Design, Synthesis, and Antiproliferative Activity of Benzopyran-4-one-Isoxazole Hybrid Compounds

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Table of Contents:

Figure S1.1. ^{1}H NMR spectra of compound 2a

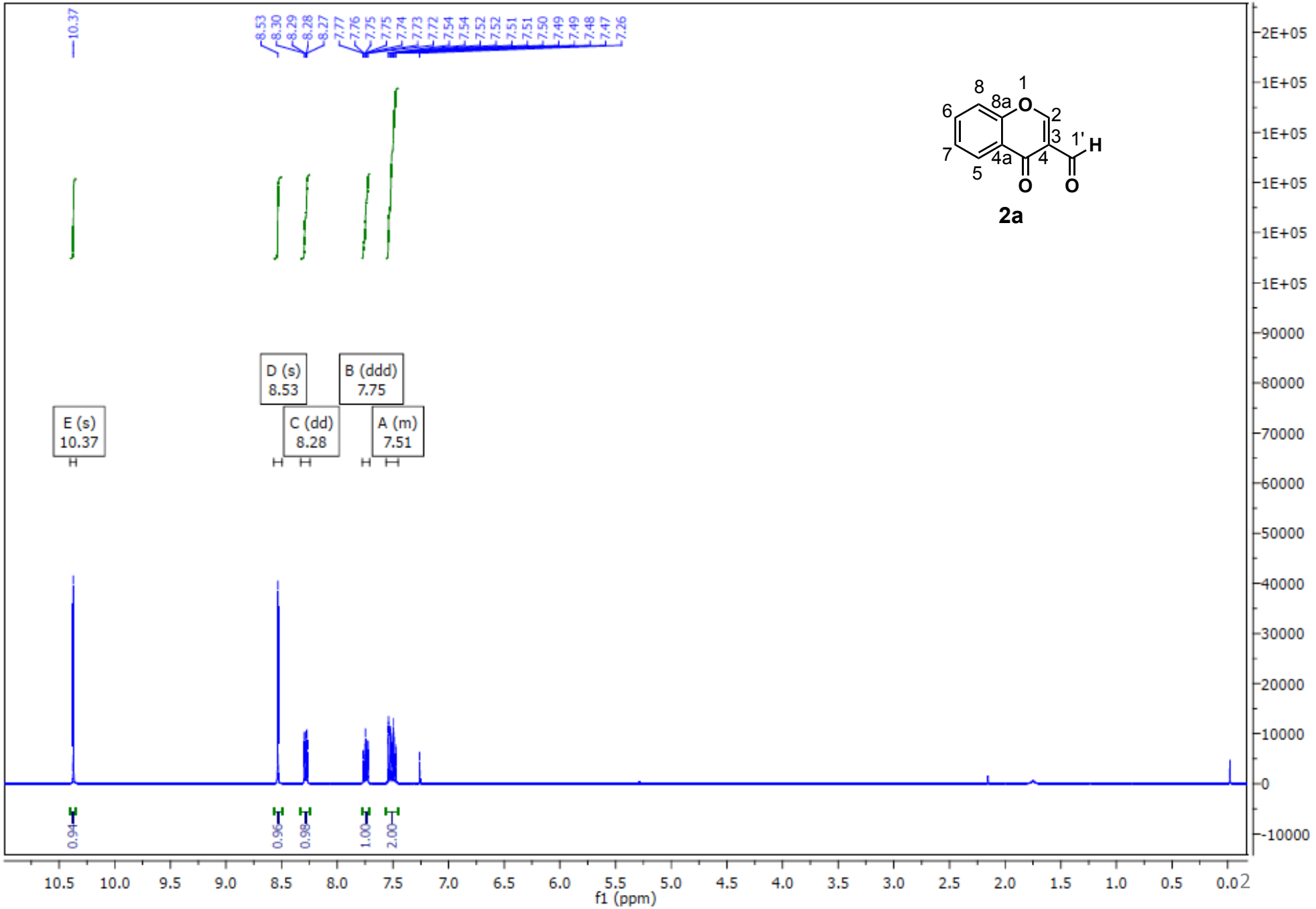


Figure S1.2. ^{13}C NMR spectra of compound 2a

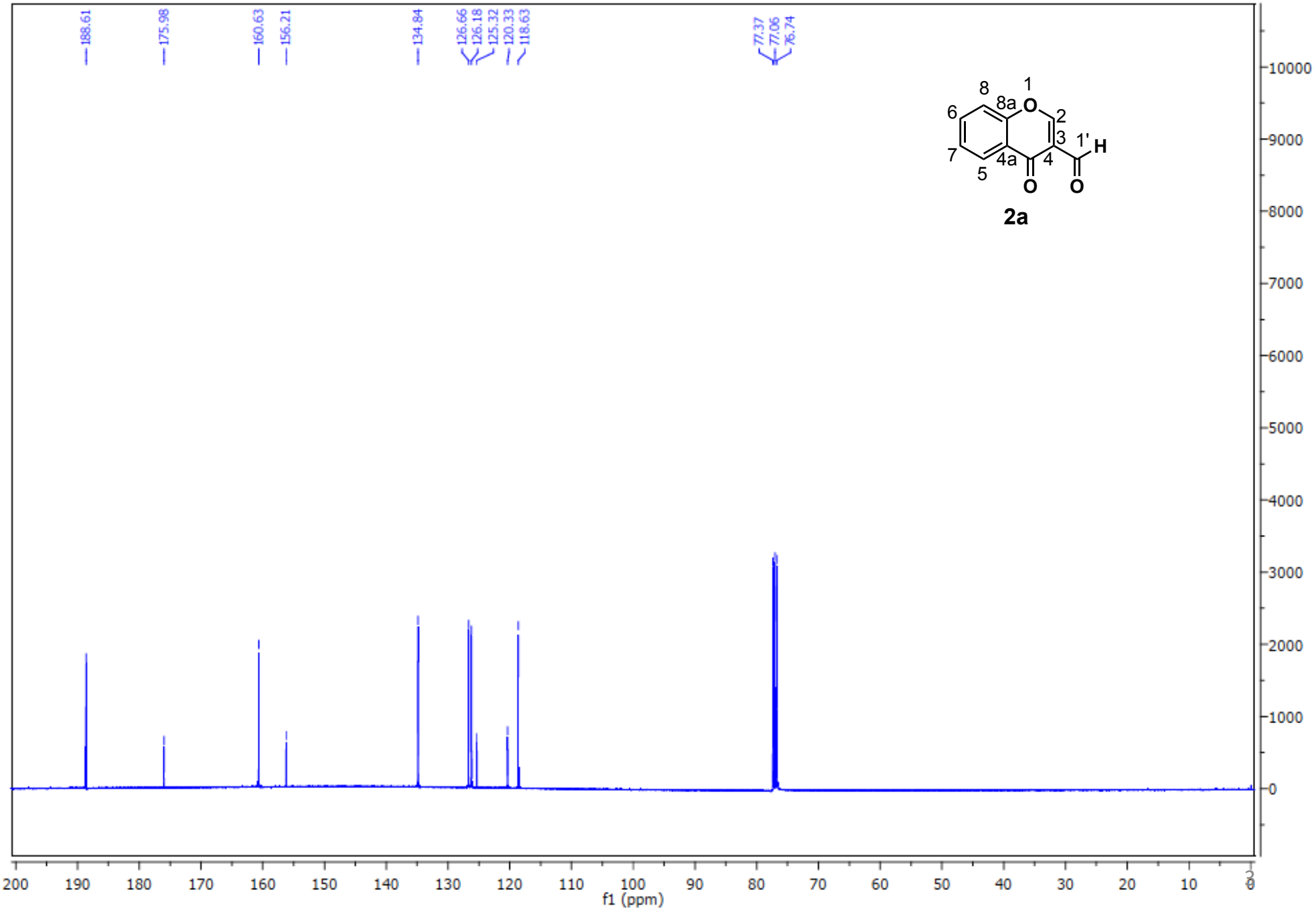


Figure S2.1. ^{1}H NMR spectra of compound 2b

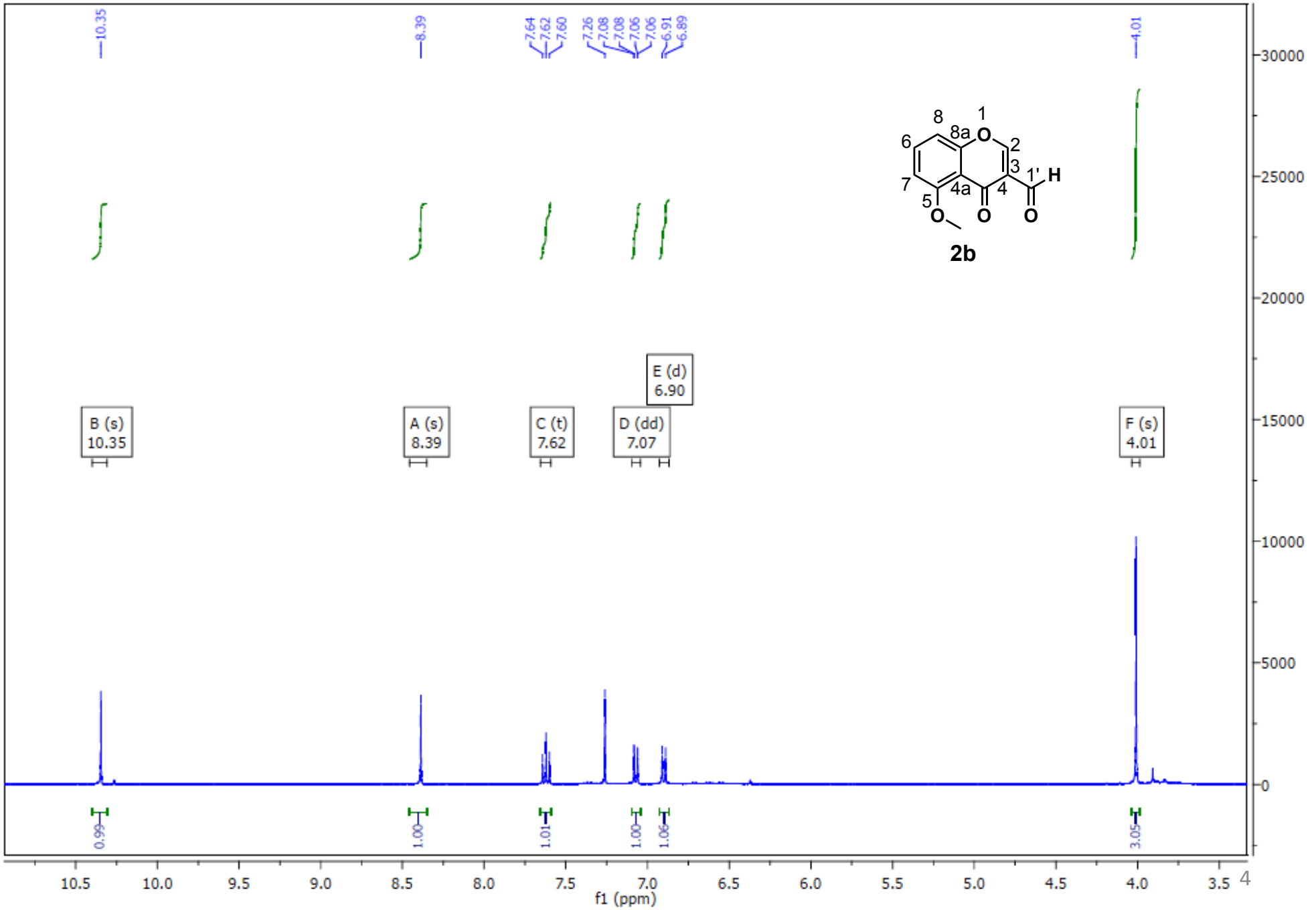


Figure S2.2. ^{13}C NMR spectra of compound 2b

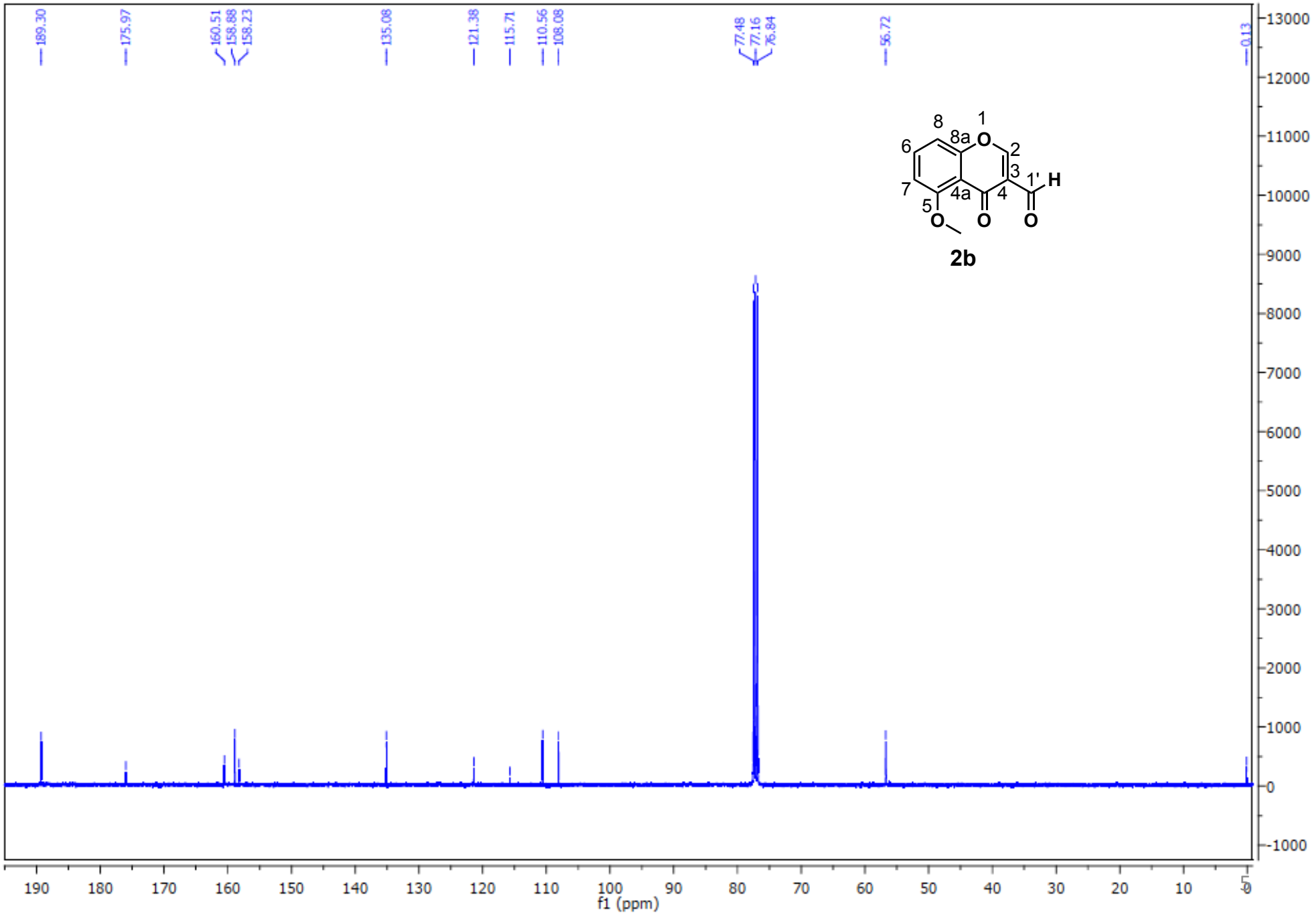


Figure S3.1. ^{1}H NMR spectra of compound 2c

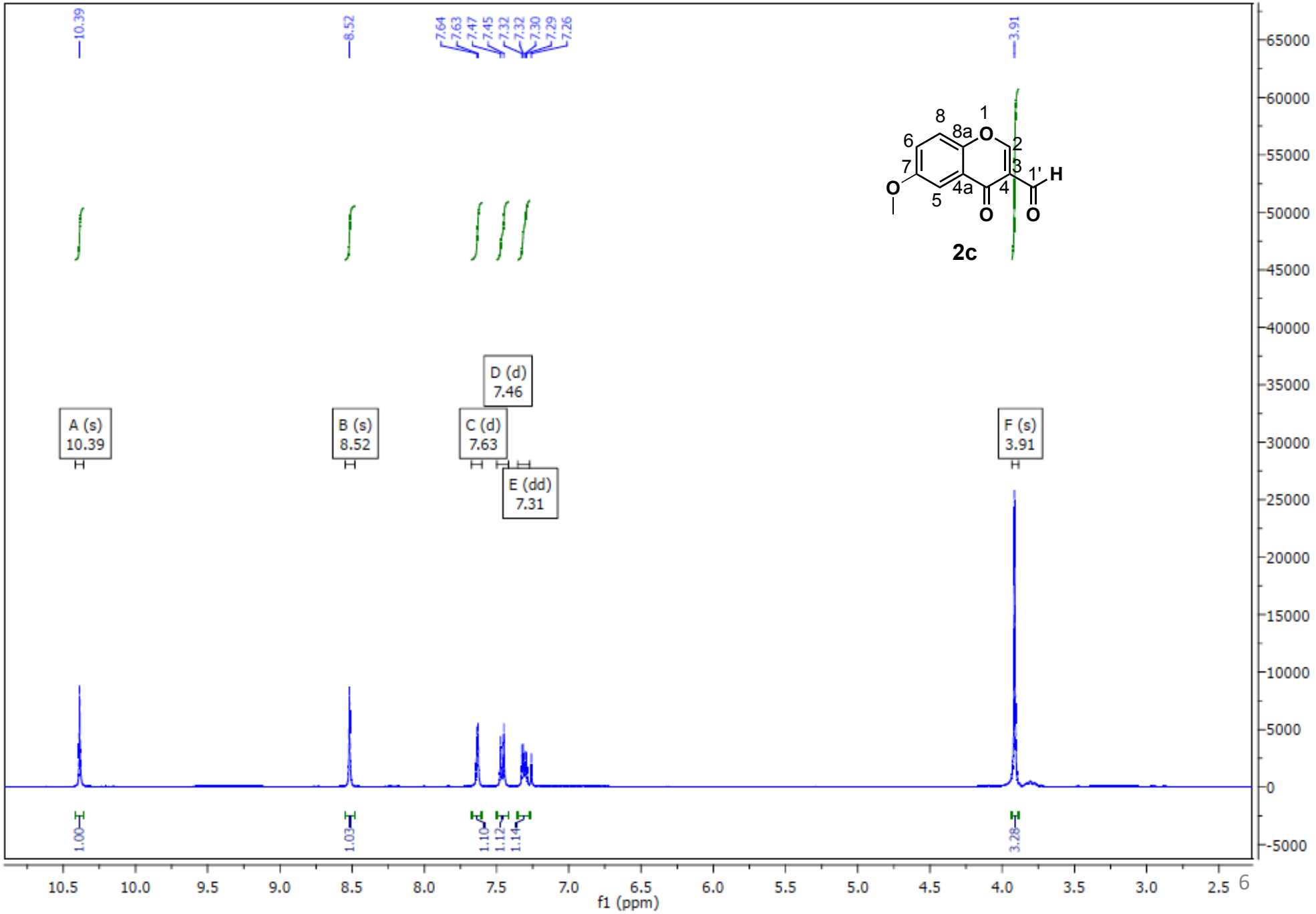


Figure S3.2. ^{13}C NMR spectra of compound 2c

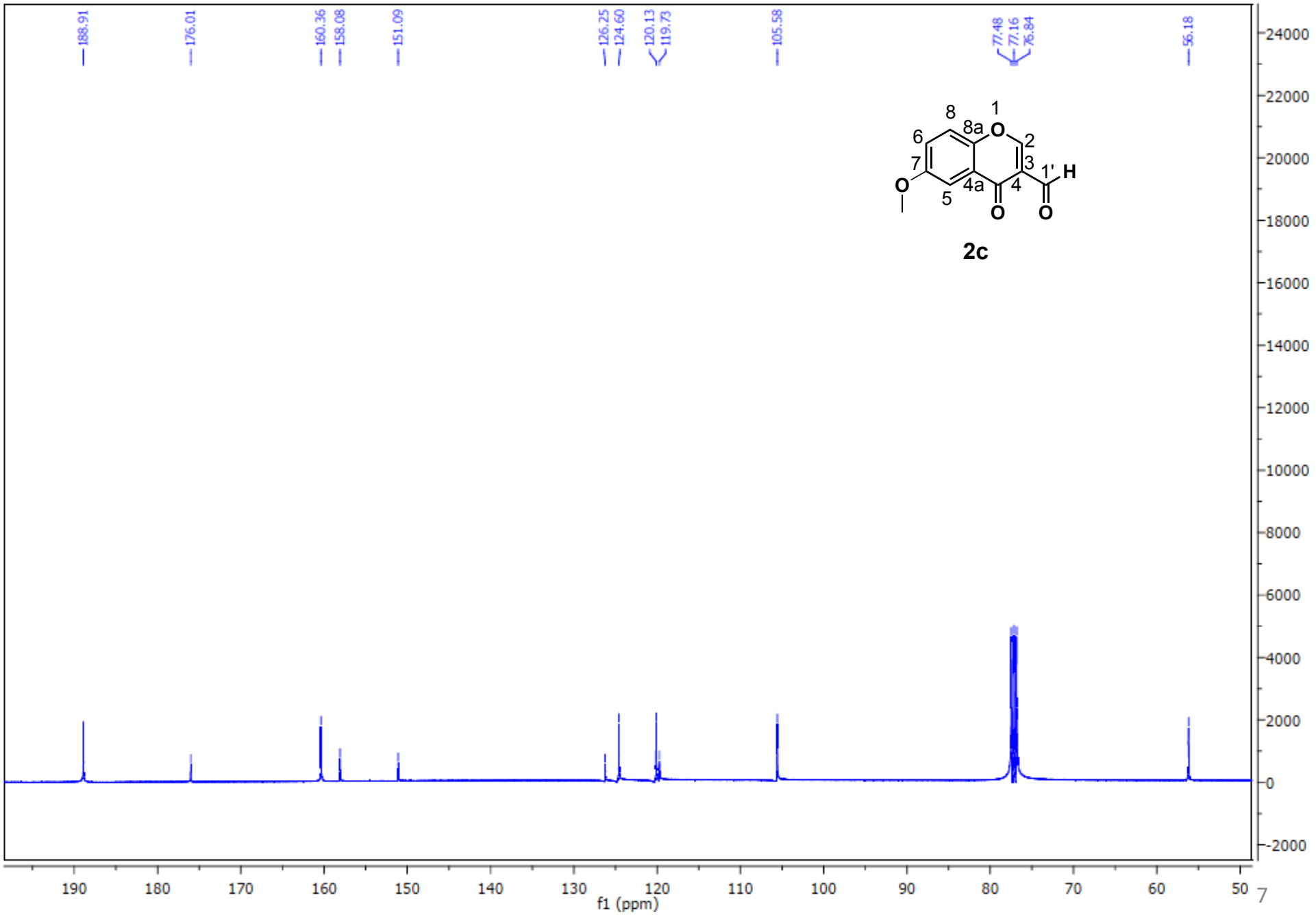


Figure S4.1. ^{1}H NMR spectra of compound 2d intermediate

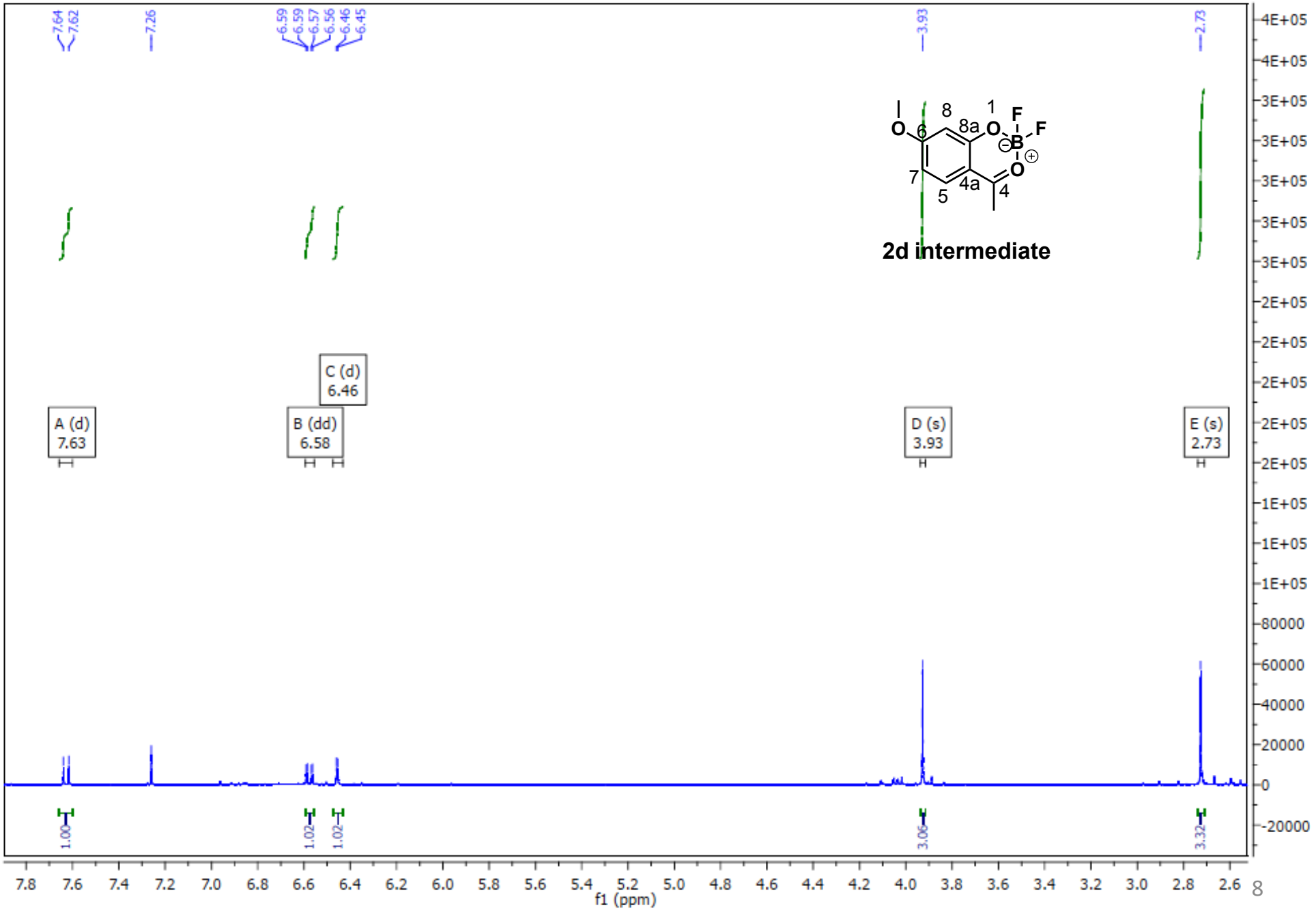


Figure S4.2. ^{1}H NMR spectra of compound 2d

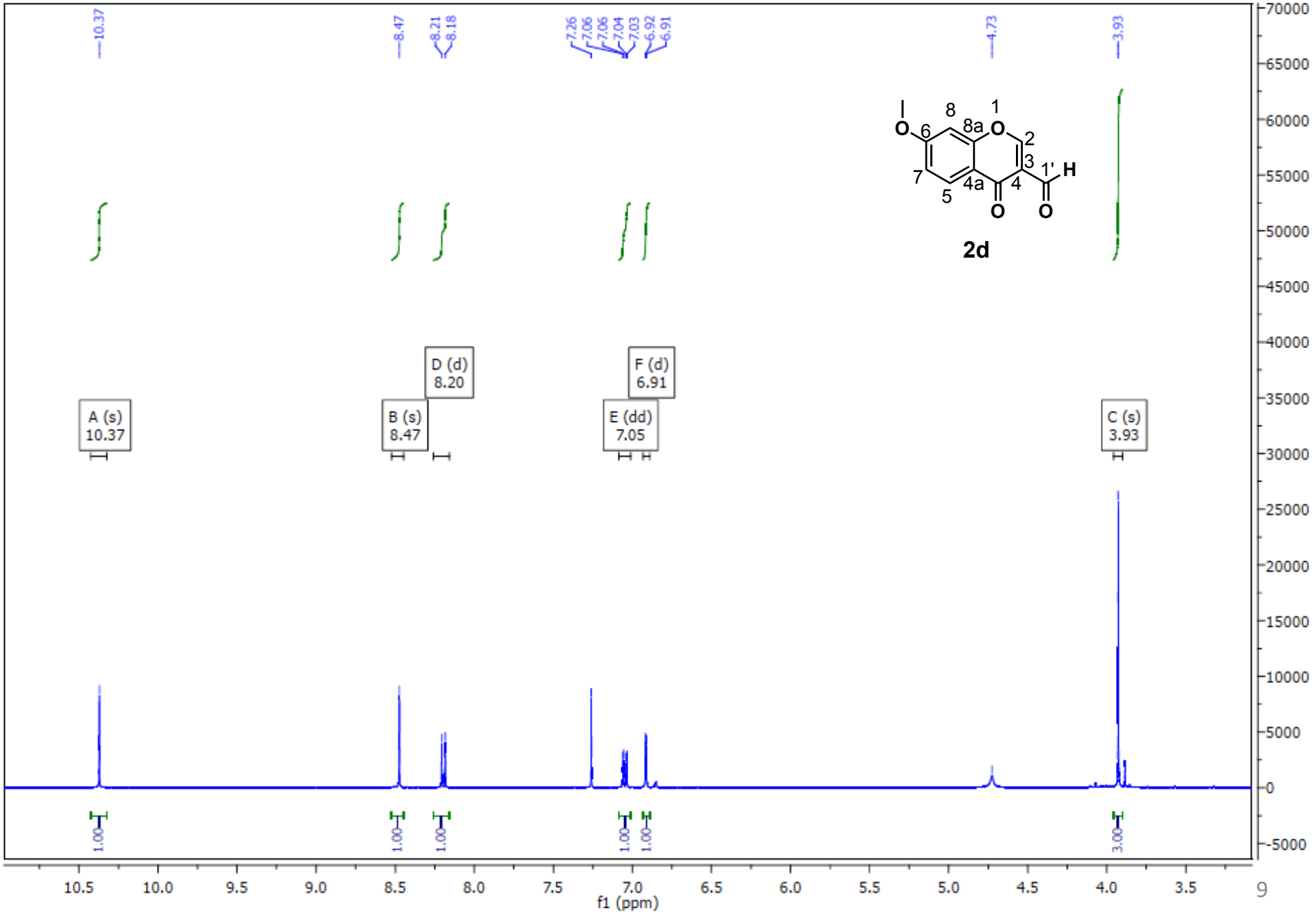


Figure S4.3. ^{13}C NMR spectra of compound 2d

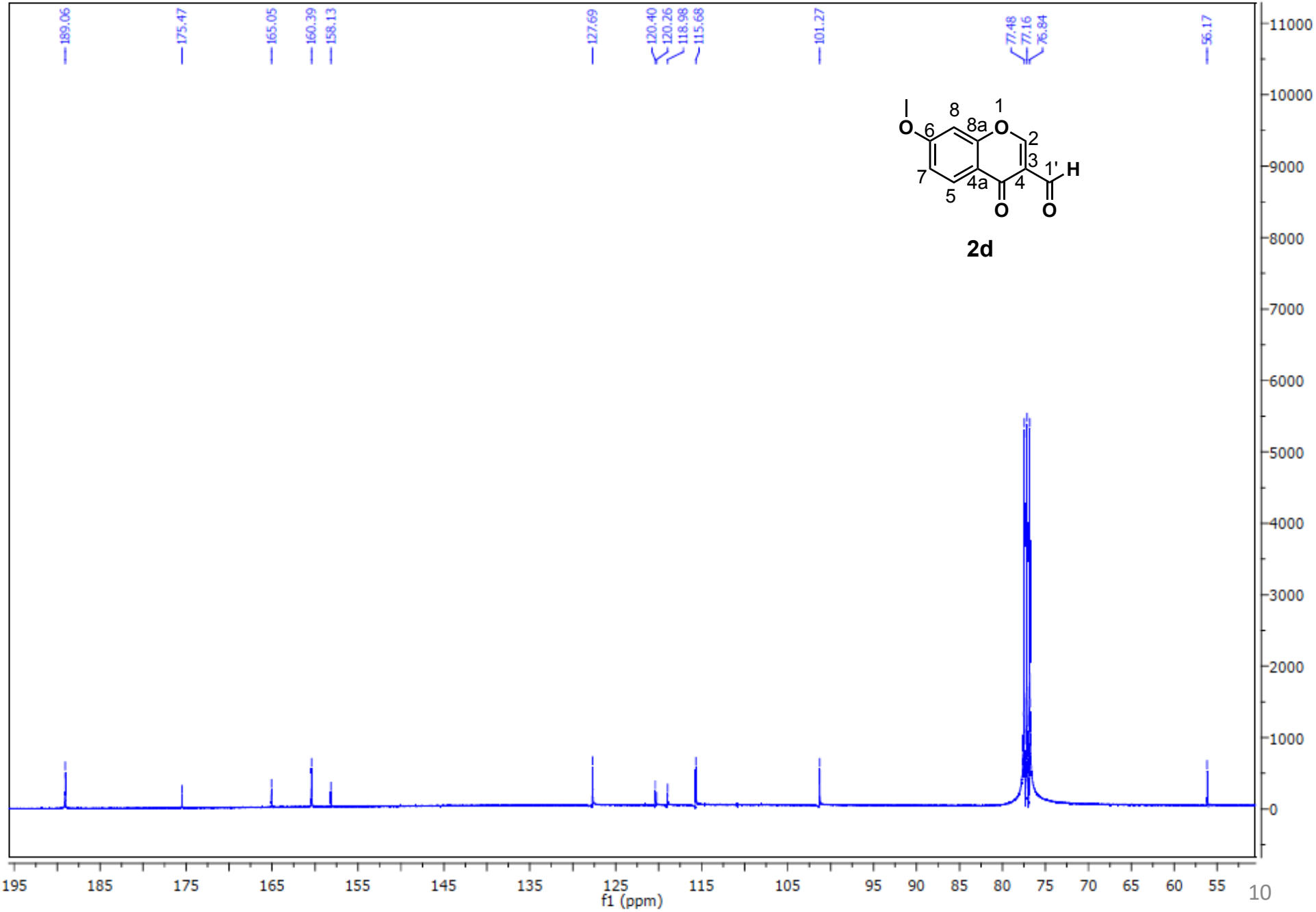


Figure S5.1. ^{1}H NMR spectra of compound 3a

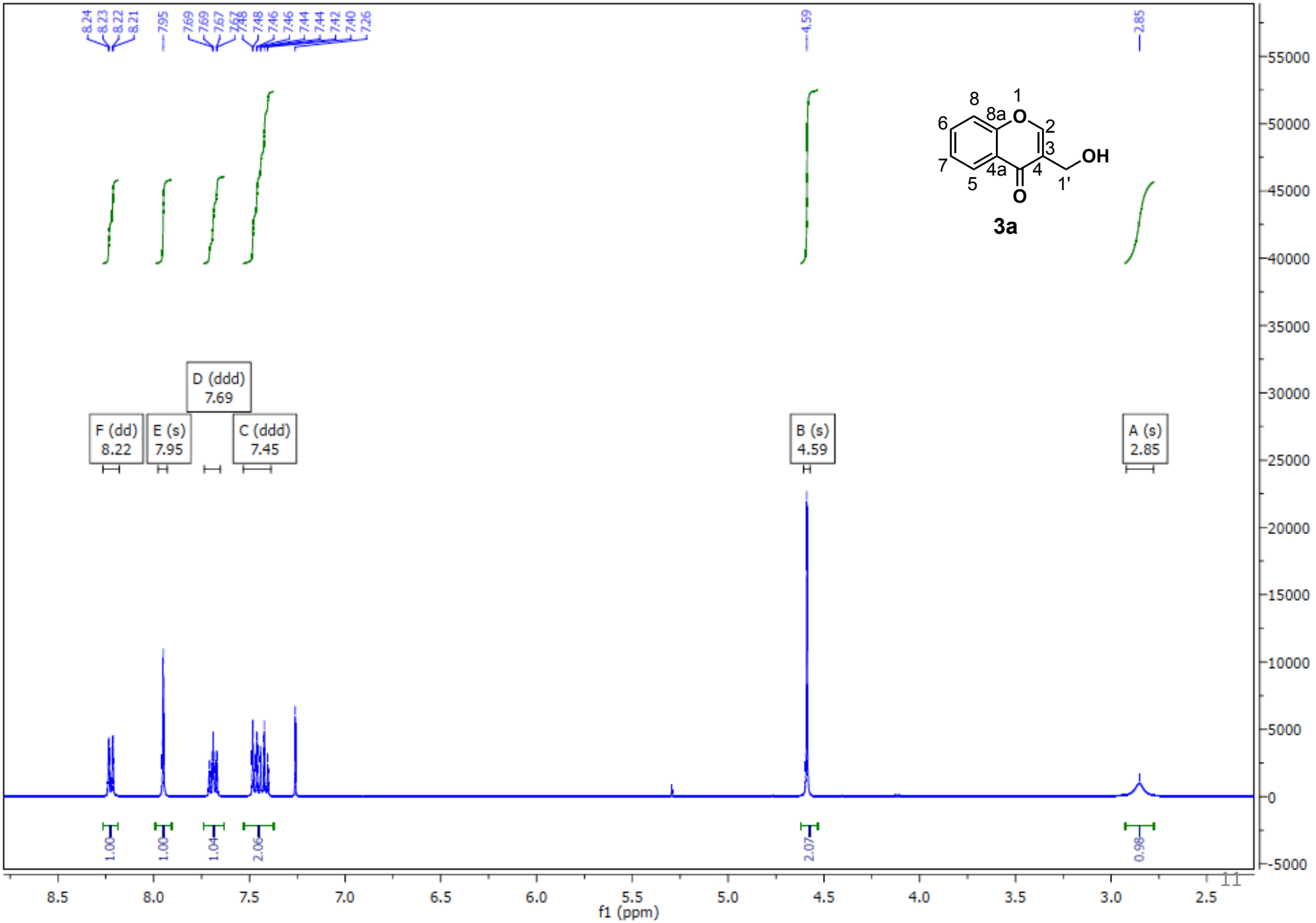


Figure S5.2. ^{13}C NMR spectra of compound 3a

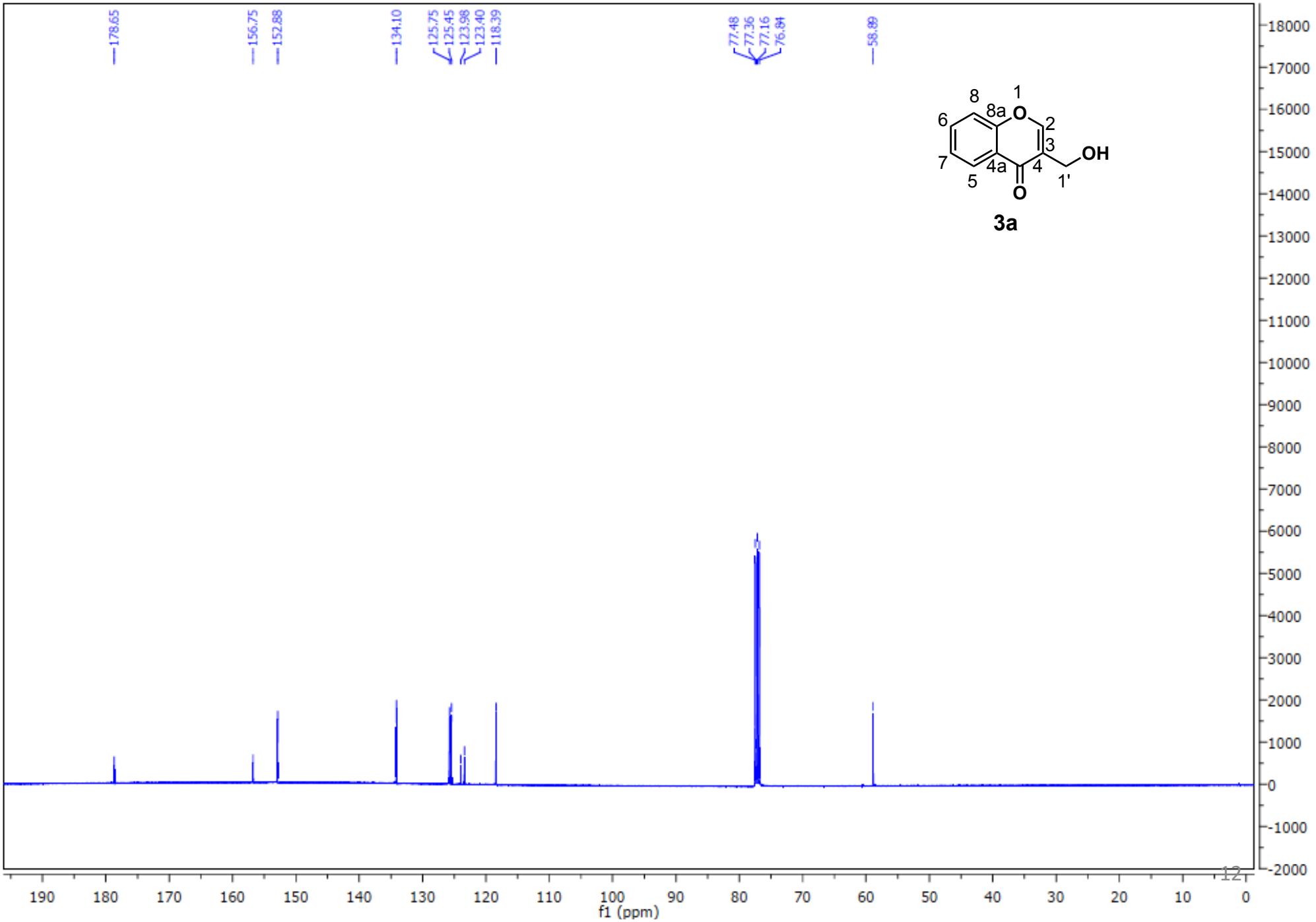


Figure S6.1. ^{1}H NMR spectra of compound 3b

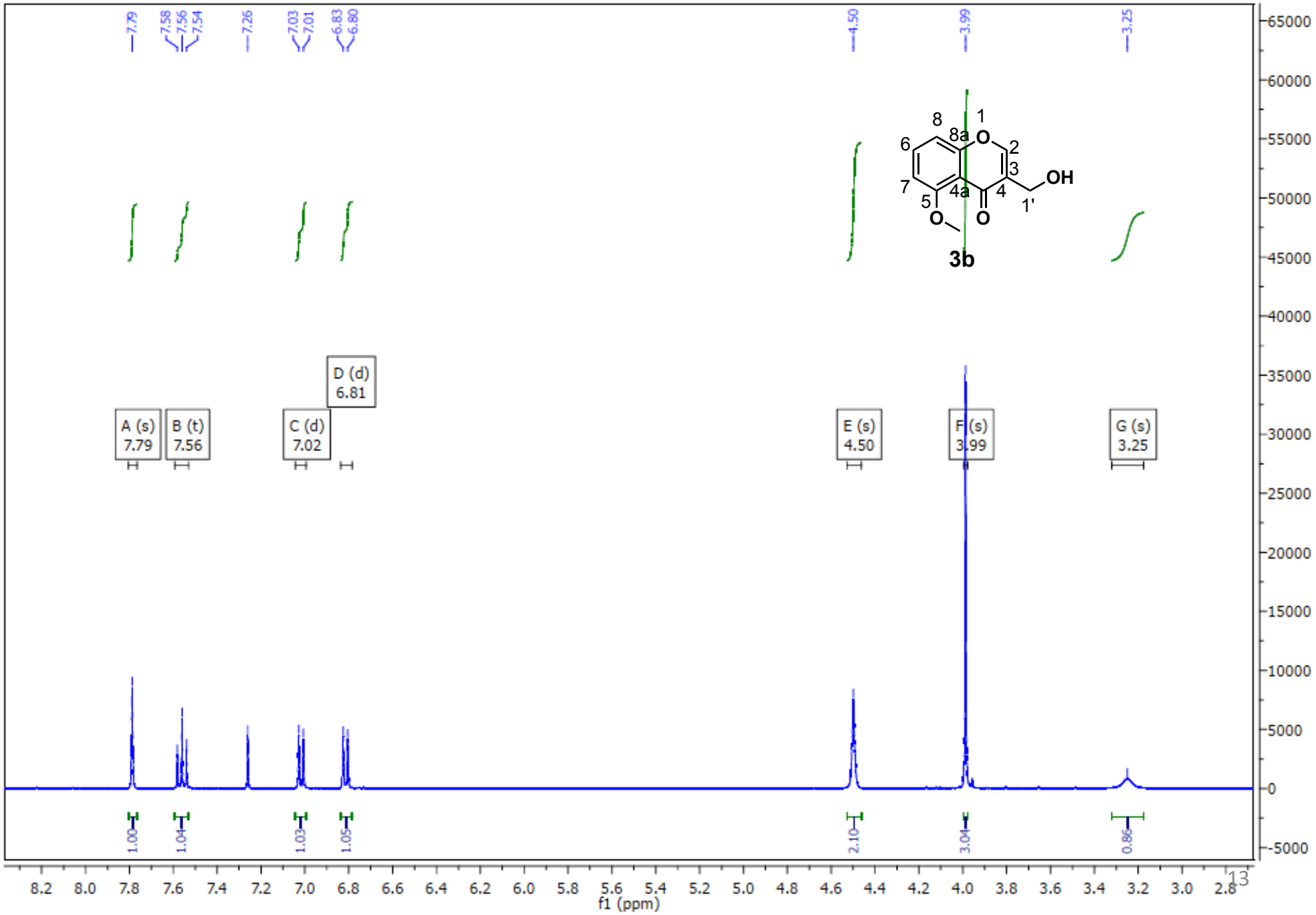


Figure S6.2. ^{13}C NMR spectra of compound 3b

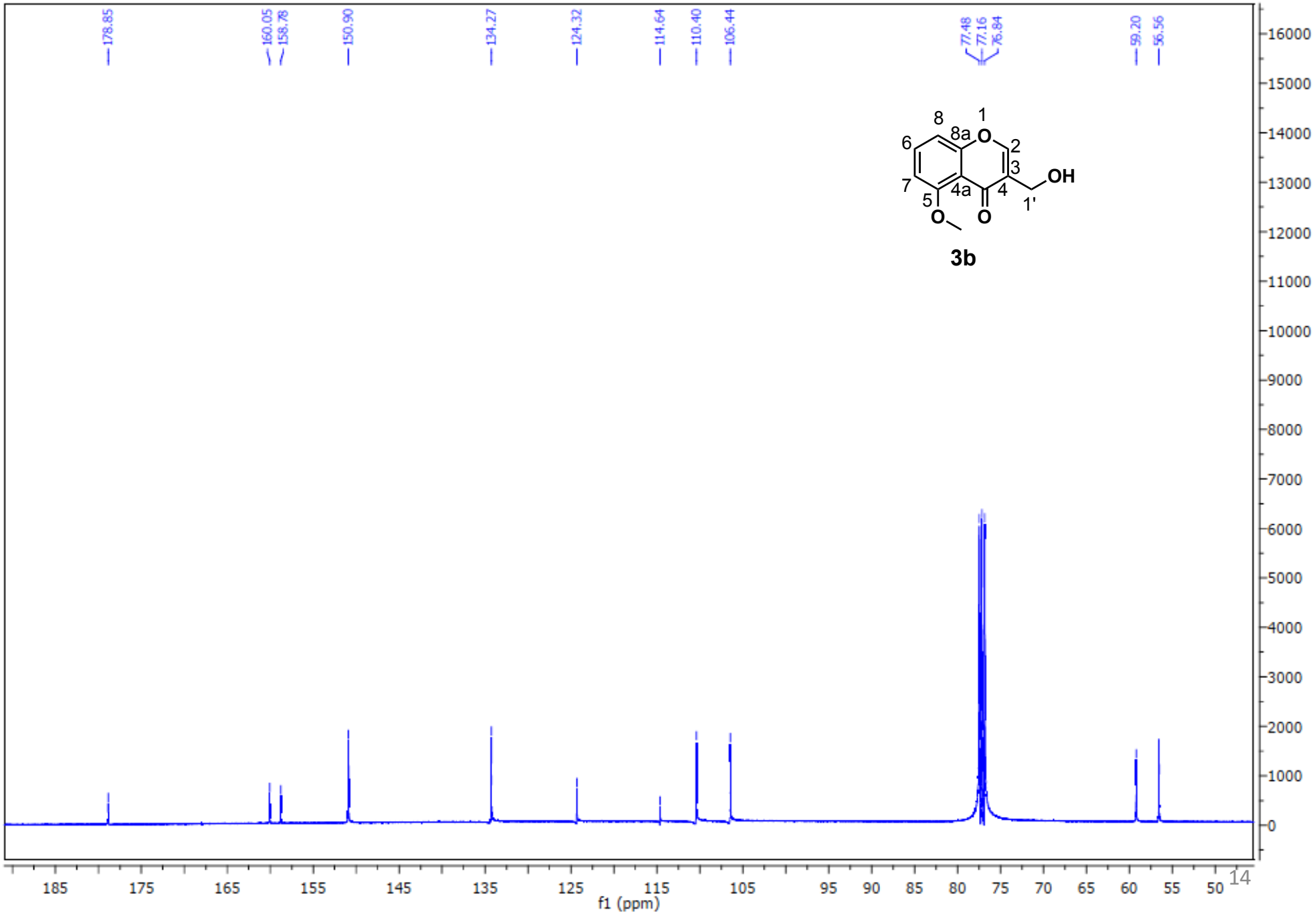


Figure S7.1. ^{1}H NMR spectra of compound 3c

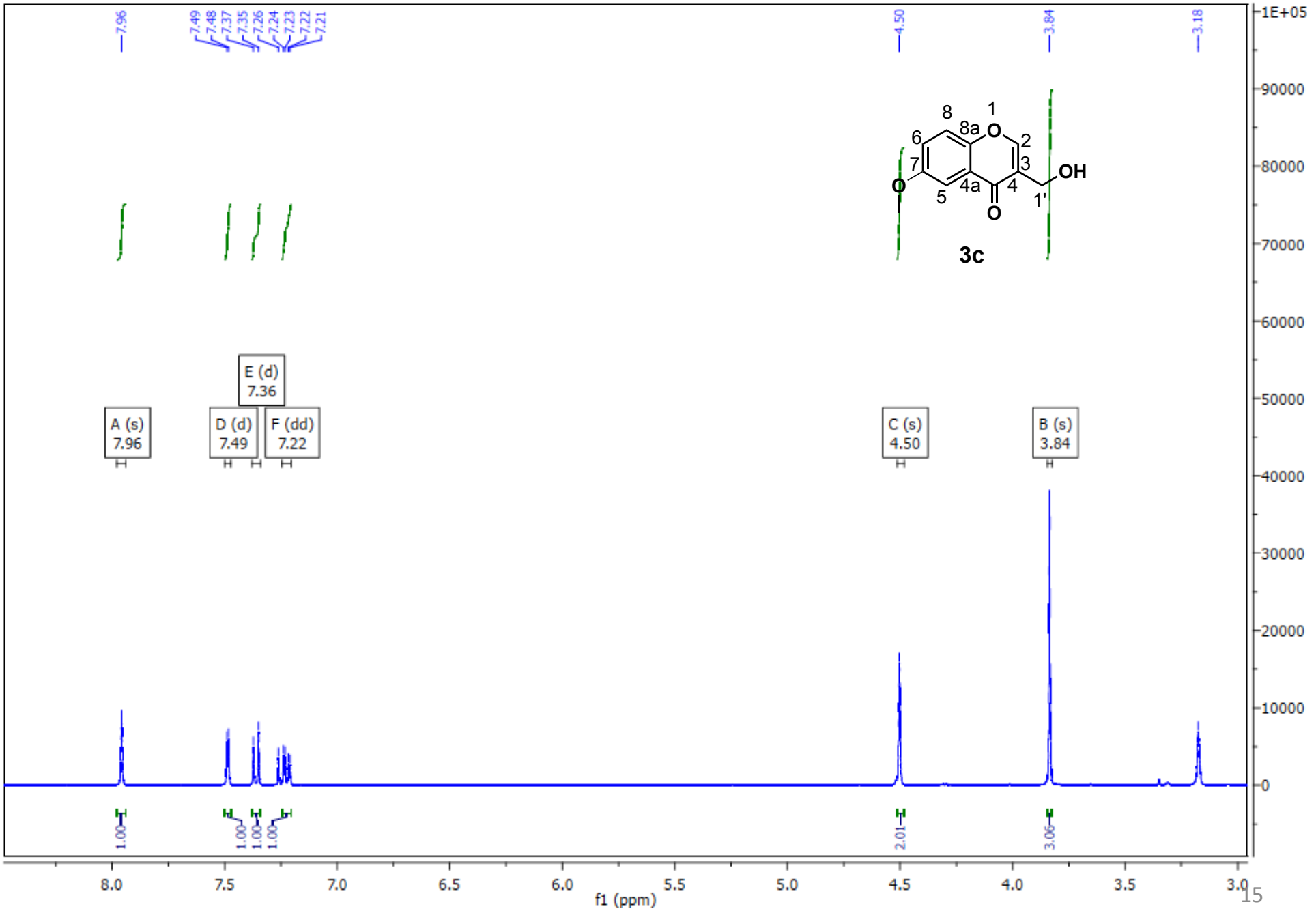


Figure S7.2. ^{1}H NMR spectra of compound 3c

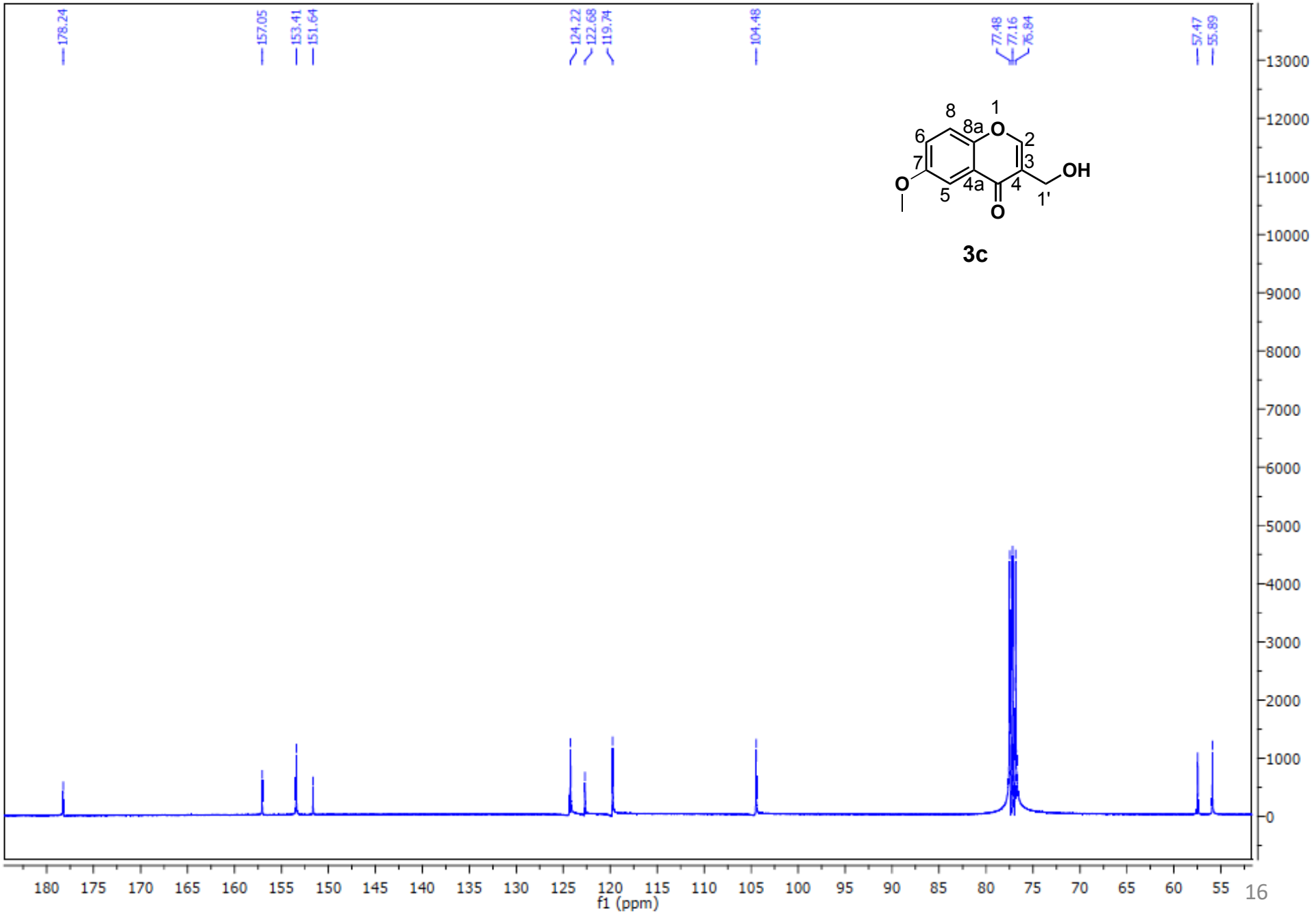


Figure S8.1. ^{1}H NMR spectra of compound 3d

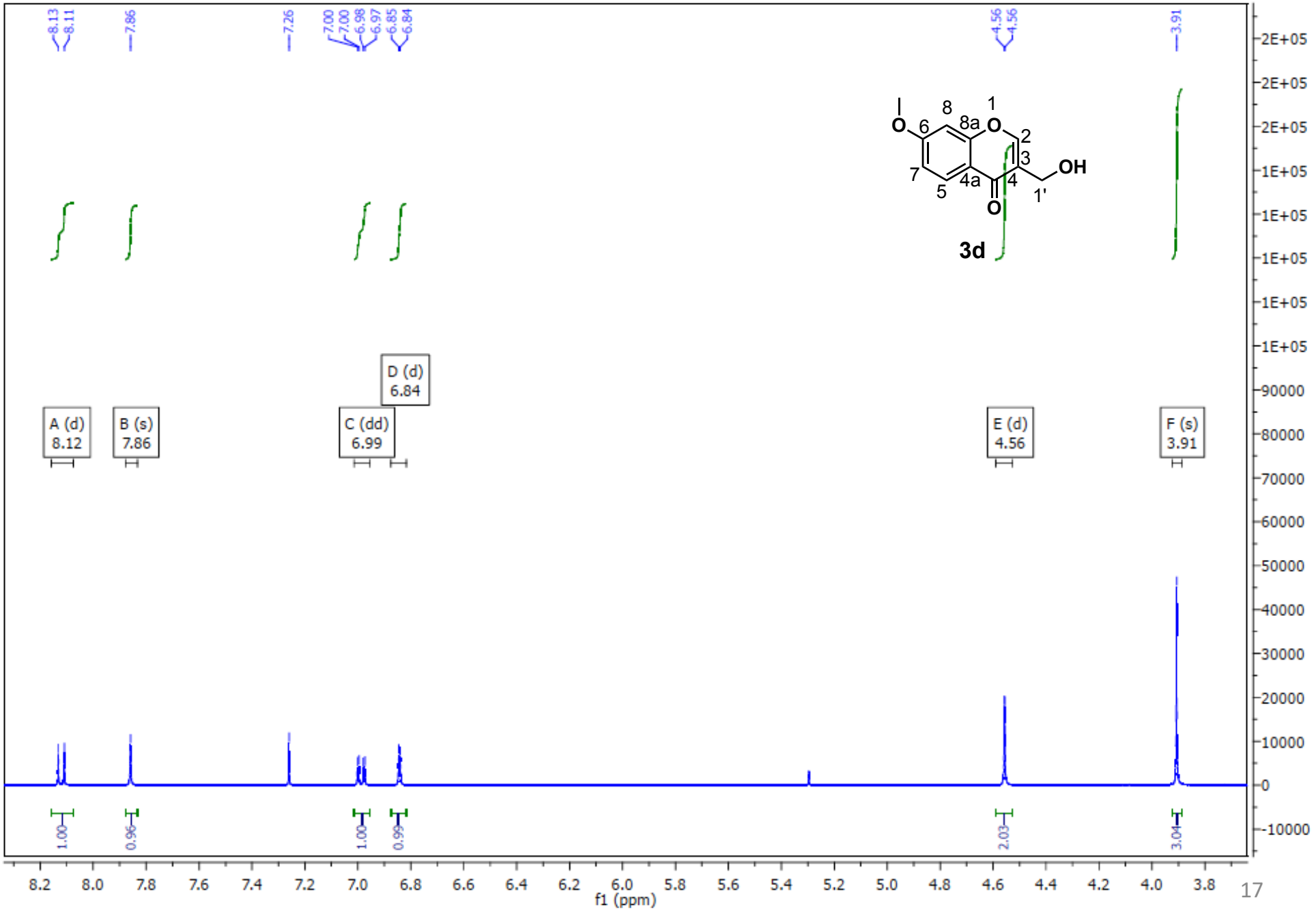


Figure S8.2. ^{13}C NMR spectra of compound 3d

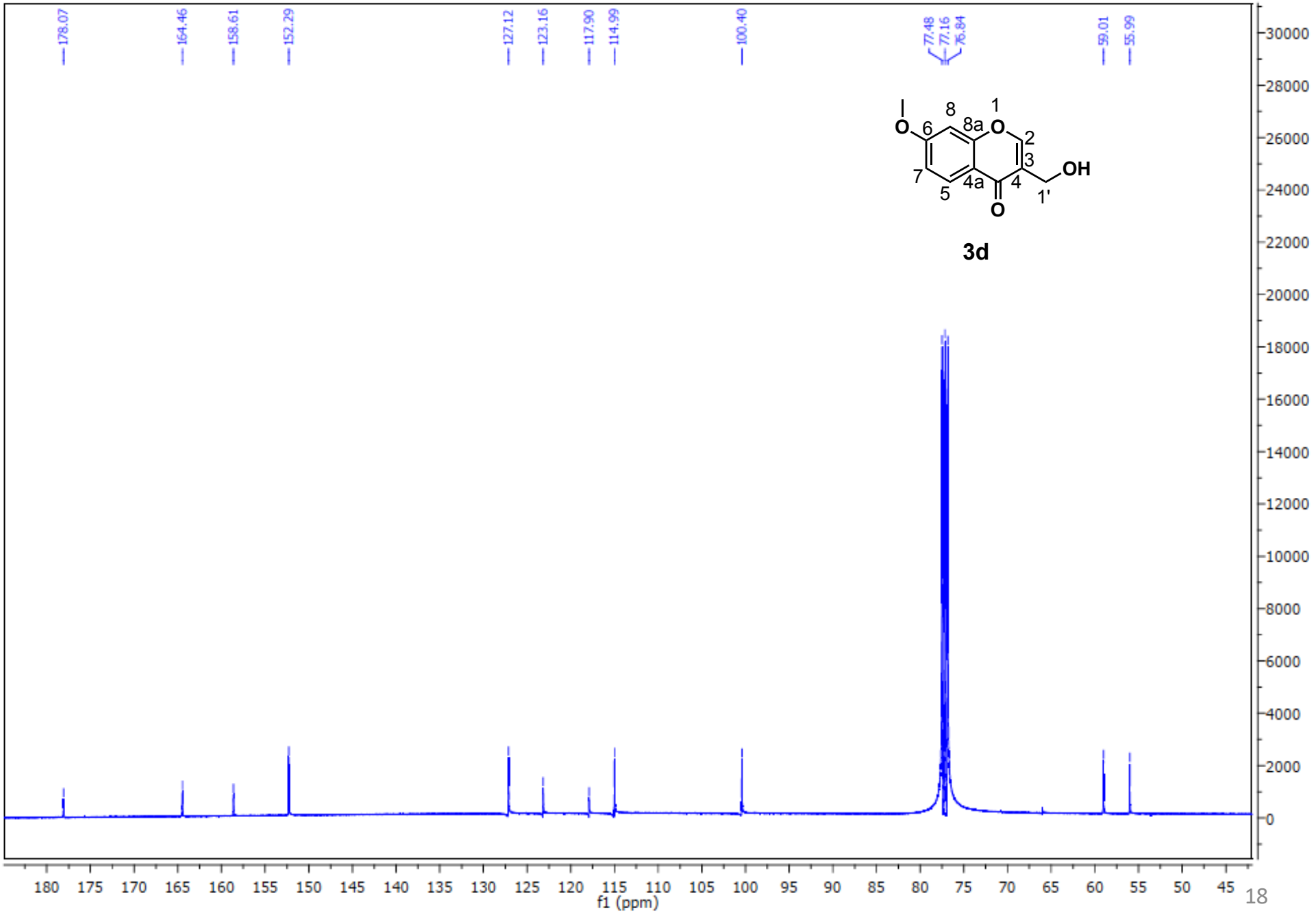


Figure S9.1. ^{1}H NMR spectra of compound 4a

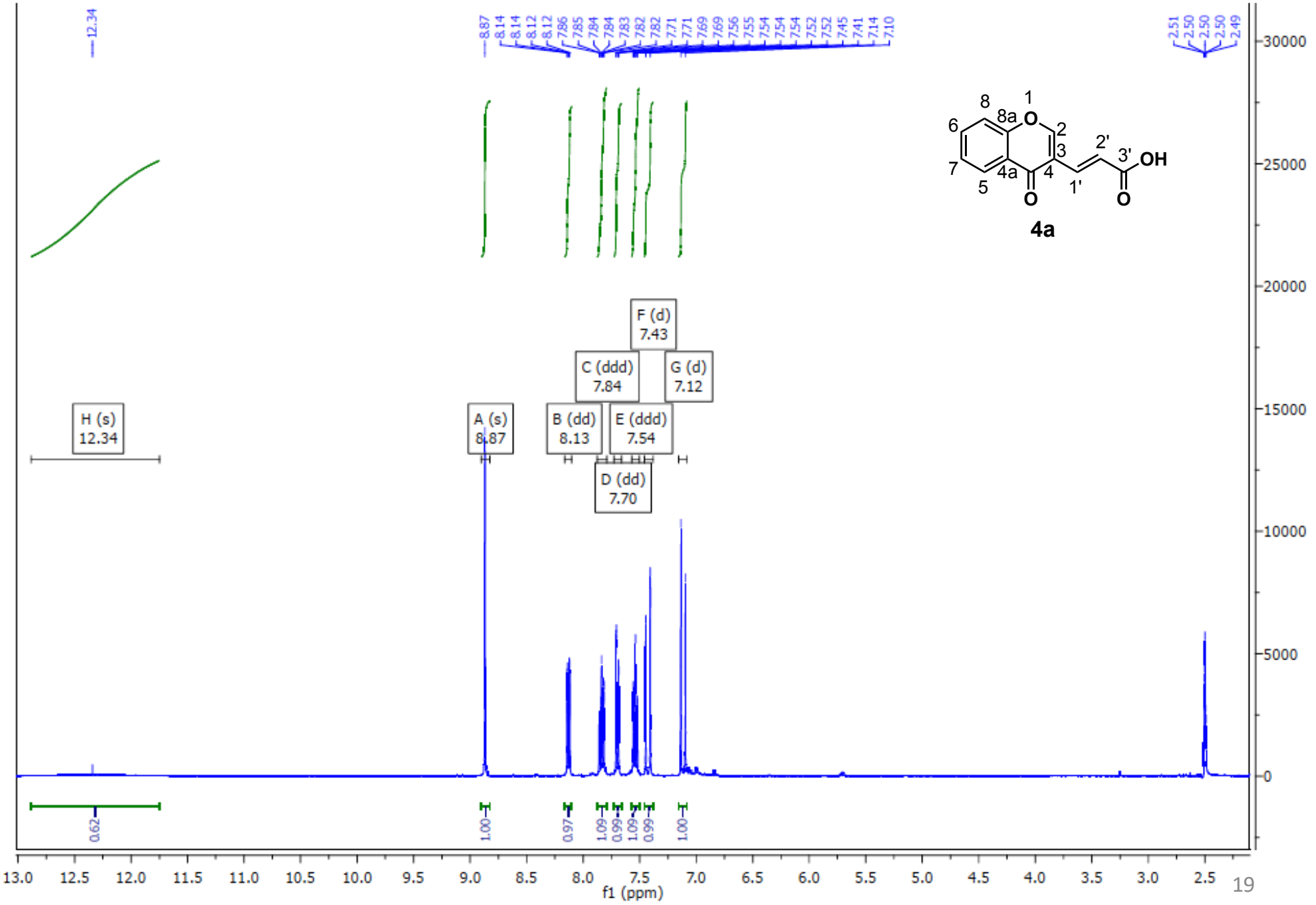


Figure S9.2. ^{13}C NMR spectra of compound 4a

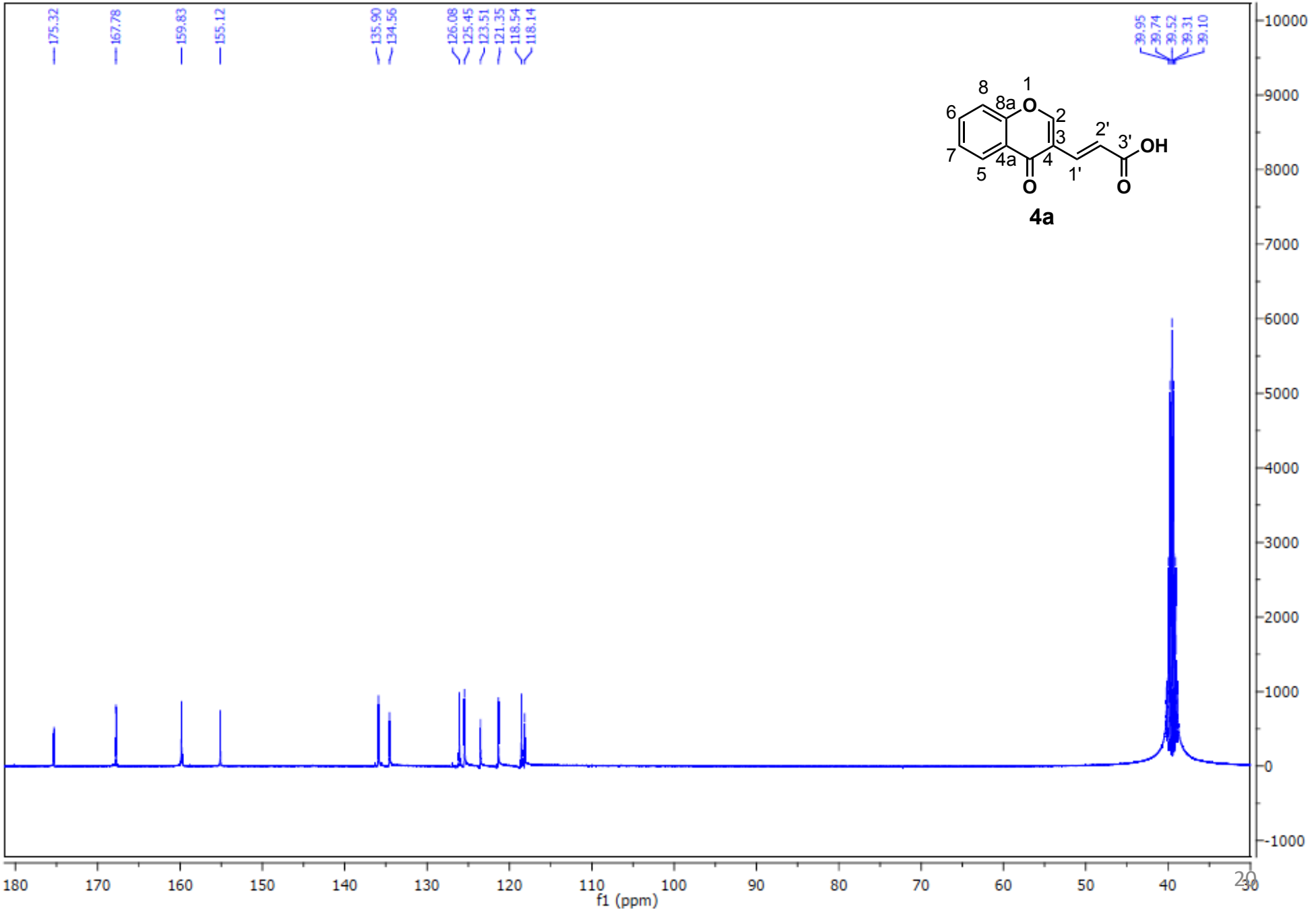


Figure S10.1. ^{1}H NMR spectra of compound 4b

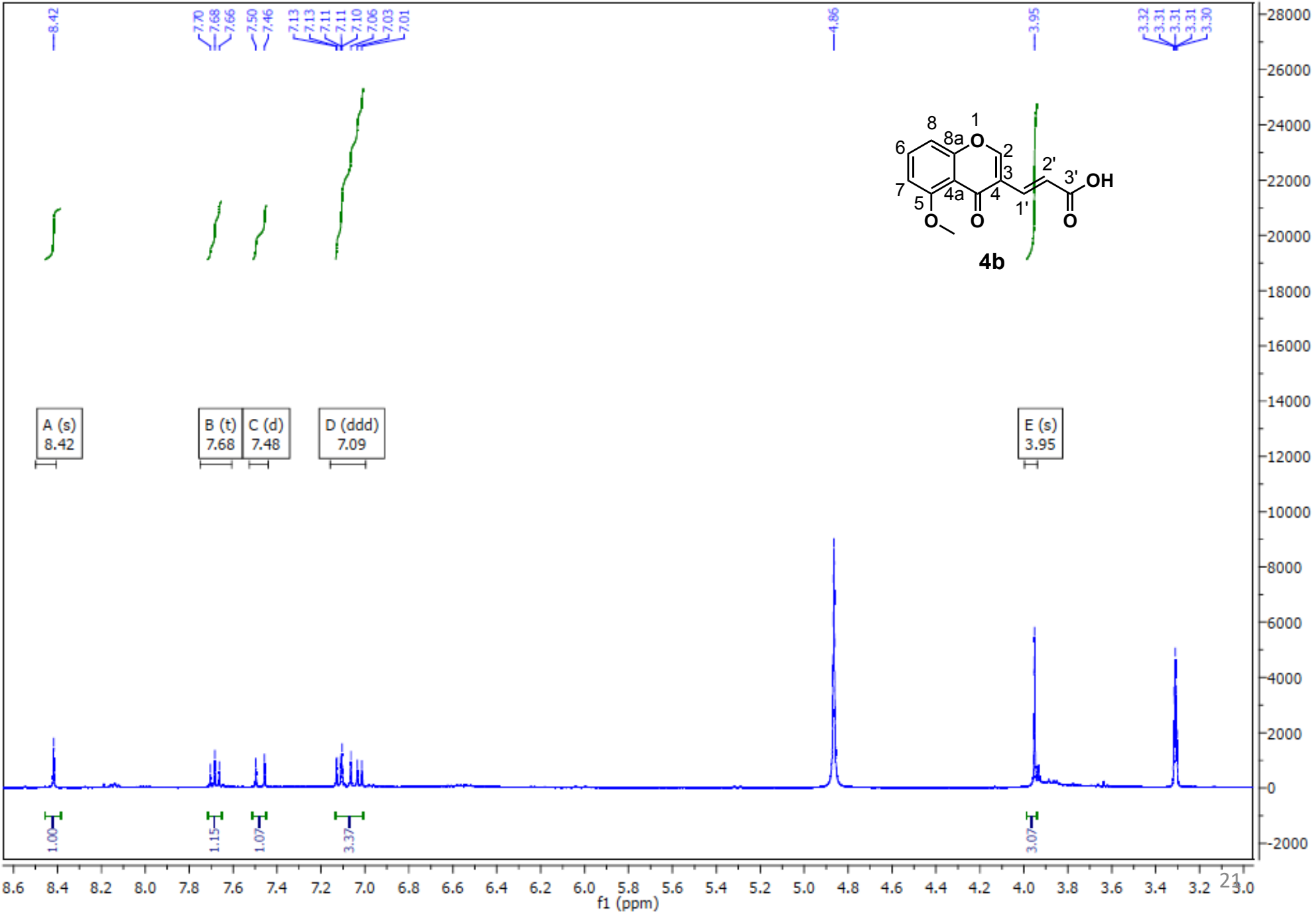


Figure S10.2. ^{13}C NMR spectra of compound 4b

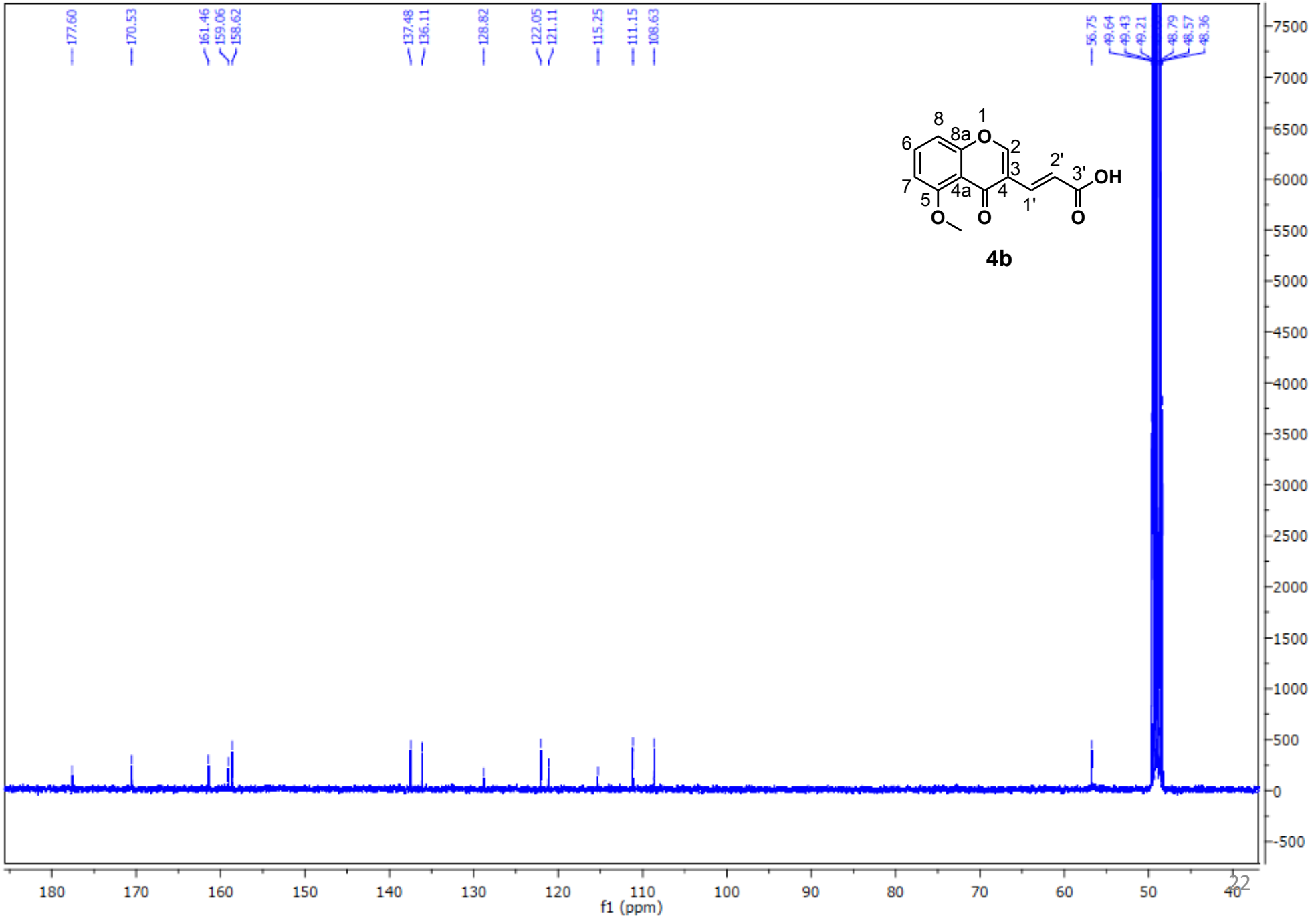


Figure S11.1. ^{1}H NMR spectra of compound 4c

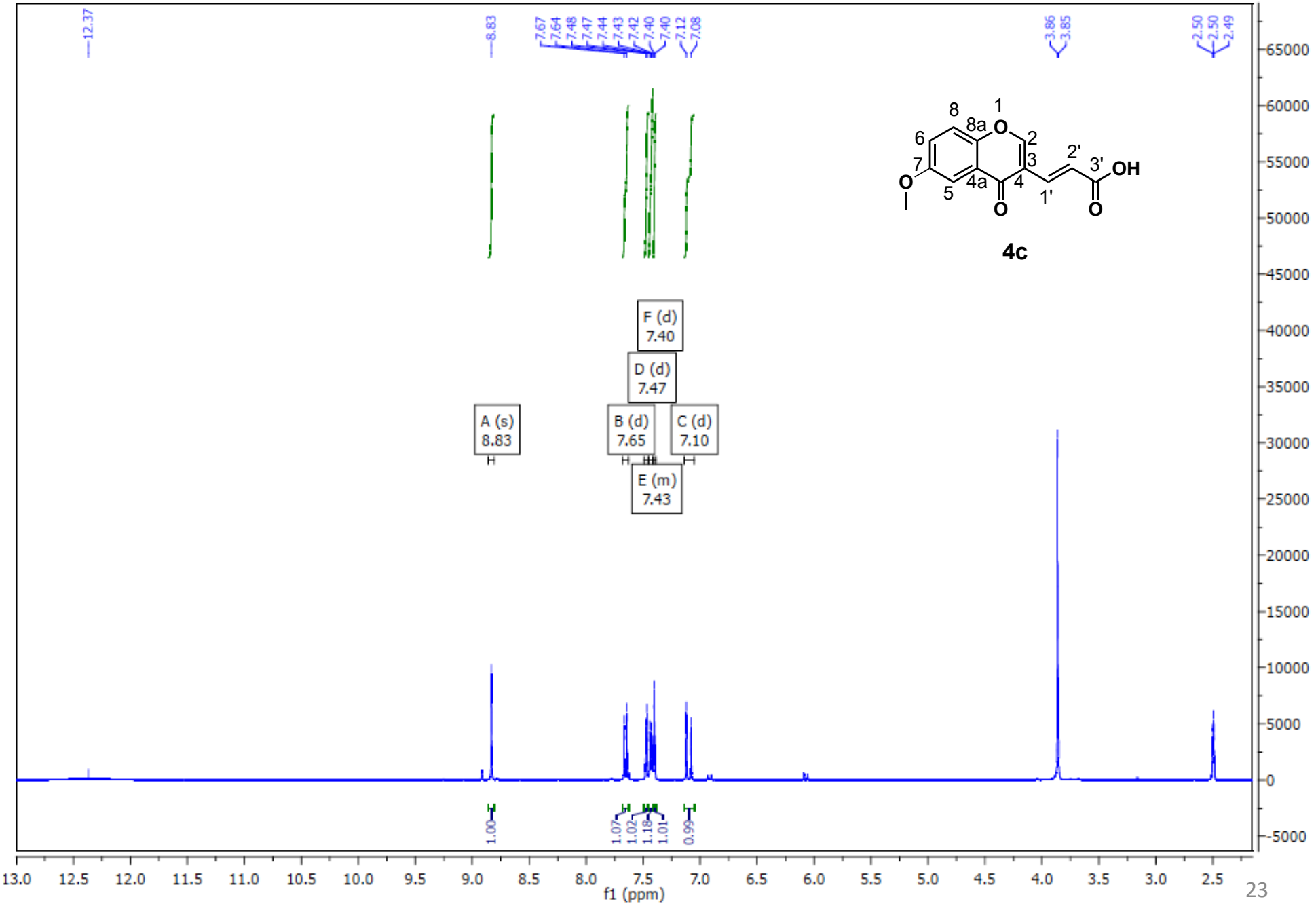


Figure S11.2. ^{13}C NMR spectra of compound 4c

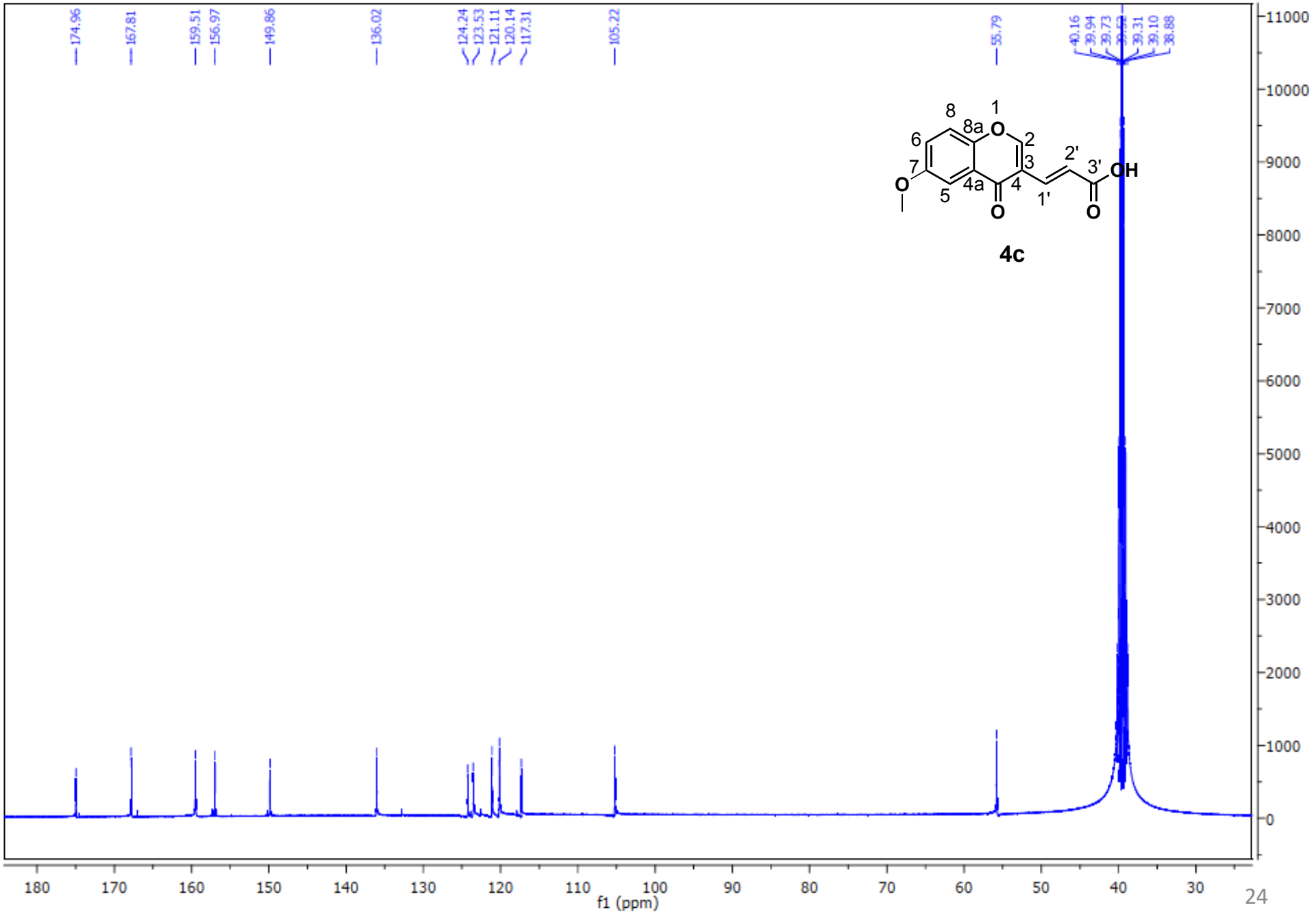


Figure S12.1. ^{1}H NMR spectra of compound 4d

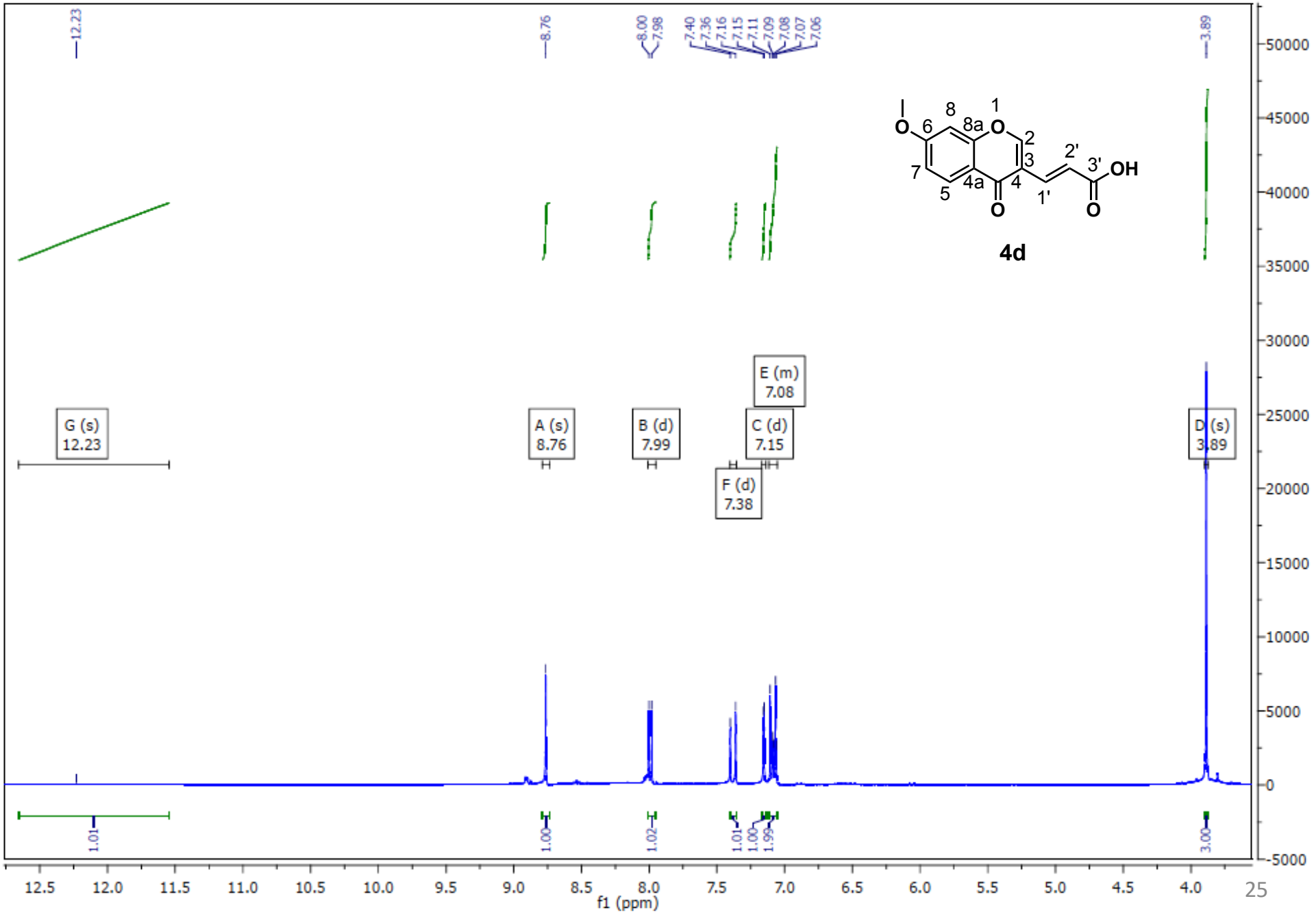


Figure S12.2. ^{13}C NMR spectra of compound 4d

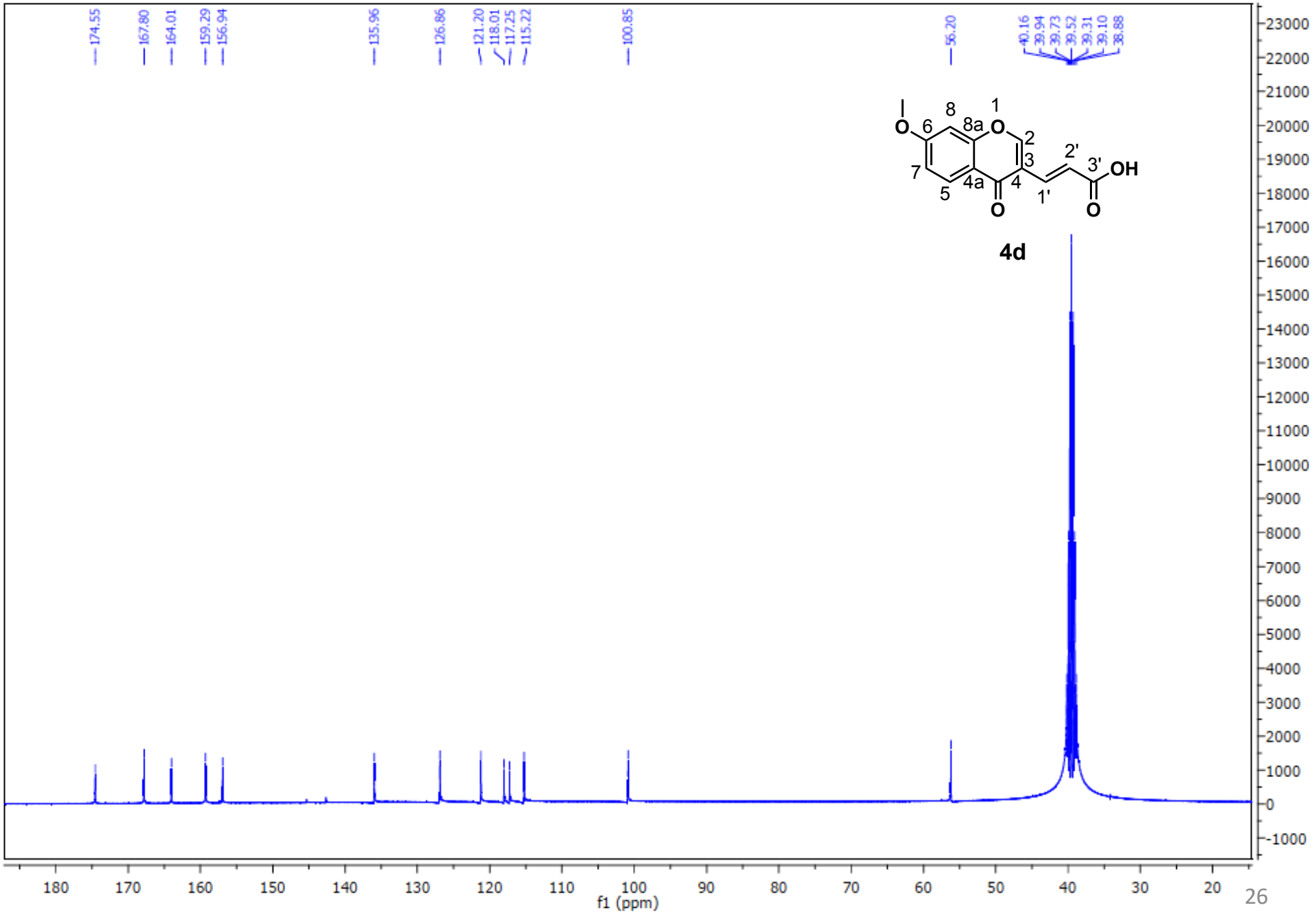


Figure S12.3. ^{13}C-DEPT NMR spectra of compound 4d

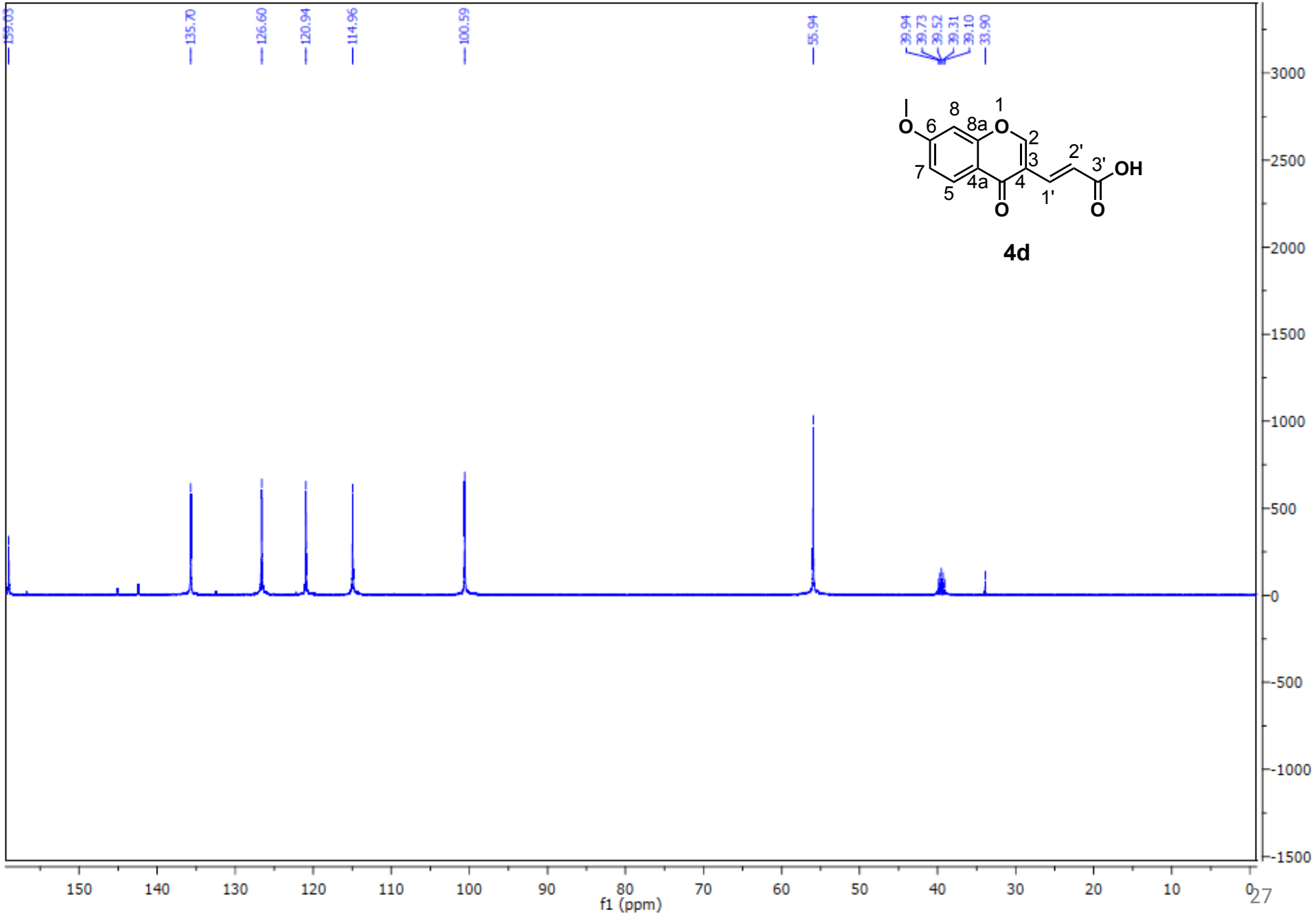
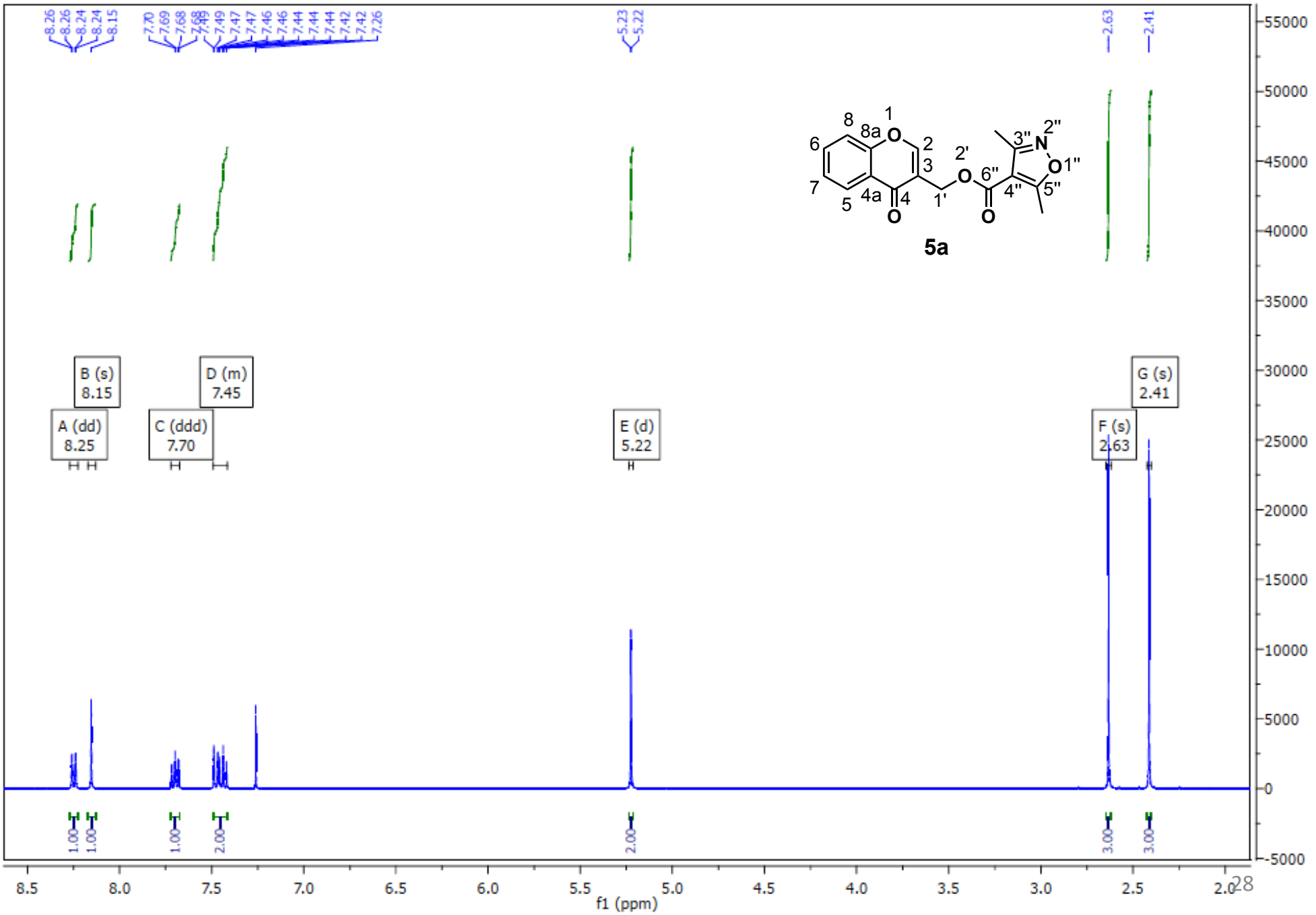


Figure S13.1. ^{1}H NMR spectra of compound 5a



5a

Figure S13.2. ^{13}C NMR spectra of compound 5a

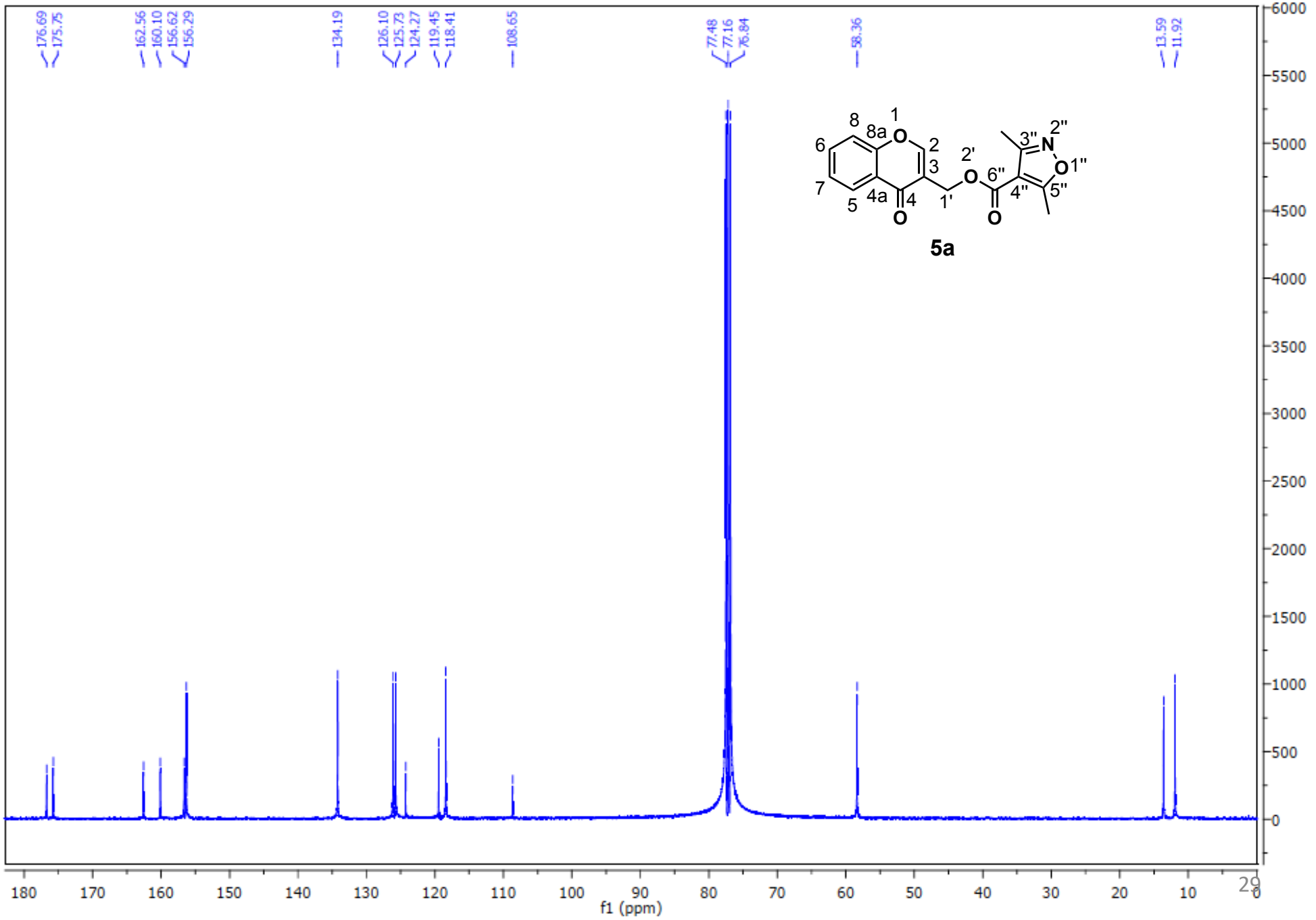


Figure S14.1. ^{1}H NMR spectra of compound 5b

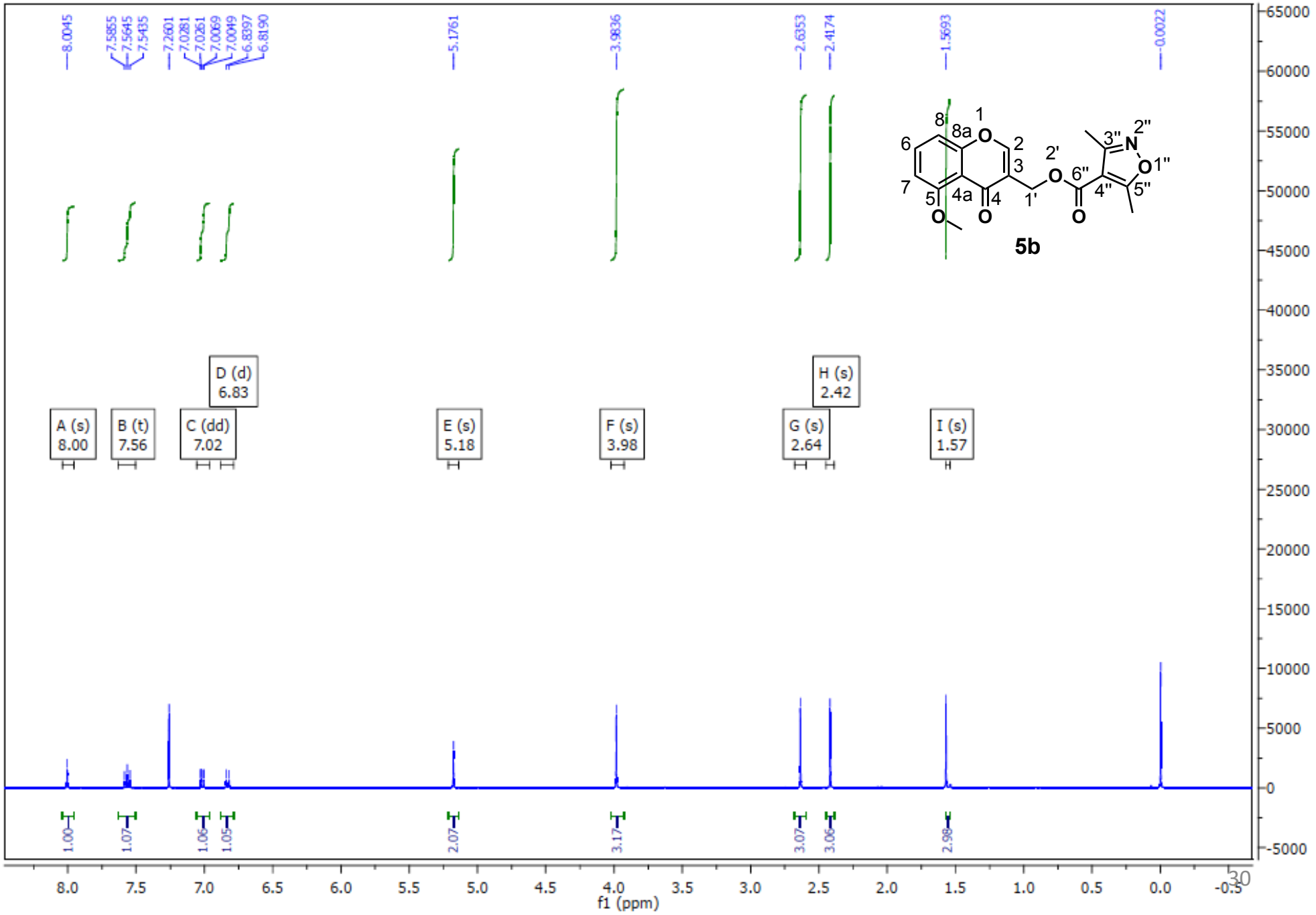


Figure S14.2. ^{13}C NMR spectra of compound 5b

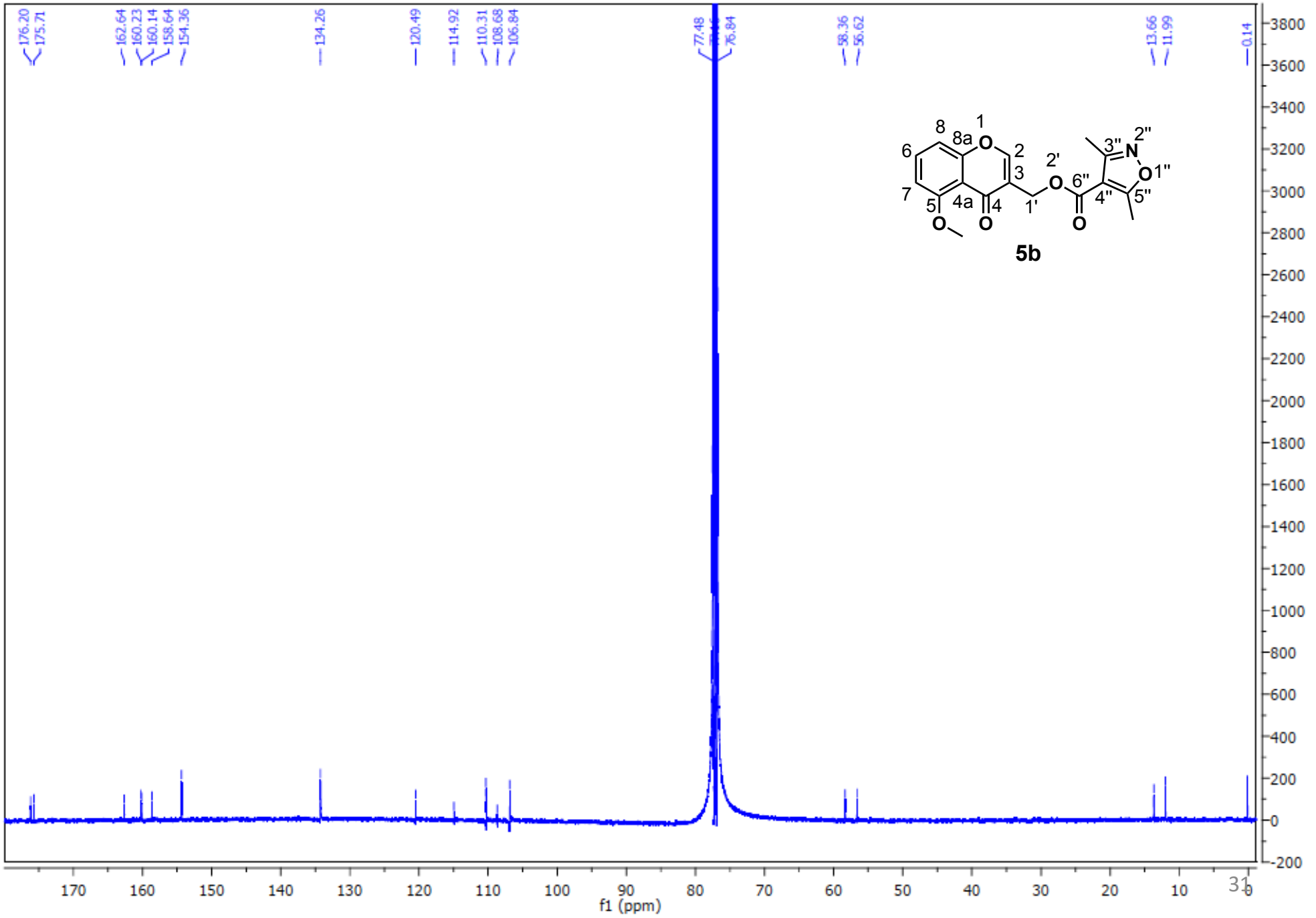


Figure S14.3. ^{13}C-DEPT NMR spectra of compound 5b

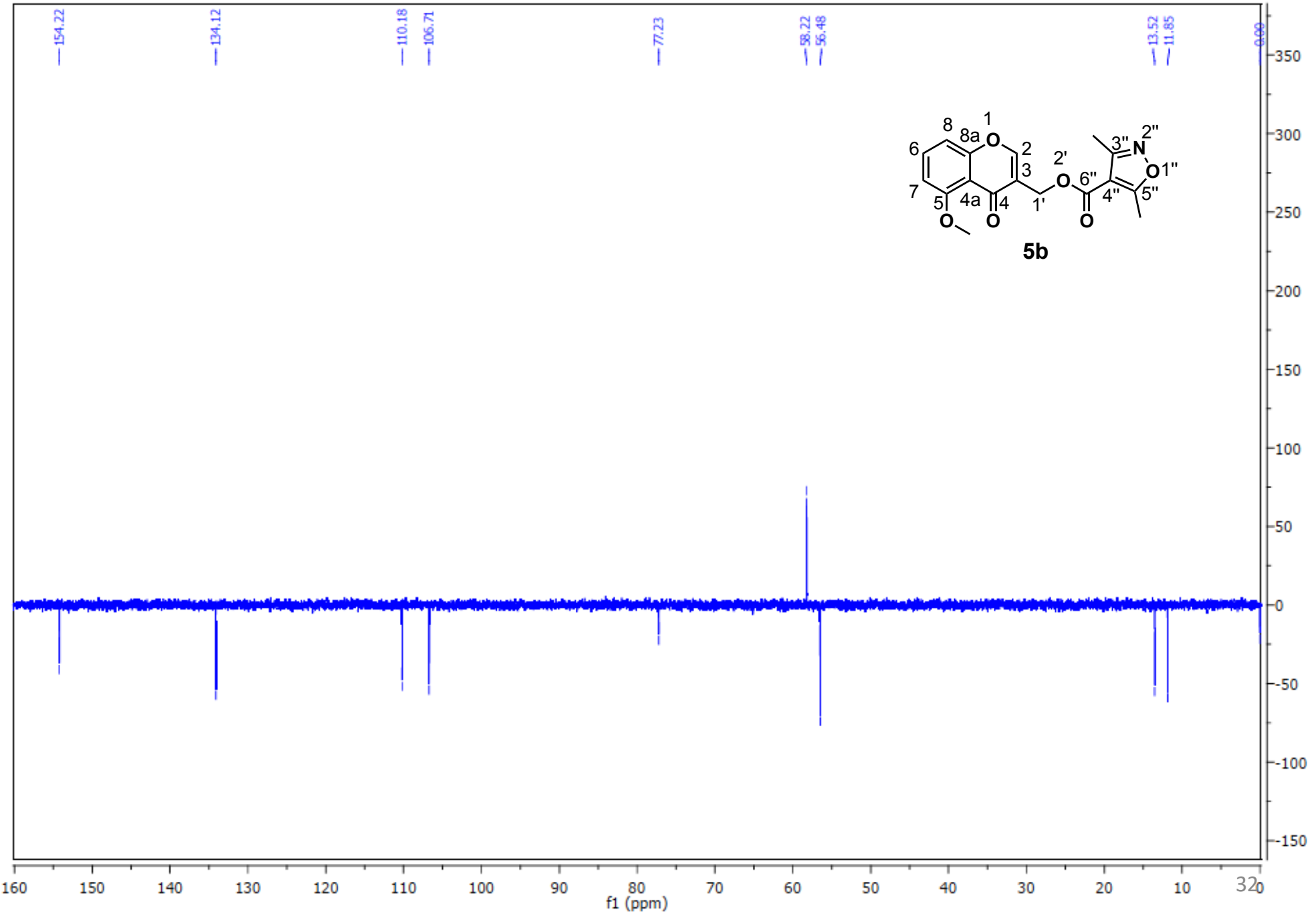


Figure S15.1. ^{1}H NMR spectra of compound 5c

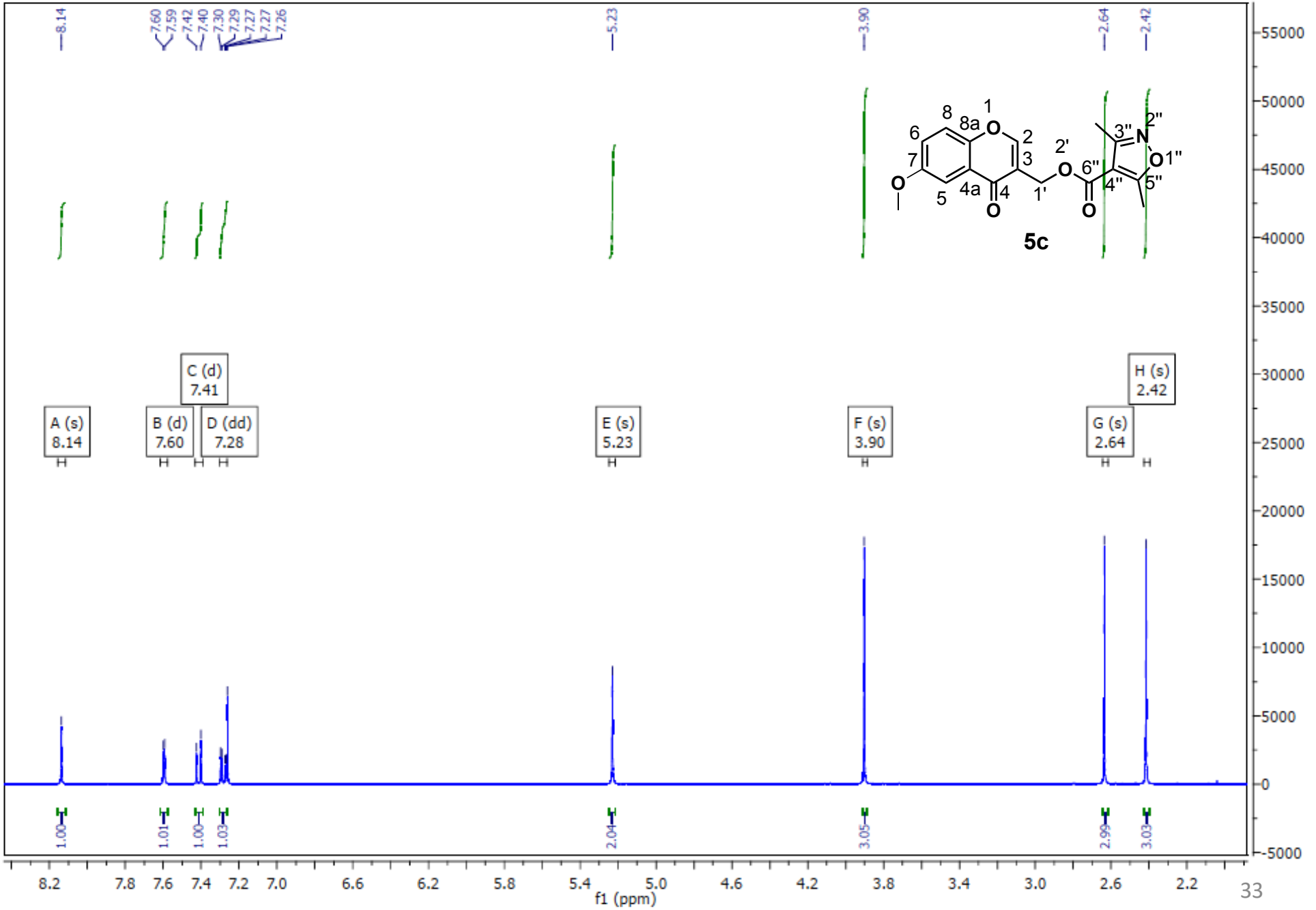


Figure S15.2. ^{13}C NMR spectra of compound 5c

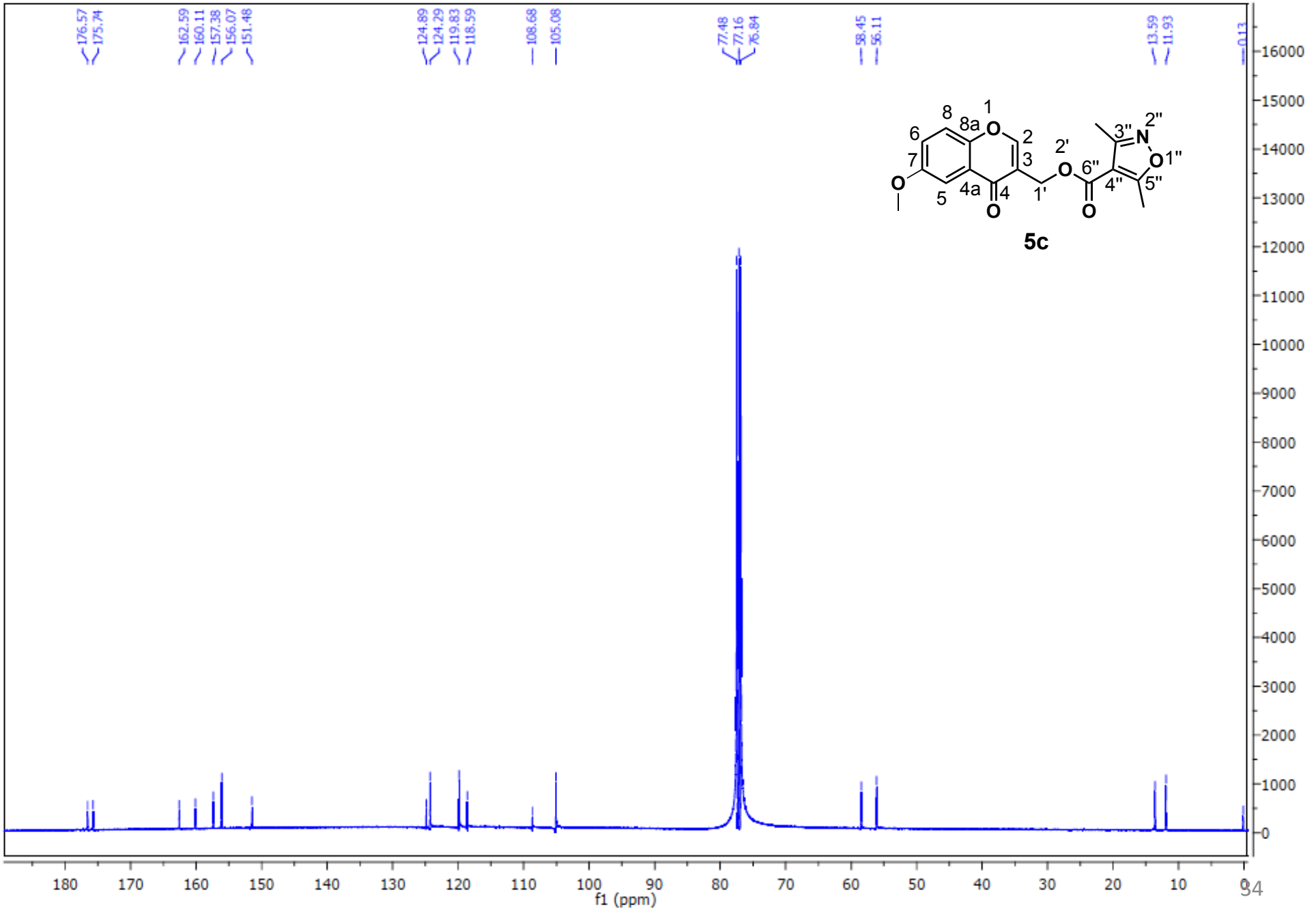


Figure S15.3. ^{13}C-DEPT NMR spectra of compound 5c

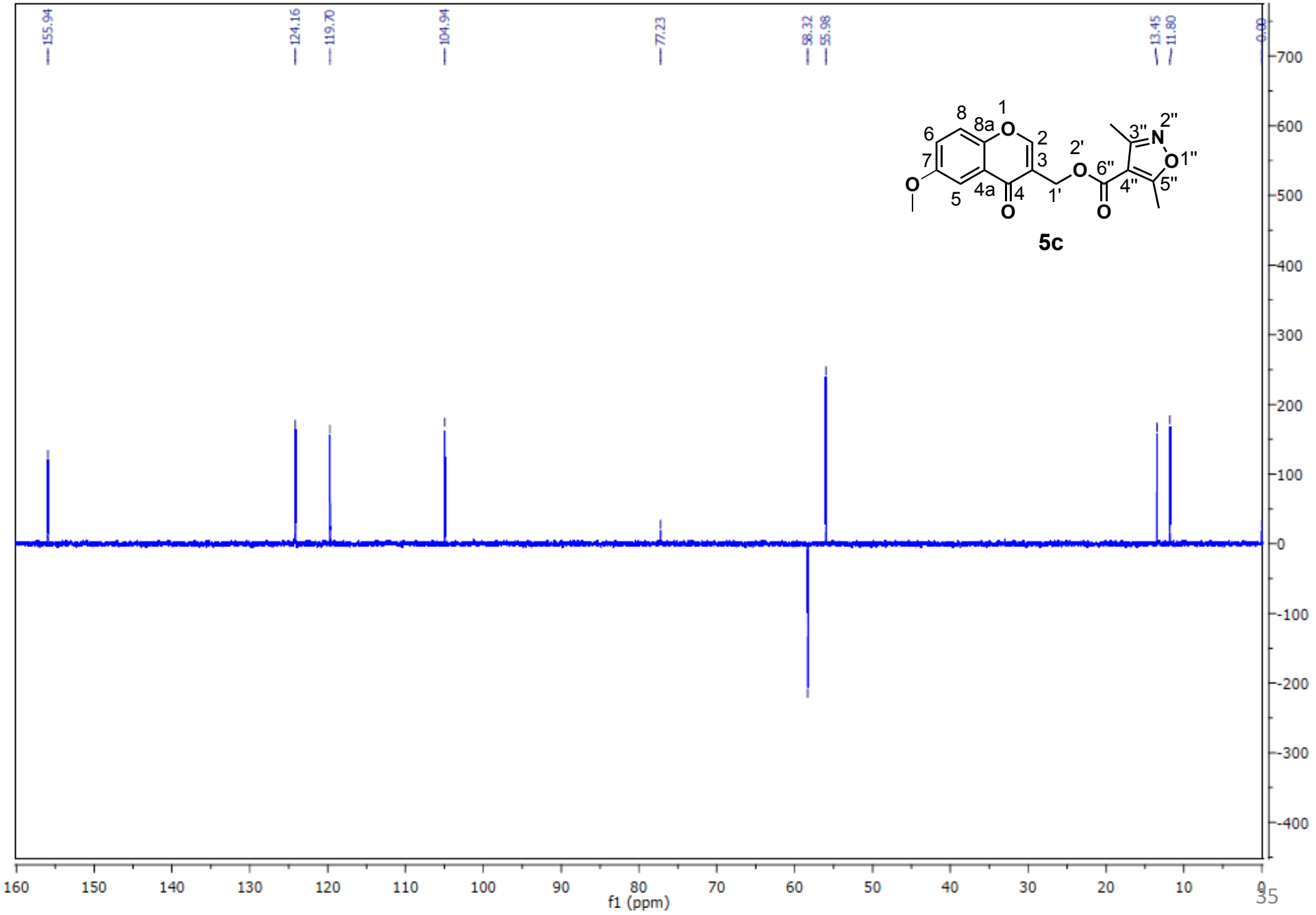


Figure S16.1. ^{1}H NMR spectra of compound 5d

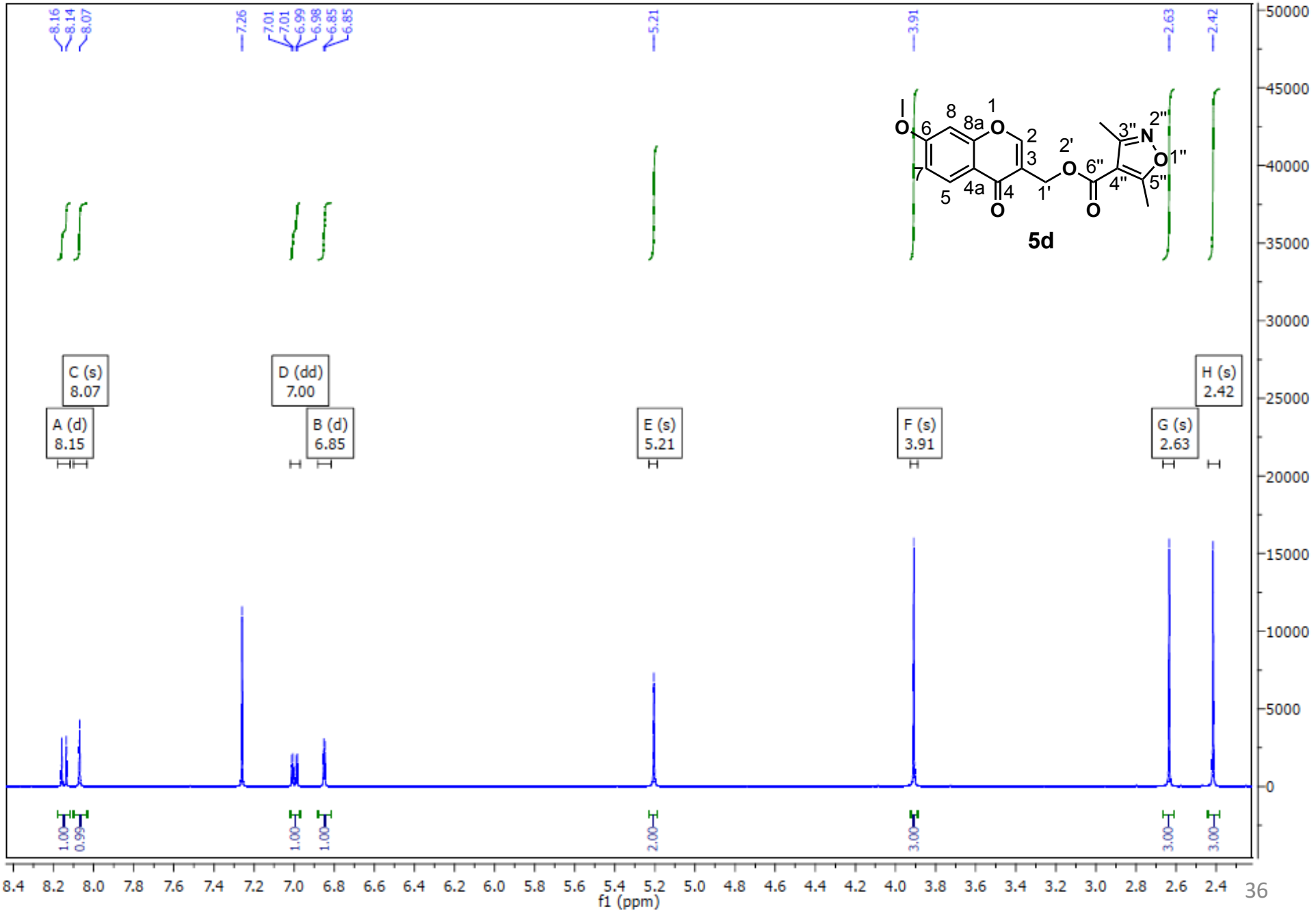


Figure S16.2. ^{13}C NMR spectra of compound 5d

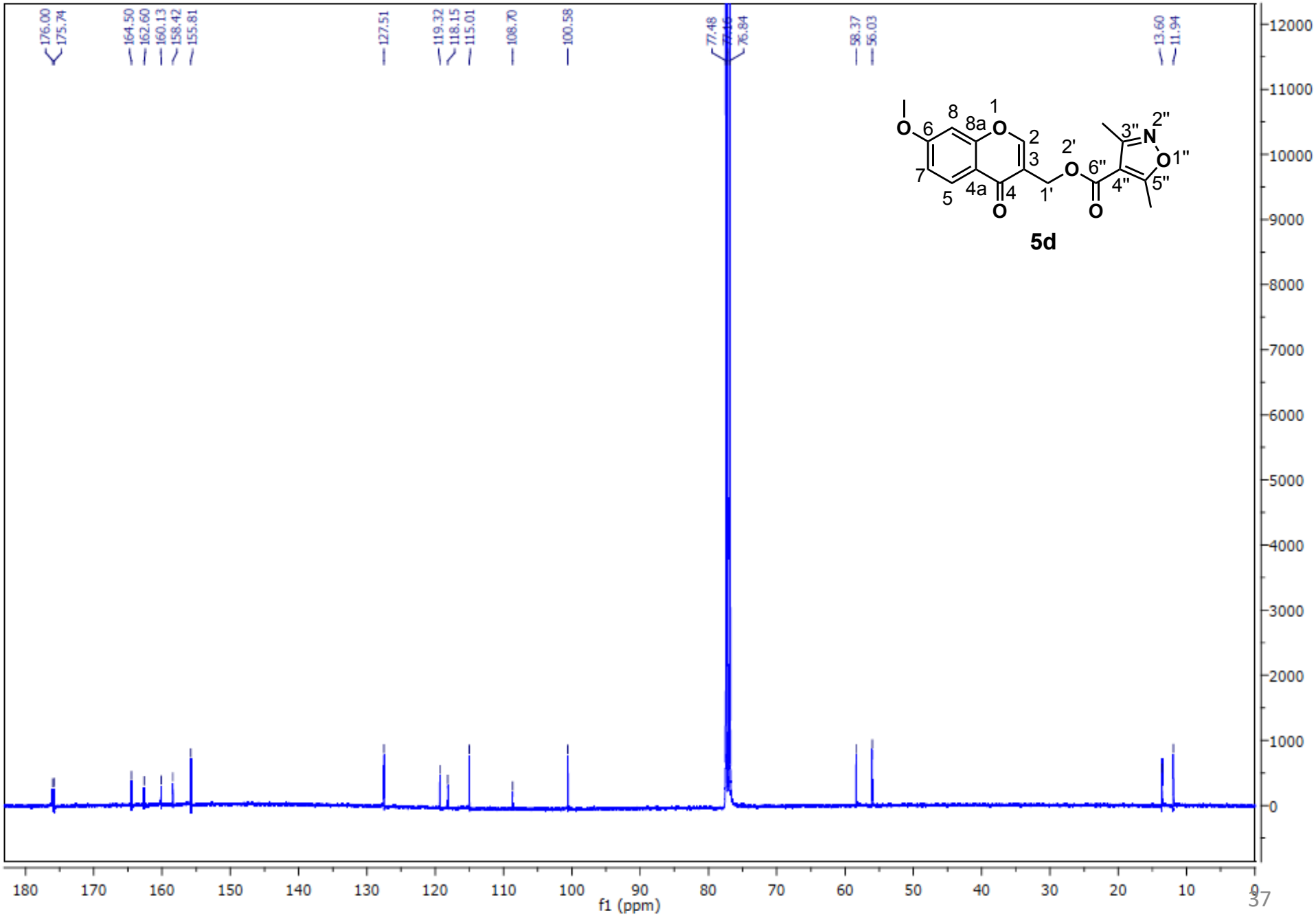
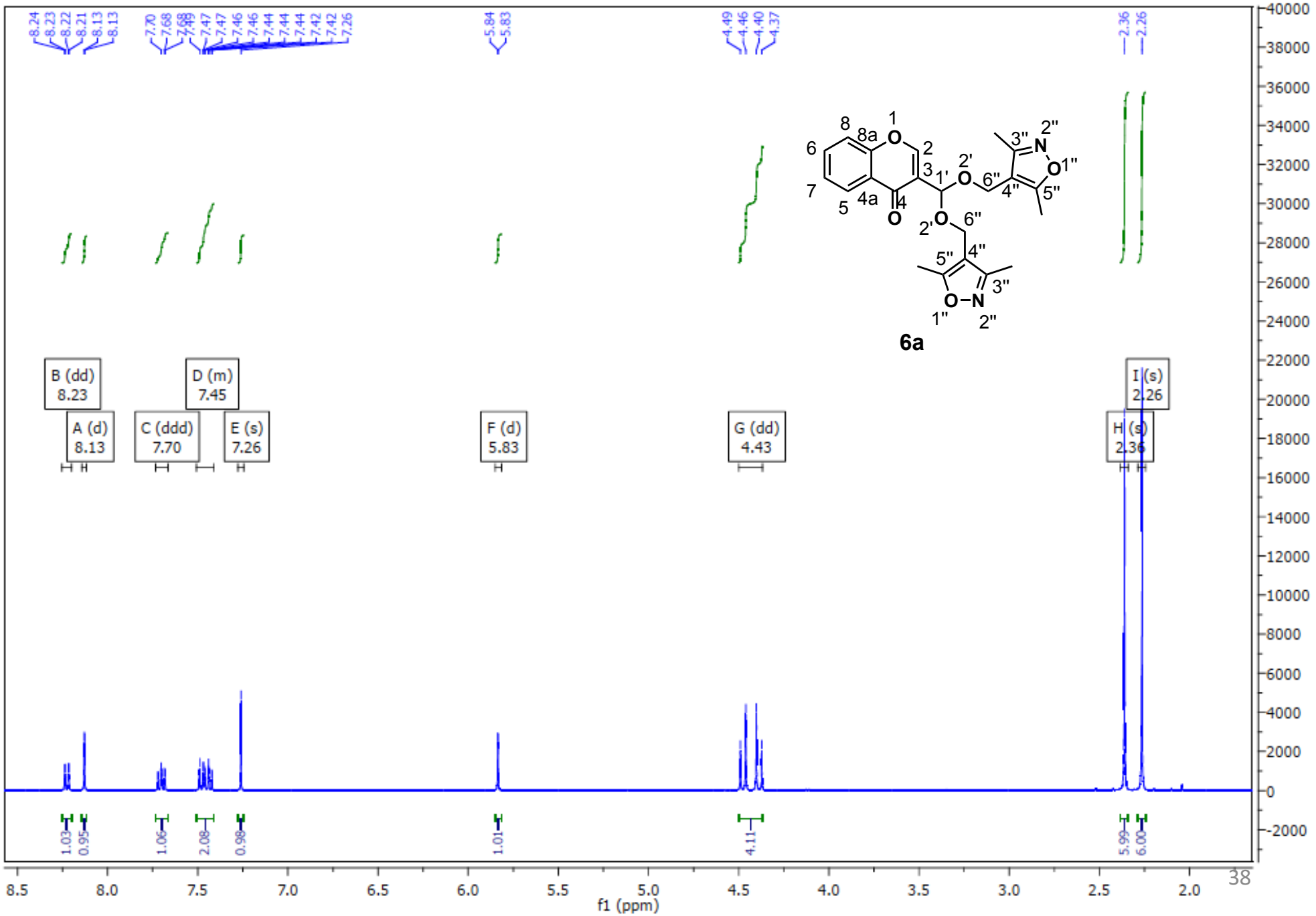


Figure S17.1. ^{1}H NMR spectra of compound 6a



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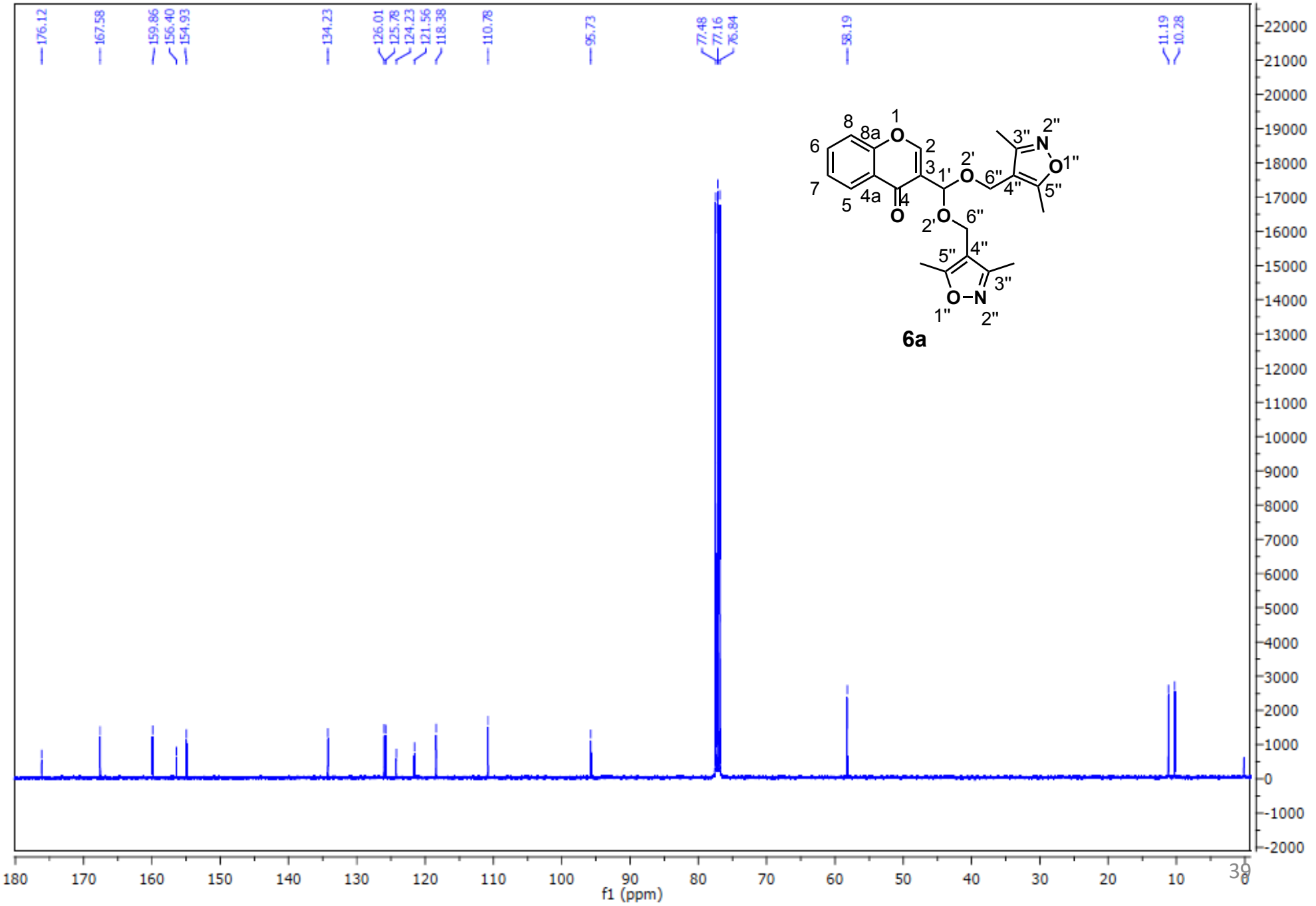


Figure S18.1. ^{1}H NMR spectra of compound 6b

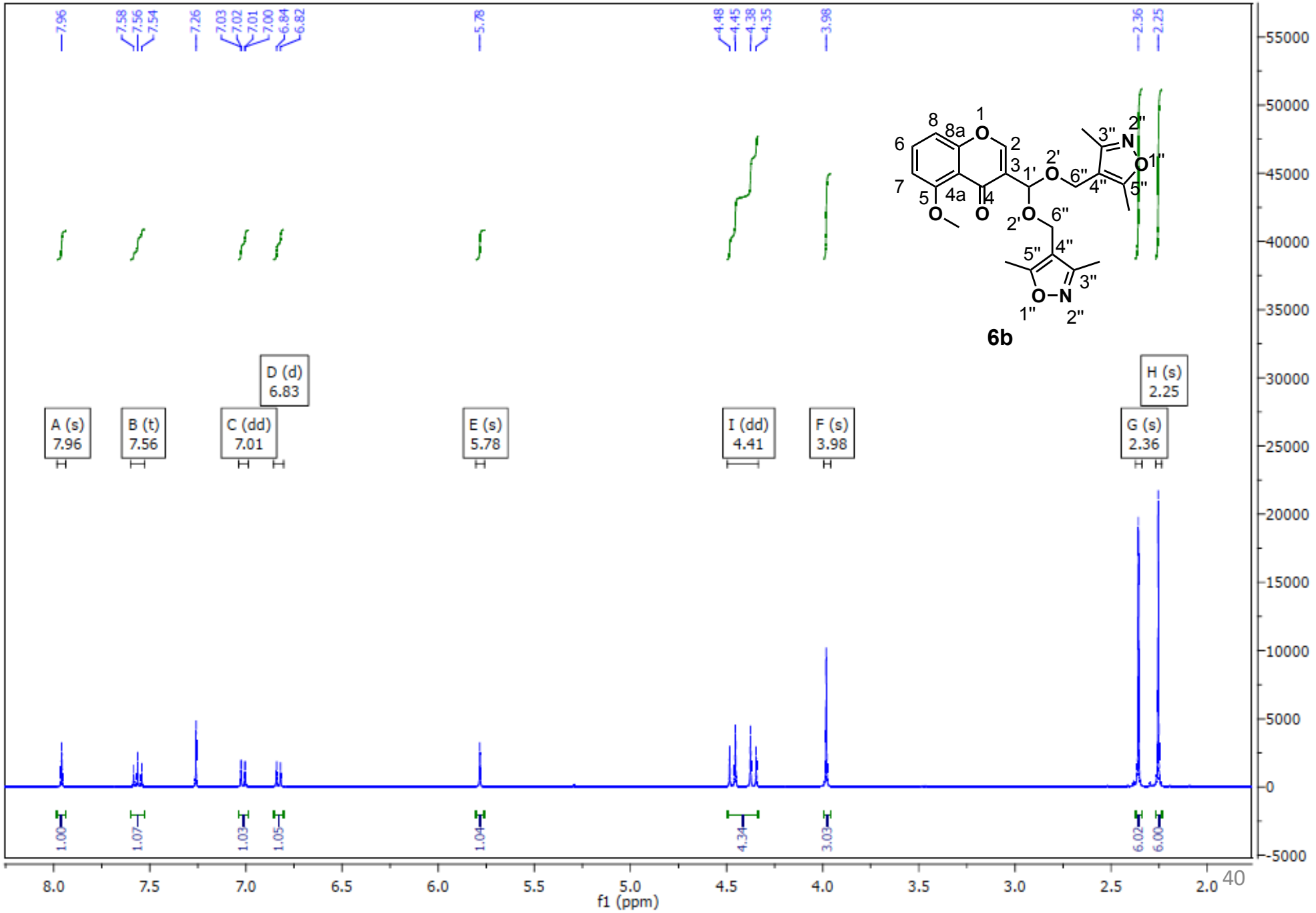


Figure S18.2. ^{13}C NMR spectra of compound 6b

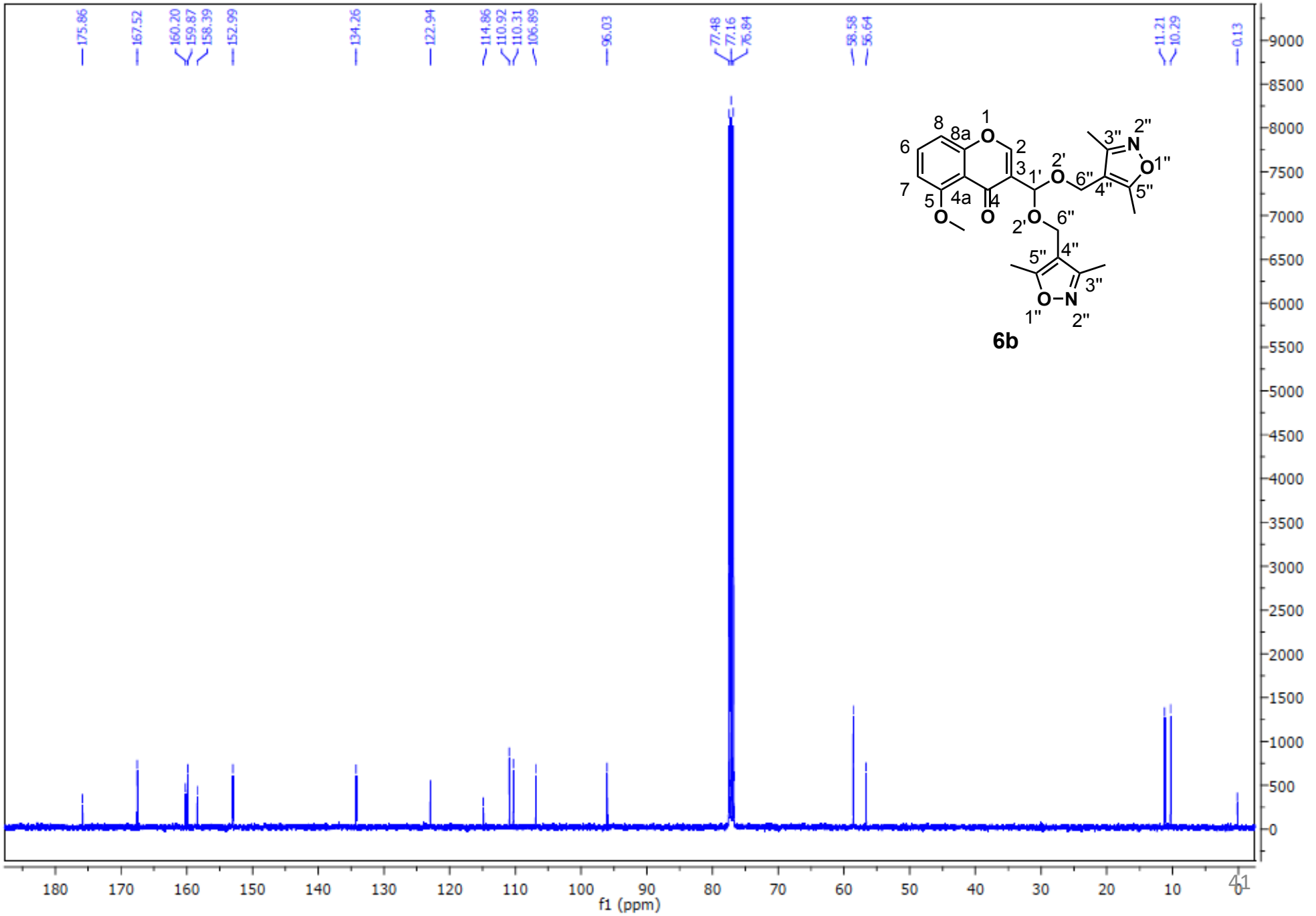


Figure S18.3. ^{13}C-DEPT NMR spectra of compound 6b

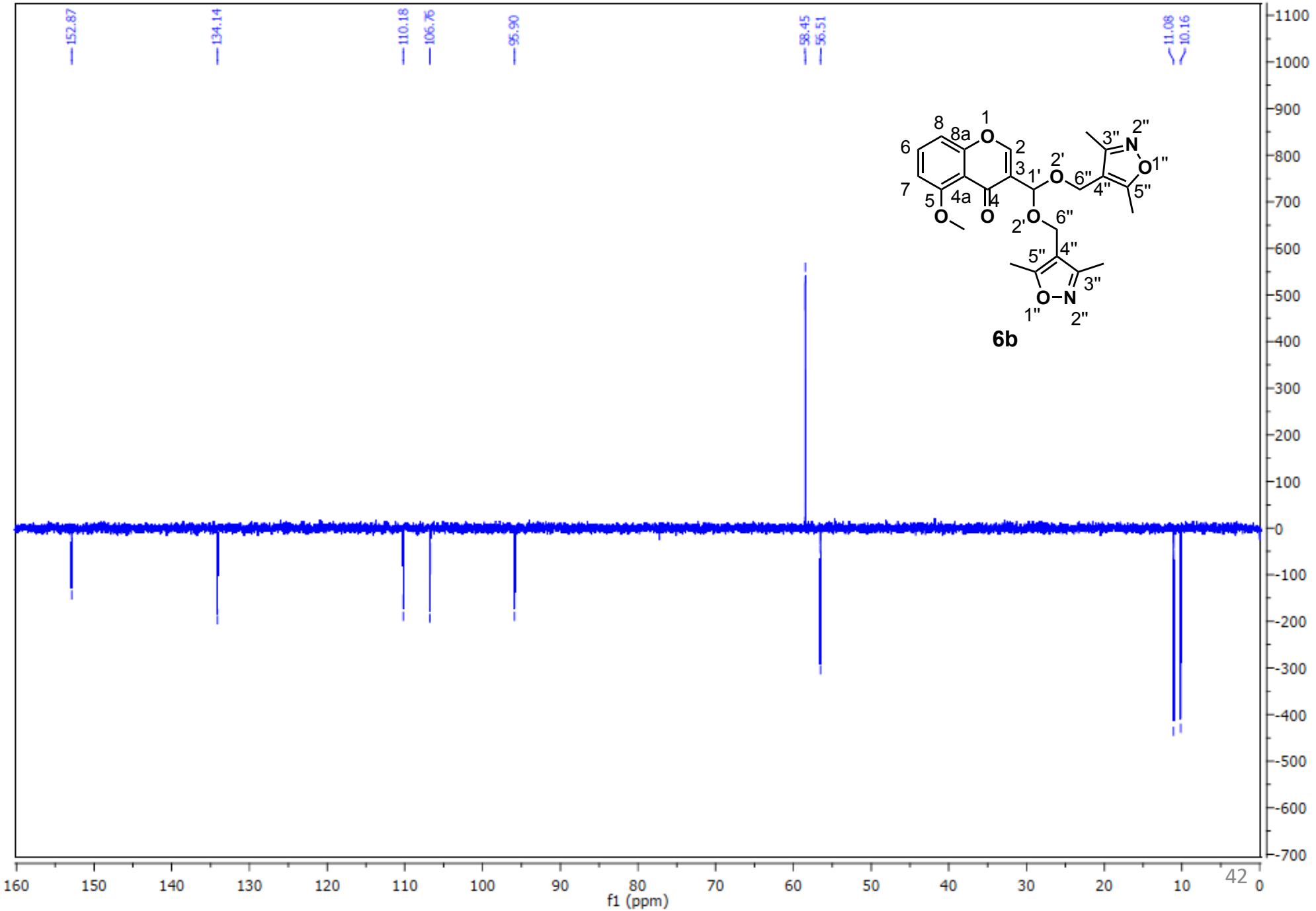


Figure S19.1. ^{1}H NMR spectra of compound 6d

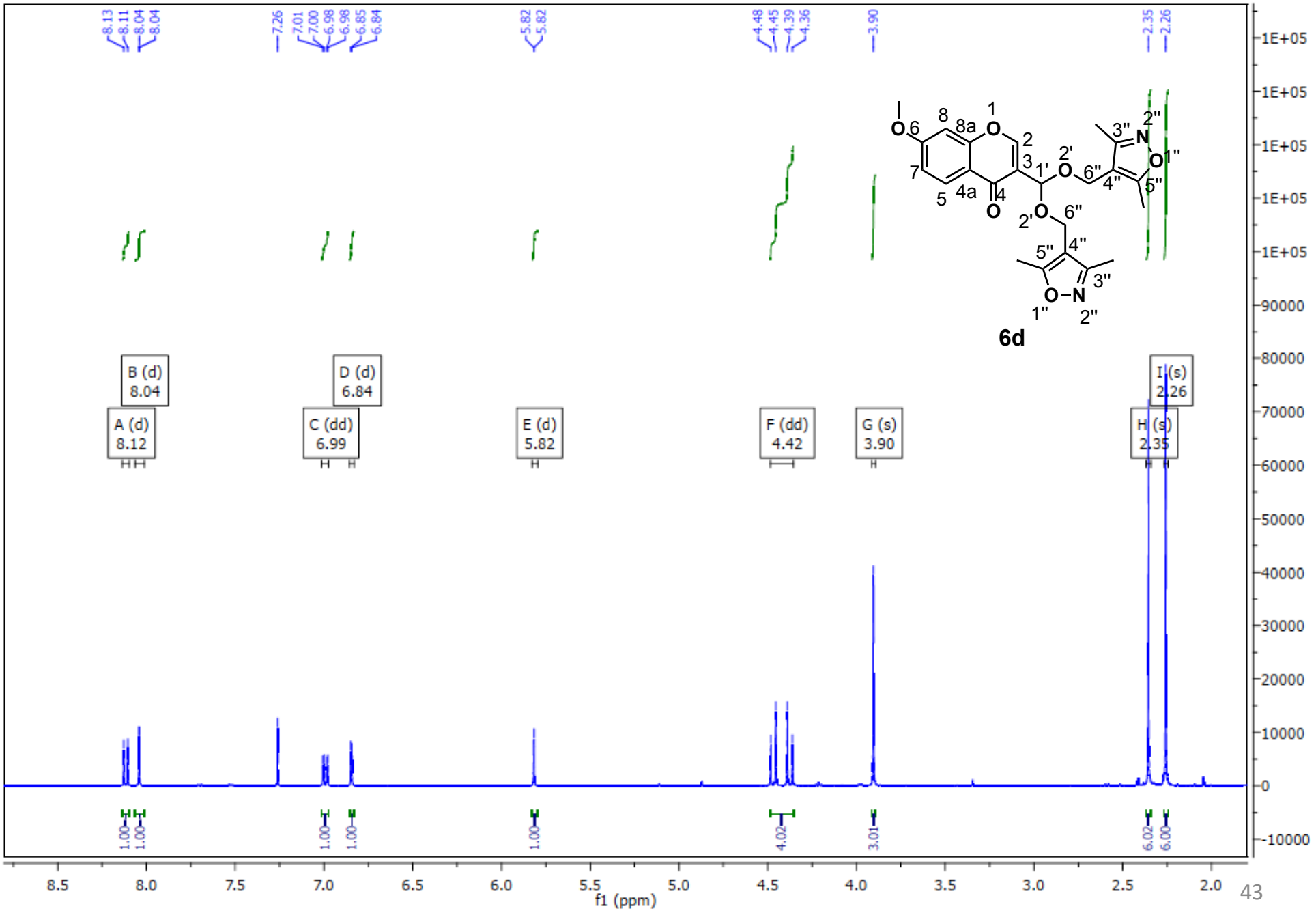


Figure S19.2. ^{13}C NMR spectra of compound 6d

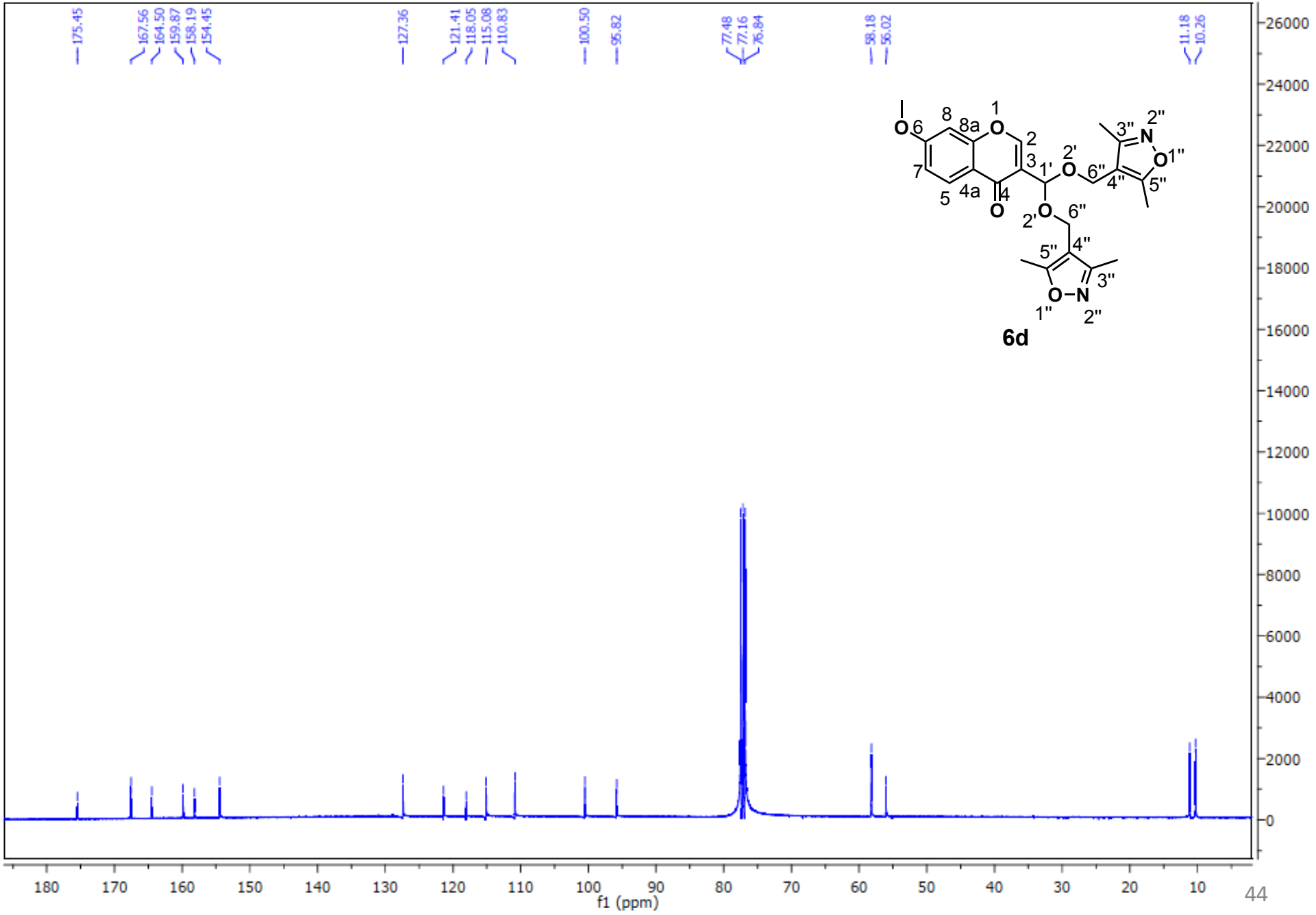
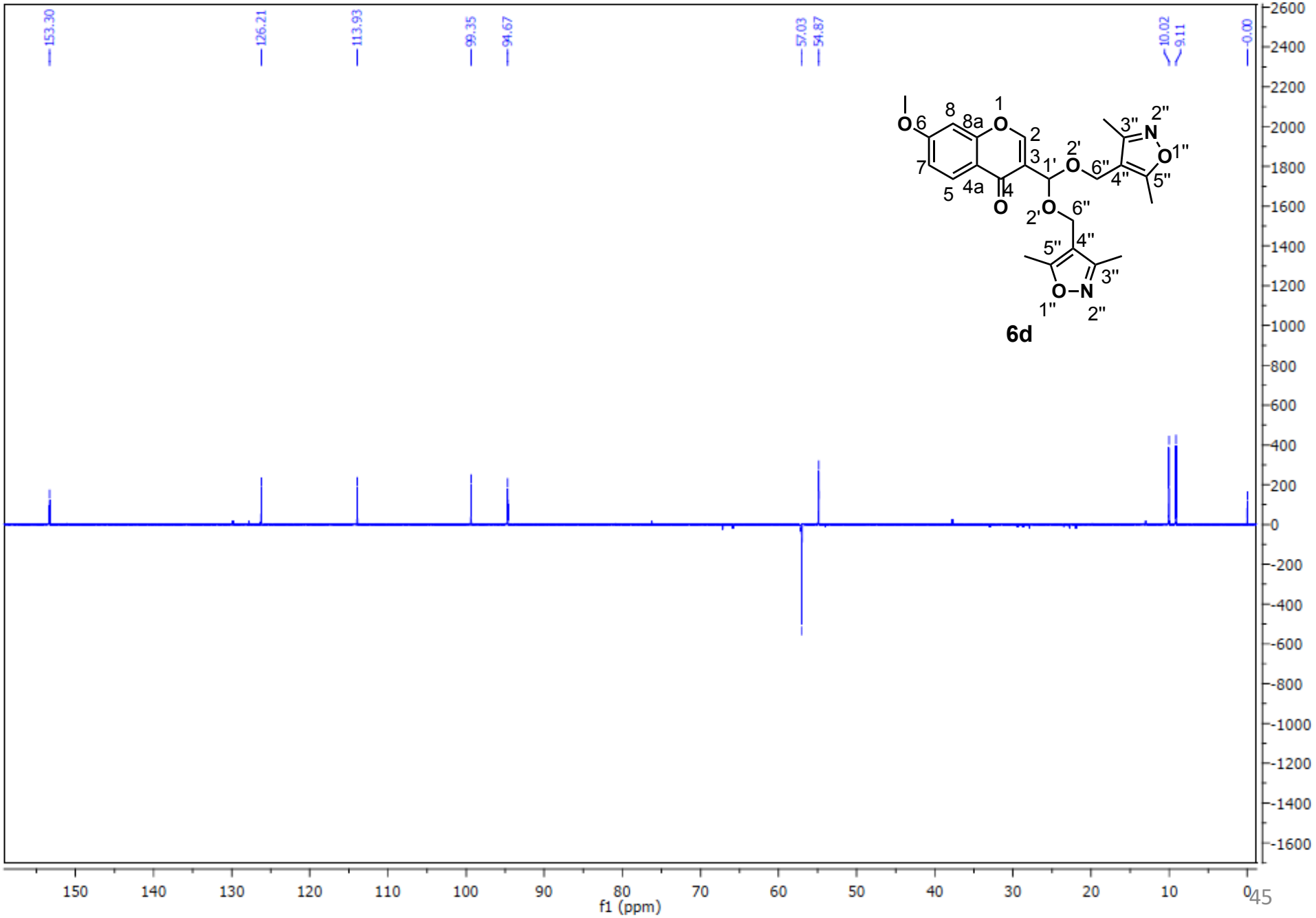


Figure S19.3. ^{13}C-DEPT NMR spectra of compound 6d



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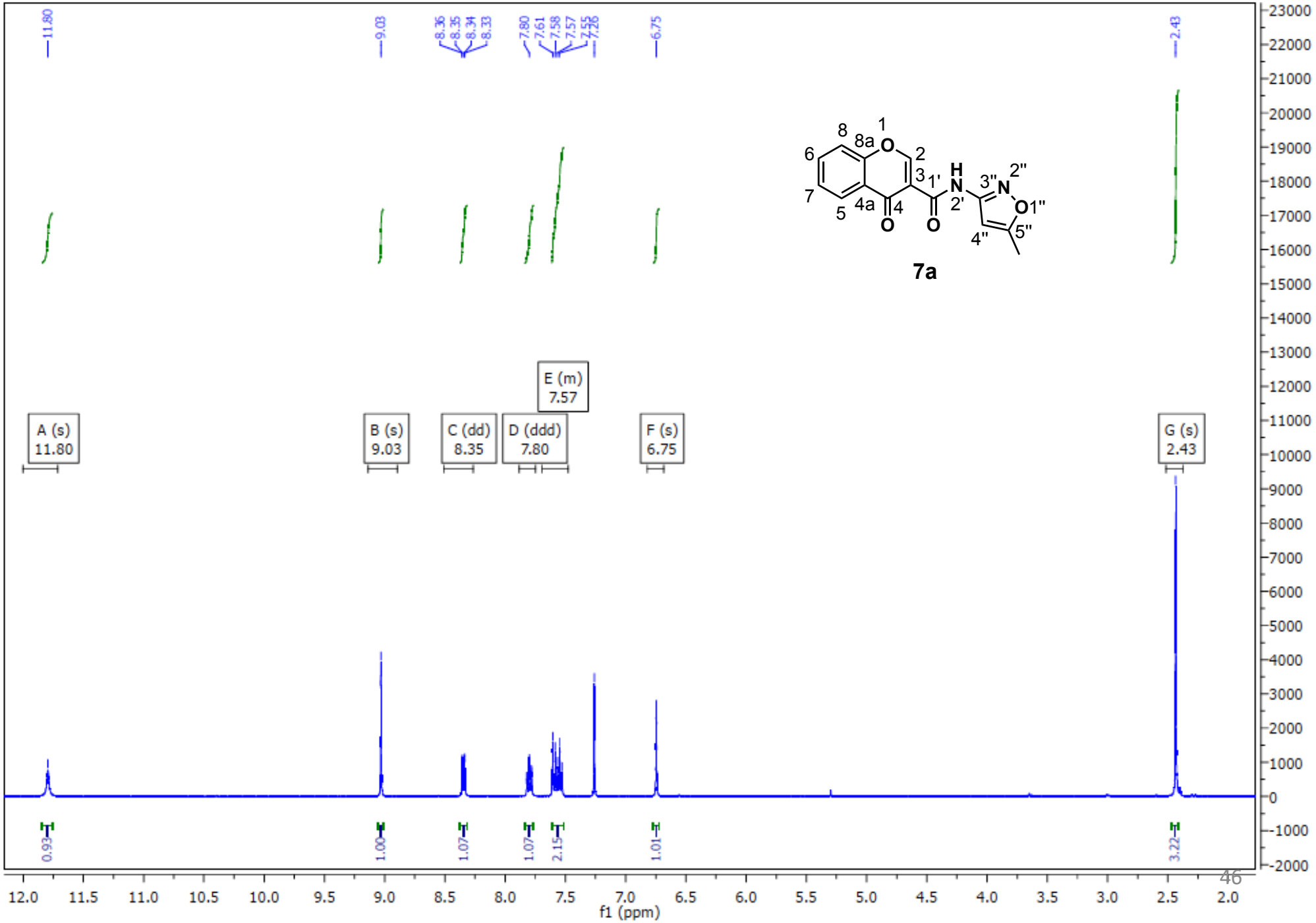


7a

Figure S20.2. ^{13}C NMR spectra of compound 7a

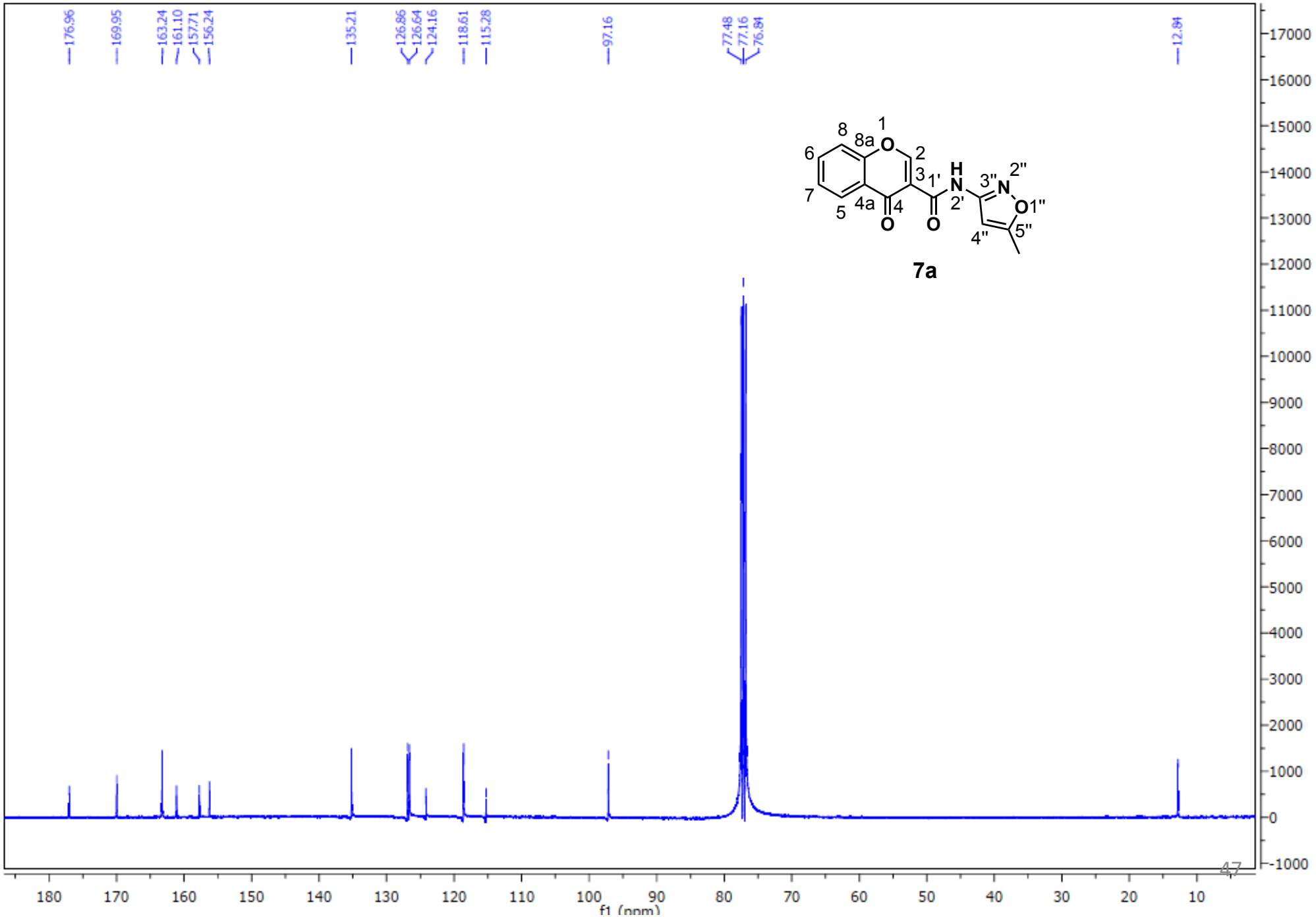


Figure S20.3. ^{13}C-DEPT NMR spectra of compound 7a

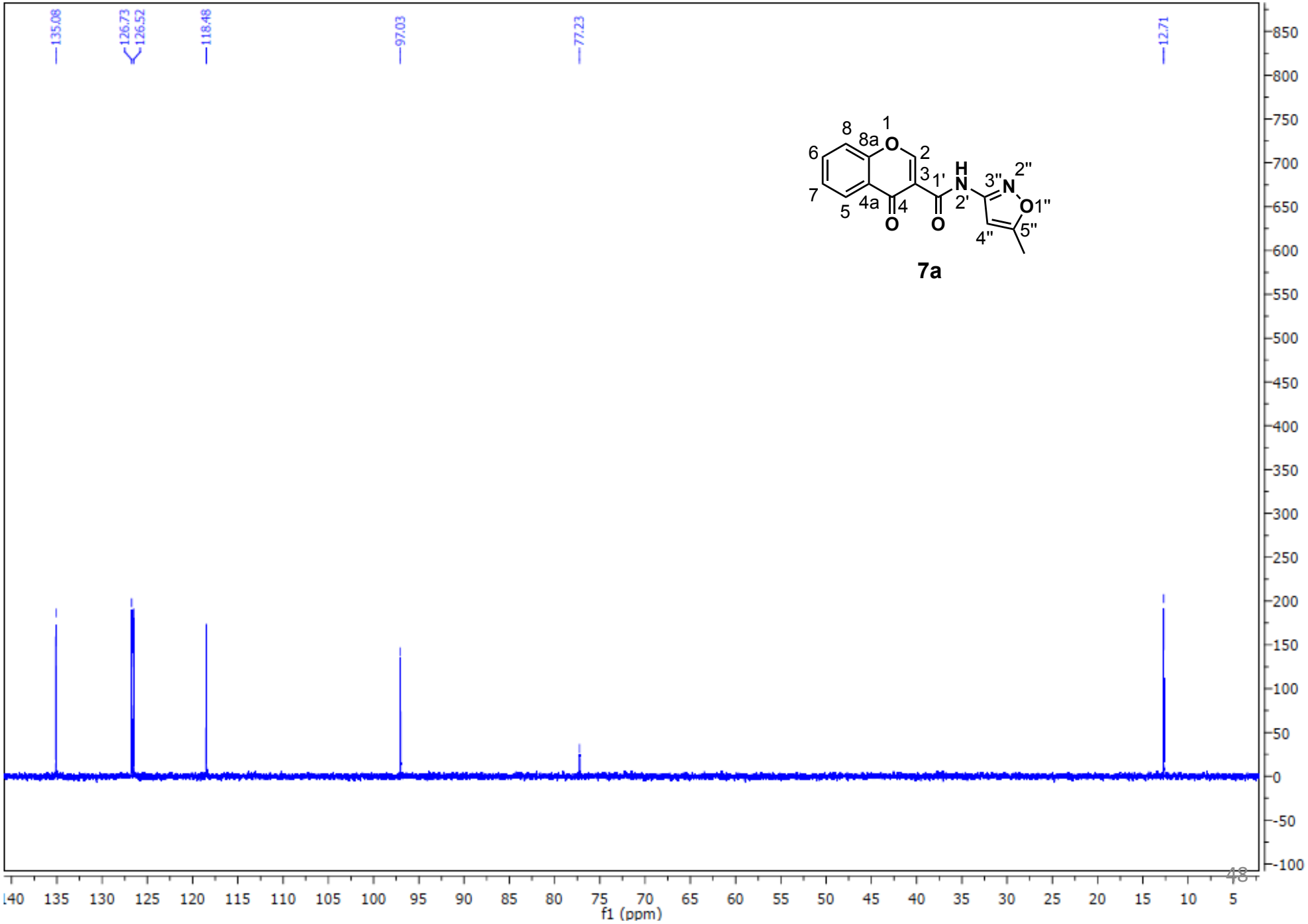


Figure S21.1. ^{1}H NMR spectra of compound 7c

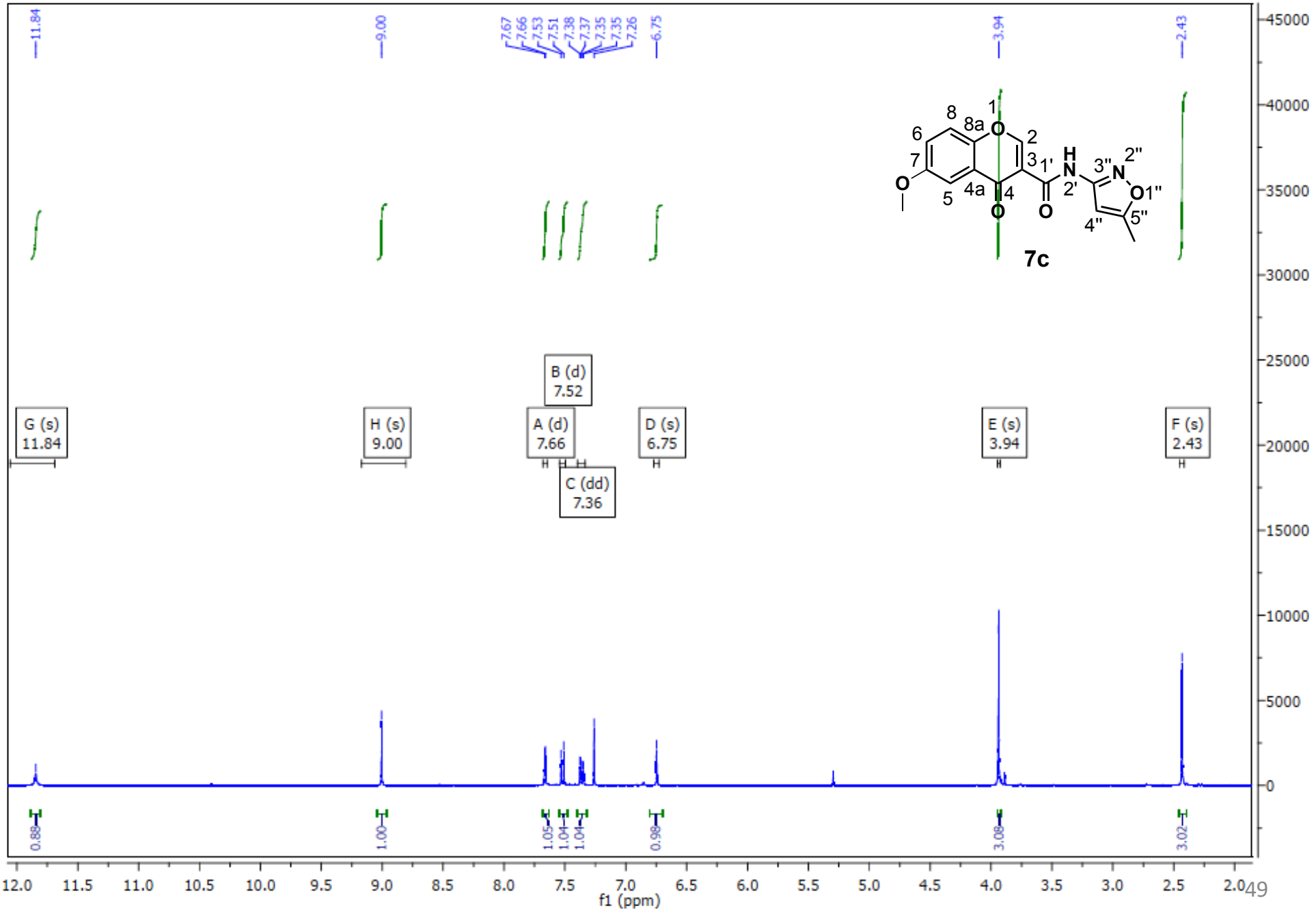


Figure S21.2. ^{13}C NMR spectra of compound 7c

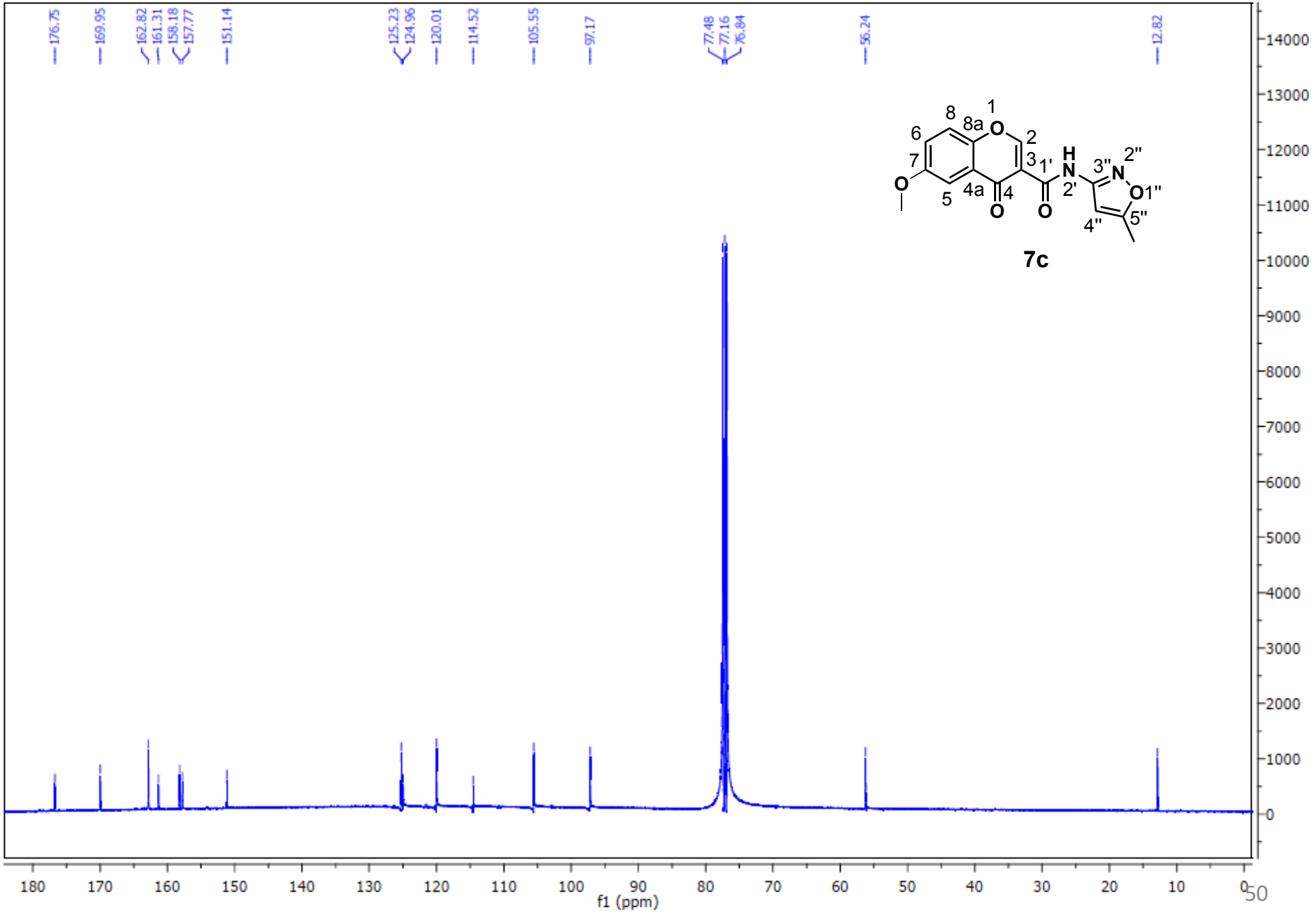


Figure S21.3. ^{13}C-DEPT NMR spectra of compound 7c

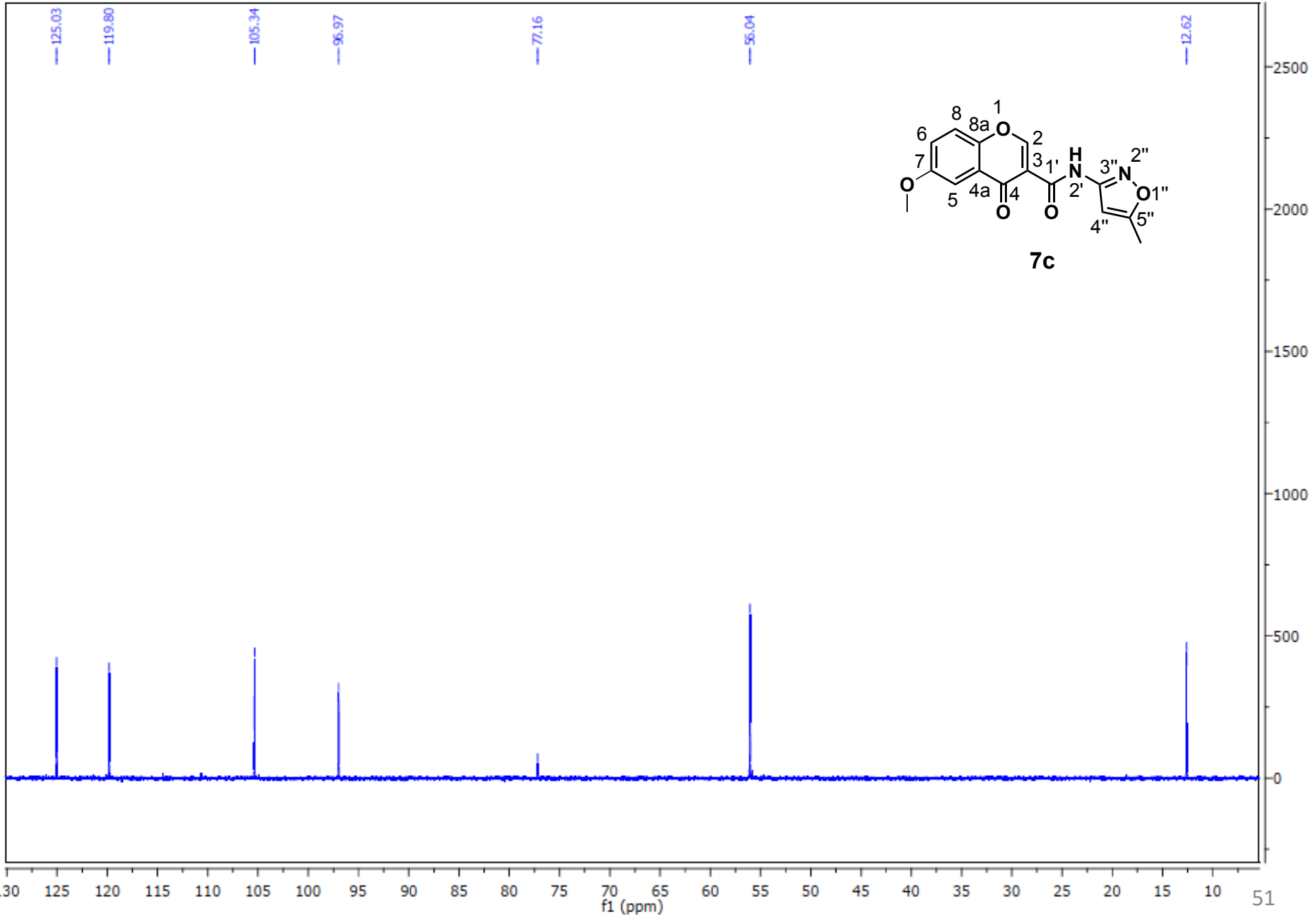


Figure S22.1. ^{1}H NMR spectra of compound 7d

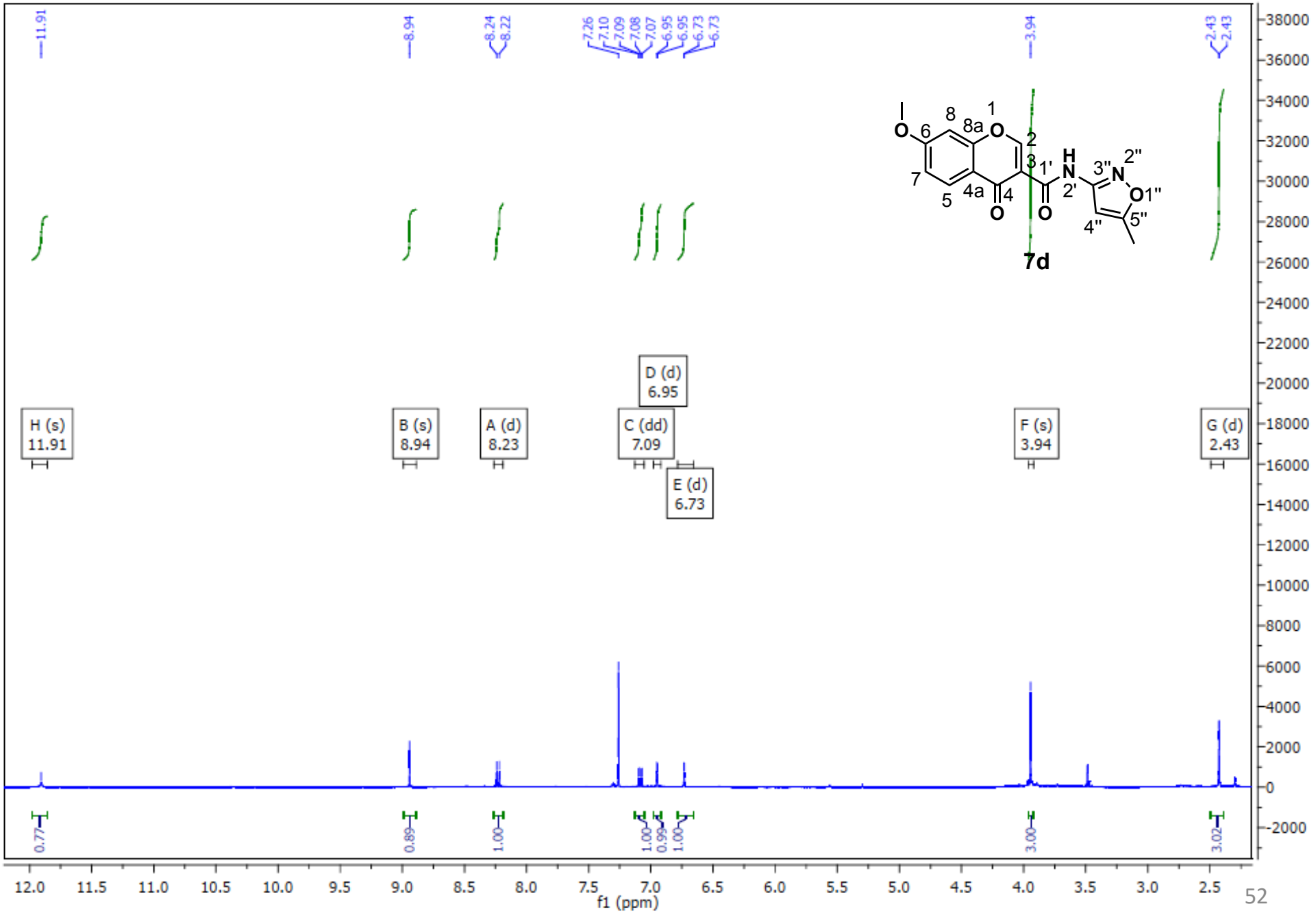


Figure S22.2. ^{13}C NMR spectra of compound 7d

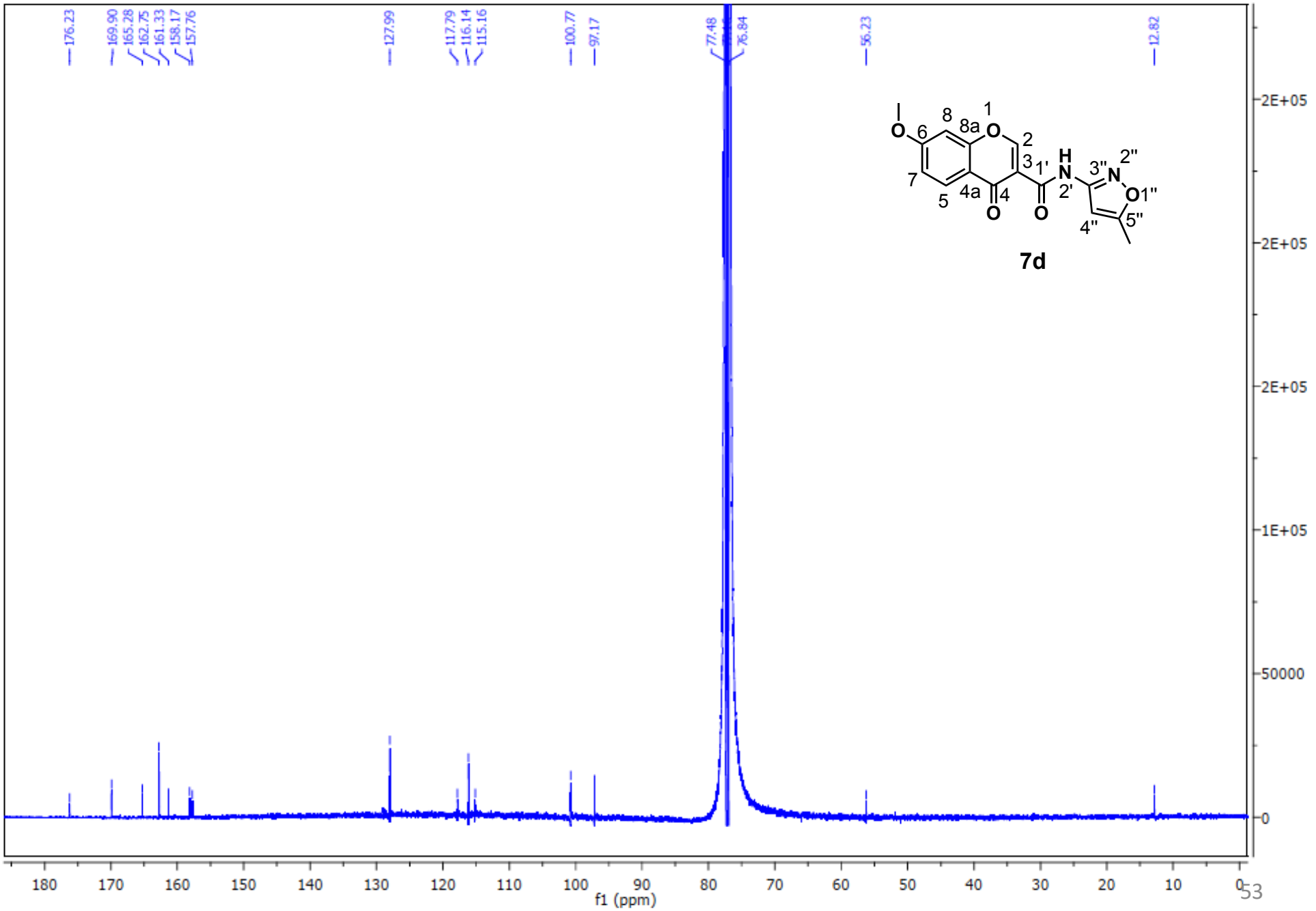


Figure S22.3. ^{13}C-DEPT NMR spectra of compound 7d

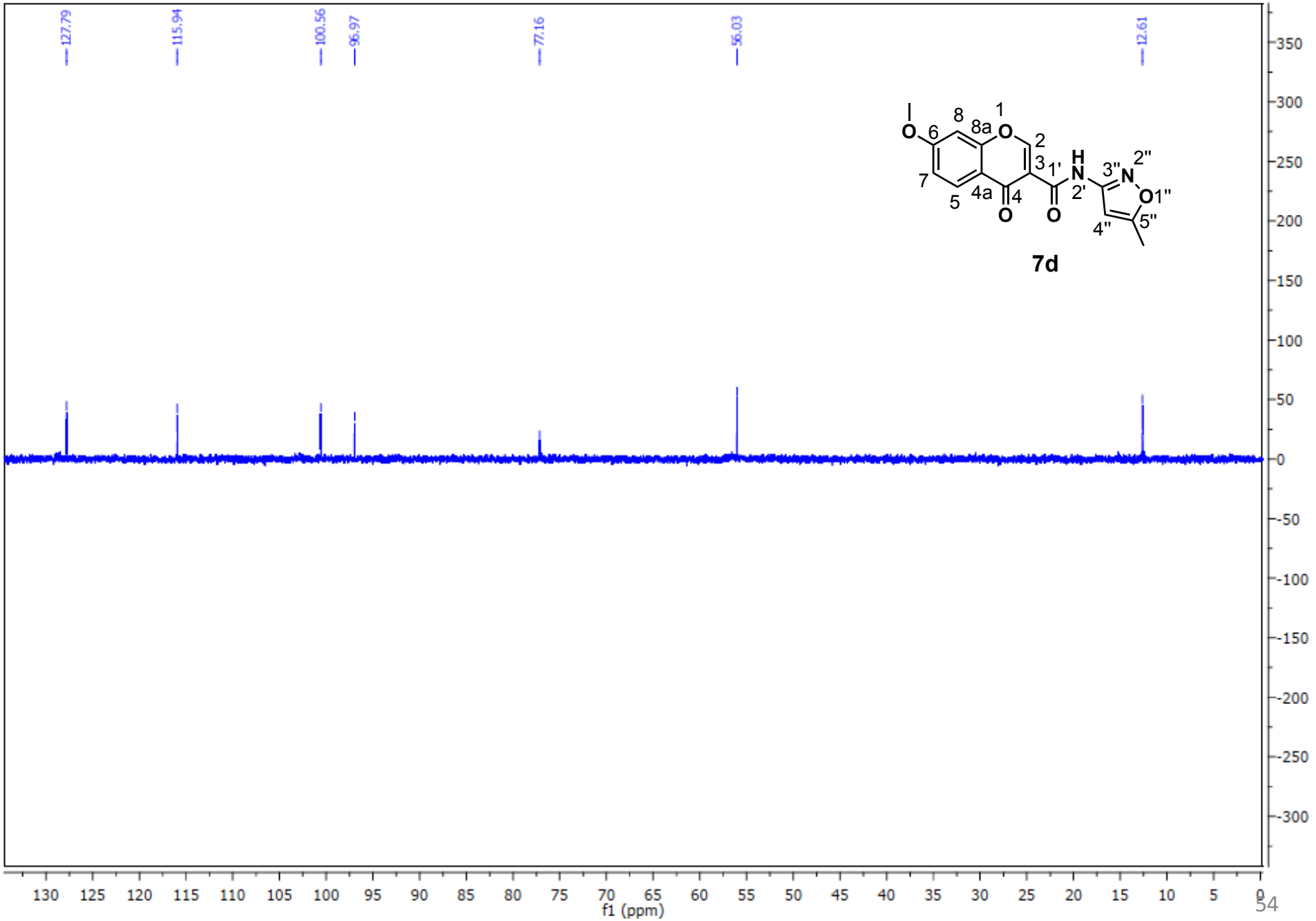
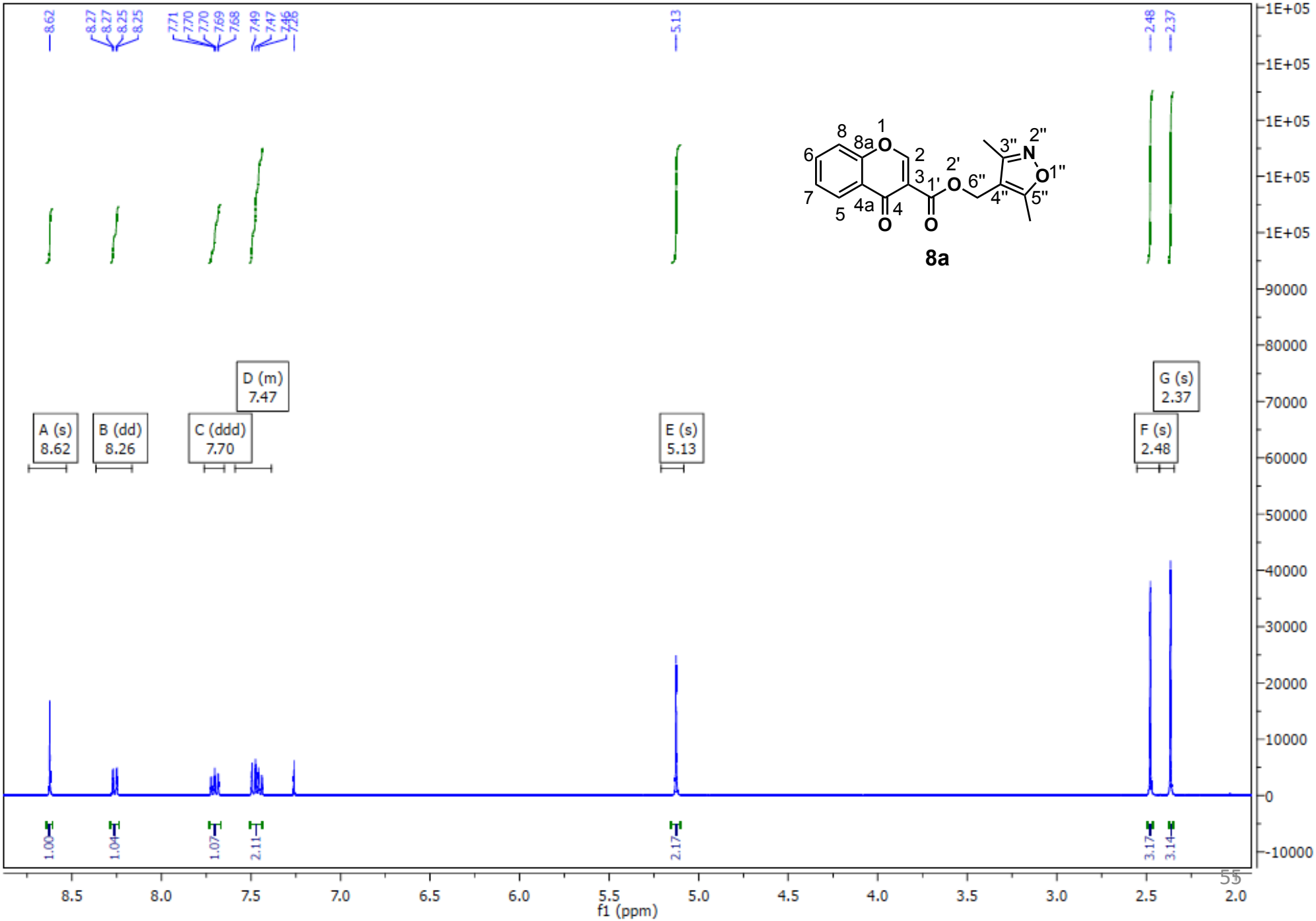


Figure S23.1. ^{1}H NMR spectra of compound 8a



8a

Figure S23.2. ^{13}C NMR spectra of compound 8a

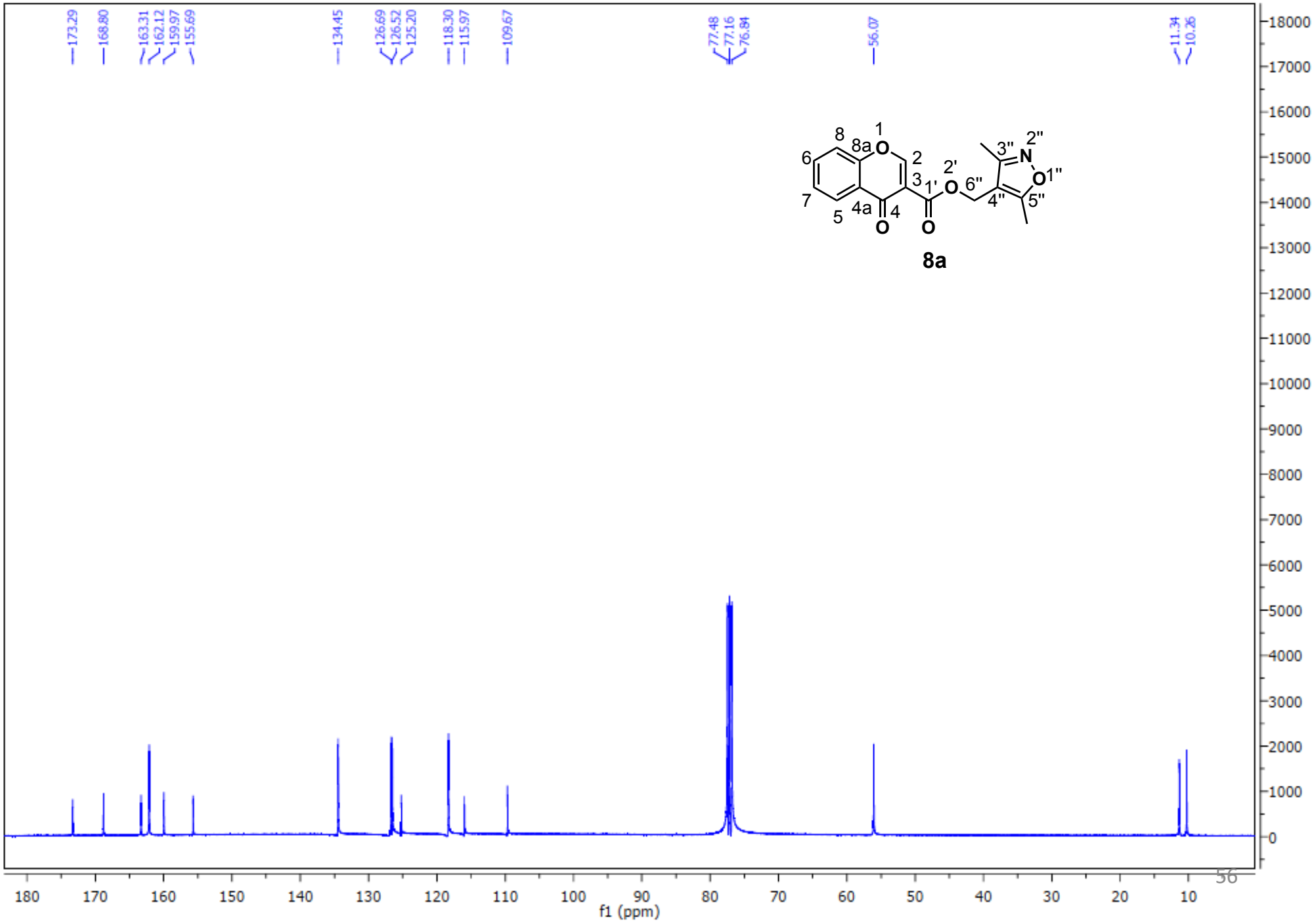


Figure S23.3. ^{13}C-DEPT NMR spectra of compound 8a

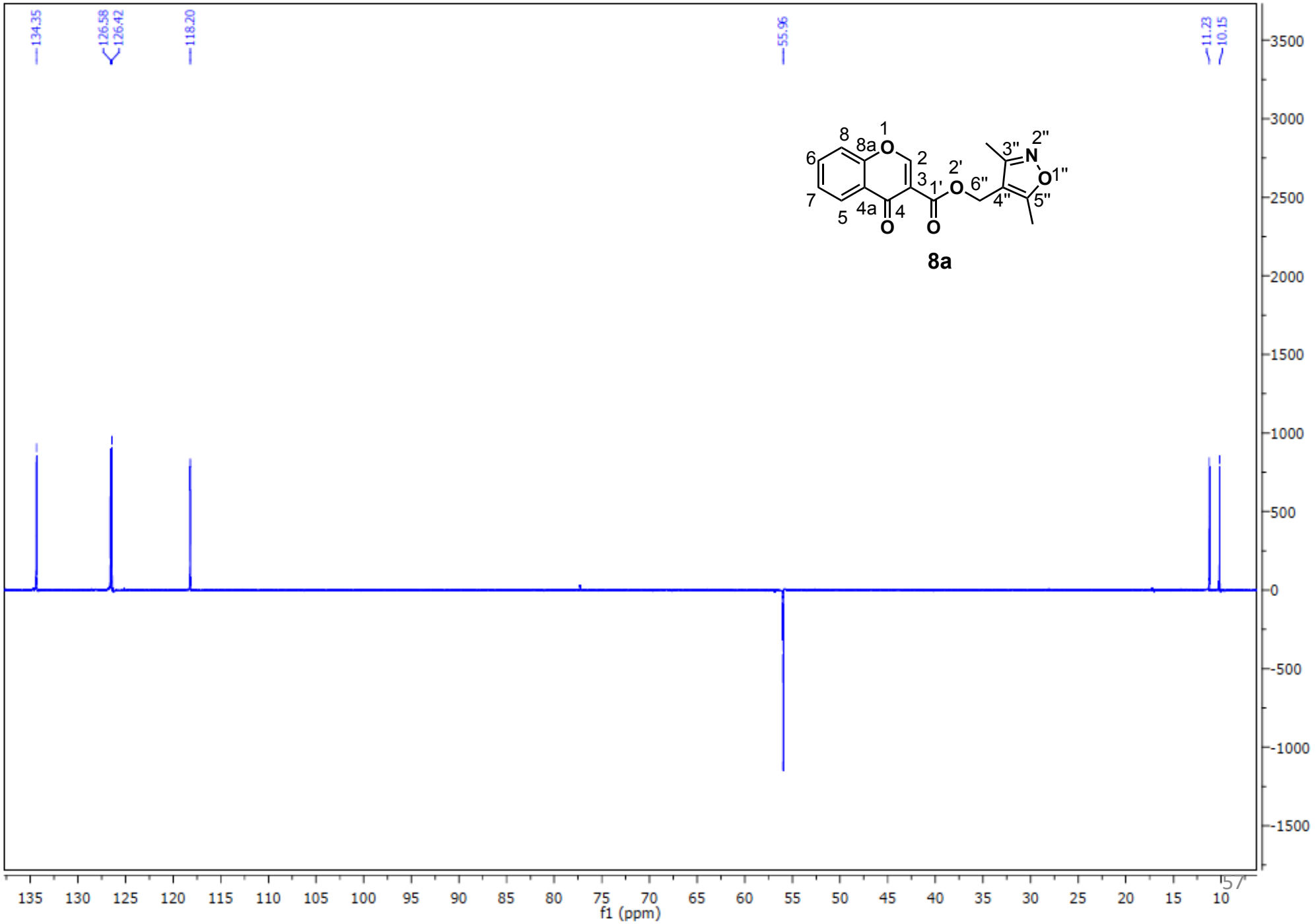
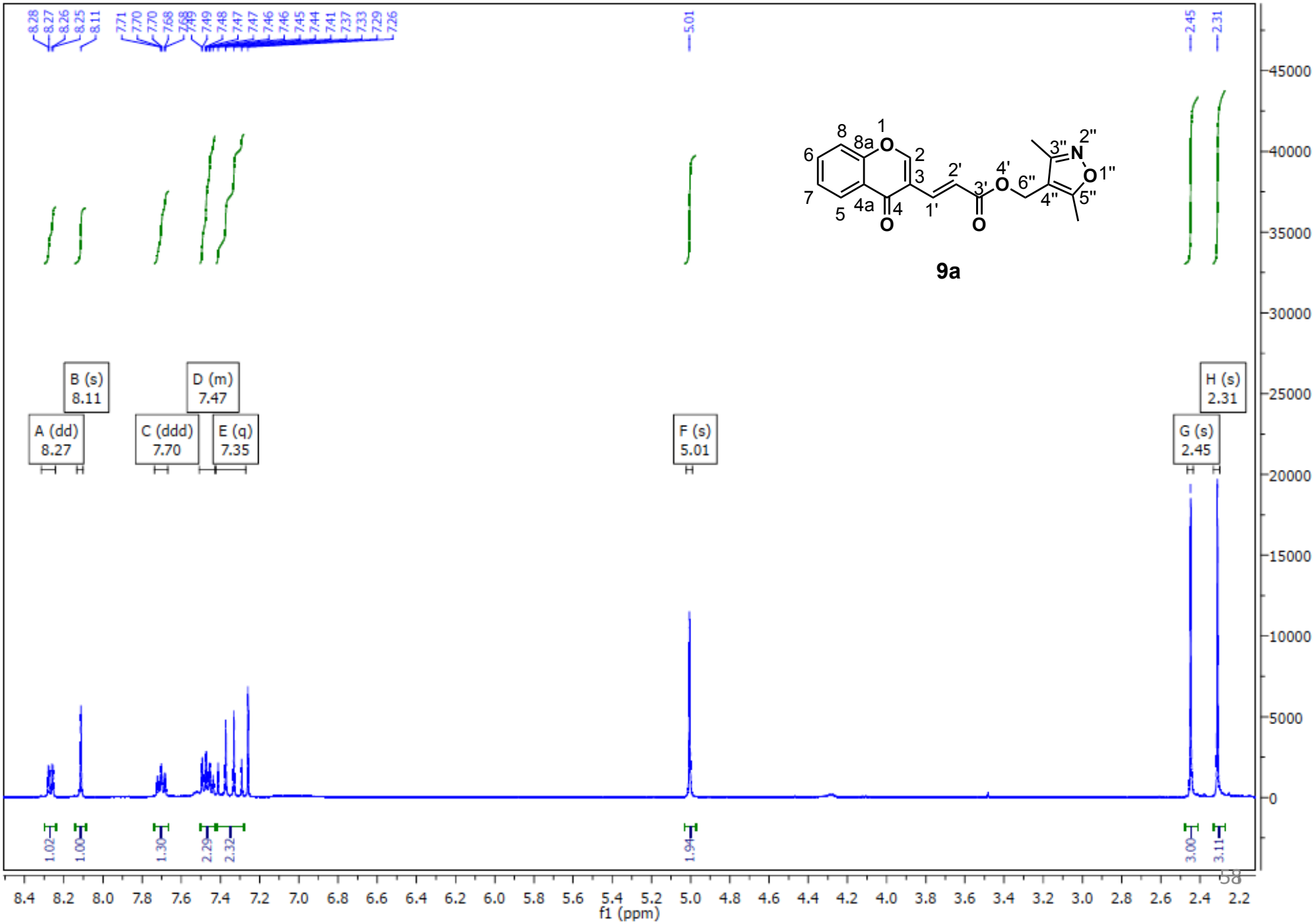


Figure S24.1. ^{1}H NMR spectra of compound 9a



9a

Figure S24.2. ^{13}C NMR spectra of compound 9a

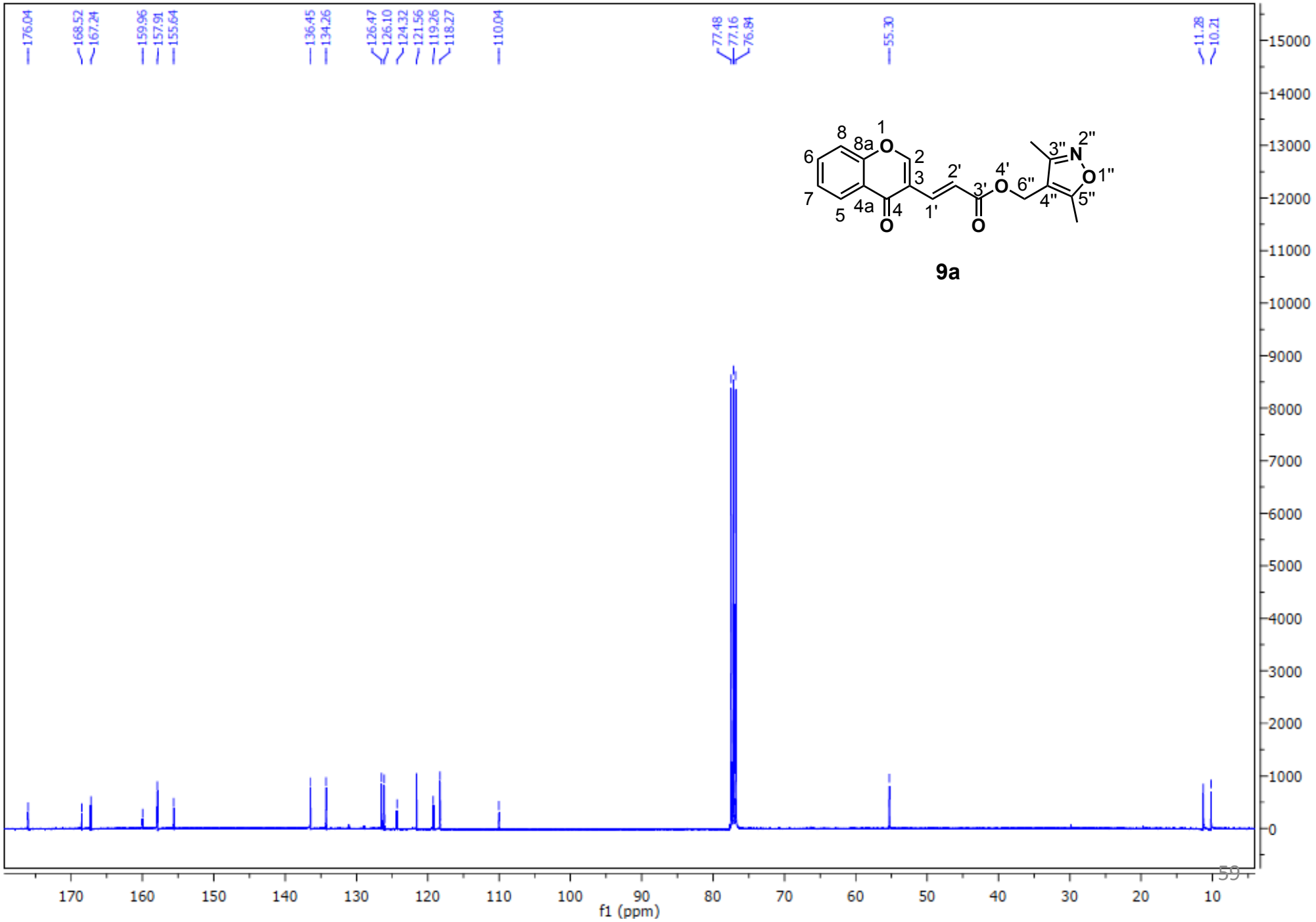


Figure S25.1. ^{1}H NMR spectra of compound 9c

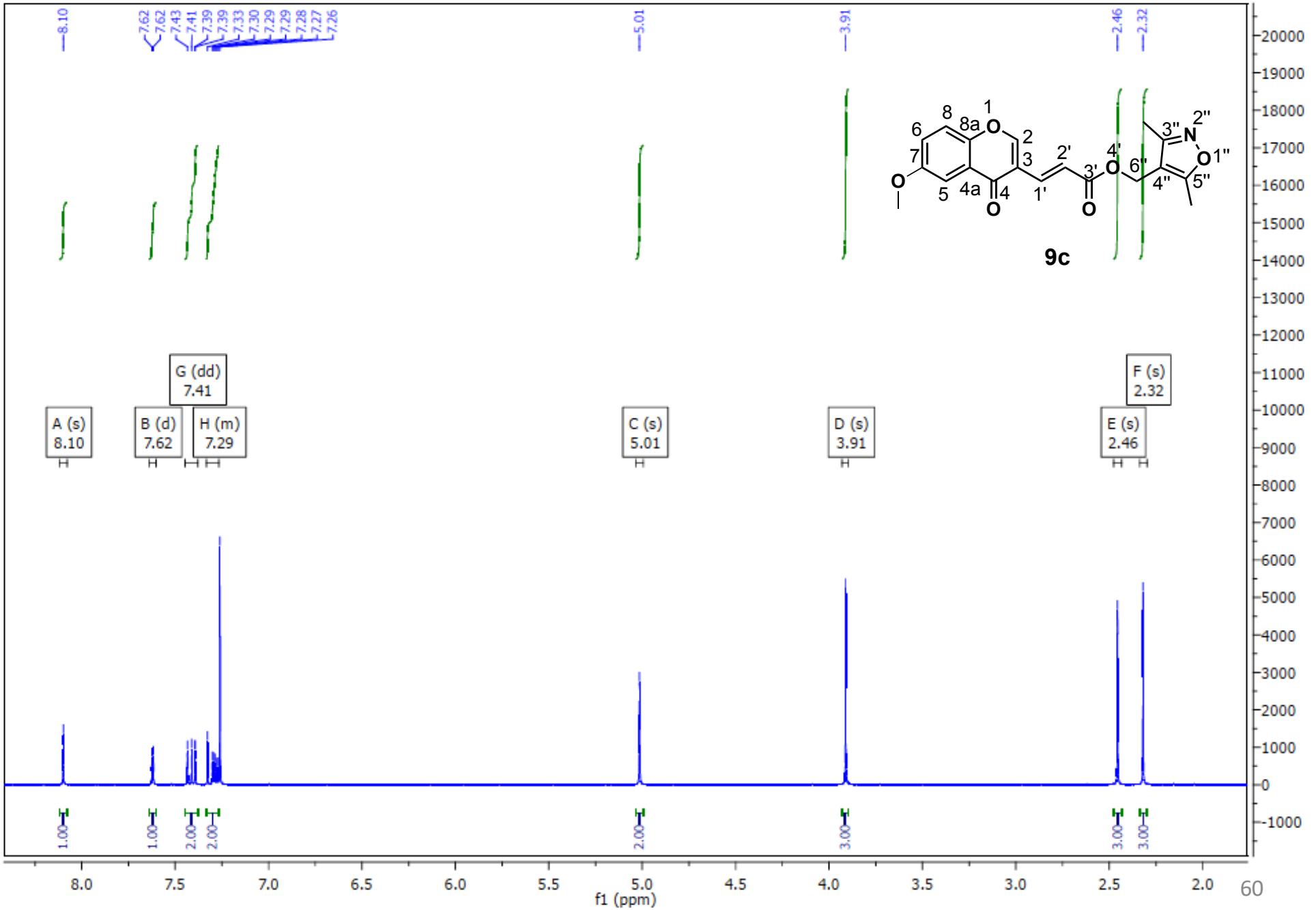


Figure S25.2. ^{13}C NMR spectra of compound 9c

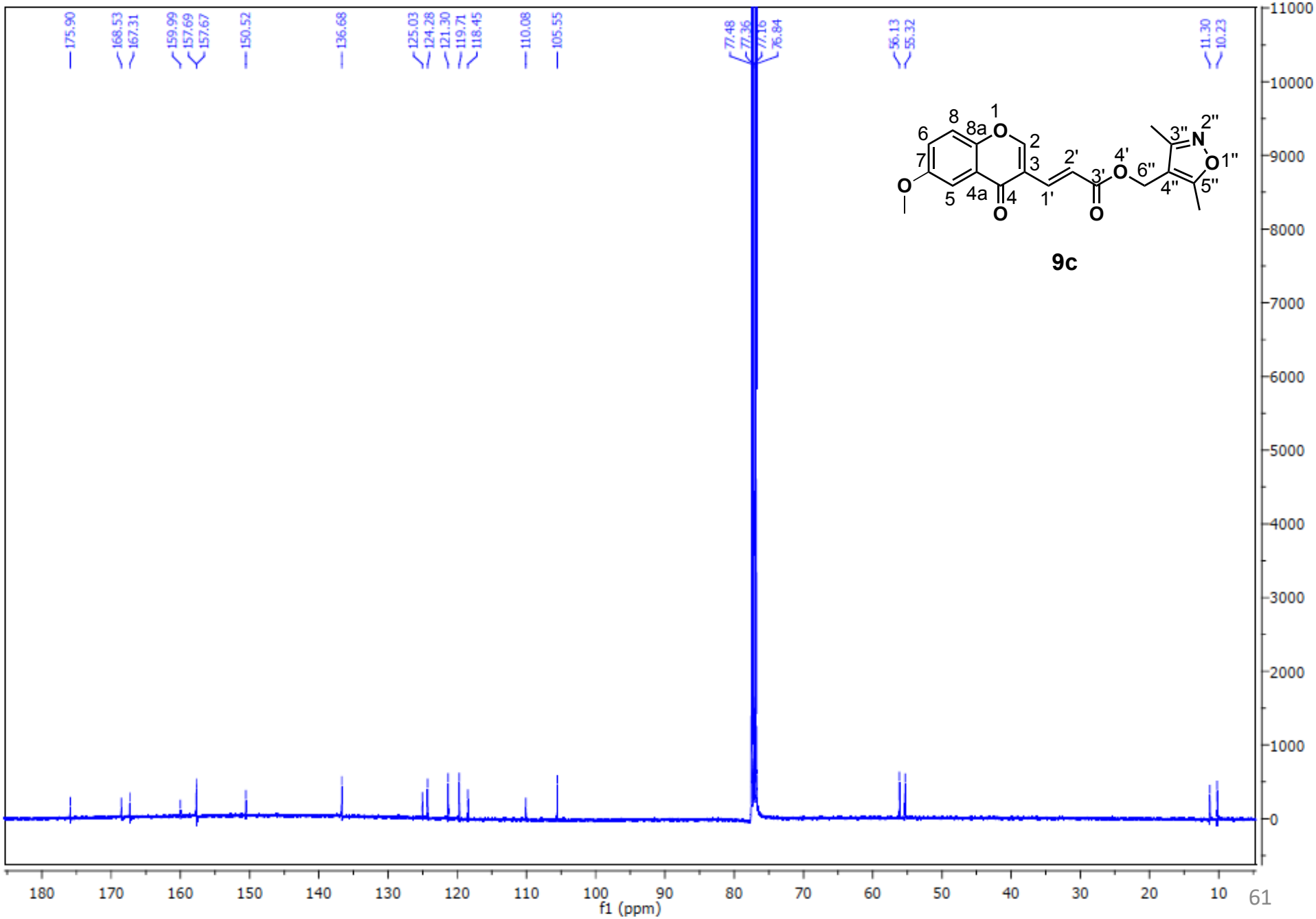


Figure S25.3. ^{13}C-DEPT NMR spectra of compound 9c

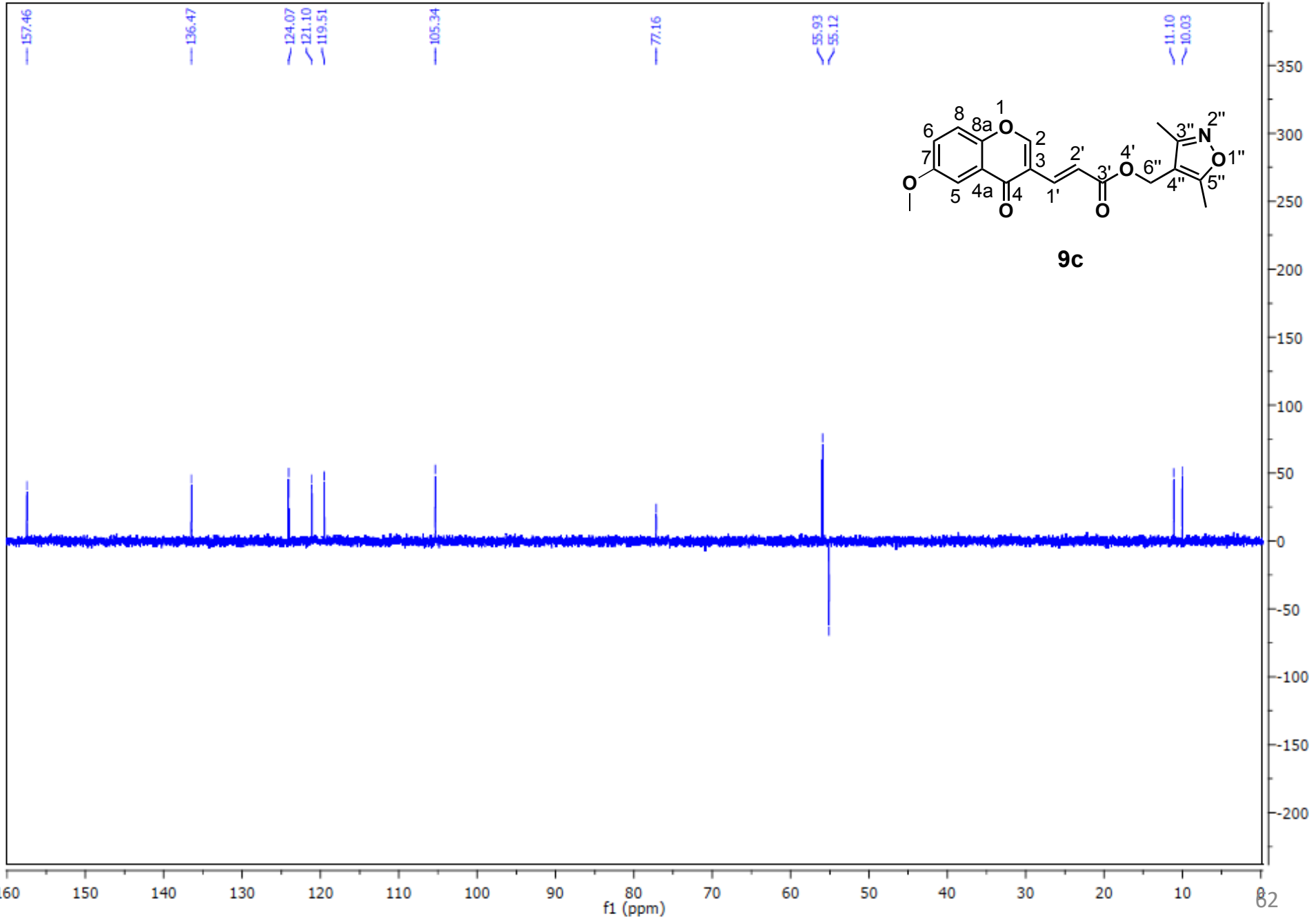


Figure S26.1. ^{1}H NMR spectra of compound 9d

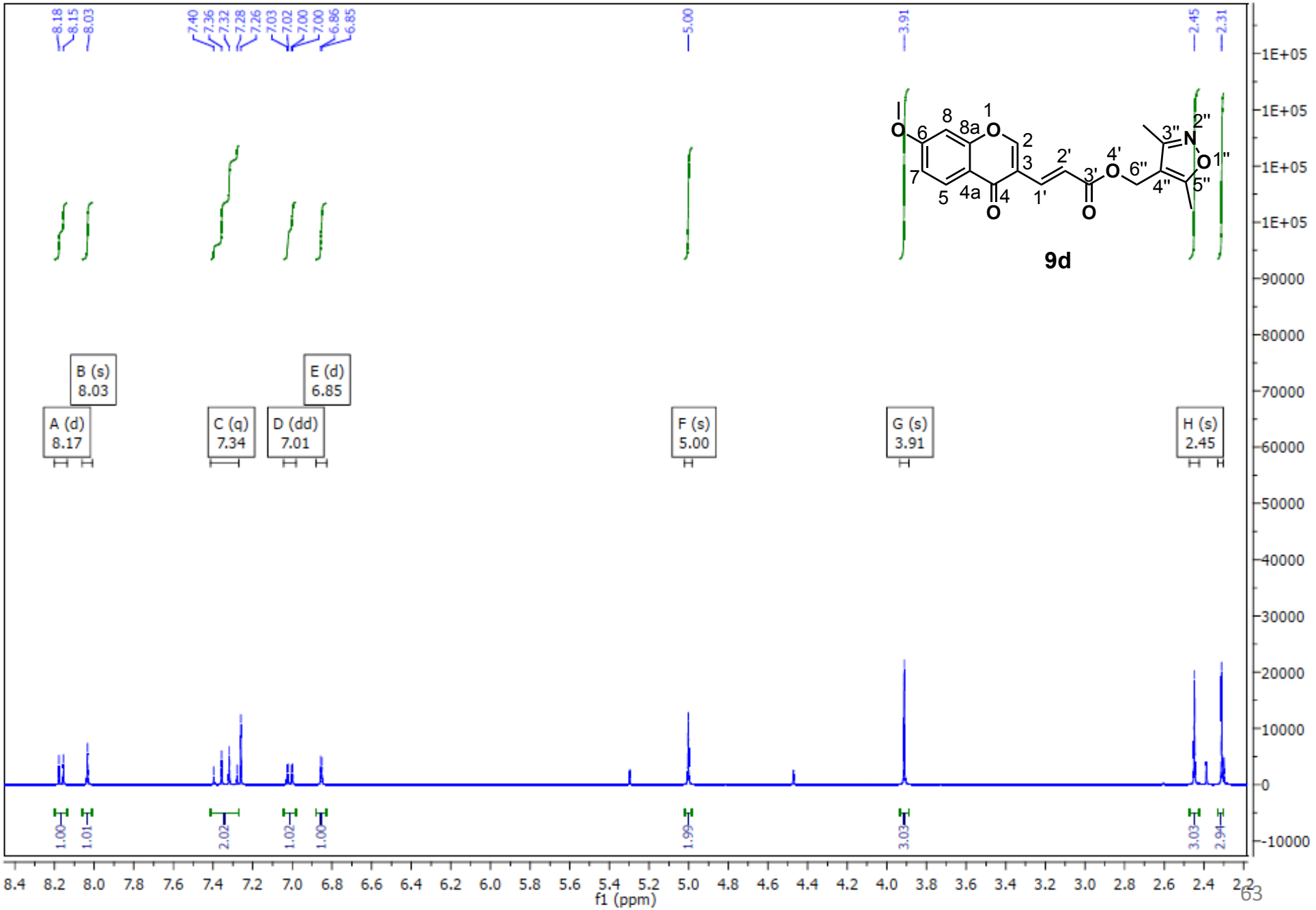


Figure S26.2. ^{13}C NMR spectra of compound 9d

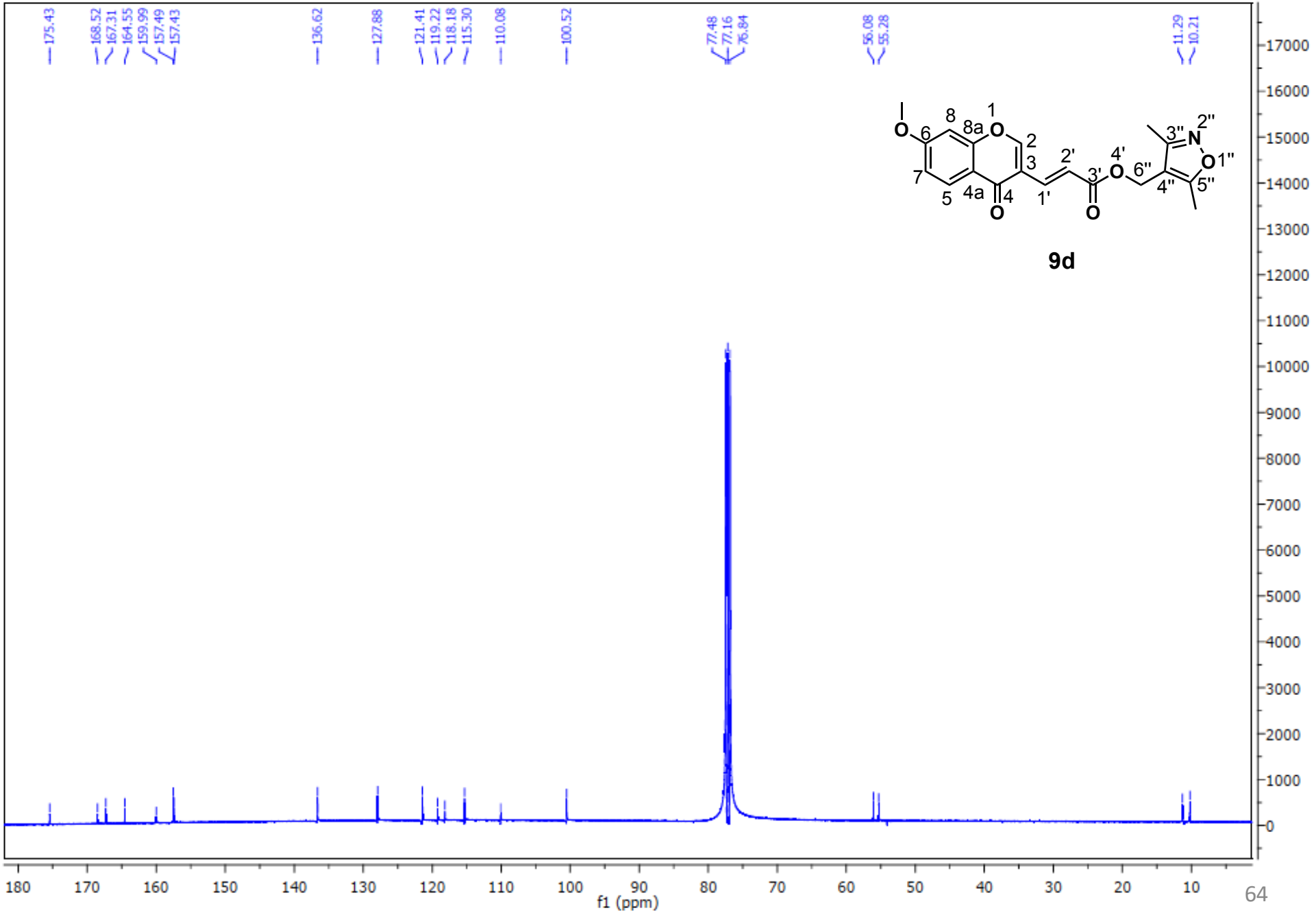


Figure S26.3. ^{13}C-DEPT NMR spectra of compound 9d

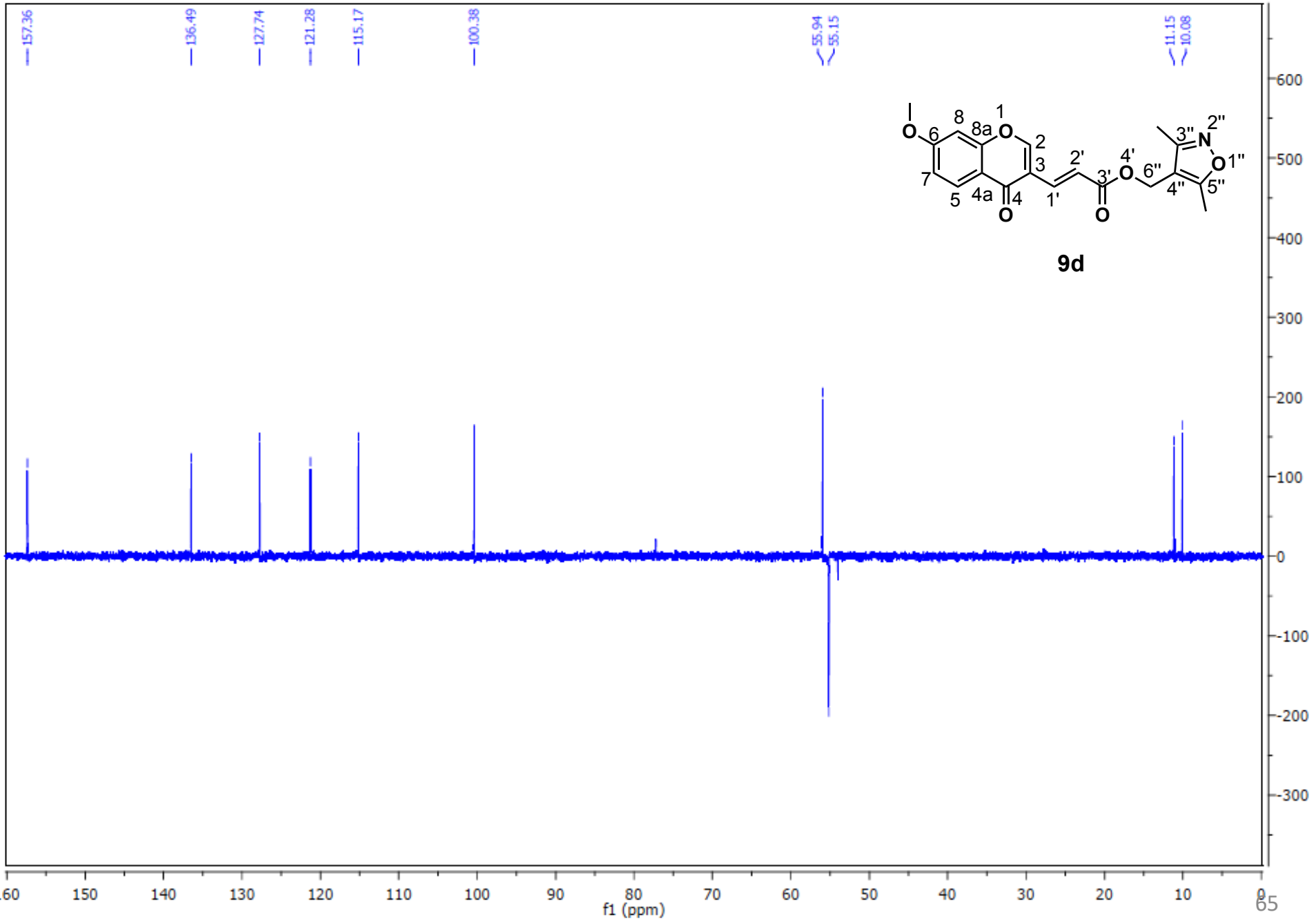


Figure S27.1. Analytical HPLC method 1

Column: C18
Solvent Flow Rate: 1 mL/min
Solvent A: Water + 0.1% TFA
Solvent B: Acetonitrile + 0.1% TFA
Detection wavelength: 254nm
Temperature of the column oven: 30°C
Gradient Method A1:

S. No.	Time	Solvent B (%)
1	0.01	10
2	10.00	10
3	40.00	60
4	42.00	100
5	50.00	100
6	55.00	10
7	60.00	10
8	65.00	STOP

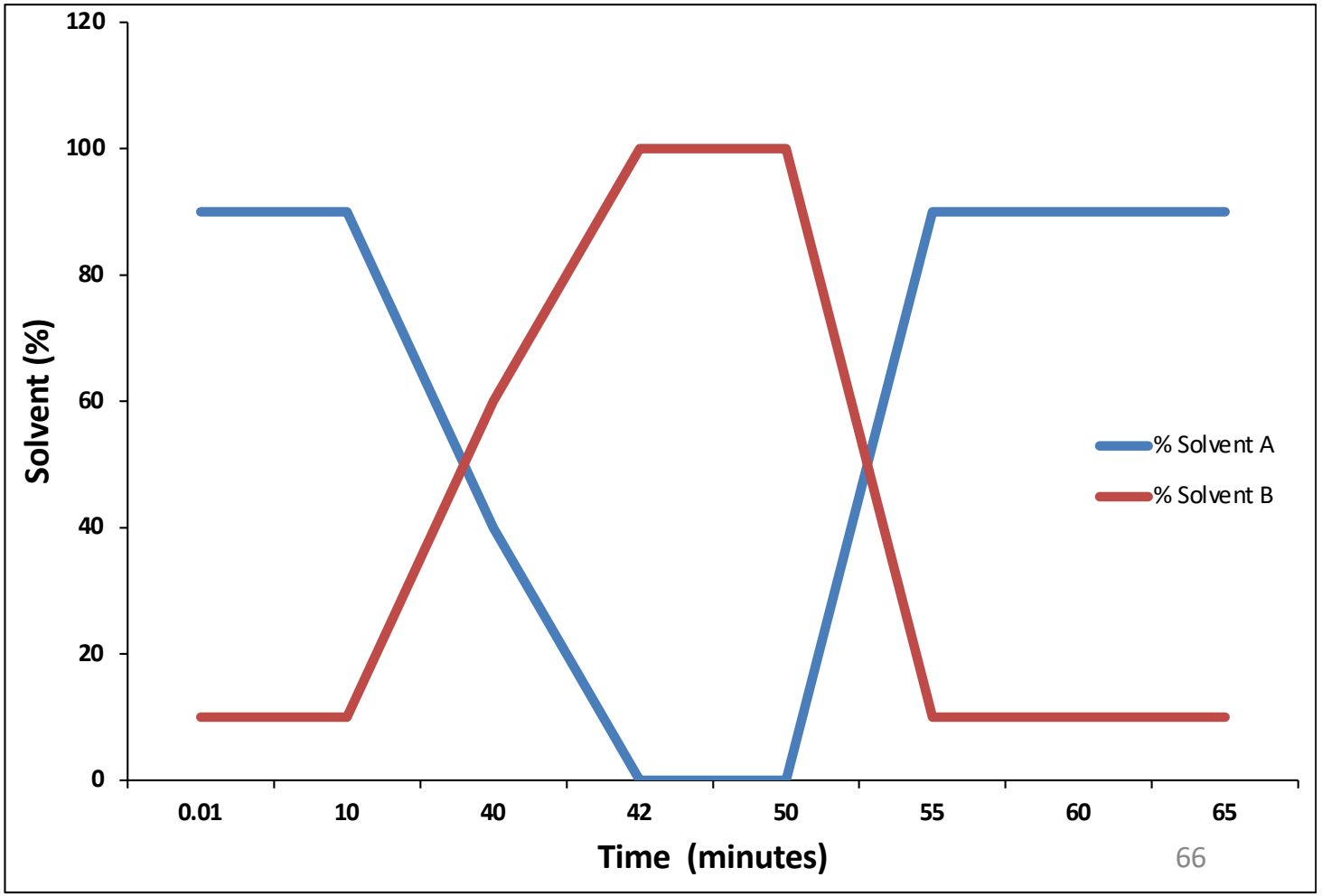


Figure S27.2. Analytical HPLC method 2

Column: C18
Solvent Flow Rate: 1 mL/min
Solvent A: Water + 0.1% TFA
Solvent B: Acetonitrile + 0.1% TFA
Detection wavelength: 254nm
Temperature of the column oven: 30°C
Gradient Method A1:

S. No.	Time	Solvent B (%)
1	0.01	10
2	10.00	10
3	40.00	60
4	42.00	100
5	50.00	100
6	55.00	10
7	60.00	10
8	65.00	STOP

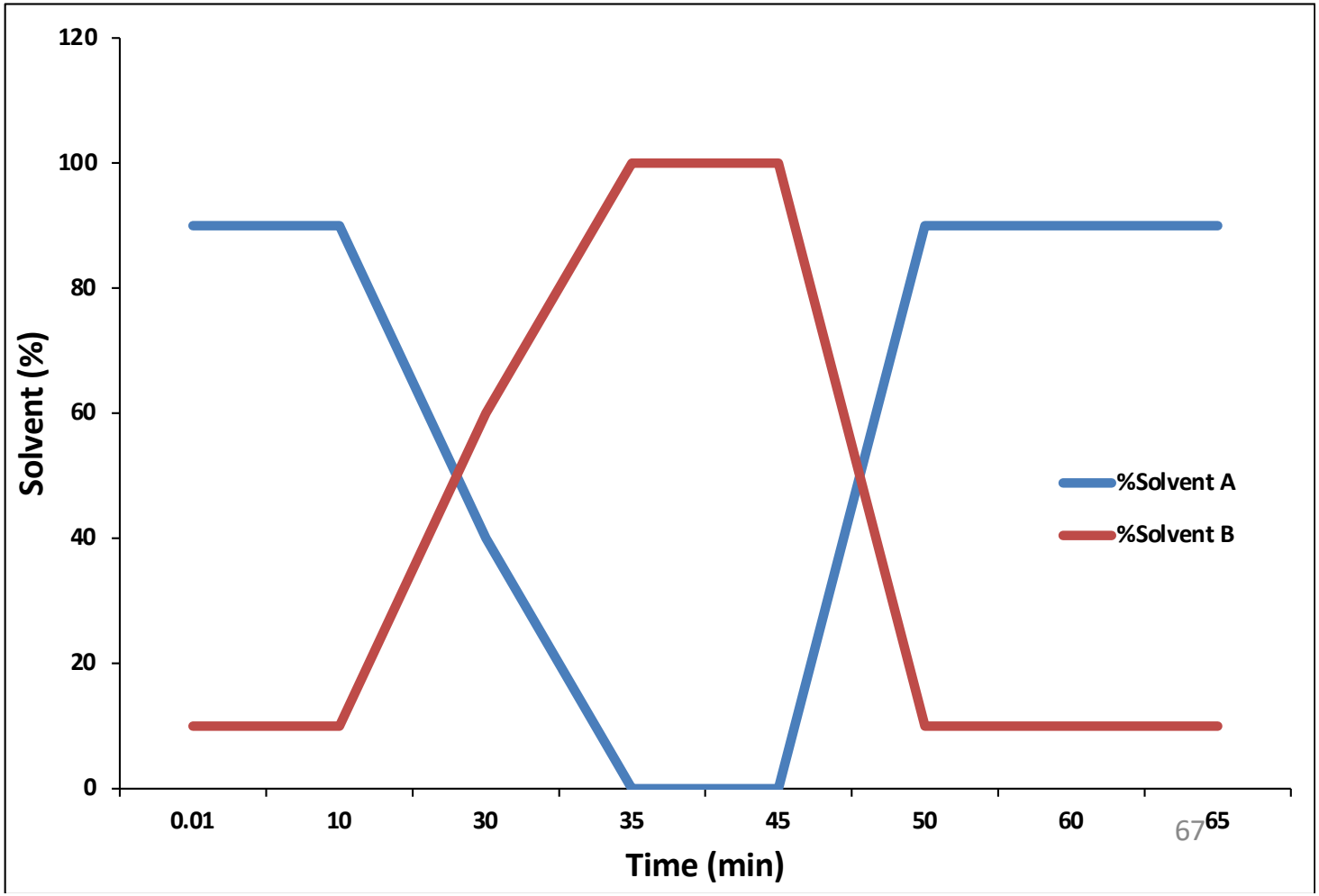
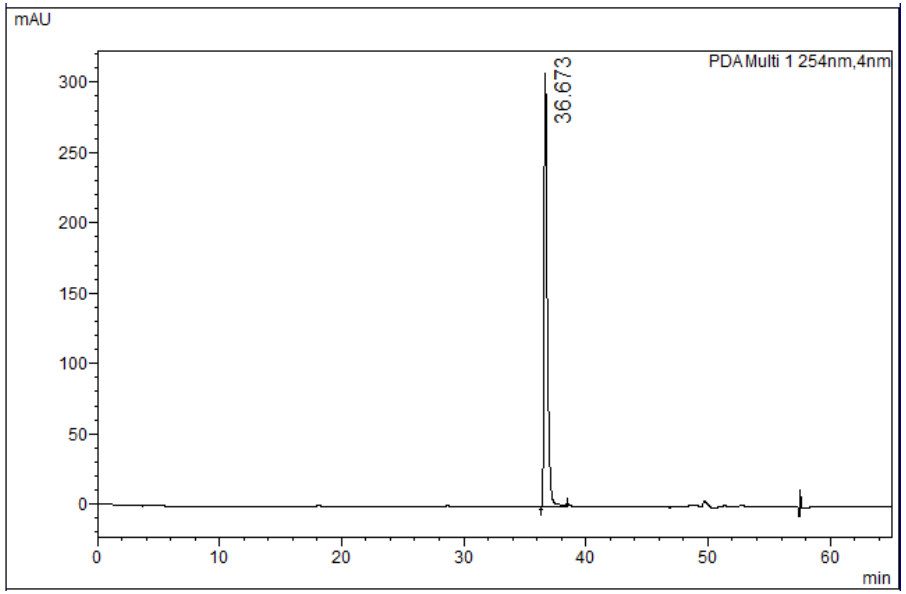
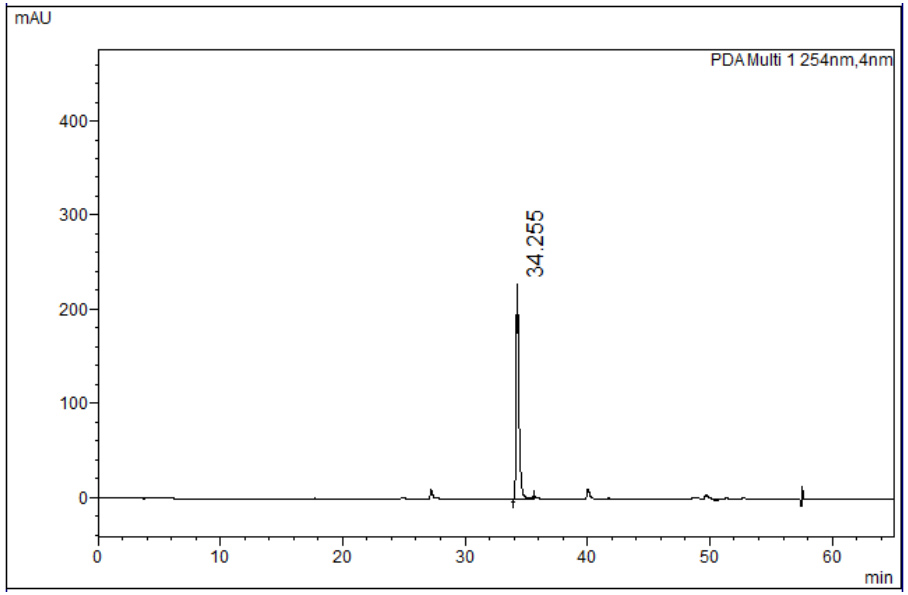


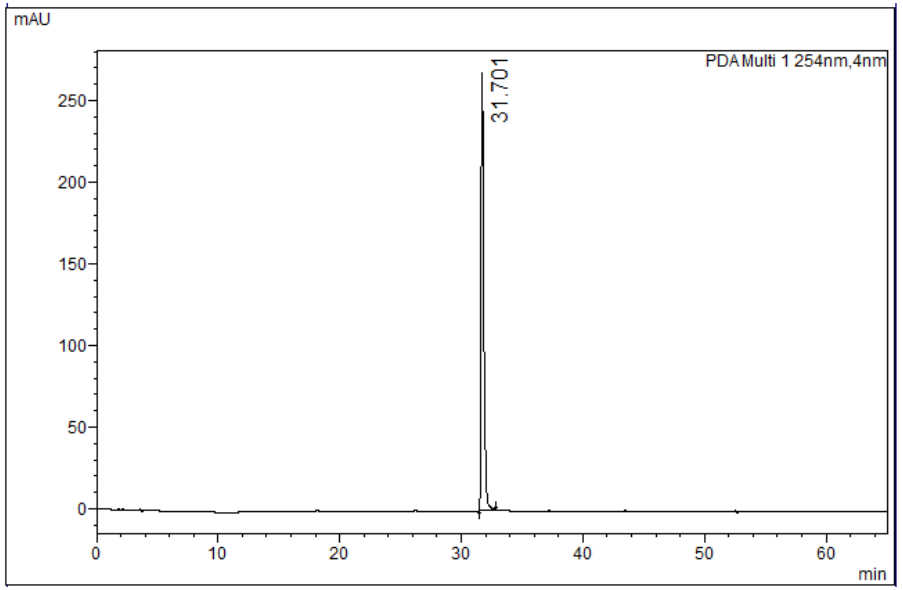
Figure S27.3. Analytical HPLC of representative compounds 5a and 5b



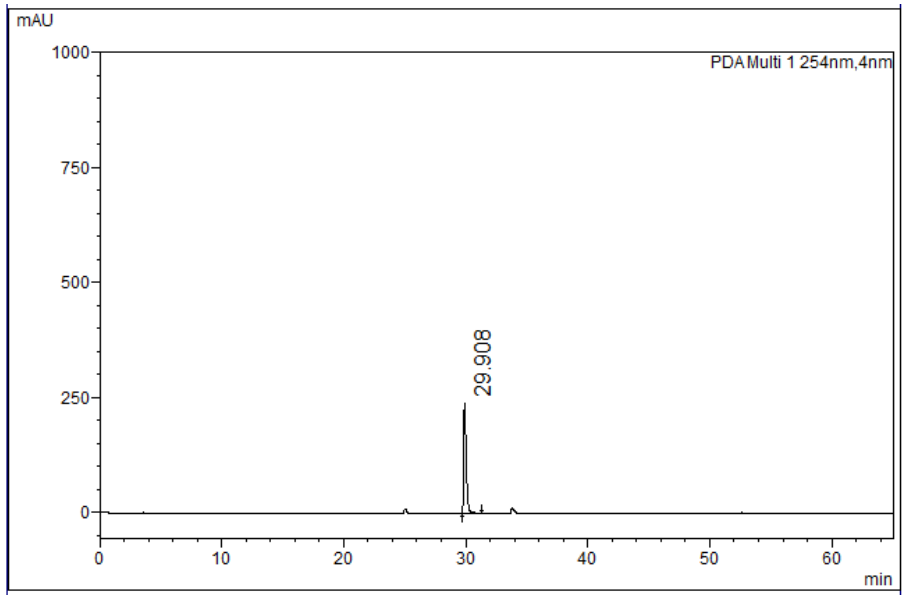
Analytical HPLC of compound 5a by Method 1



Analytical HPLC of compound 5b by Method 1

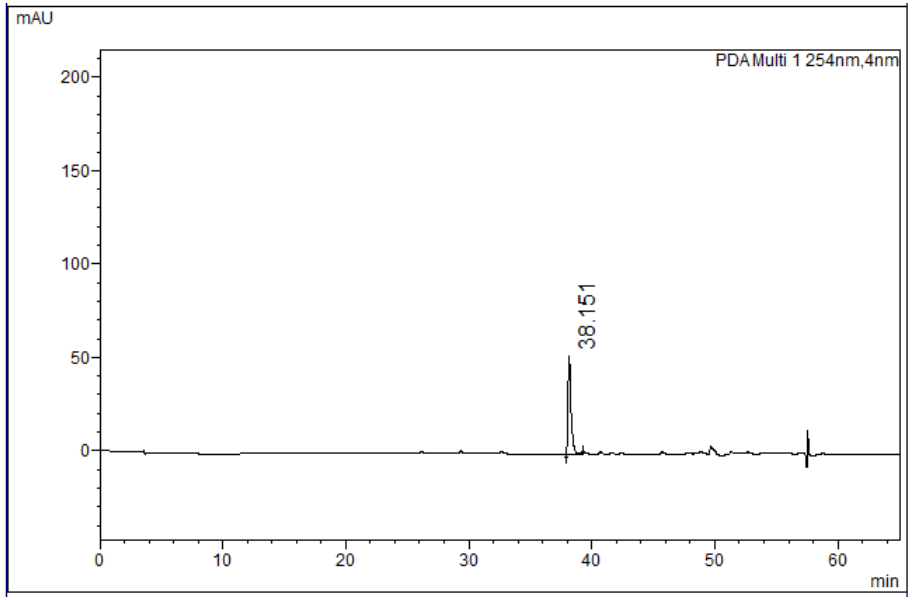


Analytical HPLC of compound 5a by Method 2

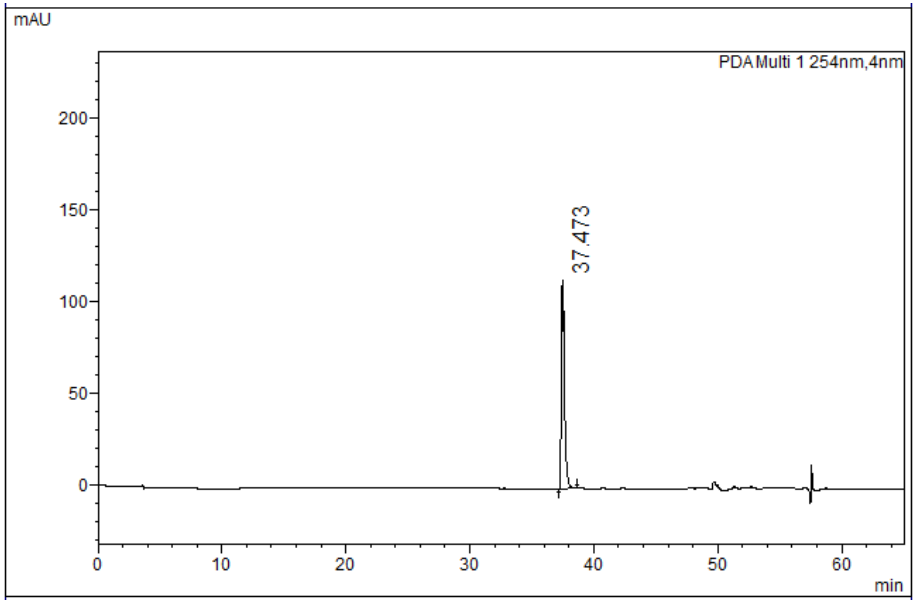


Analytical HPLC of compound 5b by Method 2

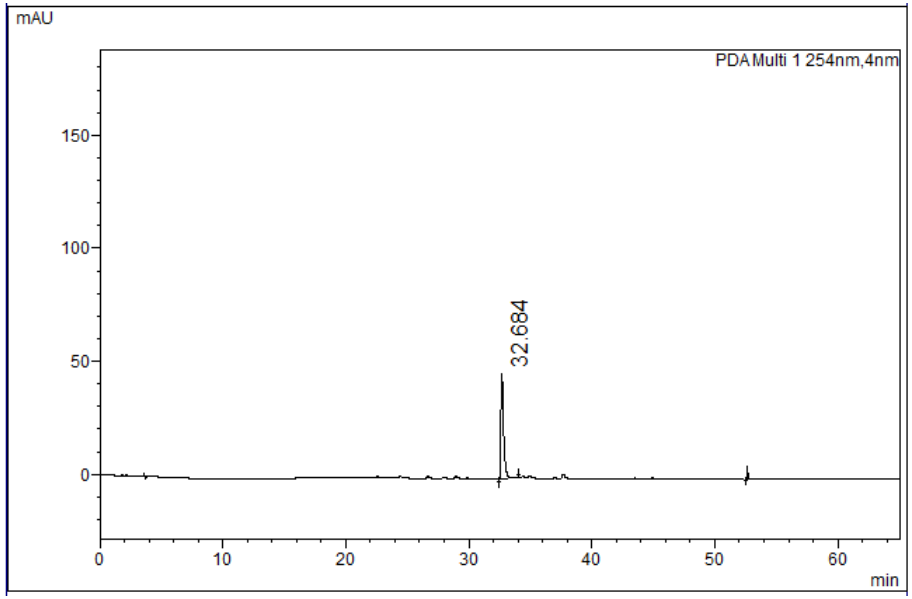
Figure S27.4. Analytical HPLC of representative compounds 5c and 5d



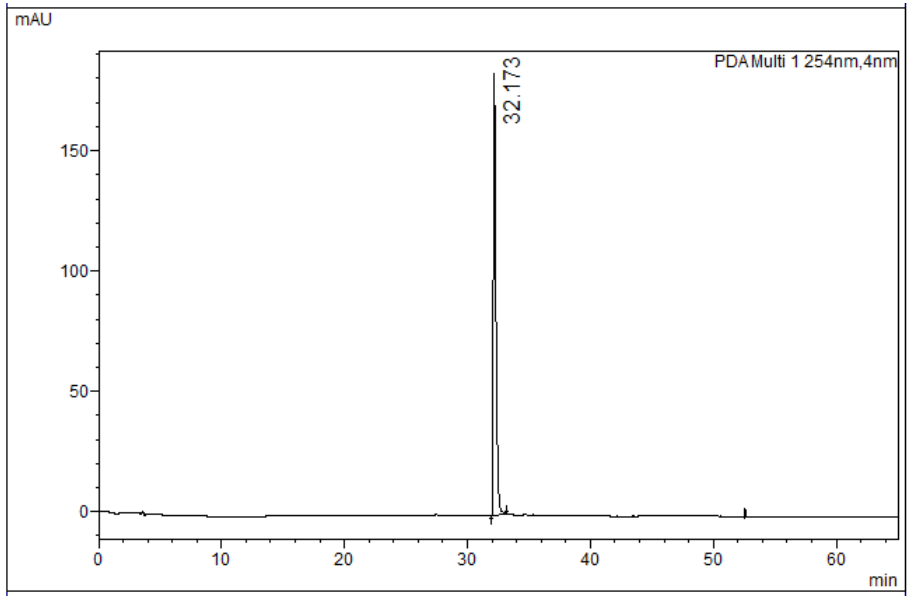
Analytical HPLC of compound 5c by Method 1



Analytical HPLC of compound 5d by Method 1



Analytical HPLC of compound 5c by Method 2



Analytical HPLC of compound 5d by Method 2

Table S1. HPLC retention time of conjugates **5a-5d** using two different methods.

	Retention time (min)	
	Method 1*	**Method 2***
5a	36.67	31.70
5b	34.25	29.90
5c	38.15	32.15
5d	37.47	32.17

* Method 1 & 2: Different gradient of solvent system used with Solvent A (water + 0.1% TFA) and Solvent B (acetonitrile + 0.1% TFA) with a flow rate of 1 mL/min (cf. Supporting Information for gradient system and HPLC chromatogram).

Figure S28.1. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards human leukemia carcinoma (CCRF-CEM) cell lines.

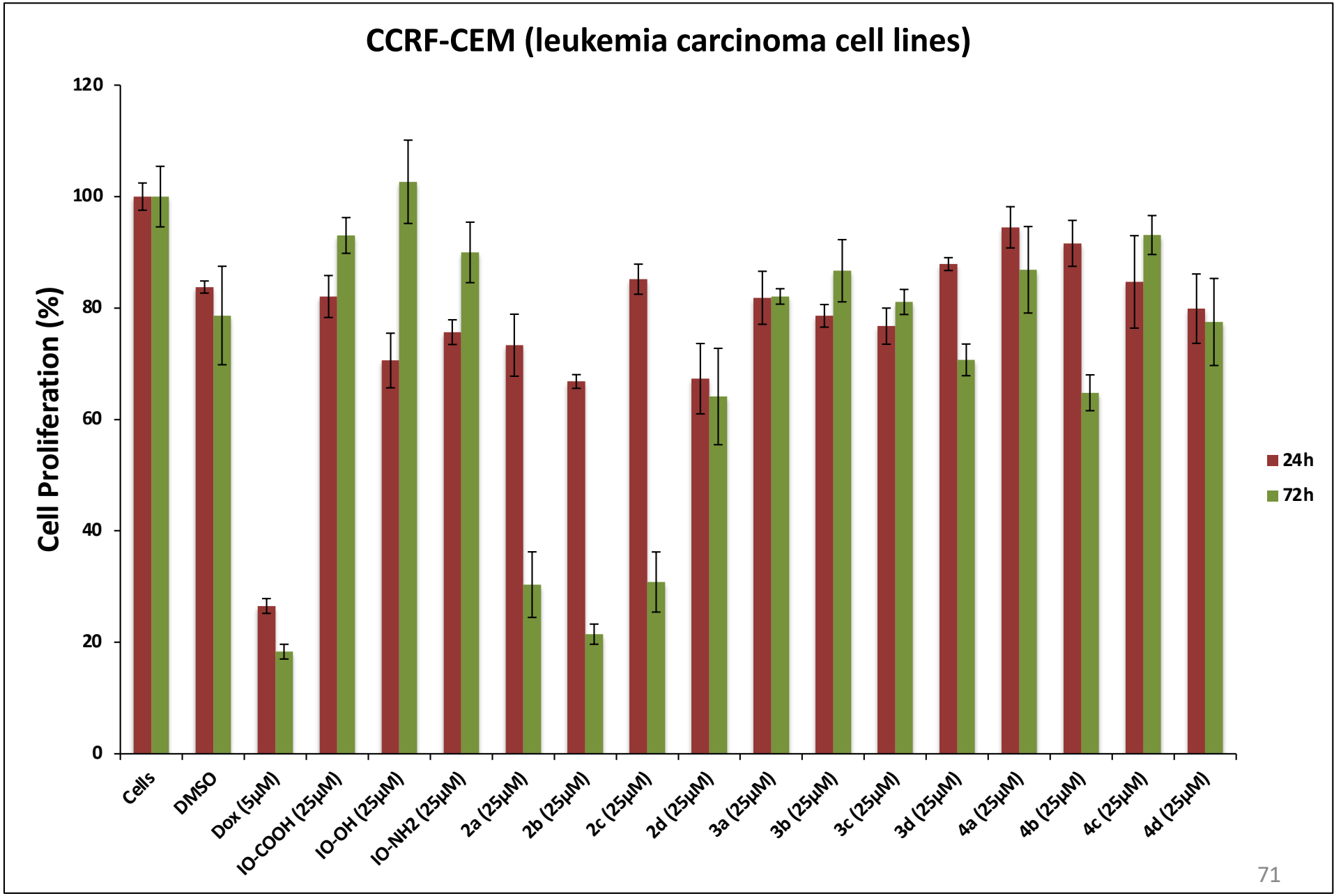


Figure S28.2. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards human ovarian adenocarcinoma (SKOV-3) cell lines.

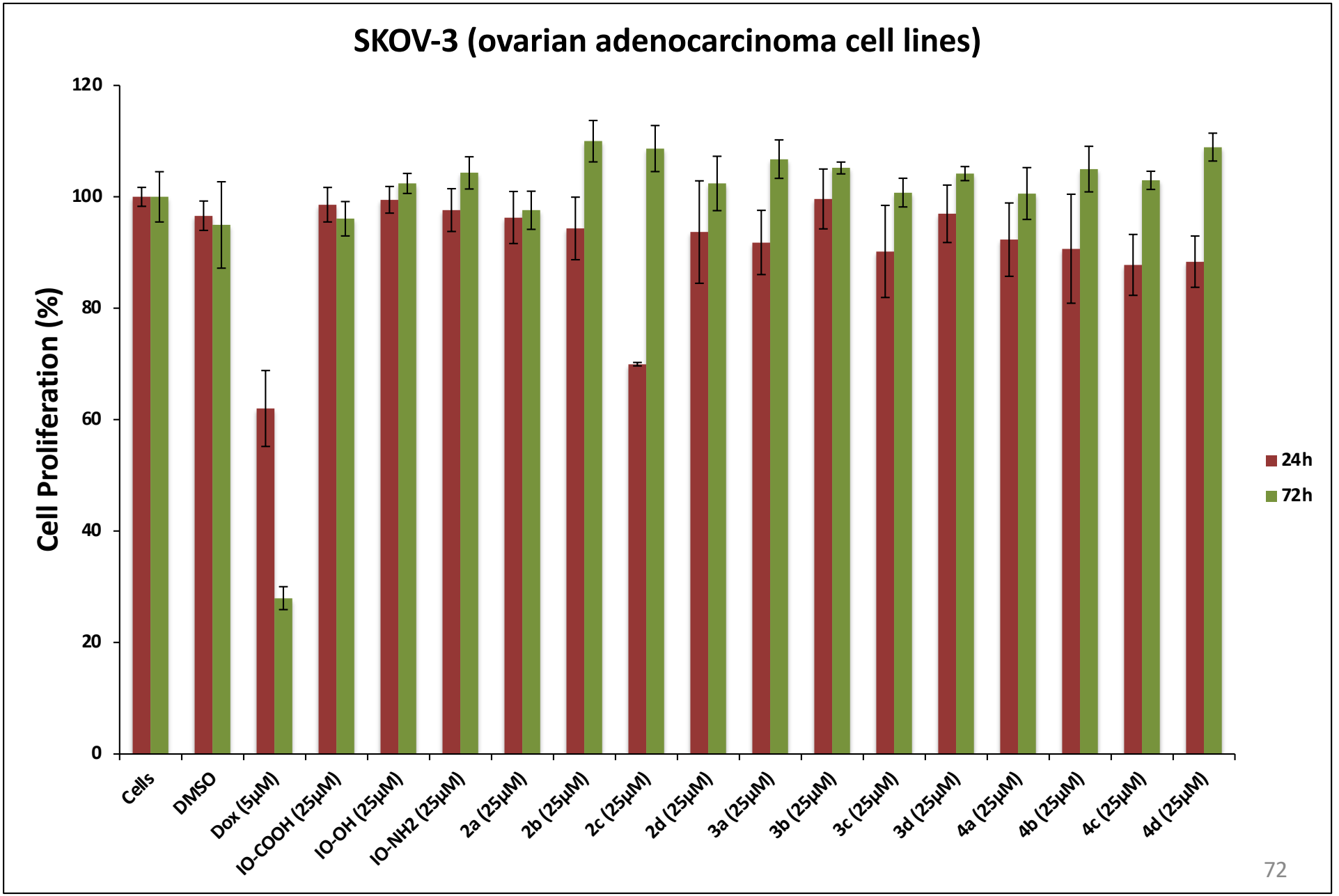


Figure S28.3. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards human breast tumor (MDA-MB-231) cell lines.

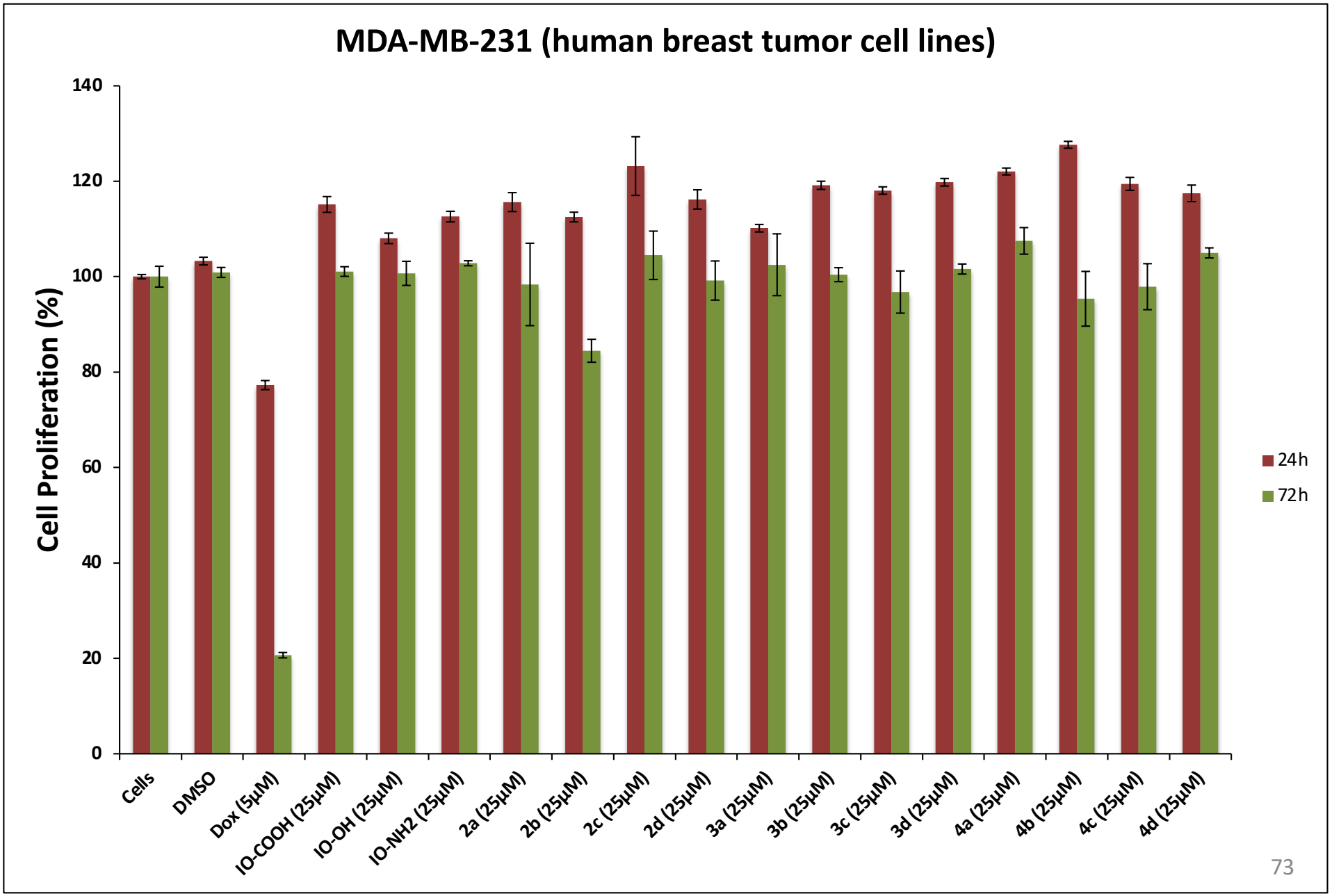


Figure S28.4. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards human prostate cancer (PC-3) cell lines.

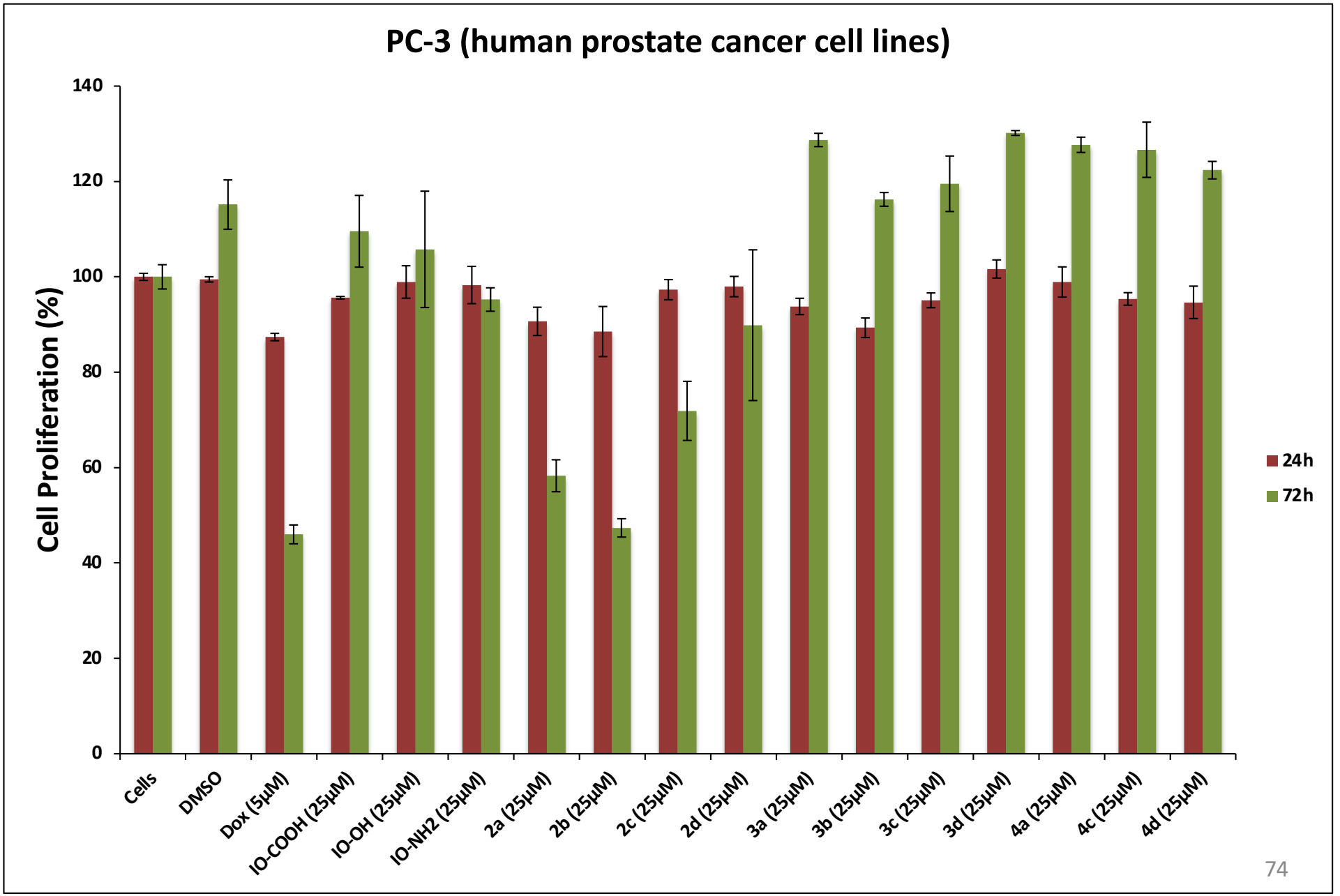


Figure S28.5. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards androgen-independent human prostate cancer (DU-145) cell lines.

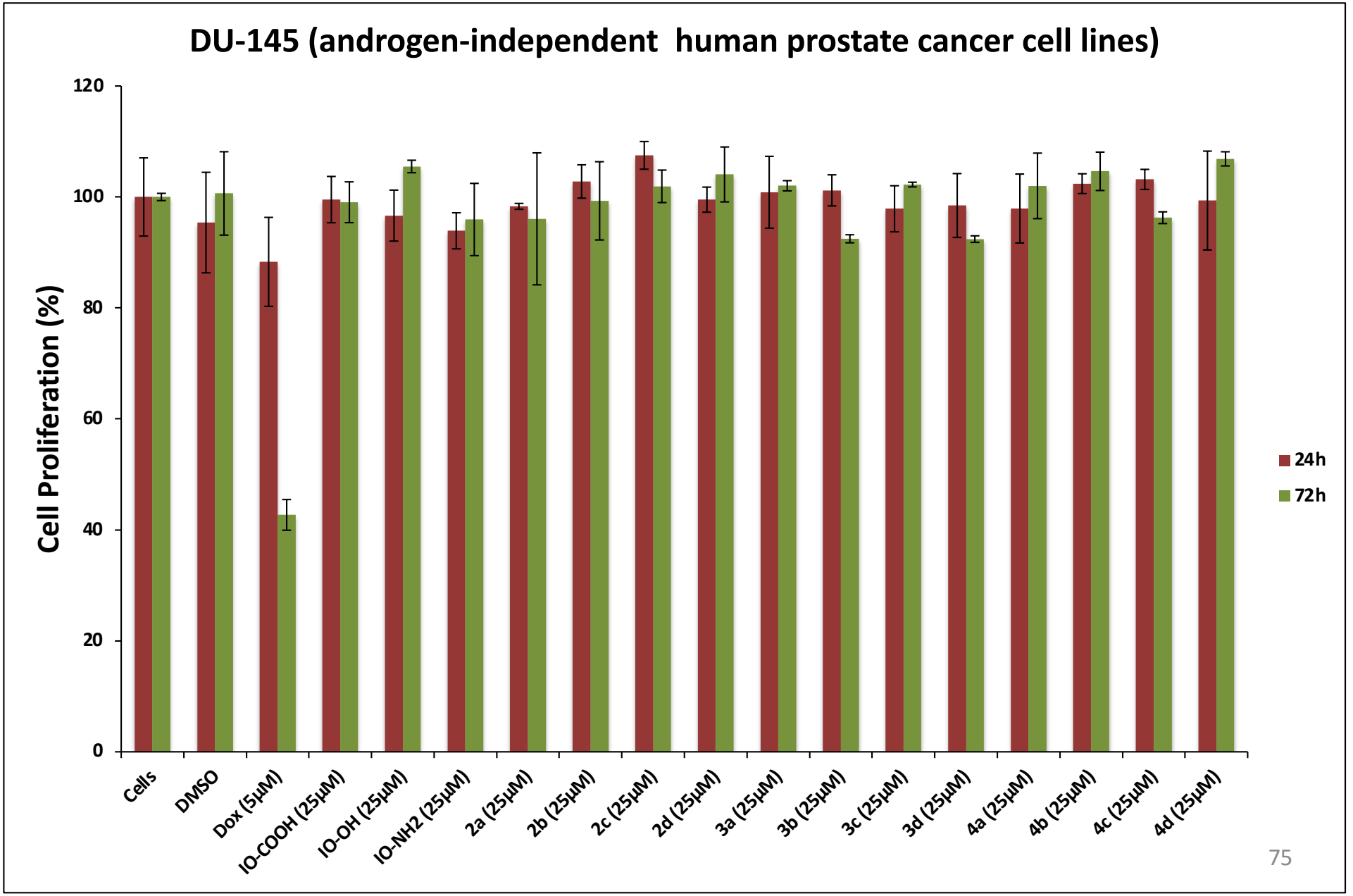


Figure S28.6. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards human renal carcinoma (iSLK) cell lines.

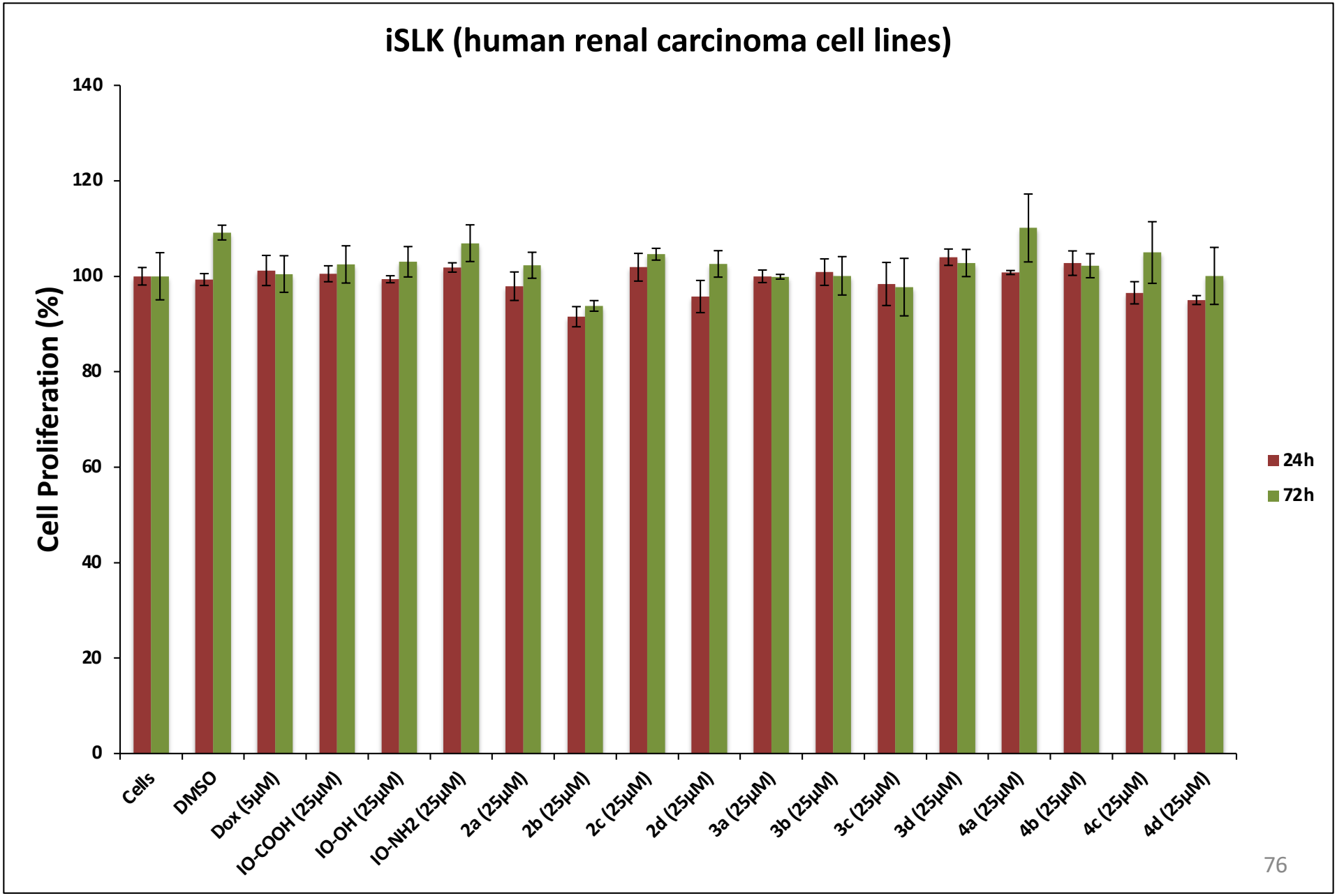


Figure S28.7. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards human embryonic kidney (HEK-293) cell lines.

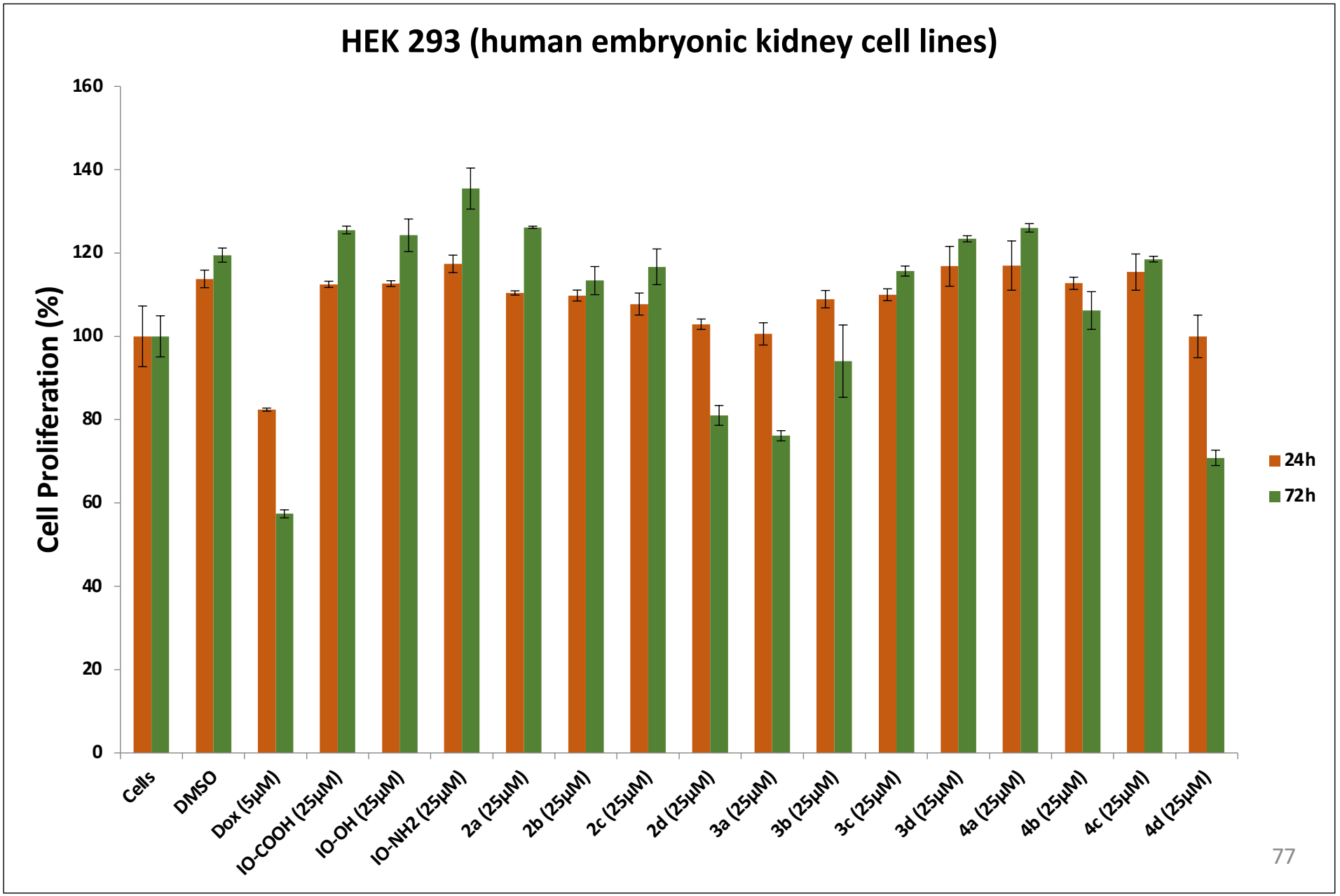


Figure S28.8. In-vitro cytotoxicity activity of benzopyran-4-one - isoxazole starting material and intermediates (at a concentration of 25 µM) towards normal mammalian kidney (LLCPK) cell lines.

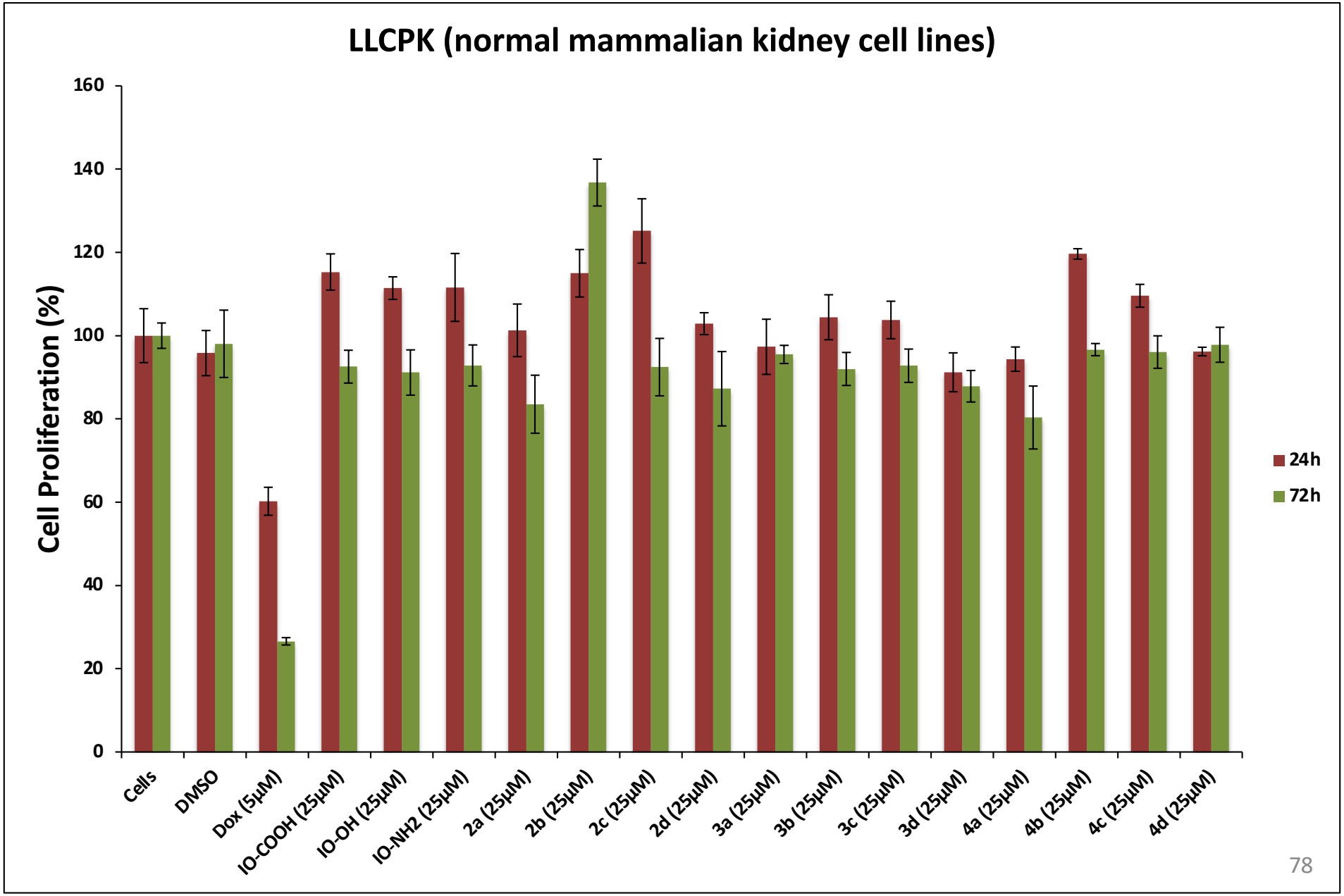


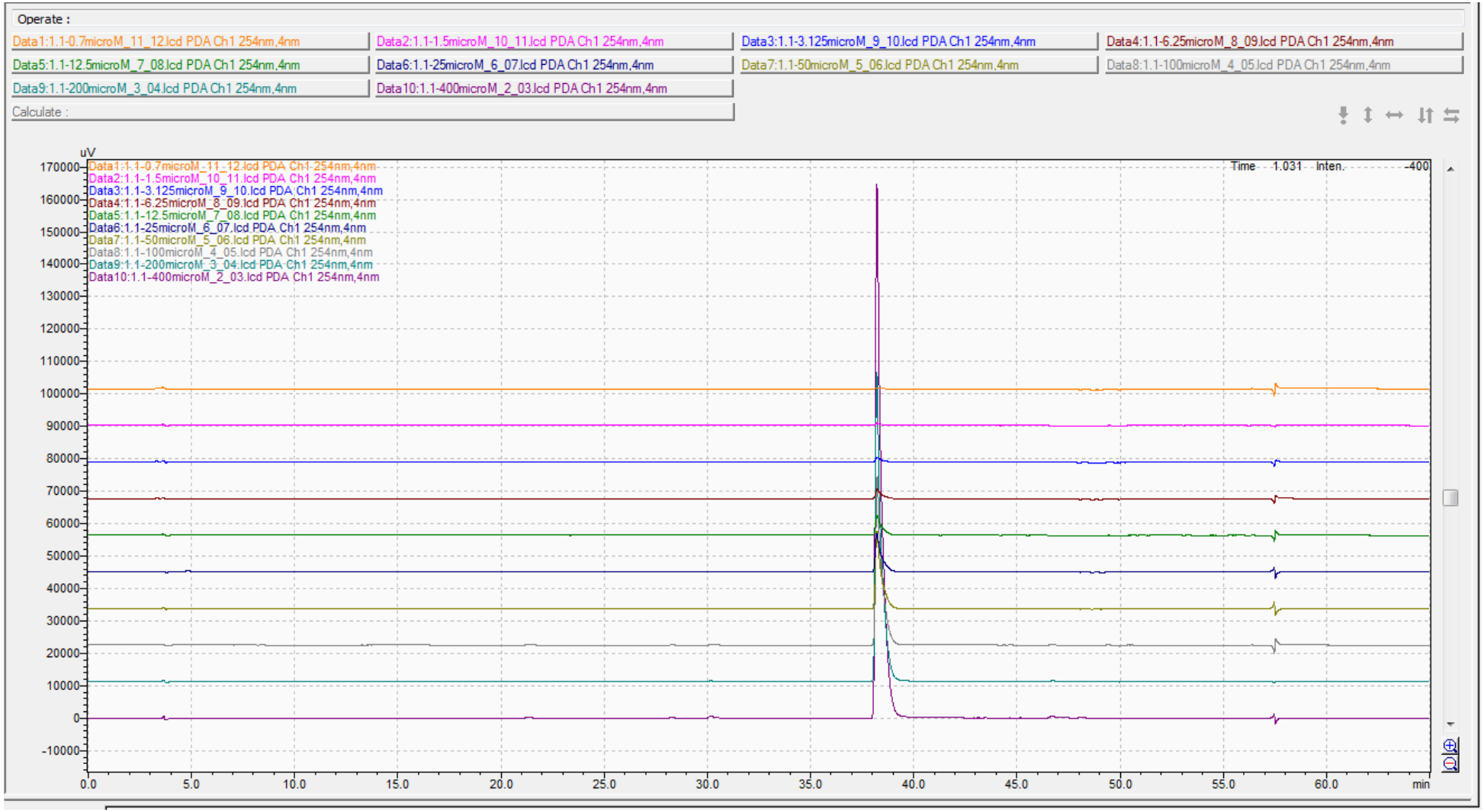
Table S2. Percentage antiproliferative activity on treatment with Dox (at a concentration of 5 µM) and conjugates **5a-5d** (at a concentration of 25 µM) 72 h of incubation.

	Inhibition of Cancer Cell Lines (%, 72 h)					
Compounds No.	**CCRF-CEM**	**SKOV-3**	**MDA-MB-231**	**PC-3**	**DU-145**	**iSLK**
Dox	82	62	73	54	57	NIL
5a	81	69	78	70	73	44
5b	83	42	60	75	50	23
5c	83	45	70	74	81	31
5d	82	48	76	75	79	42

Table S3. Percentage anti-proliferative activity of non-cancerous cell lines on treatment with doxorubicin (at a concentration of 5 µM) and conjugates 5a-5d (at a concentration of 25 µM).

	Inhibition of Non-Cancerous Cell Lines (%, 72 h)	
Compounds No.	**HEK 293**	**LLCPK**
Dox	43	74
5a	37	57
5b	34	NIL
5c	38	NIL
5d	40	18

Figure S29.1 HPLC data comparison for Standard Curve of 5a



Conc. 5a (µM)	400	200	100	50	25	12.5	6.25	3.12	1.56	0.78
AUC (at 254nm)	3239256	1892892	1034689	482517	236578	122631	59281	28929	14181	4769

Figure S29.2 Plot of area under the curve vs. conc. of 5a

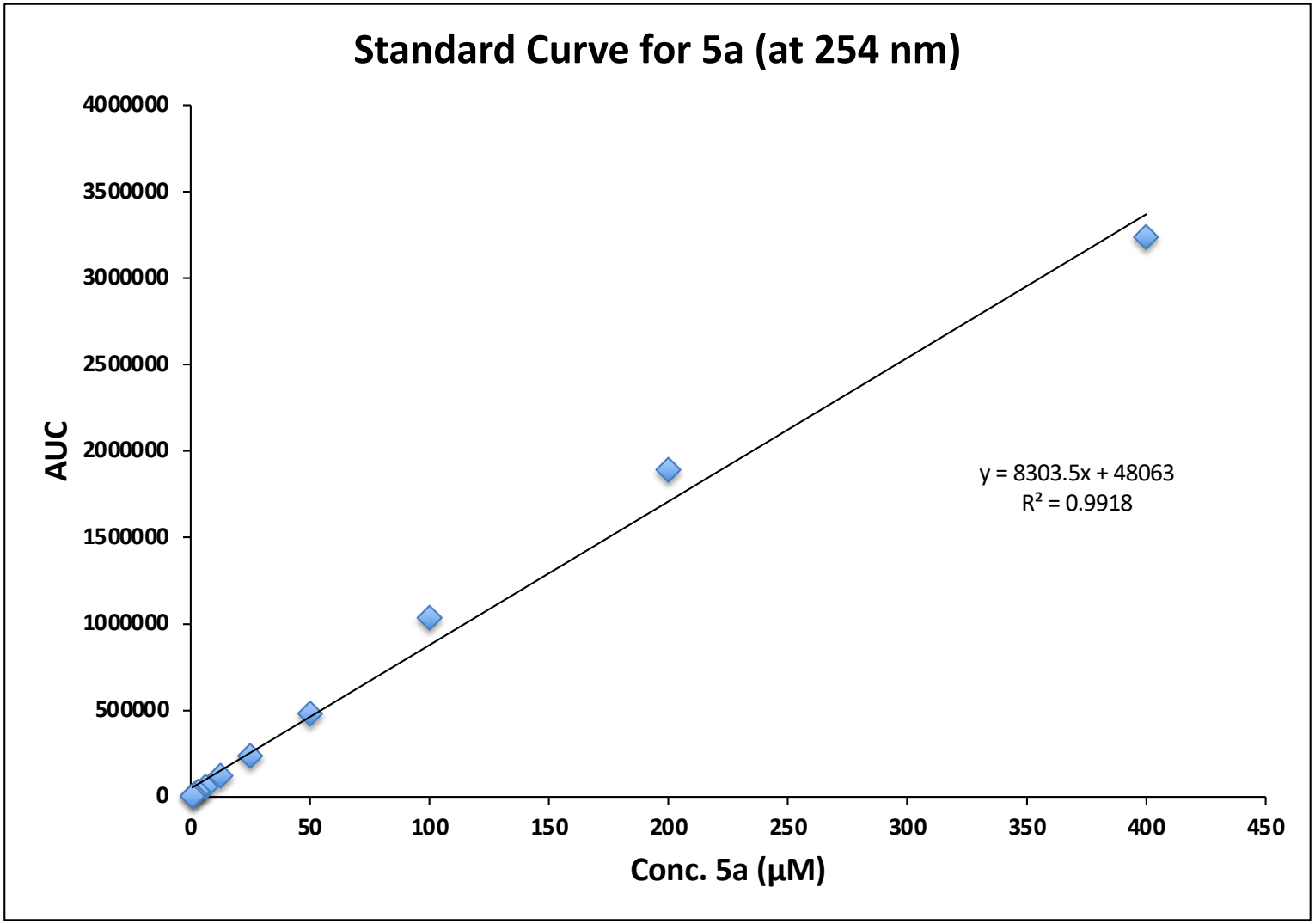


Table S4. Decrease in conc. of 5a on incubation with human serum at different time points (set of 3 independent experiments)

	Conc 5a (µM)					
Time (min)	**Expt 1**	**Expt 2**	**Expt 3**	**Average**	**Std dev**	**ln (average)**
0	76,84	81,37	84,65	100	3,921636563	4,605170186
2	64,28	54,99	50,4	69,86329575	7,071381289	4,246540415
5	52,92	51,81	58,93	67,38861899	3,830722299	4,210476146
10	40,01	43,71	42,37	51,91880098	1,873285883	3,949680978
20	34,49	24,27	34,58	38,4336655	5,926671354	3,648933781
40	18,56	11,93	8,4	16,01334103	5,158219977	2,773422189
80	9,63	0,8	8,3	7,712262212	4,760738738	2,042811557

Calculation of Half life of 5a in Human serum stability

y = -0.031x + 4.3339, where y = ln conc. of 5a and x = time (minutes)

X = (y-4.3339)/-0.031

For $t_{1/2}$

$x_{1/2}$ = (ln 50 – 4.3339)/-0.031

= (3.912-4.3339)/-0.031

= 13.609 min

Figure S30. A plot of human serum stability of **5a** is plotted as a logarithm of conc. of **5a** (μM) vs. time in minutes.