Supplementary information for

A ^{19}F-MRI probe for the detection of Fe(II) ions in an aqueous system

Ryo Kakiuchi,[1] Tasuku Hirayama,[1,]* Daijiro Yanagisawa,[2] Ikuo Tooyama,[2] and Hideko Nagasawa[1]

[1] Laboratory of Pharmaceutical and Medicinal Chemistry, Gifu Pharmaceutical University, 1-25-4, Daigaku-nishi, Gifu-shi, Gifu, 501-1196, Japan

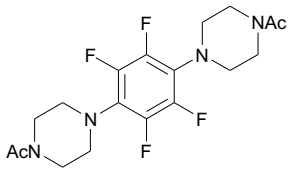
[2] Molecular Neuroscience Research Center, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan

Contents

General: All chemicals used in this study were commercial products of the highest available purity and were further purified by the standard methods, if necessary. ^{1}H-NMR spectra were obtained on a JEOL ECA-500 spectrometer at 500 MHz and JEOL ECZ-400 spectrometer at 400 MHz. ^{13}C-NMR spectra were obtained on a JEOL ECA-500 spectrometer at 125 MHz and a JEOL ECZ-400 spectrometer at 100 MHz. ^{19}F-NMR spectra were obtained on a JEOL ECA-500 spectrometer at 471 MHz and a JEOL ECZ-400 spectrometer at 376 MHz. Chemical shifts of ^{1}H-NMR are referenced to tetramethylsilane (TMS). Chemical shifts of ^{13}C-NMR are referenced to $CDCl_3$ (77.0), CD_3OD (49.0) or DMSO-d_6 (39.5). Chemical shifts of ^{19}F-NMR are referenced to hexafluorobenzene (−164.9). Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. Data for ^{1}H-NMR, ^{13}C-NMR or ^{19}F-NMR are reported as follows: chemical shift (multiplicity, coupling constants where applicable, number of hydrogens). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublet), dt (doublet of triplet), td (triplet of doublet), q (quartet), m (multiplet), br (broad). ESI-mass spectra were measured on a JEOL JMS-T100TD mass spectrometer. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-T100TD by using polyethyleneglycol (PEG) or YOKUDELNA (JEOL) as an internal standard. Anhydrous organic solvents were prepared by distillation with appropriate drying reagents before every use. Reactions were monitored by TLC silica gel (Merck, Silica gel 60 F_{254} or Silica gel 60 RP-18 F_{254S}) with visualization of components by UV light (254 nm) or a visualization reagent (molybdophosphoric acid) or with visual observation of the dye spots. Products were purified on a silica gel column chromatography (Taiko-shoji, AP-300S) or an ODS silica gel column chromatography (Fuji Silysia, CHROMATOREX, ODS-DM1020T).

1. Synthesis

Preparation of 1,1′-(4,4′-(tetrafluoro-1,4-phenylene)bis(piperazine-4,1-diyl))dimethanone (1)[1]



1

A solution of hexafluorobenzene (1.00 g, 5.37 mmol) and 1-acetylpiperazine (5.51 g, 43.0 mmol, 8.0 eq.) in 1,3-dimetyl-2-imidazolidinone (DMEU) (200 μL) was heated in a sealed tube at 95 °C for 24 h. The reaction mixture was then poured into water (100 mL), and the mixture was extracted with $CHCl_3$ (100 mL × 3). The combined organic layers were washed with water (100 mL × 3), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 30: 1) to afford **1** as a white solid (1.23 g, 57%).

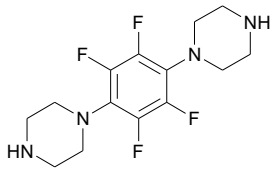
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.73 (t, J = 5.2 Hz, 4H), 3.58 (t, J = 5.2 Hz, 4H), 3.19 (t, J = 5.2 Hz, 4H), 3.14 (t, J = 5.2 Hz, 4H), 2.14 (s, 6H).

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 169.0, 143.5 (dd, J_{C-F} = 248.3, 10.8 Hz), 124.9 (m), 51.1, 50.9, 46.9, 42.0, 21.4.

^{19}F-NMR ($CDCl_3$,471 MHz): δ −154.41 (s, 4F).

HRMS (ESI+): *m/z* calcd for $C_{18}H_{22}F_4N_4NaO_2^+$:425.1571, found 425.1560.

Preparation of 1,4-di(piperazin-1-yl)-2,3,5,6-tetrafluorobenzene (2)



2

A round-bottom flask was charged with a solution of **1** (894 mg, 2.22 mmol) in THF (150 mL) and 2 M aq. HCl (150 mL). Then, the mixture was stirred at 80 °C for 24 h. The reaction mixture was washed with CH_2Cl_2 (100 mL × 3). The aqueous phase was basified by 2 M aq. NaOH (pH > 11) and extracted with $CHCl_3$ (200 mL × 3). The combined organic layer was dried over $MgSO_4$ and evaporated to afford **2** as a white solid (634 mg, 90%). This material was used for the next step without further purification.

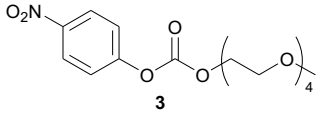
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.14 (t, J = 4.6 Hz, 8H), 2.98 (t, J = 4.6 Hz, 8H).

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 143.5 (d, J_{C-F} = 248.3 Hz), 125.3 (m), 52.4, 46.6.

^{19}F-NMR ($CDCl_3$, 471 MHz): δ −155.17 (s, 4F).

HRMS (ESI+): *m/z* calcd for $C_{14}H_{19}F_4N_4^+$: 319.1540, found 319.1569.

Preparation of 2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethyl (4-nitrophenyl) carbonate (3) (CAS: 143127-79-1)[2]

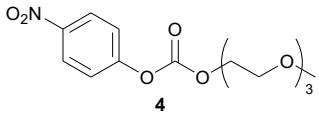


3

To a solution of 2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethanol (1.00 g, 939 μL, 4.802 mmol) and diisopropylethylamine (3.10 g, 4.15 mL, 24.0 mmol) were dissolved in dry CH_2Cl_2 (20 mL), then 4-nitrophenyl chloroformate (1.16 g, 5.76 mmol) was added at 0 °C. After stirring at room temperature for 12 h under N_2 atmosphere, the mixture was diluted with CH_2Cl_2 (15 mL) and washed with 1 M phosphate buffer (pH 7, 20 mL× 3), brine (20 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (Hexane: AcOEt = 1: 4) to afford **3** as a yellow liquid (673 mg, 36%).

^{1}H-NMR (400MHz, $CDCl_3$): δ 8.29 (d, J = 9.2 Hz, 2H), 7.40 (d, J = 9.2 Hz, 2H), 4.45 (m, 2H), 3.82 (m, 2H), 3.73-3.63 (m, 10H), 3.56 (m, 2H), 3.38 (s, 3H).

Preparation of 2-(2-(2-methoxyethoxy)ethoxy)ethyl (4-nitrophenyl) carbonate (4) (CAS: 122011-38-5)[2]

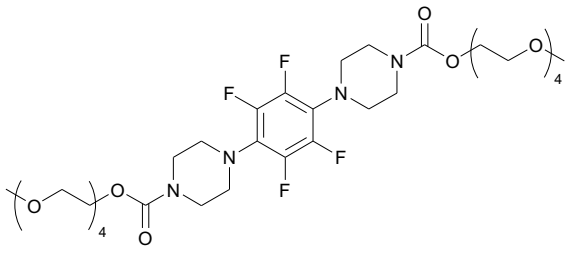


4

To a solution of 2-(2-(2-methoxyethoxy)ethoxy)ethanol (1.00 g, 952 μL, 6.09 mmol) and diisopropylethylamine (3.94 g, 5.22 mL, 30.5 mmol) were dissolved in dry CH_2Cl_2 (20 mL), then 4-nitrophenyl chloroformate (1.47 g, 7.31 mmol) was added at 0 °C. After stirring at room temperature for 13 h under N_2 atmosphere, the mixture was diluted with CH_2Cl_2 (30 mL) and washed with 1 M phosphate buffer (pH 7, 50 mL× 3), brine (50 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by

column chromatography on silica gel (Hexane: AcOEt = 1: 2) to afford **4** as a yellow liquid (1.11 g, 55%).
^{1}H-NMR (500MHz, $CDCl_3$): δ 8.28 (dt, *J* = 9.2, 2.6 Hz, 2H), 7.39 (dt, *J* = 9.2, 2.6 Hz, 2H), 4.45 (m, 2H), 3.82 (m, 2H), 3.75-3.64 (m, 6H), 3.56 (m, 2H), 3.39 (s, 3H).

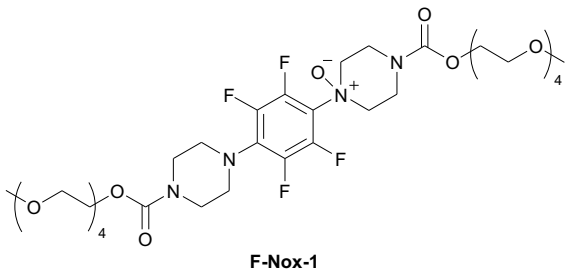
Preparation of F-1



F-1

A suspension of **2** (100 mg, 0.314 mmol) and diisopropylethylamine (97.4 mg, 129 μL, 0.754 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C under N_2 atmosphere. Then, 2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethyl (4-nitrophenyl) carbonate (282 mg, 0.754 mmol) was added to the suspension at 0 °C. After stirring at room temperature for 24 h, the reaction mixture was evaporated. THF (20 mL) and 2 M aq. NaOH (20 mL) were added to the residue. After stirring for 1 h, the reaction mixture was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was evaporated, and the residue was dissolved in CH_2Cl_2 (20 mL). Then, the mixture was washed with sat. aq. $NaHCO_3$ (20 mL × 3) and brine (20 mL), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20: 1) to afford **F-1** as a pale yellow solid (232 mg, 94%).
^{1}H-NMR (500MHz, $CDCl_3$): δ 4.27 (m, 4H), 3.72 (m, 4H), 3.71–3.62 (br, 20H), 3.60 (t, *J* = 4.9 Hz, 8H), 3.55 (m, 4H), 3.38 (s, 6H), 3.14 (br, 8H).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 155.2, 143.4 (dd, *J* = 247.1, 9.6 Hz), 125.2 (m), 71.8, 70.5, 70.4, 69.5, 64.6, 58.9, 50.8, 44.3 (br).
^{19}F-NMR (471 MHz, $CDCl_3$): δ −154.56 (s, 4F).
HRMS (ESI+): *m/z* calcd for $C_{34}H_{54}F_4N_4NaO_{12}^+$: 809.3567, found 809.3551.

Preparation of F-Nox-1



F-Nox-1

To a solution of **F-1** (144 mg, 0.183 mmol) in CH_2Cl_2 (2 mL), *m*-CPBA (*m*-chloroperbenzoic acid) (37.9 mg, 0.219 mmol) and $NaHCO_3$ (18.4 mg, 0.219 mmol) were added at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was diluted with CH_2Cl_2 (18 mL). The diluted mixture was washed with sat. $NaHCO_3$ aq. (20 mL × 3) and brine (20 mL), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 5: 1) to afford **F-Nox-1** as a white solid (71.4 mg, 49%).

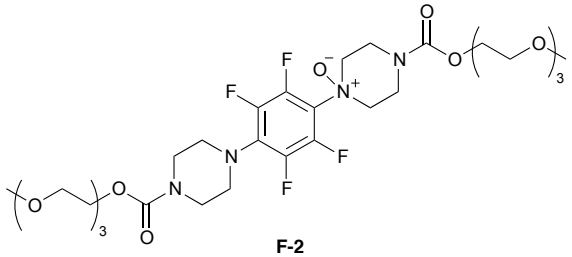
^{1}H-NMR (500MHz, $CDCl_3$): δ 4.28 (m, 4H), 4.12 (br, 4H), 3.95 (br, 2H), 3.81 (br, 2H), 3.72 (m, 4H), 3.69–3.58 (br, 24H), 3.55 (m, 4H), 3.38 (m, 6H), 3.27 (br, 4H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 155.1, 154.6, 142.5 (dd, J_{C-F} = 248.3, 15.6 Hz), 141.6 (dd, J_{C-F} = 254.3, 15.6 Hz), 130.5 (t, J_{C-F} = 10.8 Hz), 125.6 (t, J_{C-F} = 7.2 Hz), 71.8, 70.5, 70.4, 69.4, 69.3, 67.8, 65.0, 64.7, 59.0, 50.3, 44.1 (br), 38.5 (br).

^{19}F-NMR (471 MHz, $CDCl_3$): δ −144.42 (d, J_{F-F} = 18.3 Hz, 2F), −151.92 (d, J_{F-F} = 18.3 Hz, 2F).

HRMS (ESI+): *m/z* calcd for $C_{34}H_{54}F_4N_4NaO_{13}^+$: 825.3516, found 825.3490.

Preparation of F-2



F-2

A suspension of compound **2** (50.0 mg, 0.157 mmol) and diisopropylethylamine (48.7 mg, 64.6 μL, 0.377 mmol) in dry CH_2Cl_2 (5 mL) was cooled to 0 °C under N_2 atmosphere. Then, 2-(2-(2-methoxyethoxy)ethoxy) ethyl (4-nitrophenyl) carbonate (124 mg, 0.377 mmol) was added to the suspension at 0 °C. After stirring at room temperature for 12 h, the reaction mixture was evaporated. The residue was diluted with THF (25 mL) and 2 M aq. NaOH (25 mL). After stirring for 1 h, the reaction mixture was extracted with CH_2Cl_2 (50 mL). The organic layer was evaporated, and the residue was diluted with CH_2Cl_2 (20 mL), then the mixture was washed with sat. $NaHCO_3$ aq. (20 mL × 3) and brine (20 mL), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20: 1) to afford **F-2** as a white solid (79.4 mg, 72%).

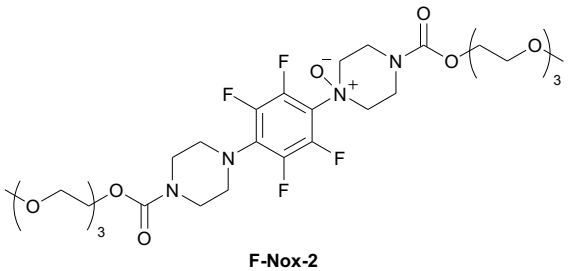
^{1}H-NMR (500MHz, $CDCl_3$): δ 4.27 (t, *J* = 5.2 Hz, 4H), 3.72 (t, *J* = 5.2 Hz, 4H), 3.71–3.62 (br, 12H), 3.60 (t, *J* = 5.2 Hz, 8H), 3.56 (m, 4H), 3.39 (s, 6H), 3.14 (br, 8H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 155.2, 143.4 (dd, J_{C-F} = 247.1, 9.6 Hz), 125.0 (m), 71.9, 70.56, 70.52, 70.48, 69.5, 64.6, 59.0, 50.9, 44.4 (br).

^{19}F-NMR (471 MHz, $CDCl_3$): δ −154.57 (s, 4F).

HRMS (ESI+): *m/z* calcd for $C_{30}H_{46}F_4N_4NaO_{10}^+$: 721.3042, found 721.3021.

Preparation of F-Nox-2



F-Nox-2

To a solution of **F-2** (100 mg, 0.143 mmol) in CH_2Cl_2 (10 mL), *m*-CPBA (*m*-chloroperbenzoic acid) (29.6 mg,

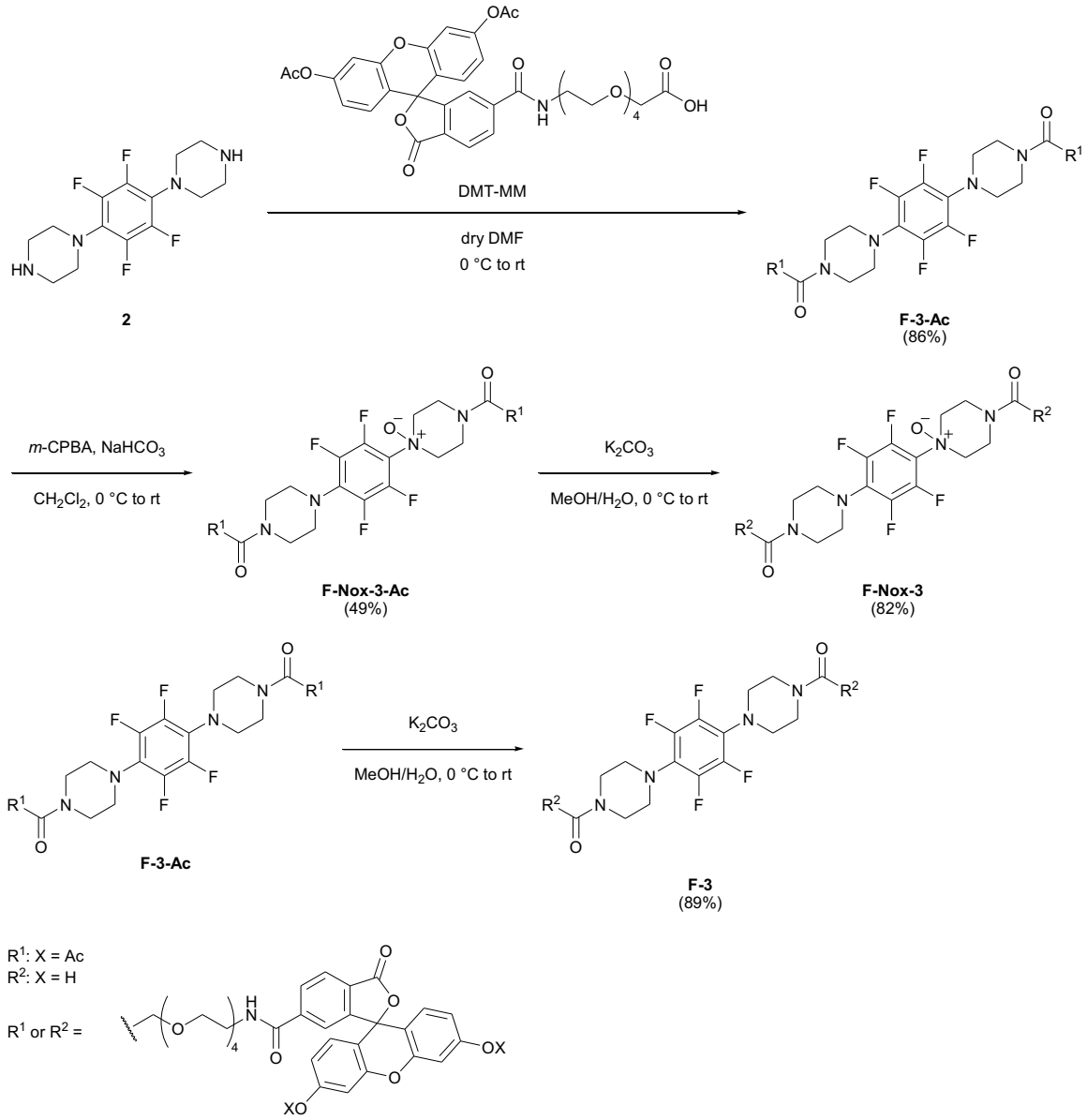
0.172 mmol) and $NaHCO_3$ (14.4 mg, 0.172 mmol) were added at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was diluted with CH_2Cl_2 (10 mL). The diluted mixture was washed with sat. $NaHCO_3$ aq. (20 mL × 3) and brine (20 mL), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 5: 1) to afford **F-Nox-2** as a white solid (44.6 mg, 44%).

^{1}H-NMR (500MHz, $CDCl_3$): δ 4.29 (m, 4H), 4.12 (br, 4H), 3.96 (br, 2H), 3.83 (br, 2H), 3.73 (m, 4H), 3.70–3.59 (br, 16H), 3.55 (m, 4H), 3.38 (m, 6H), 3.27 (br, 4H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 155.1, 154.6, 142.5 (dd, J_{C-F} = 248.3, 15.6 Hz), 141.5 (dd, J_{C-F} = 254.3, 15.6 Hz), 130.6 (t, J_{C-F} = 10.8 Hz), 125.4 (br), 71.8, 70.5, 70.45, 70.40, 69.4, 69.3, 67.7, 65.0, 64.7, 59.0, 50.3, 44.1 (br), 38.5 (br).

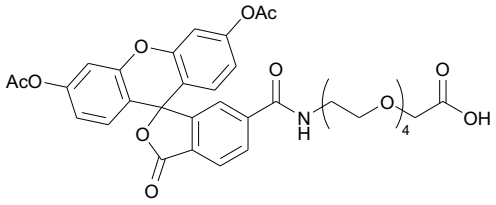
^{19}F-NMR (471 MHz, $CDCl_3$): δ −144.39 (d, J_{F-F} = 14.67 Hz, 2F), −151.90 (d, J_{F-F} = 14.67 Hz, 2F).

HRMS (ESI+): *m/z* calcd for [$C_{34}H_{54}F_4N_4NaO_{13}^+$: 737.2991, found 737.2992.



Scheme S1. Synthesis of **F-Nox-3-Ac** and **F-Nox-3**.

Preparation of TEG-Ac-fluorescein



TEG-Ac-fluorescein

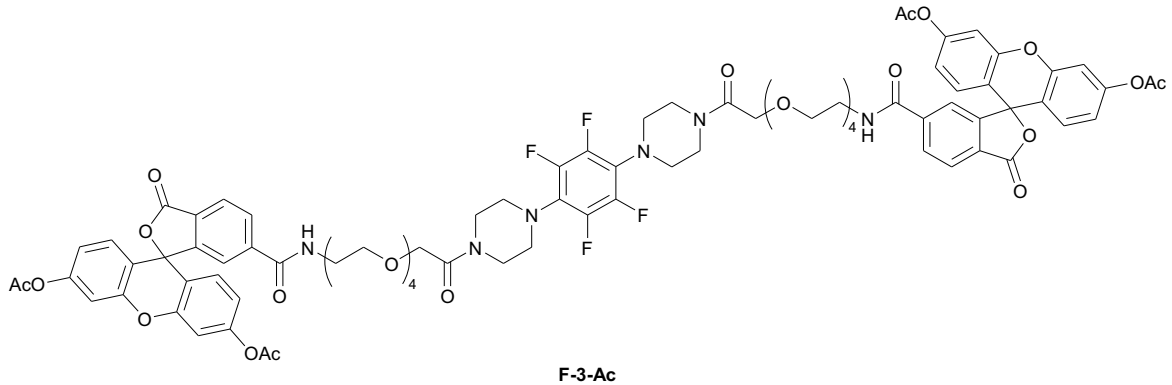
A solution of 14-amino-3,6,9,12-tetraoxadecanoic acid (1.51 g, 6.00 mmol) in dry CH_2Cl_2 (20 mL) was cooled to 0 °C, and 6-carboxyfluorescein diacetate *N*-hyroxysuccinimidyl ester (3.90 g, 7.20 mmol) was added. Then, the mixture was stirred at room temperature for 6 h under N_2 atmosphere. The reaction mixture was washed with 0.1 M aq. HCl (20 mL × 3) and brine (20 mL), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 5: 1) to afford **TEG-Ac-fluorescein** as a white solid (644 g, 15%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.17 (d, *J* = 8.0, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.72 (s, 1H), 7.53 (t, *J* = 5.2 Hz, 1H), 7.10 (m, 2H), 6.83 (m, 4H), 4.03 (s, 2H), 3.81–3.44 (br, 16H), 2.32 (s, 6H).

^{13}C-NMR ($CDCl_3$, 500 MHz): δ 172.0, 168.8, 168.4, 165.8, 152.8, 152.1, 151.5, 141.3, 129.4, 129.0, 128.2, 125.4, 123.2, 117.8, 115.9, 110.4, 82.0, 70.9, 70.3, 70.12, 70.06, 69.99, 69.7, 68.8, 40.1, 21.1.

HRMS (ESI−): *m/z* calcd for $C_{35}H_{34}NO_{14}^-$: 692.1985, found 692.2004.

Preparation of F-3-Ac



F-3-Ac

A solution of **2** (100 mg, 0.314 mmol) and **TEG-Ac-fluorescein** (523 mg, 0.754 mmol) in dry DMF (5 mL) was cooled to 0 °C. Then, DMT-MM (209 mg, 0.754 mmol) was added to the mixture. After stirring at room temperature for 4 h, the reaction mixture was poured into water (20 mL), and the mixture was extracted with AcOEt (20 mL × 3). The combined organic layers were washed with water (30 mL × 3) and brine (30 mL), dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20: 1) to afford **F-3-Ac** as a white solid (450 mg, 86%).

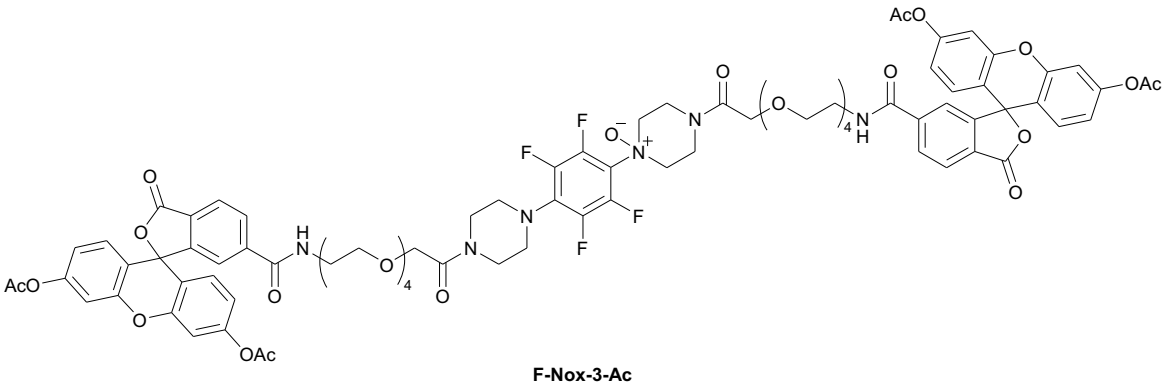
^{1}H-NMR ($CDCl_3$, 500MHz): δ 8.15 (dd, *J* = 8.0 Hz, 2H), 8.08 (d, *J* = 8.0 Hz, 2H), 7.38 (t, *J* = 5.2 Hz, 2H), 7.70 (s, 2H), 7.10 (m, 4H), 6.82 (m, 8H), 4.14 (s, 4H), 3.75–3.47 (m, 44H), 3.23-3.07 (br, 8H), 2.31 (s, 12H).

^{13}C-NMR ($CDCl_3$, 125MHz): δ 168.7, 168.3, 167.7, 165.5, 153.1, 152.1, 151.4, 143.5 (d, J_{C-F} = 249.5 Hz), 141.4, 129.4, 129.0, 128.0, 125.3, 124.8 (br), 123.0, 117.9, 115.8, 110.4, 81.8, 70.5, 70.4, 70.32, 70.29, 70.18, 70.10, 70.06, 69.5, 51.1, 50.9, 45.6, 42.3, 40.2, 21.1.

^{19}F-NMR ($CDCl_3$,471 MHz): δ −154.32 (s, 4F).

HRMS (ESI+): *m/z* calcd for $C_{84}H_{84}F_4N_6Na_2O_{26}^{2+}$: 857.2578, found 857.2556.

Preparation of F-Nox-3-Ac



F-Nox-3-Ac

To a solution of **F-3-Ac** (450 mg, 0.270 mmol) in CH_2Cl_2 (10 mL), *m*-CPBA (*m*-chloroperbenzoic acid) (55.8 mg, 0.324 mmol) and $NaHCO_3$ (27.2 mg, 0.324 mmol) were added at 0 °C. After stirring at room temperature for 3.5 h, the mixture was evaporated, and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 5: 1) to afford **F-Nox-3-Ac** as a white solid (223 mg, 49%).

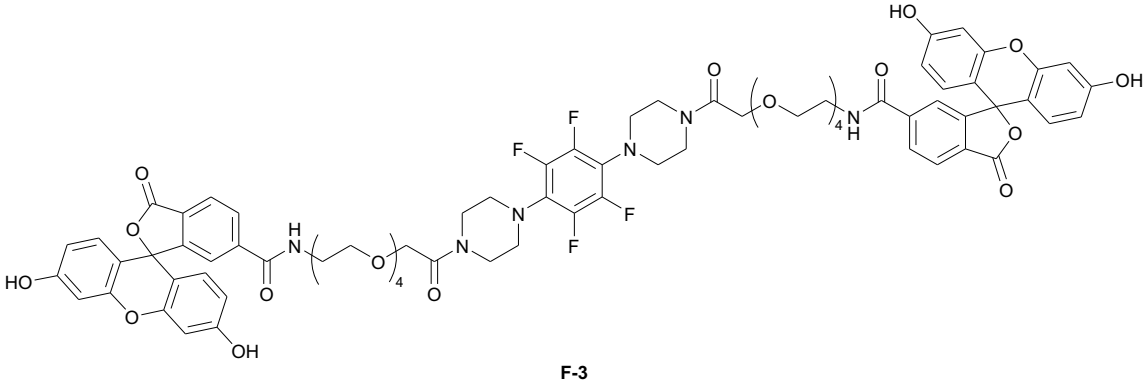
^{1}H-NMR ($CDCl_3$, 400MHz): δ 8.66 (t, *J* = 5.0 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.15 (d, *J* = 8.2 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.85 (s, 1H), 7.72 (s, 1H), 7.44 (t, *J* = 5.5 Hz, 1H), 7.15-7.02 (m, 4H), 6.88-6.70 (m, 8H), 4.43 (d, *J* = 12.8 Hz, 1H), 4.33–3.93 (m, 8H), 3.92–3.40 (m, 36H), 3.26 (m, 4H), 2.31 (m, 12H).

^{13}C-NMR ($CDCl_3$, 100MHz): δ 168.8, 168.7, 168.5, 168.3, 167.8, 167.4, 165.8, 165.6, 153.1, 152.8, 152.1, 152.0, 151.5, 151.4, 142.4 (d, J_{C-F} = 250.5 Hz),141.5 (d, J_{C-F} = 254.3 Hz), 141.4, 141.4, 141.1, 140.2, 130.2, 129.7, 129.4, 129.1, 129.0, 128.9, 128.0, 127.9, 125.6, 125.53, 125.49, 125.3, 124.8, 123.7, 123.1, 117.9, 117.7, 116.0, 115.8, 110.4, 110.4, 110.3, 81.9, 81.8, 70.7, 70.5, 70.4, 70.33, 70.27, 70.24, 70.1, 70.03, 69.99, 69.5, 68.1, 50.7, 50.4, 45.4, 42.0, 40.5, 40.1, 40.0, 36.6, 21.1.

^{19}F-NMR ($CDCl_3$, 376 MHz): δ −143.89 (d, J_{F-F} = 14.5 Hz, 2F), −151.99 (d, J_{F-F} = 14.5 Hz, 2F).

HRMS (ESI+): *m/z* calcd for $C_{84}H_{84}F_4N_6Na_2O_{27}^{2+}$: 865.2553, found 865.2532.

Preparation of F-3



F-3

To a solution of **F-3-Ac** (100 mg, 0.060 mmol) in MeOH (5 mL), K_2CO_3 (66.2 mg, 0.479 mmol) in water (2 mL) was added at 0 °C. After stirring at room temperature for 3.5 h, the mixture was neutralized by aq. HCl (1 M) and then evaporated. The residue was purified by column chromatography on ODS silica gel (water: MeOH = 1: 3) to afford to **F-3** as a red solid (80 mg, 89%).

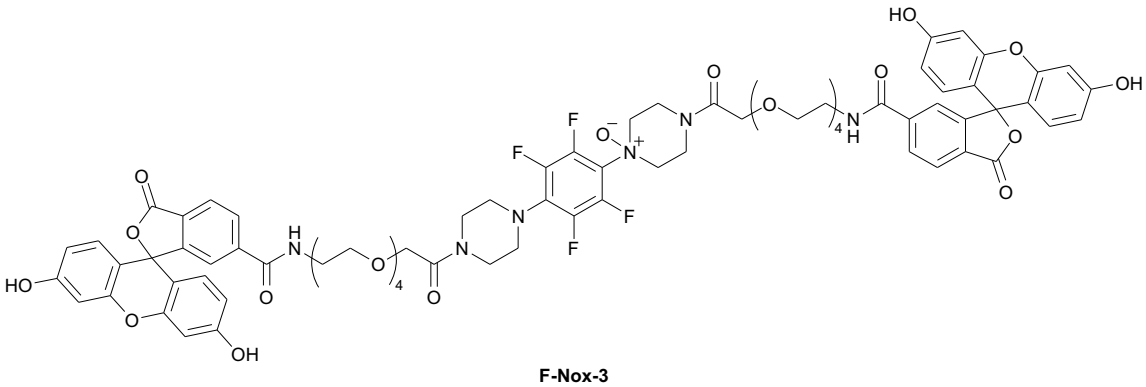
^{1}H-NMR (CD_3OD, 500MHz): δ 8.05 (d, J = 8.0 Hz, 2H), 8.02 (dd, J = 8.0, 1.7 Hz, 2H), 7.69 (m, 2H), 7.00 (d, J = 9.2 Hz, 4H), 6.49 (dd, J = 9.2, 1.7 Hz, 2H), 6.47 (d, J = 1.7 Hz, 2H), 4.16 (s, 4H), 3.71–3.47 (m, 36H), 3.37 (t, J = 5.2 Hz, 4H), 3.13–2.99 (br, 8H).

^{13}C-NMR (DMSO-d_6, 125MHz): δ 180.2, 168.8, 167.5, 165.6, 157.6, 154.9, 144.2, 143.0 (dd, J_{C-F} = 247.1, 10.8 Hz), 133.7, 133.2, 130.1, 129.7, 127.8, 126.8, 124.6 (br), 122.8, 108.7, 102.5, 69.7, 69.6, 69.5, 69.2, 68.9, 50.9, 50.6, 45.0, 41.7.

^{19}F-NMR (DMSO-d_6 ,471 MHz): δ −153.61 (s, 4F).

HRMS (ESI−): m/z calcd for $C_{76}H_{74}F_4N_6O_{22}^{2-}$: 749.2402, found 749.2408.

Preparation of F-Nox-3



F-Nox-3

To a solution of **F-Nox-3-Ac** (56 mg, 0.046 mmol) in MeOH (2 mL), K_2CO_3 (36 mg, 0.26 mmol) in water (1 mL) was added at 0 °C. After stirring at room temperature for 30 min, the mixture was neutralized by 1 M aq. HCl and then evaporated. The residue was purified by column chromatography on ODS silica gel (water: MeOH = 1: 1) to afford to **F-Nox-3** as a red solid (40 mg, 82%).

^{1}H-NMR (CD_3OD, 500 MHz): δ 8.04 (d, J = 8.0 Hz, 3H), 8.01 (dd, J = 8.0, 1.7 Hz, 3H), 7.69 (d, J = 1.7 Hz, 1H), 7.66 (m, 1H), 7.05–6.98 (m, 4H), 6.55–6.48 (m, 8H), 4.51 (d, J = 14.3 Hz, 1H), 4.34 (d, J = 14.3 Hz, 1H), 4.23 (d, J = 14.3 Hz, 1H), 4.18 (s, 3H), 4.14–4.02 (m, 2H), 3.92–3.83 (m, 3H), 3.73–3.51 (m, 35H), 3.43 (m, 2H), 3.25 (m, 2H), 3.19 (m, 2H).

^{13}C-NMR (DMSO-d_6, 100 MHz): δ 180.3, 168.8, 167.6, 167.5, 165.7, 157.7, 155.1, 142.0 (dd, J_{C-F} = 244.7, 15.4 Hz), 141.1 (dd, J_{C-F} = 251.4, 15.51 Hz), 133.7, 133.2, 130.2, 130.0, 129.7, 129.3 (m), 127.9, 126.8, 126.1 (m), 122.8, 108.8, 102.6, 69.7, 69.6, 69.53, 69.50, 69.46, 69.2, 69.0, 68.9, 66.9, 55.1, 50.3, 49.9, 48.6, 44.8, 41.68, 39.5, 36.1.

^{19}F-NMR (CD_3OD, 471 MHz): δ −141.78 (d, J_{F-F} = 14.67 Hz, 2F), −150.06 (d, J_{F-F} = 14.67 Hz, 2F).

HRMS (ESI−): m/z calcd for $C_{76}H_{74}F_4N_6O_{23}^{2-}$: 753.2376, found 757.2358.

2. ^{19}F NMR analyses

^{19}F-NMR measurements for Fe(II)-detection studies were performed with a JEOL ECZ-400 spectrometer at 376 MHz using sodium trifluoroacetate (TFA•Na) as the internal standard (−76.0 ppm) and 10%(v/v) D_2O as the deuterated solvent solvents unless otherwise mentioned.

2-1. Response of F-Nox-1 to Fe(II)

F-Nox-1 or **F-1** (final 300 μM, from stock solution of 30 mM in DMSO) and ferrous ammonium sulfate (FAS, final concentrations: 1, 0.6, 0.5, 0.3, 0.1, 0.075, 0.05, 0.025, and 0.01 mM, from 100, 60, 50, 30, 10, 5 and 1 mM stock solution in D_2O) were mixed in HEPES buffer (50 mM, pH 7.4) (total: 1.0 mL). The mixture was incubated at room temperature for 30 min. Then, a slurry of Chelex® 100 Resin (BioRad) (50 mg) was added to the mixture. After mixing for 1 min, the resins were removed by centrifugation at 15,000 rpm for 3 min. The supernatants (800 μL) was transferred into new tubes. After addition of TFA•Na in D_2O (300 μM, from 60 mM stock solution in D_2O) and D_2O (100 μL), the samples were subjected to ^{19}F NMR analysis (128 scans or 32 scans). The same amounts of the vehicles were added instead of FAS for the control experiments. For the no-Chelex® condition, the samples were subjected to ^{19}F NMR analysis (32 scans) withoutu the treatment with Chelex®.

2-2. Metal selectivity test

Aqueous solutions of **F-Nox-1** (300 μM) in HEPES buffer (1 mL, 50 mM, pH 7.4, 1%DMSO as colsolvent) were prepared. To the probe solutions was added each aqueous solution of transition metal ion species (stock solutions: 50 mM for $MnCl_2$, $FeCl_3$, $CoSO_4$, $NiSO_4$, $CuSO_4$, and $ZnSO_4$ in D_2O) (final concentration: 500 μM) in the presence or absence of Fe(II) (as FAS). After incubation for 30 min at room temperature, the reaction mixture was added to a slurry of Chelex® 100 Resin (50 mg). After mixing for 1 min, the resins were removed by centrifugation at 15,000 rpm for 3 min. The supernatants (800 μL) were carefully transferred into new tubes. After addition of TFA•Na in D_2O (300 μM, from 60 mM stock solution in D_2O) and D_2O (100 μL), the samples were subjected to ^{19}F NMR analysis (32 scans).

2-3. Stability test against reactive oxygen species, biological thiols, and reductants

Aqueous solutions of **F-Nox-1** (300 μM) in HEPES buffer (1 mL, 50 mM, pH 7.4, 1%DMSO as colsolvent) were prepared. The probe solutions were incubated with each chemical species for 30 min as follows.

L-Cysteine : 500 μM from 50 mM stock solution in D_2O
Sodium L-ascorbate : 300 μM from 50 mM stock solution in D_2O
H_2O_2 : 500 μM from 50 mM stock solution in D_2O
NaOCl : 500 μM from 50 mM stock solution in D_2O
Glutathione : 500 μM from 50 mM stock solution in NaOD/D_2O (50 mM)
Glutathione + $CuSO_4$: 500 μM from 50 mM stock solution in NaOD/D_2O (50 mM)

After incubation for 30 min at room temperature, the reaction mixture was added to a slurry of Chelex® 100 Resin (50 mg). After mixing for 1 min, the resins were removed by centrifugation at 15,000 rpm for 3 min. The supernatants (800 μL) were carefully transferred into new tubes. After addition of TFA•Na in D_2O (300

μM, from 60 mM stock solution in D_2O) and D_2O (100 μL), the samples were subjected to ^{19}F NMR analysis (32 scans).

2-4. ^{19}F NMR detection of Fe(II) in FBS

A solution of **F-Nox-1** (600 μM) and FAS (600 μM, from 60 mM stock solution in D_2O) in fetal bovine serum (GE healthcare, cat.#SH30910.03) (FBS, 1.0 mL, 1% DMSO as co-solvent) was incubated at room temperature for 30 min. After incubation for 30 min at room temperature, the reaction mixture was added to a slurry of Chelex® 100 Resin (50 mg). After mixing for 1 min, the resins were removed by centrifugation at 15,000 rpm for 3 min. The supernatants (800 μL) were carefully transferred into new tubes. After addition of TFA•Na in D_2O (300 μM, from 60 mM stock solution in D_2O) and D_2O (100 μL), the samples were subjected to ^{19}F NMR analysis (2048 scans).

3. Magnetic resonance imaging

The samples for ^{19}F-MR imaging were prepared as follows. Solutions of **F-Nox-1** (500 μM) ± FAS (500 μM, from 100 mM stock solution in water) in water (1.0 mL, 1% DMSO as co-solvent) were incubated at room temperature for 30 min. A solution of **F-1** (500 μM) in water (1.0 mL, 1% DMSO as co-solvent) was used as the authentic sample to monitor ^{19}F-signal of the deoxygenated compound. Each sample (100 μL) was transferred to a flat bottom screw vial (200 μL volume) (Agilent Technologies, P/N 5183-2090) just before the measurements.

Solutions of **F-Nox-1** (600 μM) ± FAS (600 μM, from 100 mM stock solution in water) in FBS (1.0 mL, 1% DMSO as co-solvent) were incubated at room temperature for 30 min. A solution of **F-1** (600 μM) in FBS (1.0 mL, 1% DMSO as co-solvent) was used as the authentic sample to monitor ^{19}F-signal of the deoxygenated compound. Each sample (100 μL) was transferred to a flat bottom screw vial (200 μL volume) (Agilent Technologies, P/N 5183-2090) just before the measurements.

In this investigation, a 7.0 T horizontal-bore MR scanner (Unity Inova; Agilent Technologies, Santa Clara, CA) was employed. A custom-built circular-type surface coil measuring 1.6 cm in diameter and tuned to both the ^{1}H and ^{19}F frequencies (300 MHz and 282 MHz, respectively) was used to collect the data (Takashima seisakusho, Hino, Japan). The radio frequency coil was carefully adjusted at each resonance frequency using the tuning and matching capacitors just before the ^{1}H or ^{19}F study. Gradient-echo sequence was used to obtain ^{1}H-MR images with 150-ms repetition time (TR), 3-ms echo time, 60° flip angle, 1.5-mm slice thickness, 24 mm × 24 mm field of view and 128 × 128 resolution. A nonlocalized ^{19}F-NMR spectrum was obtained using a single pulse sequence with 8,192 data points, 40,000-Hz spectral width, 1-s TR and 600 acquisitions (for 10 min). To obtain ^{19}F-MR images, free induction decay data of ^{19}F chemical shift imaging were collected with a 40,000-Hz spectral width, 24 mm × 24 mm field of view in the coronal plane, 1-s TR, 200-μs phase-encoding time and 68 acquisitions for each central 44 phase-encoding step out of 8 × 8 steps. For the residual 20 phase-encoding steps in the periphery of k-space, zero data were used. The total acquisition time for one data set was 50 min. A slice-selective pulse was not used. Whole signals covered by the coil sensitivity were acquired. The raw data were processed by 3D-Fourier transformation with 40-Hz line broadening and zero filling and finally converted to 32 × 32 spectral data sets. The ^{19}F-MR image was constructed by integrating the ^{19}F signal

intensities of compound peaks in individual pixels.

4. Product analysis by LC-MS

To a solution of **F-Nox-1** (300 μM) in water (1% DMSO as co-solvent) was added each solution of FAS (0.01, 0.05, 0.1, 0.3, 0.6, and 1 mM). After incubation for 30 min at room temperature, the reaction mixture was added to a slurry of Chelex® 100 Resin (50 mg). After mixing for 1 min, the resins were removed by centrifugation at 15,000 rpm for 3 min.. Then, the supernatants (300 μL) were carefully transferred into new tubes and diluted with water (600 μL).

Products were filtered by syringe filter and analyzed with LC-MS system (Chromaster®5110, Hitachi High-tech) equipped with a photodiode-array detector (Chromaster®5430, Hitachi High-tech) and a mass spectrometer (Chromaster®5610 MS Detector, Hitachi High-tech) and with CAPCELL PAK C_{18} column (3 μm, 4.6 × 100 mm) eluted with a gradient system consisting of water (solvent A) and CH_3CN (solvent B) containing 0.05% formic acid; 5–20 min, 20–80% B. The absorbance at 250 nm was monitored. The retention times were compared with those of the deoxygenated compounds. Assignments of the compounds were based on the observed *m/z* values at each peak.

5. Cell culture

Human hepatocellular carcinoma cells (HepG2, purchased from RIKEN Bioresource Research Center) were cultured in modified essential medium (MEM, Gibco) containing 10% fetal bovine serum (FBS, Biosera), 1% Antibiotic-Antimyocotic (ABAM, Gibco), and 2 mM glutamine at 37 °C in a 5% CO_2/ 95% air incubator.

6. Detection of Fe(II) in cell lysate by ^{19}F-NMR spectroscopy

HepG2 cells (5×10^4 cells per well) were seeded on three dishes (Φ 96 x 21 mm, TPP) and cultured in complete medium at 37 °C for 48 h in a 5% CO_2/95% air incubator. Then, the medium was replaced by fresh medium (5 mL) containing 10% fetal bovine serum (FBS, Gibco), and the cells were treated with or without FAS (final concentration 0.3 mM) for 1 h. Next, the medium was removed, and the cells were washed with EDTA (0.3 mM) in Hank's Balanced Salt Solution (HBSS, Gibco) three times. Then, the cells were treated with **F-Nox-1** (final concentration 0.3 mM) in HBSS containing DMSO (1%) with or without Bpy (final concentration 1 mM) for 2 h. The cells were harvested from the culture dishes and centrifuged at 1,000 rpm for 2 min. After removing the supernatant, the obtained cell pellets were suspended in cold phosphate-buffered saline (PBS, 1 mL) and centrifuged at 1,000 rpm for 2 min. After removing the supernatant, the obtained cell pellets were suspended in a mixed solution of MeOH (180 μL) and water (20 μL). The cell pellets were dissolved by pipetting. The lysates were centrifuged at 15,000 rpm for 15 min, and the supernatants (200 μL) were carefully transferred into new tubes. Then, water (200 μL), D_2O (100 μL) and TFA•Na (100 μM) were added to the supernatant, and the samples were subjected to ^{19}F-NMR analysis (ECZ-400 at 376 MHz).

7. Confocal fluorescence microscopy

Confocal fluorescence images were acquired by using an Olympus IX83 microscope equipped with a 130 W mercury lump and a laser diode illuminator (89 North, LDI with 7 laser lines), an EMCCD camera (Hamasmatsu Photonics, ImageEM), and a disk scan confocal unit (DSU, Olympus). Experiments were

performed with 20 × objective lens. For imaging experiment, Hank's Balanced Salt Solution (HBSS, Gibco) containing calcium and magnesium without phenol red was used. HepG2 cells (5×10^4 cells/mL) were seeded on an Advanced TC glass-bottomed dishes ($CELL_{view}^{TM}$ CellCulture Dish, Greiner) and cultured in complete medium at 37 °C for 48 h in a 5% CO_2/95% air incubator. After incubation for 48 h, the cells were washed with HBSS (×2), and then 5 μM **F-Nox-3-Ac** in HBSS was added. After incubation for 30 min at 37 °C, the cells were washed with HBSS (×2), and imaged with an FITC filter set (excitation = 465–642 nm, emission = 516–566 nm, and dichronic mirror = 495 nm).

8. MTT assay

Evaluation of cytotoxicity of F-Nox-1

MTT assay was carried out according to the conditions of the cell lysate experiment as above. HepG2 cells (5×10^3 cells per well) were seeded on a 96-well plastic dish (TPP) and cultured in complete medium at 37 °C for 48 h in a 5% CO_2/95% air incubator. Then, the cells were treated with various concentration of **F-Nox-1** (5, 10, 50, 100, 300 or 600 μM) containing DMSO (1%) in HBSS for 2 h. Next, the cells were treated with thiazolyl blue tetrazolium bromide (MTT reagent, Sigma Aldrich, final: 0.5 mg mL^{-1} (form 5 mg mL^{-1} stock)) at room temperature for 4 h, and then the media were removed. DMSO (100 μL) was added to each well, and absorption at 560 nm was measured to calculate cell viability.

9. References

1. V. I. Sorokin, V. A. Ozeryanskii, G. S. Borodkin, A. V. Chernychev, M. Muir, J. Z. Baker, *Naturforsch*, 2006, **64**, 615–625.
2. J. Luo, R. Uprety, Y. Naro, C. Chow, S. P. Nguyen, J. W. Chin, A. Deitres, *J. Am. Chem. Soc.*, 2014, **136**, 15551–15558.

Supplementary figures

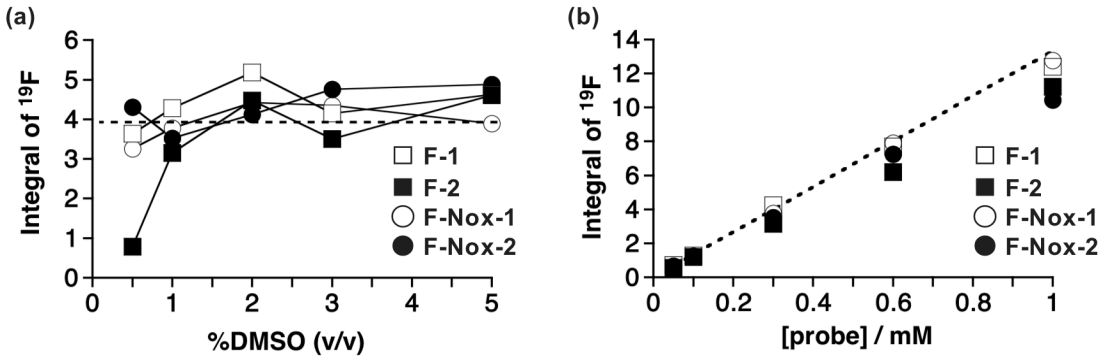


Figure S1. (a) The effect of contents of DMSO as co-solvent on the ^{19}F-signal integration values of **F-1** (open square), **F-2** (filled square), **F-Nox-1** (open circle), and **F-Nox-2** (filled circle), (300 μM) in D_2O. (b) Plots of ^{19}F-signal integration values of **F-1** (open square), **F-2** (filled square), **F-Nox-1** (open circle), and **F-Nox-2** (filled circle) at various concentrations. The co-solvent (DMSO) concentration was kept at 1%(v/v). The integration values were acquired for the peaks at the corresponding chemical shifts (−151.3 ppm for **F-1** and **F-2**, −141.9 and −149.1 ppm for **F-Nox-1** and **F-Nox-2**) and calculated by using TFA (300 μM, −76.0 ppm, 3F) as the internal standard.

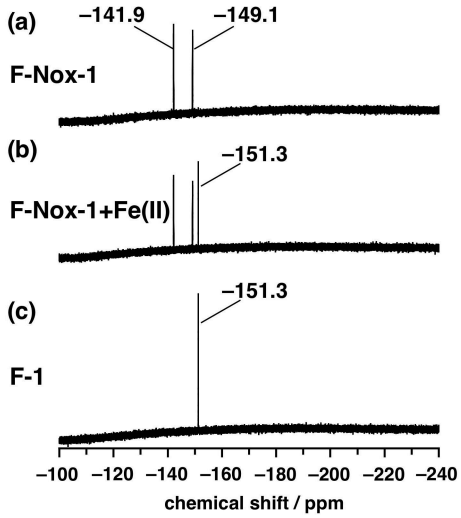


Figure S2. ^{19}F-NMR spectra (32 scans) of (a) **F-Nox-1** (300 μM), (b) the reaction mixture of **F-Nox-1** (300 μM) and Fe(II) (500 μM, supplemented as $(NH_4)_2Fe(SO_4)_2•6H_2O$), FAS) (room temperature, 30 min), and (c) **F-1** (300 μM) in HEPES buffer (50 mM, pH 7.4, 1%DMSO, 10%D_2O).

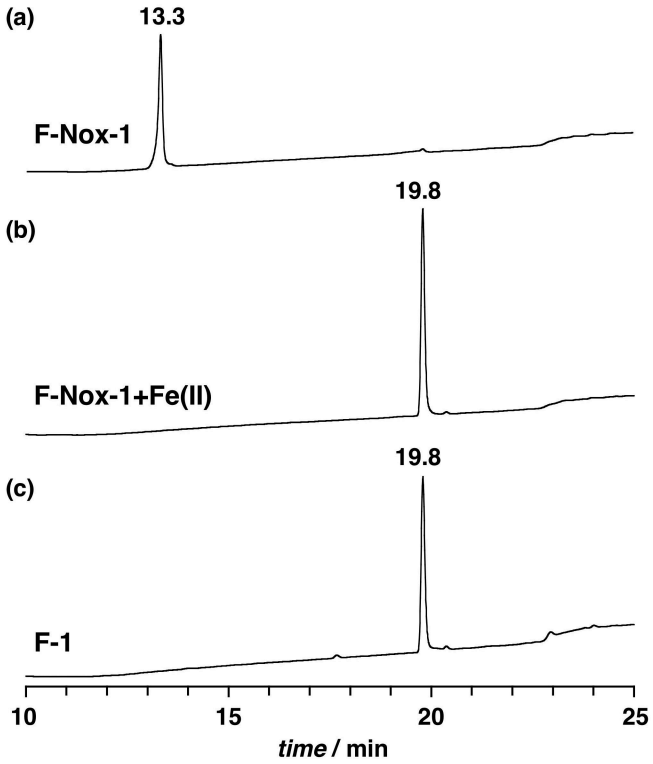


Figure S3. LC-MS analysis of the Fe(II)-mediated deoxygenation reaction of **F-Nox-1** (300 μM) with 1 mM Fe(II) (FAS) in water (1% DMSO) at room temperature for 30 min. HPLC charts of (a) **F-Nox-1**, (b) the reaction mixture of **F-Nox-1** and Fe(II), and (c) **F-1**. The absorbance at 250 nm was monitored. Total ion mass spectra were simultaneously measured to give mass peaks of 825.3 (calcd. for [**F-Nox-1**+Na]$^+$ = 825.4) at 13.3 min and 809.0 (calcd. for [**F-1**+Na]$^+$ = 809.4) at 19.8 min.

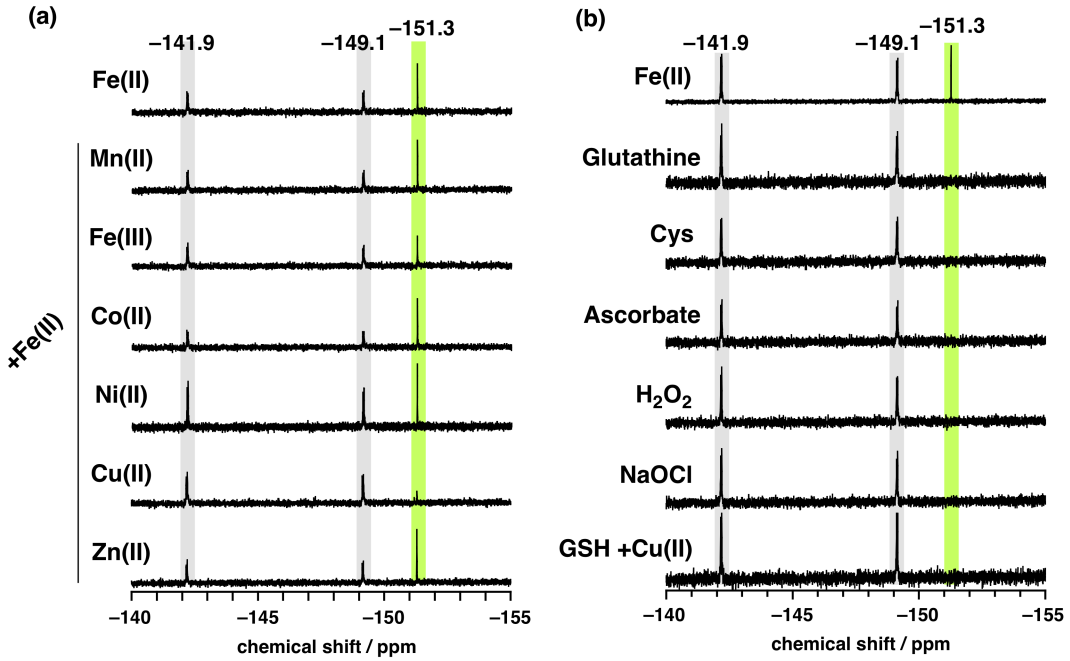


Figure S4. (a) ^{19}F-NMR spectra of **F-Nox-1** (300 μM) after reaction with various metals (500 μM) in the presence of Fe(II) (500 μM) at room temperature for 30 minutes. Cu(II) catalytically oxidizes Fe(II) to Fe(III) in the presence of oxygen (C. J. Matocha, *et al*. *J. Environ. Qual.*, 2005, **34**, 1539–1546), which deprived Fe(II) before the reaction with the probe. (b) ^{19}F-NMR spectra of **F-Nox-1** (300 μM) after reaction with various biological molecules (see experimental details for the concentration of each reagent) at room temperature for 30 min. All the data were acquired in HEPES buffer (50 mM, pH 7.4, 1%DMSO, 10%D_2O) with 32 scans.

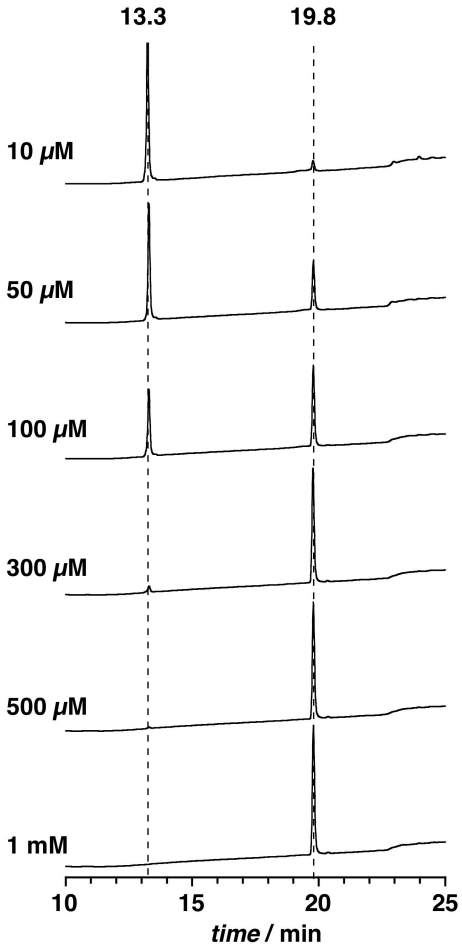


Figure S5. LC-MS analysis of the **F-Nox-1** (300 μM) after the reaction with various concentrations of Fe(II) (FAS) in water (1% DMSO) at room temperature for 30 min. The absorbance at 250 nm was monitored. Total ion mass spectra were simultaneously measured to give mass peaks of 825.3 (calcd. for [**F-Nox-1**+Na]$^+$ = 825.4) at 13.3 min and 809.0 (calcd. for [**F-1**+Na]$^+$ = 809.4) at 19.8 min.

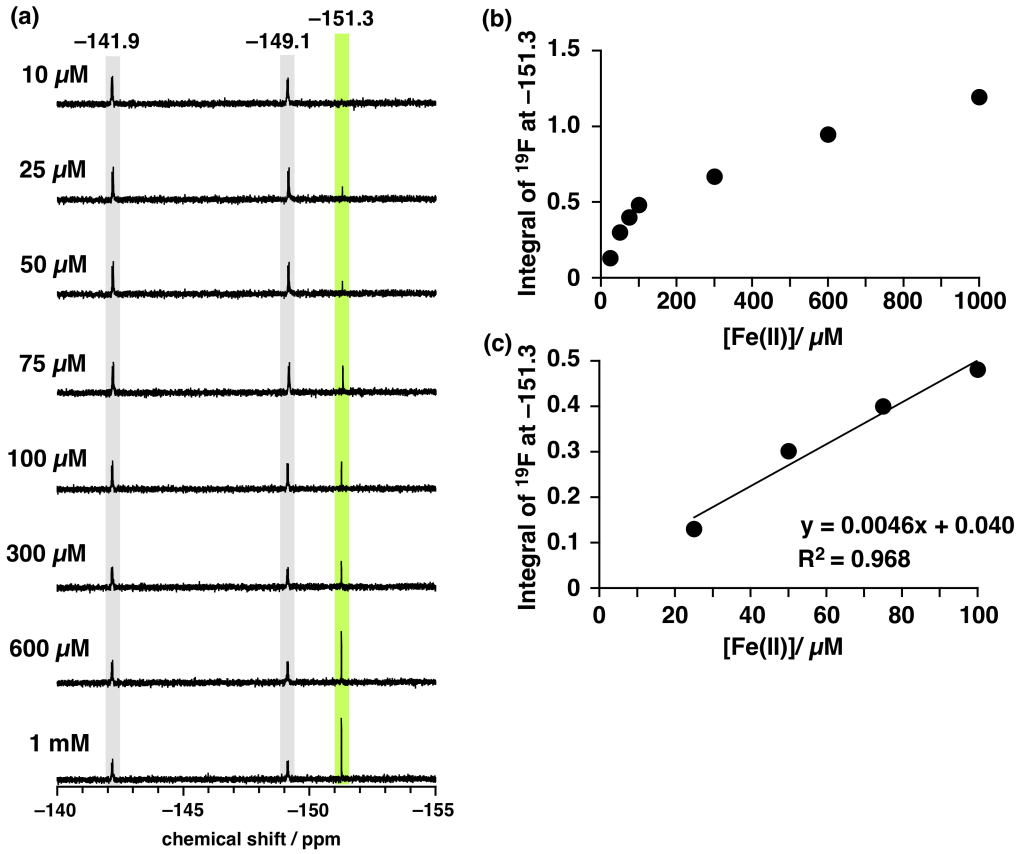


Figure S6. (a) ^{19}F-NMR spectra (32 scans) of **F-Nox-1** (300 μM) after the reaction with various concentrations of Fe(II) (FAS) at room temperature for 30 min in HEPES buffer (50 mM, pH 7.4, 1%DMSO, 10%D_2O) followed by removal of the residual metal ions. (b) A plot of integral values of the ^{19}F-signals at −151.3 ppm (standardized by internal standard, TFA•Na (3F)) observed in (a) against the concentration of Fe(II). (c) A magnified plot of (b) at 0–100 μM.

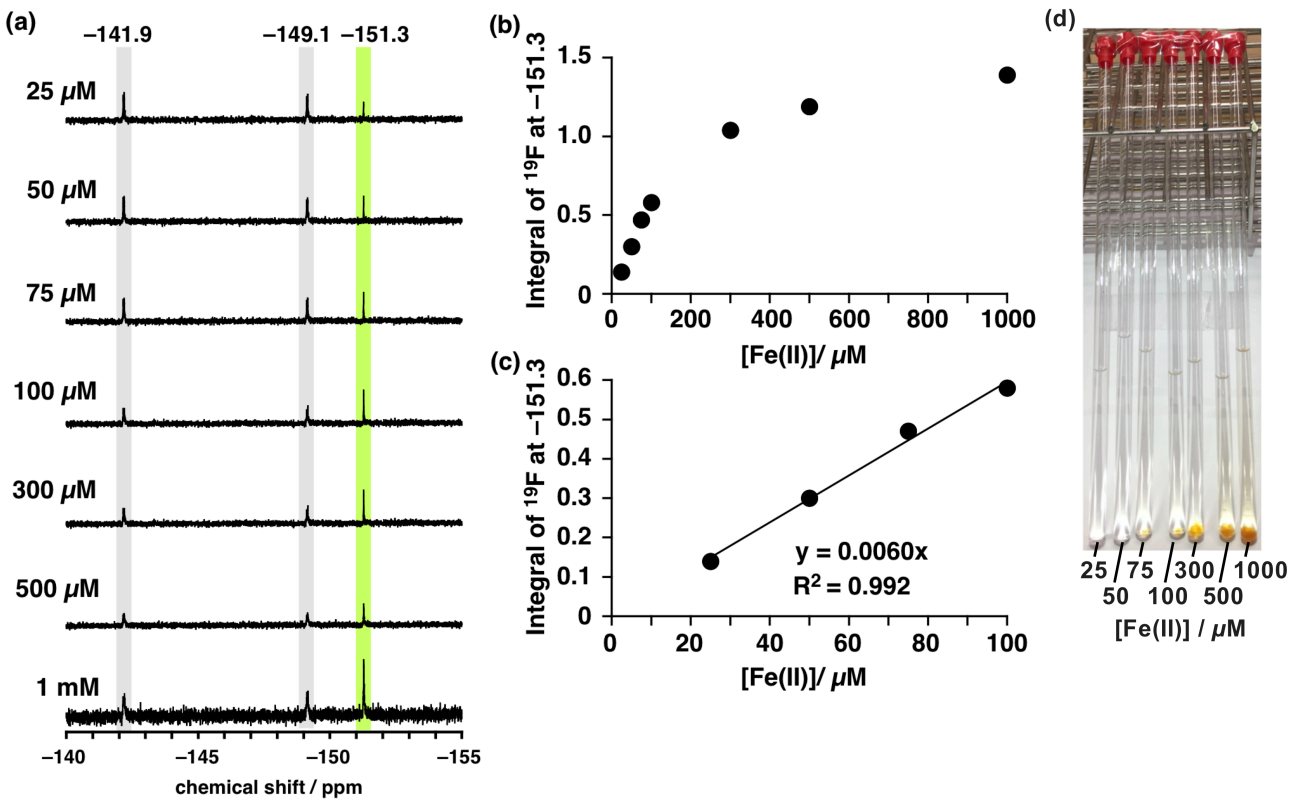


Figure S7. (a) ^{19}F-NMR spectra (32 scans) of **F-Nox-1** (300 μM) after the reaction with various concentrations of Fe(II) (FAS) at room temperature for 30 min in HEPES buffer (50 mM, pH 7.4, 1%DMSO, 10%D_2O) in the presence of the residual metal ions. (b) A plot of integral values of the ^{19}F-signals at −151.3 ppm (standardized by internal standard, TFA•Na (3F)) observed in (a) against the concentration of Fe(II). (c) A magnified plot of (b) at 0–100 μM. (d) Photographs of the NMR tubes of the samples containing the reaction mixtures which were subjected to ^{19}F-NMR measurements.

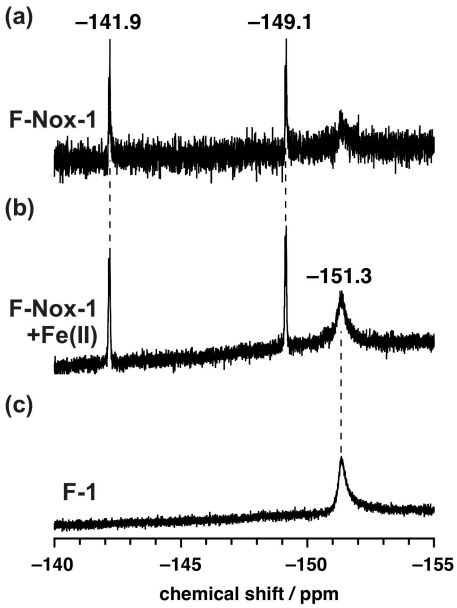


Figure S8. ^{19}F-NMR spectra (2048 scans) of (a) **F-Nox-1** (300 μM), (b) the reaction mixture of **F-Nox-1** (300 μM) and Fe(II) (600 μM, FAS), and (c) **F-1** (300 μM) in fetal bovine serum (1%DMSO, 10%D_2O).

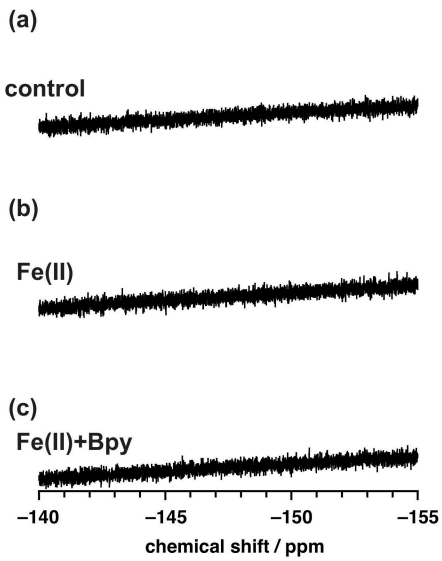


Figure S9. ^{19}F-NMR spectra (2048 scans) of cell lysate of HepG2 cells treated with **F-Nox-1** (300 μM). (a) Control cells, (b) the cells supplemented with Fe(II) (FAS, 300 μM), and (c) the cells supplemented with Fe(II) (FAS, 300 μM) and 2,2′-bipyridyl (Bpy, 1 mM). The cells were incubated with **F-Nox-1** at 37 °C for 2 h after the incubation of the cells with Fe(II) ± Bpy at 37 °C for 1 h.

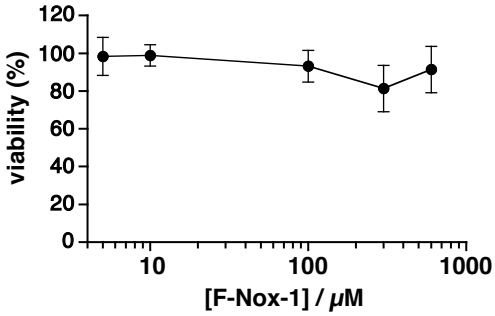


Figure S10. Cell viability assay of HepG2 cells treated with various concentrations of **F-Nox-1** at 37 °C for 2 h for 2 h. Error bars indicate ± s.d. (n=3). No significant cytotoxicity was observed.

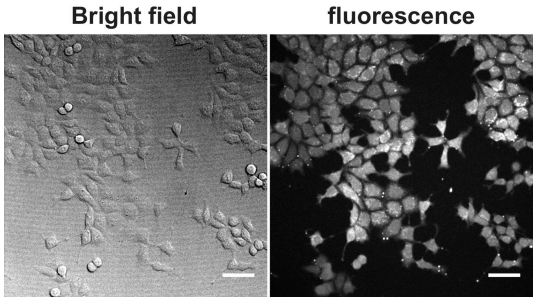
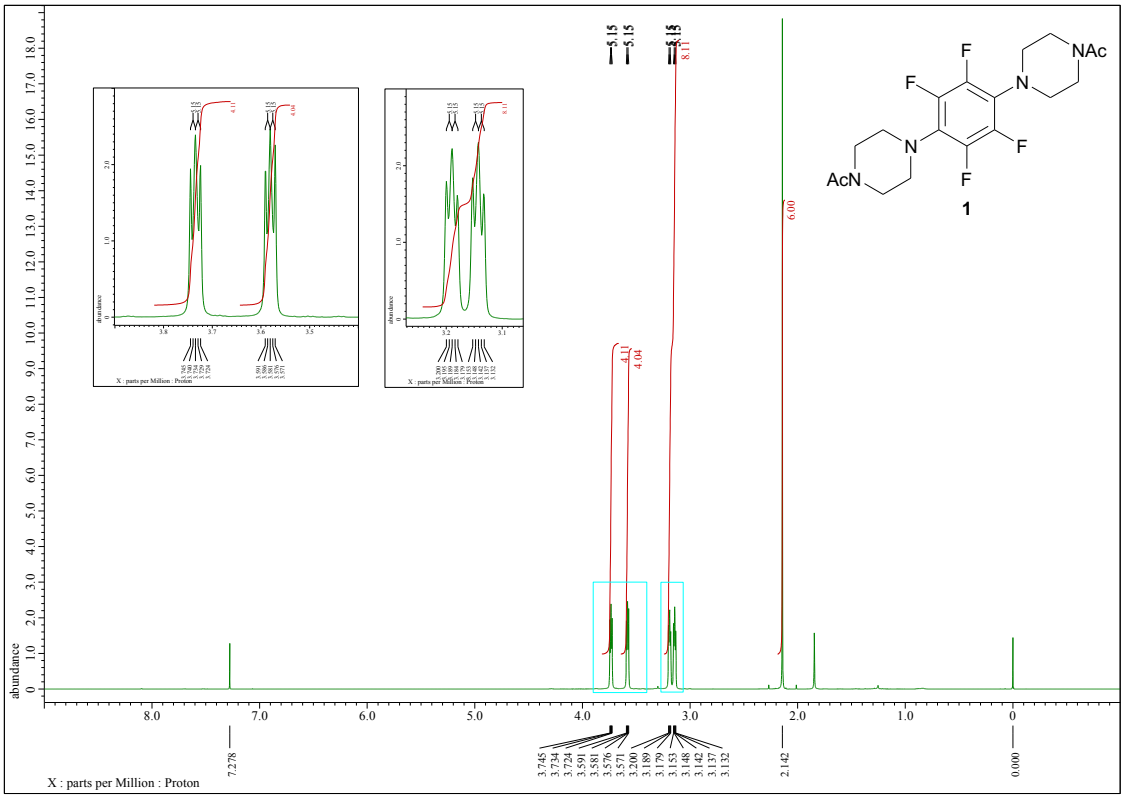


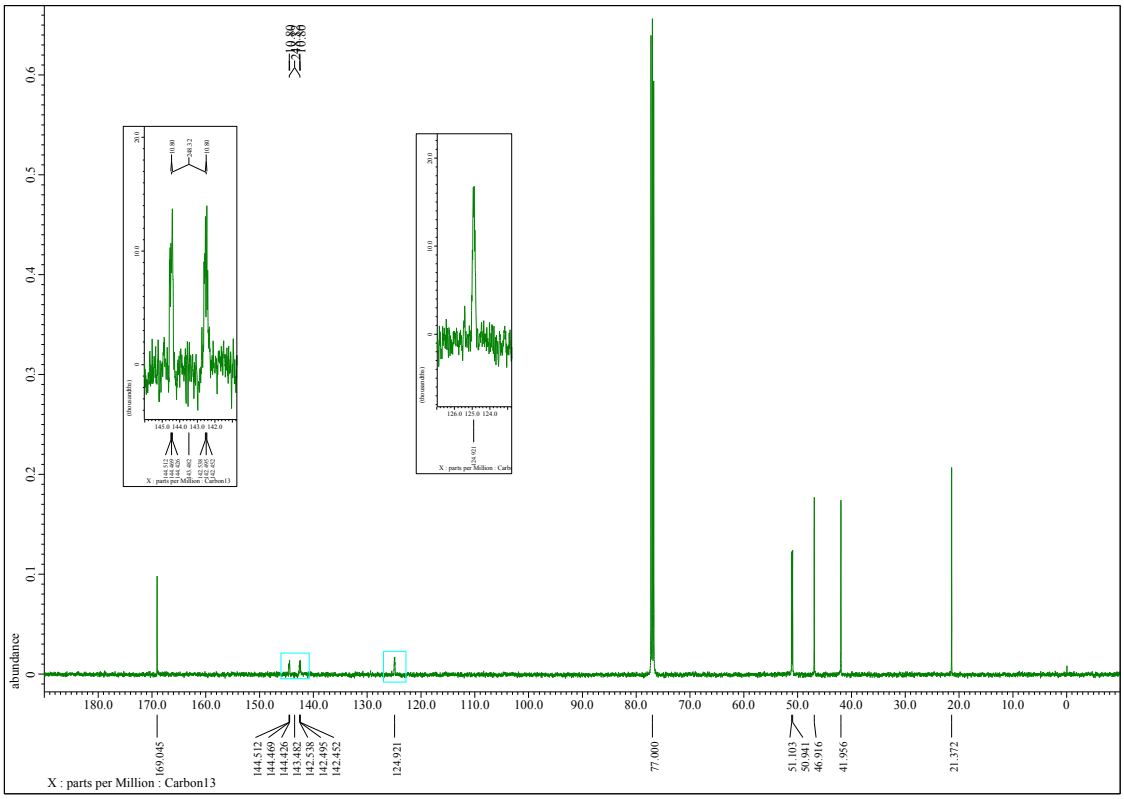
Figure S11. Bright field (left) and fluorescence (right) microscopic images of HepG2 cells treated with F-Nox-3-Ac (5 μM) at 37 °C for 30 min. Scale bars indicate 50 μm.

^{1}H-, ^{13}C-, and ^{19}F-NMR spectra of the newly synthesized compounds

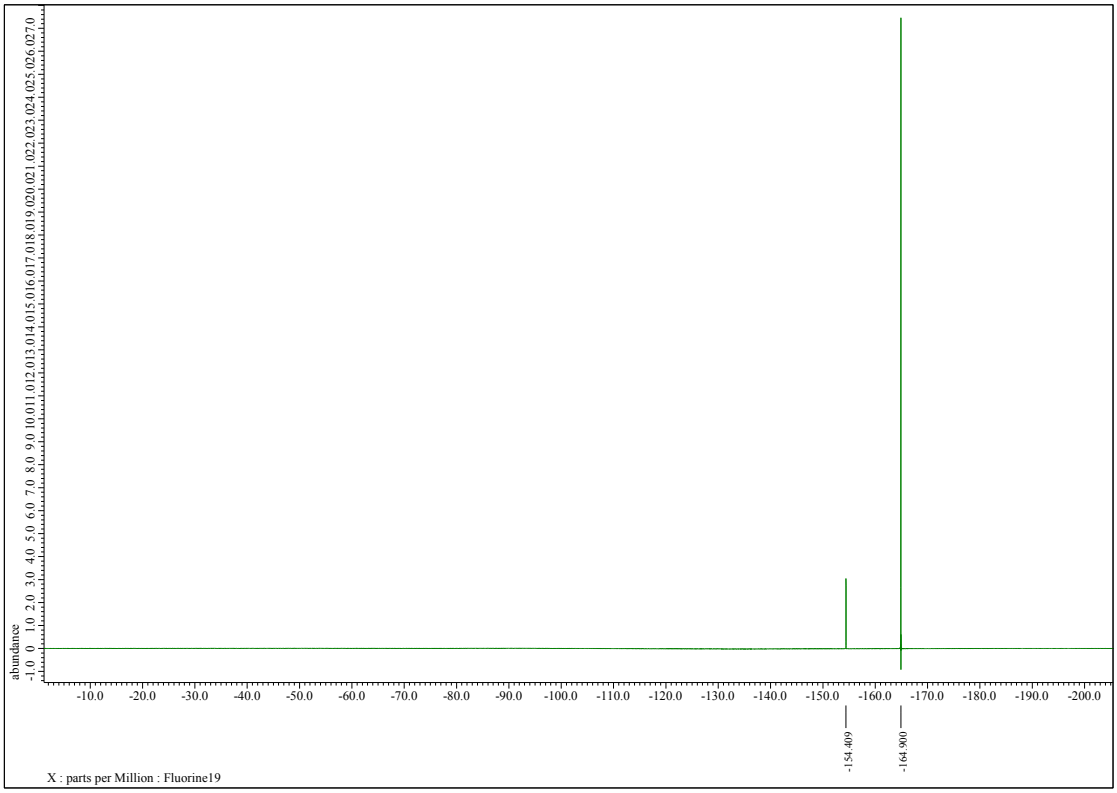
^{1}H-NMR ($CDCl_3$) spectrum of **1**



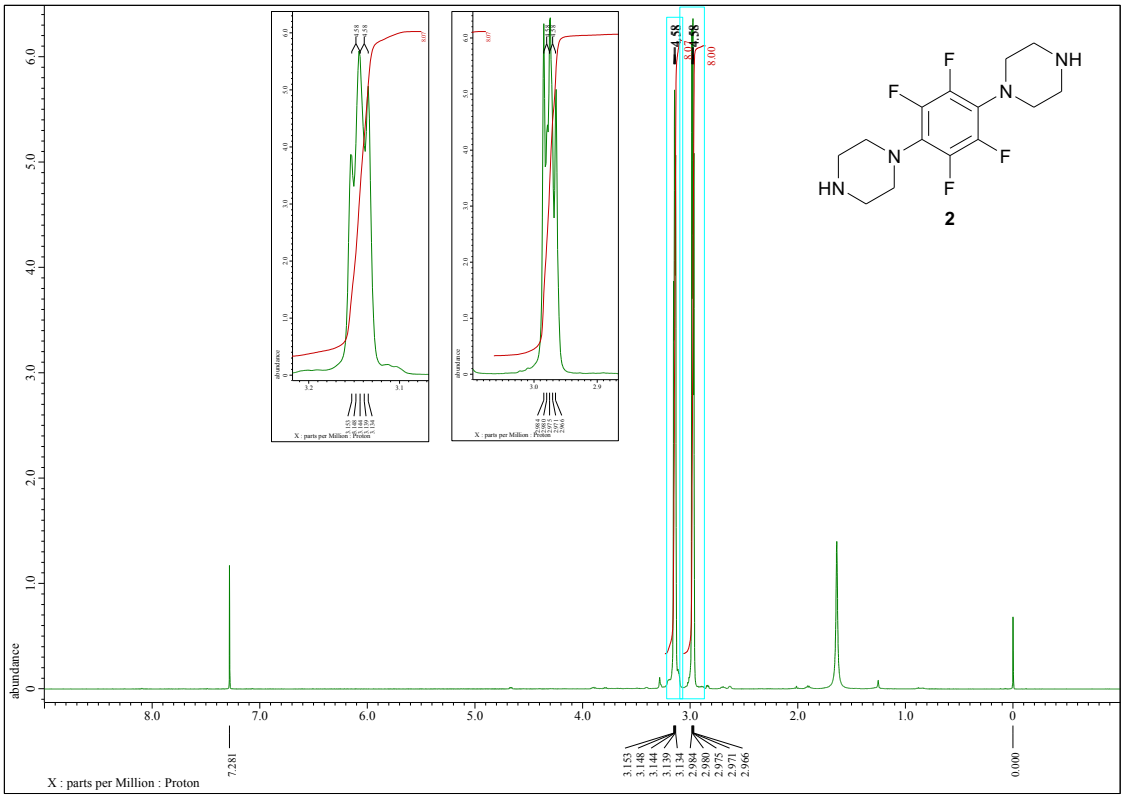
^{13}C-NMR ($CDCl_3$) spectrum of **1**



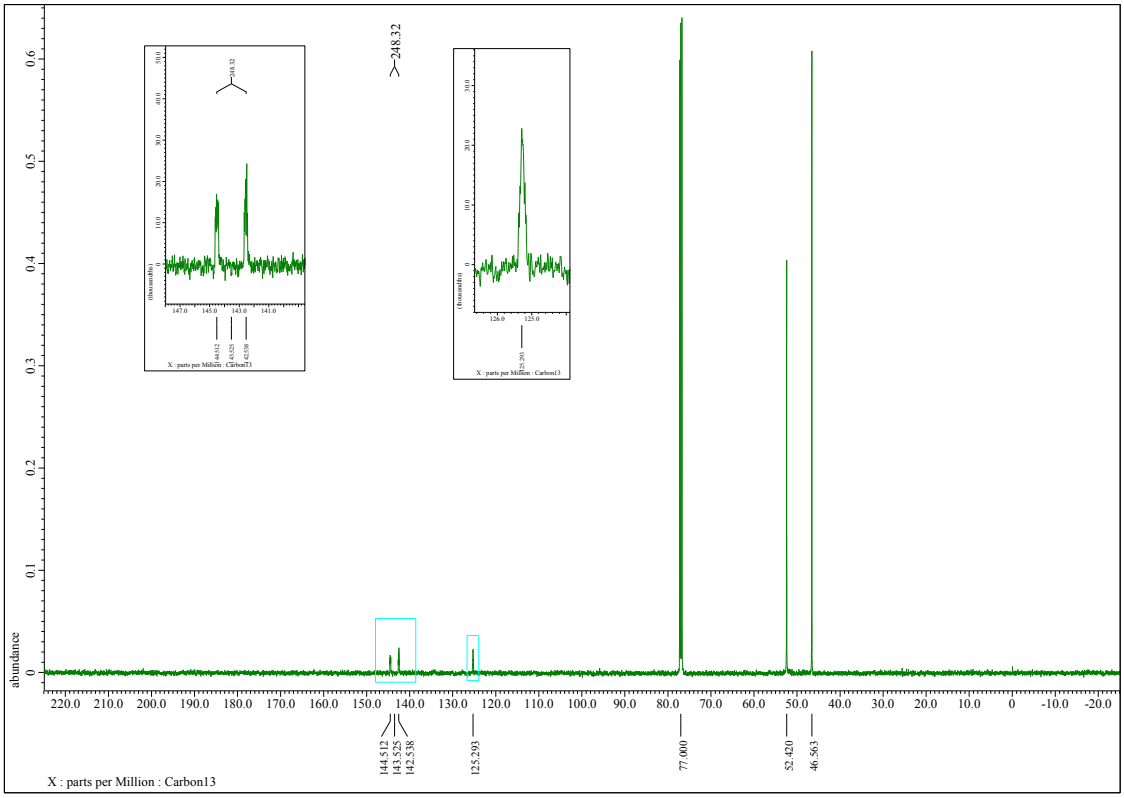
^{19}F-NMR ($CDCl_3$) spectrum of **1**



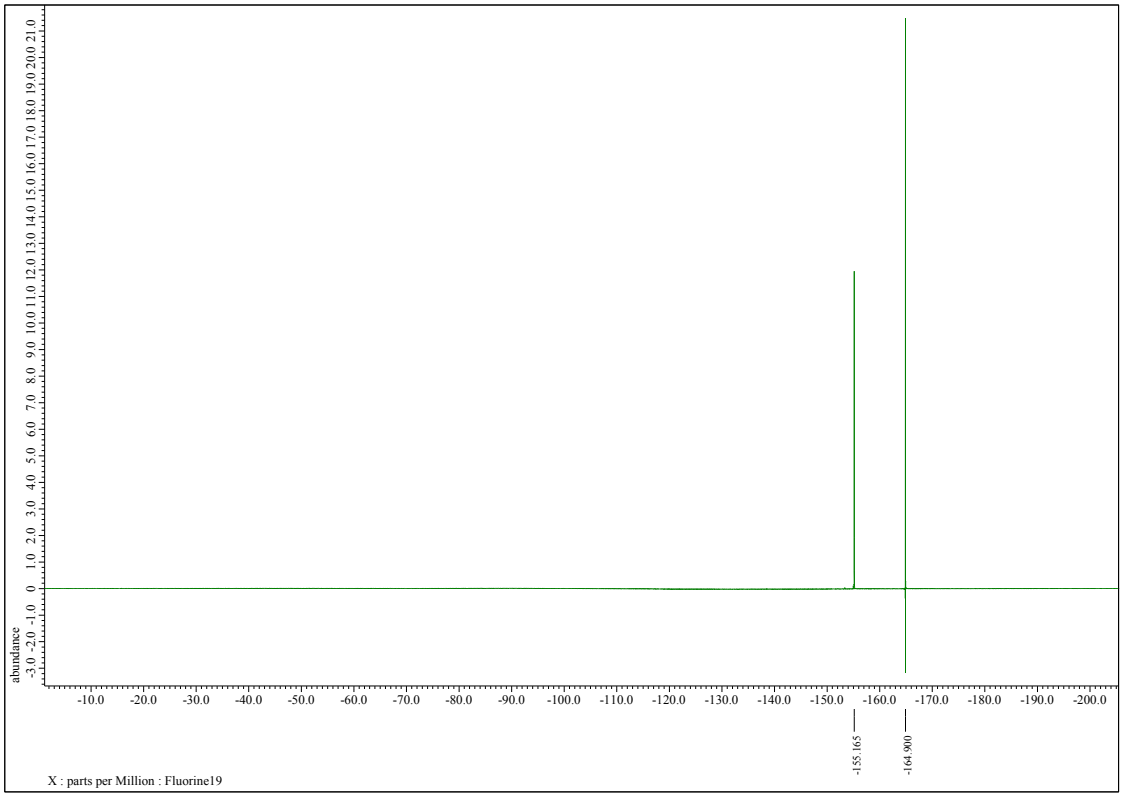
^{1}H-NMR ($CDCl_3$) spectrum of **2**



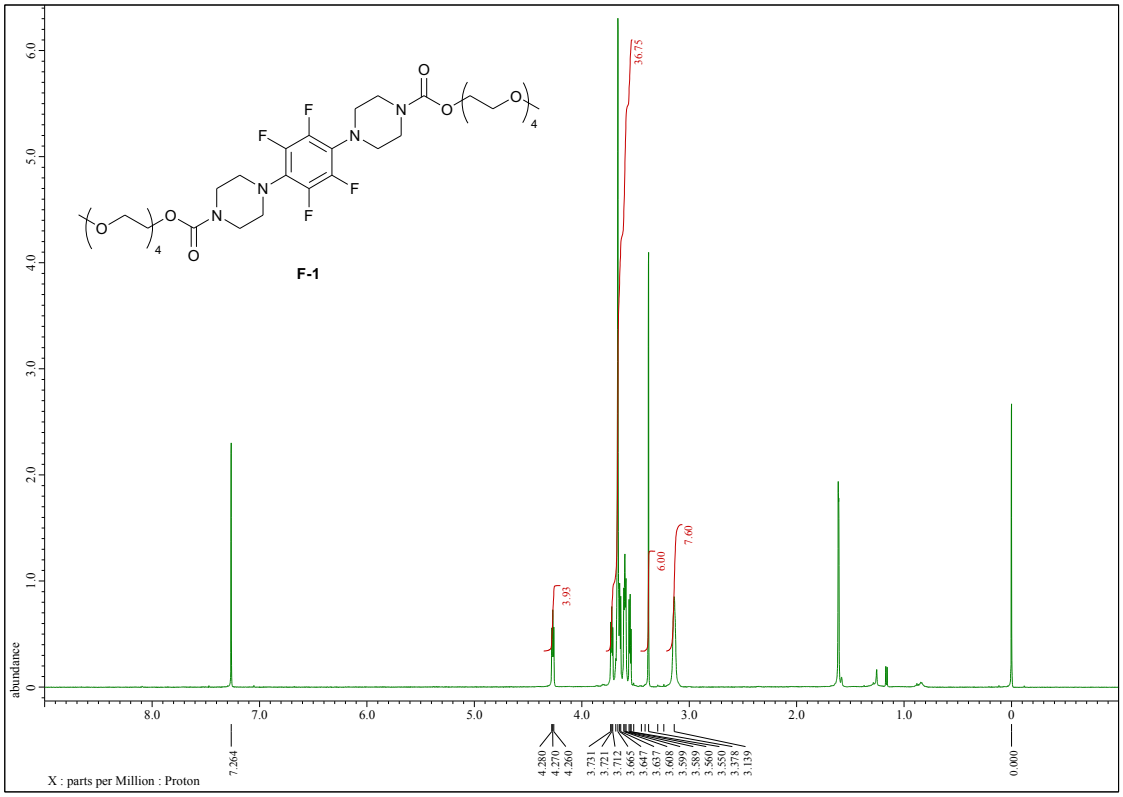
^{13}C-NMR ($CDCl_3$) spectrum of **2**



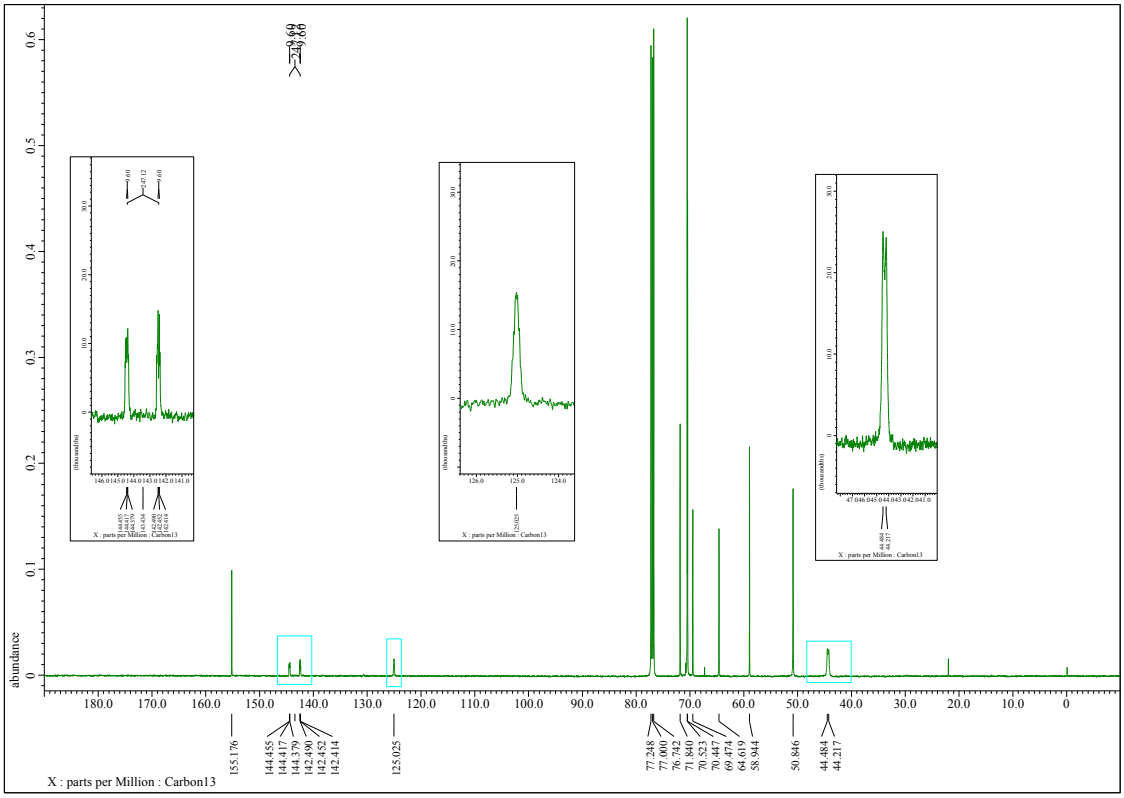
^{19}F-NMR ($CDCl_3$) spectrum of **2**



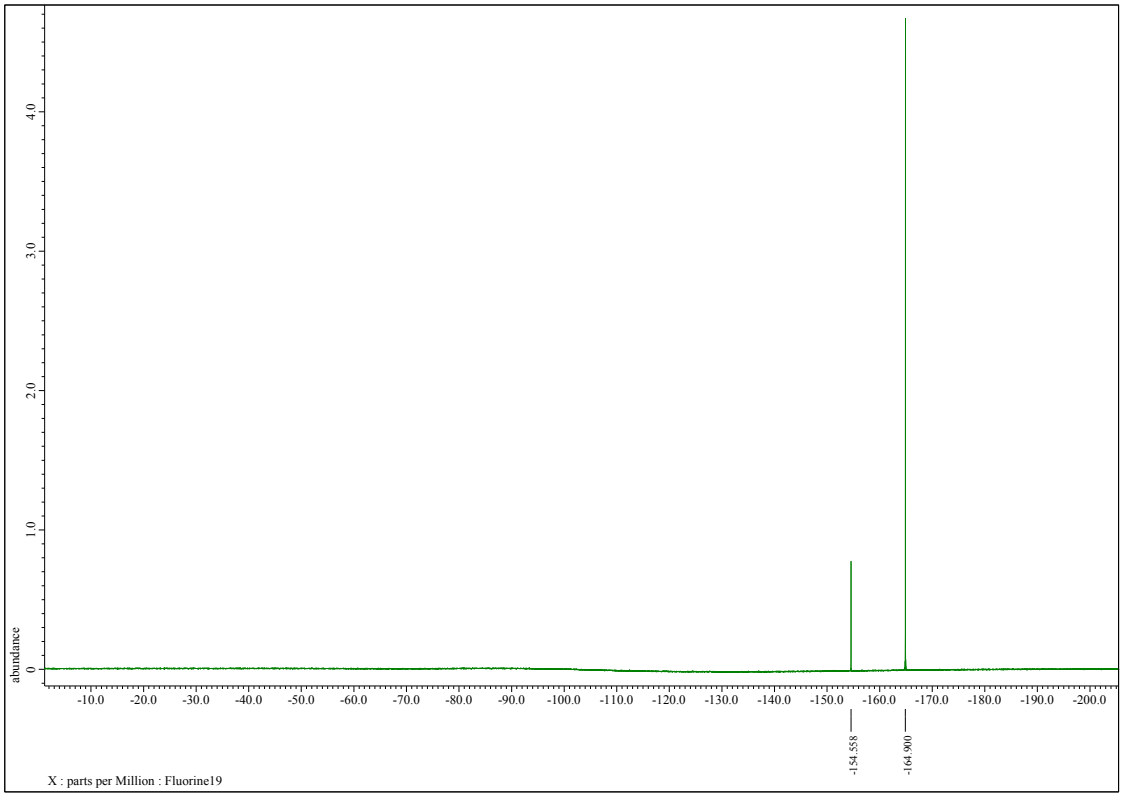
^{1}H-NMR ($CDCl_3$) spectrum of **F-1**



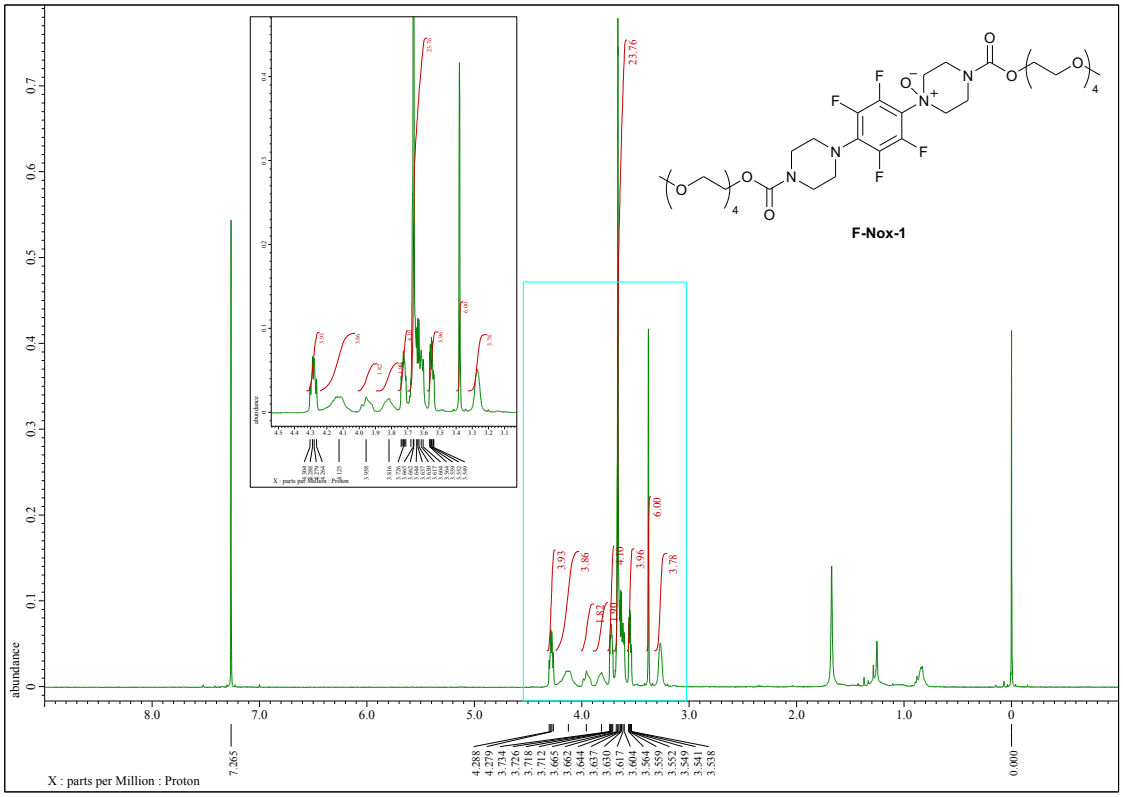
^{13}C-NMR ($CDCl_3$) spectrum of **F-1**



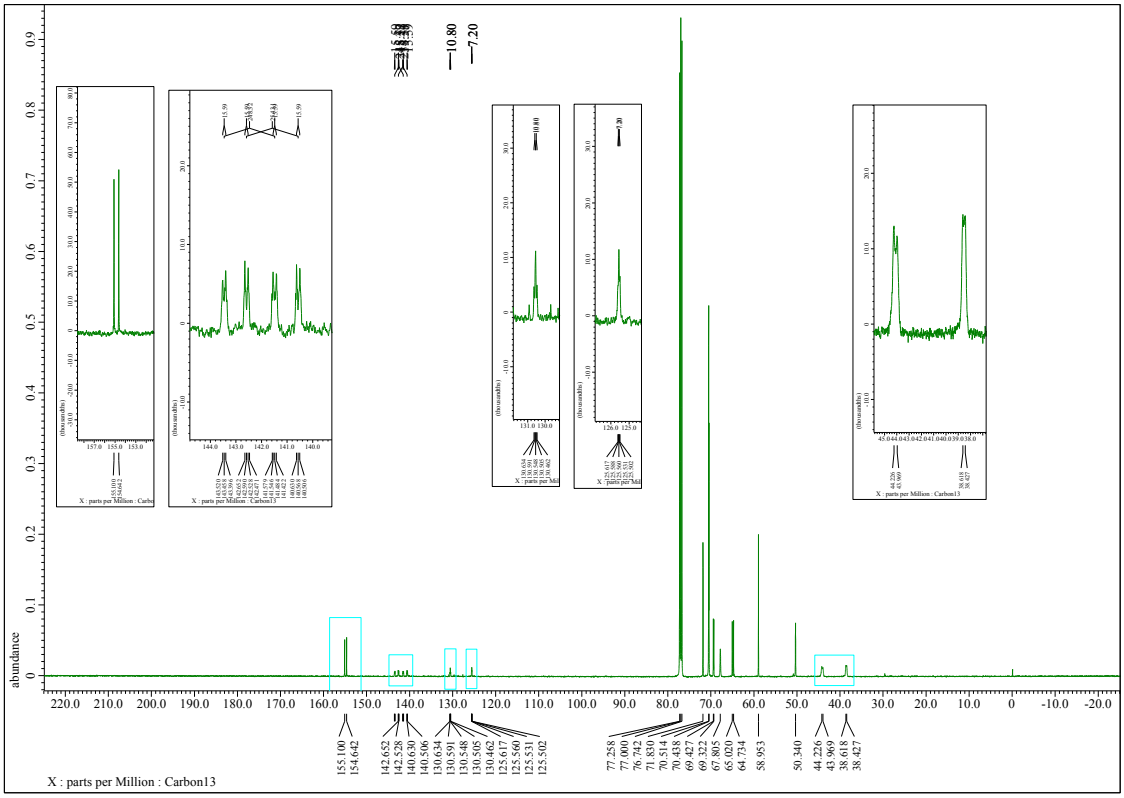
^{19}F-NMR ($CDCl_3$) spectrum of **F-1**



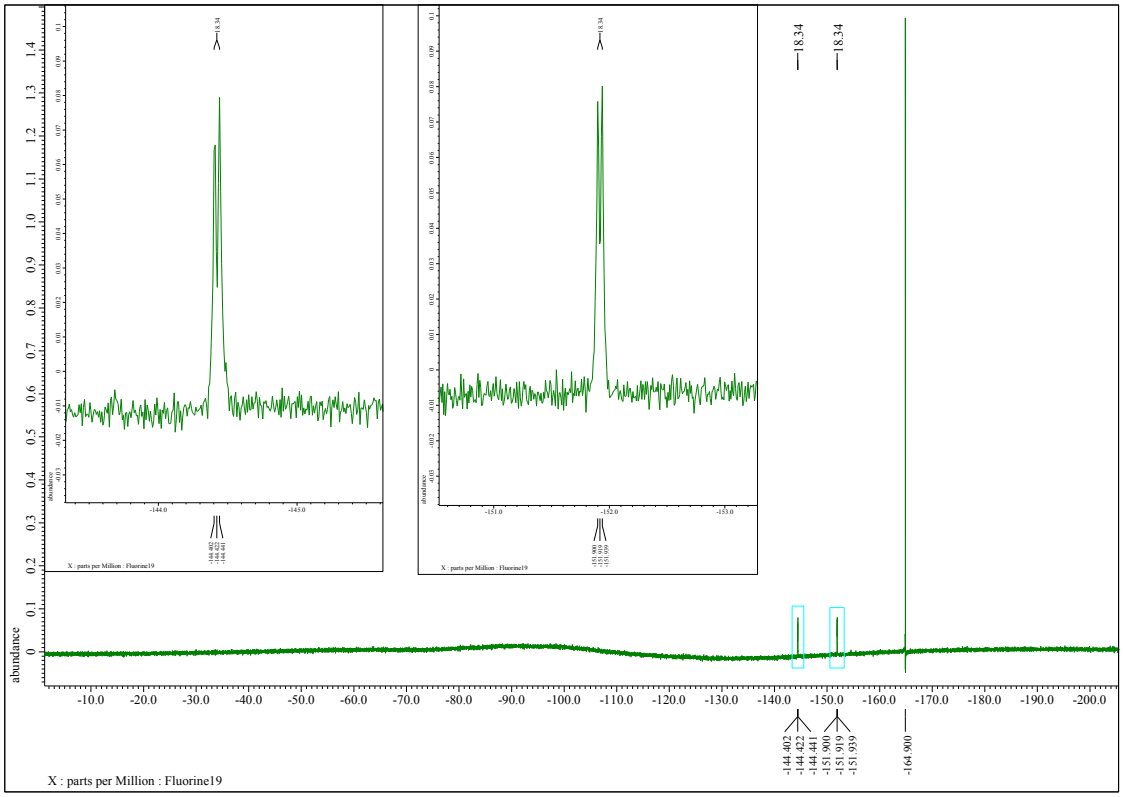
^{1}H-NMR ($CDCl_3$) spectrum of **F-Nox-1**



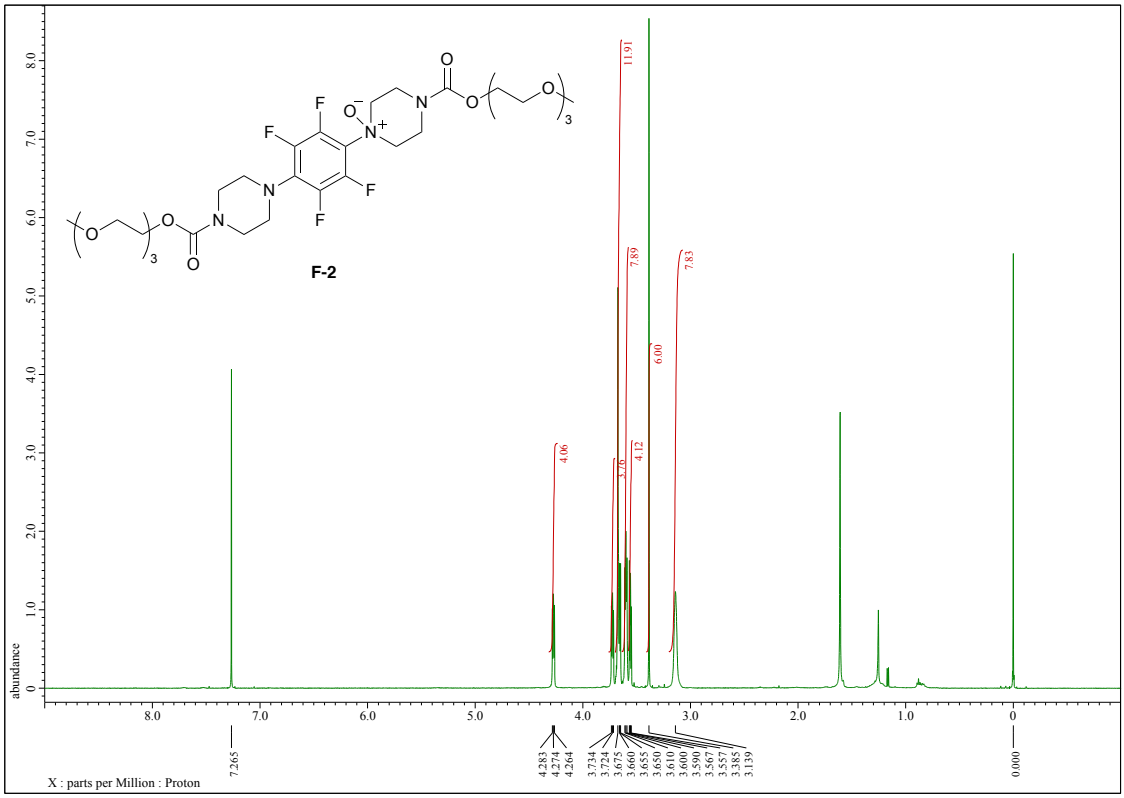
^{13}C-NMR ($CDCl_3$) spectrum of **F-Nox-1**



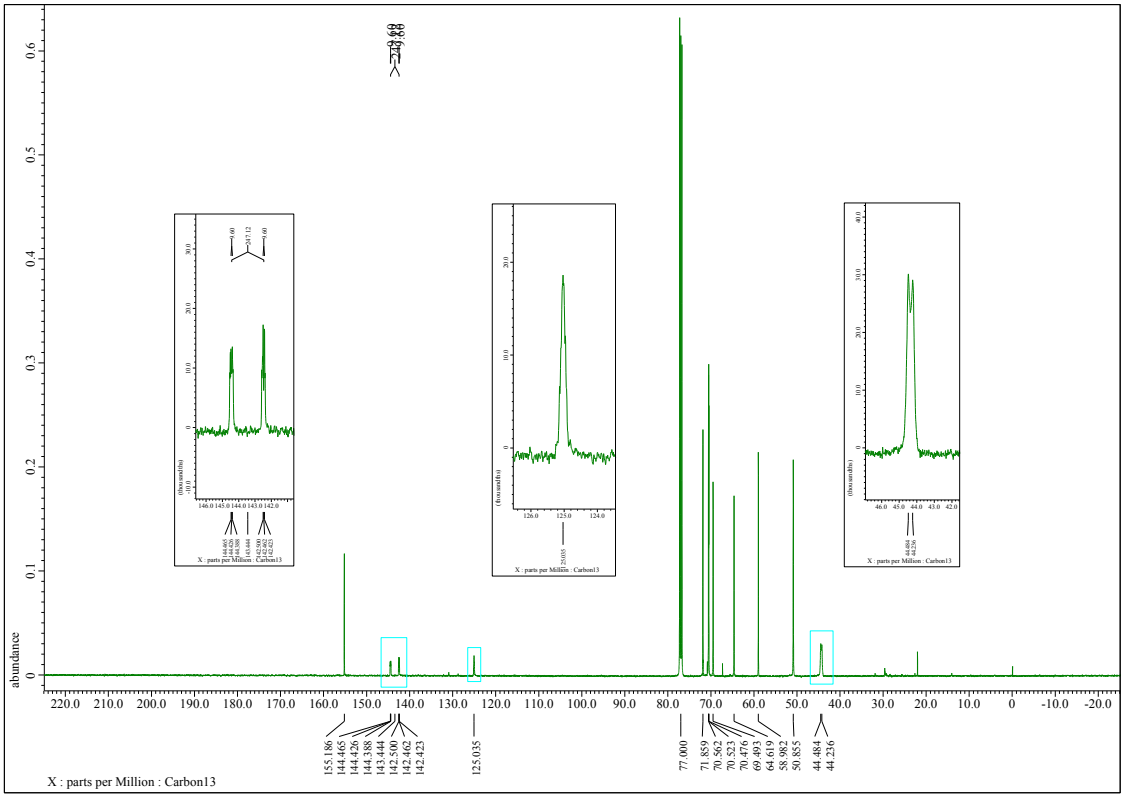
^{19}F-NMR ($CDCl_3$) spectrum of **F-Nox-1**



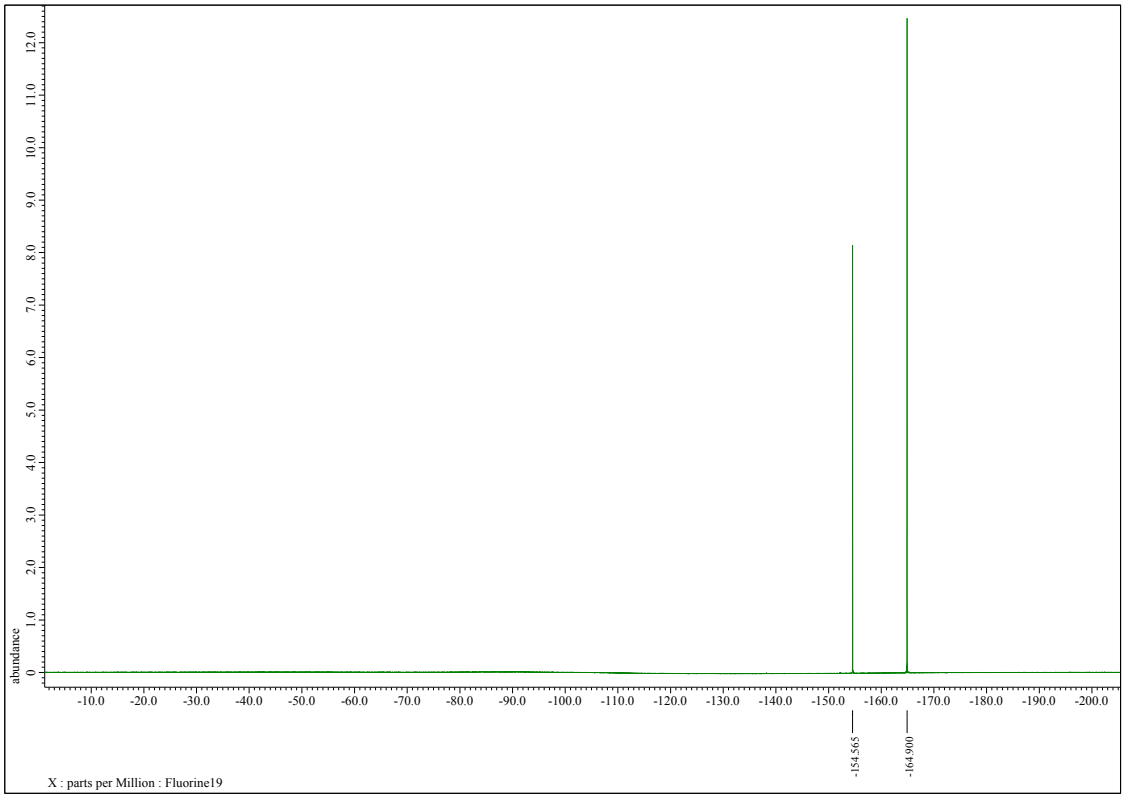
^{1}H-NMR ($CDCl_3$) spectrum of **F-2**



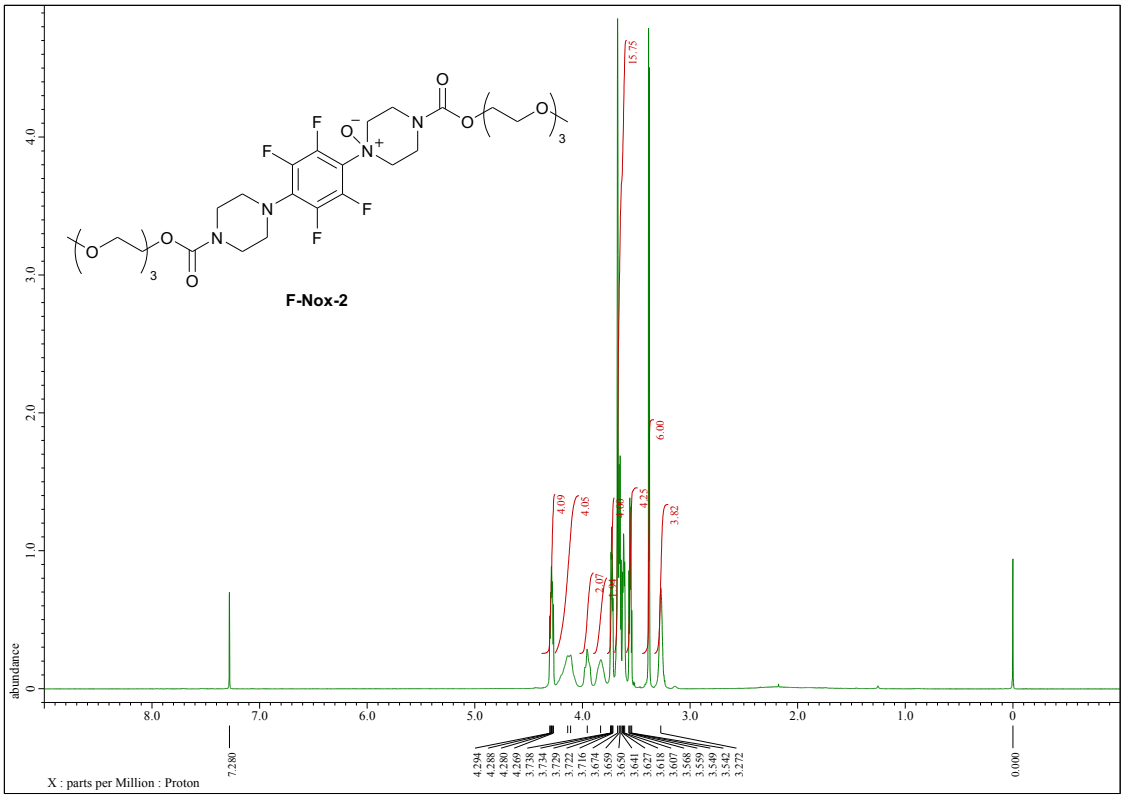
^{13}C-NMR ($CDCl_3$) spectrum of **F-2**



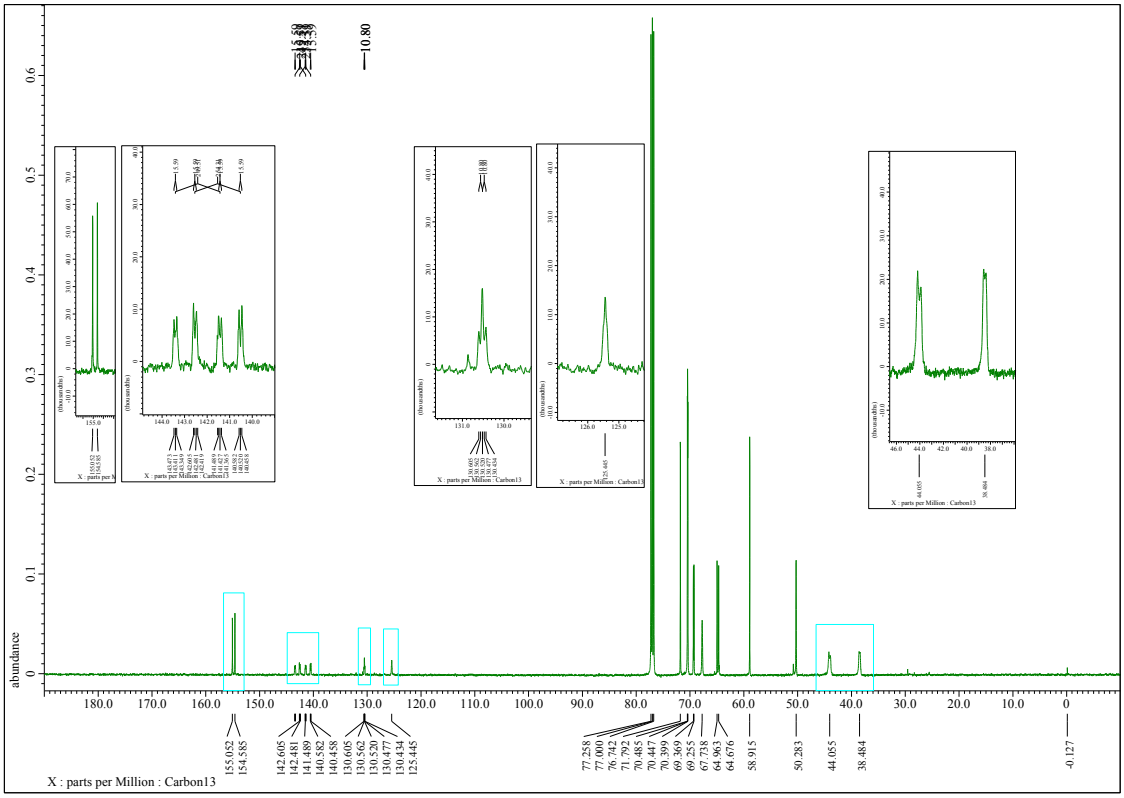
^{19}F-NMR ($CDCl_3$) spectrum of **F-2**



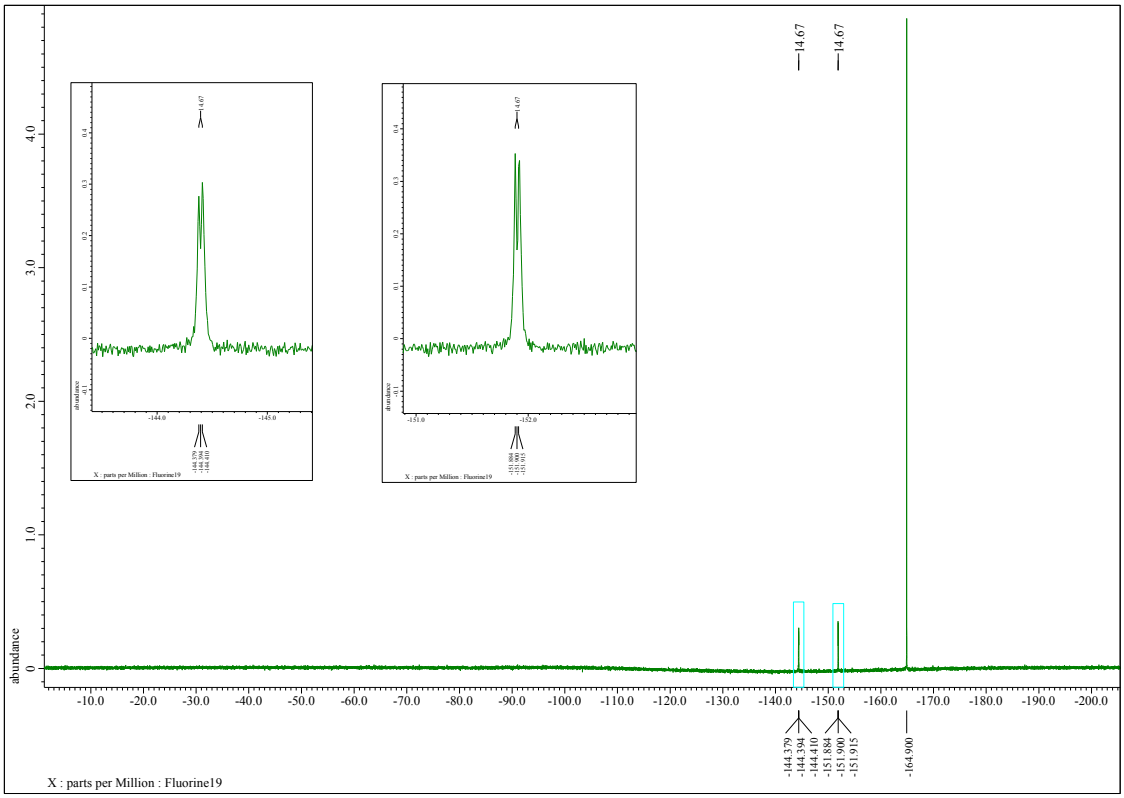
^{1}H-NMR ($CDCl_3$) spectrum of **F-Nox-2**



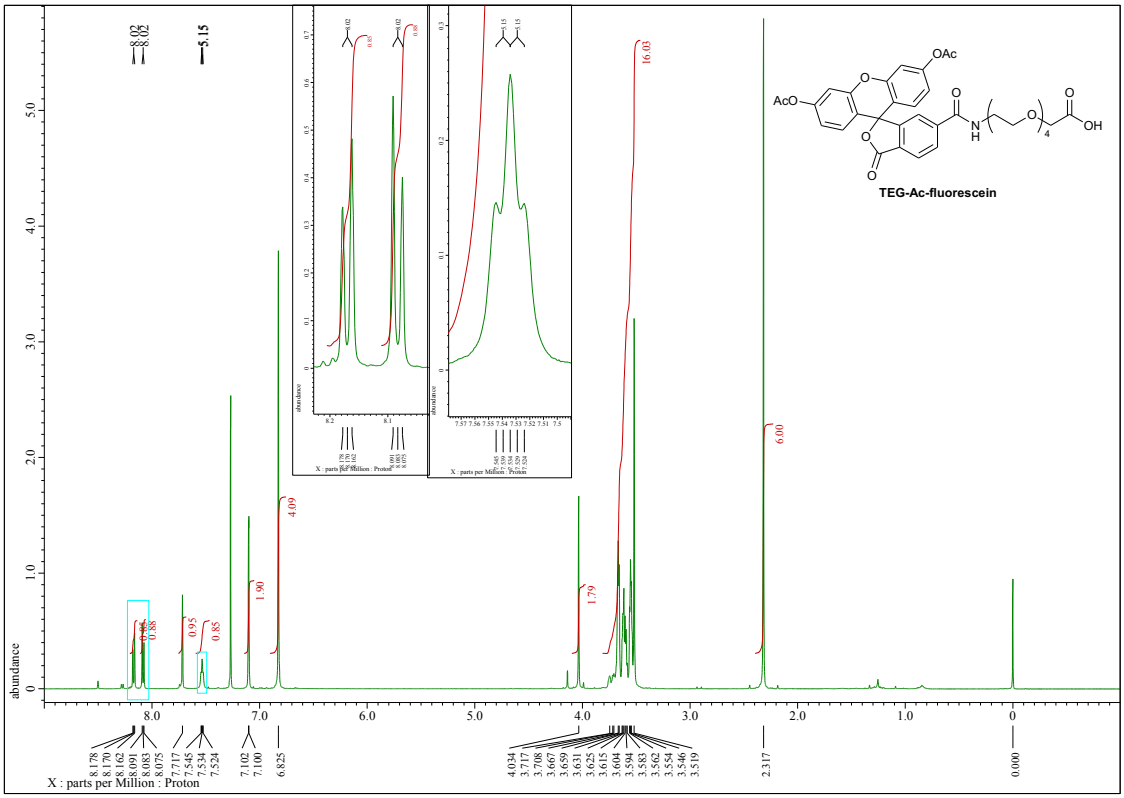
^{13}C-NMR ($CDCl_3$) spectrum of **F-Nox-2**



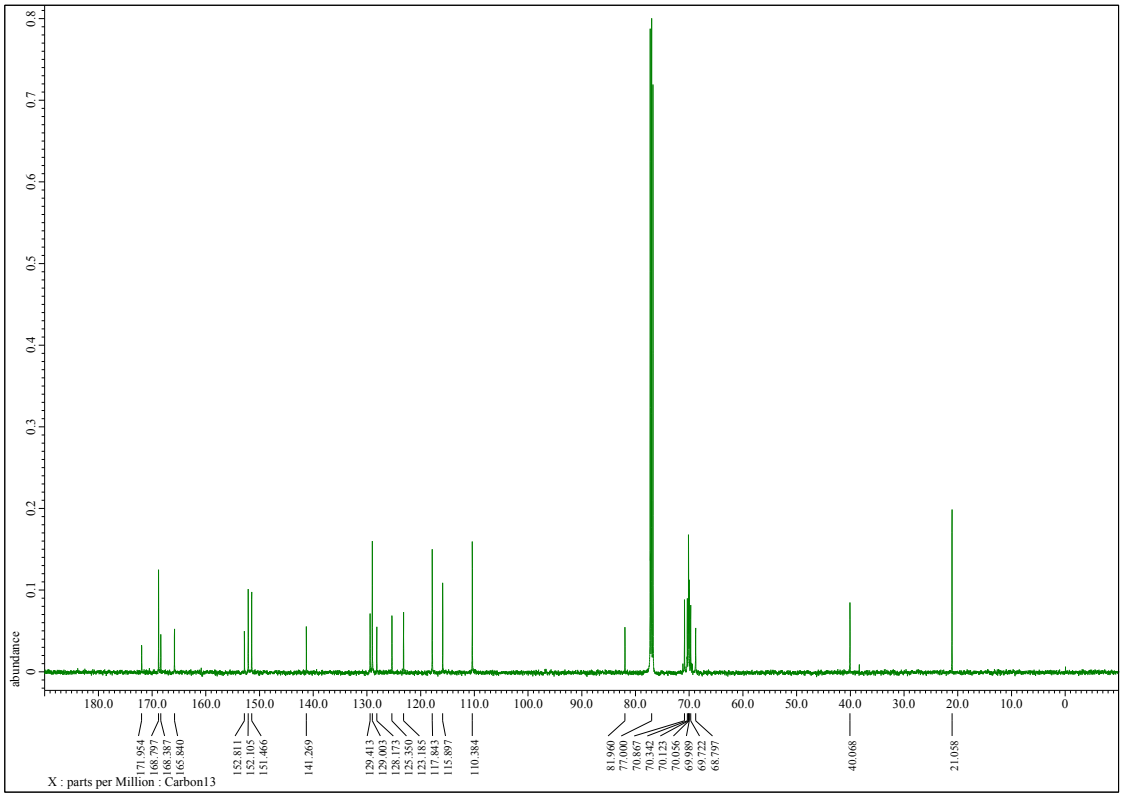
^{19}F-NMR ($CDCl_3$) spectrum of **F-Nox-2**



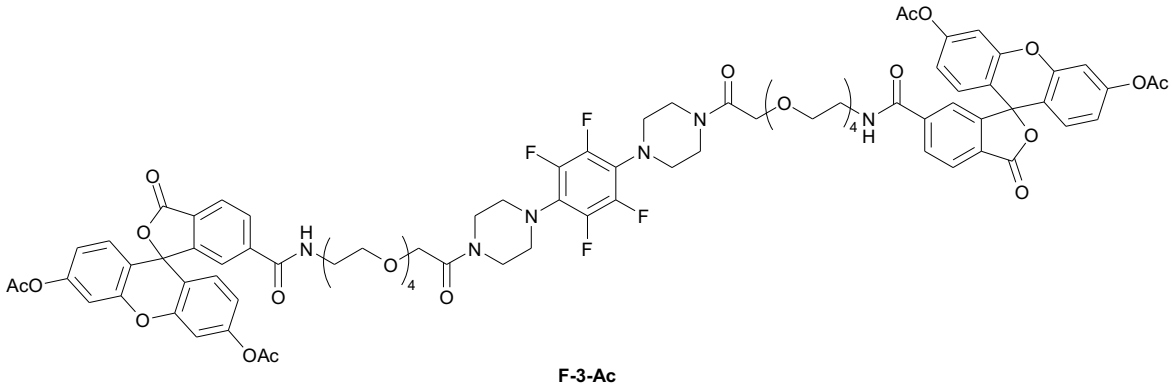
^{1}H-NMR ($CDCl_3$) spectrum of **TEG-Ac-fluorescein**



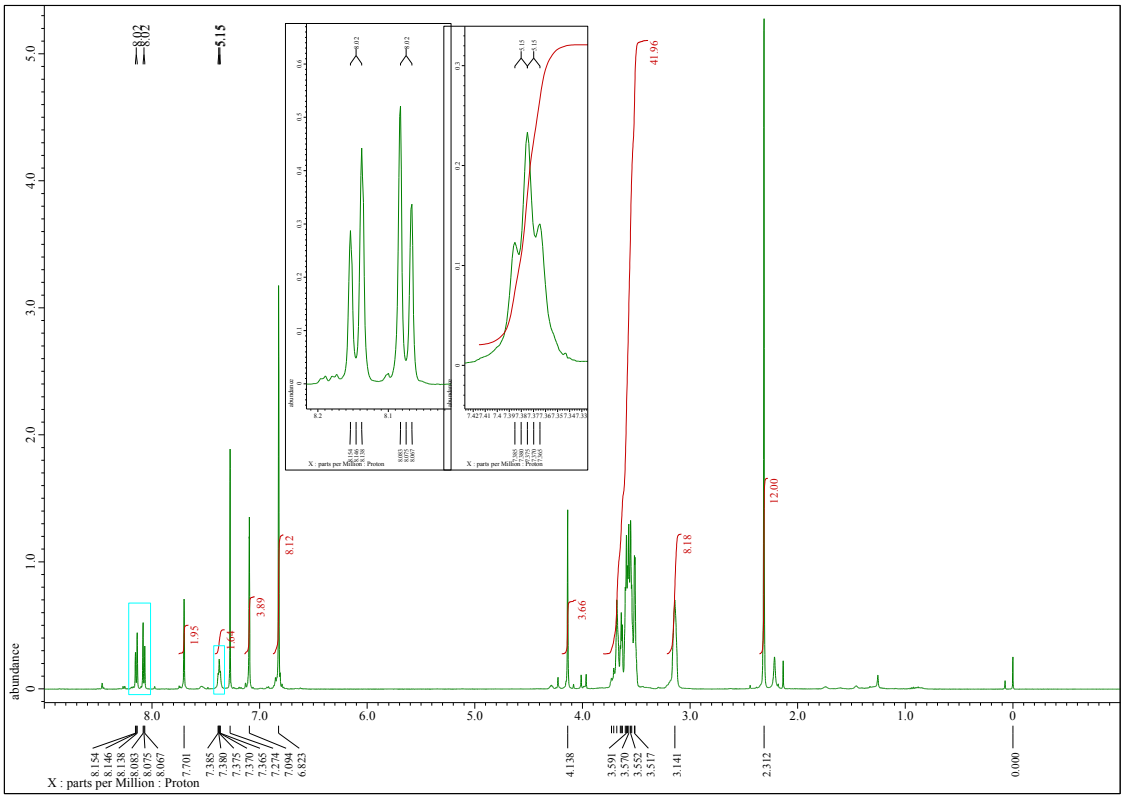
^{13}C-NMR ($CDCl_3$) spectrum of **TEG-Ac-fluorescein**



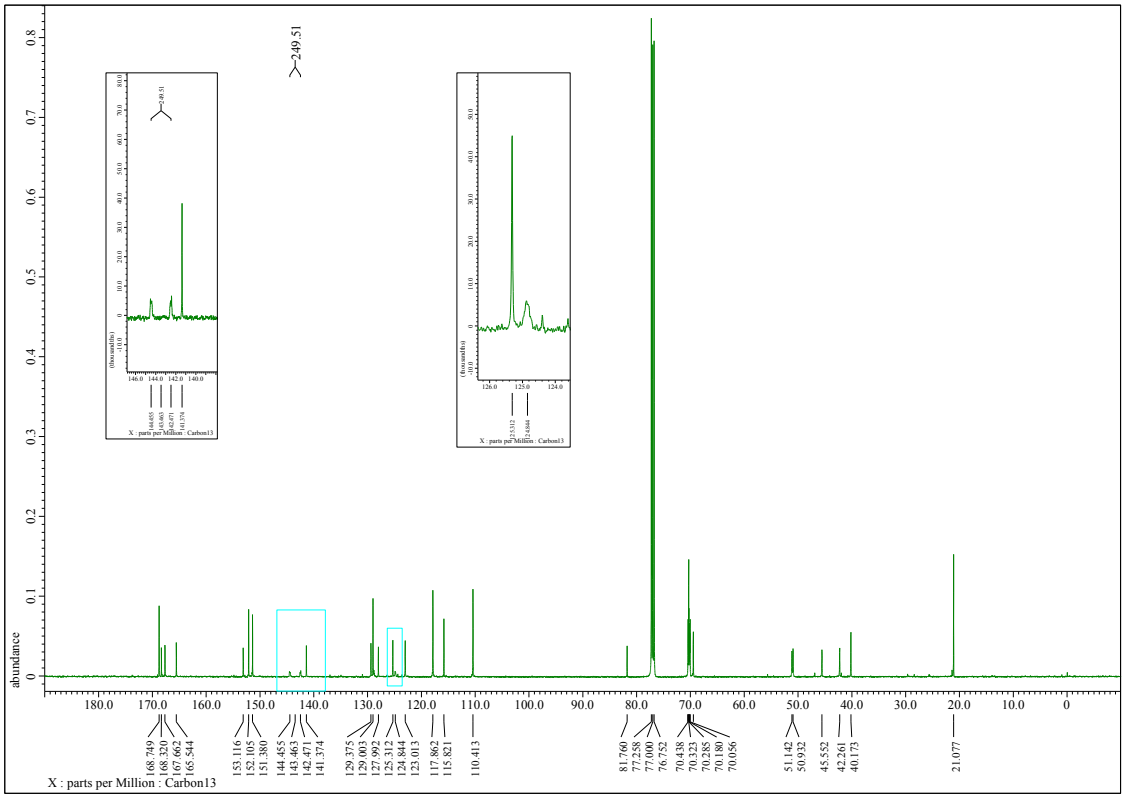
^{1}H-NMR ($CDCl_3$) spectrum of **F-3-Ac**



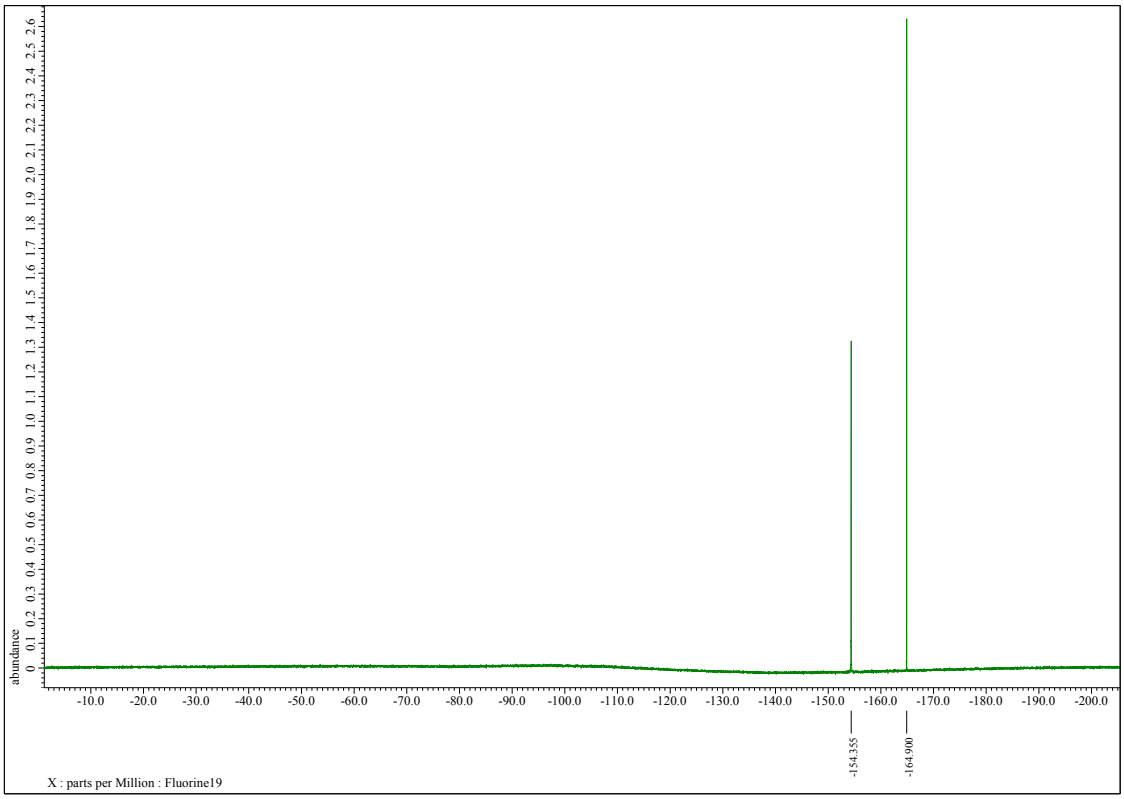
F-3-Ac



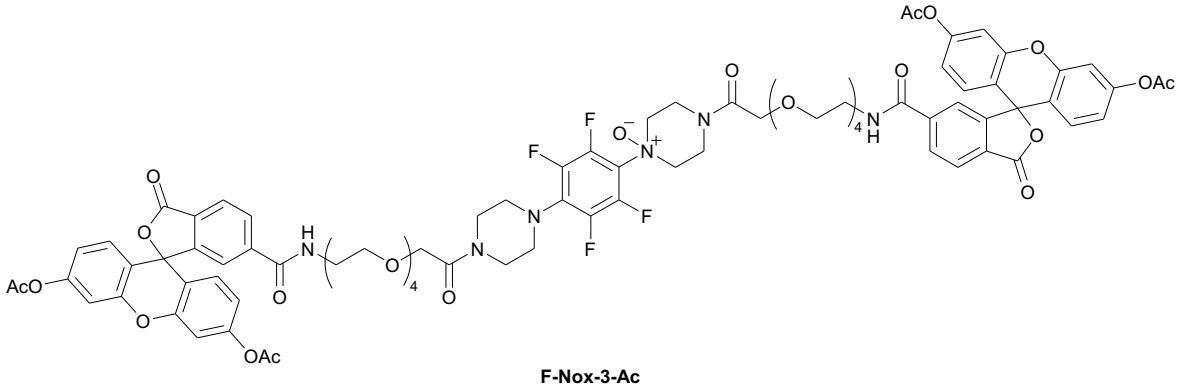
^{13}C-NMR ($CDCl_3$) spectrum of **F-3-Ac**



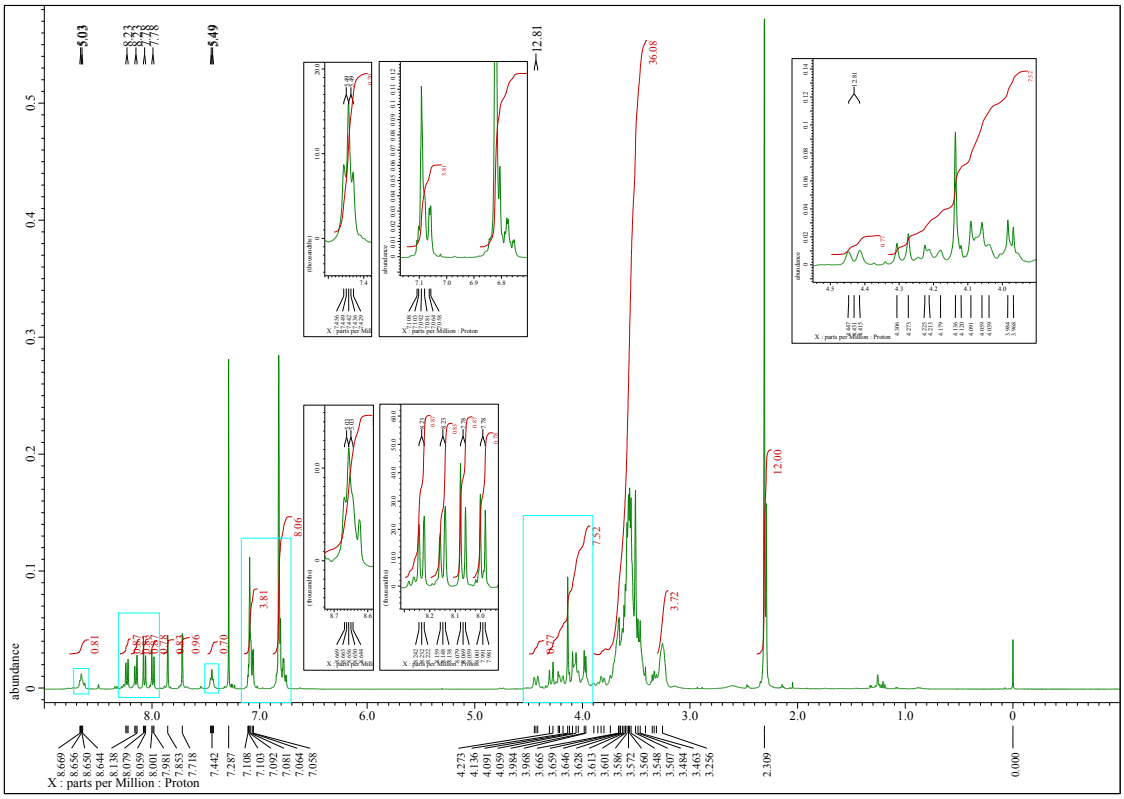
^{19}F-NMR ($CDCl_3$) spectrum of **F-3-Ac**



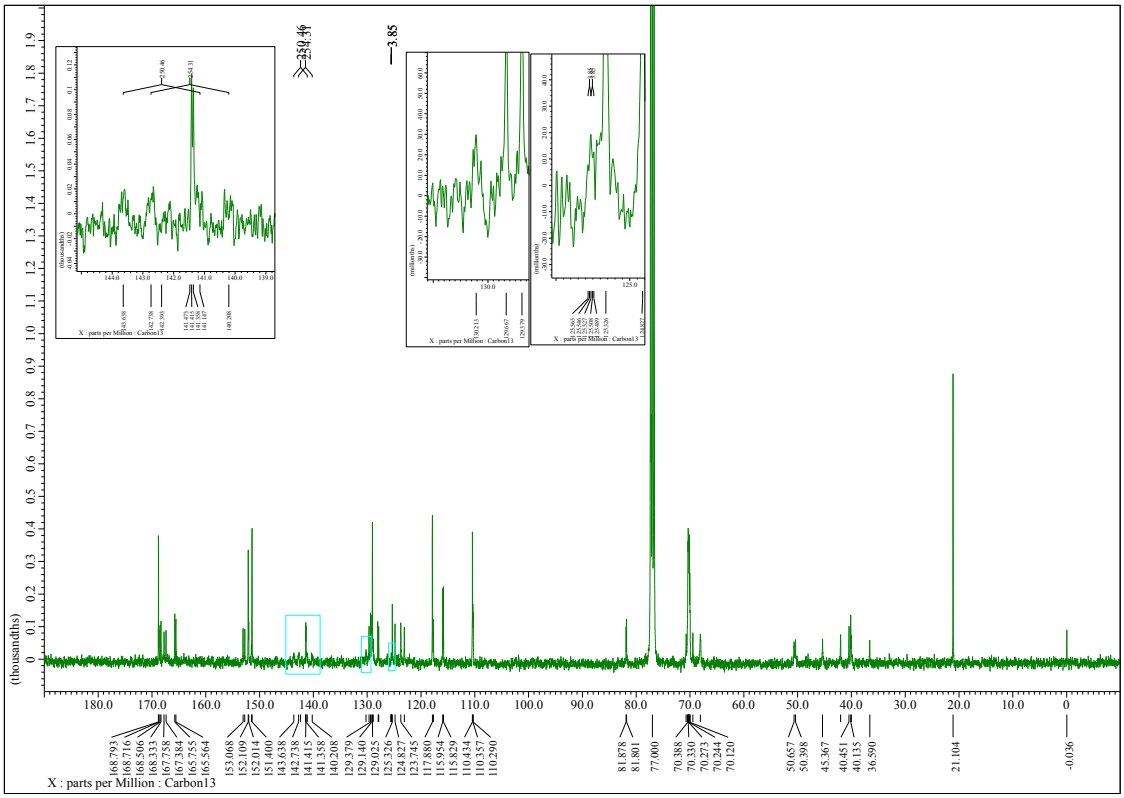
^{1}H-NMR ($CDCl_3$) spectrum of **F-Nox-3-Ac**



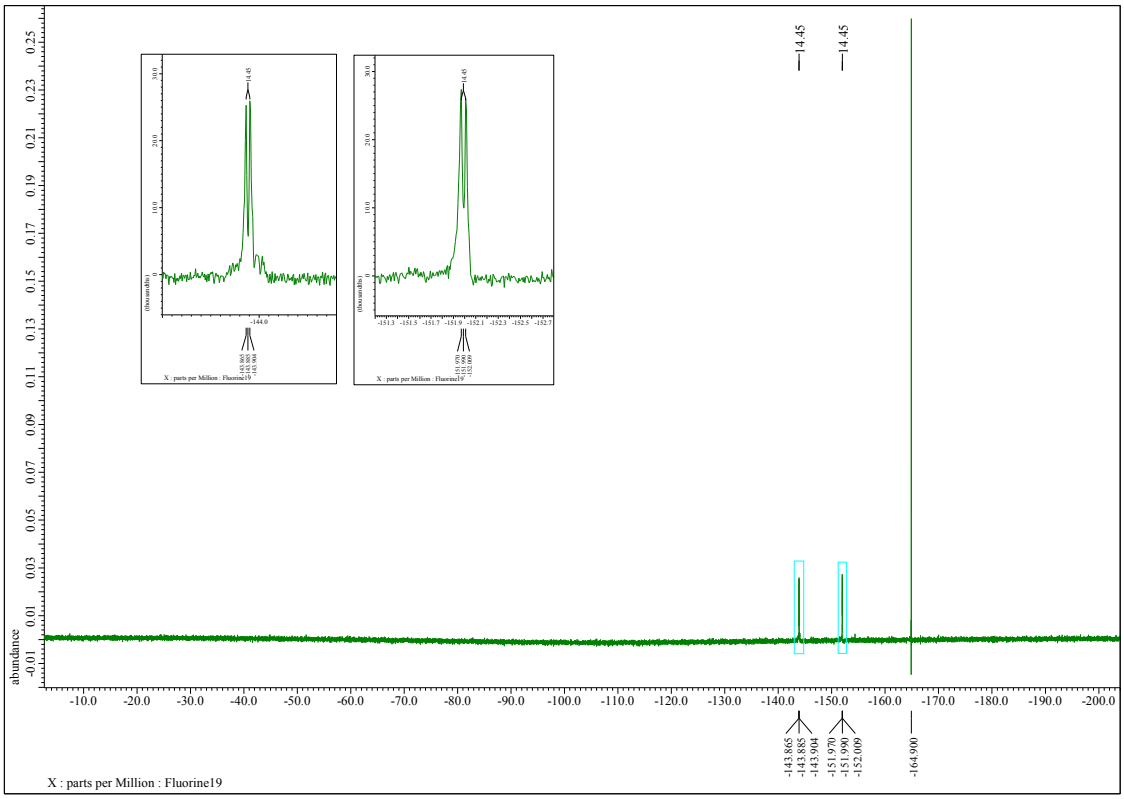
F-Nox-3-Ac



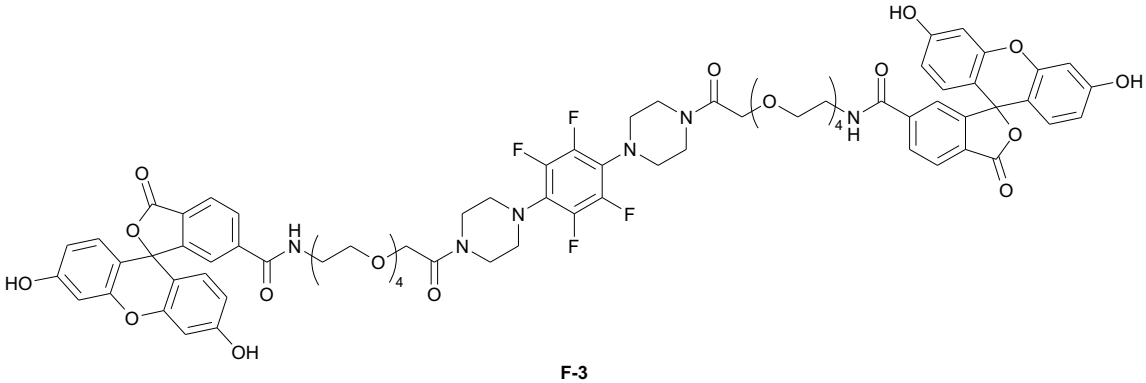
^{13}C-NMR ($CDCl_3$) spectrum of **F-Nox-3-Ac**



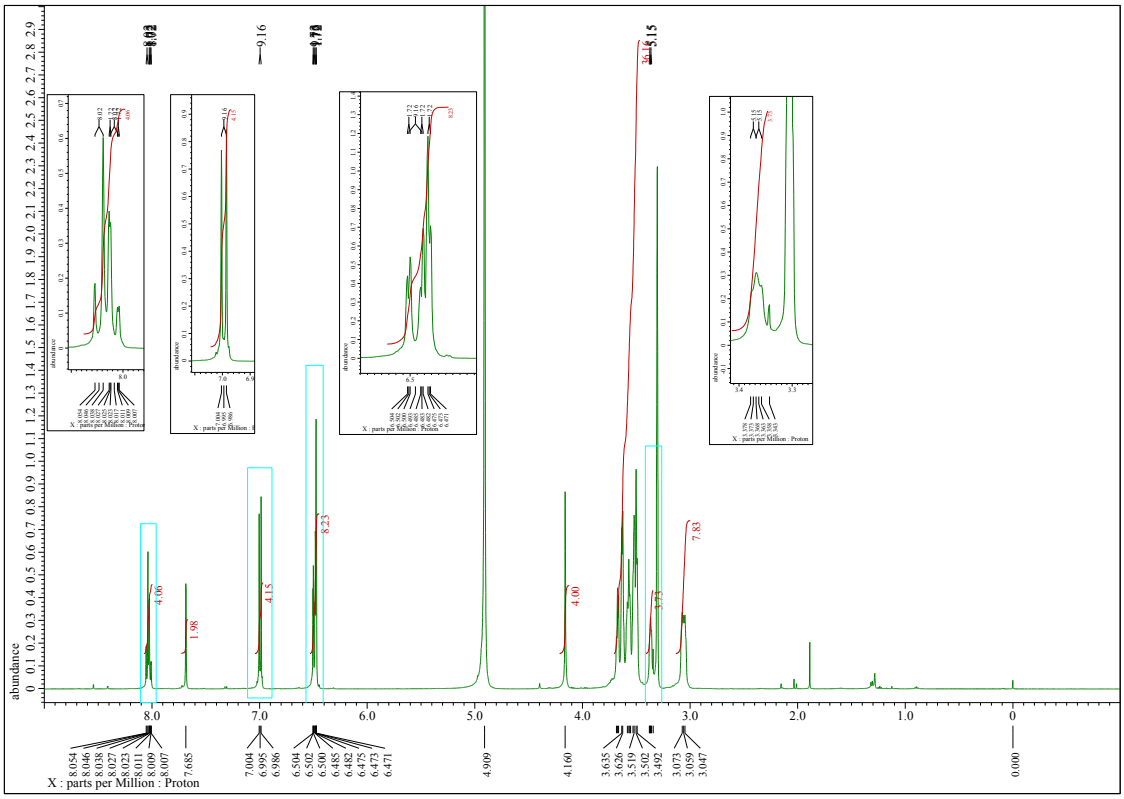
^{19}F-NMR ($CDCl_3$) spectrum of **F-Nox-3-Ac**



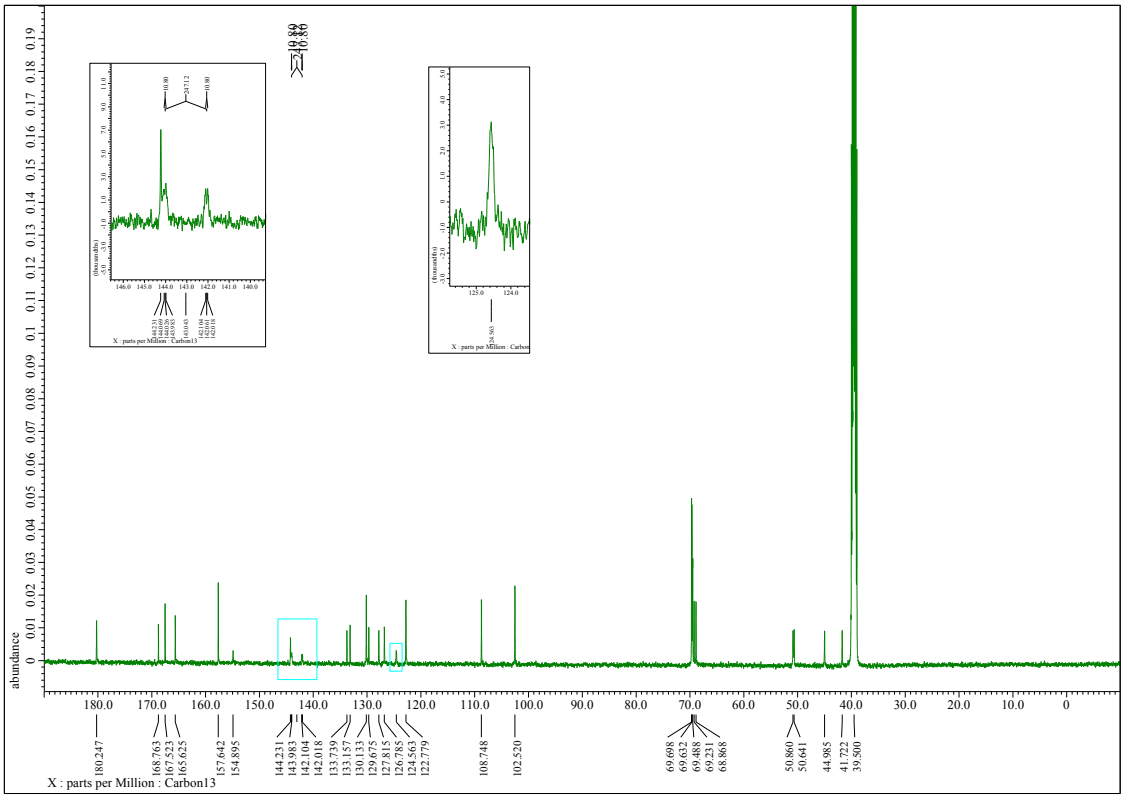
^{1}H-NMR (CD_3OD) spectrum of **F-3**



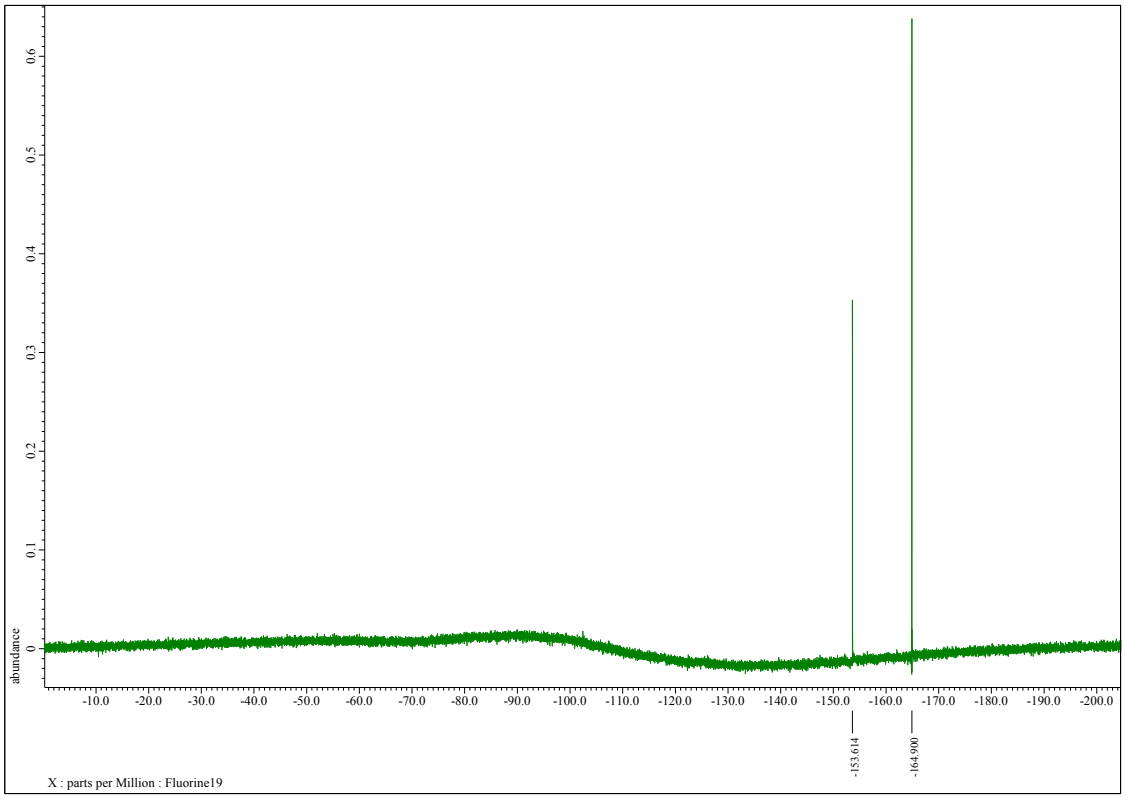
F-3



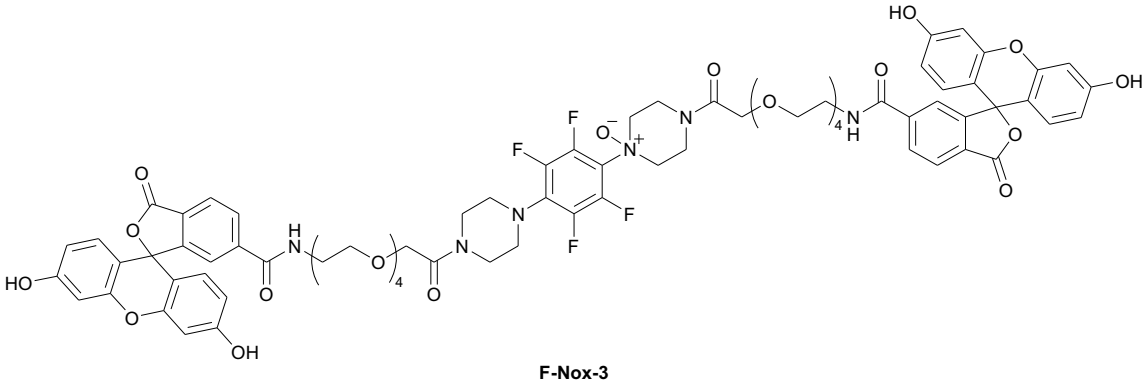
^{13}C-NMR (DMSO-d_6) spectrum of **F-3**



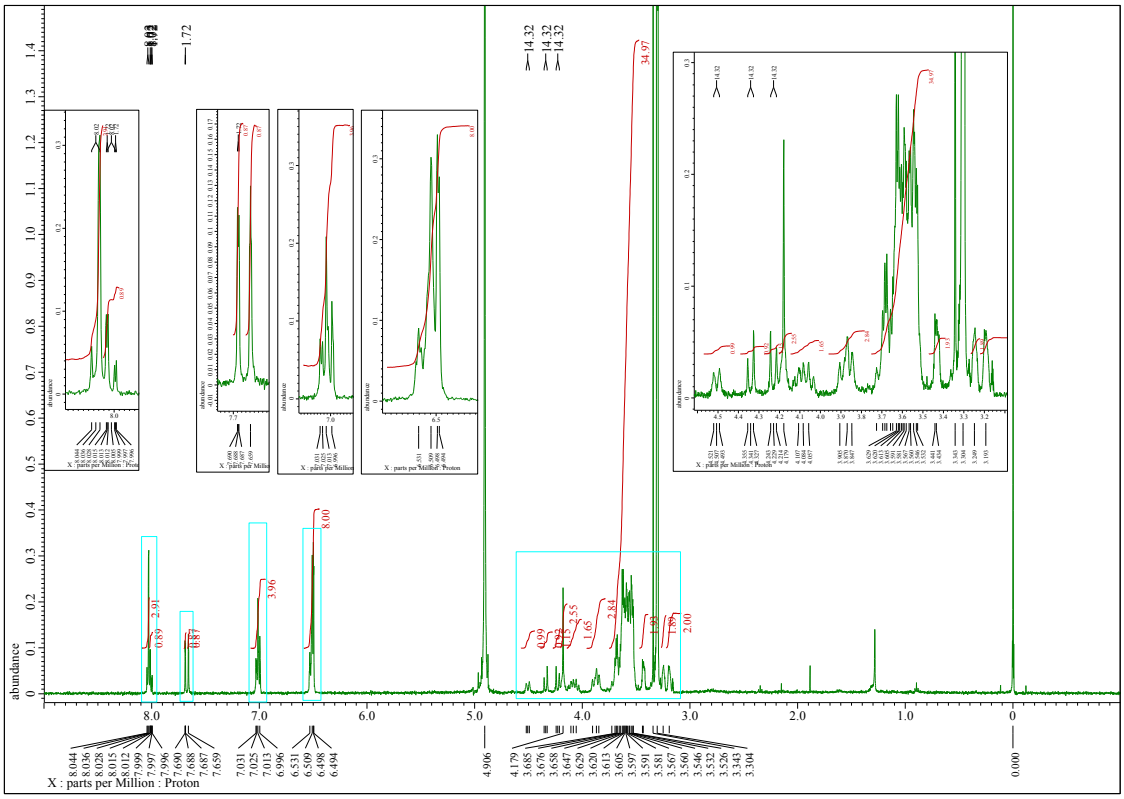
^{19}F-NMR (DMSO-d_6) spectrum of **F-3**



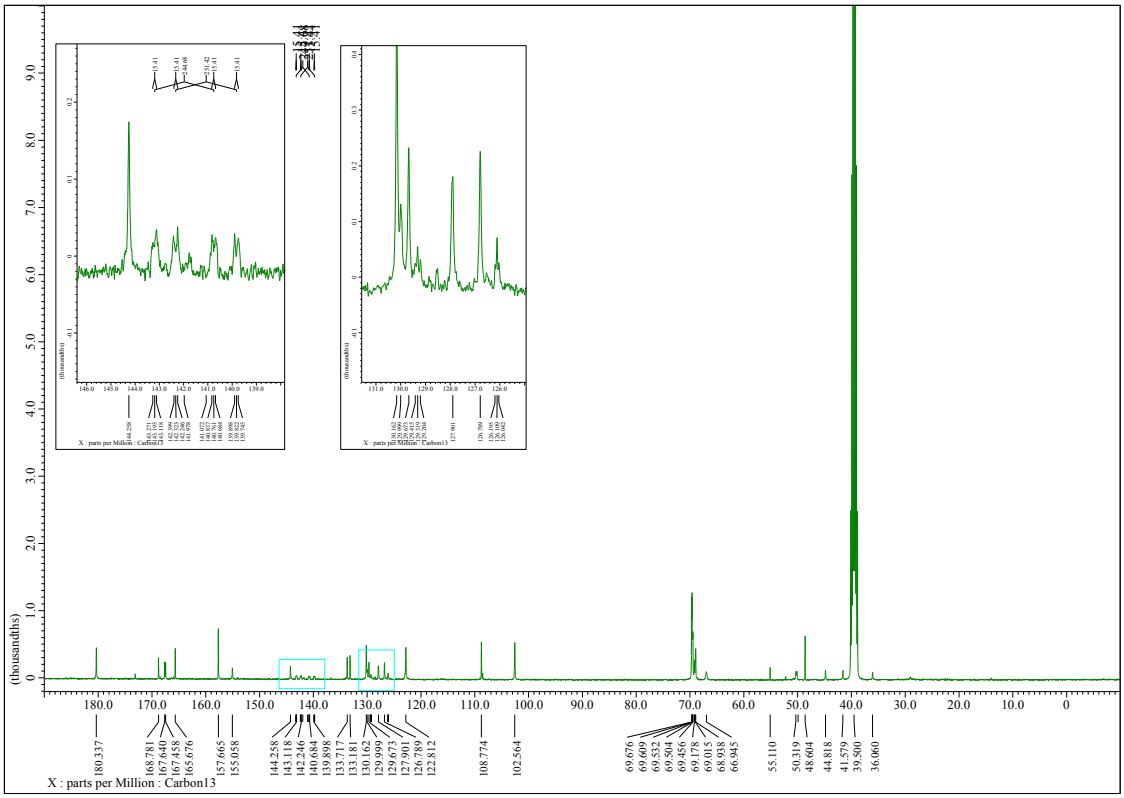
^{1}H-NMR (CD_3OD) spectrum of **F-Nox-3**



F-Nox-3



^{13}C-NMR (DMSO-d_6) spectrum of **F-Nox-3**



^{19}F-NMR (CD_3OD) spectrum of **F-Nox-3**