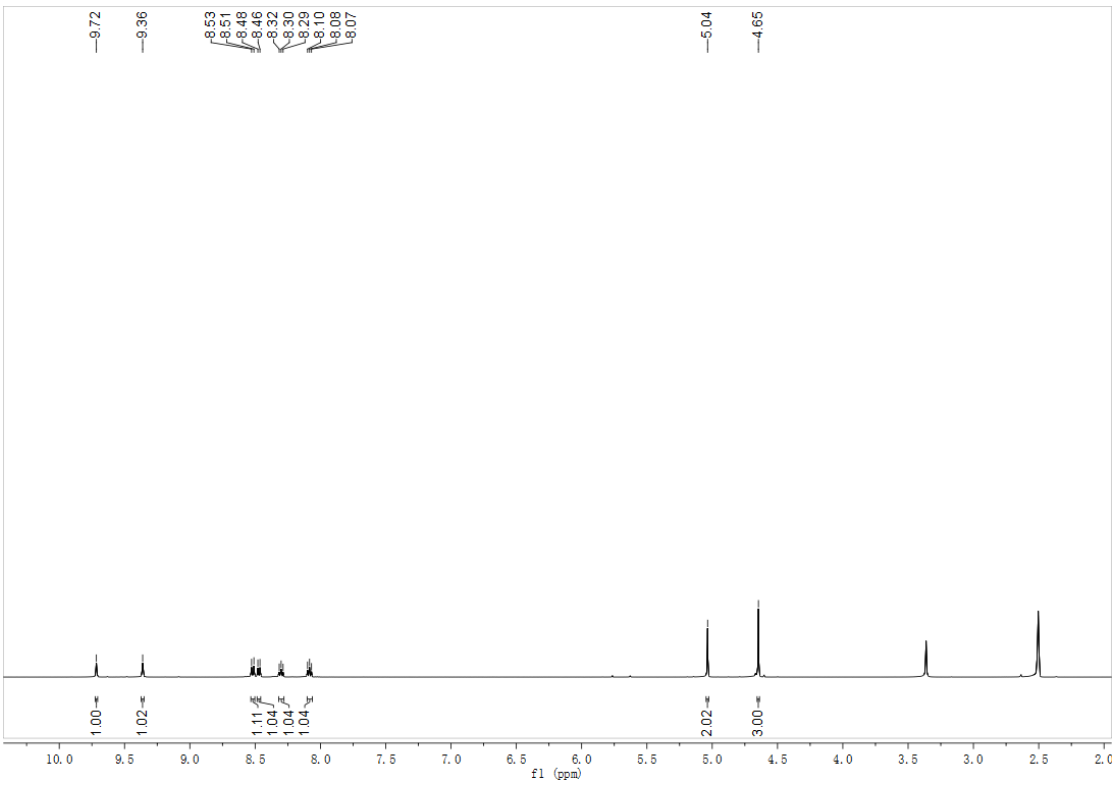


Fig. S24 The ^{1}HNMR spectrum of compound **3**.

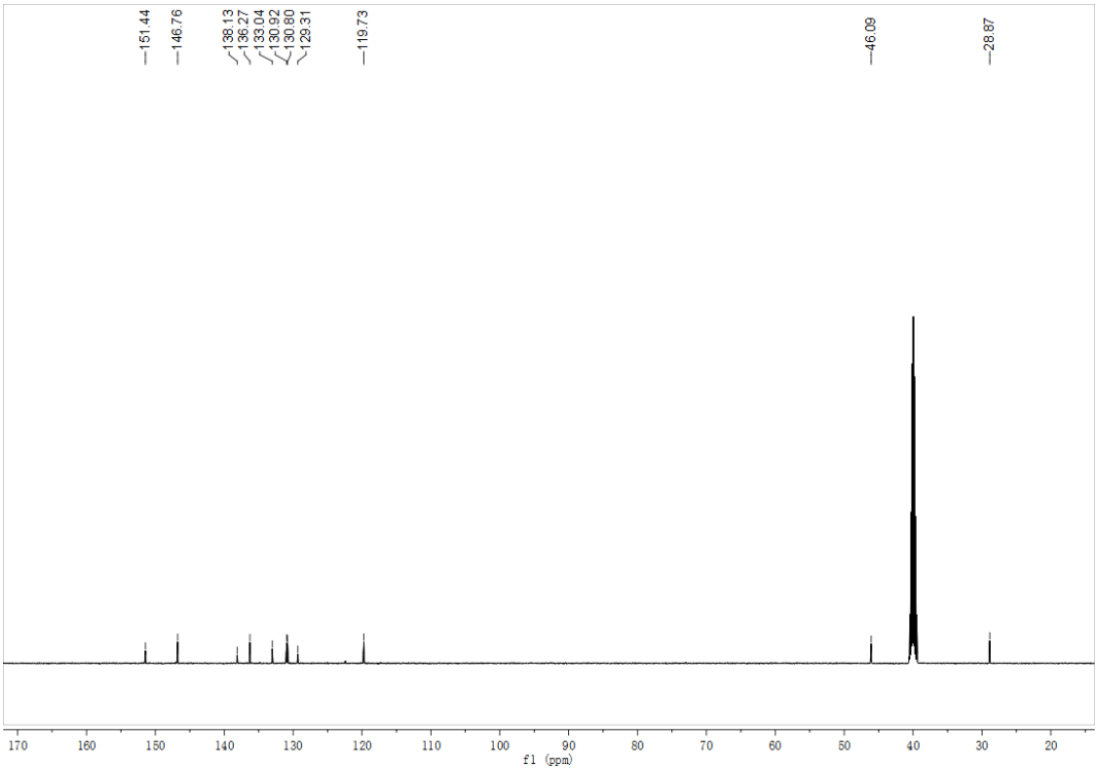


Fig. S25 The ^{13}CNMR spectrum of compound **3**.

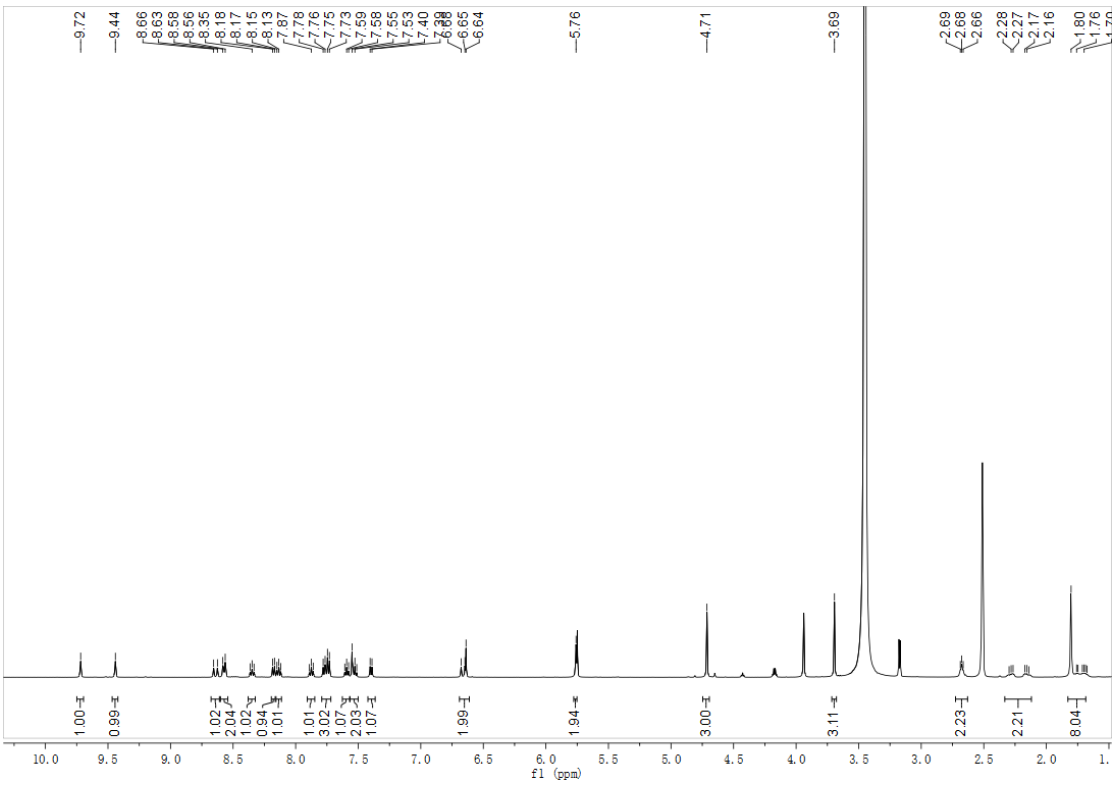


Fig. S26 The ^{1}HNMR spectrum of compound **NCCN**.

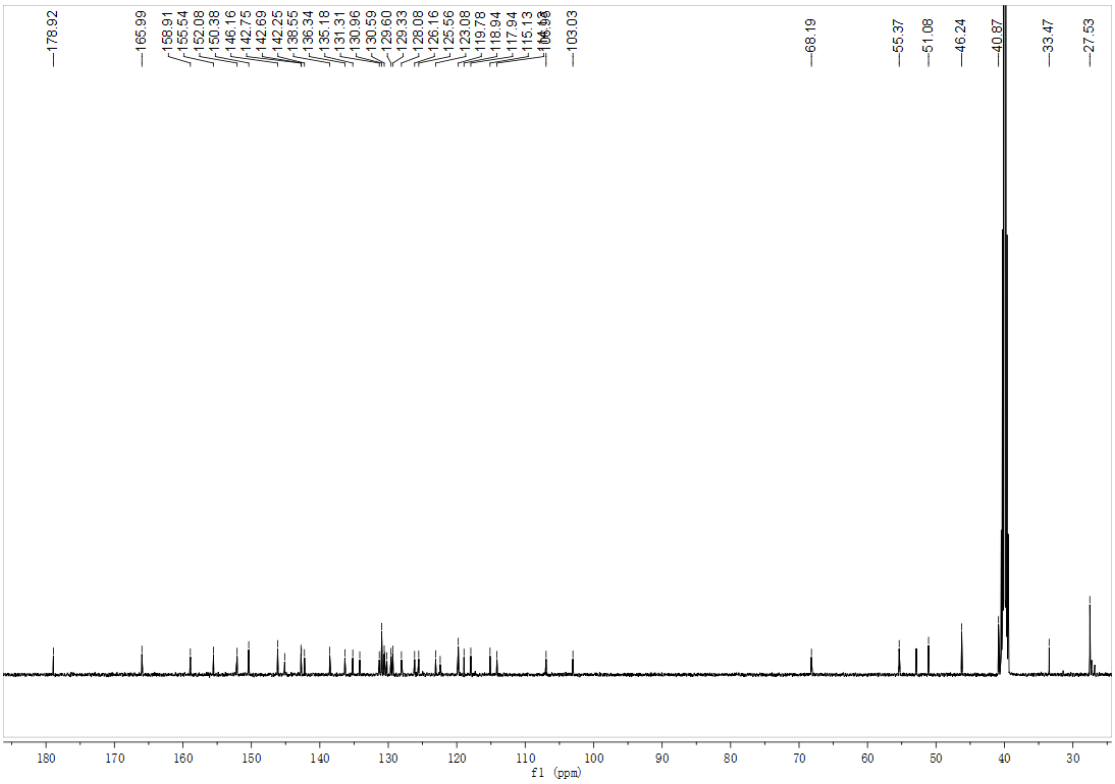


Fig. S27 The ^{13}CNMR spectrum of compound **NCCN**.

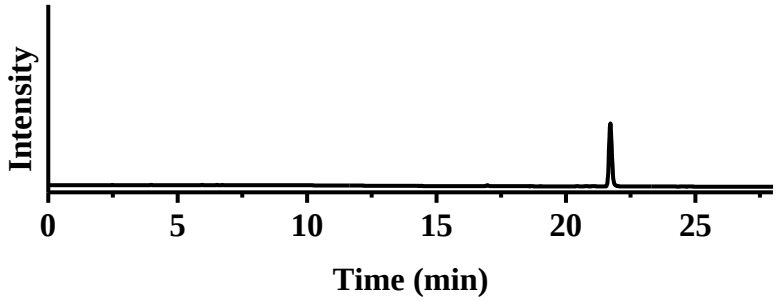


Fig. S28 The HPLC analysis of compound **NCCN** by Waters e2695 liquid chromatography (Waters, Milford, USA)

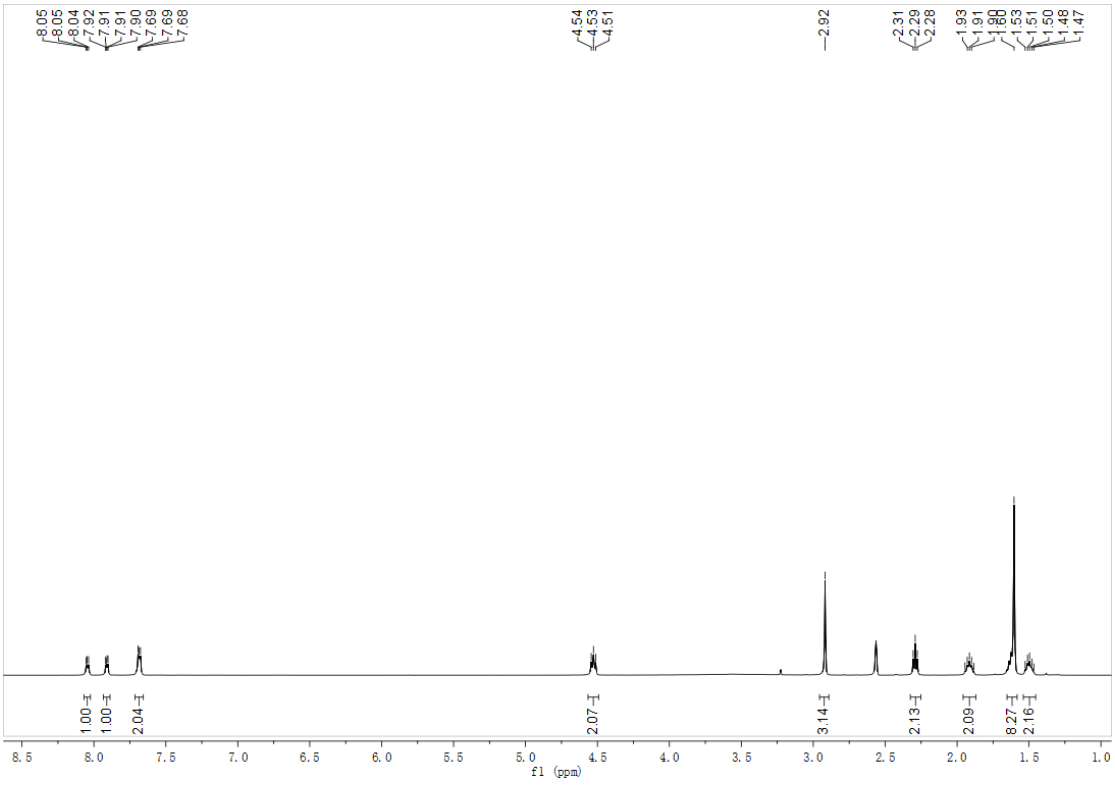


Fig. S29 The ^{1}HNMR spectrum of compound **6**.

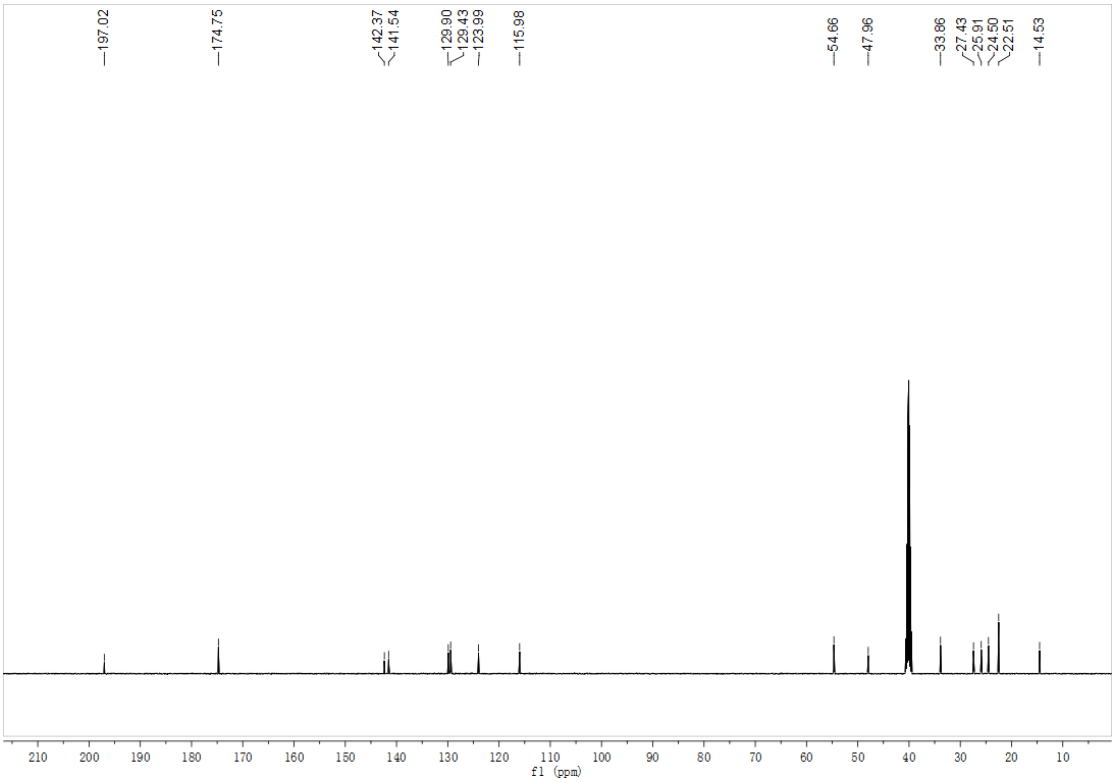


Fig. S30 The ^{13}CNMR spectrum of compound **6**.

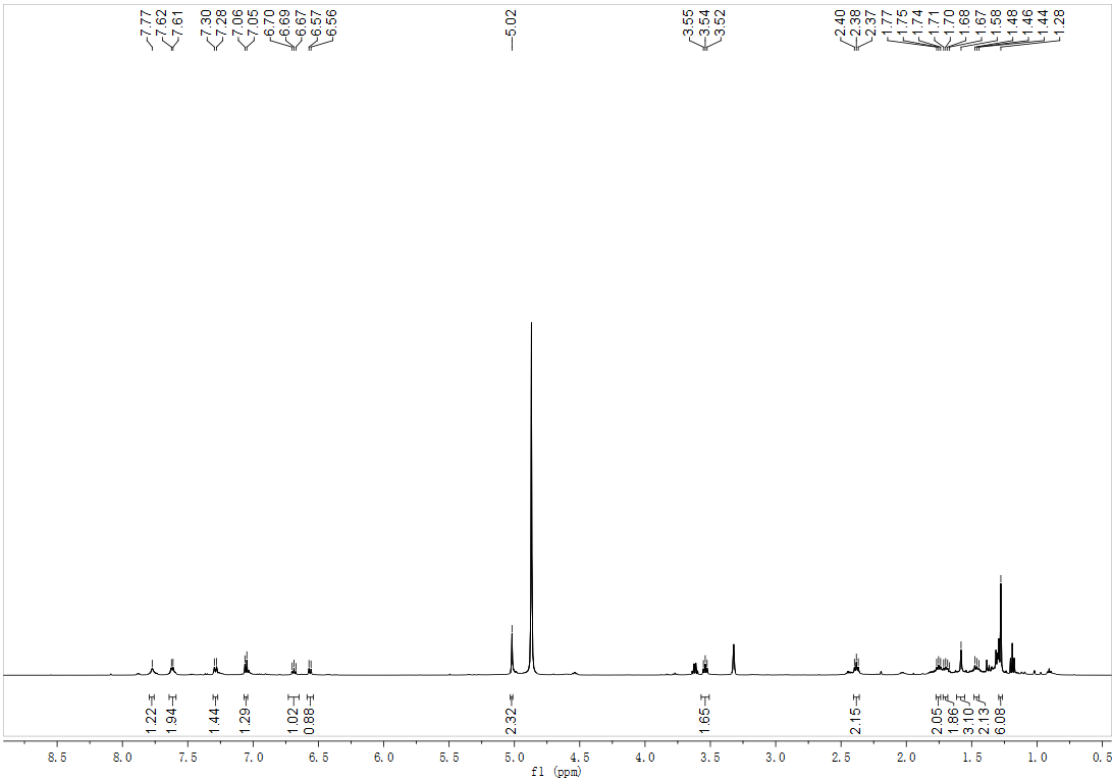


Fig. S31 The ^{1}HNMR spectrum of compound **7**.

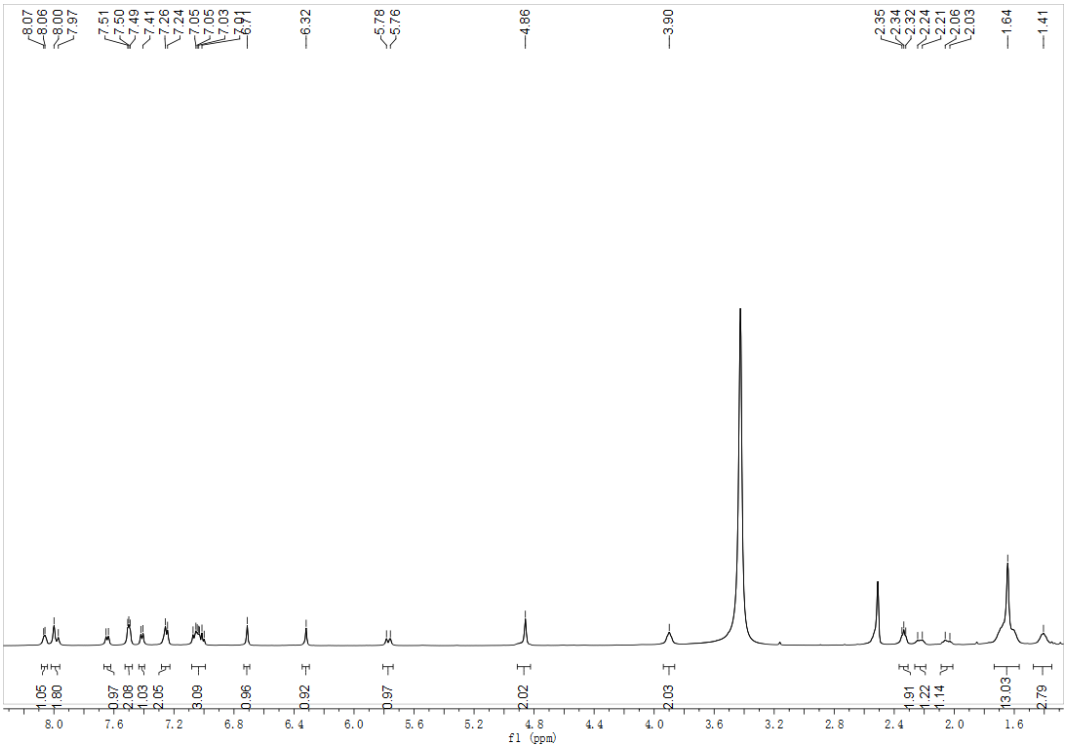


Fig. S32 The ^{1}HNMR spectrum of compound **NBCS**.

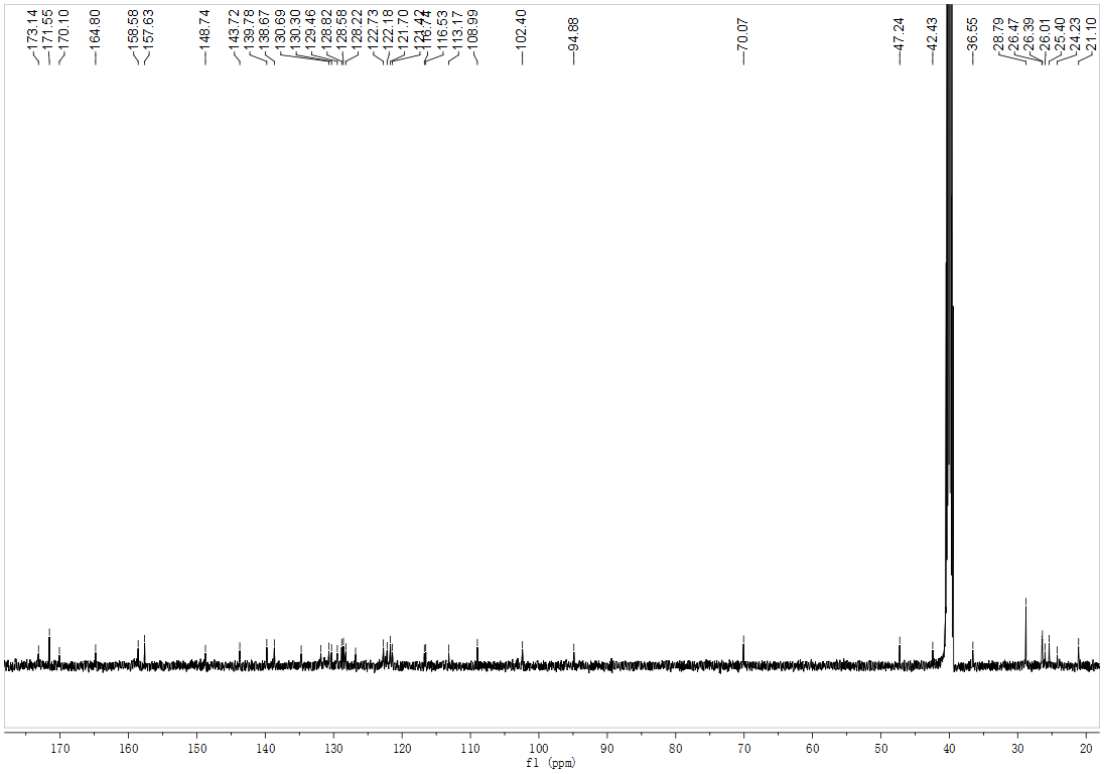


Fig. S33 The ^{13}CNMR spectrum of compound **NBCS**.

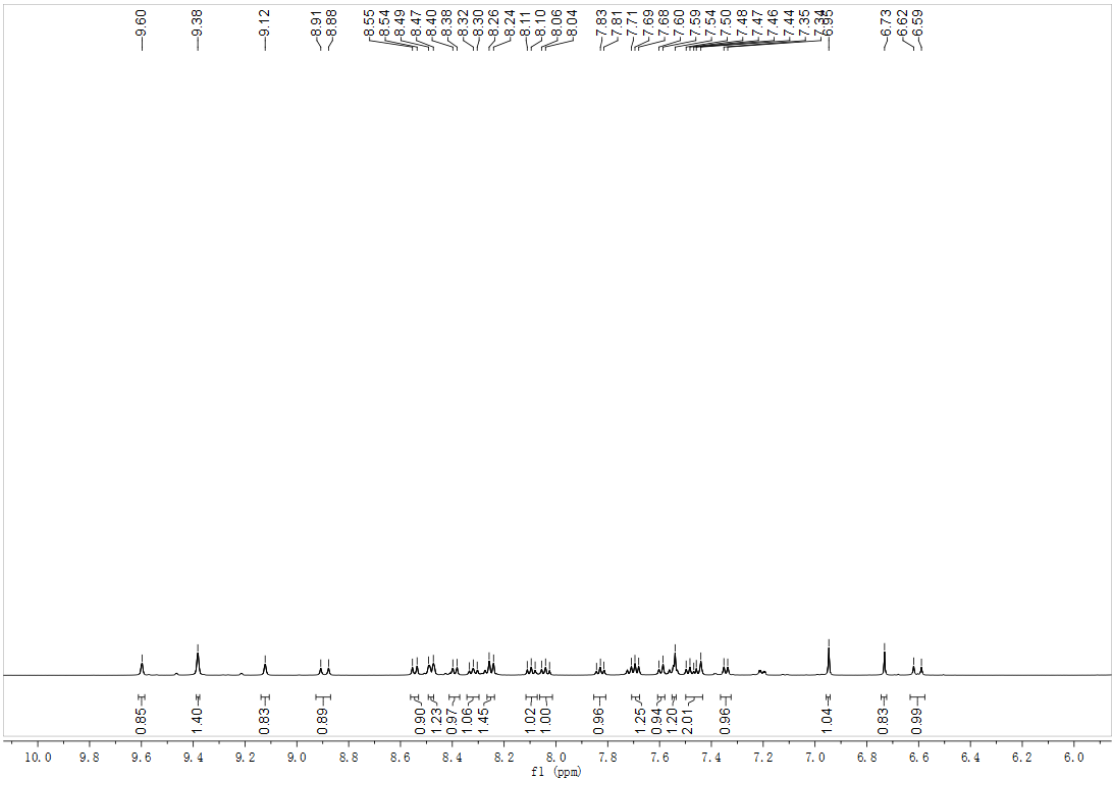


Fig. S34 The ^{1}HNMR spectrum of probe **NBON**.

17. References

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