Electronic Supplementary Information (ESI)

for

In vivo Two-photon Fluorescent Imaging of Fluoride with a Desilylation-based Reactive Probe

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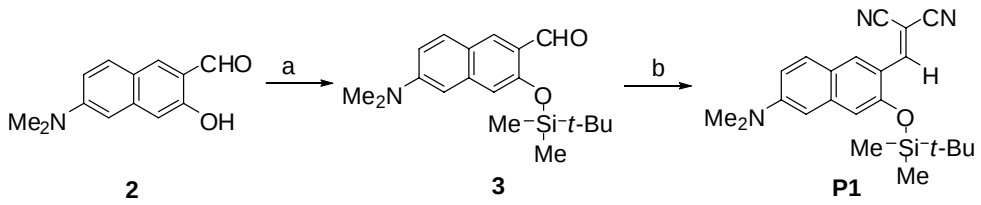
Scheme S1



Reagents and conditions: (a) *t*-butyldimethylsilyl chloride, DMAP, Et_3N, CH_2Cl_2; (f) malononitrile, piperidine, EtOH.

Synthesis of 1 and P1:

Compound **2** was prepared according to the reported procedure: I. Kim *et al, Asian. J. Org. Chem.* **2012**. DOI:10.1002/ajoc.201200034. Iminocoumarin **1**: D. Kim *et al, Chem. Commun*. **2012**, *48*, 6833-6835.

Preparation of compound 3: Compound **2** (300 mg, 1.394 mmol), 4-dimethylaminopyridine (DMAP, 5 mg, 0.019 mmol) and Et_3N (0.2 mL, 1.39 mmol) were combined in anhydrous dichloromethane (10 mL) at 0 °C, under a nitrogen atmosphere. After being stirred for 10 min, to the mixture was added *t*-butyldimethylsilyl chloride (252 mg, 1.67 mmol) in dichloromethane (5 mL) dropwise at the same temperature. The mixture was stirred at room temperature for 12 h, and then treated with a saturated $NaHCO_3$ solution (10 mL). The two layers were separated, and the aqueous layer was extracted with dichloromethane (20 mL × 3). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (eluent EtOAc/hexane = 2:8) to afford compound **3** as a yellow solid (416 mg, 91%). ^{1}H NMR ($CDCl_3$, 300 MHz, 293 K): δ 10.45 (s, 1H), 8.21 (s, 1H), 7.72–7.69 (d, 1H), 7.02–6.98 (dd, 1H), 6.92 (s, 1H), 6.66–6.65 (d, 1H), 3.10 (s, 6H), 1.05 (s, 9H), 0.32 (s, 6H). ^{13}C NMR(CDCl3, 75 MHz, 293 K): δ 190.07, 154.90, 150.57, 139.88, 131.25, 130.49, 124.02, 121.39, 111.72, 112.57, 103.61, 40.35, 25.80, 18.41, and 4.25. HRMS: *m*/*z* calcd. for $C_{19}H_{27}NO_2Si$, 329.1811; found, 329.1808.

Preparation of P1: To a stirred solution of alcohol **3** (416 mg, 1.26 mmol) and malononitrile (83.4 mg, 1.26 mmol) in ethanol (9 mL) at room temperature under argon was added piperidine (1.0 mL, 12.6 mmol). The reaction mixture was allowed to stir at

room temperature for 3 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent EtOAc/hexane = 2:8) to afford **P1** as a red solid (333.4 mg, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz, 293 K): δ 8.70 (s, 1H), 8.24 (s, 1H), 7.70–7.67 (d, 1H), 7.02–6.98 (dd, 1H), 6.88 (s, 1H), 6.60–6.59 (d, 1H), 3.14 (s, 6H), 1.05 (s, 9H), 0.31 (s, 6H). ^{13}C NMR(CDCl3, 75 MHz, 293 K): δ 154.13, 152.53, 151.30, 140.20, 131.43, 131.09, 121.46, 119.27, 115.40, 114.88, 114.30, 111.78, 103.41, 40.29, 25.79, 18.38, 4.23. HRMS: *m*/*z* calcd. for $C_{22}H_{27}N_3OSi$, 377.1923; found, 377.1923.

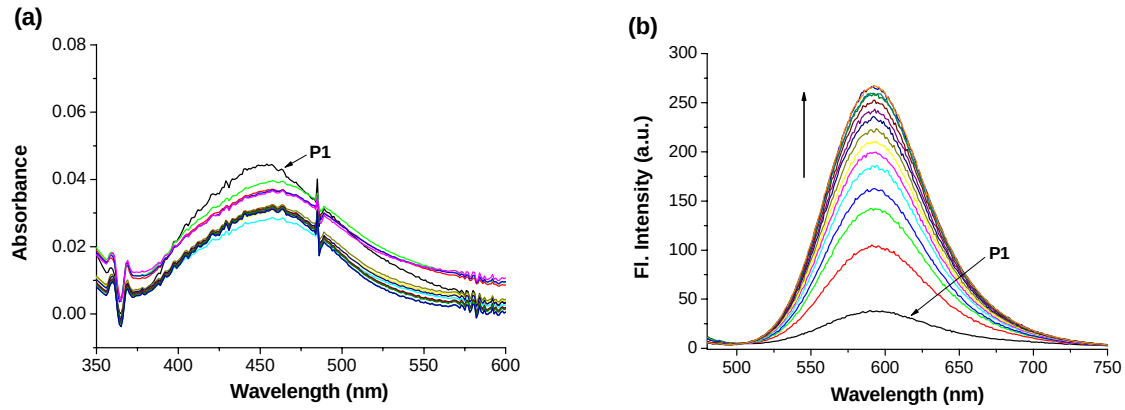


Fig. S1 Time–dependent (a) absorption and (b) emission spectra of **P1** (20 μM) treated with NaF (20 mM) in pH 7.4 buffer (10 mM HEPES containing 20% CH_3CN). Each spectra was recorded at 3, 5, 7, and 10–60 min with 5–min interval. Excitation wavelength: 460 nm.

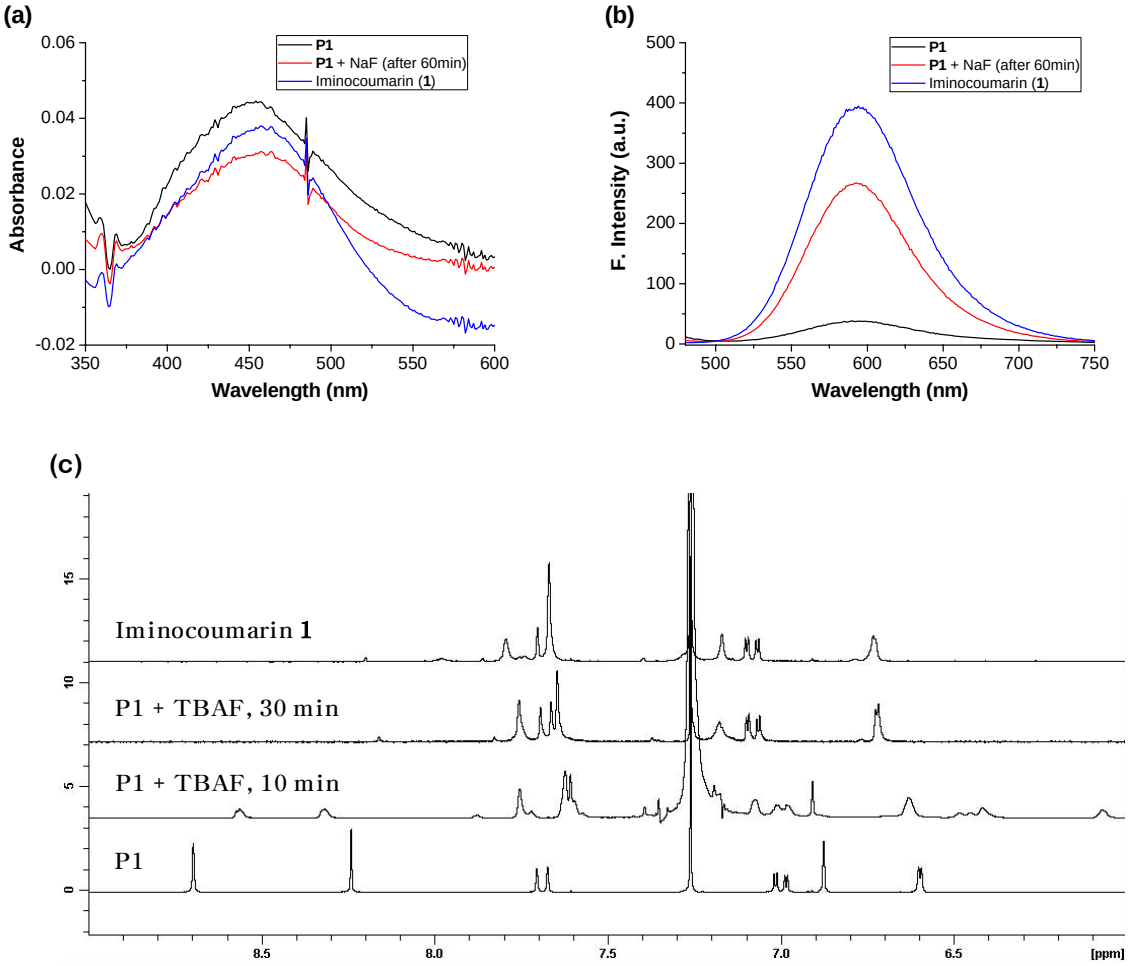


Fig. S2 (a, b) Absorption and emission spectra of **P1** (20 μM) and its mixture with NaF (20 mM), and **1** (20 μM) dissolved in pH 7.4 buffer (10 mM HEPES containing 20% CH_3CN). Excitation wavelength: 460 nm. (c) ^1H NMR titration of **P1** with TBAF (5 equiv.) in $CDCl_3$: from the bottom, **P1** only; **P1** treated with TBAF, after 10 min, **P1** treated with TBAF, after 30 min; iminocoumarin **1**.

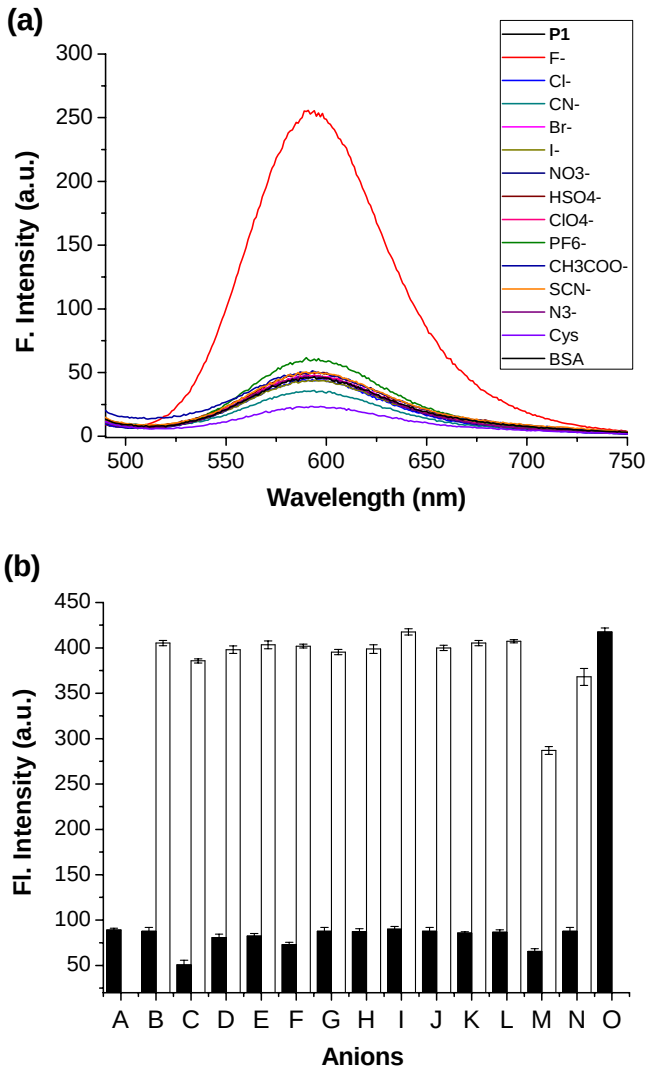


Fig. S3 (a) Fluorescence emission data for a mixture of **P1** (20 μM) and each of the various metal ions (F^-, Cl^-, CN^-, Br^-, I^-, NO_3^-, HSO_4^-, ClO_4^-, PF_6^-, CH_3COO^-, SCN^-, N_3^-; 20 mM as tetrabutylammonium salts), Cys (20 mM), and 1 mg/mL BSA in pH 7.4 buffer (10 mM HEPES containing 20% CH_3CN), obtained after 60 min of mixing. Excitation wavelength: 460 nm. (b) Fluorescence changes (emission intensity at 600 nm measured with a VICTOR multilabel counter) of **P1** (20 μM) upon addition of each of the anions (20 mM as Bu_4N^+ salts, 20 mM Cys, 1 mg/mL BSA, filled bars) followed by F^- (2 mM, empty bars) in pH 7.4 buffer (10 mM HEPES containing 20% CH_3CN), measured after 1 h (excitation wavelength: 480 nm): (A) **P1**, (B) Cl^-, (C) CN^-, (D) Br^-, (E) I−, (F) NO_3^-, (G) HSO_4^-, (H) ClO_4^-, (I) PF_6^-, (J) CH_3COO^-, (K) SCN^-, (L) N_3^-, (M) Cys, (N) BSA, and (O) F^-.

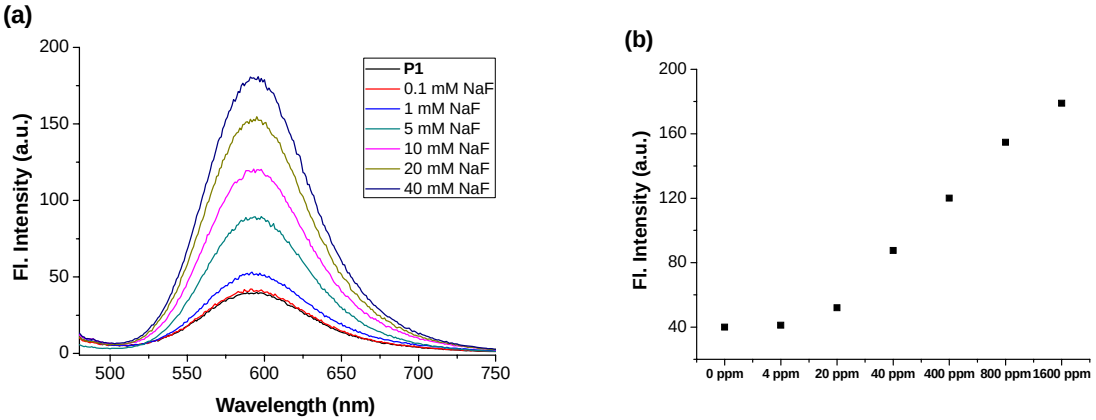


Fig. S4 (a) Fluorescence spectra of **P1** (20 μM) in the presence of NaF at different concentrations (0.1, 1, 5, 10, 20, and 40 mM) in pH 7.4 buffer (10 mM HEPES containing 20% CH_3CN), acquired after 60 min of mixing under excitation at 460 nm. (b) A plot of fluorescence intensity depending on the concentration of NaF in the range of 0 to 1600 ppm. Each data point was measured by mixing **P1** (20 μM) and NaF in pH 7.4 buffer (10 mM HEPES containing 20% CH_3CN) after 60 min of mixing. Fluorescence intensity was recorded with a VICTOR multilabel counter under excitation at 480 nm and detection at 600 nm.

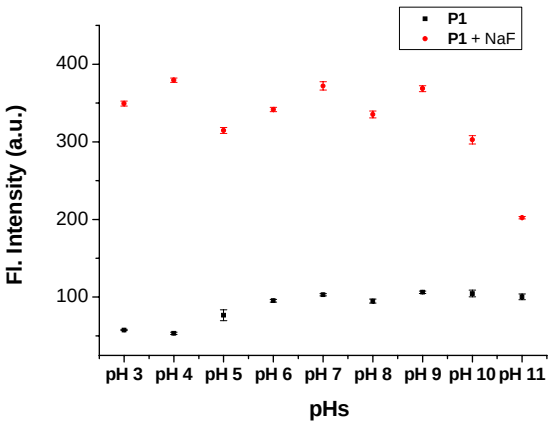


Fig. S5 Fluorescence intensity data obtained for a series of mixture of **P1** (20 μM) and NaF (20 mM) in buffer solutions containing 20% CH_3CN at various pHs (3, 4, 5, 6, 7, 8, 9, 10, and 11). Fluorescence intensity was recorded with a VICTOR multilabel counter under excitation at 480 nm and detection at 600 nm.

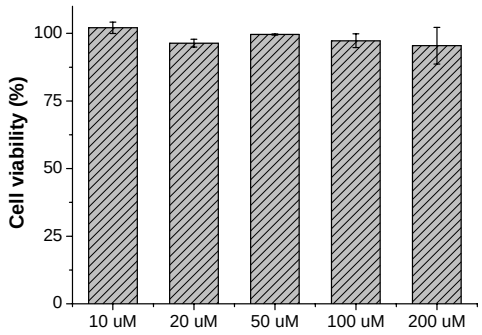


Fig. S6 Cell viability data for **P1** in B16F10 cell line determined by the CCK-8 method. Cells cultured with 5% CH_3CN were used as a control.

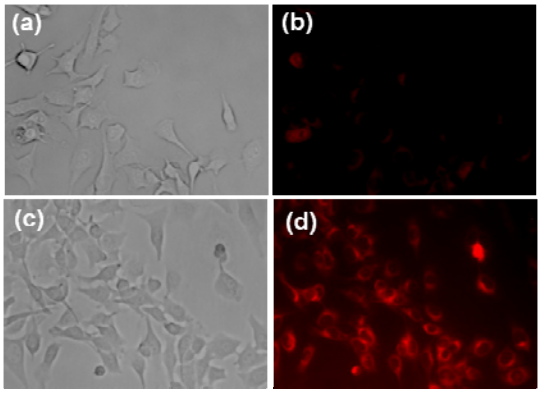


Fig. S7 (a, c) Bright field and (b, d) one-photon excitation fluorescence images of B16F10 cell line (mouse skin cancer) treated with (a, b) **P1** (20 μM) and with (c, d) **P1** (20 μM) followed by NaF (20 mM).

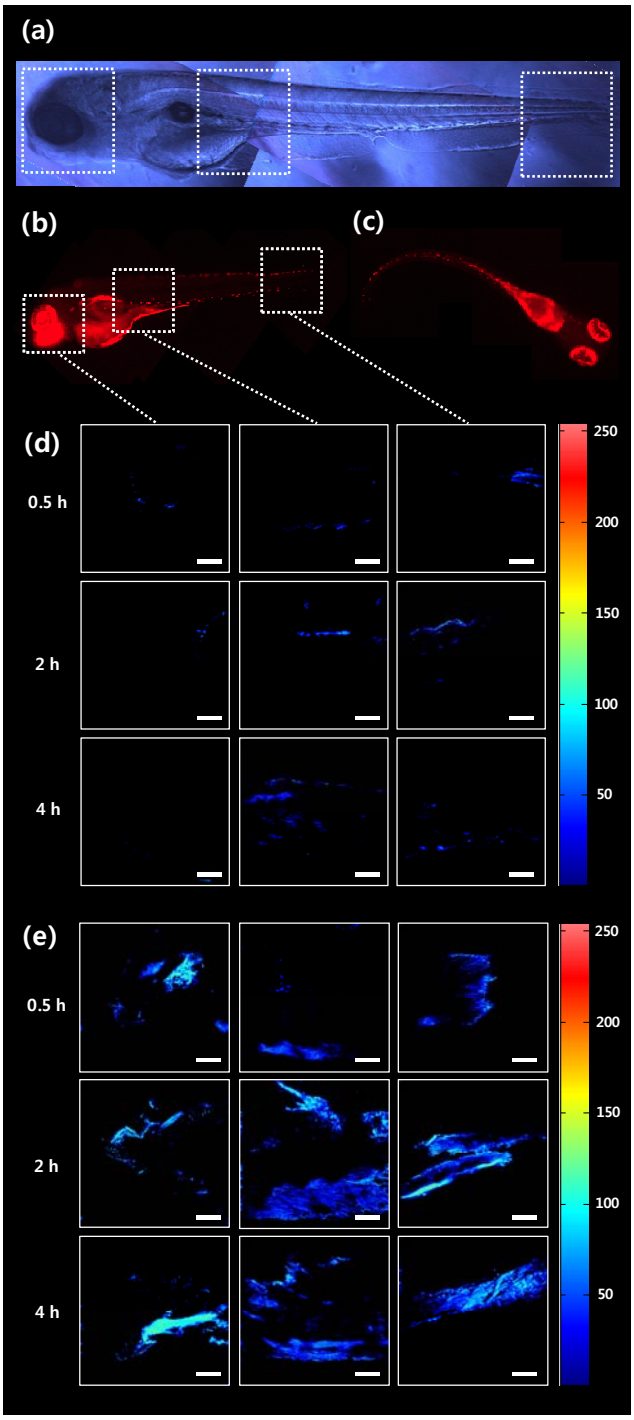


Fig. S8 Time- and depth-dependent fluorescent imaging data of F^- in live 4-day-old albino zebrafish: (a) Microscopic image; (b, c) OPM images of a side and an upper views of zebrafish incubated with **P1** (20 μM) and then with F^- (5 mM); (d) TPM images taken at specific depths (given in Fig. S9) of zebrafish treated only with **P1** for the given periods; images for the head, abdomen, and tail parts are shown; (e) TPM images of the three parts taken for the zebrafish treated with **P1** (30-min incubation) followed by F^- for different periods (30 min, 2 h, and 4 h).

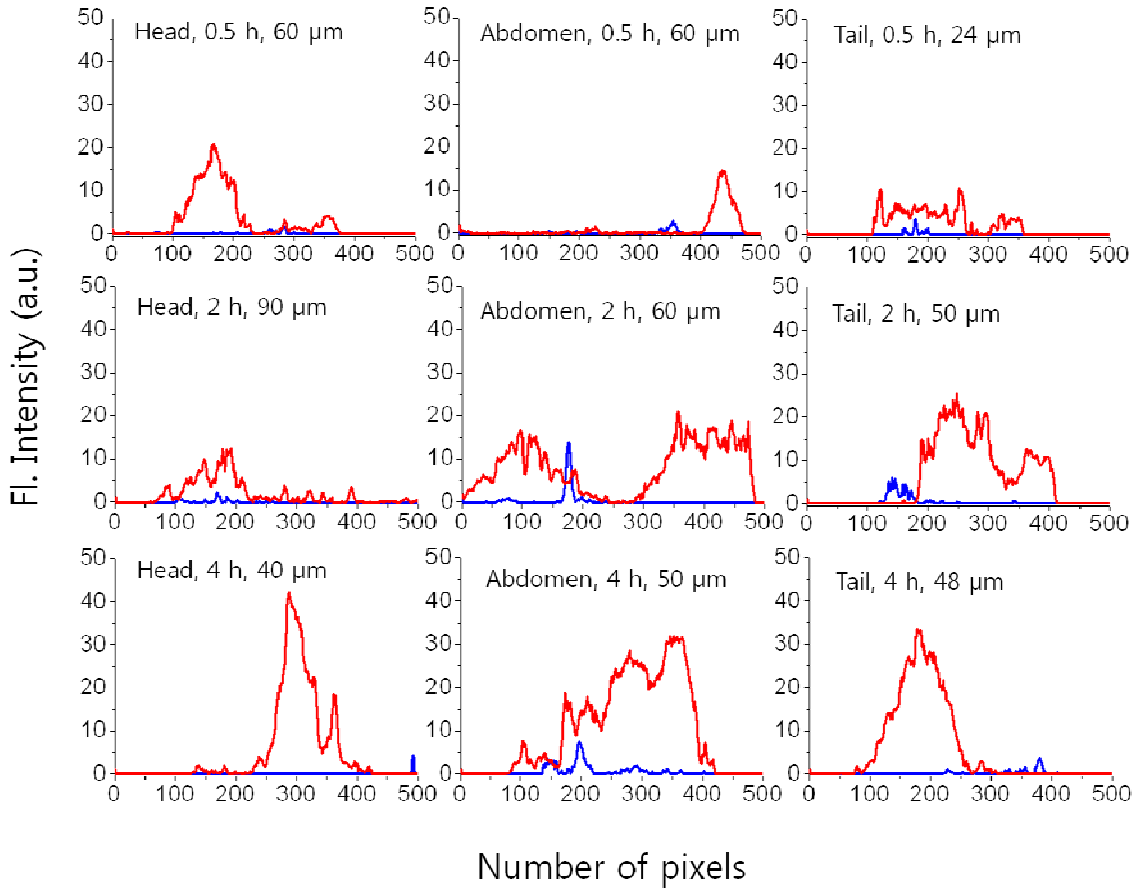


Fig. S9 Fluorescence intensity as function of pixel: blue line for Fig. S8d; red line for Fig. S8e. The depth values given for the same part are not the same because a different zebrafish is used for each data set (total 18 sets); an intensity maximum depth for a given part was selected. Scale bar: 50 μm. View area: 300 μm × 300 μm.

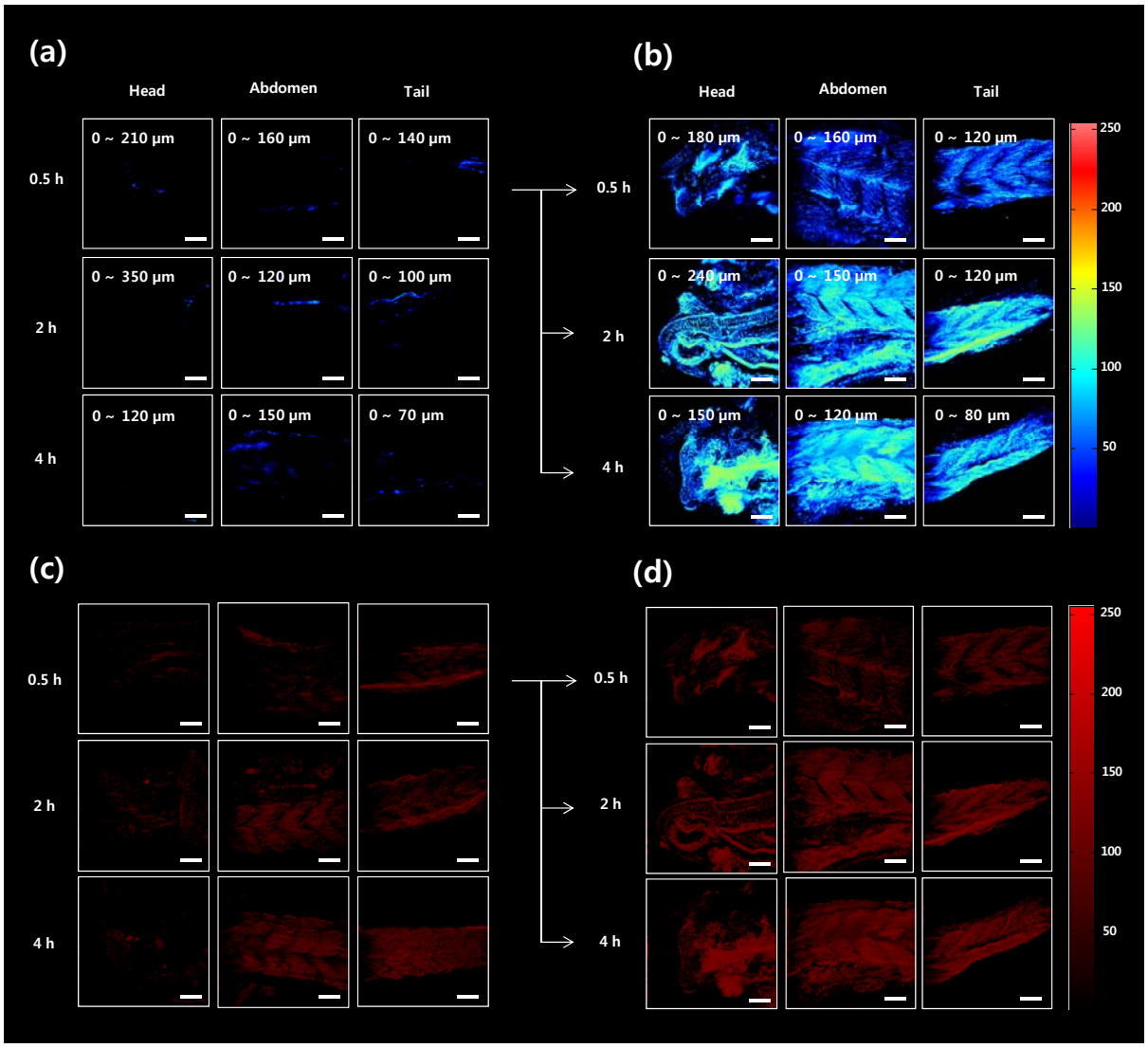


Fig. S10 Accumulated TPM fluorescent images of zebrafish in three parts: (a, b) intensity data; (c, d) fluorescent images of (a, b). Each image was constructed by image stacking for 0 – 350 μm depth, with 2 μm imaging depth step. (a, c) incubated only with **P1** (20 μM) for 30 min, 2 h, and 4 h respectively at 29 °C. (b, d) incubated with **P1** (20 μM) for 30 min at 29 °C followed by further incubation with F^- (5 mM) for 30 min, 2 h, and 4 h, respectively at 29 °C. Scale bar: 50 μm. View area: 300 μm × 300 μm.

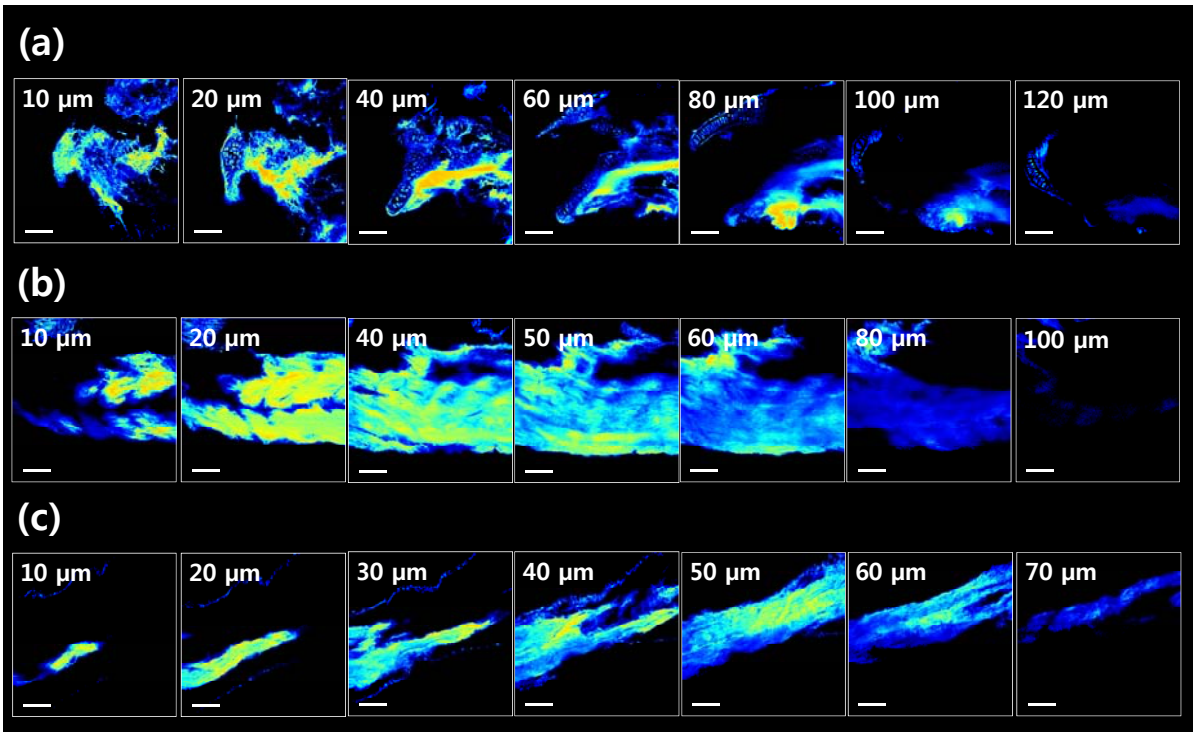


Fig. S11 Captured shots of video clips of two-photon fluorescence images of zebrafish, depending on depth. Zebrafish was treated with **P1** for 30 min followed by further incubation with NaF for 4 h. (a) Head, (b) abdomen, and (c) tail parts. Scale bar: 50 μm. View area: 300 μm × 300 μm.

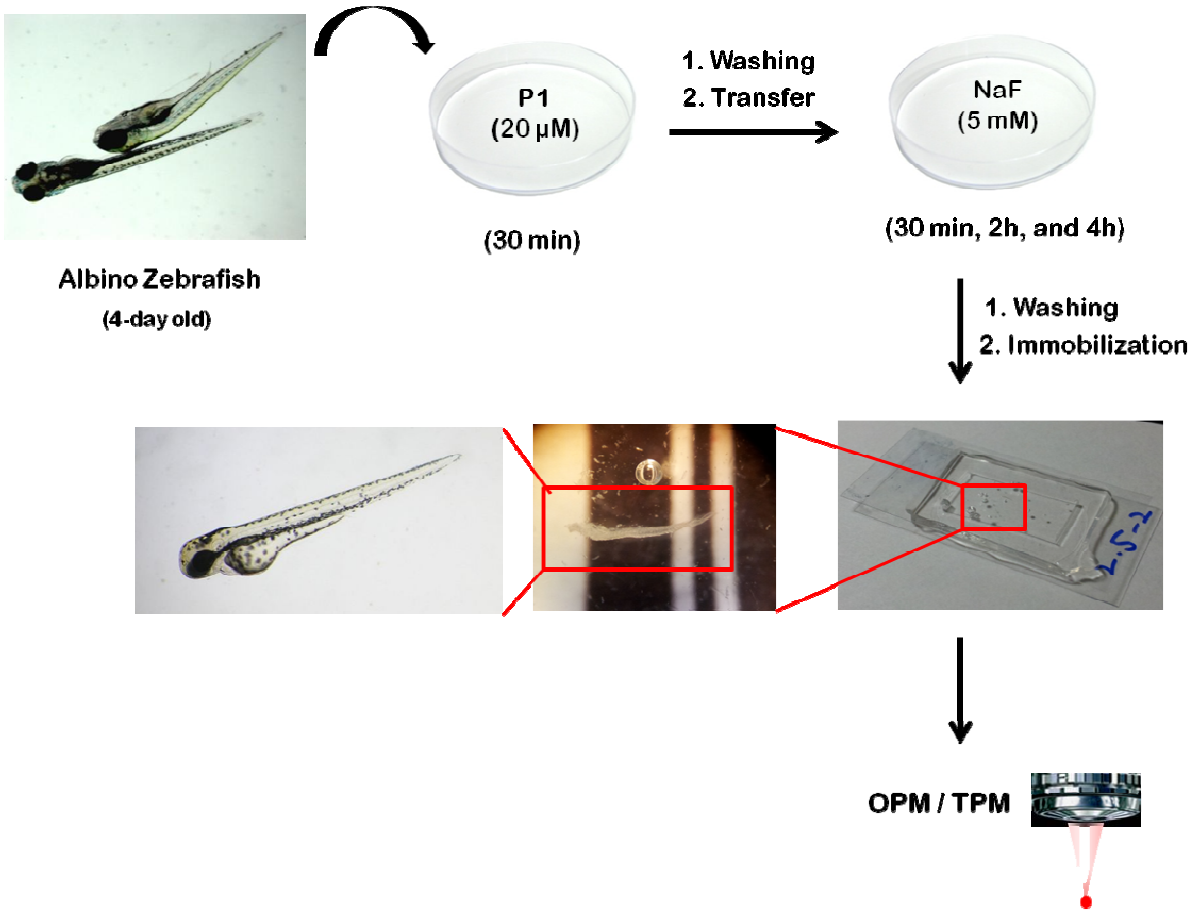
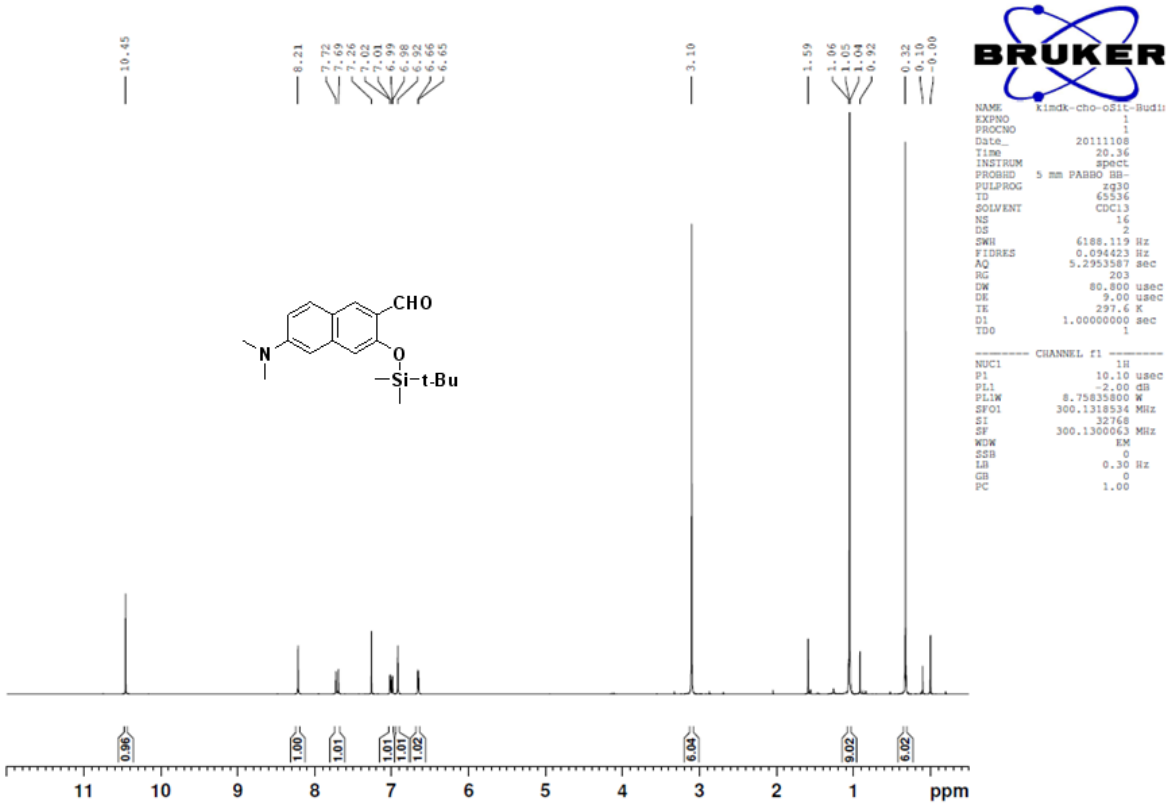
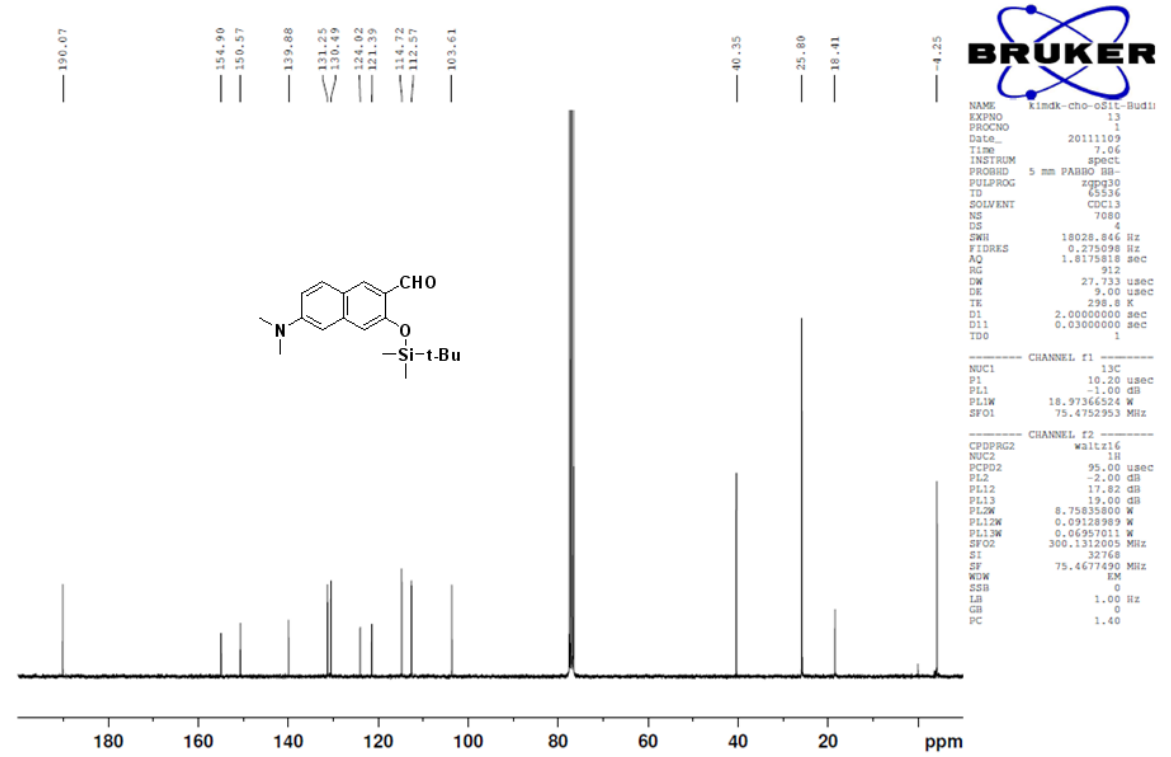


Fig. S12 Experimental set-up for *in vivo* zebrafish imaging.

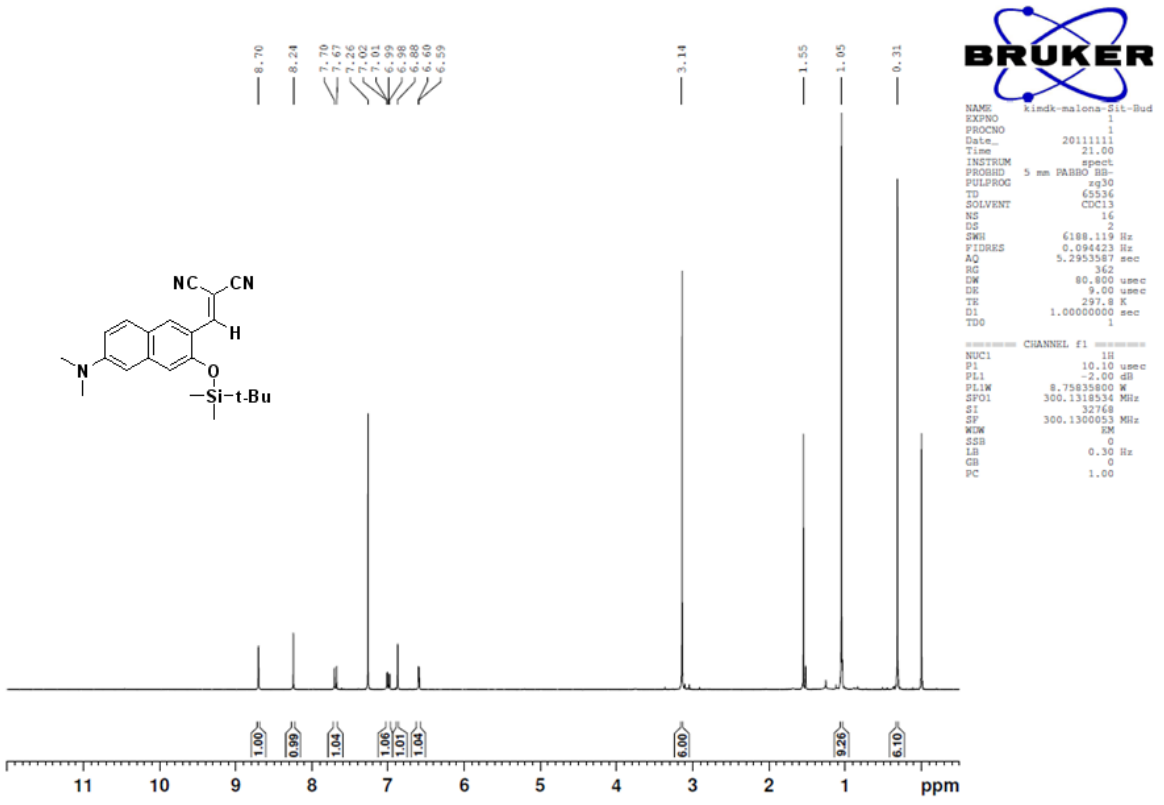
NMR spectra for the compounds synthesized

Compound **3**





P1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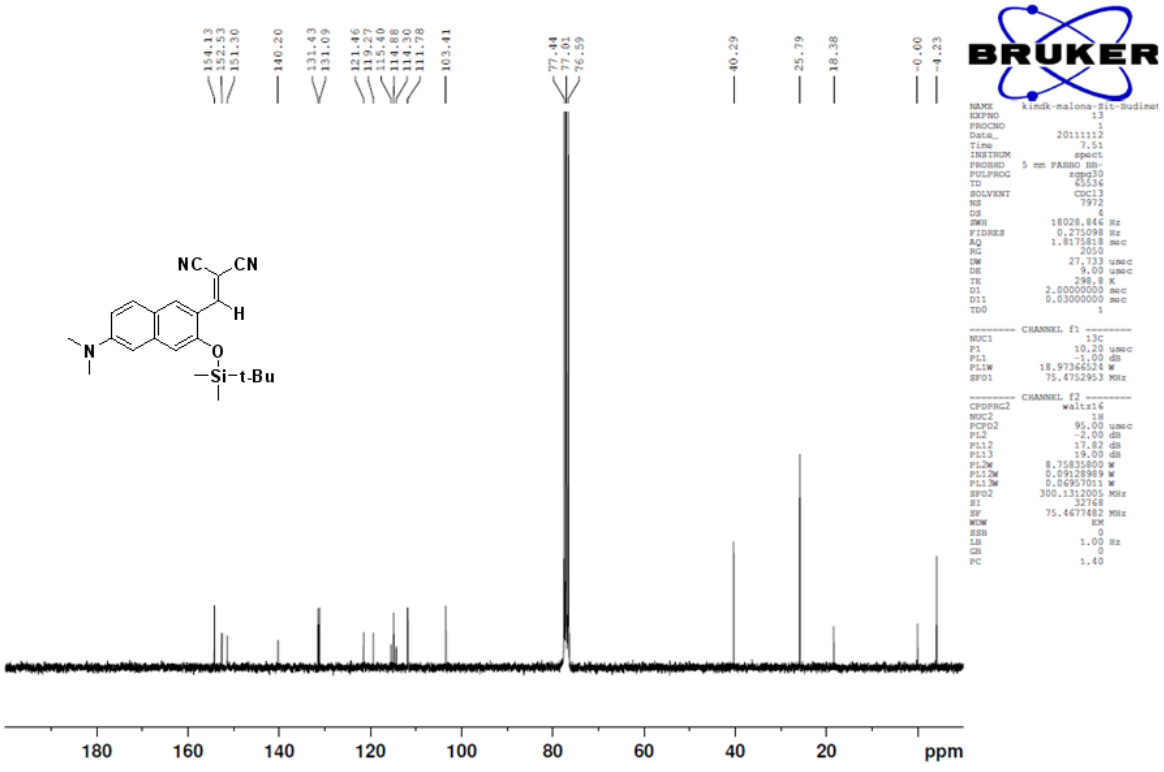
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