Supporting Information for

Marineosins A and B, cytotoxic spiroaminals from a marine-derived actinomycete.

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Table S1. NMR spectral data for Marineosin A (**1**) in acetone-d_6

#	δ_C		δ_H	*J* (Hz)	COSY	HMBC	NOESY
1	122.5	CH	6.97 dd	1.5, 2.5	2	C-2, C-3, C-4	2
2	109.9	CH	6.11 dd	2.5, 3.5	1, 3	C-1, C-3, C-4	1, 3
3	113.5	CH	6.37 dd	1.5, 3.5	2	C-1, C-2, C-4	2, 6b
4	129.1	C					
5	164.4	C					
6 a	39.0	CH_2	1.88 dd	8.5, 16	7	C-5, C-7	
b			2.88 dd	8.5, 16	7	C-5, C-7, C-8	3
7	89.9	CH	3.85 t	8.5	6	C-6, C-8, C-25	9, 25
8	106.1	C					
9	45.9	CH	2.91 d	12	21	C-7, C-8, C-10, C-11, C-20, C-21, C-22	7, 11, 20b, 22a
10	129.9	C					
11	110.1	CH	5.68 t	3	12	C-10, C-12, C-13	9, 12
12	104.7	CH	5.44 t	3	11, 14	C-10, C-11, C-13	11, 14
13	131.6	C					
14	28.6	CH_2	2.23 m		12, 15	C-12, C-13, C-15, C-16	12
15 a	25.5	CH_2	1.37 m		14		
b			1.64 m		14		
16 a	28.0	CH_2	1.28 m		17	C-14, C-18	
b			1.34 m		17	C-14, C-18	
17 a	25.5	CH_2	0.52 m		16, 18		
b			0.69 m		16, 18		
18 a	25.7	CH_2	1.02 m		17, 19	C-16, C-17, C-20	
b			1.52 m		17, 19		10-NH
19 a	25.4	CH_2	0.88 m		18, 20		
b			1.18 m		18, 20		
20 a	32.0	CH_2	0.88 m		19, 21		
b			1.35 m		19, 21		9
21	29.6	CH	2.32 m		9, 20, 22		10-NH, 22b, 24
22 a	39.6	CH_2	1.61 td	6.5, 12	21, 23	C-9, C-20, C-21, C-23, C-24	9, 20a
b			1.77 m	12	21, 23	C-9, C-21	20a, 21
23	70.4	CH	4.23 m	6.5	22, 24	C-8, C-21, C-22, C-24	
24	22.8	CH_3	1.51 d	7	23	C-22, C-23	10-NH, 21
25	58.5	CH_3	3.40 s			C-7	7
1-NH			10.93 br				
10-NH			8.23 brs				18b, 21, 24

Table S2. NMR spectral data for Marineosin B (**2**) in acetone-d_6

#	δ_C		δ_H	*J* (Hz)	COSY	HMBC	ROESY
1	122.6	CH	6.95 dd	1.5, 2.5	2	C-2, C-3, C-4	2
2	109.7	CH	6.10 dd	2.5, 3.5	1, 3		1, 3
3	113.4	CH	6.39 dd	1.5, 3.5	2		2, 6
4	128.7	C					
5	162.8	C					
6 a	41.0	CH_2	2.27 dd	6.0, 16.5	7	C-5	3, 25
b			2.58 dd	2.5, 17.0	7	C-5, C-7, C-8	3, 25
7	82.5	CH	4.03 dd	2.0, 6.0	6	C-5, C-25	10-NH, 21
8	105.9	C					
9	49.5	CH	2.71 d	13.0	21	C-7, C-8, C-10, C-11, C-21, C-22	11, 23
10	129.8	C					
11	110.4	CH	5.55 t	3.0	12, 10-NH		9
12	106.3	CH	5.57 t	2.5	11, 14, 10-NH	C-11	14a
13	132.7	C					
14 a	28.8	CH_2	2.44 m		15		12
b			2.67 m		15	C-13, C-16	10-NH
15 a	26.6	CH_2	1.44 m		14, 16		
b			1.77 m		14, 16		
16	27.4	CH_2	1.34 m		15, 17	C-14, C-18	10-NH
17 a	25.8	CH_2	0.51 m		16, 18	C-15, C-16, C-18	
b			0.72 m		16, 18		
18 a	25.8	CH_2	1.07 m		17, 19		
b			1.58 m		17, 19		
19 a	25.0	CH_2	1.00 m		18, 20		
b			1.28 m		18, 20		
20 a	33.7	CH_2	1.09 m		19, 21	C-21, C-22	
b			1.36 m		19, 21		
21	31.4	CH	2.26 m		9, 20, 22		7, 10-NH
22	38.9	CH_2	1.72 m	6.0	21, 23	C-9, C-20, C-21, C-23, C-24	23
23	66.1	CH	4.30 m	6.5	22, 24	C-8, C-21, C-24	9, 22, 24
24	22.3	CH_3	1.20 d	6.5	23	C-22, C-23	23, 25
25	57.8	CH_3	3.23 s			C-7	6, 24
1-NH			10.57 br				
10-NH			9.39 brs		11, 12		7, 14b, 16, 21

General experimental procedures

Optical rotations were measured using a Rudolph Autopol III polarimeter. UV spectra were obtained using Varian Cary 50 Bio UV-Visible spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 1600 spectrophotometer. ^{1}H, gCOSY, gHMBC, gHSQC, NOESY, TOCSY, and HSQC-TOCSY NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer. ^{13}C NMR spectra were recorded on a Varian INOVA 300 MHz spectrometer. Electron impact and high-resolution mass spectrometer measurements were obtained on ThermoFinnigan MAT900XL instrument at University of California, San Diego, CA. All solvents were distilled prior to being used.

Collection and phylogenetic analysis of strain CNQ-617

The marine actinomycete strain CNQ-617 was isolated from a marine sediment sample collected off shore of La Jolla, CA by Alejandra Prieto-Davó. The strain was designated as MAR3 clade based on 16S rDNA analysis. The phylogenetic analysis revealed that this strain showed 98% similarity to *Streptomyces cacaoi* based upon the result of NCBI blast analysis of the partial 16S rDNA. The gene sequence data is available from Genebank (deposit # EU161093). A similarity of 98% in this genus generally indicates that this is likely to be a new species.

Cultivation and extraction

Streptomyces strain CNQ-617 was cultured at 27^{o}C with shaking at 250 rpm in fifteen 2.8-L Fernbach flasks each containing 1 L of the medium A1BFe [10 g starch, 4 g yeast extract, 2 g peptone, 5 mL $Fe_2(SO_4)_3 \cdot 4H_2O$ (8 g/L in deionized water), 5mL KBr (20 g/L in deionized water), 1 L seawater]. After 5 days, the organic constituents from a

15 L culture were extracted by a solid-phase extraction method using Amberlite XAD-7 resin. Amberlite XAD-7 resin (20 g/L) was added to each 1 L culture to adsorb the organic substances. The culture and resin were shaken at 215 rpm for 2 additional hours. The resin was filtered through cheesecloth and washed with deionized water to remove salts. The resin and the cheesecloth were soaked in acetone and shaken at 215 rpm for 1 hour. The acetone extract was dried *in vacuo* to give a crude extract (1.8 g from a 15 L culture).

Isolation of marineosin A (1) and B (2)

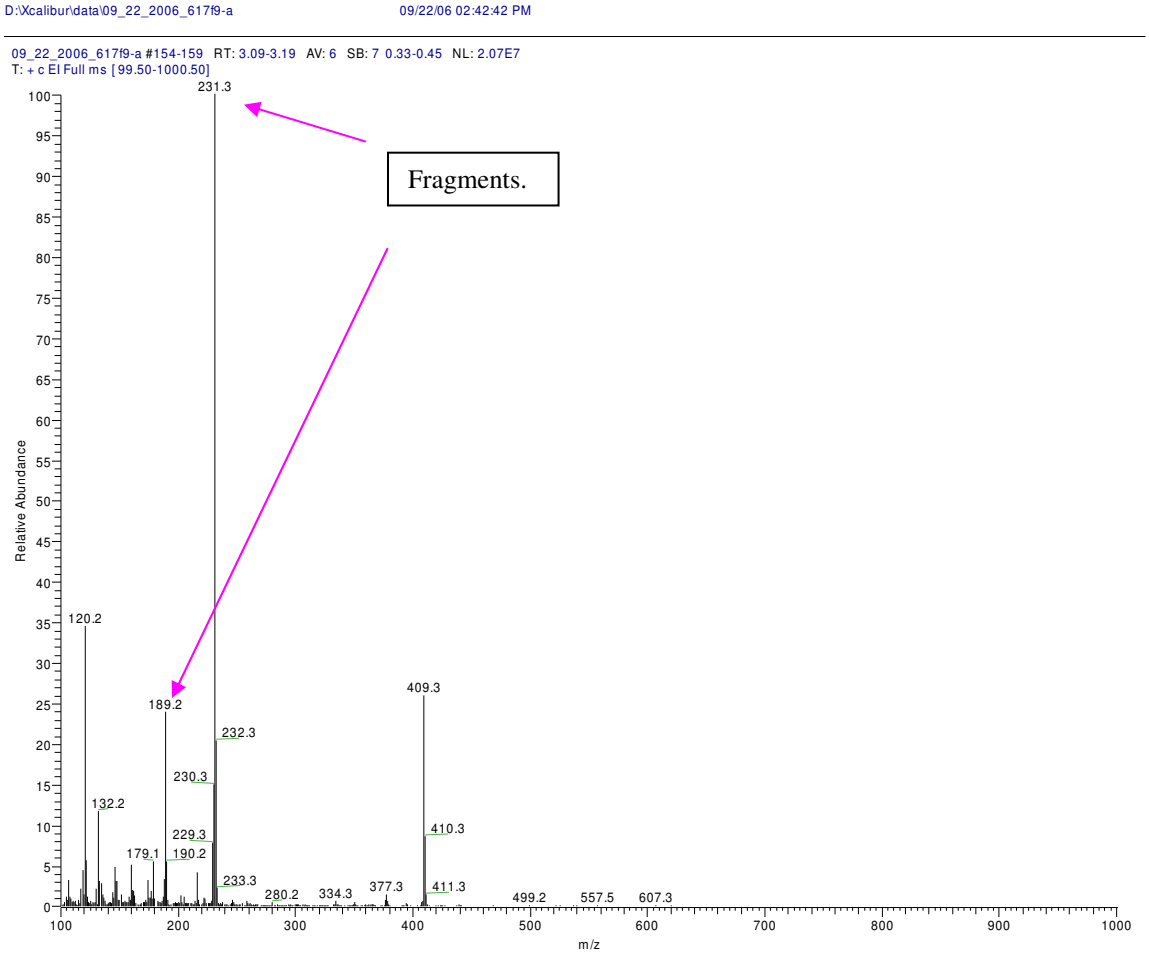
The crude acetone extract from a 15 L culture of strain CNQ-617 was dried *in vacuo* to obtain a sticky dark brown substance (1.8 g), which was partitioned by HP20SS column chromatography (acetone/water) to yield eight fractions (20%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% acetone mixtures). All eight fractions were concentrated to dryness and evaluated in the HCT-116 colon carcinoma cytotoxicity assay. The fraction eluted with 90% acetone-water (173 mg) was found to possess very potent cytotoxic activity ($IC_{50} \leq 0.8$ μg/mL), and was then fractionated by flash C18 column chromatography eluting with 30%, 50%, 75%, 80% and 100% MeOH-water mixtures. The 75% MeOH/water fraction from the flash C18 column was subjected to further purification by isocratic HPLC (Dynamax C_{18} semi-preparative, 3 mL/min, refractive index detection, 77% MeOH/H_2O over 70 min) to yield marineosins A (**1**, 4.7 mg) and B (**2**, 1.7 mg).

Antifungal assay with *Candida albicans*

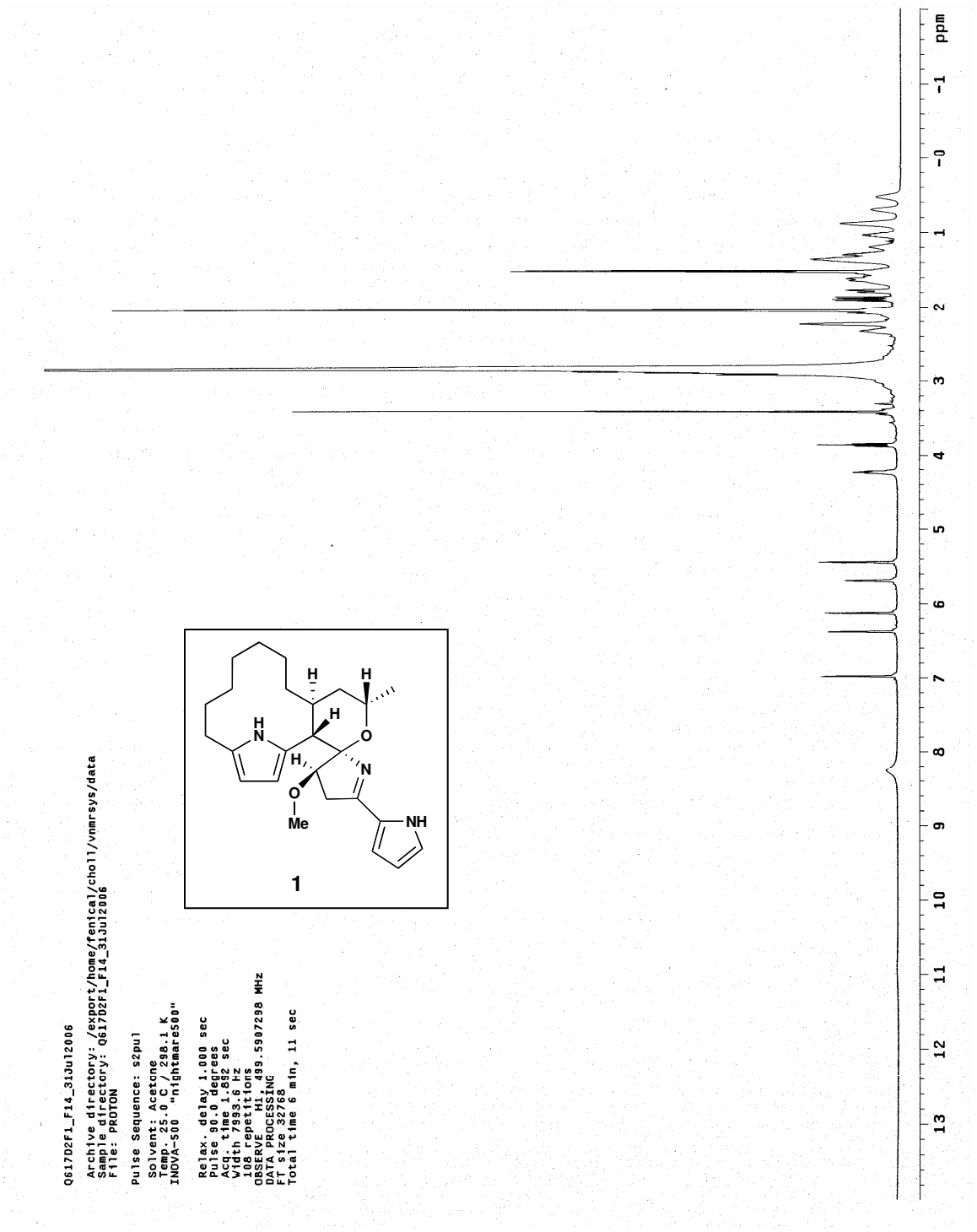
The *C. albicans* strains ATCC 32354 (wild type) and ATCC 90873 (amphotericin B-resistant) were purchased from the American Type Culture Collection (ATCC). Inhibitory activity was determined by a standard microdilution liquid antifungal assay. *C. albicans* was incubated overnight at 37 °C in RPMI 1640 media (GibcoBRL) and aliquots transferred to 96-well plates the next day. The indicator Alamar Blue was added to the *C. albicans* culture before they were transferred to the plates. Samples were added along with Amphotericin B (Sigma) and DMSO (solvent) as positive and negative controls respectively, and serially diluted. The plates were then incubated overnight for 14-16 h. Minimum inhibitory concentration (MIC) values were determined by the change in color from blue to pink of the media according to the indicator.

Human colon tumor (HCT-116) cytotoxicity assay

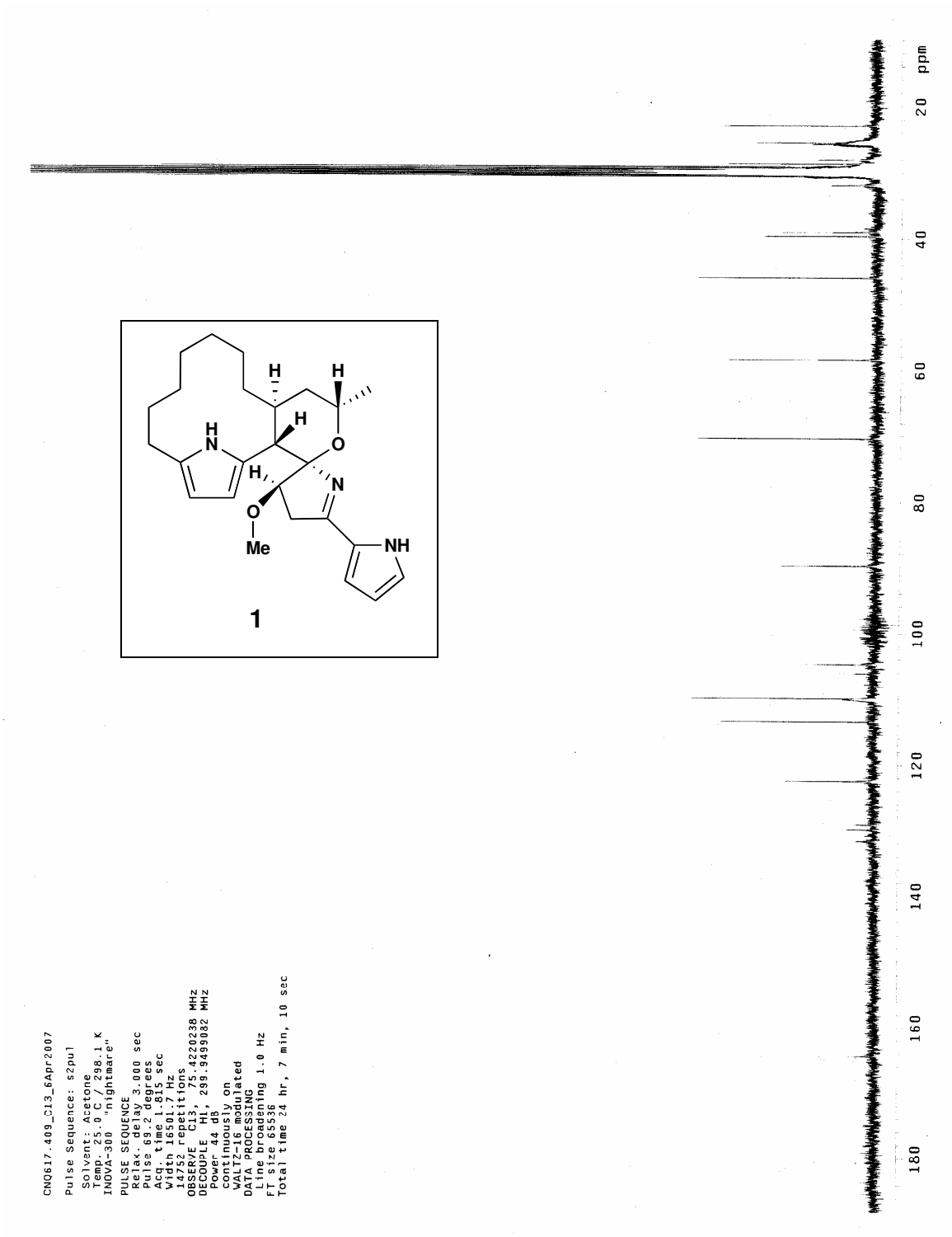
Aliquot samples of HCT-116 human colon adenocarcinoma cells were transferred to 96-well plates and incubated overnight at 37 °C in 5% CO_2/air. Test compounds were added to the plates in DMSO and serially diluted. The plates were then further incubated for another 72 h, and at the end of this period, a CellTiter 96 Aq_{ueous} non-radioactive cell proliferation assay (Promega) was used to assess cell viability. Inhibition concentration (IC_{50}) values were deduced from the bioreduction of MTS/PMS by living cells into a formazan product. MTS/PMS was first applied to the sample wells, followed by incubation for 3 h. Etoposide (Sigma) and DMSO (solvent) were used as the positive and negative controls in this assay. The quantity of the formazan product (in proportion to the number of living cells) in each well was determined by the Molecular Devices Emax microplate reader set to 490 nm wavelength. IC_{50} values were calculated using the analysis program, SOFTMax.



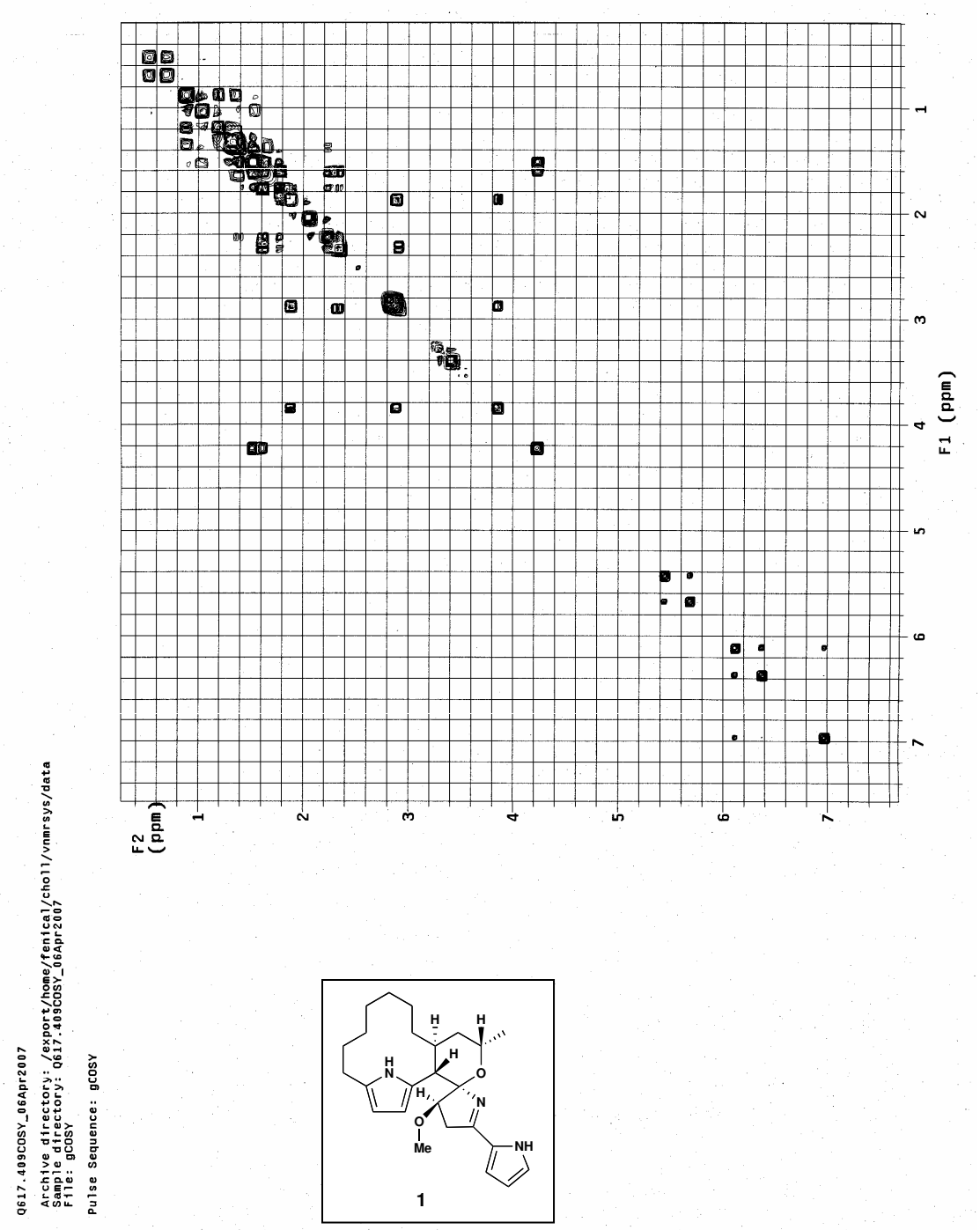
Diagnostic Fragment ions observed in the EIMS experiment performed on marineosin A (**1**)



^{1}H NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



^{13}C NMR spectrum of marineosin A (**1**) (300 MHz, acetone-d_6)



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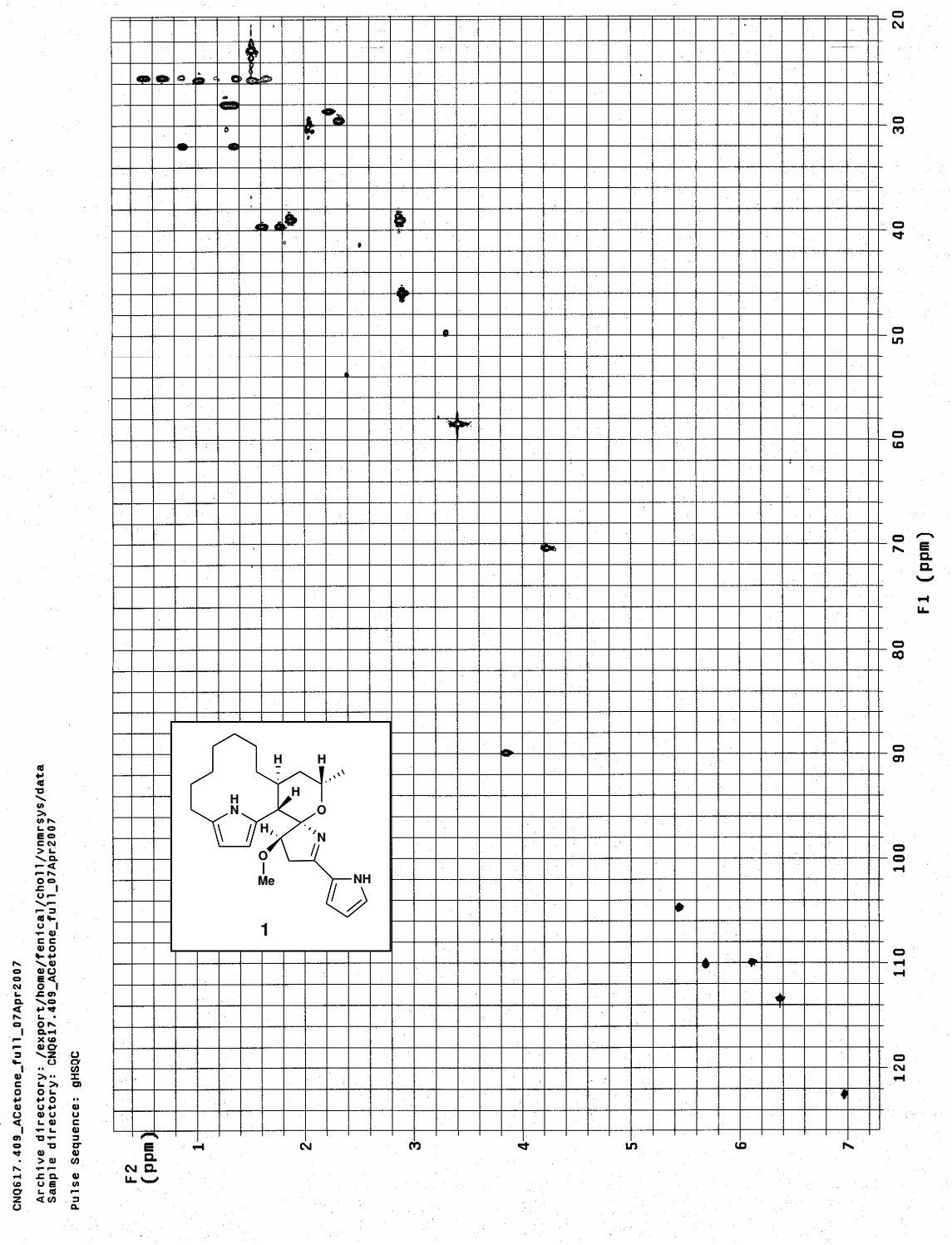
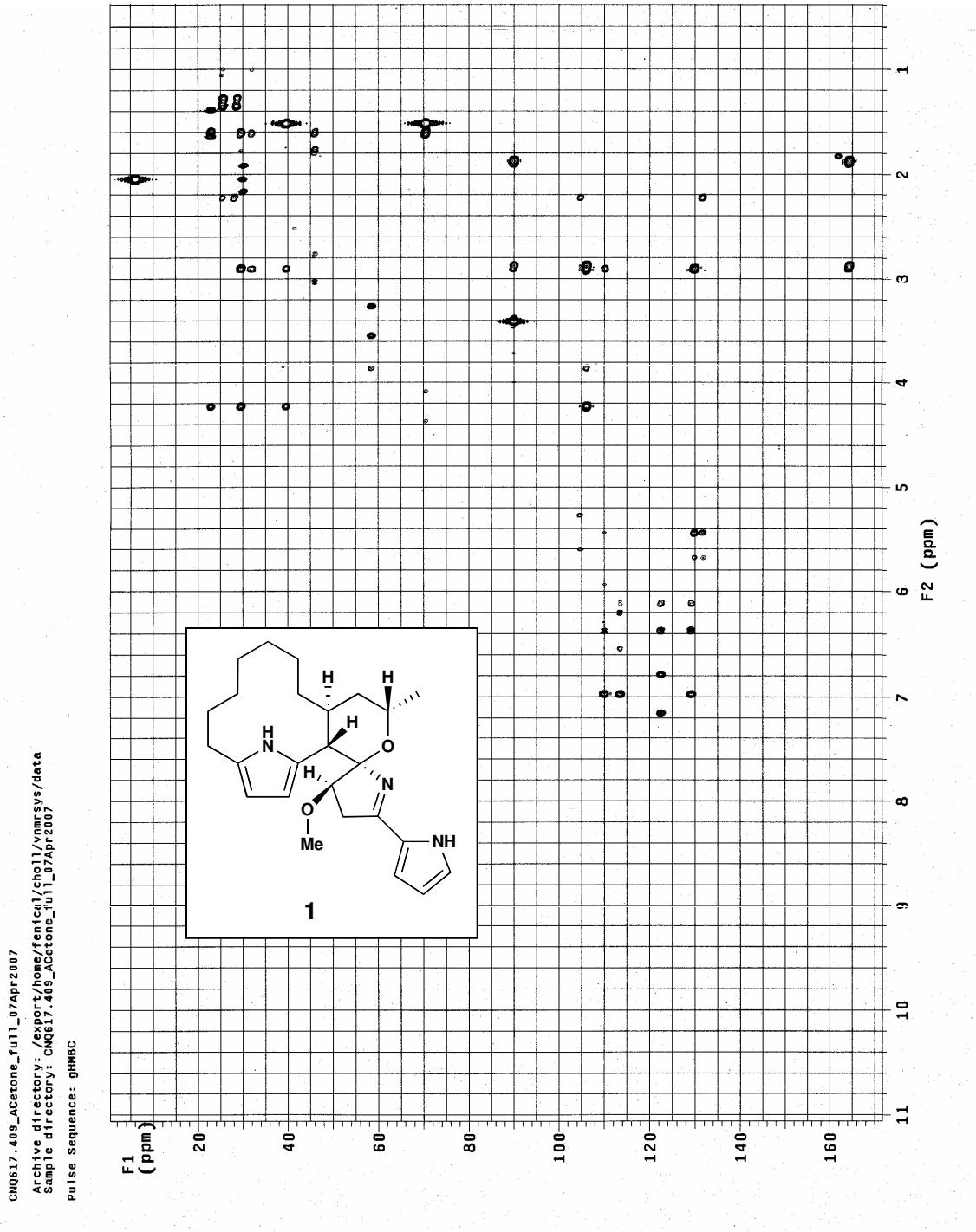
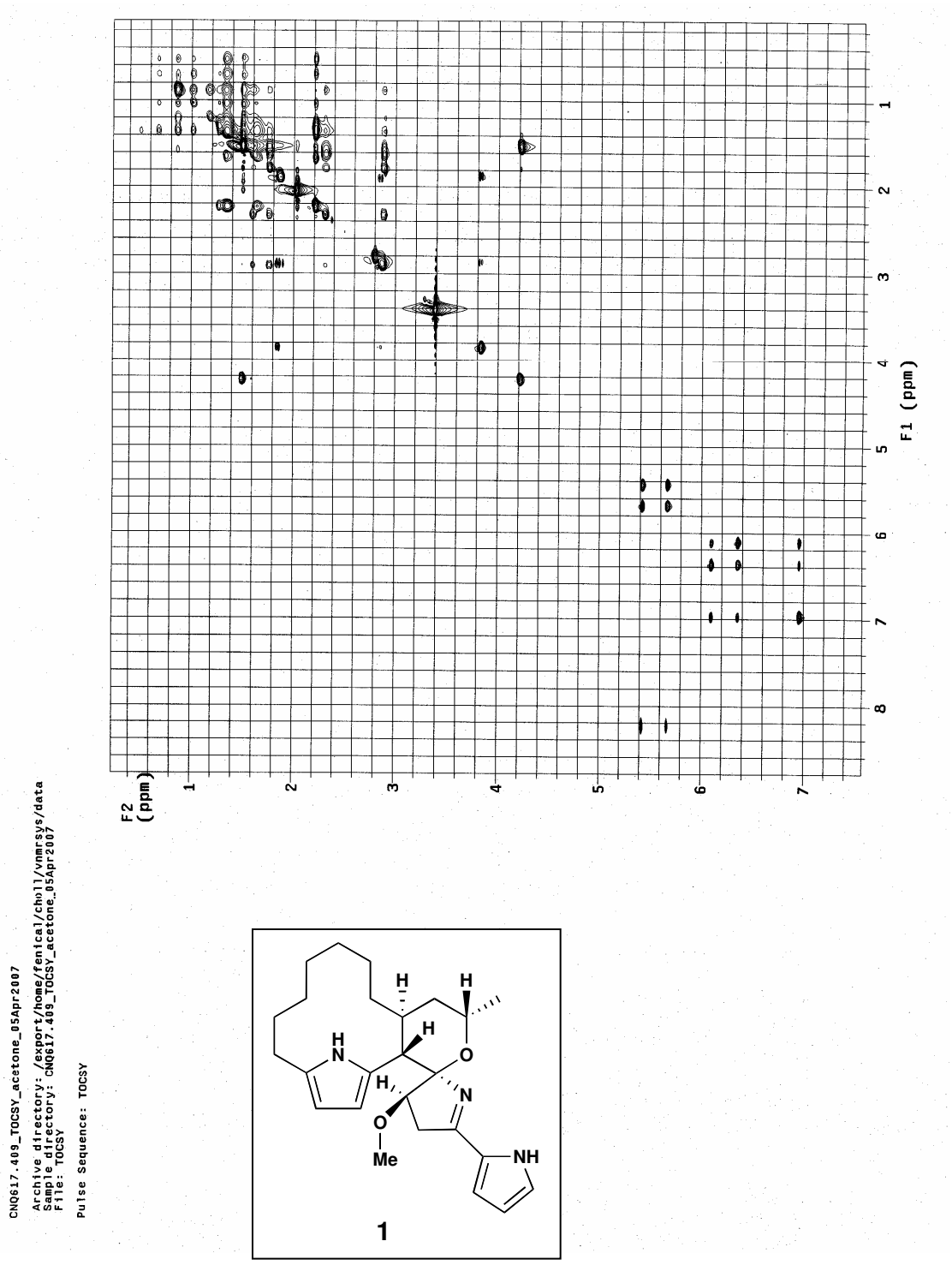
gCOSY NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



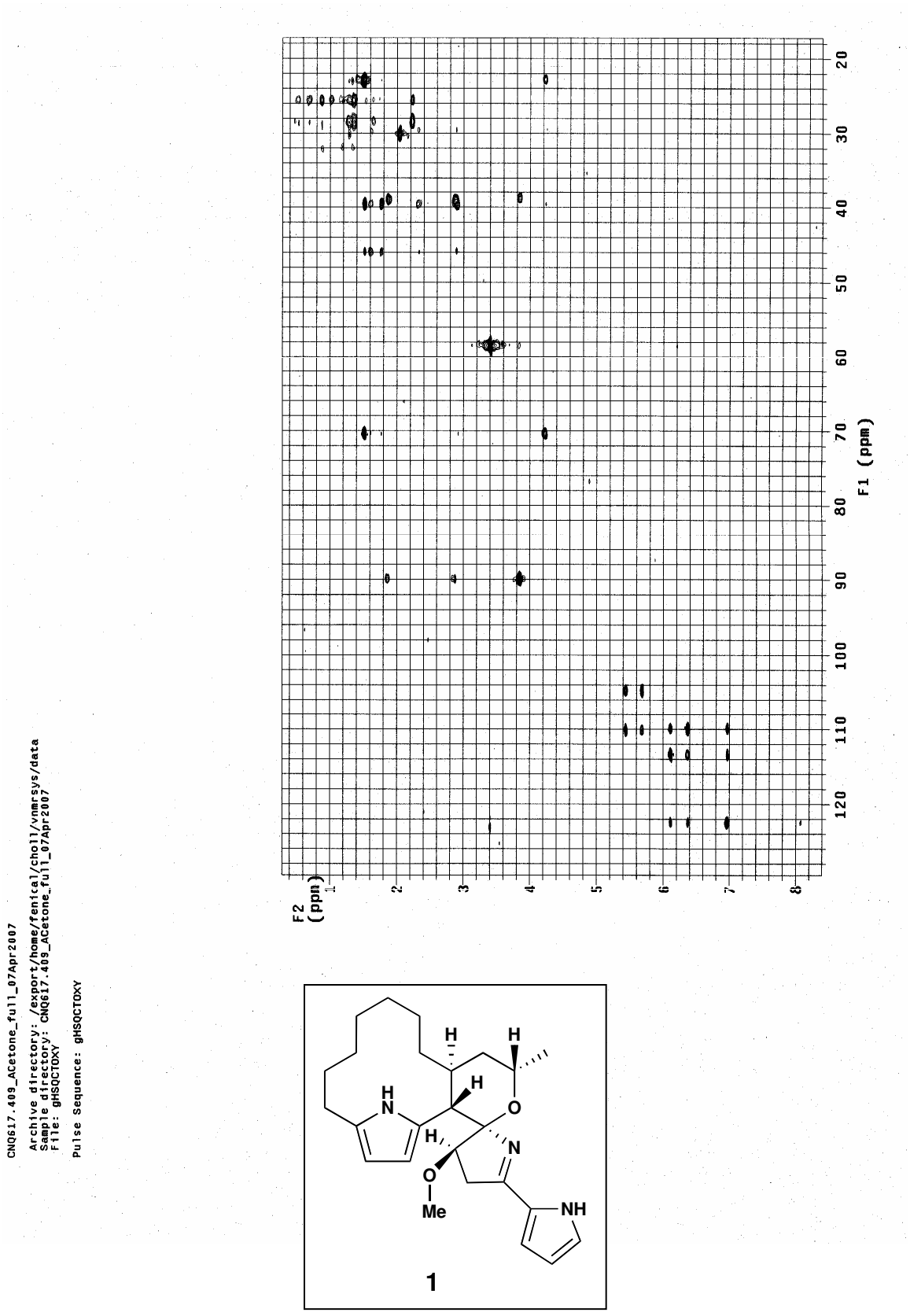
gHSQC NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



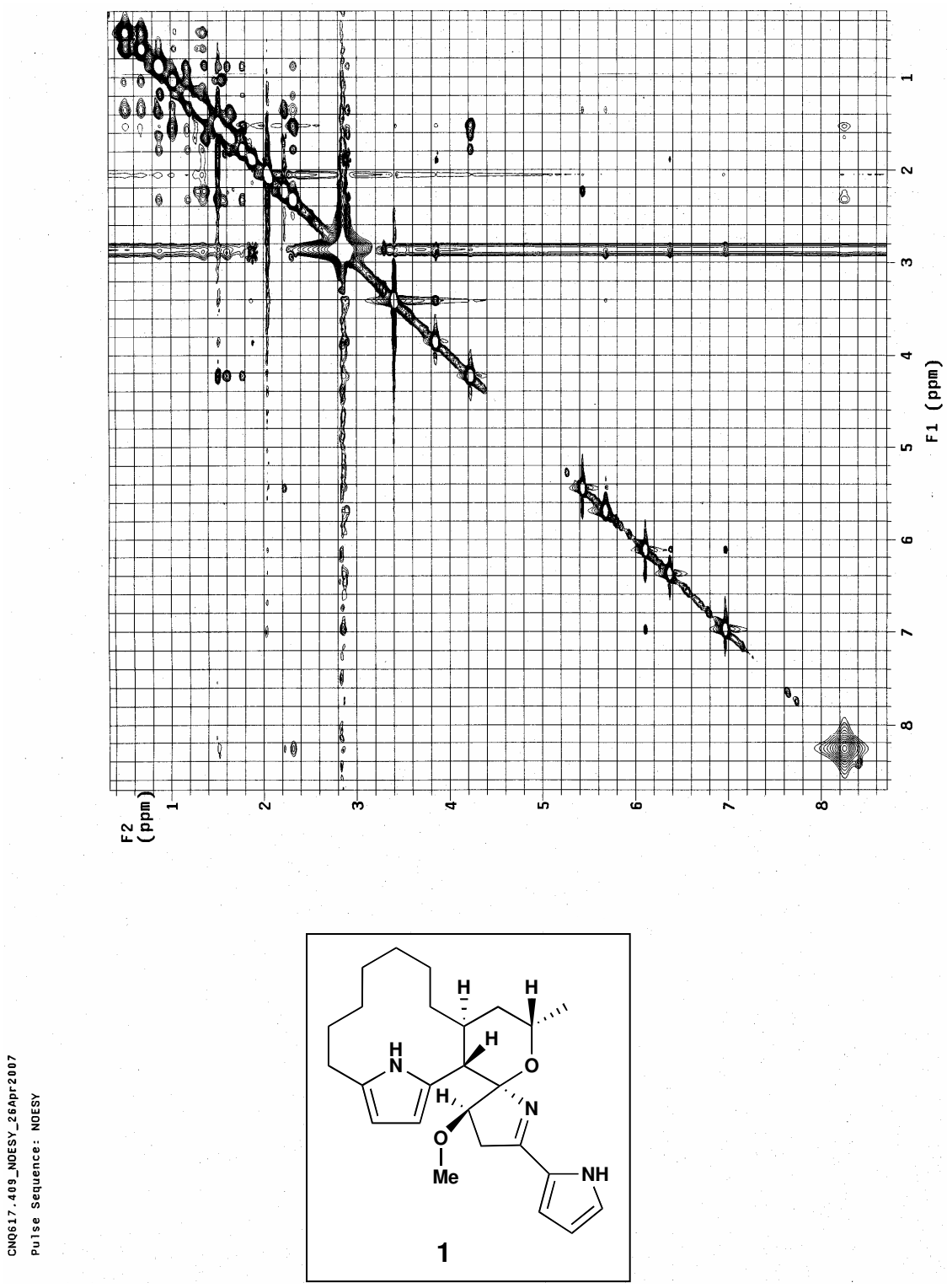
gHMBC NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



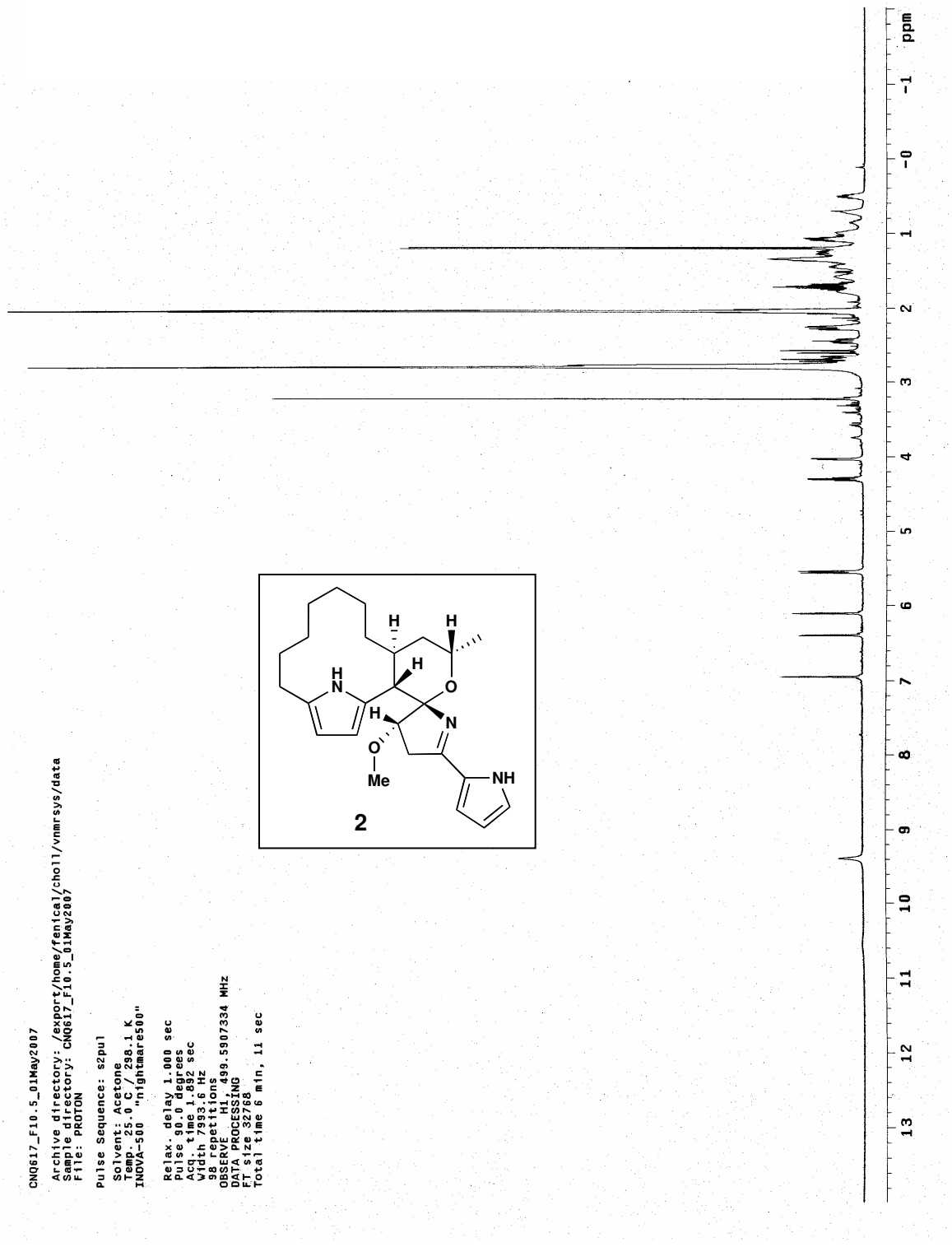
TOCSY NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



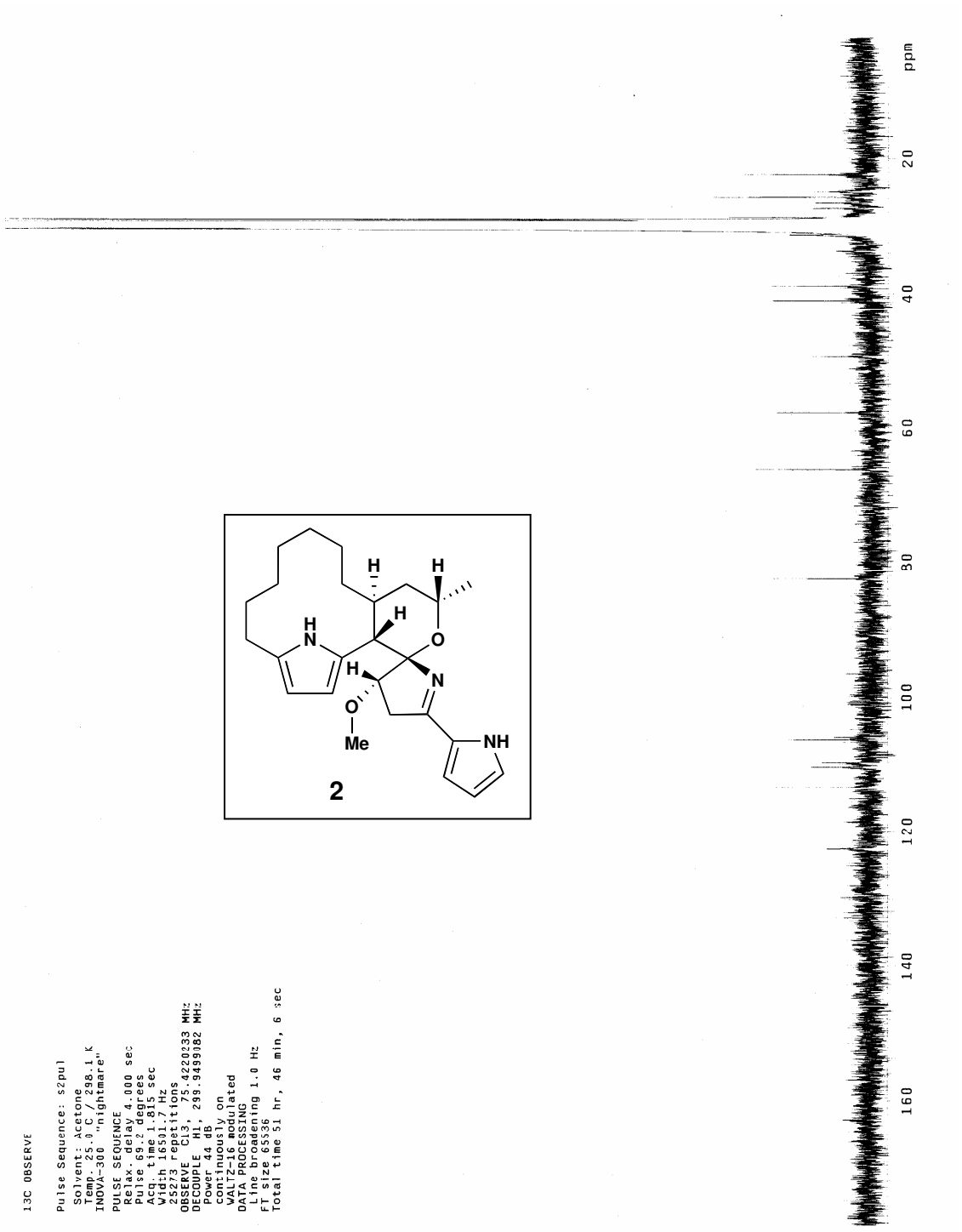
gHSQC-TOCSY NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



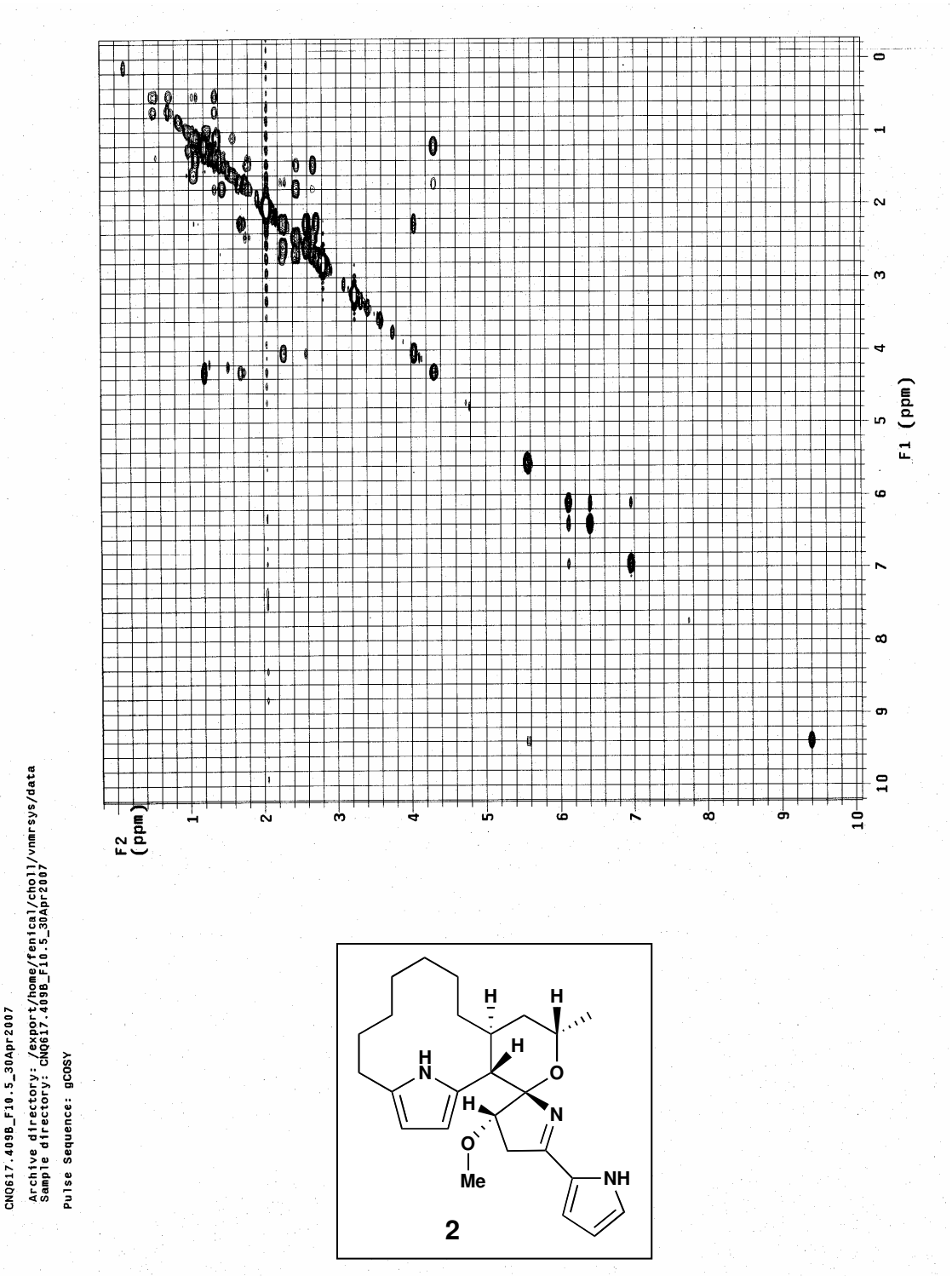
NOESY NMR spectrum of marineosin A (**1**) (500 MHz, acetone-d_6)



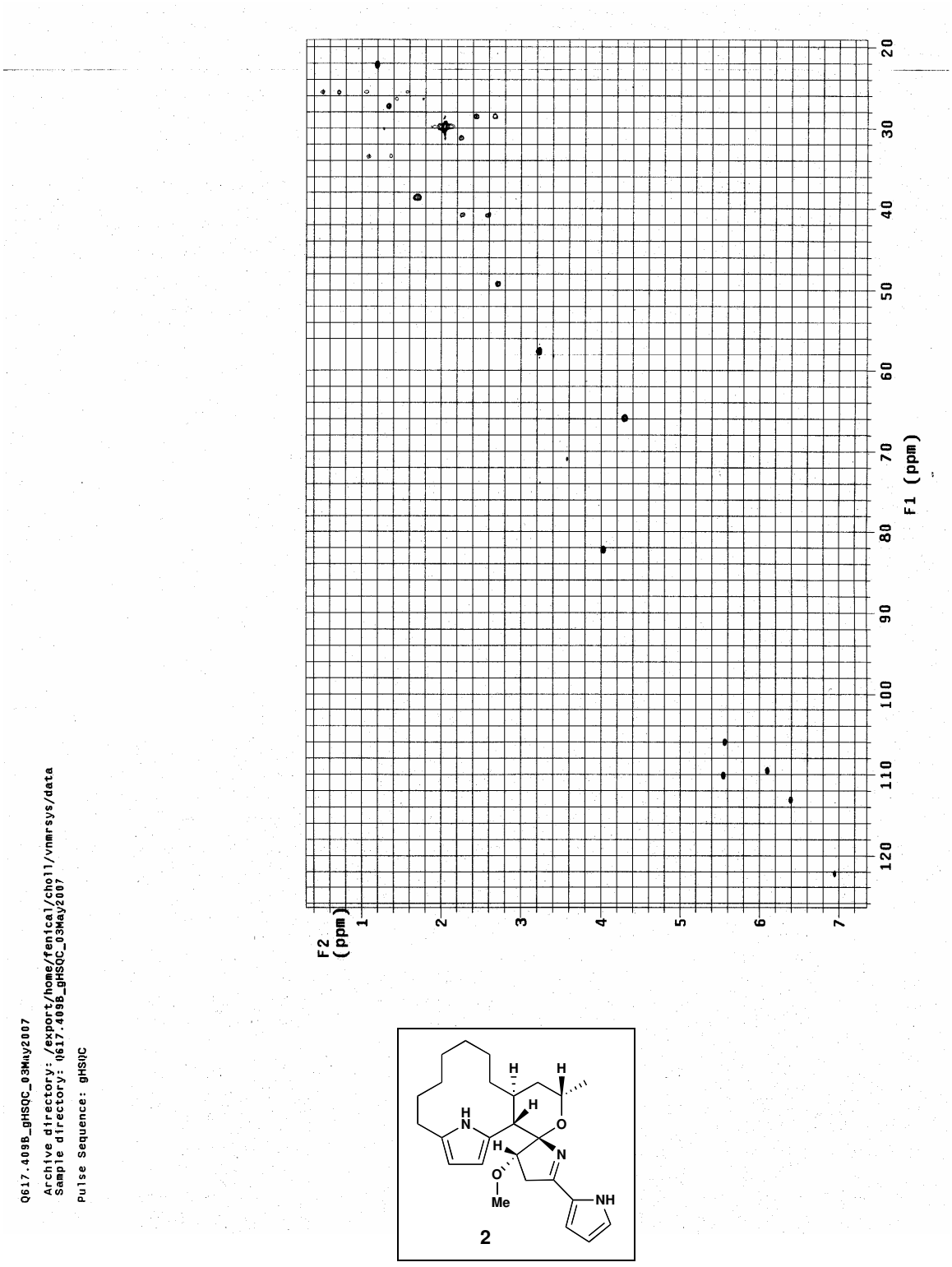
^{1}H NMR spectrum of marineosin B (**2**) (500 MHz, acetone-d_6)



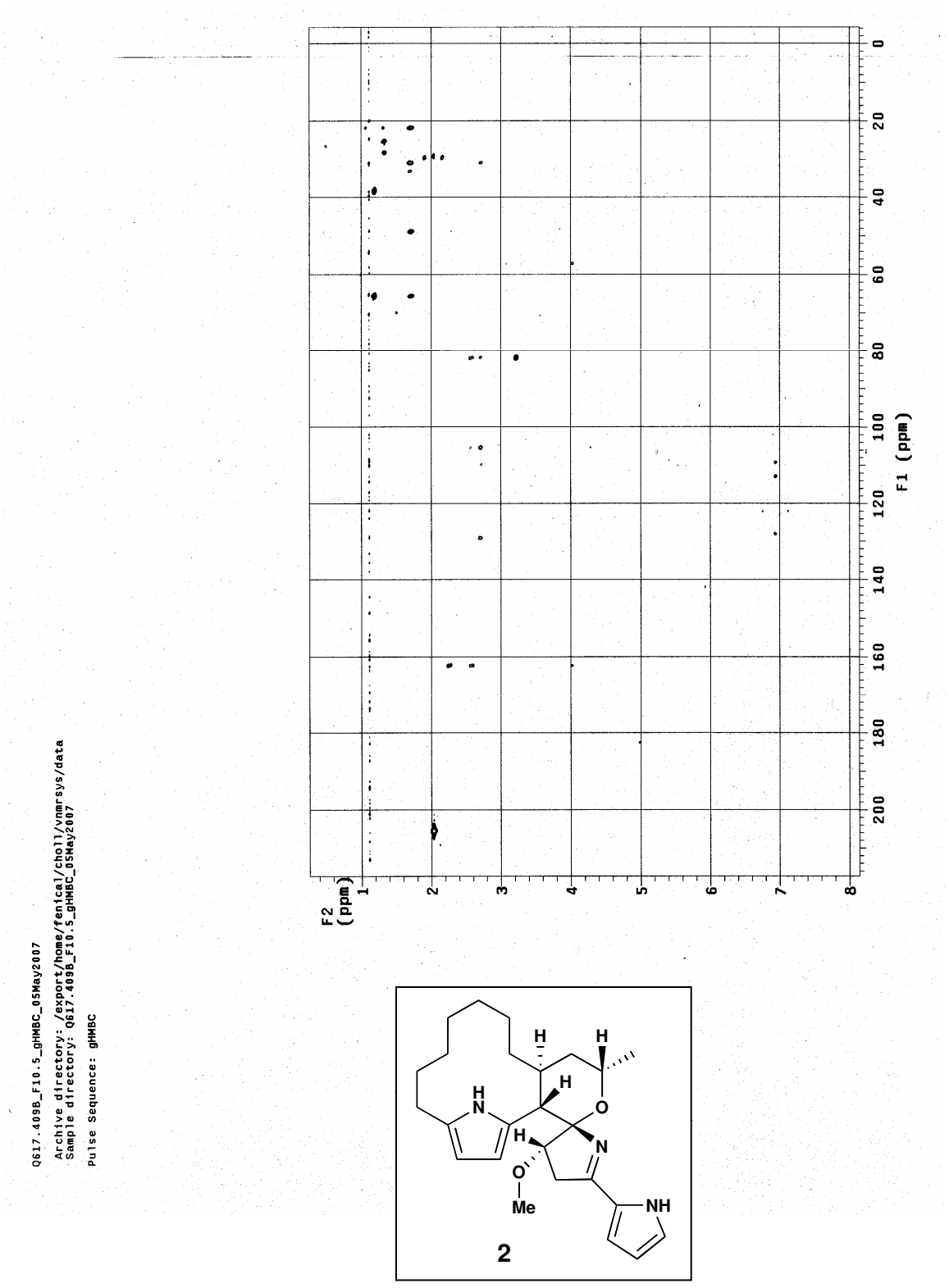
^{13}C NMR spectrum of marineosin B (**2**) (300 MHz, acetone-d_6)



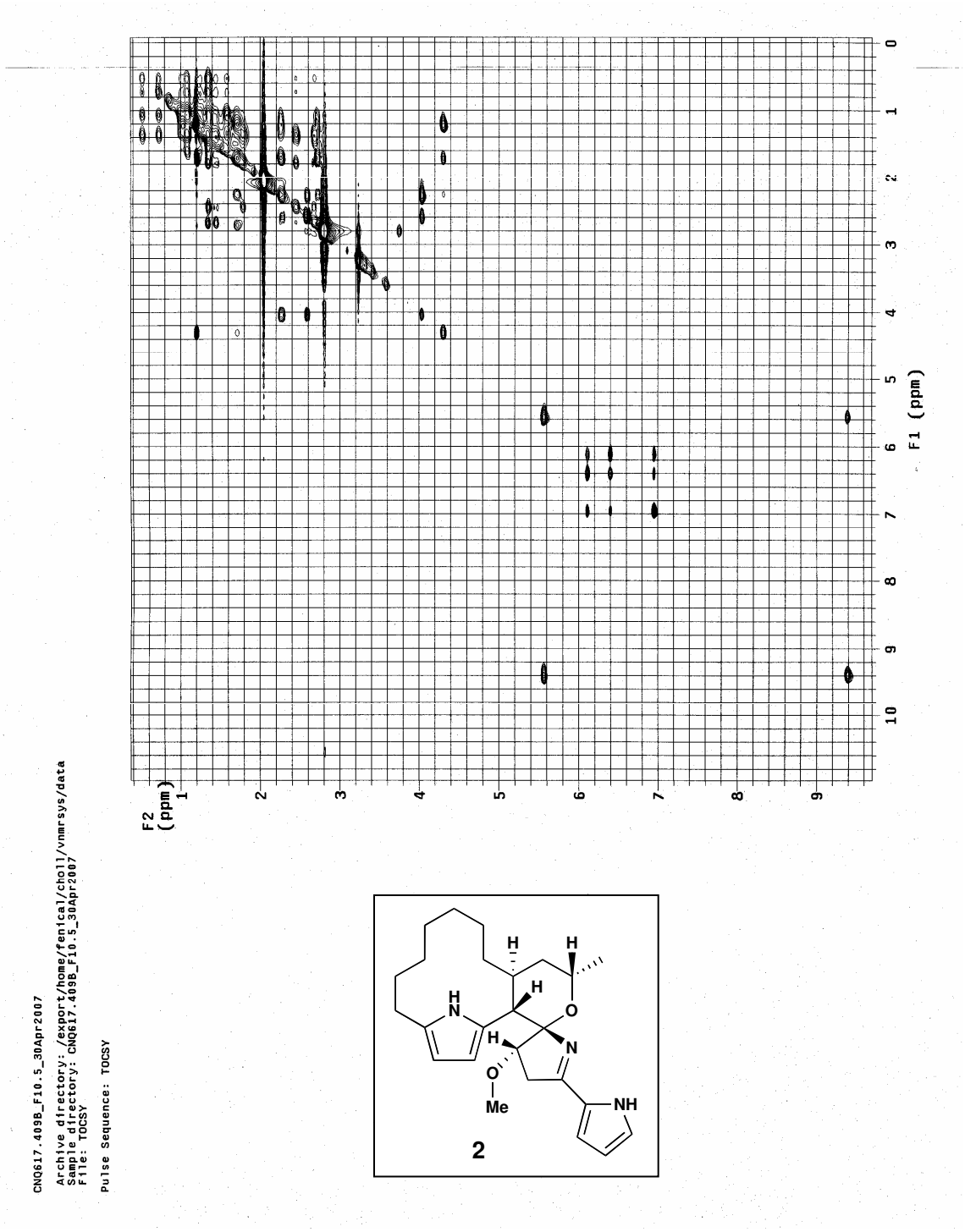
gCOSY NMR spectrum of marineosin B (**2**) (500 MHz, acetone-d_6)



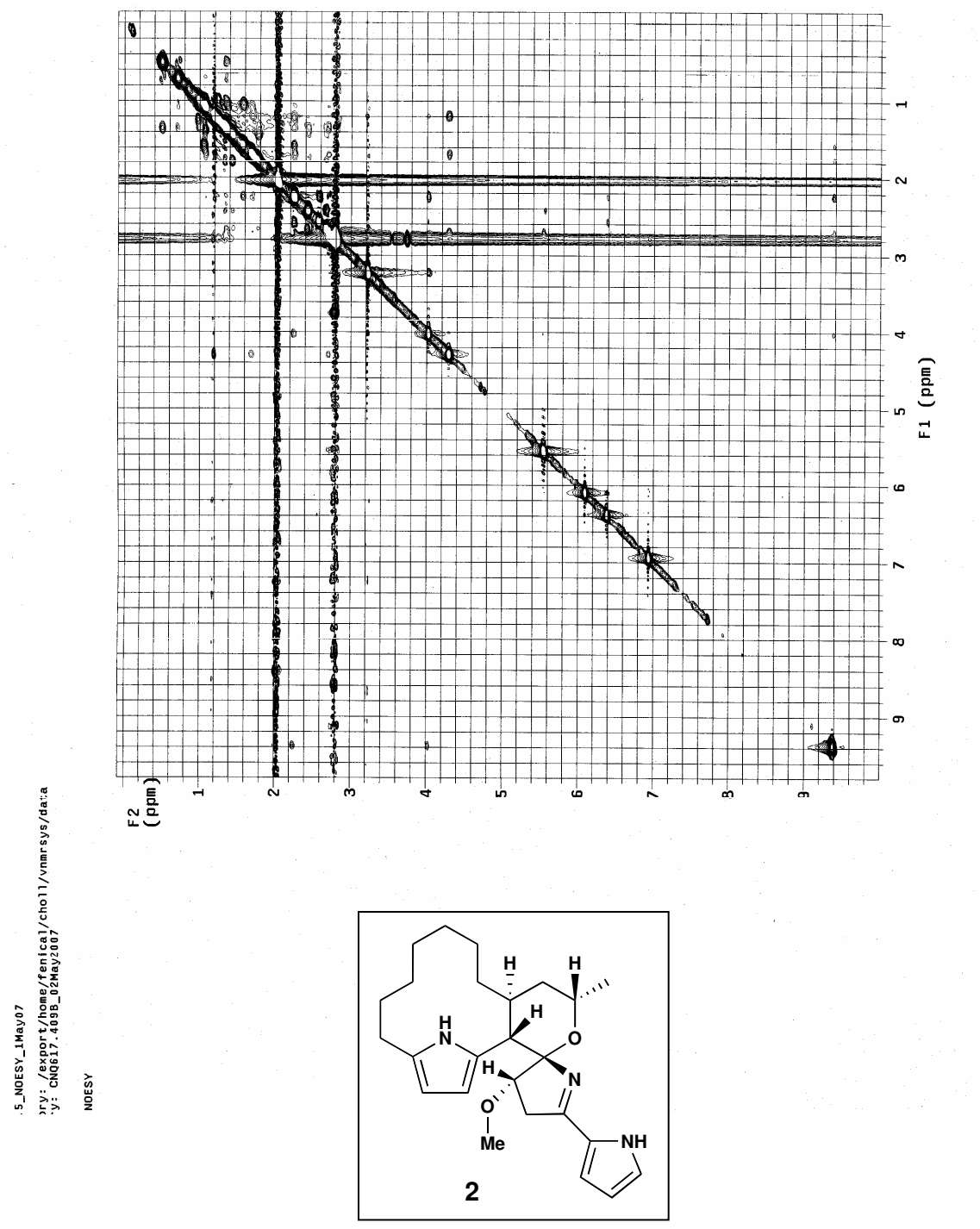
gHSQC NMR spectrum of marineosin B (**2**) (500 MHz, acetone-d_6)



gHMBC NMR spectrum of marineosin B (**2**) (500 MHz, acetone-d_6)



TOCSY NMR spectrum of marineosin B (**2**) (500 MHz, acetone-d_6)



NOESY NMR spectrum of marineosin B (**2**) (500 MHz, acetone-d_6)