

Synthesis of some new pyrimidine-based pyrene/benzochromene hybrids as EGFR kinase inhibitors in HCT-116 cancer cells through apoptosis

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Supporting Information

Table of Contents	Pages
1. Chemistry (General part).....	3
2. Characterization data.....	4
Fig. S1. IR spectrum of pyrimidine derivative 4a.....	4
Fig. S2. ¹H NMR spectrum of pyrimidine 4a (300 MHz, DMSO-<i>d</i>₆).....	5
Fig. S3. ¹³C NMR spectrum of 4a (75 MHz, DMSO-<i>d</i>₆).....	5
Fig. S4. High-resolution spectrum of 4a.....	6
Fig. S5. HPLC chromatogram of compound 4a.....	7
Fig. S6. IR spectrum of 4b.....	8
Fig. S7. ¹H NMR spectrum of pyrimidine 4b (300 MHz, DMSO-<i>d</i>₆).....	8
Fig. S8. ¹³C NMR spectrum of 4b (75 MHz, DMSO-<i>d</i>₆).....	9
Fig. S9. MALDI-TOF mass spectrum 4b	10
Fig. S10. HPLC chromatogram of compound 4b.....	11
Fig. S11. IR spectrum of 4c.....	12
Fig. S12. ¹H NMR spectrum of 4c (300 MHz, DMSO-<i>d</i>₆).....	13

Fig. S13. ^{13}C NMR spectrum of 4c (75 MHz, $\text{DMSO-}d_6$).....	14
Fig. S14. MALDI-TOF mass spectrum of 4c	15
Fig. S15. HPLC chromatogram of compound 4c	16
Fig. S16. IR spectrum of acrylamide 6	17
Fig. S17. ^1H NMR spectrum of acrylamide 6 (300 MHz, $\text{DMSO-}d_6$).....	18
Fig. S18. ^{13}C NMR spectrum of acrylamide 6 (75 MHz, $\text{DMSO-}d_6$).....	19
Fig. S19. IR spectrum of acrylamide 7	20
Fig. S20. ^1H NMR spectrum of acrylamide 7 (300 MHz, $\text{DMSO-}d_6$)	21
Fig. S21. ^{13}C NMR spectrum of acrylamide 7 (75 MHz, $\text{DMSO-}d_6$).....	22
Fig. S22. MALDI-TOF mass spectrum of acrylamide 7	23
Fig. S23. IR spectrum of 11a	24
Fig. S24. ^1H NMR spectrum of 11a (500 MHz, $\text{DMSO-}d_6$).....	25
Fig. S25. ^{13}C NMR spectrum (APT) of 11a (125 MHz, $\text{DMSO-}d_6$).....	26
Fig. S26. High resolution mass of 11a	27
Fig. S27. IR spectrum of 11c	28
Fig. S28. ^1H NMR spectrum of 11c (500 MHz, $\text{DMSO-}d_6$).....	29
Fig. S29. ^{13}C NMR spectrum of 11c (75 MHz, $\text{DMSO-}d_6$).....	30
Fig. S30. High resolution mass of 11c	31
Fig. S31. IR spectrum of 12	32
Fig. S32. ^1H NMR of compound 12 in $\text{DMSO-}d_6$	33
Fig. S33. ^{13}C NMR spectrum of 12 (125 MHz, $\text{DMSO-}d_6$).....	34
Fig. S34. High resolution Mass spectrum of 12	35
Fig. S35. IR spectrum of 15a	36
Fig. S36. ^1H NMR spectrum of 15a (300 MHz, $\text{DMSO-}d_6$).....	37
Fig. S37. ^{13}C NMR spectrum of 15a (75 MHz, $\text{DMSO-}d_6$).....	38
Fig. S38. High resolution mass of 15a	39
Fig. S39. IR spectrum of 15c	40
Fig. S40. ^1H NMR spectrum of 15c (300 MHz, $\text{DMSO-}d_6$).....	41
Fig. S41. ^{13}C NMR spectrum of 15c (75 MHz, $\text{DMSO-}d_6$).....	42
Fig. S42. High resolution mass of 15c	43
Fig. S43. IR spectrum of 16	44
Fig. S44. ^1H NMR spectrum of 16 (500 MHz, $\text{DMSO-}d_6$).....	45
Fig. S45. ^{13}C NMR spectrum of 16 (125 MHz, $\text{DMSO-}d_6$)	46

1. Chemistry

General part

Melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded on KBr disks on a Pye Unicam SP 3300 and Shimadzu FT-IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on Bruker-500 or JEOL-500 (running at 500 MHz for ^1H and 125 MHz for ^{13}C) or on Varian Mercury Vx-300 BB (running at 300 MHz for ^1H and 75 MHz for ^{13}C). Molecular weight determination using Matrix-assisted Laser Desorption Ionization Time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker, model ultraFlexXtreme) was used to determine the molecular weight of the new products. The measurements were examined in positive mode using a reflectron time-of-flight mass spectrometer. The instrument was calibrated with a peptide calibration standard. The sample was prepared as follows: the matrix DCTB dissolved in tetrahydrofuran (THF) (DCTB = Trans-2-(3-(4-tert-butylphenyl)-2-methyl-2-propenylidene)malononitrile $\geq 99.0\%$) was purchased from Sigma. All samples were dissolved in THF. The samples were deposited on the target plate as follows: 2 μL of the sample was mixed with 2 μL of the matrix solution in a 1:1 ratio using an Eppendorf Research Plus micropipette. Using a micropipette, a single drop of the solution mixture was placed on a specific labeled location in the target plate and then allowed to dry at room temperature. The most active compounds were tested for purity using HPLC (DIONEX, Ultimate 3000, Diode Array Detector, Thermo Scientific), equipped with dual (Fluorescence, Photodiode array), autosampler, detection at 272-333 nm. The column used in HPLC was C-18 (250 $\times$ 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (ACN)/ H_2O (80:20), a flow rate of 0.5 mL/min for 20 min. Their purity was recorded with a high percentage ≥ 97 , while one compound exhibited 99.5% purity. This was performed at the central laboratory of toxins and microanalytical at the Faculty of Science, Suez Canal University.

2. Characterization data

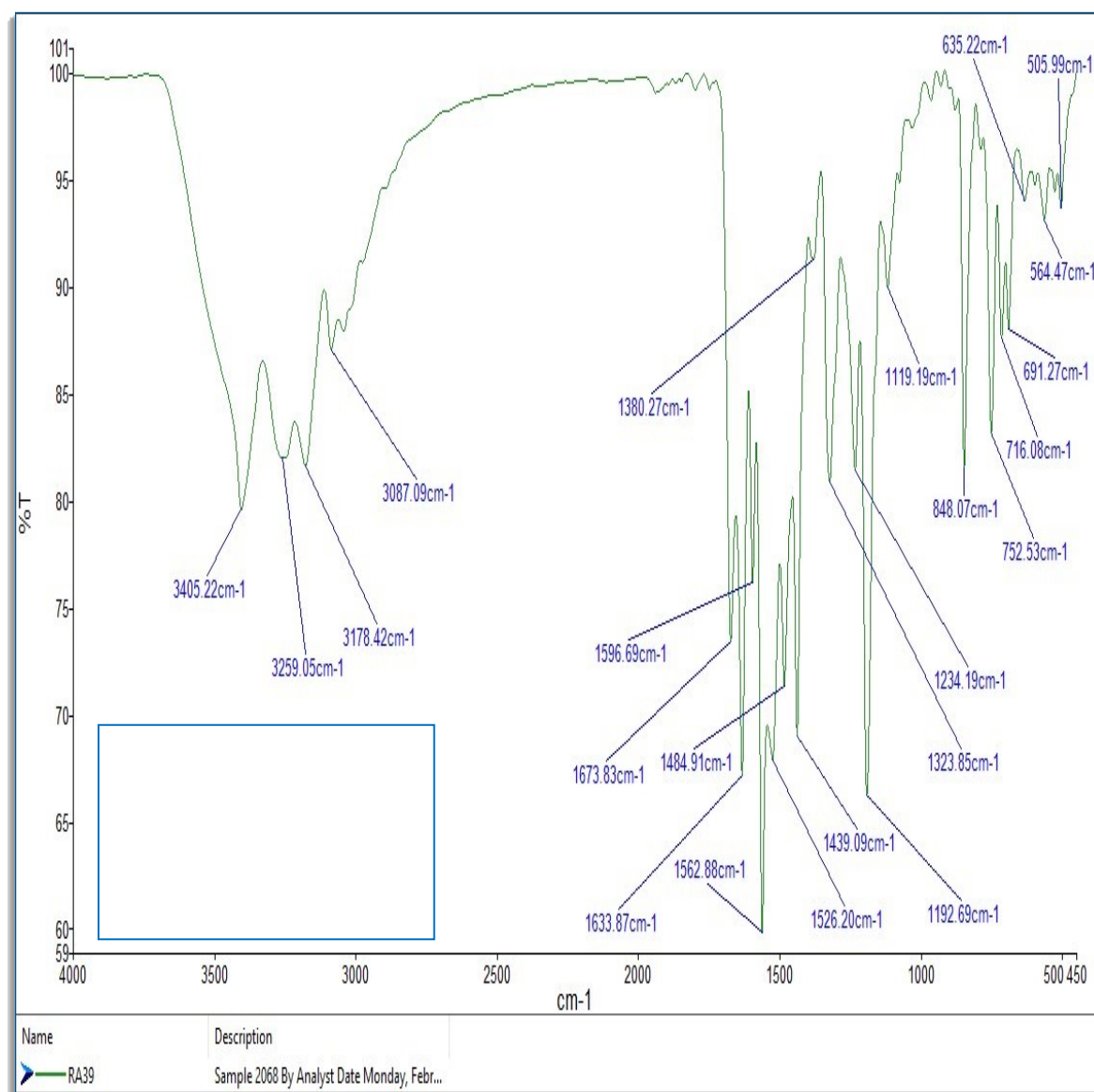


Fig. S1. IR spectrum of pyrimidine derivative **4a**

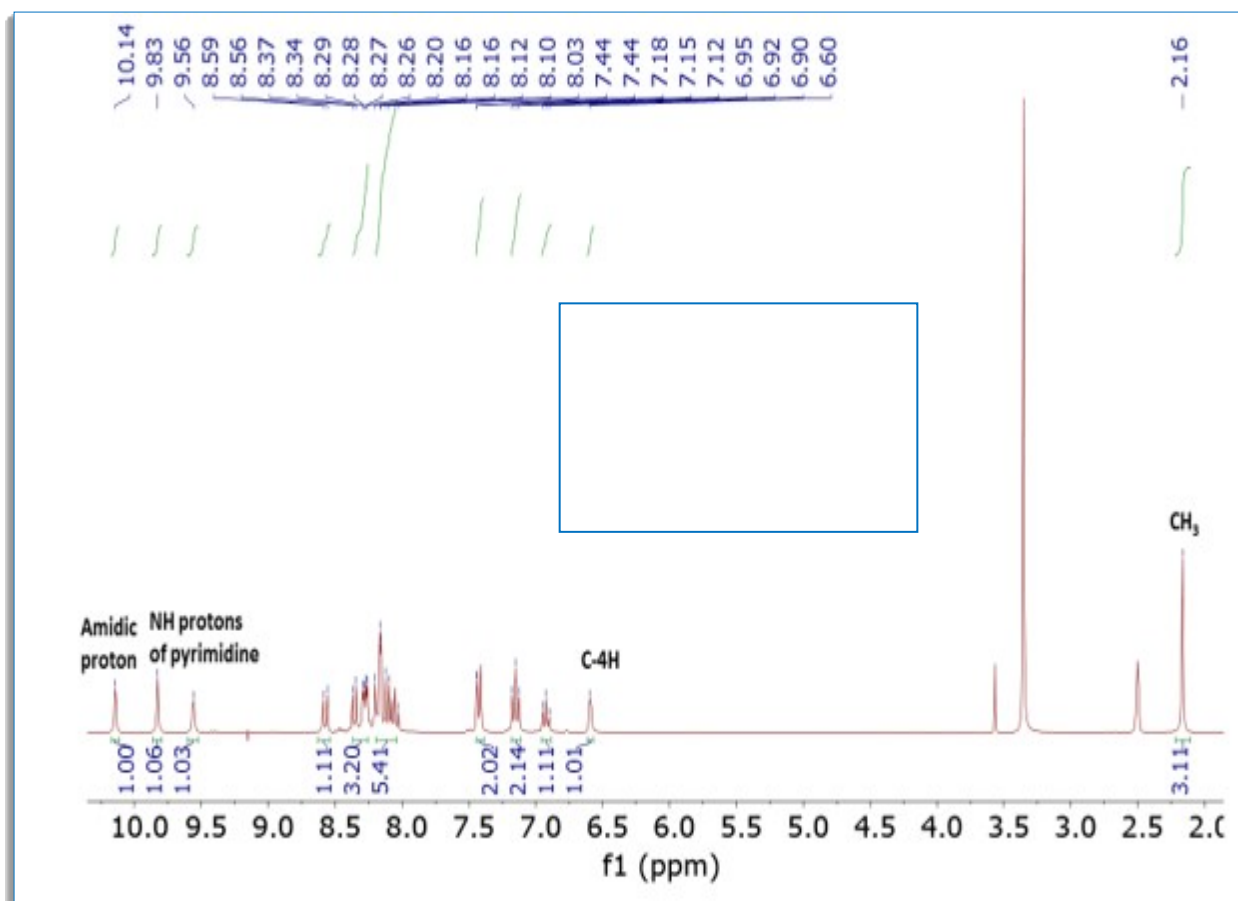


Fig. S2. ¹H NMR spectrum of pyrimidine **4a** (300 MHz, DMSO-*d*₆)

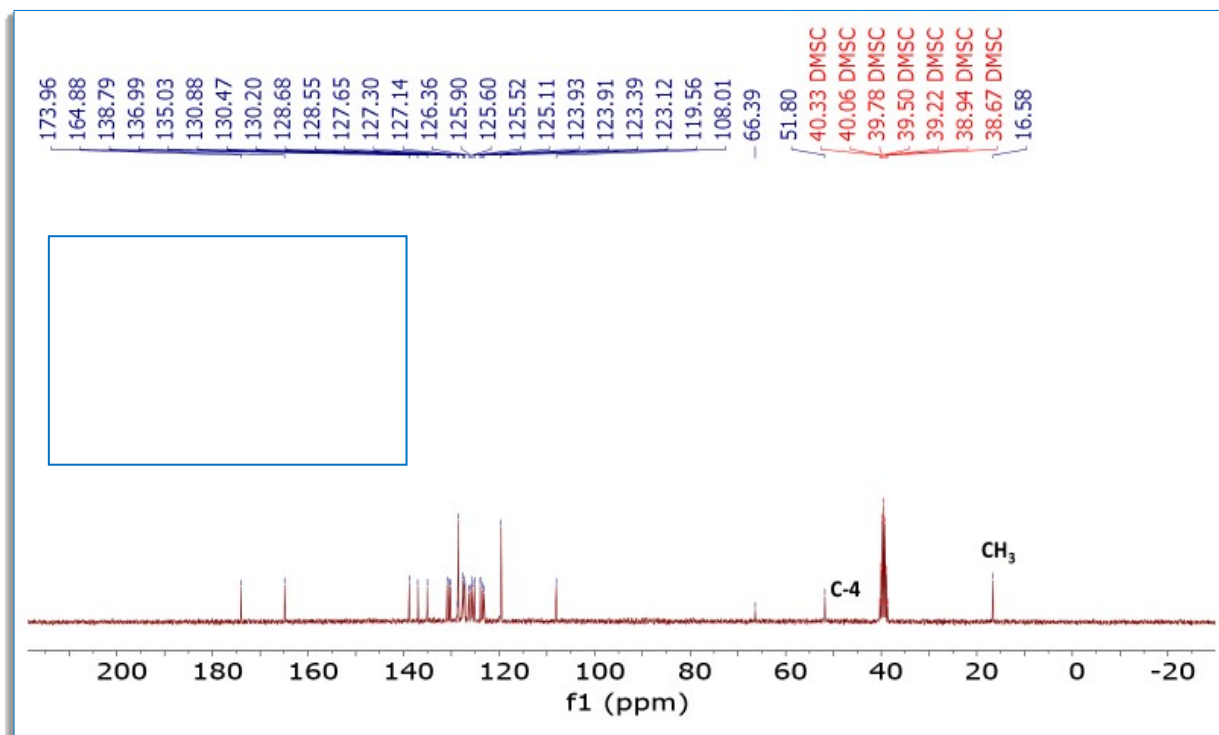


Fig. S3. ¹³C NMR spectrum of **4a** (75 MHz, DMSO-*d*₆)

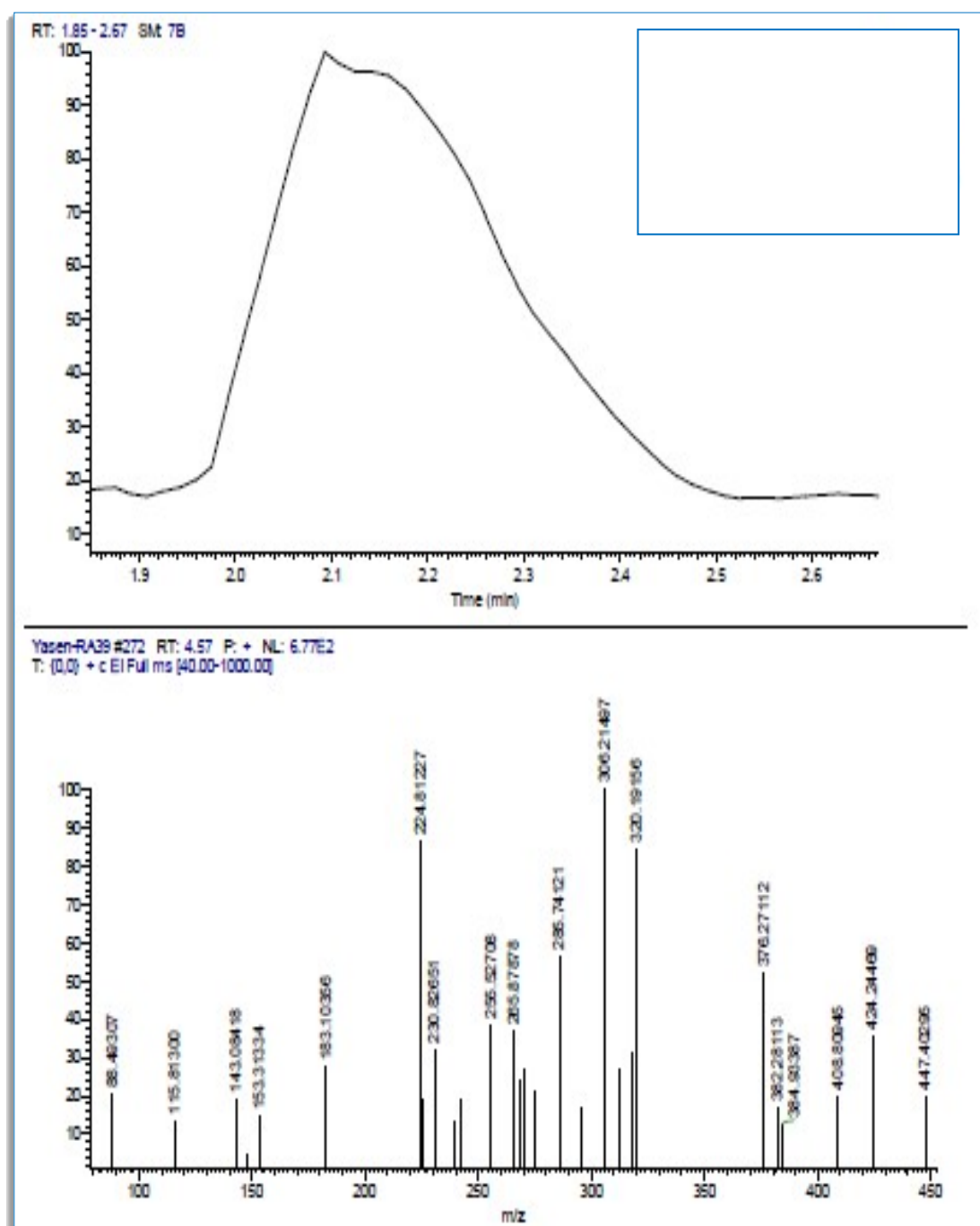
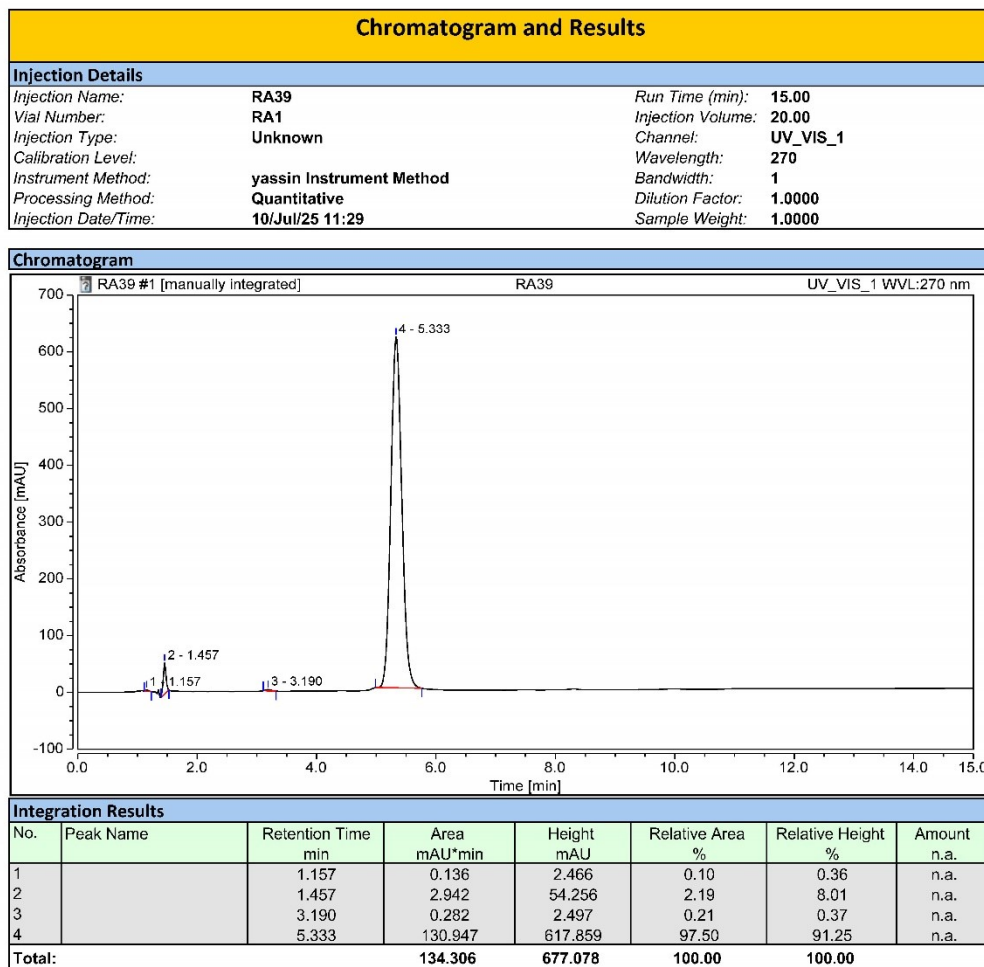


Fig. S4. High-resolution spectrum of **4a**

**Fig. S5.** HPLC chromatogram of compound **4a**.

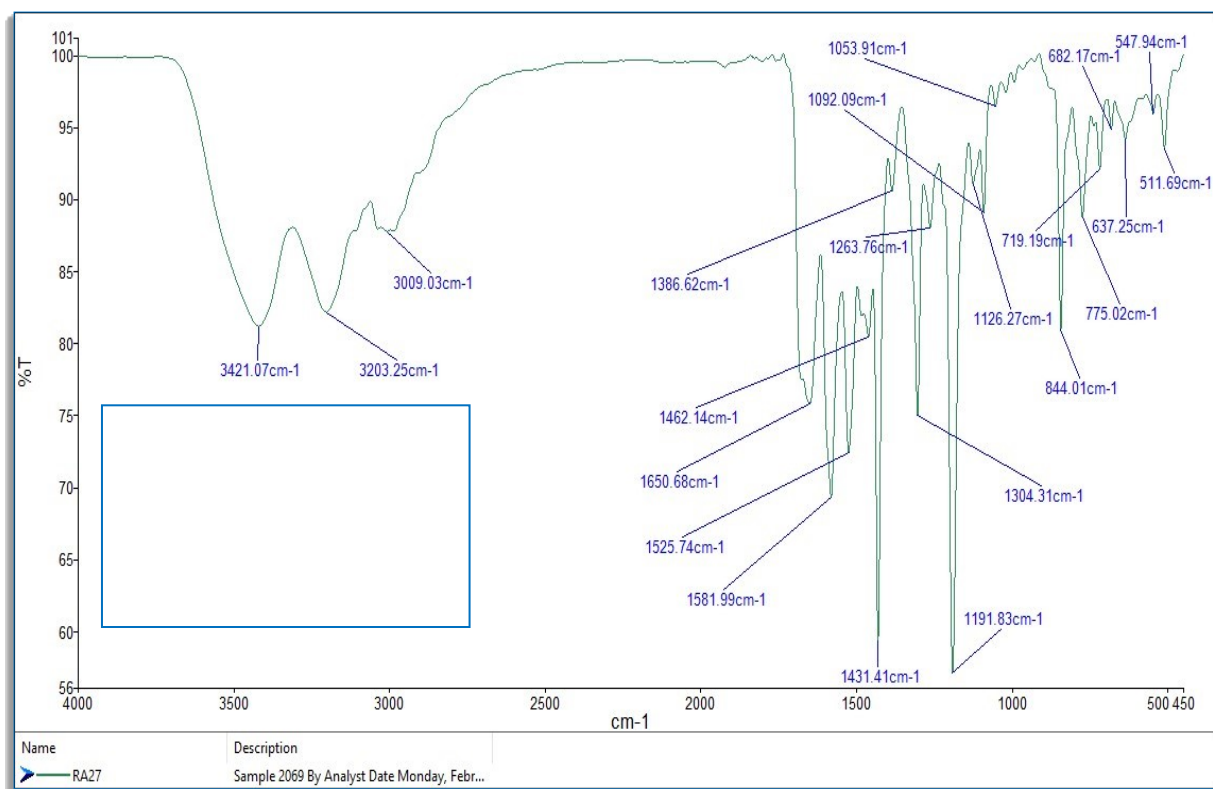


Fig. S6. IR spectrum of **4b**

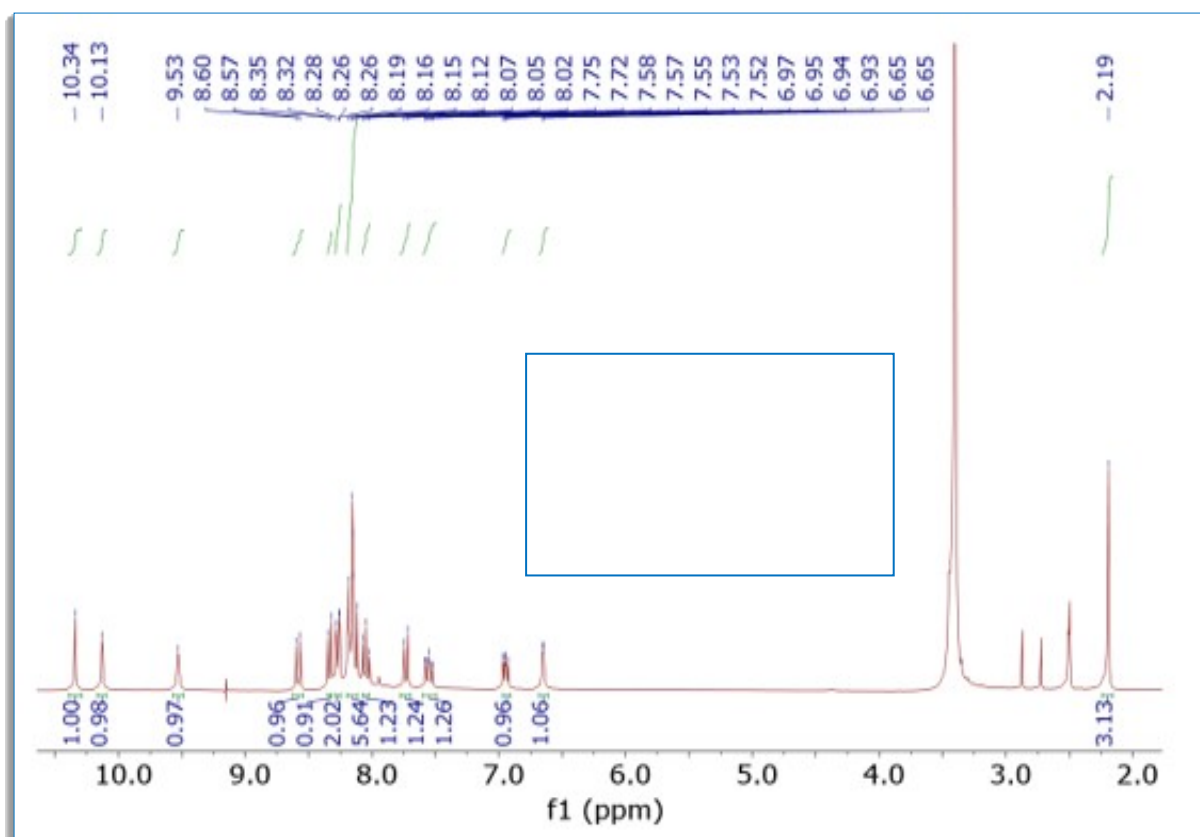


Fig. S7. ^1H NMR spectrum of pyrimidine **4b** (300 MHz, $\text{DMSO}-d_6$)

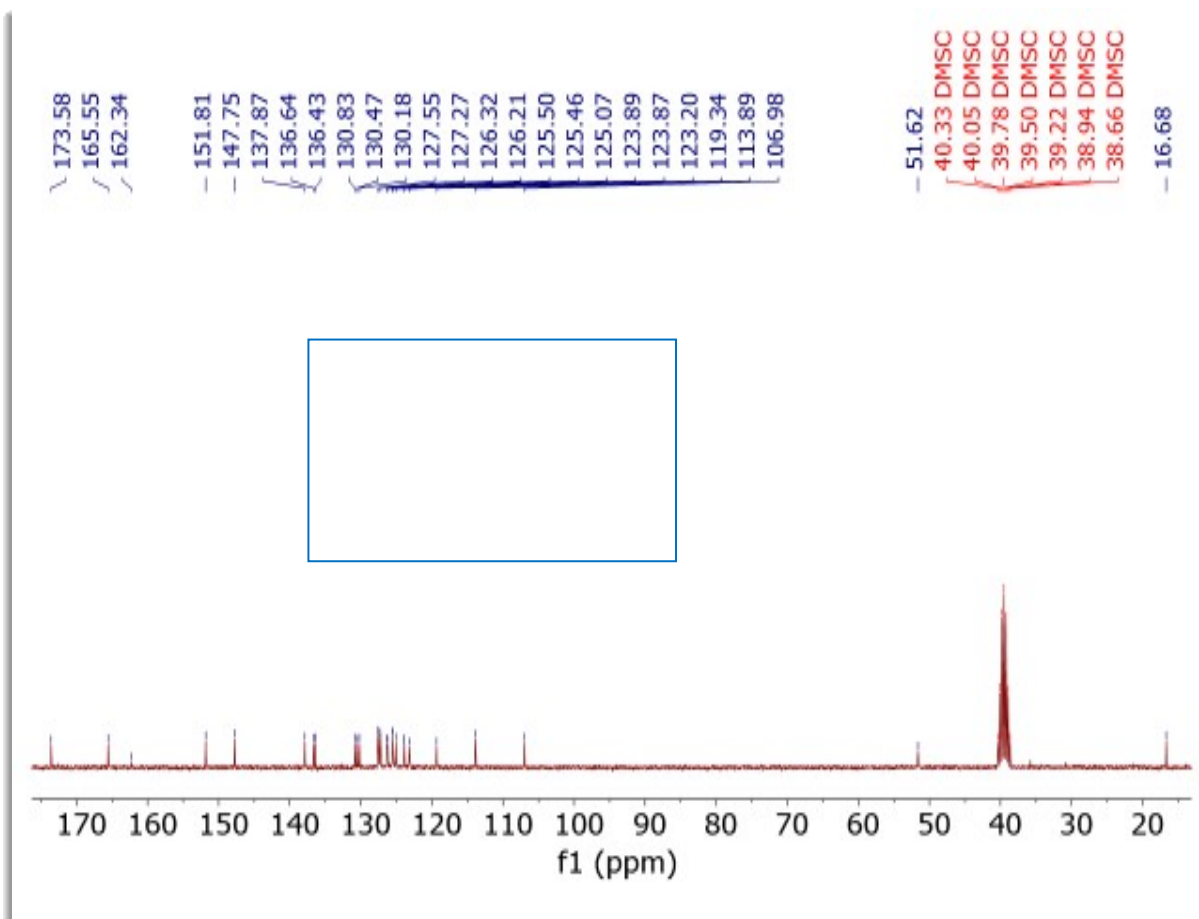


Fig. S8. ^{13}C NMR spectrum of **4b** (75 MHz, $\text{DMSO-}d_6$)

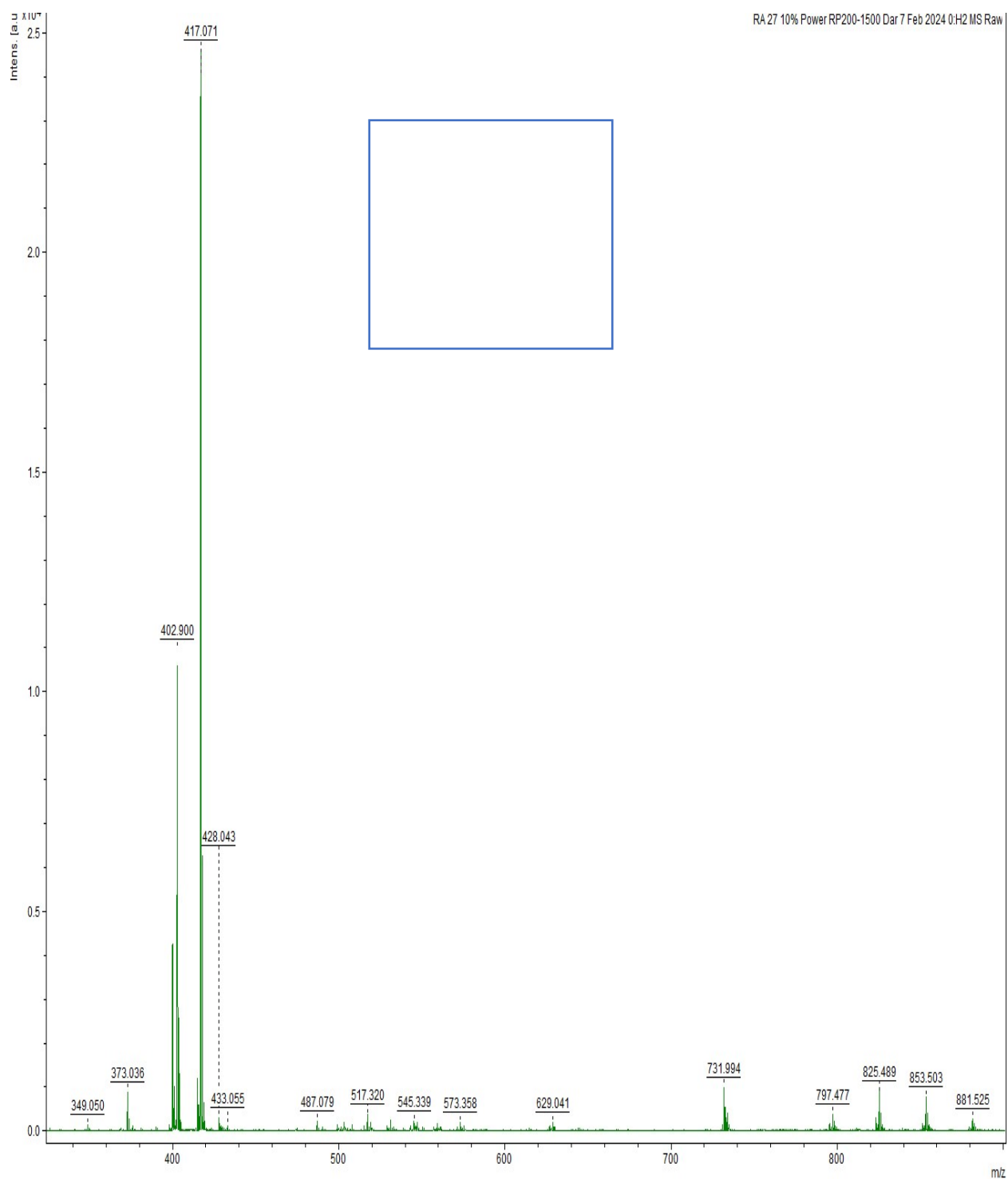
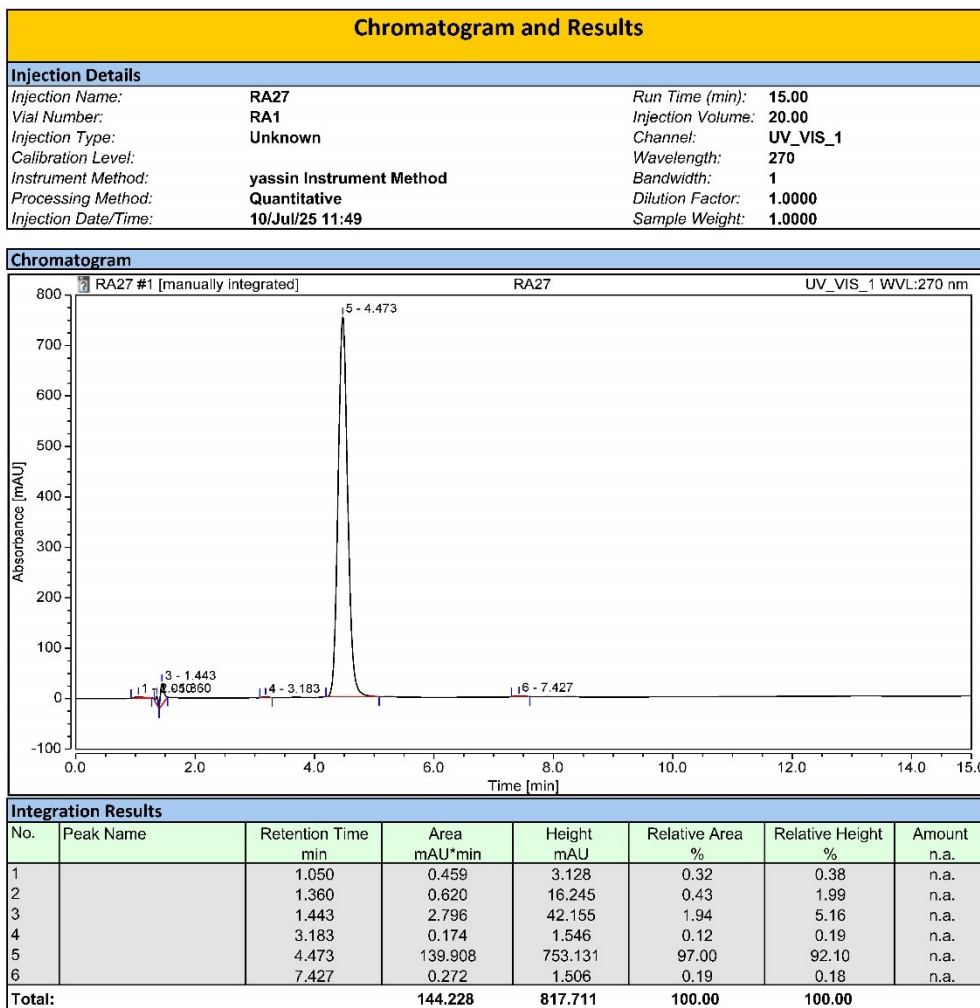


Fig. S9. MALDI-TOF mass spectrum **4b**.

Fig. S10. HPLC chromatogram of compound **4b**.

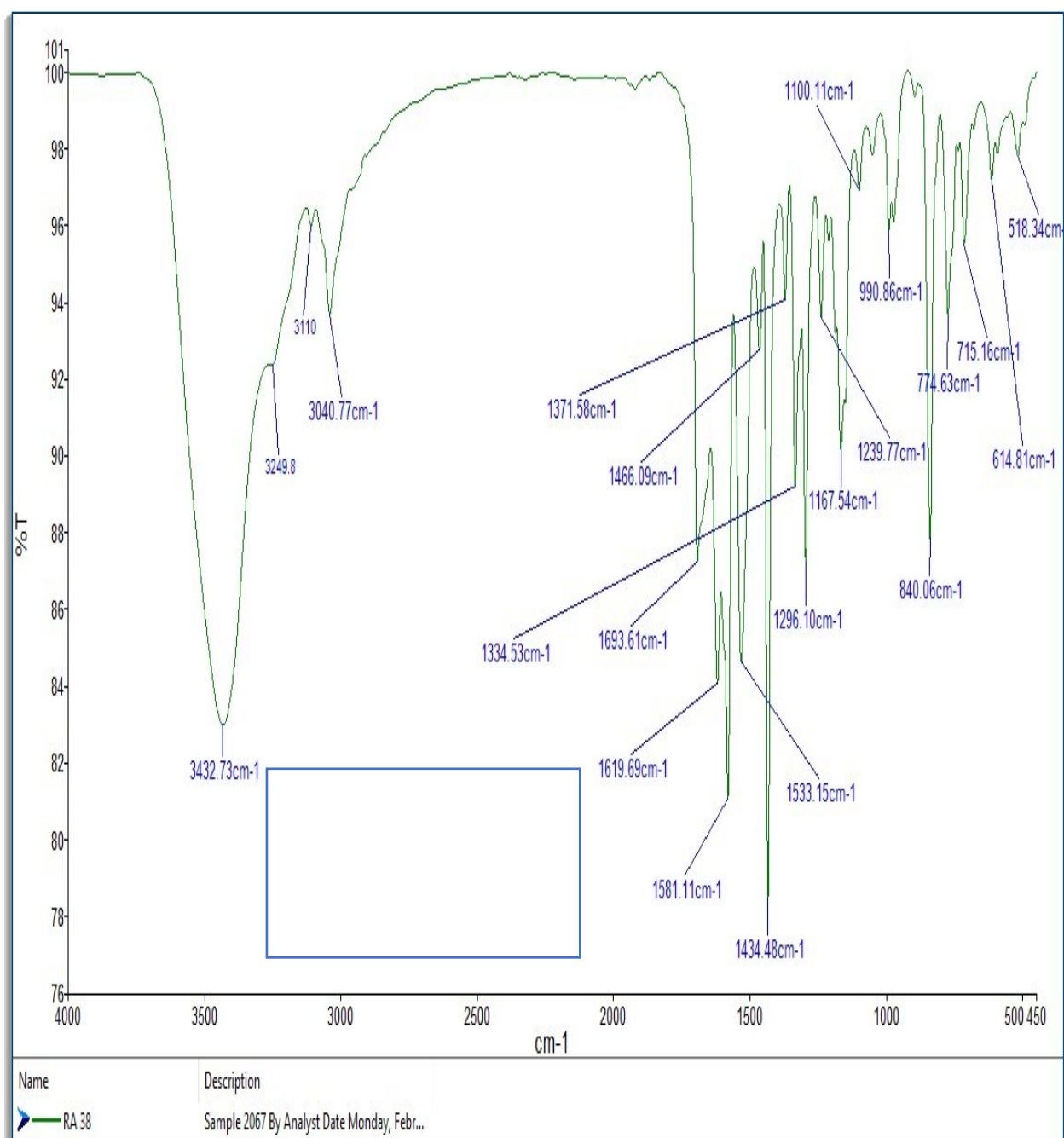


Fig. S11. IR spectrum of **4c**

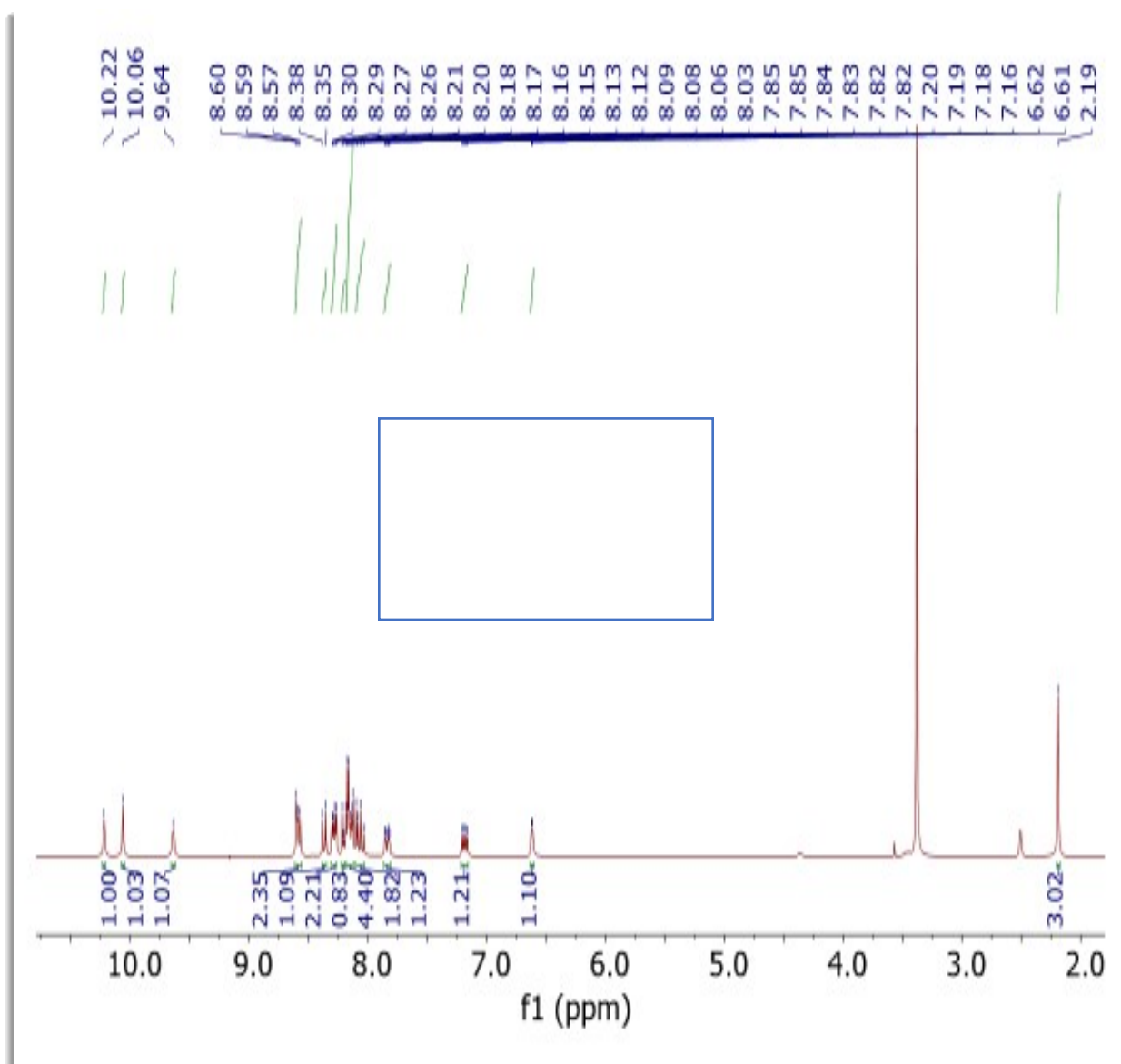


Fig. S12. ^1H NMR spectrum of **4c** (300 MHz, $\text{DMSO}-d_6$)

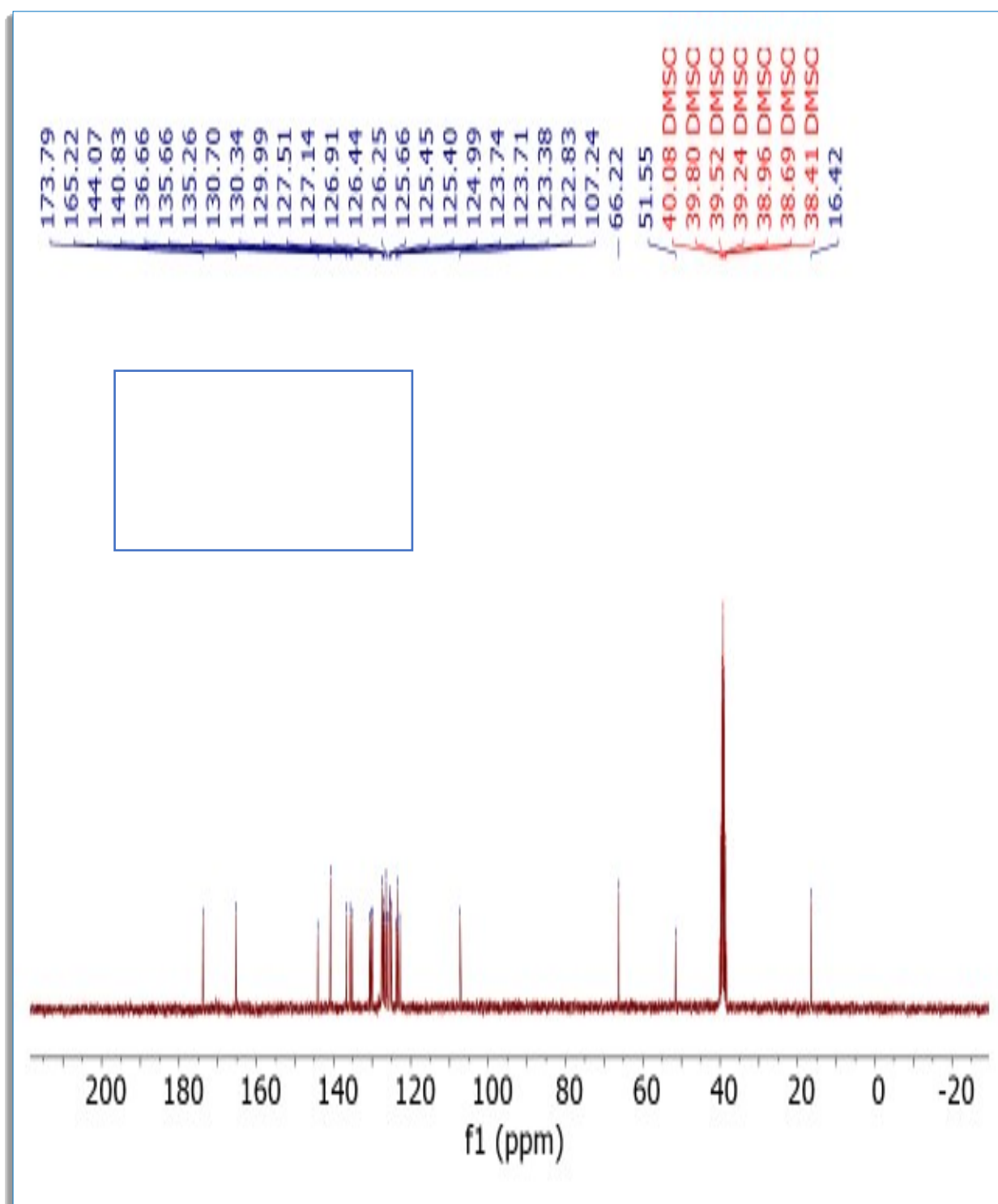


Fig. S13. ^{13}C NMR spectrum of **4c** (75 MHz, $\text{DMSO}-d_6$)

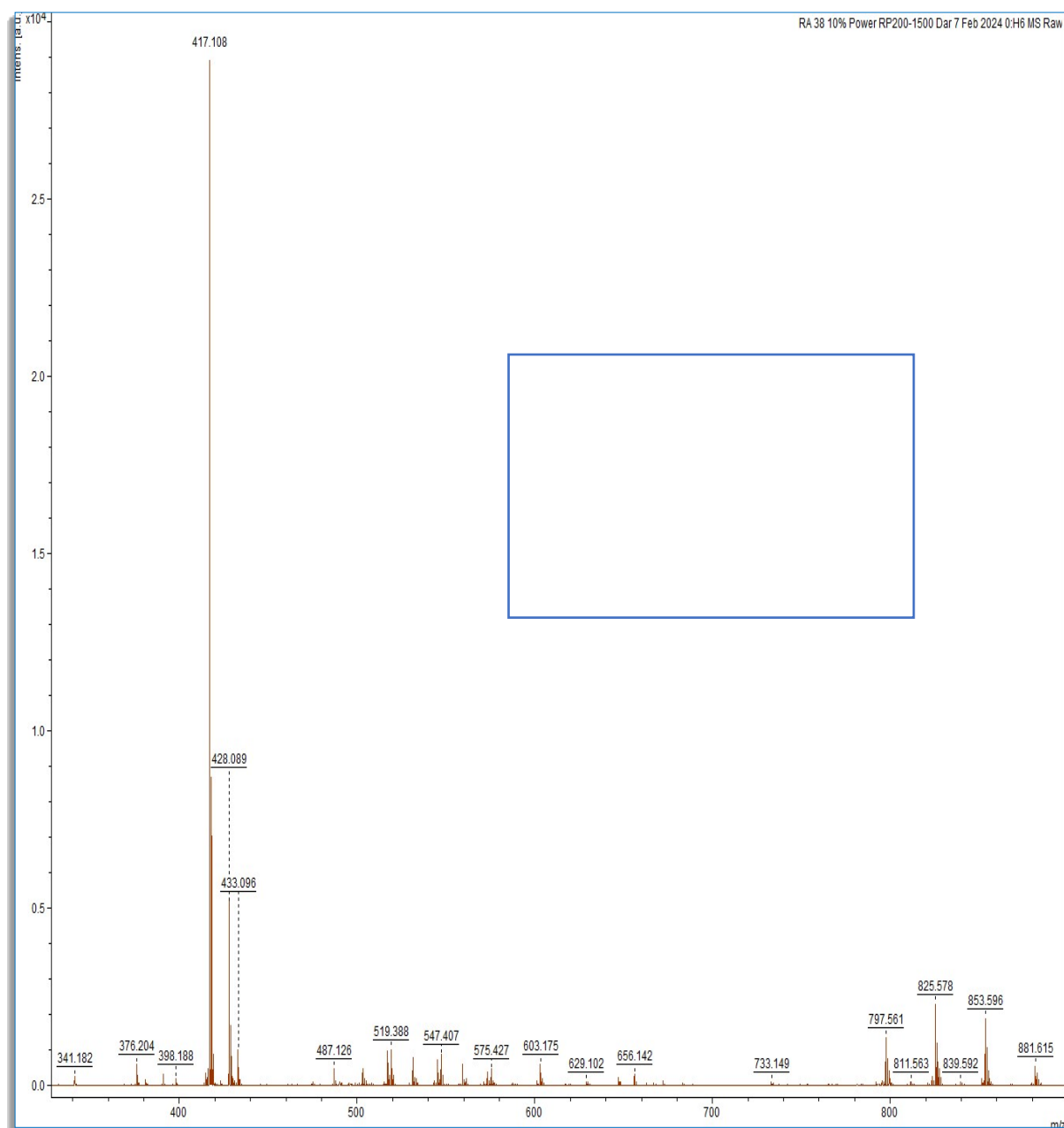


Fig. S14. MALDI-TOF mass spectrum of **4c**.

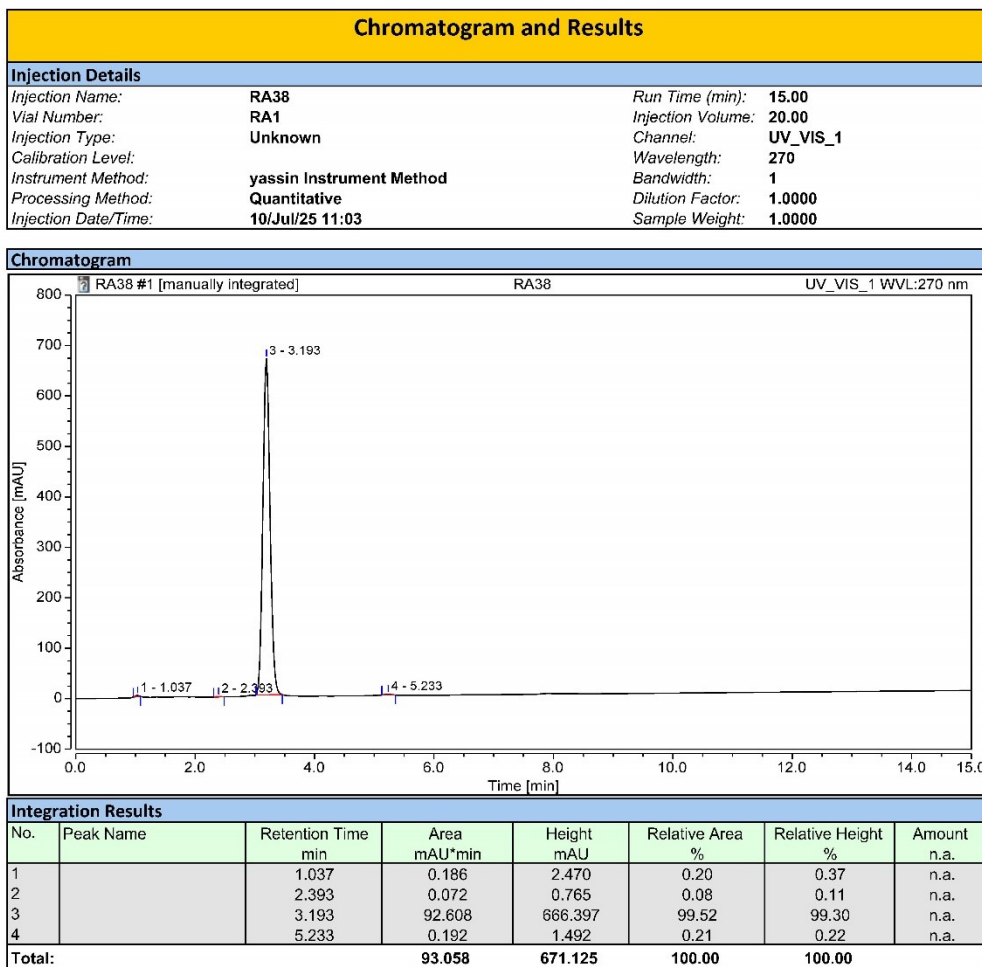
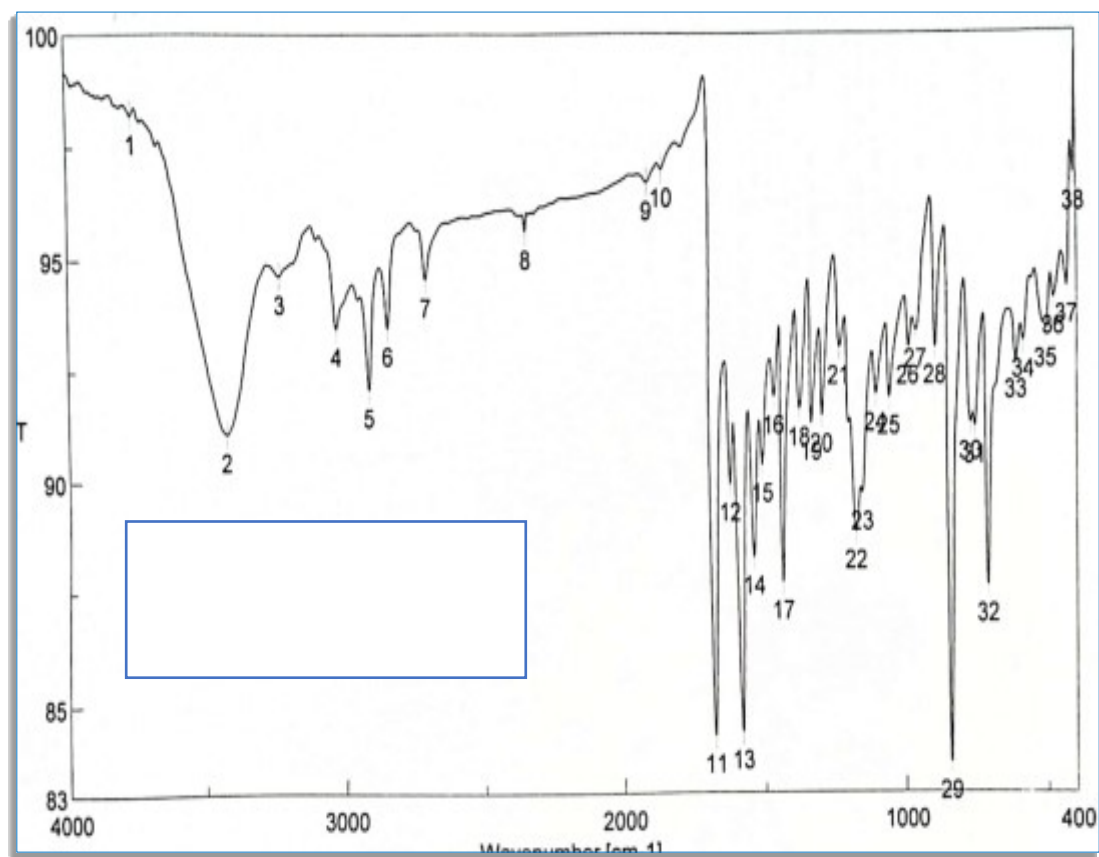


Fig. S15. HPLC chromatogram of compound 4c.



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3764.37	98.2105	2	3426.89	91.1257	3	3238.86	94.6526
4	3036.37	93.4643	5	2918.73	92.0834	6	2850.27	93.4623
7	2711.42	94.5633	8	2348.87	95.6206	9	1920.75	96.7133
10	1868.68	97.0065	11	1678.73	84.2872	12	1620.88	89.9492
13	1581.34	84.38	14	1536.99	88.2989	15	1506.13	90.3745
16	1465.63	91.9039	17	1433.82	87.7365	18	1375	91.6363
19	1332.57	91.3088	20	1294.97	91.4854	21	1236.15	93.0041
22	1173.47	88.8682	23	1150.33	89.7396	24	1103.08	91.9484
25	1055.84	91.8694	26	990.268	92.9858	27	965.198	93.3518
28	896.737	92.9819	29	839.847	83.6733	30	770.423	91.2809
31	754.995	91.2007	32	708.712	87.6595	33	612.288	92.6265
34	587.218	93.0843	35	508.151	93.3118	36	482.117	94.0348
37	435.834	94.2887	38	410.763	96.7997			

Fig. S16. IR spectrum of acrylamide 6

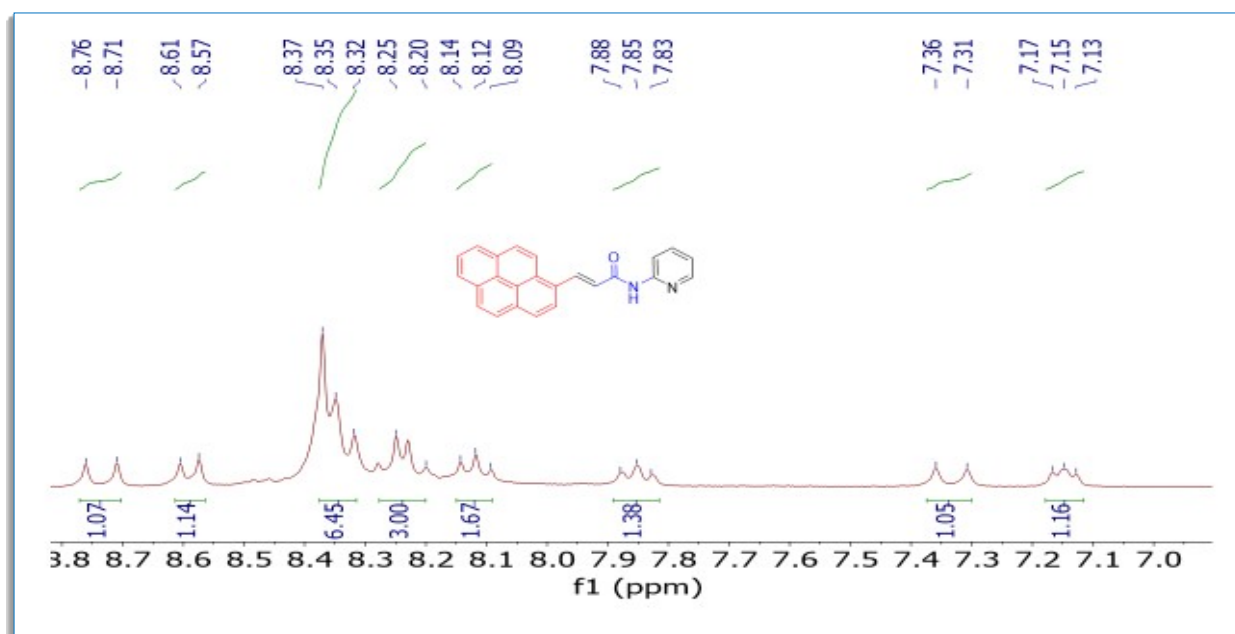
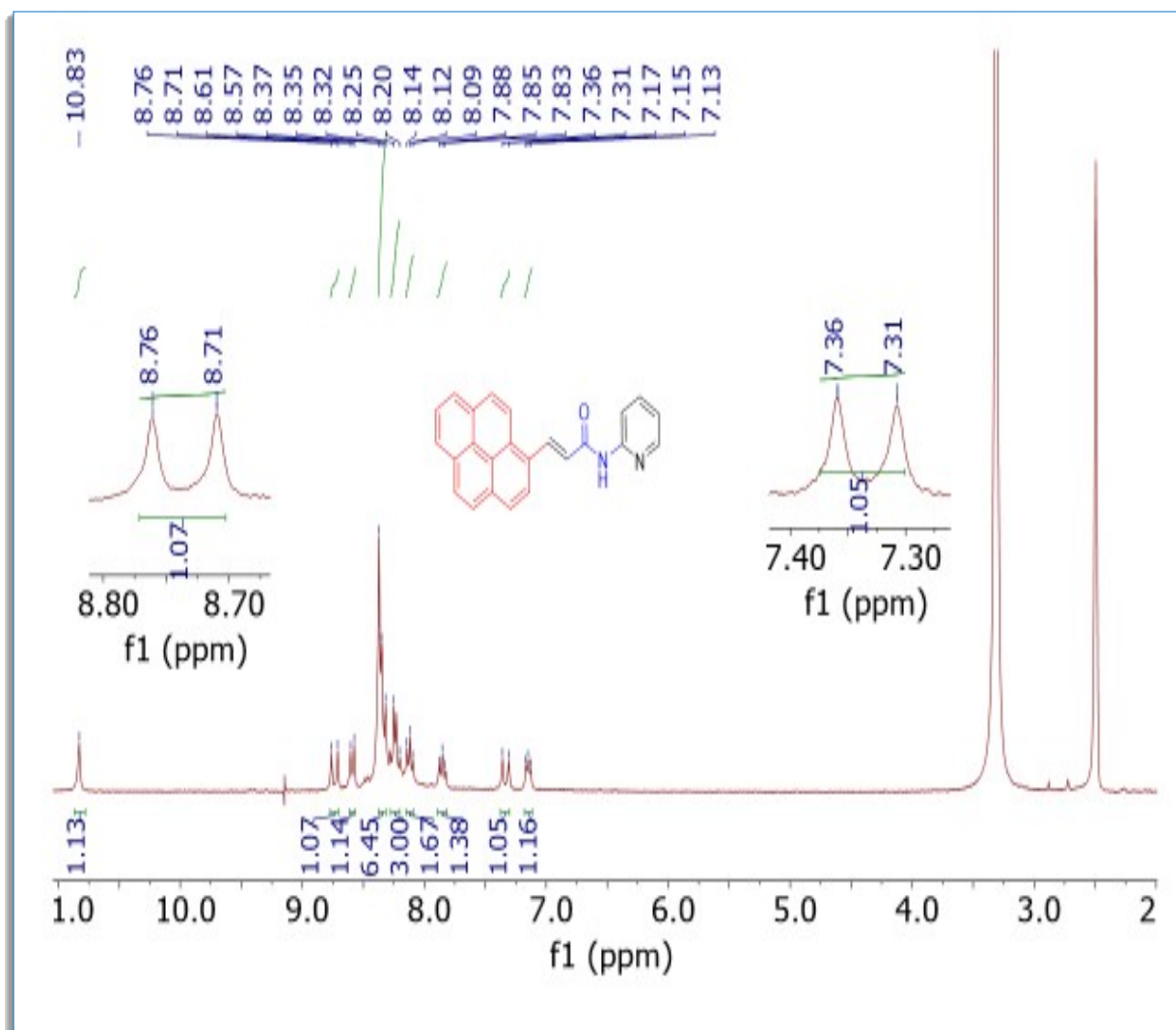


Fig. S17. ¹H NMR spectrum of acrylamide **6** (300 MHz, DMSO-*d*₆)

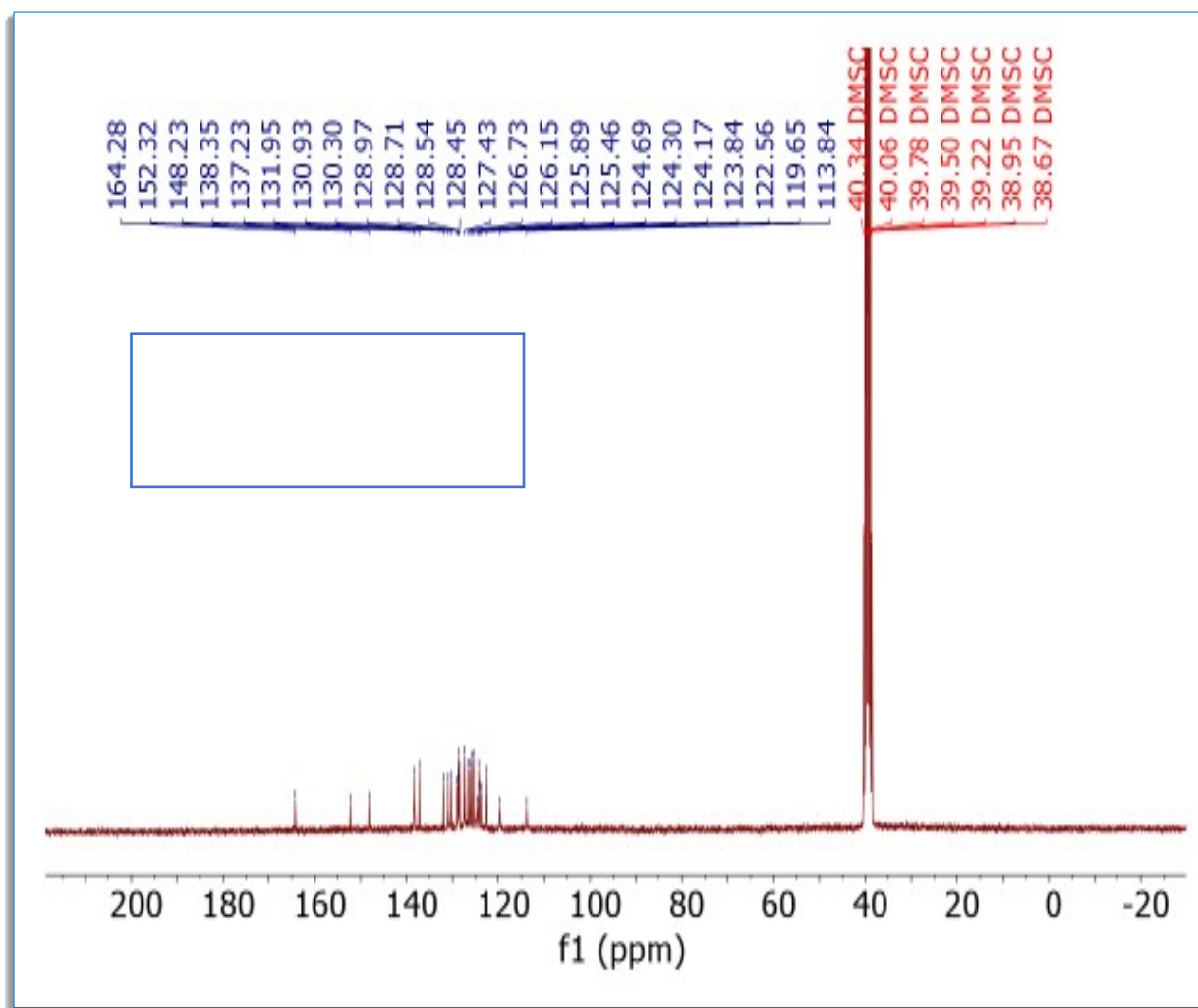


Fig. S18. ^{13}C NMR spectrum of acrylamide **6** (75 MHz, $\text{DMSO}-d_6$)

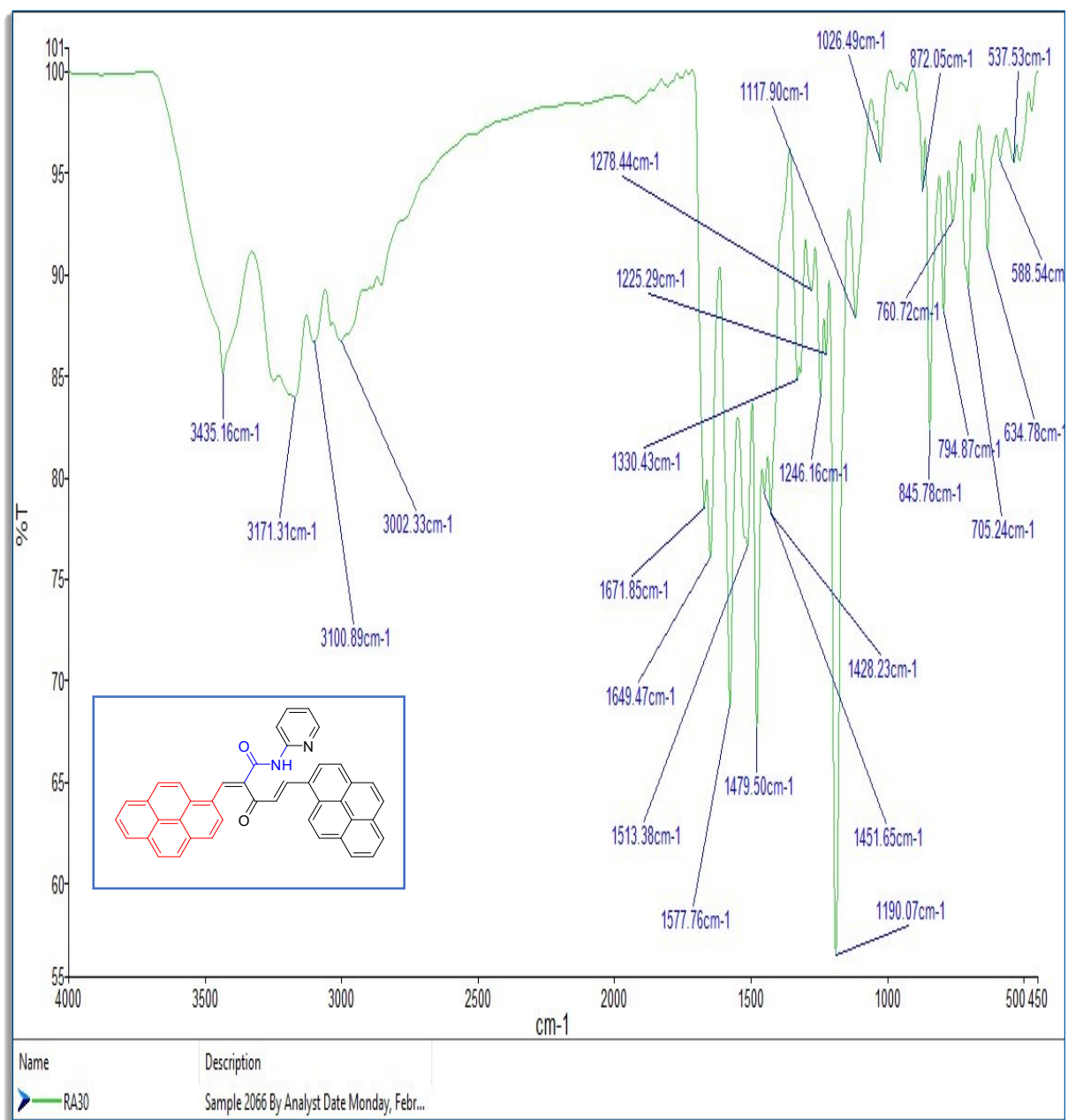


Fig. S19. IR spectrum of acrylamide **7**

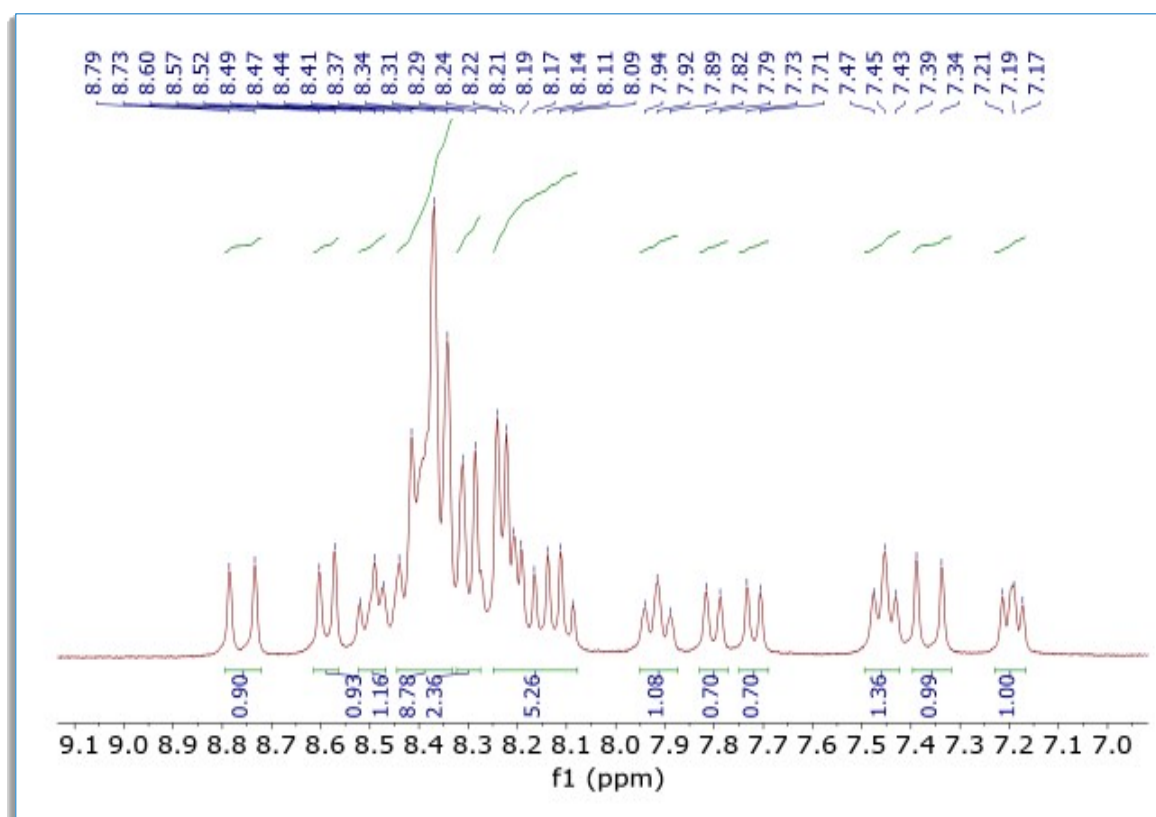
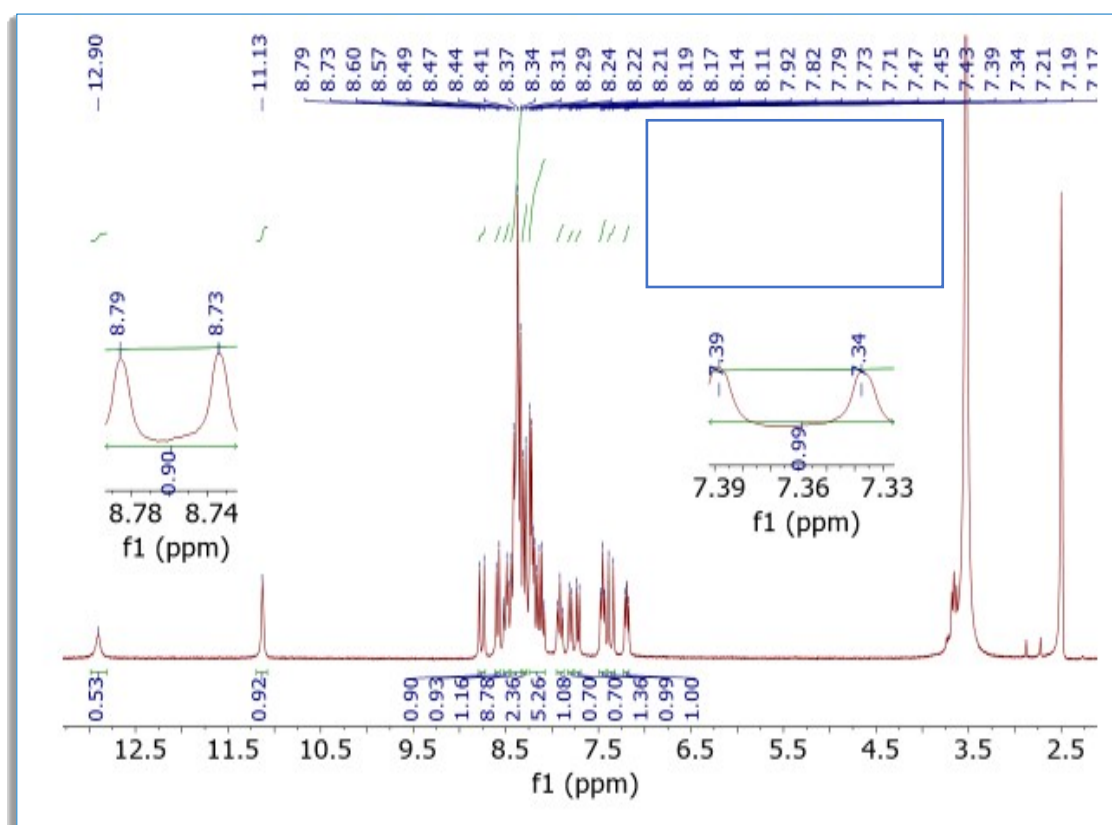


Fig. S20. ^1H NMR spectrum of acrylamide **7** (300 MHz, $\text{DMSO}-d_6$)

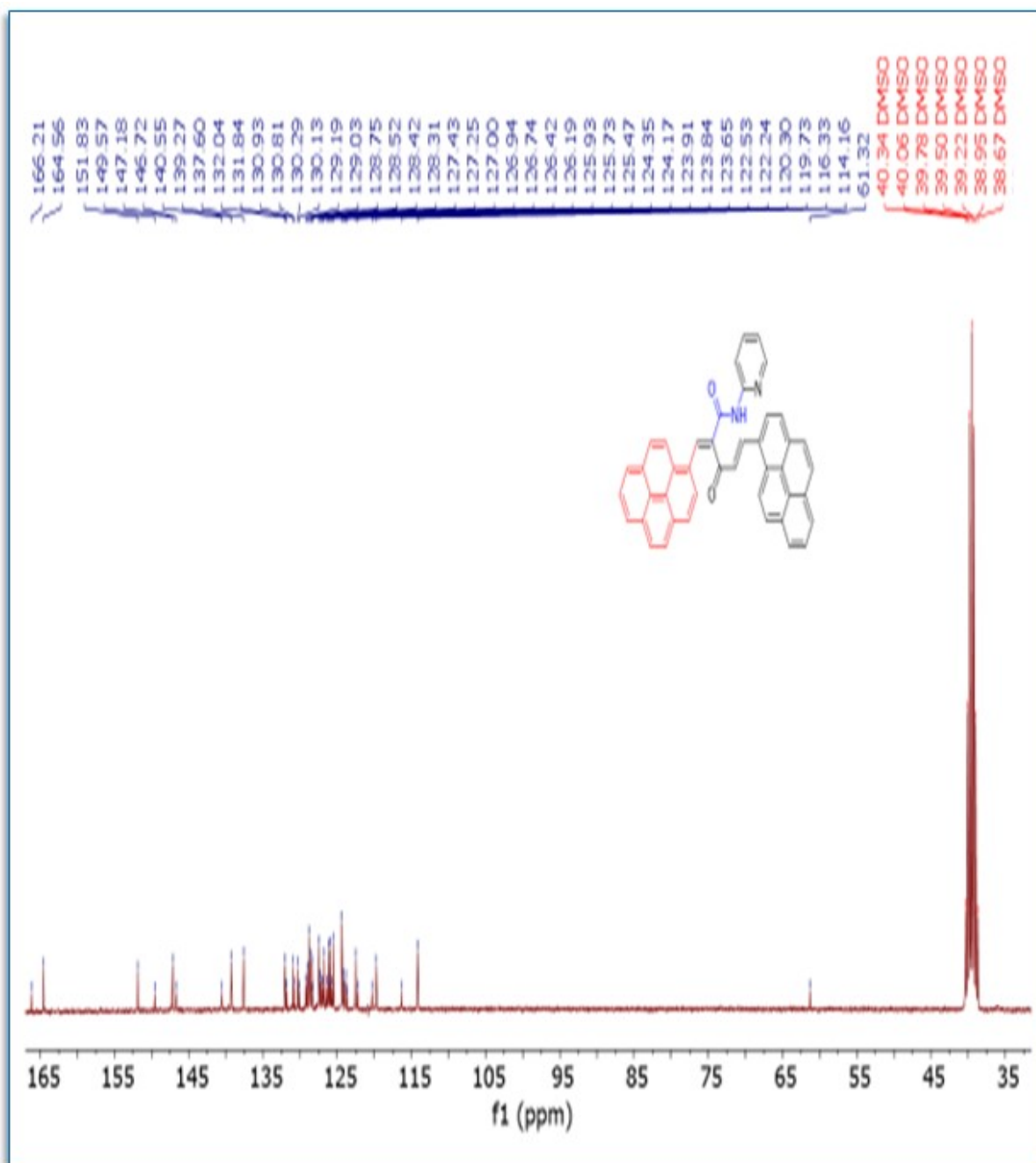


Fig. S21. ^{13}C NMR spectrum of acrylamide **7** (75 MHz, $\text{DMSO-}d_6$)

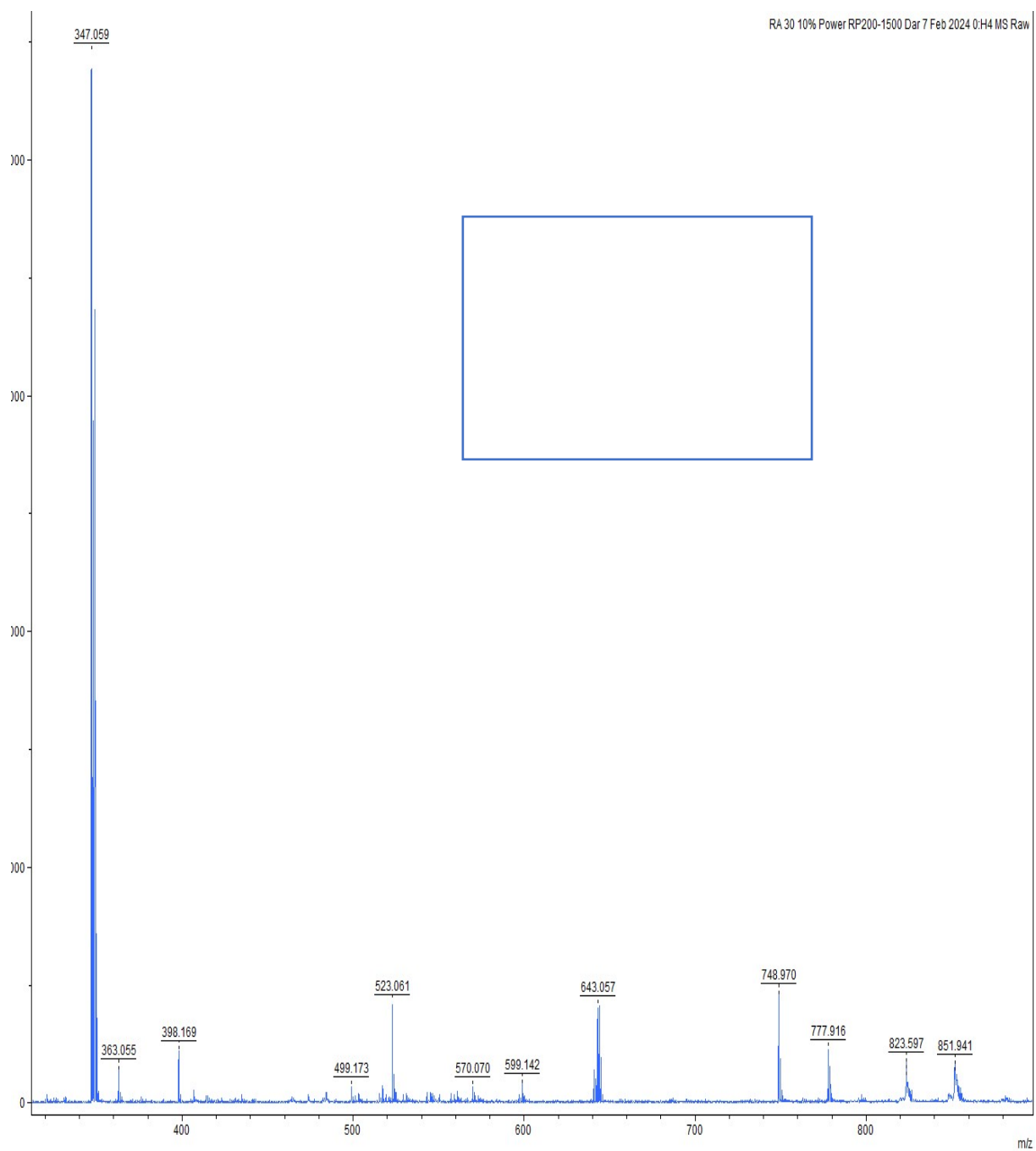
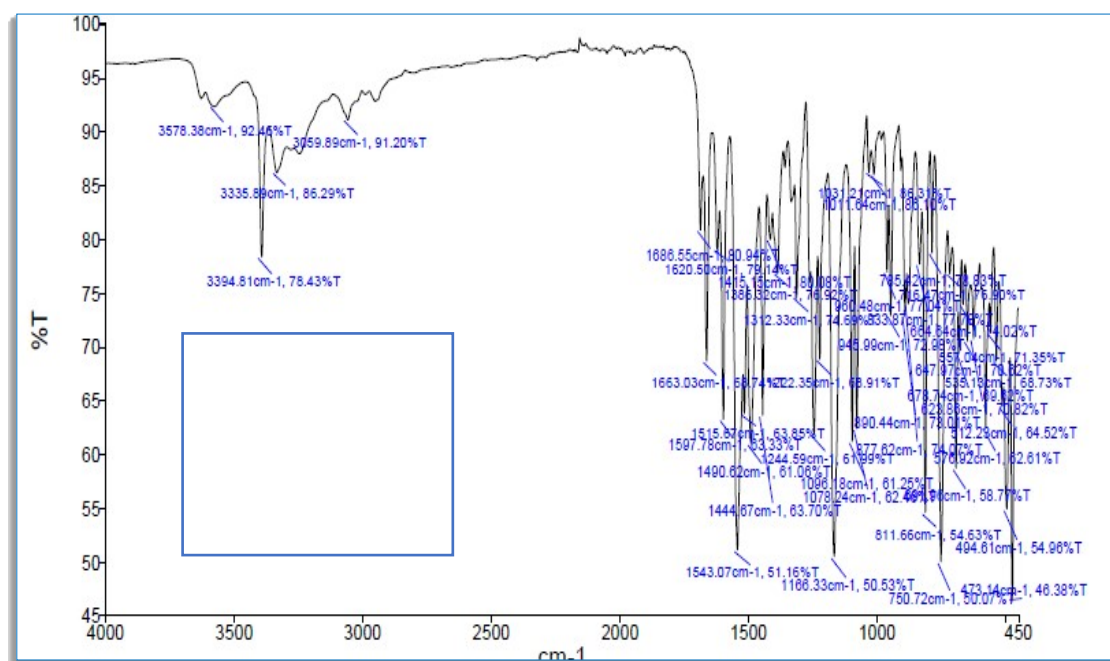


Fig. S22. MALDI-TOF mass spectrum of acrylamide **7**



List of Peak Area/Height		
Peak Number	X (cm-1)	Y (%T)
1	3578.38	92.46
2	3394.81	78.43
3	3335.89	86.29
4	3059.89	91.20
5	1686.55	80.94
6	1663.03	68.74
7	1620.50	79.14
8	1597.78	63.33
9	1543.07	51.16
10	1515.67	63.85
11	1490.62	61.06
12	1444.67	63.70
13	1415.15	80.08
14	1386.32	76.92
15	1312.33	74.69
16	1244.59	61.99
17	1222.35	68.91
18	1166.33	50.53
19	1096.18	61.25
20	1078.24	62.46
21	1031.21	86.31
22	1011.64	86.10
23	960.48	77.04
24	945.99	72.98
25	890.44	73.01
26	877.62	74.07
27	833.87	77.78
28	811.66	54.63
29	785.42	78.83
30	750.72	50.07

Fig. S23. IR spectrum of **11a**

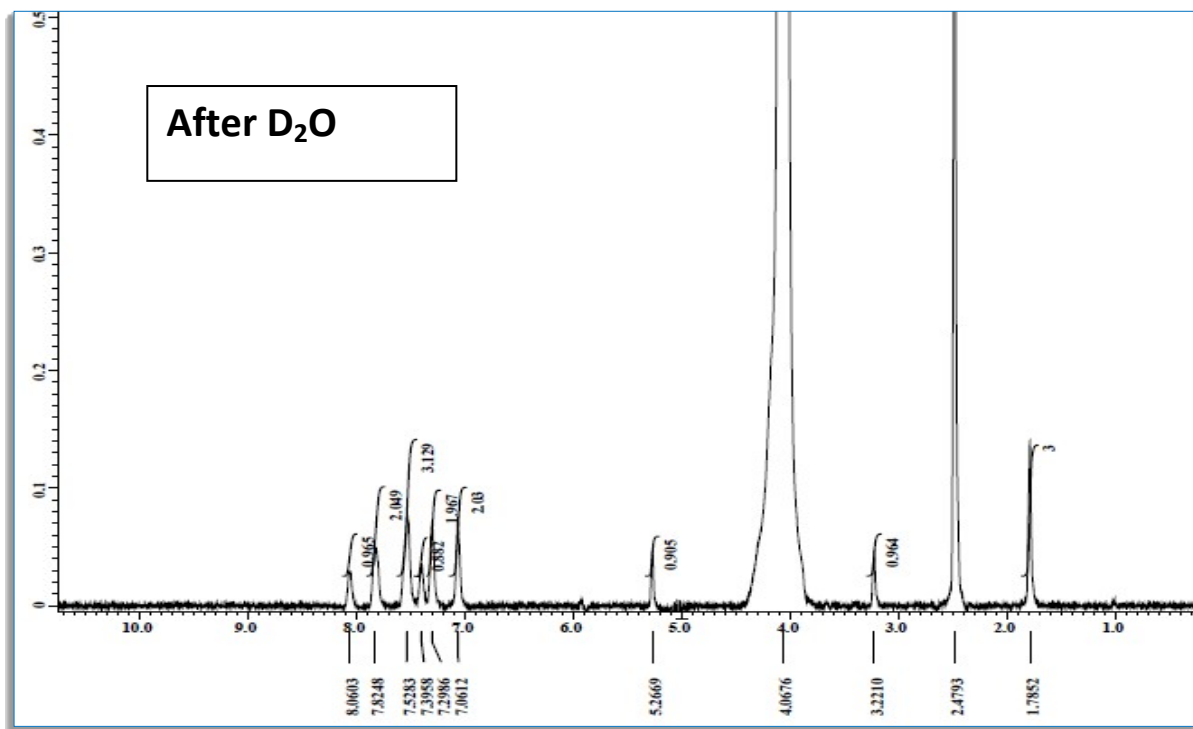
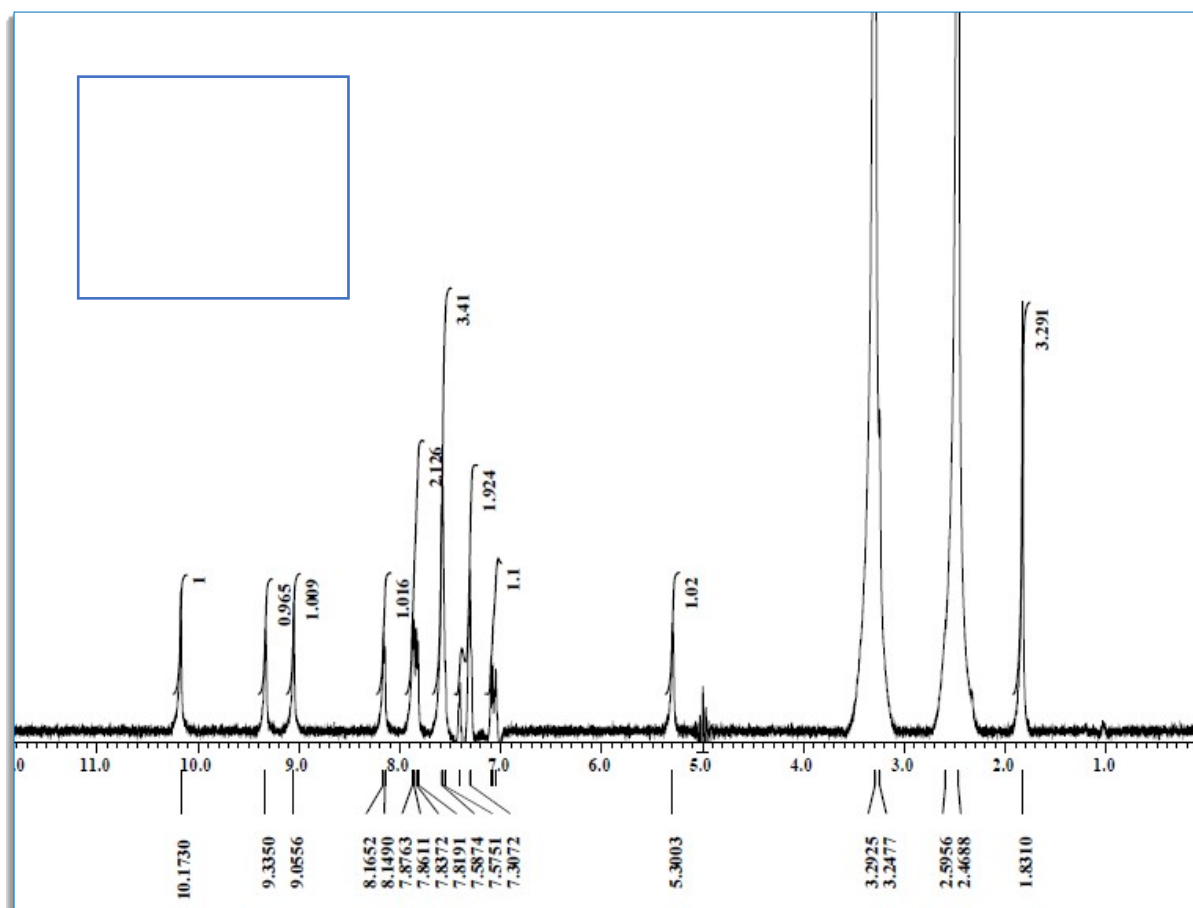


Fig. S24. ^1H NMR spectrum of **11a** (500 MHz, $\text{DMSO}-d_6$)

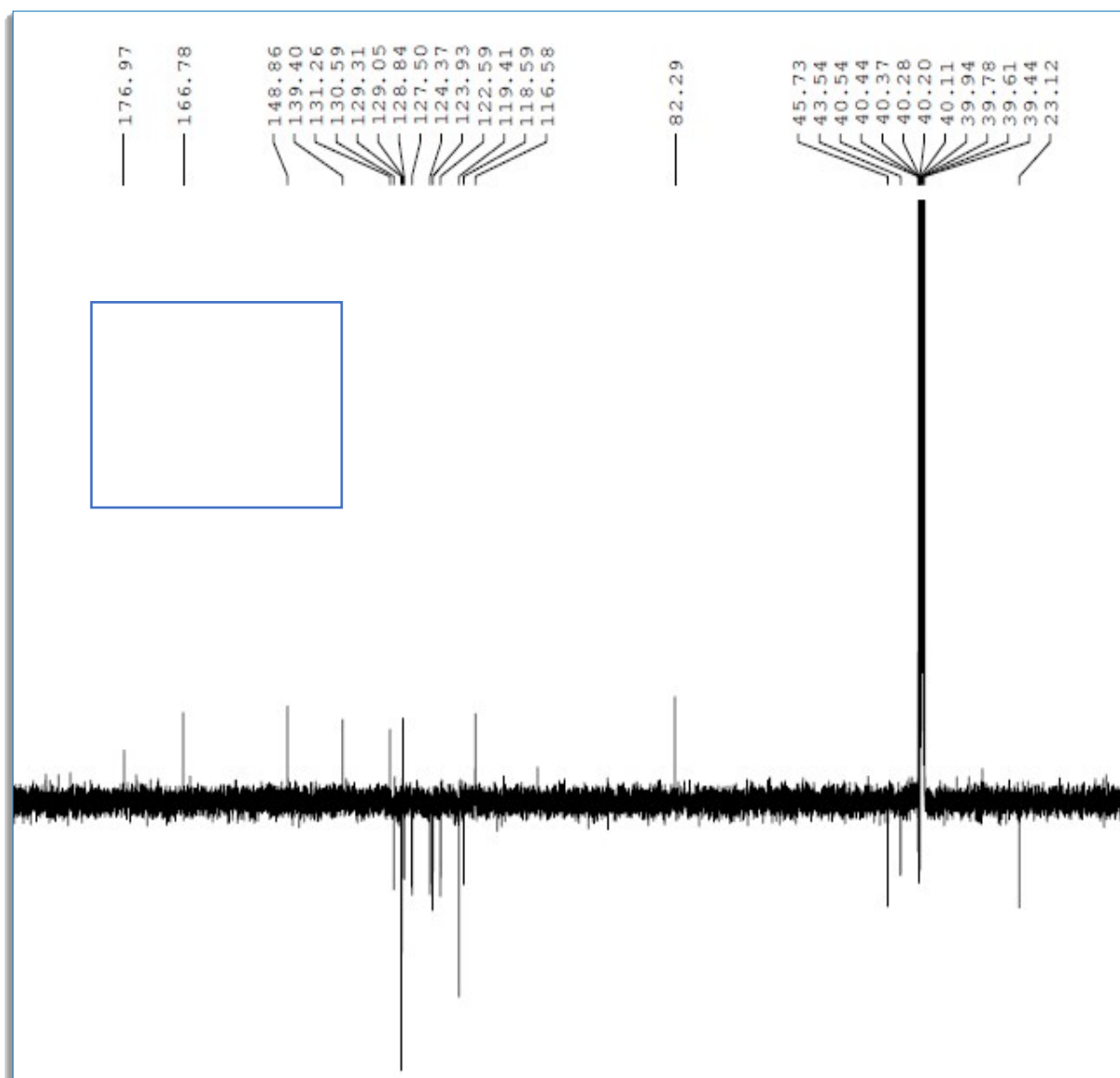


Fig. S25. ¹³C NMR spectrum (APT) of **11a** (125 MHz, DMSO-*d*₆)

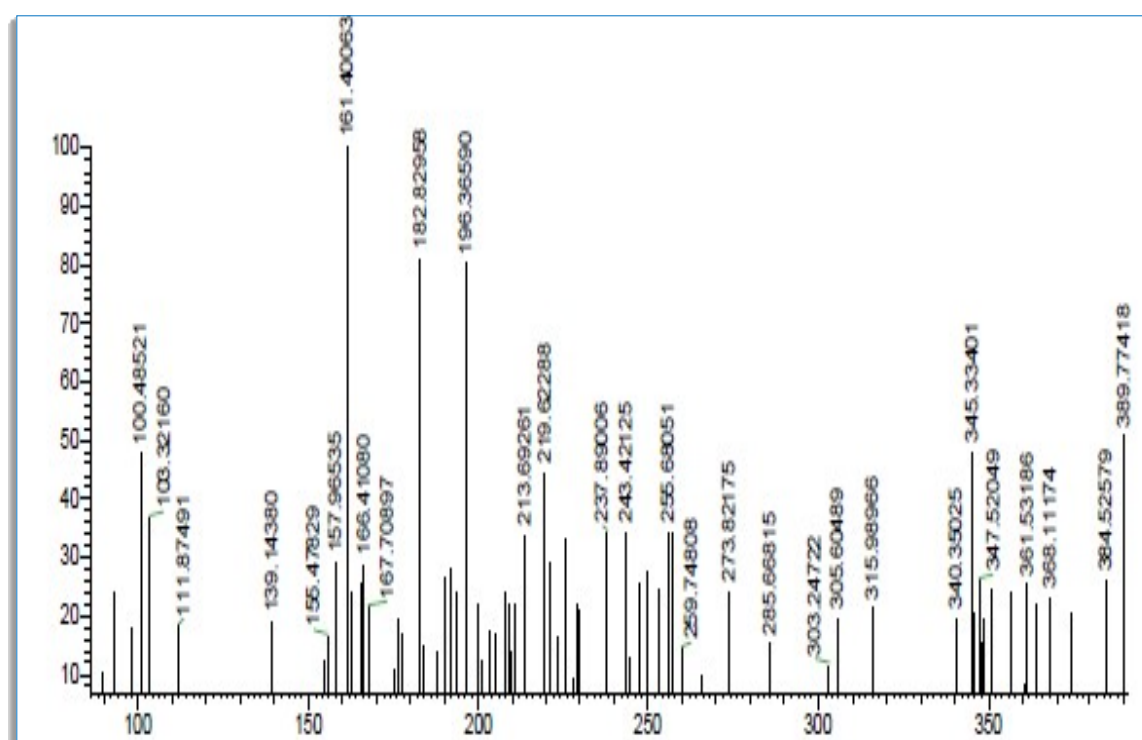
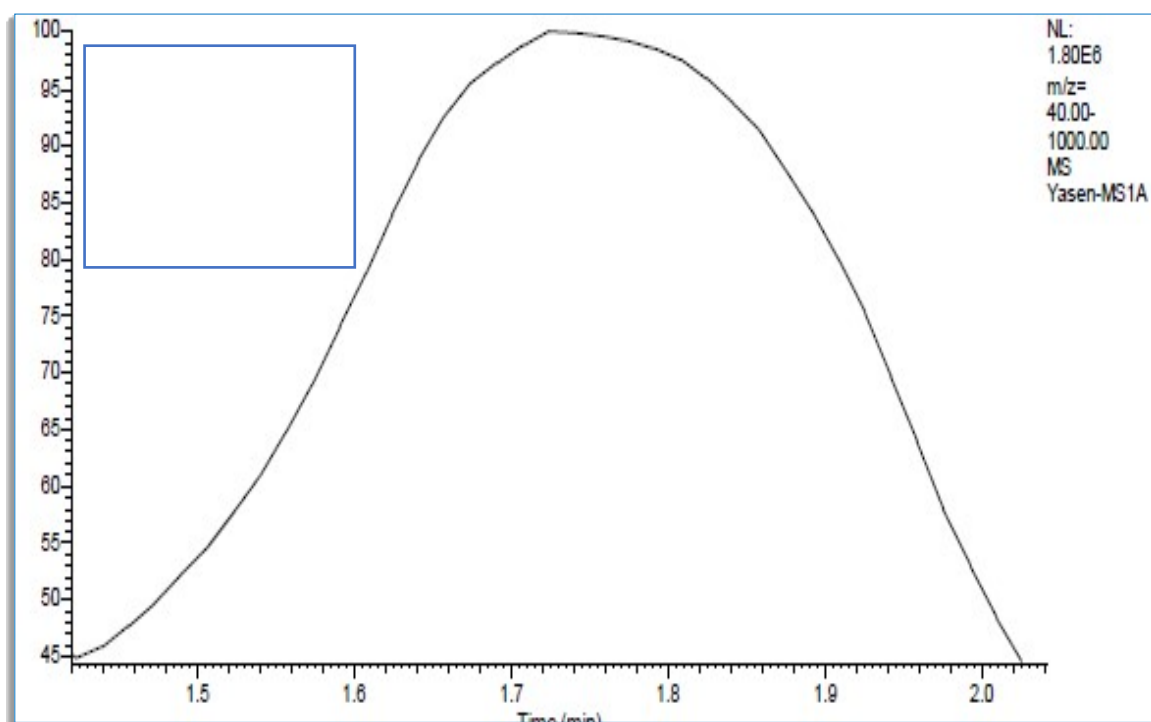
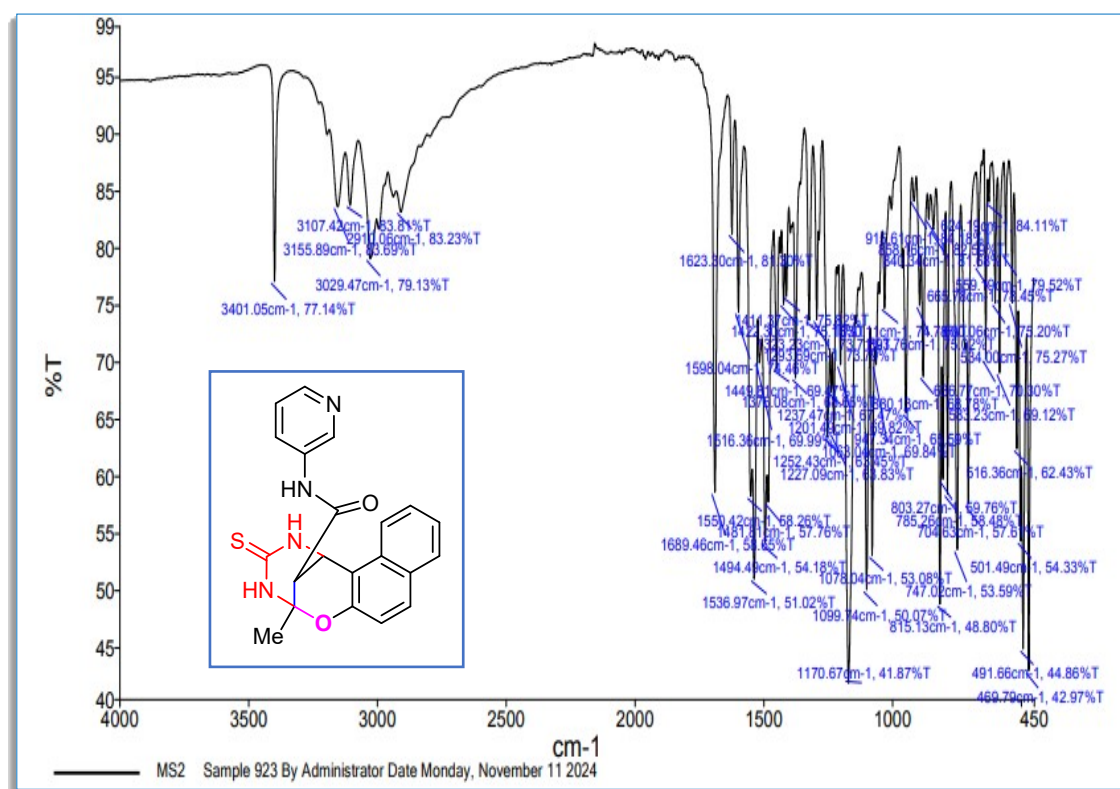


Fig. S26. High resolution mass of **11a**



List of Peak Area/Height		
Peak Number	X (cm-1)	Y (%T)
1	3401.05	77.14
2	3155.89	83.69
3	3107.42	83.81
4	3029.47	79.13
5	2910.06	83.23
6	1689.46	58.65
7	1623.30	81.30
8	1598.04	74.46
9	1550.42	58.26
10	1536.97	51.02
11	1516.36	69.99
12	1494.49	54.18
13	1481.81	57.76
14	1449.81	69.47
15	1422.30	75.15
16	1411.37	75.82
17	1376.08	68.66
18	1323.23	73.71
19	1293.69	73.79
20	1252.43	63.45
21	1237.47	67.47
22	1227.09	63.83
23	1201.49	69.82
24	1170.67	41.87
25	1099.74	50.07
26	1078.04	53.08
27	1063.04	69.84
28	1030.11	74.78
29	947.34	65.59
30	915.61	84.18
31	893.76	75.02
32	880.13	68.78

Fig. S27. IR spectrum of 11c

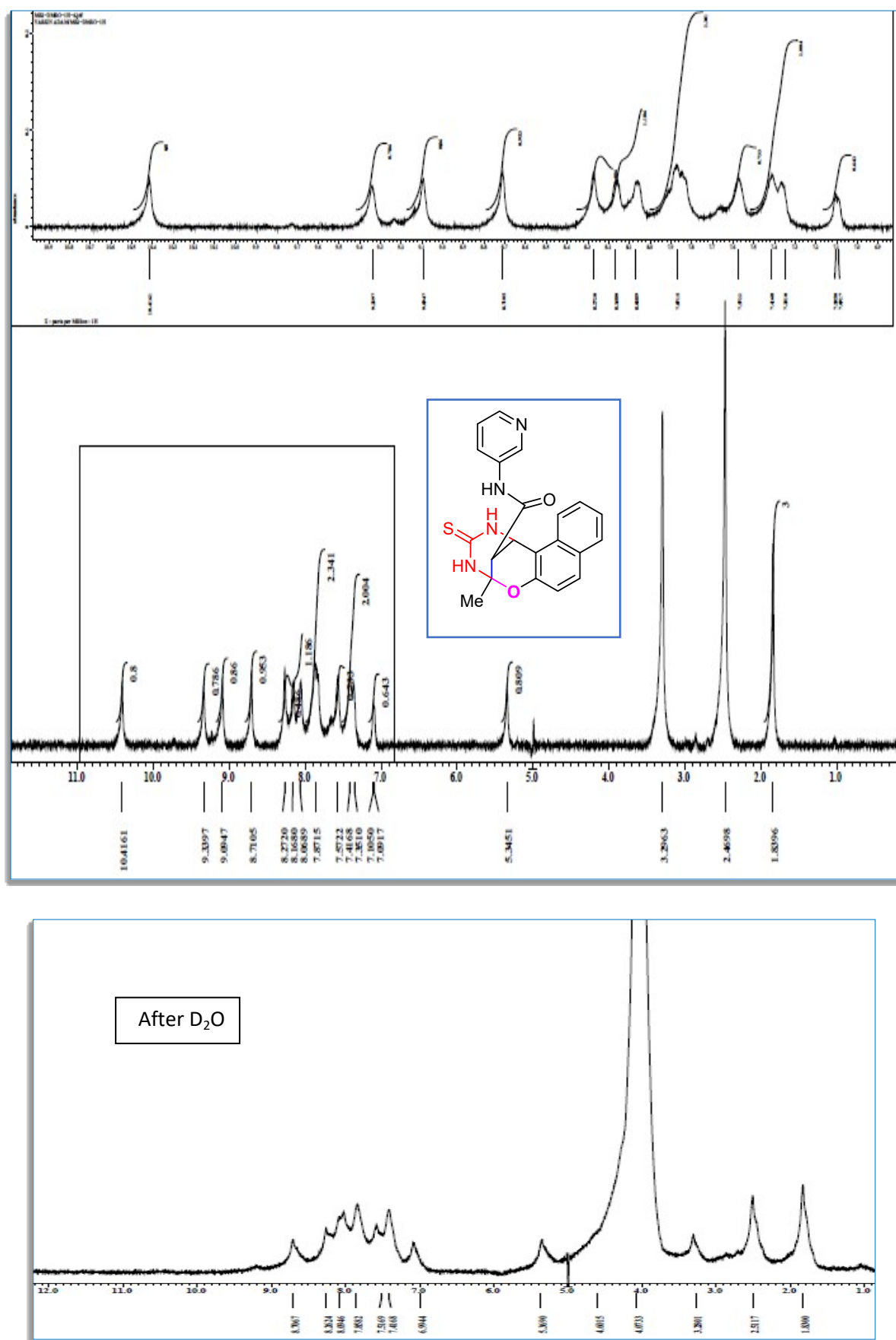


Fig. S28. ^1H NMR spectrum of **11c** (500 MHz, $\text{DMSO}-d_6$)

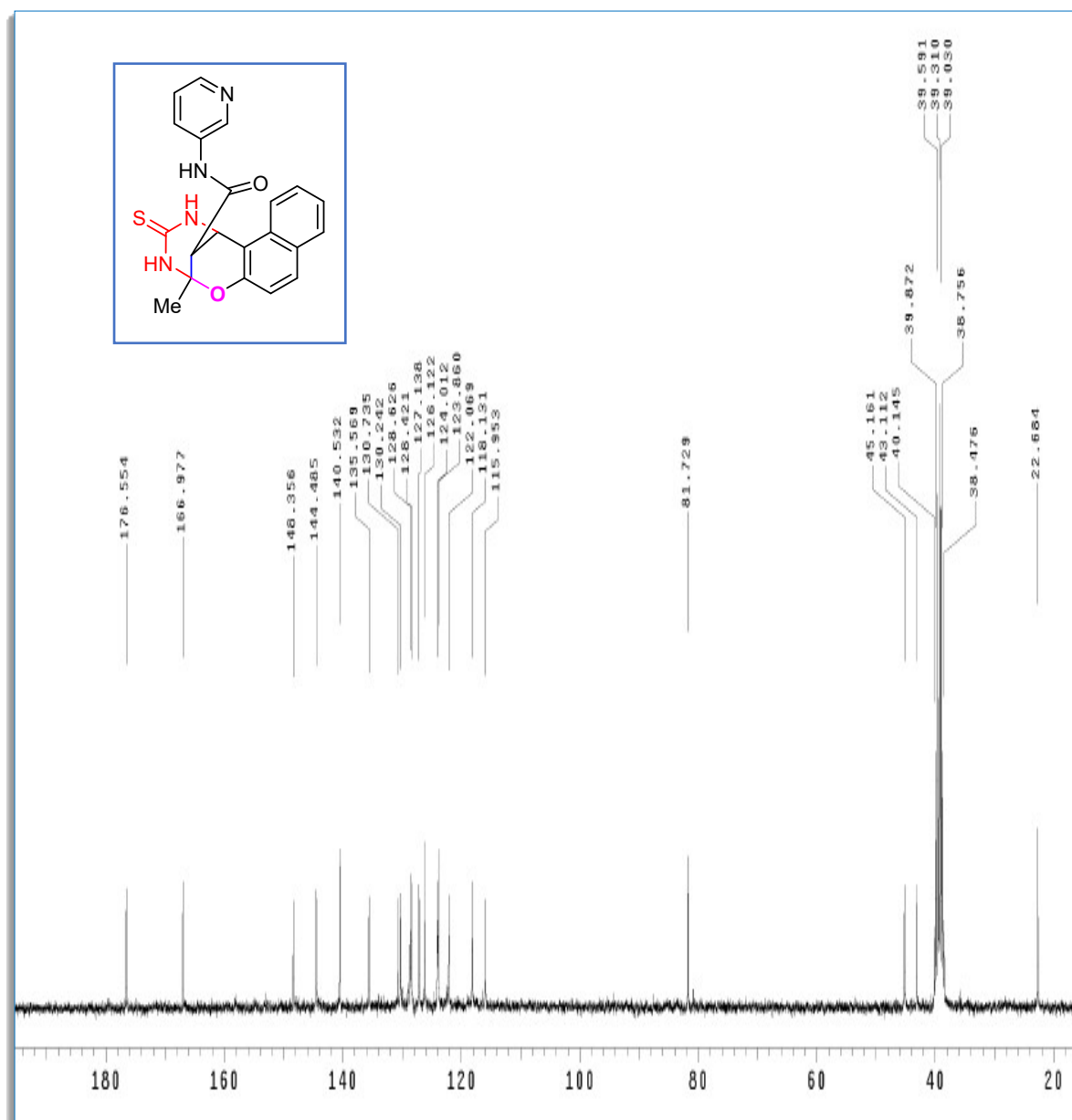


Fig. 29. ^{13}C NMR spectrum of **11c** (75 MHz, $\text{DMSO}-d_6$)

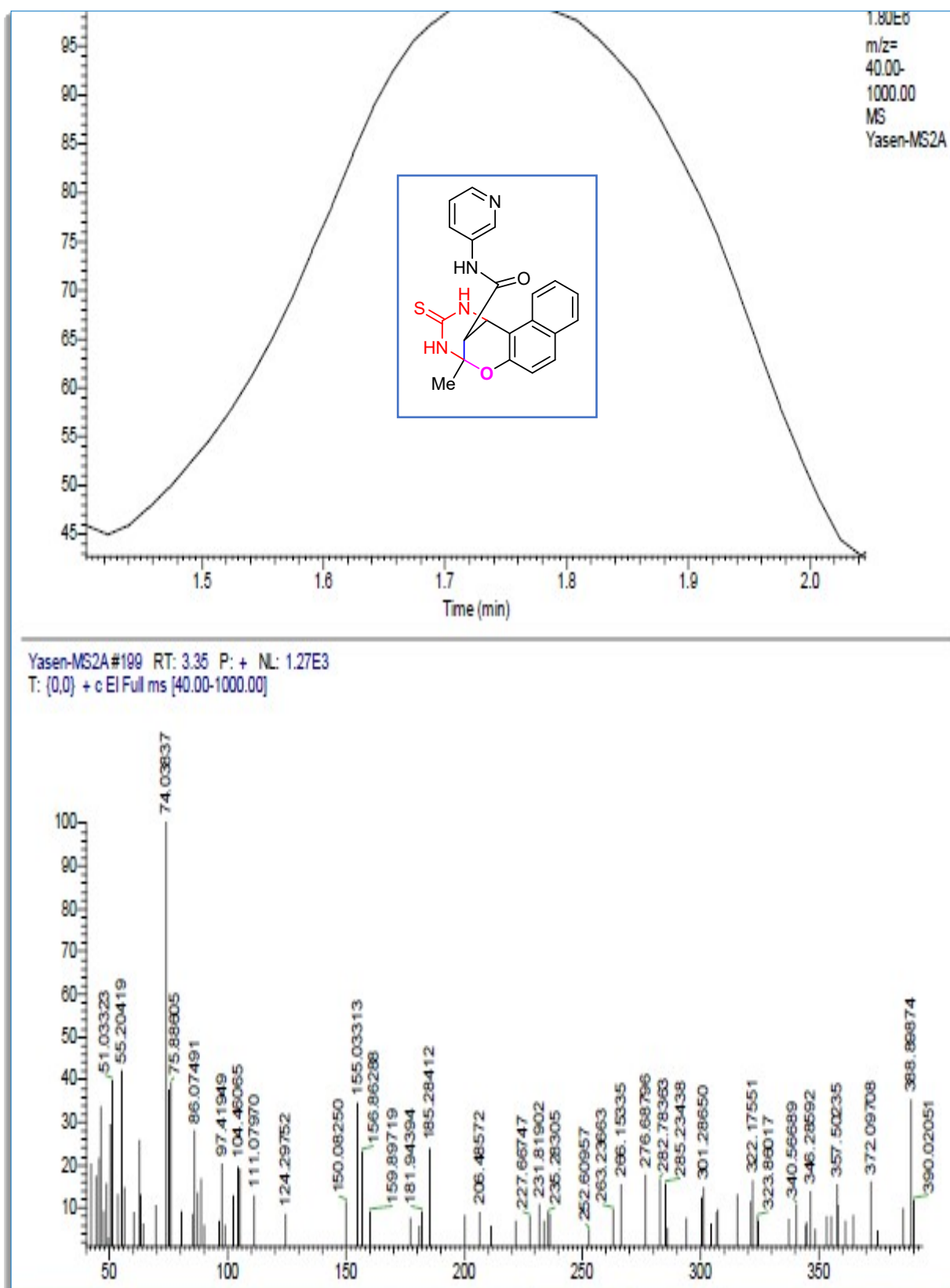
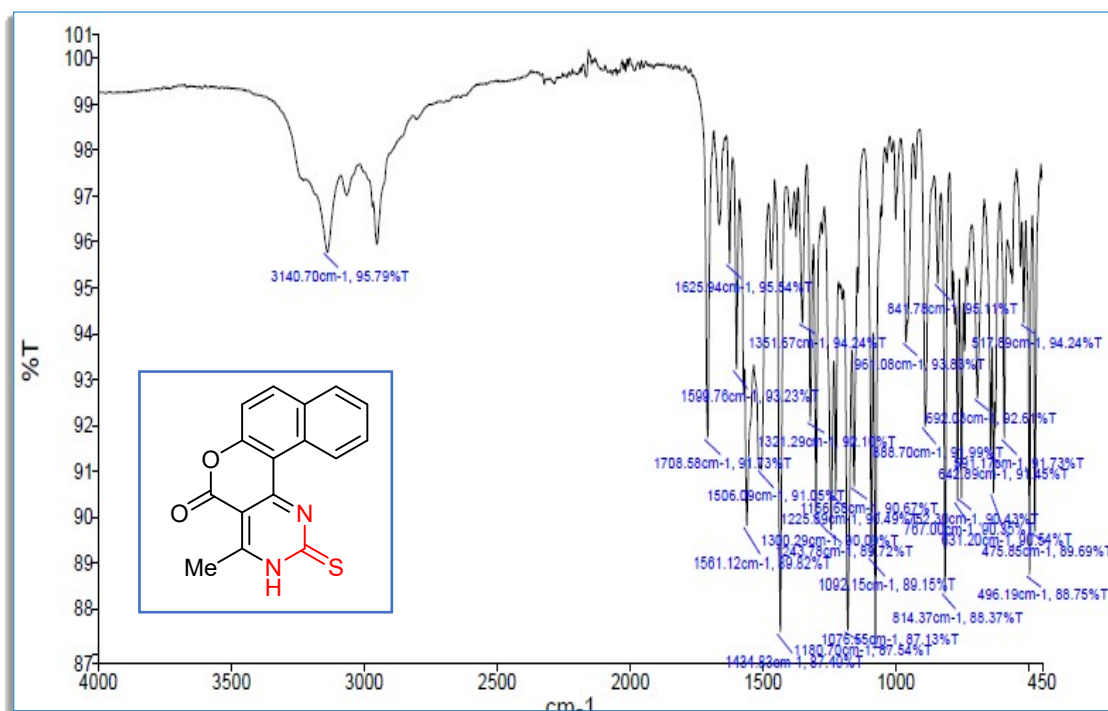
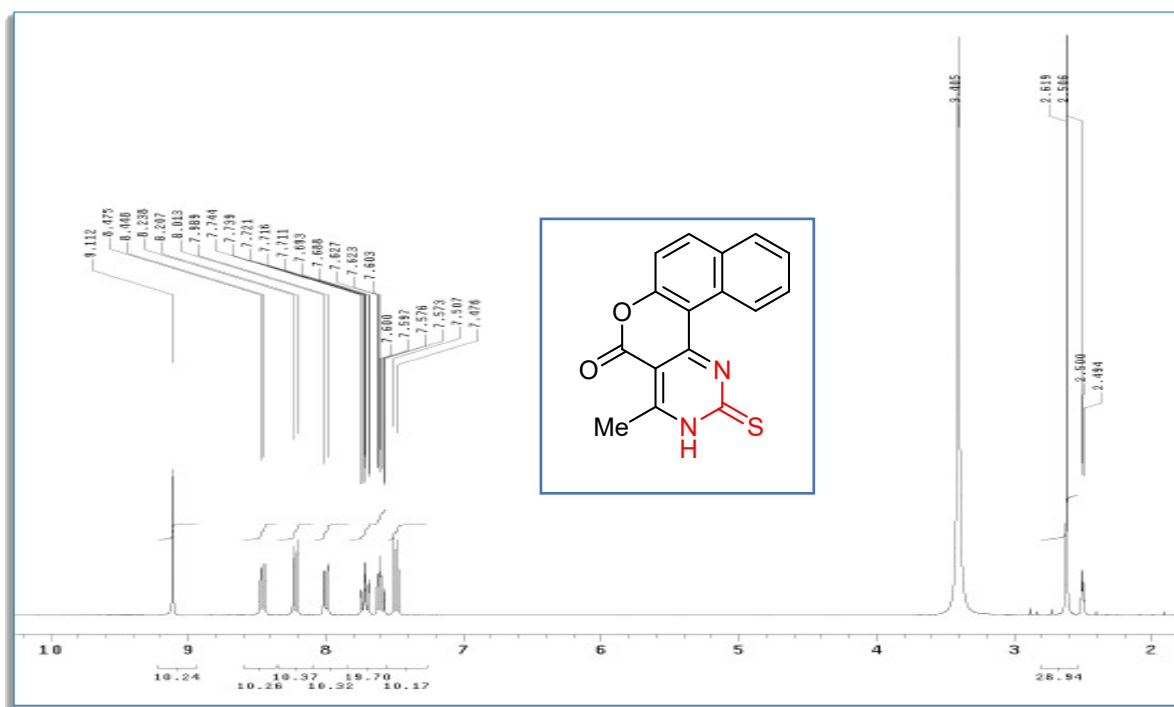


Fig. S30. High resolution mass of **11c**

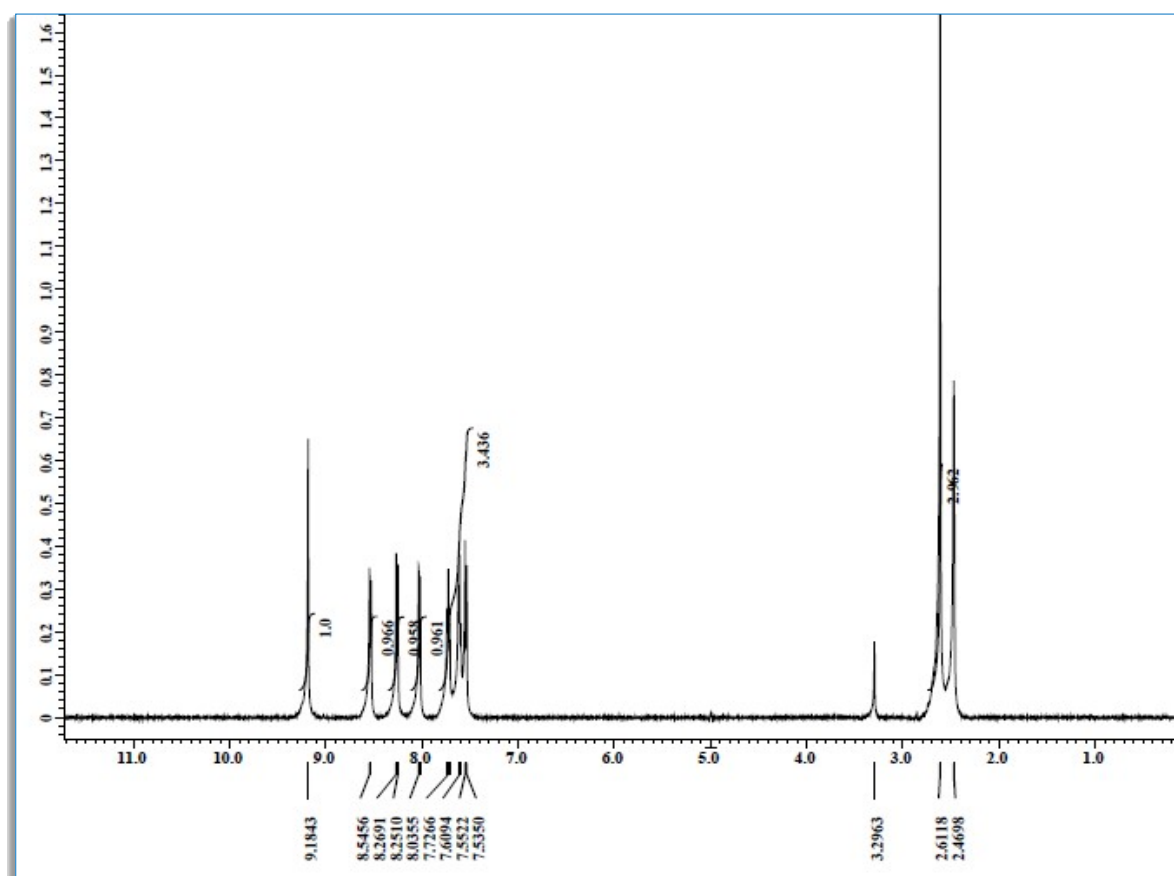


List of Peak Area/Height		
Peak Number	X (cm-1)	Y (%T)
1	3140.70	95.79
2	1708.58	91.73
3	1625.94	95.54
4	1599.76	93.23
5	1561.12	89.82
6	1506.09	91.05
7	1434.83	87.49
8	1351.67	94.24
9	1321.29	92.10
10	1300.29	90.09
11	1243.78	89.72
12	1225.89	90.49
13	1180.70	87.54
14	1156.68	90.67
15	1092.15	89.15
16	1076.55	87.13
17	961.08	93.83
18	888.70	91.99
19	841.78	95.11
20	814.37	88.37
21	767.00	90.35
22	752.30	90.43
23	692.03	92.61
24	642.89	91.45
25	631.20	90.54
26	591.17	91.73
27	517.89	94.24
28	496.19	88.75
29	475.85	89.69

Fig. S31. IR spectrum of 12



(A) (300 MHz, DMSO-*d*₆)



(B) (500 MHz, DMSO-*d*₆)

Fig. S32. ¹H NMR of compound 12

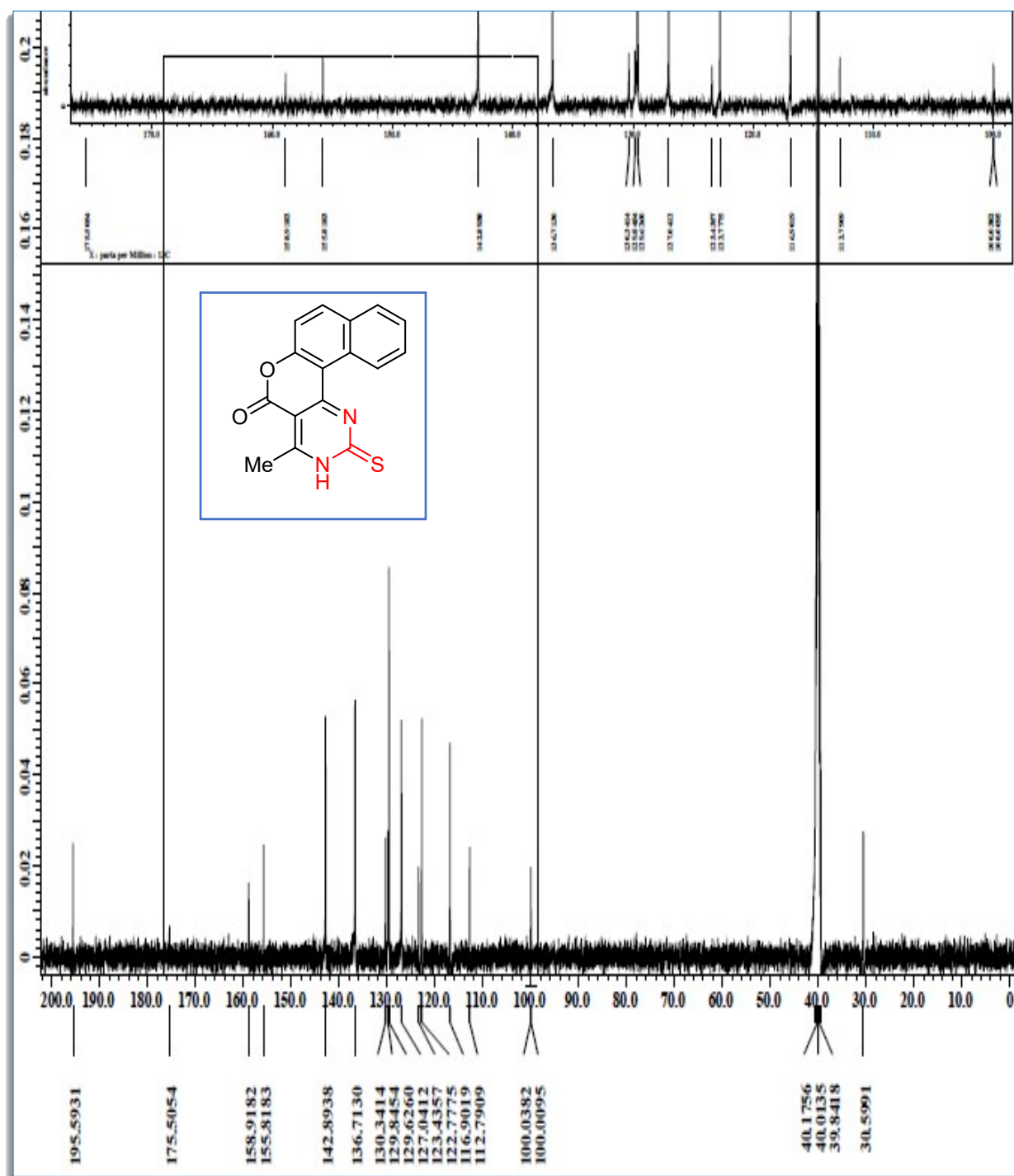


Fig S33. ¹³C NMR spectrum of **12** (125 MHz, DMSO-*d*₆)

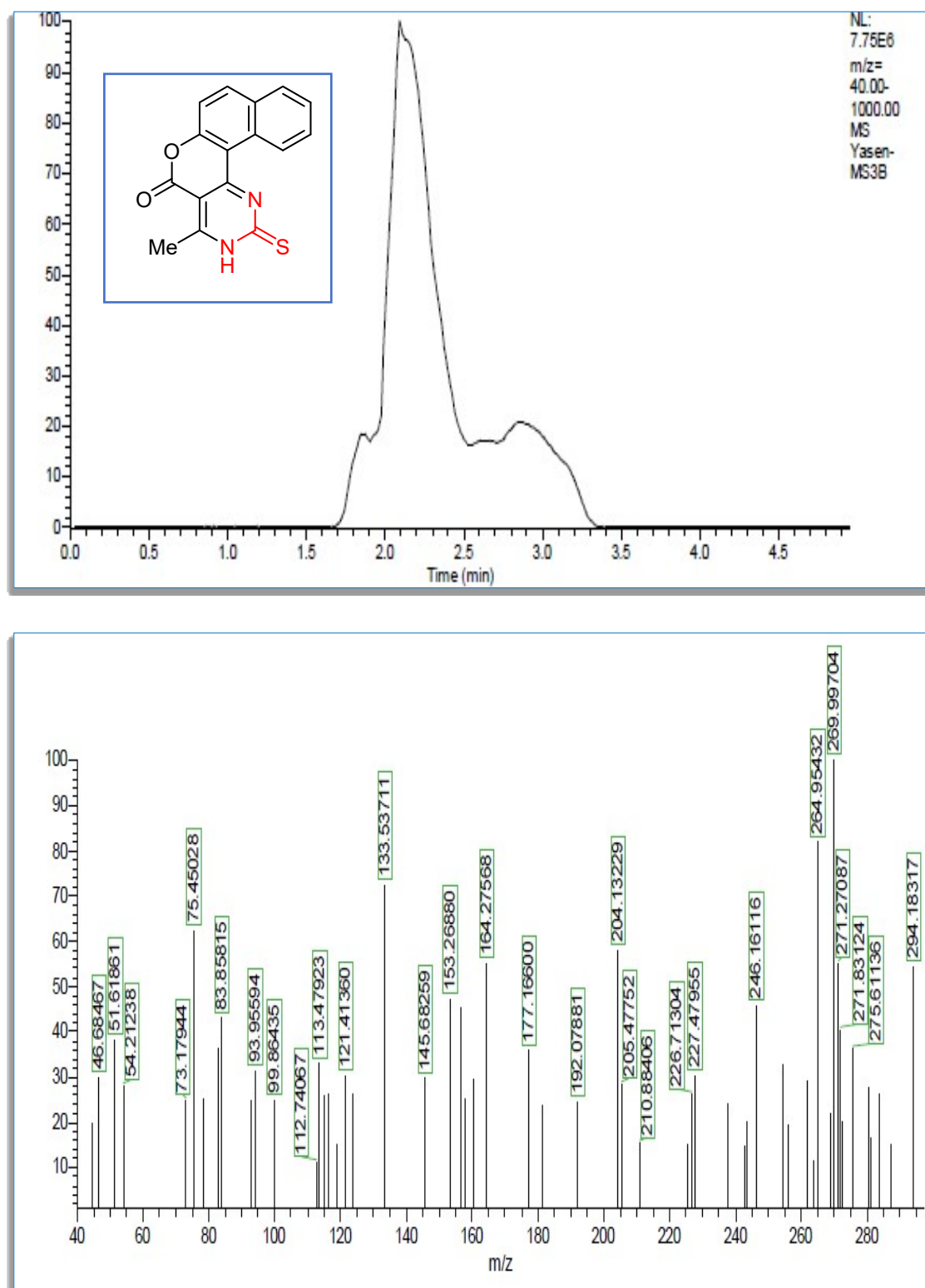


Fig. S34. High resolution Mass spectrum of 12

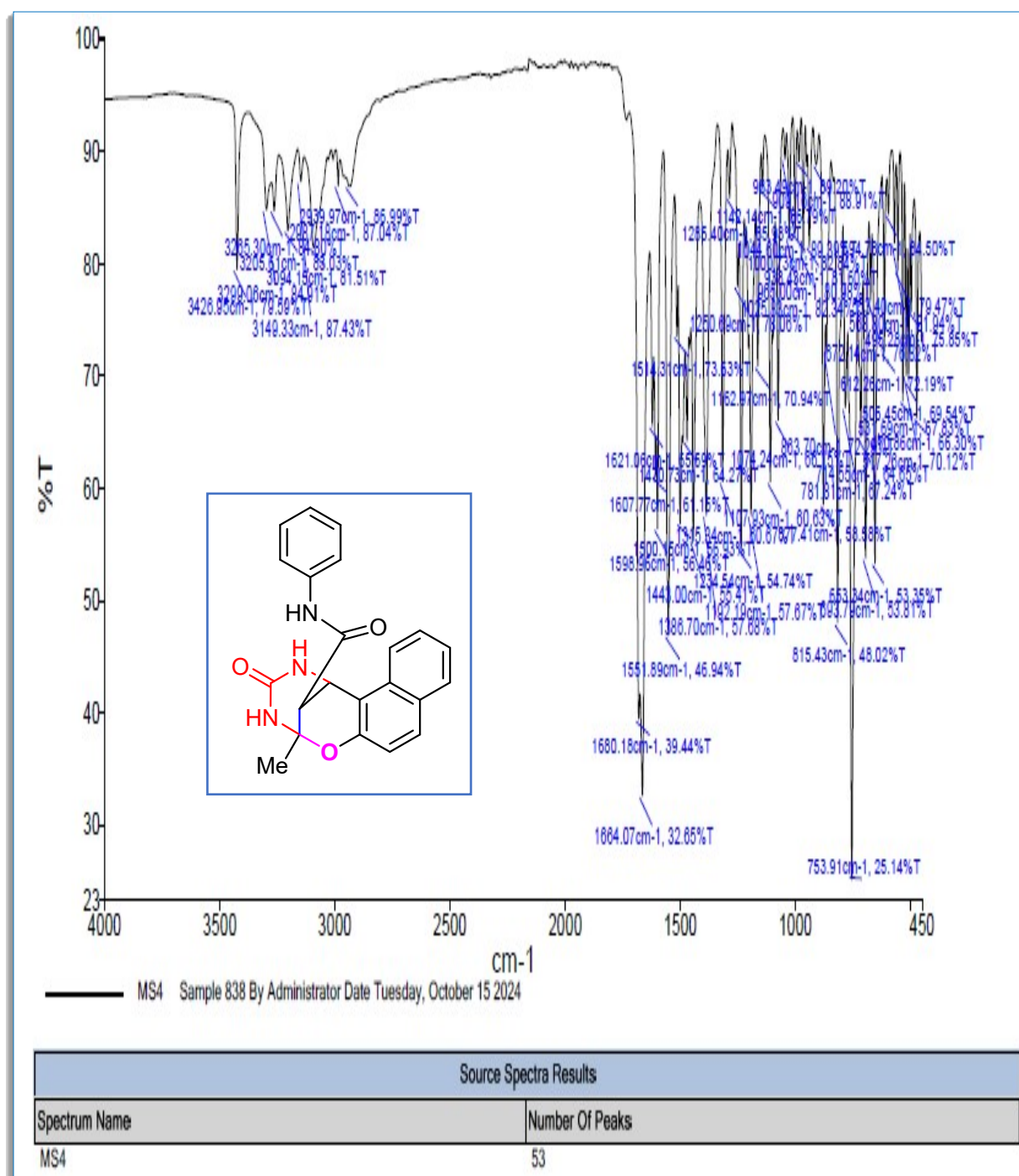


Fig. S35. IR spectrum of **15a**

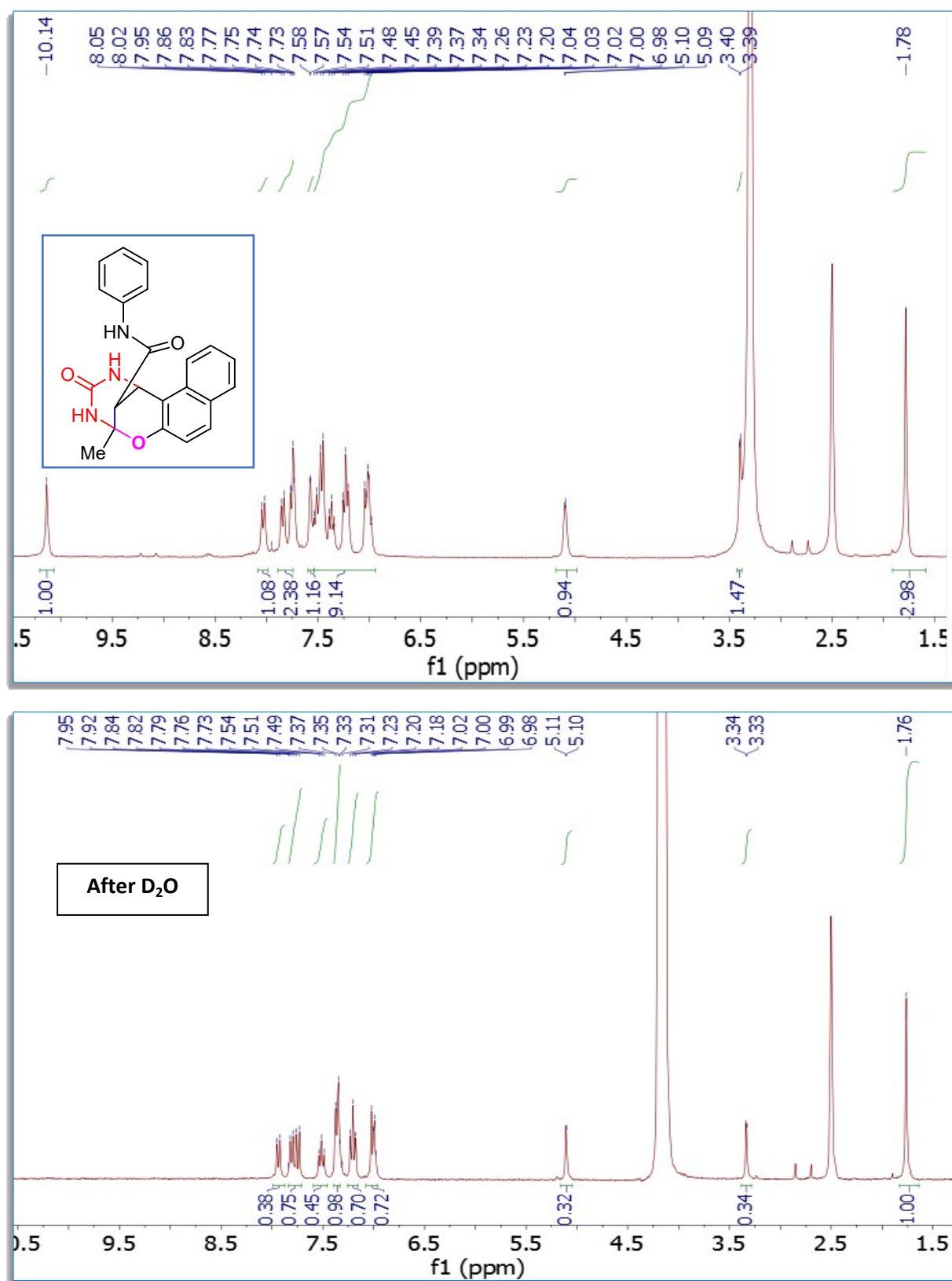


Fig. S36. ^1H NMR spectrum of **15a** (300 MHz, $\text{DMSO}-d_6$)

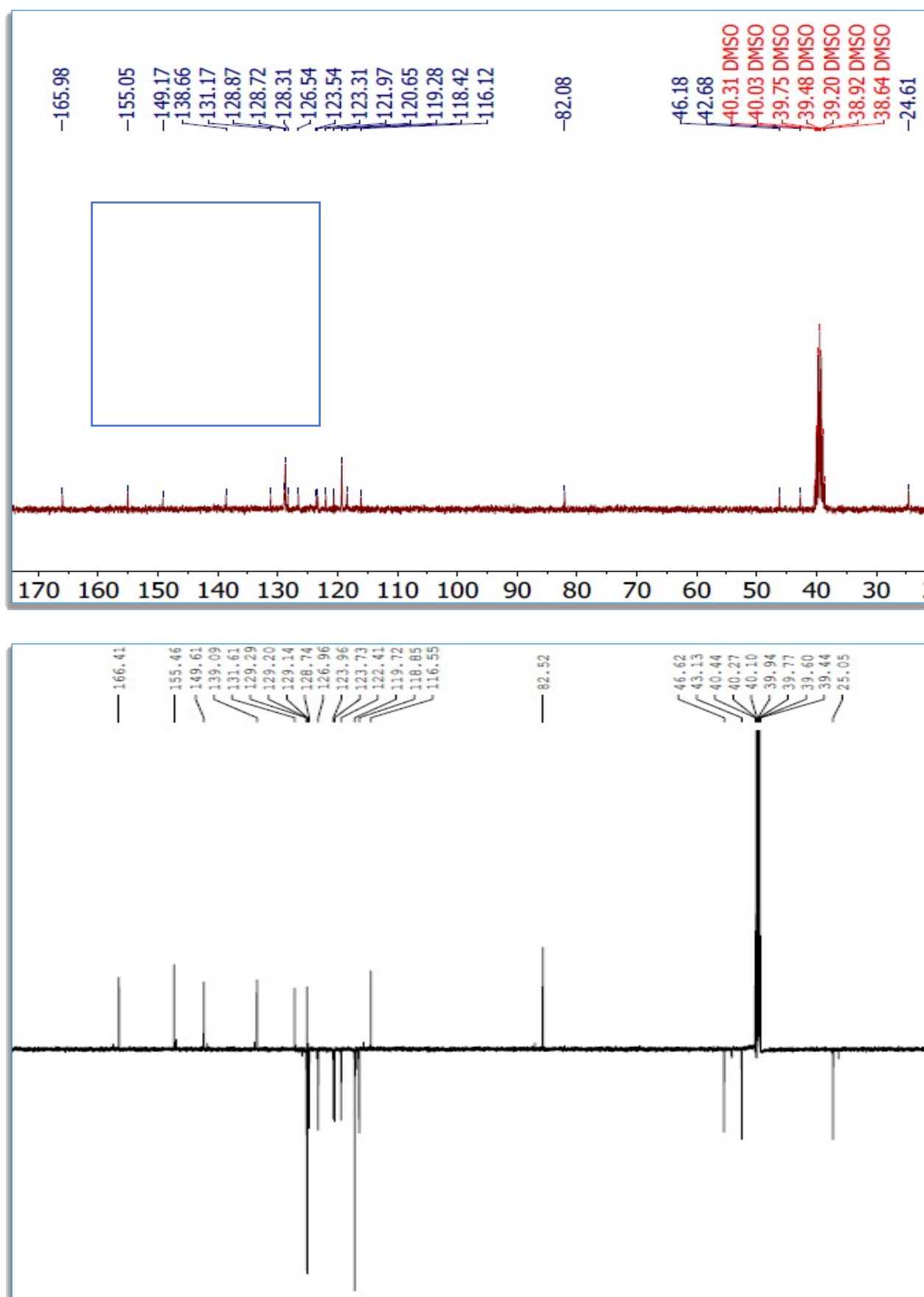


Fig. S37. ^{13}C NMR spectrum of **15a**

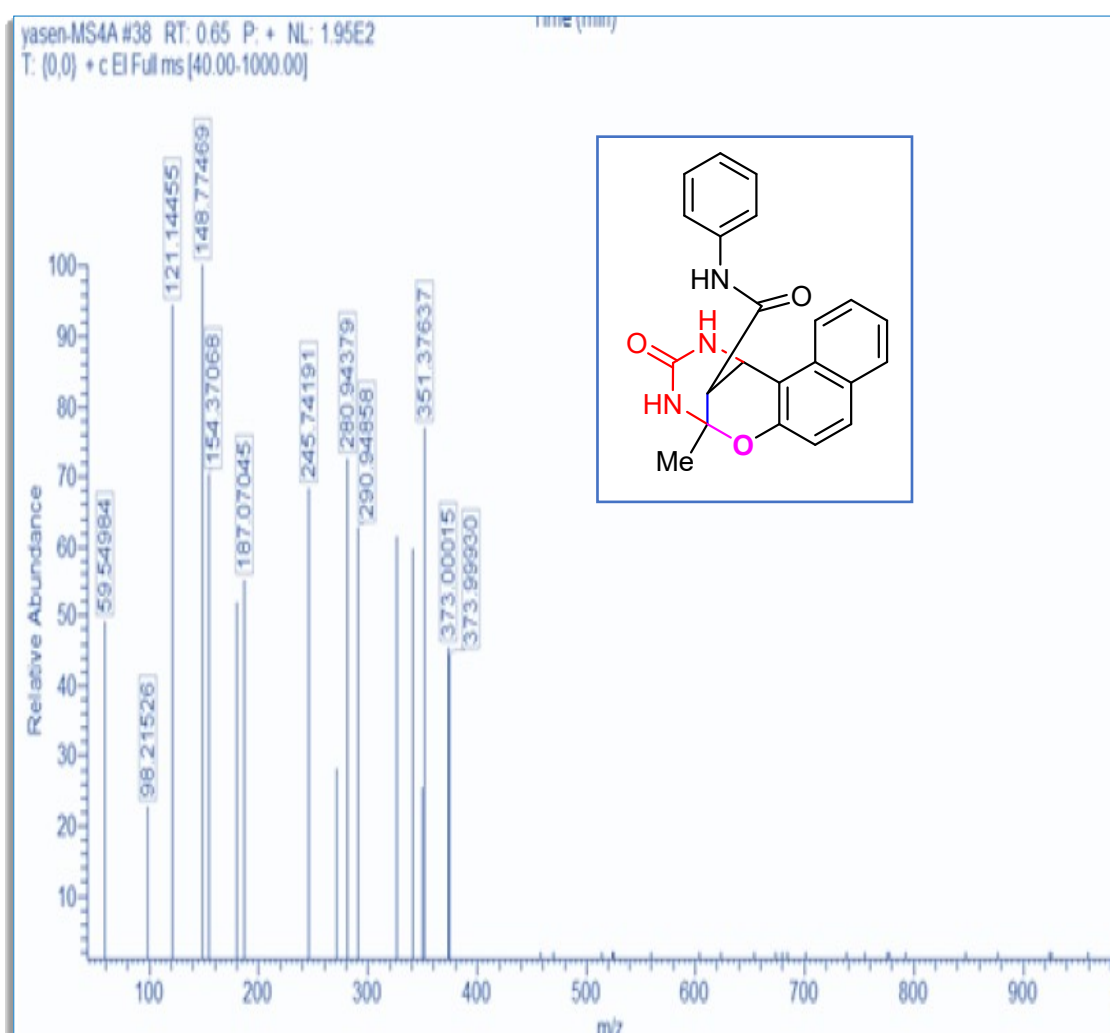
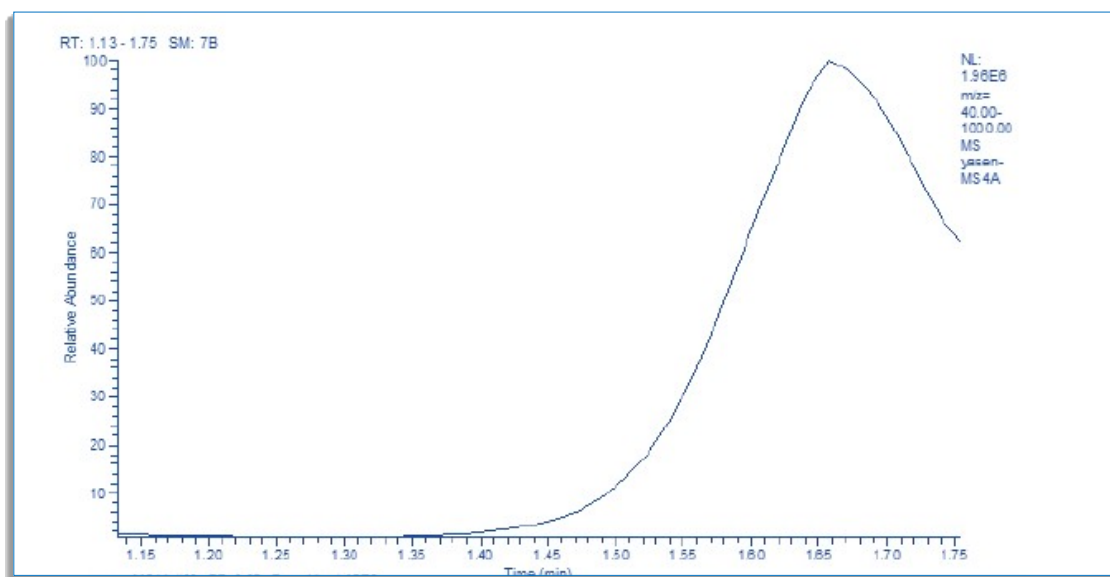
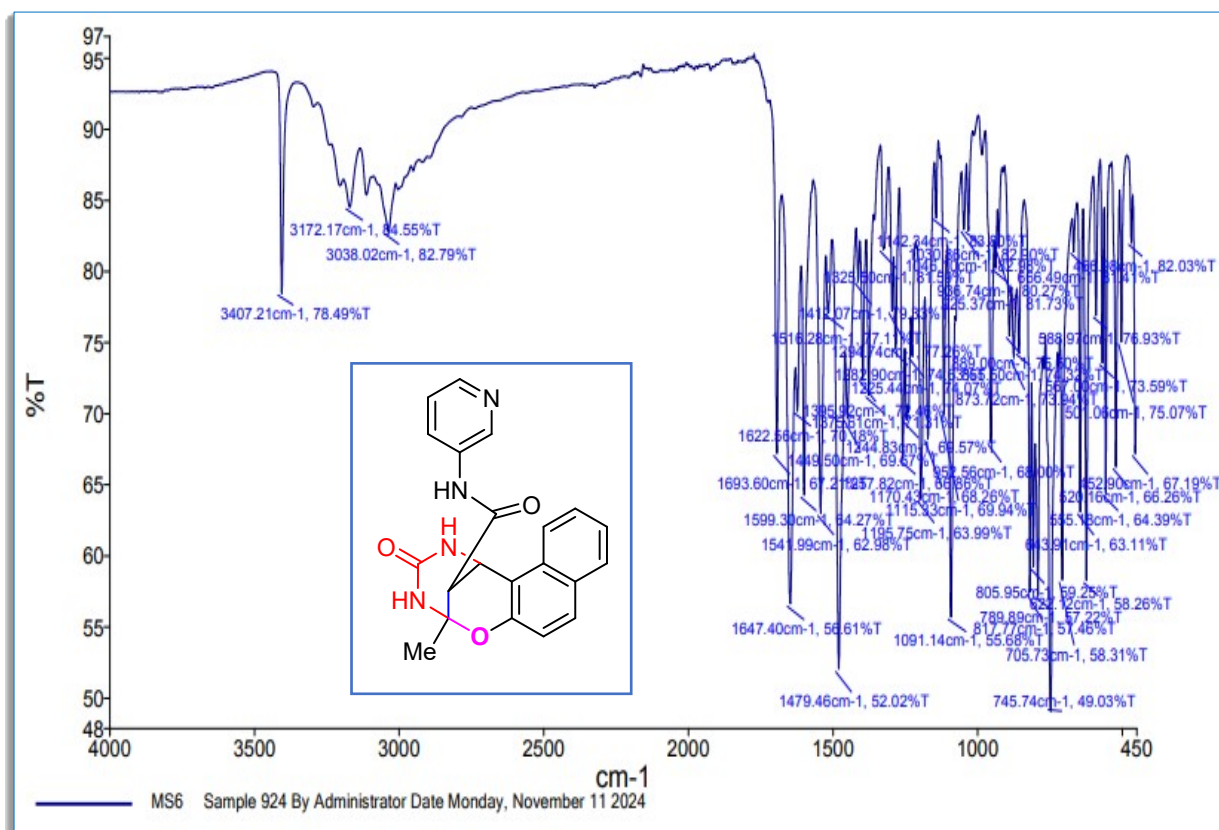


Fig. S38. High resolution mass of **15a**



List of Peak Area/Height		
Peak Number	X (cm-1)	Y (%T)
1	3407.21	78.49
2	3172.17	84.55
3	3038.02	82.79
4	1693.60	67.21
5	1647.40	56.61
6	1622.56	70.18
7	1599.30	64.27
8	1541.99	62.98
9	1516.28	77.11
10	1479.46	52.02
11	1449.50	69.67
12	1412.07	79.33
13	1395.92	72.46
14	1375.61	71.31
15	1325.50	81.59
16	1294.74	77.26
17	1282.90	74.63
18	1257.82	66.86
19	1244.83	69.57
20	1225.44	74.07
21	1195.75	63.99
22	1170.43	68.26
23	1142.34	83.80
24	1115.33	69.94
25	1091.14	55.68
26	1046.10	82.98
27	1030.86	82.90
28	952.56	68.00
29	936.74	80.27
30	925.37	81.73
31	889.00	75.50
32	873.72	73.94

Fig. S39. IR spectrum of 15c

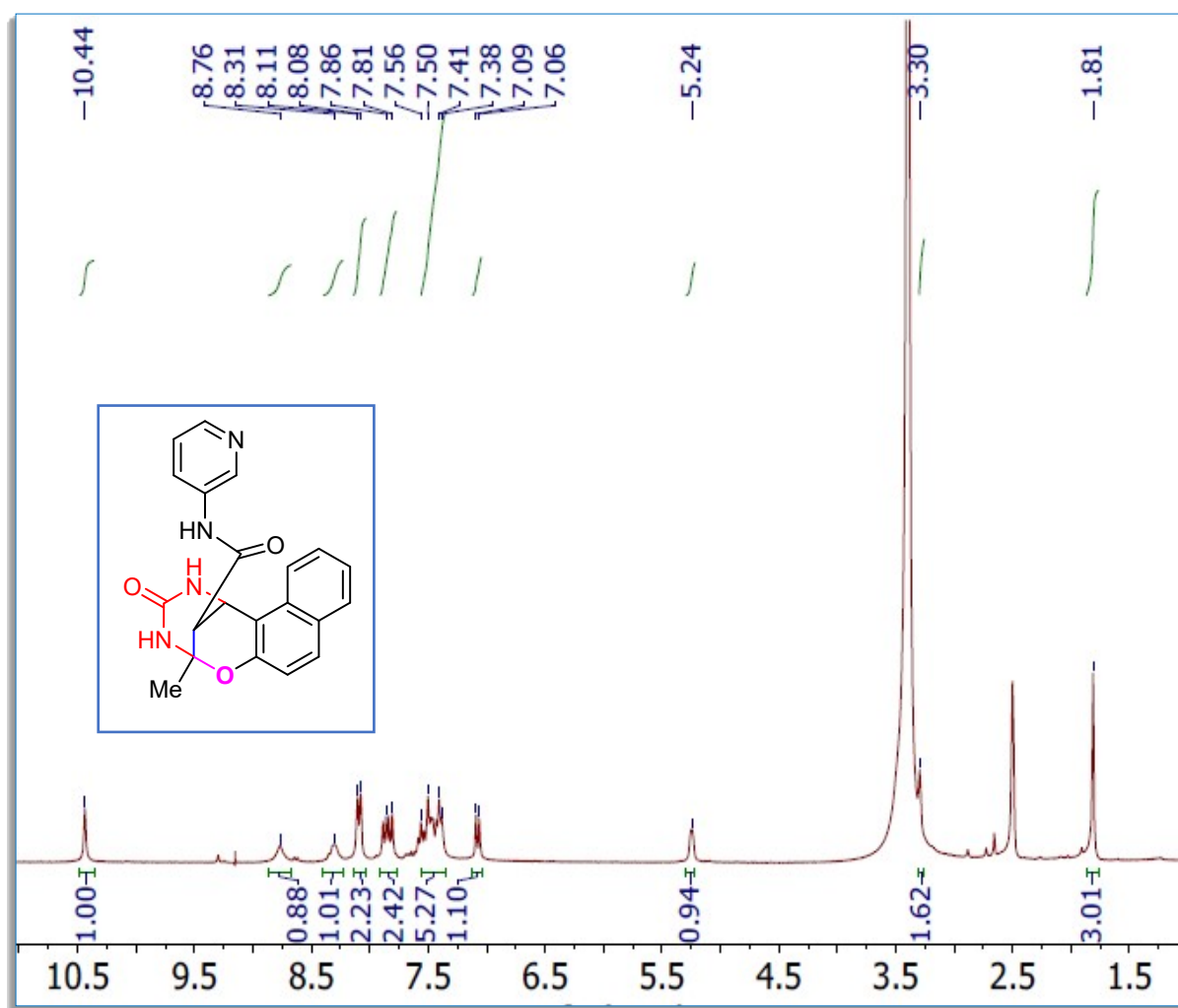


Fig S40. ^1H NMR spectrum of **15c** (300 MHz, $\text{DMSO}-d_6$)

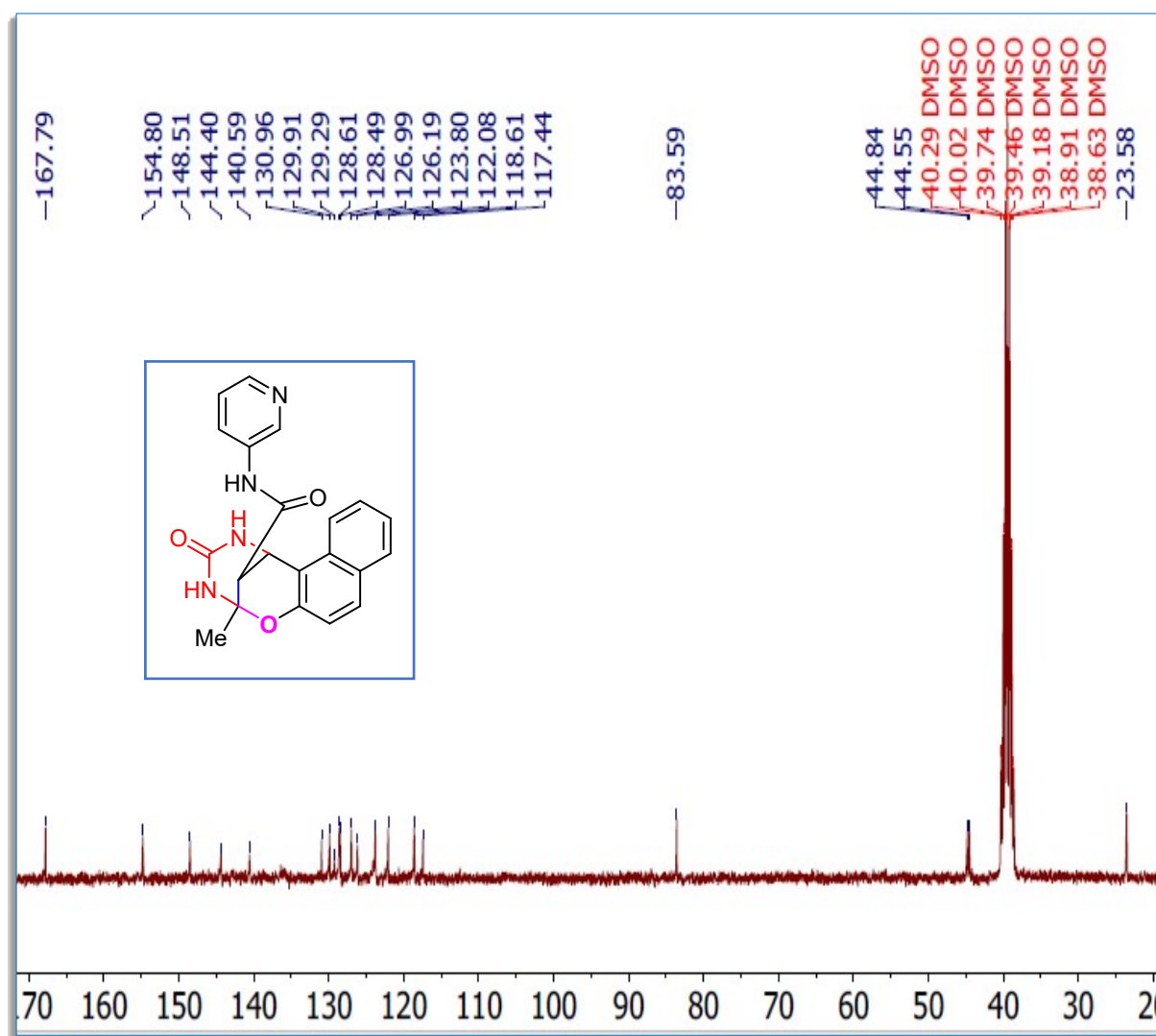


Fig S41. ^{13}C NMR spectrum of **15c** (75 MHz, $\text{DMSO}-d_6$)

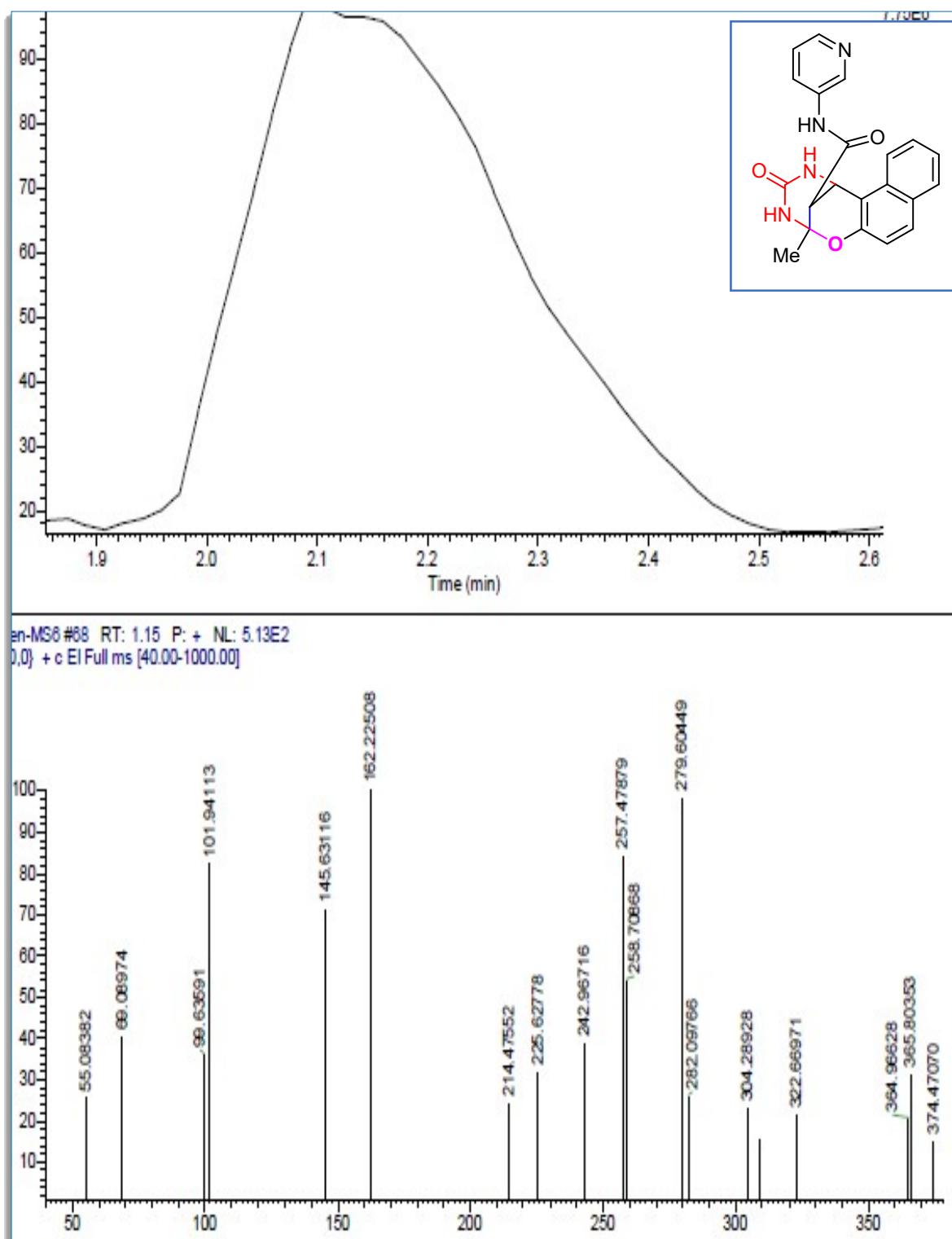
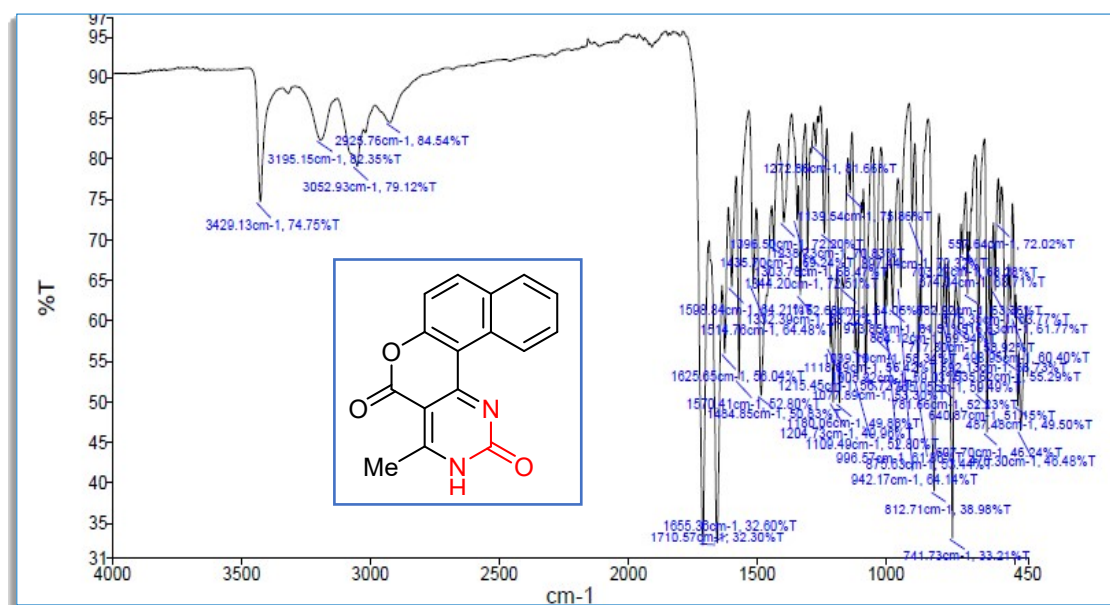


Fig. S42. High resolution mass of **15c**



List of Peak Area/Height		
Peak Number	X (cm-1)	Y (%T)
1	3429.13	74.75
2	3195.15	82.35
3	3052.93	79.12
4	2925.76	84.54
5	1710.57	32.30
6	1655.36	32.60
7	1625.65	56.04
8	1598.84	64.21
9	1570.41	52.80
10	1514.76	64.48
11	1484.85	50.83
12	1435.70	69.24
13	1396.50	72.20
14	1344.20	72.51
15	1332.39	63.22
16	1303.78	68.47
17	1272.86	81.66
18	1238.73	70.83
19	1215.45	56.72
20	1204.73	49.96
21	1180.06	49.88
22	1162.68	64.05
23	1139.54	75.86
24	1118.89	56.42
25	1109.49	52.80
26	1077.89	53.30
27	1039.10	58.34
28	1005.32	59.03
29	996.57	61.86
30	973.85	61.51
31	942.17	64.14

Fig. S43. IR spectrum of 16

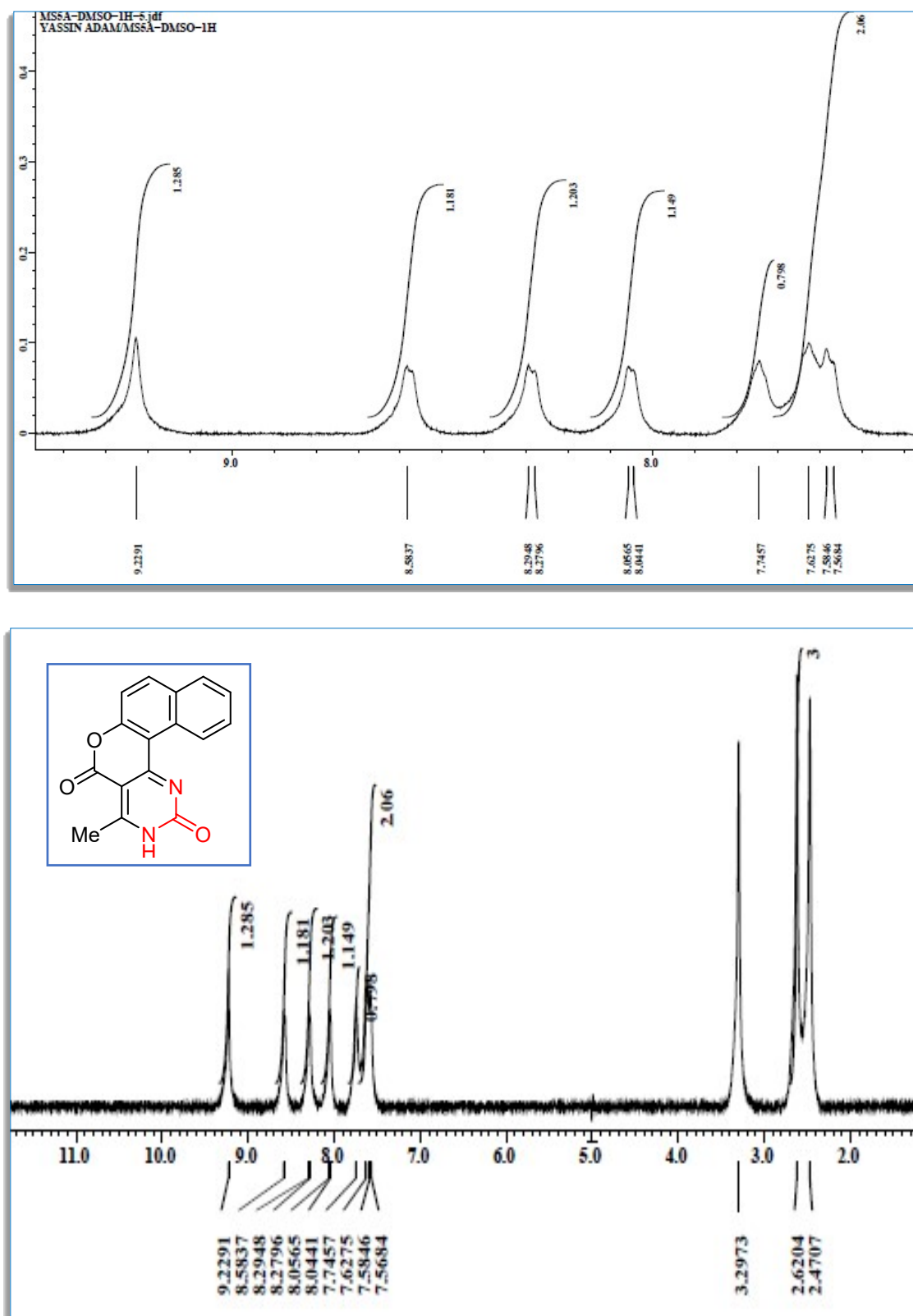


Fig. S44. ^1H NMR spectrum of **16** (500 MHz, $\text{DMSO}-d_6$)

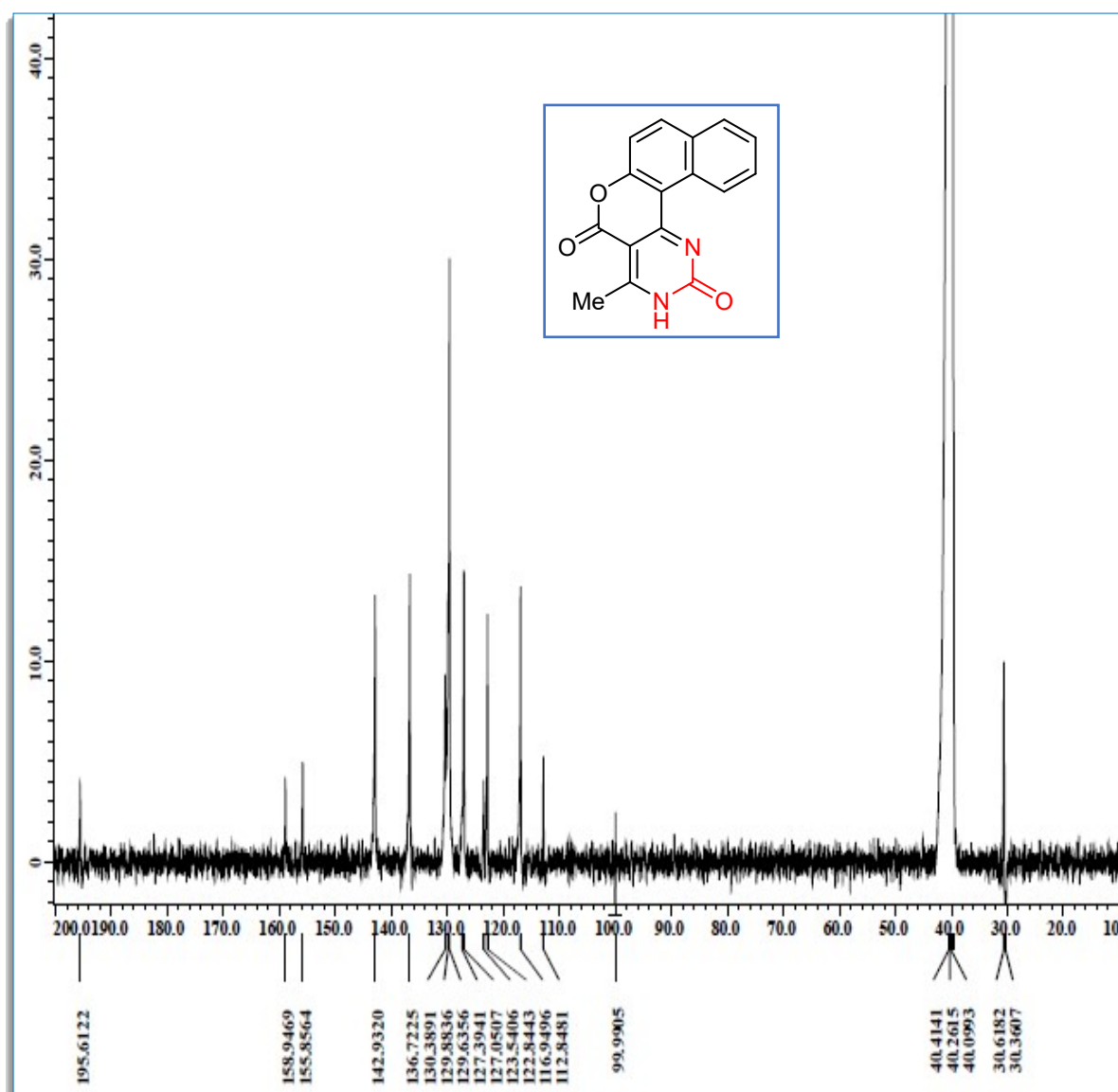


Fig. S45. ^{13}C NMR spectrum of **16** (125 MHz, $\text{DMSO}-d_6$)