Supporting information

Visualizing Hydrogen Sulfide in Mitochondria and Lysosome of Living Cells and in Tumor of Living Mice with Positively Charged Fluorescent Chemosensors

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General measurement

Preparation of the test solution

The stock solution of three probes (**Mito-HS**, **Lyso-HS**, **Con-HS**) were prepared at 1 mM in DMSO. The solution of various testing species were prepared from NaF, NaCl, NaBr, NaAcO, KSCN, Na_2CO_3, $NaHCO_3$, Na_2SO_4, $NaHSO_4$, $MgCl_2$, $CaCl_2$, ZnCl, NaClO, H_2O_2, t-BuOOH, GSH, Cys, Hcy and NaHS in distilled water. O_2^- was generated from KO_2. Single Oxygen($^1O_2$) was generated from ClO^- and H_2O_2.

Absorption Analysis

Absorption spectra were obtained with 1.0-cm Quartz cells. Both **Mito-HS** and **Lyso-HS** (DMSO, 10 μL, 1.0 mM) were added to a 1.0-mL color comparison tube. After dilution to 10 μM with 20 mM PBS buffer, then 50 eq NaHS was added. The mixture was equilibrated for 60 min followed by absorbance measurements.

Fluorescence Analysis

Unless otherwise noted, for all measurements, the excitation wavelength was 435 nm, the excitation slit widths were 5 nm, and emission slit widths were 10 nm. In kinetic studies, **Mito-HS**, **Lyso-HS,** and **Con-HS** (DMSO, 10 μL, 1.0 mM) were added to PBS buffer at a concentration of 10 μM, then NaHS (250 μM) was added. The rates of fluorescence enhancement in reaction solutions were immediately recorded by fluorimeter. The apparent rate constant k′ for the reaction of three probes with NaHS solution (250 μM) was determined by fitting the fluorescence intensities to the pseudo-first-order equation[1]:

$$\mathrm{Ln}\,[(F_{max} - F_t)/F_{max}] = -k't \qquad (1)$$

Where F_t and F_{max} are the fluorescence intensities at 540 nm at a time t and the maximum value obtained after the reaction was complete.

In sensitivity studies, **Mito-HS** and **Lyso-HS** were added to PBS buffer at the concentration of 10 μM, then various concentration of NaHS (0~100 μM) were added and incubated for 60 min followed by fluorescence measurement. In selectivity studies, various testing species were added in a similar way.

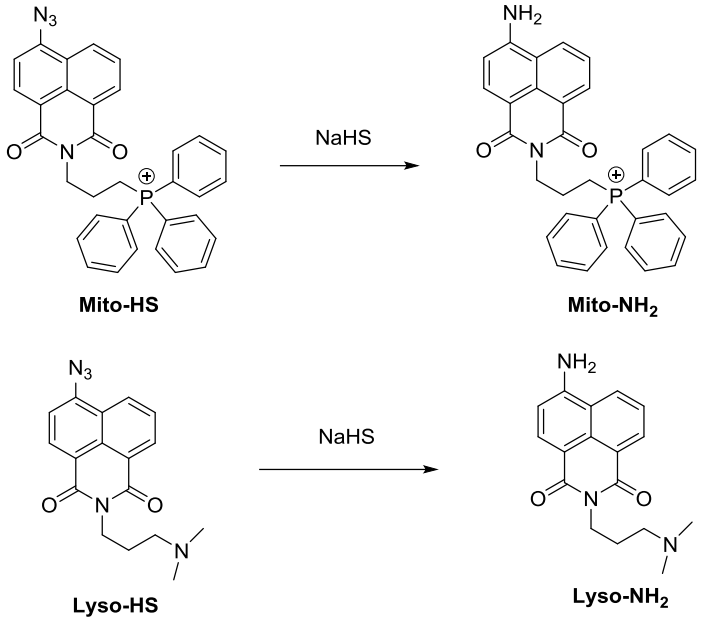
Fluorescence quantum yields

Fluorescence quantum yields were determined in pH 7.4 PBS buffer (20 mM) using fluorescein (0.1M NaOH, Φ = 0.95) as a standard according to a published method using the following equation:

$$\Phi = \Phi_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2} \qquad (2)$$

Where A_R and A are the absorbance of reference and sample solutions at the same excitation wavelength. I and IR are the corresponding integrated fluorescence intensity. And ŋ and $ŋ_R$ are the

solvent refractive indexes of samples and reference, respectively. Quantum yields: **Mito-HS**: Φ= 0.0021; **Mito-NH$_2$**: Φ = 0.12; **Lyso-HS**: Φ = 0.0022; **Lyso-NH$_2$**: Φ = 0.1.



Scheme S1 Structures of probes (**Mito-HS**, **Lyso-HS**) and their products upon reaction with NaHS.

Cytotoxicity assays

HeLa cells were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 at 37 ℃. Immediately before the experiments, the cells were grown in a 96-well plate, followed by addition of various concentrations of **Mito-HS** or **Lyso-HS** (0~100 μM). The cells were then incubated at 37 ℃ in an atmosphere of 5% CO_2 for 24 h, followed by SRB assays.

Imaging of H_2S in HeLa cells

HeLa cells were seeded on glass coverslip for confocal imaging in DMEM supplemented with 10% fetal bovine serum (FBS), and 1% antibiotics (penicillin/streptomycin, 100 U/ml) in an atmosphere of 5% CO_2 at 37 ℃. To start the experiment, HeLa cells were preloaded with or without PMA (1 μg/mL) for 30 minutes, and then **Mito-HS** or **Lyso-HS** at a concentration of 10 μM were added and further incubation for 30 min. The cells were washed three times and PMA-preloaded cells were treated with or without NaHS solutions (200 μM) for 60 min. After final washing, the cells were then subjected to imaging using confocal laser-scanning microscope with an objective lens (60×). LysoTracker Red and MitoTracker Red were used for staining lysosome and mitochondria, respectively. Emission was collected at 500~550 nm (excited at 488 nm) for green channel. LysoTracker Red and MitoTracker Red were collected at 570~620 nm (excited at 561 nm).

In vivo imaging

For in vivo imaging, BALB/C nude mice were selected and inoculated with breast tumor cells. After that, breast tumor (region A) and non-cancerous tissue (region B) were both given a skip-pop injection with probe **Mtio-HS** (50 μM, 150 μL, aqueous solution). Images were taken after incubation for 2 h by using a CRI Maestro small animal *in vivo* imaging system, with excitation of blue filter (435 nm~480 nm) and an emission filter of 500~720 nm.

Detection of hydrogen sulphide using a sulfide-specific electrode

BALB/C nude mice were selected and inoculated with breast tumor cells. After that, breast tumor tissue and non-cancerous tissue were cut down in the same mass and made to homogenate followed by centrifugation. The supernatant was analyzed by a sulfide-specific electrode.

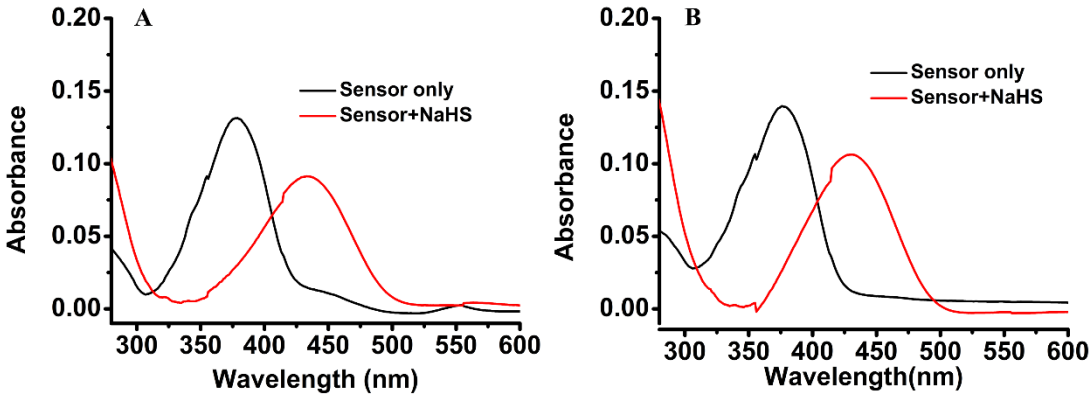


Figure S1. Absorbance changes of probes (10 μM) in the presence of NaHS (50 eq), (A): **Mito-HS**; (B): **Lyso-HS**.

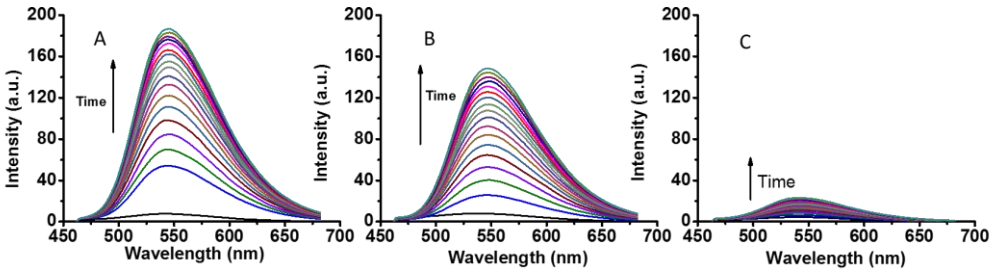


Figure S2. Time-dependent fluorescence spectral changes of probes (10 μM) in the presence of NaHS (25 eq), (A): **Mito-HS**; (B): **Lyso-HS**; (C)**: Con-HS**, the experiments were performed in degassed PBS buffer (20 mM, pH 7.4), λ_{ex} = 435 nm, slit width (ex/em): 5 nm/10 nm.

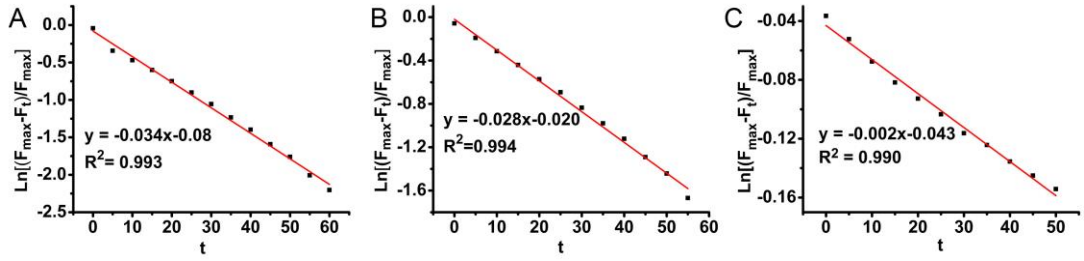


Figure S3. Pseudo-first-order kinetic rate constants: **A**: **Mito-HS**: 0.034min^{-1}; **B**: **Lyso-HS**: 0.028 min^{-1}; **C**: **Con-HS:** 0.002min^{-1}

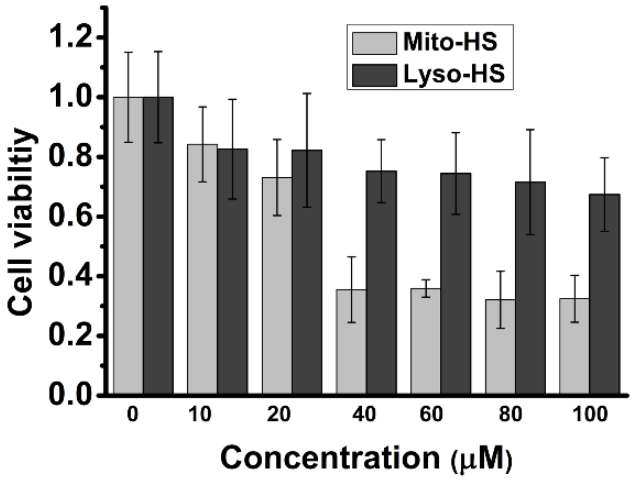


Figure S4. SRB assay of HeLa cells cultured for 24 h in media containing various concentration of **Mito-HS** and **Lyso-HS** (0~100 μM).

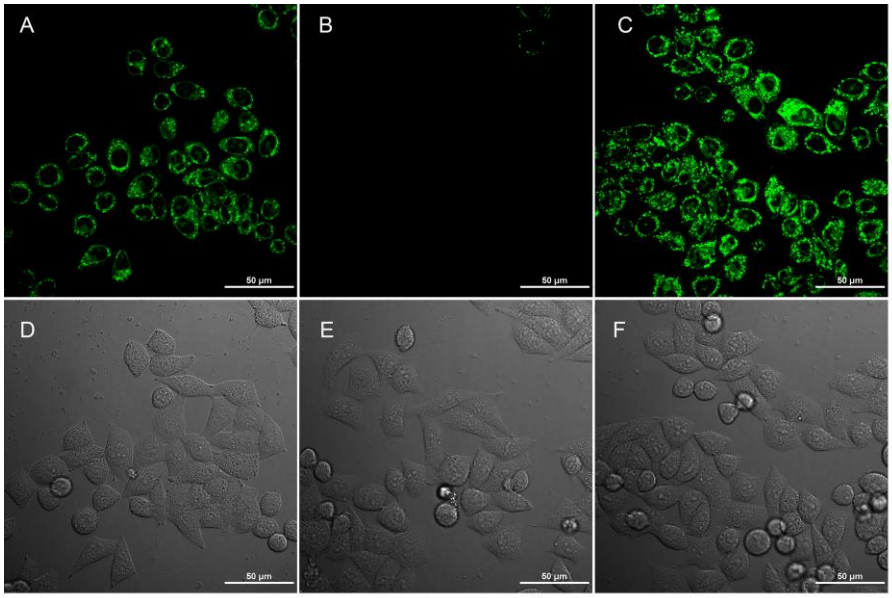


Figure S5. Fluorescence images of H_2S in HeLa cells using probe **Mito-HS**. (A, D): Cells were incubated with 10 μM probe only; (B, E): Cells were incubated with PMA (1 μg/mL) for 30 min, and then treated with **Mito-HS** for another 30 min; (C, F): Cells were pre-stimulated by PMA (1 μg/ ml) for 30 min, then were treated with with **Mito-HS** (10 μM, 30 min), followed by incubation with NaHS (200 μM, 60 min). Scale bar: 50 μm.

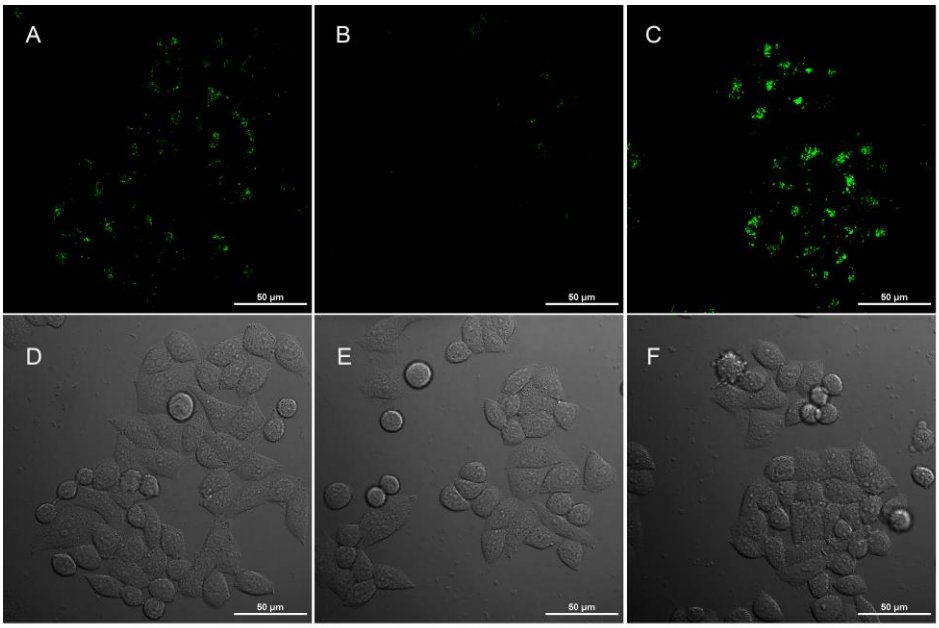


Figure S6. Fluorescence images of H_2S in HeLa cells using probe **Lyso-HS**. (A, D): Cells were incubated with 10 μM probe only; (B, E): Cells were incubated with PMA (1 μg/mL) for 30 min, and then treated with **Lyso-HS** for another 30 min; (C, F): Cells were pre-stimulated by PMA (1 μg/ ml) for 30 min, then were treated with **Lyso-HS** (10 μM, 30 min), followed by incubation with NaHS (200 μM, 60 min). Scale bar: 50 μm.

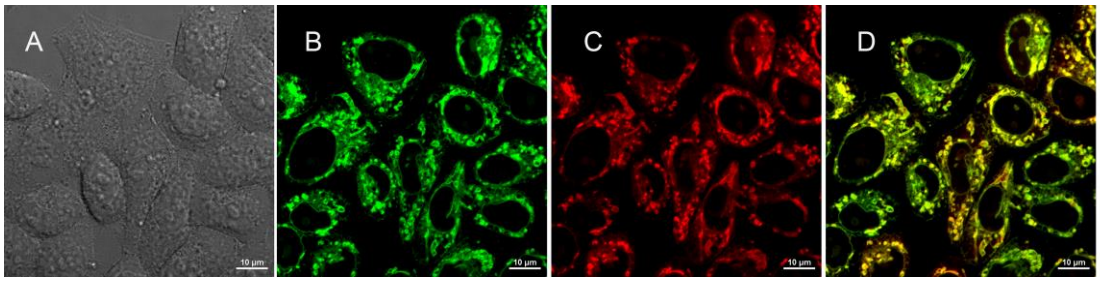


Figure S7. Probe **Mito-HS** colocalizes to mitochondria in HeLa cells. (A) Bright-field images; (B) **Mito-HS**, λ_{ex} = 488 nm, λ_{em} = 500−550 nm; (C): MitoTracker Red, λ_{ex} = 561 nm, λ_{em} = 570−620 nm; (D) overlay of A， B， and C, Scale bar: 10 μm.

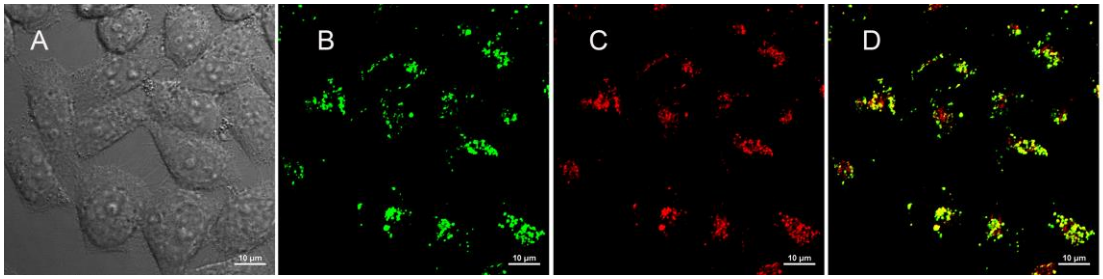


Figure S8. Probe **Lyso-HS** colocalized to lysosome in HeLa cells. (A): Bright field; (B) **Lyso-HS**, λ_{ex} = 488 nm, λ_{em} = 500−550 nm); (C): LysoTracker Red, λ_{ex} = 561 nm, λ_{em} = 570−620 nm); (D): Overlay of A, B and C. Scale bar: 10 μm.

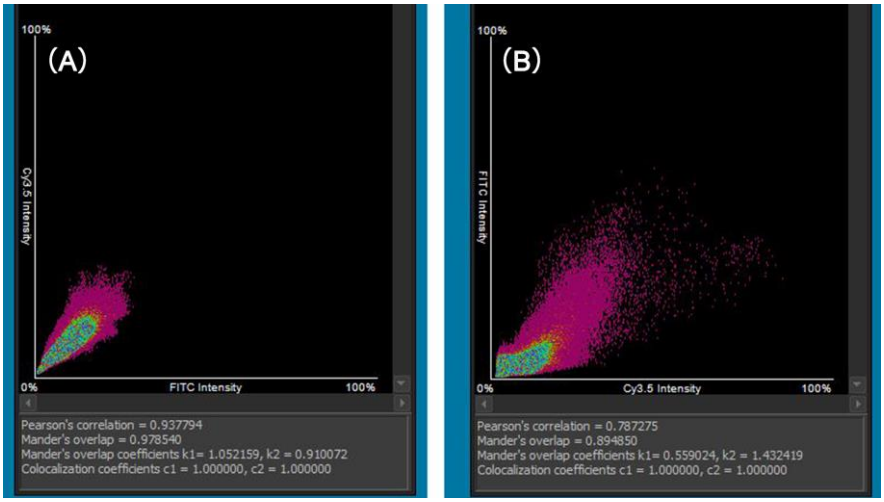


Figure S9. (A) Intensity scatter plot of **Mito-HS** and Mitotracker Red. X axis represent **Mito-HS** intensity and Y axis represent MitoTracker intensity; (B) Intensity scatter plot of **Lyso-HS** and Lysotracker Red, X axis represent LysoTracker intensity and Y axis represent **Lyso-HS**.

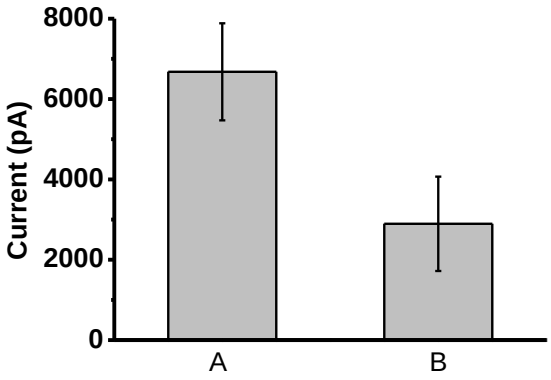


Figure S10 Electronic current of tumor tissue (A) and no-cancerous tissue (B) with a sulfide specific electrode.

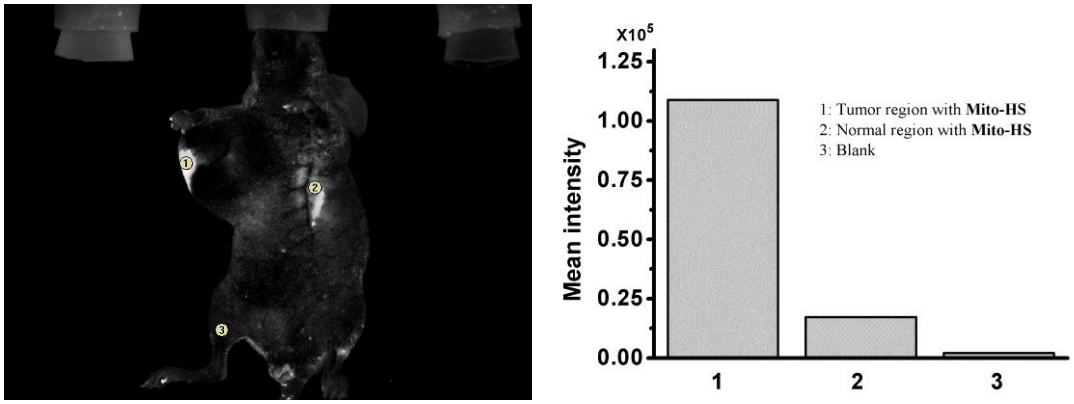
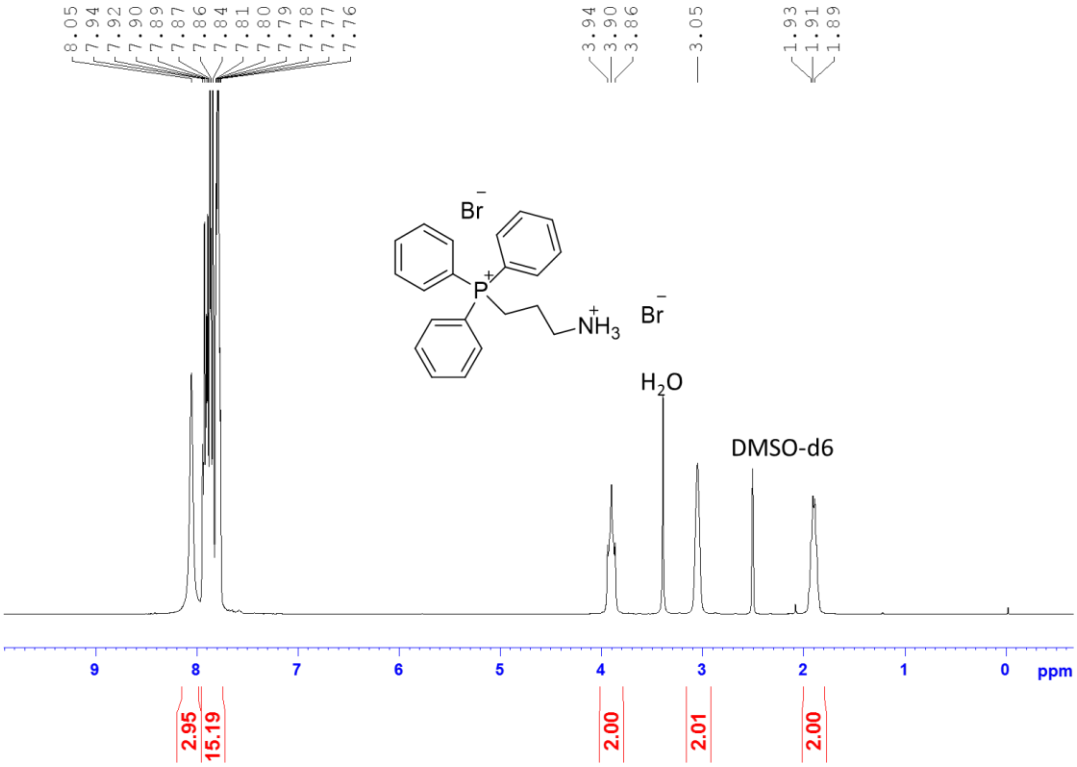
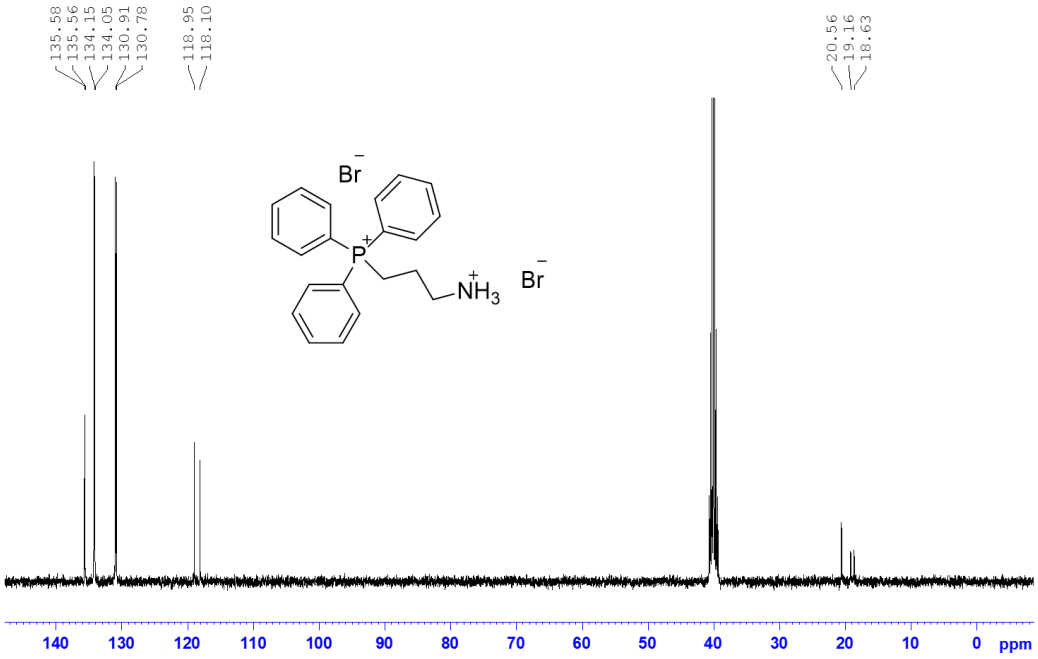


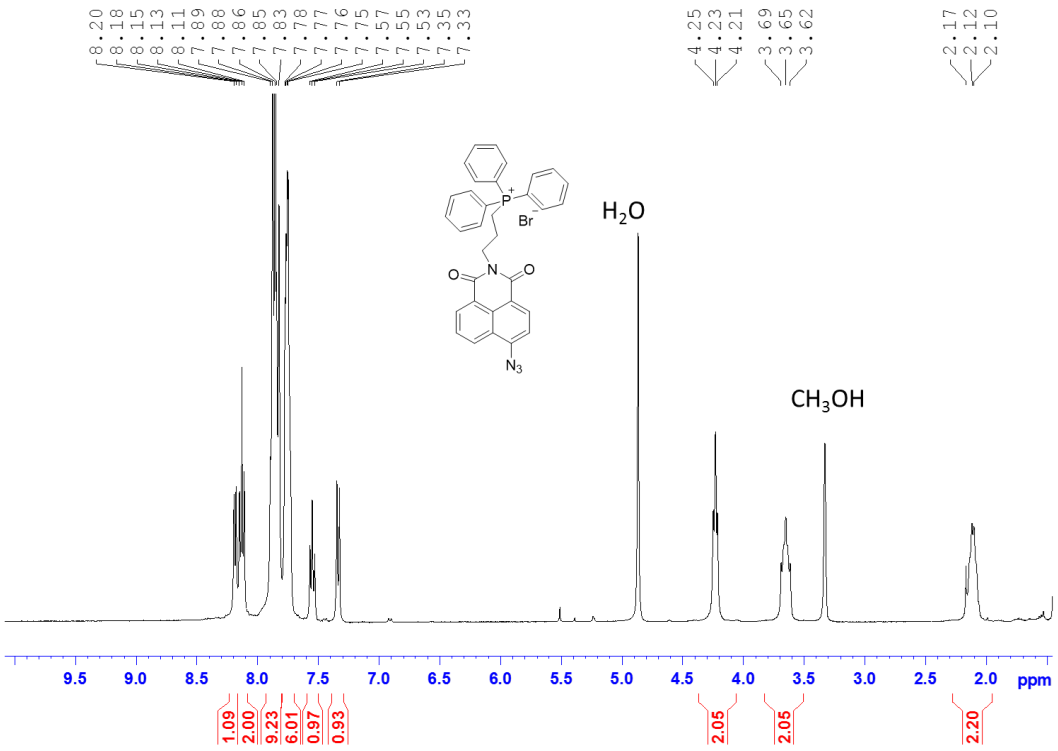
Figure S11 The signal to noise ratio of different region. The probe fluorescence intensity in injected tumor area (1) is 51.75 times as much as that of blank area (3), and the probe fluorescence intensity in injected normal area (2) is 8.20 times as much as that of blank (3).

1. Yang, S.; Qi, Y.; Liu, C.; Wang, Y.; Zhao, Y.; Wang, L.; Li, J.; Tan, W.; Yang, R. *Anal. Chem.* **2014**, *86*, 7508-7515.

NMR and MS of synthetic intermediates and probes



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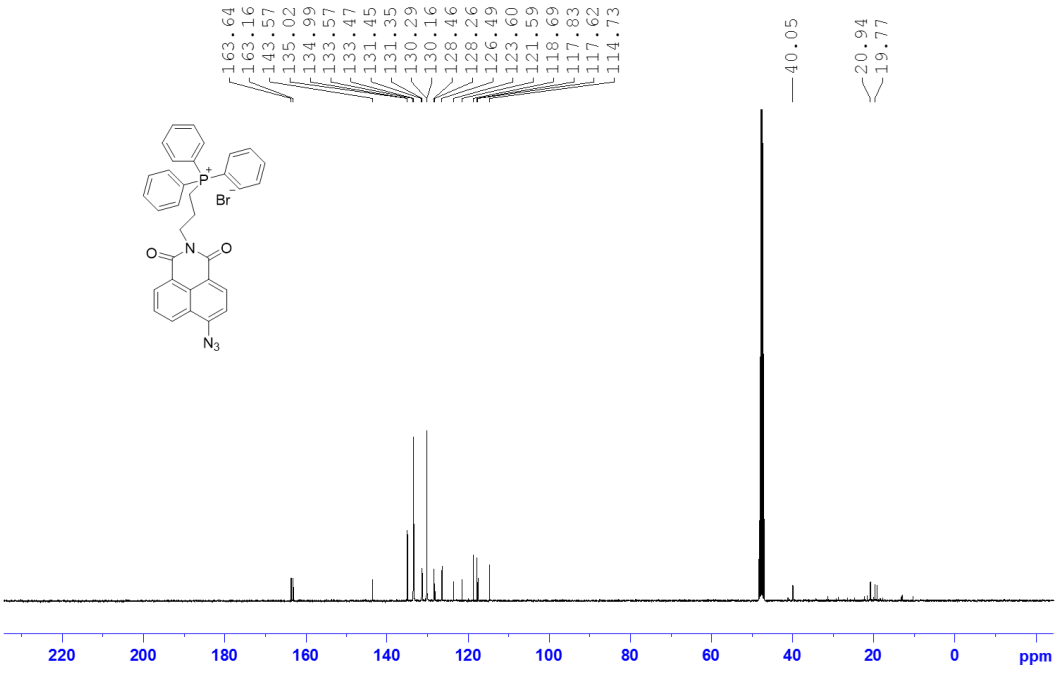
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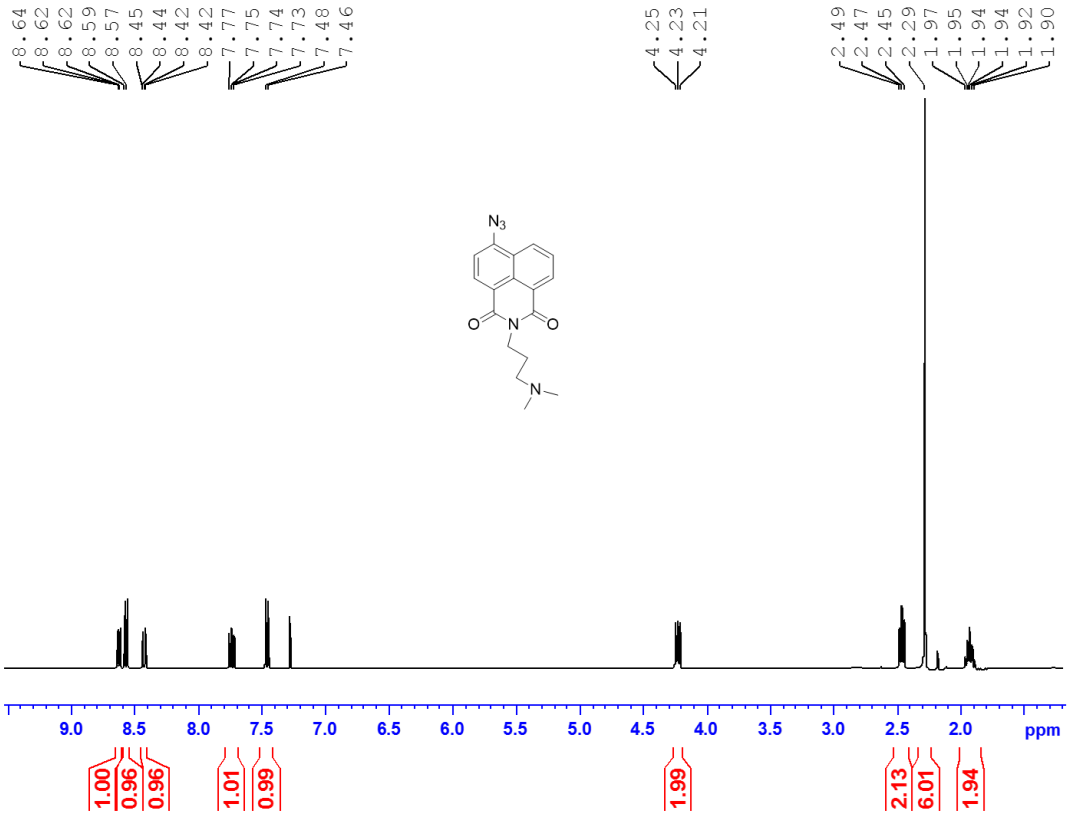
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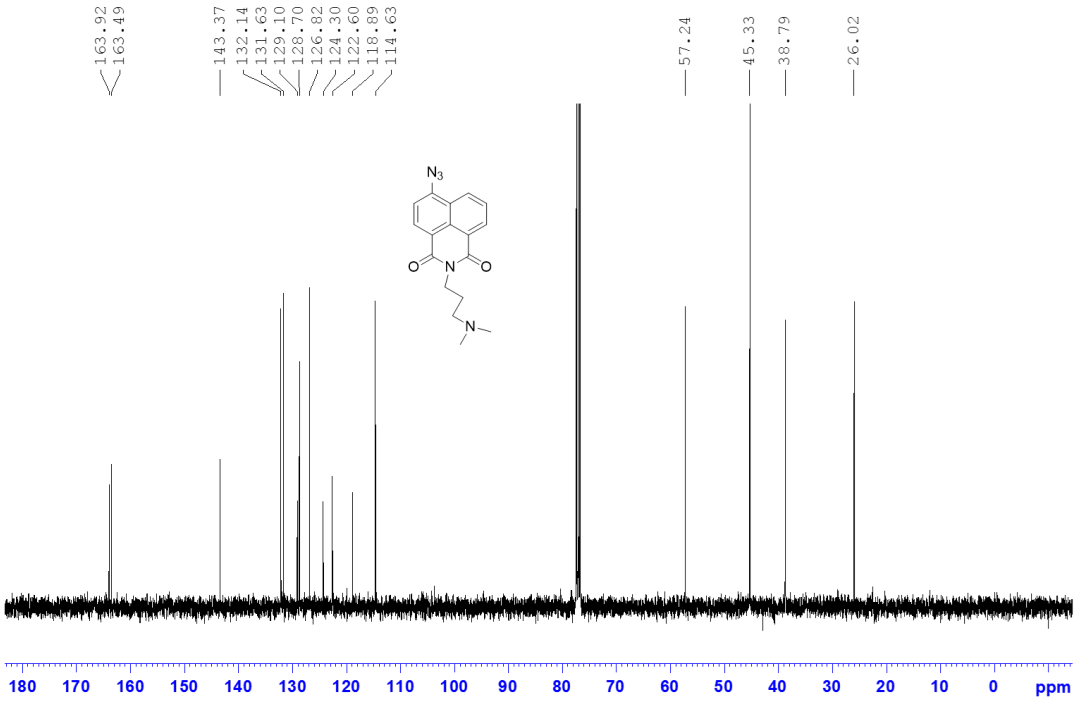
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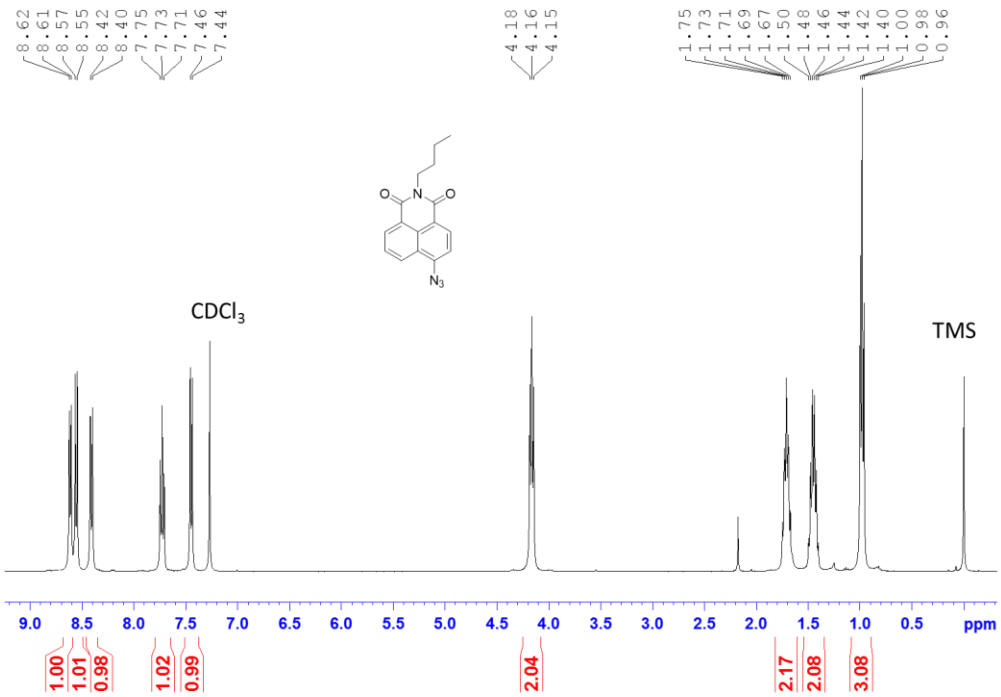
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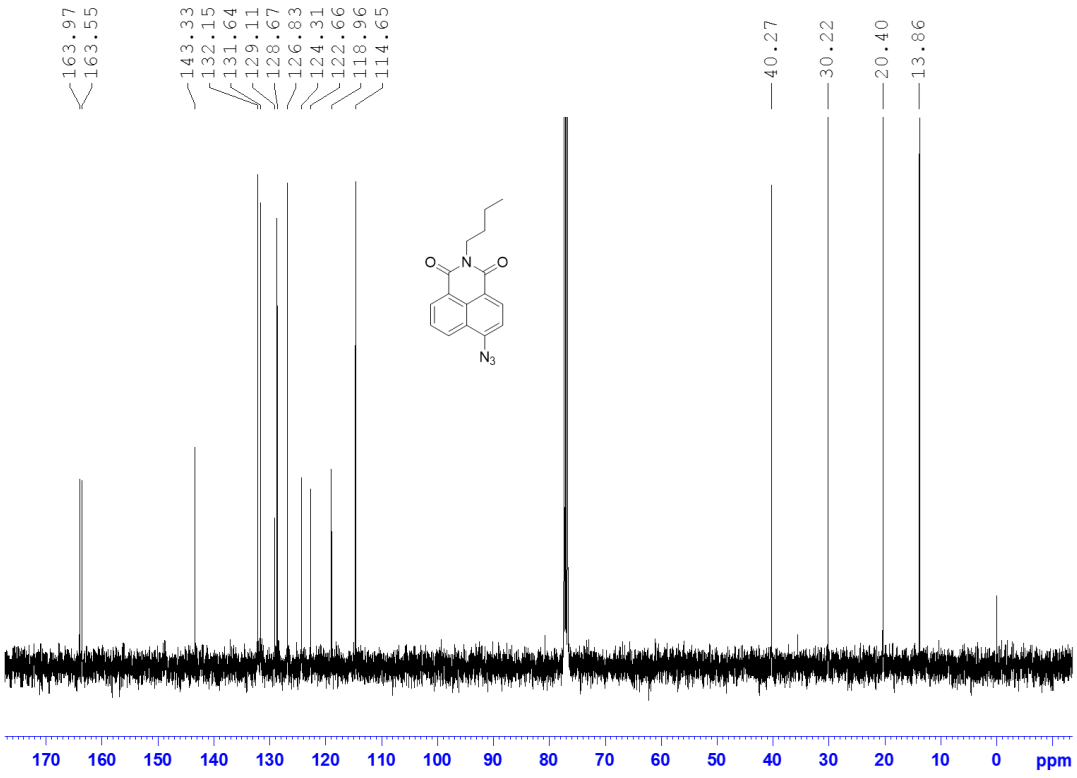
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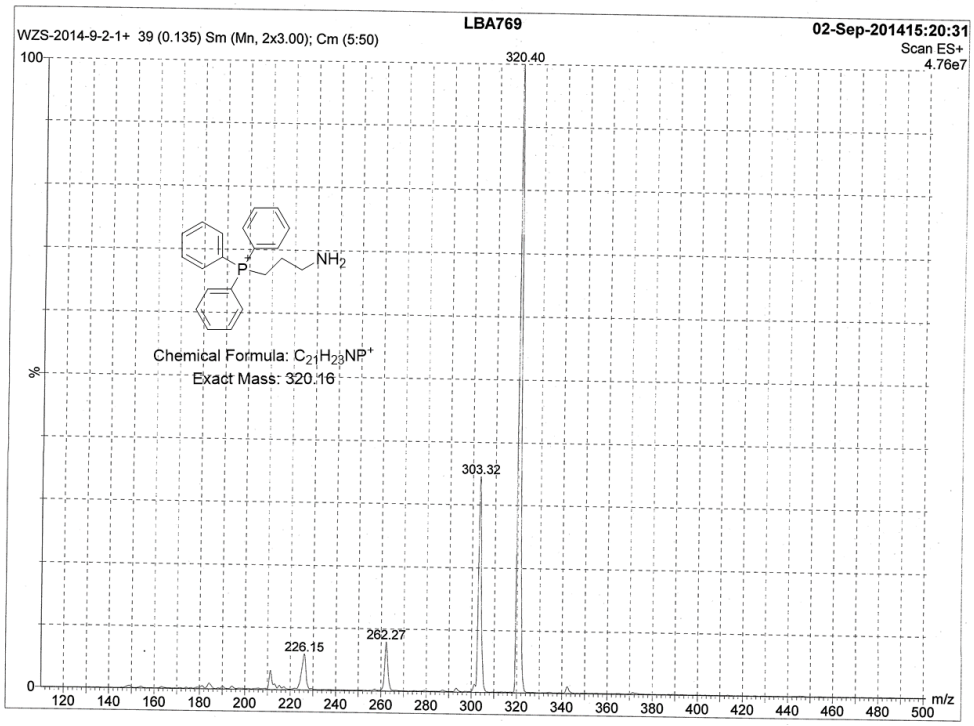
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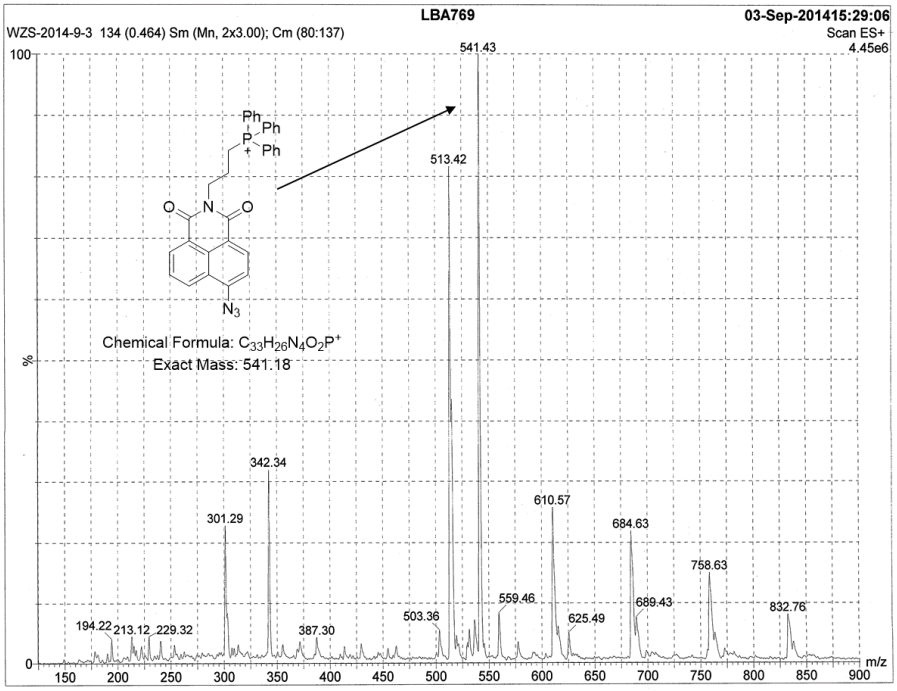
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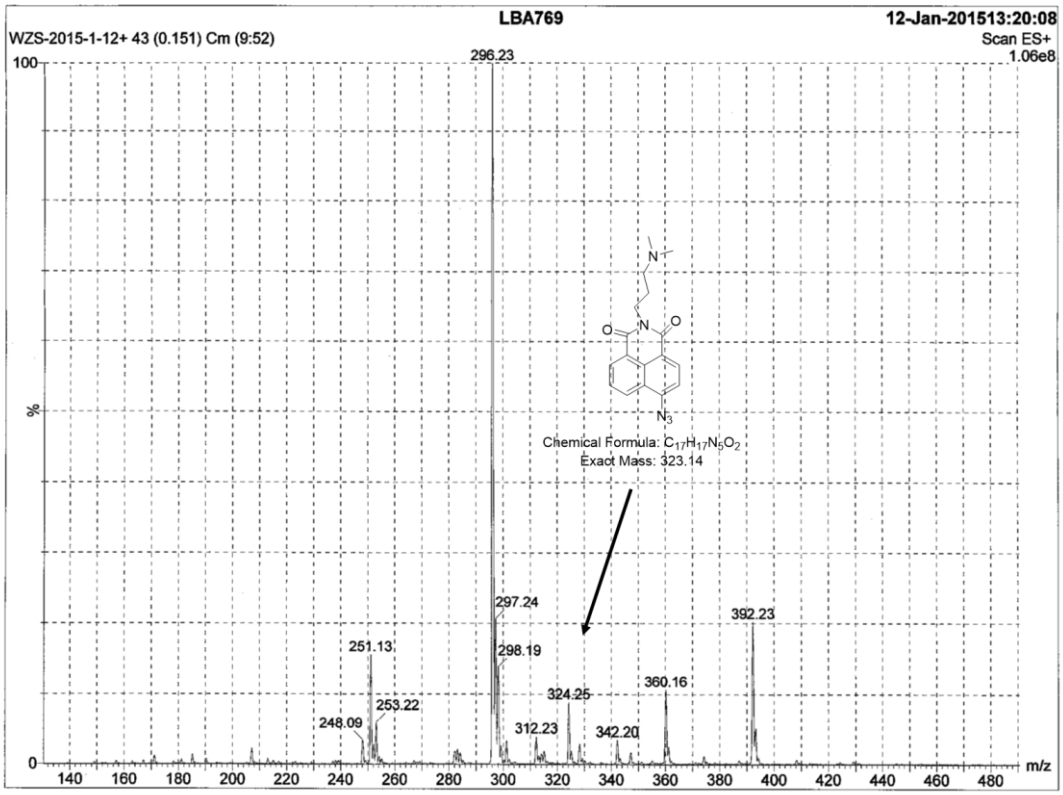
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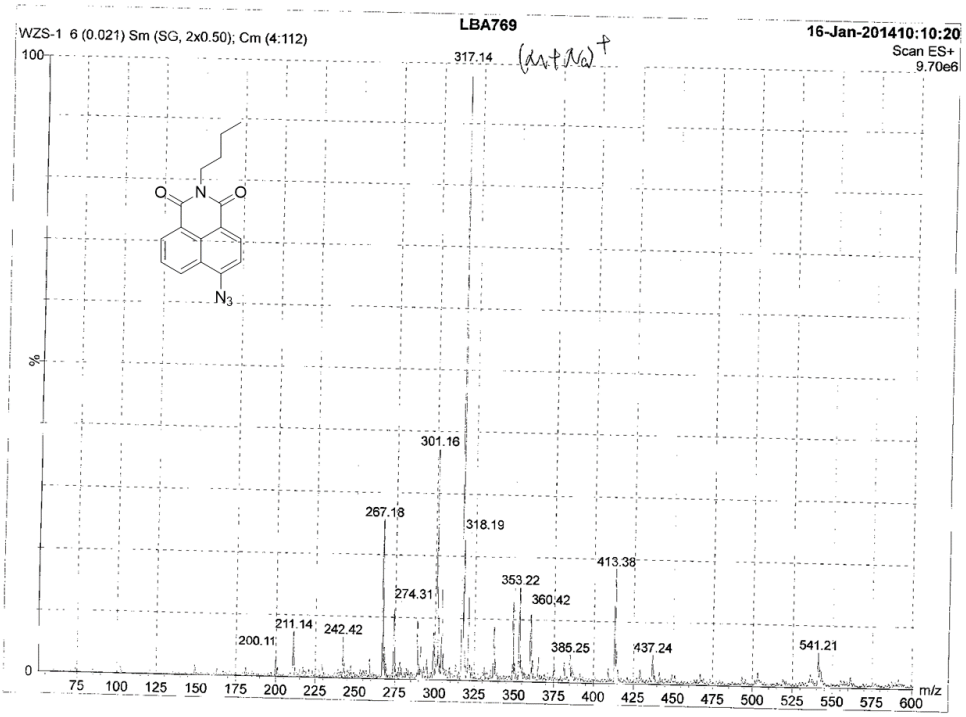
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