Supporting Information

HKOCl-4: A Rhodol-based Yellow Fluorescent Probe for Detection of Hypochlorous Acid in Living Cells and Tissues

Xiaoyu Bai,[a] Bowei Yang, [a] Hansen Chen,[b] Jiangang Shen[b] and Dan Yang*[a]

[a] Morningside Laboratory for Chemical Biology and Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, P. R. China.

[b] School of Chinese Medicine, The University of Hong Kong, Sassoon Road, Hong Kong, P. R. China

Table of Contents

1. General methods

Compound characterization

Xanthine, xanthine oxidase, SNP (sodium nitroferricyanide(III) dihydrate), 2,2'-azobis(2-amidinopropane) dihydrochloride, *tert*-butyl hydroperoxide solution, hydrogen peroxide solution and *N*-acetylcysteine (NAC) were purchased from Sigma-Aldrich. Peroxynitrite was synthesized as reported.[1] Peroxynitrite solution was split into small aliquots and frozen at below –18 °C. All other chemicals used were of analytical grade and were purchased from Acros or Sigma-Aldrich. NMR spectra were recorded in deuteriochloroform unless otherwise stated, with tetramethylsilane (TMS) as internal reference at ambient temperature, mainly on a Bruker Avance DPX 300 Fourier Transform Spectrometer operating at 300 MHz for ^{1}H and at 75 MHz for ^{13}C and Bruker Avance DPX 400 Fourier Transform Spectrometer operating at 400 MHz for ^{1}H and100 MHz for ^{13}C. Mass spectra were recorded with a Thermo Scientific DFS High Resolution Magnetic Sector mass spectrometer for both low resolution and high resolution mass analysis.

Fluorometric analysis

All fluorescence measurements were carried out at room temperature on a Hitachi F-7000 fluorescence spectrophotometer. The testing solutions of the probe were excited at 530 nm with the excitation and emission slit widths set at 2.5 nm. The emission spectrum was scanned from 540 to 600 nm at 1200 nm/min and the photomultiplier voltage was set at 800 V. The probe was dissolved in DMF to make a 5 mM stock solution, which was diluted to the required concentration of testing solution for measurement. Aliquots of analyte solutions were slowly added to probe testing solution (5 mL) with vigorous stirring at room temperature in the dark. The volume changes after addition of analyte solutions were less than 1%. The fluorescence intensities of the testing solutions were recorded after 30 min.

Preparation of analyte solutions

HOCl: NaOCl solution (5 mM) was added directly. **$^{\bullet}$OH**: Hydroxyl radical was generated by Fenton reaction or TCBQ/H_2O_2. To generate $^{\bullet}$OH, ferrous solution was added to H_2O_2 solution. The concentration of $^{\bullet}$OH was equal to the Fe (II) concentration (5 mM). **ROO$^{\bullet}$**: Alkylperoxyl radical was generated from 2,2'-azobis(2-amidinopropane) dihydrochloride (5 mM), which was added into the testing solutions directly. **$^{1}O_2$**: Singlet oxygen was generated from 3,3'-(naphthalene-1,4-diyl) dipropionic acid (5 mM). **H_2O_2**: H_2O_2 solution (5 mM) was added directly. **TBHP**: *tert*-Butyl hydroperoxide solution (5 mM) was added into the testing solutions directly. **$^{\bullet}$NO**: Nitric oxide was generated from SNP (sodium nitroferricyanide(III) dihydrate) (5 mM). $O_2^{\bullet-}$: Superoxide was generated from xanthine/xanthine oxidase system. Xanthine oxidase (0.01 U/mL) was added before addition of xanthine (30 mM). **ONOO^{-}**: Peroxynitrite solution was synthesized according to literature report.[1] Briefly, a mixture of sodium nitrite (0.6 M) and hydrogen peroxide (0.7 M) was acidified with hydrochloric acid (0.6 M), and sodium hydroxide (1.5 M) was added within 1–2 s to make the solution alkaline. The excess hydrogen peroxide was removed by passing the solution through a short column of manganese dioxide. The resulting solution was split into small aliquots and stored at below −18 °C. The aliquots were thawed immediately before use, and

the concentration of peroxynitrite was determined by measuring the absorption of the solution at 302 nm. The extinction coefficient of peroxynitrite solution in 0.1 M NaOH is 1670 M^{-1} cm^{-1} at 302 nm.

Cell culture

RAW264.7 cells, a mouse monocytic macrophage cell line, were obtained from ATCC (American Type Culture Collection), and maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 0.5 mM sodium pyruvate, 10% heat-inactivated FBS (fetal bovine serum) and 1% penicillin/streptomycin. For regular confocal imaging, RAW264.7 cells at 80% confluence were harvested by trypsinization for cell counting.

Imaging of endogenous HOCl in RAW264.7 macrophages

RAW264.7 cells were then seeded into a 35-mm confocal culture dish (Mat-Tek) at a density of about 2×10^5 cells/mL in 2-mL seeding volume and cultured overnight. For untreated group, cells were incubated with **HKOCl-4r** (5 μM) or **HKOCl-4m** (5 μM) for 30 min. For PMA (500 ng/mL) treated group, PMA was co-incubated with probes for 30 min. For LPS (500 ng/mL)/IFN-γ (50 ng/mL) stimulated group, LPS/IFN-γ were added and incubated for 16 h and then stained with **HKOCl-4r** or **HKOCl-4m** for 30 min. For the scavenger group, NAC (1 mM) was preloaded for 20 min before probe staining. For the MPO inhibitor treatment group, ABAH (50 μM) was added in medium for 1 h before stimulants were introduced. Cells were then subjected to fluorescence imaging by Zeiss LSM780 Inverted Confocal Microscope. For confocal imaging of the distribution of **HKOCl-4m**, cells were stained with **HKOCl-4m** (10 μM) together with the Mito Tracker Green FM (100 nM) for 30 min.

Rat Middle cerebral artery occlusion (MCAO) model

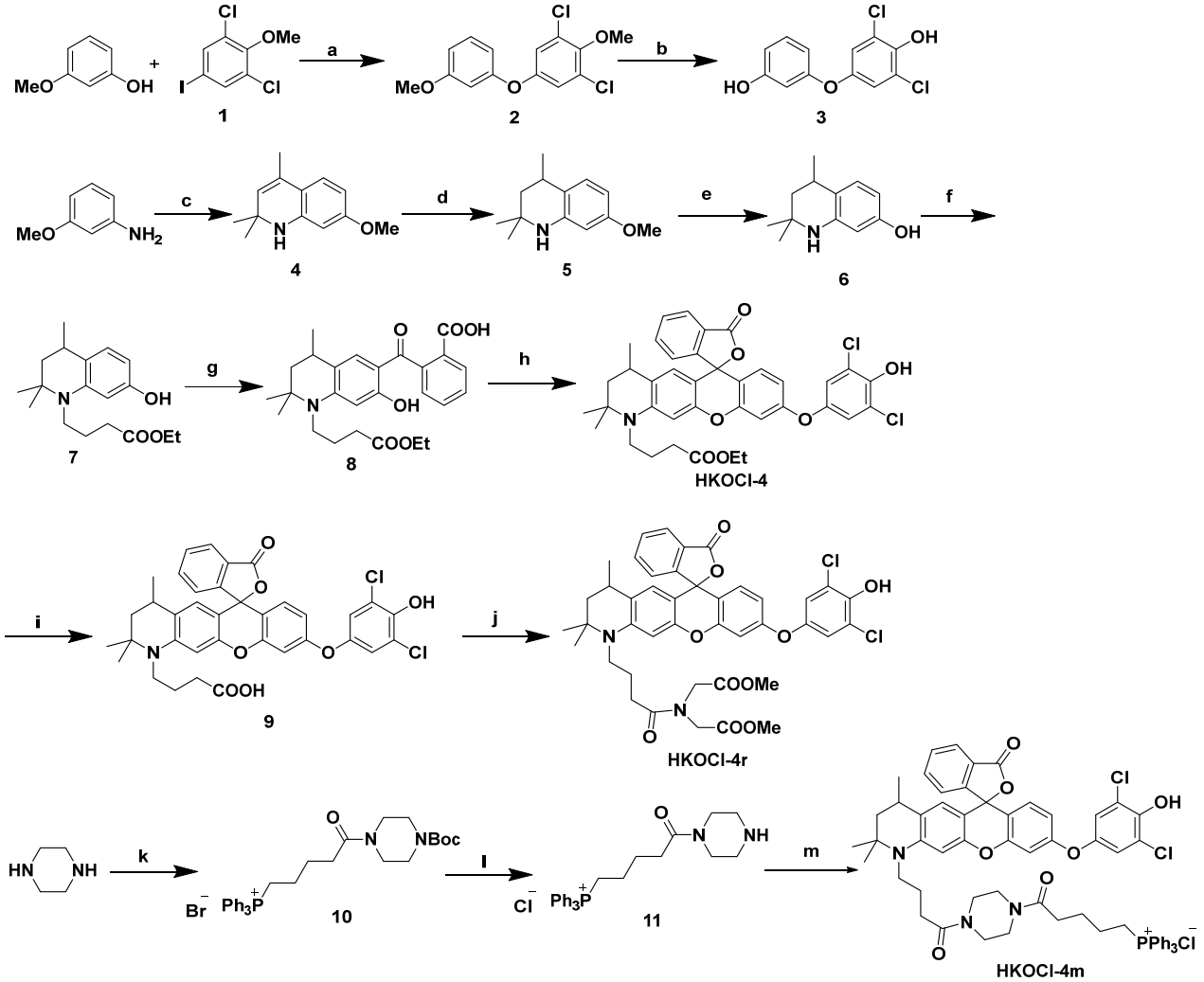
Male Sprague-Dawley (SD) rats weighing from 270-290 g were obtained from the Laboratory Animal Unit, The University of Hong Kong. Animals were housed in temperature and humidity controlled environment under 12-hour light/dark cycle, with free access to food and water. Animal experiment protocol was approved and regulated by the Committee on the Use of Live Animals in Teaching and Research (CULATR), The University of Hong Kong.

To mimic ischemic stroke, we adopted middle cerebral artery occlusion (MCAO) 5 hours plus reperfusion 19 hours model. Briefly, rats were anesthetized with 4% isofluorane and maintained at 2% isofluorane via inhalation. Silicon coated suture (Doccol, Redlands, CA, USA) was inserted from the external carotid artery (ECA) to the internal carotid artery (ICA) to occlude the middle cerebral artery (MCA). Sham control rats underwent the same anesthesia and surgical procedure but without MCA occlusion.

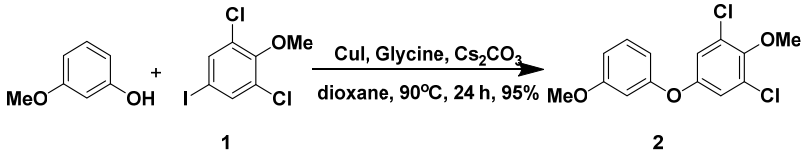
Probe staining in rat brain tissue

At 24 hours after ischemic onset or sham operation, rats were sacrificed after deep anesthesia. Brain samples were collected and immediately subjected to the frozen section. Brain slices at 6 mm away from the frontal tip were then stained with the HKOCl-4r probe at a concentration of 10 uM for 30 min at room temperature. The brain slices were then fixed with 4% PFA. Fluorescence was observed in a confocal microscope Carl Zeiss LSM 780.

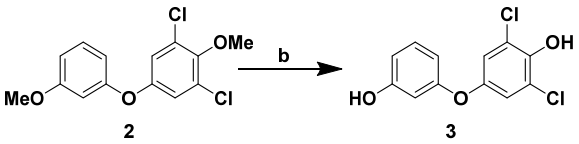
2. Preparation of HKOCl-4



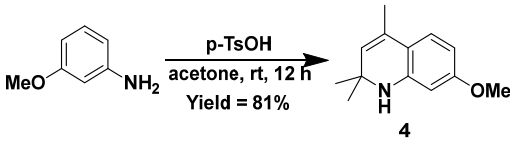
Scheme S1. Synthesis of **HKOCl-4**. Reagents and conditions: (a) CuI, *N*,*N*-dimethylglycine hydrochloride, Cs_2CO_3, 1,4-dioxane, 100 ºC, 24 h, 95%; (b) HBr, acetic acid, reflux, 12 h, 91%; (c) *p*-TsOH, acetone, rt, 12 h, 87%; (d) Pd/C, H_2, EtOH, rt, 12 h; 97%; (e) HBr, HOAc, reflux, 12 h, 87%; (f) ethyl 4-bromobutyrate, proton sponge, NaI, MeCN, reflux, 12 h, 30%; (g) phthalic anhydride, toluene, reflux, 24 h, 50 %; (h) **3**, TFA, 100 ºC, 3 h, 55 %; (i) NaOH, MeOH/H_2O, rt, 4 h, 83%; (j) $HN(CH_2COOMe)_2$, HOAt, EDC•HCl, DMF, rt, 24 h, 64%; (k) Boc_2O, CH_2Cl_2, rt, 12 h, then (4-carboxybutyl)triphenylphosphonium bromide, EEDQ, CH_2Cl_2, rt, 24 h, 90%; (l) TFA, CH_2Cl_2, rt, 3 h, Amberlite IRA-400 (Cl), rt, 30 min, then sat. K_2CO_3 (aq), 10 min, 85%; (m) **9**, HOAt, EDC•HCl, DMF, rt, 12 h, 34%.



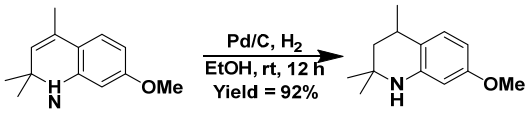
Compound **1** was synthesized according to the reported method.[2] The mixture of 3-methoxyphenol (2.09 mL, 19.8 mmol), compound **1** (3.000 g, 9.9 mmol), CuI (188 mg, 0.99 mmol), glycine (415 mg, 2.97 mmol) and Cs_2CO_3 (6.450 g, 19.8 mmol) were dissolved in anhydrous 1,4-dioxane in a sealed flask. The reaction mixture was heated to 90 °C and stirred for 24 h under Ar. After cooled to room temperature, the reaction mixture was diluted with EtOAc, and washed sequentially with 1N HCl, water and brine. The organic layer was then dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The target compound **2** as isolated as a pale yellow solid (2.800 g, 95%) after flash column chromatography on silica gel by using EtOAc:Hexane (1:9) as an eluent. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28–7.22 (m, 1H), 6.97 (s, 2H), 6.76–6.63 (m, 1H), 6.62–6.54 (m, 2H), 3.88 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8, 157.0, 153.1, 147.8, 130.1, 129.4, 118.7, 111.0, 109.5, 105.1, 60.4, 55.0; LRMS (EI, 20 eV) *m/z* (%) 298 (M^+; 100), 283 (92); HRMS (EI): calcd for $C_{14}H_{12}O_3Cl_2$ (M^+): 298.0163, found: 298.0159.



Compound **2** (2.8 g, 9.3 mmol) was dissolved in a mixture of HBr (48% wt in water) and HOAc (40 mL, 1:1). The reaction mixture was refluxed overnight. After cooled to room temperature, the resulting mixture was treated with H_2O, extracted with EtOAc, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The target compound **3** was isolated as a pale yellow solid (2.0 g, 79%) after flash column chromatography on silica gel by using EtOAc:Hexane (3:7) as an eluent. ^{1}H NMR (400 MHz, CD_3OD) δ 7.06 (t, J = 8.1 Hz, 1H), 6.88 (s, 2H), 6.54 (dd, J = 8.1, 1.5 Hz, 1H), 6.44–6.35 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 158.6, 158.5, 149.8, 145.6, 130.7, 122.8, 119.8, 111.1, 109.9, 106.0; LRMS (EI, 20 eV) *m/z* (%) 270 (M^+; 100), 272 (66); HRMS (EI): calcd for $C_{12}H_8O_3Cl_2$ (M^+): 269.9850, found: 269.9845.

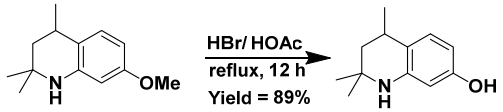


To a solution 3-methoxyaniline (5.00 g, 40.6 mmol) in acetone (150 mL) was added *p*-TsOH (1.55 g, 8.1 mmol). The solution was stirred at room temperature overnight under Ar, then concentrated *in vacuo*. The target compound **4** was isolated as a brown sticky solid (6.7 g, 81% yield) after flash column chromatography on silica gel by using Et_2O:Hexane (2:8) as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 8.3 Hz, 1H), 6.33 (d, J = 7.8 Hz, 1H), 6.15 (s, 1H), 5.29 (s, 1H), 3.88 (br, 1H), 3.82 (s, 3H), 2.08 (s, 3H), 1.35 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8, 144.5, 127.7, 125.7, 124.2, 114.9, 101.9, 98.2, 54.5, 51.4, 30.6, 18.2; LRMS (EI, 20 eV) *m/z* (%) 203 (M^+; 8), 188 (100); HRMS (EI): calcd for $C_{13}H_{17}NO$ (M^+): 203.1310, found: 203.1306.



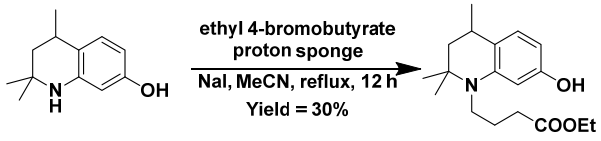
4 5

To a solution of compound **4** (2.30 g, 10.5 mmol) in degassed EtOH (80 mL) was added Pd/C (10% on carbon, 1.11 g, 1.05 mmol) slowly. The mixture was hydrogenated with a H_2 balloon at room temperature for 12 h. The mixture was then filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The target compound **5** was isolated as a brown oil (2.25 g, 97% yield) after flash column chromatography on silica gel by using EtOAc:Hexane (1:9) as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 8.4 Hz, 1H), 6.33 (dd, J = 8.4, 2.2 Hz, 1H), 6.09 (d, J = 2.2 Hz, 1H), 3.80 (s, 3H), 3.62 (br, 1H), 3.01–2.87 (m, 1H), 1.79 (dd, J = 12.8, 5.5 Hz, 1H), 1.49 (t, J = 12.5 Hz, 1H), 1.39 (d, J = 6.7 Hz, 3H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 144.6, 127.6, 117.9, 102.6, 99.1, 54.8, 49.2, 44.5, 31.3, 27.6, 26.9, 20.4; LRMS (EI, 20 eV) *m/z* (%) 205 (M^+; 41), 190 (100); HRMS (EI): calcd for $C_{13}H_{19}NO$ (M^+): 205.1467, found: 205.1465.



5 6

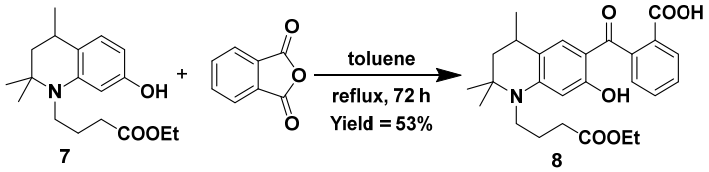
Compound **5** (500 mg, 2.27 mmol) was dissolved in a mixture of HBr (48% wt in water) and HOAc (8 mL, 1:1). The reaction mixture was heated under reflux overnight. After the reaction was cooled to room temperature, the mixture was treated with saturated $NaHCO_3$ to pH 5~7 and then extracted with EtOAc. The combined organic layers were washed with H_2O and brine. The organic layer was then dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The target compound **6** was isolated as a brown oil (380 mg, 89%) after flash column chromatography on silica gel by using EtOAc:Hexane (2:8) as an eluent**.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (d, J = 8.3 Hz, 1H), 6.21 (dd, J = 8.3, 2.0 Hz, 1H), 5.95 (d, J = 2.1 Hz, 1H), 5.04 (br, 2H), 2.95–2.73 (m, 1H), 1.71 (dd, J = 13.0, 5.6 Hz, 1H), 1.41 (t, J = 12.6 Hz, 1H), 1.28 (d, J = 6.6 Hz, 3H), 1.22 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.6, 144.2, 128.0, 118.5, 105.2, 101.5, 49.6, 44.4, 31.1, 27.3, 27.0, 20.5; LRMS (EI, 20 eV) *m/z* (%) 191 (M^+ ; 29), 176 (100); HRMS (EI): calcd for $C_{12}H_{17}NO$ (M^+): 191.1310, found: 191.1304.



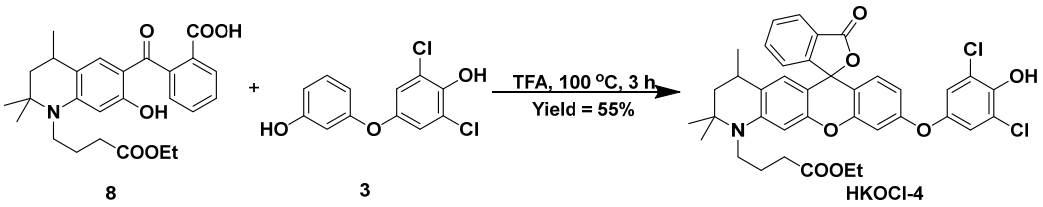
6 7

To a solution of compound **6** (1.34 g, 7.0 mmol) in dry MeCN (50 mL) were added NaI (210 mg, 1.40 mmol), proton sponge (3.0 g, 14 mmol) and ethyl-4-bromobutylate (1.52 mL, 10.5 mmol). The reaction mixture was heated under reflux for 48 h. After cooling down to room temperature, the reaction mixture was filtered through a pad of celite, then diluted with EtOAc, and washed with H_2O and brine. The organic layer was then dried over anhydrous magnesium sulfate and

concentrated *in vacuo*. The target compound **7** was isolated as a brown oil (320 mg, 30%) after flash column chromatography on silica gel by using EtOAc:Hexane (2:8) as an eluent. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (d, *J* = 7.5 Hz, 1H), 6.26–5.98 (m, 2H), 5.45 (br, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.38–3.23 (m, 1H), 3.11–2.98 (m, 1H), 2.89–2.72 (m, 1H), 2.37 (t, *J* = 6.7 Hz, 2H), 2.05–1.79 (m, 2H), 1.69 (dd, *J* = 12.9, 4.7 Hz, 1H), 1.50 (t, *J* = 12.8 Hz, 1H), 1.35–1.23 (m, 9H), 1.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 155.1, 146.0, 126.6, 120.4, 102.1, 98.6, 60.6, 54.5, 47.2, 44.5, 31.6, 29.6, 26.7, 24.9, 24.1, 20.2, 14.2; LRMS (EI, 20 eV) *m/z* (%) 305 (M^+ ; 52), 290 (100); HRMS (EI): calcd for $C_{18}H_{27}O_3N$ (M^+): 305.1991, found: 305.1983.

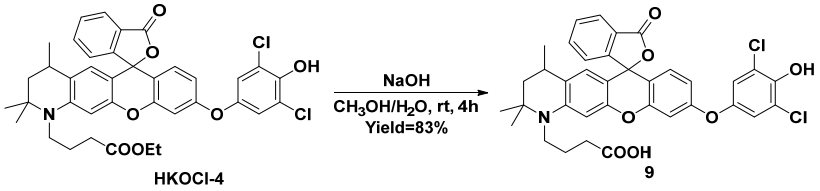


Compound **7** (150 mg, 0.49 mmol) and phthalic anhydride (87 mg, 0.58 mmol) were dissolved in dry toluene (5 mL). The suspension was heated under reflux for 48 h. After cooled down to room temperature, the solvent was removed *in vacuo*. The target compound **8** was isolated as an orange sticky solid (120 mg, 53%) after flash column chromatography on silica gel by using EtOAc:Hexane (2:8) + 1% acetic acid as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H), 8.94 (br, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.61 (t, *J* = 7.2 Hz, 1H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.35 (d, *J* = 7.3 Hz, 1H), 6.73 (s, 1H), 6.08 (s, 1H), 4.18 (q, *J* = 7.0 Hz, 2H), 3.50–3.35 (m, 1H), 3.27–3.15 (m, 1H), 2.60–2.55 (m, 1H), 2.38 (t, *J* = 6.4 Hz, 2H), 2.05–1.85 (m, 2H), 1.72–1.60 (m, 1H), 1.72–1.60 (m, 1H), 1.34 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.19 (s, 3H), 0.99 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.1, 173.0, 170.5, 164.0, 151.9, 141.1, 132.4, 130.9, 129.8, 129.1, 128.0, 119.9, 109.4, 97.4, 60.6, 55.7, 46.0, 44.6, 31.5, 29.4, 26.0, 25.6, 23.4, 19.4, 14.2; LRMS (EI, 20 eV) *m/z* (%) 453 (M^+; 9), 71 (100); HRMS (EI): calcd for $C_{26}H_{31}O_6N$ (M^+): 453.2151, found: 453.2142.

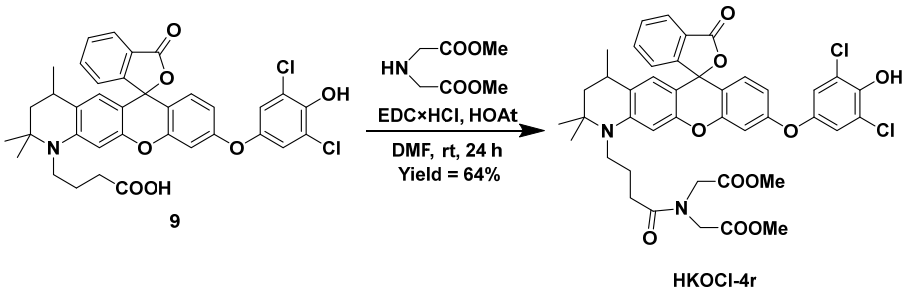


Compound **8** (230 mg, 0.51 mmol) and compound **3** (137 mg, 0.51mmol) were dissolved in TFA in a sealed tube. The reaction mixture was heated to 100 °C and stirred for 3 h. After cooled to room temperature, the solvent was removed *in vacuo*. **HKOCl-4** (193 mg, 55%) was isolated as red sticky solid after flash column chromatography on silica gel by using MeOH:CH_2Cl_2 (1:99) as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.4 Hz, 1H), 7.67 (t, *J* = 7.4 Hz, 1H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.22 (dd, *J* = 7.6 Hz, 1H), 7.03 (s, 2H), 6.76 (s, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 6.61 (d, *J* = 8.7 Hz, 1H), 6.39 (s, 1H), 6.37 (s, 1H), 5.97 (br, 1H), 4.19 (q, *J* = 6.9 Hz, 2H), 3.45–3.34 (m, 1H), 3.22–3.06 (m, 1H), 2.75–2.58 (m, 1H), 2.41 (t, *J* = 6.8 Hz, 2H), 2.04–1.87 (m, 2H), 1.70–1.60 (m, 1H), 1.50–1.39 (m, 1H), 1.38–1.21 (m, 6H), 1.16 (s, 3H), 1.05 (d, *J* = 6.5 Hz, 2H), 0.95

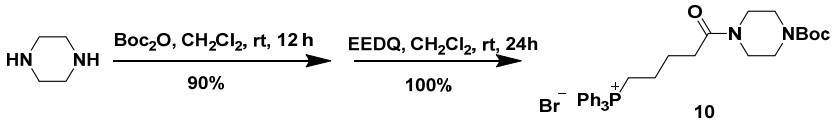
(d, J = 6.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$; values are given for one isomer with those of the second isomer in brackets) δ 173.19 (173.16), 169.61, 158.83 (158.78), 152.94 (152.87), 152.77, 151.04 (150.95), 148.54, 147.13, 146.91, 145.07, 134.81 (134.75), 129.57 (129.51), 127.21, 127.03, 125.66, 125.21, 124.89, 124.75, 124.04, 121.58, 120.22, 114.61 (114.55), 113.21, 105.28, 104.57, 97.68 (97.65), 60.58, 54.97 (54.83), 46.42 (46.34), 44.58 (44.36), 31.63 (31.61), 29.42 (29.28), 26.78 (26.60), 25.59, 25.09, 23.52 (23.34), 19.66 (19.47), 14.23; LRMS (EI, 20 eV) *m/z* (%) 687 (M^+; 0.7), 85 (100); HRMS (EI): calcd for $C_{38}H_{35}O_7N^{35}Cl_2$ (M^+): 687.1791, found: 687.1773.



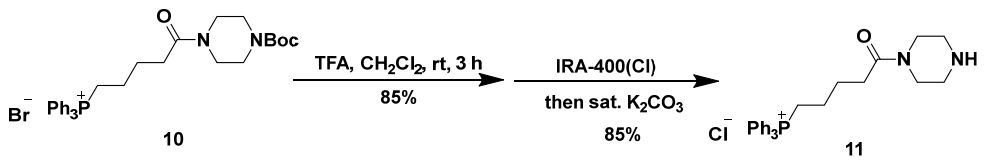
HKOCl-4 (24 mg, 0.035 mmol) was dissolved in MeOH (3 mL) and then a solution of NaOH (8 mg, 0.21 mmol) in H_2O (3 mL) was added. The resulting mixture was stirred at room temperature for 4 h. Then the reaction mixture was diluted with EtOAc and washed sequentially with 1 N HCl, water and brine. The organic layer was dried over magnesium sulfate and concentrated in vacuo. **HKOCl-4** (19 mg, 83%) was isolated as red sticky solid after flash column chromatography on silica gel by using MeOH:CH_2Cl_2 (1:50) as an eluent. Yield: 19 mg (83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J = 7.6 Hz, 1H), 7.68 (td, J = 7.4, 1.2 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.24–7.18 (m, 1H), 7.04 (s, 2H), 6.79 (dd, J = 1.4 Hz, 1H), 6.72 (dd, J = 8.8, 4.2 Hz, 1H), 6.62 (dt, J = 8.8, 2.4 Hz, 1H), 6.43 (s, 1H), 6.40 (d, J = 5.0 Hz, 1H), 3.45–3.37 (m, 1H), 3.15 (t, J = 11.2 Hz, 1H), 2.76–2.58 (m, 1H), 2.47 (td, J = 7.0, 2.5 Hz, 2H), 2.04–1.87 (m, 2H), 1.70–1.64 (m, 2H), 1.50–1.40 (m, 2H), 1.30 (d, J = 4.8 Hz, 3H), 1.16 (s, 3H), 1.06 (d, J = 6.6 Hz, 1.5H), 0.95 (d, J = 6.6 Hz, 1.5H). ^{13}C NMR (100 MHz, $CDCl_3$; values are given for one isomer with those of the second isomer in brackets) δ 178.78 (178.73), 169.76, 159.15 (159.10), 153.23 (153.17), 152.35, 151.49 (151.40), 148.74, 147.60, 147.37, 147.16, 145.17 (144.99), 134.91 (134.85), 129.81 (129.77), 129.72, 125.33, 124.99, 124.41, 124.27, 121.72, 120.36, 114.90 (114.85), 113.59, 105.53, 105.29 (105.26), 98.00 (97.94), 55.32 (55.17), 46.52 (46.44), 44.80 (44.56), 31.73 (31.27), 29.63 (29.45), 26.98 (26.79), 25.81 (25.29), 19.81 (19.61), 14.28; LRMS (ESI) 660.06 $(M+H)^+$; HRMS (ESI): calcd for $C_{36}H_{32}NO_7{}^{35}Cl_2$ $(M+H)^+$: 660.1478, found: 660.1543.



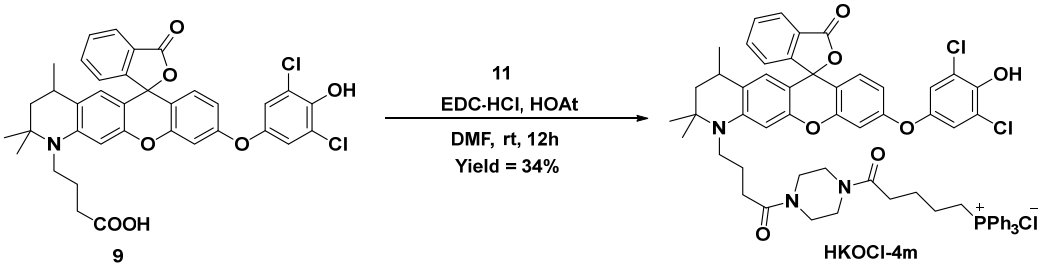
To a solution of compound **9** (18 mg, 27 μmol) and the $HN(CH_2COOMe)_2$ (5 mg, 33 μmol) in anhydrous DMF (1 mL) was added HOAt (5 mg, 33 μmol), and the resulting mixture was stirred at room temperature for 30 min under argon atmosphere. Then the mixture was added EDC·HCl (7 mg, 35 μmol) and stirred at room temperature for 24 h. Then the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The target compound **HKOCl-4r** was isolated as a red sticky solid by flash column chromatography on silica gel by using MeOH:CH_2Cl_2 (75:1) as an eluent. Yield: 14 mg (64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J = 7.6 Hz, 1H), 7.68 (tdd, J = 7.5, 3.0, 1.1 Hz, 1H), 7.62 (tdd, J = 7.5, 2.5, 0.9 Hz, 1H), 7.24–7.18 (m, 1H), 7.05 (s, 2H), 6.77 (t, J = 2.9 Hz, 1H), 6.71 (dd, J = 8.7, 2.2 Hz, 1H), 6.61 (dt, J = 8.7, 2.1 Hz, 1H), 6.40–6.37 (m, 2H), 5.85 (br, 1H), 4.24 (s, 2H), 4.19 (s, 2H), 3.78 (d, J = 3.3 Hz, 3H), 3.74 (s, 3H), 3.46–3.37 (m, 1H), 3.19–3.10 (m, 1H), 2.73–2.58 (m, 1H), 2.41 (t, J = 6.7 Hz, 2H), 1.99–1.93 (m, 2H), 1.69–1.66 (m, 1H), 1.45–1.40 (m, 1H), 1.31 (d, J = 4.1 Hz, 3H), 1.16 (s, 3H), 1.05 (d, J = 6.6 Hz, 2H), 0.95 (d, J = 6.6 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$; values are given for one isomer with those of the second isomer in brackets) δ 172.96 (172.92), 169.96 (169.95), 169.76 (169.74), 169.47, 158.98 (158.92), 153.23 (153.15), 153.07 (153.02), 151.21 (151.12), 148.85, 147.35, 147.12, 145.12 (145.11), 140.72, 134.95 (134.89), 129.80 (129.76), 129.69 (129.64), 127.43 (127.23), 125.03, 124.84, 124.24, 121.67, 120.44, 114.87 (114.82), 113.40, 105.51, 104.59, 97.97 (97.90), 55.20 (55.06), 52.75 (52.74), 52.38, 50.13, 48.06 (48.04), 46.72 (46.63), 30.10 (30.01), 29.84, 29.62 (29.45), 27.02 (26.82), 25.91 (25.35), 23.44 (23.27), 19.79 (19.63). LRMS (ESI) 803.18 $(M+H)^+$; HRMS (ESI): calcd for $C_{42}H_{41}N_2O_{10}{}^{35}Cl_2$ $(M+H)^+$: 803.2060, found: 803.2115.



To a solution of 1,4-piperazine (3.40 g, 40 mmol) in CH_2Cl_2 (50 mL), was added a solution of Boc_2O (4.40 g, 20 mmol) in CH_2Cl_2 (100 mL) dropwise. The reaction mixture was stirred at room temperature for 12 h. Then resulting solution was washed with water for three times followed by brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The product (3.40 g, 90%) was obtained as a white solid**.** To a solution of (4-carboxybutyl) triphenylphosphonium bromide (1.00 g, 2.26 mmol) in anhydrous CH_2Cl_2 (20 mL) was added EEDQ (837 mg, 3.39 mmol). The solution was stirred at room temperature for 30 min. Then the above piperazine compound (420 mg, 2.26 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. When the reaction was completed, the resulting mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ to remove the unreacted carboxylic acid. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The desired product **10** was isolated as a white sticky solid (1.4 g, 100%) after flash column chromatography on silica gel, by using MeOH:CH_2Cl_2 (1:9) as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.39 (m, 9H), 7.37–7.29 (m, 6H), 3.48–3.38 (m, 2H), 3.27–3.16 (m, 2H), 3.16–3.03 (m, 4H), 2.35–2.20 (m, 2H), 2.98–2.91 (m, 2H), 1.63–1.51 (m, 2H), 1.47–1.31 (m, 2H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 154.0, 134.5 (d, $^4J_{C,P}$ = 2.0 Hz), 133.1 (d, $^3J_{C,P}$ = 10.0 Hz), 129.9 (d, $^2J_{C,P}$ = 12.0 Hz), 118.1 (d, $^1J_{C,P}$ = 85.0 Hz), 79.4, 44.8, 44.3, 42.7, 40.7, 31.6, 27.8, 25.0 (d, $^2J_{C,P}$ = 17.0 Hz), 22.0, (d, $^1J_{C,P}$ = 51.0 Hz) 21.5 (d, $^3J_{C,P}$ = 3.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) 24.1; LRMS (ESI): 531.28 (M^+); HRMS (ESI): calcd for $C_{32}H_{40}N_2O_3P^+$ (M^+): 531.2771, found: 531.2779.



Compound **10** (1.5 g, 2.4 mmol) was dissolved in a mixture of CH_2Cl_2 and TFA (2 mL:2 mL), and then the solution was stirred at room temperature for 3 h. After the reaction was complete, the solvent was evaporated off on a rotary evaporator. Amberlite IRA-400 (Cl) (2 g) was stirred in brine for 30 min, filtered and dried in air. To the crude product in MeOH (20 mL) at room temperature was added the pretreated Amberlite IRA-400 (Cl), and the mixture was stirred for 30 min, followed by filtration. The filtrate was concentrated *in vacuo* and resuspended in saturated K_2CO_3 solution for 10 min. It was then extracted with CH_2Cl_2 for three times and the combined organic layers were dried over anhydrous magnesium sulfate and concentrated *in vacuo* to obtain crude compound **11**. The target compound **11** (900 mg; 85%) was obtained as a colorless oil and could be directly used in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.42 (m, 9H), 7.42–7.35 (m, 6H), 3.46 (s, 1 H), 3.33–3.22 (m, 2H), 3.22–3.14 (m, 4H), 2.56–2.40 (m, 2H), 2.49–2.41 (m, 2H), 2.20–2.11 (m, 2H), 1.66–1.51 (m, 2H), 1.50–1.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 136.0, 134.3 (d, $^2J_{C,P}$ = 10.0 Hz), 131.4 (d, $^3J_{C,P}$ = 13.0 Hz), 118.9 (d, $^1J_{C,P}$ = 86.0 Hz), 47.0, 46.7, 46.3, 42.9, 32.8, 26.5 (d, $^2J_{C,P}$ = 17.0 Hz), 22.9 (d, $^3J_{C,P}$ = 3.0 Hz), 22.8 (d, $^1J_{C,P}$ = 51.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) 23.3; LRMS (ESI): 431.29 (M^+); HRMS (ESI): calcd for $C_{27}H_{32}N_2OP^+$ (M^+): 431.2247, found: 431.2226.



To a solution of compound **9** (40 mg, 61 μmol) and compound **11** (37 mg, 73 μmol) in anhydrous DMF (3 mL) was added EDC·HCl (15 mg, 79 μmol). The resulting mixture was stirred at room temperature under argon atmosphere for 12 h. Then the reaction mixture was diluted with ethyl acetate and washed with 1N HCl, water and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The target compound **HKOCl-4m** was isolated as a red sticky solid by flash column chromatography on silica gel for by using MeOH:CH_2Cl_2 = 1:20 as an eluent. Yield: 23 mg (34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J = 7.5 Hz, 1H), 7.87–7.75 (m, 9H), 7.74–7.65 (m, 7H), 7.61 (t, J = 7.4 Hz, 1H), 7.21 (t, J = 8.3 Hz, 1H), 7.03 (s, 2H), 6.73–6.61 (m, 3H), , 6.43–6.32 (m, 2H), 5.30 (s, 1H), 3.78–3.55 (m, 9H), 3.51–3.37 (m, 2H), 3.20–3.09 (m, 1H), 2.88–2.58 (m, 2H), 2.53–2.38 (m, 2H), 2.33 (t, J = 8.0, 7.3 Hz, 1H), 1.95 (m, 4H), 1.81 (m, 2H), 1.65 (dd, J = 8.9, 4.6 Hz, 1H), 1.51–1.42 (m, 1H), 1.35–1.30 (m, 3H), 1.17 (s, 3H), 1.05 (d, J = 6.5 Hz, 2H), 0.96 (d, J = 6.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 171.3, 169.8, 159.6, 153.14 (153.10), 153.0, 151.2, 148.0, 146.5, 135.2, 135.0, 134.3 (d, $^3J_{C,P}$ = 10.1 Hz), 133.6 (d, $^3J_{C,P}$ = 12.6 Hz), 129.64 (129.61), 127.3, 125.0, 124.8, 124.3, 124.2, 123.1, 120.97 (120.93), 118.9 (d, $^1J_{C,P}$ = 86.0 Hz), 114.3, 113.2, 104.6, 97.8, 68.4, 68.2, 55.0, 54.9, 46.6, 46.5, 45.8, 45.6, 41.8, 41.6, 41.50 (41.48), 32.0, 30.7, 29.82 (29.78), 29.7, 29.6, 29.5, 26.8 (d, $^2J_{C,P}$ =

17.0 Hz), 25.8, 25.6, 25.4, 23.7, 23.6, 22.2 (d, $^{1}J_{C,P}$ = 50.0 Hz), 22.0 (d, $^{3}J_{C,P}$ = 3.1 Hz), 19.8, 19.7, 19.0; ^{31}P NMR (162 MHz, $CDCl_3$) 24.5; LRMS (ESI): 1072.17 (M^+); HRMS (ESI): calcd for $C_{63}H_{61}O_7{}^{35}Cl_2N_3P^+$ (M^+): 1072.3619, found: 1072.3586.

3. Absorption spectra of HKOCl-4 before and after treatment with HOCl

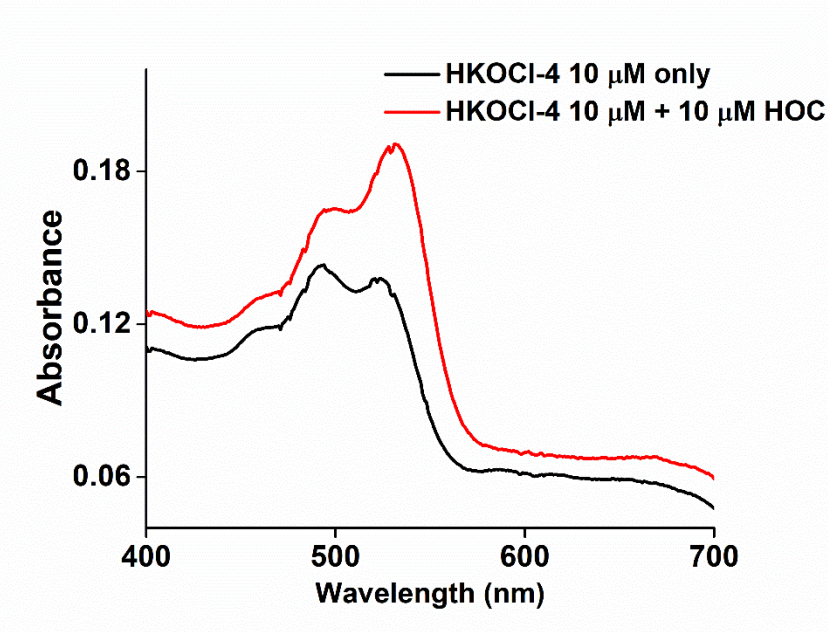


Figure S1. Absorption spectra of **HKOCl-4** (10 μM) in 0.1 M potassium phosphate buffer (0.1% DMF, pH 7.4) before and after treatment with 1 equiv of HOCl.

4. Detection of products for the reaction of HKOCl-4 with HOCl

The probe **HKOCl-4** was dissolved in DMF and then diluted with 0.1 M potassium phosphate buffer (pH 7.4) to get a 10 μM testing solution. Then 1 equiv of HOCl was added to the testing solution with vigorous stirring in the dark. The reaction mixture was extracted with ethyl acetate after 30 min. The combined organic layers were concentrated and analyzed by ESI-MS.

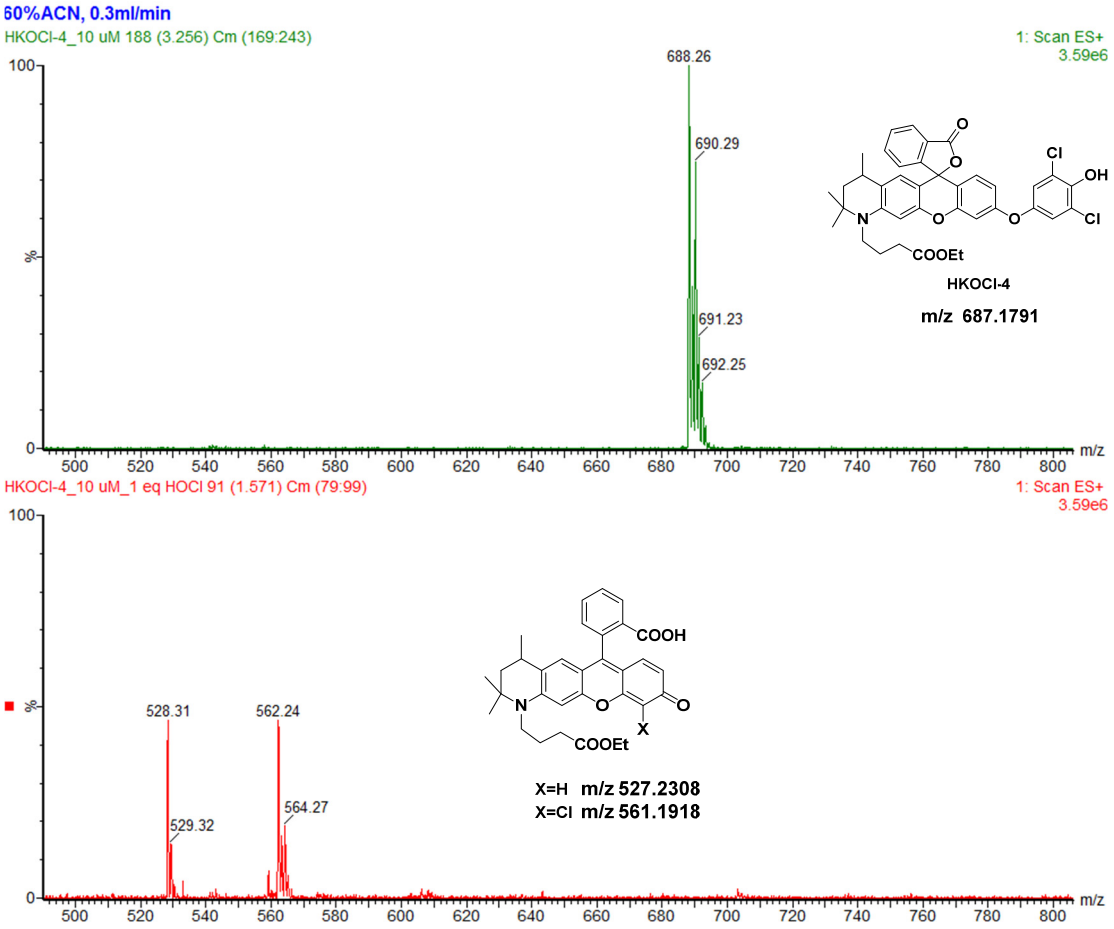
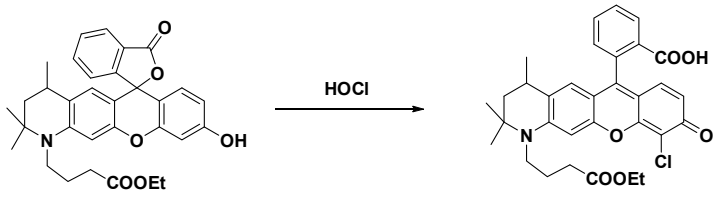


Figure S2. Detection of products obtained after reaction of **HKOCl-4** with HOCl.

From the above LC-MS analysis, it is apparent that two reaction products were obtained, i.e., the rhodol compound and its mono-chlorinated product, after treatment with 1 equiv of HOCl.



5 equiv of HOCl was slowly added to the testing solution of **HKOCl-4** with vigorous stirring at room temperature in the dark. After 30 min, the reaction mixture was extracted with ethyl acetate. The mono-chlorinated product was purified by column (using CH_2Cl_2:CH_3OH=10:1 as an eluent; 61% yield), and it chemical structure was determined by NMR and MS analysis.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, J = 7.5 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 7.61 (t, J = 7.4 Hz, 1H), 7.18 (dd, J = 7.5 Hz, 1H), 6.68 (d, J = 8.6 Hz, 1H), 6.62 (d, J = 8.6 Hz, 1H), 6.54 (s, 1H),

6.47 (d, J = 6.2 Hz, 1H), 4.24 (qd, J = 7.1, 2.3 Hz, 2H), 3.49–3.42 (m, 1H), 3.25–3.19 (m, 1H), 2.74–2.62 (m, 1H), 2.45 (td, J = 7.0, 3.0 Hz, 2H), 2.05–1.92 (m, 2H), 1.69 (dd, J = 13.0, 4.4 Hz, 1H), 1.51–1.42 (m, 1H), 1.34–1.32 (m, 3H), 1.25 (s, 3H), 1.19 (s, 3H), 1.06 (d, J = 6.5 Hz, 2H), 0.97 (d, J = 6.5 Hz, 1H); LRMS (ESI): calcd for $C_{32}H_{32}O_6N^{35}Cl$ (M^+): 561.1918, found: 561.4639.

5. Stability of HKOCl-4 toward pH changes

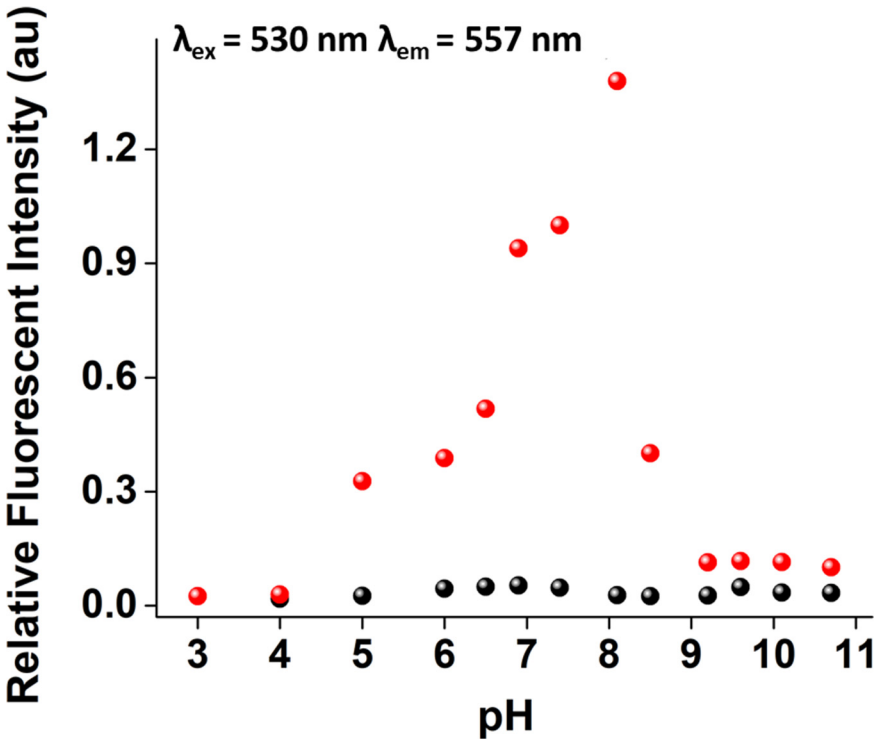


Figure S3. Investigation of **HKOCl-4** response across pH 3–11 with (red) or without (black) adding HOCl. The fluorescent intensity of **HKOCl-4** (5 μM) with 1 equiv of HOCl at pH 7.4 was normalized as 1. The fluorescence spectra of **HKOCl-4** were recorded at 30 min with an excitation at 530 nm.

6. Characterization of HKOCl-4r and HKOCl-4m in chemical system

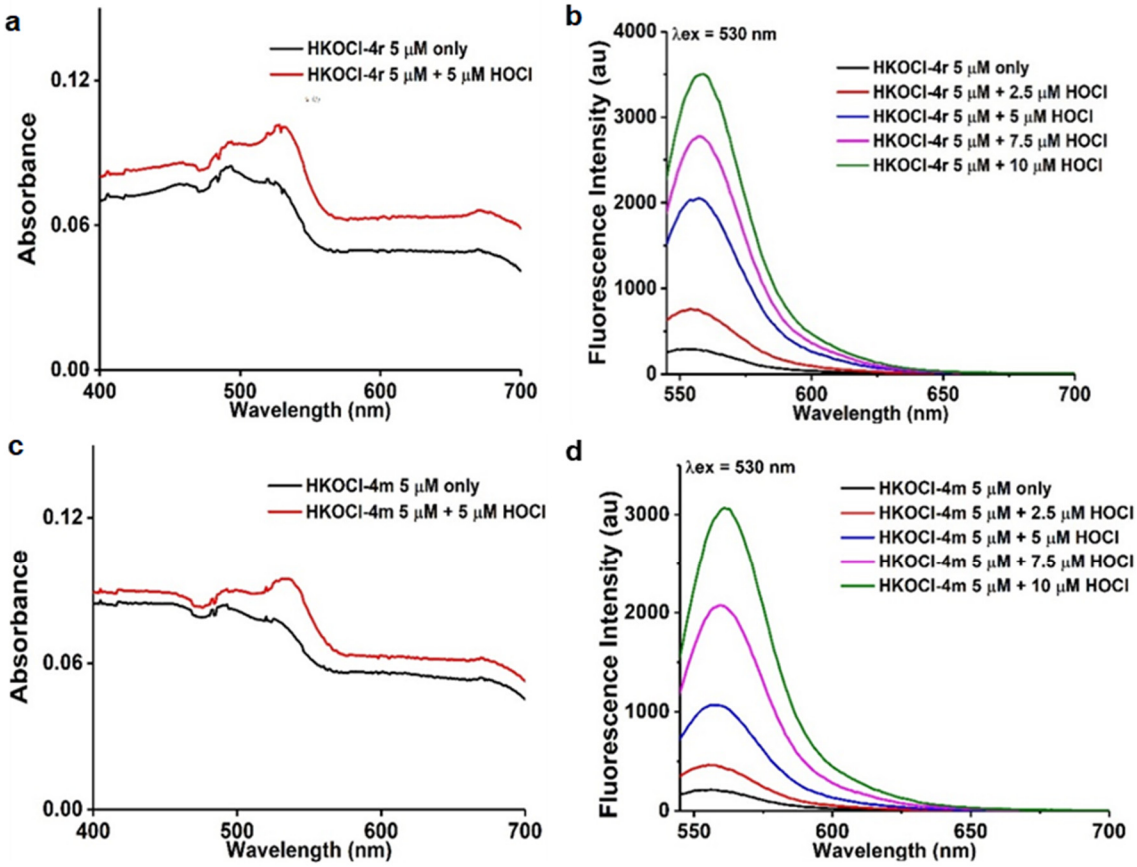


Figure S4. Characterization of **HKOCl-4r** and **HKOCl-4m** in chemical system. The fluorescent probe was dissolved in 0.1 M potassium phosphate buffer at pH 7.4 to a final concentration of 5 μM (containing 0.1% DMF). (a) Absorption spectra of **HKOCl-4r** before and after treatment with 1 equiv of HOCl. (b) Fluorescence emission spectra of **HKOCl-4r** upon addition of different amounts of HOCl. (c) Absorption spectra of **HKOCl-4m** before and after treatment with 1 equiv of HOCl. (d) Fluorescence emission spectra of **HKOCl-4m** upon treatment with different amounts of HOCl.

7. Cytotoxicity assay of HKOCl-4

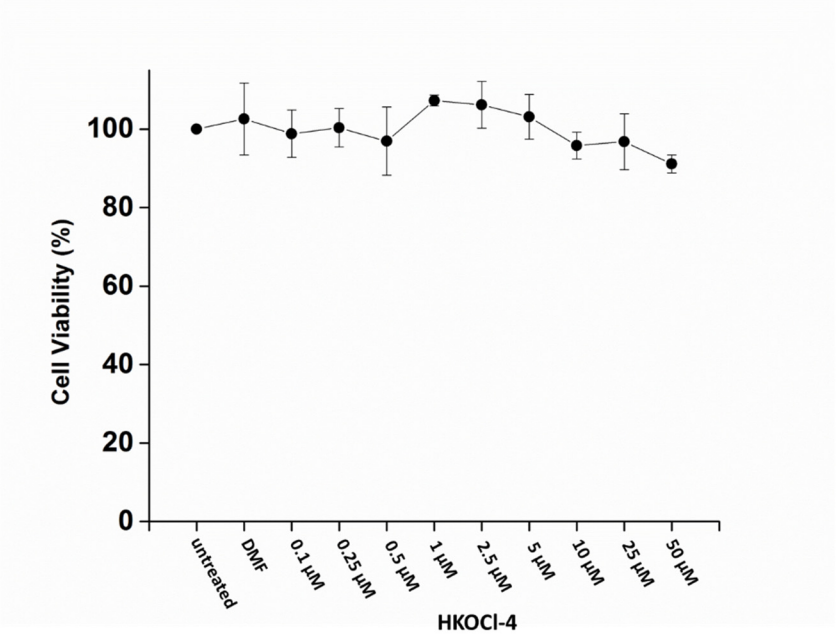


Figure S5. Cytotoxicity of **HKOCl-4** in RAW264.7 cells. RAW264.7 cells were allowed to incubate with increasing amounts of the probe for 24 h. The probe showed negligible or no cytotoxicity after 24 h incubation. Data represent mean ±s.e.m for Cell-Titer Glo assays performed in triplicates.

8. Monitoring HOCl in PMA or LPS/IFNγ stimulated RAW264.7 cells in lower magnification

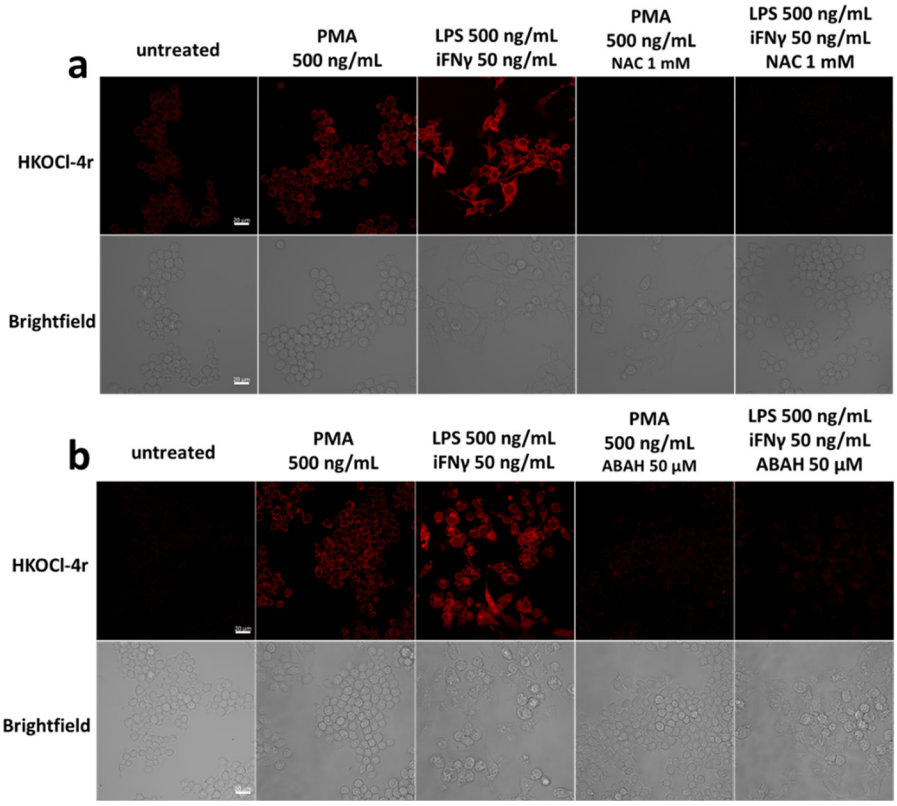
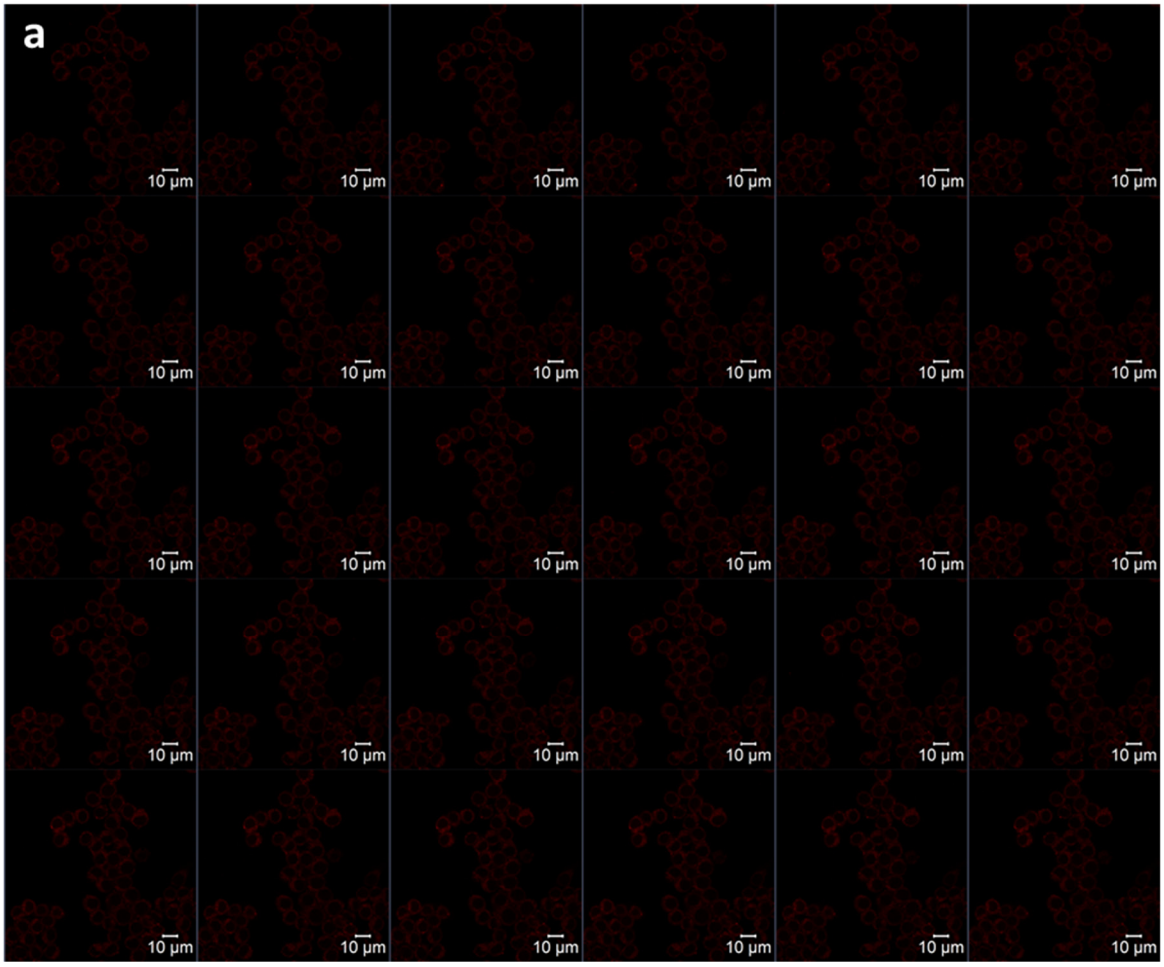


Figure S6. Confocal fluorescence imaging of endogenous HOCl with **HKOCl-4r** in RAW264.7 mouse macrophages. Cell were stimulated with PMA (500 ng/mL) for 30 min or LPS (500 ng/mL)/IFN-γ (50 ng/mL) for 16 h before staining with **HKOCl-4r** (5 μM) for 30 min. (a) For the scavenger groups, NAC (1 mM) was preloaded for 20 min before probe staining. (b) For the MPO inhibitor treatment group, ABAH (50 μM) was added in culture medium for 1 h before the stimulant was introduced. Results are representative of at least three independent experiments. Scale bar: 20 μm.

9. Time lapse monitoring of HKOCl-4r in PMA stimulated RAW264.7 cells



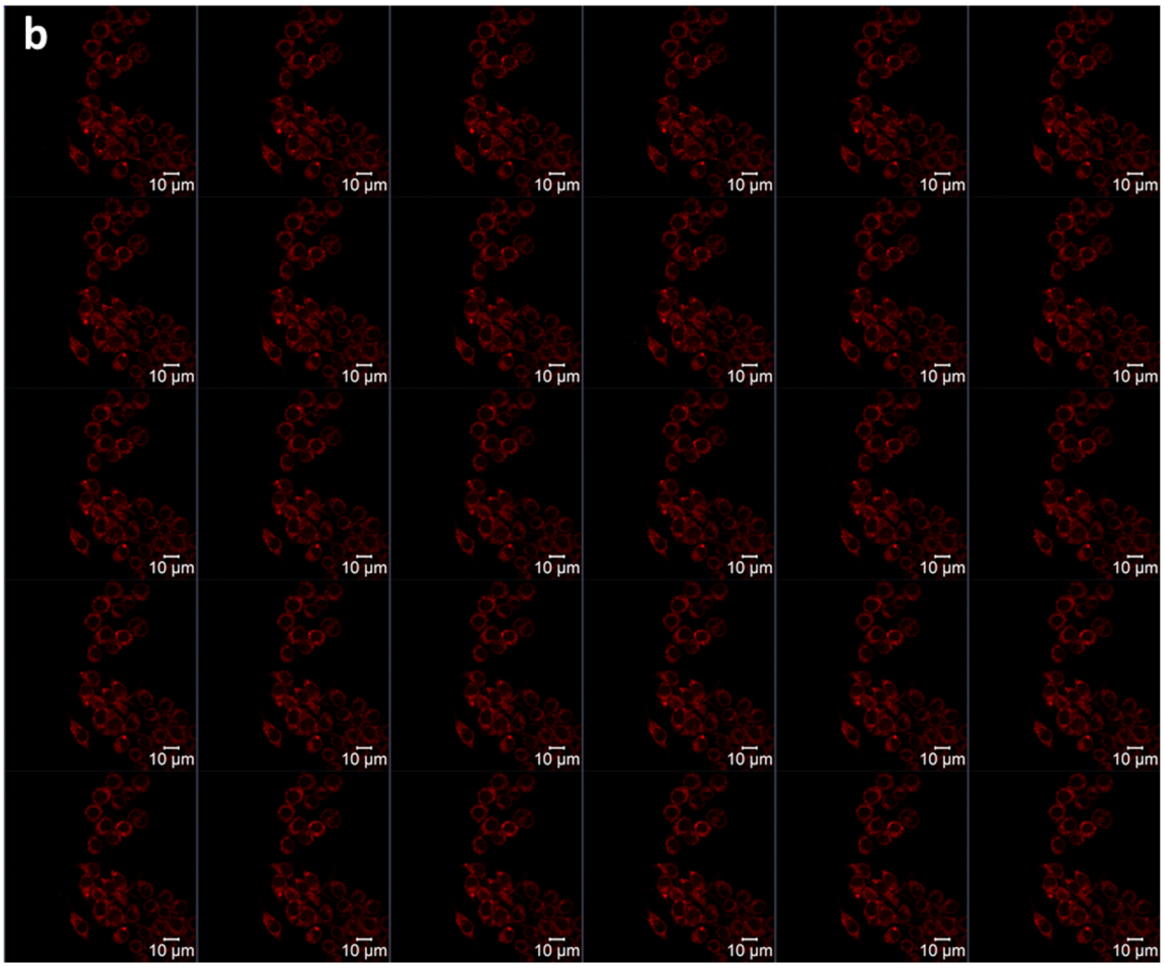


Figure S7. Time lapse monitoring of **HKOCl-4r** in PMA stimulated RAW264.7 cells. (a) Cells were incubated with **HKOCl-4r** (5 µM) alone, then monitored for 5 min. (b) Cells were co-incubated with **HKOCl-4r** (5 µM) and PMA (500 ng/mL), then monitored for 5 min. The images were recorded every 10 s per frame. Results are representative of at least three independent experiments. Scale bar: 10 µm.

10. Colocalization of HKOCl-4m and Mito Tracker Green FM

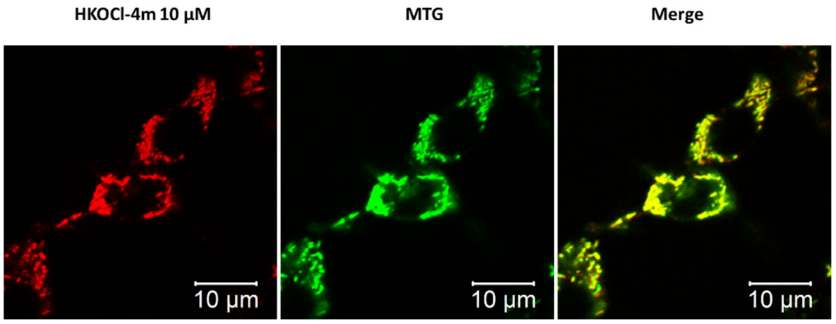
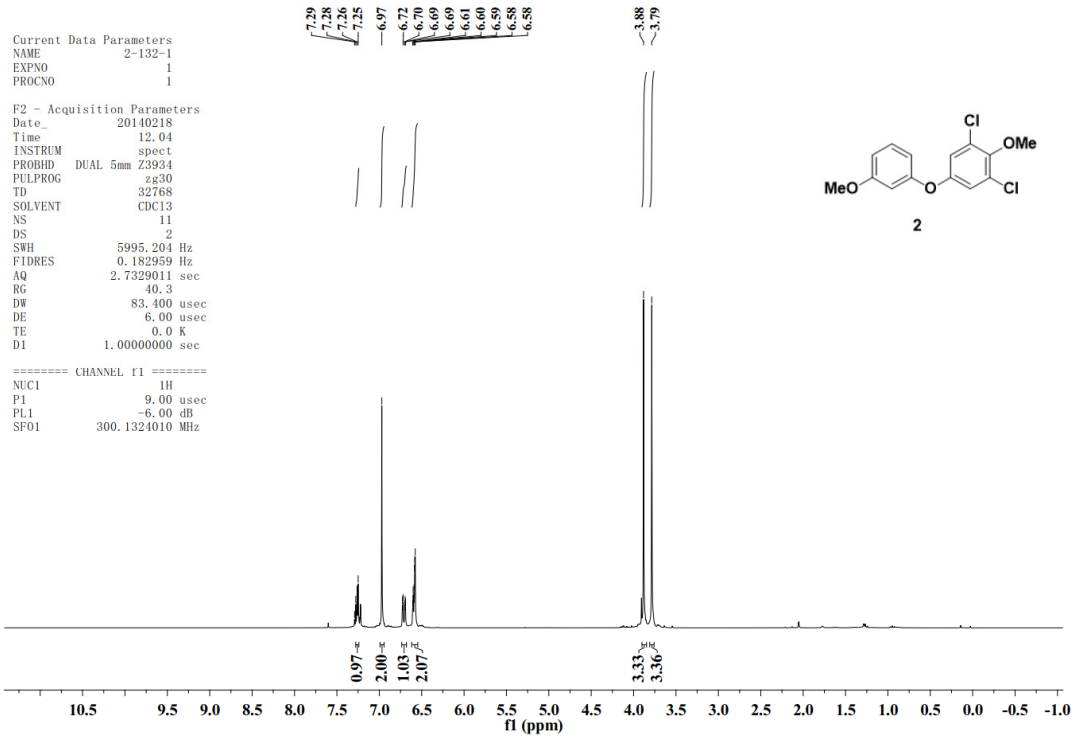
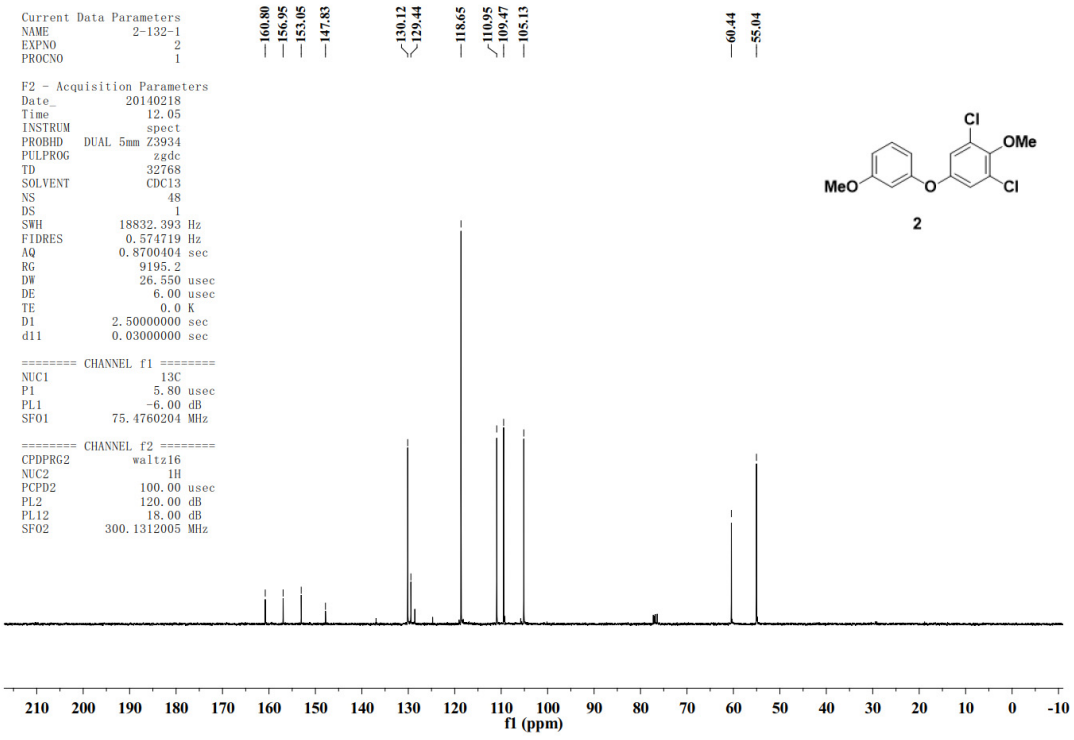
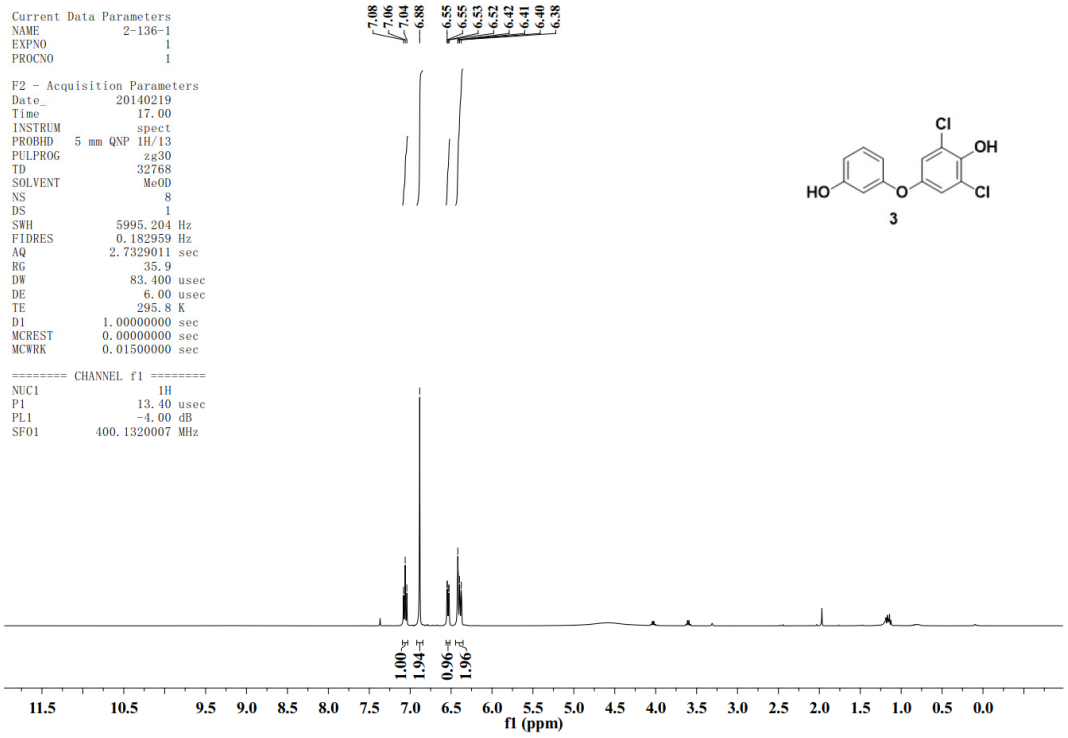


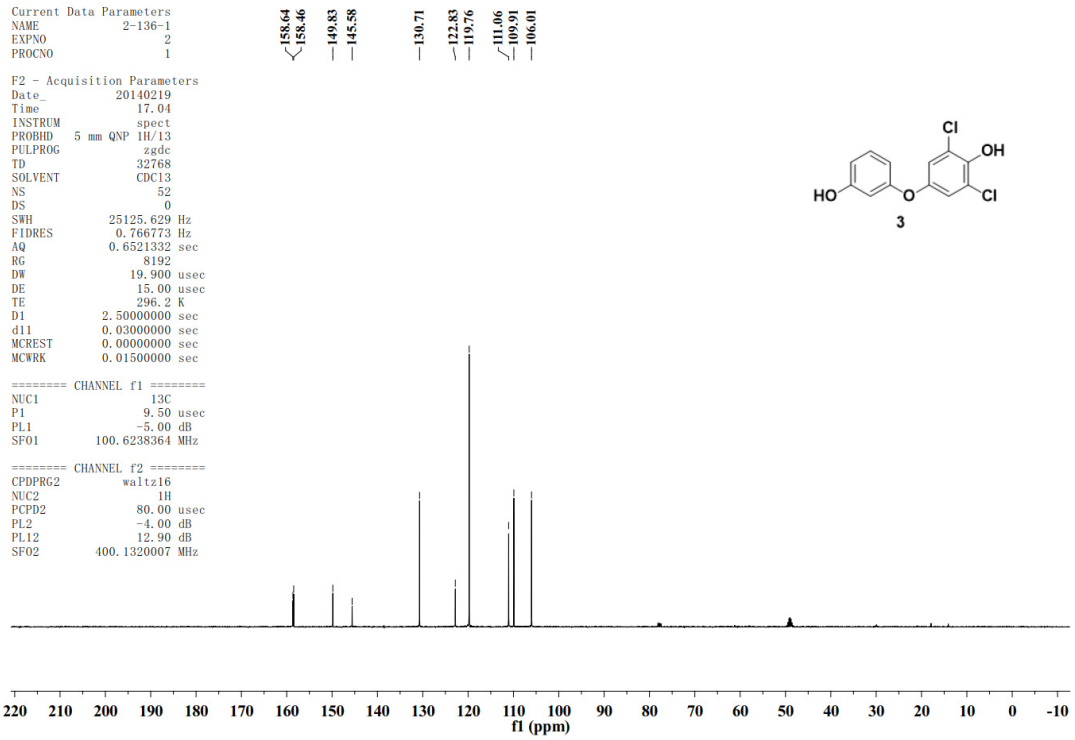
Figure S8. Colocalization of **HKOCl-4m** and Mito Tracker Green FM. **HKOCl-4m** (10 μM) was co-stained with MTG (100 nM) for 30 min. The confocal imaging was performed on LSM 710 with a higher laser intensity (Laser excitation at 453 nm: 20%, for normal use: 9 %). Results are representative of at least three independent experiments. Scale bar: 10 μm.

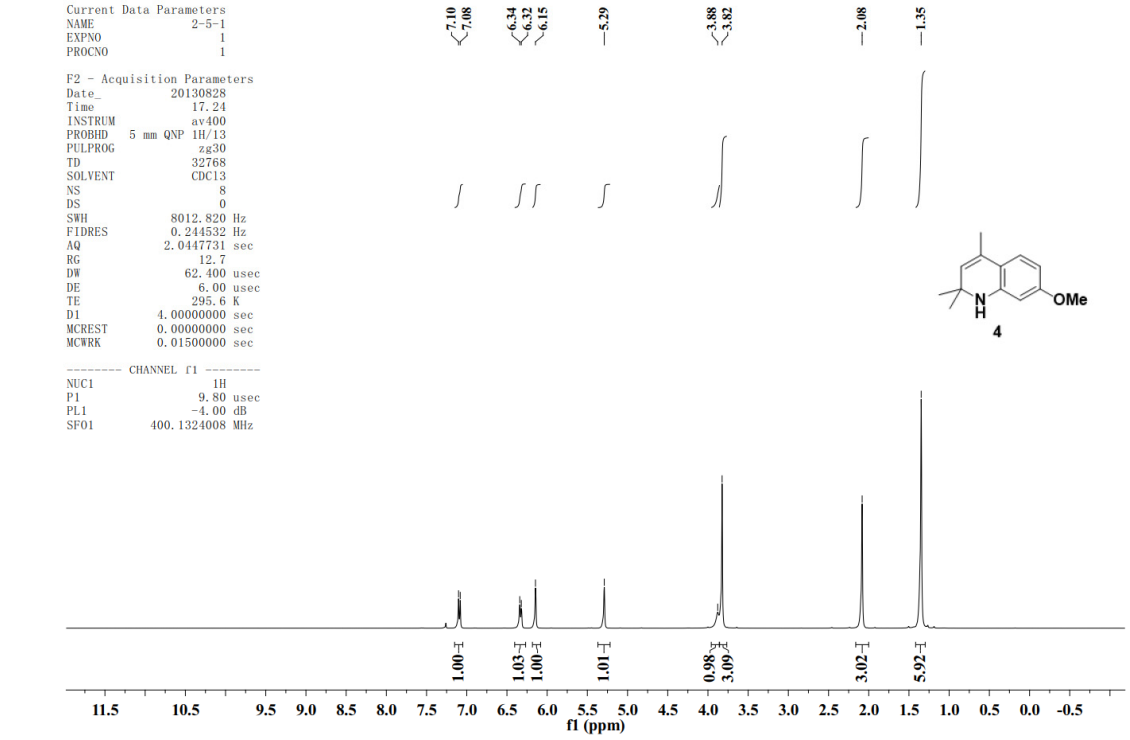
11. NMR spectra

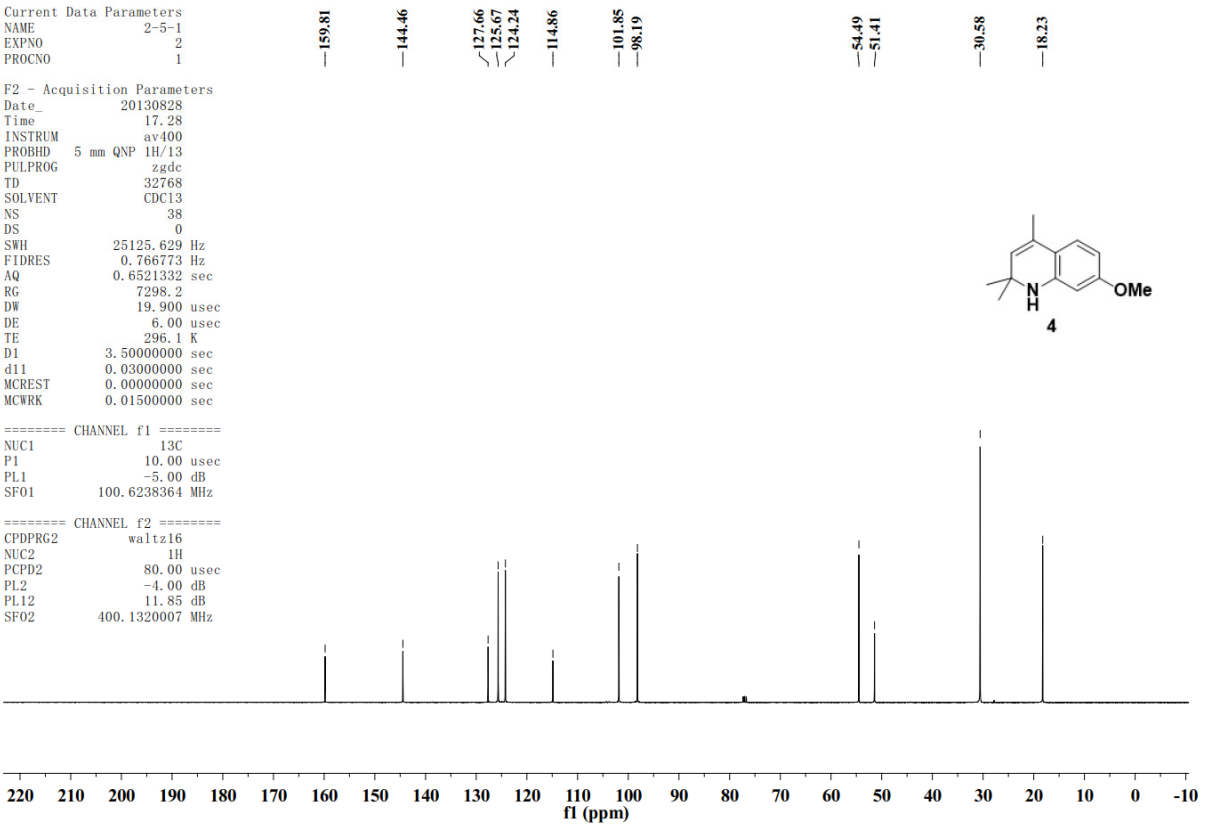

Current Data Parameters
NAME 2-132-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140218
Time 12.04
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zg30
TD 32768
SOLVENT CDC13
NS 11
DS 2
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 40.3
DW 83.400 usec
DE 6.00 usec
TE 0.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 -6.00 dB
SF01 300.1324010 MHz
7.29
7.28
7.26
7.25
6.97
6.72
6.70
6.69
6.69
6.61
6.60
6.59
6.58
6.58
3.88
3.79
0.97
2.00
1.03
2.07
3.33
3.36
Cl
OMe
MeO
O
Cl
2
10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

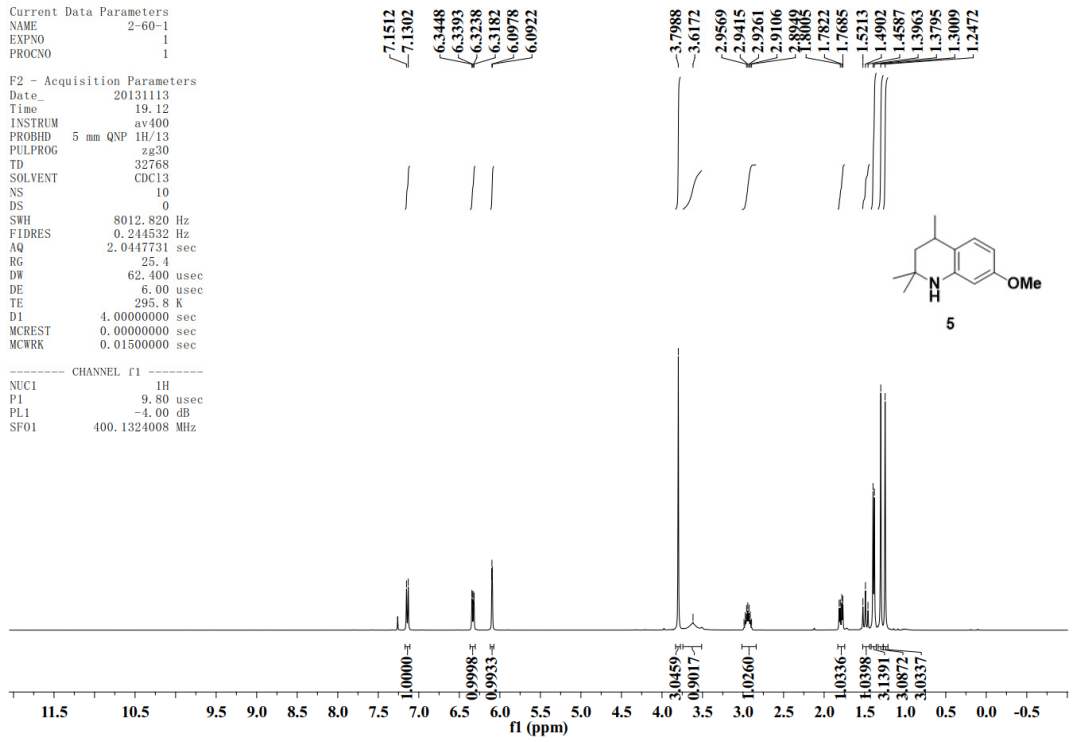

Current Data Parameters
NAME 2-132-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140218
Time 12.05
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 48
DS 1
SWH 18832.393 Hz
FIDRES 0.574719 Hz
AQ 0.8700404 sec
RG 9195.2
DW 26.550 usec
DE 6.00 usec
TE 0.0 K
D1 2.50000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SF01 75.4760204 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 120.00 dB
PL12 18.00 dB
SF02 300.1312005 MHz
160.80
156.95
153.05
147.83
130.12
129.44
118.65
110.95
109.47
105.13
60.44
55.04
Cl
OMe
MeO
O
Cl
2
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

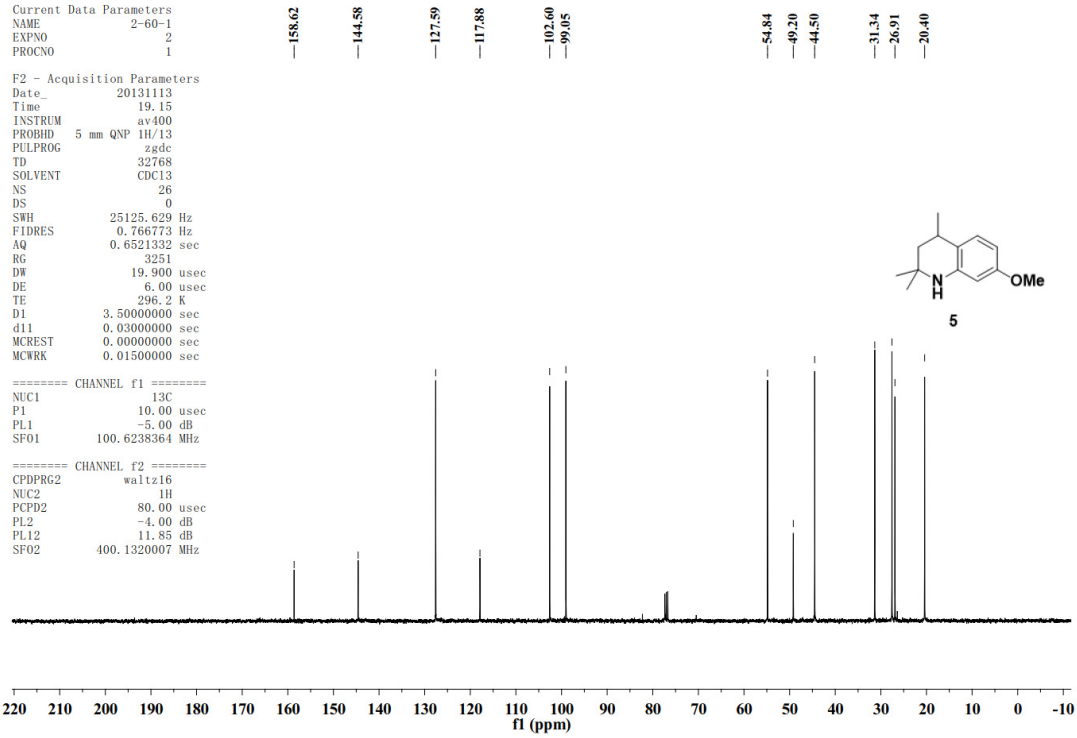

Current Data Parameters
NAME 2-136-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140219
Time 17.00
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT MeOD
NS 8
DS 1
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 35.9
DW 83.400 usec
DE 6.00 usec
TE 295.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1320007 MHz
7.08
7.06
7.04
6.88
6.55
6.55
6.53
6.52
6.42
6.41
6.40
6.38
Cl
OH
HO
O
Cl
3
1.00
1.94
0.96
1.96
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

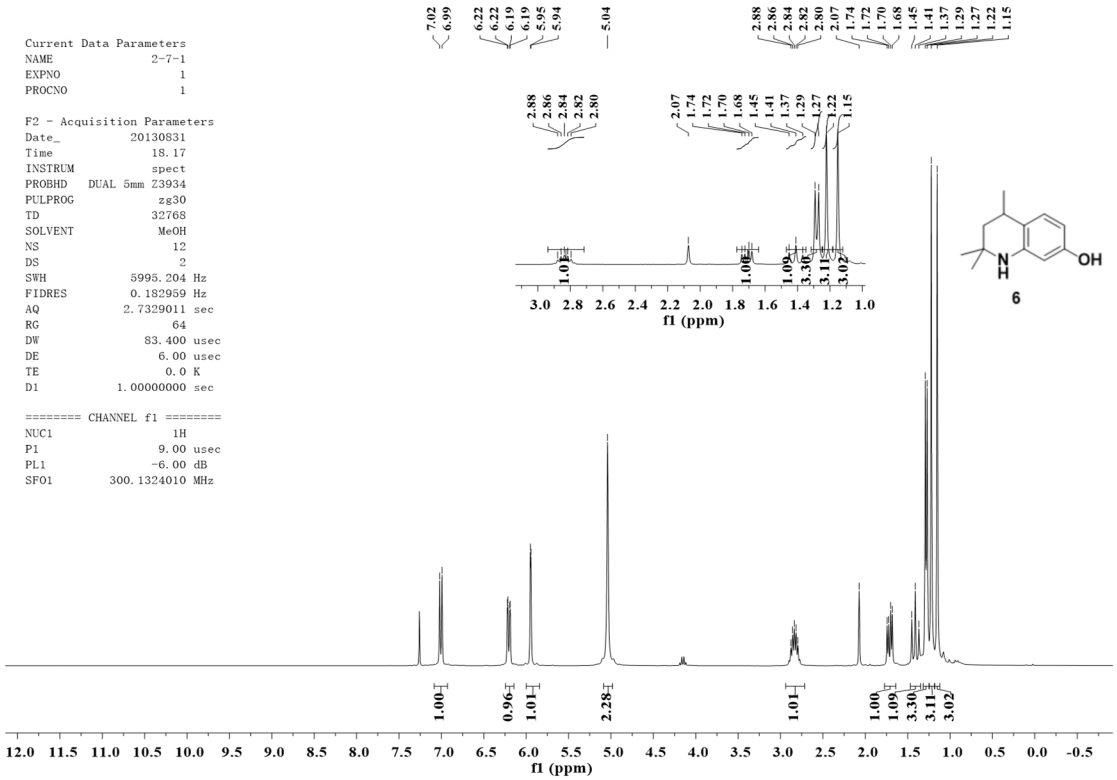

Current Data Parameters
NAME 2-136-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140219
Time 17.04
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 52
DS 0
SWH 25125.629 Hz
FIDRES 0.766773 Hz
AQ 0.6521332 sec
RG 8192
DW 19.900 usec
DE 15.00 usec
TE 296.2 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6238364 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
158.64
158.46
149.83
145.58
130.71
122.83
119.76
111.06
109.91
106.01
Cl
OH
HO
O
Cl
3
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

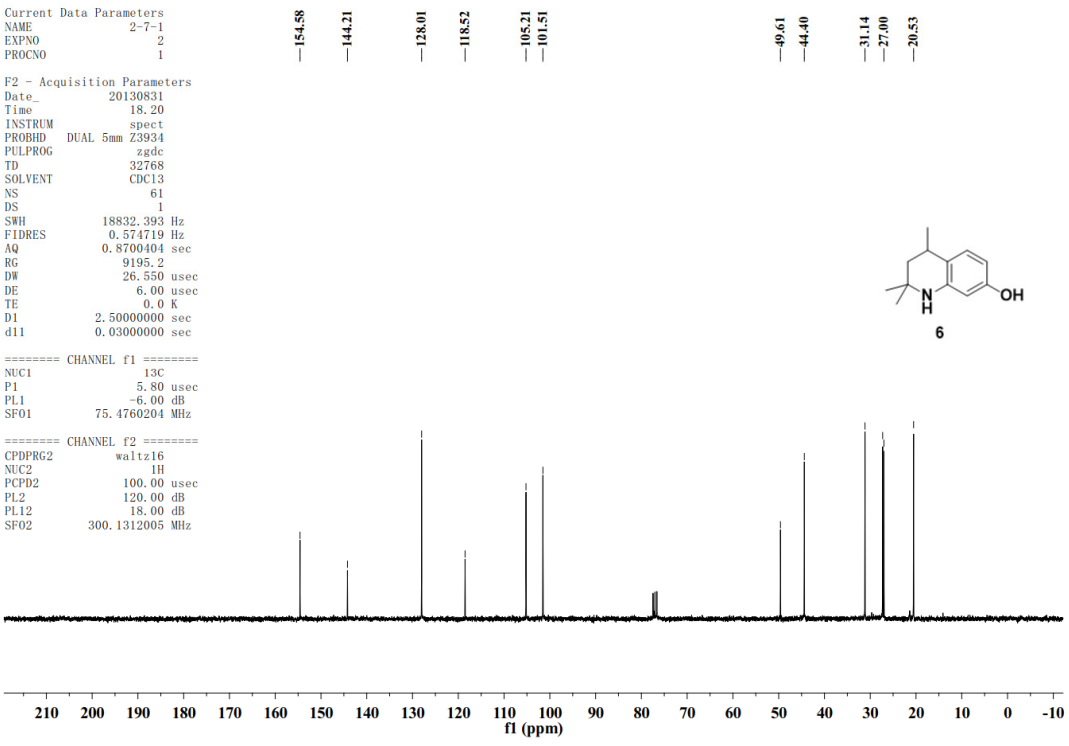

Current Data Parameters
NAME 2-5-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130828
Time 17.24
INSTRUM av400
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDC13
NS 8
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 12.7
DW 62.400 usec
DE 6.00 usec
TE 295.6 K
D1 4.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
-------- CHANNEL f1 --------
NUC1 1H
P1 9.80 usec
PL1 -4.00 dB
SF01 400.1324008 MHz
7.10
7.08
6.34
6.32
6.15
5.29
3.88
3.82
2.08
1.35
OMe
N
H
4
1.00
1.03
1.00
1.01
0.98
3.09
3.02
5.92
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

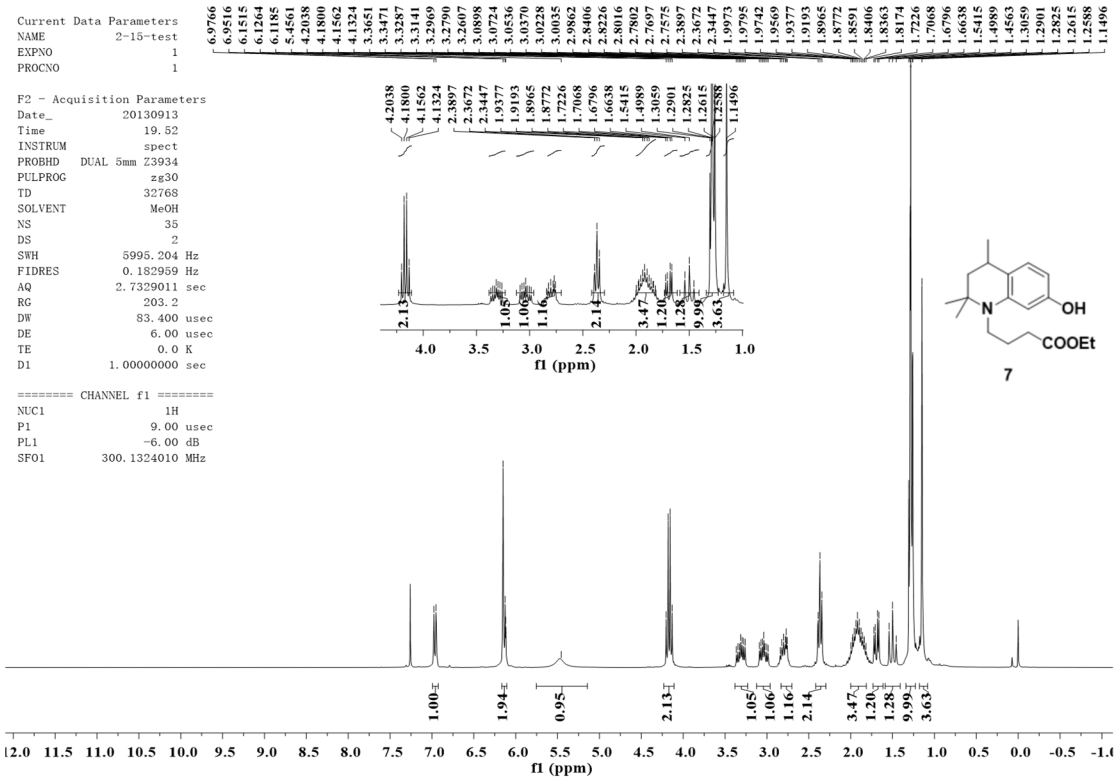

Current Data Parameters
NAME 2-5-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130828
Time 17.28
INSTRUM av400
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 38
DS 0
SWH 25125.629 Hz
FIDRES 0.766773 Hz
AQ 0.6521332 sec
RG 7298.2
DW 19.900 usec
DE 6.00 usec
TE 296.1 K
D1 3.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PL1 -5.00 dB
SF01 100.6238364 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 11.85 dB
SF02 400.1320007 MHz
159.81
144.46
127.66
125.67
124.24
114.86
101.85
98.19
54.49
51.41
30.58
18.23
OMe
N
H
4
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

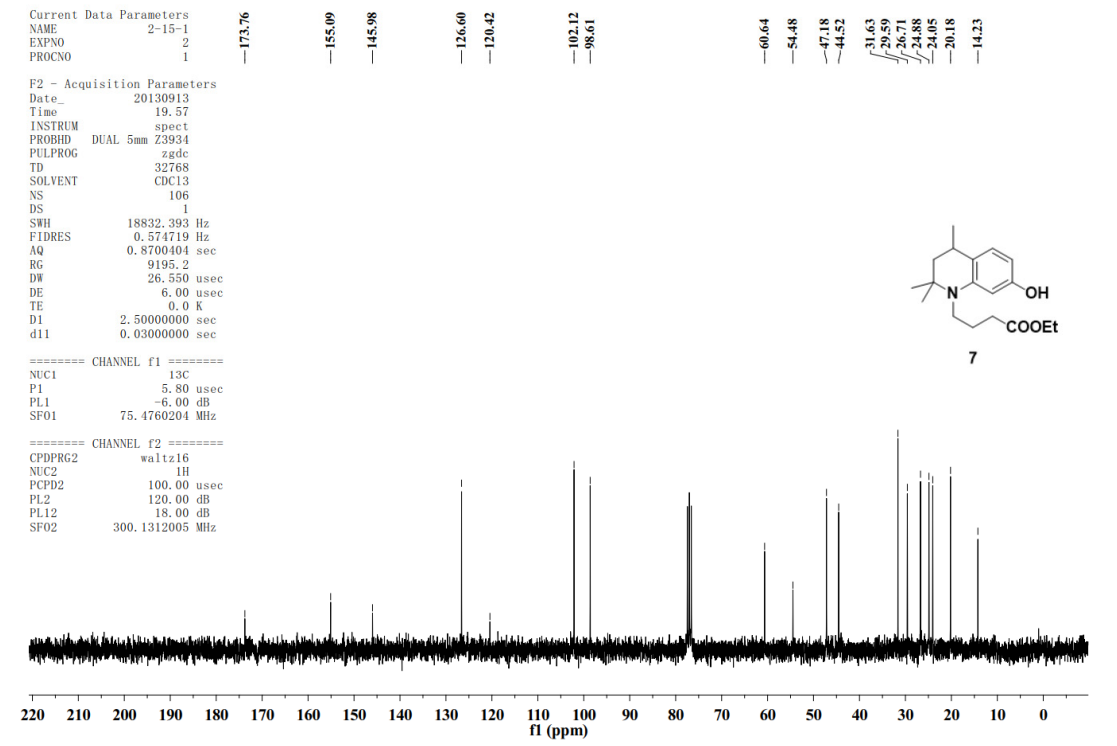

Current Data Parameters
NAME 2-60-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131113
Time 19.12
INSTRUM av400
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDC13
NS 10
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 25.4
DW 62.400 usec
DE 6.00 usec
TE 295.8 K
D1 4.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
-------- CHANNEL f1 --------
NUC1 1H
P1 9.80 usec
PL1 -4.00 dB
SFO1 400.1324008 MHz
7.1512
7.1302
6.3448
6.3393
6.3238
6.3182
6.0978
6.0922
3.7988
3.6172
2.9569
2.9415
2.9261
2.9106
2.8949
2.8805
1.7822
1.7685
1.5213
1.4902
1.4587
1.3963
1.3795
1.3009
1.2472
OMe
5
1.0000
0.9998
0.9933
3.0459
0.9017
1.0260
1.0336
1.0398
3.1391
3.0872
3.0337
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

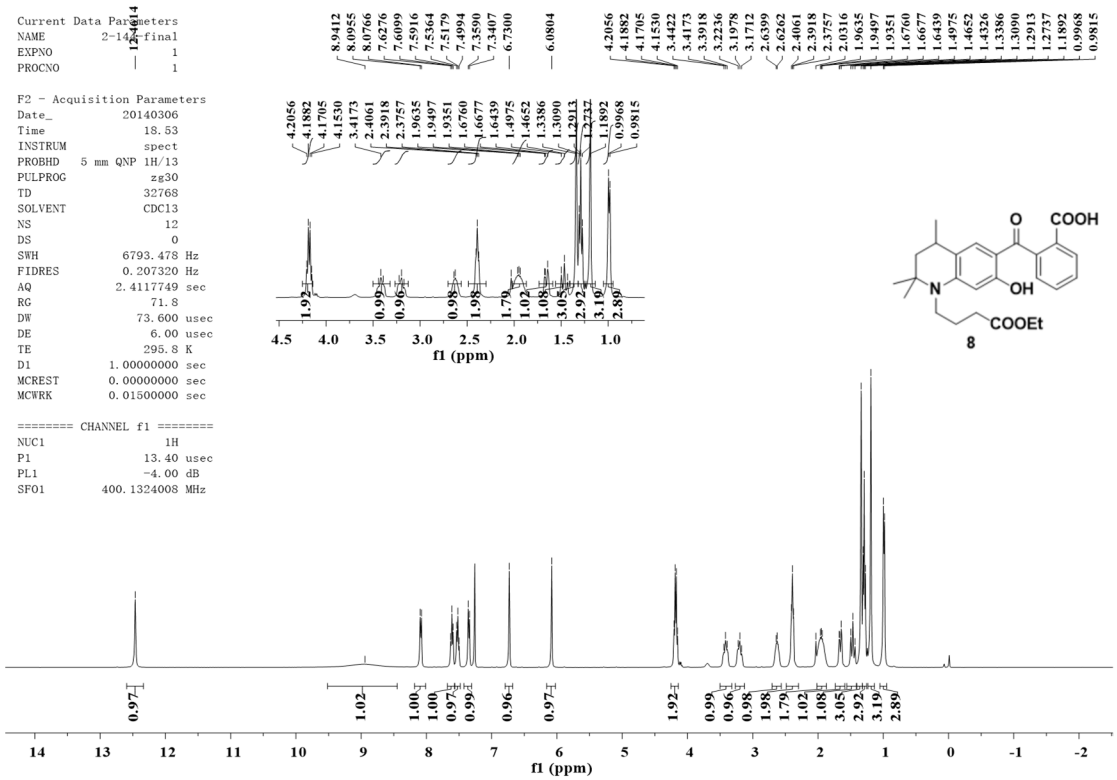

Current Data Parameters
NAME 2-60-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20131113
Time 19.15
INSTRUM av400
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 26
DS 0
SWH 25125.629 Hz
FIDRES 0.766773 Hz
AQ 0.6521332 sec
RG 3251
DW 19.900 usec
DE 6.00 usec
TE 296.2 K
D1 3.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PL1 -5.00 dB
SFO1 100.6238364 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 11.85 dB
SFO2 400.1320007 MHz
158.62
144.58
127.59
117.88
102.60
99.05
54.84
49.20
44.50
31.34
26.91
20.40
OMe
5
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

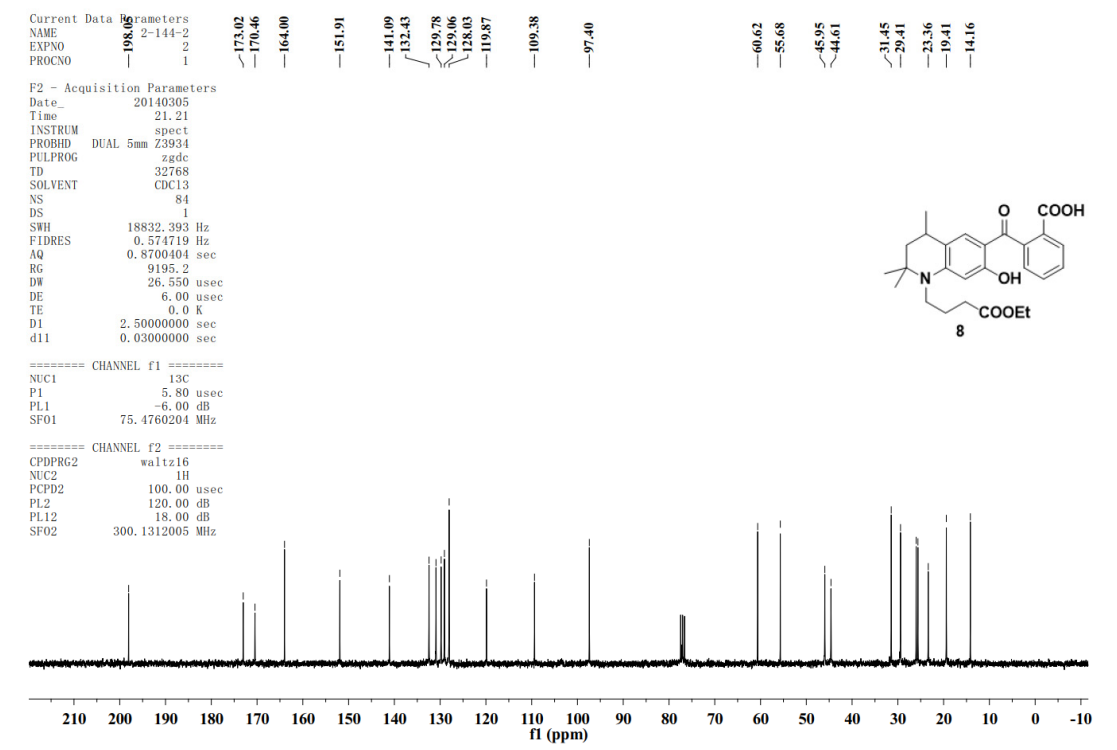

Current Data Parameters
NAME 2-7-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130831
Time 18.17
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zg30
TD 32768
SOLVENT MeOH
NS 12
DS 2
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 64
DW 83.400 usec
DE 6.00 usec
TE 0.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 -6.00 dB
SFO1 300.1324010 MHz
7.02
6.99
6.22
6.22
6.19
6.19
5.95
5.94
5.04
2.88
2.86
2.84
2.82
2.80
2.07
1.74
1.72
1.70
1.68
1.45
1.41
1.37
1.29
1.27
1.22
1.15
1.00
0.96
1.01
2.28
1.01
1.00
1.09
3.30
3.11
3.02
f1 (ppm)
OH
N
H
6

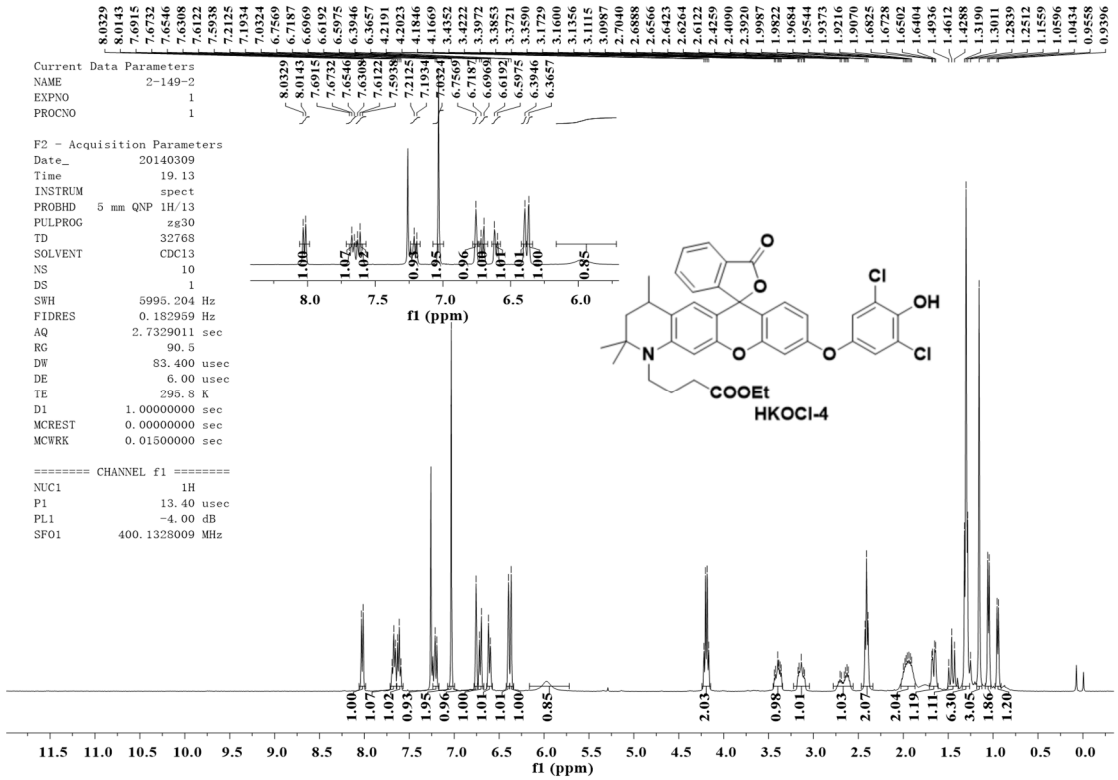

Current Data Parameters
NAME 2-7-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130831
Time 18.20
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 61
DS 1
SWH 18832.393 Hz
FIDRES 0.574719 Hz
AQ 0.8700404 sec
RG 9195.2
DW 26.550 usec
DE 6.00 usec
TE 0.0 K
D1 2.50000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 75.4760204 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 120.00 dB
PL12 18.00 dB
SFO2 300.1312005 MHz
154.58
144.21
128.01
118.52
105.21
101.51
49.61
44.40
31.14
27.00
20.53
f1 (ppm)
OH
N
H
6

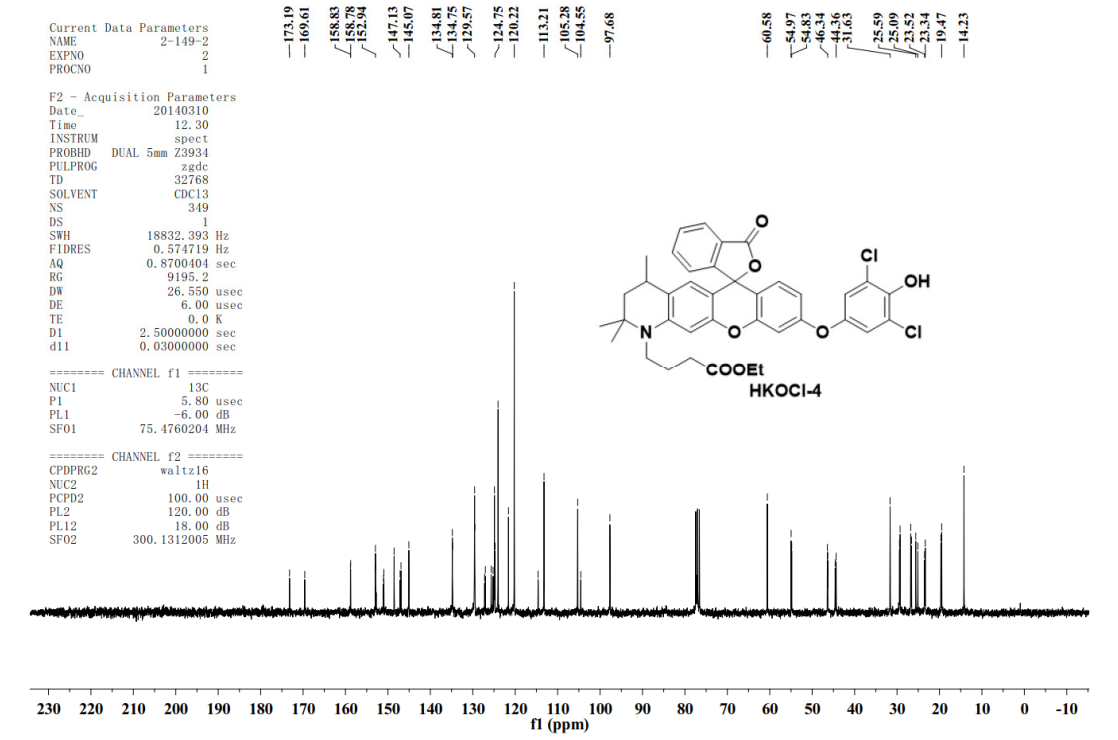

Current Data Parameters
NAME 2-15-test
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130913
Time 19.52
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zg30
TD 32768
SOLVENT MeOH
NS 35
DS 2
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 203.2
DW 83.400 usec
DE 6.00 usec
TE 0.0 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 -6.00 dB
SFO1 300.1324010 MHz
f1 (ppm)
OH
COOEt
7

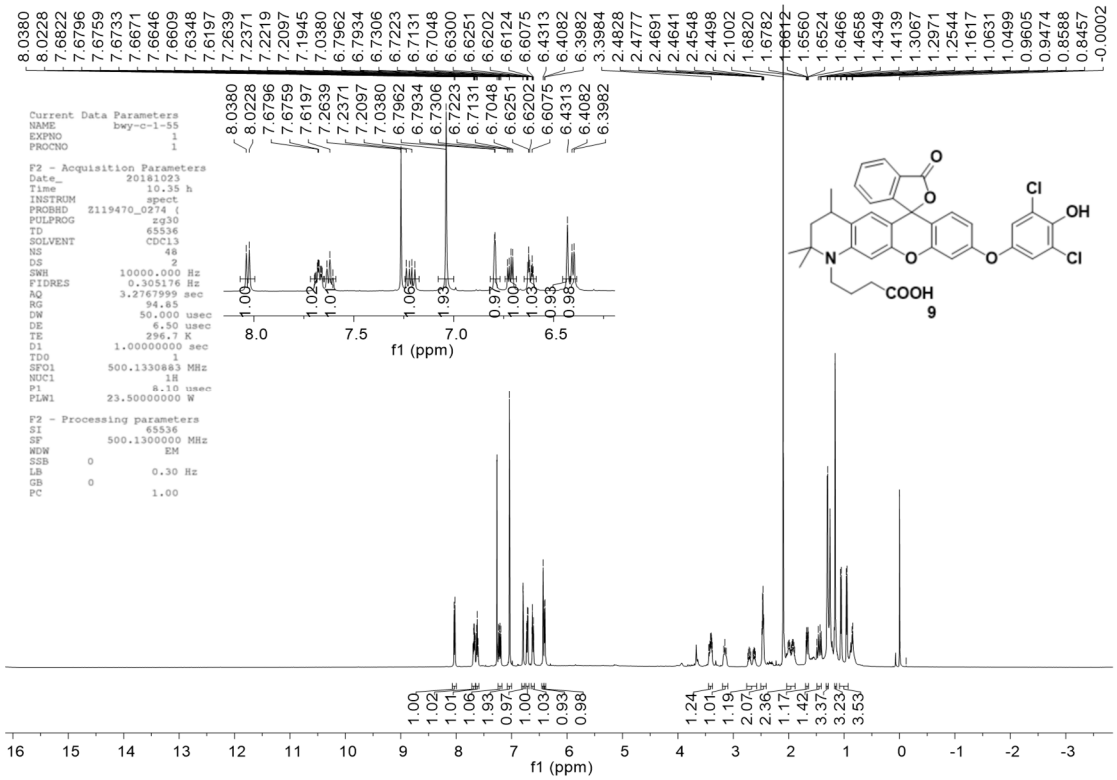

Current Data Parameters
NAME 2-15-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130913
Time 19.57
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 106
DS 1
SWH 18832.393 Hz
FIDRES 0.574719 Hz
AQ 0.8700404 sec
RG 9195.2
DW 26.550 usec
DE 6.00 usec
TE 0.0 K
D1 2.50000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 75.4760204 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 120.00 dB
PL12 18.00 dB
SFO2 300.1312005 MHz
f1 (ppm)
OH
COOEt
7

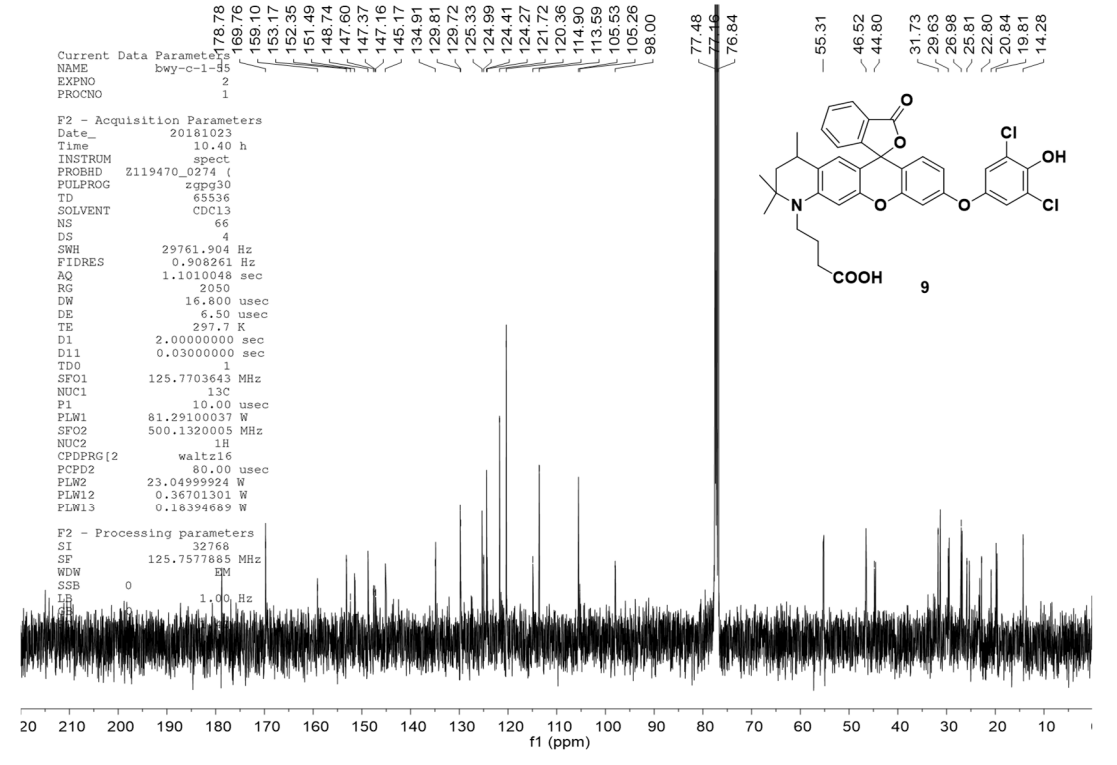

Current Data Parameters
NAME 2-144-final
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140306
Time 18.53
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDC13
NS 12
DS 0
SWH 6793.478 Hz
FIDRES 0.207320 Hz
AQ 2.4117749 sec
RG 71.8
DW 73.600 usec
DE 6.00 usec
TE 295.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1324008 MHz
f1 (ppm)
O
COOH
N
OH
COOEt
8

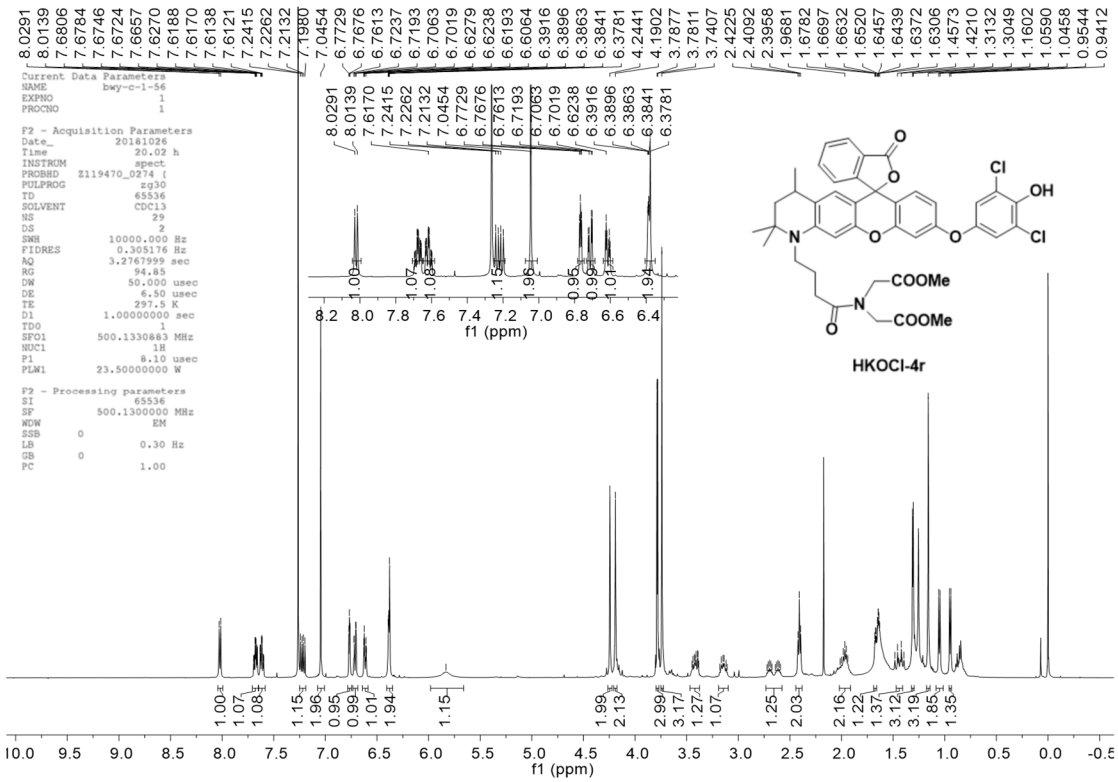

Current Data Parameters
NAME 2-144-2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140305
Time 21.21
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 84
DS 1
SWH 18832.393 Hz
FIDRES 0.574719 Hz
AQ 0.8700404 sec
RG 9195.2
DW 26.550 usec
DE 6.00 usec
TE 0.0 K
D1 2.50000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 75.4760204 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 120.00 dB
PL12 18.00 dB
SFO2 300.1312005 MHz
f1 (ppm)
O
COOH
N
OH
COOEt
8

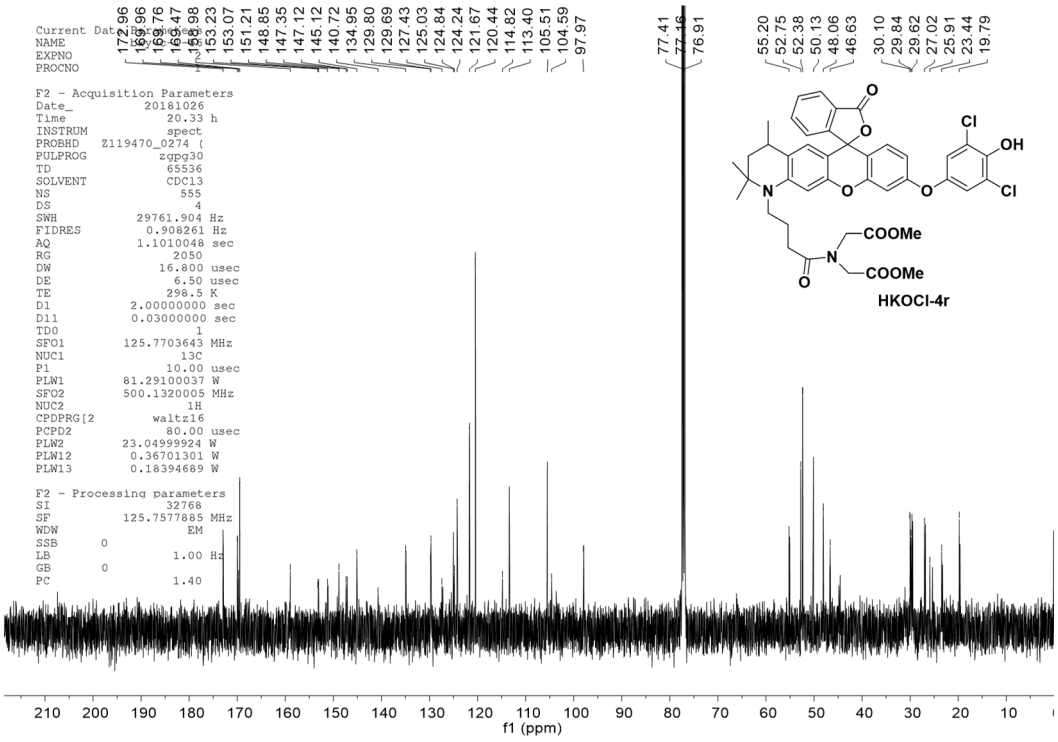

Current Data Parameters
NAME 2-149-2
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140309
Time 19.13
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 10
DS 1
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 90.5
DW 83.400 usec
DE 6.00 usec
TE 295.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1328009 MHz
f1 (ppm)
COOEt
HKOCl-4

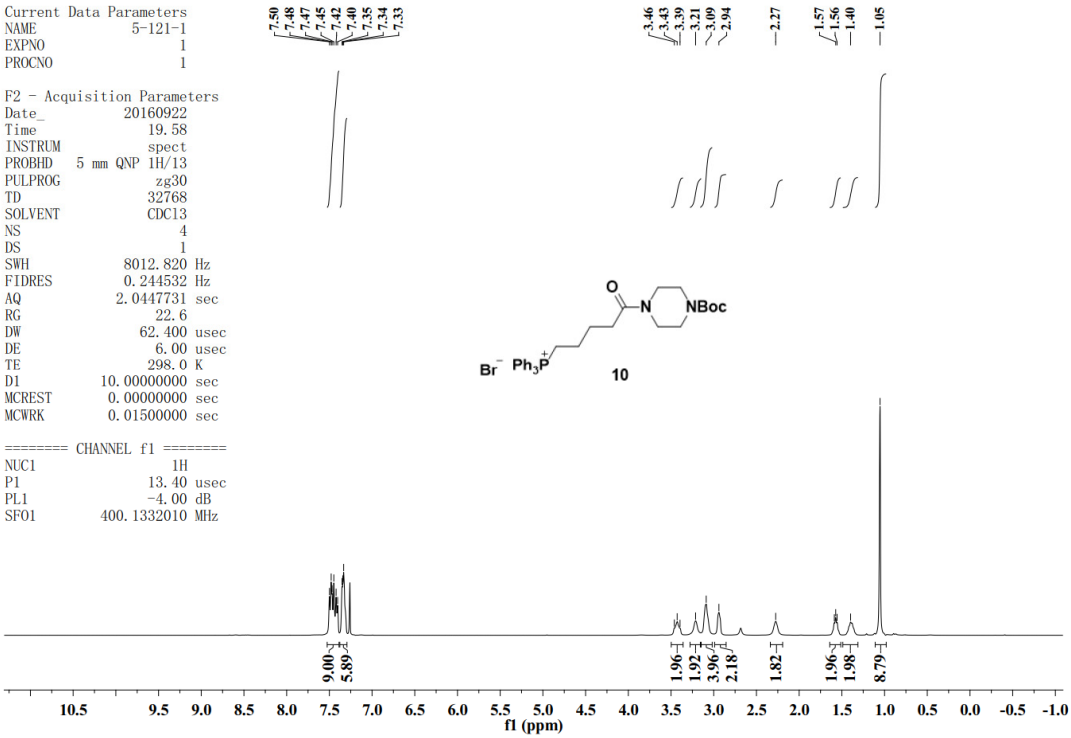

Current Data Parameters
NAME 2-149-2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140310
Time 12.30
INSTRUM spect
PROBHD DUAL 5mm Z3934
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 349
DS 1
SWH 18832.393 Hz
FIDRES 0.574719 Hz
AQ 0.8700404 sec
RG 9195.2
DW 26.550 usec
DE 6.00 usec
TE 0.0 K
D1 2.50000000 sec
d11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 5.80 usec
PL1 -6.00 dB
SFO1 75.4760204 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 120.00 dB
PL12 18.00 dB
SF02 300.1312005 MHz
f1 (ppm)
COOEt
HKOCl-4

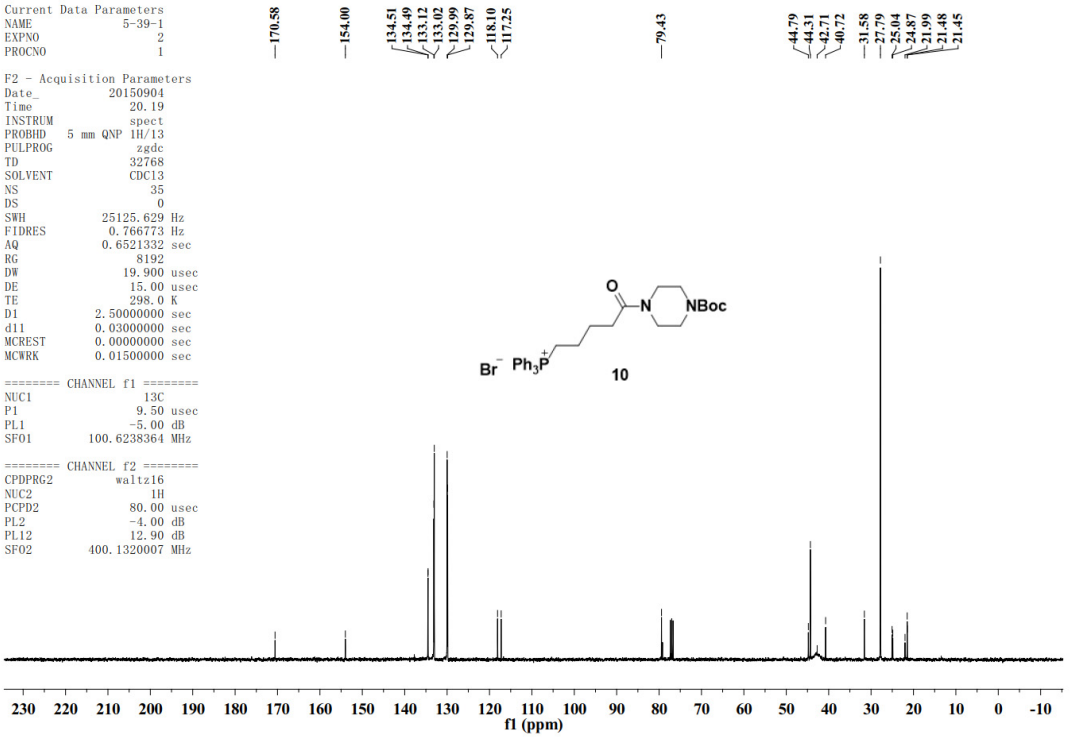

Current Data Parameters
NAME bwy-c-1-55
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181023
Time 10.35 h
INSTRUM spect
PROBHD Z119470_0274 (
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 48
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 94.85
DW 50.000 usec
DE 6.50 usec
TE 296.7 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P1 8.10 usec
PLW1 23.50000000 W
F2 - Processing parameters
SI 65536
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
f1 (ppm)
COOH
9

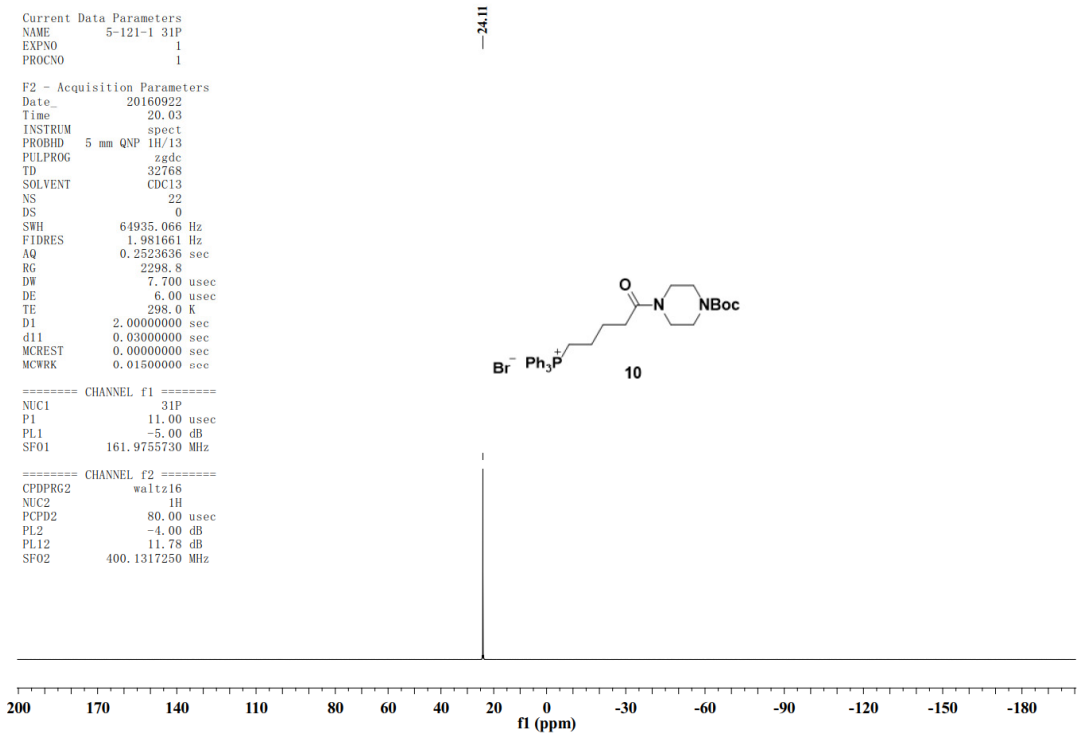

Current Data Parameters
NAME bwy-c-1-55
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181023
Time 10.40 h
INSTRUM spect
PROBHD Z119470_0274 (
PULPROG zgpg30
TD 65536
SOLVENT CDC13
NS 66
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 2050
DW 16.800 usec
DE 6.50 usec
TE 297.7 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 81.29100037 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 23.04999924 W
PLW12 0.36701301 W
PLW13 0.18394689 W
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
f1 (ppm)
COOH
9

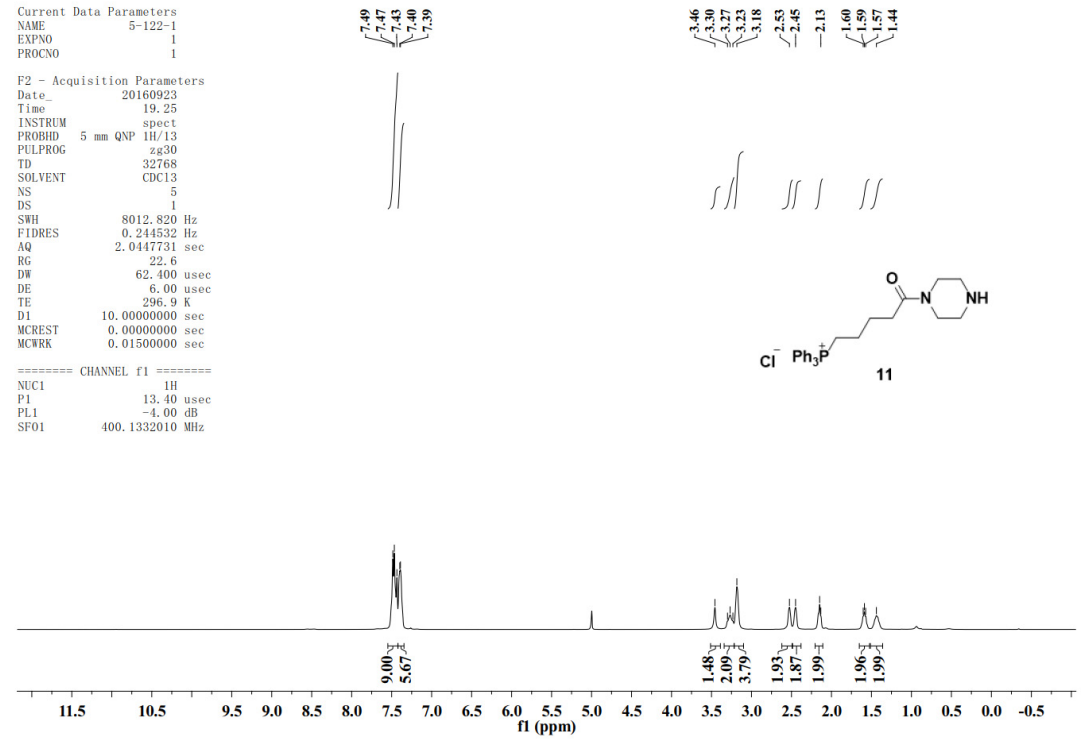

HKOCl-4r
f1 (ppm)

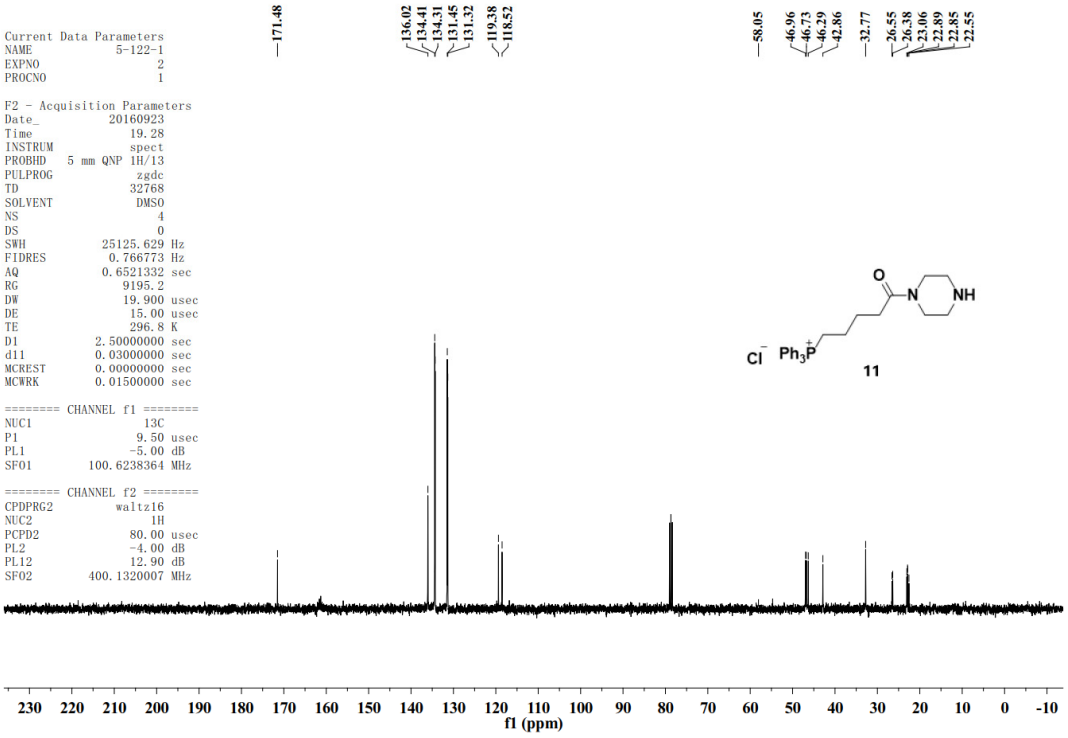

HKOCl-4r
f1 (ppm)

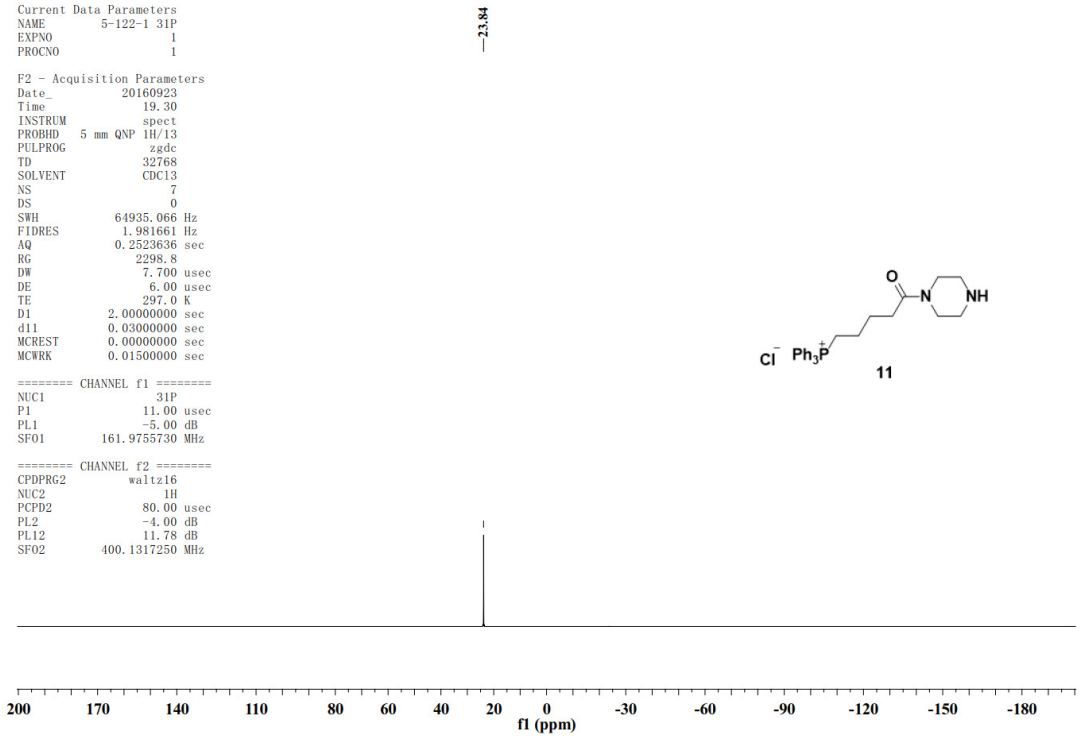

Current Data Parameters
NAME 5-121-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160922
Time 19.58
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDC13
NS 4
DS 1
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 22.6
DW 62.400 usec
DE 6.00 usec
TE 298.0 K
D1 10.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1332010 MHz
7.50
7.48
7.47
7.45
7.42
7.40
7.35
7.34
7.33
3.46
3.43
3.39
3.21
3.09
2.94
2.27
1.57
1.56
1.40
1.05
O
N
NBoc
Br⁻ Ph3P+
10
9.00
5.89
1.96
1.92
3.96
2.18
1.82
1.96
1.98
8.79
10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

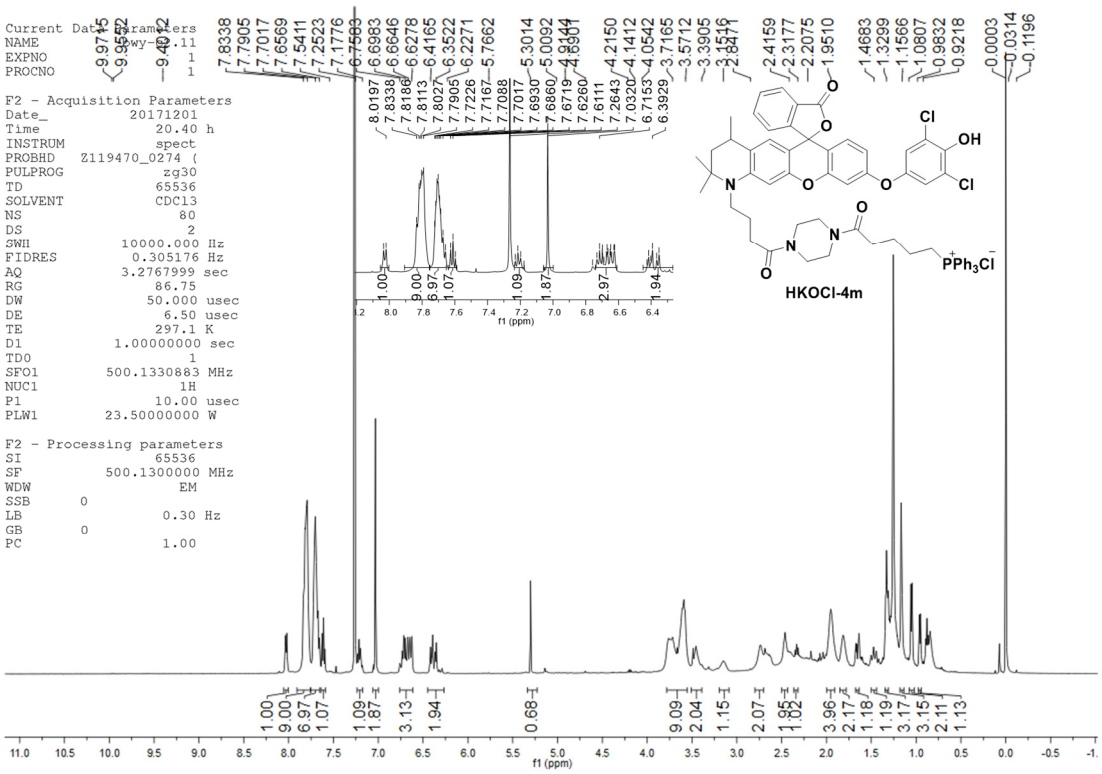

Current Data Parameters
NAME 5-39-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20150904
Time 20.19
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 35
DS 0
SWH 25125.629 Hz
FIDRES 0.766773 Hz
AQ 0.6521332 sec
RG 8192
DW 19.900 usec
DE 15.00 usec
TE 298.0 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6238364 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
170.58
154.00
134.51
134.49
133.12
133.02
129.99
129.87
118.10
117.25
79.43
44.79
44.31
42.71
40.72
31.58
27.79
25.04
24.87
21.99
21.48
21.45
O
N
NBoc
Br⁻ Ph3P+
10
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

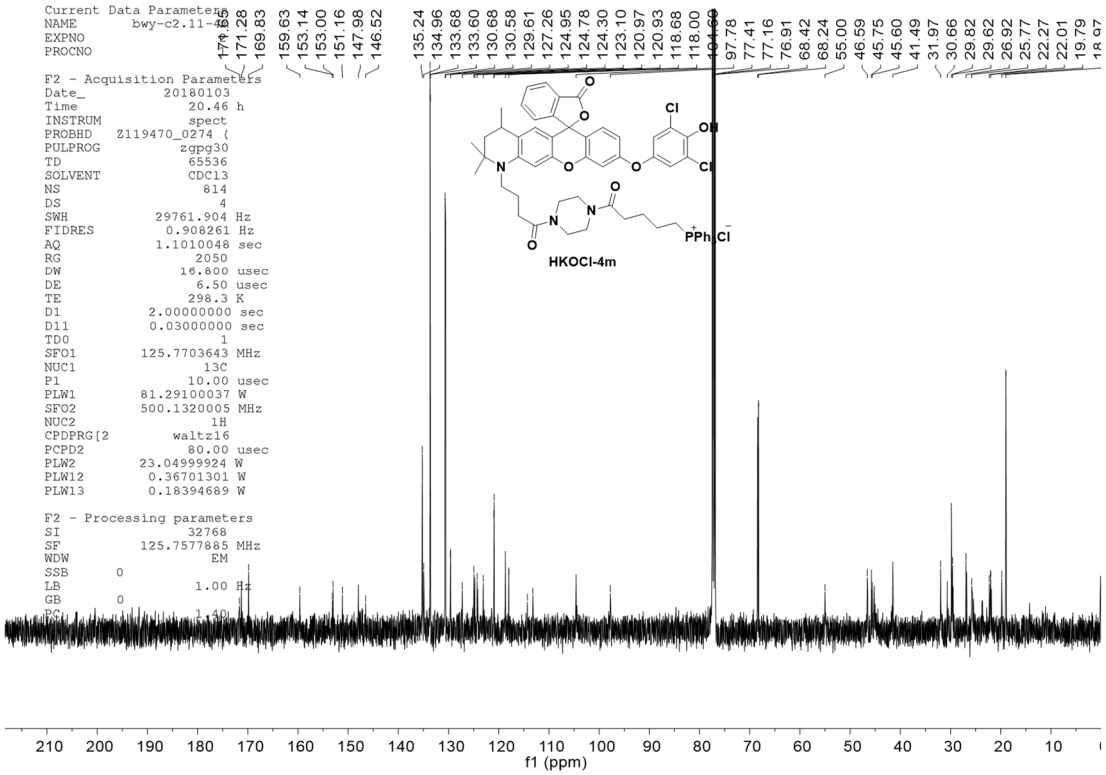

Current Data Parameters
NAME 5-121-1 31P
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160922
Time 20.03
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 22
DS 0
SWH 64935.066 Hz
FIDRES 1.981661 Hz
AQ 0.2523636 sec
RG 2298.8
DW 7.700 usec
DE 6.00 usec
TE 298.0 K
D1 2.00000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 31P
P1 11.00 usec
PL1 -5.00 dB
SFO1 161.9755730 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 11.78 dB
SFO2 400.1317250 MHz
24.11
O
N
NBoc
Br⁻ Ph3P+
10
200 170 140 110 80 60 40 20 0 -30 -60 -90 -120 -150 -180
f1 (ppm)

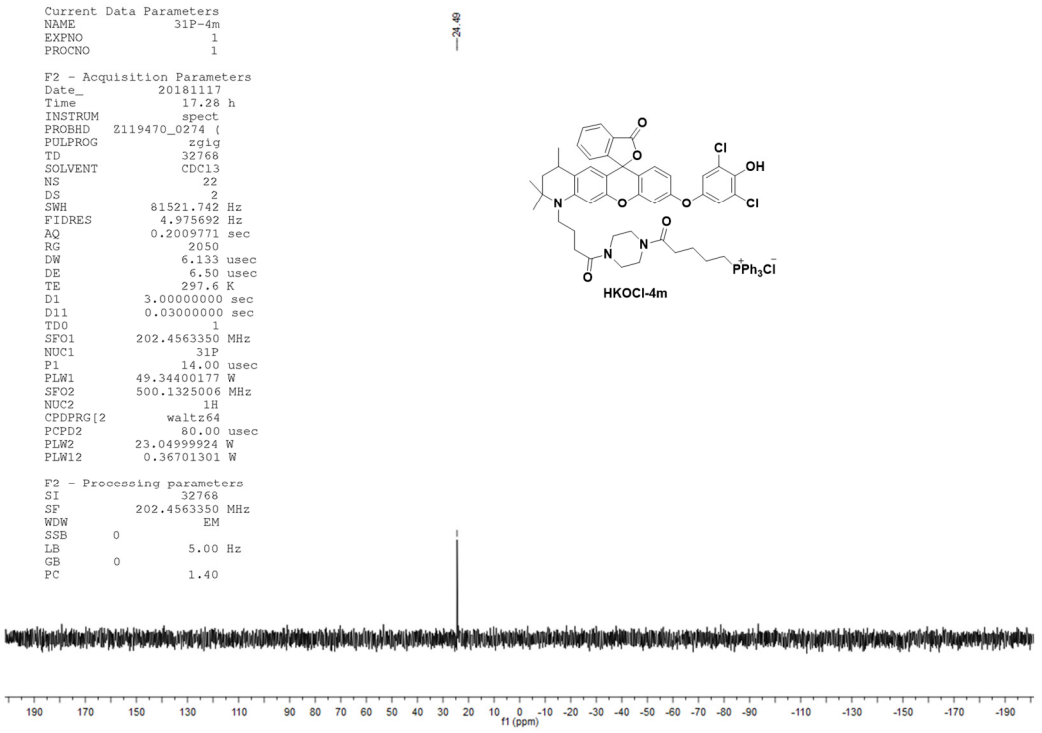

Current Data Parameters
NAME 5-122-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160923
Time 19.25
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 5
DS 1
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 22.6
DW 62.400 usec
DE 6.00 usec
TE 296.9 K
D1 10.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1332010 MHz
7.49
7.47
7.43
7.40
7.39
3.46
3.30
3.27
3.23
3.18
2.53
2.45
2.13
1.60
1.59
1.57
1.44
O
N
NH
Cl⁻ Ph3P+
11
9.00
5.67
1.48
2.09
3.79
1.93
1.87
1.99
1.96
1.99
11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

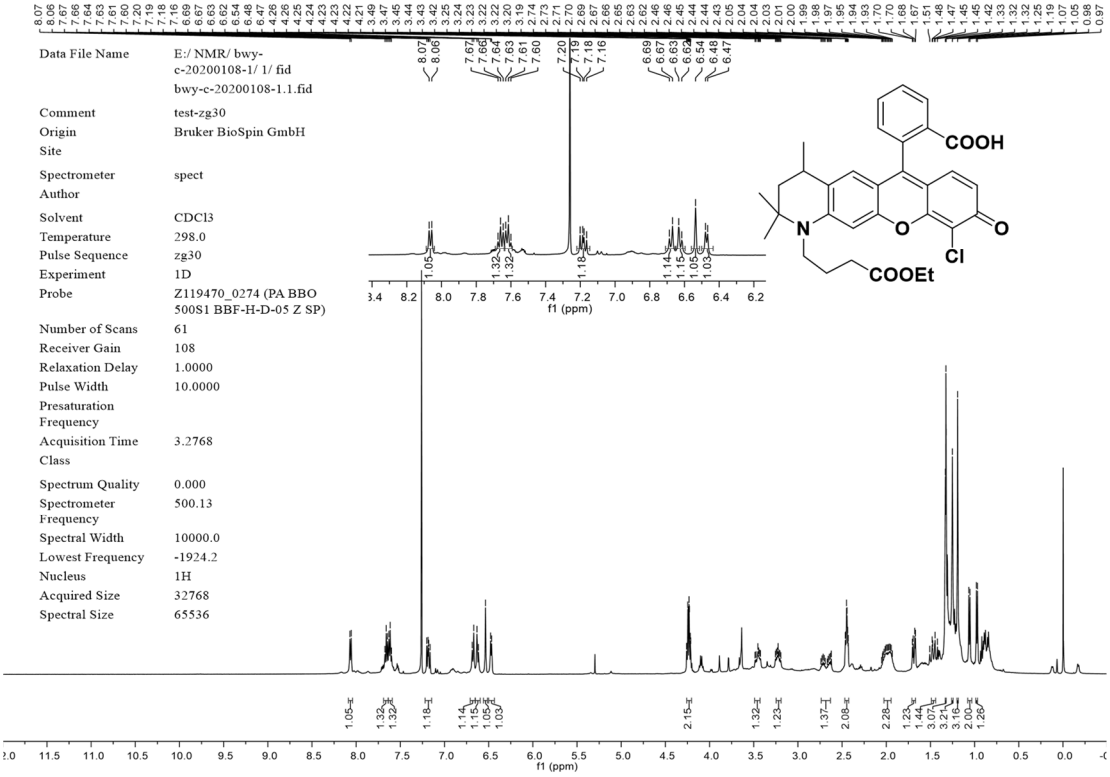

Current Data Parameters
NAME 5-122-1
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160923
Time 19.28
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT DMSO
NS 4
DS 0
SWH 25125.629 Hz
FIDRES 0.766773 Hz
AQ 0.6521332 sec
RG 9195.2
DW 19.900 usec
DE 15.00 usec
TE 296.8 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6238364 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
171.48
136.02
134.41
134.31
131.45
131.32
119.38
118.52
58.05
46.96
46.73
46.29
42.86
32.77
26.55
26.38
23.06
22.89
22.85
22.55
O
N
NH
Cl⁻ Ph3P+
11
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


Current Data Parameters
NAME 5-122-1 31P
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20160923
Time 19.30
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDC13
NS 7
DS 0
SWH 64935.066 Hz
FIDRES 1.981661 Hz
AQ 0.2523636 sec
RG 2298.8
DW 7.700 usec
DE 6.00 usec
TE 297.0 K
D1 2.00000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 31P
P1 11.00 usec
PL1 -5.00 dB
SFO1 161.9755730 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 11.78 dB
SFO2 400.1317250 MHz
23.84
O
N
NH
Cl⁻ Ph3P+
11
200 170 140 110 80 60 40 20 0 -30 -60 -90 -120 -150 -180
f1 (ppm)


HKOCl-4m
f1 (ppm)


HKOCl-4m
f1 (ppm)


Current Data Parameters
NAME 31P-4m
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181117
Time 17.28 h
INSTRUM spect
PROBHD Z119470_0274 (
PULPROG zgig
TD 32768
SOLVENT CDCl3
NS 22
DS 2
SWH 81521.742 Hz
FIDRES 4.975692 Hz
AQ 0.2009771 sec
RG 2050
DW 6.133 usec
DE 6.50 usec
TE 297.6 K
D1 3.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 202.4563350 MHz
NUC1 31P
P1 14.00 usec
PLW1 49.34400177 W
SFO2 500.1325006 MHz
NUC2 1H
CPDPRG[2 waltz64
PCPD2 80.00 usec
PLW2 23.04999924 W
PLW12 0.36701301 W
F2 - Processing parameters
SI 32768
SF 202.4563350 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40
24.49
HKOCl-4m
f1 (ppm)


Data File Name E:/ NMR/ bwy-c-20200108-1/ 1/ fid
bwy-c-20200108-1.1.fid
Comment test-zg30
Origin Bruker BioSpin GmbH
Site
Spectrometer spect
Author
Solvent CDCl3
Temperature 298.0
Pulse Sequence zg30
Experiment 1D
Probe Z119470_0274 (PA BBO 500S1 BBF-H-D-05 Z SP)
Number of Scans 61
Receiver Gain 108
Relaxation Delay 1.0000
Pulse Width 10.0000
Presaturation Frequency
Acquisition Time 3.2768
Class
Spectrum Quality 0.000
Spectrometer Frequency 500.13
Spectral Width 10000.0
Lowest Frequency -1924.2
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
COOH
COOEt
f1 (ppm)

12. References:

(1) J. W. Reed, H. H. Ho, W. L. Jolly, *J. Am. Chem. Soc.* **1974**, *96*, 1248-1249.

(2) J. J. Hu, N.-K. Wong, M.-Y. Lu, X. Chen, S. Ye, A. Q. Zhao, P. Gao, R. Yi-Tsun Kao, J. Shen, D. Yang, *Chem. Sci.* **2016**, *7*, 2094-2099.