Supporting Information

Novel reversible and selective nerve agent simulant detection in conjunction with superoxide "turn–on" probing

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Experimentals

Materials and instruments

Fluorescein (Aldrich), diethyl chlorophosphate (Aldrich), diethyl cyanophosphonate (TCI) and diethyl methylphosphonate (Aldrich) are commercially available. Fluorescein was dissolved in pH 7.4 10 mM HEPES b uffer and concentration was 10^{-6} M (1) and 0.1 M of DCP, DECP and DEMP were dissolved in acetonitrile. Fluo rescence measurements were carried out with a Shimadzu RF–5301pc spectrofluorophotometer slit width Ex, E m = 1.5 and 3. All solvents used in NMR spectral analyses were purchased commercially and were of spectrosc opy grade. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured on Bruker Avance 400 MHz spectrometer. High-resolut ion MALDI-TOF mass spectrometry was performed on an Applied Biosystem Voyager 4394 (Ionization metho d, N2 laser (337 nm, 3 ns pulse): analyzer 2.0 m linear mode; 3.0 reflector mode)

Synthetic procedure

Compound **1** (for only Mass spectrum)

Fluorescein (200 mg, 0.602 mmol) and 1,4-diazabicyclo[2,2,2]octane (135.05 mg, 1.204 mmol) were dissolved i n 15mL of dry DMF in round bottom flask under inert atmosphere of argon for 15 min. Then, DCP (0.174 mL, 1. 204 mmol) was added and the reaction was continued at room temperature for 13 hr. The reaction was quench ed by 150 mL of water and kept in refrigerator overnight. After the mixture was filtered and washed twice by wa ter and dried to get a yellow powder (184.5 mg, 50.70%)

Results

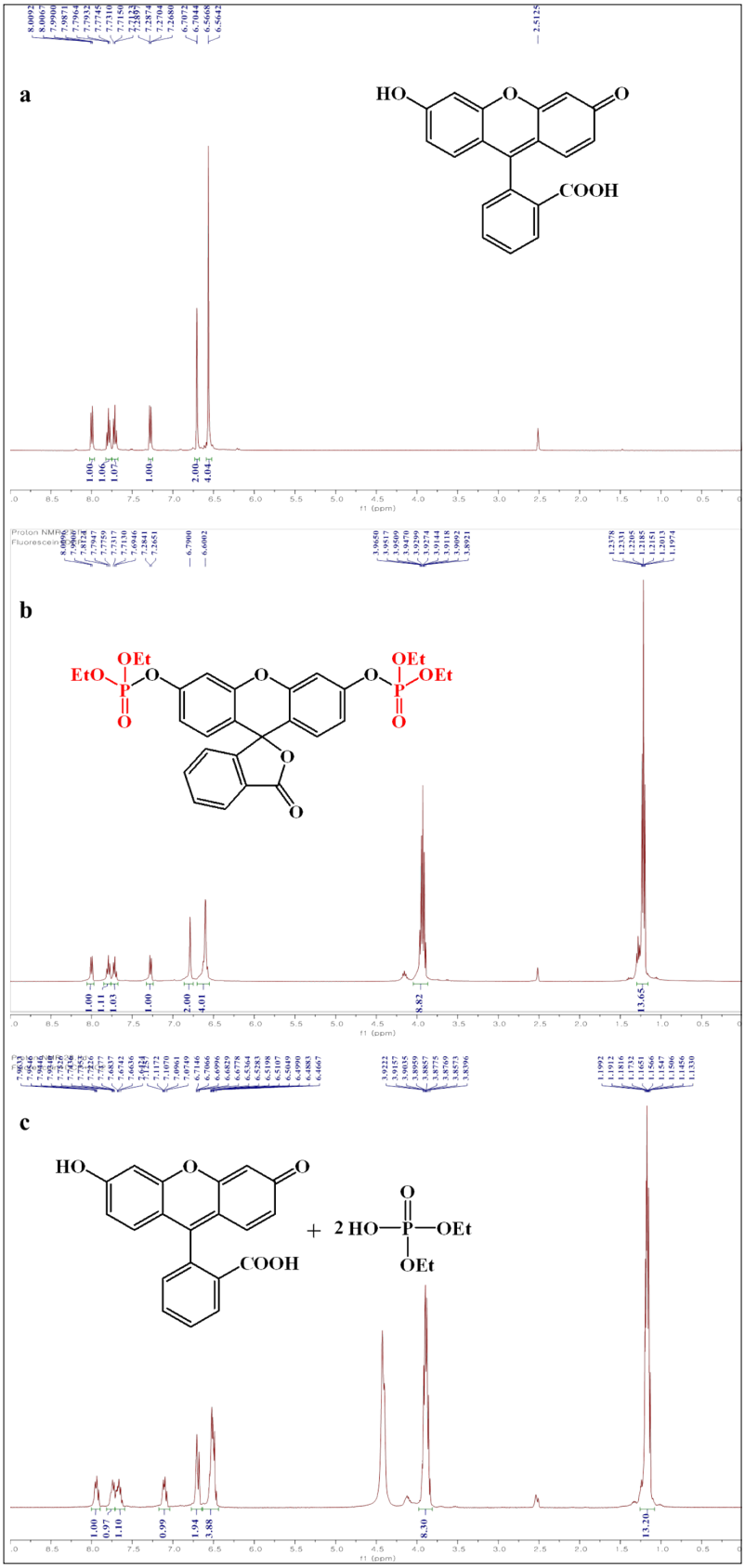

Fig. S1. ^{1}H NMR spectra of fluorescein in DMSO-d_6 (a), compound **1** (b), and compound **1** and KO_2 (c)

(a) ^{1}H NMR (400 MHz, DMSO-d_6: 2.51 ppm) δ 8.00 (dd, $^{3}J_{H\text{-}H}$ = 7.68 Hz, $^{4}J_{H\text{-}H}$ = 1.00 Hz, 1H, H_9), 7.79 (m, 1H, H_5), 7.71 (m, 1H, H_6), 7.28 (dd, $^{3}J_{H\text{-}H}$ = 7.72 Hz, $^{4}J_{H\text{-}H}$ = 0.92 Hz, 1H, H_{10}), 6.71 (s, 2H, H_{13}), 6.56 (s, 4H, $H_{7,11}$)

(b) ^{1}H NMR (400 MHz, DMSO-d_6: 2.51 ppm) δ 8.00 (d, $^{3}J_{H\text{-}H}$ = 7.60 Hz, 1H, H_9), 7.79 (t, $^{3}J_{H\text{-}H}$ = 7.08 Hz, 1H, H_5), 7.71 (t, $^{3}J_{H\text{-}H}$ = 7.48 Hz, 1H, H_6), 7.27 (d, $^{3}J_{H\text{-}H}$ = 7.6, 1H, H_{10}), 6.79 (s, 2H, H_{13}), 6.60 (s, 4H, $H_{7,11}$) 3.97–3.89 (m, 8H, $H_{15,16}$), 1.24–1.20 (m, 12H, $H_{17,18}$),

(c) ^{1}H NMR (400 MHz, DMSO-d_6 2.52 and D_2O 4.43 ppm) δ 7.96–7.91 (m, 1H, H_9), 7.76–7.64 (m, 1H, H_5), 7.70–7.64 (m, 1H, H_6), 7.13–7.07 (m, 1H, H_{10}), 6.71–6.68 (m, 2H, H_{13}), 6.54–6.47 (m, 4H, $H_{7,11}$) 3.97–3.89 (m, 8 H, $H_{15,16}$), 1.24–1.20 (m, 12H, $H_{17,18}$)

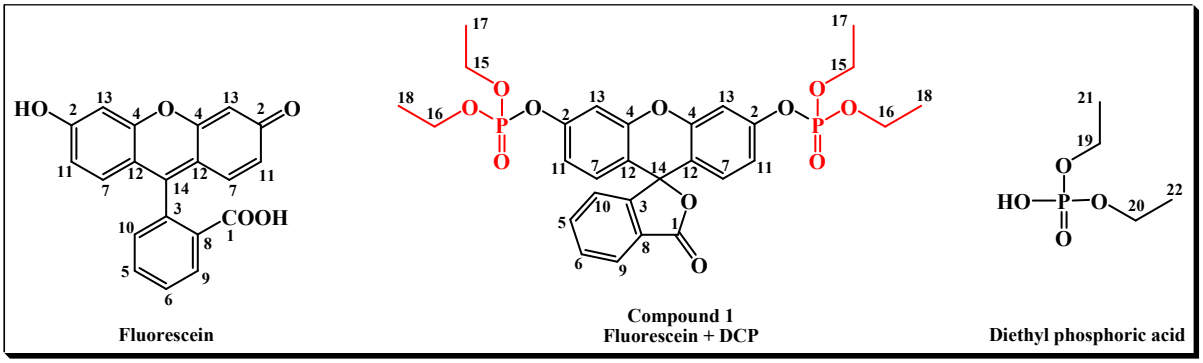

Diagram of fluorescein, compound **1,** and diethyl phosphoric acid

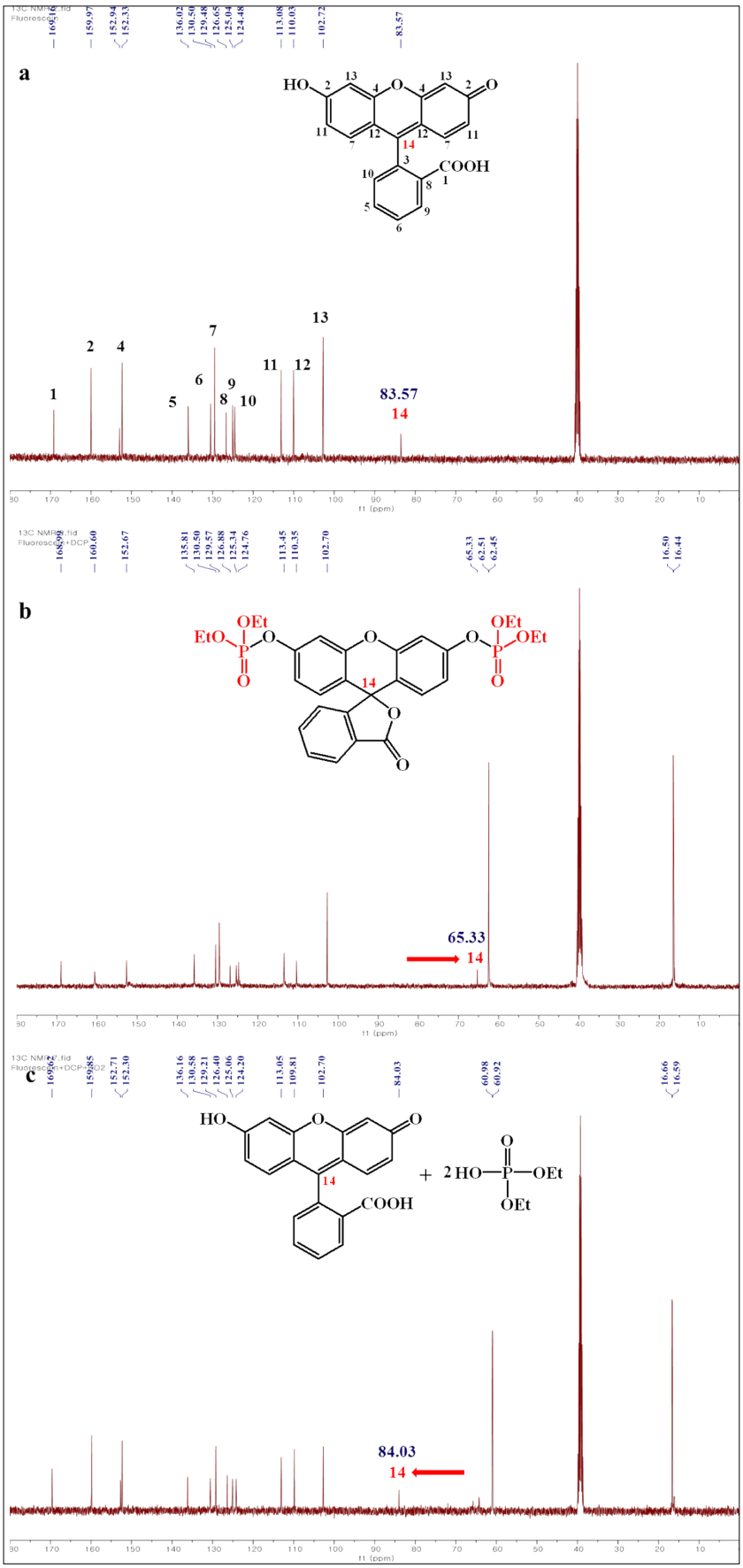
a
HO
COOH
14
1
2
4
5
6
7
8
9
10
11
12
13
83.57
14
f1 (ppm)
b
EtO
OEt
OEt
OEt
O
P
14
65.33
14
f1 (ppm)
c
HO
COOH
14
+ 2 HO—P(=O)(OEt)—OEt
84.03
14
f1 (ppm)

Fig. S2. ^{13}C NMR spectra of fluorescein (a), compound **1** (b), and compound **1** and KO_2 (c)

(a) ^{13}C NMR (100 MHz, DMSO-d_6: 3.97 ppm) δ 169.2 (C_1), 160.0 (C_2), 152.9 (C_3), 152.3 (C_4), 136.0 (C_5), 130.5 (C_6), 129.5 (C_7), 126.7 (C_8), 125.0 (C_9), 124.5 (C_{10}), 113.1 (C_{11}), 110.0 (C_{12}), 102.7 (C_{13}), 83.6 (C_{14})

(b) ^{13}C NMR (100 MHz, DMSO-d_6: 3.97 ppm) δ 169.0 (C_1), 160.6 (C_2), 152.7 (C_3), 135.8 (C_5), 130.5 (C_6), 129.6 (C_7), 126.9 (C_8), 125.3 (C_9), 124.8 (C_{10}), 113.4 (C_{11}), 110.4 (C_{12}), 102.7 (C_{13}), 65.3 (C_{14}), 62.5 (C_{15}), 62.5 (C_{16}), 16.5 (C_{17}), 16.5 (C_{18})

(c) ^{13}C NMR (100 MHz, DMSO-d_6 and D_2O: 3.97 ppm) δ 169.6 (C_1), 159.9 (C_2), 152.7 (C_3), 152.3 (C_4), 136.2 (C_5), 130.6 (C_6), 129.2 (C_7), 126.4 (C_8), 125.1 (C_9), 124.2 (C_{10}), 113.1 (C_{11}), 109.8 (C_{12}), 102.7 (C_{13}), 84.0 (C_{14}), 61.0 (C_{19}), 60.9 (C_{20}), 16.7 (C_{21}), 16.6 (C_{22})

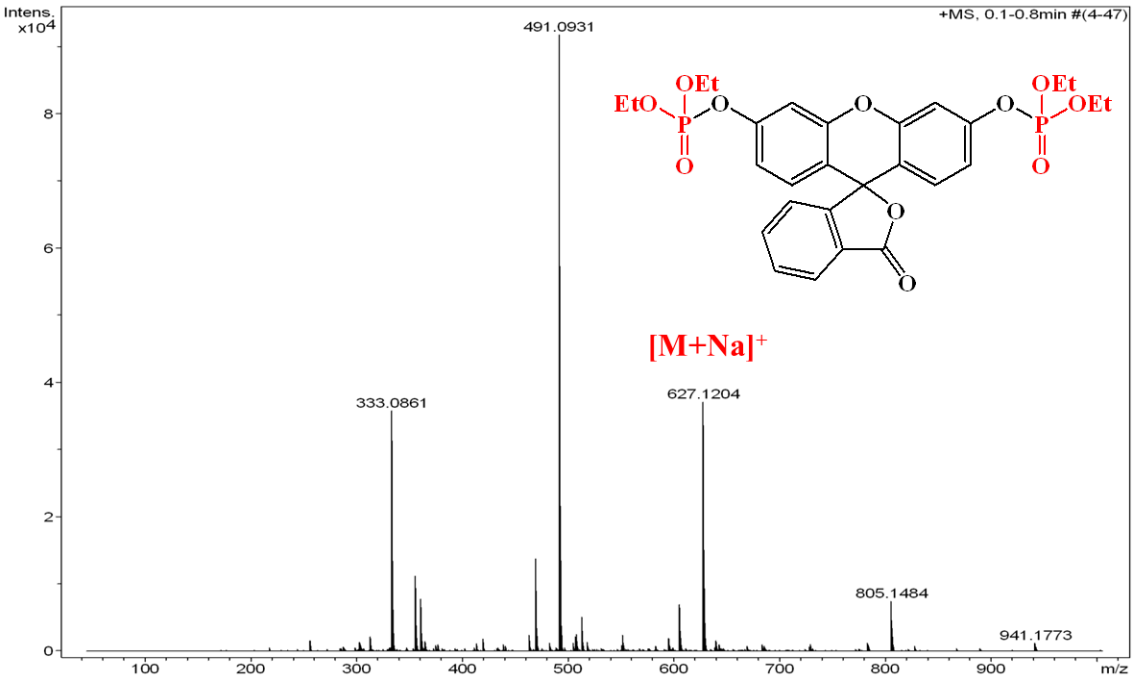

Fig. S3. HR-MS spectra of compound **1** was observed at 627.1204 and calculated for $C_{28}H_{30}O_{11}P_2Na^+$ is 627.11 56)

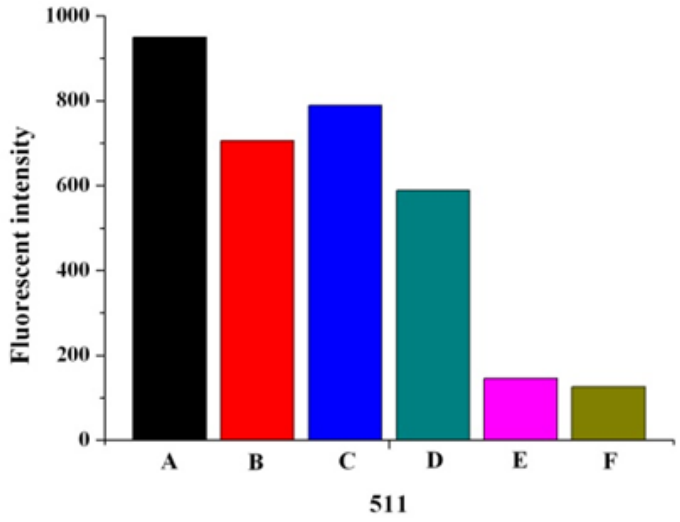

Fig. S4: Relative fluorescence intensity of compound **1** (λ_{exic} = 490 nm, λ_{emis} = 511 nm). **A** KO_2, **B** $KO_2 + H_2O_2$, **C** KO_2 + TBHP, **D** KO_2 + NaOCl, **E** KO_2 + $OH^{\cdot}$ **F** KO_2 + $OtBu^{\cdot}$.

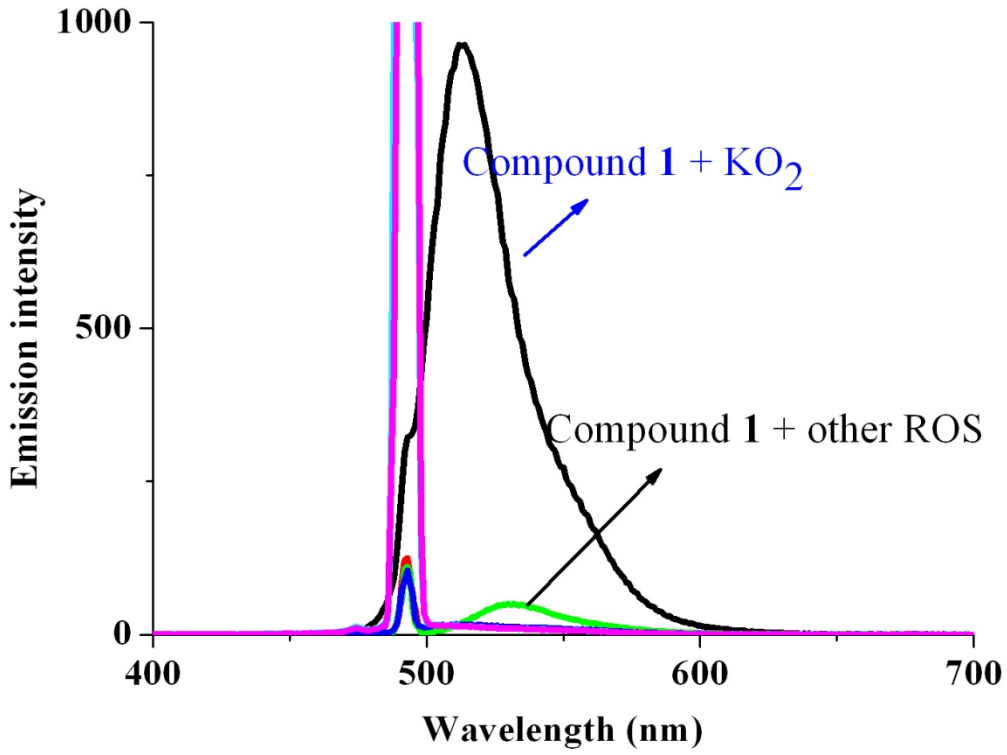

Fig. S5. Relative fluorescence intensity of compound **1** with ROS (3300 μM of KO_2, H_2O_2, TBHP, NaOCl, $OH^{\cdot}$, $OtBu^{\cdot}$ in HEPES buffer; 10 mM, pH 7.4 λ_{exic} = 490 nm, λ_{emis} = 511 nm, slit width – 3).

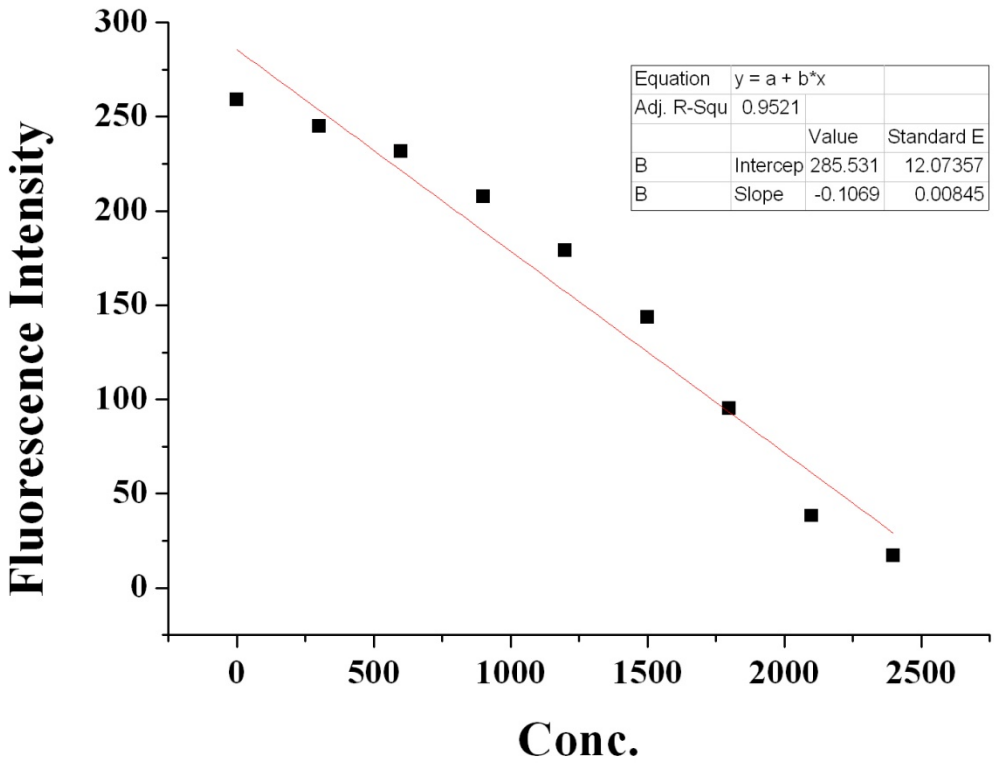

Fig. S6. Emission spectra change of **compound 1** as a function of concentration DCP (0 – 3600 μM) in HEPES buffer; 10 mM, pH 7.4). Each spectrum was recorded at real time

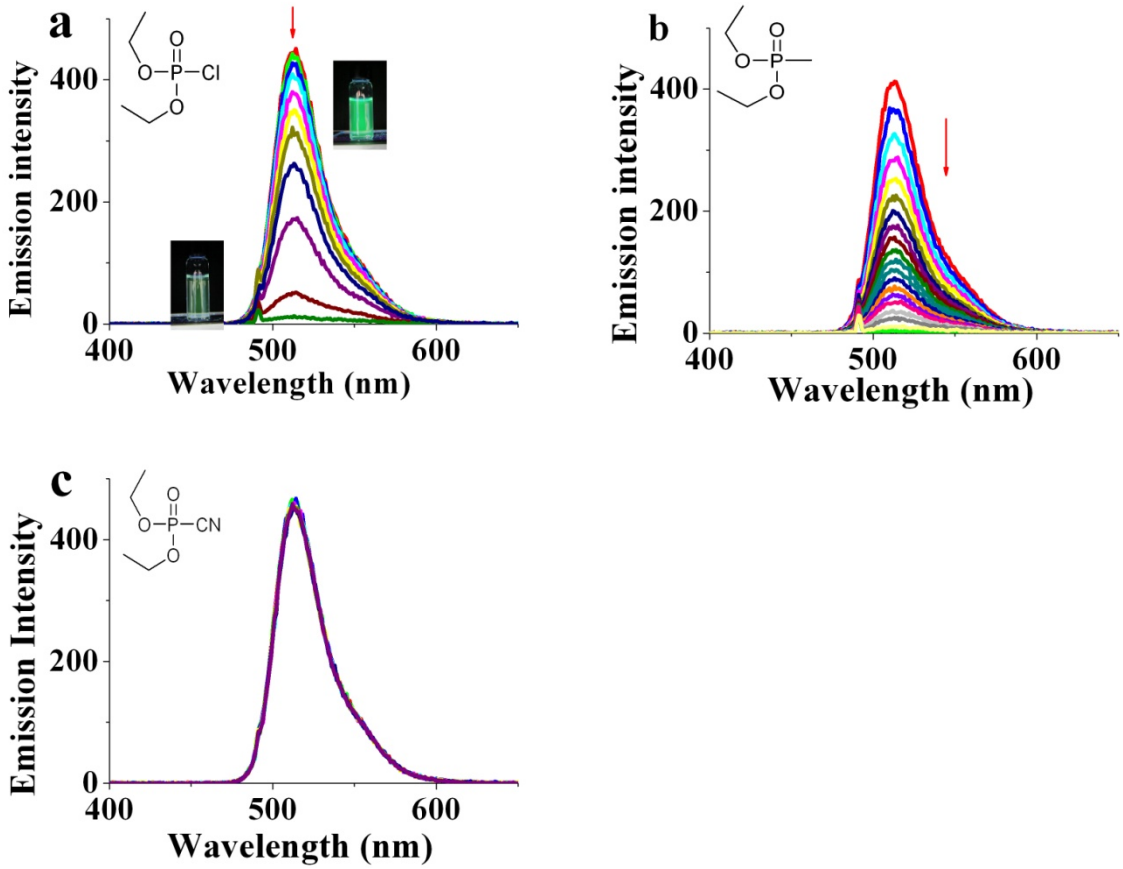

Fig. S7 Emission titration spectra of fluorescein (10^{-6} M, HEPES buffer; 10 mM, pH 7.4) with (a) DCP (0 to 3300 μM), (b) DEMP (0 to 6600 μM), and (c) DECP (0 to 3000 μM) in acetonitrile λ_{exic} = 490 nm, λ_{emis} = 511 nm (slit width – 1.5)

Nerve agent	LCt_{50} Inhalation mg·min/m^3	LD_{50} Skin mg/individual
GA	200	4000
GB	100	1700
GD	100	30
VX	50	10

Table. S1 Lethal concentration & time and lethal dose of GA, GB, GD and VX[1]

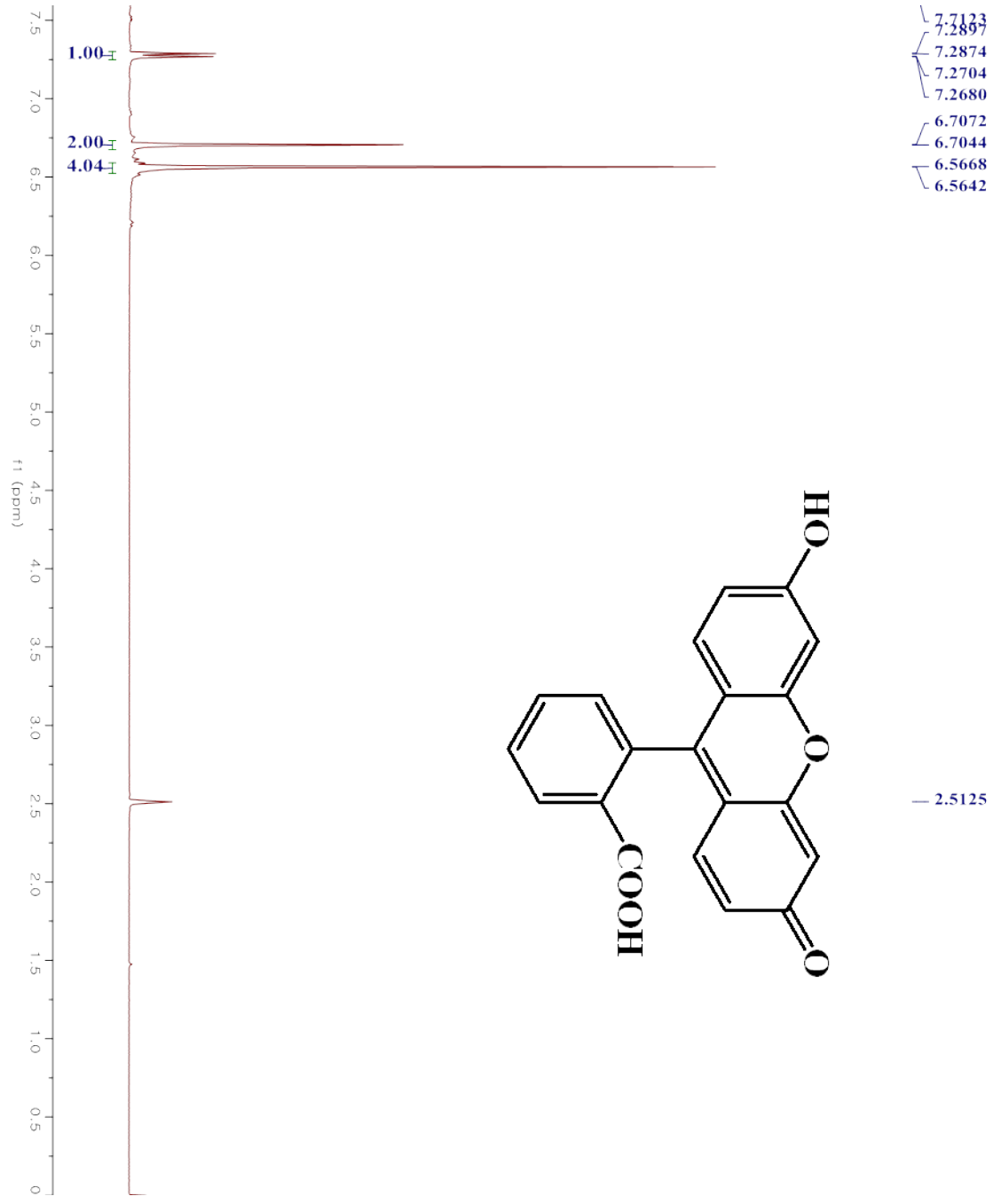

Fig. S8. ^{1}H NMR spectrum of fluorescein in DMSO-d_6

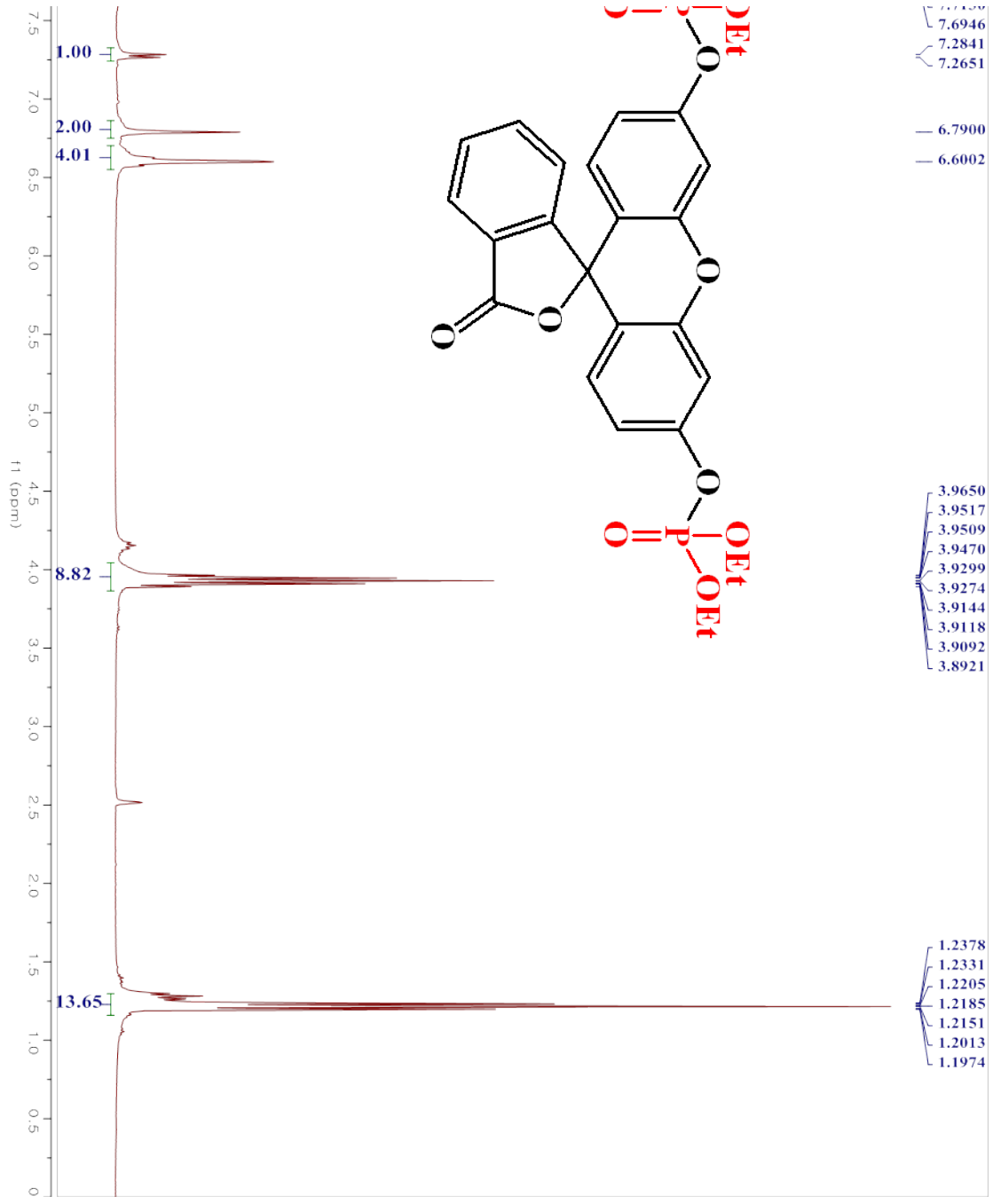

Fig. S9. ^{1}H NMR spectrum of compound **1** in DMSO-d_6

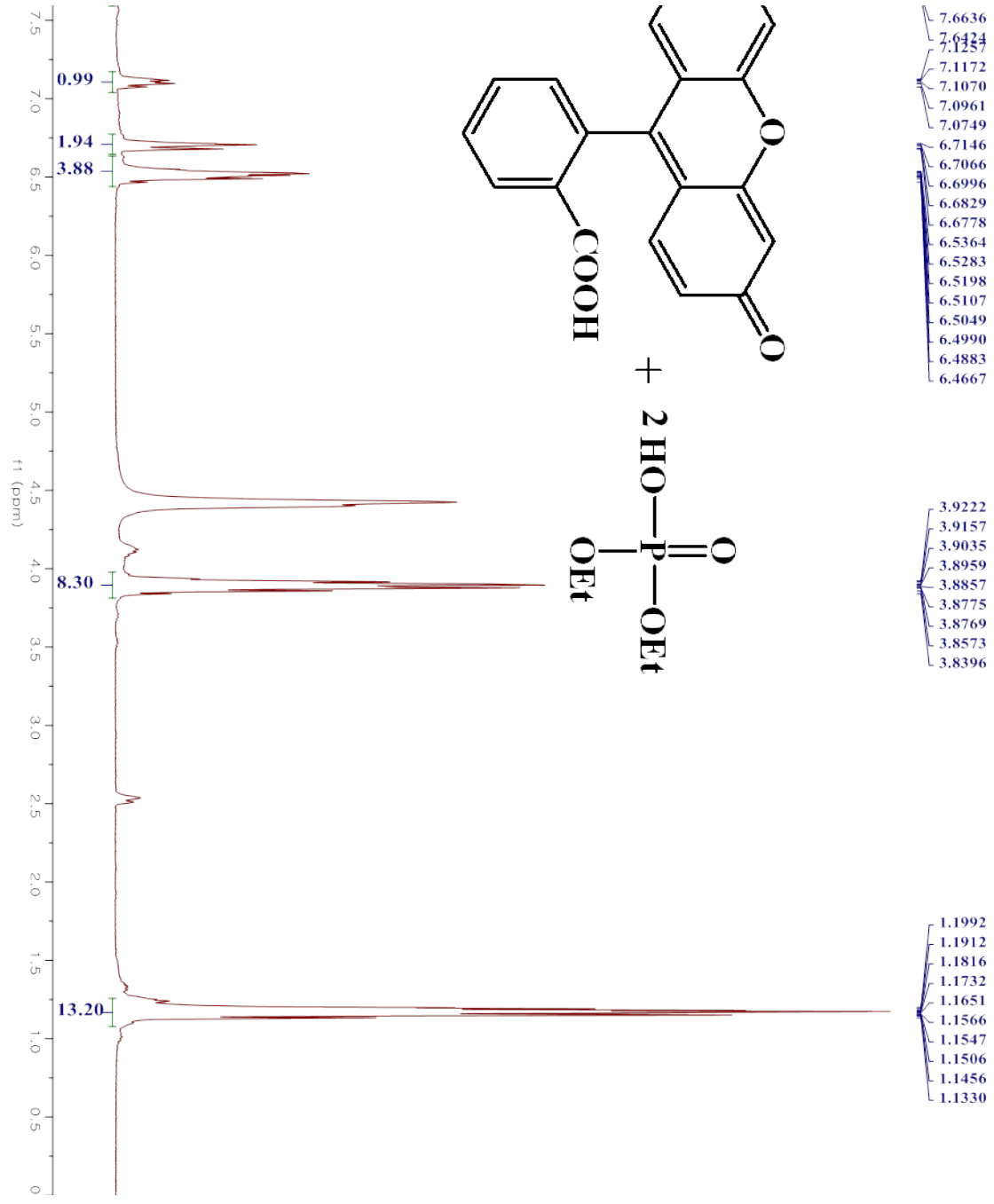

Fig. S10. ^{1}H NMR spectrum of compound **1 and KO_2** in DMSO-d_6 and D_2O

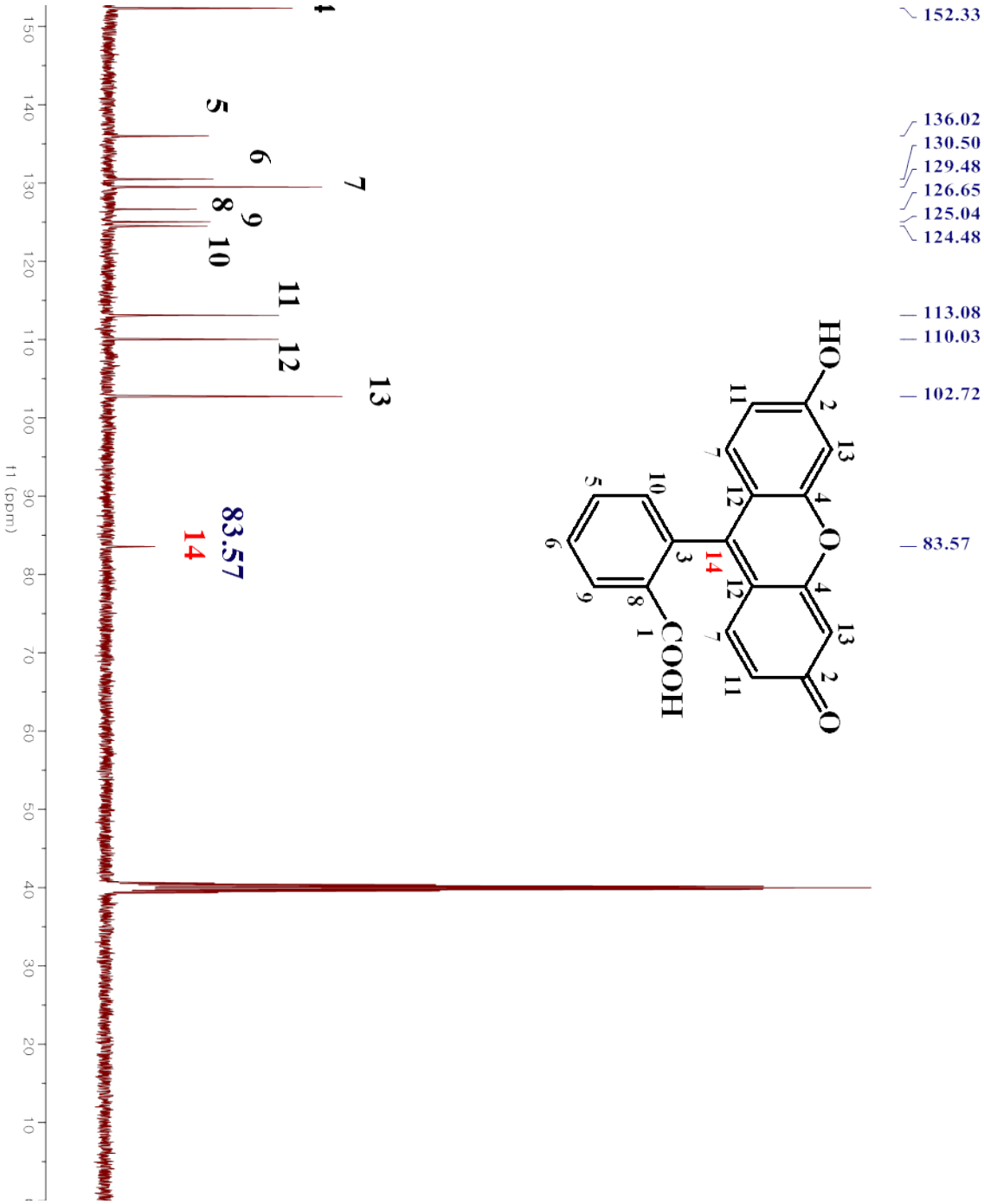

Fig. S11. ^{13}C NMR spectrum of fluorescein in DMSO-d_6

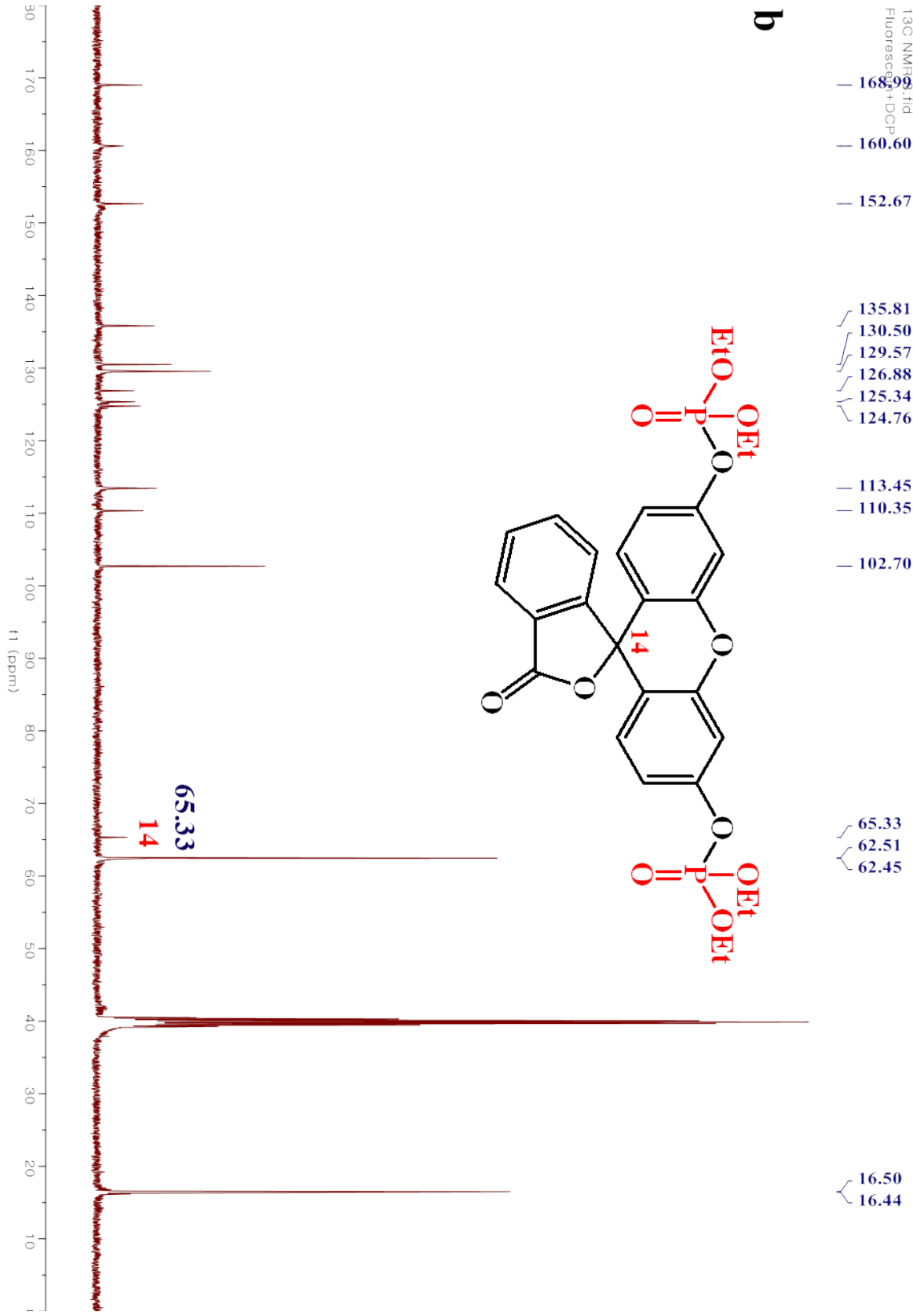

Fig. S12. ^{13}C NMR spectra of compound **1** in DMSO-d_6

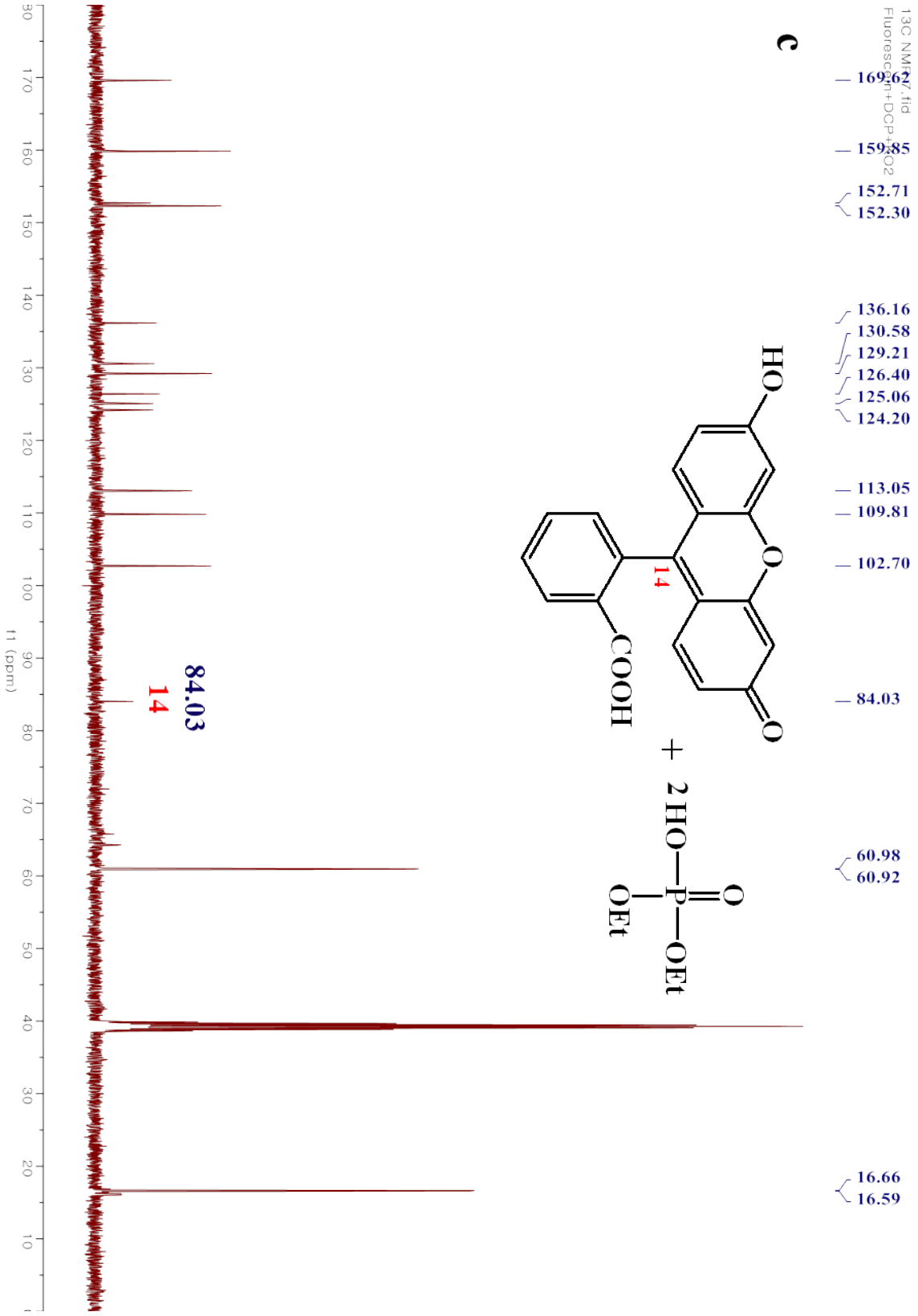

Fig. S13. ^{13}C NMR spectrum of compound **1 and KO_2** in DMSO-d_6 and D_2O

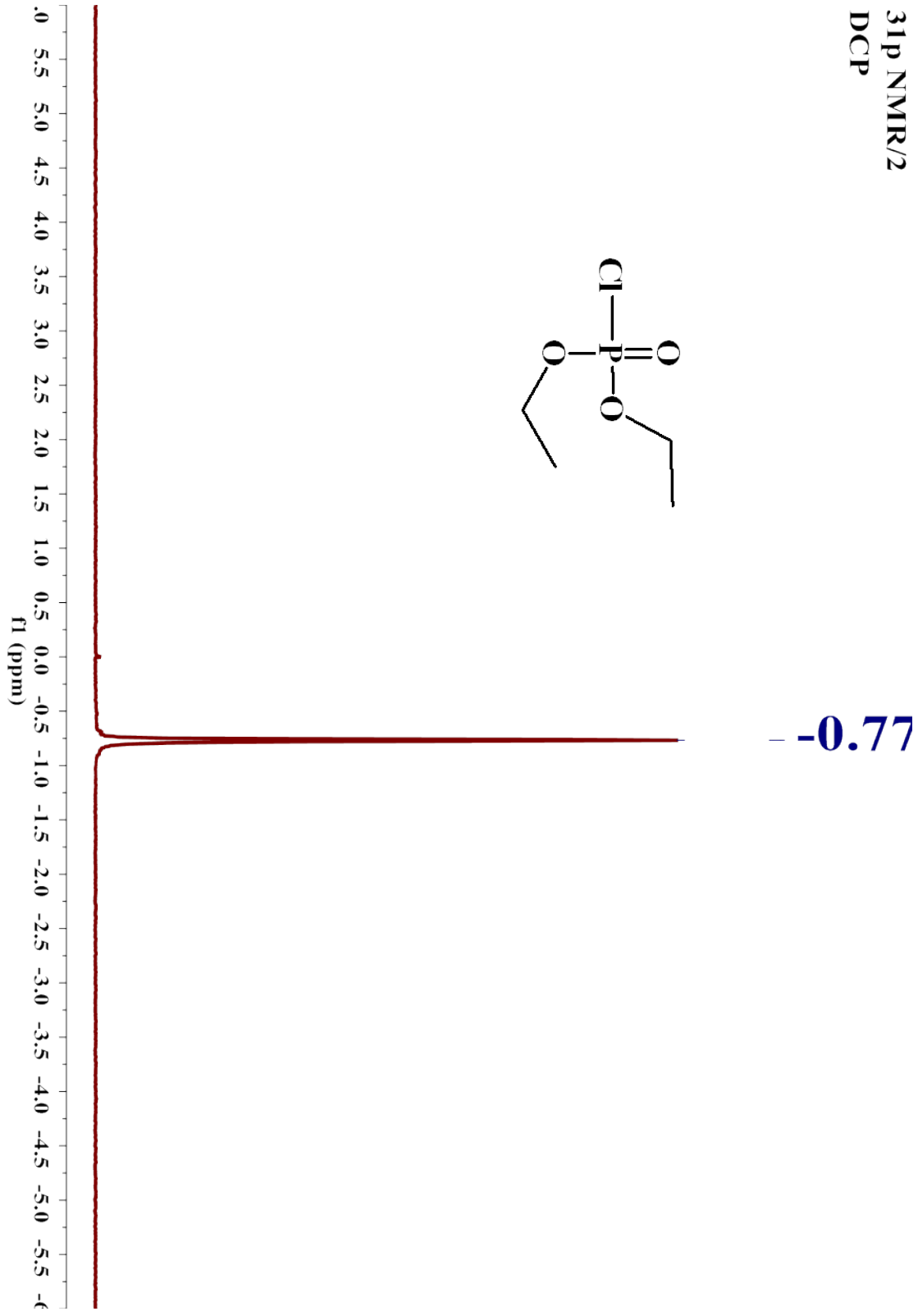

Fig. S14. ^{31}P NMR spectrum of fluorescein in DMSO-d_6

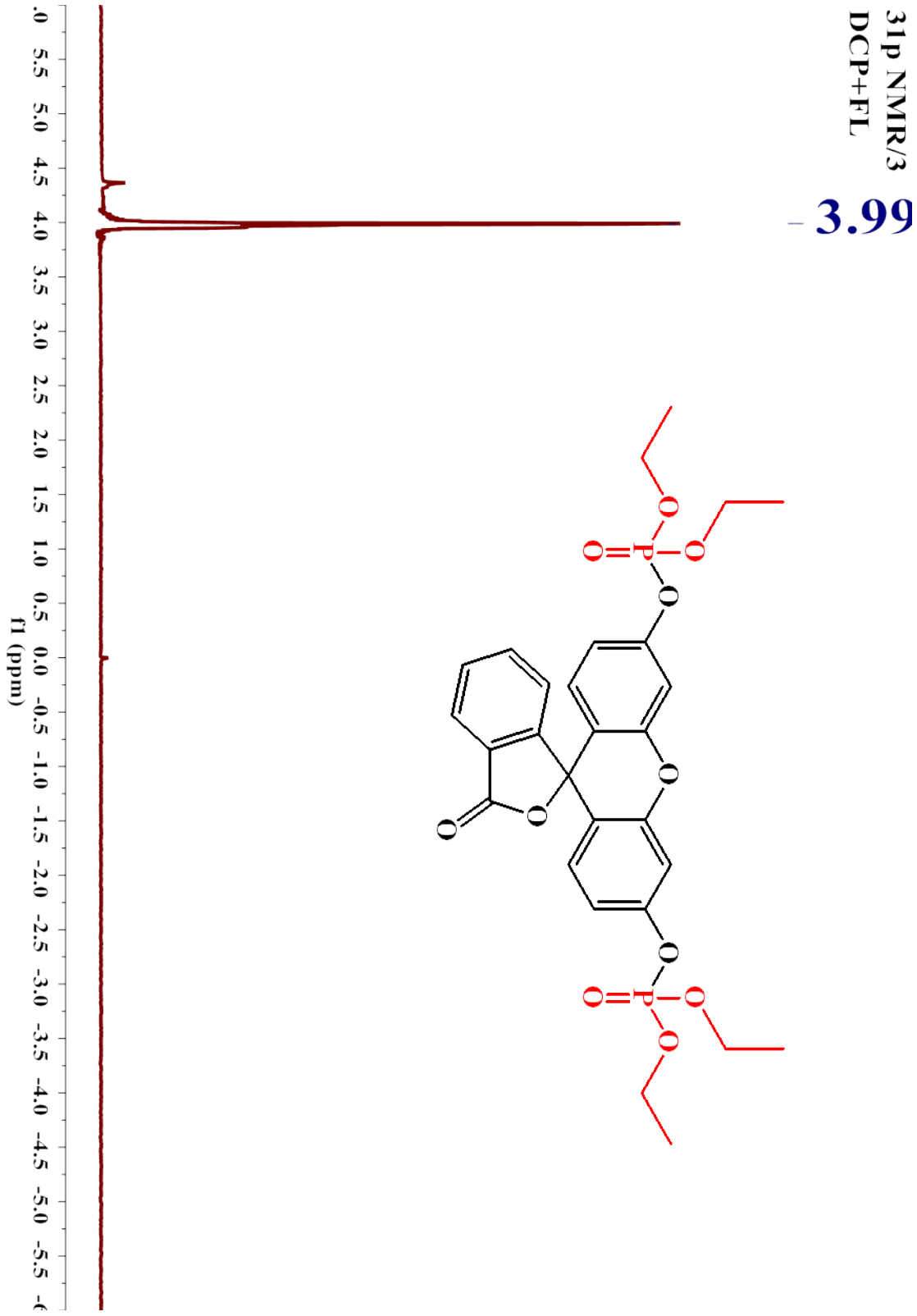

Fig. S15. ^{31}P NMR spectrum of compound **1** in DMSO-d_6

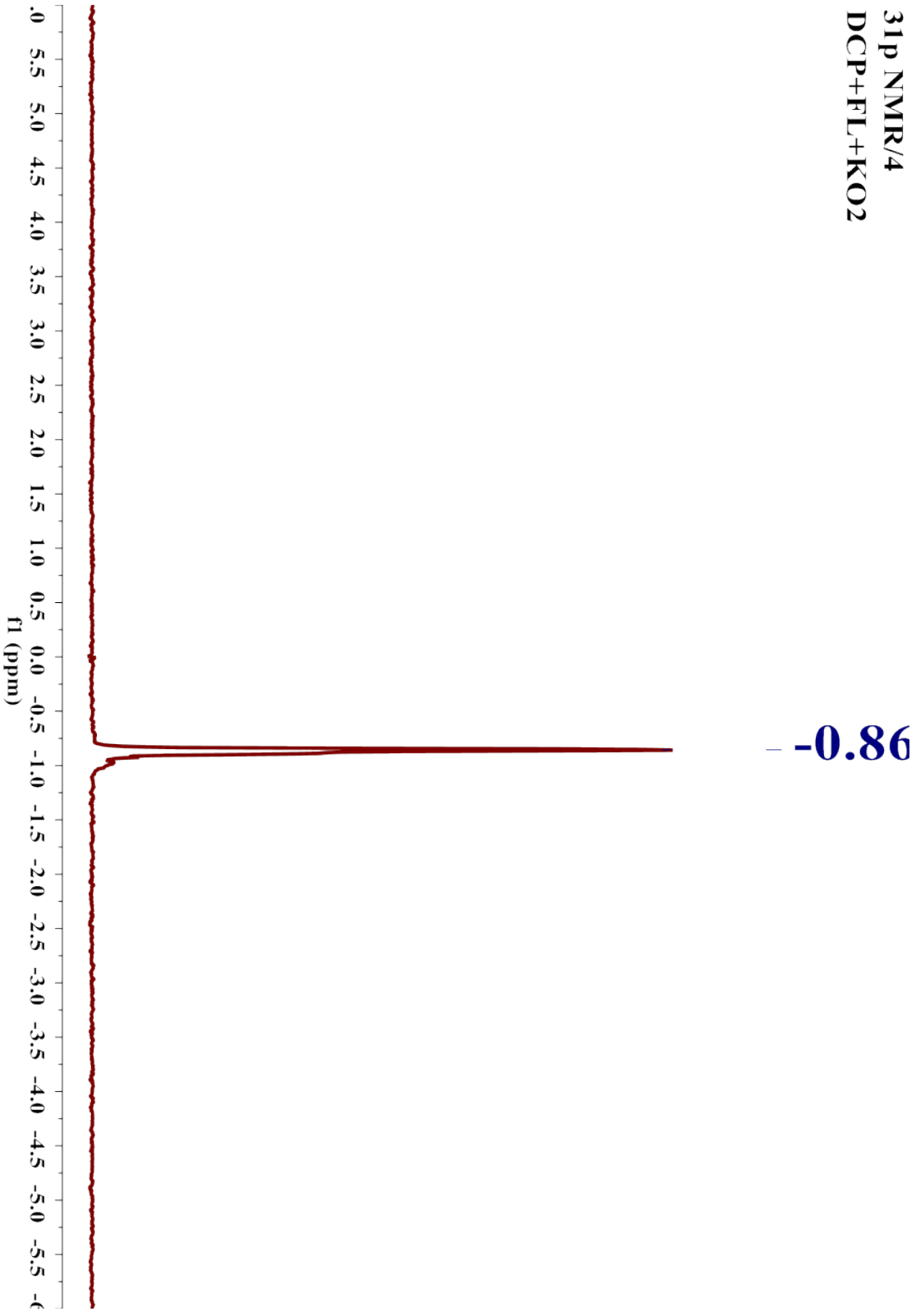

Fig. S16. ^{31}P NMR spectrum of compound **1** and KO_2 in DMSO-d_6 and D_2O

Reference

1. O. A. Sadik, W. H. Land, Jr. and J. Wang, *Electroanalysis*, 2003, **15**, 1149