Electronic Supplementary Material (ESI) for New Journal of Chemistry.

SUPPORTING INFORMATION

Fluorogenic and red-shifted diphenyl phosphinate-based probe for selective peroxynitrite detection as demonstrated in fixed cells

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Generation 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 the superoxide radical anion source.

Generation of $^\bullet$OH: Hydroxyl radical ($^\bullet$OH) was generated by the Fenton reaction. To generate $^\bullet$OH, ferrous chloride was added in the presence of 10 equiv of H_2O_2. The concentration of $^\bullet$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). Sodium hydroxide (1.5 M) was added within 1-2 s to make the solution alkaline. The resulting solution was stored at a temperature of −18 °C or lower. 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 $M^{-1}cm^{-1}$.

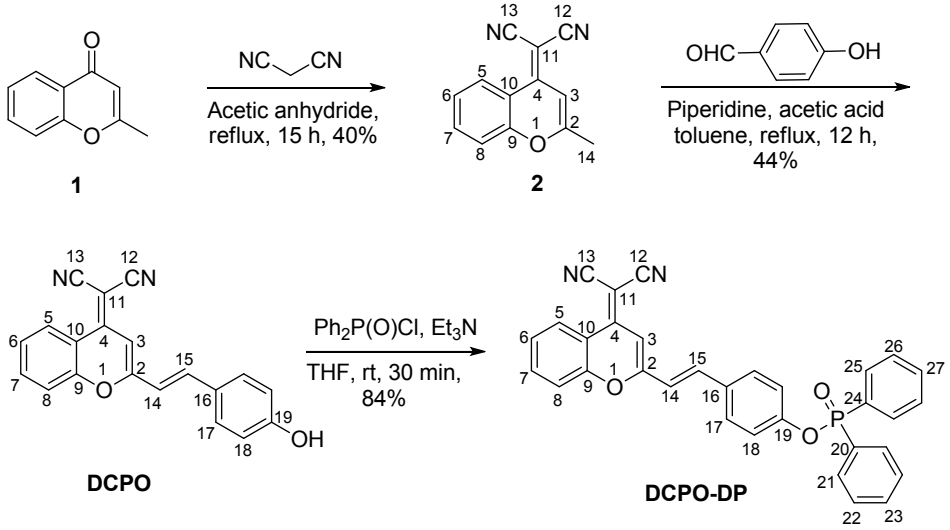
Generation of NO•: Nitric oxide was generated from SNP (sodium nitroferricyanide (III) dehydrate). Experiments were performed under anaerobic conditions. The deionized water was degassed with Ar for 30 min and then SNP was added into it under an Ar atmosphere and was stirred for 30 min at room temperature.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

Determination of the detection limit:

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectra of probe **DCPO-DP** were measured three times; the standard deviation measurement of the blank trial was also acquired. To obtain the slope, the fluorescence intensity at 690 nm was plotted as a concentration of $ONOO^-$. So the detection limit was calculated in accord with the following equation:

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

Where σ is the standard deviation of 10 blank measurements, *k* is the slope between the fluorescence intensity, versus $ONOO^-$ concentration.



Scheme 1. Synthetic scheme and numbering of atoms.

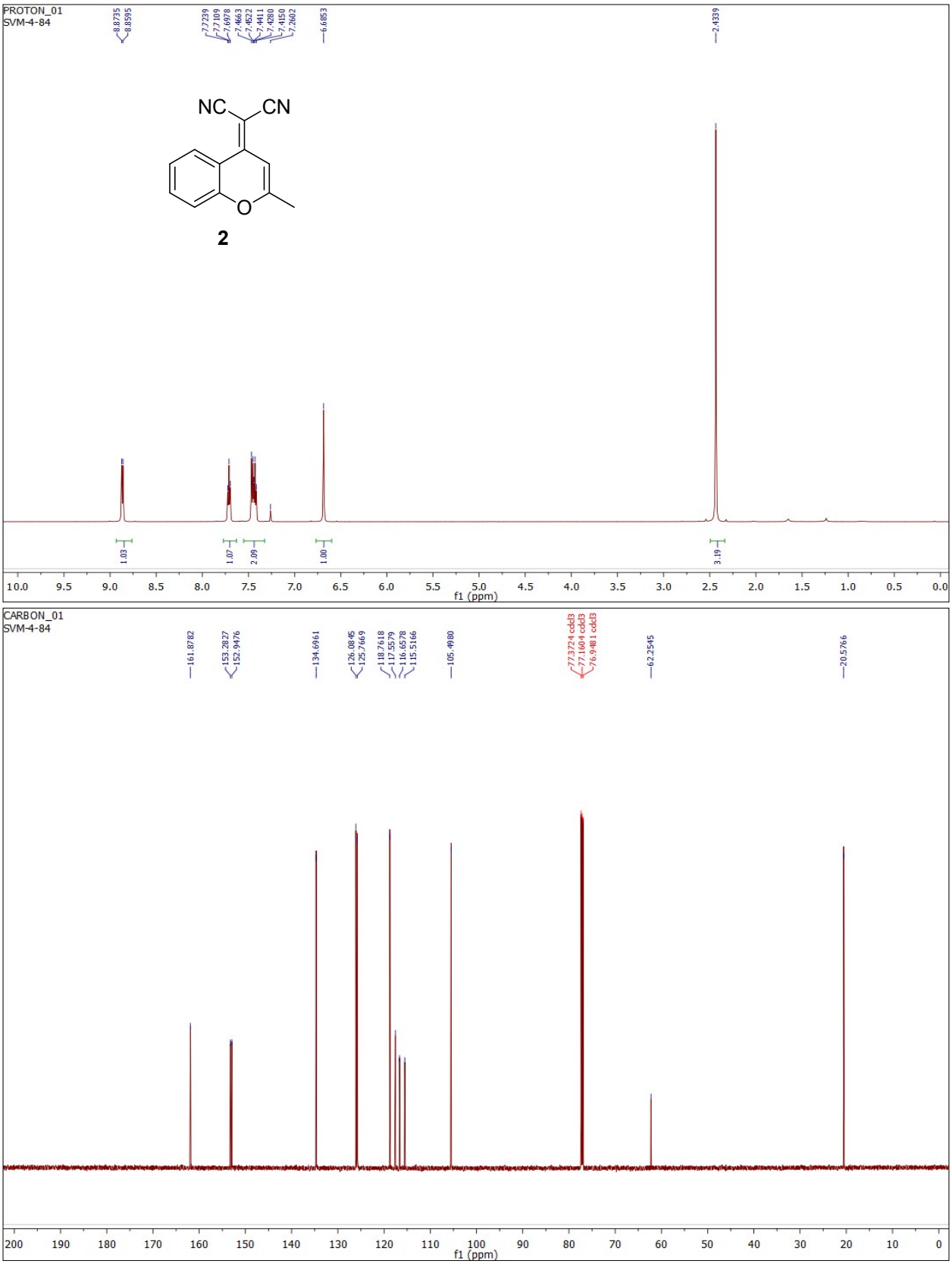


Fig. S1. (*top*) ^{1}H and (*bottom*) ^{13}C NMR spectrum of compound **2**.

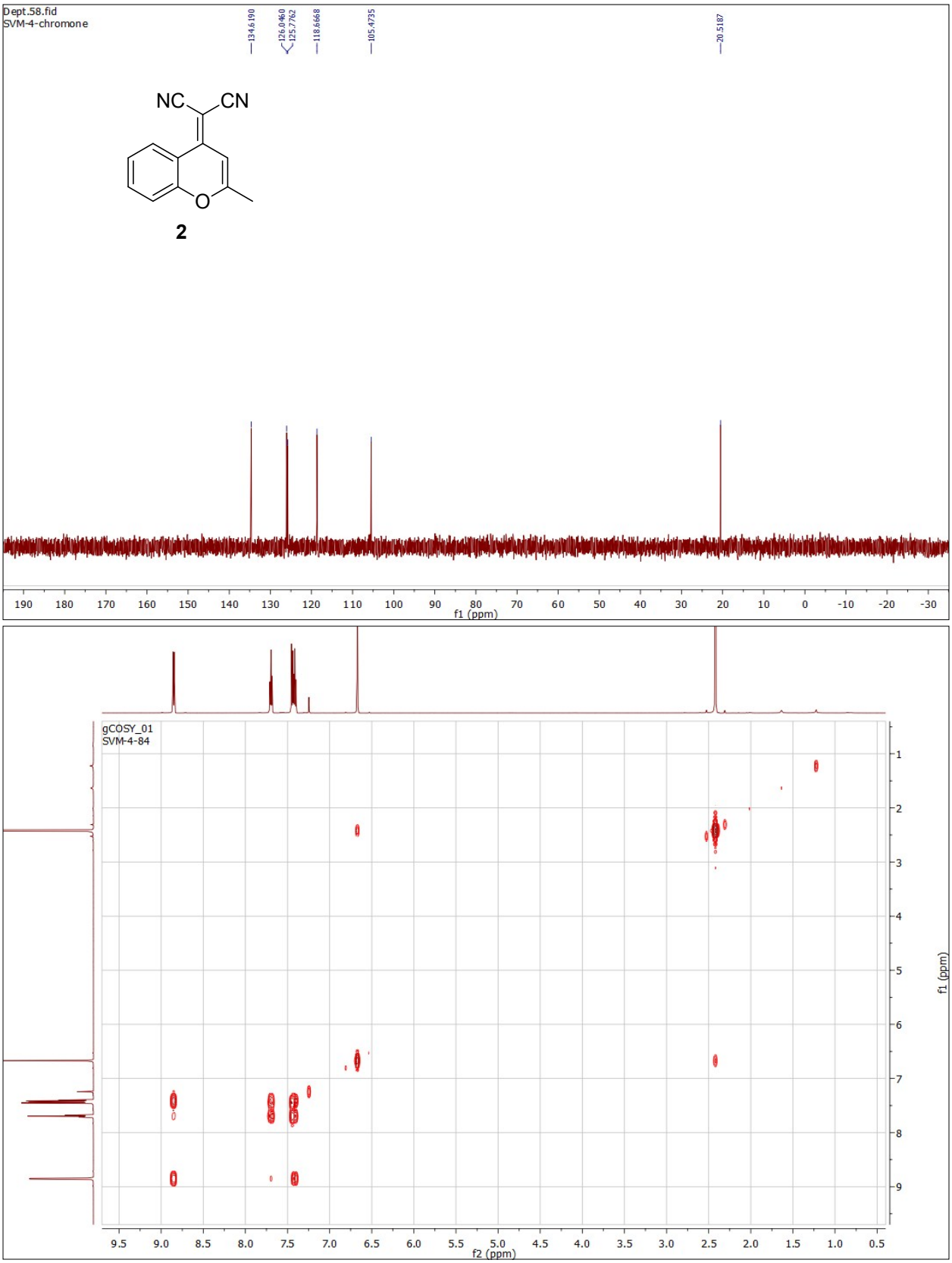


Fig. S2. (*top*) DEPT NMR and (*bottom*) COSY NMR spectrum of compound **2**.

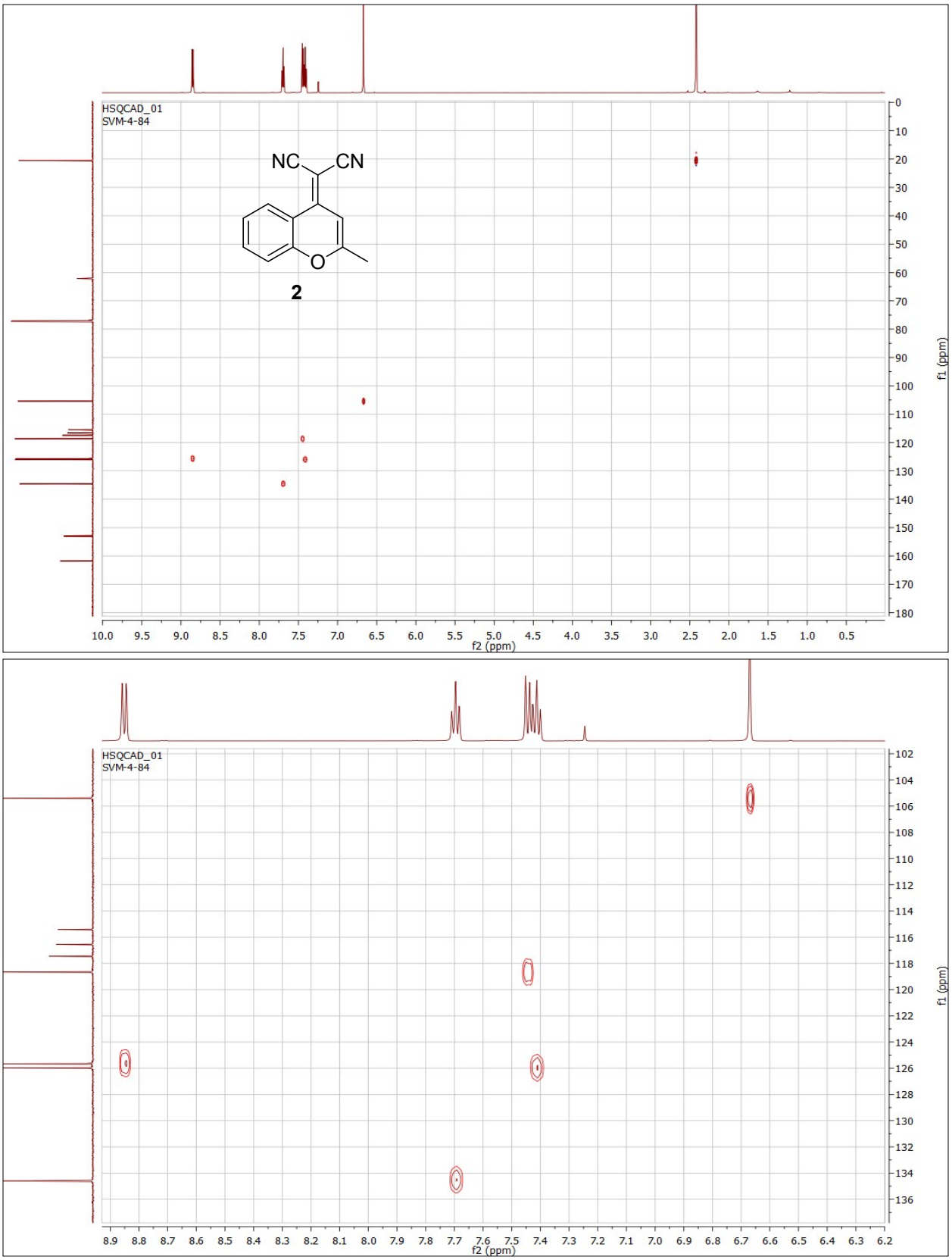


Fig. S3. (*top*) ^{1}H–^{13}C HSQC NMR spectrum of **2** and (*bottom*) expanded aromatic region.

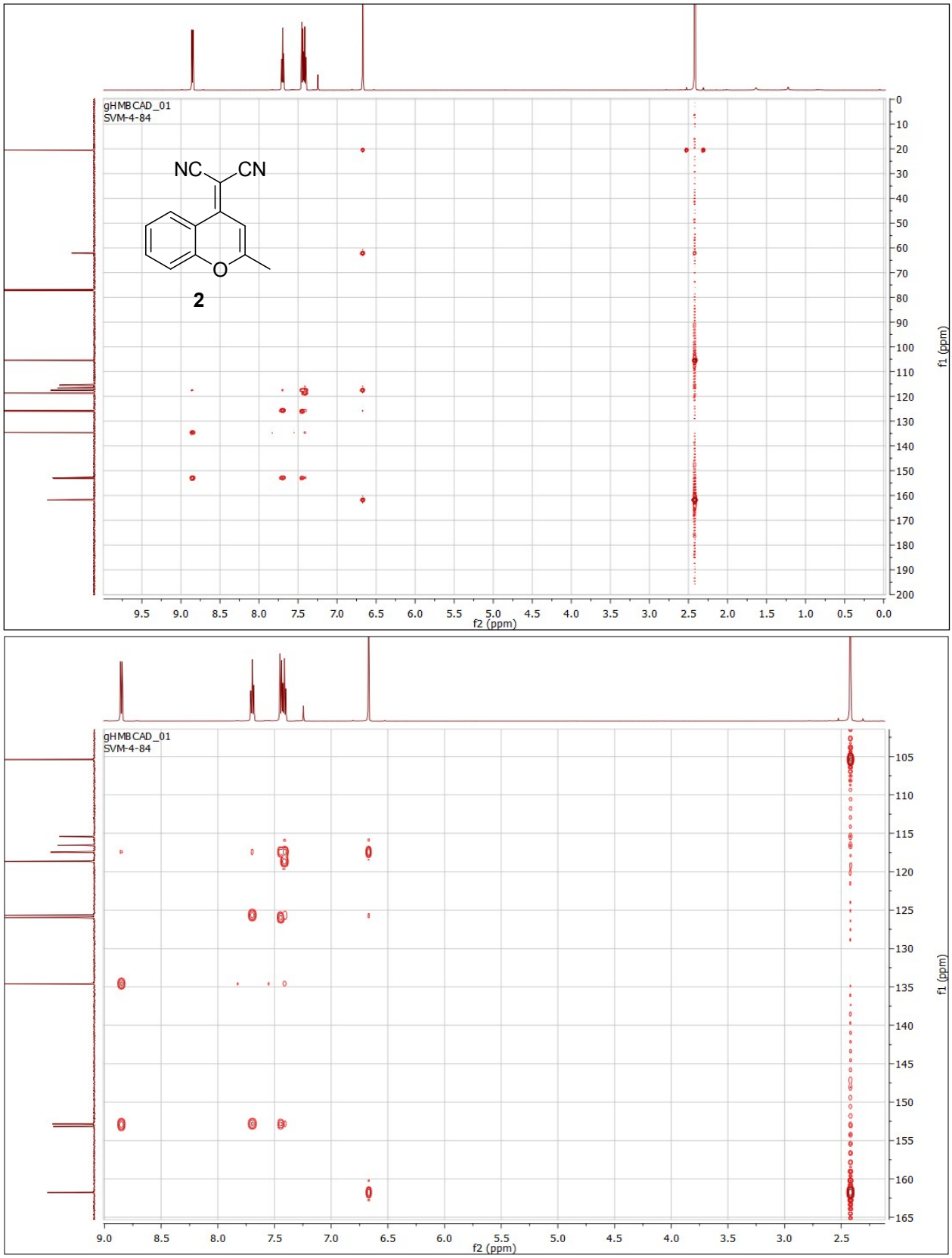
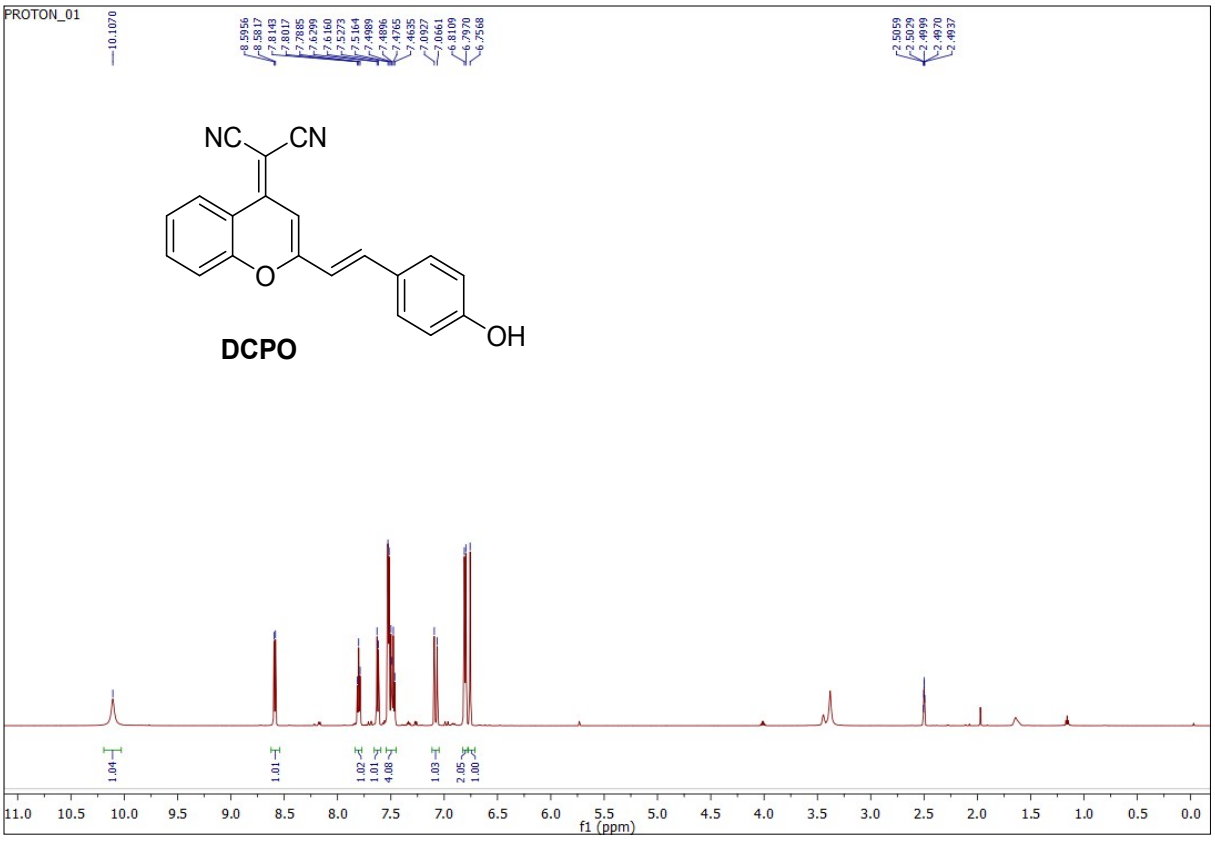
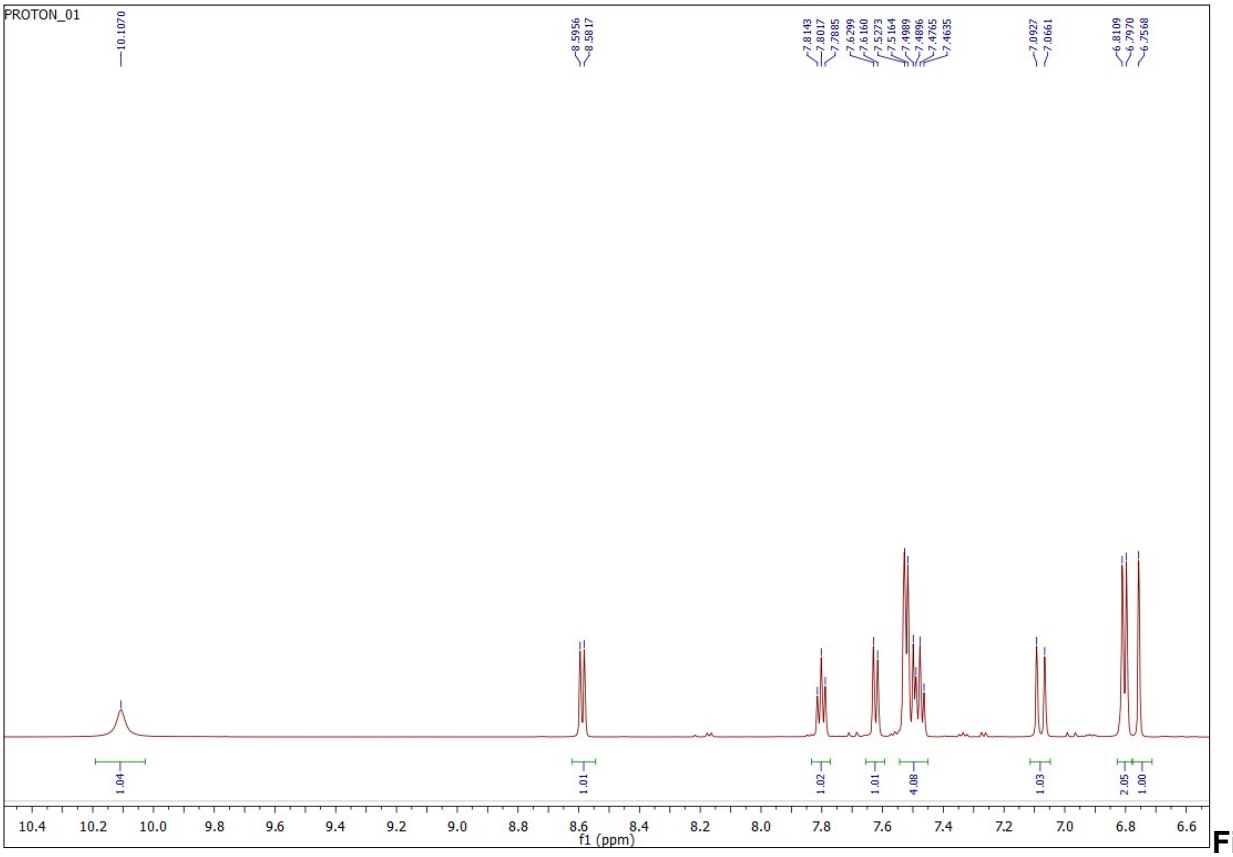


Fig. S4. (*top*) ^{1}H–^{13}C HMBC NMR spectrum of **2** and (*bottom*) expanded aromatic region.





Fi

g. S5. (*top*) ^{1}H NMR spectrum of **DCPO** and (*bottom*) expanded aromatic region.

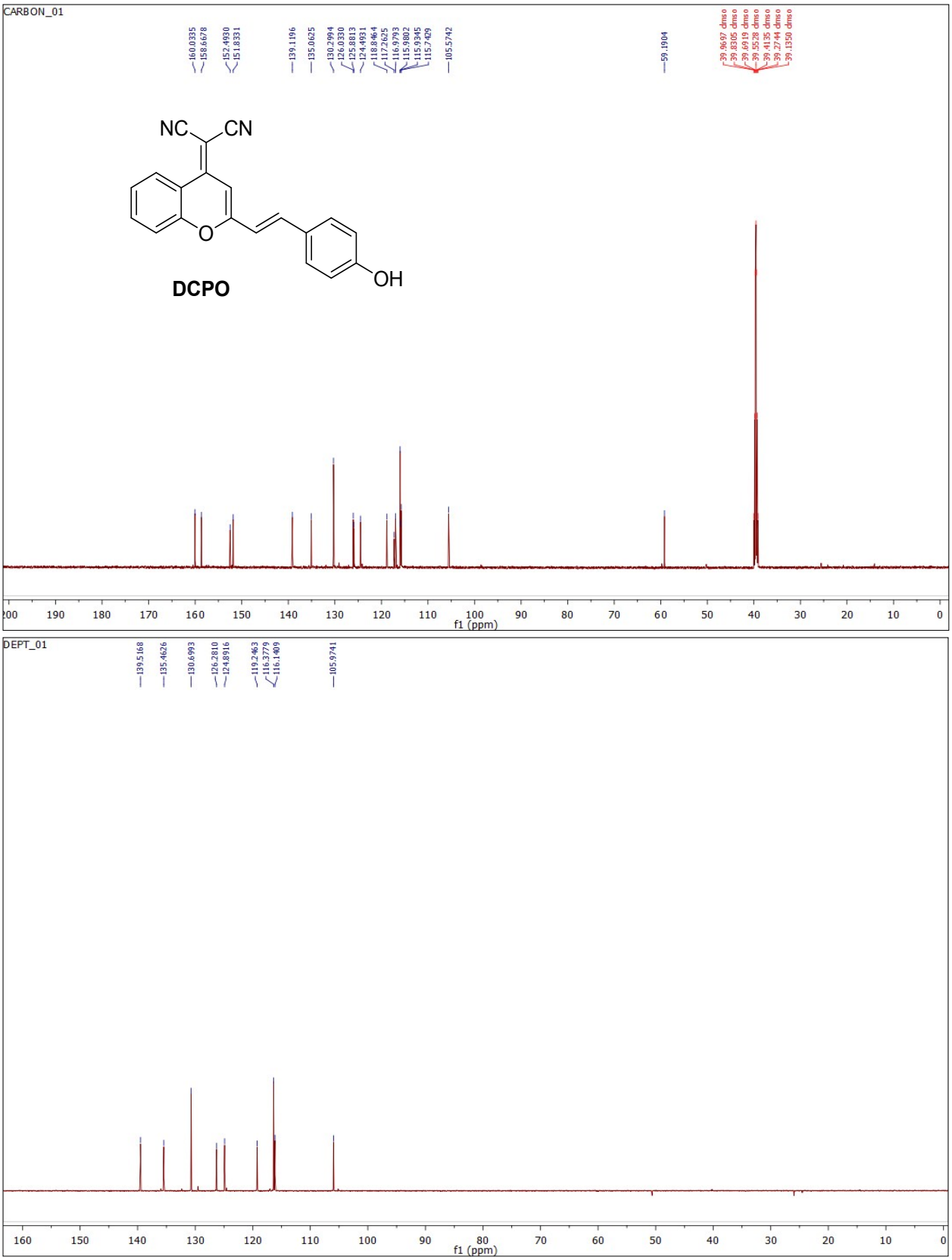


Fig. S6. (*top*) ^{13}C NMR and (*bottom*) DEPT NMR spectrum of **DCPO**.

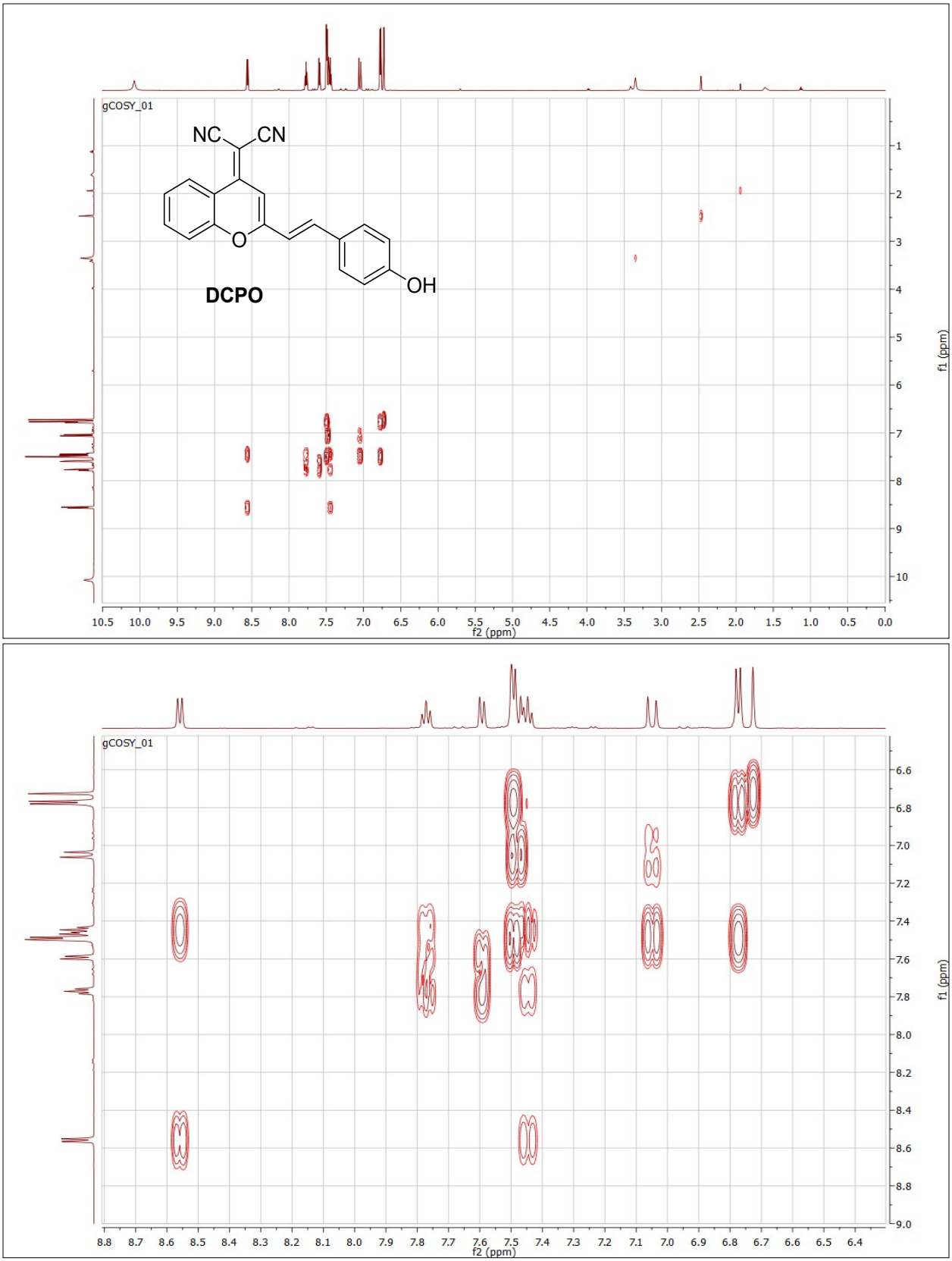


Fig. S7. (*top*) COSY NMR spectrum of **DCPO** and (*bottom*) expanded aromatic region.

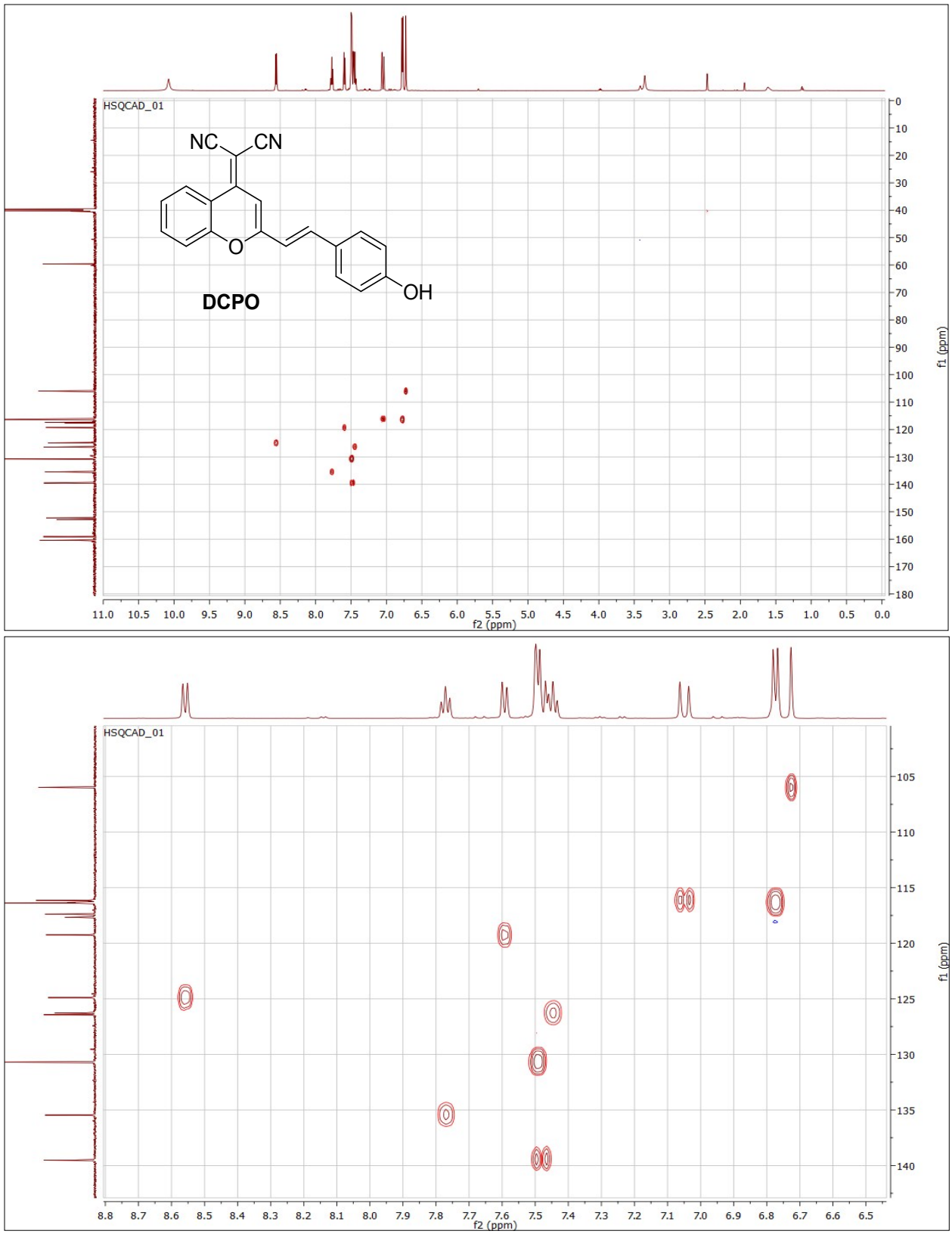


Fig. S8. (*top*) $^{1}H–^{13}C$ HSQC NMR spectrum of **DCPO** and (*bottom*) expanded aromatic region.

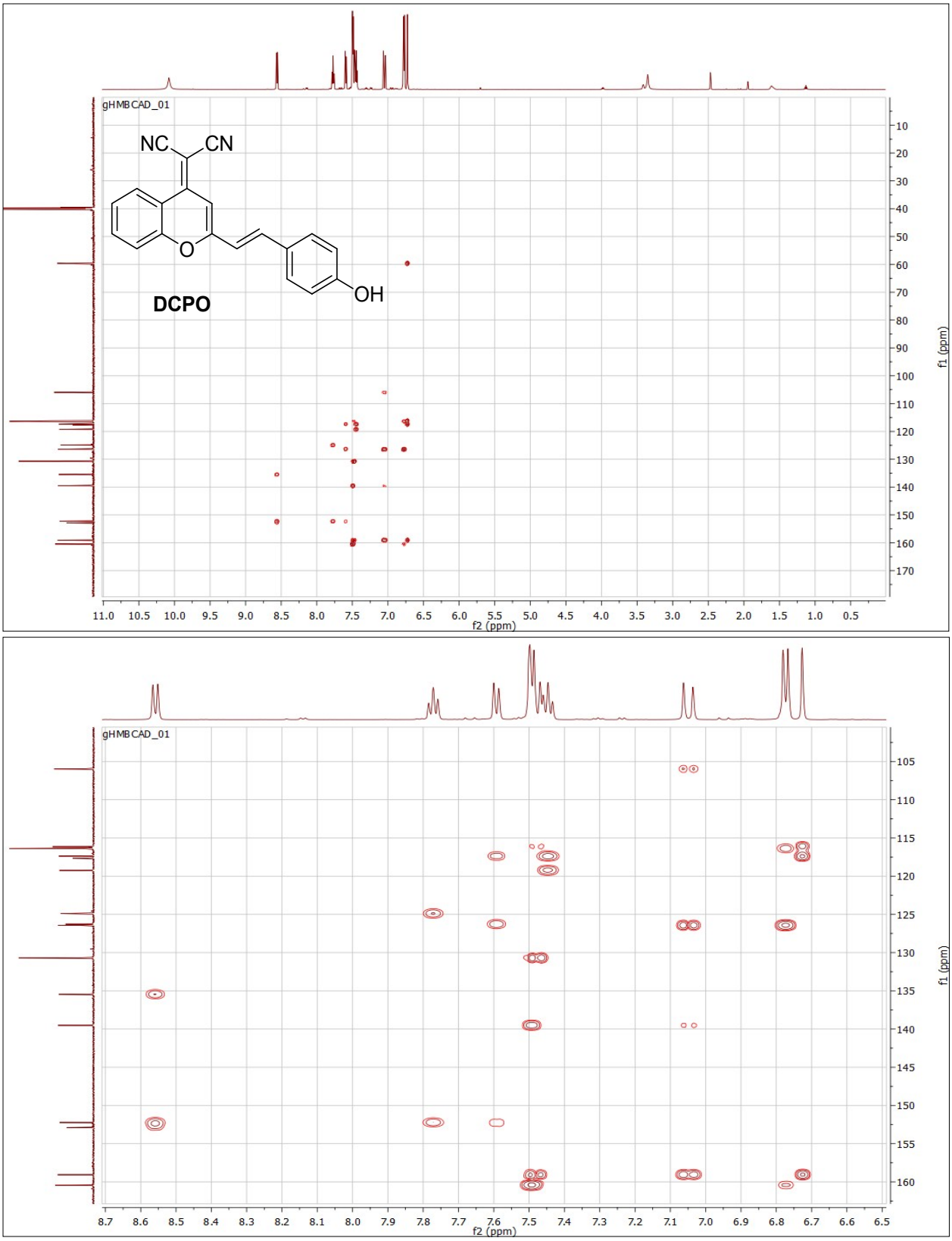


Fig. S9. (*top*) $^{1}H–^{13}C$ HMBC NMR spectrum of **DCPO** and (*bottom*) expanded aromatic region.

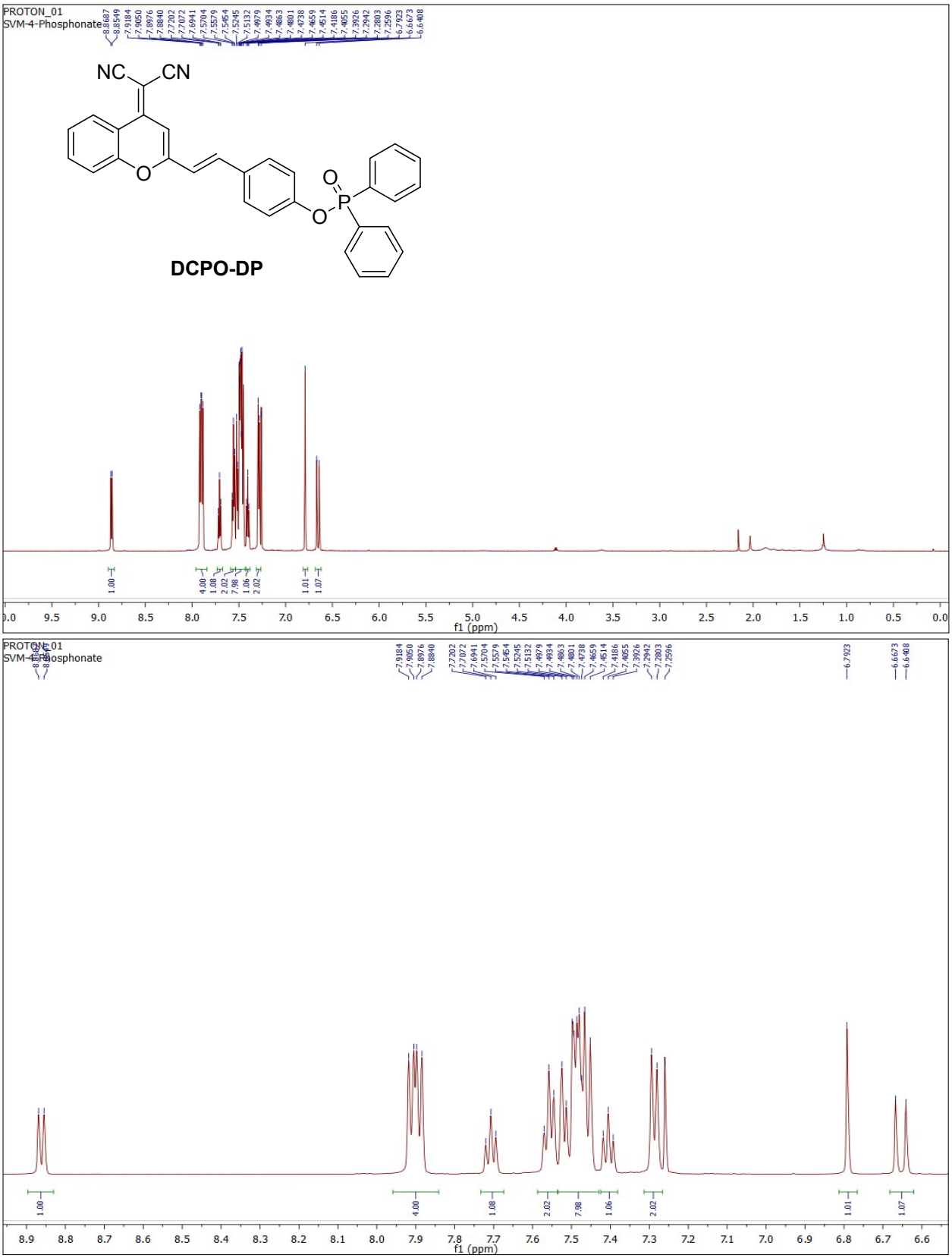


Fig. S10. (*top*) ^{1}H NMR spectrum of **DCPO-DP** and (*bottom*) expanded aromatic region.

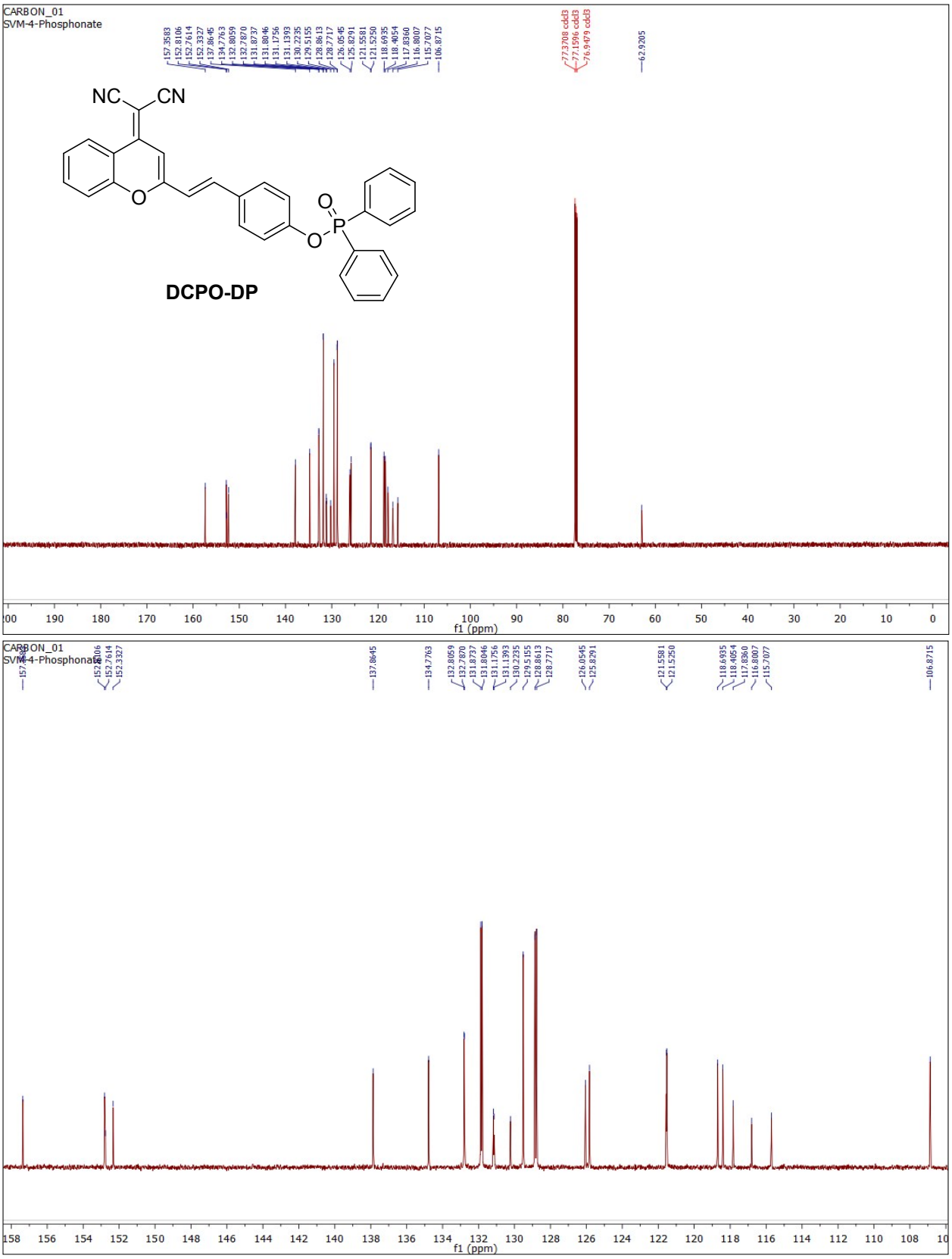
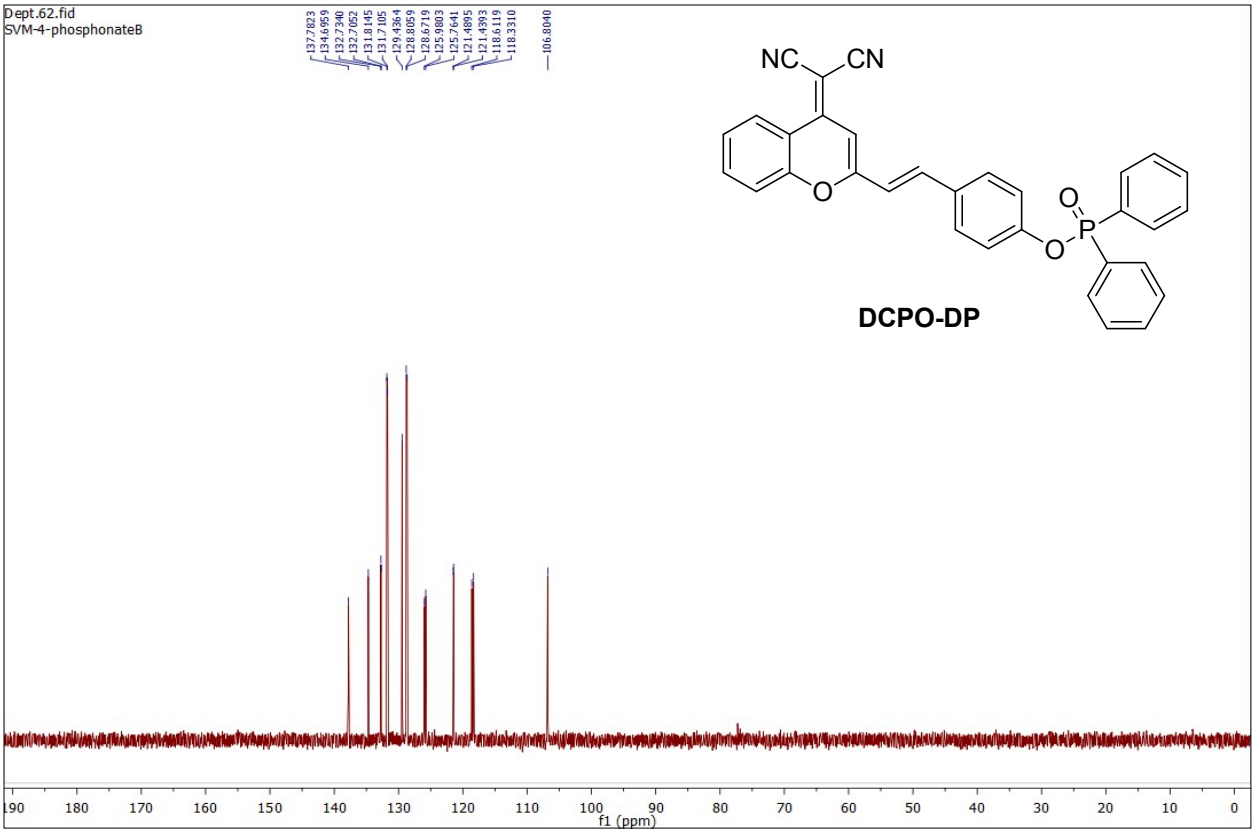


Fig. S11. (*top*) ^{13}C NMR spectrum of **DCPO-DP** and (*bottom*) expanded aromatic region.


Dept.62.fid
SVM-4-phosphonateB
137.7823
134.6959
132.7340
132.7062
131.9145
131.7105
129.9364
128.8059
128.6719
125.9803
125.7641
121.4895
121.4393
118.6119
118.3310
106.8040
NC
CN
O
O
P
O
DCPO-DP
f1 (ppm)

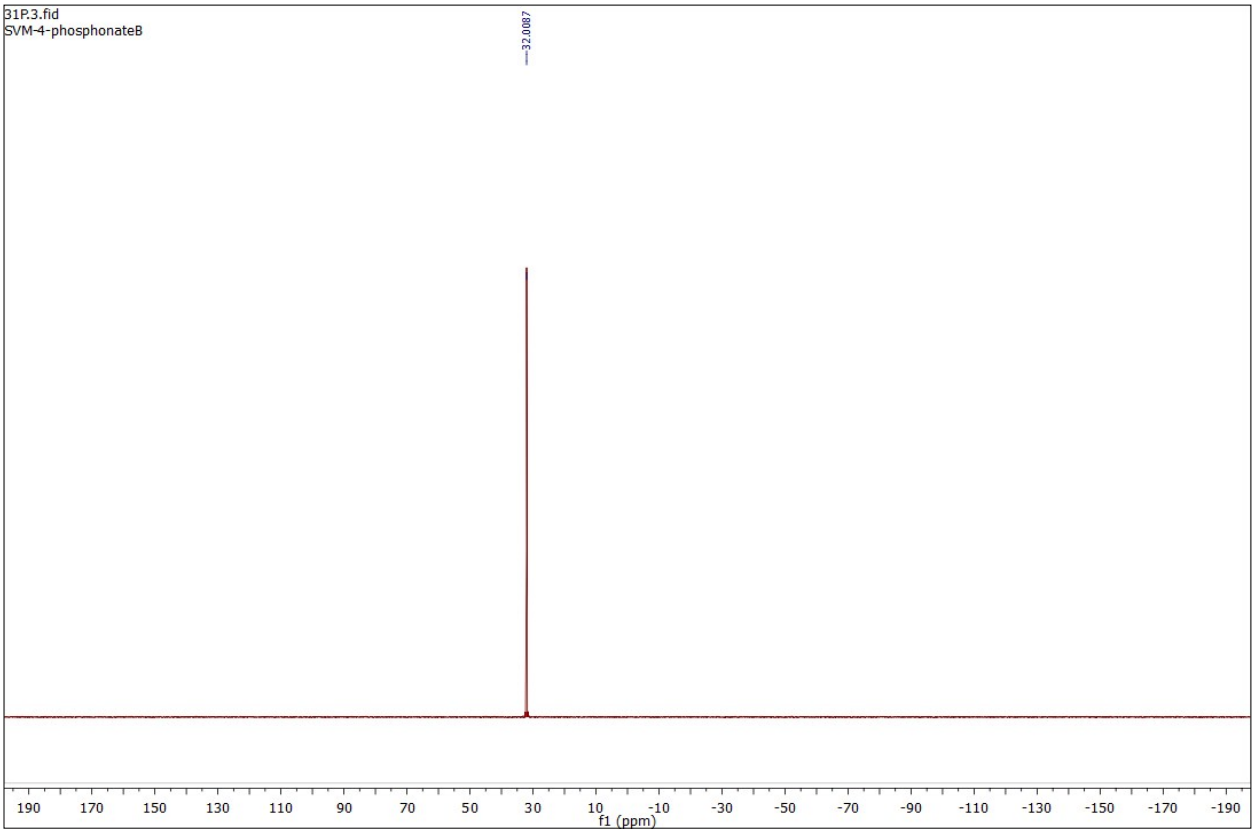

31P.3.fid
SVM-4-phosphonateB
32.0087
f1 (ppm)

Fig. S12. (*top*) DEPT NMR and (*bottom*) ^{31}P NMR spectrum of **DCPO-DP**.

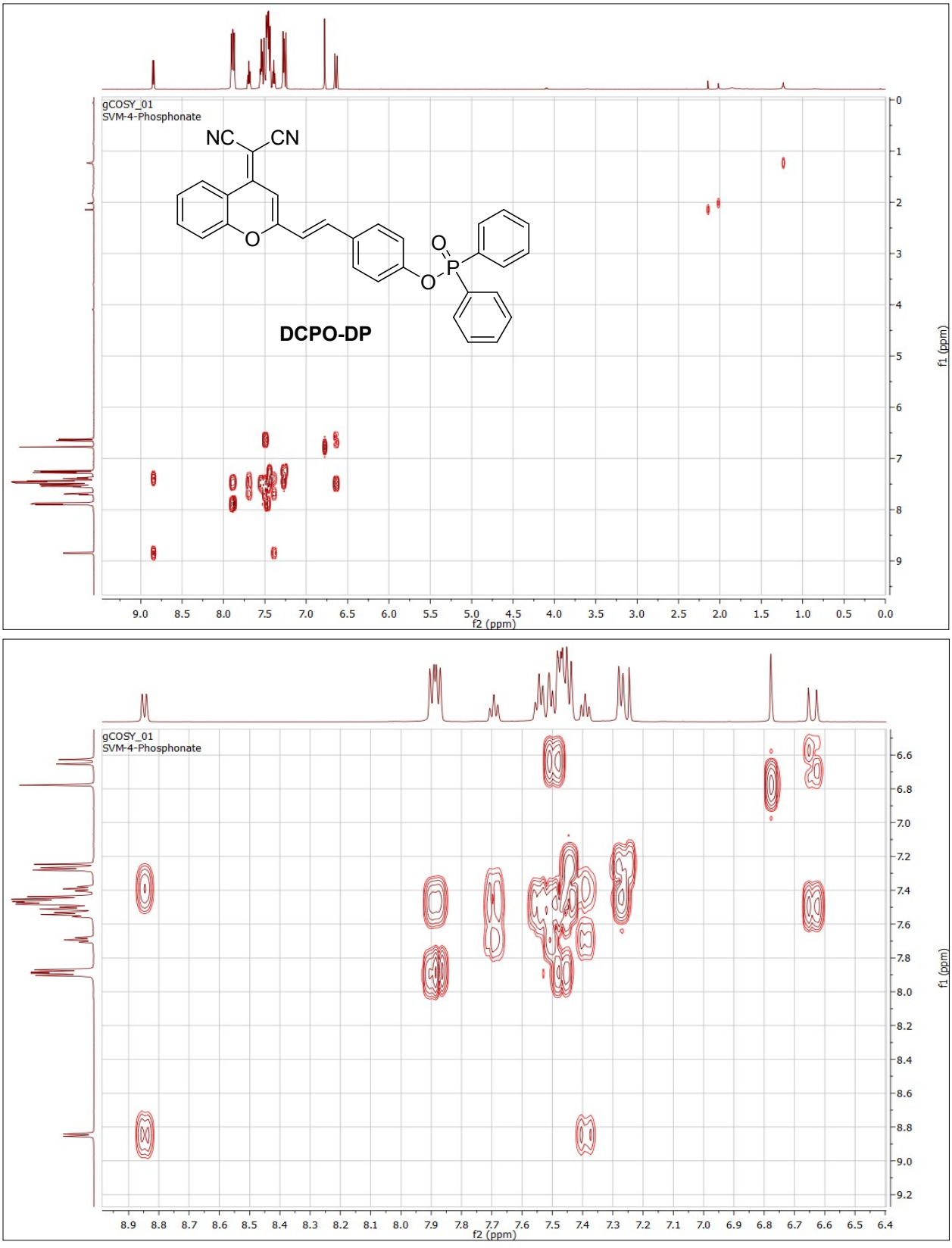


Fig. S13. (*top*) COSY NMR spectrum of **DCPO-DP** and (*bottom*) expanded aromatic region.

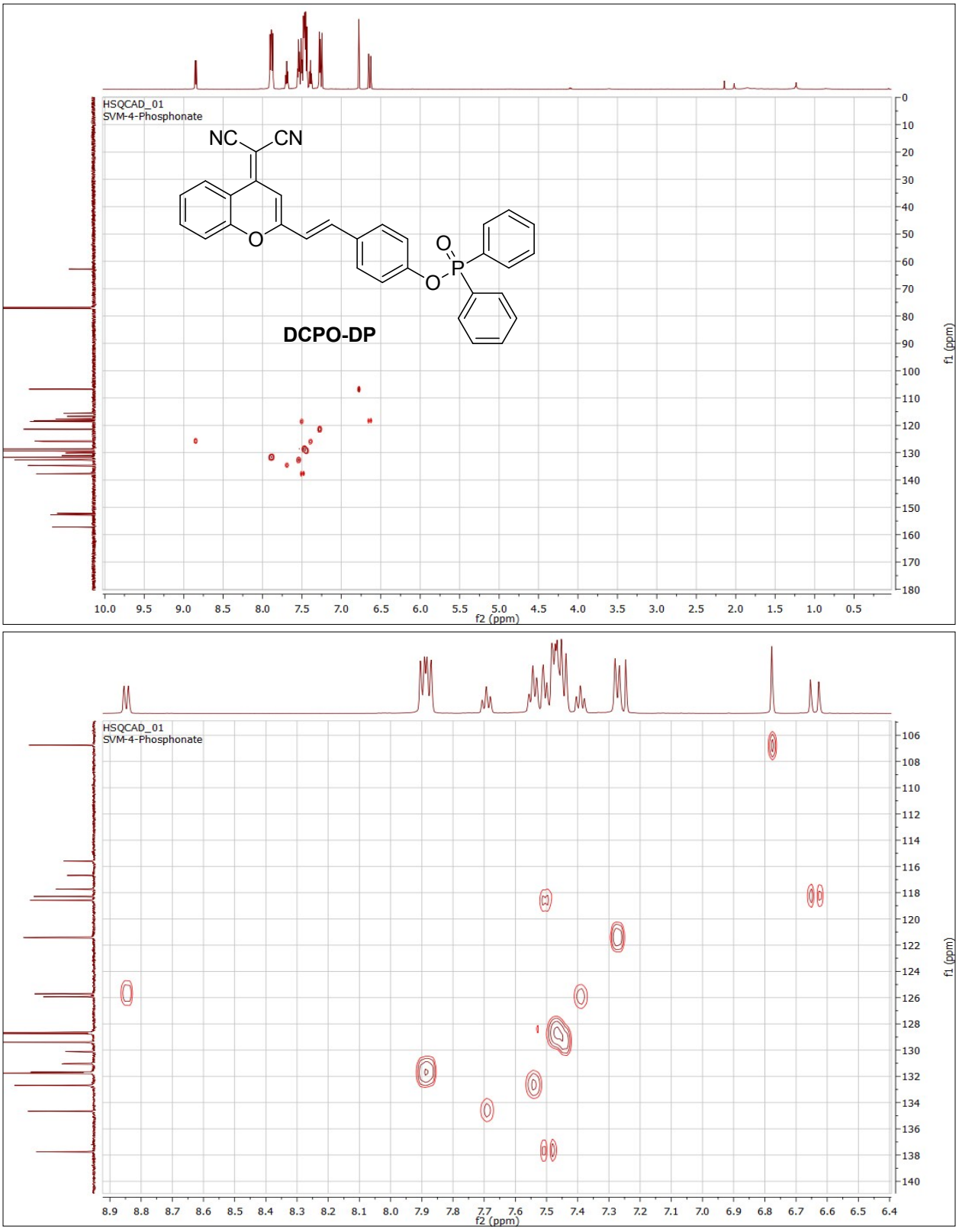


Fig. S14. (*top*) ^{1}H–^{13}C HSQC NMR spectrum of **DCPO-DP** and (*bottom*) expanded aromatic region.

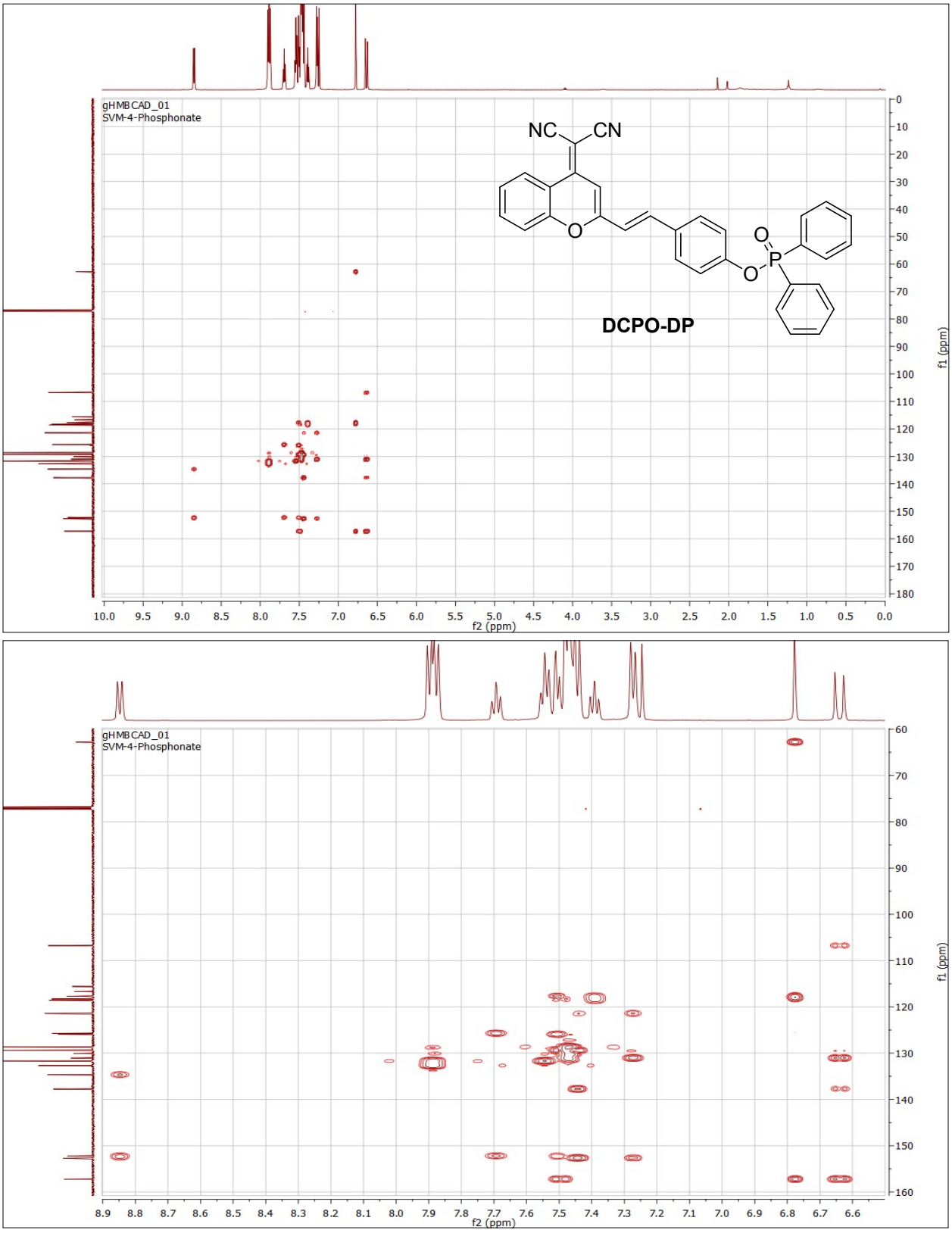


Fig. S15. (*top*) $^{1}H–^{13}C$ HMBC NMR spectrum of **DCPO-DP** and (*bottom*) expanded aromatic region.

Mass Spectrum List Report

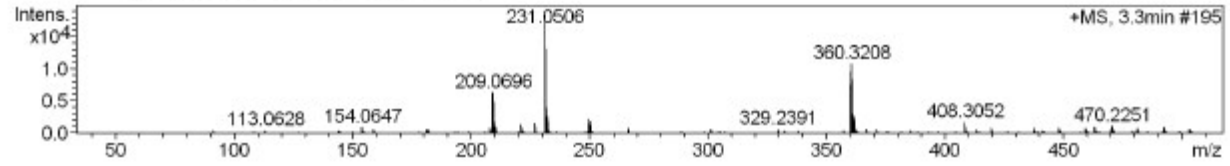
Analysis Info

Analysis Name	D:\Data\2016\1208\Sandip1.d		
Method	Tune low_pos_50_650mz_160629.m	Operator	BDAL@KR
Sample Name	Sandip1	Instrument / Ser#	micrOTOF-Q 10184
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	650 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source

Sandip1.d: BPC 48.9989-650.9910 +All MS



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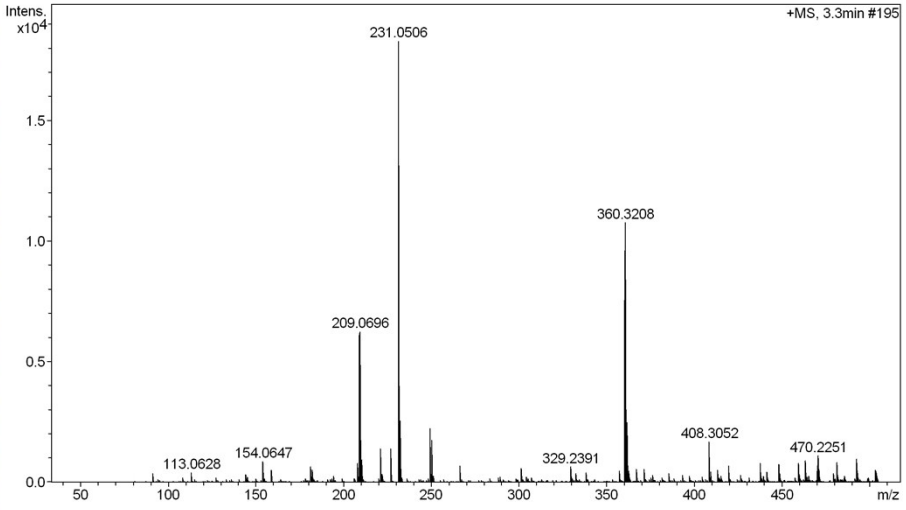
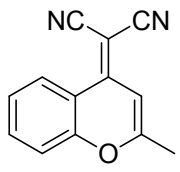
5	209.0696	6256
6	210.0726	936
7	221.1705	1381
8	226.9501	1395
9	231.0506	18297
10	232.0545	2569
11	249.0632	2236
12	250.1743	1762
13	266.1697	673
14	301.2006	566
15	329.2391	643
16	360.3208	10762
17	361.3244	2479
18	367.0519	526
19	371.1607	532
20	408.3052	1684
21	413.2649	511
22	419.2636	685
23	437.2074	799
24	448.2136	761
25	459.2295	803
26	463.2922	880
27	470.2251	1102
28	470.7275	531
29	481.2407	810
30	492.2409	956





2

Calculated $[M + Na]^+ = 231.0534$

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Fig. S16. Mass spectrum of compound **2**.

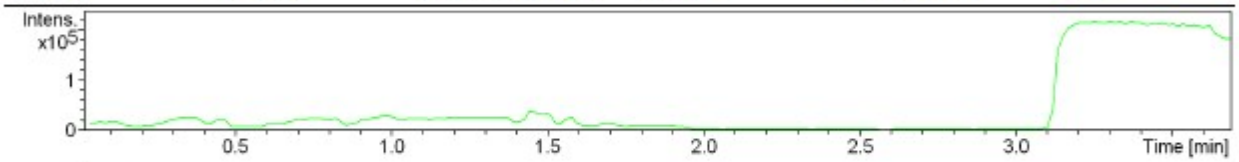
Mass Spectrum List Report

Analysis Info

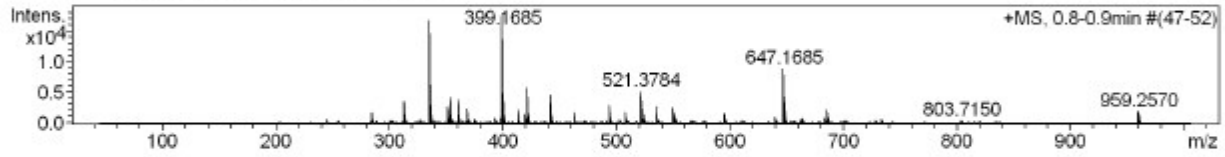
Analysis Name	D:\Data\2017\01\0117\72-1.d		
Method	Tune low_pos.m	Operator	BDAL@KR
Sample Name	72-1	Instrument / Ser#	micrOTOF-Q 10184
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	320.0 Vpp	Set Divert Valve	Source



72-1.d: BPC 49.0041-1000.9811 +All MS



#	m/z	I
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4	336.0800	3356
5	351.0520	2721
6	353.0861	4161
7	360.3211	3873
8	368.4223	2220
9	399.1685	17462
10	400.1713	4552
11	413.2656	2078
12	419.2725	1300
13	421.1509	5627
14	422.1542	1639
15	441.2950	4509
16	463.2999	1700
17	493.3463	2942
18	507.3271	1825
19	521.3784	5112
20	522.3801	1533
21	524.1941	1342
22	535.2405	2688
23	549.4108	2519
24	551.3581	1676
25	595.3776	1467
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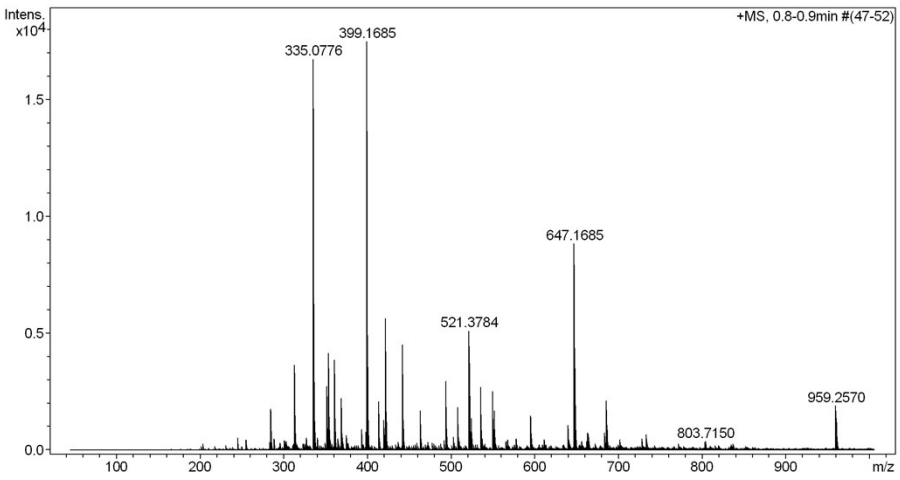
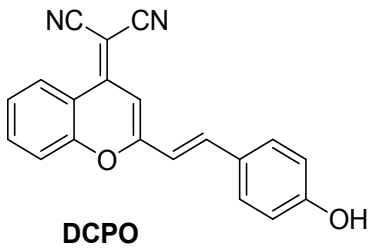
29	959.2570	1888
30	960.2565	1376





DCPO

Calculated $[M + Na]^+ = 335.0796$

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Fig. S17. Mass spectrum of compound **DCPO**.

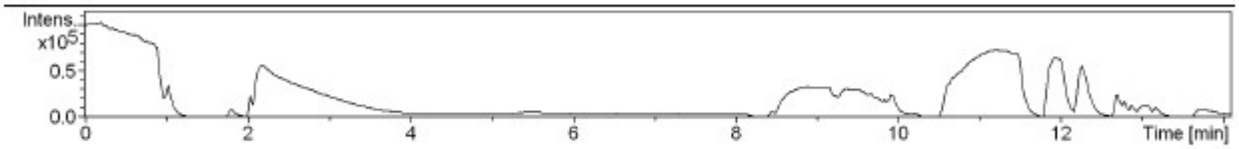
Mass Spectrum List Report

Analysis Info

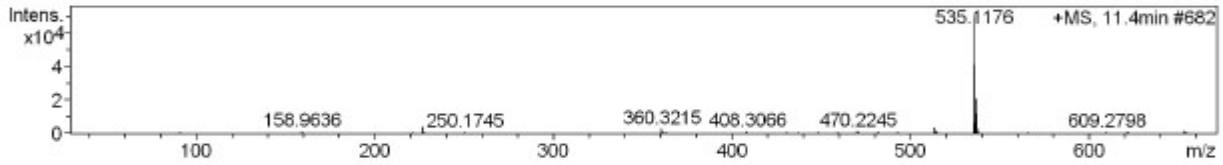
Analysis Name	D:\Data\2016\1208\Sandip3.d		
Method	Tune low_pos_50_650mz_160629.m	Operator	BDAL@KR
Sample Name	Sandip3	Instrument / Ser#	micrOTOF-Q 10184
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	650 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Sandip3.d: BPC 48.9987-650.9881 +All MS



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4	250.1745	1146
5	360.3215	2693
6	361.3264	611
7	362.9254	664
8	408.3066	1332
9	430.9125	521
10	437.2031	912
11	448.2116	1012
12	459.2229	657
13	470.2245	1245
14	470.7258	597
15	481.2362	537
16	492.2389	1033
17	492.7411	507
18	513.1326	3537
19	514.1563	1263
20	535.1176	70030
21	535.6188	2742
22	536.1187	20743
23	537.1246	3530
24	538.1278	640
25	565.2538	585
26	603.1075	508
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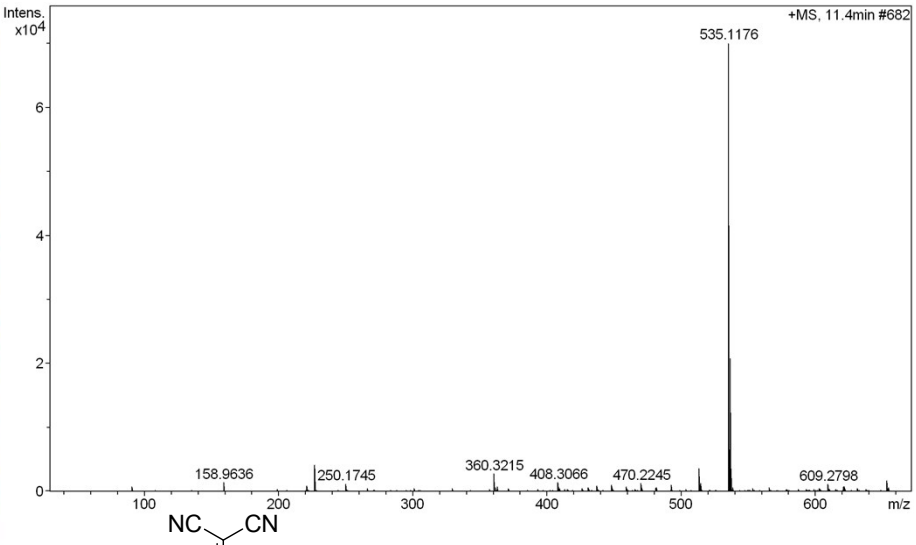
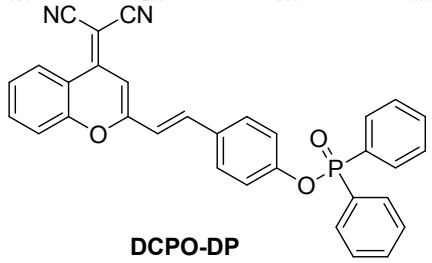
28	621.1845	773
29	653.3025	1601
30	654.3126	527





DCPO-DP

Calulated $[M + Na]^+$ = 535.1187

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Fig. S18. Mass spectrum of compound **DCPO-DP**.

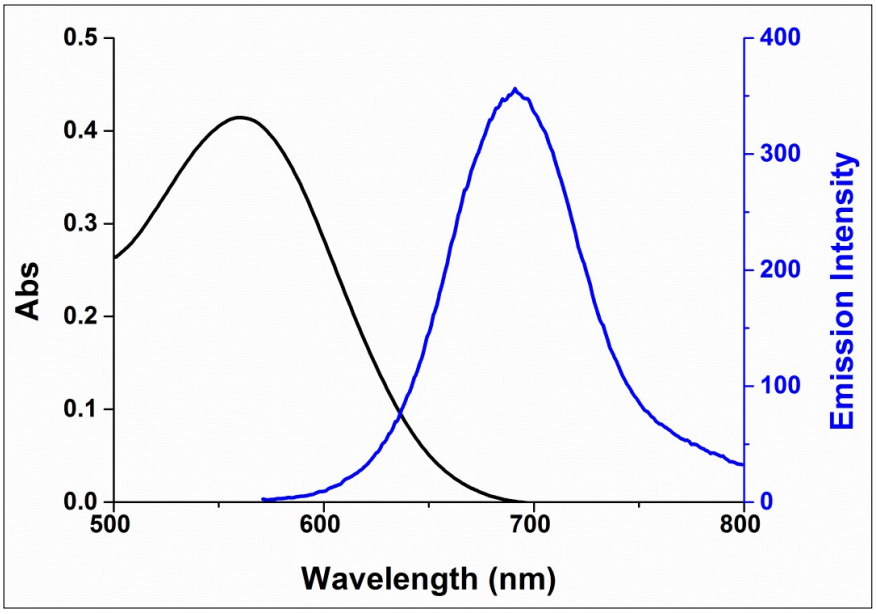


Fig. S19. Absorbance and emission spectra of **DCPO** (15.0 μM) in the mixture of solution (DMSO / 10 mM PBS, pH 7.4, 1:1 v/v).

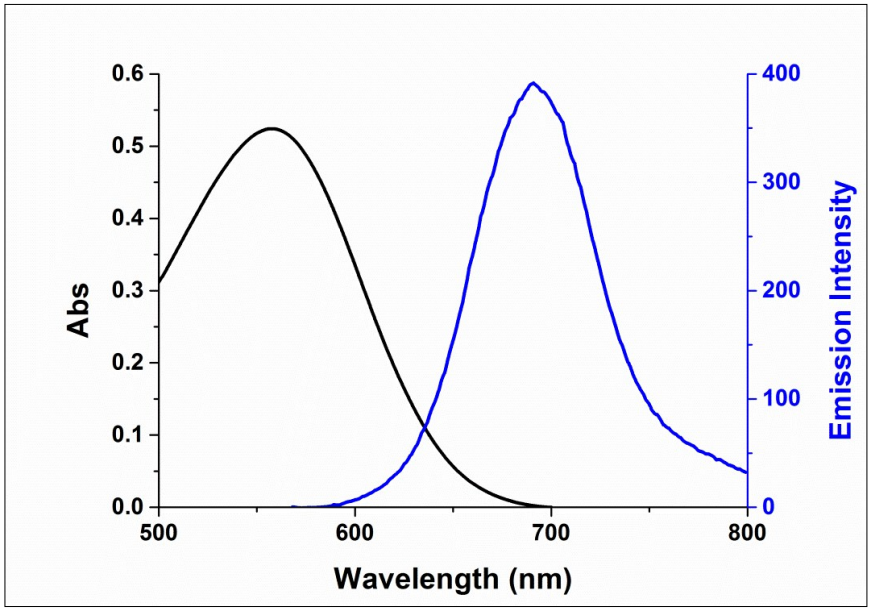


Fig. S20. Absorbance and emission spectra of **DCPO-DP** (15.0 μM) after addition of $ONOO^-$ (20.0 equiv) in the mixture of solution (DMSO / 10 mM PBS, pH 7.4, 1:1 v/v).

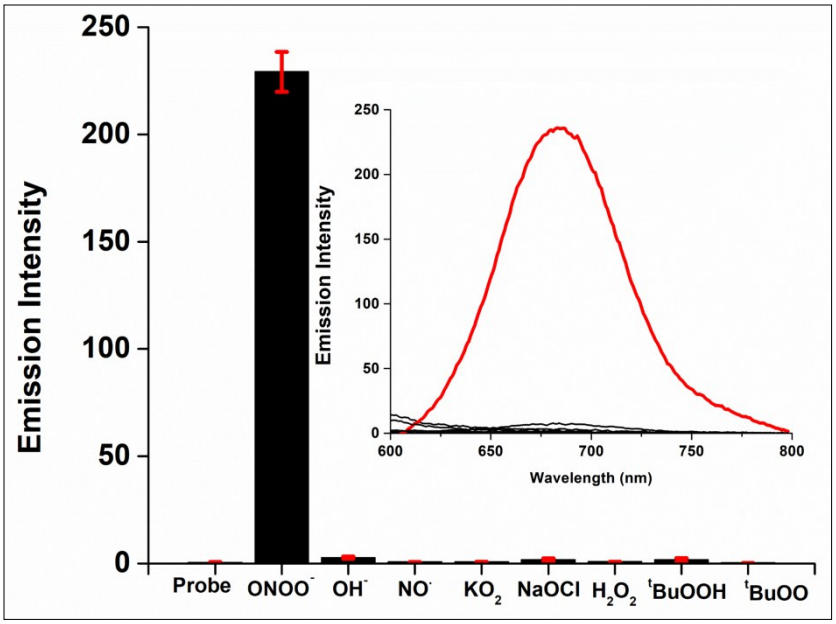


Fig.S21. Plot of relative fluorescence intensity maxima of $ONOO^-$, $O_2^{\bullet-}$, H_2O_2, NO, NaOCl, $^{\bullet}OH$, $^{t}BuO^{\bullet}$, and $^{t}BuO_2H$ (10.0 μM) trials upon addition of ROS/RNS in solution (CH_3CN / 10 mM PBS, pH 7.4, 1:1 v/v), incubation time: 20.0 min, λ_{ex}: 550 nm, λ_{em}: 690 nm, slit width 5 nm / 5 nm, (Data are expressed as mean of three experiments accompanied by a ± SD value). Inset: Selectivity of **DCPO-DP** with ROS/RNS; λ_{ex}: 550.

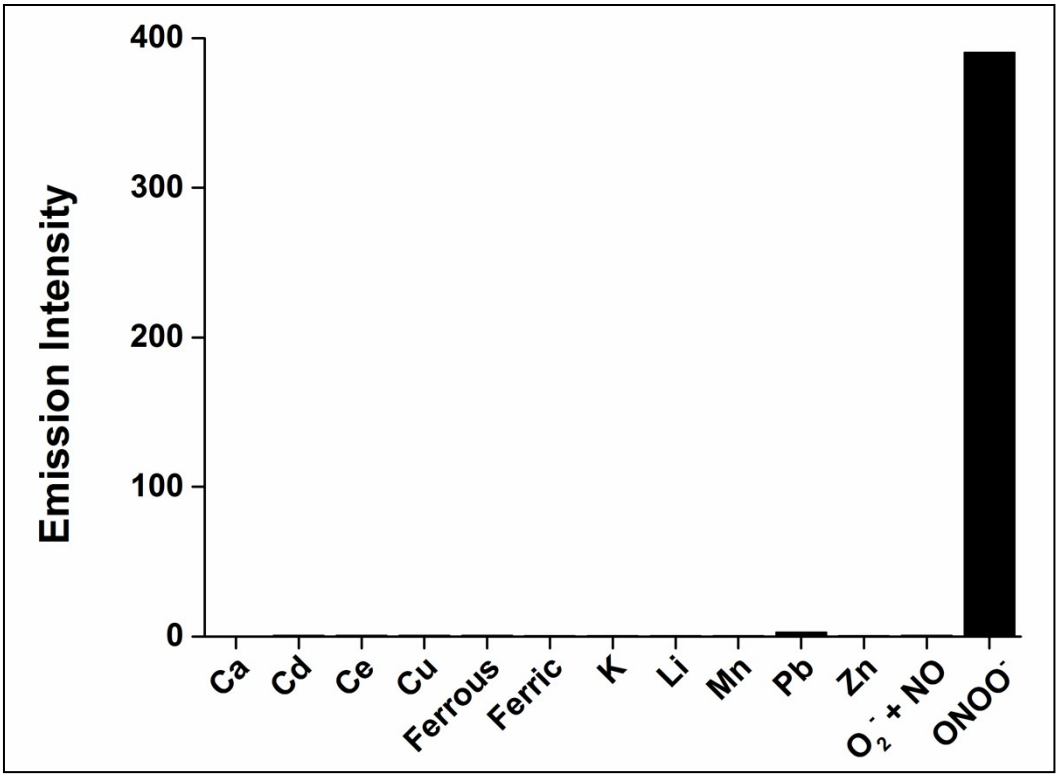


Fig. S22. Fluorescence intensity of **DCPO-DP** (15.0 μM) with various metals in the solution (DMSO / 10mM PBS pH 7.4, 1:1 v/v) incubated for 30.0 min. λ_{ex}: 555 nm, λ_{em}: 690 nm, slit width 5 nm / 5 nm.

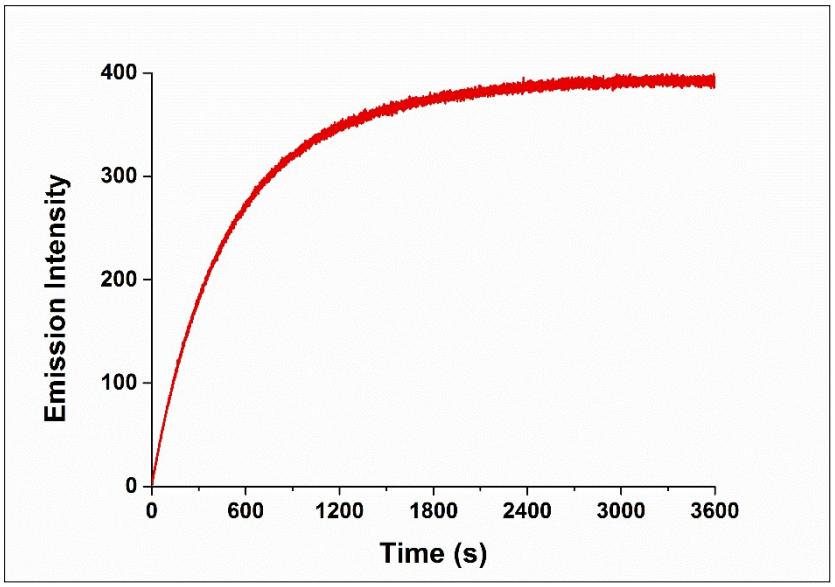


Fig. S23. Time-dependent emission spectral changes of **DCPO-DP** (15.0 μM) with 30.0 equiv of $ONOO^-$ in solution (DMSO / 10 mM PBS, pH 7.4, 1:1 v/v); λ_{ex}: 555 nm, λ_{em}: 690 nm, slit width 5 nm / 5 nm.

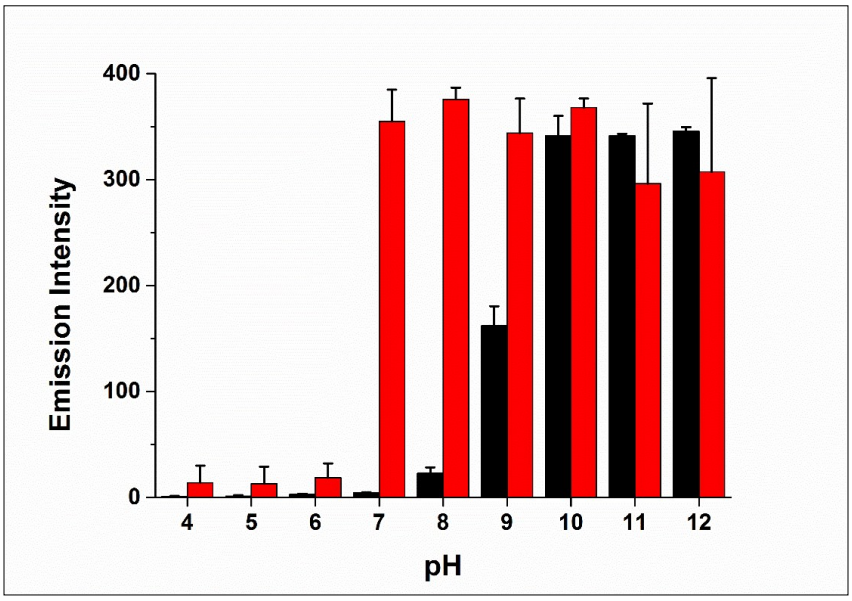


Fig. S24. Fluorescence emission intensity by **DCPO-DP** (15.0 μM, *black*) and **DCPO-DP** (15.0 μM) with 20.0 equiv. of $ONOO^-$ (*red*) under various different pH environments, λ_{ex}: 555 nm, λ_{em}: 690 nm, slit width 5 nm/ 5 nm.

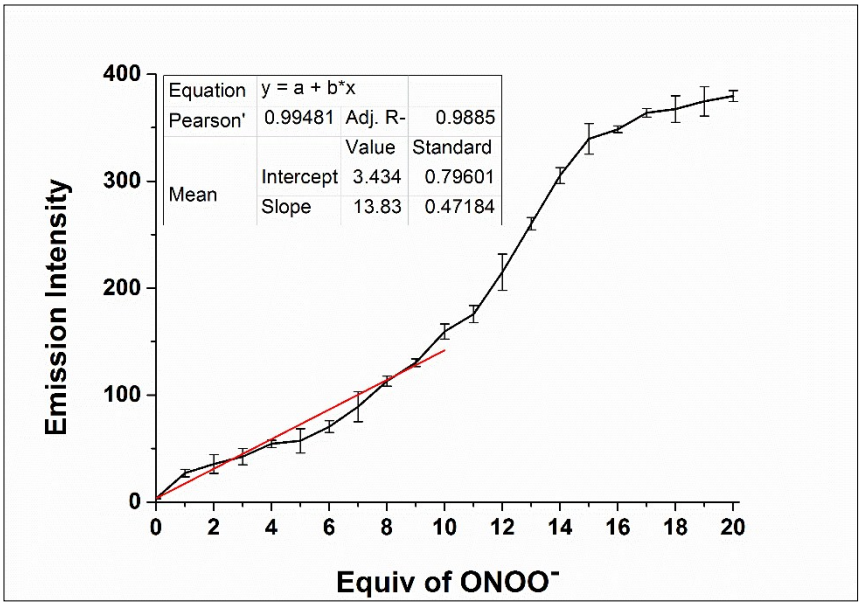


Fig. S25. Plot for the calculation of limit of detection from the emission of **DCPO-DP** (15.0 μM, DMSO/ 10 mM PBS pH 7.4, 1:1 v/v) with increasing concentration of $ONOO^-$ (0.0 to 20.0 equiv) incubated for 30.0 min at r.t., Slit width for λ_{ex} and λ_{em} = 5 nm, (average of three experiments).

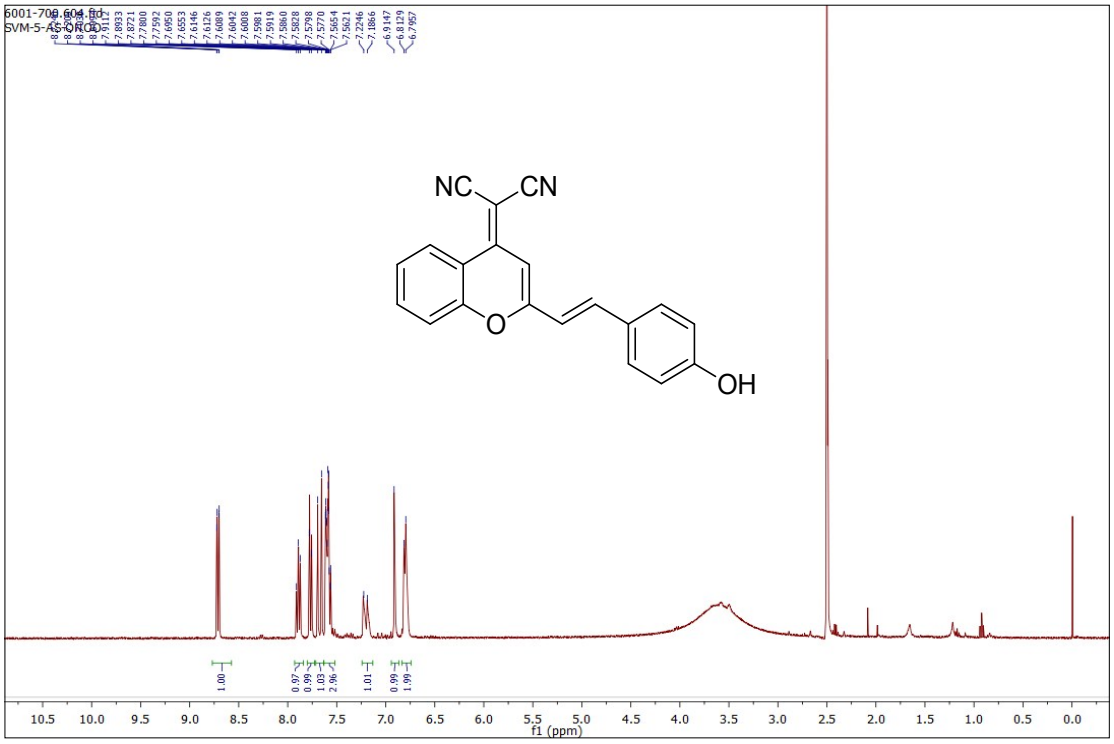


Fig. S26. ^{1}H NMR spectrum of the isolated product from the reaction of **DCPO-DP** and $ONOO^-$ in DMSO-*d6*.

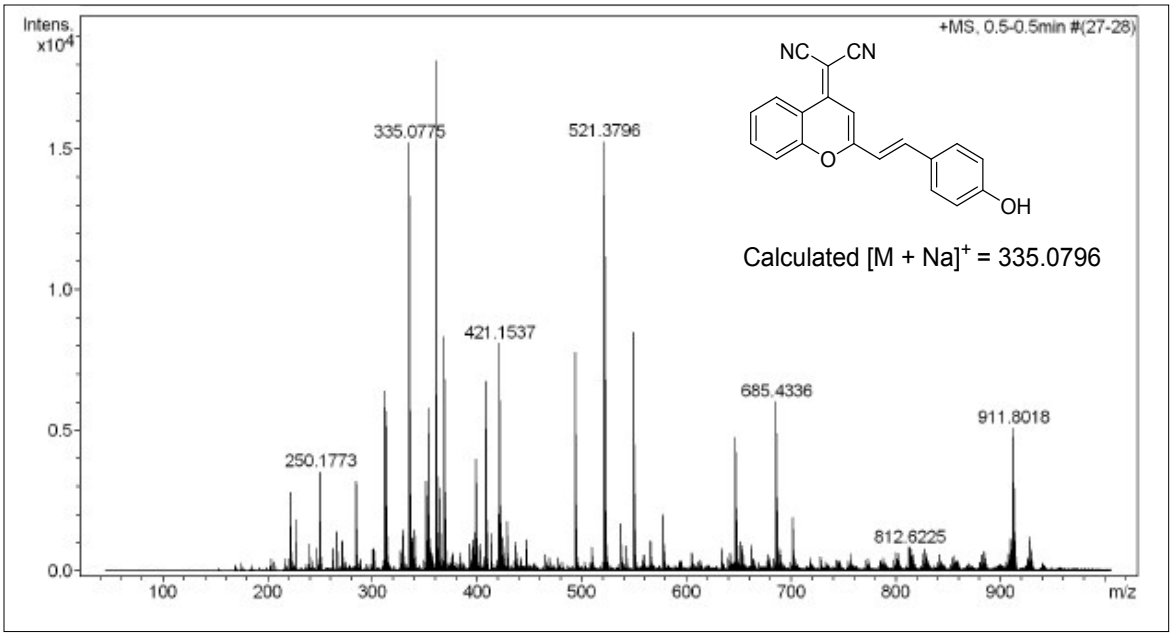


Fig. S27. HR-MS spectrum of the isolated product from the reaction of of **DCPO-DP** and $ONOO^-$.

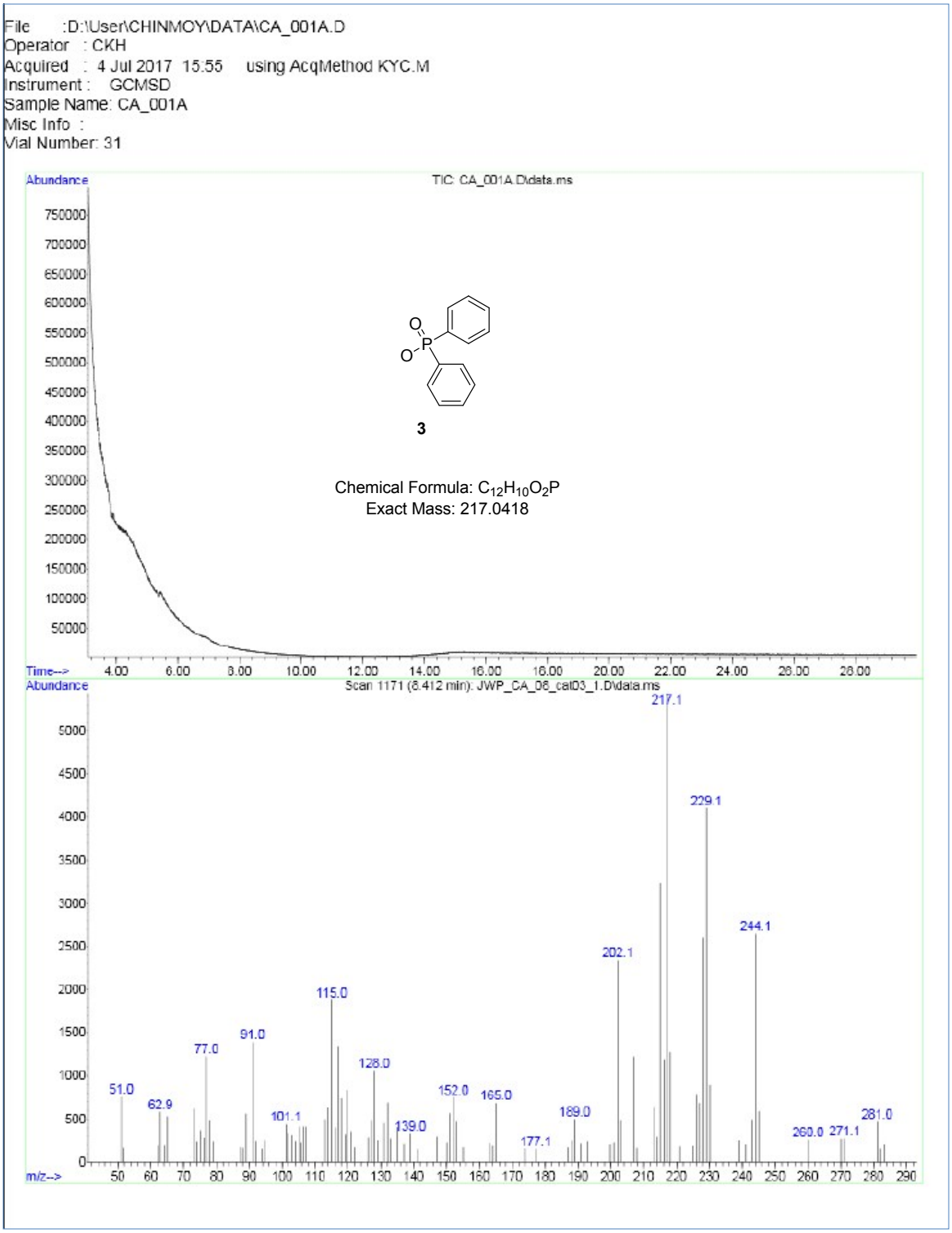


Fig. S28. GS-MS spectrum of the crude product from the reaction of **DCPO-DP** and $ONOO^-$.

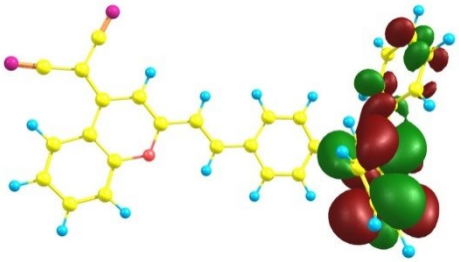
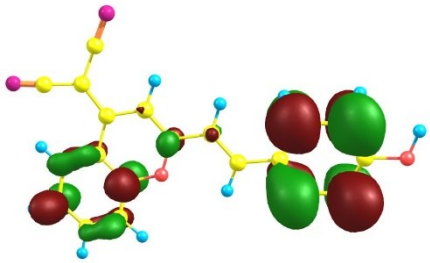
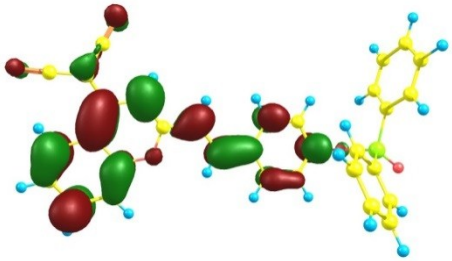
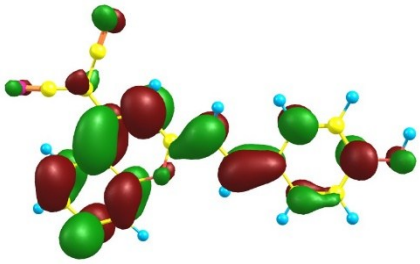
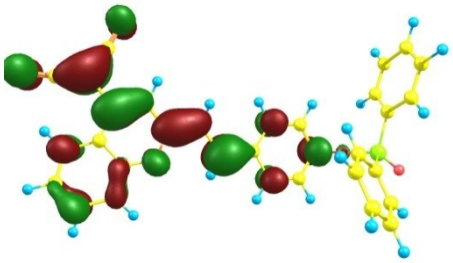
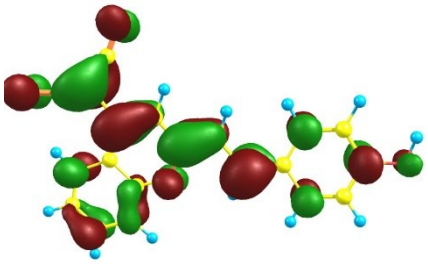
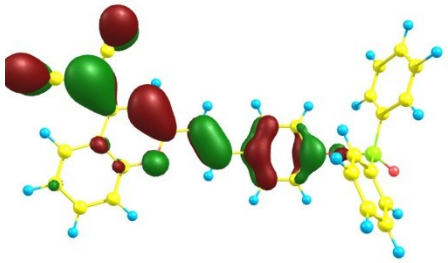
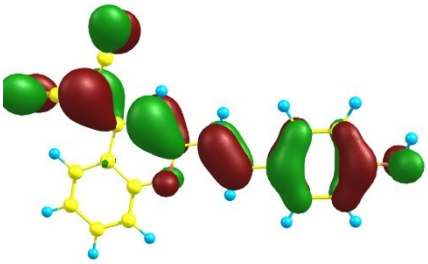
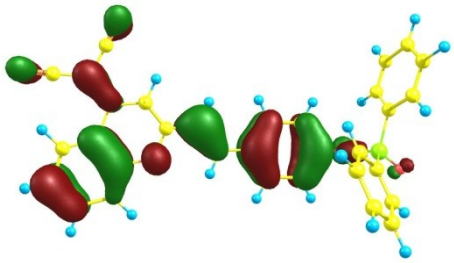
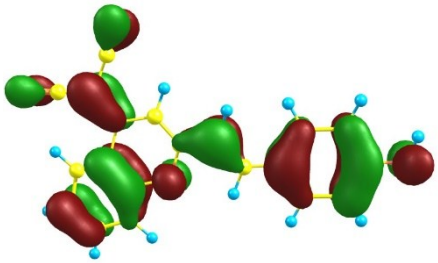
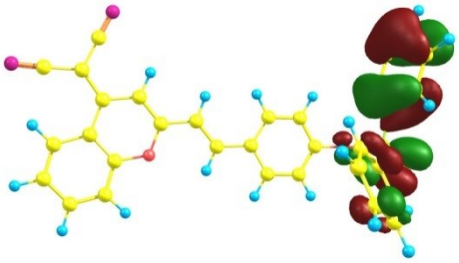
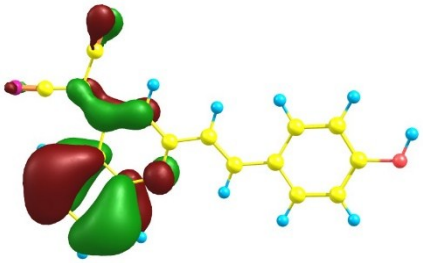
	DCPO-DP	DCPO
LUMO+2		
LUMO+1		
LUMO		
HOMO		
HOMO-1		
HOMO-2		

Fig. S29. Graphical display of HOMO-LUMO levels of DFT-optimized geometry of **DCPO** and **DCPO-DP** (B3LYP/6-31g* basis set, G09).

Table S1. Absorption energies with largest oscillator strength for **DCPO** and **DCPO-DP** (B3LYP/6-31g* basis set, G09).

	f	Composition	CI (%)
DCPO	1.0601	HOMO-1 → LUMO	9.7
		HOMO → LUMO	**89.6**
DCPO-DP	0.3771	HOMO-2 → LUMO	6.9
		HOMO-1 → LUMO	**87.9**
		HOMO → LUMO	4.5
	0.6474	**HOMO-2 → LUMO**	**83.2**
		HOMO-2 → LUMO+1	5.3
		HOMO-1 → LUMO	6.3
		HOMO-1 → LUMO+1	2.4

Table S2. Results of experimental determination of log P value by the (shake flask) method for **DCPO-DP**

C_1	V	A	Ā	C_2	P	Log P
1.3×10^{-4}	40	0.4581 0.4813 0.4862	0.4752	3.37×10^{-5}	111.6	2.04
1.6×10^{-4}	50	0.4544 0.4582 0.4521	0.4549	3.22×10^{-5}	149.7	2.18
2.0×10^{-4}	60	0.4077 0.3941 0.4052	0.4023	2.85×10^{-5}	225.6	2.35

C_1 = Concentration ($mol \cdot L^{-1}$) of 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 ($mol \cdot L^{-1}$) in buffer solution after partitioning; P = partition coefficient; log P = logarithm of the partition coefficient.

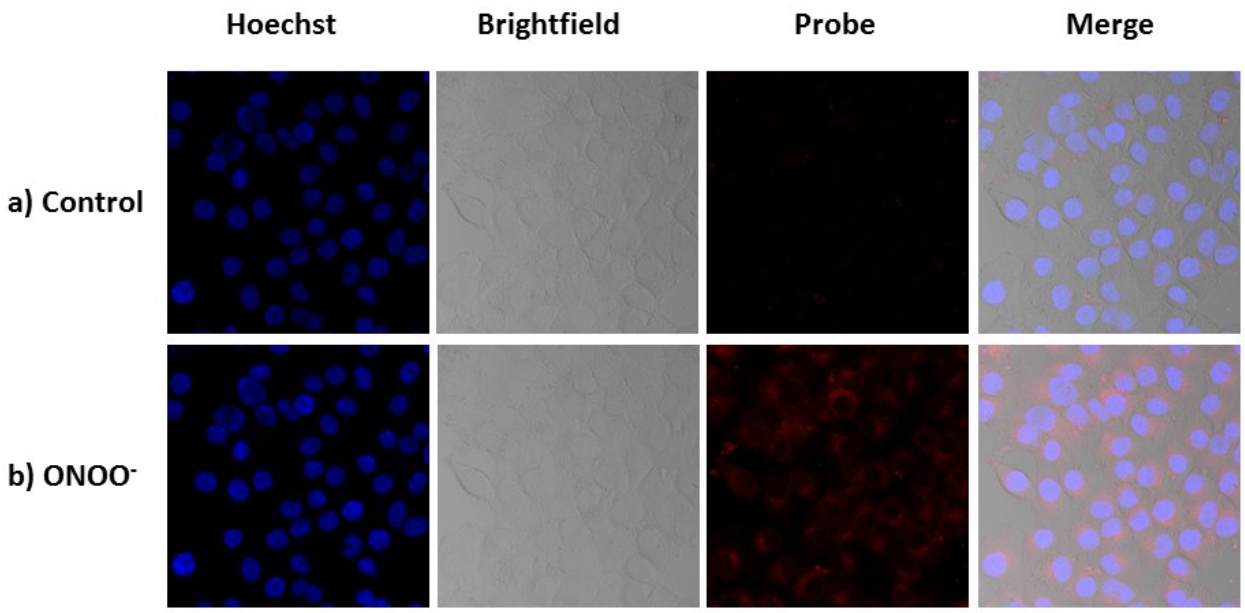


Fig. S30. Confocal fluorescence images of exogenous $ONOO^-$ in HeLa cells; a) cells pretreated with Probe (40 μM) for 30 min; b) cells were treated with Probe (40 μM) for 30 min washed by D-PBS after fixing and followed by incubation with $ONOO^-$ (400 μM) for 30 min; Scale bar: 20 mm.

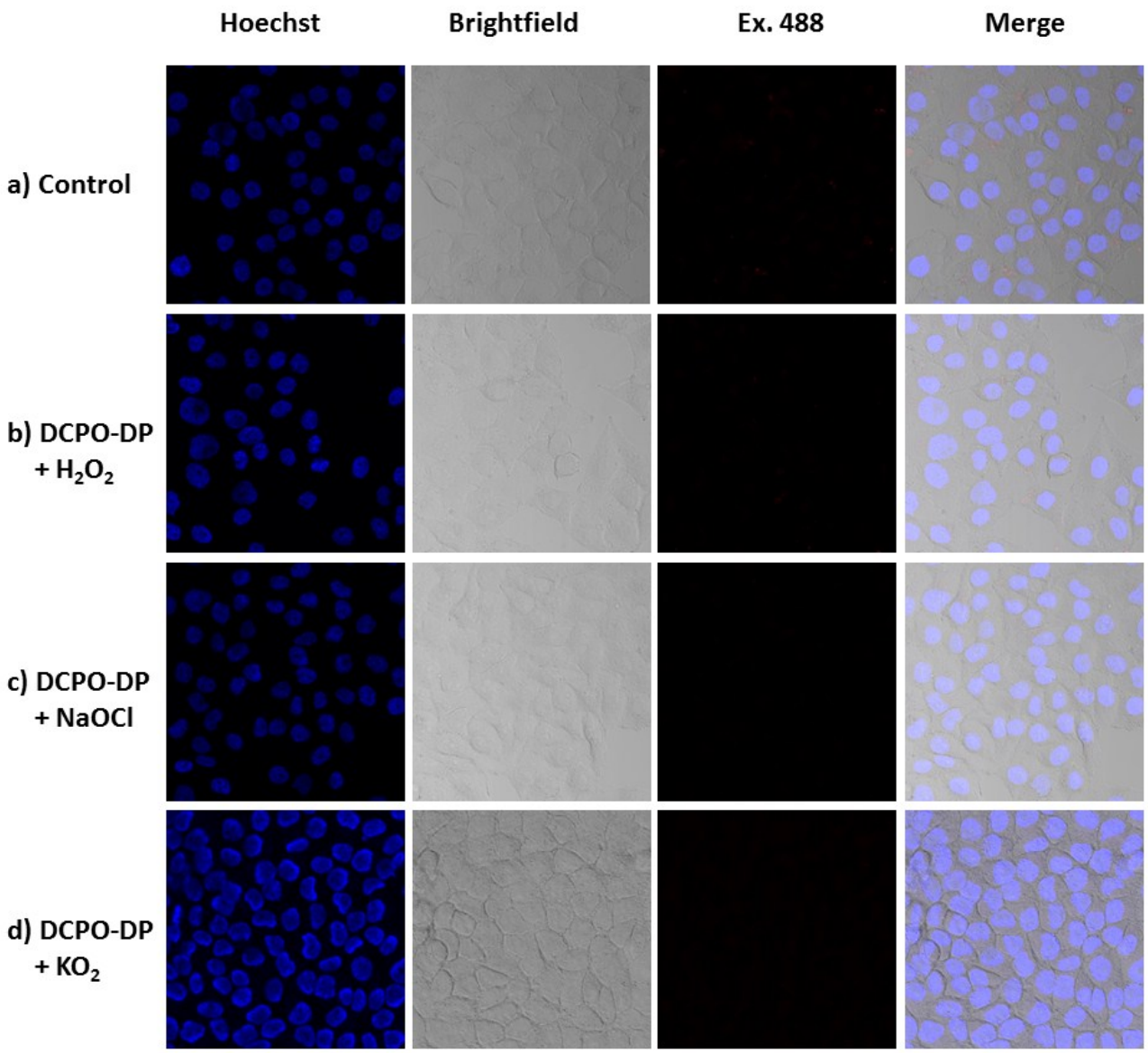


Fig. S31. Confocal fluorescence images of HeLa cells; a) cells pretreated with **DCPO-DP** (40 μM) for 30 min; in the cases of (b), (c), and (d) cells were treated with **DCPO-DP** (40 μM) for 30 min washed by D-PBS. After fixing, followed by incubation with H_2O_2, NaOCl, and KO_2 (250 equiv) for 1 min, respectively; length of scale bar = 20 μm.

Table S3. Information of the **DCPO-DP** and **DCPO** calculated through 'molinspiration property engine v2011.04' at the website, http://www.molinspiration.com.

Properties (DCPO-DP) (NOTE: abbreviation same as website)	Value (DCPO-DP)	Value (DCPO-DP)
milogP	7.58	4.30
TPSA	87.03	80.95
Natom	38	24
MW	512.50	312.30
nON	5	4
nOHNH	0	1
nviolations	2	0
nrotb	6	2
volume	449.71	277.58

※ log P = octanol-water partition coefficient, PSA = molecular polar surface area, n atoms = number of atoms without hydrogen, MW = molecular weight, n ON = number of O atoms and N atoms, n OHNH = number of OHNH functional groups, n violations = number of violations against 'Rule of 5', n rotb = number of rotatable bonds, volume; molecular volume.

※ Rule of 5: Lipinski suggested 4 properties regarding the 'drug-like' of molecules; log P value should be less than 5, molecular weight should be less than 500, number of hydrogen bond acceptors should be less than 10, number of hydrogen bond donors should be less than 5.[1]

References:

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

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