Supporting Information

Targeted discovery of polycyclic tetramate macrolactams from an environmental *Streptomyces* strain

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List of Supporting Information

Materials and Methods

General experimental procedures. All NMR experiments were carried out on a Varian INOVA 600 MHz spectrometer. Clifednamides A (**4**) and B (**5**) were purified from active fractions on an Agilent 1100 series HPLC (Agilent Technologies) using a preparative Phenomenex C18 column (Luna, 25 cm × 21.2 mm, 5 μm particle size). IR spectrum was obtained on a Perkin Elmer 1600 series FT-IR spectrometer. $[\alpha]_D$ measurements were obtained using a Jasco P-2000 digital polarimeter with a sodium lamp.

***Streptomyces* sp. JV178 culturing and extraction of the clifednamides.** The isolation of *S*. sp. JV178 as a putative PTM producer was previously described.[1] JV178 was grown for 2 days in 15 mL MYG (10g malt extract, 4g glucose, 4g yeast extract per liter, pH 7.2) liquid medium while enclosed in a 125 mL flask (equipped with 6mm glass beads to provide baffles) at 30 °C on a flatbed shaker operating at 250 rpm. After 2 days growth, 0.2 mL of the mycelial suspension was plated for confluent growth to Difco ISP4 agar plates (Becton, Dickinson &Co., Sparks MD) containing ~30 ml solid medium. After 6 days of growth at 30 °C, the agar from the plates was diced and extracted with ethyl acetate overnight at room temperature. The liquid extract containing clifednamides A and B was filtered through Whatman paper to remove small agar pieces and spore material washed from the plates. The extract was dried under vacuum while the reserved agar pieces were again extracted for an additional 2.5 hours and the resulting extract was processed as above.

Separation. The EtOAc extract was chromatographed on a C18 HPLC column (Phenomenex, Luna, 250 × 21.2 mm, 5 μ) using 60% MeCN-H_2O (9 mL/min) with 0.1% formic acid to yield compounds, **4** (t_R 16.5 min, 2 mg) and fraction D (t_R 12 min). From fraction D, compound **5** (t_R 11 min, 0.8 mg) was obtained using a phenyl-hexyl HPLC column (Phenomenex, Luna, 250 × 10 mm, 5 μ; 60% MeOH/H_2O with 0.1 % formic acid; 2 mL/min).

Reference

(1) Blodgett, J. A. V; Oh, D. C.; Cao, S.; Currie, C. R.; Kolter, R.; Clardy, J. *Proc. Natl. Acad. Sci. U S A* **2010**, *107*, 11692-11697.

Additional structural data for clifednamides A and B.

Clifednamide A **(4)**: colorless powder; $[\alpha]^{23}_{D}$ +96 (*c*, 0.1, 50% MeOH/$CHCl_3$); UV (50% MeCN/H_2O) λ_{max} 236 (4.22), 327 (4.03) nm; IR (film) 3331, 1706, 1655, 1638, 1609, 1504, 1234, 756; ^{1}H NMR (600 MHz, 90% pyridine-d_5/CD_3OD) and ^{13}C NMR (150 MHz, 90% pyridine-d_5/CD_3OD): see Table S1; HRMS *m/z* 493.2697 ([M+H]), calcd for $C_{29}H_{37}N_2O_5$, 493.2702).

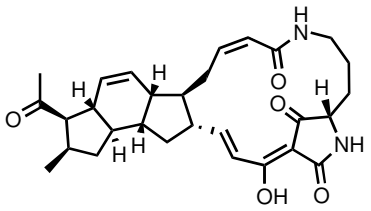
Clifednamide B **(5)**: colorless powder; $[\alpha]^{23}_{D}$ +122 (*c*, 0.1, 50% MeOH/$CHCl_3$); UV (50% MeCN/H_2O) λ_{max} 236 (4.23), 327 (4.05) nm; IR (film) 3306, 1706, 1654, 1636, 1607, 1506, 1235, 770; ^{1}H NMR (600 MHz, 90% pyridine-d_5/CD_3OD) and ^{13}C NMR (150 MHz, 90% pyridine-d_5/CD_3OD): see Table S1; HRMS *m/z* 509.2662 ([M+H]), calcd for $C_{29}H_{37}N_2O_6$, 509.2652).

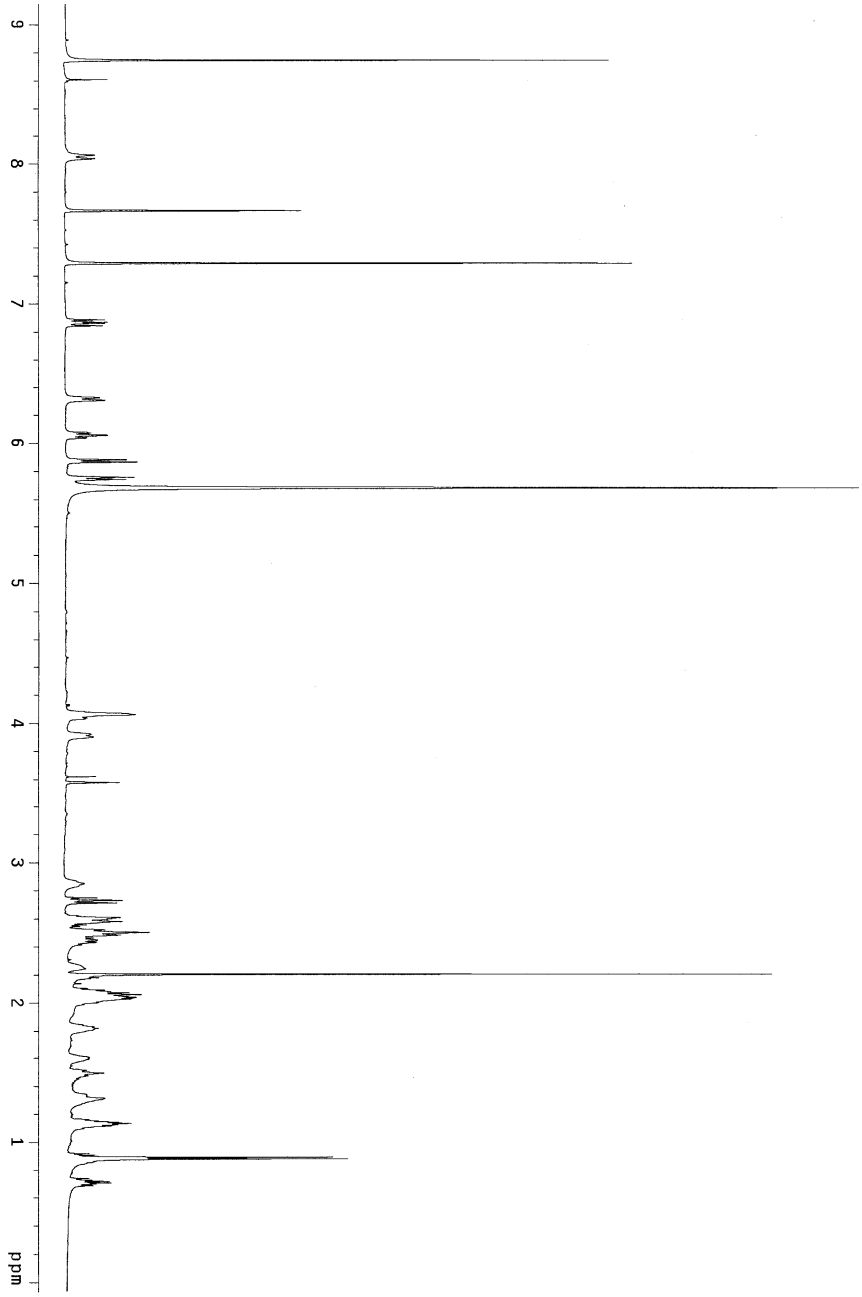
Table S1. $^1H^a$ and $^{13}C^b$ NMR Data (in 90% pyridine-d_5/CD_3OD) of Compounds **4** and **5**

	^{1}H		^{13}C		
#	**4**	**5**	#	**4**	**5**
1			1	167.6	168.2
2	6.32 (d 11.1)	6.40 (d 10)	2	125.8	126.4
3	6.06 (td 11.1, 2.4)	6.11 (t 10)	3	139.9	140.0
4	4.05 (m)	4.21 (m)	4	26.2	26.5
	2.60 (m)	2.60 (m)			
5	1.49 (m)	1.46 (m)	5	48.5	49.0
6	2.50 (m)	2.55 (m)	6	43.2	43.3
7	5.75 (br d, 9.6)	5.76 (br d, 9.6)	7	129.7	130.5
8	5.87 (br d, 9.6)	5.86 (br d, 9.6)	8	130.5	130.7
9	2.50 (m)	2.55 (m)	9	43.7	44.2
10	2.73 (dd 11.4, 10.8)	2.70 (t 10.8)	10	59.4	59.9
11	2.58 (m)	2.55 (m)	11	34.2	34.6
12	2.06 (m)	2.04 (m)	12	39.3	39.7
	0.72 (ddd 19.2, 12.0, 7.2)	0.69 (m)			
13	1.13 (m)	1.13 (m)	13	48.2	48.5
14	2.06 (m)	2.05 (m)	14	41.8	42.3
15	2.06 (m)	2.02 (m)	15	37.4	38.2
	1.13 (m)	1.12 (m)			
16	2.45 (m)	2.50 (m)	16	49.4	49.0
17	6.86 (dd 15.6, 10.2)	6.85 (dd 14.4, 10.8)	17	147.5	143.1
18	8.05 (d 15.6)	8.51 (d 14.4)	18	124.0	134.0
19			19	174.3	180.3
20			20	103.2	104.0
21			21	178.0	185.3
22			22		
23	4.05 (m)	4.47 (br s)	23	61.4	68.7
24			24	197.6	196.1
25	2.24 (m)	4.94 (m)	25	28.3	73.1
	2.06 (m)				
26	1.82 (m)	1.60 (m)	26	22.3	33.6
	1.60 (m)				
27	3.91 (m)	4.21 (m)	27	39.5	38.3
	2.85 (m)	3.3 (m)			
28					
29			29	209.9	210.2
30	2.21 (s)	2.19 (s)	30	31.6	32.1
31	0.88 (d 7.2)	0.88 (d 7.2)	31	19.3	19.8

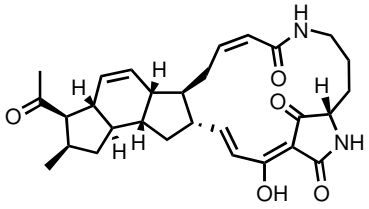
[a]δ (ppm) 600 MHz; multiplicities; *J* values (Hz) in parentheses. [b]δ (ppm) 150 MHz.

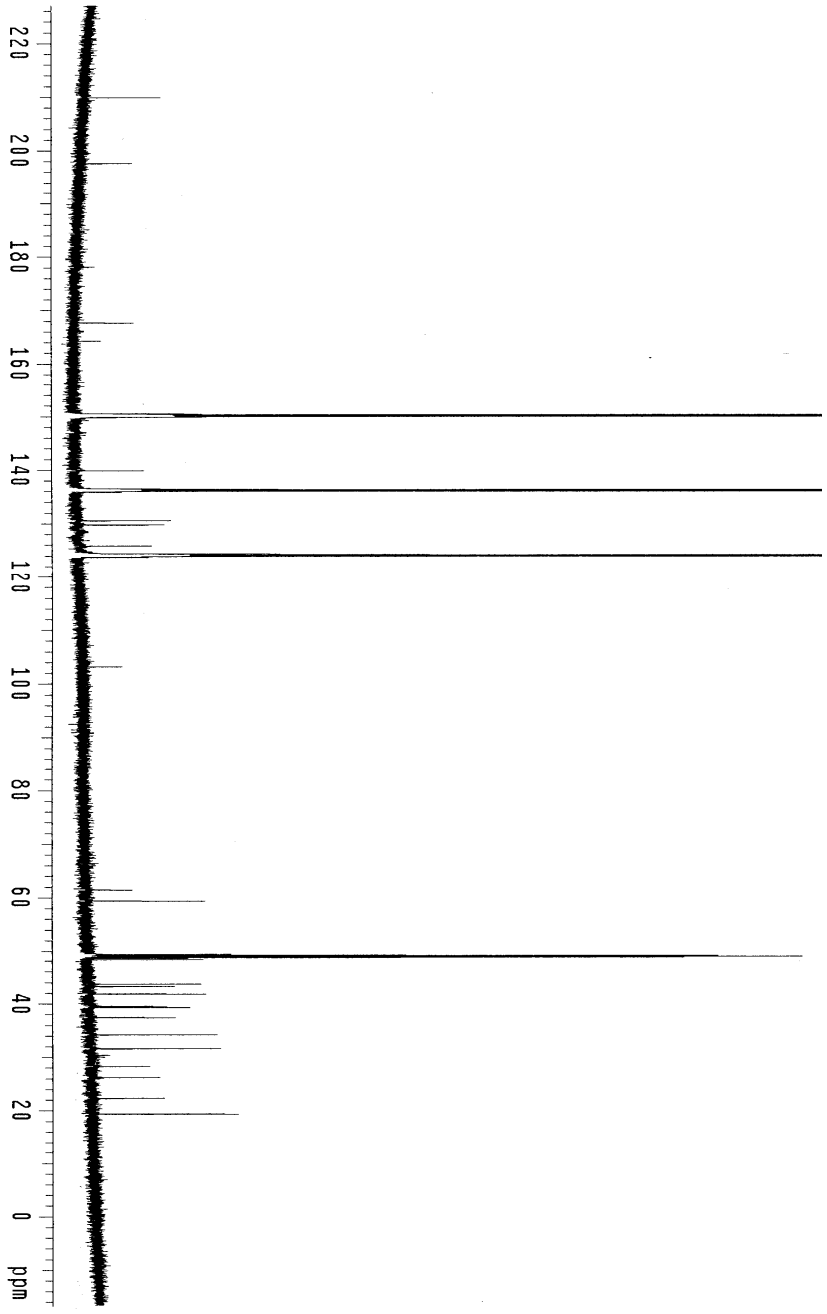
^{1}H NMR spectrum of **4**



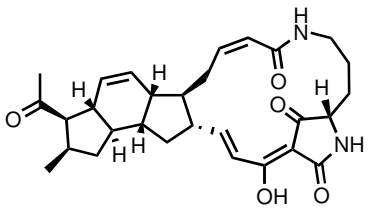


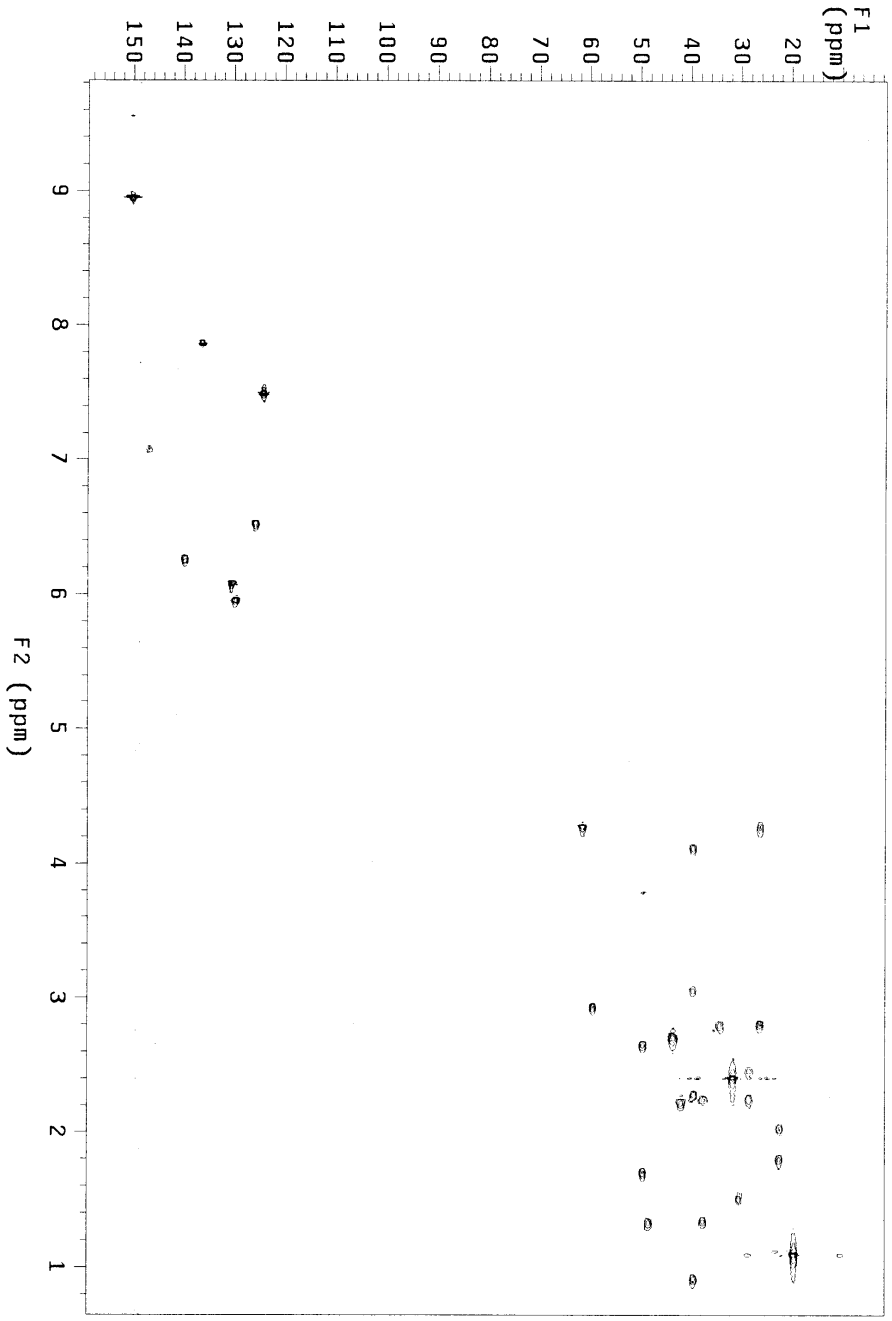
^{13}C NMR spectrum of **4**



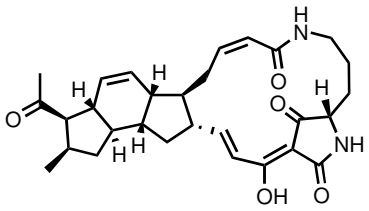


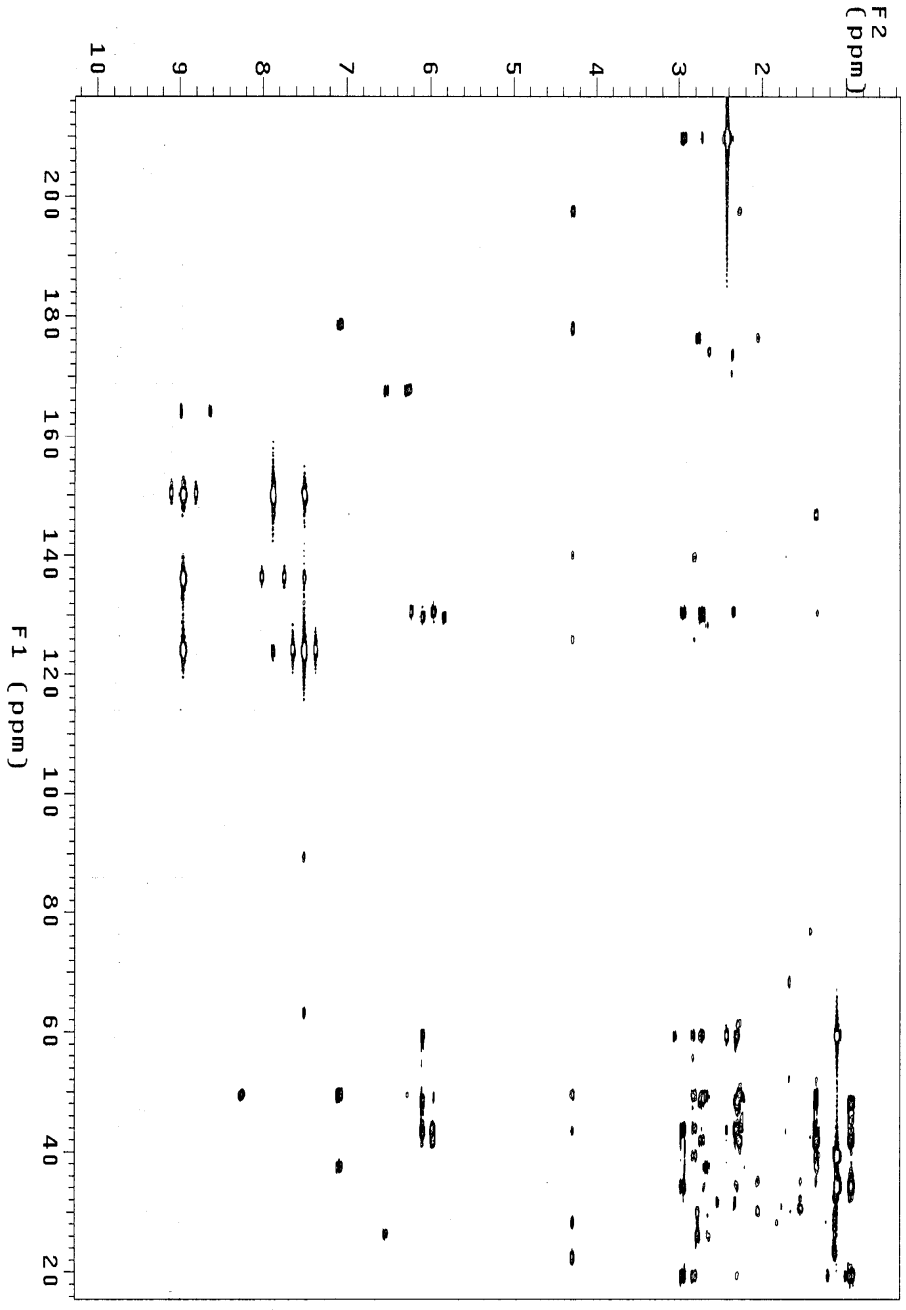
gHSQC spectrum of **4**



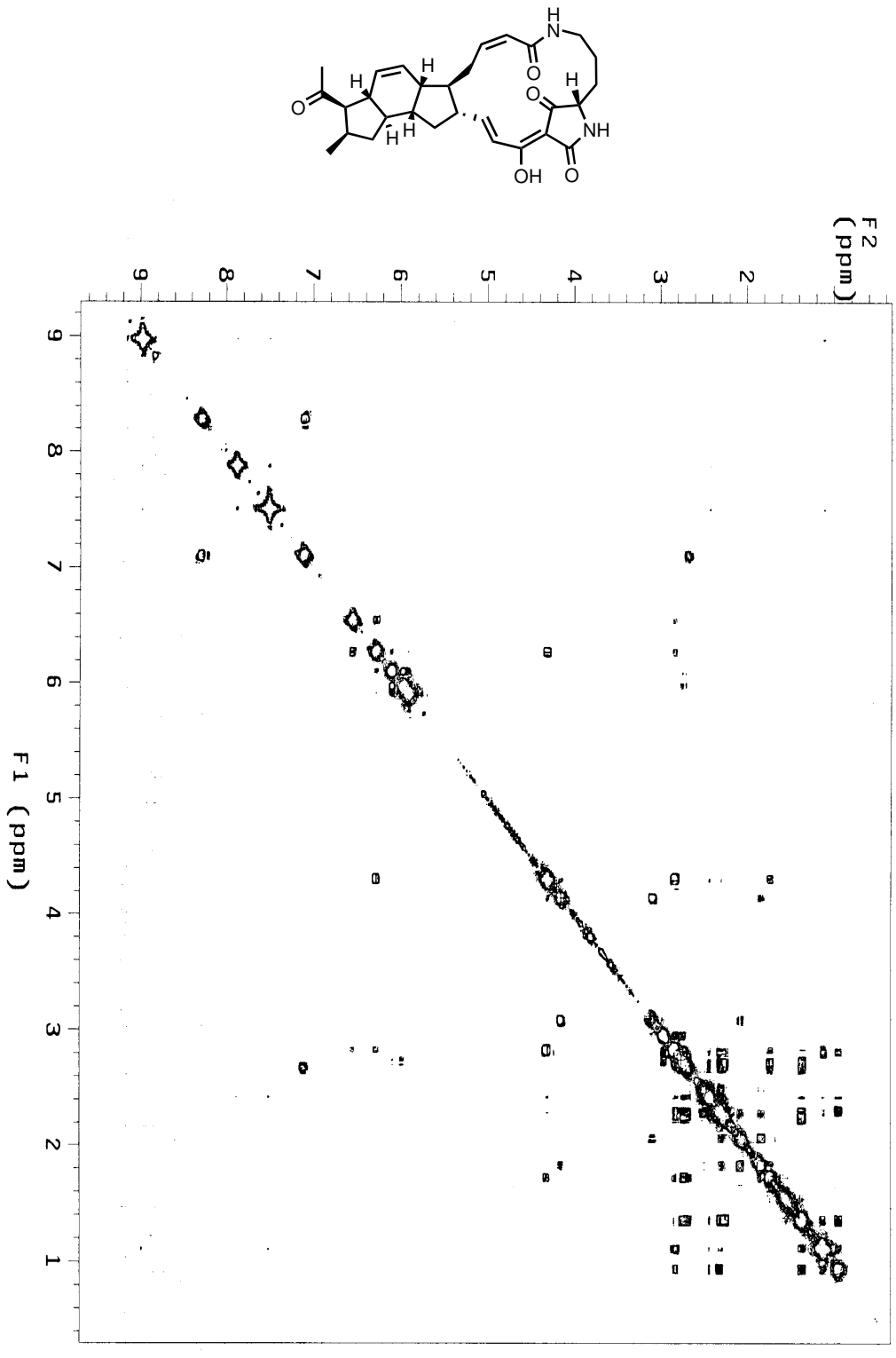


gHMBC spectrum of **4**

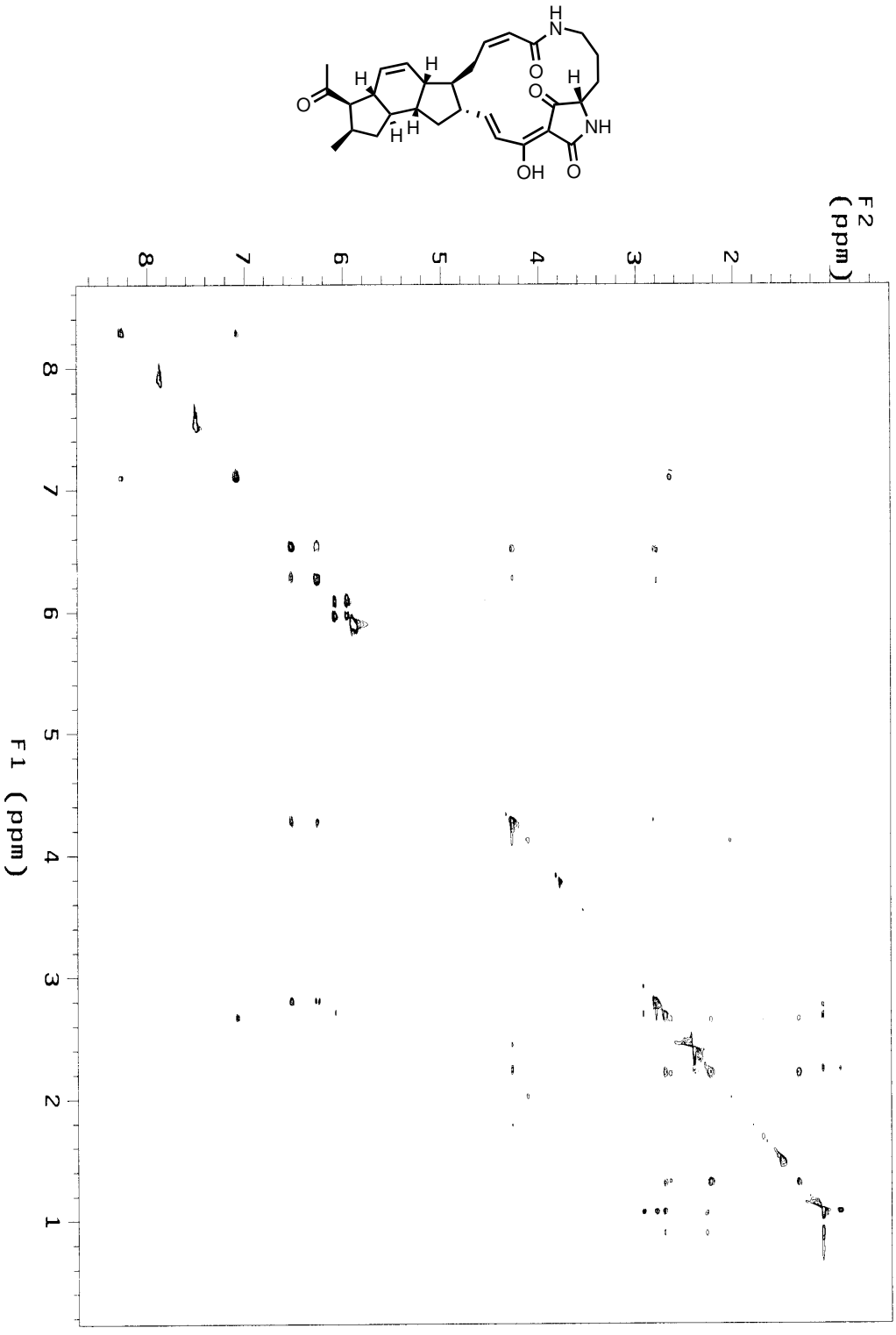




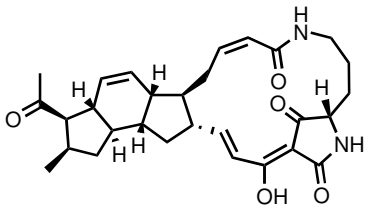
gCOSY spectrum of **4**

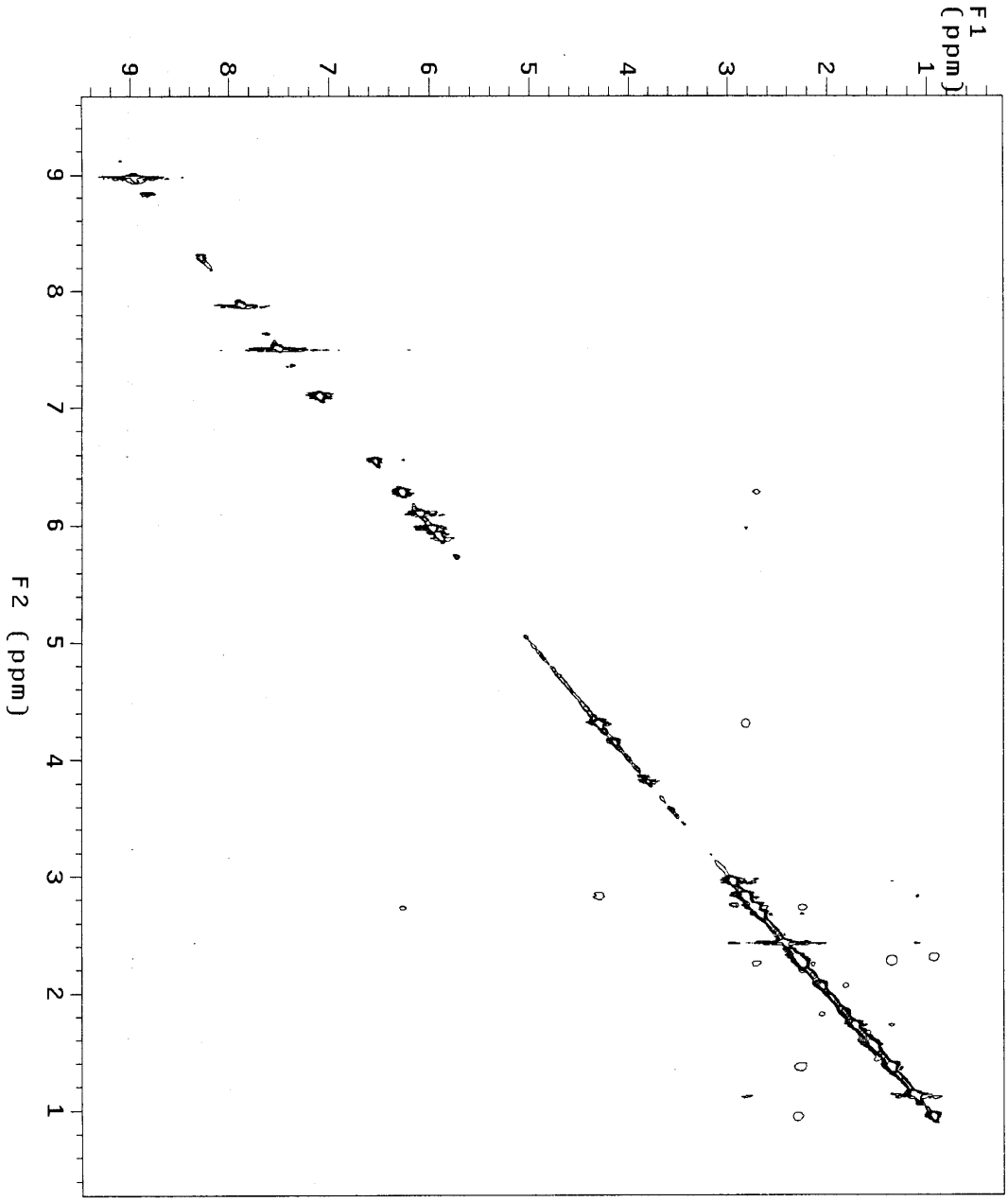


TOCSY spectrum of **4**

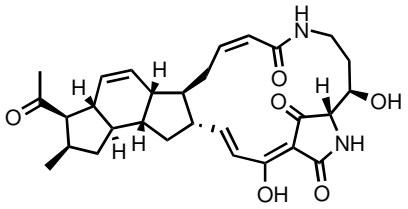


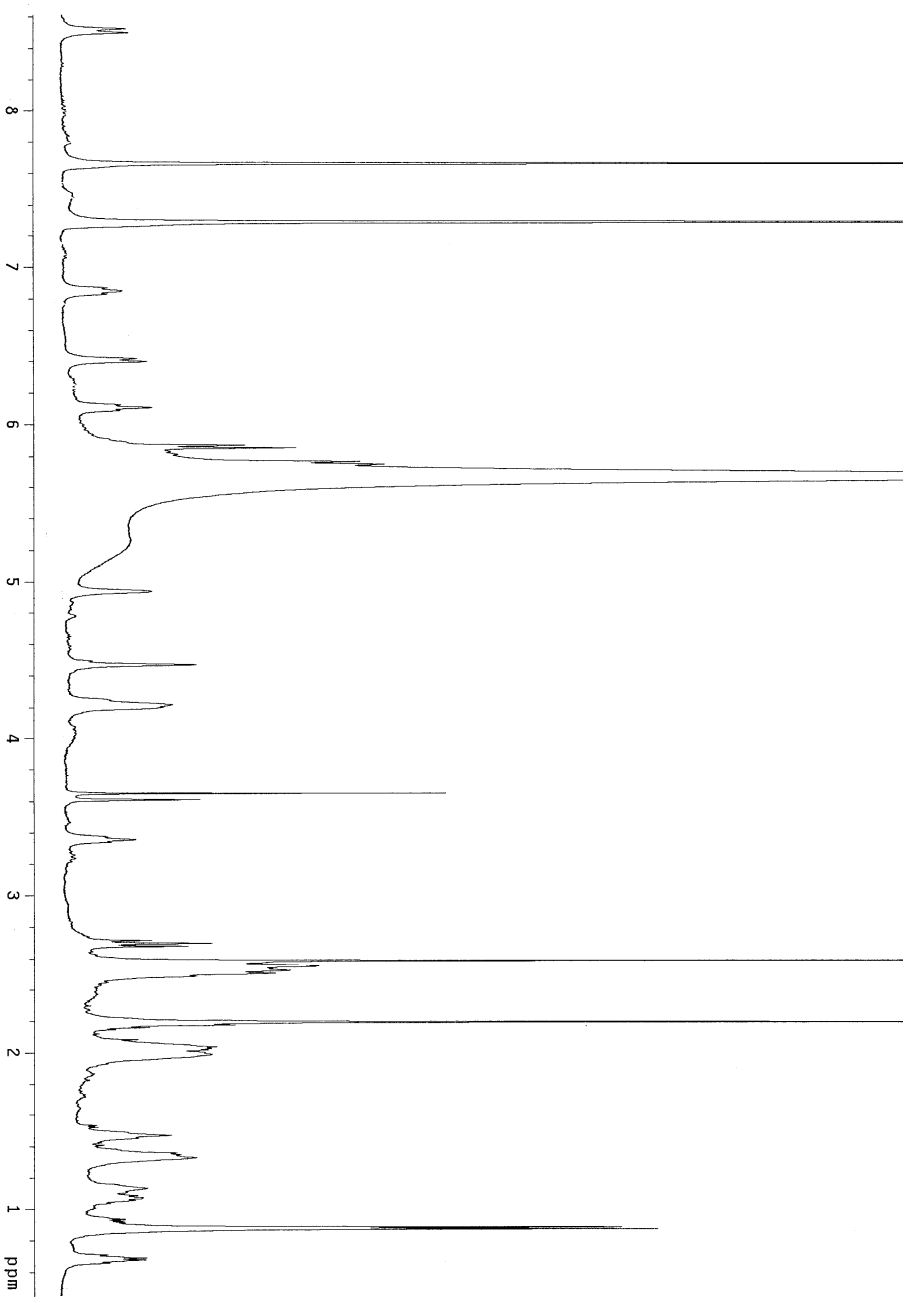
ROESY spectrum of **4**



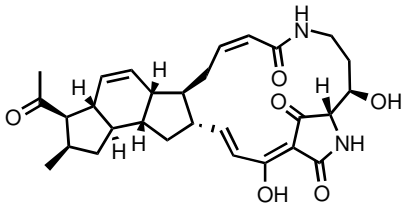


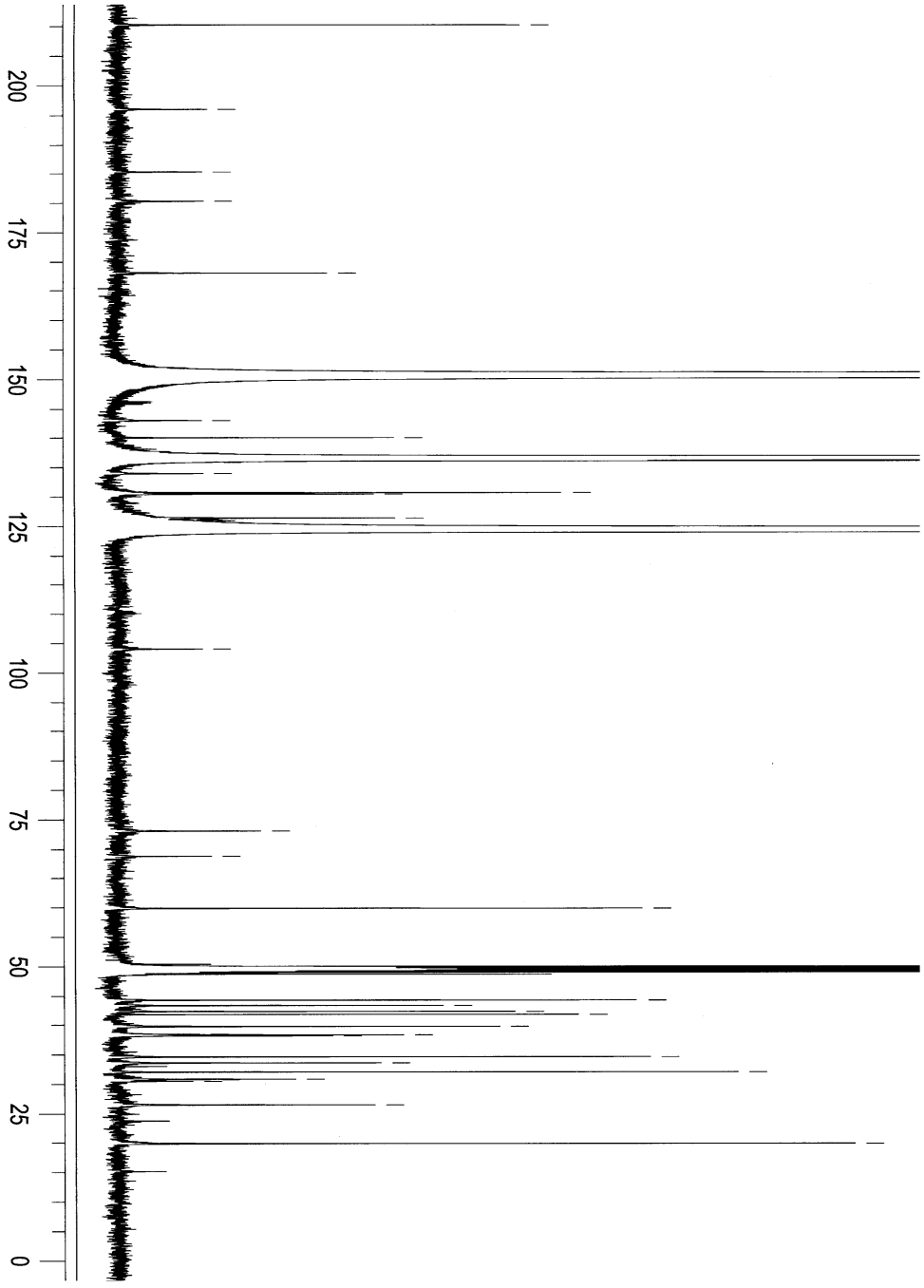
^{1}H NMR spectrum of **5**



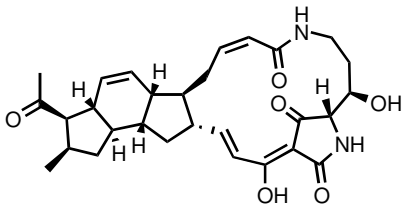


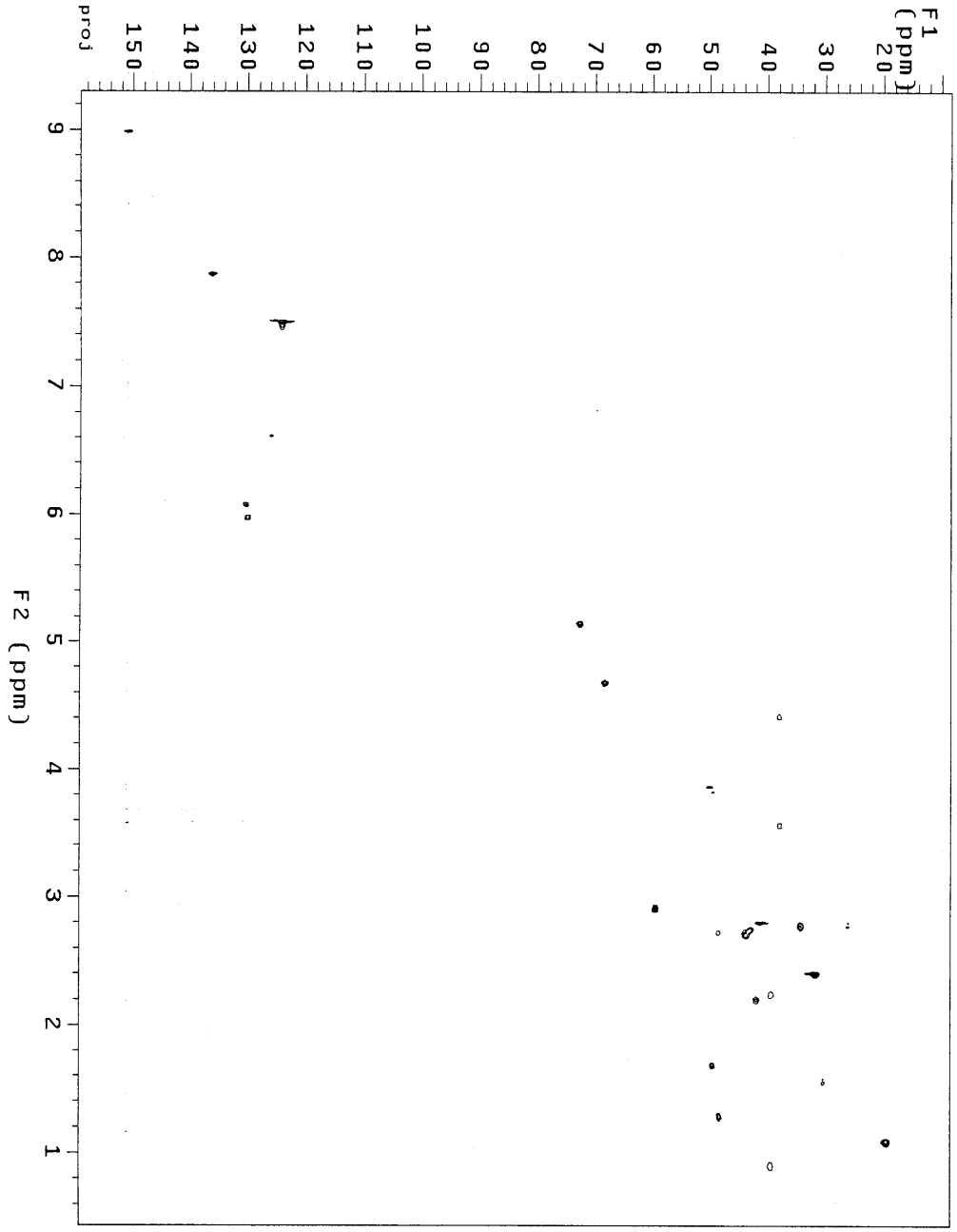
^{13}C NMR spectrum of **5**



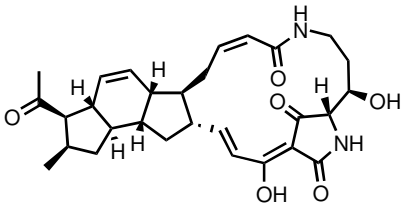


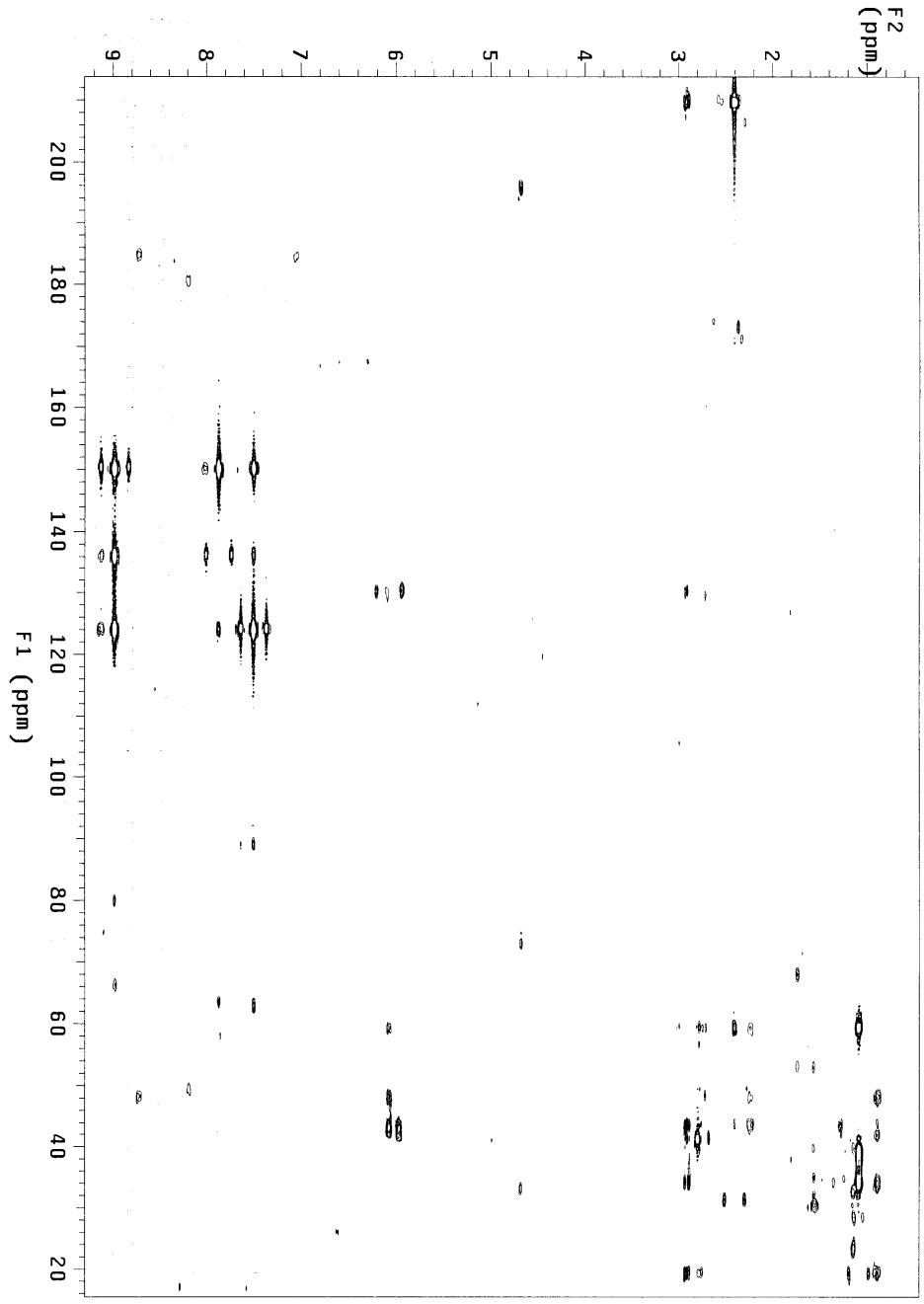
gHSQC spectrum of **5**



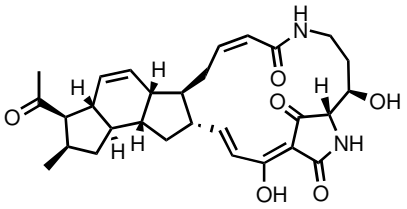


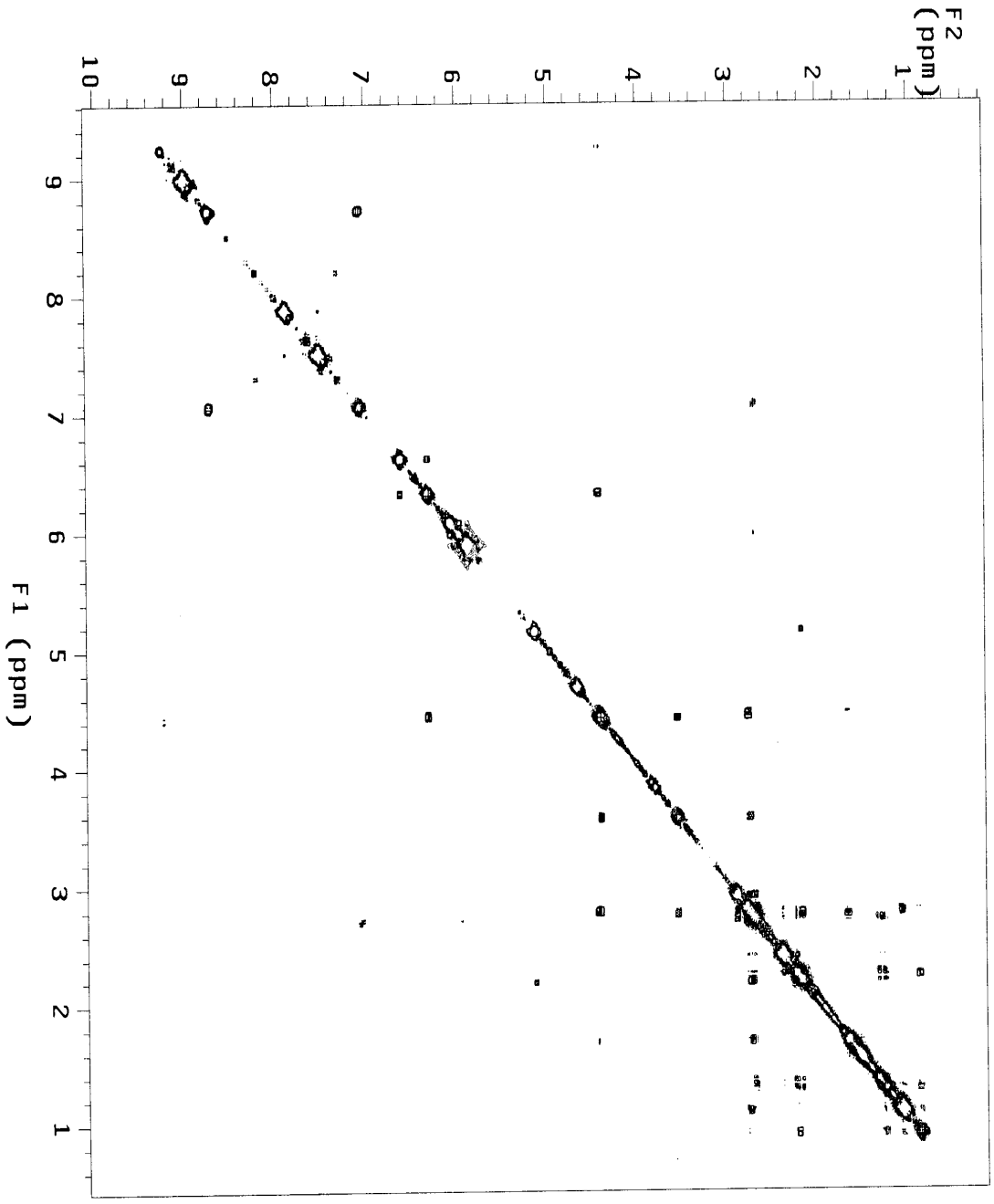
gHMBC spectrum of **5**



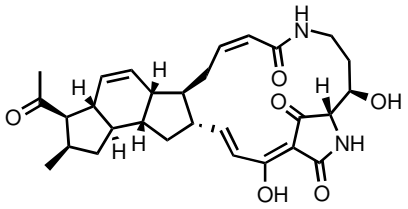


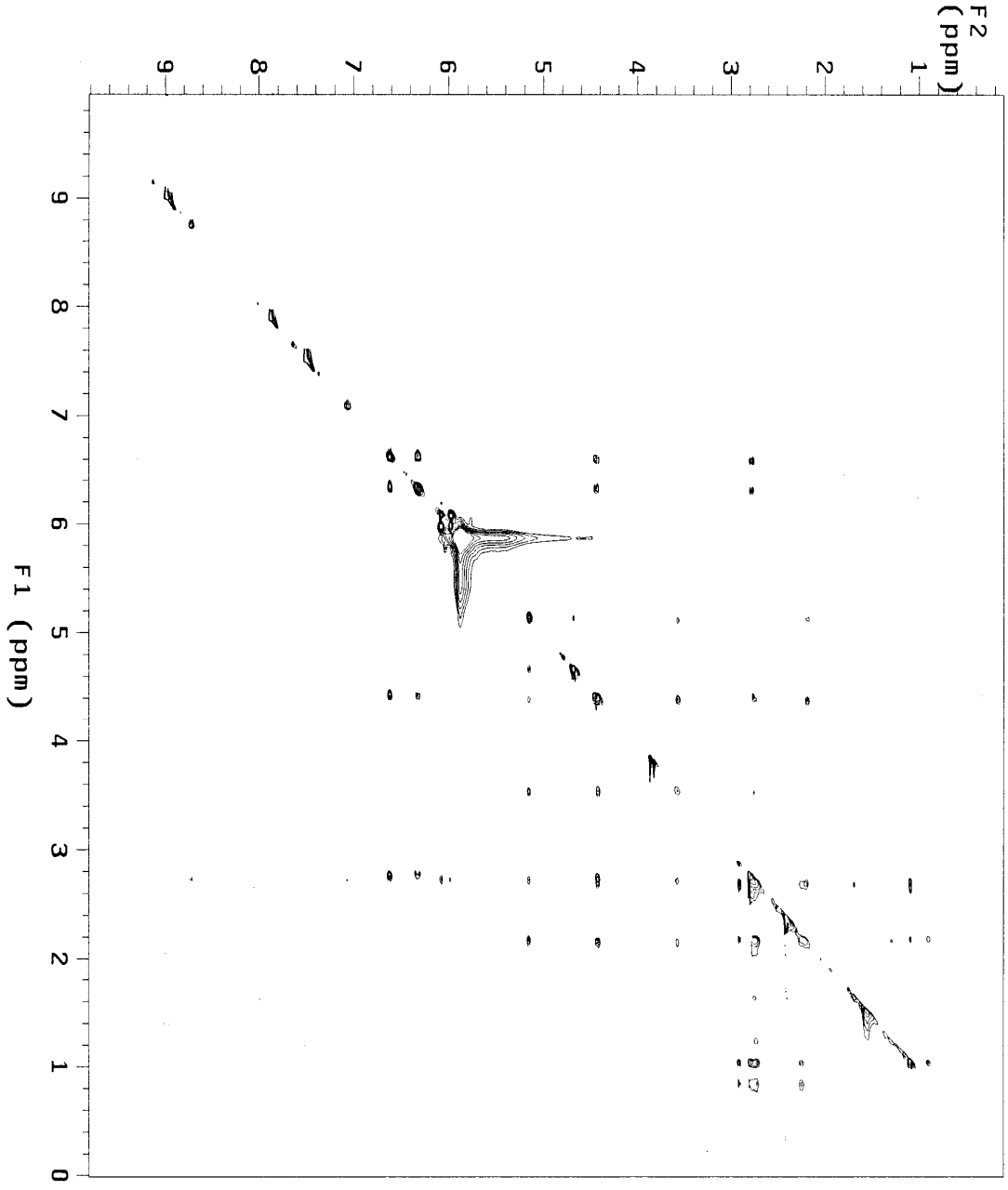
gCOSY spectrum of **5**



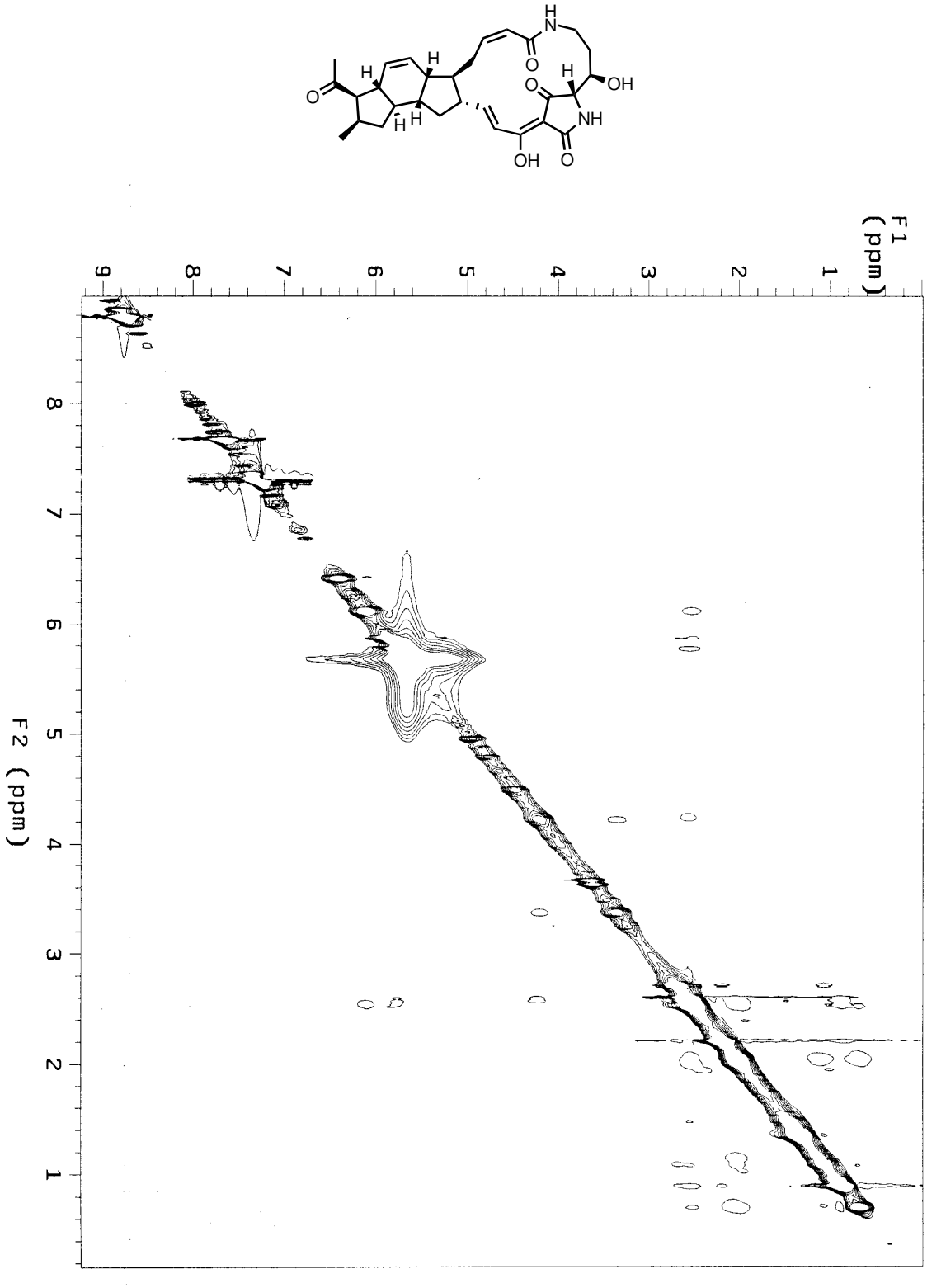


TOCSY spectrum of **5**

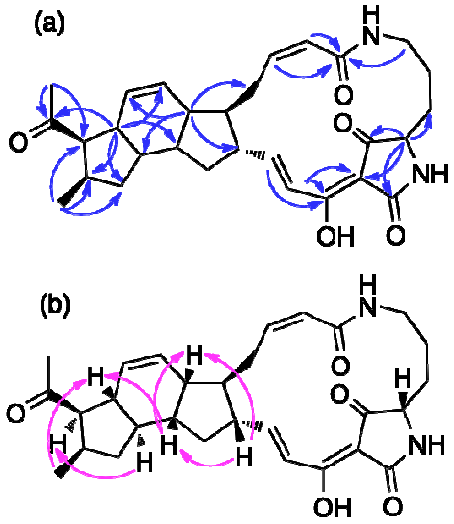




ROESY spectrum of **5**



Key HMBC (a) and NOESY (b) correlations of **4**



Relative stereochemistry of **5**