

## **Supporting Information for**

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

*Chollaratt Boonlarppradab, Christopher A. Kauffman, Paul R. Jensen, and William Fenical\**

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography,  
University of California at San Diego, La Jolla, California 92093-0204, USA

\*Corresponding author: [wfenical@ucsd.edu](mailto:wfenical@ucsd.edu)

## Table of Contents

Table of Contents.....	S1
Table S1. NMR spectral data for Marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S3
Table S2. NMR spectral data for Marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S4
General experimental procedures.....	S5
Collection and phylogenetic analysis of strain CNQ-617.....	S5
Cultivation and extraction.....	S5
Isolation of marineosins A ( <b>1</b> ) and B ( <b>2</b> ).....	S6
Antifungal assay with <i>Candida albicans</i> .....	S6
Human colon tumor (HCT-116) cytotoxicity assay.....	S7
Fragment ions from the EIMS experiment performed on marineosin A.....	S8
<sup>1</sup> H NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S9
<sup>13</sup> C NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S10
gCOSY NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S11
gHSQC NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S12
gHMBC NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S13
TOCSY NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S14
gHSQC-TOCSY NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S15
NOESY NMR spectrum of marineosin A ( <b>1</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S16
<sup>1</sup> H NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S17
<sup>13</sup> C NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S18
gCOSY NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S19
gHSQC NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S20
gHMBC NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S21

TOCSY NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S22
NOESY NMR spectrum of marineosin B ( <b>2</b> ) in acetone- <i>d</i> <sub>6</sub> .....	S23

**Table S1.** NMR spectral data for Marineosin A (**1**) in acetone-*d*<sub>6</sub>

#	$\delta_C$		$\delta_H$		<i>J</i> (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 <sub>2</sub>	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 <sub>2</sub>	2.23	m		12, 15	C-12, C-13, C-15, C-16	12
15 a	25.5	CH <sub>2</sub>	1.37	m		14		
b			1.64	m		14		
16 a	28.0	CH <sub>2</sub>	1.28	m		17	C-14, C-18	
b			1.34	m		17	C-14, C-18	
17 a	25.5	CH <sub>2</sub>	0.52	m		16, 18		
b			0.69	m		16, 18		
18 a	25.7	CH <sub>2</sub>	1.02	m		17, 19	C-16, C-17, C-20	
b			1.52	m		17, 19		10-NH
19 a	25.4	CH <sub>2</sub>	0.88	m		18, 20		
b			1.18	m		18, 20		
20 a	32.0	CH <sub>2</sub>	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 <sub>2</sub>	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 <sub>3</sub>	1.51	d	7	23	C-22, C-23	10-NH, 21
25	58.5	CH <sub>3</sub>	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*<sub>6</sub>

#	$\delta_C$		$\delta_H$	<i>J</i> (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 <sub>2</sub>	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 <sub>2</sub>	2.44 m		15		12
b			2.67 m		15	C-13, C-16	10-NH
15 a	26.6	CH <sub>2</sub>	1.44 m		14, 16		
b			1.77 m		14, 16		
16	27.4	CH <sub>2</sub>	1.34 m		15, 17	C-14, C-18	10-NH
17 a	25.8	CH <sub>2</sub>	0.51 m		16, 18	C-15, C-16, C-18	
b			0.72 m		16, 18		
18 a	25.8	CH <sub>2</sub>	1.07 m		17, 19		
b			1.58 m		17, 19		
19 a	25.0	CH <sub>2</sub>	1.00 m		18, 20		
b			1.28 m		18, 20		
20 a	33.7	CH <sub>2</sub>	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 <sub>2</sub>	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 <sub>3</sub>	1.20 d	6.5	23	C-22, C-23	23, 25
25	57.8	CH <sub>3</sub>	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.  $^1\text{H}$ , gCOSY, gHMBC, gHSQC, NOESY, TOCSY, and HSQC-TOCSY NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer.  $^{13}\text{C}$  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 Genbank (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°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  $\text{Fe}_2(\text{SO}_4)_3 \cdot 4\text{H}_2\text{O}$  (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 ( $IC_{50} \leq 0.8 \mu\text{g/mL}$ ), 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<sub>18</sub> semi-preparative, 3 mL/min, refractive index detection, 77% MeOH/H<sub>2</sub>O over 70 min) to yield marineosins A (**1**, 4.7 mg) and B (**2**, 1.7 mg).

### **Antifungal assay with *Candida albicans***

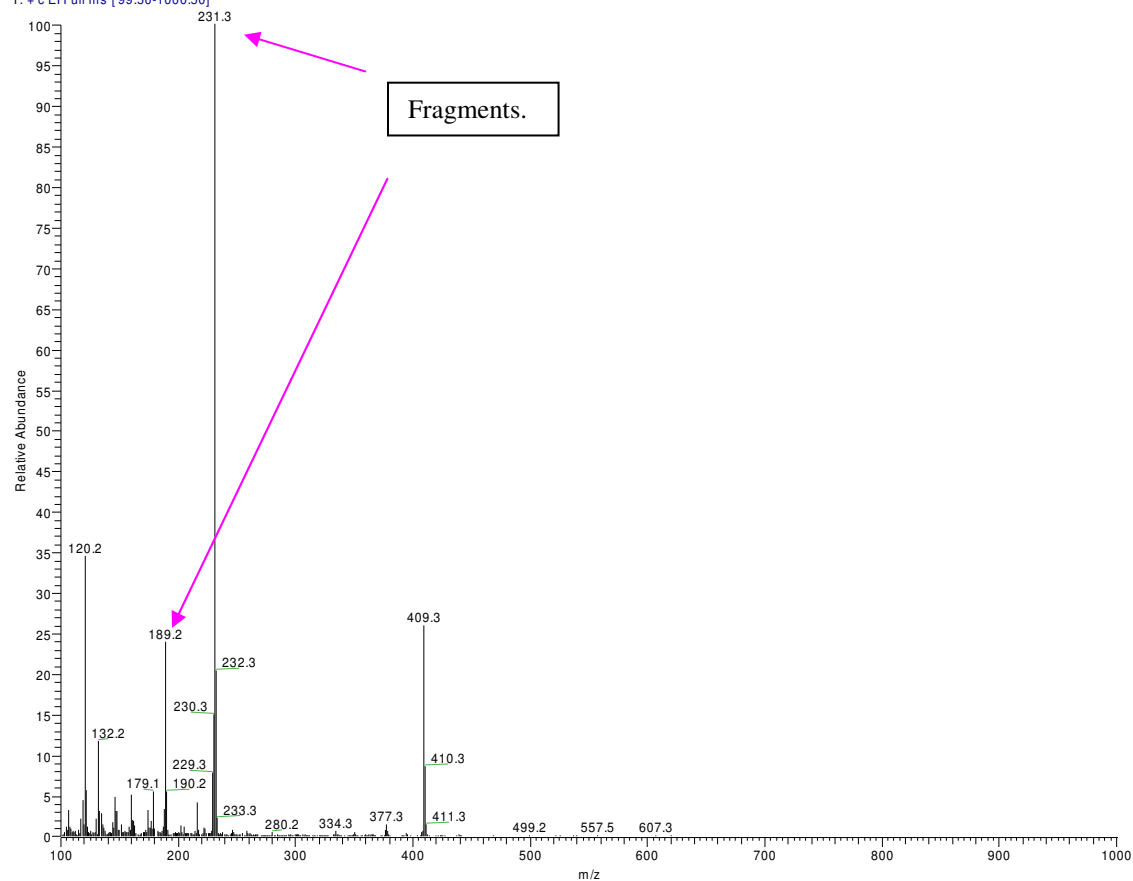
The *C. albicans* strains ATCC 32354 (wild type) and ATCC 90873 (amphotericin B-resistant) were purchased from the American Type Culture Collection (ATCC). Inhibitory activity was determined by a standard microdilution liquid antifungal assay. *C. albicans* was incubated overnight at 37 °C in RPMI 1640 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<sub>2</sub>/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 Aqueous non-radioactive cell proliferation assay (Promega) was used to assess cell viability. Inhibition concentration (IC<sub>50</sub>) 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<sub>50</sub> values were calculated using the analysis program, SOFTMax.



09\_22\_2006\_61719-a #154-159 RT: 3.09-3.19 AV: 6 SB: 7 0.33-0.45 NL: 2.07E7  
T: +c EI Full ms [99.50-1000.50]

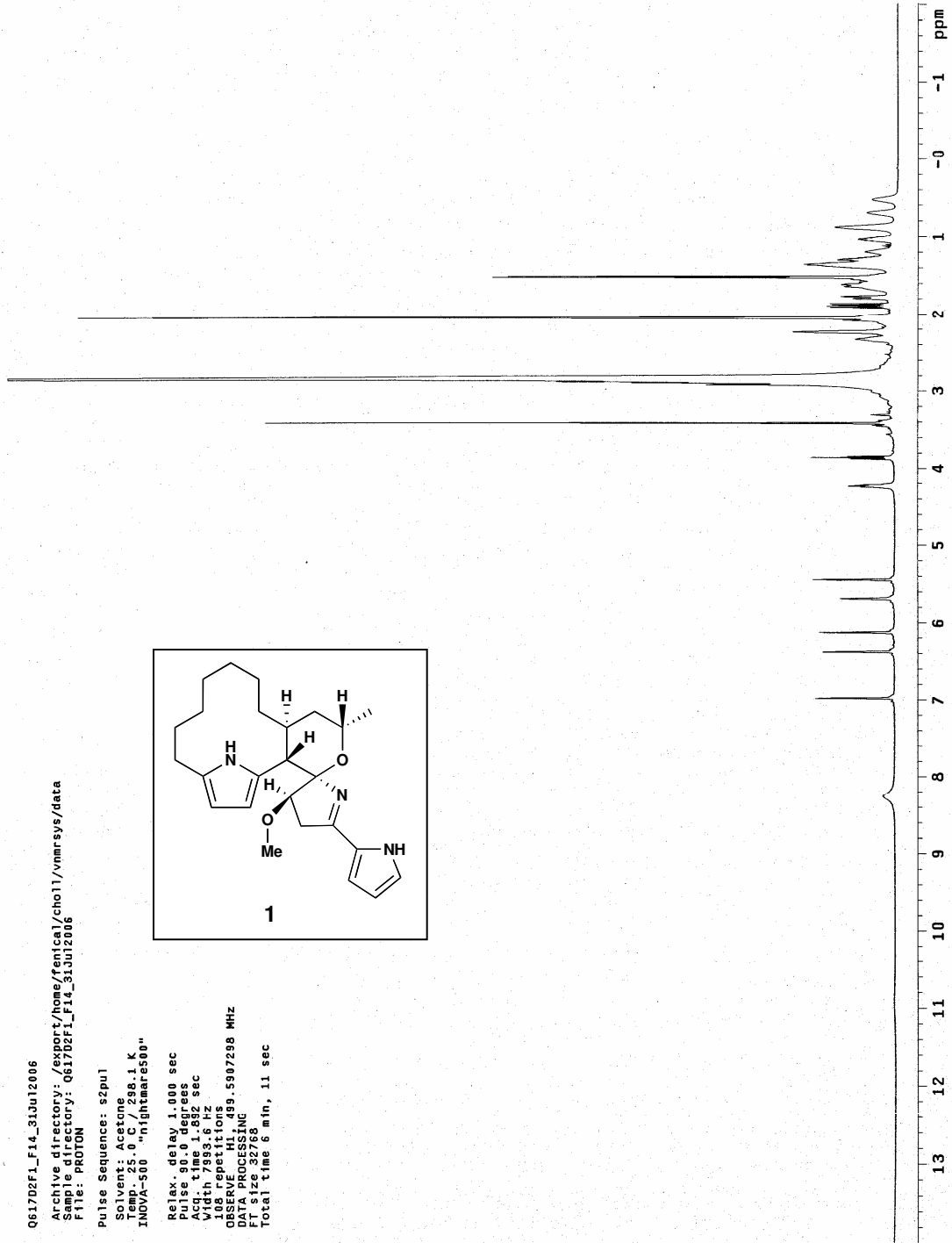
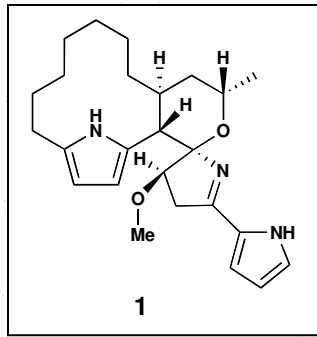


Diagnostic Fragment ions observed in the EIMS experiment performed on marineosin A

(1)

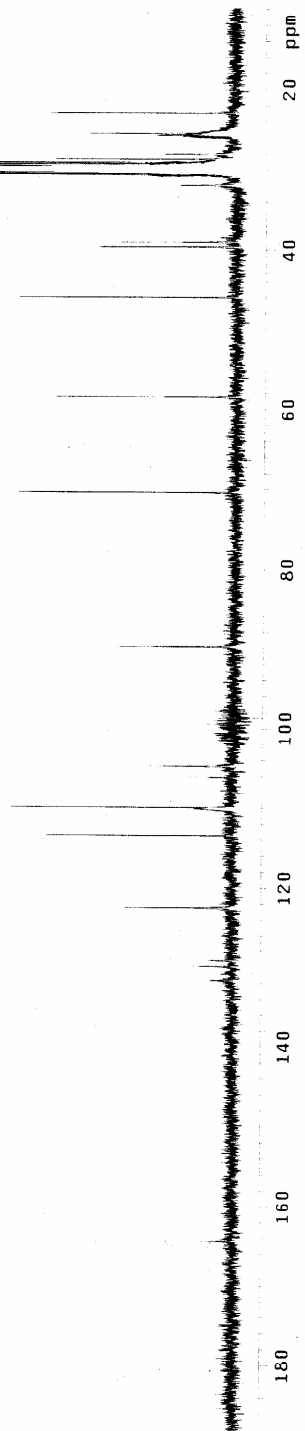
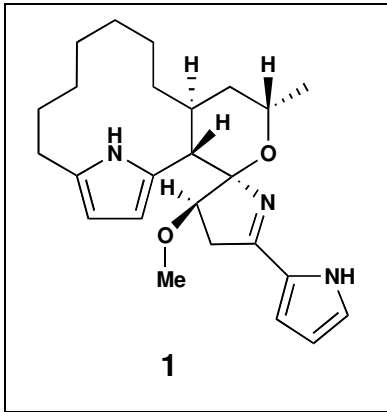
Q61702F1\_F14\_31Jul2006  
Archive directory: /export/home/fenical/cho11/vnmrSYS/data  
Sample directory: Q61702F1\_F14\_31Jul2006  
File: PROTON

Pulse Sequence: s2pul  
Solvent: Acetone  
Temperature: 298.1 K  
INDVA=500 "nightmares00"  
Relax. delay 1.000 sec  
Pulse 90.0 degrees  
Acq. time 1.862 sec  
Width 7893.6 Hz  
Obs. repetitions 99.5907298 MHz  
SOLVENT  
DATA PROCESSING  
FT size 32768  
Total time 6 min, 11 sec



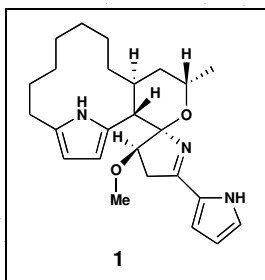
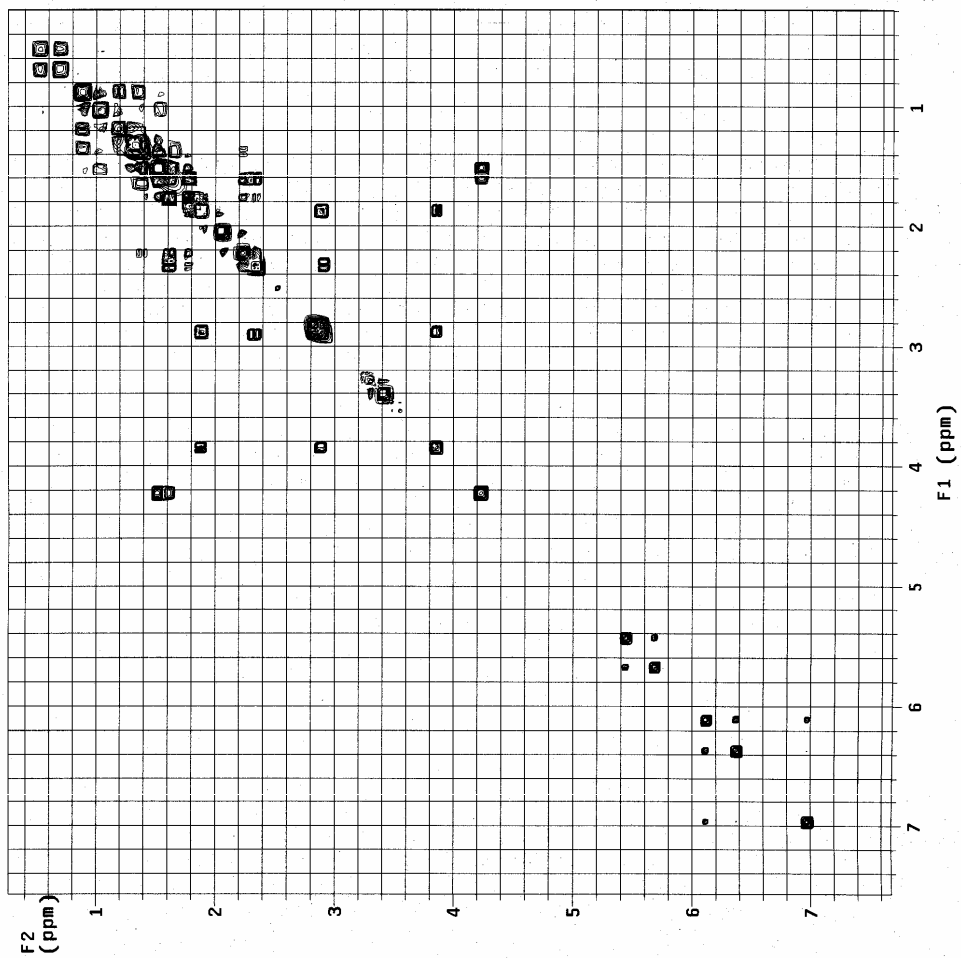
<sup>1</sup>H NMR spectrum of marineosin A (**1**) (500 MHz, acetone-*d*<sub>6</sub>)

CN0617\_408\_C13\_6Apr2007  
 Pulse Sequence: s2pul  
 Solvent: Acetone  
 Temp.: 25.0 C / 298.1 K  
 INOVA-300 "nightmare"  
 PULSE SEQUENCE  
 Relax. delay 3.000 sec  
 Pulse 69.2 degrees  
 Acq. time 1.815 sec  
 Width 16501.7 Hz  
 41 Channels  
 OBSERVE C13, 25.4220238 MHz  
 DECOUPLE H1, 299.9499082 MHz  
 Power 44 db  
 Continuously on  
 WALTZ-16 modulated  
 DATA PROCESSING  
 FT 1.0 hr, 65556  
 Total time 24 hr, 7 min, 10 sec



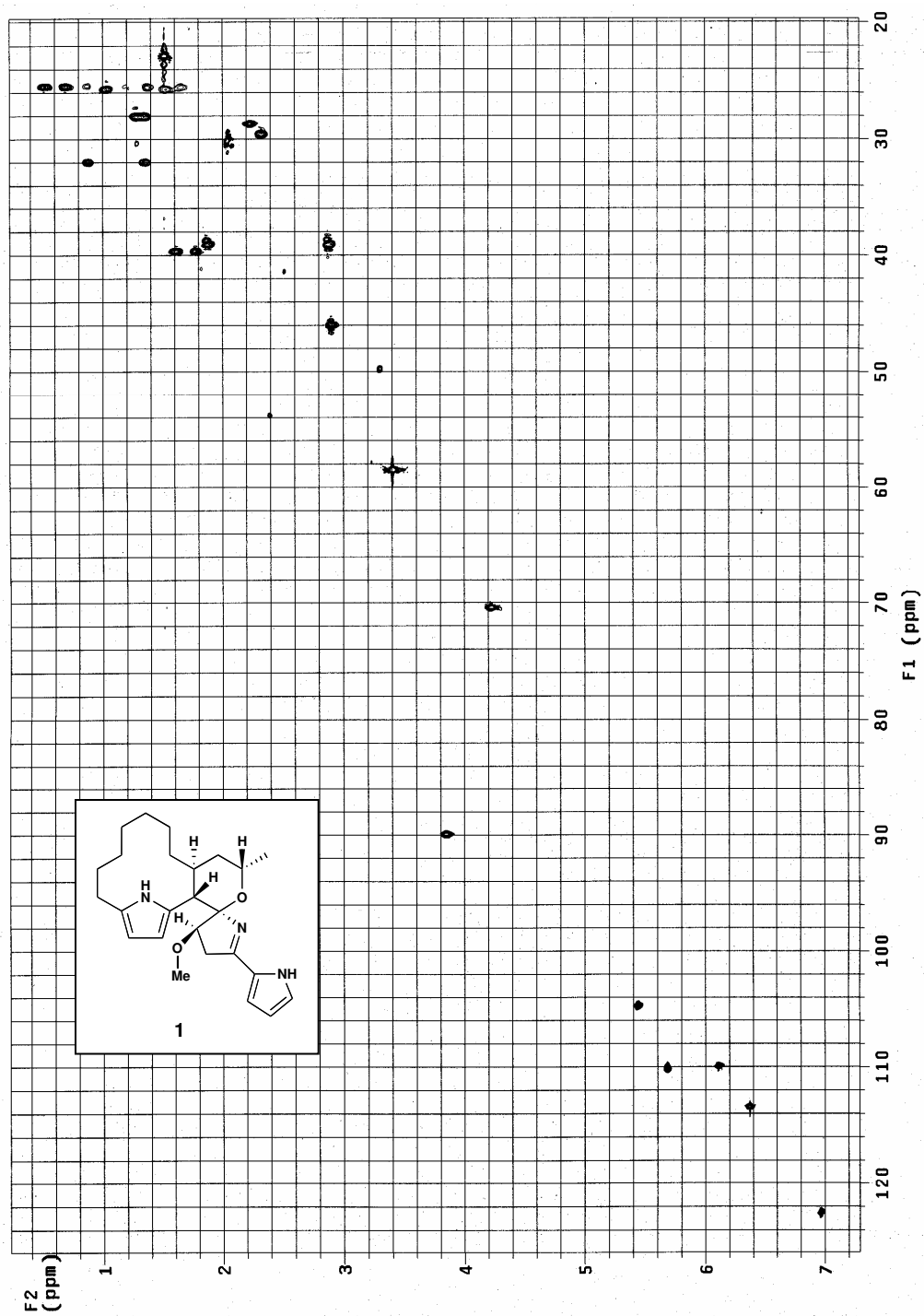
<sup>13</sup>C NMR spectrum of marineosin A (1) (300 MHz, acetone-d<sub>6</sub>)

0617.409COSY\_06Apr2007  
Archive directory: /export/home/fenical/cho11/vnmrsys/data  
Sample directory: 0617.409COSY\_06Apr2007  
File: gCOSY  
Pulse Sequence: gCOSY



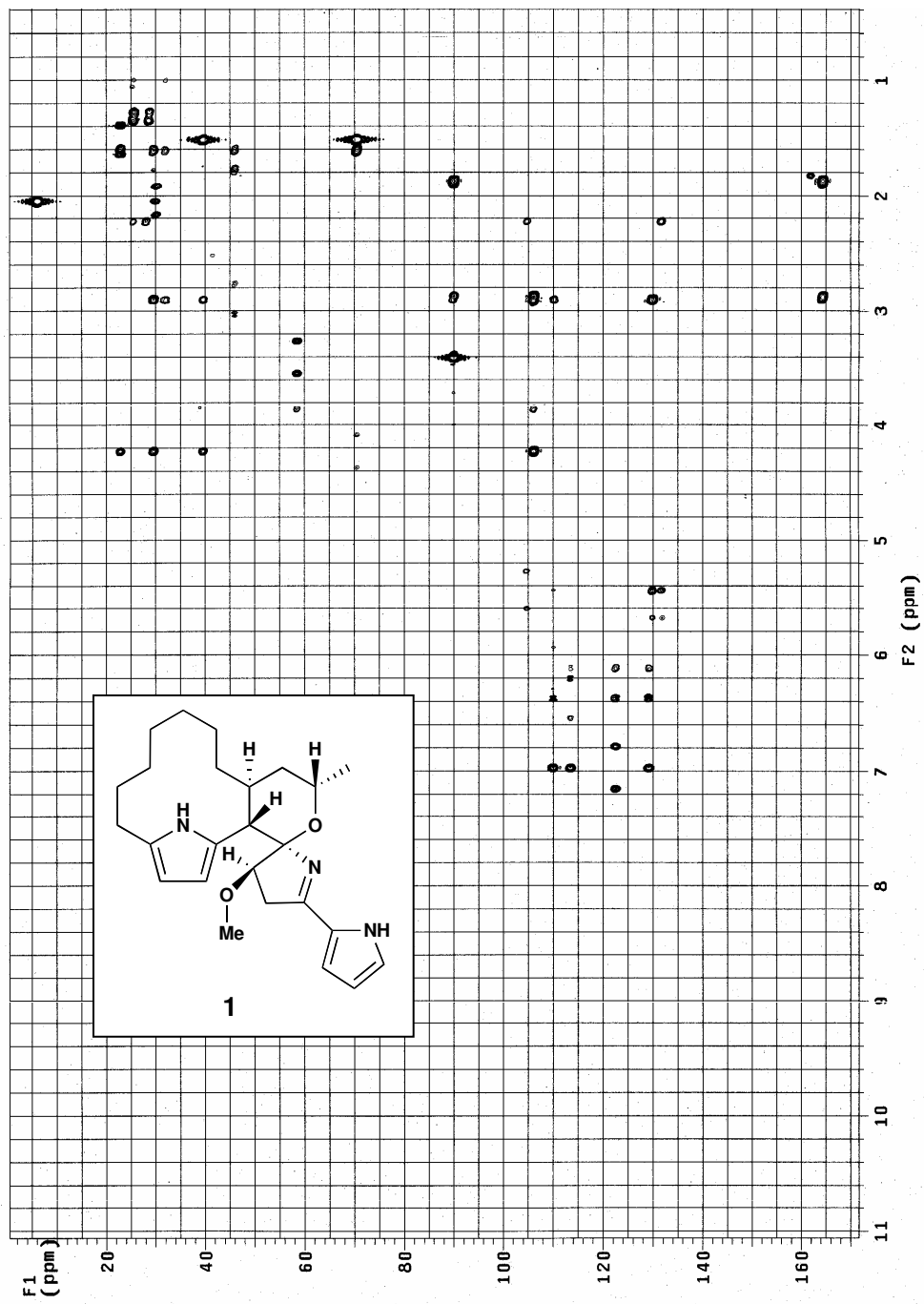
gCOSY NMR spectrum of marineosin A (**1**) (500 MHz, acetone-*d*<sub>6</sub>)

CN0617.409\_Acetone\_full\_07Apr2007  
Archive directory: /export/home/fenical/choi/vnmrsys/data  
Sample directory: CN0617.409\_Acetone\_full\_07Apr2007  
Pulse Sequence: gHSQC



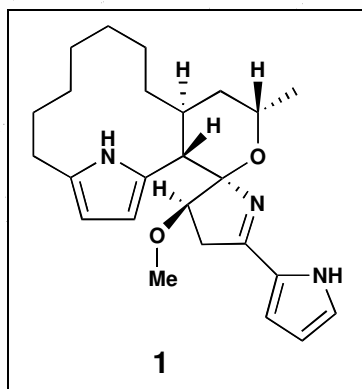
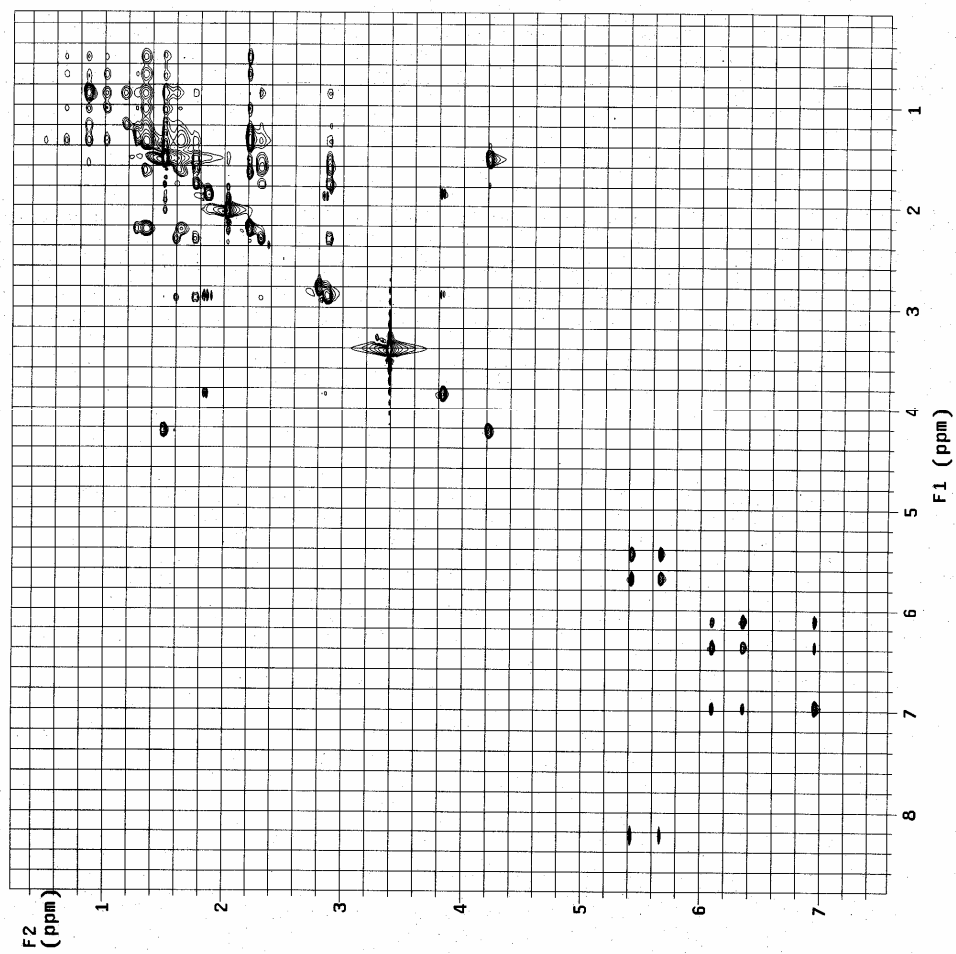
gHSQC NMR spectrum of marineosin A (1) (500 MHz, acetone-*d*<sub>6</sub>)

CN0617.409\_Acetone\_full\_07Apr2007  
Archive directory: /export/home/fenical/cho1/vnmrsys/data  
Sample directory: CN0617.409\_Acetone\_full\_07Apr2007  
Pulse sequence: gHMBC



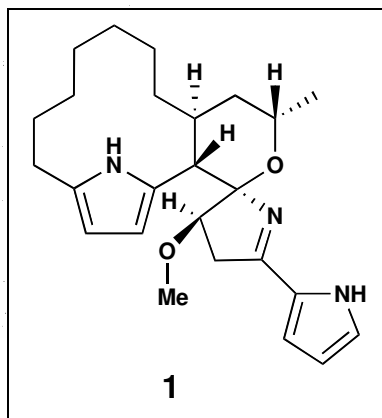
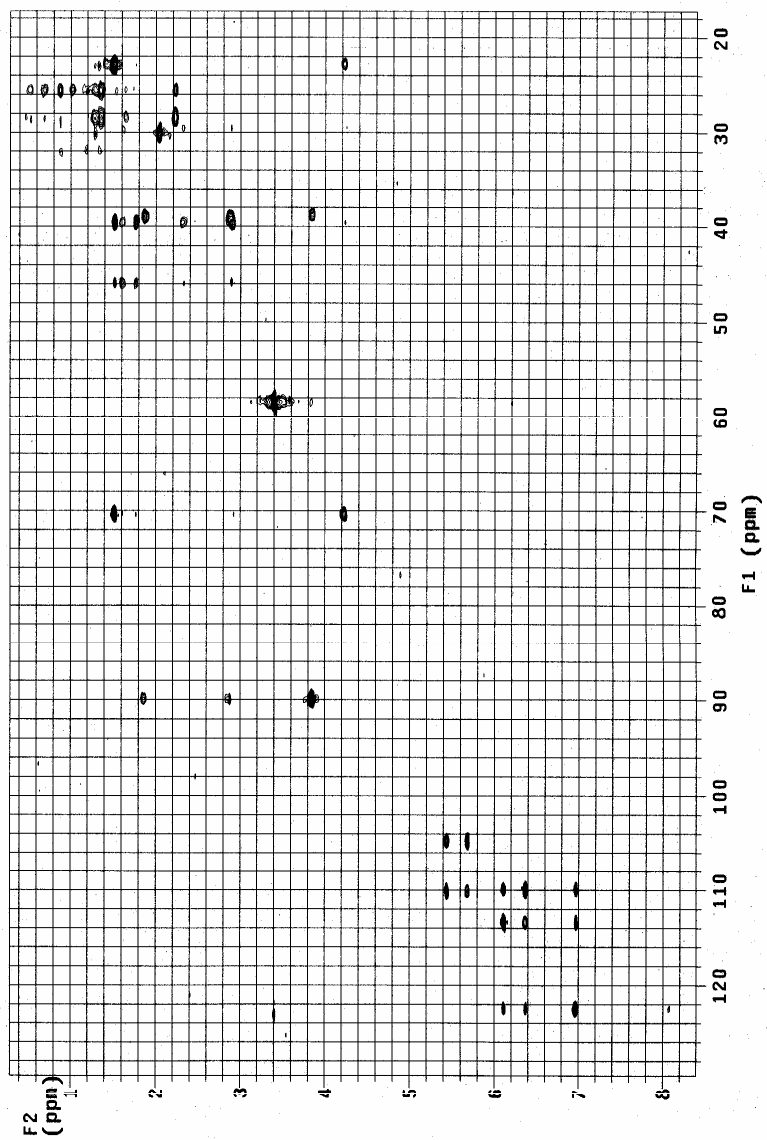
gHMBC NMR spectrum of marineosin A (**1**) (500 MHz, acetone-*d*<sub>6</sub>)

CN0617.409\_TOCSY\_acetone\_05Apr2007  
Archive directory: /export/home/fenical/cho11/vnmrsys/data  
Sample directory: CN0617.409\_TOCSY\_acetone\_05Apr2007  
File: TOCSY  
Pulse Sequence: TOCSY



TOCSY NMR spectrum of marineosin A (**1**) (500 MHz, acetone-*d*<sub>6</sub>)

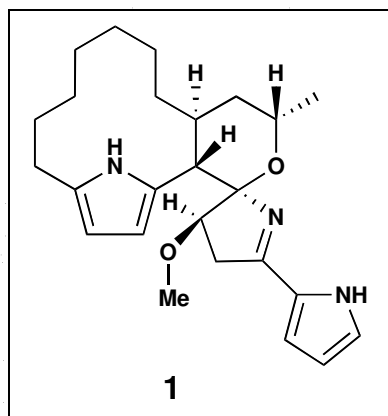
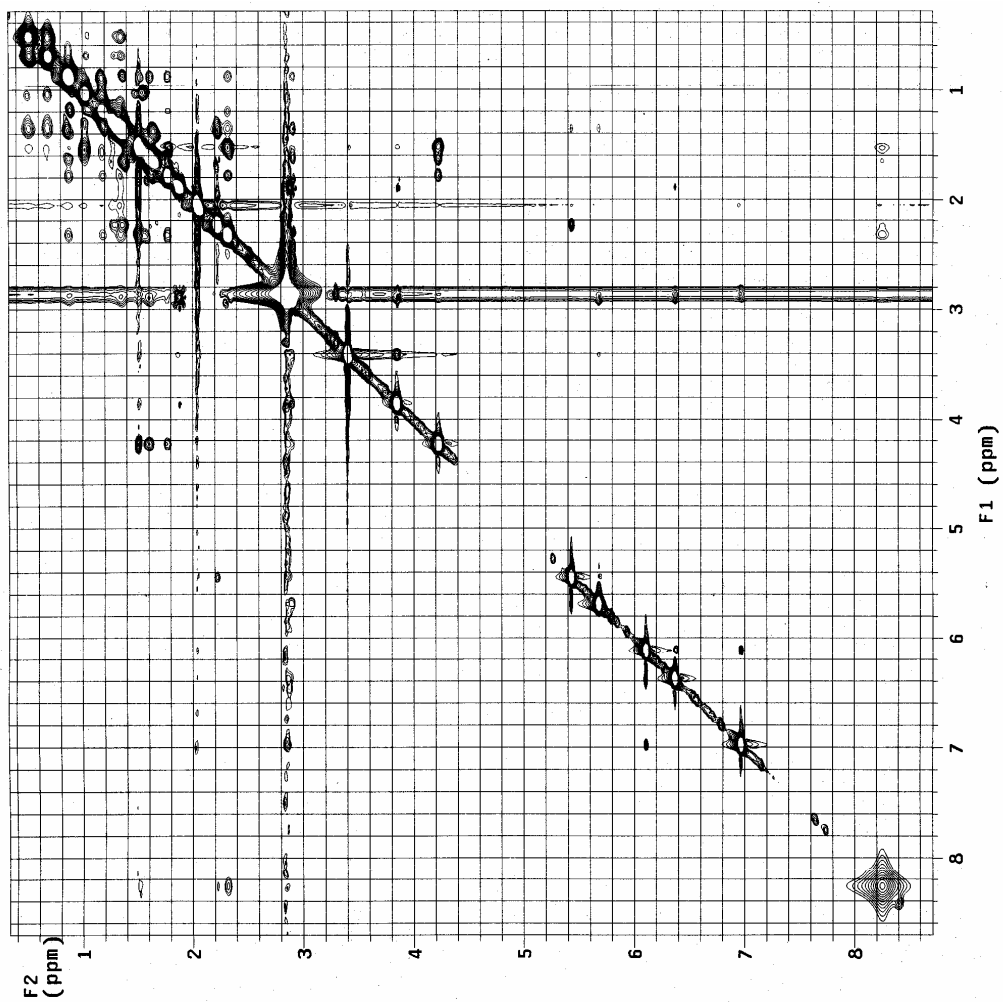
CN0617.409\_Acetone\_full\_07Apr2007  
Archive directory: /export/home/fenical/cho11/vnmrsys/data  
Sample directory: CN0617.409\_Acetone\_full\_07Apr2007  
File: gHSQCTOXY  
Pulse Sequence: gHSQCTOXY



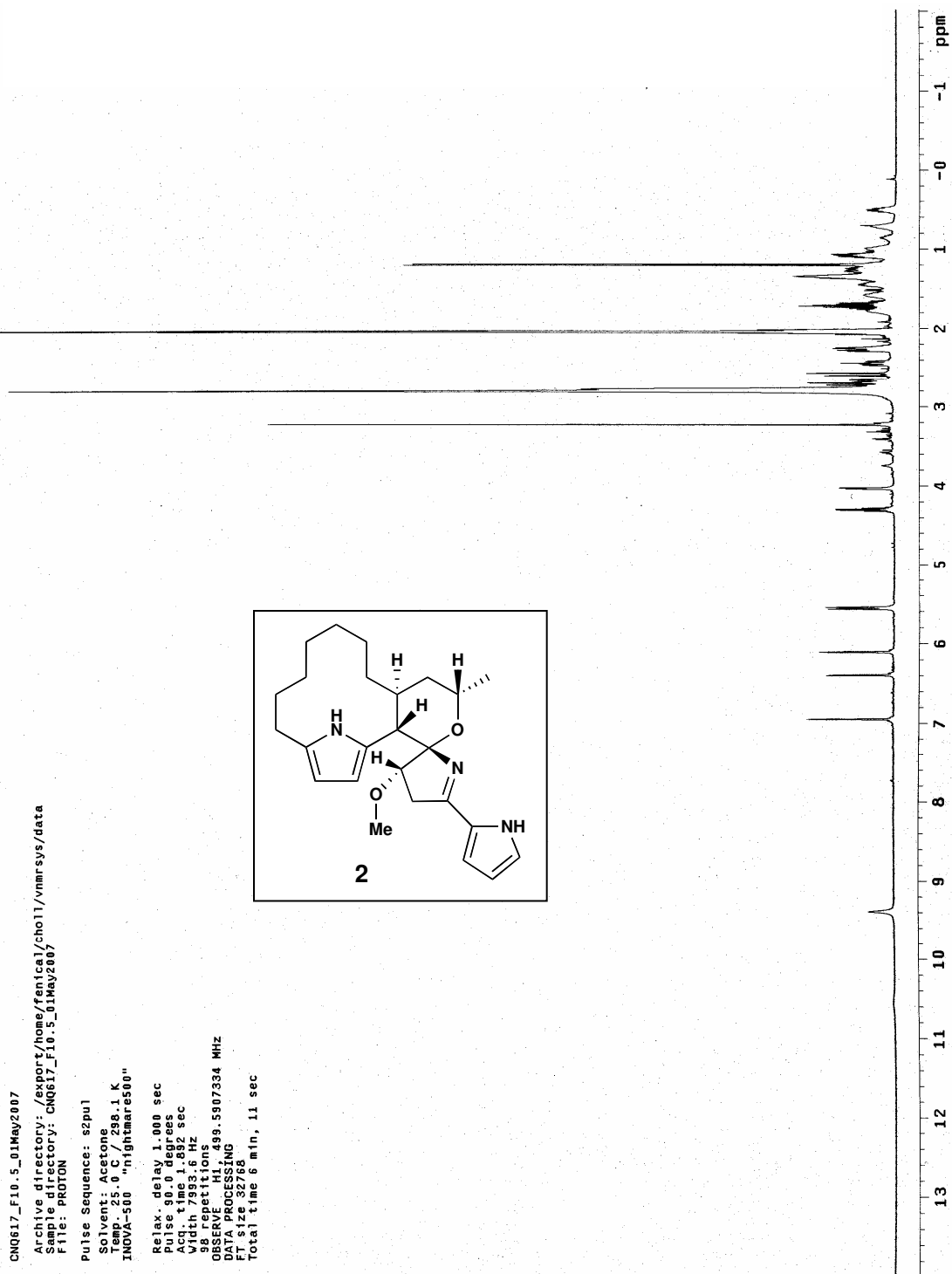
gHSQC-TOCSY NMR spectrum of marineosin A (**1**) (500 MHz, acetone- $d_6$ )



CN0617\_409\_NOESY\_26Apr2007  
Pulse Sequence: NOESY



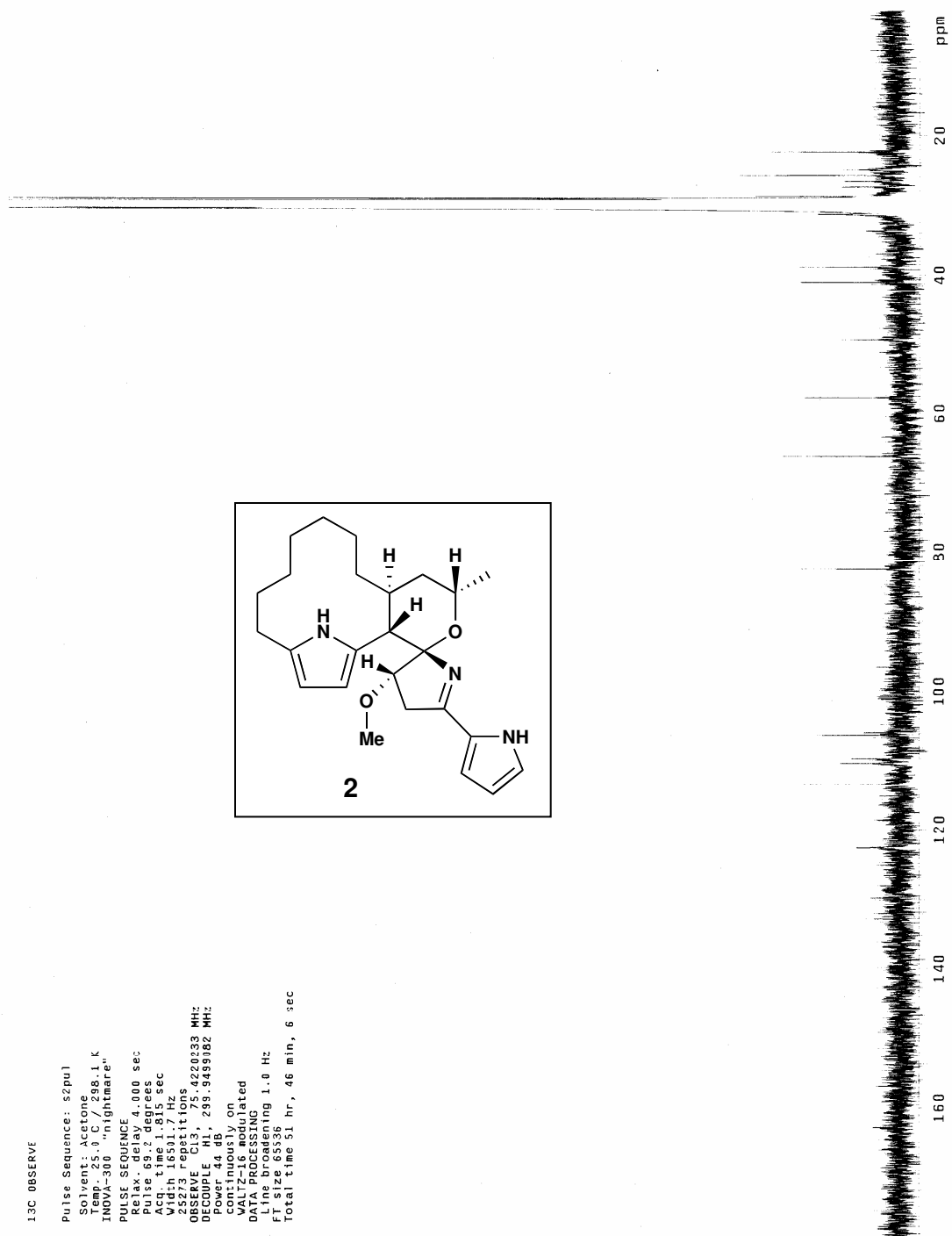
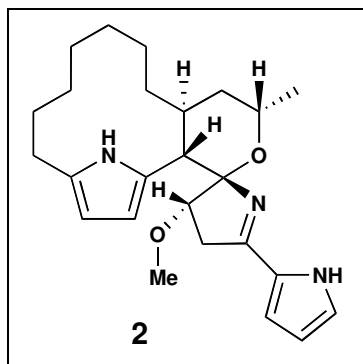
NOESY NMR spectrum of marineosin A (1) (500 MHz, acetone- $d_6$ )



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

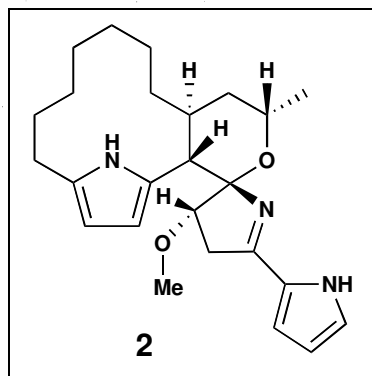
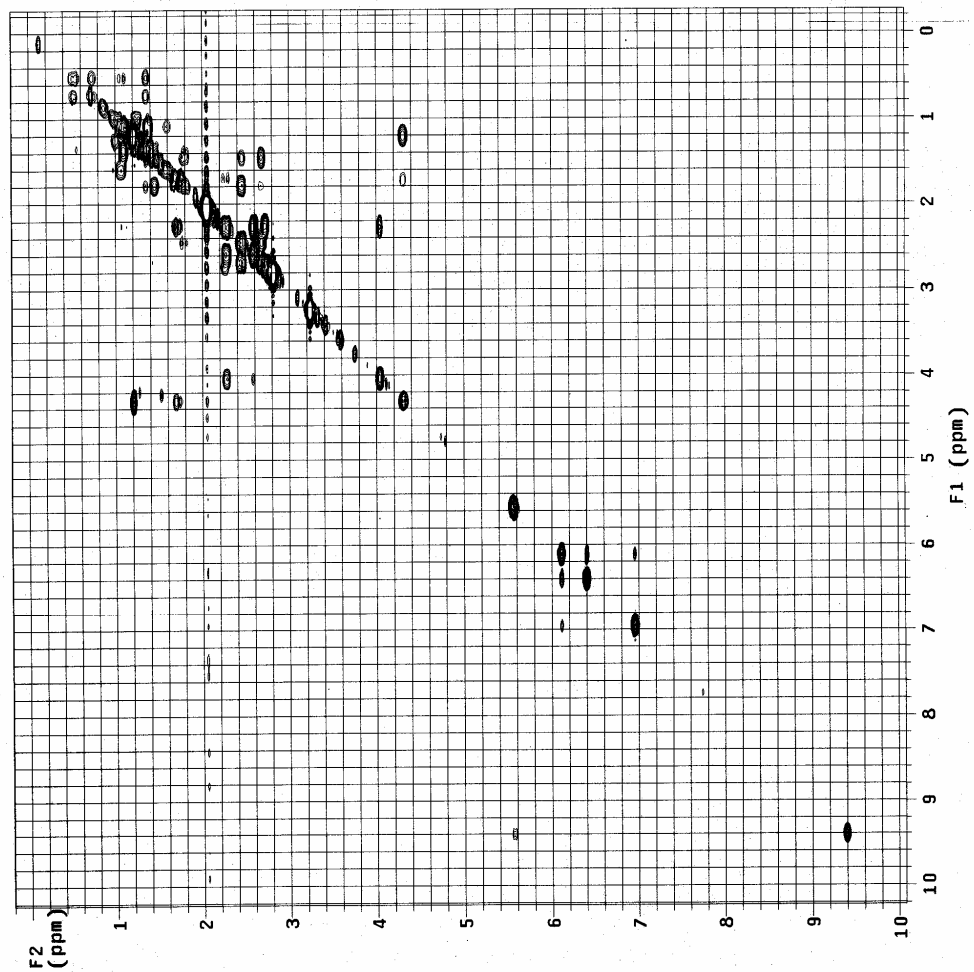
13C OBSERVE

Pulse Sequence: s2pul  
Solvent: Acetone  
Channel 1: CPDPR8.1.K  
INVA: 300. "nightmare"  
PULSE\_SEQUENCE 4.000 sec  
PCPD: det  
Pulse: 69.2 degrees  
Acq. time: 1.815 sec  
Width: 16501.7 Hz  
25273 repetitions  
DECUPLE CH1: 5.4200333 MHz  
DECUPLE CH2: 239.3499782 MHz  
Power: 44 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line processing: 1.0 Hz  
F1: 125.000 MHz  
Total time: 51 hr, 46 min, 6 sec



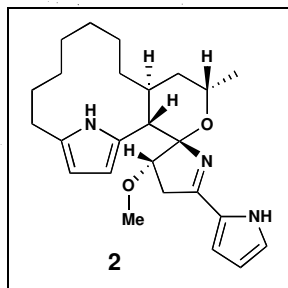
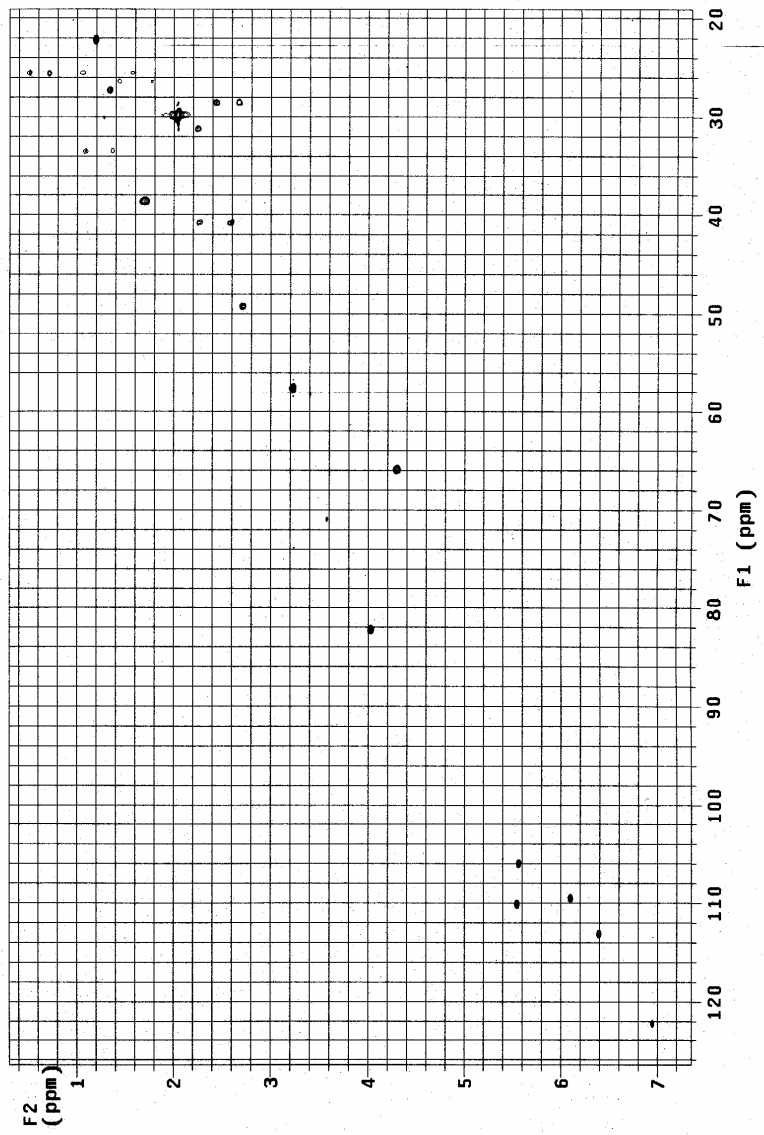
<sup>13</sup>C NMR spectrum of marineosin B (2) (300 MHz, acetone-*d*<sub>6</sub>)

CN0617\_4098\_F10\_5\_30Apr2007  
Archive directory: /export/home/fenical/cho1/vnmr/sy/data  
Sample directory: CN0617\_4098\_F10\_5\_30Apr2007  
Pulse Sequence: gCOSY



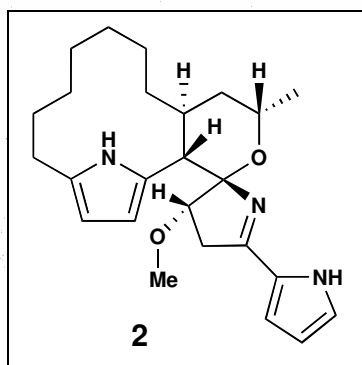
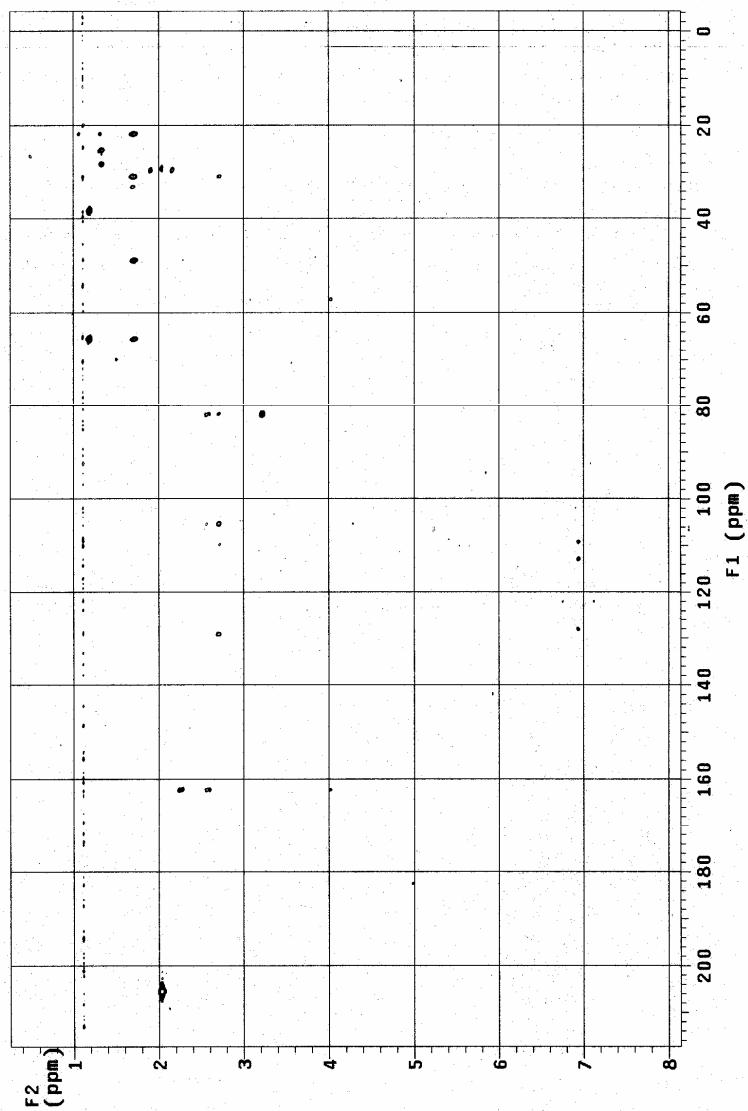
gCOSY NMR spectrum of marineosin B (**2**) (500 MHz, acetone-*d*<sub>6</sub>)

0617\_409B\_gHSQC\_03May2007  
Archive directory: /export/home/fenical/cho11/vnmrsys/data  
Sample directory: 0617\_409B\_gHSQC\_03May2007  
Pulse Sequence: gHSQC



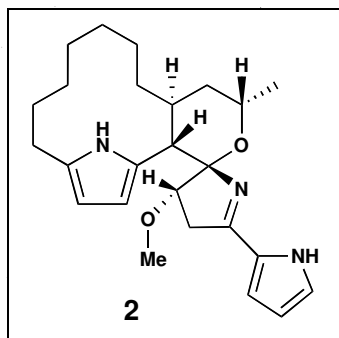
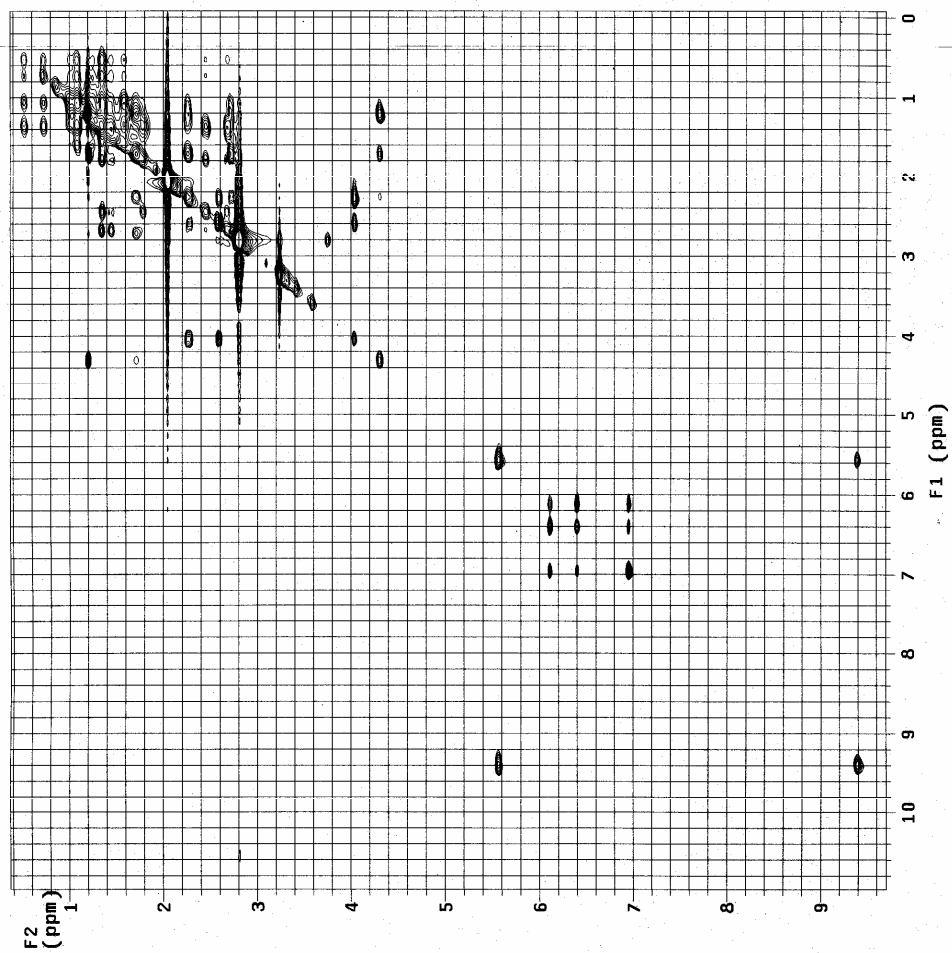
gHSQC NMR spectrum of marineosin B (**2**) (500 MHz, acetone-*d*<sub>6</sub>)

0617\_409B\_F10.5\_gHMBC\_05May2007  
Archive directory: /export/home/fenical/cho11/vmr-sys/data  
Sample directory: 0617\_409B\_F10.5\_gHMBC\_05May2007  
Pulse Sequence: gHMBC



gHMBC NMR spectrum of marineosin B (**2**) (500 MHz, acetone-*d*<sub>6</sub>)

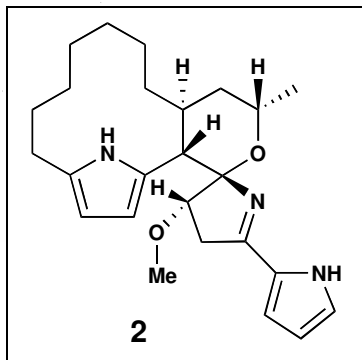
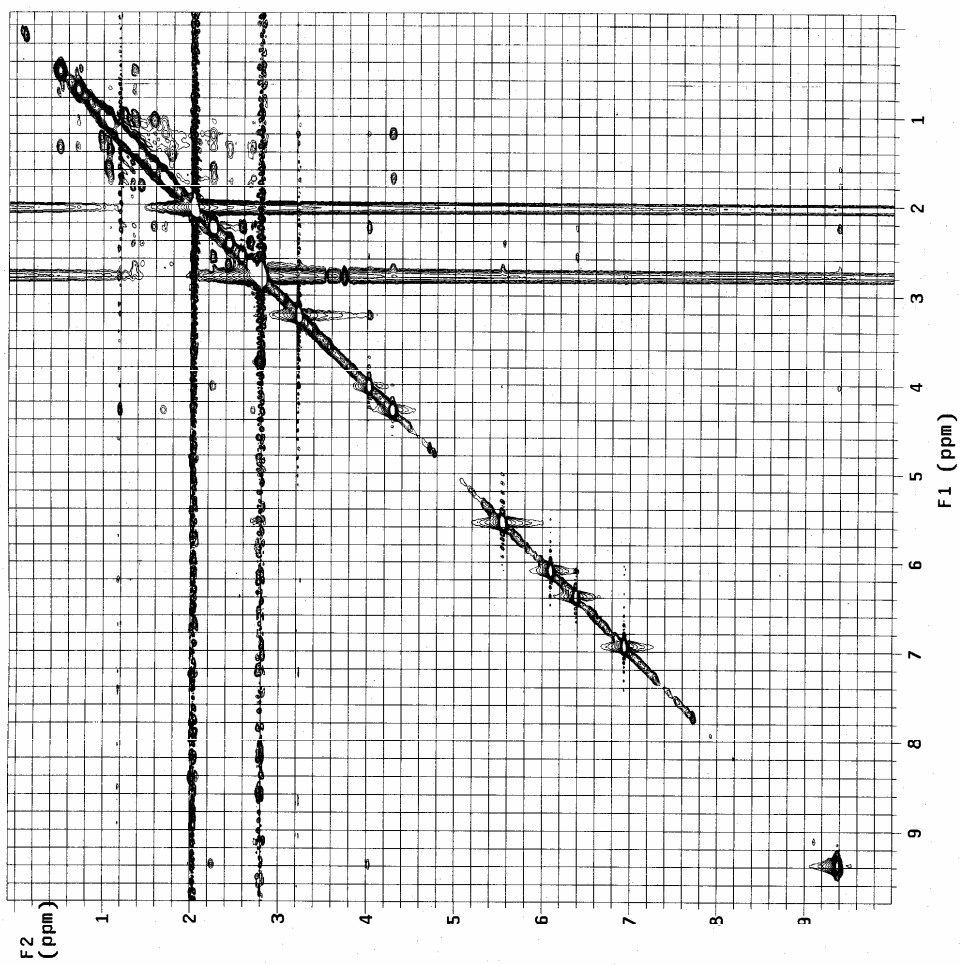
CN0617.409B\_F10\_5\_30Apr2007  
Archive directory: /export/home/fentical/choi1/vnmrsys/data  
Sample directory: CN0617.409B\_F10\_5\_30Apr2007  
File: TOCSY  
Pulse Sequence: TOCSY



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

.5\_NOESY\_1May07  
xy: /export/home/ferica/cho11/vnmr/sys/data  
y: CNG817.4098\_02May2007

NOESY



NOESY NMR spectrum of marineosin B (**2**) (500 MHz, acetone-*d*<sub>6</sub>)