Isolation and characterization of karlotoxin 1, a new amphipathic toxin from *Karlodinium veneficum*—Supporting Information

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Experimental Methods

Culture growth and solid-phase extraction.

Four 10 L cultures of K. veneficum were grown at the FDA in seawater from Indian River, DE that was sterilized by autoclaving (121 ℃, 15 psi) and filtration (0.22 µm) prior to dilution to 15 PSU with double deionized water, and supplemented with f/2-Si nutrient mixture.[1] Cultures were maintained at 20 ℃ under a 14/10 light dark cycle and were harvested in late exponential phase. Toxins were isolated from culture supernatants after removal of cells by vacuum filtration through 90 mm glass fiber filters (Whatman GF/F) as previously described.[2] Filtrates were chilled on ice and passed at a flow rate of approximately 1 L/hr, under vacuum, through a large (200 mL bed volume) solid phase extraction column that had been pre-conditioned with 600 mL each of methanol and HPLC grade water. After desalting with 400 mL of HPLC grade water, total lipids were eluted with 400 mL of methanol followed by 200 mL of 1:1 (v/v) acetone/methanol. The organic lipid solubles were then concentrated by drying under N_2 at 50 ℃, dissolved in 100 mL methanol, filtered through a pre-rinsed glass fiber filter (Whatman GF/F) to remove white un-dissolved particulates, and dried again under N_2 at 50 ℃. Solid phase extraction of karlotoxin from the culture filtrate and flash chromatography were carried out using BakerBond Octadecyl Prep LC 40 µm packing material (J.T. Baker, Inc).

Analytical characterization.

NMR spectra were recorded on a Varian Inova 800 MHz spectrometer located in the Duke NMR Center at Duke University. NMR spectra were obtained at 298K using a Varian cold probe and a Dell 390 computer with VNMRJ software. The 1D and 2D NMR data were analyzed using Topspin V. 2.0 (Bruker BioSpin, Inc). Scalar coupling constants in the ^{1}H NMR spectrum were measured when possible using the Daisy package of Topspin with manual optimization of lineshape fitting. Low-resolution mass spectra were acquired on a Waters ZQ instrument using electrospray ionization and interfaced to an Agilent 1100 HPLC system with a Waters XTerra C18 column (2.3 × 30 mm, 2.5 µm). High-resolution mass spectra were acquired on a Waters QTof Ultima spectrometer using electrospray ionization by the Mass Spectrometry Laboratory at the University of Illinois at Urbana-Champaign. Tandem MS/MS experiments were acquired on an Applied Biosystems, Inc. QTRAP spectrometer in enhanced product ion mode with a collision energy of 130. Preparative HPLC was carried out using a system consisting of two Waters 515 pumps and a Waters 2487 UV-vis detector with a Waters SunFire C18 column (4.6 × 150 mm, 3.5 µm). UV-vis spectra were acquired on a Beckman DU640B Spectrophotometer. Infrared spectra were acquired on a Nicolet 6700 FT-IR (Thermo Scientific, Inc.) using OMNIC acquisition software. Optical rotation measurements were made on an Autopol III polarimeter (Rudolph Research Analytical) at ambient temperature.

Hemolysis assays.

Human red blood cells were obtained from the American Red Cross. Erythrocytes were washed, centrifuged and diluted to 4.2-4.7 × 10^4 cells/mL with ELA buffer. The modified ELA[3] is performed in 96-well V-bottom microtitre plates (Costar) using 125 µL of erythrocyte suspension and 125 µL of compound diluted with ELA buffer. Saponin was used as a positive control. Samples at each concentration were tested in triplicate. Plates containing sample and erythrocytes were incubated in sealed plates at 4 ℃ for 24 h, centrifuged, and the supernatants analyzed for optical densities at 415 nm. EC50 values were determined using non-linear regression analysis in GraphPad Prism.

References

[1] Guillard, R. R. L., *Culture of marine invertebrate animals*, Plenum Press, **1975**, pp. 26–60.

[2] Deeds, J. R.; Terlizzi, D. E.; Adolf, J. E.; Stoecker, D. K.; Place, A. R., *Harmful Algae* **2002**, 1, 169–189.

[3] Eschbach, E.; Scharsack, J. P.; John, U.; Medlin, L. K., *J. Appl. Toxicol.* **2001**, 21, 513–519.

NMR assignments for KmTx 1 (1)

Table 1. ^{1}H and ^{13}C NMR data for KmTx1338 in 2:1 CD_3OD/Pyridine-d_5

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
1a	3.59	(dd, 4.6, 11.0)	66.8	1b, 2	1b, 2, 3a, 3b, 4, 5	2, 3	
1b	3.55	(dd, 6.3, 11.1)		1a, 2	1a, 2, 3a, 3b	2, 3	2, 3
2	3.73	m	72.8	1a, 1b, 3a, 3b	1a, 1b, 3a, 3b, 4, 5	1, 3	
3a	2.32	m	37.4	2, 3b, 4	1a, 1b, 2, 3b, 4, 5, 6	1, 2, 4, 5	1, 2, 4, 5
3b	2.22	m		2, 3a, 4	1a, 1b, 2, 3a, 4, 5, 6	1, 2, 4, 5	1, 2, 4, 5
4	5.75	(dt, 15.5, 7.3)	127.9	3a, 3b, 5	1a, 1b, 2, 3a, 3b, 5, 6, 7a	2, 3, 5	2, 3, 5
5	5.59	(dd, 15.5, 6.7)	136.9	4, 6	1a, 1b, 2, 3, 4, 6, 7a, 7b	3, 4, 6	3, 4, 6
6	4.05	(q, 6.7)	73.1	5, 7a, 7b	1a, 1b, 2, 3a, 3b, 4, 5, 7a, 7b, 8a, 8b	4, 5, 7, 8	4, 5, 8
7a	1.55	m	38.0	5, 6, 7b*, 8a*, 8b*	3a, 3b, 4, 5, 6, 7b*, 8a*, 8b*, 10	6, 8	6, 8
7b	1.51	m		5, 6, 7a*, 8a*, 8b*	3a, 3b, 4, 5, 6, 7b*, 8a*, 8b*, 10	8	6, 8
8a	1.60	m	22.8	7a*, 7b*, 8b*, 9*	5, 6, 7a*, 7b*, 8b*, 9*, 10	7, 10	
8b	1.42	m		5, 6, 7a*, 7b*, 8a*, 9*, 10	7, 10	10	
9	1.45	m	38.1	8a*, 8b*, 10	6, 7a*, 7b*, 8a*, 8b*, 10	7*, 8*, 10	7*, 8*, 10

* Ambiguous due to spectral overlap.

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Table 1 – continued from previous page

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
10	3.56	m	72.0	9, 11	7a, 7b, 8a, 8b, 9, 11, 12a, 12b*	8, 9, 12	
11	1.41	m	38.1	10, 12*	7a*, 7b*, 8a*, 8b*, 9*, 10, 12*, 14	10, 12	10
12a	1.66	m	23.0	11*, 12b, 13*	10, 11*, 13*, 14*	11, 13	
12b	1.42	m		11*, 12a, 13*	10, 11*, 12a, 13*, 14*, 15b	11, 13	
13	1.47	m	39.8	12a*, 12b*, 14	12a*, 12b*, 14, 15a, 15b, 16, 17, 66	12	11, 12
14	3.68	m	70.1	13, 15a	12a*, 12b*, 13*, 15a, 15b, 16, 66	15, 66	
15a	1.76	m	41.0	14, 15b, 16	13, 14, 15b, 16, 17, 66	16, 66	16, 66
15b	1.29	m		14, 15a, 16	13, 14, 15a, 16, 17, 66	16, 66	16, 66
16	2.09	m	33.3	15b, 66, 17	12a, 12b, 13, 14, 15a, 15b, 17, 18, 66	15, 66	
17	3.19	(dd, 4.16, 6.10)	79.6	16, 18	13, 14, 15a, 15b, 16, 18, 19, 66	15, 16, 18, 66	
18	3.68	m	72.8	17, 19a, 19b	15a*, 15b*, 16, 17, 19a*, 19b, 20b, 21	16*, 17, 20, 66, 67	

* Ambiguous due to spectral overlap.

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Table 1 – continued from previous page

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
19a	1.70	m	32.0	18, 19b*, 20a*, 20b*	17, 18, 19b*, 20a*, 20b*, 21, 67		
19b	1.62	m		18, 19a*, 20a*, 20b	17, 18, 19a*, 20a*, 20b, 21, 67		18
20a	1.72	m	31.4	19, 20b, 21	17, 18, 19, 20b, 21, 67	18, 19, 67	
20b	1.45	m		19, 20a, 21	17, 18, 19, 20a, 21, 67	19	18
21	2.02	m	34.8	20a, 20b, 22, 67	17, 18, 19, 20a, 20b, 22, 23, 67	67	
22	3.86	(dd, 2.3, 9.0)	73.8	21, 23	19, 20a, 20b, 21, 23, 24, 67		20, 23, 24, 67
23	3.47	(dd, 0.9, 9.0)	73.6	22, 24	20a, 20b, 22, 23, 24, 25b, 67		21, 22
24	4.02	m	71.4	23, 25a, 25b	22, 23, 25a, 25b	23, 21*, 25, 26 27*	
25a	1.78	m	35.4	24, 25b, 26a*, 26b	24, 25b, 26a*, 26b, 27a, 29, 68	24, 26, 27*	
25b	1.58	m		24, 25a, 26a*, 26b	24, 25a, 26a*, 26b, 27a, 29, 68	24, 26, 27*	
26a	1.53	m	24.2	25a, 25b*, 26b*	24*, 25a, 25b, 26b, 27a, 29, 68	27*	
26b	1.46	m		25a, 25b, 26a*, 27a	24*, 25a, 25b, 26a, 27b, 68	25, 27*, 24*	
27a	1.72*	m	32.7	26a*, 26b*, 27b, 28*	25a*, 25b*, 26a, 26b, 27b, 28*, 29, 30*, 68		

* Ambiguous due to spectral overlap.

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Table 1 – continued from previous page

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
27b	1.27	m		26b, 27a, 1.76	25a, 25b, 26a, 26b, 27a, 28, 29, 68		
28	1.78	m	36.4	27b, 29, 68	25b, 26a, 26b, 27b, 29, 30	29, 68	68
29	3.12	(dd, 3.3, 7.2)	79.9	28, 30	26a, 26b, 27a, 27b, 28, 30, 31a, 32, 68	68	27
30	4.03	m	68.1	29, 31a, 31b	27a*, 27b*, 28, 29, 31a, 31b, 32, 33, 68	26, 27, 31, 32	
31a	1.93	m	34.6	30, 31b, 32	29, 30, 31b, 32, 33		
31b	1.80	m		30, 31a, 32	29, 30, 31a, 32, 33		
32	4.34	(dt, 10.9, 3.1)	74.6	31a, 31b, 33	29, 30, 31a, 31b, 33, 34, 35	30, 31	33
33	3.88	(dd, 3.1, 3.1)	73.0	32	30, 31a, 31b, 32, 35, 36, 38a	32, 35, 36	
34	3.91	(dd, 3.3, 7.8)	73.0	35	31a, 31b, 32, 35, 36, 38a, 39a, 39b	36, 35	
35	4.11	(dd, 7.8, 7.8)	71.2	34, 36	32, 33, 34, 36, 38a, 38b, 39a, 39b	34, 36	34, 36

* Ambiguous due to spectral overlap.

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Table 1 – continued from previous page

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
36	3.57	m	77.9	35, 36	34, 35, 36, 38a, 38b, 39a, 39b	35	34, 38
37	4.19	m	71.7	36*, 38a, 38b	34, 36, 38a, 38b, 39a, 39b		
38a	2.16	m	31.8	37, 38b, 39a, 39b	36, 37, 38b, 39a, 39b, 69a, 69b	39	
38b	1.82	m		37, 38a, 39a, 39b	36, 37, 38a, 39a, 39b, 69a, 69b	39	
39a	2.63	(ddd, 5.0, 10.8, 15.8)	27.4	38a, 38b, 39b	36, 37, 38a, 38b, 39b, 69a, 69b	38	
39b	2.26	(ddd, 6.0, 10.0, 15.8)		38a, 38b, 39a	36, 37, 38a, 38b, 39a, 69a, 69b	38	40, 69
40	.		151.7				
41	4.39	(d, 8.7)	76.7	42	42, 43, 44a, 69a	42	39, 42, 43, 69
42	3.51	(dd, 1.1, 8.8)	74.9	41, 43	41, 43, 44a, 44b, 69a	41	40, 41
43	4.21	(dt, 12.2, 1.8)	70.7	42, 44a, 44b	41, 42, 44a, 44b	44, 45	
44a	2.32	m	31.7	43, 44b, 45	43, 44b, 45, 4.30	43, 45, 48	43, 45, 48
44b	1.63	m		43, 44a, 45	43, 44b, 45, 46	43, 45	48
45	4.17	m	67.2	44a, 44b, 46	44a, 44b, 46	43, 44, 46	

* Ambiguous due to spectral overlap.

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Table 1 – continued from previous page

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
46	4.31	(t, 2.3)	68.5	45, 47	44a, 44b, 45, 47	43, 44, 45	44, 45
47	3.99	(dd, 1.2, 10.0)	80.6	46, 48	46, 48, 49, 50, 51, 52, 1.68	49	45, 48, 49
48	4.19	m	72.5	47, 49	46, 47, 49, 50, 51, 52		47
49	4.61	(dd, 2.9, 7.4)	74.1	48, 50	47, 48, 50, 51, 52, 53, 54	47, 50, 51, 52	51
50	5.74	(dd, 8.1, 15.6)	128.6	49, 51	47, 48, 49, 51, 52, 53, 54	49, 51	51, 52
51	5.81	(dt, 15.6, 6.8)	135.1	50, 52	47, 48, 49, 50, 52, 53, 54	49, 50	49, 50, 52, 53
52	1.94	m	33.4	51, 53	48, 49, 50, 51, 53, 54	49, 50	49, 50, 51, 53
53	1.26	m	30.1	52, 54	49, 50, 51, 52, 54		52, 54
54	1.12–1.20	m	29.0–31.4				
55	1.12–1.20	m	29.0–31.4				
56	1.12–1.20	m	29.0–31.4				
57	1.12–1.20	m	29.0–31.4				
58	1.12–1.20	m	29.0–31.4				
59	1.12–1.20	m	29.0–31.4				
60	1.27	m	30.0	59, 61	59, 61, 62, 63, 64, 65a, 65b	61	58
61	1.98	m	33.4	60, 62	59, 60, 62, 63, 64, 65a, 65b	60	60, 62, 63
62	5.63	(dt 15.0, 7.0)	136.2	61, 63	59, 60, 61, 63, 64, 65a, 65b		60, 61, 64

* Ambiguous due to spectral overlap.

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Table 1 – continued from previous page

position	δ_H	(m, Hz)	δ_C	COSY	TOCSY	HSQC-TOCSY	HMBC
63	6.00	(dd, 10.5, 15.0)	132.1	62, 64	59, 60, 61, 62, 64, 65a, 65b	62, 65	61
64	6.27	(ddd, 10.3, 10.5, 17.0)	138.5	63, 65a, 65b	60, 61, 62, 63, 65a, 65b	65	62
65a	5.03	(dd, 1.3, 17.0)	114.7	64, 65b	60, 61, 62, 63, 64, 65b		63
65b	4.88	(dd, 1.3, 10.3)		64, 65a	61, 62, 63, 64, 65a	64, 64	63
66	0.98	(d, 7.0)	17.8	16	13, 14, 15a, 15b, 16, 17	14, 15, 16, 17	15, 16, 17
67	0.97	(d, 7.0)	13.5	21	19, 20a, 20b, 21, 22, 23	20, 21	20, 21, 22
68	0.92	(d, 6.7)	16.8	28	26a, 26b, 27a, 27b, 28, 29, 30	26, 27, 28, 29	27, 28, 29
69a	5.10	s	112.9	69b	38a, 38b, 39a, 39b, 41, 42, 69a		39, 40, 41
69b	5.01	s		69a	38a, 38b, 39a, 39b	39	39, 40

* Ambiguous due to spectral overlap.

NMR spectra of KmTx 1

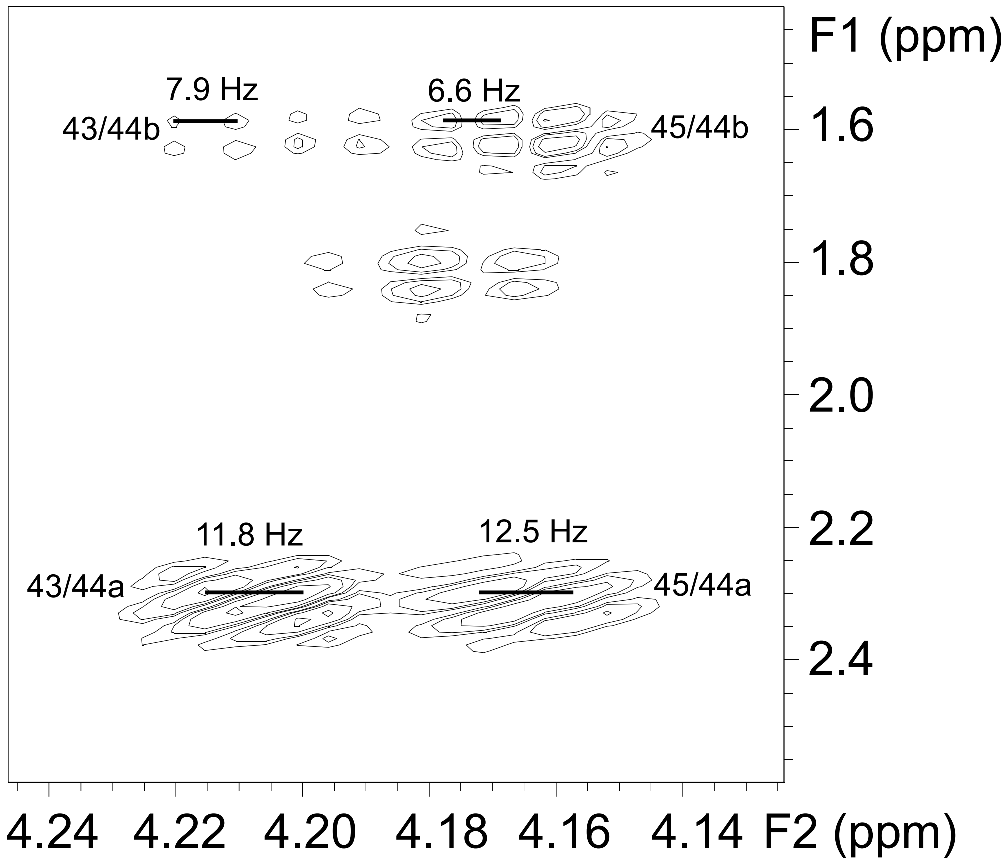
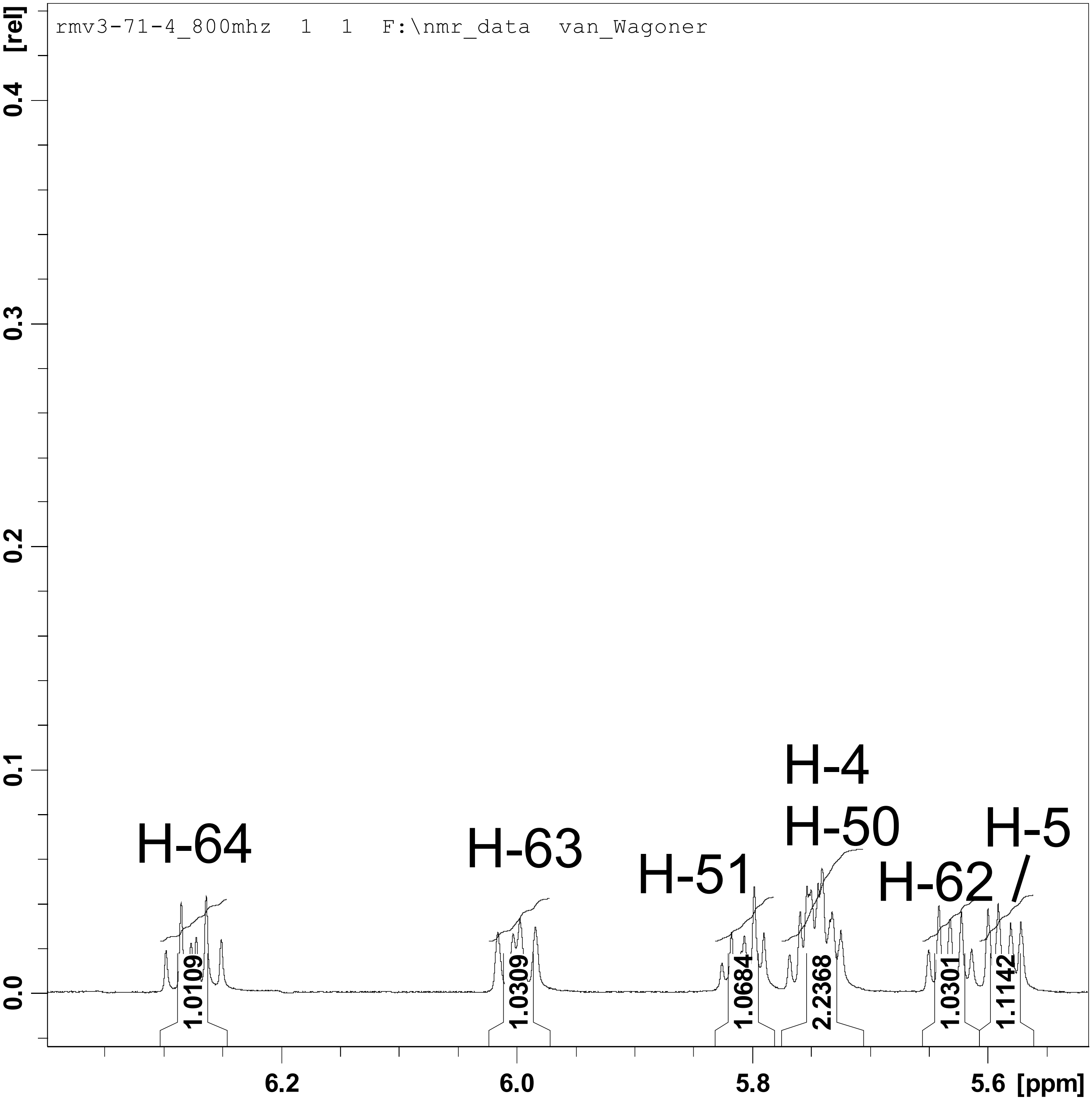
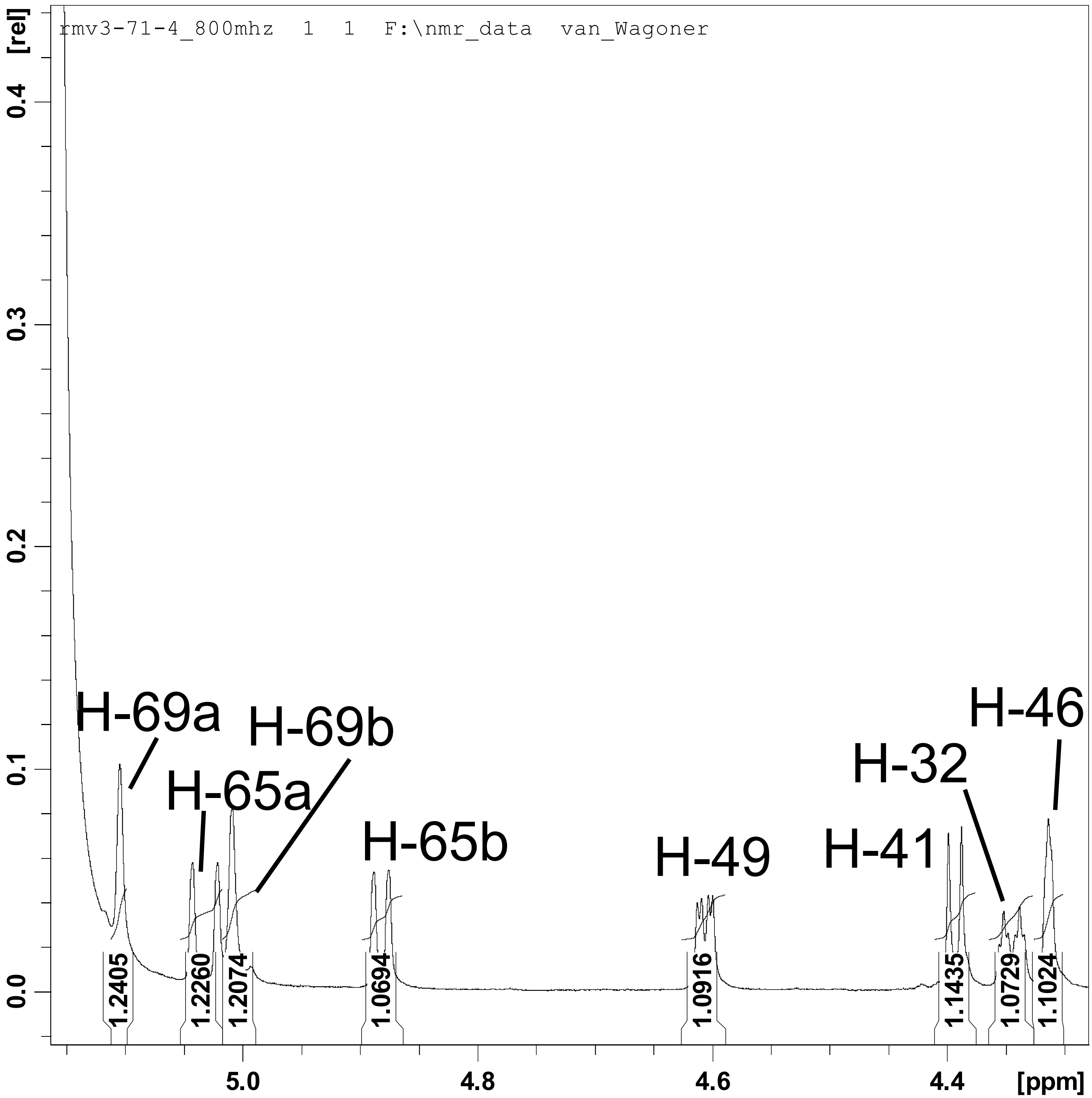


Figure S1. DQF-COSY spectrum of the 43/44 and 45/44 cross peaks for **1**. The similarity in antiphase peak separation for H-43 and H-45 with H-44a indicates that each of them couple strongly to H-44a, supporting an axial location for all three protons. Note that the apparent active coupling for H-43/H-44b is larger than was measured in the ^{1}H NMR spectrum due to the combined effects of digital resolution and linewidths.



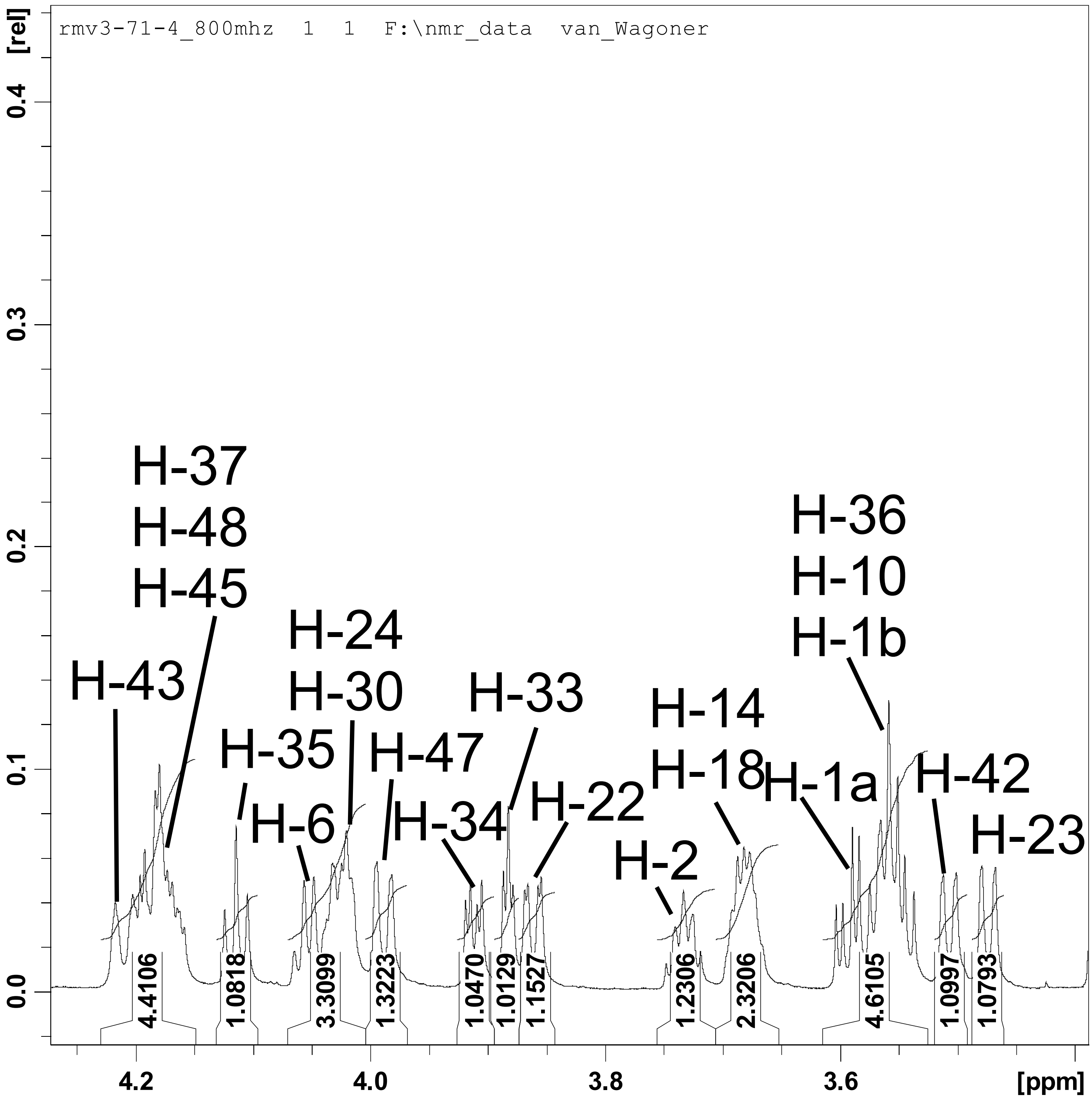
(a)

Figure S2. ^{1}H NMR spectrum of KmTx 1 (**1**). a–f- Details from various regions.



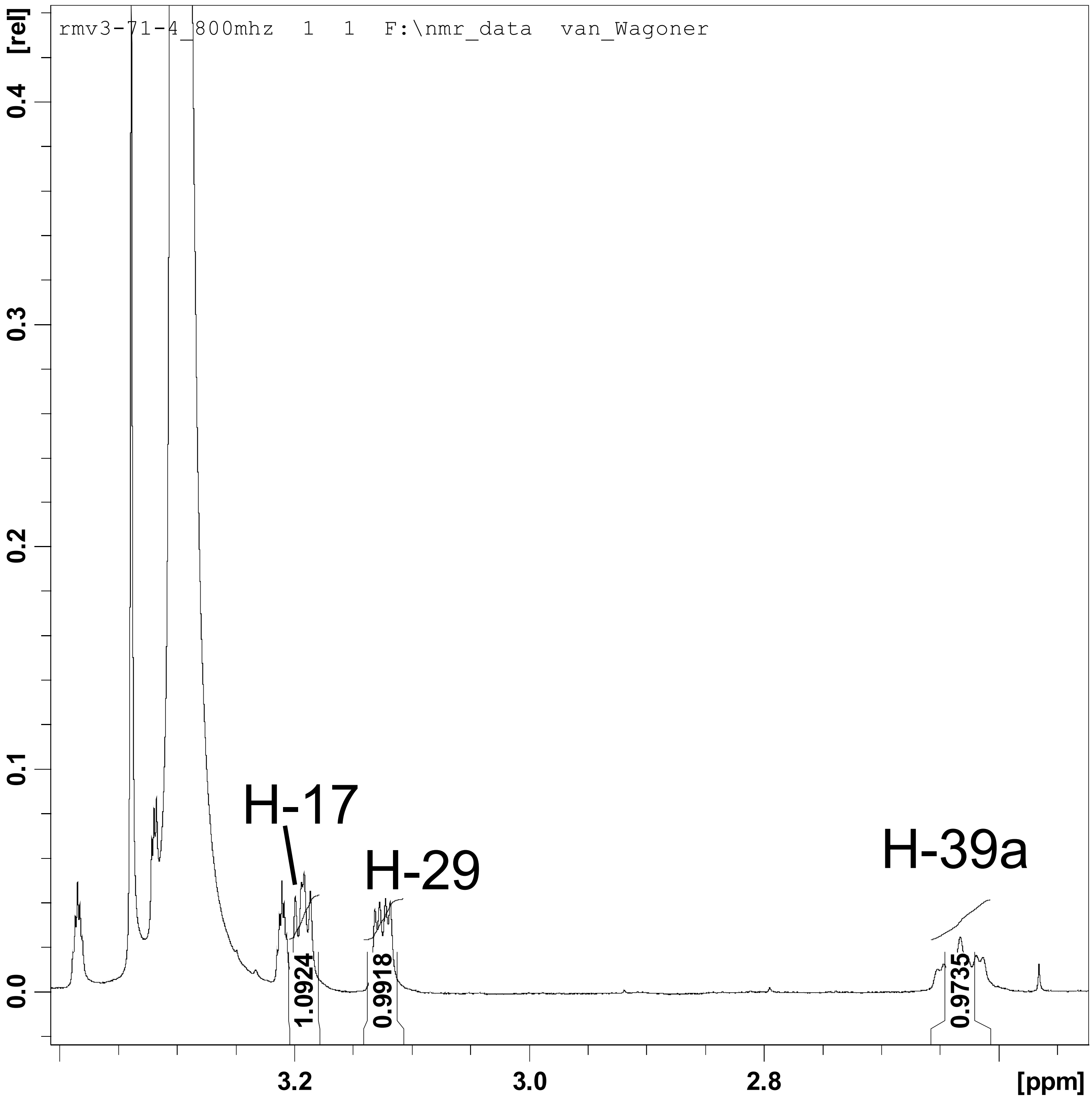
(b)

Figure S2 continued.



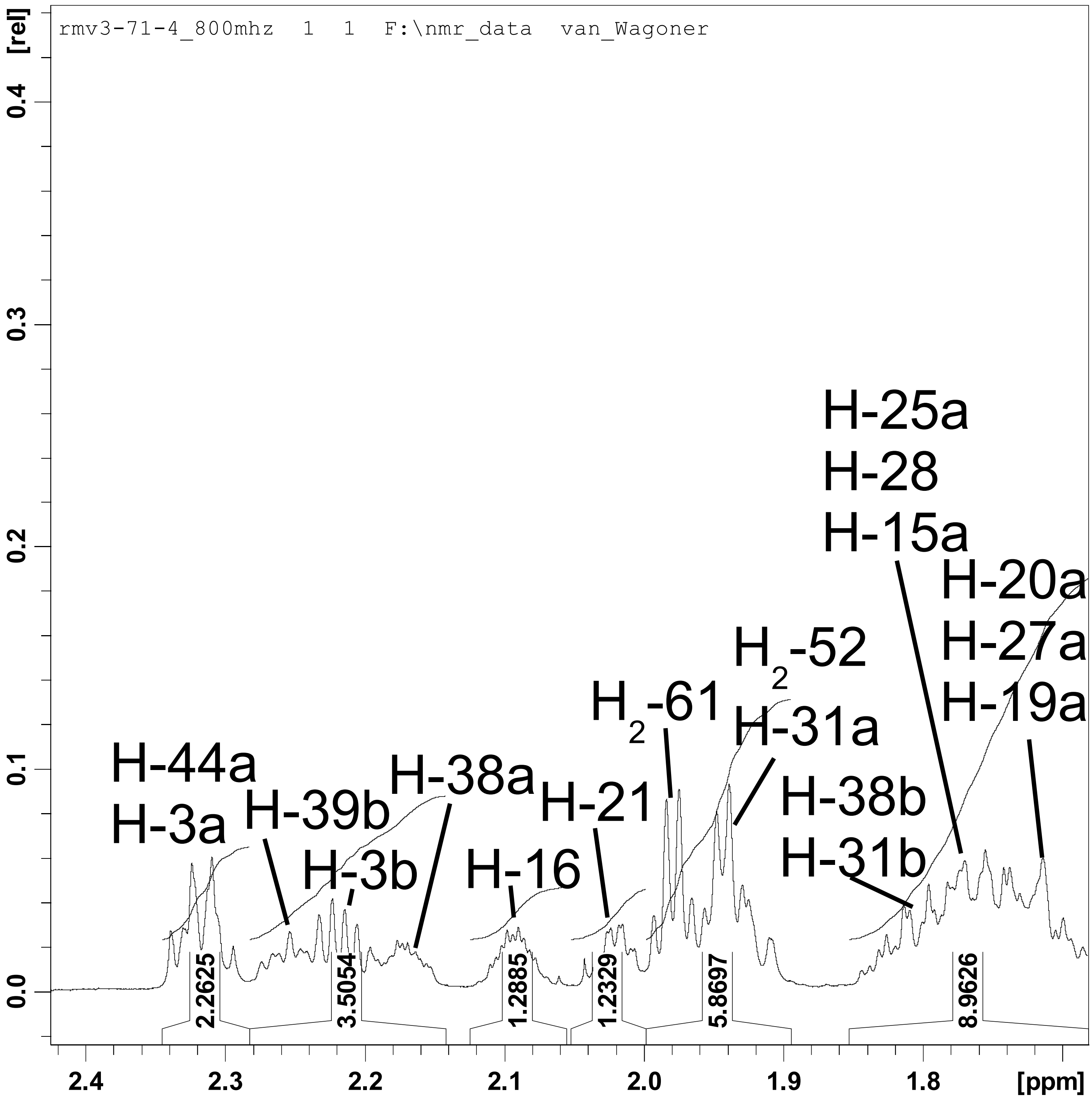
(c)

Figure S2 continued.



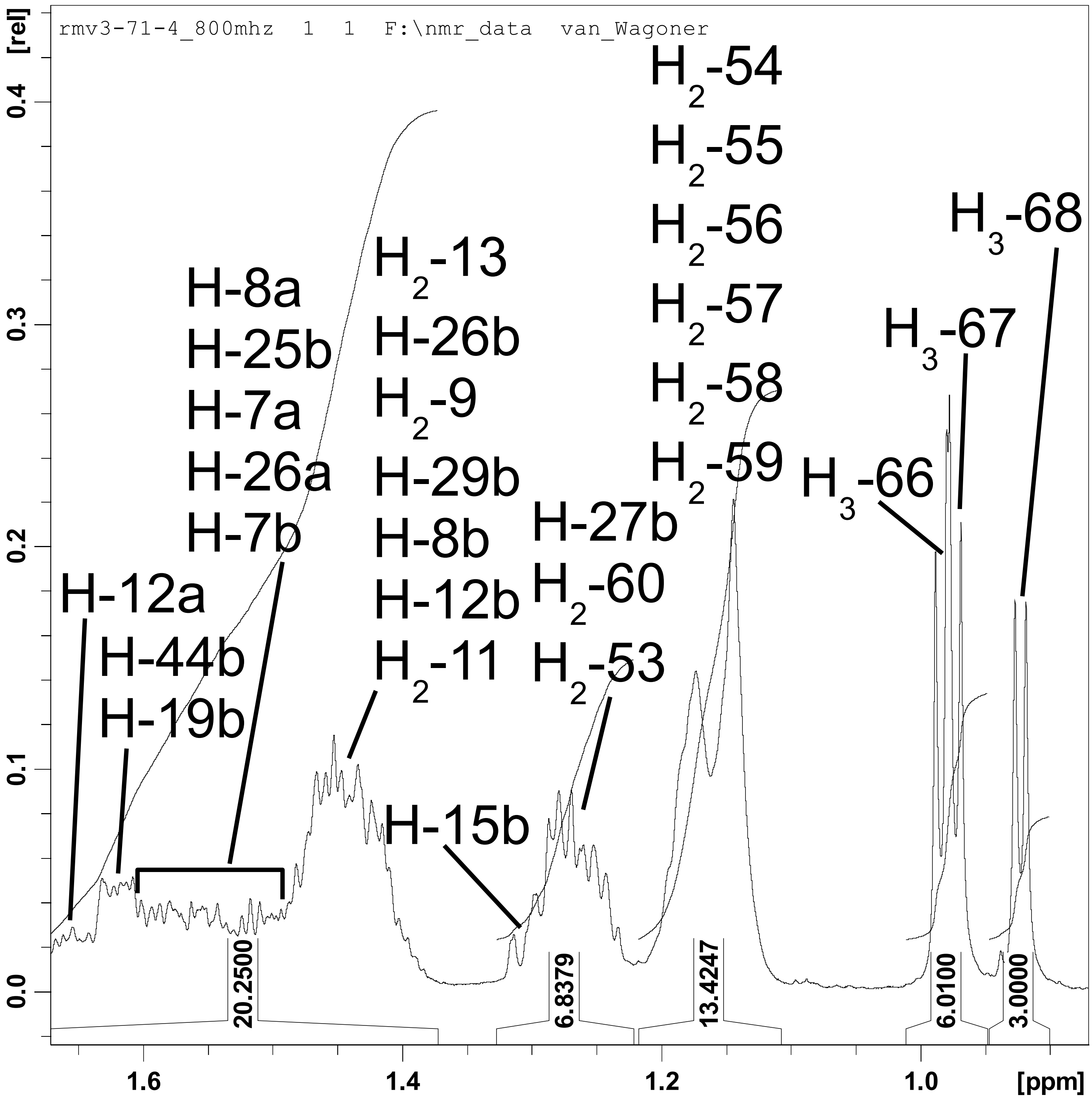
(d)

Figure S2 continued.



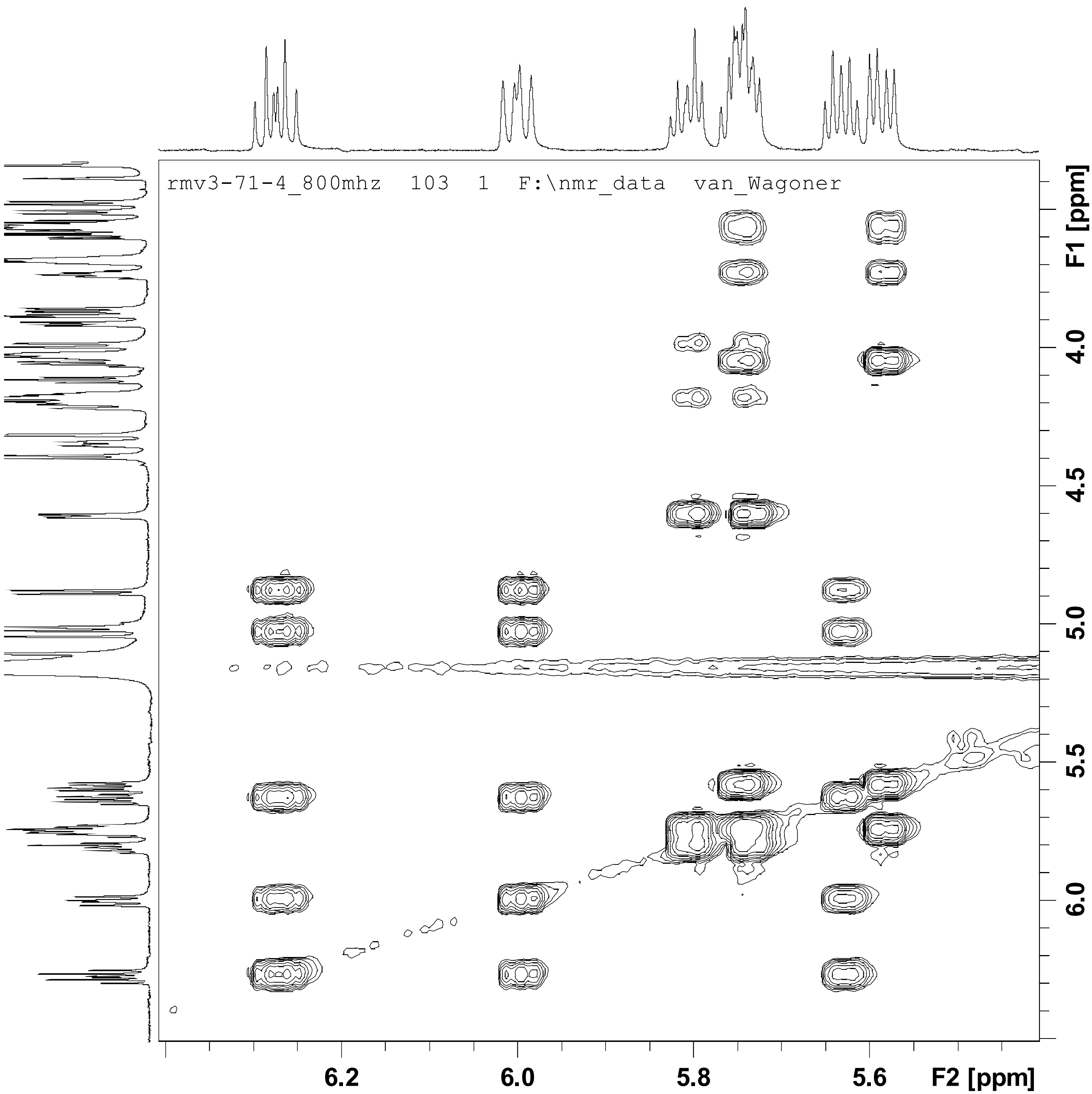
(e)

Figure S2 continued.



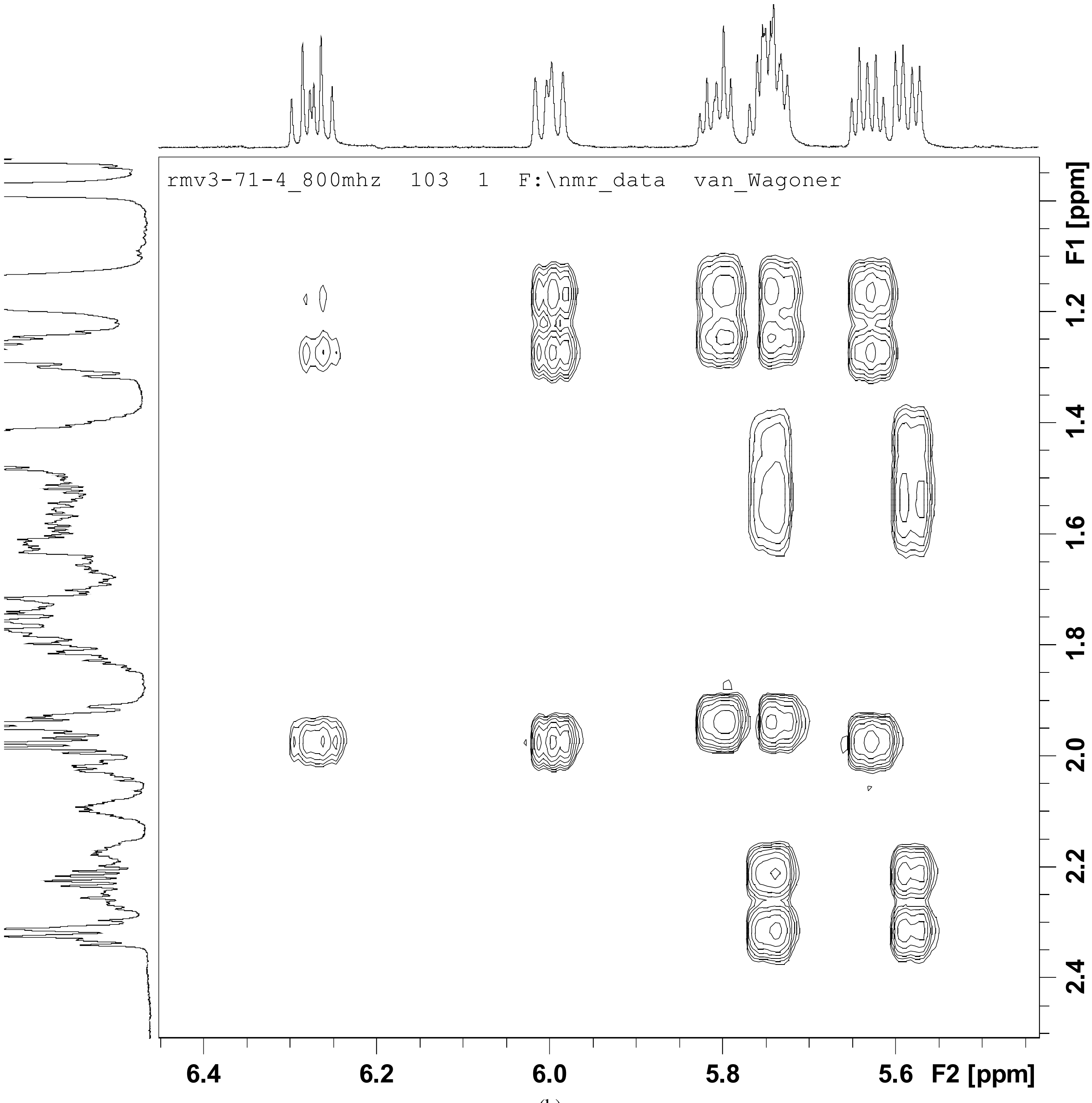
(f)

Figure S2 continued.



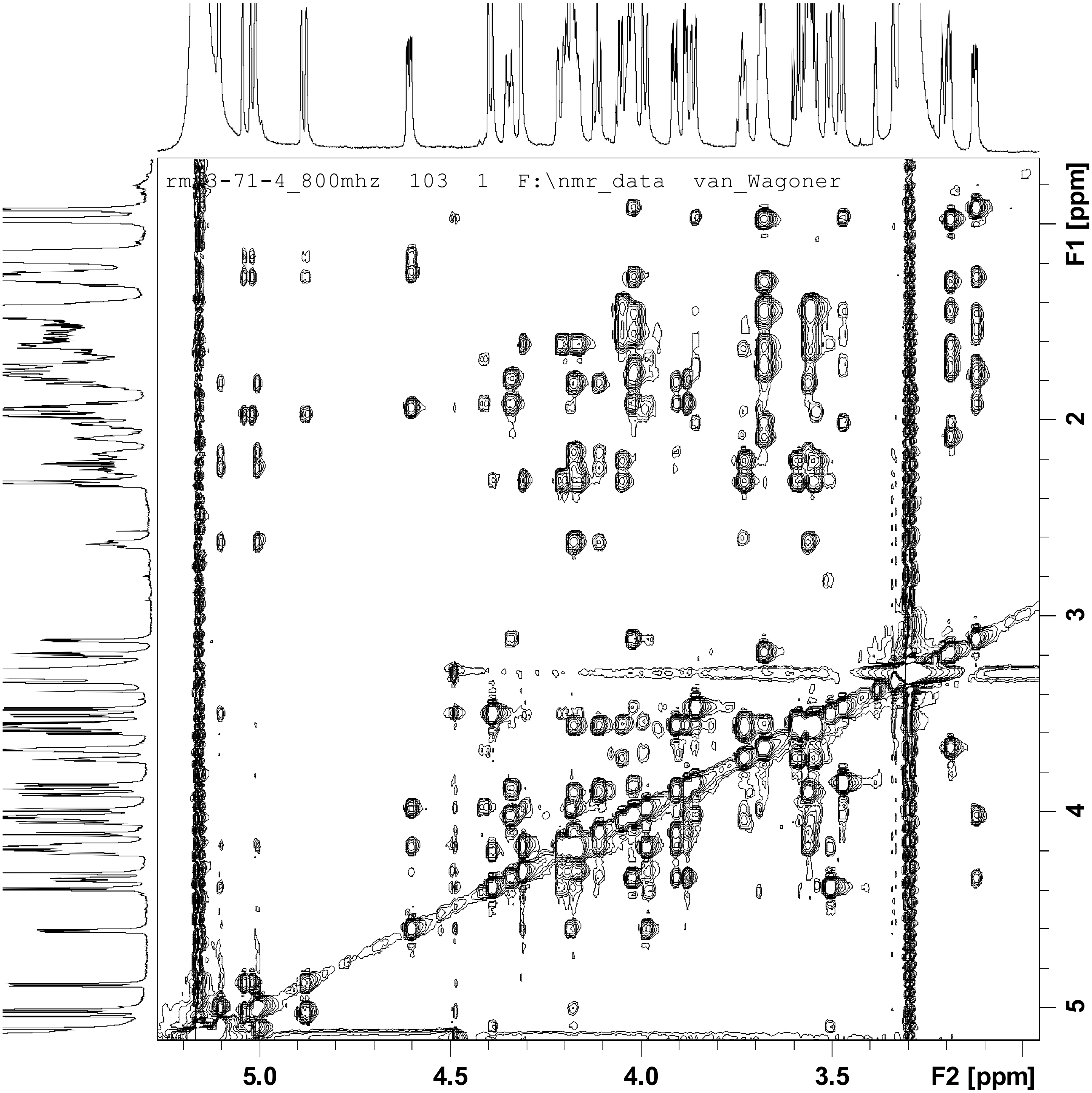
(a)

Figure S3. TOCSY spectrum of KmTx 1 (**1**). a–d- Details from various regions.



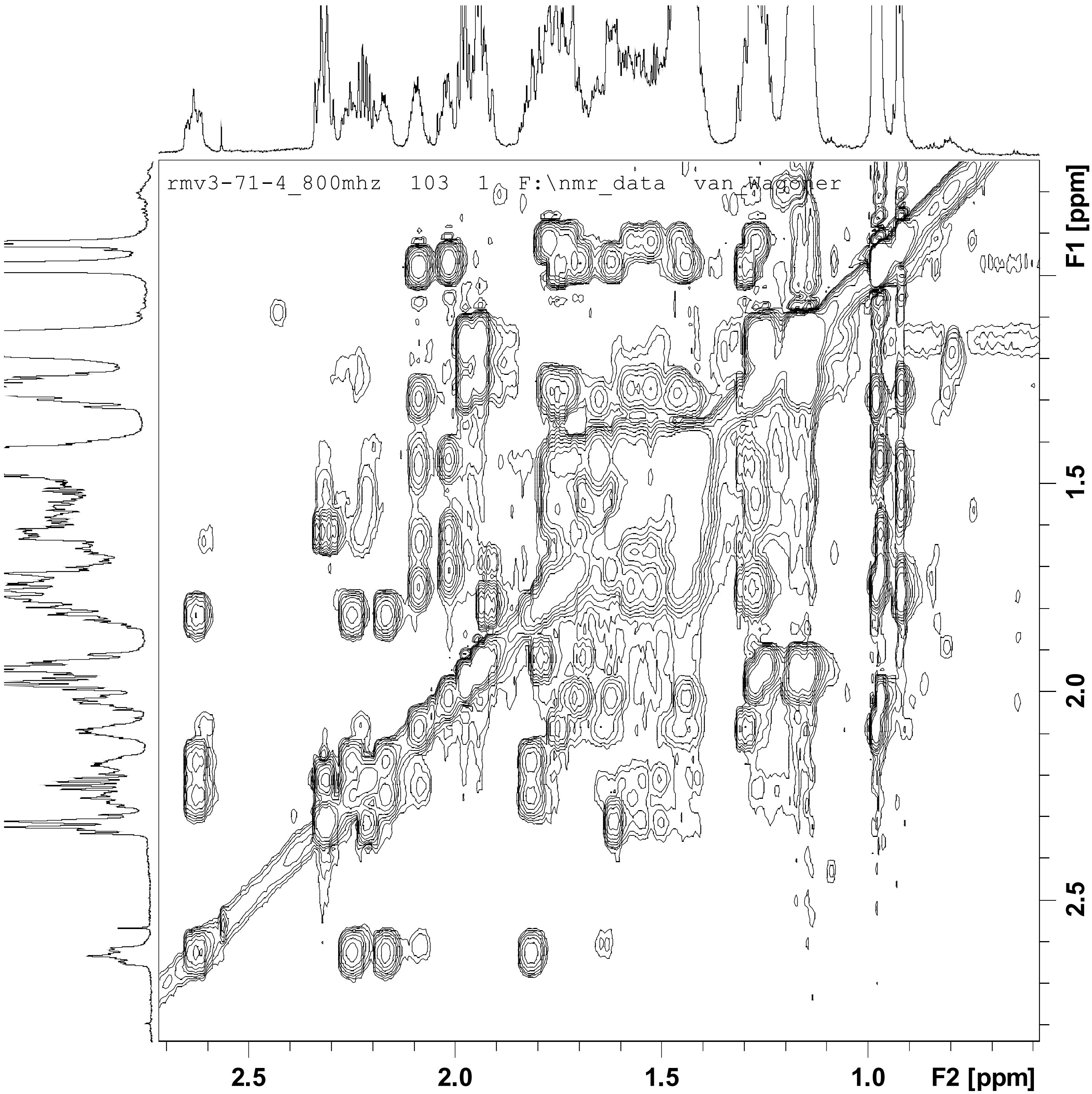
(b)

Figure S3 continued.



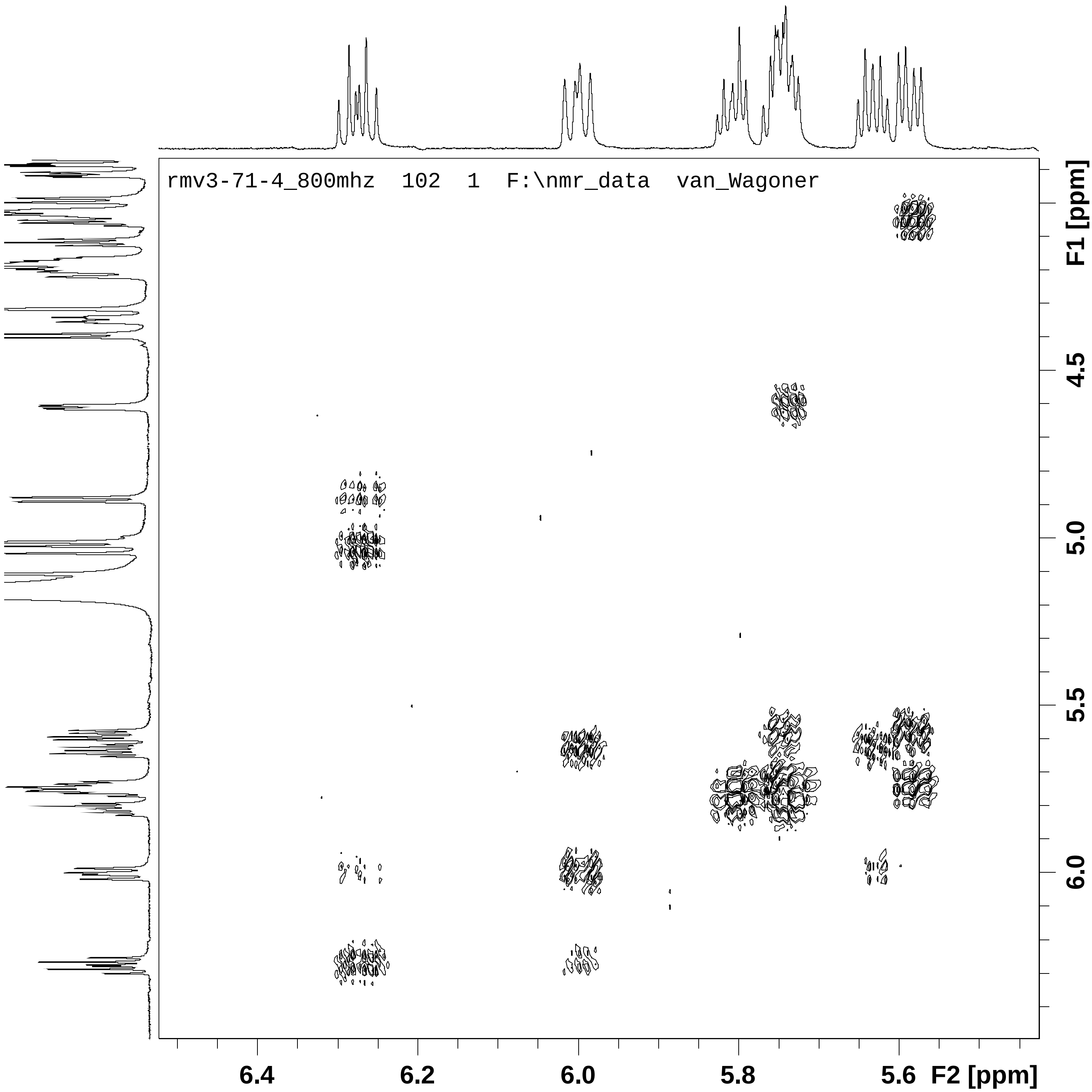
(c)

Figure S3 continued.



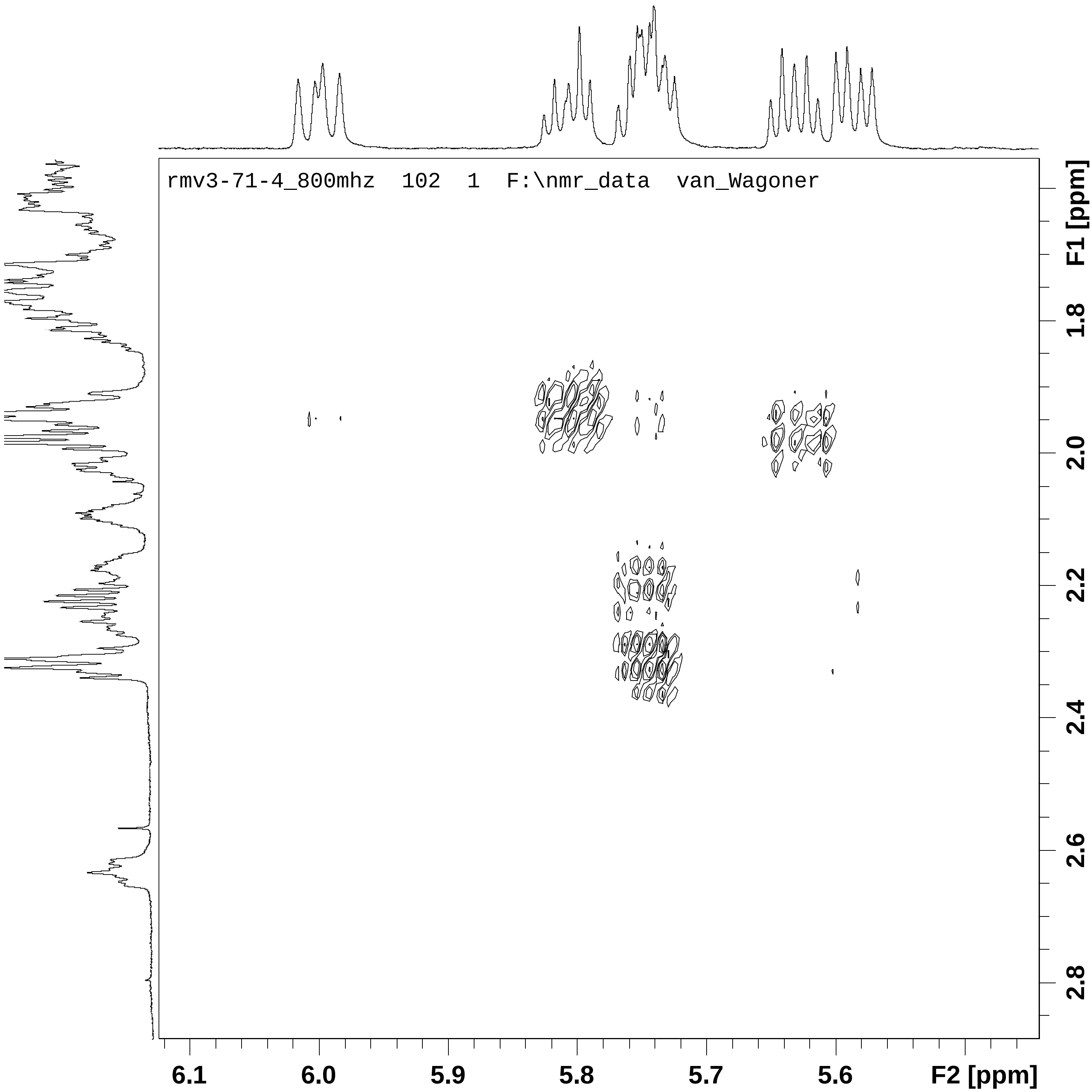
(d)

Figure S3 continued.



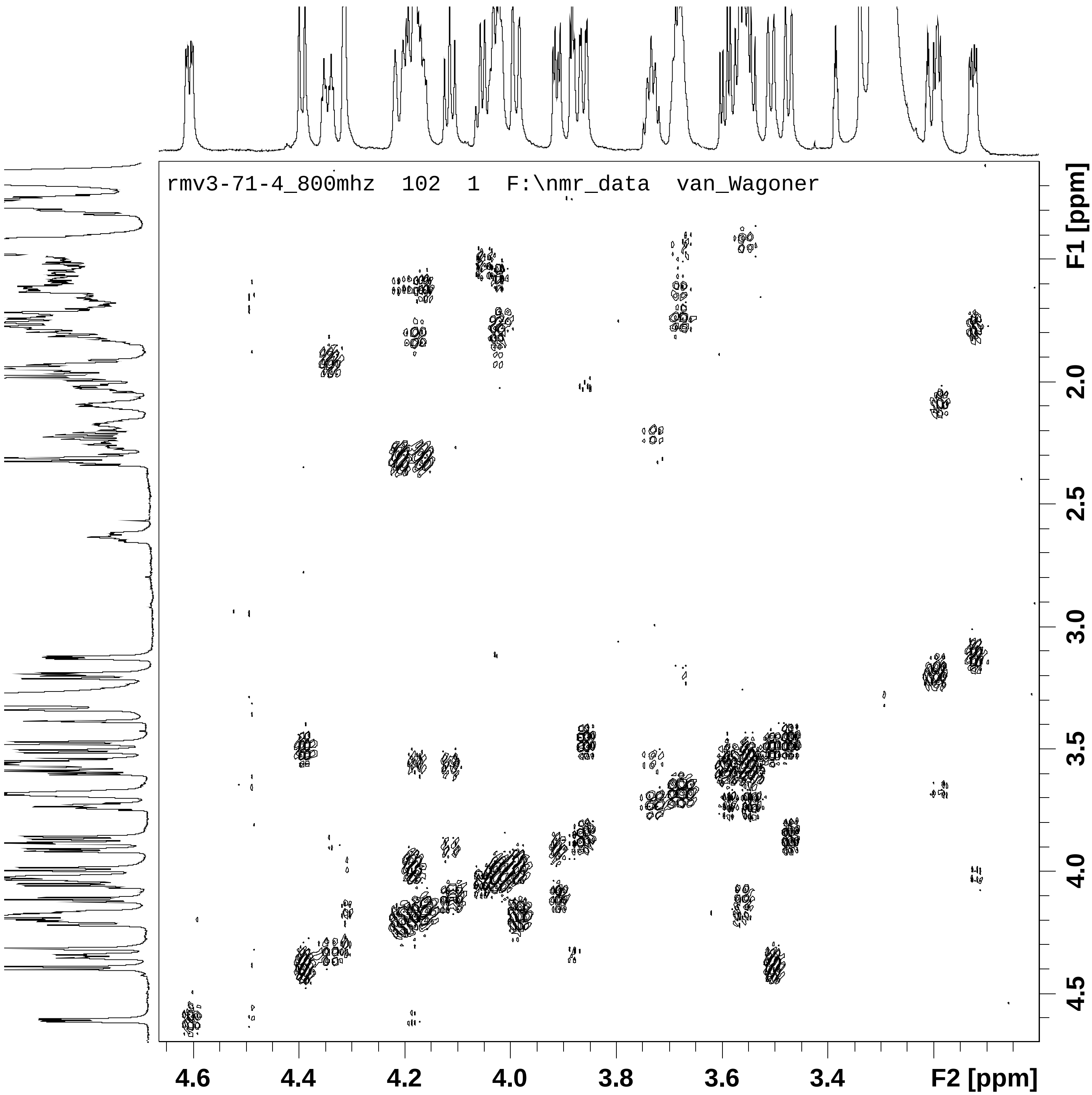
(a)

Figure S4. DQF-COSY of KmTx 1 (**1**) recorded at 800 MHz. a–d- Details from various regions.



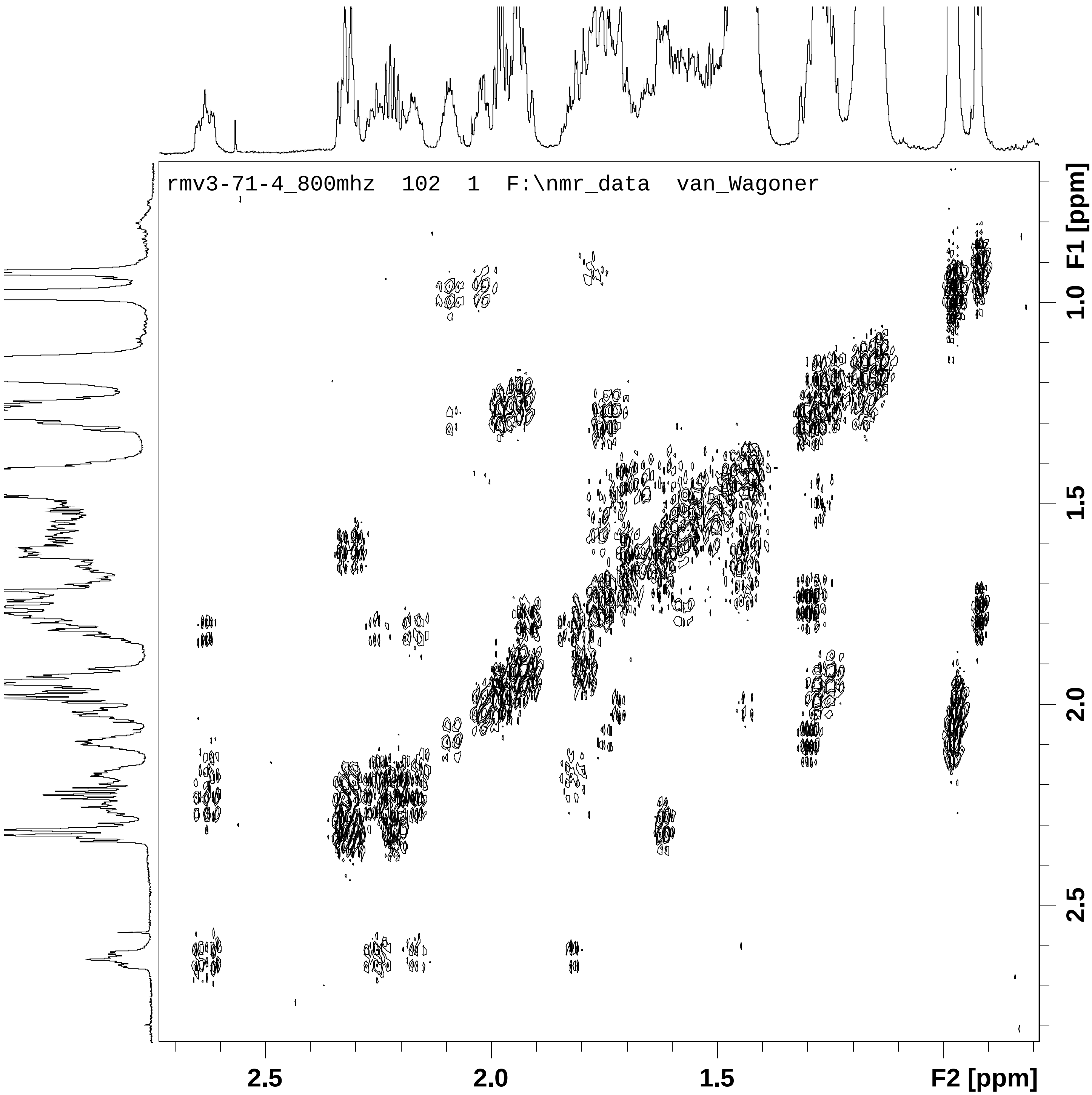
(b)

Figure S4 continued.



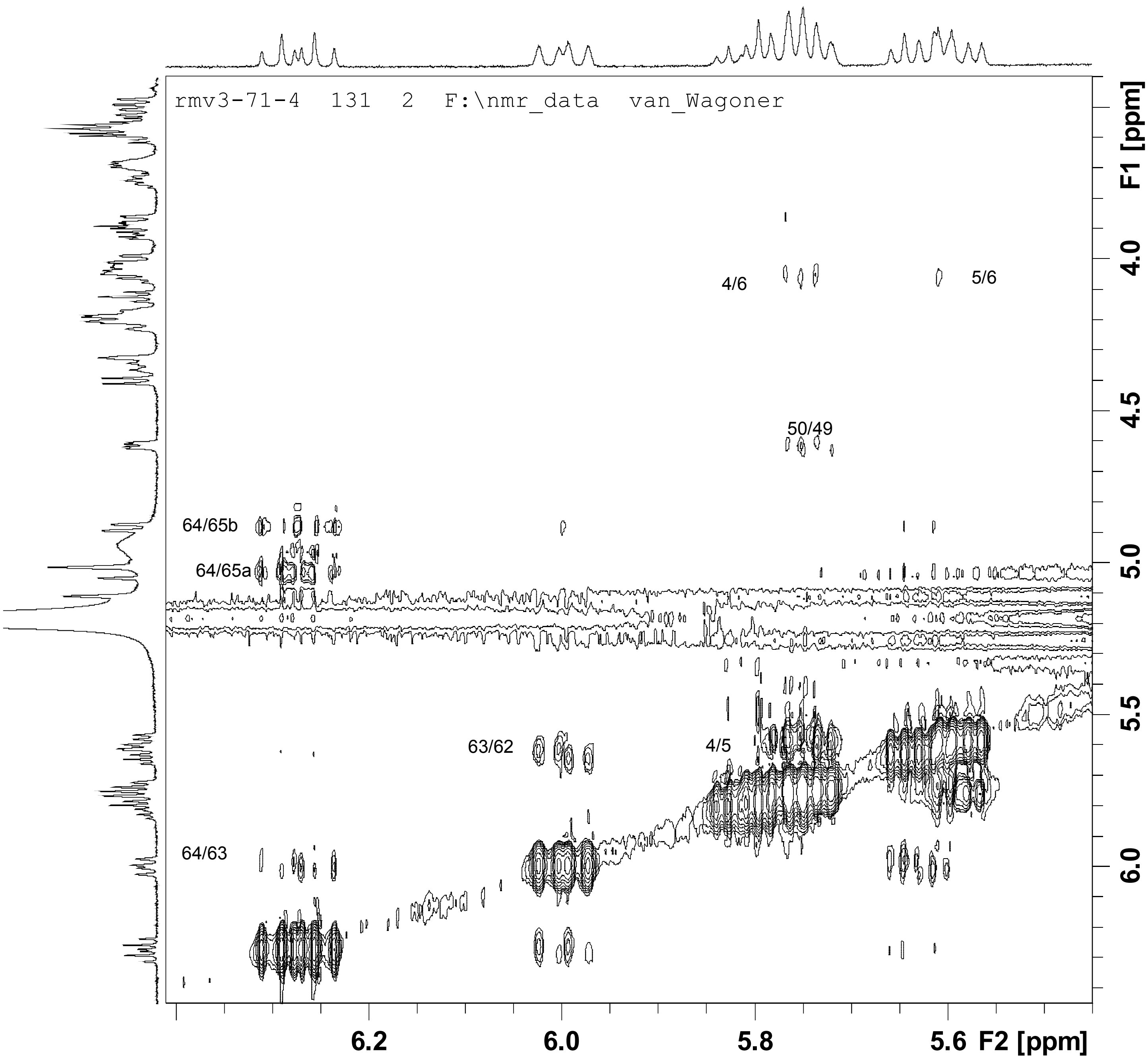
(c)

Figure S4 continued.



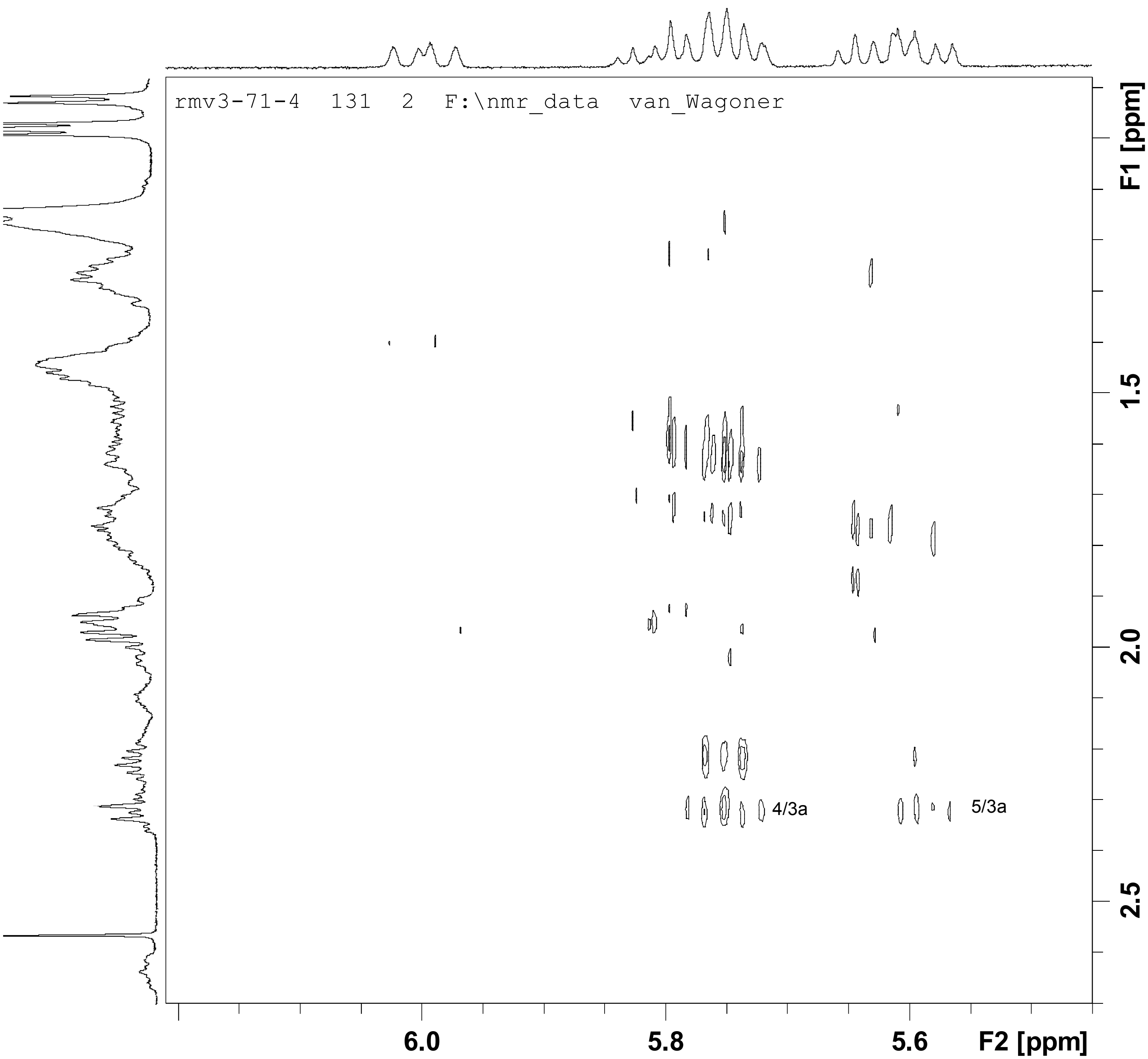
(d)

Figure S4 continued.



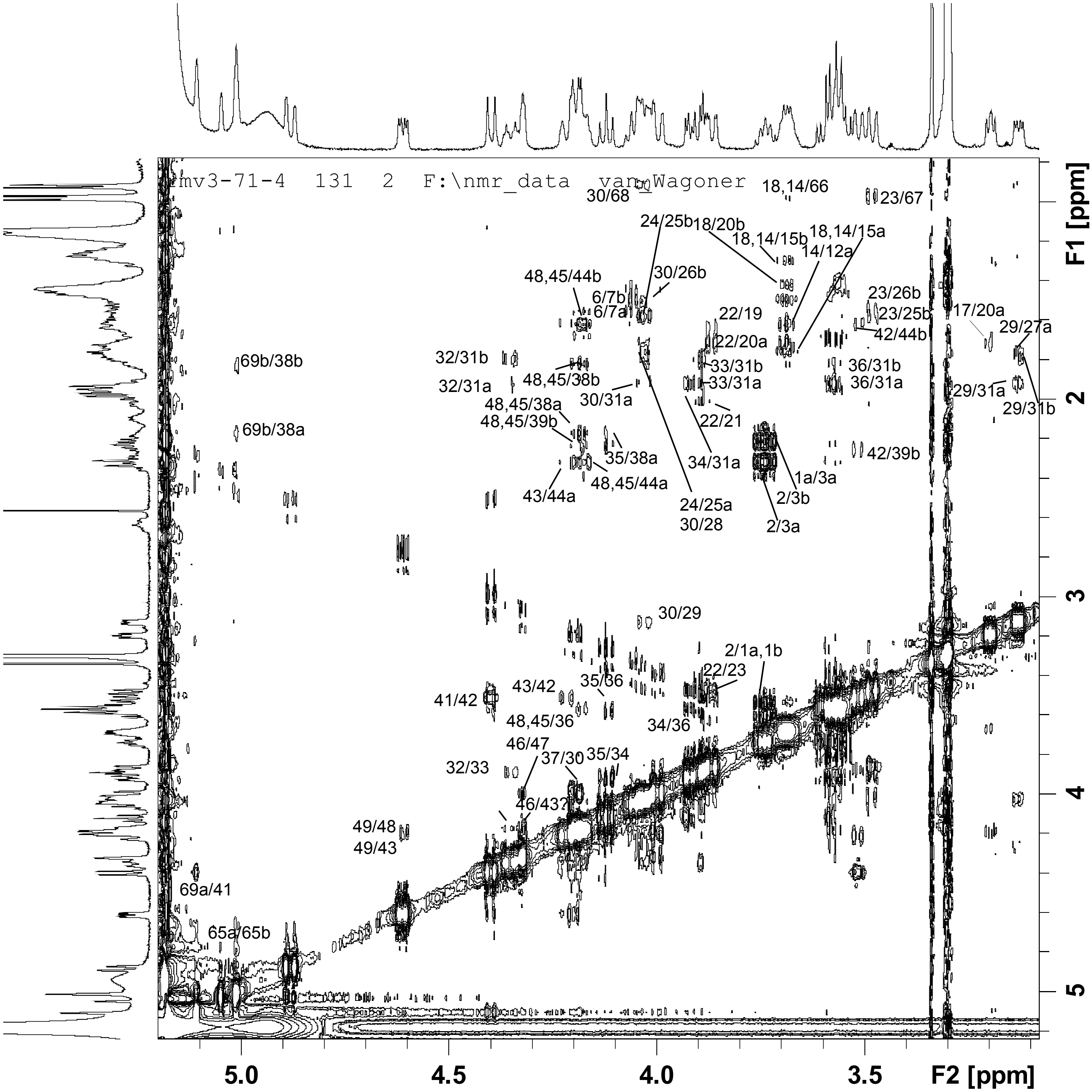
(a)

Figure S5. NOESY spectrum of KmTx 1 (**1**) recorded at 500 MHz, 200 ms mixing time. a–d- Details from various regions.



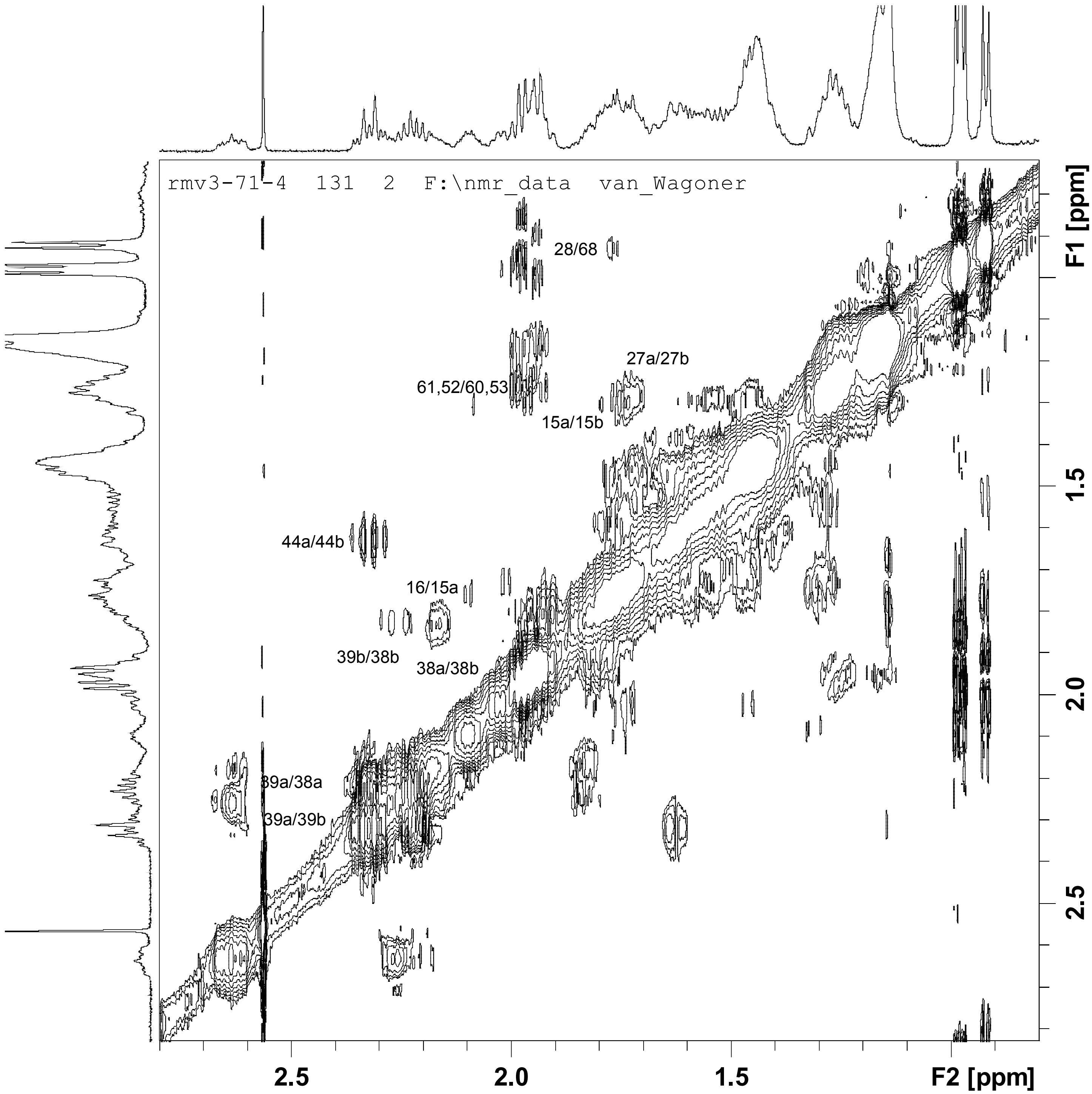
(b)

Figure S5 continued.



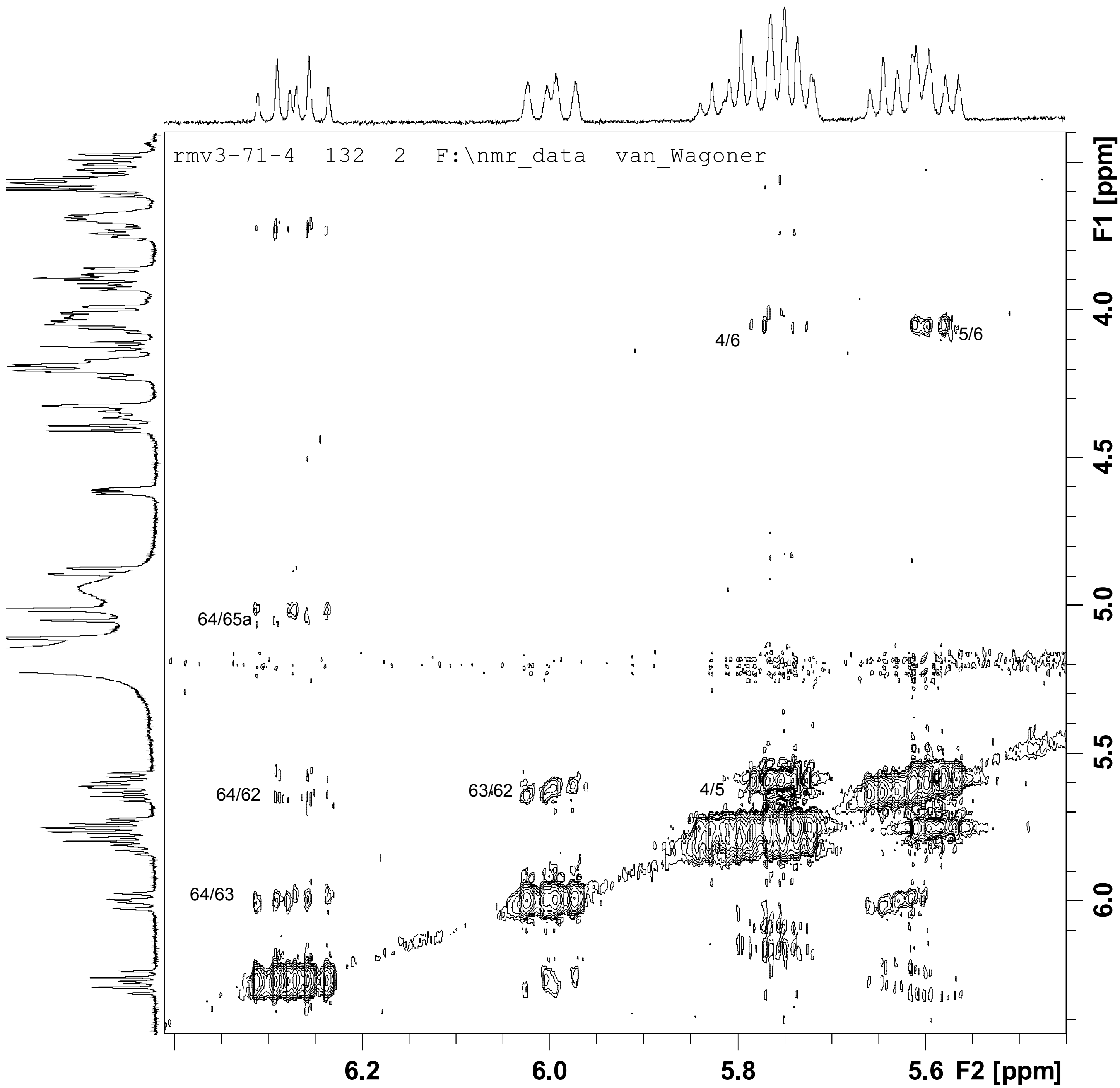
(c)

Figure S5 continued.



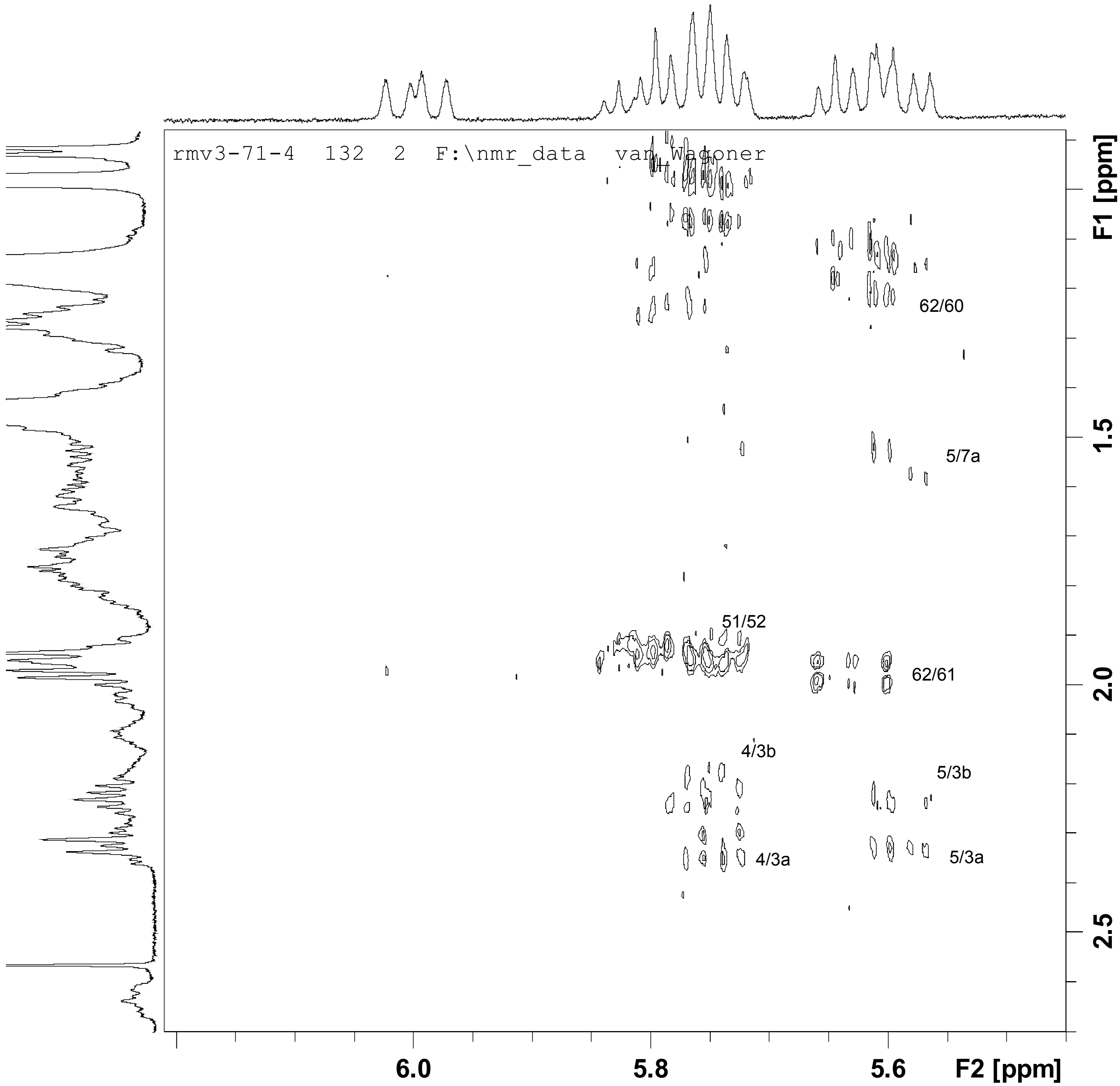
(d)

Figure S5 continued.



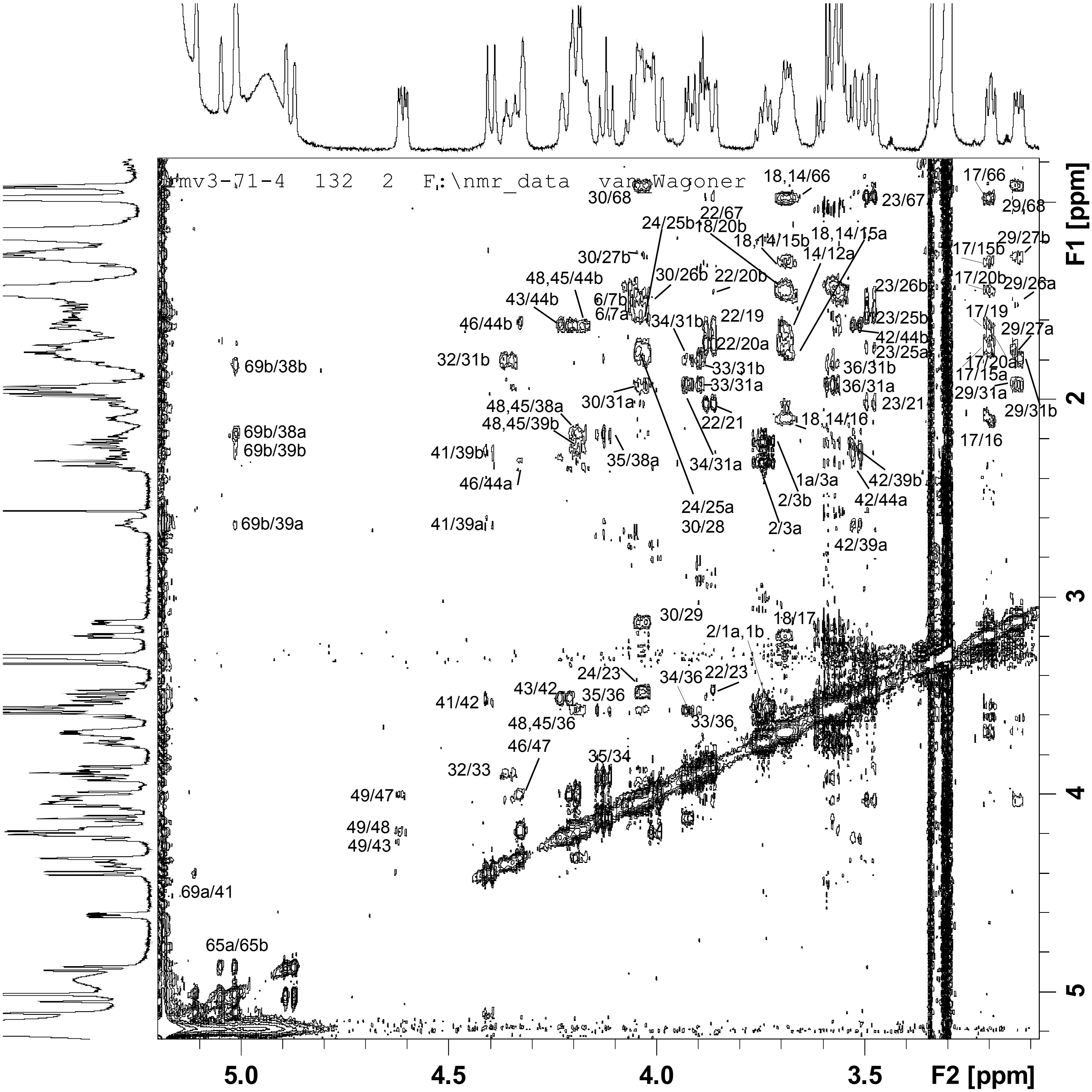
(a)

Figure S6. ROESY spectrum of KmTx 1 (**1**) recorded at 500 MHz, 200 ms mixing time. a–d- Details from various regions.



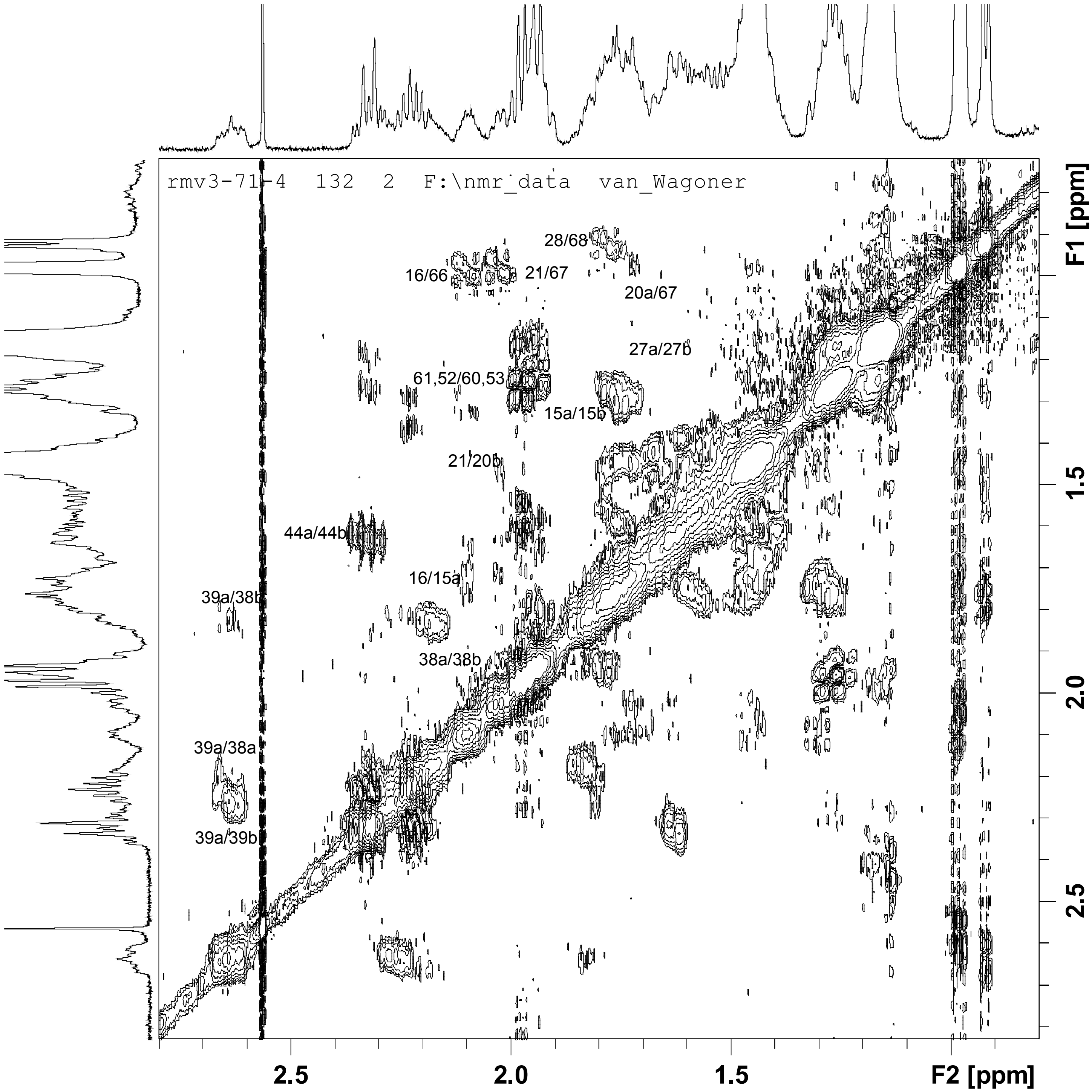
(b)

Figure S6 continued.



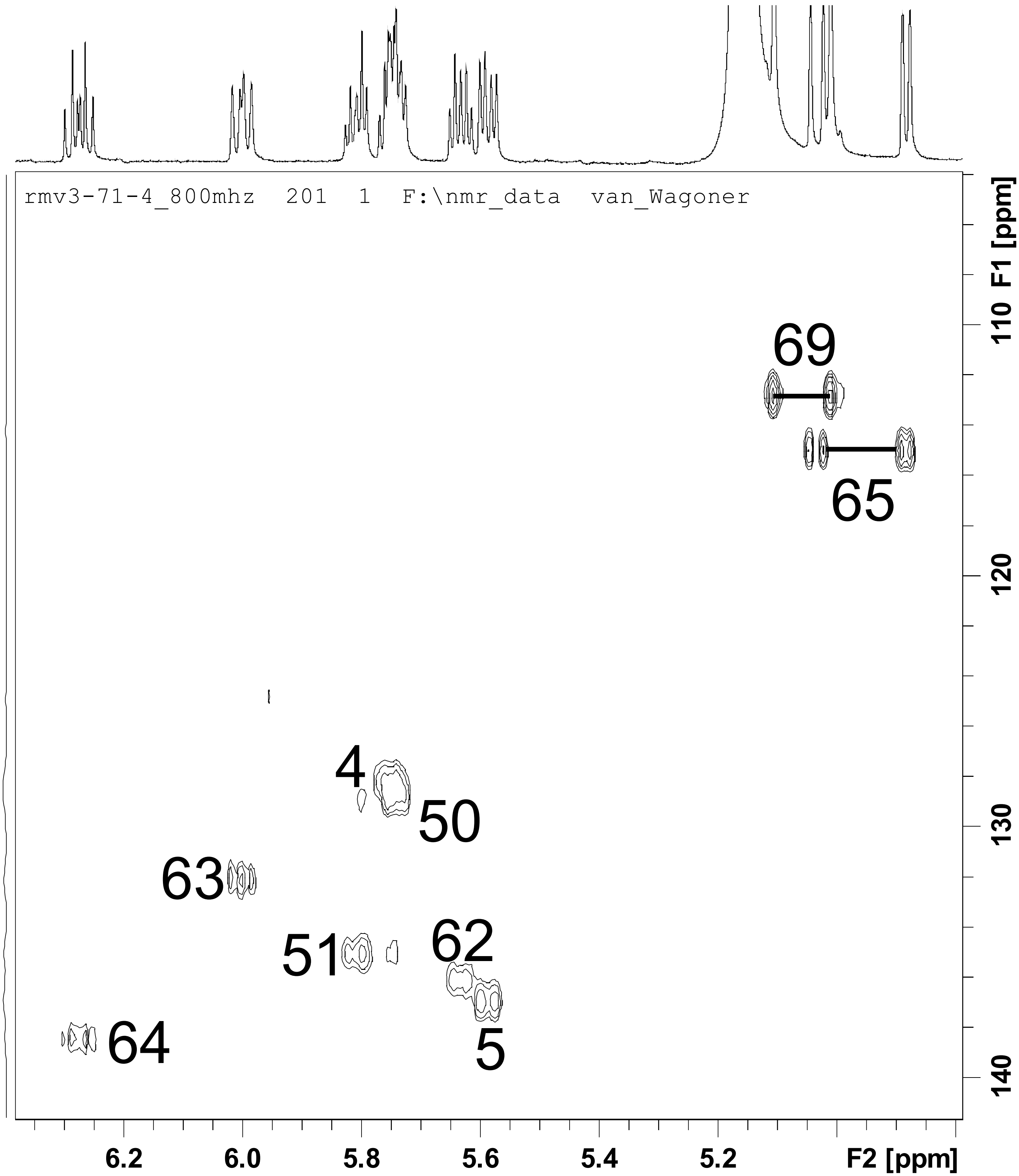
(c)

Figure S6 continued.



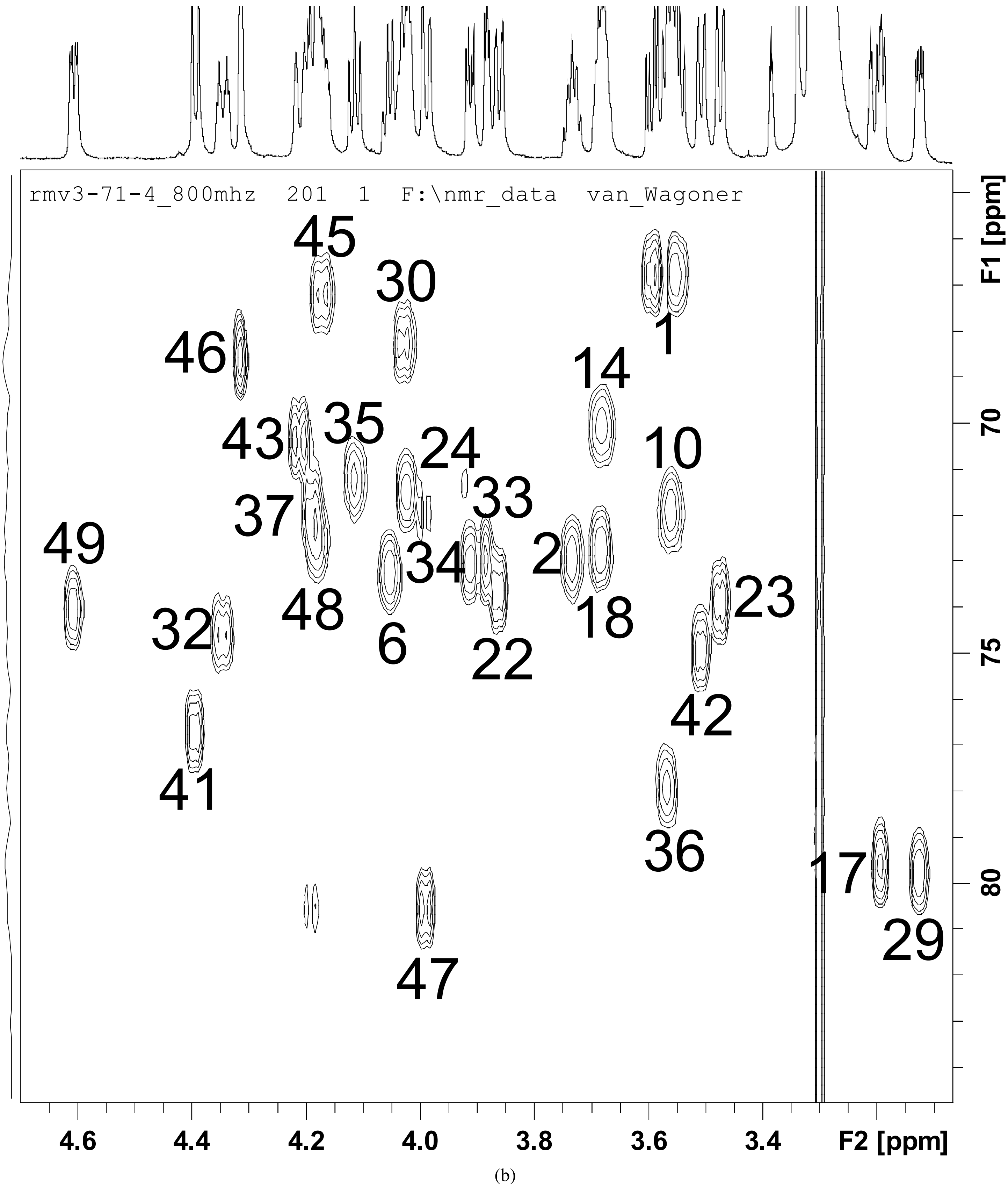
(d)

Figure S6 continued.



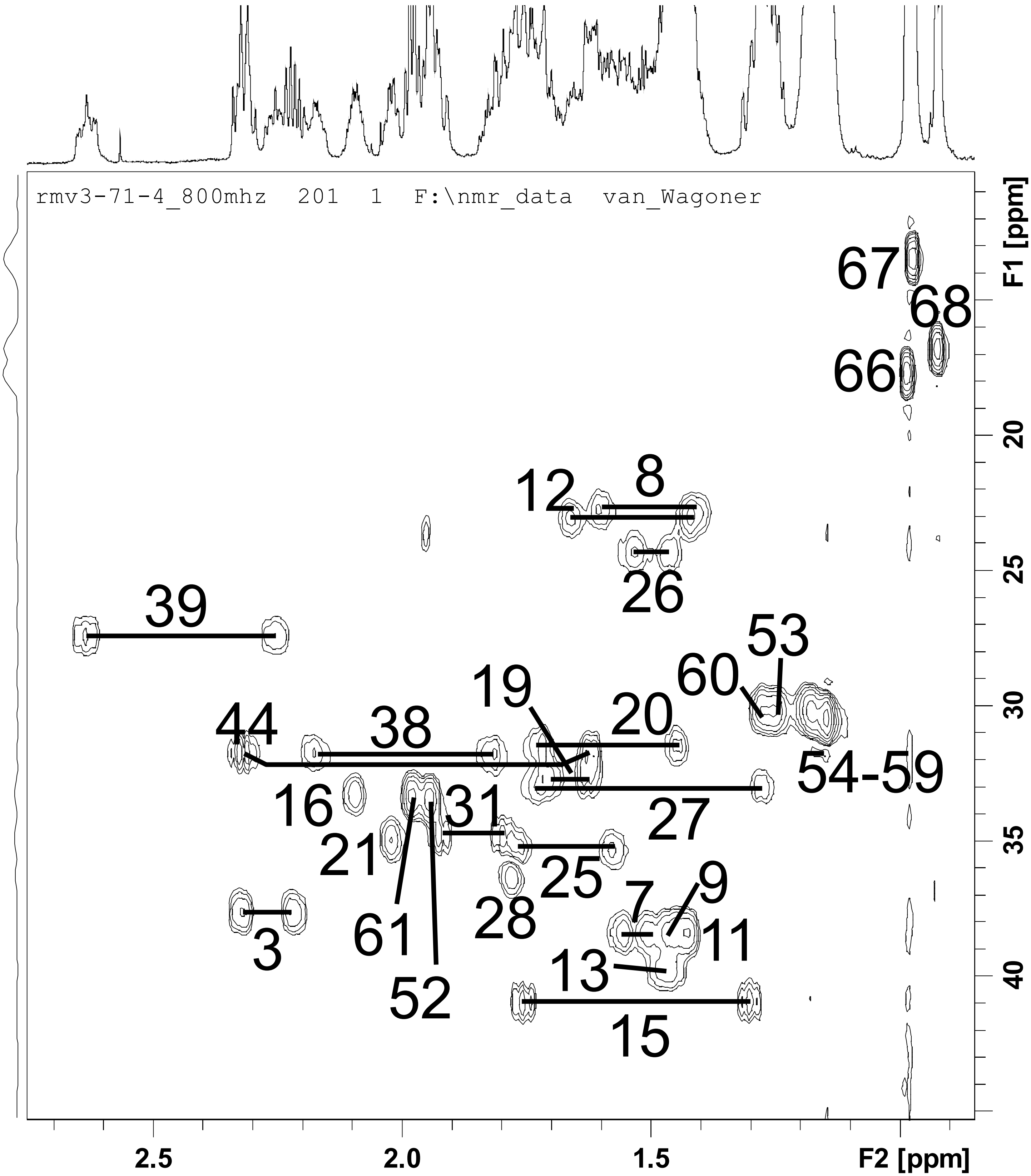
(a)

Figure S7. HSQC spectrum of KmTx 1 **1**. a–c- Details from various regions.



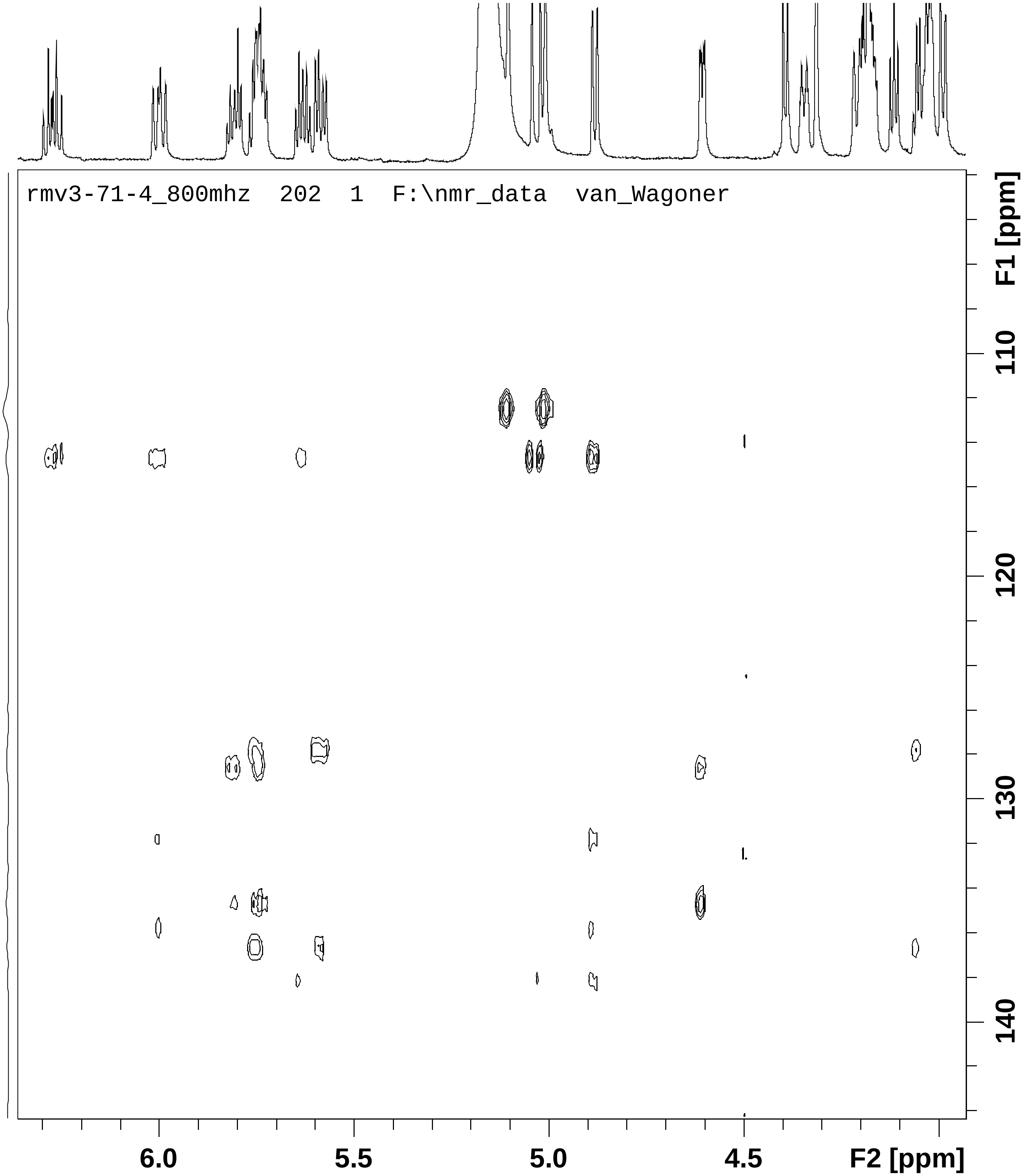
(b)

Figure S7 continued.



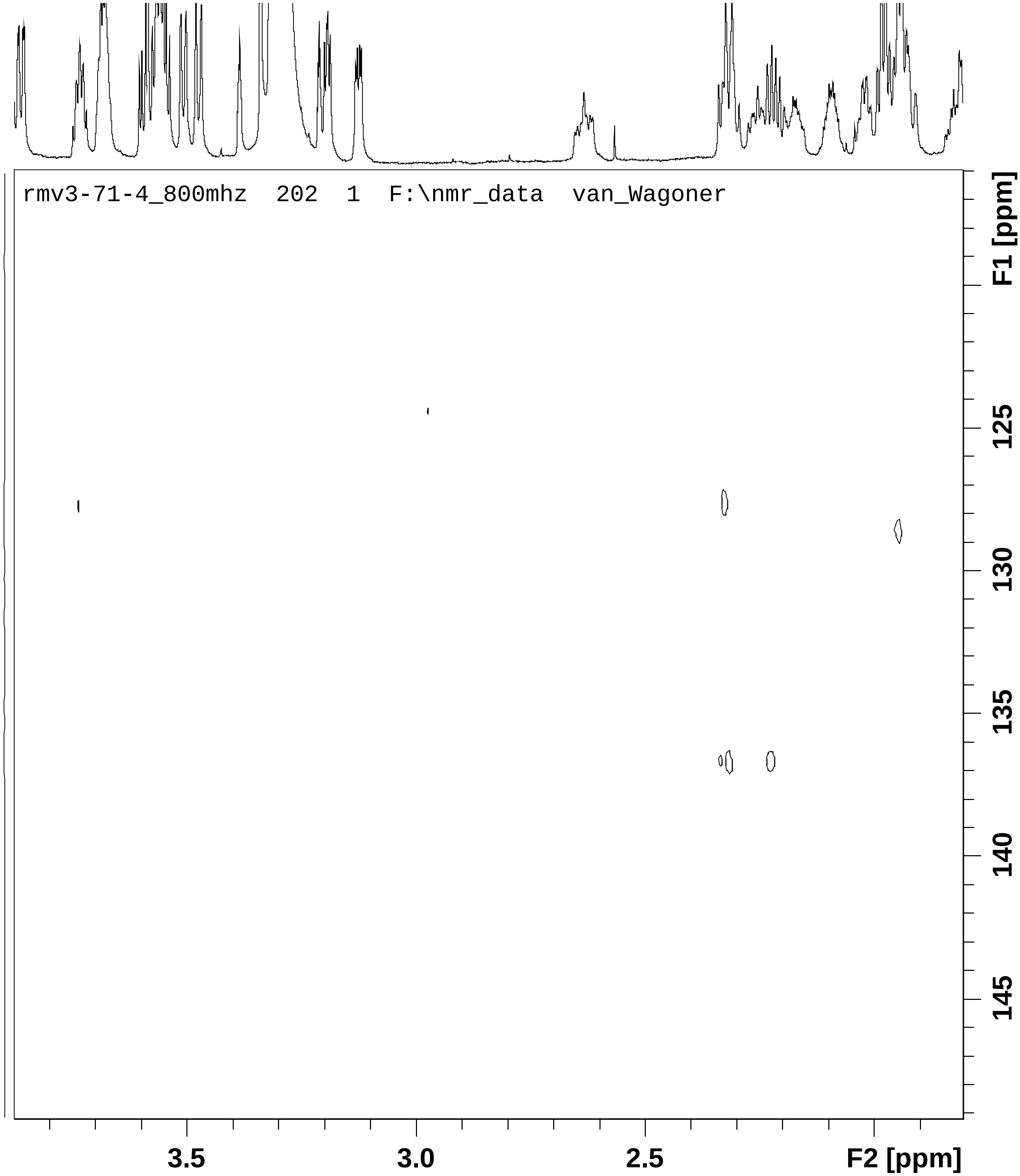
(c)

Figure S7 continued.



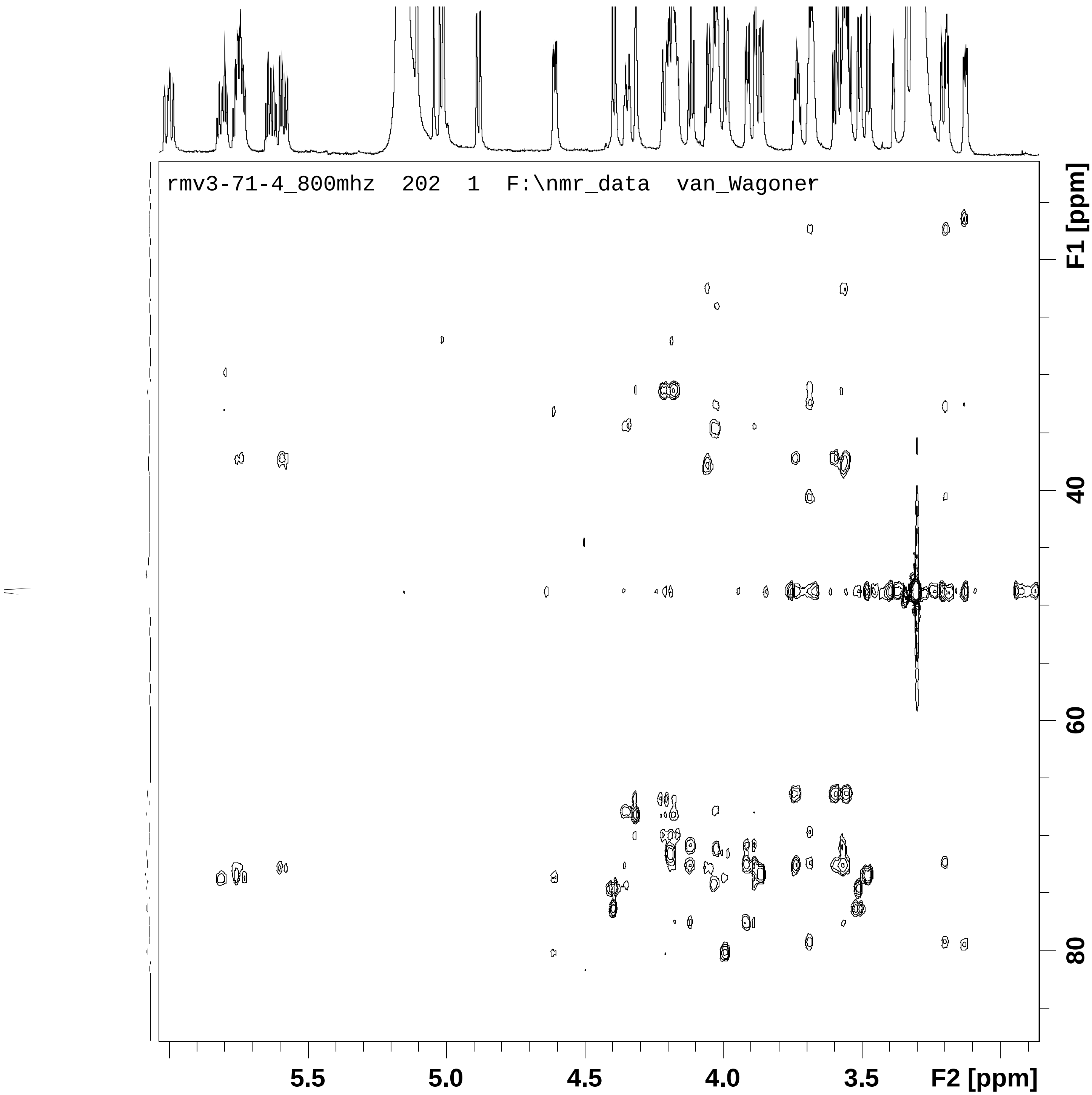
(a)

Figure S8. 2D HSQC-TOCSY of KmTx 1 (**1**) recorded at 800 MHz. a–d- Details from various regions.



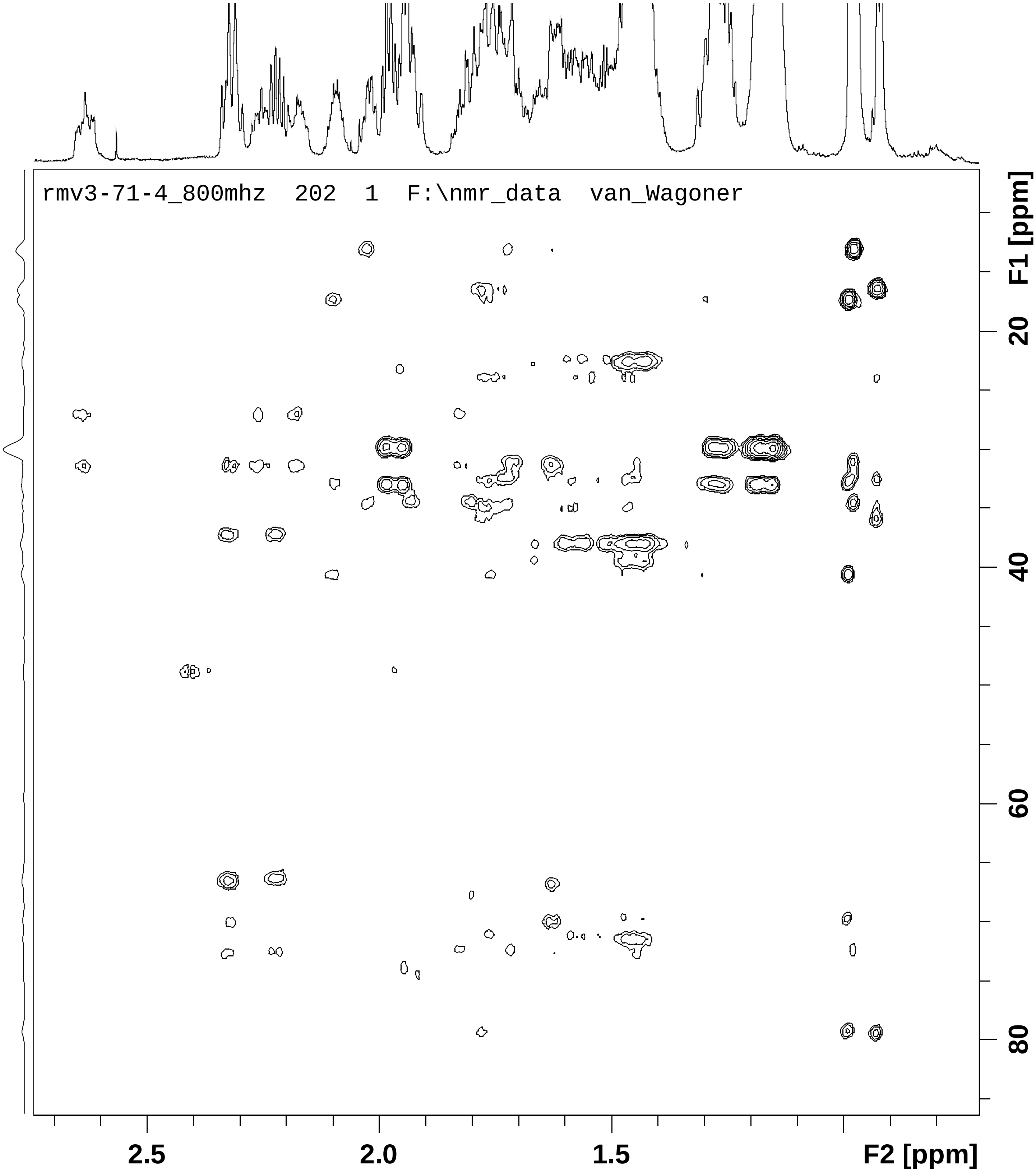
(b)

Figure S8 continued.



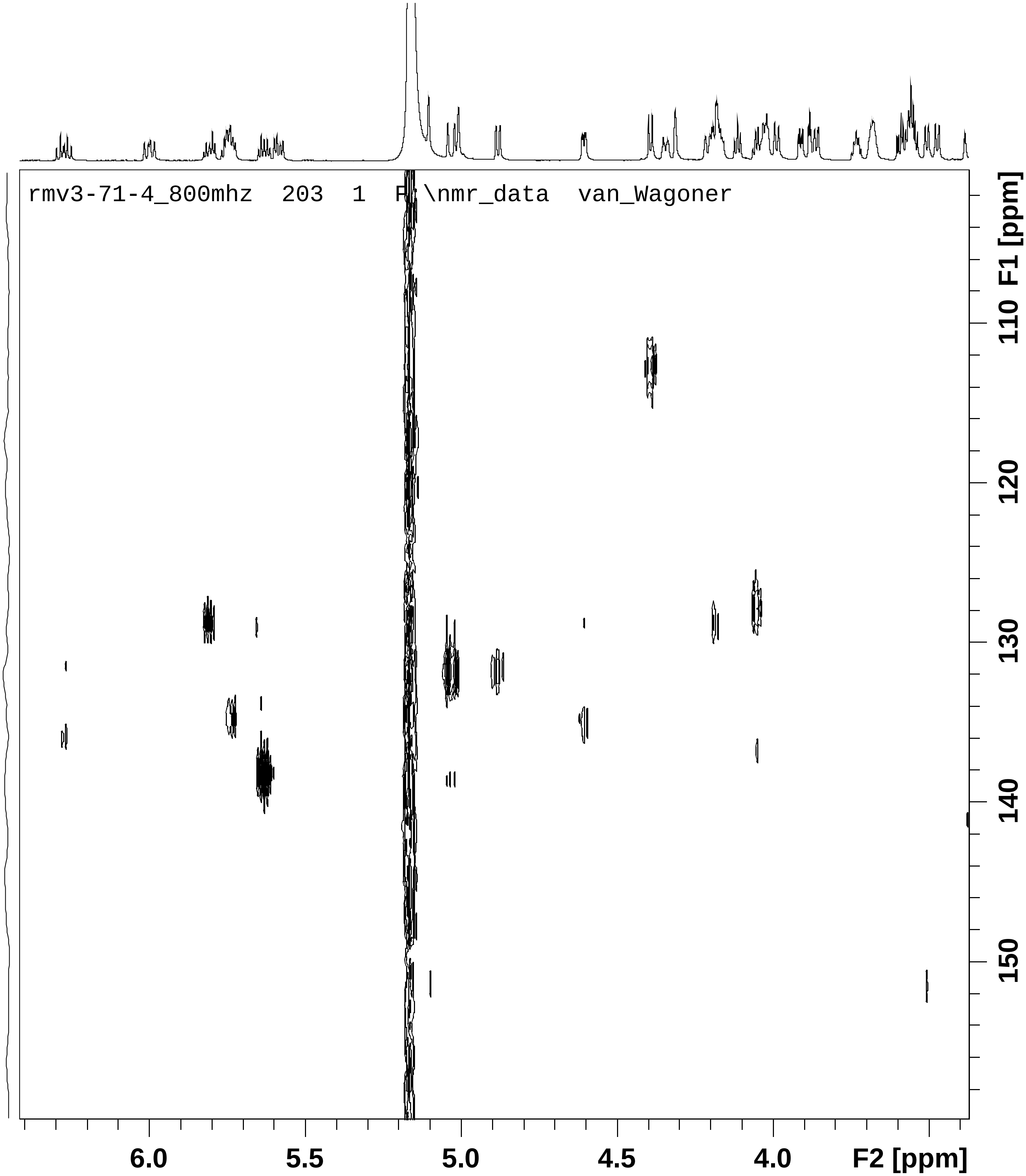
(c)

Figure S8 continued.



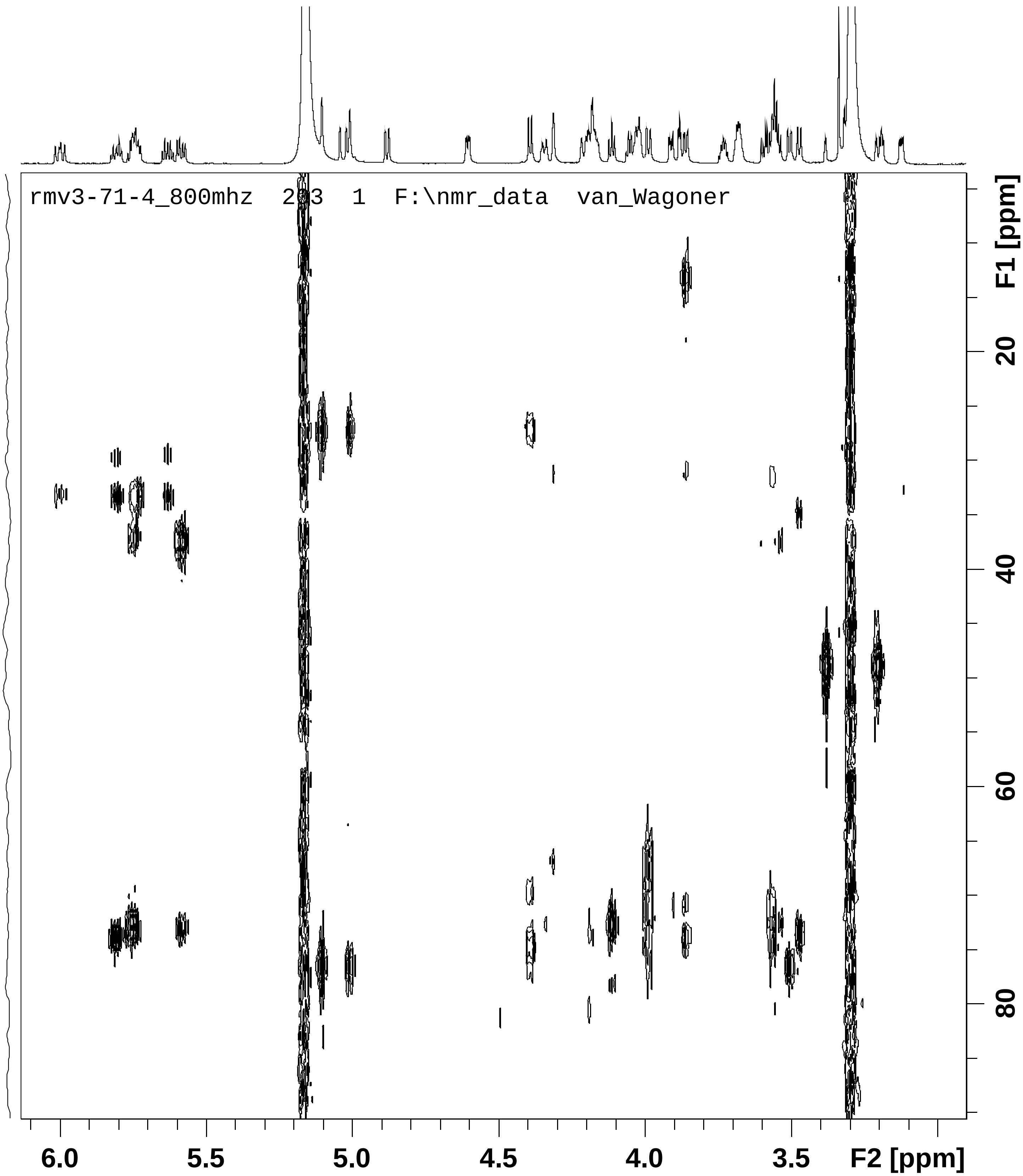
(d)

Figure S8 continued.



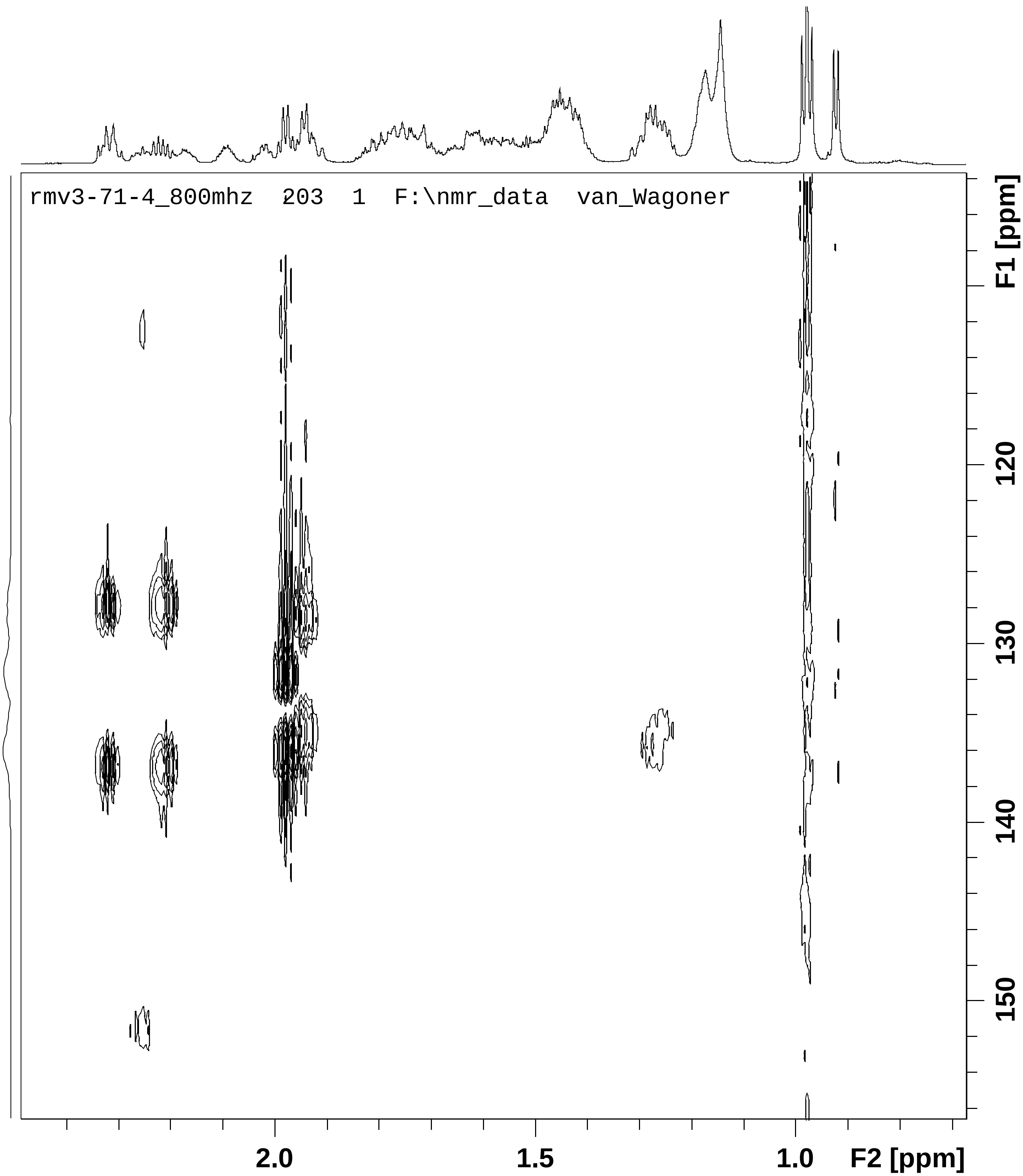
(a)

Figure S9. HMBC of KmTx 1 (**1**) recorded at 800 MHz. a–d- Details from various regions.



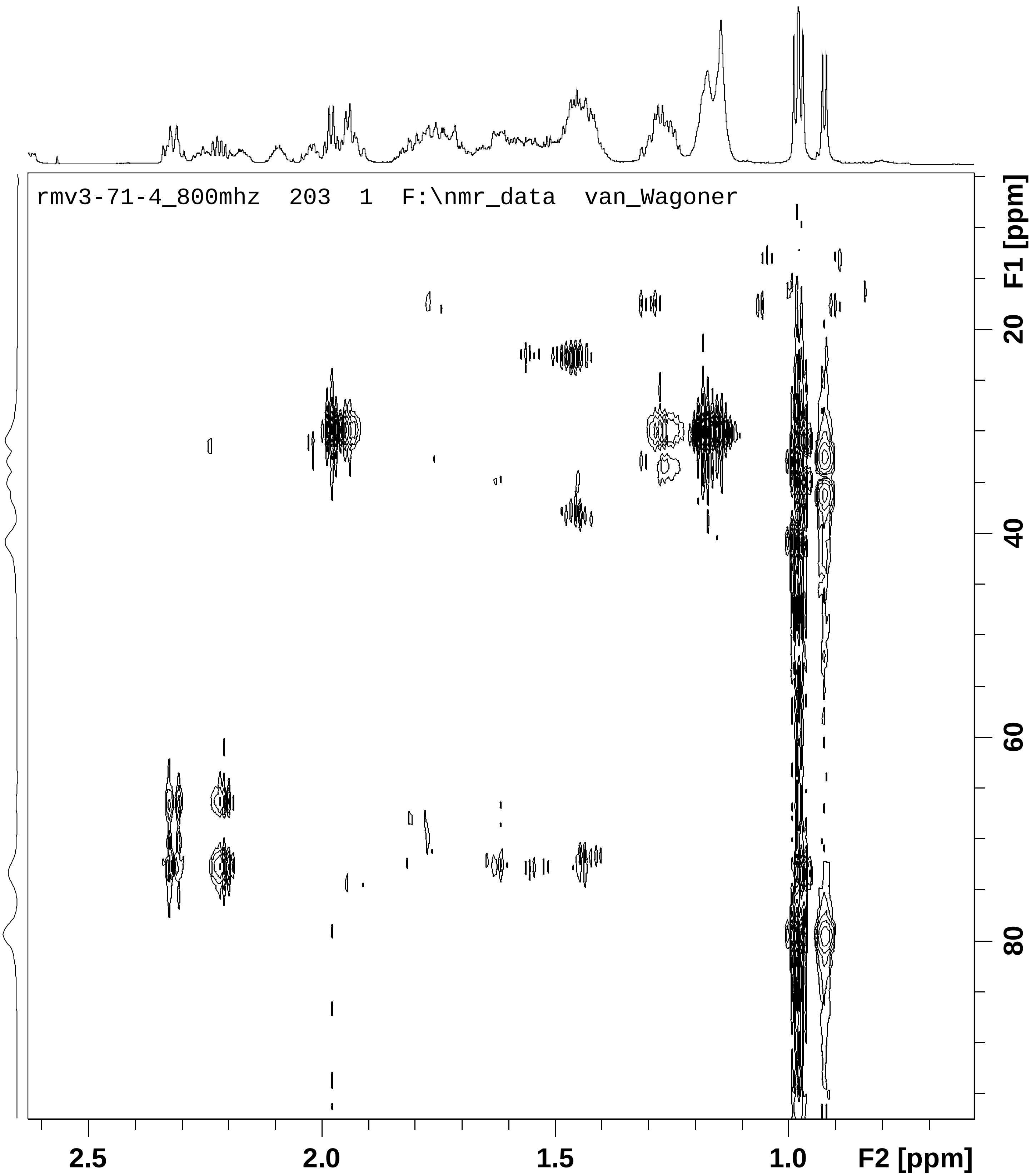
(b)

Figure S9 continued.



(c)

Figure S9 continued.



(d)

Figure S9 continued.