SUPPORTING INFORMATION

Overriding Phthalate Decomposition when Exploring Mycophenolic acid Intermediates as Selenium-based ROS Biological Probes

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Contents

Standard protocol for preparation of Various ROS and RNS:

Generation of OCl^-: The source of NaOCl was commercial bleach. The concentration of the OCl^- stock solution was determined by measuring the absorbance at 209 nm with a molar extinction coefficient of 350 M^{-1} cm^{-1}.

Generation of H_2O_2: H_2O_2 solution was added directly. The concentration of H_2O_2 was determined by measuring the absorbance at 240 nm with a molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$.

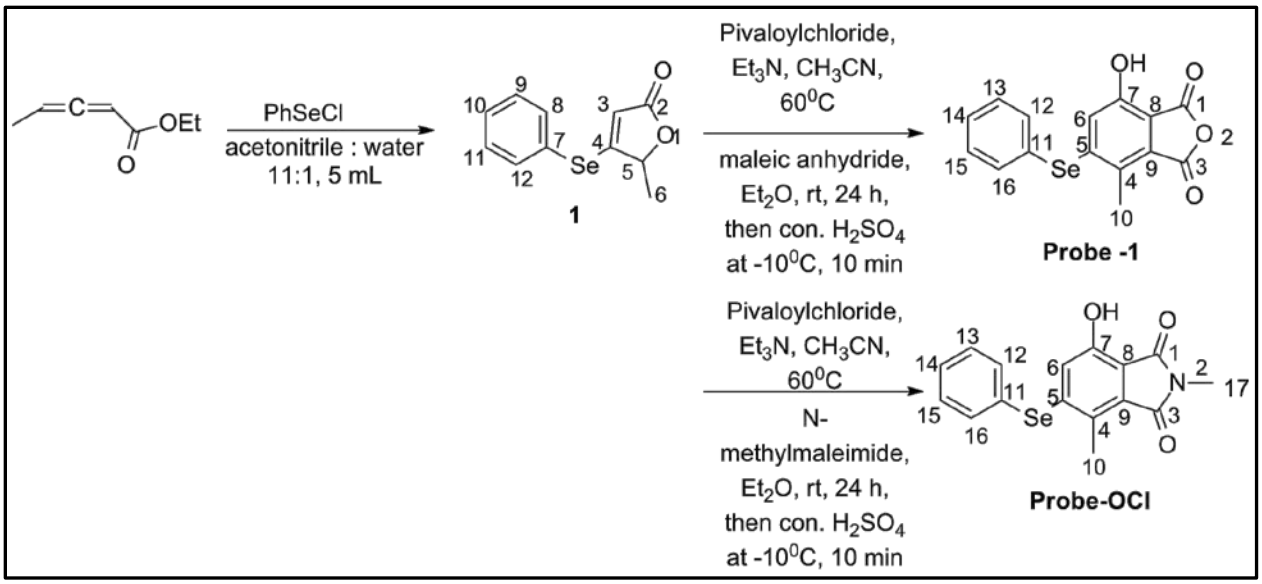
Generation of ^{t}BuOOH: The commercial available *tert*-Butyl hydroperoxide solution was diluted with deionized water.

Generation of $O_2^{\bullet-}$: Solid potassium superoxide was used as superoxide radical anion source.

Generation of •OH: Hydroxyl radical (•OH) was generated by the Fenton reaction. To generate •OH, ferrous chloride was added in the presence of 10 equiv of H_2O_2. The concentration of •OH was equal to the Fe (II) concentration.

Generation of peroxynitrite ($ONOO^-$): 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 resulting solution was stored at lower than -18 °C. The solution was thawed immediately before use. The concentration of the stock solution was determined in 0.1 M NaOH by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 $M^{-1}cm^{-1}$.

Generation of NO•: Nitric oxide was generated from SNP (Sodium nitroferricyanide (III) dehydrate). The experiments were performed under anaerobic conditions. The deionized water was degassed with Ar for 30 min and then SNP was added into it under Ar atmosphere and stirred for 30 min at room temperature. The probe solution was also degassed before the reaction with NO•.



Scheme 1. Synthesis of **Probe-1** and **Probe-OCl.**

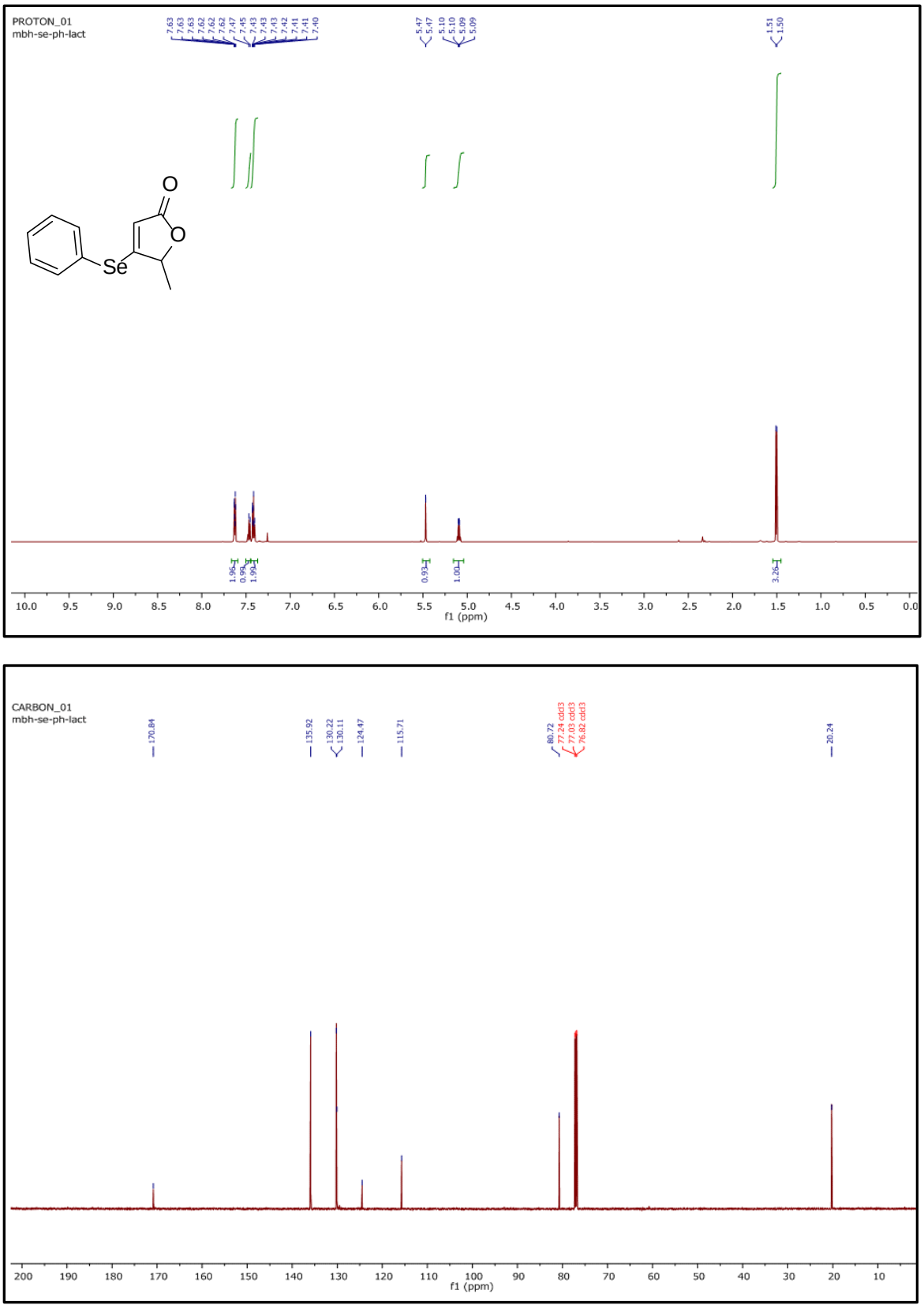


Figure S1. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **1**.

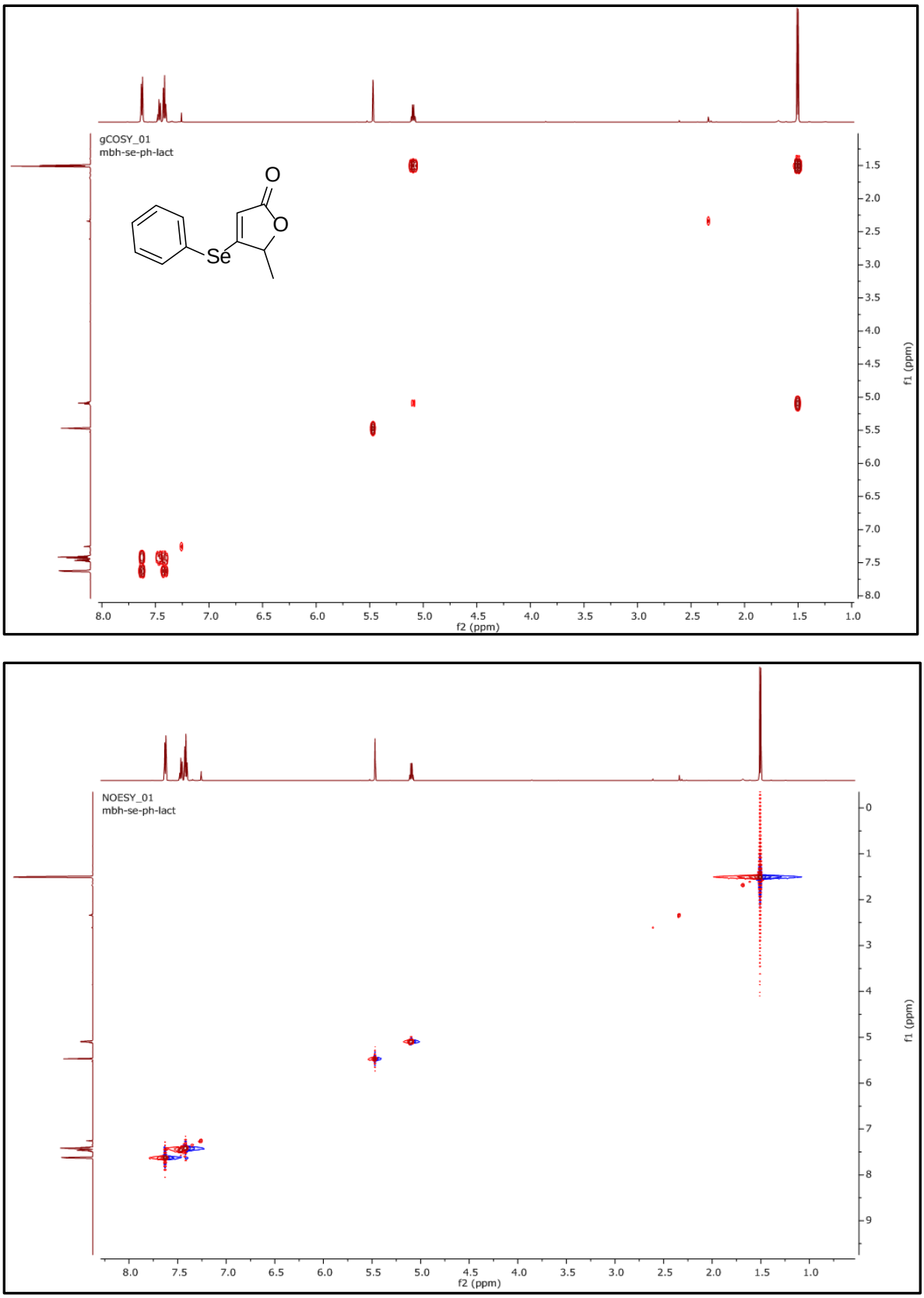
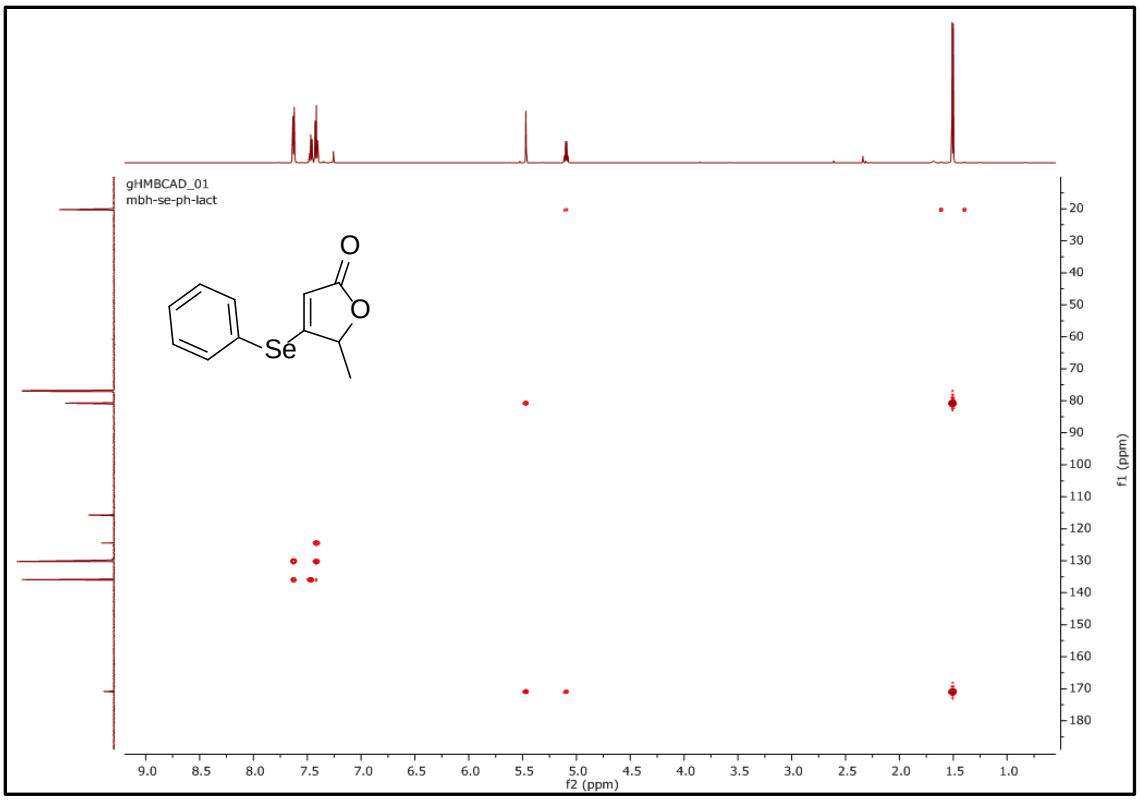


Figure S2. (top) COSY and (bottom) ^{1}H NOESY NMR spectrum of **1**.



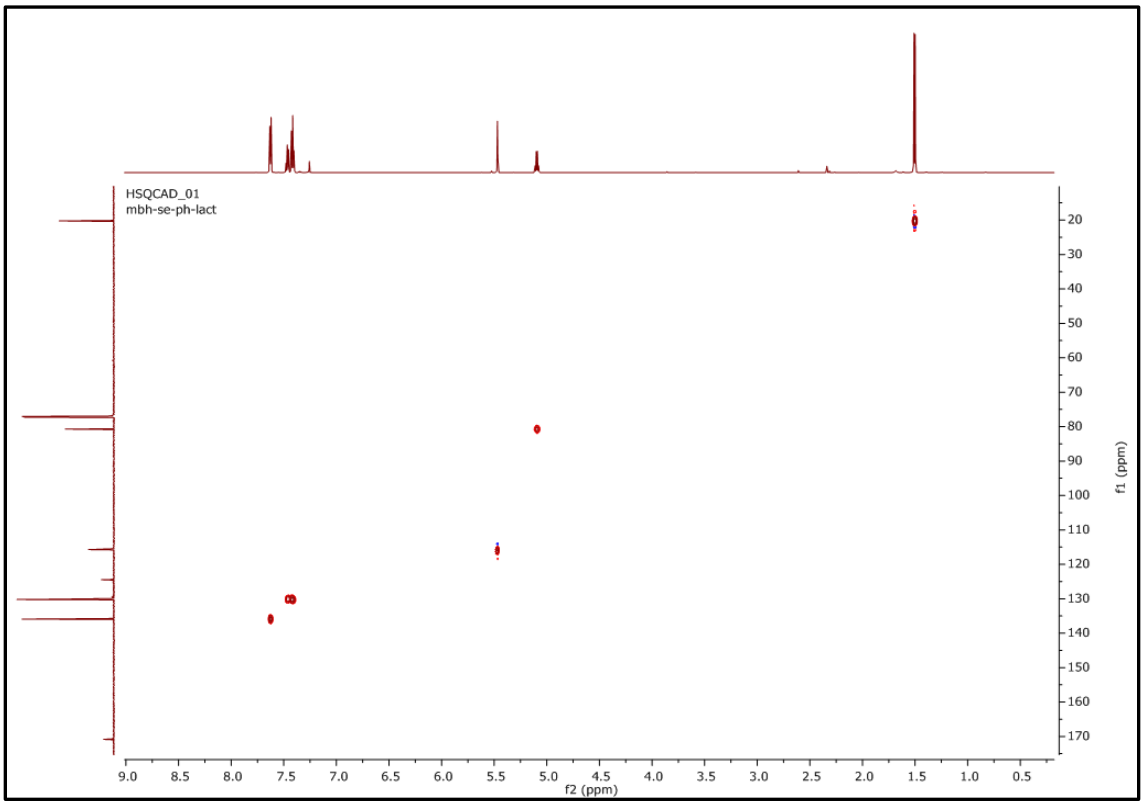


Figure S3. (top) ^{1}H – ^{13}C HMBC NMR spectrum of **1** and (bottom) ^{1}H – ^{13}C HSQC NMR spectrum of **1**.

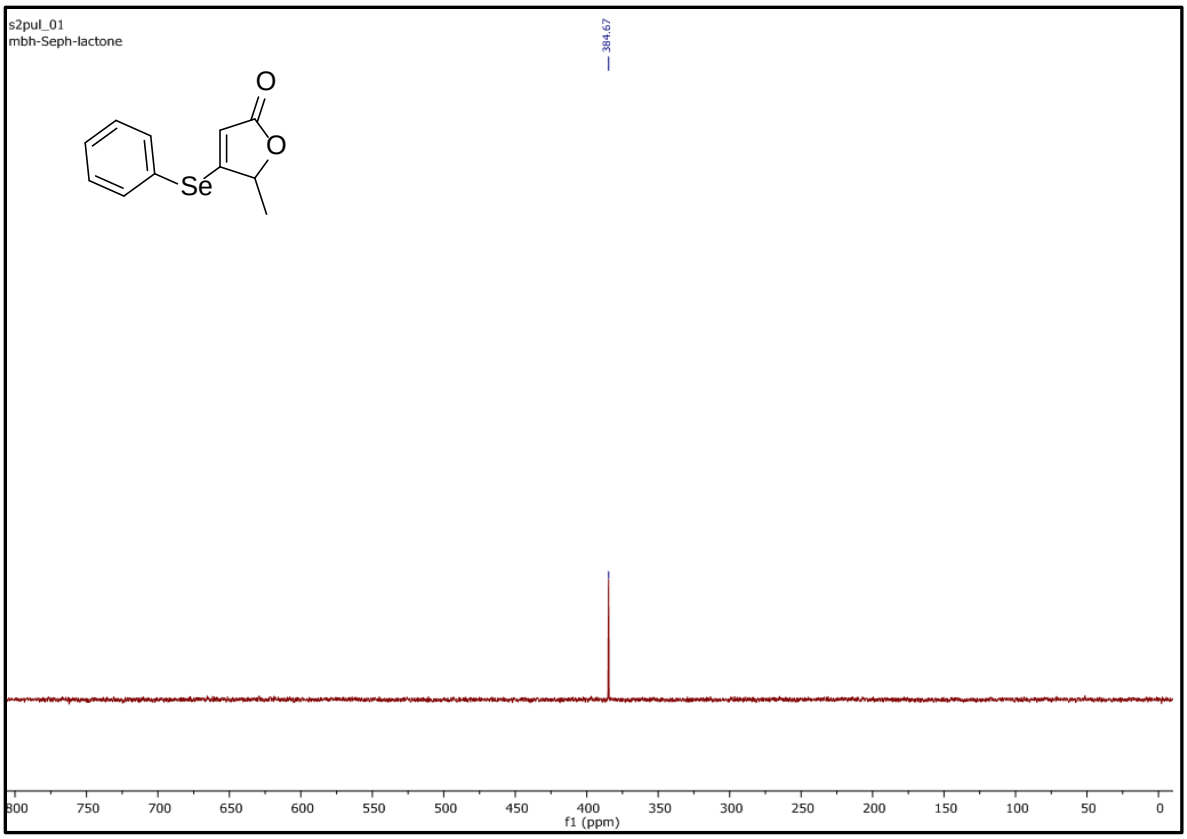


Figure S4. ^{77}Se NMR spectrum of **1.**

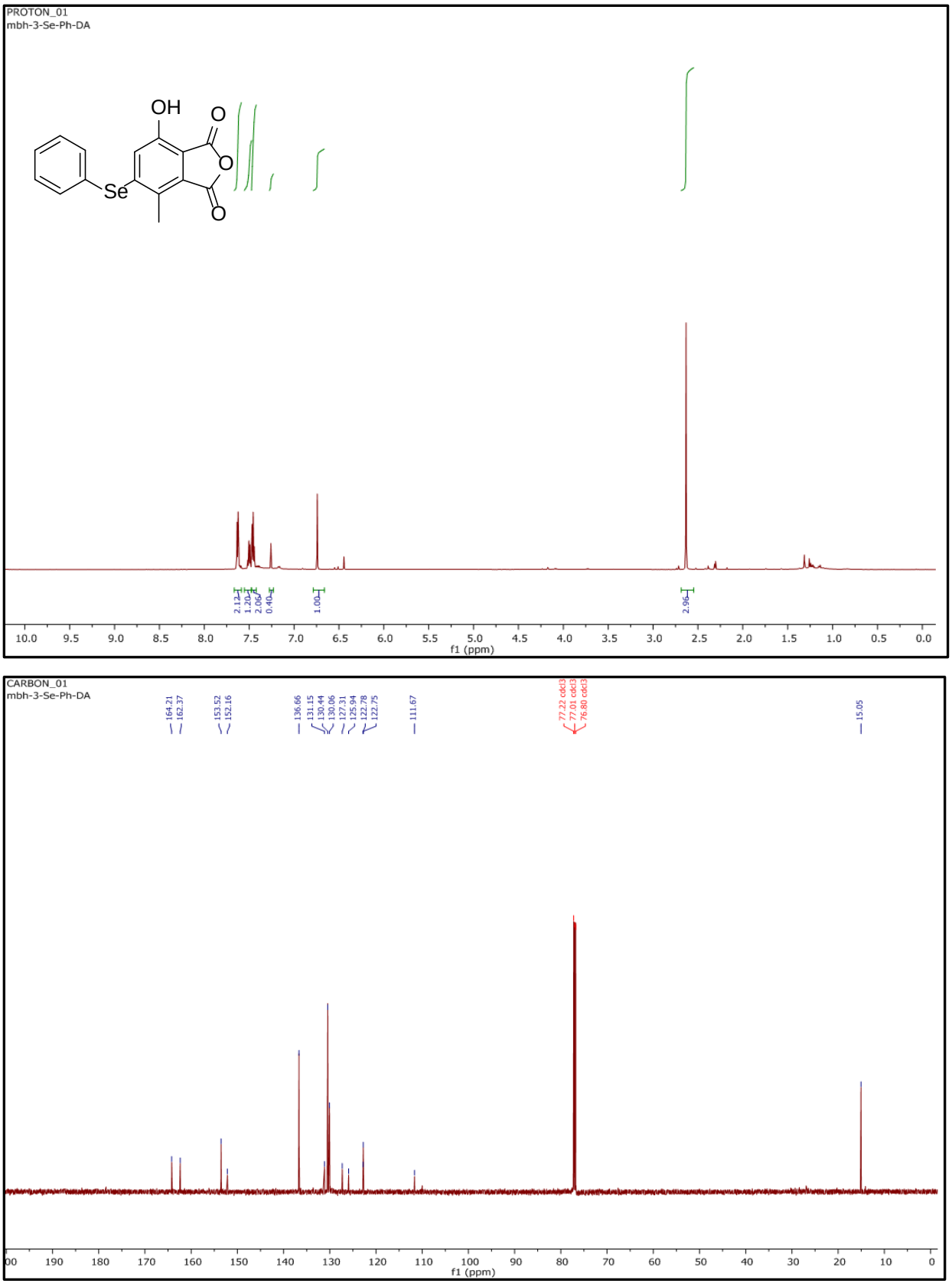


Figure S5. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **Probe-1**.

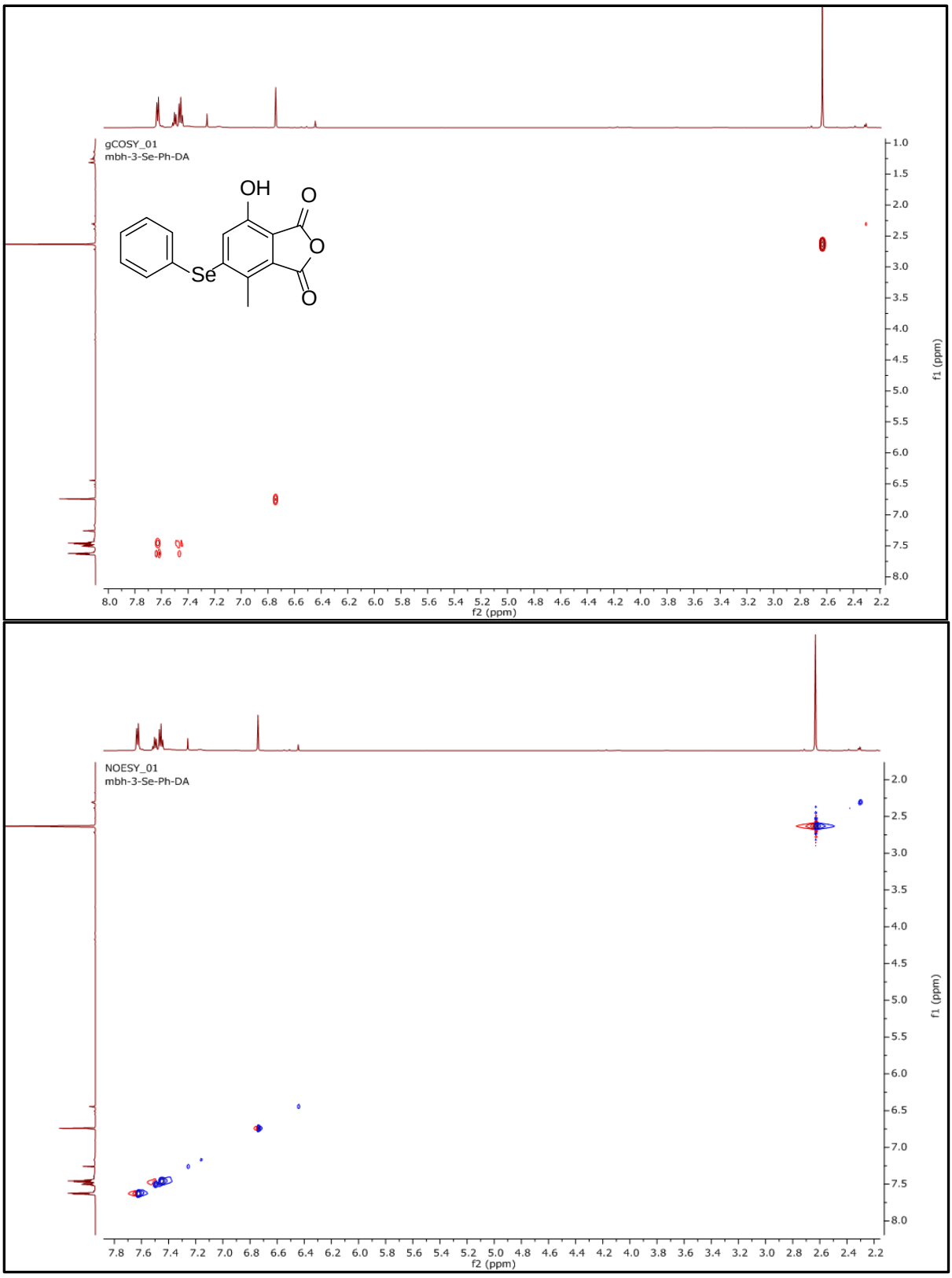


Figure S6. (top) ^{1}H COSY NMR spectrum of **Probe-1** and (bottom) ^{1}H NOESY NMR spectrum of **Probe-1.**

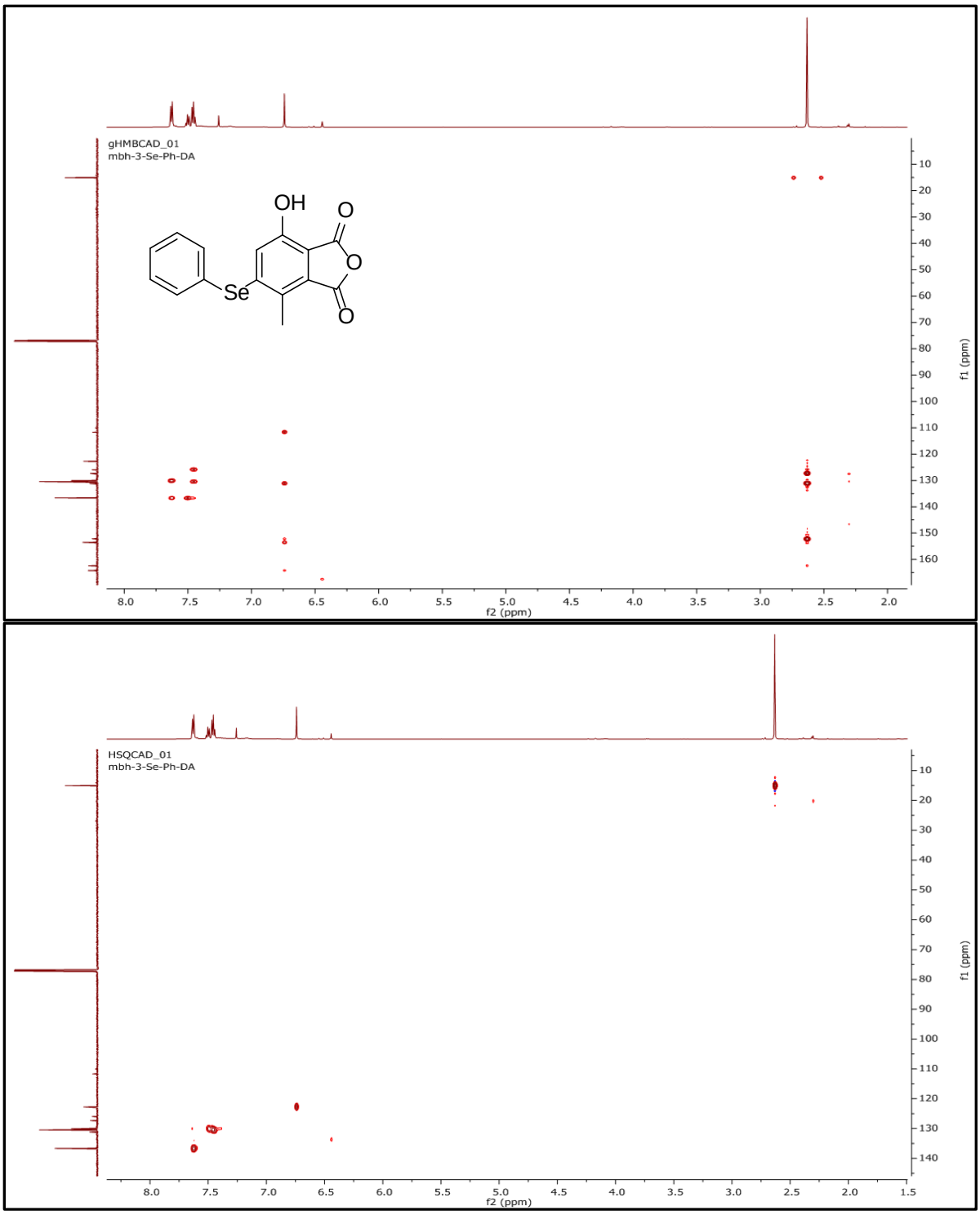


Figure S7. (top) ^{1}H – ^{13}C HMBC NMR spectrum of **2** and (bottom) ^{1}H – ^{13}C HSQC NMR spectrum of **Probe-1**.

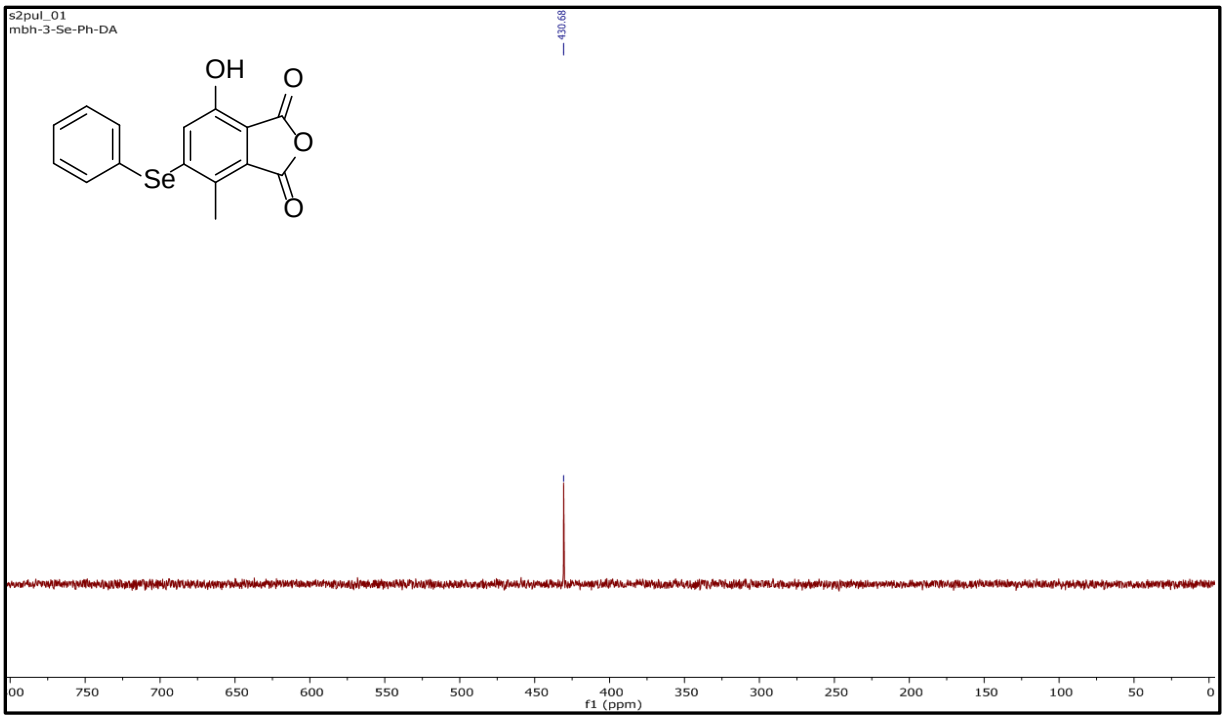
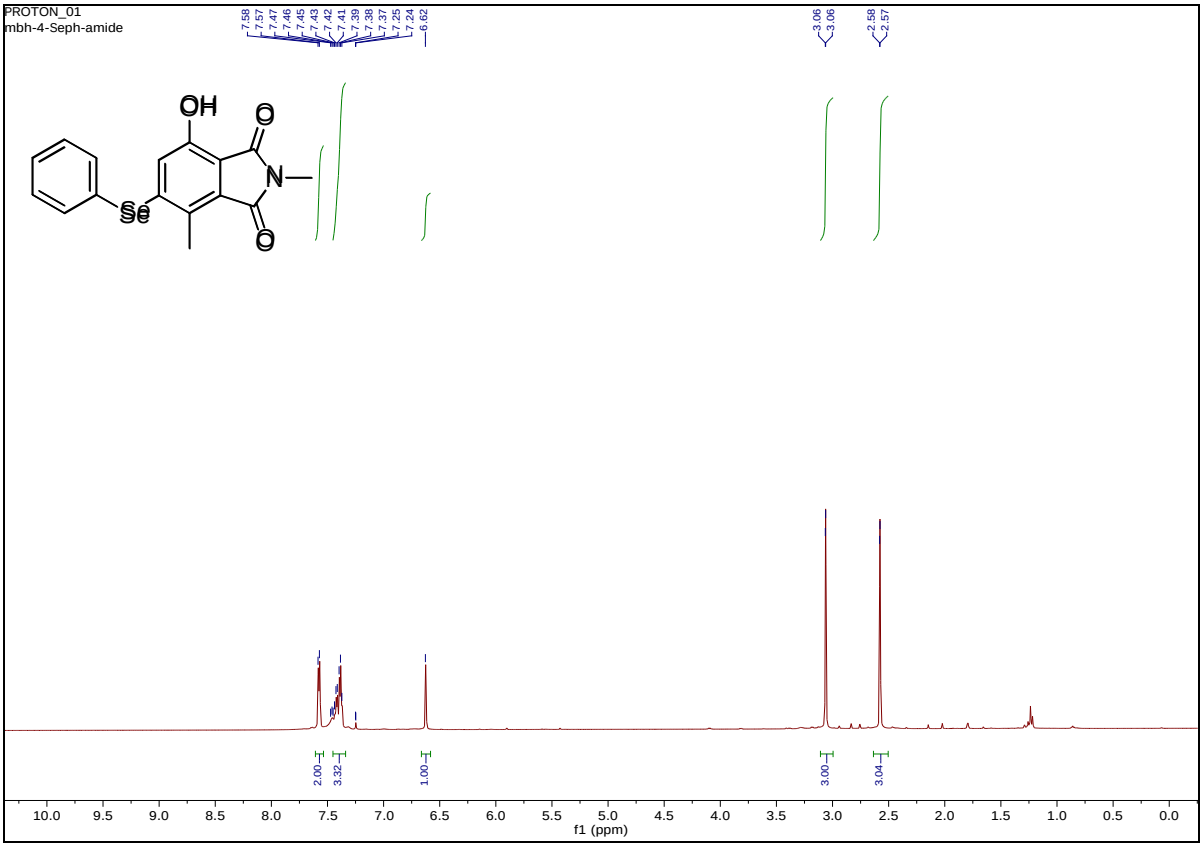


Figure S8.^{77}Se NMR spectrum of **Probe-1**.



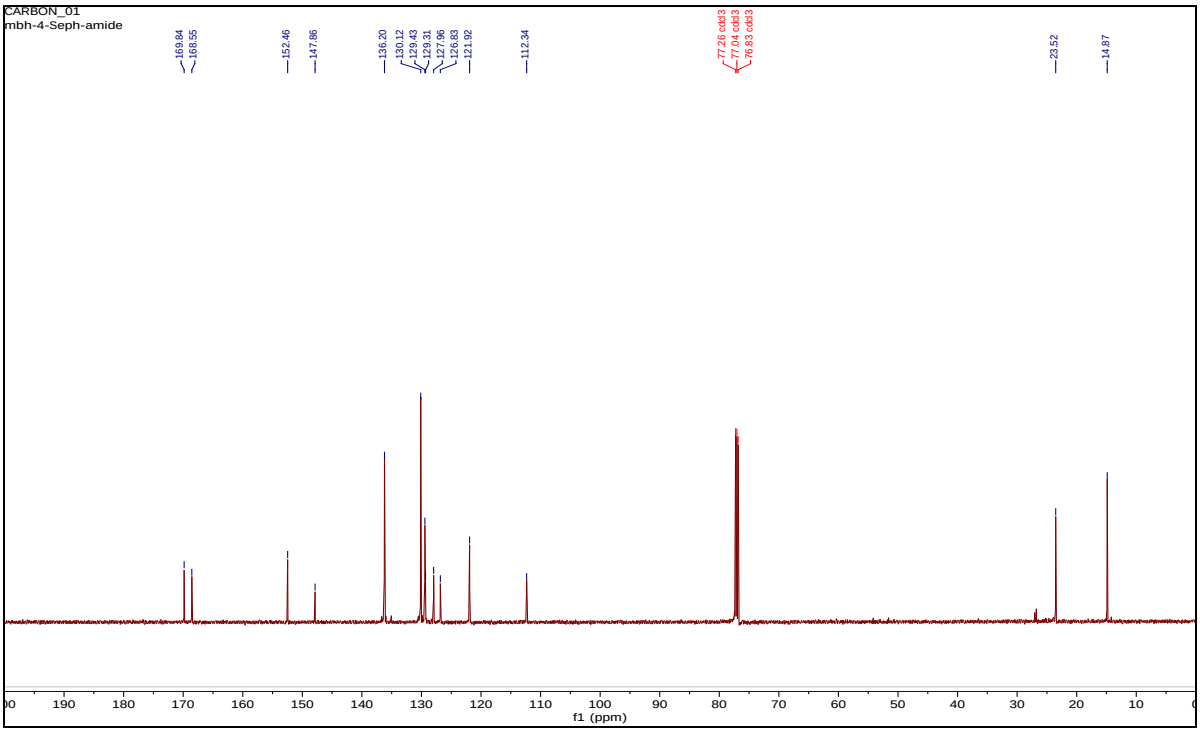
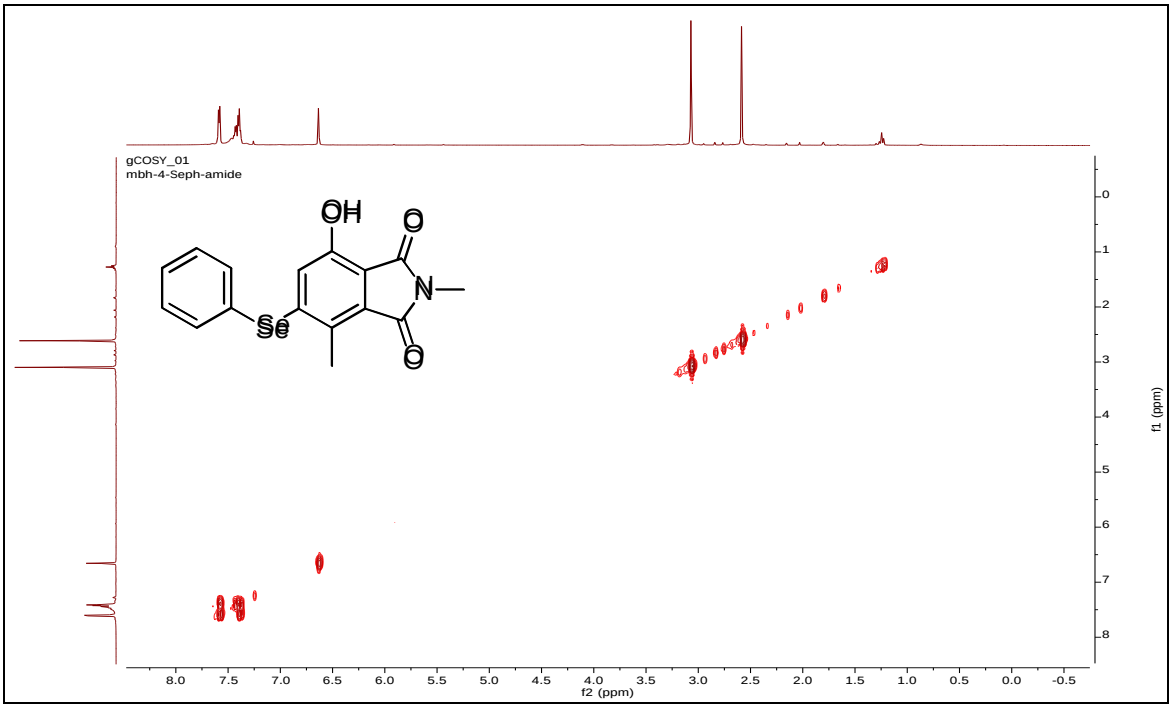


Figure S9. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **Probe-OCl**.



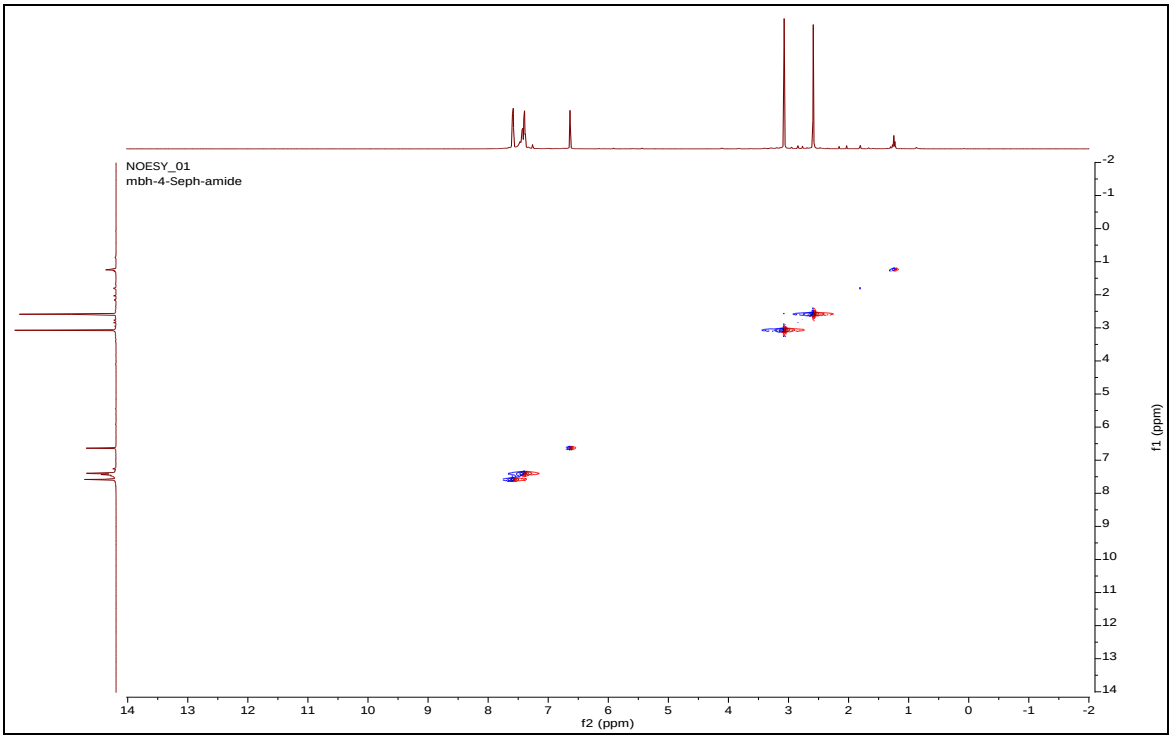


Figure S10. (top) ^{1}H COSY NMR spectrum of **Probe-OCl** and (bottom) ^{1}H NOESY NMR spectrum of **Probe-OCl**

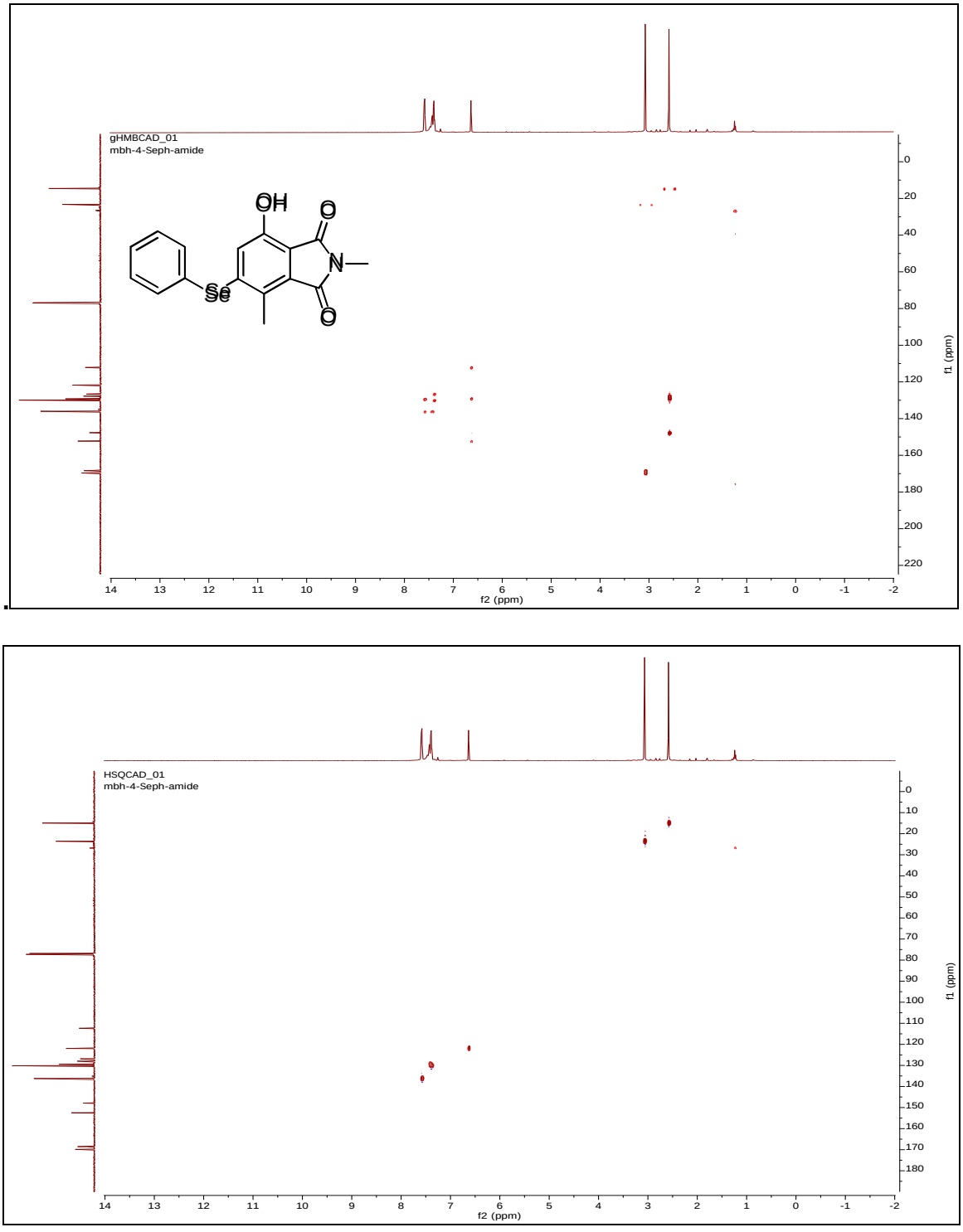
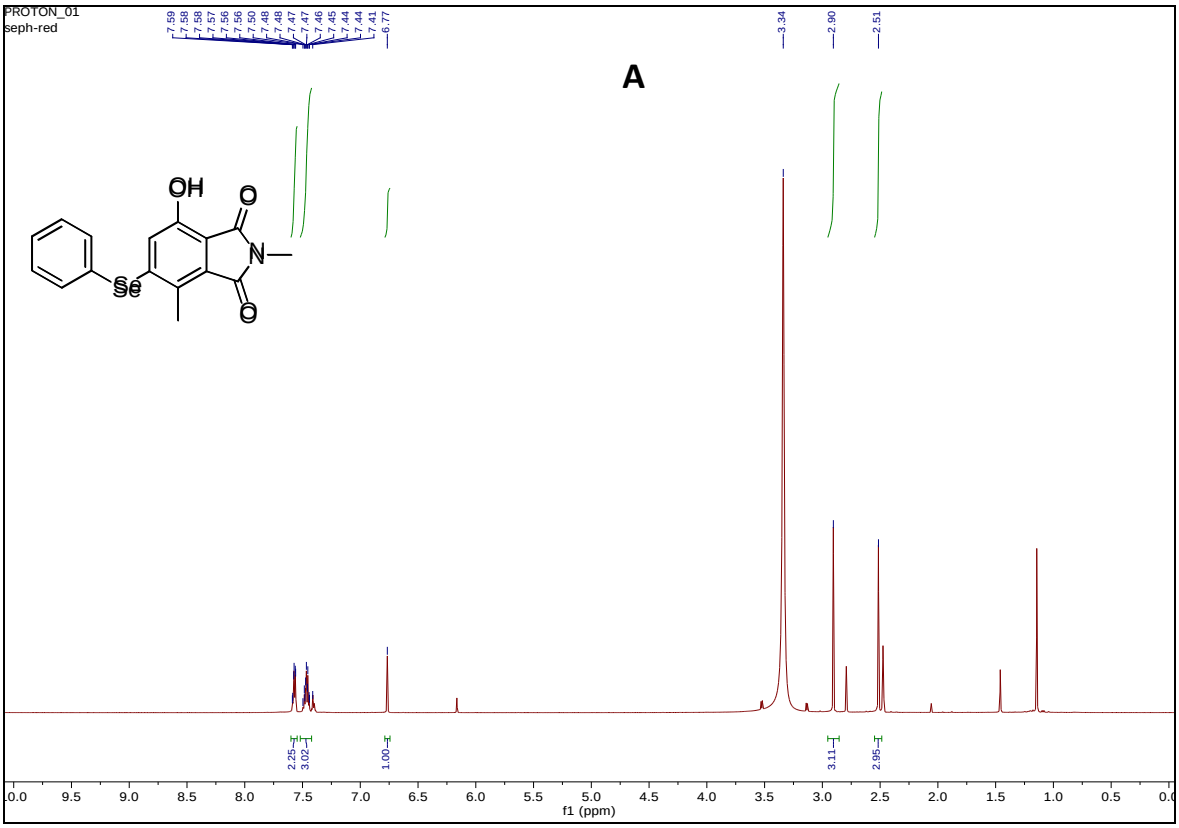
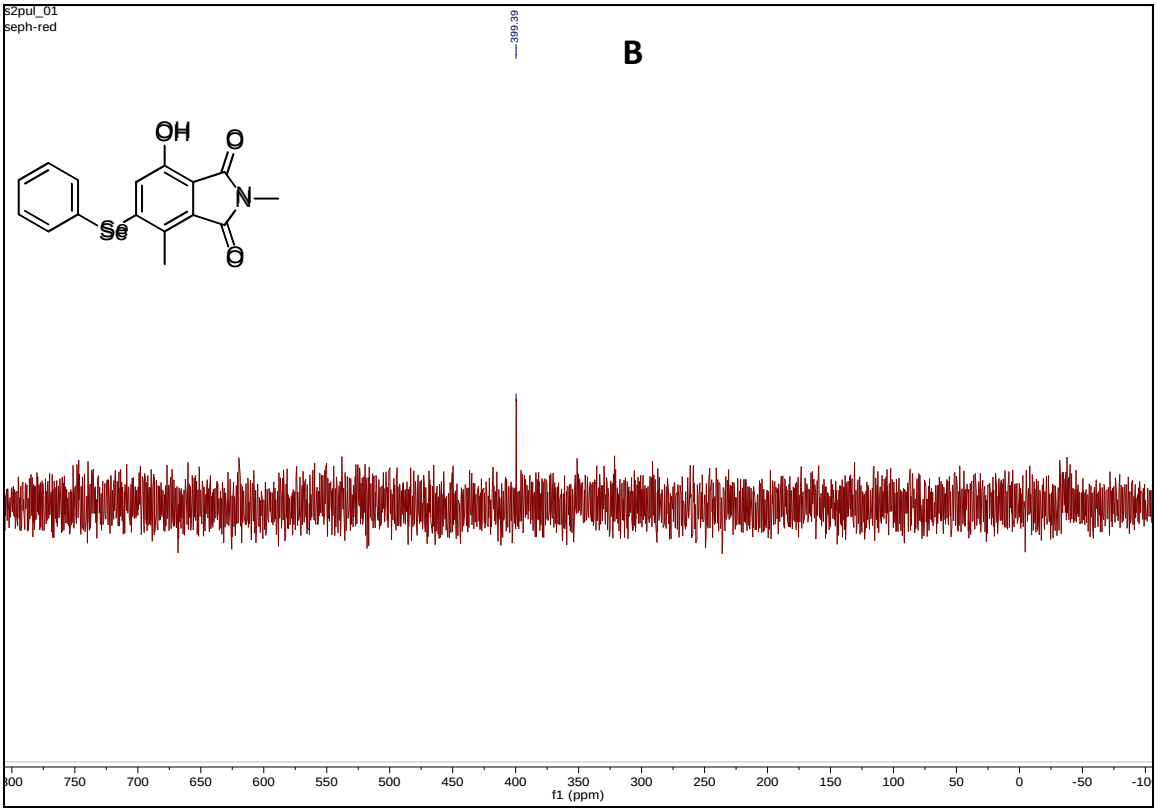


Figure S11. (top) ^{1}H – ^{13}C HMBC NMR spectrum of **Probe-OCl** and (bottom) ^{1}H – ^{13}C HSQC NMR spectrum of **Probe-OCl.**


PROTON_01
seph-red
A
7.59
7.58
7.58
7.57
7.56
7.56
7.50
7.48
7.48
7.47
7.47
7.46
7.45
7.44
7.44
7.41
6.77
3.34
2.90
2.51
OH
O
N
Se
O
2.25
3.02
1.00
3.11
2.95
f1 (ppm)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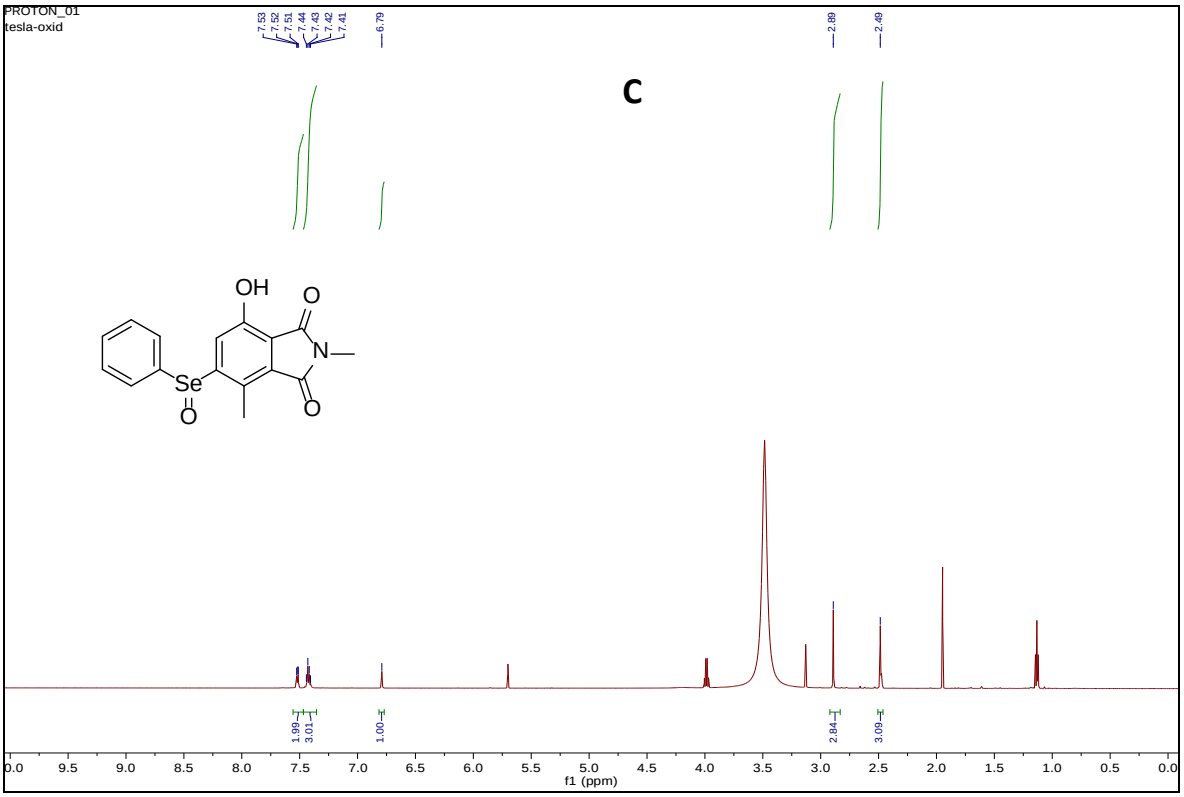
seph-red
B
399.39
OH
O
N
Se
O
f1 (ppm)



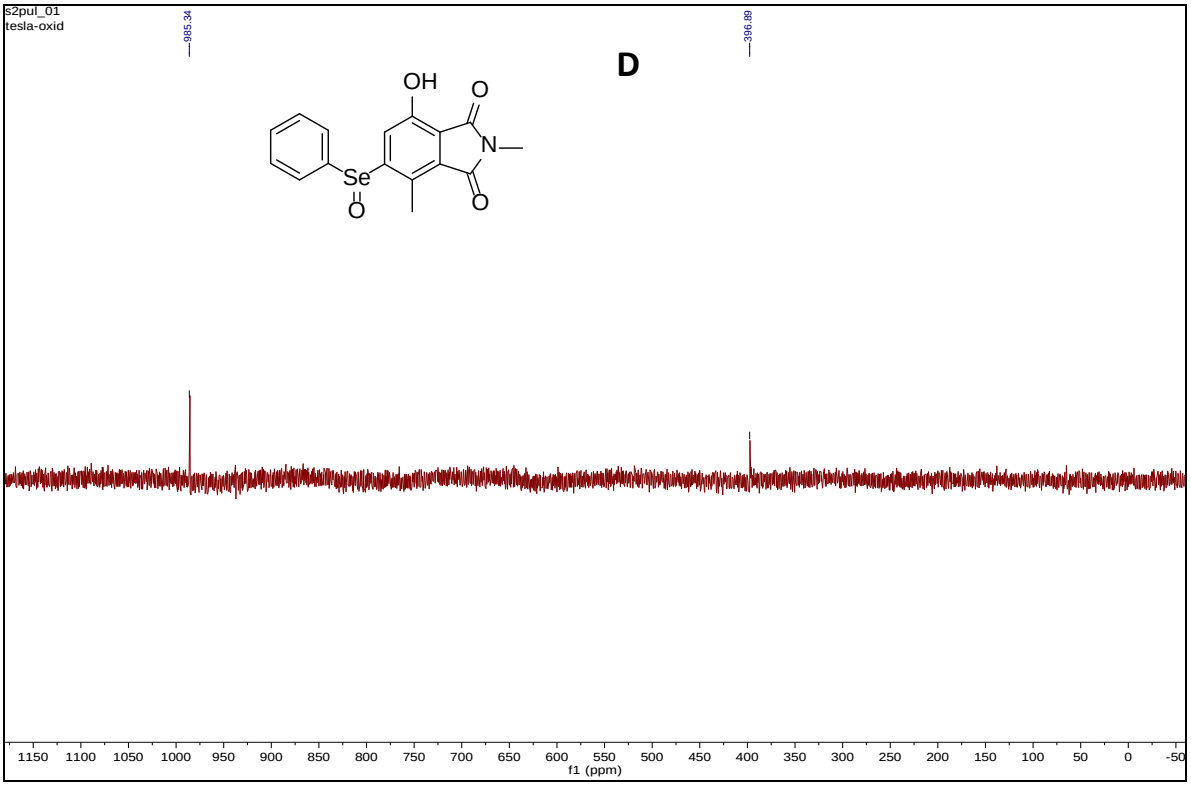


Figure S12. (A) ^{1}H NMR spectrum of **Probe-OCl** and (B) ^{77}Se NMR spectrum of **Probe-OCl** (C) ^{1}H NMR spectrum of **Probe-OCl[O]** (D) ^{77}Se NMR spectrum of **Probe-OCl[O]**

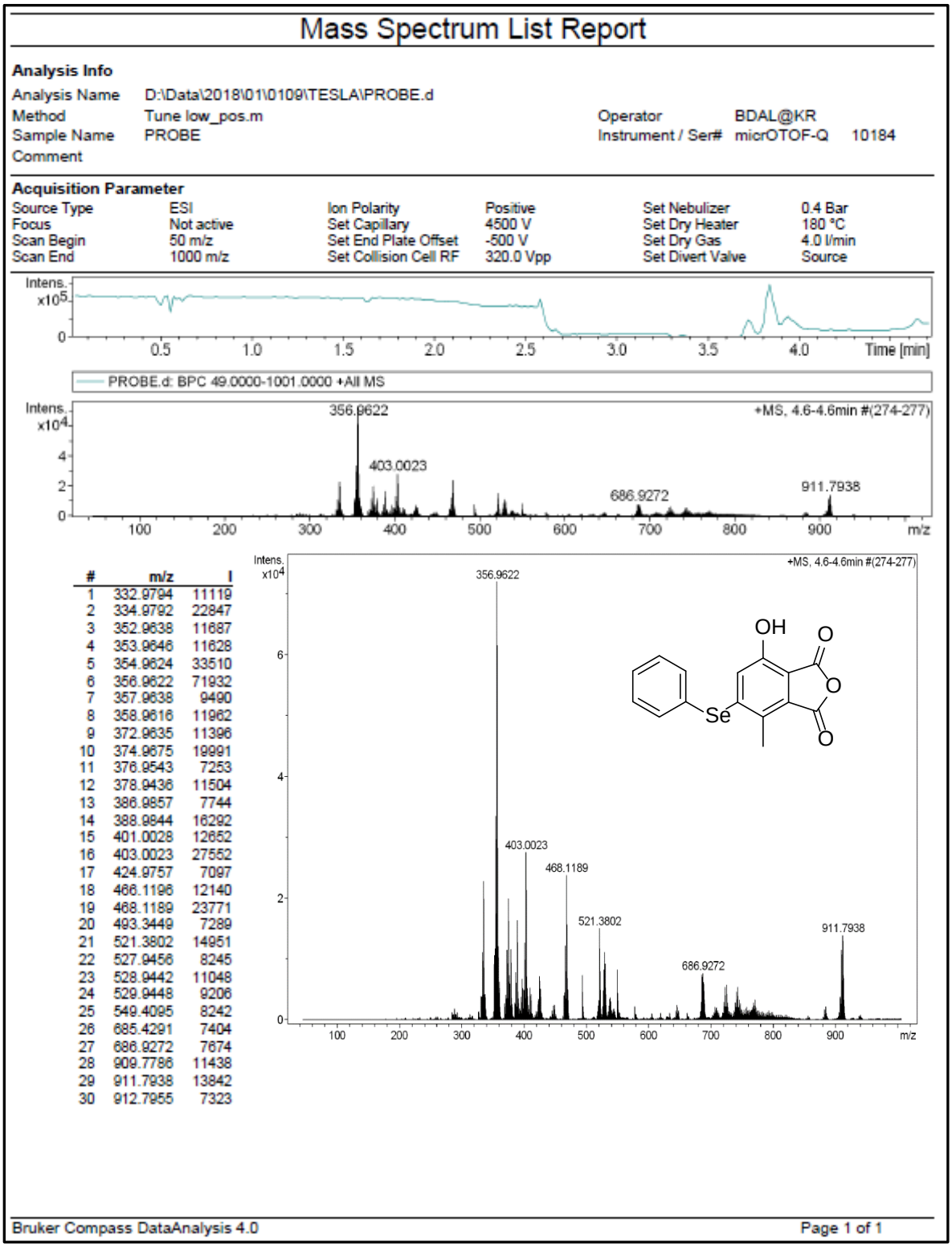


Figure S13. HR-MS (ESI) spectrum of **Probe-1**.

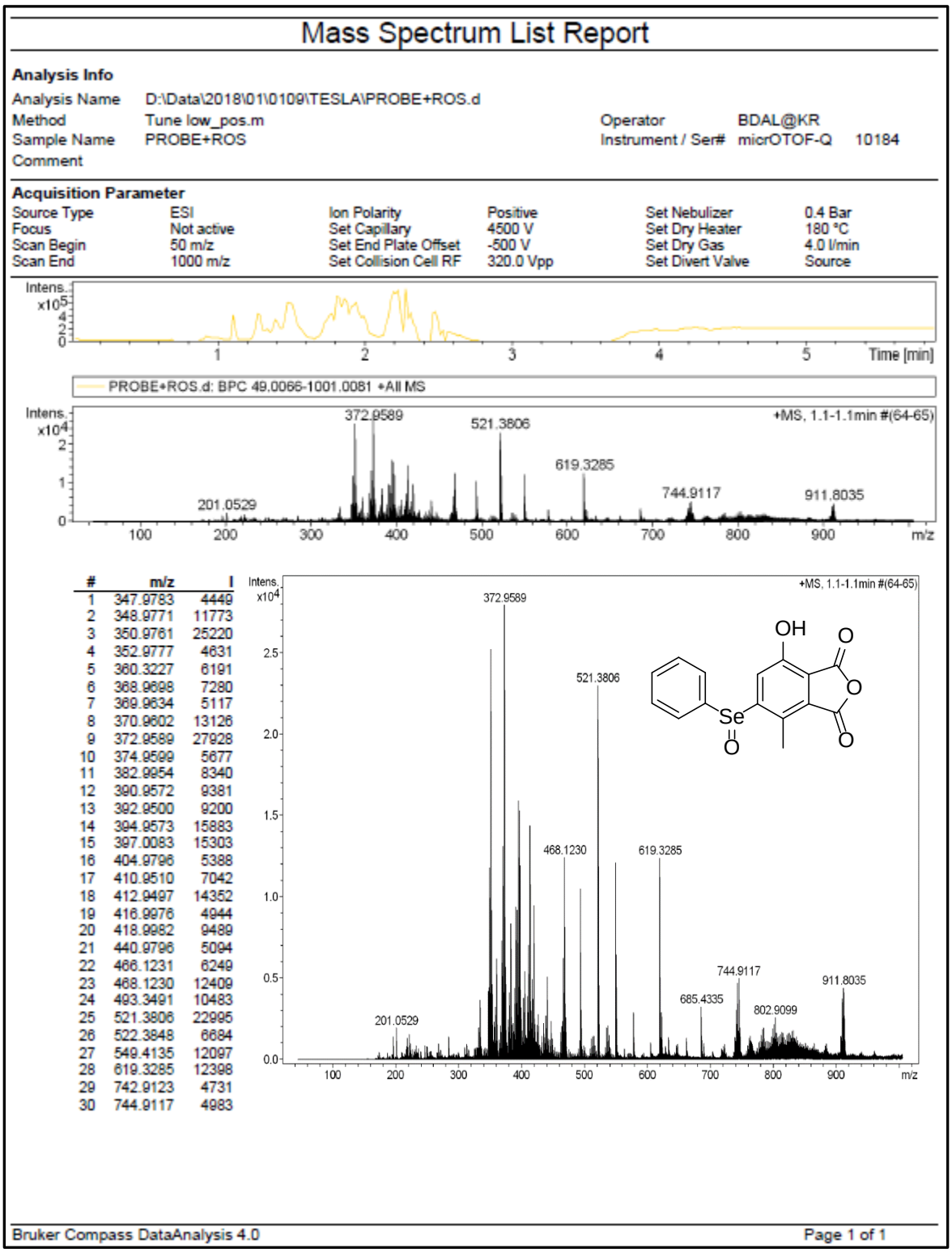


Figure S14. HR-MS (ESI) spectrum of **Probe-1+OCl$^-$.**

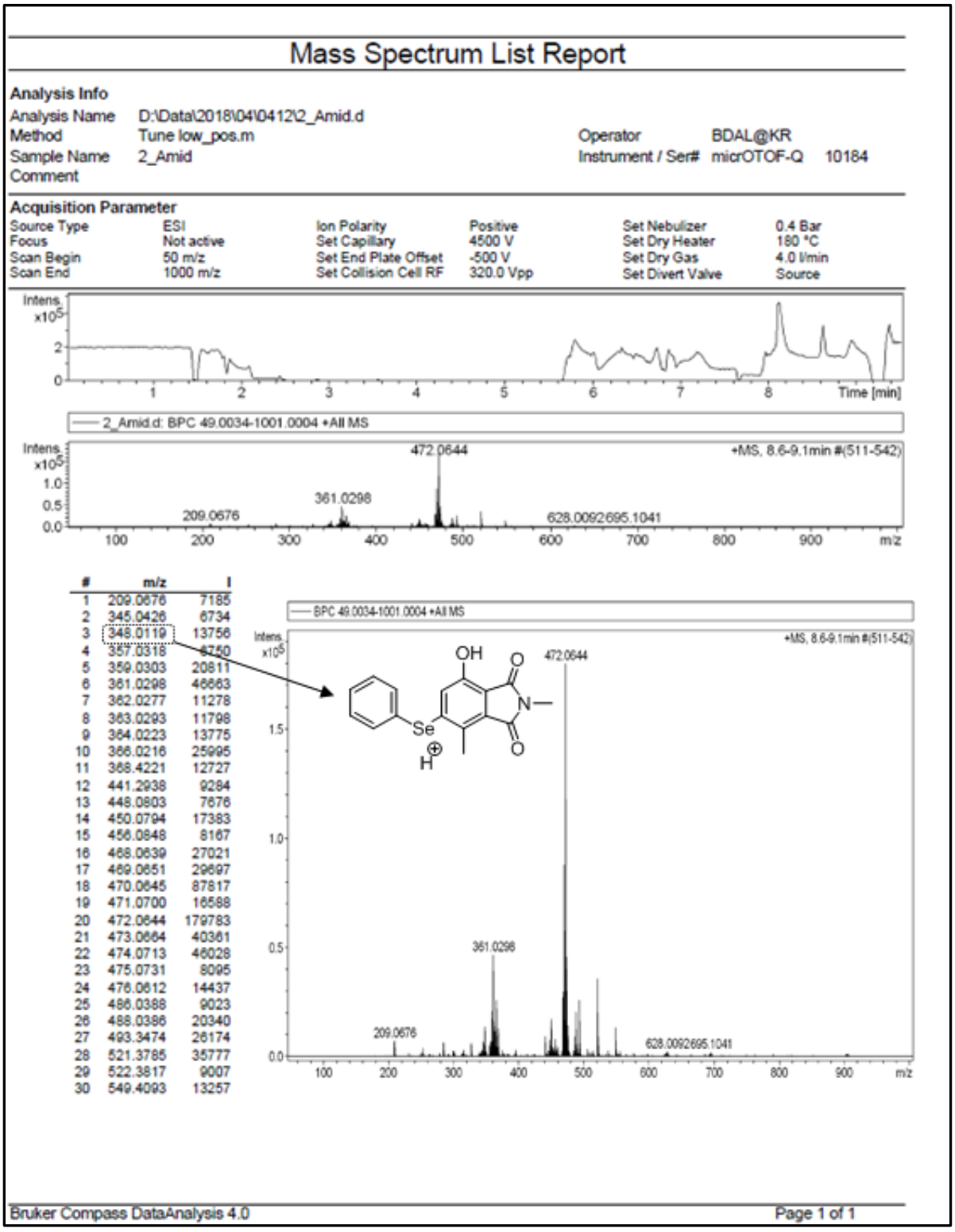


Figure S15. HR-MS (ESI) spectrum of **Probe-OCl.**

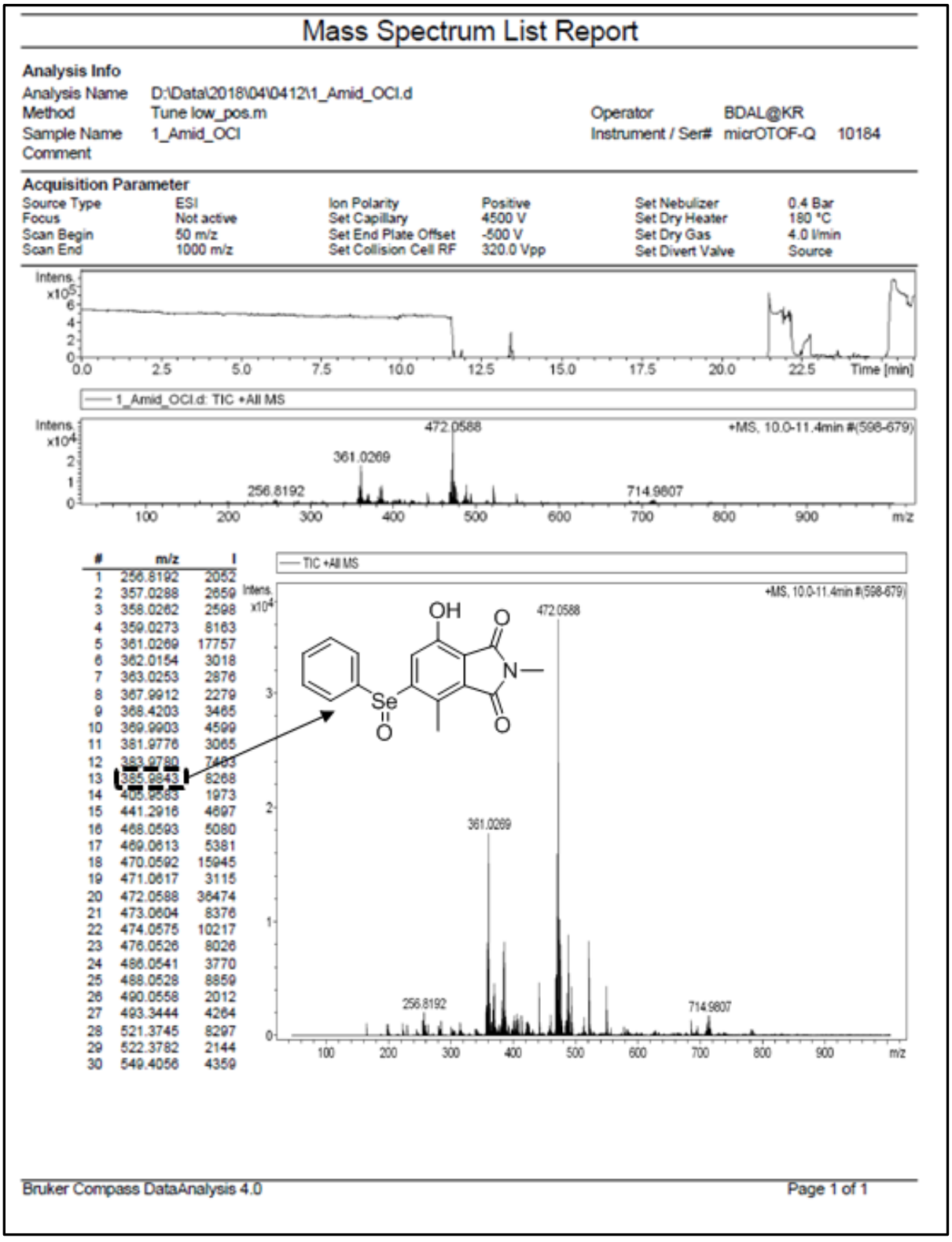


Figure S16. HR-MS (ESI) spectrum of **Probe-OCl+ OCl**$^{-}$

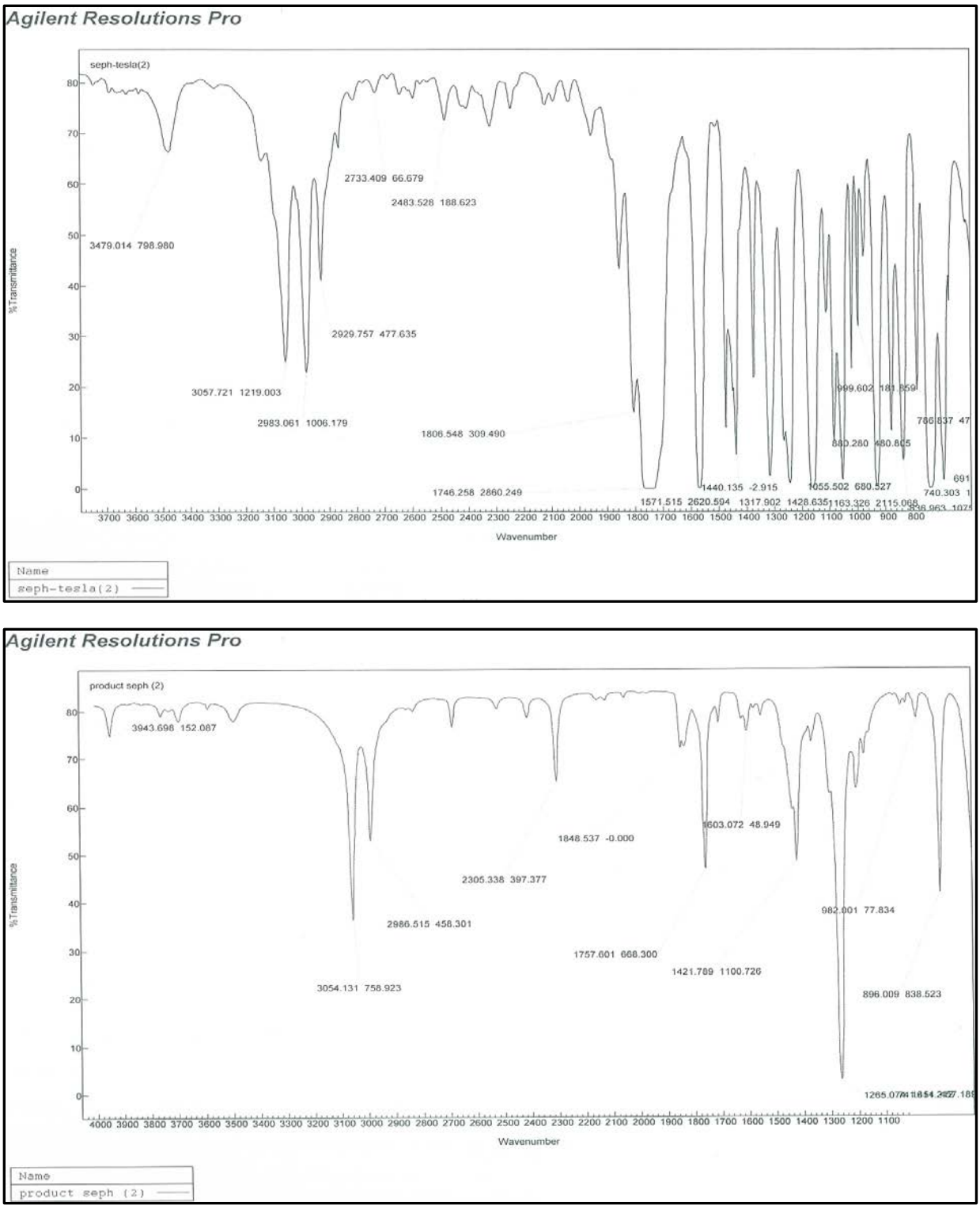


Figure S17. (top) IR spectrum of **1** and (bottom) **Probe-1**.

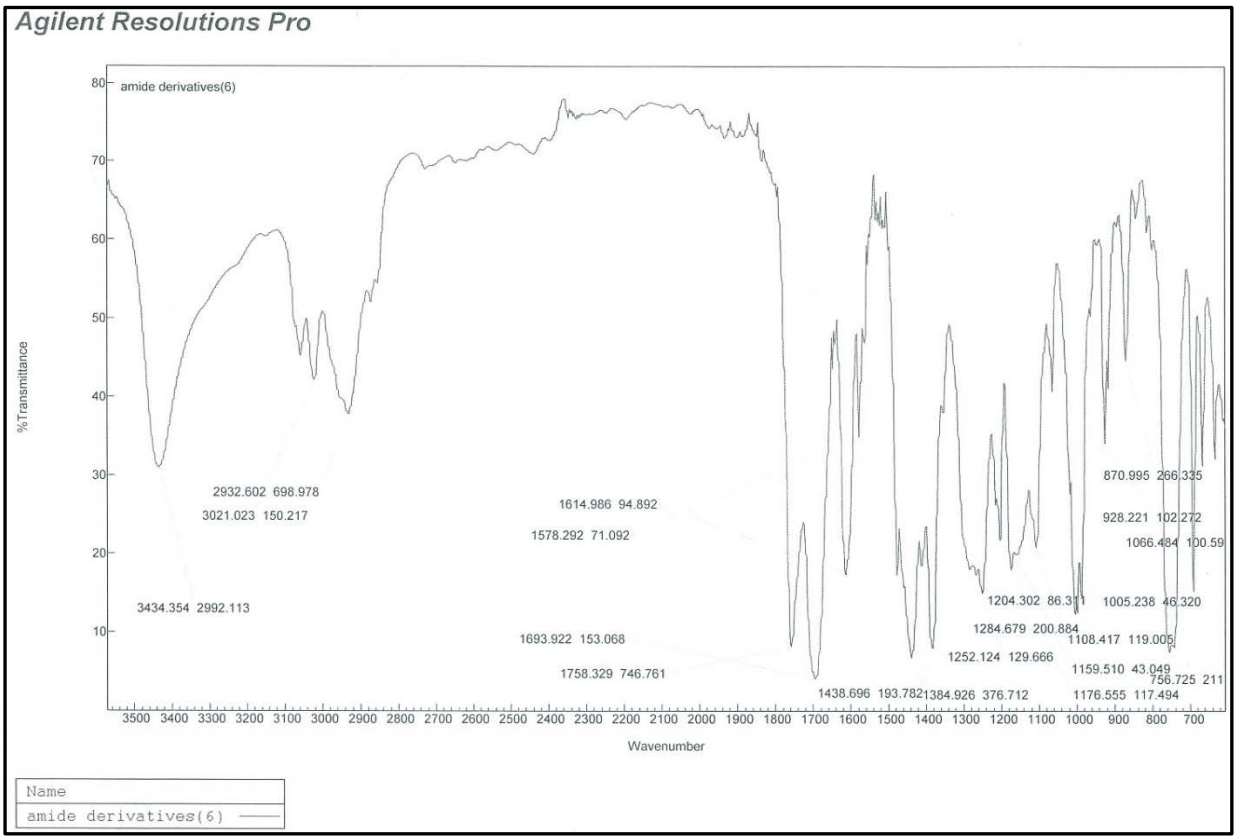


Figure S18. IR spectrum of **Probe-OCl**.

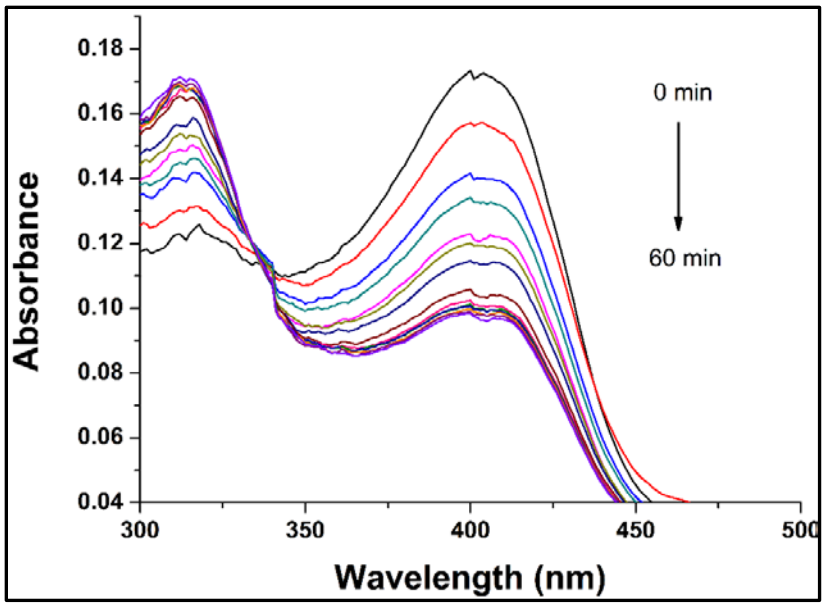


Figure S19. Absorption spectra of the **Probe-1** (15.0 μM) with 1.0 equiv OCl^- in time from 0 to 60 minutes in the solution (10 mM PBS pH 7.4) incubated for 1.0 min at r.t.

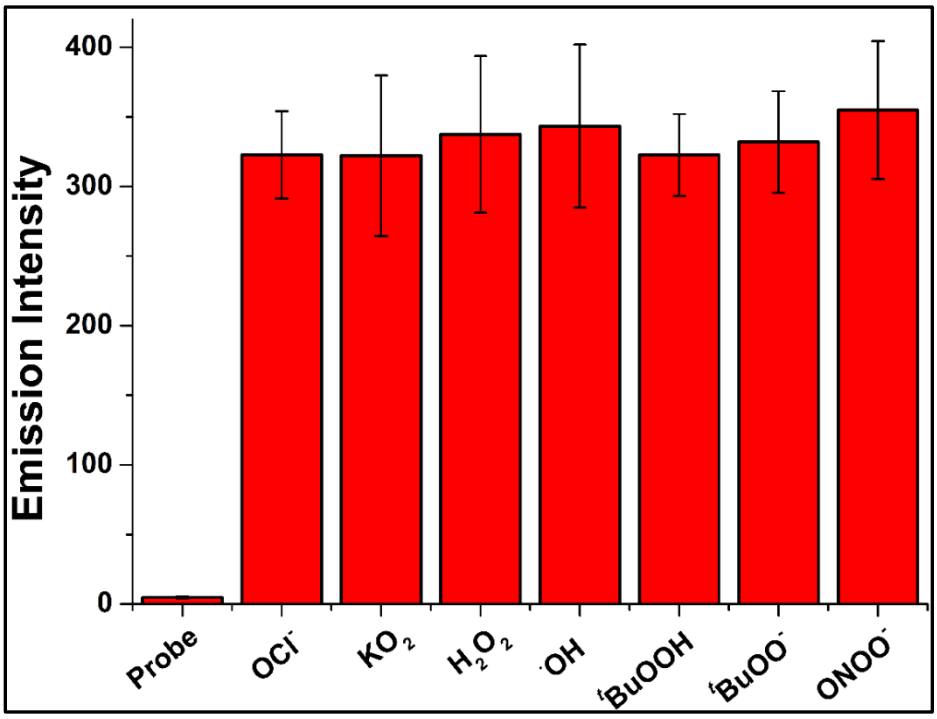


Figure S20. Fluorescence intensity of **Probe-1** (15.0 μM) with ROS/RNS in the solution (10 mM PBS pH 7.4) incubated for 2.0 min. λ_{ex}: 404 nm, λ_{em}: 502 nm, slit width 3.0 nm/ 3.0 nm.

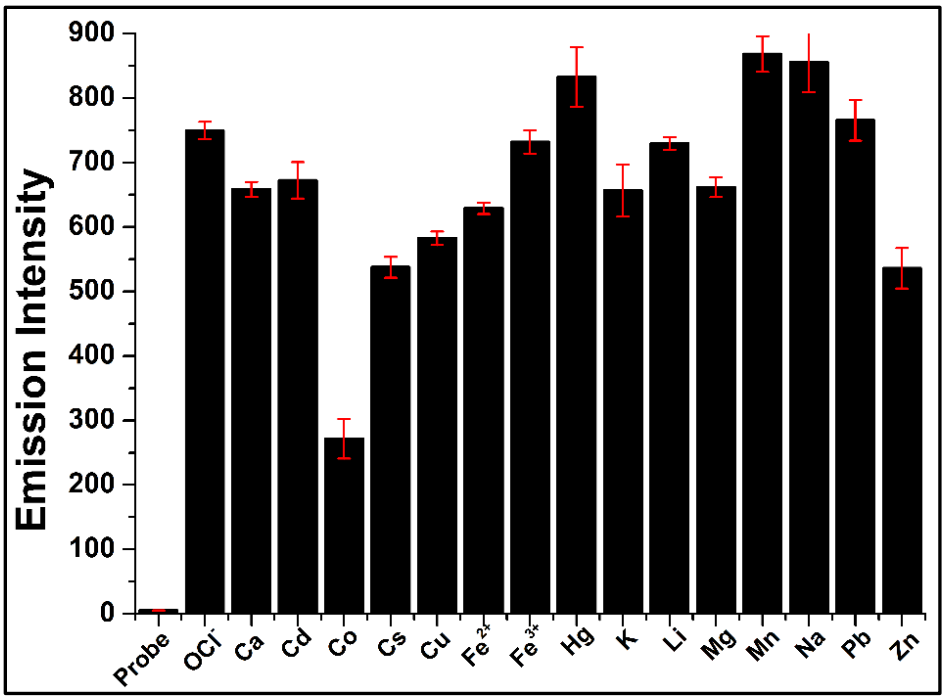


Figure S21. Fluorescence intensity of **Probe-1** (15.0 µM) with various metals in solution (10 mM PBS pH 7.4) incubated for 1.0 min. λ_{ex}: 404 nm, λ_{em}: 512 nm, slit width 3.0 nm/3.0nm.

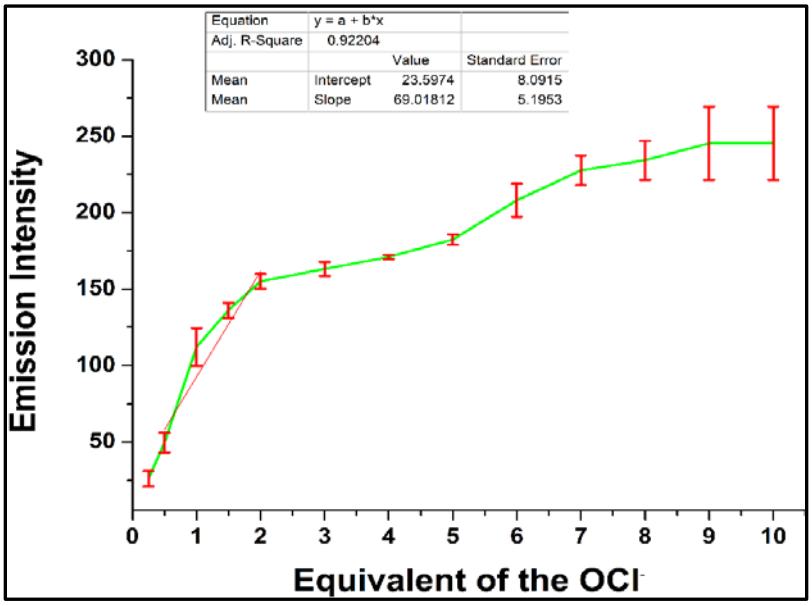


Figure S22. Plot for the calculation of limit of detection from the emission of **Probe-1** (15.0 µM) in the solution (10 mM PBS pH 7.4, 3:7 v/v) with increasing concentration of OCl^- (0.0 to 10.0 µM in water) incubated for 1.0 min at r.t, λ_{ex}. 404 nm and λ_{em}. 502 nm slith width 3.0 nm/ 3.0 nm (average of three experiments).

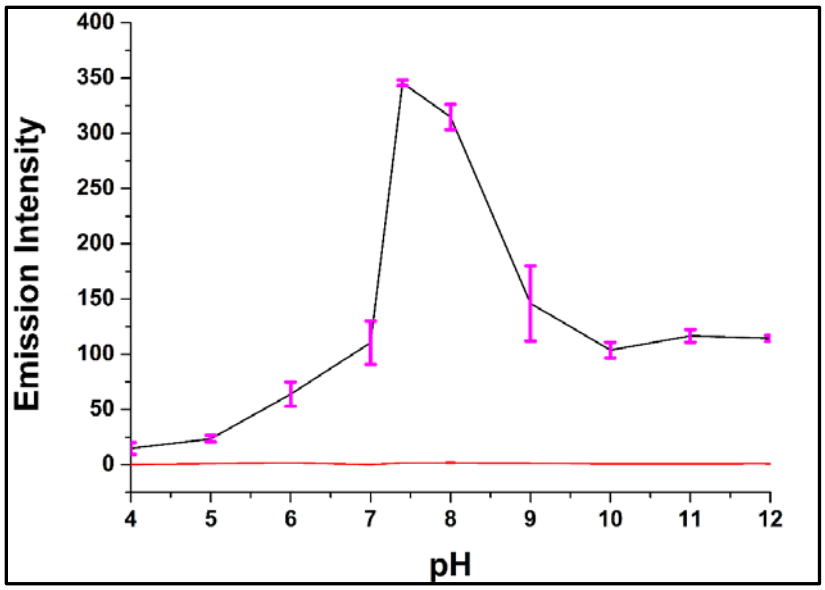


Figure S23. pH-dependent spectral changes of **Probe-1** (15.0 μM) with 1.0 equiv of OCl^- in various pH values in solution (10 mM PBS, pH 7.4); λ_{ex}: 502 nm, λ_{em}: 512 nm. Slit width 3,0 nm/3.0 nm.

Table S1. Results of experimental determination of log P value by the "shake flask" method for the **Probe-1**

C_1	V	A	$\bar{A}$	C_2	P	Log P
1.3×10^{-4}	35	0.3289 0.28425 0.24004	0.2844	7.49×10^{-5}	29.7	1.47
1.6×10^{-4}	48	0.25045 0.25801 0.20986	0.23944	6.21×10^{-5}	59.1	1.77
2.0×10^{-4}	60	0.25146 0.26715 0.27875	0.2658	7.44×10^{-5}	63.3	1.80

※ C_1 = Concentration (mol L^{-1}) of the stock solution in *n*-octanol before partition; V = volume (μL) of stock solution; A = absorbance in buffer solution after the partition (λ = 500 nm); $\bar{A}$ = arbitrary absorbance in buffer solution after partitioning (λ = 500 nm); C_2 = concentration (mol L^{-1}) in buffer solution after partitioning; P = partition coefficient; log P = logarithm of the partition coefficient.

Table S2. Information of the **Probe-1** calculated through the 'molinspiration property engine v2011.04' at the website, http://www.molinspiration.com.

Properties (Probe-1) (NOTE: abbreviation same as website)	Value
milogP	**3.41**
TPSA	**67.51**
Natom	**20**
MW	**333.20**
nON	**4**
nOHNH	**1**
nviolations	**0**
nrotb	**2**
volume	**239.63**

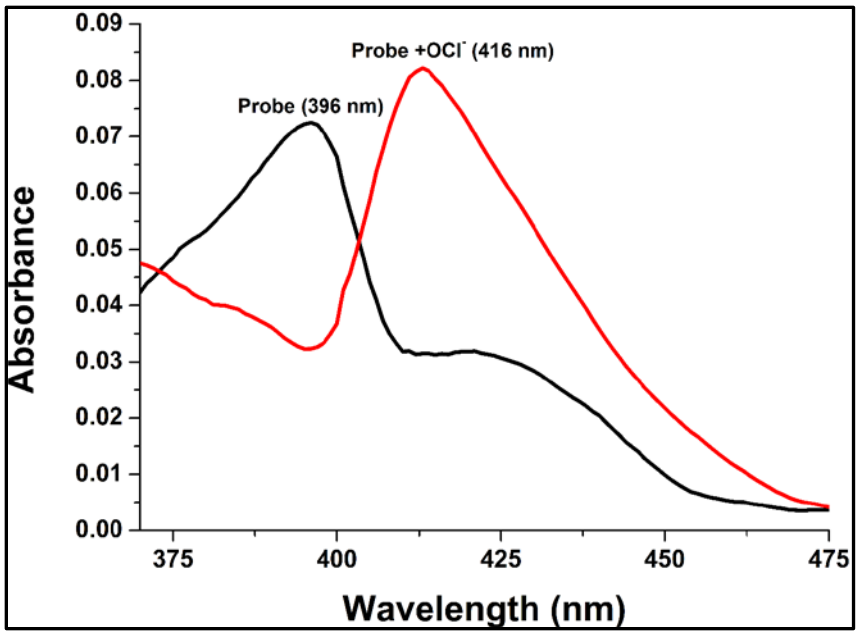


Figure S24. Absorption spectra of the **Probe-OCl** (15.0 μM) and **Probe-OC**l + OCl^-(15.0 μM with 1.0 equiv OCl^-) in the solution (10 mM PBS pH 7.4) incubated for 1.0 min at r.t.

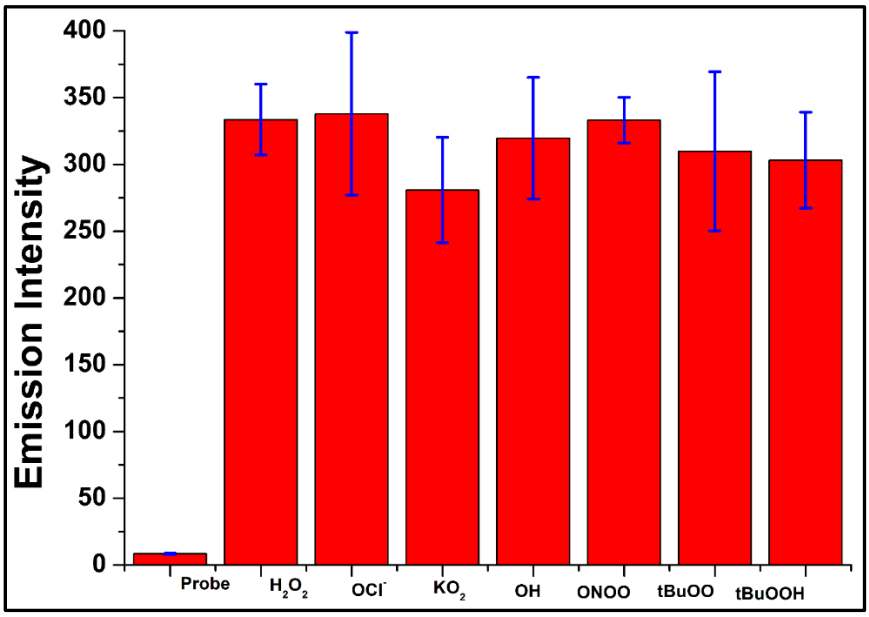


Figure S25. Fluorescence intensity of **Probe-OCl** (15.0 μM) with addition of OCl^- followed by addition of other ROS/RNS in solution (10 mM PBS pH 7.4) incubated for 1.0 min. λ_{ex}: 416 nm, λ_{em}: 523 nm, slit width 3.0 nm/ 3.0 nm.

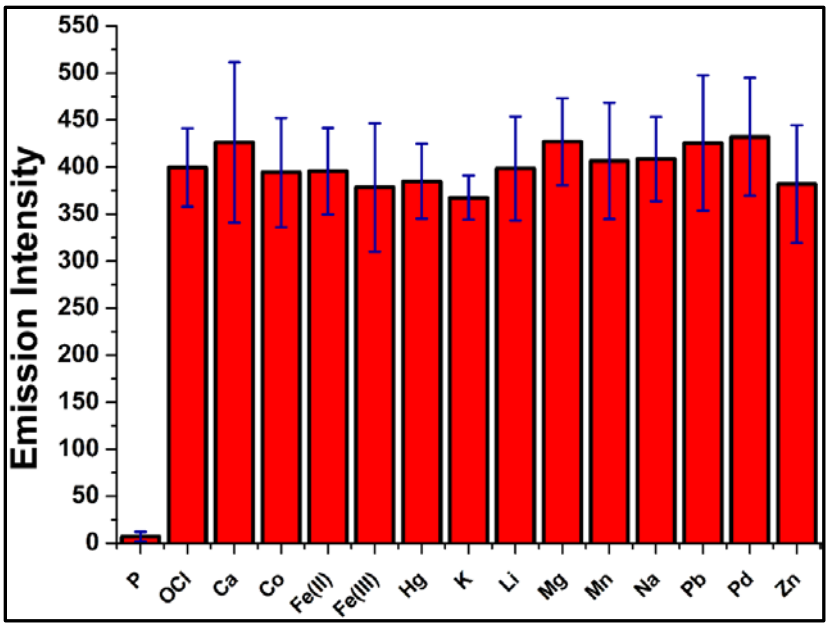


Figure S26. Fluorescence intensity of **Probe-OCl** (15.0 µM) with addition of OCl^- followed by the addition of various metals in solution (10 mM PBS pH 7.4) incubated for 1.0 min. λ_{ex}: 416 nm, λ_{em}: 523 nm, slit width 3.0 nm/3.0 nm.

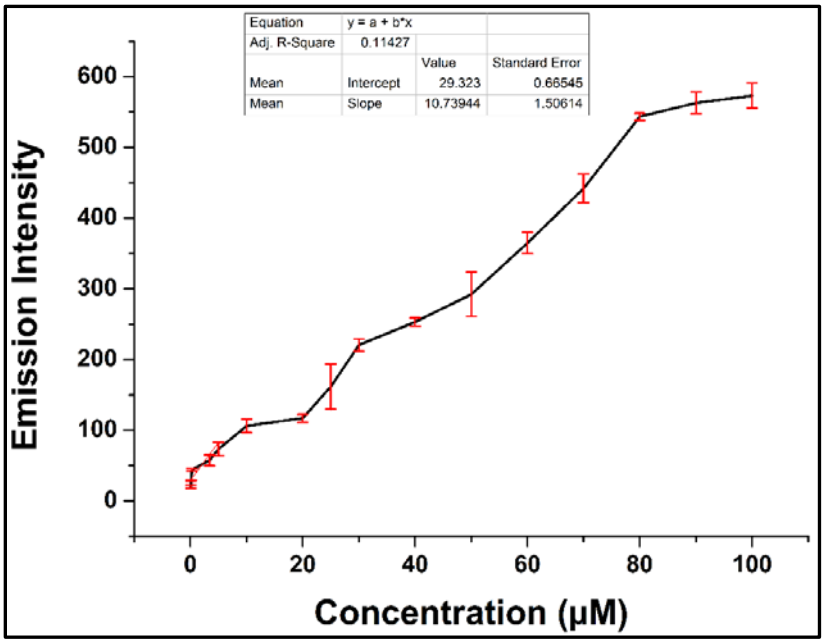


Figure S27. Plot for the calculation of limit of detection from the emission of **Probe-OCl** (15.0 µM) in the solution (10 mM PBS pH 7.4, 3:7 v/v) with increasing concentration of OCl^- (0.0 to 100.0 µM in water) incubated for 1.0 min at r.t, λ_{ex}. 416 nm and λ_{em}. 523 nm slit width 3.0 nm/ 3.0 nm (average of three experiments).

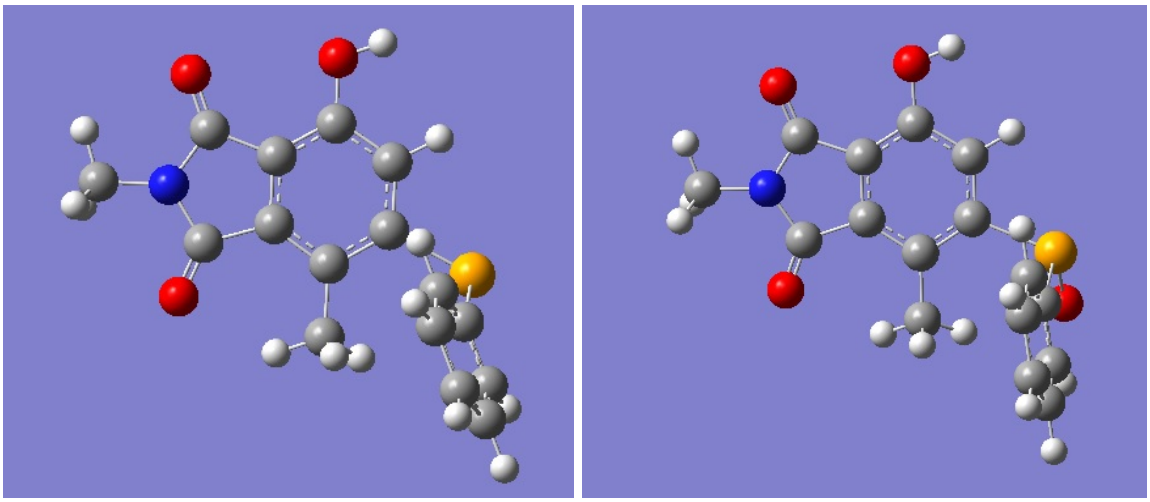
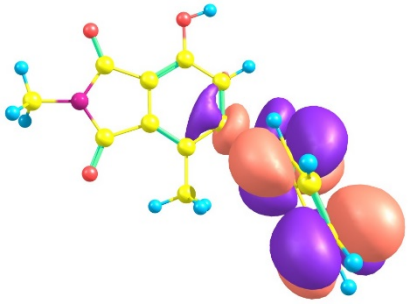
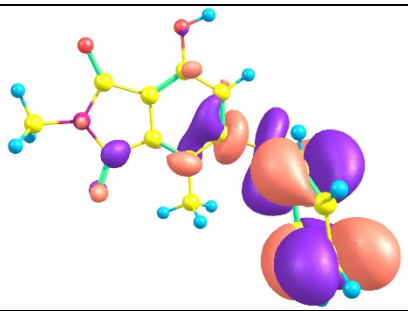
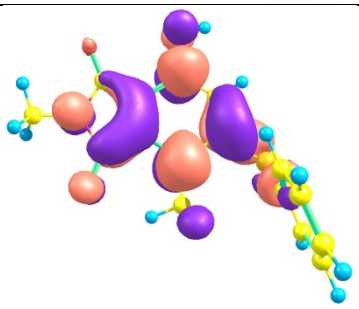
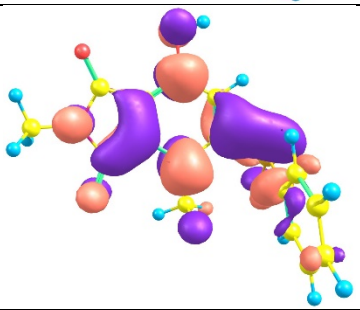


Figure S28. DFT-optimized geometries of (left) probe, and (right) oxidized probe (B3LYP/6-31g* basis set, G09).

	Probe	Oxidized probe
LUMO+2		
LUMO+1		

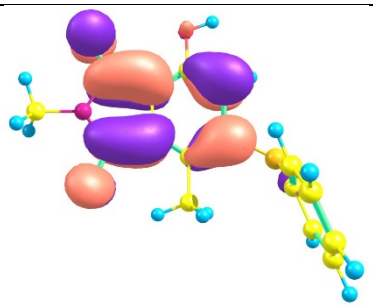
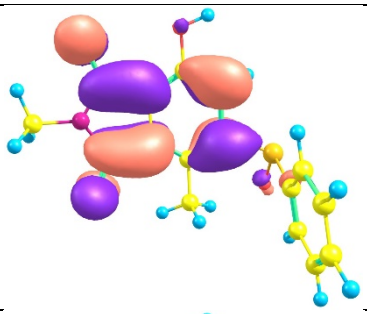
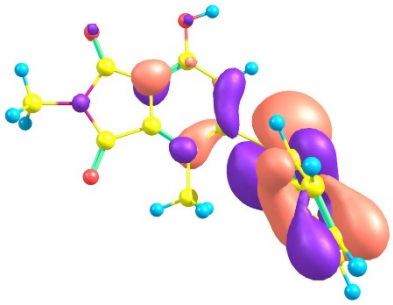
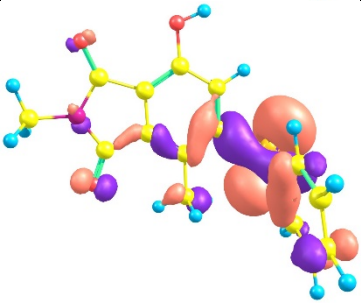
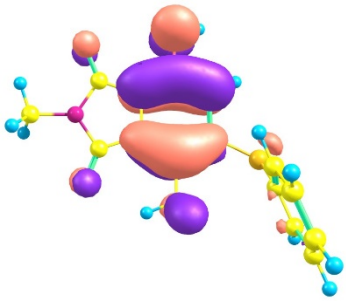
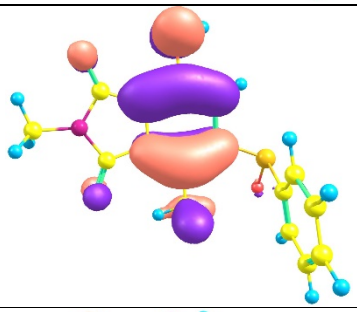
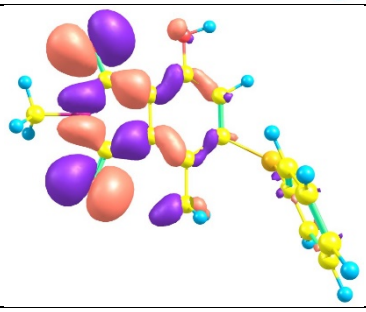
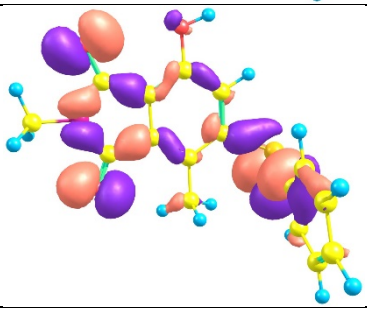
LUMO		
HOMO		
HOMO-1		
HOMO-2		

Figure S30 HOMO-LUMO of DFT-optimized geometries of probe and oxidized probe (B3LYP/6-31g* basis set and 6-311g* only for Se, G09).

	f	Composition	CI(%)
Probe	0.0358	**HOMO → LUMO**	**98.4**
	0.1158	HOMO-2 → LUMO	2.37
		HOMO-1 → LUMO	**86.7**
		HOMO → LUMO+1	5.29
Oxidized probe	0.0020	HOMO-2 → LUMO	7.47
		HOMO → LUMO	**88.1**
	0.1438	**HOMO-1 → LUMO**	**90.7**

Table S3. Absorption energies with largest oscillator strength for Probe and oxidized Probe (B3LYP/6-31g* basis set, G09)

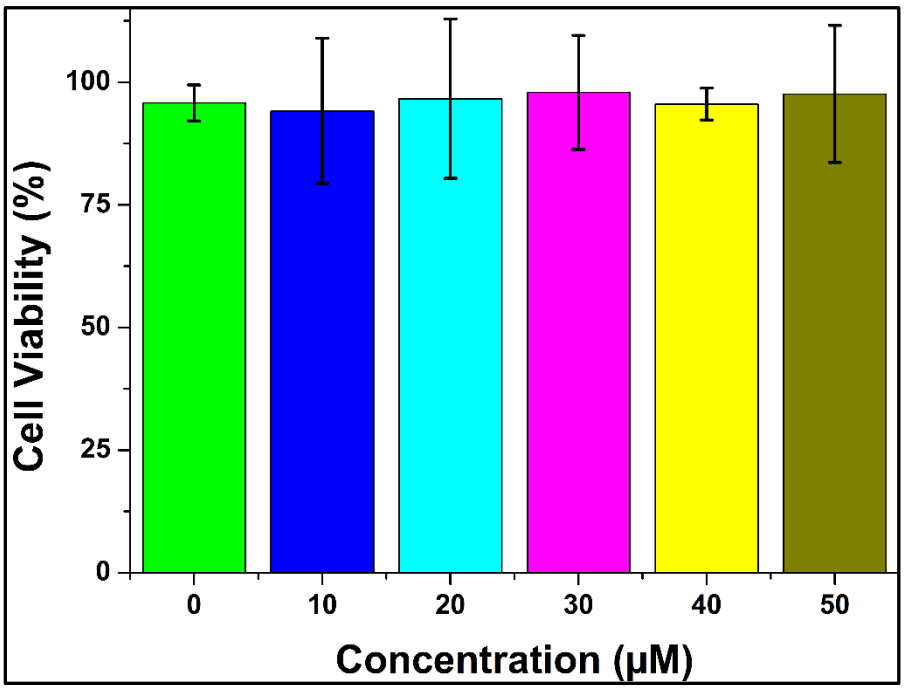


Figure S31. Cell viability was measured by MTT assay for concentration of **Probe-OCl** at 0 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM in 1 hour incubation in U-2 OS cells. Absorbance was determined at 540 nm. Data expressed as a mean ± SD for four experiments.

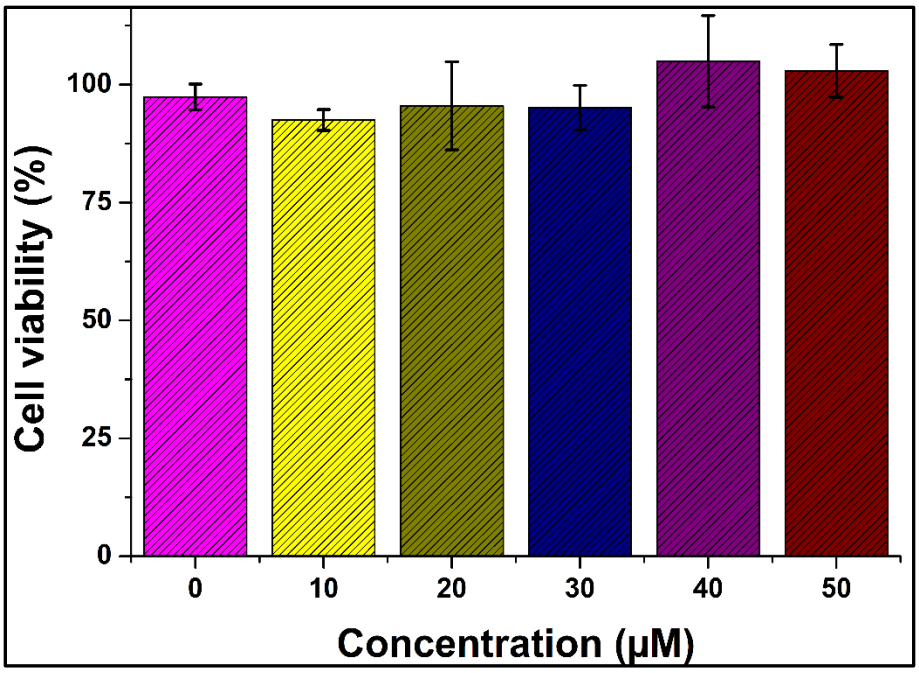


Figure S32. Cell viability was measured by MTT assay for concentration of **Probe-OCl** at 0 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM after 6 hours of incubation with U-2 OS cells. Absorbance was determined at 540 nm. Data is expressed as mean ± SD for four experiments.

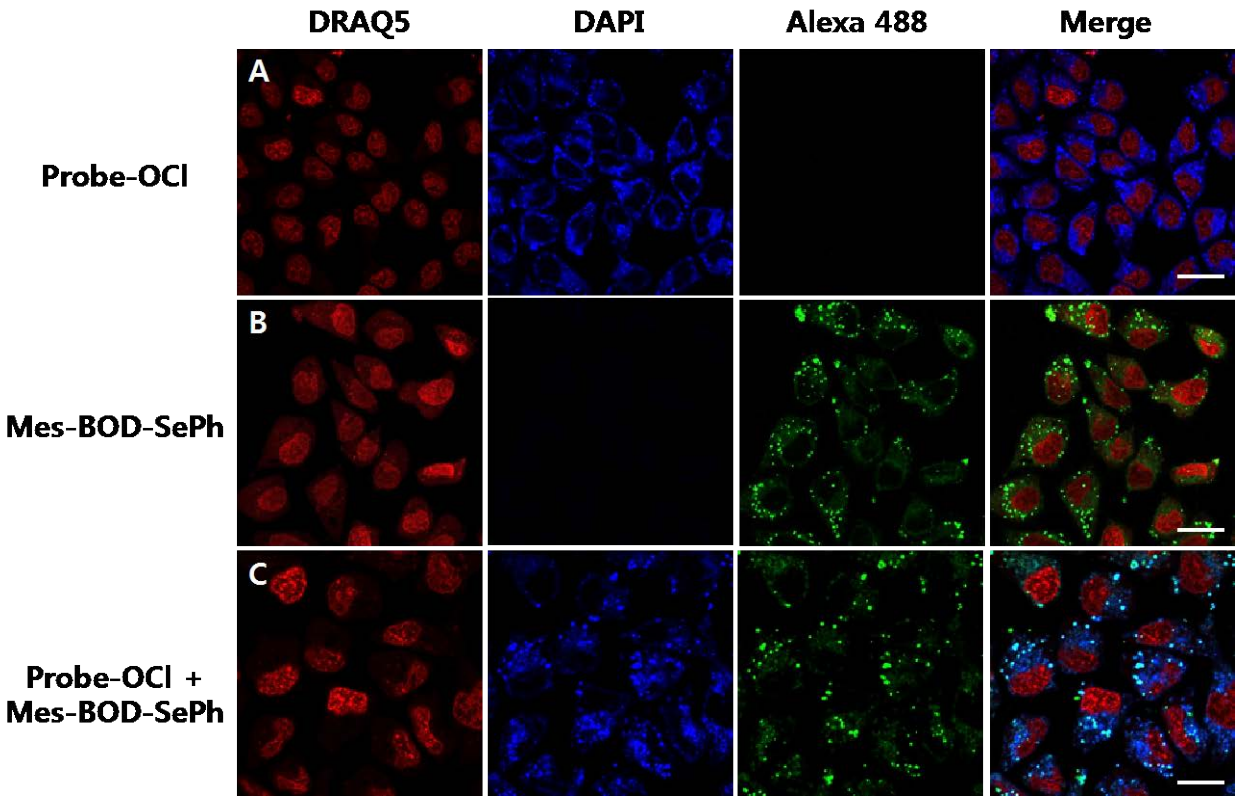


Figure S32. Live cell imaging in HeLa cells with **Probe-OCl** and **Mes-BOD-SePh**. [A] HeLa cells were incubated for 10 min with 25 µM of **Probe-OCl** (blue) in PBS [B] 25 µM **Mes-BOD-SePh** (green) [C] and counterstained for 10 min. **Probe-OCl** signals were consistent with lipid droplet staining dye **MES-BOD-SePh**. Scale bar, 10 µm

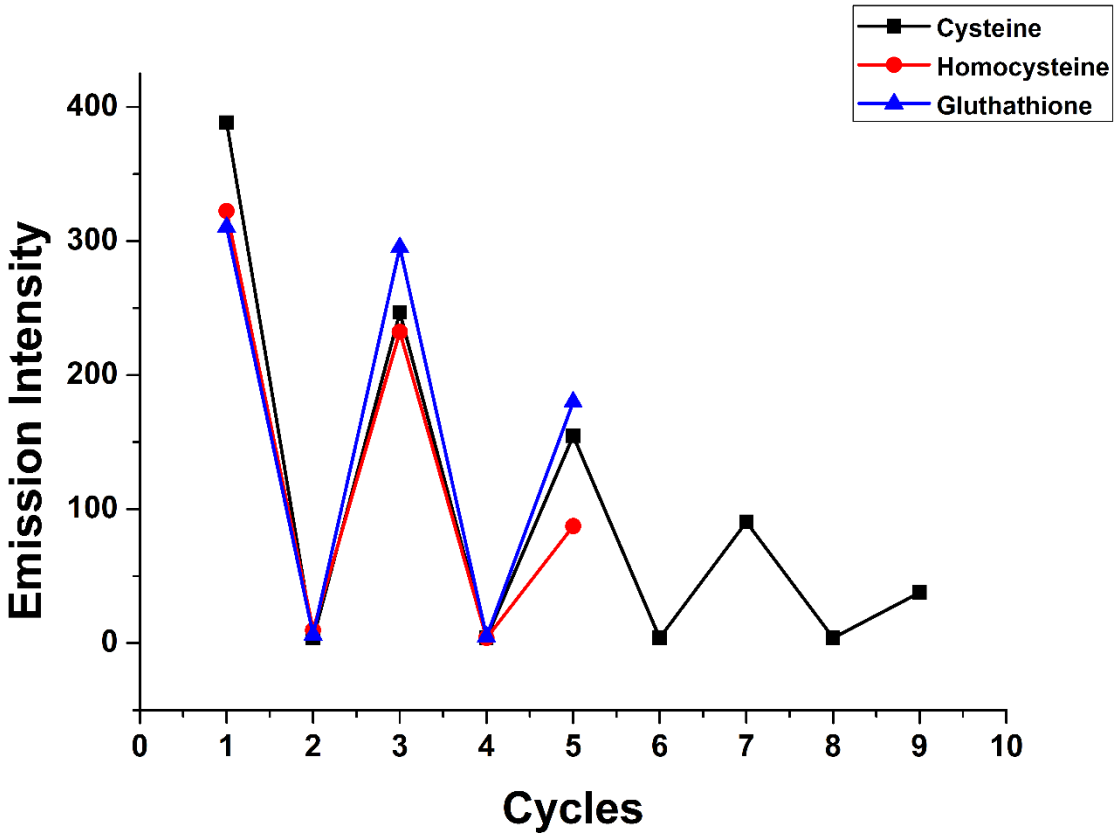


Figure S33. Reversibility of the **Probe-OCl** (15 μM) with addition of 10 equiv. of L-cysteine (5 minutes incubation time), homocysteine, and gluthathione (5 hours incubation time) in solution (10 mM PBS pH 7.4) incubated for 5 hours. λ_{ex}: 416 nm, λ_{em}: 523 nm. Width 3.0 nm/ 3.0 nm.

Reversibility of **Probe-OCl** was studied by added OCl^- (5 equiv) in three vials with 5 minutes of incubation time. The solution become green fluorescent (measured with 365 nm UV lamp) following the addition of cysteine, homocysteine, and gluthathione with an incubation time of 5 minutes for cysteine and 5 hours for homocysteine and gluthathione. We observed a significant decrease in fluorescence intensity after the addition of cysteine into the vial after 4 cycles.

Table S4. Results of experimental determination of log P value by the shake flask method for the **Probe-OCl**

C_1	V	A	Ā	C_2	P	Log P
1.3×10^{-5}	3.5	0.0399 0.0491 0.0454	0.0415	1.24×10^{-5}	1.89	0.276
1.6×10^{-5}	4.8	0.0475 0.0429 0.0461	0.0455	1.36×10^{-5}	6.96	0.842
2.0×10^{-5}	6.0	0.0439 0.0409 0.0440	0.0428	1.28×10^{-5}	22.20	1.34

※ C_1 = Concentration (mol L^{-1}) of the stock solution in *n*-octanol before partition; V = volume (μL) of stock solution; A = absorbance in buffer solution after the partition (λ = 500 nm); Ā = arbitrary absorbance in buffer solution after partitioning (λ = 500 nm); C_2 = concentration (mol L^{-1}) in buffer solution after partitioning; P = partition coefficient; log P = logarithm of the partition coefficient.

Properties (Probe-1) (NOTE: abbreviation same as website)	Value
milogP	**3.37**
TPSA	**59.30**
Natom	**21**
MW	**346.24**
nON	**4**
nOHNH	**1**
nviolations	**0**
nrotb	**2**
volume	**259.99**

Table S5 . Information of the **Probe-OCl** calculated through 'molinspiration property engine v2011.04' at the website, http://www.molinspiration.com.

References:

1. C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Del. Rev.*, **1997**, *23*, 3-25.