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Supporting Information

In Vivo Nitroreductase Imaging via Fluorescence and Chemical Shift Dependent ^{19}F NMR

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SUPPORTING INFORMATION

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Experimental Procedures

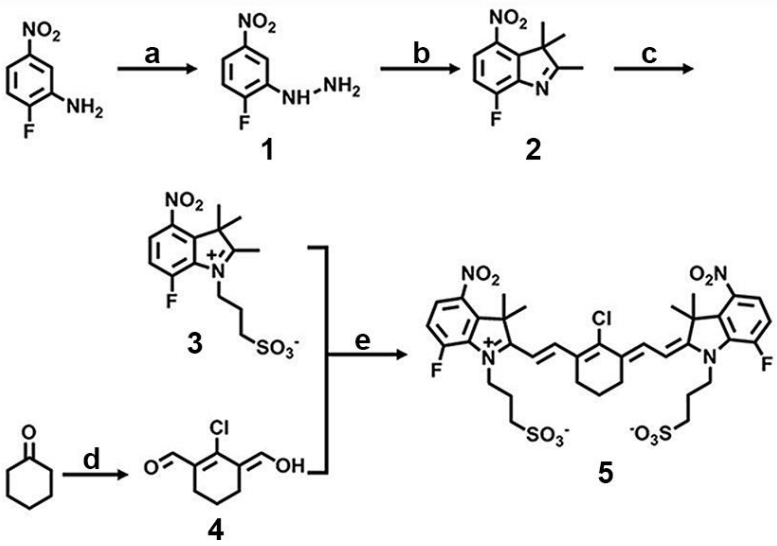
1. Materials and Instruments

2-fluoro-5-nitroaniline, 3-methyl-2-butanone, 1,3-propane sulfonic acid lactone, stannous chloride dihydrate, and cyclohexanone were purchased from J&K Scientific Ltd.; Concentrated hydrochloric acid, acetic acid, acetic anhydride, and other solvents were purchased from Chinese medicine reagents Co. Ltd.; Nitroreductase from *Escherichia coli* and *β*-Nicotinamide adenine dinucleotide disodium salt hydrate (NADH) were purchased from Sigma-Aldrich Co. Ltd.; The 200-300 mesh TLC silica-gel powder was purchased from Qingdao Foreign Chemical Industry Co. Ltd. All reagents were of analytical grade. NTR freeze-dried powder was dissolved in pure water, divided into 20 equal parts of 1 mL, and stored at -20°C. Pure water/ultra-pure water was prepared and purified by a Milli-Q reference system (Millipore). The bimodal molecular probe was dissolved in purified water or PBS (pH=7.4), and 1 mM FCy7-NO_2 was prepared and stored at 4°C for later use.

The compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and high-performance liquid chromatography-tandem quadrupole mass spectrometry (HRMS). A Thermo EVOLUTION-220 UV−vis spectrometer was used to measure the UV−vis absorption spectra. An Edinburgh FS5 fluorescence spectrometer with a 150 W xenon lamp was utilized to obtain the steady-state emission spectra. ^{1}H MRI and ^{19}F MRI were measured on Bruker 400 MHz wide-bore nuclear magnetic resonance spectrometer. *In vivo* fluorescence imaging was performed with small animals living fluorescence imaging system by PerkinElmer Co. Ltd.

2. Synthesis Procedures

Referring to the previously reported method,[1] we modified the synthesis of molecular probes as follows (Scheme S1).



Scheme S1. Synthesis route of FCy7-NO_2. Reagents and conditions: (a) concentrated hydrochloric acid, $NaNO_2$, then $SnCl_2{\cdot}2H_2O$, -25°C, 3 h; (b) 3-methyl-2-butanone, concentrated hydrochloric acid, glacial acetic acid, 95°C, 3 h; (c) 1,3-propane sultone, 110°C, 12 h; (d) $POCl_3$, DMF, 50°C, 10 h; (e) anhydrous sodium acetate, acetic anhydride, 60°C, 12 h.

2.1. Synthesis of Compound 1. 2-fluoro-5-nitroaniline (20.00 g, 128.1 mmol, 1.0 eq.) was dissolved in 160 mL of concentrated hydrochloric acid (12 mol L^{-1}), and the reaction was stirred for 30 min at room temperature. Then the reaction was transferred to a low-temperature reaction bath (-25°C) and stirred for 10 min. $NaNO_2$ (10.62 g, 153.6 mmol, 1.2 eq.) was dissolved in pure water (30 mL) and added dropwise with a constant pressure drop funnel. Then the reaction continued to stir for 1 h. Subsequently, stannous chloride dihydrate (57.77 g, 255.7 mmol, 2.0 eq.) was dissolved in 150 mL of concentrated hydrochloric acid and added slowly to the reaction mixture. After stirring for 10 min, the reaction was transferred to room temperature and continued to stir for 1 h. After filtration, the residue was washed with concentrated hydrochloric acid, ether, and dichloromethane, respectively. Finally, the solvent was removed by a rotary evaporator and concentrated *in vacuo* to obtain compound **1** as a yellow solid (16.93 g, 75.4% yield).
^{1}H NMR (500 MHz, CD_3OD) δ 8.08 (d, *J* = 5.1 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.45 (t, *J* = 9.6 Hz, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 153.9, 153.9, 144.7, 134.0, 118.4, 116.3. 116.1, 110.2. ^{19}F NMR (471 MHz, CD_3OD) δ -121.81 (d, *J* = 10.4Hz). HRMS (ESI) calc for $C_6H_6N_3O_2F$, $[M-H]^-$ = 170.03713, found 170.03569 (Figure S1-S4).

2.2 Synthesis of Compound 2. Compound **1** (3.42 g, 20.0 mmol, 1.0 eq.), 3-methyl-2-butanone (3.45g, 40.0 mmol, 2.0 eq.), and sodium acetate (3.32g, 40.0 mmol, 2.0 eq.) were stirred at 50 mL of glacial acetic acid at 95°C for 3 h under N_2 atmosphere. After cooling to room temperature, the solvent was removed under reduced pressure. And the residue was dissolved with CH_2Cl_2. The organic phase was washed with 1 M $NaHCO_3$ aqueous solution until no bubbles, and the organic layer was collected and dried over

anhydrous sodium sulfate. After filtration, the solvent was concentrated, and the residue was purified by column chromatography (ethyl acetate: n-hexane = 1:20, v/v) to afford compound **2** as a reddish oil (2.10 g, 47.3% yield).
^{1}H NMR (500 MHz, CD_3OD) δ 8.13 (dd, *J* = 9.2, 4.2 Hz, 1H), 7.39 (t, *J* = 9.0 Hz, 1H), 2.40 (s, 3H), 1.56 (d, *J* = 2.0 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 193.0, 157.6, 155.6, 143.0. 142.3, 141.7, 123.6, 116.2, 116.0, 58.3, 18.6, 14.2. ^{19}F NMR (471 MHz, CD_3OD) δ -119.65 (dd, *J* = 8.8, 4.2 Hz). HRMS (ESI) calc for $C_{11}H_{11}N_2O_2F$, $[M-H]^-$ = 221.07318, found 221.08510 (Figure S5-S8).

2.3 Synthesis of Compound 3. Compound **2** (1.63 g, 7.2 mmol, 1.0 eq.) and 1,3-propane sultone (1.94 g, 15.8 mmol, 2.2 eq.) were suspended in 1,2-dichlorobenzene (20 mL) and stirred at 110°C overnight under N_2 atmosphere. After cooling to room temperature, the reaction was filtrated and washed with CH_2Cl_2 three times. The residue was purified by column chromatography (CH_3OH: CH_2Cl_2=1:8, v/v). Compound **3** was obtained as a light pink solid (1.21 g, 48.4% yield).
^{1}H NMR (500 MHz, CD_3OD) δ 8.52 (dd, *J* = 9.3, 3.7 Hz, 1H), 7.80 (d, *J* = 1.1 Hz, 1H), 3.57 (t, *J* = 6.3 Hz, 1H), 3.08 – 2.98 (m, 2H), 2.93 (dd, *J* = 17.3, 10.3 Hz, 1H), 2.53 – 2.38 (m, 2H), 2.11 – 2.02 (m, 1H), 1.84 (s, 5H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.9, 152.2, 150.4, 145.9, 142.3, 137.9, 131.7, 130.2, 122.6, 118.0, 101.3, 52.1, 45.7, 26.2, 24.7, 23.7, 20.6. ^{19}F NMR (471 MHz, CD_3OD) δ -117.79 (s). HRMS (ESI) calc for $C_{14}H_{17}N_2SO_5F$ $[M-H]^-$ = 343.07694, found 343.08580 (Figure S9-S12).

2.4 Synthesis of Compound 4. Anhydrous DMF (50 mL) was added in a 150 mL round bottom flask at 0°C. Then $POCl_3$ (35 mL, 380.0 mmol, 3.8 eq.) was slowly added and stirred for 30 min. Next, cyclohexanone (9.98 g, 100.0 mmol, 1.0 eq.) was added by syringe, and the reaction was stirred at 50°C for 10 h. After cooling to room temperature, the red mixture was poured into 400 g ice and stood overnight. The yellow precipitate was collected, washed with H_2O, and triturated with CH_2Cl_2. The product was collected by filtration and dried *in vacuo* to obtain compound **4** as luminous yellow powder (7.74 g, 45.8% yield).
^{1}H NMR (500 MHz, DMSO-d6) δ 8.81 (s, 2H), 2.36 (t, *J* = 6.2 Hz, 4H), 1.67-1.48 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 150.7, 148.7, 141.8, 139.0, 120.8, 112.9, 111.4. 111.2. HRMS (ESI) calc for $C_8H_9O_2Cl$ $[M-H]^-$= 173.03638, found 172.94569 (Figure S13-15).

2.5 Synthesis of Compound 5. Compound **3** (690.0 mg, 2.0 mmol, 2.0 eq.), compound **4** (191.4 mg, 1.1 mmol, 1.1 eq.), and anhydrous sodium acetate (88.4 mg, 1.0 mmol, 1.0 eq.) were added in acetic anhydride (20 mL) and stirred at 60°C overnight under N_2 atmosphere. After cooling to room temperature, the mixture was precipitated with Et_2O and dried *in vacuo*. The crude product was purified by column chromatography (CH_3OH: CH_2Cl_2=1:5, v/v). The final compound **5** (FCy7-NO_2) was obtained as a dark green with metallic gloss solid (416.5 mg, 48.2% yield).
^{1}H NMR (500 MHz, CD_3OD) δ 8.59 (d, *J* = 14.1 Hz, 2H), 8.01 (dd, *J* = 9.2, 3.8 Hz, 2H), 7.52 (dd, *J* = 11.3, 9.2 Hz, 2H), 6.60 (d, *J* = 14.1 Hz, 2H), 4.65-4.48 (m, 4H), 3.73 (s, 1H), 2.99 (t, *J* = 6.9 Hz, 4H), 2.86 (t). *J* = 6.0 Hz, 4H), 2.43-2.29 (m, 4H), 1.99 (s, 13H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.9, 152.2, 150.4, 145.9, 142.4, 137.8, 131.7, 130.2, 122.6, 118.0, 101.3, 52.1, 28.2, 26.2, 24.7, 23.7, 20.6. ^{19}F NMR (471 MHz, CD_3OD) δ 124.59 (d, *J* = 9.6 Hz). HRMS (ESI) calc for $C_{36}H_{38}N_4O_{10}S_2F_2Cl$ $[M]^-$ = 823.16914, found 823.17143 (Figure S16-19).

3. *In Vitro* Studies of FCy7-NO_2

3.1 Docking Calculations.

The ligands of FCy7-NO_2 and Cy7-NO_2 were studied by the PM3 method to calculate their electronic structures using Gaussian 16. The binding affinity calculations between probes and NTR protein (PDB ID: 4DN2) were carried out on AutoDock vina software (1.2.0 version).[2] The crystal structure of NTR was obtained from the protein databank, www.pdb.org. All the binding affinity calculations and the graphics were generated using AutoDock and PyMOL software.

3.2 Comparison of Fluorescence Stability between FCy7-NO_2 and Commercial Dyes.

Unless otherwise specified, all UV-vis and fluorescence spectra experiments were carried out in 10 mM PBS (pH 7.4). FCy7-NO_2 (200 μL, 1 mM) was diluted to 40 mL with PBS (the final concentration of FCy7-NO_2 was 5 μM) and packed in two glass bottles. One group was placed in normal condition (room temperature, sunlight). The other group was irradiated under an ultraviolet lamp (UV lamp power was 8 W, and the glass bottle was about 15 cm from the UV lamp). Then the fluorescence intensity of the FCy7-NO_2 solution (2 mL, 5 μM) was measured at different time points. The excitation wavelength was 770 nm, the emission wavelength was 600-1000 nm, and the bandwidth was 5 nm. The same experiments with ICG and Cy7 were also performed.[3] The concentration of the experimental group and the control group was 5 μM and irradiated under sunlight and ultraviolet light, respectively, and samples were taken at different time points (sunlight irradiation in days, 0, 1, 2, 3, 4, 5, 6, 7 days; UV irradiation in min, respectively, at 0, 10, 20, 30, 40, 50, 60, 70 min). Finally, the maximum fluorescence intensity in the fluorescence emission curve of the probe was measured.

3.3 Conventional NTR Optical Spectral Detection and ^{19}F NMR Detection.

FCy7-NO_2 (0.1 mL, 1 mM), NTR (0.5 mL), and PBS (0.4 mL) were mixed in the 2 mL EP tube, then 5 times equivalent of NADH was added. The mixture was incubated in a 37°C water bath, and 200 μL of the reaction solution was diluted to 4 mL with PBS at specific time points. A 10 × 10 mm four-way colorimetric plate was used for the ultraviolet absorption and fluorescence emission spectrum. Similarly, FCy7-NO_2 (1 mL, 10 mM, 1.0 eq.), NTR (4 mL), PBS (5 mL) were added to 15 mL EP tube, and subsequently, NADH (1 mL, 20 mM, 2.0 eq.) was added. The mixture was incubated in a water bath at 37°C. At different time points, 450 μL of the reaction solution was added to 50 μL of D_2O. ^{19}F NMR spectrum was performed on a Bruker 500 MHz NMR spectrometer, and the probe temperature was maintained at 0°C.

3.4 Cell Culture.

A549 cells, derived from the Institute of Biochemistry and Cell Biology, SIBS, Chinese Academy of Sciences, were cultured in F12K medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C, 5% CO_2, and normal oxygen concentration (20%). In addition, the A549 cells were cultured in a three-gas incubator under hypoxia such as 10%, 5%, and 1% oxygen concentration according to specific experimental requirements with other conditions unchanged.

3.5 Toxicity Analysis.

In vitro toxicity analysis was performed in A549 cells by the standard MTT method.[4] Briefly, the logarithmic phase cells in the culture flask of T25 were dispensed into 96-well plates, and the number of cells per well was controlled at about 1×10^4. After the cells adhered, 100 μL of F12K culture medium containing the FCy7-NO_2 probe (5-50 μM) was added to each well, with PBS added into the control group. The cells were cultured at 37°C and 5% CO_2 for 12 h and 24 h, respectively. Then MTT solution was added and cultured for 4 hours and then washed 3 times with PBS. Finally, DMSO (100 μL) was added to each well, and the plates were incubated for 15 min until all the crystals were dissolved. An enzyme-linked immunosorbent assay (Elisa) was used to test at 490 nm. The cell survival rate = $A/A_0 \times 100\%$ (A is the absorbance value measured in the experimental group, and A_0 is the absorbance value measured in the PBS control group).

3.6 Confocal Fluorescence Imaging for Cells.

A549 cells (about 1×10^5) were plated on 14 mm glass coverslips and adhered for 24 h. The cells were incubated with 10 μM FCy7-NO_2 in F12K for 4 h at 37°C and then washed with PBS. The nucleus was then stained with DAPI. Cell imaging was then carried out after washing cells with pure water (2 mL × 3 times), followed by fixation with 4% paraformaldehyde. Fluorescence imaging was performed with a Zeiss LSM810 two-photon confocal fluorescence microscope with a 40 × oil immersion objective lens. The fluorescence signal of cells incubated with FCy7-NO_2 was collected at 800 ± 30 nm, using CW two-photon laser at 745 nm as an excitation resource.

3.7 H&E Staining.

The major organs (heart, liver, spleen, lung, and kidney) were dissected and fixed in 4% paraformaldehyde and subjected to hematoxylin and eosin (H&E) staining assays.

3.8 ^{19}F MRI *In Vitro*.

In vitro ^{19}F MRI has been completed on a Bruker 400 MHz wide-bore nuclear magnetic resonance spectrometer. A ^{19}F (376.5 MHz) coil with an inner diameter of 10 mm was used for RF transmission and reception. The probes were imaged in solution and cells. ^{19}F MR imaging was acquired using a RARE sequence. The parameters were set as follows: repetition time (TR) = 3000 ms, effective time (TE) = 3 ms, rare factor =4, matrix size = 64 × 64, number of average = 64, field of view (FOV) = 37 mm × 37 mm, slice thickness (SI) = 10 mm, bandwidth=5400 Hz, the total scan time was approximately 30 min.

4. *In Vivo* Imaging with FCy7-NO_2

4.1 Animals and Cancer Model mice.

All experimental protocols involving animals were approved by the Animal Welfare and Research Ethics Committee at Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences (APM21018T). All BALB/c male nude mice (5-6 weeks of age, approximately 20 g) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. and fed on SPF grad mice feed. In situ lung cancer model mice were produced according to related reports.[5] About 1×10^6 A549 cells in 0.5 mL F12K culture medium were mixed with an equal volume of matrix glue. When the left lung was cut open, 50 μL cell gel was injected into the left lung of nude mice. After surgery, the physiological health status of nude mice was monitored to avoid infection. In addition, the size of the tumor was monitored by CT and ^{1}H MRI every week.

4.2 *In Vivo* NIR Fluorescence Imaging.

Prior to imaging, the in-situ lung cancer mice were anesthetized by isoflurane. And then, 100 μL of FCy7-NO_2 (100 μM) in PBS buffer was injected by posterior venous plexus. After 5 min, the NIR fluorescence imaging was measured with excitation at 745 nm using an in-vivo imaging system (PerkinElmer). The 745 nm CW laser with adjustable power of 0-1.3 W is used as the excitation light source, and the fluorescence signal is collected by Andor DU897 EMCCD with Semrock 800 ± 12 nm bandpass filter.

4.3 ^{19}F MRI *In Vivo*.

^{1}H/^{19}F MRI was performed on a 400 MHz wide-bore nuclear magnetic resonance spectrometer. 30 mm inner diameter ^{1}H (400 MHz) and ^{19}F (376.5 MHz) coils were used for radio frequency transmission and reception. Isoflurane was used for anesthesia during the experiment. ^{1}H MRI was carried out using the RARE (rapid acquisition with refocused echoes) method. The imaging parameters were set as follows: number of average = 4, repetition time (TR) = 6000 ms, effective time (TE) = 56 ms, field of view (FOV) = 30 mm × 30 mm, slice thickness (SI) = 1 mm, matrix size = 256 × 256. ^{19}F MRI was acquired using the RARE sequence. The parameters were set as follows: number of average = 256, repetition time (TR) = 3000 ms, effective time (TE) = 3 ms, matrix size = 64 × 64, field of view (FOV) = 37 mm × 37 mm, slice thickness (SI) = 1.235 mm, rare factor =4, bandwidth=2000 Hz, the total scan time was approximately 2 hours. All raw data were processed using MATLAB (R2018a, MathWorks, Natick, MA).

5. Characterization Data

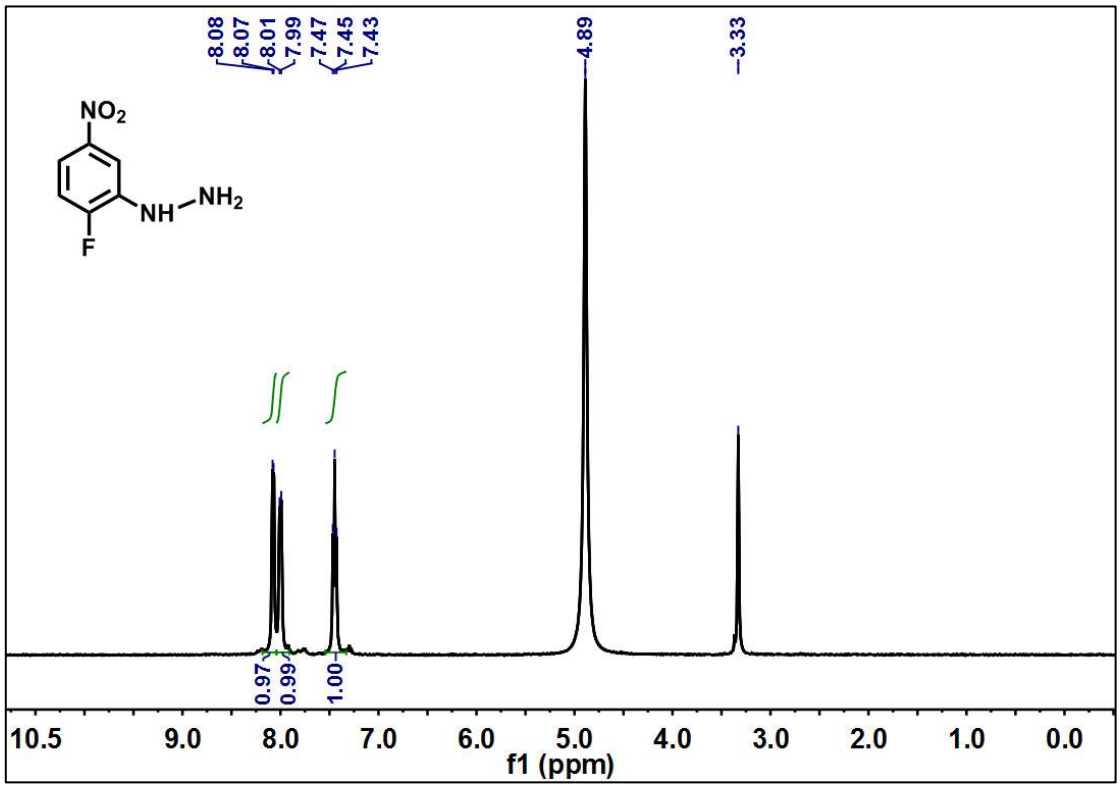


Figure S1. ^{1}H NMR spectra of compound **1** in CD_3OD.

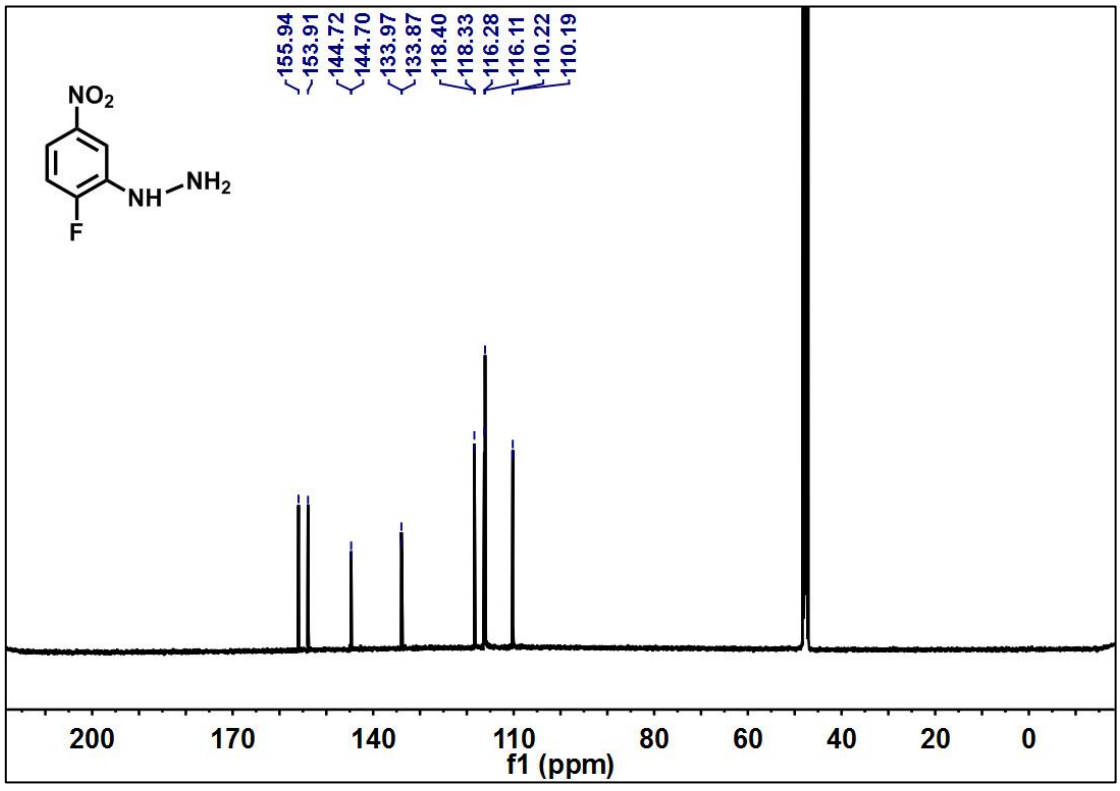


Figure S2. ^{13}C NMR spectra of compound **1** in CD_3OD.

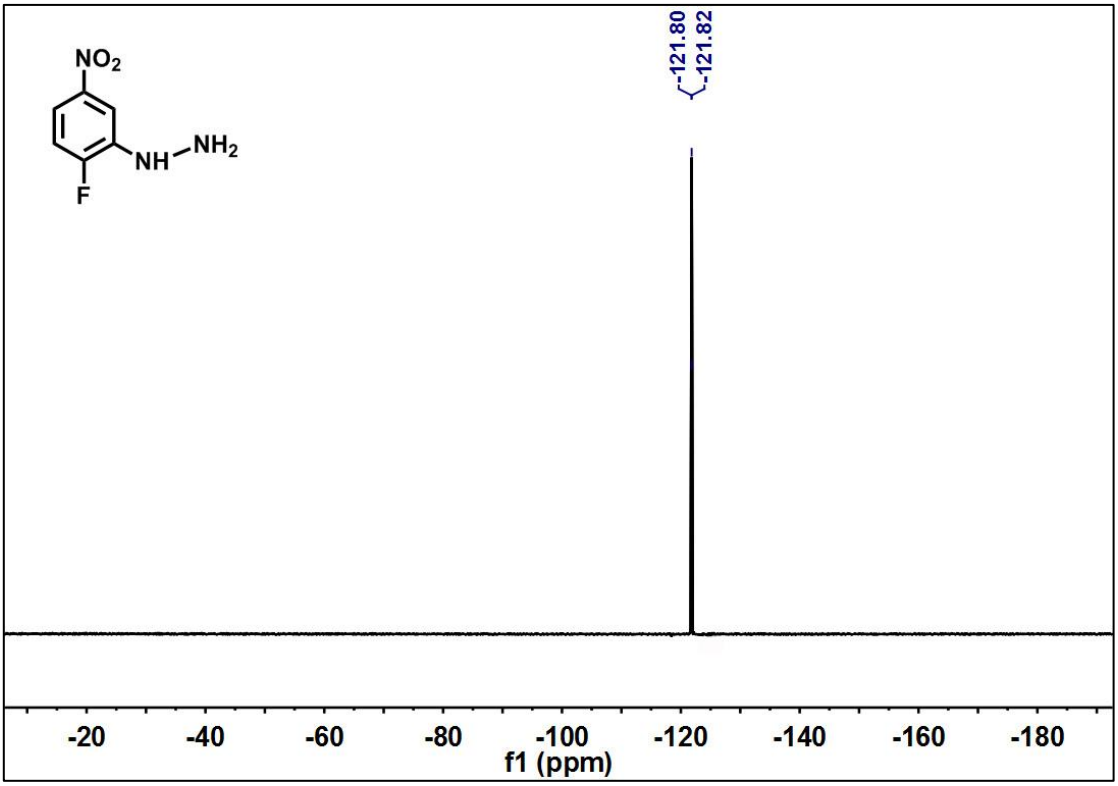


Figure S3. ^{19}F NMR spectra of compound **1** in CD_3OD.

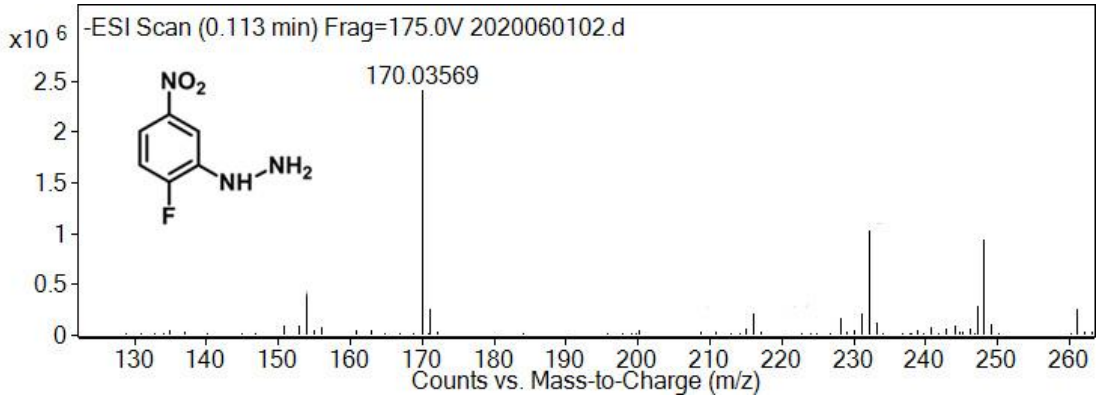


Figure S4. HRMS spectra of compound **1**.

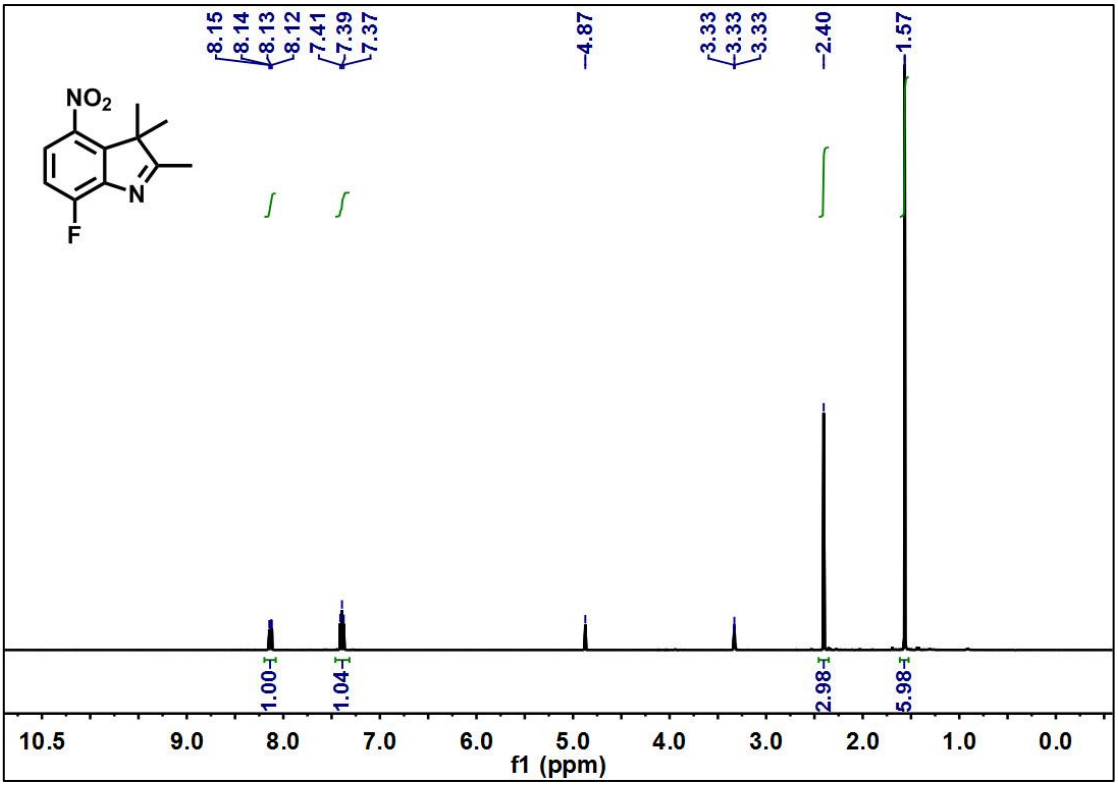


Figure S5. ^{1}H NMR spectra of compound **2** in CD_3OD.

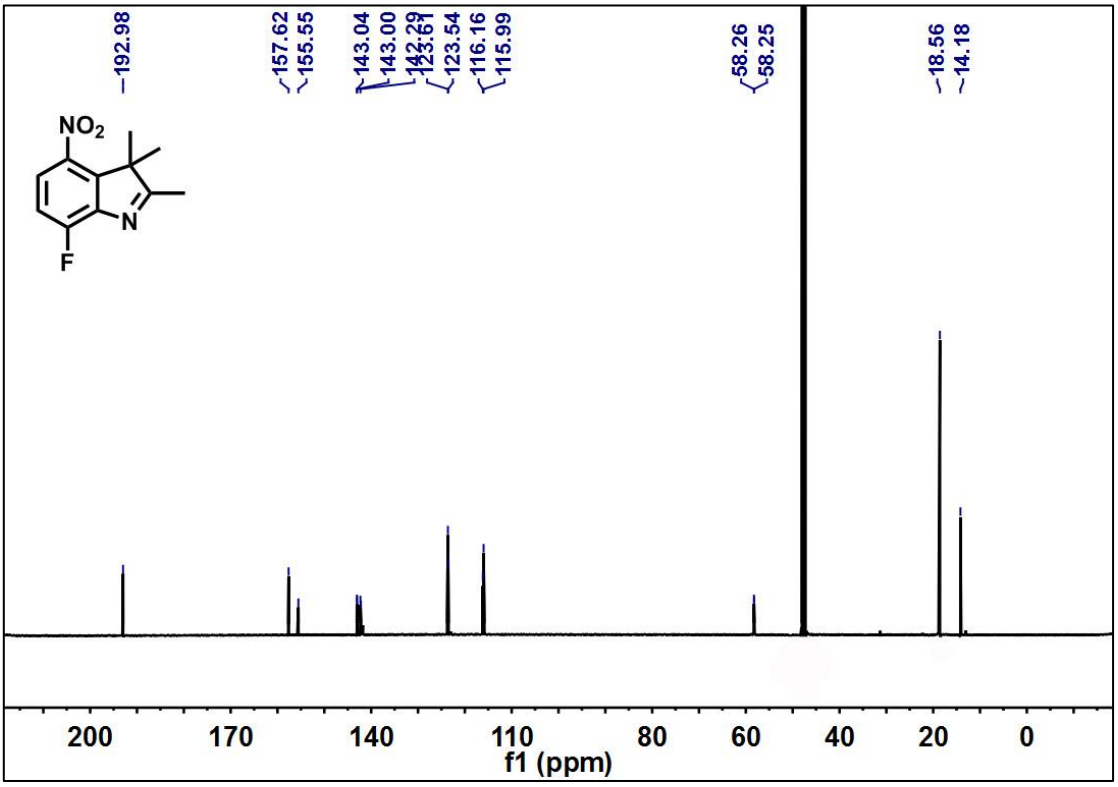


Figure S6. ^{13}C NMR spectra of compound **2** in CD_3OD.

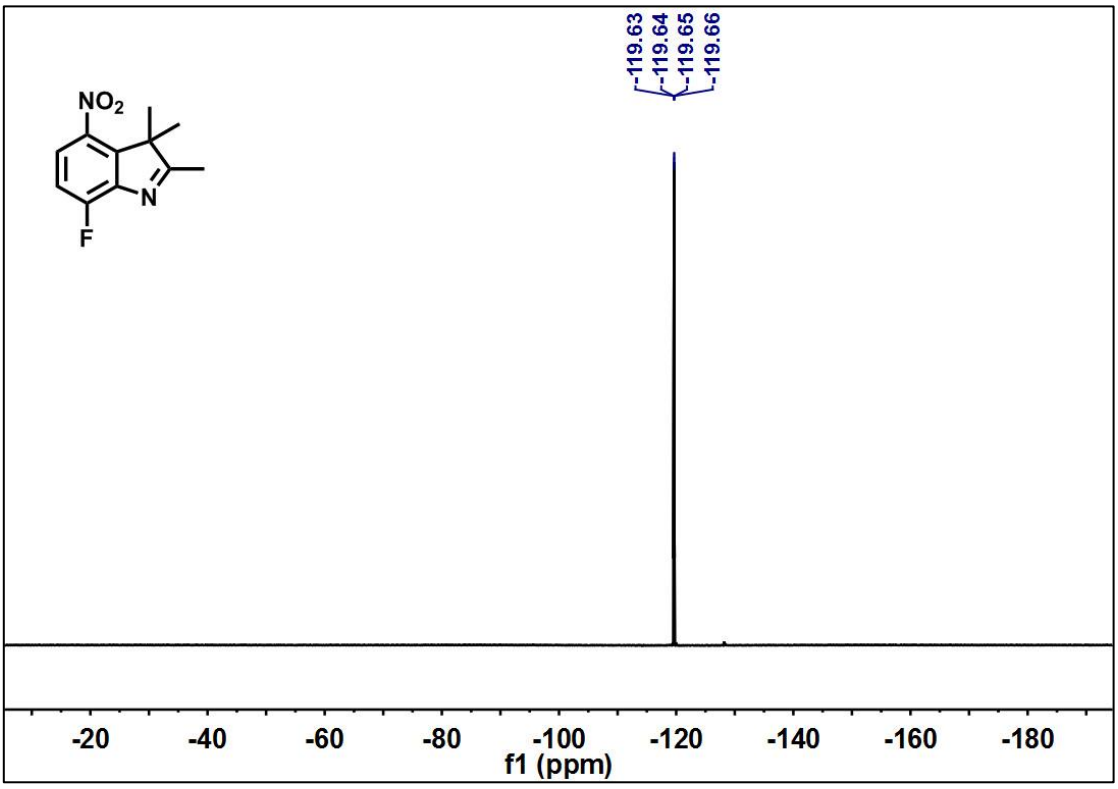


Figure S7. ^{19}F NMR spectra of compound **2** in CD_3OD.

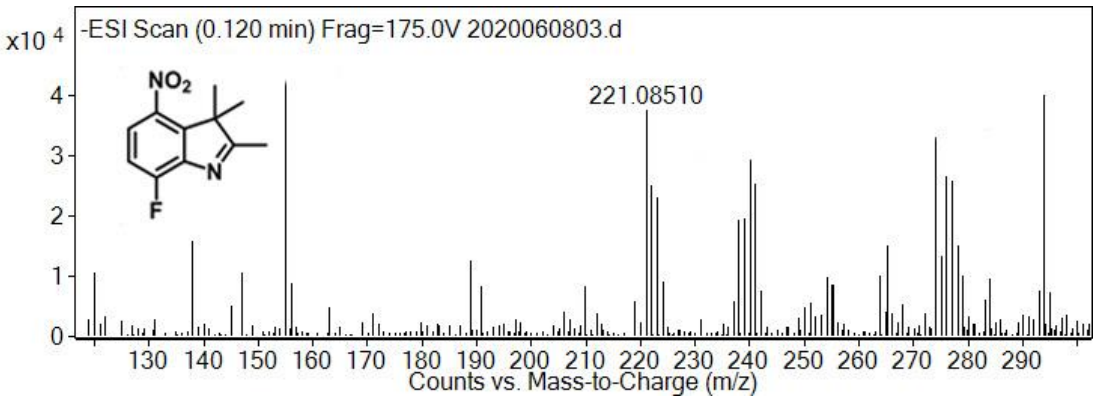


Figure S8. HRMS spectra of compound **2**.

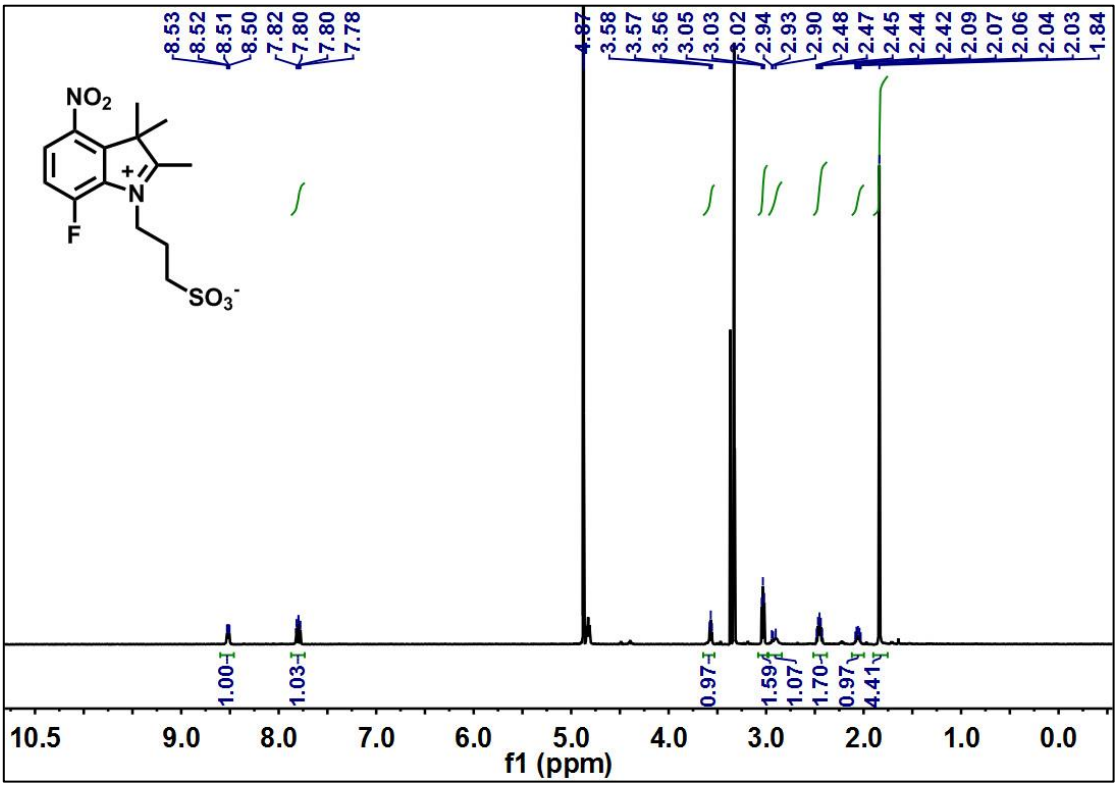


Figure S9. ^{1}H NMR spectra of compound **3** in CD_3OD.

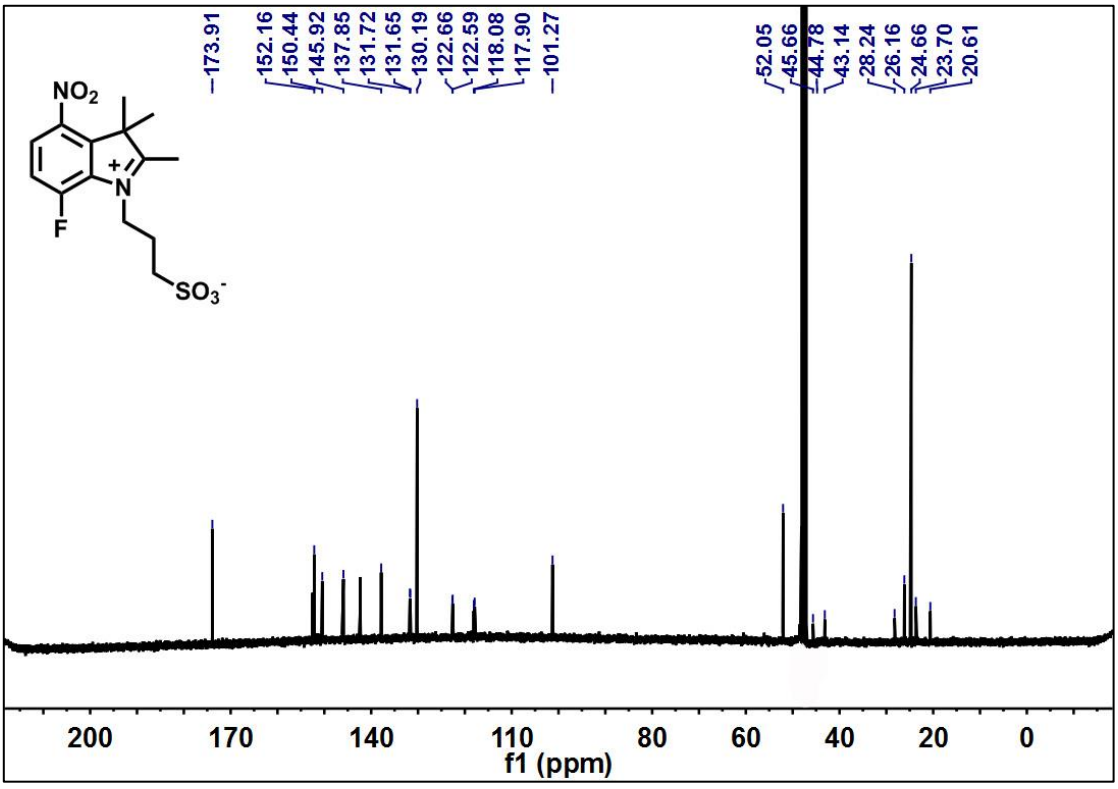


Figure S10. ^{13}C NMR spectra of compound **3** in CD_3OD.

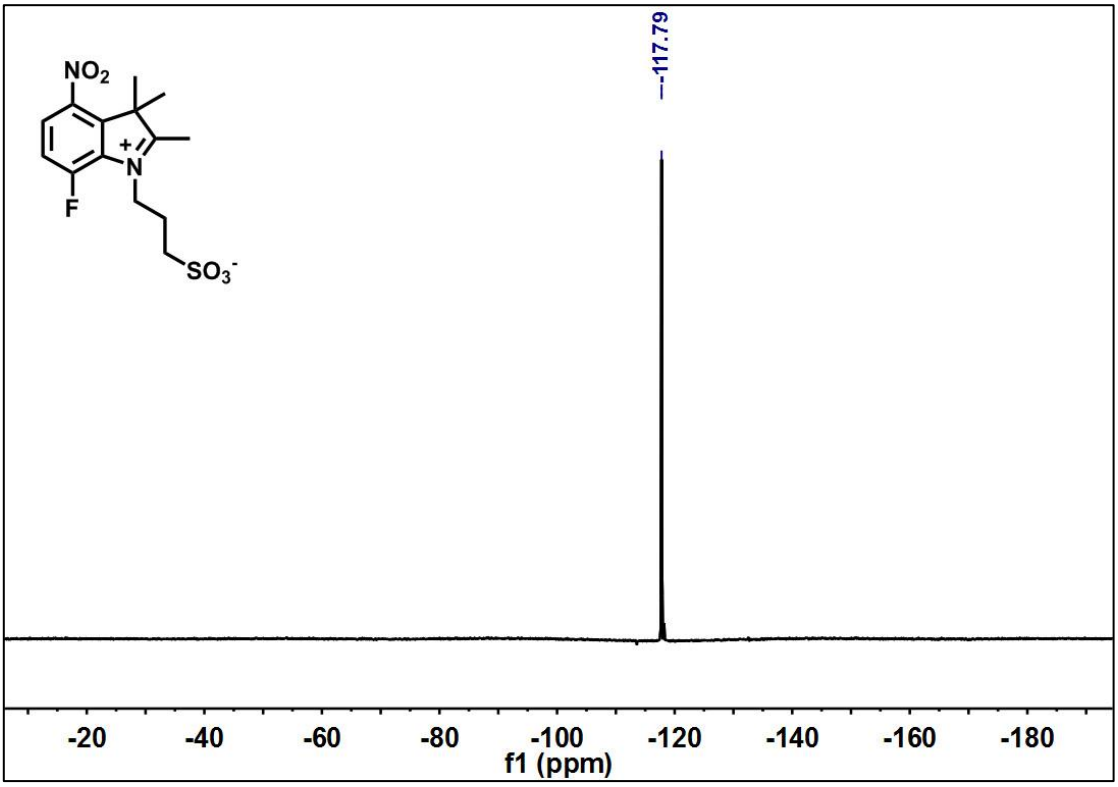


Figure S11. ^{19}F NMR spectra of compound **3** in CD_3OD.

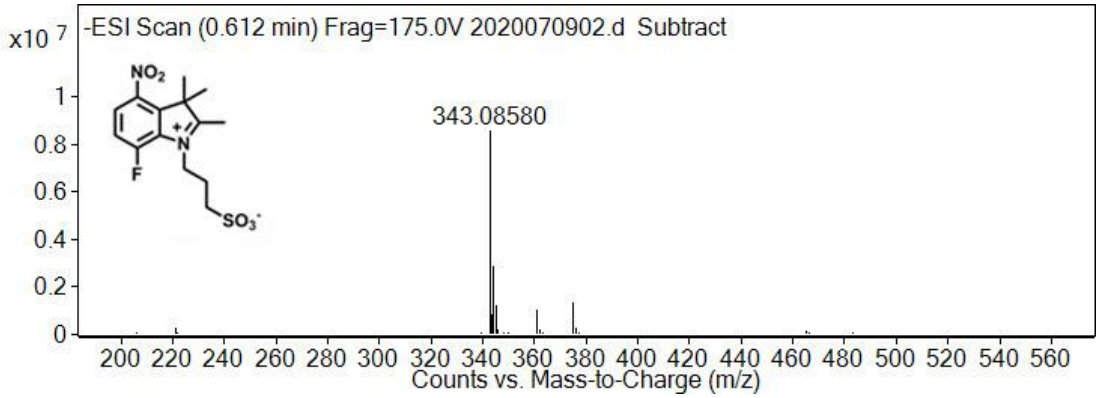


Figure S12. HRMS spectra of compound **3**.

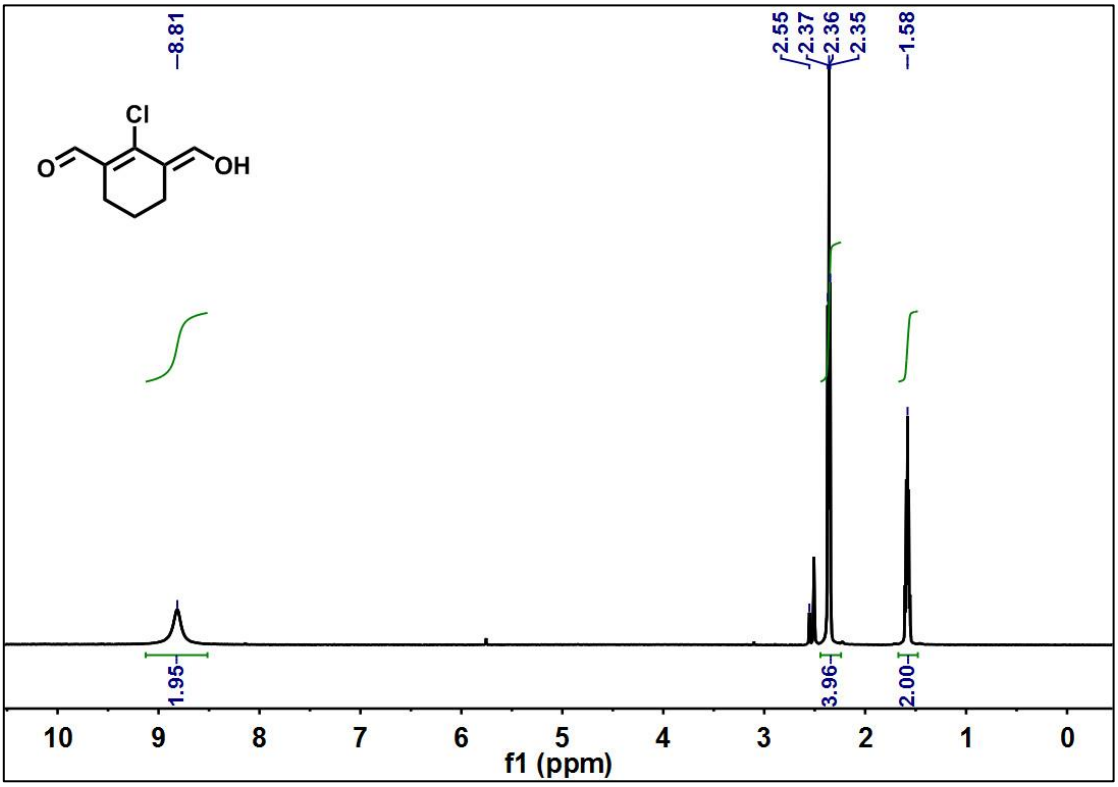


Figure S13. ^{1}H NMR spectra of compound **4** in DMSO-d6.

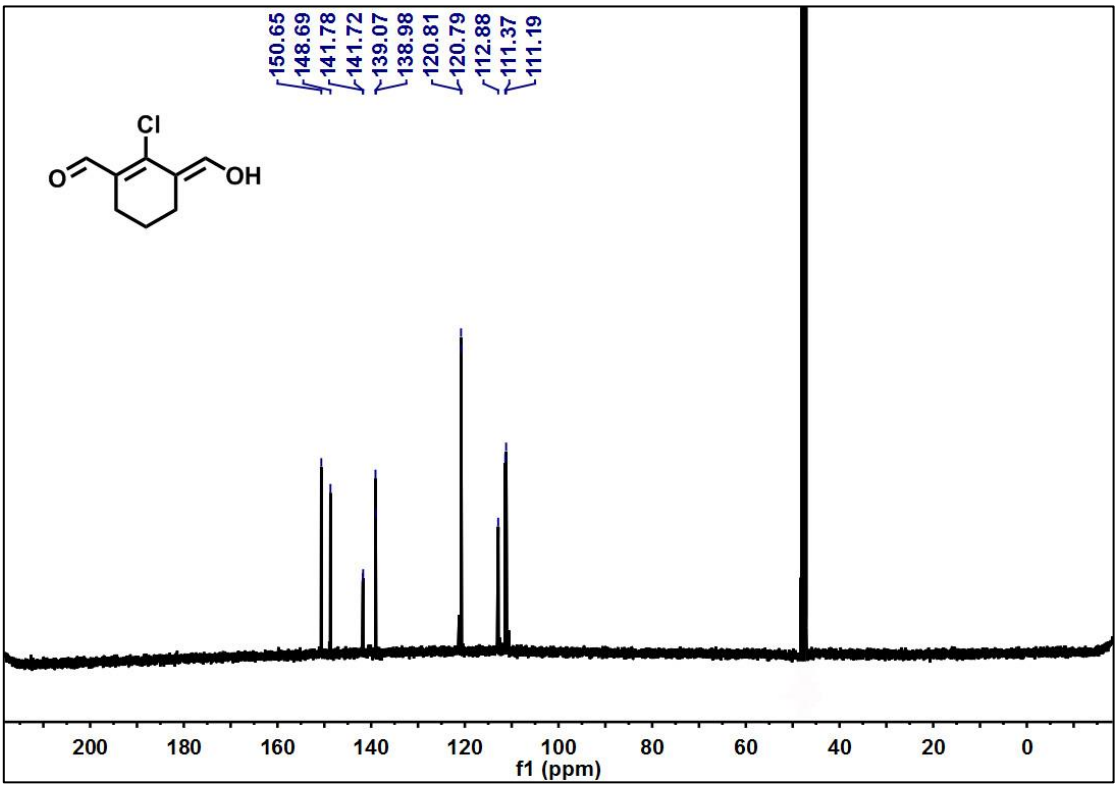


Figure S14. ^{13}C NMR spectra of compound **4** in CD_3OD.

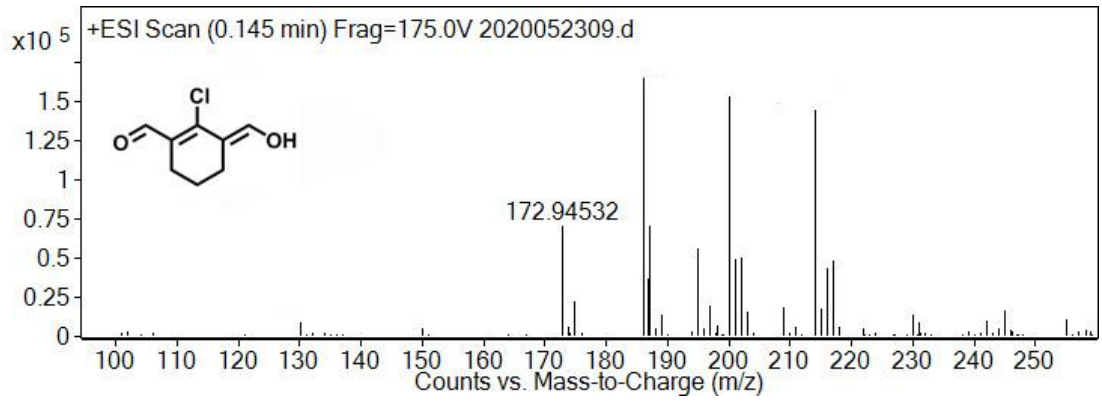


Figure S15. HRMS spectra of compound **4**.

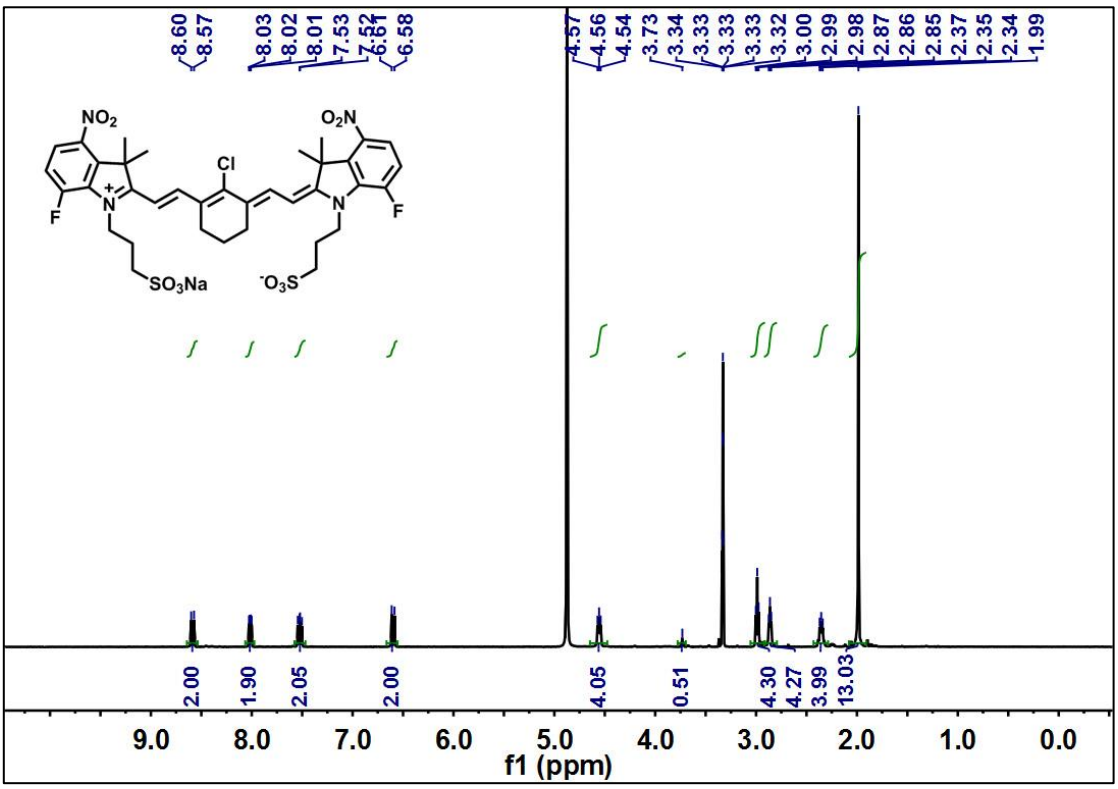


Figure S16. ^{1}H NMR spectra of compound **5** in CD_3OD.

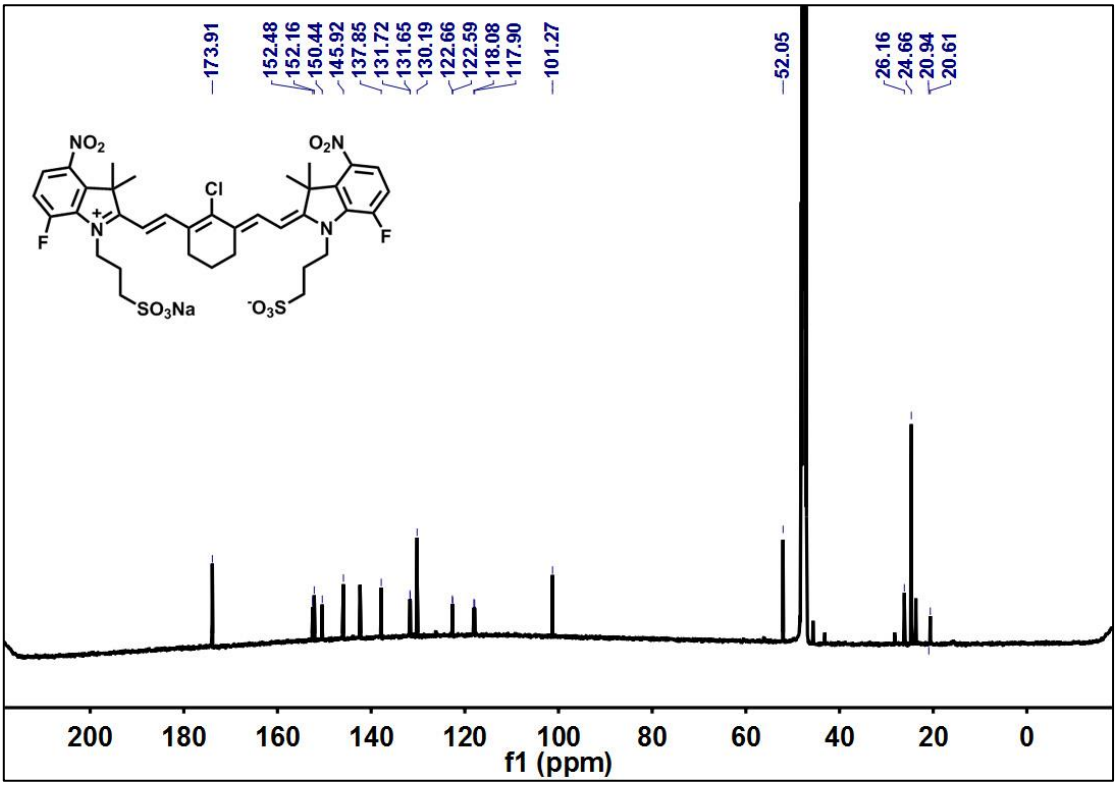


Figure S17. ^{13}C NMR spectra of compound **5** in CD_3OD.

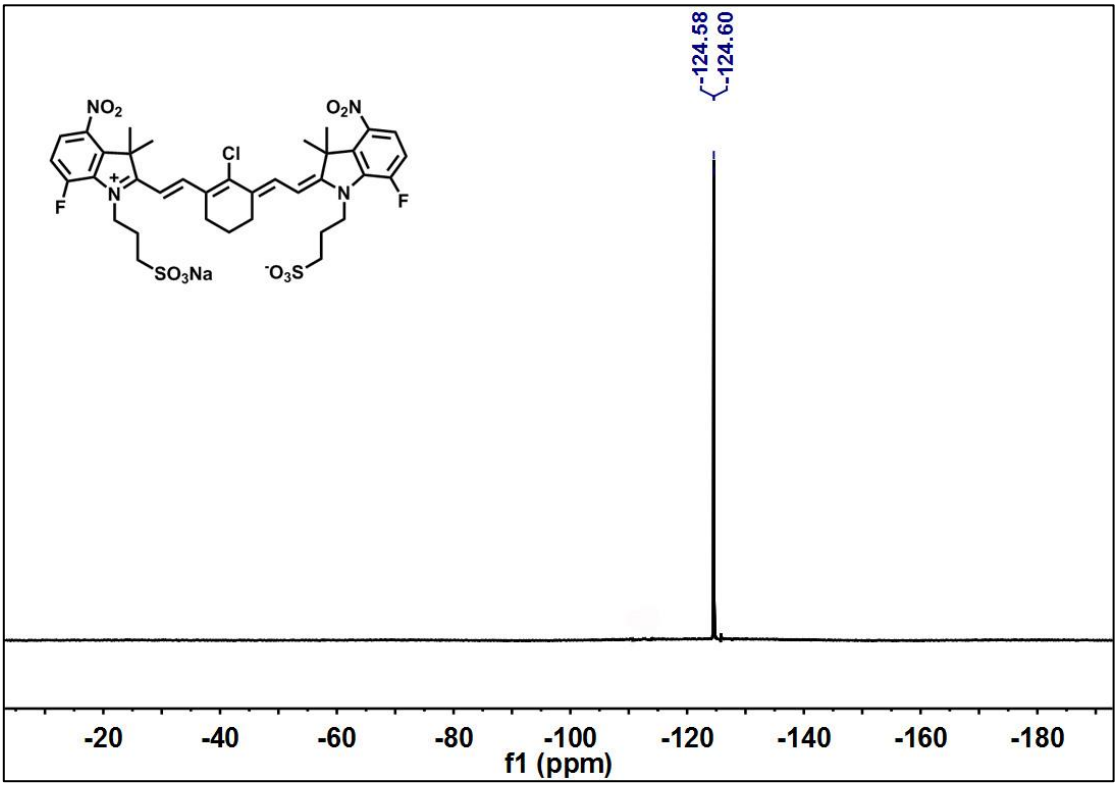


Figure S18. ^{19}F NMR spectra of compound **5** in CD_3OD.

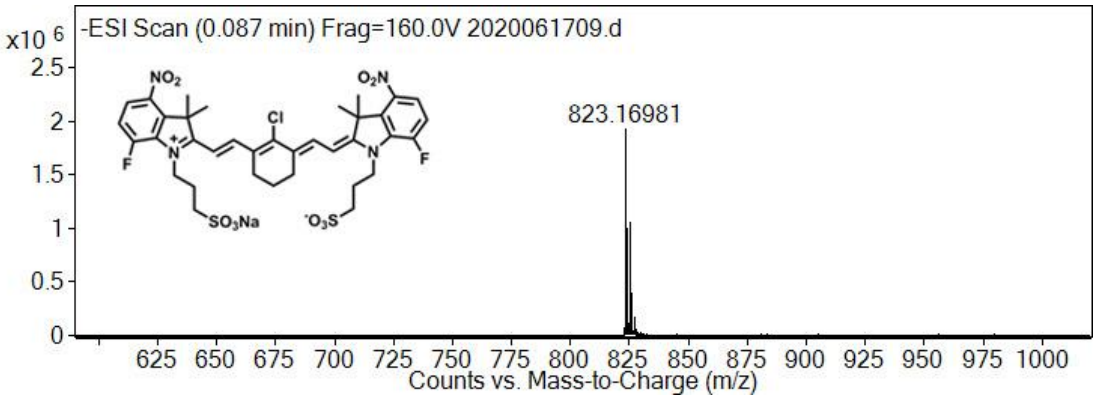


Figure S19. HRMS spectra of compound **5**.

6. Supplementary Figures

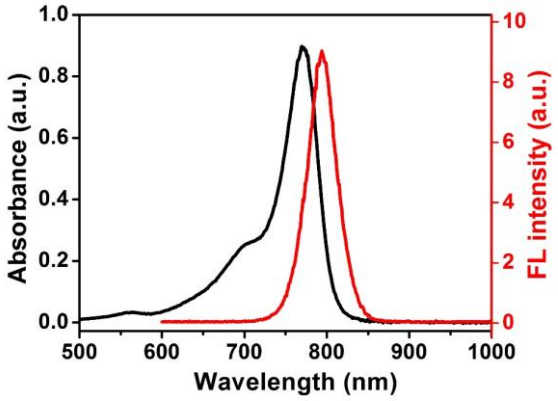


Figure S20. UV-vis absorption spectra (black) and fluorescence emission spectra (red) of FCy-NO_2 (5 μM) in 10 mM PBS, pH=7.4.

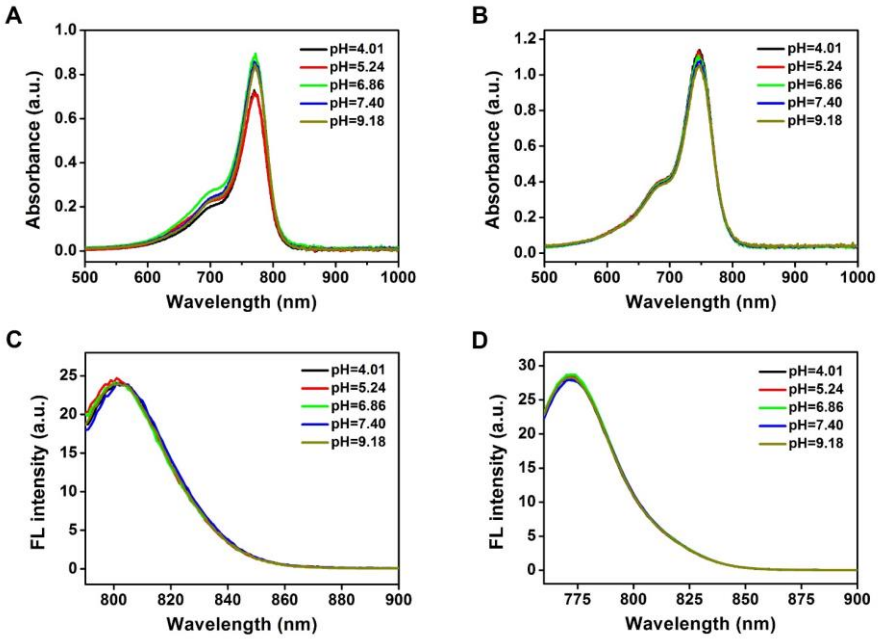


Figure S21. The UV-vis absorption spectra of (A) FCy7-NO_2 and (B) FCy7-NH_2 under different pH value (pH=4.01, 5.24, 6.86, 7.40, and 9.18, respectively). The fluorescence n spectra of (C) FCy7-NO_2 and (D) FCy7-NH_2 under different pH value (pH=4.01, 5.24, 6.86, 7.40, and 9.18, respectively).

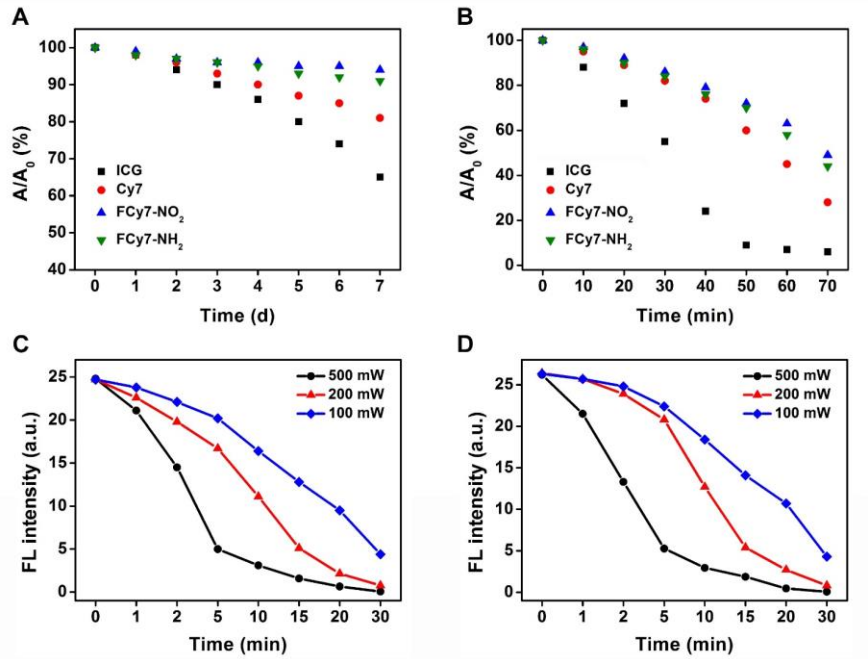


Figure S22. The photostability experiments of FCy7-NO_2 and FCy7-NH_2. The fluorescence emission spectra of ICG (5 μM, monitored at 805 nm, black square), Cy7 (5 μM, monitored at 748 nm, red dot), FCy7-NO_2 (5 μM, monitored at 770 nm, blue triangle), and FCy7-NH_2 (5 μM, monitored at 745 nm, green triangle) in PBS were measured after the sample was exposed to (A) sunlight and (B) ultraviolet light at a specific time. A_0 was the maximum fluorescence intensity at the initial moment and A was the maximum fluorescence intensity to be measured. (C) FCy7-NO_2 (5 μM, irradiated at 770 nm) and (D) FCy7-NH_2 (5 μM, irradiated at 745 nm) fluorescence intensities remained after irradiating a certain period of time at different laser intensities, respectively.

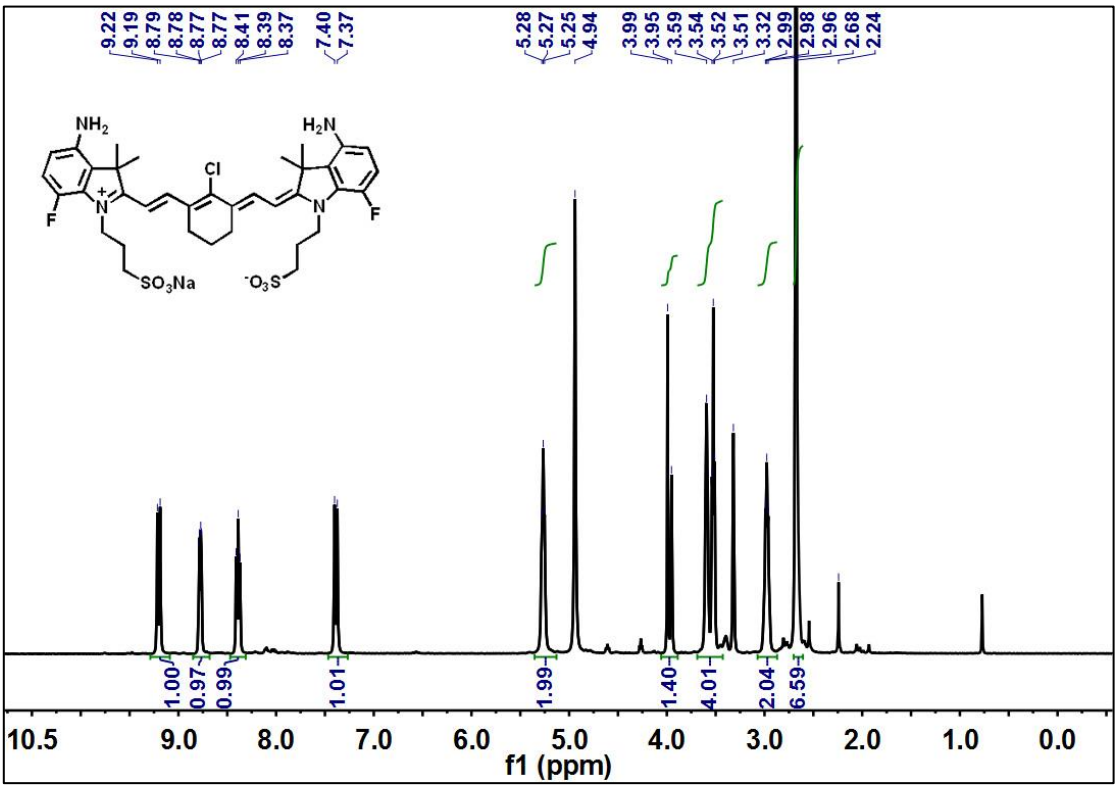


Figure S23. ^{1}H NMR spectra of FCy7-NH_2 in CD_3OD.

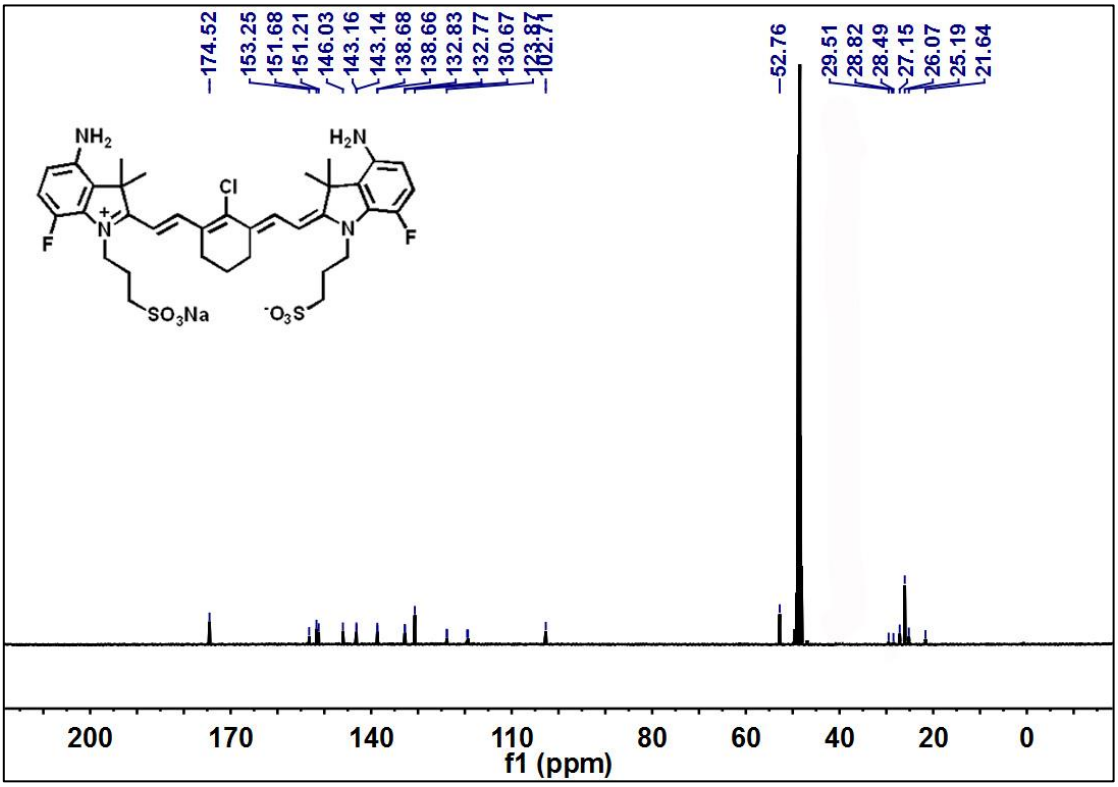


Figure S24. ^{13}C NMR spectra of FCy7-NH_2 in CD_3OD.

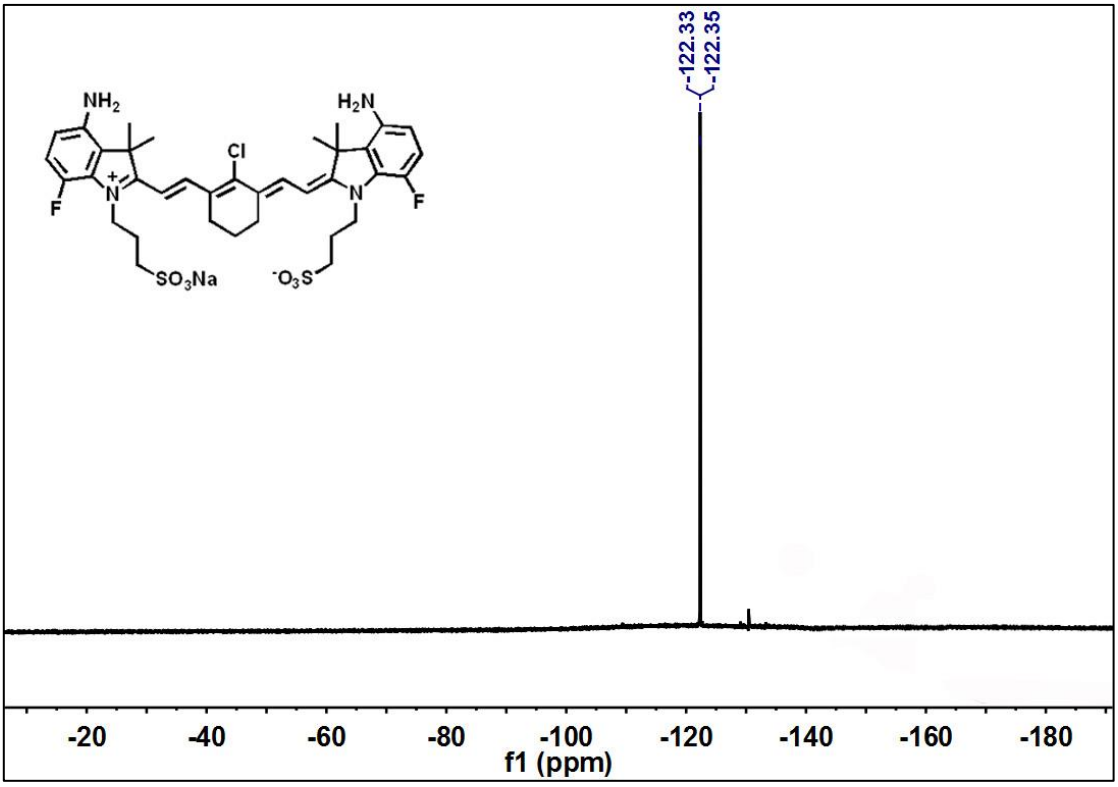


Figure S25. ^{19}F NMR spectra of FCy7-NH_2 in CD_3OD.

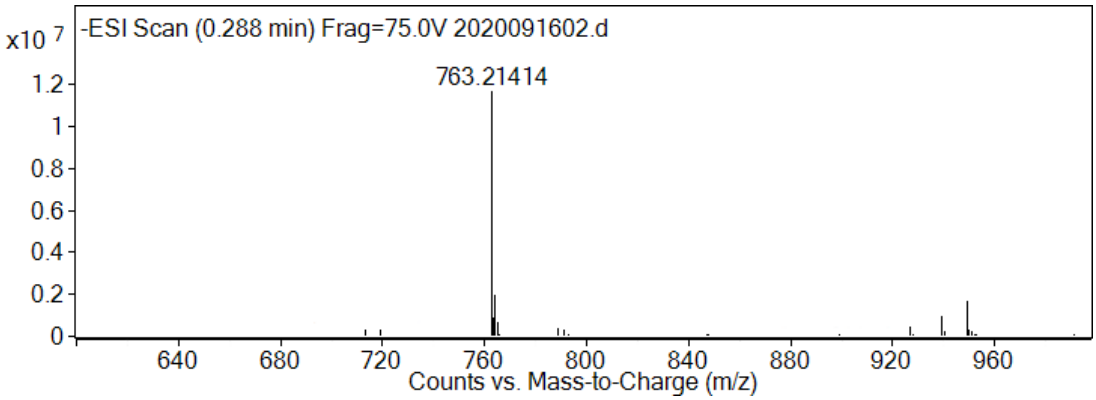


Figure S26. HRMS spectra of target compound FCy7-NH_2, HRMS (ESI) calcd for $C_{36}H_{42}N_4O_6S_2F_2Cl$ $[M]^+$=763.21969, found 763.21414.

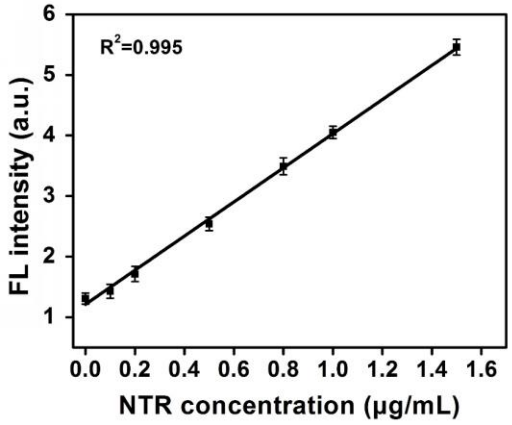


Figure S27. The maximum fluorescence intensity of FCy7-NO_2 (10 μM, PBS) was catalyzed by different NTR concentrations (0-1.5 μg mL^{-1}). The corresponding detection limit based on the calculation of the standard deviation was as low as 16.7 ng mL^{-1}.

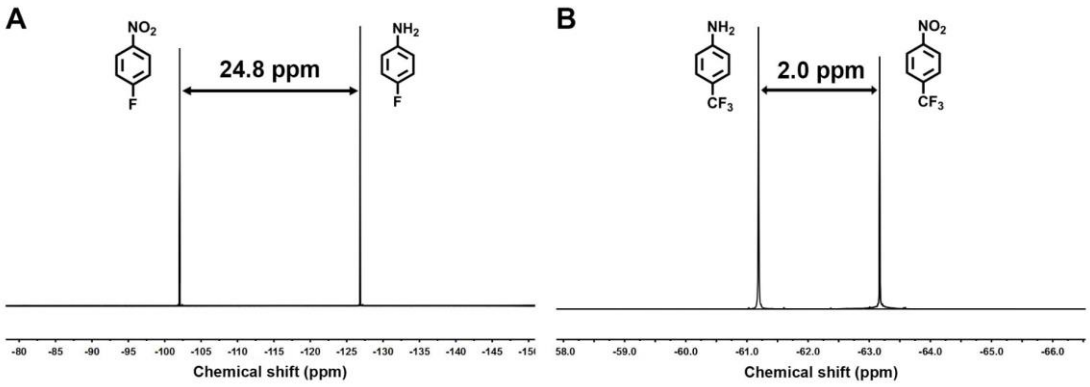


Figure S28. The effect of the length of the carbon chain on the chemical shift of ^{19}F when the nitro group became an amino group. (A) -F was directly attached to the benzene, and (B) -CF_3 was attached to the benzene.

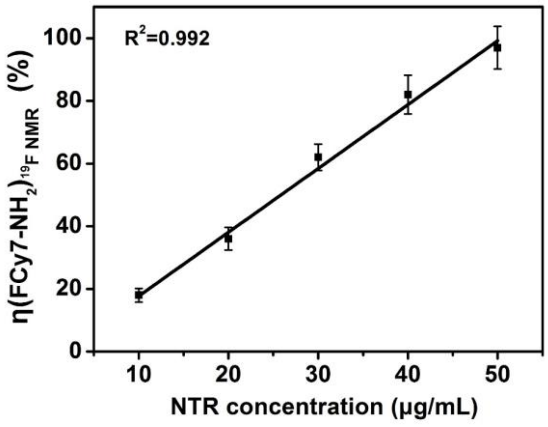


Figure S29. The ratio of ^{19}F signal in FCy7-NH_2 to all ^{19}F signals as a function of NTR concentration. The Y-axil $\eta(FCy7\text{-}NH_2)_{^{19}F\ NMR}$ represented the ratio of the ^{19}F signal of FCy7-NH_2 to the total F signal in FCy7-NH_2 and FCy7-NO_2 that obtained from ^{19}F NMR spectra at 1 hour.

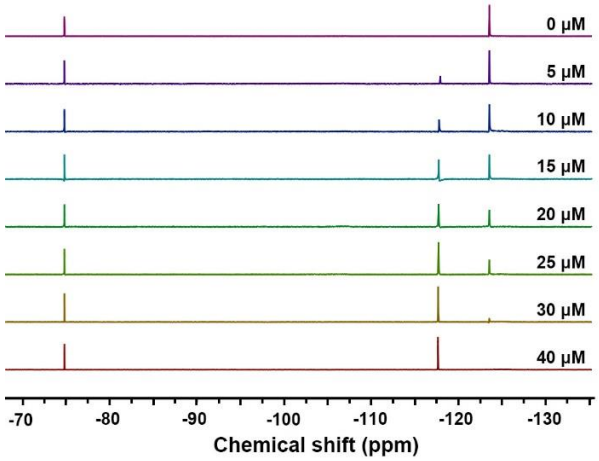


Figure S30. Monitoring the inhibition of NTR enzyme activity with dicoumarin by ^{19}F NMR method. 50 μg mL^{-1} NTR was added to the FCy7-NO_2 solution, and then different concentrations of dicoumarin as indicated were added to the mixture and reacted for 1h. 0.5 mM CF_3COONa was used as a chemical shift reference.

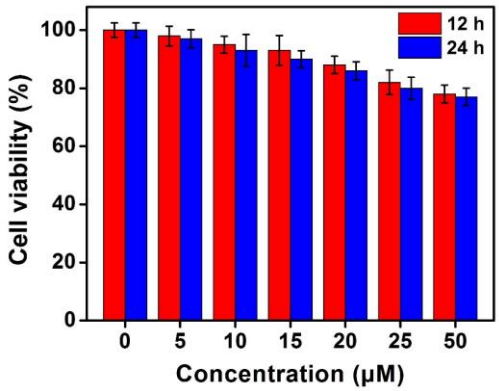


Figure S31. Cell viabilities (%) estimated by MTT proliferation tests versus incubation concentrations of FCy7-NO_2. A549 cells were incubated with 0-50 μM FCy7-NO_2 at 37°C for 12 h (red) and 24 h (blue).

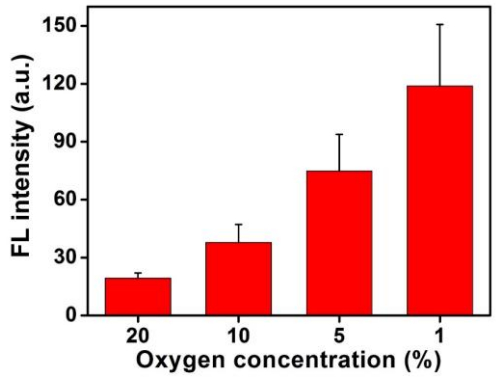


Figure S32. The fluorescence intensity of A549 cells corresponded to different oxygen concentrations. The fluorescence imaging was collected at the near-IR channel (800 ± 30 nm, λ_{ex} = 745 nm CW laser).

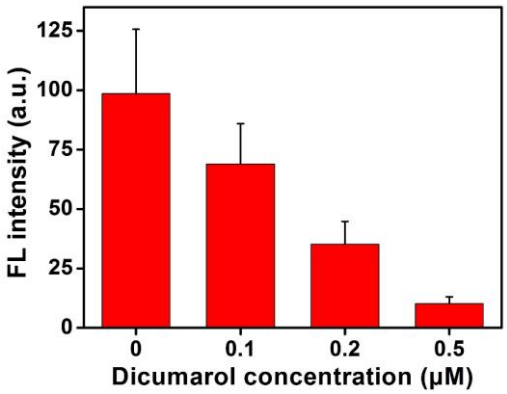


Figure S33. The fluorescence intensity of A549 cells corresponded to different dicumarol concentrations. The fluorescence imaging was collected at the near-IR channel (800 ± 30 nm, λ_{ex} = 745 nm CW laser).

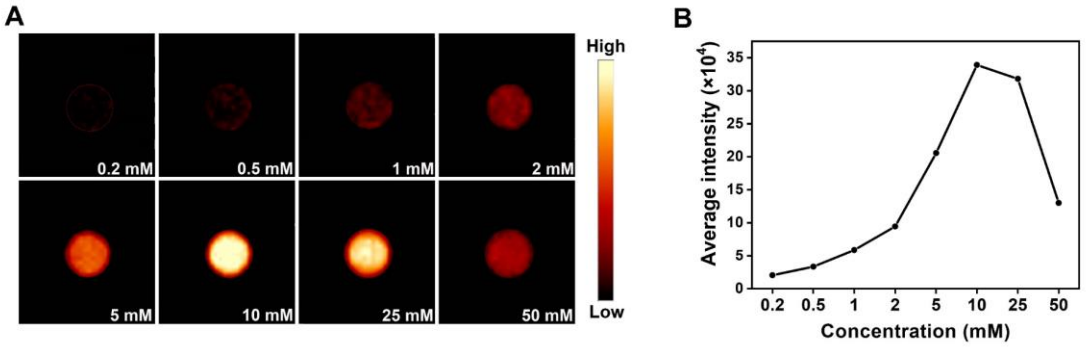


Figure S34. *In vitro* ^{19}F MRI of FCy7-NO_2. (A) ^{19}F MR phantom images of FCy7-NO_2 at different concentrations (0.2, 0.5, 1, 2, 5, 10, 25, 50 mM). (B) Concentration-dependent ^{19}F MRI intensity of FCy7-NO_2.

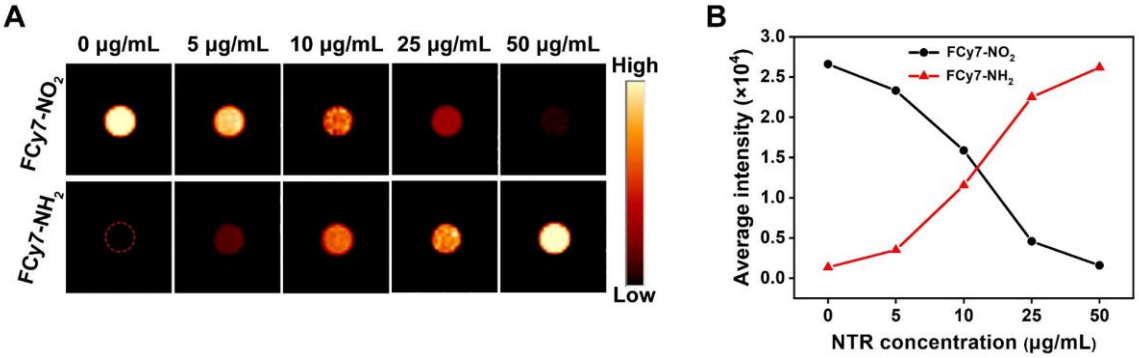


Figure S35. *In vitro* ^{19}F MRI of FCy7-NO_2 incubated with different concentrations NTR. (A) NTR concentration-dependent ^{19}F MR phantom images of FCy7-NO_2 (1 mM) with variable concentrations of NTR (0-50 μg mL^{-1}) and NADH (5 mM) at 37°C in PBS for 1 h. Top, FCy7-NO_2; bottom, FCy7-NH_2. (B) NTR concentration-dependent ^{19}F MRI intensity of FCy7-NO_2 and FCy7-NH_2.

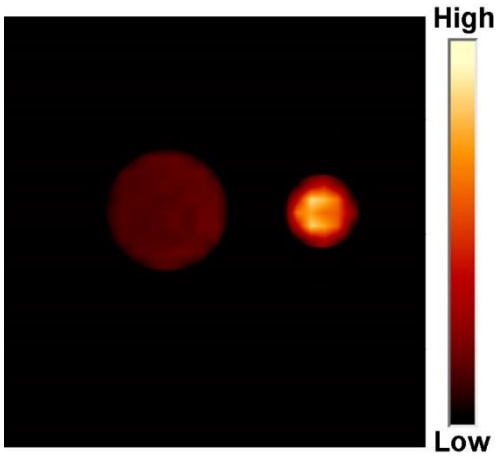


Figure S36. ^{19}F MR phantom images of A549 cells incubated with 0.5 mM FCy7-NO_2 in 1% O_2 concentration for 4 h (left, δ = -123.8 ppm), and 0.1 mM CF_3COONa was used as a reference (right).

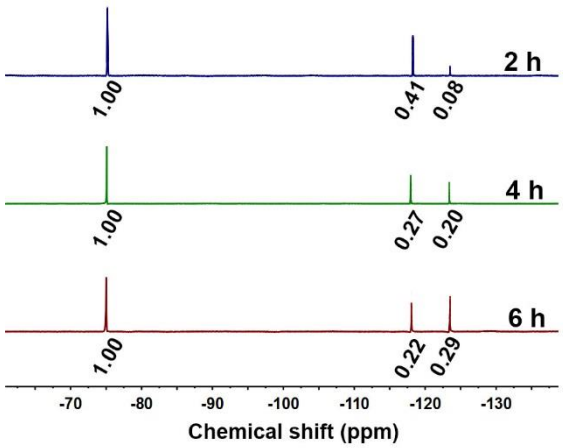


Figure S37. ^{19}F NMR spectra of FCy7-NO_2 incubating with A549 cells in hypoxia (1% oxygen concentration) for different times (2, 4, and 6 h), and 0.1 mM CF_3COONa (-75.3 ppm) was used as a reference, respectively.

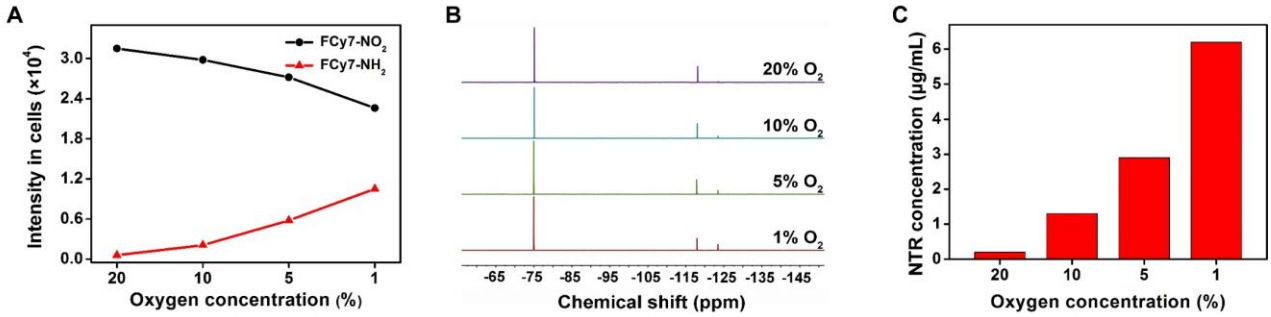


Figure S38. ^{19}F NMR quantitative analysis of NTR concentration in A549 cells. (A) Comparison of two ^{19}F MRI signal intensities after lysis of A549 cells under culture with different oxygen concentrations. (B) ^{19}F NMR signal measurement, CF_3COONa at -75.3 ppm as the internal standard. (C) According to the ^{19}F signal intensity ratio, the NTR concentration in A549 cells under different concentration conditions was obtained.

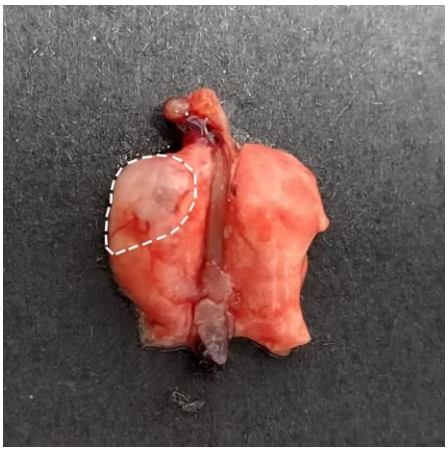


Figure S39. Photograph of the lung without fluorescence.

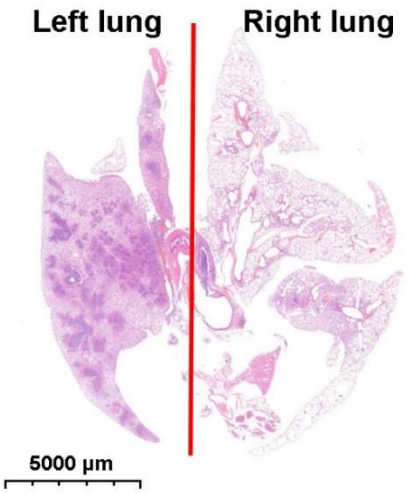


Figure S40. H&E staining of lungs of nude mice with lung cancer in situ. Scale bar = 5000 μm.

7. References

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