Gargantulide A, a complex 52-membered macrolactone showing antibacterial activity from *Streptomyces* sp.

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Contents

General Experimental Method

General Instruments

Optical rotation was measured on a JASCO P-1010 polarimeter with a 5 cm cell. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer. All NMR spectra were measured on a Unity INOVA 500 and a Bruker Avance II 900 MHz spectrometers in a solvent of MeOH-d_4 in reference to residual solvent peaks at δ_H 3.30 and δ_C 49. High-resolution ESI –FT mass spectrum was acquired using a Thermo LTQ-XL Orbitrap. The HPLC was performed using an Agilent 1200 system using the YMC ODS-A column (10 × 250 mm, S-5μm). All solvents were distilled prior to use.

Biological Source Material

Strain ID: A42983
Taxonomy: *Stretomyces* sp.

Strain A42983 was sub-cultured on ISP4 agar (Difco) for 10 days at 28 °C. The subculture was inoculated into 250 mL Erlenmeyer flasks, each containing 50 mL of seed medium, composed of 1.5% glucose (Sigma), 1.5% glycerol solution (Merck), 1.5% soya peptone (Oxoid), 0.1% $CaCO_3$ (BDH) and reverse osmosis water (1L). The pH of the medium was adjusted to 7 prior to sterilization (autoclave 121 °C for 20 min). The seed

culture was incubated for 3 days at 28 °C on a rotary shaker at 200 rpm and then inoculated into 250 mL Erlenmeyer flasks, each containing 50 mL of liquid medium. The liquid medium composed of 2.1% Mops, 0.1 % TES-1, 0.2% KH_2PO_4, 0.05% $MgSO_4$, 0.1% NaCl, 0.5% $CaCO_3$, 0.5% NH_4Cl, 2% glycerol, 0.2% L-proline, reverse osmosis water (1L) and 2% glucose which was sterilized and added separately. The pH of the medium was adjusted to 7 prior to sterilization (autoclave 121 °C for 20 min). The fermentation was carried out for 14 days at 28°C at 200 rpm.

Extraction and Isolation

The culture broth (15 L) of *Streptomyces* sp A42983 was centrifuged to separate the mycelial cake and supernatant. The supernatant (12 L) was passed through a column packed with Waters Prep C18, 55-105 μm, 125 Å (Vacuum Liquid Chromatography). The column was eluted with mixtures of water and methanol, both containing 0.1% formic acid. The column was initially washed with 100% water, followed by stepped gradient elution with the solvent system of 10 to 100% MeOH/H_2O to afford 10 fractions (1 L each), each of the fractions was analyzed by HR-ESI-MS (Bruker microQTOF). Fractions 5 – 8 (50% to 80% MeOH/H_2O) containing gargantulide A were combined and concentrated under reduced pressure. The combined fraction was dissolved in MeOH and subjected to reverse phase preparative Gilson GX-281 HPLC [Waters NovaPak C-18 radial cartridge column, 6 μm, 40 ×100 mm; mobile phase: solvent A: H_2O/formic acid (0.1%), solvent B: acetonitrile/formic acid (0.1%)] at a flow rate 18 mL/min with a mobile phase gradient of the solvent B from 15% to 23% in 10 min, 23% to 33% in 40 min to afford gargantulide A (1) (1.5 g, RT 29 min).

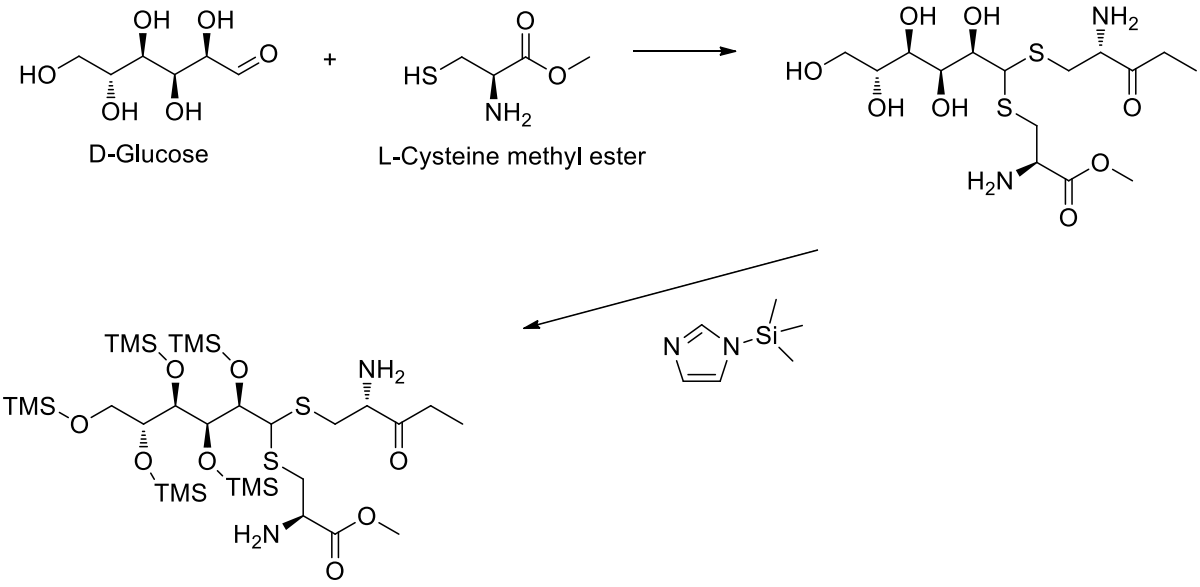
Gargantulide A (**1**): white powder, m.p.= 157 ℃, $[\alpha]^{25}$ -36.5 (*c* 0.30, MeOH); IR ν_{max} 3331, 2933, 1715, 1588 and 1456 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1S; positive HR-ESI-FT-MS *m/z* = 1049.6974 for $[M + 2H]^{2+}$ (Calcd for $C_{105}H_{202}N_2O_{38}$, 1049.6968).

Determination of Absolute Configuration of Glucose and Mannose

Purified glucose and mannose were each dissolved in 2 N trifluoroacetic acid and incubated at 110 °C for 24 h. The hydrolysate was dried under N_2 (g). The residue (0.5 mg) was resuspended in 0.1 mL of pyridine. To a solution of hydrolysate was add L-cysteine methyl ester hydrochloride in pyridine (0.1 M, 0.05 mL) and the reaction mixture was incubated at 60 °C for 2 h. Trimethylsilylimidazole solution (0.1 mL) was added to the reaction mixture and then incubated at 60 °C for 1.5 h. To this solution, 200 μL of hexanes

and 200 μL of deionized water were added. The organic layer was injected into the GC-MS (DB-5MS, Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temperature was 150 °C, held for 1 min, followed by a ramp from 150 °C to 250 °C at a rate of 2 °C/min and held at 250 °C for 10 min.

Authentic standards were also prepared by the same procedure. Retention times of the sugar derivatives: D-mannose derivative (36.43), L-mannose derivative (36.19), D-glucose derivative (36.39), L-glucose derivative (37.02), Natural mannose derivative (36.44), and natural glucose derivative (36.42)



Scheme S1. Preparation of the trimethylsilylimidazole derivative of D-glucose.

Table S1. ^{1}H and ^{13}C NMR data for gargantulide A (**1**) recorded in MeOH-d_4

no	^{1}H	^{13}C	no	^{1}H	^{13}C
1		175.1, C	45	4.21, m	72.5, CH_2
2	2.35, m	35.4, CH_2	46	1.56, m	40.4, CH
3	1.61, m	26.7, CH_2	47	4.05, m	71.42, CH
4	a 1.32, m; b 1.42, m	28.4, CH_2	48	a 1.56, m; b 1.65, m	38.0, CH_2
5	a 1.19, m; b 1.48, m	34.22, CH_2	49	3.67, m	76.9, CH
6	1.47, m	39.8, CH	50	2.30, m	38.3, CH
Me-6	0.87, d	14.5, CH_3	Me-50	0.92, d	10.7, CH_3

7	3.41, m	76.1, CH	51	4.88, m	76.8, CH
8	1.44, m	32.7, CH_2	52	1.51, m	40.87, CH
9	a 1.38, m; 1.46, m	32.4, CH_2	53	a 1.19, m; b 1.79, m	24.5, CH_2
10	1.64, m	36.1, CH	54	a 1.43, m; b 1.88, m	31.7, CH_2
Me-10	0.85, d	13.2, CH_3	55	3.67, m	83.7. CH
11	3.11, dd(8.3, 2.1)	79.9, CH	56	1.71, m	43.1, CH
12	1.54, m	37.5, CH	Me-56	0.93, d	10.4, CH_3
Me-12	0.85, d	16.6, CH_3	57	4.18, m	68.4, CH
13	a 1.08, m; b 1.76, m	34.01, CH_2	58	a 1.41, m; b 1.67, m	43.9, CH_2
14	a 1.21, m; b 1.49, m	28.6, CH_2	59	4.01, m	68.8, CH
15	a 1.30, m; b 1.56, m	27.1, CH_2	60	a 1.59, m; b 1.63, m	45.6, CH
16	a 1.32, m; b 1.59, m	35.9, CH_2	61	3.79, m	71.49, CH
17	3.59, m	74.7, CH	62	a 1.42, m; b 1.52, m	38.7, CH_2
18	2.66, dd(6.8, 7.3)	53.8, CH	63	a 1.38, m; b 1.61, m	22.7, CH_2
Me-18	0.98, d	13.5, CH_3	64	a 1.40, m; b 1.49, m	38.5, CH_2
19		214.4, C	65	3.49, m	72.8, CH
20	a 2.81, dd(17.9, 6.6)	49.9, CH_2	66	a 1.36, m; b 1.51, m	35.92, CH_2
	b 2.86, dd(17.9, 6.6)		67	a 1.13, m; b 1.51, m	34.04, CH_2
21	4.46, d(6.6)	73.9, CH	68	1.43, m	34.07, CH
22		146.5, C	Me-68	0.91, d	20.1, CH_3
Me-22	1.77, s	11.8, CH_3	69	a 1.20, m; b 1.38, m	37.6, CH_2
23	5.21, d(9.8)	123.3, CH	70	a 1.38, m; b 1.44, m	25.0, CH_2
24	4.80, dd(9.8, 8.3)	75.7, CH	71	1.62, m	29.0, CH_2
25	3.61, dd(8.3, 2.9)	80.3, CH	72	2.91, t(7.7)	40.78, CH_2
26	1.73, m	39.2, CH_2	1’	a 1.07, m; b 1.35, m	34.18, CH_2
Me-26	1.06, d	11.7, CH_3	2’	a 1.27, m; b 1.48, m	21.5, CH_2
27	4.10	69.6, CH	3’	0.88, t(7.1)	14.7, CH_3
28	a 1.26, m; b 1.57, m	43.8, CH_2	Man-1	4.57, br s	96.8, CH
29	3.69, m	69.0, CH	Man-2	3.80, m	72.9, CH
30	a 1.41, m; b 1.44, m	39.4, CH_2	Man-3	3.45, m	75.2, CH
31	1.50, m	23.2, CH_2	Man-4	3.60, t(9.3)	68.6, CH
32	a 1.43, m; b 1.49, m	39.3, CH_2	Man-5	3.15, m	78.5, CH
33	3.82, m	69.3, CH	Man-6	a 3.74, m; b 3.87, m	62.8, CH_2
34	a 1.48, m; b 1.56, m	46.1, CH_2	Glc-1	4.21, d(7.3)	102.3, CH
35	4.09, m	66.35, CH	Glc-2	3.18, m	74.8, CH
36	1.55, m	46.7, CH_2	Glc-3	3.34, m	78.0, CH
37	4.09, m	66.4, CH	Glc-4	3.30, m	71.7, CH
38	1.57, m	46.8, CH_2	Glc-5	3.20, m	78.1, CH
39	4.06, m	66.4, CH	Glc-6	a 3.66, m; b 3.82, m	62.7, CH_2
40	a 1.57, m; b 1.61, m	44.0, CH_2	maG-1	4.44, d(7.3)	104.3, CH
41	4.03, m	73.7, CH	maG-2	3.47, dd(10.2, 7.3)	70.6, CH
42	1.62, m	40.82, CH	maG-3	2.98, t(10.2)	66.2, CH
Me-42	0.94, d	6.68, CH_3	maG-4	3.34, m	71.52, CH
43	3.80, m	77.5, CH	maG-5	3.39, dd(8.8, 6.3)	74.2, CH
44	1.66, m	41.8, CH	maG-6	1.32, d(6.3)	18.2, CH_3
Me-44	0.83, d	10.8, CH_2	N-Me	2.78, s	31.4, CH_3

Table S2. MIC (μg/mL, n = 5 strains) ranges of gargantulide A (**1**) and comparator antibiotics against multi-drug resistant Gram-positive pathogens.[a]

Compound	MRSA	MRSE	VRE	PRSP
Gargantulide A	1-2	1-1	0.25-1	0.125-0.5
Oxacillin	32->128	16->128	16->128	8-64
Penicillin G	64-128	16-16	2->128	1-16
Vancomycin	2-4	2-2	128->128	0.25-1
Linezolid	1-4	1-2	2-4	0.5-1
Trimethoprim	0.5-64	>128	≤0.125->128	32-128
Erythromycin	2->128	0.5->128	4->128	≤0.125->128

[a] MRSA methicillin-resistant *S. aureus*, MRSE methicillin-resistant *S. epidermidis*, VRE vancomycin-resistant enterococci, PRSP penicillin-resistant *S. pneumoniae*.

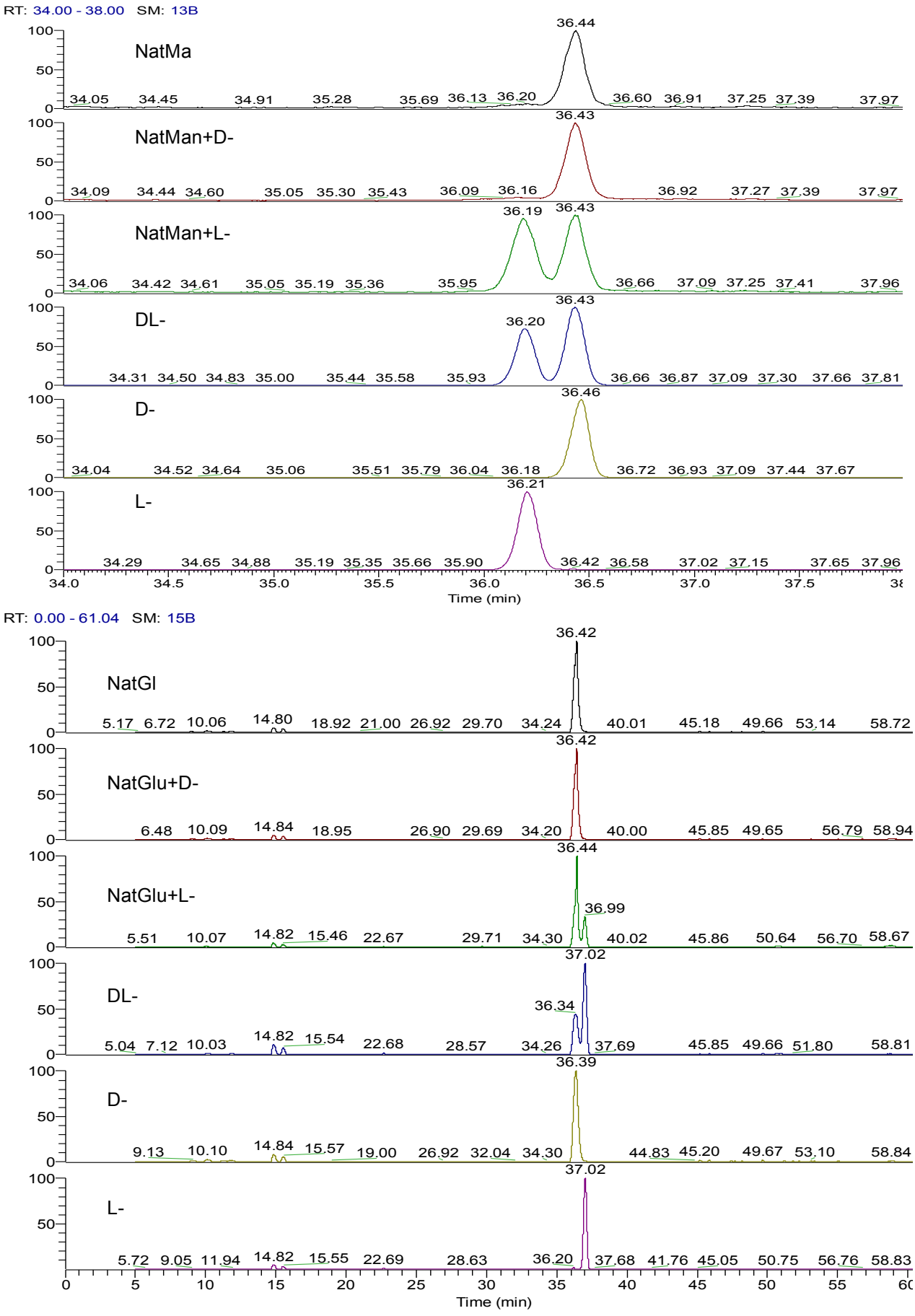


Figure S1. GC/MS analysis after additional acid hydrolysis and trimethylsilylation.

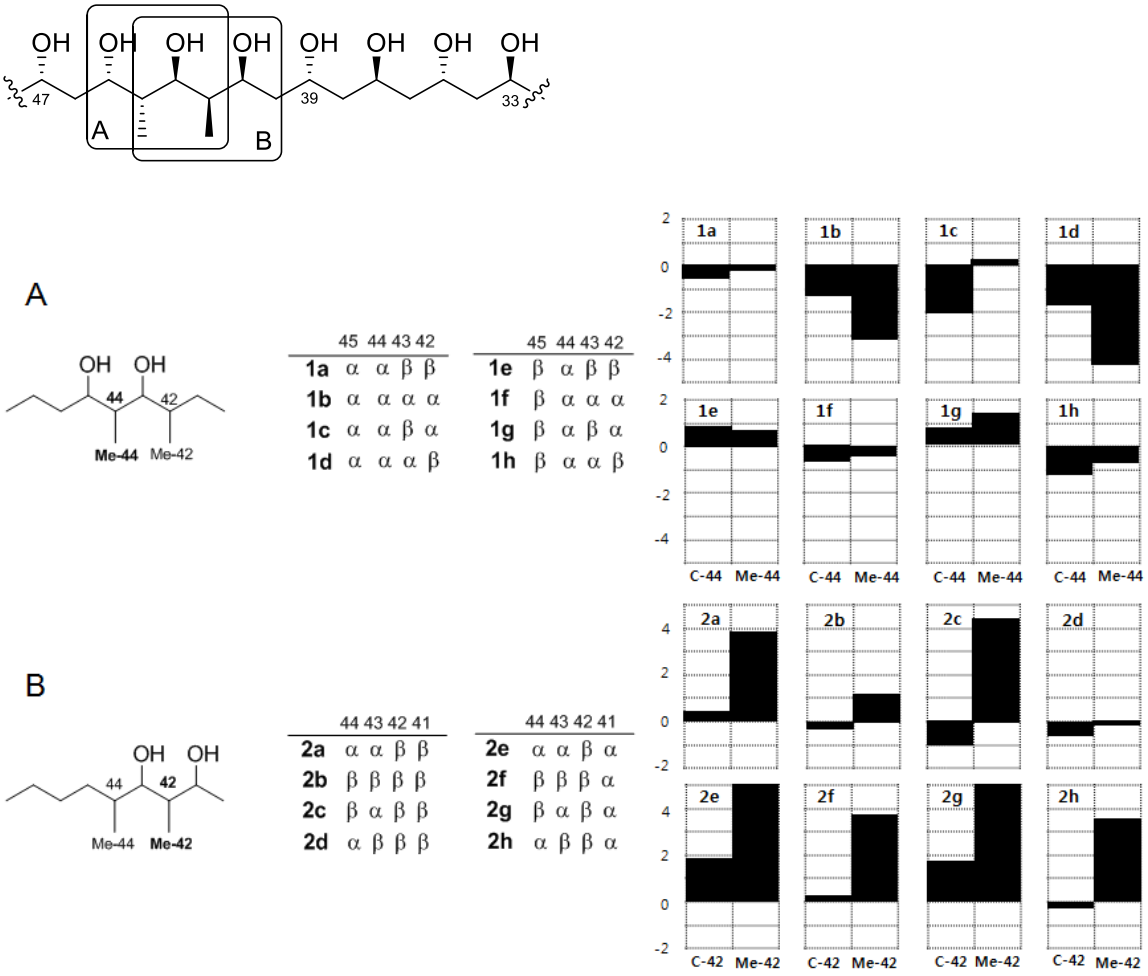


Figure S2. Application of Kishi's NMR database for the consecutive hydroxyl/methyl/hydroxyl/methyl substituents from C-42 to C-45.

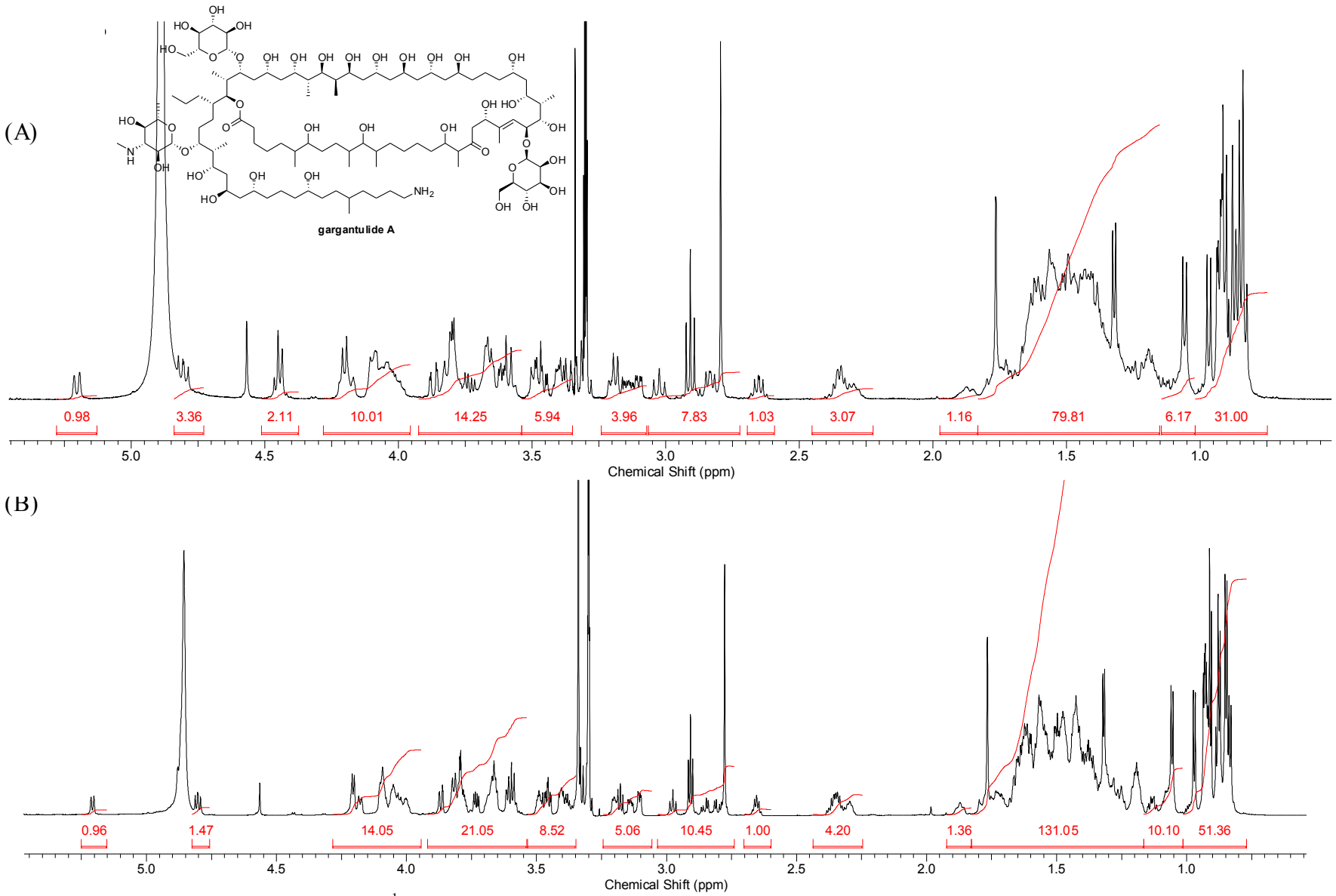


Figure S3. ^{1}H NMR spectra of gargantulide A (**1**) at 500MHz (A) and 900MHz (B).

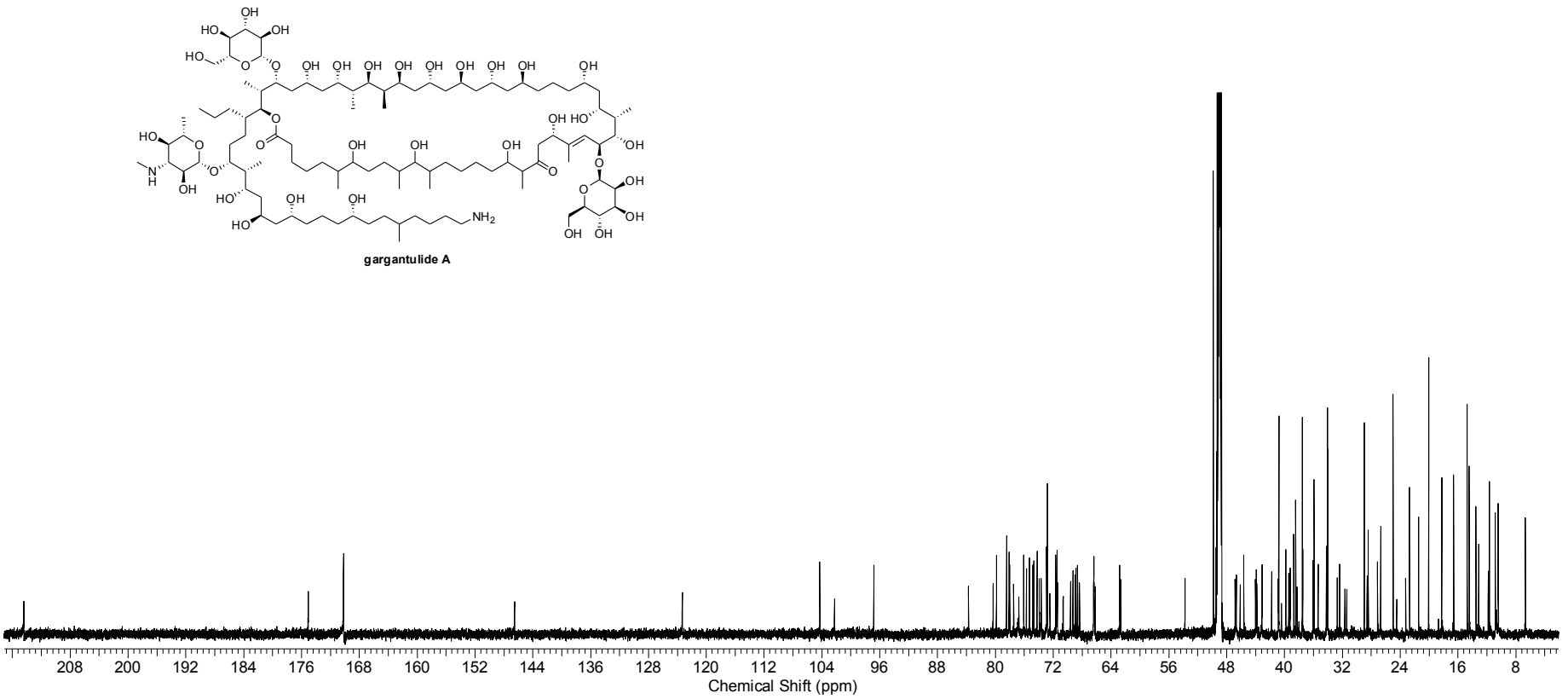


Figure S4. ^{13}C NMR spectrum of gargantulide A (**1**) at 900MHz.

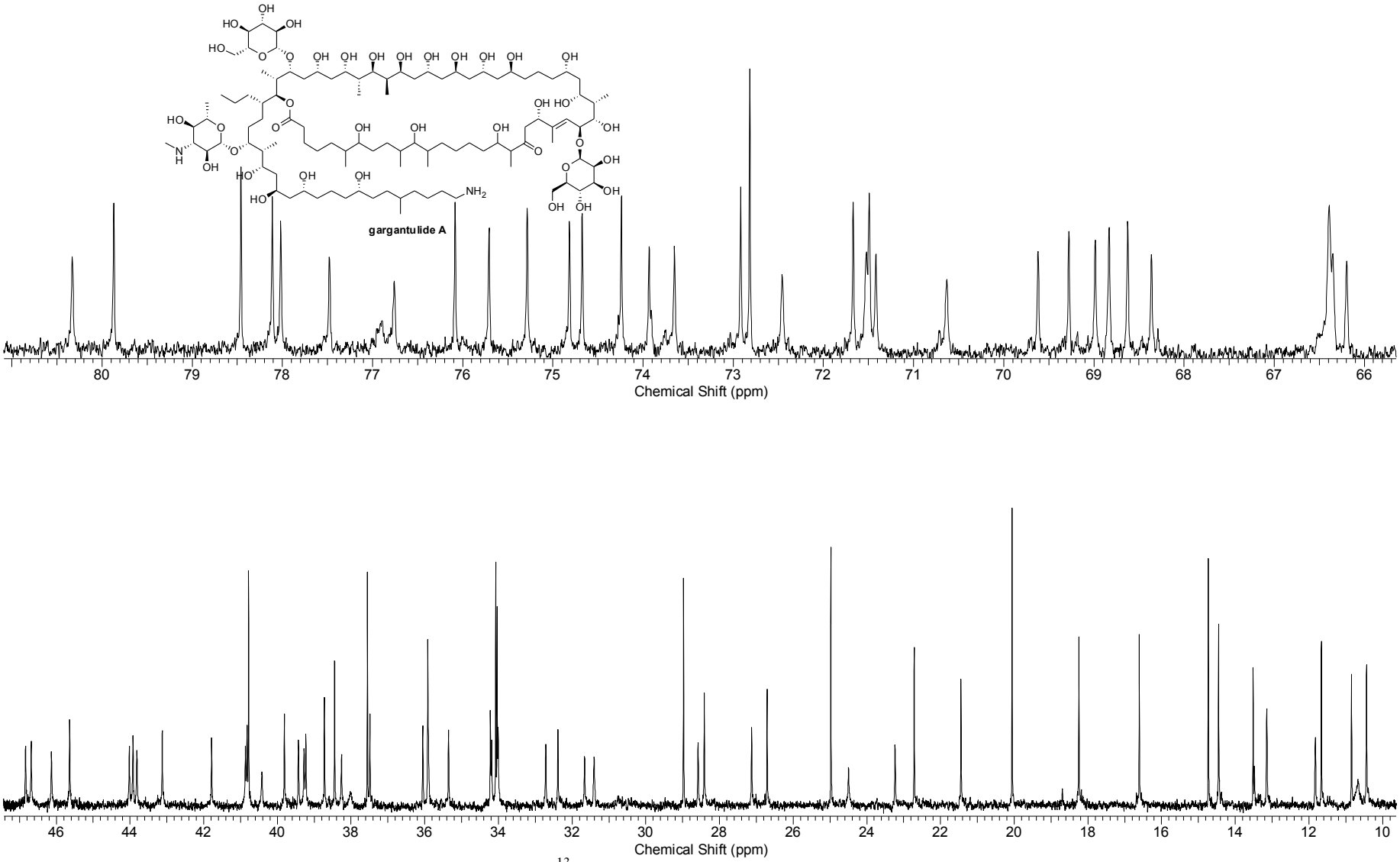


Figure S5. Expanded ^{13}C NMR spectrum of gargantulide A (**1**) at 900MHz.

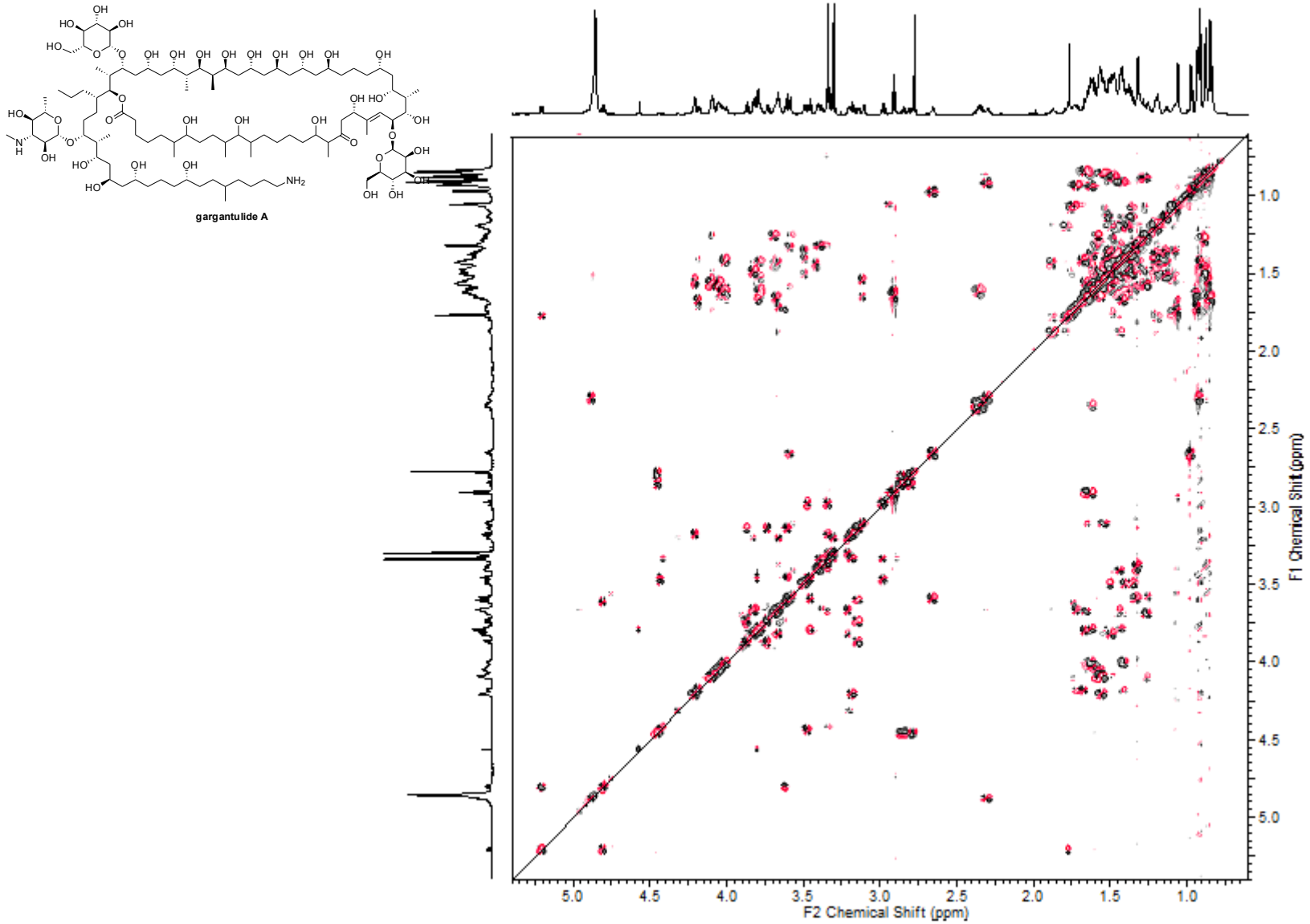


Figure S6. DQF-COSY NMR spectrum of gargantulide A (**1**) at 900MHz

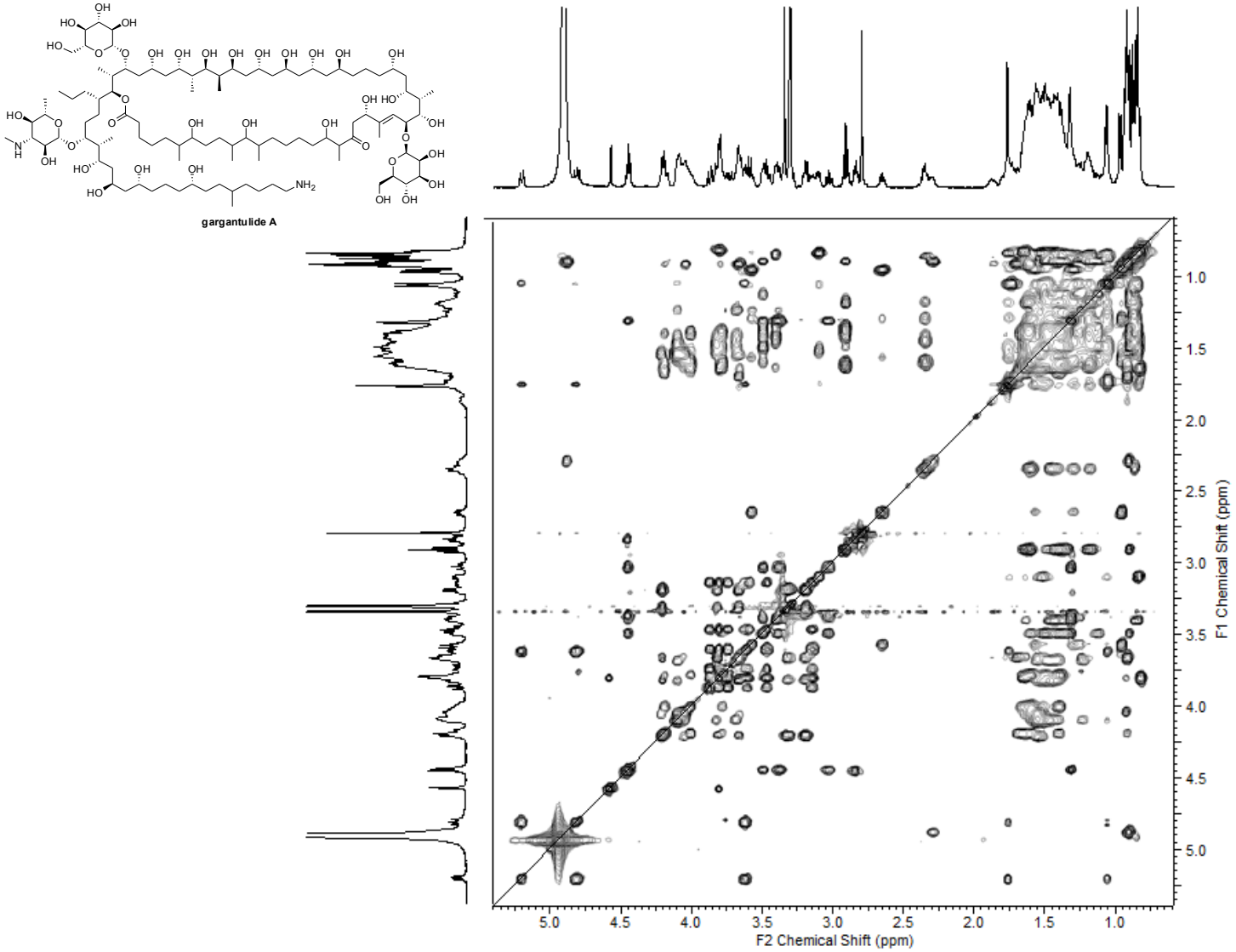


Figure S7. TOCSY spectrum of gargantulide A (**1**) at 500MHz

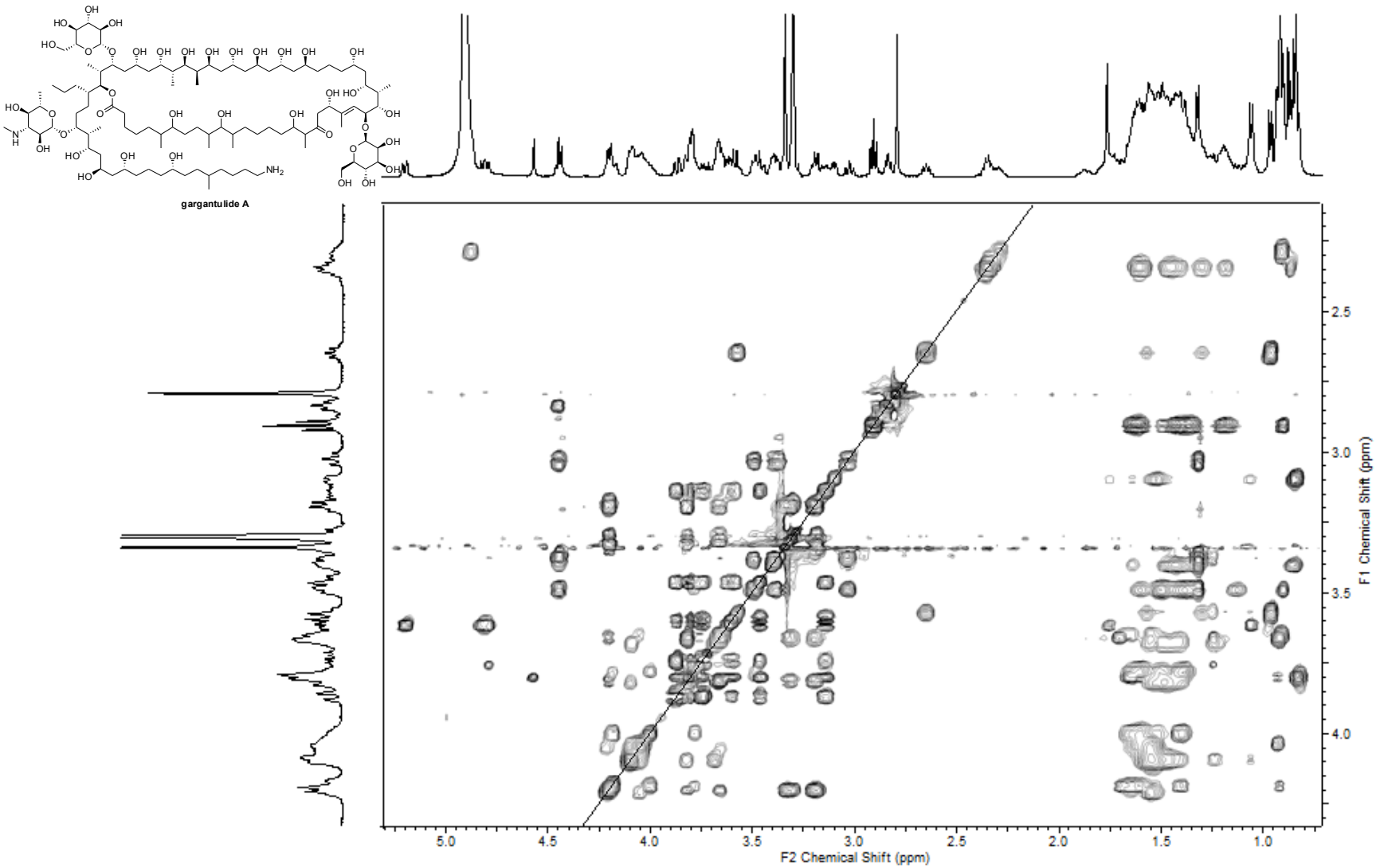


Figure S8. Expanded TOCSY spectrum of gargantulide A (**1**) at 500MHz.

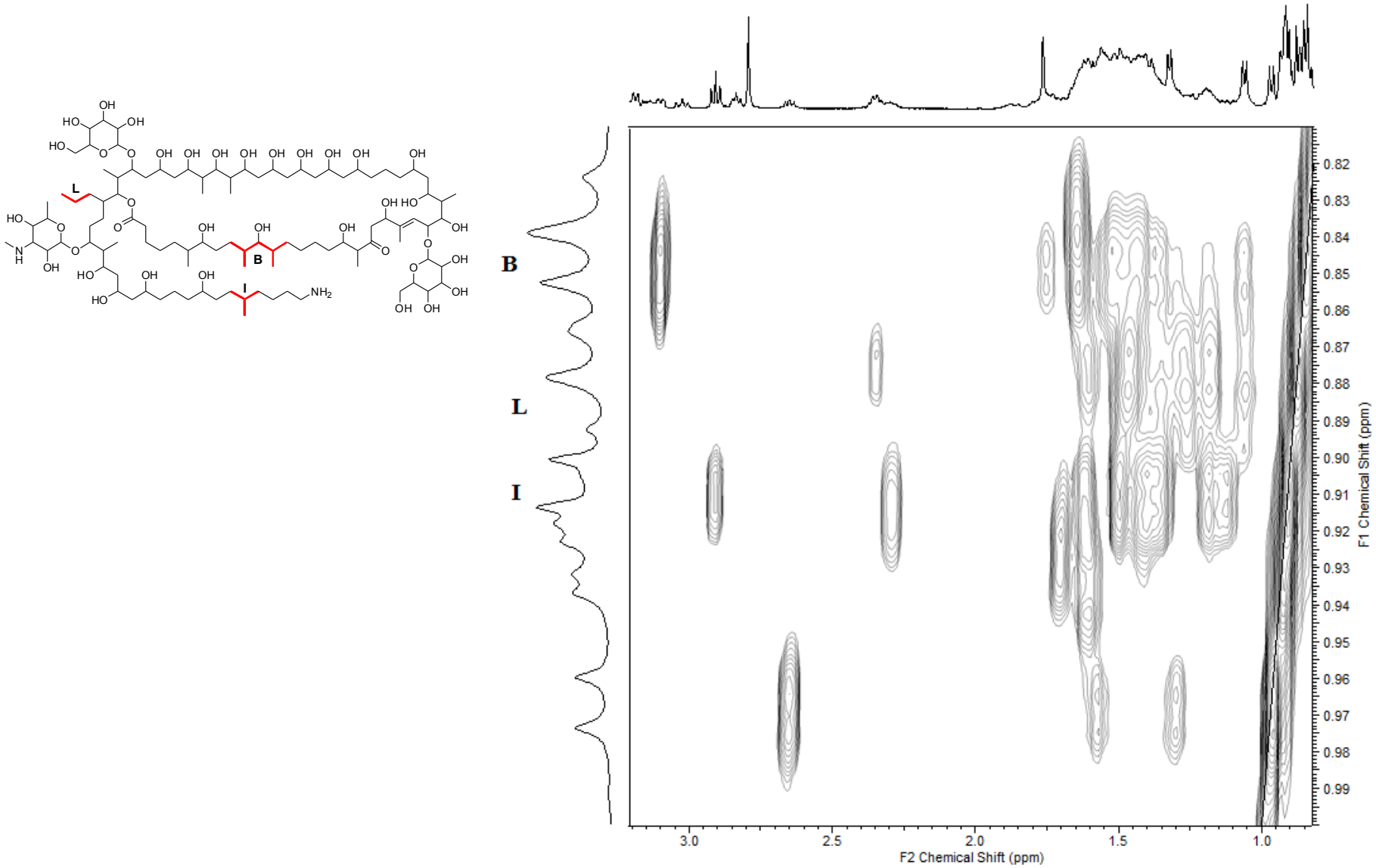


Figure S9. Expanded TOCSY spectrum of gargantulide A (**1**) at 500MHz.

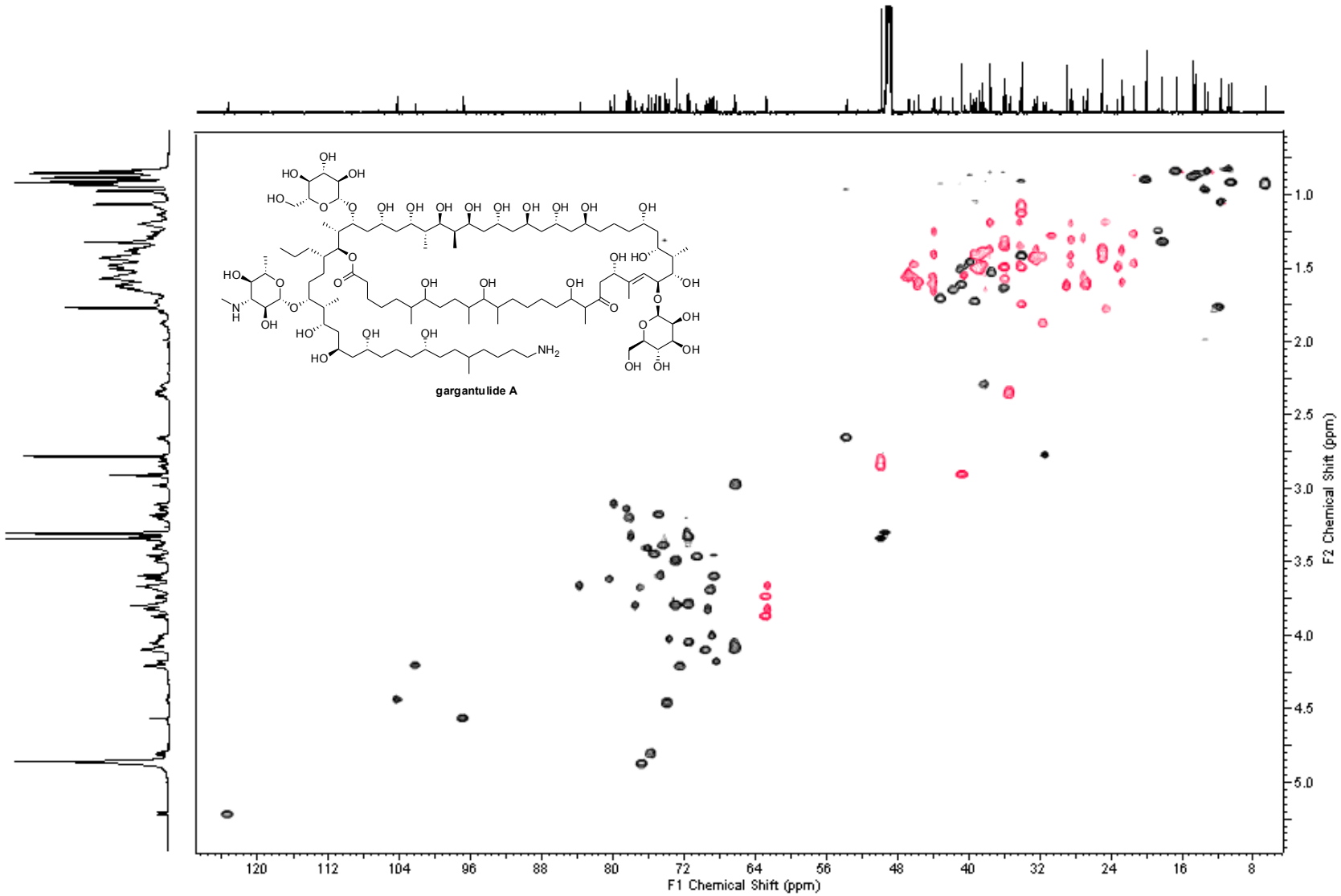


Figure S10. HSQC spectrum of gargantulide A (**1**) [Red : CH_2, Black: CH + CH_3] at 900 MHz.

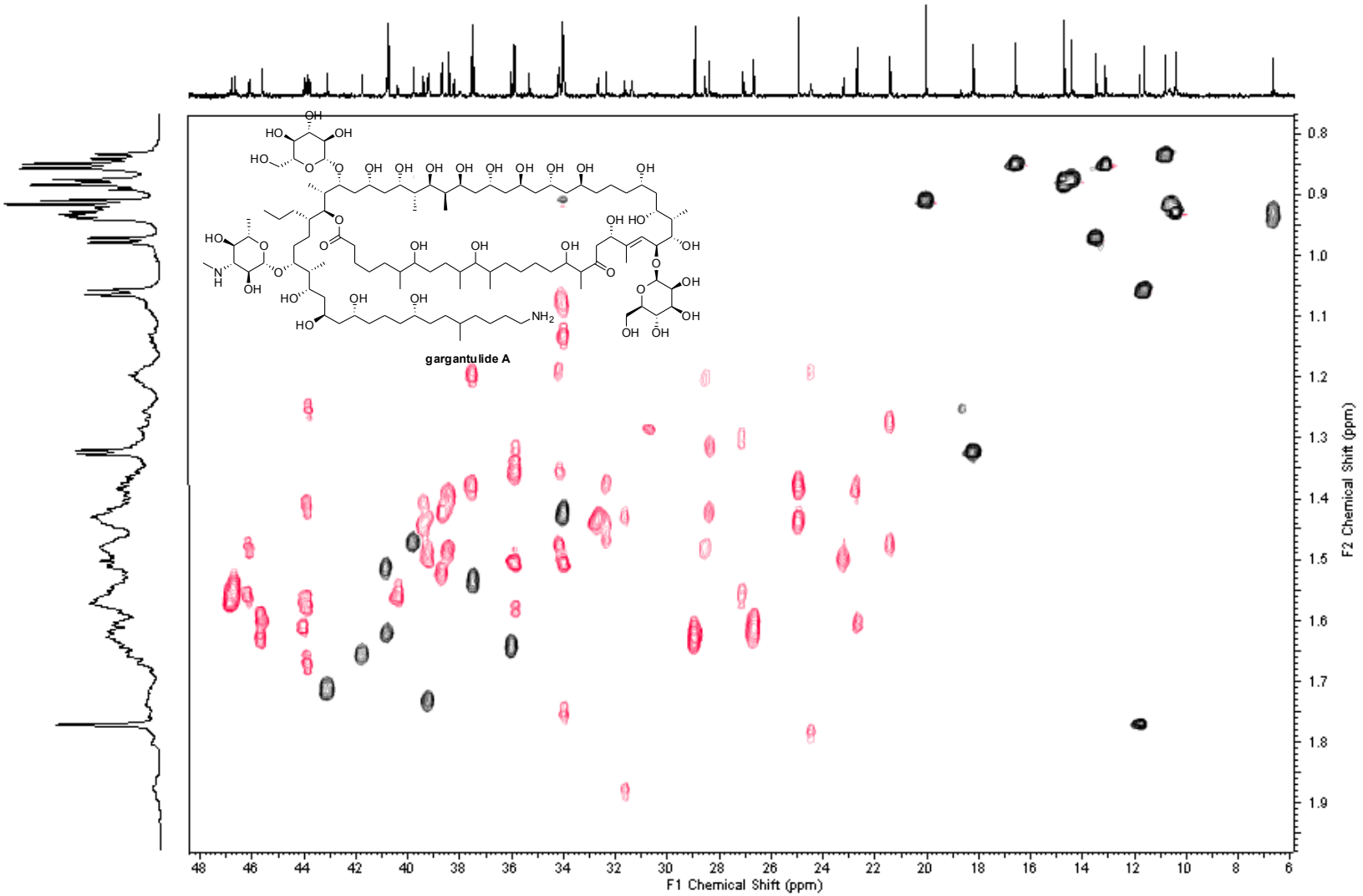


Figure S11. Expanded HSQC spectrum of gargantulide A (**1**) [Red : CH_2, Black: CH + CH_3] at 900 MHz..

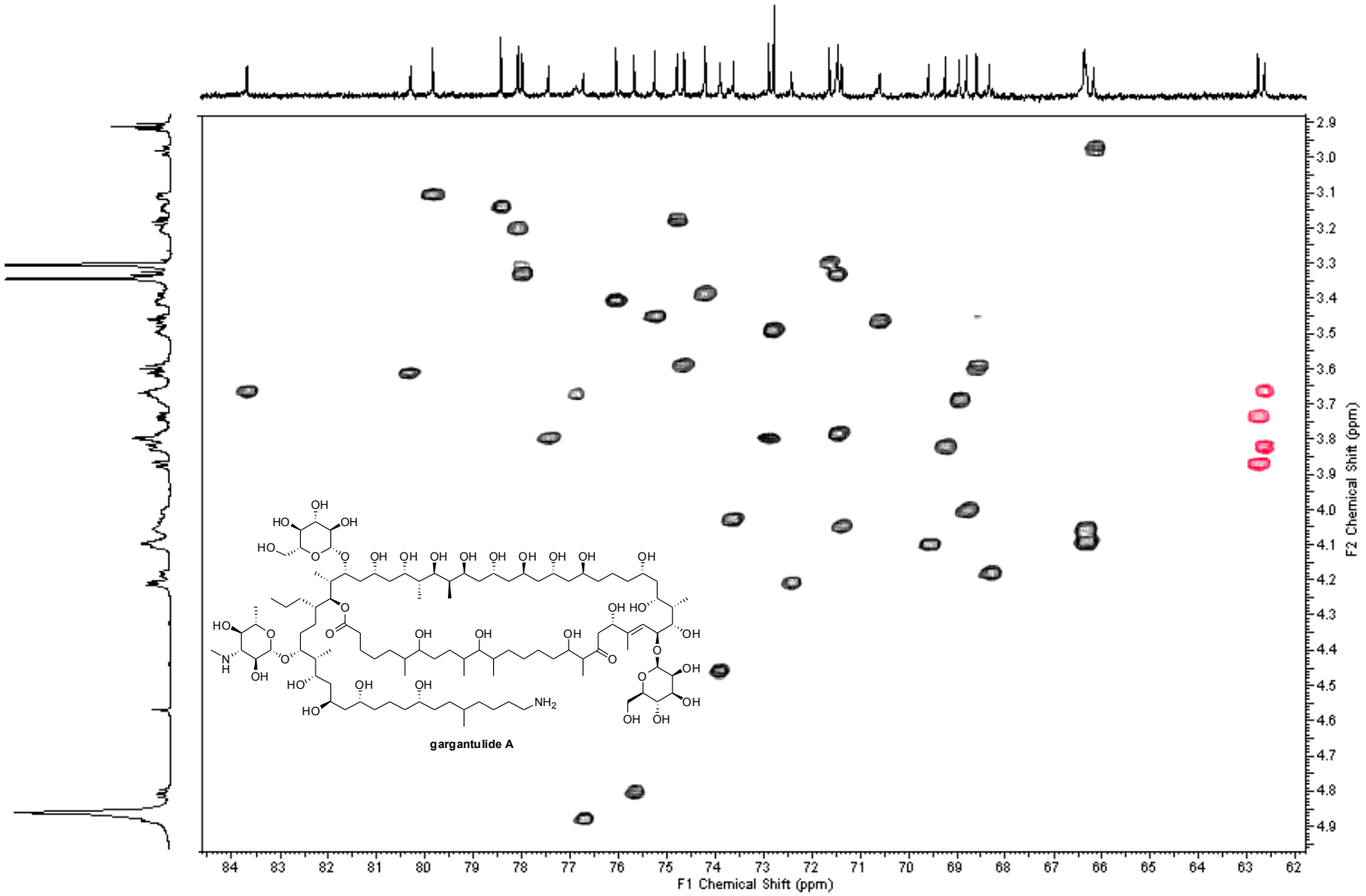


Figure S12. Expanded HSQC spectrum of gargantulide A (**1**) [Black : CH, Red: CH_2] at 900 MHz..

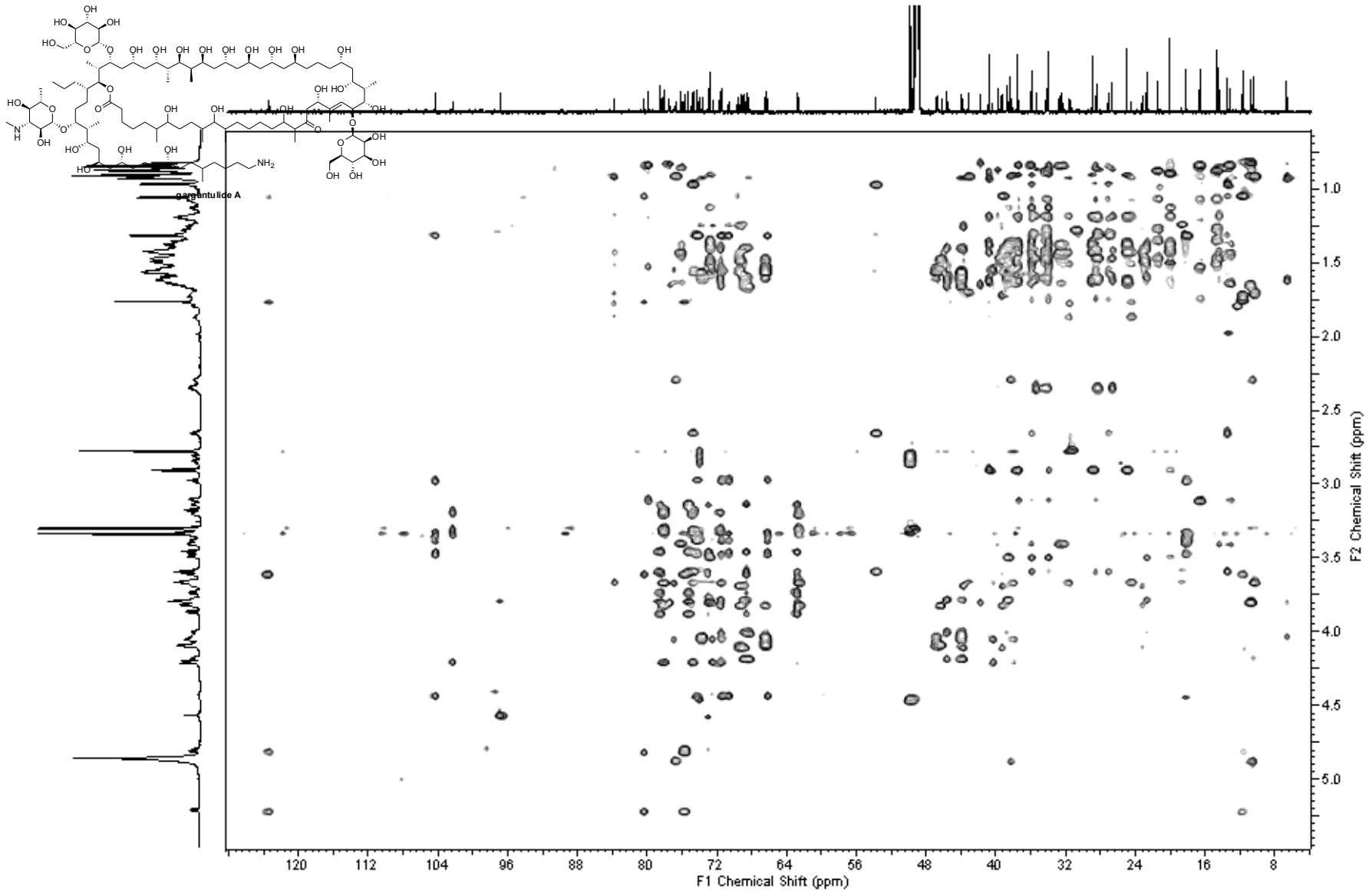


Figure S13. HSQC-TOCSY spectrum of gargantulide A (**1**) at 900 MHz.

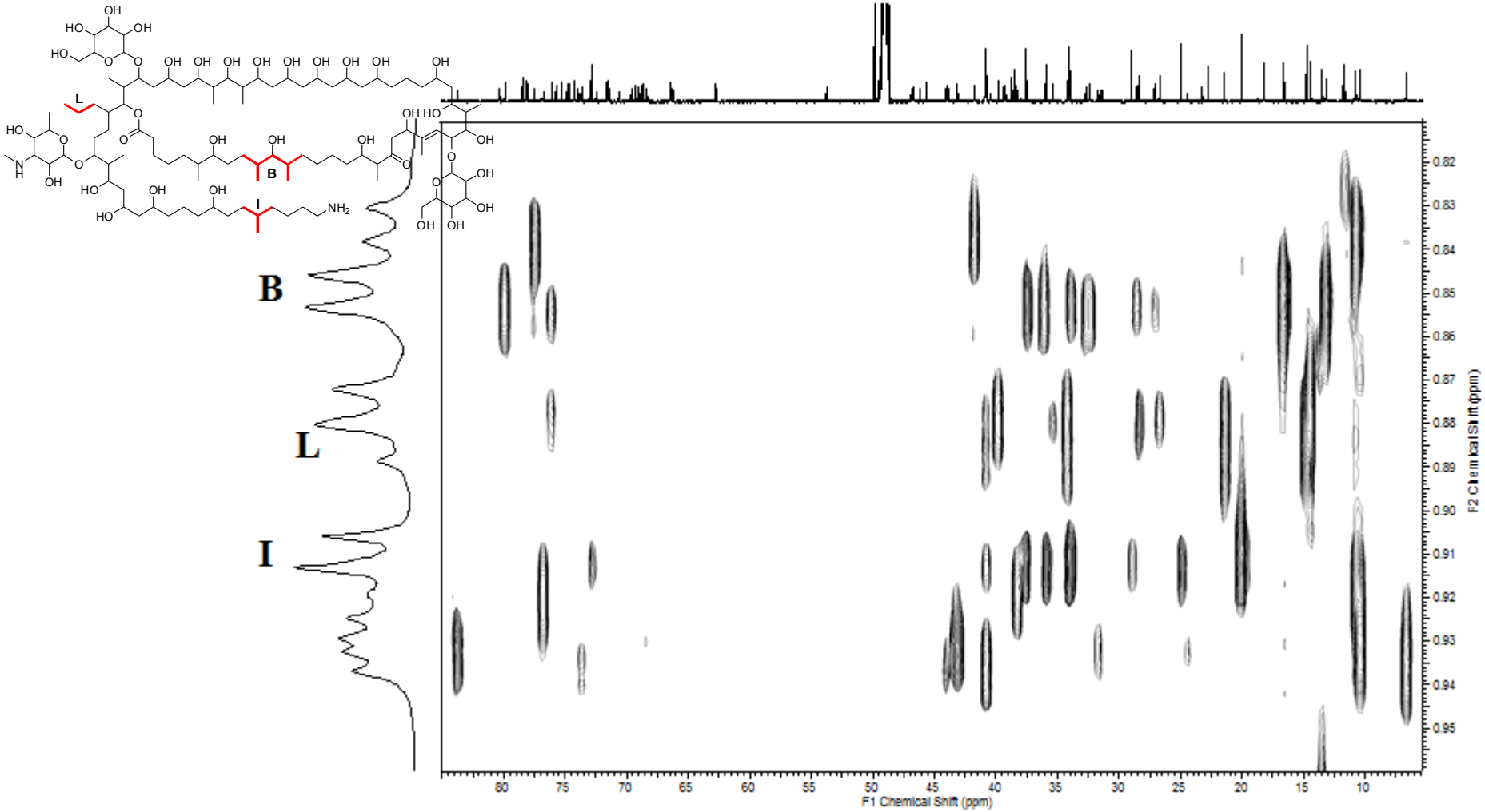


Figure S14. Expanded HSQC-TOCSY spectrum of gargantulide A (**1**) at 900 MHz.

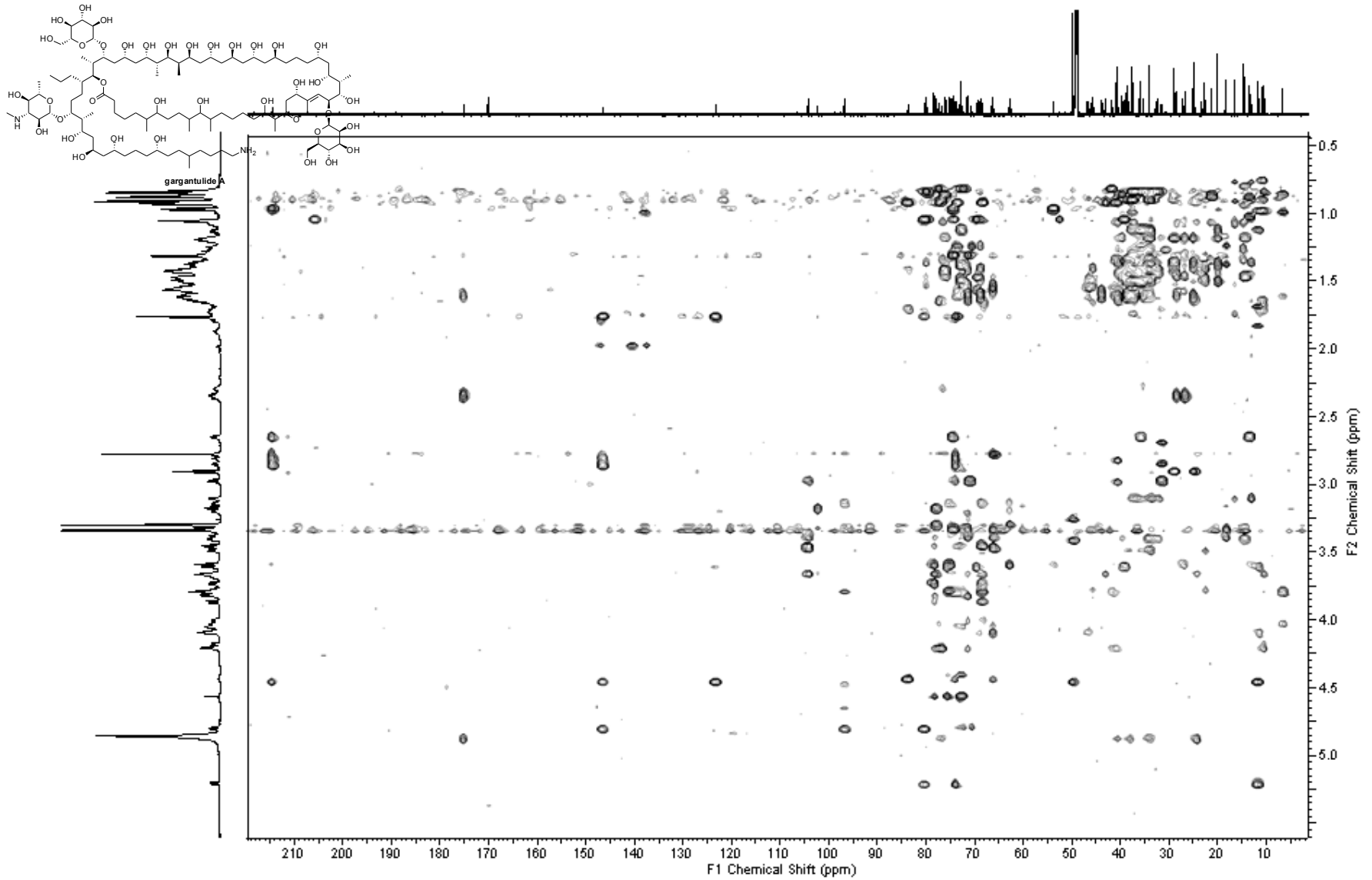


Figure S15. HMBC spectrum of gargantulide A (**1**) at 900 MHz.

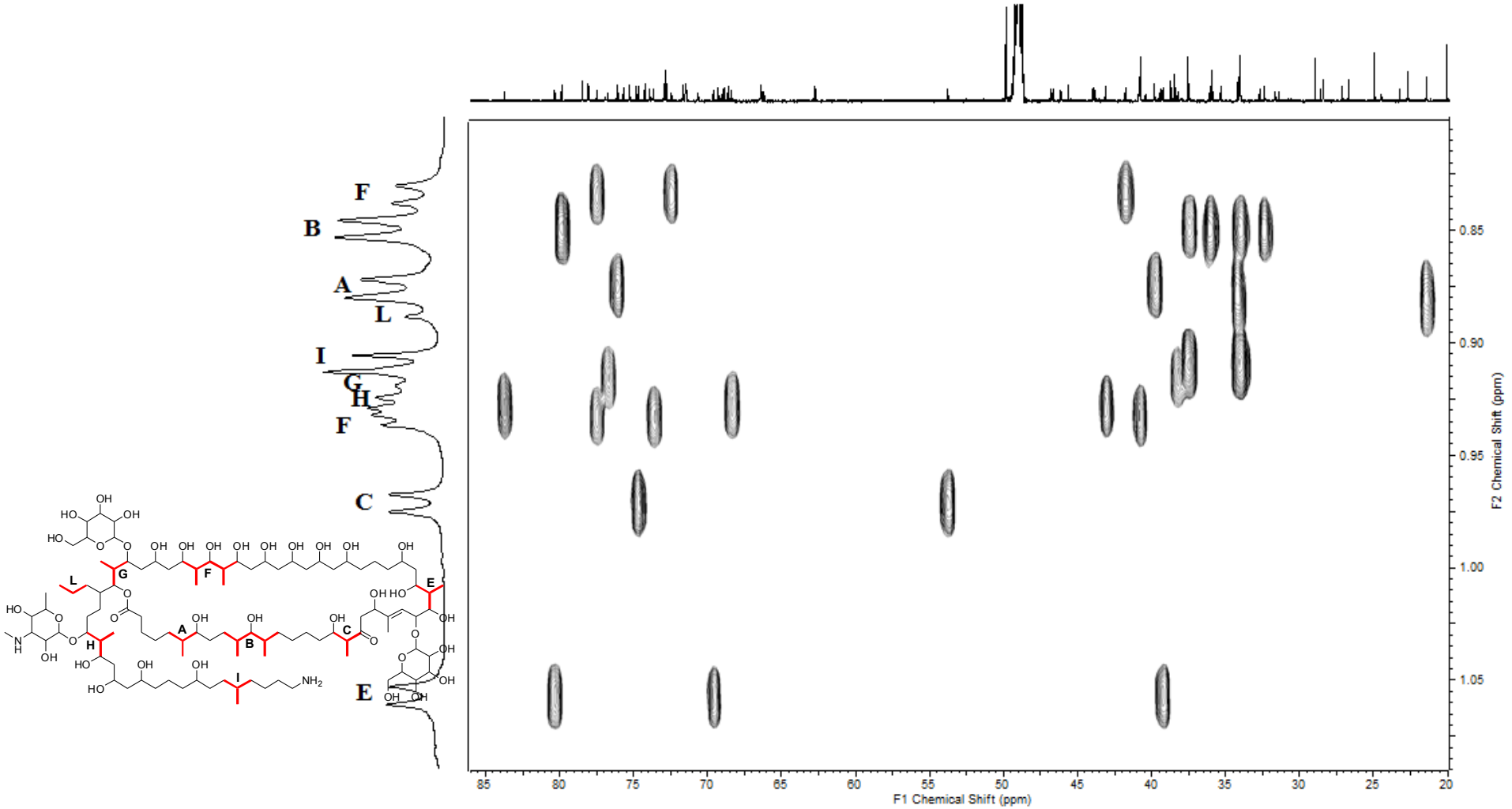


Figure S16. Expanded HMBC spectrum of gargantulide A (**1**) at 900 MHz

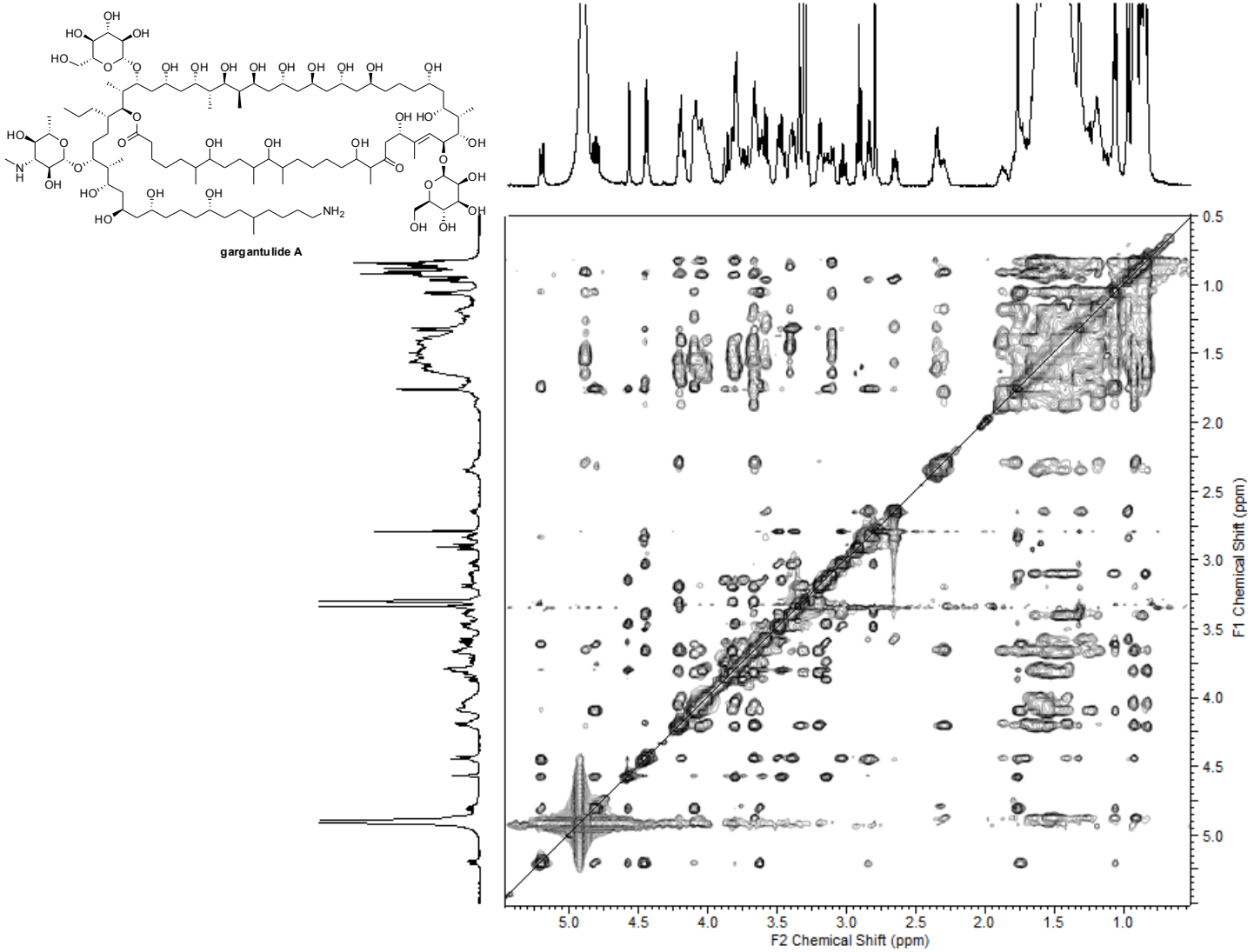


Figure S17. NOESY spectrum of gargantulide A (**1**) at 500 MHz.

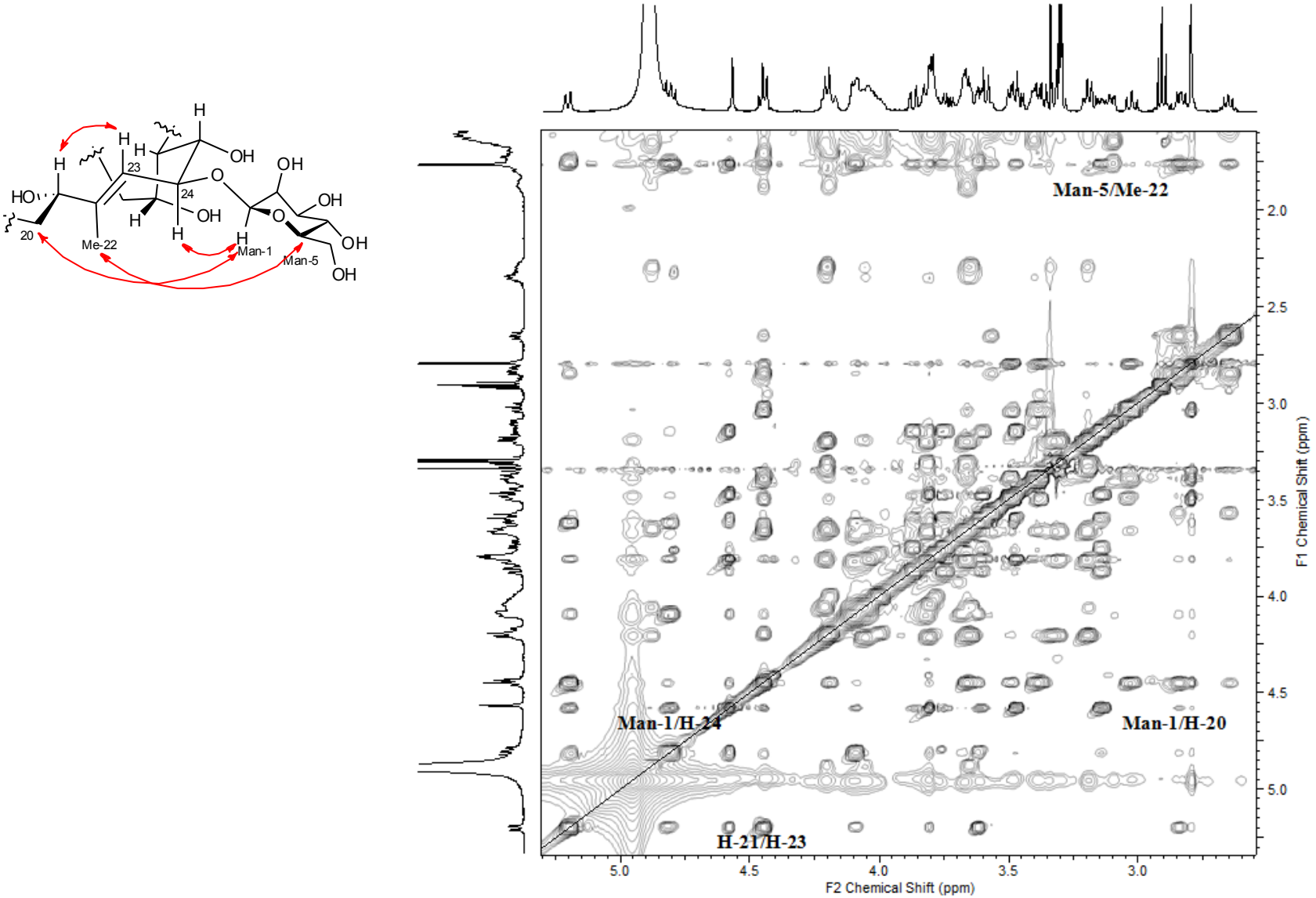


Figure S18. Expanded NOESY spectrum of gargantulide A (**1**) at 500 MHz.

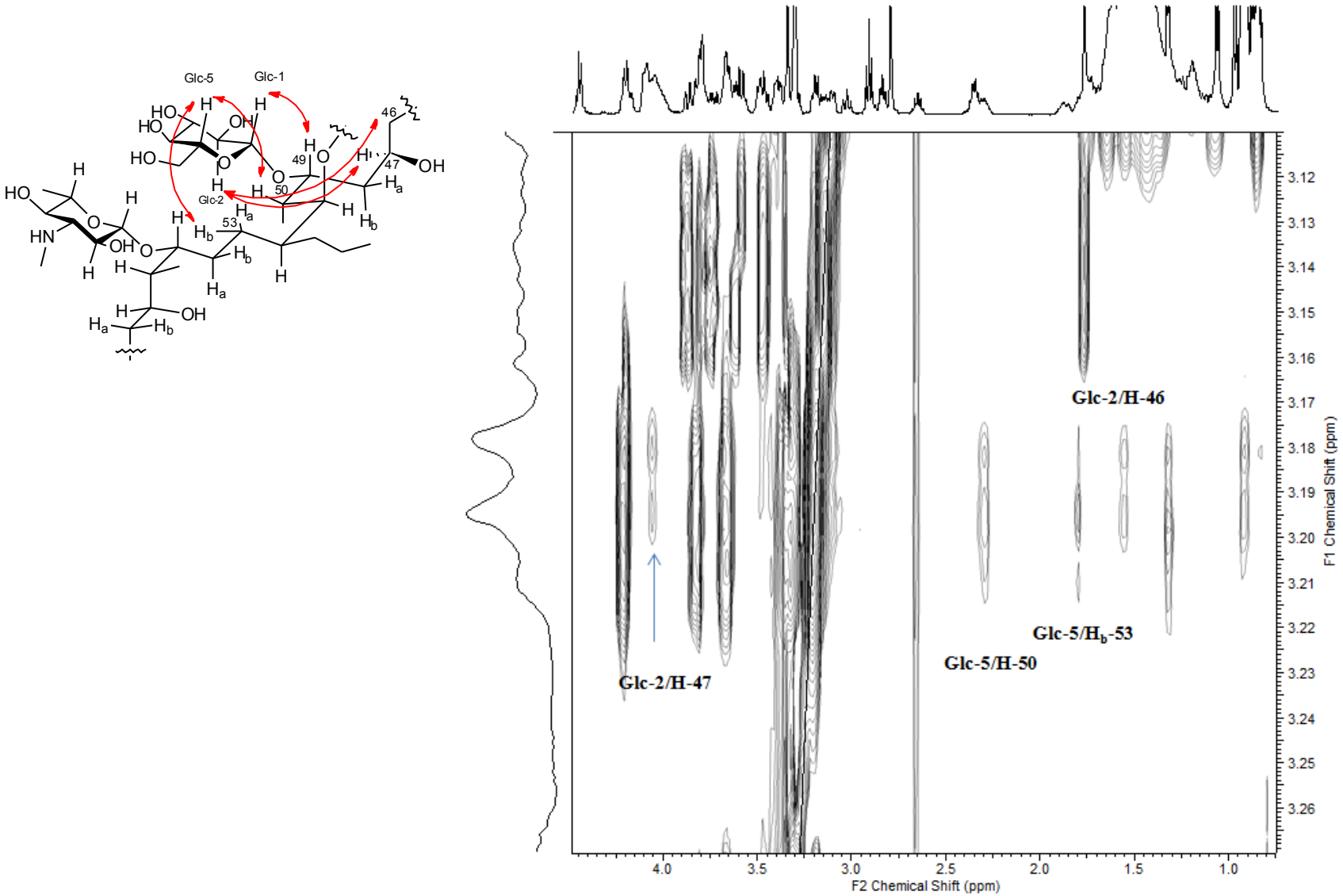


Figure S19. Expanded NOESY spectrum of gargantulide A (**1**) at 500 MHz.

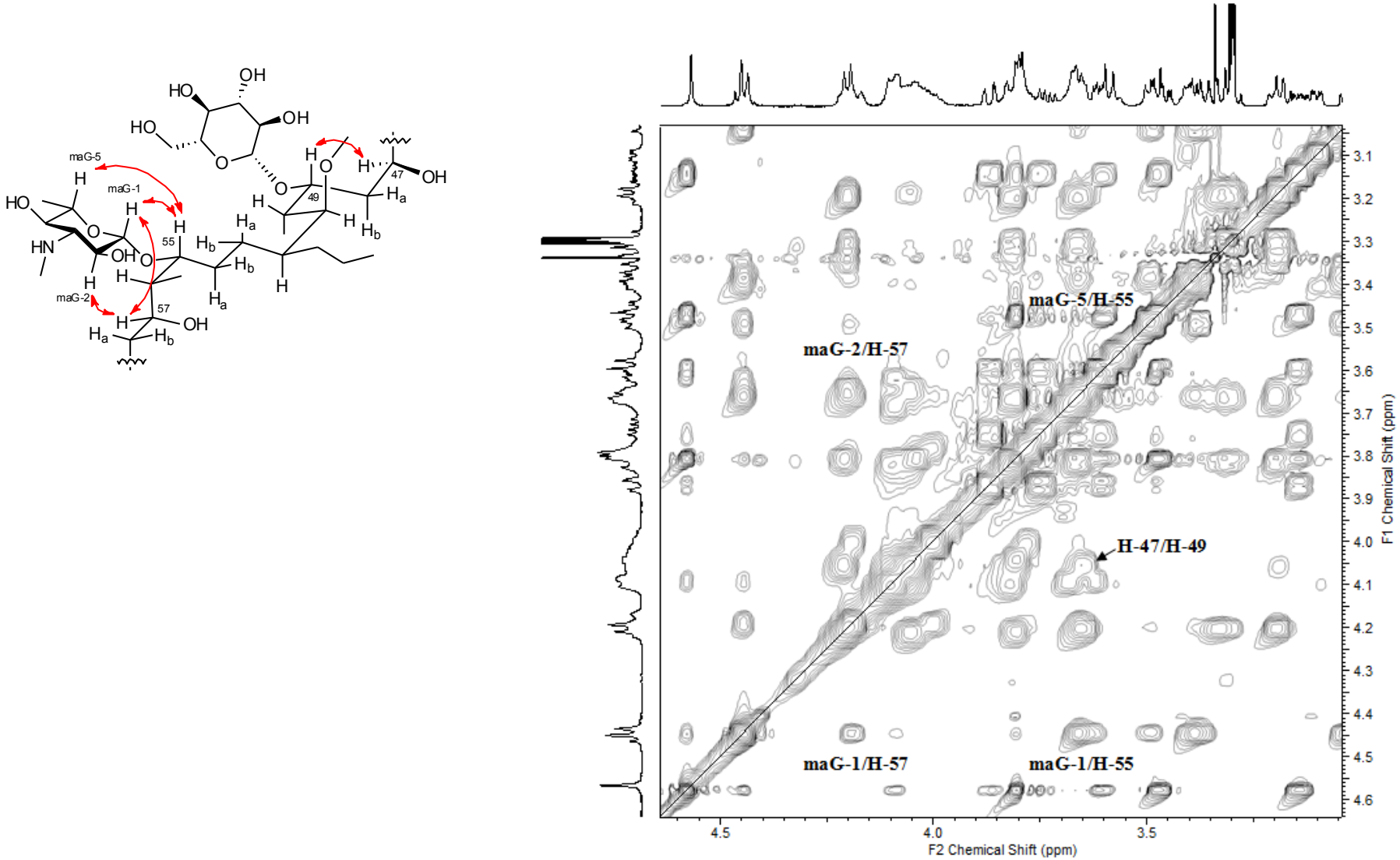


Figure S20. Expanded NOESY spectrum of gargantulide A (**1**) at 500 MHz.

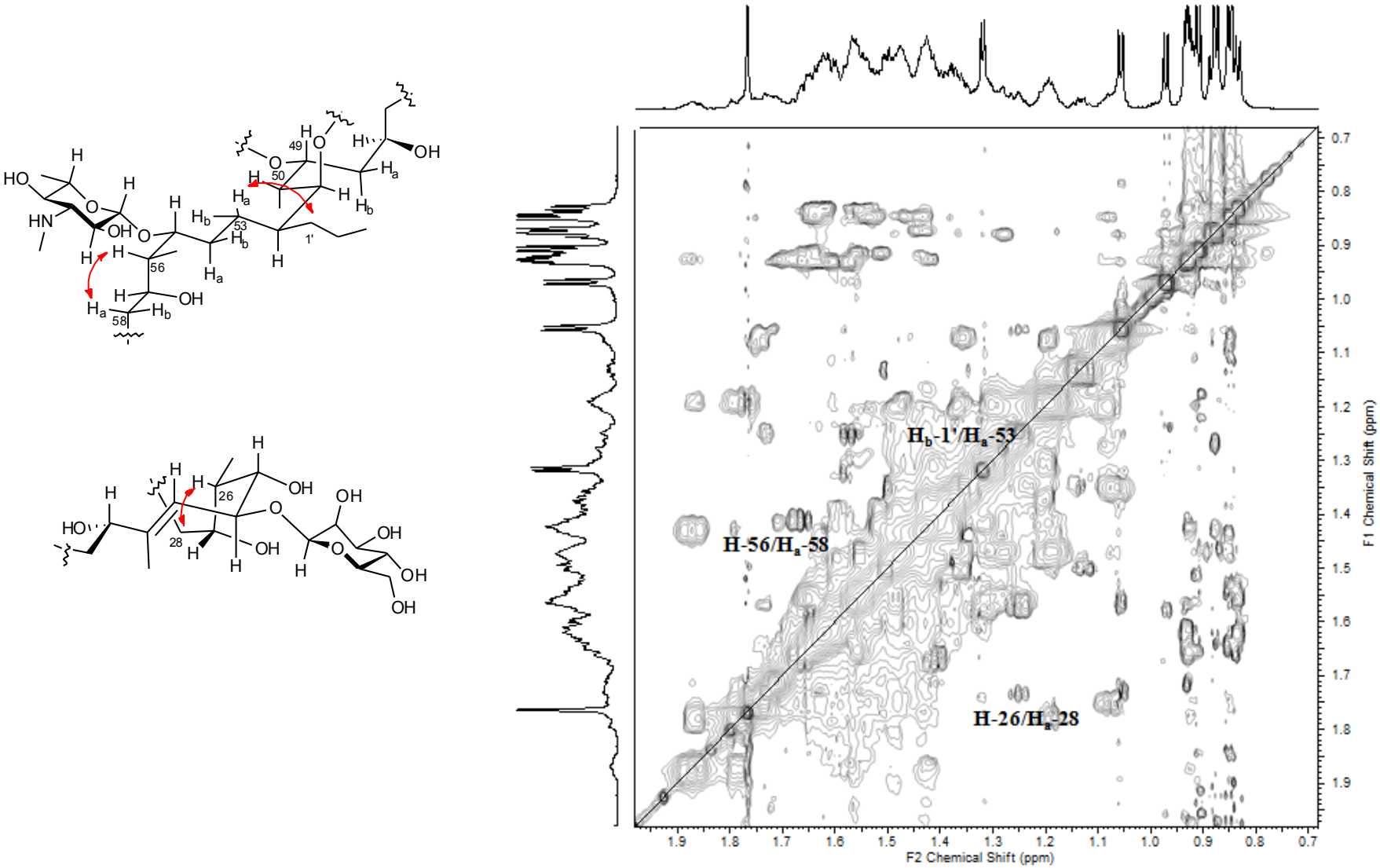


Figure S21. Expanded NOESY spectrum of gargantulide A (**1**) at 900 MHz.

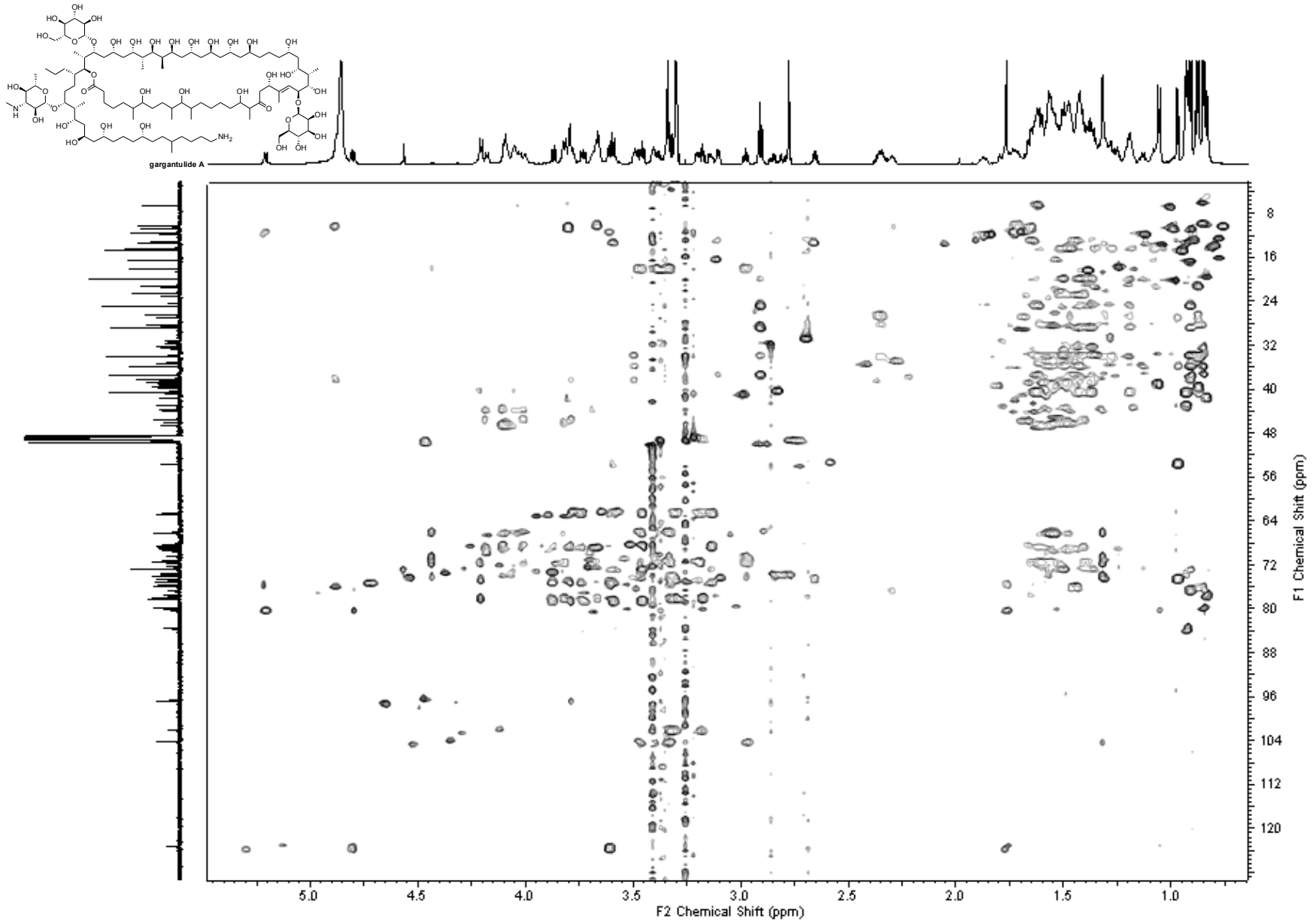


Figure S22. HECADE spectrum of gargantulide A (**1**) at 900 MHz.