Supplementary materials

Structure and toxicity of AZA-59, an azaspiracid shellfish poisoning toxin produced by *Azadinium poporum* (Dinophyceae)

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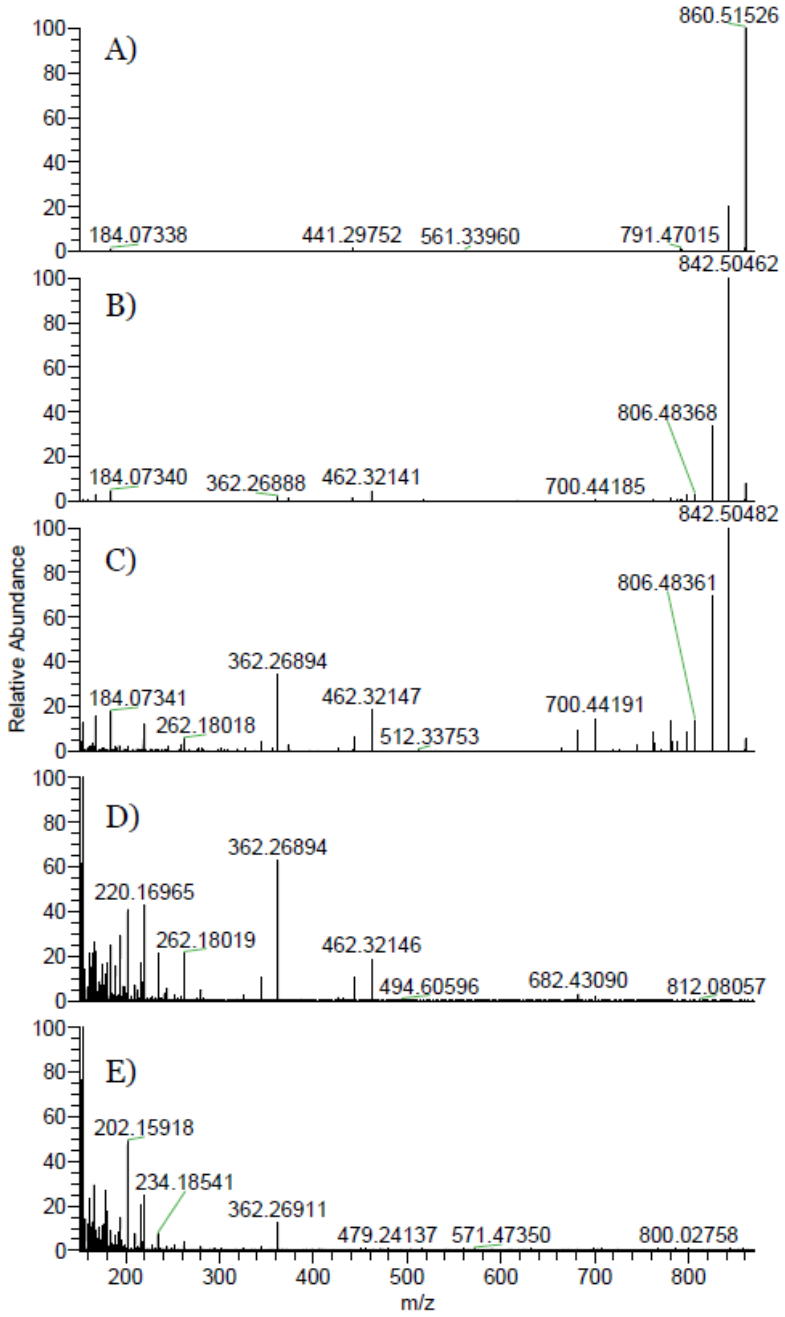

Figure S1: HRMS/MS spectra for AZA-59 at A) 15, B) 30, C) 40, D) 55 and E) 65 NCE, respectively.

Table S1: Observed and theoretical mass-to-charge (*m/z*) ratios, intensity, relative intensity, elemental composition, and deviation from the calculated mass in ppm for AZA-59 at 40 NCE.

Observed mass (*m/z*)	Intensity	Relative intensity	Elemental omposition	Theoretical mass (*m/z)*	Delta (ppm)
132.1021	3628.5	7.19	C6 H14 O2 N	132.10191	1.22
152.1071	2161.3	4.29	C9 H14 O N	152.10699	0.45
154.1227	6520.2	12.93	C9 H16 O N	154.12264	0.29
168.1384	8188.2	16.24	C10 H18 O N	168.13829	0.38
184.0734	9258.3	18.36	C9 H12 O4	184.07301	2.25
220.1696	6581	13.05	C14 H22 O N	220.16959	0.09
262.1802	2772.6	5.5	C16 H24 O2 N	262.18016	0.21
344.2584	2524.3	5.01	C22 H34 O2 N	344.25841	0.00
362.2689	18168.1	36.02	C22 H36 O3 N	362.26897	-0.08
444.3109	2970.8	5.89	C27 H42 O4 N	444.31084	0.21
462.3215	9940.7	19.71	C27 H44 O5 N	462.3214	0.15
682.4314	5301.3	10.51	C40 H60 O8 N	682.43134	0.09
700.442	7133.9	14.15	C40 H62 O9 N	700.44191	0.14
744.4833	1527.4	3.03	C46 H66 O7 N	744.48338	-0.15
762.4939	4223.6	8.37	C46 H68 O8 N	762.49394	-0.01
764.4729	1782	3.53	C45 H66 O9 N	764.47321	-0.38
780.5043	7658.4	15.18	C46 H70 O9 N	780.50451	-0.29
782.4835	2278.4	4.52	C45 H68 O10 N	782.48377	-0.4
788.4732	2079.7	4.12	C47 H66 O9 N	788.47321	-0.05
798.5148	4716.2	9.35	C46 H72 O10 N	798.51507	-0.36
806.4838	7525.6	14.92	C47 H68 O10 N	806.48377	-0.01
824.4944	35819.7	71.02	C47 H70 O11 N	824.49434	0.07
842.5049	50434.2	100	C47 H72 O12 N	842.5049	-0.06
860.5153	3115.2	6.18	C47 H74 O13 N	860.51547	-0.22

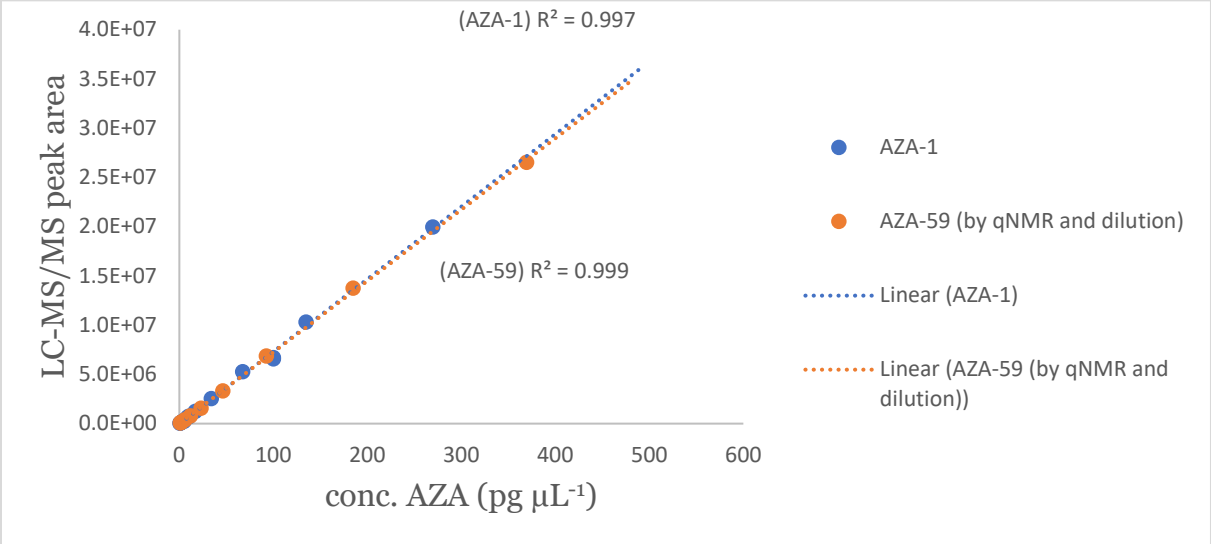

Figure S2: Calibration curves for standard dilution series of AZA-59 and AZA-1 (concentration determined by qNMR in pg µL^{-1} and dilution calculation). The area for the transition of the first waterloss (AZA-59: *m/z* 860>842, AZA-1 *m/z* 842>824) are shown.

Table S2: AZA-59 concentration when calculated from the calibration curve equations of AZA-59 and AZA-1 and % difference to the expected result

AZA-59 STD concentration (qNMR)	AZA-59 concentration calc. via AZA-1 calib. equation	% difference to expected (qNMR) value	AZA-59 concentration calc. via AZA-59 calib. equation	% difference to expected (qNMR) value
[fmol µL^{-1}]	[fmol µL^{-1}]	via AZA-1 calib.	[fmol µL^{-1}]	via AZA-59 calib.
0.84	2.43	289.67	0.53	63.61
1.68	3.15	188.22	1.26	74.92
3.35	4.68	139.68	2.78	82.84
6.68	7.95	119.04	6.03	90.32
13.41	14.39	107.27	12.44	92.75
26.83	26.81	99.94	24.81	92.48
53.65	55.36	103.19	53.24	99.23
107.30	112.53	104.87	110.16	102.66
214.60	223.43	104.11	220.57	102.78
429.21	429.49	100.06	425.73	99.19

Table S3: Collated NMR data for AZA-59 and structurally related AZA-1, -2, -36, -37, 37-*epi* AZA-1 and 37-*epi* AZA-2

	AZA-1[1]			**AZA-2**[1]			**AZA-36**[2]			**AZA-37**[2]			**37-*epi* AZA-1**[3]			**37-*epi*-AZA-2**[3]			**AZA-59**		
No.	δC	δH	δH'	δC	δH		δC	δH	δH'	δC	δH	δH'				δC	δH	δH'	δC	δH	δH'
1a	180.3			177.8			180.3	-	-	180.3	-	-	181.3						178.5		
2	37.4	2.31	2.31	35.6	2.34	2.34	46	2.34	2.39	46.1	2.33	-	38.4	2.25					44.6	2.39	
3	30.3	2.33	2.33	29.5	2.31	2.31	71.4	4.43	-	71.4	4.39	-	30.3	2.33					70.4	4.45	
4	133.8	5.74		132.8	5.68		135.5	5.75	-	134.6	5.7	-	133.7	5.75					133.6	5.72	
5	131.8	5.46		132.1	5.42		132.1	5.64	-	133.1	5.65	-	130.7	5.44					132.4	5.68	
6	73.2	4.81		73.3	4.72		72.8	4.79	-	73.3	4.35	-	72.5	4.8					72.4	4.38	
7*	**130.1**	**5.65**		123.6	5.32		123.4	5.36	-	**38.4**	**1.43**	**1.87**	129.4	5.64					**32.0**	**1.68**	**1.34**
8*	**124.1**	**5.76**		132.8			132.1	-	-	**22.2**	**1.7**	**1.77**	123.3	5.73					**21.4**	**1.93**	**1.76**
8-Me				23.8	1.67		23	1.7	-	-	-	-	35.8	2.48	2.12						
9*	36.5	2.49	2.15	41.1	2.42	1.97	41.1	2	2.44	36.6	1.7	1.83							35.8	1.7	
10	107.9			108.3			108.3	-	-	109.1	-	-	107.3						108.3		
11	33.9	2.33	1.68	34	2.33	1.65	34	1.71	2.33	33.9	1.69	2.33	37.6	2.14	1.95				37.6	2.08	1.9
12	38.3	2.16	1.97	38.3	2.16	1.96	38.3	1.99	2.18	32.8	1.83	2.03	33.3	2.33	1.65				33.1	2.31	1.64
13	112.1			112.1			112.2	-	-	111.8	-	-	111.4						111.0		
14	31.7	2.02		31.7	2		32.1	2.02	-	31.8	2.01	-	31.1	2.03			2.01		31.0	2.01	
14-Me	17.4	0.94		17.4	0.93		17.5	0.94	-	17.5	0.9	-	16.7	0.95		n.r.	0.94		16.6	0.91	
15	33.4	1.85	1.77	33.4	1.83	1.73	33.3	1.76	1.86	33.5	1.76	1.87	32.7	1.83	1.76		1.82	1.75	32.8	1.88	1.82
16	79.1	3.89		79	3.87		78.9	3.91	-	78.9	3.94	-	78.1	3.91		78.1	3.91		78.2	3.95	
17	74.2	4.25		74.2	4.2		74.2	4.23	-	74.3	4.29	-	73	4.2		73	4.17		73.4	4.29	
18	37.8	2.01	2	37.7	1.98	1.98	37.5	1.99	2.06	37.6	2	2.07	39	2.07	2		2.06	2	37.0	2.11	2.03
19	79.9	4.44		79.9	4.42		79.9	4.43	-	79.9	4.44	-	79.2	4.43			4.43		79.1	4.45	
20	77.6	3.94		77.6	3.93		77.5	3.9	-	77.4	3.93	-	77	3.5		77	3.5		76.7	3.96	
21	101.1			101			101.1	-	-	101	-	-	99.9			99.8			100.1		
22	37.6	2.09		37.6	2.07		37.8	2.06	-	37.6	2.07	-	35.9	2.3		35.9	2.3		36.7	2.05	
22-Me	17.2	0.91		17.2	0.89		17.2	0.92	-	17.2	0.92	-	16.6	0.9		16.5	0.9		16.4	0.94	
23	38.9	1.44	1.44	39	1.43	1.43	39.1	1.44	1.44	39.1	1.43	1.43	38.6	1.42	1.42		1.41	1.41	38.2	1.45	
24	43.1	1.35		43.1	1.33		43	1.35	-	43	1.35	-	42.2	1.36			1.35		42.4	1.36	

	AZA-1[1]			AZA-2[1]			AZA-36[2]			AZA-37[2]			37-*epi* AZA-1[3]			37-*epi*-AZA-2[3]			AZA 59		
No.	δC	δH	δH'	δC	δH		δC	δH	δH'	δC	δH	δH'				δC	δH	δH'	δC	δH	δH'
24-Me	18.8	0.84		18.9	0.83		18.9	0.84	-	18.9	0.84	-	18.1	0.82		18.1	0.82		18.1	0.86	
25	80.4	4		80.4	3.97		80.4	4	-	80.3	4	-	79.9	3.87		79.8	3.88		79.6	4	
26	149.1			149.1			149	-	-	149	-	-	148.1			148.1			148.4		
`=CH2	117.2	5.36	5.18	118.1	5.35	5.17	117.8	5.16	5.33	117.8	5.15	5.33	115.5	5.28	5.13	115.7	5.28	5.13	117.0	5.37	5.19
27	50.4	2.43	2.26	50.1	2.42	2.24	50.4	2.26	2.43	50.4	2.25	2.42	49	2.37	2.14	49.2	2.37	2.14	49.4	2.44	2.27
28	99.5			99.5			99.4	-	-	99.4	-	-	98.3						98.7		
29	44.9	2.05	1.37	44.9	2.03	1.36	45	1.37	2.05	45	1.36	2.05	44.6	2	1.32	44.1	2	1.32	44.1	2.07	1.4
30	27.2	2.23		27.2	2.22		27.2	2.23	-	27.2	2.23	-	26.7	2.26			2.26		26.3	2.25	
30-Me	24.3	0.96		24.1	0.93		24.3	0.96	-	24.3	0.96	-	23.9	0.94		23.9	0.94		23.5	0.97	
31	36.1	1.84	1.54	36.1	1.82	1.51	36.2	1.52	1.83	36.1	1.52	1.84	35.5	1.78	1.5		1.78	1.5	35.2	1.86	1.54
32	73.6	4.38		73.6	4.35		73.7	4.37	-	73.7	4.37	-	72.2	4.24		72.7	4.24		72.8	4.38	
33	82.3	4.08		82.4	4.06		82.1	4.05	-	82.1	4.05	-	78.8	3.81		79	3.81		81.6	4.09	
34	75.6	5.02		75.6	5		75.7	4.99	-	75.7	5	-	75.4	4.83		75.4	4.82		74.8	5.03	
35	42.5	2.64	2.5	42.4	2.62	2.49	42.8	2.49	2.61	42.7	2.49	2.6	45.1	2.11			2.13	n.r.	41.7	2.66	2.51
36	97.4			97.5			98	-	-	98	-	-	96.8			96.6			96.7		
37	36.4	1.99		36.5	1.97		37.5	1.99	-	36.7	1.98	-	**39.1**	**1.78**		37.3	n.r.		35.7	2.03	
37-Me	16.2	0.98		16.2	0.97		16.4	0.98	-	16.4	0.97	-	15.8	1.06		15.9	1.06		15.5	1.01	
38	38.4	1.7	1.31	38.4	1.68	1.29	29.8	1.61	1.68	29.7	1.63	1.67	36.8	1.73	1.45	38.9	1.73	1.45	37. 6	1.73	1.32
39	30.2	1.89		30.2	1.86		23.8	1.7	1.81	23.8	1.7	-	25	1.84			1.84		29.4	1.93	
39-Me	**19.3**	**0.95**		19.3	0.94								19.3	0.88		19.3	0.88		**18.5**	**0.98**	
40	46.9	2.91	2.84	46.9	2.91	2.83	41.3	2.98	3.17	41.2	2.99	3.17	47.8	2.67	2.62	47.9	2.67	2.62	46.1	2.92	2.85

1 Ofuji, K. *et al.* Two analogs of azaspiracid isolated from mussels, *Mytilus edulis*, involved in human intoxication in Ireland. *Natural Toxins* **7**, 99-102 (1999).

2 Krock, B. *et al.* Structure Elucidation and in Vitro Toxicity of New Azaspiracids Isolated from the Marine Dinoflagellate *Azadinium poporum*. *Mar Drugs* **13**, 6687-6702 (2015).

3 Kilcoyne, J. *et al.* Epimers of Azaspiracids: Isolation, Structural Elucidation, Relative LC-MS Response, and in Vitro Toxicity of 37-*epi*-Azaspiracid-1. *Chemical Research in Toxicology* **27**, 587-600 (2014).

4 Nicolaou, K. C. *et al.* Structural revision and total synthesis of azaspiracid-1, part 2: Definition of the ABCD domain and total synthesis. *Angewandte Chemie-International Edition* **43**, 4318-4324 (2004).

5 Nicolaou, K. C. *et al.* Structural revision and total synthesis of azaspiracid-1, part 1: Intelligence gathering and tentative proposal. *Angewandte Chemie-International Edition* **43**, 4312-4318 (2004).

* Shift data from Ofiji et al. (1999) shown with numbering according to the revised position of the double bond ([4,5]).

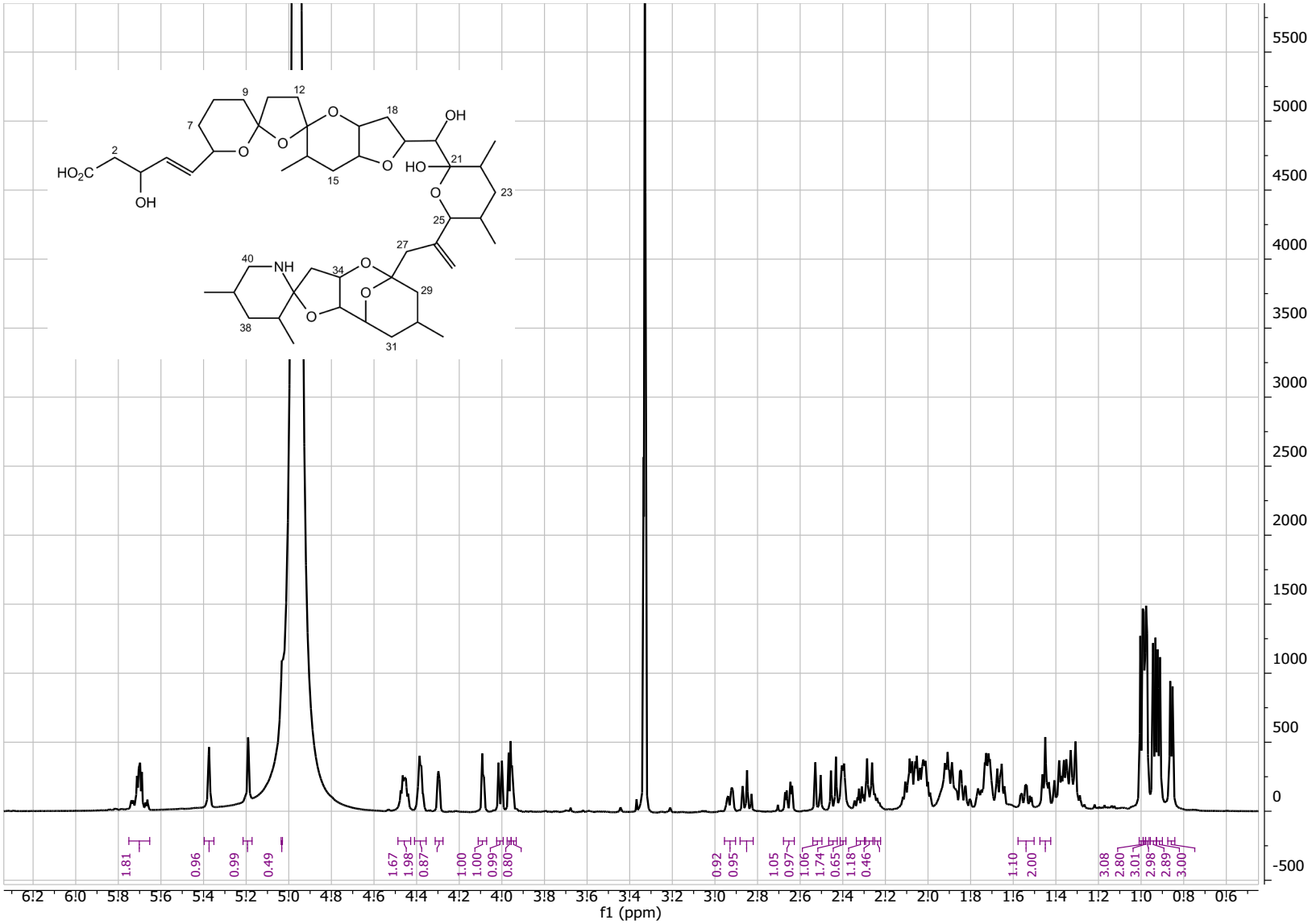

Figure S3: 1D proton spectra of AZA-59 (600 MHz, d4-methanol). Insert: Structure and numbering.

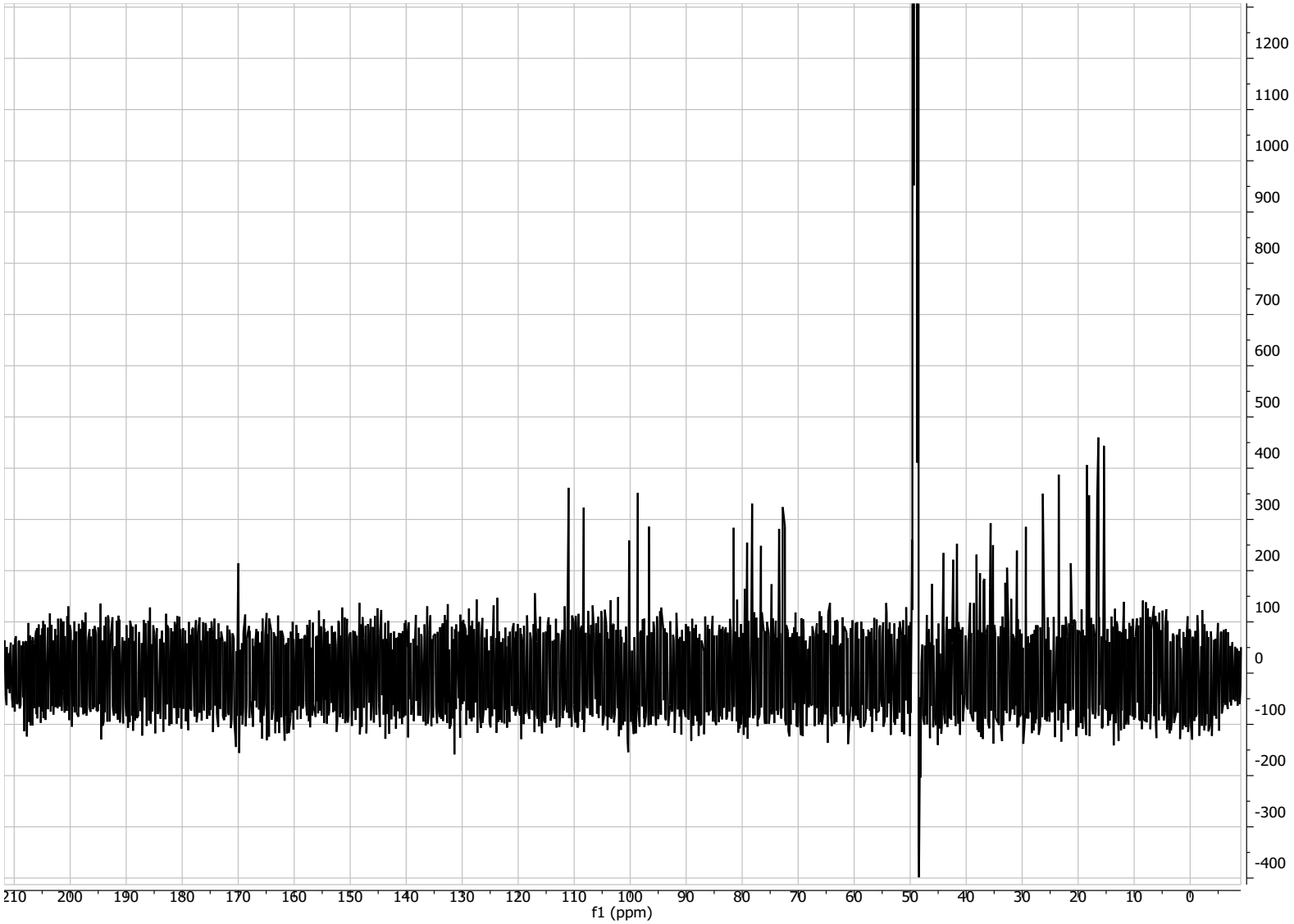

Figure S4: 1D carbon spectra of AZA-59 (150 MHz, d4-methanol).

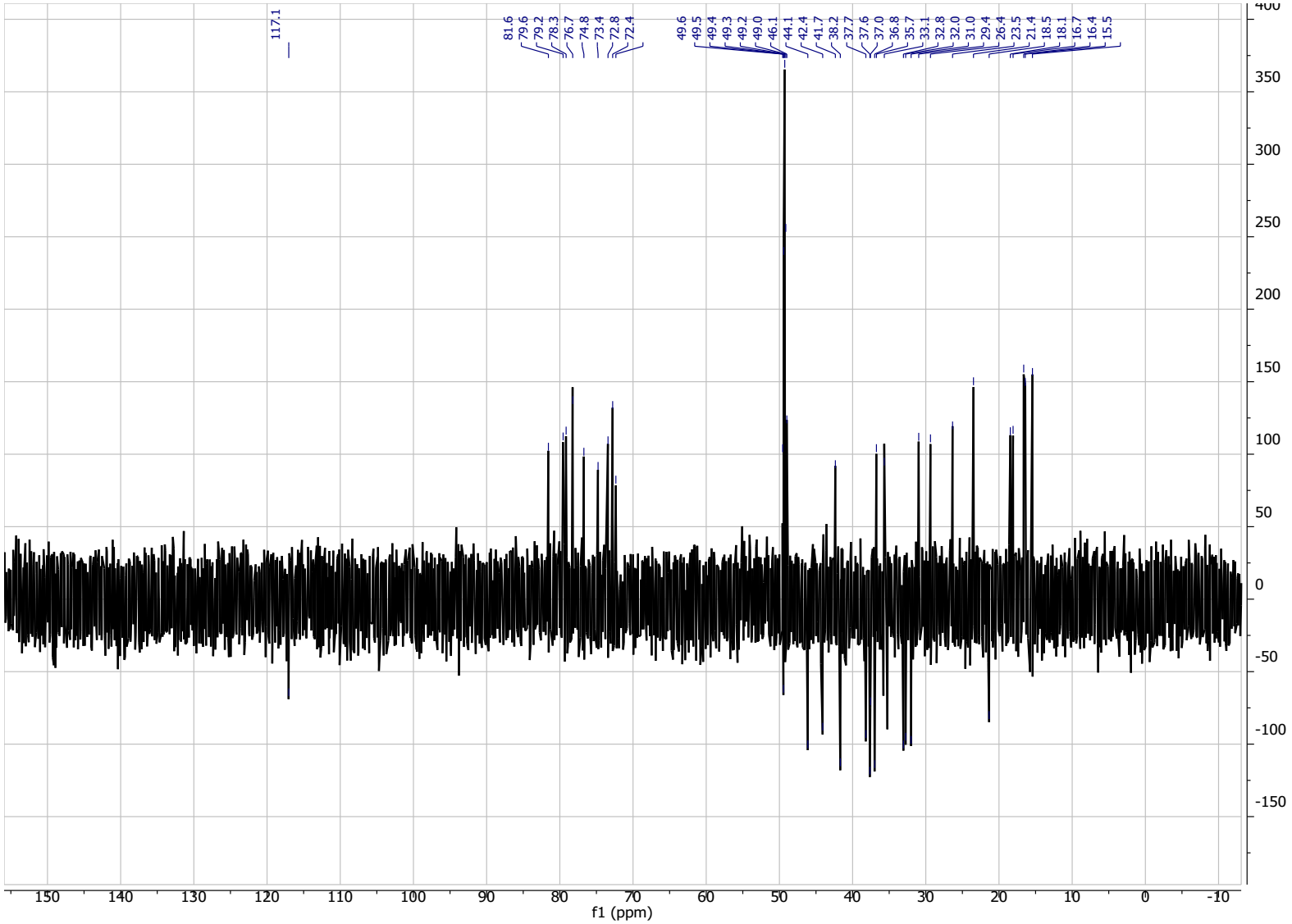

Figure S5: 1D DEPT spectra of AZA-59 (150 MHz, d4-methanol).

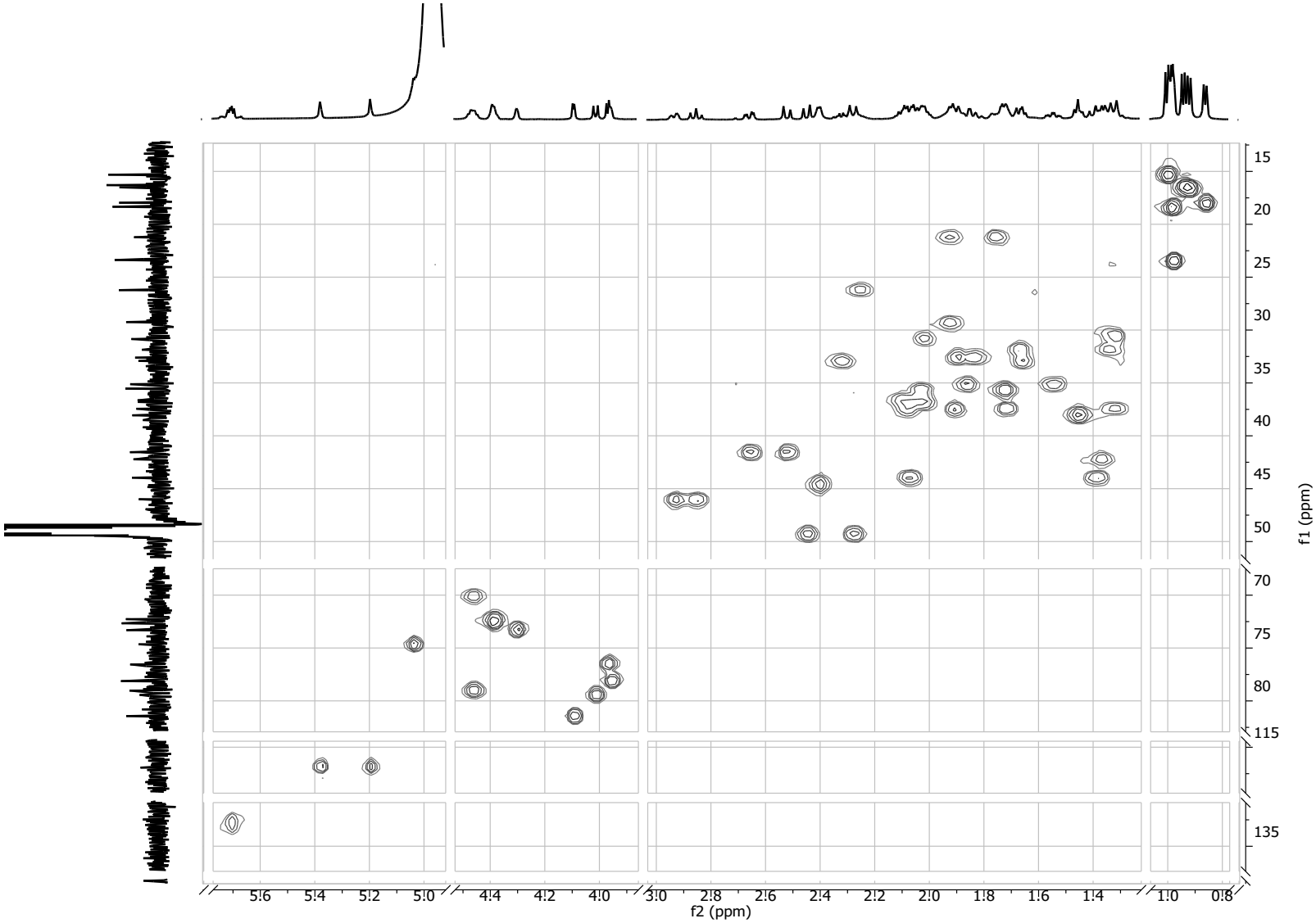

Figure S6: 2D HSQC spectra of AZA-59 (600 MHz Proton frequency, for picked peaks see Figure S 5 and Figure S 6).

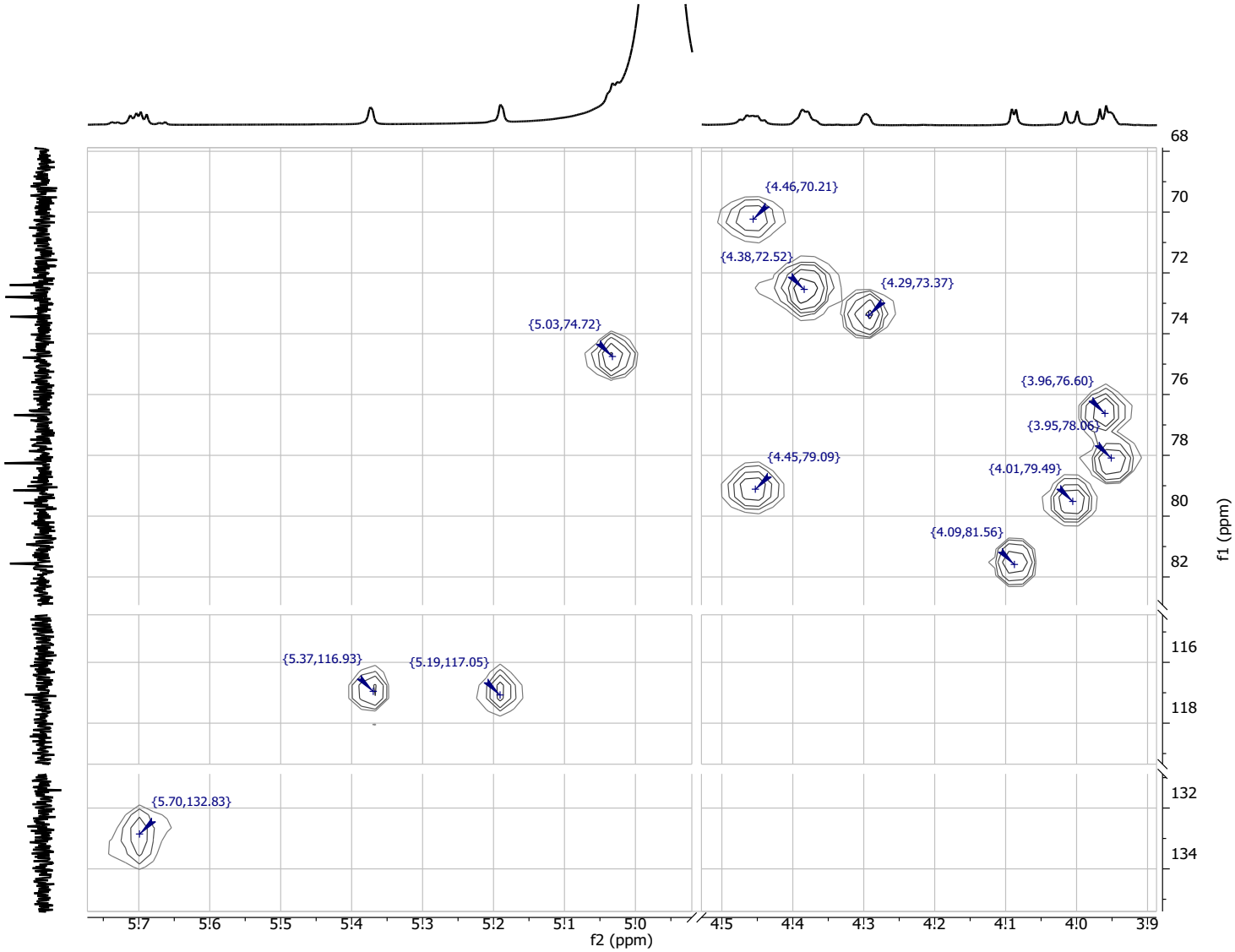

Figure S7: Slice 1 of 2D HSQC spectra and picked peaks of AZA-59 (600 MHz Proton frequency, d4-methanol).

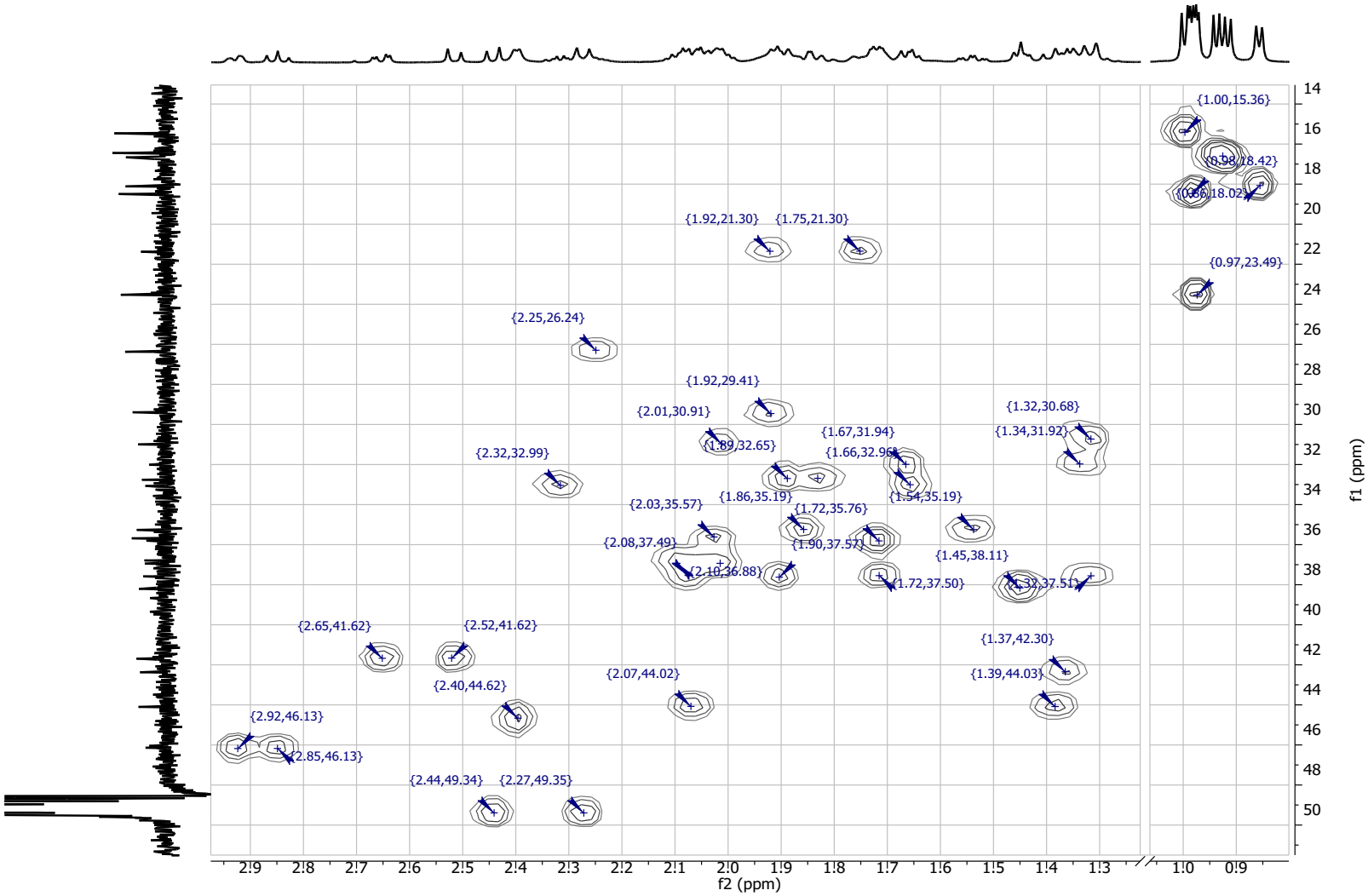

Figure S8: Slice 1 of 2D HSQC spectra and picked peaks of AZA-59 (600 MHz Proton frequency, d4-methanol).

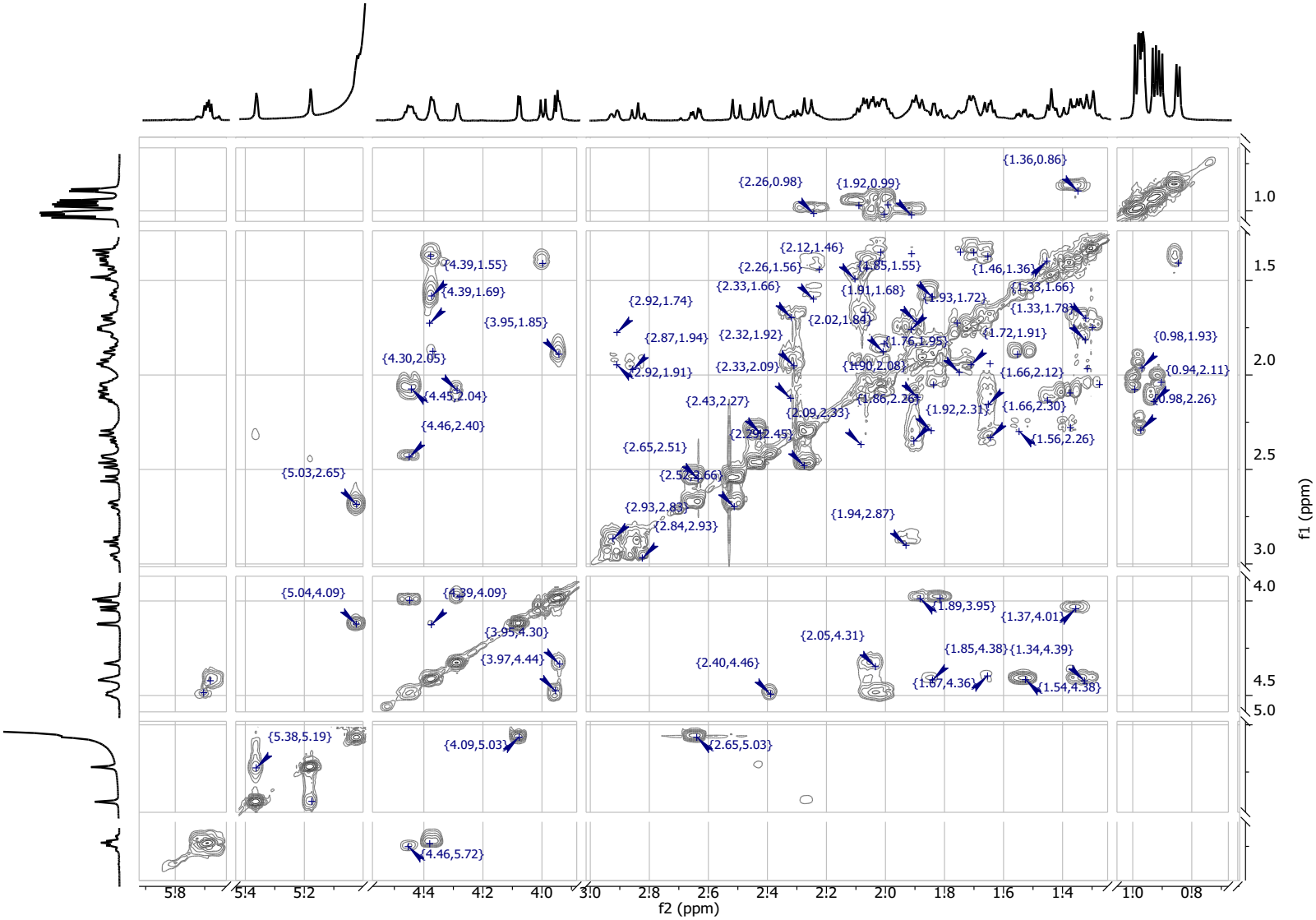

Figure S9: 2D COSY spectra of AZA-59 (600 MHz Proton frequency, d4-methanol).

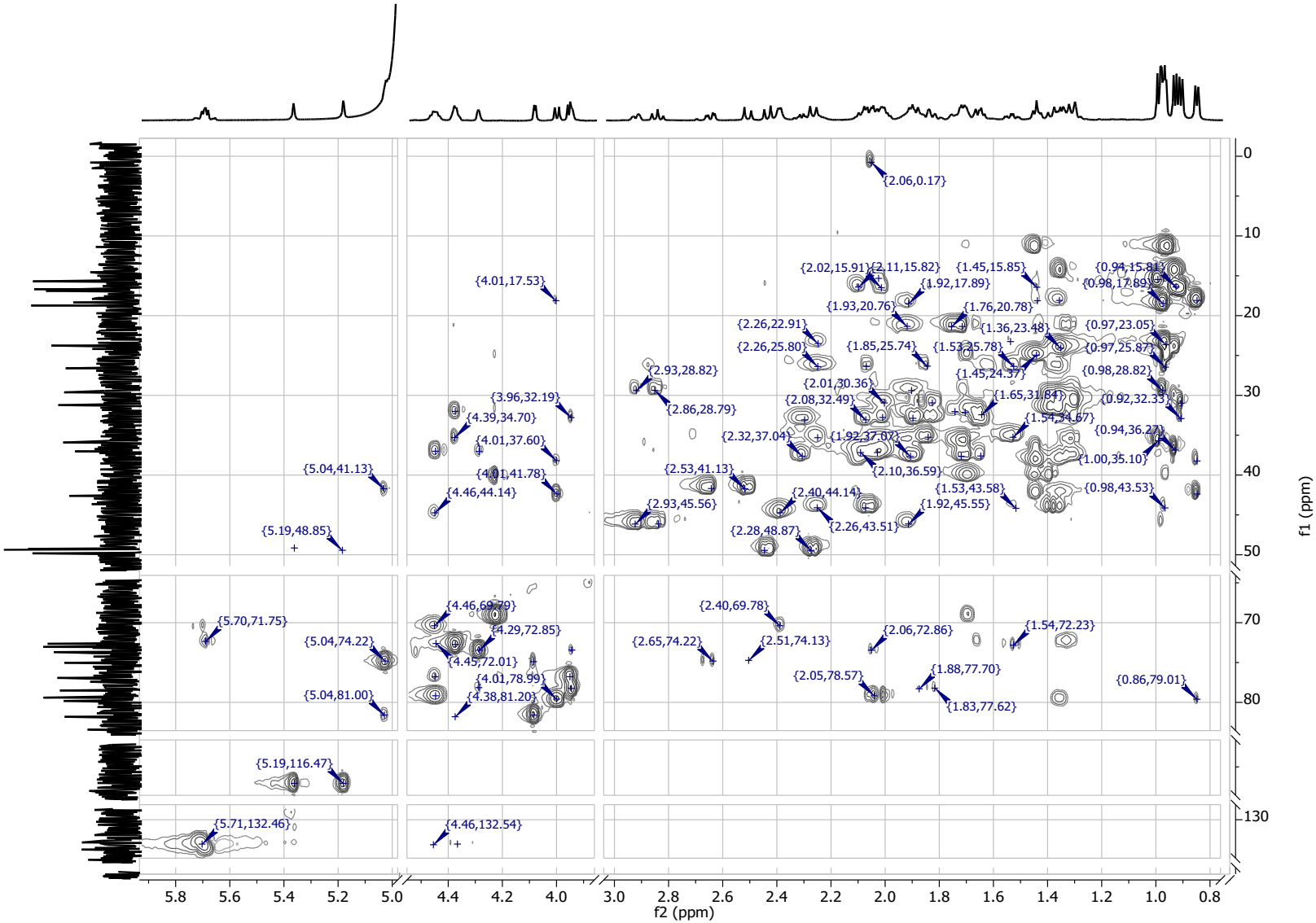

Figure S10: 2D HSQC-TOCSY spectra of AZA-59 (600 MHz Proton frequency, d4-methanol, mixing time 10 ms).

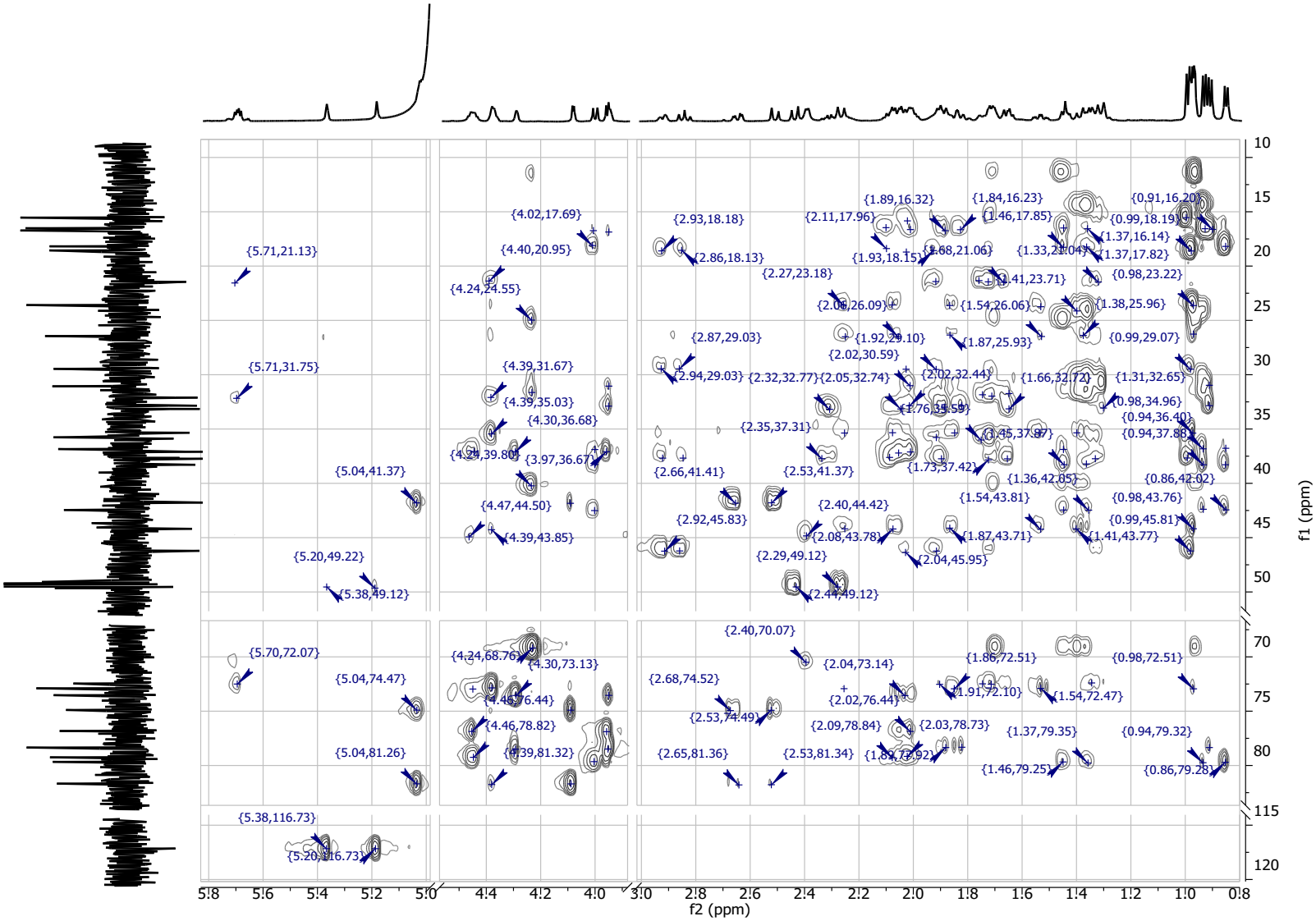

Figure S11: 2D HSQC-TOCSY spectra of AZA-59 (600 MHz Proton frequency, d4-methanol, mixing time 60 ms).

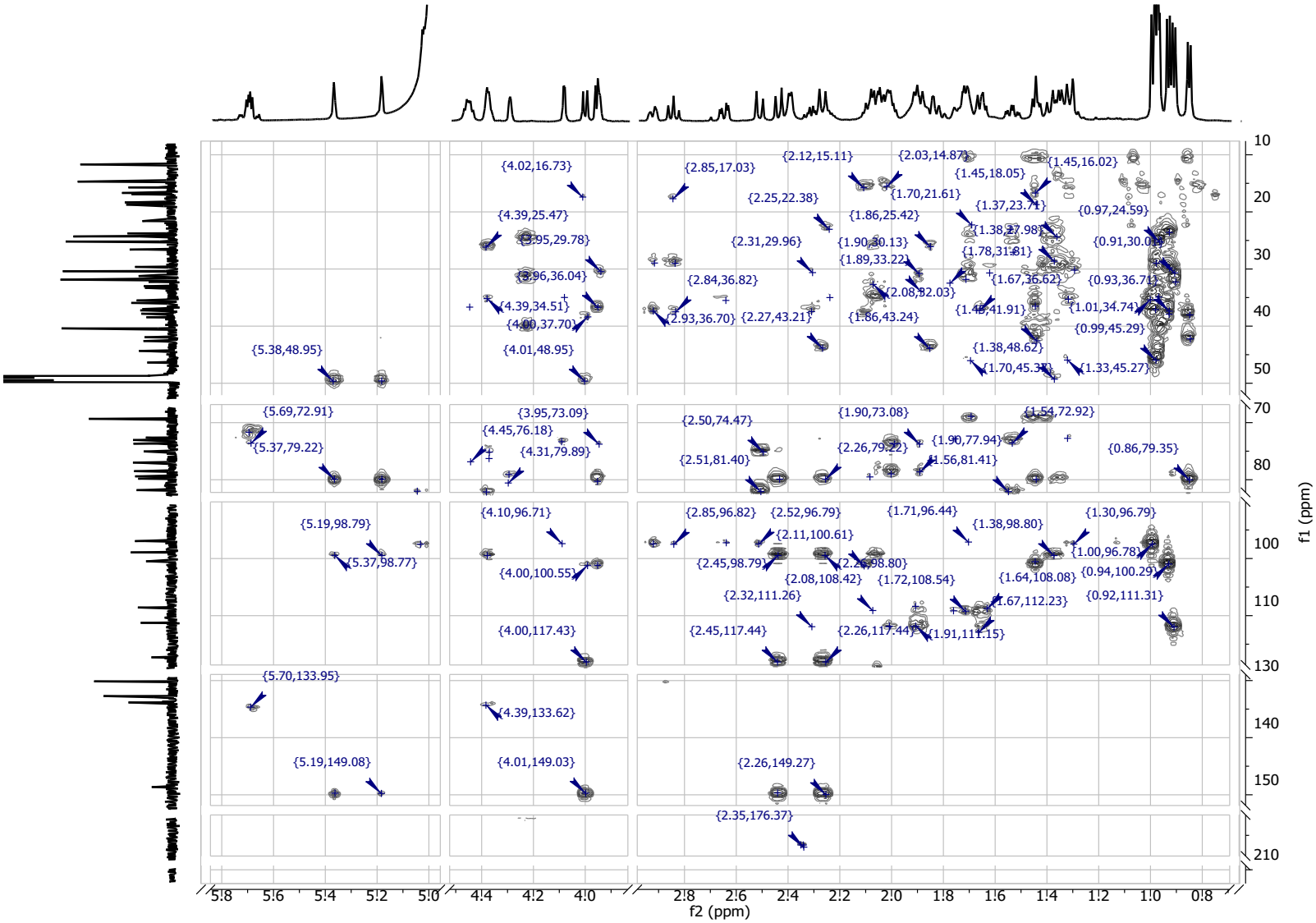

Figure S12: 2D HMBC spectra of AZA-59 (600 MHz Proton frequency, d4-methanol).

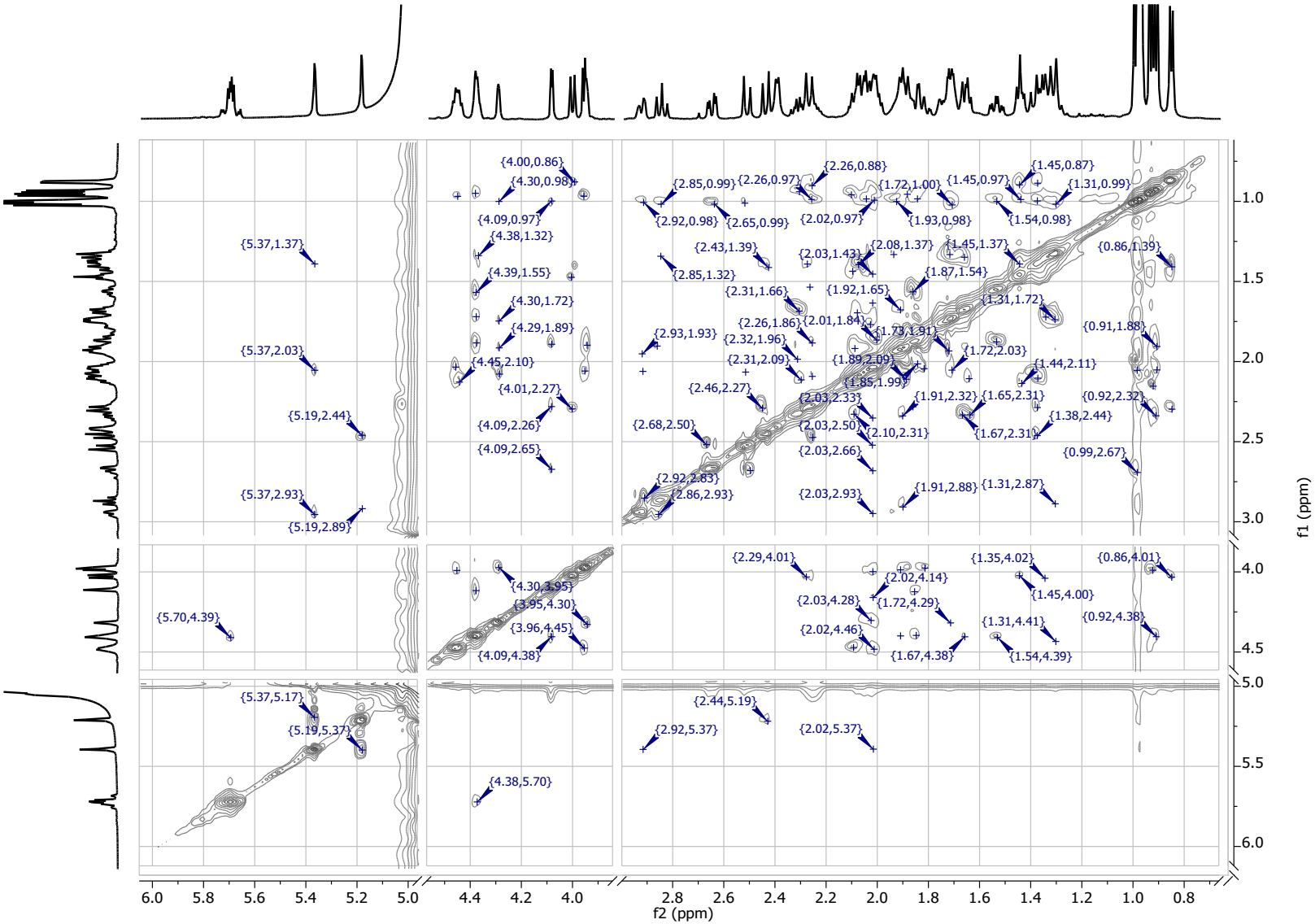

Figure S13: 2D ROESY spectra of AZA-59 (600 MHz Proton frequency, d4-methanol).