Supporting Information

Cyclotheonellazoles A-C, Potent Protease Inhibitors from the Marine Sponge *Theonella* aff. *swinhoei*

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Figure S1. ^{1}H-NMR Spectrum of Cyclotheonellazole A (**1**) in DMSO-d_6

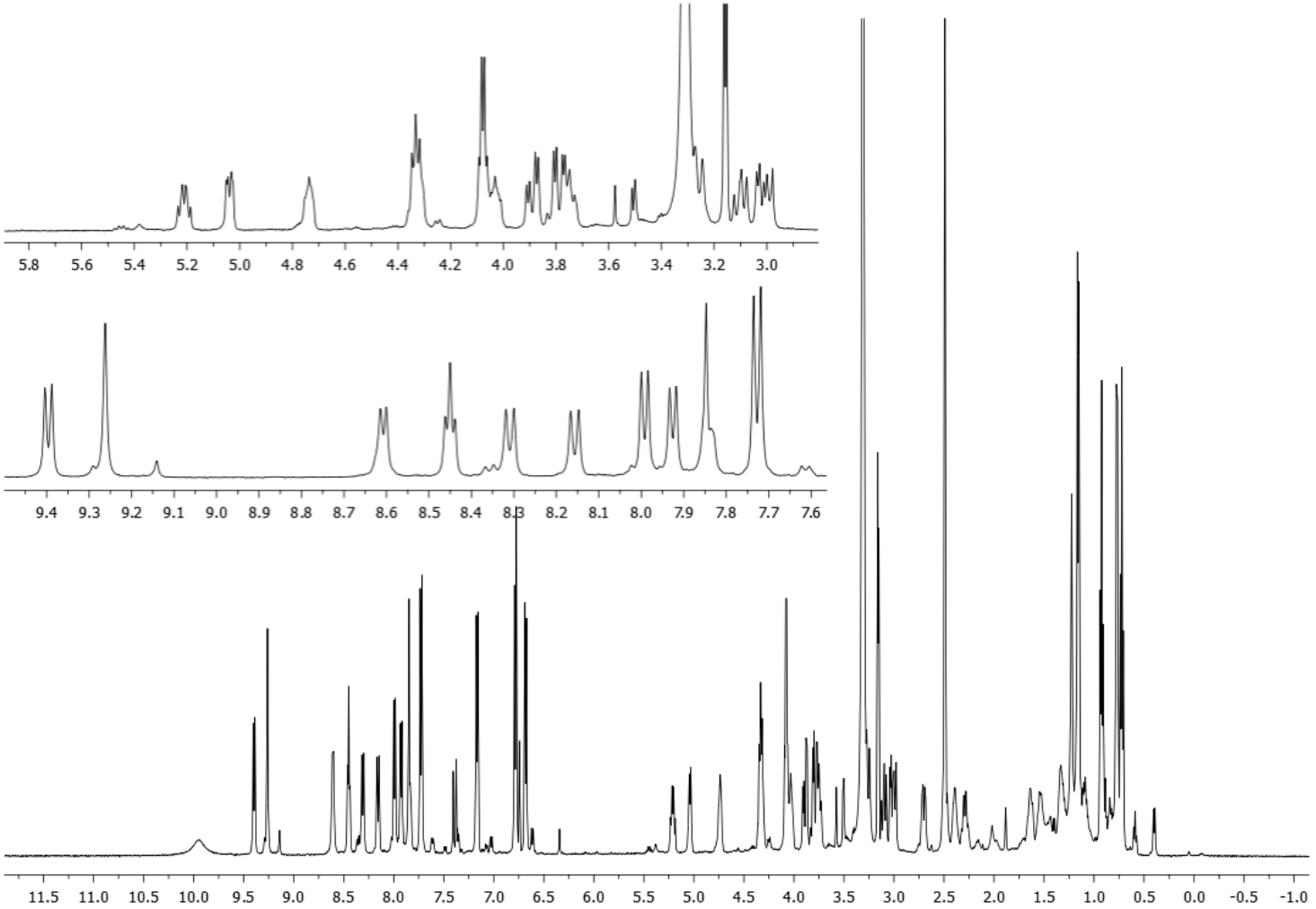


Figure S2. ^{13}C-NMR Spectrum of Cyclotheonellazole A (**1**) in DMSO-d_6

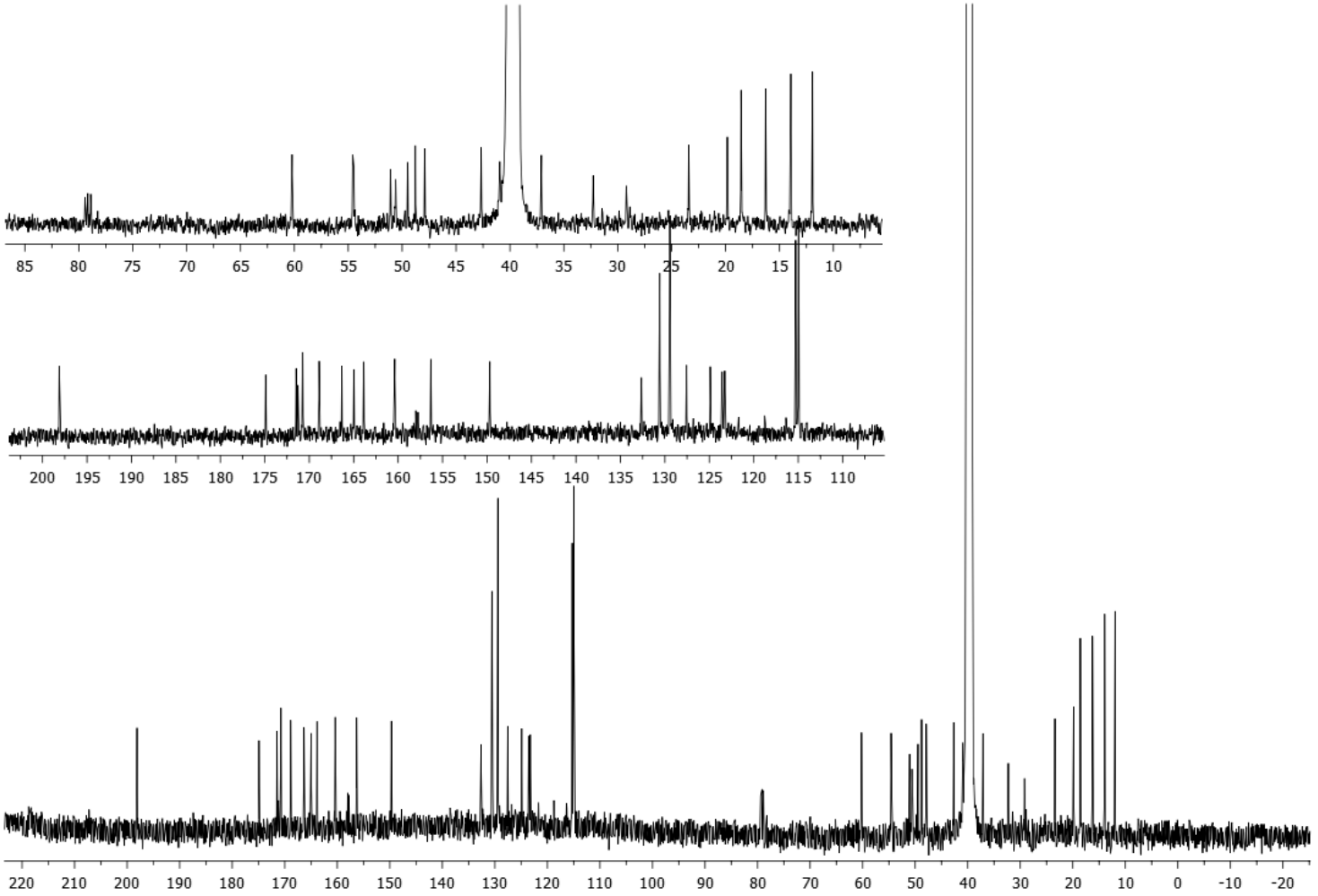


Figure S3. HSQC Spectrum of Cyclotheonellazole A (**1**) in DMSO-d_6

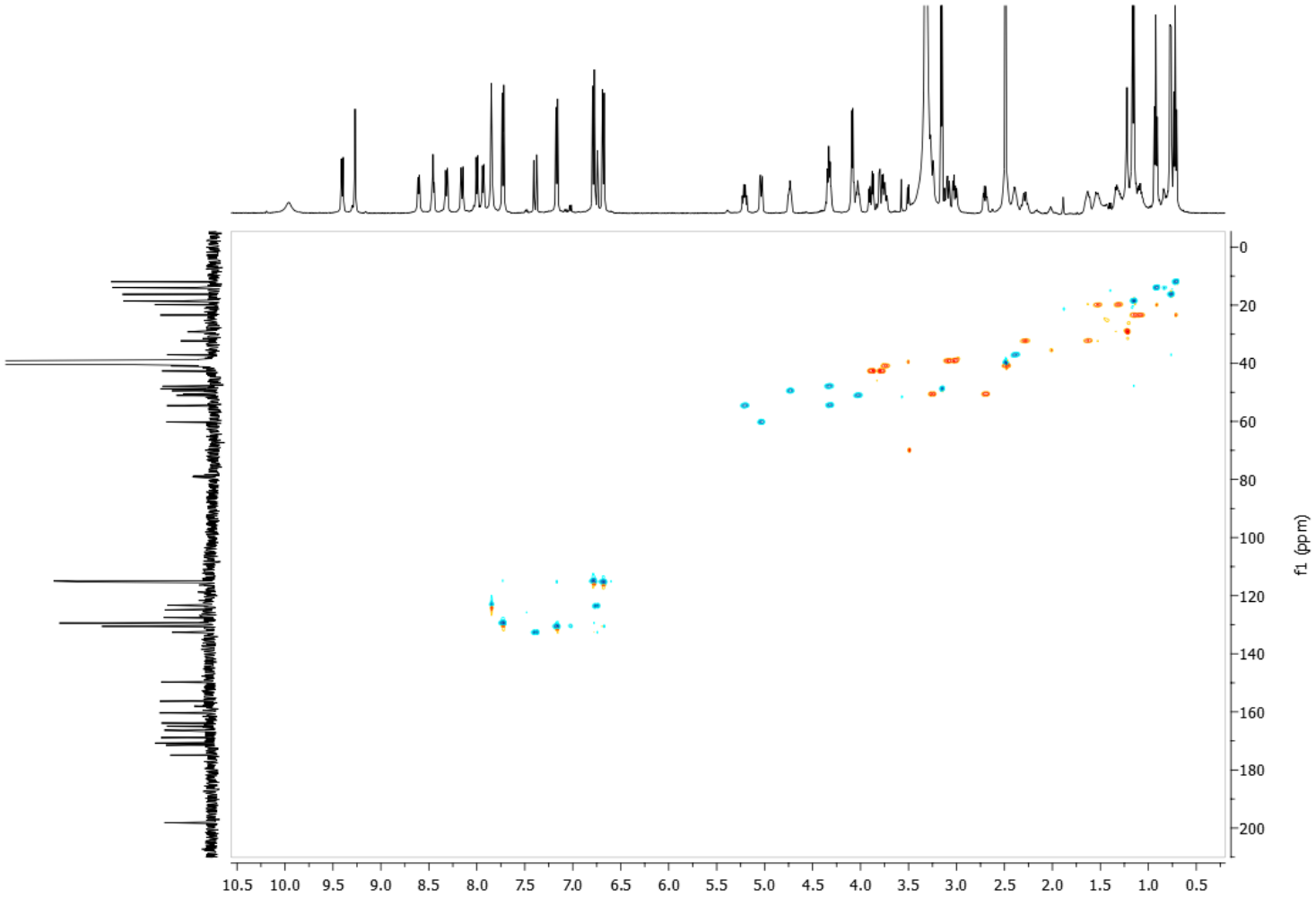


Figure S4. HMBC Spectrum of Cyclotheonellazole A (**1**) in DMSO-d_6

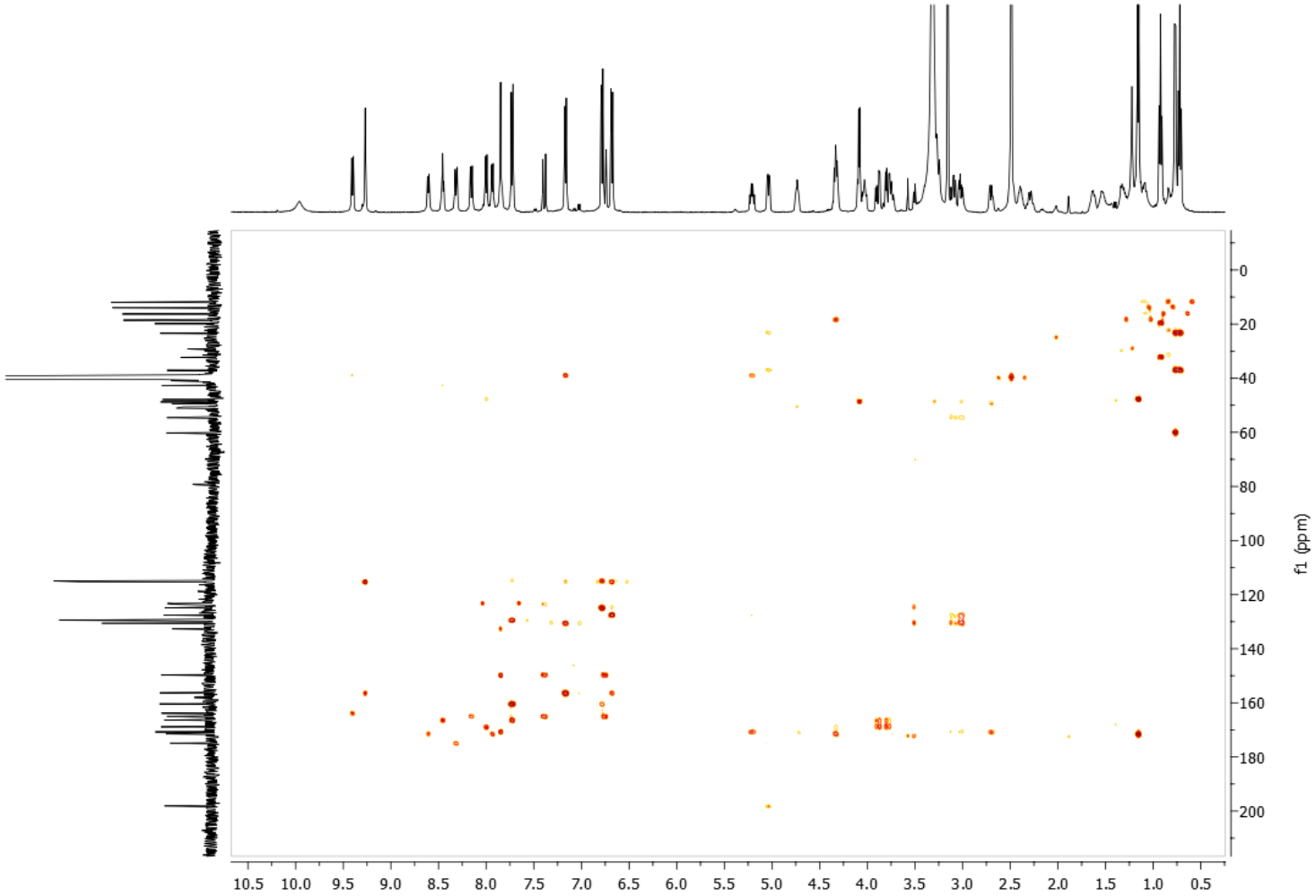


Figure S5. COSY Spectrum of Cyclotheonellazole A (**1**) in DMSO-d_6

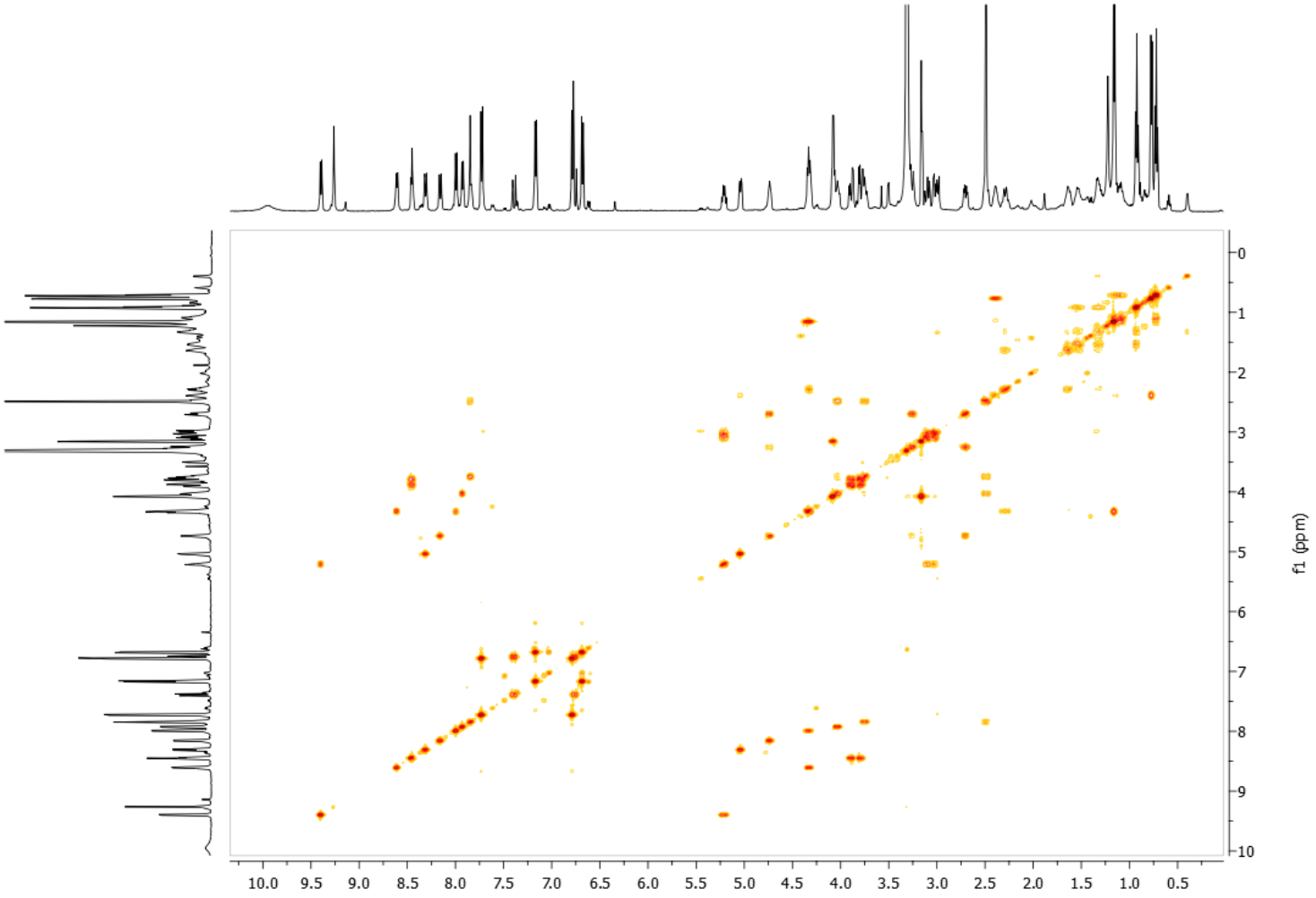


Figure S6. HR ESI MS of Cyclotheonellazole A (**1**)

Elemental Composition Report **Page 1**

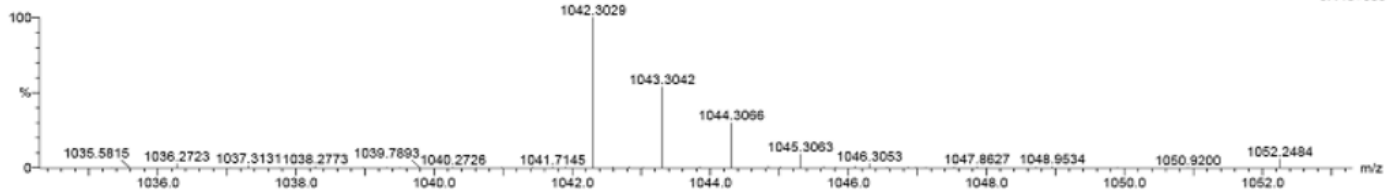
Single Mass Analysis
Tolerance = 1.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
932 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 40-50 H: 50-60 N: 5-15 O: 10-20 Na: 0-3 S: 1-3

MI-075/3
carmeli540b 2 (0.104) Cm (2)

Michal Issac

1: TOF MS ES+
3.14e+003



Minimum:				-1.5			
Maximum:		10.0	1.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1042.3029	1042.3028	0.1	0.1	27.5	36.8	3.8	C50 H52 N5 O18 S
	1042.3027	0.2	0.2	21.5	34.6	1.6	C44 H54 N9 O14 Na2 S2
	1042.3035	-0.6	-0.6	23.5	34.4	1.4	C43 H52 N11 O16 S2
	1042.3022	0.7	0.7	18.5	34.5	1.5	C42 H56 N7 O20 S2
	1042.3038	-0.9	-0.9	19.5	34.2	1.2	C45 H57 N5 O18 Na S2

Figure S7. ^{1}H-NMR Spectrum of Cyclotheonellazole B (**2**) in DMSO-d_6

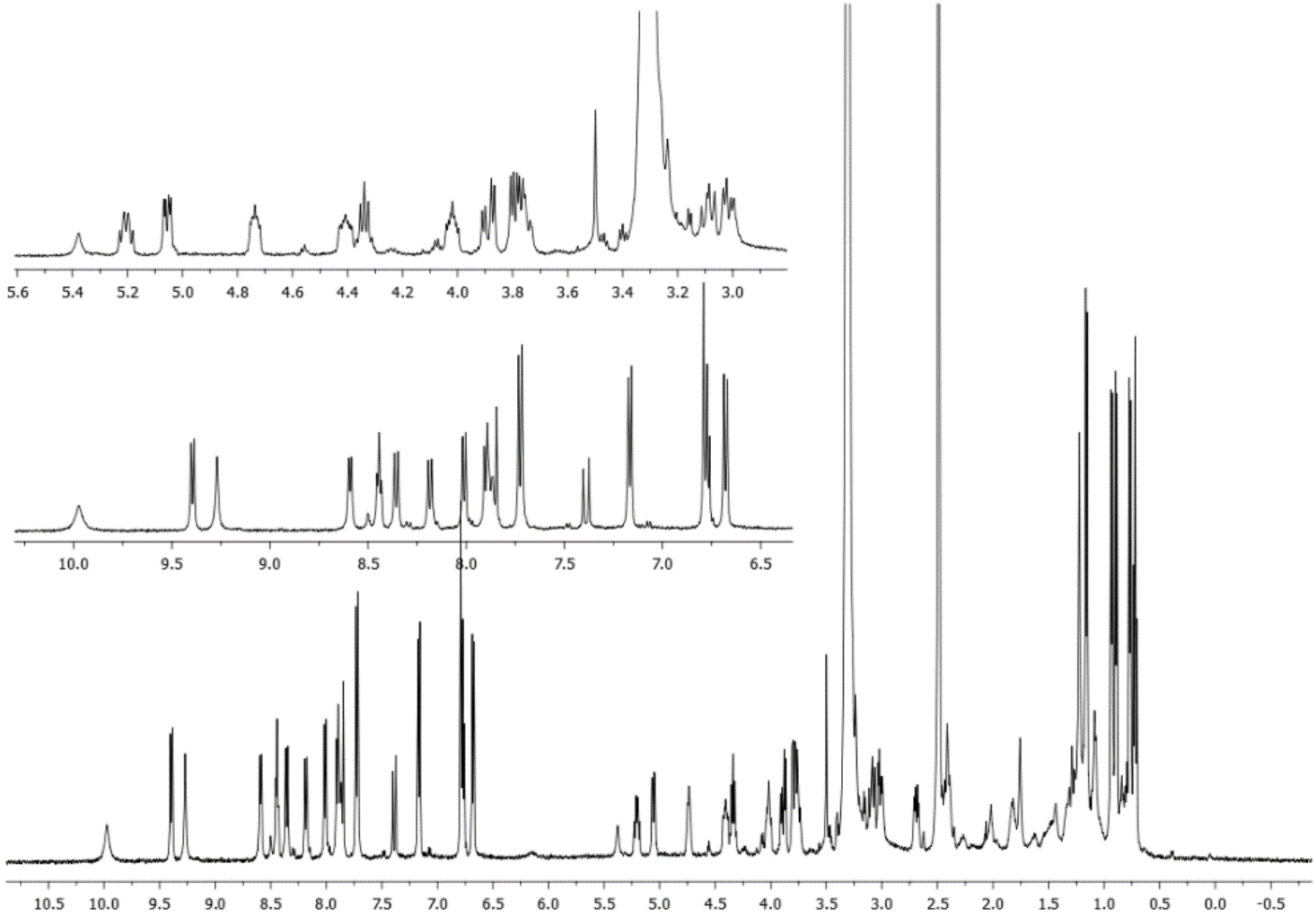


Figure S8. ^{13}C-NMR Spectrum of Cyclotheonellazole B (**2**) in DMSO-d_6

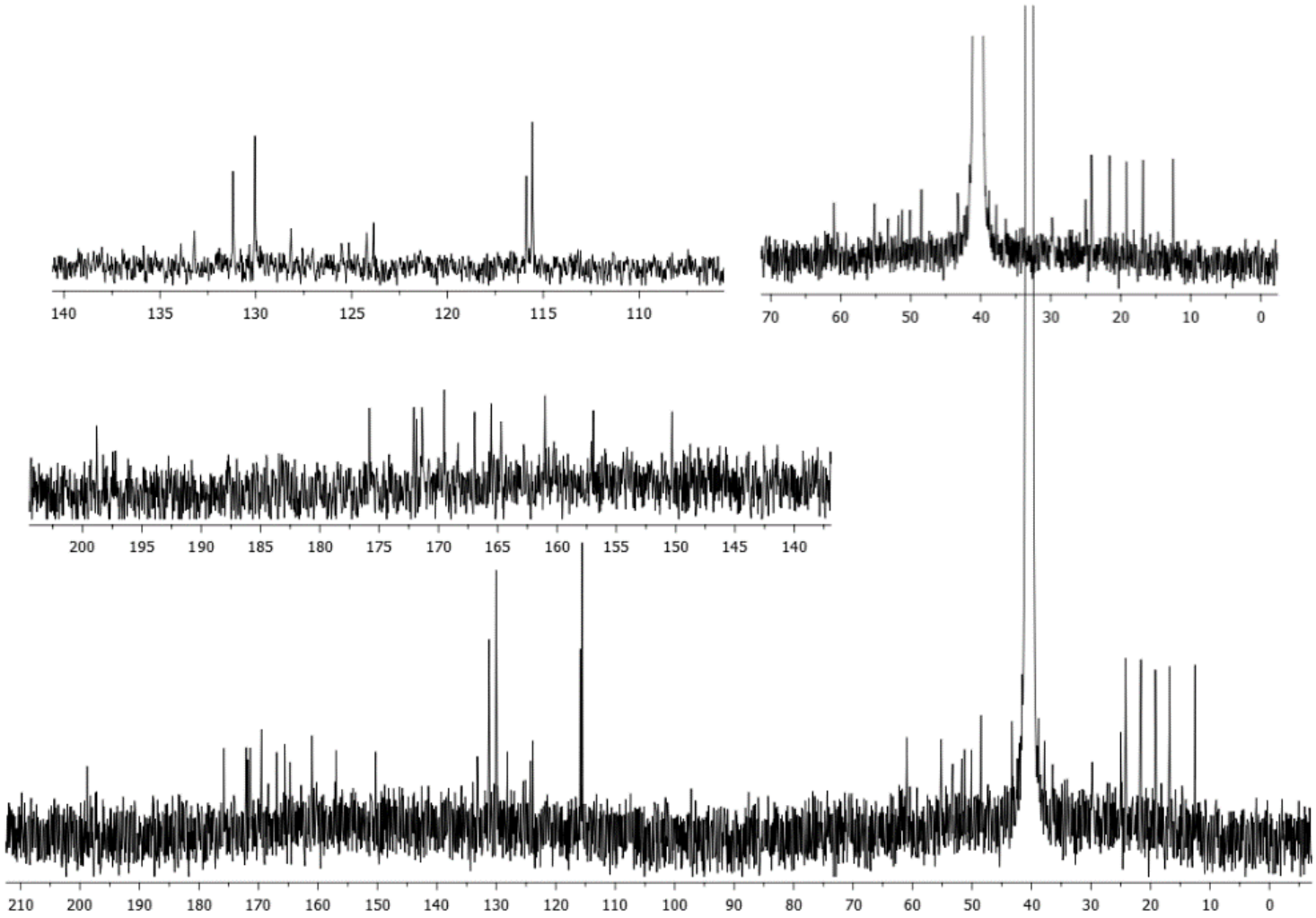


Figure S9. HSQC Spectrum of Cyclotheonellazole B (**2**) in DMSO-d_6

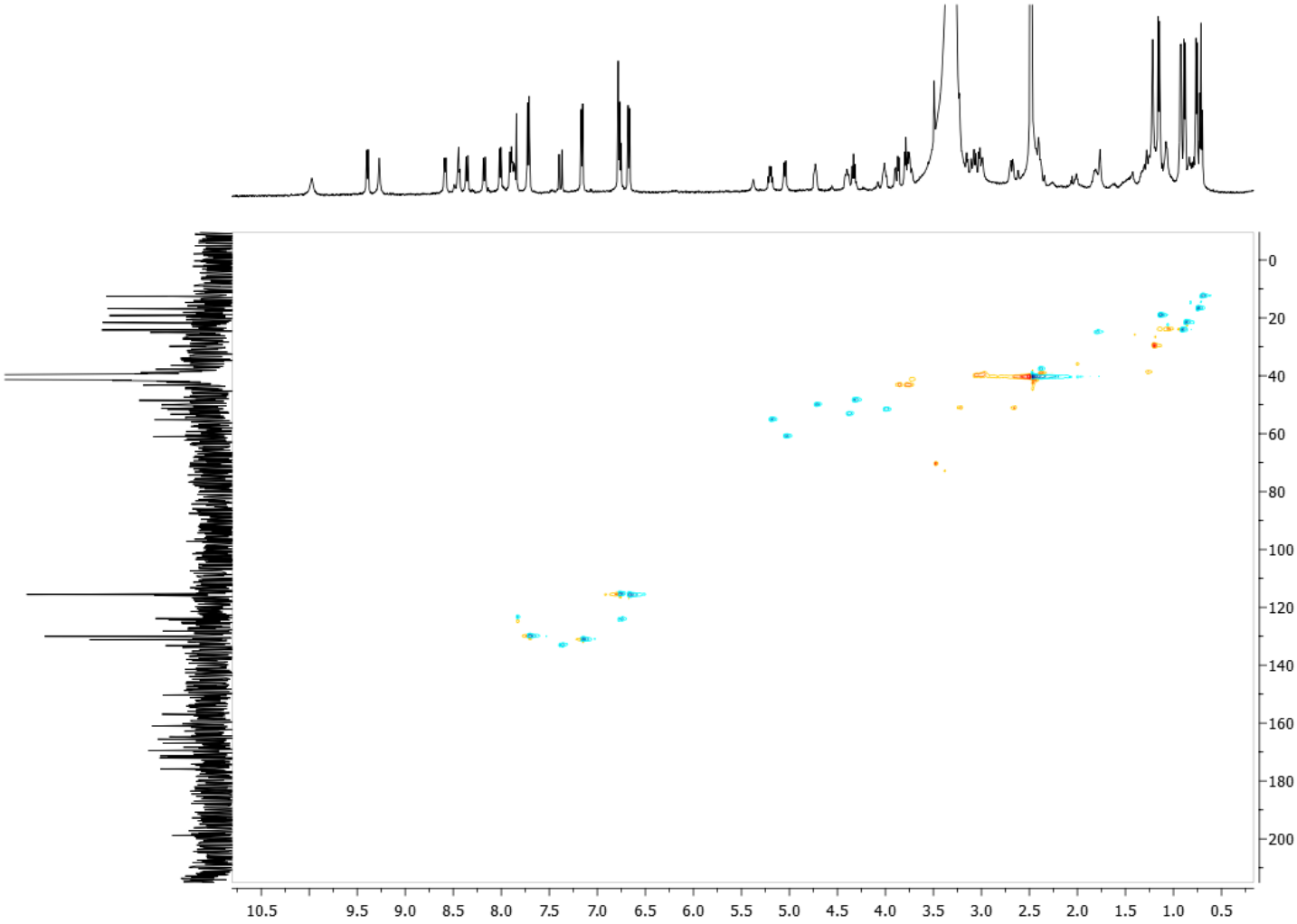


Figure S10. HMBC Spectrum of Cyclotheonellazole B (**2**) in DMSO-d_6

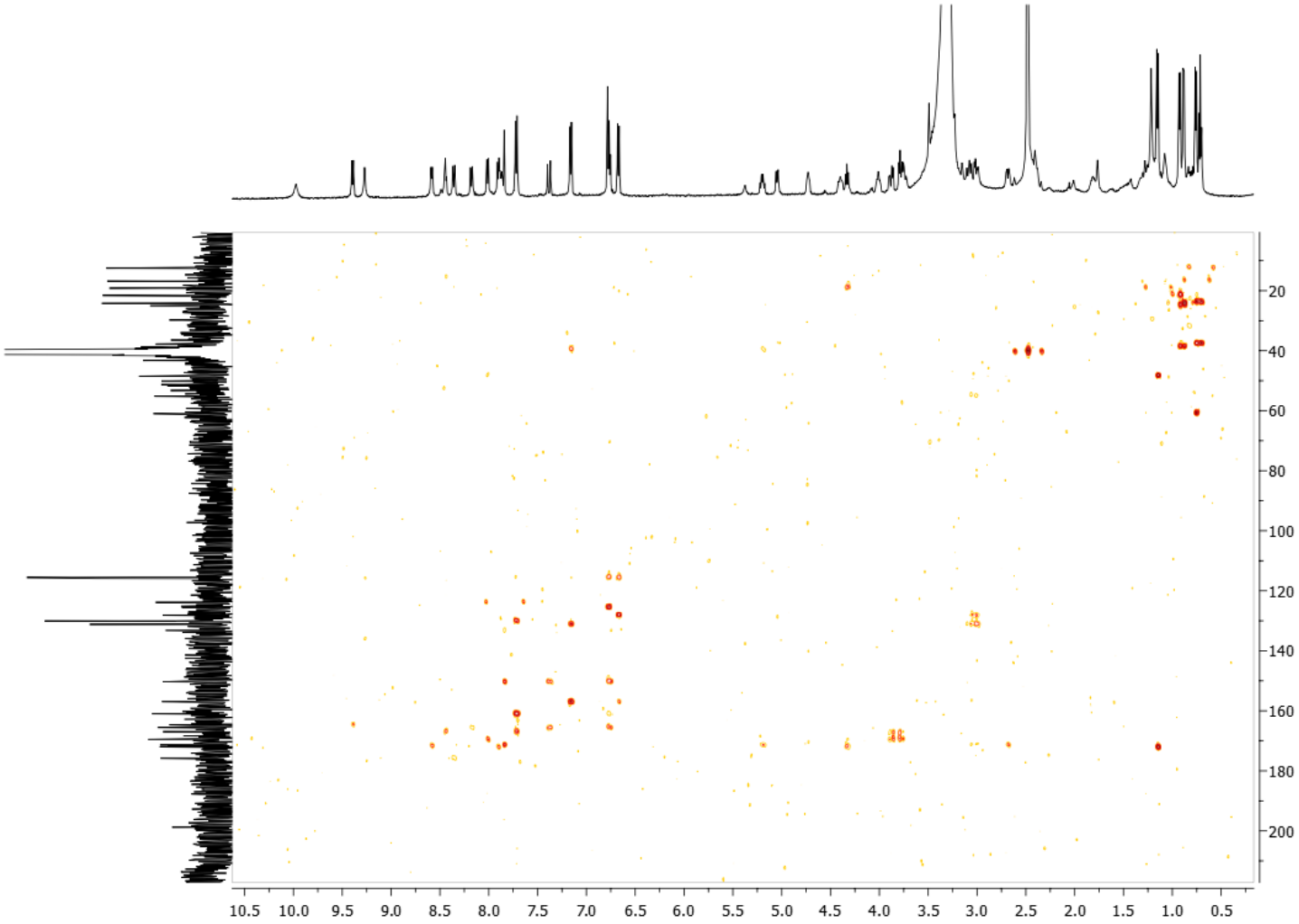


Figure S11. COSY Spectrum of Cyclotheonellazole B (**2**) in DMSO-d_6

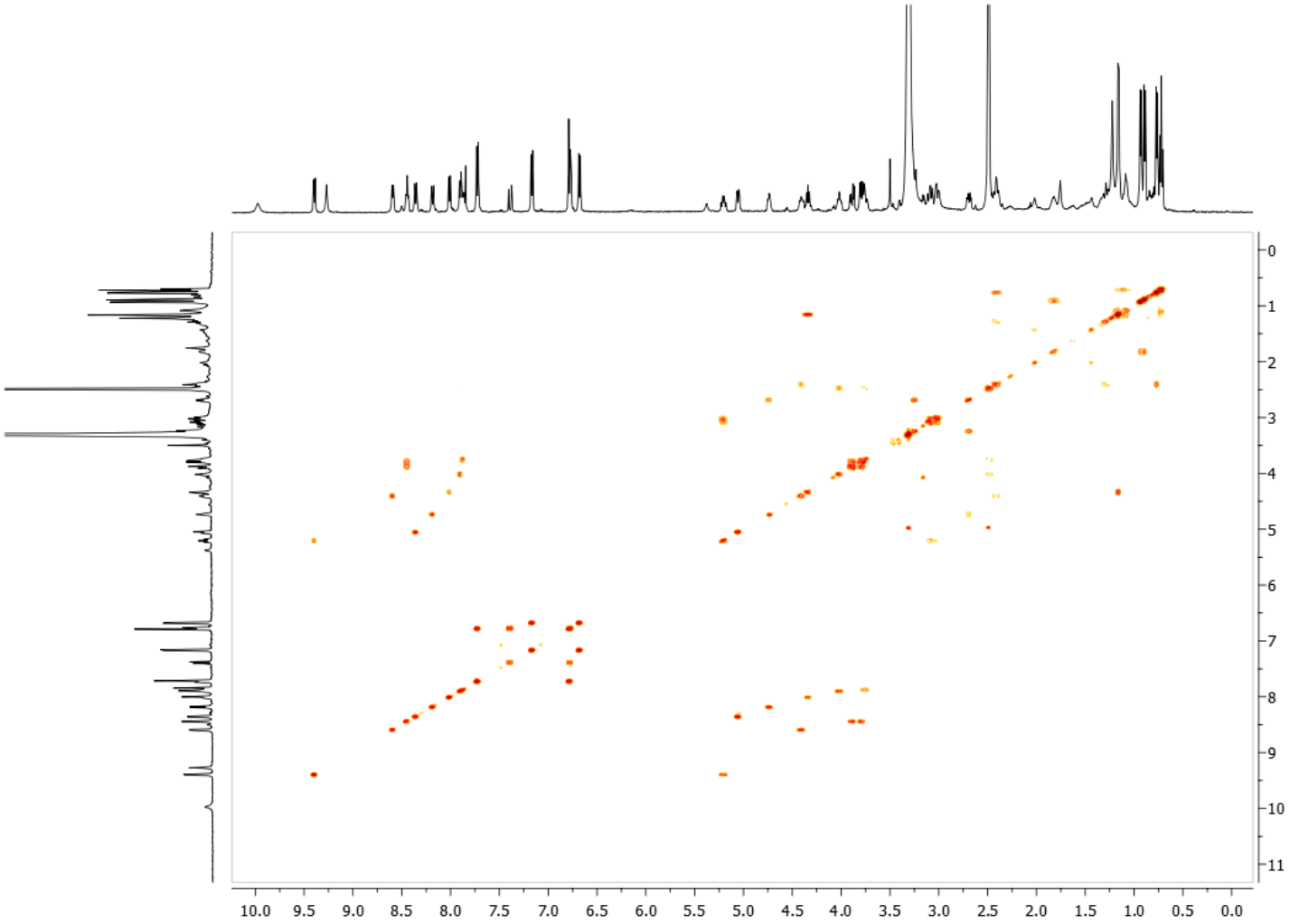


Table S1. NMR Data (DMSO-d_6) of Cyclotheonellazole B (**2**).[a]

Position	δ_C, type[b]	δ_H (*J* in Hz)	HMBC correlations[c]	NOE correlations[d]
Hbza 1	166.3, C		Hbza-3,3' Gly-2a,2b,NH	
2	124.9, C		Hbza-4,4'	
3,3'	129.4, CH	7.72, d (8.5)		Hbza-4,4', Gly-2a,2b,NH
4,4'	115.0, CH	6.78, d (8.5)		Hbza-3,3'
5	160.4, C		Hbza-3,3',4,4'	
5-OH		9.97 brs		
Gly 1	168.9, C		Gly-2a,2b, Ala-NH	
2	42.7, CH_2	3.78, dd (16.0,6.0)	Ala-2	Hbza-3,3', Gly-NH, Ala-NH
		3.88, dd (16.0,6.0)		Hbza-3,3', Gly-NH, Ala-NH
NH		8.45, t (6.0)		Hbza-3,3', Gly-2a,2b
Ala 1	172.1, C		Ala-2,3, Dpr-α-NH	
2	48.5, CH	4.33, dq (7.5,7.0)	Ala-3	Ala-3, Dpr-α-NH
3	19.2, CH_3	1.15, d (7.0)	Ala-2	Ala-2,NH
NH		8.01, d (7.5)		Ala-2, Gly-2a,2b

Dpr 1	171.2, C		Dpr-3b, Leu-NH	
2	51.2, CH	4.01, m		Dpr-3a,3b,α-NH, Leu-NH
3	41.0, CH_2	2.45, m		Dpr-2,3b,β-NH
		3.76, m		Dpr-2,3a,β-NH
α-NH		7.90, d (7.5)		Dpr-2, Ala-2
β-NH		7.84, brs		Dpr-3a,3b, Cya-2
Leu 1	175.2, C		Leu-2, Amoha-NH	
2	52.7, CH	4.40, m		Leu-3b,4,5,6,NH, Amoha-NH
3	38.2, CH_2	1.26, m	Leu-5,6	Leu-3b,NH
		2.38, m		Leu-2,3a,NH
4	24.4, CH	1.79, m	Leu-2,5	Leu-2,5
5	11.7, CH_3	0.92, d (6.5)	Leu-6	Leu-2,4
6	11.7, CH_3	0.88, d (6.5)	Leu-5	Leu-2
NH		8.58, d (7.0)		Dpr-2, Leu-2,3a,3b,
Amoha 1	164.1, C		Ptt-NH	
2	198.9, C			

3	60.4, CH	5.05, dd (9.5,3.5)	Amoha-5	Amoha-4,5,NH
4	38.1, CH	2.39, m	Amoha-5,7	Amoha-3,5
5	16.2, CH_3	0.76, d (6.5)		Amoha-3,4,NH
6	23.5, CH_2	1.07, m	Amoha-5,7	
		1.15, m		
7	11.9, CH_3	0.71, t (7.5)		
NH		8.36, d (9.5)		Amoha-3,5, Leu-2
Ptt 1	165.0, C		Ptt-2,3, Cya-NH	
2	123.6, CH	6.76, d (15.0)	Ptt-3	Ptt-3, Cya-NH
3	132.6, CH	7.38, d (15.0)	Ptt-5	Ptt-2,5
4	149.7, C		Ptt-2,3,5	
5	123.2, CH	7.84, brs		Ptt-3
6	170.8, C		Ptt-5,7	
7	54.6, CH	5.20, q (7.5)	Ptt-8a,8b	Ptt-8a,8b,10,10',NH
8	39.1, CH_2	3.01, m	Ptt-10,10'	Ptt-7,10,10',11,11',NH
		3.08, m		Ptt-7,10,10',11,11',NH
9	127.6, C		Ptt-11,11'	

10,10’	130.6, CH	7.16, d (8.0)	Ptt-8a,8b	Ptt-7,8a,8b,11,11’
11,11’	115.3, CH	6.67, d (8.0)	Ptt-10,10’, 12-OH	Ptt-8a,8b,10,10’
12	156.3, C		Ptt-10,10’,11,11’	
12-OH		9.27, s		
NH		9.39, d (8.0)		Ptt-7,8a,8b,
Cya 1	170.9, C		Cya-2,3a	
2	49.5, CH	4.73, m		Cya-3a,3b,NH, Drp-β-NH
3	50.7, CH_2	2.68, dd (13.5,5.5)		Cya-2,3b,NH
		3.25, m		Cya-2,3a
NH		8.18, d (9.0)		Cya-2,3a, Ptt-2

[a]500 MHz for ^{1}H, 100 MHz for ^{13}C. [b]Multiplicity and assignment from HSQC experiment. [c]Determined from HMBC experiment, $^{n}J_{CH}$=8 Hz, recycle time 1s, are from carbon(s) stated to the indicated proton(s). [d]Selected NOE’s from ROESY experiment.

Figure S12. HR ESI MS of Cyclotheonellazole B (**2**)

Elemental Composition Report **Page 1**

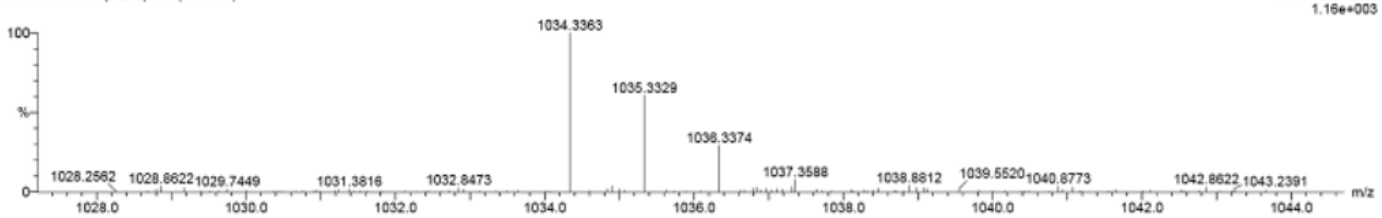
Single Mass Analysis
Tolerance = 1.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
880 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 40-50 H: 55-65 N: 4-14 O: 10-20 Na: 0-1 S: 0-5

MI-075/4
carmeli538 141 (6.208) Cm (141:143)

Michal Issac

1: TOF MS ES+
1.16e+003



Minimum:				-1.5			
Maximum:		10.0	1.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1034.3363	1034.3364	-0.1	-0.1	21.5	73.8	0.1	C45 H57 N9 O14 Na S2
	1034.3359	0.4	0.4	15.5	76.8	3.1	C45 H65 N5 O13 Na S4
	1034.3357	0.6	0.6	19.5	76.3	2.6	C43 H60 N11 O11 S4

Figure S13. ^{1}H-NMR Spectrum of Cyclotheonellazole C (**3**) in DMSO-d_6

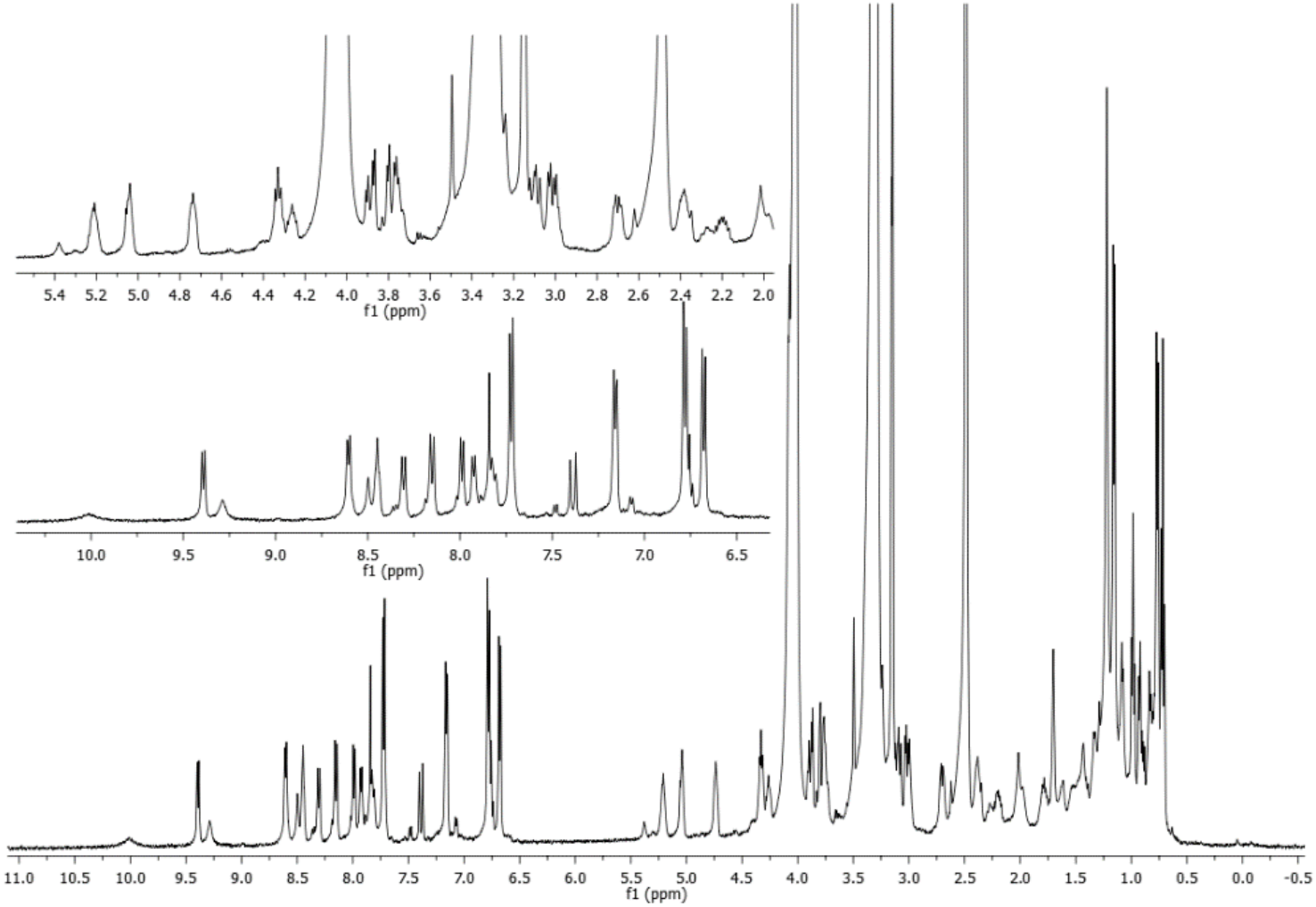


Figure S14. ^{13}C-NMR Spectrum of Cyclotheonellazole C (**3**) in DMSO-d_6

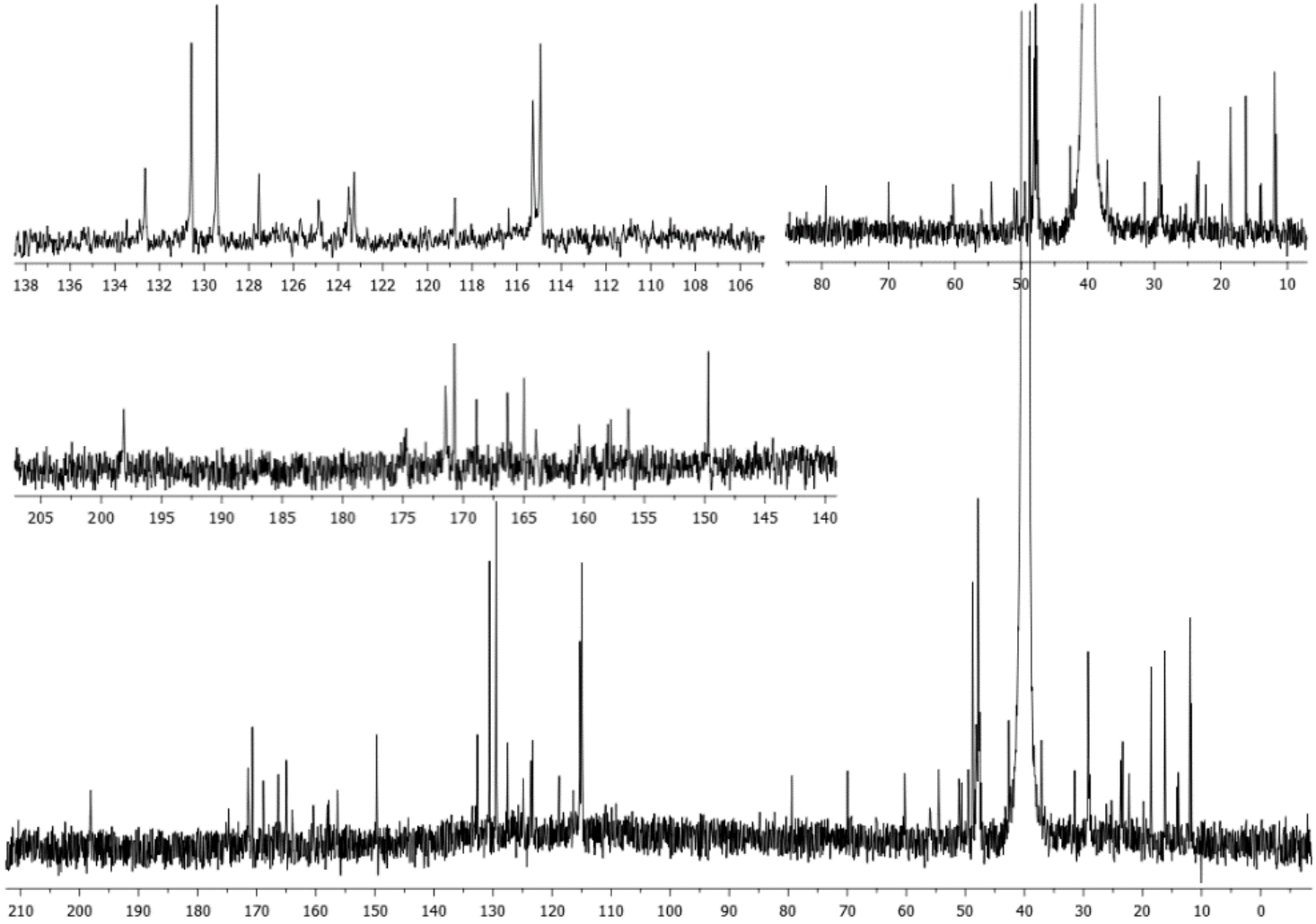


Figure S15. HSQC Spectrum of Cyclotheonellazole C (**3**) in DMSO-d_6

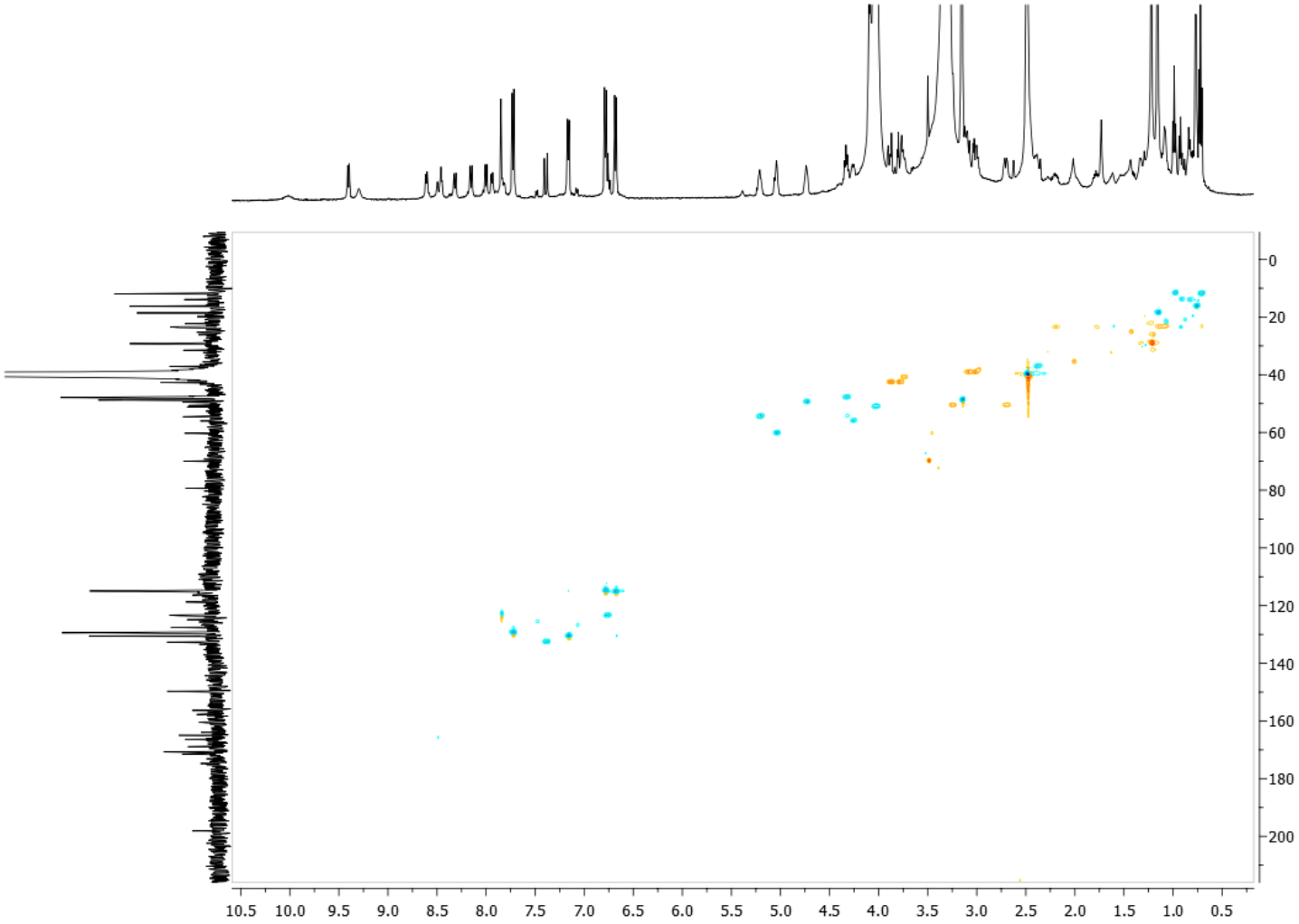


Figure S16. HMBC Spectrum of Cyclotheonellazole C (**3**) in DMSO-d_6

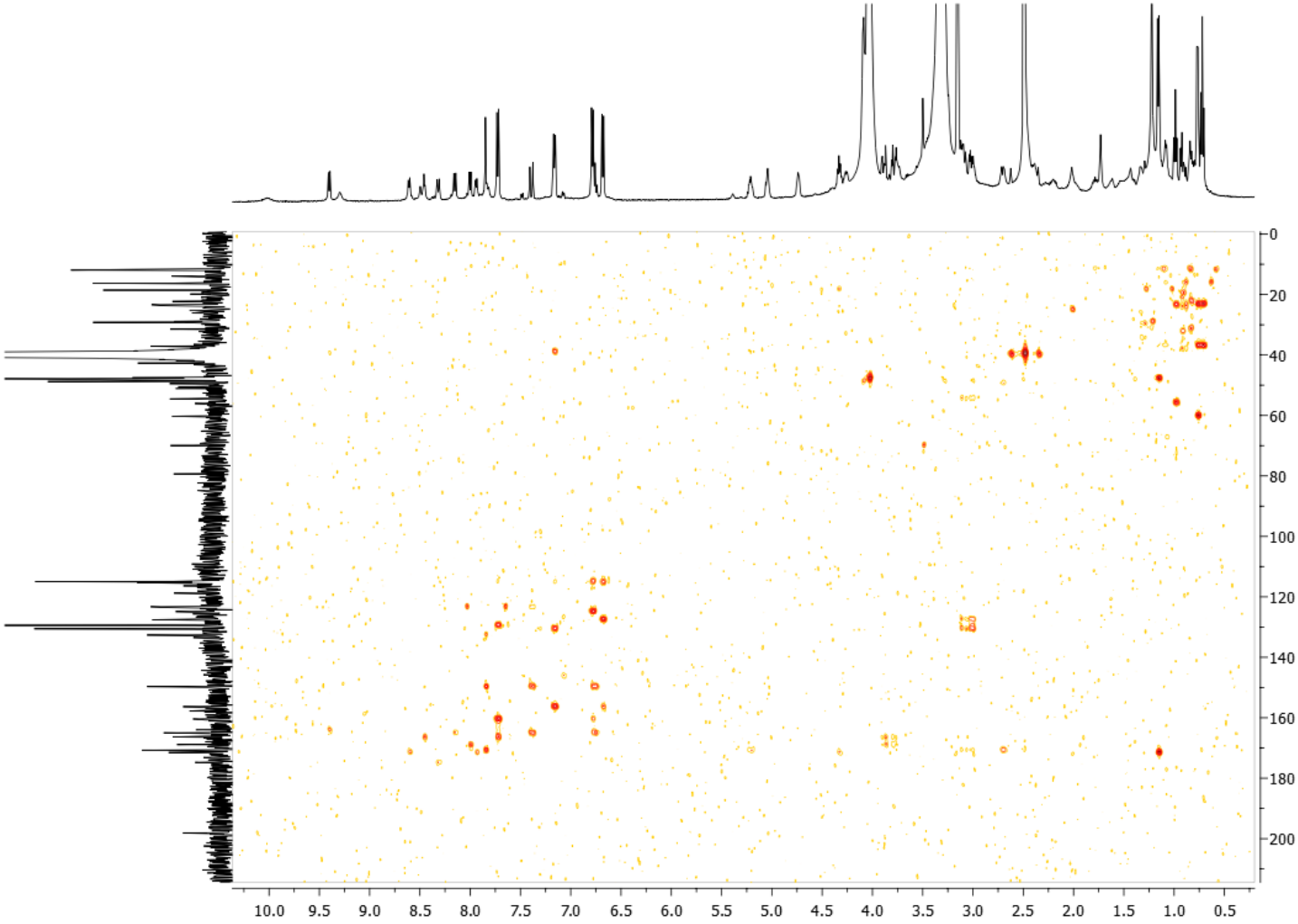


Figure S17. COSY Spectrum of Cyclotheonellazole C (**3**) in DMSO-d_6

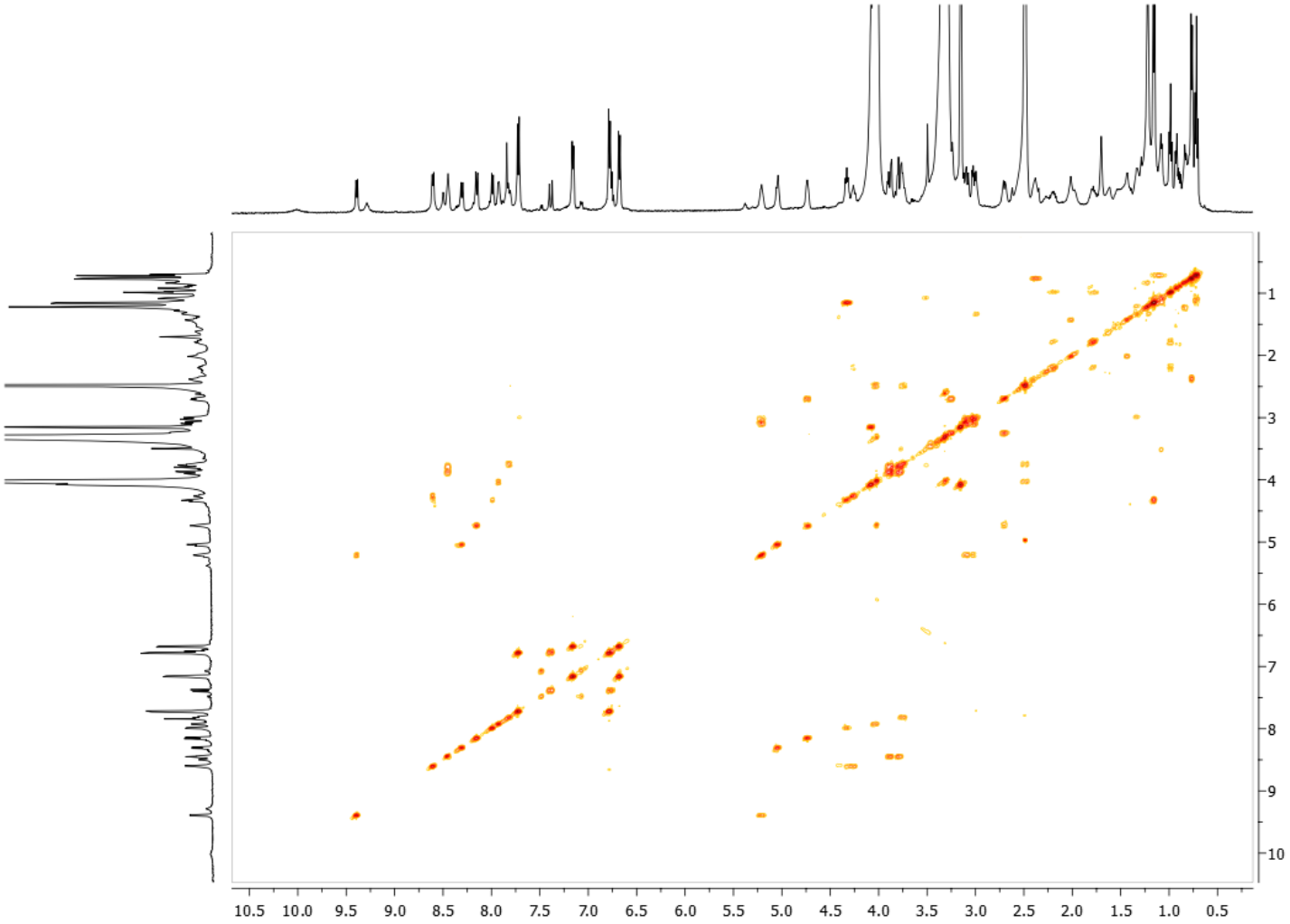


Table S2. NMR Data (DMSO-d_6) of Cyclotheonaellazole C (**3**).[a]

Position	δ_C, type[b]	δ_H (J in Hz)	HMBC correlations[c]	NOE correlations[d]
Hbza 1	166.4, C		Hbza-3,3' Gly-NH	
2	124.9, C		Hbza-4,4'	
3,3'	129.4, CH	7.73, d (8.5)		Hbza-4,4', Gly-NH,
4,4'	114.9, CH	6.78, d (8.5)		Hbza-3,3'
5	160.4, C		Hbza-3,3',4,4'	
5-OH		9.95 brs		
Gly 1	168.9, C		Gly-2a,2b, Ala-NH	
2	42.7, CH_2	3.78, m		Gly-NH, Ala-NH
		3.89, m		
NH		8.46, t (5.5)		Hbza-3,3', Gly-2a,2b
Ala 1	171.5, C		Ala-2,3, Dpr-α-NH	
2	47.7, CH	4.33, dq (7.5,7.0)	Ala-3	Ala-3,NH
3	18.5, CH_3	1.16, d (7.0)	Ala-2	Ala-2,NH
NH		8.00, d (7.5)		Ala-2,3, Gly-2a,2b

Dpr 1	171.4, C		hAla-NH	
2	51.1, CH	4.03, m		Dpr-β-NH, hAla-NH
3	40.6, CH_2	2.46, m	hAla-2	Dpr-3b,β-NH
		3.77, m		Dpr-3a,α-NH
α-NH		7.94, d (7.5)		Dpr-3b, hAla-2
β-NH		7.83, brs		Dpr-2,3a, Cya-NH
hAla 1	174.8, C		Amoha-NH	
2	55.9, CH	4.27, m	hAla-4	hAla-3a,4, Dpr-α-NH, Amoha-NH
3	23.3, CH_2	1.78, m	hAla-4	hAla-2,3b,4
		2.19, m		hAla-3a,4,NH
4	11.7, CH_3	0.99, t (7.0)		hAla-2,3a,3b,NH
NH		8.61, d (7.5)		hAla-3b,4, Dpr-2,3b
Amoha 1	163.9, C		Ptt-NH	
2	198.1, C			
3	60.2, CH	5.04, m	Amoha-5	Amoha-4,5,NH
4	37.1, CH	2.39, m	Amoha-5,7	Amoha-3,7

5	16.3, CH_3	0.77, d (6.5)	Amoha-6a	Amoha-3,6a,6b
6	23.4, CH_2	1.08, m	Amoha-5,7	Amoha-5,7
		1.14, m		Amoha-5,7
7	11.9, CH_3	0.72, t (7.5)	Amoha-6a	Amoha-4,6a,6b
NH		8.32, d (9.5)		Amoha-3, hAla-2
Ptt 1	165.0, C		Ptt-2,3, Cya-NH	
2	123.5, CH	6.76, m		Ptt-3, Cya-NH
3	132.7, CH	7.39, d (15.0)	Ptt-5	Ptt-2,5, Cya-NH
4	149.7, C		Ptt-3,5	
5	123.3, CH	7.85, brs	Ptt-3	Ptt-3
6	170.7, C		Ptt-5,7	
7	54.5, CH	5.21, m	Ptt-8a,8b	Ptt-8a,8b,10,10',NH
8	39.2, CH_2	3.02, dd (14.0,6.0)	Ptt-10,10'	Ptt-7,10,10',NH
		3.09, m		Ptt-7,10,10',NH
9	127.6, C		Ptt-8a,8b,11,11'	
10,10'	130.6, CH	7.16, d (8.5)		Ptt-7,8a,8b,11,11'
11,11'	115.3, CH	6.68, d (8.5)	Ptt-10,10'	Ptt-10,10'

12	156.3, C		Ptt-10,10',11,11'	
12-OH		9.26, s		
NH		9.40, d (8.0)		Ptt-7,8a,8b,
Cya 1	170.7, C		Cya-3a	
2	49.5, CH	4.74, m	Cya-3a	Cya-3a,3b,NH
3	50.7, CH_2	2.70, dd (14.0,6.0)		Cya-2,3b
		3.25, m		Cya-2,3a
NH		8.15, d (9.5)		Cya-2, Dpr-β-NH, Ptt-2,3

[a]500 MHz for ^{1}H, 100 MHz for ^{13}C. [b]Multiplicity and assignment from HSQC experiment. [c]Determined from HMBC experiment, $^{n}J_{CH}$=8 Hz, recycle time 1s, are from carbon(s) stated to the indicated proton(s). [d]Selected NOE's from ROESY experiment.

Figure S18. HR ESI MS of Cyclotheonellazole C (**3**)

Elemental Composition Report **Page 1**

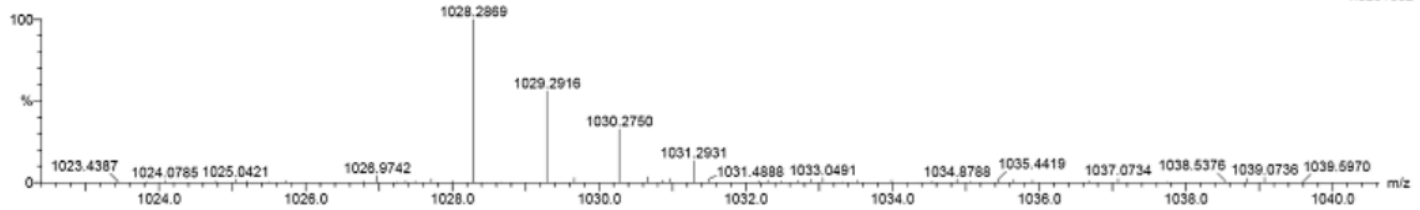
Single Mass Analysis
Tolerance = 1.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
948 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 40-50 H: 50-60 N: 5-15 O: 10-20 Na: 0-3 S: 1-3

MI-075/2
carmeli541 98 (4.313) Cm (98)

Michal Issac

1: TOF MS ES+
4.82e+002



Minimum:				-1.5			
Maximum:		10.0	1.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1028.2869	1028.2871	-0.2	-0.2	21.5	26.4	1.2	C43 H52 N9 O14 Na2 S2
	1028.2872	-0.3	-0.3	27.5	27.7	2.4	C49 H50 N5 O18 S
	1028.2865	0.4	0.4	18.5	26.5	1.2	C41 H54 N7 O20 S2
	1028.2878	-0.9	-0.9	23.5	26.5	1.2	C42 H50 N11 O16 S2