Erythronolides H and I, New Erythromycin Congeners from a New Halophilic Actinomycete *Actinopolyspora* sp. YIM90600

Sheng-Xiong Huang,[†,⊥] Li-Xing Zhao,[†,∥] Shu-Kun Tang,[∥] Cheng-Lin Jiang,[∥] Yanwen Duan,[⊥] Ben Shen[*,†,‡,§]

[†]*Division of Pharmaceutical Sciences,* [‡]*University of Wisconsin National Cooperative Drug Discovery Group, and* [§]*Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53705, USA;* [⊥]*Hunan Engineering Research Center of Combinatorial Biosynthesis and Natural Product Drug Discovery, Changsha, Hunan 410329, China; and* [∥]*Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, China*

bshen@pharmacy.wisc.edu

Supporting Information

Table of Contents

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 241 polarimeter at the sodium D line (589 nm). ^1H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Unity Inova 500 instrument operating at 500 MHz for ^1H and 125 MHz for ^{13}C nuclei. The chemical shifts were referenced to residual solvent signals: δ_H 7.26 and δ_C 77.29 for $CDCl_3$, and δ_H 2.46 and δ_C 40.19 for DMSO–d_6. ^1H-^1H gCOSY (mixing time = 80 ms), gHMQC ($^1J_{CH}$ = 140 Hz), and *g*HMBC ($^{2\text{-}3}J_{XH}$ = 8.0 Hz) spectra were performed using standard VARIAN pulse sequences. Electrospray ionization-mass (ESI-MS) spectra and LC–MS data were obtained on an Agilent 1100 HPLC–MSD SL quadrupole mass spectrometer. High resolution mass spectral analyses were acquired on an IonSpec HiResMALDI FT–Mass spectrometer with a 7 tesla superconducting magnet. Semipreparative HPLC was performed on a Varian liquid chromatograph system with an Altima-C18, 9.4 mm × 25 cm column. Column chromatography were performed either on silica gel (230–400 mesh, Natland International Corporation, Research Triangle Park, NC), Lichroprep RP-18 gel (40–63 μm, Merck, Dramstadt, Germany), or Sephadex LH-20 (Pharmacia, Kalamazoo, MI).

Isolation of YIM90600. The *Actinopolyspora* sp. YIM90600 strain was isolated from a saline soil on the bed of dried salt lake in Xingjiang province, northwest of China, after 3 weeks incubation at 37 ºC on Cellulose-casein multi-salt medium (microcrystalline cellulose 1%, casein 0.03%, KNO_3 0.02%, K_2HPO_4 0.05%, $CaCO_3$ 0.002%, $FeSO_4$ 0.001%, NaCl 10%, $MgCl_2{\cdot}6H_2O$ 3%, KCl 2%, agar 1.5%).[S1] All salts used were sterilized separately before addition to the medium. The pH of the medium was adjusted to pH 7.5 with 1 N NaOH and isolates maintained on ISP 4 agar slants containing 10 % (w/v) NaCl at 4 ºC or as suspensions of mycelial fragments in 20 % (v/v) glycerol.

Strain identification. Strain YIM90600 was identified on the basis of polyphasic taxonomy. Strain YIM90600 is a moderately halophilic actinomycete displaying optimal growth characteristics at 37 ºC, pH 7~8 and 10~15 % (w/v) NaCl; containing *meso*-diaminopimelic acid as the diagnostic diamino acid and galactose, glucose, arabinose and ribose as the major whole-cell sugar. The major phospholipids were phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinones were MK-9(H_4) and MK-10(H_4). The major fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. Phylogenetic analysis based on 16S rRNA gene sequences revealed low 16S rRNA similarity (< 96%) with other species of the genus *Actinopolyspora*. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of the members of the family *Pseudonocardiaceae* showed that stain YIM90600, *A. halophila* and *A. mortivallis* belong to the same lineage within the genus of *Actinopolyspora*. On the base of the polyphasic evidence, YIM90600 was assigned to the genus *Actinopolyspora*. Strain YIM90600 (accession number EF116937) has been deposited at Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, P. R. China**.**

Fermentation and Isolation. The inoculum was prepared by introducing the periphery of 7-day-old petri dish cultures of *Actinopolyspora* sp. YIM90600 into 250-mL flasks containing 50 mL of the broth (soluble starch 0.5%, glucose 2%, peptone 0.2%, yeast extract 0.2%, soybean flour 1%, NaCl 10%, K_2HPO_4 0.05%, $MgSO_4{\cdot}7H_2O$ 0.05%, $CaCO_3$ 0.2% in tap water, pH 7.8, adjusted with 1 N NaOH), followed by shaking (250 rpm) continuously for 5 days at 28 ± 0.5 °C. The follow-up fermentation was accomplished by adding the inoculum (50 mL) into 2-L flasks containing 500 mL of the same culture broth (10 × 0.5 L), and then shaking for 28 days under the same condition. Solid phase extraction of the broth using resin (HP-20), filtration through cheesecloth, and elution of the resin with acetone afforded, after solvent removal under vacuum, a gummy extract was obtained. The extract (15 g) was chromatographed on silica gel column

using $CHCl_3$–MeOH (100:0, 50:1, 20:1, 10:1, 5:1, 1:1 and 0:100, 1.5 L each) as the mobile phase to get seven fractions A-G. Fraction B (50:1) (500 mg) was further chromatographed over Sephadex LH-20 column and eluted with methanol to yield three subfractions A1-A3. Subfraction A2 was finally purified by semi-preparative HPLC to afford compounds **2** (5 mg) and **3** (6 mg). Fraction C (20:1) (380 mg) was chromatographed on RP-18 gel eluted in a step gradient manner with methanol/water (from 20:80 to 100:0) to get compound **4** (35 mg) as a colorless crystal. Compounds **5** (300 mg) and **6** (35 mg) were obtained from fraction F (1:1) by repeated chromatography on silica gel and Sephadex LH-20 columns.

Erythronolide H (2): white solid; $[\alpha]_D^{19}$+20.76 (*c* 0.25, $CHCl_3$); UV (MeOH) end absorption; APCIMS *m*/*z* 417 (7), 399 (100), 381 (52), 341 (10), 285 (41), 267 (22); HR-MALDIMS (positive ion) *m*/*z* 439.2292 (calcd for $C_{21}H_{36}O_8Na$ $[M+Na]^+$, 439.2302).

Erythronolide I (3): amorphous powder; $[\alpha]_D^{19}$+4.76 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} log (ε) 256 (3.56) nm; APCIMS (positive ion) *m*/*z* 383 (10), 347 (40), 307 (100), 289 (25), 251 (48), 123 (10); HR-MALDIMS (positive ion) *m*/*z* 405.2244 (calcd for $C_{21}H_{34}O_6Na$ $[M+Na]^+$, 405.2248).

References:

S1. Tang, S. -K.; Tian, X. -P.; Zhi, X. -Y.; Cai, M.; Wu, J. -Y.; Yang, L. -L.; Xu, L. -H.; Li, W. -J. *Int. J. Syst. Evol. Microbiol*. **2008**, *58*, 2075–2080.

Table 1. ^{1}H and ^{13}C NMR Data of erythronolide H (**2**) and I (**3**)[a]

	2[b]		**3**[c]	
No.	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H (mult., *J* in Hz)	^{13}C
1		175.8 (s)		166.7 (s)
2α	2.60 (m)	44.2 (d)		126.8 (s)
3	3.73 (d, 10.8)	77.7 (d)	6.63 (dd, 6.5, 1.5)	146.4 (d)
4	1.88 (m)	37.7 (d)	2.56 (m)	31.1 (d)
5	4.00 (br s)	71.6 (d)	4.34 (d, 3.0)	84.8 (d)
6		58.8 (s)		82.3 (s)
7a	2.38 (br d, 13.8)	37.3 (t)	1.94 (t, 12.0)	40.0 (t)
7b	0.83 (br d, 13.8)		1.75 (br d, 12.0)	
8	2.91 (m)	38.5 (d)	2.16 (m)	36.8 (d)
9		212.9 (s)		113.8 (s)
10	2.71 (m)	44.5 (d)	2.03 (m)	45.4 (d)
11	3.64 (m)	71.5 (d)	3.93 (dd, 9.6, 5.5)	86.4 (d)
12	1.58 (m)	38.7 (d)		82.1 (s)
13	5.12 (d, 9.1)	78.2 (d)	3.56 (br d, 10.5)	77.3 (d)
14a	3.71 (m)	67.0 (d)	1.72 (m)	23.6 (t)
14b			1.45 (m)	
15	1.00 (d, 6.8)	20.7 (q)	1.00 (t, 6.4)	11.5 (q)
16	1.11 (d, 6.8)	15.3 (q)	1.90 (br s)	17.2 (q)
17	0.89 (d, 6.8)	5.9 (q)	1.07 (d, 7.0)	13.7 (q)
18a	2.76 (d, 7.0)	46.9 (t)	1.25 (s)	24.0 (q)
18b	2.27 (d, 7.0)			
19	0.95 (d, 6.2)	14.5 (q)	0.98 (d, 6.4)	13.0 (q)
20	0.77 (d, 6.4)	6.7 (q)	1.03 (d, 7.0)	11.5 (q)
21	0.79 (d, 6.4)	10.5 (q)	1.27 (s)	21.9 (q)
OH-3	-[d]			
OH-5	-[d]			
OH-11	-[d]		3.05 (d, 5.5)	
OH-13			2.98 (br s)	
OH-14	-[d]			

[a] Signals were assigned with aid of ^{1}H-^{1}H COSY, HMQC, and HMBC experiments.
[b] Data were recorded in DMSO-d_6.
[c] Data were recorded in $CDCl_3$.
[d] Not observed.

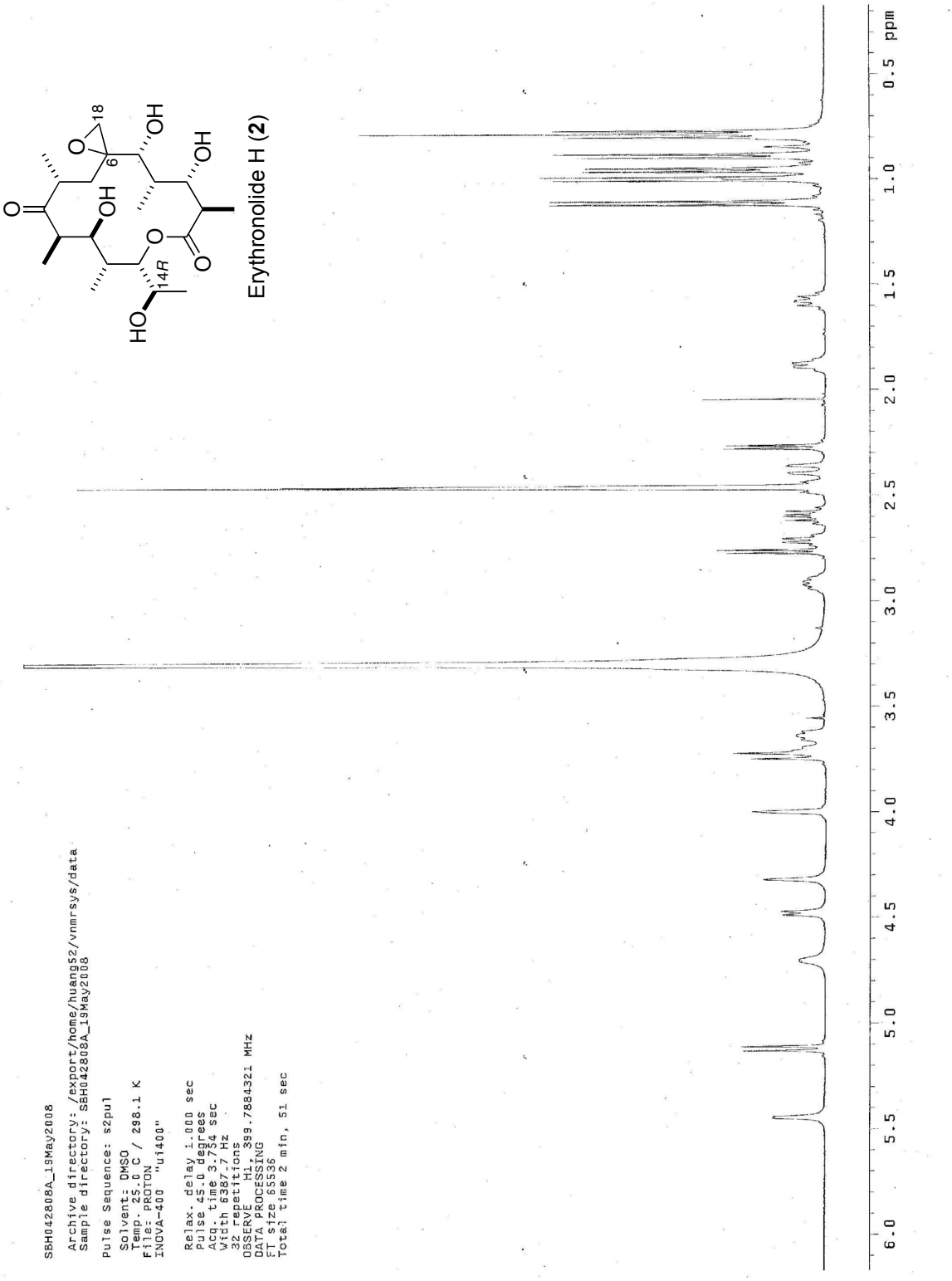

Figure S1. ^{1}H NMR spectrum of erythronolide H (**2**)

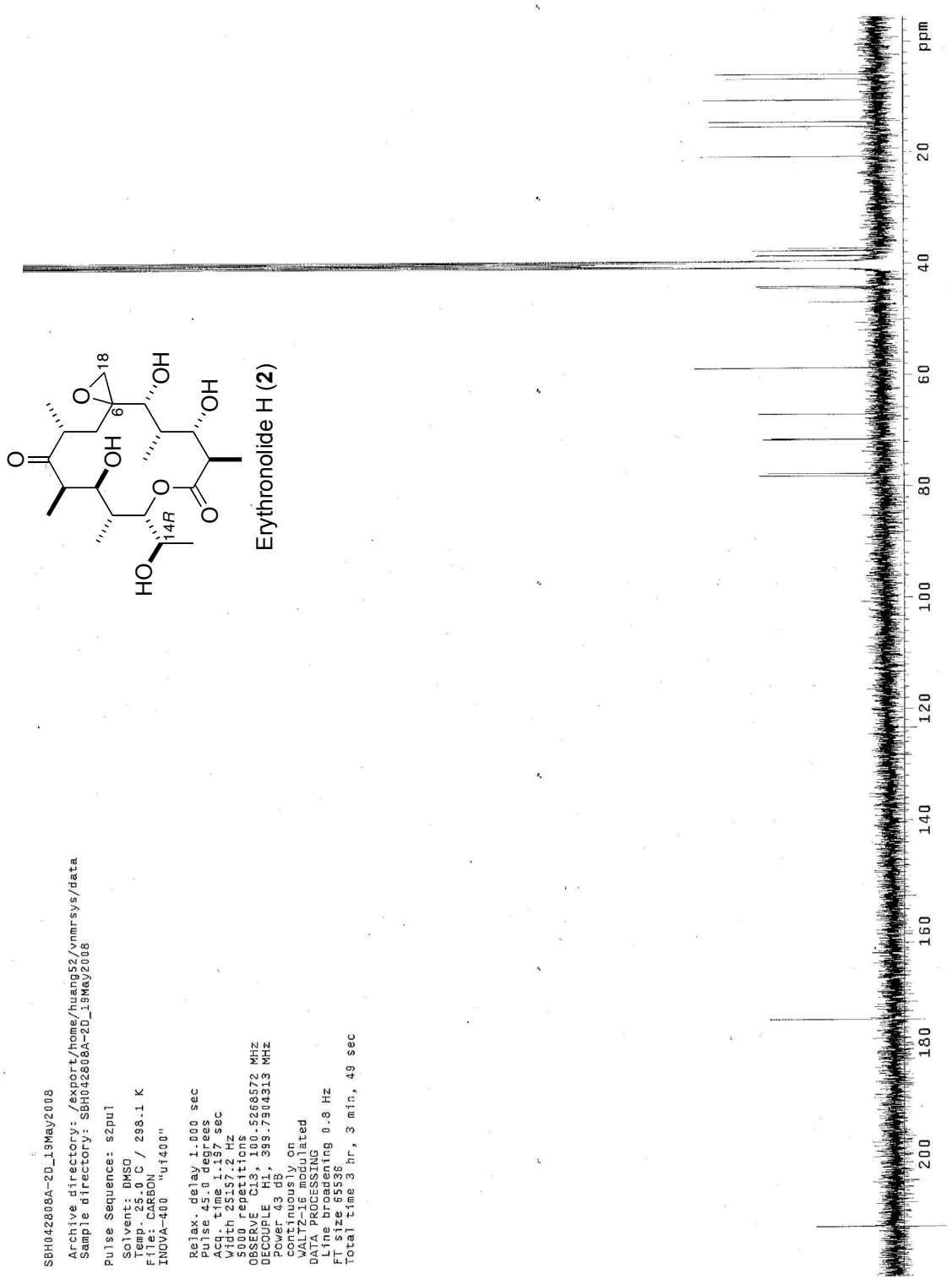

Figure S2. ^{13}C NMR spectrum of erythronolide H (**2**)

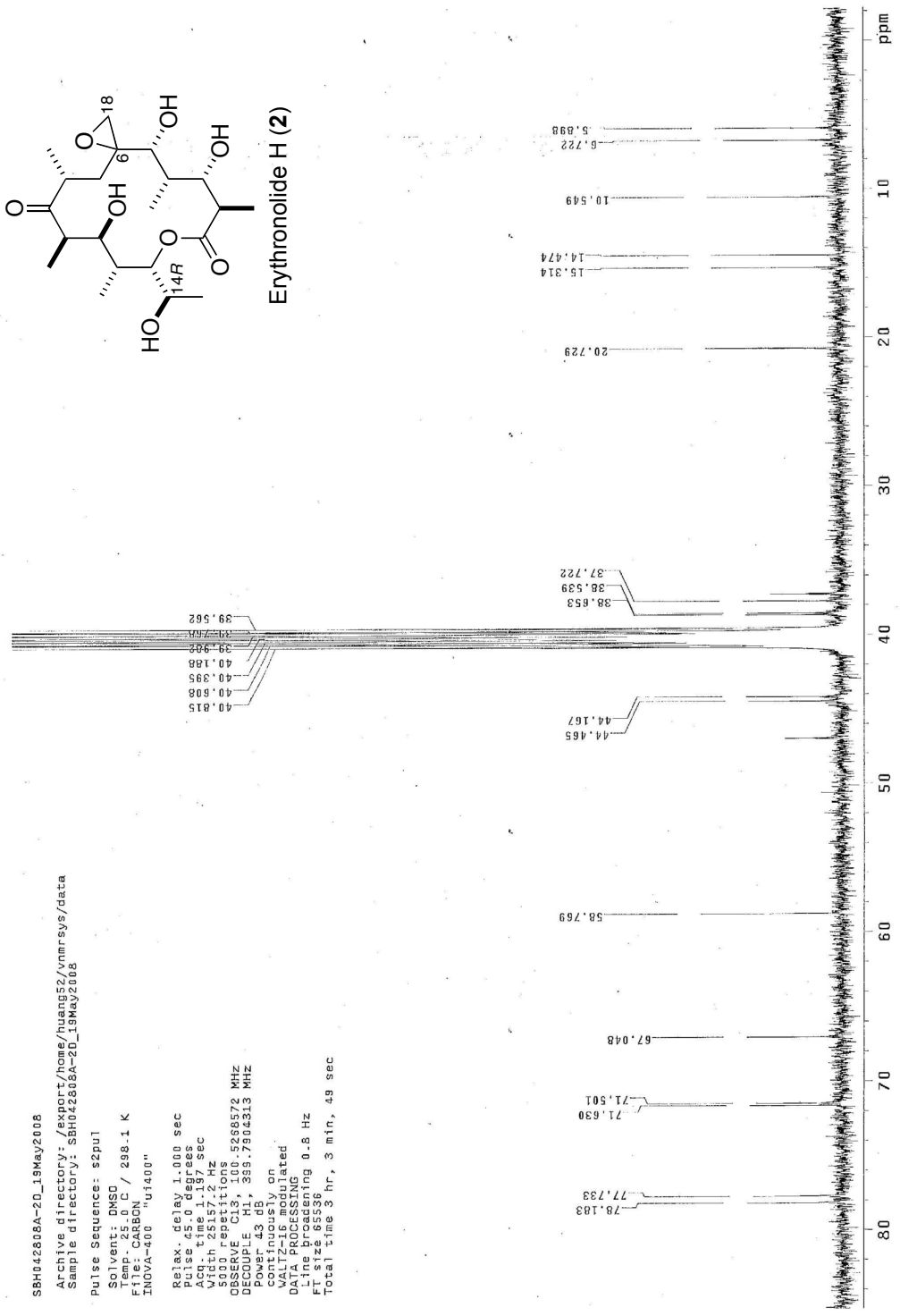

Figure S3. Expanded ^{13}C NMR spectrum of erythronolide H (**2**)

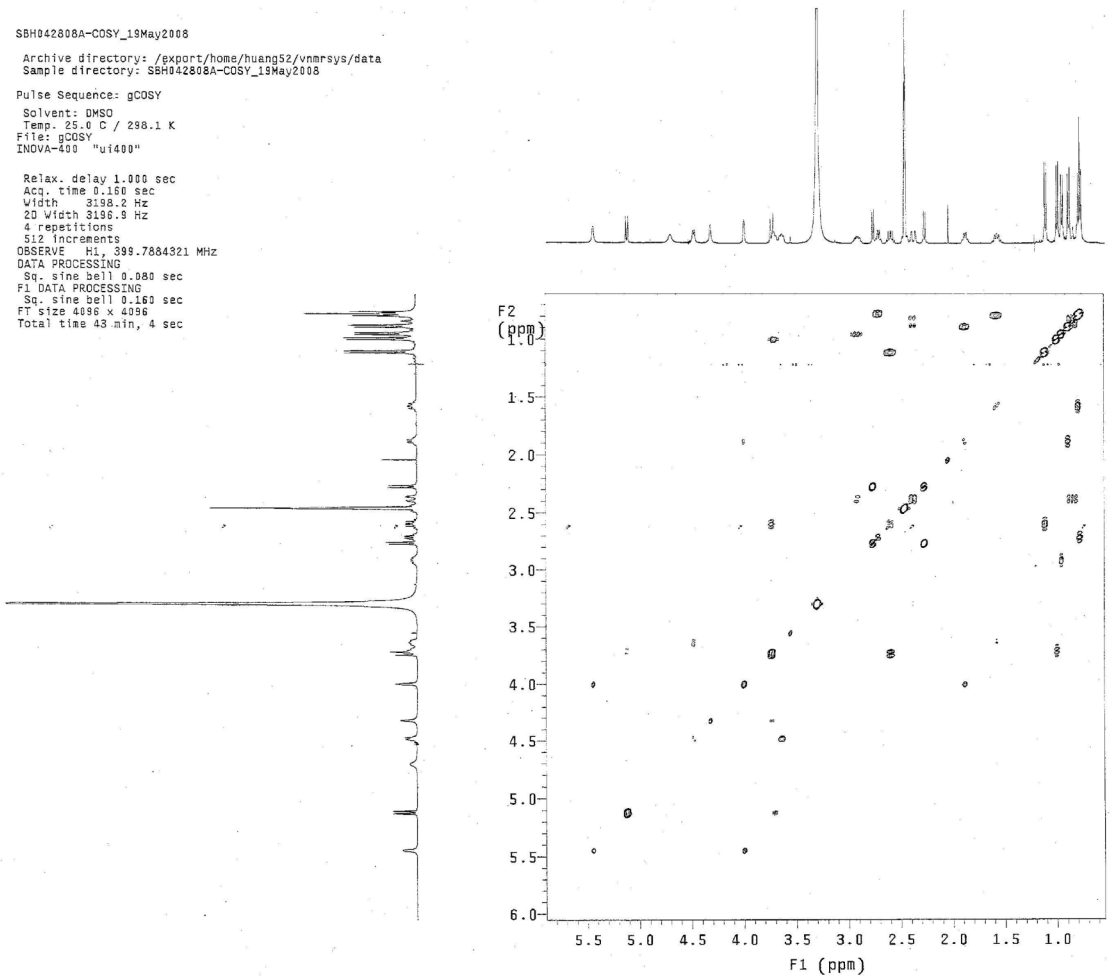

Figure S4. ^{1}H-^{1}H gCOSY NMR spectrum of erythronolide H (**2**)

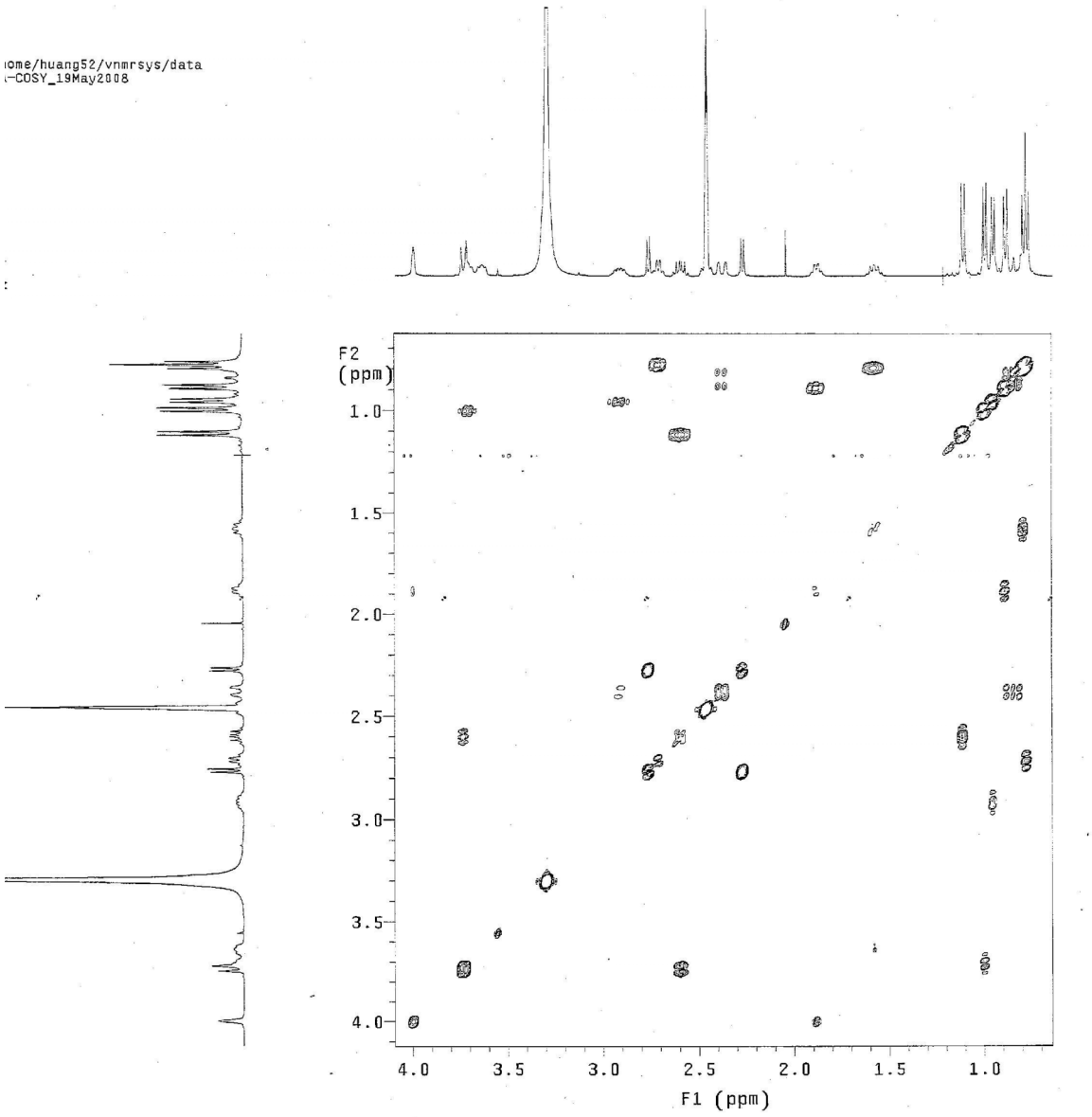

Figure S5. Expanded ^{1}H-^{1}H gCOSY spectrum of **2**

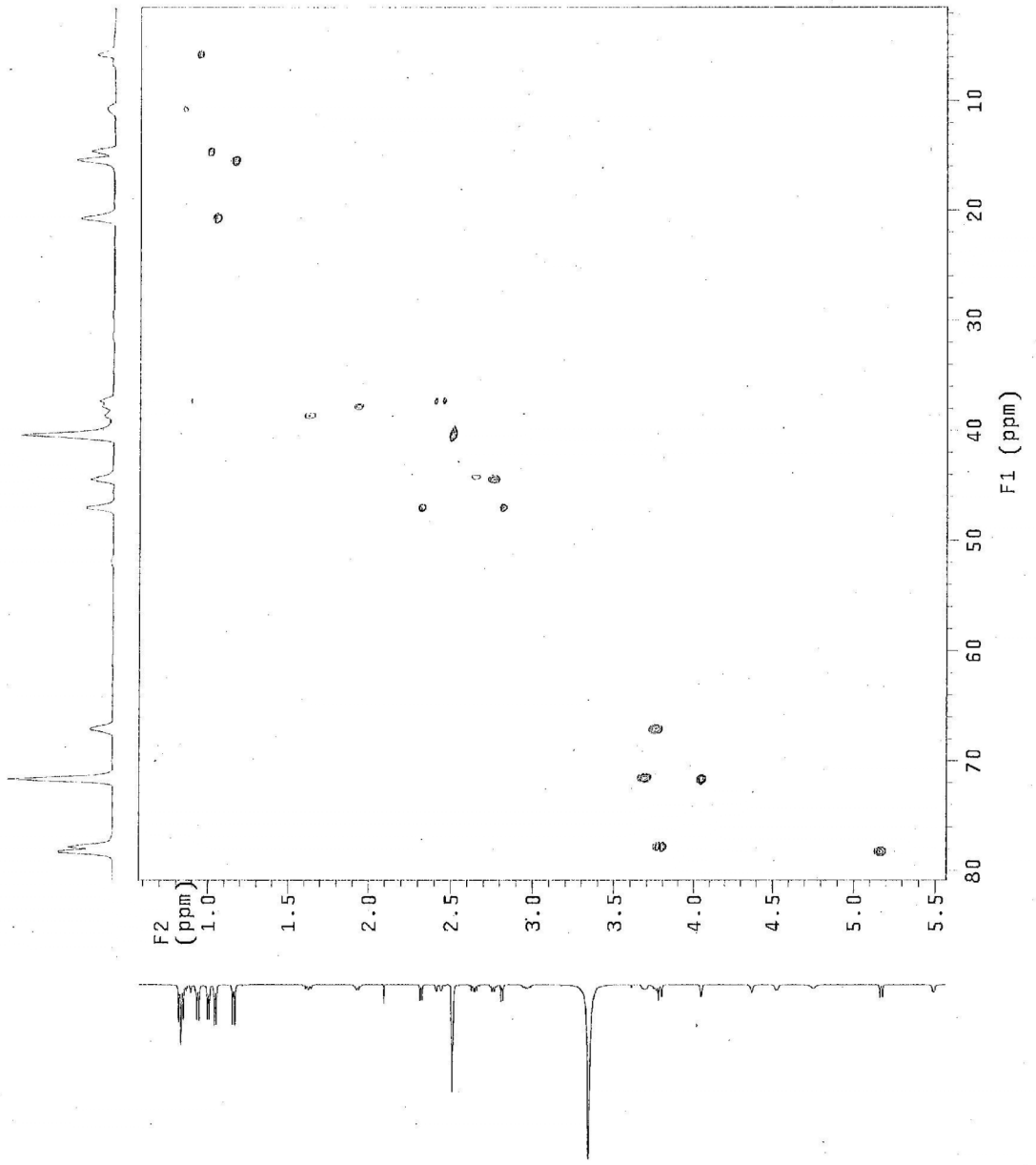

Figure S6. gHMQC NMR spectrum of erythronolide H (**2**)

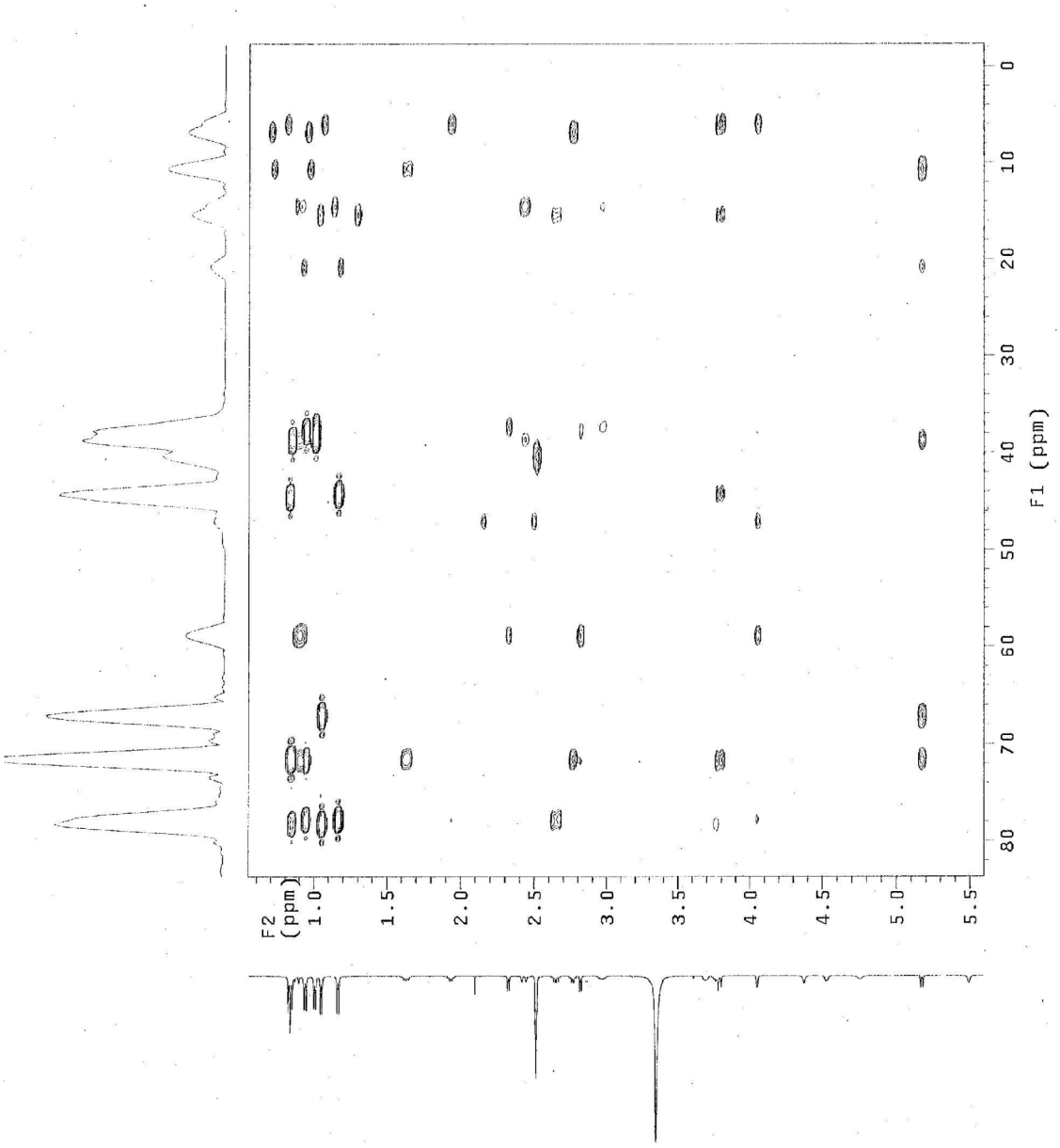

Figure S7. Expanded gHMBC spectrum of erythronolide H (**2**)

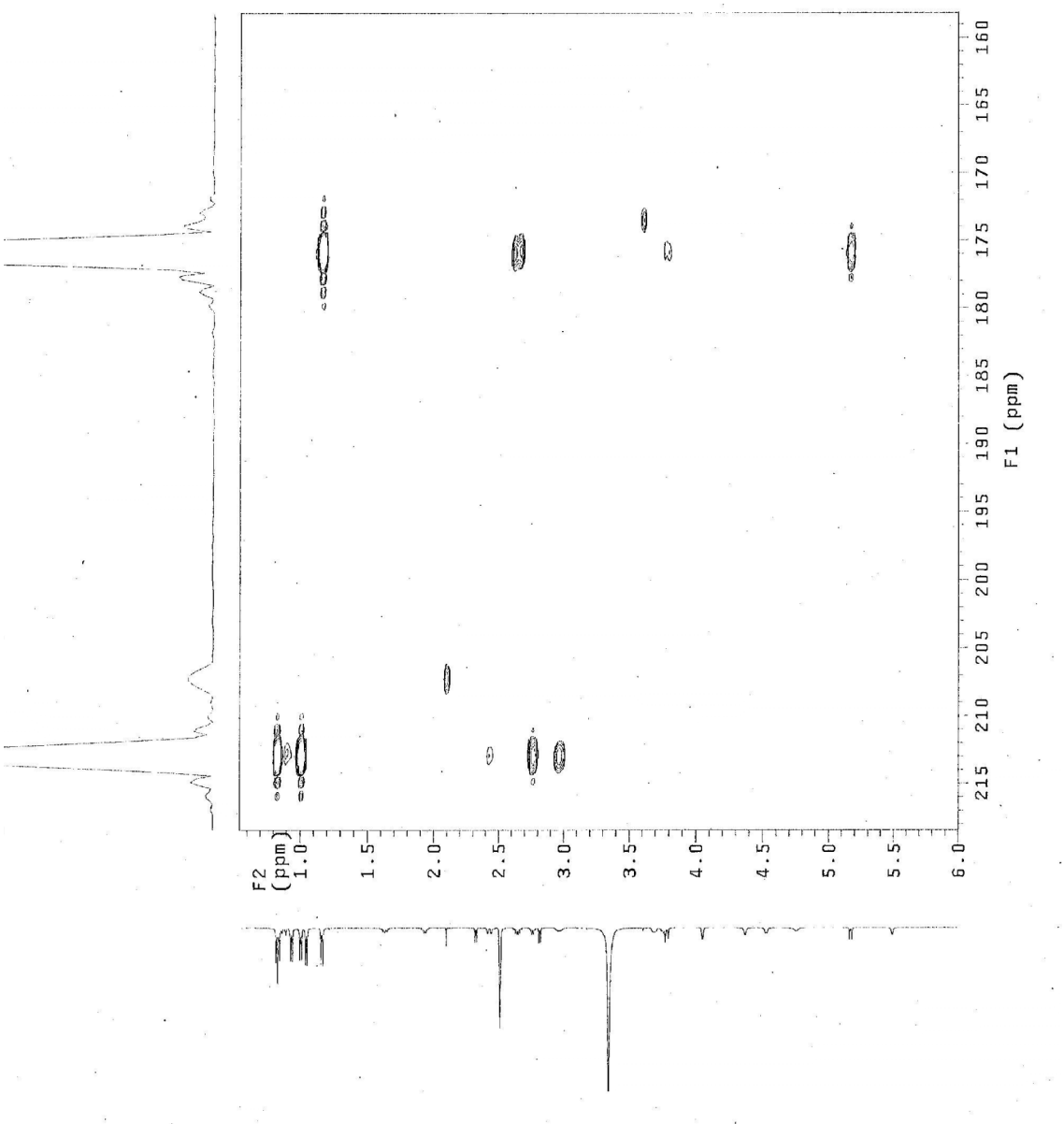

Figure S8. Expanded gHMBC spectrum of erythronolide H (**2**)

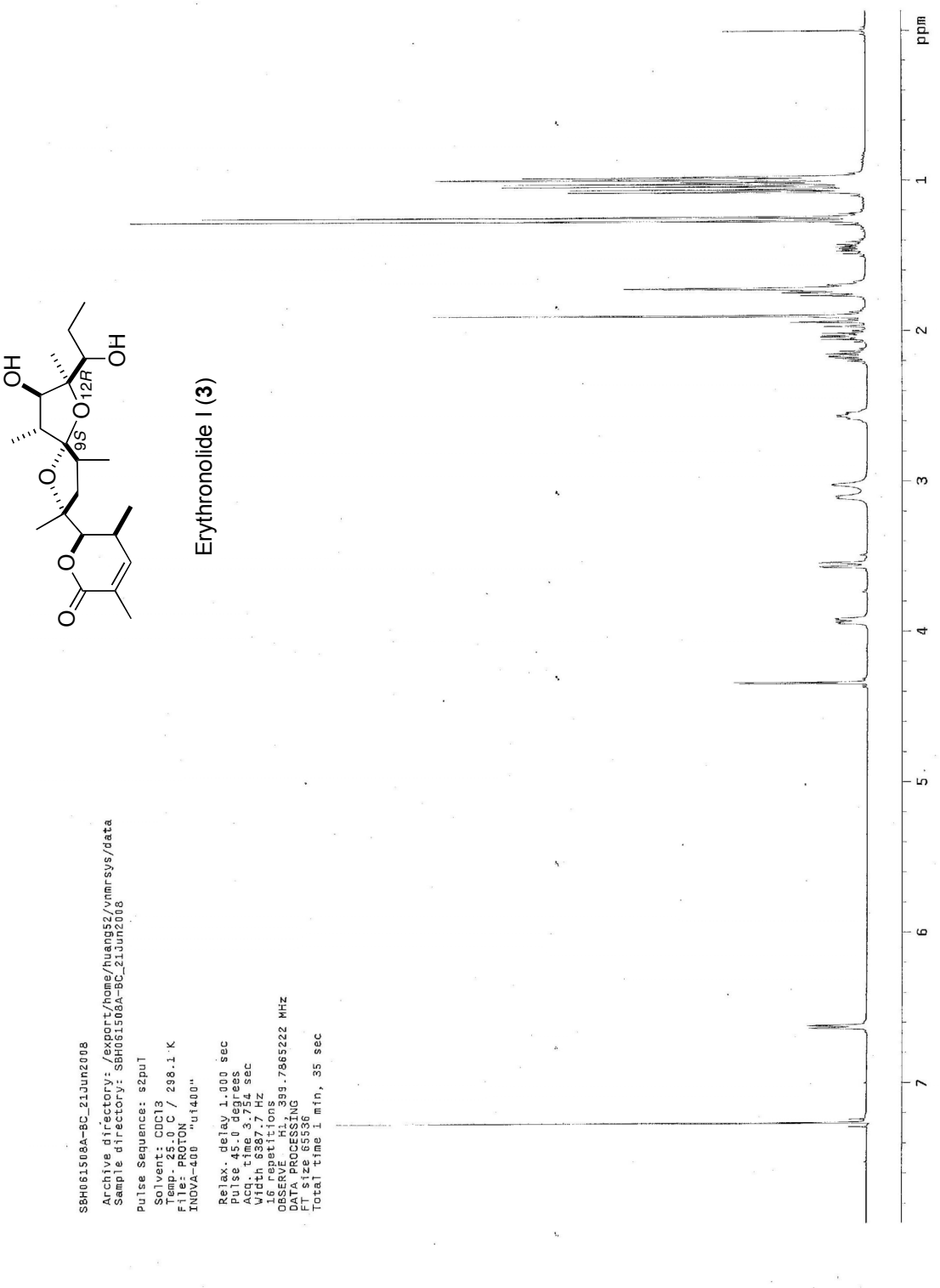

Figure S9. ^{1}H NMR spectrum of erythronolide I (**3**)

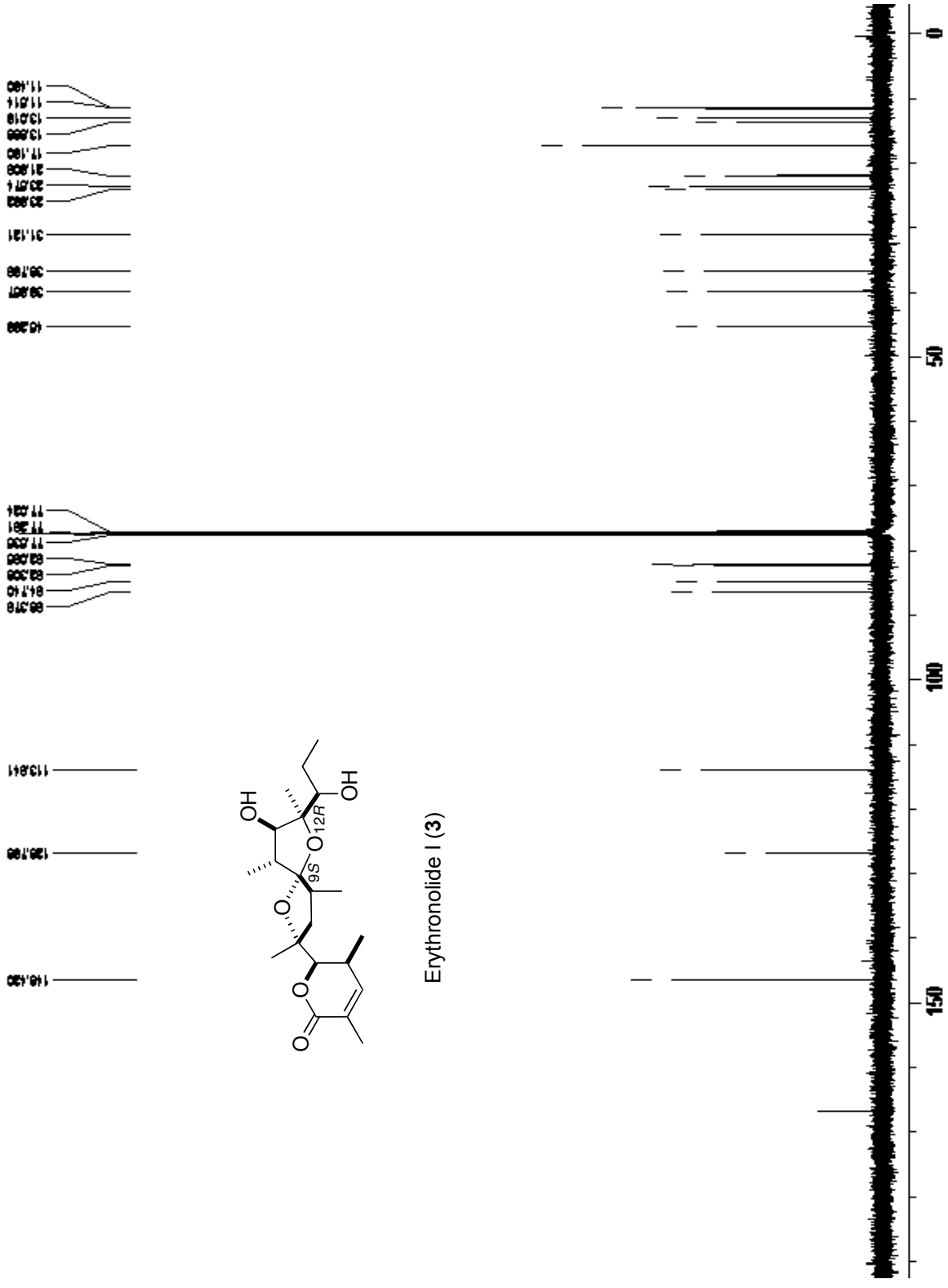

Figure S10. ^{13}C NMR spectrum of erythronolide I (**3**)

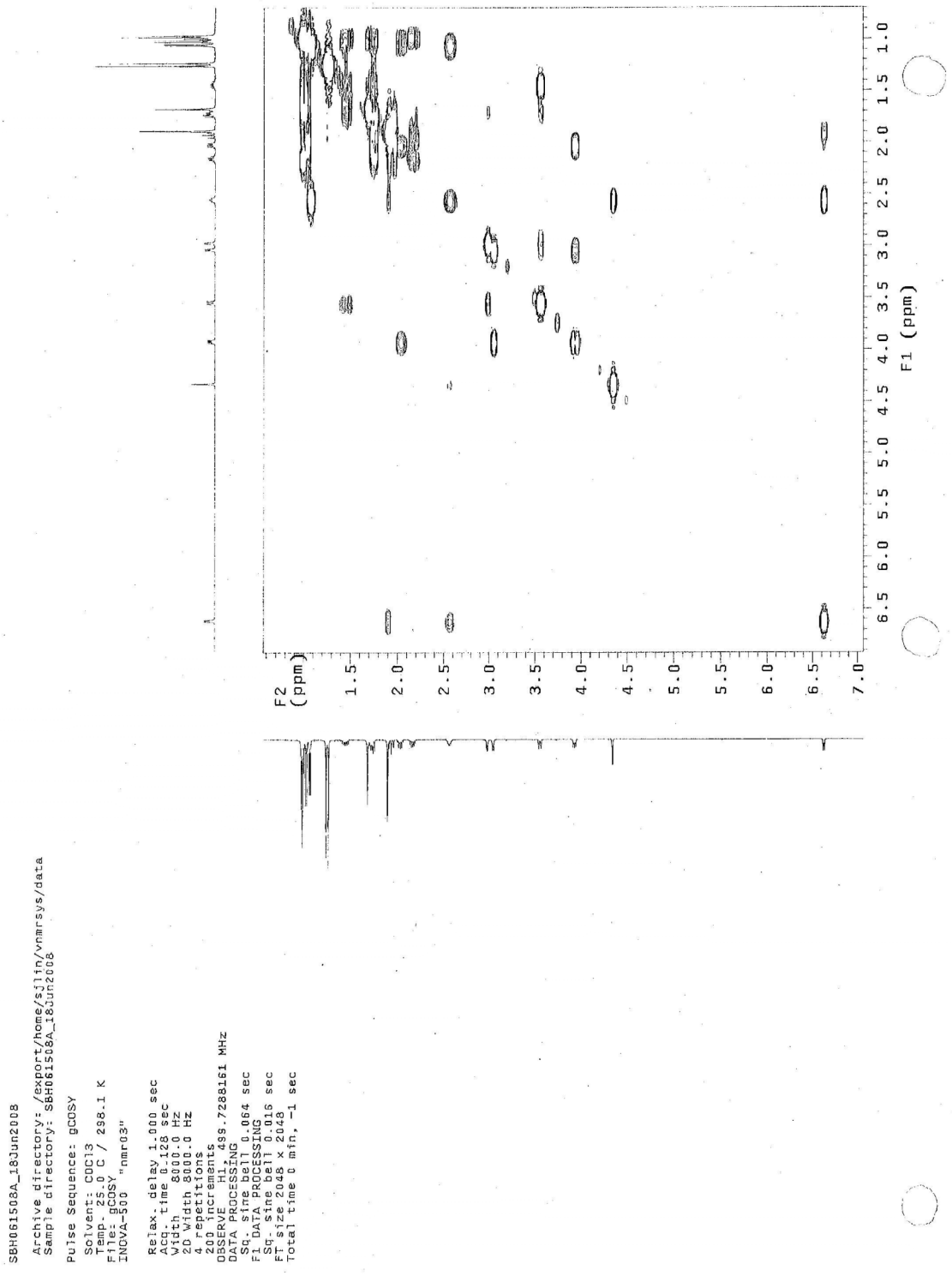

Figure S11. ^{1}H-^{1}H gCOSY NMR spectrum of erythronolide I (**3**)

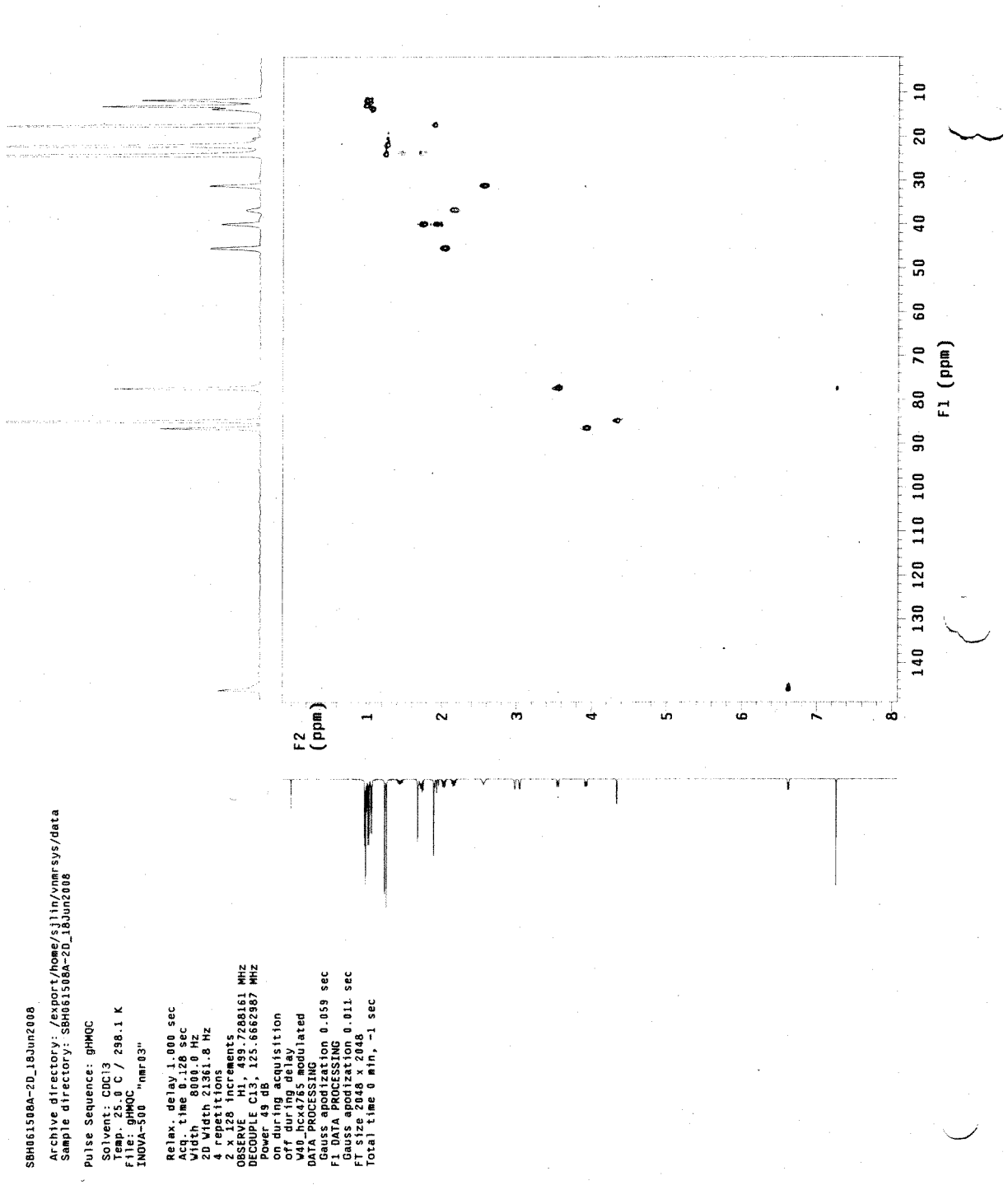

Figure S12. gHMQC NMR spectrum of erythronolide I (**3**)

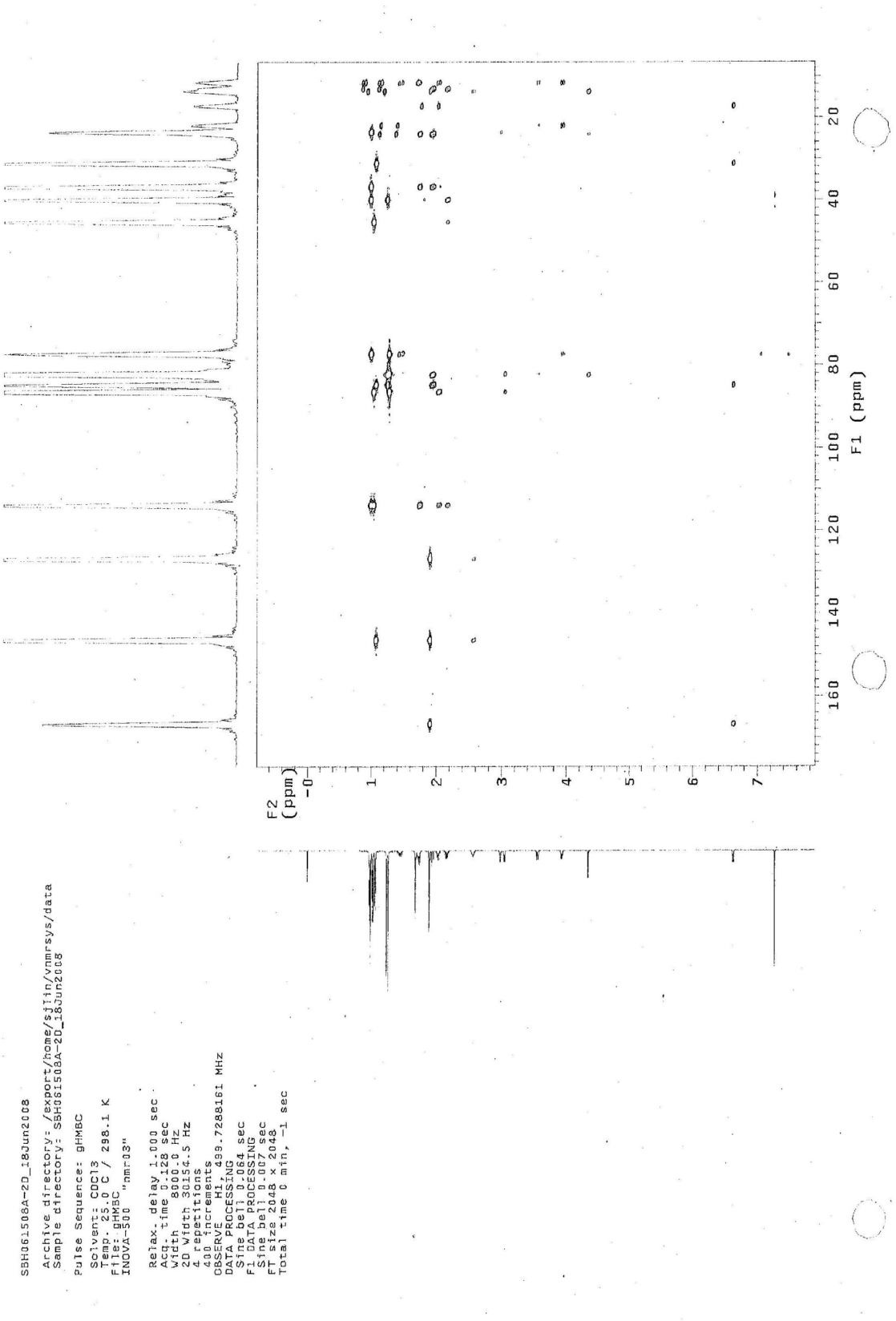

Figure S13. gHMBC NMR spectrum of erythronolide I (**3**)

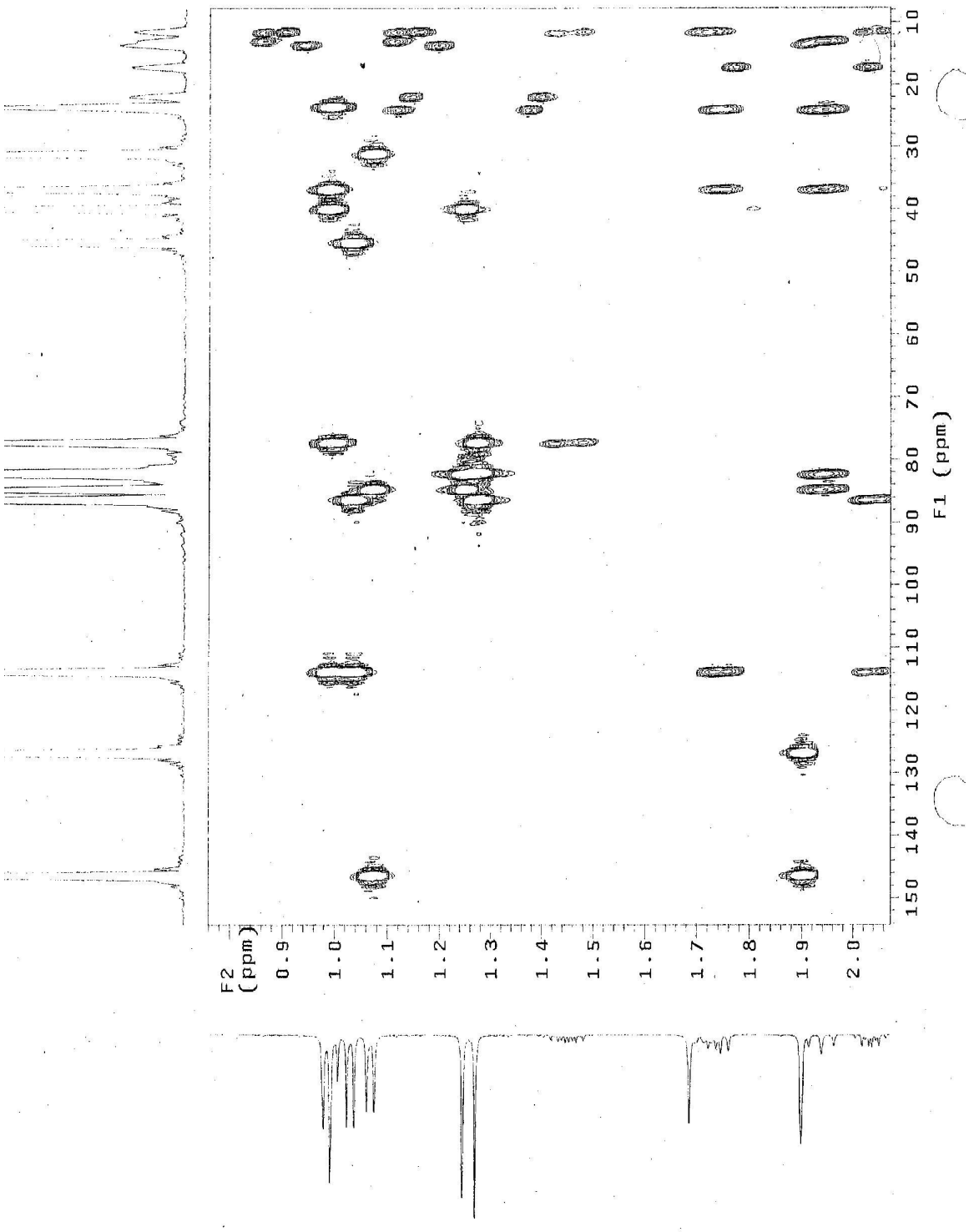

Figure S14. Expanded gHMBC spectrum of erythronolide I (**3**)

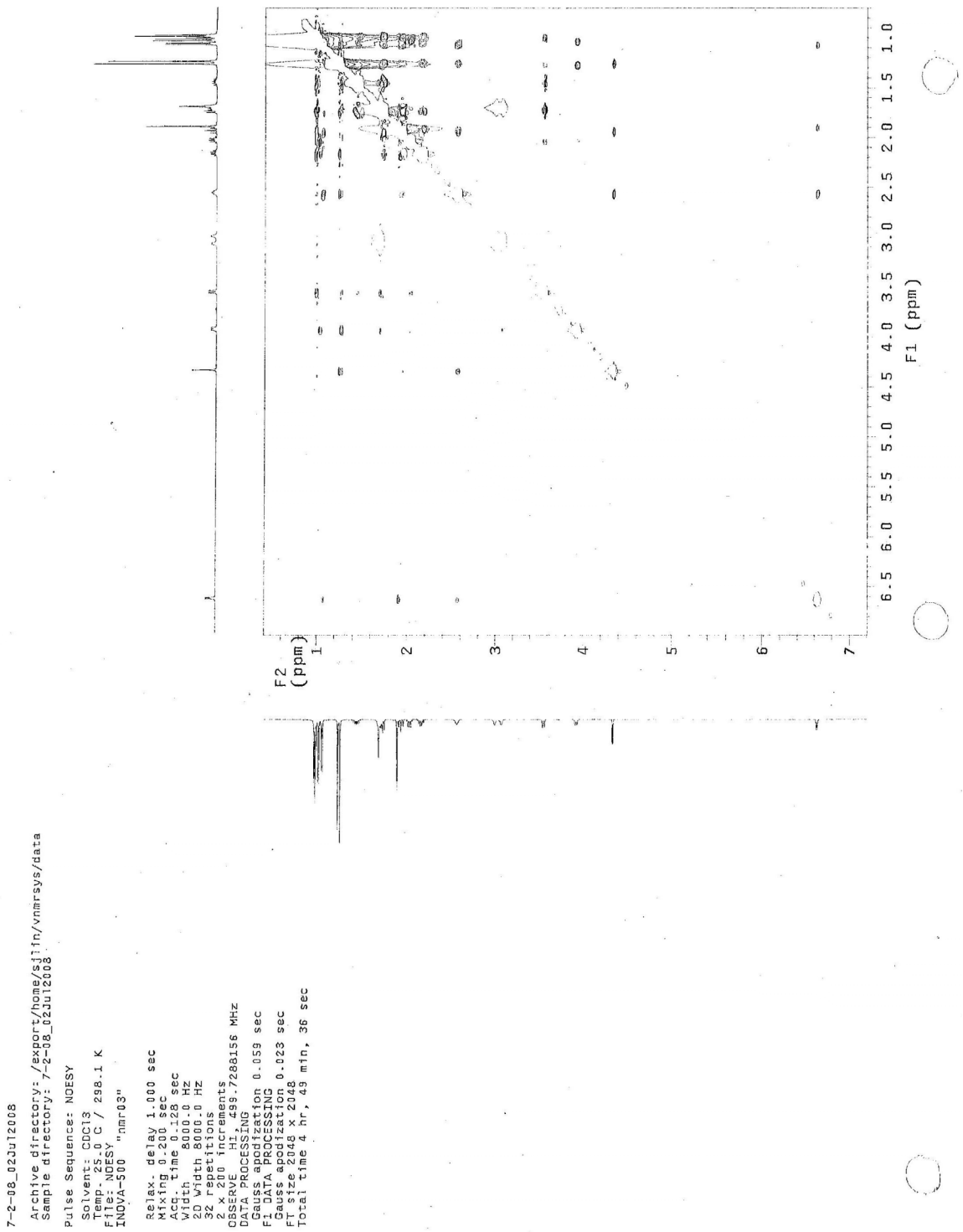

Figure S15. ROESY NMR spectrum of erythronolide I (**3**)