Labdane diterpenoids from Leonotis ocymifolia with selective cytotoxic activity against HCC70 breast cancer cell line

Jane Busisiwe Ncongwane 1*, Vuyelwa Jacqueline Tembu1, Comfort Nkambule 1, Douglas Kemboi 1, 4, Gerda Fouche 2, Nyeleti Vukea 3 and Jo-Anne de la Mare 3

1 Department of Chemistry, Tshwane University of Technology, Private Bag X680 Pretoria, 0001, South Africa ; ncongwanejb@tut.ac.za (J.B.N); TembuVJ@tut.ac.za (V.J.T.); nkambulecm@tut.ac.za (C.N); kemboidouglas01@gmail.com (D.K.M.)
2 Department of Chemistry, University of Pretoria, Pretoria, 0001, South Africa; gerda.fouche@up.ac.za
3 Department of Biochemistry and Microbiology, Biomedical Biotechnology Research Unit, Rhodes University, Grahamstown, 6140, South Africa
4 School of Science and Technology. University of Kabianga, 2030-20200, Kericho, Kenya
* Correspondence: ncongwanejb@tut.ac.za; TembuVJ@tut.ac.za (V.J.T.); Tel.: +27-12-382-6288 (V.J.T.)

Abstract

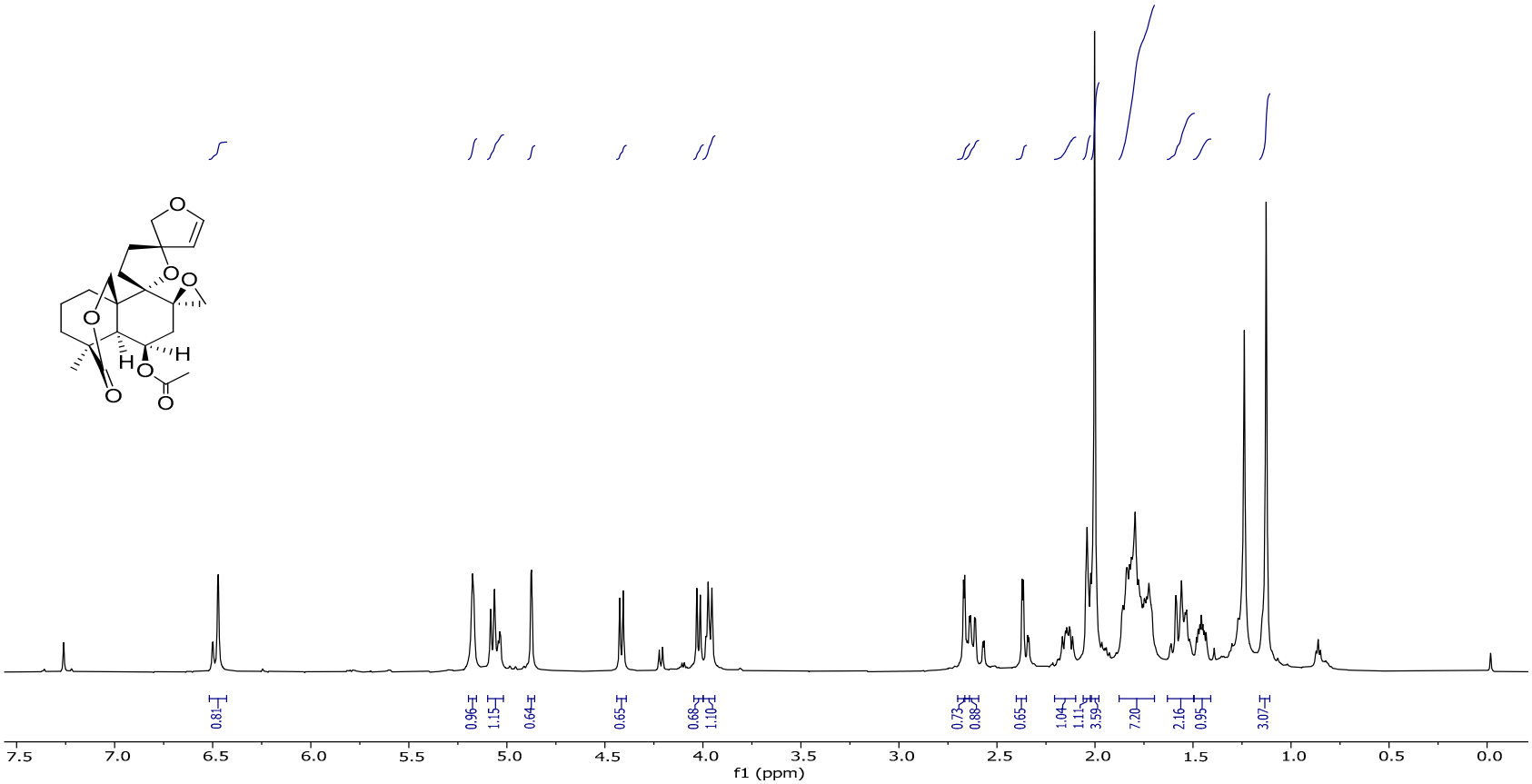
Leonotis ocymifolia is a shrub widely used in traditional medicine to alleviate illnesses such as wounds, abdominal pain, headache, hypertension, malaria, asthma, diabetes, and eczema. Compounds from *Leonotis ocymifolia* were characterised using spectroscopic data such as IR, 1D and 2D NMR and MS spectrometry and evaluated for cytotoxic activity against triple negative breast cancer (HCC70), hormone receptor-positive breast cancer (MCF-7), and non-tumorigenic mammary epithelial cell lines (MCF-12A). A novel bis-spirolabdane, 13S-nepetaefolin (**1**), together with five known labdane diterpenoids, nepetaefolin (**2**), dubiin (**3**), nepetaefuran (**4**), leonotin (**5**) and leonotinin (**6**) from the genus Leonotis were isolated. Overall, the labdane diterpenoids showed selective activity toward triple negative breast cancer cells (HCC70). Of the compounds extracted, 13S-nepetaefolin demonstrated the greatest cytotoxic activity with an IC_{50} of 24.65 μM against HCC70 cells, however, it was equally cytotoxic to non-tumorigenic MCF-12A breast cells (IC_{50} of 26.55 μM) whereas its isomer showed no activity. This suggests that stereochemistry might have an effect on the cytotoxic activity of the bis-spirolabdane diterpenoids.

Keyword

Leonotis ocymifolia, labdane diterpenoids, MCF-7, HCC70, cytotoxic activity

Contents

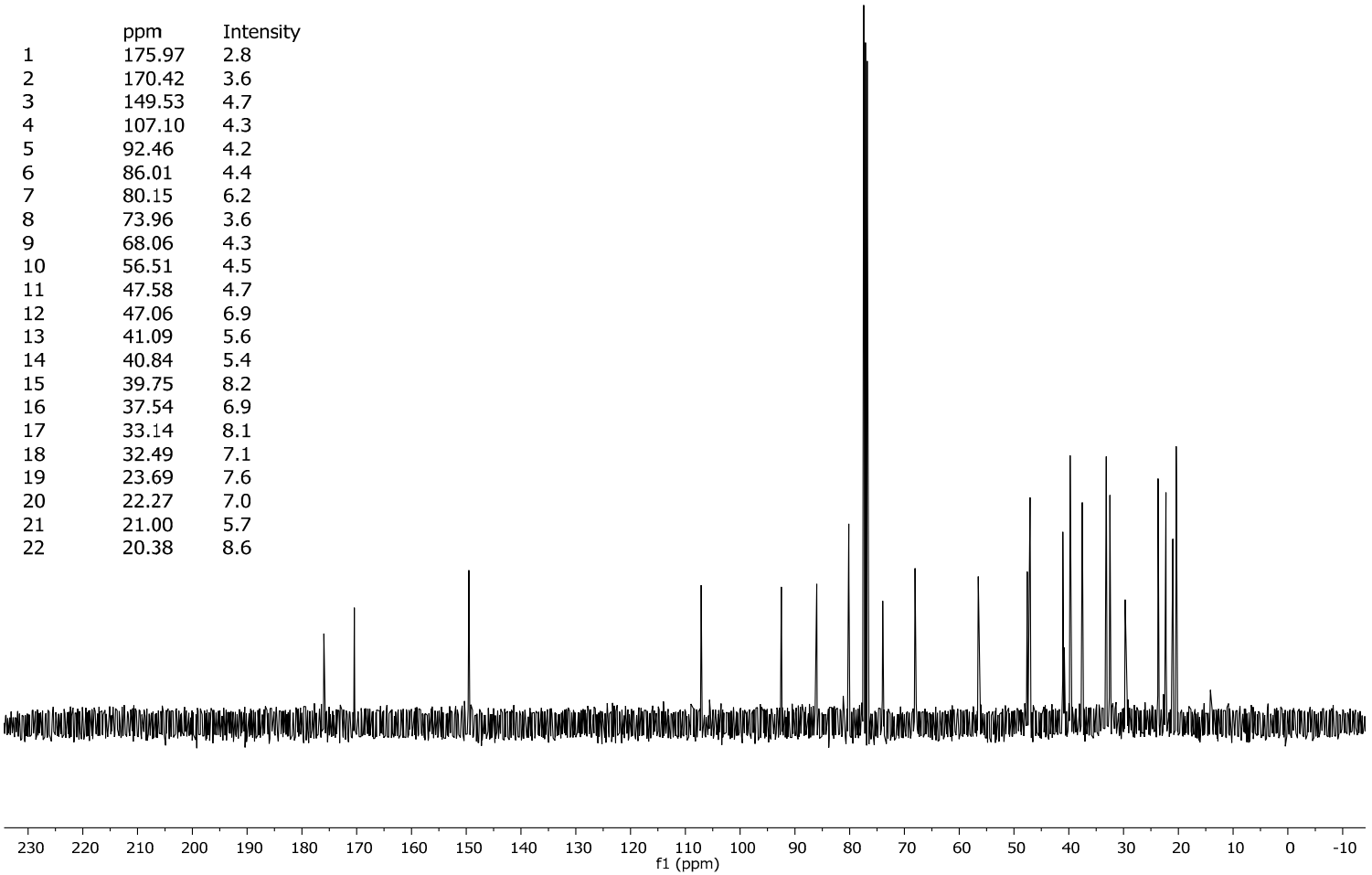
Appendix 1: ^{1}H NMR spectrum of 13*S*-nepetaefolin (1) in $CDCl_3$



Spectrum A1: ^{1}H NMR spectrum of compound 1 (13*S*-Nepetaefolin)

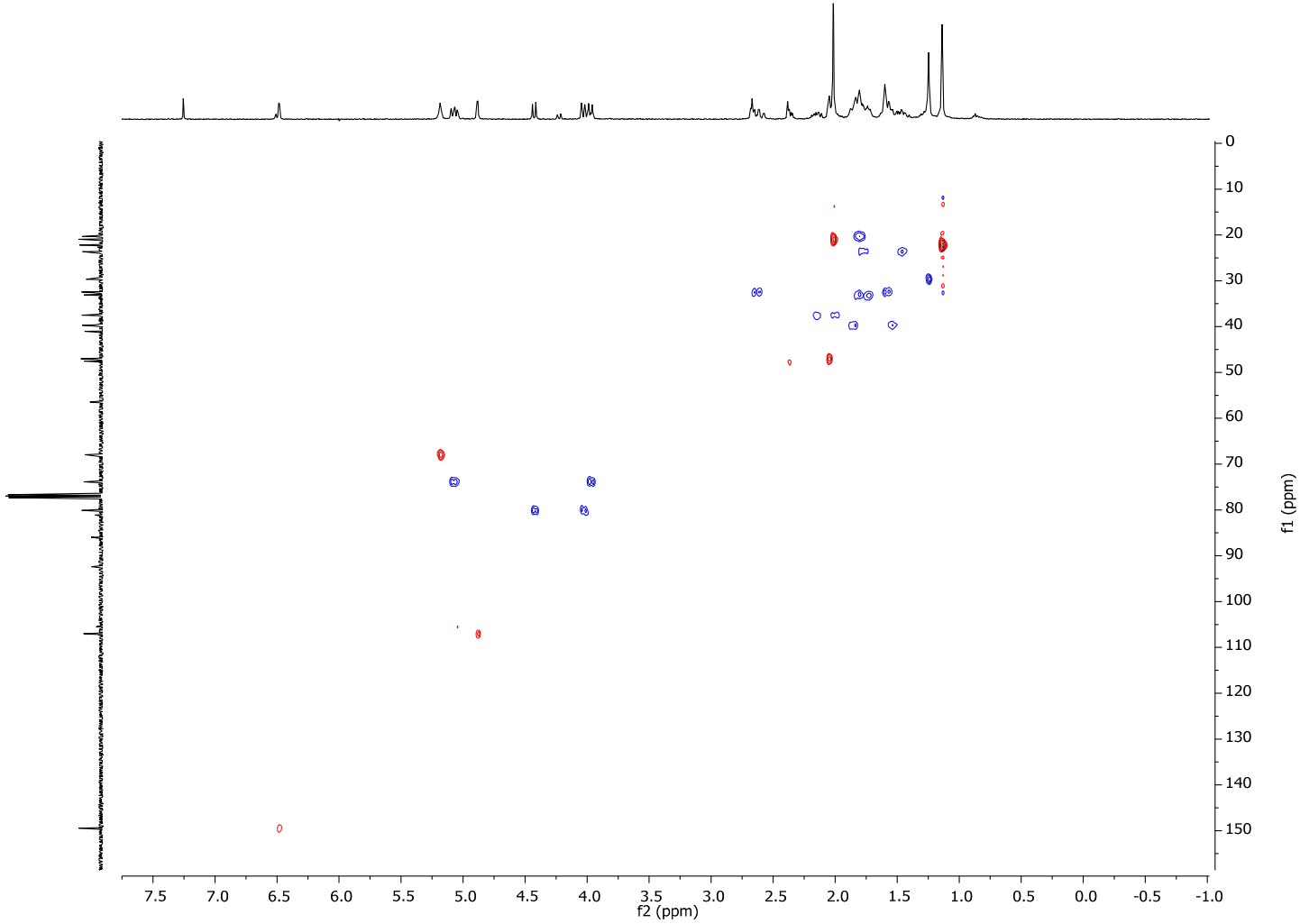
Appendix 2: ^{13}C NMR spectrum of 13S nepetaefolin (1) in $CDCl_3$

	ppm	Intensity
1	175.97	2.8
2	170.42	3.6
3	149.53	4.7
4	107.10	4.3
5	92.46	4.2
6	86.01	4.4
7	80.15	6.2
8	73.96	3.6
9	68.06	4.3
10	56.51	4.5
11	47.58	4.7
12	47.06	6.9
13	41.09	5.6
14	40.84	5.4
15	39.75	8.2
16	37.54	6.9
17	33.14	8.1
18	32.49	7.1
19	23.69	7.6
20	22.27	7.0
21	21.00	5.7
22	20.38	8.6



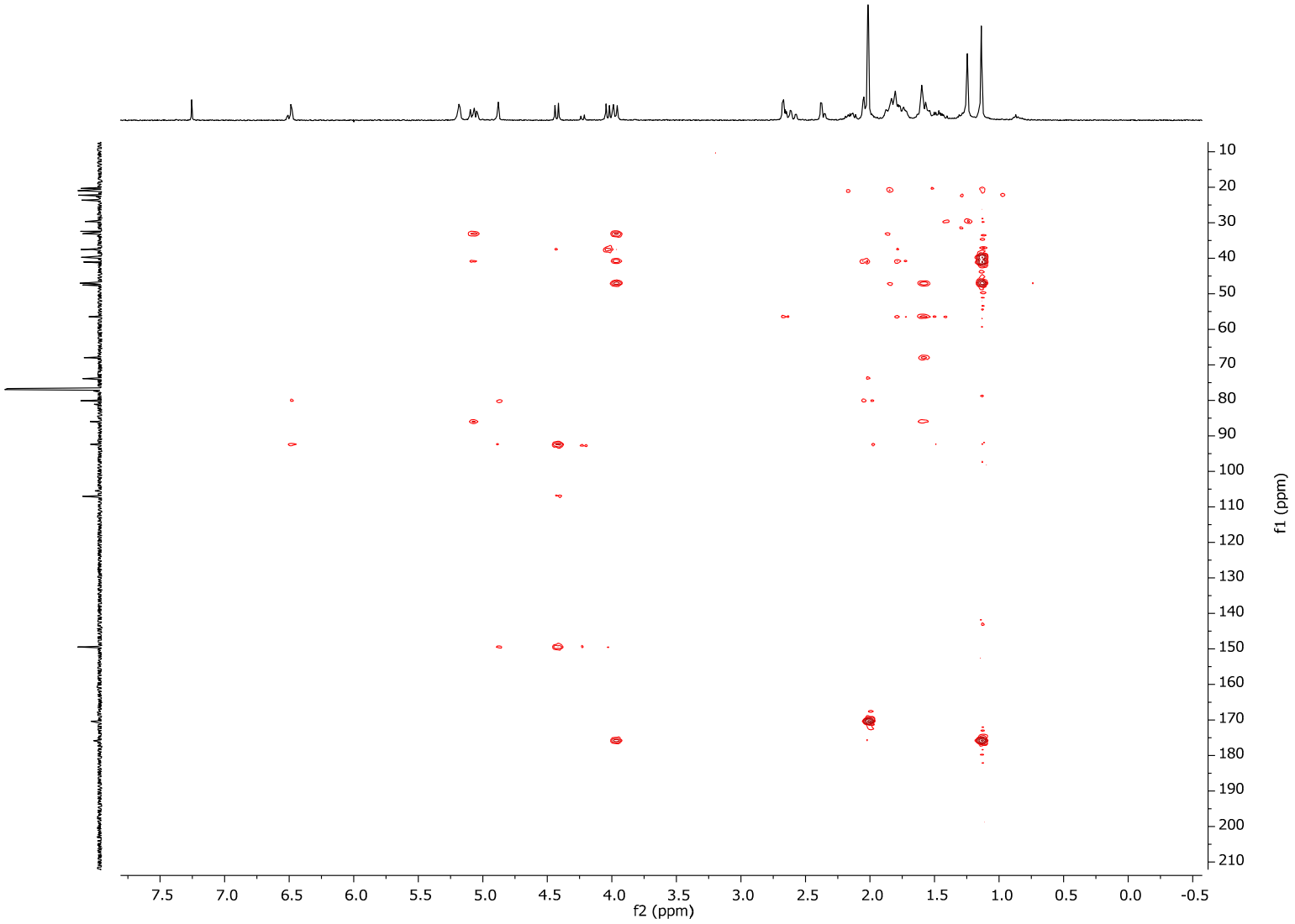
Spectrum A2: ^{13}C NMR spectrum of compound 1 (13*S*-Nepetaefolin)

Appendix 3: HSQC spectrum of 13S nepetaefolin (1) in $CDCl_3$



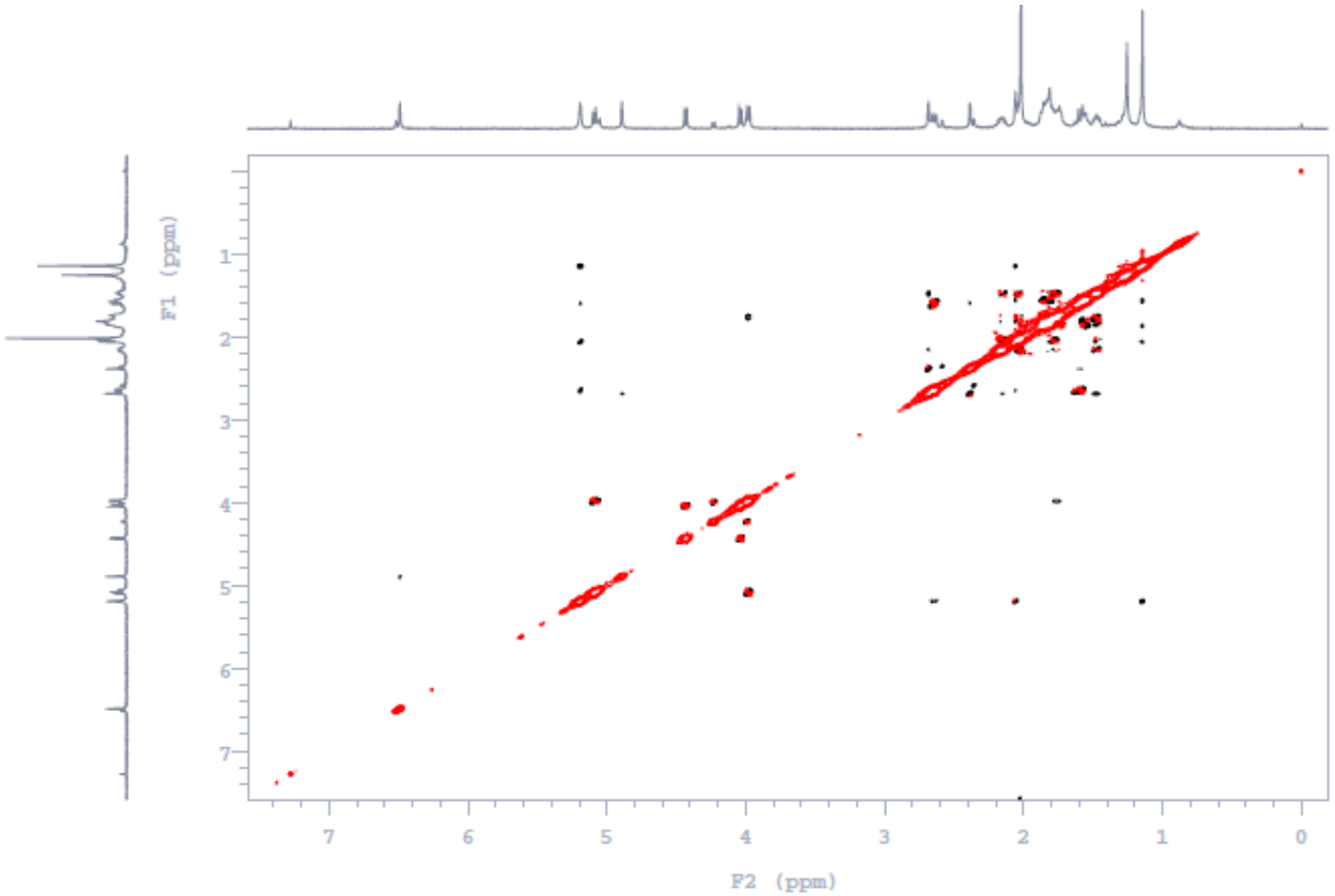
Spectrum A3: HSQC spectrum of compound 1 (13*S*-Nepetaefolin)

Appendix 4: HMBC spectrum of 13S nepetaefolin (1) in $CDCl_3$



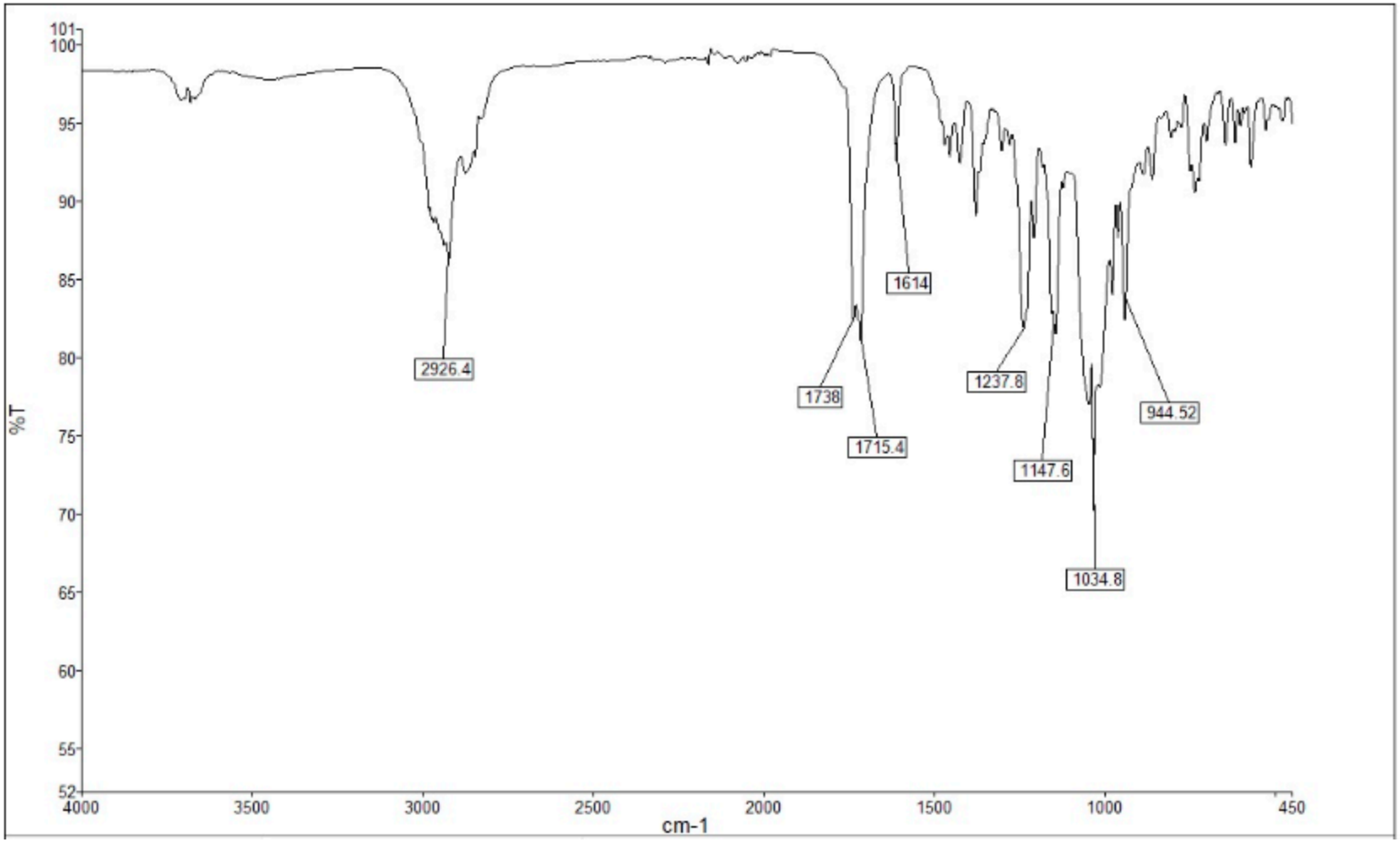
Spectrum A4: HMBC NMR spectrum of compound 1 (13S-Nepetaefolin)

Appendix 5: NOESY spectrum of 13S nepetaefolin (1) in $CDCl_3$



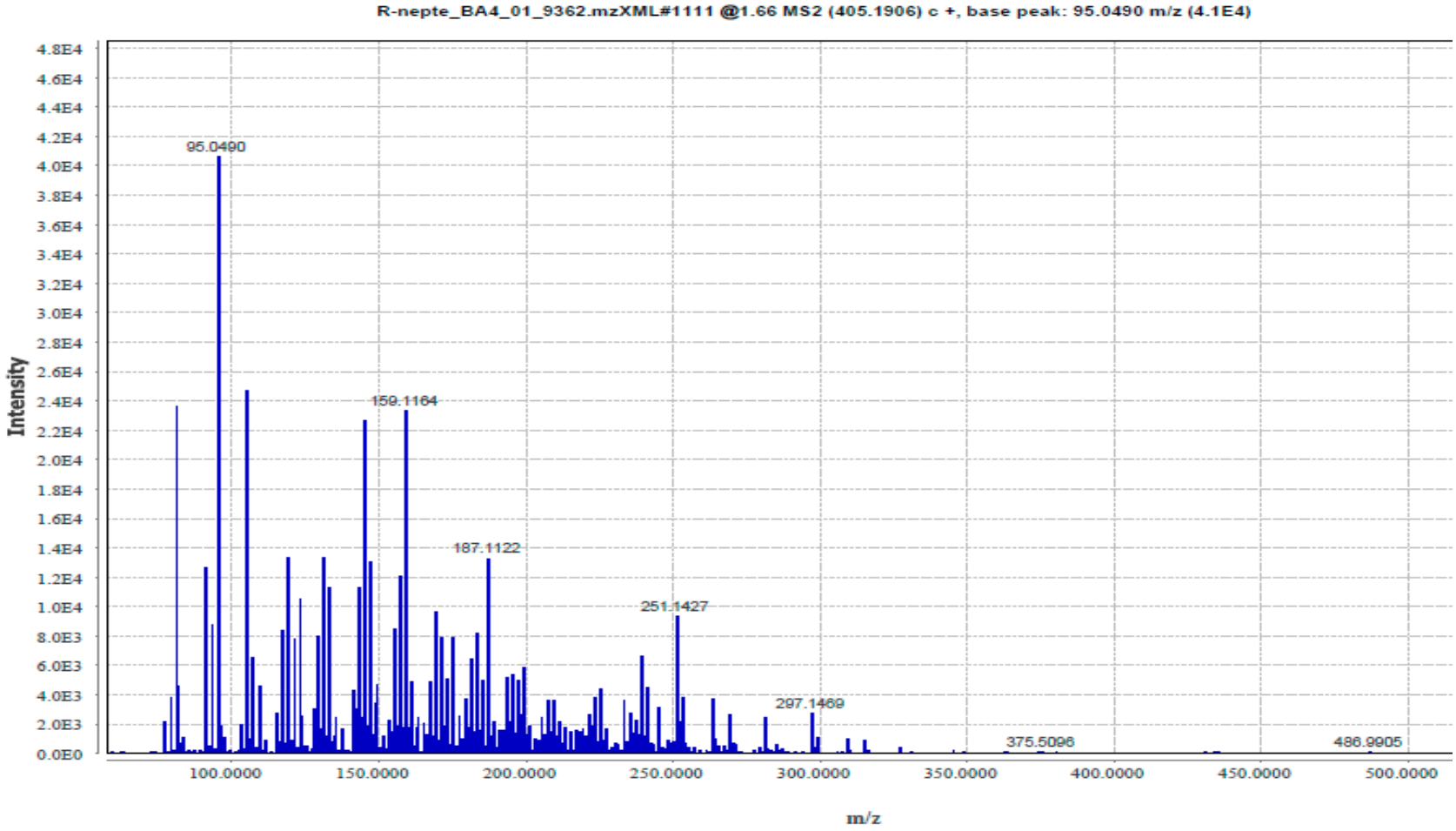
Spectrum A6: NOESY NMR spectrum of compound 1 (13*S*-Nepetaefolin)

Appendix 6: IR spectrum of 13S nepetaefolin (1)



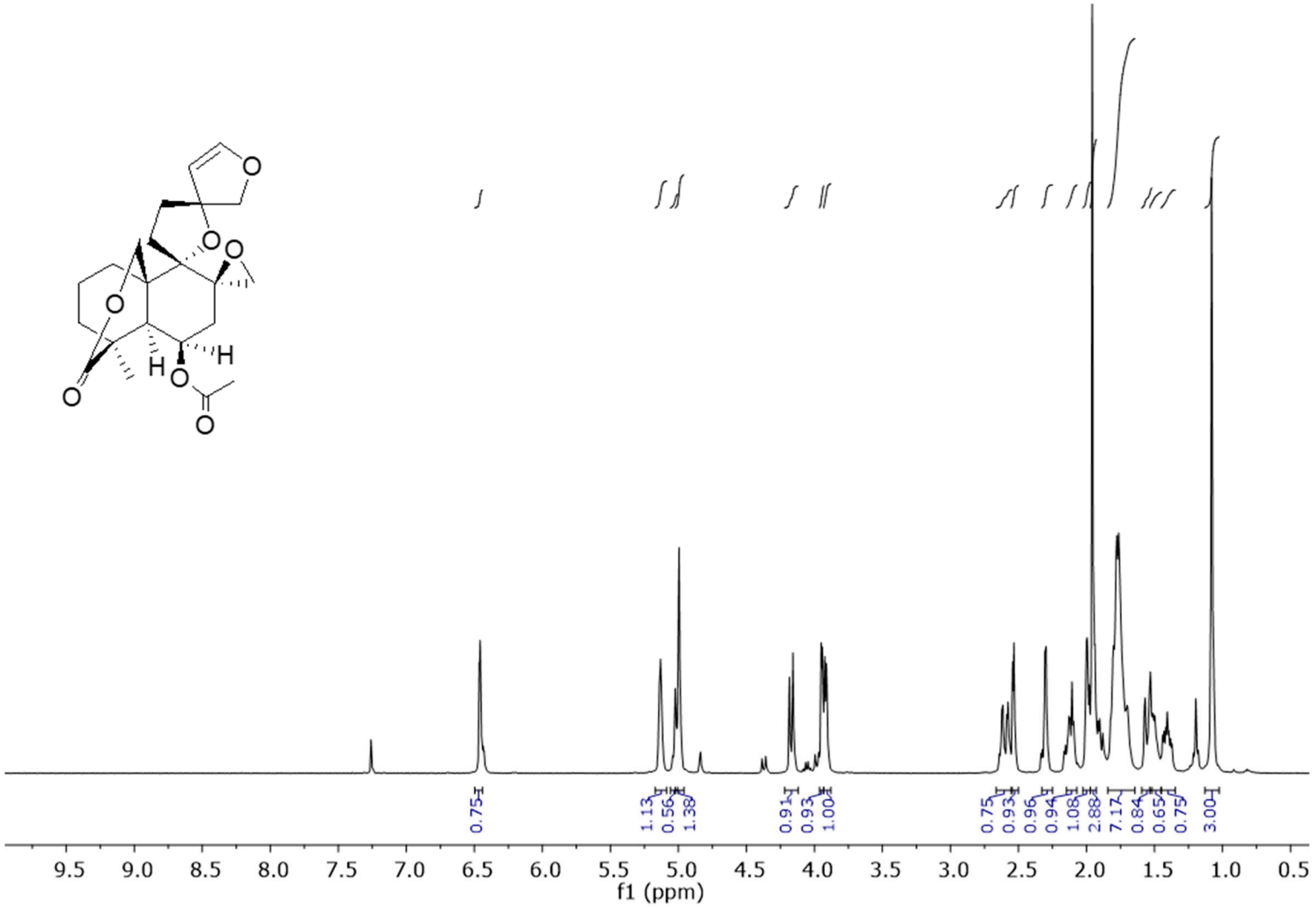
Spectrum A7: IR spectrum of compound 1 (13*S*-Nepetaefolin)

Appendix 7: MS spectrum of 13S nepetaefolin (1)



Spectrum A8: MS spectrum of compound 1 (13S-Nepetaefolin)

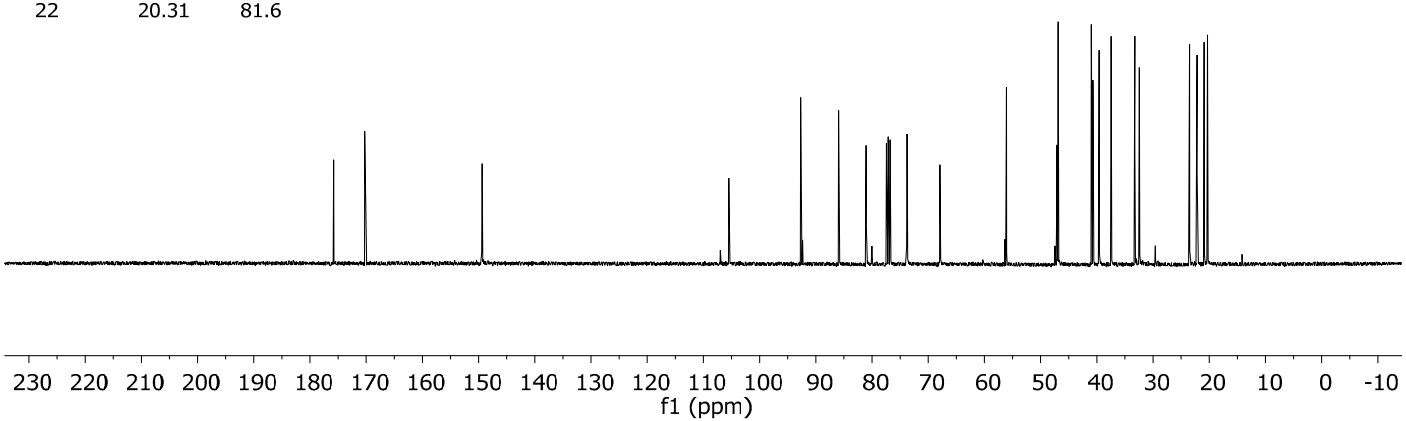
Appendix 8: ^{1}H NMR spectrum of nepetaefolin (2) in $CDCl_3$



Spectrum B1: ^{1}H NMR spectrum of compound 2 (nepetaefolin)

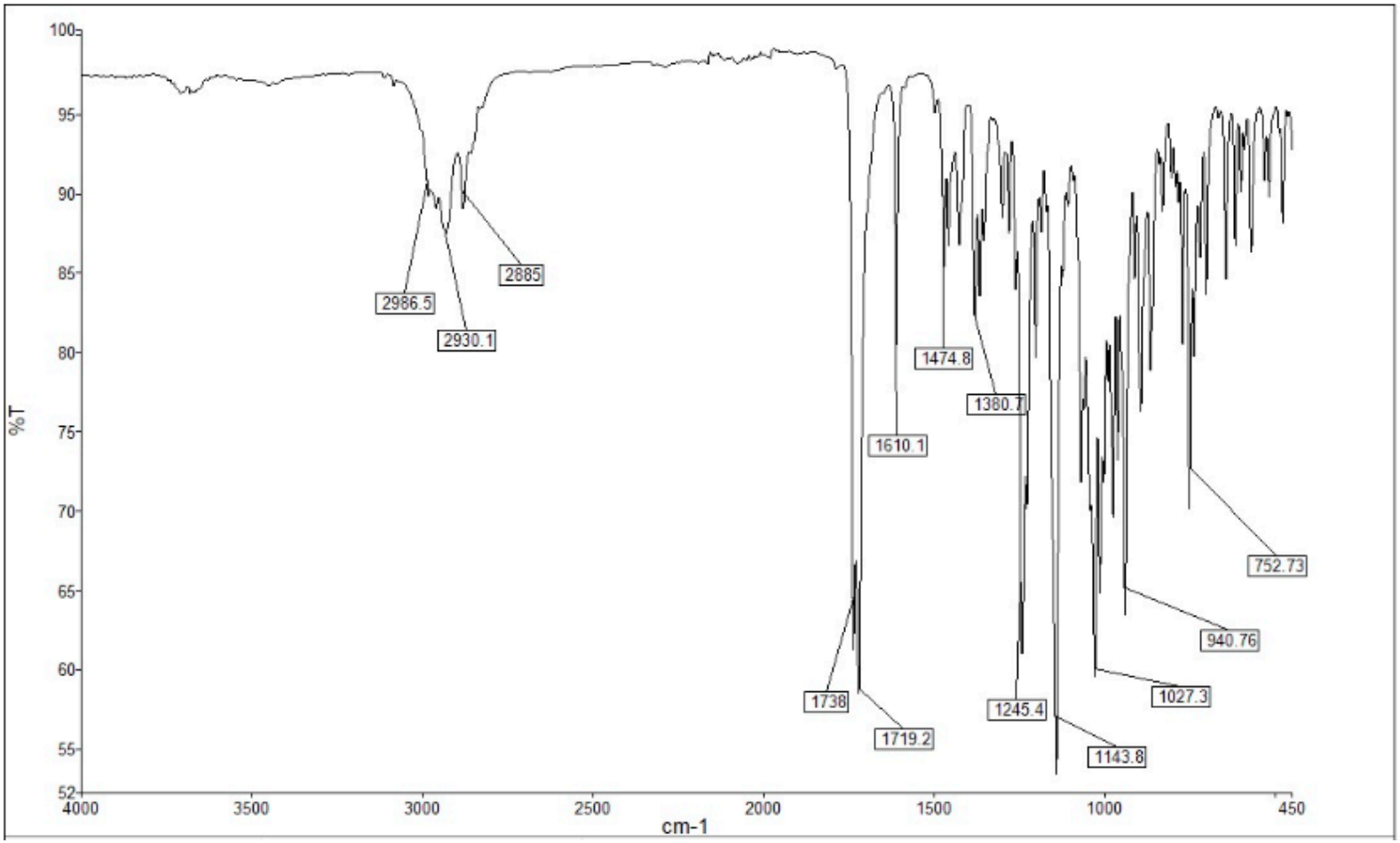
Appendix 9: ^{13}C NMR spectrum of nepetaefolin (2) in $CDCl_3$

	ppm	Intensity
1	175.79	37.8
2	170.26	47.1
3	149.37	35.3
4	105.44	28.4
5	92.71	59.2
6	85.95	55.3
7	81.06	41.7
8	73.77	45.7
9	67.87	32.1
10	56.07	62.3
11	47.12	41.9
12	46.89	85.2
13	40.99	84.5
14	40.71	66.7
15	39.59	76.0
16	37.46	80.1
17	33.24	80.5
18	32.45	69.7
19	23.50	77.7
20	22.17	73.5
21	20.90	78.3
22	20.31	81.6



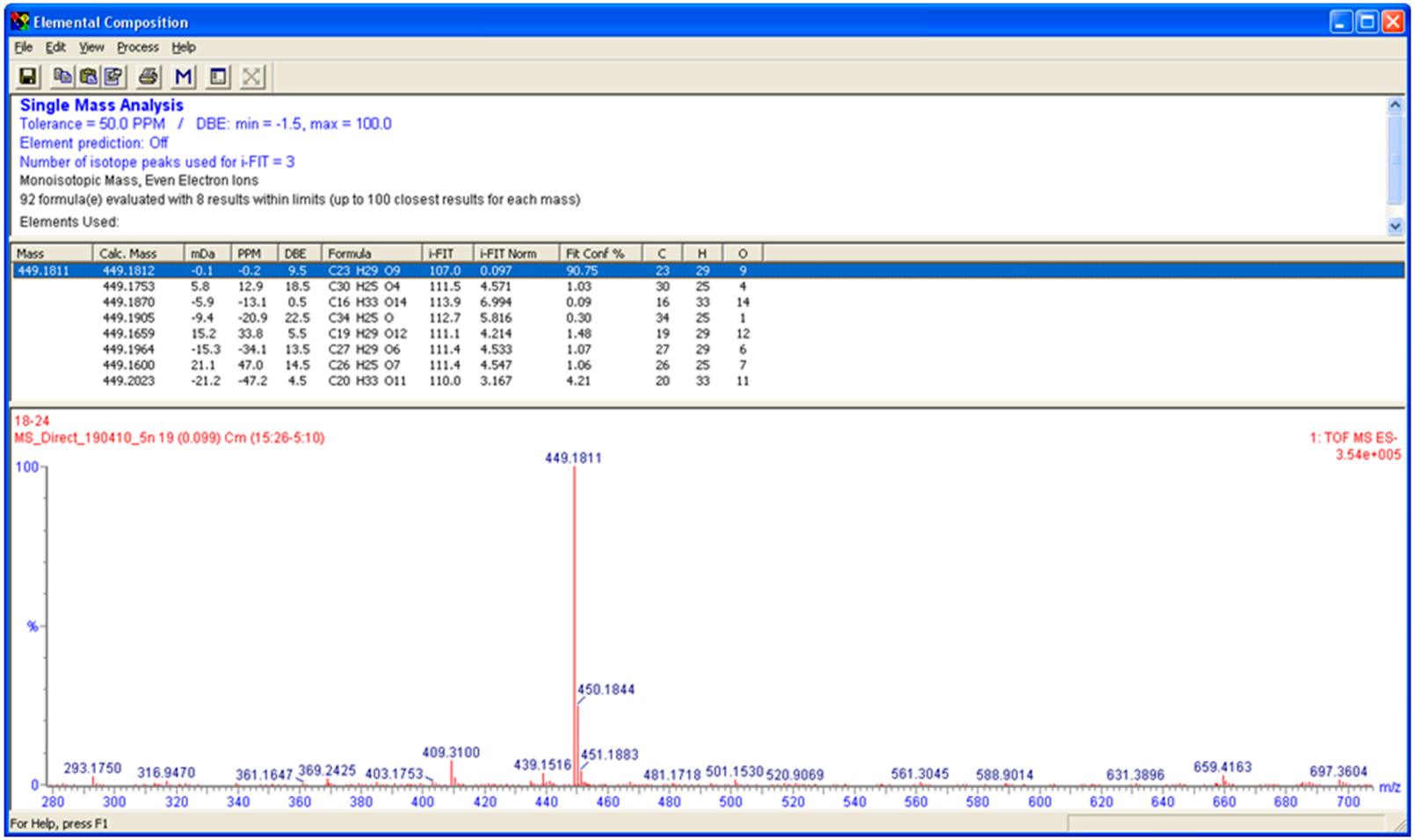
Spectrum B2: ^{13}C NMR spectrum of compound 2 (nepetaefolin)

Appendix 10: IR spectrum of nepetaefolin (2)



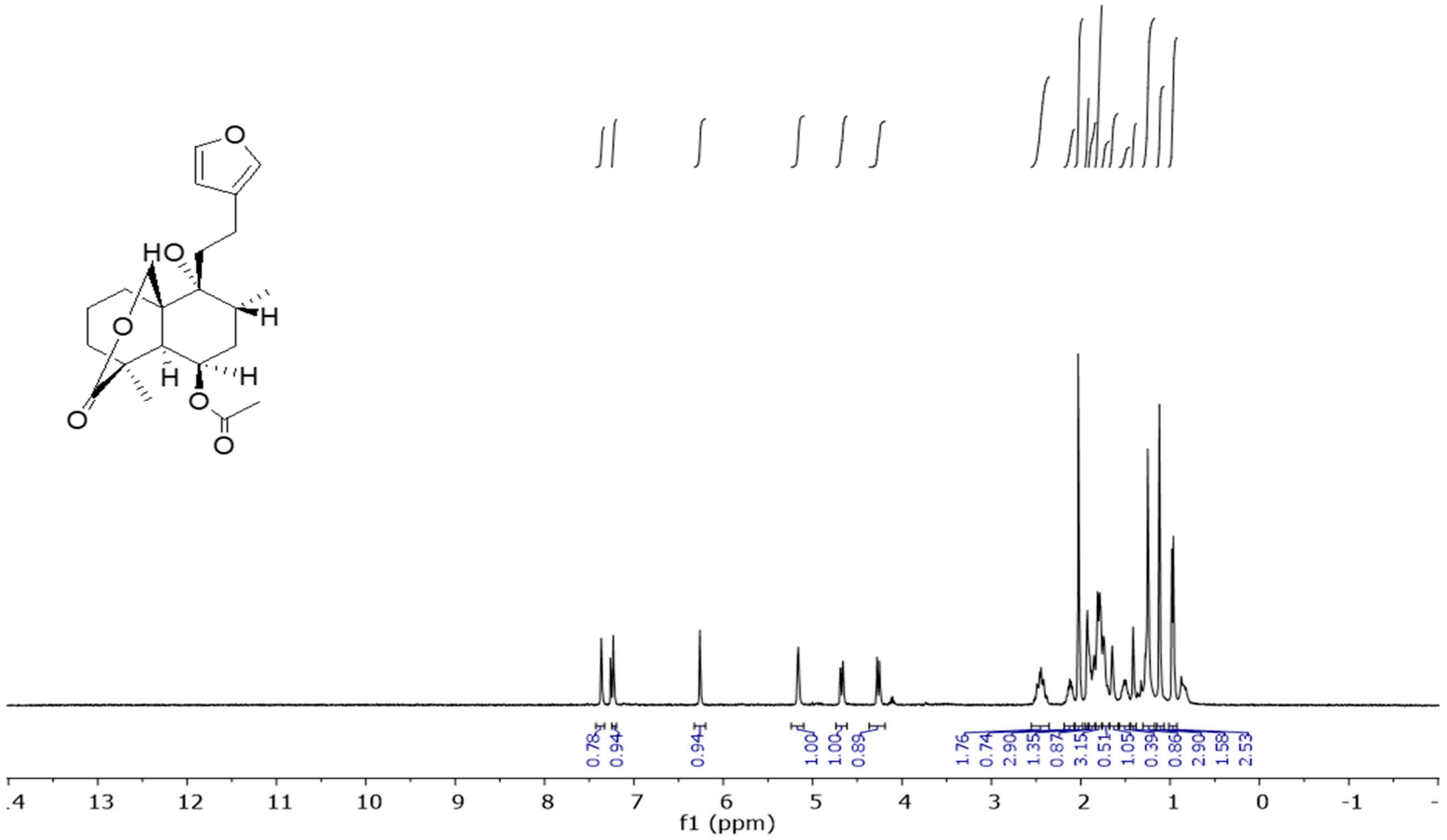
Spectrum B3: IR spectrum of compound 2 (nepetaefolin)

Appendix 11: MS spectrum of nepetaefolin (2)



Spectrum B4: TOF-MS spectrum of compound 2 (nepetaefolin)

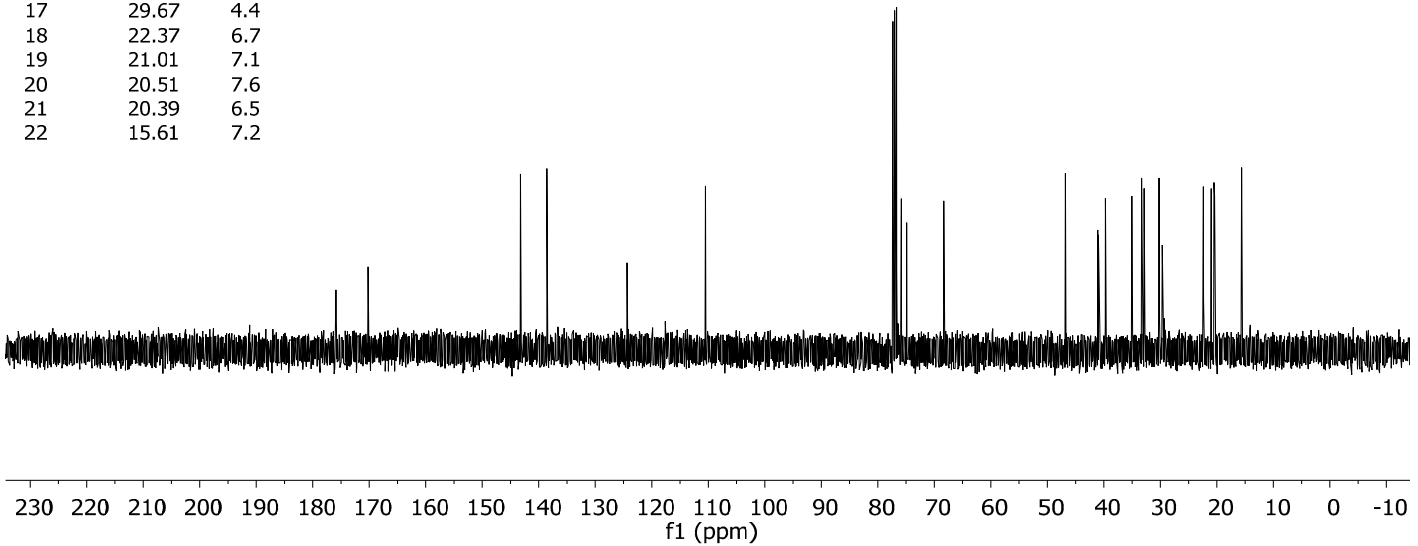
Appendix 12: ^{1}H NMR spectrum of dubiin (3) in $CDCl_3$



Spectrum C1: ^{1}H NMR spectrum of compound 3 (Dubiin)

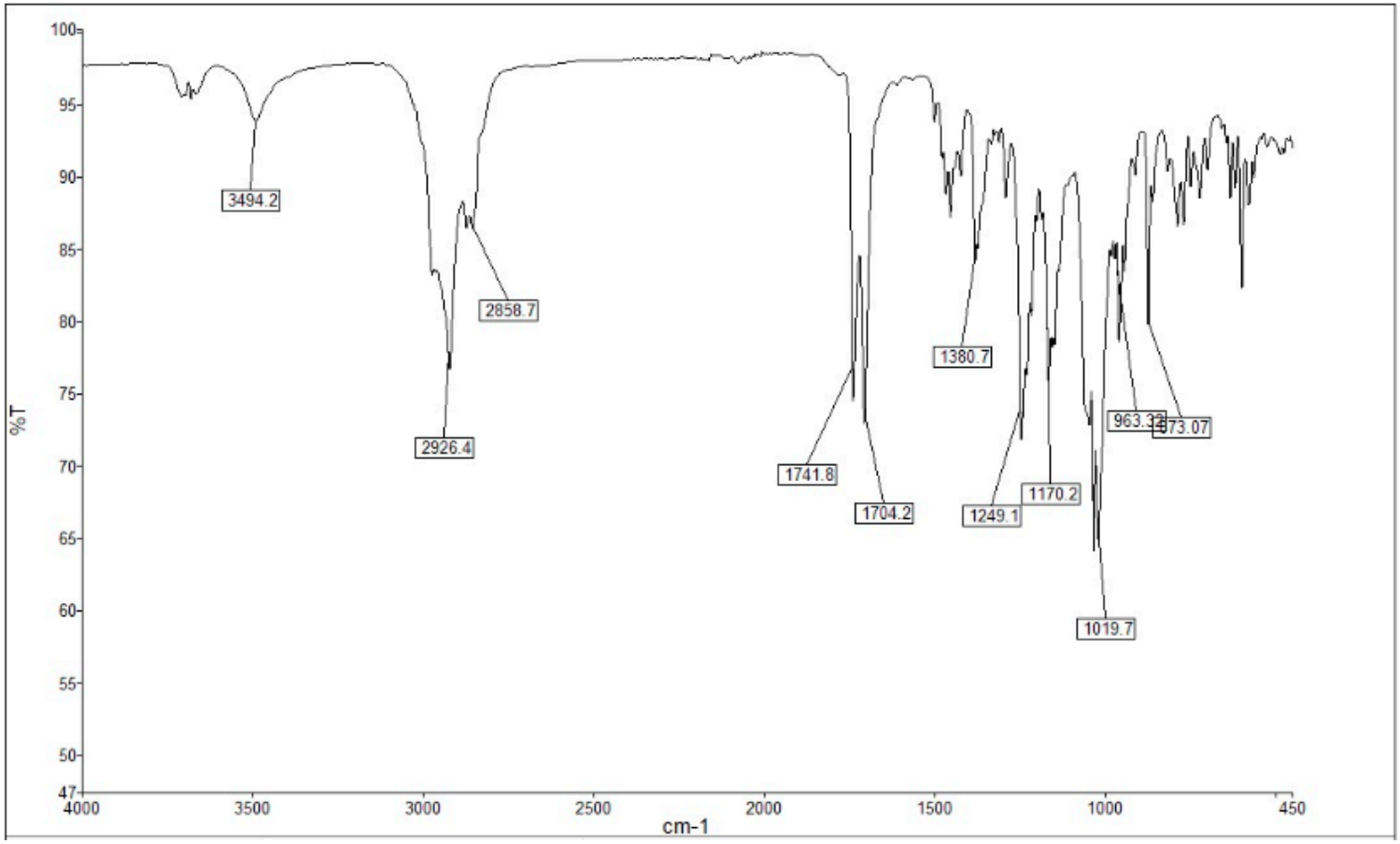
Appendix 13: ^{13}C NMR spectrum of dubiin (3) in $CDCl_3$

	ppm	Intensity
1	170.20	3.6
2	143.24	7.5
3	138.55	7.7
4	124.39	3.6
5	110.50	6.8
6	75.83	6.5
7	74.91	5.4
8	68.33	6.5
9	46.80	7.7
10	41.09	5.1
11	40.97	4.9
12	39.72	6.5
13	35.05	6.7
14	33.33	7.1
15	32.87	6.8
16	30.22	7.2
17	29.67	4.4
18	22.37	6.7
19	21.01	7.1
20	20.51	7.6
21	20.39	6.5
22	15.61	7.2



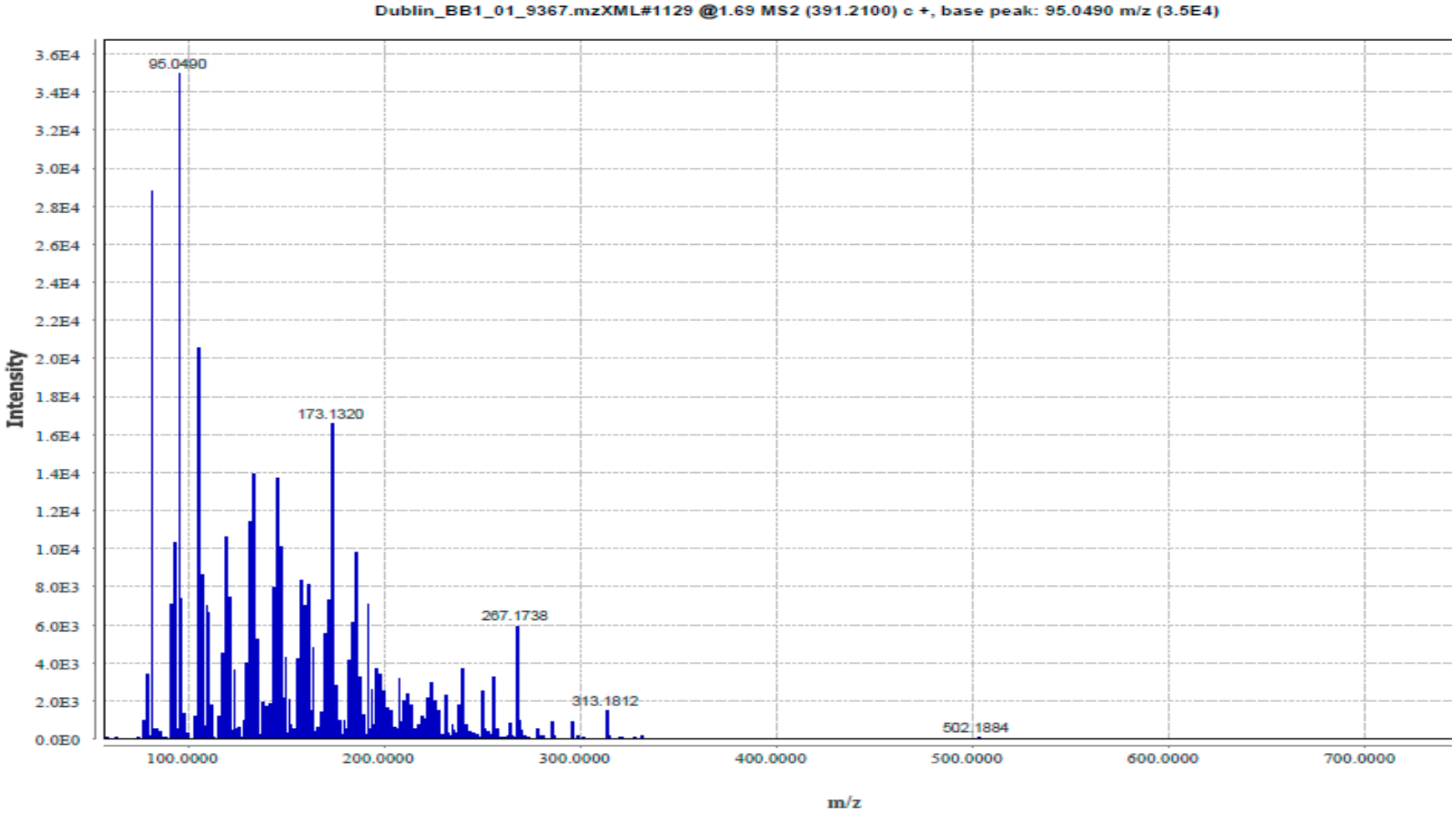
Spectrum C2: ^{13}C NMR spectrum of compound 3 (Dubiin)

Appendix 14: IR spectrum of dubiin (3)



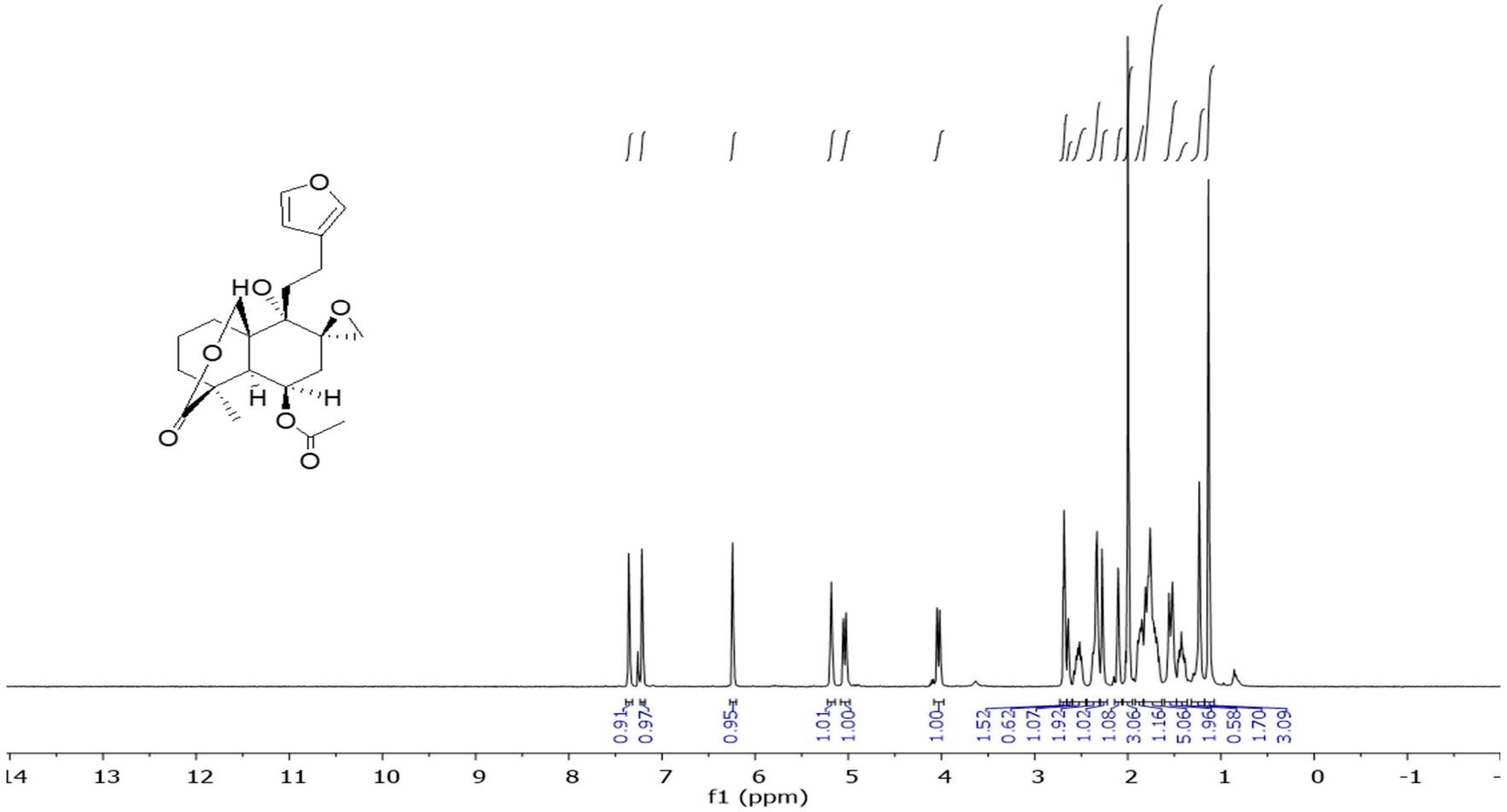
Spectrum C3: IR spectrum of compound 3 (Dubiin)

Appendix 15: MS spectrum of dubiin (3)



Spectrum C4: MS spectrum of compound 3 (Dubiin)

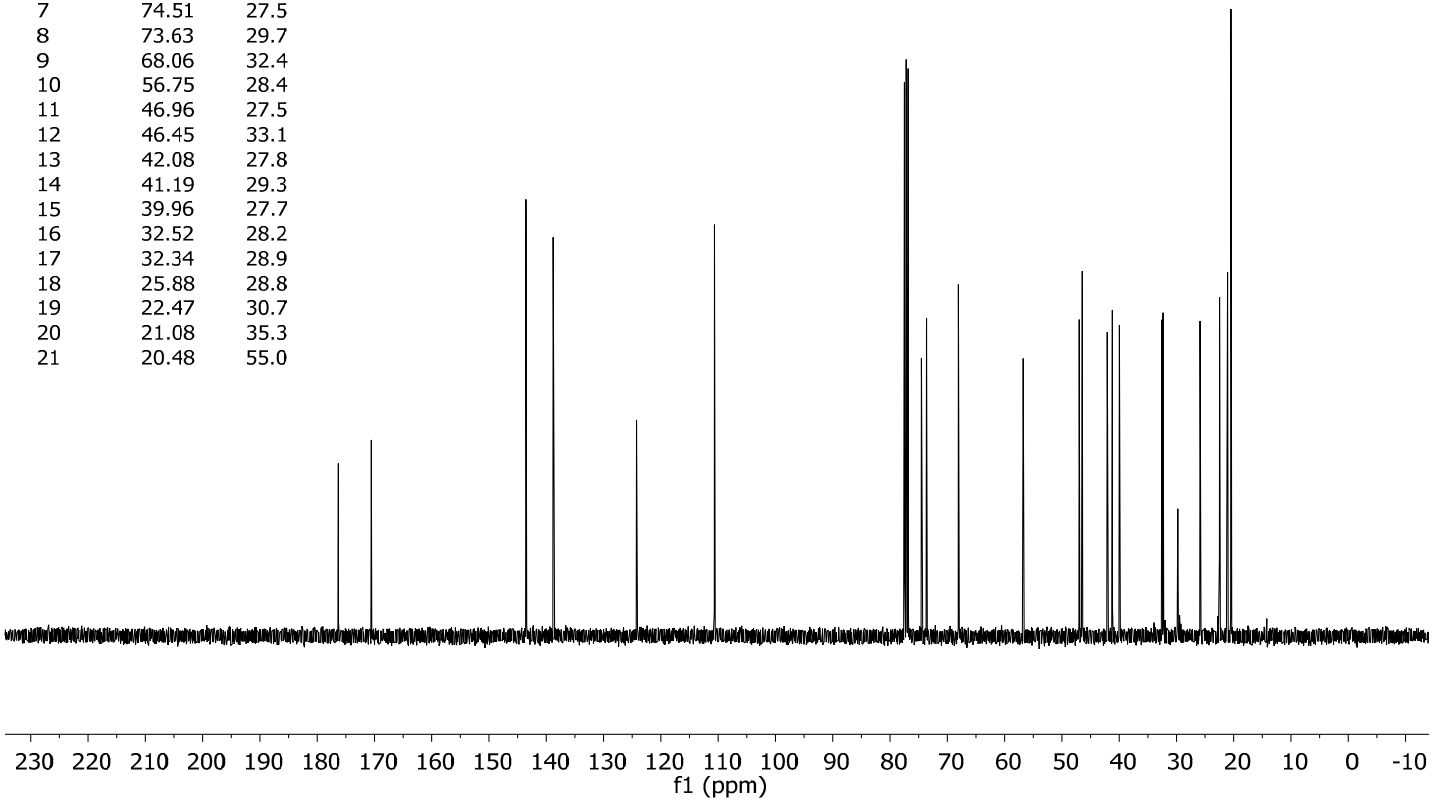
Appendix 16: ^{1}H NMR spectrum of nepetaefuran (4) in $CDCl_3$



Spectrum D1: ^{1}H NMR spectrum of compound 4 (nepetaefuran)

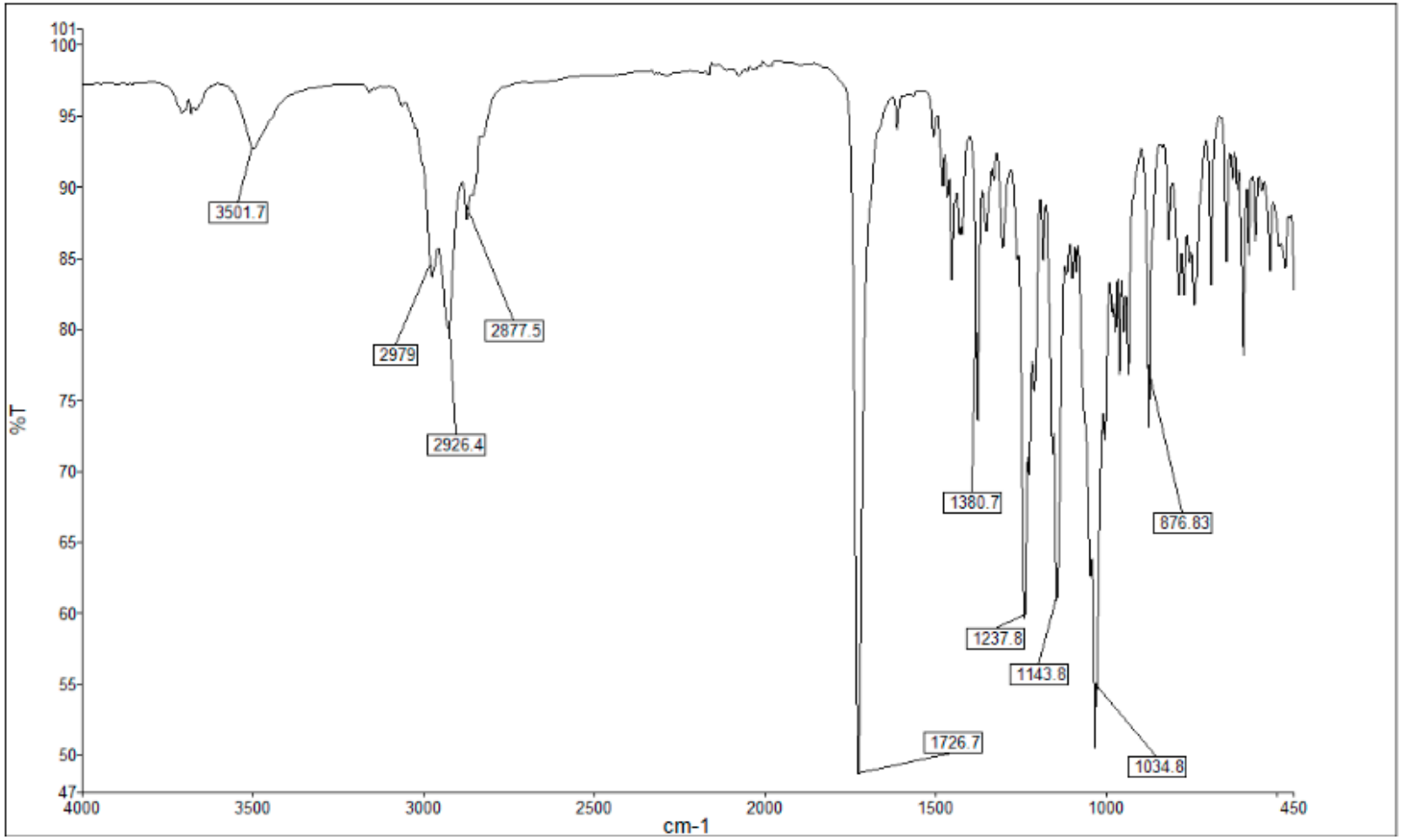
Appendix 17: ^{13}C NMR spectrum of nepetaefuran (4) in $CDCl_3$

	ppm	Intensity
1	176.30	15.4
2	170.55	17.1
3	143.56	38.8
4	138.81	38.3
5	124.24	19.2
6	110.66	36.8
7	74.51	27.5
8	73.63	29.7
9	68.06	32.4
10	56.75	28.4
11	46.96	27.5
12	46.45	33.1
13	42.08	27.8
14	41.19	29.3
15	39.96	27.7
16	32.52	28.2
17	32.34	28.9
18	25.88	28.8
19	22.47	30.7
20	21.08	35.3
21	20.48	55.0



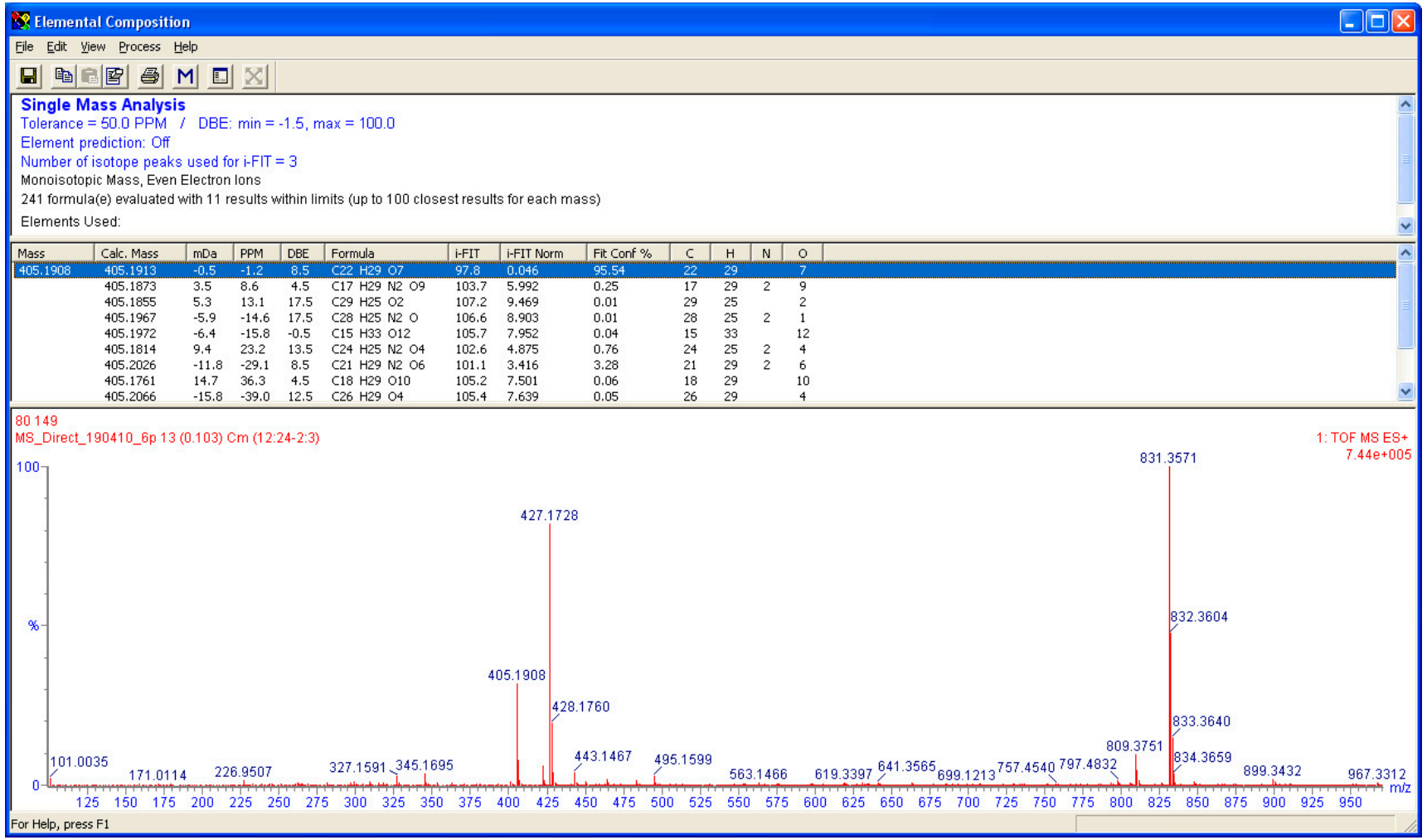
Spectrum D2: ^{13}C NMR spectrum of compound 4 (nepetaefuran)

Appendix 18: IR spectrum of nepetaefuran (4)



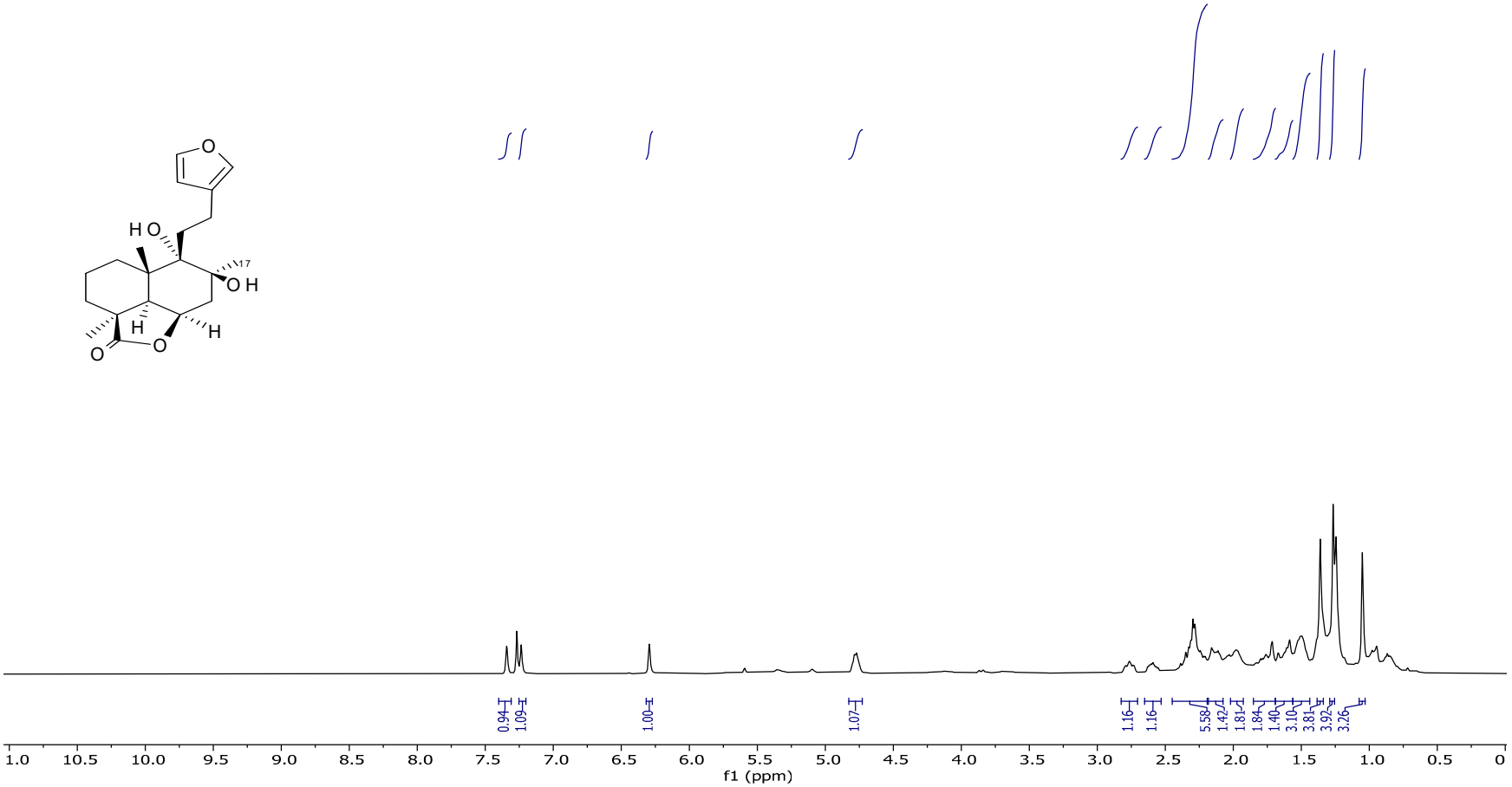
Spectrum D3: IR spectrum of compound 4 (nepetaefuran)

Appendix 19: MS spectrum of nepetaefuran (4)



Spectrum D4: TOF-MS spectrum of compound 4 (nepetaefuran)

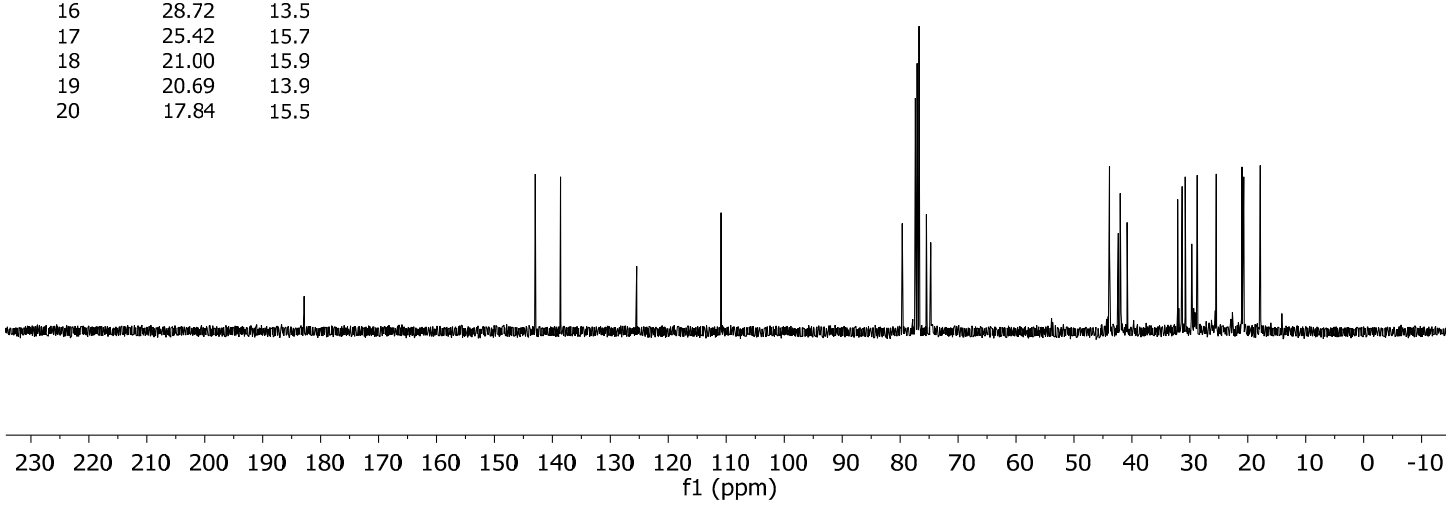
Appendix 20: ^{1}H NMR spectrum of leonotin (5) in $CDCl_3$



Spectrum E1: ^{1}H NMR spectrum of compound 5 (Leonotin)

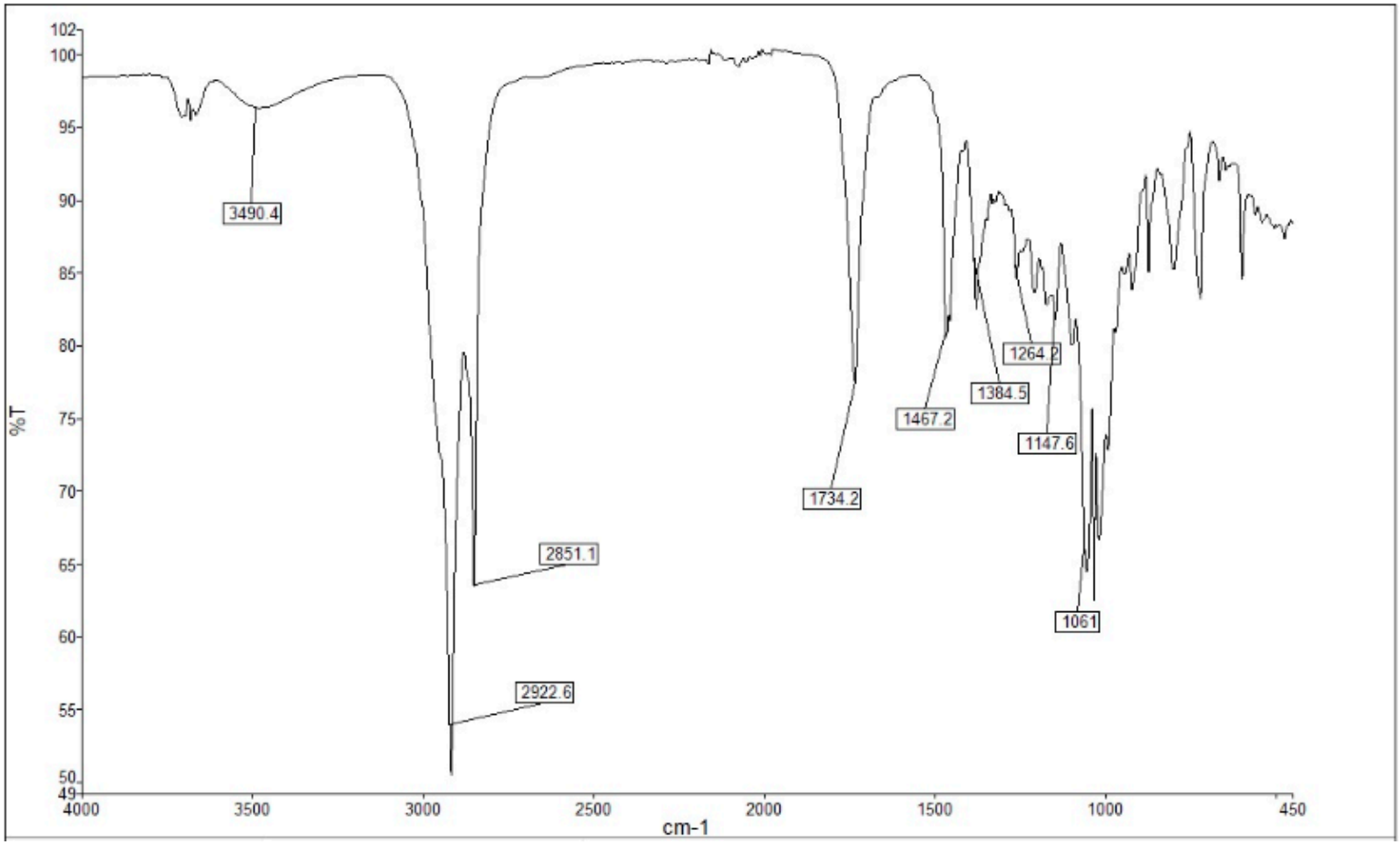
Appendix 21: ^{13}C NMR spectrum of leonotin (5) in $CDCl_3$

	ppm	Intensity
1	182.85	2.9
2	142.97	15.1
3	138.60	15.7
4	125.47	5.9
5	110.91	11.3
6	79.64	10.2
7	75.47	11.0
8	74.70	8.2
9	43.86	14.8
10	42.34	9.3
11	42.01	12.5
12	40.81	10.1
13	32.10	12.5
14	31.33	13.6
15	30.79	14.9
16	28.72	13.5
17	25.42	15.7
18	21.00	15.9
19	20.69	13.9
20	17.84	15.5



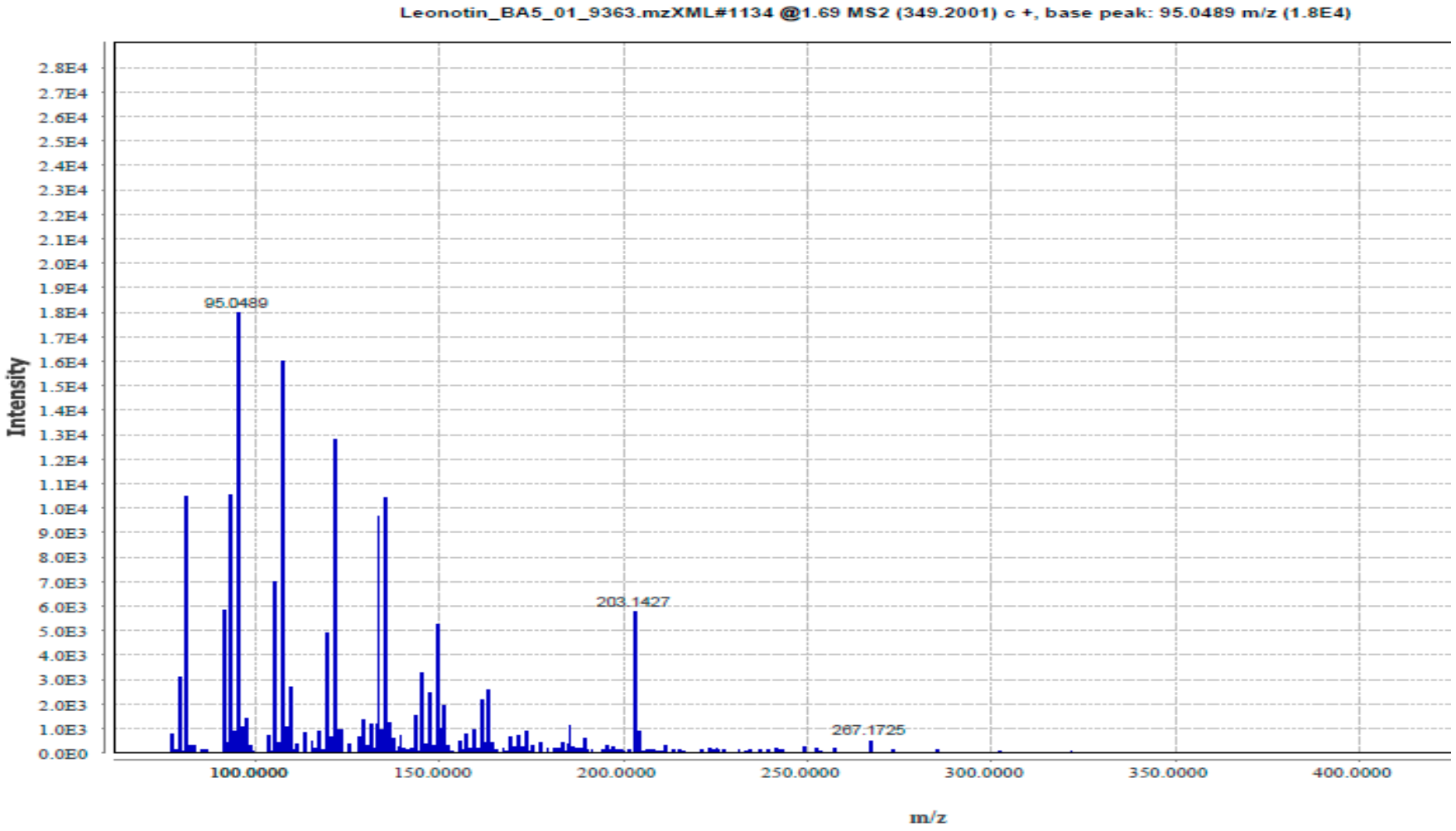
Spectrum E2: ^{13}C NMR spectrum of compound 5 (Leonotin)

Appendix 22: IR spectrum of leonotin (5)



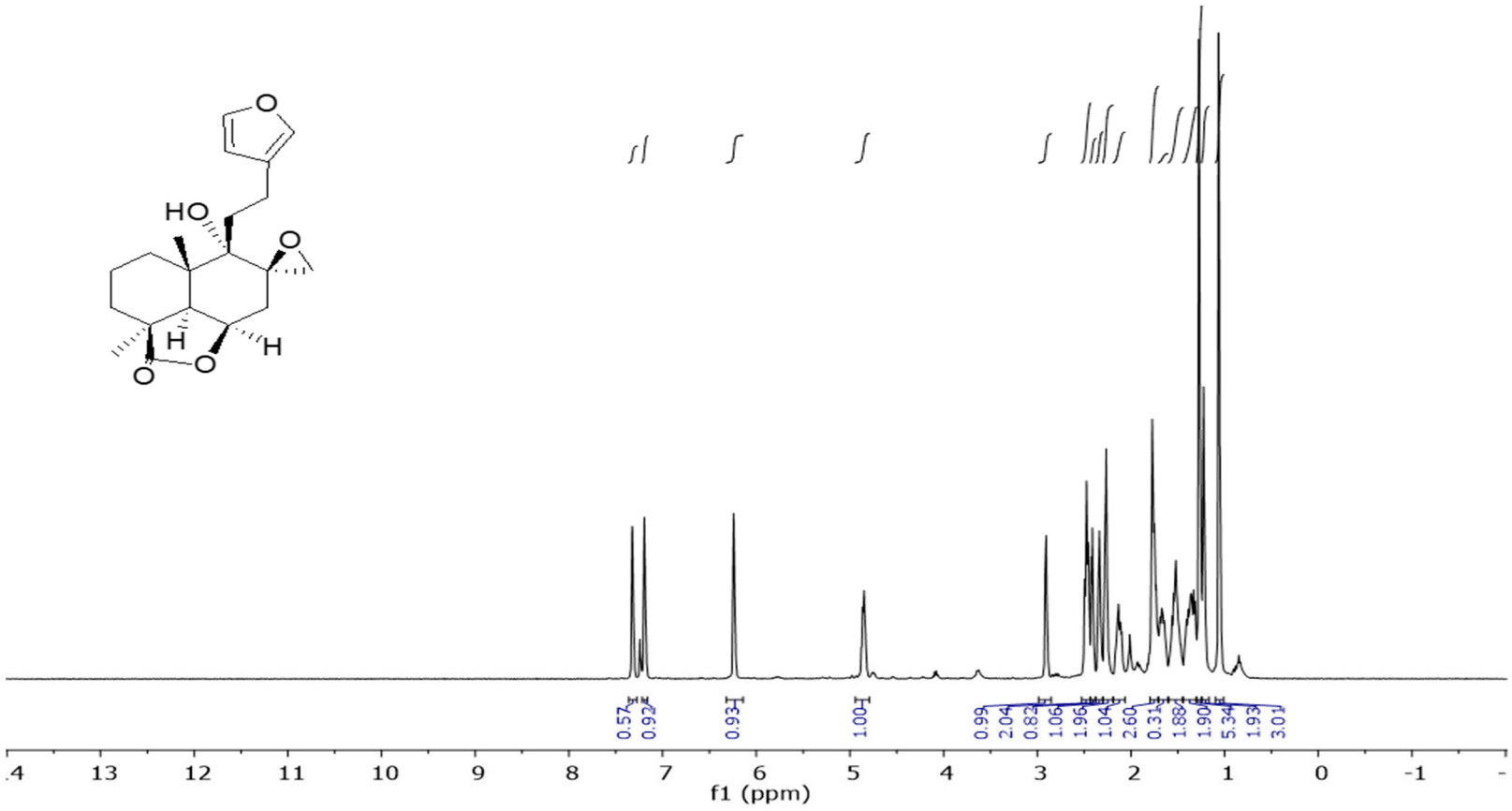
Spectrum E3: IR spectrum of compound 5 (Leonotin)

Appendix 23: MS spectrum of leonotin (5)



Spectrum E4: MS spectrum of compound 5 (Leonotin)

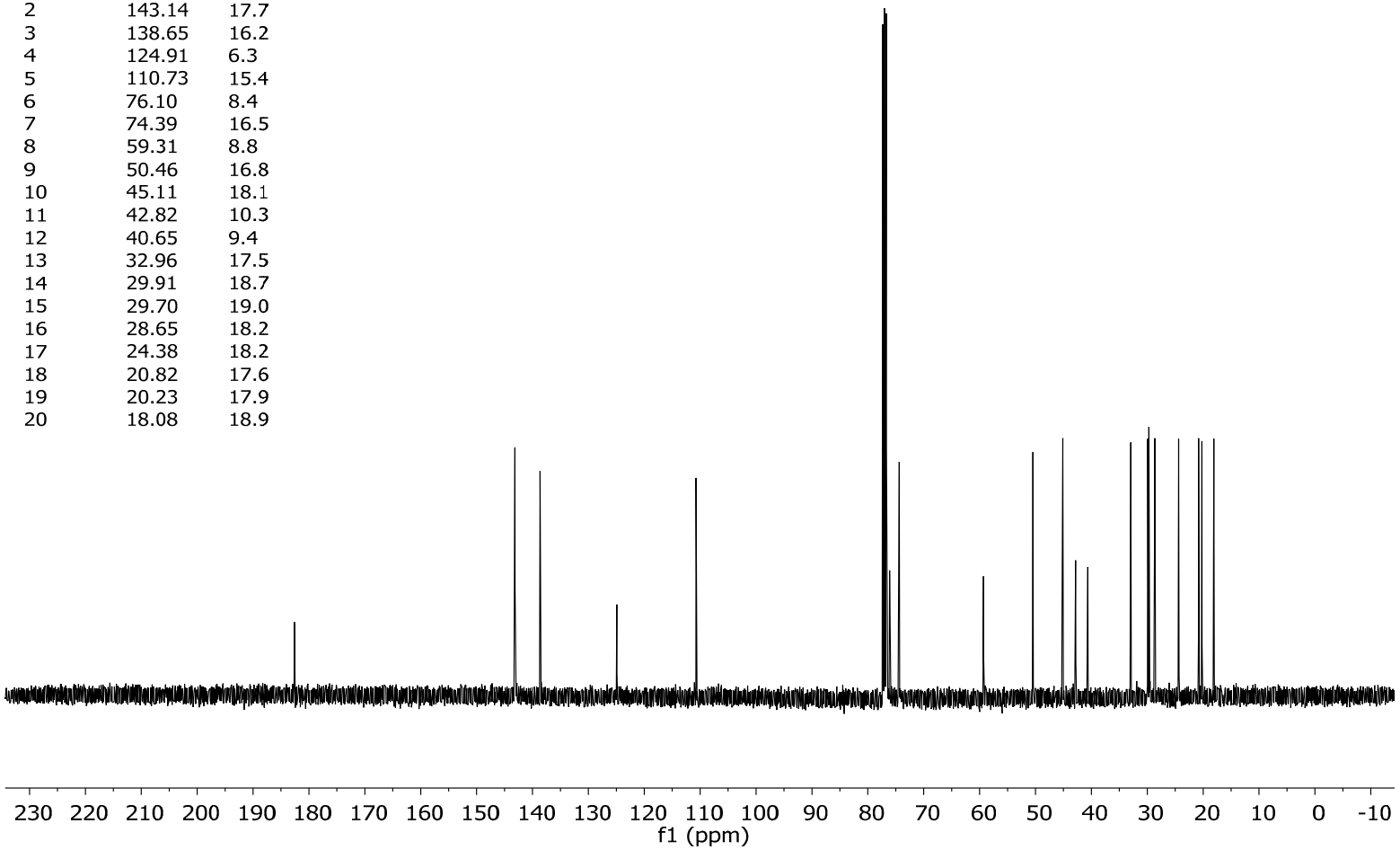
Appendix 24: ^{1}H NMR spectrum of leonotinin (6) in $CDCl_3$



Spectrum F1: ^{1}H NMR spectrum of compound 6 (Leonotinin)

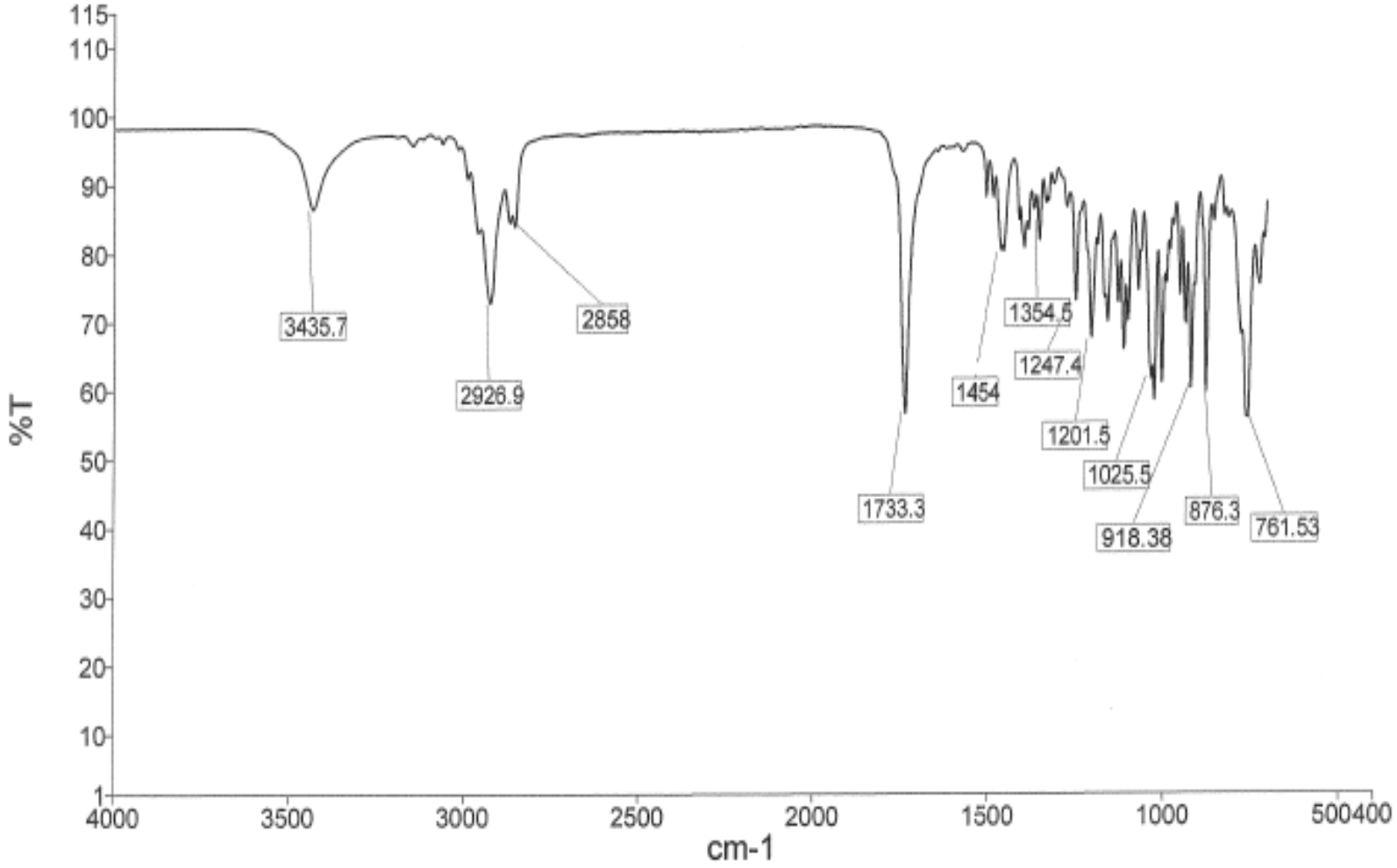
Appendix 25: ^{13}C NMR spectrum of leonotinin (6) in $CDCl_3$

	ppm	Intensity
1	182.60	5.1
2	143.14	17.7
3	138.65	16.2
4	124.91	6.3
5	110.73	15.4
6	76.10	8.4
7	74.39	16.5
8	59.31	8.8
9	50.46	16.8
10	45.11	18.1
11	42.82	10.3
12	40.65	9.4
13	32.96	17.5
14	29.91	18.7
15	29.70	19.0
16	28.65	18.2
17	24.38	18.2
18	20.82	17.6
19	20.23	17.9
20	18.08	18.9



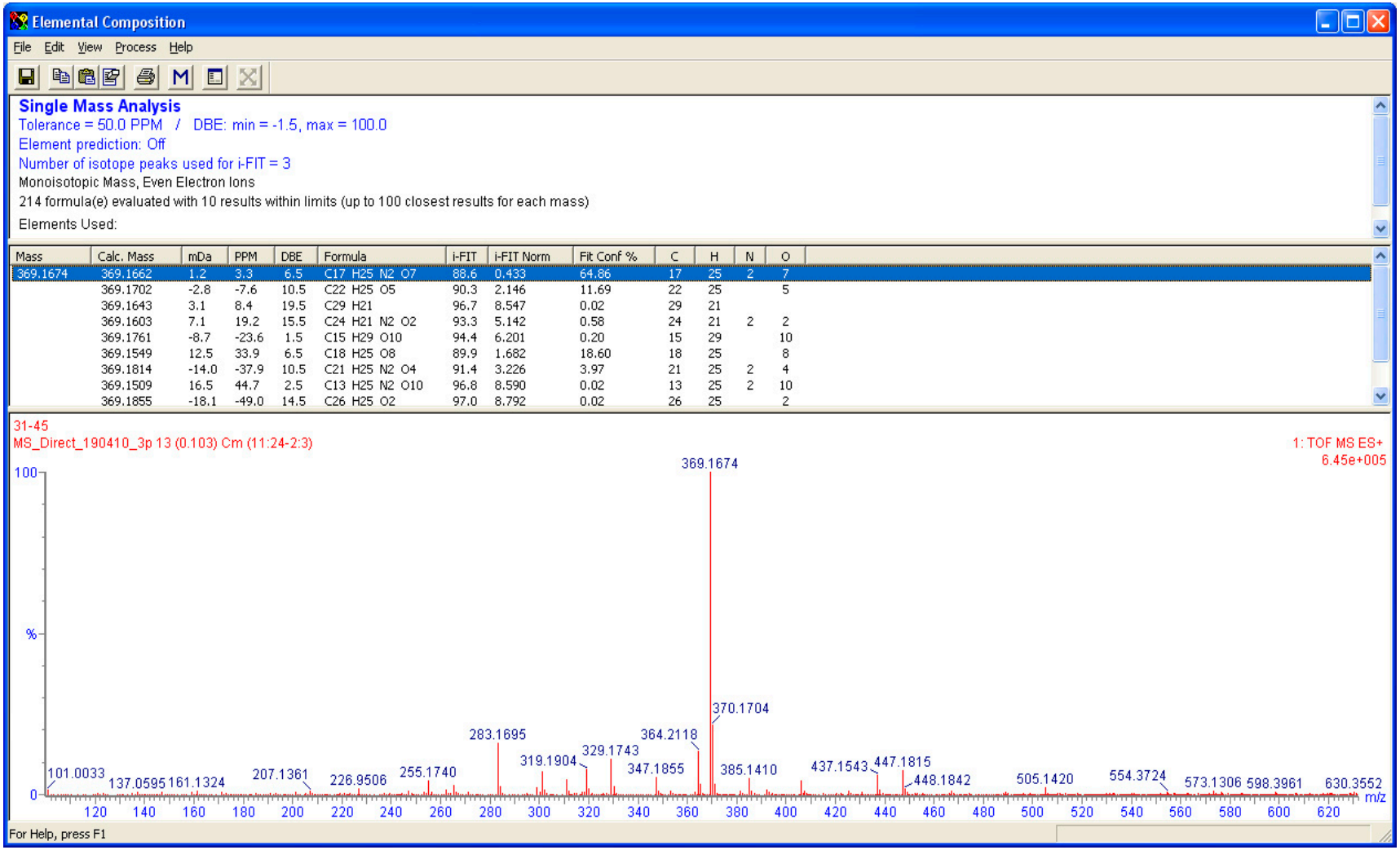
Spectrum F2: ^{13}C NMR spectrum of compound 6 (Leonotinin)

Appendix 26: IR spectrum of leonotinin (6)



Spectrum F3: IR spectrum of compound 6 (Leonotinin)

Appendix 27: MS spectrum of leonotinin (6)



Spectrum F4: MS spectrum of compound 6 (Leonotinin)

Table S1 ^{13}C (δ_c) (100.6 MHz) NMR data for compounds **1-6** in $CDCl_3$.

NO.	1	2	3	4	5	6
1	33.07	33.29	32.86	32.52	31.44	29.85
2	20.32	20.35	20.39	20.48	17.95	18.22
3	39.69	39.61	39.71	39.96	28.83	28.79
4	40.77	40.74	41.08	42.08	42.45	42.96
5	46.98	46.91	46.76	46.45	43.97	45.25
6	67.97	67.92	68.36	68.06	74.81	74.53
7	32.43	32.47	33.32	32.34	42.12	33.10
8	56.44	56.07	30.16	56.75	75.58	59.45
9	85.92	85.95	74.87	74.51	79.75	76.24
10	41.02	41.03	40.96	41.19	40.92	40.79
11	23.63	23.54	35.04	25.88	32.21	30.05
12	37.46	37.51	20.49	20.36	20.80	20.37
13	92.41	92.73	124.42	124.24	125.58	125.05
14	107.01	105.50	110.51	110.66	111.01	110.87
15	149.43	149.45	143.21	143.56	143.08	143.29
16	80.07	81.11	138.54	138.81	138.71	138.79
17	47.53	47.16	15.60	46.96	30.90	50.60
18	22.21	22.21	22.37	22.47	25.53	24.52
19	175.91	175.85	175.95	176.29	182.96	182.75
20	73.90	73.80	75.88	73.63	21.10	20.96
21	170.33	170.34	170.21	170.55		
22	20.95	20.95	21.01	21.08		

Table S2: ^{1}H (δ_H, *mult*, (*J*, Hz) NMR (400 MHz) data for compounds **1-6** in $CDCl_3$.

	1	2	3	4	5	6
1α	1.80 (1H, m)	1.76 (1H, m)	1.88 (1H, m)	1.86 (1H, m)	1.50 (1H, m)	1.52 (1H, m)
1B	1.73 (1H, m)	1.72 (1H, m)	1.78 (1H, m)	1.74 (1H, m)	1.39 (1H, m)	1.33 (1H, m)
2A	1.80 (1H, m)	1.76 (1H, m)	1.77 (1H, m)	1.76 (2H, m)	1.63 (1H, m)	1.51 (1H, m)
2B	1.80 (1H, m)	1.75 (1H, m)	1.77 (1H, m)		1.51 (1H, m)	1.67 (1H, m)
3A	1.54 (1H, m)	1.50 (1H, m)	1.5 (1H, m)	1.50 (1H, m)	1.35 (1H, m)	1.37 (1H, m)
3B	1.84 (1H, m)	1.81 (1H, m)	1.83 (1H, m)	1.82 (1H, m)	2.12 (1H, m)	2.13 (1H, m)
4	-	-	-	-	-	-
5	2.01 (1H, d, *J* = 3.6 Hz)	2.00 (1H, d, *J* = 3.5 Hz)	2.06 (1H, m)	2.11 (1H, d, *J* = 3.3 Hz)	2.29 (1H, d, *J* = 6.1 Hz)	2.33 (1H, d, *J* = 4.9 Hz)
6	5.18 (1H, d, *J* = 3.4 Hz)	5.12 (1H, t, *J* = 3.3 Hz)	5.15 (1H, q, *J* = 3.3 Hz)	5.16 (1H, q, *J* = 3.2 Hz)	4.78 (1H, dt, *J* = 8.8, 6.2 Hz)	4.85 (1H, dt, *J* = 7.5, 5.0 Hz)
7A	2.63 (1H, dd, *J* = 15.4, 3.4 Hz)	2.65 (1H, (dd, *J* = 15.2, 3.4 Hz)	1.75 (1H, m)	2.64 (1H, d, *J* = 3.2 Hz)	2.34 (1H, m)	2.27 (2H, brd, *J* = 5.23 Hz)
7B	1.59 (1H, dd, *J* = 15.4, 3.4 Hz)	1.61 (1H, dd, *J* = 15.2, 3.4 Hz)	1.71 (1H, m)	1.52 (1H, dd, *J* = 15.6, 2.9 Hz)	2.34 (1H, m)	
8	-	-	2.12 (1H, dt, *J* = 12.1, 5.4 Hz)	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11A	1.45 (1H, ddd, *J* = 14.0, 9.6, 4.9 Hz)	1.44 (1H, m)	1.89 (1H, m)	1.43 (1H, ddd, *J* = 14.9, 9.8, 4.9 Hz)	1.76 (1H, m)	1.75 (2H, brd, *J* = 8.3 Hz)
11B	1.76 (1H, m)	1.79 (1H, m)	1.74 (1H, m)	1.68 (1H, m)	2.25 (1H, m)	
12A	1.95 (1H, m)	1.94 (1H, m)	2.44 (1H, tt, *J* = 20.2, 10.0 Hz)	2.34 (1H, m)	2.59 (1H, ddd, *J* = 15.0, 10.5, 5.8 Hz)	2.47 (2H, t, *J* = 8.0 Hz)
12B	2.18 (1H, m)	2.16 (1H, m)	2.44 (1H, tt, *J* = 20.2, 10.0 Hz)	2.53 (1H, m)	2.77 (1H, ddd, *J* = 15.4, 10.9, 4.7 Hz)	
13	-	-	-	-	-	-
14	4.88 (1H, d, *J* = 2.7 Hz)	5.01 (1H, d, *J* = 2.5 Hz)	6.25 (1H, brs)	6.24 (1H, m)	6.3 (1H, brs)	6.24 (1H, brs)
15	6.48 (1H, d, *J* = 2.7 Hz)	6.52 (1H, d, *J* = 2.6 Hz)	7.35 (1H, t, *J* = 1.70 Hz)	7.36 (1H, t, *J* = 1.8 Hz)	7.34 (1H, t, *J* = 1.6 Hz)	7.32 (1H, d, *J*= 1.9 Hz)
16A	4.03 (1H, d, *J* = 10.6 Hz)	3.95 (1H, d, J = 10.4 Hz)	7.22 (1H, s)	7.22 (1H, brs)	7.24 (1H, brs)	7.19 (1H, brs)
16B	4.43 (1H, d, *J* = 10.6 Hz)	4.17 (1H, d, *J* = 10.41 Hz)				

17A	2.34 (1H, d, *J* = 3.9 Hz)	2.30 (1H, d, *J* = 3.9 Hz)	0.97 (3H, d, *J* = 5.7 Hz)	2.33 (1H, d, *J* = 3.8 Hz)	1.36 (3H, s)	2.41 (1H, d, *J* = 4.6 Hz)
17B	2.67 (1H, d, *J* = 3.9 Hz)	2.65 (1H, d, *J* = 3.9 Hz)		2.68 (1H, d, *J* = 3.8 Hz)		2.9 (1H, d, *J* = 4.7 Hz)
18	1.07 (3H, s)	1.08 (3H, s)	1.25 (3H, s)	1.11 (3H, s)	1.27 (3H, s)	1.27 (3H, s)
19	-	-	-	-	-	-
20A	3.97 (1H, d, *J* = 11.7 Hz)	3.93 (1H, d, *J* = 11.6 Hz)	4.26 (1H, d, *J* = 11.2 Hz)	4.03 (1H, d, *J* = 11.7 Hz)	1.05 (3H, s)	1.06 (3H, s)
20B	5.10 (1H, d, *J* = 11.7 Hz)	5.02 (1H, d, *J* = 11.6 Hz)	4.68 (1H, d, *J* = 11.2 Hz)	5.01 (1H, d, *J* = 11.8 Hz)		
21	-	-	-	-		
22	1.98 (3H, s)	1.96 (3H, s)	2.02 (3H, s)	1.97 (3H, s)		