New pyridine and chromene scaffolds as potent vasorelaxant and anticancer agents

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Supplementary material

Spectroscopic chart captions

Fig. S72. Mass spectrum of compound **4c**.

Fig. S73. IR spectrum of compound **4d** (KBr pellet).

Fig. S74. ^{1}H-NMR spectrum of compound **4d**.

Fig. S75. ^{13}C-NMR spectrum of compound **4d**.

Fig. S76. Mass spectrum of compound **4d**.

Fig. S77. IR spectrum of compound **4e** (KBr pellet).

Fig. S78. ^{1}H-NMR spectrum of compound **4e**.

Fig. S79. ^{13}C-NMR spectrum of compound **4e**.

1. Vasodilation Activity Screening

The vasodilation activity screening was undertaken by Pharmacology Department, National Research Centre, Egypt, according to the standard in vitro bioassay technique [1-4] by testing the effects of the synthesized agents **3a-o** and **4a-e** and compared with prazosin hydrochloride (α1-AR antagonist) on isolated thoracic aortic rings of male Wistar rats (200–250 g) pre-contracted with norepinephrine hydrochloride. After light ether anesthesia, the rats were sacrificed by cervical dislocation. The aortae were immediately excised, freed of extraneous tissues, and prepared for isometric tension recording. Aorta was cut into (3-5 mm width) rings and each ring was placed in a vertical chamber "10 ml 5 jacketed automatic multi-chamber organ bath system (Model no. ML870B6/C, Panlab, Spain)" filled with Krebs solution composed of (in mM): NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 1.8; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11.0 and oxygenated with carbogen gas (95% O_2/5% CO_2) at 37 ± 0.5 °C. Each aortic ring was mounted between two stainless steel hooks passed through its lumen. The lower hook was fixed between two plates, while the upper one was attached to a force displacement transducer (Model no. MLT0201, Panlab, Spain) connected to an amplifier (PowerLab, AD Instruments Pty. Ltd.), which was connected to a computer. The Chart for windows (v 3.4) software was used to record and elaborate data. Preparations were stabilized under 2 g resting tension during 2 h, and then the contracture response to norepinephrine hydrochloride (10-6 M) was measured before and after exposure to increasing concentrations of the tested synthesized compounds (50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 μM). The tested compounds were dissolved in dimethyl sulfoxide

(DMSO) as stock solution (10 ml of 0.005 M). Control experiments were performed in the presence of DMSO alone, at the same concentrations as those used with the derivatives tested, which demonstrated that the solvent did not affect the contractile response of isolated aorta. The observed vasodilation activity screening data for the synthesized compounds **3a-o** and **4a-e** and prazosin hydrochloride are expressed as IC_{50} (μM) concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture utilizing four different replicates.

2. Cell Culture Methods

Cell culturing

The MCF-7 cells were cultured in RPMI with 5% FBS. The MDA-MB 231 cells was cultured in DMEM (with L-glutamine) with 10% FBS. All of the cells were stored in an incubator at 37 °C with 5% CO_2.

The cells were subcultured at 80-90 % confluency. For MCF-7 and MDA-MB 231, the old medium was removed and discarded, and the flask was washed with 5 ml, phosphate buffer solution (PBS). 1 mL of trypsin was then added to the flask and allowed to incubate for 3 min before 4 mL of complete medium was added to deactivate the trypsin. The solution was then removed from the flask and put into a falcon tube and the cells were separated from the medium by centrifuging at 215 x g for 5 min. The old medium was removed and discarded, and fresh medium was added and the cells suspended, and new flasks were seeded at 1:10 cells to complete medium.

Cell counting

To count the cells, the same procedure for detaching cells as subculturing was used. After the cells were resuspended in fresh medium, a 100 μL aliquot was added to 100 μL of Trypan blue (TB) solution. The cells were then counted using a haemocytometer. The haemocytometer has a neubauer chamber, which contains two sets of 9 squares in a grid. Using the haemocytometer, 5 squares from each set were counted and all 10 squares averaged, and the concentration of cells in the solution was calculated using the following equation:

Concentration (cells/mL) = 2 x Cells/square average x 10^4

In this equation, 2 is the dilution factor from the addition of the TB solution and 10^4 is the converted value of the volume each square contains. Using the concentration of cells that was determined, an aliquot of the cell suspension was diluted with fresh medium to the desired density for seeding a 96 well plate. Plates were seeded 24 hours before they were treated with the test compounds.

Phosphate Buffer Saline Preparation

Phosphate buffer saline (PBS) was prepared by adding 4.4 g NaCl, 7.5 g Na_2HPO_4, and 2.1 g Na_2HPO_4 to a 1 L media bottle containing approximately 750 mL deionized water and stirred until completely dissolved. The pH was checked to confirm it was 7.4, and then the volume was brought up to 1 L with deionized water. The buffer was then decanted into smaller bottles before autoclaving.

Test compounds stock solutions

The compounds were provided in powdered form, and to assess the compounds' activities against various cell lines, stock solutions were prepared by dissolving them in DMSO to a concentration of 4 mM. The stock solutions were stored in the freezer at -20 °C.

MTT cell viability assay

Cytotoxicity of the compounds was tested using the MTT assay. MTT (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide) is a salt that is enzymatically reduced by live cells, resulting in the formation of formazan crystals which can then be read by a spectrophotometer.

To test the compounds against MCF-7 and NIDA-MB 231 cells, solutions were prepared from the 4 mM stock solutions. They were diluted to 20 μM in complete medium to contain 1% DMSO. MCF-7 and MDA-MB 231 were screened against all compounds at a concentration of 10 μM by adding 100 μL of the diluted solution to each well. The four compounds that exhibited the highest cytotoxic effect were selected to find the IC_{50} for each of the cancer cell lines in the MCF-7 and MDA-MB 231 experiments were selected. Each experiment was replicated three times, and the results were expressed as the mean ± the standard error of the mean.

After the initial screening, the selected compounds were again plated and screened at concentrations ranging from 0.01 μM to 20 μM, and the MTT assay was carried out

again. Each experiment was replicated three times, and the results were expressed as the mean ± the standard error of the mean. The results were plotted in graph pad in order to determine the IC_{50} values.

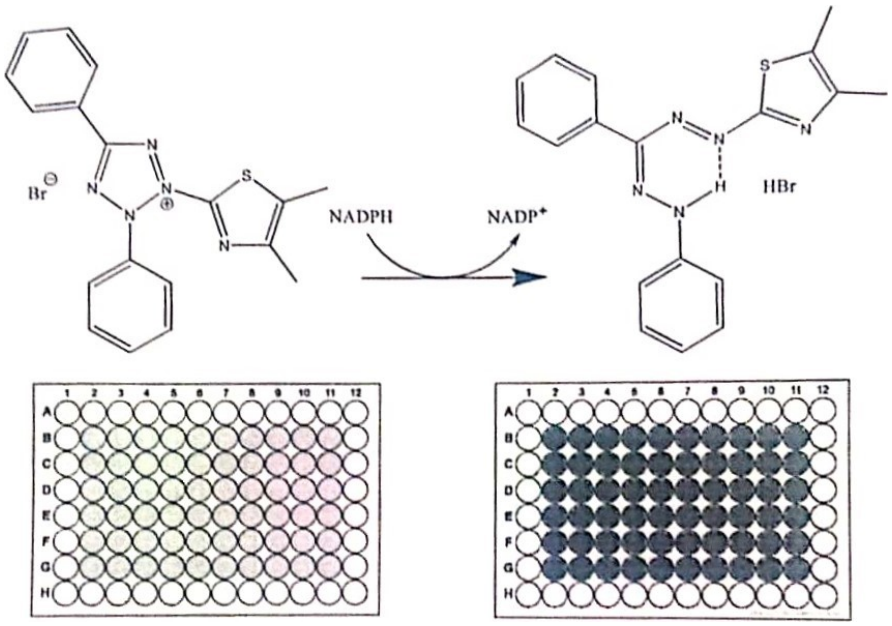
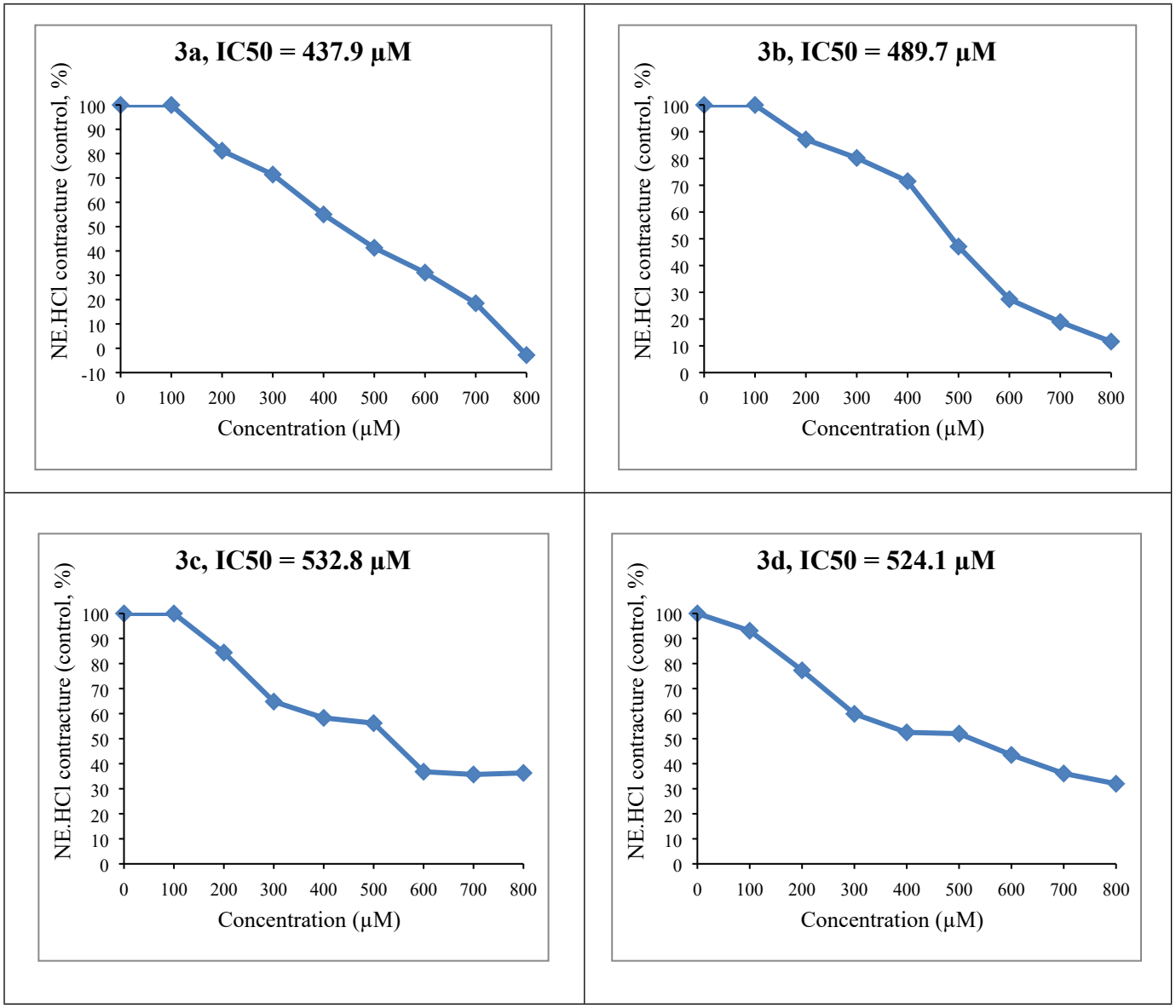
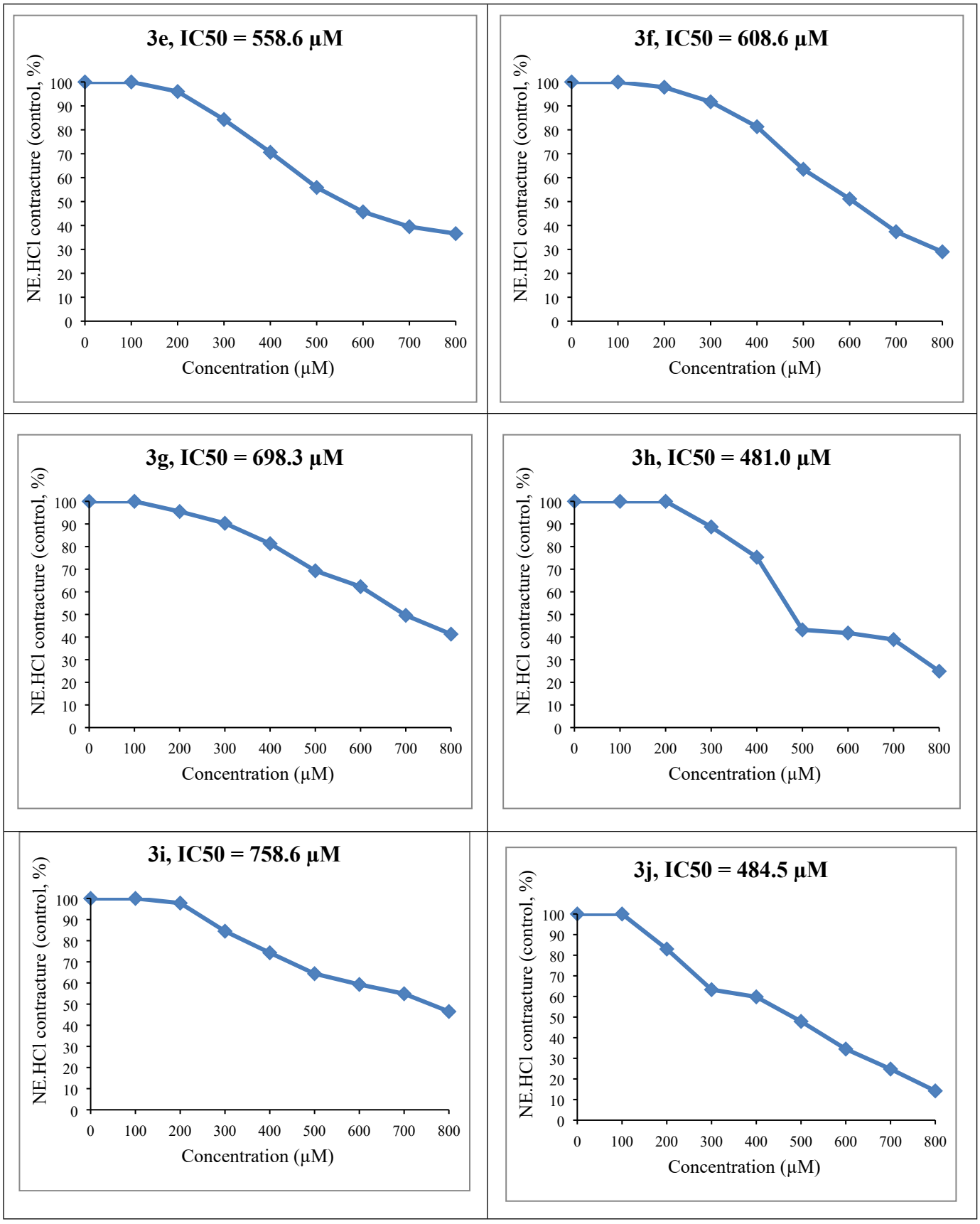


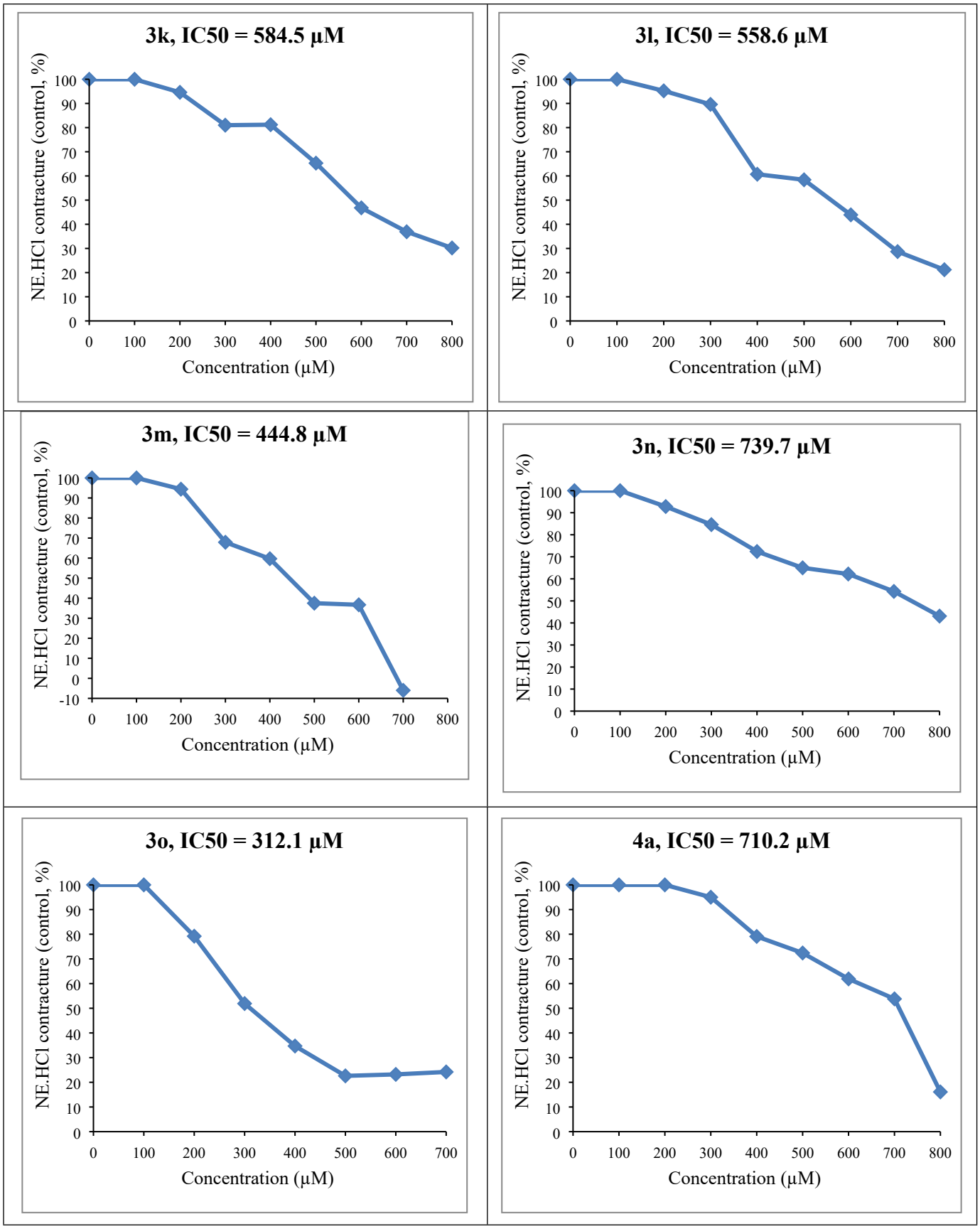
Fig. 1: MTT is reduced by NADPH to form purple formazan crystals

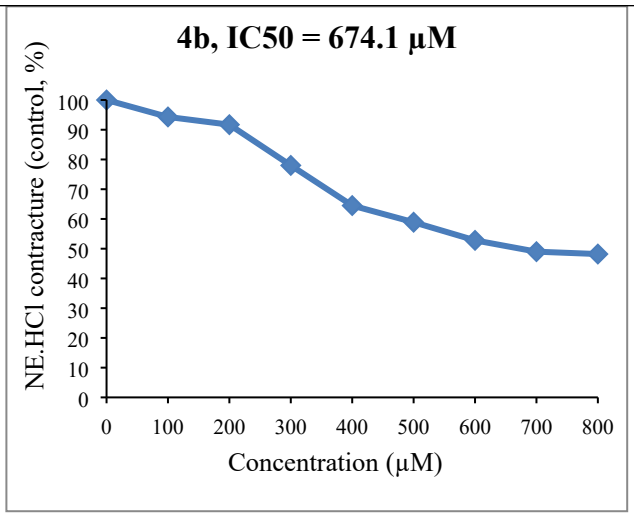
An MTT solution was prepared by dissolving MTT in PBS at a concentration of 5 mg/mL. 20 μL of the solution was added to each of the wells treated with a compound, as well as to the control, and allowed to incubate for 5 h. After 5 h, the media in the wells was removed and the formazan crystals were dissolved in 100 μL of DMSO. The plate was then incubated for 30 min to allow the formazan crystals to dissolve, and the plate was read on a spectrophotometer at 570 nm, with a reference wavelength of 450 nm. Each experiment was replicated three times, and the results were expressed as the mean ± the standard error of the mean.

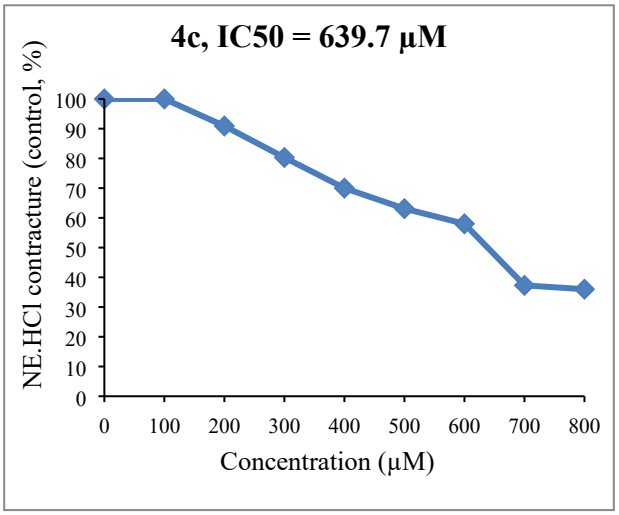
Table S1. Dose response curves the tested compounds **3a-o** and **4a-e.**

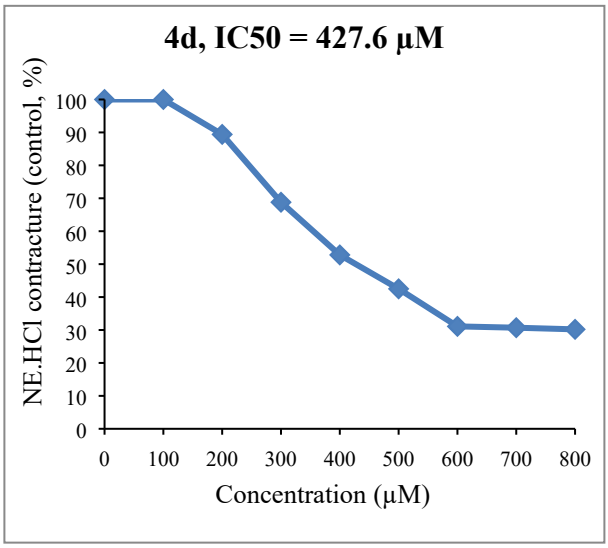


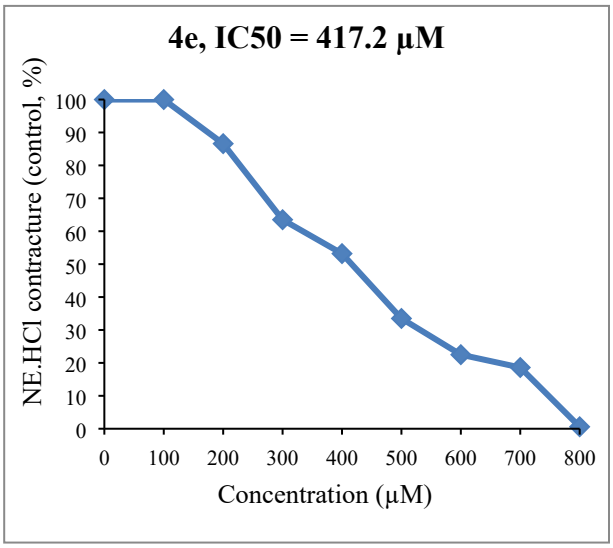

3e, IC50 = 558.6 µM
3f, IC50 = 608.6 µM
3g, IC50 = 698.3 µM
3h, IC50 = 481.0 µM
3i, IC50 = 758.6 µM
3j, IC50 = 484.5 µM
NE.HCl contracture (control, %)
Concentration (µM)
0
10
20
30
40
50
60
70
80
90
100
0
100
200
300
400
500
600
700
800


3k, IC50 = 584.5 µM
NE.HCl contracture (control, %)
Concentration (µM)
3l, IC50 = 558.6 µM
NE.HCl contracture (control, %)
Concentration (µM)
3m, IC50 = 444.8 µM
NE.HCl contracture (control, %)
Concentration (µM)
3n, IC50 = 739.7 µM
NE.HCl contracture (control, %)
Concentration (µM)
3o, IC50 = 312.1 µM
NE.HCl contracture (control, %)
Concentration (µM)
4a, IC50 = 710.2 µM
NE.HCl contracture (control, %)
Concentration (µM)


4b, IC50 = 674.1 µM
NE.HCl contracture (control, %)
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Concentration (µM)


4c, IC50 = 639.7 µM
NE.HCl contracture (control, %)
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Concentration (µM)


4d, IC50 = 427.6 µM
NE.HCl contracture (control, %)
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Concentration (µM)


4e, IC50 = 417.2 µM
NE.HCl contracture (control, %)
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Concentration (µM)

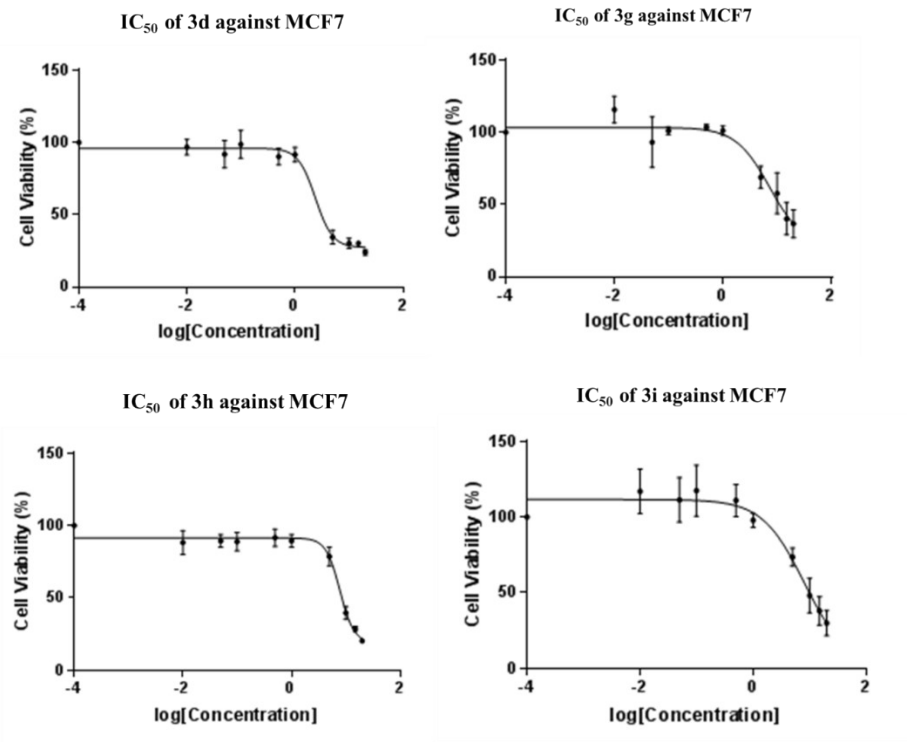


Fig. 2. IC_{50} curves of compounds **3d**, **3g**, **3h** and **3i** on MCF-7 cell line

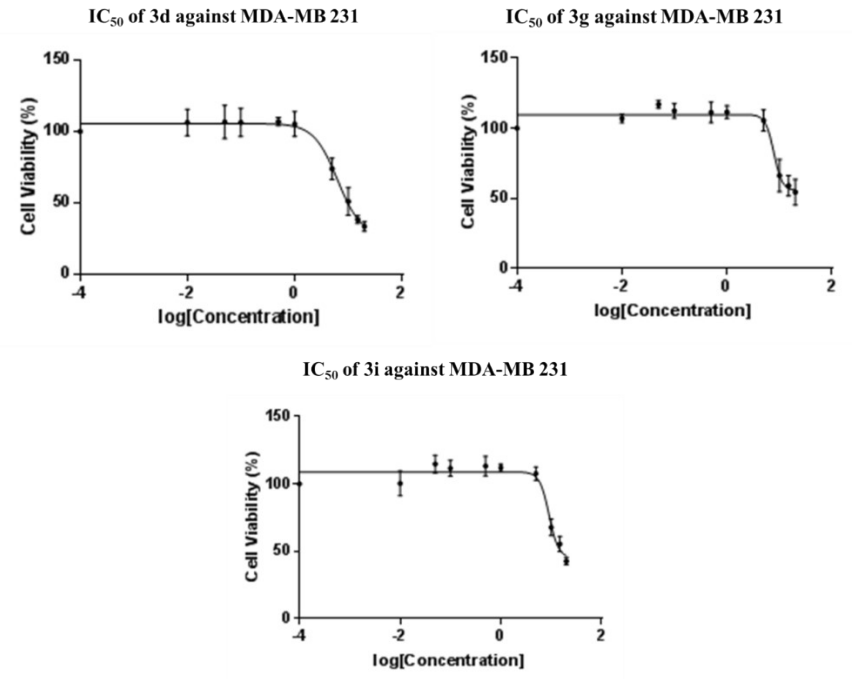
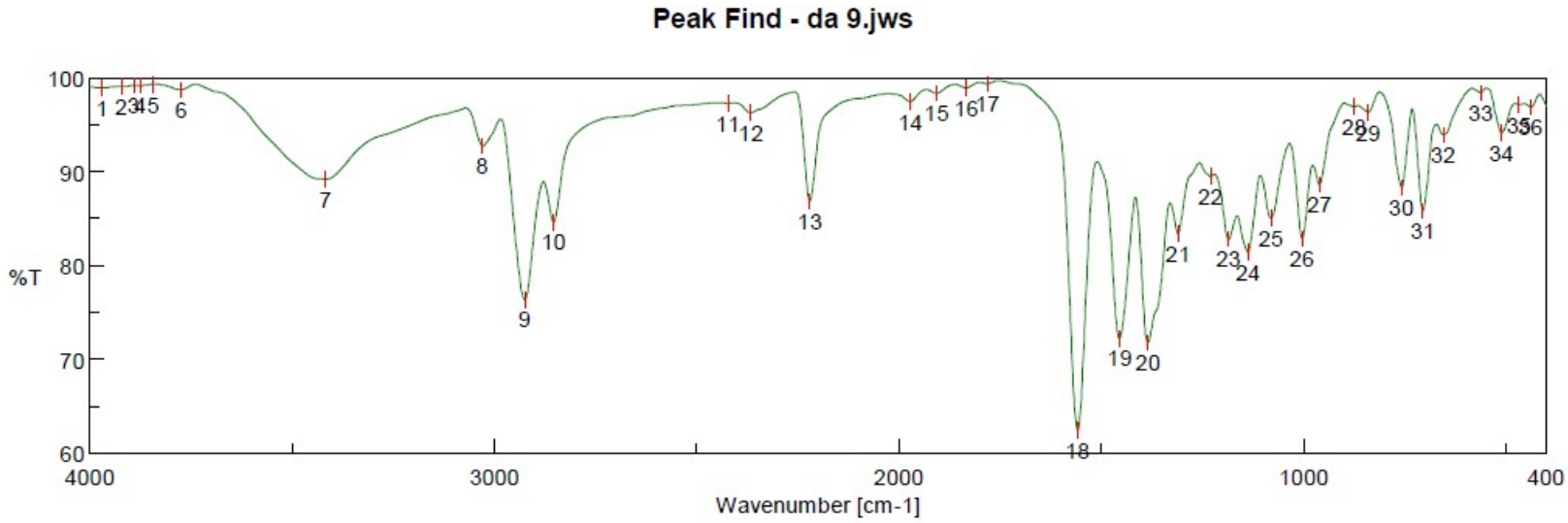


Fig. 3. IC_{50} curves of compounds **3d**, **3g** and **3i** on MDA-MB 231 cell line



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3969.75	98.9253	2	3922.5	99.0548	3	3890.68	99.1379	4	3874.29	99.1263
5	3842.47	99.2688	6	3774.01	98.6978	7	3417.24	89.1819	8	3028.66	92.7156
9	2925.48	76.3217	10	2854.13	84.5752	11	2423.12	97.2736	12	2366.23	96.2709
13	2220.63	86.8103	14	1971.86	97.453	15	1906.29	98.3406	16	1833.97	98.8997
17	1781.9	99.359	18	1558.2	62.4467	19	1455.03	72.2026	20	1384.64	71.7551
21	1310.39	83.3971	22	1230.36	89.4741	23	1185.04	82.7703	24	1136.83	81.3836
25	1080.91	85.0524	26	1003.77	82.8602	27	960.377	88.5958	28	875.524	96.9262
29	839.847	96.2915	30	756.923	88.2719	31	704.855	85.8251	32	654.715	93.9258
33	562.148	98.3853	34	511.044	94.1183	35	467.653	97.1015	36	438.726	96.859

Fig. S1. IR spectrum of compound **3a** (KBr pellet).

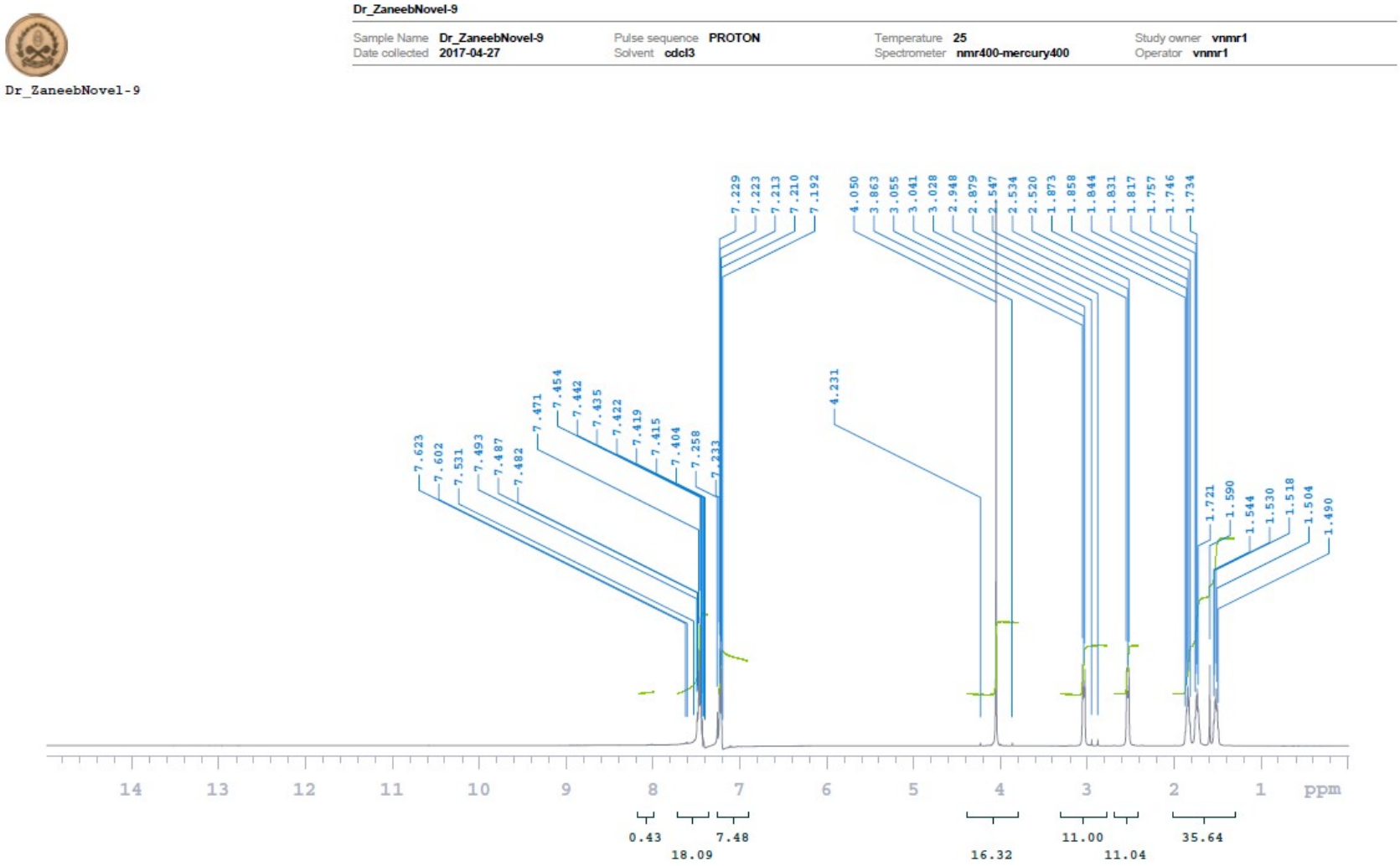


Fig. S2. ^{1}H-NMR spectrum of compound **3a**.

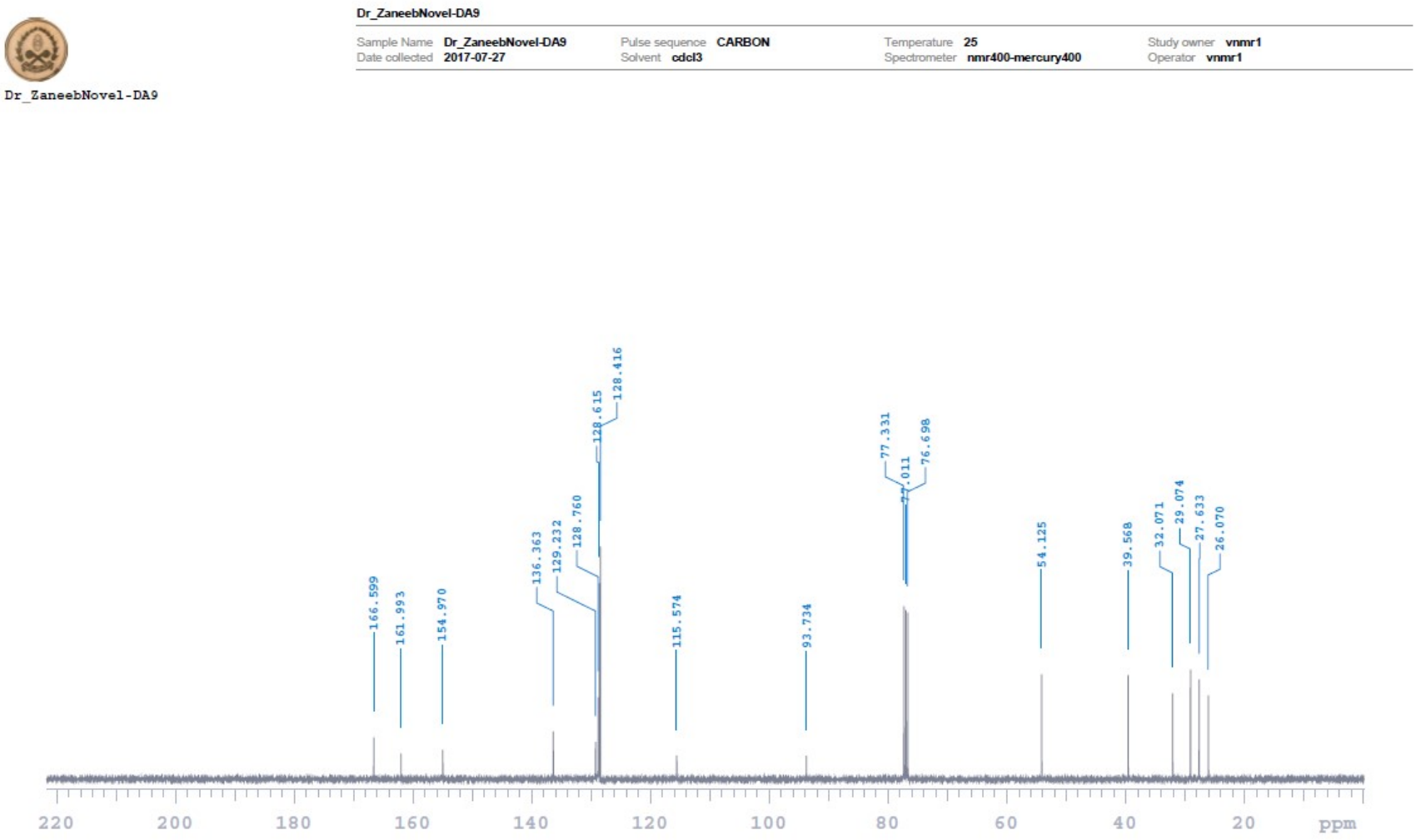


Fig. S3. ^{13}C-NMR spectrum of compound **3a**.

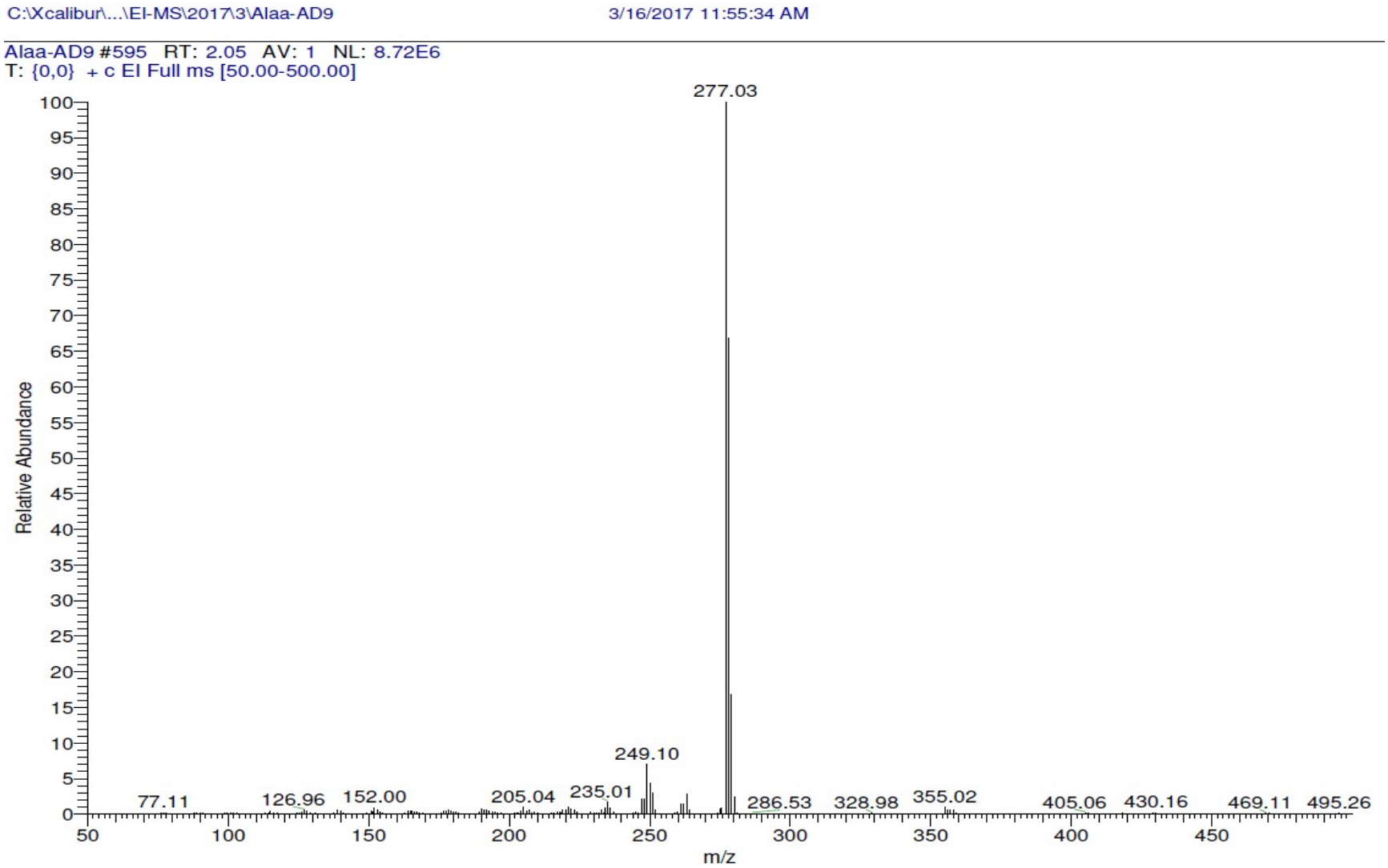
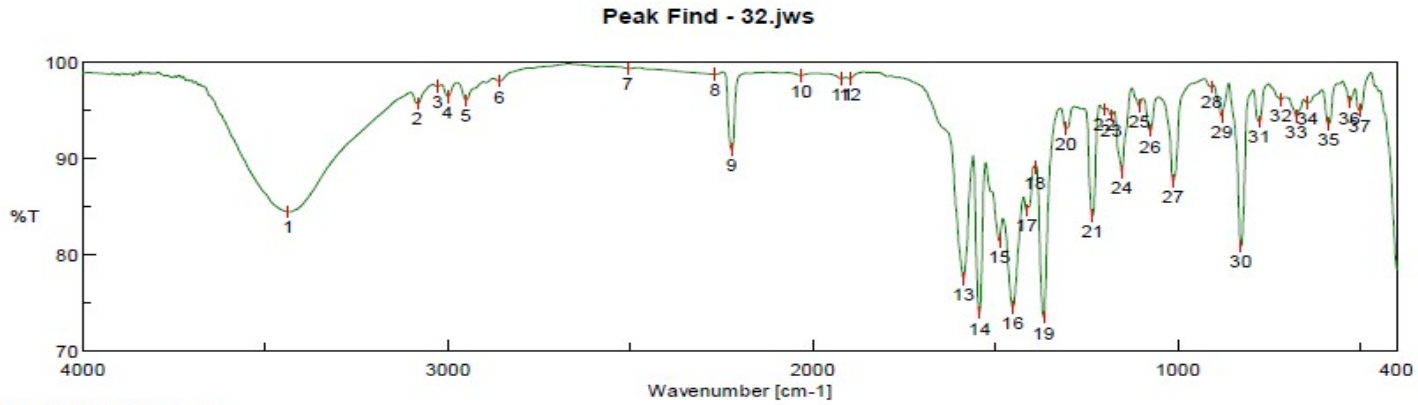


Fig. S4. Mass spectrum of compound **3a**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3437.49	84.42	2	3082.65	95.6428	3	3027.69	97.4571	4	2999.73	96.3541
5	2949.59	96.0082	6	2857.99	97.9645	7	2506.04	99.2832	8	2267.88	98.6527
9	2221.59	90.8439	10	2030.68	98.5783	11	1919.79	98.2453	12	1897.61	98.2513
13	1587.13	77.4515	14	1542.77	73.899	15	1488.78	81.3552	16	1451.17	74.4785
17	1414.53	84.5567	18	1391.39	88.9907	19	1367.28	73.4164	20	1305.57	93.0496
21	1233.25	83.9898	22	1200.47	95.0508	23	1182.15	94.4199	24	1153.22	88.5703
25	1105.01	95.3655	26	1075.12	92.8099	27	1012.45	87.5735	28	906.379	97.3733
29	879.381	94.3395	30	826.348	80.818	31	776.208	93.7644	32	716.425	96.023
33	674.963	94.3191	34	644.108	95.7263	35	586.254	93.5051	36	529.364	95.7666
37	500.437	94.901									

Fig. S5. IR spectrum of compound **3b** (KBr pellet).

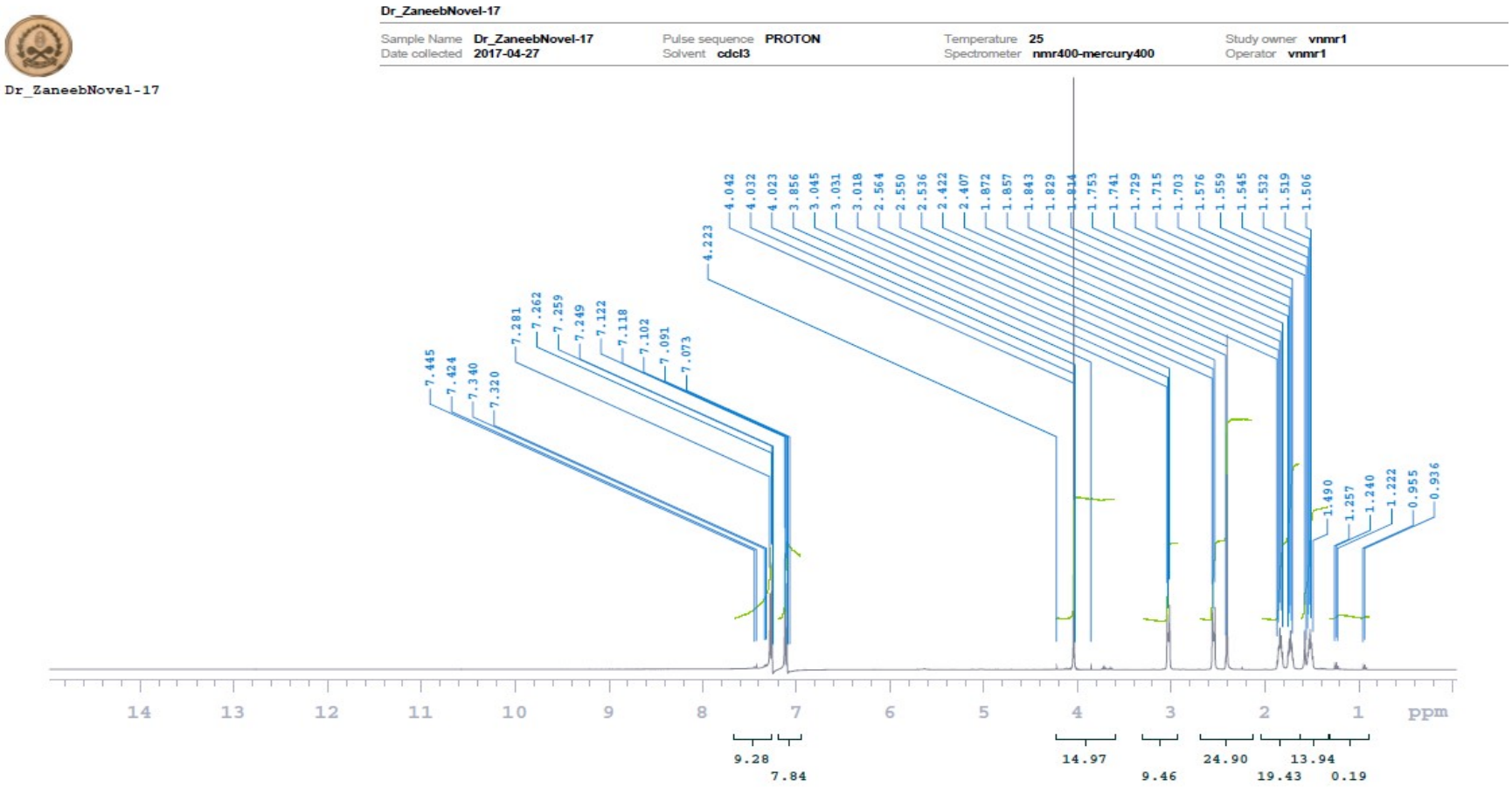


Fig. S6. [1]H-NMR spectrum of compound **3b**.

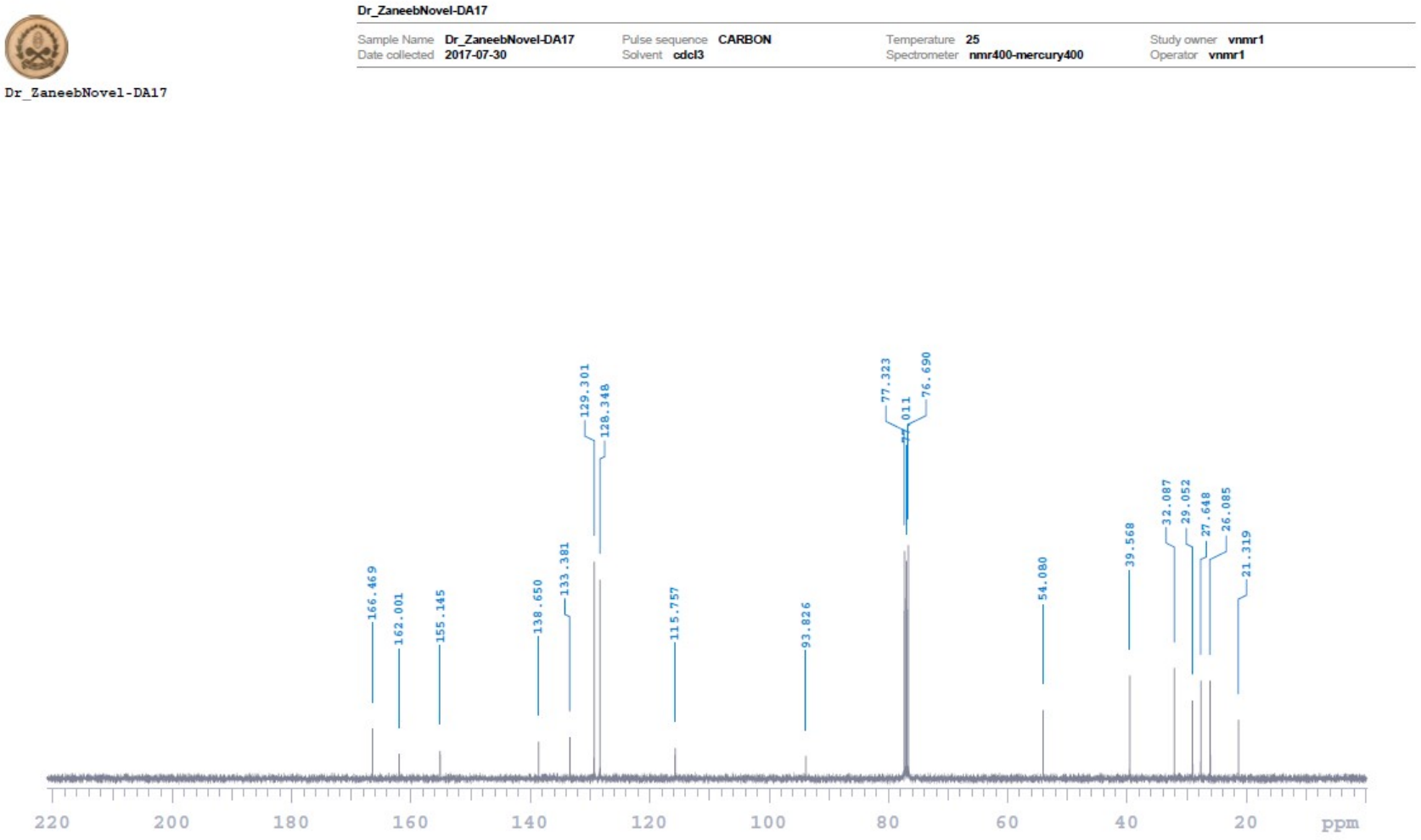


Fig. S7. ^{13}C-NMR spectrum of compound **3b**.

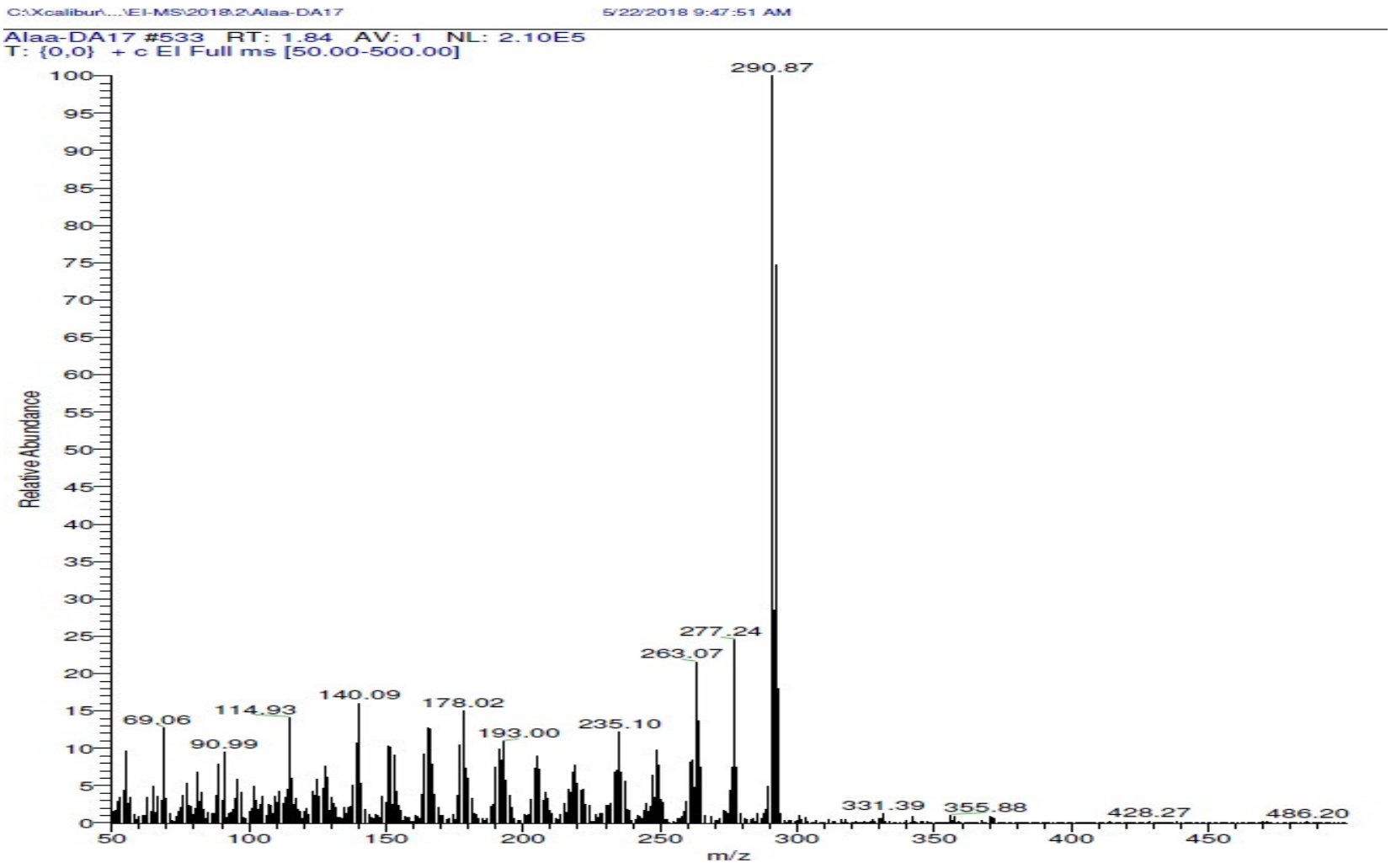
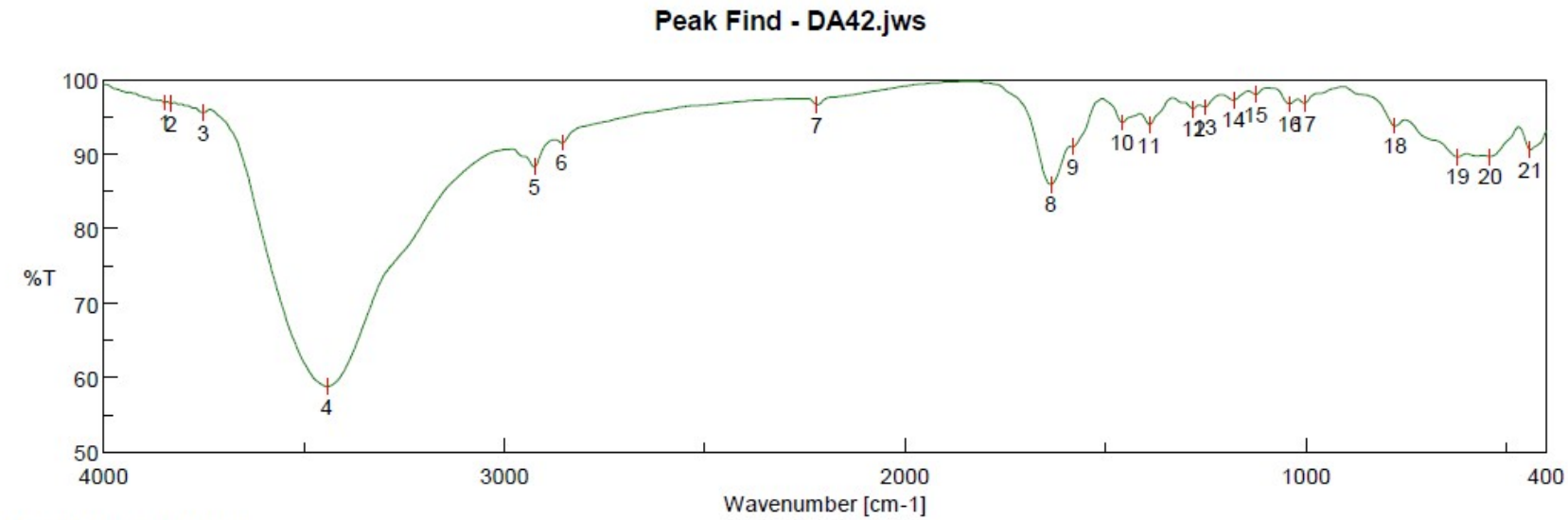


Fig. S8. Mass spectrum of compound **3b**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3847.29	96.8641	2	3831.86	96.7884	3	3750.87	95.6042	4	3442.31	58.7584
5	2925.48	88.2794	6	2856.06	91.4975	7	2219.67	96.599	8	1635.34	85.936
9	1581.34	90.964	10	1457.92	94.2459	11	1390.42	93.9422	12	1280.5	96.045
13	1251.58	96.3446	14	1180.22	97.2394	15	1124.3	98.0304	16	1041.37	96.7536
17	1002.8	96.8377	18	777.172	93.7493	19	620.966	89.6129	20	539.971	89.6452
21	439.69	90.5672									

Fig. S9. IR spectrum of compound **3c** (KBr pellet).

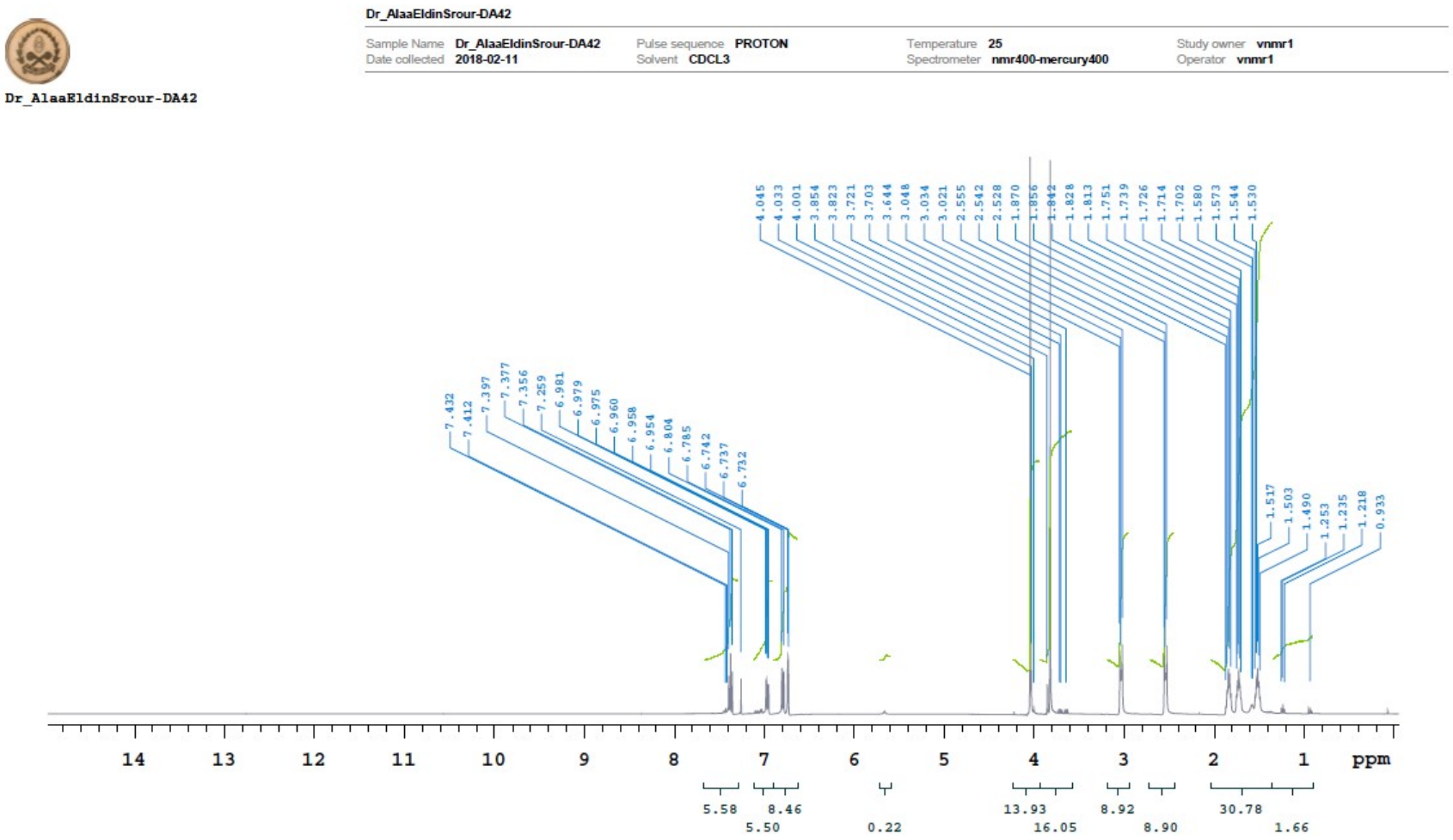


Fig. S10. [1]H-NMR spectrum of compound **3c**.

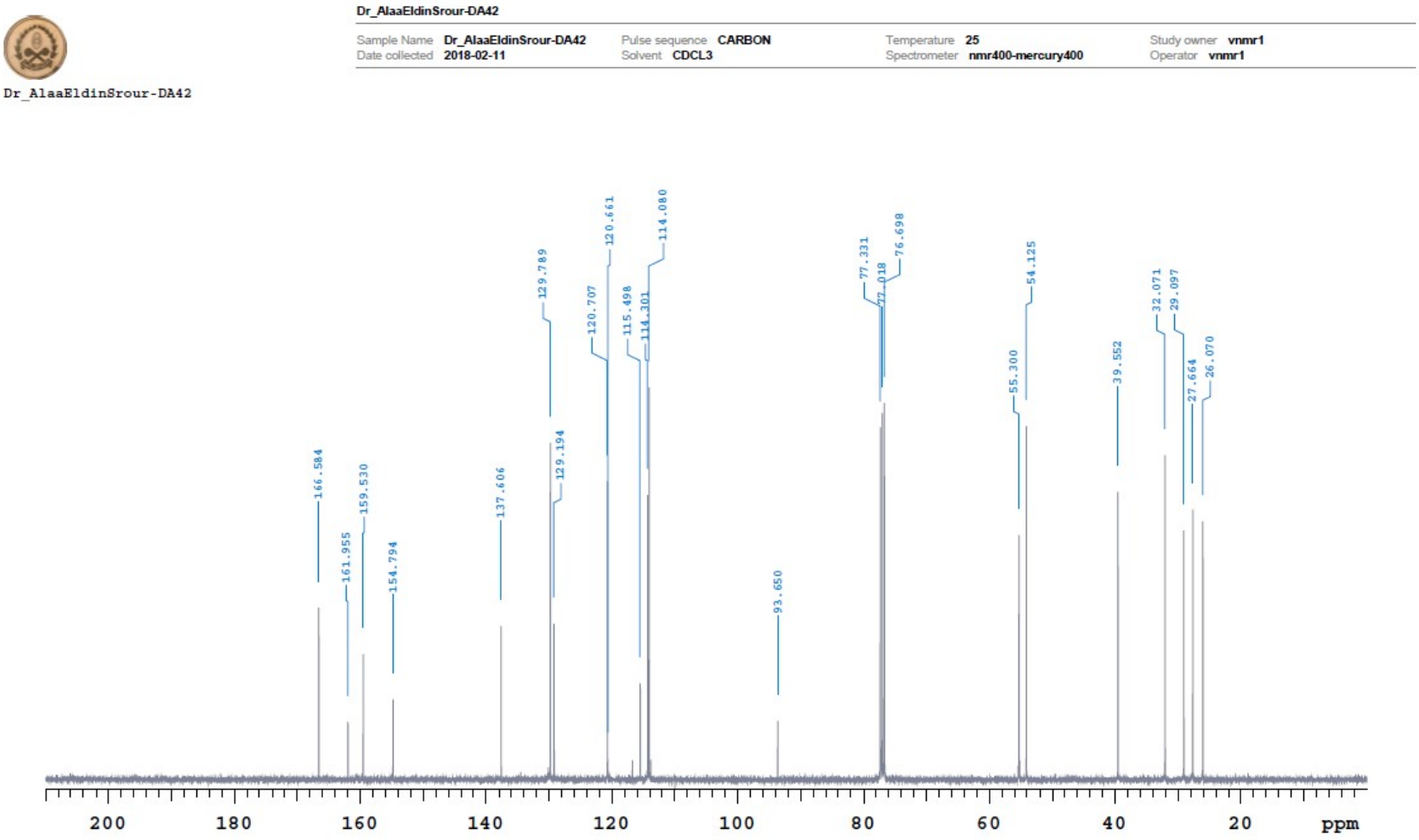


Fig. S11. ^{13}C-NMR spectrum of compound **3c**.

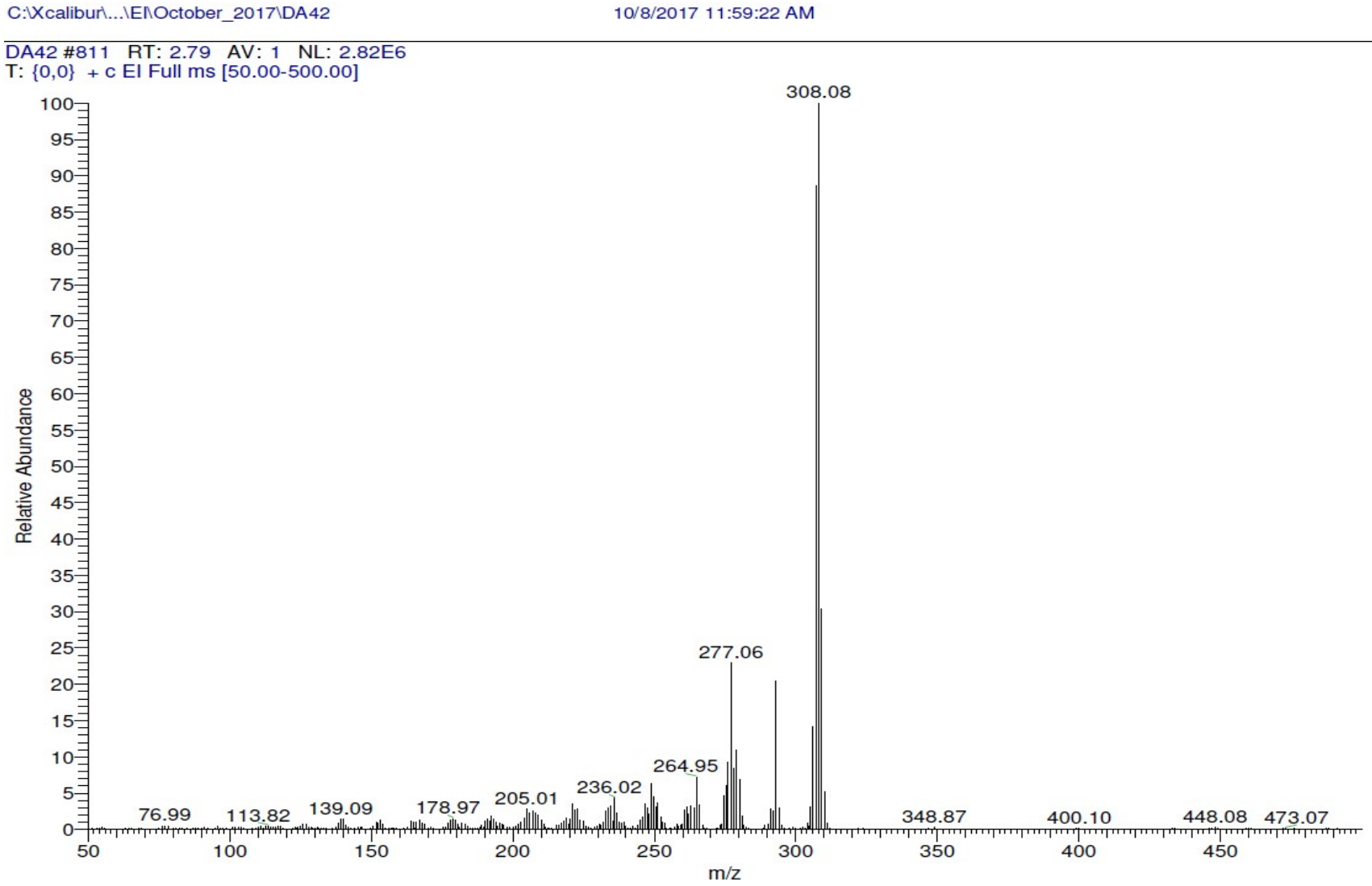
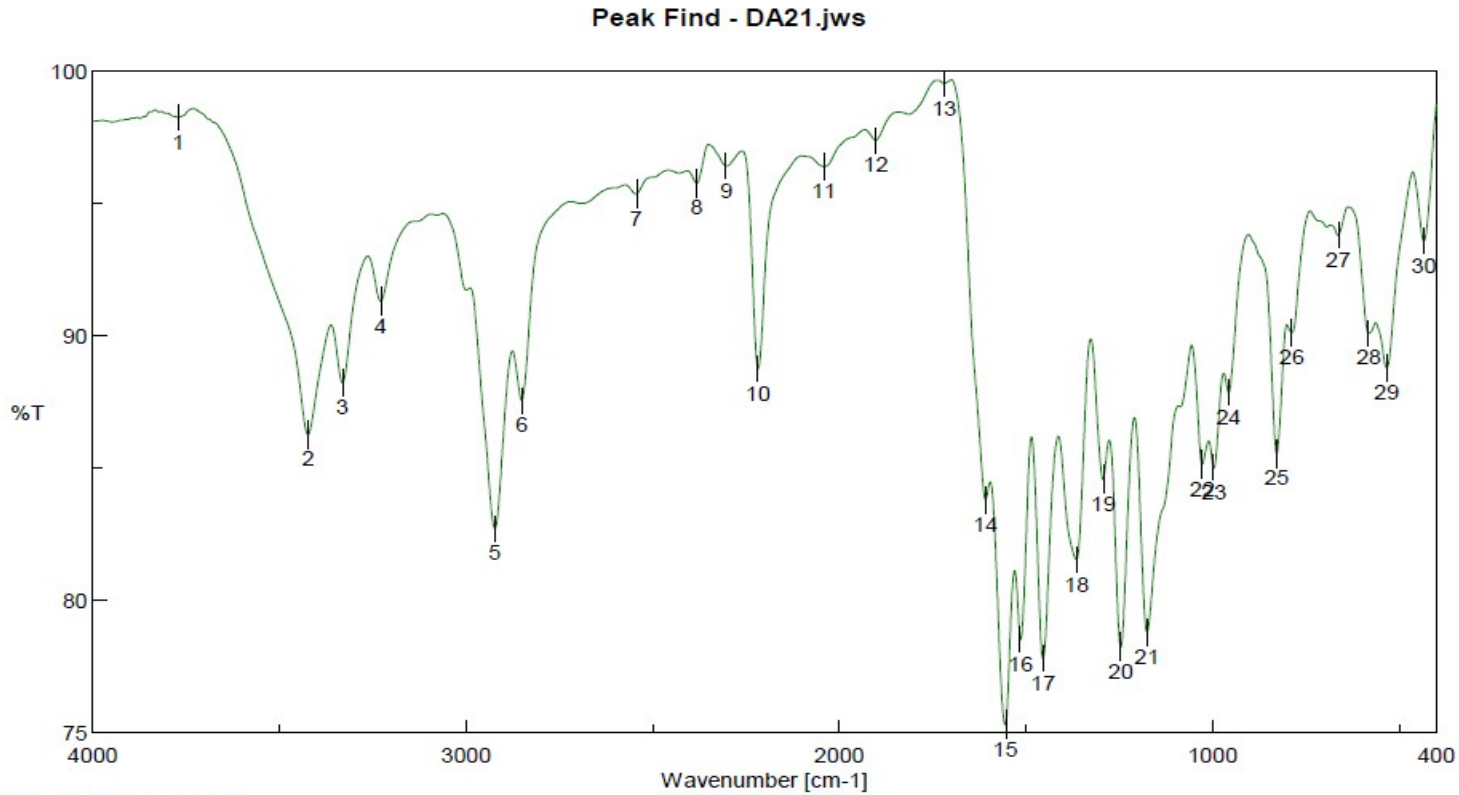


Fig. S12. Mass spectrum of compound **3c**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3772.08	98.2336	2	3423.03	86.259	3	3331.43	88.2079	4	3229.22	91.2961
5	2922.59	82.7008	6	2851.24	87.5587	7	2545.58	95.3499	8	2381.66	95.7496
9	2301.63	96.4068	10	2217.74	88.7428	11	2041.28	96.391	12	1902.43	97.3677
13	1719.23	99.5181	14	1608.34	83.7968	15	1555.31	75.2932	16	1513.85	78.5161
17	1454.06	77.7852	18	1364.39	81.5332	19	1293.04	84.5622	20	1245.79	78.2198
21	1175.4	78.8032	22	1027.87	85.1375	23	996.053	84.9943	24	956.52	87.8492
25	828.277	85.5454	26	786.815	90.1235	27	663.393	93.7796	28	581.433	90.0868
29	533.221	88.7856	30	433.905	93.5624						

Fig. S13. IR spectrum of compound **3d** (KBr pellet).

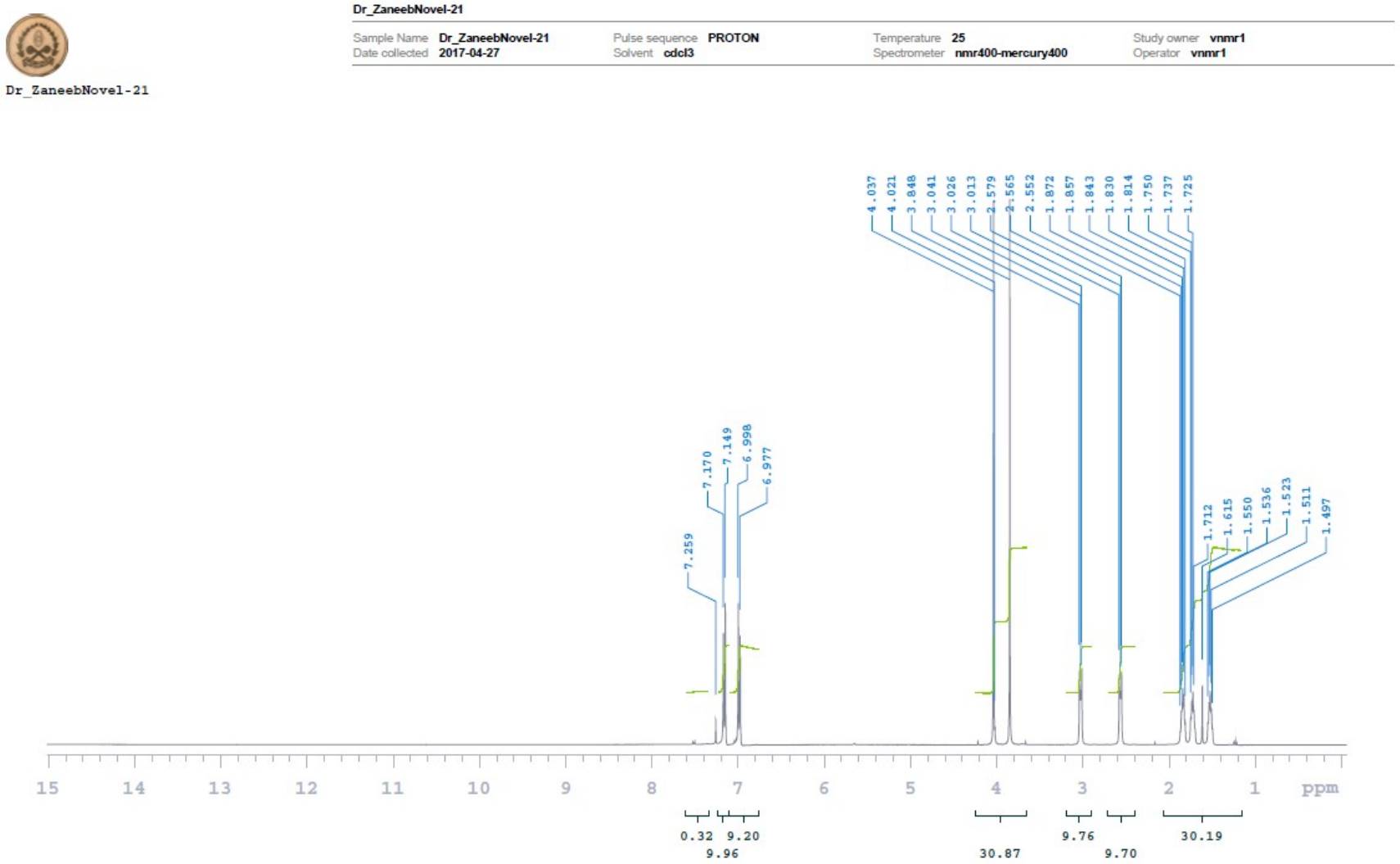


Fig. S14. ^{1}H-NMR spectrum of compound **3d**.

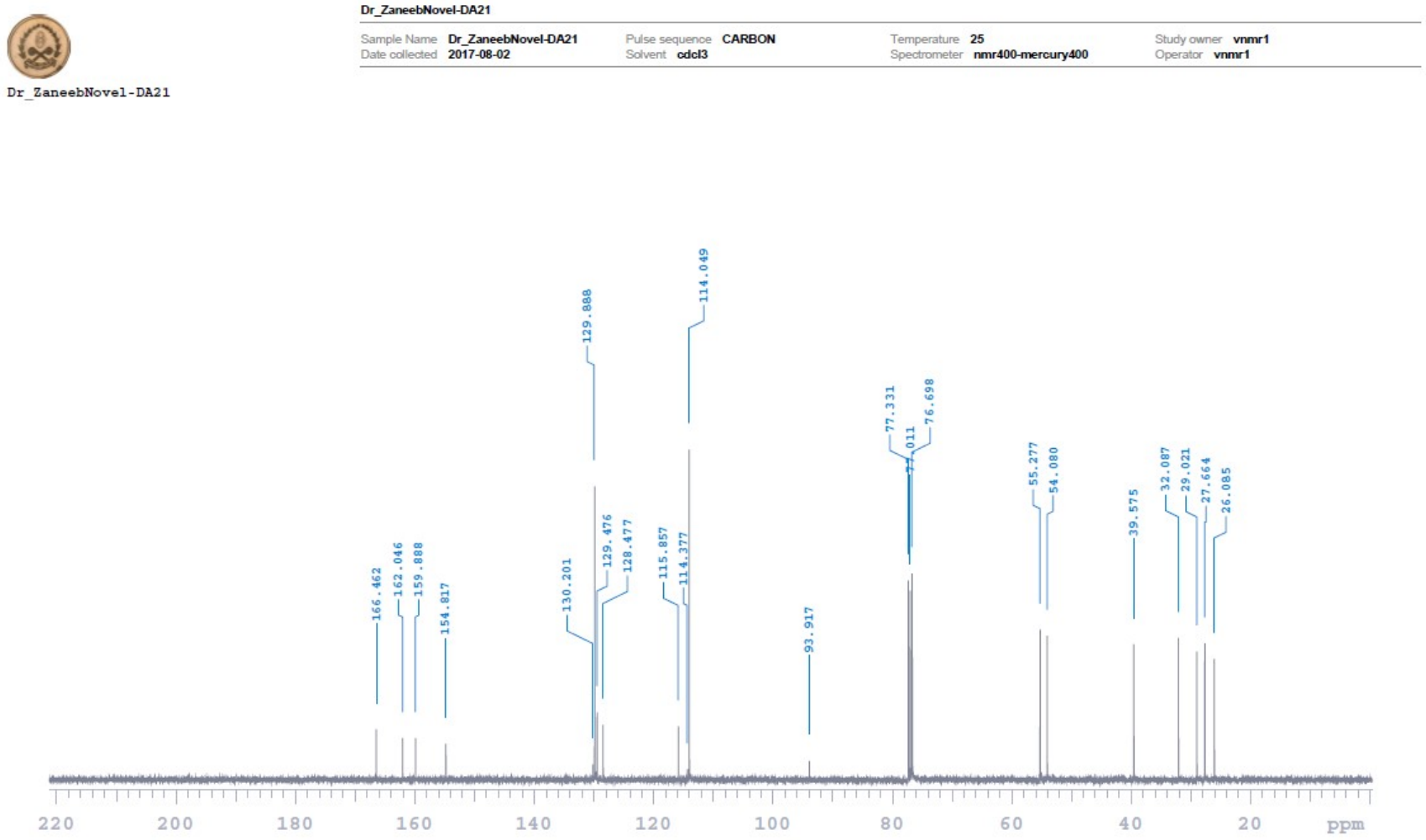


Fig. S15. ^{13}C-NMR spectrum of compound **3d**.

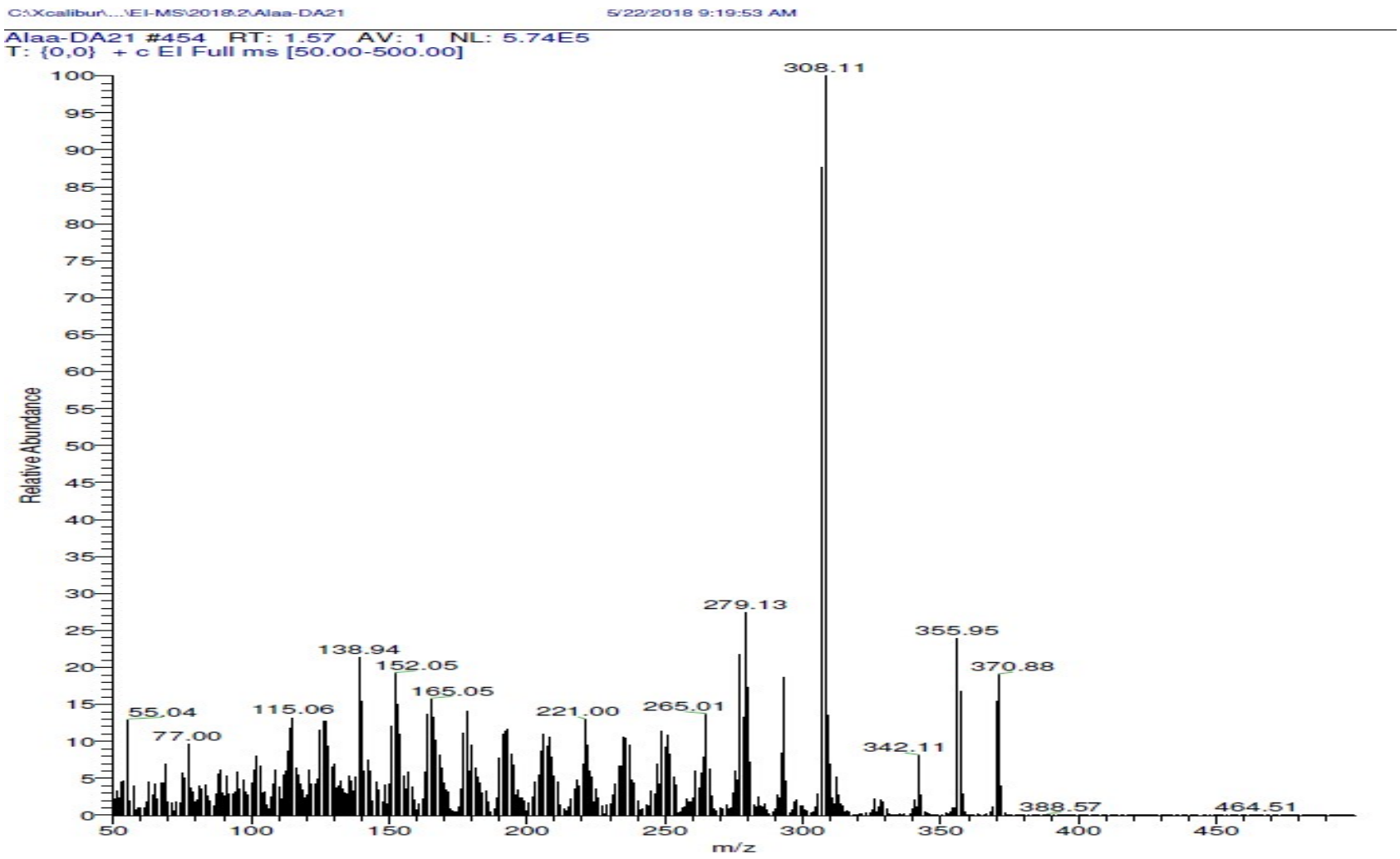
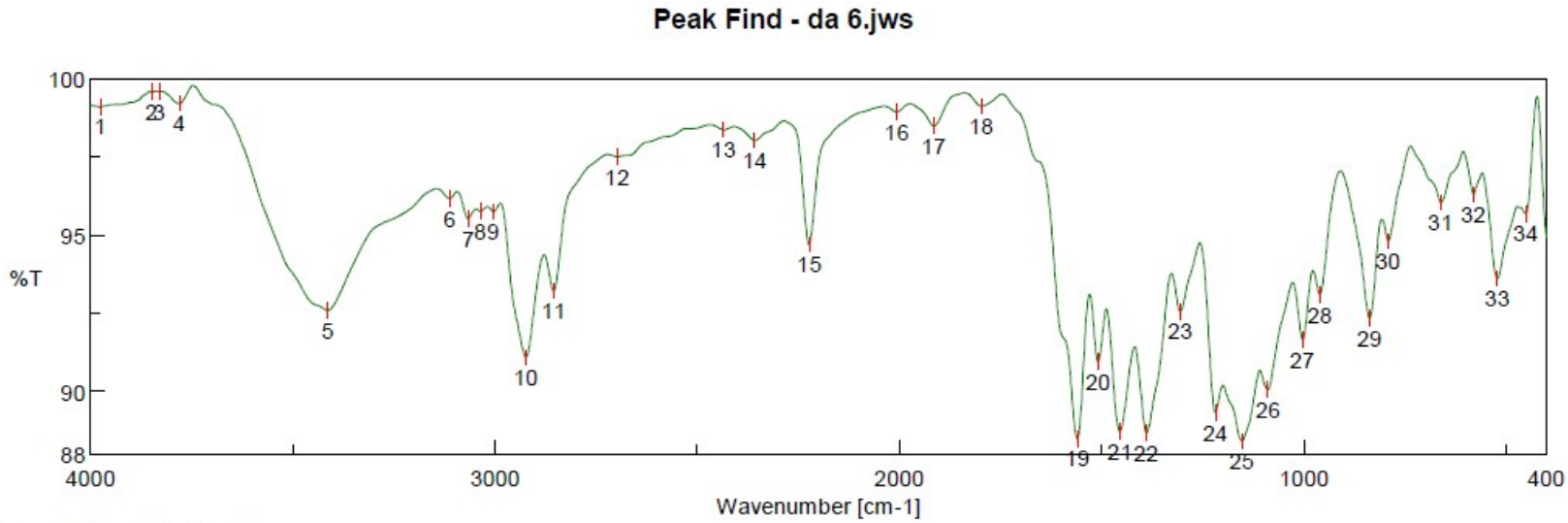


Fig. S16. Mass spectrum of compound **3d**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3976.5	99.0938	2	3849.22	99.5602	3	3829.94	99.5634	4	3780.76	99.1966
5	3413.39	92.5884	6	3111.58	96.1627	7	3066.26	95.5242	8	3036.37	95.7567
9	3003.59	95.7376	10	2923.56	91.0914	11	2855.1	93.2081	12	2698.89	97.498
13	2434.69	98.3555	14	2357.55	98.0131	15	2223.52	94.6953	16	2007.53	98.9327
17	1914.97	98.4806	18	1797.33	99.1086	19	1560.13	88.4876	20	1509.03	90.9518
21	1454.06	88.7255	22	1389.46	88.6727	23	1305.57	92.5561	24	1218.79	89.3275
25	1153.22	88.4185	26	1088.62	90.0541	27	1002.8	91.6451	28	960.377	93.0884
29	836.955	92.3338	30	790.671	94.8083	31	661.464	96.024	32	580.469	96.2947
33	521.65	93.6066	34	451.261	95.6789						

Fig. S17. IR spectrum of compound **3e** (KBr pellet).

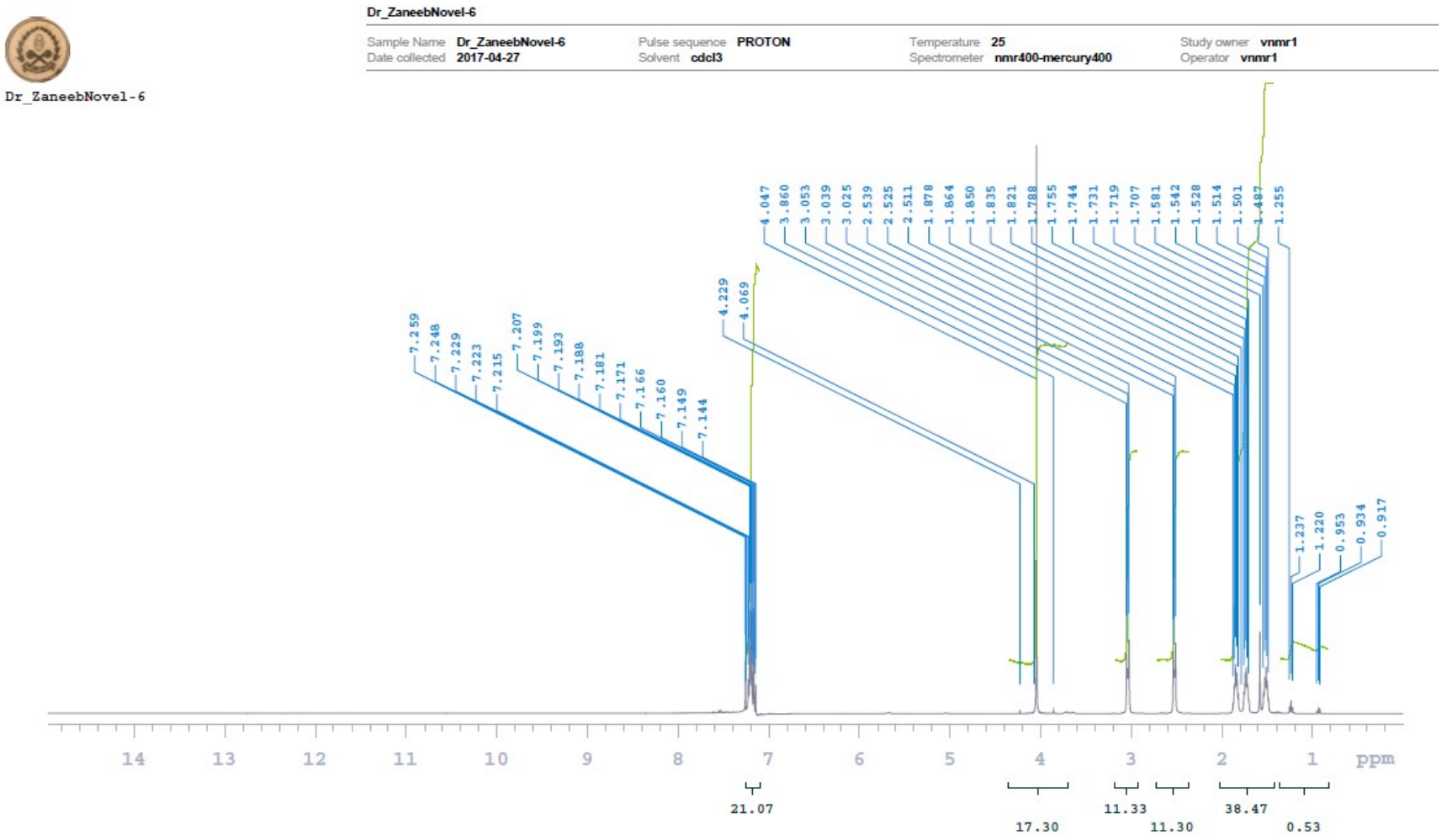


Fig. S18. [1]H-NMR spectrum of compound **3e**.

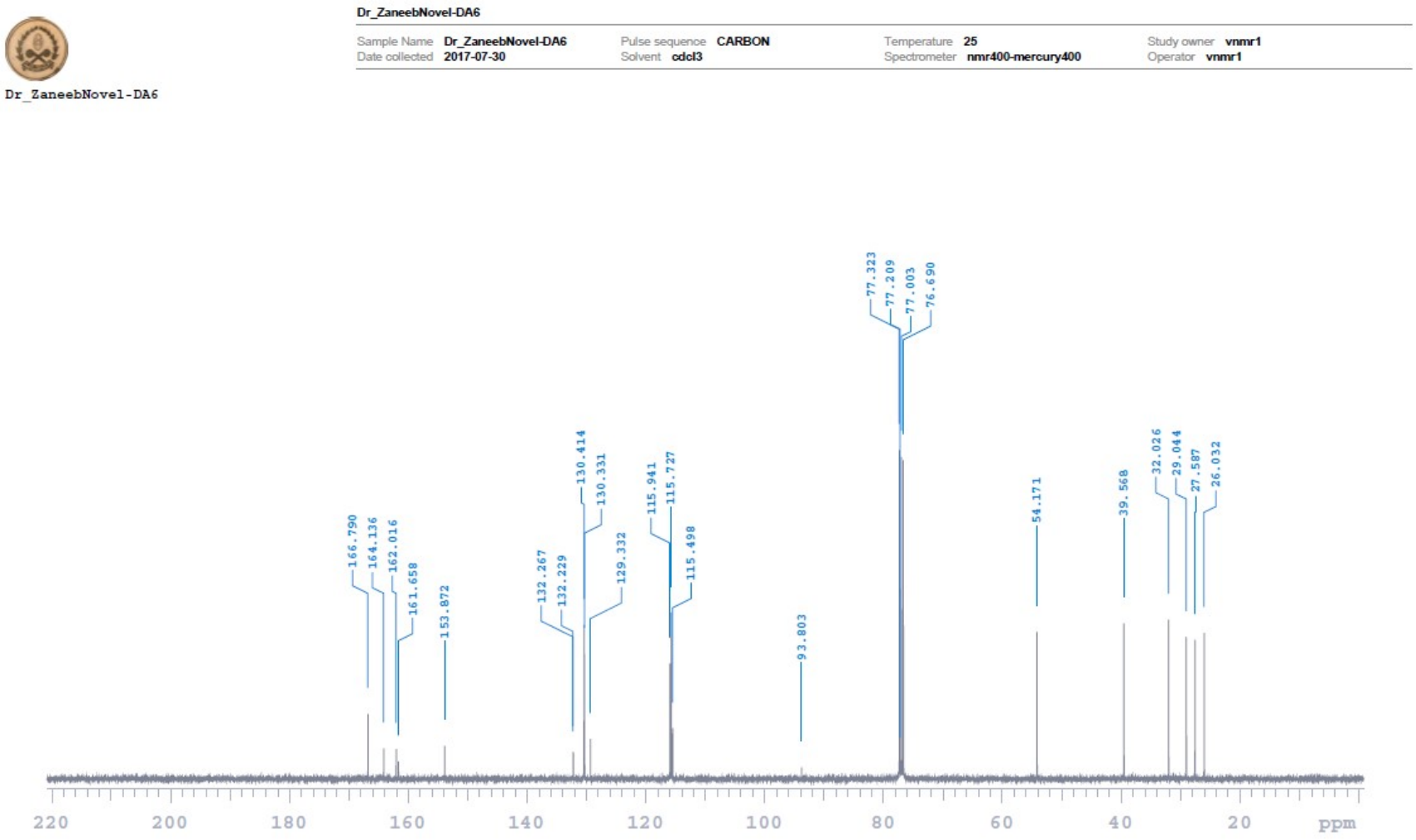


Fig. S19. ^{13}C-NMR spectrum of compound **3e**.

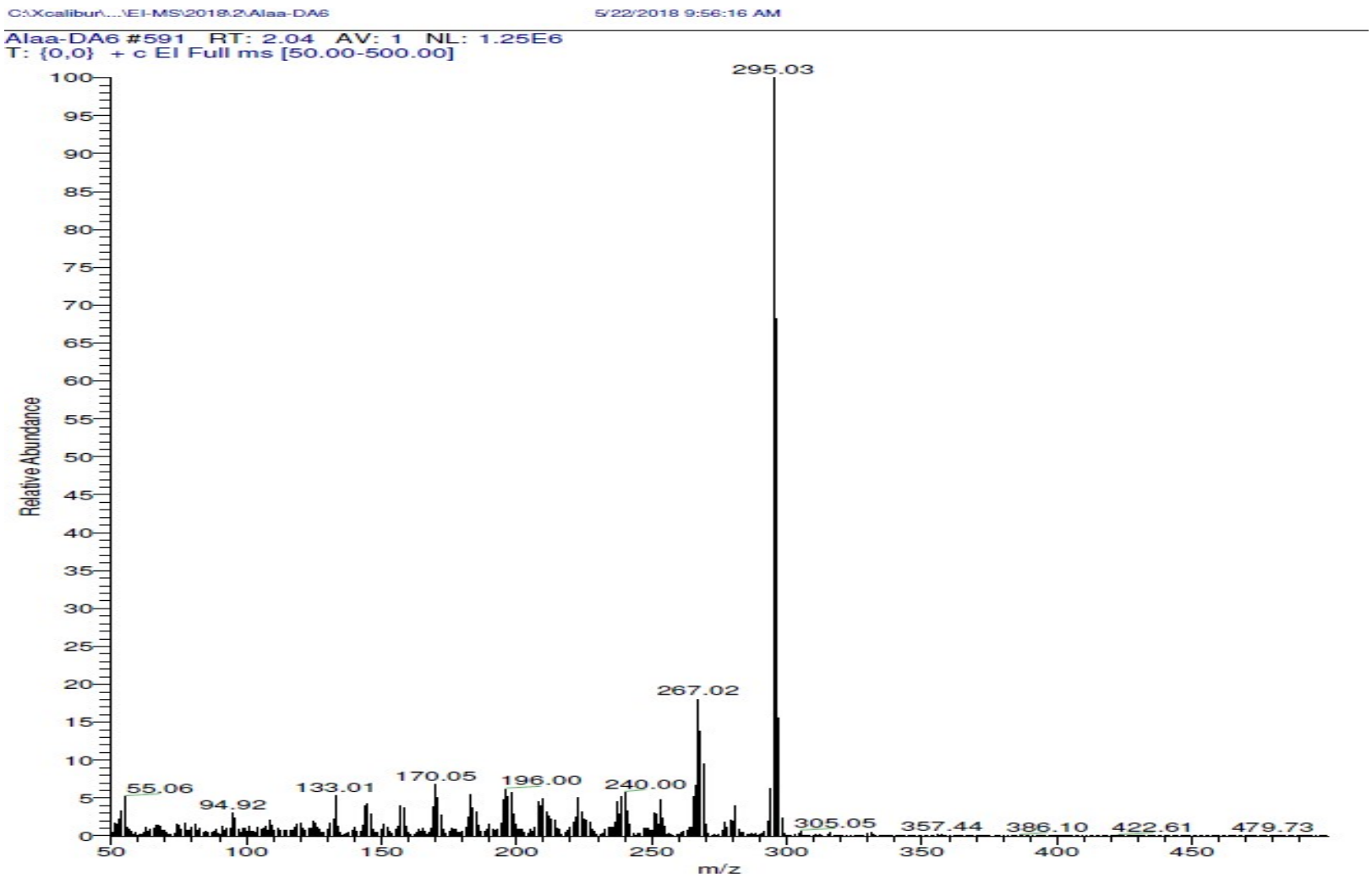
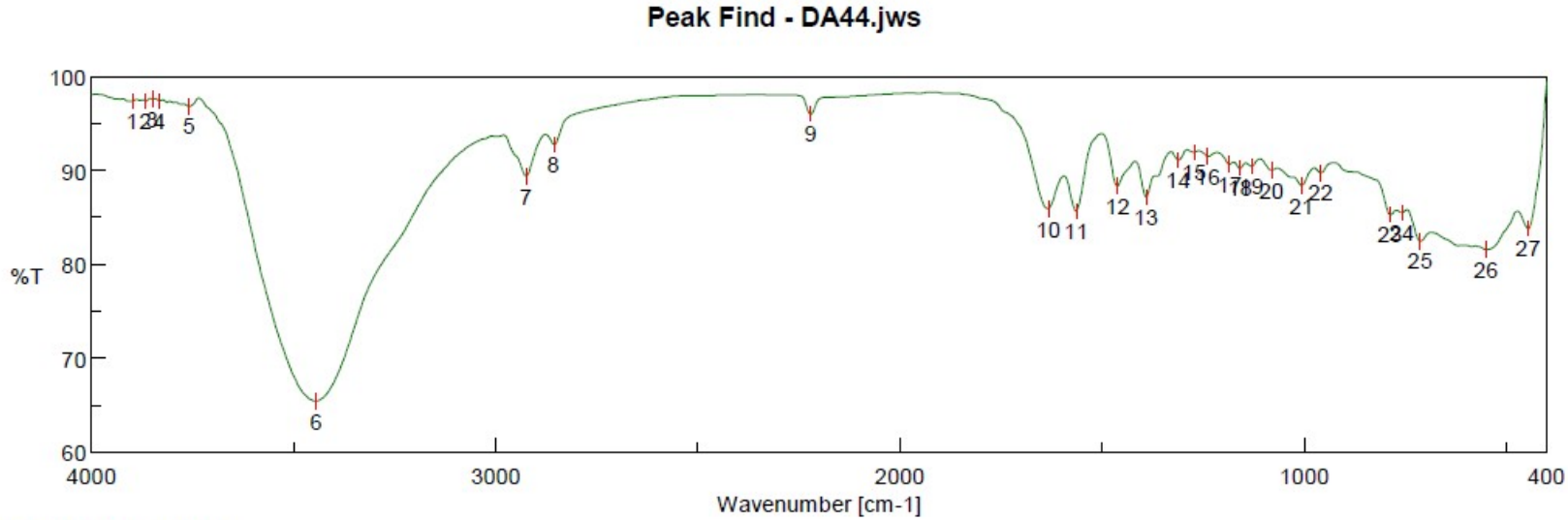


Fig. S20. Mass spectrum of compound **3e**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3899.36	97.3476	2	3864.65	97.3727	3	3849.22	97.5446	4	3831.86	97.3921
5	3758.58	96.7903	6	3444.24	65.4102	7	2925.48	89.3844	8	2856.06	92.7654
9	2219.67	95.9839	10	1633.41	85.8884	11	1563.99	85.6607	12	1461.78	88.2979
13	1390.42	87.137	14	1311.36	91.0958	15	1272.79	91.9247	16	1238.08	91.4687
17	1184.08	90.6419	18	1159.01	90.2115	19	1130.08	90.4003	20	1079.94	90.02
21	1006.66	88.3911	22	958.448	89.7328	23	786.815	85.2741	24	757.888	85.4919
25	713.533	82.4401	26	549.613	81.5578	27	445.476	83.7627			

Fig. S21. IR spectrum of compound **3f** (KBr pellet).

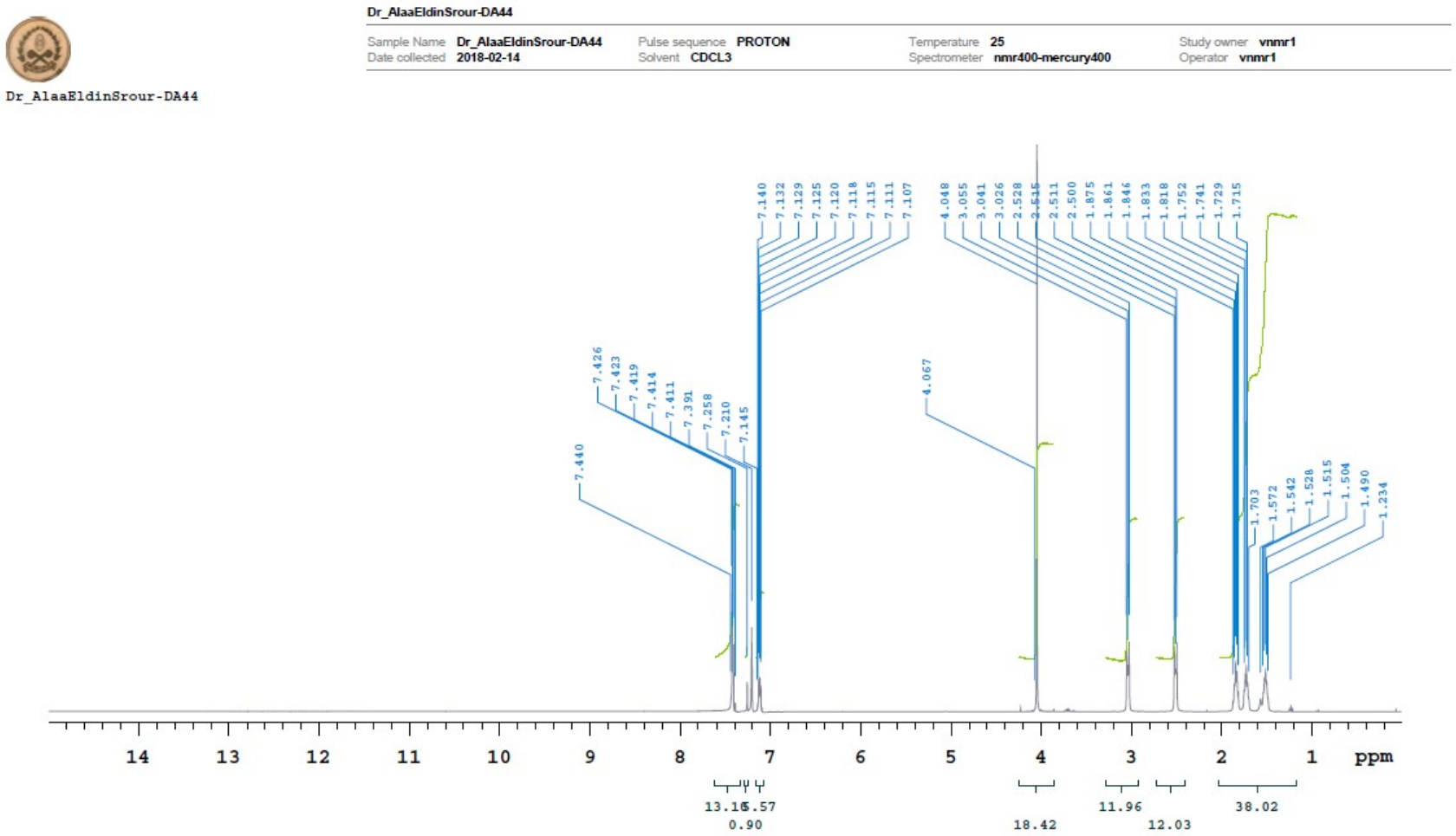


Fig. S22. ^{1}H-NMR spectrum of compound **3f.**

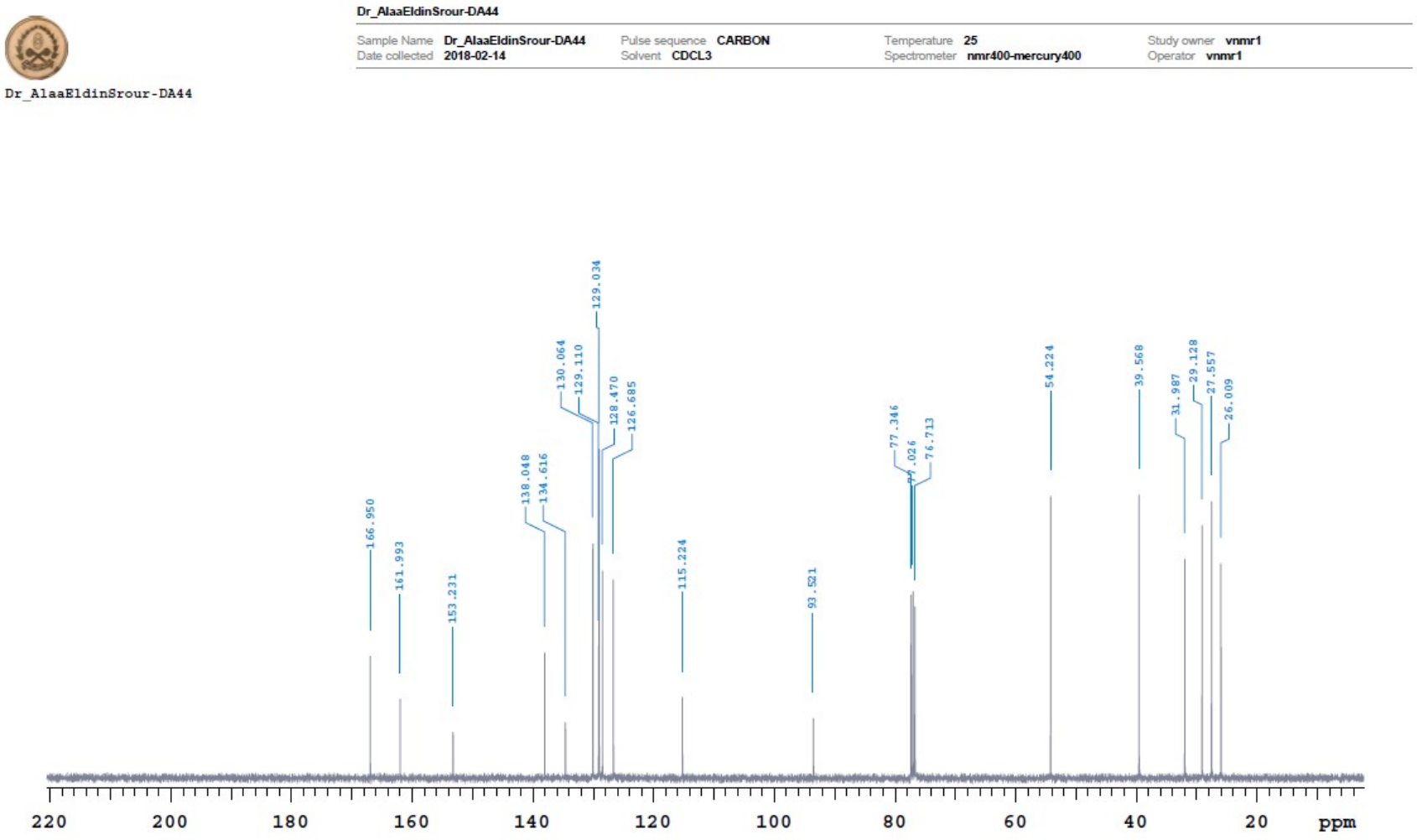


Fig. S23. ^{13}C-NMR spectrum of compound **3f**.

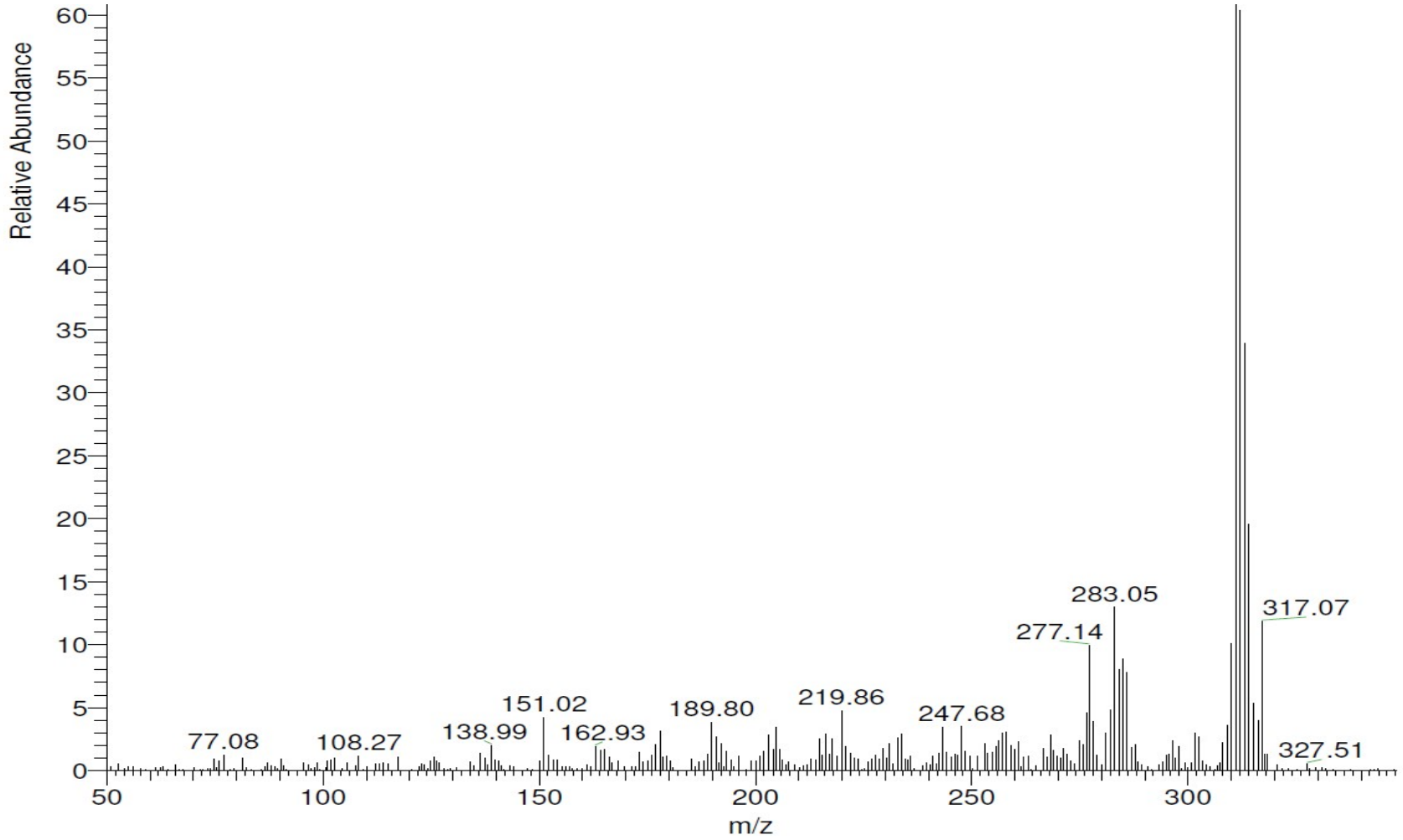
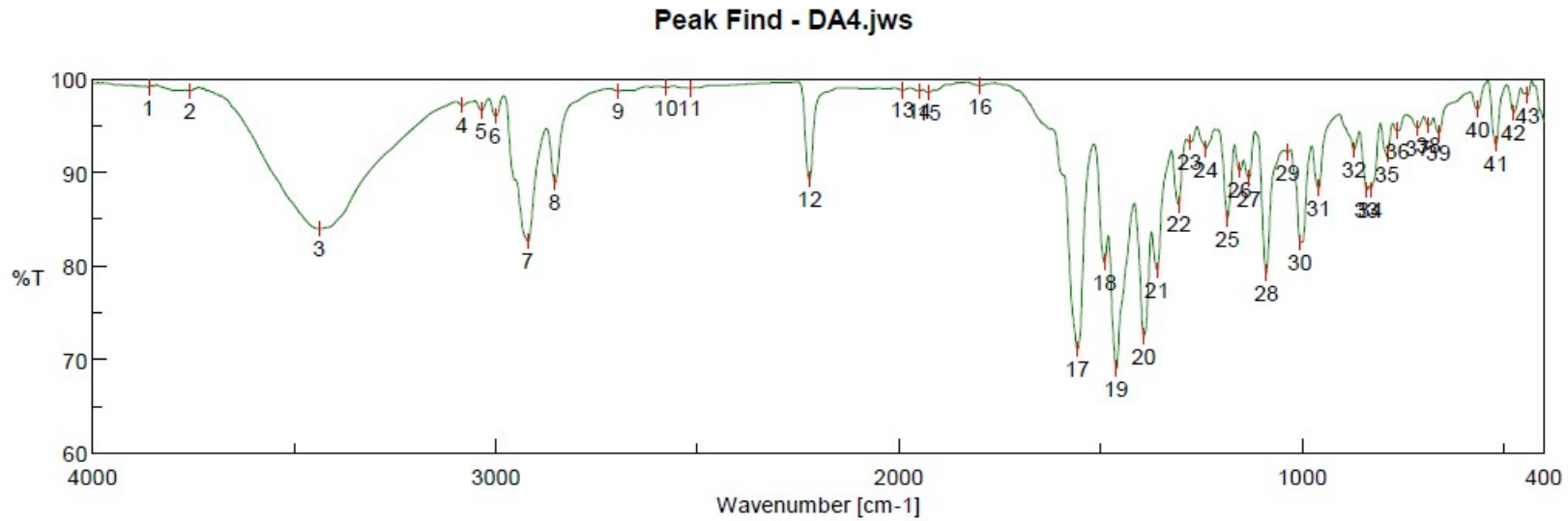


Fig. S24. Mass spectrum of compound **3f**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3860.79	99.0717	2	3758.58	98.689	3	3437.49	83.9715	4	3082.65	97.153
5	3035.41	96.6238	6	3000.69	95.99	7	2920.66	82.5909	8	2853.17	88.8938
9	2696	98.6672	10	2578.36	99.0679	11	2518.58	98.9867	12	2221.59	89.2406
13	1991.14	98.8064	14	1950.64	98.6973	15	1925.57	98.5265	16	1799.26	99.2722
17	1557.24	71.0861	18	1489.74	80.3857	19	1460.81	69.0135	20	1392.35	72.4848
21	1359.57	79.5247	22	1306.54	86.5699	23	1278.57	93.2272	24	1239.04	92.5295
25	1185.04	85.0389	26	1154.19	90.2149	27	1132.01	89.3383	28	1088.62	79.2286
29	1035.59	92.147	30	1004.73	82.4747	31	959.412	88.3325	32	870.703	92.4181
33	839.847	88.1219	34	830.205	88.1387	35	788.743	91.878	36	762.709	94.4349
37	712.569	94.7473	38	686.534	95.0373	39	661.464	94.2506	40	565.041	96.7741
41	518.758	93.0068	42	475.367	96.3587	43	439.69	98.2441			

Fig. S25. IR spectrum of compound **3g** (KBr pellet).

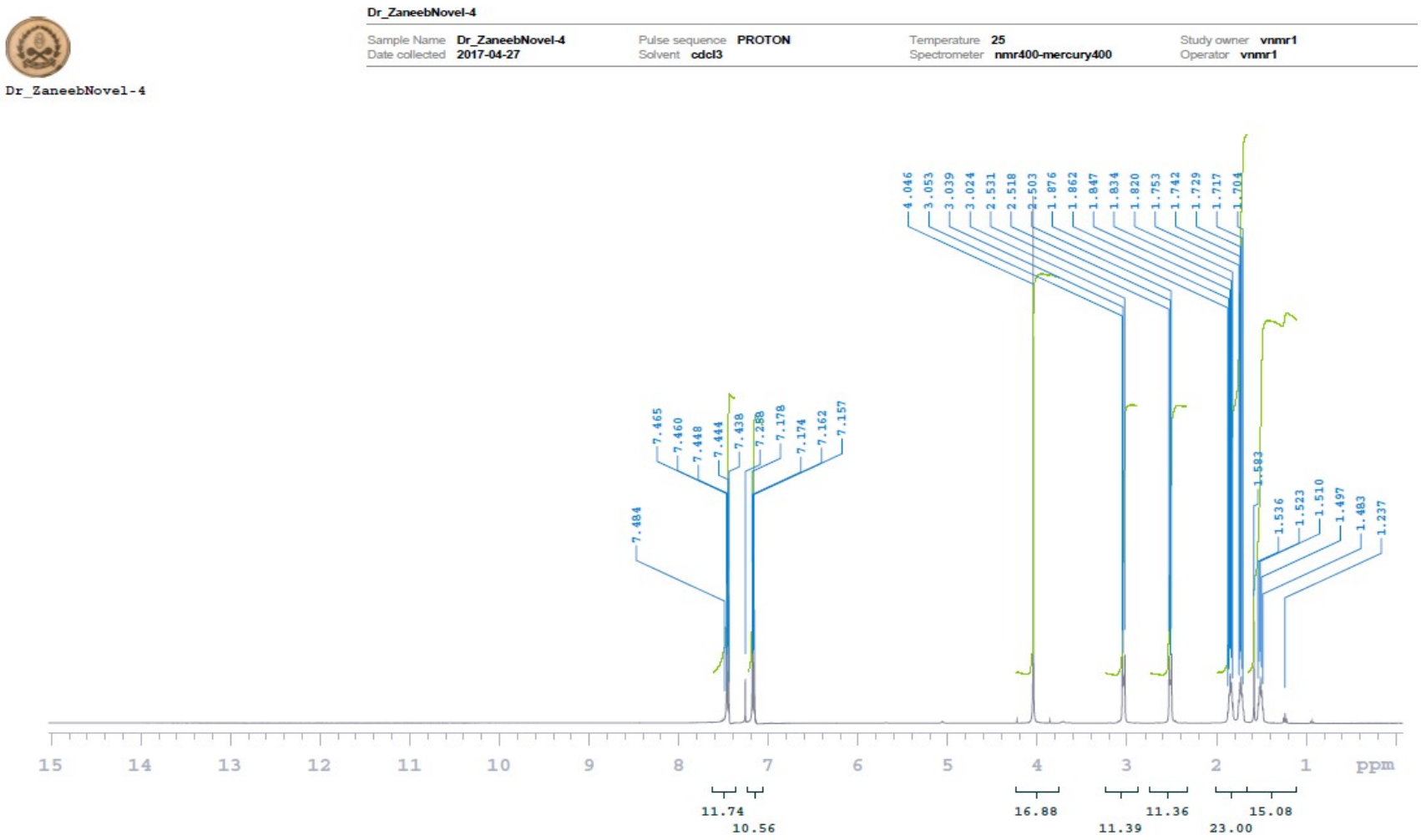


Fig. S26. ^{1}H-NMR spectrum of compound **3g**.

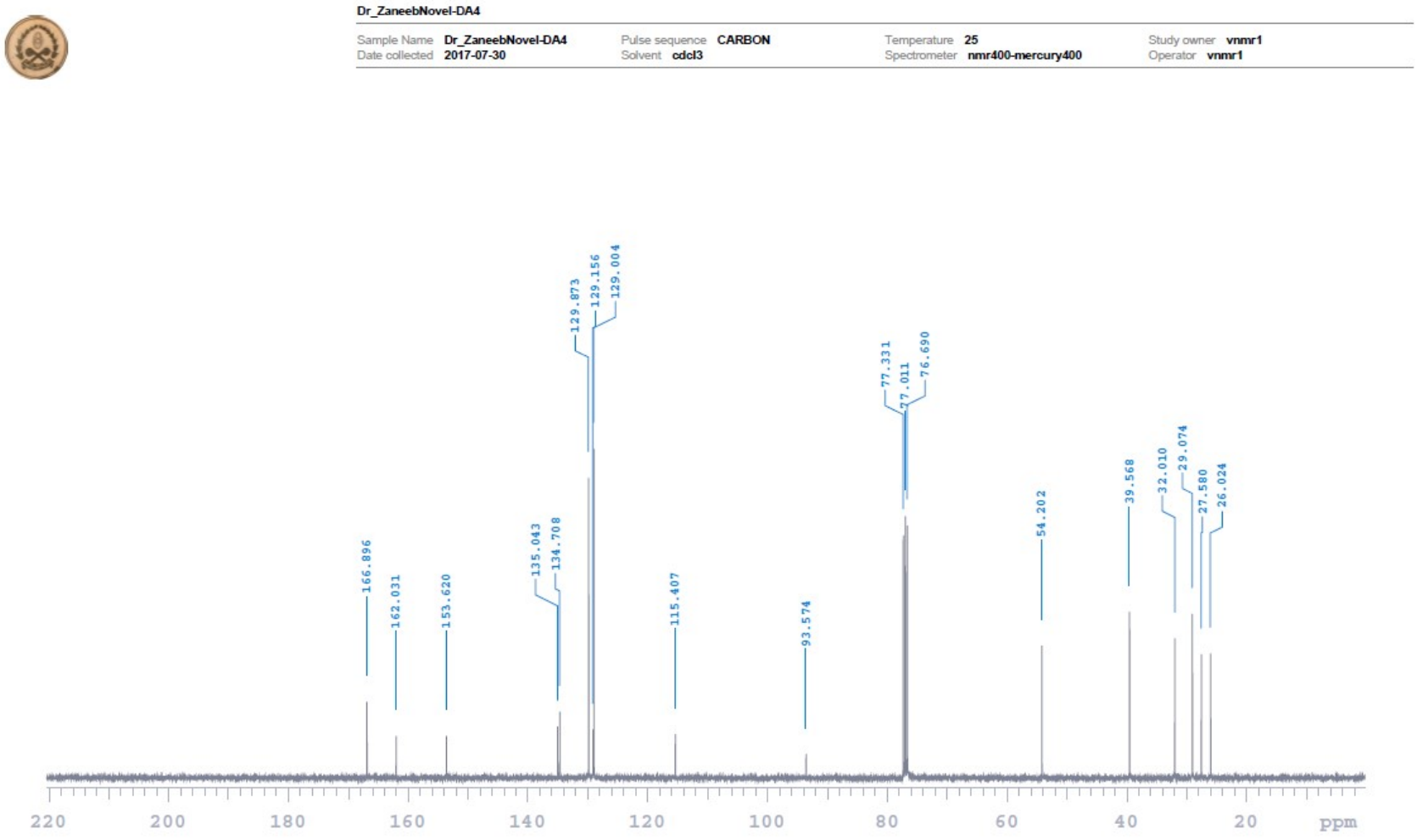


Fig. S27. ^{13}C-NMR spectrum of compound **3g**.

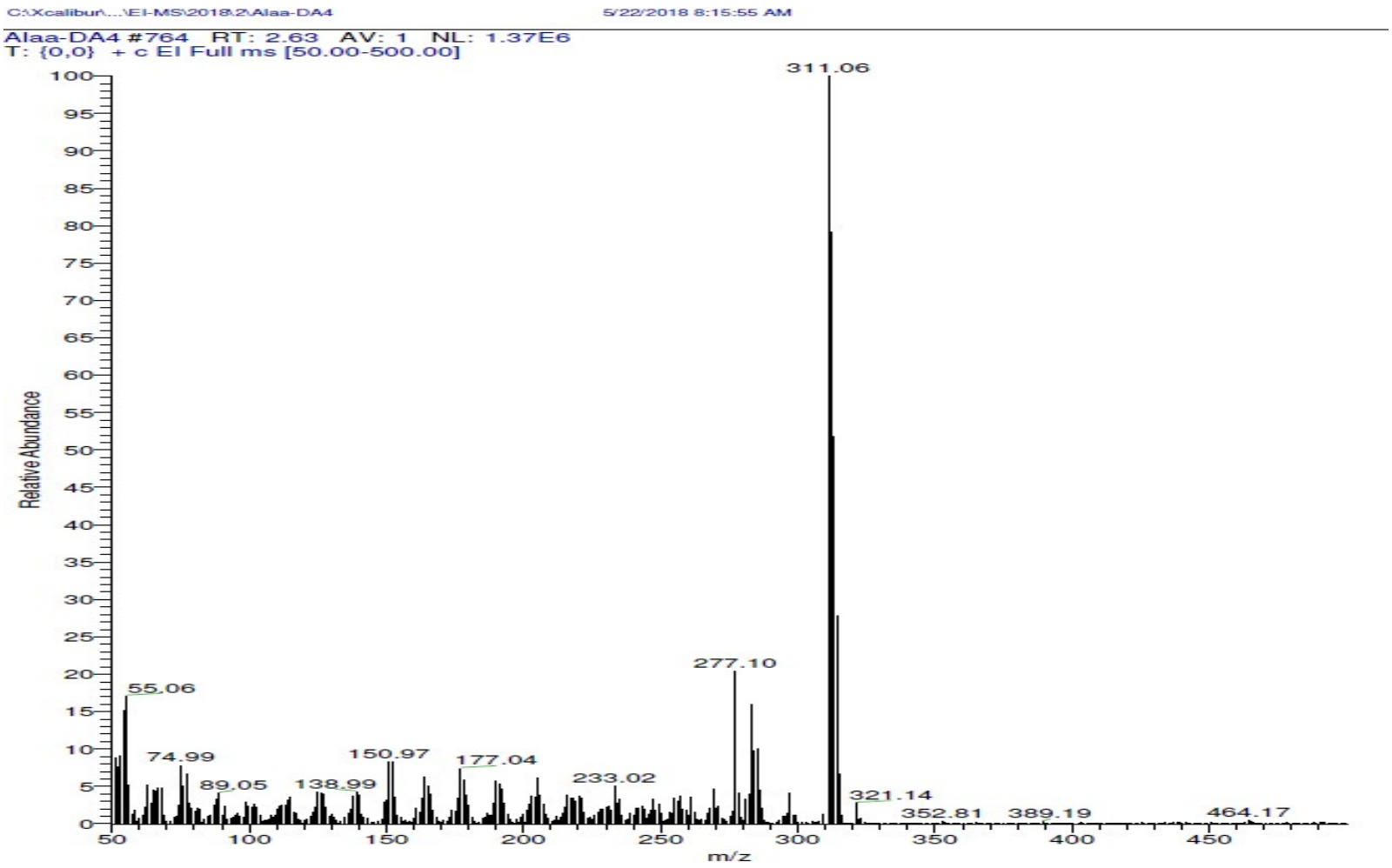
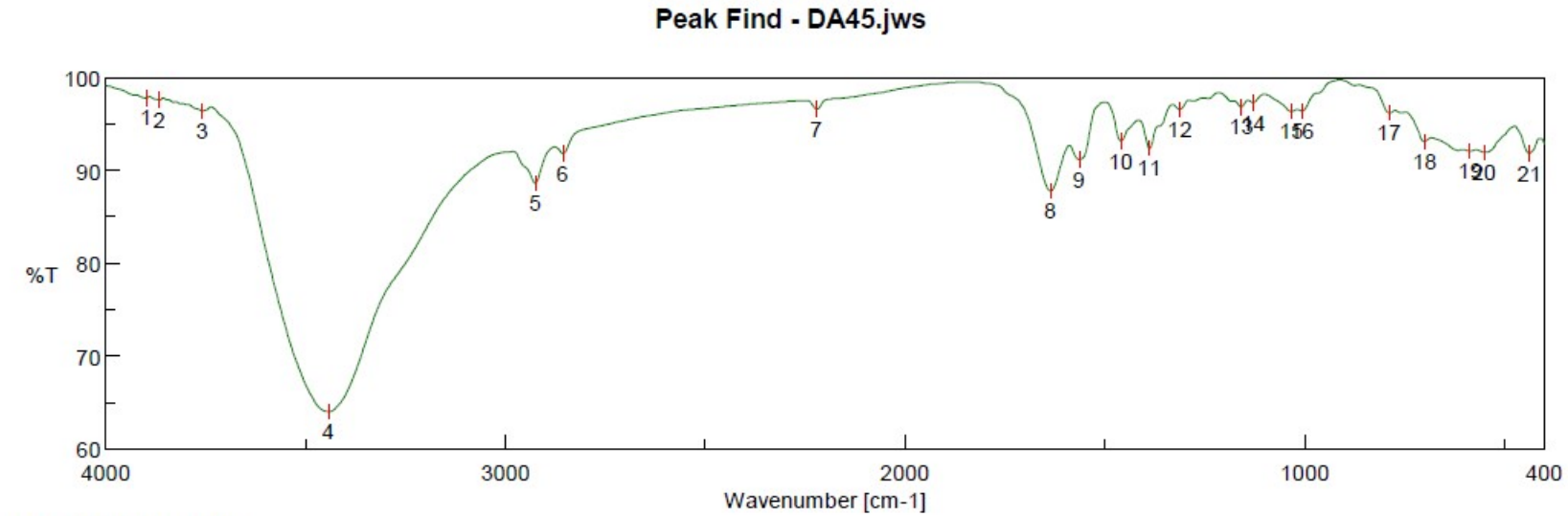


Fig. S28. Mass spectrum of compound **3g**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3899.36	97.7987	2	3864.65	97.5661	3	3758.58	96.3737	4	3442.31	63.9729
5	2925.48	88.6056	6	2856.06	91.7969	7	2221.59	96.5837	8	1635.34	87.7782
9	1563.99	91.159	10	1459.85	93.1623	11	1388.5	92.3666	12	1313.29	96.5502
13	1159.01	96.831	14	1128.15	97.2734	15	1033.66	96.3203	16	1006.66	96.3928
17	788.743	96.1955	18	700.034	93.0945	19	586.254	92.0608	20	551.542	91.9394
21	437.762	91.8065									

Fig. S29. IR spectrum of compound **3h** (KBr pellet).

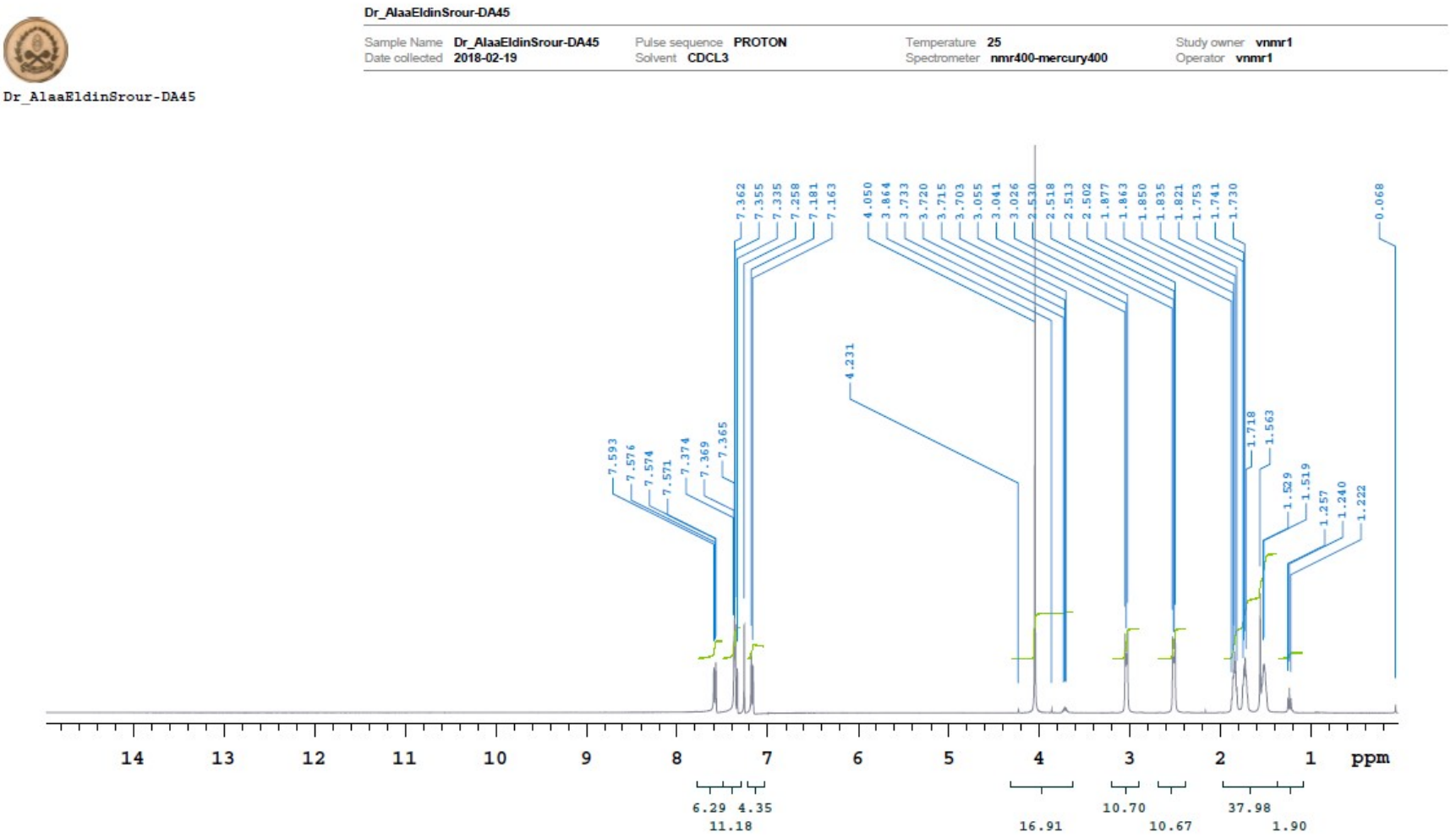


Fig. S30. ^{1}H-NMR spectrum of compound **3h**.

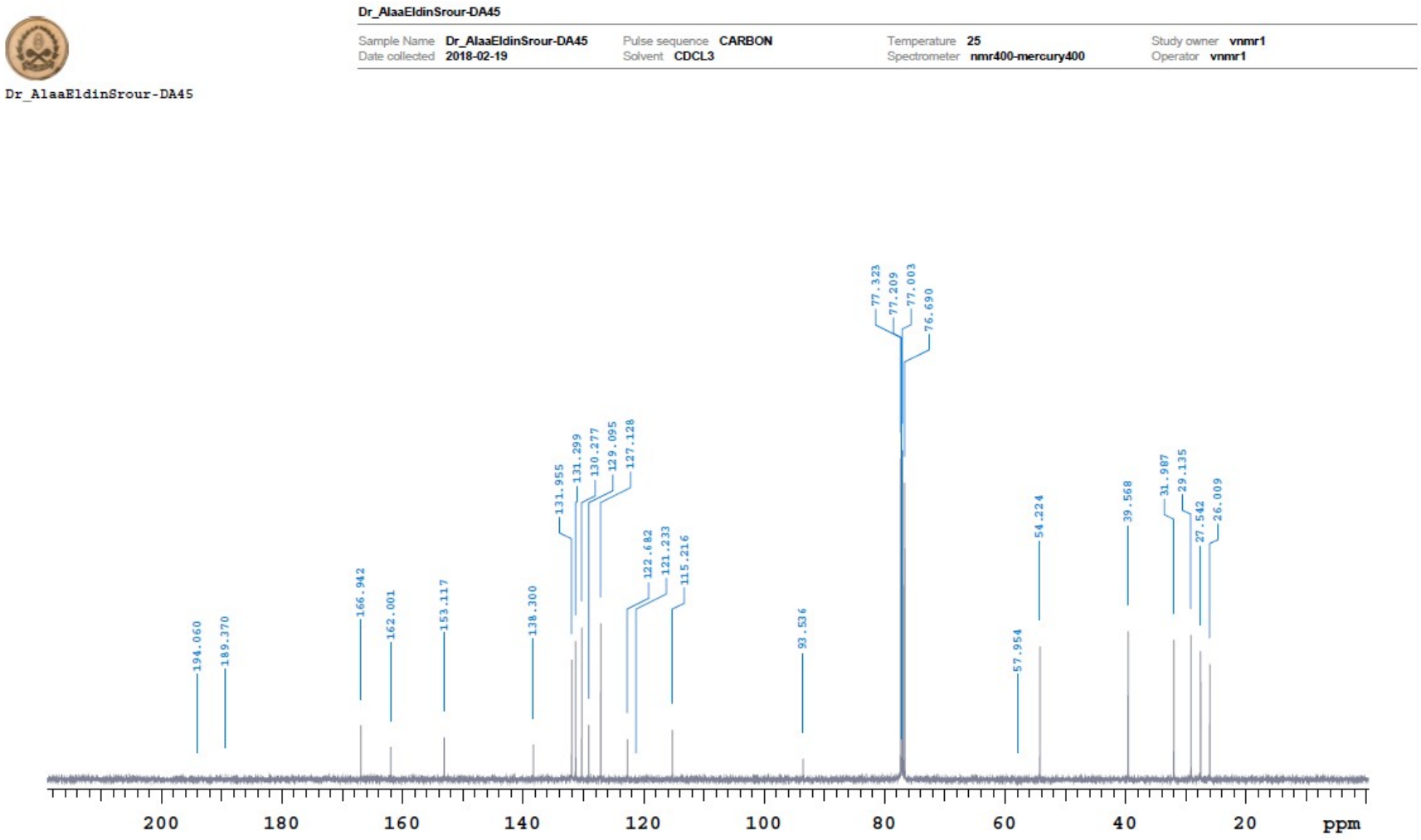


Fig. S31. ^{13}C-NMR spectrum of compound **3h**.

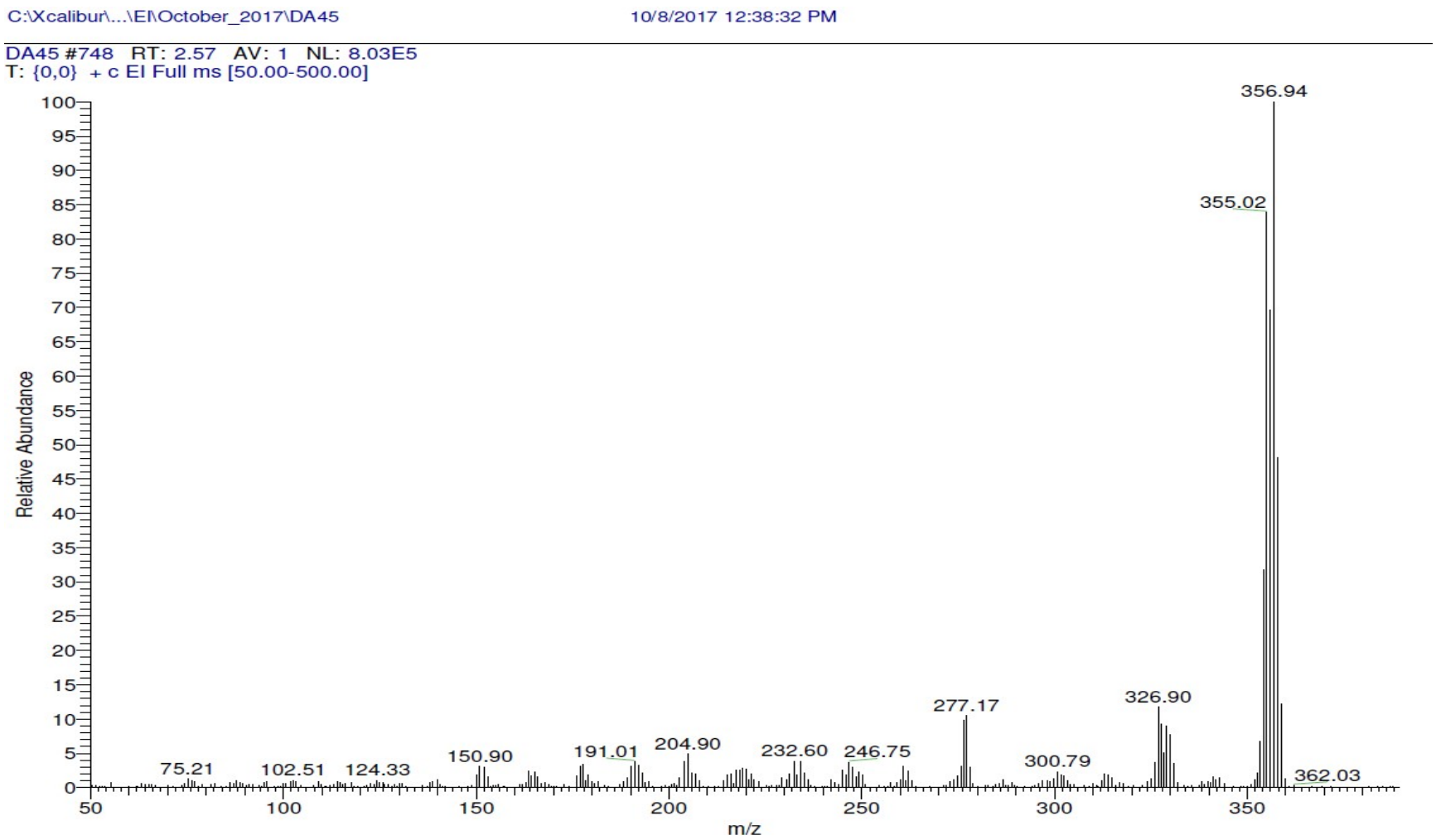
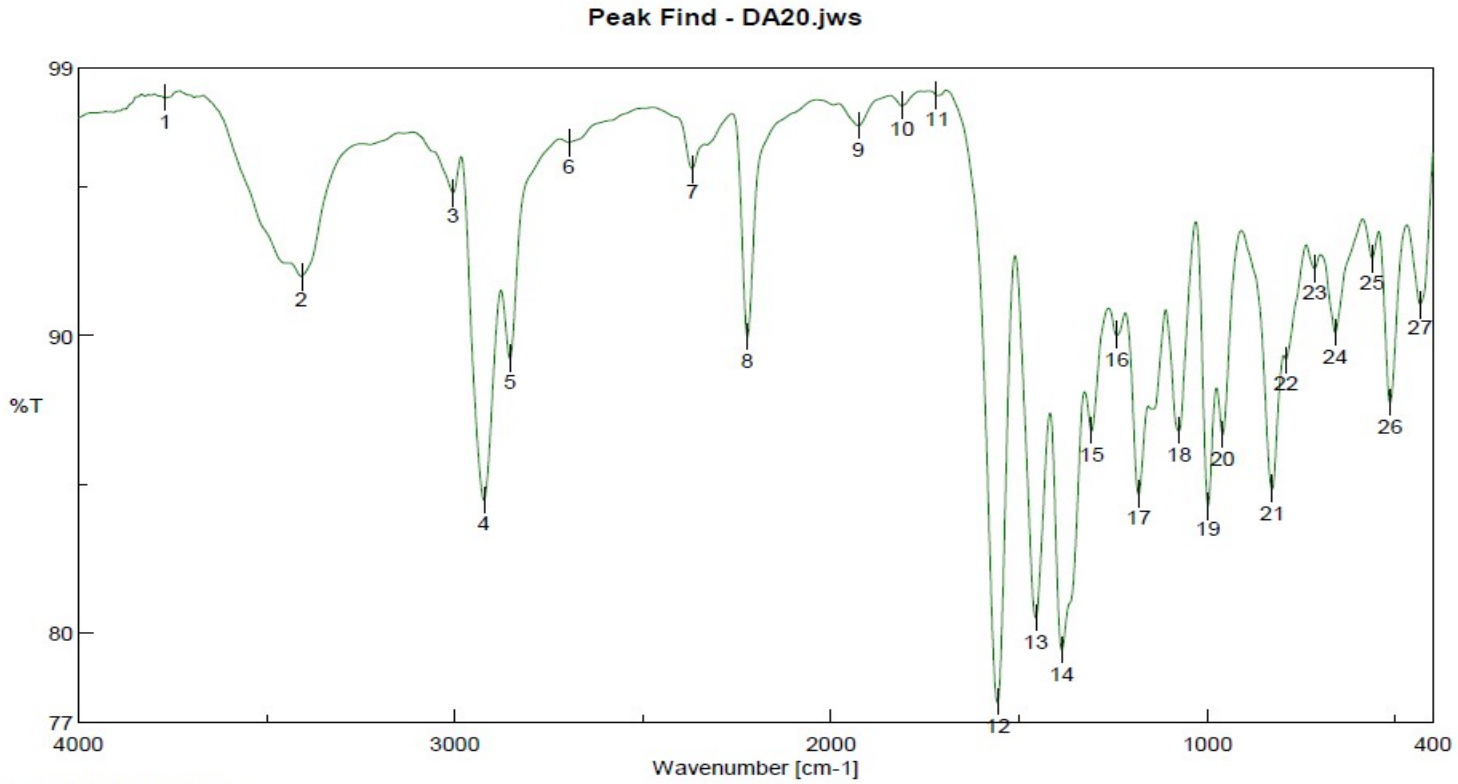


Fig. S32. Mass spectrum of compound **3h**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3770.15	97.9866	2	3408.57	91.9936	3	3004.55	94.8054	4	2922.59	84.4686
5	2853.17	89.2255	6	2697.93	96.4971	7	2369.12	95.6231	8	2222.56	89.9538
9	1927.5	97.0597	10	1811.79	97.7274	11	1718.26	98.0631	12	1557.24	77.67
13	1455.99	80.5124	14	1386.57	79.4276	15	1307.5	86.7831	16	1240.97	90.0013
17	1182.15	84.6582	18	1075.12	86.7866	19	997.982	84.2855	20	959.412	86.6754
21	827.312	84.8343	22	790.671	89.1878	23	714.497	92.2491	24	659.536	90.0921
25	562.148	92.5971	26	513.936	87.7294	27	433.905	91.048			

Fig. S33. IR spectrum of compound **3i** (KBr pellet).

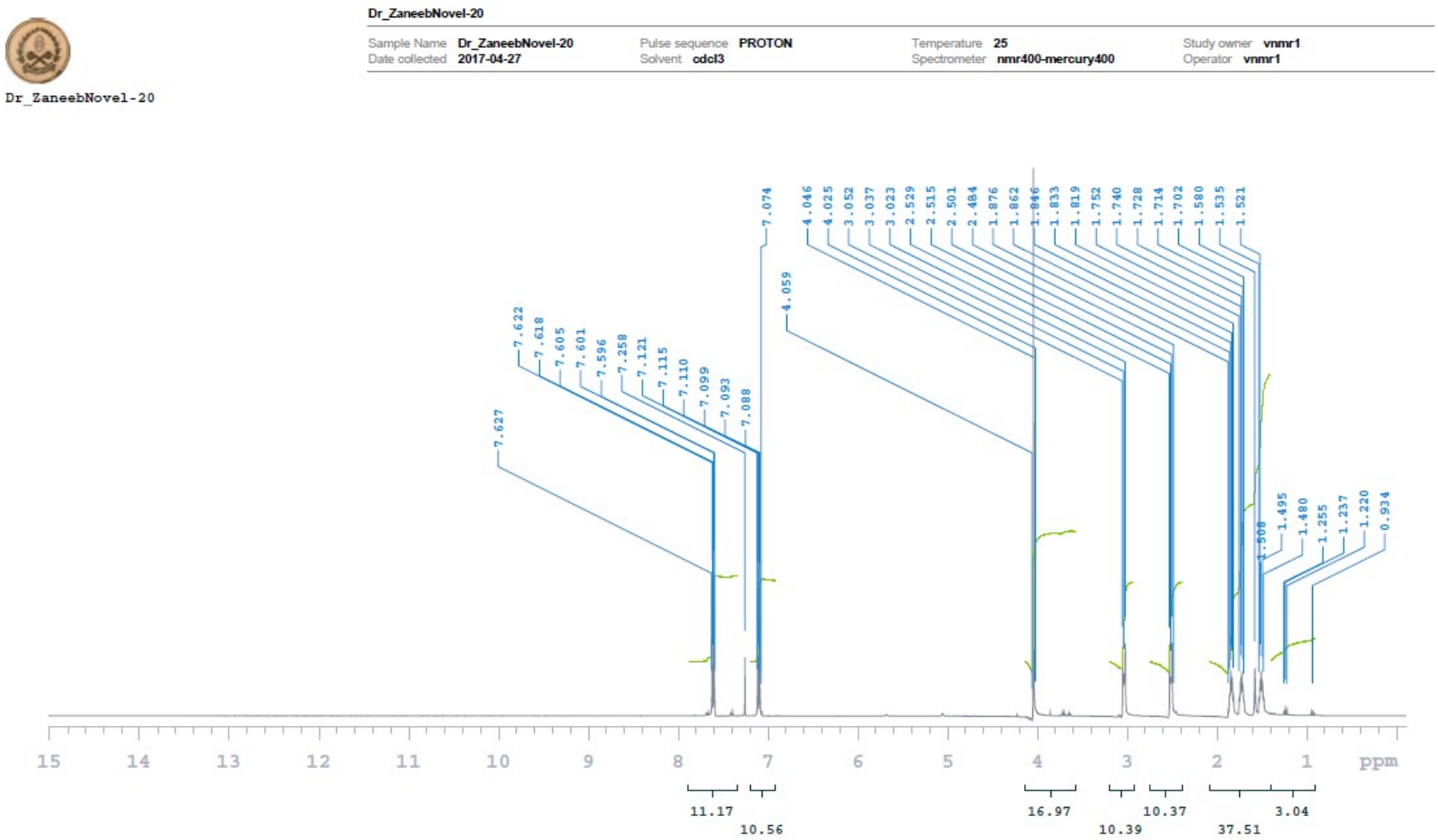


Fig. S34. [1]H-NMR spectrum of compound **3i**.

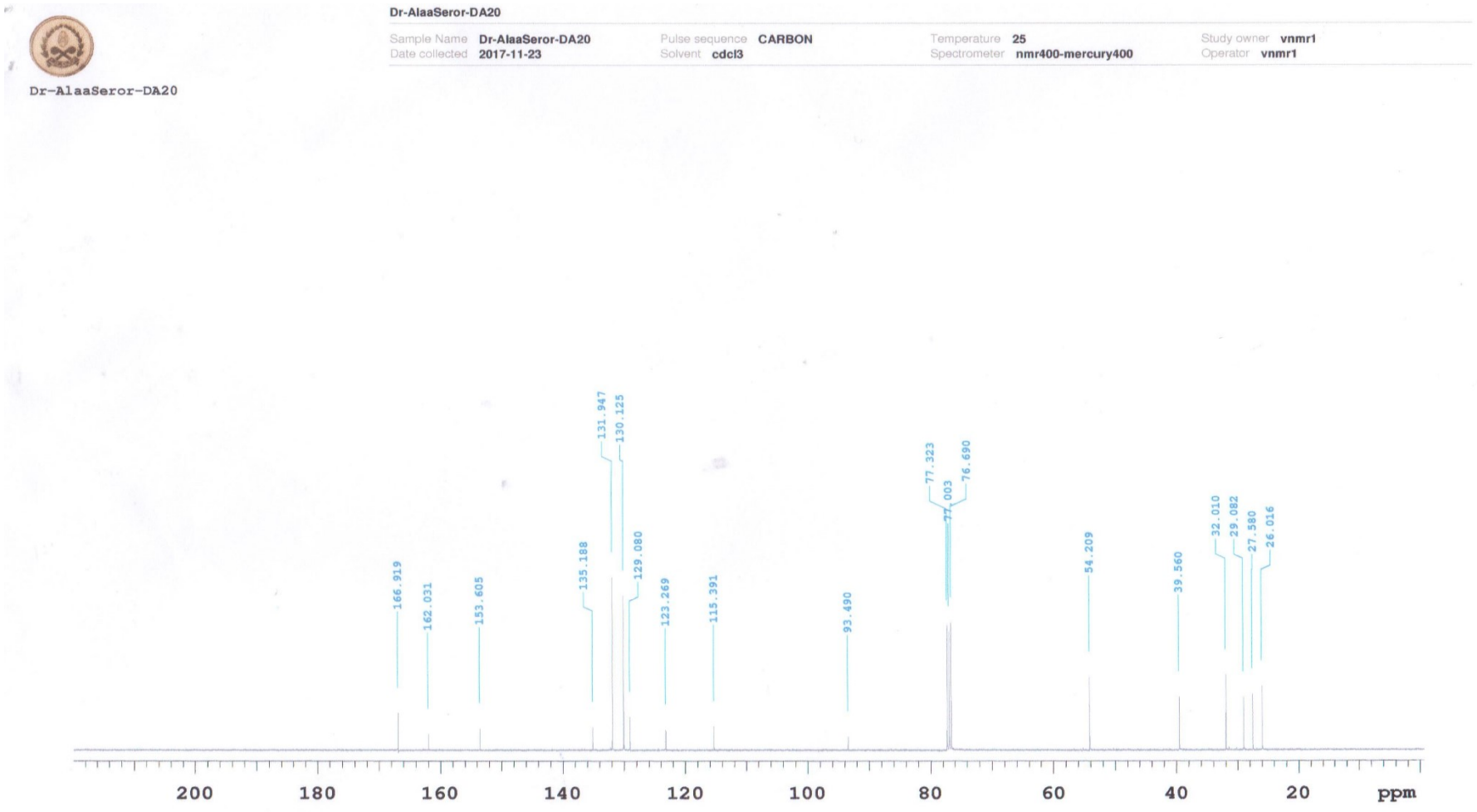


Fig. S35. ^{13}C-NMR spectrum of compound **3i**.

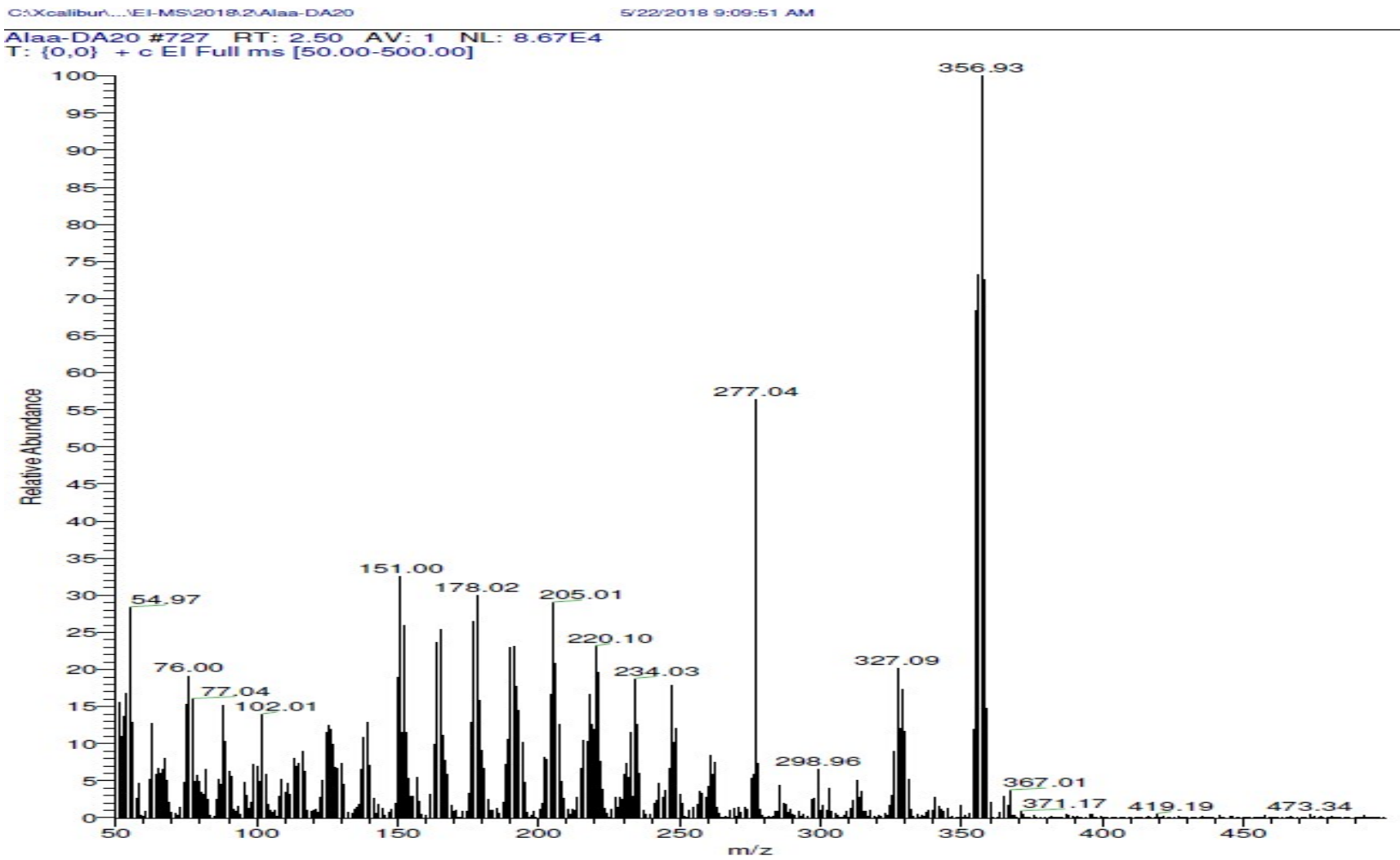
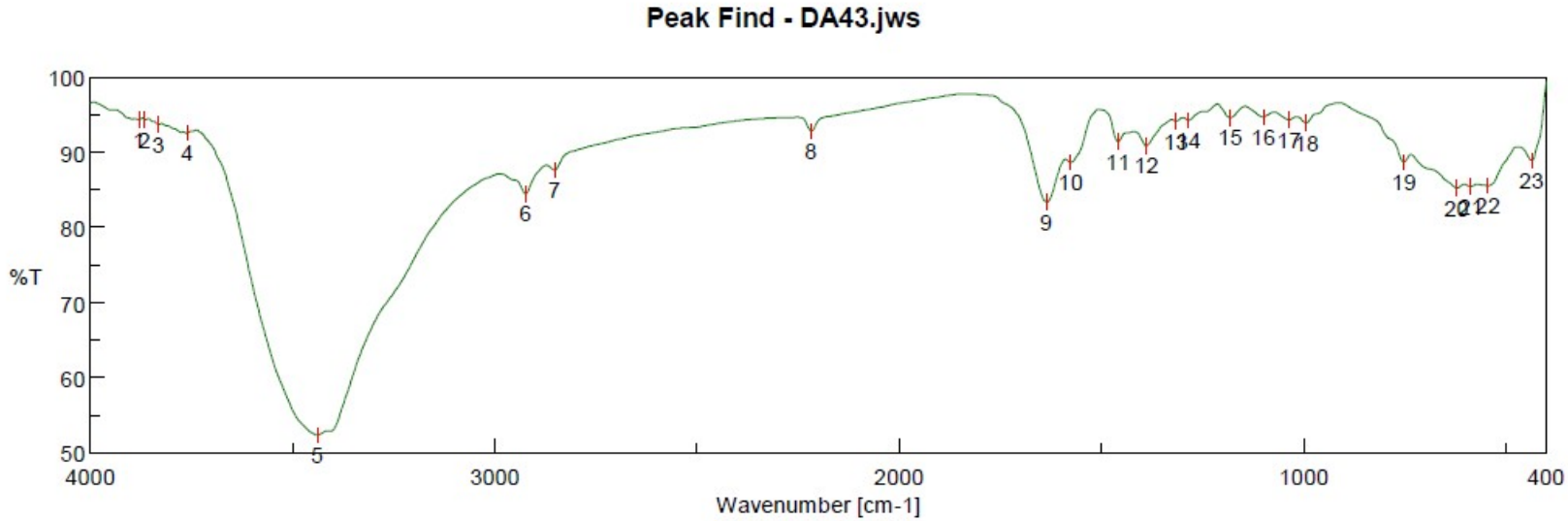


Fig. S36. Mass spectrum of compound **3i**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3880.08	94.3346	2	3864.65	94.3254	3	3831.86	93.7073	4	3760.51	92.498
5	3438.46	52.3201	6	2923.56	84.4766	7	2852.2	87.6113	8	2217.74	92.8349
9	1635.34	83.3429	10	1575.56	88.6322	11	1457.92	91.4122	12	1388.5	90.8307
13	1315.21	94.1082	14	1284.36	94.2627	15	1182.15	94.5749	16	1099.23	94.6607
17	1035.59	94.3143	18	993.16	93.9012	19	752.102	88.6809	20	620.966	85.2241
21	586.254	85.4394	22	543.828	85.5131	23	435.834	88.9224			

Fig. S37. IR spectrum of compound **3j** (KBr pellet).

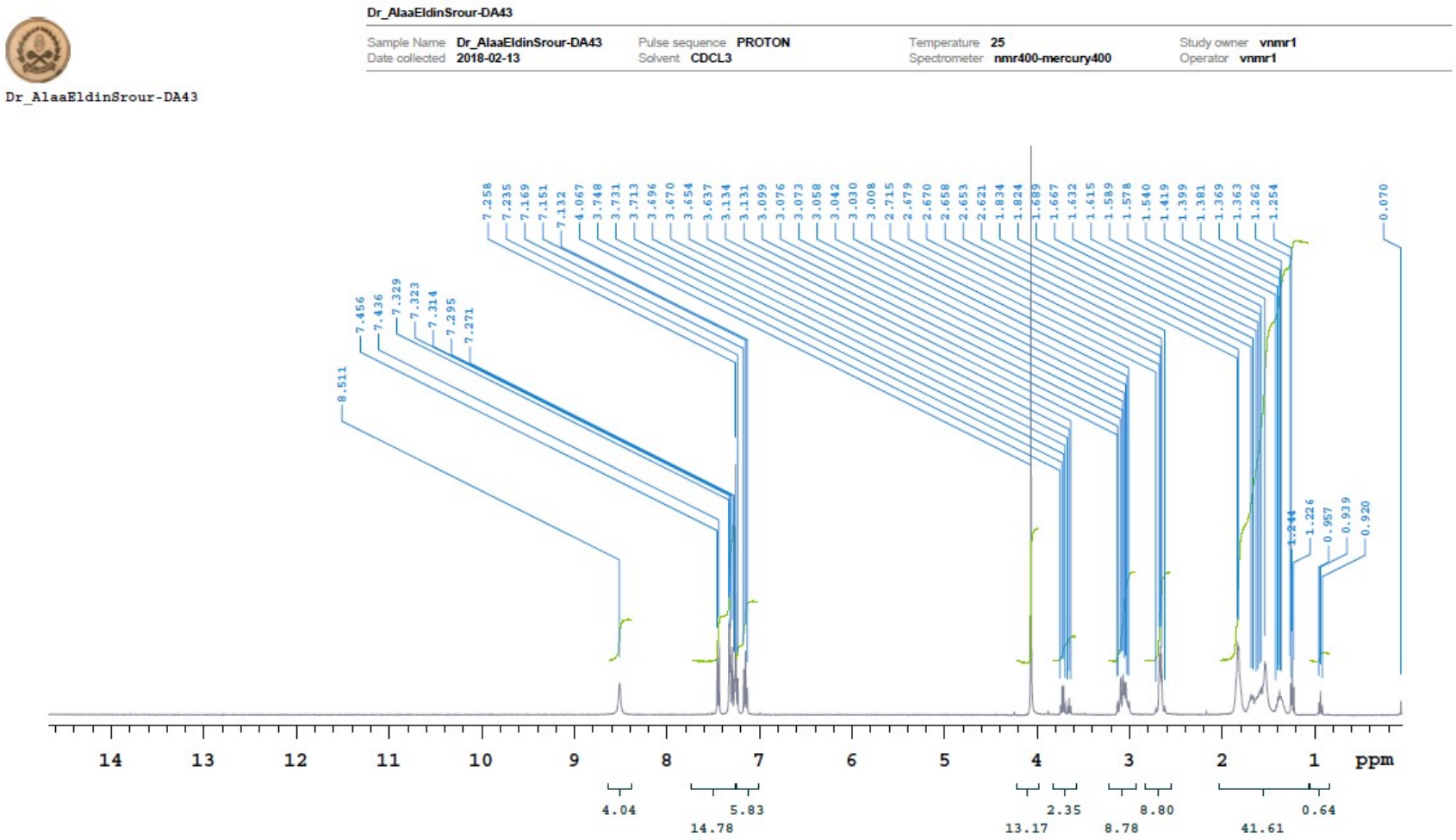


Fig. S38. ^{1}H-NMR spectrum of compound **3j**.

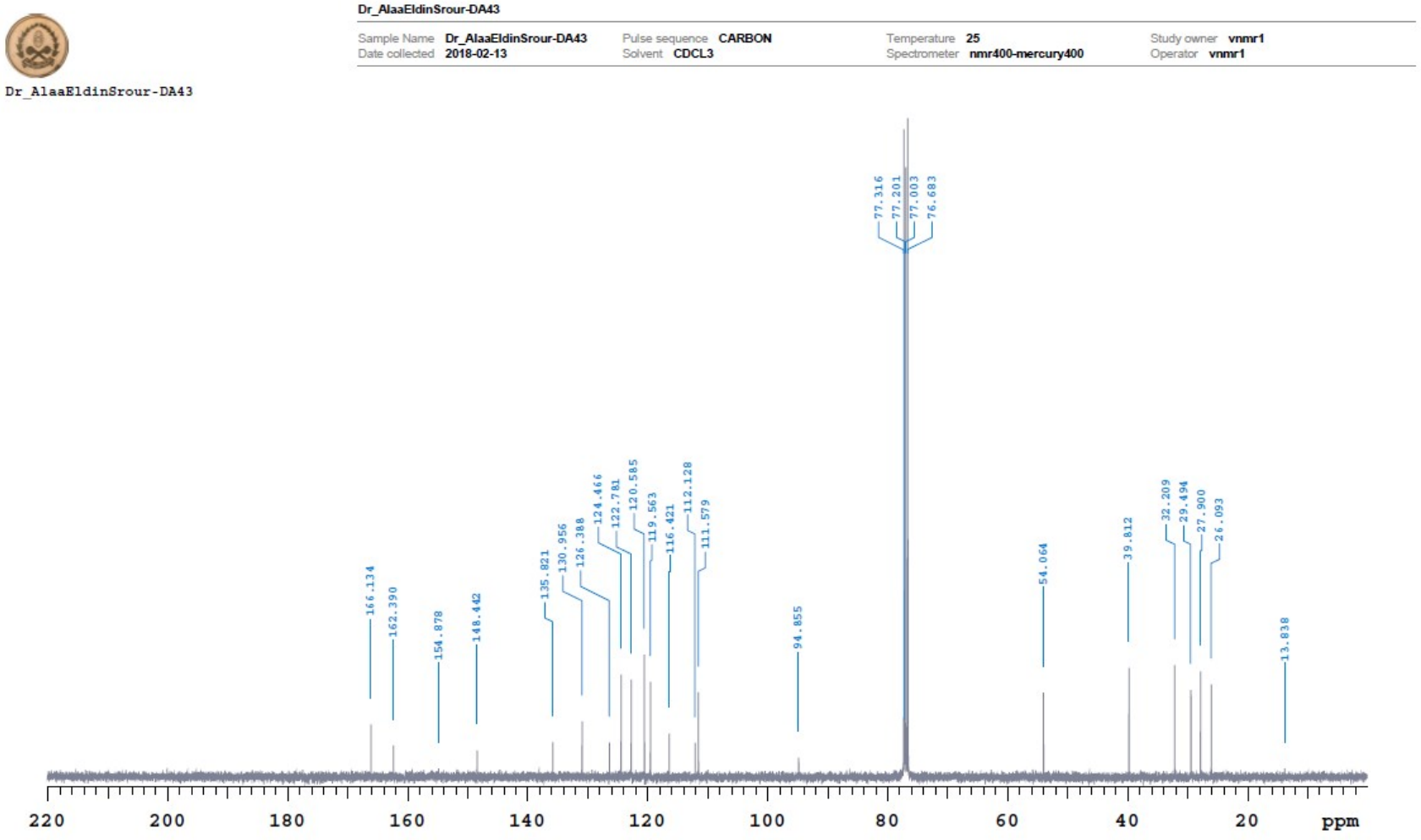


Fig. S39. ^{13}C-NMR spectrum of compound **3j**.

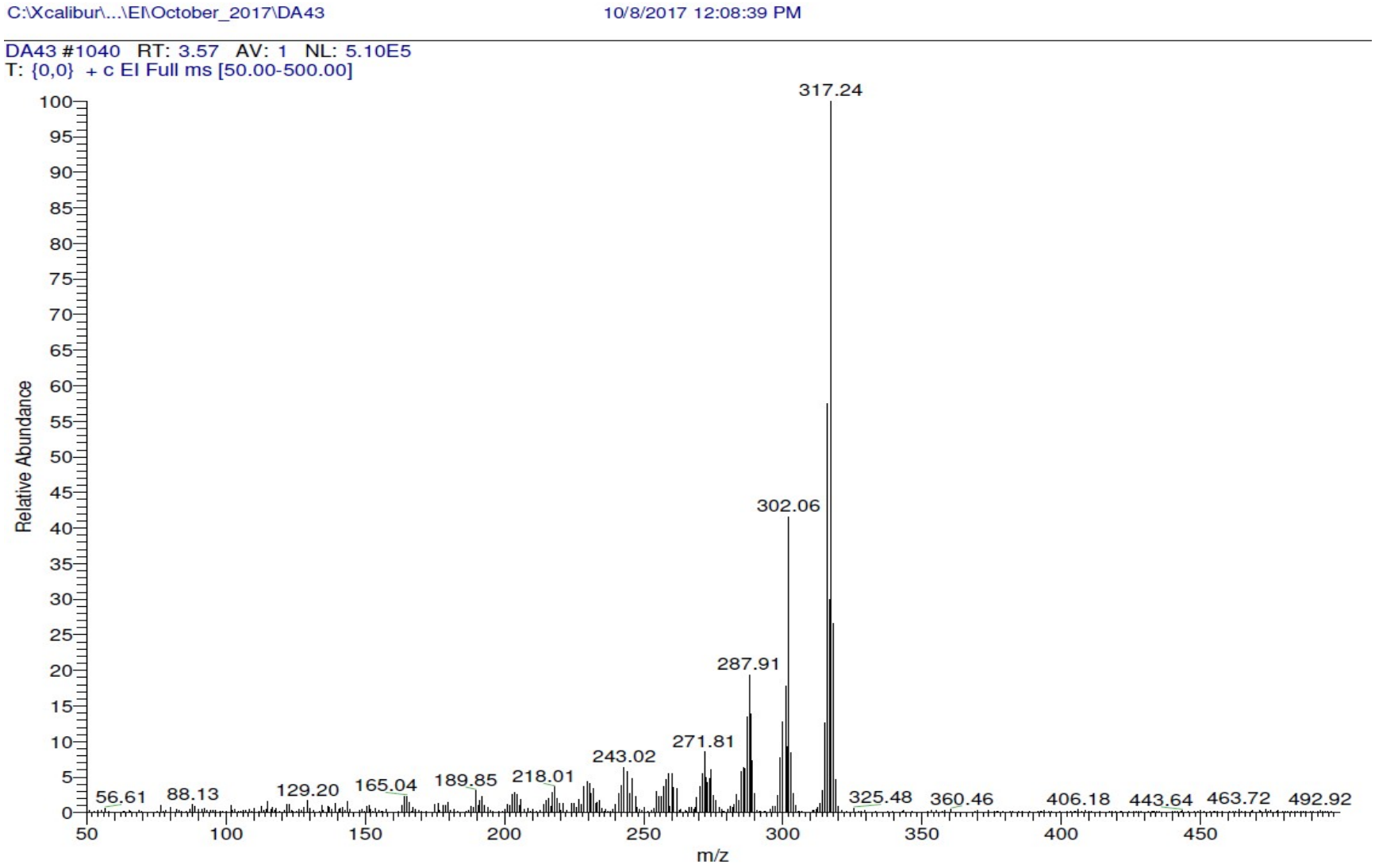
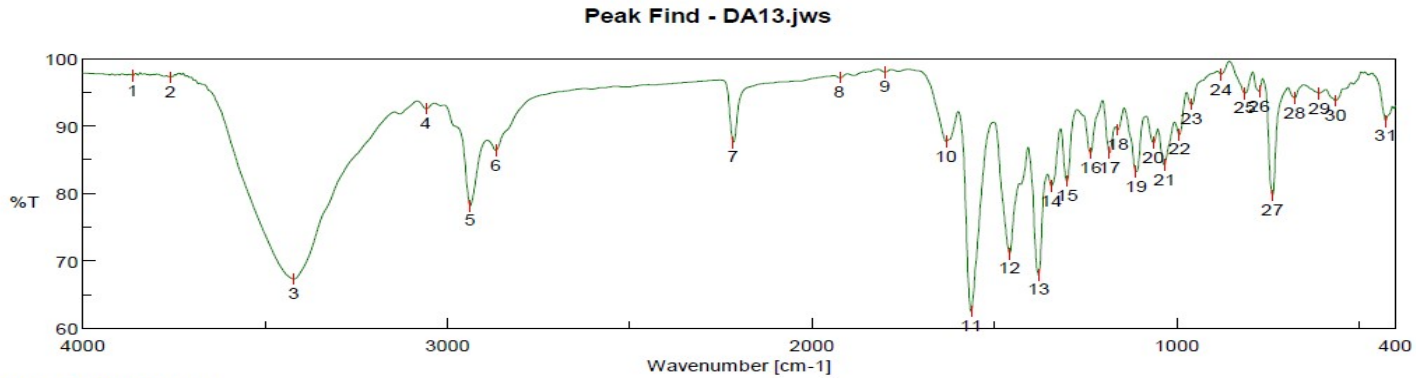


Fig. S40. Mass spectrum of compound **3j**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3862.72	97.4377	2	3759.55	97.2406	3	3421.1	67.2685	4	3058.55	92.5383
5	2937.06	78.1484	6	2867.63	86.3657	7	2215.81	87.5908	8	1924.61	97.1455
9	1800.22	97.963	10	1632.45	87.6664	11	1563.99	62.4717	12	1457.92	71.0682
13	1378.85	67.8793	14	1344.14	81.0809	15	1300.75	81.8005	16	1236.15	85.9377
17	1184.08	86.037	18	1160.94	89.4016	19	1110.8	83.2278	20	1063.55	87.4972
21	1033.66	84.1988	22	992.196	88.7148	23	958.448	93.1825	24	878.417	97.6438
25	812.849	94.8353	26	773.315	95.112	27	736.674	79.7234	28	675.928	94.1492
29	609.396	94.8024	30	565.041	93.7502	31	426.191	90.7289			

Fig. S41. IR spectrum of compound **3k** (KBr pellet).

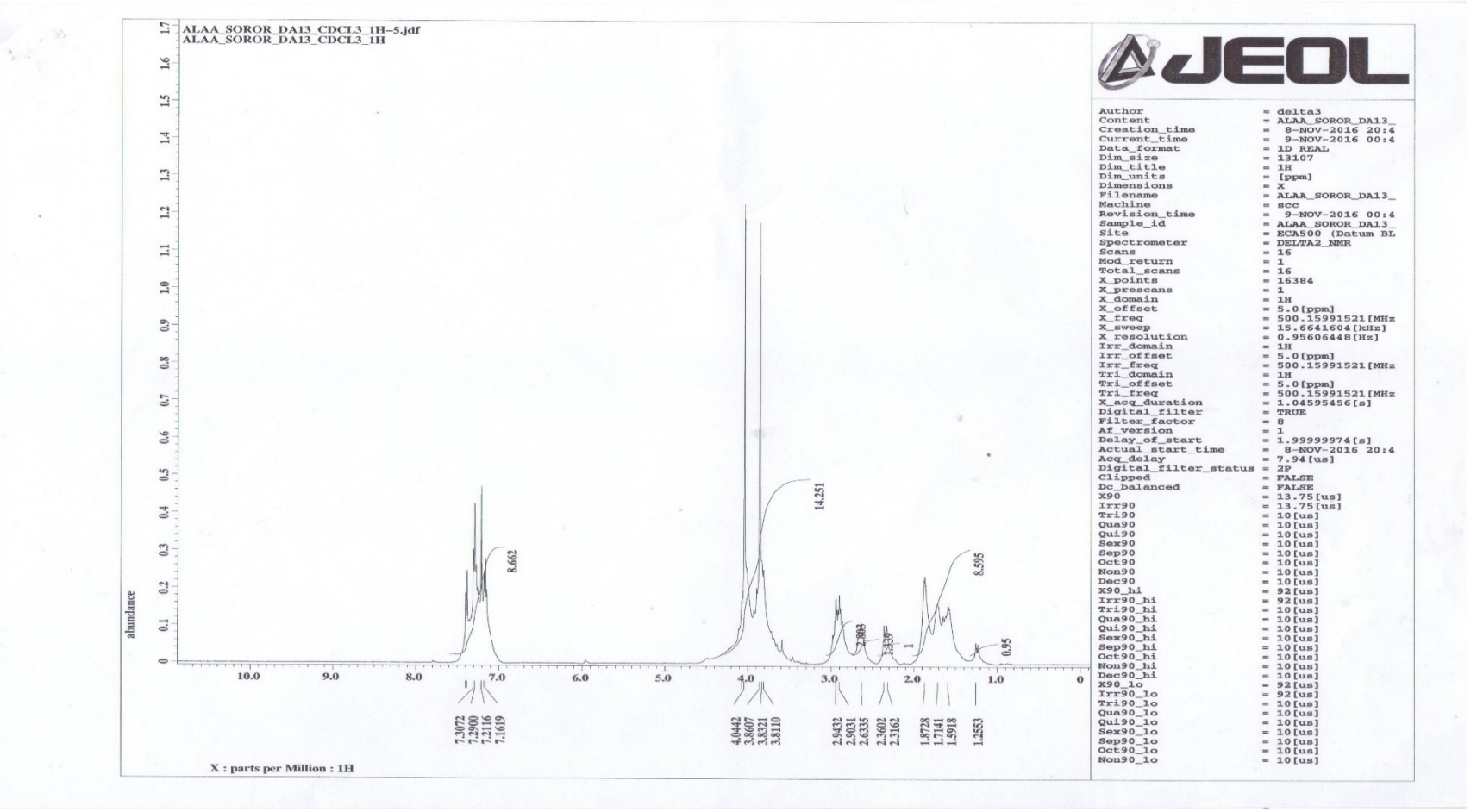


Fig. S42. ^{1}H-NMR spectrum of compound **3k**.

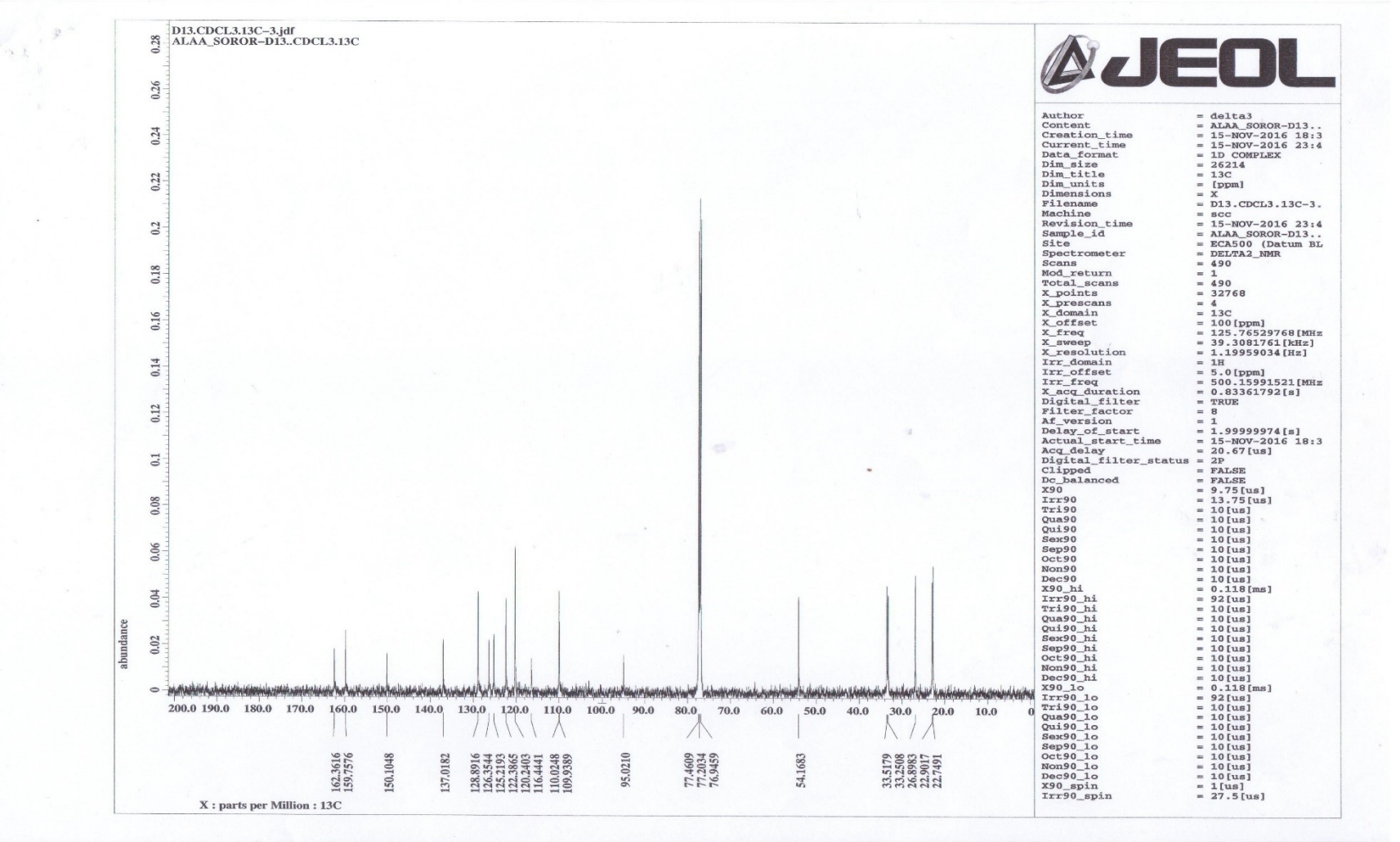


Fig. S43. ^{13}C-NMR spectrum of compound **3k**.

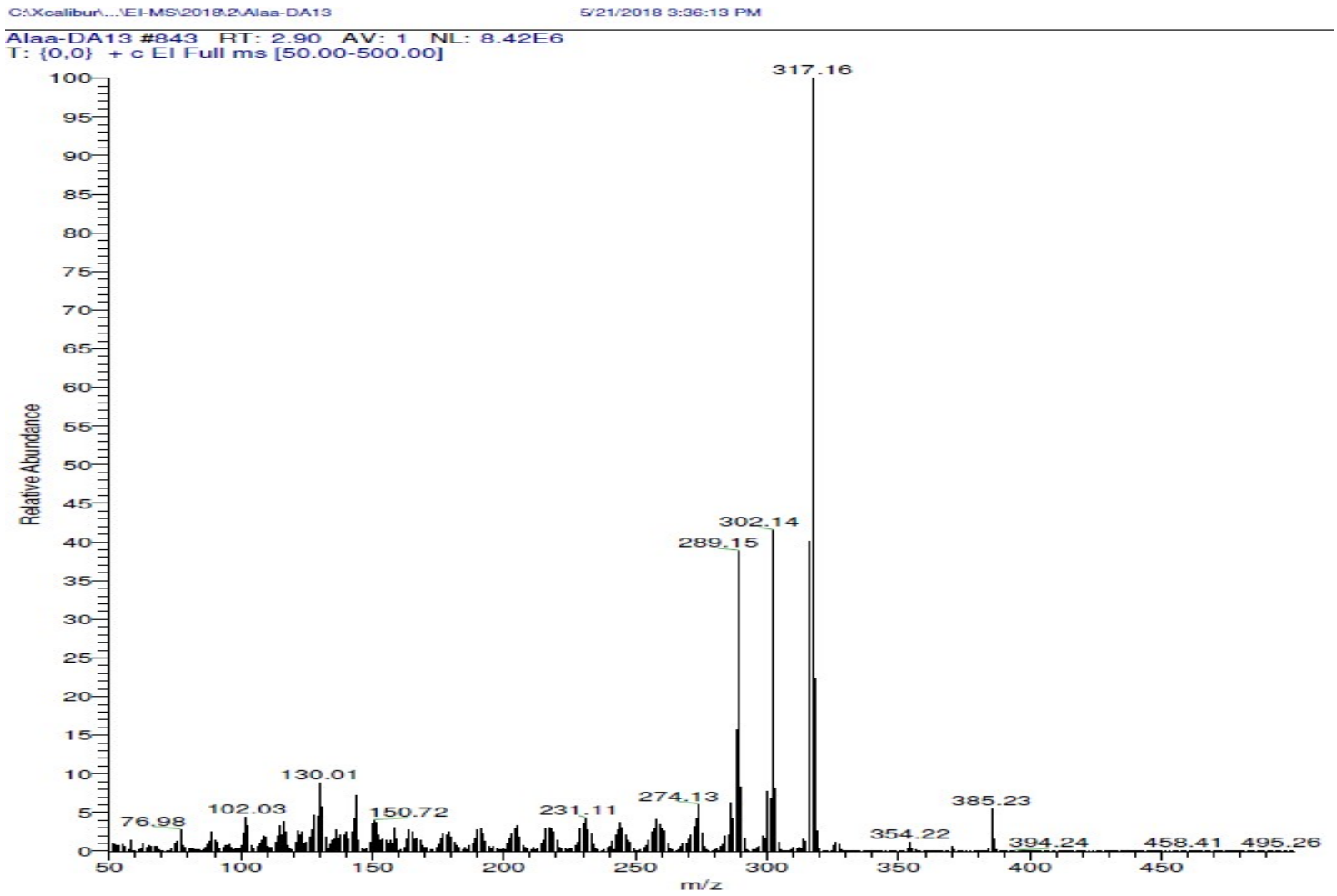
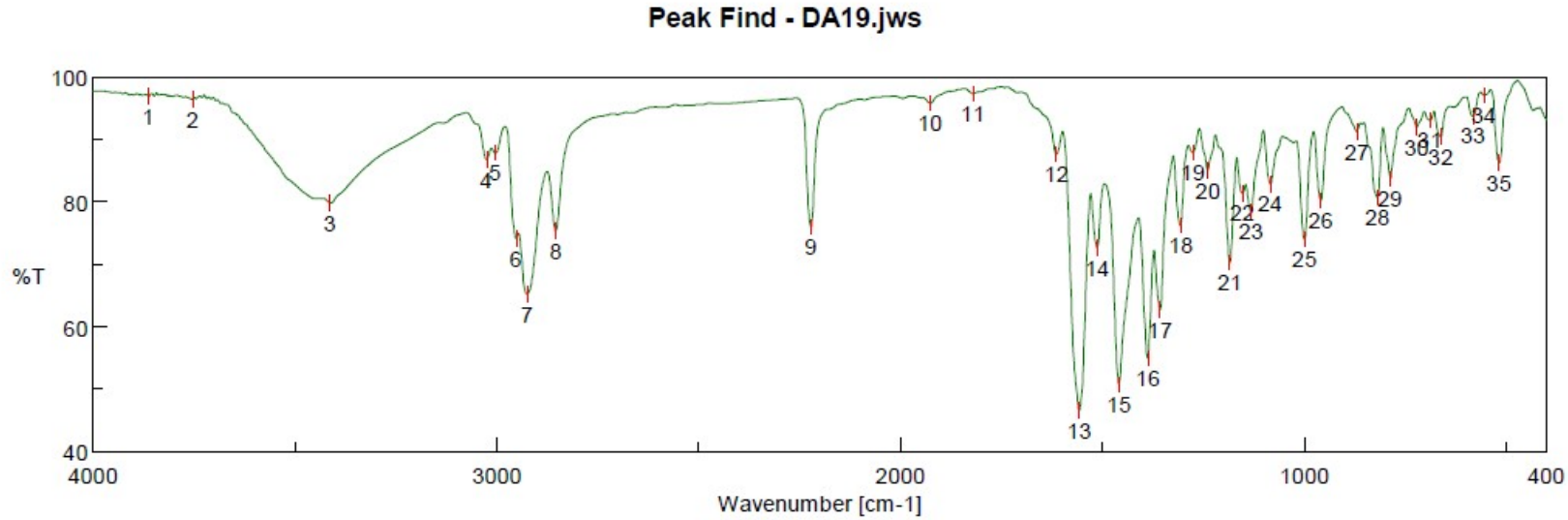


Fig. S44. Mass spectrum of compound **3k**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3862.72	96.8456	2	3753.76	96.348	3	3412.42	79.8083	4	3025.76	86.7409
5	3003.59	87.7712	6	2952.48	74.1214	7	2925.48	65.2341	8	2854.13	75.3186
9	2221.59	75.9331	10	1926.54	95.7912	11	1820.47	97.309	12	1614.13	87.6203
13	1557.24	46.6109	14	1512.88	72.621	15	1458.89	50.7286	16	1387.53	54.94
17	1356.68	62.6757	18	1306.54	76.1939	19	1276.65	87.8667	20	1239.04	85.0329
21	1184.08	70.2193	22	1153.22	81.3172	23	1132.01	78.4503	24	1083.8	82.8254
25	999.91	73.9312	26	958.448	80.1968	27	869.739	91.1954	28	818.634	80.6971
29	786.815	83.6782	30	721.247	91.9305	31	688.463	93.0484	32	661.464	90.3985
33	582.397	93.7241	34	554.434	97.045	35	516.829	86.273			

Fig. S45. IR spectrum of compound **3l** (KBr pellet).

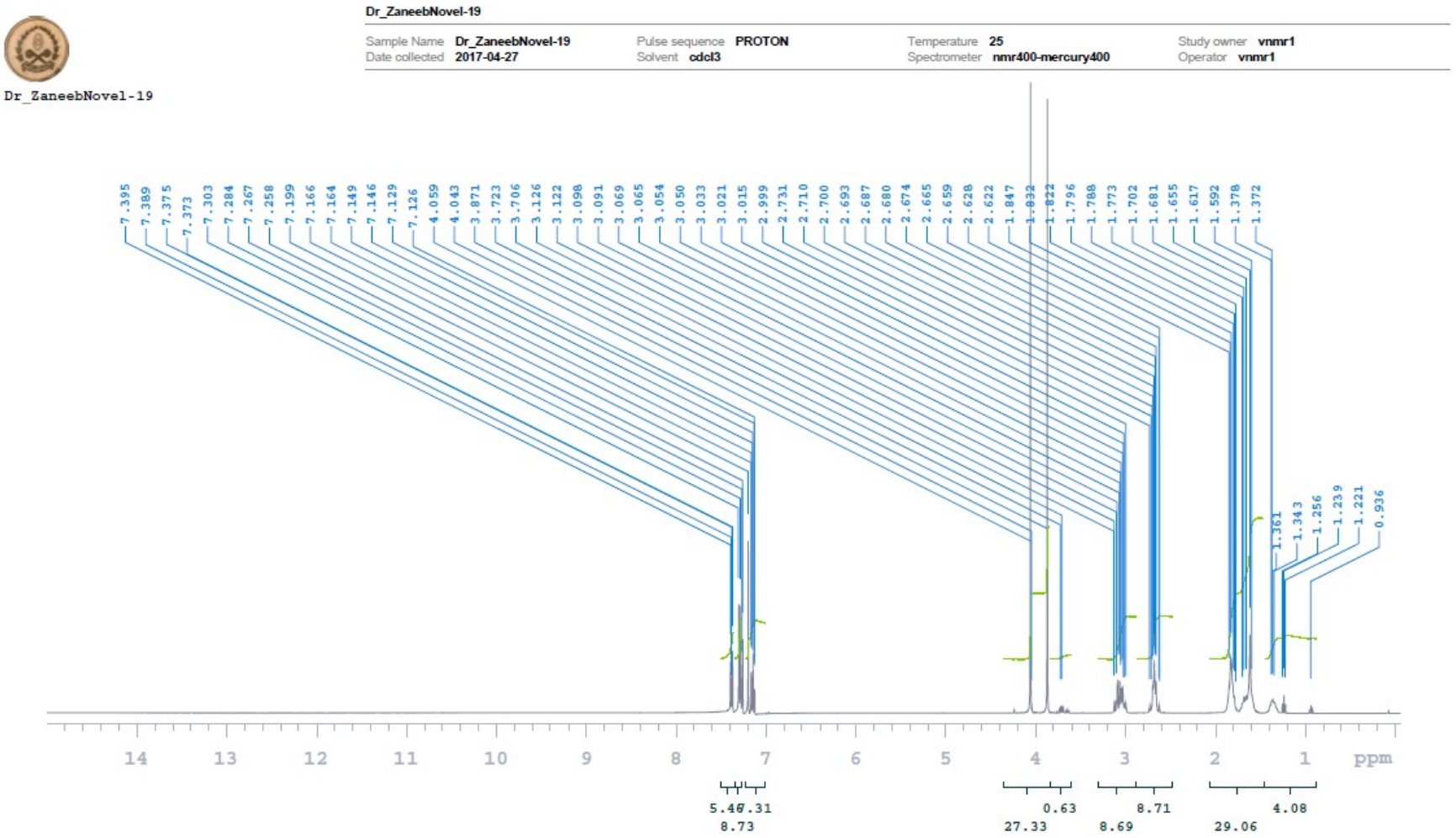


Fig. S46. [1]H-NMR spectrum of compound **3l**.

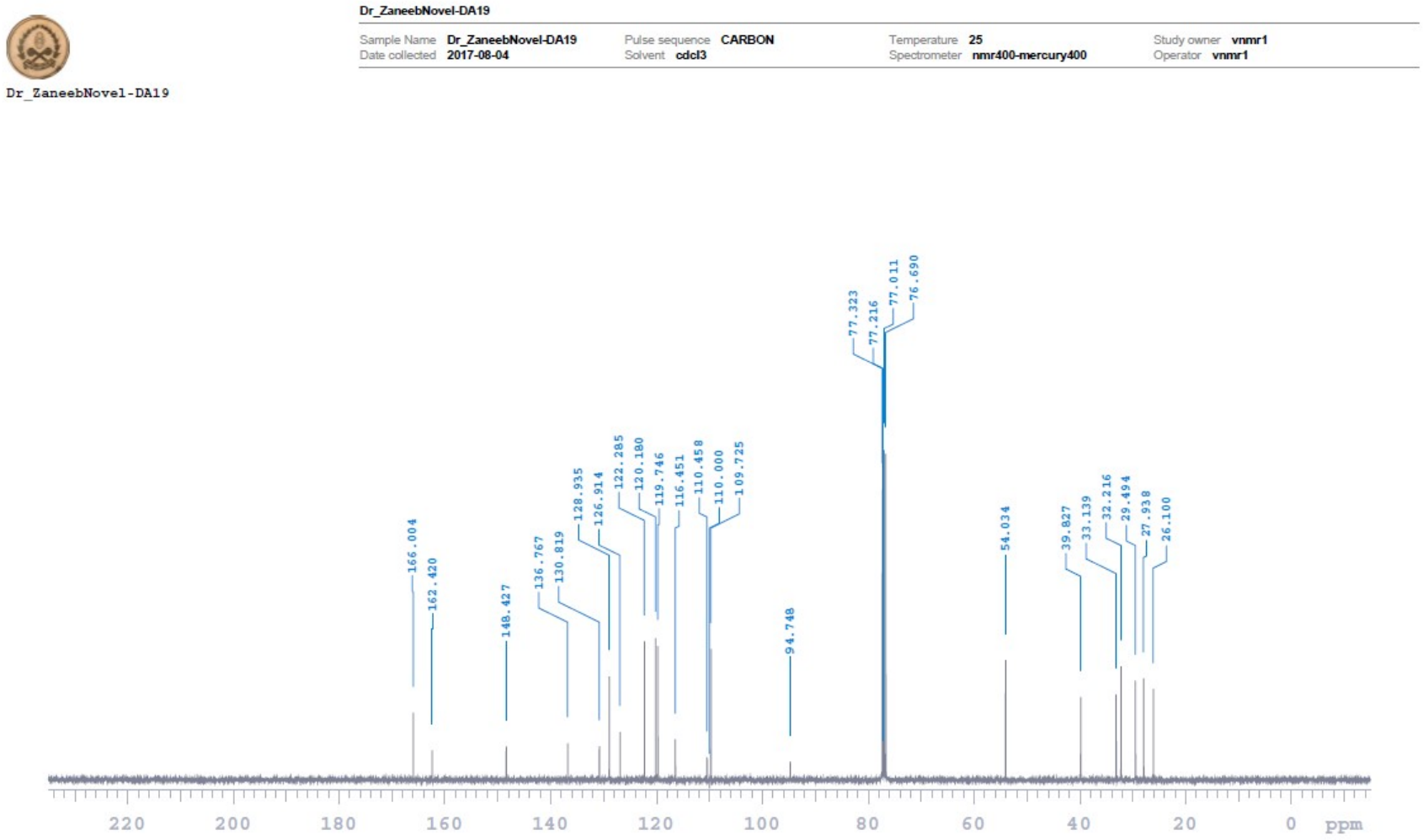


Fig. S47. ^{13}C-NMR spectrum of compound **3l**.

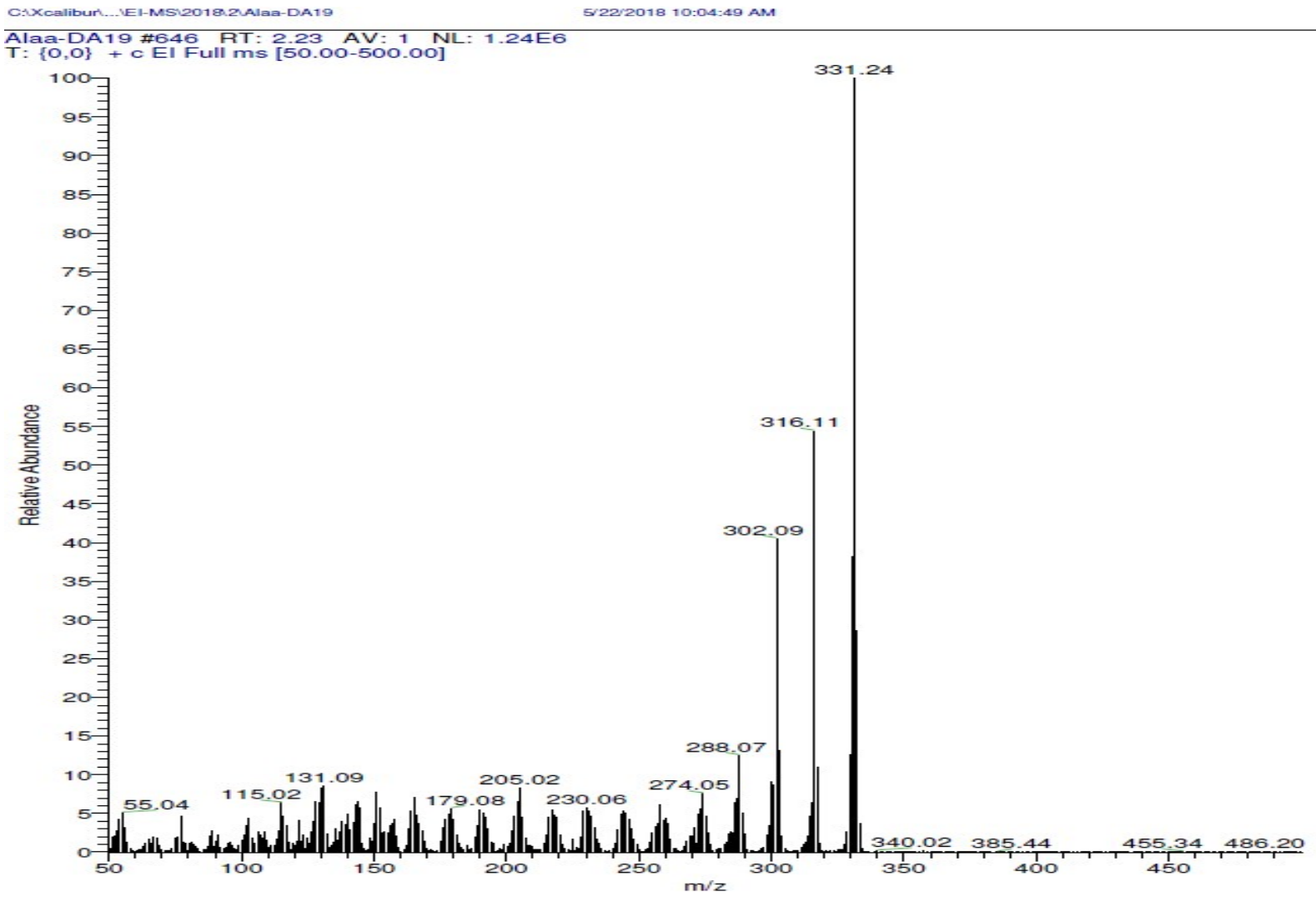
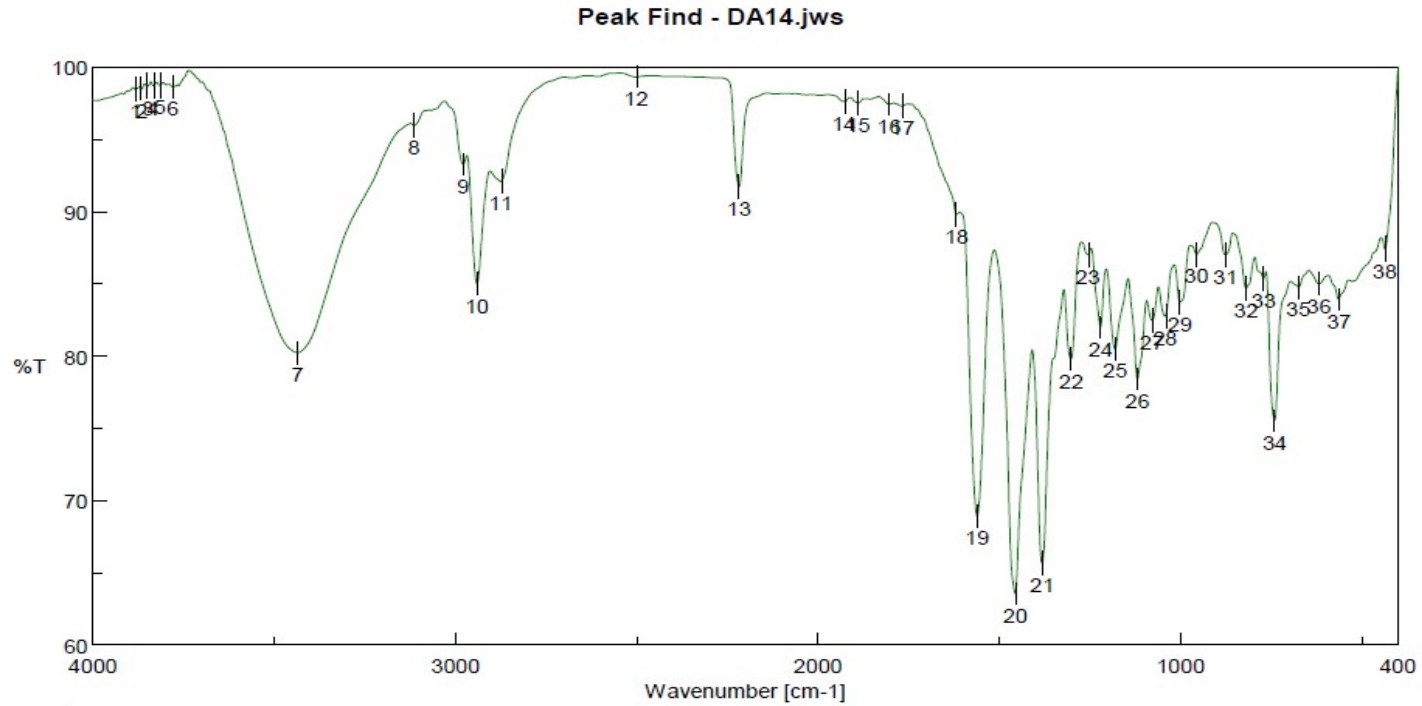


Fig. S48. Mass spectrum of compound **3l**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3882.97	98.4512	2	3865.61	98.5005	3	3849.22	98.6985	4	3833.79	98.7127
5	3815.47	98.7639	6	3779.8	98.6295	7	3435.56	80.2385	8	3114.47	95.9632
9	2979.48	93.2915	10	2940.91	85.008	11	2875.34	92.0903	12	2502.19	99.3211
13	2217.74	91.727	14	1928.47	97.6273	15	1888.93	97.5305	16	1804.08	97.4192
17	1766.48	97.3184	18	1617.98	89.8006	19	1561.09	68.9301	20	1455.99	63.571
21	1381.75	65.6771	22	1301.72	79.8012	23	1255.43	87.0269	24	1220.72	82.0044
25	1180.22	80.4943	26	1118.51	78.4204	27	1078.98	82.4966	28	1042.34	82.7739
29	1000.87	83.7298	30	955.555	87.0525	31	875.524	87.0339	32	818.634	84.6954
33	770.423	85.3799	34	739.567	75.5586	35	673.999	84.7639	36	618.074	85.0238
37	563.112	83.9345	38	435.834	87.4195						

Fig. S49. IR spectrum of compound **3m** (KBr pellet).

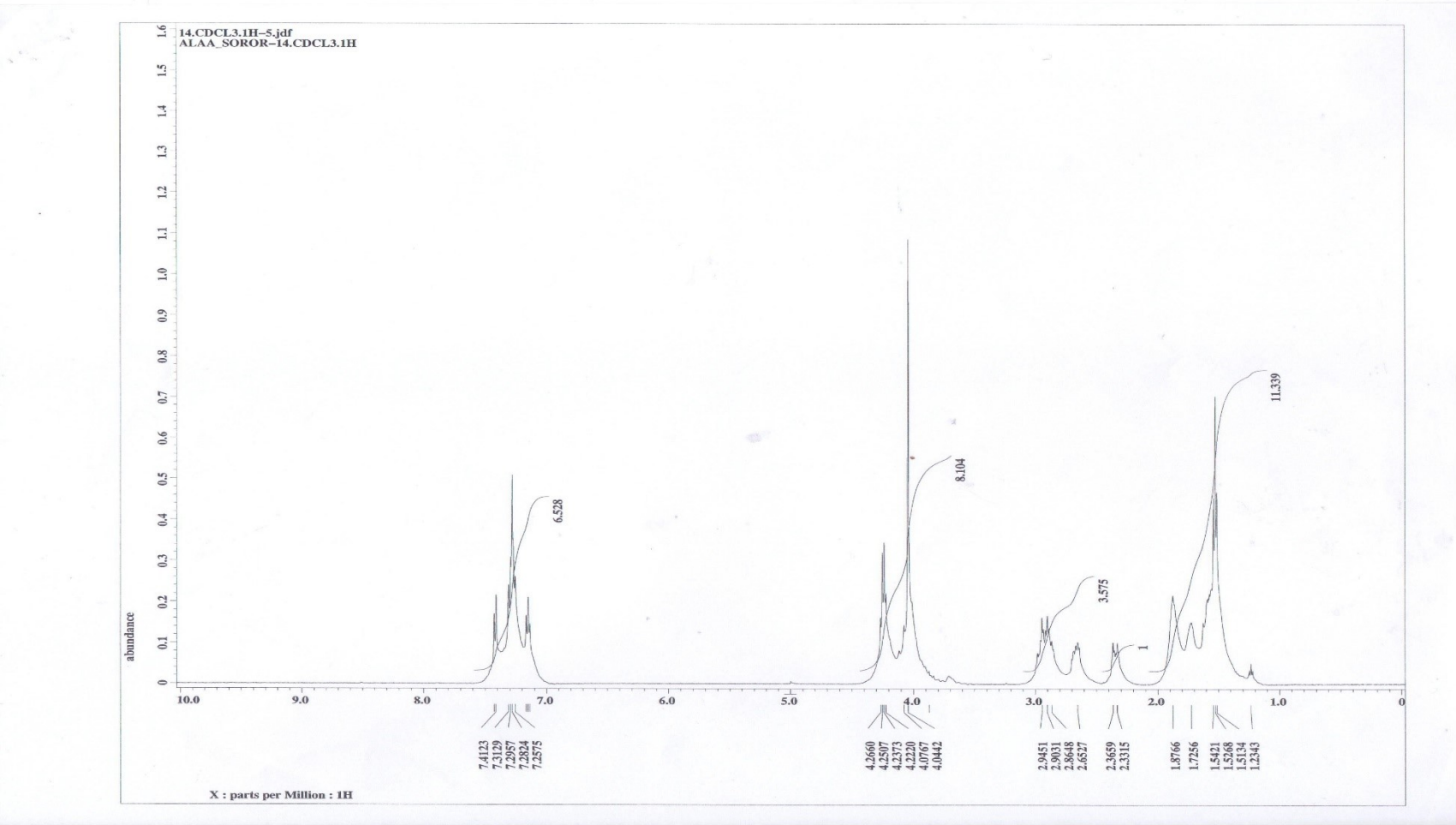


Fig. S50. ^{1}H-NMR spectrum of compound **3m**.

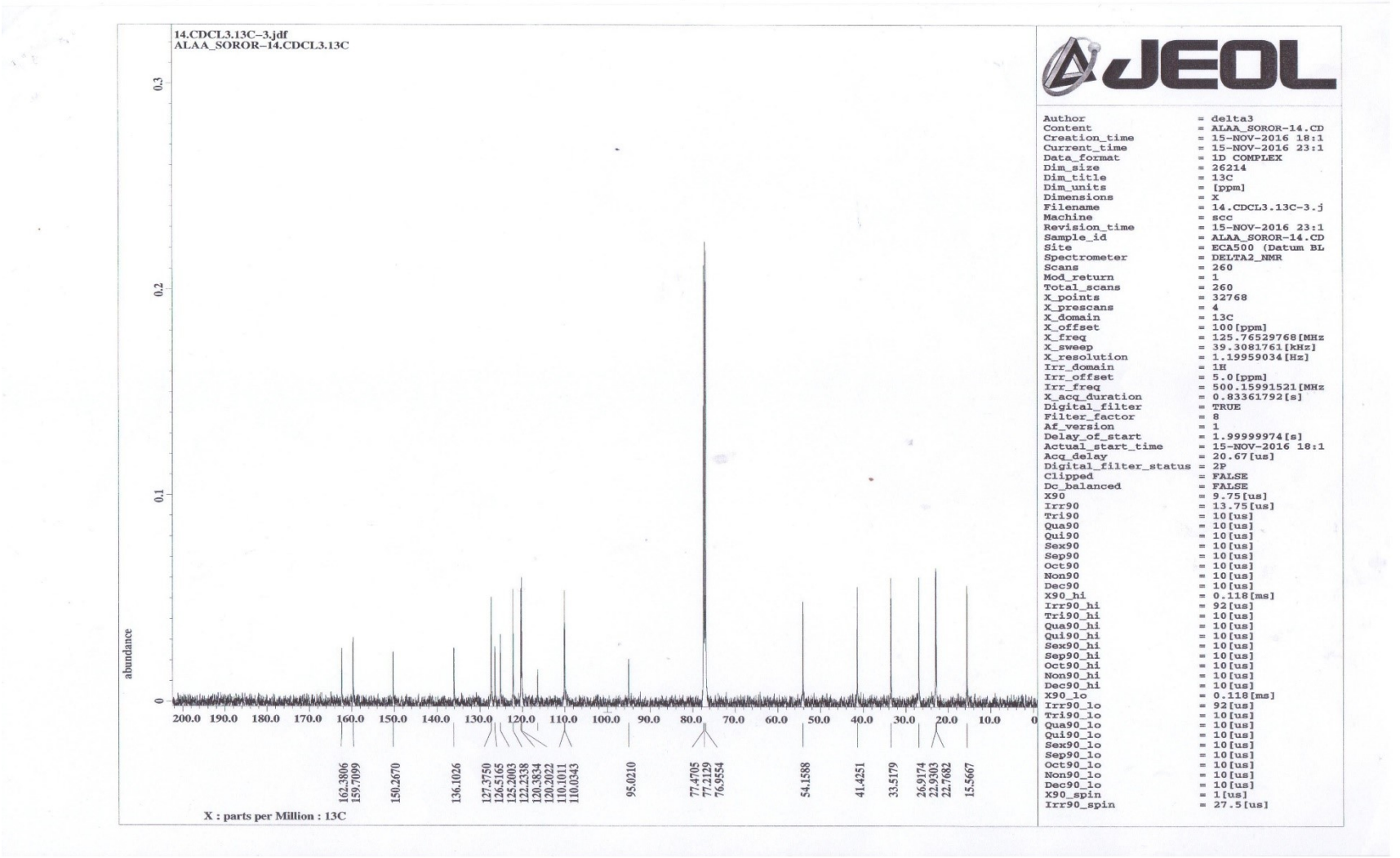


Fig. S51. ^{13}C-NMR spectrum of compound **3m**.

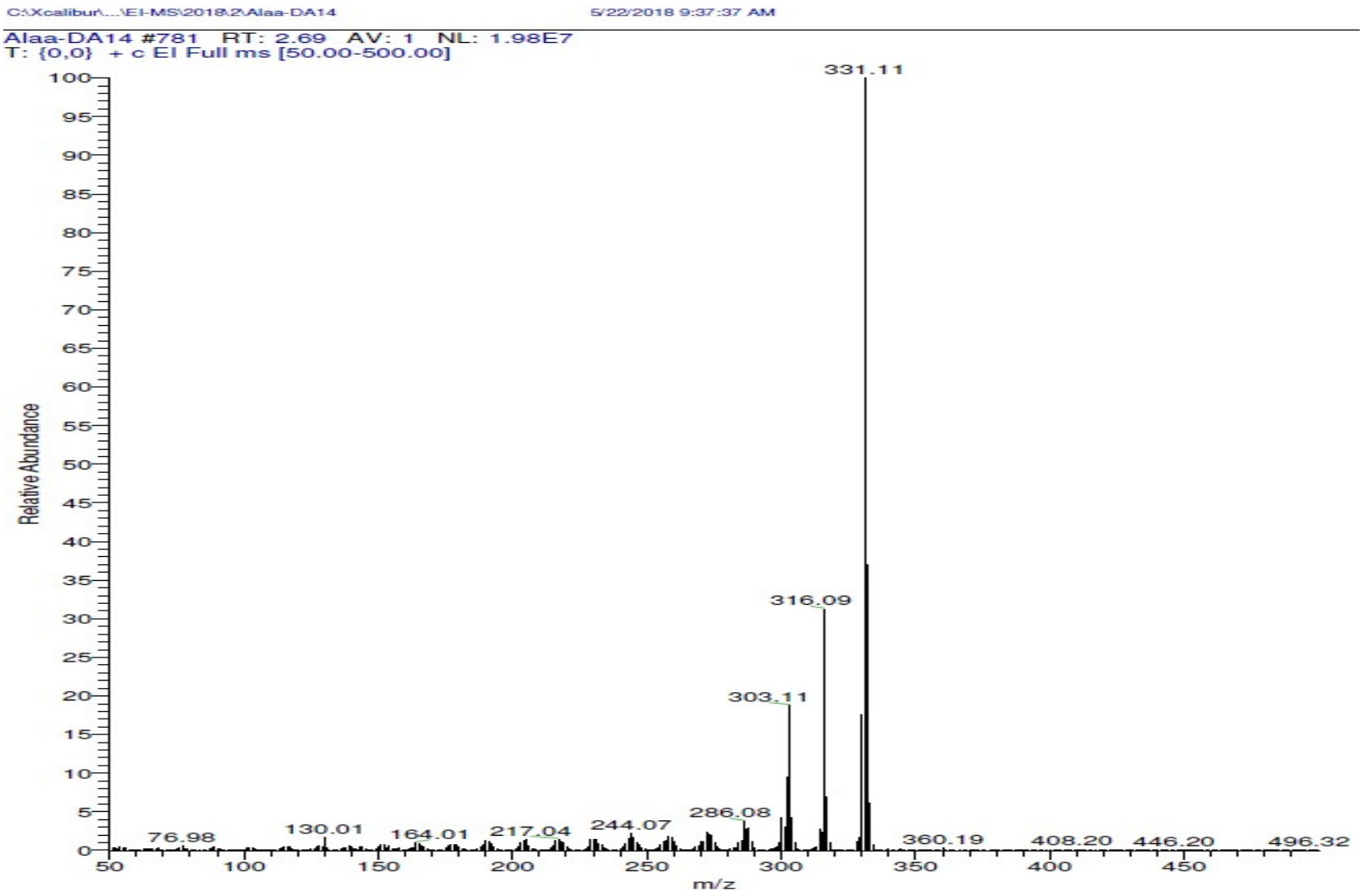
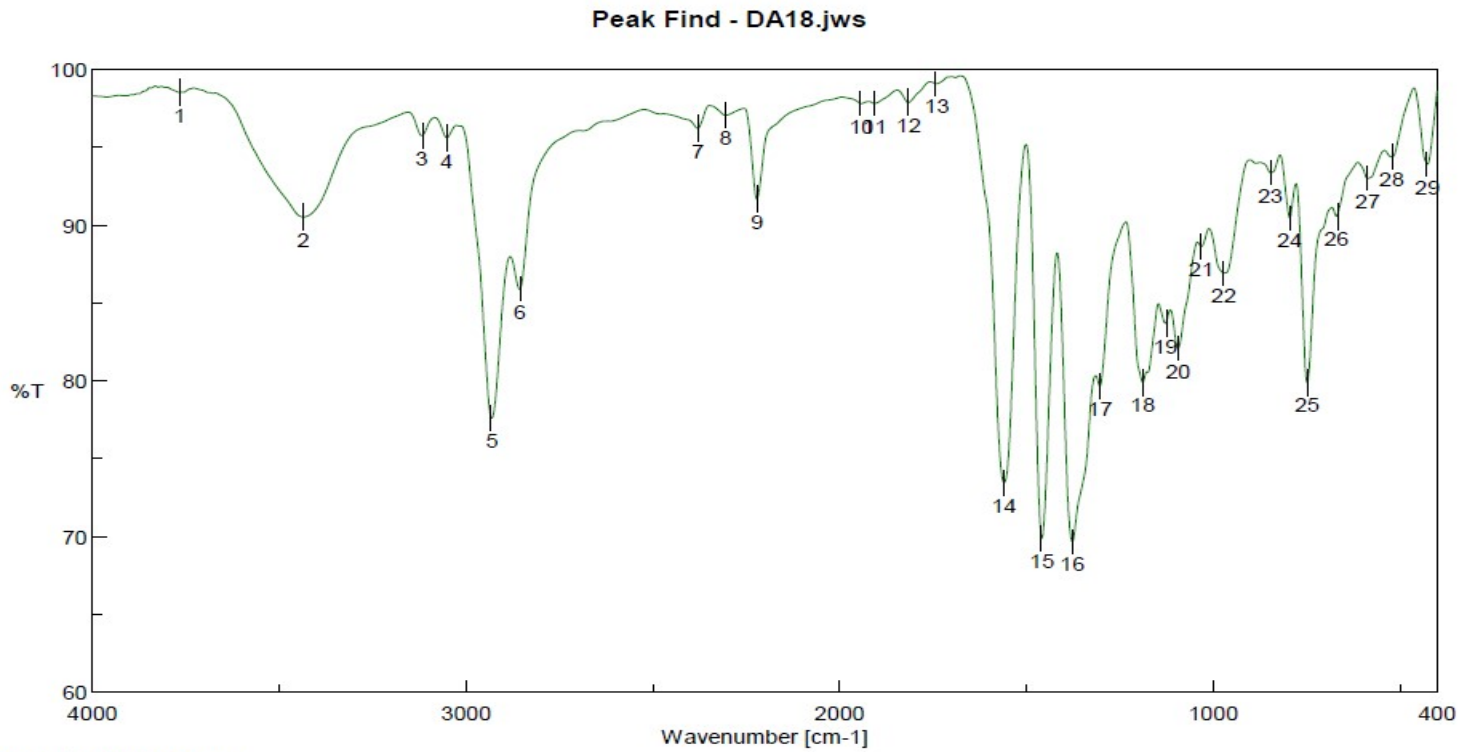


Fig. S52. Mass spectrum of compound **3m**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3767.26	98.5222	2	3435.56	90.5107	3	3118.33	95.736	4	3051.8	95.5955
5	2931.27	77.5995	6	2856.06	85.8632	7	2379.73	96.2084	8	2304.52	97.0434
9	2221.59	91.6613	10	1941.97	97.7959	11	1906.29	97.8006	12	1815.65	97.8589
13	1739.48	99.0745	14	1560.13	73.4488	15	1458.89	69.8711	16	1376.93	69.6532
17	1302.68	79.6422	18	1187.94	79.9132	19	1128.15	83.6761	20	1093.44	82.0867
21	1031.73	88.6189	22	970.983	86.9092	23	846.597	93.3549	24	794.528	90.4566
25	748.245	79.8727	26	670.142	90.5609	27	586.254	92.9907	28	521.65	94.3747
29	426.191	93.9059									

Fig. S53. IR spectrum of compound **3n** (KBr pellet).

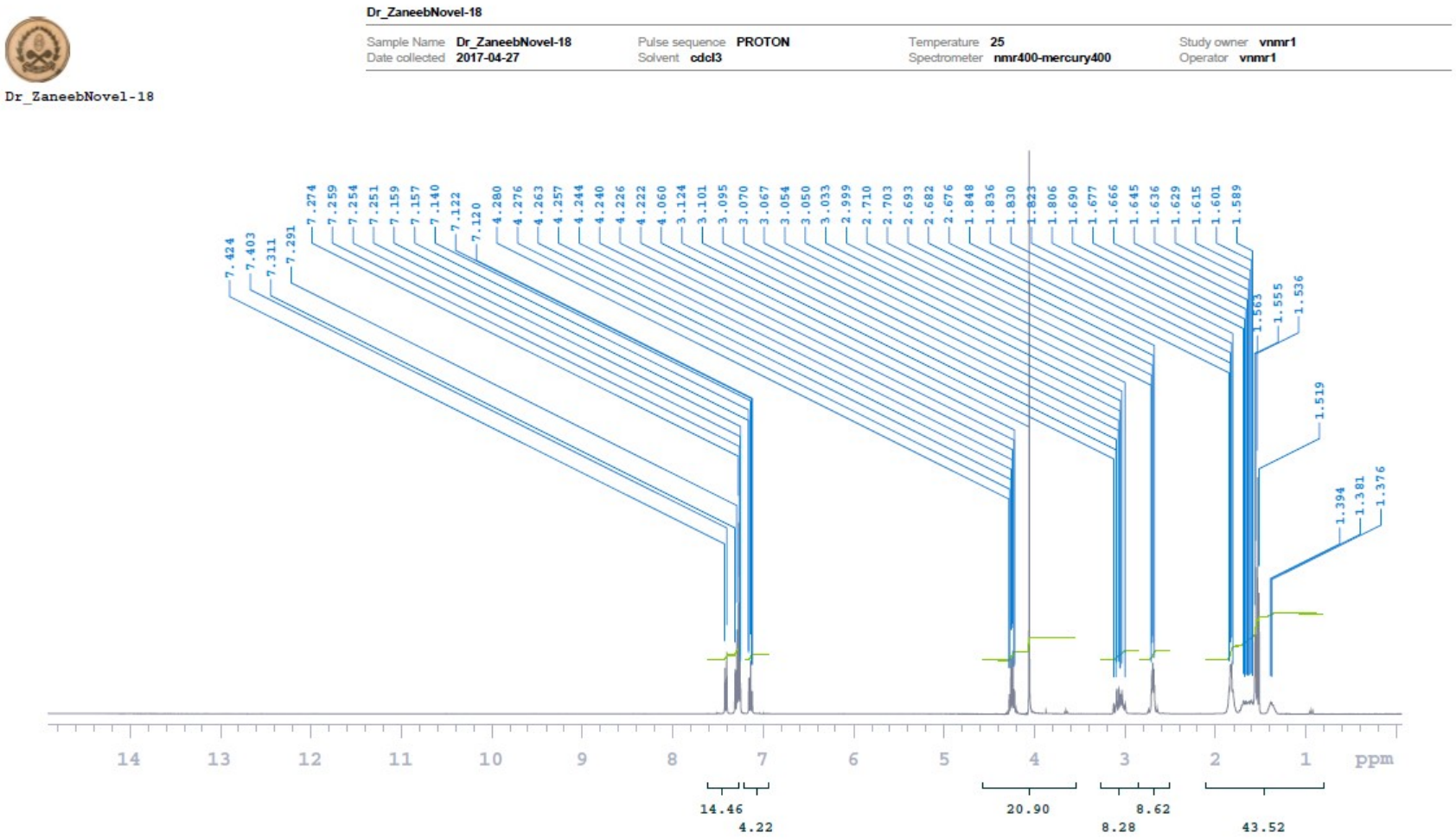


Fig. S54. ^{1}H-NMR spectrum of compound **3n**.

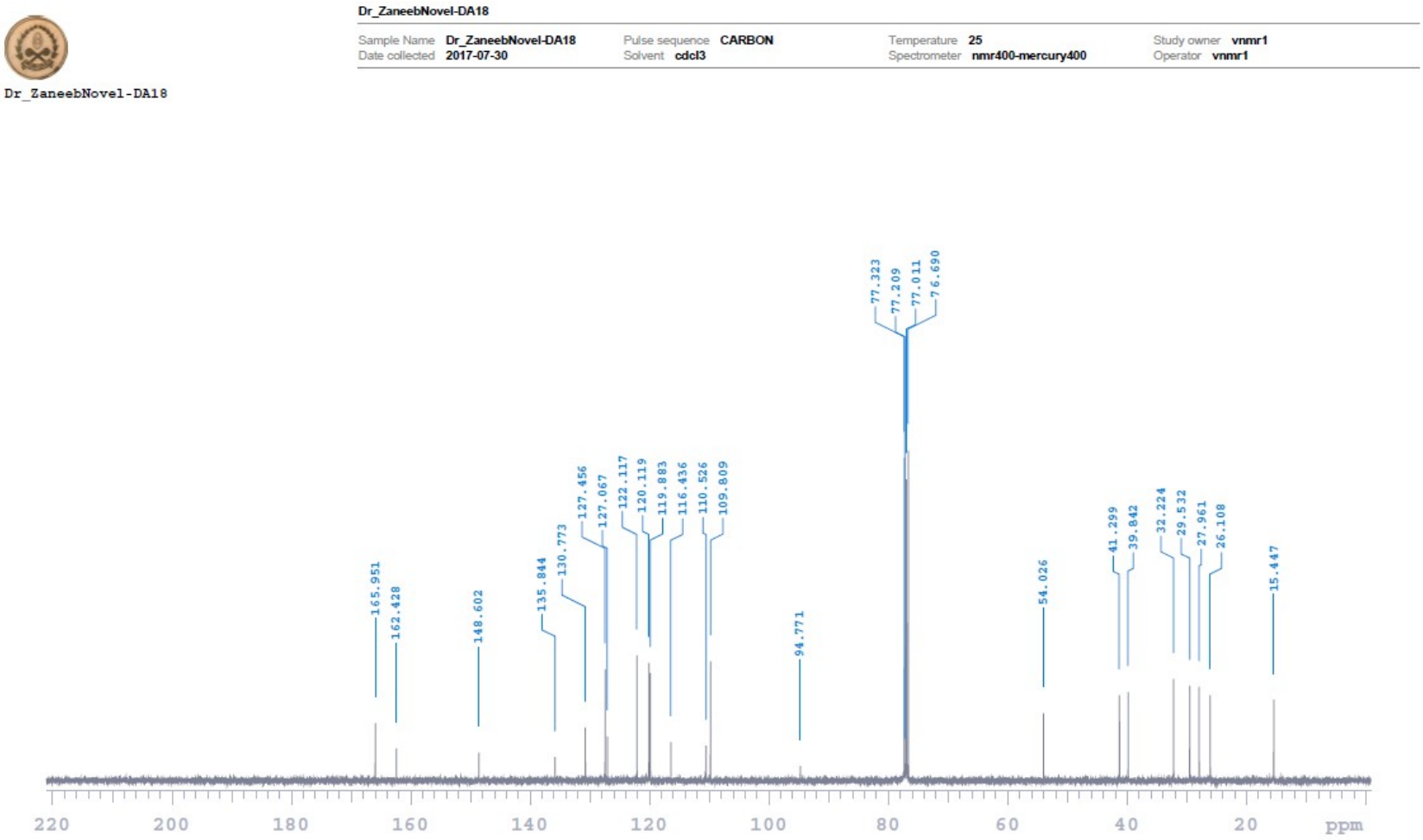


Fig. S55. ^{13}C-NMR spectrum of compound **3n**.

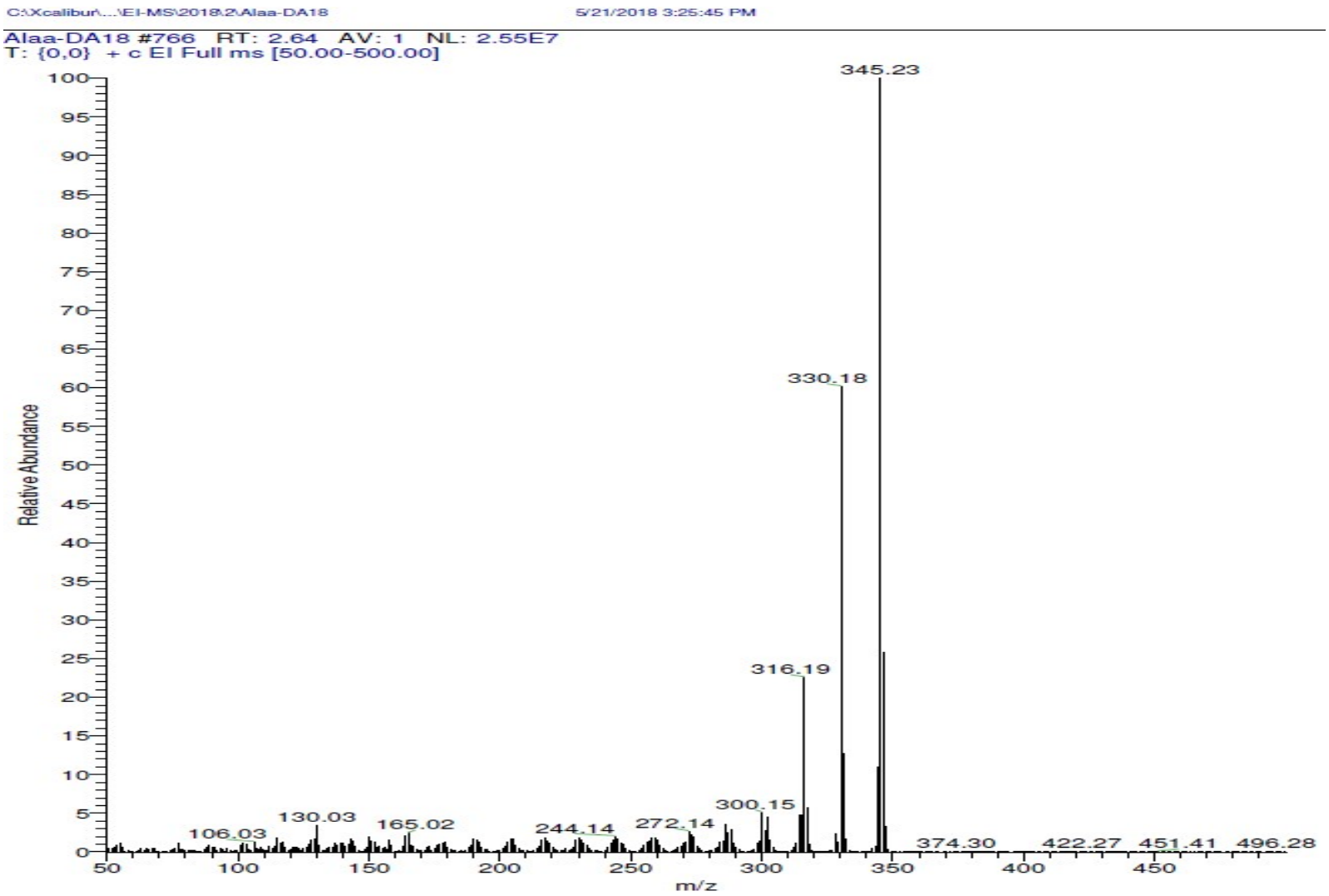
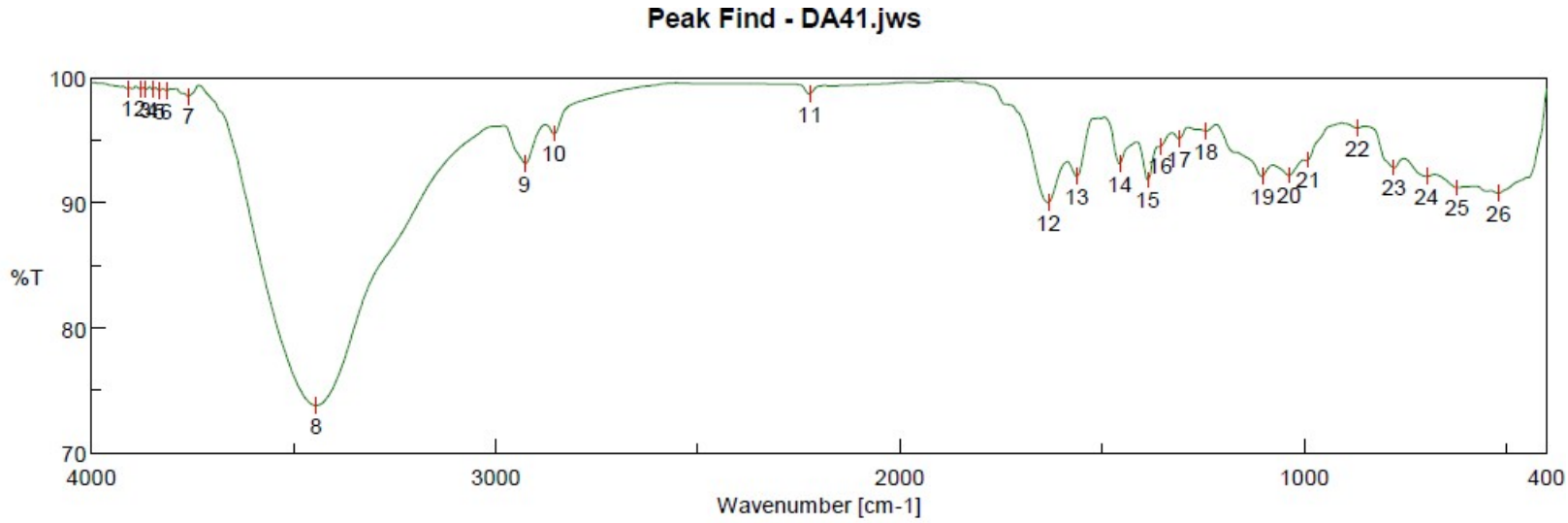


Fig. S56. Mass spectrum of compound **3n**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3910.93	99.1108	2	3880.08	99.0868	3	3864.65	99.0432	4	3849.22	99.0428
5	3831.86	98.9487	6	3814.51	98.9494	7	3760.51	98.4767	8	3444.24	73.7942
9	2927.41	93.1381	10	2856.06	95.5153	11	2223.52	98.6905	12	1633.41	90.0008
13	1562.06	92.1336	14	1455.99	93.1264	15	1386.57	91.8487	16	1355.71	94.4913
17	1309.43	95.1197	18	1241.93	95.7198	19	1103.08	92.1092	20	1037.52	92.1804
21	989.304	93.4313	22	867.81	95.9626	23	777.172	92.799	24	696.177	92.1016
25	620.966	91.1981	26	518.758	90.7549						

Fig. S57. IR spectrum of compound **3o** (KBr pellet).

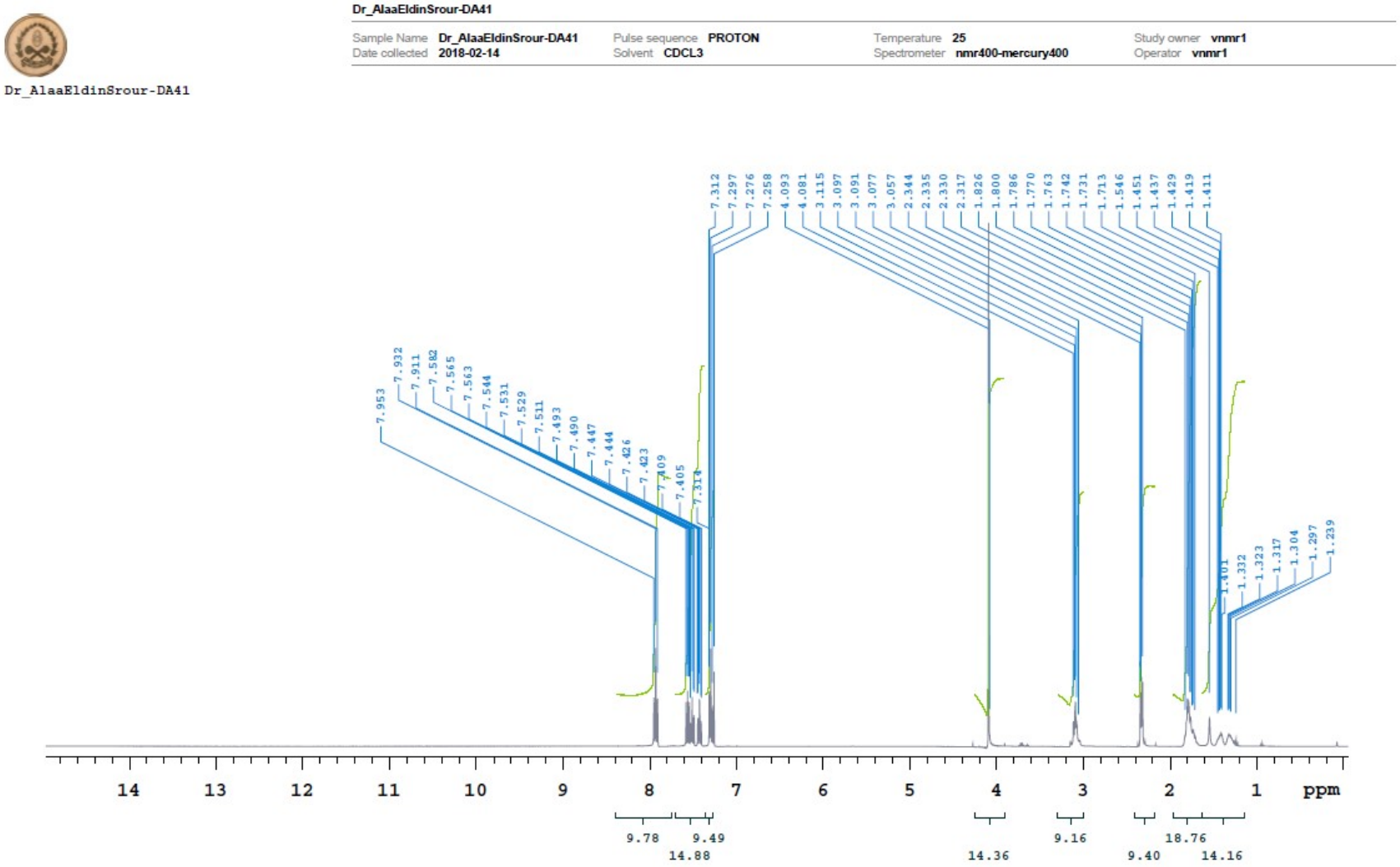


Fig. S58. [1]H-NMR spectrum of compound **3o**.

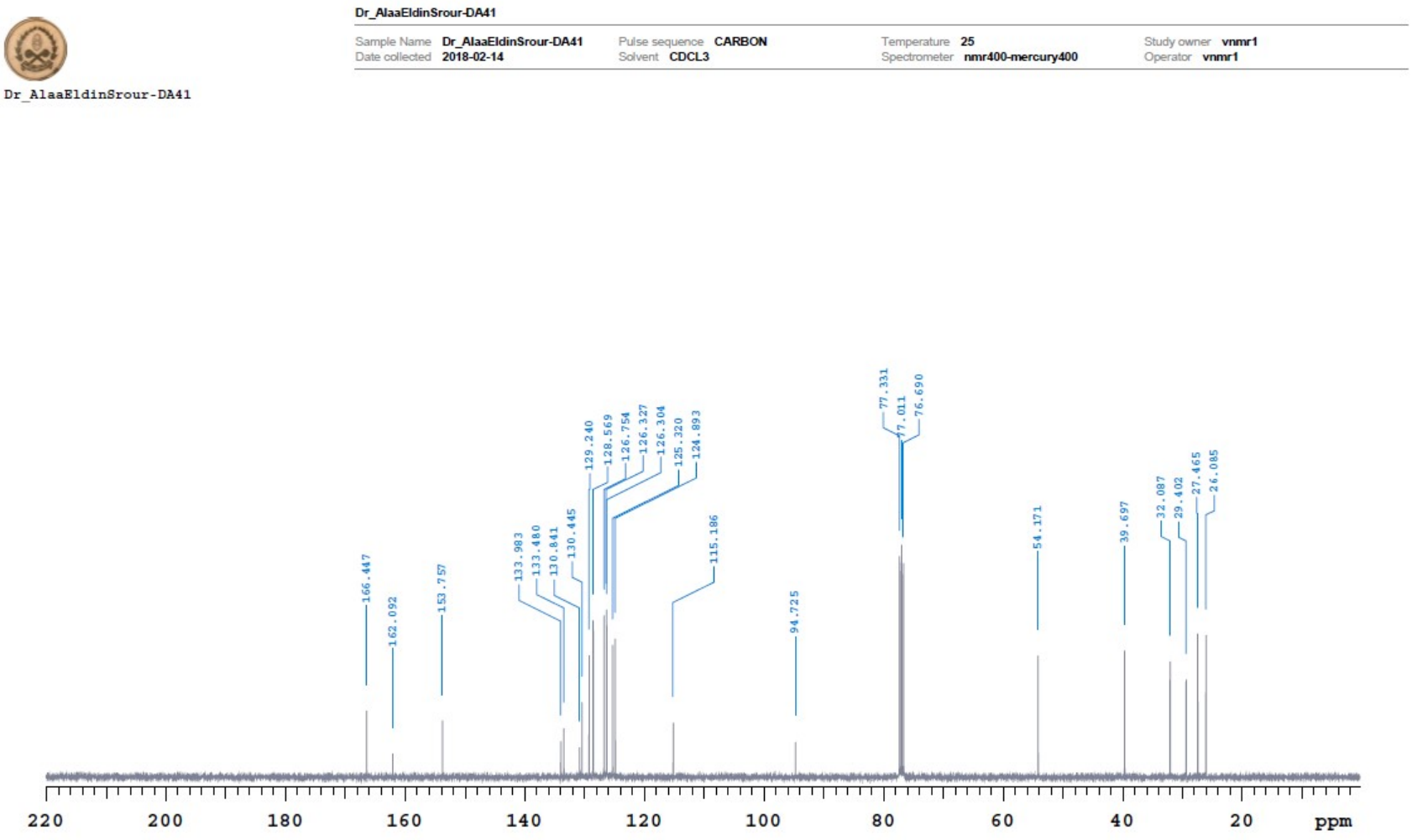


Fig. S59. ^{13}C-NMR spectrum of compound **3o**.

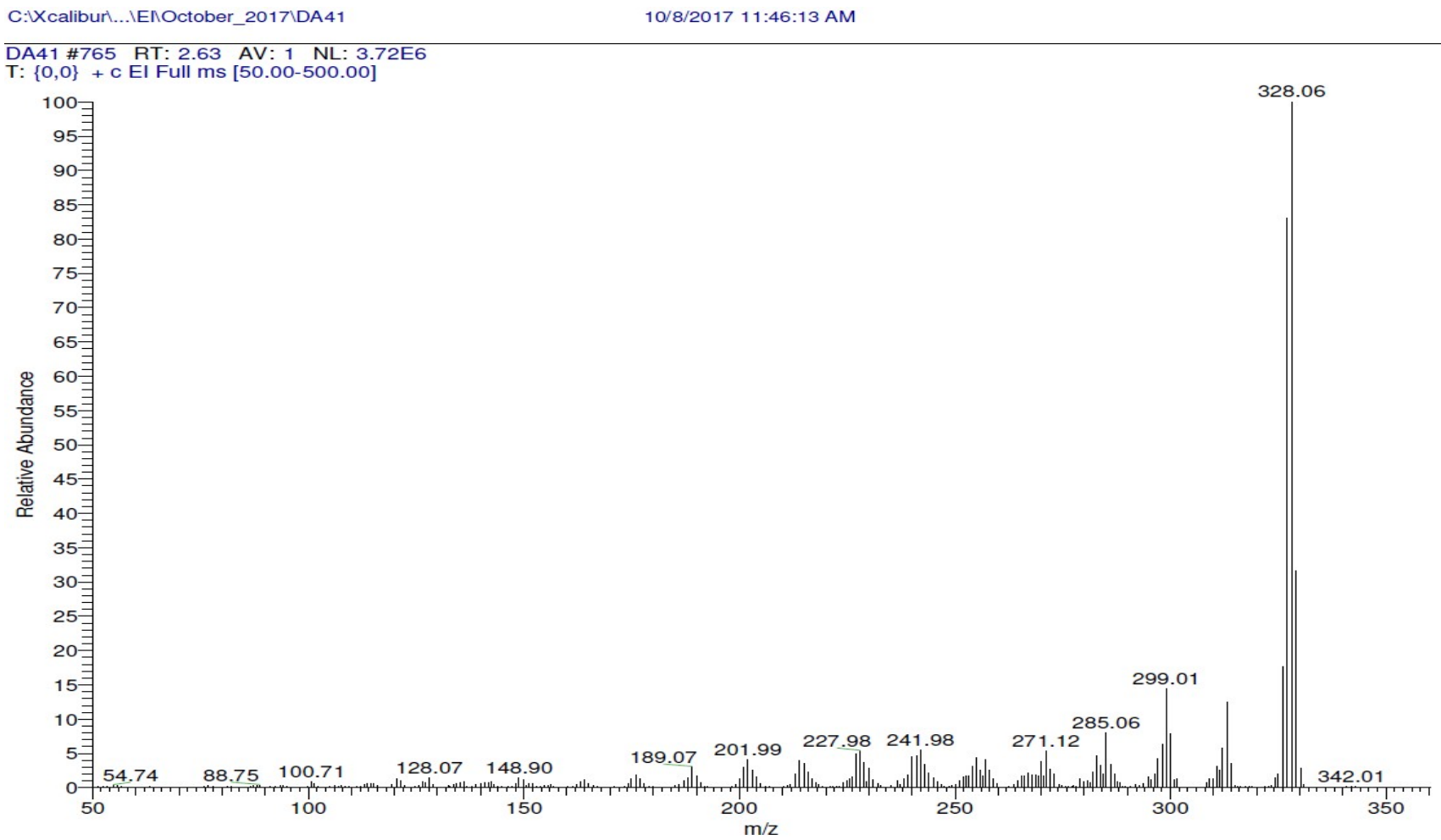
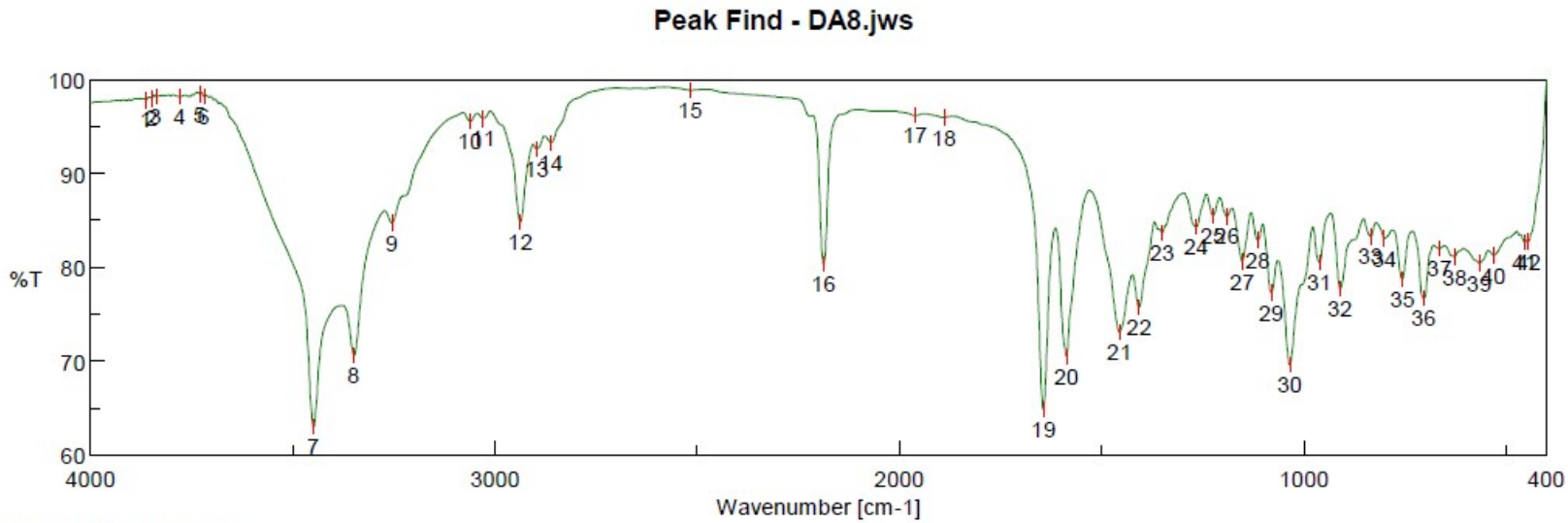


Fig. S60. Mass spectrum of compound **3o**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3862.72	97.8019	2	3849.22	97.983	3	3837.65	98.1861	4	3778.83	98.1459
5	3729.66	98.4447	6	3719.05	98.1816	7	3449.06	62.9924	8	3348.78	70.6009
9	3255.25	84.6855	10	3061.44	95.5514	11	3029.62	95.847	12	2937.06	84.8162
13	2896.56	92.5933	14	2861.84	93.2857	15	2517.61	98.843	16	2187.85	80.3227
17	1961.25	96.154	18	1889.9	95.9632	19	1644.02	64.8166	20	1587.13	70.4615
21	1455.99	73.0759	22	1406.82	75.7209	23	1350.89	83.6421	24	1266.04	84.2865
25	1224.58	85.4917	26	1189.86	85.4086	27	1152.26	80.599	28	1113.69	82.9005
29	1079.94	77.2507	30	1034.62	69.5687	31	961.341	80.5217	32	910.236	77.646
33	834.062	83.2646	34	800.314	83.0671	35	755.959	78.7408	36	702.926	76.7203
37	664.357	81.9508	38	626.752	81.1138	39	566.005	80.4052	40	530.328	81.2352
41	454.154	82.6534	42	444.512	82.749						

Fig. S61. IR spectrum of compound **4a** (KBr pellet).

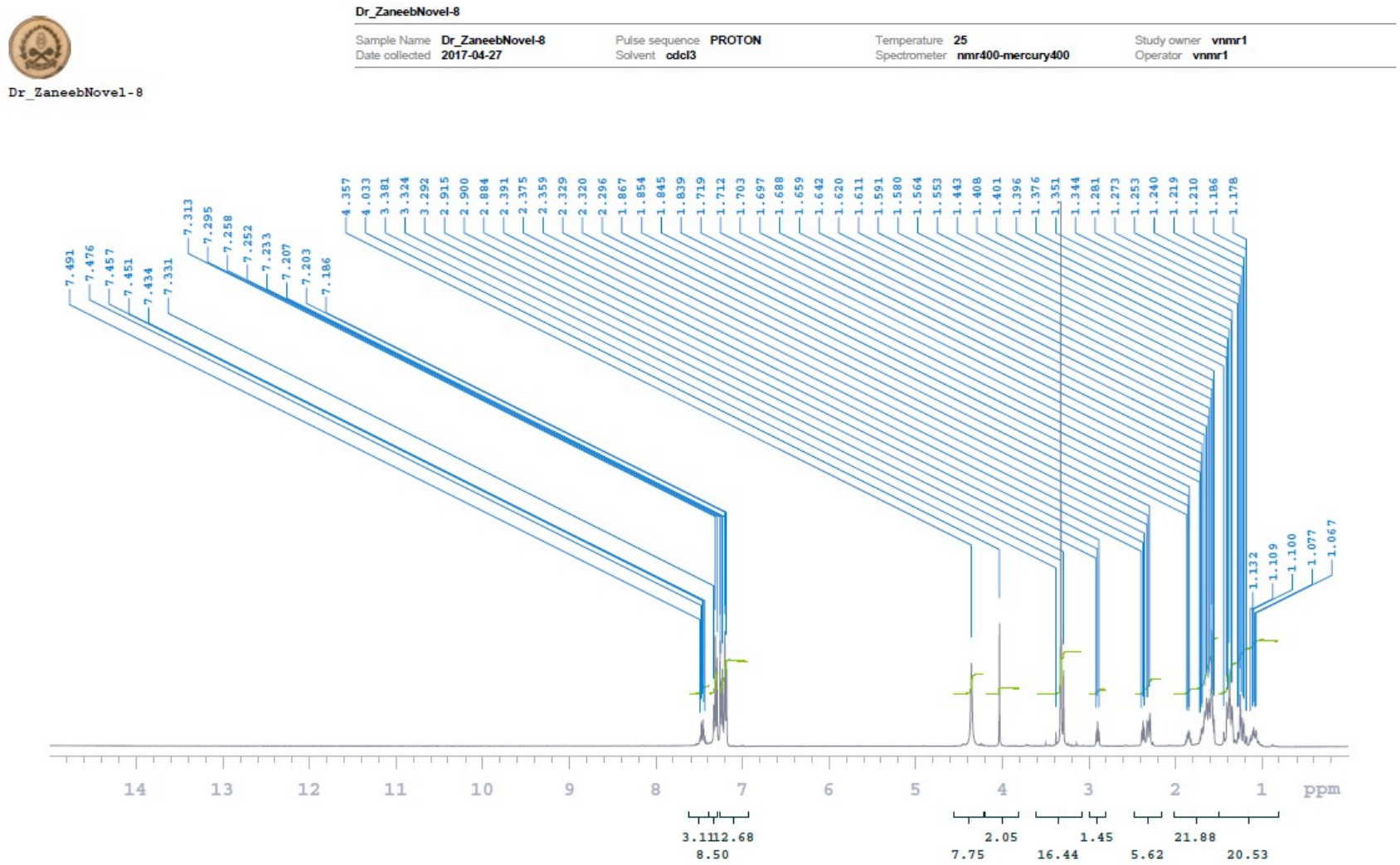


Fig. S62. ^{1}H-NMR spectrum of compound **4a**.

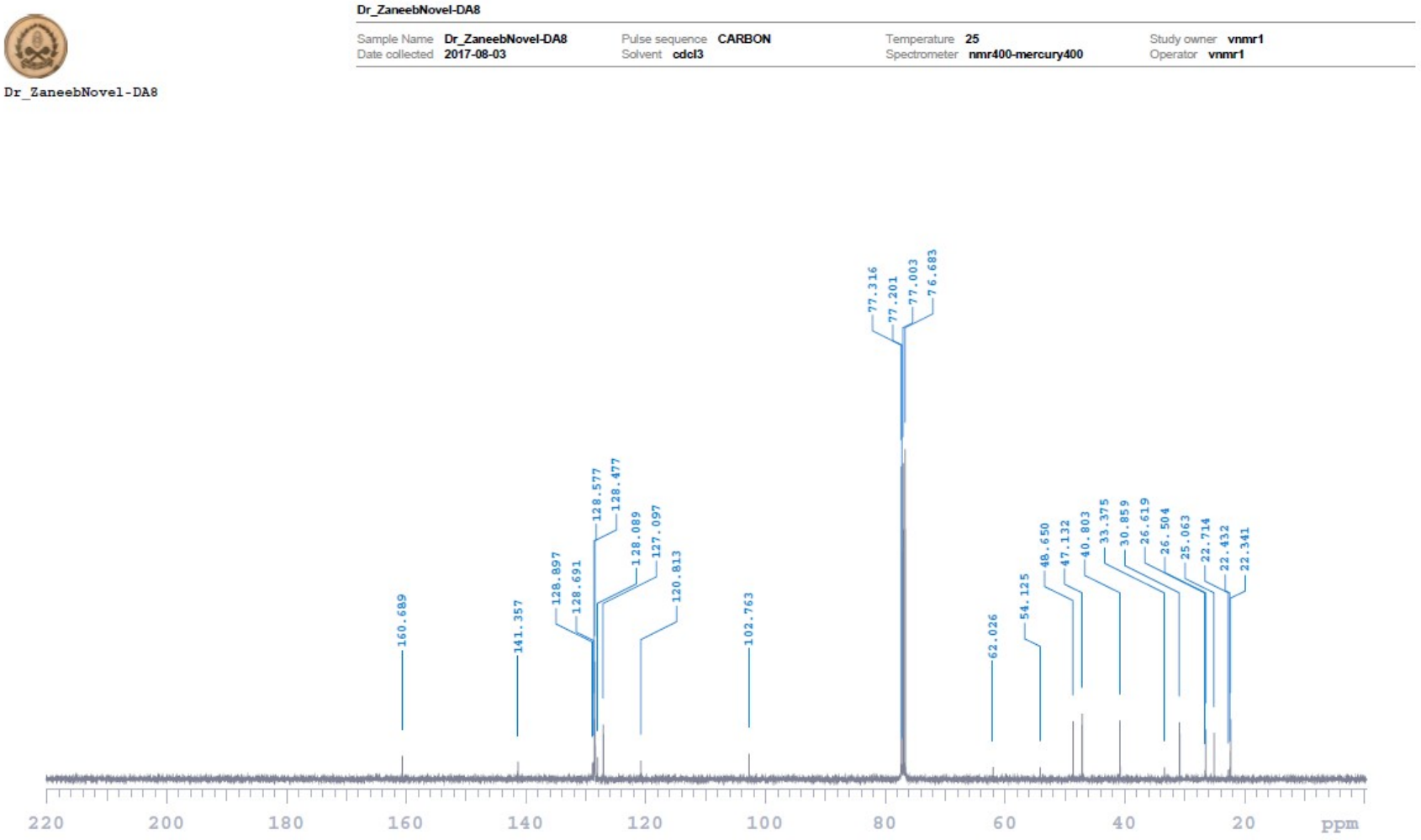


Fig. S63. ^{13}C-NMR spectrum of compound **4a**.

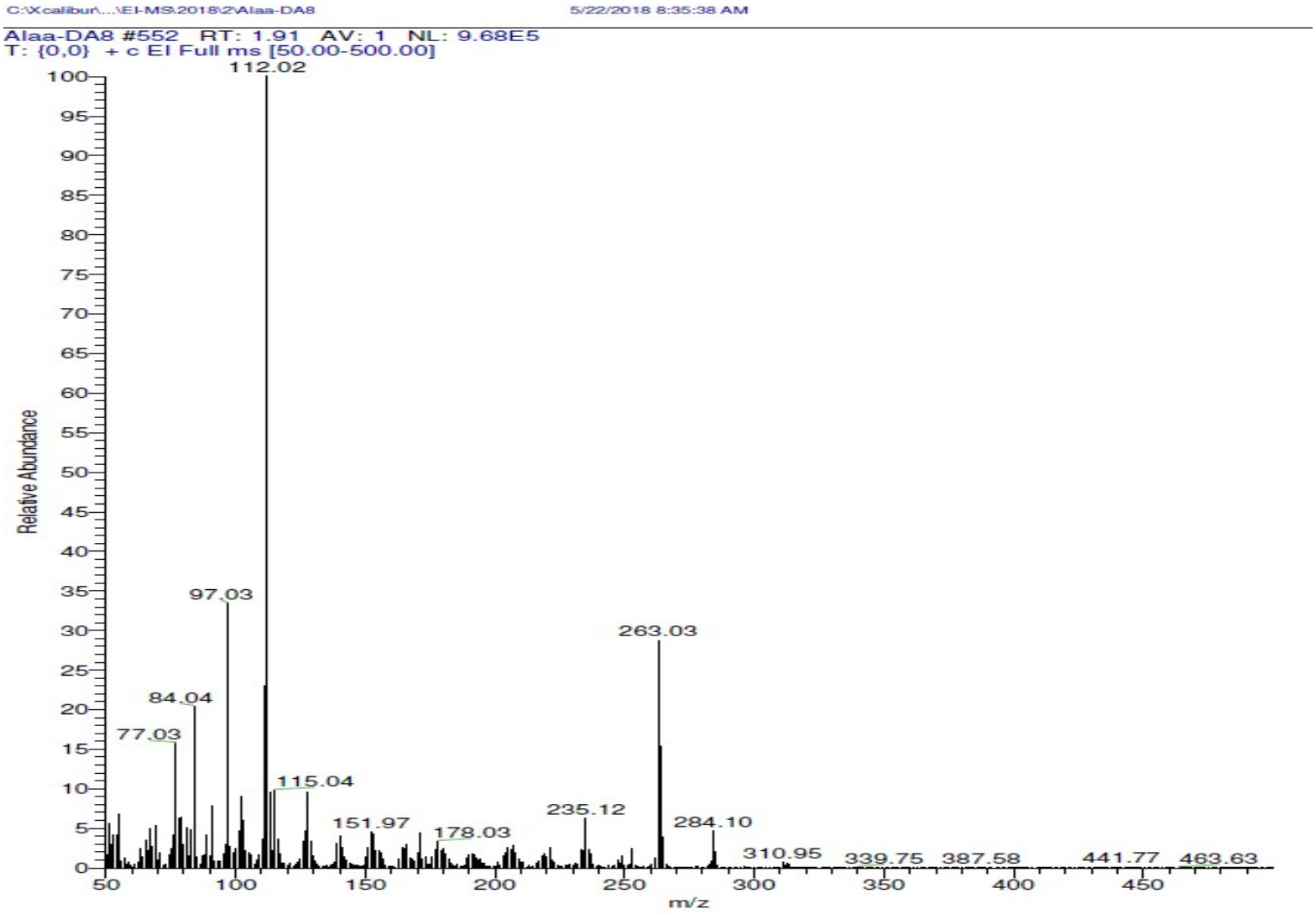
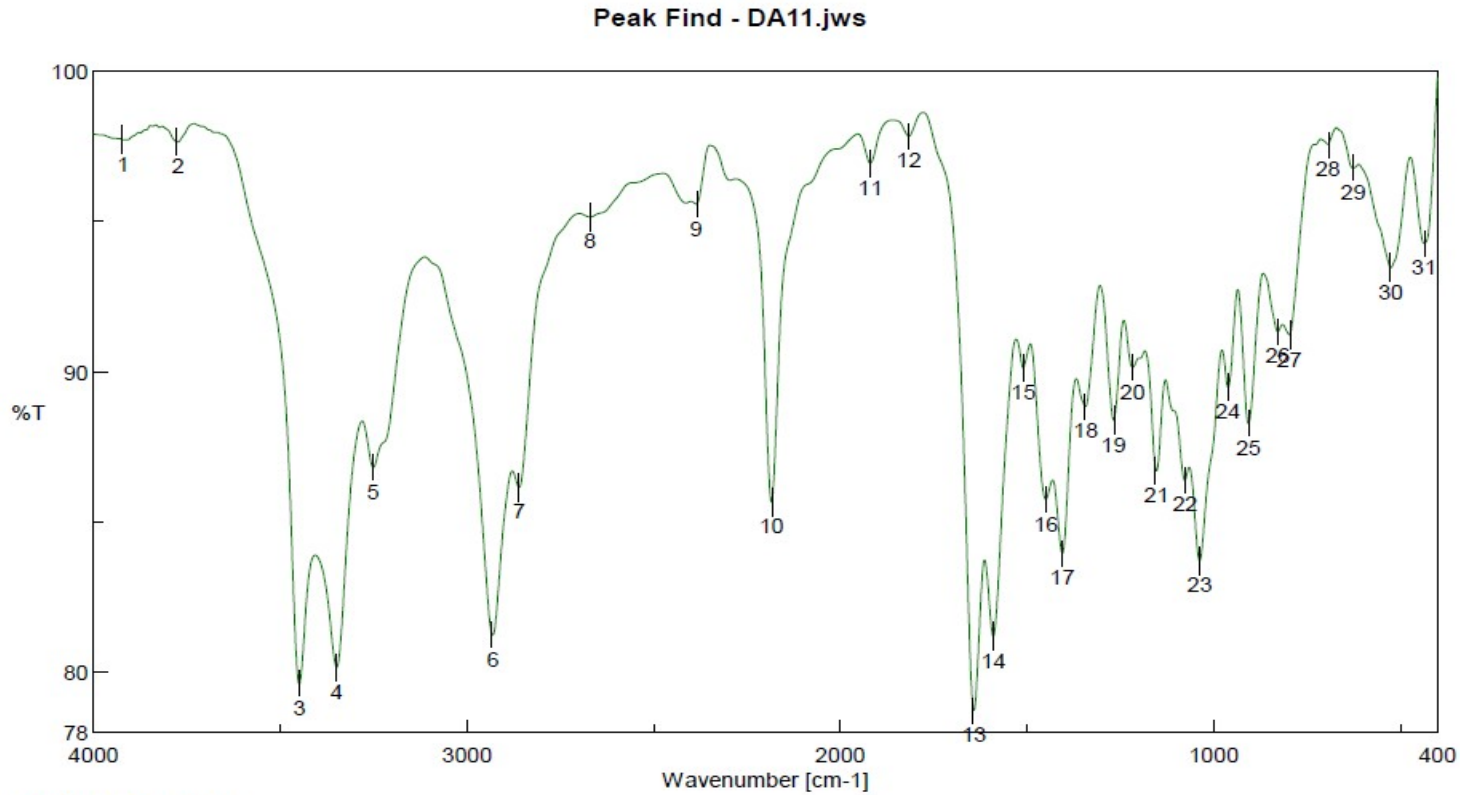


Fig. S64. Mass spectrum of compound **4a**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3920.57	97.7102	2	3774.01	97.6344	3	3449.06	79.6114	4	3348.78	80.1684
5	3251.4	86.817	6	2931.27	81.2399	7	2859.92	86.1451	8	2670.93	95.1361
9	2386.48	95.576	10	2184.95	85.6659	11	1919.79	96.9261	12	1816.62	97.8241
13	1642.09	78.7363	14	1590.02	81.1977	15	1509.99	90.1287	16	1449.24	85.7486
17	1404.89	83.9529	18	1343.18	88.8356	19	1267.97	88.3874	20	1216.86	90.1472
21	1153.22	86.7013	22	1077.05	86.388	23	1037.52	83.7099	24	961.341	89.4753
25	906.379	88.2704	26	828.277	91.3246	27	797.421	91.2274	28	694.248	97.5597
29	625.788	96.7734	30	525.507	93.4575	31	435.834	94.2594			

Fig. S65. IR spectrum of compound **4b** (KBr pellet).

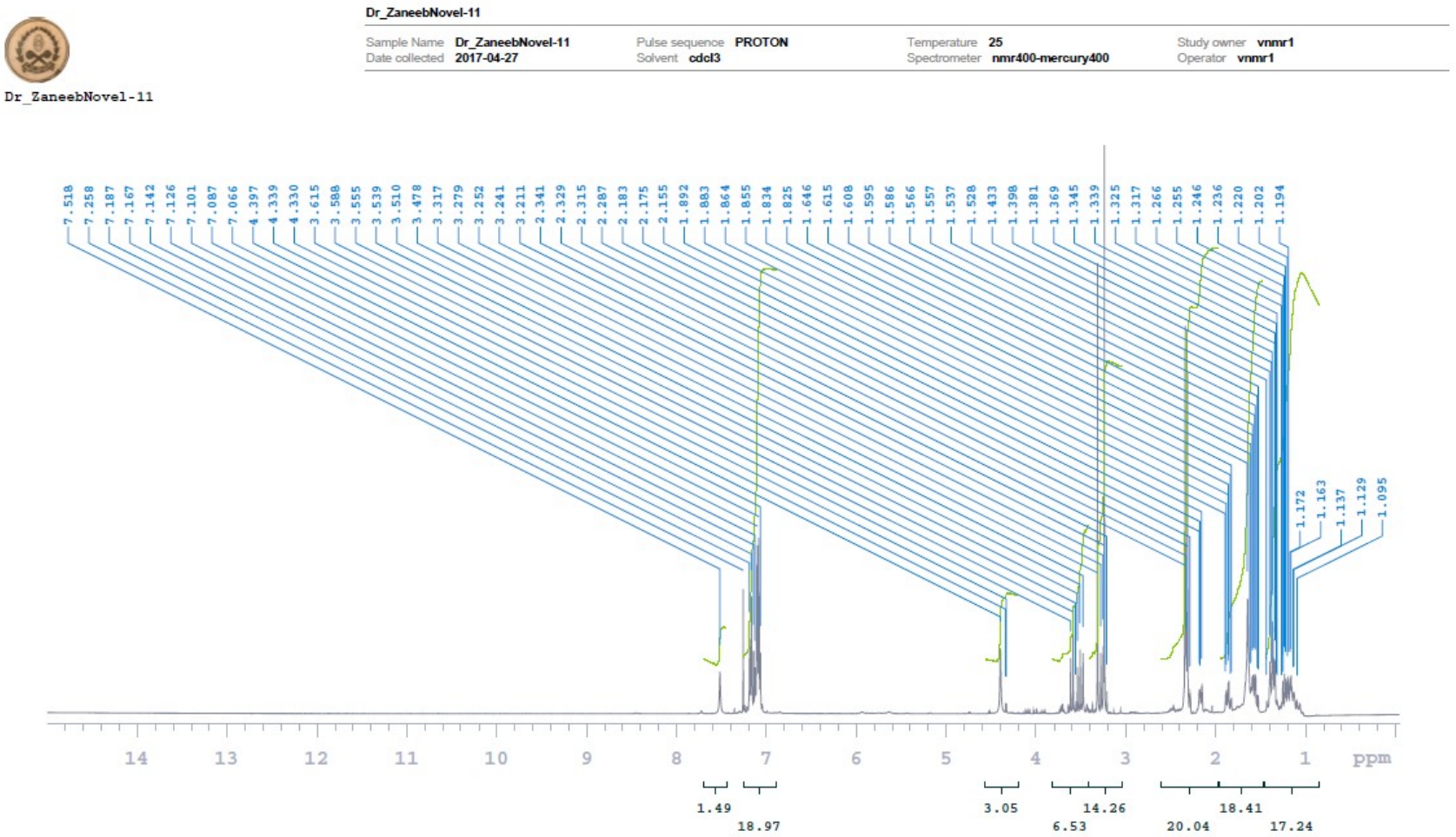


Fig. S66. [1]H-NMR spectrum of compound **4b**.

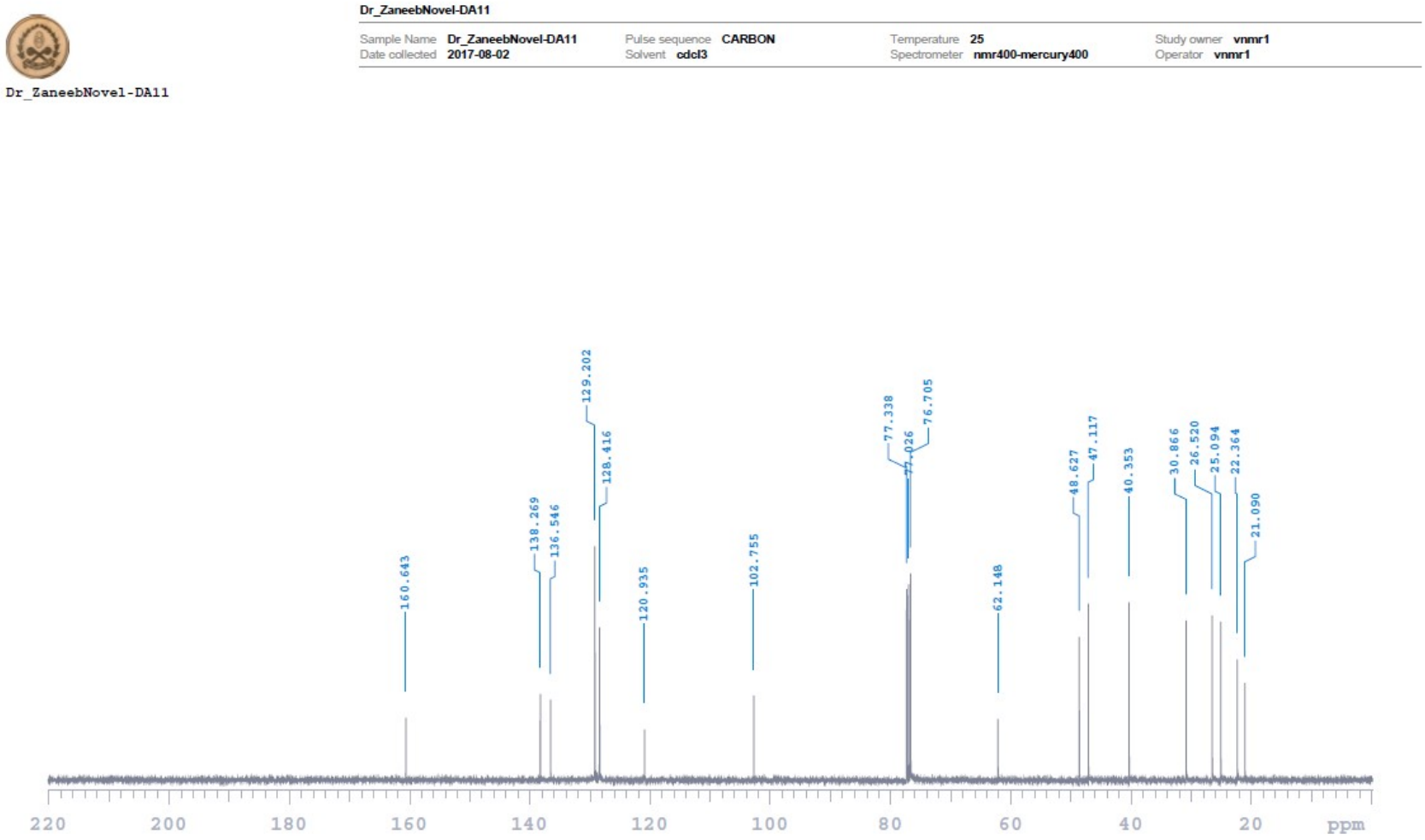


Fig. S67. ^{13}C-NMR spectrum of compound **4b**.

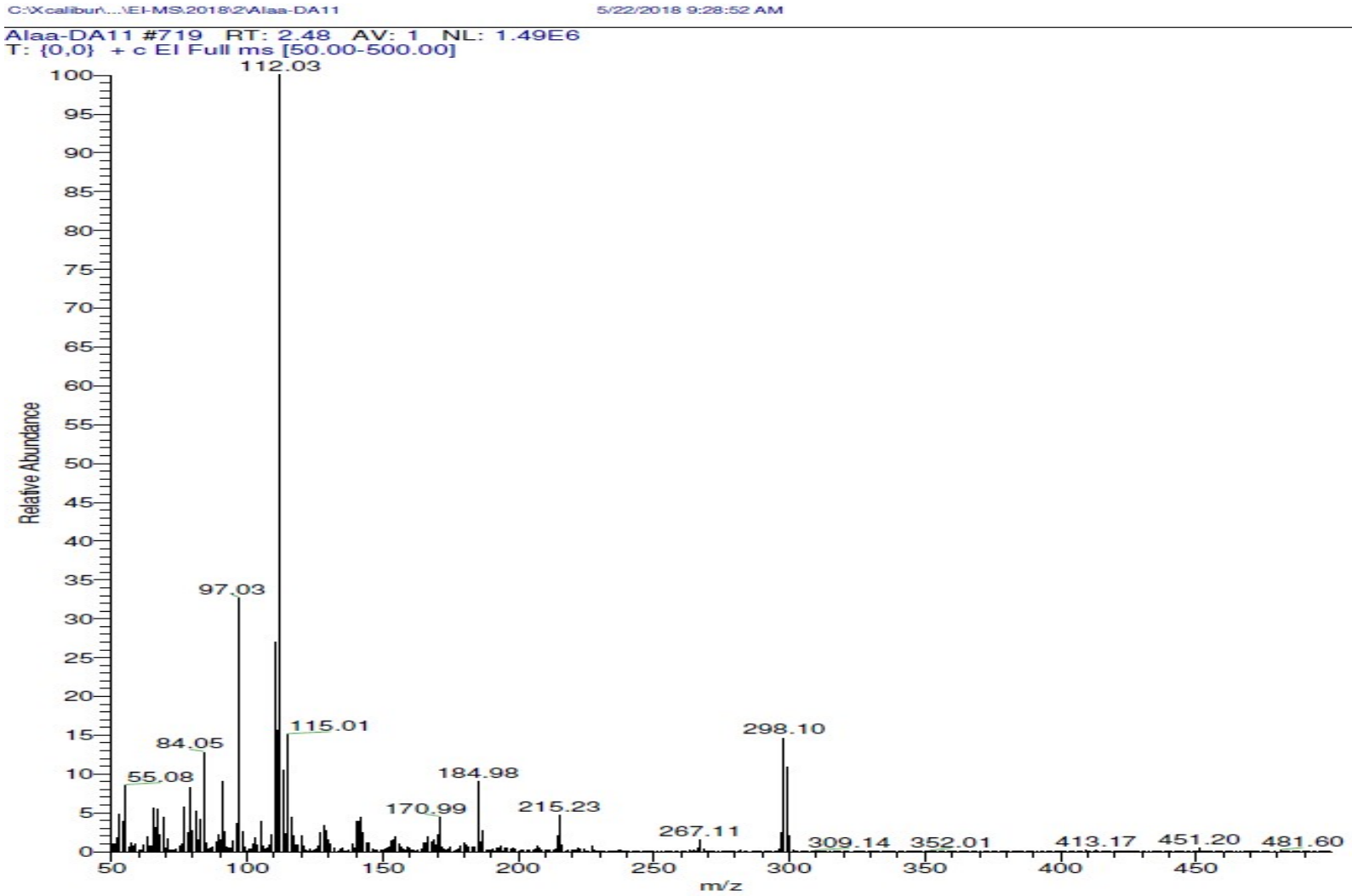
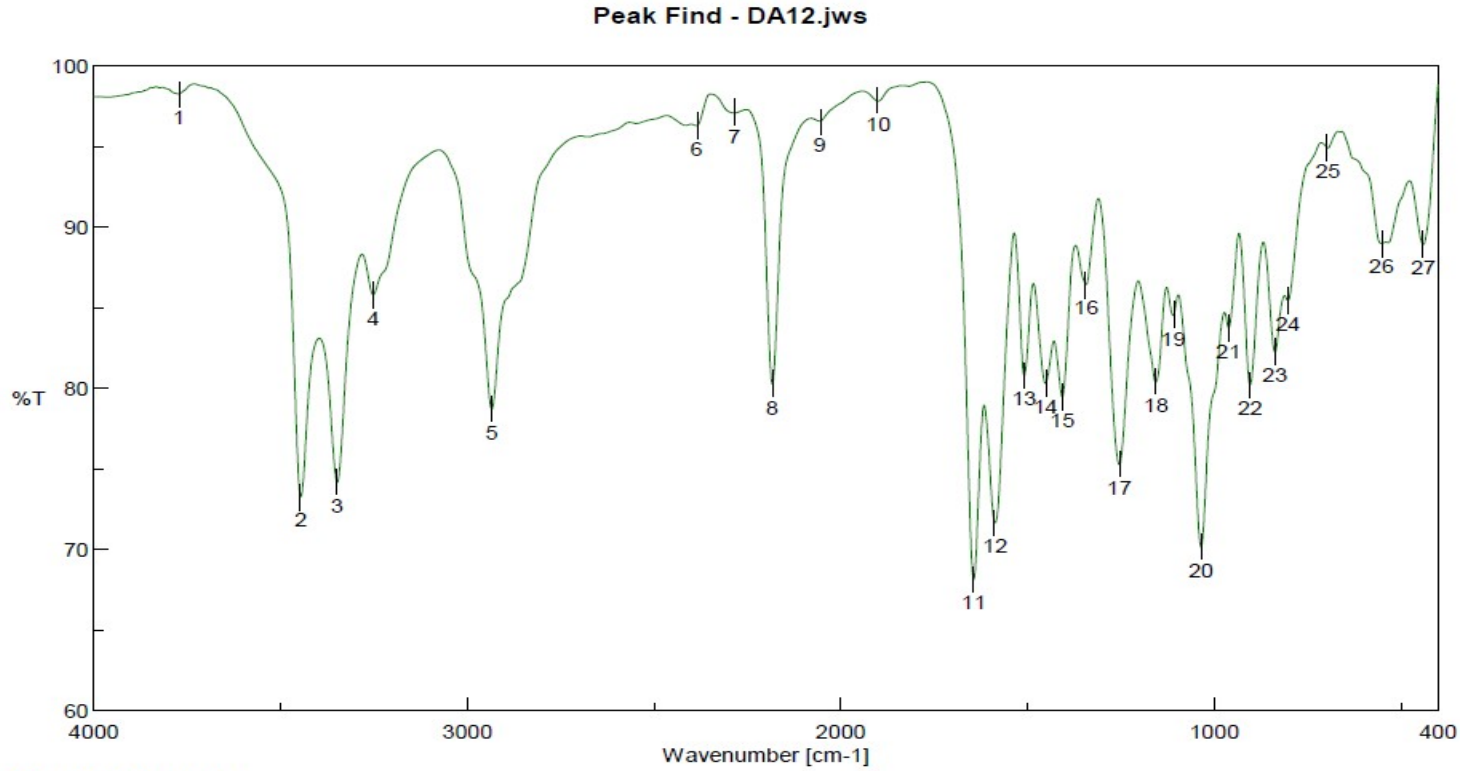


Fig. S68. Mass spectrum of compound **4b**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3773.05	98.2361	2	3446.17	73.256	3	3348.78	74.1493	4	3252.36	85.7805
5	2935.13	78.6892	6	2386.48	96.2685	7	2285.23	97.058	8	2183.02	80.2346
9	2056.71	96.5515	10	1899.54	97.7939	11	1644.02	68.1237	12	1587.13	71.6381
13	1509.03	80.7791	14	1452.14	80.2817	15	1406.82	79.4432	16	1343.18	86.4173
17	1254.47	75.2463	18	1156.12	80.3586	19	1109.83	84.4893	20	1034.62	70.1172
21	961.341	83.7293	22	903.487	80.1895	23	836.955	82.2213	24	802.242	85.4182
25	695.212	94.8828	26	551.542	88.9669	27	439.69	88.889			

Fig. S69. IR spectrum of compound **4c** (KBr pellet).

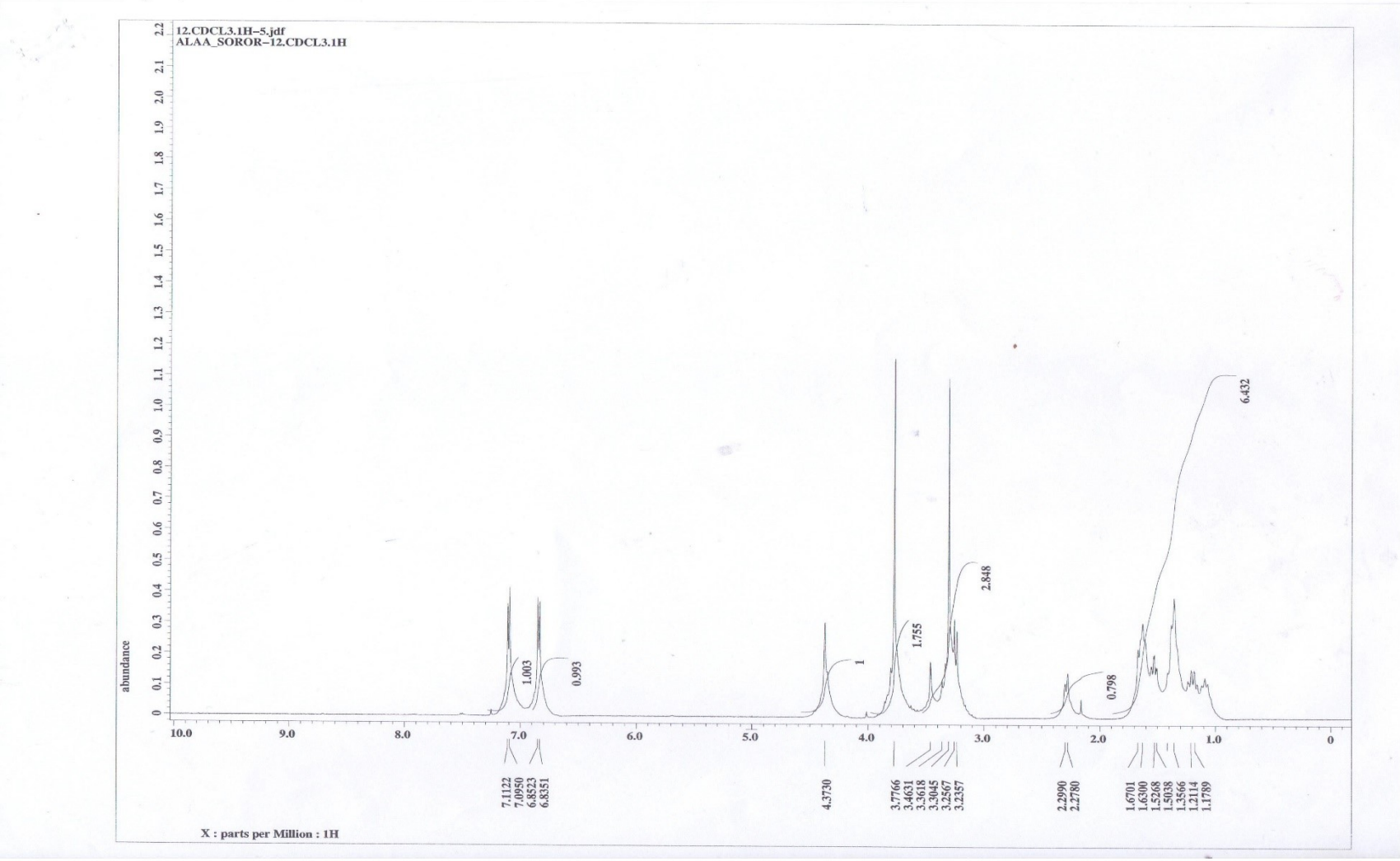


Fig. S70. [1]H-NMR spectrum of compound **4c**.

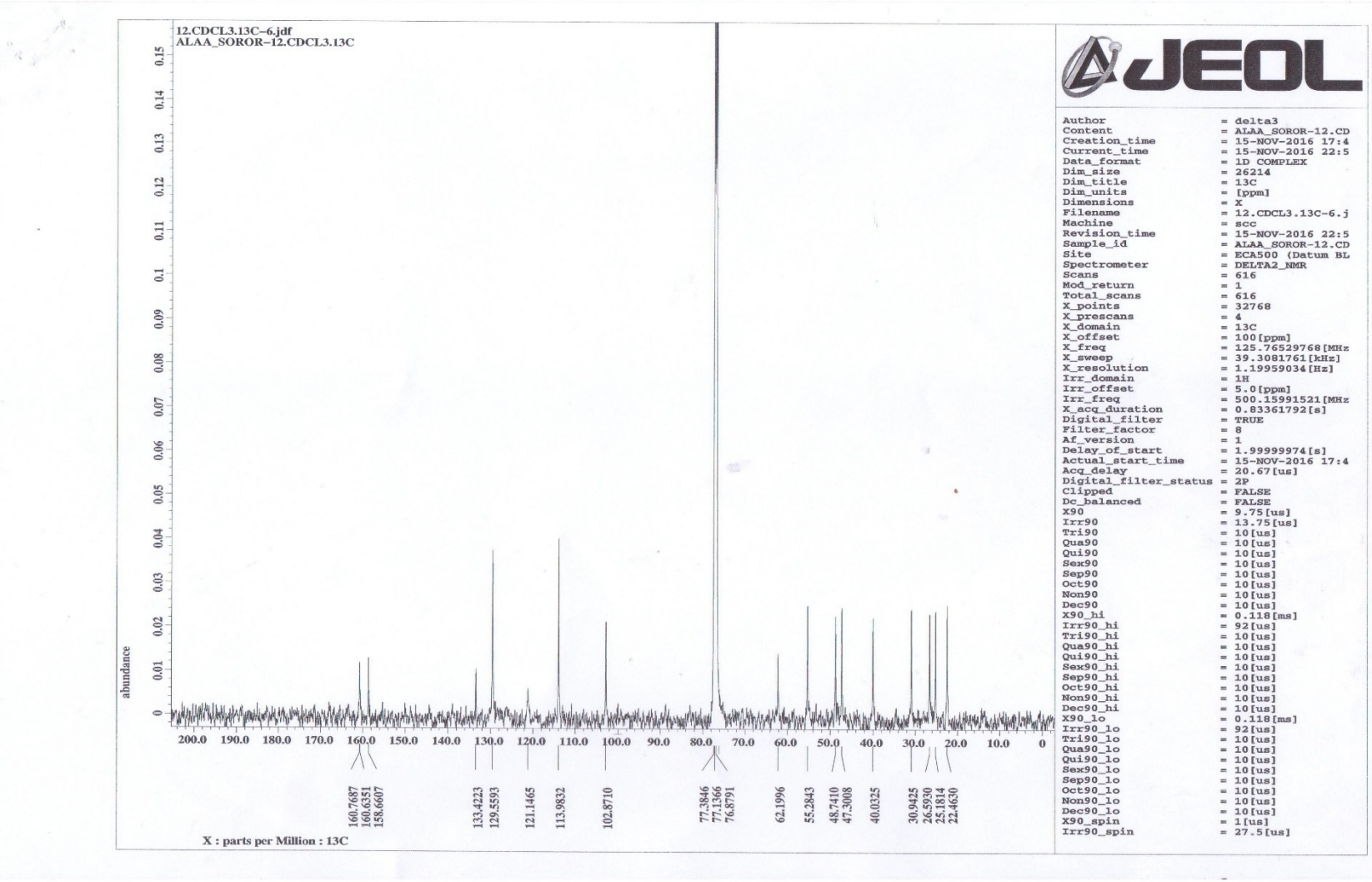


Fig. S71. ^{13}C-NMR spectrum of compound **4c**.

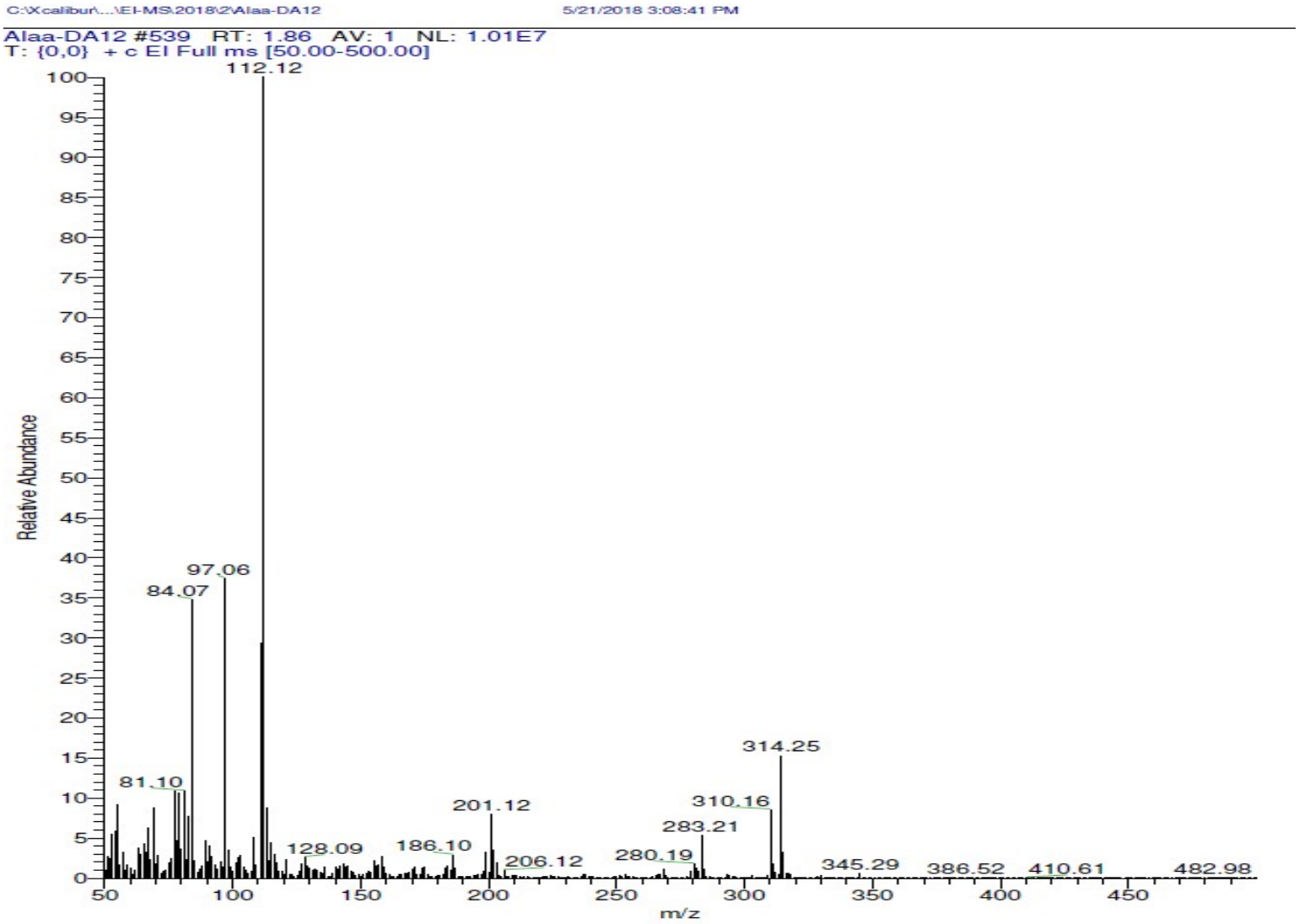
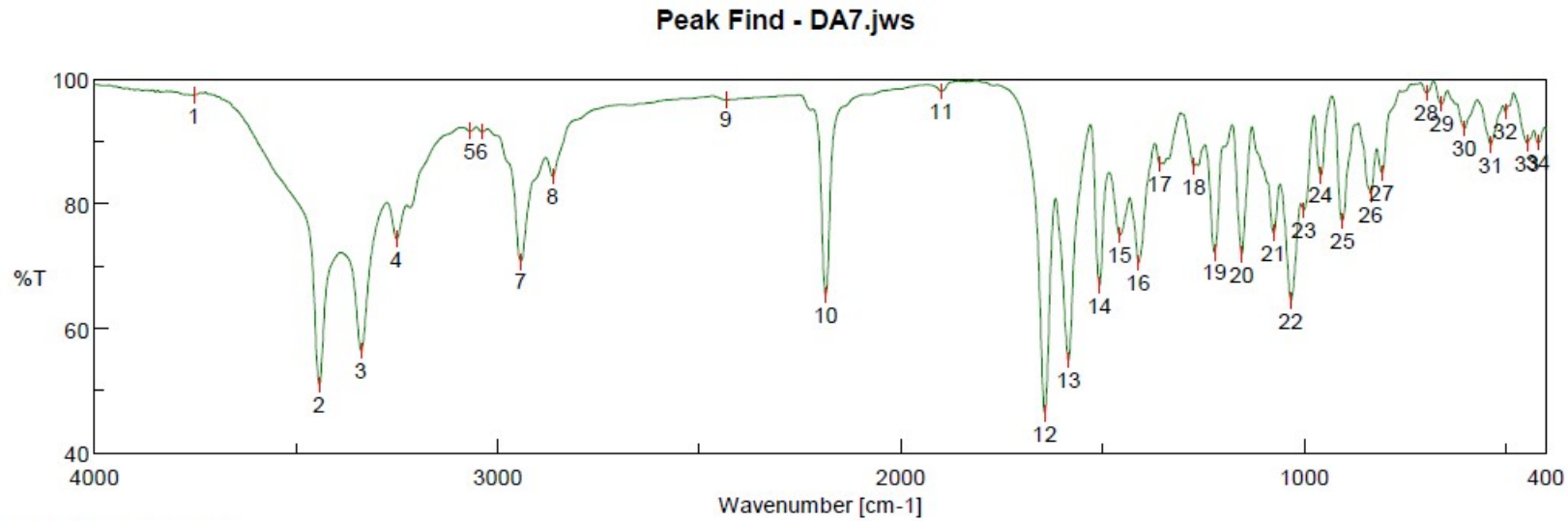


Fig. S72. Mass spectrum of compound **4c**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3753.76	97.3842	2	3442.31	50.989	3	3338.18	56.3791	4	3250.43	74.3288
5	3069.16	91.6051	6	3038.3	91.5202	7	2942.84	70.7409	8	2863.77	84.3654
9	2433.73	96.6704	10	2186.88	65.3249	11	1899.54	98.0079	12	1644.02	46.3515
13	1584.24	54.8659	14	1507.1	66.9234	15	1456.96	74.965	16	1410.67	70.5228
17	1358.6	86.3548	18	1273.75	85.9462	19	1222.65	72.1927	20	1154.19	71.8595
21	1076.08	75.3517	22	1032.69	64.4342	23	1000.87	78.9852	24	958.448	84.6174
25	905.415	77.2752	26	835.026	81.5303	27	807.063	84.952	28	697.141	97.786
29	659.536	95.9883	30	601.682	92.1171	31	537.078	89.441	32	501.401	94.7629
33	447.404	89.7041	34	420.406	89.7728						

Fig. S73. IR spectrum of compound **4d** (KBr pellet).

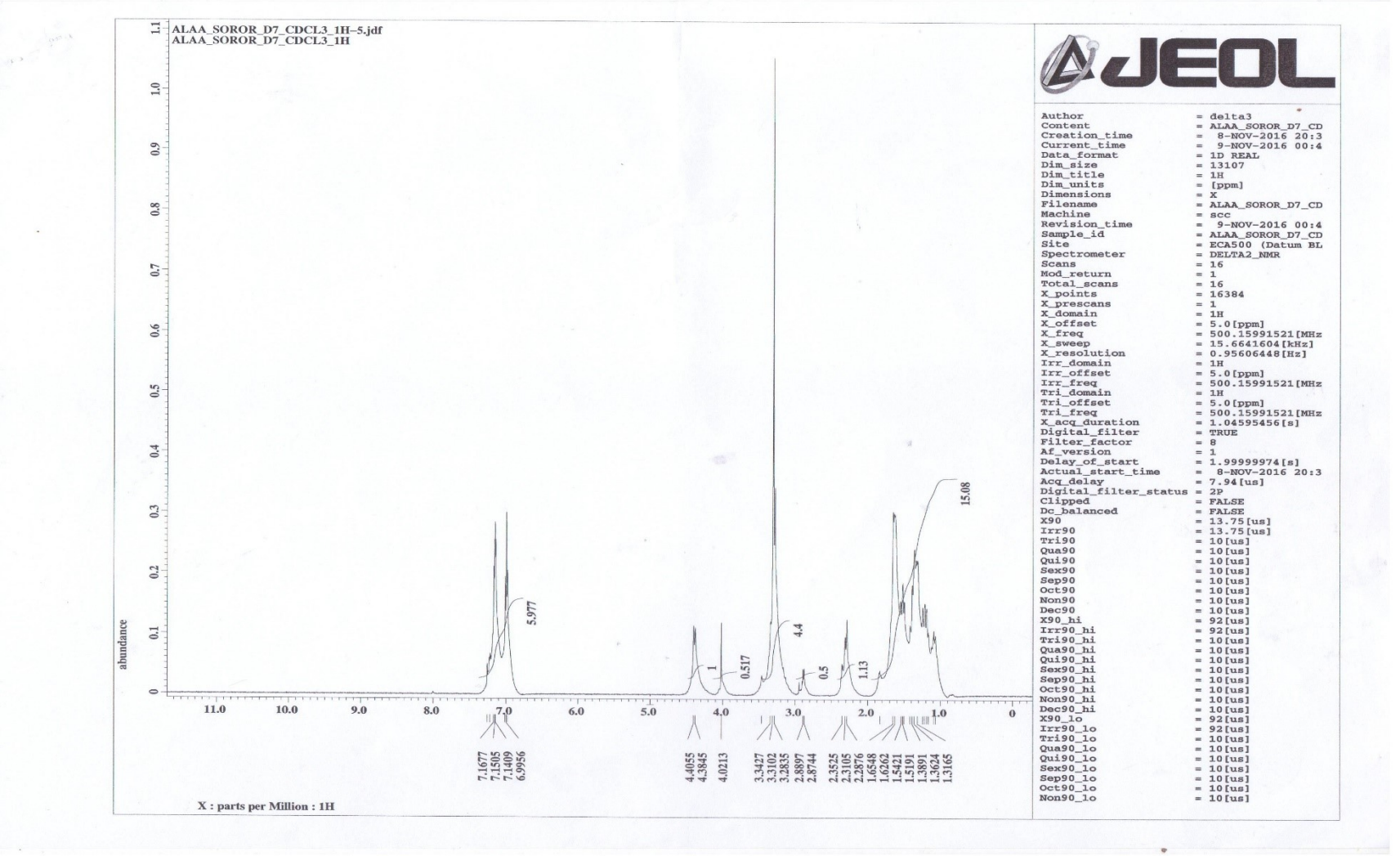


Fig. S74. ^{1}H-NMR spectrum of compound **4d**.

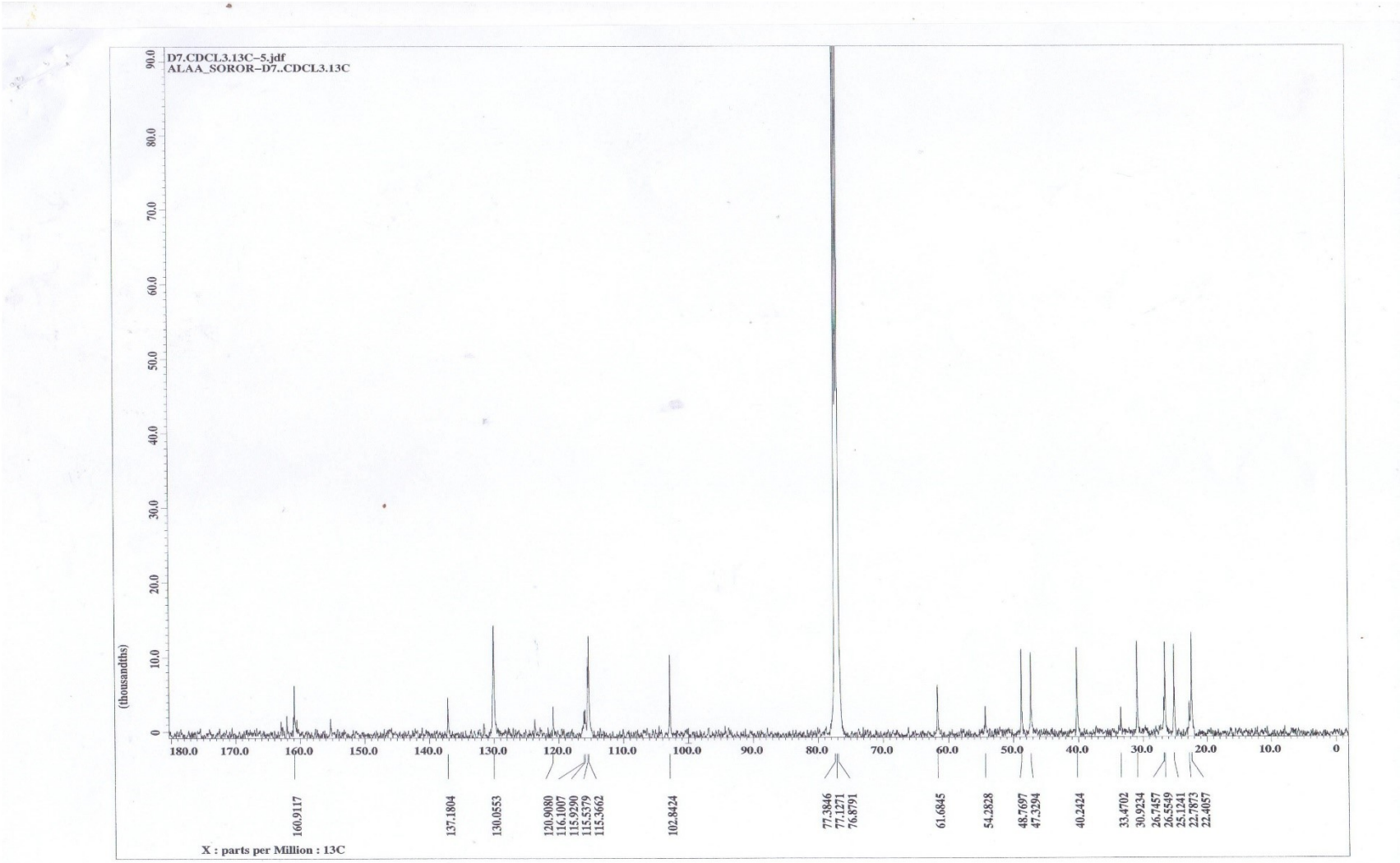


Fig. S75. ^{13}C-NMR spectrum of compound **4d**.

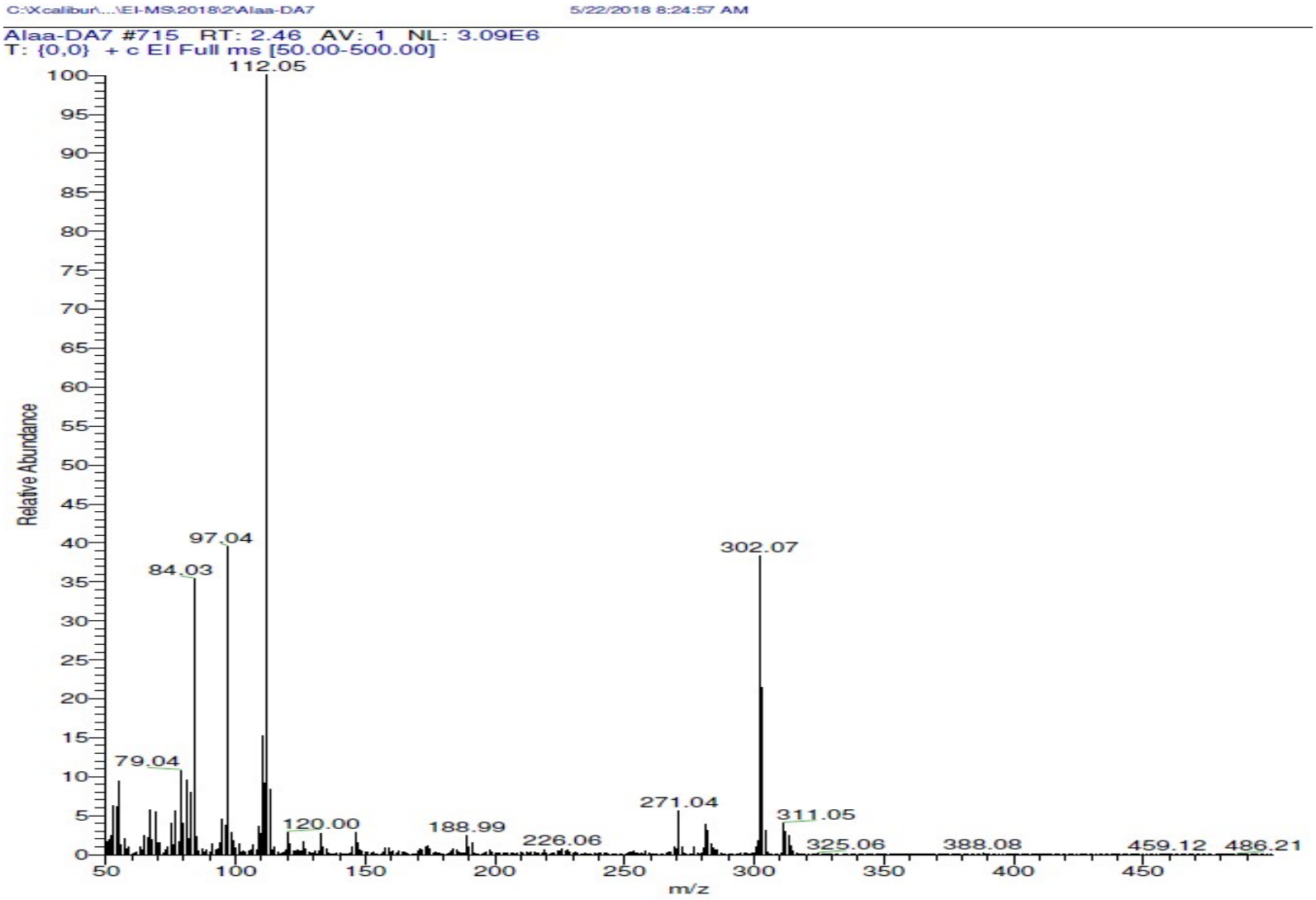
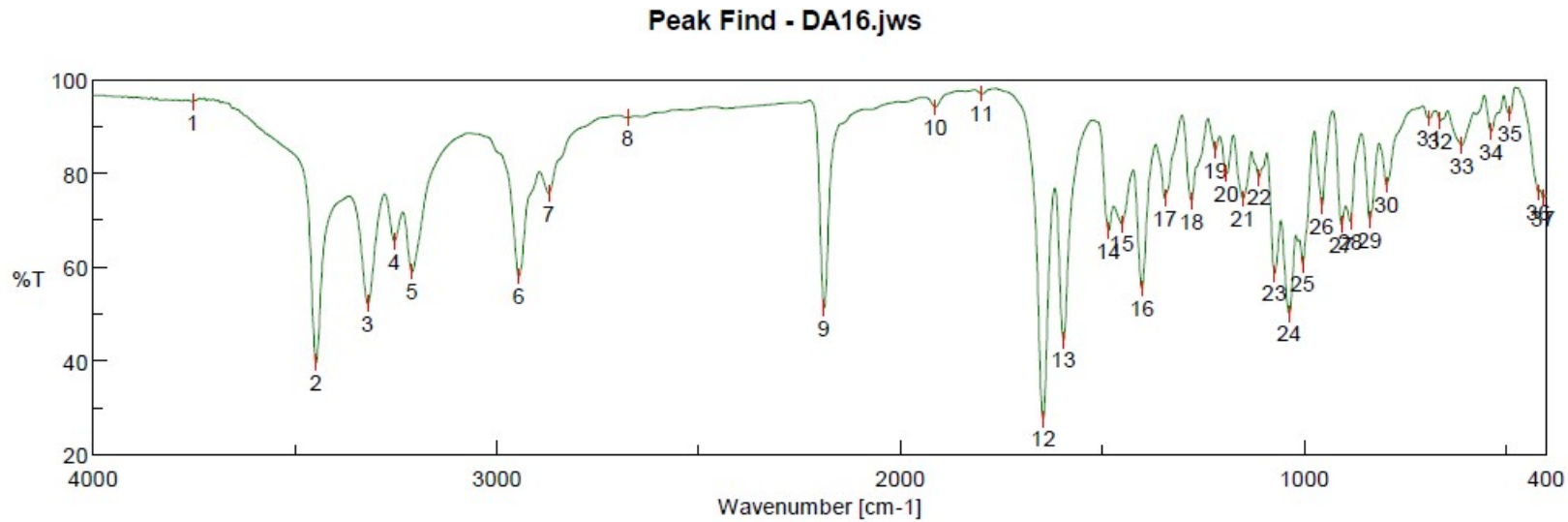


Fig. S76. Mass spectrum of compound **4d**.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3753.76	95.2633	2	3448.1	39.6083	3	3319.86	52.1672	4	3253.32	65.5215
5	3209.93	58.9237	6	2945.73	58.0336	7	2871.49	75.726	8	2675.75	91.9164
9	2188.81	51.2432	10	1913.04	94.1455	11	1798.3	96.9494	12	1645.95	27.5204
13	1595.81	44.3579	14	1483.96	67.7793	15	1452.14	69.1581	16	1402	55.4149
17	1345.11	74.7185	18	1278.57	74.0667	19	1219.76	84.9991	20	1191.79	79.9716
21	1151.29	74.5874	22	1111.76	79.0627	23	1073.19	58.6047	24	1036.55	49.9891
25	1002.8	60.5719	26	955.555	73.0408	27	907.344	69.0725	28	885.166	69.7611
29	837.919	70.1178	30	795.493	77.5145	31	689.427	91.7541	32	662.428	91.1311
33	609.396	85.9427	34	536.114	88.966	35	490.795	92.7079	36	419.442	75.9275
37	407.871	74.7009									

Fig. S77. IR spectrum of compound **4e** (KBr pellet).

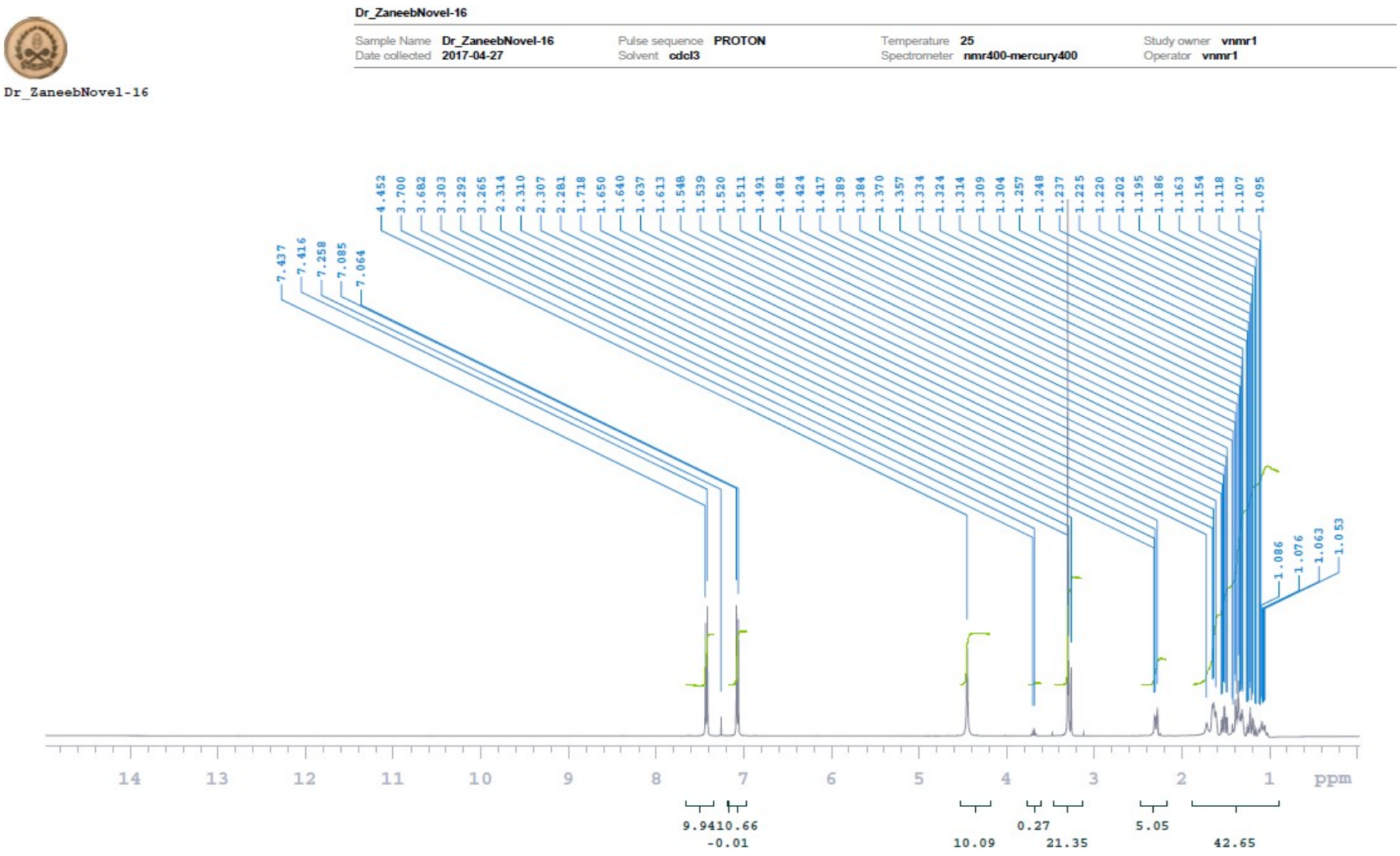


Fig. S78. [1]H-NMR spectrum of compound **4e**.

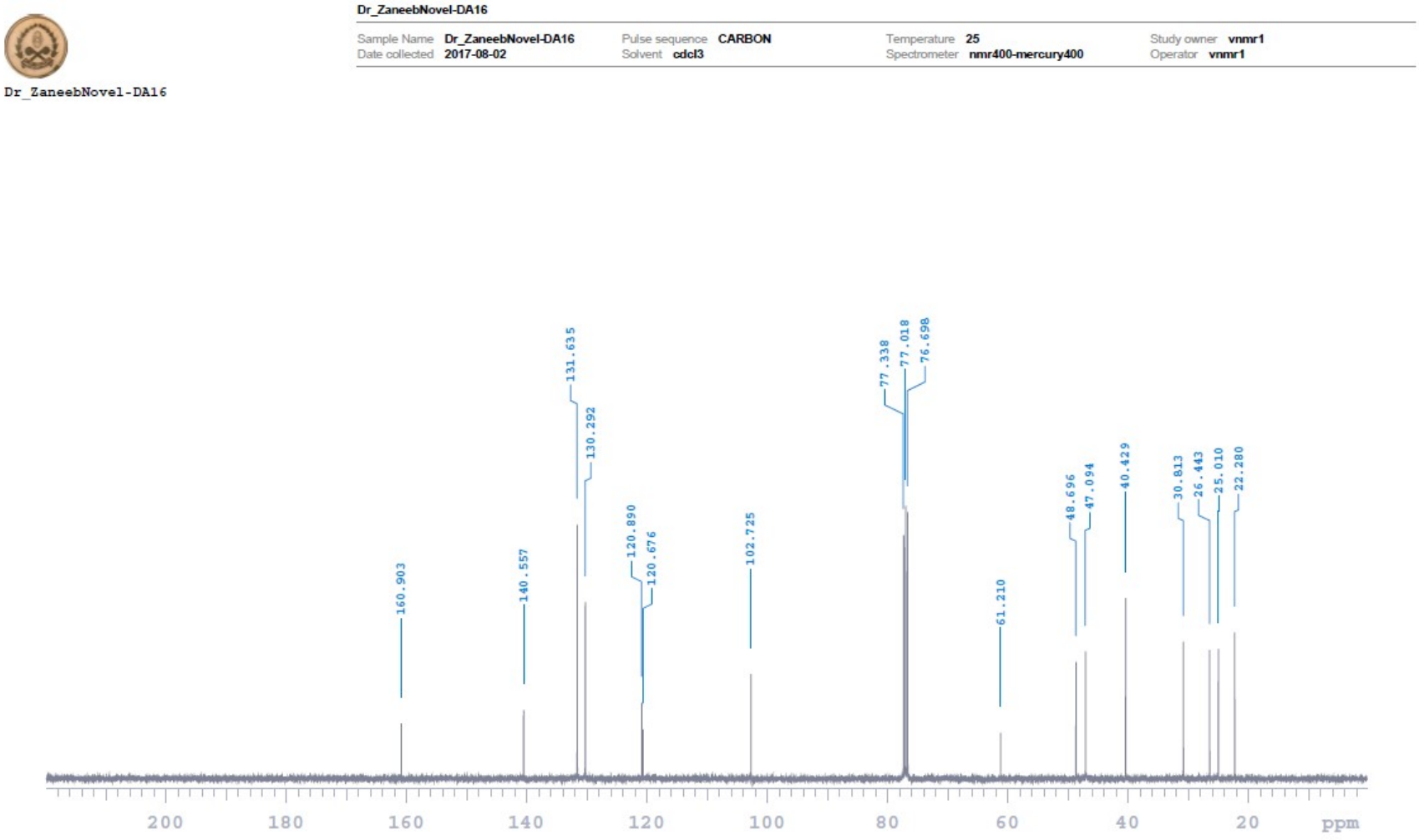


Fig. S79. ^{13}C-NMR spectrum of compound **4e**.

[1] A.S. Girgis, A. Kalmouch, M. Ellithey, Synthesis of novel vasodilatory active nicotinate esters with amino acid function. *Bioorg. Med. Chem*. 14 (**2006**) 8488-8494.

[2] Z.M. Nofal, A.M. Srour, W.I. El-Eraky, D.O. Saleh, A.S. Girgis. Rational design, synthesis and QSAR study of vasorelaxant active 3-pyridinecarbonitriles incorporating 1H-benzimidazol-2-yl function. *Eur. J. Med. Chem*. 63 (**2013**) 14-21.

[3] A.M. Srour, S.S. Abd El-Karim, D.O. Saleh, W.I. El-Eraky, Z.M. Nofal, Rational design, synthesis and 2D-QSAR study of novel vasorelaxant active benzofuran-pyridine hybrids. *Bioorg. Med. Chem. Lett*. 26 (**2016**) 2557-2561.

[4] N.M. Khalifa, A.M. Srour, S.S. AbdEl-Karim, D.O. Saleh, M. A. Al-Omar, Synthesis and 2D-QSAR Study of Active Benzofuran-Based Vasodilators, *Molecules*, 22 (**2017**) 1820.

[5] M. R. Boyd, K.D. Paull, *Drug Rev. Res.* **1995**, 34, 91–109.

[6] B. S. El-Menshawi, W. Fayad, K. Mahmoud, S. M. El-Hallouty, M. El-Manawaty, M. H. Olofsson, *Indian J. Exp. Biol.* **2010**, 48, 258–264.

[7] M. I. Thabrew, R. D. Hughes, I. G. McFarlane*, J. Pharm. Pharmacol.* **1997**, 49, 1132–1135.