Two New Troponoides with Anti-inflammatory Activity from the Stems of *Juniperus formosana* Hayata

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ABSTRACT: Two new troponoides (**1**–**2**) were isolated from a 95% ethanol extract of the stems of *Juniperus formosana* (Cupressaceae), together with six known compounds (**3**–**8**). The structures of the new compounds were comprehensively characterized by high resolution electrospray ionization-mass spectrometry (HR-ESI-MS), 1D and 2D nuclear magnetic resonance (NMR). All isolated compounds were evaluated for their anti-inflammatory against the expression of IL-1β, IL-6 and TNF-α in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. The new compounds showed moderate anti-inflammatory effect, while other compounds did show no activity.

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Content   Page

</div>

Table S1. ^{1}H (C$_5$D$_5$N, 600 MHz) and ^{13}C NMR (150 MHz) data of the new compounds **1-2**.

No.	1		2	
	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	173.8		174.3	
2	168.8		167.9	
3	120.8	7.54 (1H, s)	127.3	7.33 (1H, br. s)
4	155.5		147.9	
5	138.8		138.0	
6	140.5	7.32 (1H, d, J = 12.1 Hz)	140.0	7.32 (1H, d, J = 12.1 Hz)
7	126.2	7.34 (1H, d, J = 12.1 Hz)	122.9	7.34 (1H, d, J = 12.1 Hz)
1'	35.3	3.44 (2H, br. d, J = 6.8 Hz)	34.9	3.37 (1H, br. d, J = 7.2 Hz)
2'	121.9	5.74 (1H, br. t, J = 6.5 Hz)	122.4	5.76 (1H, br. t, J = 6.9 Hz)
3'	137.7		137.8	
4'	67.2	4.32 (2H, br. s)	67.3	4.32 (2H, br. s)
5'	13.9	1.88 (3H, br. s)	13.9	1.84 (3H, br. s)
1"	32.2	3.24 (1H, spt, J = 6.8 Hz)	151.0	
2"	22.9	1.13 (3H, d, J = 6.8 Hz)	114.5	5.12 (1H, br. s) & 4.84 (1H, br. s)
3"	22.9	1.13 (3H, d, J = 6.8 Hz)	24.1	1.93 (3H, br. s)

Table S2. Cytotoxicity were evaluated by MTT assay in RAW264.7 cells.

	concentration	cell viability	SD
Blank		100.00%	15.52%
LPS	1 μg/ml	98.50%	1.92%
1	20 μM	27.02%	0.69%
2	20 μM	10.52%	1.36%
3	20 μM	90.97%	0.49%
4	20 μM	67.57%	1.44%
5	20 μM	80.38%	1.07%
6	20 μM	95.92%	10.28%
7	20 μM	95.57%	0.28%

The results were showed as means ± SD of at least three independent experiments.

Table S3. The primer gene sequence of TNF-α, IL-6, IL-1β, and GAPDH.

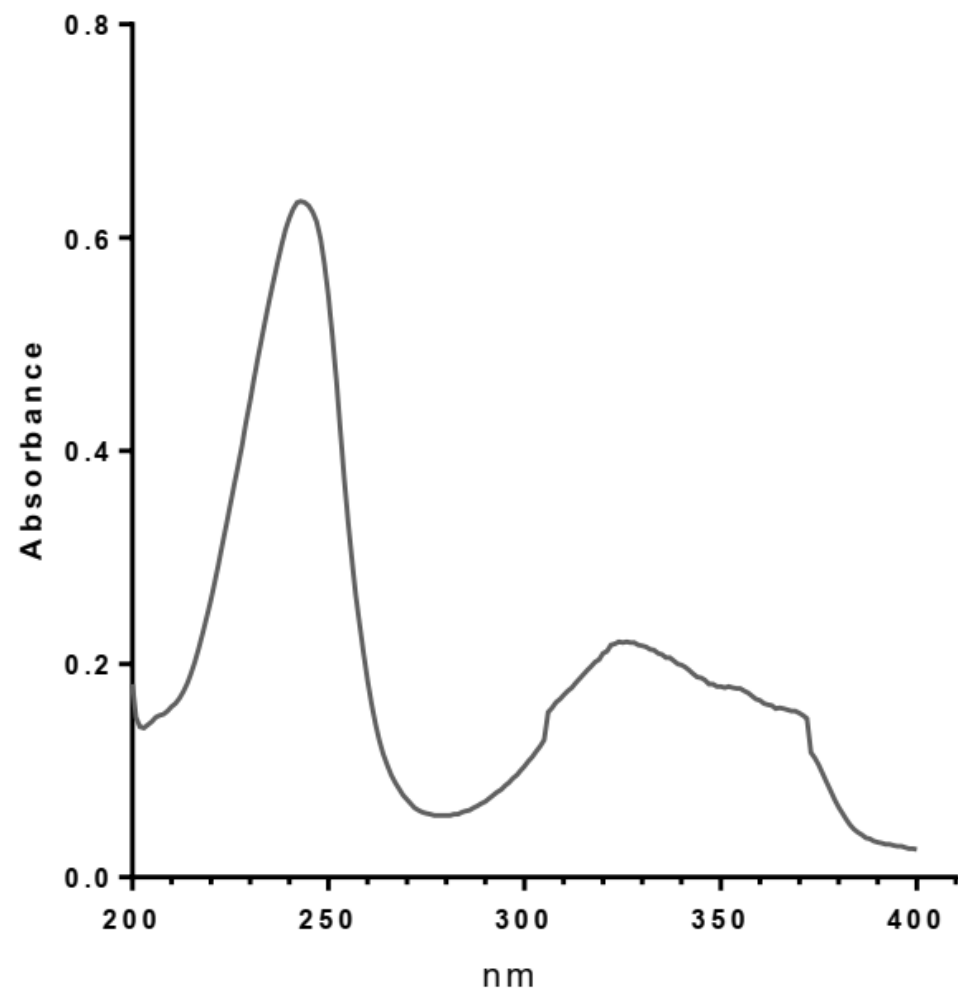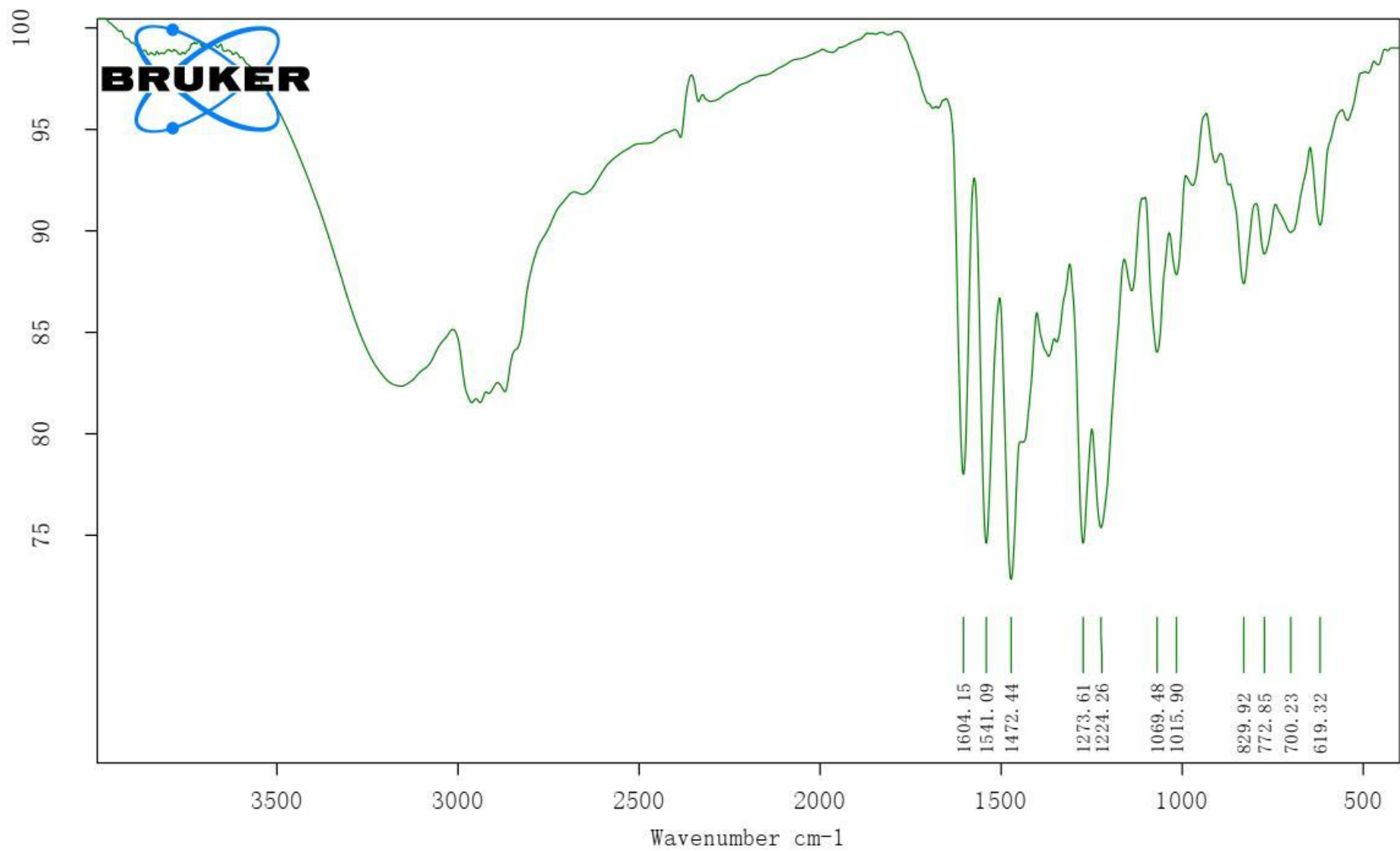| Gene	Sense	Antisense
TNF-α	5'-GAACTGGCAGAAGAGGCACT-3'	5'-AGGGTCTGGGCCATAGAACT-3'
IL-1β	5'-AGAGCATCCAGCTTCAAAT-3'	5'-CATCTCGGAGCCTGTAGTG-3'
IL-6	5'-AGTTGCCTTCTTGGGACTGA-3'	5'-TCCACGATTTCCCAGAGAAC-3'
GAPDH	5'-CCTTCCGTGTTCCTACCC-3'	5'-CAACCTGGTCCTCAGTGTAG-3'

Figure S 1. UV spectrum of compound **1**

Figure S 2. IR spectrum of compound **1**

249.1478 — C15H21O3 (-2.7)

C15H20NaO3 (-2.4)

250.1511

271.1298

272.1331

282.2787

231.1376

497.2884 — 498.2919

519.2703

ESI+

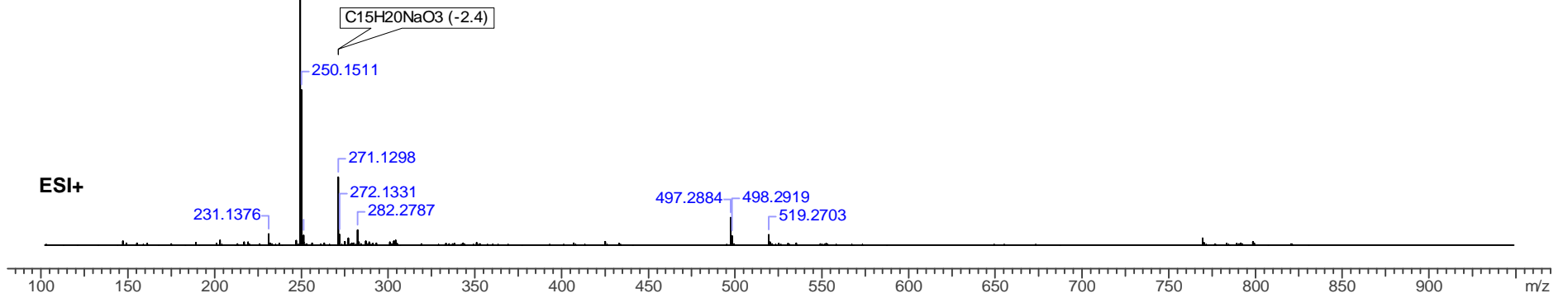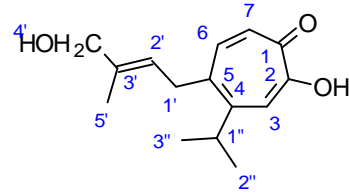100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900   m/z
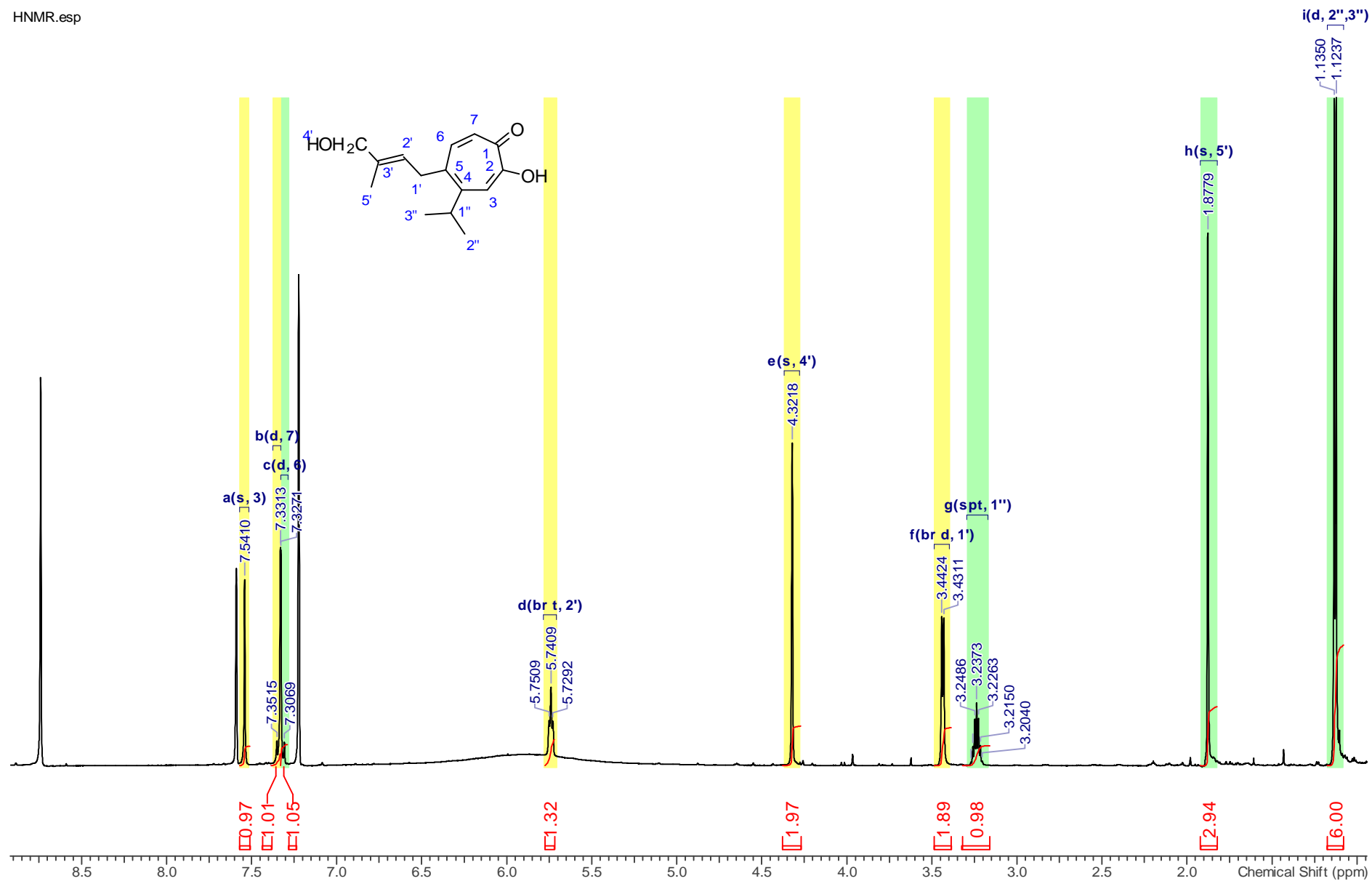
Figure S 3. HR-ESI-MS spectra of compound **1**
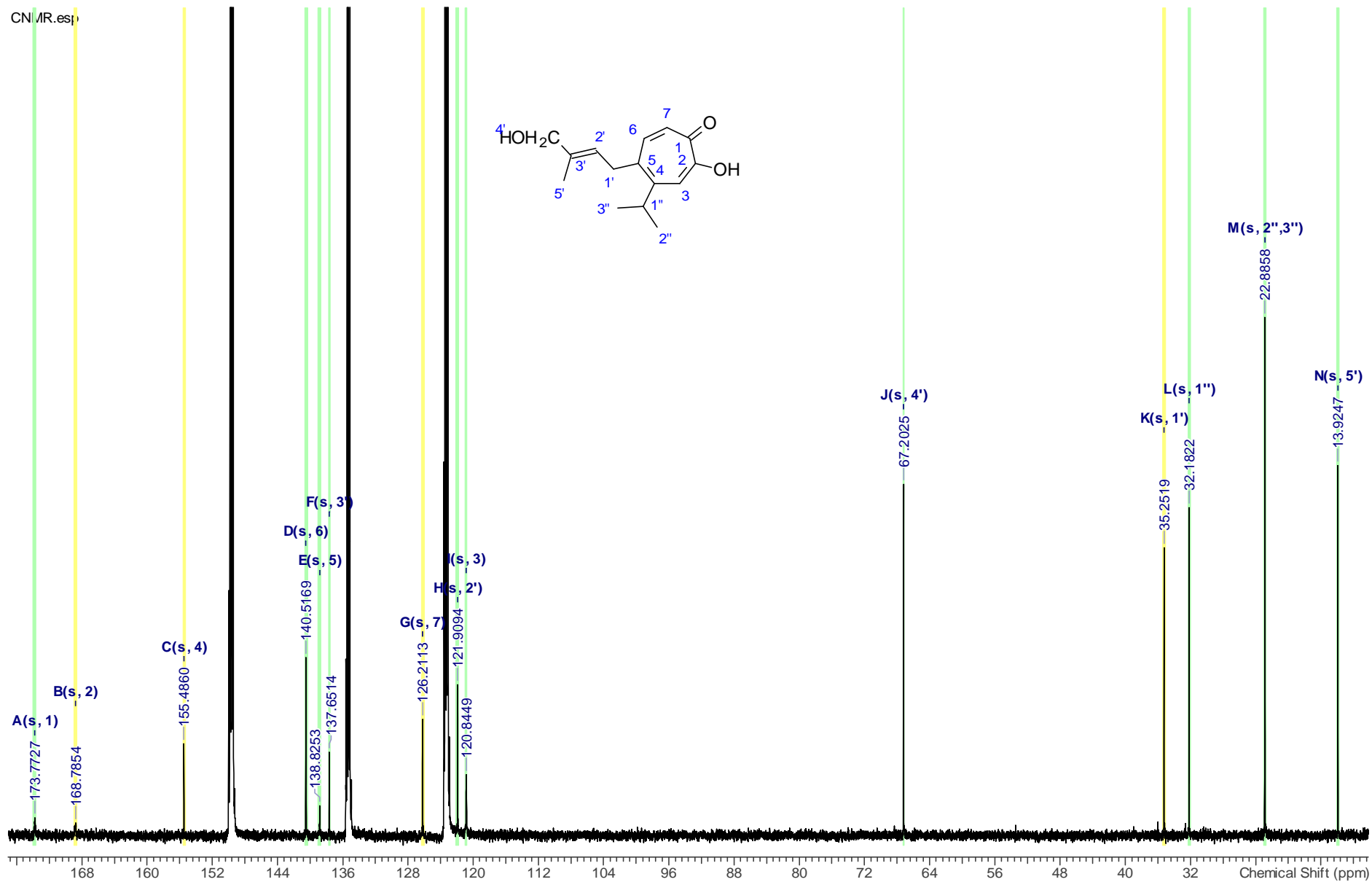
Figure S 4. ^{1}H-NMR spectrum of compound **1**
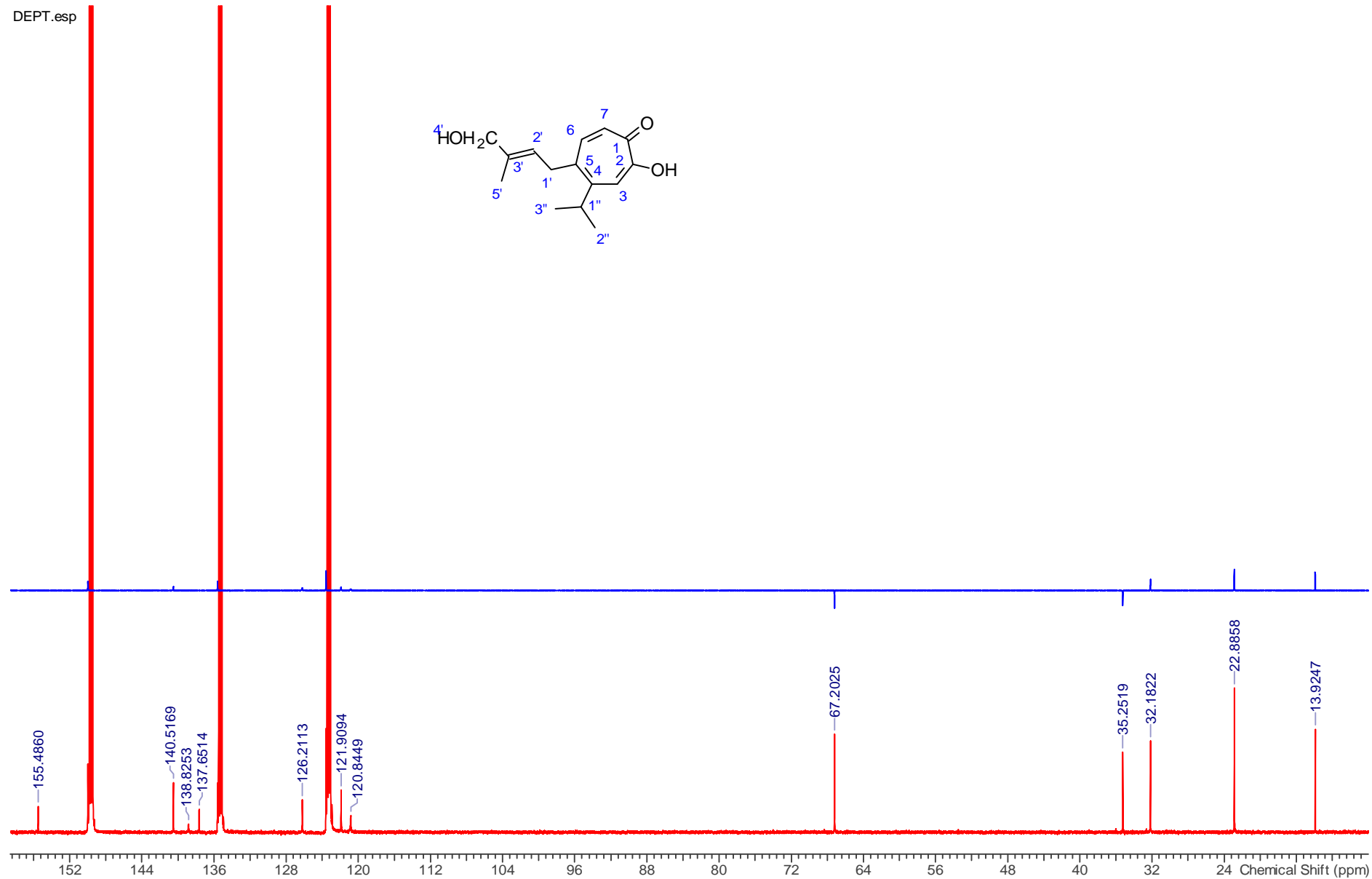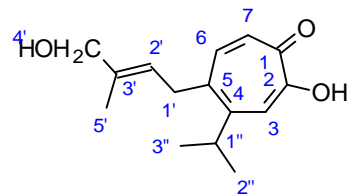
Figure S 5. ^{13}C-NMR spectrum of compound **1**

Figure S 6. DEPT spectrum of compound **1**
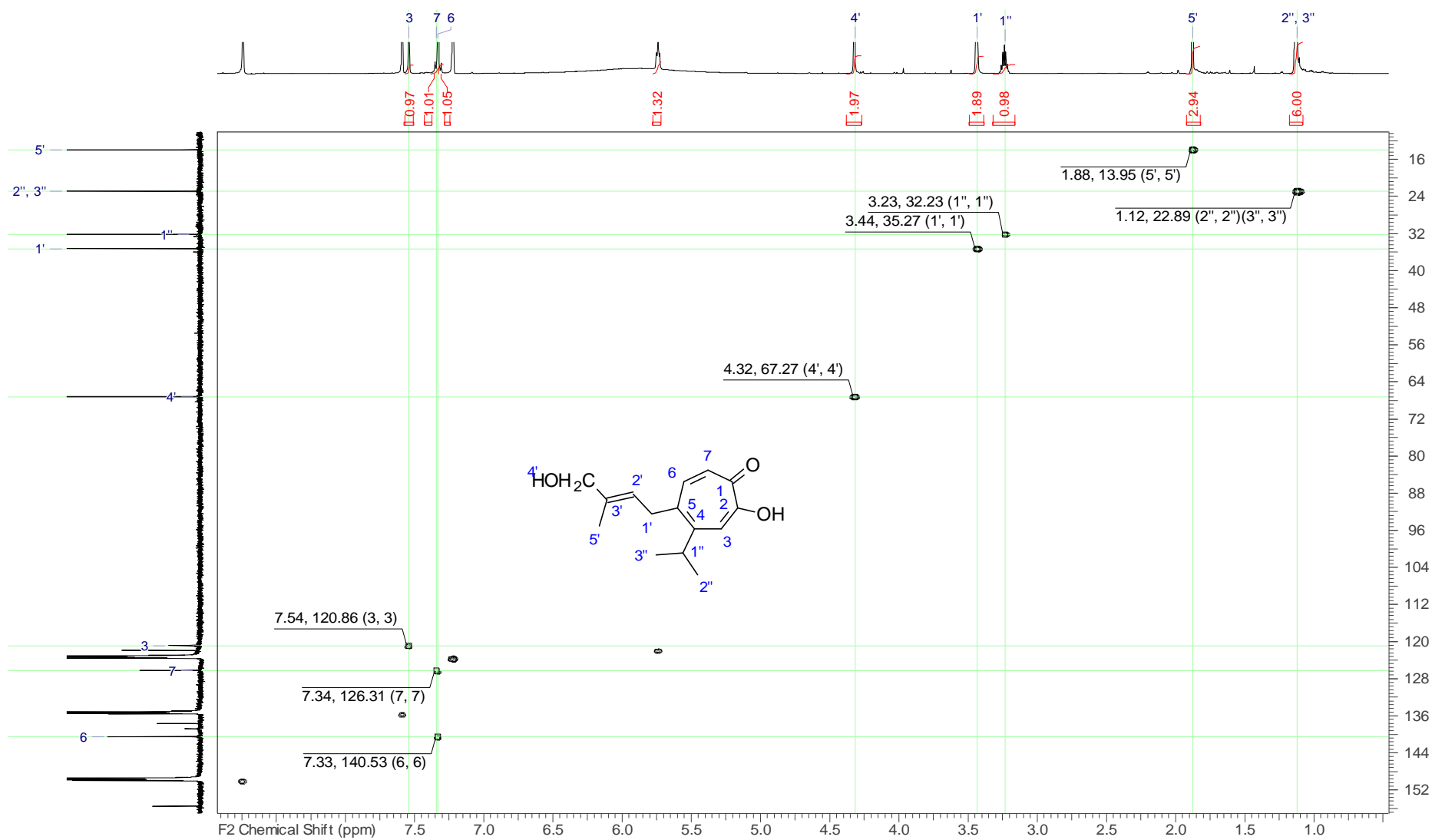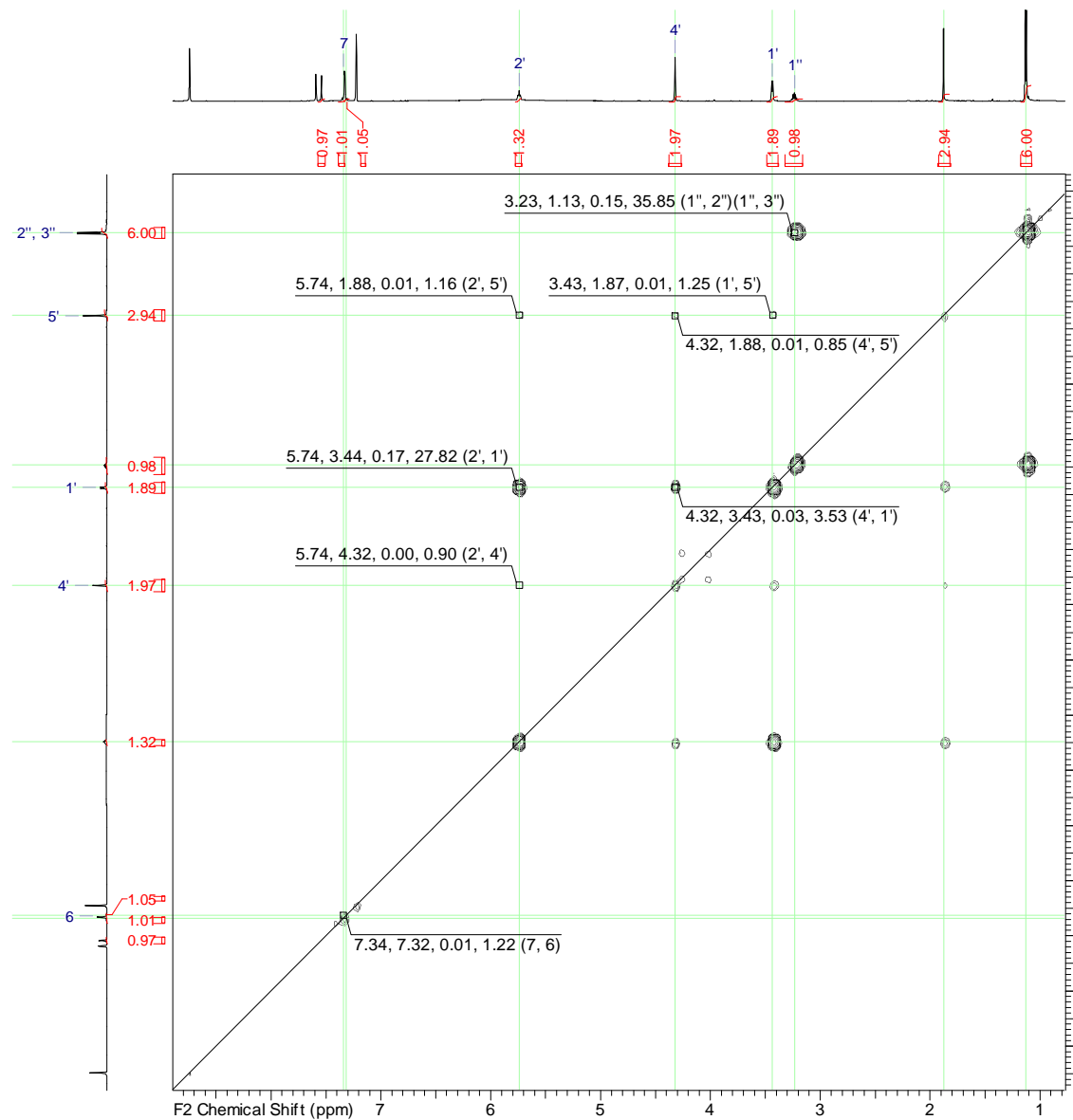
Figure S 7. HSQC spectrum of compound **1**

Figure S 8. ^{1}H-^{1}H COSY spectrum of compound **1**

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Origin   spect
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Owner   nmr
Points Count   (2048, 1024)
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Spectrum Type   COSY
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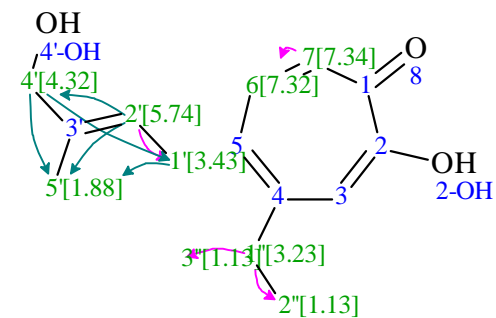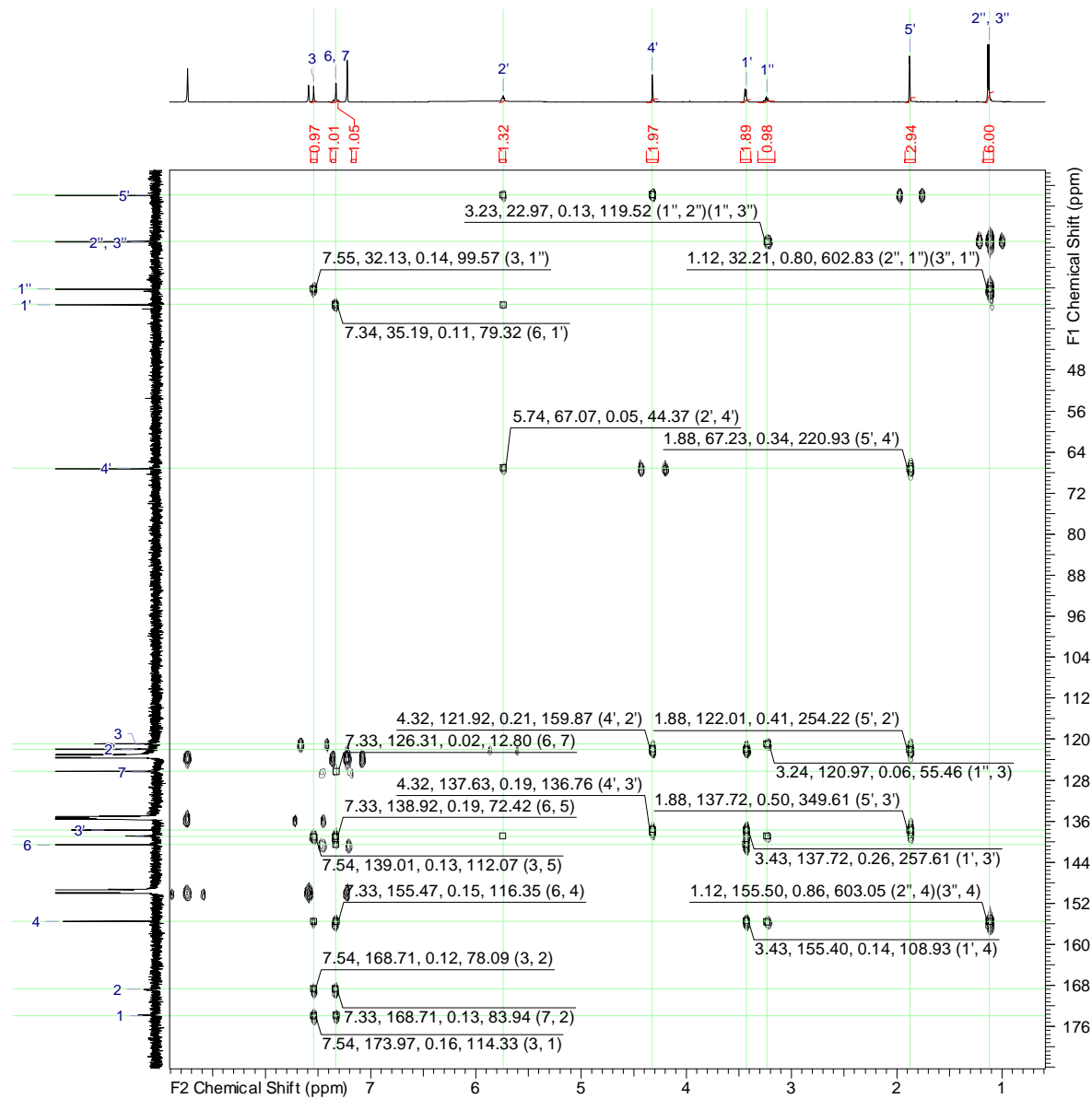3.23, 1.13, 0.15, 35.85 (1", 2")(1", 3")
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3.43, 1.87, 0.01, 1.25 (1', 5')
4.32, 1.88, 0.01, 0.85 (4', 5')
5.74, 3.44, 0.17, 27.82 (2', 1')
4.32, 3.43, 0.03, 3.53 (4', 1')
5.74, 4.32, 0.00, 0.90 (2', 4')
7.34, 7.32, 0.01, 1.22 (7, 6)

13

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Owner: nmr
Points Count: (1024, 1024)
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Solvent: Pyr
Spectrum Type: HMBC
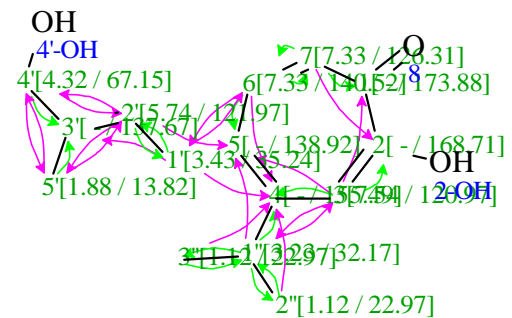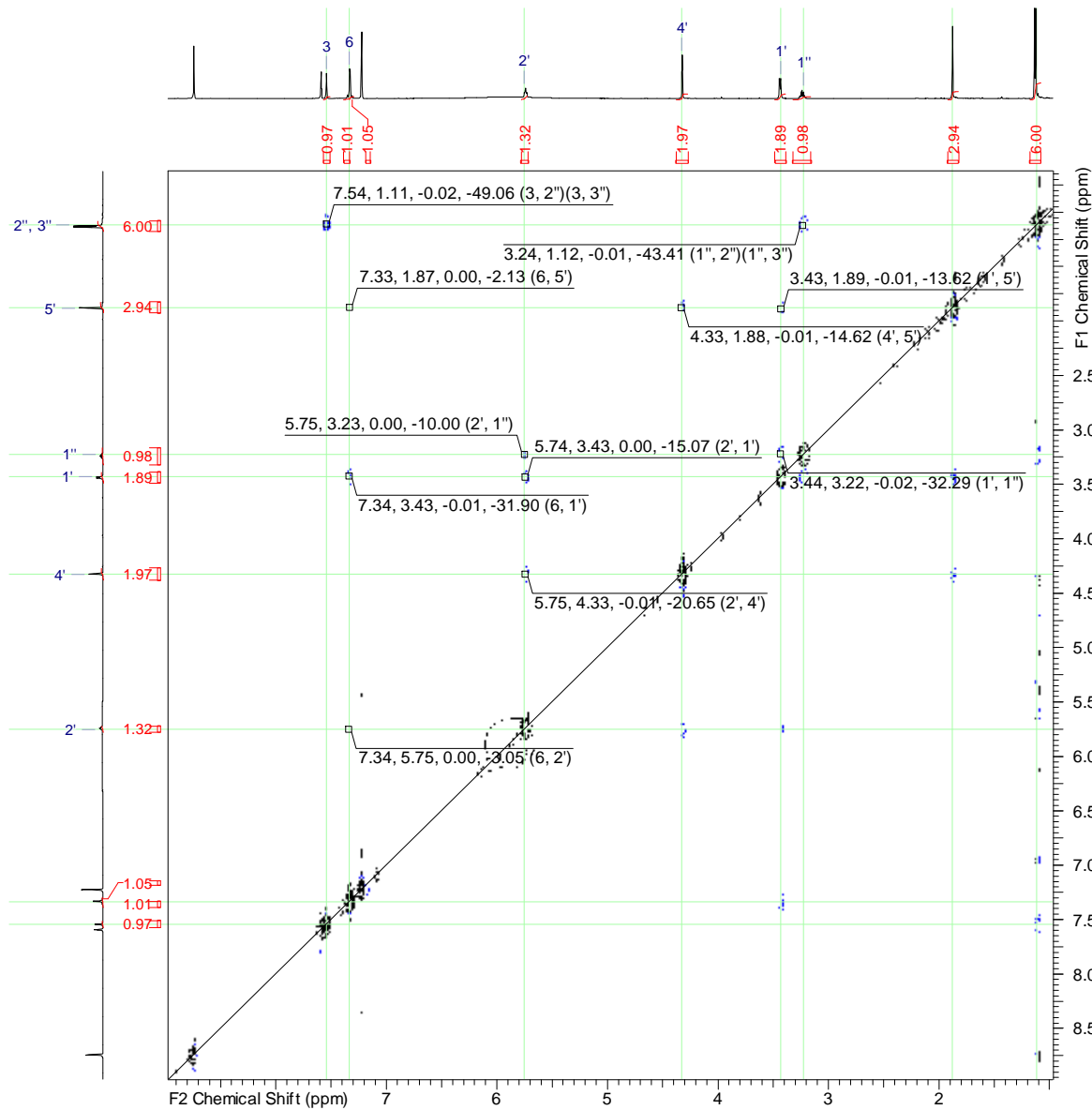Sweep Width (Hz): (6660.16, 33524.28)
Temperature (degree C): 24.040

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1.12, 32.21, 0.80, 602.83 (2", 1")(3", 1")
7.34, 35.19, 0.11, 79.32 (6, 1')
5.74, 67.07, 0.05, 44.37 (2', 4')
1.88, 67.23, 0.34, 220.93 (5', 4')
4.32, 121.92, 0.21, 159.87 (4', 2') 1.88, 122.01, 0.41, 254.22 (5', 2')
7.33, 126.31, 0.02, 12.80 (6, 7)
3.24, 120.97, 0.06, 55.46 (1", 3)
4.32, 137.63, 0.19, 136.76 (4', 3') 1.88, 137.72, 0.50, 349.61 (5', 3')
7.33, 138.92, 0.19, 72.42 (6, 5)
7.54, 139.01, 0.13, 112.07 (3, 5)
3.43, 137.72, 0.26, 257.61 (1', 3')
7.33, 155.47, 0.15, 116.35 (6, 4) 1.12, 155.50, 0.86, 603.05 (2", 4)(3", 4)
3.43, 155.40, 0.14, 108.93 (1', 4)
7.54, 168.71, 0.12, 78.09 (3, 2)
7.33, 168.71, 0.13, 83.94 (7, 2)
7.54, 173.97, 0.16, 114.33 (3, 1)

F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

OH
4'-OH
4'[4.32 / 67.15]
3'[7.67]
5'[1.88 / 13.82]
2'[5.74 / 14.97]
1'[3.43 / 55.54]
7[7.33 / 126.31]
6[7.33 / 140.52]
5[7.54 / 138.92]
3[7.54 / 126.97]
2[- / 168.71]
1[7.54 / 173.88]
1"[3.23 / 32.17]
3"[1.12 / 22.97]
2"[1.12 / 22.97]
OH
O

Figure S 9. HMBC spectrum of compound **1**

Figure S 10. NOESY spectrum of compound **1**

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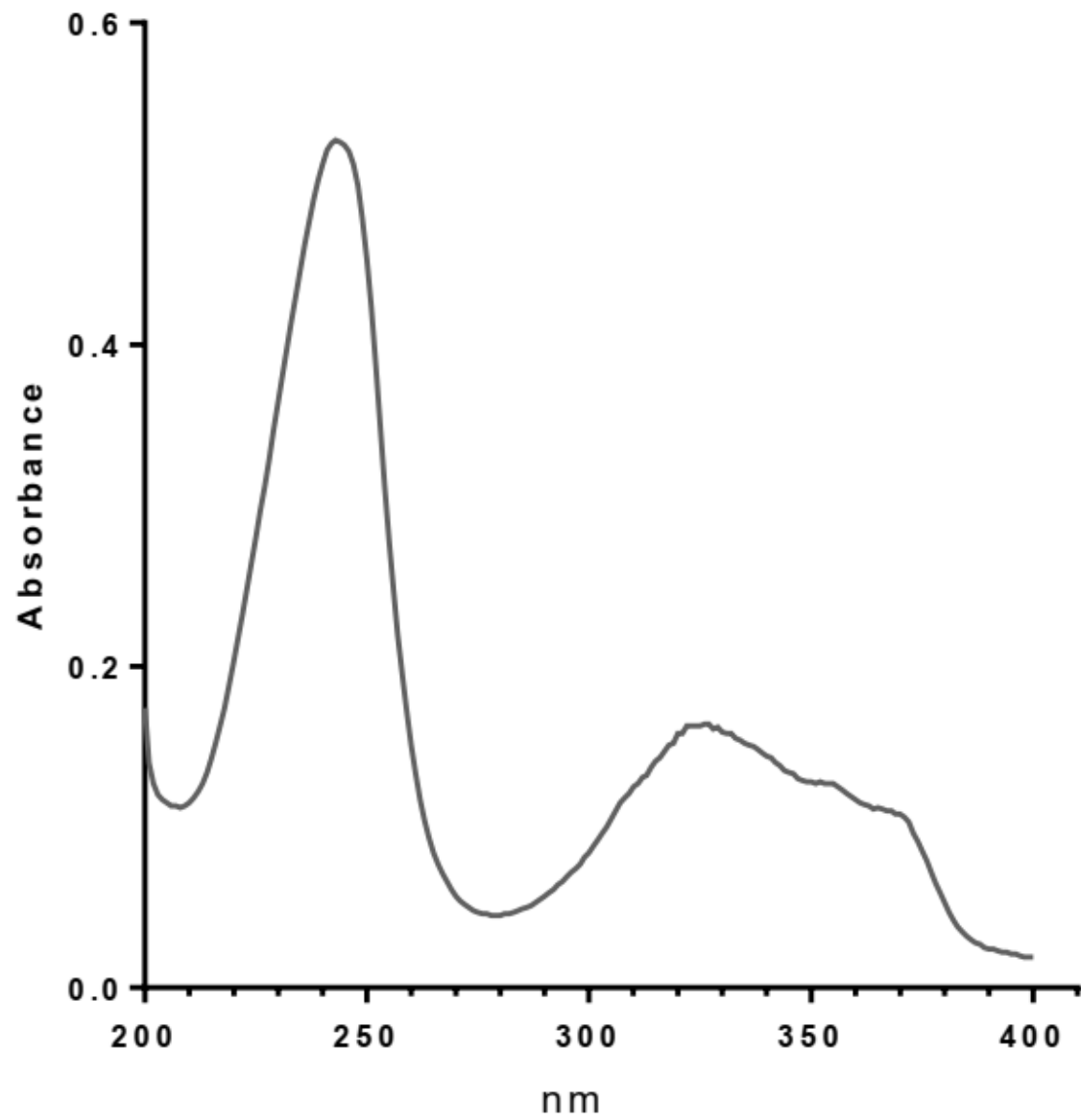Figure S 11. UV spectrum of compound **2**

Figure S 12. IR spectrum of compound **2**
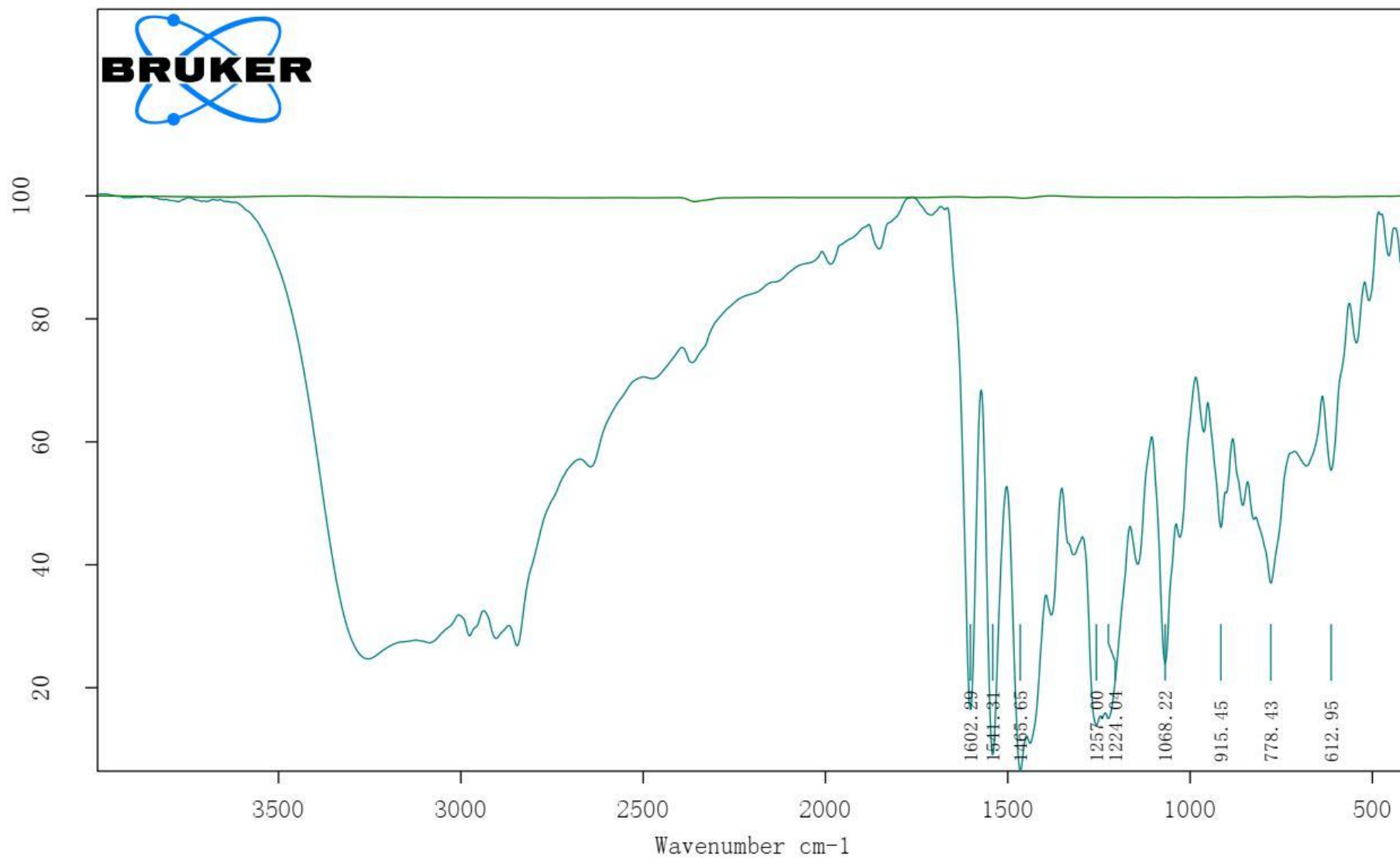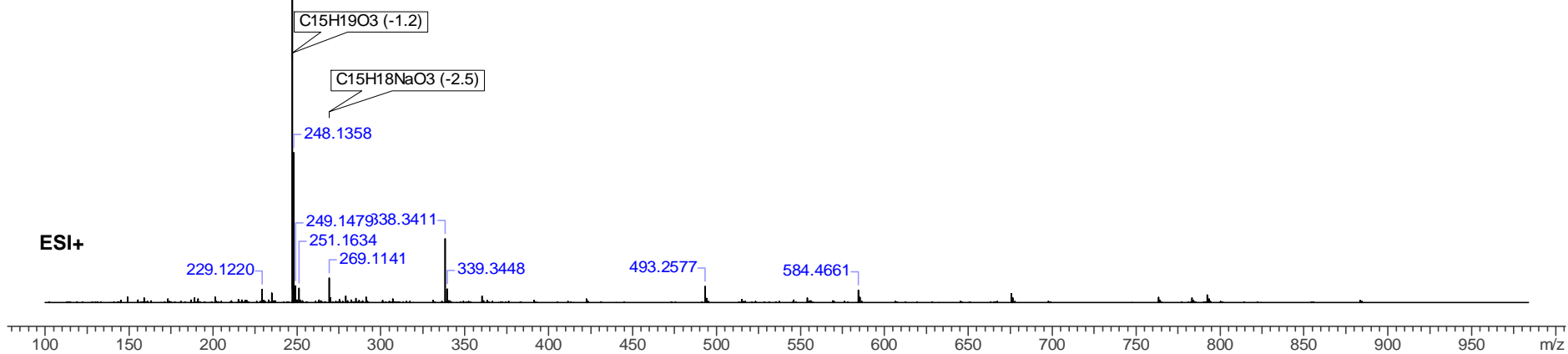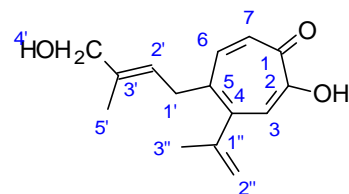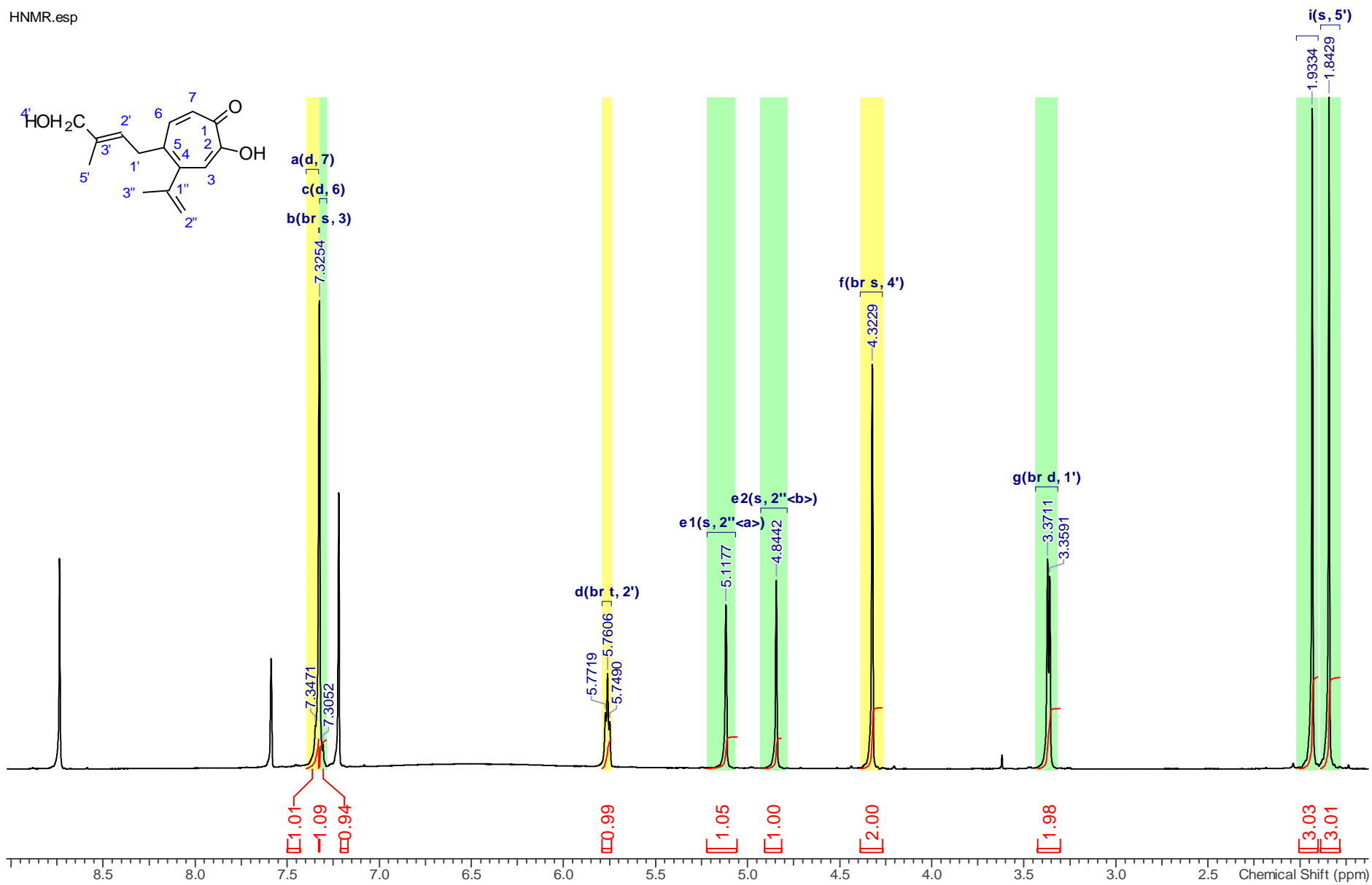
Figure S 13. HR-ESI-MS spectra of compound **2**

Figure S 14. ^{1}H-NMR spectrum of compound **2**
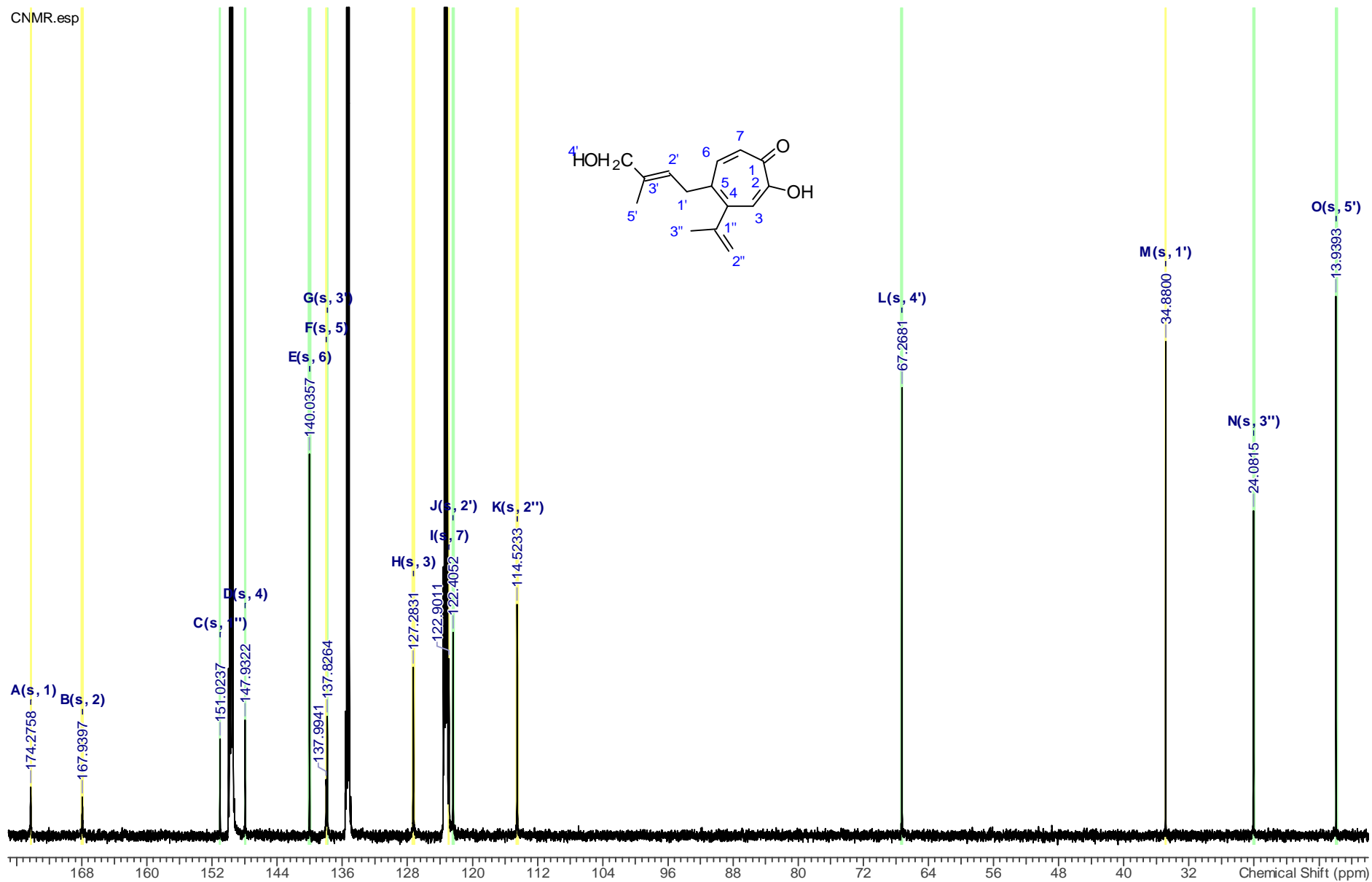
Figure S 15. ^{13}C-NMR spectrum of compound **2**
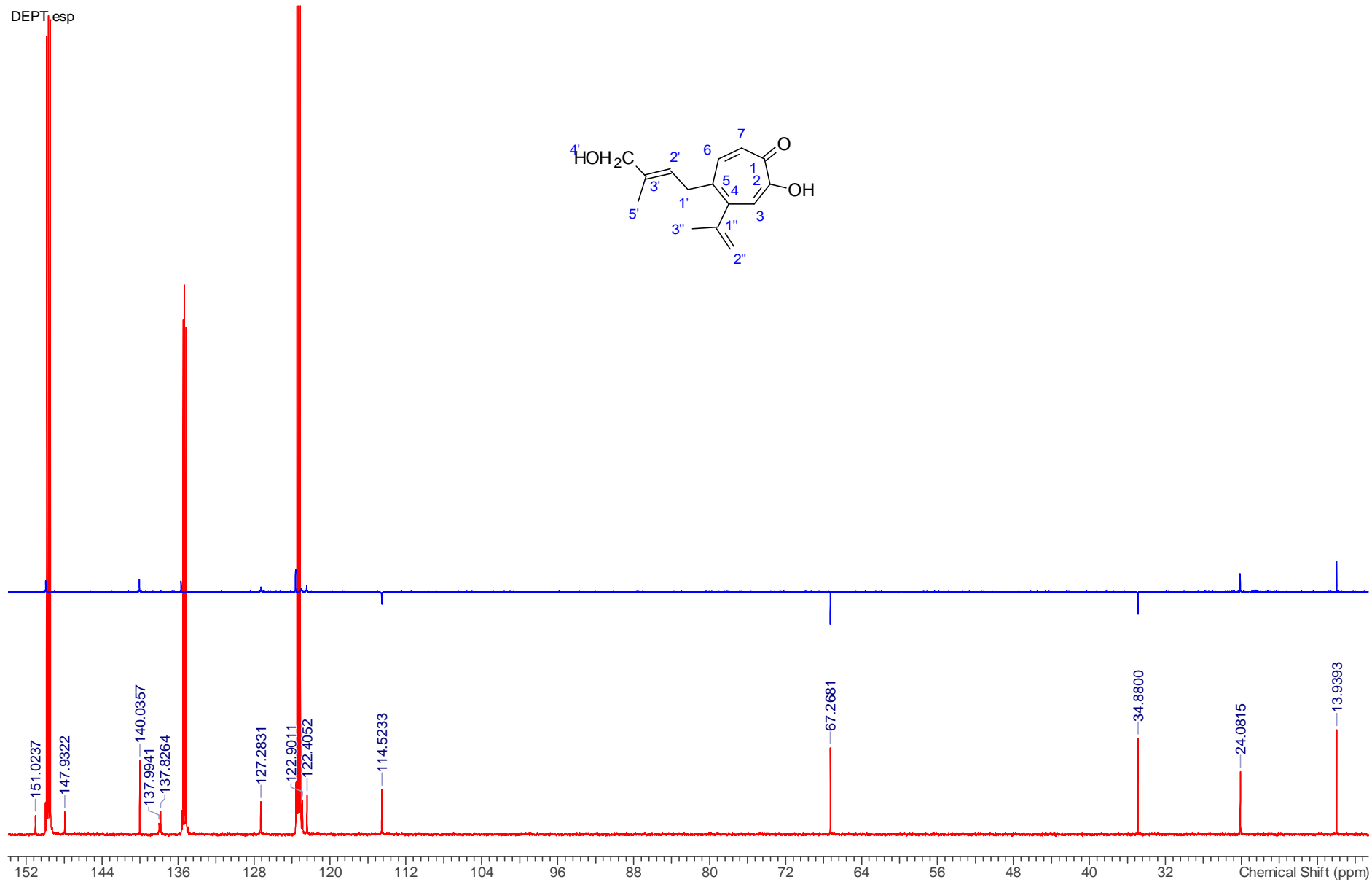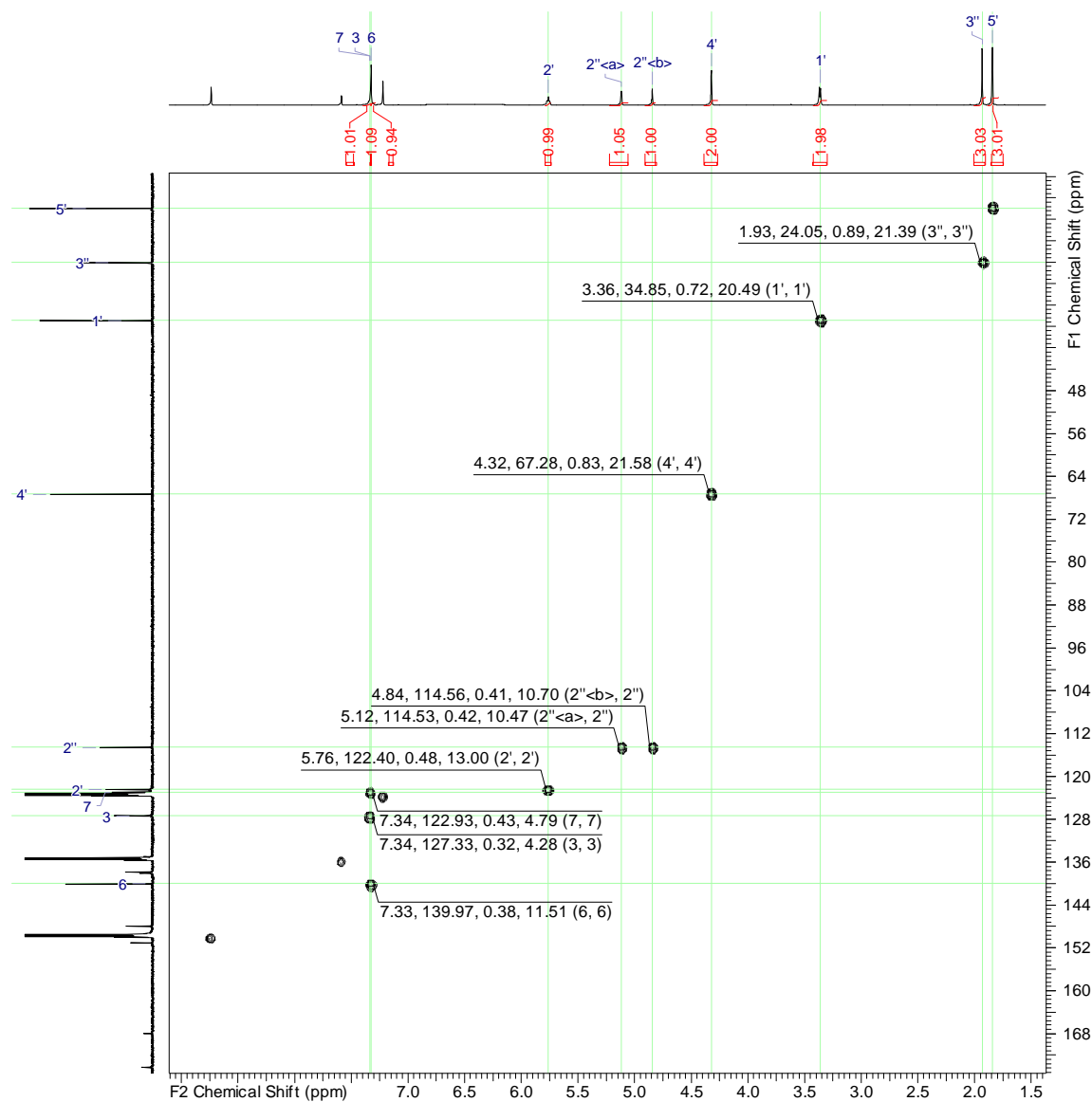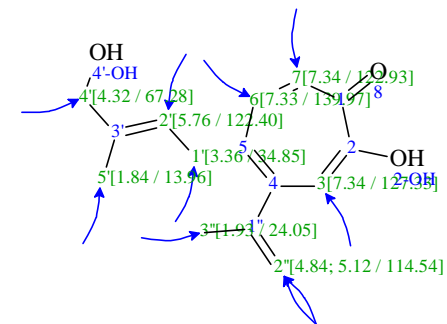
Figure S 16. DEPT spectrum of compound **2**

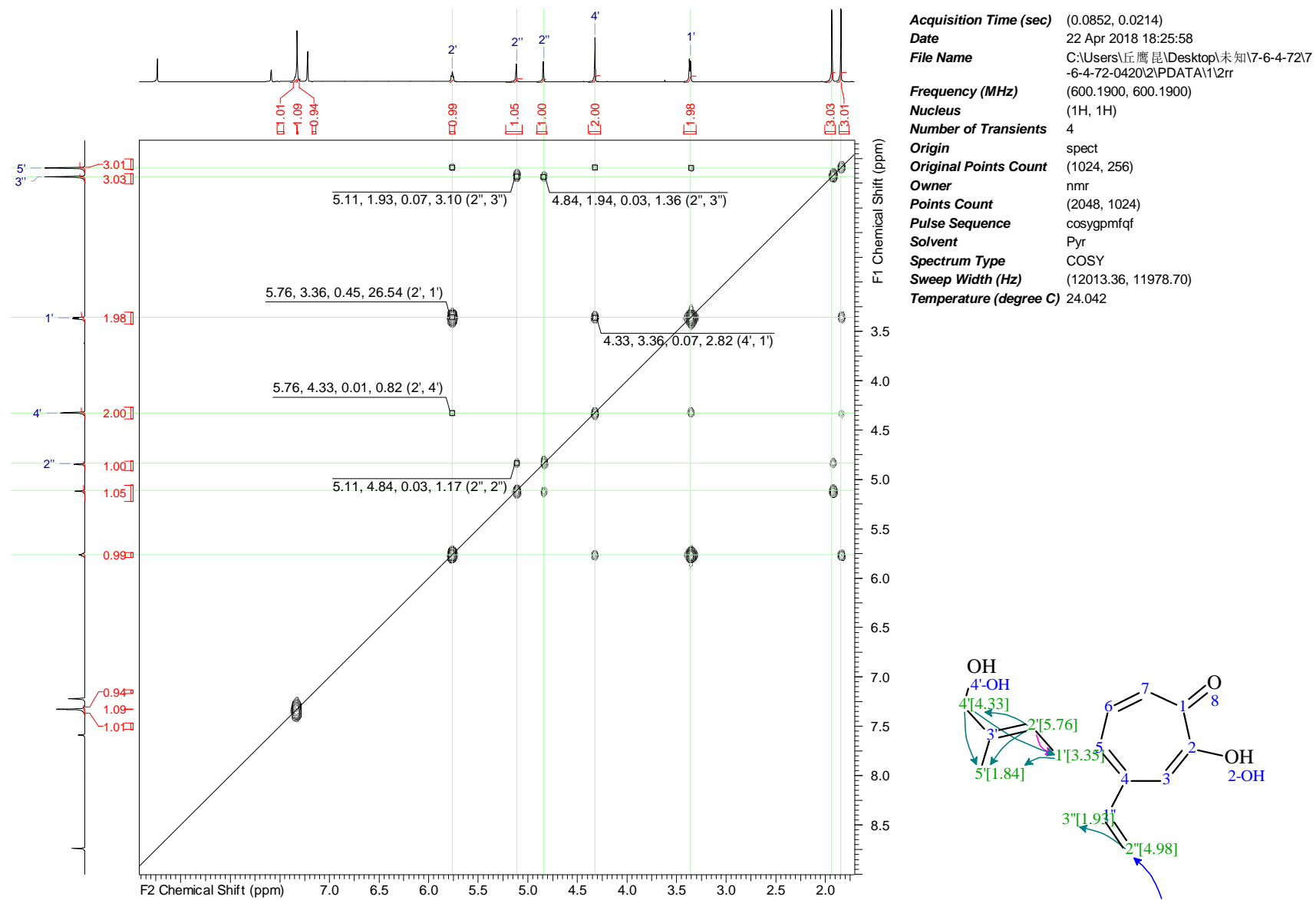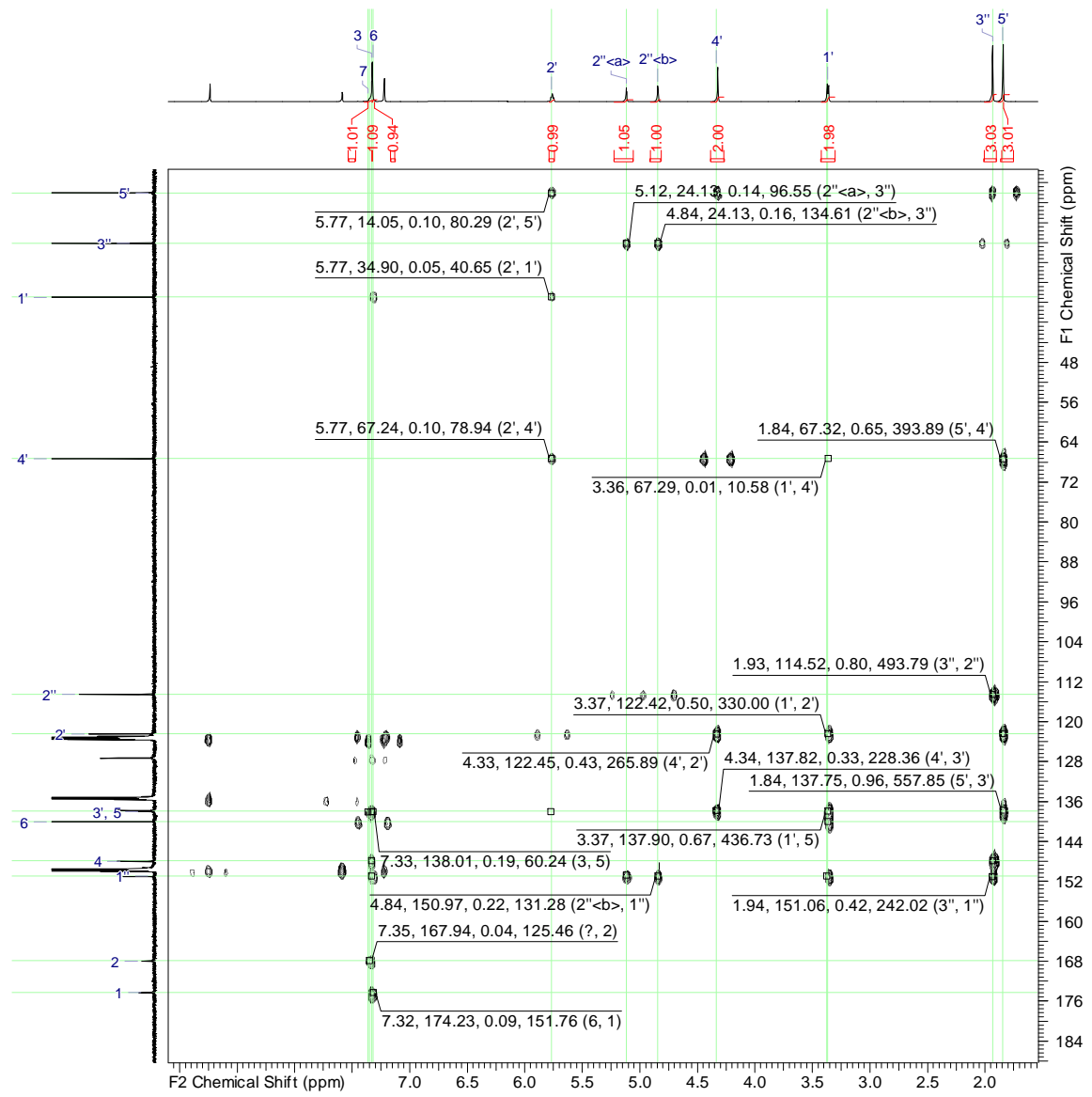Figure S 17. HSQC spectrum of compound **2**

Figure S 18. ^{1}H-^{1}H COSY spectrum of compound **2**

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Original Points Count   (2048, 128)
Owner   nmr
Points Count   (1024, 1024)
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Solvent   Pyr
Spectrum Type   HMBC
Sweep Width (Hz)   (7135.88, 33524.28)
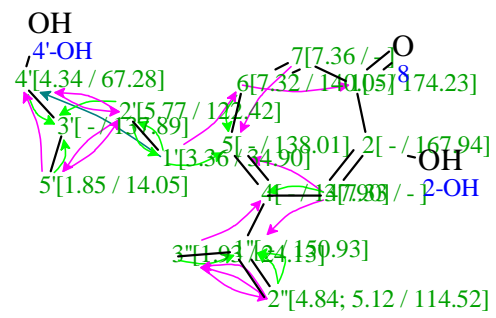Temperature (degree C)   24.041

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5.77, 34.90, 0.05, 40.65 (2', 1')
5.77, 67.24, 0.10, 78.94 (2', 4')
1.84, 67.32, 0.65, 393.89 (5', 4')
3.36, 67.29, 0.01, 10.58 (1', 4')
1.93, 114.52, 0.80, 493.79 (3'', 2'')
3.37, 122.42, 0.50, 330.00 (1', 2')
4.33, 122.45, 0.43, 265.89 (4', 2')
4.34, 137.82, 0.33, 228.36 (4', 3')
1.84, 137.75, 0.96, 557.85 (5', 3')
3.37, 137.90, 0.67, 436.73 (1', 5)
7.33, 138.01, 0.19, 60.24 (3, 5)
4.84, 150.97, 0.22, 131.28 (2''<b>, 1'')
1.94, 151.06, 0.42, 242.02 (3'', 1'')
7.35, 167.94, 0.04, 125.46 (?, 2)
7.32, 174.23, 0.09, 151.76 (6, 1)

F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

OH
4'-OH
4'[4.34 / 67.28]
3'[- / 137.89]
2'[5.77 / 122.42]
5'[1.85 / 14.05]

7[7.36 / -]
6[7.32 / 174.05]
8[174.23]
5[- / 138.01]
1'[3.36 / 34.90]
4[- / 79.93]
2[- / 167.94]
OH
2-OH

3''[1.93 / 24.13]
2''[4.84; 5.12 / 114.52]
1''[/ 150.93]

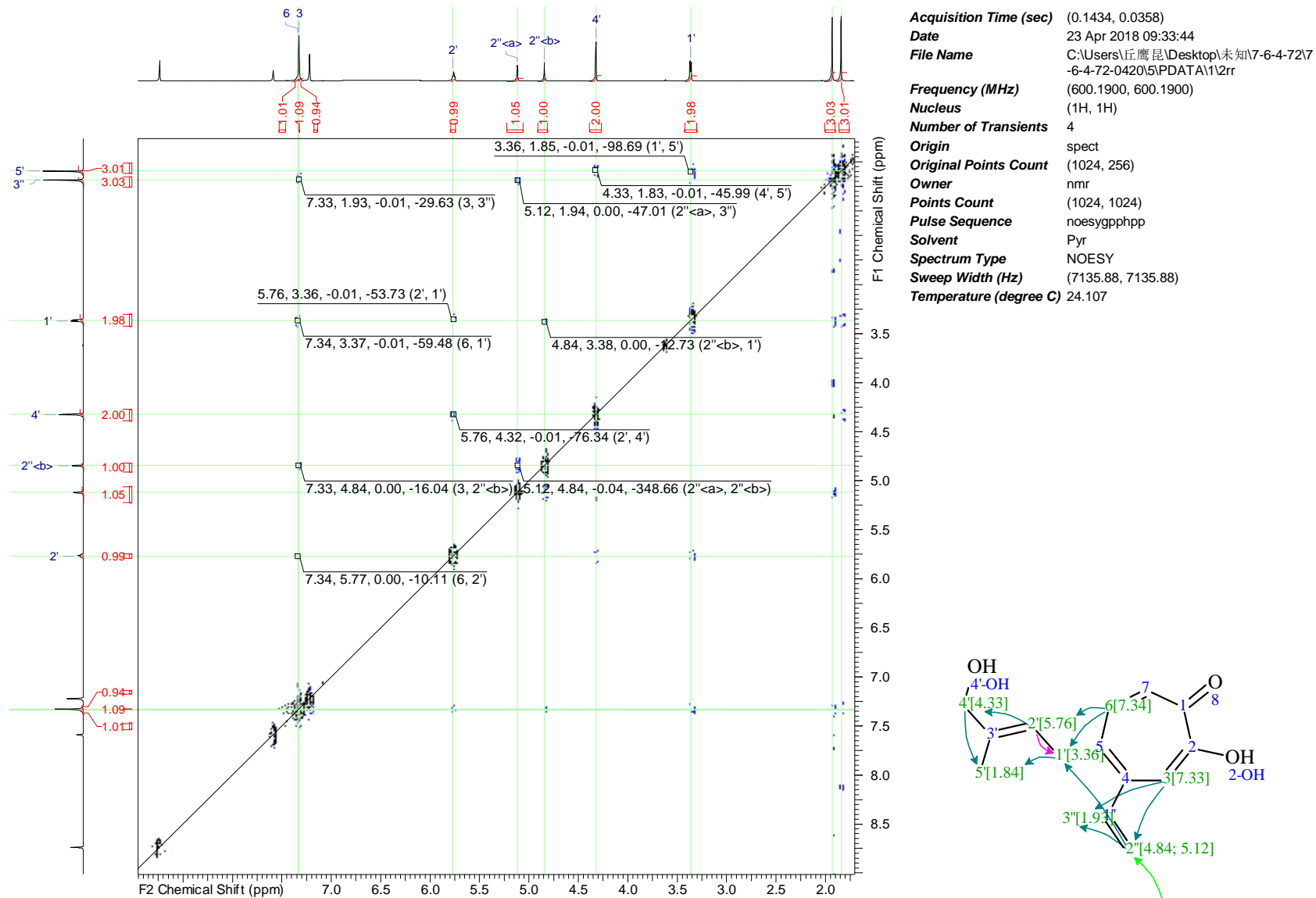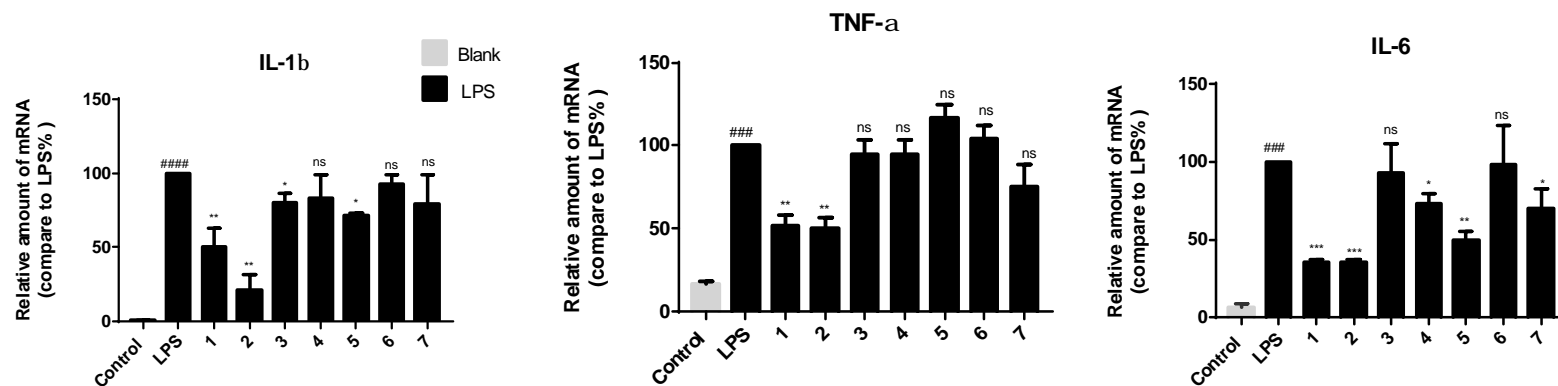Figure S 19. HMBC spectrum of compound **2**

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Figure S 20. NOESY spectrum of compound **2**

Figure S 21. Inhibitory effect of compounds **1**-**7** on the mRNA transcription of important pro-inflammatory proteins, including TNF-α, IL-1β, IL-6 in LPS-stimulated macrophages. RAW 264.7 cells were pretreated with compounds (20 μM) for 0.5 h. Thereafter, cells were stimulated with or without LPS (1 μg/ml) for 12 h; Total RNA was extracted and subjected to real time-qPCR for TNF-α, IL-6 and IL-1β. The values were normalized to GAPDH, and compared with the LPS group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, vs. LPS, ### $p < 0.001$ compared with the blank group.
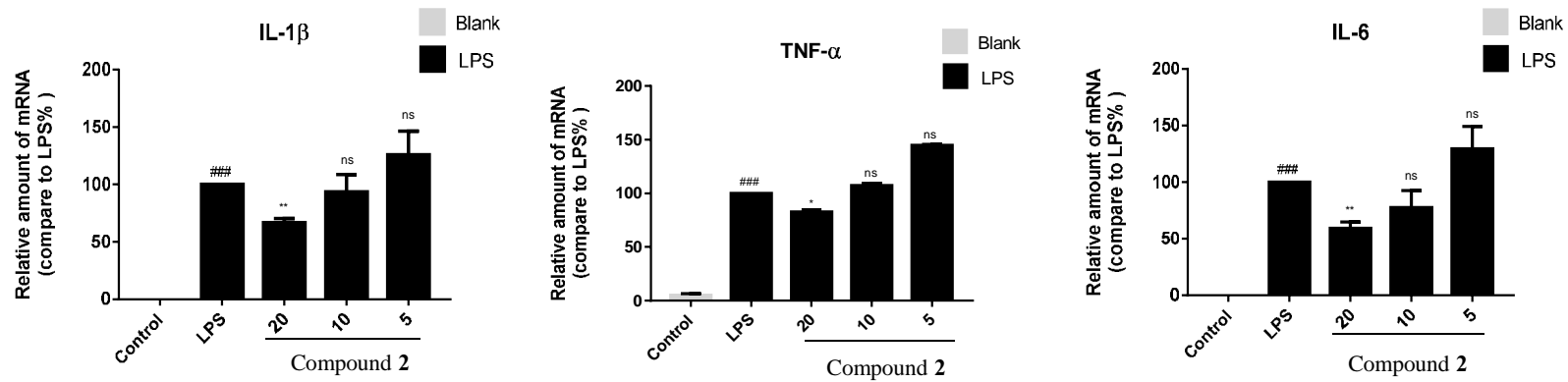
Figure S 22. Inhibitory effect of compound **2** on the mRNA transcription of important pro-inflammatory proteins, including TNF-α, IL-1β, IL-6 in LPS-stimulated macrophages. RAW 264.7 cells were pretreated with the indicated doses of compound **2** for 0.5h. Cells were incubated with LPS for 124 h. Total RNA was extracted and subjected to real time-qPCR for TNF-a, IL-6 and IL-1b. The values were normalized to GAPDH, and compared with the LPS group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, vs. LPS, ### $p < 0.001$ compared with the blank group.