Electronic Supplementary Information

Detection of Boronic Acid Derivatives in Cells Using Fluorescent Sensor

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Supporting Information

General

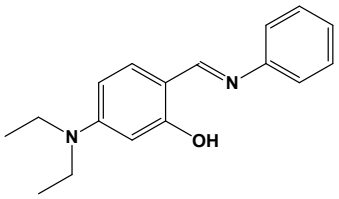
L-^{10}BPA was provided by Stella Pharma Corporation (Osaka, Japan). Flash SiO_2 column chromatography was carried out using Isolera Spektra (Biotage Sweden AB, Uppsala, Sweden) with a SNAP Ultra cartrige (Biotage Sweden AB, Uppsala, Sweden). ^{1}H NMR and ^{13}C NMR spectra were measured on a JMTC-400/54/SS (400 MHz, JEOL Ltd., Tokyo, Japan). Fluorescent spectra were measured on a FP-8200 (JASCO Corporation, Tokyo, Japan).

Synthesis

General procedure for the synthesis of *o*-iminophenol 1-7

To a solution of 4-diethylaminosalicylaldehyde (400mg, 2.07 mmol) in MeOH (5mL) was added amine (2.07 mmol, 1.00eq.), and refluxed for 24 h. The reaction mixture was concentrated in vacuo, the residue was purified by flash SiO_2 column chromatography eluting with hexane/AcOEt.

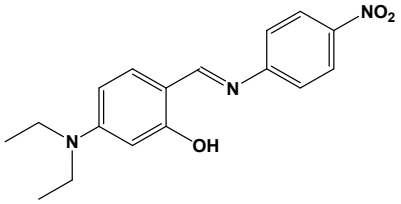
5-(diethylamino)-2-((phenylimino)methyl)phenol (1)



1

Yellow crystals. Yield: 84%. ^{1}H-NMR($CDCl_3$): δ =13.86 (br, 1H,), 8.39 (s, 1H), 7.12-7.37 (m, 5H), 6.18-6.23 (m, 5H), 3.36 (q, *J* = 7.2Hz, 4H), 1.18 (t, *J* =7.2Hz, 6H). ^{13}C-NMR($CDCl_3$): δ = 164.24, 160.32, 151.70, 148.62, 133.68, 129.15, 125.36, 120.66, 108.97, 103.65,97.63, 44.47, 12.60.

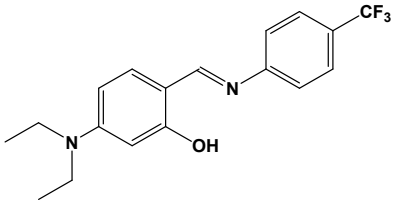
5-(diethylamino)-2-(((4-nitrophenyl)imino)methyl)phenol (2)



2

Red crystals. Yield: 89%. ^{1}H-NMR($CDCl_3$): δ =13.27 (br, 1H,), 8.43-8.46 (m, 1H), 8.23 (d, *J* =8.8Hz, 2H), 7.28 (d, *J* =8.8Hz, 2H),7.17 (d, *J* =8.8Hz, 1H), 6.26-6.29 (m, 1H), 6.17-6.19 (m, 1H), 3.41 (q, *J* = 7.2Hz, 4H), 1.22 (t, *J* =7.2Hz, 6H). ^{13}C-NMR($CDCl_3$): δ = 164.20, 162.28, 154.83, 152.69, 144.71, 134.58, 125.13, 121.22, 108.90, 104.46, 97.39, 44.67, 12.62.

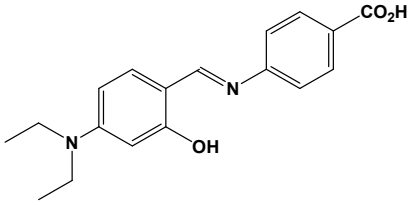
5-(diethylamino)-2-(((4-(trifluoromethyl)phenyl)imino)methyl)phenol (3)



3

Yellow crystals. Yield: 81%. ^{1}H-NMR($CDCl_3$): δ =13.44 (br, 1H,), 8.41 (s, 1H), 7.61 (d, *J* =8.0Hz, 2H), 7.28 (d, *J* =8.0Hz, 2H), 7.16 (d, *J* =8.8Hz, 1H), 6.25 (*J* =8.8Hz, 2.0Hz, 1H), 6.18 (d, *J* =2.0Hz, 1H), 3.40 (q, *J* = 7.2Hz, 4H), 1.21 (t, *J* =7.2Hz, 6H). ^{13}C-NMR($CDCl_3$): δ = 164.00, 161.88, 152.22, 152.13, 134.17, 127.05, 126.38, 122.95, 121.03, 108.87, 104.05, 97.55, 44.62, 12.65.

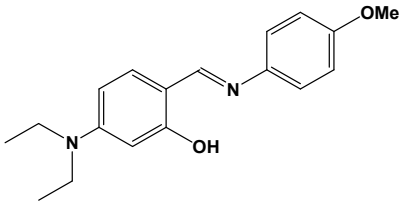
4-((4-(diethylamino)-2-hydroxybenzylidene)amino)benzoic acid (4)



4

Yellow crystals. Yield: 87%. ^{1}H-NMR(DMSO-d_6): δ =13.38 (br, 1H,), 13.00 (br, 1H,), 8.75 (s, 1H), 7.97 (d, *J* =8.0Hz, 2H), 7.39 (d, *J* =8.0Hz, 2H), 7.36 (d, *J* =8.8Hz, 1H), 6.34 (*J* =8.8Hz, 2.0Hz, 1H), 6.09 (s, 1H), 3.40 (q, *J* = 7.2Hz, 4H), 1.24 (t, *J* =7.2Hz, 6H). ^{13}C-NMR(DMSO-d_6): δ = 167.03, 163.67, 162.60, 152.26, 152.02, 134.64, 130.78, 127.33, 120.86, 108.61, 104.21, 96.73, 44.00, 12.56.

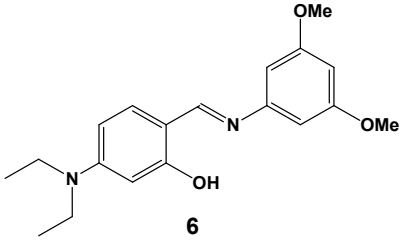
5-(diethylamino)-2-(((4-methoxyphenyl)imino)methyl)phenol (5)



5

Yellow crystals. Yield: 65%. ^{1}H-NMR($CDCl_3$): δ =13.93 (br, 1H,), 8.38 (s, 1H), 7.29 (d, *J* =8.8Hz, 2H), 7.13 (d, *J* =8.8Hz, 1H), 6.90 (d, *J* =8.8Hz, 2H), 6.26-6.29 (dd, *J* =8.8Hz, 1.6Hz, 1H), 6.18 (d, *J* =1.6Hz, 1H), 3.81 (s, 3H), 3.38 (q, *J* = 7.2Hz, 4H), 1.20 (t, *J* =7.2Hz, 6H). ^{13}C-NMR($CDCl_3$): δ = 163.89, 158.97, 157.64, 151.42, 141.93, 133.36, 121.64, 114.42, 109.08, 103.52, 97.76, 55.46, 44.50, 12.66.

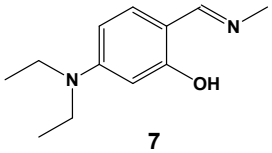
5-(diethylamino)-2-(((3,5-dimethoxyphenyl)imino)methyl)phenol (6)



6

Yellow crystals. Yield: 71%. ^{1}H-NMR($CDCl_3$): δ =13.74 (br, 1H,), 8.39 (s, 1H), 7.14 (d, *J* =8.8Hz, 1H), 6.39 (d, *J* =2.0Hz, 2H), 6.30-6.32 (m, 1H), 6.24 (dd, *J* =8.8Hz, 2.0Hz, 1H), 6.17 (d, *J* =2.0Hz, 1H), 3.81 (s, 6H), 3.39 (q, *J* = 7.2Hz, 4H), 1.20 (t, *J* =7.2Hz, 6H). ^{13}C-NMR($CDCl_3$): δ = 164.42, 161.25, 160.46, 151.89, 150.73, 133.82, 108.86, 103.79, 98.96, 97.76, 55.41, 44.56, 12.66.

5-(diethylamino)-2-((methylimino)methyl)phenol (7)



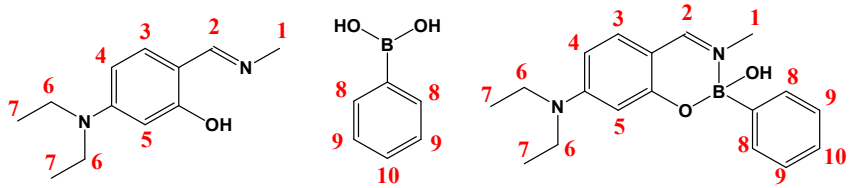
7

Brown oil. Yield: 99%. ^{1}H-NMR($CDCl_3$): δ =13.73 (br, 1H,), 7.91 (s, 1H), 6.94 (d, *J* =8.8Hz, 1H), 6.11 (dd, *J* =8.8Hz, 2.0Hz, 1H), 6.06 (d, *J* =2.0Hz, 1H), 3.34 (q, *J* = 7.2Hz, 4H), 3.31 (s, 3H), 1.17 (t, *J* =7.2Hz, 6H). ^{13}C-NMR($CDCl_3$): δ = 168.01, 163.62, 151.71, 132.68, 108.22, 98.42, 44.32, 42.49, 12.64.

Preparation of boron-sensor-PhB(OH)$_2$ complex

To a solution of PhB(OH)$_2$ (1 mmol) in Acetone (2.5 mL) was added a solution of boron-sensor (1 mmol) in acetone. The mixture was permitted to stand for 20 min at r.t., the solution was used for measurement.

Table S1. ^{1}H NMR chemical shift of compound **7,** $PhB(OH)_2$ and **7**-$PhB(OH)_2$ complex in acetone-d_6.



Proton	Chemical shift (ppm)		
	7	**$PhB(OH)_2$**	**7-$PhB(OH)_2$ Complex**
1	3.34 (s)	-	3.12 (s)
2	8.21 (s)	-	8.08 (s)
3	7.07 (d)	-	7.07-7.20 (m)
4	6.22 (dd)	-	6.26 (dd)
5	6.04 (d)	-	5.98 (d)
6	1.15 (t)	-	1.17 (t)
7	3.40 (q)	-	3.36-3.53 (m)
8	-	7.87 (dd)	7.49 (dd)
9	-	7.32-7.36 (m)	7.07-7.20 (m)
10	-	7.39-7.44 (m)	7.07-7.20 (m)

Measurement of fluorescent spectra

Preparation of sensor-BPA complex

To a solution of BPA (10 mM in PBS, 500 μL) was added a solution of boron-sensor (10 mM in DMSO, 500 μL) and PBS (4 mL). The mixture was permitted to stand for 2 h at 37 ^{o}C, the solution was used for measurement.

Preparation of sensor-Bortezomib complex

To a solution of Bortezomib (20 mM in DMSO, 250 μL) was added a solution of boron-sensor (20 mM in DMSO, 250 μL), and PBS (4.5 mL). The mixture was permitted to stand for 2 h at 37 ^{o}C, the solution was used for measurement.

Relationship between BPA concentration and emitted fluorescence following staining with 7 (Fig 6)

To a solution of BPA (10 mM, 5 mM, 2.5 mM in PBS, 500 μL) was added a solution of boron-sensor (10 mM in DMSO, 500 μL) and PBS (4 mL). The mixture was permitted to stand for 2 h at 37 ^{o}C, the solution was used for measurement.

Cells and cell culture

The C6 (rat glioma) cell line used in the cytotoxicity analyses, boron incorporation, tumor cell killing studies, and immunostaining, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and 24 mM sodium hydrogen carbonate at 37°C in a 5% CO_2 atmosphere. Cells in the mono-layer were harvested with 0.25% trypsin/0.02% ethylenediaminetetraacetic acid (EDTA) in Ca^{2+}-free phosphate-buffered saline (PBS). Matrigel (growth factor reduced type) was purchased from BD Science (SanJose, CA, US).

Cell staining with boron-sensor.

Glass coverslips coated with Matrigel (3.5 □g/cm^2 protein) were seeded with C6 (rat glioma) cells (0.8×10^5 cells suspended in 3 mL of DMEM), and allowed to settle for 1 h at 37 ^{o}C. The medium was replaced with an equivalent medium containing compound L-BPA (the final concentration was 1.0 mM), and the cells were cultured for 24 h at 37°C. After washing with DMEM, C6 cells were fixed with 10% paraformaldehyde in PBS for 10 min at room temperature. The cells were rinsed with PBS, and incubated with compound **7** in 10% DMSO/PBS (1 mM) for 20 min at 37°C. After washing with PBS, the cells were mounted with Permafluor (Immunotech, Marseille, France)) and then photographed with a microscope (Bz-9000, Keyence, Osaka).

Fluorescent spectra

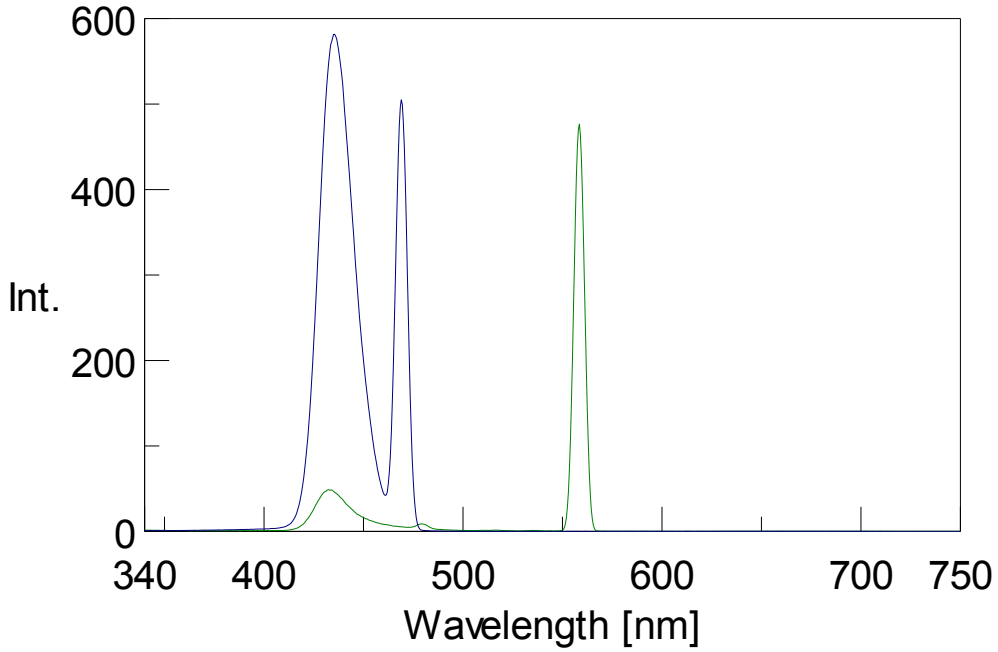


Figure S1a. Excitation spectra of compound **1** (green line, em: 559 nm) and $PhB(OH)_2$-**1** complex(blue line, em: 469 nm) in acetone at 25°C.

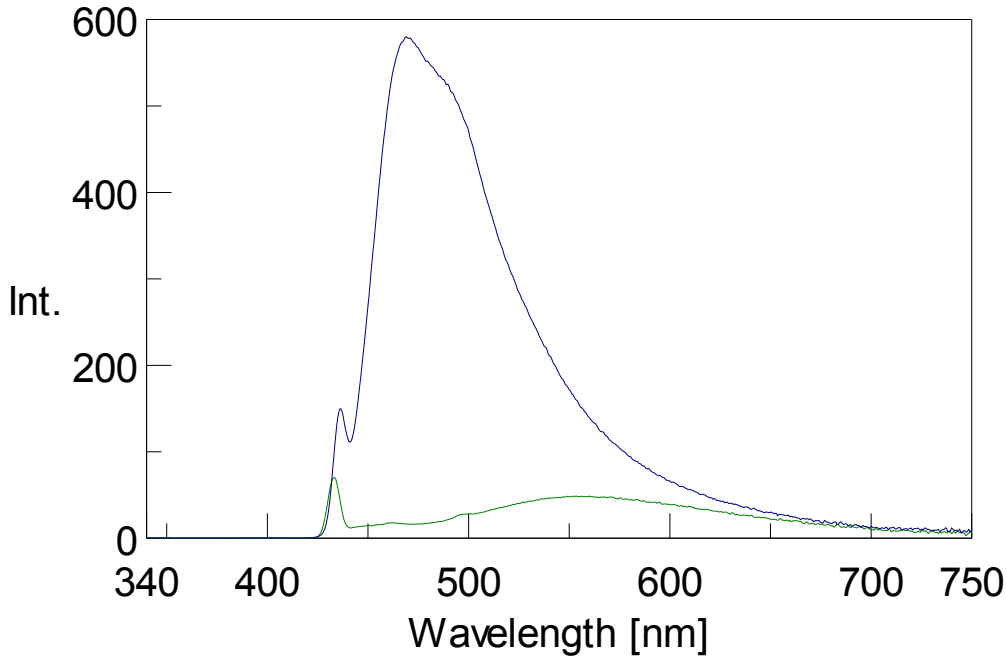


Figure S1b. Fluorescence spectra of compound **1** (green line, ex 433nm) and $PhB(OH)_2$-**1** complex (blue line, ex 436 nm) in acetone at 25°C.

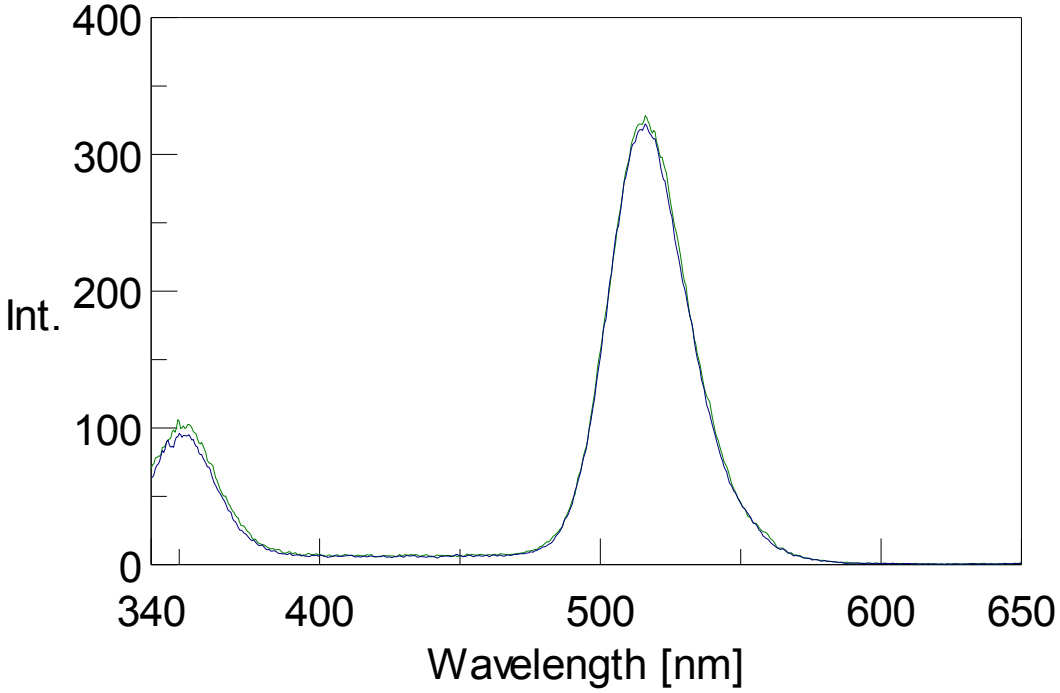


Figure S2a. Excitation spectra of compound **2** (green line, em: 668 nm) and $PhB(OH)_2$-**2** complex (blue line, em: 663 nm) in acetone at 25°C.

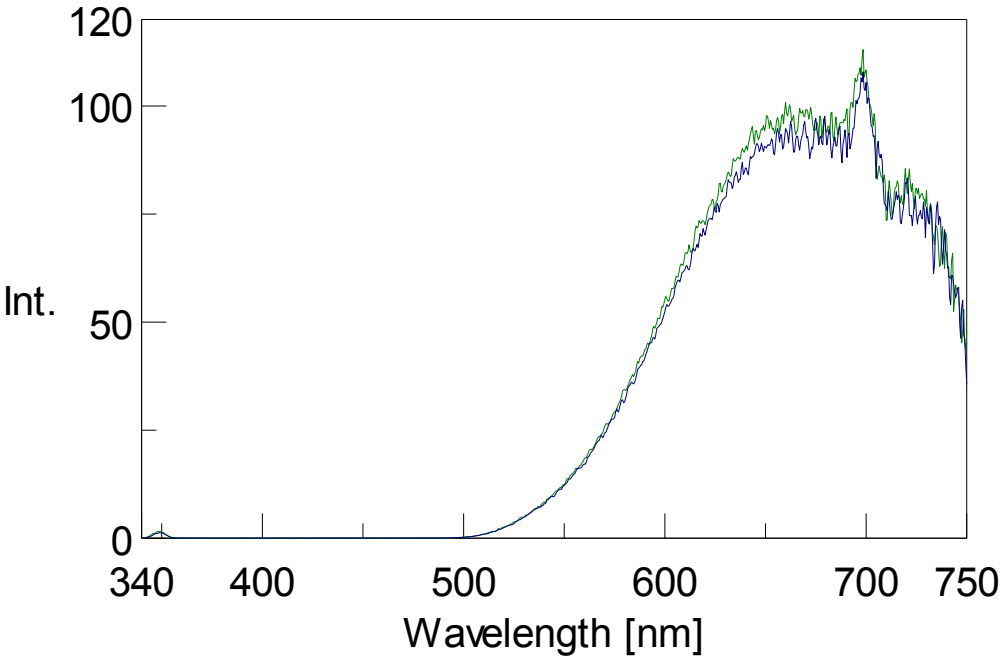


Figure S2b. Fluorescence spectra of compound **2** (green line, ex 350 nm) and $PhB(OH)_2$-**2** complex (blue line, 350 nm) in acetone at 25°C.

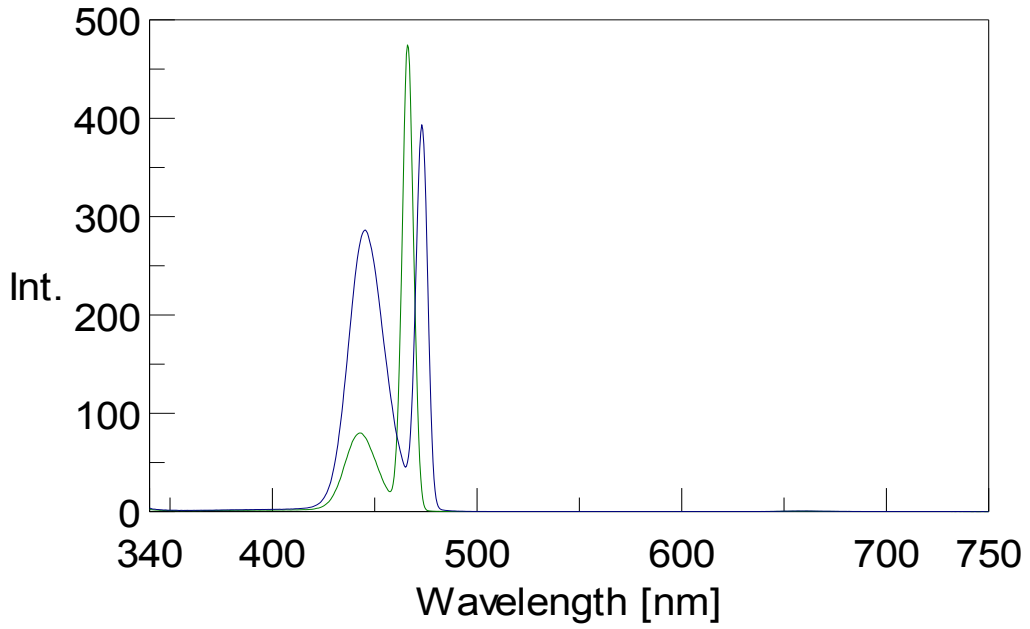


Figure S3a. Excitation spectra of compound **3** (green line, em: 465 nm) and $PhB(OH)_2$-**3** complex(blue line, em: 474 nm) in acetone at 25°C.

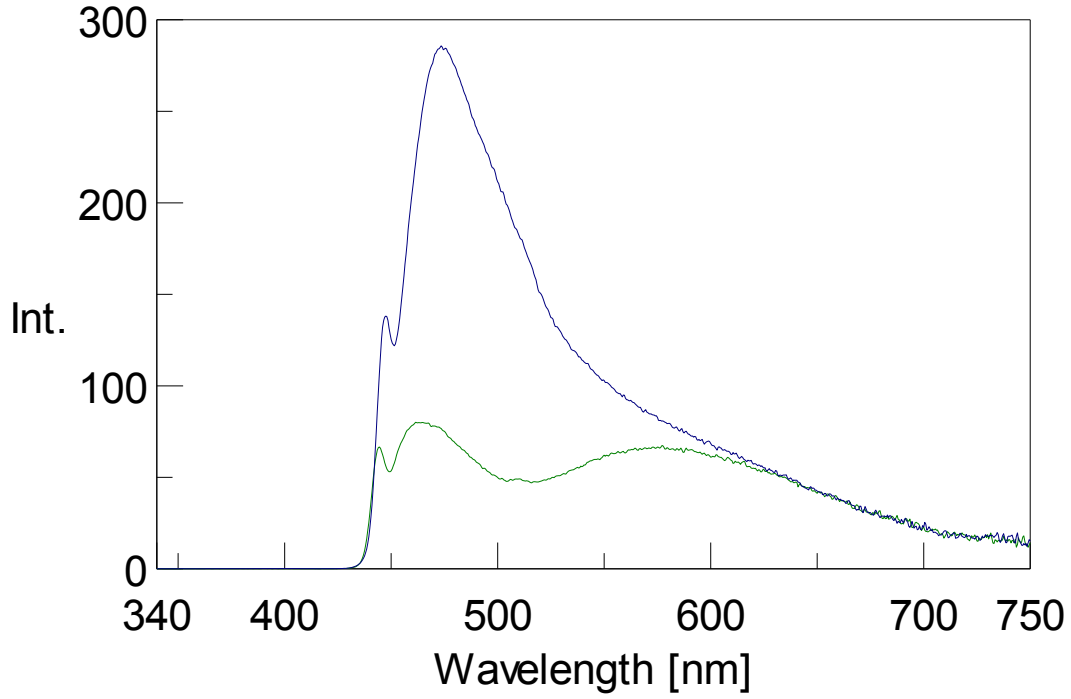


Figure S3b. Fluorescence spectra of compound **3** (green line, ex 443 nm) and $PhB(OH)_2$-**3** complex (blue line, ex 446 nm) in acetone at 25°C.

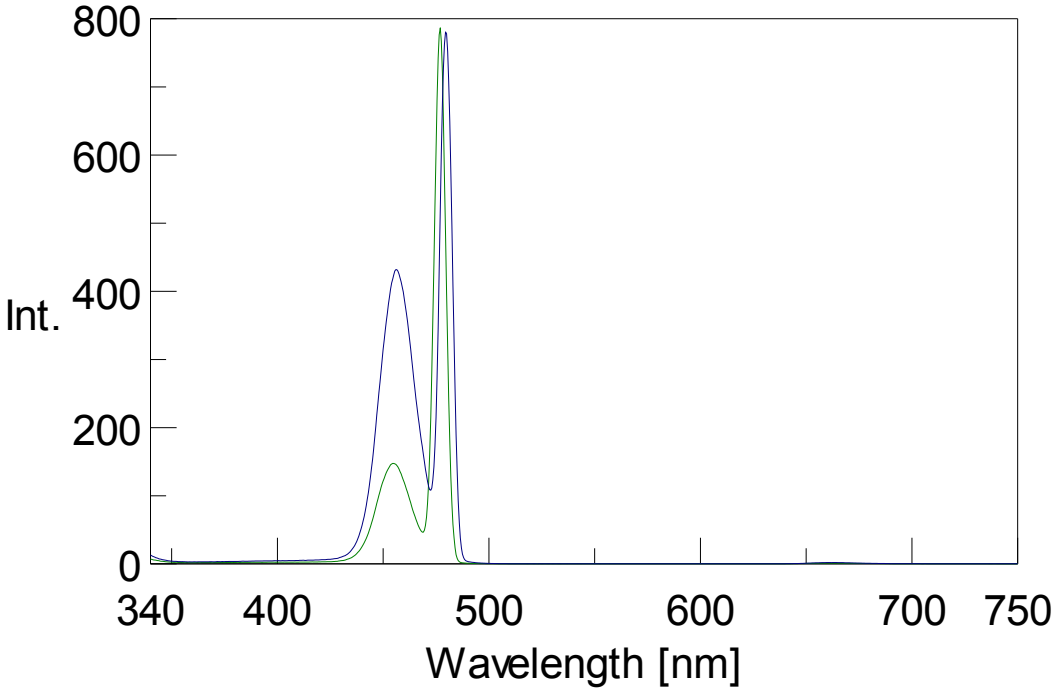


Figure S4a. Excitation spectra of compound **4** (green line, em: 479 nm) and $PhB(OH)_2$-**4** complex (blue line, em: 481 nm) in acetone at 25°C.

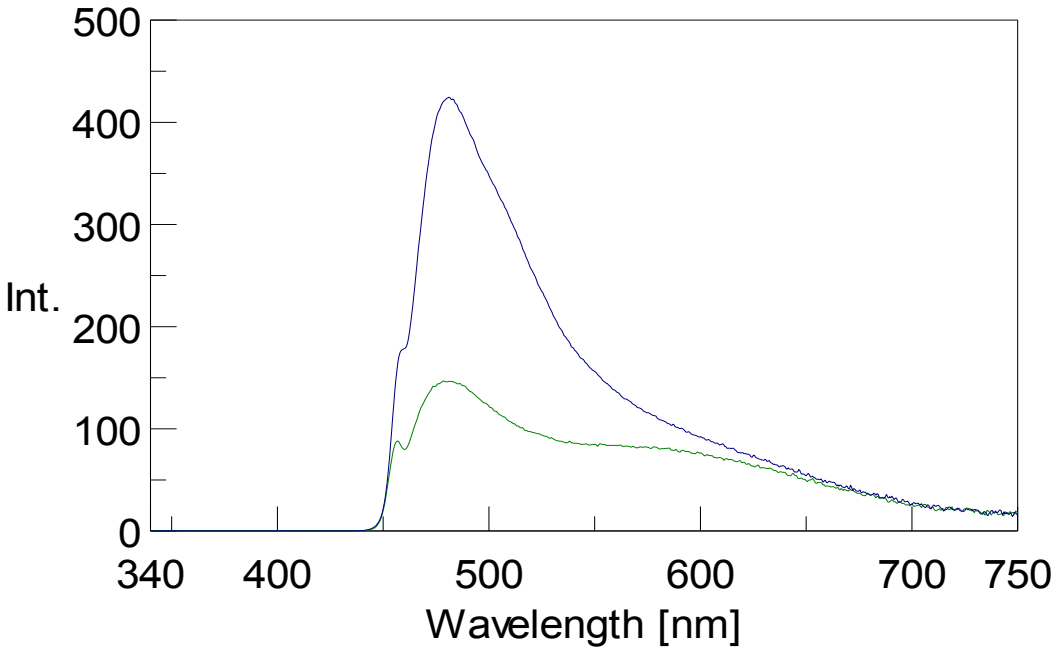


Figure S4b. Fluorescence spectra of compound **4** (green line, ex 455 nm) and $PhB(OH)_2$-**4** complex (blue line, ex 456 nm) in acetone at 25°C.

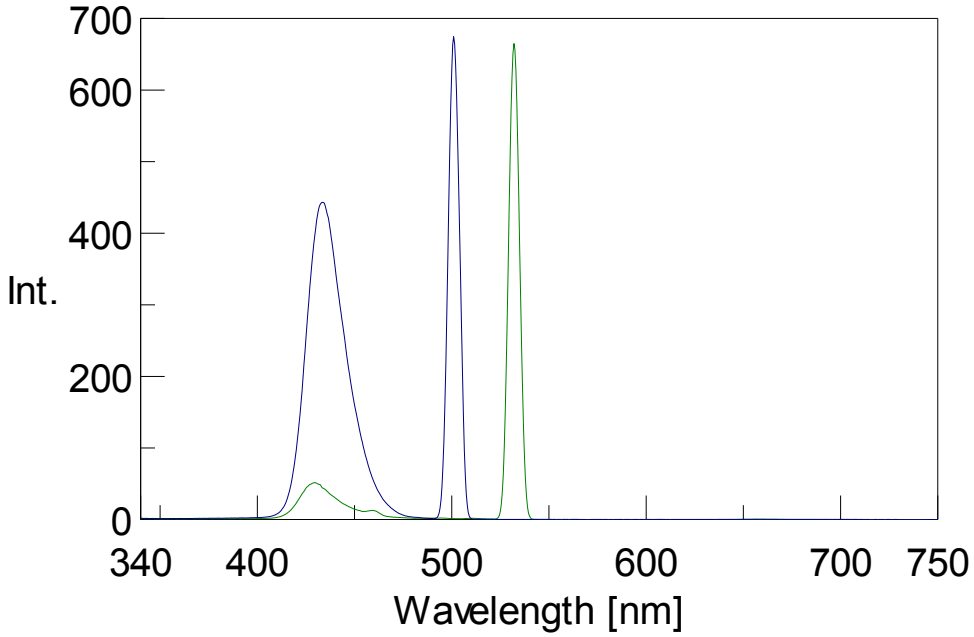


Figure S5a. Excitation spectra of compound **5** (green line, em: 533 nm) and $PhB(OH)_2$-**5** complex (blue line, em: 500 nm) in acetone at 25°C.

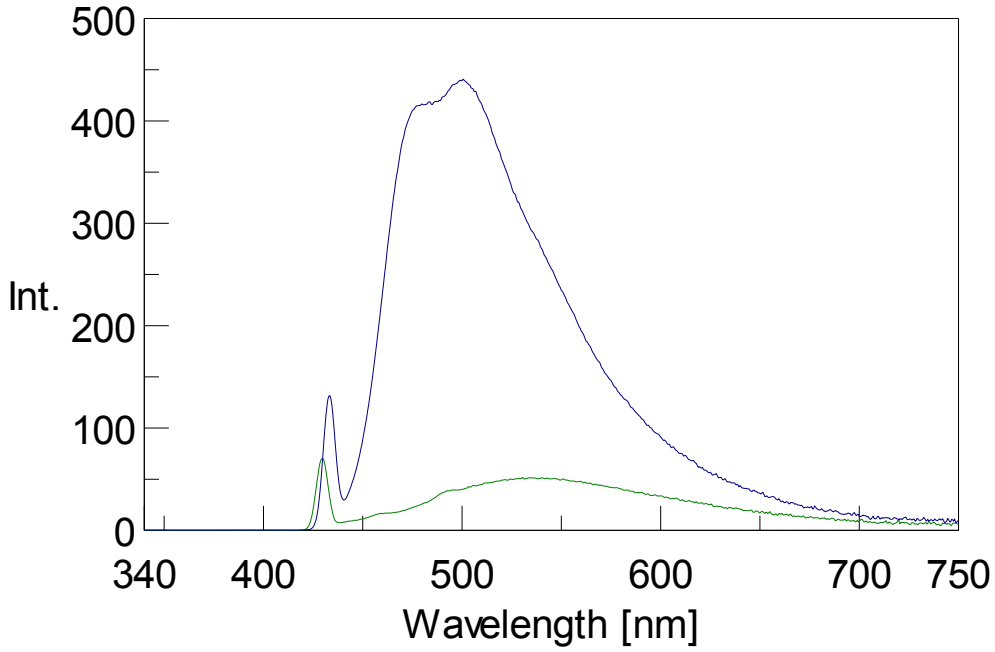


Figure S5b. Fluorescence spectra of compound **5** (green line, 430 nm) and $PhB(OH)_2$-**5** complex (blue line, 434 nm) in acetone at 25°C.

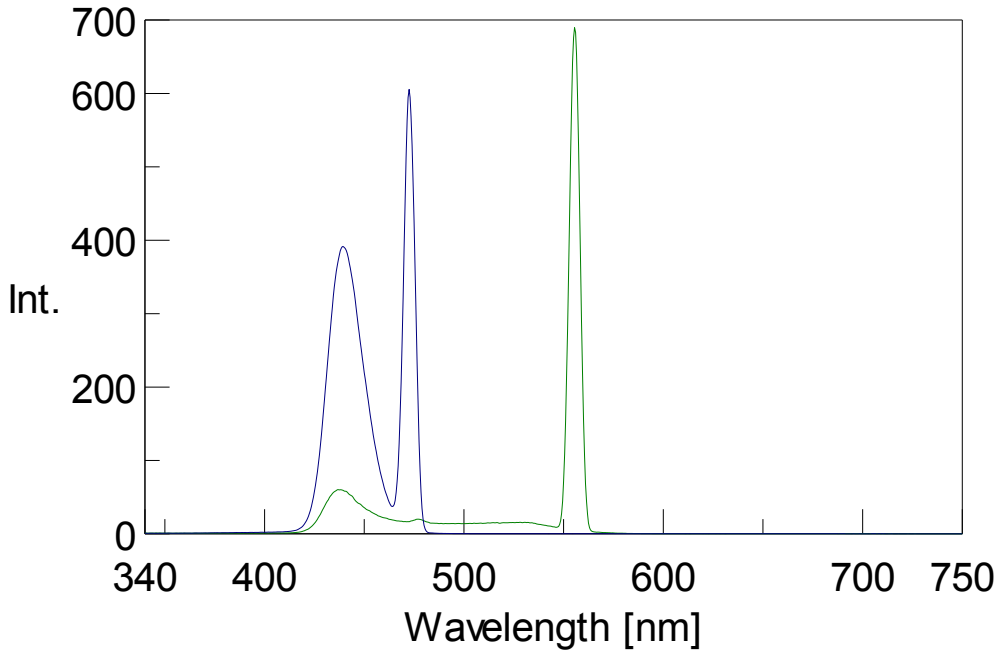


Figure S6a. Excitation spectra of compound **6** (green line, em: 556nm) and $PhB(OH)_2$-**6** complex(blue line, em: 473 nm) in acetone at 25°C.

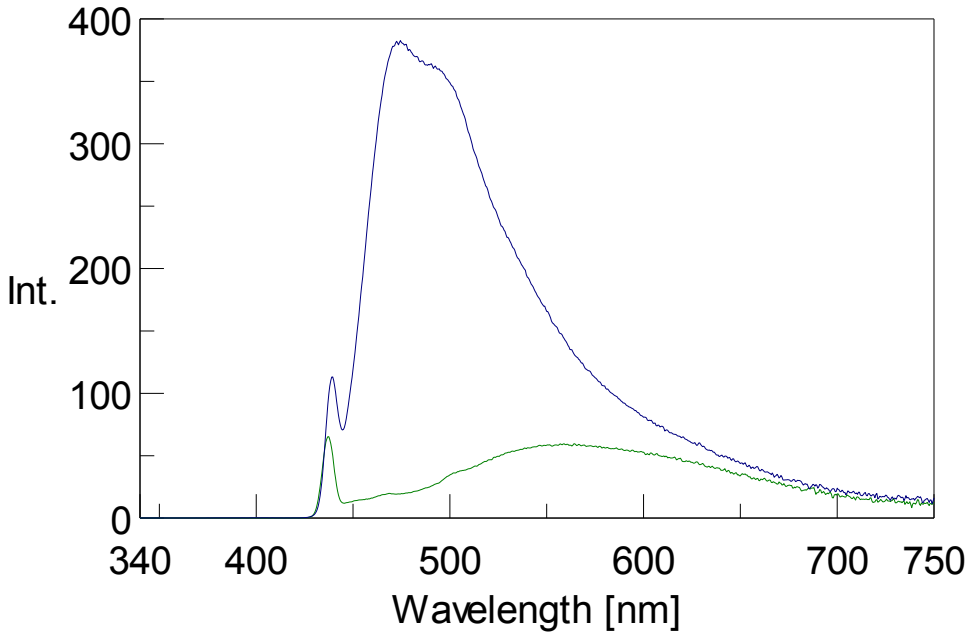


Figure S6b. Fluorescence spectra of compound **6** (green line, ex 437 nm) and $PhB(OH)_2$-**6** complex (blue line, ex 439 nm) in acetone at 25°C.

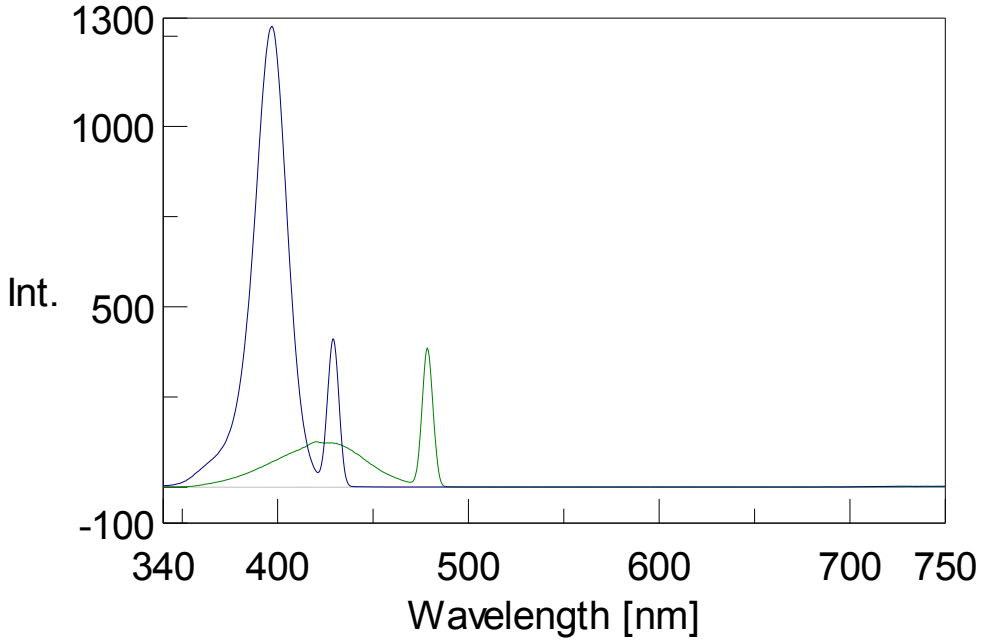


Figure S7a. Excitation spectra of compound **7** (green line, em: 481 nm) and $PhB(OH)_2$-**7** complex (blue line, em: 431nm) in acetone at 25°C.

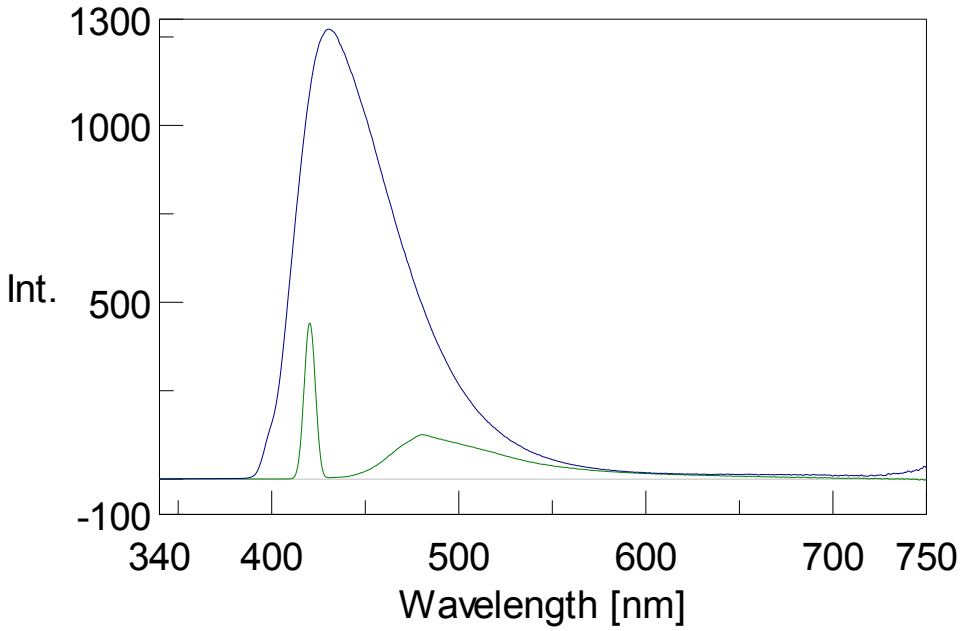


Figure S7b. Fluorescence spectra of compound **7** (green line, ex 420 nm) and $PhB(OH)_2$-**7** complex (blue line, ex 397 nm) in acetone at 25°C.

NMR Spectra

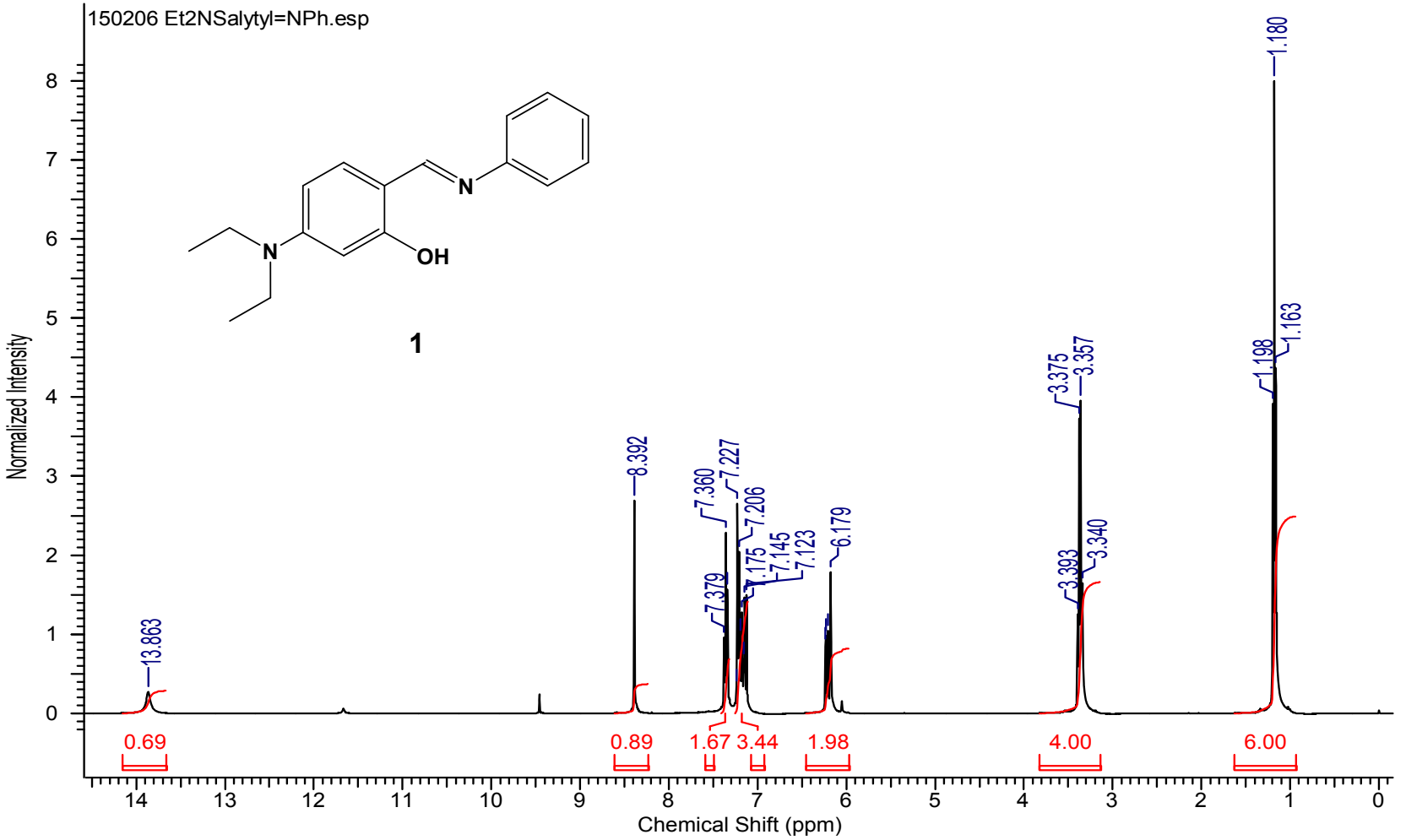


Figure S8a ^{1}H NMR spectrum of compound **1** ($CDCl_3$).

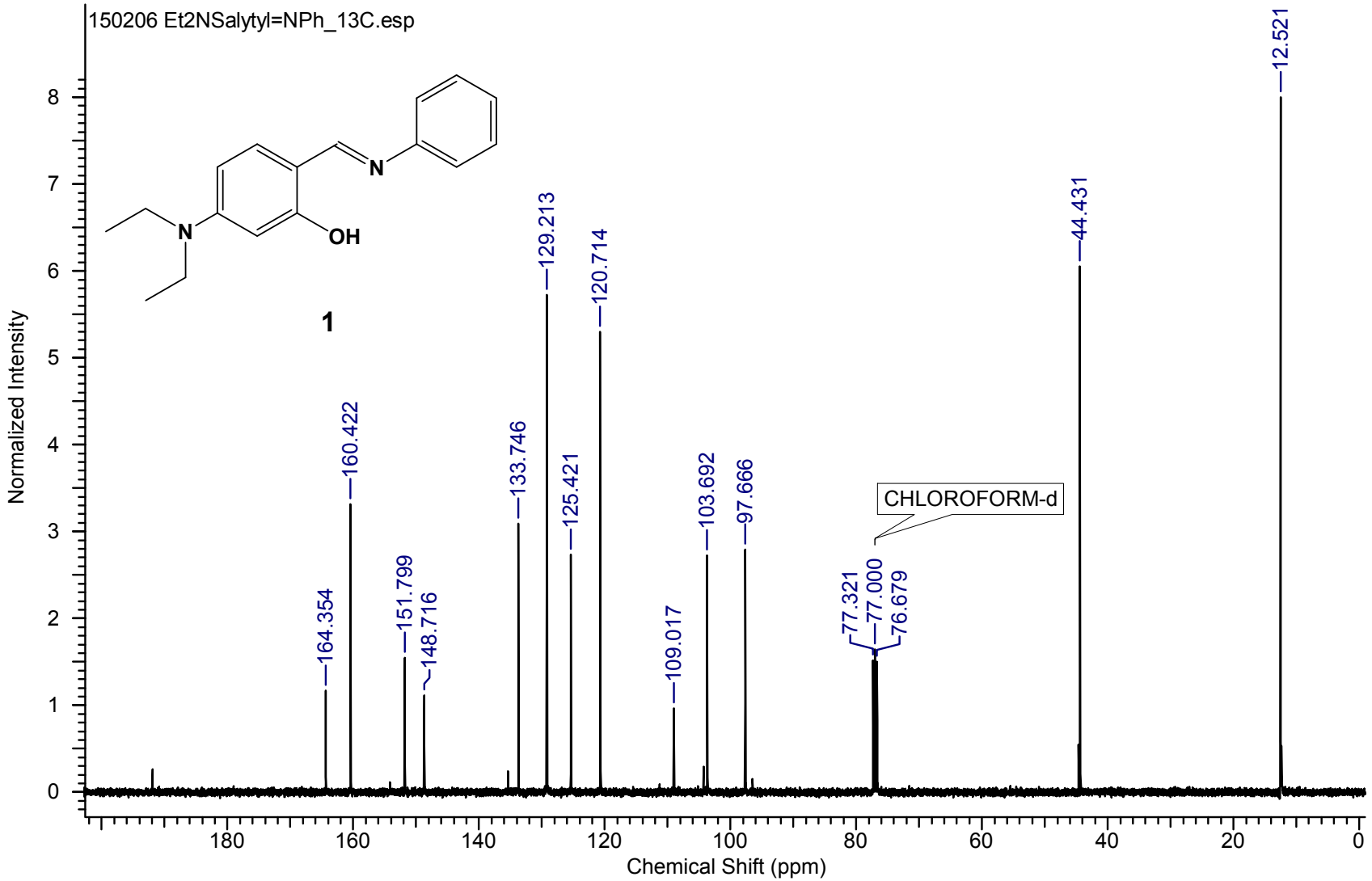


Figure S8b ^{13}C NMR spectrum of compound **1** ($CDCl_3$).

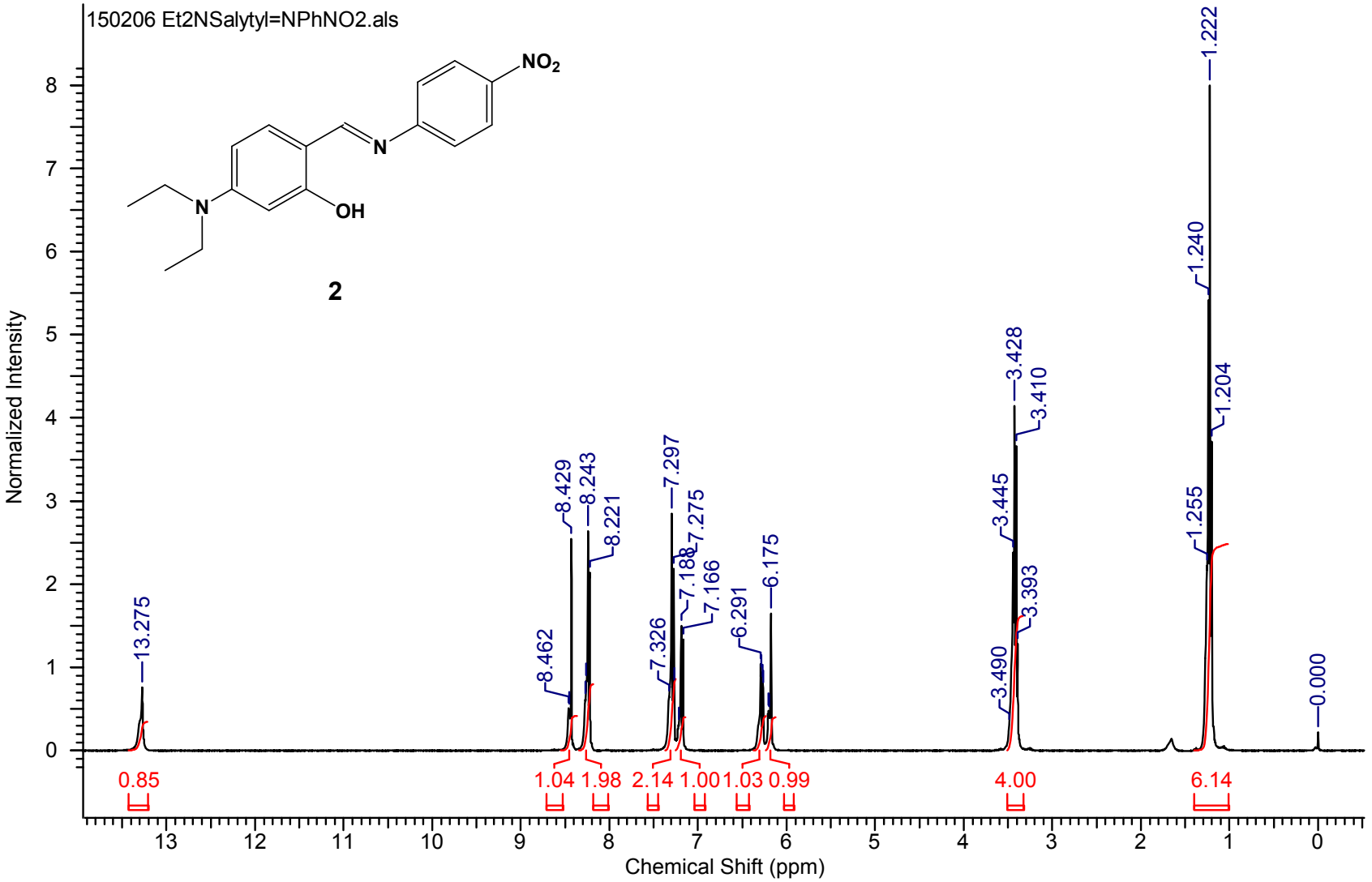


Figure S9a ^{1}H NMR spectrum of compound **2** ($CDCl_3$).

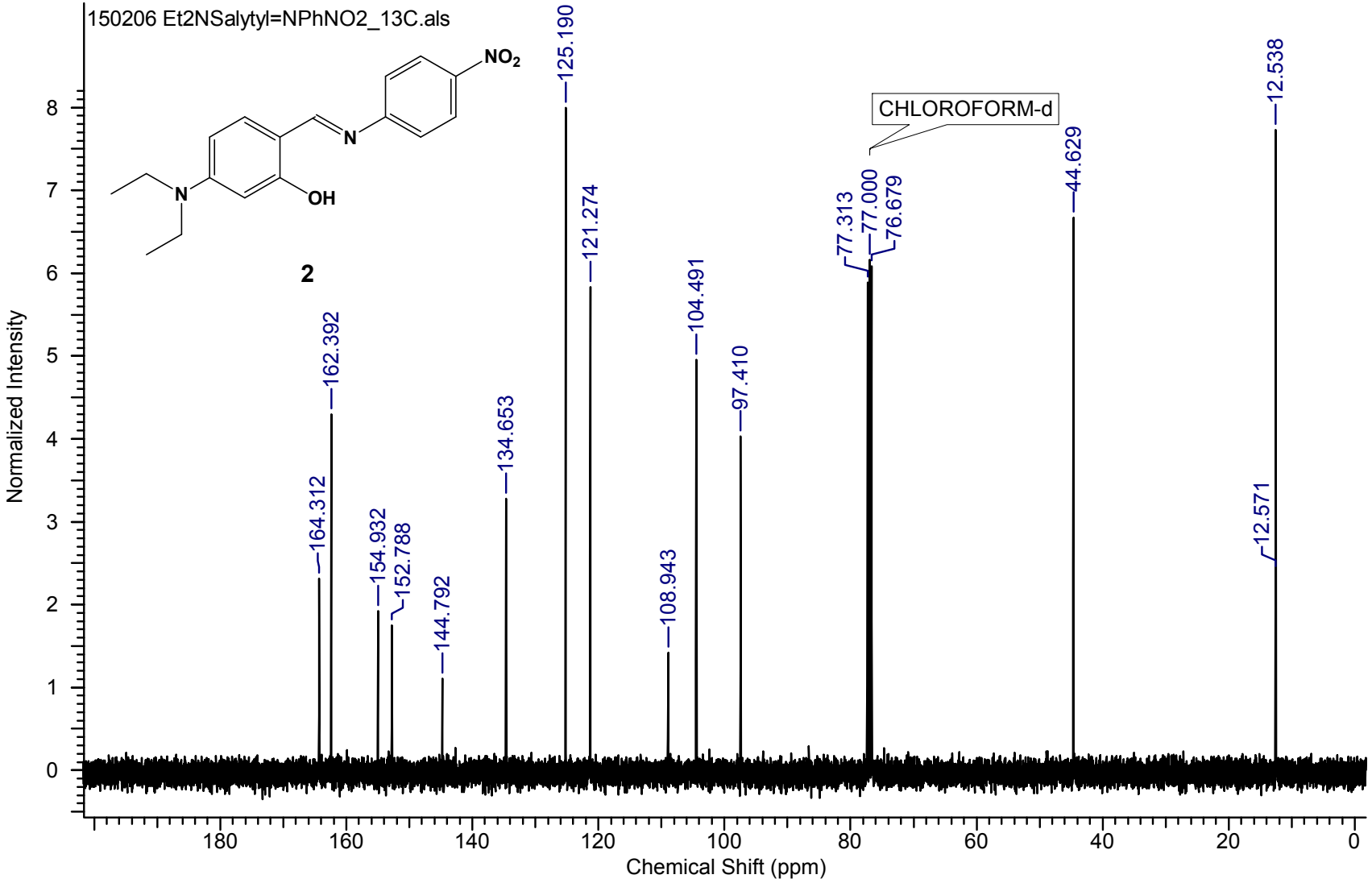


Figure S9b ^{13}C NMR spectrum of compound **2** ($CDCl_3$).

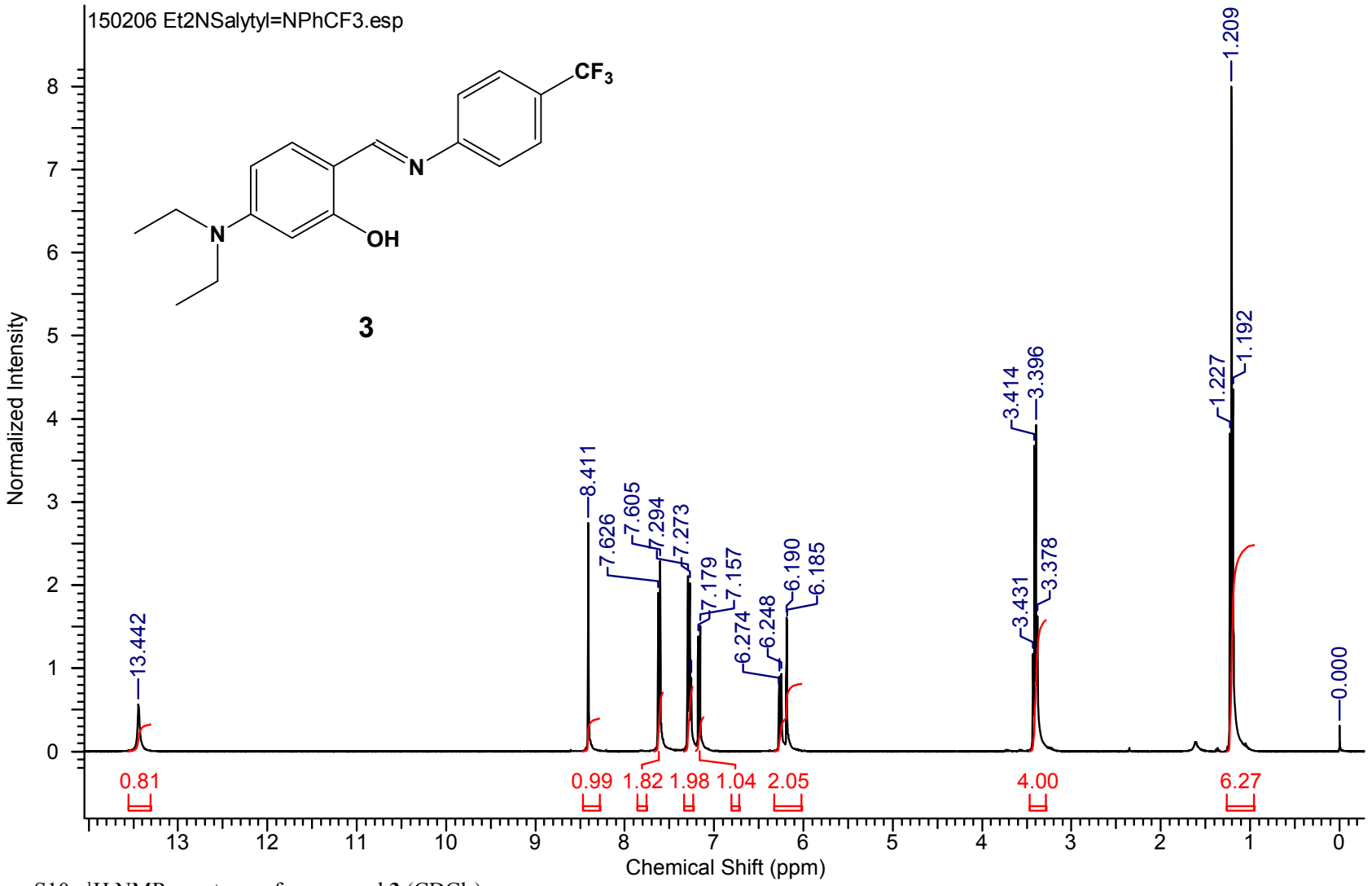


Figure S10a ^{1}H NMR spectrum of compound **3** ($CDCl_3$).

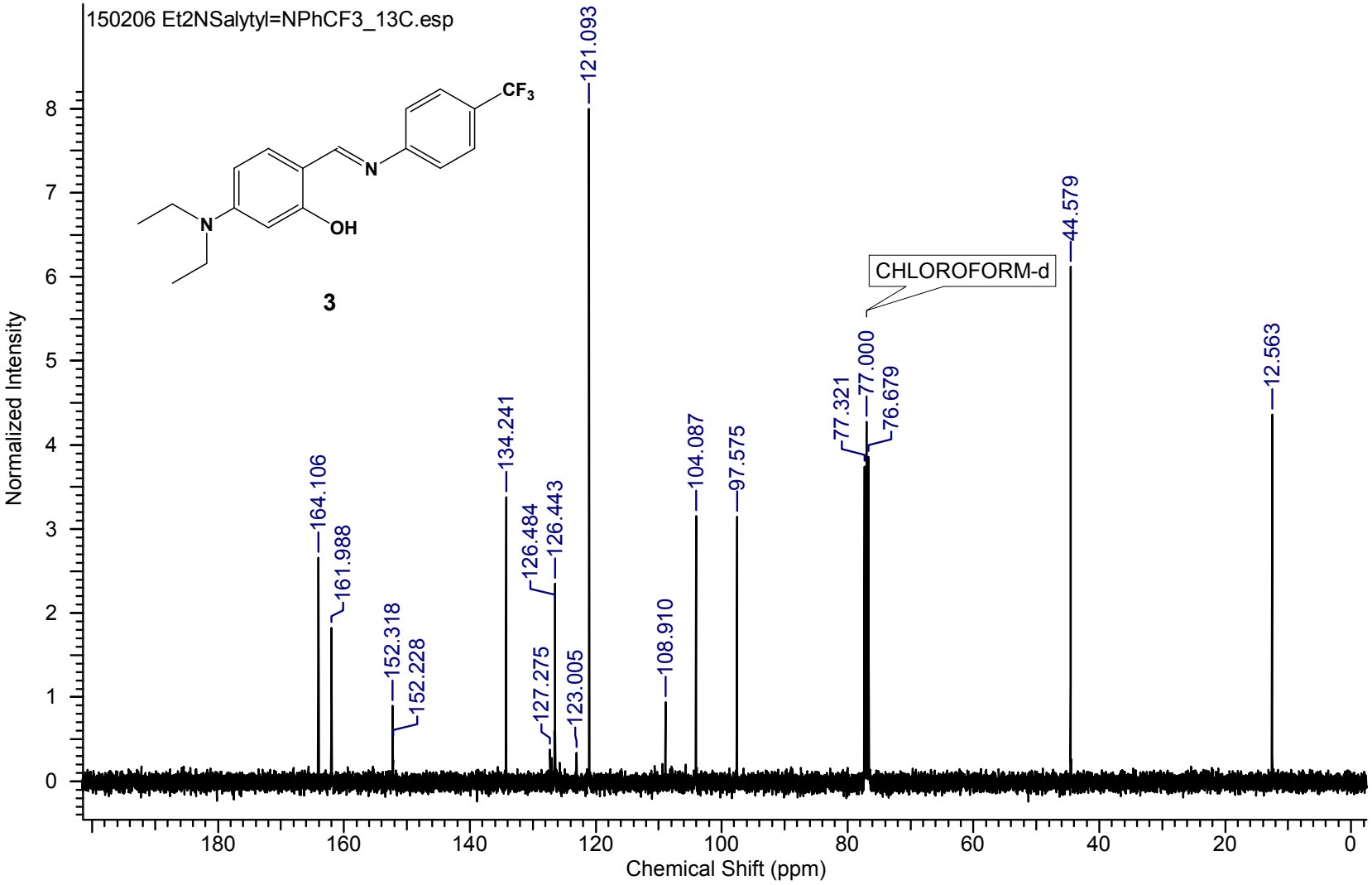


Figure S10b ^{13}C NMR spectrum of compound **3** ($CDCl_3$).

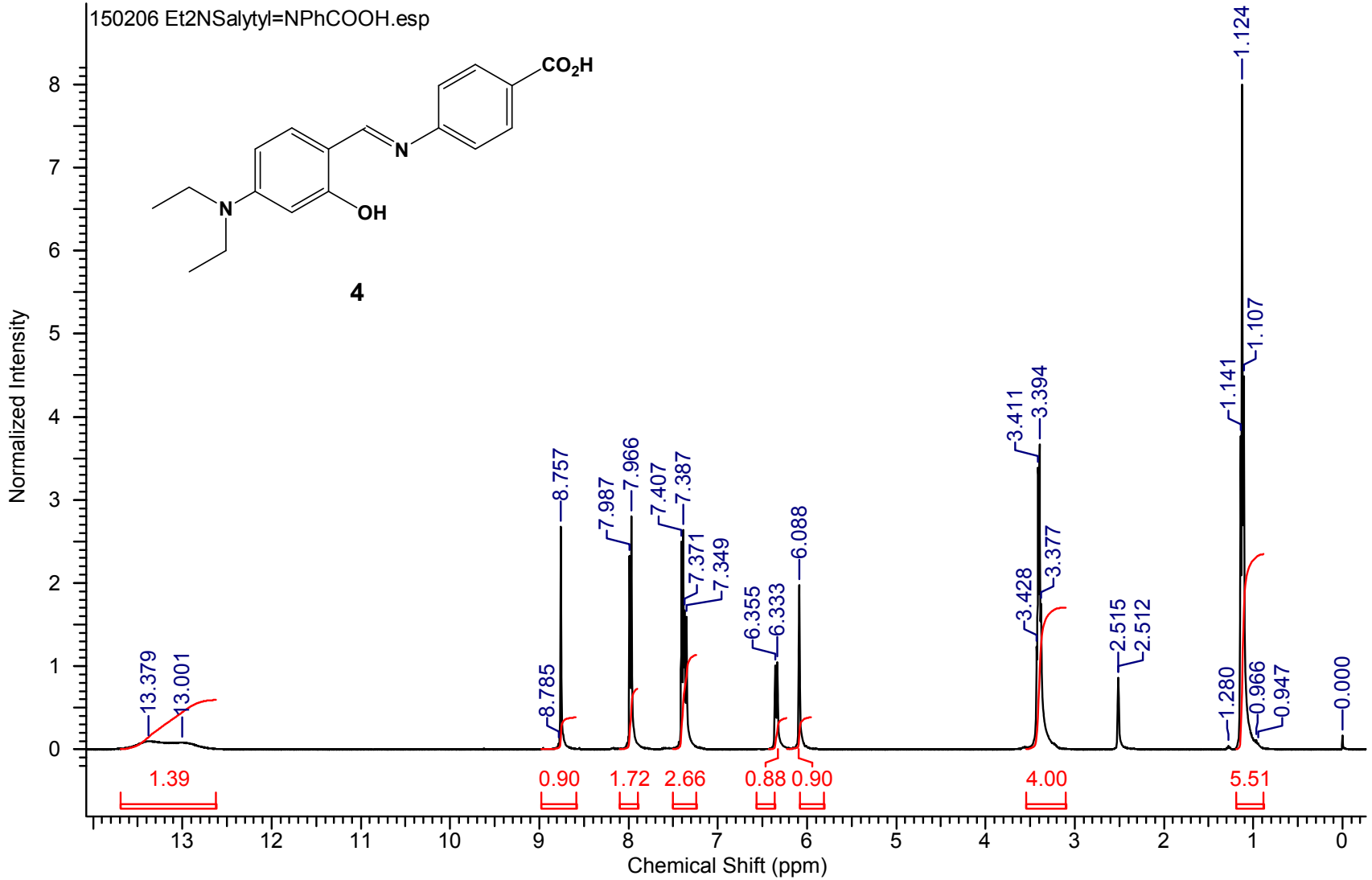


Figure S11a ^{1}H NMR spectrum of compound **4** (DMSO-d_6).

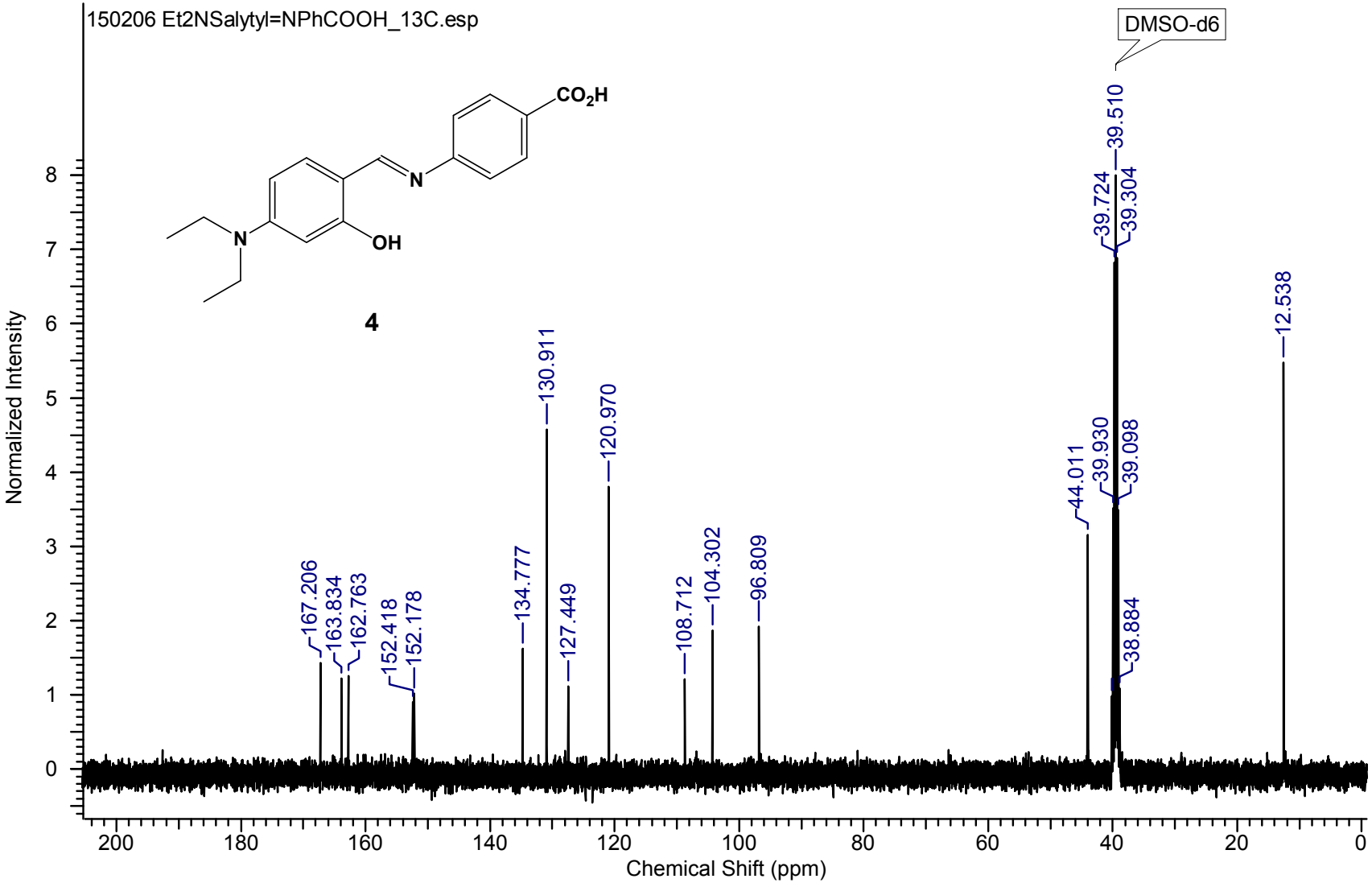


Figure S11b ^{13}C NMR spectrum of compound **4** (DMSO-d_6).

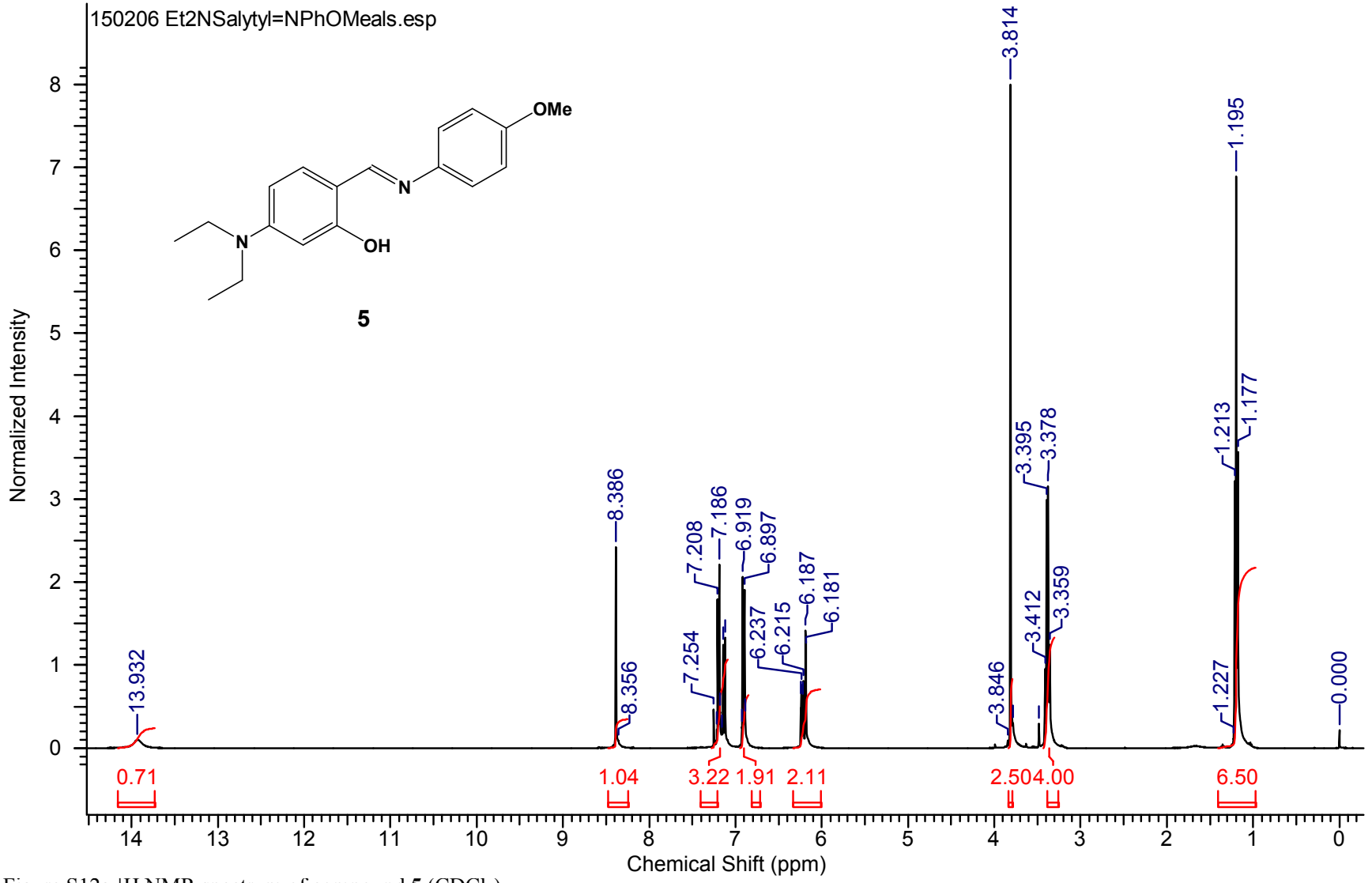


Figure S12a ^{1}H NMR spectrum of compound **5** ($CDCl_3$).

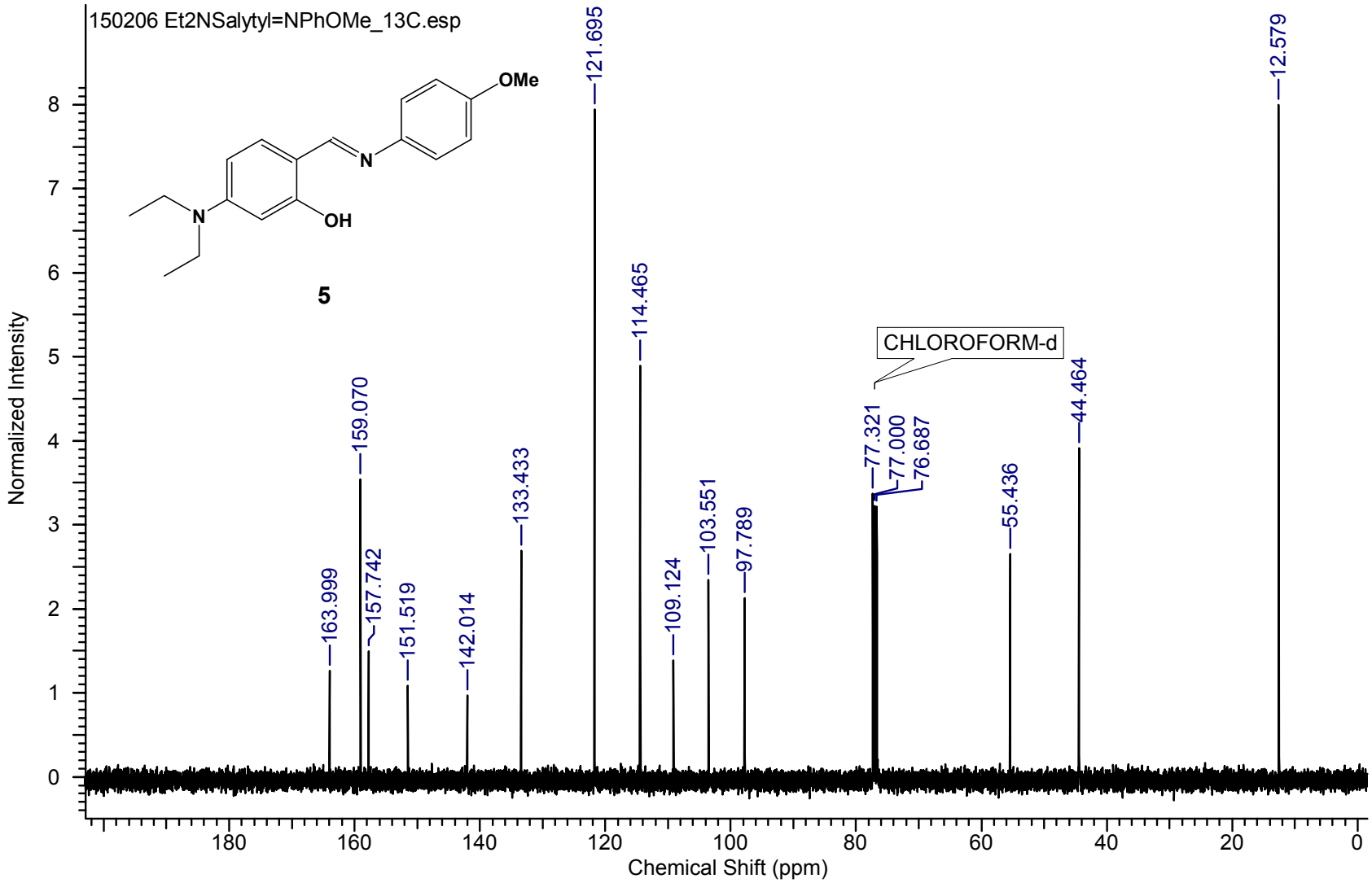


Figure S12b ^{13}C NMR spectrum of compound **5** ($CDCl_3$).

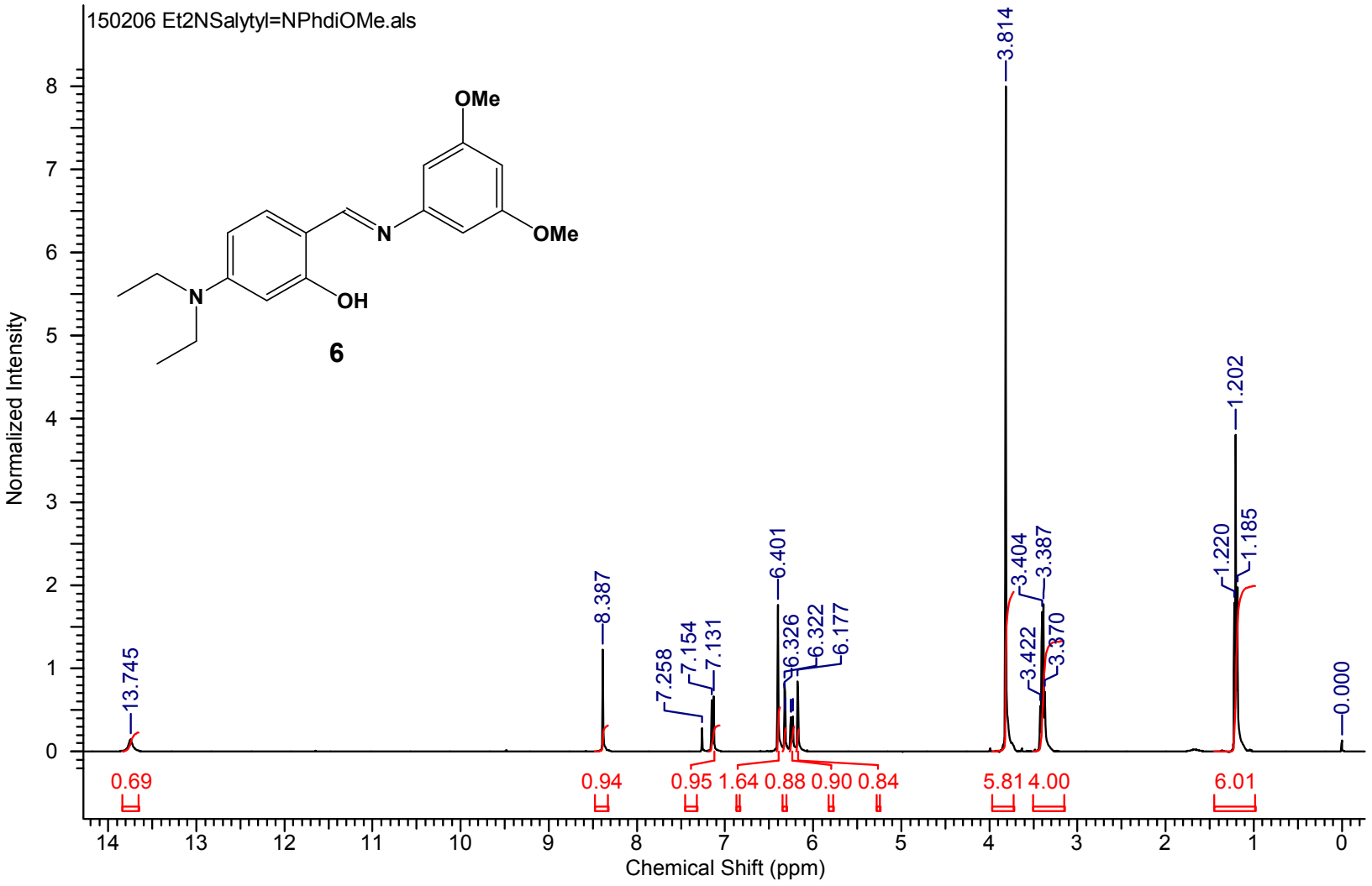


Figure S13a ^{1}H NMR spectrum of compound **6** ($CDCl_3$).

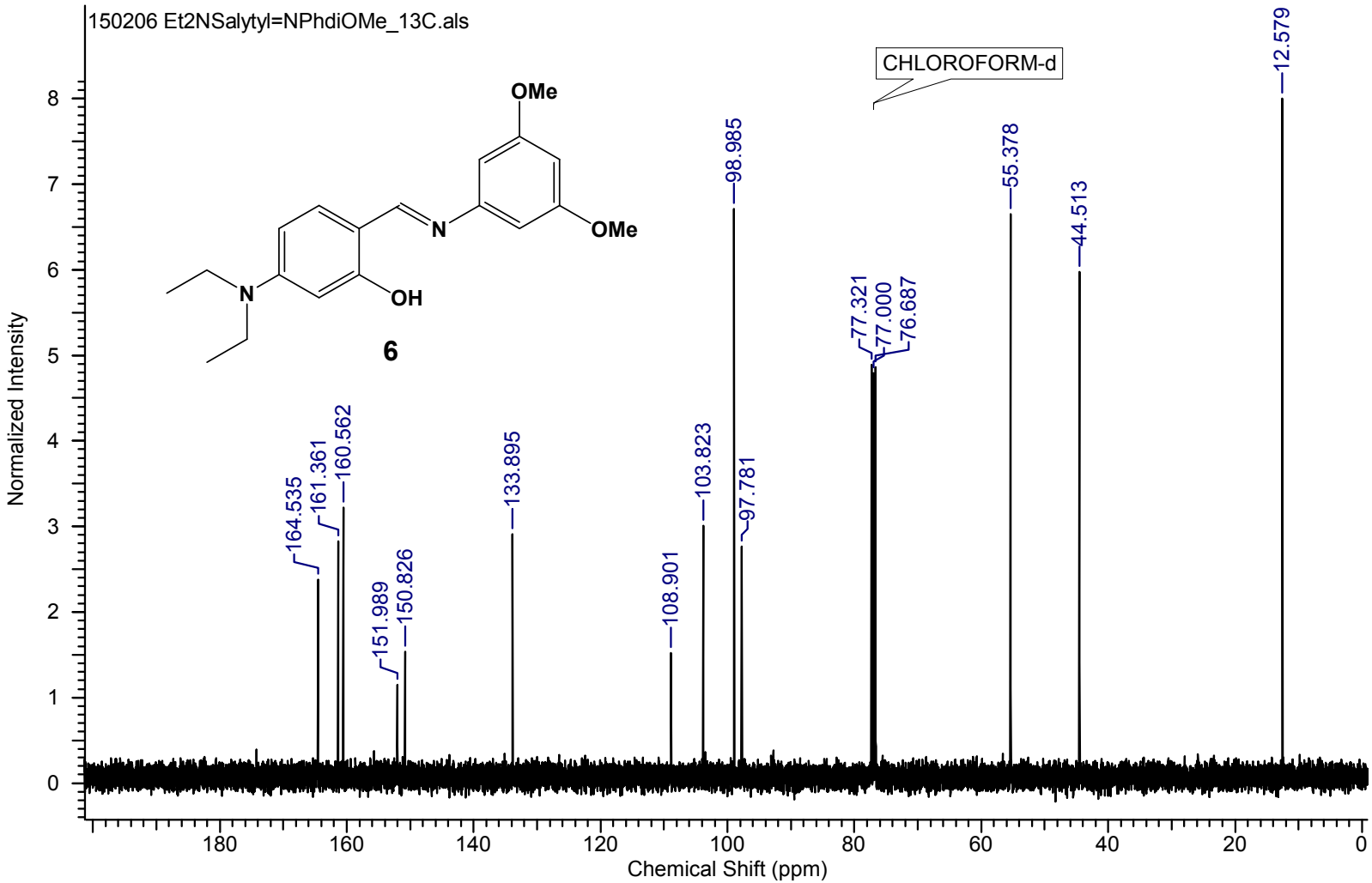


Figure S13b ^{13}C NMR spectrum of compound **6** ($CDCl_3$).

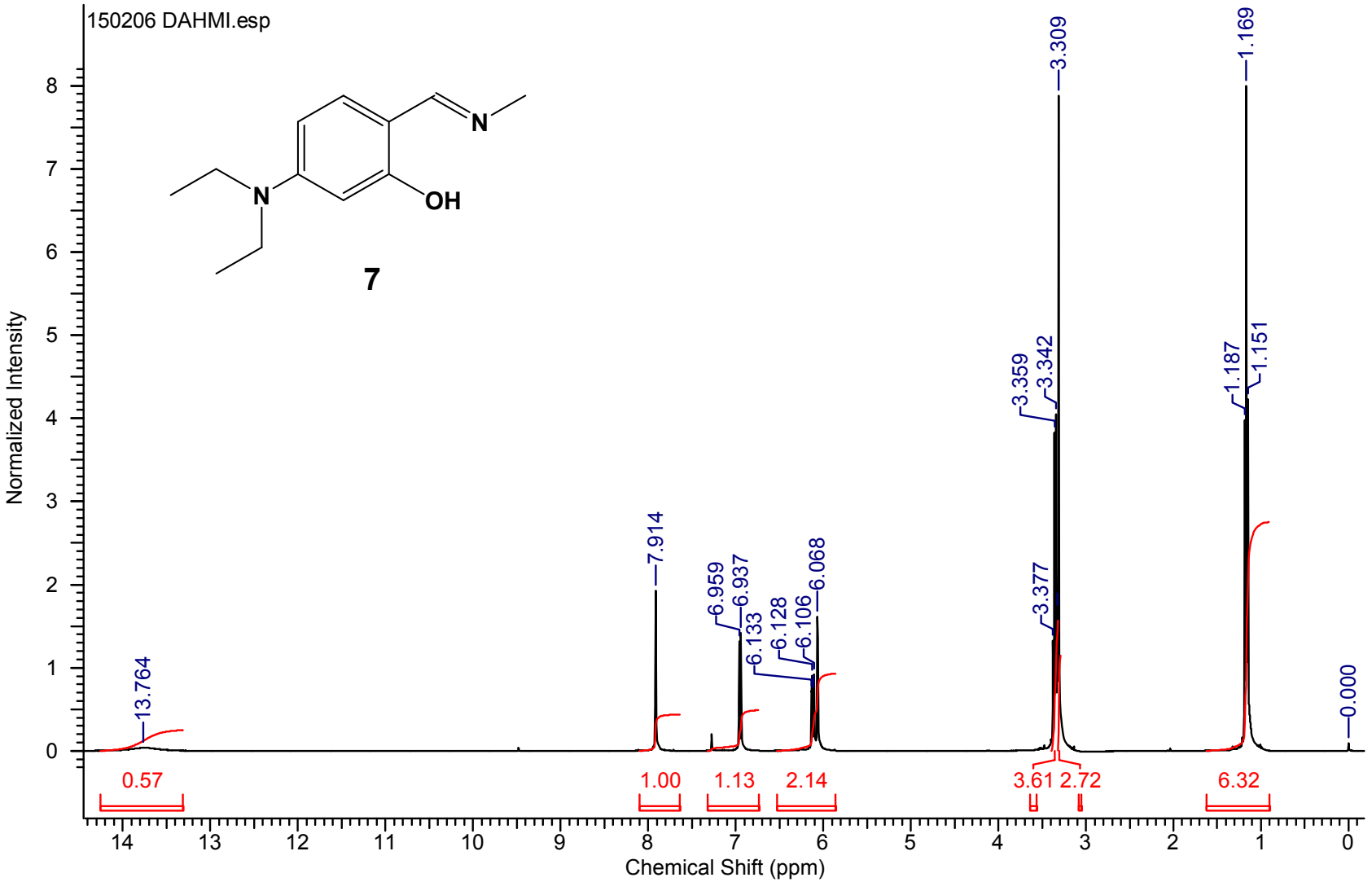


Figure S14a ^{1}H NMR spectrum of compound **7** ($CDCl_3$).

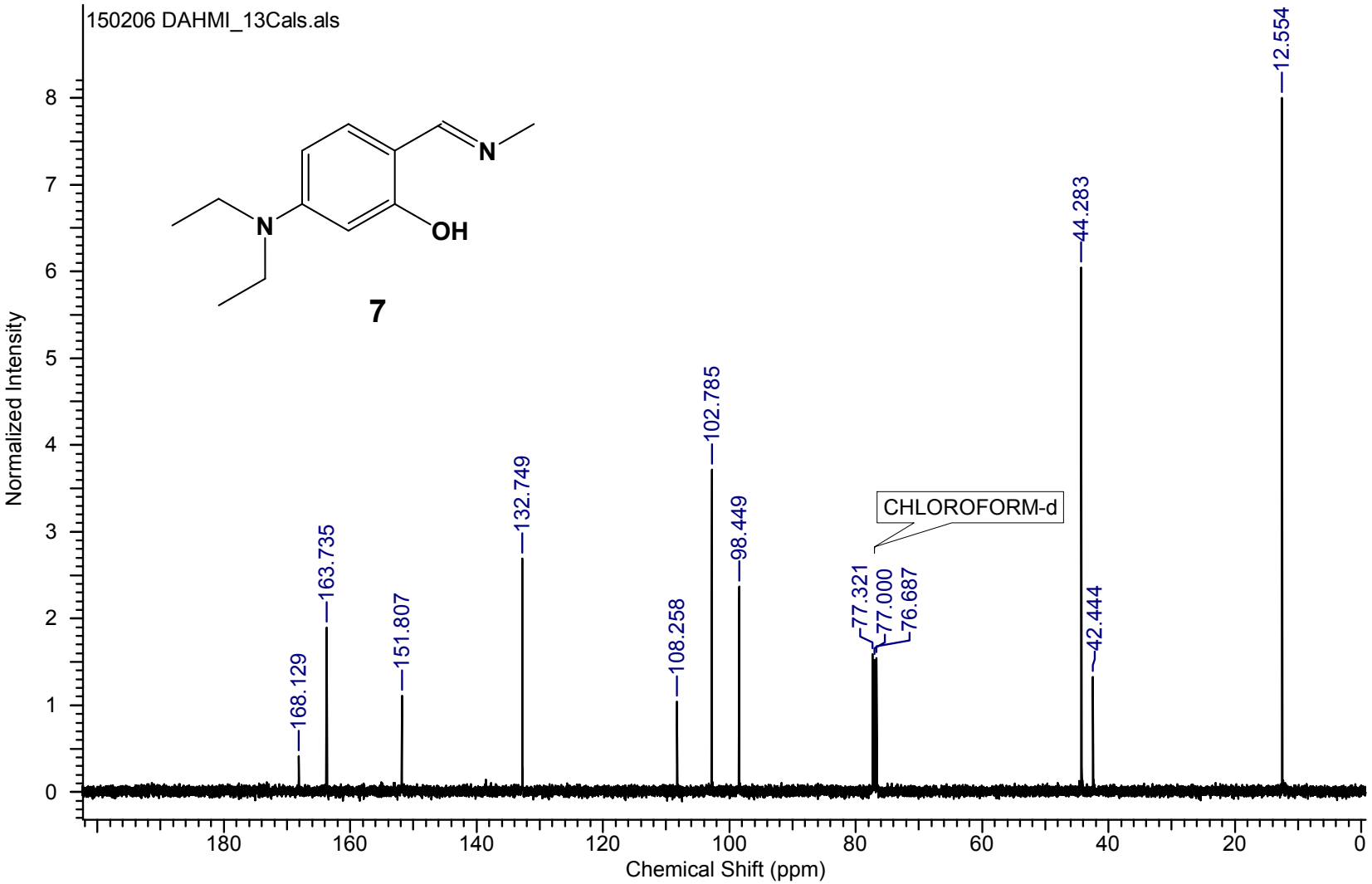


Figure S14b ^{13}C NMR spectrum of compound **7** ($CDCl_3$).

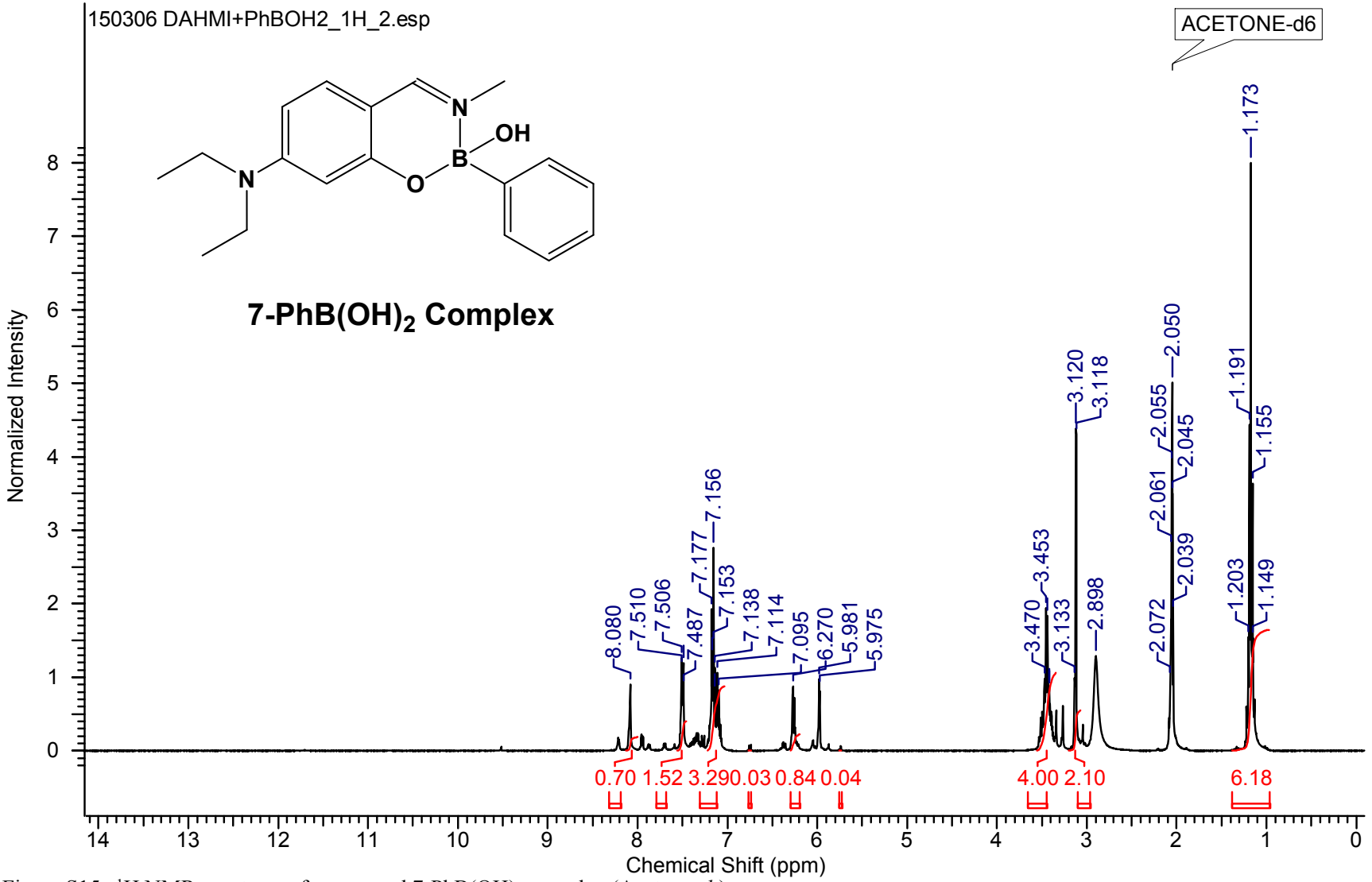


Figure S15a ^{1}H NMR spectrum of compound **7**-PhB(OH)$_2$ complex (Acetone-d_6).

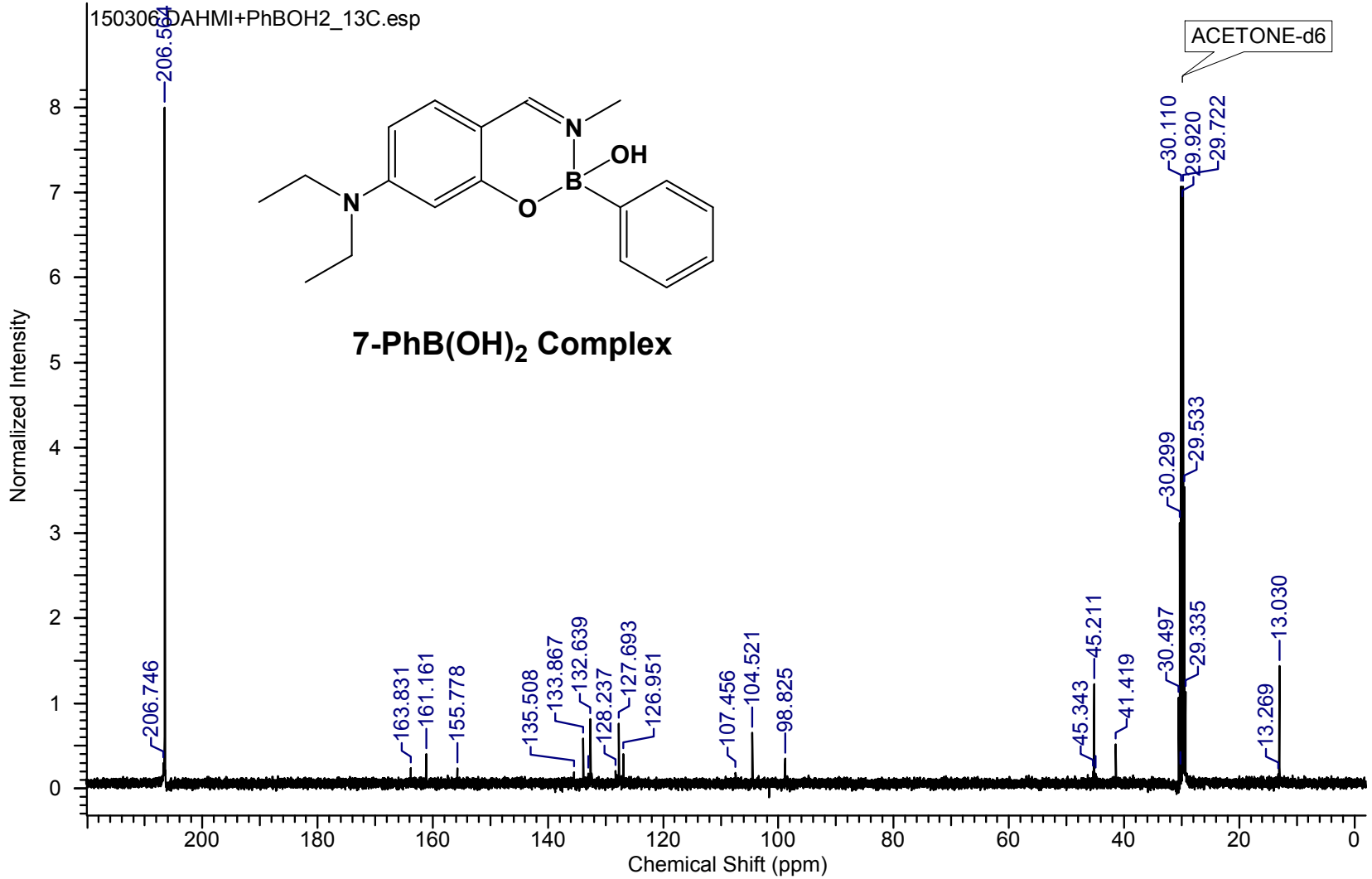


Figure S15b ^{13}C NMR spectrum of compound **7**-PhB(OH)$_2$ complex (Acetone-d_6).