Supporting Information

Biosynthetic Multitasking Facilitates Thalassospiramide Structural Diversity in Marine Bacteria

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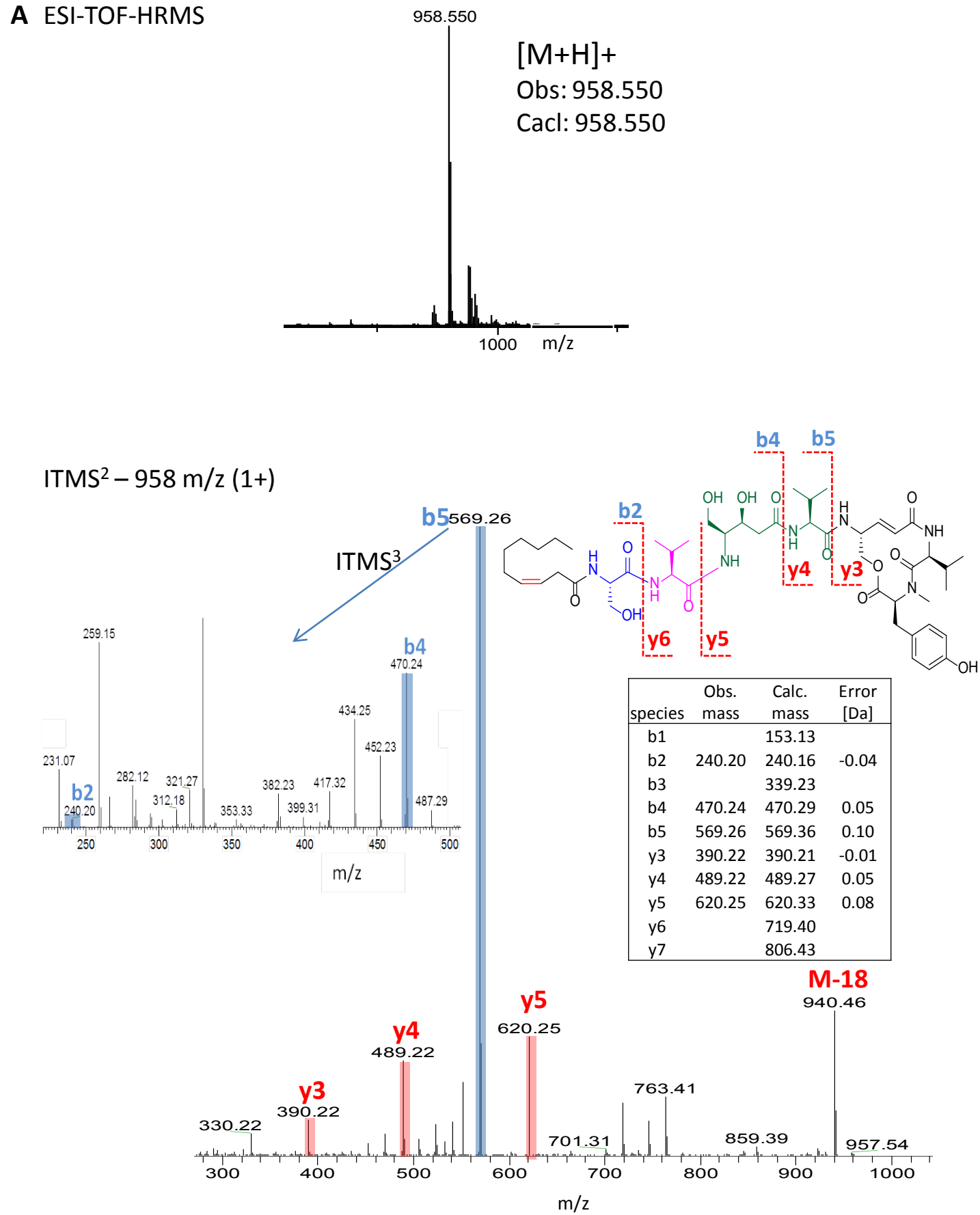


Figure S1. Characterization of thalassospiramide A (1). (**A**) MS^n analysis.

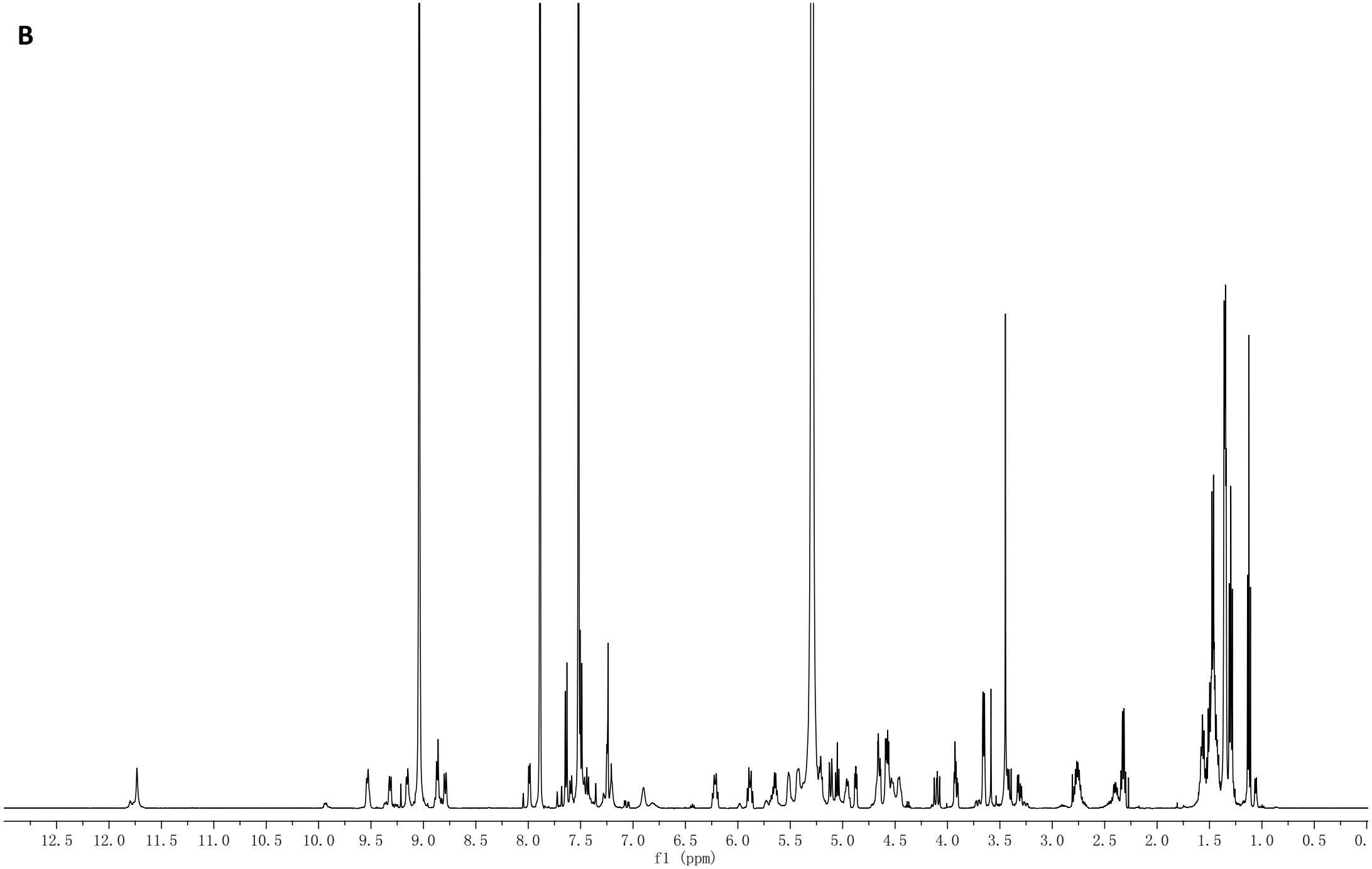


Figure S1. Characterization of thalassospiramide A (1). (**B**) ^{1}H spectrum in pyridine-d_5.

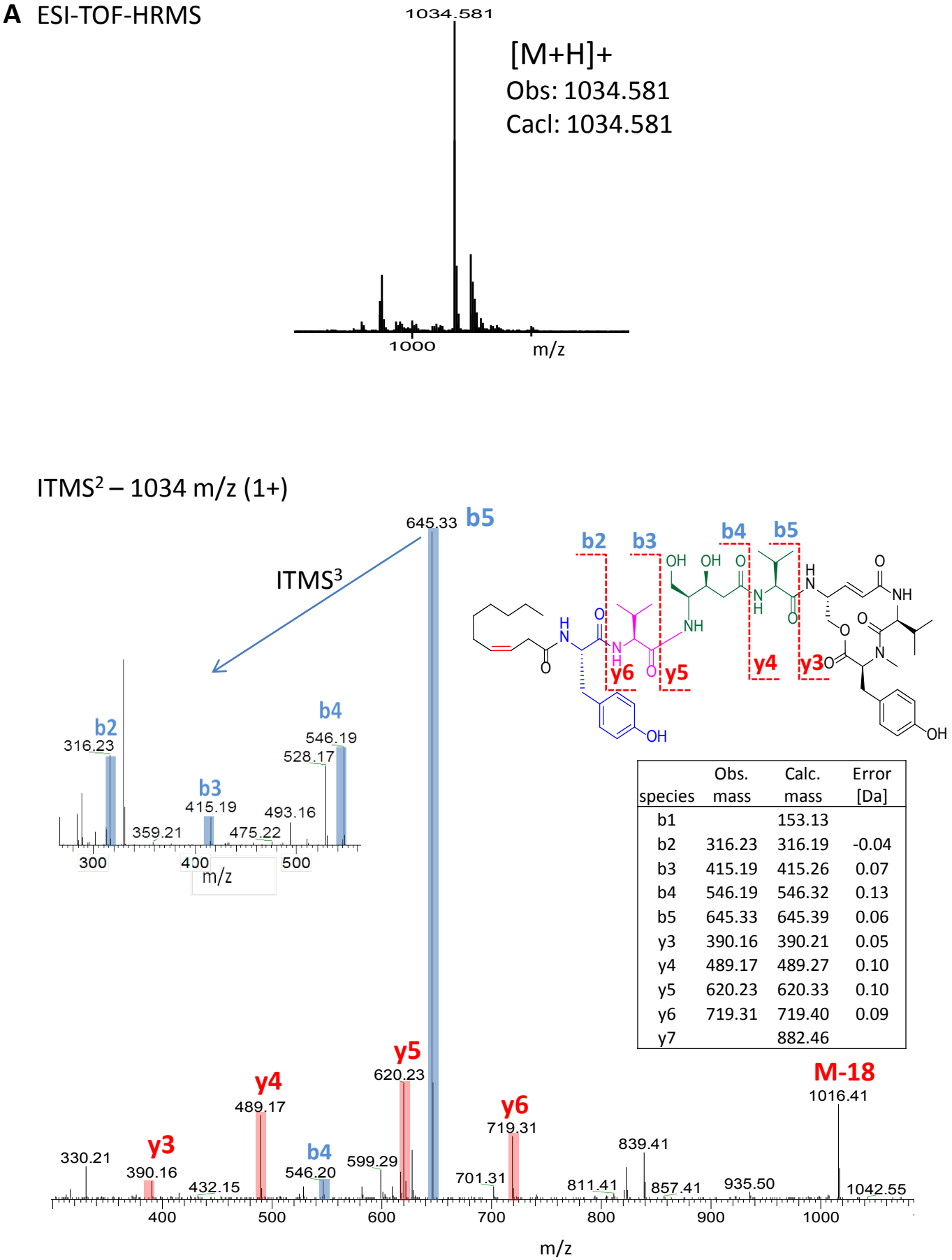


Figure S2. Characterization of thalassospiramide A1 (3). (**A**) MS^n analysis.

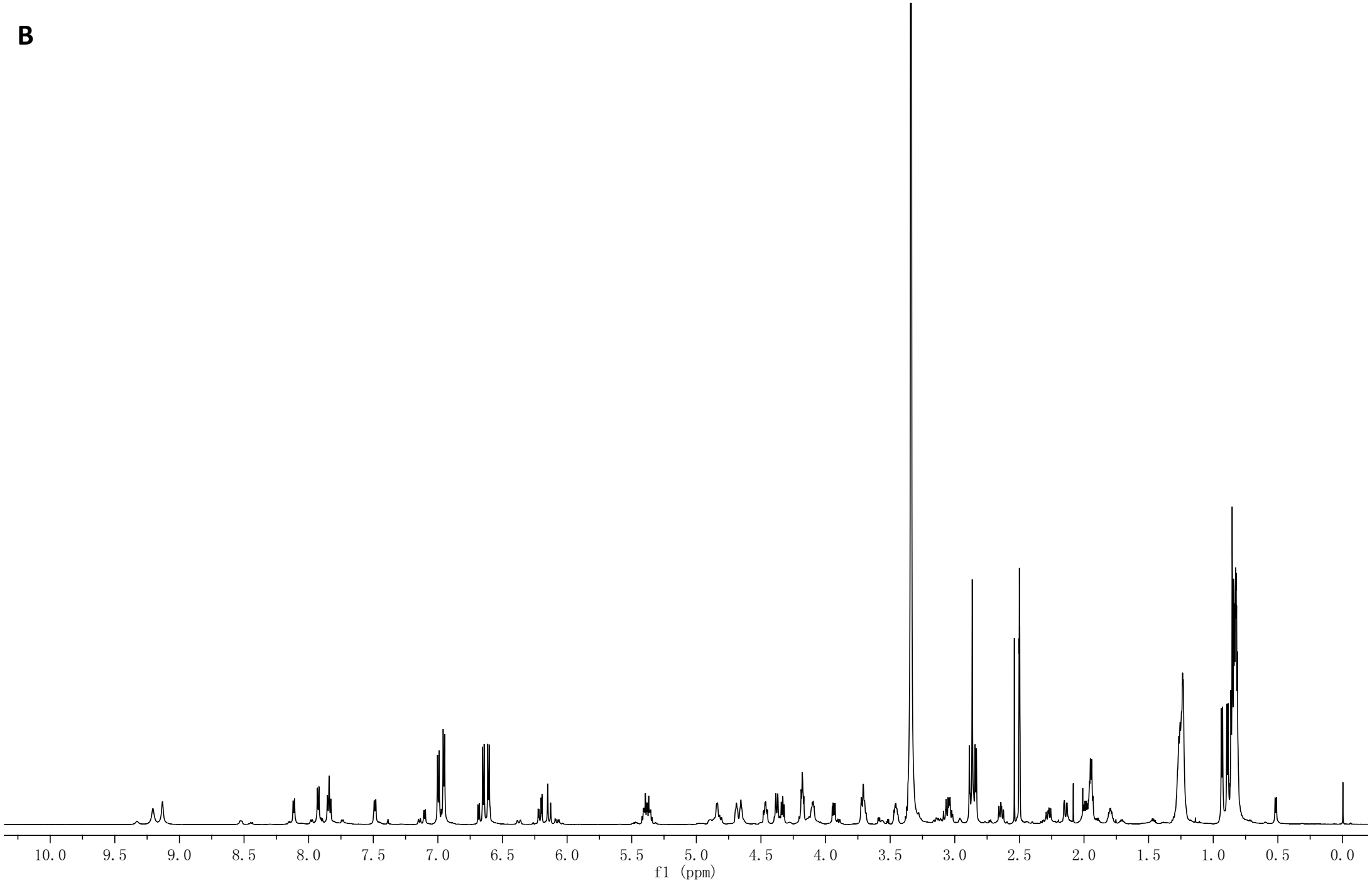


Figure S2. Characterization of thalassospiramide A1 (3). (**B**) ^{1}H spectrum in pyridine-d_5.

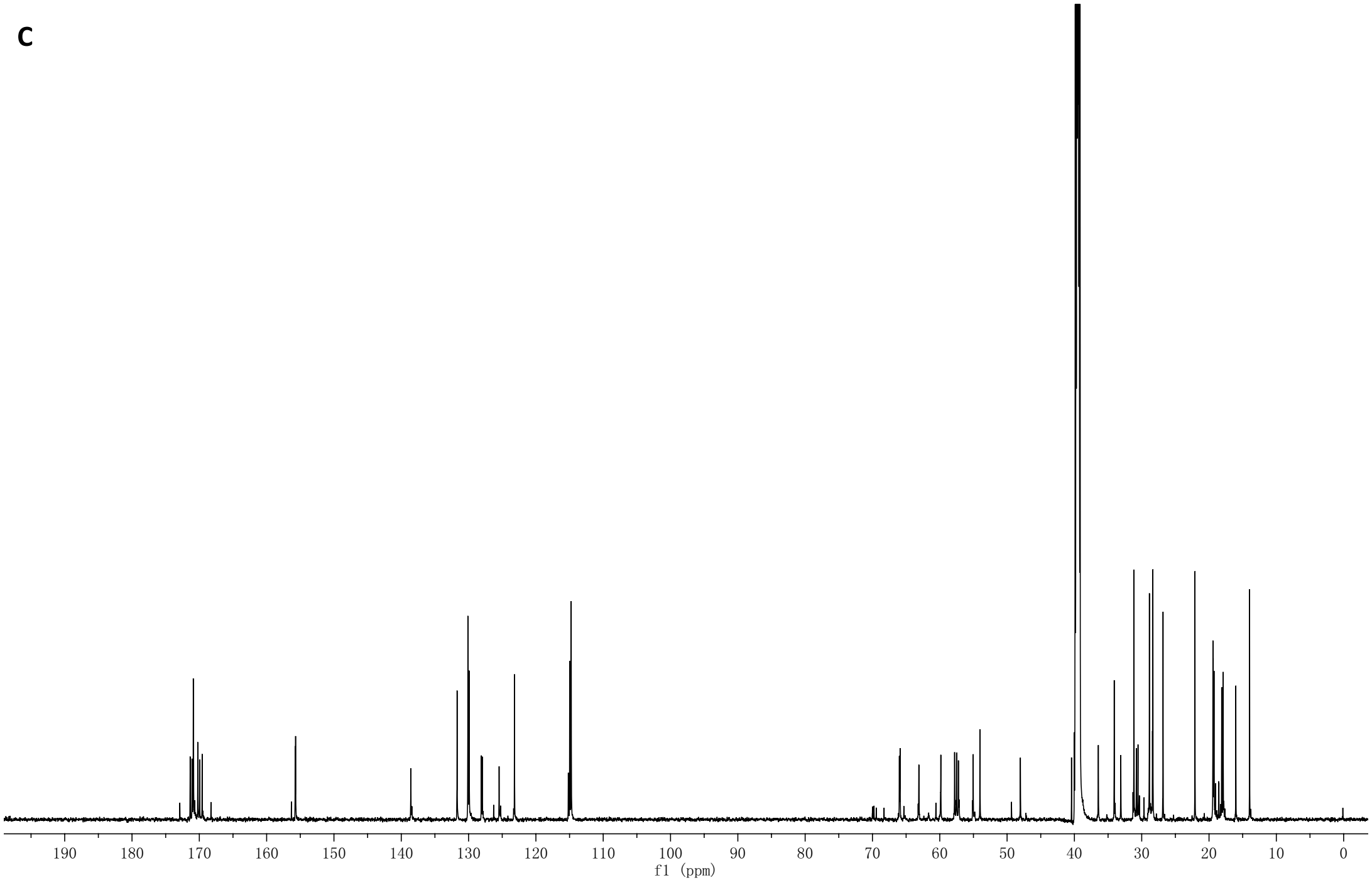


Figure S2. Characterization of thalassospiramide A1 (3). (C) ^{13}C spectrum in pyridine-d_5.

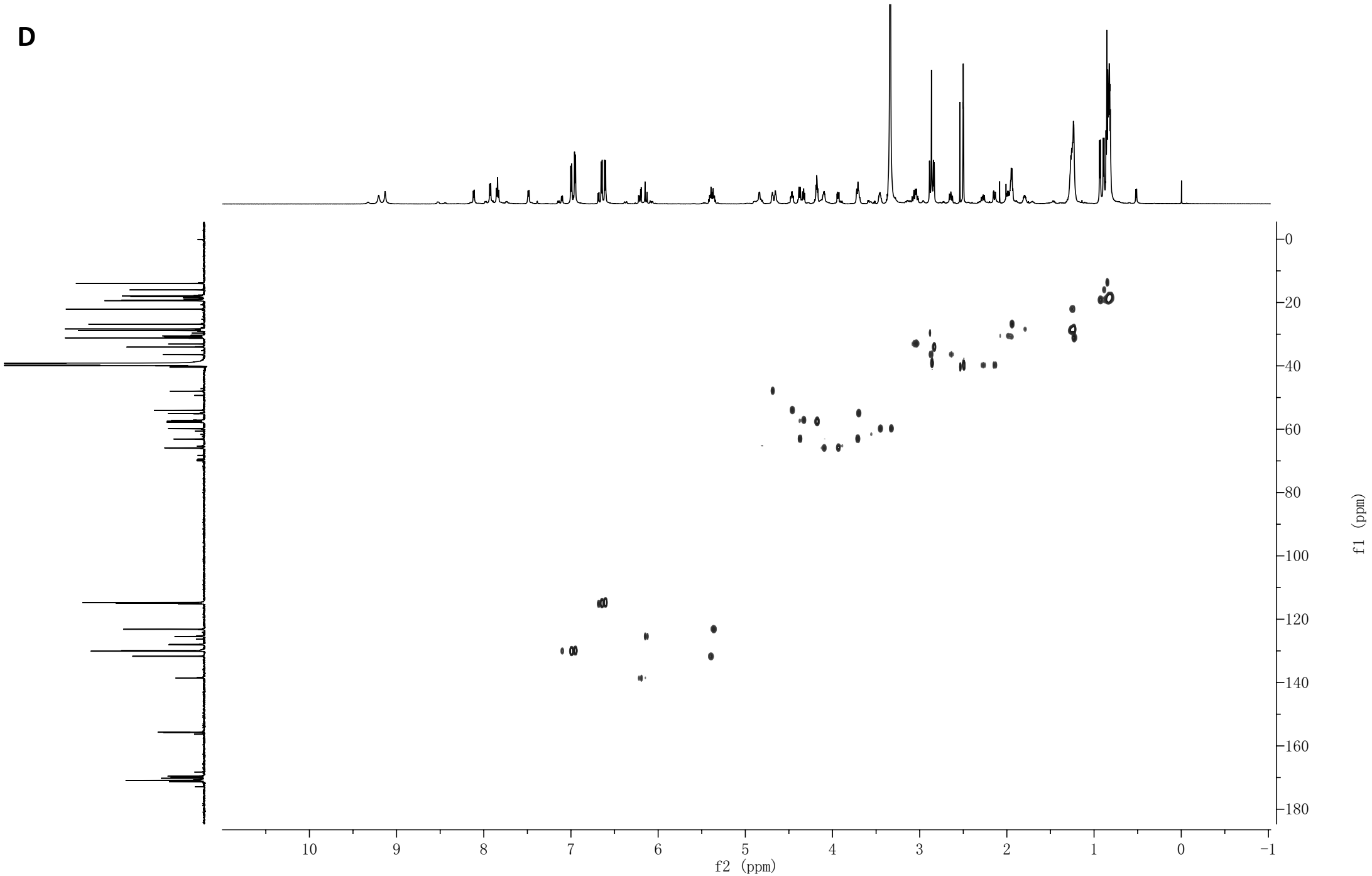


Figure S2. Characterization of thalassospiramide A1 (3). (**D**) HSQC spectrum in pyridine-d_5.

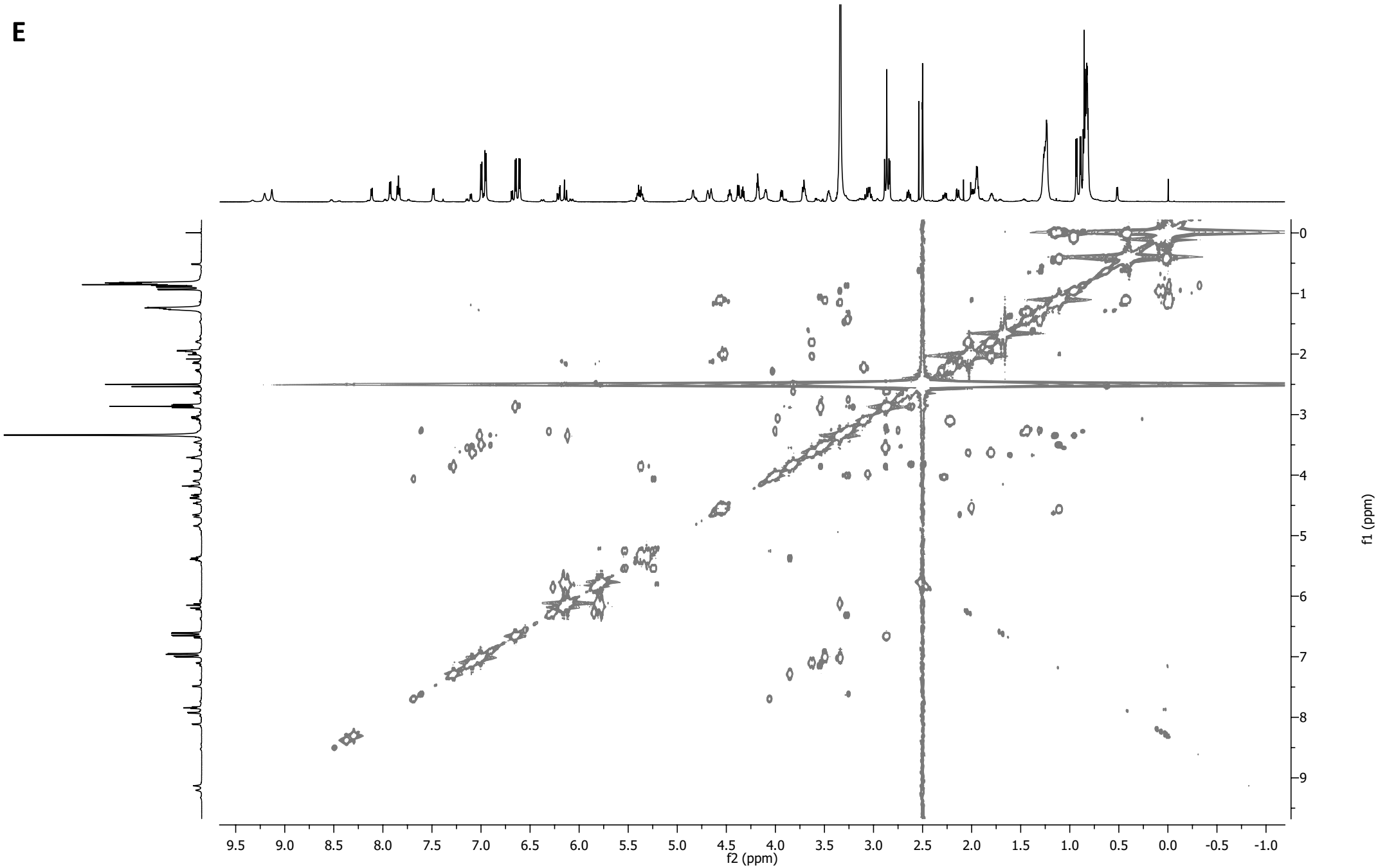


Figure S2. Characterization of thalassospiramide A1 (3). (**E**) ^{1}H-^{1}H COSY spectrum in pyridine-d_5.

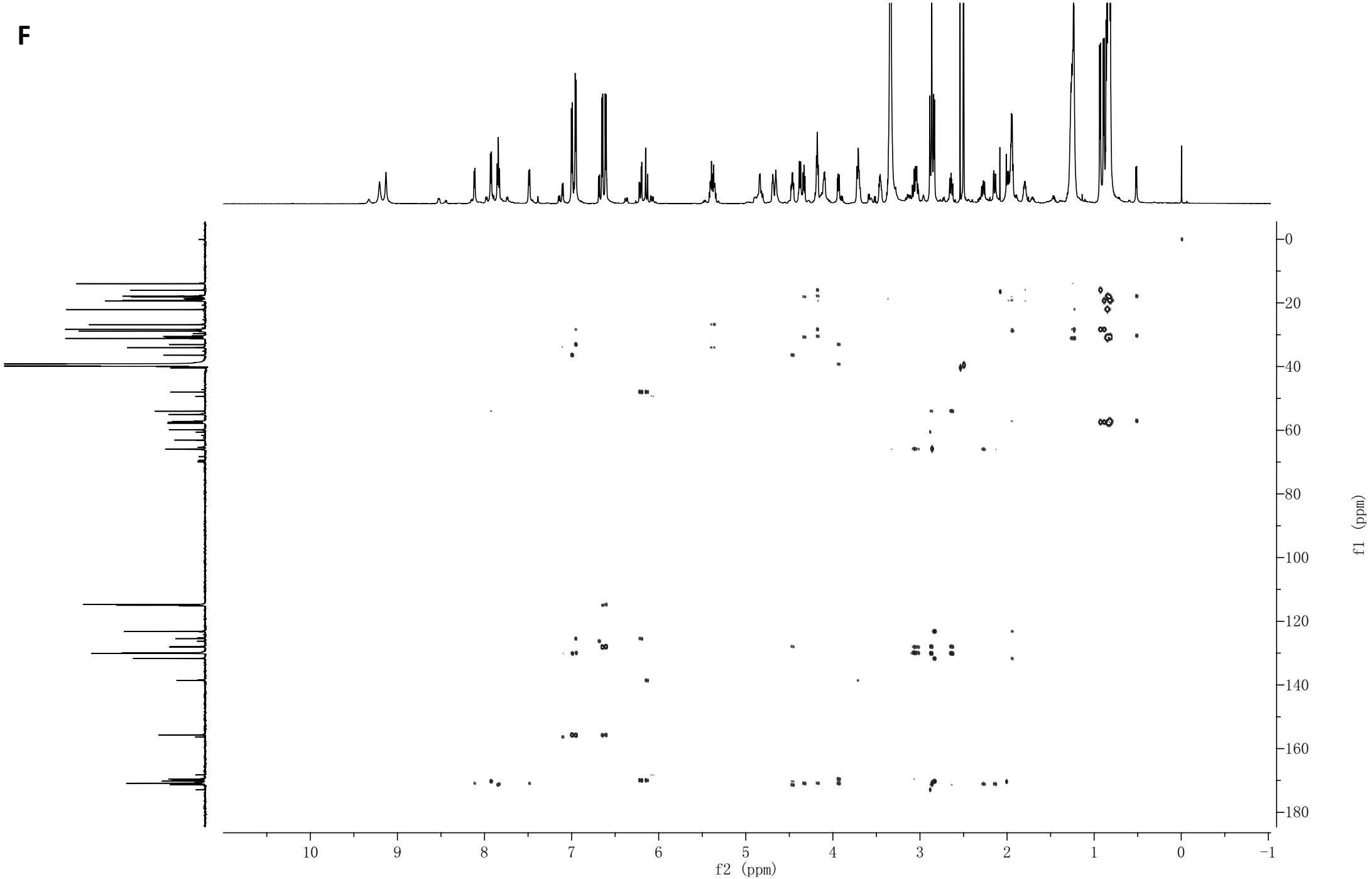
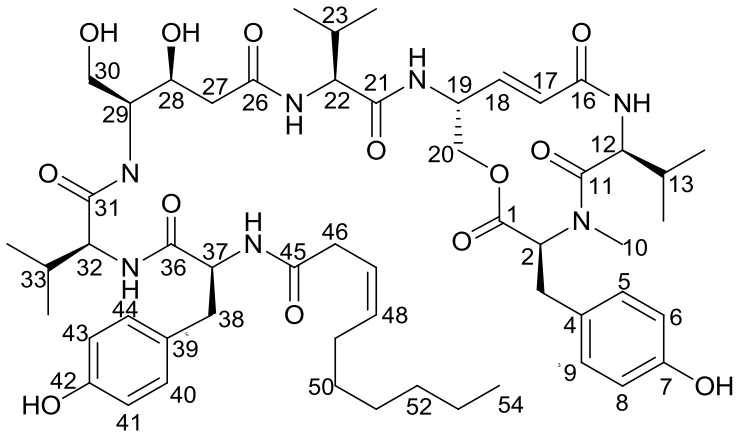


Figure S2. Characterization of thalassospiramide A1. (**F**) HMBC spectrum in pyridine-d_5.

G



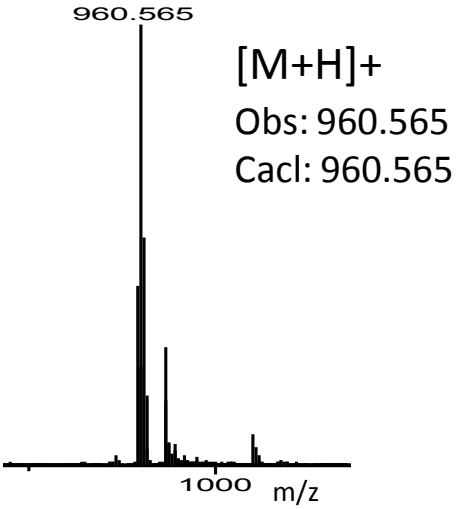
NMR Spectral Data (700 MHz (^{1}H), 175 MHz (^{13}C) in DMSO-d_6) of thalassospiramide A1 (**3**)

C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1				C	
2	3.93	dd(10.6,5.0)	65.9	CH	1,3,4,10
3a	3.07	dd(14.0,10.7)	33.1	CH_2	
3b	3.03	dd(13.8,4.7)			
4			128	C	
5	6.95	d(8.4)	129.9	CH	3,6,7
6	6.65	d(8.4)	115	CH	4,7
7			155.8	C	
7-OH	6.68	d(8.4)			6,7,8
8	6.65	d(8.4)	115	CH	4,7
9	6.95	d(8.4)	129.9	CH	3,7,8
10	2.86	s	40	CH_3	
11			170.9	C	
12	4.18	m	57.4	CH	11,13,15
12-NH	6.96	d(7.0)			12,13,16,17
13	1.8	m	28.4	CH	
14	0.93	d(6.9)	19.4	CH_3	
15	0.89	d(6.9)	16	CH_3	
16			170	C	
17	6.14	d(15.8)	125.5	CH	
18	6.21	dd(15.8,4.9)	138.6	CH	
19	4.69	m	48.4	CH	
19-NH	8.12	d(7.3)			19,20,21
20a	4.38	d(10.5)	63.1	CH_2	
20b	3.71	m			
21			170.9	C	
22	4.18	m	57.7	CH	
22-NH	7.83	d(9.3)			22,23,26
23	1.99	m	30.5	CH	
24	0.84	m	19.4	CH_3	
25	0.84	m	18.1	CH_3	
26			171.1	C	
27a	2.28	dd(14.4,9.8)	40.4	CH_2	
27b	2.15	dd(14.3,2.7)			
28	4.1	m	66.1	CH	

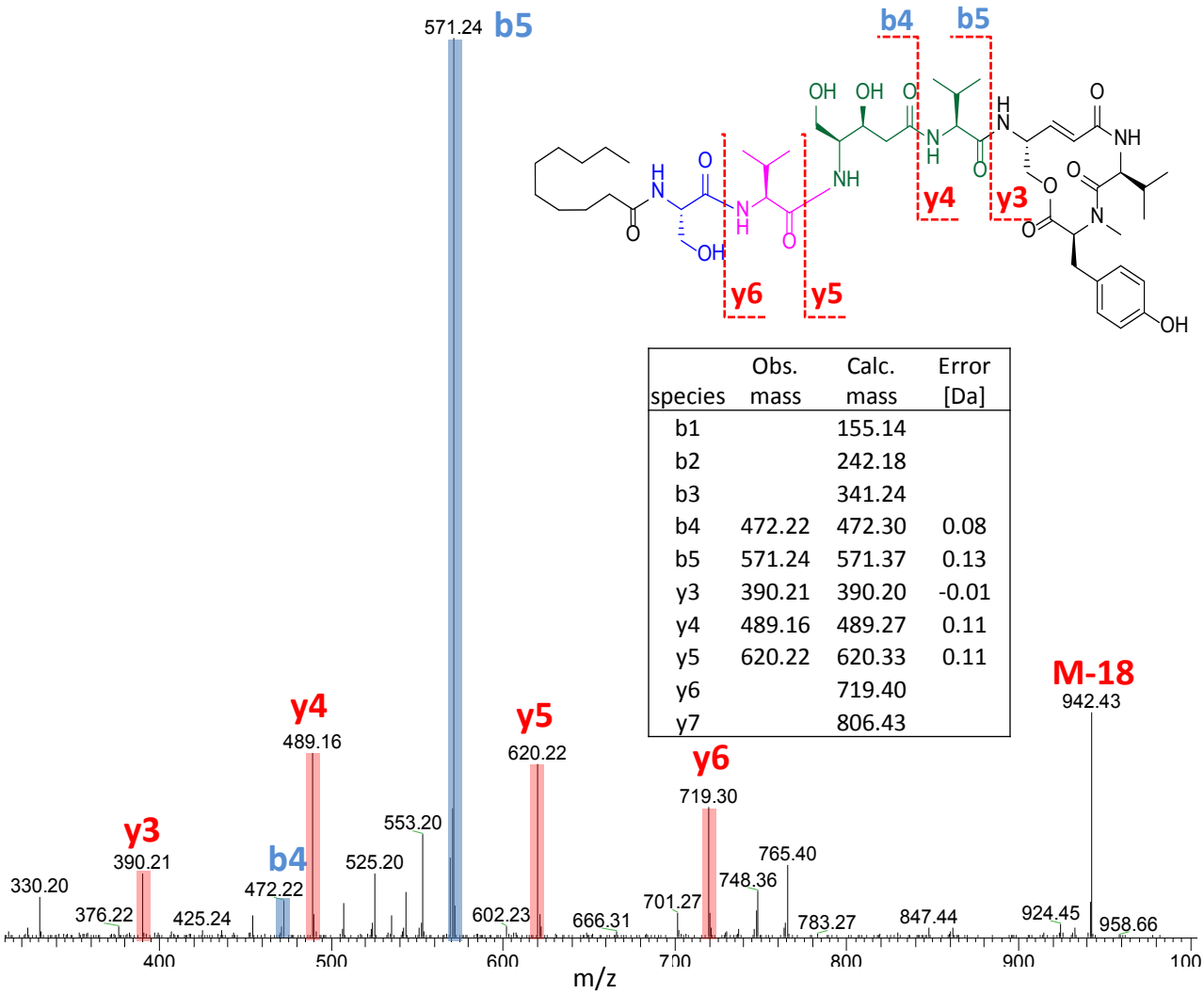
28-OH	4.84				27,28,29
29	3.7	m	55	CH	
29-NH	7.49	d(8.5)			28,29,30,31
30a	3.33	m	59.9	CH_2	
30b	3.46	m			
30-OH	4.66				29,30
31			170.9	C	
32	4.33	dd(8.6,7.3)	57.1	CH	
32-NH	7.85	d(4.8)			32,33,36
33	1.96	m	30.8	CH	
34	0.84	m	19.3	CH_3	
35	0.84	m	17.9	CH_3	
36			171.4	C	
37	4.47	td(9.1,4.1)	54	CH	
37-NH	7.93	d(8.2)			37,38,45
38a	2.64	dd(13.8,10.0)	36.5	CH_2	
38b	2.89	m			
39			127.7		
40	7	d(8.4)	130.1	CH	38,41,42
41	6.61	d(8.4)	114.7	CH	39,42
42			155.7		
42-OH	7.1	d(7.3)			41,42,43
43	6.61	d(8.4)	114.7	CH	39,42
44	7	d(8.4)	130.1	CH	38,42,43
45			170.2		
46	2.84	d(6.7)	34.1	CH_2	
47	5.4	m	131.7	CH	
48	5.37	m	123.2	CH	
49	1.94	m	26.8	CH_2	
50	1.26	m	28.9	CH_2	
51	1.24	m	28.3	CH_2	
52	1.23	m	31.2	CH_2	
53	1.26	m	22.1	CH_2	
54	0.86	t(7.0)	14	CH_3	

Figure S2. Characterization of thalassospiramide A1 (3). (**G**) NMR assignment.

A ESI-TOF-HRMS



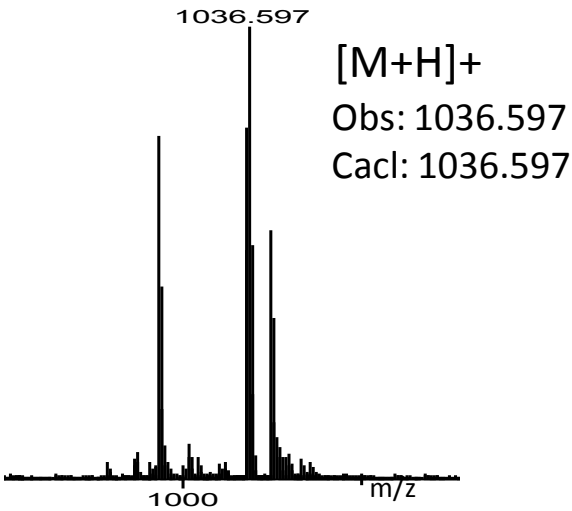
ITMS^2 – 960 m/z (1+)

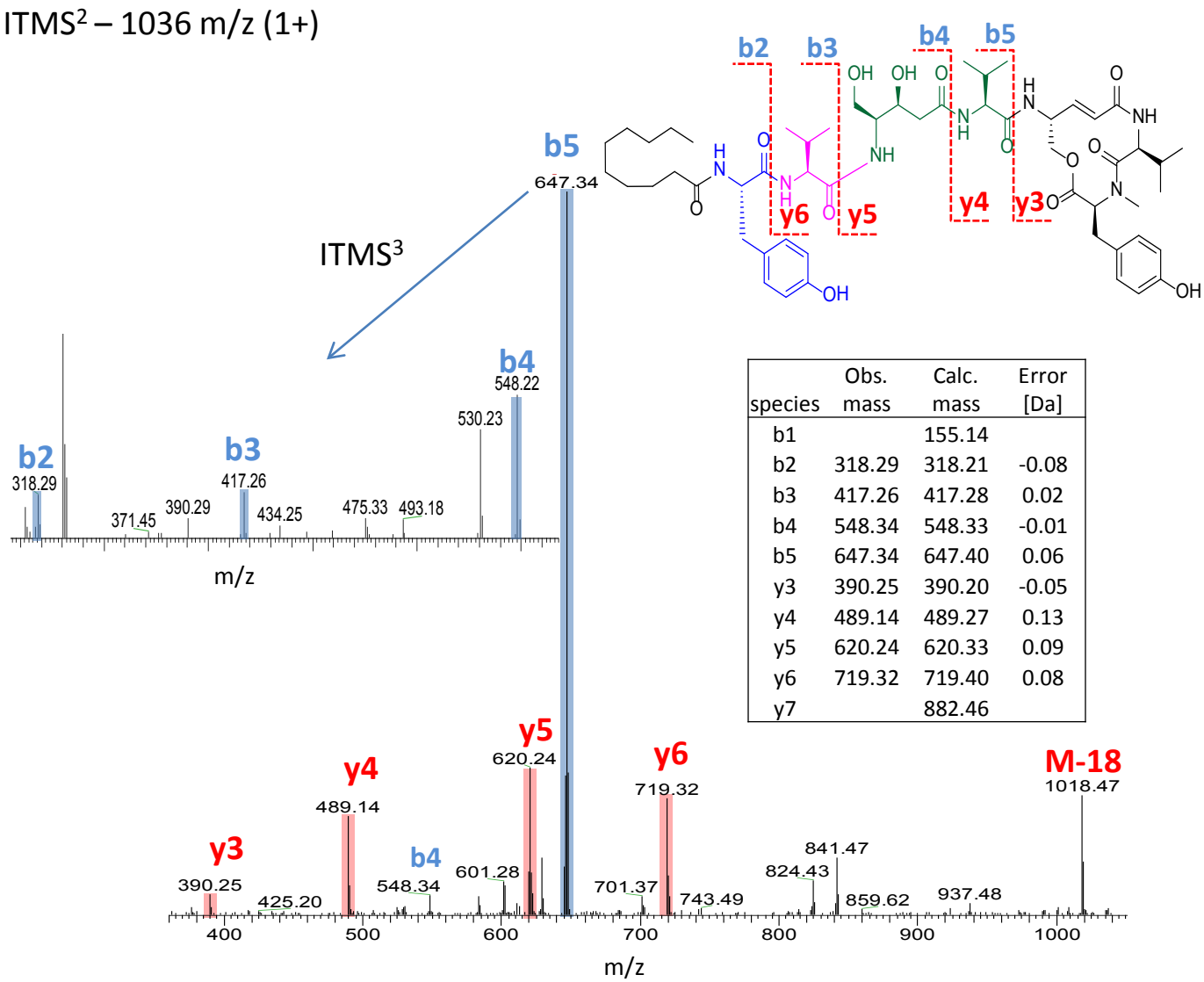


species	Obs. mass	Calc. mass	Error [Da]
b1		155.14	
b2		242.18	
b3		341.24	
b4	472.22	472.30	0.08
b5	571.24	571.37	0.13
y3	390.21	390.20	-0.01
y4	489.16	489.27	0.11
y5	620.22	620.33	0.11
y6		719.40	
y7		806.43	

Figure S3. Characterization of thalassospiramide A2 (4). (**A**) MS^n analysis.

A ESI-TOF-HRMS

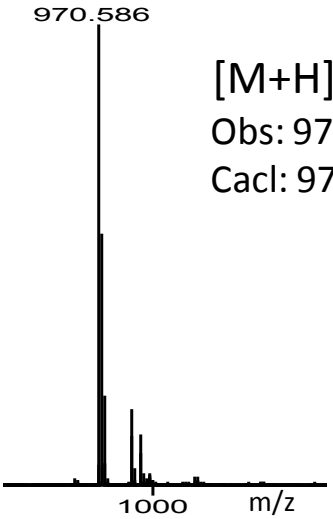


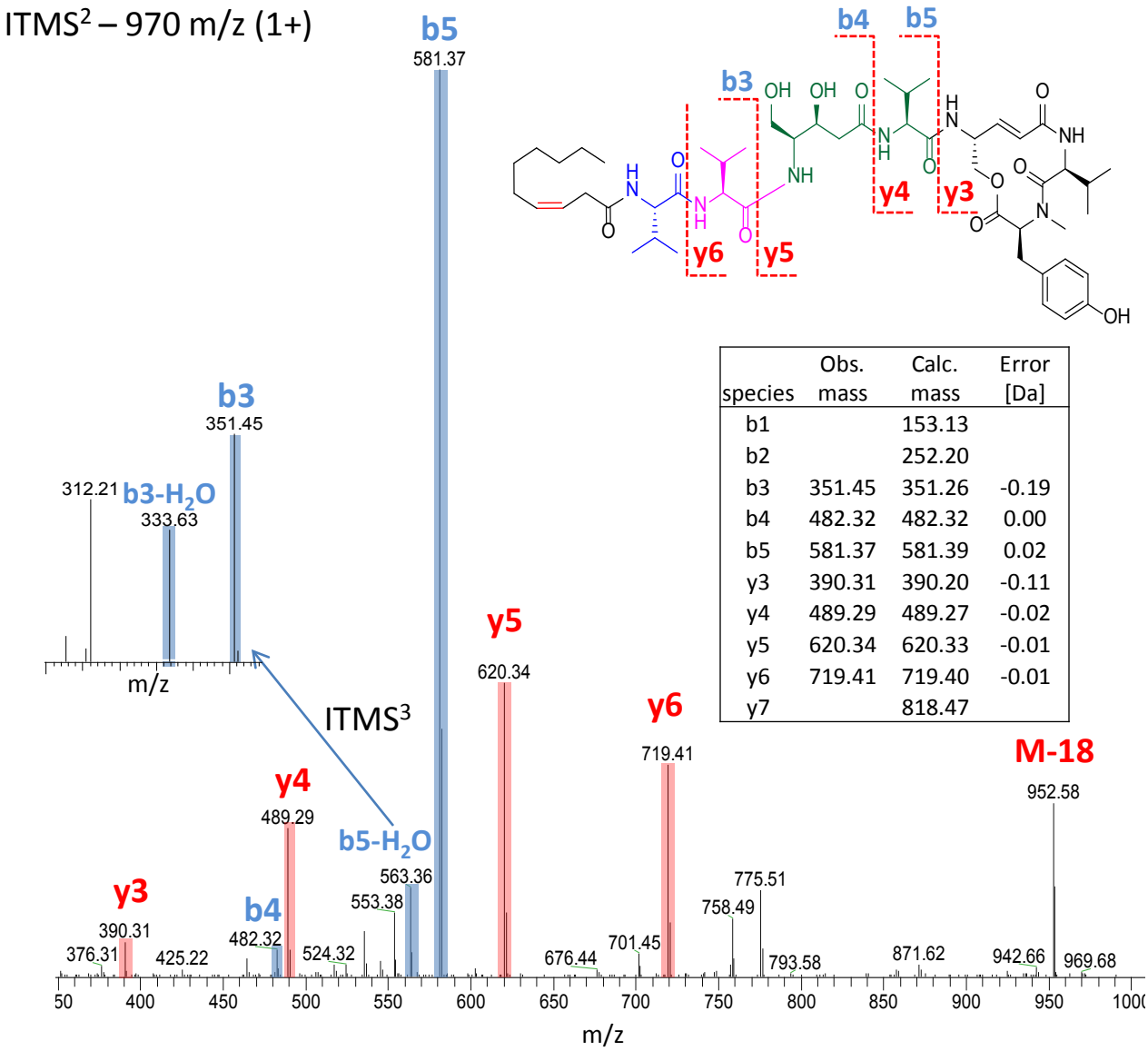


species	Obs. mass	Calc. mass	Error [Da]
b1		155.14	
b2	318.29	318.21	-0.08
b3	417.26	417.28	0.02
b4	548.34	548.33	-0.01
b5	647.34	647.40	0.06
y3	390.25	390.20	-0.05
y4	489.14	489.27	0.13
y5	620.24	620.33	0.09
y6	719.32	719.40	0.08
y7		882.46	

Figure S4. Characterization of thalassospiramide A3 (5). (**A**) MS^n analysis.

A ESI-TOF-HRMS





species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2		252.20	
b3	351.45	351.26	-0.19
b4	482.32	482.32	0.00
b5	581.37	581.39	0.02
y3	390.31	390.20	-0.11
y4	489.29	489.27	-0.02
y5	620.34	620.33	-0.01
y6	719.41	719.40	-0.01
y7		818.47	

Figure S5. Characterization of thalassospiramide A4 (6). (**A**) MS^n analysis.

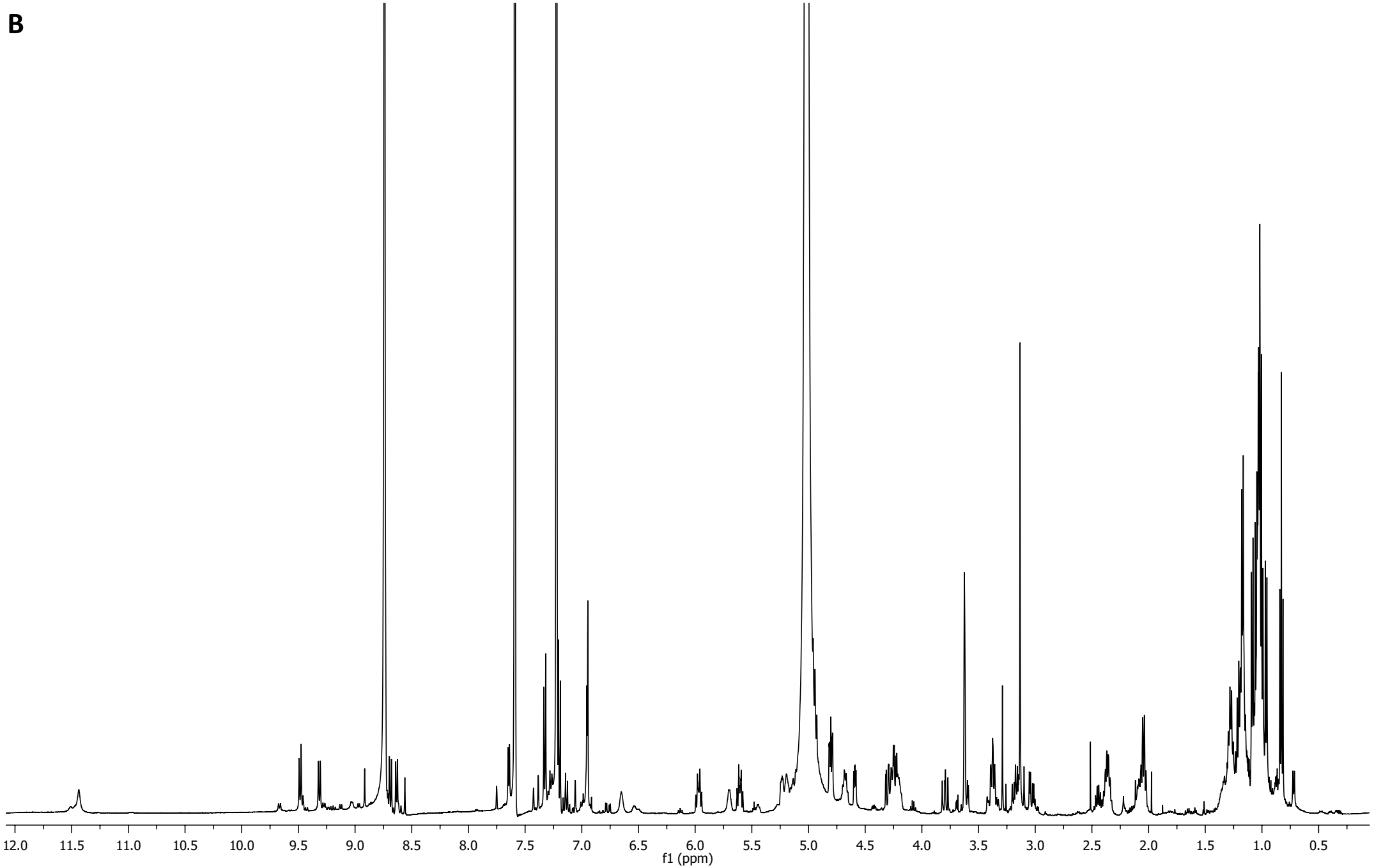


Figure S5. Characterization of thalassospiramide A4 (6). (**B**) ^{1}H spectrum in pyridine-d_5.

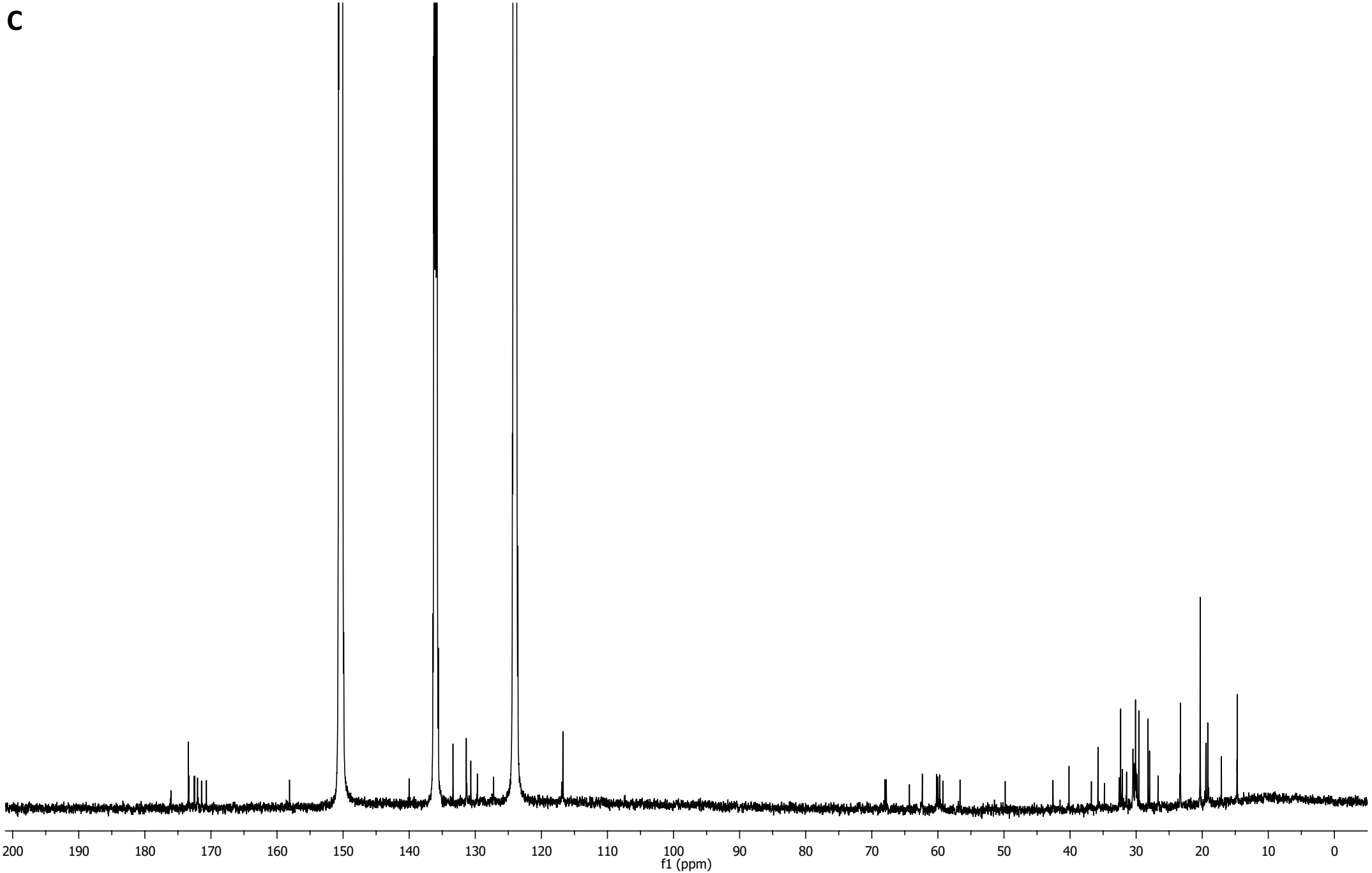


Figure S5. Characterization of thalassospiramide A4 (6). (**C**) ^{13}C spectrum in pyridine-d_5.

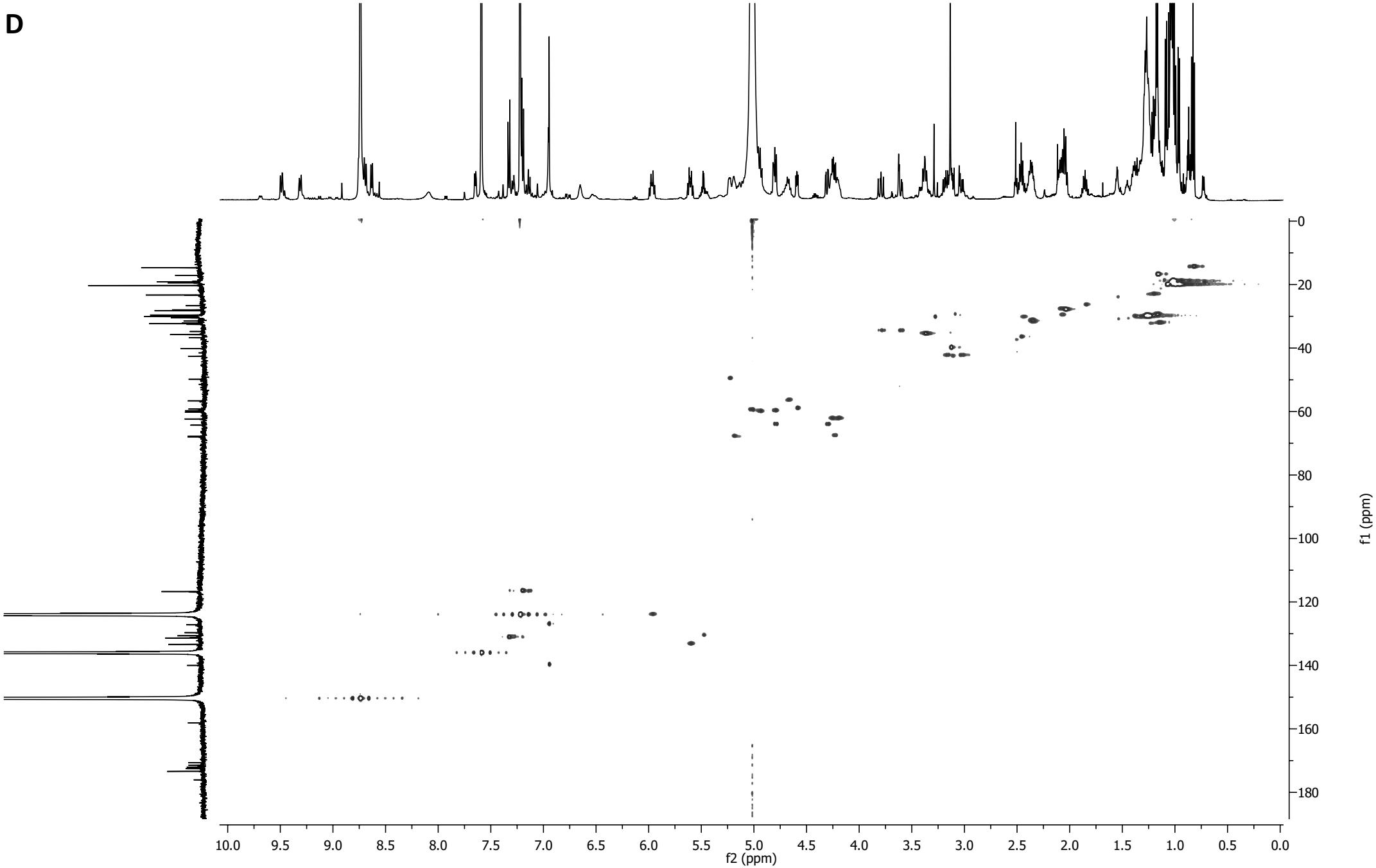


Figure S5. Characterization of thalassospiramide A4 (6). (**D**) HSQC spectrum in pyridine-d_5.

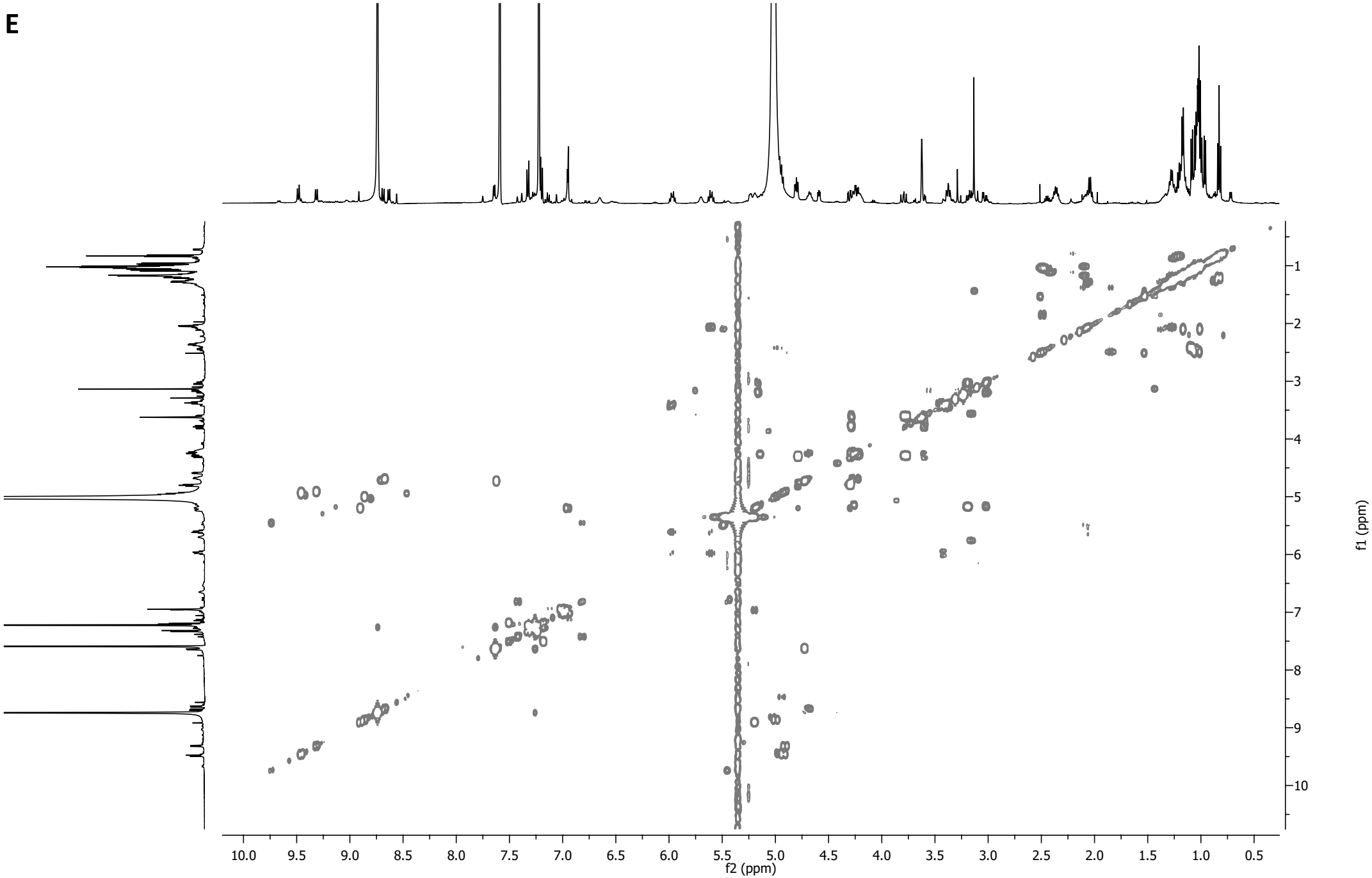


Figure S5. Characterization of thalassospiramide A4 (6). (**E**) ^{1}H-^{1}H COSY spectrum in pyridine-d_5.

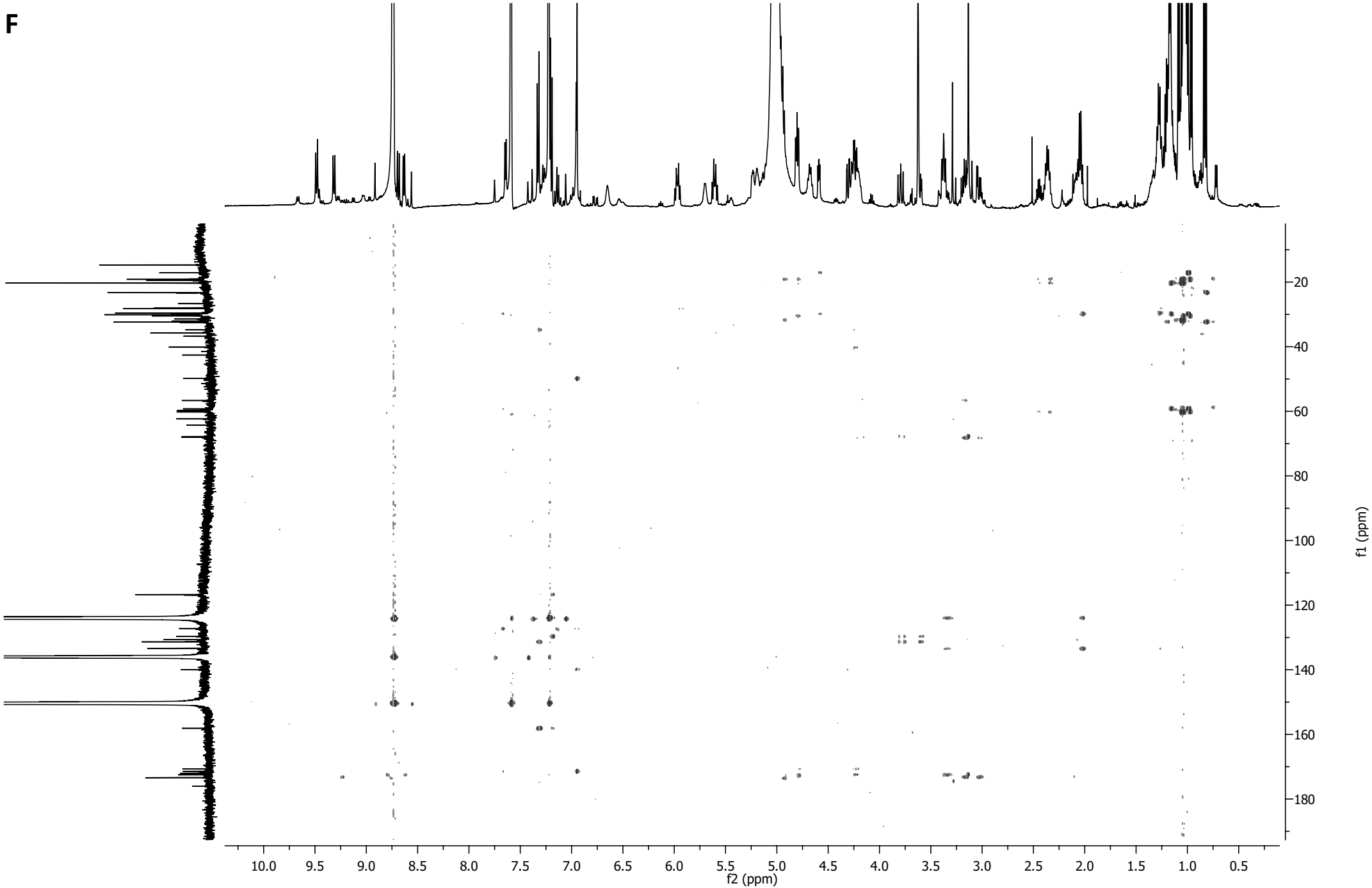


Figure S5. Characterization of thalassospiramide A4 (6). (**F**) HMBC spectrum in pyridine-d_5.

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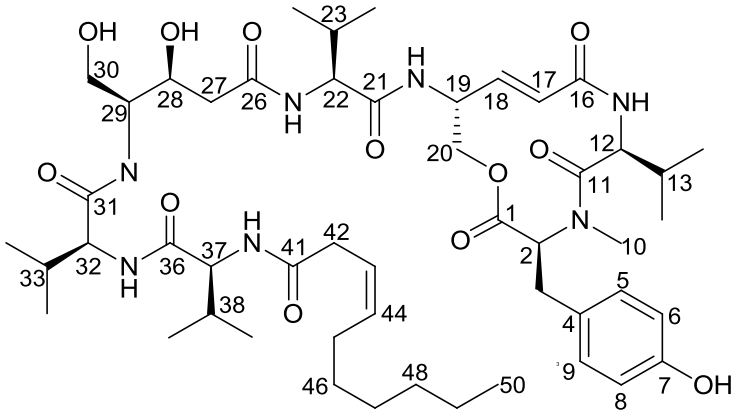


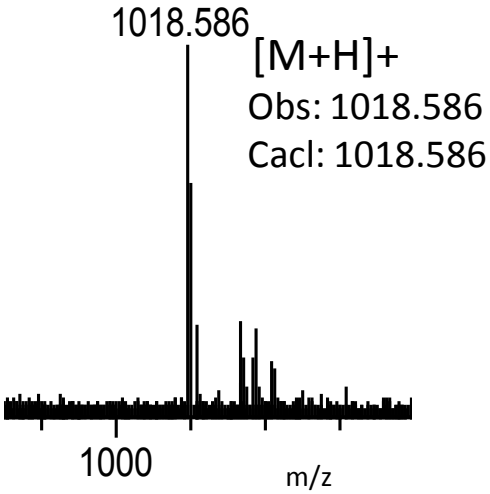
NMR Spectral Data (500 MHz (^{1}H), 125 MHz (^{13}C) in pyridine-d_5) of thalassospiramide A4 (**6**)

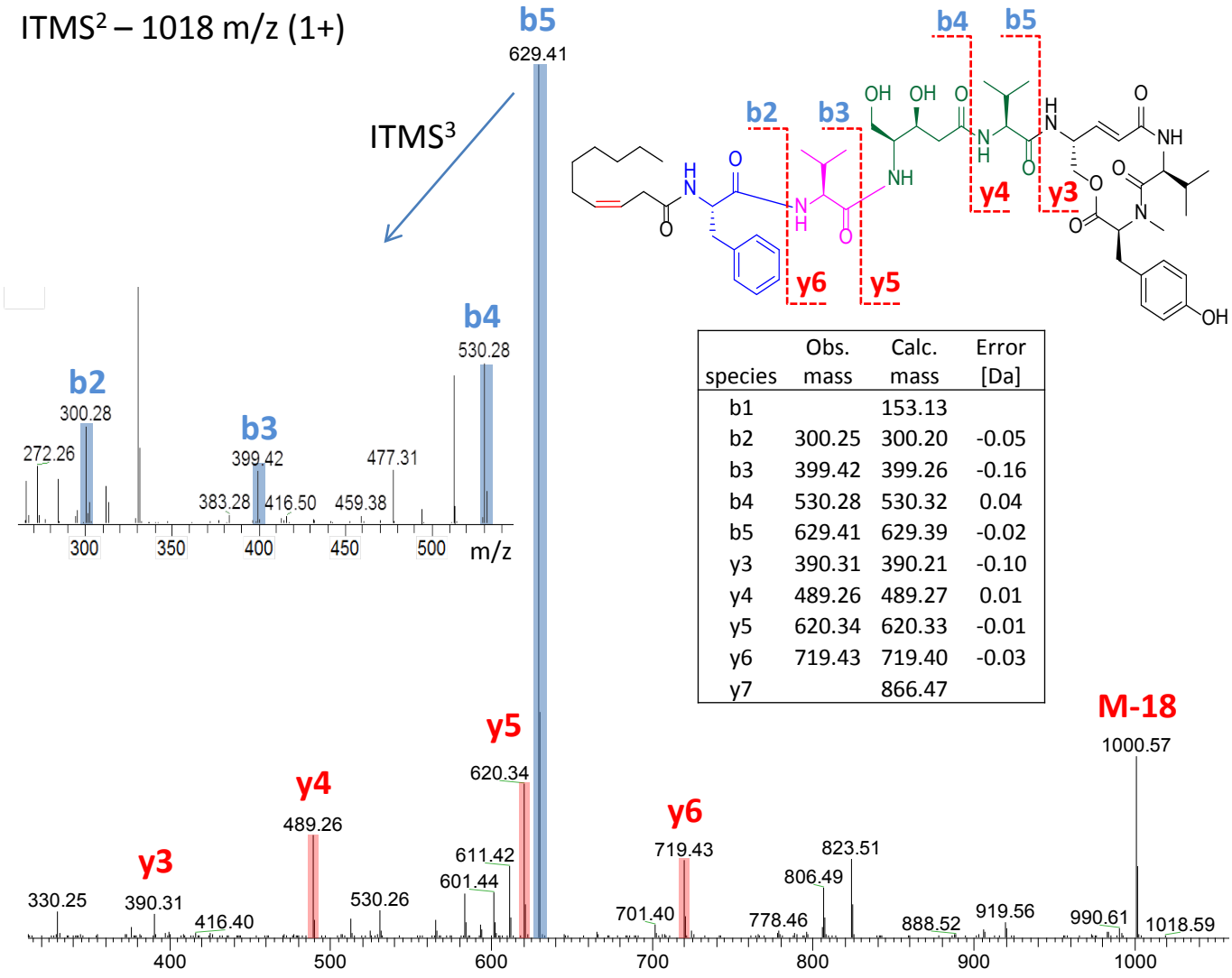
C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1			170.7	C	
2	4.26	m	67.8	CH	1, 10
3a	3.61	dd (14.0, 4.0)	34.8	CH_2	4
3b	3.79	dd (14.0, 11,0)			4
4			129.7	C	
5	7.33	d (8.3)	131.4	CH	3
6	7.19	d (8.3)	116.7	CH	3
7			158.0	C	
7-OH					
8	7.19	d (8.3)	116.7	CH	3
9	7.33	d (8.3)	131.4	CH	3
10	3.12	s	40.0	CH_3	2, 11
11			172.5	C	
12	4.58	dd (6.2, 4.1)	59.2	CH	
12-NH	7.64	d (6.3)			16, 17
13	2.08	m	29.6	CH	15
14	0.99	d (7.0)	20.2	CH_3	
15	1.17	d (7.0)	17.0	CH_3	
16			171.4	C	
17	6.94	m	127.2	CH	16, 18, 19
18	6.95	m	139.8	CH	16, 18, 19
19	5.19	m	49.8	CH	
19-NH	8.63	d (7.5)			
20a	4.81	m	64.2	CH_2	1
20b	4.31	d (11.2, 2.3)			1
21			172.5	C	
22	4.79	m	60.0	CH	
22-NH	9.30				26
23	2.44	m	30.1	CH	
24	0.97	d (6.8)	20.2	CH_3	
25	1.03	d (6.8)	19.2	CH_3	
26			173.4	C	
27a	3.19	dd (14.2, 8.2)	42.6	CH_2	26
27b	3.03	dd (14.3, 5.3)			
28	5.11	m	68.0	CH	
28-OH					
29	4.65	m	56.7	CH	

29-NH					
30a	4.24	dd (11.0, 4.3)	62.4	CH_2	
30b	4.30	m			
30-OH	6.61				
31			172.0	C	
32	5.00	m	59.7	CH	
32-NH	8.78				36
33	2.33	m	31.5	CH	
34	1.04	d (6.9)	19.2	CH_3	
35	1.05	d (6.9)	19.5	CH_3	
36			173.4	C	
37	4.93	m	60.2	CH_2	36
37-NH	8.78	m			41
38	2.33	m	32.1	CH	
39	1.05	d (7.0)	19.2	CH_3	
40	1.04	d (7.0)	20.2	CH_3	
41			172.5	C	
42	3.37	m	35.8	CH_2	
43	5.94	dtt(10.7, 7.0, 2.0)	123.5	CH	
44	5.59	dtt(10.7, 7.0, 1.5)	133.4	CH	
45	2.01	m	28.2	CH_2	
46	1.27	m	29.9	CH_2	
47	1.17	m	29.6	CH_2	
48	1.15	m	32.3	CH_2	
49	1.17	m	23.4	CH_2	
50	0.80	t (7.0)	14.6	CH_3	

Figure S5. Characterization of thalassospiramide A4 (6). (**G**) NMR assignment.

A ESI-TOF-HRMS





species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2	300.25	300.20	-0.05
b3	399.42	399.26	-0.16
b4	530.28	530.32	0.04
b5	629.41	629.39	-0.02
y3	390.31	390.21	-0.10
y4	489.26	489.27	0.01
y5	620.34	620.33	-0.01
y6	719.43	719.40	-0.03
y7		866.47	

Figure S6. Characterization of thalassospiramide A5 (11). (**A**) MS^n analysis.

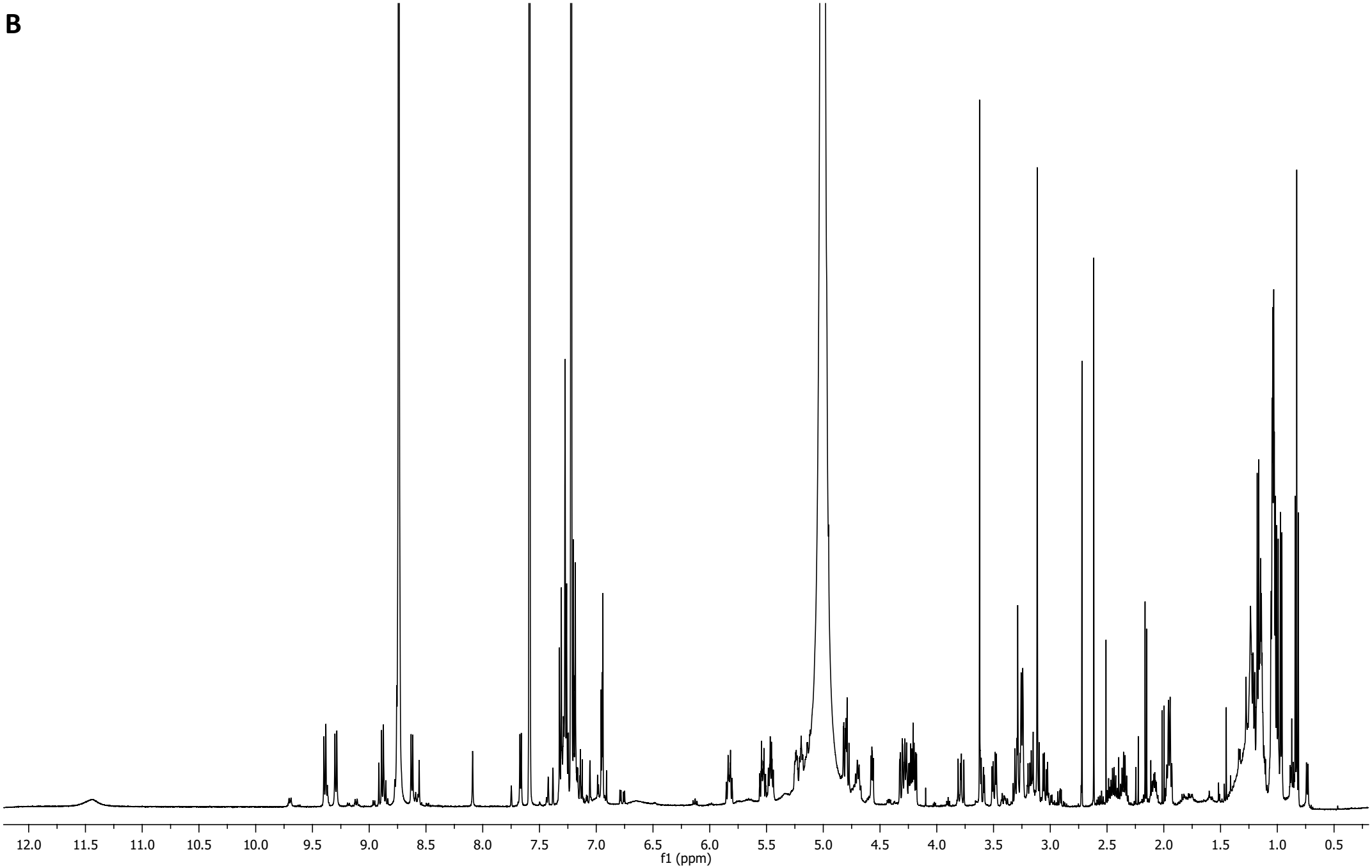


Figure. S6. Characterization of thalassospiramide A5 (11). (**B**) ^{1}H spectrum in pyridine-d_5.

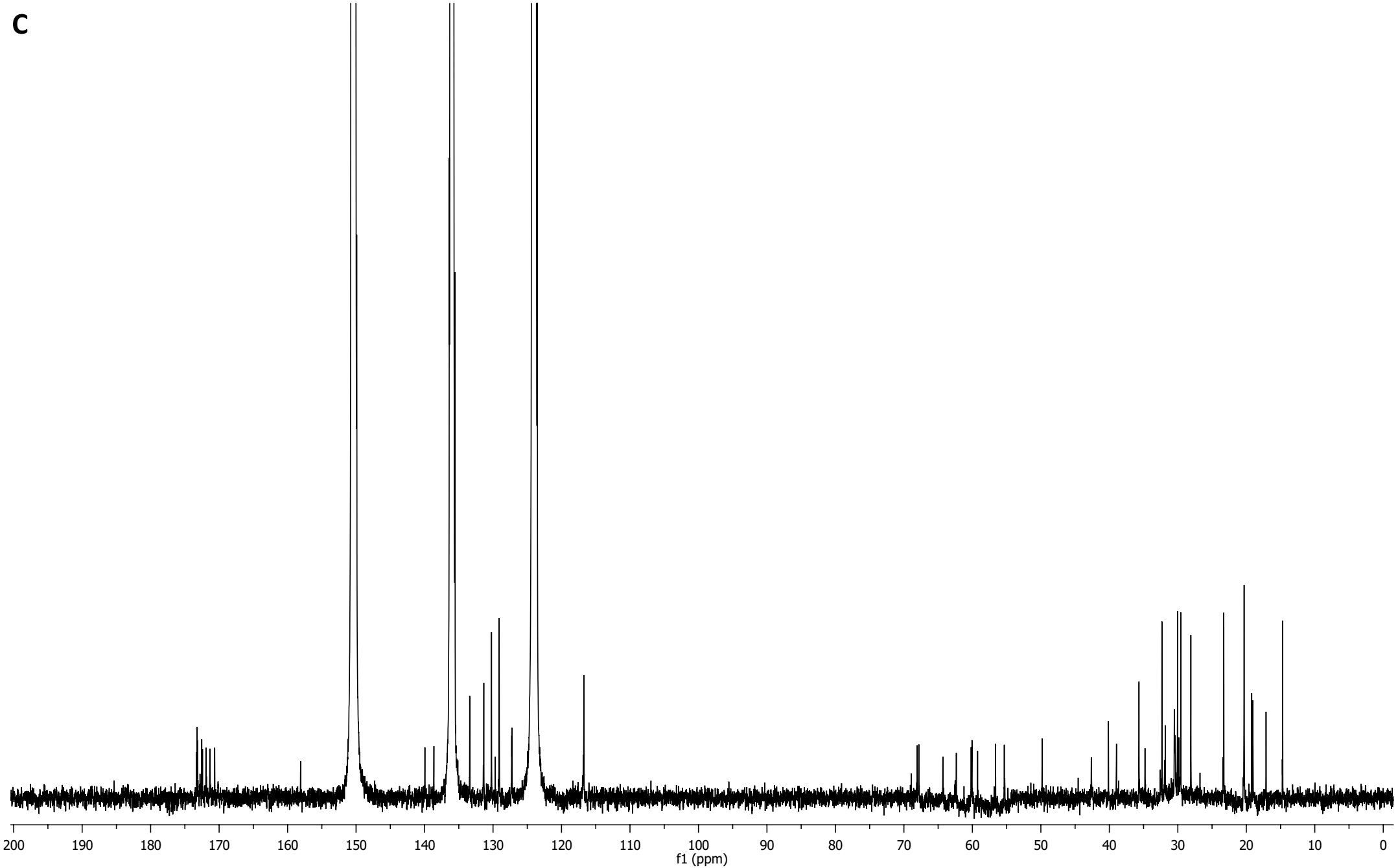


Figure S6. Characterization of thalassospiramide A5 (11). (**C**) ^{13}C spectrum in pyridine-d_5.

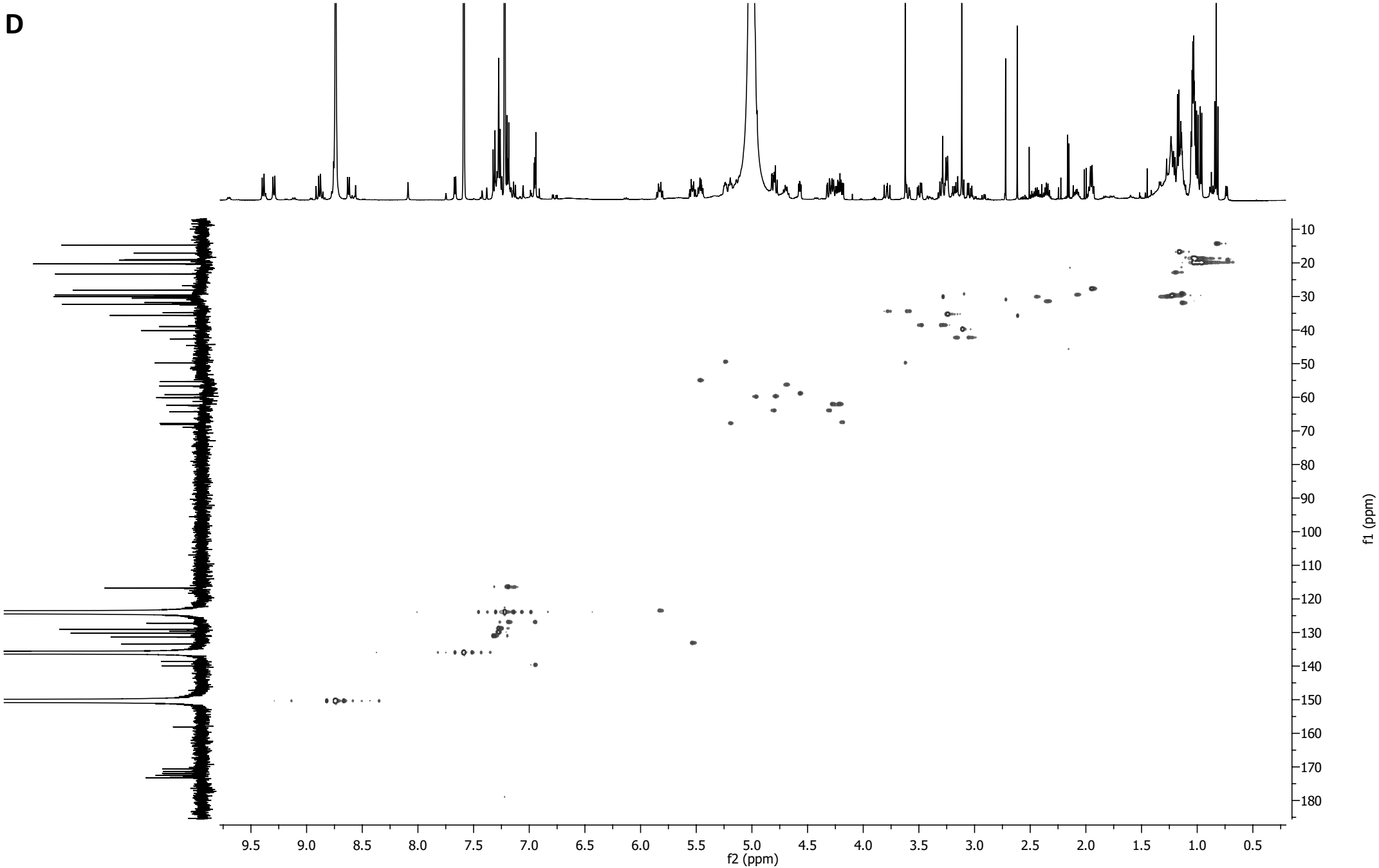


Figure S6. Characterization of thalassospiramide A5 (11). (**D**) HSQC spectrum in pyridine-d_5.

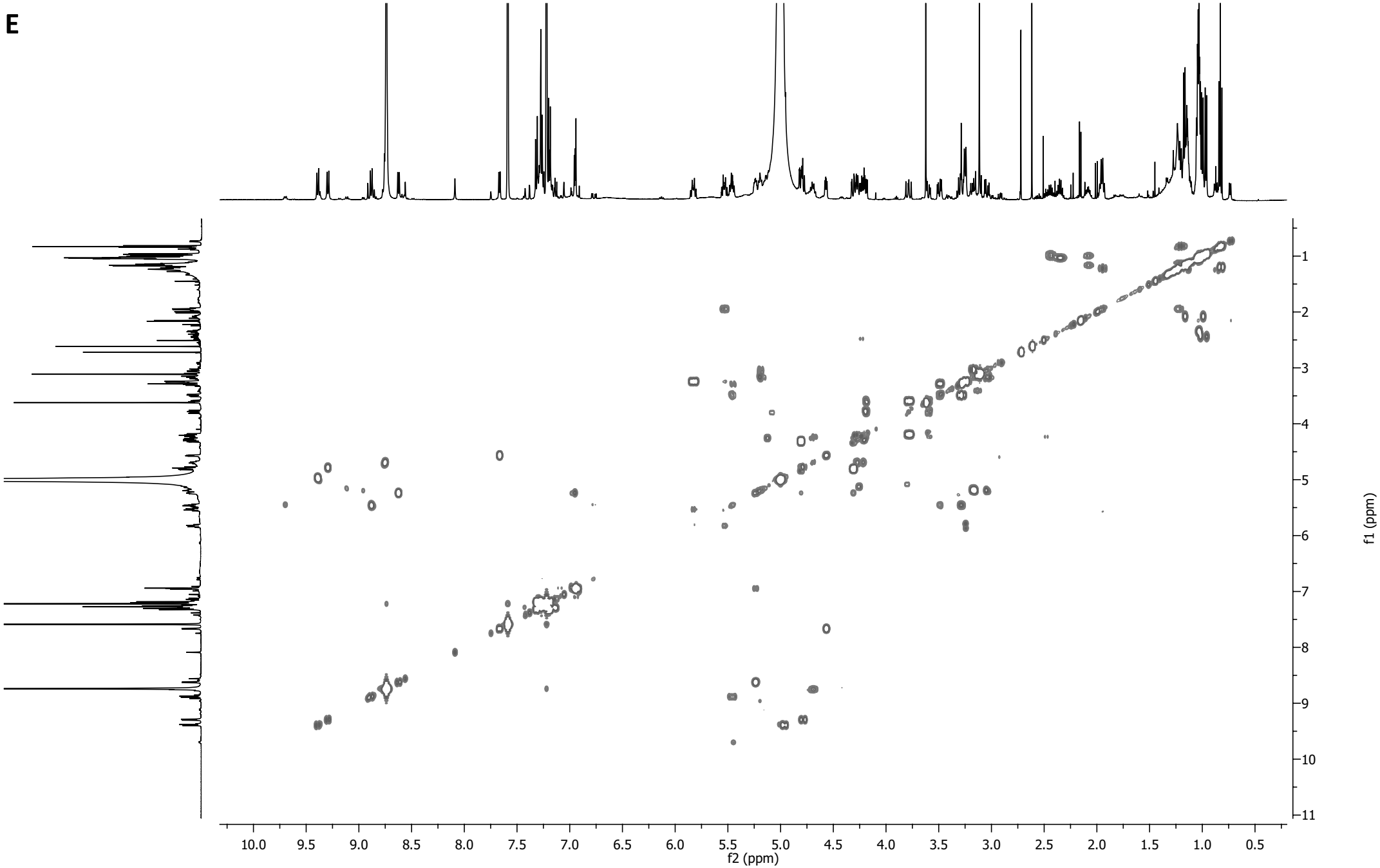


Figure S6. Characterization of thalassospiramide A5 (11). (**E**) ^{1}H-^{1}H COSY spectrum in pyridine-d_5.

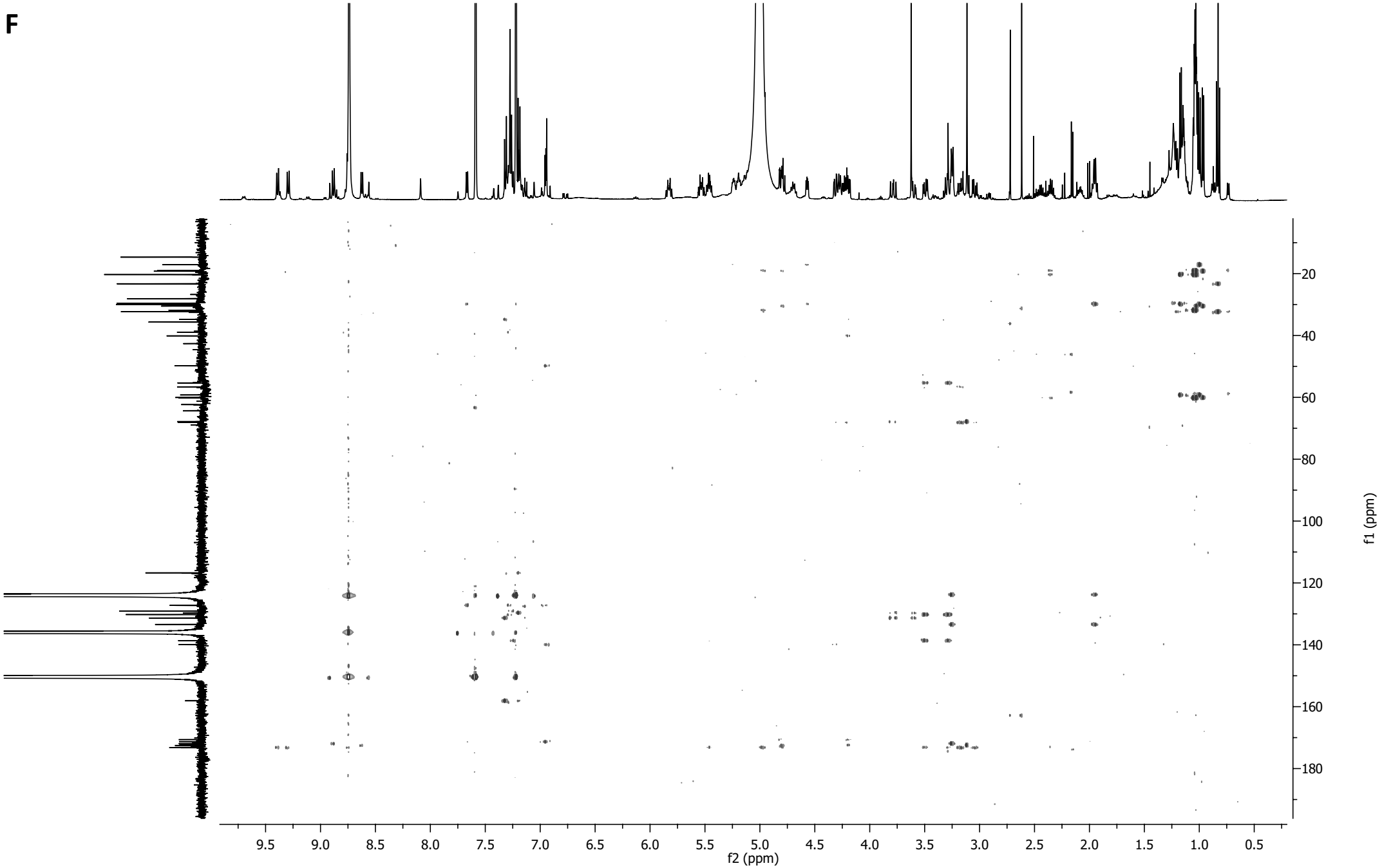


Figure S6. Characterization of thalassospiramide A5 (11). (**F**) HMBC spectrum in pyridine-d_5.

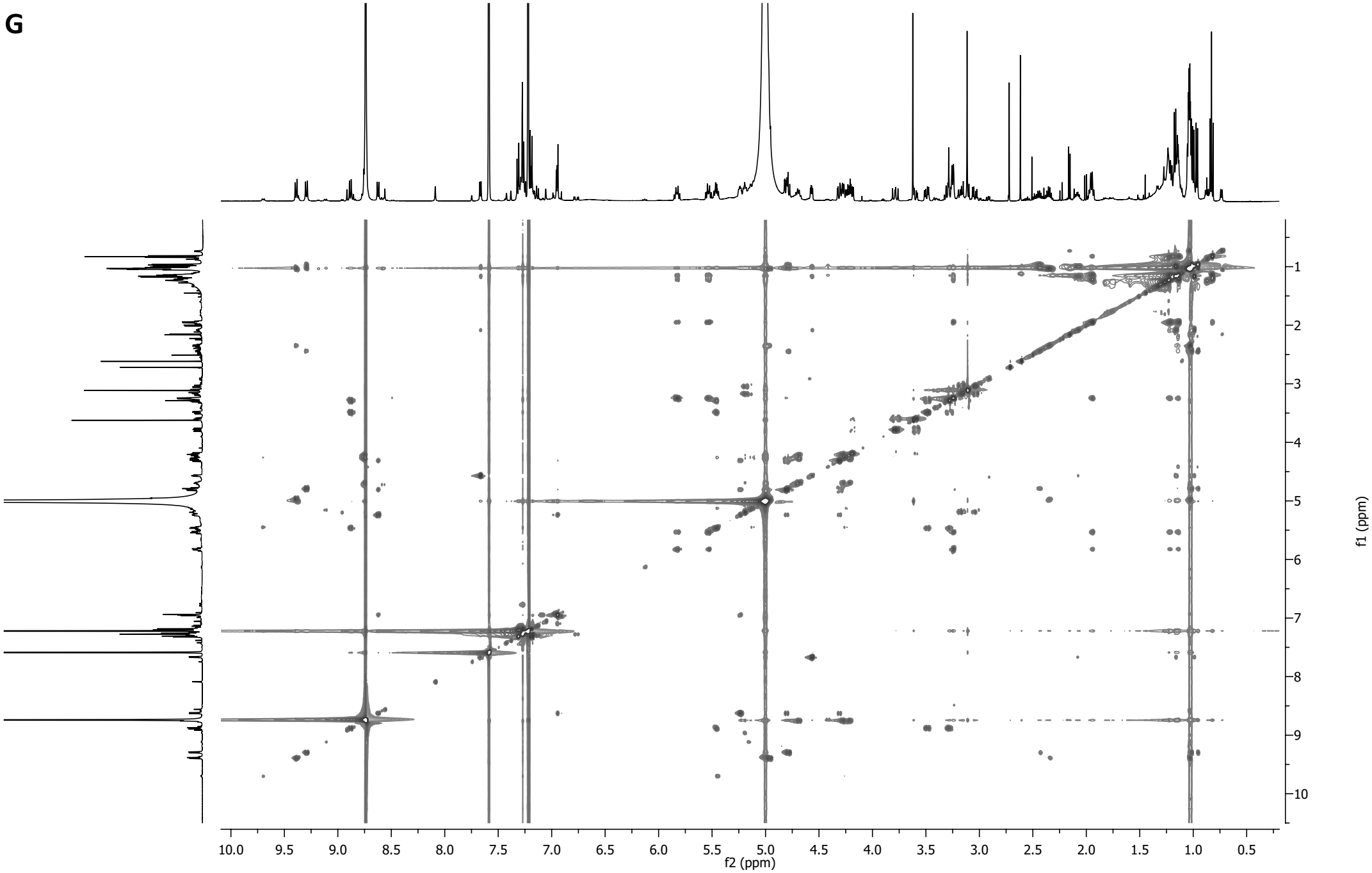
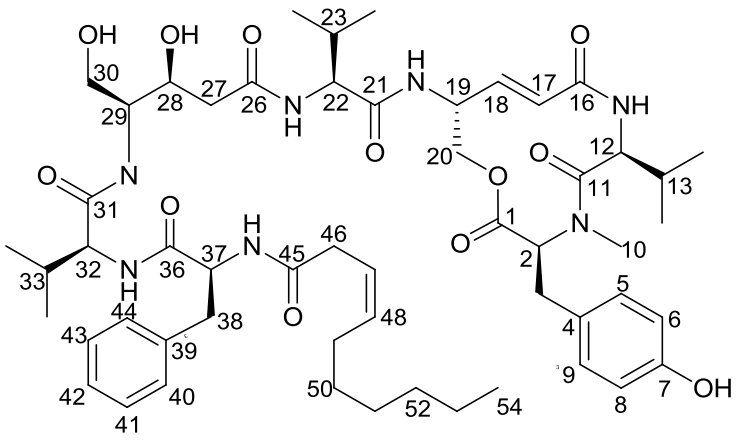


Figure S6. Characterization of thalassospiramide A5 (11). (**G**) ^{1}H-^{1}H TOCSY spectrum in pyridine-d_5.

H



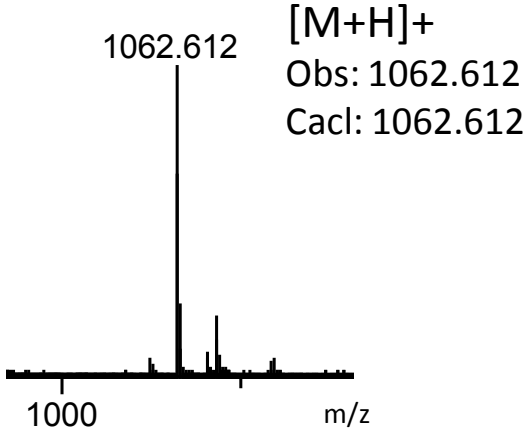
NMR Spectral Data (500 MHz (^{1}H), 125 MHz (^{13}C) in pyridine-d_5) of thalassospiramide A5 (**11**)

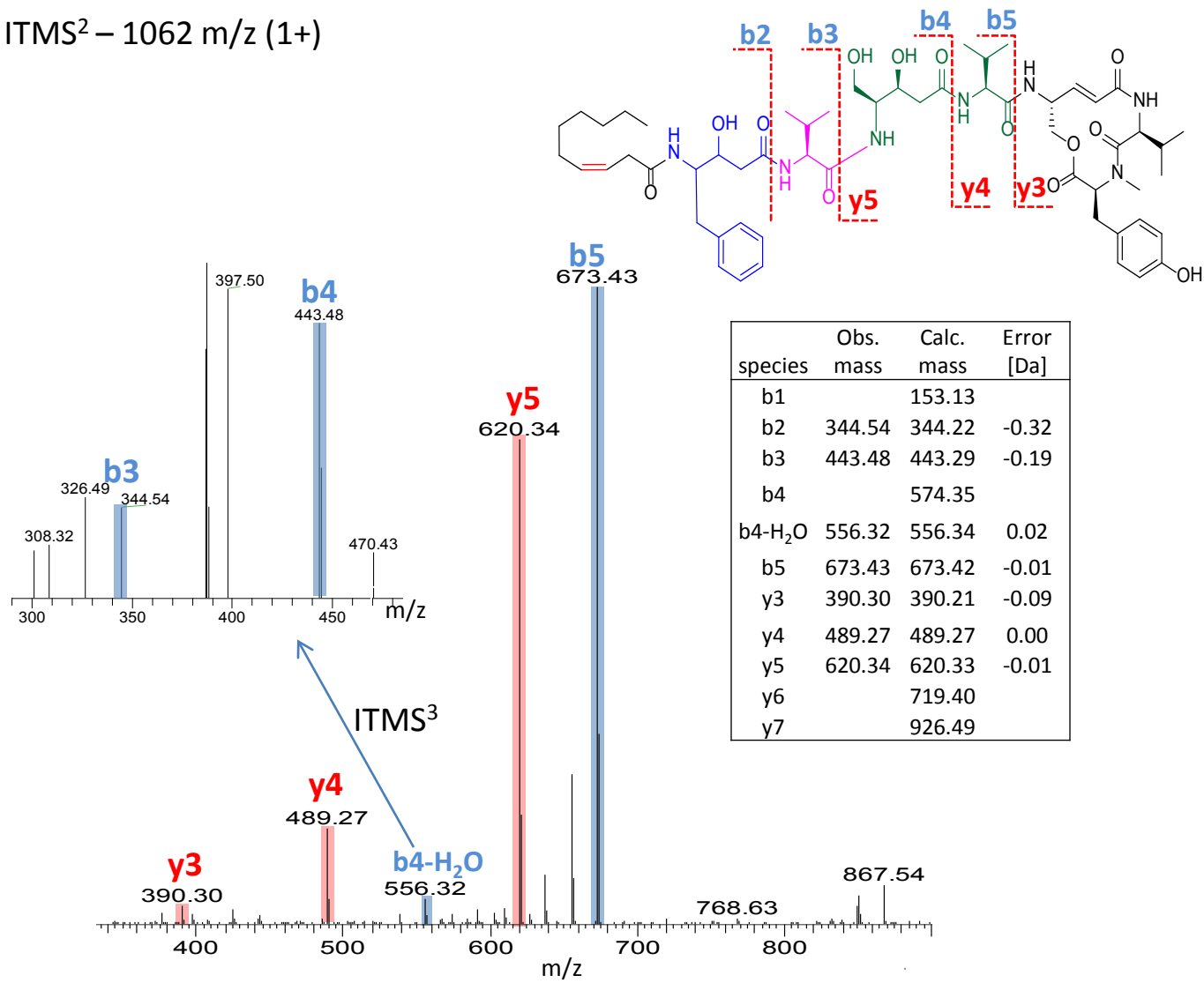
C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1				C	
2				CH	
3a	3.60	dd (14.2, 4.3)	34.8	CH_2	
3b	3.79	dd (14.2, 11.0)			
4			129.6	C	
5	7.31	d (8.4)	131.4	CH	
6	7.19	d (8.4)	116.8	CH	
7			158.1	C	
7-OH					
8	7.19	d (8.4)	116.8	CH	
9	7.31	d (8.4)	131.4	CH	
10	3.12	s	40.1	CH_3	2, 11
11			172.4	C	
12	4.57	dd (6.2, 4.2)	59.2	CH	
12-NH	7.67	dd (6.2)			16
13	2.09	m	29.6	CH	
14	1.00	dd (6.9)	20.3	CH_3	
15	1.17	dd (6.9)	17.1	CH_3	
16			171.4	C	
17	6.93	d (15.8)	127.2	CH	16
18	6.97	d (15.8, 4.3))	140.0	CH	
19	5.24	m	49.8	CH	
19-NH	8.62	d (7.5)			21
20a	4.31	m	64.3	CH_2	
20b	4.82	m			
21			172.5	C	
22	4.79	m	60.1	CH	
22-NH	9.30				26
23	2.44	m	30.0	CH	
24	0.97	d (6.7)	20.3	CH_3	
25	1.02	m	19.2	CH_3	
26			173.3	C	
27a	3.17	dd (14.2, 8.3)	42.6	CH_2	26
27b	3.04	dd (14.2, 5.5)			
28	5.19	m	68.1	CH	
28-OH					

29	4.69	m	56.6	CH	
29-NH	8.74	m			31
30a	4.22	m	62.4	CH_2	
30b	4.28	m			
30-OH					
31			173.2	C	
32	4.98	m	60.2	CH	31
32-NH	9.39	d (8.5)			36
33	2.35	m	31.8	CH	
34	1.04	m	20.3	CH_3	
35	1.05	m	19.1	CH_3	
36			173.2	C	
37	5.46		55.3	CH	
37-NH	8.89				45
38a	3.31	dd (14.0, 5.9)	39.0	CH_2	
38b	3.49	dd (14.0, 5.4)			
39			138.6	C	
40	7.28	m	130.2	CH	
41	7.27	m	129.1	CH	
42	7.25	m	129.1	CH	
43	7.27	m	129.1	CH	
44	7.28	m	130.2	CH	
45			171.9	C	
46	3.25	d (7.2)	35.7	CH_2	45
47	5.83	dtt (10.5, 7.2, 1.0)	133.4	CH	
48	5.53	dtt (10.5, 7.2, 1.5)	123.9	CH	
49	1.95	m	28.1	CH_2	
50	1.22	m	29.9	CH_2	
51	1.26	m	30.5	CH_2	
52	1.13	m	32.3	CH_2	
53	1.20	m	23.3	CH_2	
54	0.83	t (7.0)	14.7	CH_3	

Figure S6. Characterization of thalassospiramide A5 (11). (**H**) NMR assignment.

A ESI-TOF-HRMS





species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2	344.54	344.22	-0.32
b3	443.48	443.29	-0.19
b4		574.35	
b4-H_2O	556.32	556.34	0.02
b5	673.43	673.42	-0.01
y3	390.30	390.21	-0.09
y4	489.27	489.27	0.00
y5	620.34	620.33	-0.01
y6		719.40	
y7		926.49	

Figure S7. Characterization of thalassospiramide B (2). (**A**) MS^n analysis.

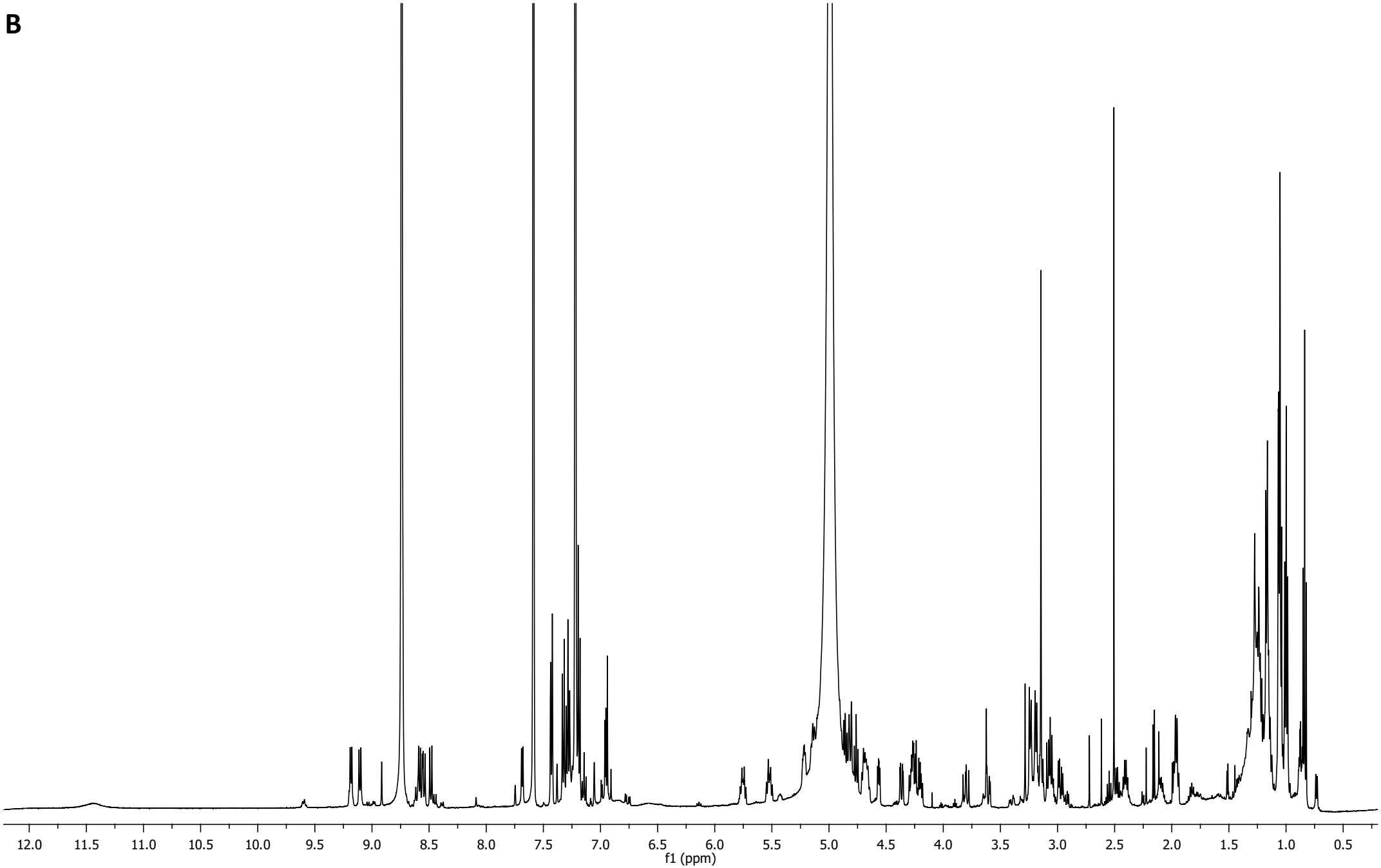
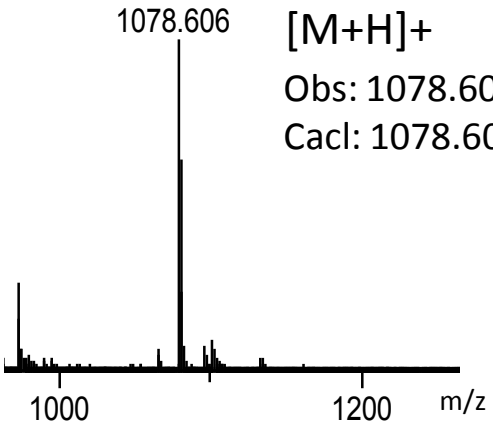
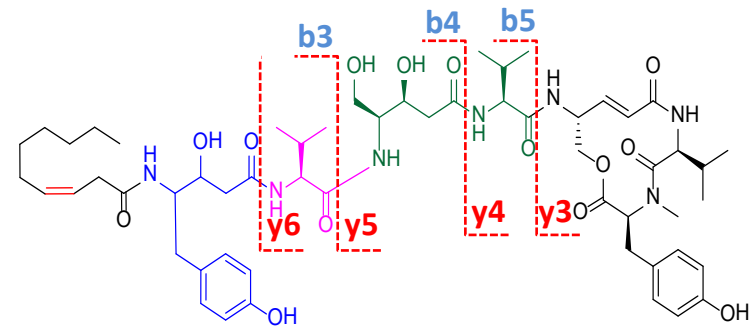


Figure S7. Characterization of thalassospiramide B (2). (**B**) ^{1}H spectrum in pyridine-d_5.

A ESI-TOF-HRMS



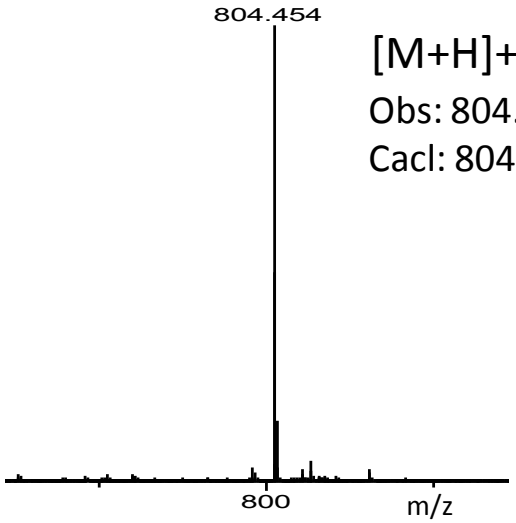
ITMS^2 – 1078 m/z (1+)



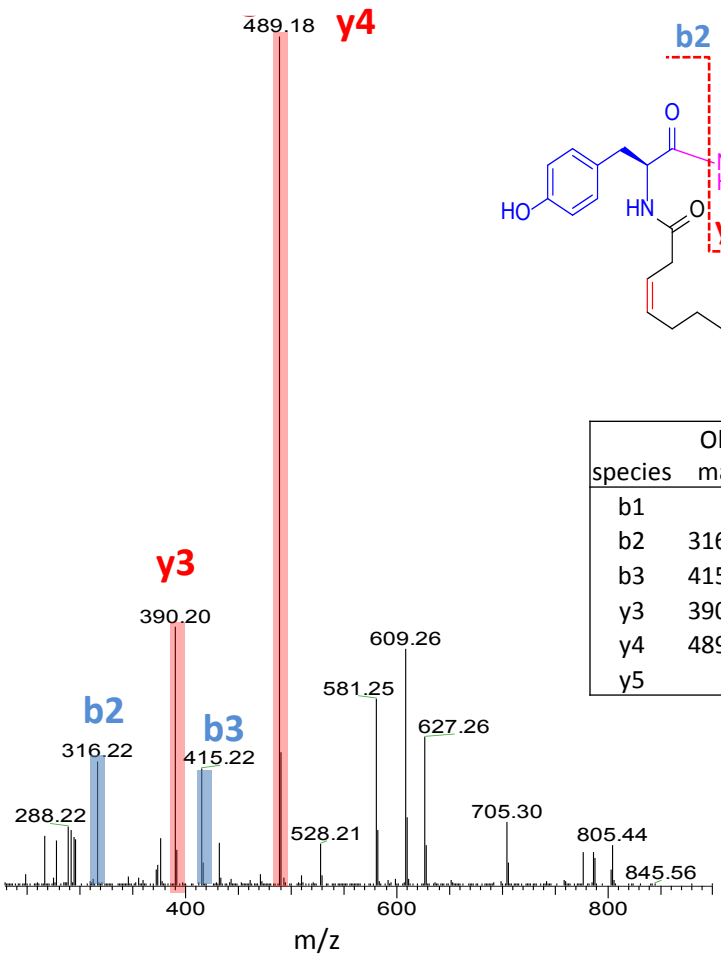
species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2		360.22	
b3		459.29	
b3-H_2O	441.31	441.28	-0.03
b4		590.34	
b4-H_2O	572.33	572.33	0.00
b5	689.42	689.41	-0.01
b5-H_2O	671.41	671.40	-0.01
y3	390.33	390.21	-0.12
y4	489.25	489.27	0.02
y5	620.33	620.33	0.00
y6	719.47	719.40	-0.07
y7		926.49	

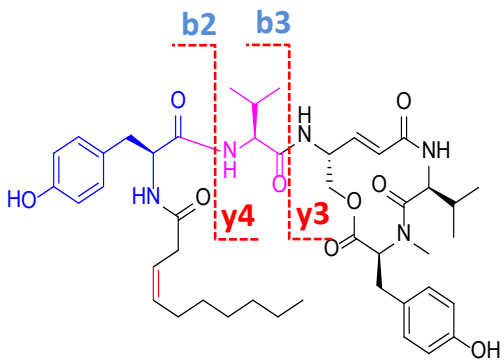
Figure S8. Characterization of thalassospiramide B1 (12). (**A**) MS^n analysis.

A ESI-TOF-HRMS



ITMS^2 – 804 m/z (1+)





species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2	316.22	316.19	-0.03
b3	415.22	415.26	0.04
y3	390.20	390.21	0.01
y4	489.18	489.27	0.09
y5		652.33	

Figure S9. Characterization of thalassospiramide C (7). (**A**) MS^n analysis.

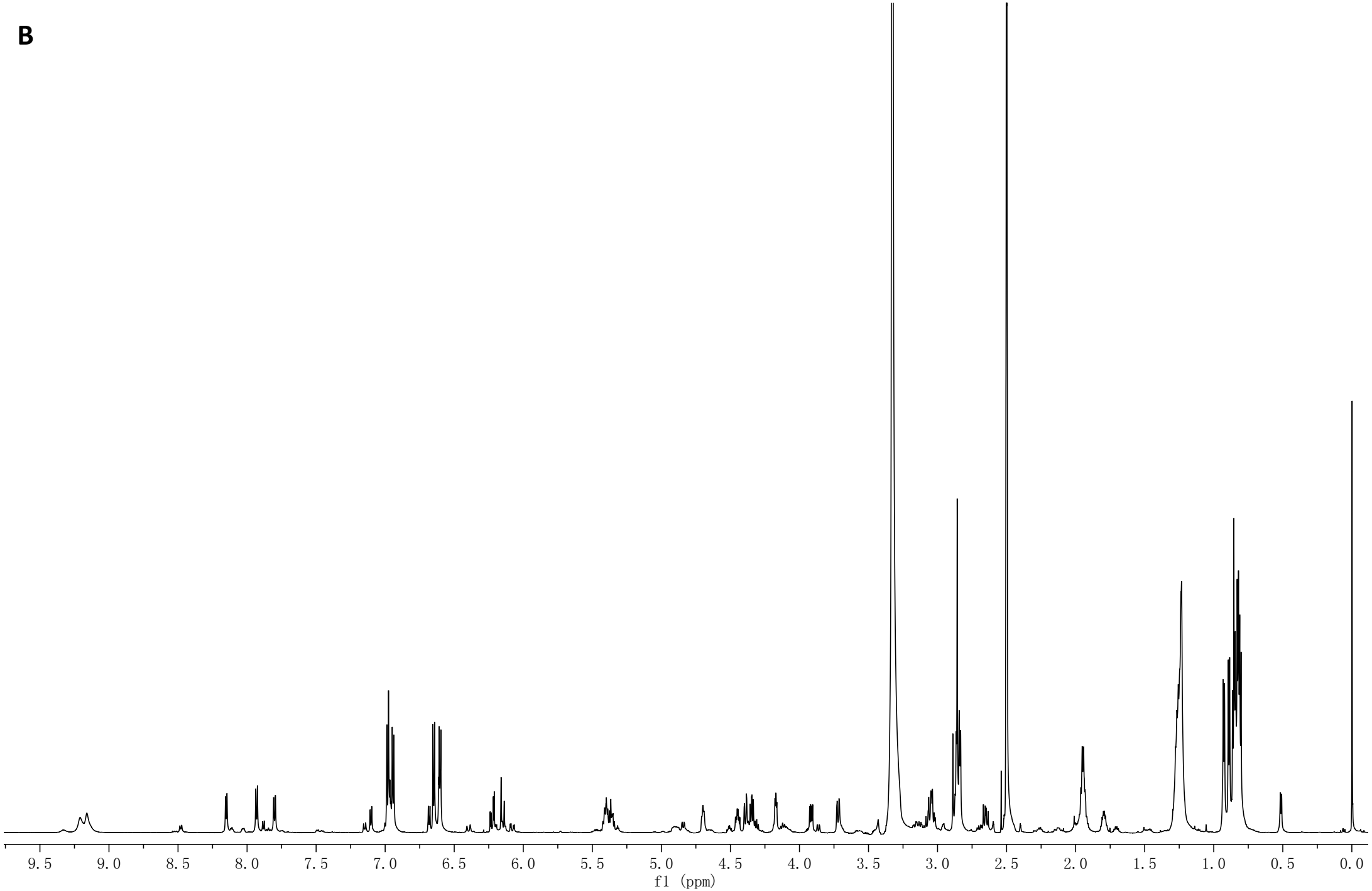


Figure S9. Characterization of thalassospiramide C (7). (**B**) ^{1}H spectrum in DMSO-d_5.

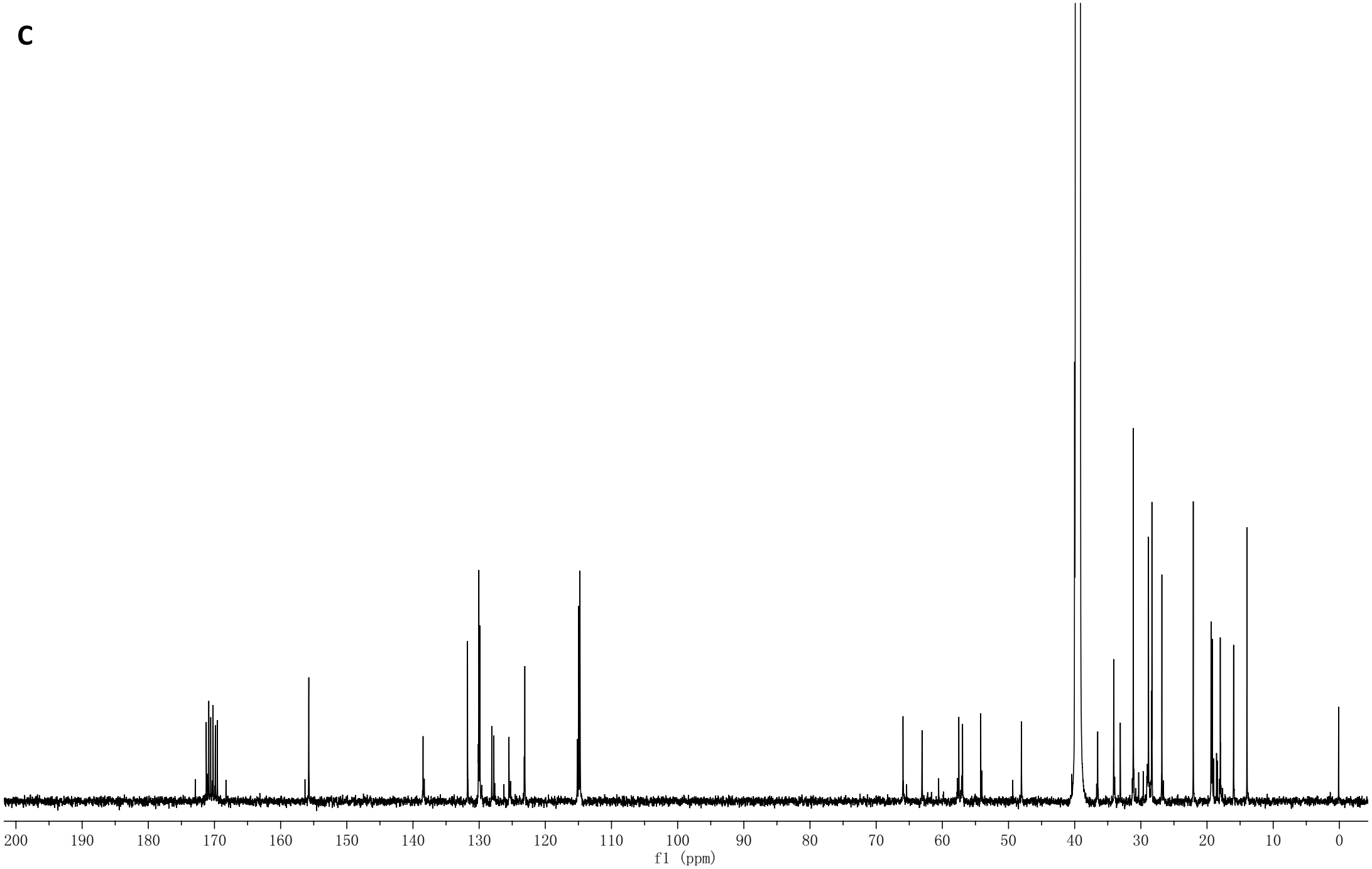


Figure S9. Characterization of thalassospiramide C (7). (**C**) ^{13}C spectrum in DMSO-d_5.

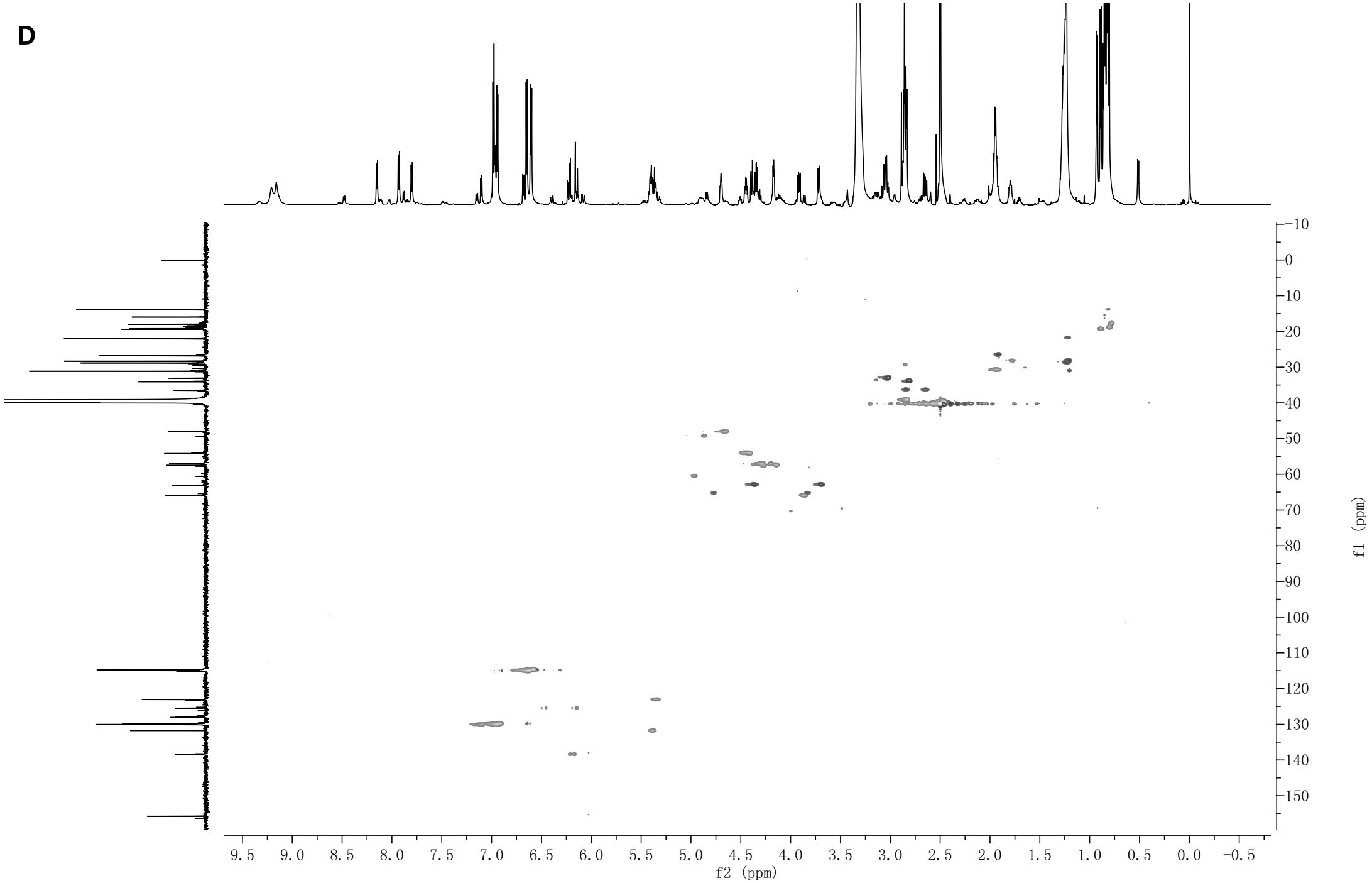


Figure S9. Characterization of thalassospiramide C (7). (**D**) HMQC spectrum in DMSO-d_5.

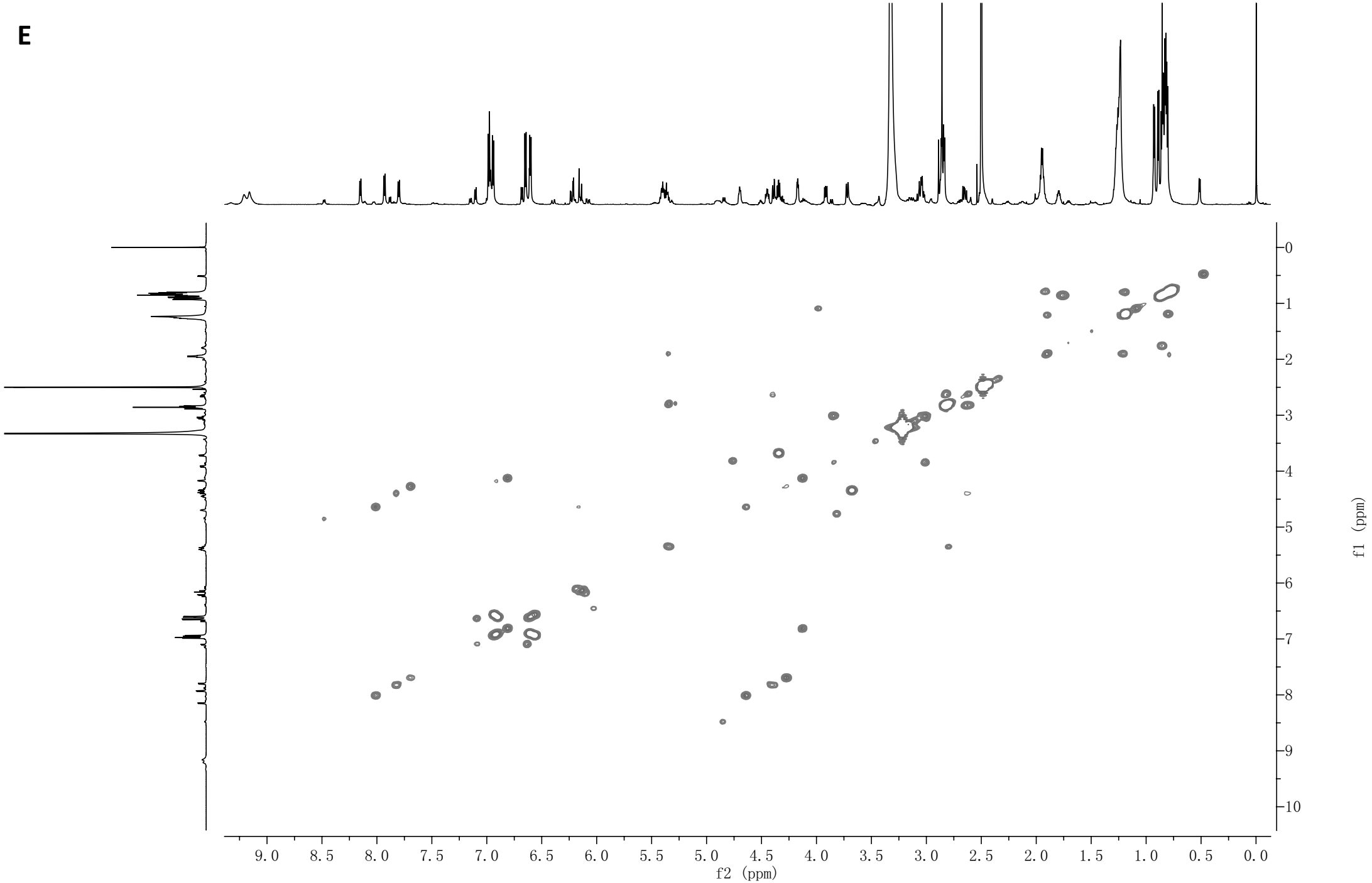


Figure S9. Characterization of thalassospiramide C (7). (**E**) ^{1}H-^{1}H COSY spectrum in DMSO-d_5.

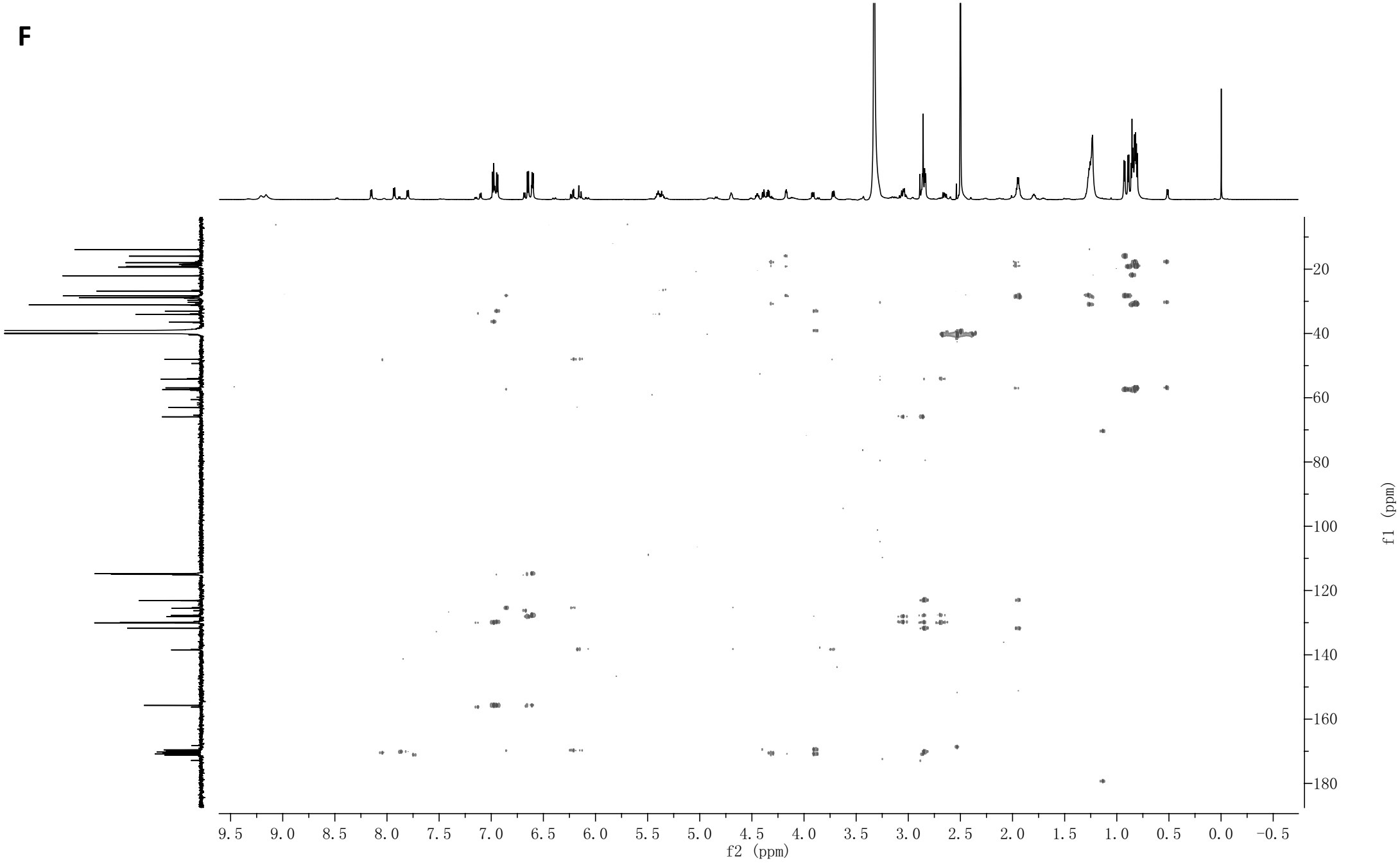


Figure S9. Characterization of thalassospiramide C (7). (**F**) HMBC spectrum in DMSO-d_5.

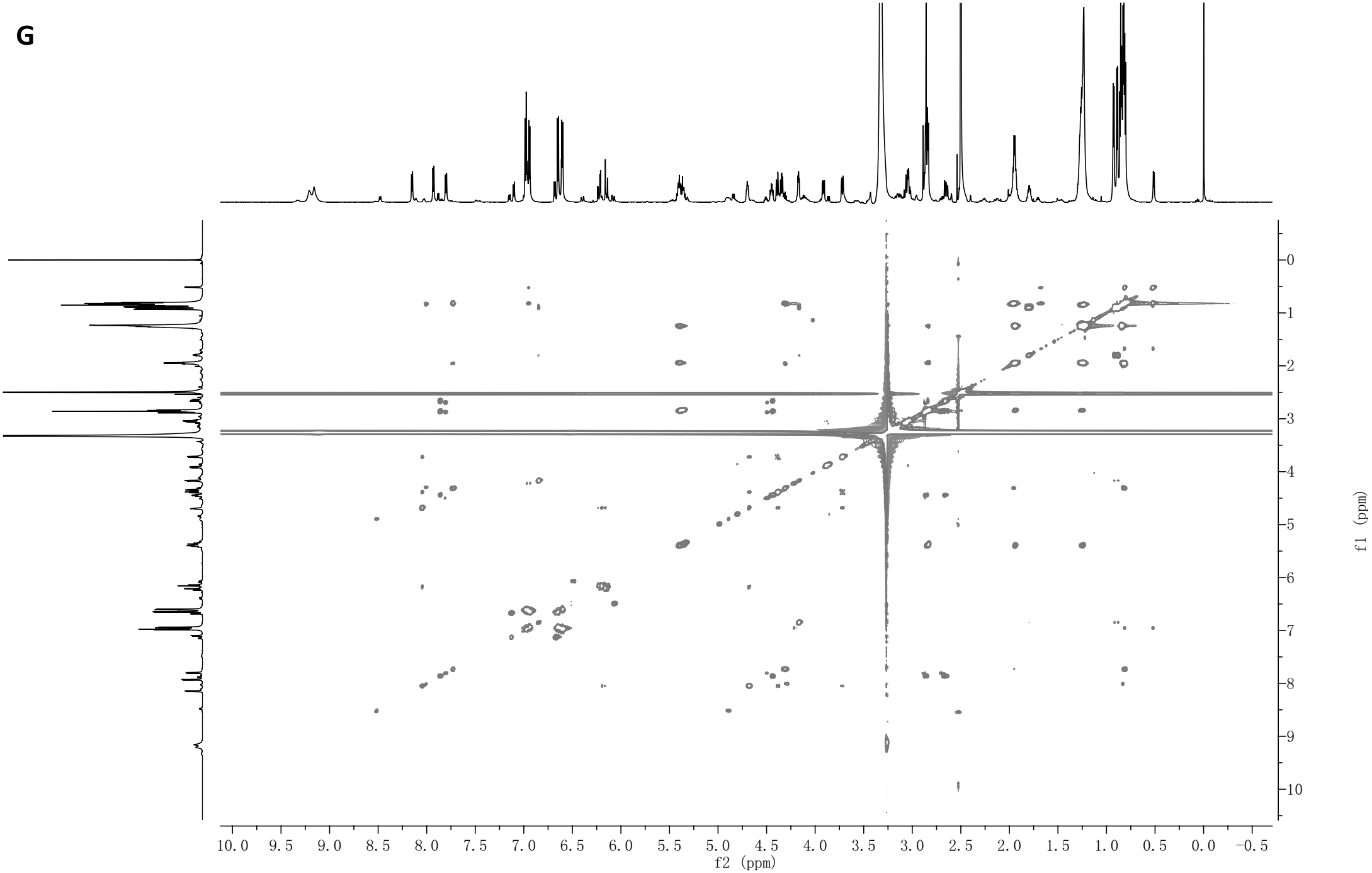
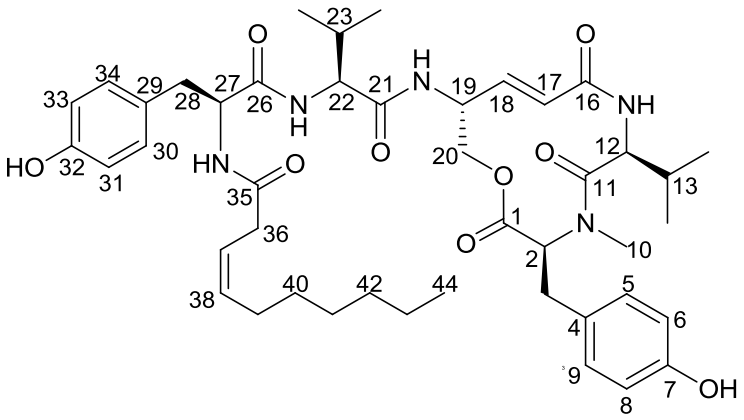


Figure S9. Characterization of thalassospiramide C (7). (**G**) ^{1}H-^{1}H TOCSY spectrum in DMSO-d_5.

H



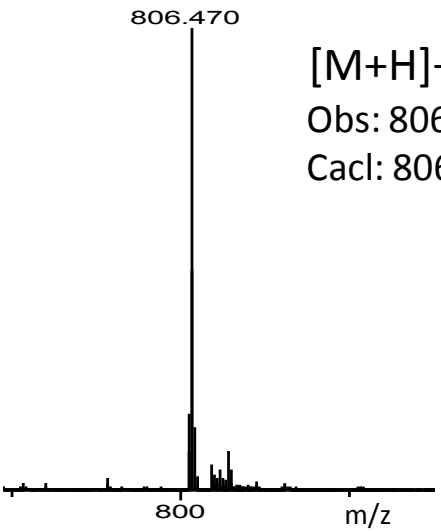
NMR Spectral Data (700 MHz (^{1}H), 175 MHz (^{13}C) in DMSO-d_6) of thalassospiramide C (**7**)

C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1			169.6	C	
2	3.9	dd(10.4,5.1)	66	CH	1,3,10
3a	3.03	dd(14.1,4.4)	33.15	CH_2	2,5,9
3b	3.06	dd(14.1,10.9)			2,5,9
4			128.1	C	
5	6.94	d(8.4)	129.9	CH	3,9
6	6.65	d(8.4)	115	CH	4,7,8
7			155.8	C	
7-OH	6.68	d(8.4)			6,7,8
8	6.65	d(8.4)	115	CH	4,6,7
9	6.94	d(8.4)	129.9	CH	3,5
10	2.85	s	40.1	CH_3	2,11
11			170.9	C	
12	4.17	dd(6.0,4.5)	57	CH	11,13,14,15
12-NH	6.98	d(7.0)			12,13,16,17
13	1.82	m	28.6	CH	
14	0.93	d(7.0)	19.2	CH_3	12,13,15
15	0.89	d(7.0)	16	CH_3	12,13,14
16			169.9	C	
17	6.16	d(15.4)	125.4	CH	16,18,19
18	6.22	dd(16.1,4.9)	138.5	CH	16,17,19
19	4.7	m	48.1	CH	17,18
19-NH	8.16	d(7)			19,21
20a	3.72	dd(11.6,2.2)	63.1	CH_2	18
20b	4.38	dd(11.6,2.2)			1
21			170.6	C	
22	4.34	dd(7.7,6.3)	57.5	CH	21,23,24
22-NH	7.8	d(7.0)			26
23	1.98	m	31.2	CH	22,24,25
24	0.83	m	19.4	CH_3	22,23,24
25	0.83	m	18	CH_3	22,23,24
26			171.24	C	
27	4.45	m	53.8	CH	
27-NH	7.93	d(7.0)			35
28a	2.66	dd(13.9,9.7)	36.5	CH_2	27,29,30,34
28b	2.84	m			29,30,34

29			127.7		
30	6.98	d(8.5)	130	CH	28,34
31	6.6	d(8.5)	114.8	CH	29,32,33
32			155.8		
32-OH	7.1	d(7.3)			30,32,34
33	6.6	d(8.5)	114.8	CH	29,31,32
34	6.98	d(8.5)	130	CH	28,30
35			170.2		
36	2.88	m	33.9	CH_2	35,37,38
37	5.4	dtt(11.9,8,2.7)	131.7	CH	36
38	5.37	dtt(11.9,8,2.7)	123.1	CH	39
39	1.95	m	26.8	CH_2	37,38,40
40	1.24	m	28.8	CH_2	41
41	1.24	m	28.2	CH_2	42
42	1.24	m	31.2	CH_2	41
43	1.25	m	22.1	CH_2	42
44	0.83	t (7.0)	13.9	CH_3	42,43

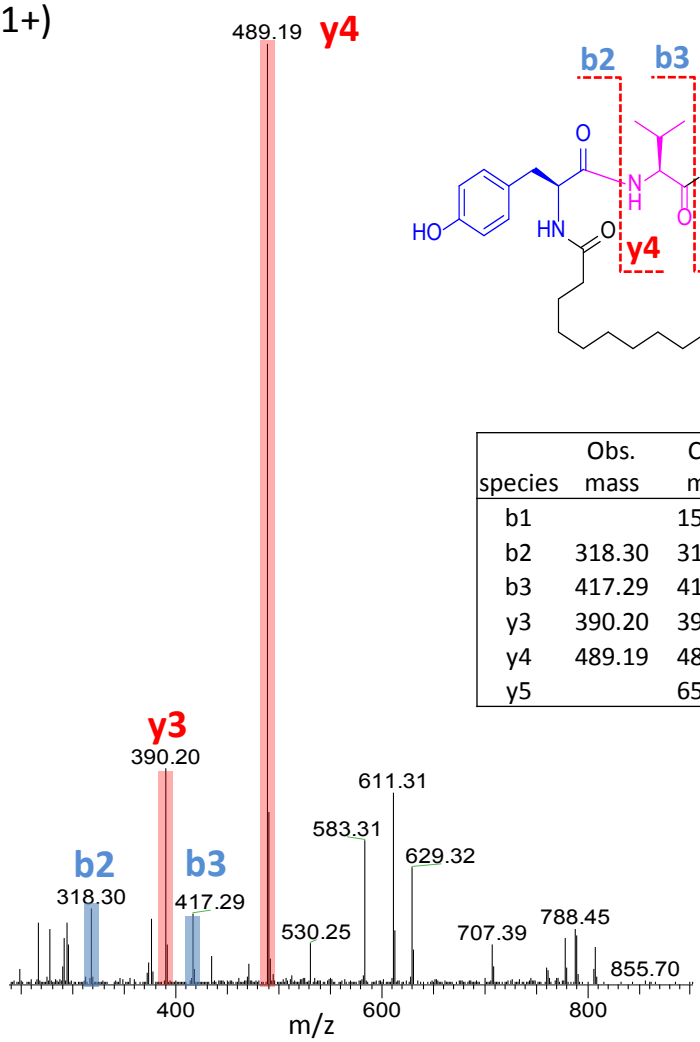
Figure S9. Characterization of thalassospiramide C. (**H**) NMR assignment.

A ESI-TOF-HRMS



[M+H]+
Obs: 806.470
Cacl: 806.470

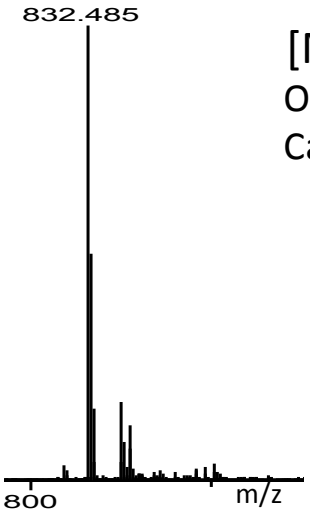
ITMS^2 – 806 m/z (1+)



species	Obs. mass	Calc. mass	Error [Da]
b1		155.14	
b2	318.30	318.21	-0.09
b3	417.29	417.28	-0.01
y3	390.20	390.21	0.01
y4	489.19	489.27	0.08
y5		652.33	

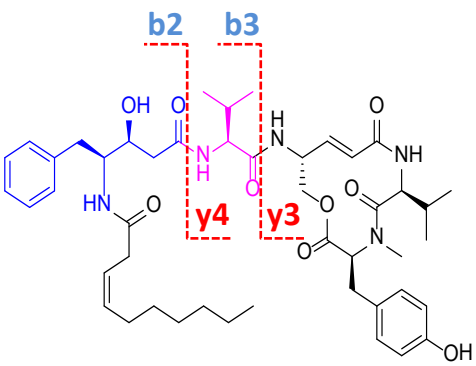
Figure S10. Characterization of thalassospiramide C1 (8). (**A**) MS^n analysis.

A ESI-TOF-HRMS



[M+H]+
Obs: 832.485
Cacl: 832.486

ITMS^2 – 832 m/z (1+)



species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2		344.22	
b2-H_2O	326.29	326.21	-0.08
b3	443.33	443.30	-0.03
y3	390.26	390.21	-0.05
y4	489.31	489.27	-0.04
y5		680.37	

Figure S11. Characterization of thalassospiramide D (14). (**A**) MS^n analysis.

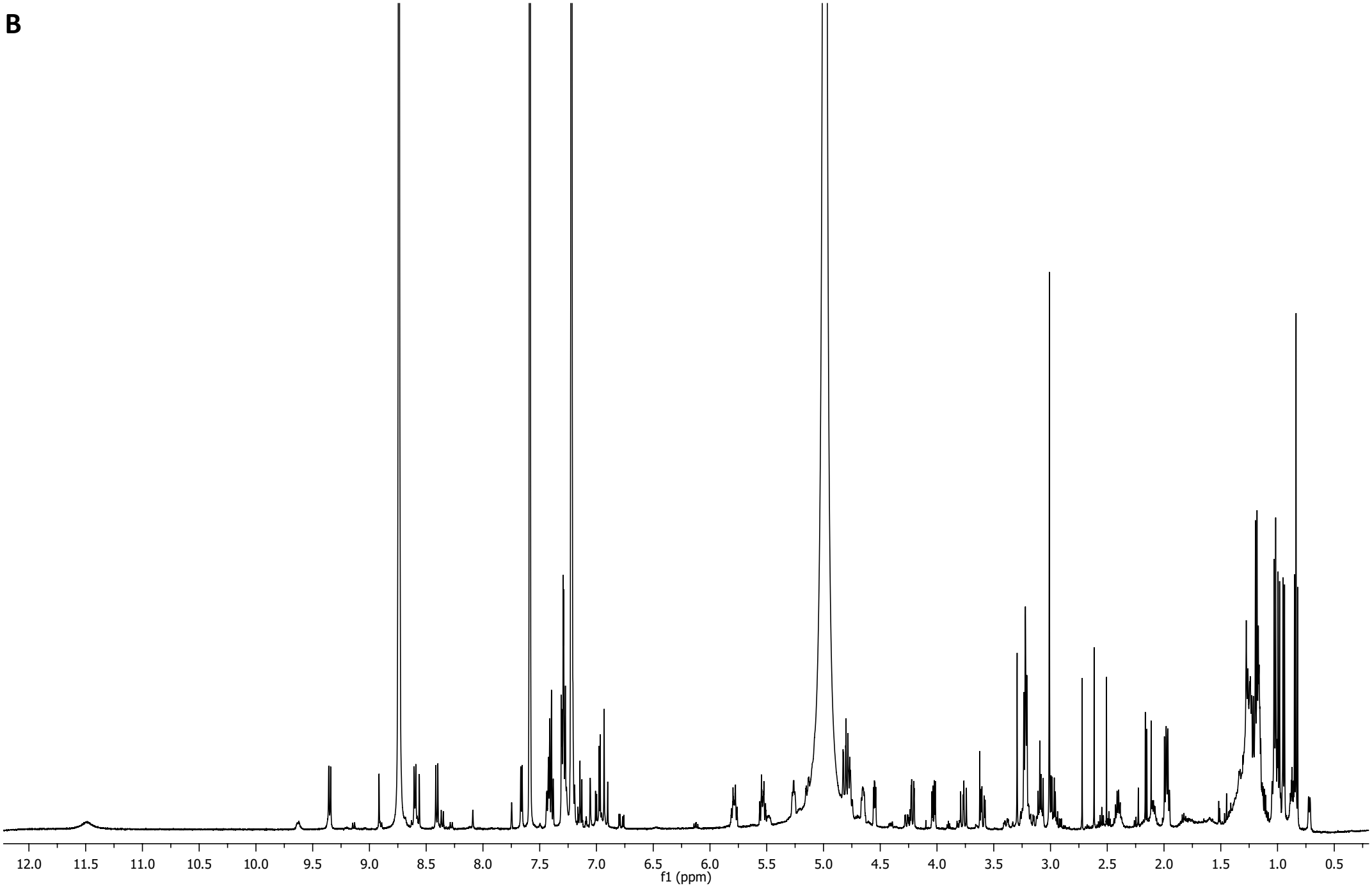


Figure S11. Characterization of thalassospiramide D (14). (**B**) ^{1}H spectrum in pyridine-d_5.

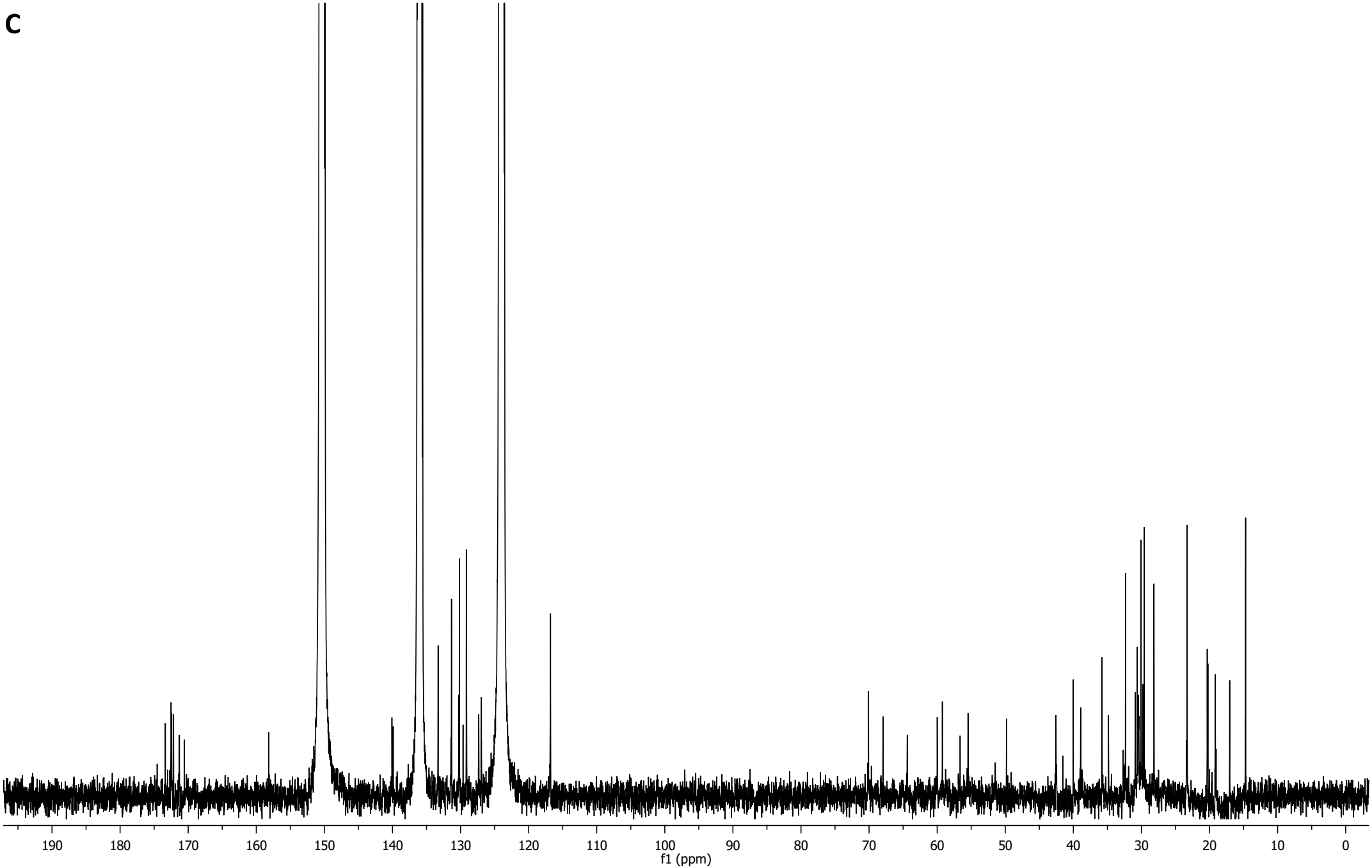


Figure S11. Characterization of thalassospiramide D (14). (**C**) ^{13}C spectrum in pyridine-d_5.

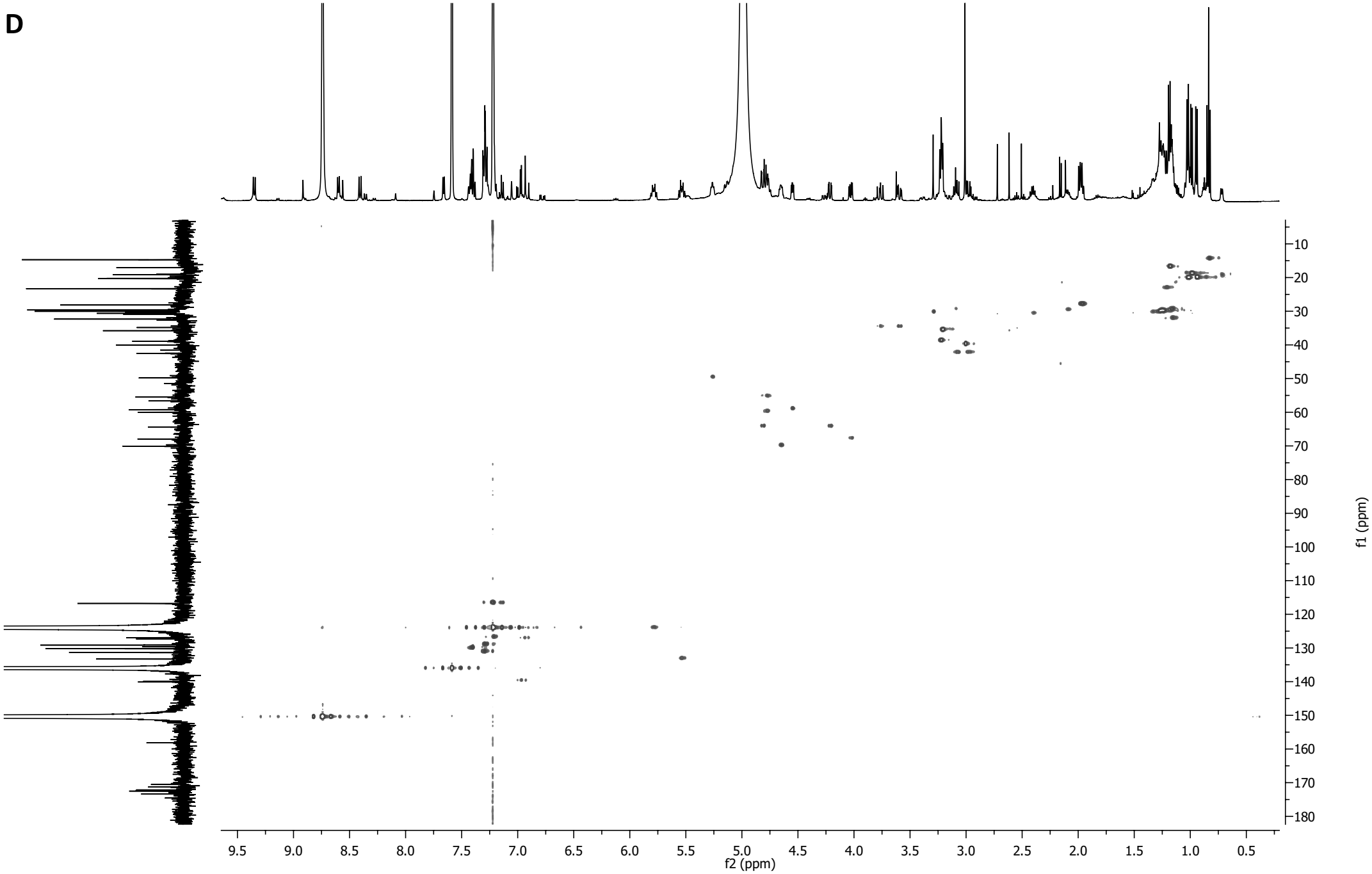


Figure S11. Characterization of thalassospiramide D (14). (**D**) HMQC spectrum in pyridine-d_5.

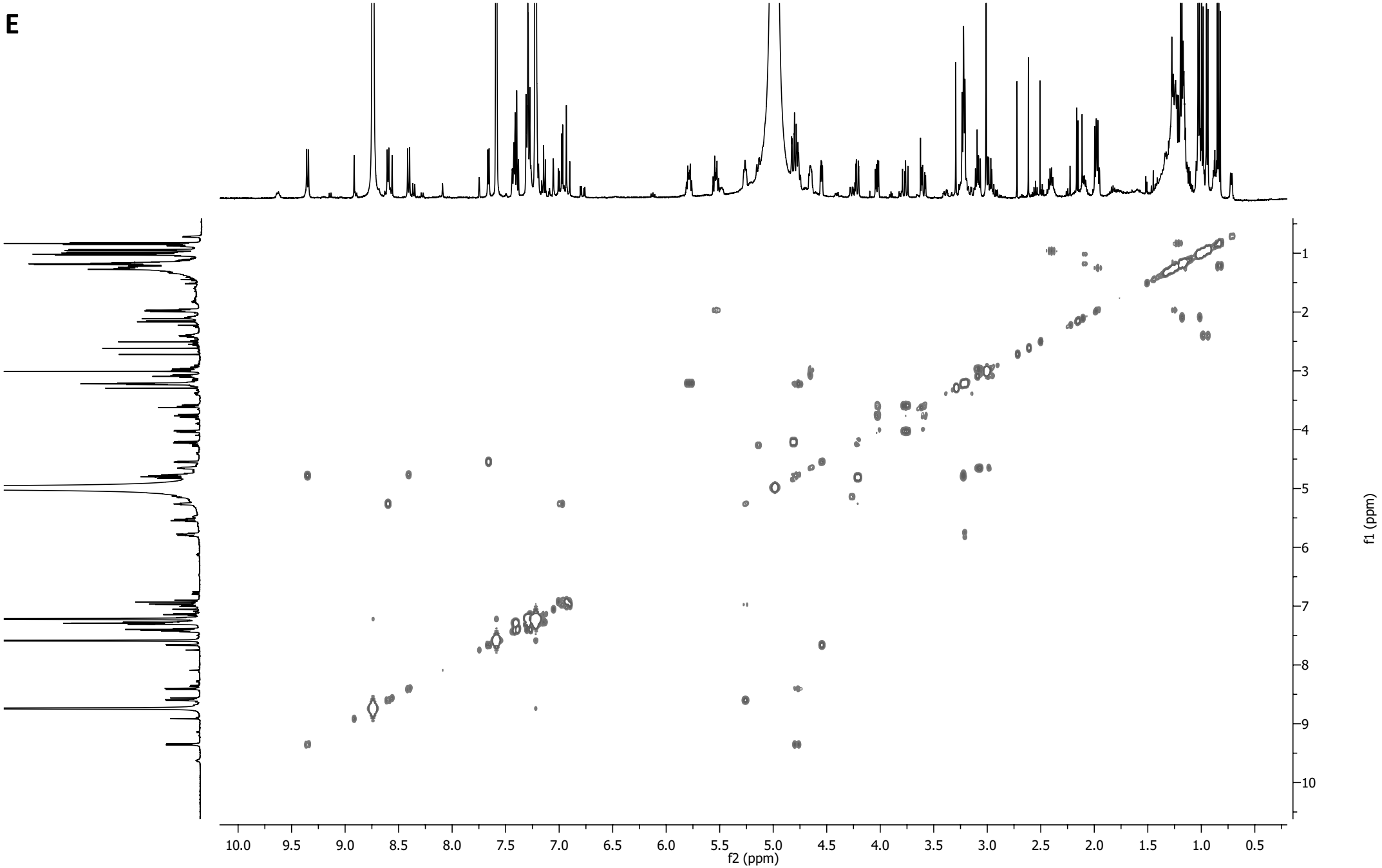


Figure S11. Characterization of thalassospiramide D (14). (**E**) ^{1}H-^{1}H COSY spectrum in pyridine-d_5.

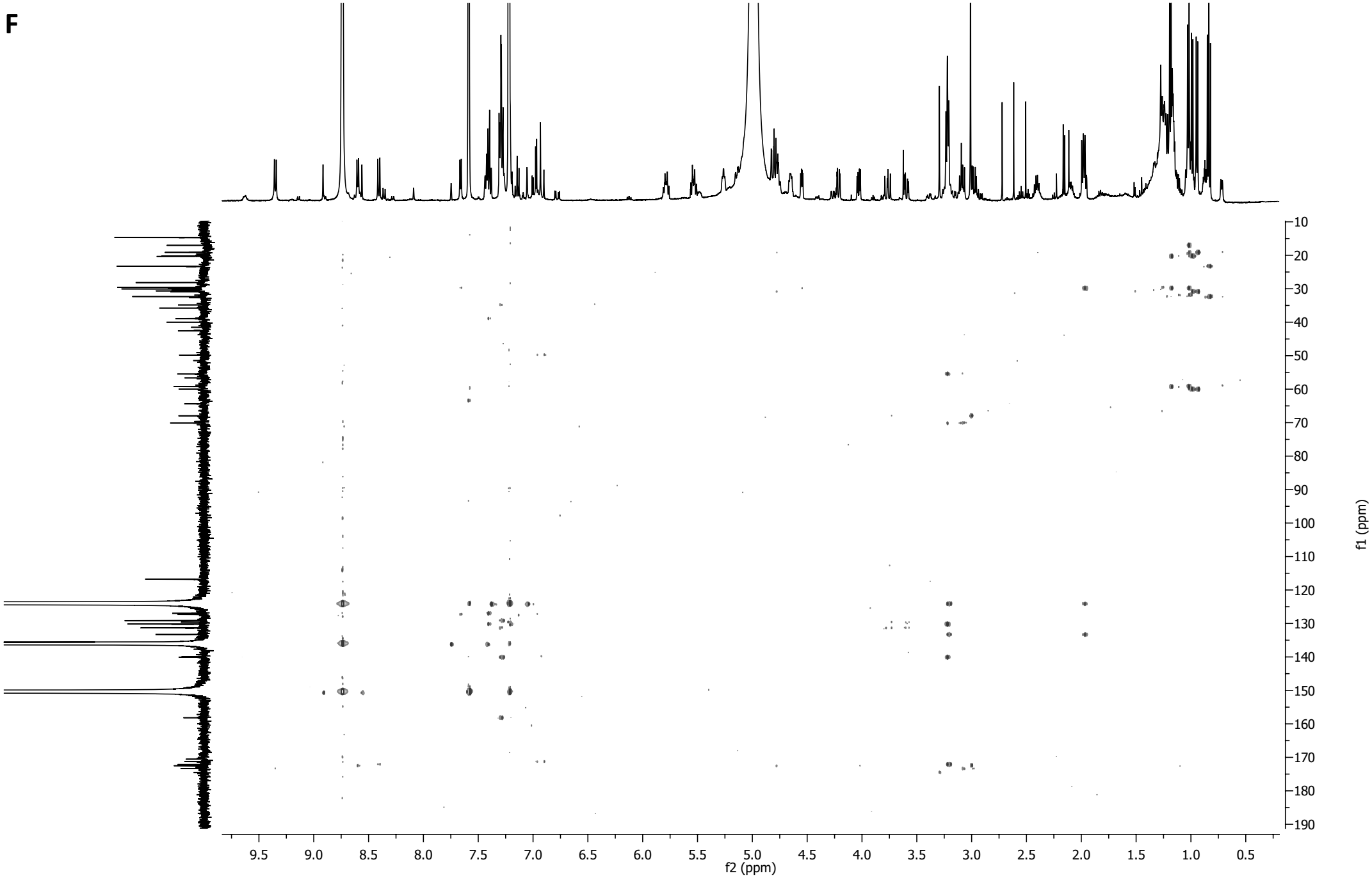
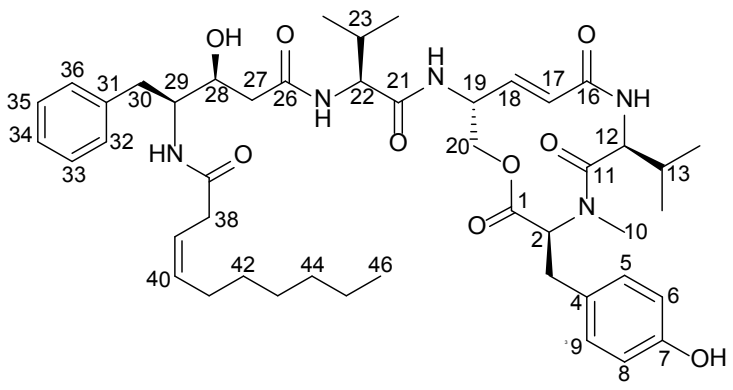


Figure S11. Characterization of thalassospiramide D (14). (**F**) HMQC spectrum in pyridine-d_5.

G



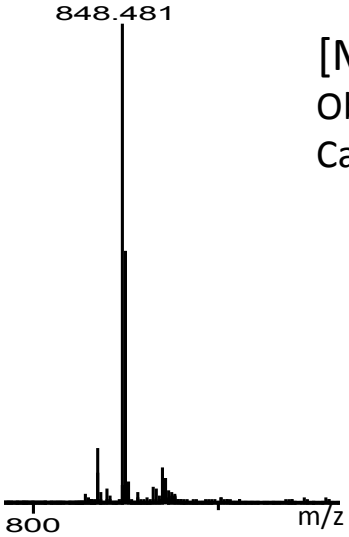
NMR Spectral Data (500 MHz (^{1}H), 125 MHz (^{13}C) in pyridine-d_5) of thalassospiramide D (**14**)

C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1			170.6	C	
2	4.03	dd (10.8, 4.4)	68.0	CH	1
3a	3.59	dd (14.0, 4.4)	34.8	CH_2	
3b	3.77	dd (14.0, 11.0)			
4			129.6	C	
5	7.30	m	131.3	CH	7
6	7.22	m	116.8	CH	4
7			158.2	C	
7-OH					
8	7.22	m	116.8	CH	4
9	7.30	m	131.3	CH	7
10	3.01	s	40.0	CH_3	2
11			172.4		
12	4.55	dd (6.2, 4.0)	59.2	CH	
12-NH	7.66	dd (6.3)			
13	2.09	m	29.8	CH	
14	1.02	d (7.0)	20.3	CH_3	
15	1.19	d (7.0)	17.0	CH_3	
16			171.3	C	
17	6.92	d (15.8)	127.3	CH	16
18	6.99	dd (15.8, 5.0)	139.9	CH	16
19	5.26	m	49.8	CH	
19-NH	8.60	d (7.5)			21
20a	4.21	dd (11.3, 2.4)	64.4	CH_2	
20b	4.82	dd (11.3, 2.0)			1
21			172.5	C	
22	4.77	m	60.0	CH	
22-NH	9.35	d (8.5)			26
23	2.41	m	30.7	CH	21
24	0.95	d (6.8)	20.2	CH_3	22
25	0.99	d (6.8)	19.1	CH_3	22
26			173.4	C	
27a	2.99	dd (14.5, 4.5)	42.5	CH_2	
27b	3.08	dd (14.5, 8.3)			
28	4.65	m	70.1	CH	

28-OH					
29	4.79	m	55.8	CH	30
29-NH	8.41	d (9.1)			37
30	3.22	m	38.9	CH_2	
31			140.1	C	
32	7.40	m	130.2	CH	
33	7.28	m	129.2	CH	31
34	7.42	m	130.2	CH	
35	7.28	m	129.2	CH	
36	7.40	m	130.2	CH	
37			172.1	C	
38	3.21	m	35.8	CH_2	37
39	5.79	dtt (11, 7.0, 1.5)	123.8	CH	
40	5.54	dtt (11, 7.0, 2.0)	133.3	CH	
41	1.98	m	28.2	CH_2	
42	1.27	m	30.1	CH_2	
43	1.17	m	29.6	CH_2	
44	1.15	m	32.3	CH_2	
45	1.22	m	23.3	CH_2	
46	0.84	t (7.1)	14.7	CH_3	

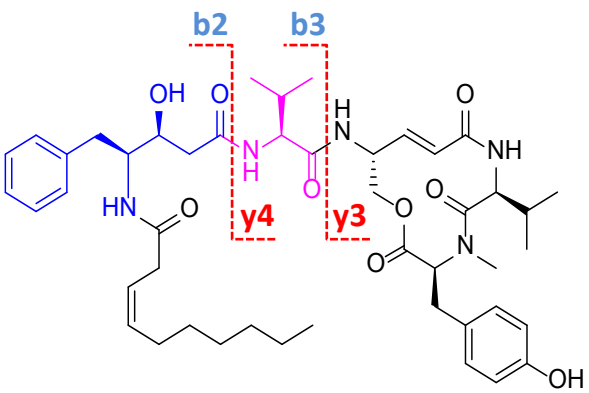
Figure S11. Characterization of thalassospiramide D (14). (**G**) NMR assignment.

A ESI-TOF-HRMS



[M+H]+
Obs: 848.481
Cacl: 848.480

ITMS^2 – 848 m/z (1+)



species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2		360.22	
b2-H_2O	342.26	342.21	-0.05
b3	459.33	459.29	-0.04
y3	390.28	390.21	-0.07
y4	489.24	489.27	0.03
y5		696.36	

Figure S12. Characterization of thalassospiramide D1 (15). (**A**) MS^n analysis.

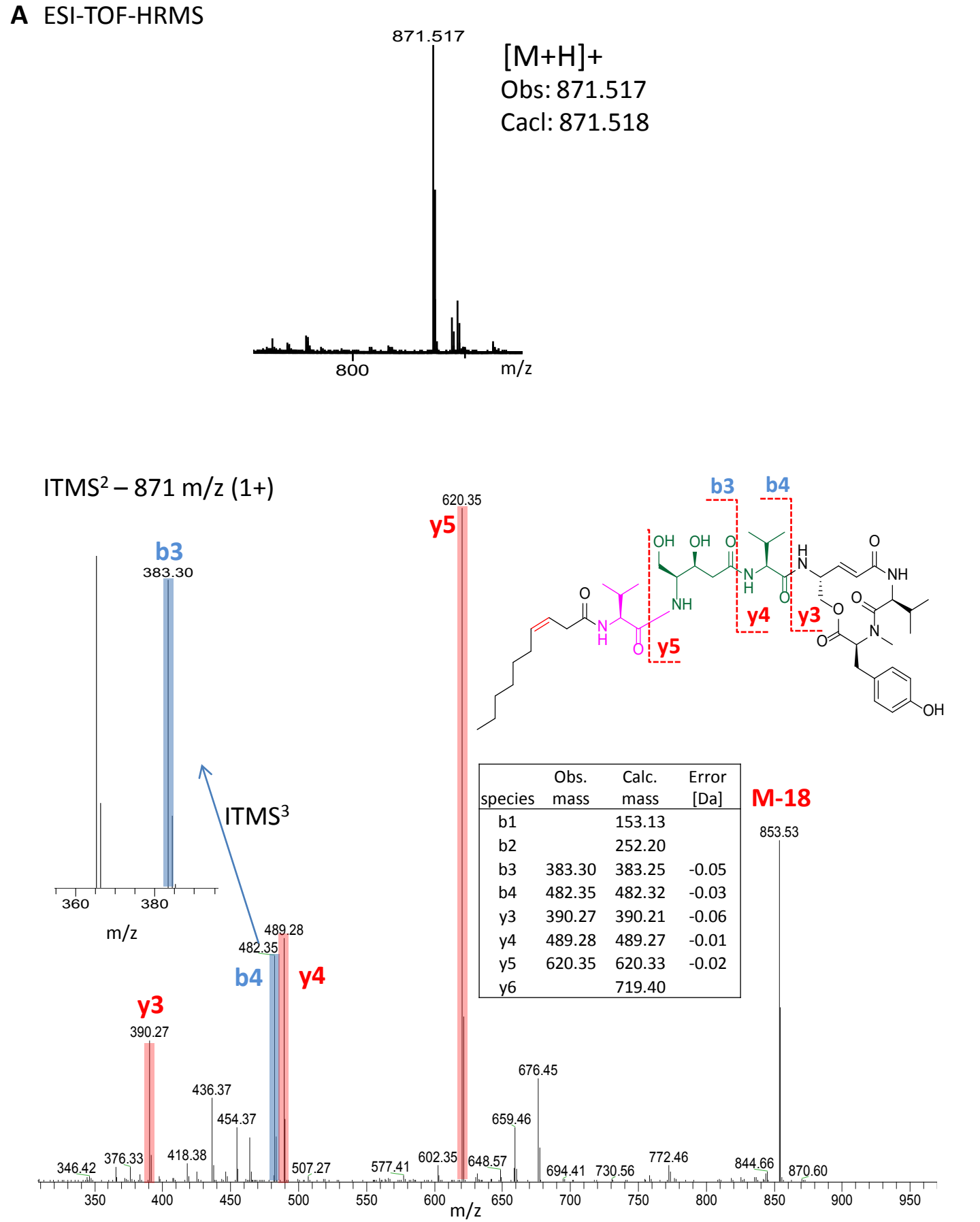


Figure S13. Characterization of thalassospiramide E (9). (**A**) MS^n analysis.

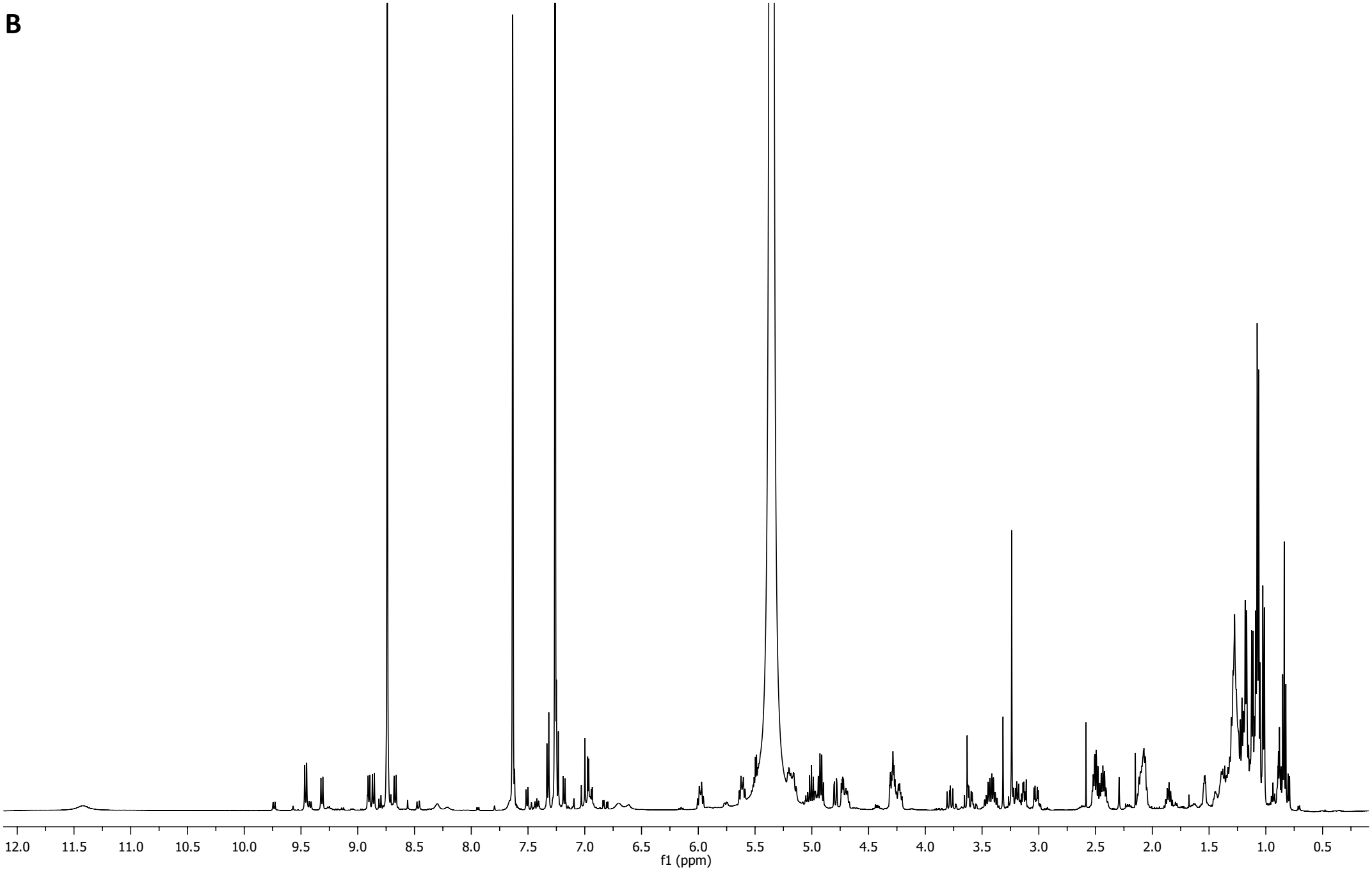


Figure S13. Characterization of thalassospiramide E (9). (**B**) ^{1}H spectrum in pyridine-d_5.

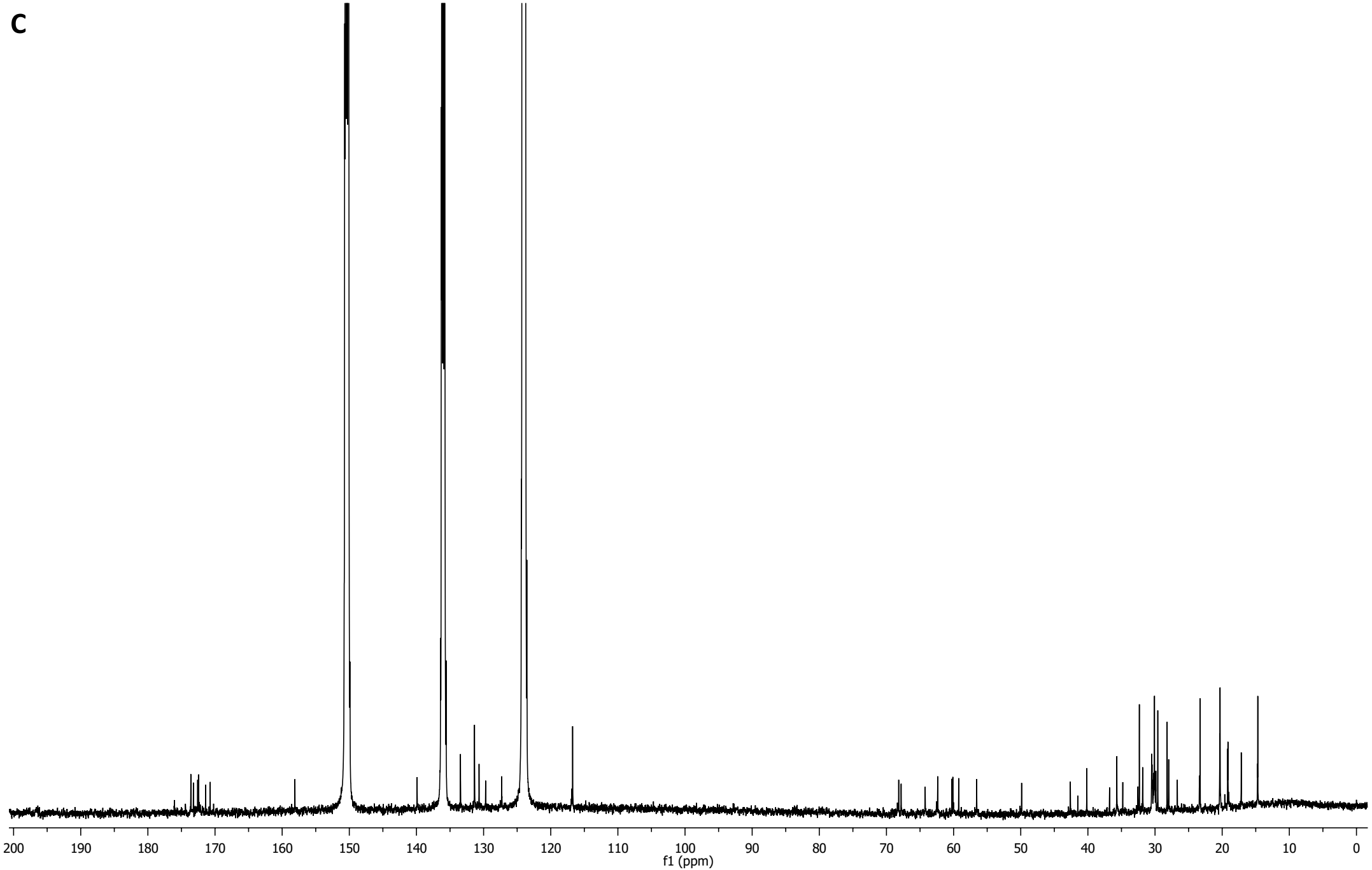


Figure S13. Characterization of thalassospiramide E (9). (**C**) ^{13}C spectrum in pyridine-d_5.

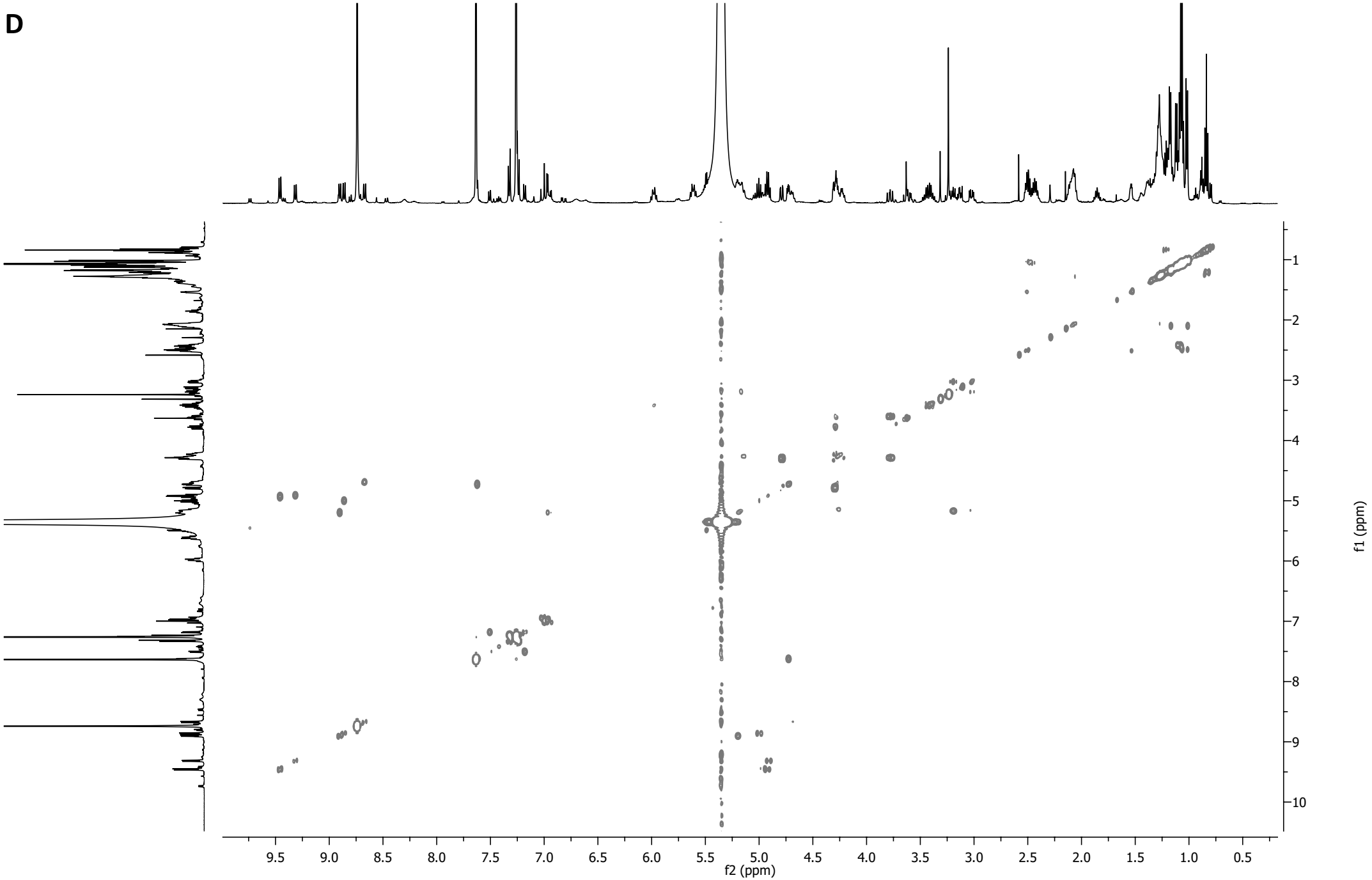


Figure S13. Characterization of thalassospiramide E (9). (**D**) ^{1}H-^{1}H COSY spectrum in pyridine-d_5.

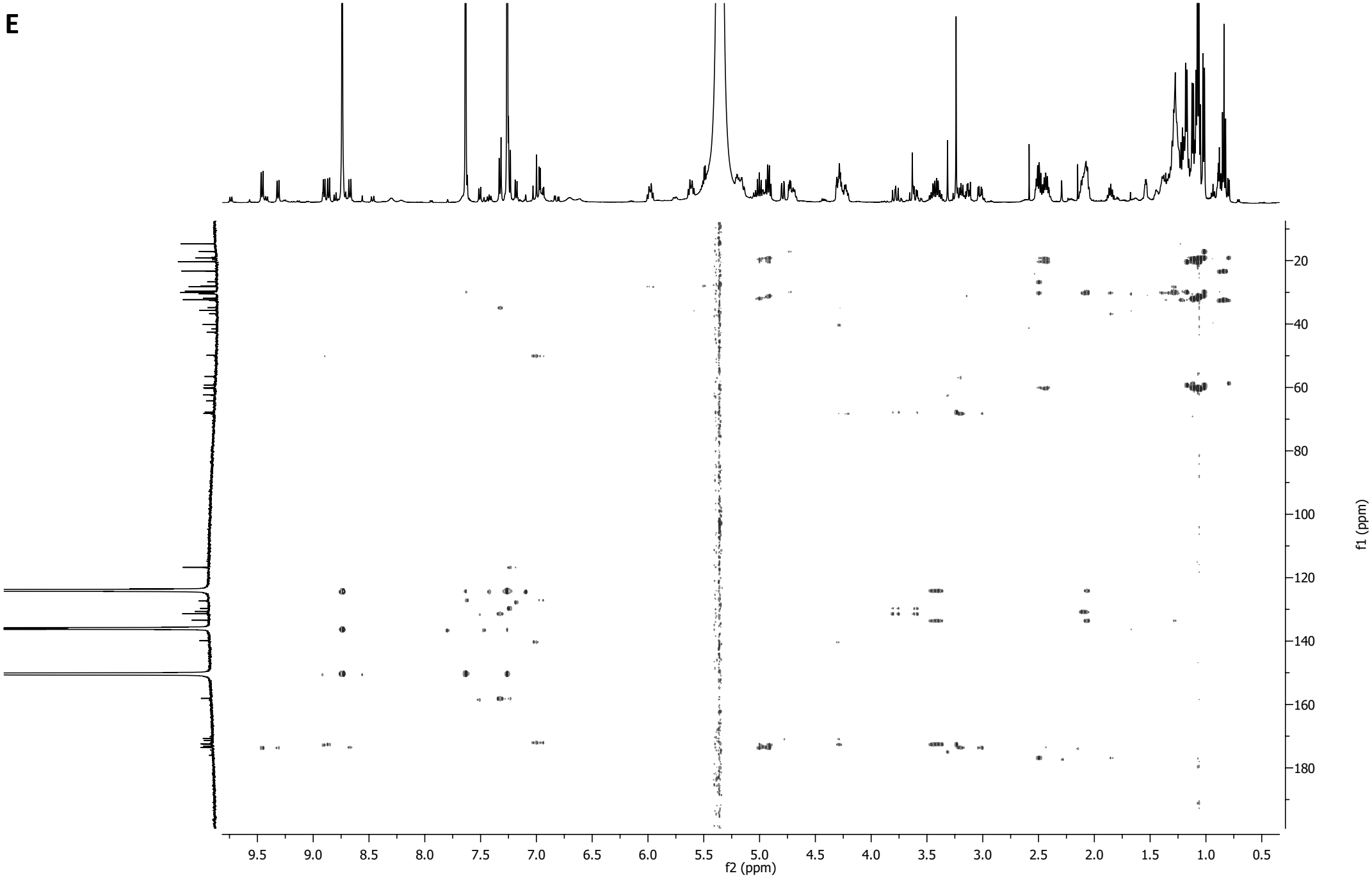
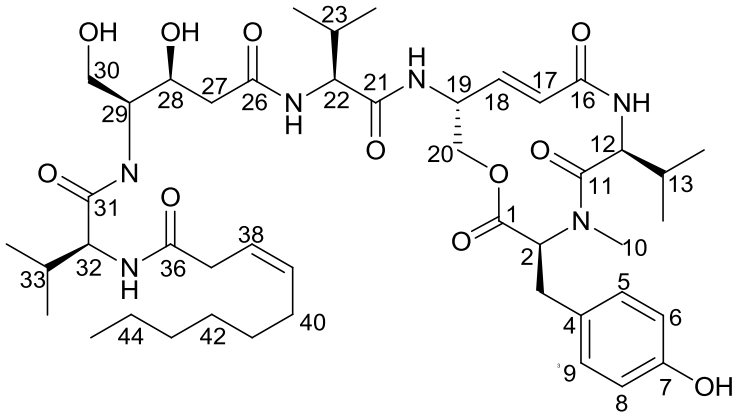


Figure S13. Characterization of thalassospiramide E (9). (**E**) HMQC spectrum in pyridine-d_5.

F



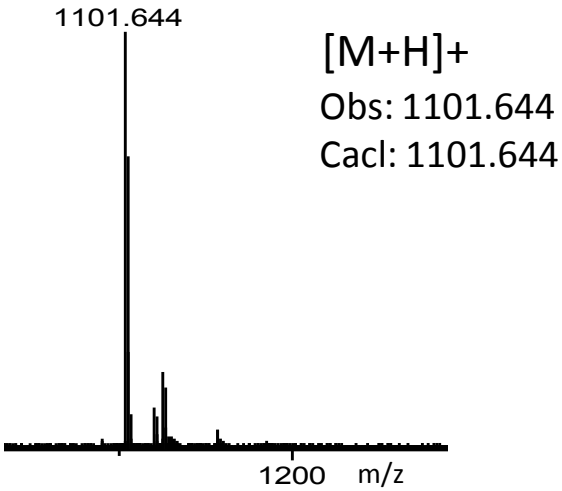
NMR Spectral Data (500 MHz (^{1}H), 125 MHz (^{13}C) in pyridine-d_5) of thalassospiramide E (**9**)

C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1			170.7	C	
2	4.26	m	67.8	CH	1, 10
3a	3.61	dd (14.0, 4.5)	34.8	CH_2	4
3b	3.79	dd (14.0, 11.0)			4
4			129.7	C	
5	7.33	d (8.4)	131.3	CH	3
6	7.24	d (8.4)	116.7	CH	3
7			158.0	C	
7-OH					
8	7.24	d (8.4)	116.7	CH	3
9	7.33	d (8.4)	131.3	CH	3
10	3.24	s	40	CH_3	2, 11
11			172.5		
12	4.73	dd (6.0, 4.1)	59.2	CH	
12-NH	7.62				16
13	2.09	m	29.8	CH	
14	1.02	d (6.8)	20.2	CH_3	
15	1.18	d (6.8)	17.0	CH_3	
16			171.4	C	
17	7.01	d (15.8)	127.3	CH	16, 19
18	6.95	dd (15.7, 4.7)	139.9	CH	16
19	5.18	m	49.8	CH	
19-NH	8.90	d (7.2)			21
20a	4.79	dd (11.0, 1.5)	64.2	CH_2	1
20b	4.31	m			1
21			172.5	C	
22	4.92	dd (6.0, 4.1)	60.2	CH	
22-NH	9.46	d (8.5)			21, 26
23	2.44	m	30.3	CH	21
24	1.07	d (7.0)	20.3	CH_3	
25	1.12	d (7.0)	19.2	CH_3	
26			173.6	C	
27a	3.19	dd (14.0, 5.2)	42.6	CH_2	
27b	3.03	dd (14.0, 8.5)			
28	5.18	m	68.2	CH	
28-OH					
29	4.69	m	56.6	CH	30

29-NH	8.68	d (8.9)			31
30a	4.20	m	62.4	CH_2	
30b	4.30	m			
30-OH					
31			173.6	C	
32	5.01	m	60.1	CH	
32-NH	8.86	d (8.7)			31, 36
33	2.51	m	32.2	CH	
34	1.02	d (6.7)	19.2	CH_3	
35	1.05	d (6.5)	20.2	CH_3	
36			172.5	C	
37	3.42	m	31.8	CH_2	
38	5.98	dtt (11.0, 7.5, 2.0)	123.5	CH	
39	5.61	dtt (11.0, 7.5, 1.5)	133.4	CH	
40	2.07	m	28.2	CH_2	
41	1.27	m	29.9	CH_2	
42	1.17	m	29.6	CH_2	
43	1.30	m	30.5	CH_2	
44	1.22	m	23.3	CH_2	
45	0.84	t (7.0)	14.7	CH_3	

Figure S13. Characterization of thalassospiramide E (9). (**F**) NMR assignment.

A ESI-TOF-HRMS



ITMS^2 – 1101 m/z (1+)

species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2		252.20	
b3		383.25	
b4	482.48	482.32	-0.16
b5		613.38	
b5-H_2O	595.48	595.37	-0.11
b6	712.49	712.45	-0.04
y3	390.24	390.21	-0.03
y4	489.24	489.27	0.03
y5	620.30	620.33	0.03
y6	719.45	719.40	-0.05
y7	850.46	850.46	0.00
y8		949.52	

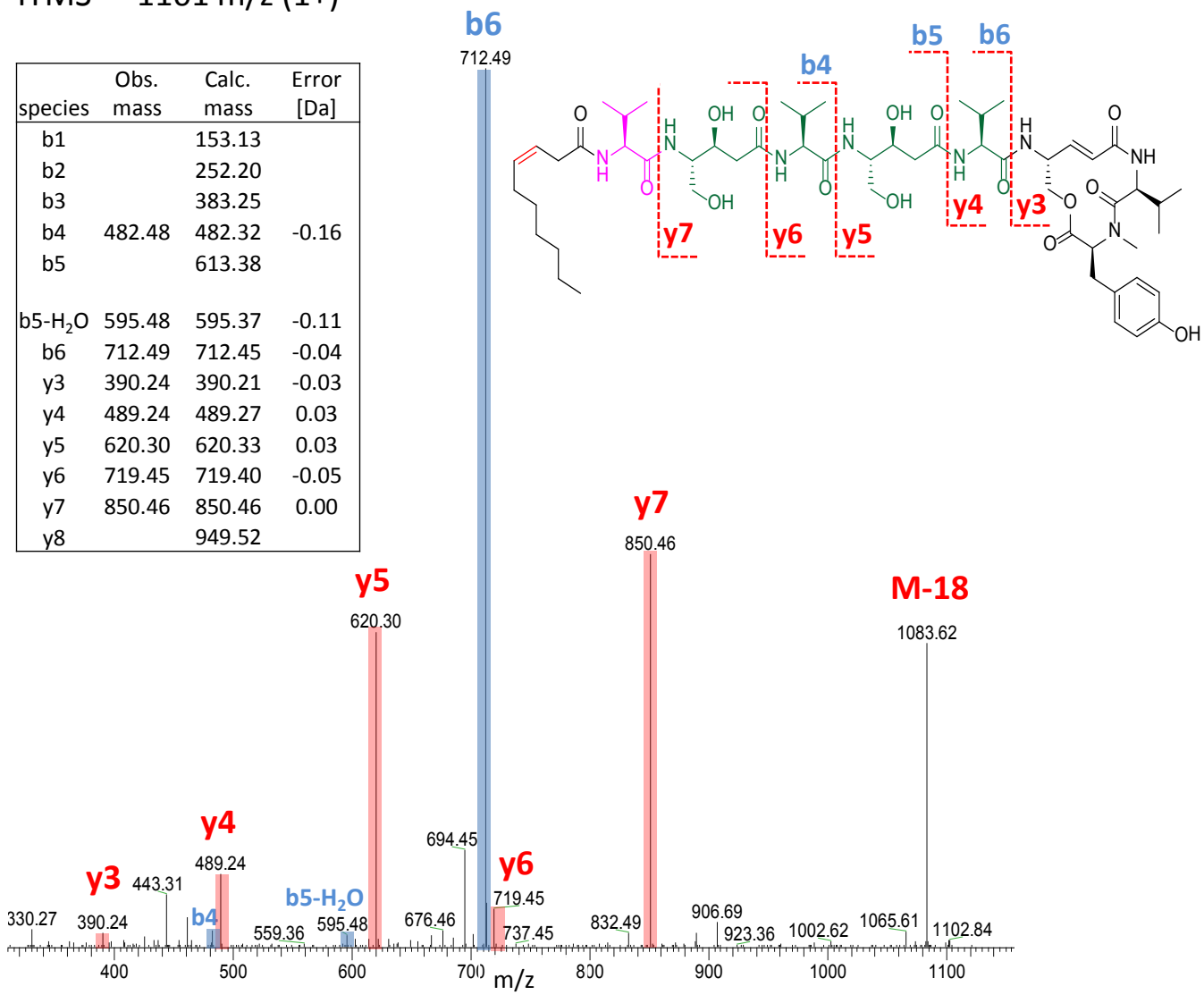
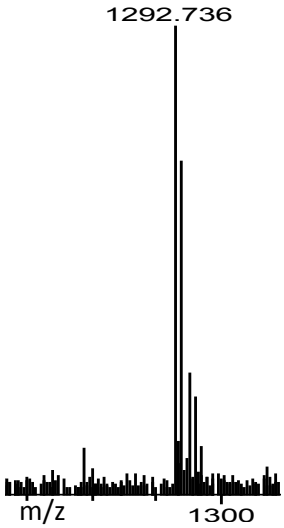


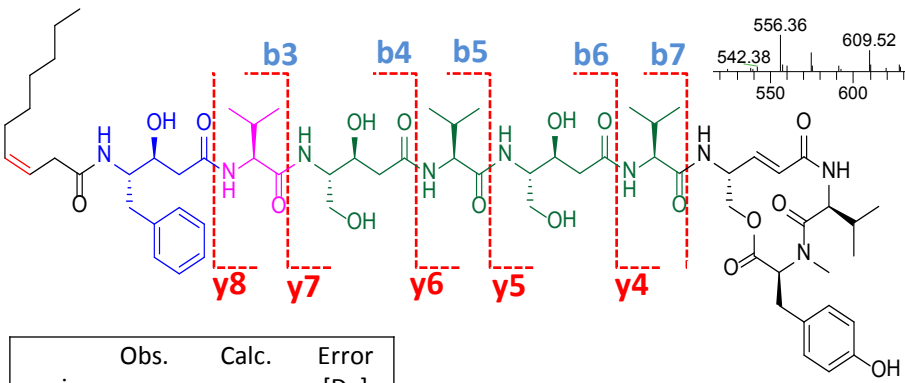
Figure S14. Characterization of thalassospiramide E1 (10). (**A**) MS^n analysis.

A ESI-TOF-HRMS

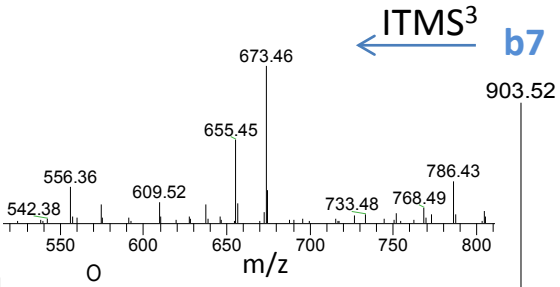


[M+H]+
Obs: 1292.736
Cacl: 1292.739

ITMS^2 – 1293 m/z (1+)



ITMS^3



species	Obs. mass	Calc. mass	Error [Da]
b1		153.13	
b2		344.22	
b3	443.31	443.29	0.02
b4		574.35	
b4-H_2O	556.36	556.34	0.02
b5	673.42	673.42	0.00
b6		804.48	
b6-H_2O	786.43	786.47	-0.04
b7	903.52	903.54	-0.02
y3		390.21	
y4	489.26	489.27	-0.01
y5	620.34	620.33	0.01
y6	719.40	719.40	0.00
y7	850.45	850.46	-0.01
y8	949.56	949.52	0.04
y9		1140.62	

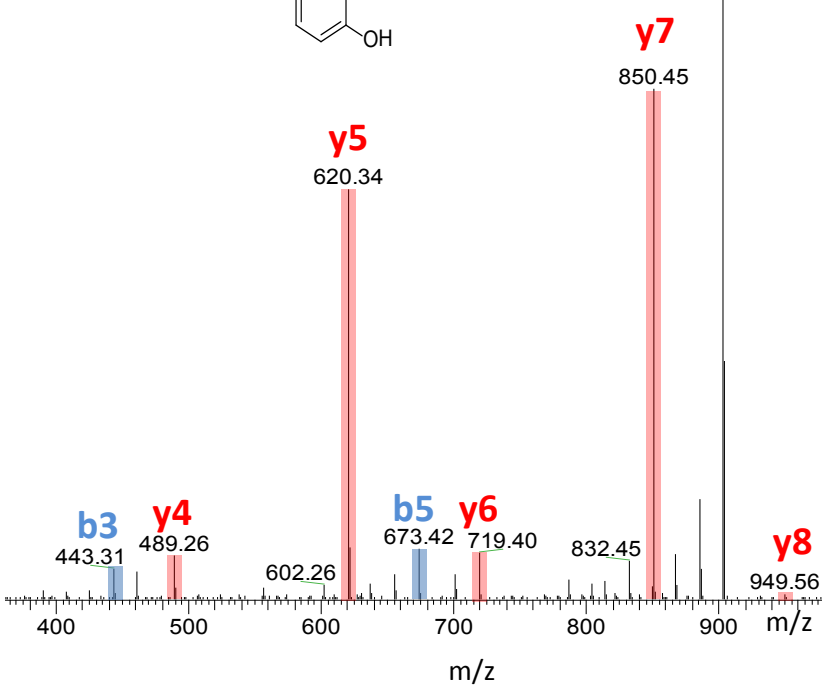


Figure S15. Characterization of thalassospiramide F (16). (**A**) MS^n analysis.

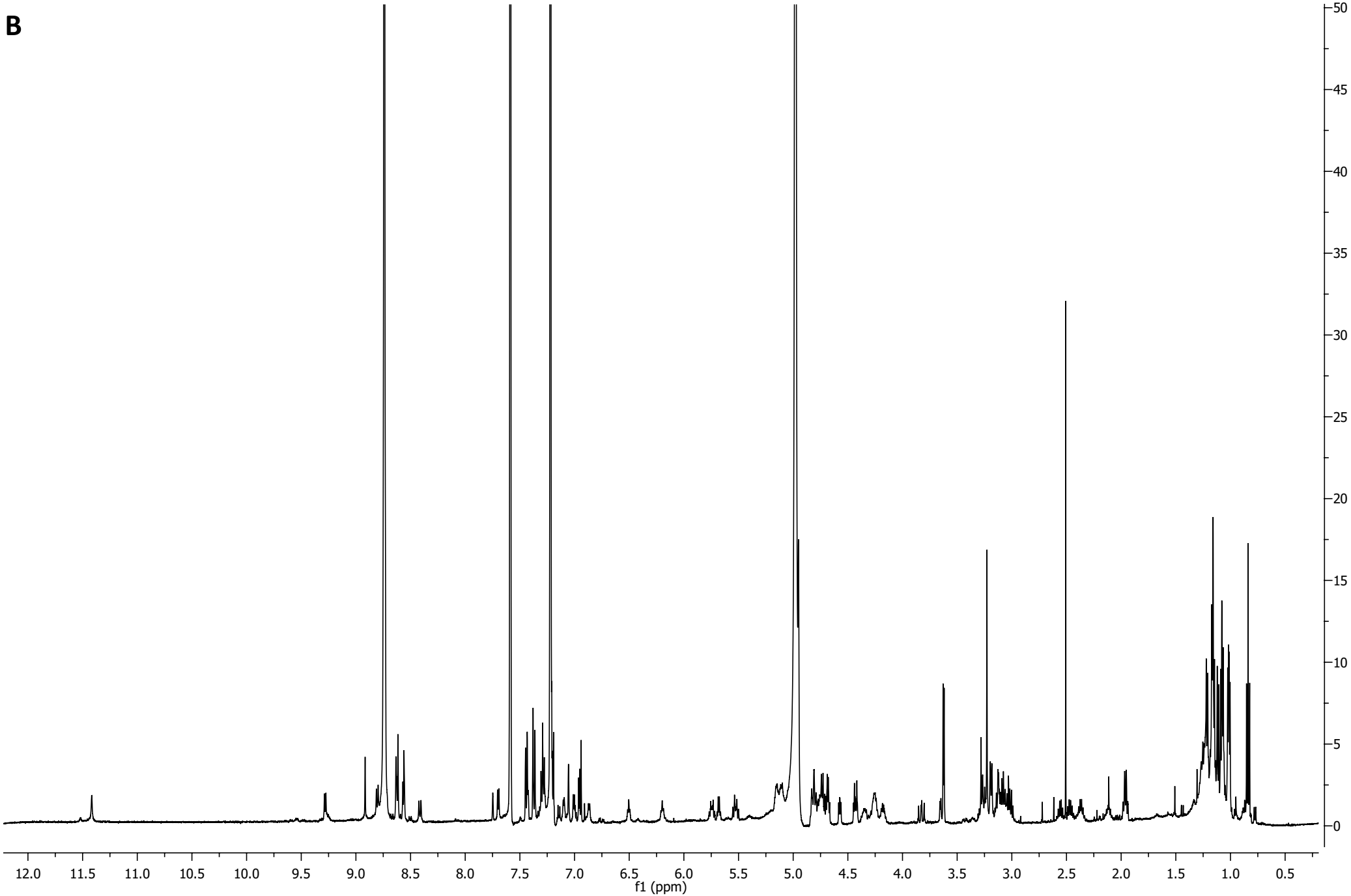


Figure S15. Characterization of thalassospiramide F. (**B**) ^{1}H spectrum in pyridine-d_5.

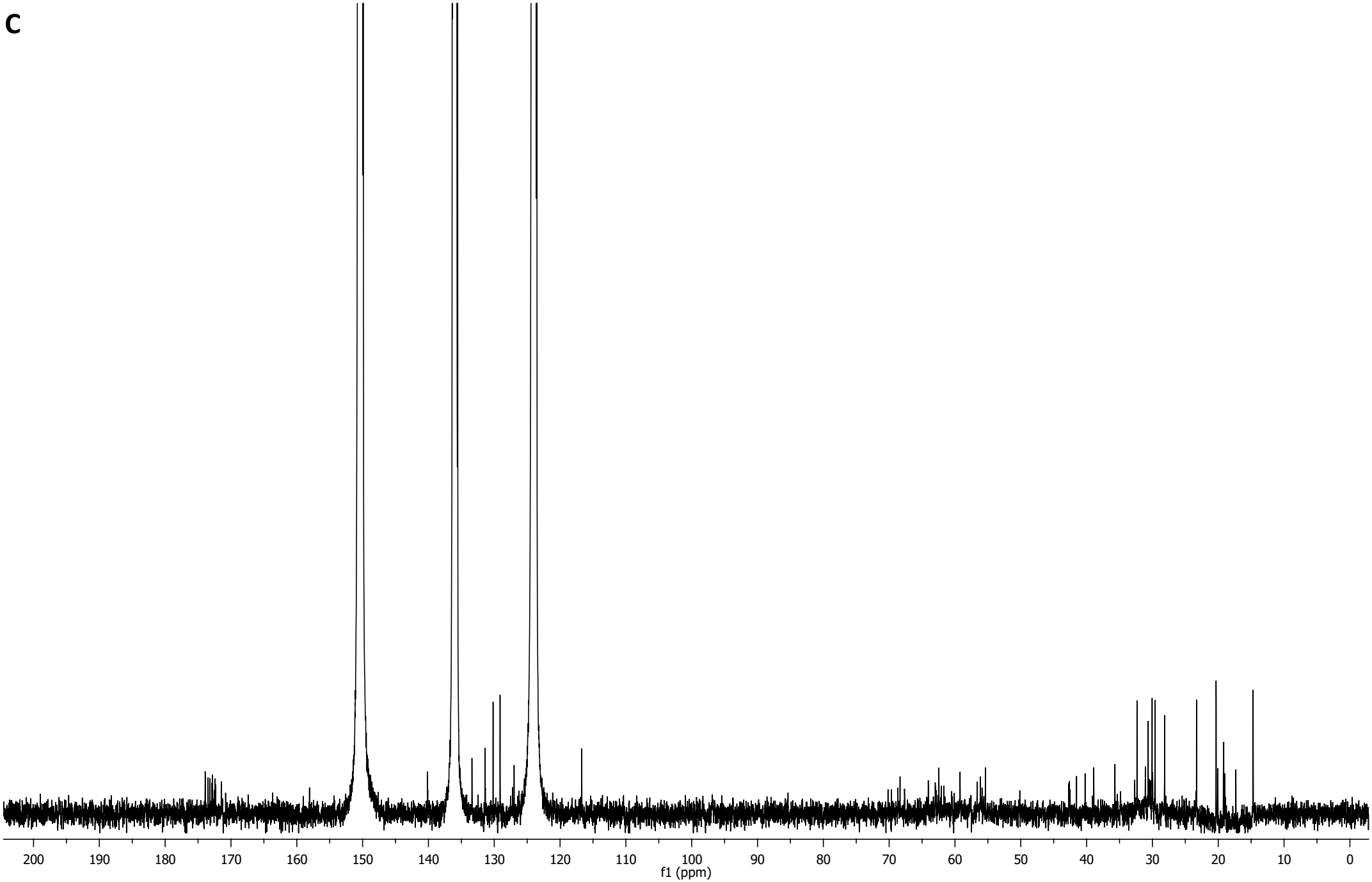


Figure S15. Characterization of thalassospiramide F (16). (**C**) ^{13}C spectrum in pyridine-d_5.

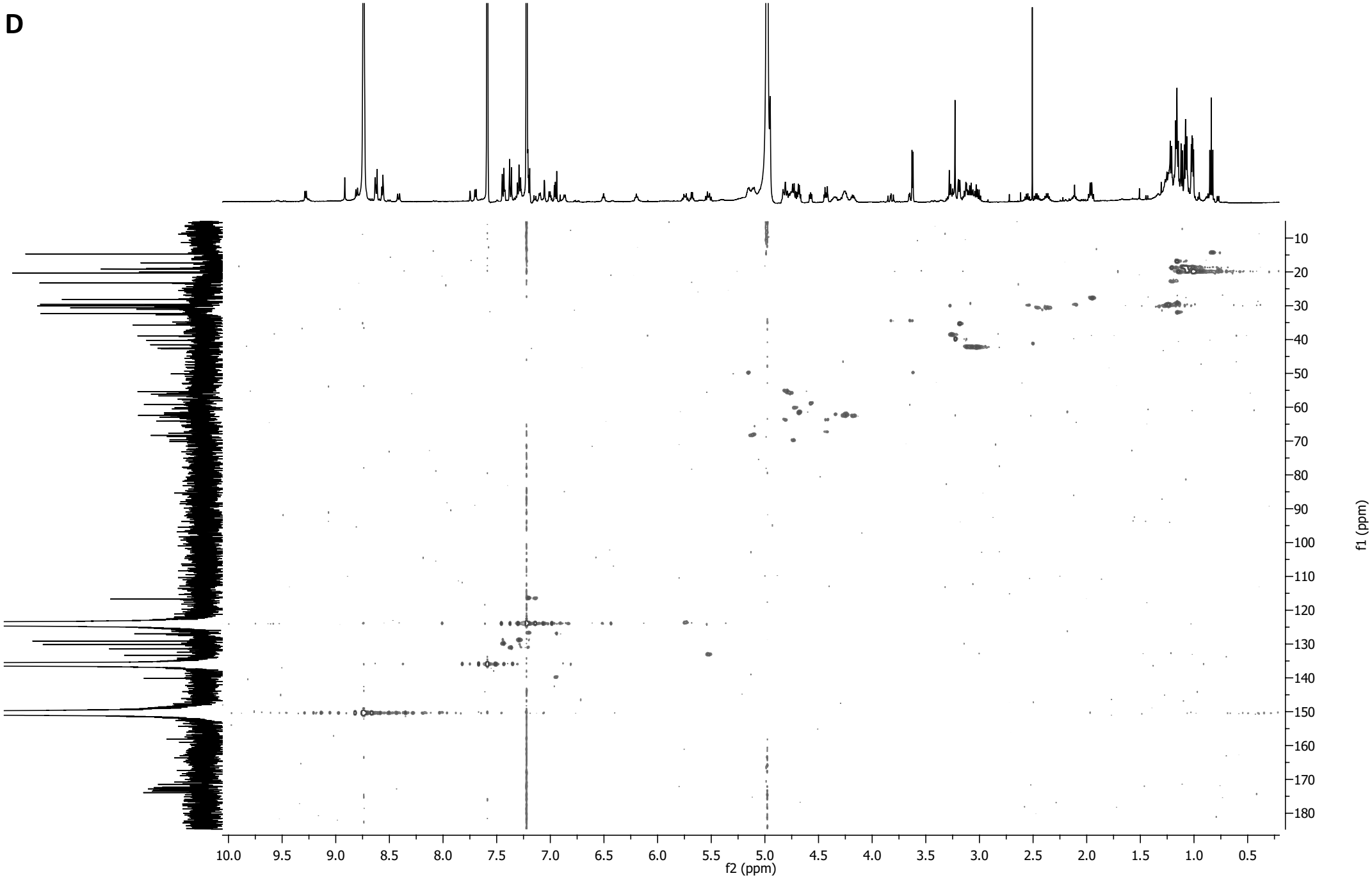


Figure S15. Characterization of thalassospiramide F (16). (**D**) HMQC spectrum in pyridine-d_5.

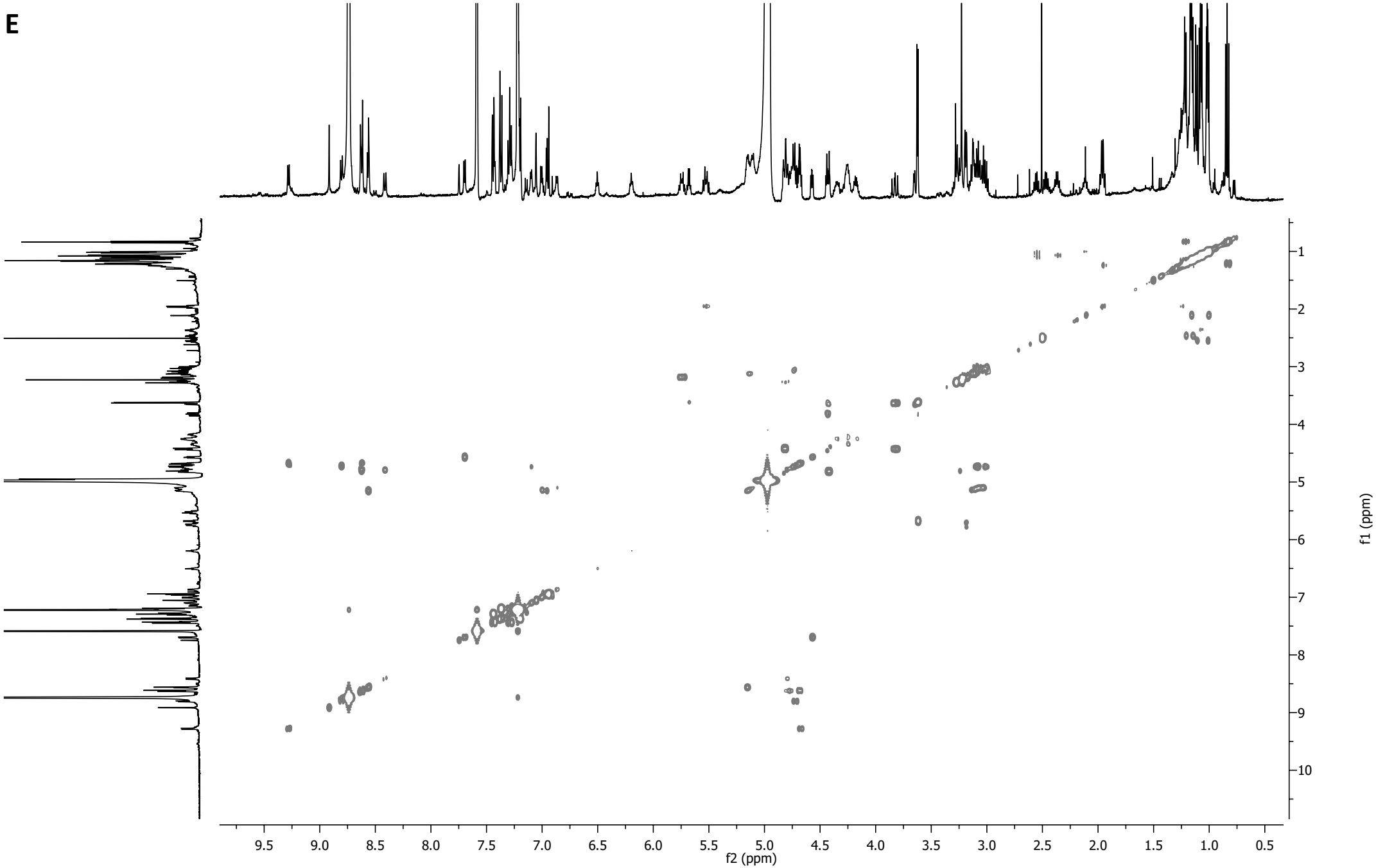


Figure S15. Characterization of thalassospiramide F (16). (**E**) ^{1}H-^{1}H COSY spectrum in pyridine-d_5.

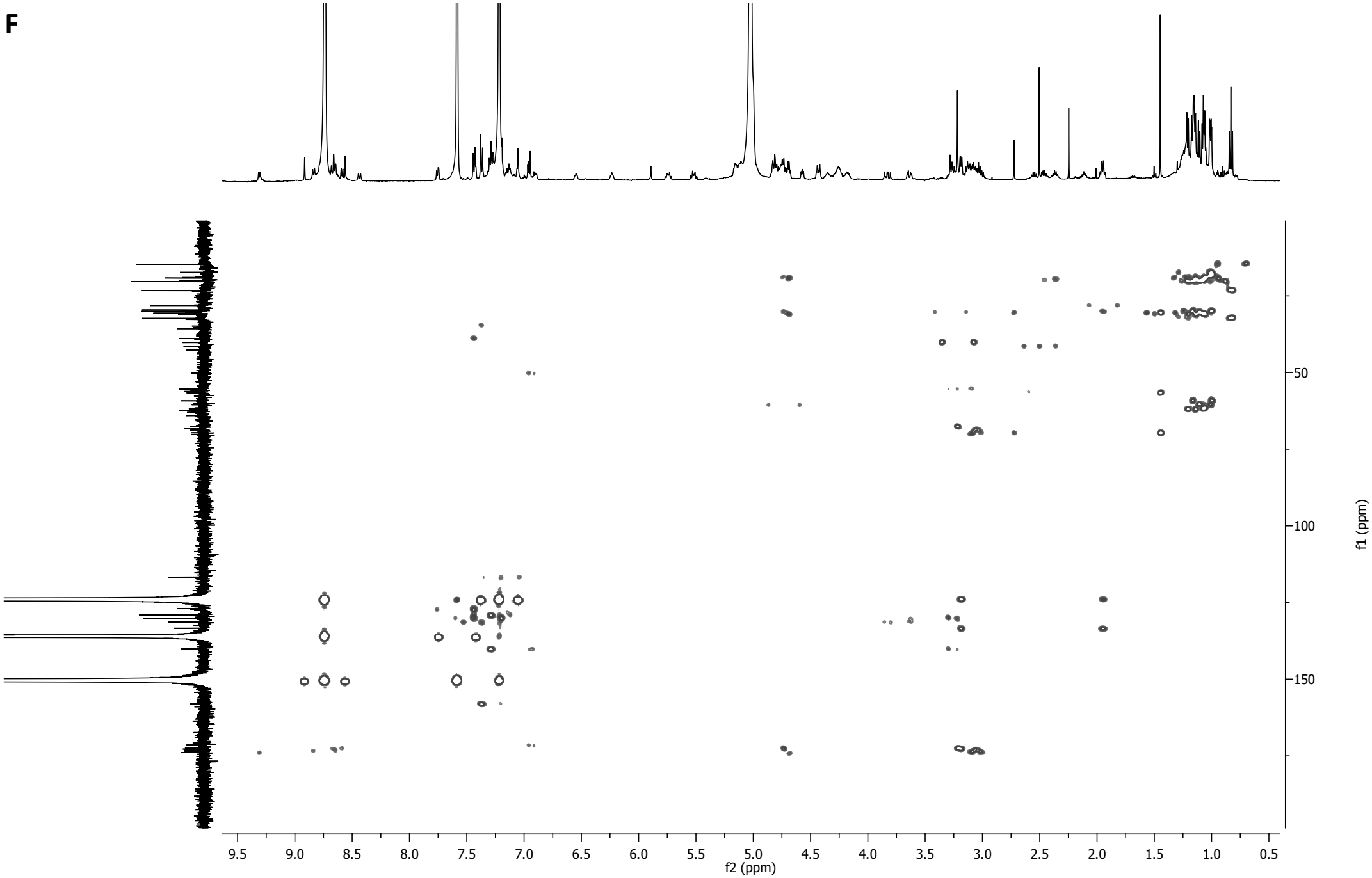


Figure S15. Characterization of thalassospiramide F (16). (**F**) HMQC spectrum in pyridine-d_5.

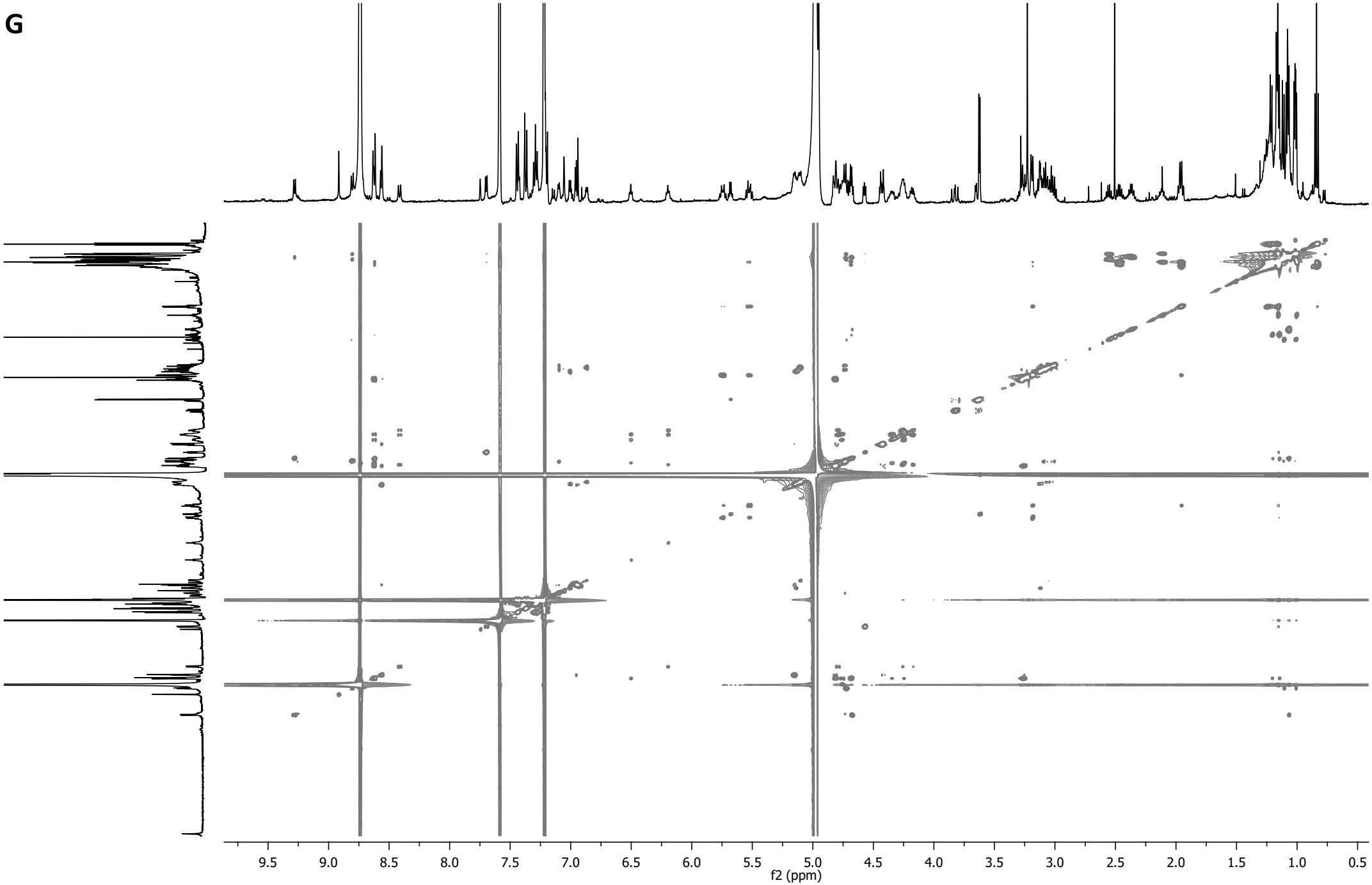
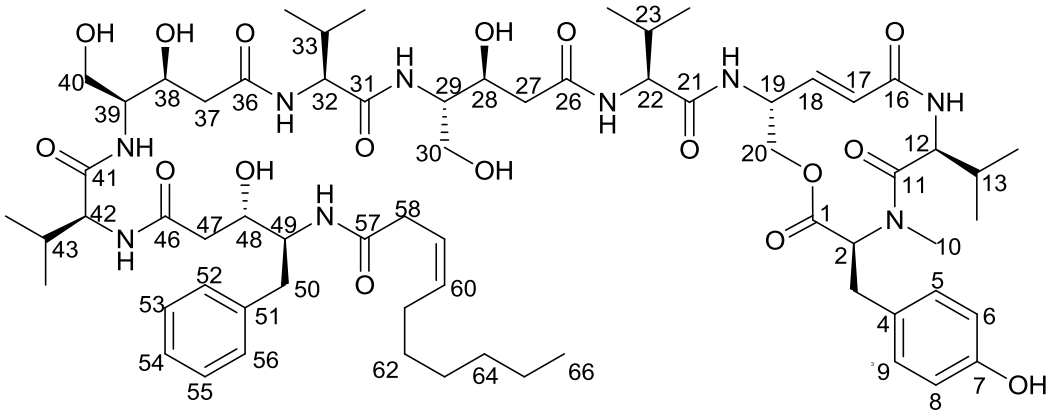


Figure S15. Characterization of thalassospiramide F (16). (**G**) ^{1}H-^{1}H TOCSY spectrum in pyridine-d_5.

H



NMR Spectral Data (500 MHz (^{1}H), 125 MHz (^{13}C) in pyridine-d_5) of thalassospiramide F (**16**)

C/H	δ_H	mult (J in Hz)	δ_C		key HMBC
1			170.8	C	
2	4.43	m	67.7	CH	
3a	3.64	dd (14.2, 4.7)	34.8	CH_2	
3b	3.83	dd (14.2, 10.9)			
4			127.0	C	
5	7.37	d (8.6)	131.4	CH	
6	7.20	d (8.6)	116.8	CH	
7			158.1	C	
7-OH					
8	7.20	d (8.6)	116.8	CH	
9	7.37	d (8.6)	131.4	CH	
10	3.23	s	40.2	CH_3	11
11			172.3	C	
12	4.55	dd (6.0, 4.5)	59.2	CH	
12-NH	7.70	d (6.0)			16
13	2.11	m	30.0	CH	
14	1.01	d (6.5)	20.3	CH_3	
15	1.16	d (6.5)	17.3	CH_3	
16			171.5	C	
17	6.94	d (15.8)		CH	
18	6.99	m		CH	
19	5.15	m	50.2	CH	
19-NH	8.57	d (5.0)			21
20a	4.44	m	64.1	CH_2	1
20b	4.81	m			1
21			172.4	C	
22	4.68	m	61.7	CH	
22-NH	9.28	d (6.5)			26
23	2.37	m	31.1	CH	
24	1.06	d (6.5)	20.1	CH_3	
25	1.08	d (6.5)	19.2	CH_3	
26			173.9	C	
27	3.04		42.7	CH_2	
28	5.10		68.3	CH	
29	4.82		56.1	CH	
29-NH	8.41	d (9.5)			

30a	4.18	m	63.0	CH_2	
30b	4.25	m			
30-OH	6.20				
31			173.9	C	29
32	4.68	m	62.1	CH	
32-NH	8.63	m			36
33	2.47	m	30.5	CH	
34	1.15	d (6.4)	20.3	CH_3	
35	1.21	d (6.8)	19.2	CH_3	
36			173.2	C	
37	3.06		42.6	CH_2	
38	5.10		68.7	CH	
39	4.78		55.4	CH	
39-NH	8.63	m			39
40a	4.25	m	62.5	CH_2	
40b	4.35	m			
40-OH	6.50				
41			172.8	C	
42	4.73	m	60.5	CH	
42-NH	8.81	d (8.0)			
43	2.55	m	30.3	CH	
44	1.02		20.3	CH_3	
45	1.12	d (6.5)	19.0	CH_3	
46		d (6.8)	173.5	C	
47a	3.01		42.5	CH_2	
47b	3.09				
48	5.11		70.2	CH	
49	4.80		55.4	CH	
49-NH	8.63	m			
50a	3.22		38.9	CH_2	
50b	3.30				
51			140.1	C	
52	7.44		129.8	CH	
53	7.30		129.1	CH	
54	7.30		129.1	CH	
55	7.30		129.1	CH	
56	7.44		129.8	CH	
57			172.9	C	
58	3.19	m	35.7	CH_2	57
59	5.74	dtt(10.5, 7.5, 1.5)	123.7	CH	
60	5.52	dtt(10.5, 7.5, 1.0)	133.4	CH	
61	1.96	m	28.1	CH_2	
62	1.24	m	30.0	CH_2	
63	1.17	m	29.6	CH_2	
64	1.15	m	32.3	CH_2	
65	1.20	m	23.2	CH_2	
66	0.84	t (7.0)	14.7	CH_3	

Figure S15. Characterization of thalassospiramide E. (**H**) NMR assignment.

Table S1: Stereochemical characterization of amino acid residues of thalassopiramides using advanced Marfey's reagents

i) thalassospiramide A (1)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Ser	399.1	11.8	12.1	L
Met-tyr	489.2	11.4	11.6	L
Ahpa	427.2	12.5	12.3	R

ii) thalassospiramide A1 (3)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Tyr	475.2	11.2	11.3	L
Met-tyr	489.2	11.4	11.6	L
Ahpa	427.2	12.5	12.3	R

iii) thalassospiramide A4 (6)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Met-tyr	489.2	11.4	11.6	L
Ahpa(19C)	427.2	12.5	12.3	R

iv) thalassospiramide A5 (11)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Met-tyr	489.2	11.4	11.6	L
Phe	459.2	14.6	16.0	L
Ahpa(19C)	427.2	12.5	12.3	R

v) thalassospiramide B (2)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Met-Tyr	489.2	11.4	11.6	L
Ahpa(19C)	427.2	12.5	12.3	R

vi) thalassospiramide C (7)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Tyr	475.2	11.2	11.3	L
Met-tyr	489.2	11.4	11.6	L
Ahpa(19C)	427.2	12.5	12.3	R

vii) thalassospiramide D (14)

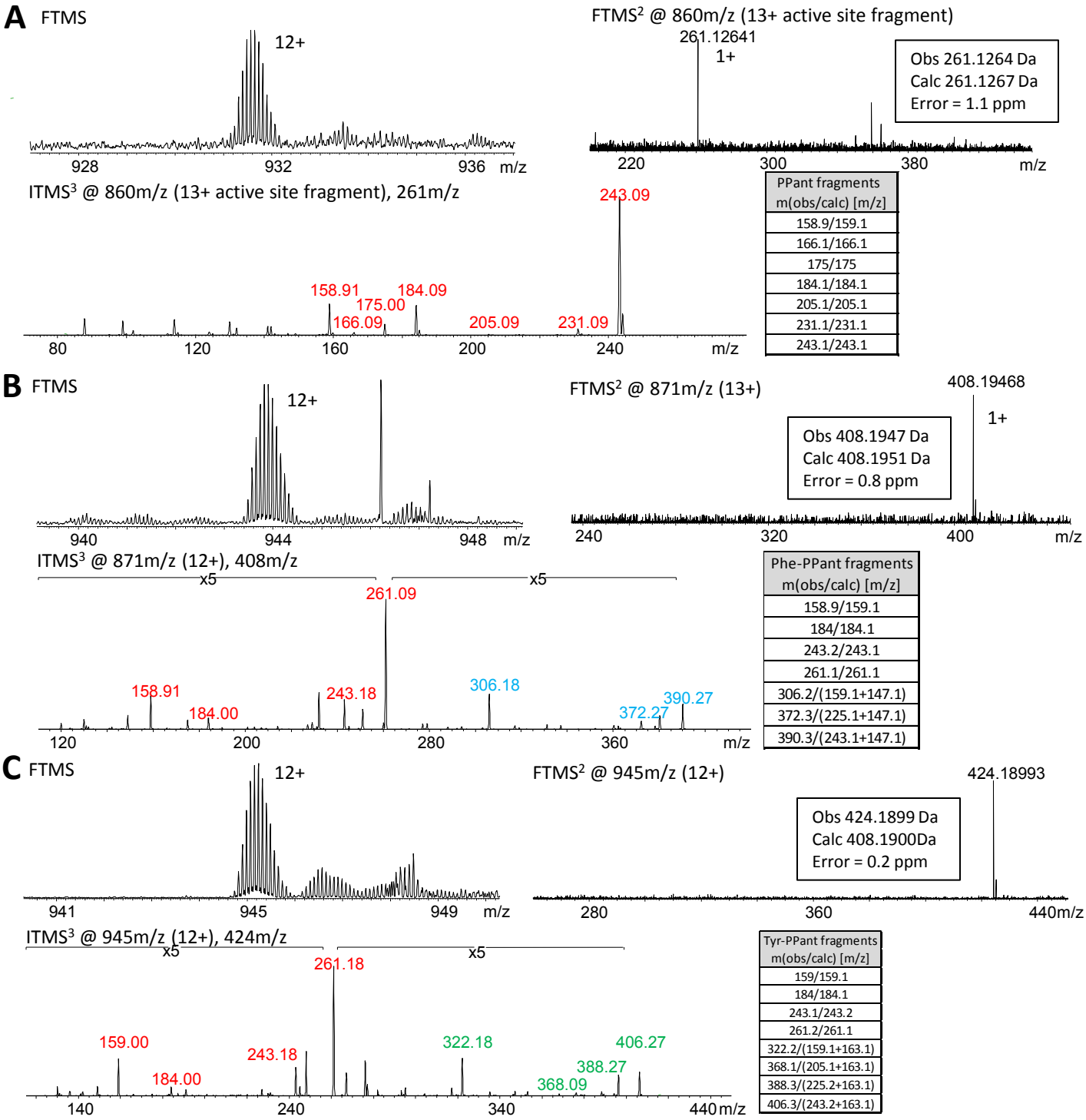
Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Met-Tyr	489.2	11.4	11.6	L
Ahpa(19C)	427.2	12.5	12.3	R

viii) thalassospiramide E (9)

Fragment	MW	L-FDLA	D-FDLA	configuration
Valine	411.2	13.8	15.5	L
Met-Tyr	489.2	11.4	11.6	L
Ahpa(19C)	427.2	12.5	12.3	R

ix) thalassospiramide F (16)

Fragment	MW	L-FDLA	D-FDLA	configuration
valine	411.2	13.8	15.5	L
met-Tyr	489.2	11.4	11.6	L
Ahpa(19C)	427.2	12.5	12.3	R

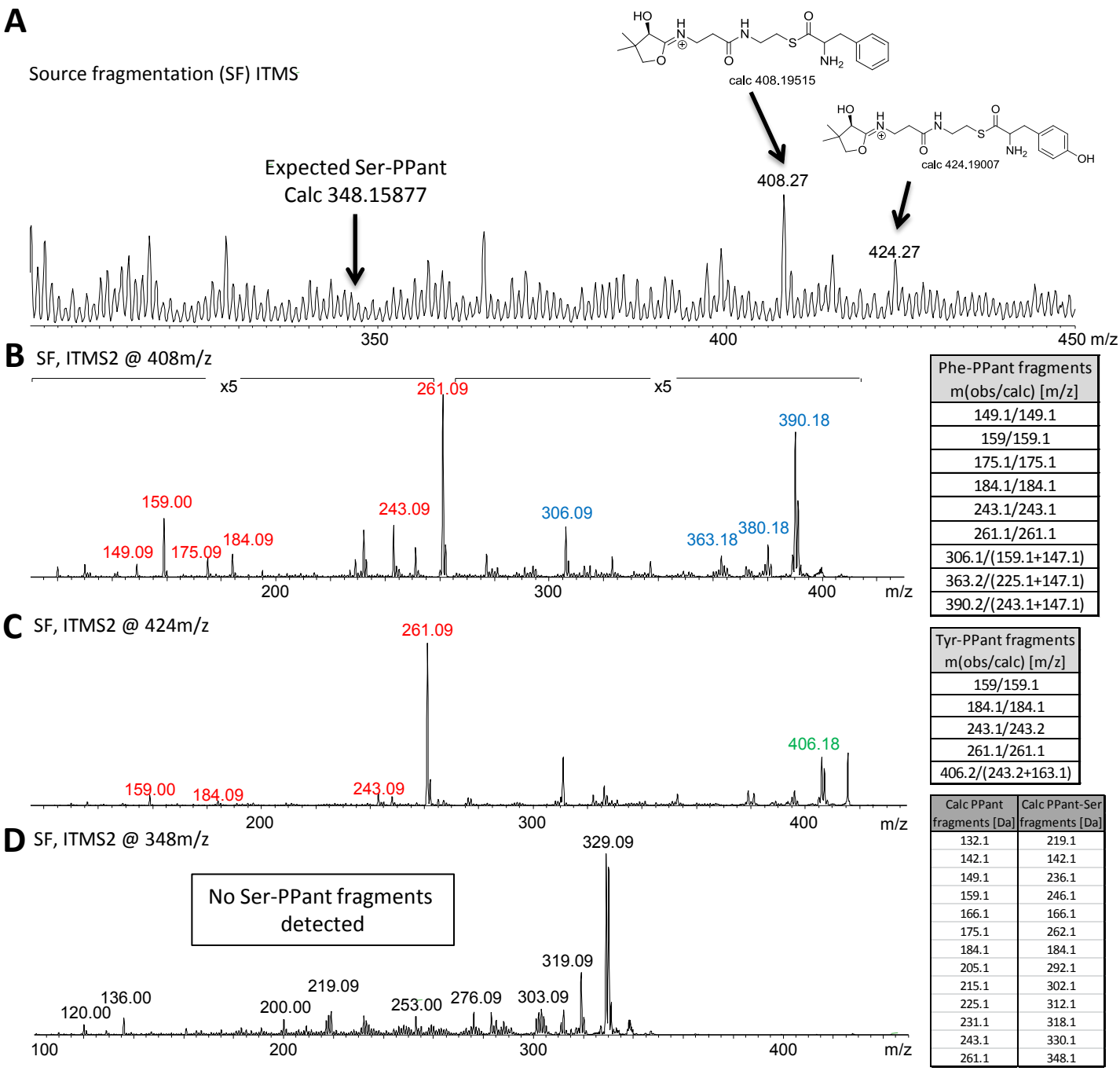


D

Species	obs [Da]	calc [Da]	Error [ppm]
apo T active site	10820.547*	10820.556	0.8
holo T active site	11160.647	11160.656	0.8
Phe-loaded T active site	11307.719	11307.724	0.4
Tyr-loaded T active site	11323.713	11323.719	0.5

* apo active site mapped as (R[1016-1111]Q) of used CAT construct by observed holo active site fragment subtracted from 340.1 (PPant)

Figure S16. Active site mapping and substrate identification of the TtcA CAT tridomain of the *Thalassospira sp.* CNJ-328 thalassospiramide pathway by ESI-FTMS, PPant ejection and PPant fragmentation. Observed Ppant fragments are listed next to the corresponding MS^3 spectra. (**A**) Characterization of the holo active site fragment of the T domain by ESI-FTMS, PPant ejection (MS^2 of 13+ active site fragment) and PPant fragmentation (MS^3 @ 261 m/z). (**B**) Characterization of phenylalanine loading on the active site fragment of the T domain by ESI-FTMS, PPant ejection (MS^2 of Phe-loaded active site fragment) and PPant fragmentation (MS^3 @ 408 m/z, Phe-PPant). (**C**) Characterization of tyrosine loading on the active site fragment of the T domain by ESI-FTMS, PPant ejection (MS^2 of Tyr-loaded active site fragment) and PPant fragmentation (MS^3 @ 424 m/z, Tyr-PPant). (**D**) Observed active site fragments of the T domain. The mapped active site fragment of the tryptic digest was R[1016-1111]Q.



Phe-PPant fragments m(obs/calc) [m/z]
149.1/149.1
159/159.1
175.1/175.1
184.1/184.1
243.1/243.1
261.1/261.1
306.1/(159.1+147.1)
363.2/(225.1+147.1)
390.2/(243.1+147.1)

Tyr-PPant fragments m(obs/calc) [m/z]
159/159.1
184.1/184.1
243.1/243.2
261.1/261.1
406.2/(243.2+163.1)

Calc PPant fragments [Da]	Calc PPant-Ser fragments [Da]
132.1	219.1
142.1	142.1
149.1	236.1
159.1	246.1
166.1	166.1
175.1	262.1
184.1	184.1
205.1	292.1
215.1	302.1
225.1	312.1
231.1	318.1
243.1	330.1
261.1	348.1

Figure S17.Substrate screening of the TtcA CAT tridomain of the *Thalassospira* sp. CNJ-328 thalassospiramide pathway by source fragmentation-PPant ejection and PPant fragmentation identifies phenylalanine and tyrosine as substrates, but not serine or any other amino acids. Observed and calculated PPant fragments are listed next to the corresponding MS^2 spectra. (**A**) Source fragmentation spectrum of active site HPLC fraction of substrate screening reaction in substrate-loaded PPant mass range. Putative Tyr-PPant and Phe-PPant ejected ions, but no Ser-PPant ejected ion could be detected. (**B**) Phe-PPant fragmentation from source fragmentation spectrum. (**C**) Tyr-PPant fragmentation from source fragmentation spectrum. (**D**) Fragmentation of expected Ser-PPant ion yielded no Ser-PPant fragments.

```
TistMA5    -----TVLLTGWAILLSRLTGRDDITVALPVNTRAAAGF--DGVVGMHAALVPVRMQVPADGSGAALLAHIRDRQAGALSHHALSLGRMLAEFQAPAMPE 93
TistBA5    -----SVLLAAWAVLLARLAGNDDVTIAVPVNTRGRAGF--DGAVAMHAALVPVRLAISGDLSGAALLDHVRSRHGGALAHHALPLGRLLTELKAPAMPE 93
ThalA5     -----SLMMATWFAVLGRIAATDDLVISVPVNQRDGAGF--RNVPGMMVSLLPLRMQIADDETFASLLHKVQNHHIDALRHRAYFLDLLLDDLSPPAEPE 93
TistMA2    -----VWTAIATTALWRILEDTQRLSAGIAFAGRSRRDT--TDAIGCFVNLLPVVVEPTAGEALPQLARRIGRDISQVLTVQDYPLSRLAADWAQAVPAR 93
TistBA2    ---TAIWTAIVATALTRLIDDTPRLAFGFPFAGRNRGGT--ADAIGCFVNLLPVIAAPEDGDTLTTLGRRIGLELSRVLAVQDYPLSRLTADWSRAVPDR 95
ThalA2     -----LWASLTIAVIARHKENGT-AIIGLPFAGRRDKET--MQSMGCFVNVLPVAIKPDMADNLDALTRQVGHEVLSILDVQDYPLSRLVHDLTKVAQGP 92
TistMA3    -----TVLTALLQALLHRLTGARDIALGLLVAGRGAPVP--DDLVGFLVNTVVLRQSVDPGARFADHLAITTDRLAEASADQDAPFEAVLQTLALDRDPA 93
TistBA3    -----TVMAALLQILLHRMTGQTDIALGLLVGGRAAPVP--QDVVGFLVNTVVLRQPVDPAAAFTTHLAVTRDRLAQAIADQDAPFEAVVASLGADRRAD 93
ThalA3     ------ILTALVQILMYRQSGQTDIPLGMLVAGREQTAL--DDVVGFFVNTVVLRQNVDPDAGFDDHLTQTGKTIIDALSDQAAPFENVVAAVNAPRDPA 92
2VSQ       HTTLSTALQAVWSVLISRYQQSGDLAFGTVVSGRPAEIKGVEHMVGLFINVVPRRVKLSEGITFNGLLKRLQEQSLQSEPHQYVPLYDIQSQADQPKLID 350
1AMU       ---------------------------------------------------------------------------------------------------- 
TistMA1    -----SLALALFLVLLGRVAGTDTPVCNTPLGNRMGRAE--RRTPGEFAYTVPTGTRIDGTESLAGLARRLDAMTRRDLRHMRLAPMRWPEAGFIPDRP- 92
TistBA1    ------VMLALFLALLGRSVGTDTPSCTTLLSNRLTRAD--RRTLGQFAYTVPFWTPIDPAESLEDLARRVDSIARRDLRHMRLAPVRWREAGFTPAAA- 91
ThalA1     ----TAIVMAAYFILLGQITHIKHPTVTMPLLNRLGRAE--RQTPGAFSYIVPFDADLGKHARFSDLARDIFARARRDVRHMRLGAPRMRAARLGARGLS 94


TistMA5    RSPLSEVSISYMNHAATAAGTLD-AGGFRLRGLTRADGKNDLSVFIADKGEGFGLVLEYDTGLFDAARIEALGPMLQRILAGLTADPDQ---PVGRLPLL 189
TistBA5    RSPLSEVSISYMNHASVAADAVG-AAGFGVRGLARPAGKNDLSVFLADHGEAMGLAIEYDTALFDAWRIAAFAPMLRTLLTALIADPDR---PIGRLPLL 189
ThalA5     RTLLSEVSLSYMNYAQGDYGPDDQAKSMRPIGLGRQQCKNDLAIFVRDLPERMVISFDYYADMFDRDRMIELGHIFTKTLQKLVATDTASTAPIASLDLL 193
TistMA2    GPTPVDLVCTLENDLSA----------LEAADISFATGKFPLLIGLMWQGDAAALNIEYDPSRFGSAWIGRLQTVITRLIAAAAADPAT--PIDRLDLLD 181
TistBA2    GPAPFDVVCTLEGDLAA----------LDSADLAIATGKFPLLIGLMWQGDAAALNIEHDPARFGTAWVDRLARVLQAVIMAAAADPAT--PLDRLSLLD 183
ThalA2     TGQPFDAVHVMEISDSP----------FMIEDLDFGAGKFPLMVGMTKKGAETSLAVEYQSNIYGEAWIERFAKRFLTFARTLAQNPEM--PLGLVDILP 180
TistMA3    RNPLFDVLVTWQDDMPAMPAMAG--LTVRPLDCVLPFAKFDLSFHFQRDGGRIRLHLEYATELFETATVRRLADRLAVLAAAPGLEG----SAIGALPLM 187
TistBA3    RNPIFDVLVTWQDDMPAMPPMGG--LQVVPVETVLPFAKFDLSFHIQREGAAIRLHVEYAIELFDAATVQALIDRLAVLAQALPSS-----APVAALPLL 186
ThalA3     RNPLFDVLVAWQDSIPDLGKLGD--AELSLLKTEFPFSKFDLAFYFSKQNDALLCQIEFDTDLFDKSTIQAIFNRLEILAAAALSTSSS--AKIAELPIL 188
2VSQ       HIIVFENYPLQDAKNEESSENGF--DMVDVHVFEKSNYDLNL---MASPGDEMLIKLAYNENVFDEAFILRLKSQLLTAIQQLIQNPD---QPVSTINLV 442
1AMU       ---------------------------------------------MVNSSKSILIHAQNKNGTHEE---------------------------------- 21
TistMA1    ---------------------------------------------------ASRGALFNAMDLPATYG---------------------------------- 109
TistBA1    ---------------------------------------------------IARGATFNAMDFPATLG---------------------------------- 108
ThalA1     GGGAFFNSLDGADPLSFAGLDTR--RVNIYNGPVTDLGLIYMMQALSPDRQEAEIIWQYDTACHDVKSVTRIADRFRHFLSRALDDPEQEIATIAAPPLD 192


TistMA5    DDETRGLLAAREAAEG---------------------------------------------------------------------------------- 205
TistBA5    DDTARAIITGYETRHG---------------------------------------------------------------------------------- 205
ThalA5     PDEQKANIEIWEQGPEPDTRLDTDANAGIFAIFARQASITPDAPAIRDTNGTWTFGELHANACAIAHCLQTAGIEPGDLVALHIERGRPAIAAILGITAI 293
TistMA2    AGDR-AVIAAANATEFTWPRDGGLGRQLDQVLR-----ADPDRIVLADDARALTARDLRARLGGVAAALAAVGVEPGTPVALAVERDPDGLTALMGIIWA 275
TistBA2    DADR-ALIDAVNATTTPYPREQGLGPQLDAVLT-----ADPARPVIADDDQTLDAGTLRARLGAVASALEDAGVAPGAVVGLAVERDLGGIIGLMGIIWA 277
ThalA2     EDERDQLSISLNDTASDYPRDCGLGELLARQITN---PANAGKIALQDRDHKITYSALGQRVAAISTGLDALDVTPGSIVALAADRSLDAIITLLGIAWH 277
TistMA3    TDIDRAALARWNDTARALDTTRPLPTLR---HA-----GDPSAPALIAPGLTLDNQGFDRRVGGLARRLLAAGVRRGDVVALVLPRSPDLLIAVHAVLAA 279
TistBA3    TADDRRHLAAWNRTGQALDTRRSIPMVWRDAVA-----AMPDAPALISDDITLNHRAFAARVDALAARLHAAGVAGGDVVAVALARGADMLIAIHAVLRA 281
ThalA3     PADERTRIDQFNATDLDLAIERSISEPFLDQVR-----ATPTAPAVIGTSETLSYEQFAPACRRIAAKLRAASVGPGDVIGVAVQRSIDMLAAIHGILLC 283
2VSQ       DDREREFLLTGLNPPAQAHETKPLTYWFKEAVN-----ANPDAPALTYSGQTLSYRELDEEANRIARRLQKHGAGKGSVVALYTKRSLELVIGILGVLKA 537
1AMU       ----EQYLFAVNNTKAEYPRDKTIHQLFEEQVS-----KRPNNVAIVCENEQLTYHELNVKANQLARIFIEKGIGKDTLVGIMMEKSIDLFIGILAVLKA 112
TistMA1    ---------------------------------------------------------------------------------------------------- 
TistBA1    ---------------------------------------------------------------------------------------------------- 
ThalA1     ELAVIRKLETGPAFTRPVPELLIPDRIQHAISS-----RPGDIALITDDGREVTFATLGHMTNAVAHHLRQNGIAAGDFVGMNVSPSAQQIAAVIGILKC 287


TistMA5    ---------------------------------------------------------------------------------------------------- 
TistBA5    ---------------------------------------------------------------------------------------------------- 
ThalA5     GAGYVPLDPAYPAARNQFILADSGAKLVLVDDAGHDALDALGALSESGHPSPQRINIGGIAPAPTDFKLPELTNADSLPAYIMYTSGSTGTPKGVLIEQG 393
TistMA2    GNAFLPLAATFPVATARELLATAGCTVLVADDAGLERWAALAPGITLIPLPTG------------RADLPPAPRGPEDPACLLYTSGSTGMPKGVVVPHR 363
TistBA2    GGAYLPLATTFPIDTARRLLANAGCTVLLADDAGLAHWAALAPDITLVPLTAAMP----VASAHDRPDRAPAQRGPEDLACILYTSGSTGEPKGVAIPQR 373
ThalA2     GAAYLPIDKSLPGDAIAALMEECGANVLFCDNTEFDRLSGISGRVRIAYLPDAN----------ATSNASFAKRTGDDLAYVMFTSGSTGAPKGVLIPNR 367
TistMA3    GAAWCPLGPDLPPQRRAHMIEDLGDPWFLSDAAHGTDLP-----ADRLILI-----------GDDLADAPVVPAGPDALAYVLFTSGSTGRPKGVEITRG 363
TistBA3    GAAWCPLDPALPERRAHMIEDLGHPWFLCDAAGATAFP-----ADRSVWVDAA-------DGTAAATPPPLPSDPDALAYVLFTSGSTGRPKGVEIAHH 369
ThalA3     GAAYSPLDPDHPEQRRMDMLDDLGYPRVITTADLADLFD-----ANNVLIID----------GSEDADIPDAINAPDDLAYVLFTSGSTGRPKGVEIAHR 368
2VSQ       GAAYLPVDPKLPEDRISYMLADSAAACLLTHQEMKEQAAELPYTGTTLFIDDQT-------RFEEQASDPATAIDPNDPAYIMYTSGTTGKPKGNITTHA 630
1AMU       GGAYVPIDIEYPKERIQYILDDSQARMLLTQKHLVHLIHNIQFNGQVEIFEEDT-------IKIREGTNLHVPSKSTDLAYVIYTSGTTGNPKGTMLEHK 205
TistMA1    ---------------------------------------------------------------------------------------------------- 
TistBA1    ---------------------------------------------------------------------------------------------------- 
ThalA1     GAVCVQLDPLQPVARNASMTRHLDCRLVLSDSTDAWCEIGNTTITVKDINAVPG------IDGRTVLEDVLADICGDDIAFVFHTSGSTGQPKPVPVHHQ 381


TistMA5    ---------------------------------------------------------------------------------------------------- 
TistBA5    ---------------------------------------------------------------------------------------------------- 
ThalA5     AVRRLASGADYAKIVASD------VVAQAGPLAFDASTFEIWATLLRGVEIAVIDRNDLLDPAKFGDALTQFGVTKMFMSVGLFNRQVDHDPQ------T 481
TistMA2    AILRLAYGD---FCTPGG------CMAQAAPMAFDAISLELWAPLLNHGRVRILPATTMFDPPLLERRIRDDGIDTMWVTASLFNQIVDERPG------T 448
TistBA2    AIARLAHGG---LCRPGQ------TMAQAAPIAFDAICLELWAPLLNHGRVRIIPAMTMFDPPLLERCLRDARVDVAWVTVSLLNRIIDDRPQ------T 458
ThalA2     AVARLVVNNRALAFDDTD------VMAQAASLGFDAATLEIWAPLLNGGKLVLIDNETLFDPAALRNALEDGCVTTMWLTASLFNRIADDAPG------C 455
TistMA3    ALANRILWMQQTFPIGPASIDAGDVILQKTPAGFDVSVWELVWWAWSGAAVAVPPPGIERDPQALAAAIRDHRVTVIHFVPAMLRAFLDALDDGRIEGAH 463
TistBA3    SVLNRILWMQATFPIGP-----GDVILQKTPAGFDVSVWELLWWSWTGAAVAMPPPGVERDPQALADAIAGHGVTVIHFVPAMLRAFLNAIEDGIVDAGR 464
ThalA3     GVLNRILWMQDAFPIGP-----DDVILQKTPITFDVSVWELFWWSWTGARVVLPDPGVERDPQQLAQAIRDHSVTTMHFVPSMLASFLFSVEHGLVDIEH 463
2VSQ       NIQGLVKHVD-YMAFSD-----QDTFLSVSNYAFDAFTFDFYASMLNAARLIIADEHTLLDTERLTDLILQENVNVMFATTALFNLLTDAGED------W 718
1AMU       GISNLKVFFENSLNVTE-----KDRIGQFASISFDASVWEMFMALLTGASLYIILKDTINDFVKFEQYINQKEITVITLPPTYVVHLDPE---------R 291
TistMA1    ---------------------------------------------------------------------------------------------------- 
TistBA1    ---------------------------------------------------------------------------------------------------- 
ThalA1     SLADKIDVAIAQFGIGSHENGNPETMGLFPSLGFDPWMHQFCVGLICGHRMWLMTHAILSDGHKFWNGVARHHVTHLMSVPSFYESVIDAIPADG----- 476


TistMA5    ----------------------------------------------------------------------VSVLDDRGQPVPPGVWGRLHRLG--- 228
TistBA5    ----------------------------------------------------------------------LAVLDPAGAPAPVGVWGALHRIG--- 228
ThalA5     LAGLDILMIGGDAISKQHVRNFINACPHVT---VLNGYGPTESTTFAVVGPITENDLSGDTDATIIGKSIAHTKTLILDQNGQRAPVGVWGELMIGGSRL 578
TistMA2    FAGLKRLMTGGEVISPDHVRRVMAACPG---VVLYNGYGPTENGTLTTIHRITAADLEGGP--VTIGRPVPGTRVHIVDGRGQPLPIGVWGELVTAGDGL 543
TistBA2    FAGLSLLITGGEVVSPGHIARLIDACPG---ITVLNGYGPTENGTFTTTHIVTSADLDGGP--VPIGRPVANTRIHVVDDRGRPVPPGVWGNLLAAGDGL 553
ThalA2     FAPLKRLLSGGEALSPTHLQKVMTACPG---LALINGYGPTENTTFTCIHPITPQDVKSAN--IPIGRPIGNTRTYILDAGGQPVPTGVWGELYAAGDGL 550
TistMA3    LATLRLVFASGEALDAATAARFDRLLHARFGTALHNLYGPTEATVDVTWQPCTPWDPAAKT--VPIGRPVANTRVLILDRAGRALPPGVAGEIVLAGPQV 561
TistBA3    LAGLRLVFASGEALDAATVRRFNHLLHARFGTMLHNLYGPTEATVDVTWQPCTPWAADSQI--VPIGRPVANTRVLILDDAGRPVPPGGVGEIVLAGPQV 562
ThalA3     LGSLKRVFASGEALDPALVKRFNDLLFDRFGTELHNLYGPTEATVDVTWHACSPLE-NPDI--VPIGKPIANTTIRILDDRLGDMPIGIAGEIVLGGPQI 560
2VSQ       MKGLRCILFGGERASVPHVRKALRIMGPGK---LINCYGPTEGTVFATAHVVHDLPDSISS--LPIGKPISNASVYILNEQSQLQPFGAVGELCISGMGV 813
1AMU       ILSIQTLITAGSATSPSLVNKWKEKVT------YINAYGPTETTICATTWVATKETIGHS---VPIGAPIQNTQIYIVDENLQLKSVGEAGELCIGGEGL 382
TistMA1    ----------------------------------------------------------------------FDGLQVRVADSIFG---------- 123
TistBA1    ----------------------------------------------------------------------FDGLSVAVRNAVFG---------- 122
ThalA1     NFKLRRMIIGGEVLQPTLSRRLHSVFES---ADIWNCYGPTEATIHATTHLVPREIKTKN---IPLGHVDKGAFVRILDEDGKRVTLGVPGEIYLGGTGL 570
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TistMA5    ------------------------DDGWQPTGDRARWRLDLTAIETGGRLDRLTLWQGHLLDPALIEA-QLSGLPGVAALRILPRPGRLVAAITAAGPRP 303
TistBA5    ------------------------DDGRAPTGLRARWRADG-EIETSGRLDRVTRWQGHLLDPAAIEARLLAALPHLAGLRIAVGDDALRAAVLAAPSTP 303
ThalA5     AREYWQRSDLTGERFIPDP--DQPDQRLYRTGDLARWTRDG-RIEFGGRHDNQIKLRGFRIELDEIEQQLQSAPGIKNAVALFDVNAPDGGAIIGCIQTT 675
TistMA2    ATGYAGRPELTAAAFVTLD--GLDEPLVYRTGDRARWRADG-RIDFGGRRDGQLKIRGQRIETAAVEAILAAAPGVRDAVVAGIGHG--ADQILAALVAA 638
TistBA2    AIGYVGRPDLTARSFVTLP--DIDEPLLYRTGDRVRLRADG-VLEFGGRRDGQLKIRGQRIETAAIEAALTARPGVRDAMVVALGAG--ADQTLAALVAA 648
ThalA2     ALGYSGAPERTAKAFVTFD--HLPETRLYKTGDRARWRADG-VIEFGGRRDGQVKIRGHRIETAAIEKRLSQIDGIRNACVMSVGSG--ADAFLGAAIAA 645
TistMA3    ARGYRGRPDLTADRFRPDP--EIAGARVYHTGDLGRWTADG-VVEYLGRIDDQVKIGGVRLEPAEVEAALDACPGVVRGLVRVGRRD-GLAELEAWVMGA 657
TistBA3    ARGYRGRPGLTAERFRPDPDADHPGARVYHTGDLGRWTADG-AVAYLGRADDQVKIGGVRLELAEVETALDACPGVVRGLARVATRD-GLTELHAYVLGA 660
ThalA3     ACGYRNRPELTAEKFPDDP--RKPGHKLYRTGDLGRFLAGG-SVEYLGRIDHQVKIRGFRIECGEVETTIESHDLVERALVKAVRVG-DLDELHAFVLGE 656
2VSQ       SKGYVNRADLTKEKFIENP--FKPGETLYRTGDLARWLPDG-TIEYAGRIDDQVKIRGHRIELEEIEKQLQEYPGVKDAVVVADRHESGDASINAYLVNR 910
1AMU       ARGYWKRPELTSQKFVDNP--FVPGEKLYKTGDQARWLSDG-NIEYLGRIDNQVKIRGHRVELEEVESILLKHMYISETAVSVHKDHQEQPYLCAYFVSE 479
TistMA1    ----------------------------ELHDVMACLRTYG----AADASDMQWSFPPARIDRARVESLAAAFEAMLDAALADPTQP--VDHLPAGDLPS 189
TistBA1    ----------------------------PLDDVLACLRTYG----TAGGADMQWSFPPARIDASAVEALADSYDRMLDAALADPSQP--LRQIQA----- 183
ThalA1     NRGYINMPAVTAEKFVKDP-TSETDETFYRSGDLASWGEDG-LLYYHGRTDEQVKIRGQRIEIGEIEYQLSRVPGVGQGAVLYVKNN-HGGELIGYLLPD 667

TistMA5    --------DPAHLRRAAGAILPPVLVPGAWHLLDALPEDDAALAAGVAATPSLEEAA----------------------------EAATERLATVLAVV 366
TistBA5    PLSSTPPLSATELHAVAAALLPPALVPTAWYRLDTLPEDRHALADLIAAATPLGATAGDRSSP----S------------------AAEAEAEAAVCAVF 381
ThalA5     Q---DQDIDIPALMTWLGTHLPGYMIPAKWHIVDTIPITANGKVDRKALLETVRTQSNTMALDGGTDT------------------PPANPAEELVCDIF 754
TistMA2    D-----EADEAGWRAAIAARLPAYMVPERFRLVDRLPVNANGKADRRQAAAMLAEAAP---------------------VARPAG--TGTGFERAVITAF 710
TistBA2    D-----TADPQGWHQAVADRLPAYMIPARFERVDRLPVNANGKADRAAAARLLAGAAAGVGETPAAAVVAAAMPLASGLPTRPDGPLEDDPLERAVATAF 743
ThalA2     D-----QDNSLVWMQILGRNLPDYMIPERFVVLDHLPVNVNGKIDRKQLLDTLKNTAP--------------------LVPHTGNANSSSELEQIVANHF 720
TistMA3    A-----DLTPAGLRAALADRLPAAMIPTRWFRIDQVPLSPNGKVDRKALSG--VPLAGRPAVAAAPAG--------------------HPAEAEIARIW 729
TistBA3    D-----DLTPQALRAALARHLPDAMIPSRWFRIDAVPLSANGKVDRKALTGRPFQLAAAPAPMPAHAP--------------------TSPEAGIAAIW 734
ThalA3     G-----DLTIGVLRDHLRTRLPEYMIPARFFALDHLPLTSSGKVDRKALSG--TPMAGSPKTASRKSKPAIA-------AKPDAEIHDLANLERVLQRLW 742
2VSQ       T-----QLSAEDVKAHLKKQLPAYMVPQTFTFLDELPLTTNGKVNKRLLPKPDQDQ-LAEEWIGPRNE----------------------MEETIAQIW 981
1AMU       K-----HIPLEQLRQFSSEELPTYMIPSYFIQLDKMPLTSNGKIDRKQLPEPDLTFGMRVDYEAPRNE----------------------IEETLVTIW 551
TistMA1    P-------ADTAGPVPPQPAVPLIDTTTRTALEAEVTRLFAGLLDAPQLTA---------------------------------------------- 233
TistBA1    ---------LAAGPVA-IATAPVIDDAAVWRLEDEITRIFAGLLHSPALT---------------------------------------------- 223
ThalA1     G-SHSKRPDIATVRSELARHLSDAAVPTRLEWVETLPLLPSGKIDRKALAQFAQAPGGKQPVAQETPPP----------ASRKPDQMRIHQMAEKIAKIW 756
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Figure S18. Alignment of protein sequences for the areas surrounding the first four adenylation domains in *T. mobilis* (TistMA1-5**), *T. bauzanensis* (**TistBA1-5**) and *T.* sp. CNJ-328 (ThalA1-5), to the surfactin termination module (2VSQ) and the *N*-terminal adenylation domain from the Gramicidin S cluster (1AMU), both of which have crystal structures. Sequences span the final 50 amino acid residues of the preceding condensation domain through to the first 15 amino acid residues of the following thiolation domain. Area highlighted in yellow is the *C*-terminal sub-adenylation domain, lysine shown in green is a conserved AMP binding residue, notably missing from the sub domains for the *Tistrella* modules 1 and 5.**

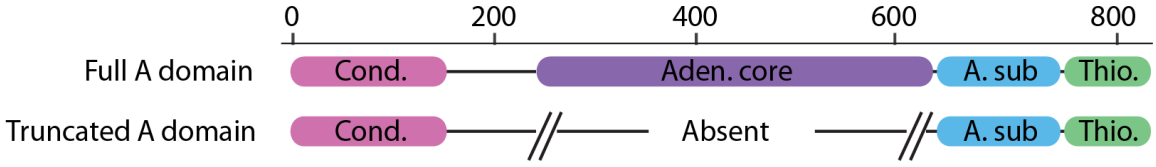

Figure S19. Simplified graphic depicting the biosynthetic domains present in the region between the condensation and thiolation domains based upon the preceding protein alignment. All *Thalassospira* regions analyzed are characterized as full A domains. For the *Tistrella* regions analyzed, portions from module 2 and 3 are characterized as full A domains, while the regions from module 1 and 5 are characterized as truncated A domains in which the 400 amino acid residues of the *N*-terminal core adenylation domain are absent but the 100 amino acid residues of the *C*-terminal sub-adenylation domain are present.