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Supplemental information for.

The yin and yang of intracellular delivery of amphipathic optical probes using n-butyl charge masking.

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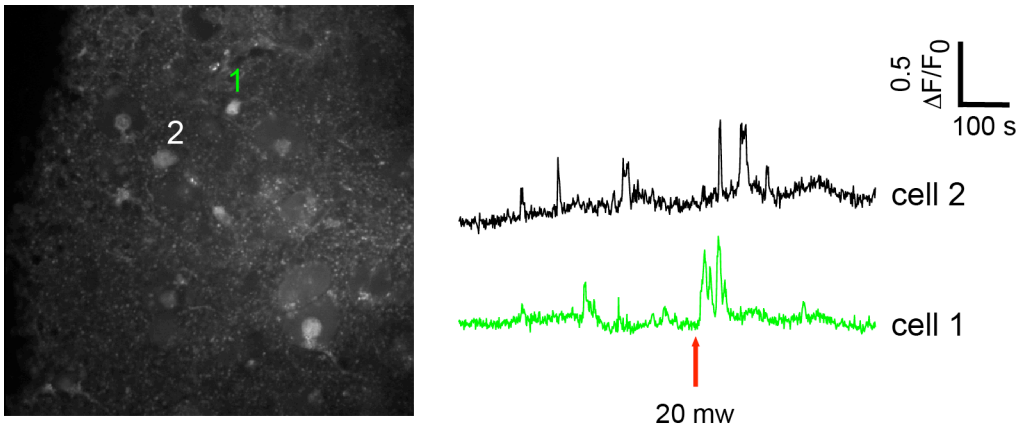
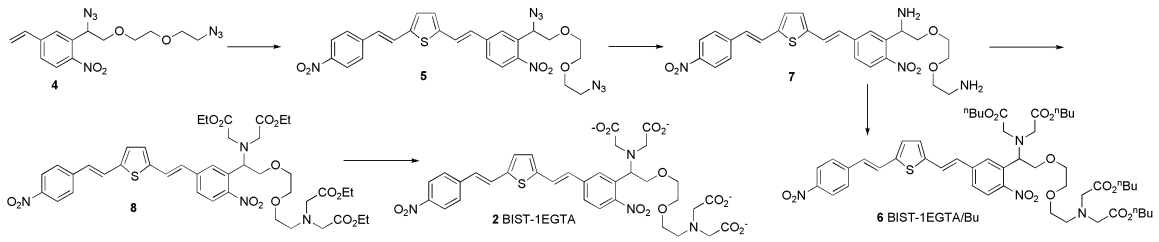


Figure S1. IP_3 photolysis after bolus injection.
Uncaging in cell 1 was with a 3x3 grid of 20 ms flashes of 720 nm light directed at the cell body. A small Ca^{2+} wave was evoked in the near by cell 2.

Synthesis.

General. All chemicals were purchased from commercial sources and used as received unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on Merck KGaA glass silica gel plates (60 F254) and were visualized with UV light or ninhydrin staining followed by heating. Flash chromatography was performed using Agela Technologies industrial grade silica (200-300 mesh, 40-60 microns). NMR spectra were recorded on a Varian 300 MHz NMR spectrometer. The chemical shifts are reported in ppm using the solvent peak as the internal standard. Peaks are reported as: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet. High resolution mass spectral data were obtained using an Agilent G1969A ToF LC-MS (Agilent, Santa Clara, CA, USA). UV-Vis absorption spectra were recorded using a Cary 50 spectrophotometer (Agilent). Photolysis used a 473 nm laser (LRS-0473-PFM-00100-05, Laserglow, Toronto, Canada) in HEPES buffer (40 mM, 100 mM KCl, pH 7.4) in a quartz cuvette (1 cm pathlength).

Synthesis of BIST-1EGTA (**2**) and BIST-1EGTA/Bu (**6**).



2-((E)-3-(1-azido-2-(2-(2-azidoethoxy)ethoxy)ethyl)-4-nitrostyryl)-5-((E)-4-nitrostyryl)thiophene (5). To a solution of 2-(4-Nitrostyryl)-5-vinylthiophene[1] (280 mg, 0.36 mmol), (**4**, 290 mg, 0.725 mmol) in DMF (5 mL) was added lithium chloride (170 mg, 4 mmol), sodium bicarbonate (336 mg, 4 mmol), and tetrabutylammonium chloride (278 mg, 1 mmol), which was then purged with N_2 for 20 min. Palladium(II) acetate (16 mg, 0.07 mmol) was added and the reaction mixture was heated at 110 °C for 1 h. Water (100 mL) was added and the reaction mixture was then extracted with ethyl acetate (3 x 100 mL). The organic layer was separated, concentrated in vacuo and purified by flash column chromatography (35% ethyl acetate in hexanes) to give 320 mg (77%) of **5** as an amorphous red solid. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.19 (d, J = 8.8 Hz, 2H, Ar H), 8.01

(d, *J* = 8.6 Hz, 1H, Ar H), 7.72 (d, *J* = 1.7 Hz, 2H, Ar H), 7.57 (d, *J* = 8.8 Hz, 2H, Ar H), 7.50 (dd, *J* = 1.8 and 8.6 Hz, 1H, Ar H), 7.33 (dd, *J* = 16 and 2.5 Hz, 2H, Ar H), 7.05-7.13 (m, 2H, thiophene-C*H*), 6.91 (dd, *J* = 8.3 and 16 Hz, 2H, Ar-H), 5.62 (dd, *J* = 3.3 and 8.0 Hz, 1H, benzylic-C*H*), 3.90 (dd, *J* = 3.5 and 10.5 Hz, 1H, -CH_2-), 3.58-3.83 (m, 9H, -CH_2-), 3.38 (t, *J* = 3.4 Hz, 2H, -C*H*2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.55, 145.98, 142.95, 142.22, 141.97, 132.92, 129.27, 129.09, 126.83, 126.56, 126.51, 126.04, 125.98, 125.85, 125.69, 125.63, 124.06, 74.97, 70.74, 70.66, 70.67, 60.36, 50.72. HRMS (m/z) for $C_{26}H_{24}N_8O_6S$. Calcd. 576.1540. Found: m/z 599.1408 $(M+Na)^+$.

3,12-bis(carboxymethyl)-4-(2-nitro-5-((E)-2-(5-((E)-4-nitrostyryl)thiophen-2-yl)vinyl)phenyl)-6,9-dioxa-3,12-diazatetradecane-1,14-dioic acid (2). To a solution of **5** (300 mg, 0.52 mmol) in THF (20 mL) and water (5 mL) was added triphenylphosphene (1.65 g, 6.25 mmol), and the reaction mixture was stirred at RT for 2 h. Sodium hydroxide solution (4 M, 5 mL) was added and the reaction mixture was stirred at RT for 18 h. Water (100 mL) was added and the reaction mixture was extracted with dichloromethane (3 x 100 mL). The organic layer was separated and extracted with acidic water (3 x 100 mL 0.1% TFA). The aqueous layer was separated and washed with dichloromethane (100 mL), dried by lyophilization and purified by C_{18}-reverse phase HPLC (isocratic elution 40% acetonitrile in water with 0.1% TFA) to give 247 mg (70%) of **7**, with trace impurities of triphenylphosphine oxide, but pure enough for the next step. ^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.75 (s, 2H, -NH_2), 8.24 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.08-8.18 (m, 2H, Ar-H), 7.68-8.04 (m, 4H, -NH_2 and Ar-H), 7.50-7.68 (m, 3H, Ar-H) 7.35 (dd, *J* = 3.6 and 16.6 Hz, 2H, Ar-H), 7.11 (dd, *J* = 16.1 and 23.7 Hz, 2H, Ar-H), 5.02-5.16 (m, 1H, benzylic-C*H*), 3.84-3.99 (m, 2H, -CH_2-), 3.56-3.76 (m, 6H, -CH_2-), 2,92-3.04 (m, 2H, -CH_2-). To a solution of **7** (45 mg, 0.086 mmol), pentamethylpiperidine (0.115 mL, 0.86 mmol) and sodium iodide (630 mg, 4.2 mmol) in anhydrous acetonitrile (50 mL) was added ethyl bromoacetate (0.9 mL, 8.44 mmol, in four equal portions over 72 h) and the reaction mixture was heated for 72 h at 60°C. The solution was concentrated in vacuo and purified by flash chromatography (5% methanol in dichloromethane) to give a red-colored amorphous solid. This was dissolved in 60% acetonitrile in water (v/v) and purified by reverse phase HPLC using acetonitrile in water (0.1 % TFA) using a linear gradient of acetonitrile of 60-80% over 1 h. The solvents were removed under reduced pressure to give 56 mg (62%) of **8**. ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.6 Hz, 2H, Ar H), 8.03-8.16 (m, 1H, Ar H), 7.83 (d, *J* = 8.5 Hz, 1H, Ar H), 7.58 (d, *J* = 8.6 Hz, 2H, Ar H), 7.45 (d, *J* = 8.1 Hz, 1H, Ar H), 7.34 (t, *J* = 16 Hz, 2H, Ar H), 7.03-7.17 (m, 2H,

thiophene-C*H*), 6.94 (dd, *J* = 16 Hz, 2H, Ar-H), 4.97-5.14 (m, 1H, benzylic-C*H*), 4.07-4.20 (m, 8H, -CH_2-), 3.43-3.73 (m, 16H, -CH_2-), 2.85-3.04 (m, 2H, -CH_2-), 1.15-1.32 (m, 12H, ethyl-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.26, 165.87, 148.12, 146.62, 143.09, 142.39, 141.95, 141.69, 136.29, 129.15, 129.06, 127.91, 126.61, 126.44, 125.79, 125.68, 125.16, 124.15, 73.29, 70.13, 70.02, 66.69, 62.45, 60.73, 59.31, 54.64, 54.60, 53.90, 53.24, 14.36, 14.11. HRMS (m/z) for $C_{42}H_{52}N_4O_{14}S$. Calcd. 868.3201. Found: m/z 869.3283 $(M+H)^+$.

Compound **8** (56 mg, 0.064 mmol) was dissolved in methanol (25 mL) and potassium hydroxide (9 M, 0.087 mL) was added to the solution. The reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated and residue was purified by reverse phase HPLC using 35% acetonitrile in water (0.1 % TFA) as the solvent system. Solvents were removed by lyophilization to give 25 mg (51%) of **2** as an amorphous red solid. ^{1}H NMR: (300 MHz, Acetone-d_6) δ 8.20 (d, *J* = 8.8 Hz, 2H, Ar H), 8.09 (d, *J* = 1.4 Hz, 1H, Ar H), 7.88 (d, *J* = 8.5 Hz, 1H, Ar H), 7.83 (d, *J* = 8.8 Hz, 2H, Ar H), 7.73 (d, *J* = 16.1 Hz, 1H, Ar H), 7.67 (d, *J* = 1.6 Hz, 1H, Ar H), 7.59 (d, *J* = 16.1 Hz, 1H, Ar H), 7.30 (dd, *J* = 4.0 and 5.4 Hz, 2H, thiophene-C*H*), 7.10 (d, *J* = 16.1 Hz, 1H, Ar-H), 7.01 (d, *J* = 16.1 Hz, 1H, Ar-H), 4.87 (dd, *J* = 4.3 and 6.2 Hz, 1H, benzylic-C*H*), 4.02 (s, 4H, -CH_2-), 3.79 (dd, *J* = 6.9 and 10.99 Hz, 1H, -CH_2-), 3.25-3.72 (m, 13H, -CH_2-). 13C NMR (75 MHz, $CDCl_3$) δ 173.34, 169.45, 148.78, 146.74, 144.11, 142.88, 142.53, 141.73, 136.79, 130.68, 130.29, 128.44, 127.87, 127.41, 127.14, 126.96, 126.63, 126.22, 125.63, 124.75, 73.04, 70.15, 69.96, 66.99, 59.27, 55.72, 55.37, 53.41. HRMS (m/z) for $C_{34}H_{36}N_4O_{14}S$. Calcd. 756.1949. Found: m/z 757.2020 $(M+H)^+$.

Dibutyl 3,12-bis(2-butoxy-2-oxoethyl)-4-(2-nitro-5-((E)-2-(5-((E)-4-nitrostyryl)thiophen-2-yl)vinyl)phenyl)-6,9-dioxa-3,12-diazatetradecane-1,14-dioate (6). To a solution of **7** (190 mg, 0.362 mmol) and DIPEA (10 mL) in anhydrous acetonitrile (100 mL) was added of n-butyl iodoacetate (7.0 mL, 28.92 mmol) in portions of 1 mL over the period of 72 h. During this period, the reaction mixture was heated at 60 °C . After 72 h the solution was concentrated in vacuo and purified by flash chromatography (5% methanol in dichloromethane) to give a red-colored amorphous solid. This was dissolved in 60% acetonitrile in water (v/v) and purified by reverse phase HPLC using acetonitrile in water (0.1 % TFA) using a linear gradient of acetonitrile of 60-80% over 1 h. The solvents were removed under reduced pressure to give 178 mg (50%) of **6**. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.6 Hz, 2H, Ar H), 8.15 (s, 1H, Ar H), 7.85 (d, *J* = 8.5 Hz, 1H, Ar H), 7.59 (d, *J* = 8.6 Hz, 2H, Ar H), 7.46 (d, *J* = 8.5 Hz, 1H, Ar H), 7.37 (t, *J* = 15.2 Hz, 2H, Ar

H), 7.07-7.15 (m, 2H, thiophene-C*H*), 6.94 (dd, *J* = 9.6 and 16 Hz, 2H, Ar-H), 5.02-5.10 (m, 1H, benzylic-C*H*), 4.26 (s, 4H, -CH_2-), 4.16 (t, *J* = 6.7 Hz, 4H, -CH_2-), 4.02-4.12 (m, 4H, -CH_2-), 3.44-3.86 (m, 12H, -CH_2-), 2.93 (dd, 1H, -CH_2-), 2.37 (dd, 1H, -CH_2-), 1.54-1.66 (m, 8H, butyl-CH_2-), 1.28-1.40 (m, 8H, butyl-CH_2-), 0.91 (t, J = 7.3 Hz, 12H, butyl-CH_3). 13C NMR (100 MHz, $CDCl_3$) δ 171.74, 171.57, 166.01, 148.17, 146.61, 143.18, 142.43, 141.98, 141.75, 136.39, 129.22, 129.07, 127.83, 126.61, 126.35, 125.84, 125.70, 125.64, 125.15, 124.12, 73.19, 69.98, 69.95, 66.22, 66.14, 64.68, 64.48, 59.24, 54.30, 53.72, 53.04, 41.92, 34.59, 30.51, 30.46, 30.18, 19.02, 18.97, 18.80, 13.55, 13.44. HRMS (m/z) for $C_{50}H_{68}N_4O_{14}S$. Calcd. 980.4453. Found: m/z 981.4584 $(M+H)^+$.

Synthesis of NV-IP3/Bu (**3**).

(±)-1,4,5-Tris[bis-n-butylphospho]-2,3-O-Cyclohexilydene-6-O-(ortho-nitroveratryl)-*myo*-inositol 1,4,5-trisphosphate (3): To a solution of (±)-2,3 :4,5-di-O-cyclohexylidene-6-O-(ortho-nitroveratryl)-*myo*-inositol[2] (0.050 g, 0.10 mmol), dibutyl-N,N diisopropylphosphoramidite (0.182 g, 0.65 mmol) in dichloromethane (4 mL) was added tetrazole (0.035 g, 0.5 mmol) in acetonitrile (1.5 mL), then stirred at RT for 18 h. The reaction mixture was cooled to 5–10°C, and *tert*-butyl hydrogen peroxide (0.25 mL of 30% solution in water) was added, then the solution stirred at RT for 4 h. Dichloromethane (20 mL) was added and washed with water (10 mL), saturated $NaHCO_3$ (10 mL), and saturated NaCl solution (20 mL), then separated and dried with $NaSO_4$, The organic layer was concentrated in vacuo and purified by flash chromatography (2% methanol in dichloromethane) to give 0.090g of **3** (79 %) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.60 (s, 1H), 5.18 (d, J = 3.9 Hz, 2H), 4.71 (d, J = 21.2 Hz, 1H), 4.57 (d, J = 33.7 Hz, 3H), 4.38 – 4.21 (m, 1H), 4.18 – 3.98 (m, 11H), 3.98 – 3.82 (m, 3H), 3.87 – 3.51 (m, 3H), 3.40 (s, 1H), 1.84 – 1.43 (m, 16H), 1.49 – 1.13 (m, 12H), 1.07 (d, J = 7.5 Hz, 3H), 0.90 (dd, J = 7.9, 3.8 Hz, 9H), 0.69 (t, J = 7.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0, 147.3, 138.1, 130.8, 111.5, 110.2, 107.2, 78.1, 78.1, 76.1, 74.9, 74.8, 73.8, 70.9, 67.8, 67.8, 67.7, 67.7, 67.6, 67.6, 67.5, 67.4, 67.3, 56.8, 56.2, 36.6, 34.4, 32.2, 32.2, 32.1, 32.1, 32.0, 31.9, 31.8, 31.8, 24.9, 23.8, 23.5, 18.6, 18.5, 18.3, 18.3, 13.5, 13.5, 13.4, 13.3, 13.3. ^{31}P NMR (121 MHz, $CDCl_3$) δ -1.54, -1.68, -2.17. HRMS: (m/z) for $C_{45}H_{80}N_4O_{19}P_3$. Calcd. 1031.4537. Found: m/z 1032.4845 $(M+H^+)$.

Ca^{2+} affinity.

To a solution of BIST-1EGTA (1 mM) in HEPES (3 mL, 40 mM, pH 7.5) and KCl (100 mM) was added $CaCl_2$ (0.003 mL, 100 mM) in sequential amounts. The [Ca^{2+}] was measured with a Ca^{2+}-selective electrode as previously described[1]. Scatchard analysis revealed the affinity was 8.9 nM. The same value was obtained in an independent titration experiment using fluorescence from X-rhod-1 the measure the change in [Ca^{2+}].

Quantum yield.
The time course of photolysis of solutions BIST-1EGTA and DEAC450-Glu[3] with 473-nm light was followed by HPLC. The former was photolyzed at a rate of 80% of the latter, corresponding to a quantum yield of 0.31.

Power dependence of Ca^{2+} uncaging.
A Ti:sapphire laser (Mira 9000F, Coherent, Santa Clara, CA, USA) pumped by 8 W solid-state Verdi V-8 (Coherent) was used for both 2P (i.e. mode-locked) and continuous wave excitation of BIST-1EGTA. Samples were irradiated at 810 nm, with pulse duration of ~120 fs, when mode-locked. Laser power was measured in the objective focal plane by a power meter (PM200 with sensor S170C, Thorlabs, Newton, New Jersey, USA). The laser beam was guided to the SIM scanner of the confocal microscope (Fluoview 1000, Olympus, Volketswil, Switzerland) operating in single point excitation mode simultaneously with the main scanner. The irradiation period was 20 ms (controlled by an electronic shutter LS3, Vincent Associates, Rochester, NY, USA) and was adjusted by neutral density and polarizing filters, to give a power train between 0.8 and 6 mW. The confocal microscope was operating in line scan mode. To record changes in Ca^{2+} concentration rhod-FF (Teflabs, Austin, TX, USA) was excited at 561 nm. Solutions used for droplet experiments was composed of (mM): 1 BIST-1EGTA K_4, 0.1 rhod-FF, 1 $CaCl_2$, 100 KCl, 10 HEPES, pH = 8.0. Recorded images were analyzed in MATLAB (MathWorks, Inc., Natick, MA, USA) and the results were fitted with a quadratic equation.

Physiological experiments.

Ethical approvals.
Housing and breeding of mice was carried out with free access to rodent laboratory chow and water. In NYC all experiments were approved by Mount Sinai IACUC review. In Bern handling was performed with permission of the State Veterinary Administration

of the Canton of Bern (BE) and in accordance with Swiss Federal Animal protection law. The animal experimentation permit (No. BE 6/2019) was issued after ethical review of our experimental and preparatory procedures, including the death of the mice (see below), by the State Committee for Animal Ethics and with endorsement by the State Veterinarian of the Canton of Bern, and after final approval by the Swiss Federal Food Safety and Veterinary Office.

Brain slices experiments.

C57BL/6J mice (2-5 weeks.) were anaesthetized with isoflurane and the brain was quickly removed. Horizontal slice sections (350 μm) were then made in ice-cold cutting solution containing (in mM): 60 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, 100 sucrose, 3 sodium pyruvate, 1.3 sodium ascorbate equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). The brain slices were then incubated for 15 min at 33°C in artificial cerebrospinal fluid (ACSF, mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, 3 sodium pyruvate, 1.3 sodium ascorbate; 95% O_2 and 5% CO_2, pH 7.4). Slices from juvenile mice (ca. 2 weeks) were placed in a small chamber with 1-2 mL of ACSF containing 50 μg each the of Ca^{2+} dye (Oregon Green BAPTA-1, OGB1) and compound **6** previously dissolved in 5 μL 20% Pluronic-F127 in DMSO. The slice was held under carbogen atmosphere to maintain the pH for 30 mins. After incubation, all brain slices were held in ACSF at room temperature > 1 hour before imaging. For young adult brain slices the probes were loaded by pressure injection of concentrated solution of dye and **6** below the surface of the slice using a piecospritzer on the microscope chamber.

Two-photon microscopy.

Brain slices were imaged on a BX-61 microscope with a LUMFLN60XW objective (1.1 numerical aperture, Olympus, Penn Valley, PA, USA) in a chamber perfused with ACSF (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, 95% O_2 and 5% CO_2, pH 7.4) at room temperature. Two-photon microscopy was performed with Ultima dual-galvo scan head (Prairie Technologies, Inc., WI, USA) controlled by Prairie View 5.3, using a Vision II laser for imaging at 820 nm or 950 nm and a Chameleon laser for uncaging at 720 nm (both Coherent, Santa Clara, CA, USA), both modulated by 350-80 Pockels cells (Conoptics, Danby, CT, USA). Hamamatsu R3896 alkali, side-on PMTs were used for detection. Uncaging power was measured in the

image plane using a Thor S120 power meter. Astrocytes were indentified according to their characteristic morphology and size.

Image analysis.
Fluorescent intensities were captured in each pixel on a 12-bit scale, and displayed in pseudo-colored mode in which cold (dark blue) and warm (red or white) colors represent a relative change in fluorescence (i.e. $\Delta F/F$, where F is the resting fluorescence and ΔF the signal increase). Baseline fluorescence was defined as the mean fluorescence intensity of the first five frames (~7s) of the image stack for each astrocyte.

Cardiac myocytes isolation
Ventricular myocytes were isolated from C57Bl/6 mice aged between 7 and 8 months following an established protocol. Adult mice were euthanized by cervical dislocation and the hearts were excised, cannulated and retrogradely perfused on a Langendorff system. Hearts were perfused at 37° C for around 15 minutes with a Ca^{2+}-free modified Tyrode solution composed of mmol/l: 140 NaCl, 5.4 KCl, 1.1 $MgCl_2$, 10 HEPES, 1 NaH_2PO_4, 10 glucose (pH 7.4). Cells were enzymatically dissociated using a cocktail of collagenase type II (160 U/mL, Worthington, Switzerland) and protease type XIV (0.21 U/mL, Sigma, Switzerland). After isolation, ventricular myocytes were kept at room temperature in a modified Tyrode containing 250 μM $CaCl_2$ and used within 6 hours.

Intact cardiac myocytes.
Intact ventricular myocytes were loaded for 90 minutes with 20 μM BIST-1EGTA butyl ester at 37°C with added 5 μM rhod-2 acetoxymethyl ester (Invitrogen, Thermo Fisher Scientific, USA) for last 10 minutes in modified Tyrode solution supplemented with 250 μM $CaCl_2$, 0.5% Bovine Serum Albumin and 0.6% Pluronic F-127 (Biotium Inc., Hayward, CA, USA). After 30 minutes of perfusion to allow for de-esterification with modified Tyrode solution (in mmol/l: 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.1 $MgCl_2$, 10 HEPES, 1 NaH_2PO_4, 10 glucose; pH 7.4) myocytes were paced at 1 Hz for 60 s. Laminin (10 μg/ml) was used to coat the coverslips to minimize motion artifacts.

Permeabilized cardiac myocytes.
Isolated ventricular myocytes were permeabilized by exposure to β-escin for 60 s (in mM: 100 K-Asp (potassium aspartate), 20 KCl, 3.7 $MgCl_2$, 0.5 EGTA, 10 HEPES, 0.005 %

β-escin; pH=7.2). Permeabilized myocytes were then placed in recording chamber in final solution containing (mM): 120 K-Asp, 3 K_2ATP, 0.1 EGTA, 3 $MgCl_2$, 10 phosphocreatine, 5 U/mL creatine phosphokinase, 10 HEPES, 1 L-Glutathione reduced, 0.04 $CaCl_2$, 0.05 rhod-2-K_3 (Biotium Inc., Hayward, CA, USA), 0.05 BIST-1EGTA-K_8, final $[Ca^{2+}]_i$ was 60 nM, pH 7.2. Free Ca^{2+} concentration was calculated using Patcher's Power Tools package for Igor Pro. In pharmacological experiments 5 μM cAMP, 1 μM cyclopiazonic acid (CPA) or 10 mM caffeine together with 20 mM 2,3-butanedione 2-monoxime (BDM) was added.

Two photon photolysis in cardiac myocytes.

A Verdi-Mira laser (Coherent) was used for 2P excitation of BIST-1EGTA. Wavelength of the laser was set to 810 nm with pulse duration of ~120 fs. Power of the laser was adjusted by neutral density and polarizing filters and was measured at the objective focal plane by a power meter (PM200 with sensor S170C, Thorlabs). The laser beam was guided to the second SIM scanner of the confocal microscope (Fluoview 1000, Olympus, Volketswil, Switzerland) operating in single point excitation mode simultaneously with the main scanner. The main scanner of the confocal microscope was operating in line scan mode. To record changes in Ca^{2+} concentration rhod-2 (Biotium Inc., Hayward, CA, USA) was excited at 561 nm and detected at 585-685 nm. Hamamatsu GaAsP H7422 PMTs were used for detection. All experiments were performed at room temperature (~22 °C). Chemicals were purchased from Sigma-Aldrich unless stated otherwise.

Image analysis.

Line-scan images were analyzed using a custom-written program in Matlab (Mathworks, USA) similarly as in[4].

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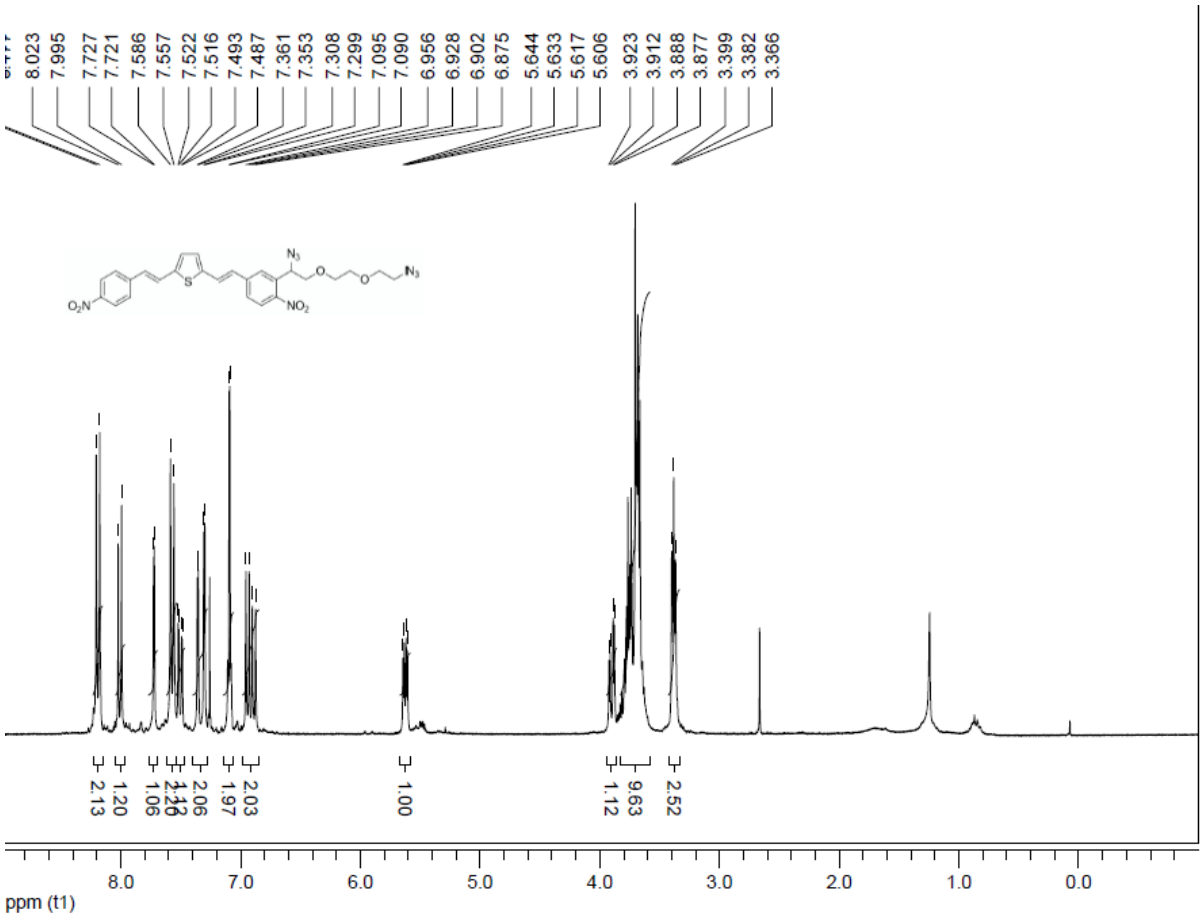
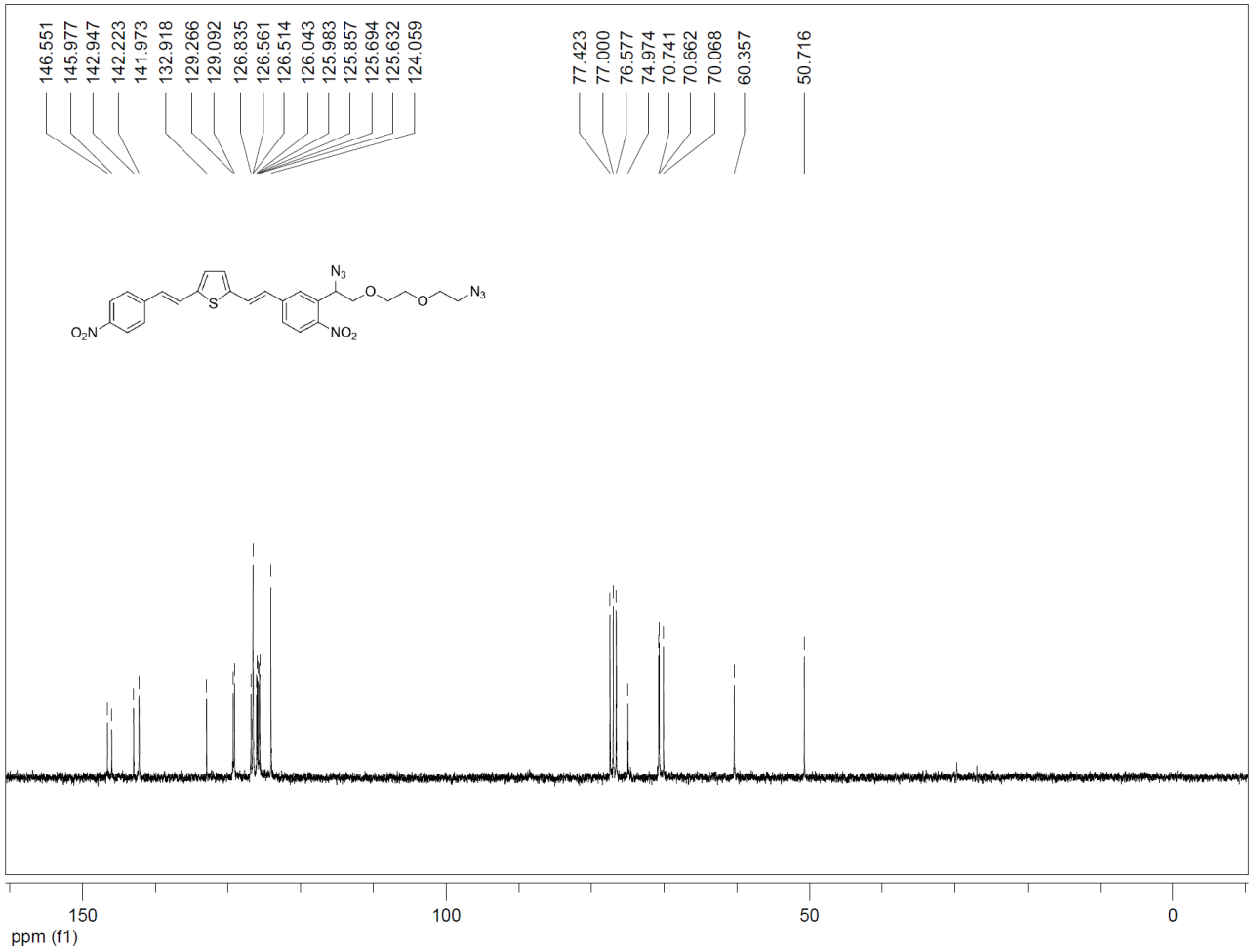
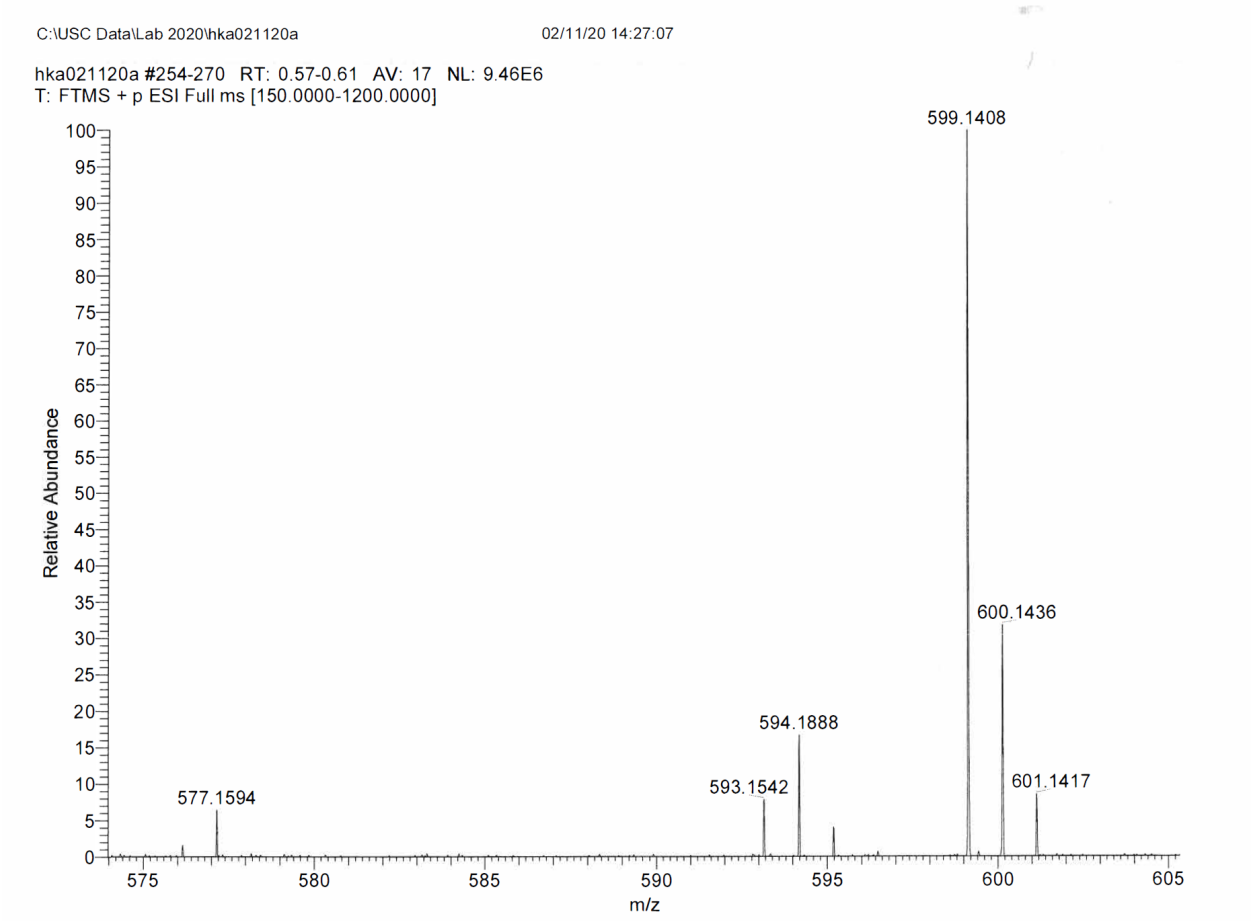


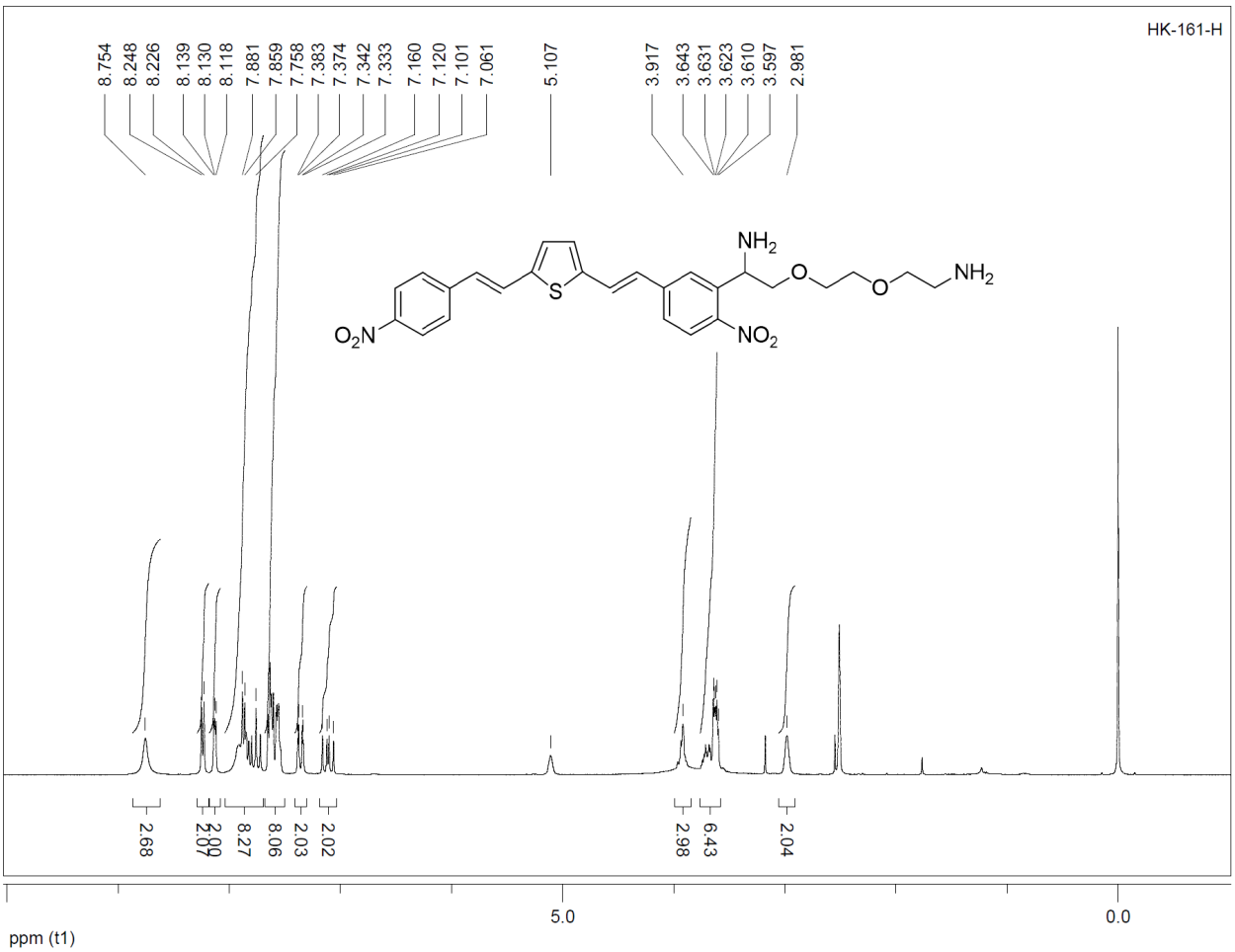
Compund 5. H-1 spectrum in CDCl3.



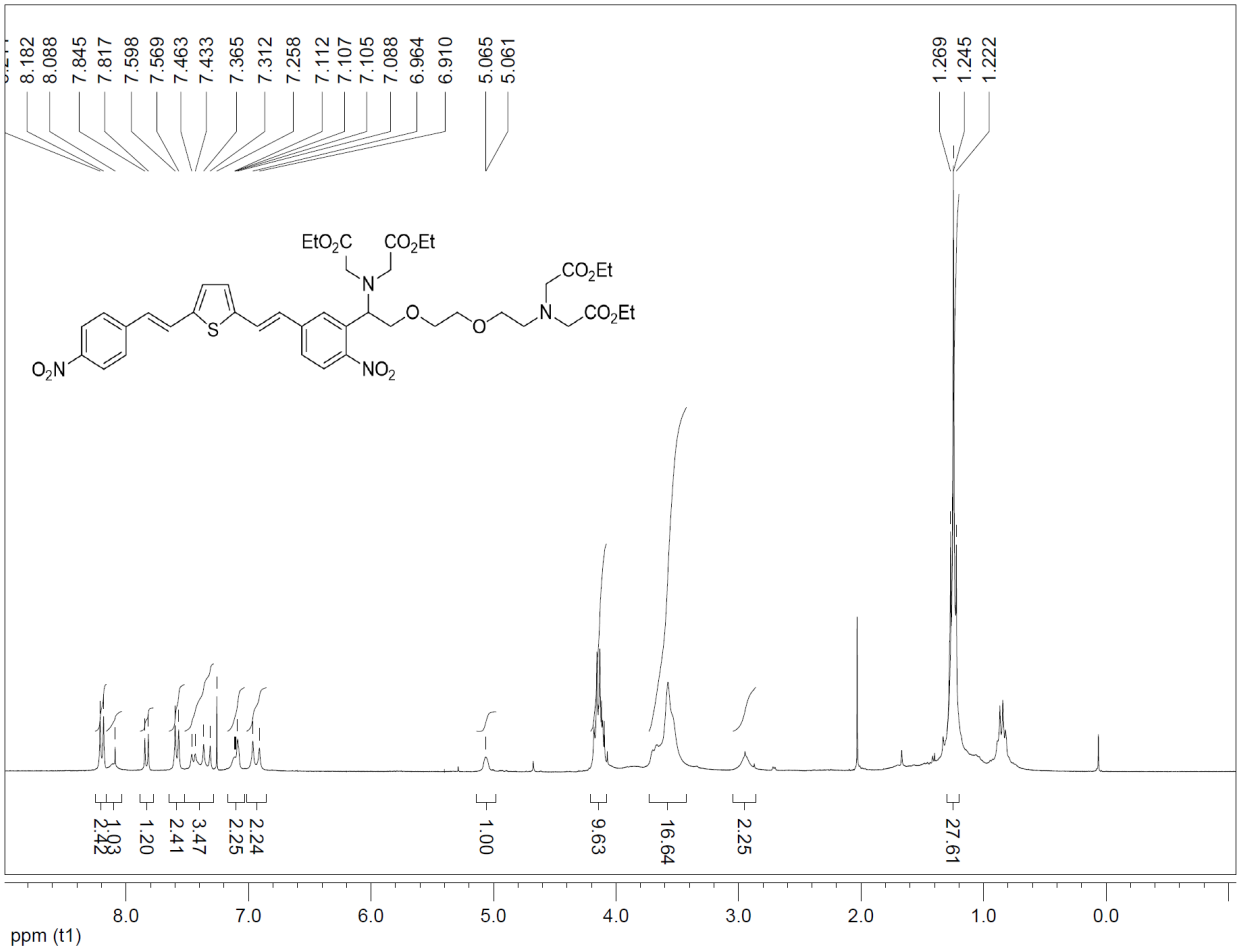
Compound 5, C-13 spectrum in CDCl3.



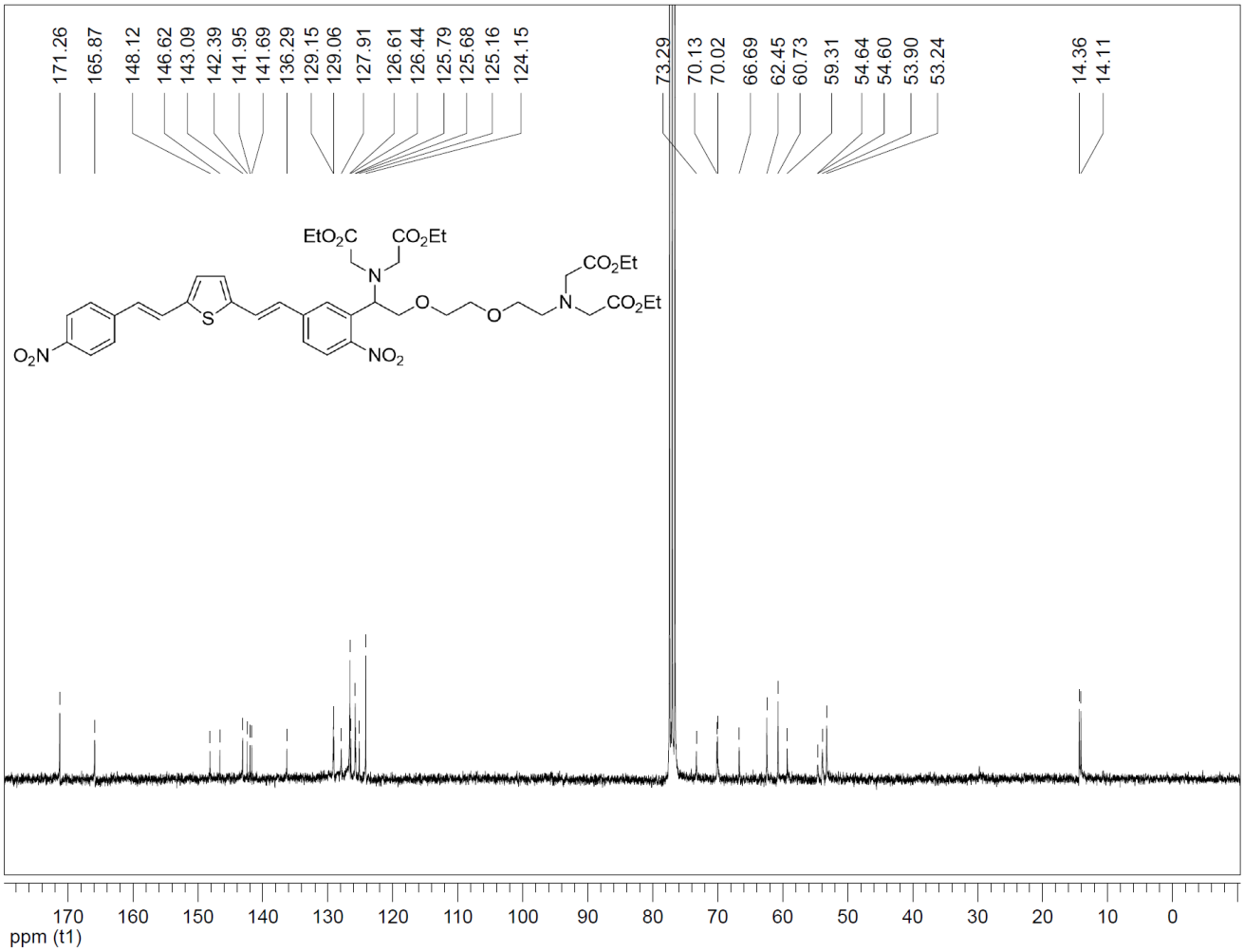
Compound 5. HRMS.



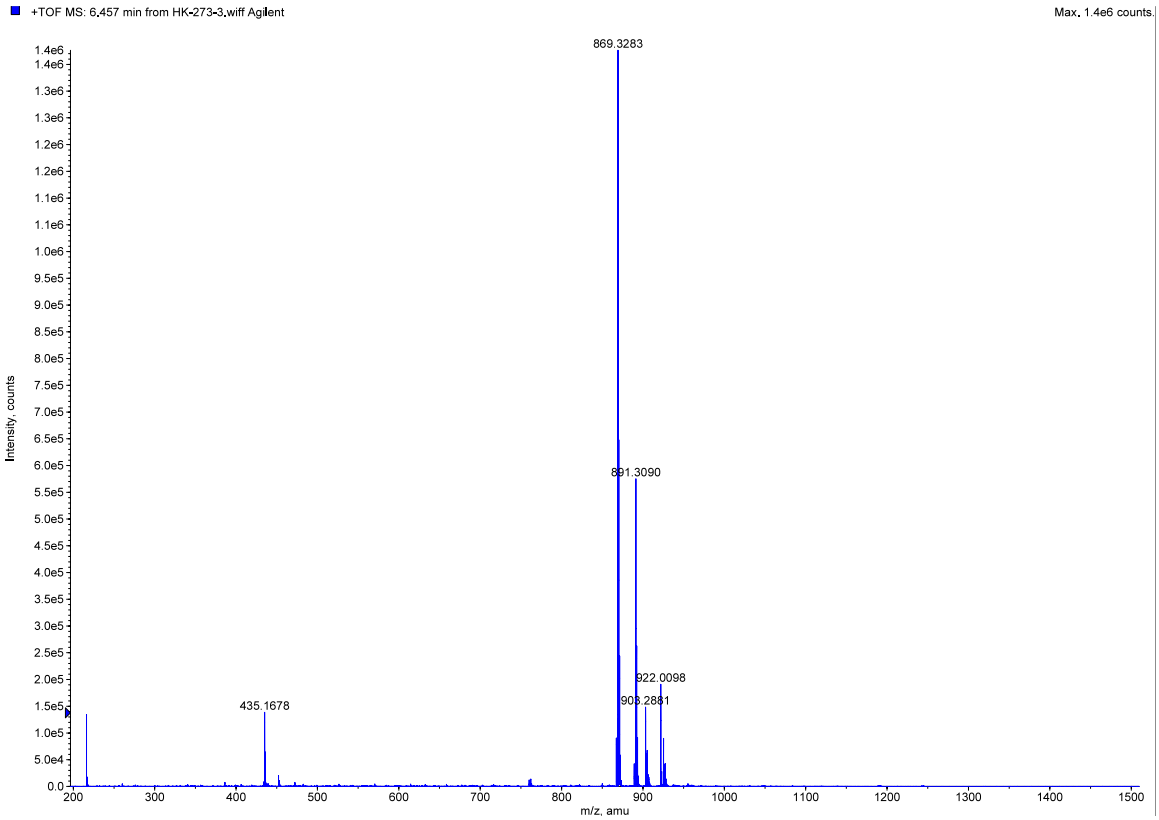
Compound 6. H-1 spectrum in DMSO-d6.



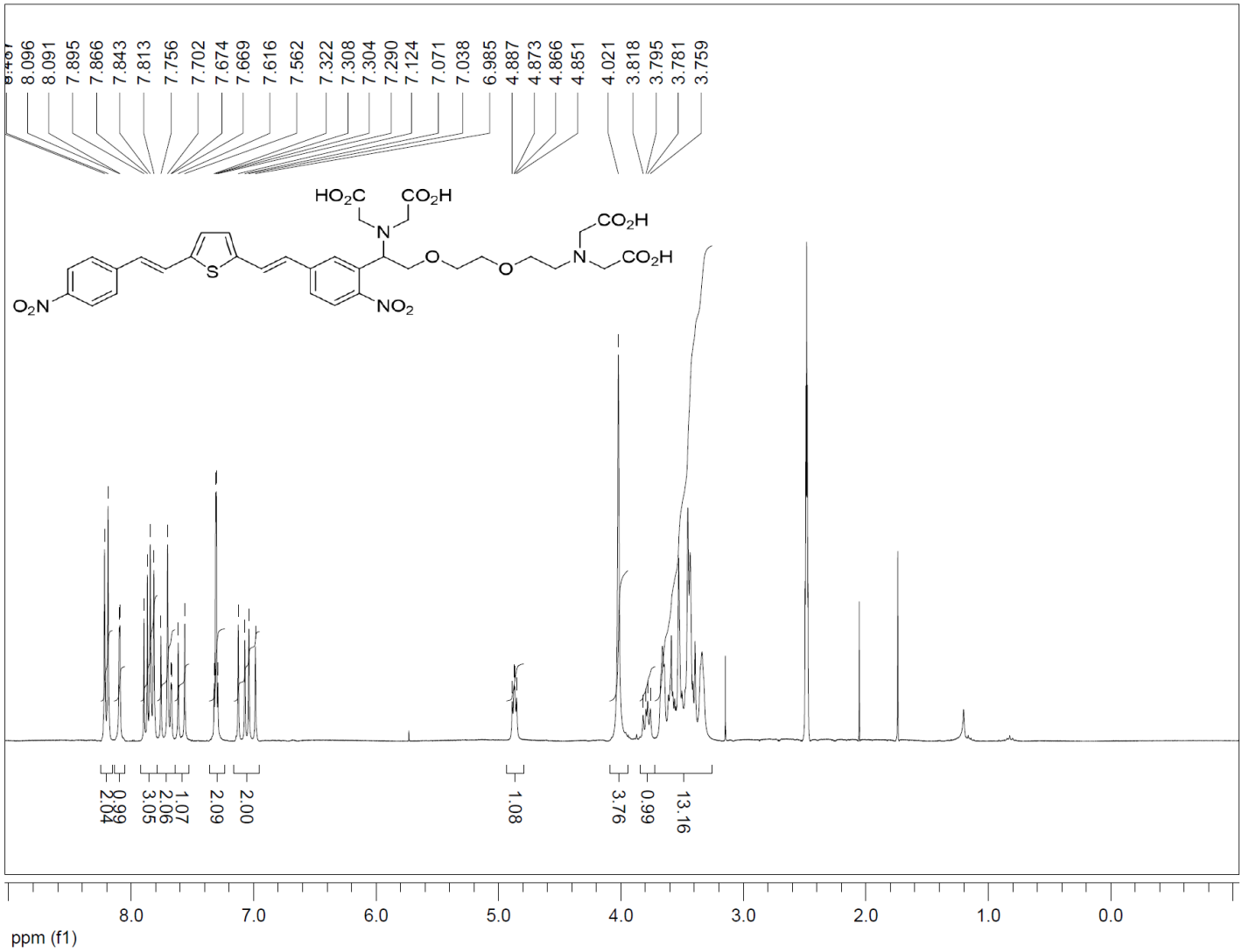
Compound 7. H-1 spectrum in CDCl3



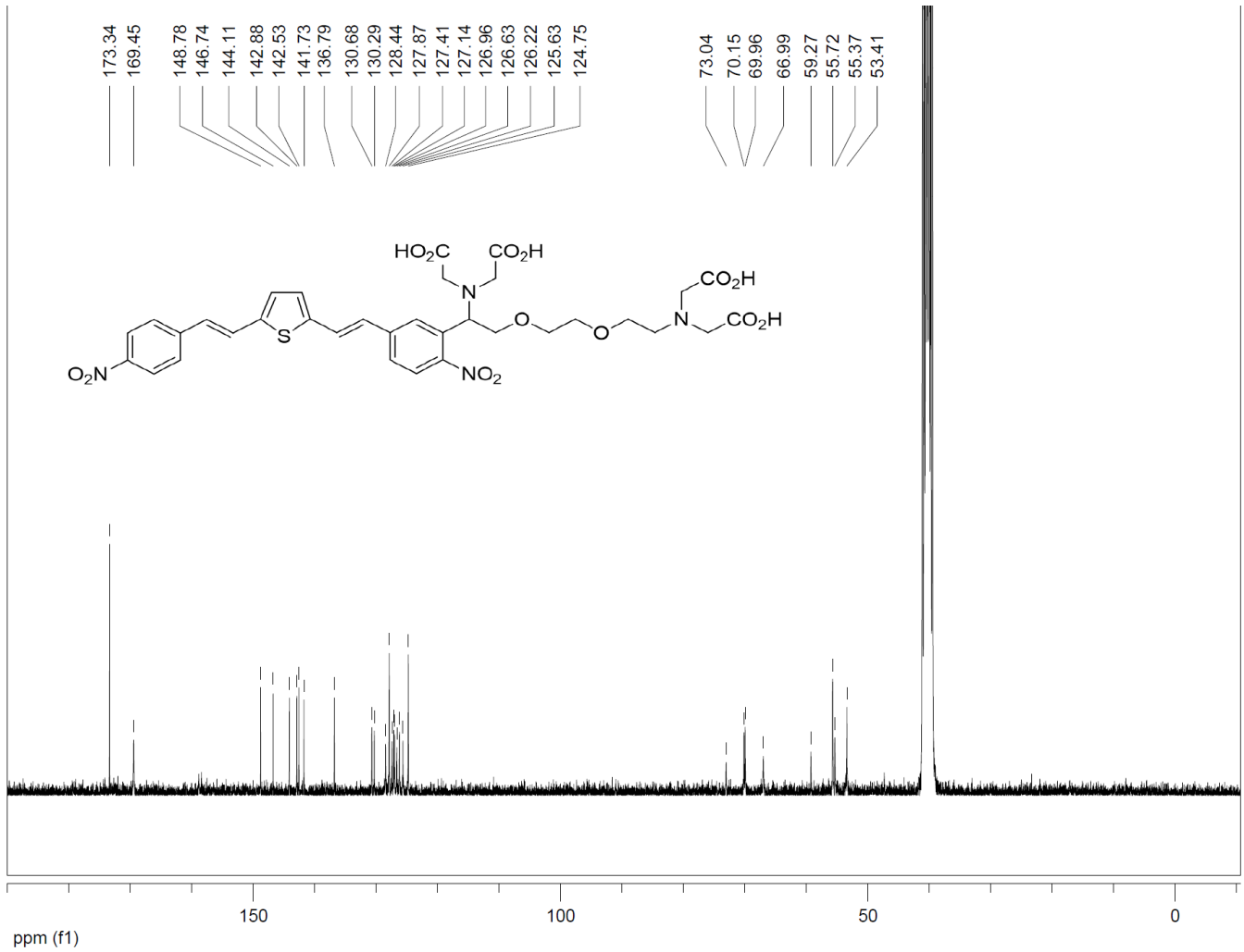
Compound 7. C-13 spectrum in CDCl3



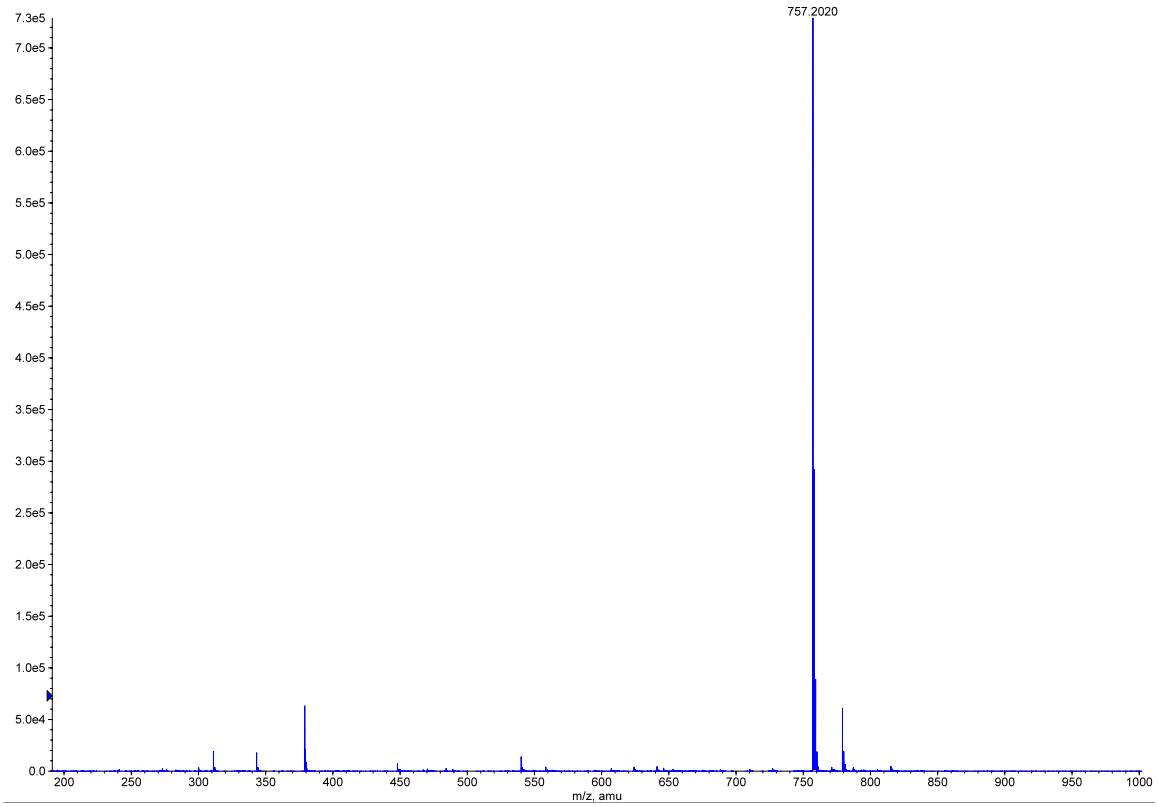
Compound 7. HRMS



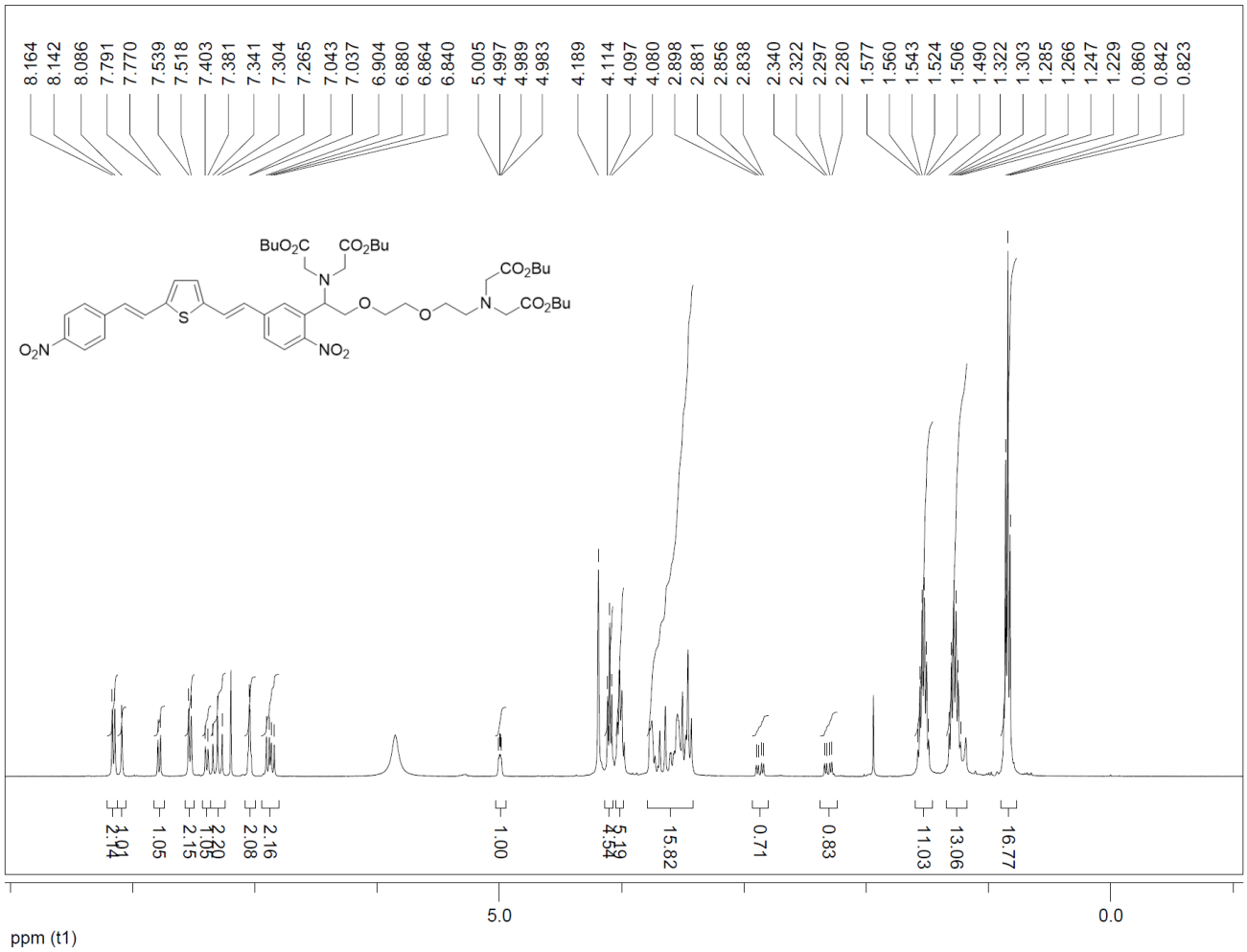
Compound 2 . H-1 spectrum in MeOH-d4



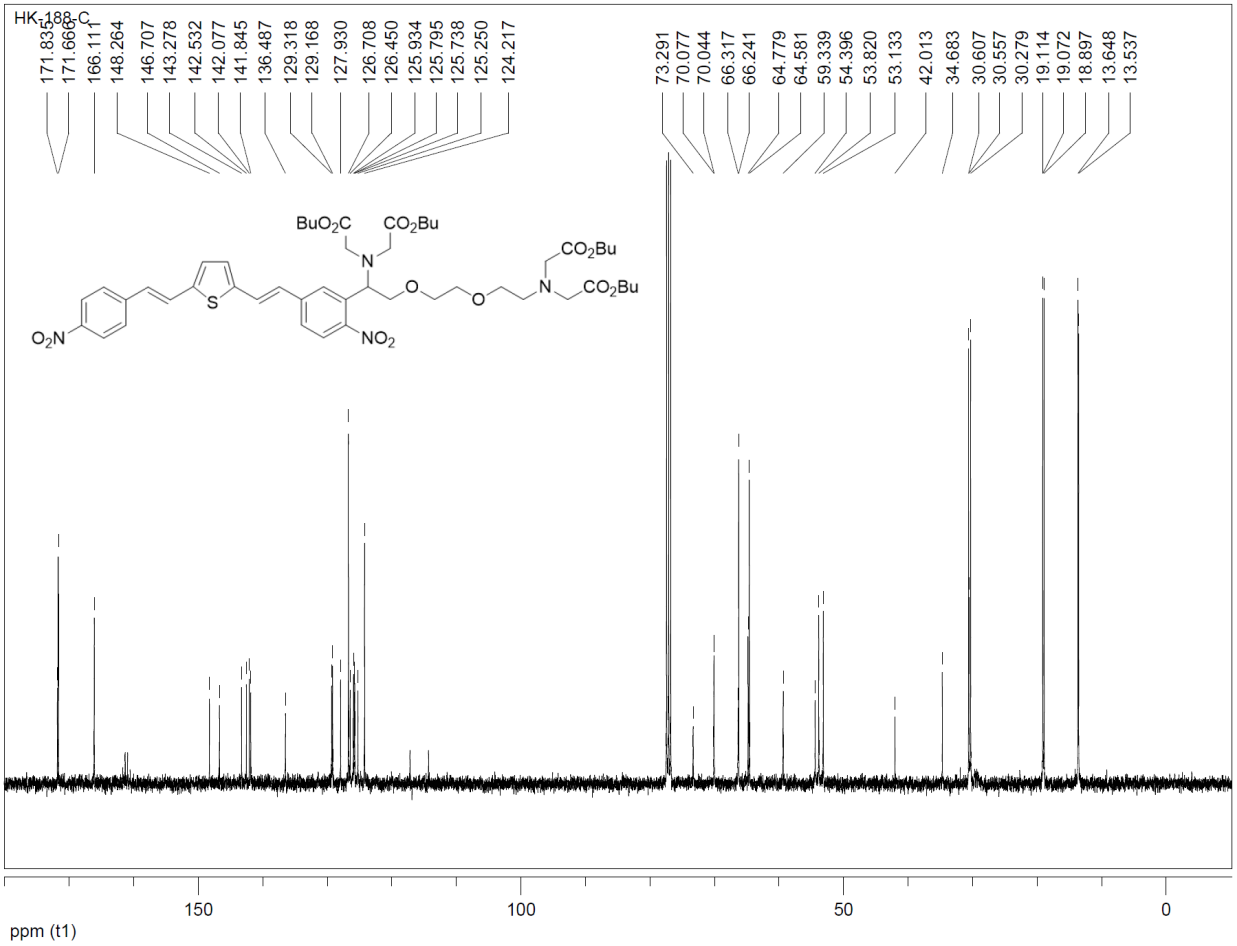
Compound 2 . C-13 spectrum in MeOH-d4



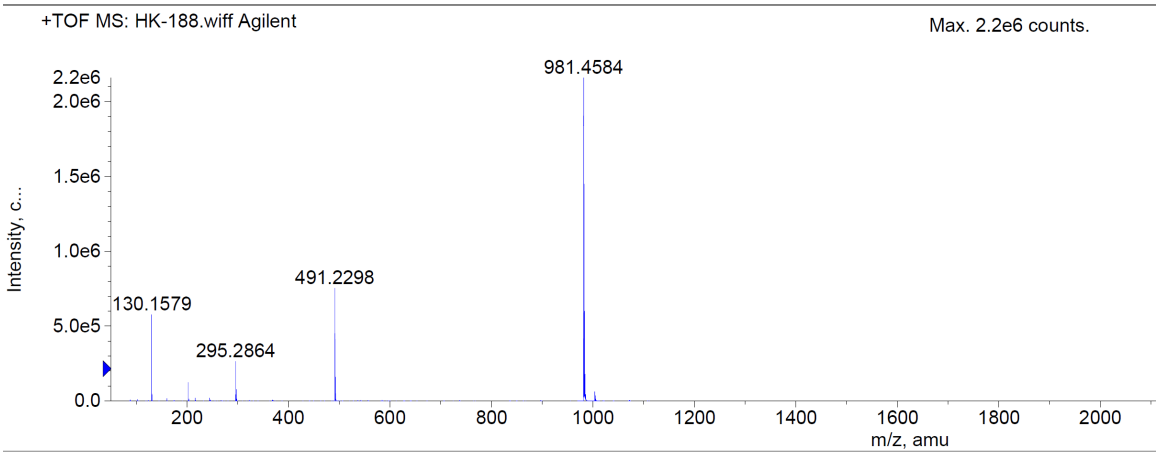
Compound 2. HRMS



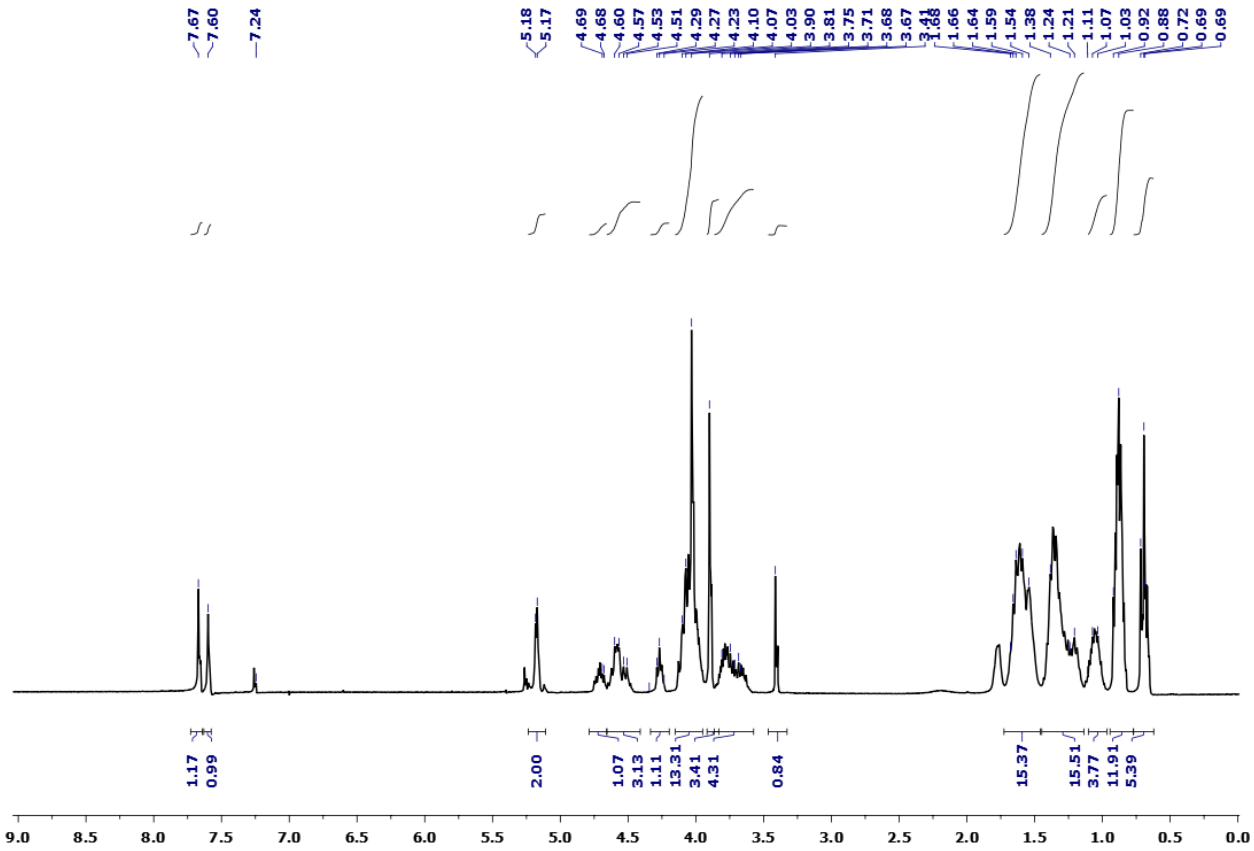
Compound 6. H-1 spectrum in CDCl3.



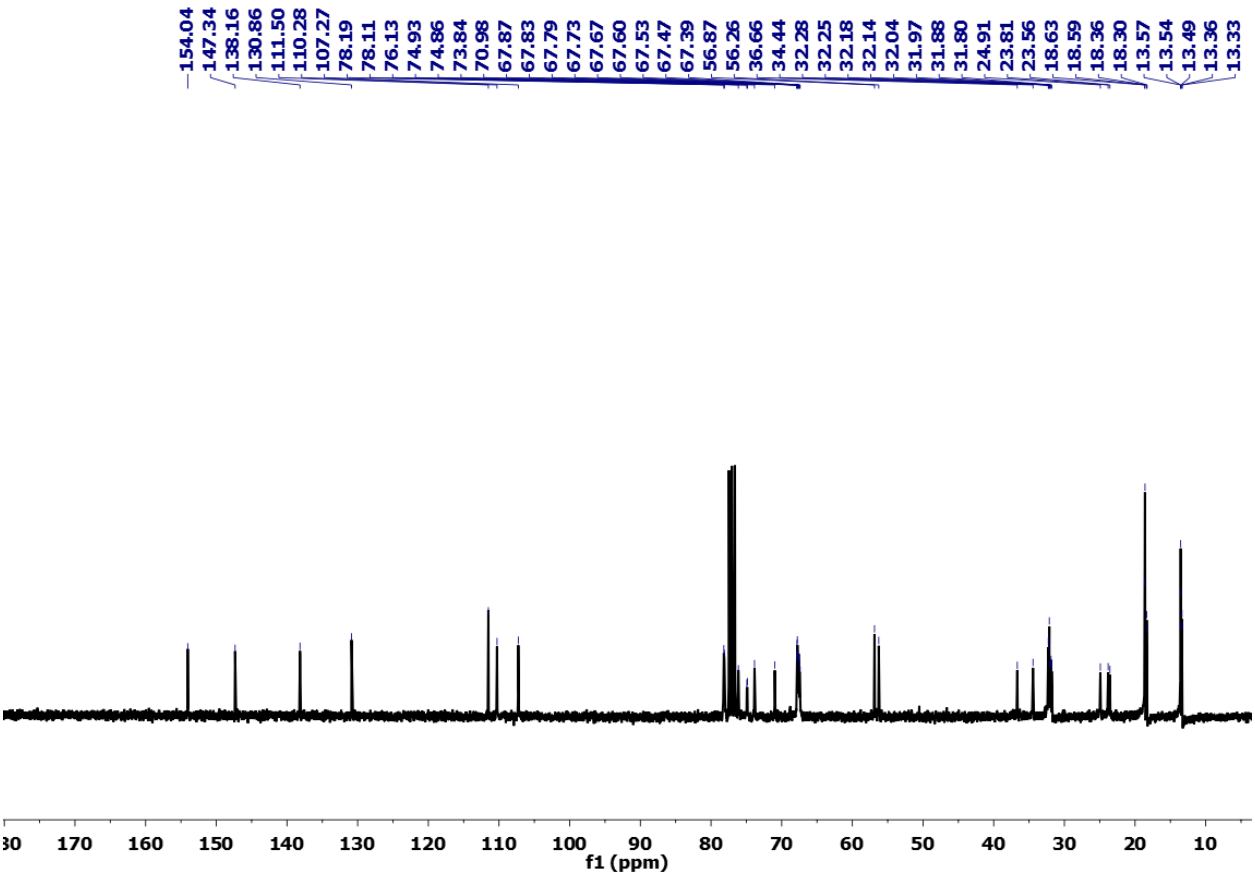
Compound 6. C-13 spectrum in CDCl3.



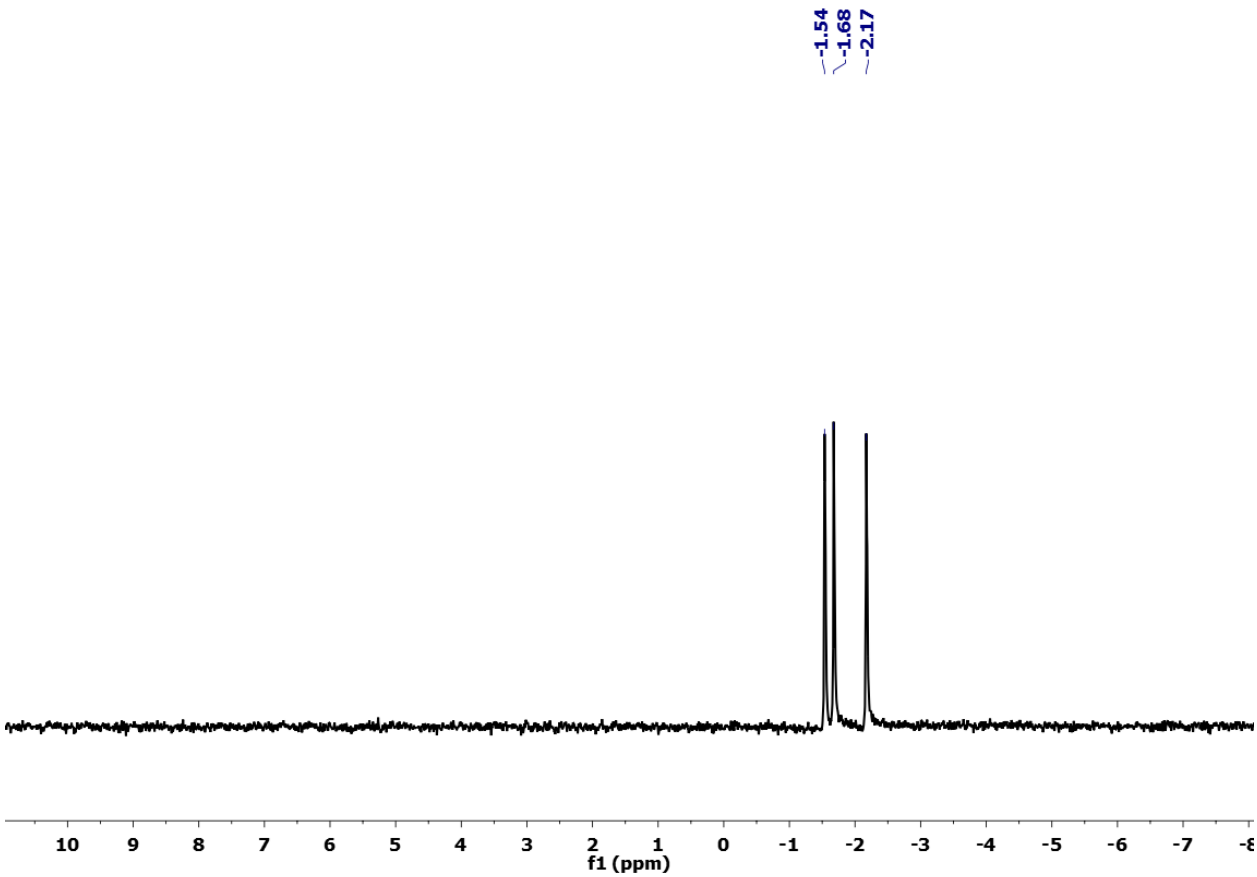
Compound 6. HRMS.



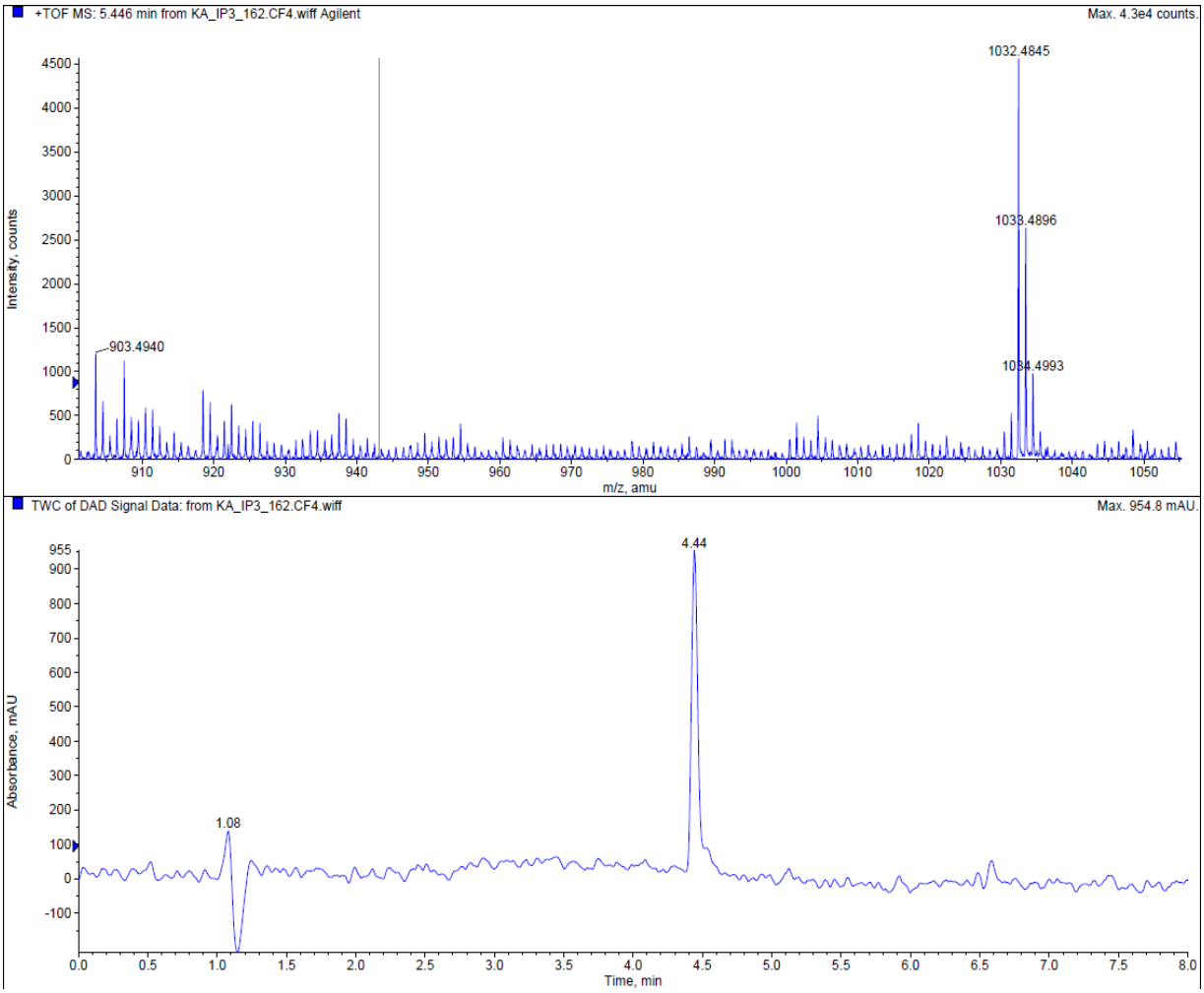
Compound 3a. H-1 spectrum in CDCl3.



Compound 3a. C-13 spectrum in CDCl3.



Compound 3a. P-31 spectrum in CDCl3.



Compound 3a. HRMS.