Isolation of Aerucyclamides C and D and Structure Revision of Microcyclamide 7806A: Heterocyclic Ribosomal Peptides from *Microcystis aeruginosa* PCC 7806 and Their Antiparasite Evaluation

Supporting Information

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[1] Ziemert, N.; Ishida, K.; Quillardet, P.; Bouchier, C.; Hertweck, C.; Tandeau de Marsac, N.; Dittmann, E. *Appl. Environ. Microbiol.* **2008**, *74*, 1791

Figure 01: ^{1}H NMR spectrum of aerucyclamide C (**6**) in DMSO-d_6 (600MHz)

Aerucyclamide C (**6**)

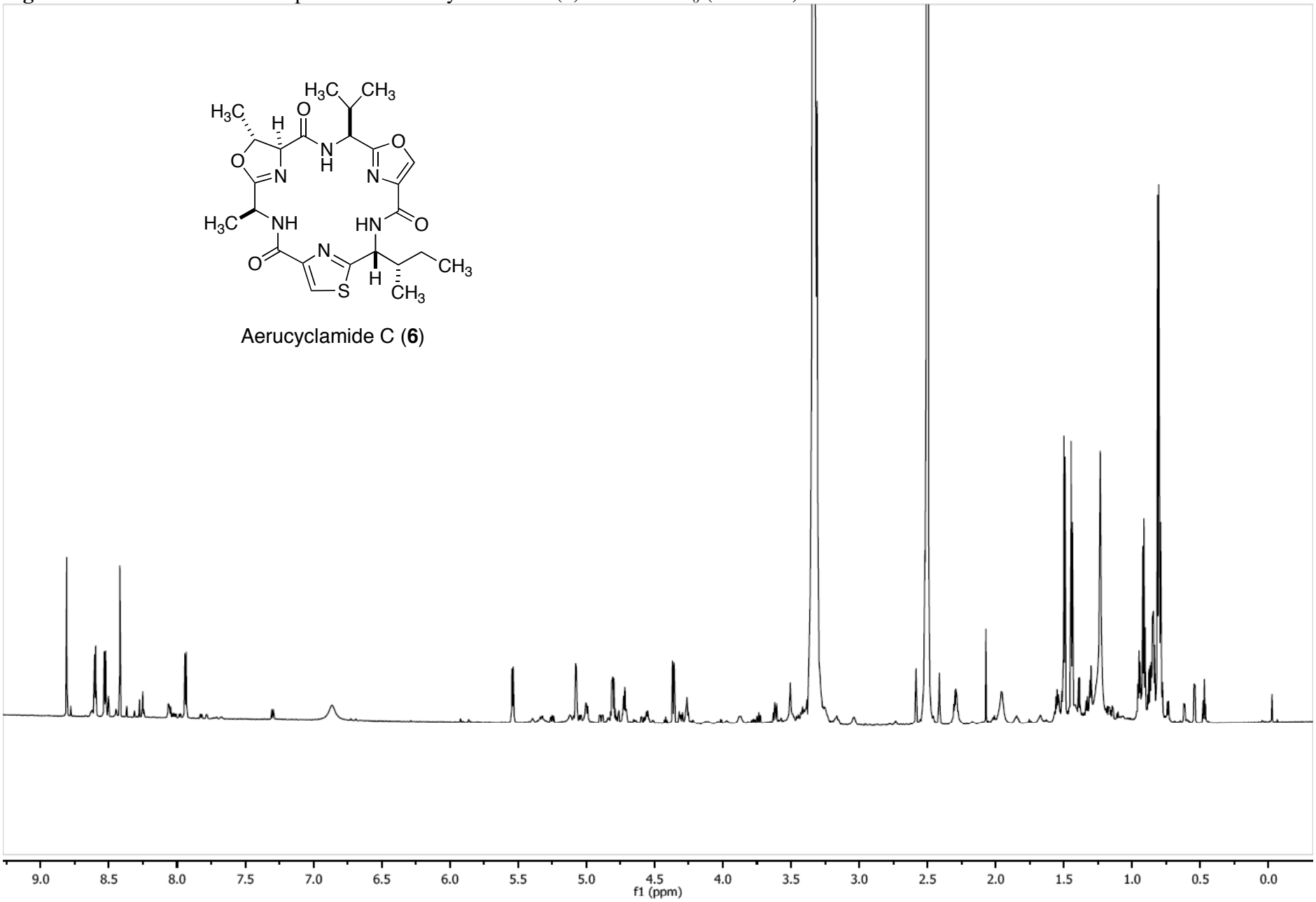


Figure 02: ^{13}C NMR spectrum of aerucyclamide C (**6**) in DMSO-d_6 (125MHz)

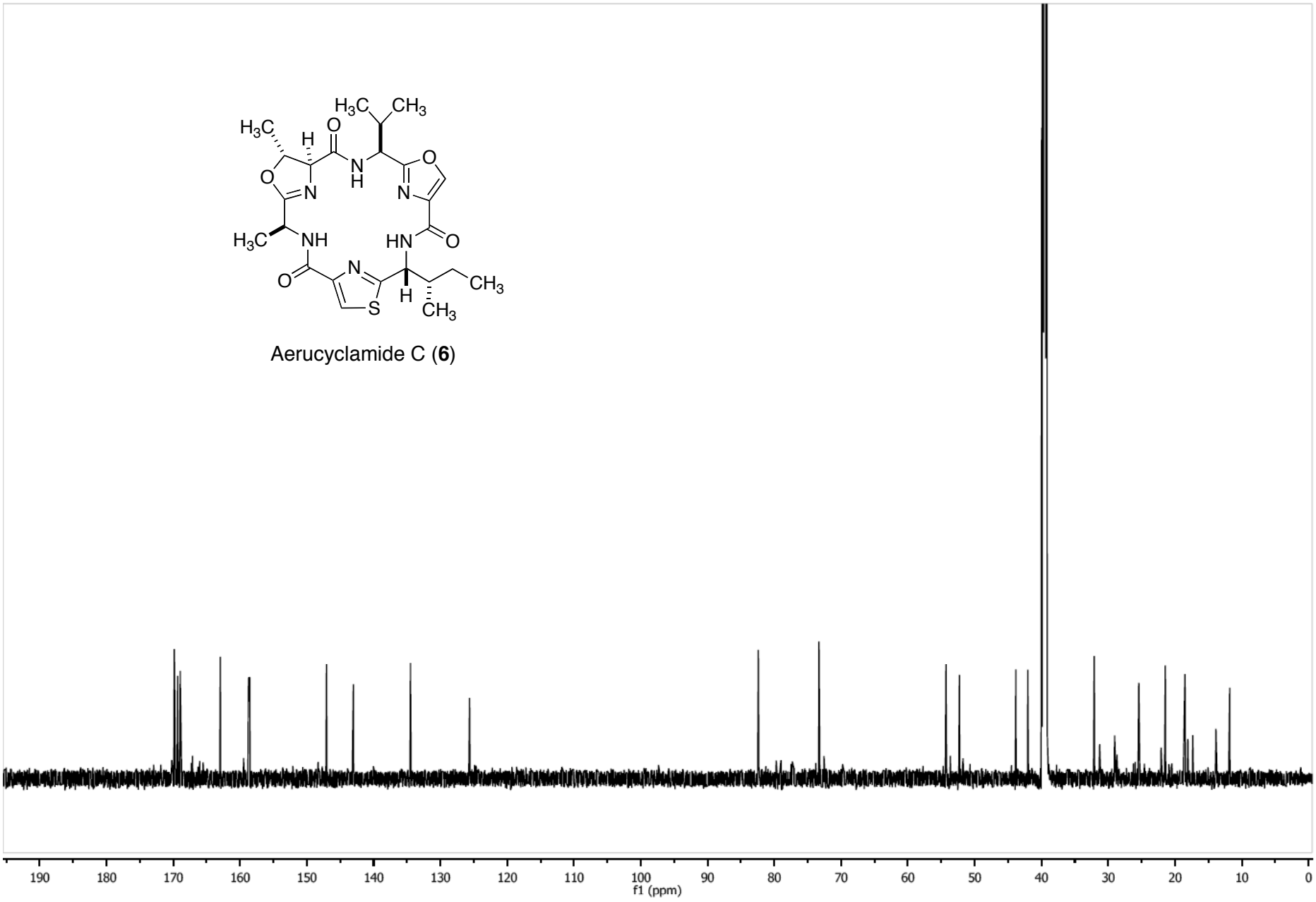


Figure 03: COSY NMR spectrum of aerucyclamide C (**6**) in DMSO-d_6 (600MHz)

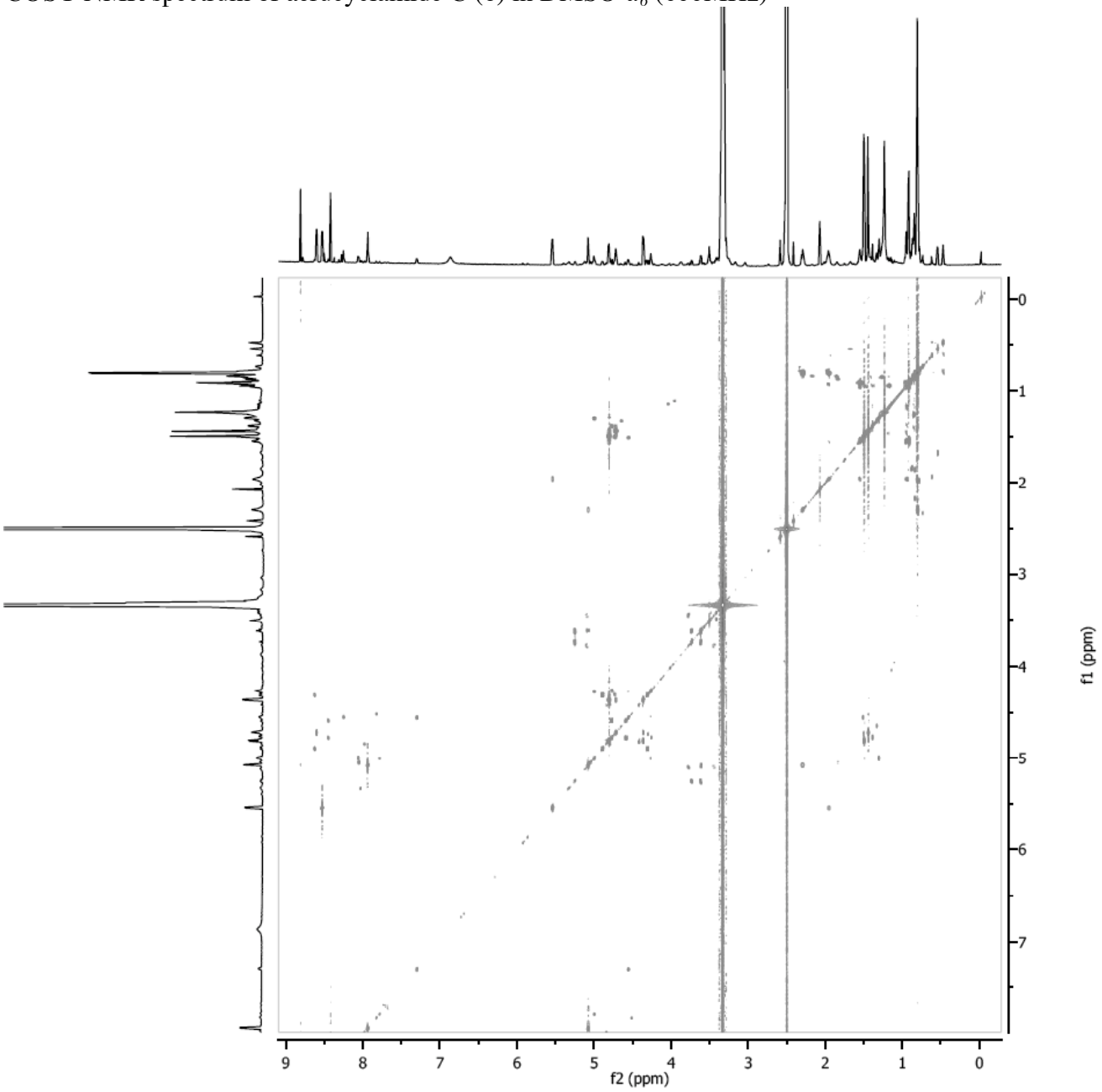


Figure 04: gHSQC NMR spectrum of aerucyclamide C (**6**) in DMSO-d_6 (600MHz)

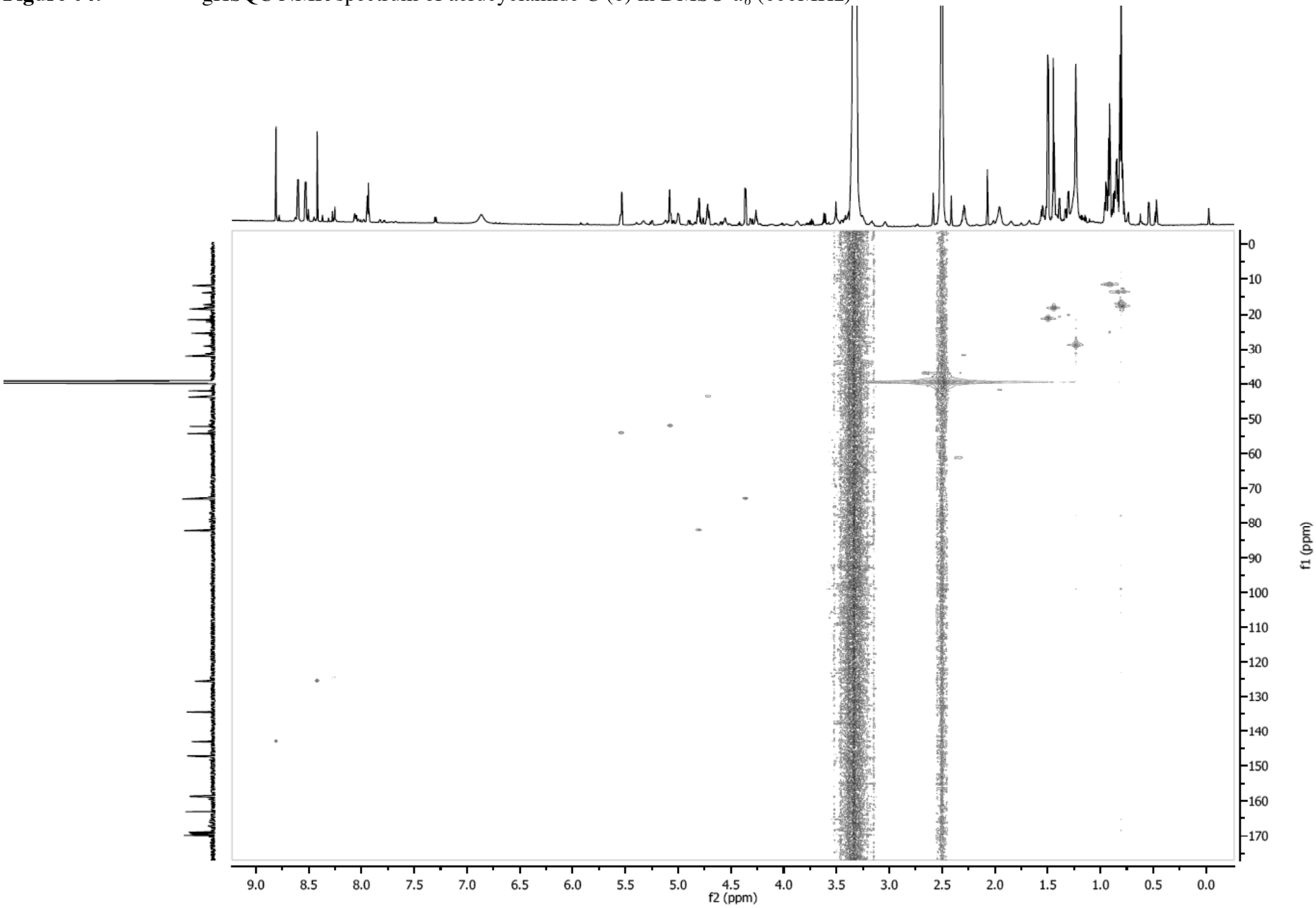


Figure 05: gHMBC NMR spectrum of aerucyclamide C (**6**) in DMSO-d_6 (600MHz)

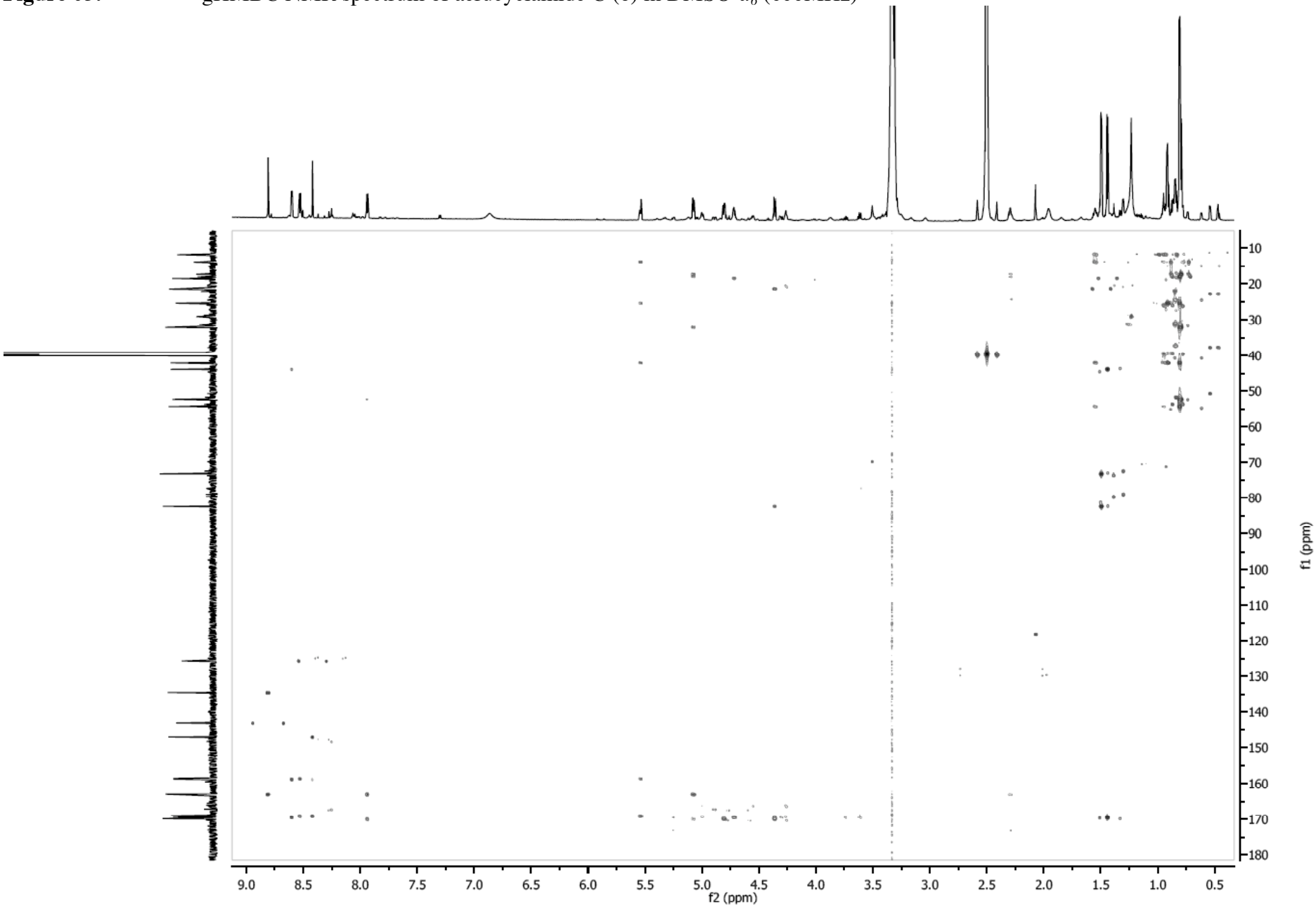


Figure 06: MS/MS of aerucyclamide C (**6**) and ^{15}N and ^{34}S labeled **6**

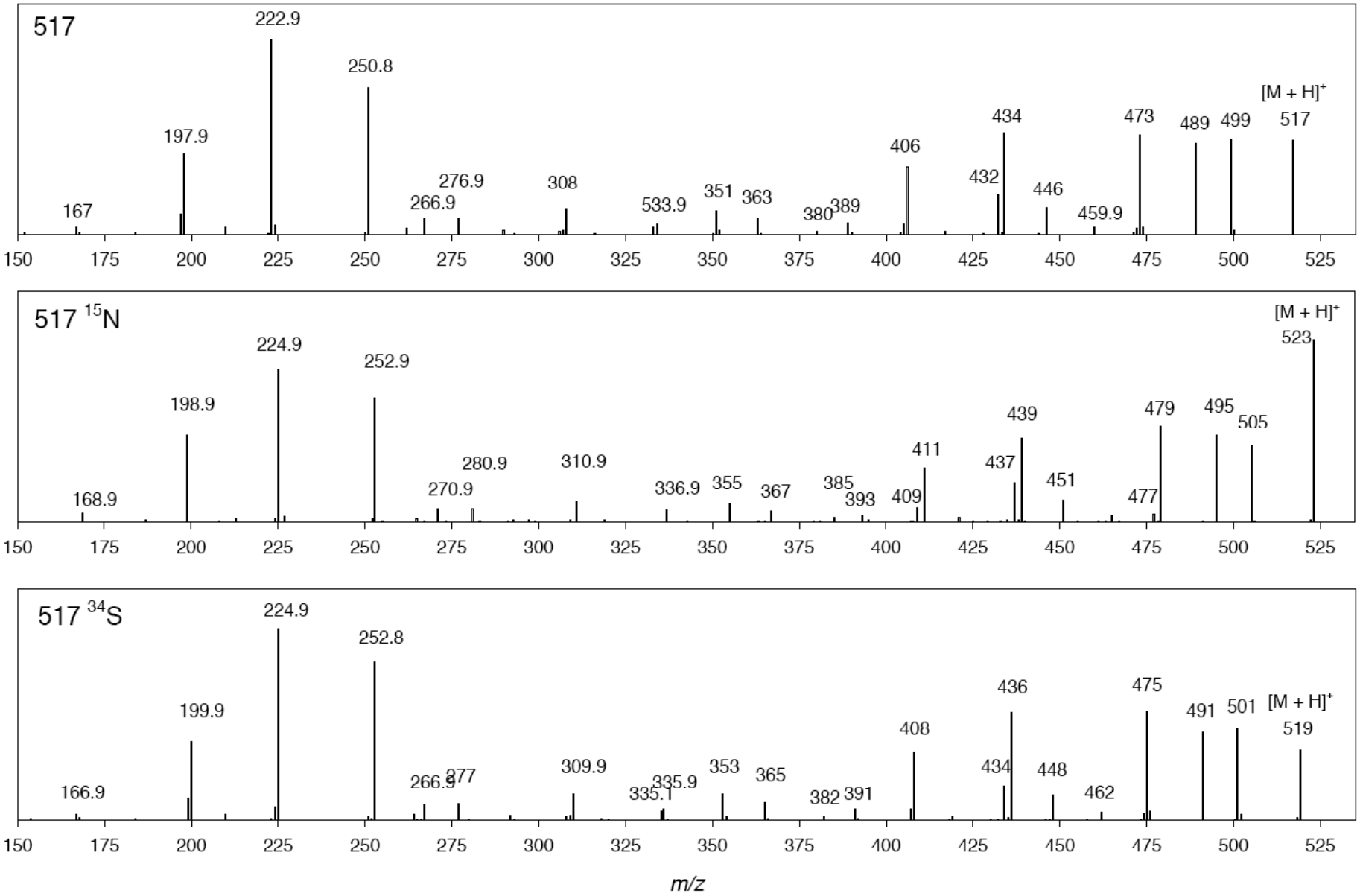


Figure 07: ^{1}H NMR spectrum of aerucyclamide D (**7**) in DMSO-d_6 (600MHz)

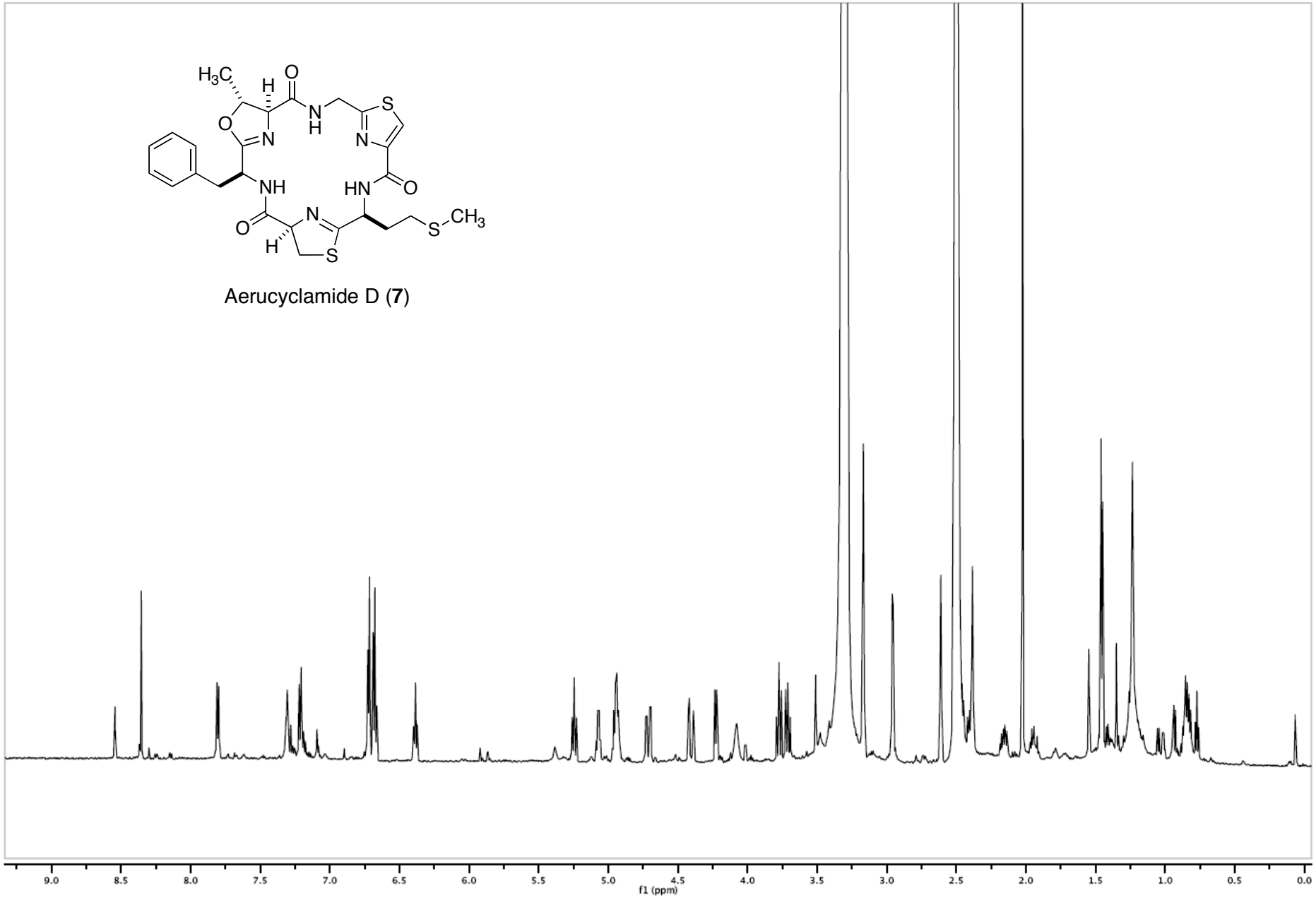


Figure 08: COSY NMR spectrum of aerucyclamide D (**7**) in DMSO-d_6 (600MHz)

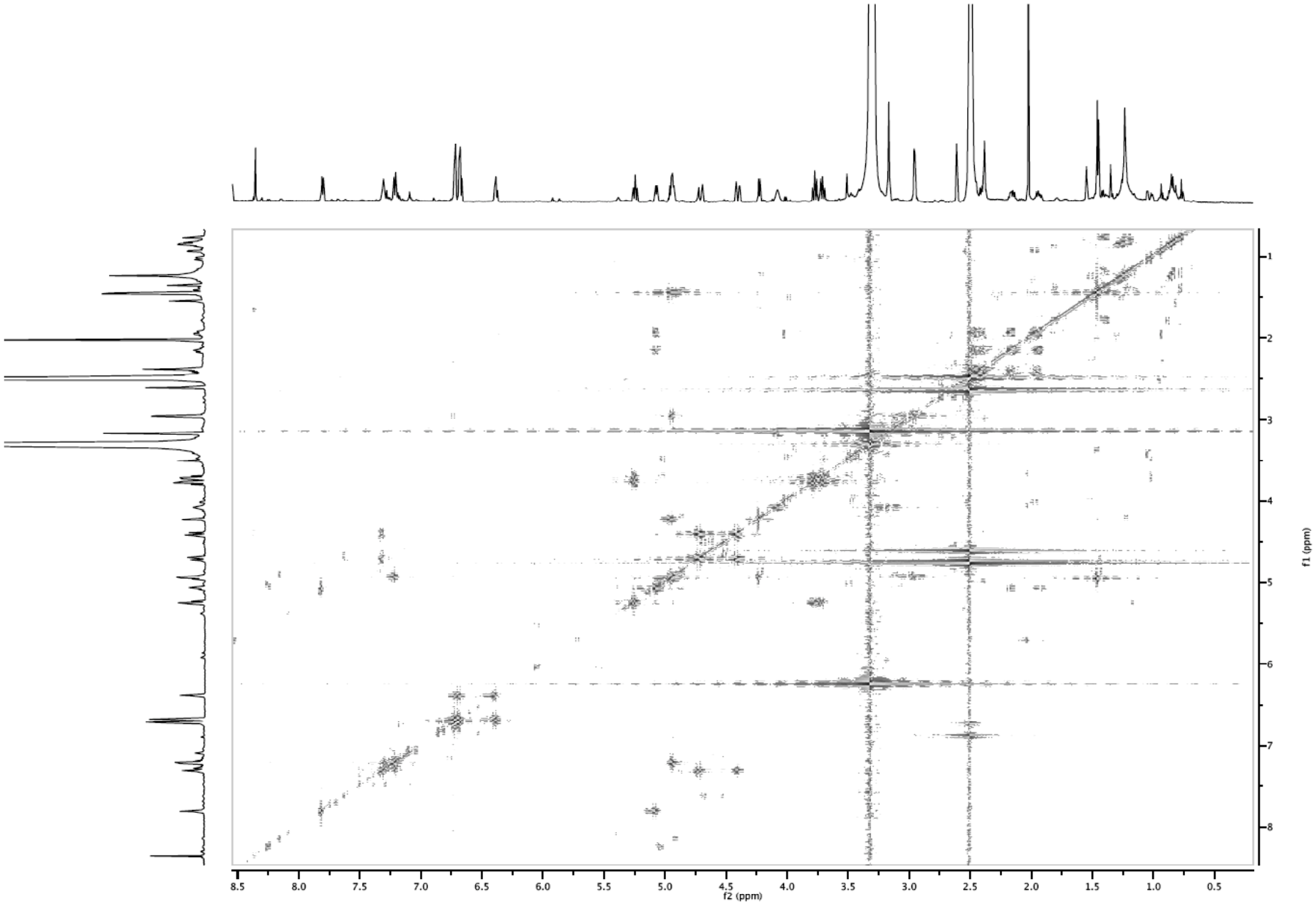


Figure 09: gHSQC NMR spectrum of aerucyclamide D (**7**) in DMSO-d_6 (600MHz)

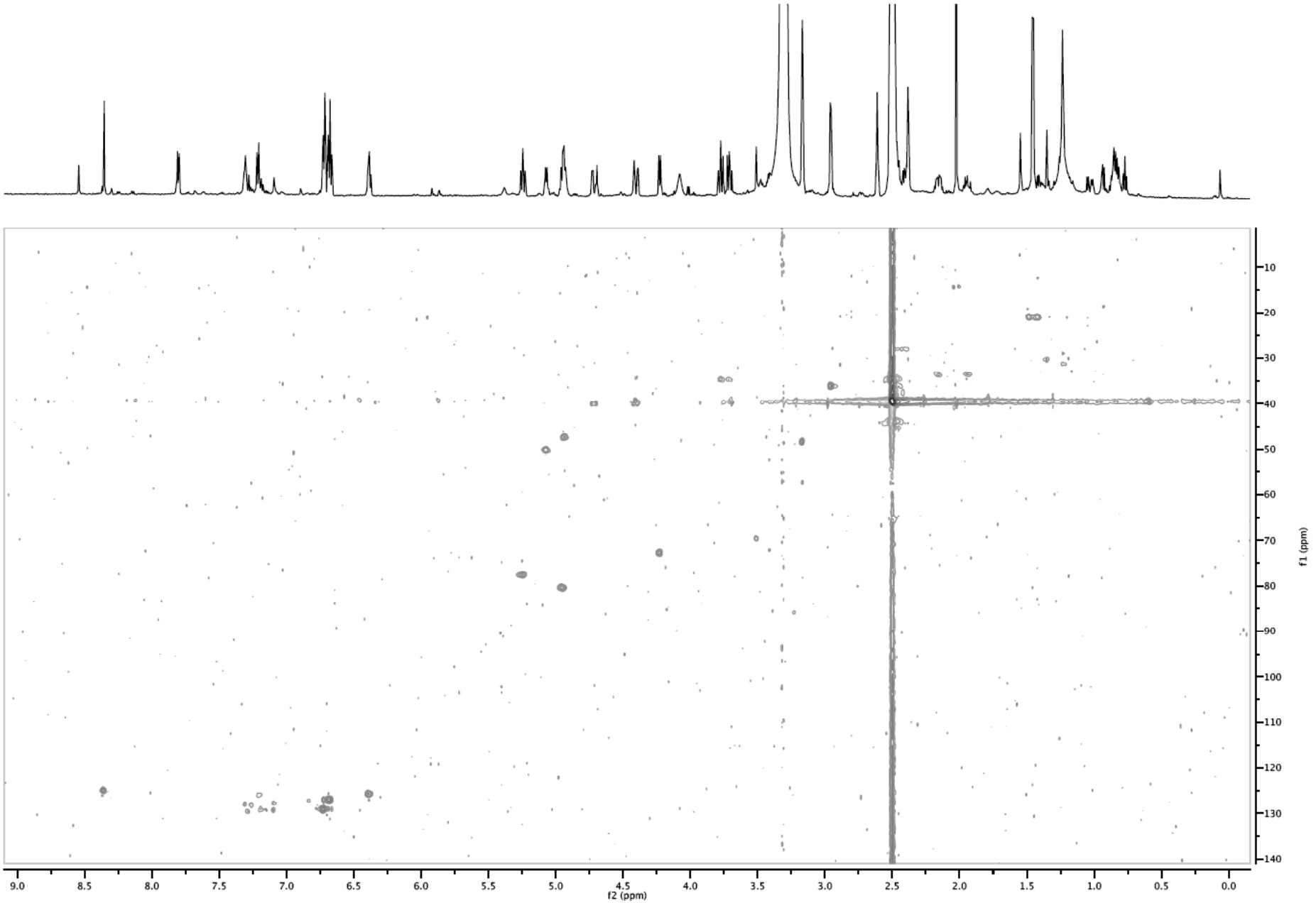


Figure 10: gHMBC NMR spectrum of aerucyclamide D (**7**) in DMSO-d_6 (600MHz)

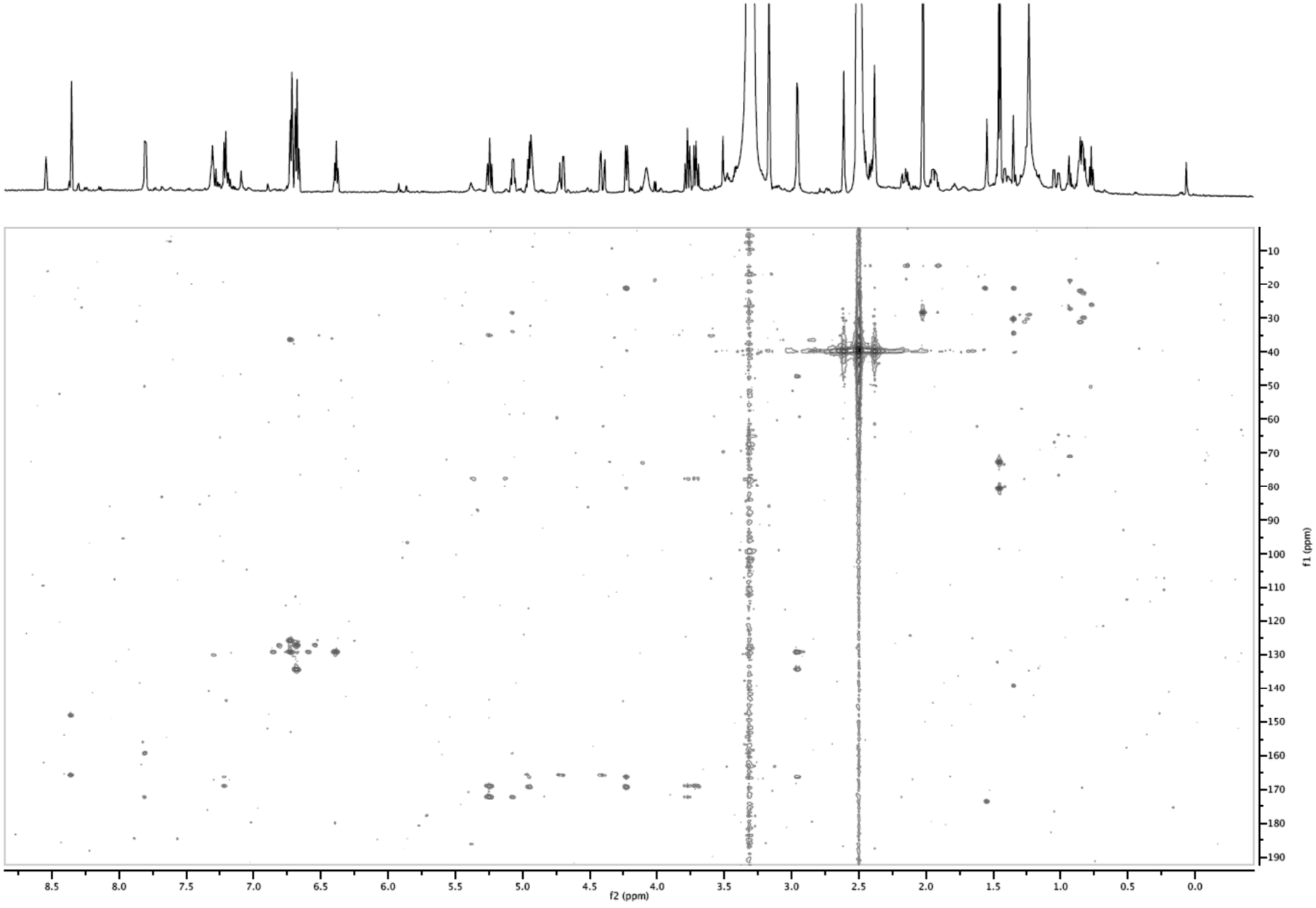


Figure 11: Aerucyclamide C (**6**), mass chromatograms monitored at *m/z* 342 for the protonated alanine Marfey's derivatives. Compound **6** was ozonized and hydrolyzed prior to the derivatisation.

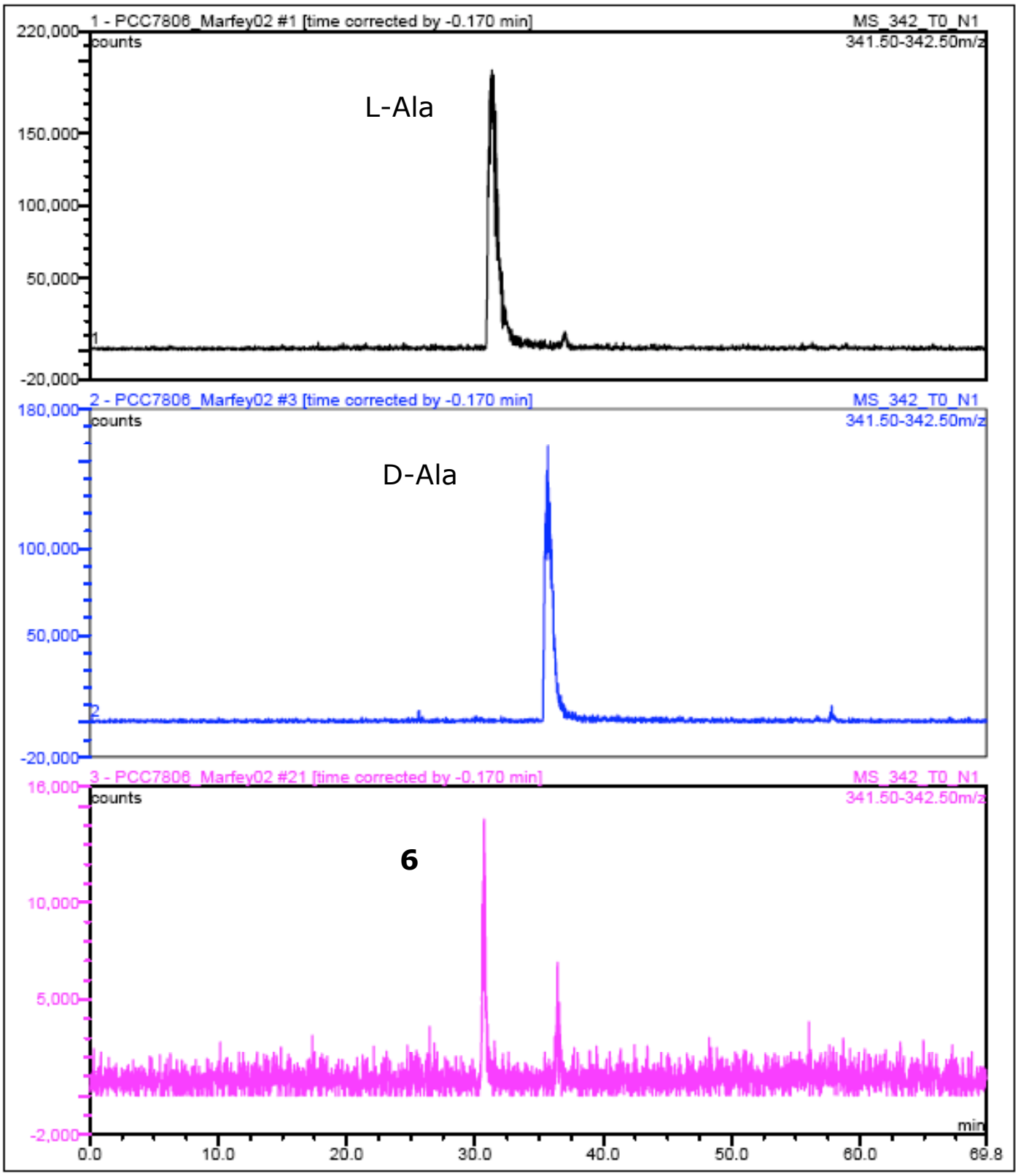


Figure 12: Aerucyclamide C (**6**), mass chromatograms monitored at *m/z* 370 for the protonated valine Marfey's derivatives. Compound **6** was ozonized and hydrolyzed prior to the derivatisation.

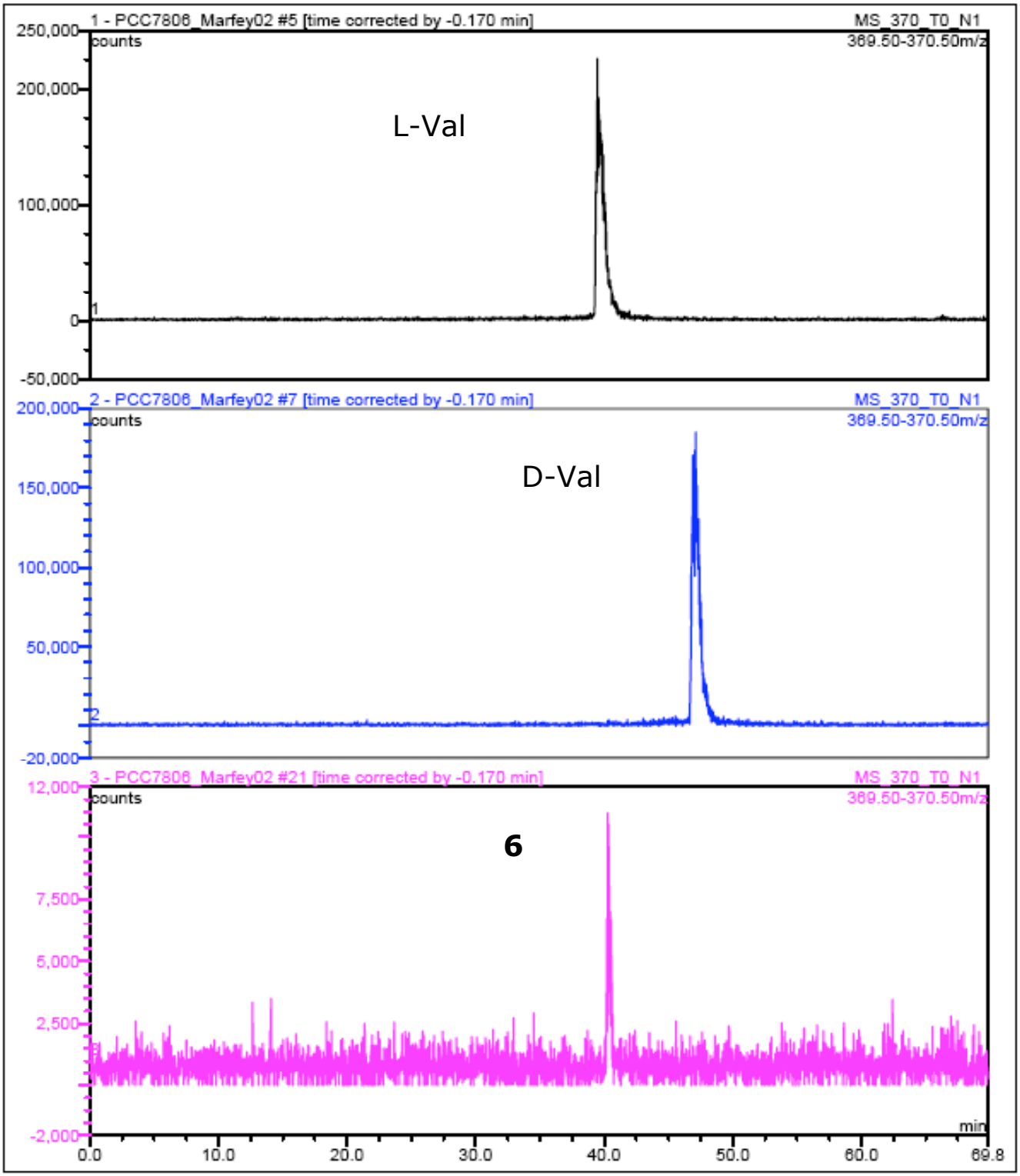


Figure 13: Aerucyclamide C (**6**), mass chromatograms monitored at *m/z* 372 for the protonated threonine Marfey's derivatives. Compound **6** was ozonized and hydrolyzed prior to the derivatisation.

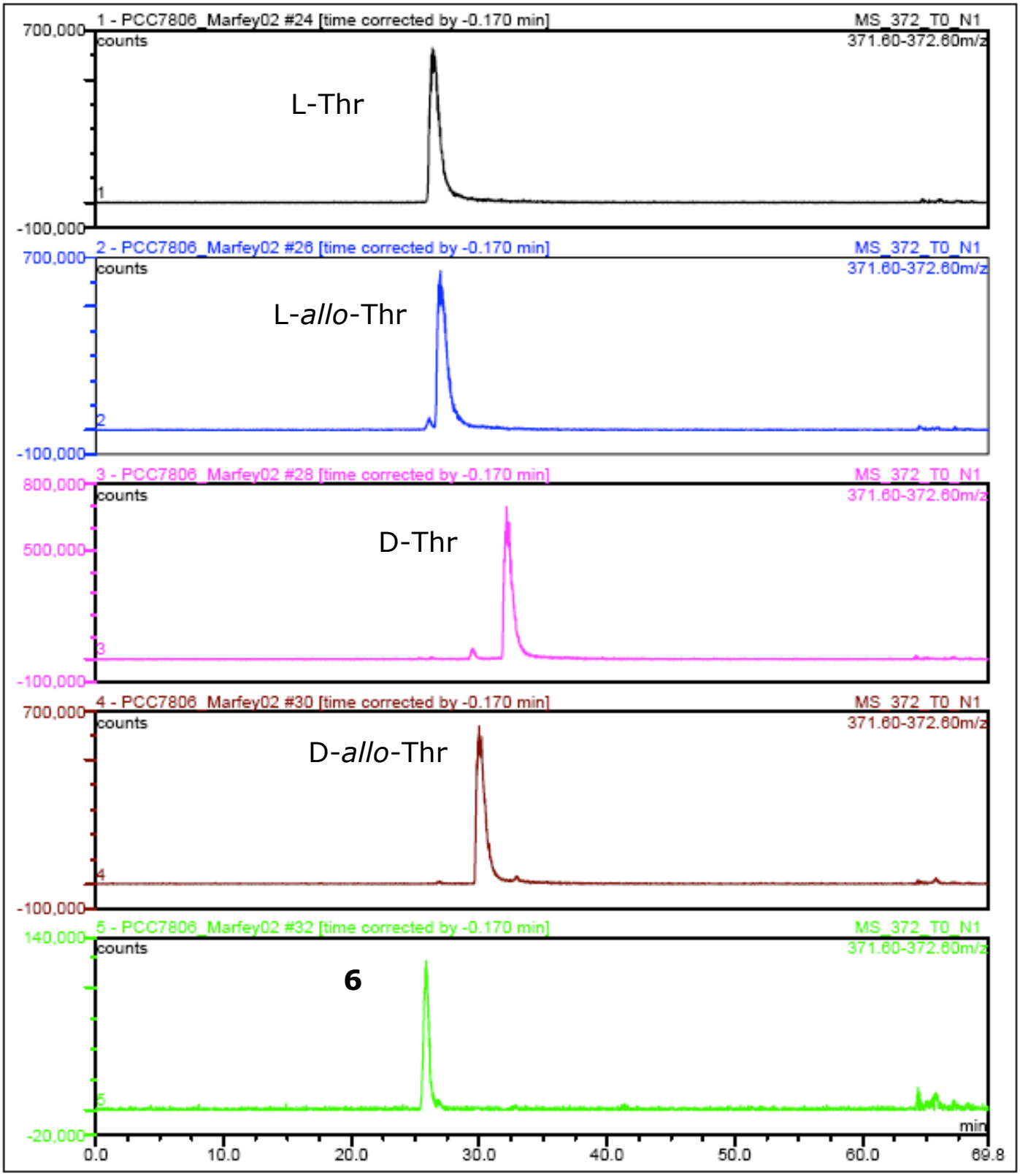


Figure 14: Coninjection of aerucyclamide C (**6**) and L-*allo*-Thr, mass chromatograms monitored at *m/z* 372 for the protonated threonine Marfey's derivatives. Compound **6** was ozonized and hydrolyzed prior to the derivatisation.

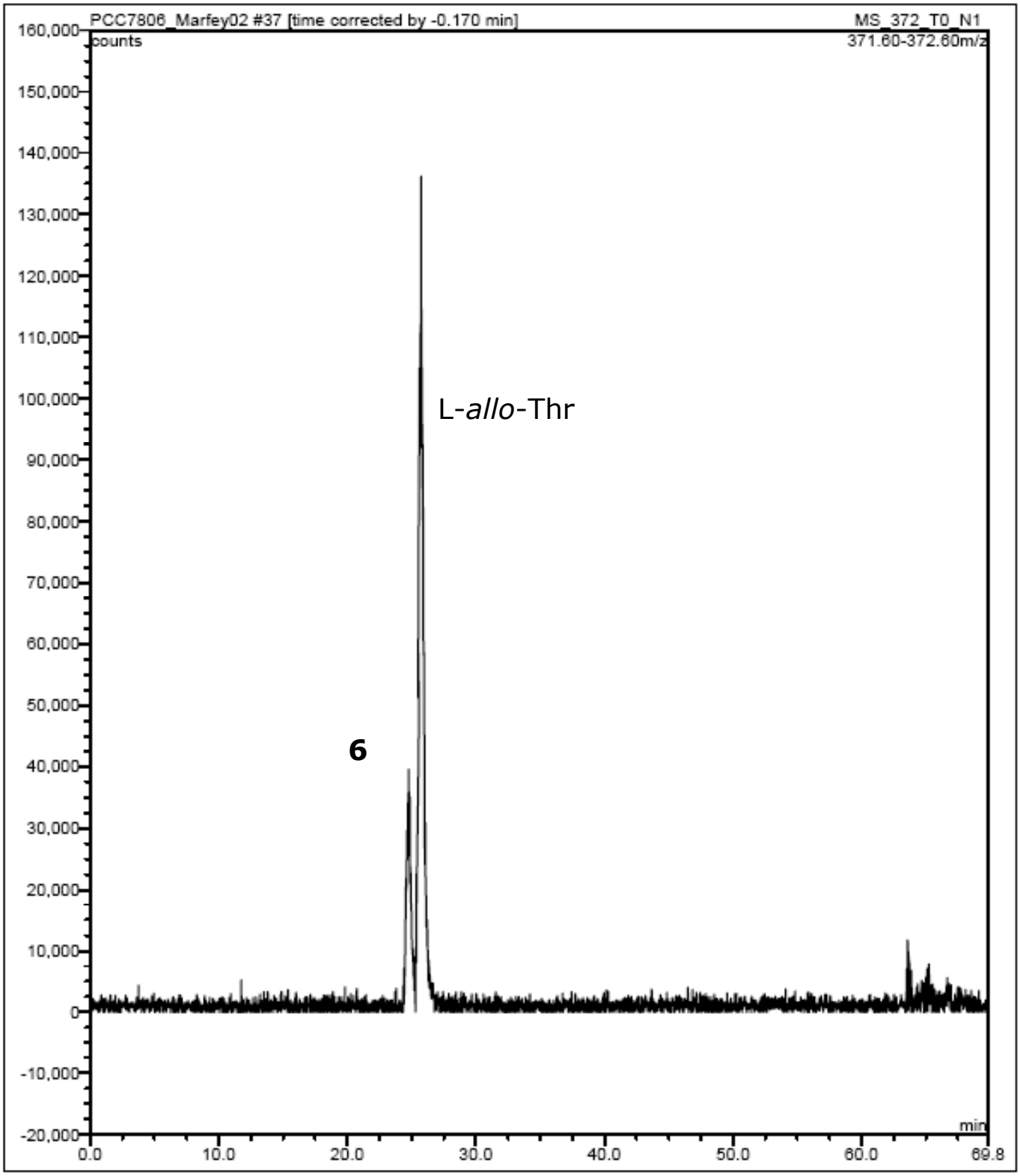
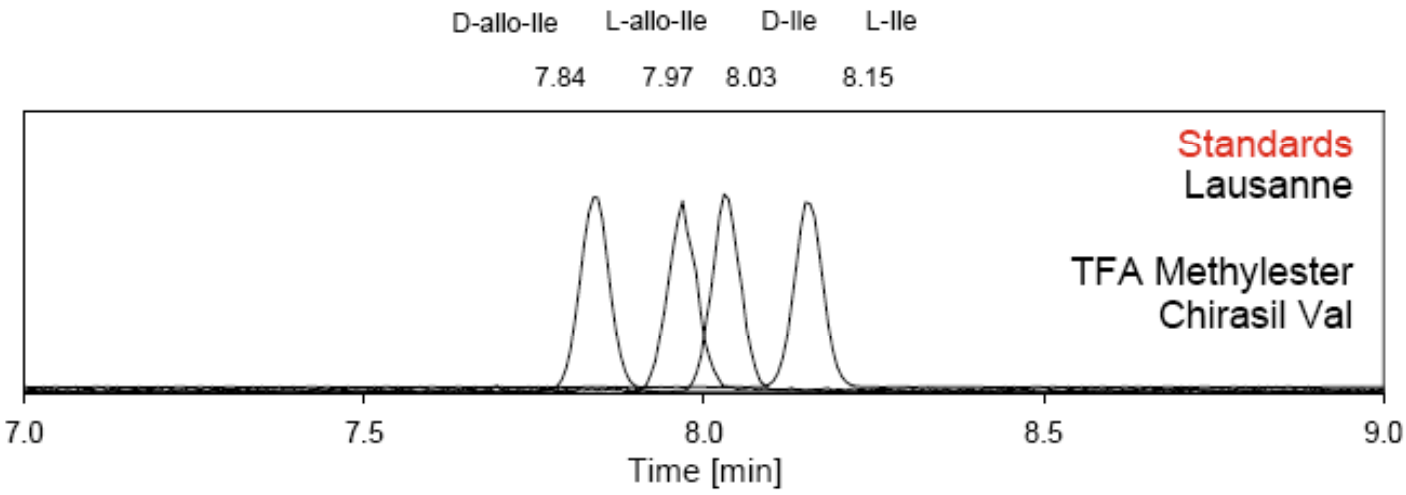


Figure 15: Aerucyclamide C (**6**), GC chromatogram of the Ile enantiomers. Compound **6** was hydrolyzed prior to derivatisation with trifluoroacetic acid.



Aerucyclamide C (**6**)

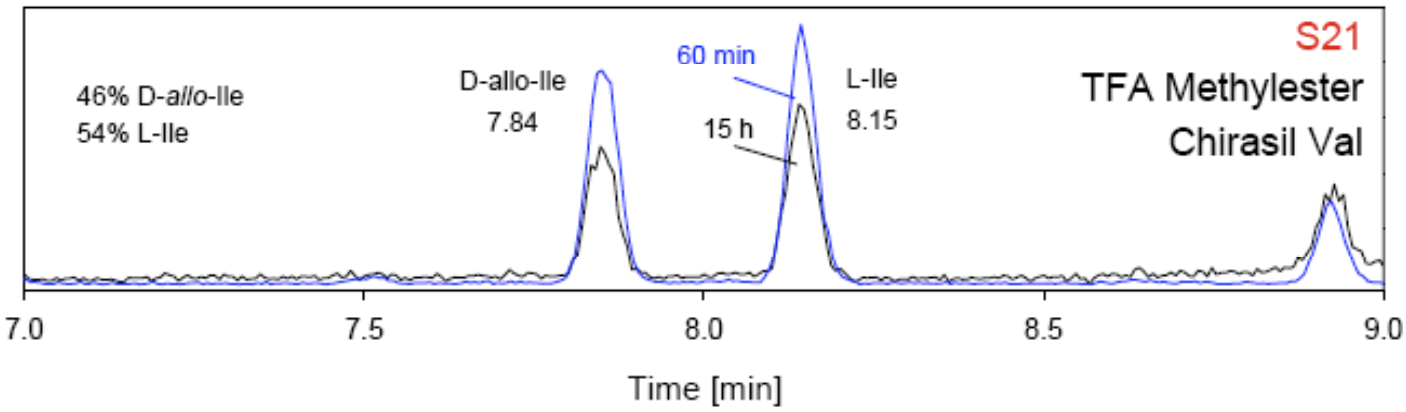


Figure 16: Aerucyclamide C (**6**), mass chromatograms monitored at *m/z* 384 for the protonated isoleucine Marfey's derivatives. Compound **6** was ozonized and hydrolyzed prior to the derivatisation.

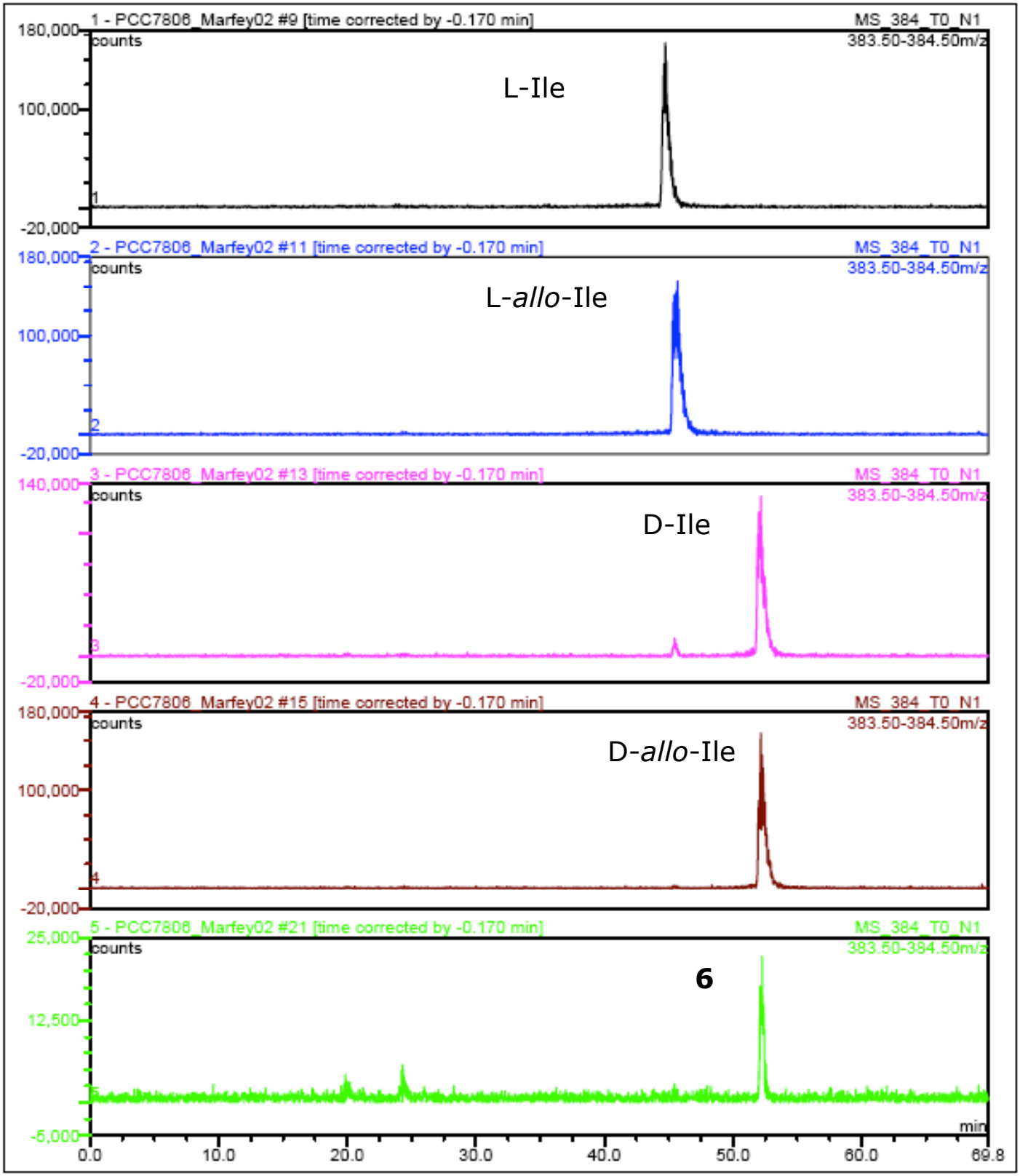


Figure 17: Aerucyclamide D (**7**), mass chromatograms monitored at *m/z* 626 for the protonated cysteine di-Marfey's derivatives. Compound **7** was ozonized and hydrolyzed prior to the derivatisation.

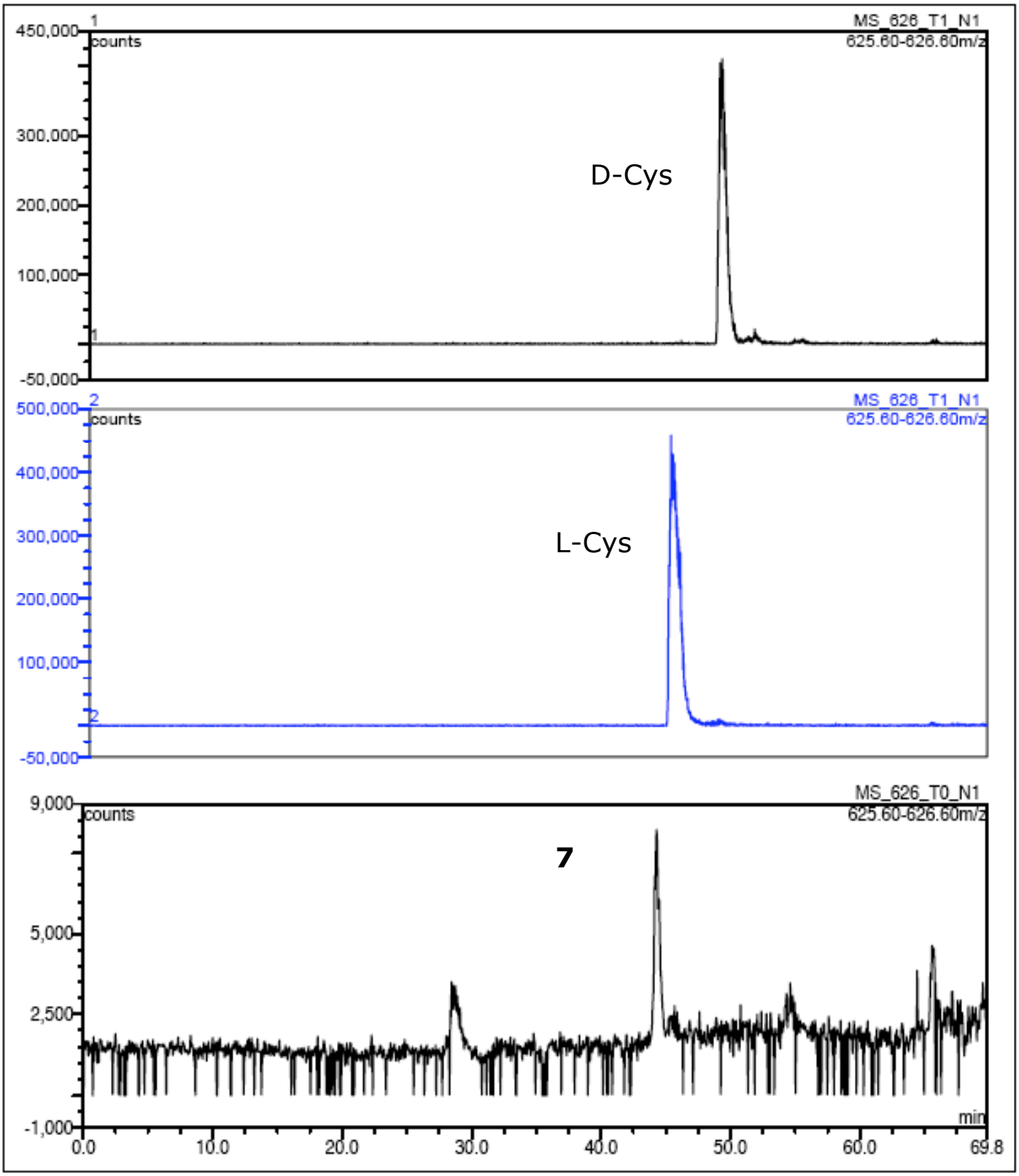


Figure 18: Aerucyclamide D (**7**), mass chromatograms monitored at *m/z* 372 for the protonated threonine Marfey's derivatives. Compound **7** was ozonized and hydrolyzed prior to the derivatisation.

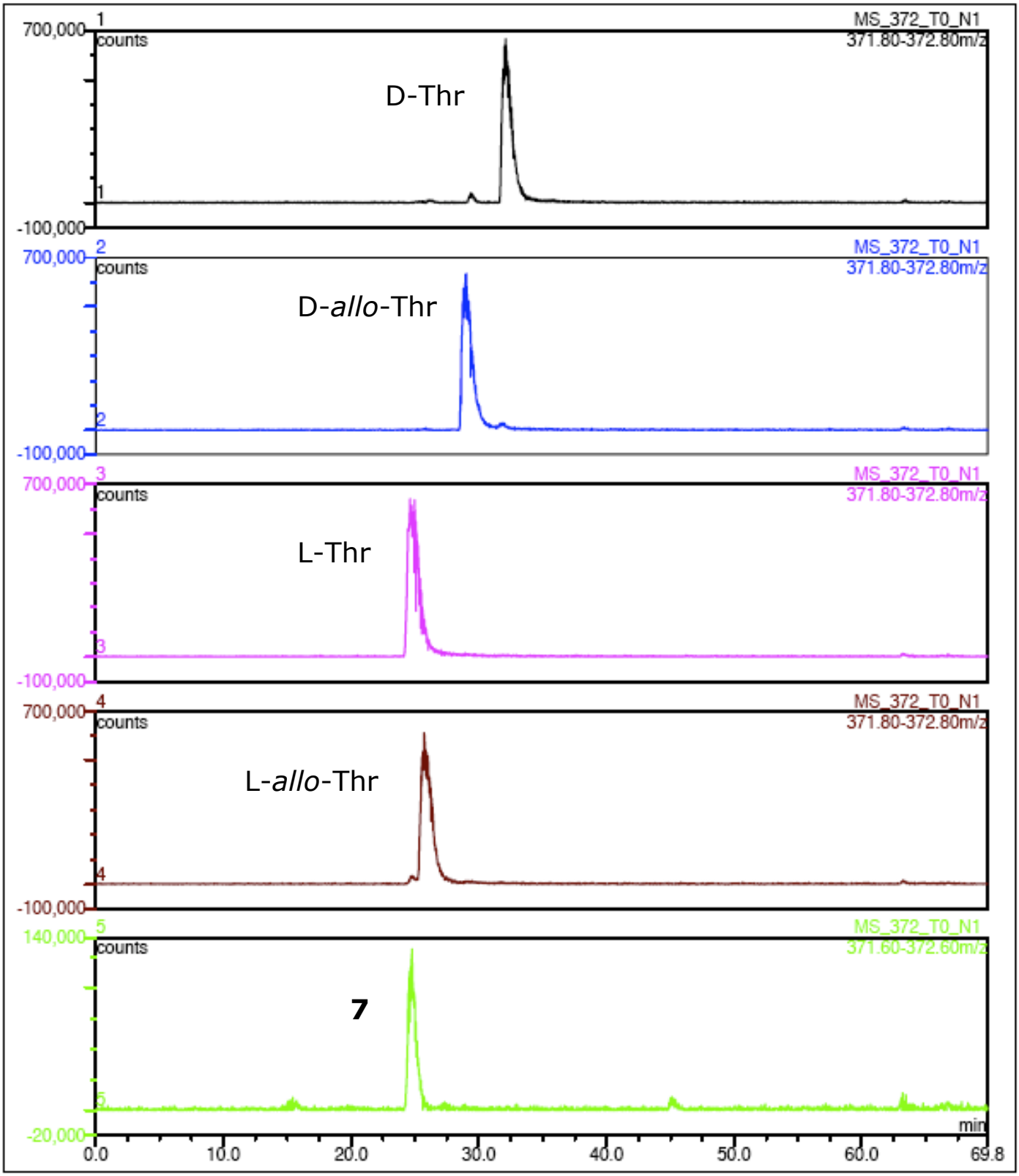


Figure 19: Coninjection of Aerucyclamide D (**7**) and L-*allo*-Thr, mass chromatograms monitored at *m/z* 372 for the protonated threonine Marfey's derivatives. Compound **7** was ozonized and hydrolyzed prior to the derivatisation.

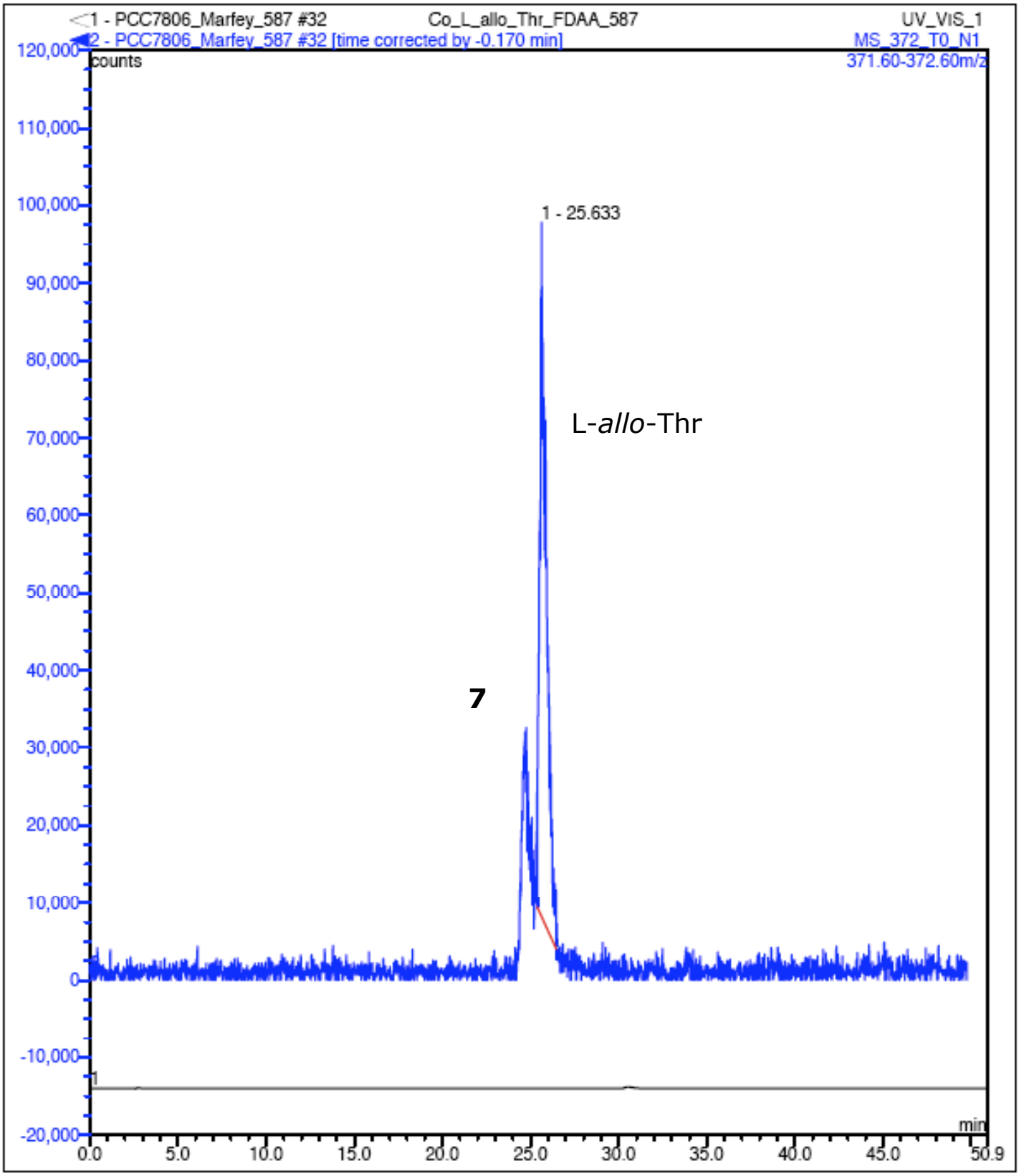


Figure 20: Aerucyclamide D (**7**), mass chromatograms monitored at *m/z* 402 for the protonated methionine Marfey's derivatives. Compound **7** was ozonized and hydrolyzed prior to the derivatisation.

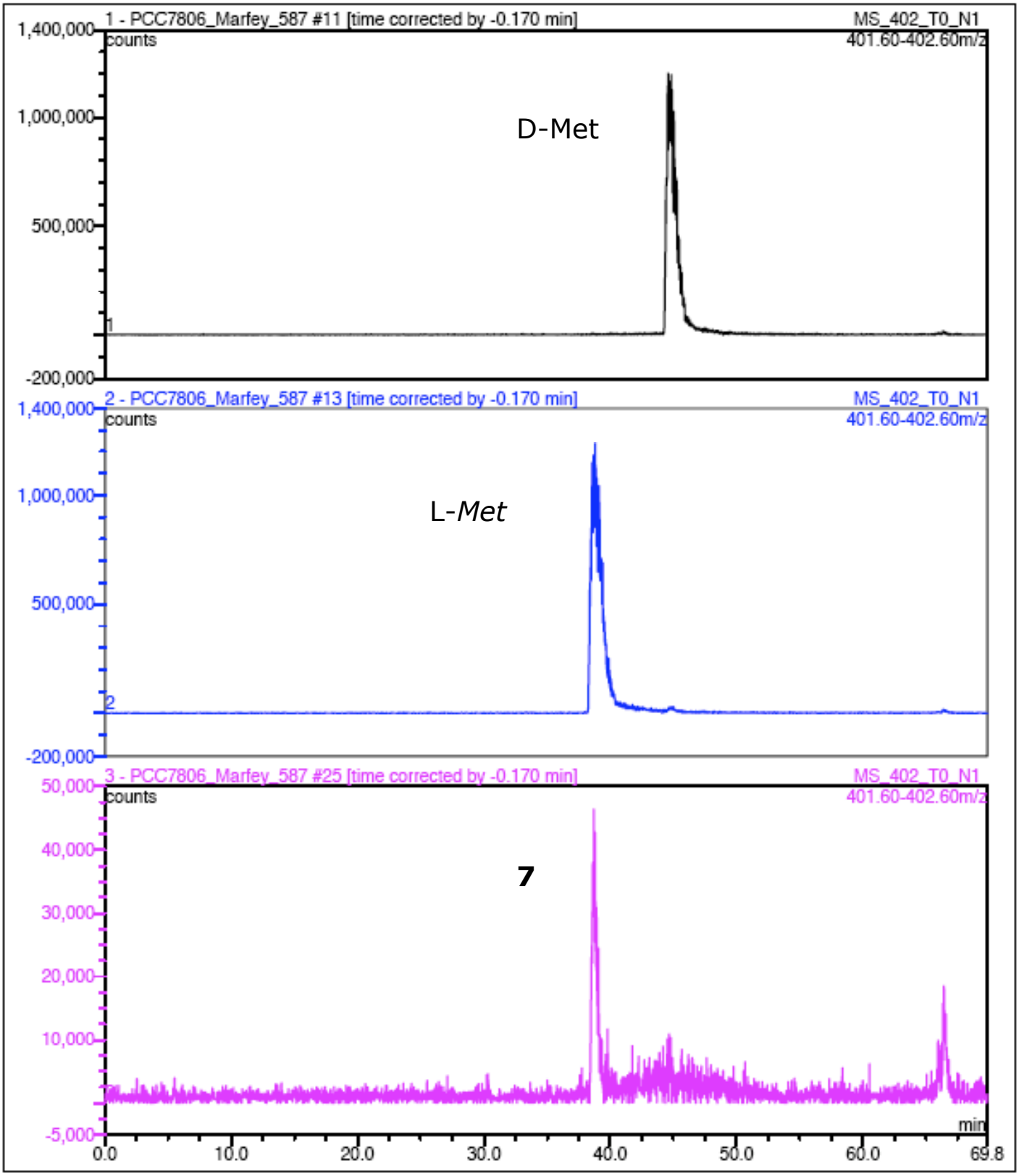


Figure 21: Aerucyclamide D (**7**), mass chromatograms monitored at *m/z* 418 for the protonated phenylalanine Marfey's derivatives. Compound **7** was ozonized and hydrolyzed prior to the derivatisation.

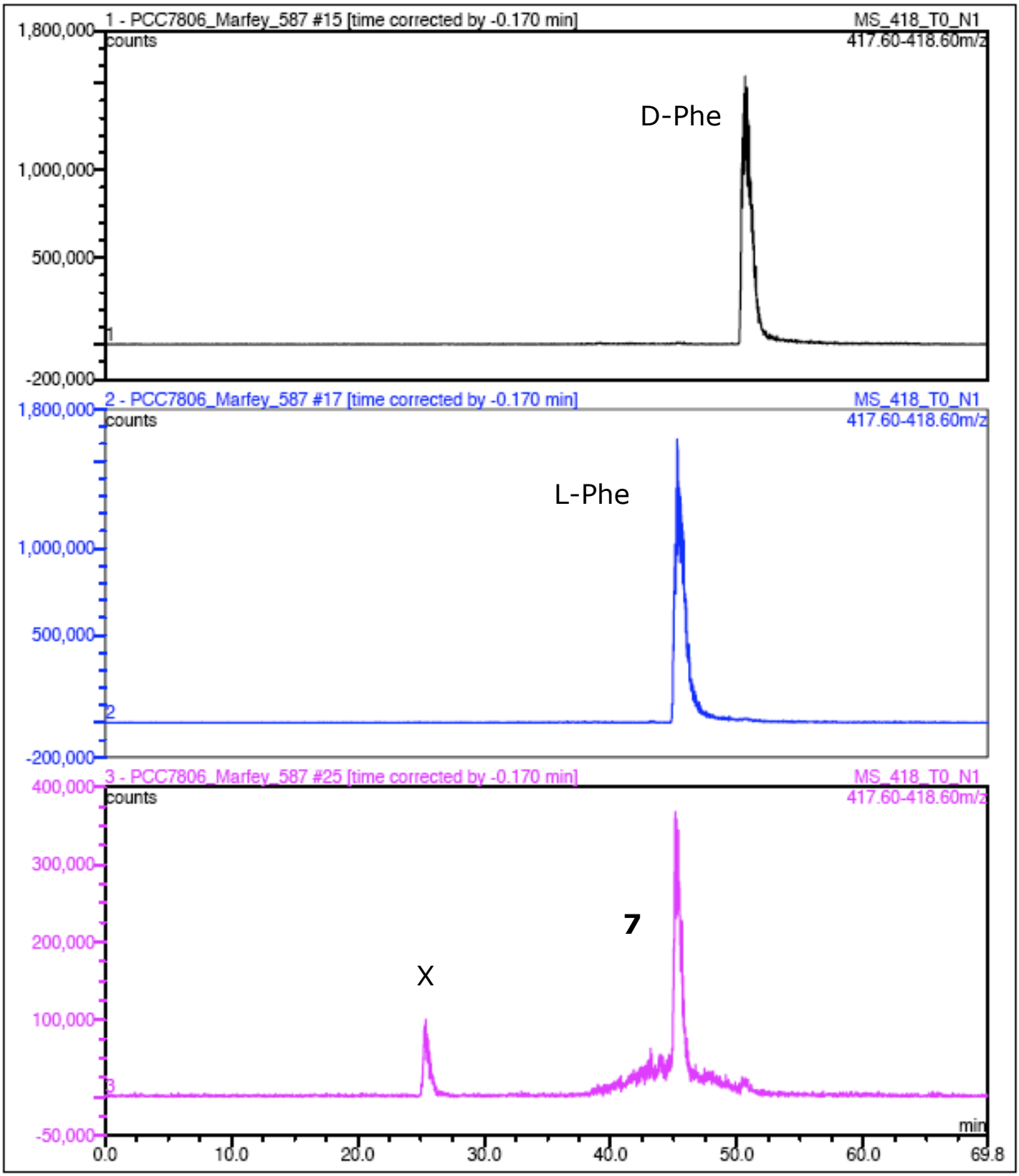
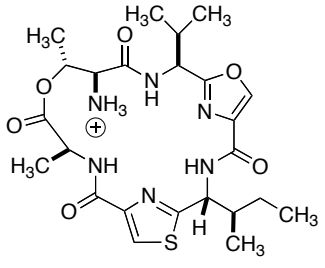


Figure 22: NMR spectroscopic data (600 MHz, DMSO-d_6) for microcyclamide 7806A revised structure (**2**).

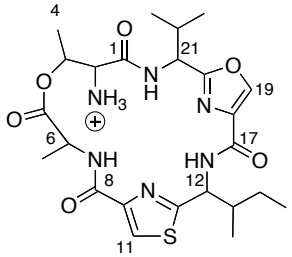
Microcyclamide 7806A revised structure (2)				
Position	δ_C	δ_H (*J* in Hz)	HMBC [a]	COSY
1	166.1, qC			
2	54.8, CH	4.12, q (5.2)	1	NH_3^+
NH_3^+		8.28, d (4.8)		2
3	69.4, CH	5.33, q (6.9)	1, 4, 5	4
4	16.9, CH_3	1.41, d (6.9)	2, 3	3
5	171.2, qC			
6	47.4, CH	4.43, dq (5.0, 7.0)	5, 7	7, NH(2)
7	17.5, CH_3	1.34, d (7.0)	5, 6	6
NH (2)		7.90, d (5.0)	5, 6, 8	6
8	158.9, qC			
9	146.7, qC			
10	124.9, CH	8.34, s	8, 9, 11	
11	171.1, qC			
12	53.9, CH	5.55, dd (10.5, 3.2)	11, 13, 14, 15, 17	13, NH(4)
13	38.7, CH	2.14, m		
14	13.4, CH_3	0.83, d (6.9)	12, 13, 15	13
15	26.4, CH_2	1.57, m	12, 13, 14, 16	13
		1.40, m	12, 13, 14, 16	
16	11.3, CH_3	1.02, m	13, 15	15
NH (4)		7.30, d (10.5)	-	12
17	161.8			
18				
19	142.9, CH	8.78, s	20	
20	161.3, qC			
21	52.7, CH	4.80, dd (9.4, 9.2)	1, 20, 22, 23	22, NH(6)
22	30.6, CH	2.15, m	21, 23	
23	18.8, CH_3	1.04, d (6.6)	21, 22	22
23'	18.6, CH_3	0.79, d (6.6)	21, 22, 23	22
NH (6)		9.22, d (9.2)	1	21

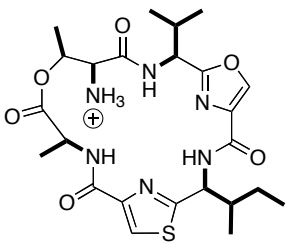
[a]HMBC correlations, are proton(s) stated to the indicated carbon

[b]This carbon atom was not visible in the HMBC spectrum. Its chemical shift was extrapolated from the NMR table of microcyclamide 7806A reported in Dittmann and co-workers, *Appl. Environ. Microbiol.* **2008**, *74*, 1791

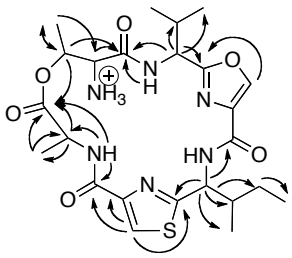


Microcyclamide 7806A
Revised Structure (**2**)



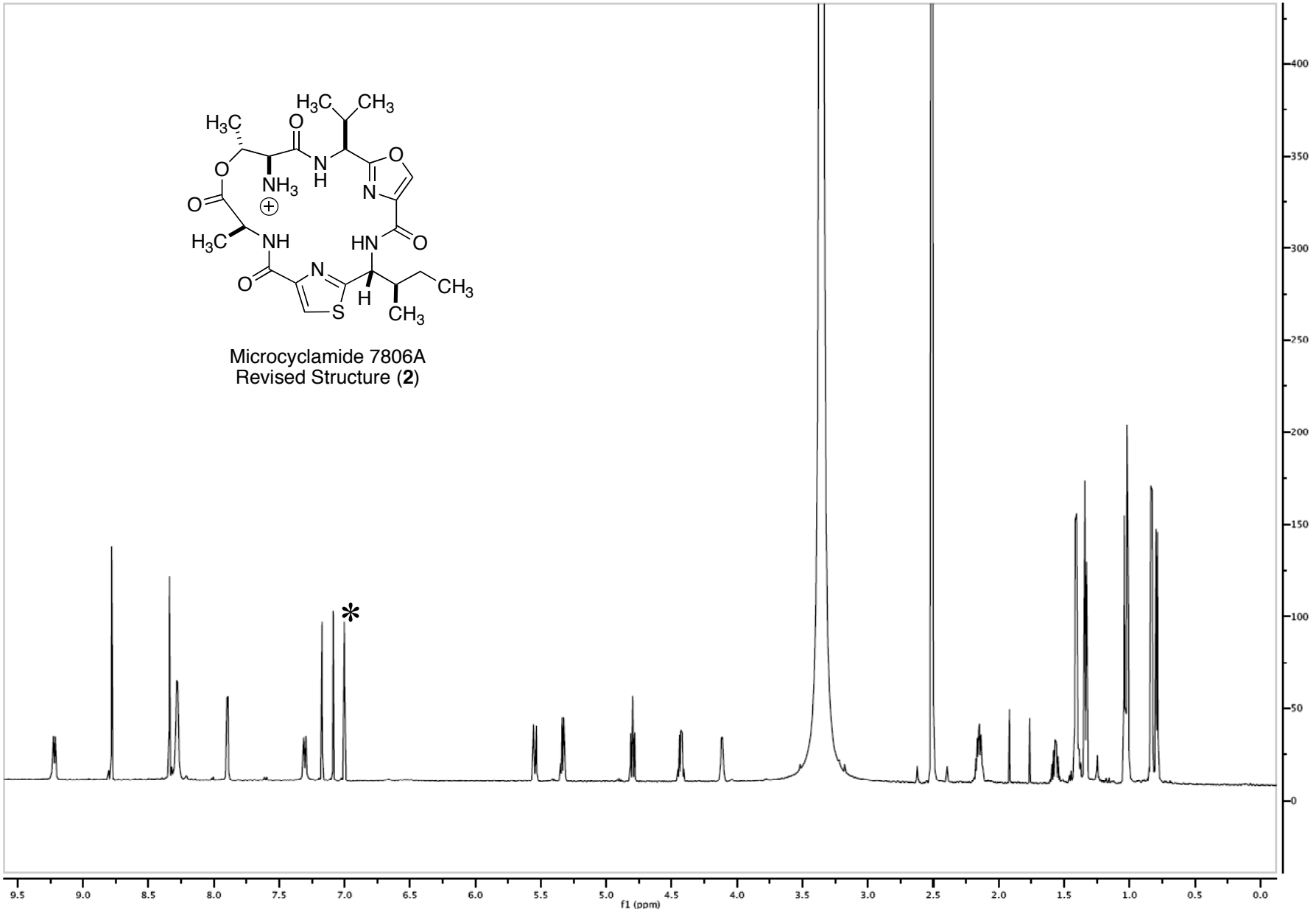


COSY



selected HMBC

Figure 23: ^{1}H NMR spectrum of microcyclamide 7806A revised structure (**2**) in DMSO-d_6 (600MHz)



* traces of NH_4^+

Figure 24: COSY NMR spectrum microcyclamide 7806A revised structure (**2**) in DMSO-d_6 (600MHz)

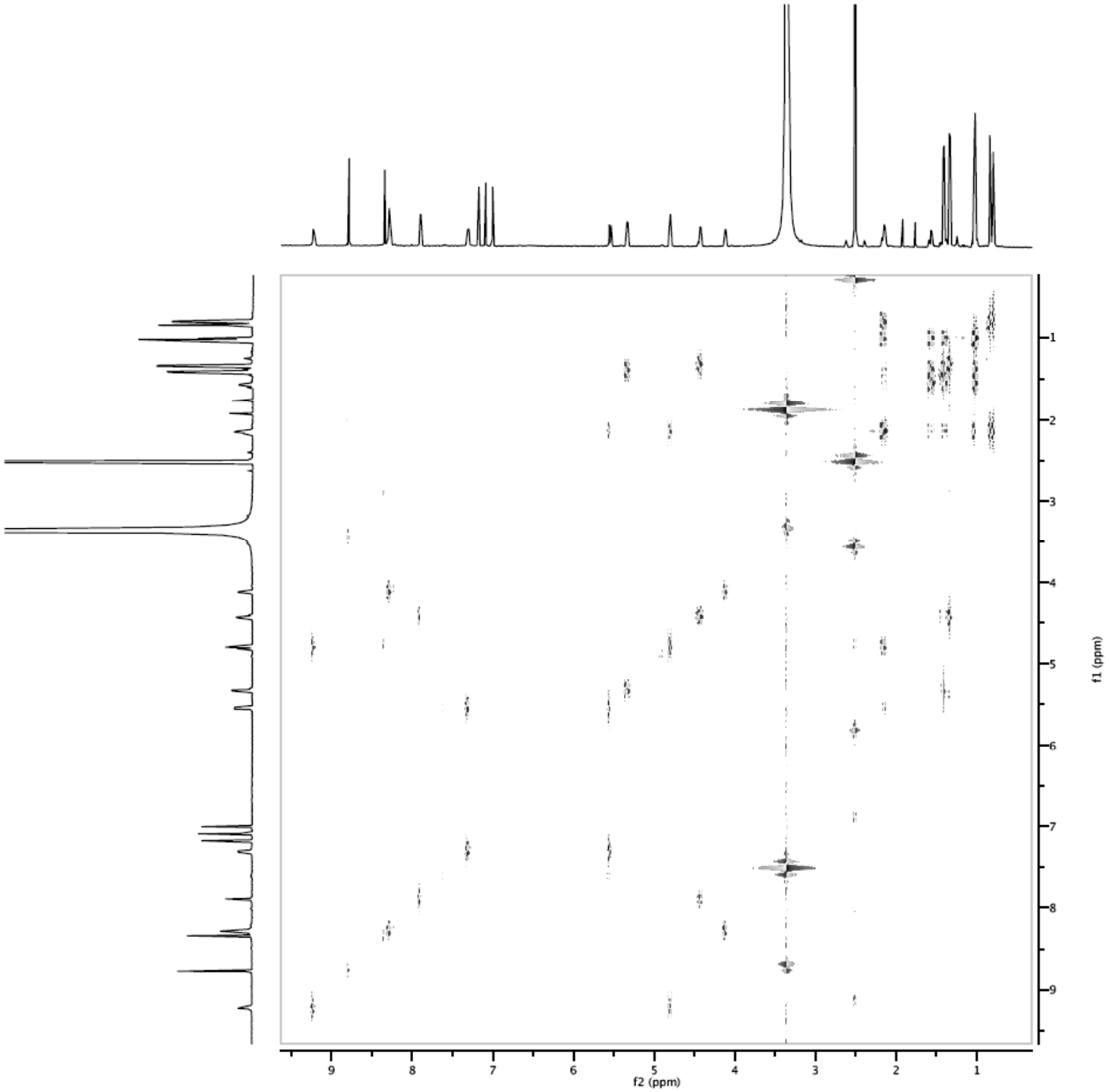


Figure 25: gHSQC NMR spectrum microcyclamide 7806A revised structure (**2**) in DMSO-d_6 (600MHz)

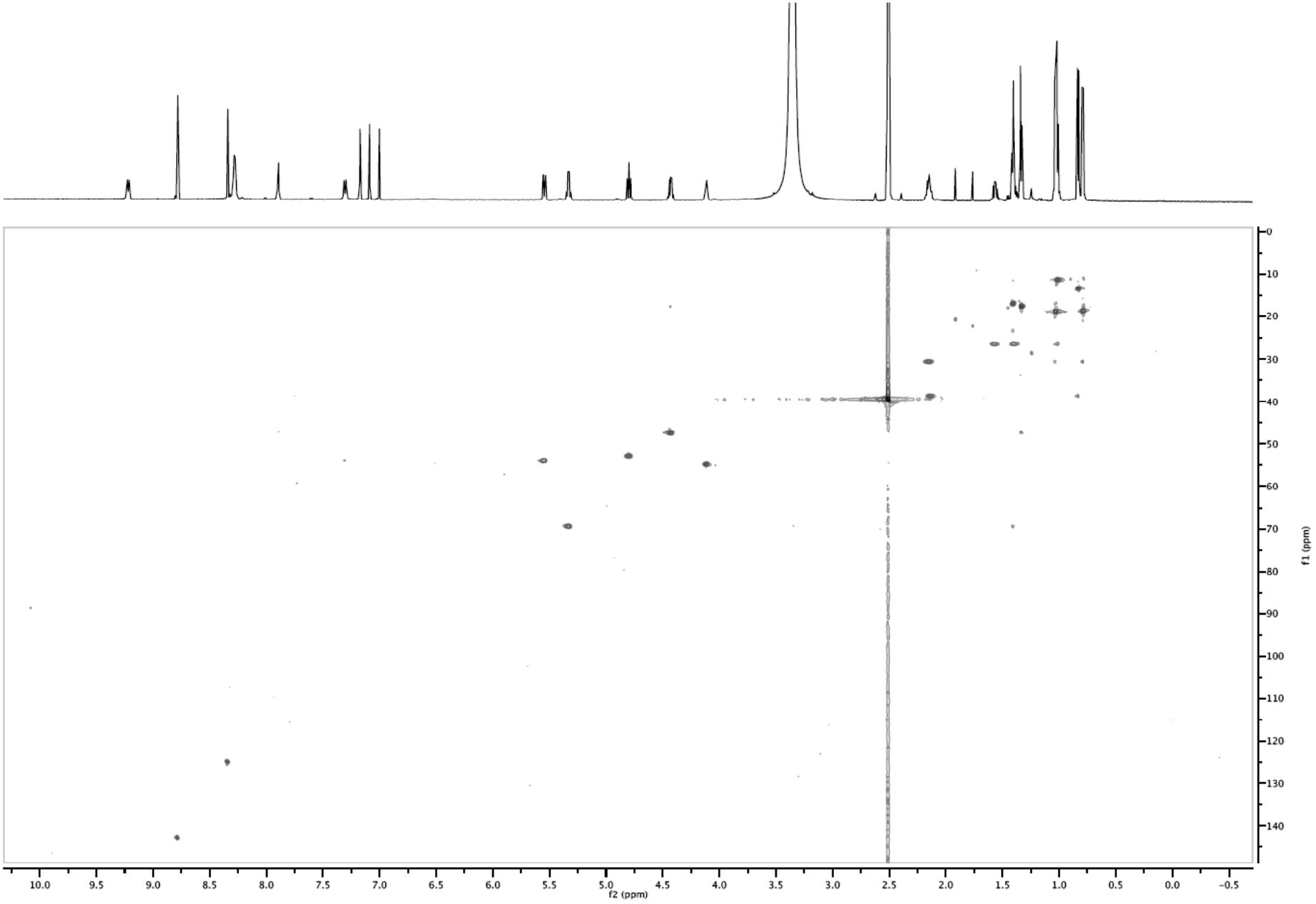


Figure 26: gHMBC NMR spectrum of microcyclamide 7806A revised structure (**2**) in DMSO-d_6 (600MHz)

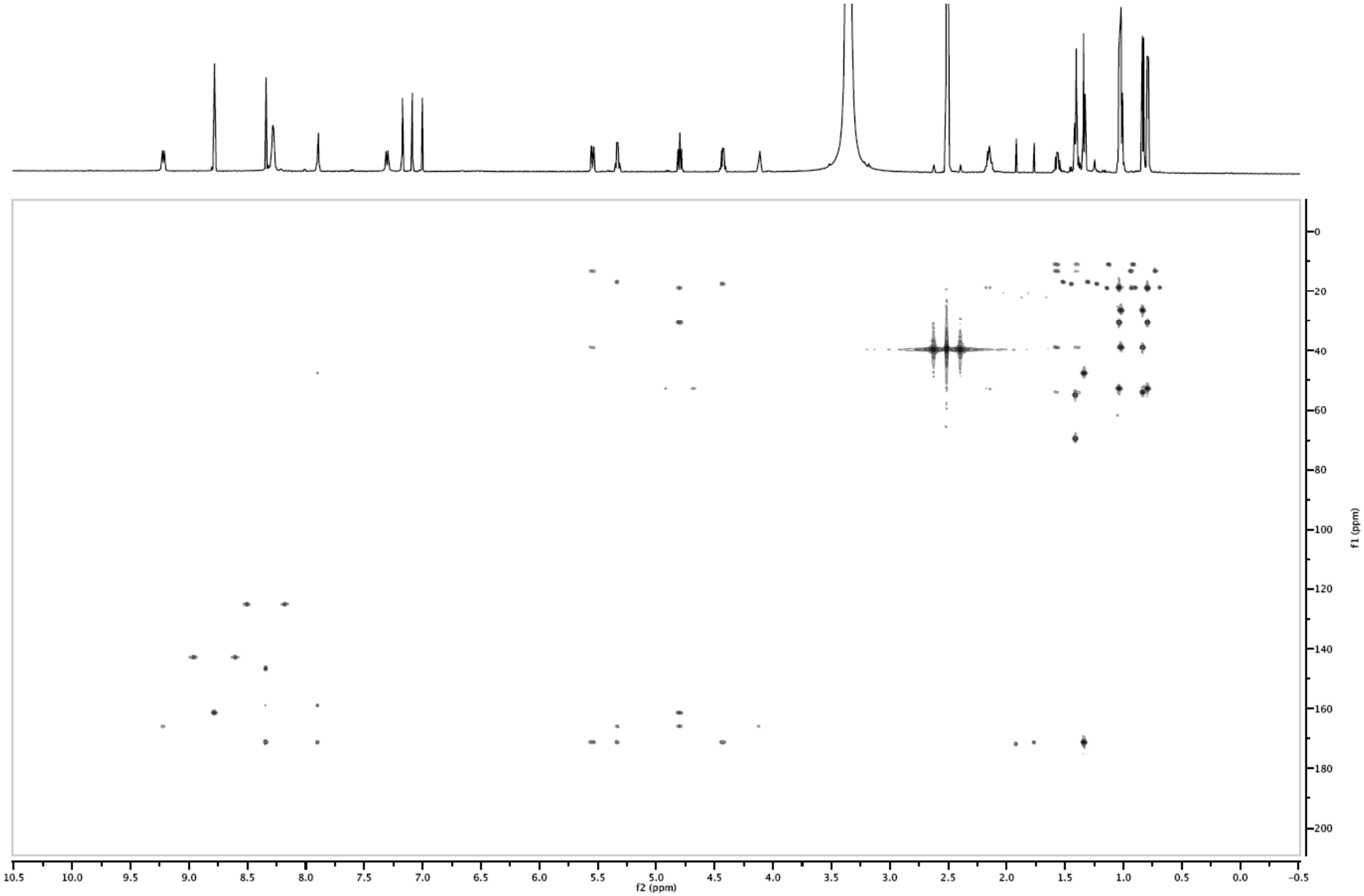
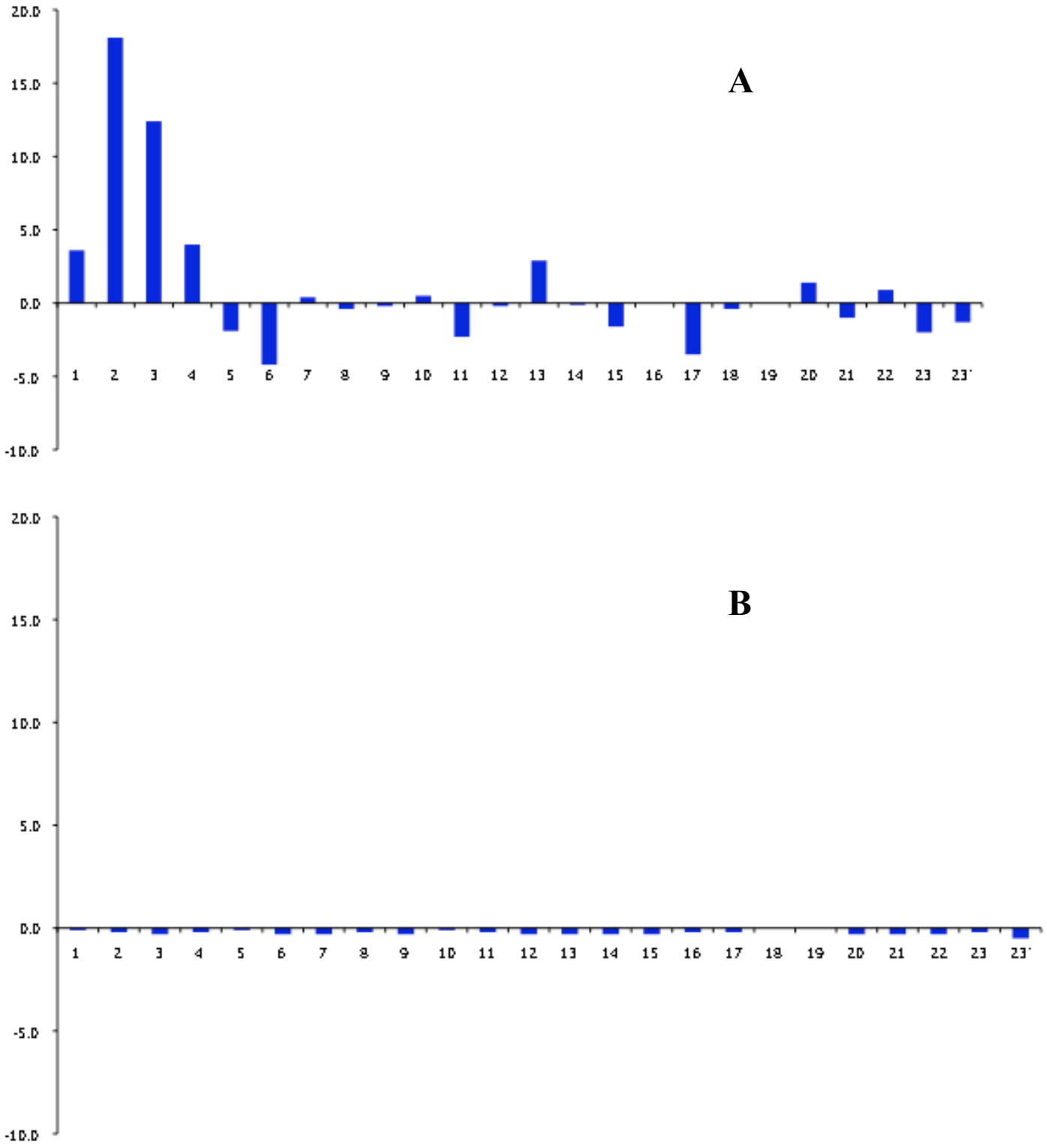
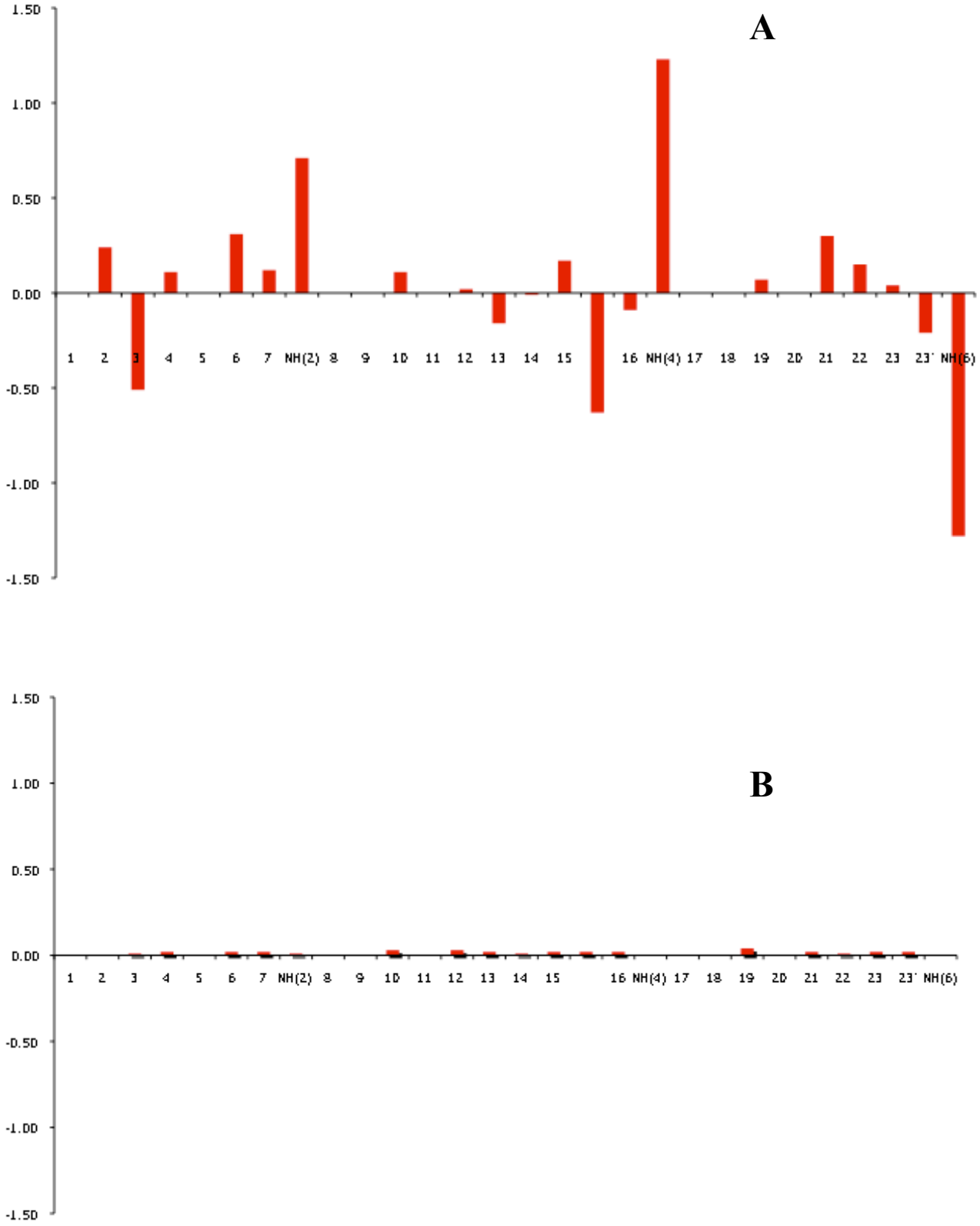


Figure 27: Comparison of the ^{13}C chemical shift data between **A:** aerucyclamide C (**6**) and microcyclamide 7806A (data reported in ref.1) and **B:** microcyclamide 7806A revised structure (**2**) (this work) and microcyclamide 7806A (data reported in ref.1)



[1] Ziemert, N.; Ishida, K.; Quillardet, P.; Bouchier, C.; Hertweck, C.; Tandeau de Marsac, N.; Dittmann, E. *Appl. Environ. Microbiol.* **2008**, *74*, 1791.

Figure 28: Comparison of the [1]H chemical shift data between **A:** aerucyclamide C (**6**) and microcyclamide 7806A (data reported in ref.1) and **B:** microcyclamide 7806A revised structure (**2**) (this work) and microcyclamide 7806A (data reported in ref.1)



[1] Ziemert, N.; Ishida, K.; Quillardet, P.; Bouchier, C.; Hertweck, C.; Tandeau de Marsac, N.; Dittmann, E. *Appl. Environ. Microbiol.* **2008**, *74*, 1791.

Figure 29: Comparison of the HPLC traces of an extract of *microcyctis aeruginosa* PCC7806; microcyclamide 7806A revised structure (**2**) and aerucyclamide C (**6**).

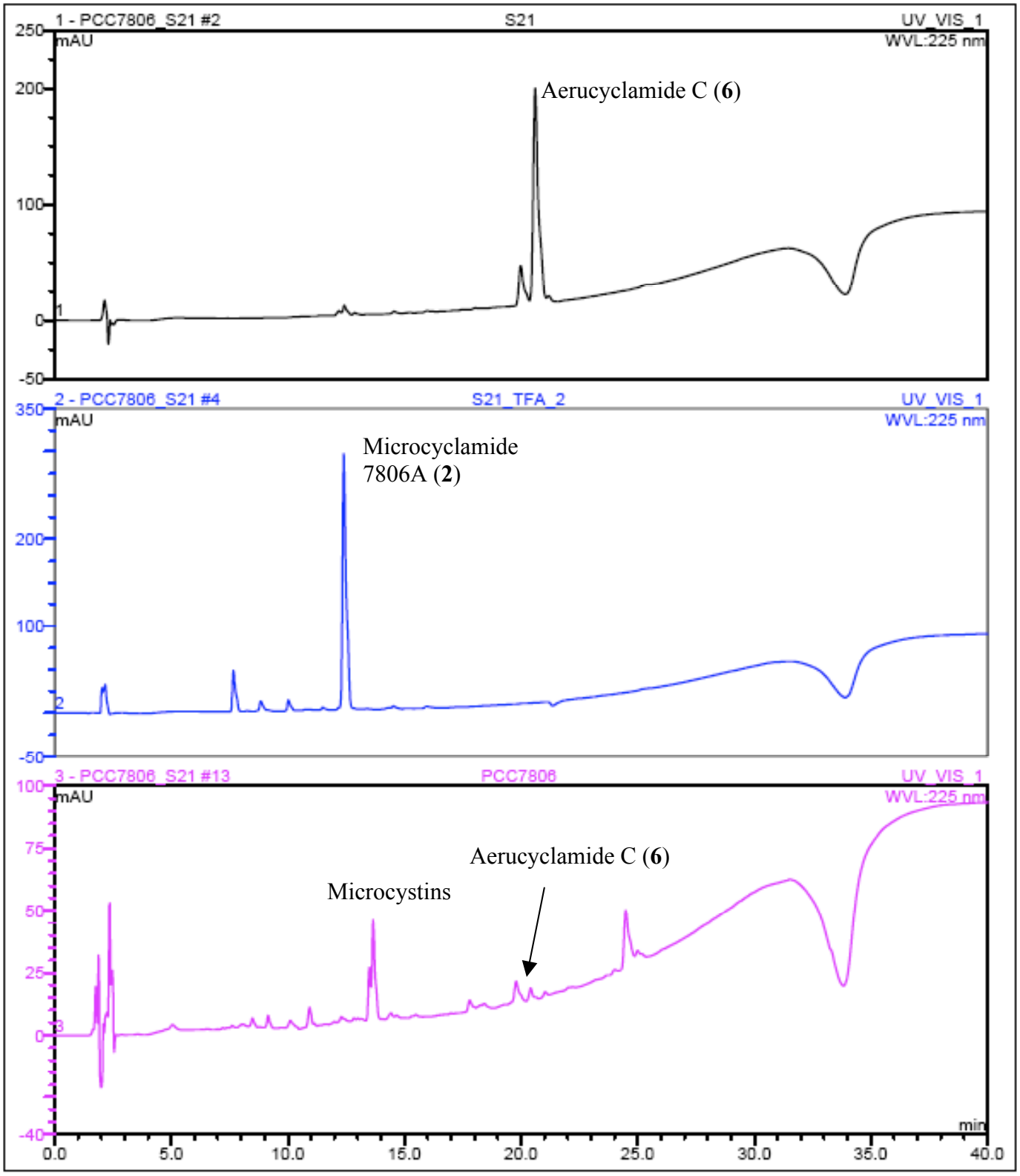


Figure 30: ^{1}H NMR spectrum of microcyclamide 7806B (**3**) in DMSO-d_6 (600MHz) obtained after treatment of microcyclamide 7806A (**2**. 0.5mg, 9.5μmol) in DMSO-d_6 d with 30 % NaOD in D_2O (3 μl) and then neutralized with 20% DCl in D_2O (2μl).

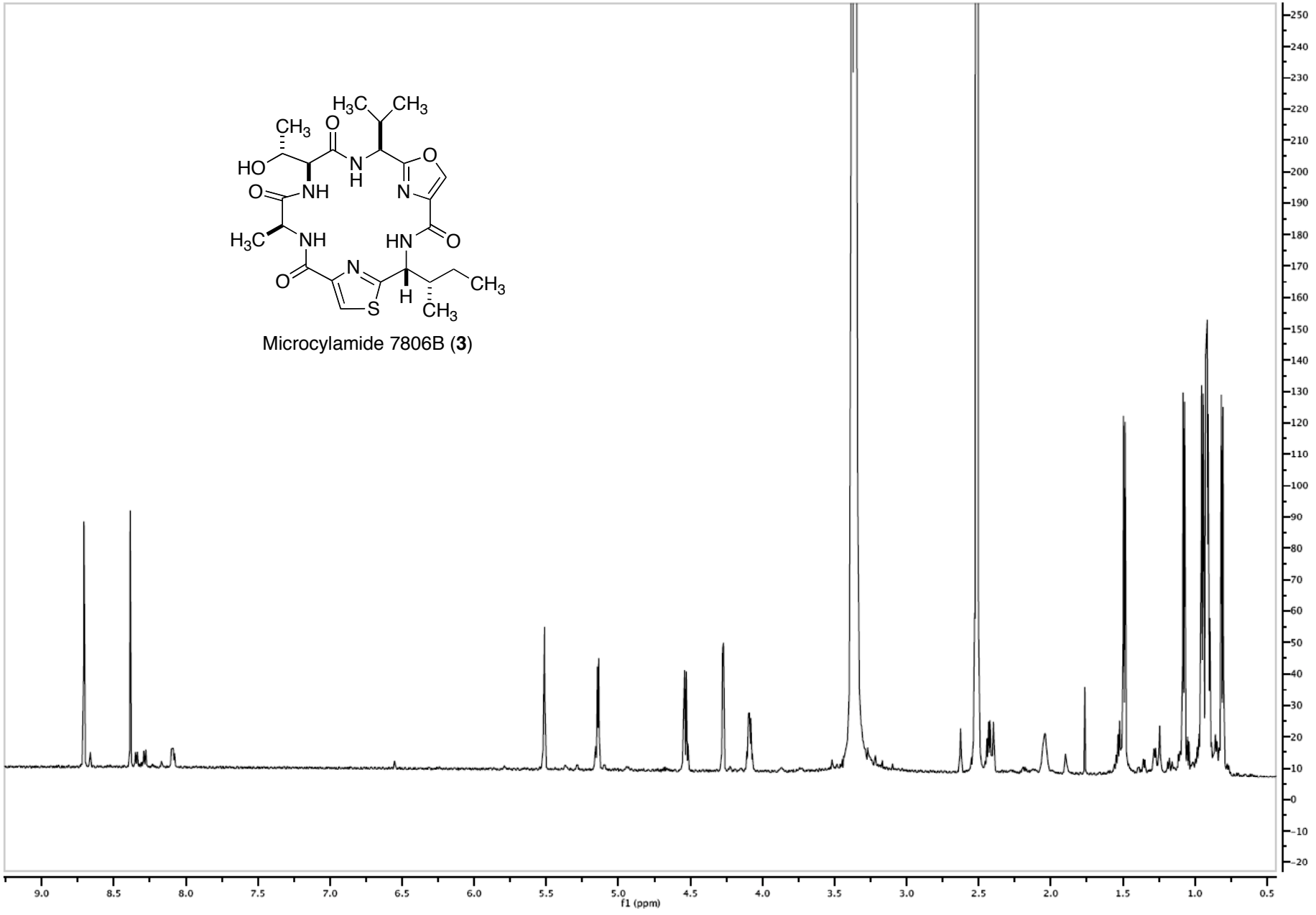


Figure 31: Dose-response curves for the mortality [%] of *Thamnocephalus platyurus* (24-h acute toxicity) exposed to different concentrations of aerucyclamide C (**6**).