Supporting information:

Visualizing Fluoride ion in Mitochondria and Lysosome of Living Cells and in Living Mice with Positively Charged Ratiometric Probes

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ABSTRACT:

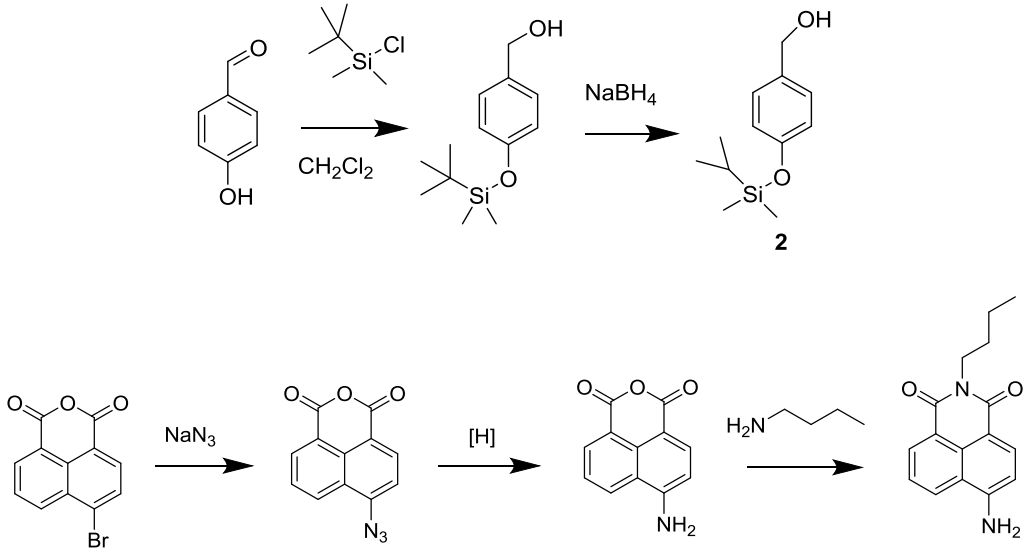
Two ratiometric probes fluoride ion, **Mito-F** and **Lyso-F**, were rationally designed and synthesized with positive charges at physiological conditions. The positive charges functioned as target moieties for subcellular mitochondria and lysosome of living cells, and effective sequesters of fluoride ion for fast and efficient fluorescent detection. In addition, in vivo imaging of fluoride ion in living mice was successfully achieved for the first time using probe **Mito-F**.

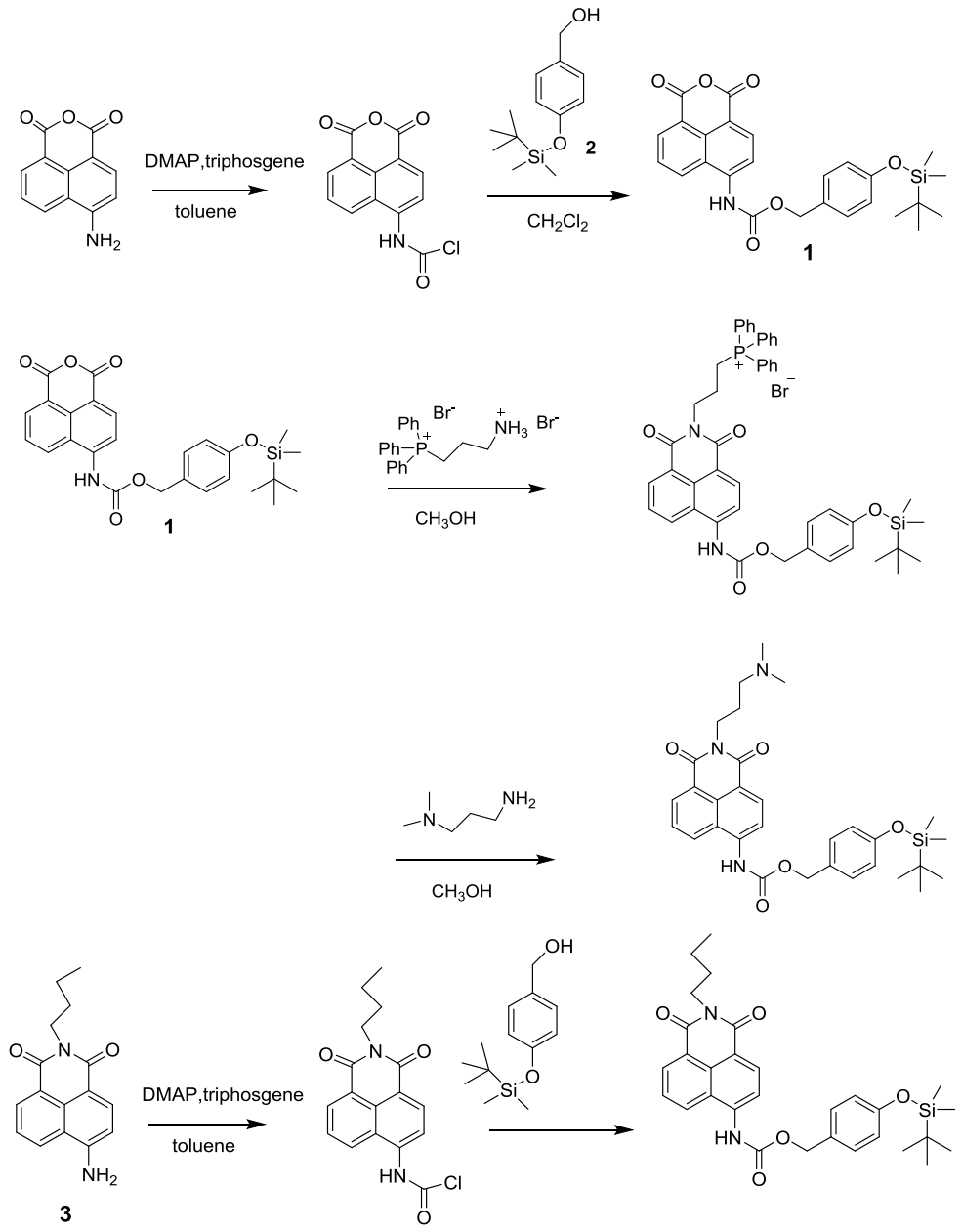
Materials and Apparatus

All chemicals were obtained from commercial suppliers and were used without further purification. The probe stock solutions were prepared in DMSO. The solutions of various testing species were prepared from NaCl, NaBr, NaI, Na_2CO_3, $NaHCO_3$, NaN_3, $NaNO_2$, AcONa, NaH_2PO_4, NaF, and TBAF at 0.1 M in distilled water. HEPES buffers were prepared using distilled water. NMR spectra were measured on Bruker instrument, operating at 400 M for ^1H NMR and 100 M for ^{13}C NMR with chemical shift was reported as ppm. Electrospray mass spectra (ESI-MS) were acquired on a Waters SQD. UV-Visible spectra were obtained on a DU800 ultraviolet spectrometer and fluorescence measurement was performed on a Cary Eclipse fluorimeter. Cell imaging was performed with a Nikon A1R confocal microscope. In vivo imaging was performed with a Carestream Health FX PRO small animal in vivo imaging system with a 430 nm excitation laser and a 535 nm emission filter.

1. Synthesis of three probes

4-amino-1,8-naphthalic anhydride was prepared from 4-bromo-1,8-naphthalic anhydride according to the literature methods[1]. N-butyl-4-amino-1, 8-napthalmide (**3**), (3-aminopropyl) triphenylphosphonium bromide and [4-(tert-butyldimethylsilyloxy) phenyl] methanol (**2**) were synthesized according to the literature methods with modifications.[2-4] Synthesis of the probes is described as Scheme S1.





Scheme S1. Synthetic methods of probe **Mito-F**, **Lyso-F** and **Con-F**.

Synthesis of compound 1:

To a mixture of 4-amino-1, 8-naphthalic anhydride (213 mg, 1 mmol) and dimethylaminopyridine (DMAP) (244 mg, 2 mmol) in toluene was added a toluene solution of triphosgene (593 mg, 2 mmol) dropwise in ice bath for 2 h. Then the resulting solution was heated to reflux for 5 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 and filtered. To the filtrate was added compound **2** and the reaction solution was stirred at room temperature for another 3 h. The solution was then concentrated, and the residue was washed with methanol

(MeOH) several times and dried to give a yellow solid (300 mg, 63% yield). ^{1}H NMR ($CDCl_3$, 400 MHZ), δ/ppm: 8.64 (t, J = 8.02 Hz, 2H), 8.54 (d, J = 8.20 Hz,1H), 8.30 (d, J = 8.48 Hz,1H), 7.84 (t, J = 7.82 Hz, 1H), 7.64 (s,1H), 7.34 (d, J = 8.04Hz, 2H), 6.87 (d, J = 8.08 Hz, 2H), 5.26 (s, 2H), 1.01(s, 9H), 0.23 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): 160.83, 160.06, 156.35, 152.78, 140.55, 135.02, 133.39, 131.21, 130.52, 127.71, 127.25, 127.00, 122.64, 120.32, 119.65, 116.46, 113.10, 68.16, 25.65, 18.22, -4.39. (ESI-MS): m/z, calculated for $(M-H)^-$: 476.15, found 476.42.

*Synthesis of probe **Mito-F**:*

(3-aminopropyl) triphenylphosphonium bromide (240 mg, 0.5 mmol) and compound **1** (200 mg, 0.4 mmol) was dissolved in MeOH, and 3 mL triethyl amine was then added. The solution was heated to reflux for 5 h. After cooling to temperature, the solvent was removed under reduced pressure. The solid residue was purified by silica gel flash column chromatograph using EtOAC-CH_3OH (v/v, from 4:1 to 1:1) as eluent to obtain probe **Mito-F** (176 mg, 51% yield). ^{1}H NMR (CD_3OD, 400 MHZ), δ/ppm: 8.17 (d, J = 7.88 Hz, 2H), 7.97 (q, J = 9.50 Hz, 2H), 7.85~7.72 (m, 16H), 7.48 (t, J = 7.88 Hz, 1H), 7.42 (d, J = 8.16 Hz, 2H), 6.89 (d, J = 8.16 Hz, 2H), 5.18 (s, 2H), 4.21 (t, J = 6.68 Hz, 2H) , 3.66~3.59 (m, 2H), 2.12~2.10 (m, 2H, 1.00 (s, 9H), 0.21 (s, 6H); ^{13}C NMR (CD_3OD, 100 MHz): 164.00, 163.40, 155.88, 154.13, 140.73, 135.00, 134.98, 133.55, 133.45, 133.36, 133.26, 131.56, 130.72, 130.27, 130.14, 130.06, 129.69, 129.15, 128.17, 125.90, 123.02, 121.77, 119.87, 118.67, 117.81, 116.53, 116.17, 114.92, 66.91, 39.99, 24.80, 20.92, 19.29, 17.71, -5.62. (ESI-MS)： m/z, calculated for $(M)^+$: 779.31, found 779.80.

*Synthesis of probe **Lyso-F**:*

A mixture of compound **1** (200 mg, 0.4 mmol) and N, N-dimethylpropane-1,3-diamine (202 mg，2 mmol) in MeOH was heated to reflux for 5 h. After cooling to room temperature, the solvent was removed. The solid residue was purified by silica gel flash column chromatography using EtOAC-CH_3OH (v/v, from 6:1 to 1:1) as eluent to obtain a yellow solid (160 mg, 71% yield.) ^{1}H NMR (400 MHz,CD_3OD), δ/ppm: 8.28 (d, J = 6 .64Hz, 1H), 8.21 (d, J = 8.48 Hz, 1H), 8.16 (d, J = 8.32 Hz, 1H), 8.03 (d, J = 8.28 Hz, 1H), 7.52 (t, J = 7.92 Hz, 1H), 7.41 (d, J= 8.48 Hz, 2H) ,6.87 (d, J = 8.48 Hz, 2H), 5.21 (s, 2H), 4.06 (t, J = 7.28 Hz, 2H), 2.63 (t, J = 7.64Hz, 2H), 2.43 (s, 6H), 1.98~1.90 (m, 2H), 0.99 (s, 9H), 0.20 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ/ppm 163.98,

163.44, 155.83, 154.19, 140.63, 131.58, 130.67, 130.00, 129.18, 128.21, 128.02, 125.87, 123.17, 126.82, 121.93, 119.83, 116.80, 116.41, 66.88, 56.45, 43.62, 37.73, 24.98, 24.79, 17.69, -5.63. ESI-MS: calculated for $(M + H)^+$: 562.27, found 562.55.

*Synthesis of probe **Con-F**:*

To a mixture of N-butyl-4-amino-1,8-napthalmide (**3**) (268 mg, 1 mmol) and dimethylaminopyridine (DMAP) (244 mg, 2 mmol) in toluene was added a toluene solution of triphosgene (593 mg, 2 mmol) dropwise in ice bath for 2 h. After cooling to room temperature, the solvent was removed. To the filtrate was added compound **2** and the reaction solution was stirred at room temperature for another 3 h. The solution was then concentrated, followed by purification by flash column chromatography using petroleum ether-EtOAc (v/v, 2:1) as eluent to obtain a yellow solid (213 mg, 40 % yield.) ^{1}H NMR (400 MHz, $CDCl_3$), δ/ppm: 8.65~8.60 (m, 2H), 8.42 (d, J = 8.24 Hz, 1H), 8.17 (d, J = 8.00 Hz, 1H), 7.78～7.74 (m, 1H), 7.49 (s, 1H), 7.41 (d, J= 8.48 Hz, 2H) ,6.88 (d, J = 8.48 Hz, 2H), 5.25 (s, 2H), 4.20 (t, J = 7.52 Hz, 2H), 1.77~1.70 (m, 2H), 1.51~1.42 (m, 2H), 1.01 (s, 9H) , 0.99 (t , J = 7.40 Hz, 3H) ,0.23 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ/ppm 164.12, 163.63, 156.24, 153.13, 138.95, 132.46, 131.17, 130.4, 128.89, 128.01, 126.53, 125.85, 123.46, 122.86, 120.28, 117.84, 116.70, 67.84, 40.22, 30.21, 25.65, 20.39, 18.21, 13.84, -4.40. ESI-MS: calculated for $(M -H)^-$: 531.23, found 531.46.

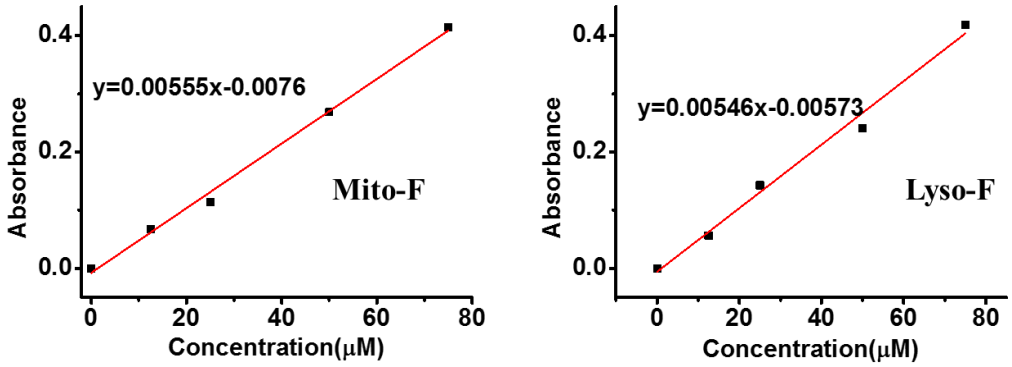
2. Study of water solubility of Mito-F and Lyso-F

Different concentrations of probe solutions (1.25 mM~7.5 mM) were prepared in CH_3OH, then 10 μL of probe solutions were added to 990 μL HEPES buffer (pH=7.4). Both **Mito-F** and **Lyso-F** can be dissolved in HEPES buffer at the low concentrations (12.5~75 μM). standard curves were obtained according to the absorbance of 376 nm (**Mito-F**) and 378 nm (**Lyso-F**), and the linear relationships indicated the probes were fully soluble at these concentrations. We then prepared the saturated solution of probes in HEPES buffer until the precipitated probes were observed. The solutions were centrifuged and the saturated supernatants were collected and further diluted with HEPES buffer followed by measurement of absorbance. The concentrations of two probe saturated solutions were calculated according to the standard curves. The experiments were performed at room temperature.

Mito-F : λ_{max}=376 nm, ε=5550 L.mol^{-1}.cm^{-1}, $C_{\text{Mito-F}}$ = 823.8 μM;

Lyso-F: λ_{max}=378 nm, ε=5460 L.mol^{-1}.cm^{-1}, $C_{\text{Lyso-F}}$ = 414.7 μM.

C represents the concentration of saturated solutions in HEPES buffer.



Standard working curves of **Mito-F** and **Lyso-F** between the concentrations of 0~75 μM.

3. Spectrophotometric Measurements.

Both the fluorescence and UV−vis absorption measurements were conducted in 20 mM HEPES buffer solution (pH = 7.4) containing 50% DMSO (for comparison to **Con-F**, which is not soluble in HEPES buffer). All the fluorescence measurements were recorded by excitation at 405 nm and scanned from 410 nm to 700 nm at 1200 nm/min. In kinetic studies, **Mito-F**, **Lyso-F**, and **Con-F** were respectively added to HEPES buffer (pH 7.4, 50% DMSO, v/v) at a concentration of 10 μM, then 10 mM NaF was added. The rates of fluorescence enhancement in reaction solutions were immediately recorded by fluorimeter. Then fluorescence ratio (F_{540}/F_{475}) change against time was acquired and the apparent rate constant k′ for the reaction of probes (10 μM) with NaF (10 mM) was determined by fitting the data of fluorescence change at 475 nm. In sensitivity studies, **Mito-F** and **Lyso-F** were added to HEPES buffer at a final concentration of 10 μM, then various concentration of NaF (0~11.4 ppm, 0~0.6 mM) were added and incubated for 60 min, followed by fluorescence measurement. In selectivity assays, the testing species (NaCl, NaBr, NaI, Na_2CO_3, $NaHCO_3$, NaN_3, $NaNO_2$, AcONa, NaH_2PO_4, NaF, and TBAF) at a concentration of 1 mM were added to probe solutions (10 μM). Then the resulting solutions were shaken well and incubated for 60 min, followed by fluorescence measurement.

*Absorbance spectra of **Mito-F** and **Lyso-F** solution upon the addition of NaF*

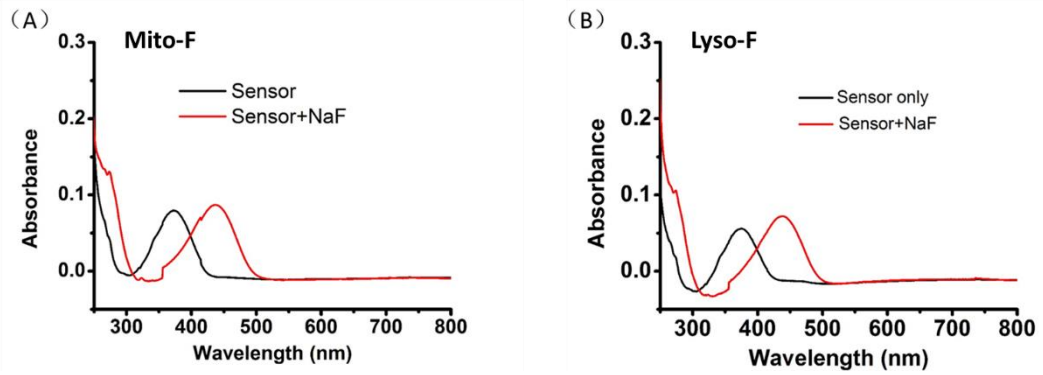


Figure S1. Absorbance spectra of 10 μM **Mito-F** (A) and **Lyso-F** (B) in the absence and presence of NaF (10 mM).

Kinetic assays

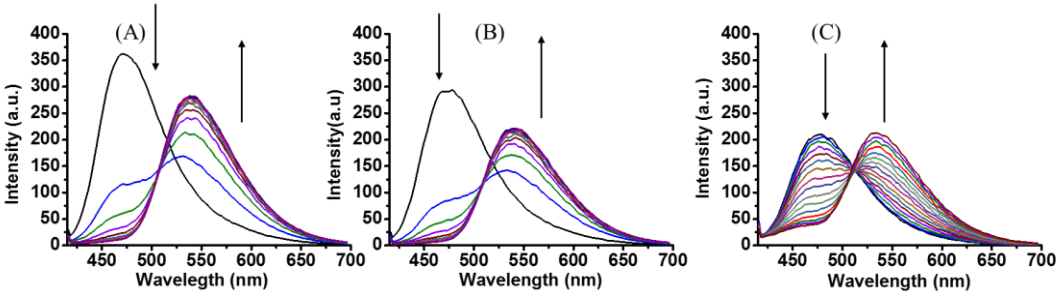


Figure S2. Time-dependent fluorescence spectral changes of probes (10 μM) in the presence of NaF (10 mM). (A) **Mito-F;** (B) **Lyso-F**; (C) **Con-F**. All experiments were performed in HEPES buffer (20 mM, pH 7.4, 50 % DMSO). Data were acquired from 0 to 30 min. λ_{ex} = 405 nm, slit width (ex/em): 5 nm/10 nm.

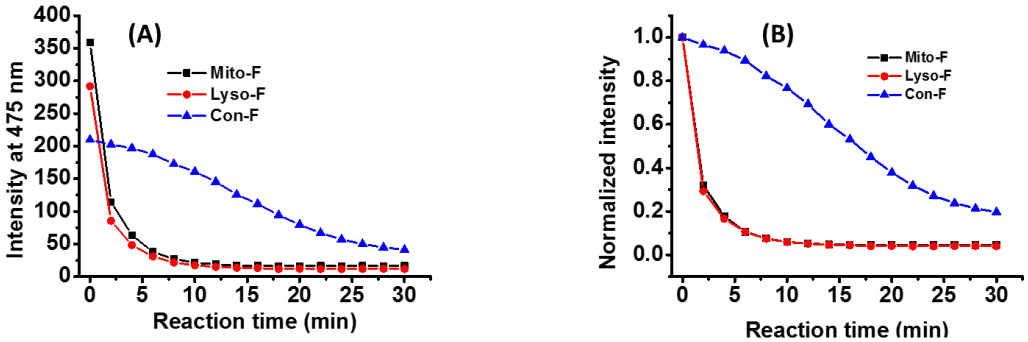
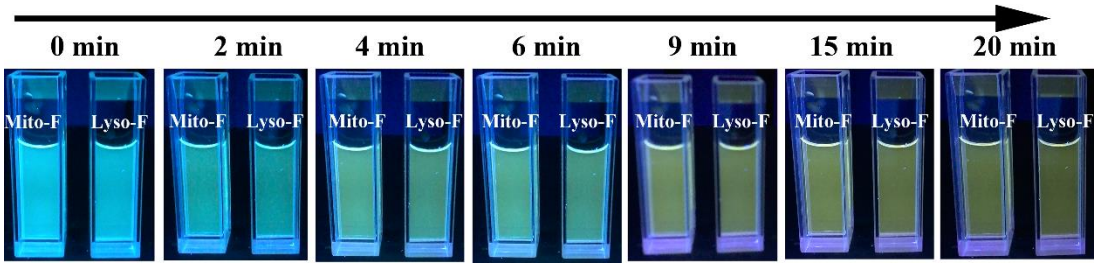


Figure S3. (A) Time-dependent fluorescence intensity (475 nm) of three probes (10 μM) in the presence of NaF (10 mM). (B) Normalized intensity at 475 nm against time from 0 to 30 min.



FigureS4. Photos of fluorescence color change of 10 μM **Mito-F** and **Lyso-F** against time upon addition of 10 mM NaF under a handhold UV light (365 nm). The experiment was performed in 20 mM HEPES buffer (7.4) containing 50 % DMSO.

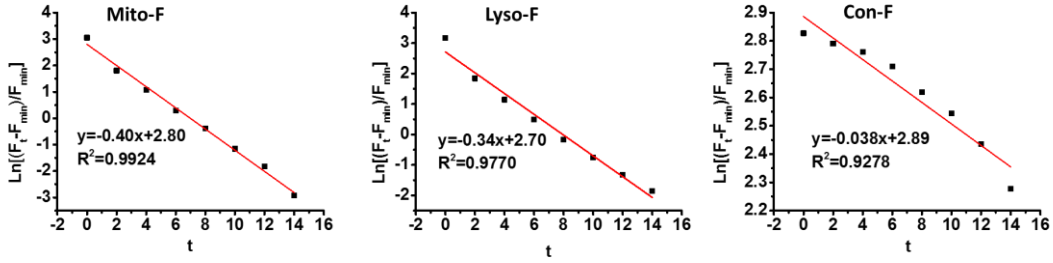


Figure S5. Kinetic plots of fluorescence of the pseudo-first order reaction (10 μM probes to 10 mM NaF), using excitation wavelength at 405 nm. The slope of the plot corresponds to the observed reaction rate. Where F_t and F_{min} are the fluorescence intensity at 475 nm (F_{475}) at a time t and the minimum value obtained after the reaction was complete.

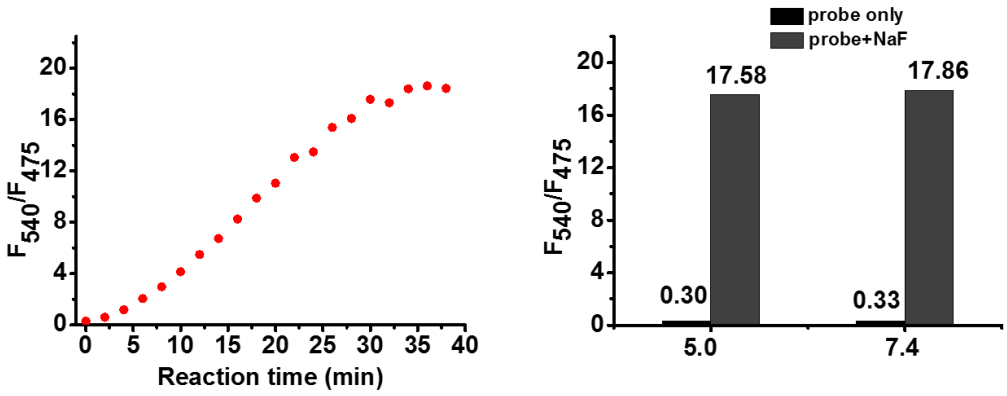


Figure S6. (A) Time-dependent fluorescence ratio changes (F_{540}/F_{475}) of **Lyso-F** (10 μM) in the presence of NaF (10 mM) at pH 5.0 HEPES buffer containing 50% DMSO. (B) Intensity ratios (F_{540}/F_{475}) for **Lyso-F** in the absence and presence of NaF after incubation for 30 min at pH 5.0 and 7.4.

Concentration-dependent assays

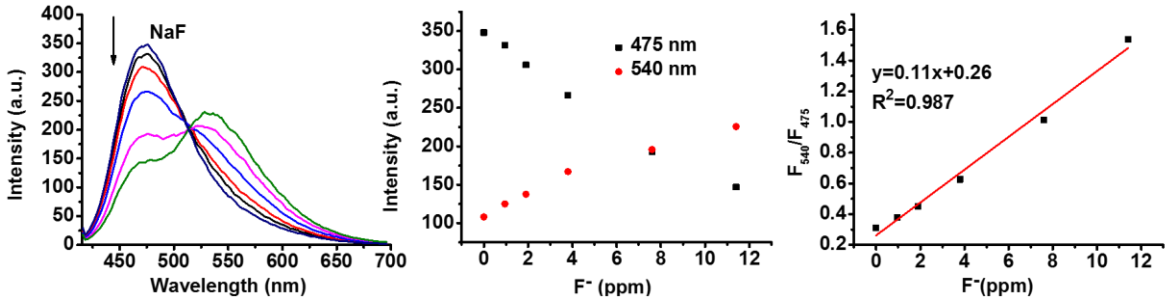


Figure S7. Titration of F^- (0~11.40 ppm) with **Mito-F** (10 μM) at pH 7.4 HEPES buffer

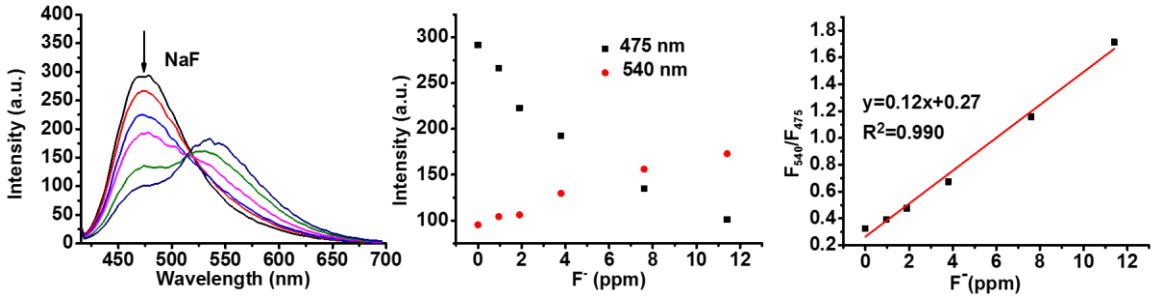


Figure S8. Titration of F^- (0~11.40 ppm) with **Lyso-F** (10 μM) at pH 7.4 HEPES buffer

Selectivity assays:

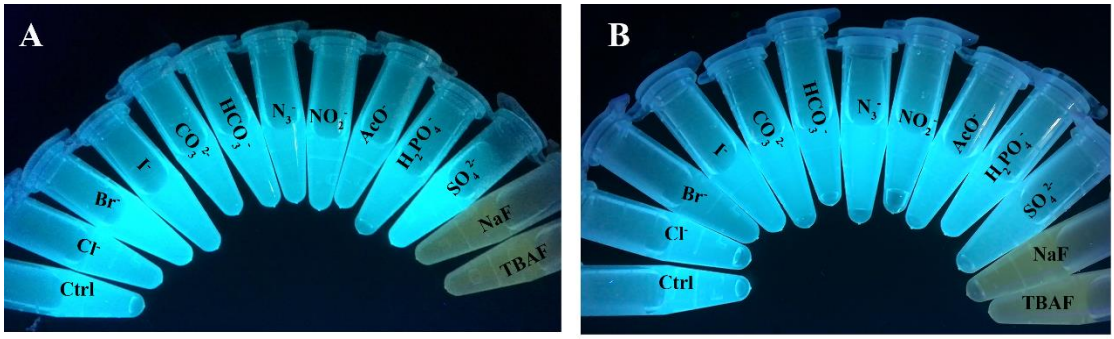


Figure S9. Photos of fluorescence color change of 10 μM **Mito-F** (A) and **Lyso-F** (B) in the presence of various species under a 365 nm light. The experiment was performed in 20 mM HEPES buffer. The relative low fluorescence with the addition of NaF and TBAF was due to the red-shift of probe absorbance.

4. Cell and Animal Imaging

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 ℃. Immediately prior to the imaging experiments, HeLa cells were incubated with **Mito-F** (10 μM) or **Lyso-F** (10 μM) for 30 minutes. The cells were washed three times with PBS and then treated with NaF (1 mM) for 120 min in an atmosphere of 5% CO_2 at 37 ℃. After final washing, the cells were then subjected to imaging using a Nikon A1R confocal laser-scanning microscope with an objective lens (40×). For co-localization experiment, HeLa cells were treated as mentioned above. The cells were then co-stained with **Mito-F**/MitoTracker Deep red or **Lyso-F**/LysoTracker Red. After final washing, the cells were then subjected to imaging using confocal laser scanning microscope.

For in vivo imaging, BALB/C mice were selected and divided to two groups. The mice in control group was intraperitoneally injected with PBS (200 μL, 10 mM). While mice in experimental group was intraperitoneally injected with NaF (200 μL, 0.5 mM NaF in 10 mM PBS solution). After continuous injection every 24 h for four days, both group were given an intraperitoneal or tail intravenous injection of probe **Mito-F** (50 μM, 200 μL, 0.2% DMSO) in the fifth day. Images were taken after incubation of **Mito-F** for 10 h by using a Carestream Health FX PRO small animal *in vivo* imaging system containing a sensitive CCD camera, with an excitation laser of 430 nm and an emission filter of 535 nm.

Cytotoxicity assays

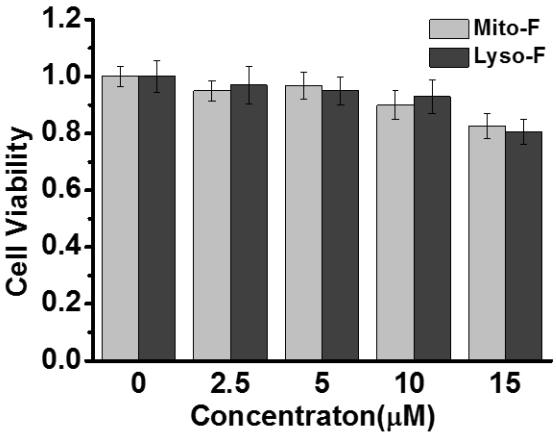


Figure S10. SRB assay of HeLa cells cultured for 24 h in DMEM containing various concentration of **Mito-F** and **Lyso-F** (0~15 μM).

Imaging F^- in living cells

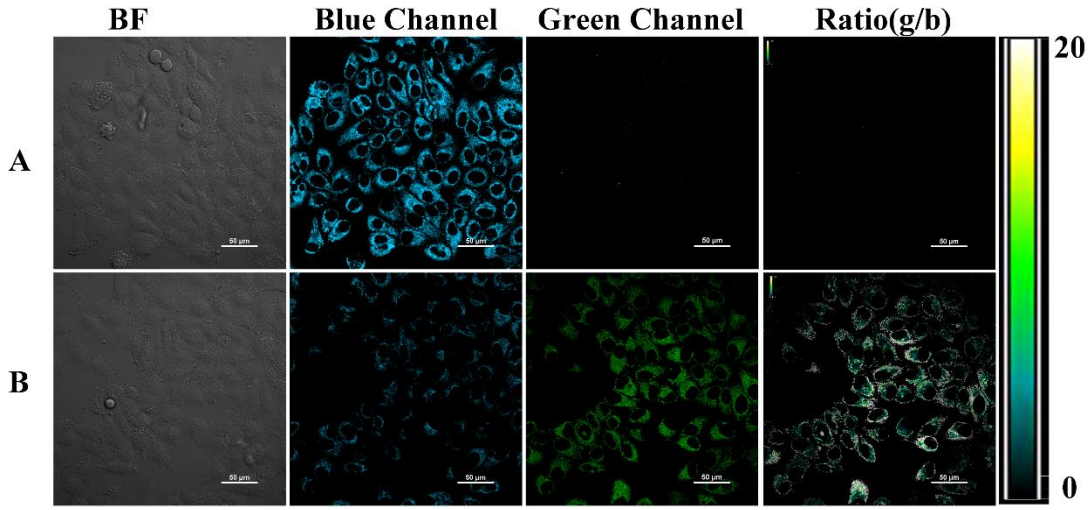
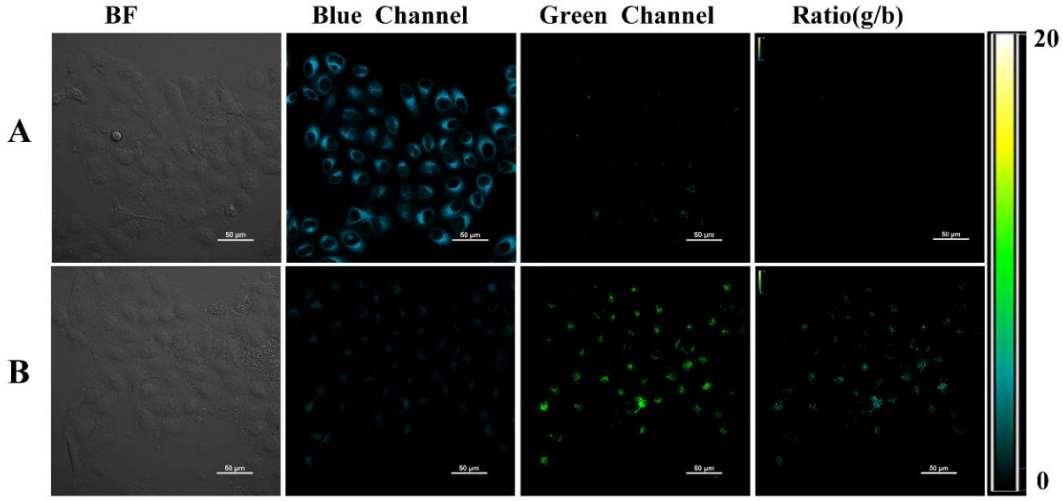


Figure S11. Ratiometric imaging of F^- in mitochondria of HeLa cells. (A) Cells were incubated with 10 μM probe **Mito-F** only; (B) Cells were treated with 1 mM (NaF) after incubation with probe **Mito-F**. Scale bars: 50 μm.



FigureS12. Ratiometric imaging of F^- in lysosome of HeLa cells. (A) Cells were incubated with 10 μM probe **Lyso-F** only. (B) Cells were treated with 1 mM NaF after incubation with probe **Lyso-F.** Scale bars: 50 μm.

Colocalization study

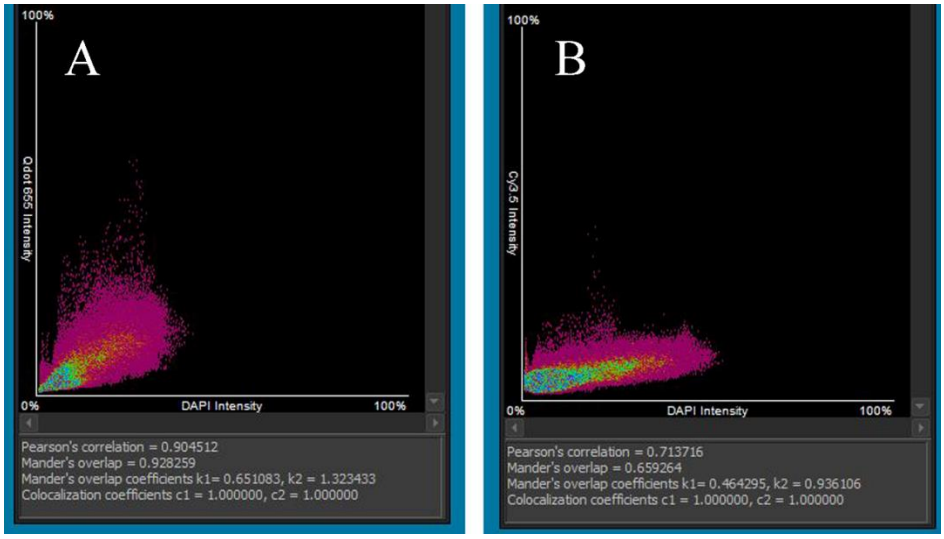


Fig.S13 (A) Correlation plot of **Mito-F** and MitoTracker Deep Red intensities. (B) Correlation plot **of Lyso-F** and LysoTracker Red intensities. The X axis represent the **Mito-F** and **Lyso-F** intensities, Y axis represent the MitoTracker and LysoTracker intensities.

In vivo imaging of F^- in living mice

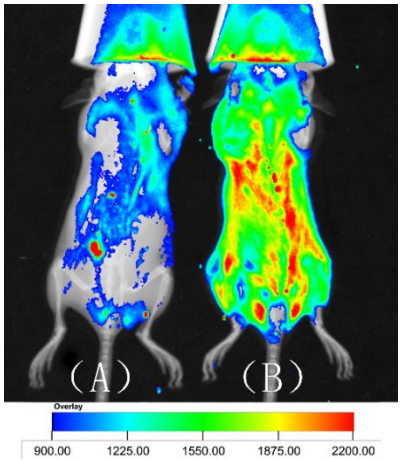


Figure S14. In vivo imaging of F^- in living mice with **Mito-F**. Mice were intraperitoneally injected with only PBS (A) or NaF in PBS (B) continuously for 4 days. **Mito-F** was then intravenously injected in fifth days.

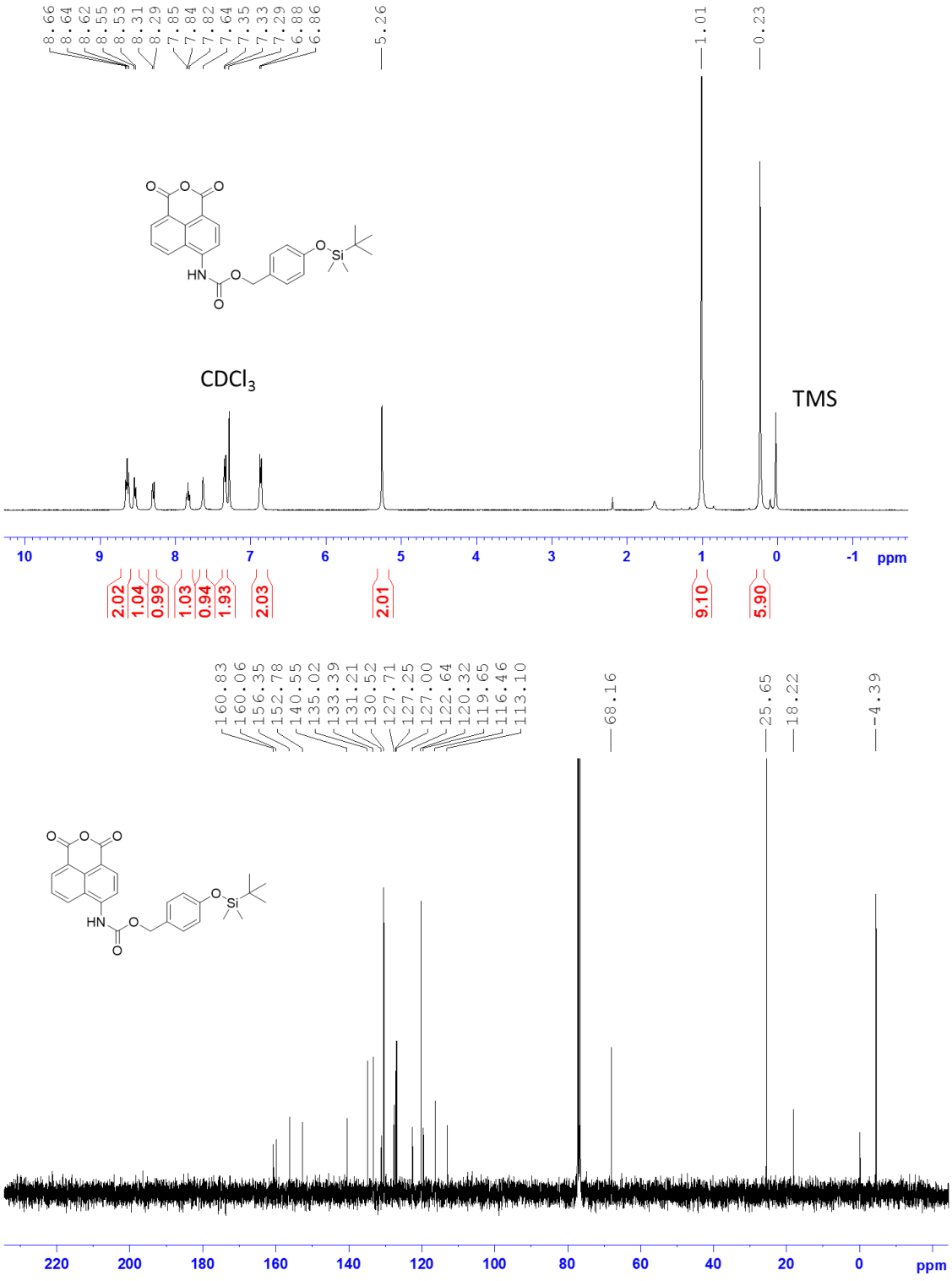
(1) Kumari, G.; Singh, R. K. *Med. Chem. Res.* **2015**, *24*, 171.

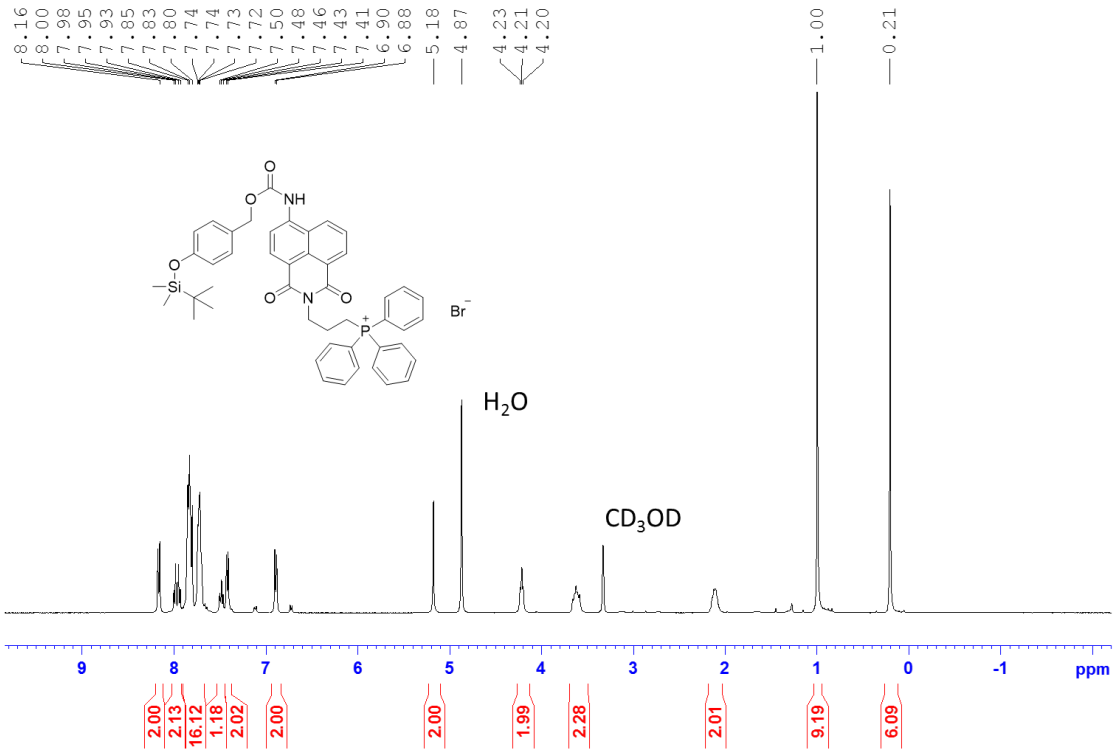
(2) Nishihara, R.; Suzuki, H.; Hoshino, E.; Suganuma, S.; Sato, M.; Saitoh, T.; Nishiyama, S.; Iwasawa, N.; Citterio, D.; Suzuki, K. *Chem. Ccommun.* **2015**, *51*, 391.

(3) Nawimanage, R. R.; Prasai, B.; Hettiarachchi, S. U.; McCarley, R. L. *Anal Chem* **2014**, *86*, 12266.

(4) Millard, M.; Gallagher, J. D.; Olenyuk, B. Z.; Neamati, N. *J. Med. Chem.* **2013**, *56*, 9170.

Characterization of probes and their intermediates

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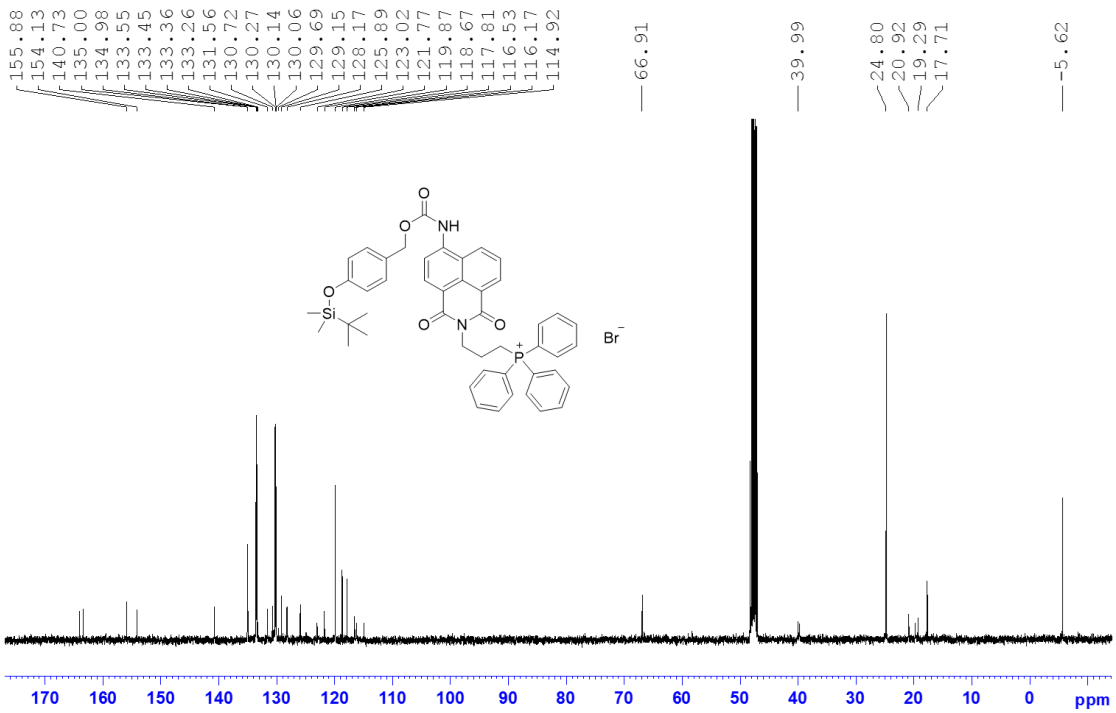
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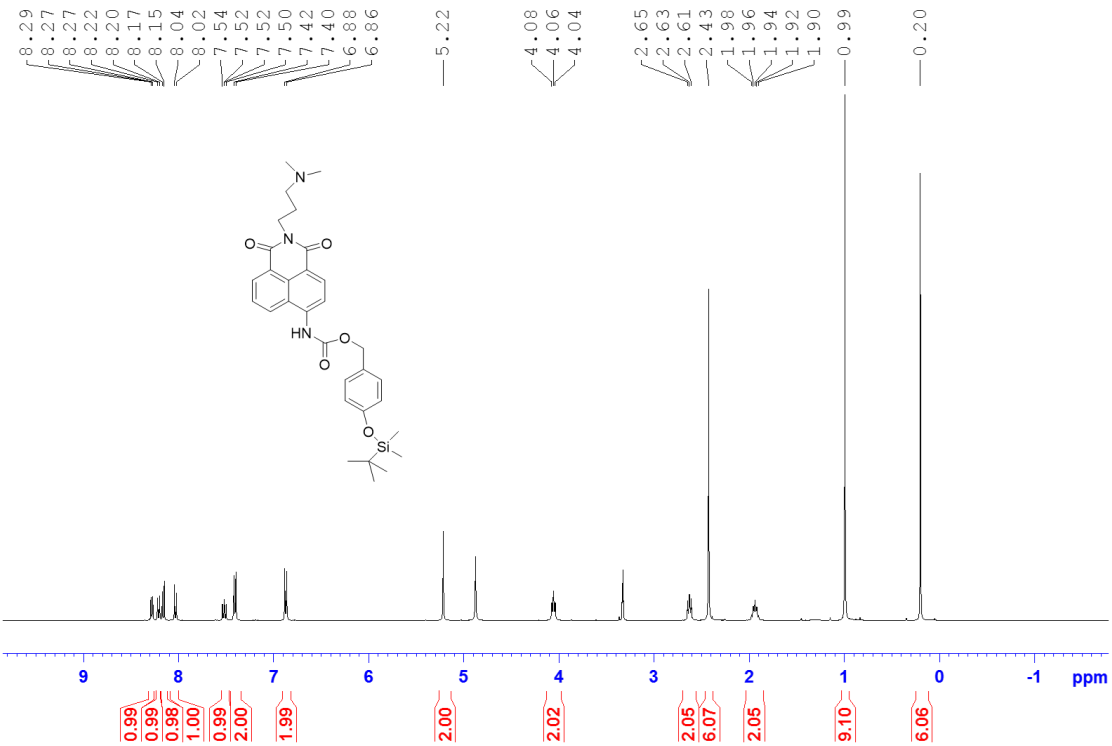
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8.17
8.15
8.04
8.02
7.54
7.52
7.52
7.50
7.42
7.40
6.88
6.86
5.22
4.08
4.06
4.04
2.65
2.63
2.61
2.43
1.98
1.96
1.94
1.92
1.90
0.99
0.20
ppm
0.99
0.99
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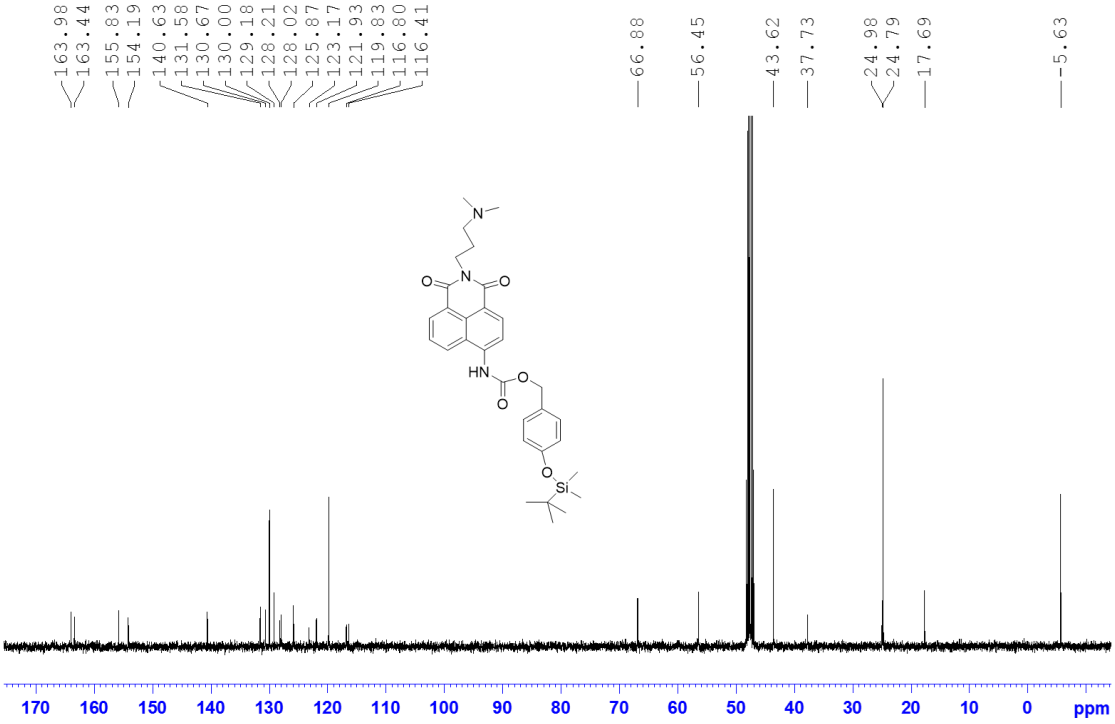
9.10
6.06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123.17
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66.88
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43.62
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24.98
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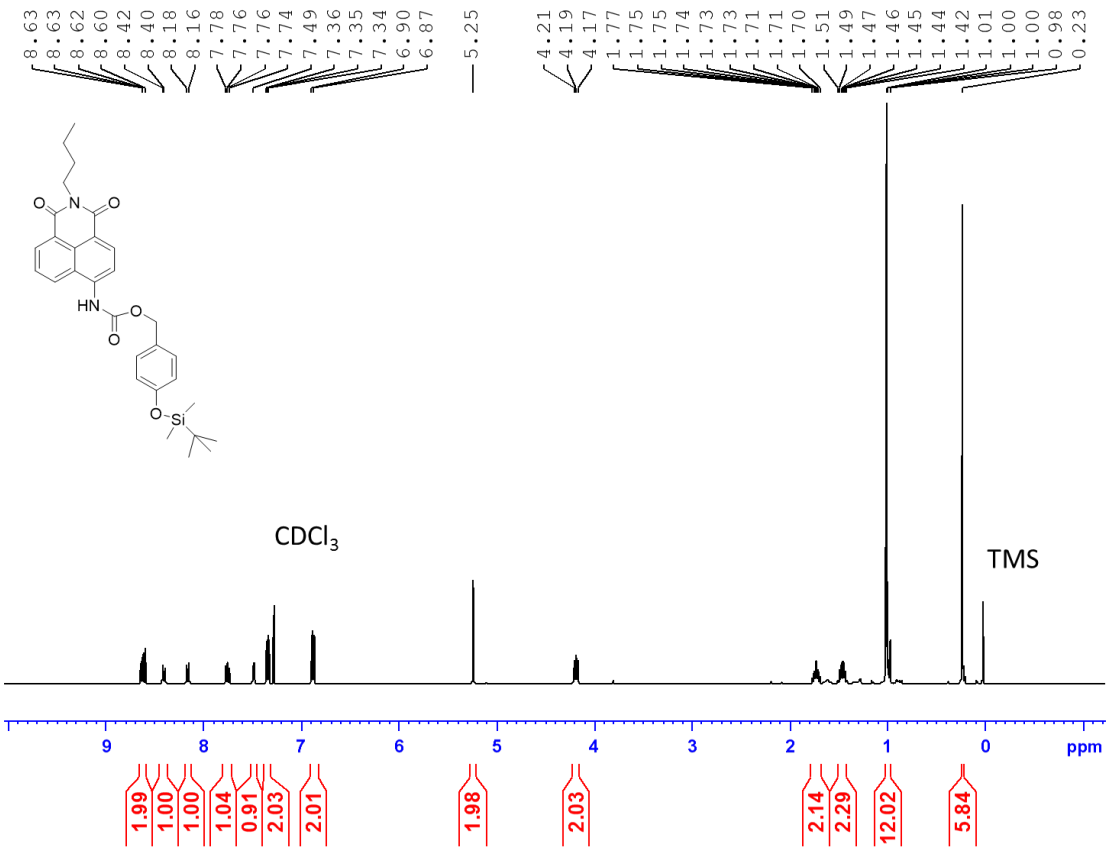
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7.35
7.34
6.90
6.87
5.25
4.21
4.19
4.17
1.77
1.75
1.75
1.74
1.73
1.73
1.71
1.71
1.70
1.51
1.49
1.47
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1.45
1.44
1.42
1.01
1.00
1.00
0.98
0.23
CDCl3
TMS
ppm
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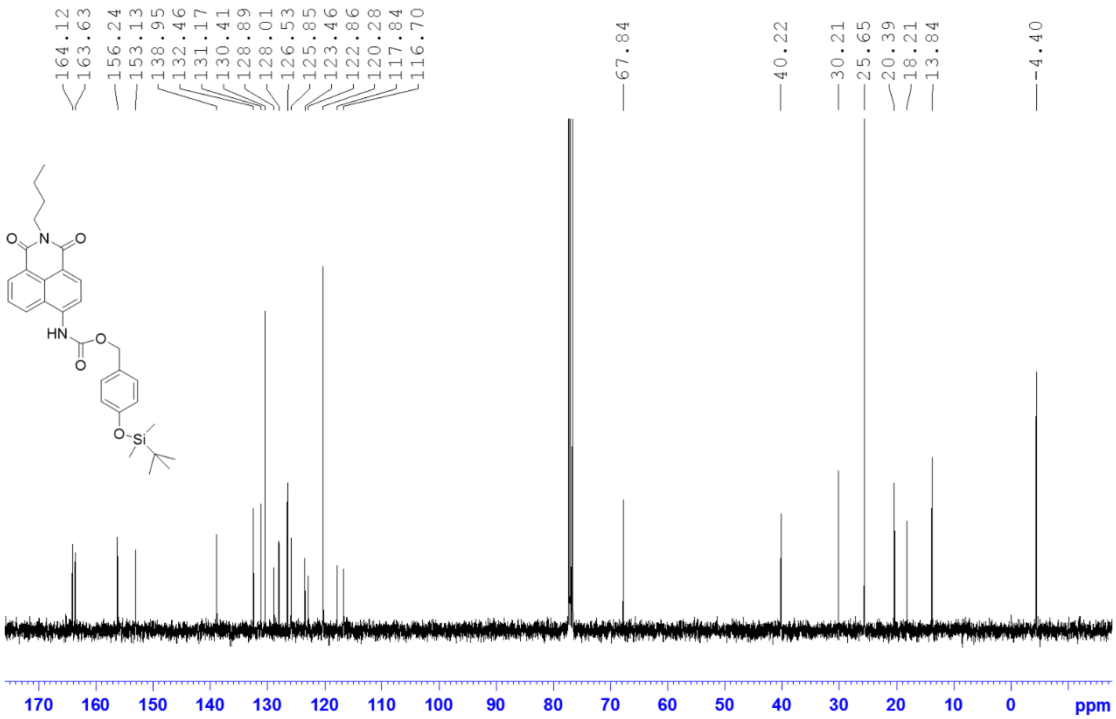
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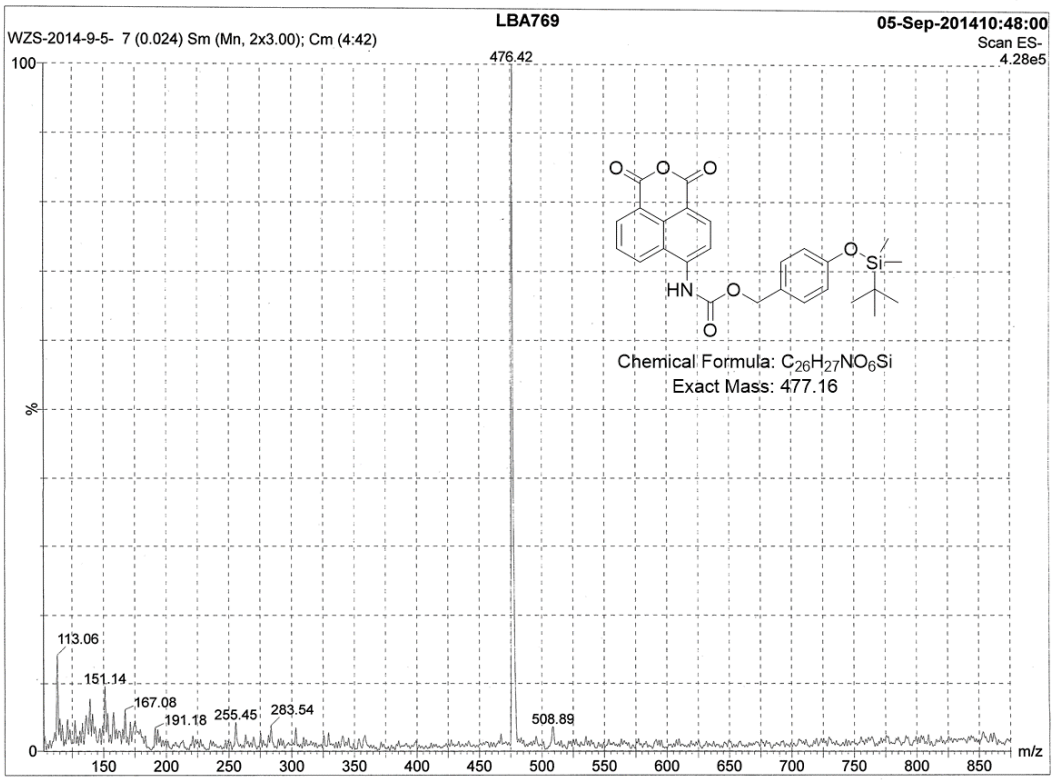
ppm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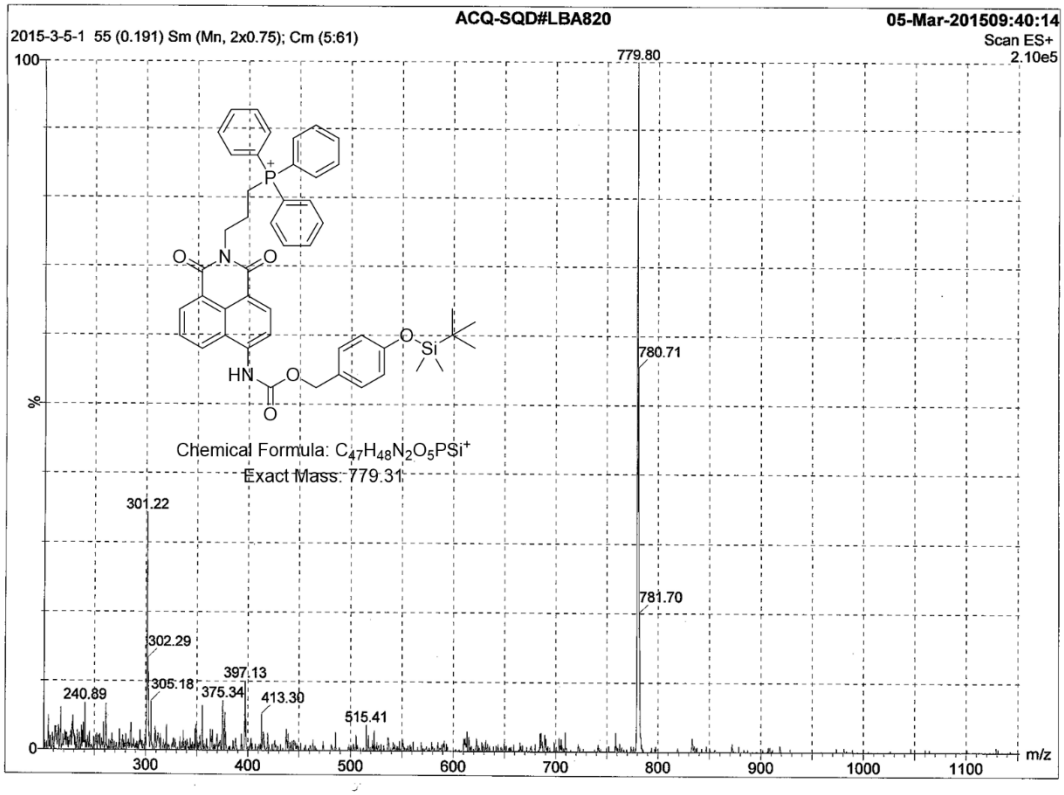
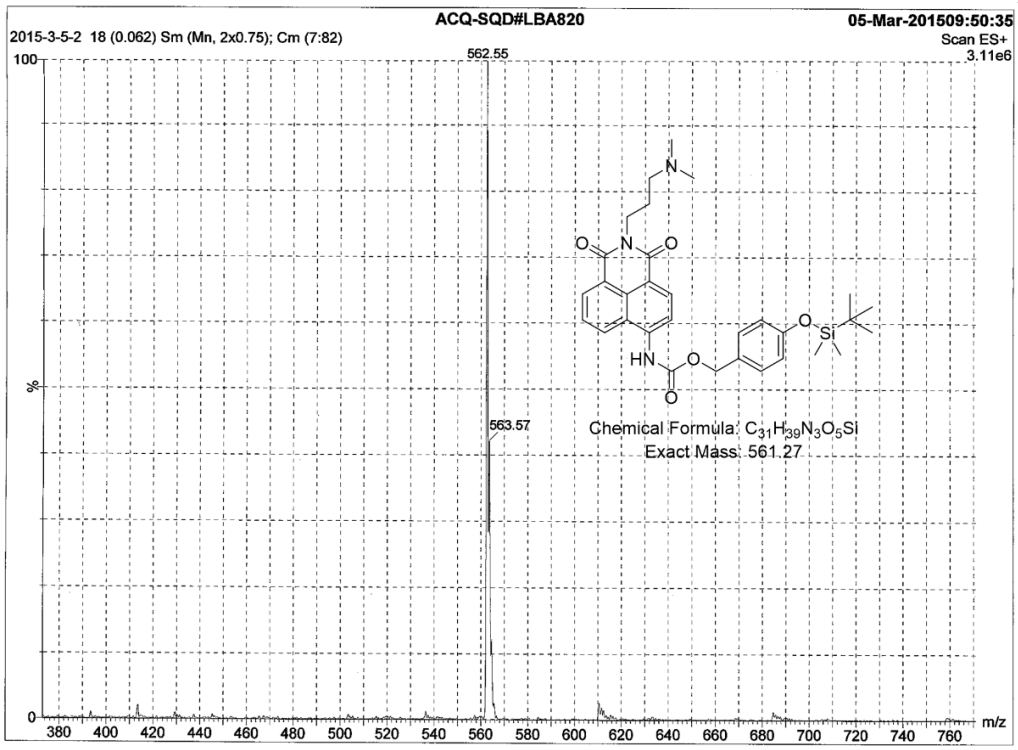
05-Sep-201410:48:00
WZS-2014-9-5- 7 (0.024) Sm (Mn, 2x3.00); Cm (4:42)
Scan ES-
4.28e5
476.42
Chemical Formula: C26H27NO6Si
Exact Mass: 477.16
113.06
151.14
167.08
191.18
255.45
283.54
508.89
m/z


ACQ-SQD#LBA820
05-Mar-201509:40:14
2015-3-5-1 55 (0.191) Sm (Mn, 2x0.75); Cm (5:61)
Scan ES+
2.10e5
779.80
780.71
781.70
Chemical Formula: C47H48N2O5PSi+
Exact Mass: 779.31
301.22
302.29
305.18
240.89
375.34
397.13
413.30
515.41
m/z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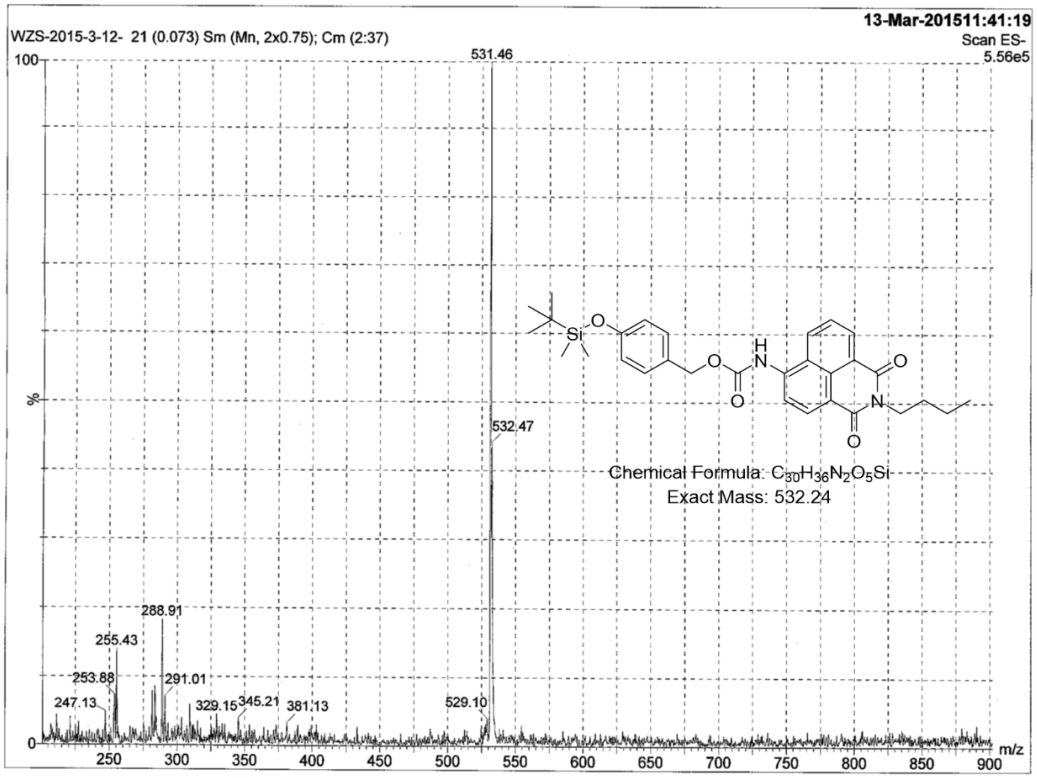
2015-3-5-2 18 (0.062) Sm (Mn, 2x0.75); Cm (7:82)
Scan ES+
3.11e6
562.55
563.57
Chemical Formula: C31H39N3O5Si
Exact Mass: 561.27
m/z


13-Mar-201511:41:19
WZS-2015-3-12- 21 (0.073) Sm (Mn, 2x0.75); Cm (2:37)
Scan ES-
5.56e5
531.46
532.47
Chemical Formula: C30H36N2O5Si
Exact Mass: 532.24
288.91
255.43
253.88
291.01
247.13
329.15
345.21
381.13
529.10
m/z