Supporting Information

Discovery of Ircinianin Lactones B and C – Two New Cyclic Sesterterpenes from the Marine Sponge *Ircinia wistarii*

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C O N T E N T

Animal Material

Spectral data for ircinianin (1)

Spectral data for oxoircinianin (3)

Spectral data for ircinianin lactam A (4)

Spectral data for oxoircinianin lactam A (5)

Spectral data for ircinianin lactone A (6)

Spectral data for ircinianin lactone B (7)

Spectral data for ircinianin lactone C (8)

Biological assay data for ircinianin (1)

Figure S1. Underwater photograph of *Ircinia wistarii* (HER 6).
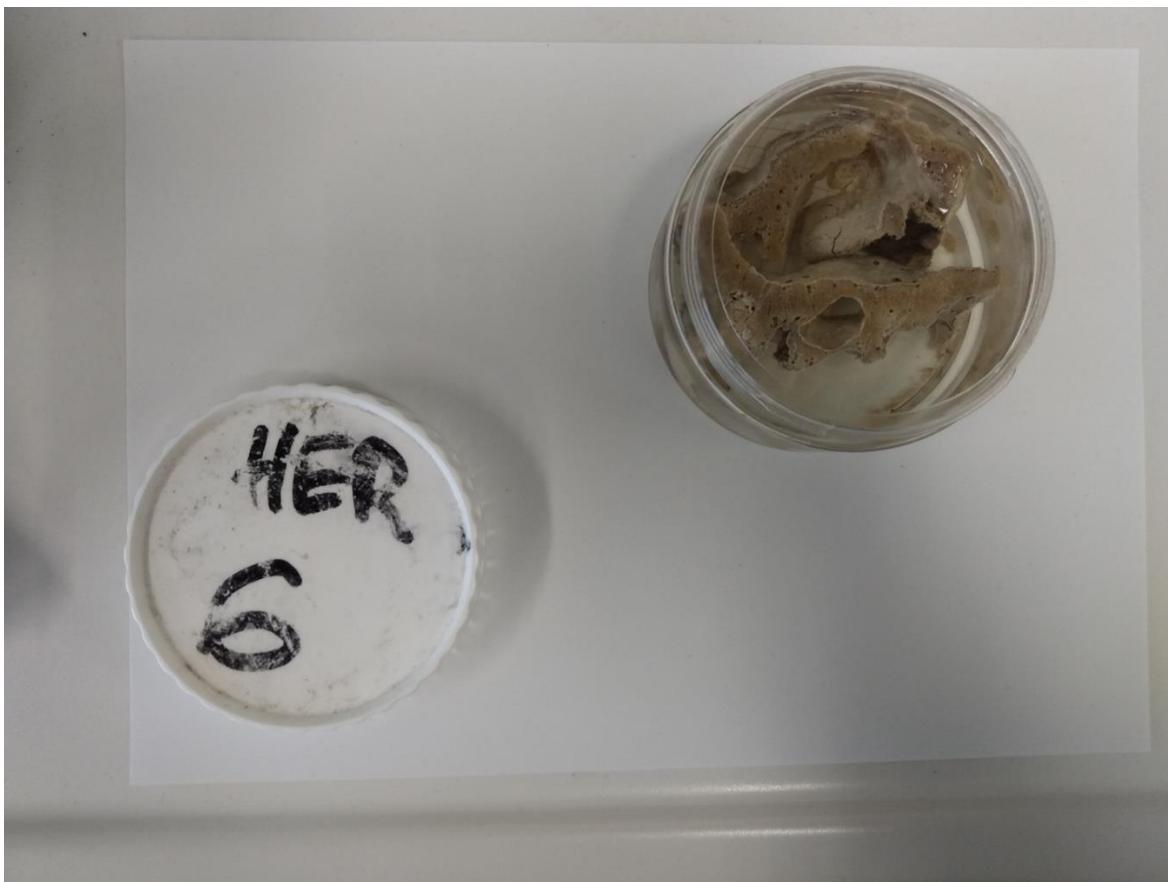
Figure S2. *Ircinia wistarii* (HER 6) – voucher sample.
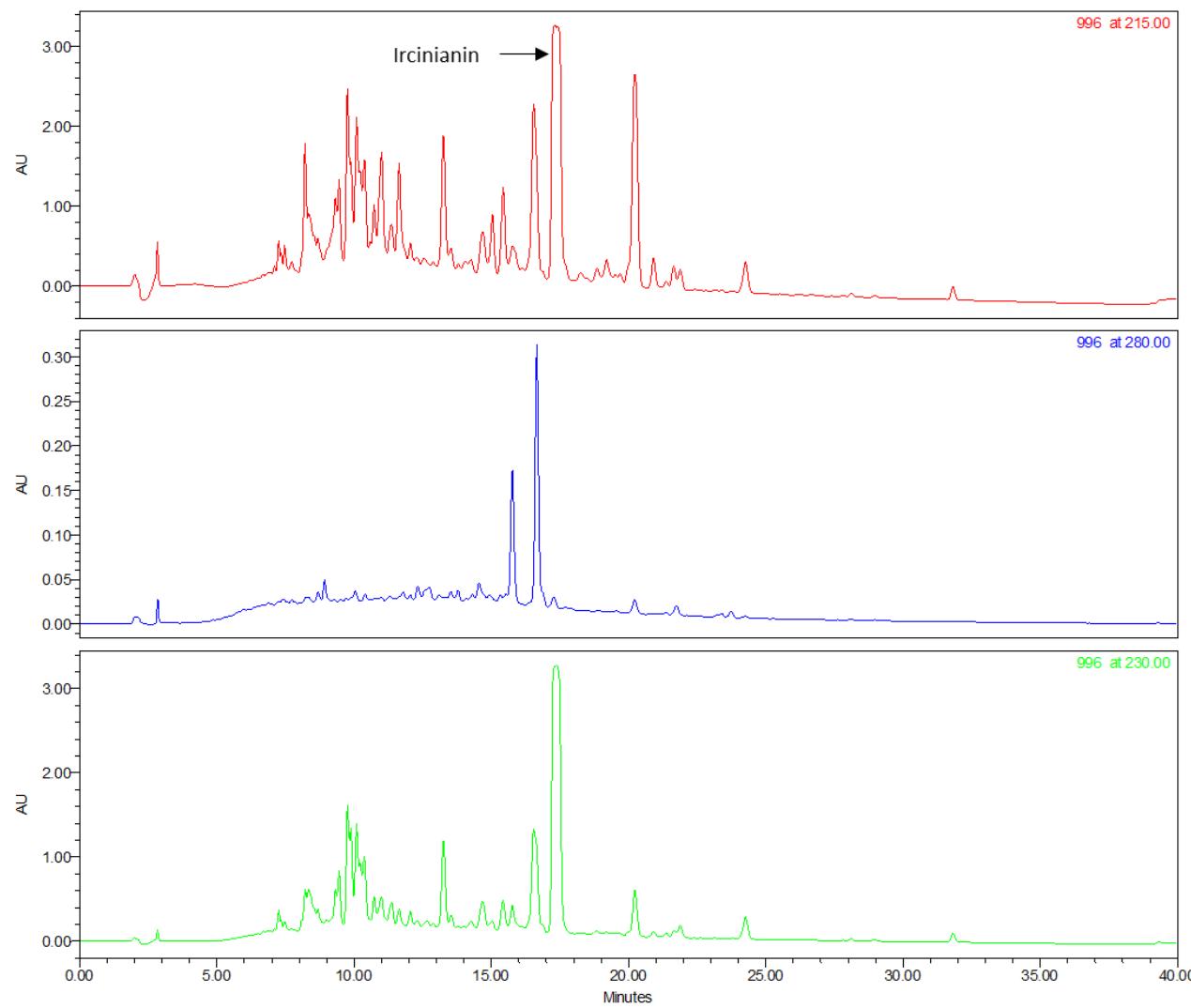
Figure S3. Chromatogram of the 90%-LC-fraction.

A

B
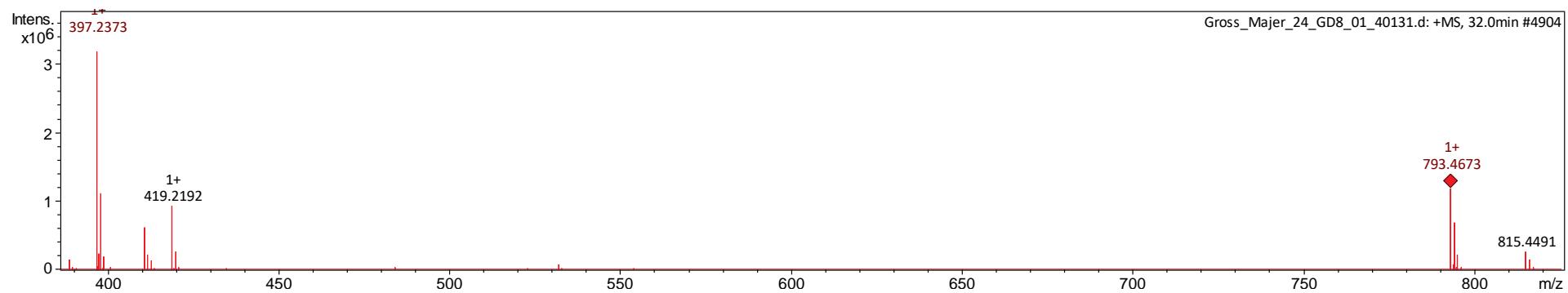
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
397.2373	1	$C_{26}H_{29}N_4$	397.2387	3.5	3.1	15.0	ok	even	29.7	43.4
397.2373	2	$C_{25}H_{33}O_4$	397.2373	0.2	0.0	10.0	ok	even	42.6	61.2
397.2373	3	$C_{21}H_{29}N_6O_2$	397.2347	-6.6	-7.5	11.0	ok	even	56.6	84.5
397.2373	4	$C_{20}H_{33}N_2O_6$	397.2333	-10.0	-10.6	6.0	ok	even	69.5	103.4
397.2373	5	$C_{14}H_{33}N_6O_7$	397.2405	8.2	6.8	2.0	ok	even	98.7	153.5
397.2373	6	$C_{11}H_{25}N_{16}O$	397.2392	4.8	1.6	8.0	ok	even	99.6	159.5
397.2373	7	$C_{10}H_{29}N_{12}O_5$	397.2378	1.4	-1.1	3.0	ok	even	112.3	180.1

C
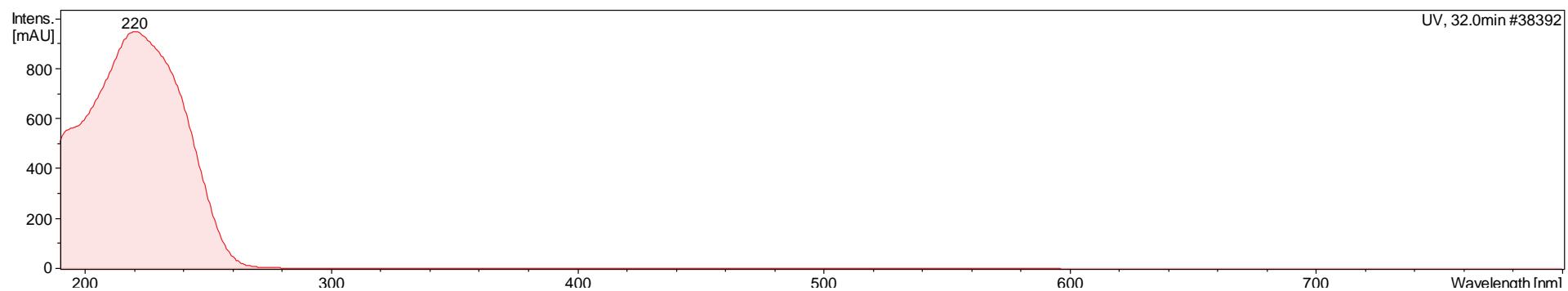
Figure S4. Ircinianin (**1**, 90%-fraction) Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.

6
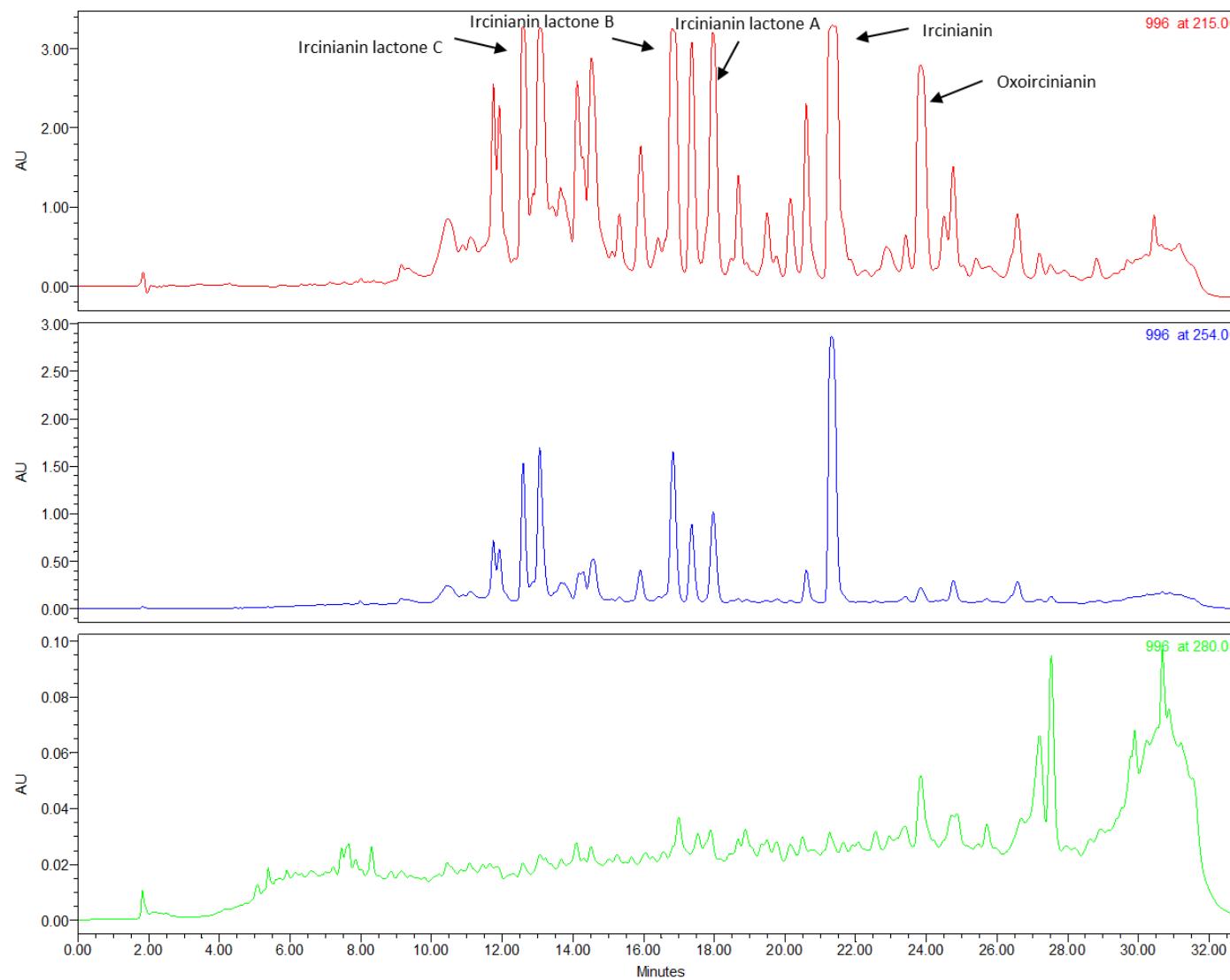
Figure S5. Chromatogram of the 100%-LC-fraction.

A

B
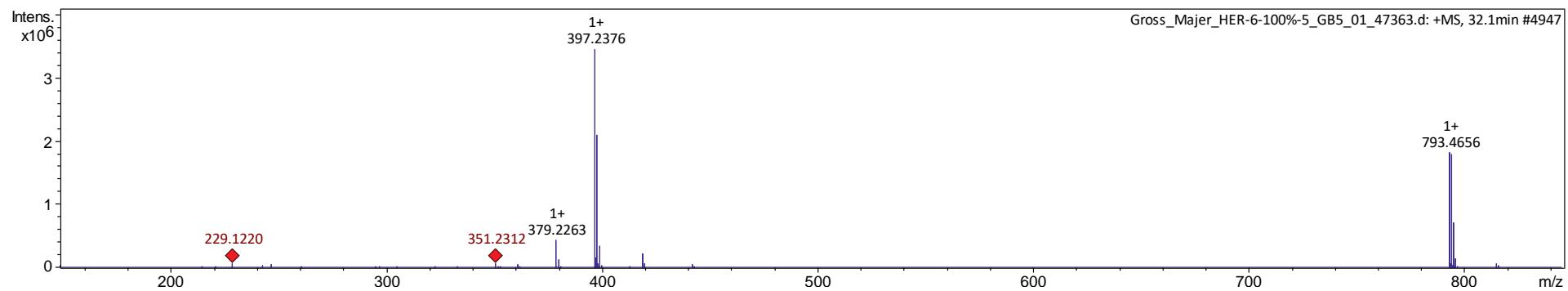
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
397.2376	1	$C_{26}H_{29}N_4$	397.2387	2.6	3.2	15.0	ok	even	180.9	209.1
397.2376	2	$C_{25}H_{33}O_4$	397.2373	-0.7	0.1	10.0	ok	even	194.0	227.3
397.2376	3	$C_{21}H_{29}N_6O_2$	397.2347	-7.5	-7.4	11.0	ok	even	207.9	250.4
397.2376	4	$C_{14}H_{33}N_6O_7$	397.2405	7.3	6.9	2.0	ok	even	250.1	319.6
397.2376	5	$C_{11}H_{25}N_{16}O$	397.2392	3.9	1.6	8.0	ok	even	250.8	325.4
397.2376	6	$C_{10}H_{29}N_{12}O_5$	397.2378	0.5	-1.1	3.0	ok	even	263.7	346.2

C
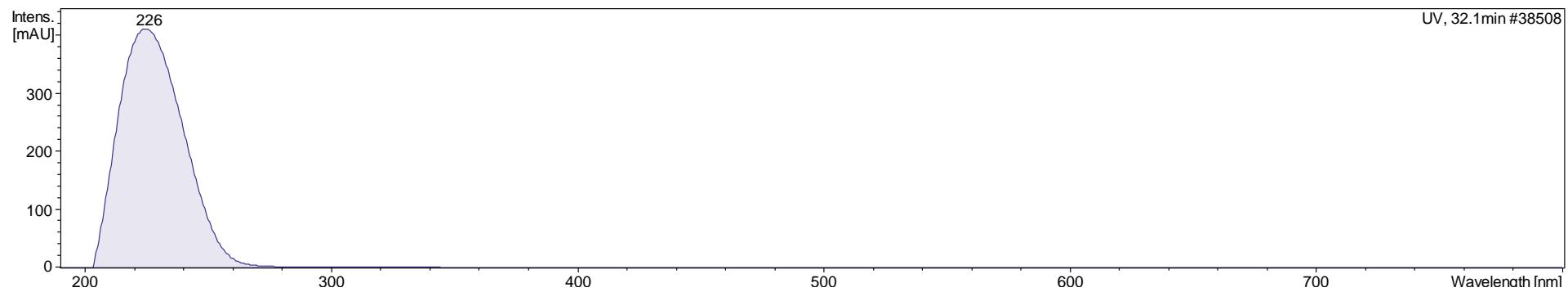
Figure S6. Ircinianin (**1**, 100%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.
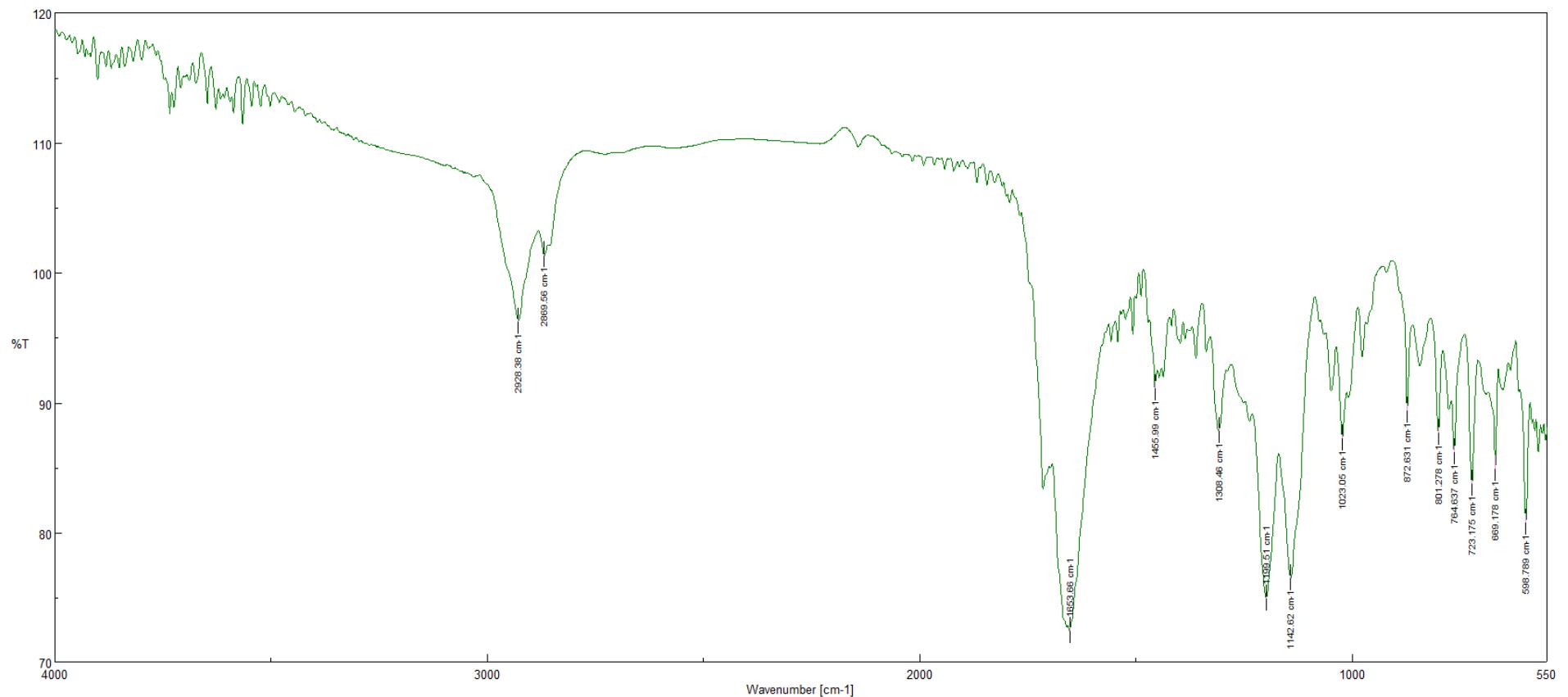
Figure S7. FT-IR spectrum of ircinianin (**1**).
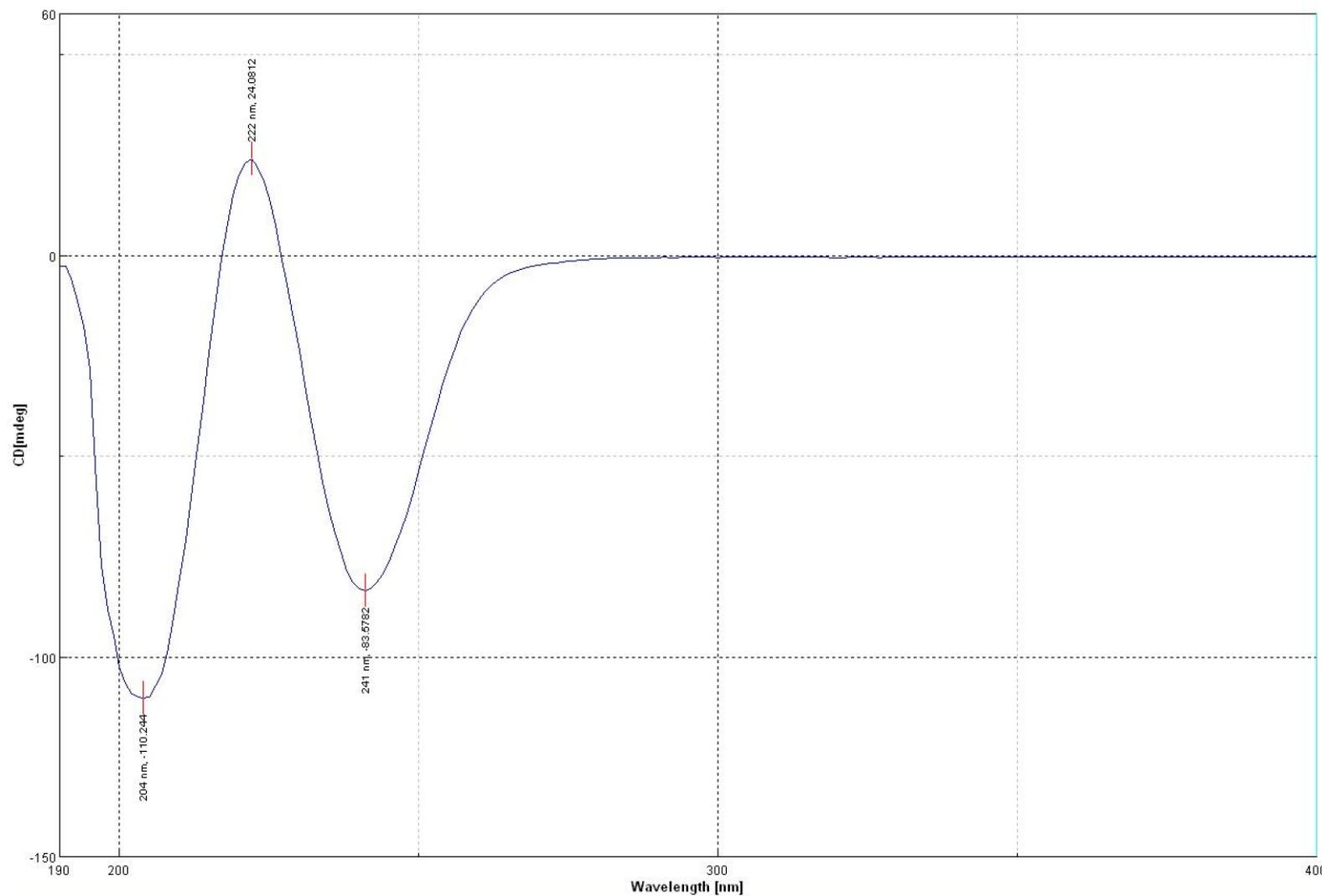
Figure S8. CD-spectrum of ircinianin (**1**) in MeOH.

Ircinianin (1) was isolated as a white powder from the 90% and 100% fraction and the molecular formula was deduced as $C_{25}H_{33}O_4$ requiring ten degrees of unsaturation – based on (+)-HRESIMS analysis ([M+H]$^+$ at *m/z* 379.2373 and 379.2376, respectively, calcd. as 379.2373, see Figures S4 and S6). ^1H, ^{13}C and DEPT 135 experiments (see Table 1, Figures S11-13) insinuated a furano sesterterpenoid with a tricyclic core. The ^1H showed three furan singlets at δ_H 7.38 (1H), 7.26 (1H) and 6.30 (1H) and additionally four signals of methyl groups (δ_H 0.92 (3H), 1.57 (3H), 1.64 (3H), 1.71 (3H)) [5]. The assumption of a mono substituted furan moiety was encouraged by four signals in ^{13}C / DEPT135 spectra (δ_C 144.0 (CH), 140.3 (CH), 126.5 (C), 112.1 (CH)), the characteristic carbon shifts of a tetronic acid pattern (δ_C 179.2 (C), 177.7 (C) and 97.5 (C)) could be also deduced [3]. Furthermore, four olefinic carbons with resonances at δ_C 123.6 (CH), 125.0 (CH), 136.6 (C), 137.1 (C) were detected, whereby the two quaternary carbons exhibited a characteristic shift caused by a methyl-substitution. Together with four methyl (δ_C 16.3, 20.7, 20.8, 6.1), five methylene (δ_C 25.3, 27.3, 29.5, 33.6, 40.5), four methine (δ_C 33.2, 46.2, 48.7, 52.0) and one quaternary carbon (δ_C 86.9), the ircinianin scaffold was completed. The subtraction of eight double bound equivalents (6x double bounds, 1x furan ring and 1x tetronic acid ring) from the total of ten degrees of unsaturation, pointed out two additional ring closures, which was also in accordance with a potential indene motive within the tricyclic indene-spirotetronic acid formation. From the results of ^1H-^1H-TOCSY (see Figure S16) together with ^1H-^1H-COSY and ^1H-^{13}C HMBC experiments, three major fragments could be assigned. One fragment (C-15 to C-20) represented the five-membered ring of the indene motif with a methyl group attached to C-18, while the second fragment (C-8 to C-14) was a combination of parts of the indene together with the alkyl linker with double bonds incorporated between C-12 to C-13 and C-8 to C-10, whereas C-8 was substituted with a further methyl group. The third fragment (C-1 to C-7) covered the furan ring together with the remaining three carbons of the alkyl linker. Diagnostic ^1H-^{13}C HMBC correlations could be used to combine these fragments to the known scaffold, whereby through space correlations of a ^1H-^1H-NOESY experiment were used to corroborate the molecular architecture in the polycyclic core unit. The absolute configuration was secured by X-ray analysis [26] and CD-spectroscopy (Figure S8) [23].
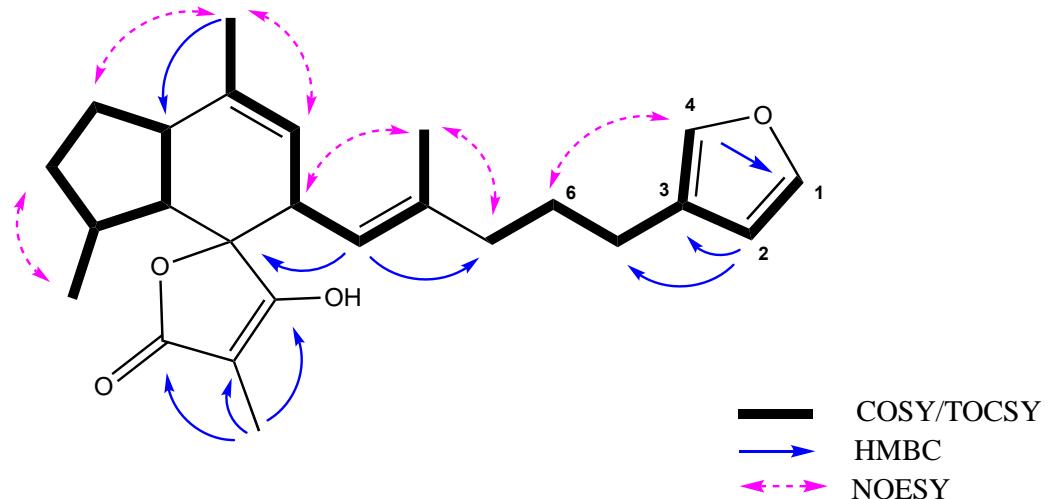
Figure S9. Key 2D-NMR correlations for **1**.
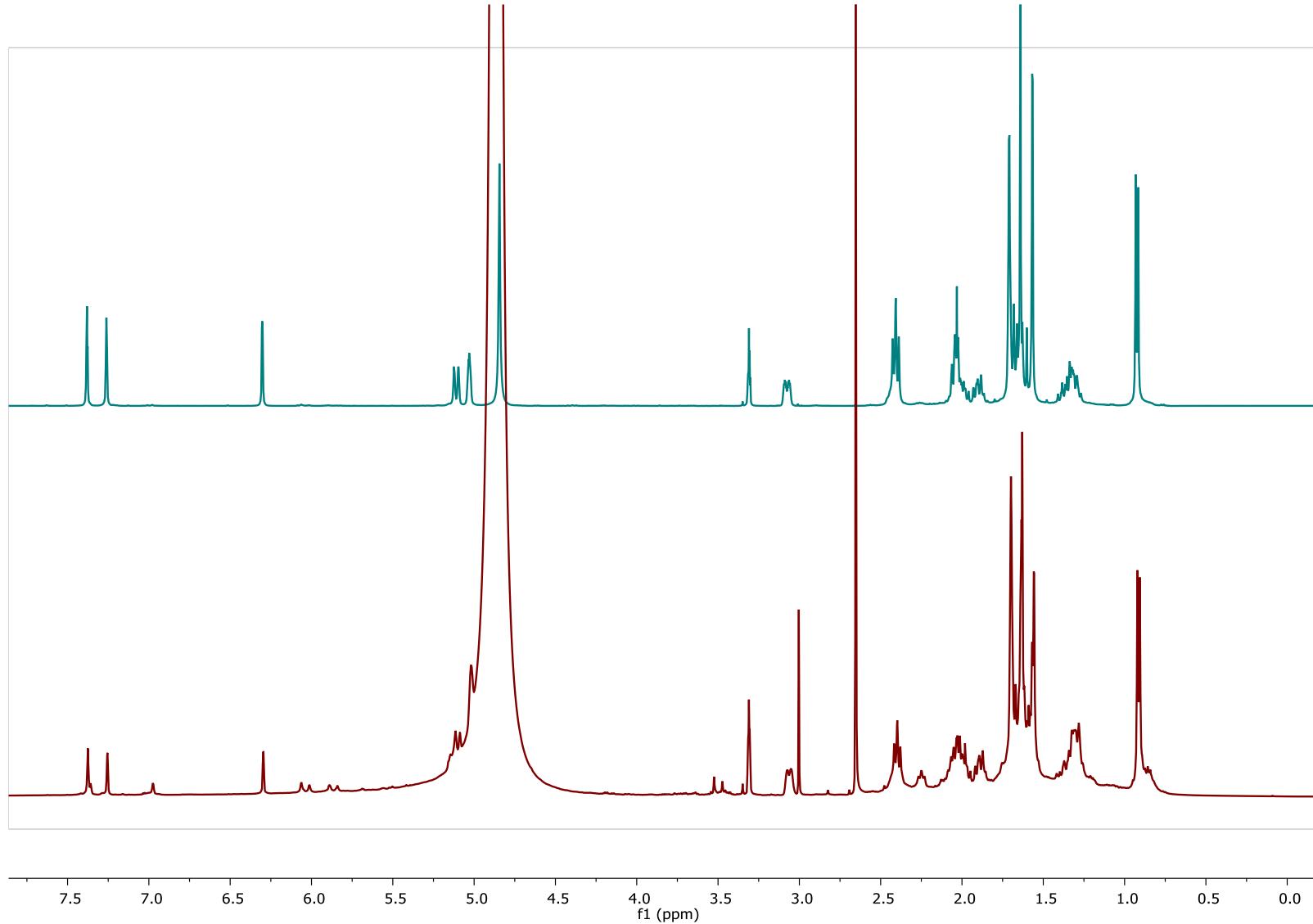
Figure S10. 400 MHz ^{1}H NMR spectrum of ircinianin (**1**, 90%-fraction, top) and ircinianin (**1**,100%-fraction, bottom) in d_4-MeOH.
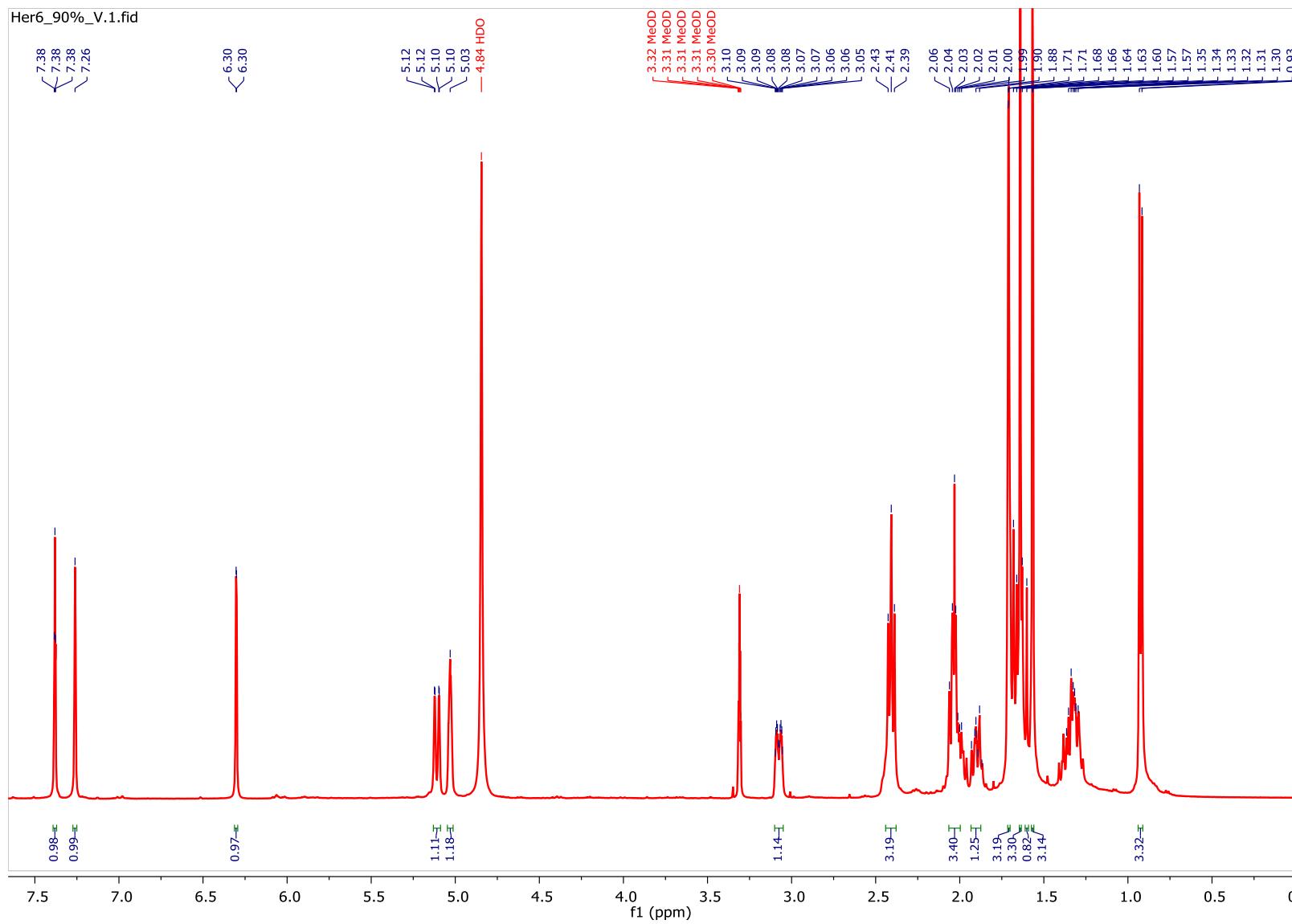
Figure S11. 400 MHz ^{1}H NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
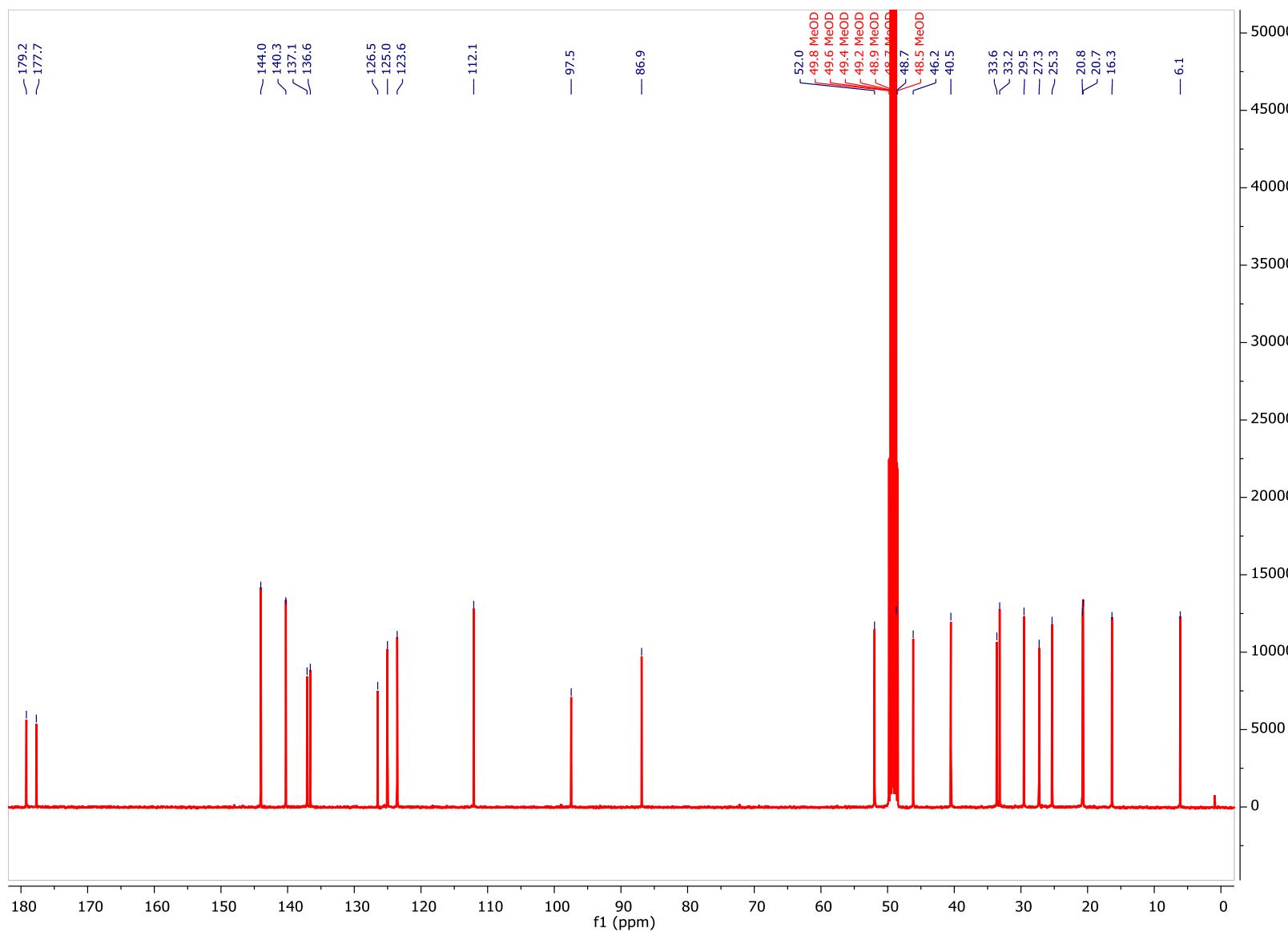
Figure S12. 100 MHz ^{13}C NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
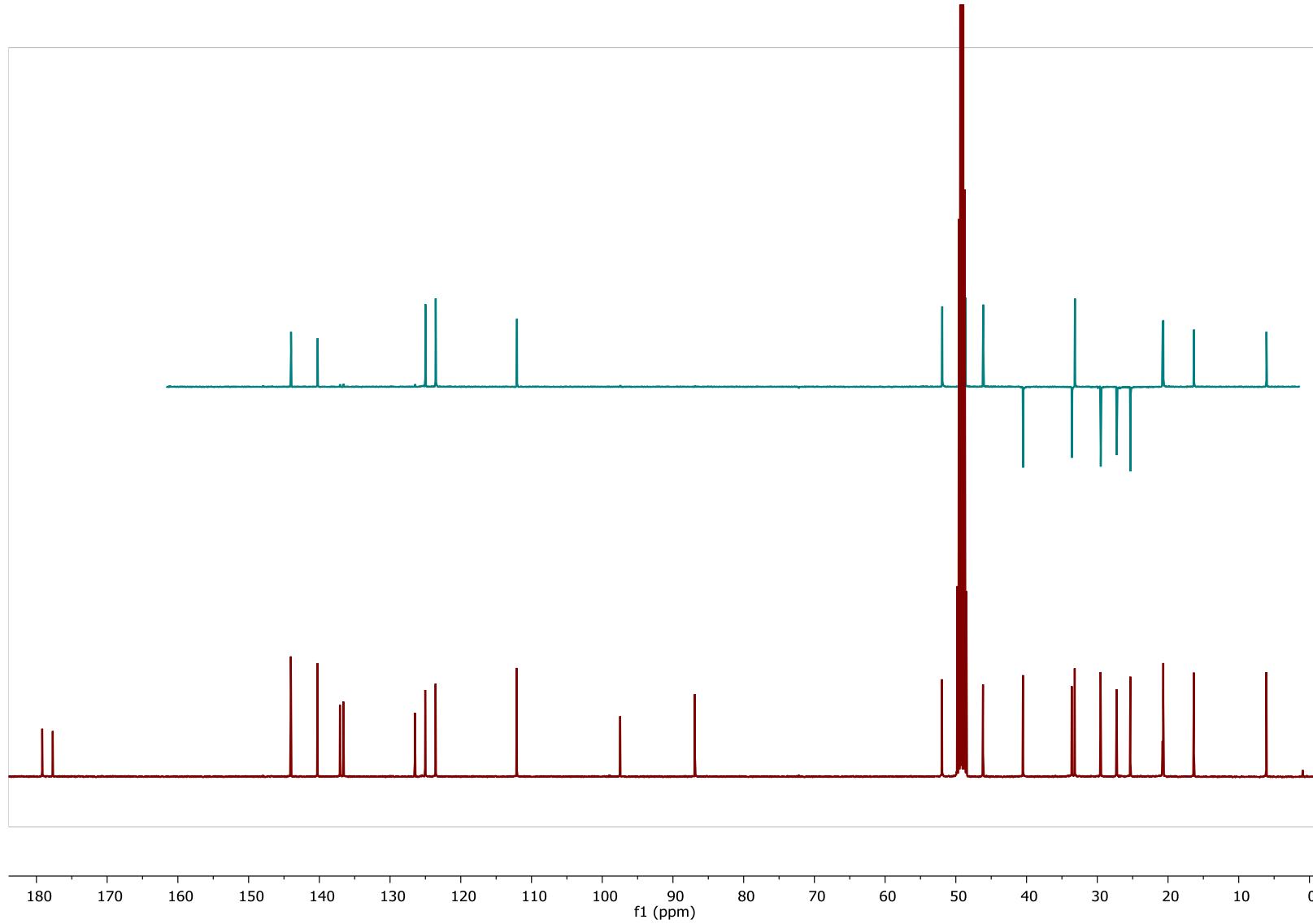
Figure 13. 100 MHz ^{13}C NMR spectrum (bottom) and 400 MHz DEPT135 NMR spectrum (top) of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
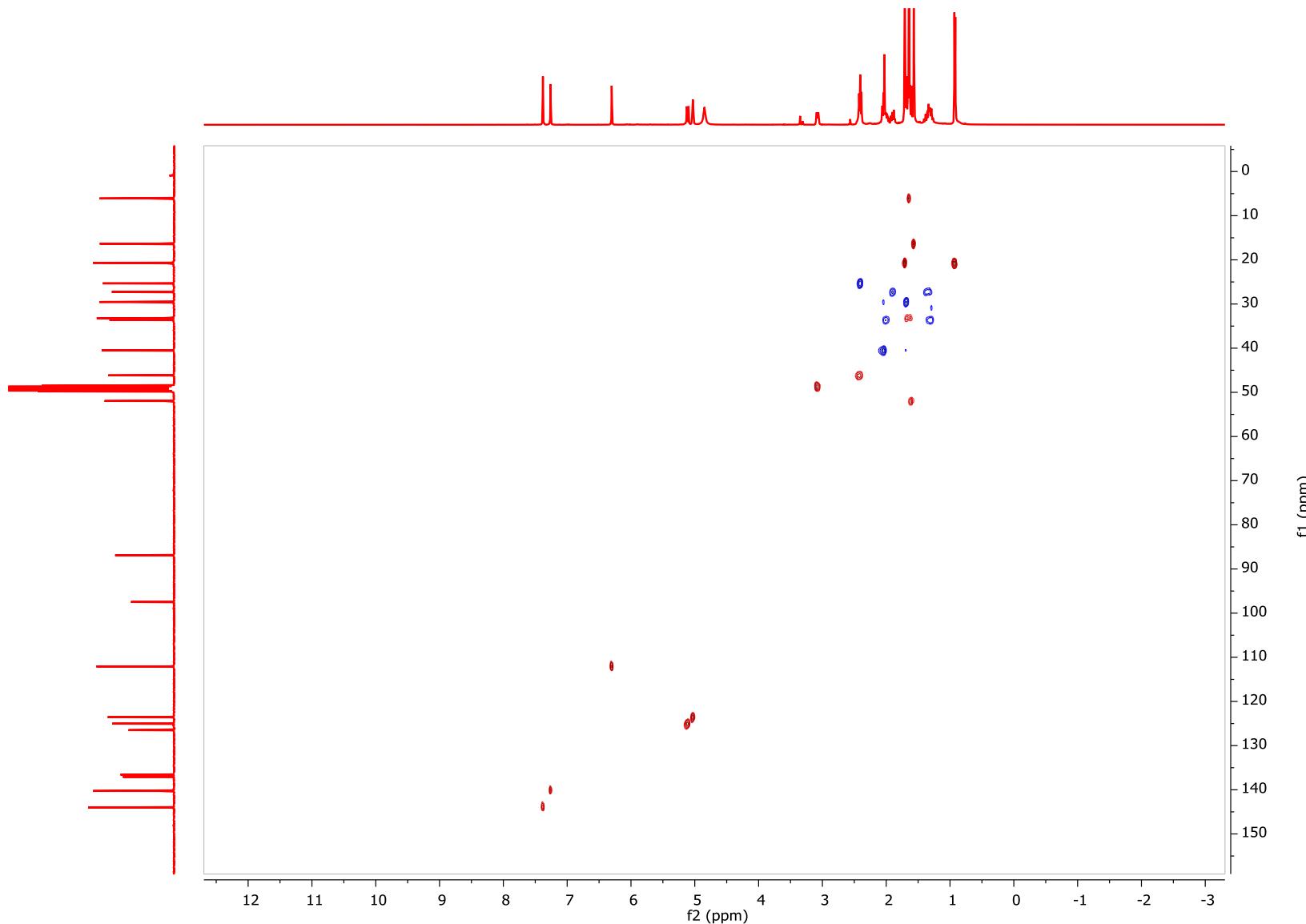
Figure S14. 400 MHz multiplicity edited ^{1}H-^{13}C HSQC NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
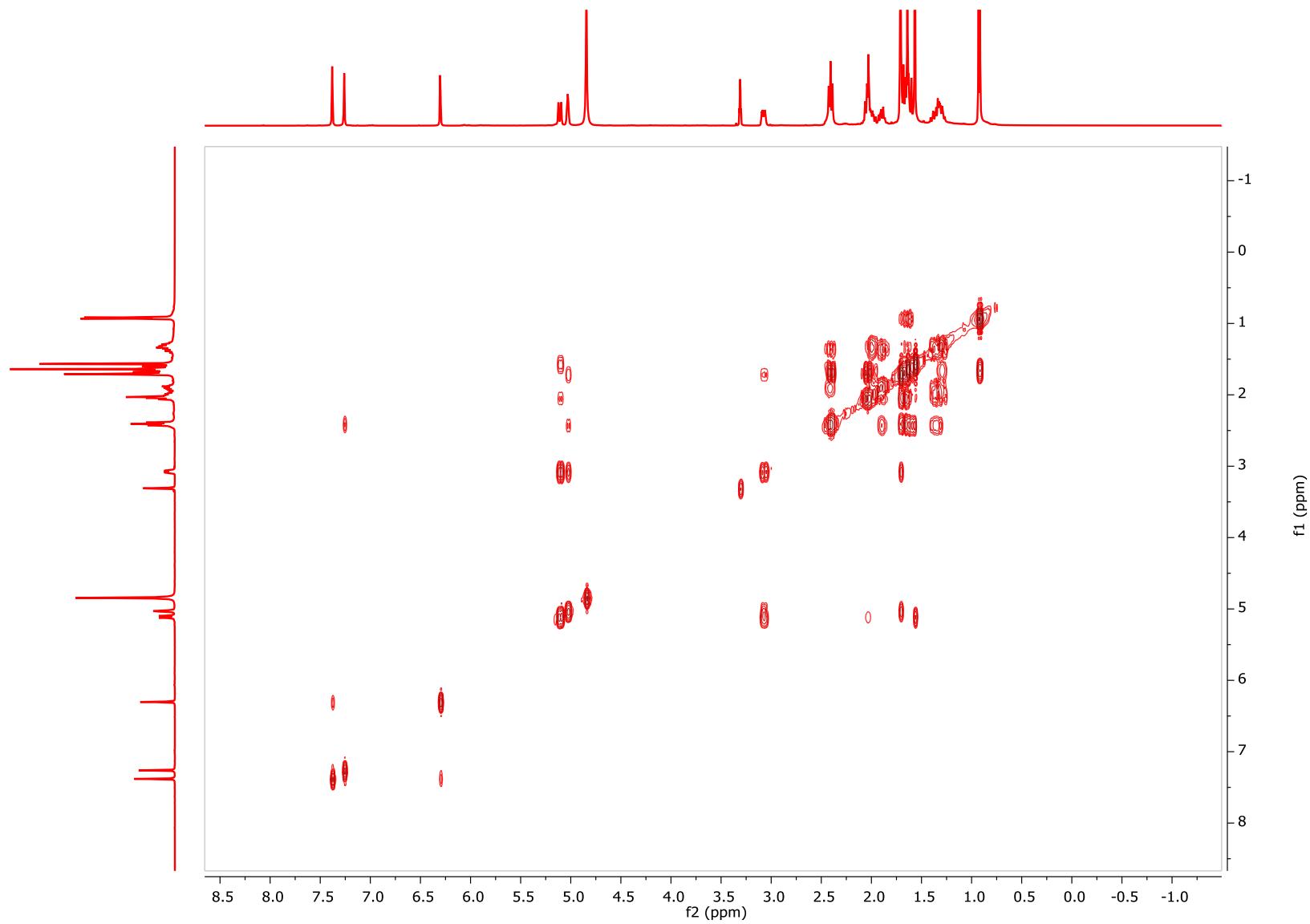
Figure S15. 400 MHz ^{1}H-^{1}H COSY NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
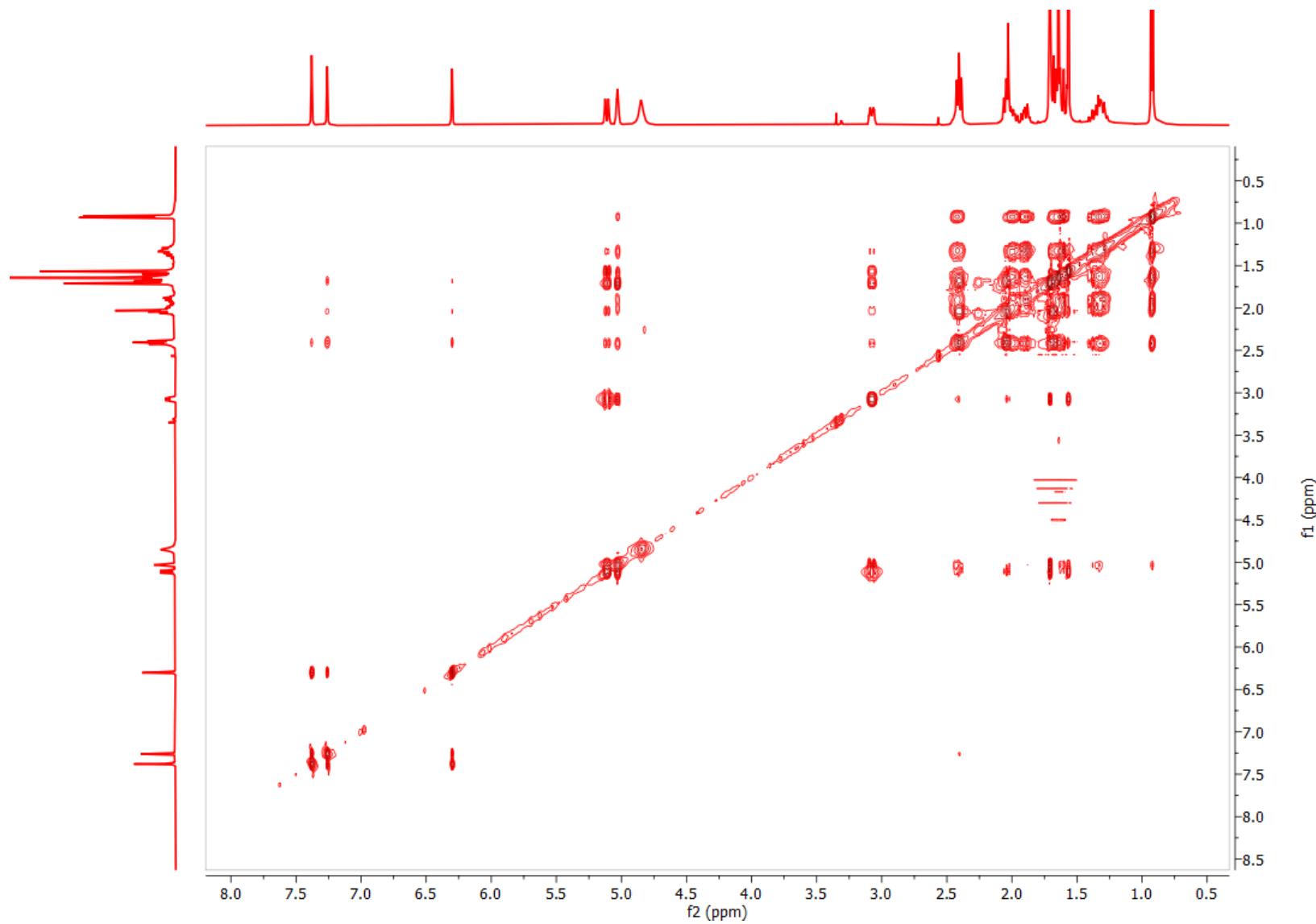
Figure S16. 400 MHz ^{1}H-^{1}H TOCSY NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
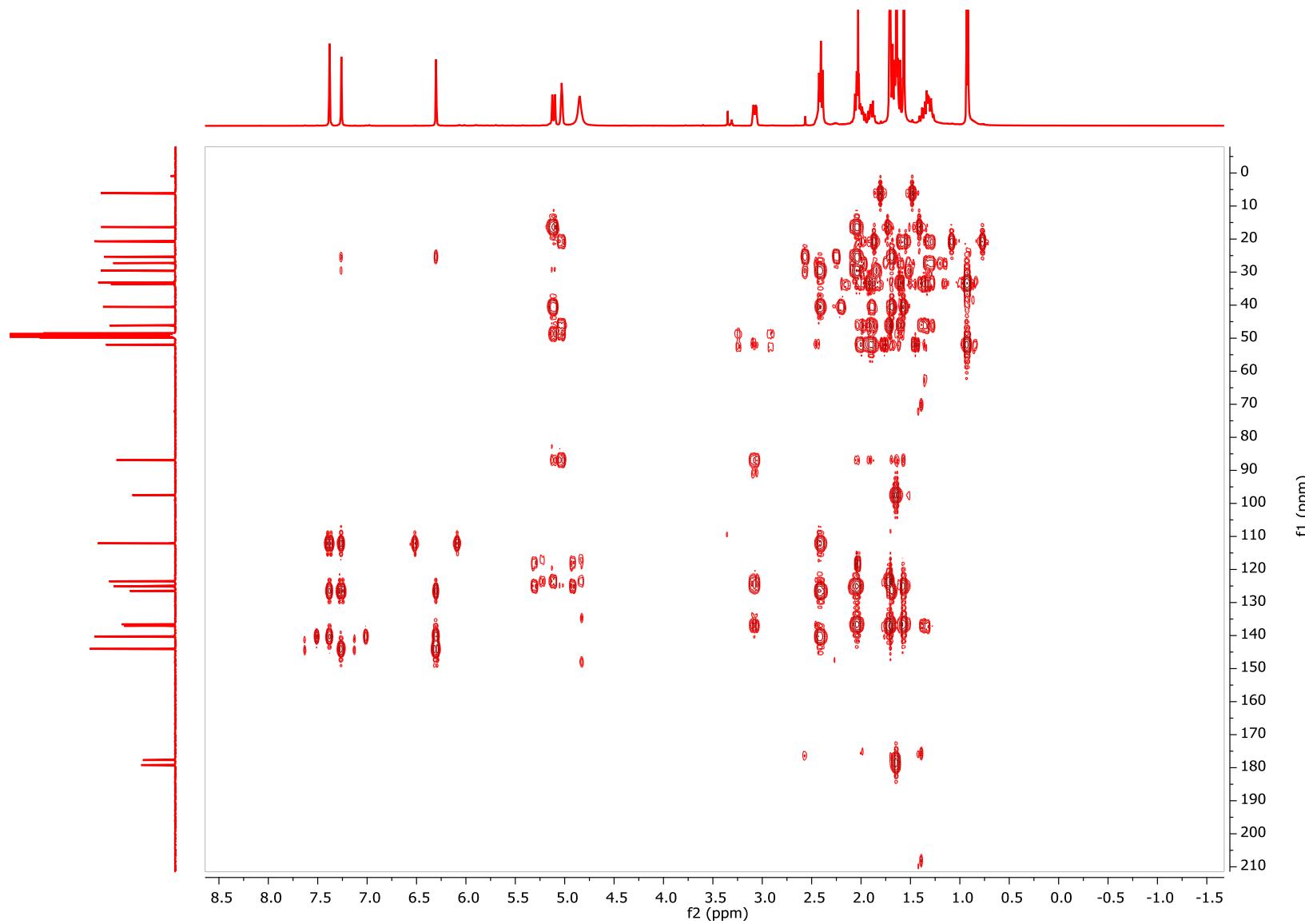
Figure S17. 400 MHz ^{1}H-^{13}C HMBC NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.
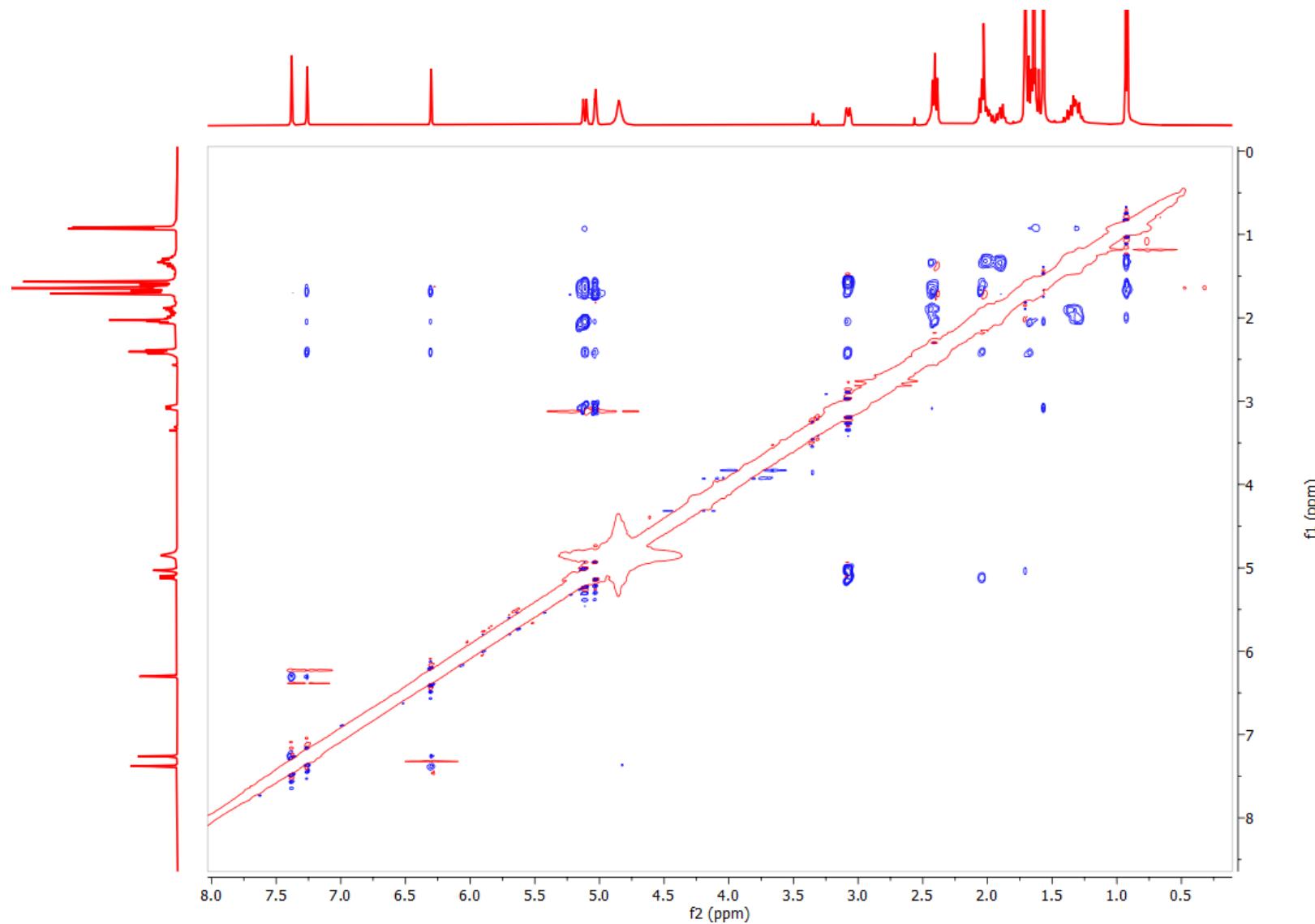
Figure S18. 400 MHz ^{1}H-^{1}H NOESY NMR spectrum of ircinianin (**1**, 90%-fraction) in d_4-MeOH.

A

B
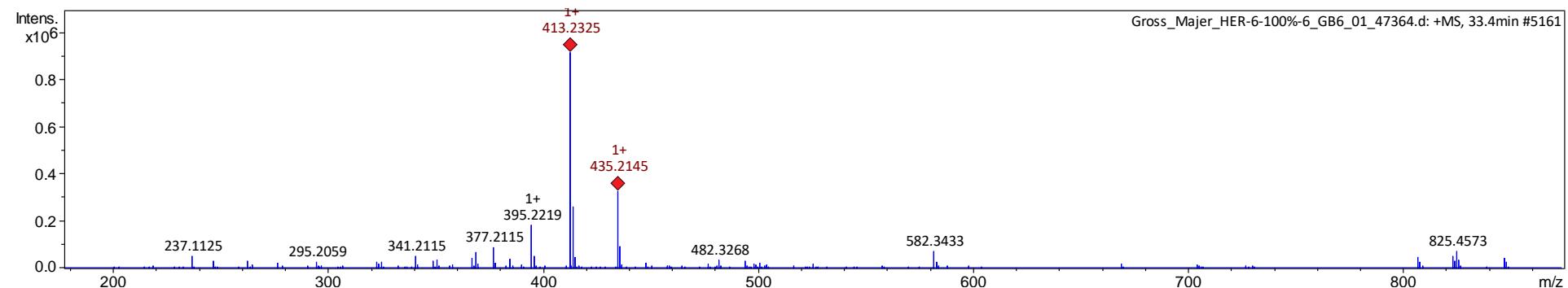
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
413.2325	1	$C_{25}H_{33}O_5$	413.2323	-0.7	-0.9	10.0	ok	even	5.5	8.9
413.2325	2	$C_{26}H_{29}N_4O$	413.2336	2.6	2.0	15.0	ok	even	9.3	11.9
413.2325	3	$C_{21}H_{29}N_6O_3$	413.2296	-7.2	-8.1	11.0	ok	even	19.7	32.5

C
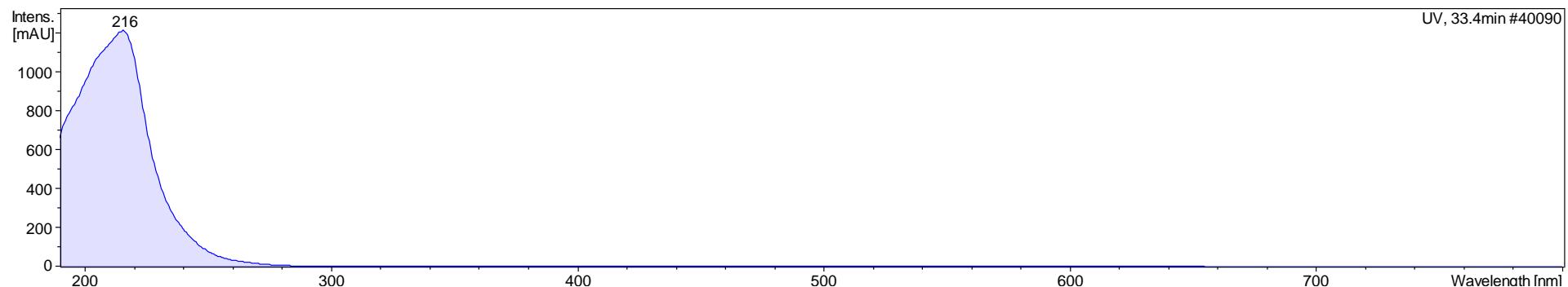
Figure S19. Oxoircinianin (**3**, 100%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.
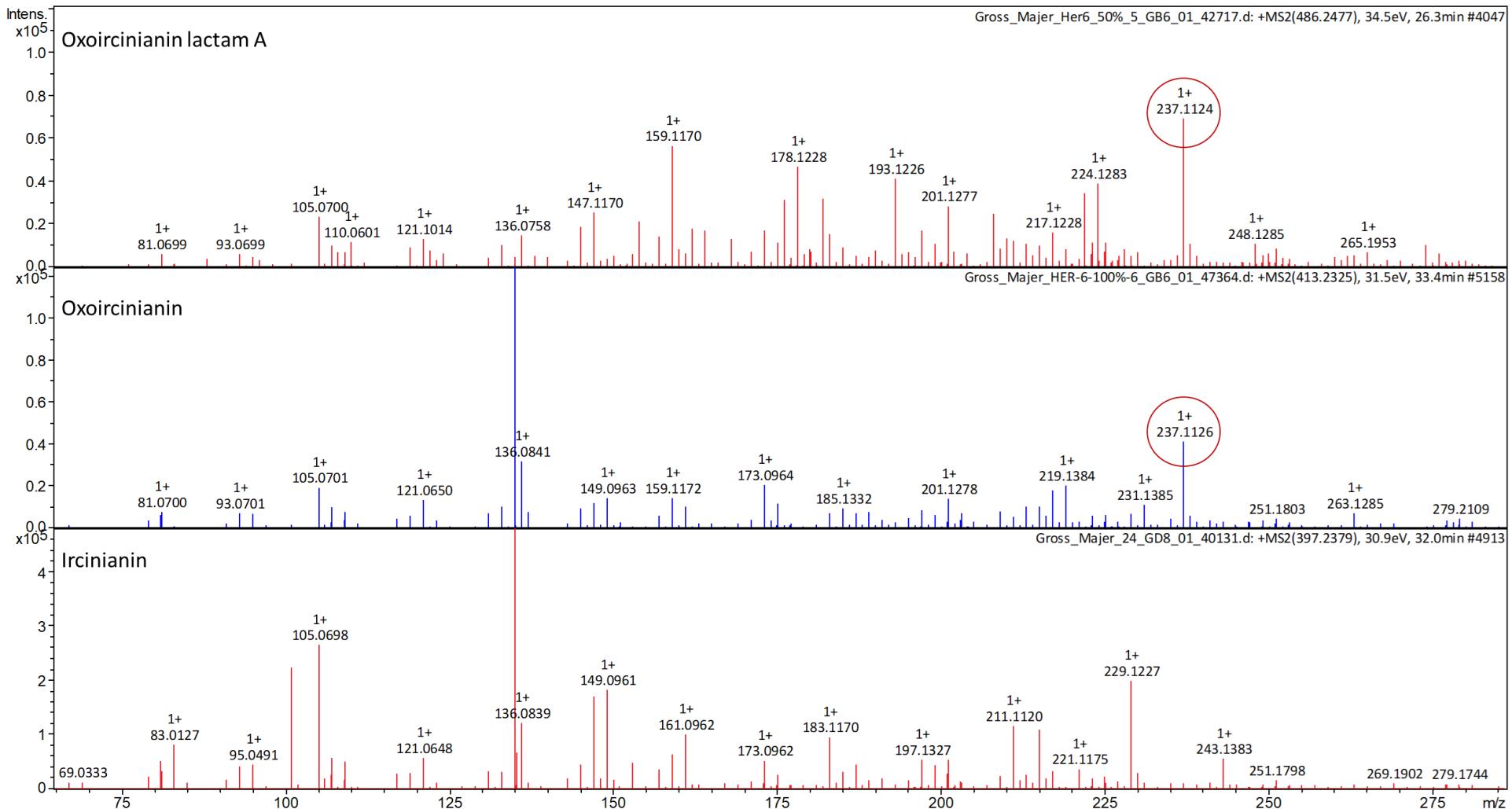
Figure S20. Comparison of the MS^2 spectra of ircinianin (**1**), oxoircinianin lactam A (**5**) and oxoircinianin (**3**) – the characteristic fragment of **5** and **3**, indicating the "oxo"-skeleton, is highlighted. Oxoircinianin (**3**) was characterized by HRESIMS. The scaffold affiliation was proven by MS^2 analysis in comparison with **1** and **5** which exhibited comparable fragmentation patterns. Furthermore, a ^{13}C NMR experiment of an oxoircinianin enriched fraction confirmed the "oxo"-molecular architecture in the tetronic acid motif.

22
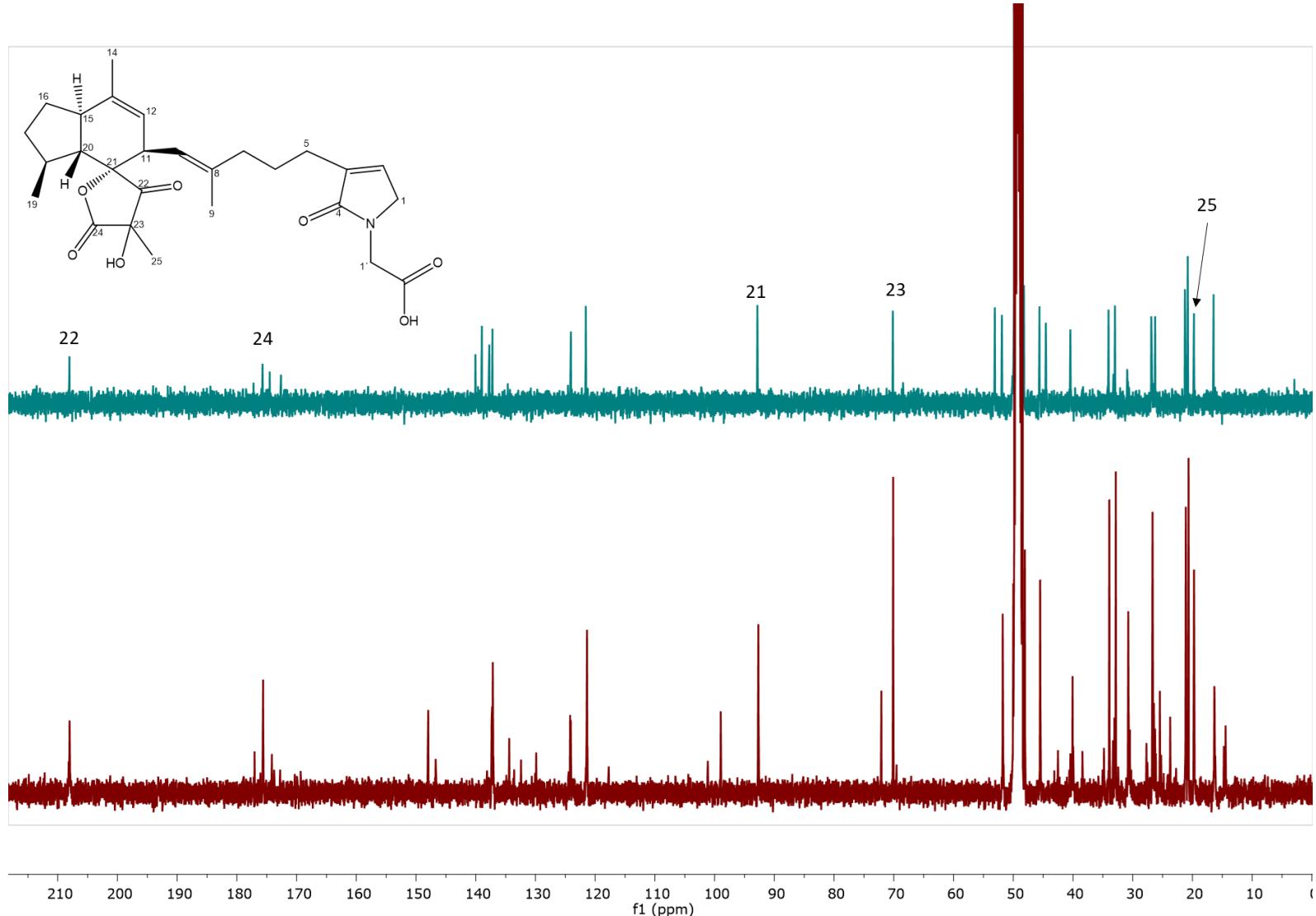
Figure S21. ^{13}C (100 MHz) of oxoircinianin lactam (**5**) A (top) and ^{13}C (100 MHz) of oxoircinianin (**3**) containing fraction (bottom) in d_4-MeOH – Peaks representing the "oxo"-form of the tetronic acid motif are labeled.
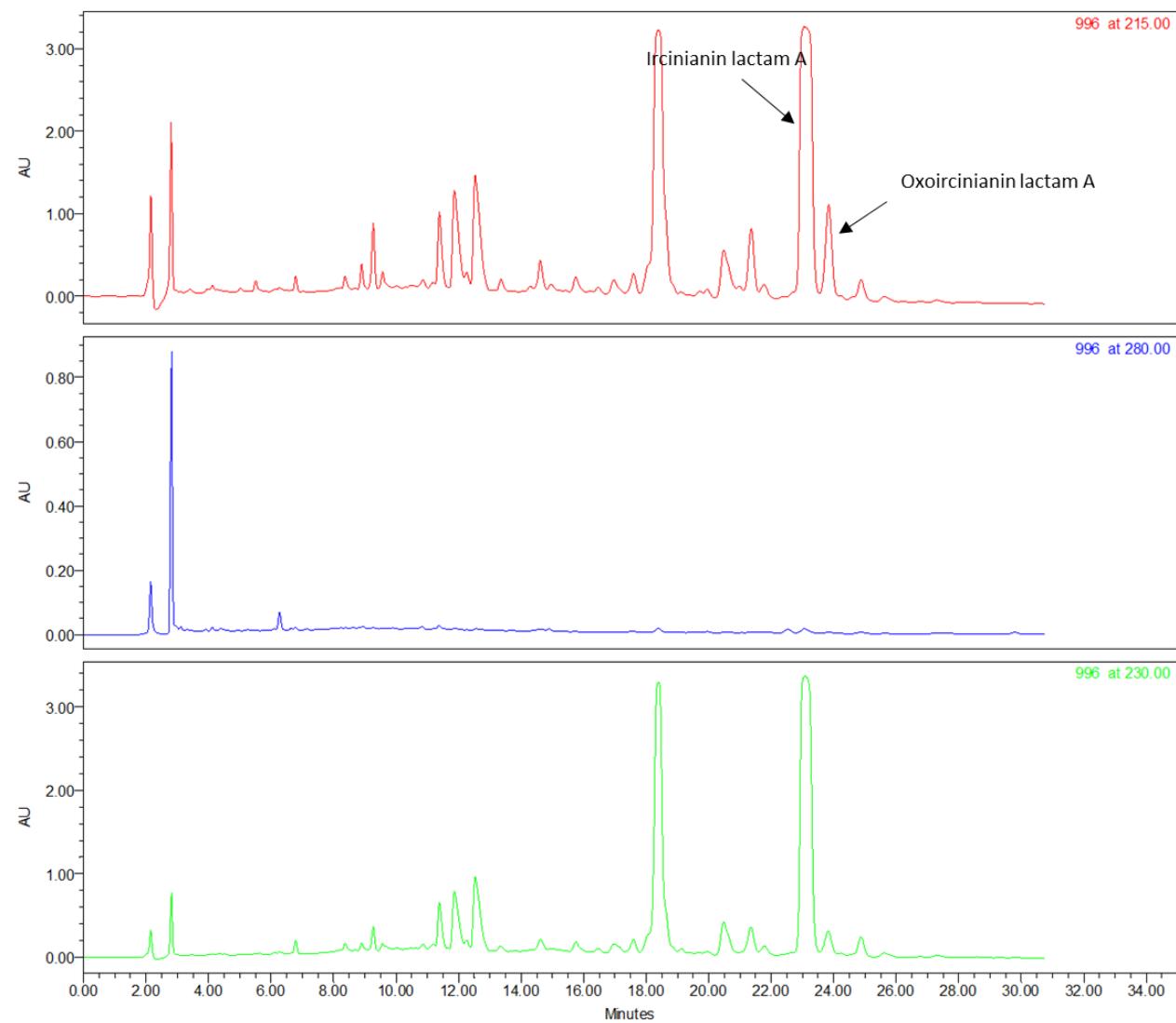
Figure S22. Chromatogram of the 50%-LC-fraction.

A

B
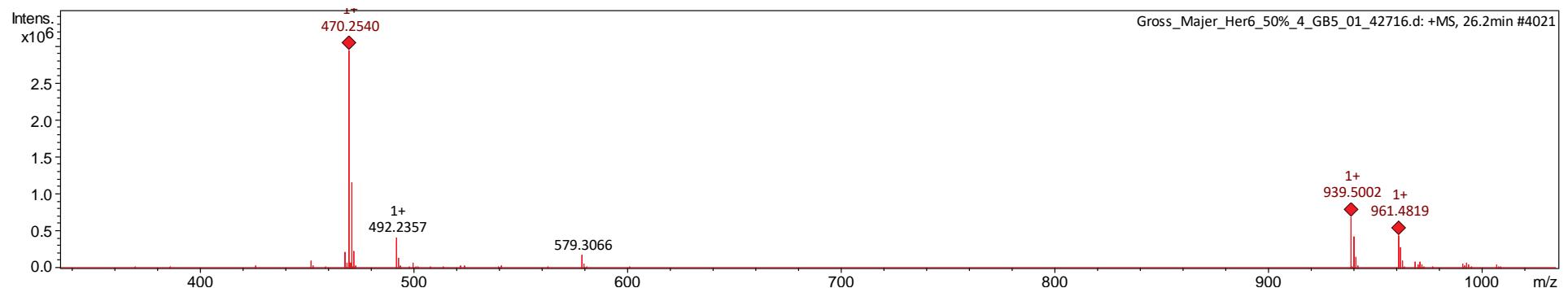
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
470.2540	1	$C_{28}H_{32}N_5O_2$	470.2551	2.3	2.3	16.0	ok	even	35.6	56.5
470.2540	2	$C_{27}H_{36}NO_6$	470.2537	-0.5	-0.3	11.0	ok	even	46.7	72.9
470.2540	3	$C_{24}H_{28}N_{11}$	470.2524	-3.4	-4.2	17.0	ok	even	55.4	77.6
470.2540	4	$C_{23}H_{32}N_7O_4$	470.2510	-6.2	-6.7	12.0	ok	even	68.0	95.0
470.2540	5	$C_{16}H_{36}N_7O_9$	470.2569	6.3	5.4	3.0	ok	even	110.2	161.1
470.2540	6	$C_{13}H_{28}N_{17}O_3$	470.2556	3.4	1.3	9.0	ok	even	111.3	166.9
470.2540	7	$C_{12}H_{32}N_{13}O_7$	470.2542	0.6	-1.2	4.0	ok	even	123.9	186.6
470.2540	8	$C_9H_{24}N_{23}O$	470.2529	-2.3	-5.8	10.0	ok	even	125.1	192.9

C
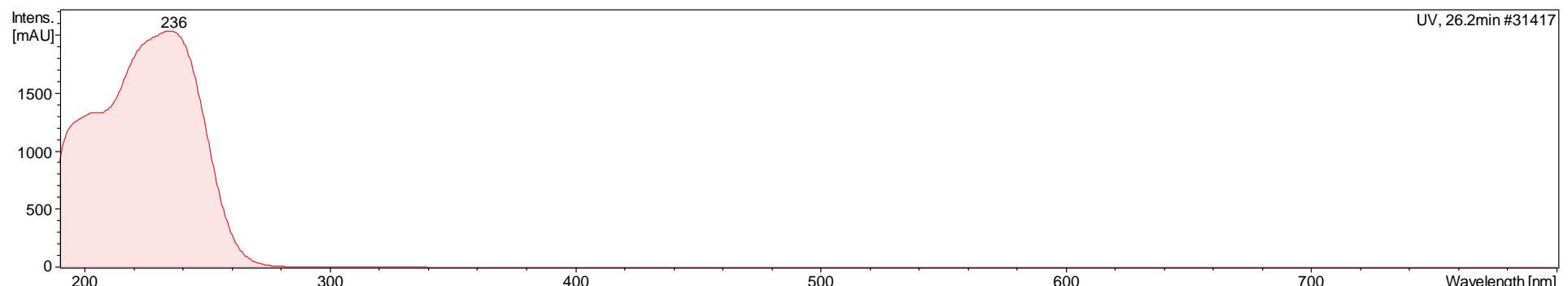
Figure S23. Ircinianin lactam A (**4**, 50%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profil.

Table S1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of ircinianin lactam A (**4**) in d_4-MeOH. Chemical shifts are given in ppm.

| Position	δ_H (mult., J [Hz])	δ_C
(-) Ircinianin lactam A (4)		
1	4.07, m^A	53.2
2	6.94, m	139.1
3	-	140.1
4	-	172.5
5	2.22-2.29, m	26.2
6	1.71, m^B	27.0
7	2.08, dd (7.2, 6.9)	40.4
8	-	136.2
9	1.57, d (1.1)	16.2
10	5.13, d (10.2)	125.5
11	3.07, dm (10.2)	48.8^E
12	5.02, m	123.5
13	-	137.0
14	1.71, m^B	20.71^F
15	2.39-2.47 (m)	46.1
16	*a.* 1.87-1.92, m^C *b.* 1.19-1.42, m^{D*}	27.3
17	*a.* 1.99, m^C *b.* 1.19-1.42, m^{D*}	33.6
18	1.66-1.69, m*	33.2
19	0.93, m*	20.71^F
20	1.58-1.64, m	51.7
21	-	86.8
22	-	179.2
23	-	97.4
24	-	177.7
25	1.65, s	6.1
1`	4.07, br s	44.4
2`	-	174.6

$^{A/B/C/D/F}$ Overlapping signals within one column.

* Overlapped by n-butanol impurity.

^{E} Overlapped by solvent peak.
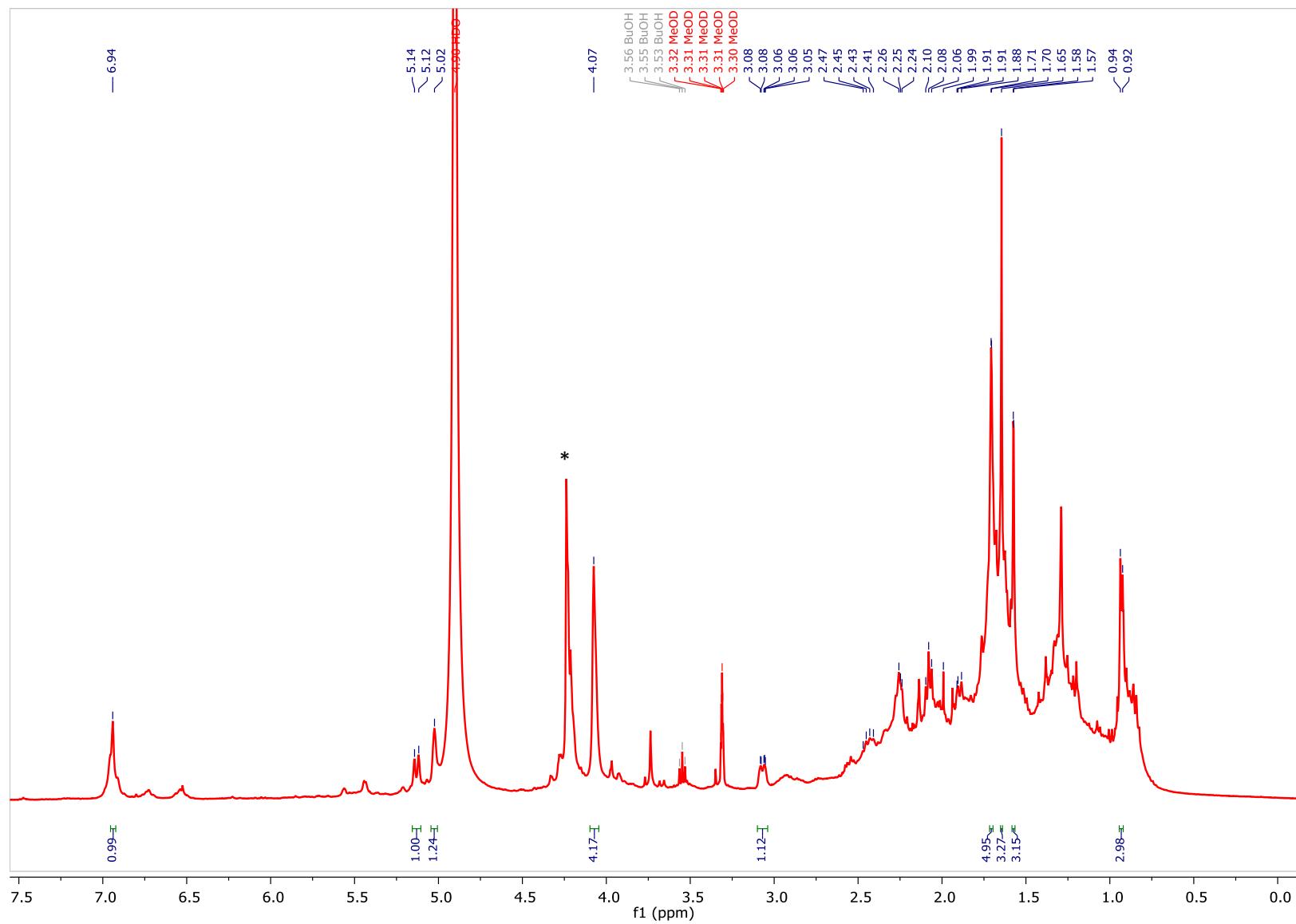
Figure S24. 400 MHz ^{1}H NMR spectrum of ircinianin lactam A (**4**, 50%-fraction) in d_4-MeOH. Asterisk denotes an impurity at 4.23 ppm.
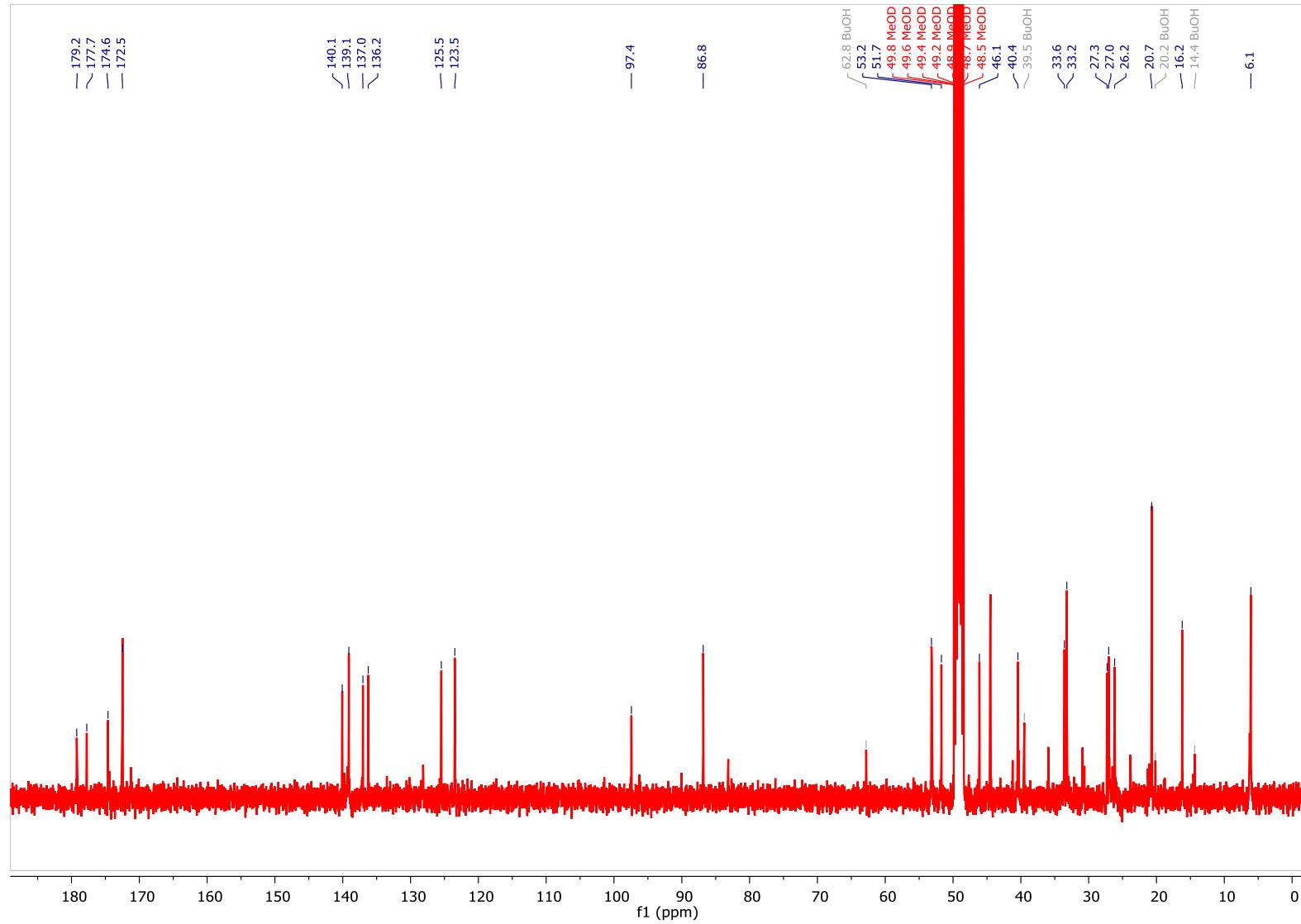
Figure S25. 100 MHz ^{13}C NMR spectrum of ircinianin lactam A (**4**, 50%-fraction) in d_4-MeOH.

A

B
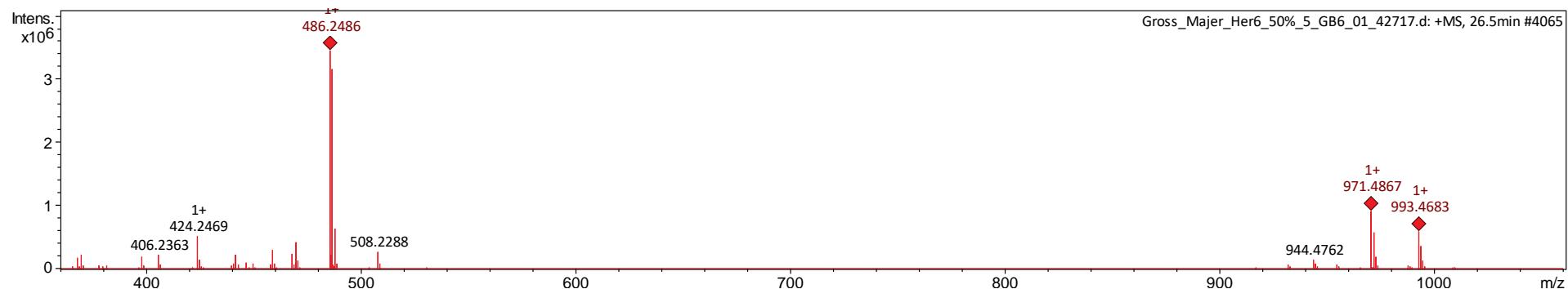
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
486.2486	1	$C_{28}H_{32}N_5O_3$	486.2500	2.9	4.1	16.0	ok	even	300.2	318.9
486.2486	2	$C_{27}H_{36}NO_7$	486.2486	0.1	1.6	11.0	ok	even	311.6	335.7
486.2486	3	$C_{23}H_{32}N_7O_5$	486.2459	-5.4	-4.7	12.0	ok	even	323.7	358.5
486.2486	4	$C_{22}H_{36}N_3O_9$	486.2446	-8.2	-7.2	7.0	ok	even	335.0	376.2
486.2486	5	$C_{24}H_{28}N_{11}O$	486.2473	-2.7	-2.2	17.0	ok	even	360.7	341.7
486.2486	6	$C_{14}H_{24}N_{21}$	486.2518	6.6	5.4	14.0	ok	even	404.1	411.7
486.2486	7	$C_{16}H_{36}N_7O_{10}$	486.2518	6.7	7.1	3.0	ok	even	416.0	425.7
486.2486	8	$C_{13}H_{28}N_{17}O_4$	486.2505	3.9	3.1	9.0	ok	even	416.9	431.1
486.2486	9	$C_{12}H_{32}N_{13}O_8$	486.2491	1.1	0.7	4.0	ok	even	429.7	451.0
486.2486	10	$C_9H_{24}N_{23}O_2$	486.2478	-1.6	-3.6	10.0	ok	even	430.7	457.0

C
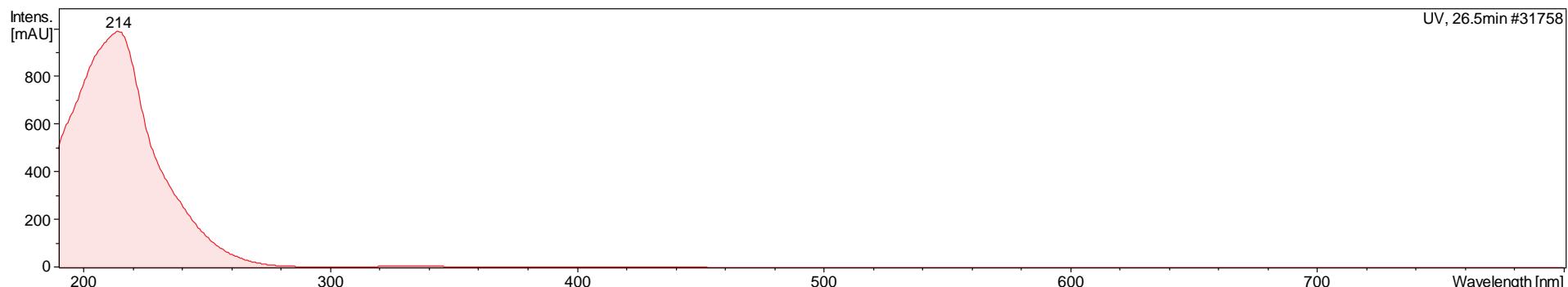
**Figure S26.** Oxoircinianin lactam A (**5**, 50%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.

Table S2. 400 MHz ^{1}H and 100 MHz ^{13}C NMR-data of oxoircinianin lactam A (**5**) in d_4-MeOH. Chemical shifts are given in ppm.

Position	δ_H (mult., J [Hz])	δ_C
**(-)-Oxoircinianin lactam A (5)**	
1	4.07, m	53.1
2	6.96, m	139.0
3	-	140.1
4	-	174.5
5	2.26, br t (7.5)	26.3
6	1.64-1.70, m*	26.9[A]
7	2.05, dd (7.3, 7,1)[B]	40.4
8	-	137.7
9	1.58, d (1.1)	16.5
10	5.00, m[C]	124.1
11	3.34, m[F]	48.2
12	5.03, m[C]	121.6
13	-	137.2
14	1.72, d (1.2)[E]	21.3
15	2.36-2.49, m	45.6
16	a. 1.89-1.95, m b. 1.28-1.36, m[D]	26.8[A]
17	a. 2.05, m[B] b. 1.28-1.36, m[D]	34.1
18	1.73, m[E]	33.0
19	0.83, d (6.1)	20.8
20	1.65-1.73, m*	51.9
21	-	92.9
22	-	208.0
23	-	70.2
24	-	175.7
25	1.38, s	19.8
1´	4.23, s	44.6
2´	-	172.6

[A] Assignments interchangeable.
* Overlapped by other signals.
[B/C/D/E] Overlapping signals within one column.
[F] Overlapped by solvent peak.
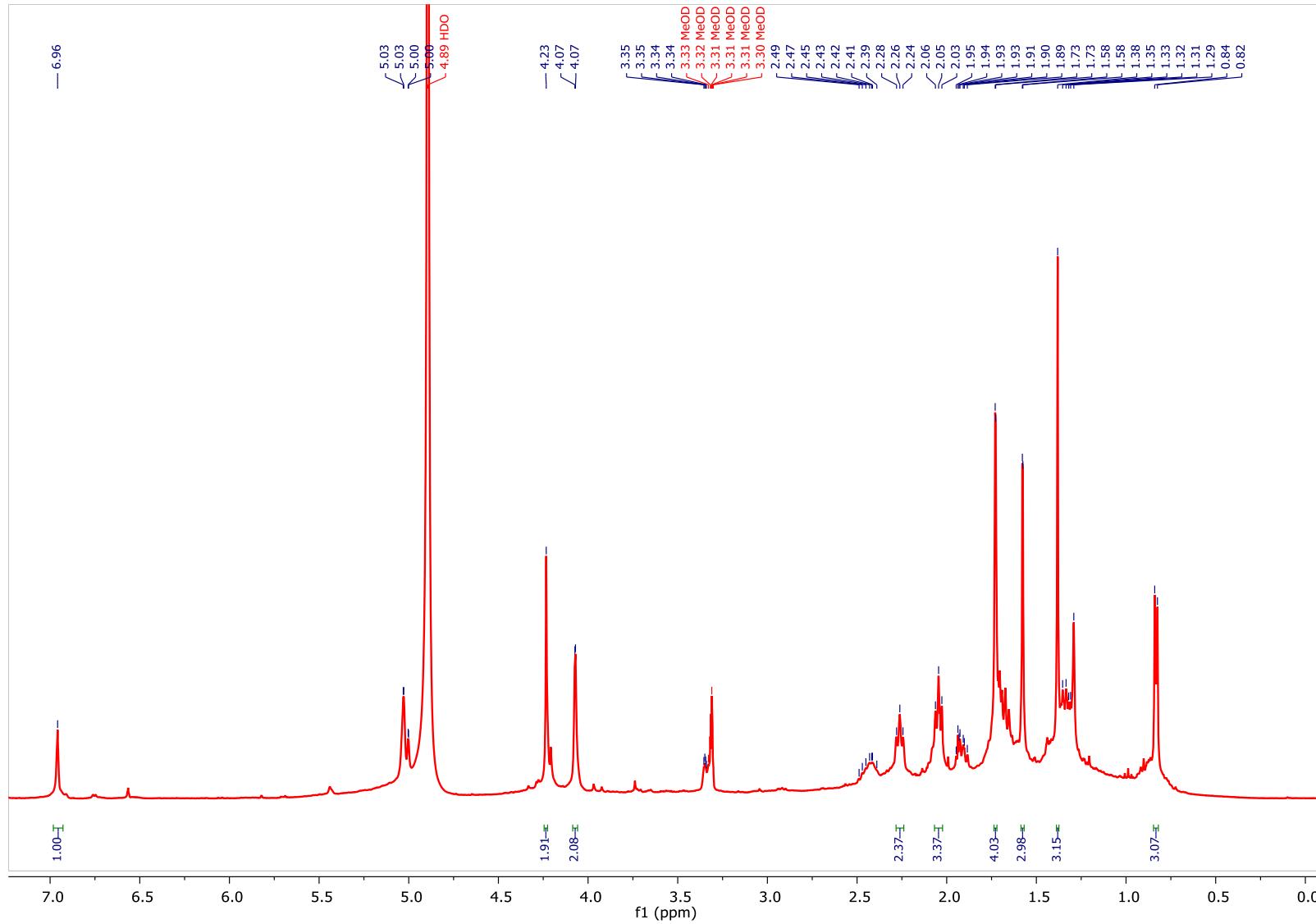
Figure S27. 400 MHz ^{1}H NMR spectrum of oxorcinianin lactam A (**5**, 50%-fraction) in d_4-MeOH.
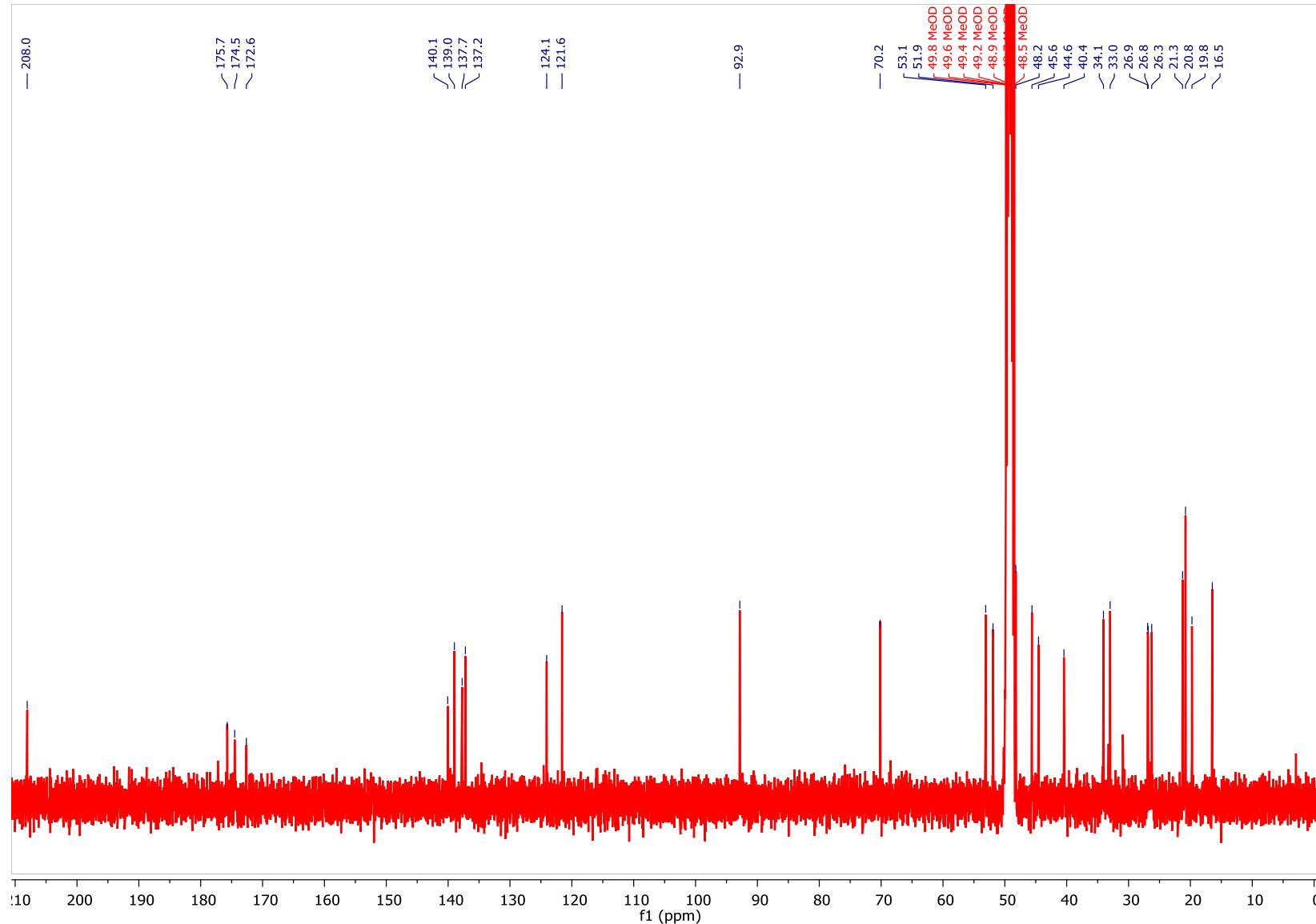
Figure S28. 100 MHz ^{13}C NMR spectrum of oxoircinianin lactam A (**5**, 50%-fraction) in d_4-MeOH.

A

B
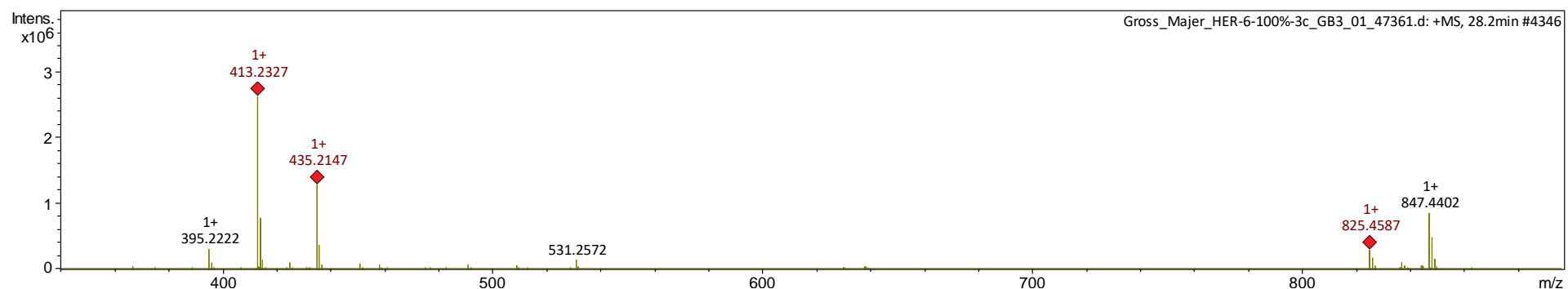
| Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
413.2327	1	$C_{26}H_{29}N_4O$	413.2336	2.1	1.5	15.0	ok	even	4.6	6.3
413.2327	2	$C_{25}H_{33}O_5$	413.2323	-1.1	-1.4	10.0	ok	even	11.6	17.7
413.2327	3	$C_{21}H_{29}N_6O_3$	413.2296	-7.6	-8.6	11.0	ok	even	25.7	41.2
413.2327	4	$C_{14}H_{33}N_6O_8$	413.2354	6.6	5.1	2.0	ok	even	67.6	109.7
413.2327	5	$C_{11}H_{25}N_{16}O_2$	413.2341	3.3	0.2	8.0	ok	even	68.6	115.8
413.2327	6	$C_{10}H_{29}N_{12}O_6$	413.2328	0.1	-2.4	3.0	ok	even	81.2	136.3
413.2327	7	$C_7H_{21}N_{22}$	413.2314	-3.2	-8.3	9.0	ok	even	82.4	143.0

C
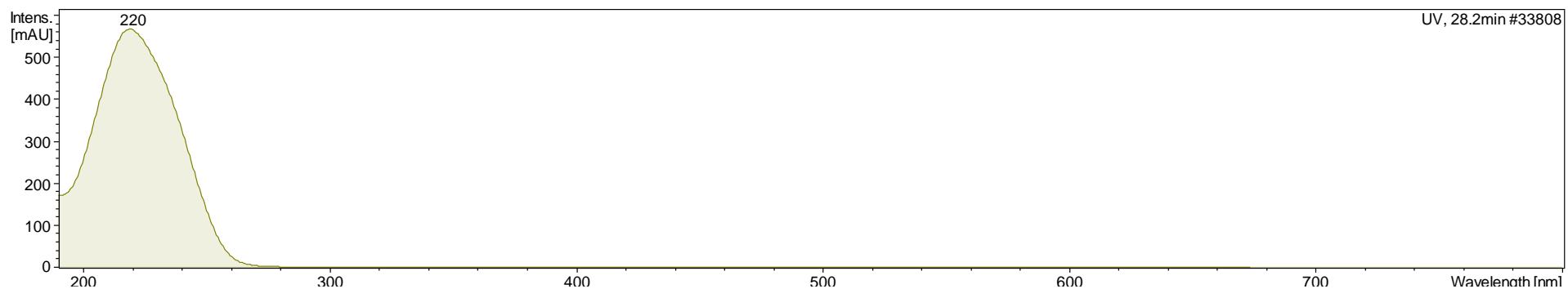
Figure S29. Ircinianin lactone A (**6**, 100%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.

Table S3. 400 MHz ^{1}H and 100 MHz ^{13}C NMR-data of ircinianin lactone A (**6**) in d_4-MeOH. Chemical shifts are given in ppm.

| (-)-Ircinianin lactone A (6)		
Position	δ_H **(mult., J [Hz])^a**	δ_C
1	4.83, br q (1.7)	72.1
2	7.37, br quin (1.4)	147.9
3	-	134.4
4	-	177.0
5	2.26, m	25.7
6	1.69, m^B	26.7
7	2.08, dd (7.6, 7.4)	40.3
8	-	136.0
9	1.58, d (1.1)	16.1
10	5.14, d (10.4)	125.3
11	3.07, dm (10.4)	48.6^F
12	5.03, m	123.3
13	-	137.0
14	1.71, d (1.1)^B	20.5^C
15	2.41, m	46.0
16	a. 1.89, m^A b. 1.32, m^C	27.1
17	a. 2.01, m^A b. 1.30, m^C	33.5
18	1.64, m^E	33.1
19	0.92, d (6.1)	20.6^C
20	1.61, m*	51.7
21	-	86.7
22	-	179.3^D
23	-	97.3
24	-	177.6^D
25	1.64, s^E	6.0
^{a} Assignments supported by ^{1}H-^{13}C multiplicity edited HSQC. $^{A/B/E}$ Overlapping signals within a column. $^{C/D}$ Assignments interchangeable. * Overlapped by other signals. ^{F} Overlapped by solvent peak.		
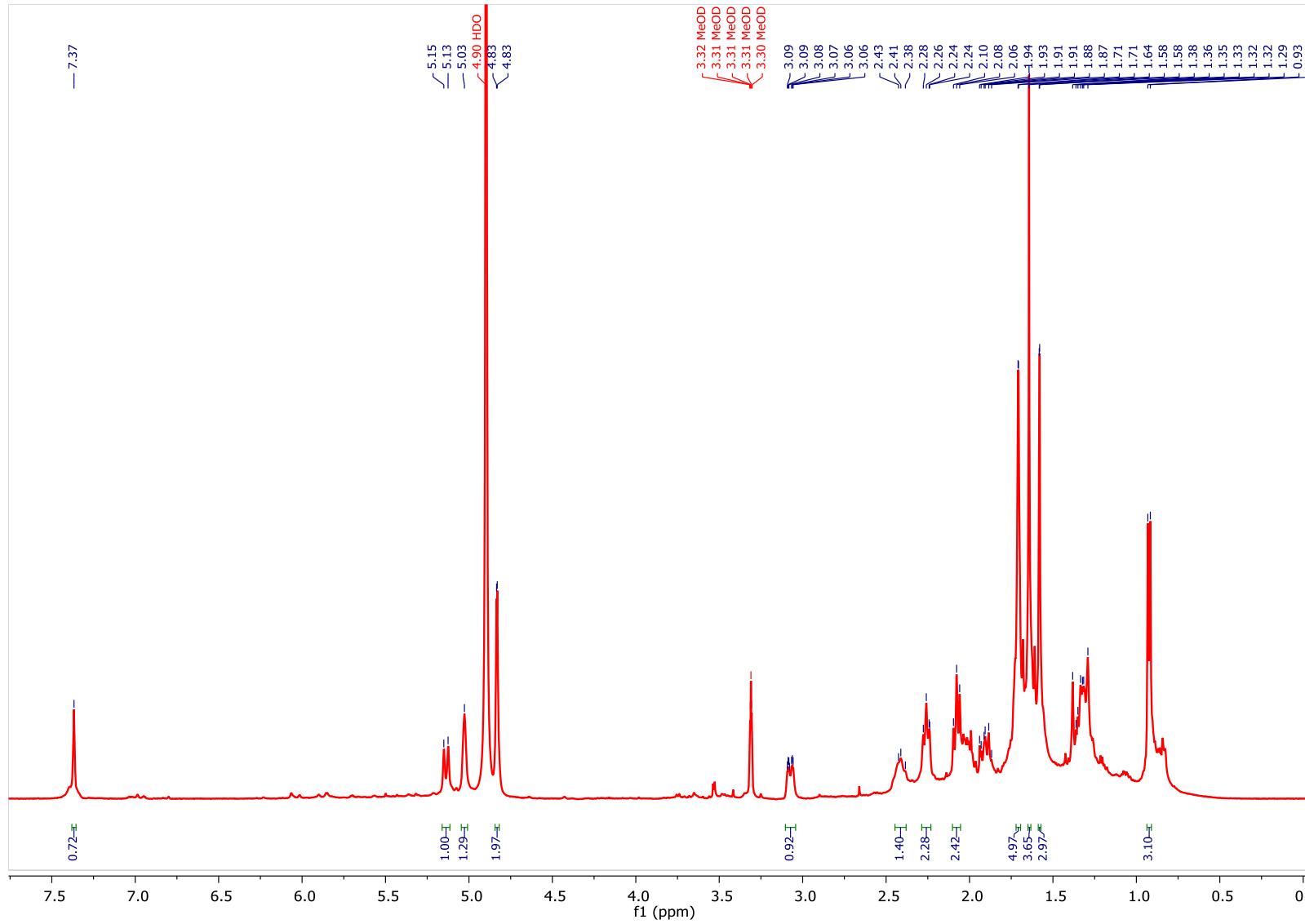
Figure S30. 400 MHz ¹H NMR spectrum of ircinianin lactone A (**6**, 100%-fraction) in d_4-MeOH.
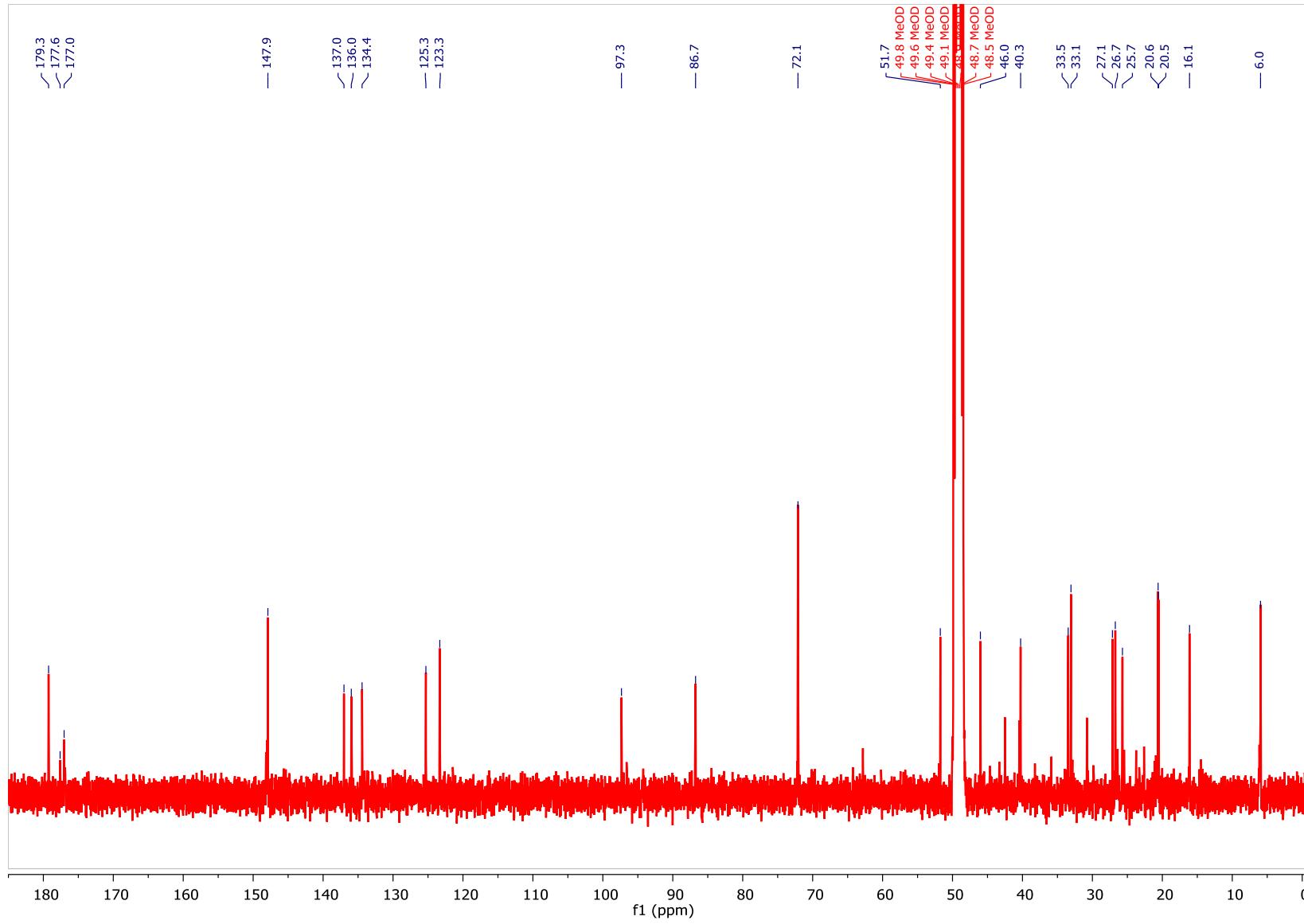
Figure S31. 100 MHz ^{13}C NMR spectrum of ircinianin lactone A (**6**, 100%-fraction) in d_4-MeOH.
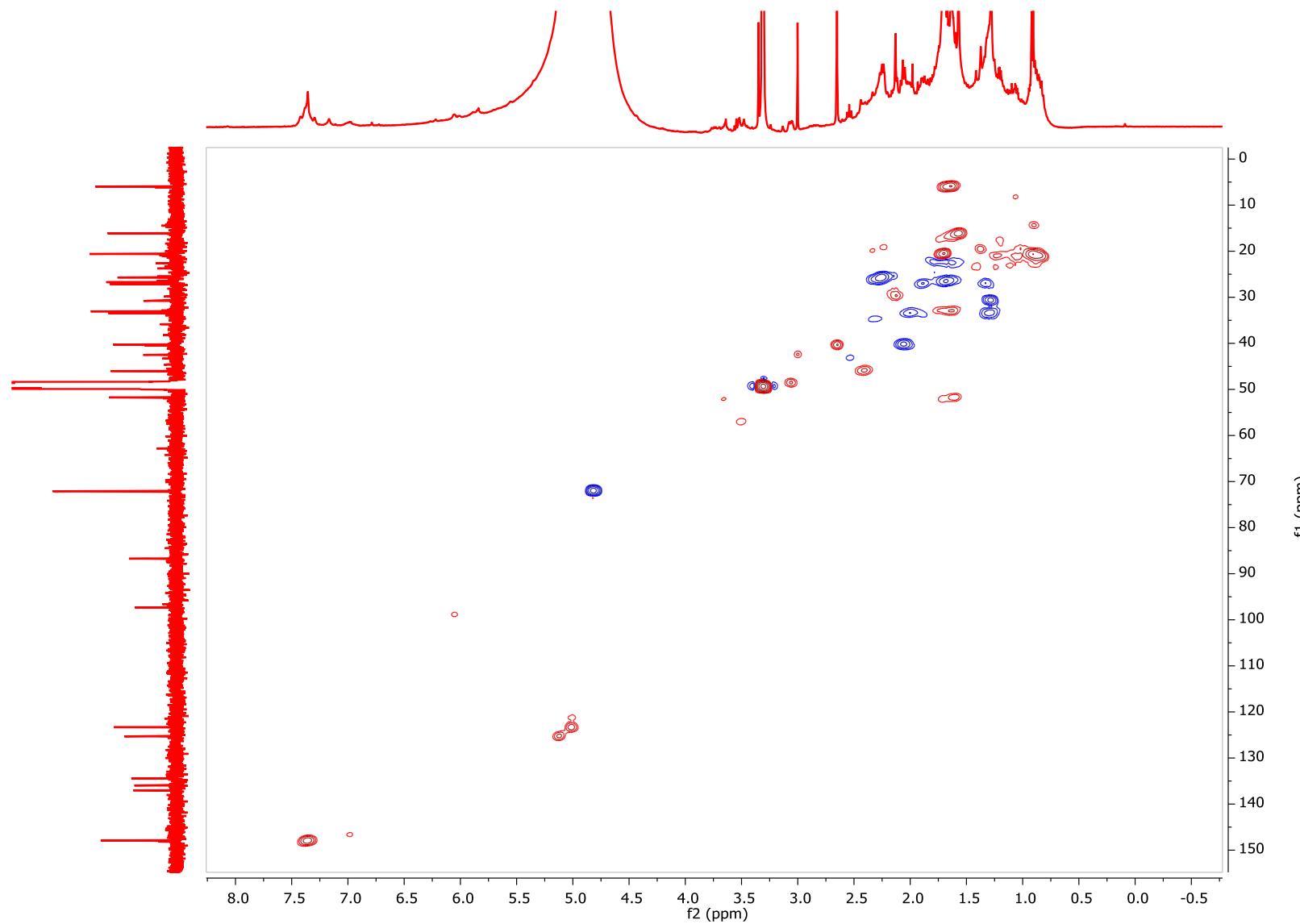
Figure S32. 400 MHz multiplicity edited ^{1}H-^{13}C HSQC NMR spectrum of ircinianin lactone A (**6**, 100%-fraction) in d_4-MeOH.

A

B
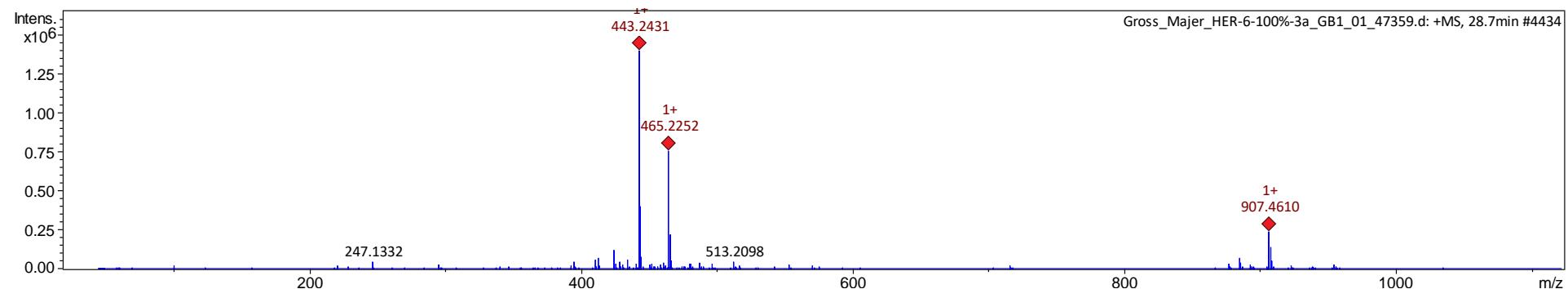
| Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I
443.2431	1	$C_{26}H_{35}O_6$	443.2428	-0.6	-2.1	10.0	ok	even	3.2	7.1
443.2431	2	$C_{23}H_{27}N_{10}$	443.2415	-3.7	-5.2	16.0	ok	even	10.1	16.3
443.2431	3	$C_{27}H_{31}N_4O_2$	443.2442	2.4	1.5	15.0	ok	even	13.3	17.0
443.2431	4	$C_{22}H_{31}N_6O_4$	443.2401	-6.7	-7.9	11.0	ok	even	16.5	27.6

C
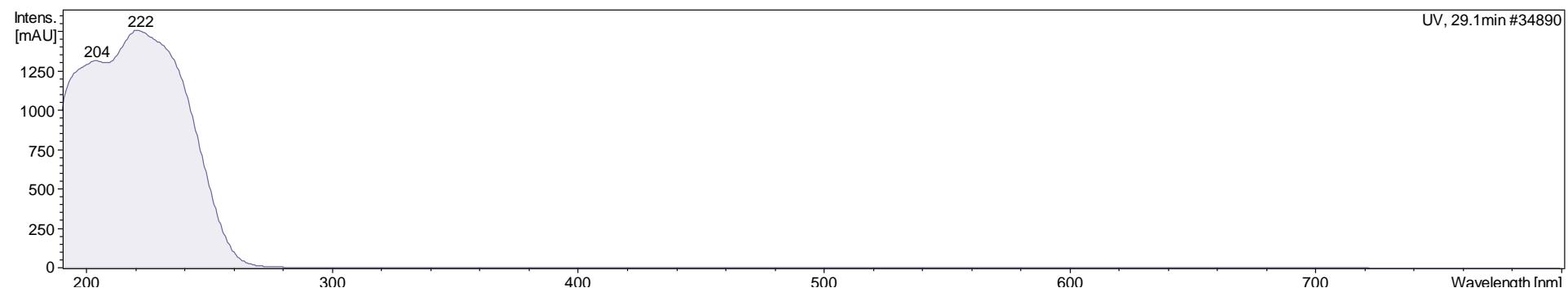
Figure S33. Ircinianin lactone B (**7**), 100%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.

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Table S4. Comparison ^{1}H (400 MHz) and ^{13}C (100 MHz) chemical shift values (ppm) of ircinianin lactone A (**6**) and ircinianin lactone B (**7**), recorded in d_4-MeOH. The column on the far right of the table indicates the shift-deviations in a color-coded way: <mark style="background:lime">no deviations</mark>; <mark style="background:yellow">minor to significant shift deviations</mark>; <mark style="background:red">strong shift deviations</mark>.

| Ircinianin lactone A (6)			Ircinianin lactone B (7)			Δ
Position	δ_H, mult. (J in Hz)	δ_C, type	Position	δ_H, mult. (J in Hz)	δ_C, type	|δ_H(6)- δ_H(7)| / |δ_C(6)- δ_C(7)|
1	4.83, br q (1.7)	72.1, CH$_2$	1	5.84, br q (1.2)	104.4, CH	1.01 / 32.3
2	7.37, br quin (1.4)	147.9, CH	2	6.98, br quin (1.2)	144.65; 144.72, CH	0.39 / 3.18 - 3.25
3	-	134.4, C	3	-	139.03; 139.08, C	- / 4.63 - 4.68
4	-	177.0, C	4	-	173.5, C	- / 3.5
5	2.26, m	25.7, CH$_2$	5	2.26, br t (7.8)	25.5, CH$_2$	0.00 / 0.2
6	1.69, m	26.7, CH$_2$	6	1.67, m	26.44; 26.47, CH$_2$	0.02 / 0.23 - 0.26
7	2.08, dd (7.6, 7.4)	40.3, CH$_2$	7	2.07, dd (7.9, 7.2)	40.2, CH$_2$	0.01 / 0.1
8	-	136.0, C	8	-	135.75; 135.78, C	- / 0.22 - 0.25
9	1.58, d (1.1)	16.1, CH$_3$	9	1.57, d (1.3)	16.06; 16.08, CH$_3$	0.01 / 0.02 – 0.04
10	5.14, d (10.4)	125.3, CH	10	5.12, m	125.41; 125.44, CH	0.02 / 0.11 – 0.14
11	3.07, dm (10.4)	48.6, CH	11	3.06, dm (10.3)	48.6, CH	0.01 / 0.0
12	5.03, m	123.3, CH	12	5.01, m	123.3, CH	0.02 / 0.0
13	-	137.0, C	13	-	137.0, C	- / 0.0
14	1.71, d (1.1)	20.5, CH$_3$	14	1.70, d (1.4)	20.56, CH$_3$	0.01 / 0.06
15	2.41, m	46.0, CH	15	2.40, m	46.0, CH	0.01 / 0.0
16	1.89, m; 1.32, m	27.1, CH$_2$	16	1.89, m; 1.32, m	27.1, CH$_2$	0.00; 0.00 / 0.0
17	2.01, m; 1.30, m	33.5, CH$_2$	17	2.00, m; 1.29, m	33.4, CH$_2$	0.01; 0.01 / 0.1
18	1.64, m	33.1, CH	18	1.63, m	33.1, CH	0.01 / 0.0
19	0.92, d (6.1)	20.6, CH$_3$	19	0.92, d (6.2)	20.62, CH$_3$	0.00 / 0.02
20	1.61, m	51.7, CH	20	1.61, m	51.7, CH	0.00 / 0.0
21	-	86.7, C	21	-	86.7, C	- / 0.0
22	-	179.3, C	22	-	179.3, C	- / 0.0
23	-	97.3, C	23	-	97.3, C	- / 0.0
24	-	177.6, C	24	-	177.6, C	- / 0.0
25	1.64, s	6.0, CH$_3$	25	1.63, br s	6.0, CH$_3$	0.01 / 0.0
1′	-	-	1′	3.52, br s	57.13; 57.16, CH$_3$	3.52 / 57.13 -57.16
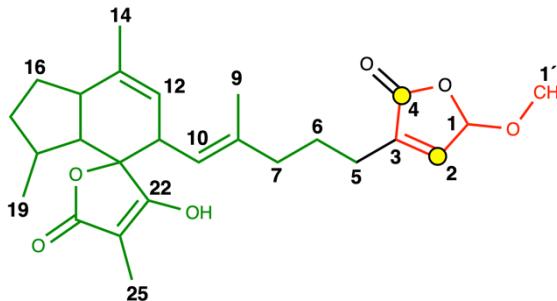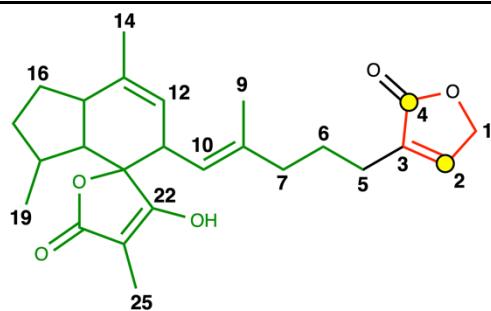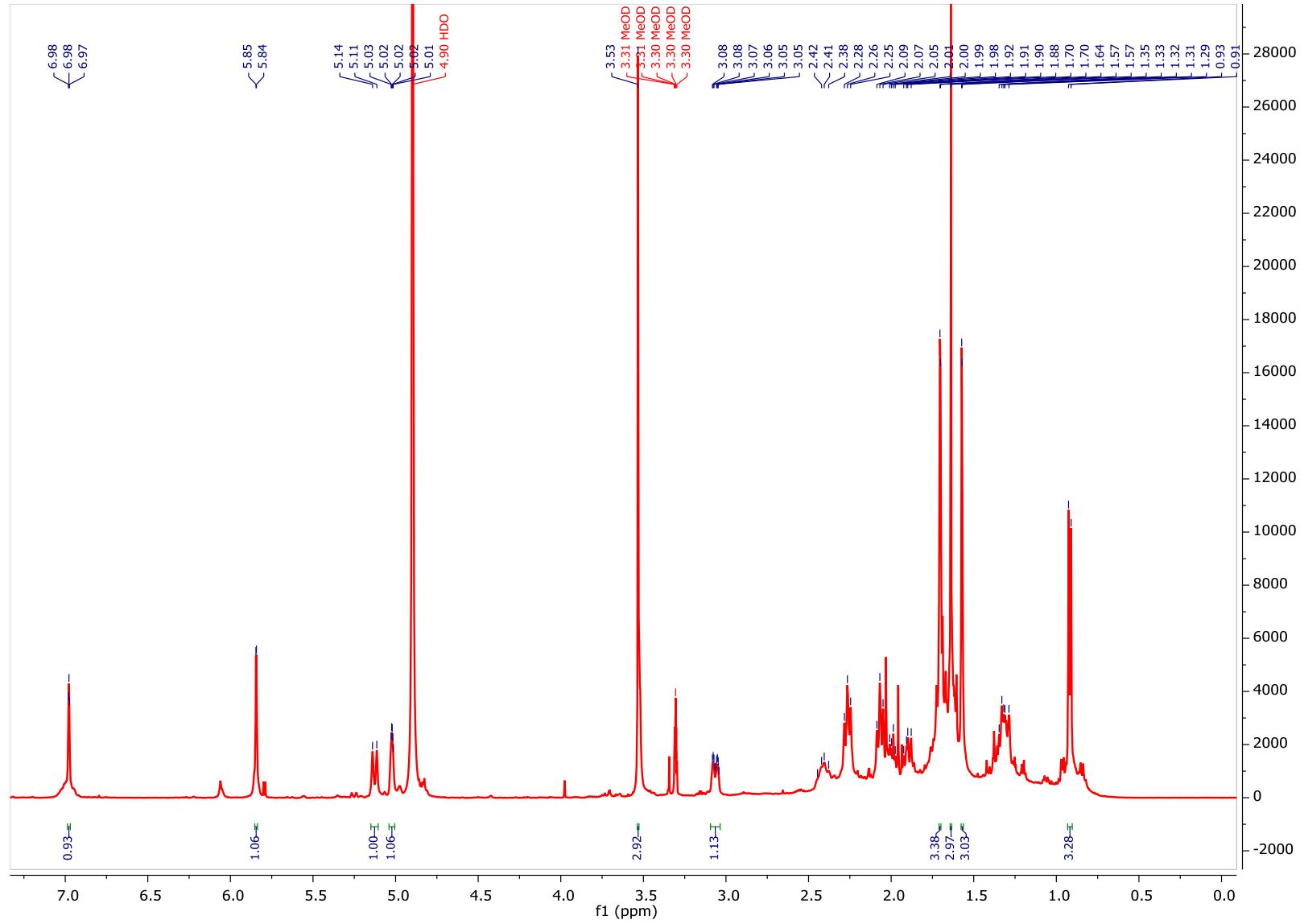
Figure S34. 400 MHz ^{1}H NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
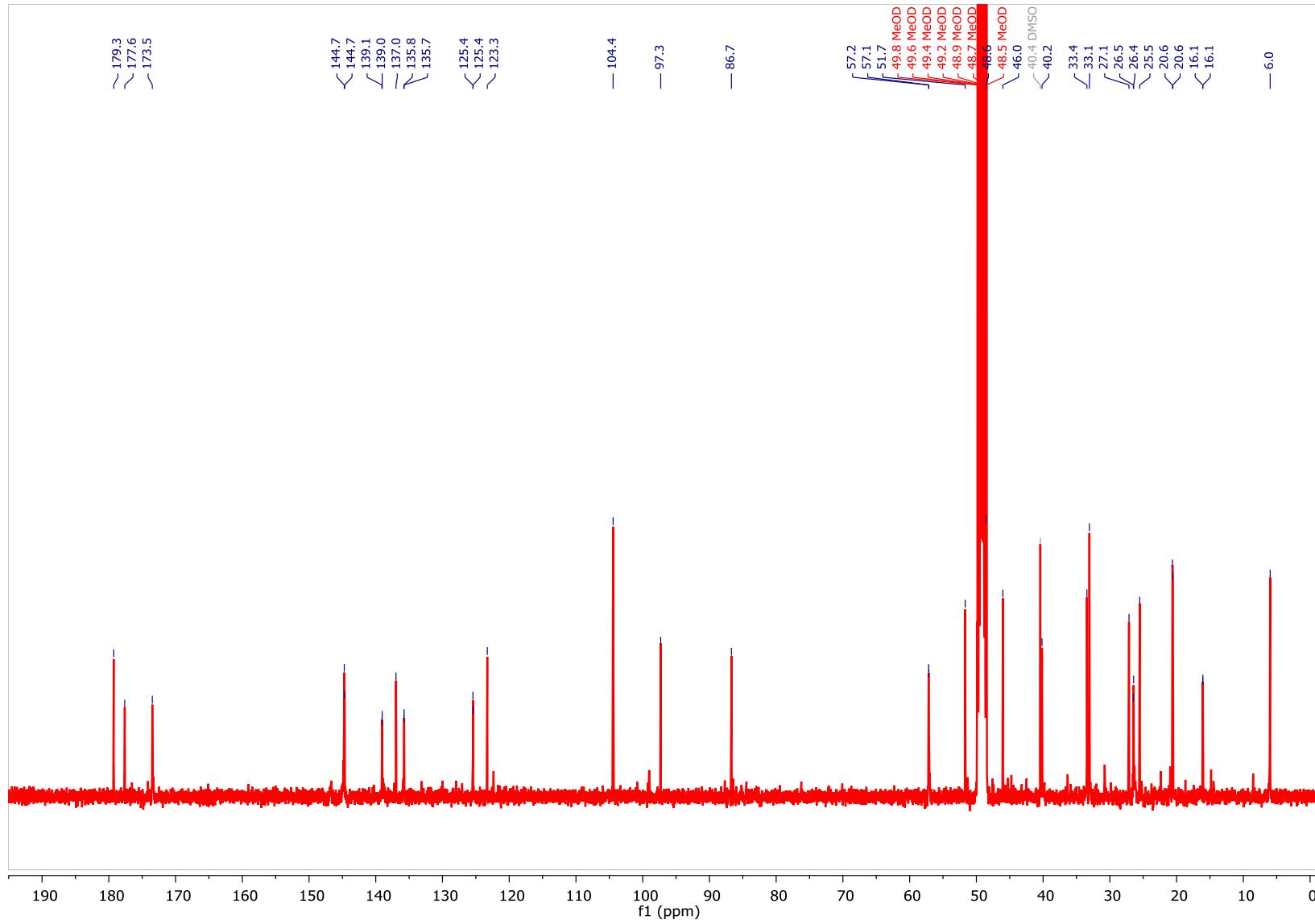
Figure S35. 100 MHz ^{13}C NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
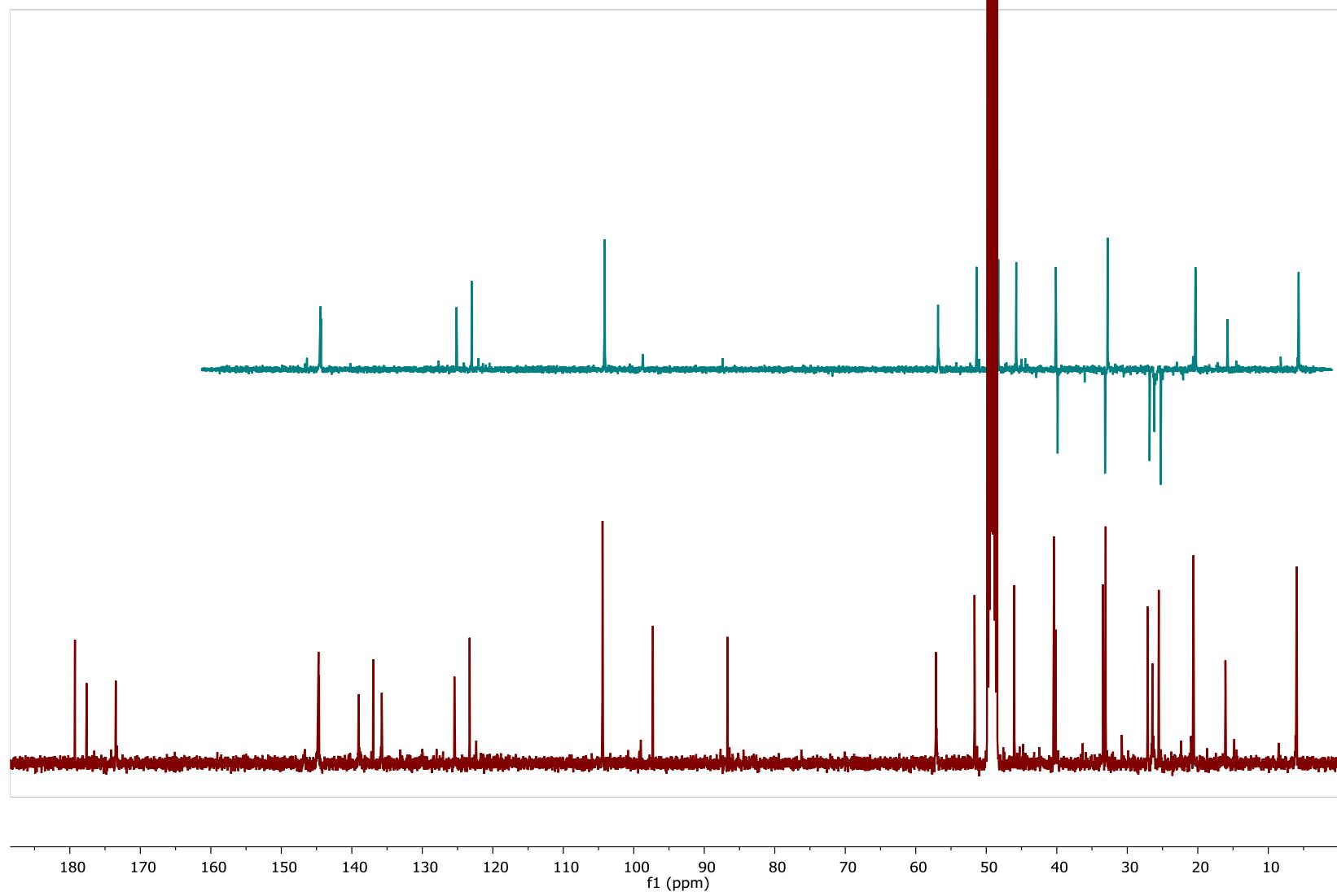
Figure S36. 100 MHz ^{13}C (bottom) and 400 MHz DEPT135 (top) NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
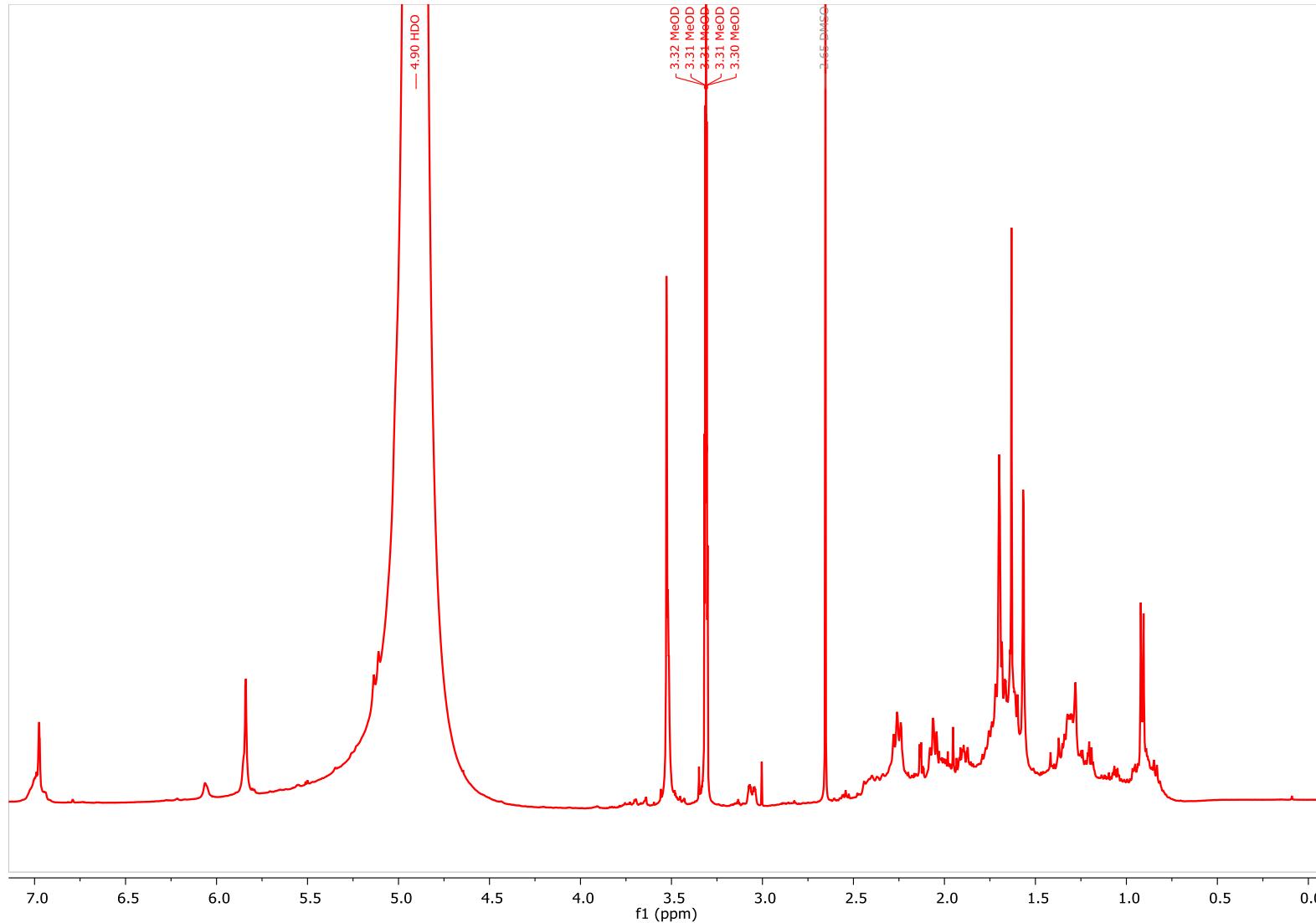
Figure S37. 400 MHz ^{1}H NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH (before 2D NMR experiments).
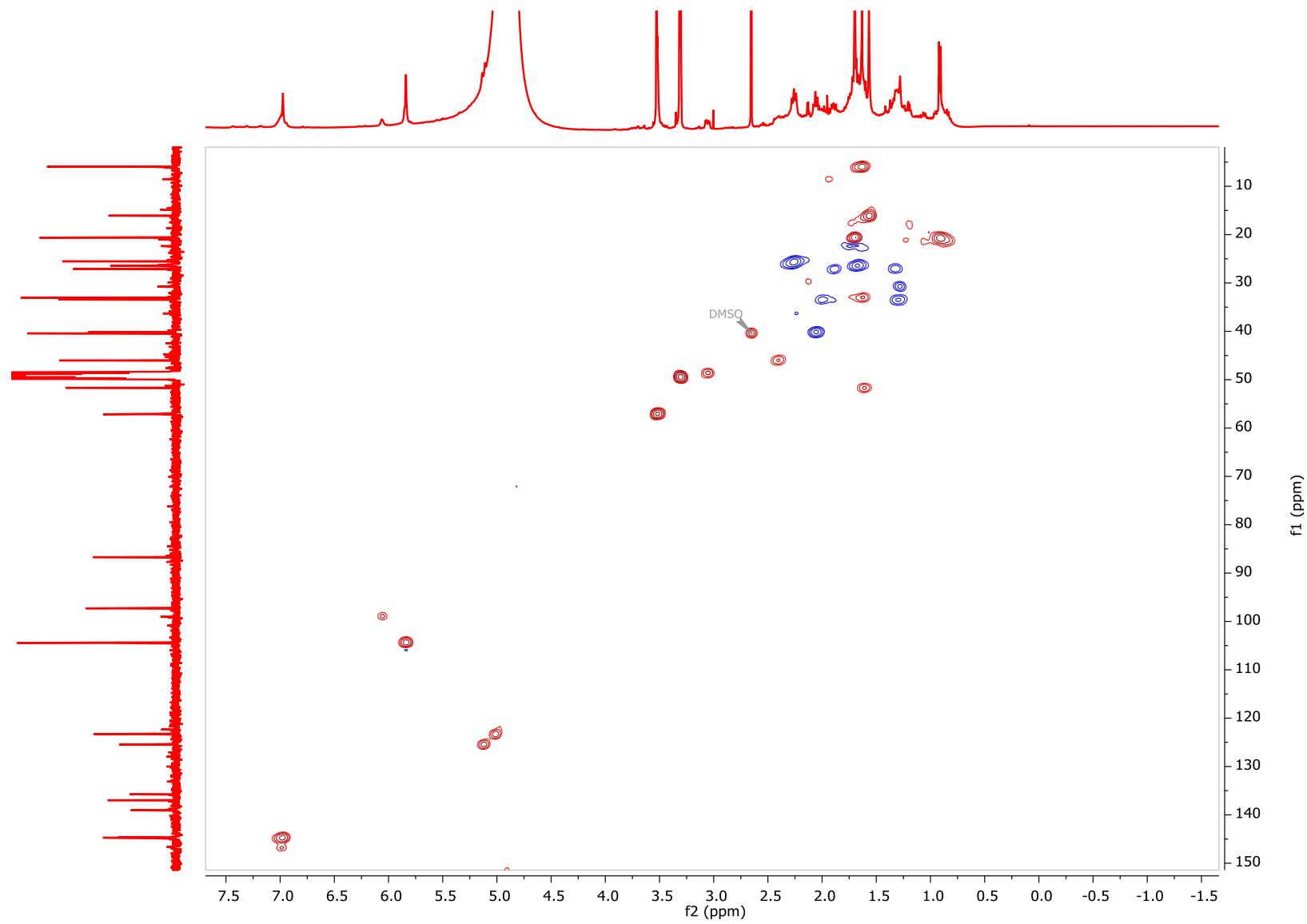
Figure S38. 400 MHz multiplicity edited ^{1}H-^{13}C HSQC NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
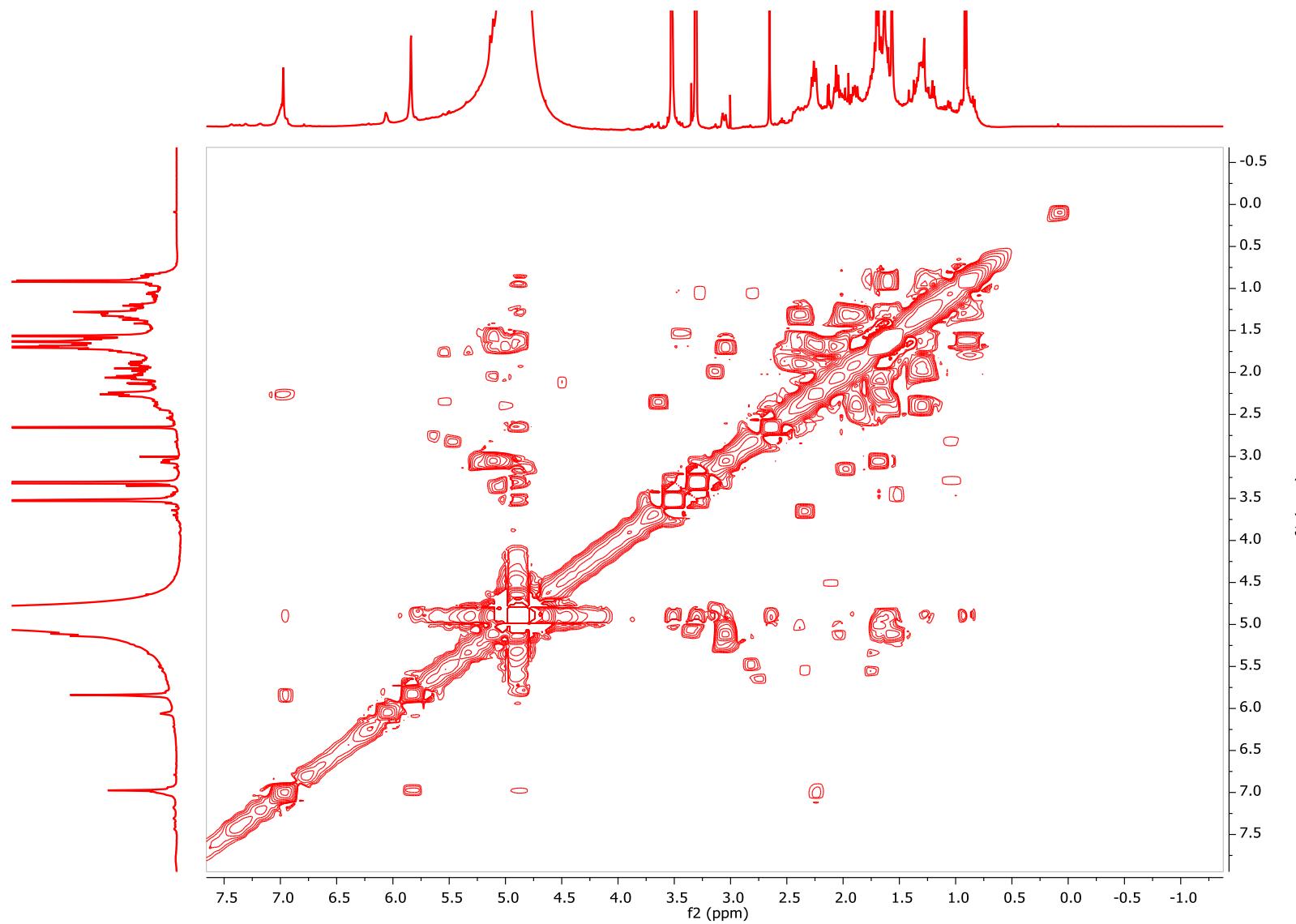
**Figure S39.** 400 MHz ^{1}H-^{1}H COSY NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
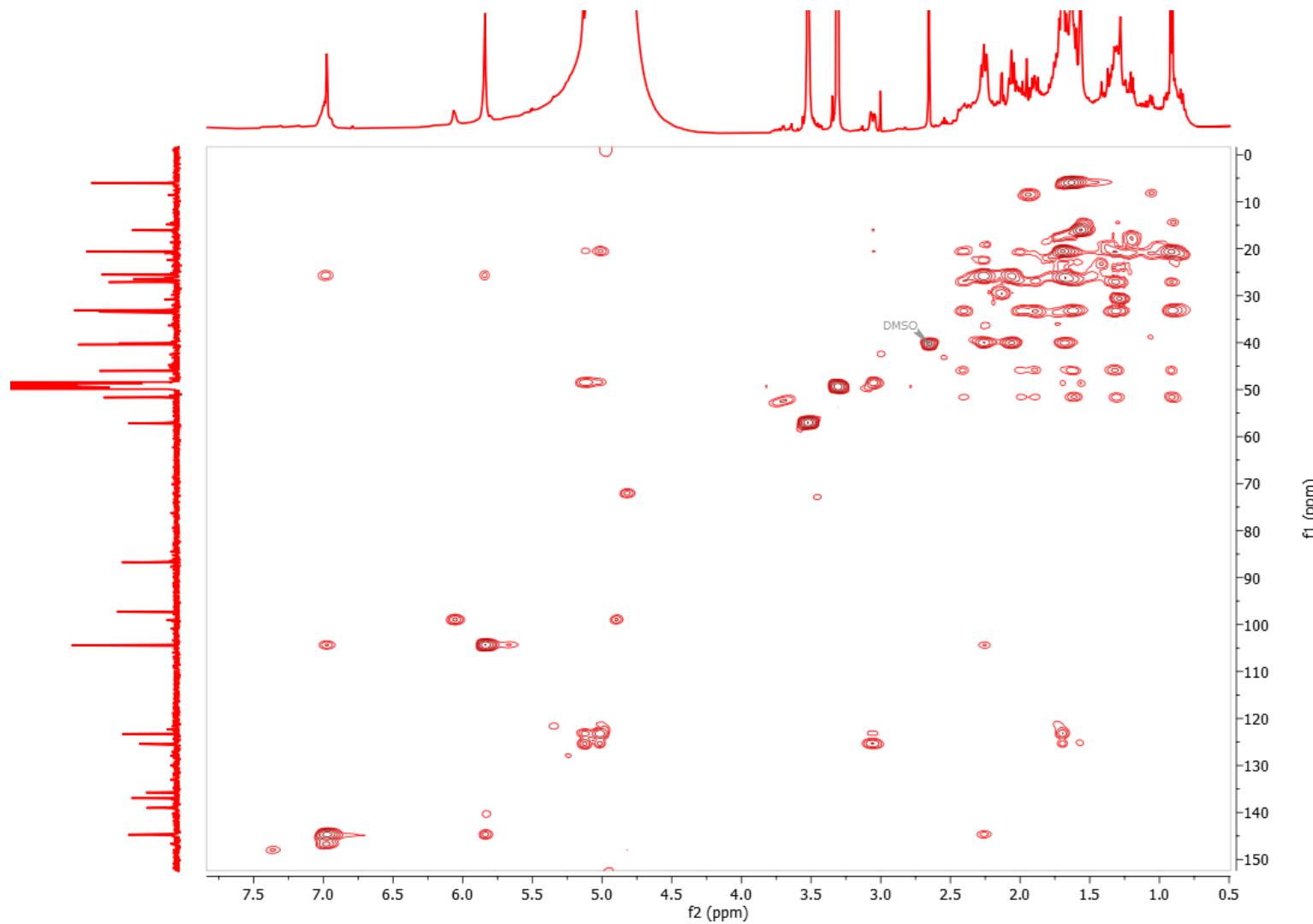
Figure S40. 400 MHz ^{1}H-^{13}C HSQC-TOCSY NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
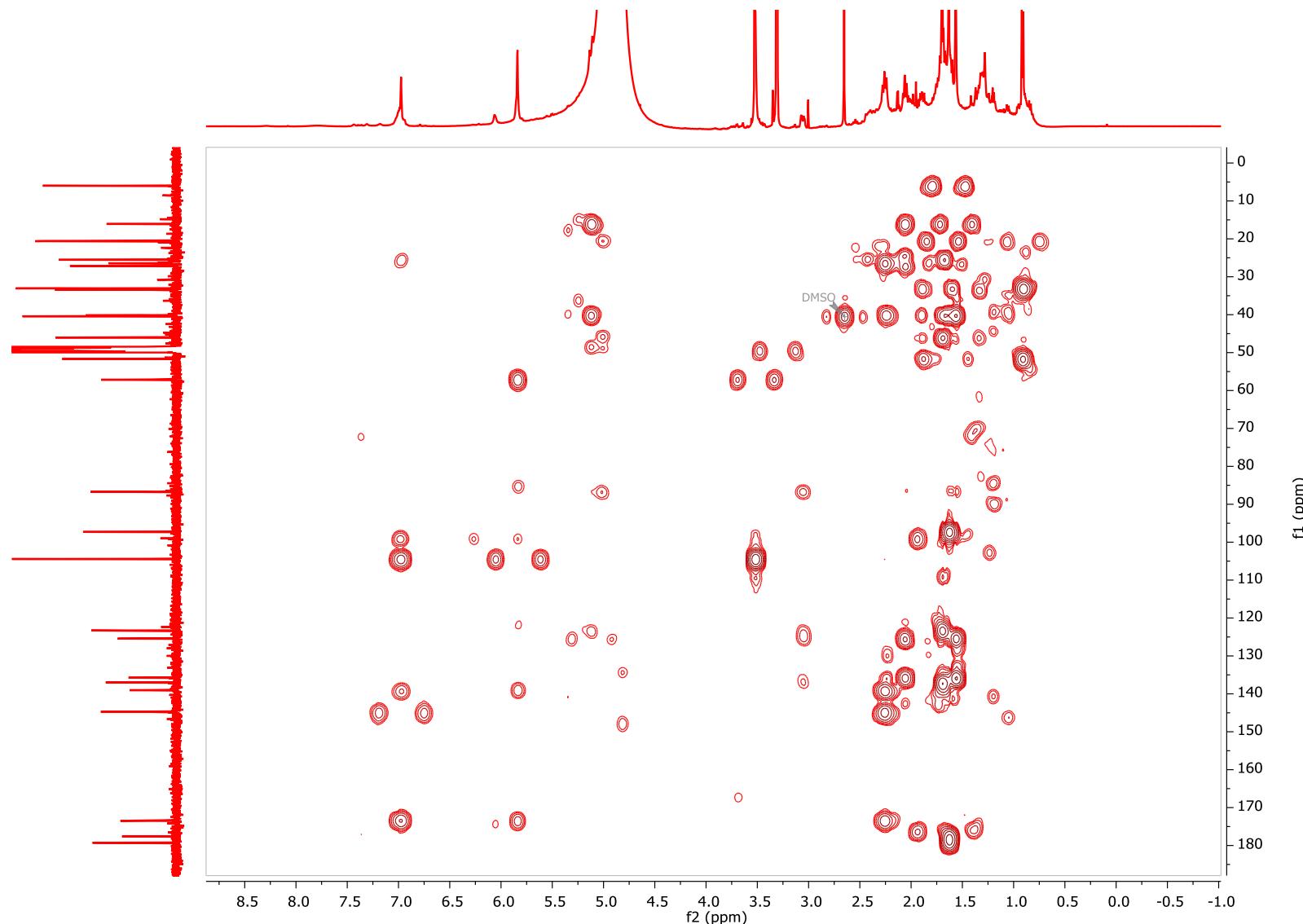
Figure S41. 400 MHz ^{1}H-^{13}C HMBC NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
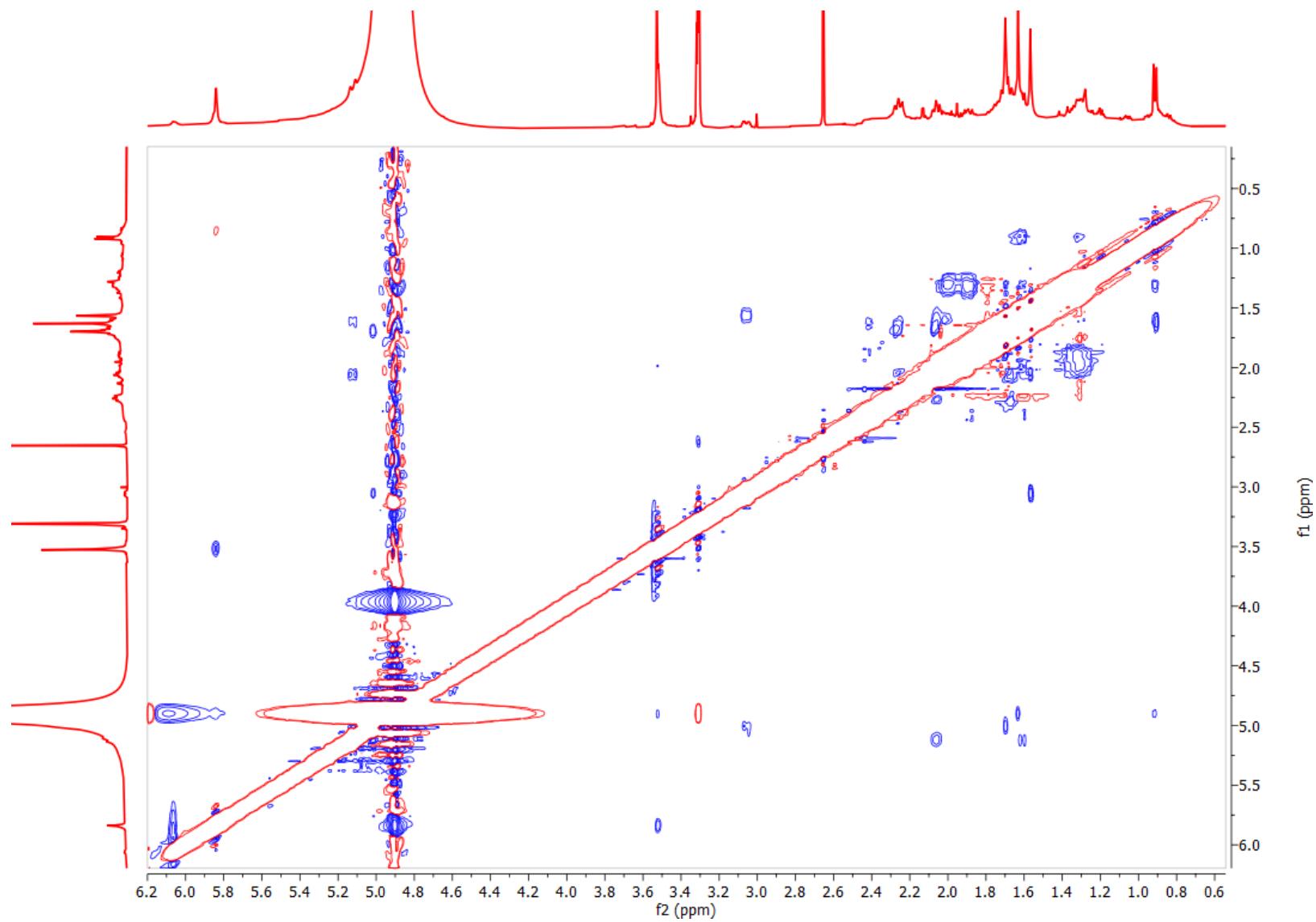
Figure S42. 400 MHz ^{1}H-^{1}H NOESY NMR spectrum of ircinianin lactone B (**7**, 100%-fraction) in d_4-MeOH.
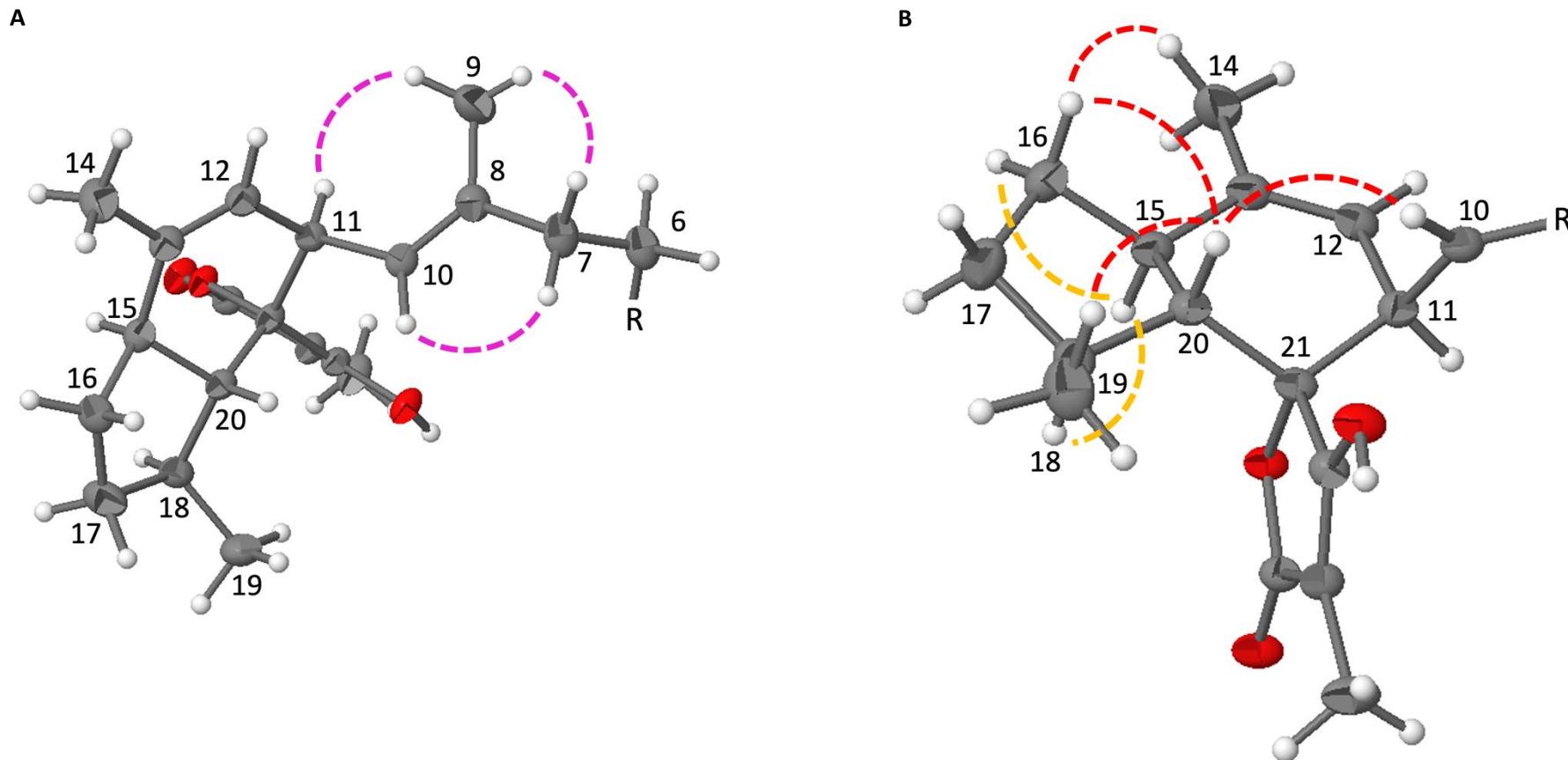
Figure S43. Visualization of the key cross peaks, observed in the ^{1}H-^{1}H NOESY NMR spectrum of **7**.
A) Key-NOE contacts, drawn in magenta dashed lines, which supported the deduction of the *E*-double bond geometry of $^{8,10}\Delta$, located in the alkene side chain.
B) Key-NOE contacts, that allowed the deduction of the relative configuration of atoms C-11, C15, C-18 and C-20. Red dashed lines indicate NOE contacts on the upper side of the molecule, while orange dashed lines represent the NOE correlations on the lower side of the molecule.

A

B
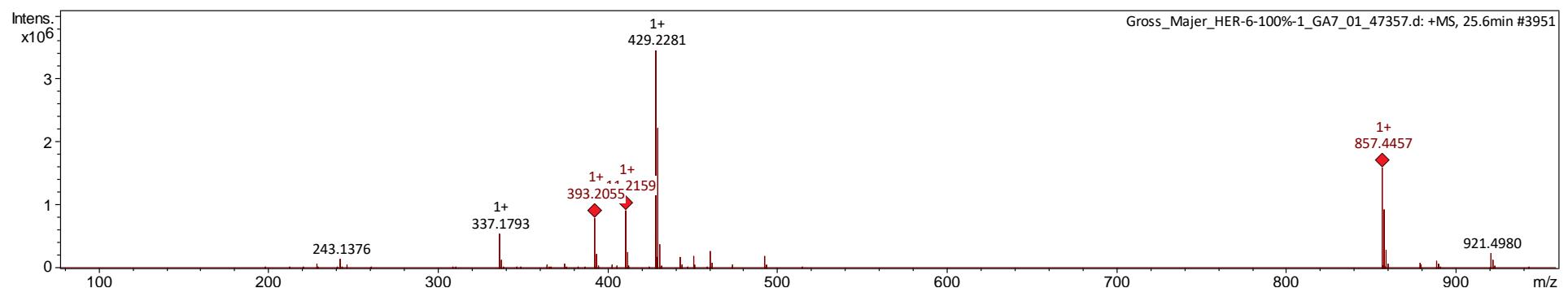
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻	Conf	mSigma	Std I
429.2281	1	$C_{25}H_{33}O_6$	429.2272	-2.3	-0.1	10.0	ok	even		185.1	242.6
429.2281	2	$C_{26}H_{29}N_4O_2$	429.2285	0.8	2.4	15.0	ok	even		200.5	225.2
429.2281	3	$C_{22}H_{25}N_{10}$	429.2258	-5.4	-4.7	16.0	ok	even		214.4	247.7
429.2281	4	$C_{21}H_{29}N_6O_4$	429.2245	-8.5	-7.5	11.0	ok	even		227.5	266.3
429.2281	5	$C_{15}H_{29}N_{10}O_5$	429.2317	8.3	8.5	7.0	ok	even		256.8	314.9
429.2281	6	$C_{14}H_{33}N_6O_9$	429.2304	5.1	5.8	2.0	ok	even		269.7	335.1
429.2281	7	$C_{11}H_{25}N_{16}O_3$	429.2290	2.0	1.1	8.0	ok	even		270.5	340.7
429.2281	8	$C_{10}H_{29}N_{12}O_7$	429.2277	-1.1	-1.5	3.0	ok	even		283.3	361.5
429.2281	9	$C_7H_{21}N_{22}O$	429.2263	-4.3	-6.9	9.0	ok	even		284.1	367.6

C
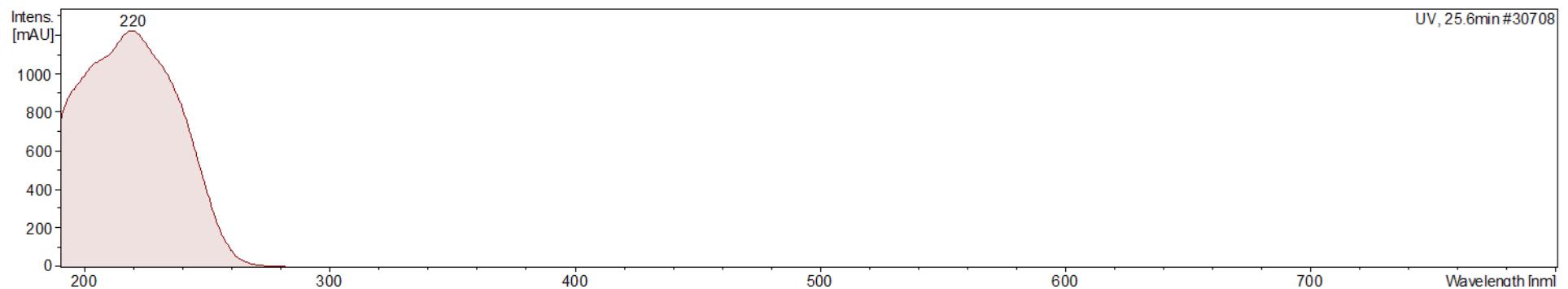
Figure S44. Ircinianin lactone C (**8**, 100%-fraction) MS-Analysis – A: HRMS-result; B: predicted molecular formula; C: extracted UV-Profile.

Table S5. Comparison ^{1}H (400 MHz) and ^{13}C (100 MHz) chemical shift values (ppm) of ircinianin (**1**) and ircinianin lactone C (**8**), recorded in d_4-MeOH. The column on the far right of the table indicates the shift-deviations in a color-coded way: no deviations; minor to significant shift deviations; strong shift deviations. Please note the different numbering schemes for the 5-membered ring systems (furan vs. ß-substituted γ-hydroxy-γ-butenolide ring).

|	Ircinianin (1)			Ircinianin lactone C (8)			Δ
Position	δH, mult. (J in Hz)	δC, type	Position	δH, mult. (J in Hz)	δC, type		|δH(1)- δH(8)| / |δC(1)- δC(8)|
1	7.38, t (1.7)	144.0, CH	4	-	173.8, C		7.38 / 29.8
2	6.30, d (0.9)	112.1, CH	3	5.90, d (2.3)	117.9, CH		0.40 / 5.8
3	-	126.5, C	2	-	172.7, C		- / 46.2
4	7.26, m	140.3, CH	1	6.02, d (2.3)	101.1, CH		1.24 / 39.2
5	2.41, br t (7.5)	25.3, CH₂	5	2.43, m	28.2, CH₂		0.02 / 2.9
6	1.68, m	29.5, CH₂	6	1.76, m	25.9, CH₂		0.08 / 3.6
7	2.04, m	40.5, CH₂	7	2.11, m	40.4, CH₂		0.07 / 0.1
8	-	136.6, C	8	-	135.9, C		- / 0.3
9	1.57, d (1.3)	16.3, CH₃	9	1.59, d	16.2, CH₃		0.02 / 0.1
10	5.11, dd (10.3, 1.1)	125.0, CH	10	5.15, d (10.3)	125.7, CH		0.04 / 0.7
11	3.08, dm (10.3)	48.7, CH	11	3.08, dm (10.1)	48.7, CH		0.00 / 0.0
12	5.03, m	123.6, CH	12	5.03, m	123.4, CH		0.00 / 0.2
13	-	137.1, C	13	-	137.2, C		- / 0.1
14	1.71, m	20.8, CH₃	14	1.71, m	20.68, CH₃		0.00 / 0.12
15	2.42, m	46.2, CH	15	2.42, m	46.2, CH		0.00 / 0.0
16	1.89, m; 1.34, m	27.3, CH₂	16	1.89, m; 1.34, m	27.3, CH₂		0.00; 0.00 / 0.0
17	2.00, m; 1.32, m	33.6, CH₂	17	2.02, m; 1.31, m	33.6, CH₂		0.02; 0.01 / 0.0
18	1.65, m	33.2, CH	18	1.64, m	33.2, CH		0.01 / 0.0
19	0.92, d (6.3)	20.7, CH₃	19	0.93, d (6.2)	20.74, CH₃		0.01 / 0.04
20	1.61, m	52.0, CH	20	1.62, m	51.8, CH		0.01 / 0.2
21	-	86.9, C	21	-	86.8, C		- / 0.1
22	-	179.2, C	22	-	179.1, C		- / 0.1
23	-	97.5, C	23	-	97.5, C		- / 0.0
24	-	177.7, C	24	-	177.7, C		- / 0.0
25	1.64, br s	6.1, CH₃	25	1.65, br s	6.1, CH₃		0.01 / 0.0
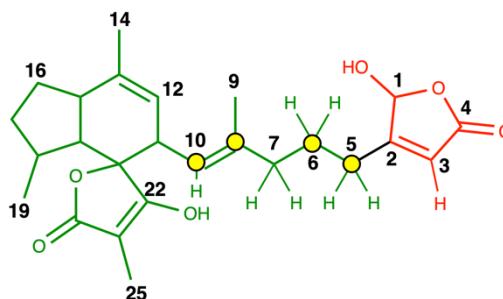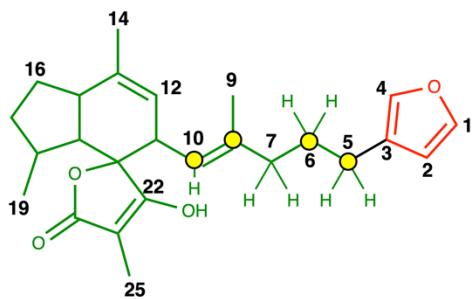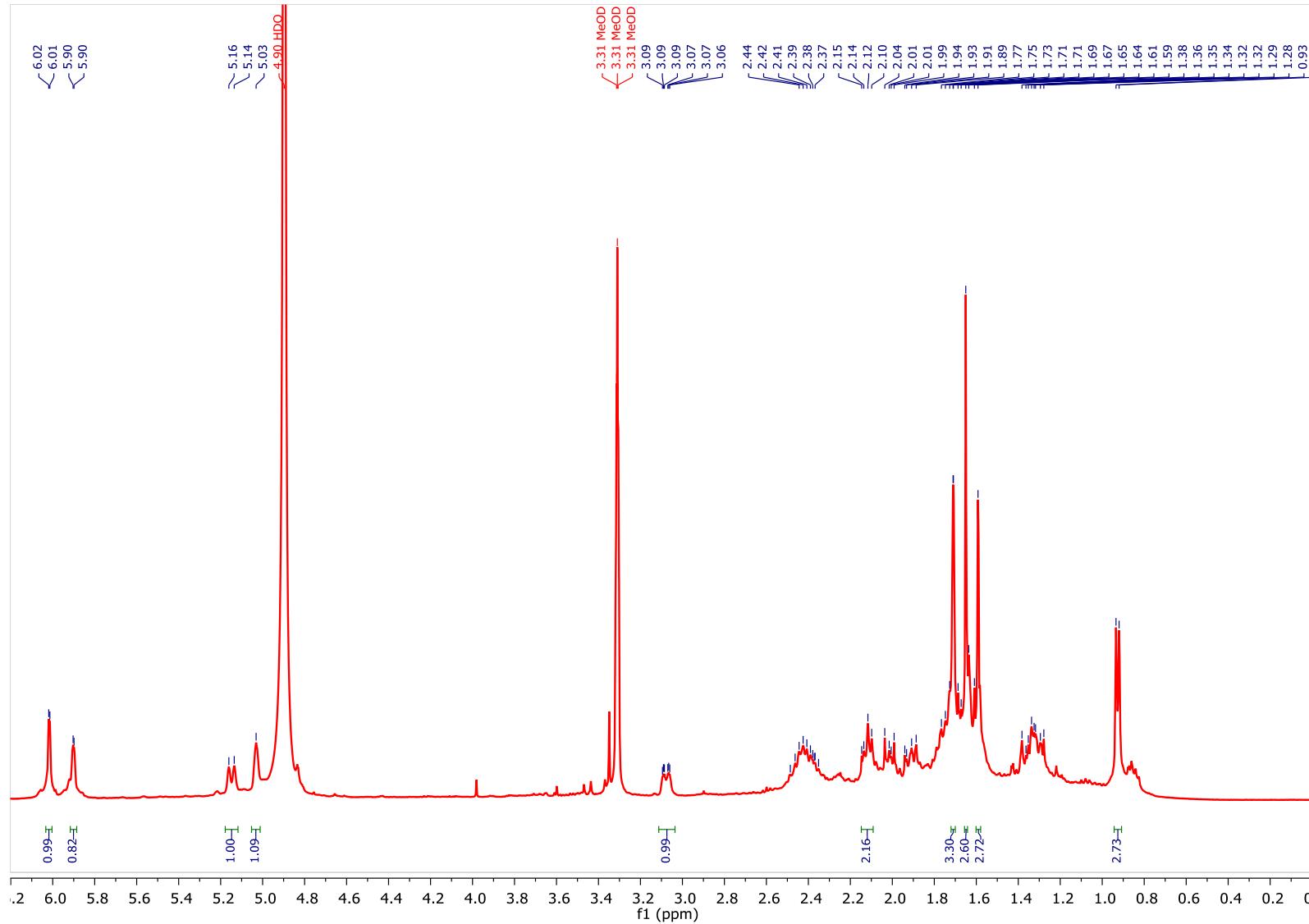
Figure S45. 400 MHz ^{1}H NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
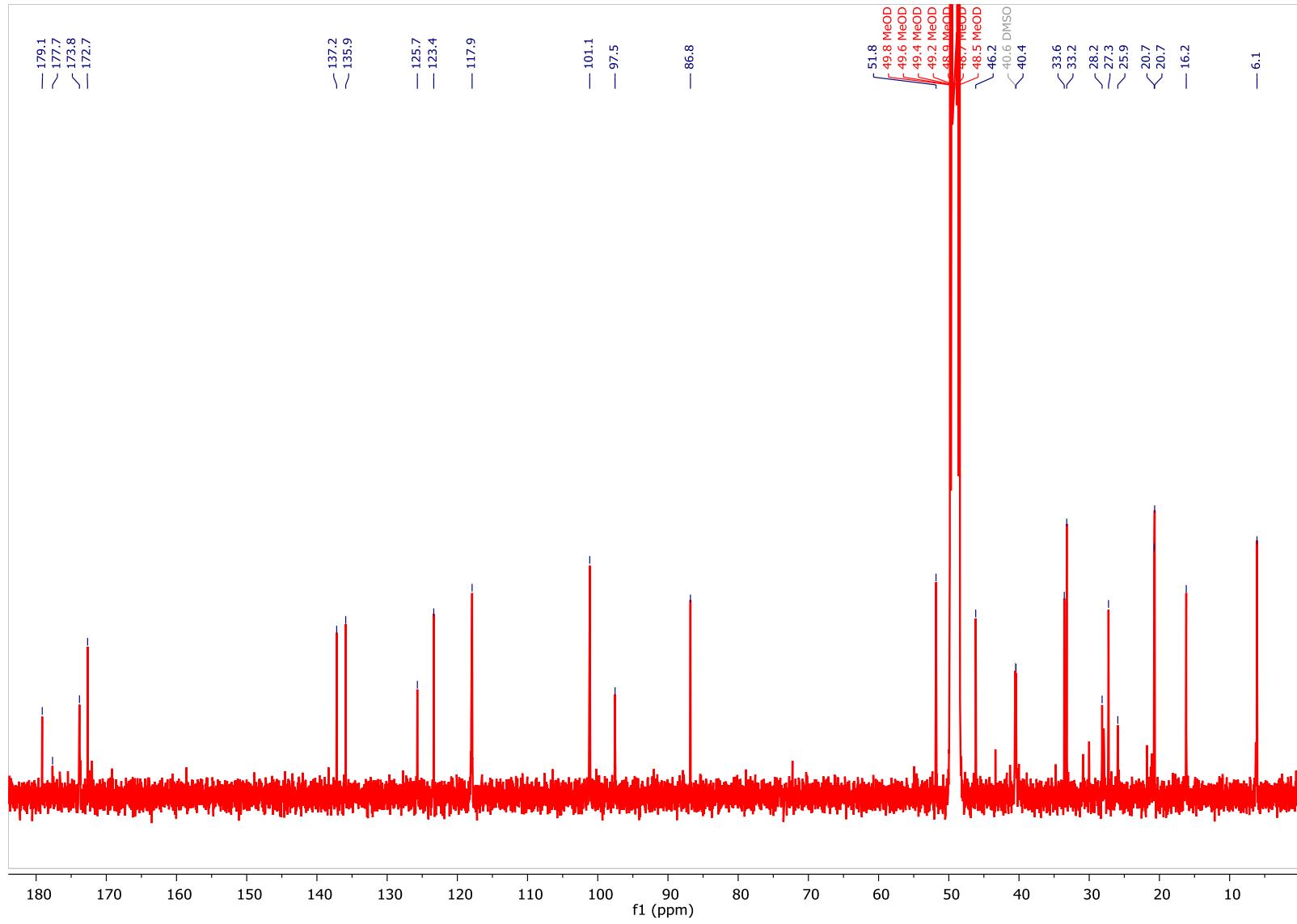
Figure S46. 100 MHz ^{13}C NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
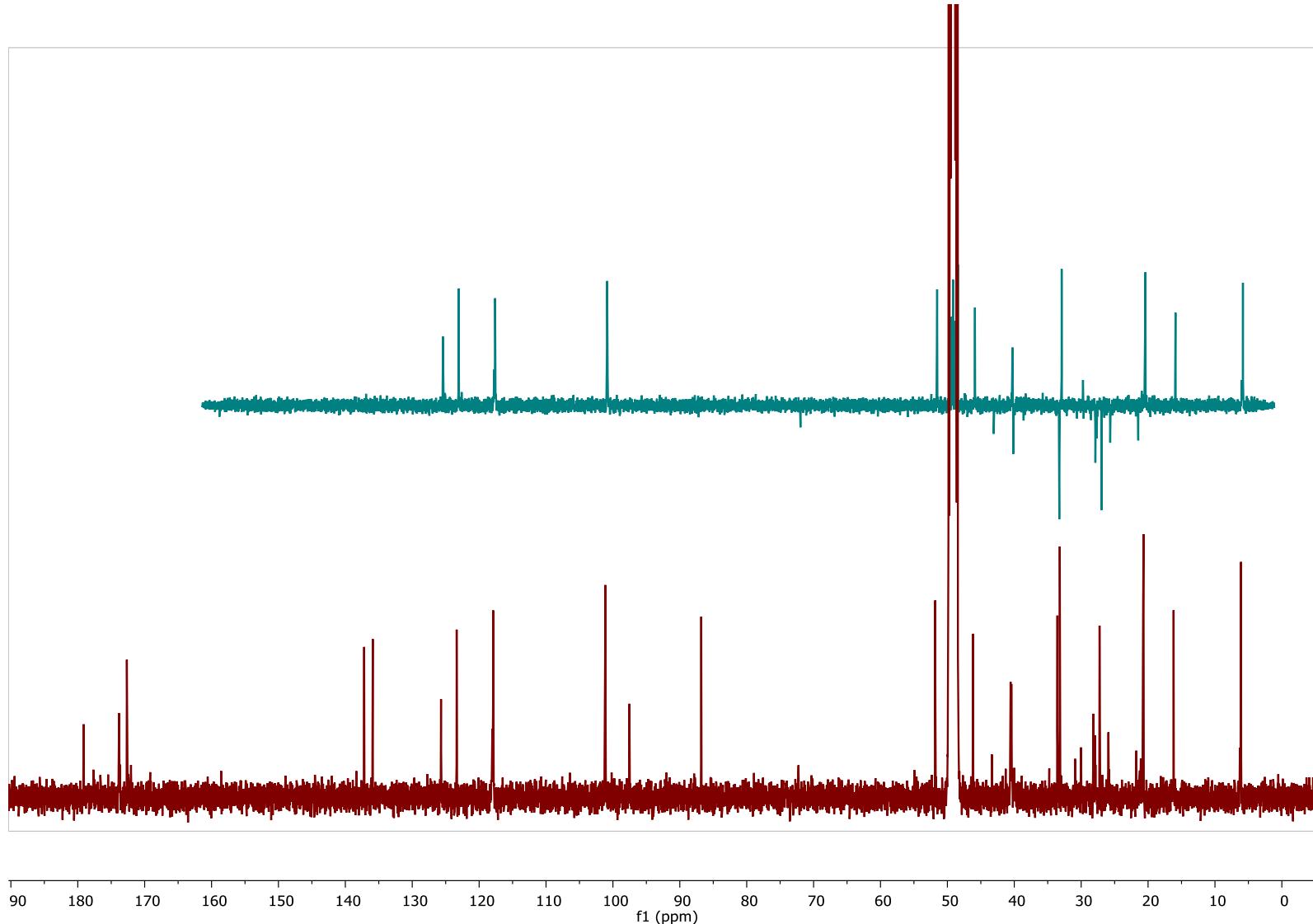
Figure S47. 100 MHz ^{13}C (bottom) and 400 MHz DEPT135 (top) NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
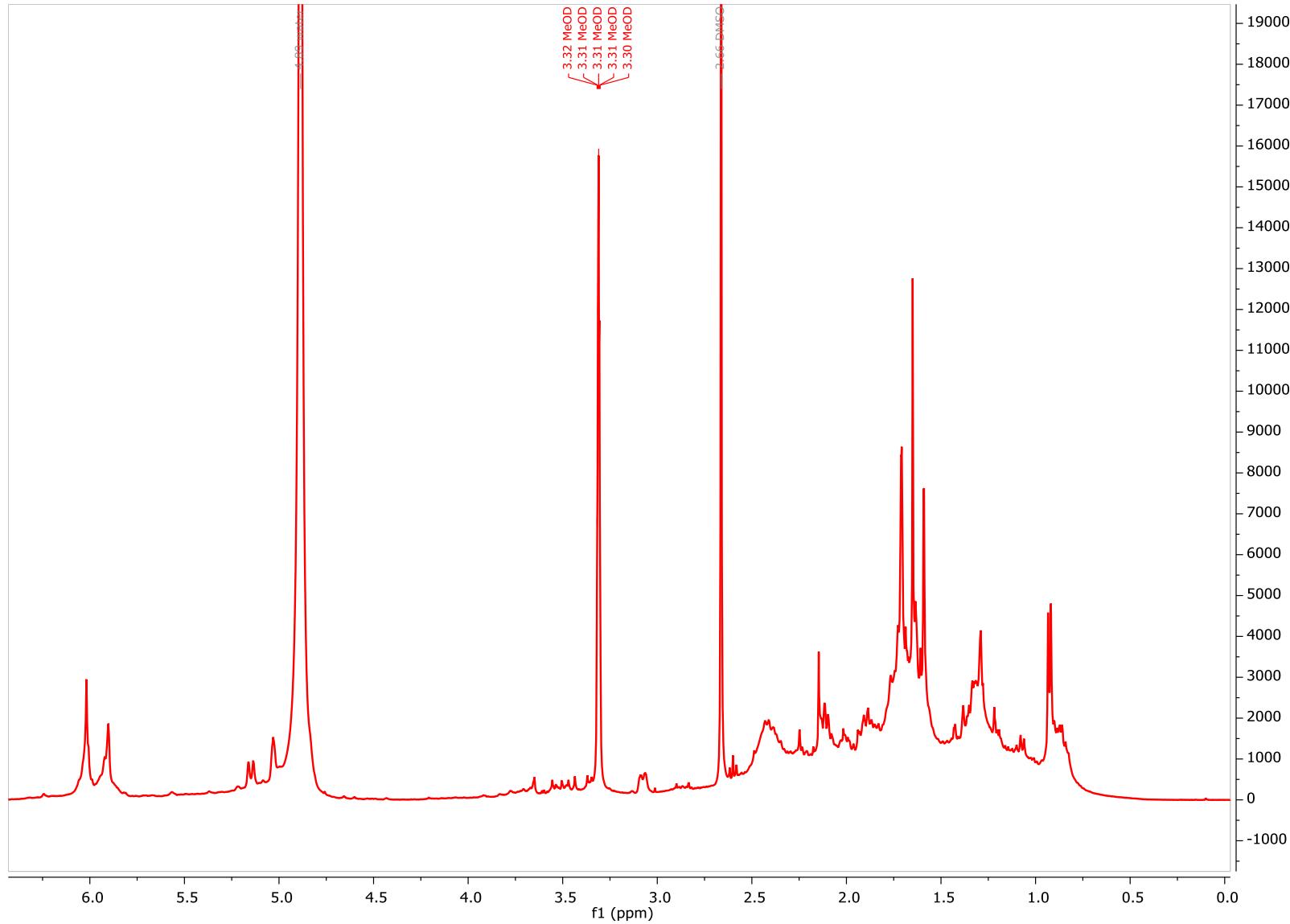
Figure S48. 400 MHz ^{1}H NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH before 2D-NMR experiments.
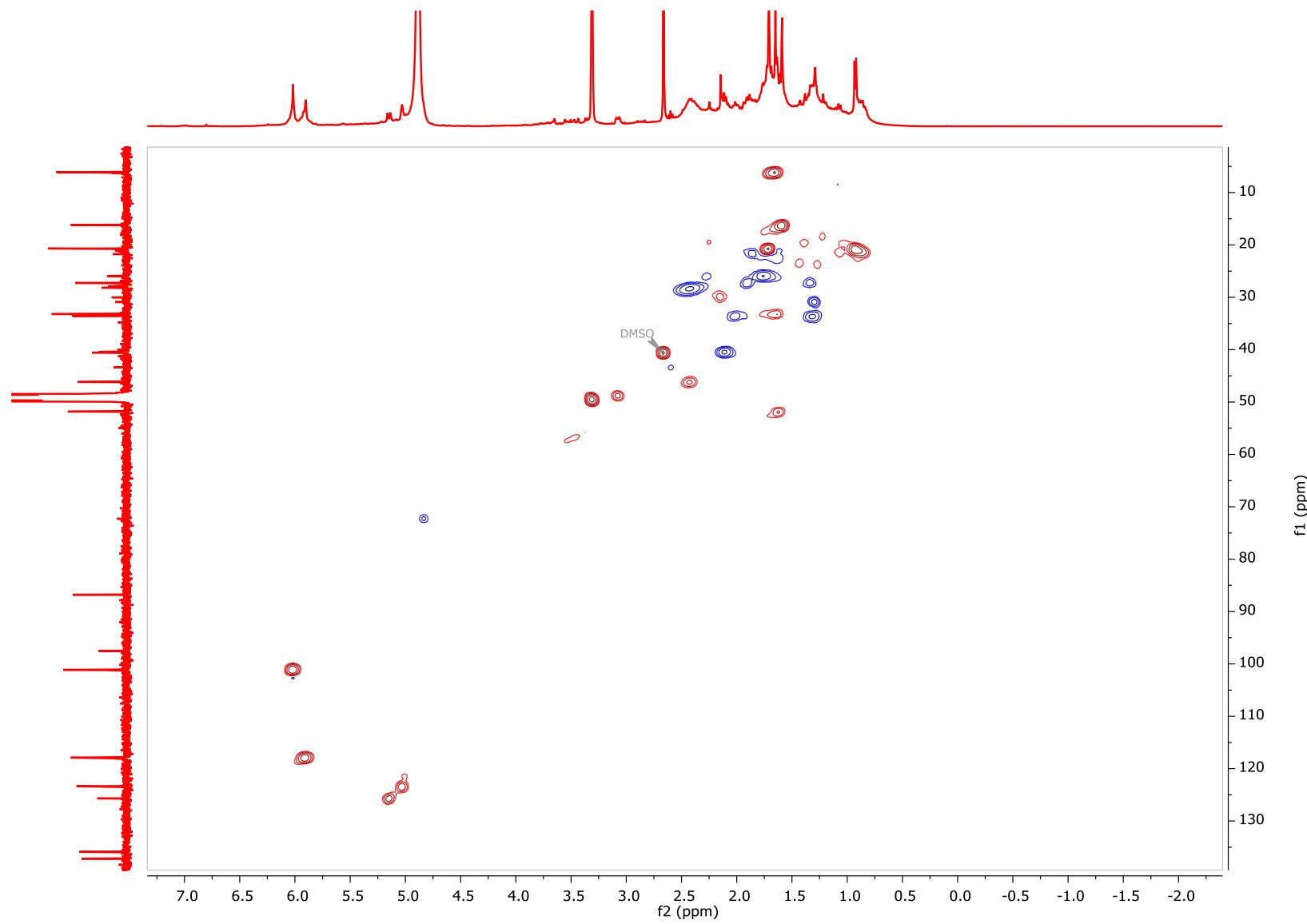
Figure S49. 400 MHz multiplicity edited ^{1}H-^{13}C HSQC NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
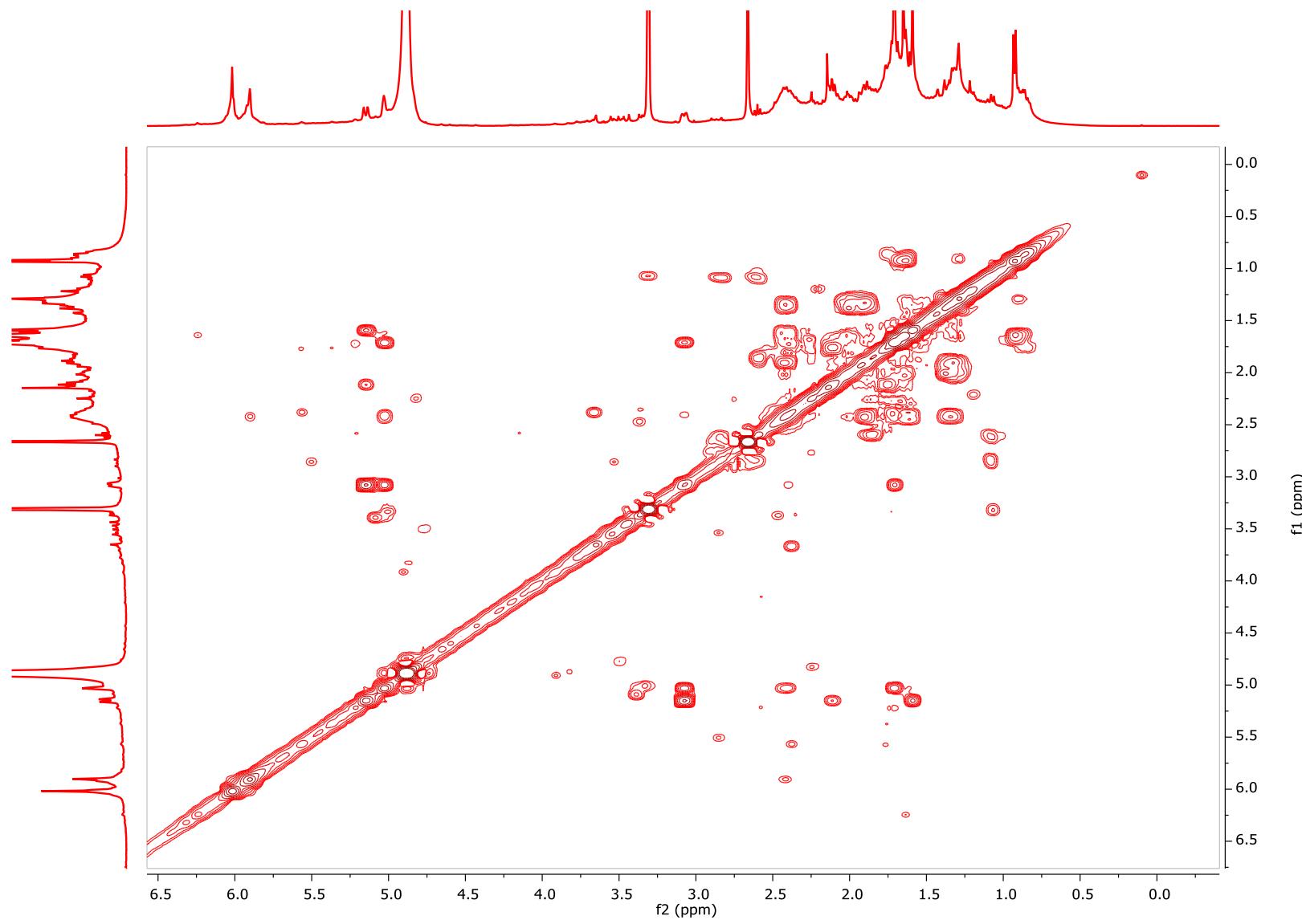
Figure S50. 400 MHz ^{1}H-^{1}H COSY NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
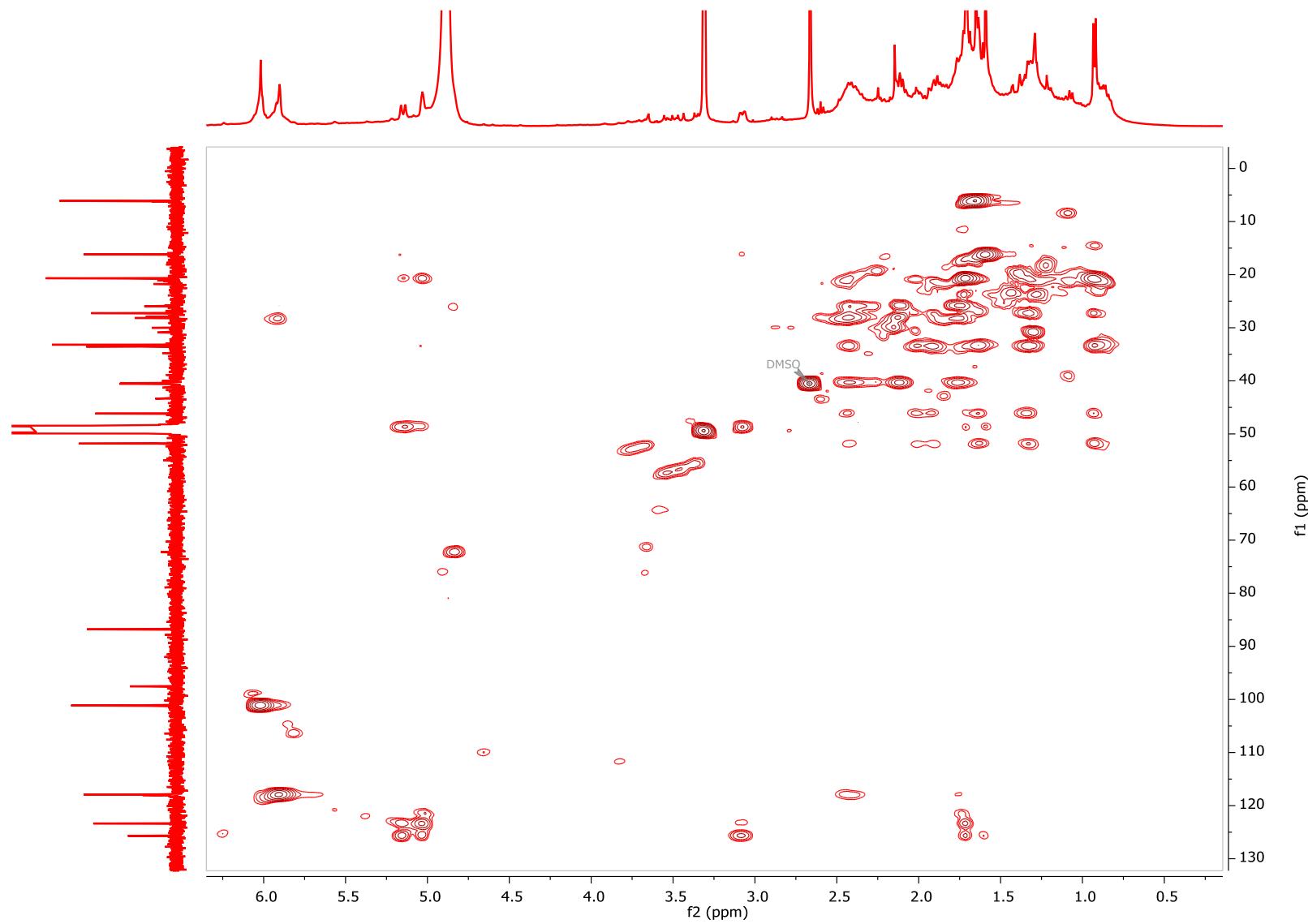
Figure S51. 400 MHz ^{1}H-^{13}C HSQC-TOCSY NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
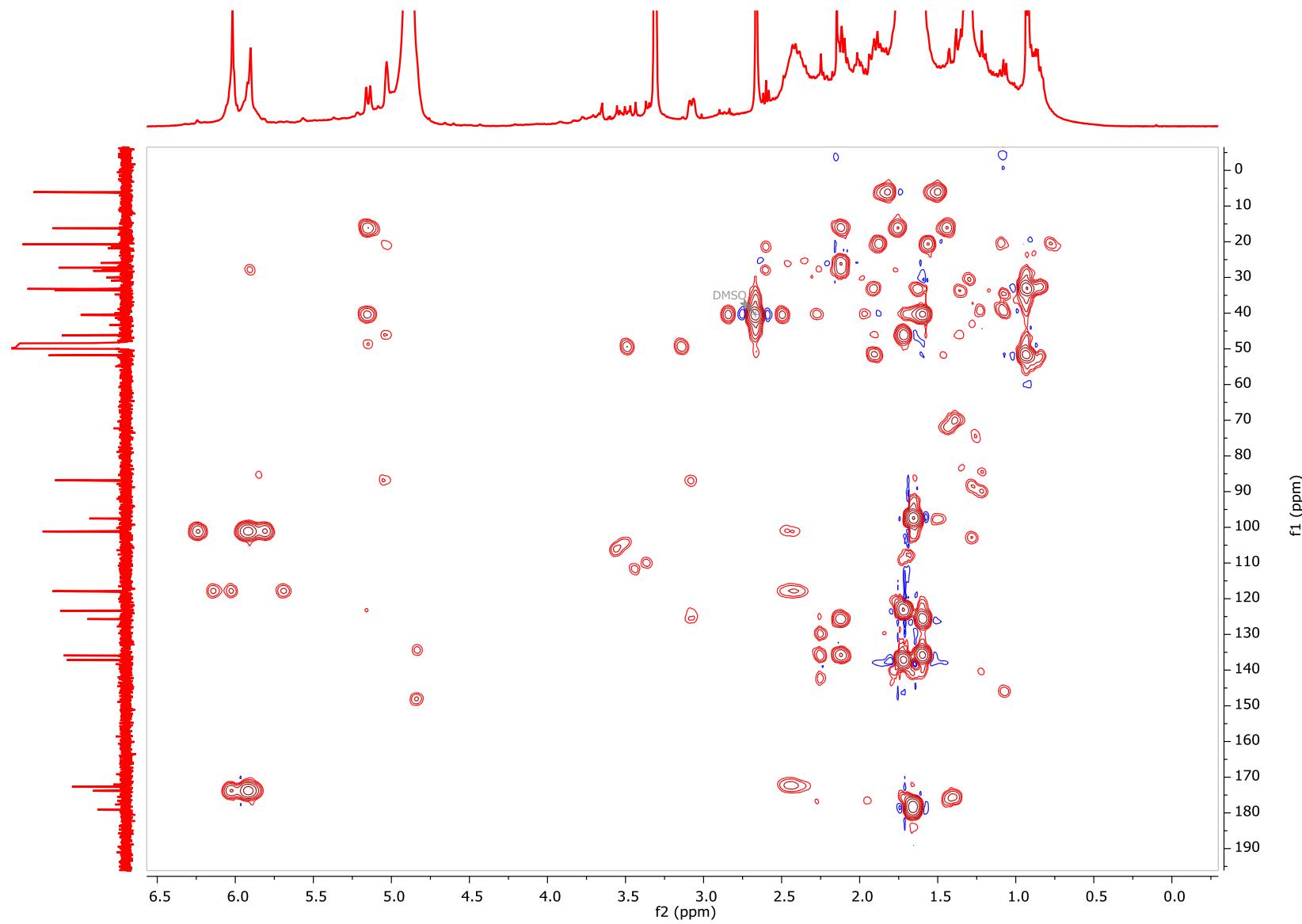
Figure S52. 400 MHz ^{1}H-^{13}C HMBC NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.
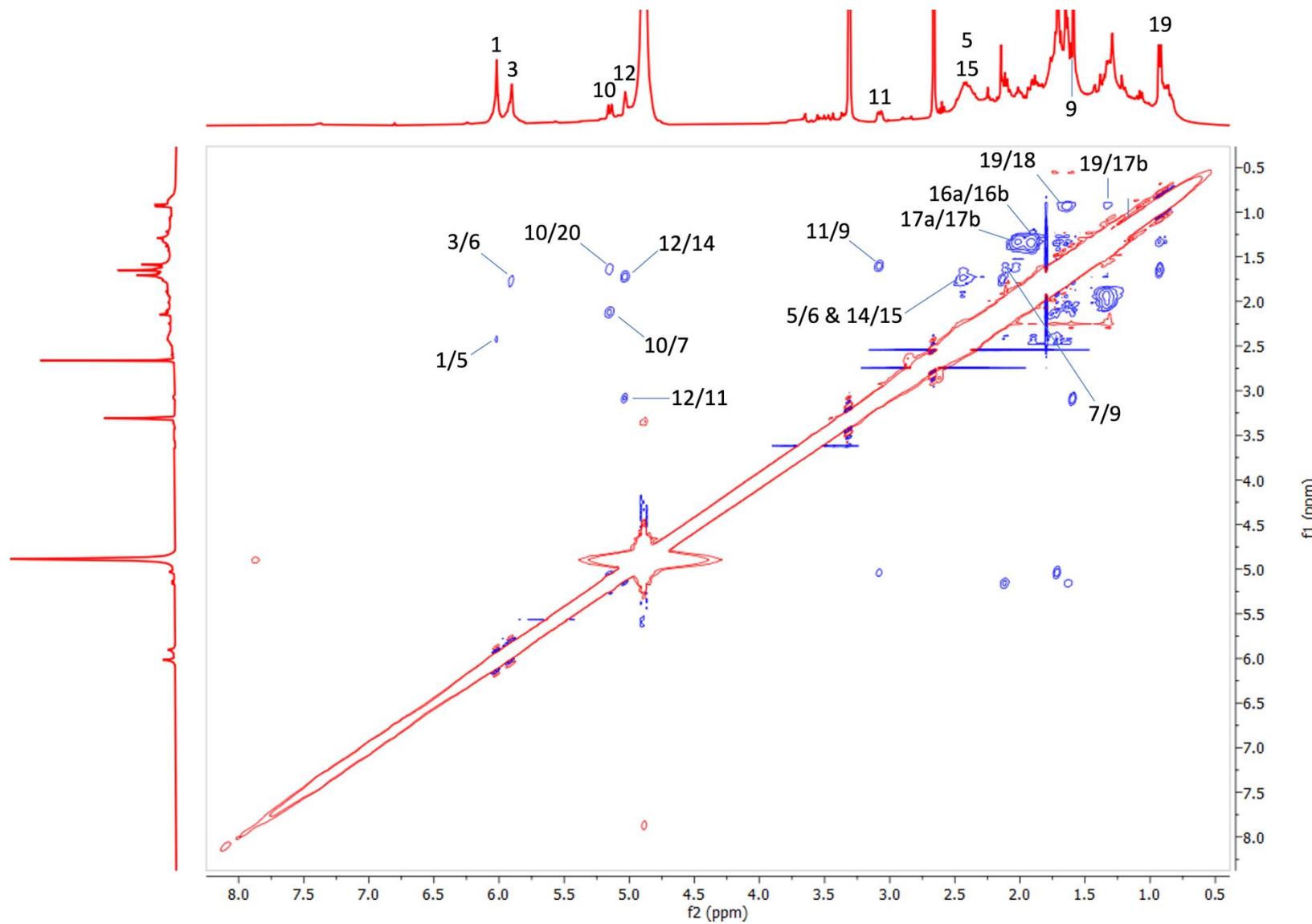
Figure S53. 400 MHz ^{1}H-^{1}H NOESY NMR spectrum of ircinianin lactone C (**8**, 100%-fraction) in d_4-MeOH.

Table S6. Results of the antimicrobial assays for ircinianin (**1**).

Antibacterial assay	
Bacterial strain	**MIC [µg/mL]**
E. faecium BM4147-1	>32
S. aureus ATCC29213	>32
K. pneumoniae ATCC12657	>32
A. baumannii 09987	>32
P. aeruginosa ATCC27853	>32
E. aerogenes ATCC13048	>32
E. coli ATCC25922	>32
B. subtilis 168	>32
M. smegmatis mc^2 155	>32

Figure S54. Results of the antiviral assay. Caco-2 cells (SARS-CoV-2) or HFF (HCMV) were infected as detailed in the M&M section. Cellular nuclei were stained with DAPI to visualize single cells and to evaluate the total number of cells. In the GFP/YFP channel infection is visualized by expression of mNeonGreen (SARS-CoV-2) or GFP (HCMV) via the viral reporter system, allowing to quantify infection levels. Images were taken with a Cytation3 multiplate reader. Images were colored with ImageJ. The scale bar represents 1mm. Evaluation of potential antiviral activity of ircinianin (10 μM) against (**A**) SARS-CoV2 or (**B**) HCMV.
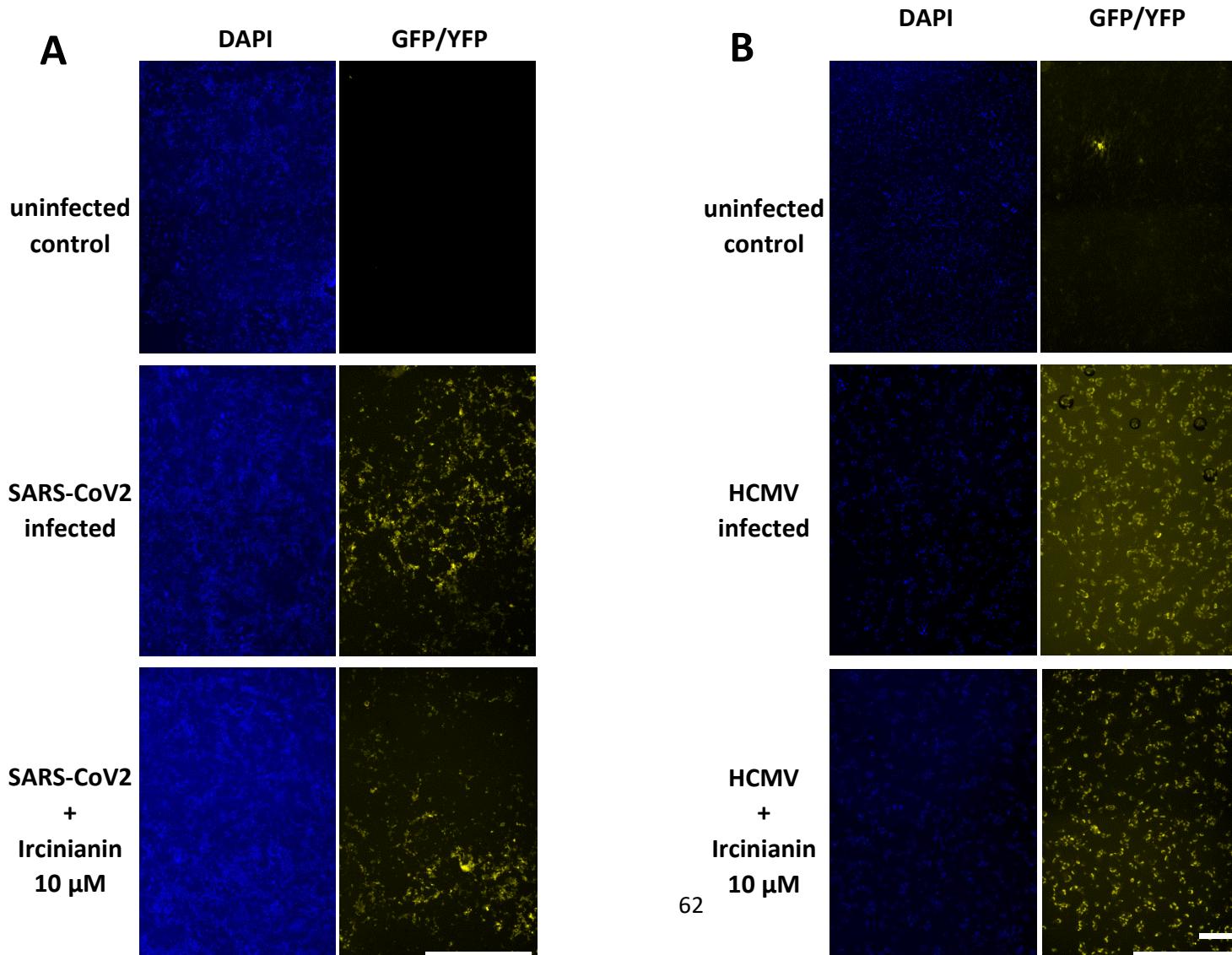
Table S7. Cytotoxocity Assays: TOP: Developmental Therapeutics Program (DTP)-One dose Mean Graph NCI-60 data for Ircinianin (**1**). BOTTOM: Single cell-line assays.
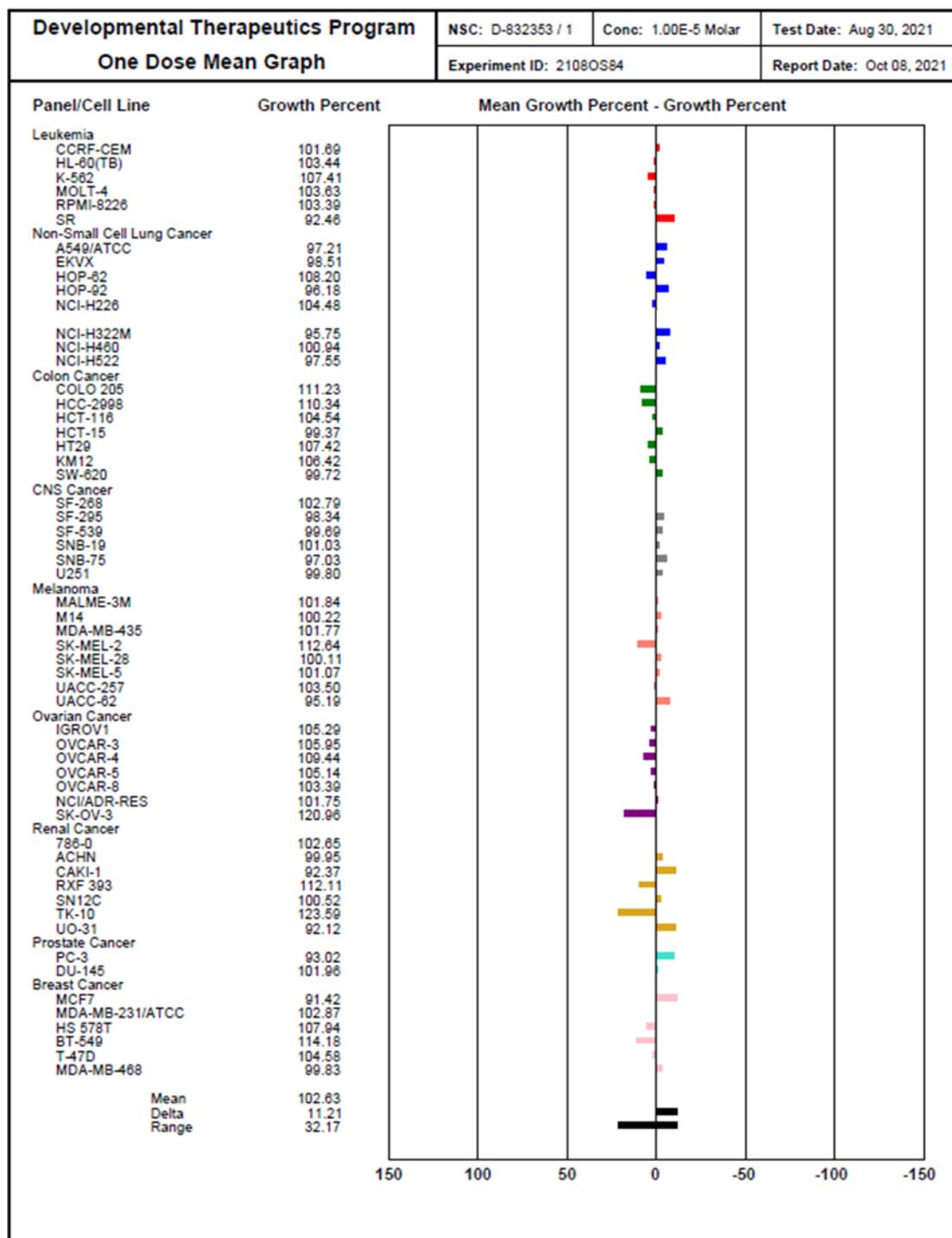
Single cell line assays	
Cell line	**IC$_{50}$ [μg/mL]**
HeLa	>64
L6	59.5