Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

Diselenide-based probe for the selective imaging of hypochlorite in living cancer cells

EXPERIMENTAL SECTION

General considerations. All chemicals used herein were used as received from commercial suppliers (Aldrich, Tokyo Chemical Industry). ^{1}H, ^{13}C, ^{77}Se NMR spectra were acquired using a Bruker Avance 400 and Agilent-NMR-vnmrs 600 MHz spectrometer. TMS and dimethyl selenide were used as external standards, respectively. ESI-mass spectrometry was performed on a BRUKER micrOTOF-QⅡ by the research support staff at KAIST. A Time-of-Flight mass spectrometer was operated at a resolution of 20,000. Absorption spectra were measured using a JASCO V–530 UV/Vis spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF–5301pc spectro-fluorophotometer. Microwave synthesis was carried out with a MWO-20M5 domestic microwave oven (Tongyang Magic). Melting points were measured by Buchi Melting Point M-565.

Synthetic procedures for compound 3. Compound **1** (5-amino-3-methyl-1-phenyl pyrazole) (0.187 g, 1.08 mmol) and compound **2** (bis(*ortho*-formylphenyl)diselenide) (0.100 g, 0.27 mmol) were ground together in a glass mortar. The mixture was placed into an unsealed pyrex glass vial and irradiated in a domestic microwave for 5 min at 500 W. The reaction was monitored by TLC. After completion of the reaction, the crude was purified by column chromatography (ethyl acetate/hexane) to afford the desired compounds **3**. Yield = 0.083 g, 29.6 %, m.p.: 250-252 ^{o}C, ^{1}H NMR ($CDCl_3$, δ 7.24, 400 MHz): 7.80 (d, $^{3}J_{H-H}$ = 8.2 Hz, 2 H_{12}), 7.48-7.39 (m, 16 $H_{13,14}$), 7.28 (td, $^{3}J_{H-H}$ = 5.6 Hz, $^{4}J_{H-H}$ = 1.2 Hz, 4 H_{17}), 7.20 (d, $^{3}J_{H-H}$ = 4.2 Hz, 4 $H_{9,11}$), 7.12 (m, 2 H_{10}), 5.38 (s, 2 H_6), 3.51 (s, 8 H (NH_2)), 2.14 (s, 12 H_{13}). ^{13}C NMR ($CDCl_3$, δ 76.9, 100 MHz) = 148.3 (C_1), 143.0 (C_4), 140.9 (C_7), 138.4 (C_{14}), 133.1 (C_8), 132.9 (C_{12}), 129.4 (C_{16}), 128.1 (C_{10}), 127.9 (C_9), 127.5 (C_{11}), 127.2 (C_{17}), 124.0 (C_{15}),

100.6 (C_5), 36.6 (C_6), 12.4 (C_{13}). ^{77}Se NMR ($CDCl_3$, δ 0.00) = 492. ESI-MS (positive mode, CH_3OH - *[Compound 3 / 2] calculated 513.1306, obtained 513.1268).*

Synthetic procedures for compound 4 (BDPP-DSe). The compound **3** (0.080 g, 0.078 mmol) was placed into an unsealed pyrex glass and irradiated in a domestic microwave oven for 15 min at 500 W. The completion of the reaction was confirmed by TLC. After completion of the reaction, the crude material was purified by column chromatography (ethyl acetate/hexane) to afford the desired compound **4 BDPP-DSe**. Yield = 0.032 g, 41.5 %, m.p.: 281-283 ^{o}C, ^{1}H NMR ($CDCl_3$, δ 7.24, 400 MHz): 8.42 (dd, $^{3}J_{H-H}$ = 8.7, $^{4}J_{H-H}$ = 1.0, 8 H_{15}), 7.67 (m, 2 H_{12}), 7.53 (td, $^{3}J_{H-H}$ = 7.4 Hz, $^{4}J_{H-H}$ = 1.2 Hz, 8 H_{16}), 7.39 (m, 4 $H_{10,11}$), 7.29 (m, 6 $H_{17,9}$), 1.99 (s, 6 H_{13}). ^{13}C NMR (100 MHz, δ 76.9, $CDCl_3$) δ 150.5 (C_1), 144.0 (C_4), 139.5 (C_{14}), 138.4 (C_6), 134.2 (C_7), 130.5 (C_{12}), 130.3 (C_{10}), 129.7 (C_8), 129.1 (C_9), 128.9 (C_{16}), 127.2 (C_{11}), 125.2 (C_{17}), 120.2 (C_{15}), 113.2 (C_5), 14.2 (C_{13}). ^{77}Se NMR ($CDCl_3$, δ 0.00) = 405. ESI-MS (positive mode, CH_3OH - *[BDPP-DSe + Na^+] calculated 1011.1666, obtained 1011.1600. [BDPP-DSe + H^+] calculated 989.1841, obtained 989.1769).*

Cell culture and confocal imaging. MCF-7 cells were cultured in RPMI-1640 medium (WELGENE) supplemented with 10% fetal bovine serum (GIBCO), 1% penicillin/streptomycin (100 U/mL, GIBCO). The MCF-7 cells seeded at a density of 1×10^5 cells/well on sterilized 18 mm cover-slips and maintained at 37 °C in a 5% CO_2 humidified incubator. Fluorescent images were acquired on Zeiss LSM 780 laser scanning confocal microscope and a 20× objective lens was used. The excitation wavelength was 355 nm and the detection wavelength was 410–571 nm. Prior to imaging, the culture medium was removed and cells were washed with D-PBS three times.

Determination of the detection limit:

The detection limit was calculated based on the fluorescence titration. The standard deviation of blank measurement (10 samples) was achieved. To gain the slops, the fluorescence intensity at 436 nm were plotted as a concentration of NaOCl. So the detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity versus NaOCl concentration.

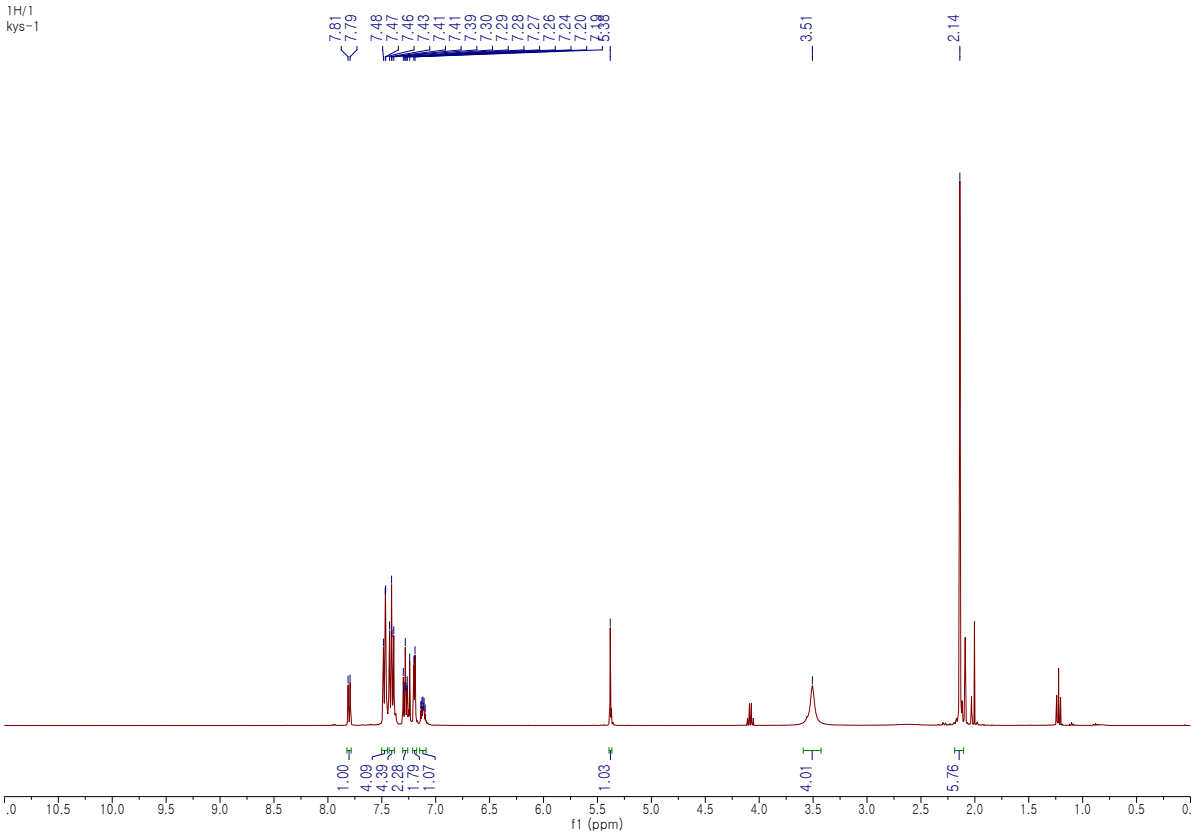


Figure S1A. ^{1}H NMR spectrum of Compound **3**.

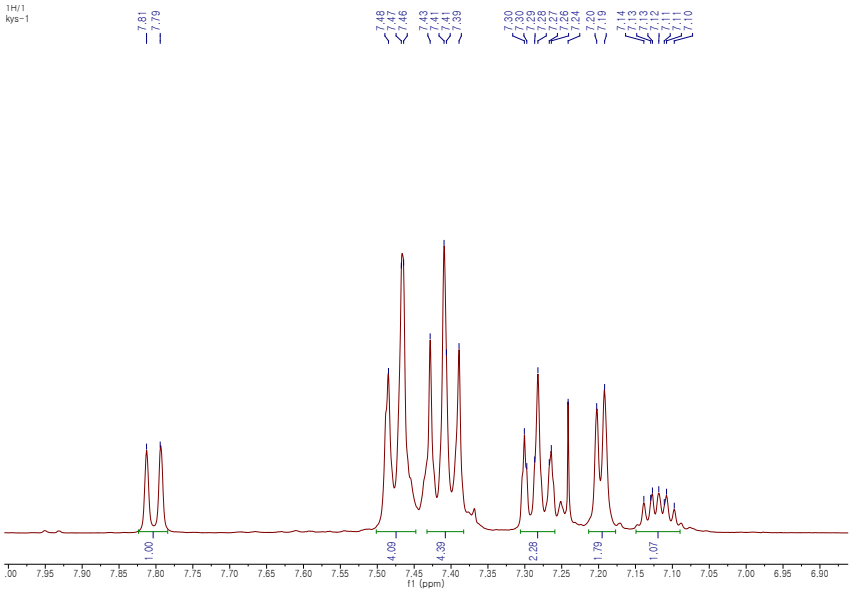


Figure S1B. ^{1}H NMR spectrum of Compound **3** (Expanded aromatic region).

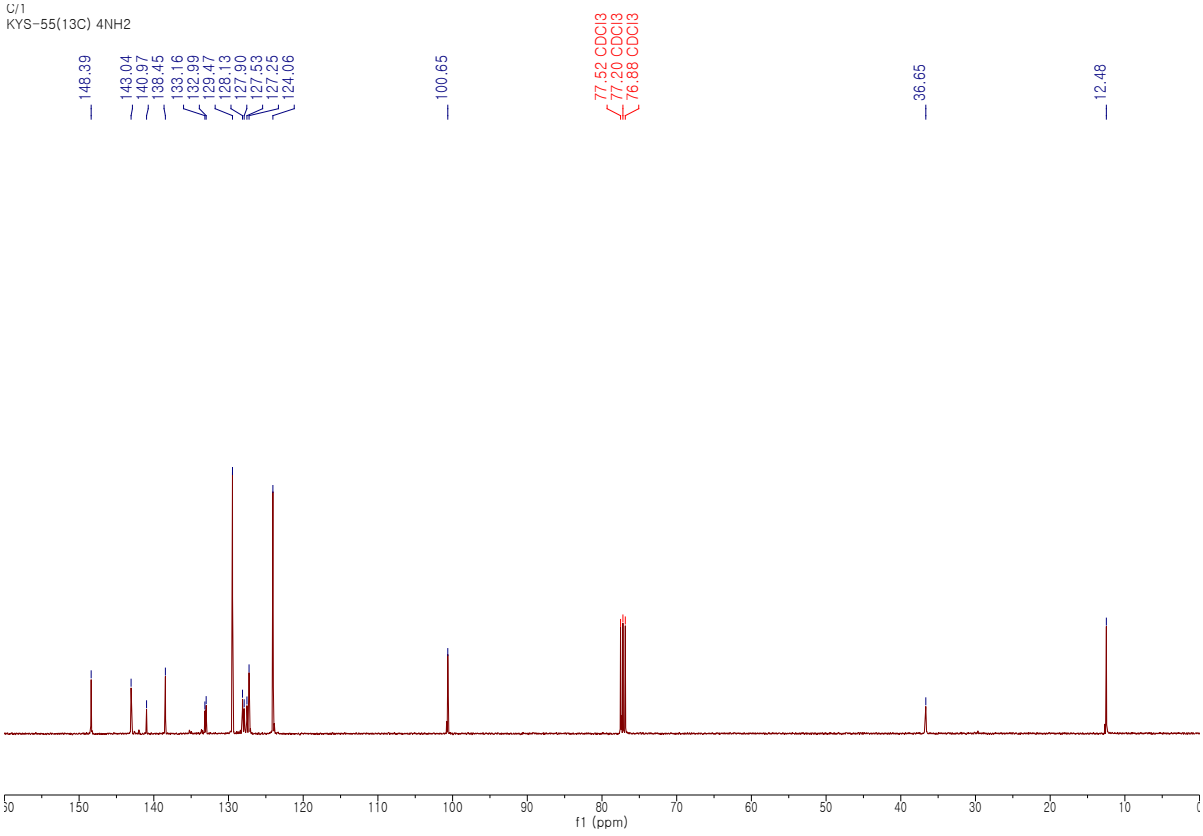


Figure S2A. ^{13}C NMR spectrum of Compound **3**.

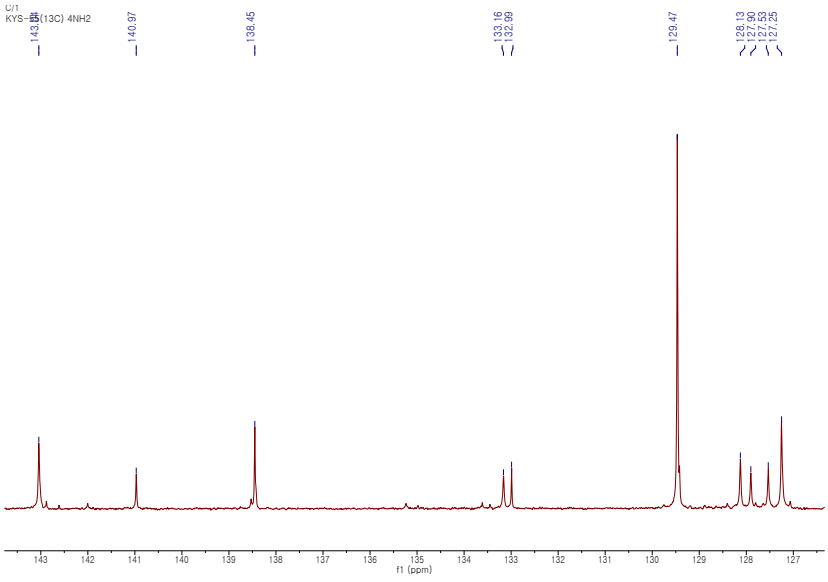


Figure S2B. ^{13}C NMR spectrum of Compound **3** (Expanded aromatic region:126–144 ppm).

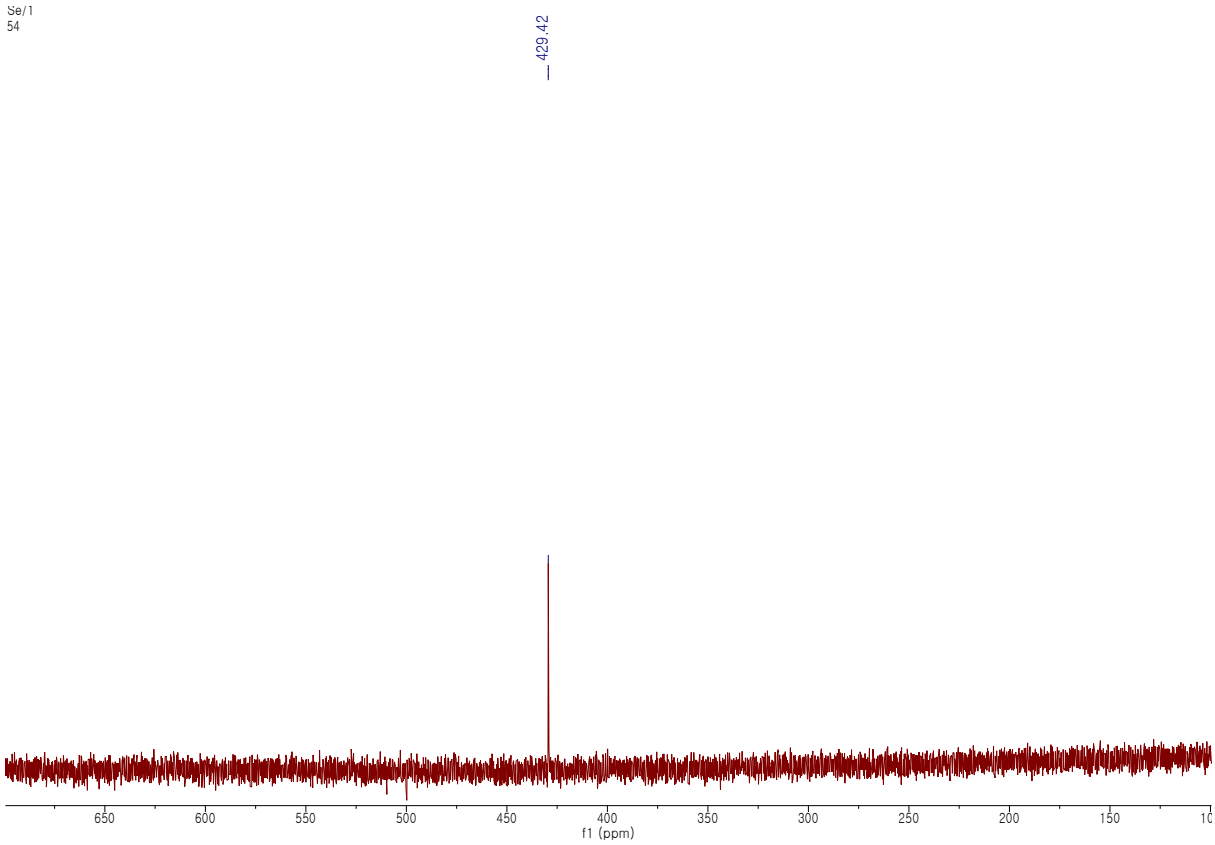


Figure S3. ^{77}Se NMR spectrum of Compound **3**.

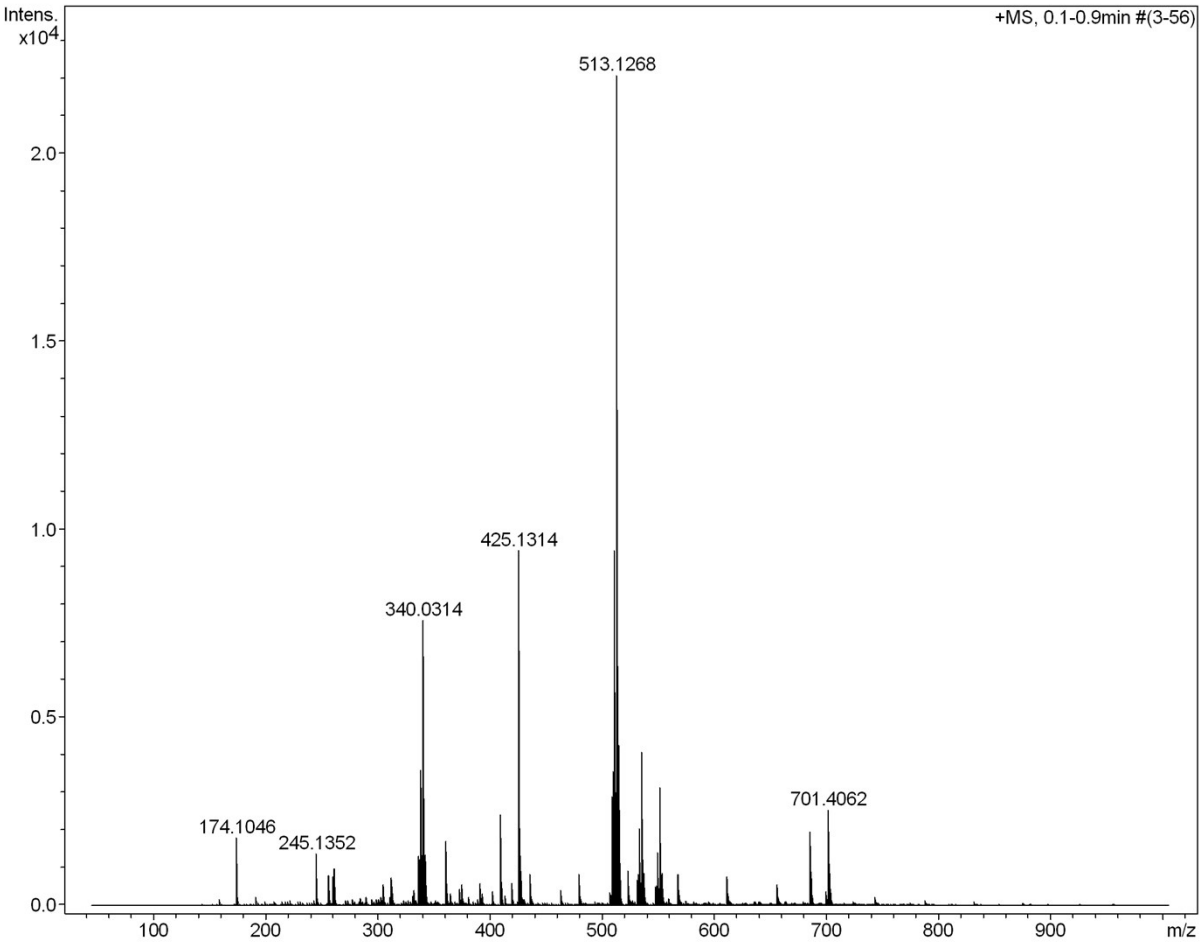


Figure S4. ESI–mass spectrum of Compound **3**.

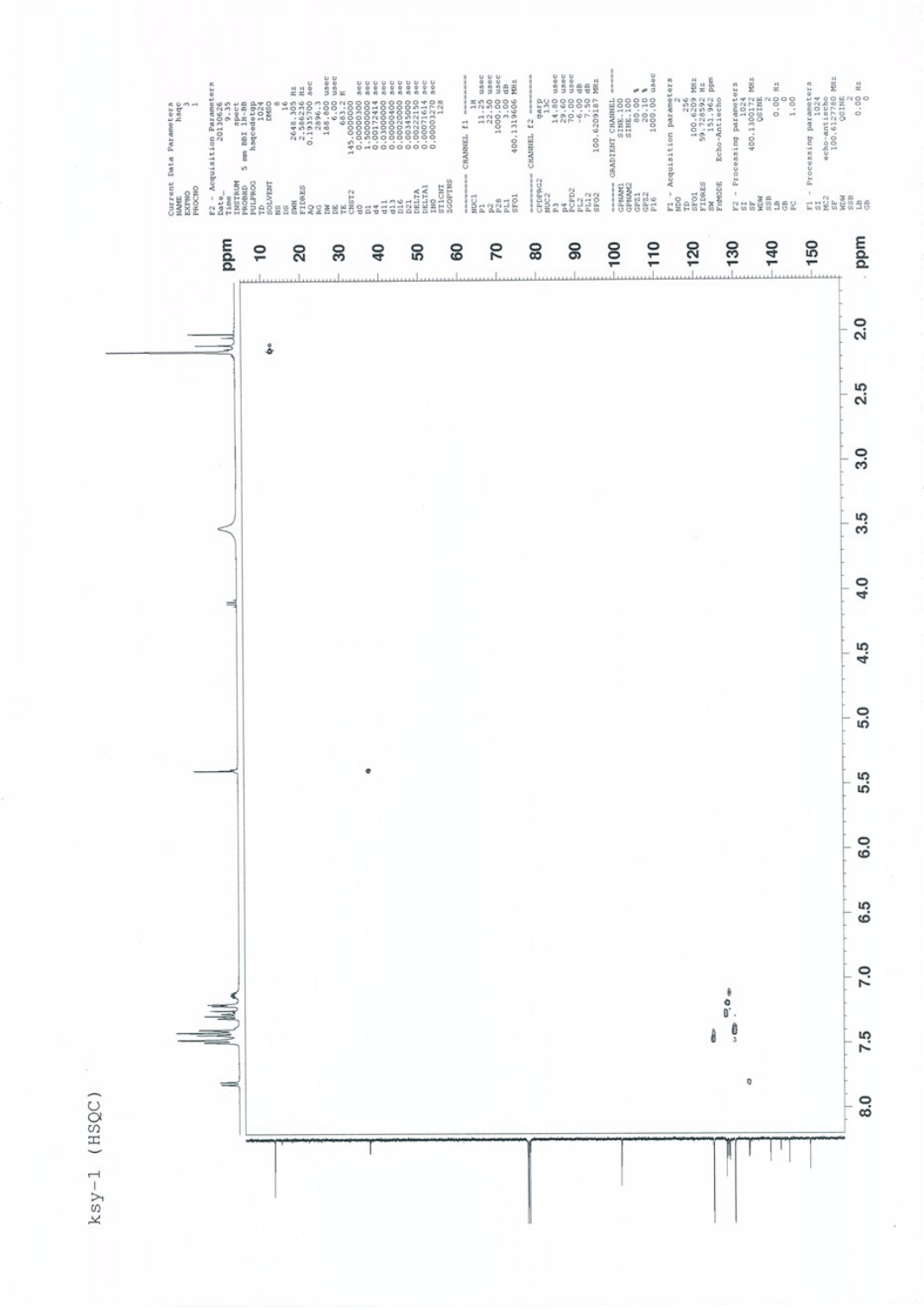


Figure S5A. $^{1}H–^{13}C$ HSQC NMR spectrum of Compound **3**.

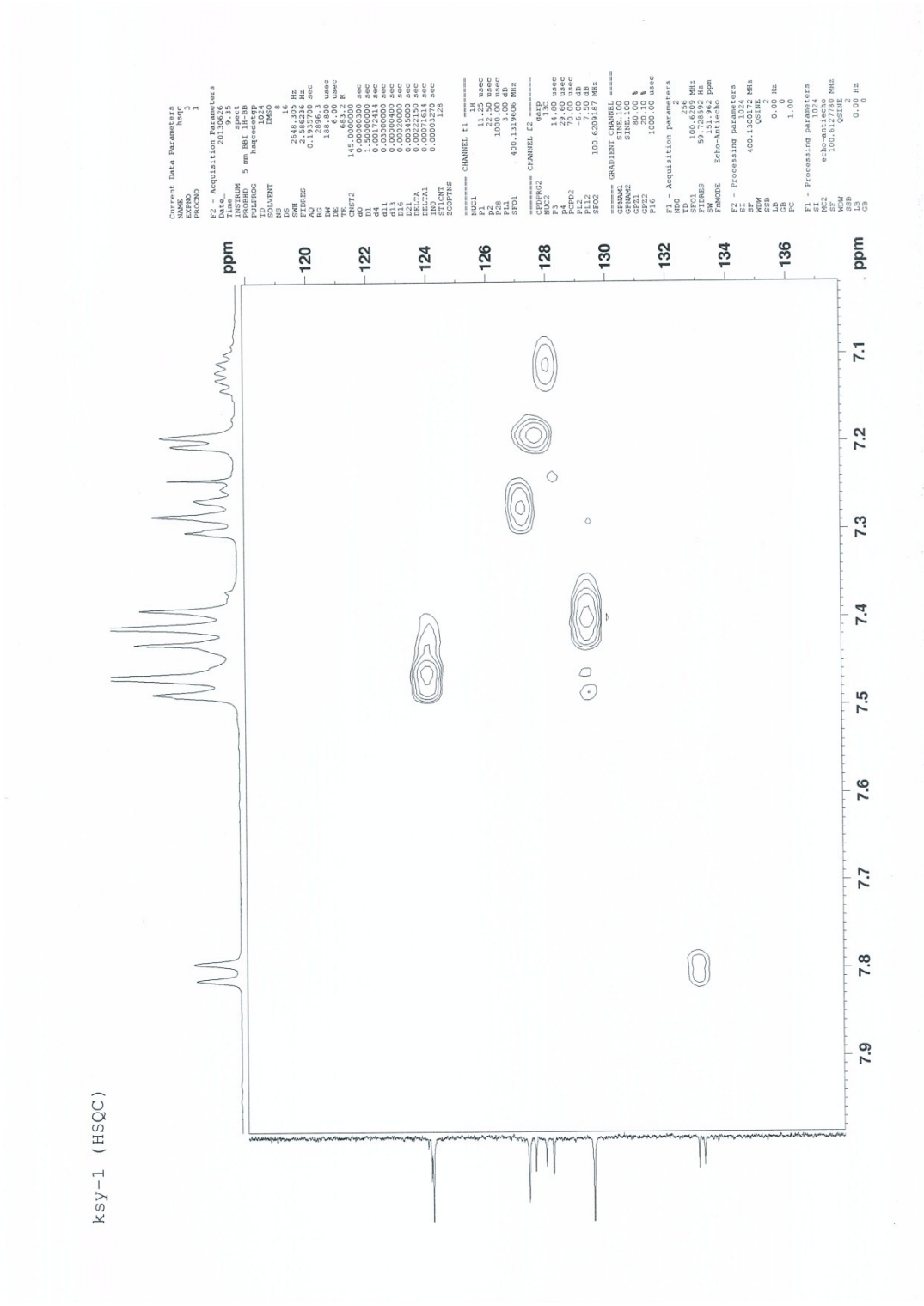


Figure S5B. ^{1}H–^{13}C HSQC NMR spectrum of Compound **3** (Expanded aromatic region).

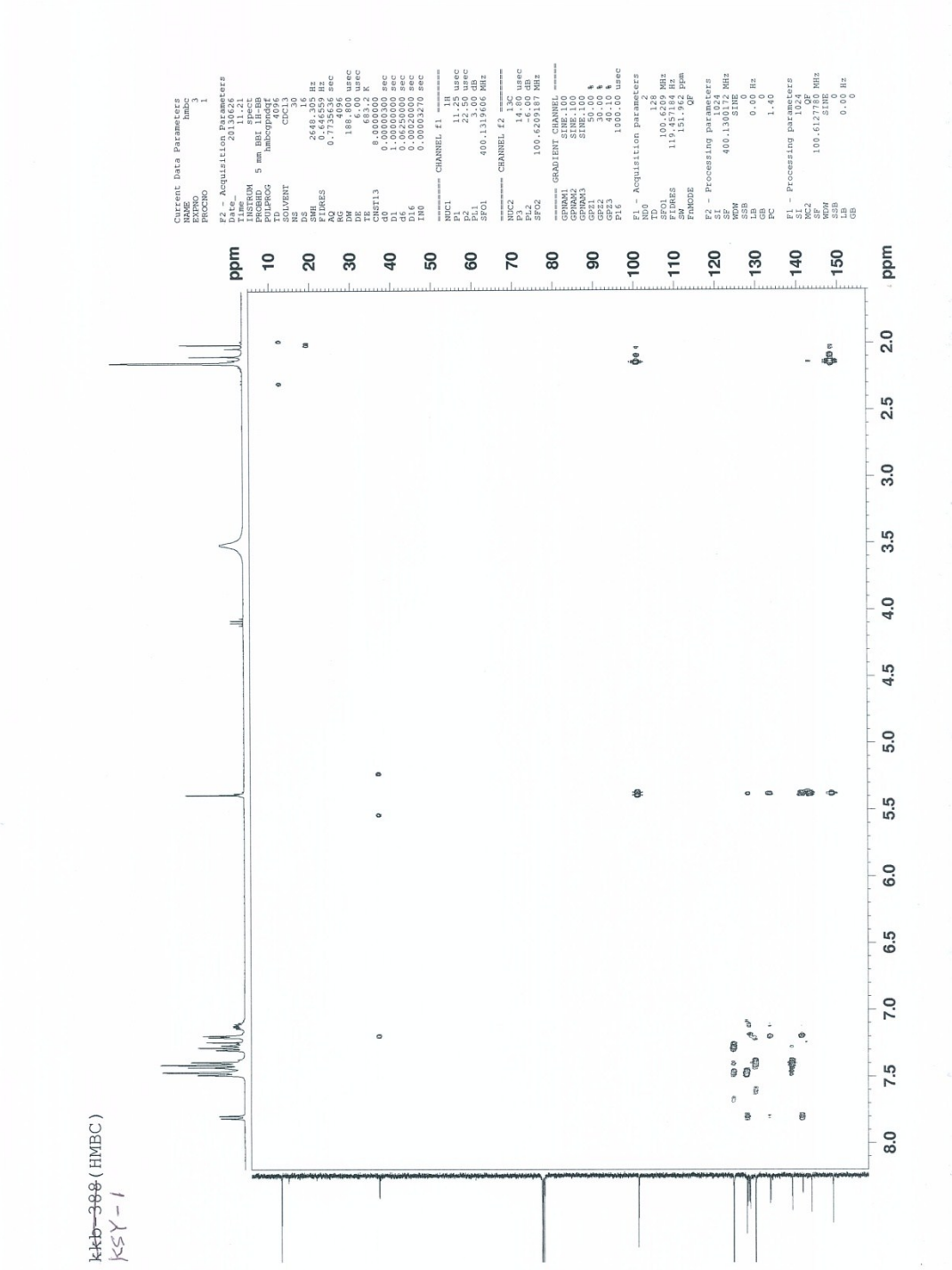


Figure S6A. ^{1}H–^{13}C HMBC NMR spectrum of Compound **3**.

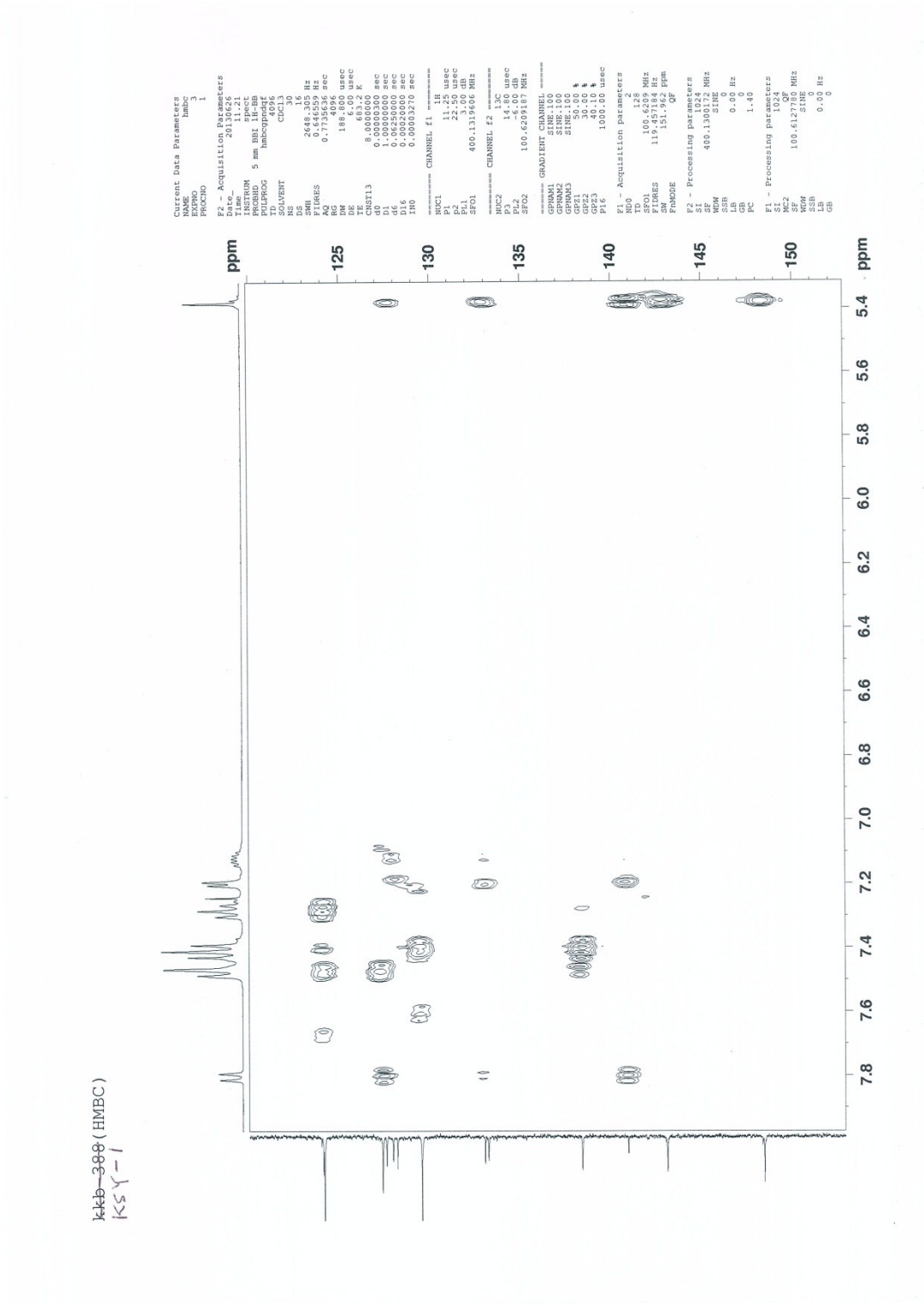


Figure S6B. ^{1}H–^{13}C HMBC spectrum of Compound **3** (Expanded aromatic region).

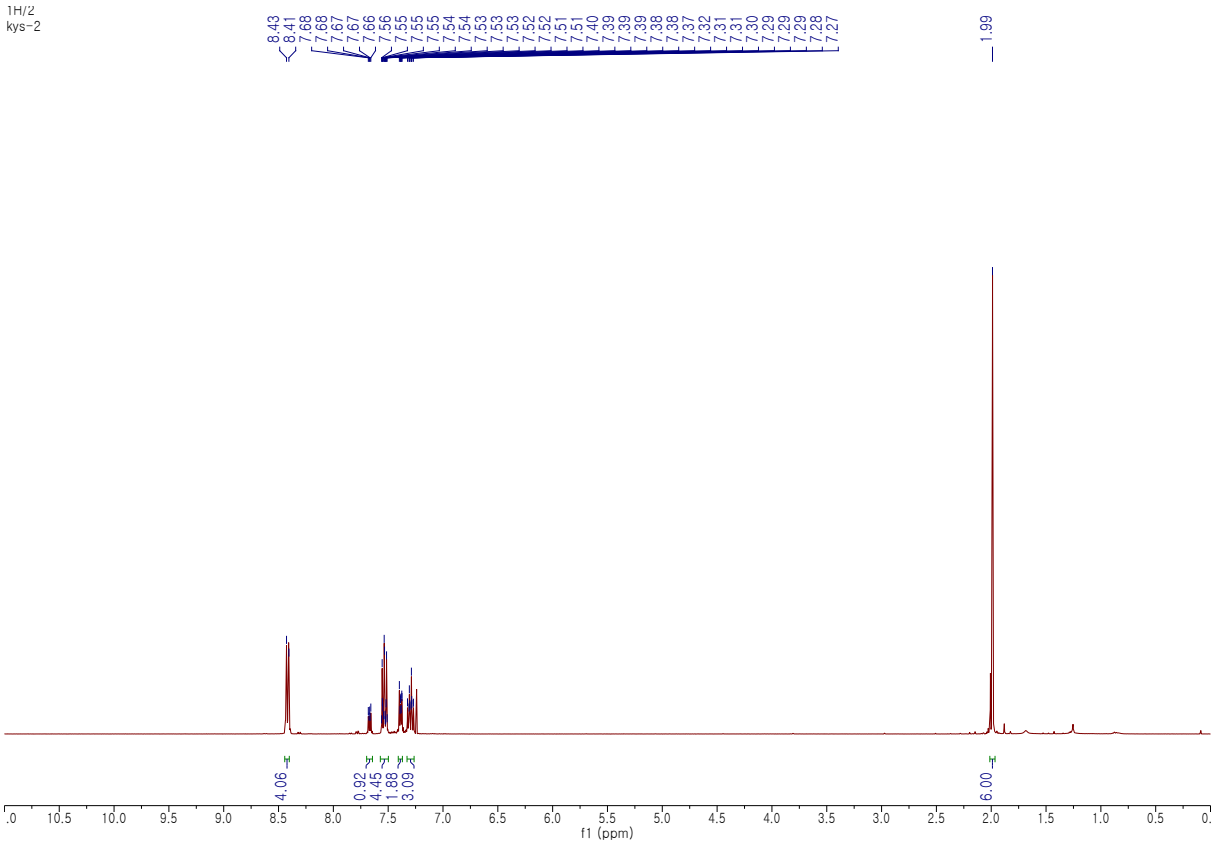


Figure S7A. ^{1}H NMR spectrum of Compound **4** [**BDPP-DSe**].

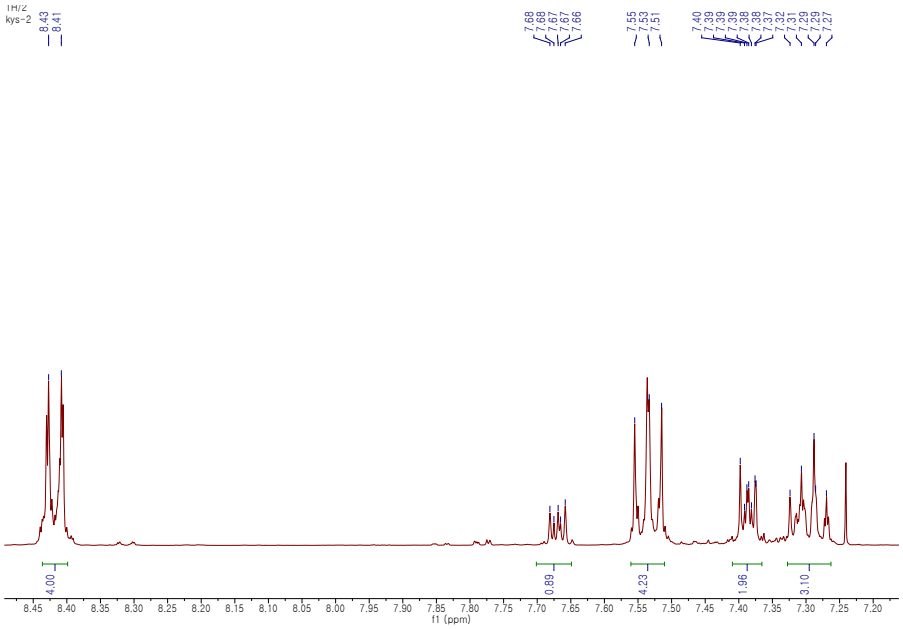


Figure S7B. ^{1}H NMR spectrum of Compound **4** [**BDPP-DSe**] (Expanded aromatic region).

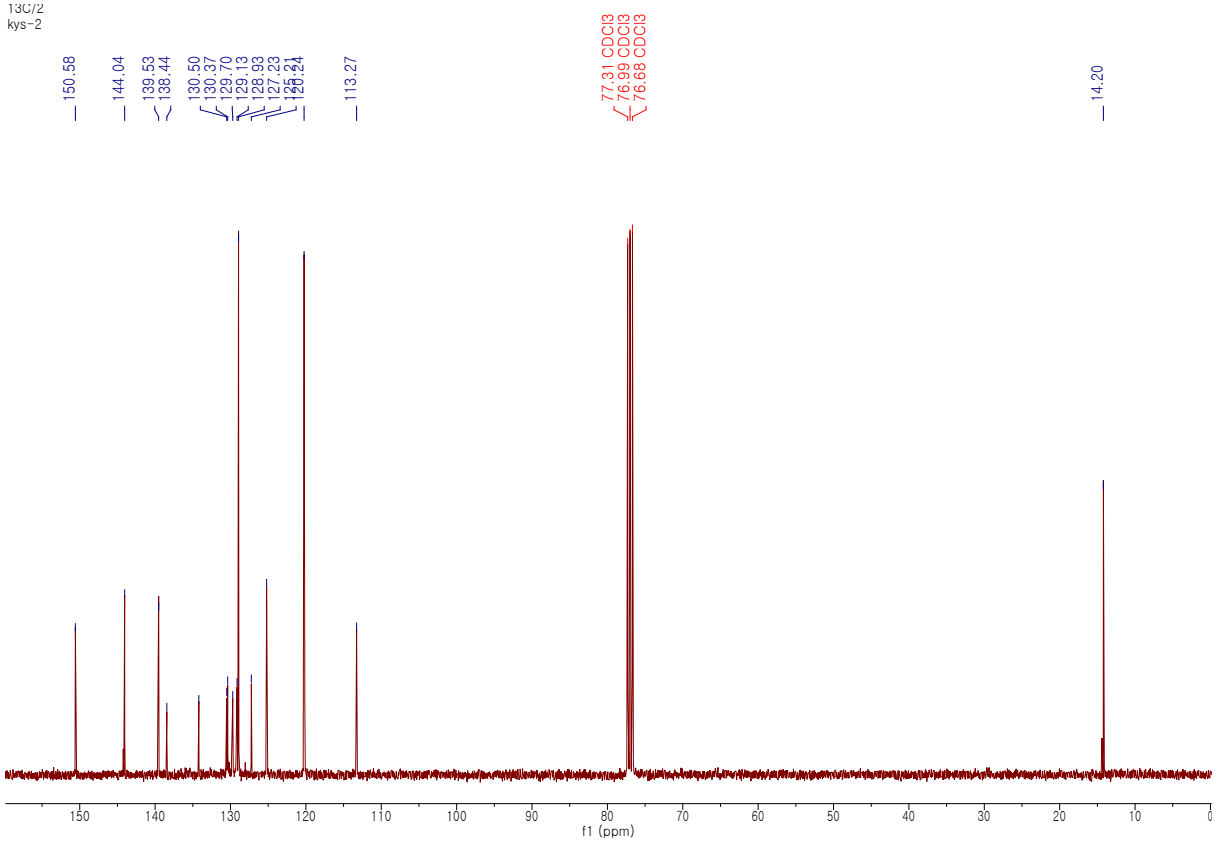


Figure S8. ^{13}C NMR spectrum of Compound **4** [**BDPP-DSe**].

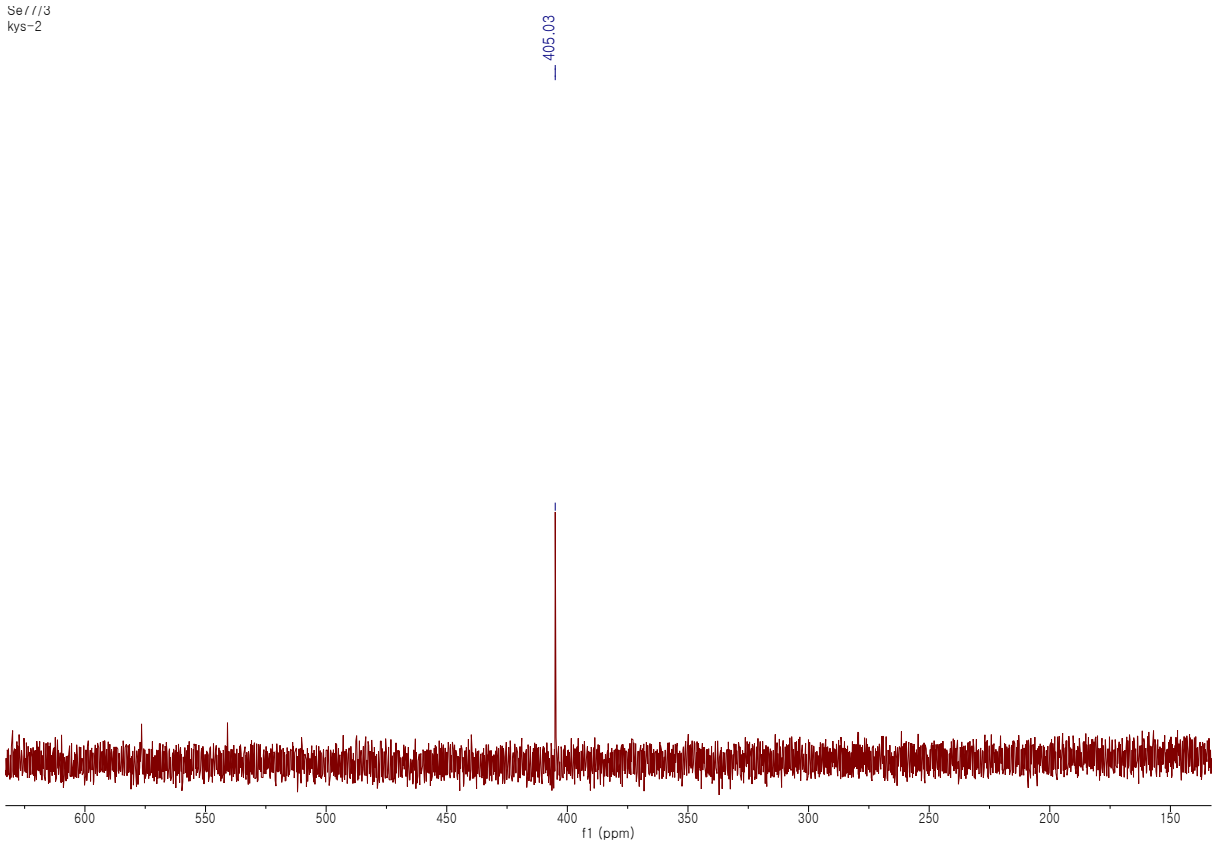


Figure S9. ^{77}Se NMR spectrum of Compound **4** [**BDPP-DSe**].

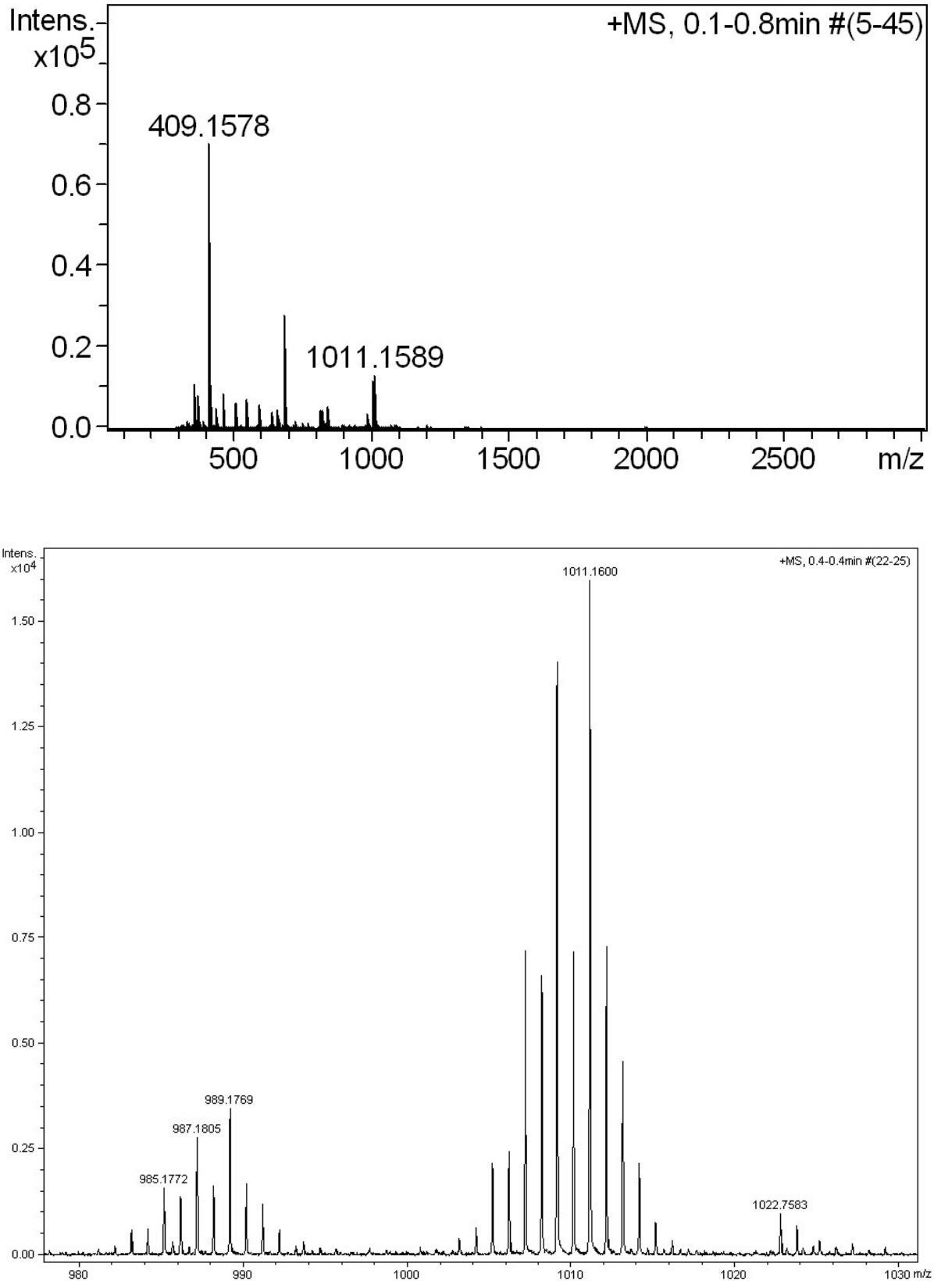


Figure S10. ESI–mass spectrum of Compound **4** [**BDPP-DSe**].

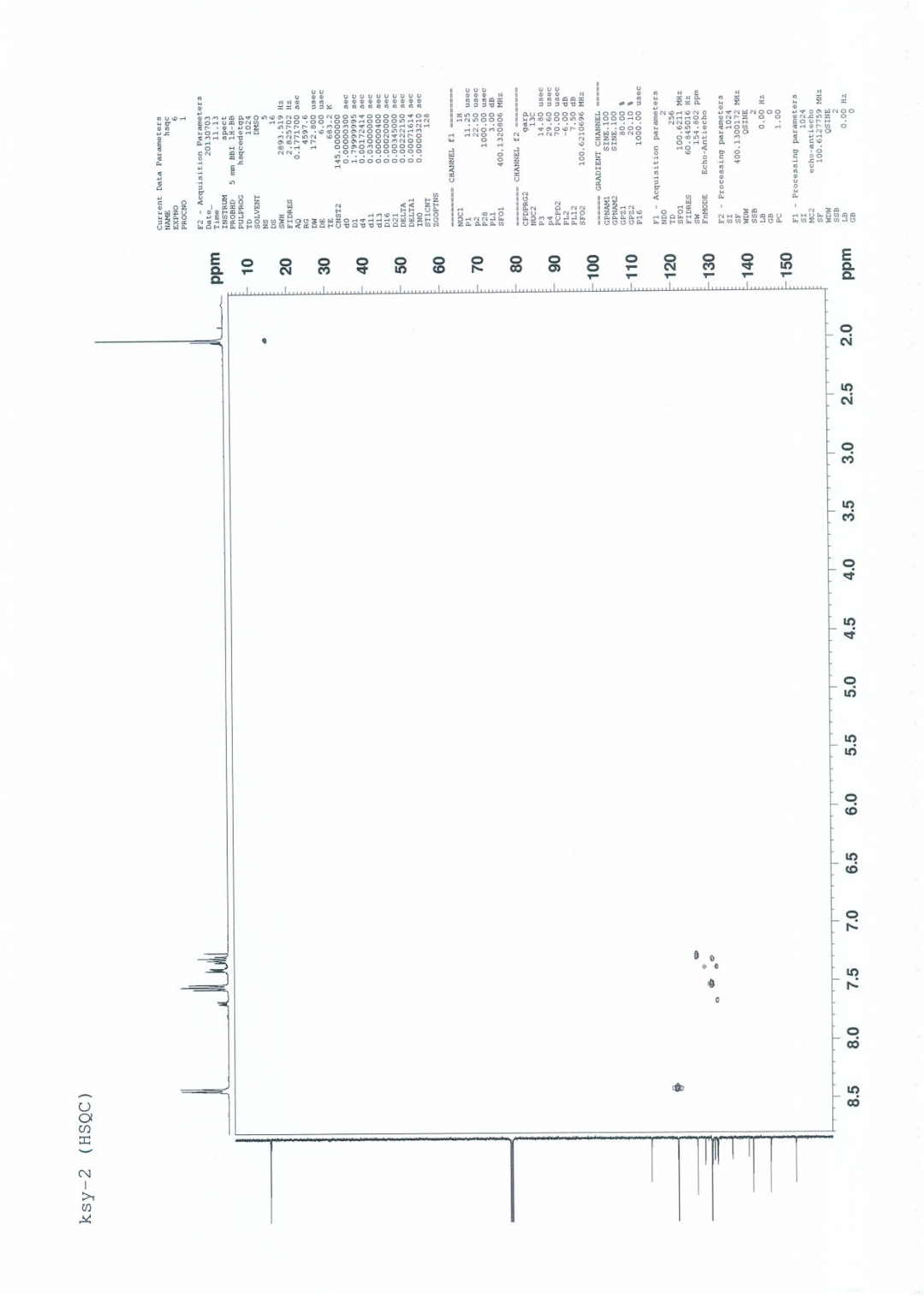


Figure S11A. ^{1}H–^{13}C HSQC NMR spectrum of Compound **4** [**BDPP-DSe**].

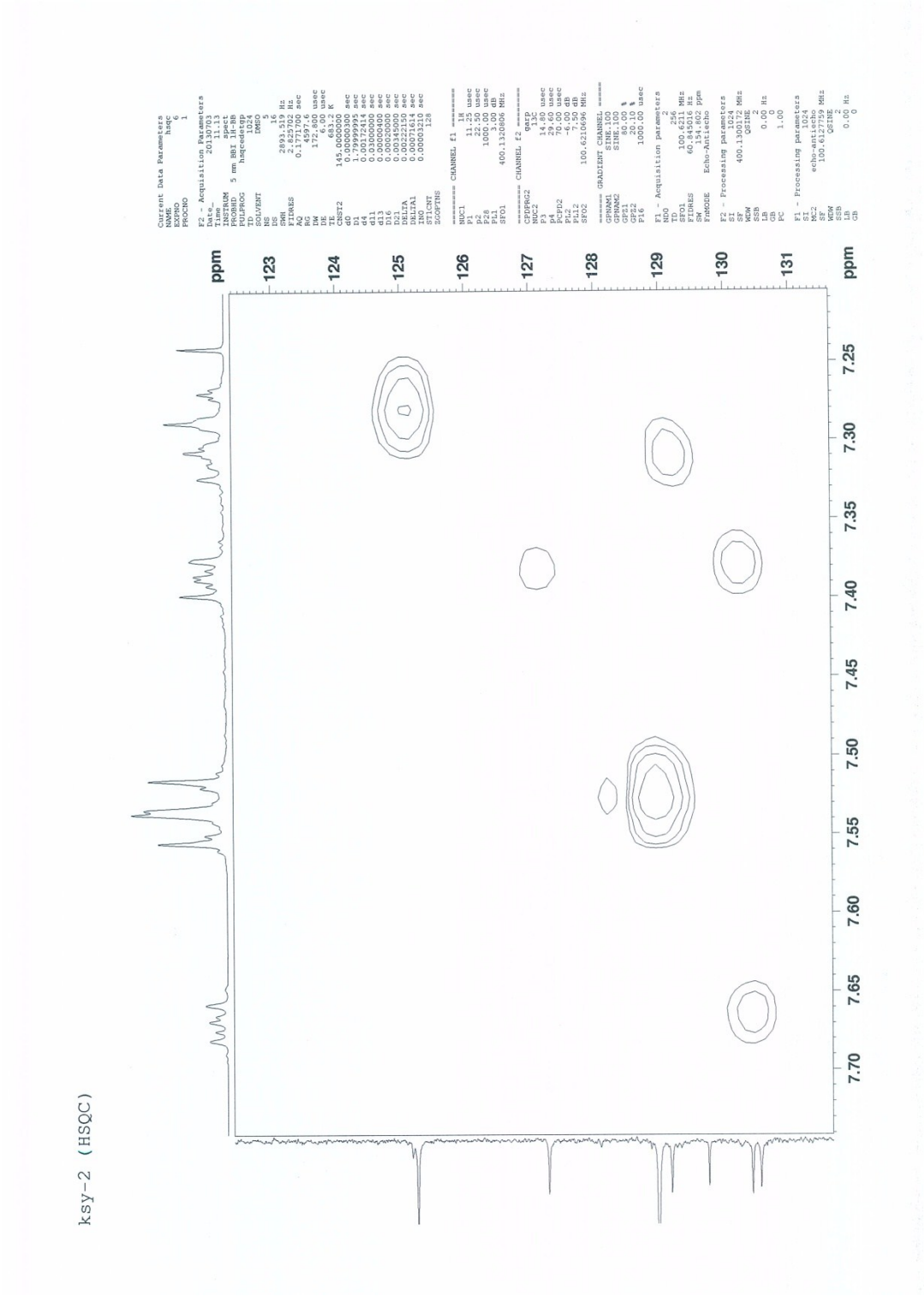


Figure S11B. $^{1}H–^{13}C$ HSQC NMR spectrum of Compound **4** [**BDPP-DSe**] (Expanded aromatic region).

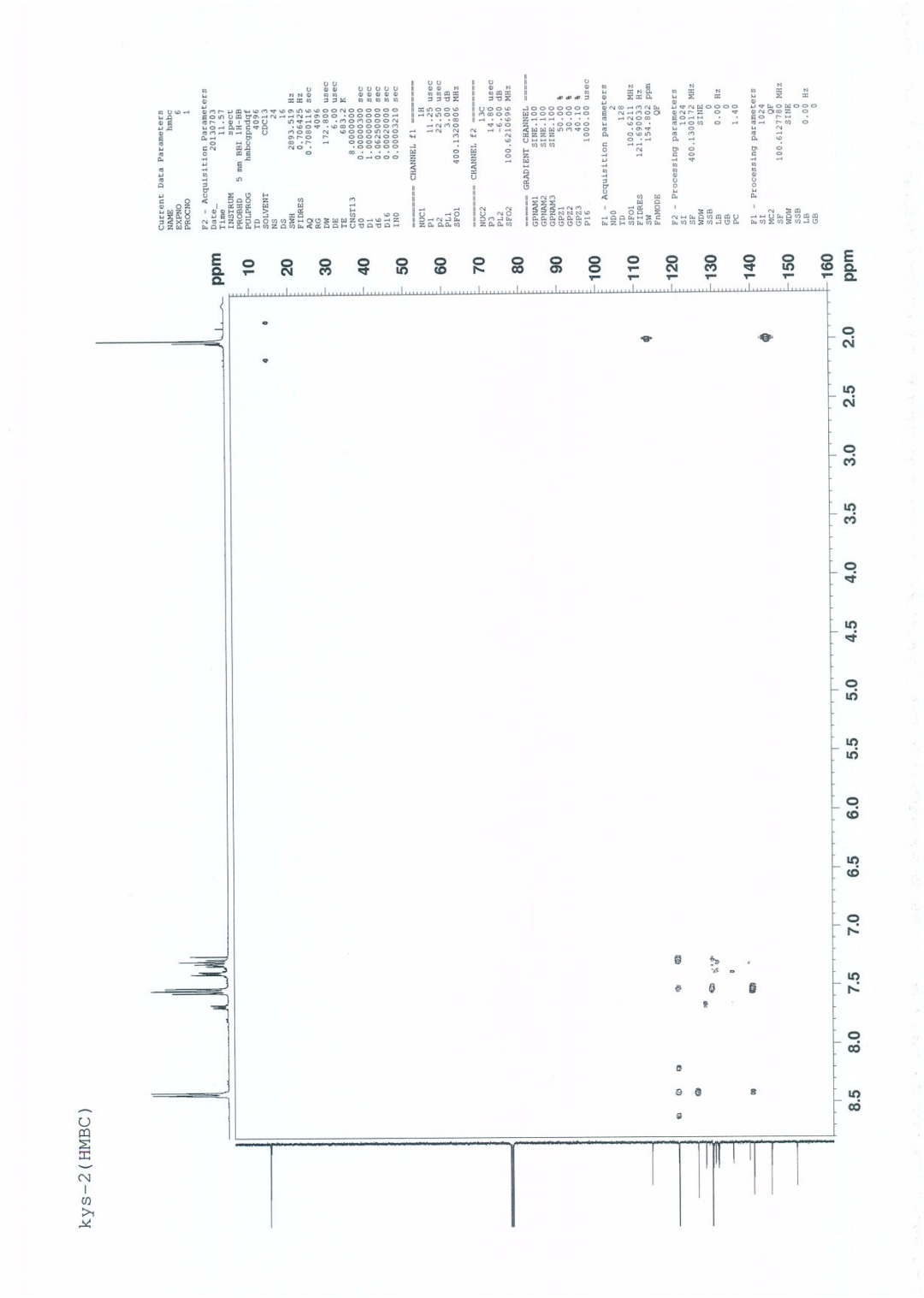


Figure S12A. $^{1}H–^{13}C$ HMBC NMR spectrum of Compound **4** [**BDPP-DSe**].

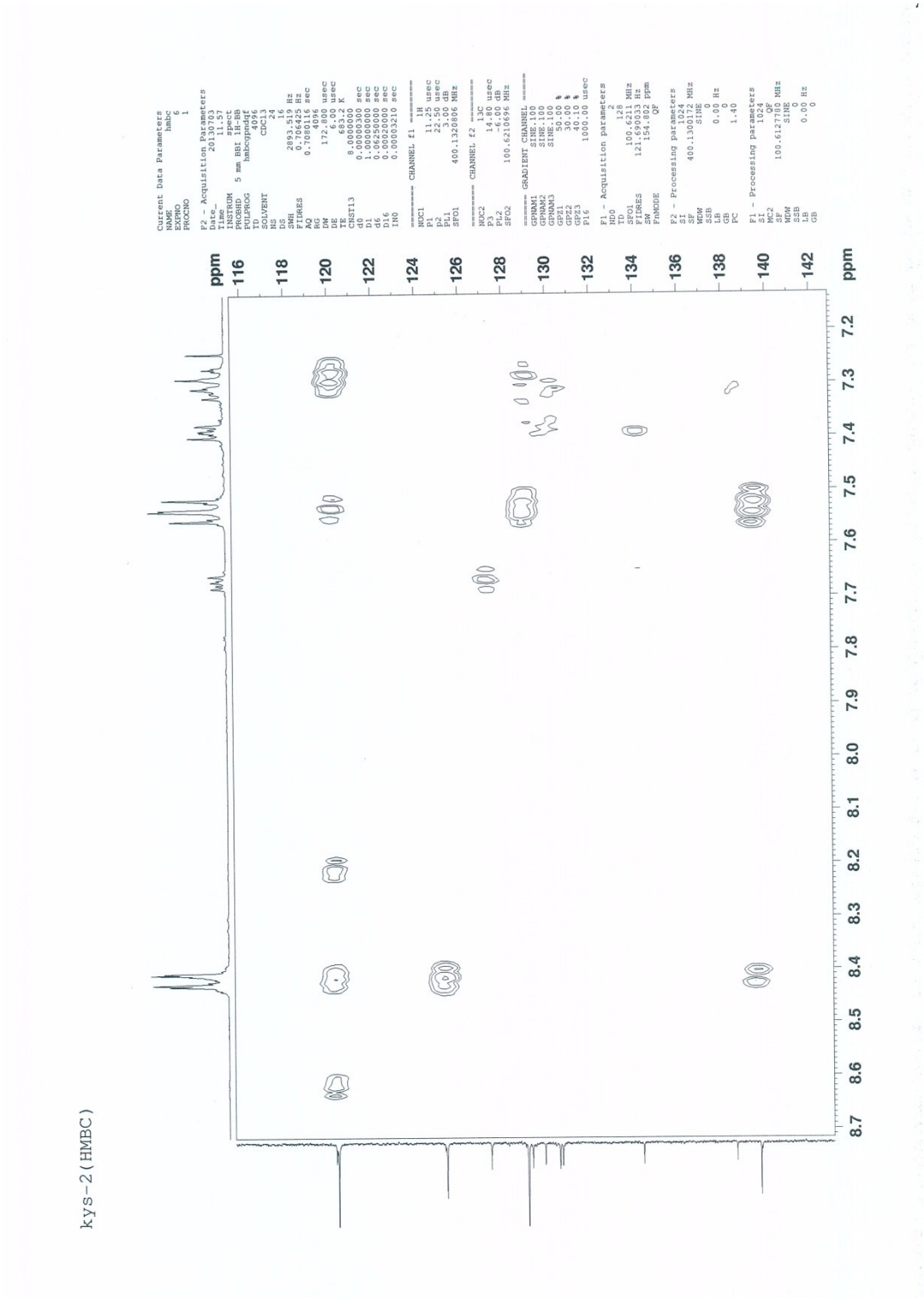


Figure S12B. ^{1}H–^{13}C HMBC NMR spectrum of Compound **4** [**BDPP-DSe**] (Expanded aromatic region).

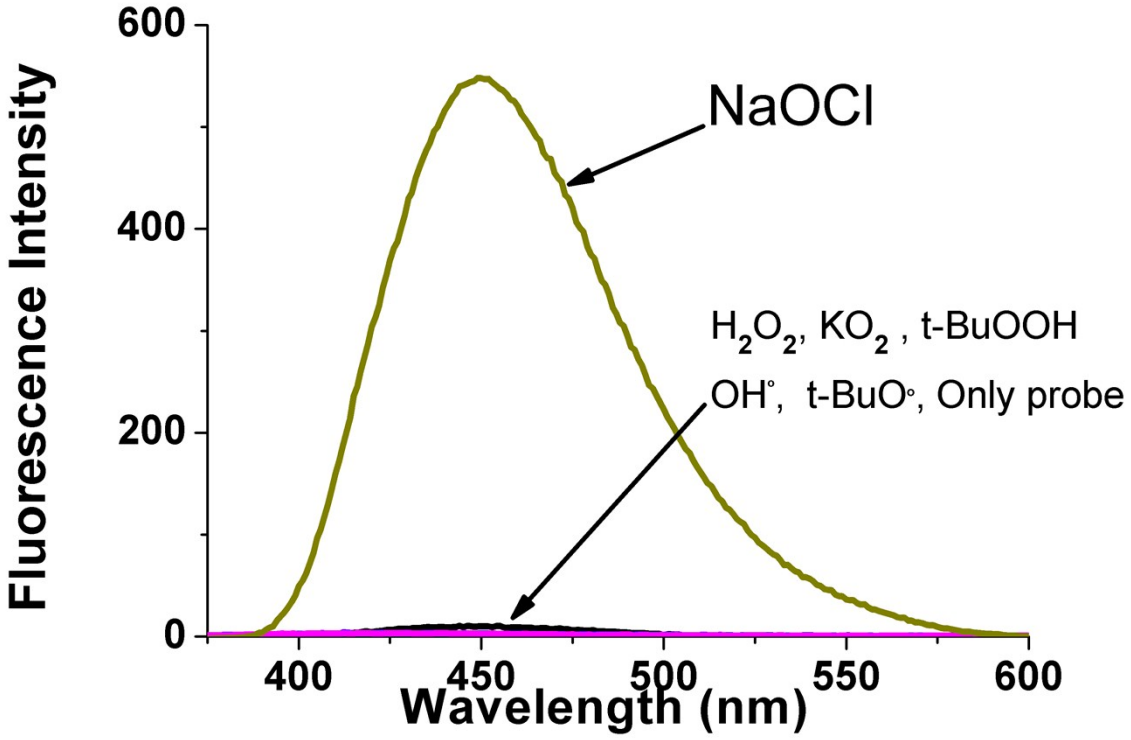


Figure S13. Emission spectra of BDPP–DSe (1.0×10^{-5} M, acetonitrile: DPBS, v/v, 8:2) with various other ROS (1.0×10^{-4} M; NaOCl, H_2O_2, KO_2, ^{t}BuOOH, OH•, ^{t}BuO•) incubated for 10 min. Excitation wavelength: 311 nm. Slit width: 3 nm / 3 nm.

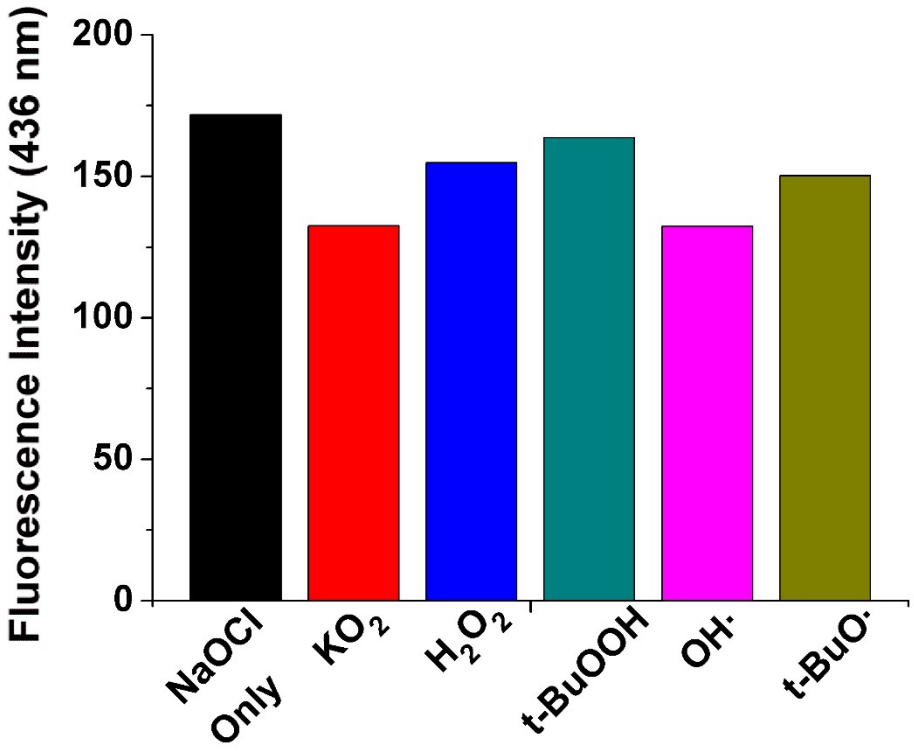


Figure S14. Relative emission fluorescence intensity of **BDPP-DSe** (1.0×10^{-5} M) with NaOCl (1.0×10^{-4} M) and **BDPP-DSe** (1.0×10^{-5} M) with other ROS (1.0×10^{-4} M) incubated for 10 min at rt and after being treated with NaOCl (1.0×10^{-4} M). Excitation wavelength: 311 nm. Emission wavelength: 436 nm. Slit width: 1.5 nm/ 1.5 nm.

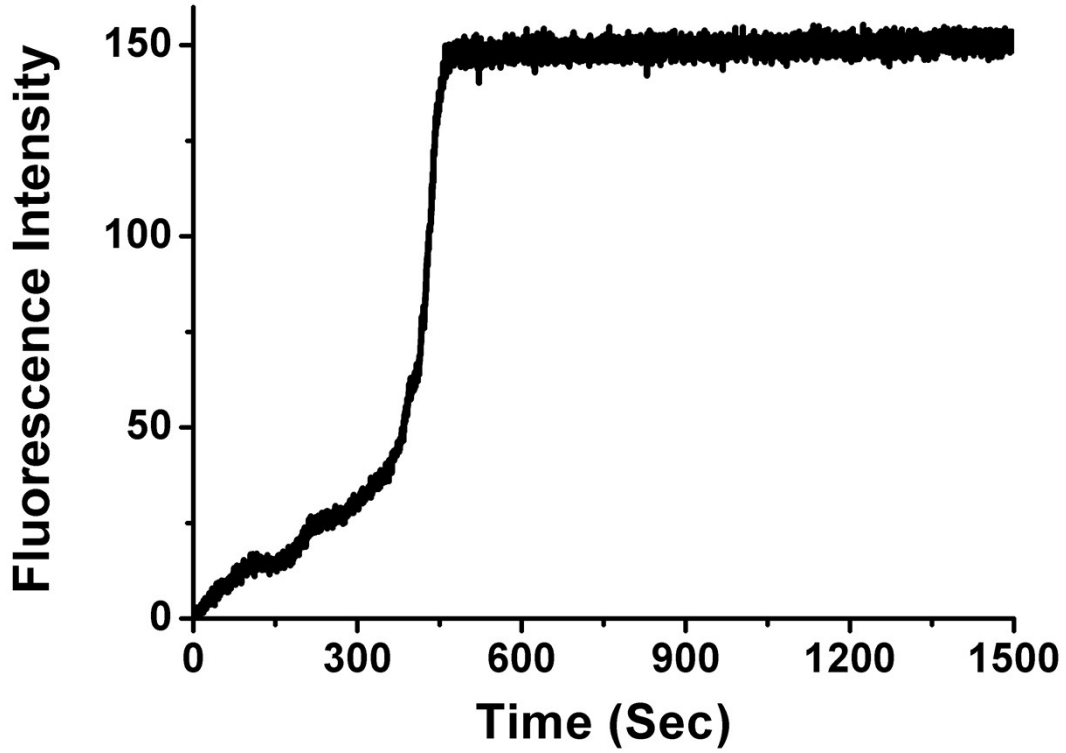


Figure S15. Time-dependent emission spectra changes of **BDPP-DSe** (1.0×10^{-5} M) with NaOCl (1.0×10^{-4} M). Excitation wavelength: 311 nm. Emission wavelength: 436 nm. Slit width: 1.5 nm/ 1.5 nm.

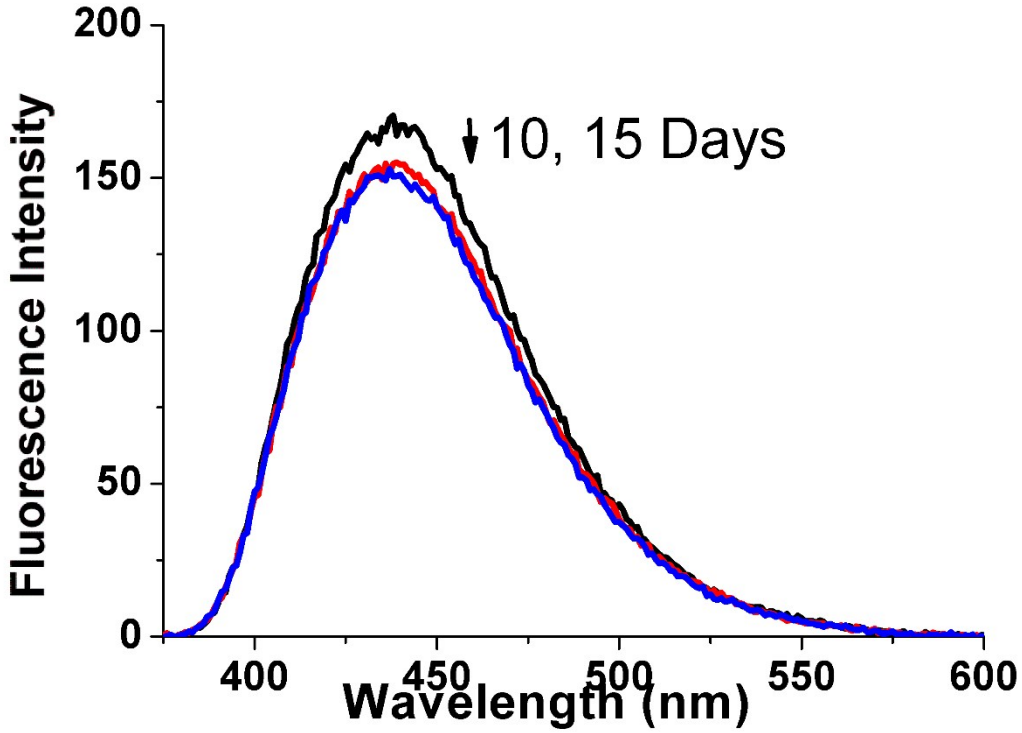


Figure S16. Emission spectrum of **BDPP-DSe** (1.0×10^{-5} M) with NaOCl (9.0×10^{-5} M) and changes after 10 (Red) and 15 days (Blue). Excitation wavelength: 311 nm. Emission wavelength: 436 nm. Slit width: 1.5 nm/ 1.5 nm.

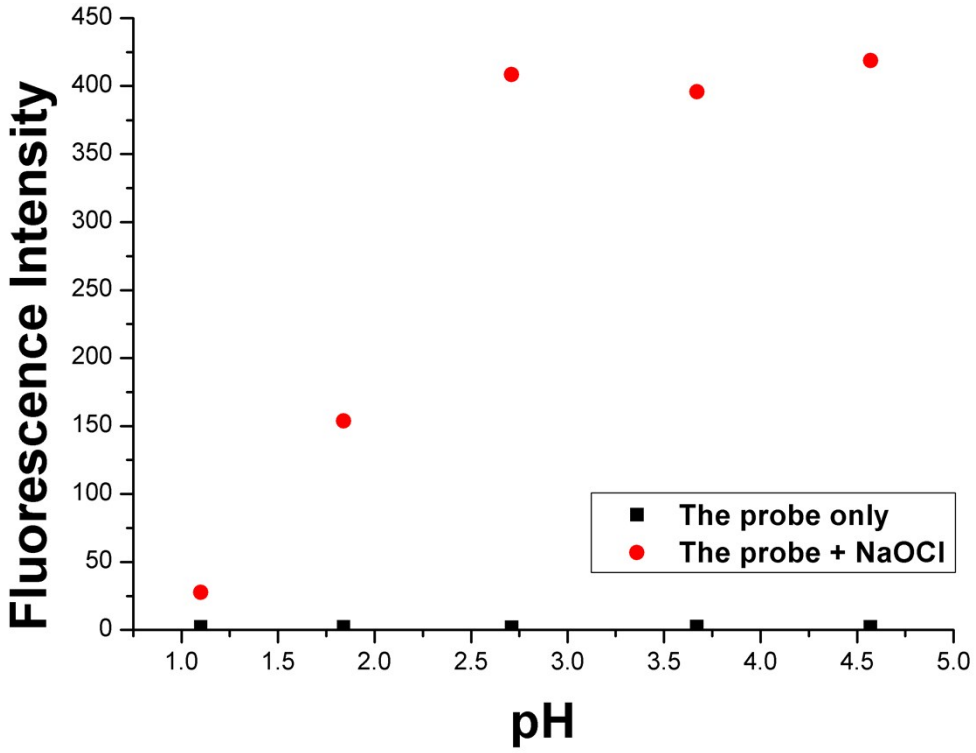


Figure S17. Emission spectra of **BDPP–DSe** (1.0×10^{-5} M, acetonitrile: water, v/v, 8:2) in acidic solution (pH 1.1 – 4.6) after incubation for 10 min. Excitation wavelength: 311 nm. Slit width: 3 nm / 3 nm.

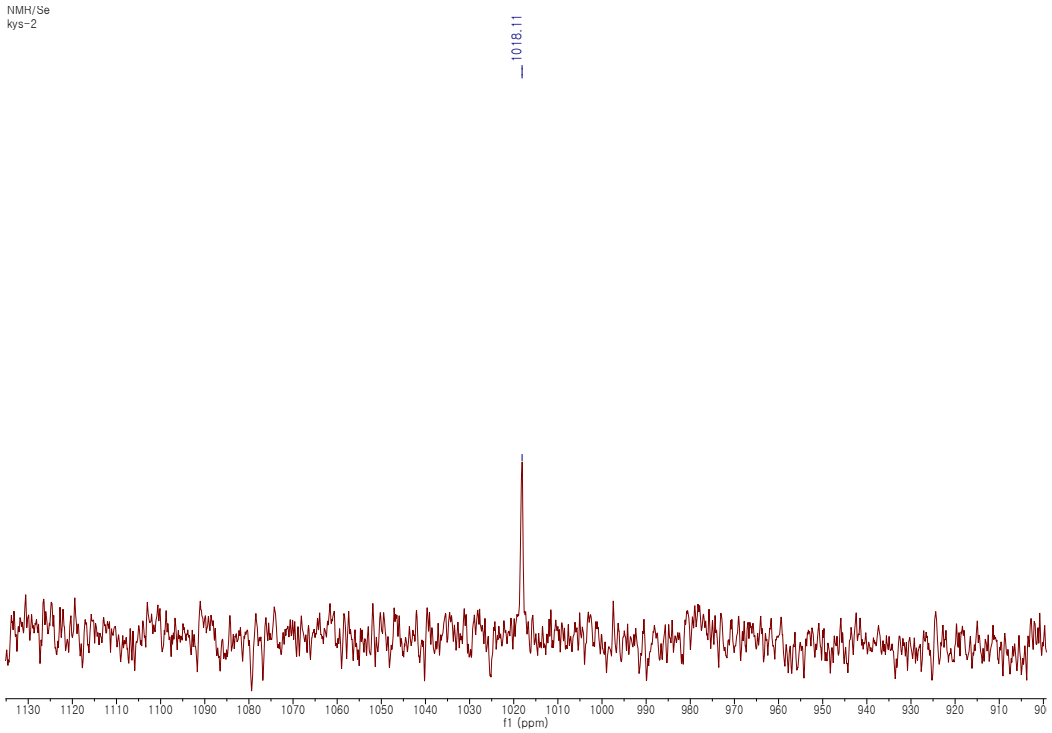


Figure S18. ^{77}Se NMR spectrum of [**BDPP-DSeO**].

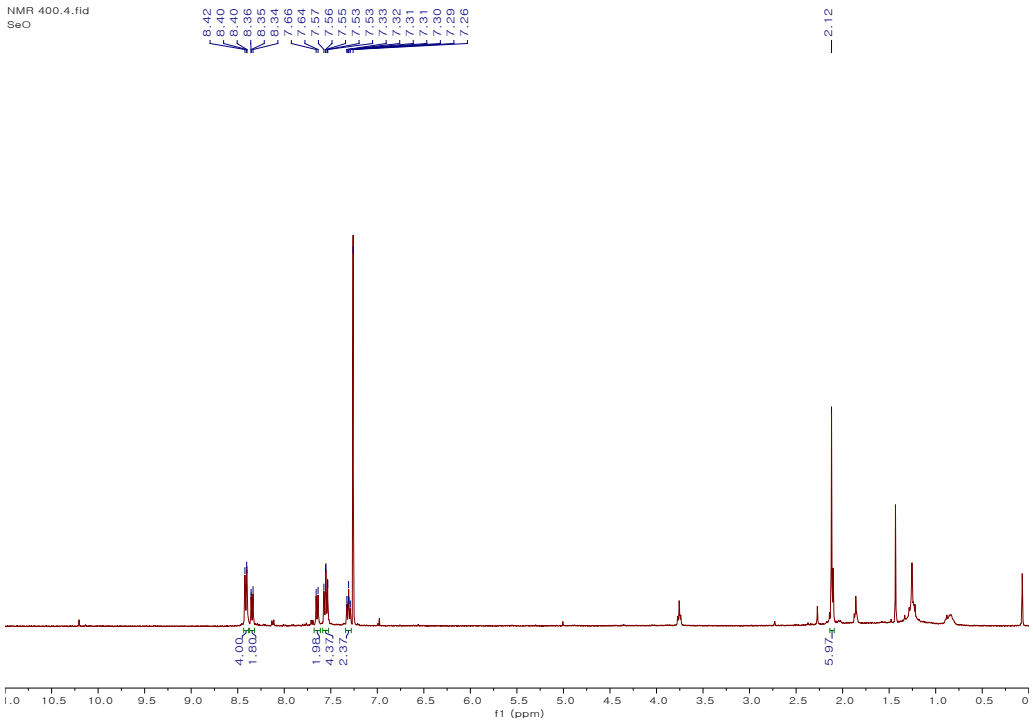


Figure S19. ^{1}H NMR spectrum of [**BDPP-DSeO**].

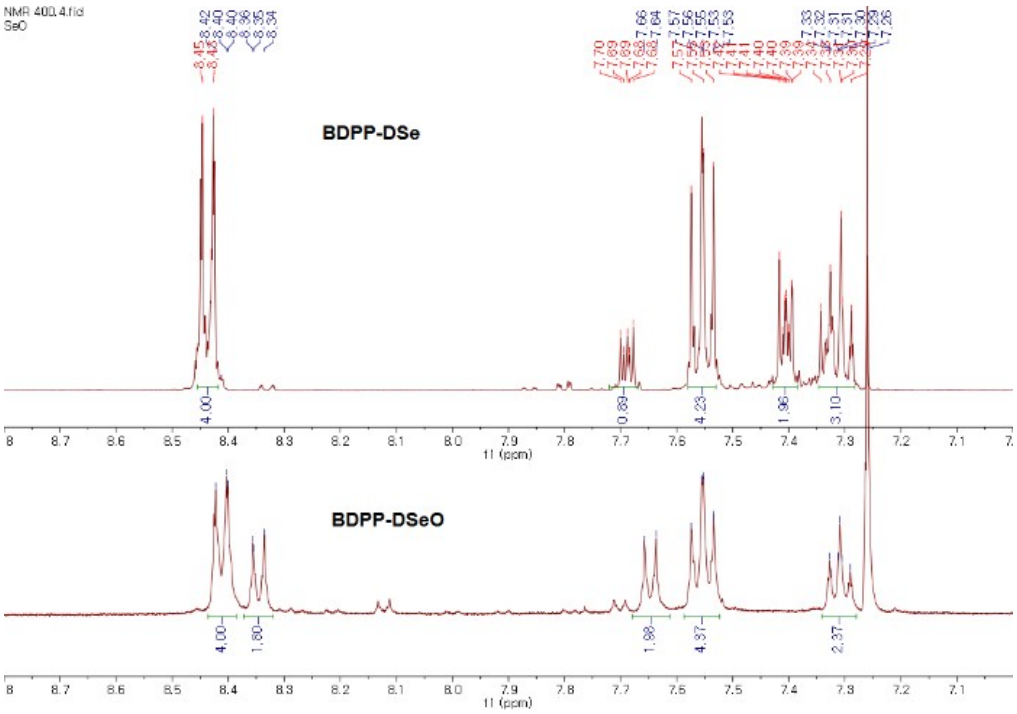


Figure S20. ^{1}H NMR spectrum of [**BDPP-DSe**] and [**BDPP-DSeO**] (expanded aromatic region).

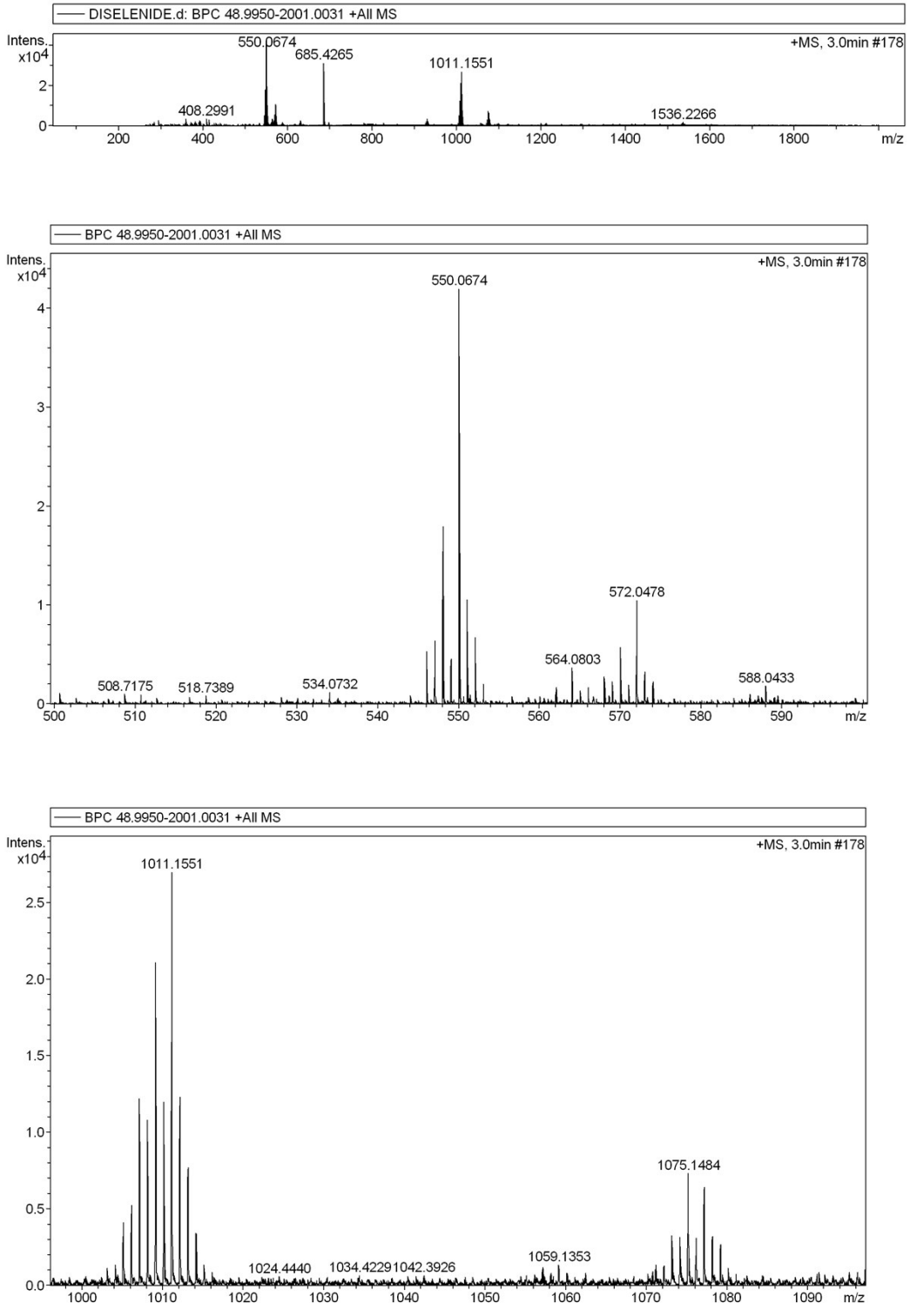
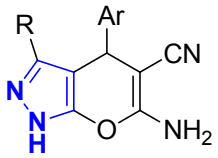
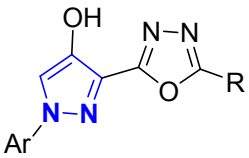


Figure S21. ESI – mass spectrum of [**BDPP-DSeO**] (positive mode, CH_3OH - *[BDPP-$DSeO_2$ + Na]$^+$ calc.*: 1075.1457*, obtained*: 1075.1484 *and [DPP-$SeHO_2$ + Na]$^+$ calc.*: 550.0753*, obtained*: 550.0674).



R = Me, Ph
Ar = 2-pyridyl, 3-pyridyl, pipronyl

LC_{50} = 35.5 - 50.6



R = Propyl, Ph
Ar = 3-Chlorophenyl

LC_{50} = 57.9 - 82.4

Figure S22. Reported LC_{50} of pyrazole moieties (μM).

END