Tirandamycins from *Streptomyces* sp. 17944 Inhibiting the Parasite *Brugia malayi* Asparagine tRNA Synthetase

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Supporting Information

General

Optical rotations were measured in ethanol with a Perkin-Elmer 241 instrument at the sodium D line (589 nm). ^{1}H and ^{13}C NMR spectra were recorded at 25°C with a Varian Unity Inova 500 instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei. High-resolution mass spectra were acquired with a Bruker Daltonics Ultra High Resolution TOF – Maxis spectrometer. Semi-preparative HPLC was performed on a Varian HPLC system with an Altima C18 column (5 μ, 10.0 x 250 mm, Alltech, Deerfield, IL). Column chromatography was performed using either silica gel (230-400 mesh, Natland International Corporation, Research Triangle Park, NC) or Sephadex LH-20 (Pharmacia, Kalamazoo, MI). All chemical regents were purchased from Sigma-Aldrich and used without further purification.

Fermentation and Isolation

The *Streptomyces* sp. 17944 was preserved as a spore solution at -80°C. A two-stage fermentation was used, each of which uses ISP2 as a medium. Thus, a 250-mL baffled Erlenmeyer flask, containing 50 mL of ISP2, was inoculated with 10 μL of the *S.* sp.17944 spore solution and incubated with shaking (250 rpm) at 28.0°C for 2 days to prepare the seed culture. Eighteen 2-L baffled Erlenmeyer flasks, each containing 400 mL of ISP2, were then inoculated with 20 mL of the seed culture and grown for 7 days under identical conditions.

The production cultures were centrifuged at 5,000 rpm and 4°C for 30 min to remove mycelia, and the broth was extracted with 3% Amberlite XAD16 resin for 4 hr at room temperature with agitation.[1] Resin was harvested by centrifugation and eluted three times with methanol. The combined methanol elution was then concentrated under reduced pressure to afford the crude extract as a dark oil.

The crude extract (4.5 g) was subjected to silica gel chromatography eluted step wisely with chloroform:methanol (100:0, 50:1, 20:1, 10:1, 5:1 and 0:100, 1-L each) as the mobile phase to afford six fractions, A-F. Fraction E (212 mg) was further chromatographed over Sephadex LH-20 column and eluted with methanol to yield three sub-fractions E1-E3. Subfraction E2 was finally purified by semi-preparative HPLC to afford **1** (2.7 mg), **2** (3.1 mg), **3** (3.0 mg), **4** (17.0 mg), and **5** (7.9 mg).

Tirandamycin E (**1**): yellow oil; $[\alpha]^{20}_{D}$ -3.3° (c 0.48, EtOH); UV (MeOH) λ_{max} at 213 and 351 nm; for complete NMR data see Table 1; HRESIMS *m/z* $[M + Na]^{+}$ 442.18355 (calcd. for $C_{22}H_{29}NO_7Na$, 442.18362).

Tirandamycin F (**2**): yellow oil; $[\alpha]^{20}_{D}$ -48.3° (c 0.34, EtOH); UV (MeOH) λ_{max} at 213 and 351 nm; for complete NMR data see Table 1; HRESIMS *m/z* $[M + Na]^{+}$ 428.16998 (calcd. for $C_{21}H_{27}NO_7Na$, 428.16797).

Tirandamycin G (**3**): yellow oil; $[\alpha]^{20}_{D}$ -3.6° (c 0.33, EtOH); UV (MeOH) λ_{max} at 213 and 351 nm; for complete NMR data see Table 1; HRESIMS *m/z* $[M + Na]^{+}$ 444.19900 (calcd. for $C_{22}H_{31}NO_7Na$, 444.19927).

AsnRS Inhibition Assay

AsnRS inhibition assays were carried out according to our recently published procedure.[2] This non-radioactive assay measures phosphate/pyrophosphate generated by the recombinant AsnRS and capitalizes on the fact that the first step of the two step aminoacylation reaction does not require tRNA to generate the pyrophosphate substrate of bacterial pyrophosphatase. Inorganic phosphate produced by the pyrophosphatase is readily detected by reaction with malachite green and absorption monitoring at 620

nm. The assay exploits the asparagine substrate mimetic L-aspartate β-hydroxamate and has been optimized for HTS of microbial extracts using both recombinant *B. malayi* and human AsnRS.[2] Thus, in the 96 well-plate format, the assay conditions were as follows: 50 mM Hepes, pH 7.4, 25 mM $Mg(C_2H_3O_2)_2$, 0.2 mM ATP, 1 U/mL pyrophosphatase, 20 mM L-Cys, 5 mM DTT, 0.38 mM AsnRS, and the natural product inhibitor (crude extracts, partially purified fractions, or pure TAMs). Enzyme and inhibitors were incubated for 10 min before addition of L-aspartate β-hydroxamate (0.2 mM final concentration). The reaction was then incubated at 37°C for 3 hr before adding 50 μL of the malachite green reagent containing 0.0876% malachite green hydrochloride, 0.05% Triton X-100, and 2.8% ammonium molybdate tetrahydrate in 0.7 N H_2SO_4. The resultant mixture was incubated at room temperature for 10 min, and the absorption at 620 nm was finally measured using the LJL Analyst AD 96-384 plate reader (Molecular Devices, Sunnyvale, CA).

Adult *B. malayi* Worm Killing Assay

Adult *B. malayi* worm killing assays were carried out according to previously published procedures.[3-6] In brief, live adult *B. malayi*, obtained from the NIH NIAID Filariasis Research Repository Resource facilities (Athens, GA), were transferred to 6-well plates (3 worms, male or female per well) containing fresh RPMI 1640 culture medium supplemented with L-glutamine and penicillin/streptomycin. AsnRS inhibitors, dissolved in 100% DMSO, were diluted serially with RPMI media to obtain a final concentration of 100 μM in 0.1% DMSO. The parasites were incubated with inhibitors at 37°C in a 5% CO_2 atmosphere for at least 120 hr. The worms were then transferred to a fresh medium for ~2 hr to examine any reversal in motility under a stereozoom dissecting microscope (NIKON, JAPAN). Control groups are subjected to DMSO alone as negative controls (worms are alive for up to three weeks *in vitro*) or 100 μM albendazole, a well-known antifilarial compound that kills worms within 14 days, as positive controls. The movement and mortality of the worm can be monitored every 24 hr for up to 20 days; worms in control wells are generally fully motile and assume a coiled morphology, whereas dead worms are clearly immobile and assume an elongated morphology (see Figures 2B and 2C).

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays, which allowed us to assess more quantitatively the viability of worms that appeared to be dead or dying as opposed to those just paralyzed (immobile), were carried out according to a literature procedure.[7] Briefly, after observing motility of the parasites, MTT assays were performed with the same worms (drug treated vs. untreated). The worms were washed in PBS, pH 7.2, quickly blotted over a filter disc, and immediately transferred to 96-well plate containing 0.1 mL MTT (0.5 mg/mL in PBS, pH 7.2). The plate was incubated at 37°C for 45 min and each worm was transferred to 0.1 mL of DMSO in another 96-well plate that was left at 37°C for 45 min for solubilization of blue formazan (the reduced product of MTT). The worms were removed, and the plate was gently agitated to disperse the color evenly. The absorbance of the solubilized formazan was measured at 510 nm using a multi-well-plate-reader (Tecan) against the DMSO blank. The absorbance value of each well was compared with the mean value of control wells. Percent inhibition in MTT reduction by the treated parasite over that of untreated control worm was then calculated.

References

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2. Danel, F.; Caspers, P.; Nuoffer, C.; Hartlein, M.; Kron, M. A.; Page, M. G. *Curr. Drug Discov. Technol*. **2010**, *8*, 66-75.
3. Fox, L. M.; Furness, B. W.; Haser, J. K.; Desire, D.; Brissau, J.-M.; Milord, M.-D.; Lafontant, J.; Lammie, P. J.; Beach, M. J. *Am. J. Tropical Med. & Hygeine* **2005**, *73*, 115-121.
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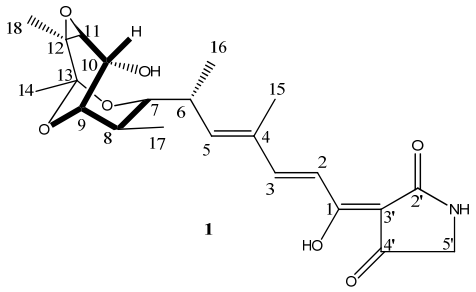
Table S1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for **1**, **4** and **5** in $CDCl_3$



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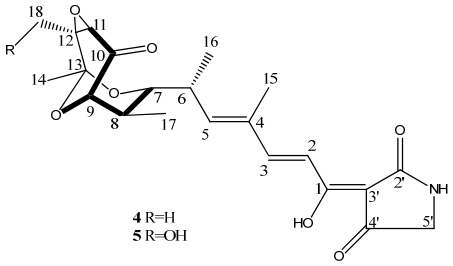
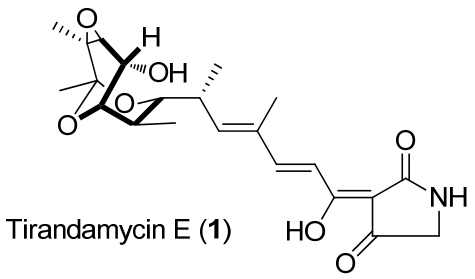


4 R=H
5 R=OH

position	**1**		**4**		**5**	
	δ_C	δ_H *mult*	δ_C	δ_H *mult*	δ_C	δ_H *mult*
1	175.7 s		175.4 s		175.4 s	
2	116.5 d	7.15 d 16.0	116.9 d	7.16 d 15.5	117.1 d	7.17 d 15.5
3	150.6 d	7.60 d 15.5	150.1 d	7.57 d 15.5	149.9 d	7.57 d 15.5
4	134.7 s		135.2 s		135.3 s	
5	145.4 d	6.29 d 9.5	144.0 d	6.22 d 10.5	143.6 d	6.19 d 10.0
6	35.0 d	2.89 m	34.8 d	2.85 m	34.8 d	2.86 m
7	76.2 d	3.92 d 10.5	77.2 d	3.58 d 11.5	77.5 d	3.67 d 11.5
8	36.6 d	2.04 m	35.0 d	1.98 m	34.8 d	1.99 m
9	71.8 d	3.98 m	79.1 d	4.02 d 6.0	79.0 d	4.05 d 6.5
10	67.3 d	4.41 d 7.0	202.8 s		201.7 s	
11	64.2 d	3.19 s	61.5 d	3.28 s	58.4 d	3.70 s
12	56.4 s		57.4 s		57.2 s	
13	96.5 s		97.1 s		96.2 s	
14	22.3 q	1.42 s	22.9 q	1.57 s	23.6 q	1.58 s
15	12.5 q	1.93 s	12.6 q	1.91 s	12.6 q	1.91 s
16	17.3 q	1.14 d 7.0	17.3 q	1.13 d 7.0	17.2 q	1.13 d 7.0
17	13.2 q	0.96 d 7.5	11.7 q	0.72 d 7.0	11.7 q	0.73 d 7.0
18	16.6 q	1.40s	15.9 q	1.47 s	59.6 t	4.00 br s
						3.99 br s
2’	176.7 s		176.8 s		176.7 s	
3’	100.1 s		100.4 s		100.4 s	
4’	192.8 s		193.0 s		192.8 s	
5’	51.8 t	3.82 s	51.9 t	3.83 s	51.9 t	3.83 s

Figure S1. ^{1}H NMR spectrum of tirandamycin E (**1**) in $CDCl_3$



Tirandamycin E (**1**)

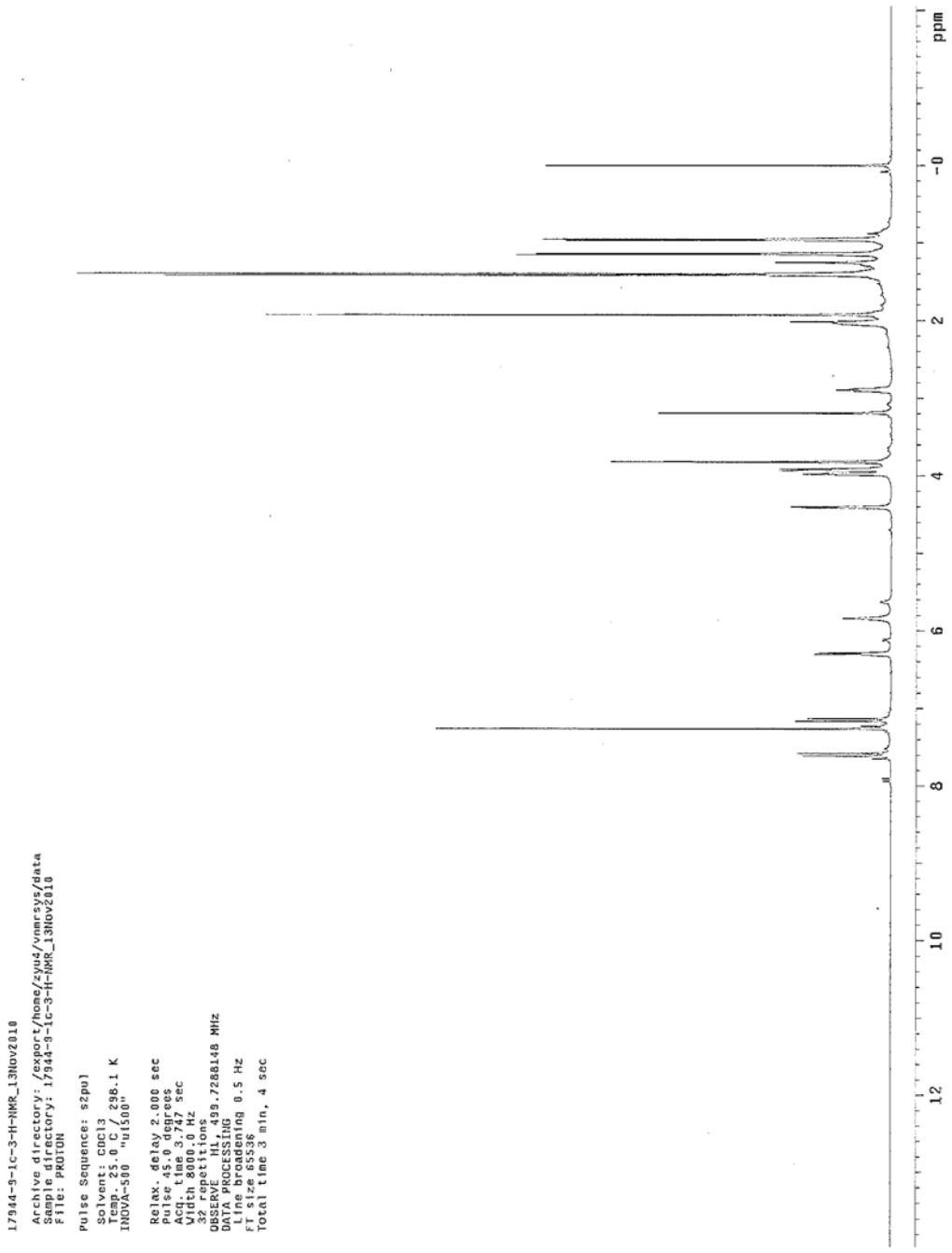
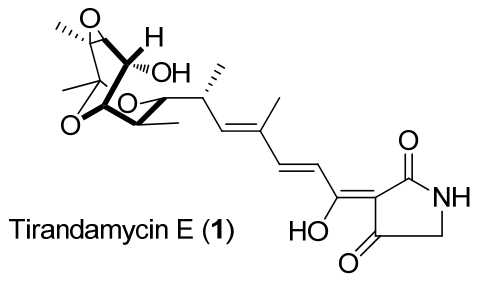


Figure S2. ^{13}C NMR spectrum of tirandamycin E (**1**) in $CDCl_3$



Tirandamycin E (**1**)

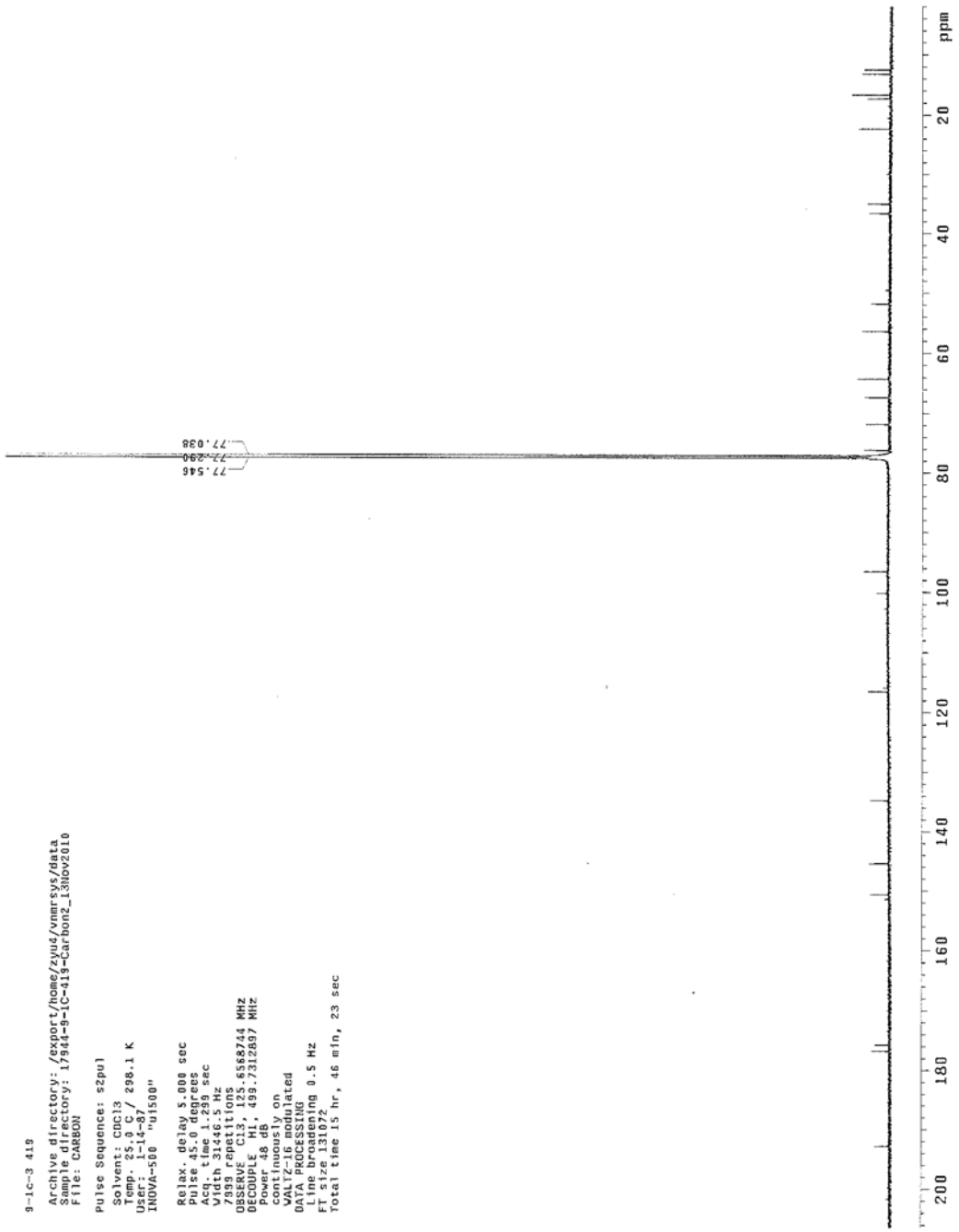
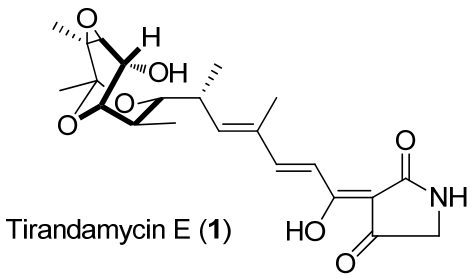


Figure S3. gHSQC spectrum of tirandamycin E (**1**) in $CDCl_3$



Tirandamycin E (**1**)

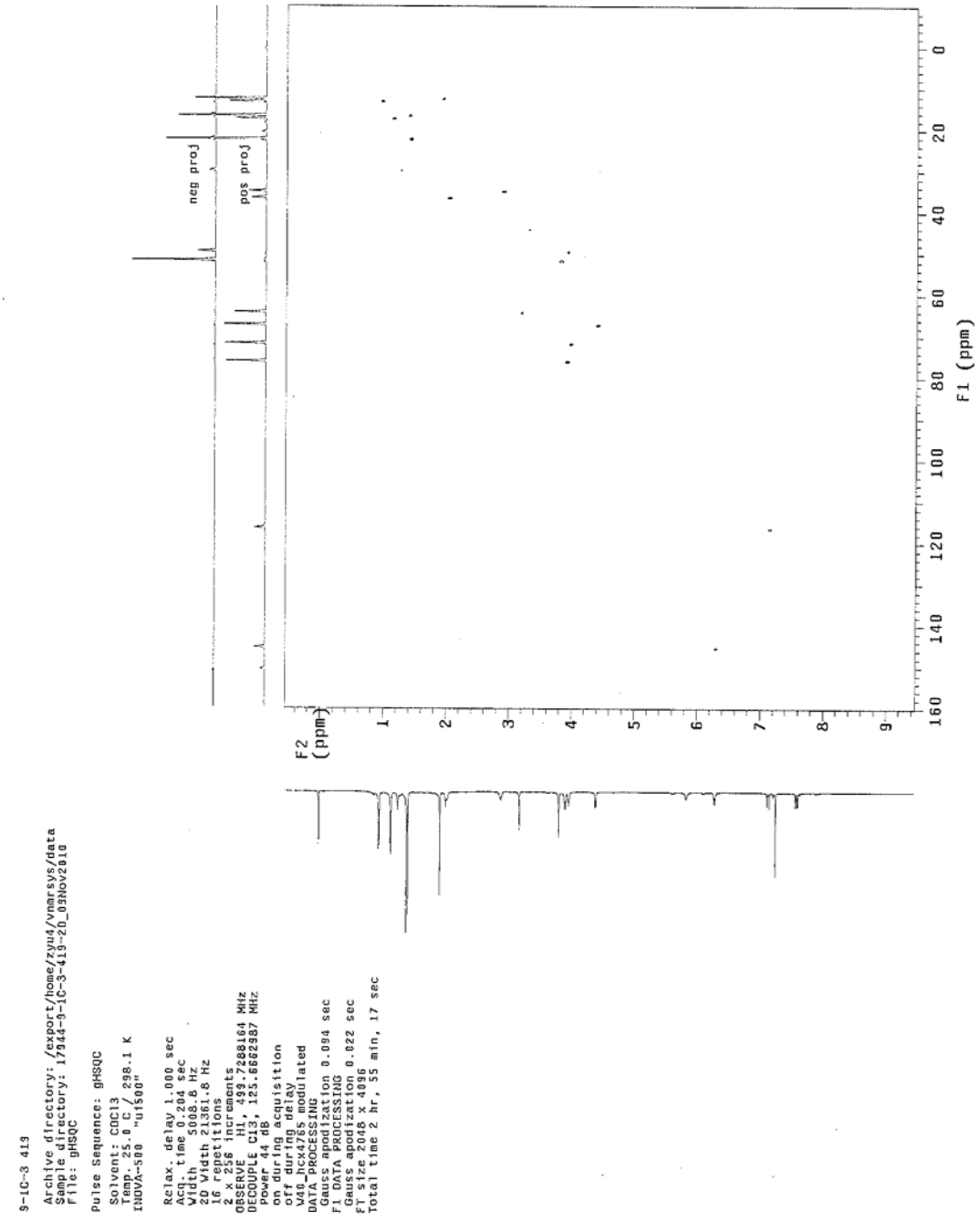


Figure S4. gHMBC spectrum of tirandamycin E (**1**) in $CDCl_3$

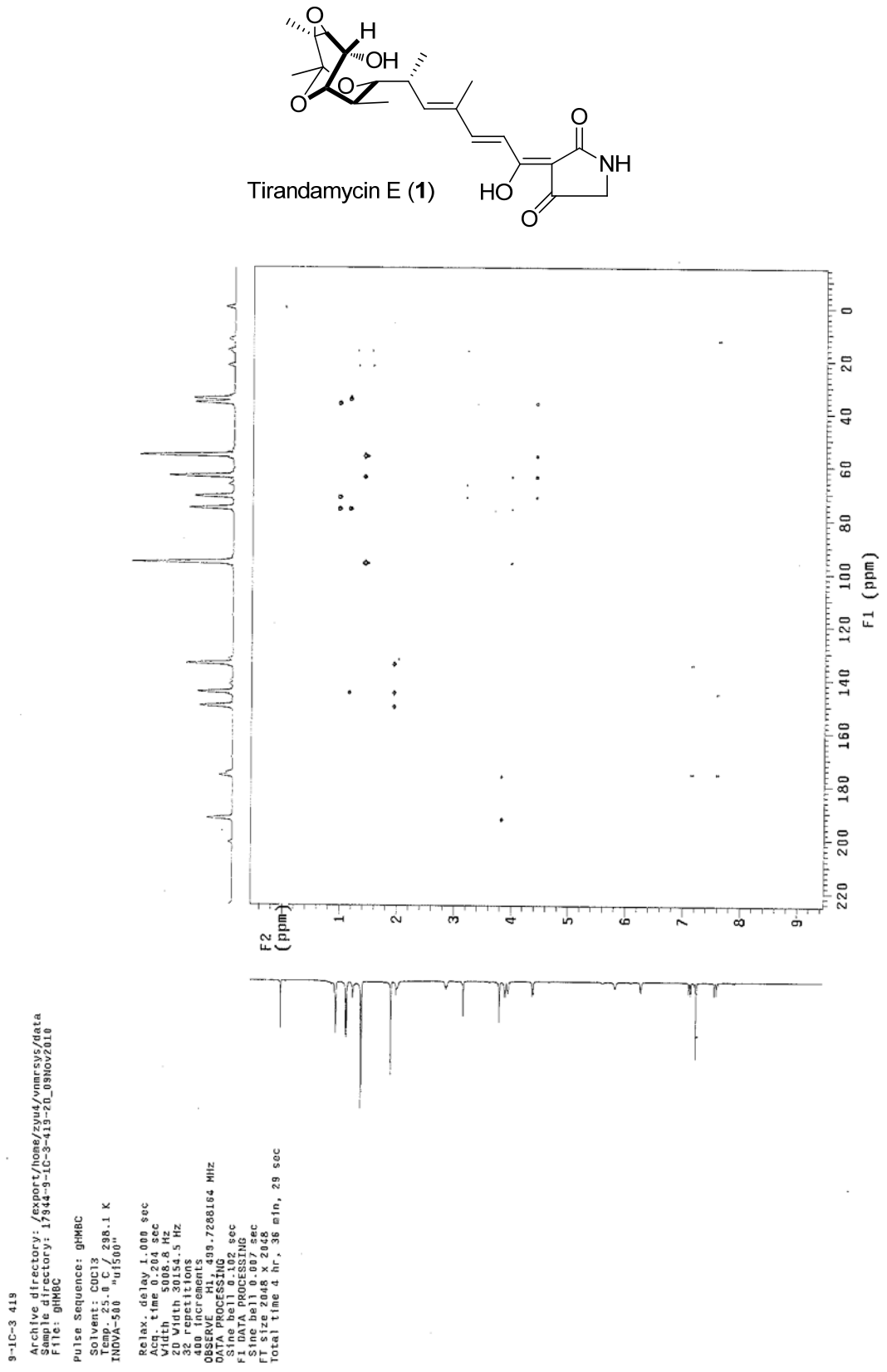


Figure S5. ^{1}H-^{1}H COSY spectrum of tirandamycin E (**1**) in $CDCl_3$

Figure S6. NOESY spectrum of tirandamycin E (**1**) in $CDCl_3$

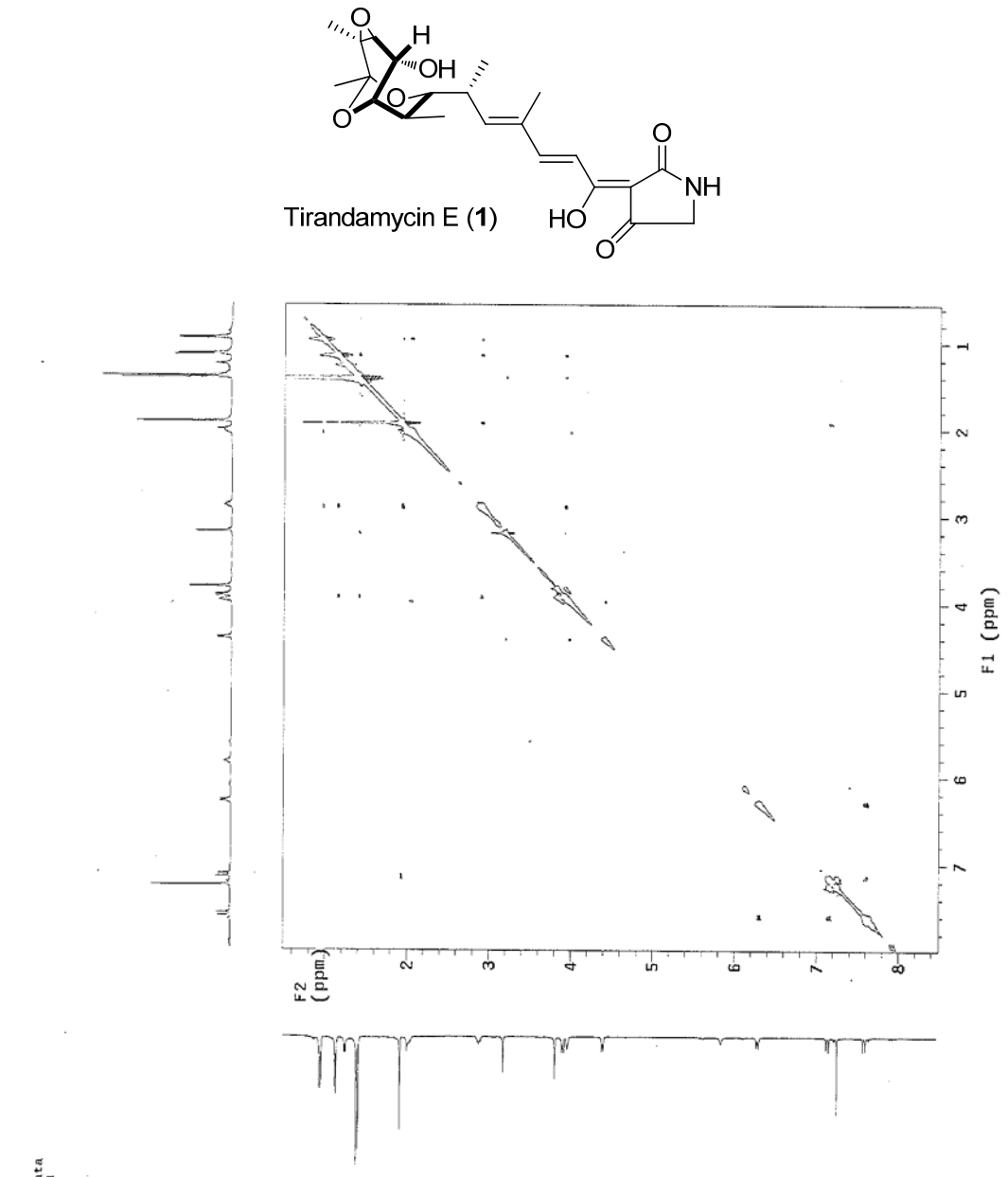
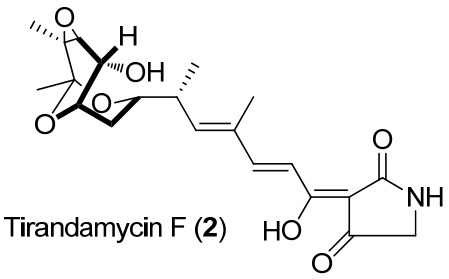


Figure S7. ^{1}H NMR spectrum of tirandamycin F (**2**) in $CDCl_3$



Tirandamycin F (**2**)

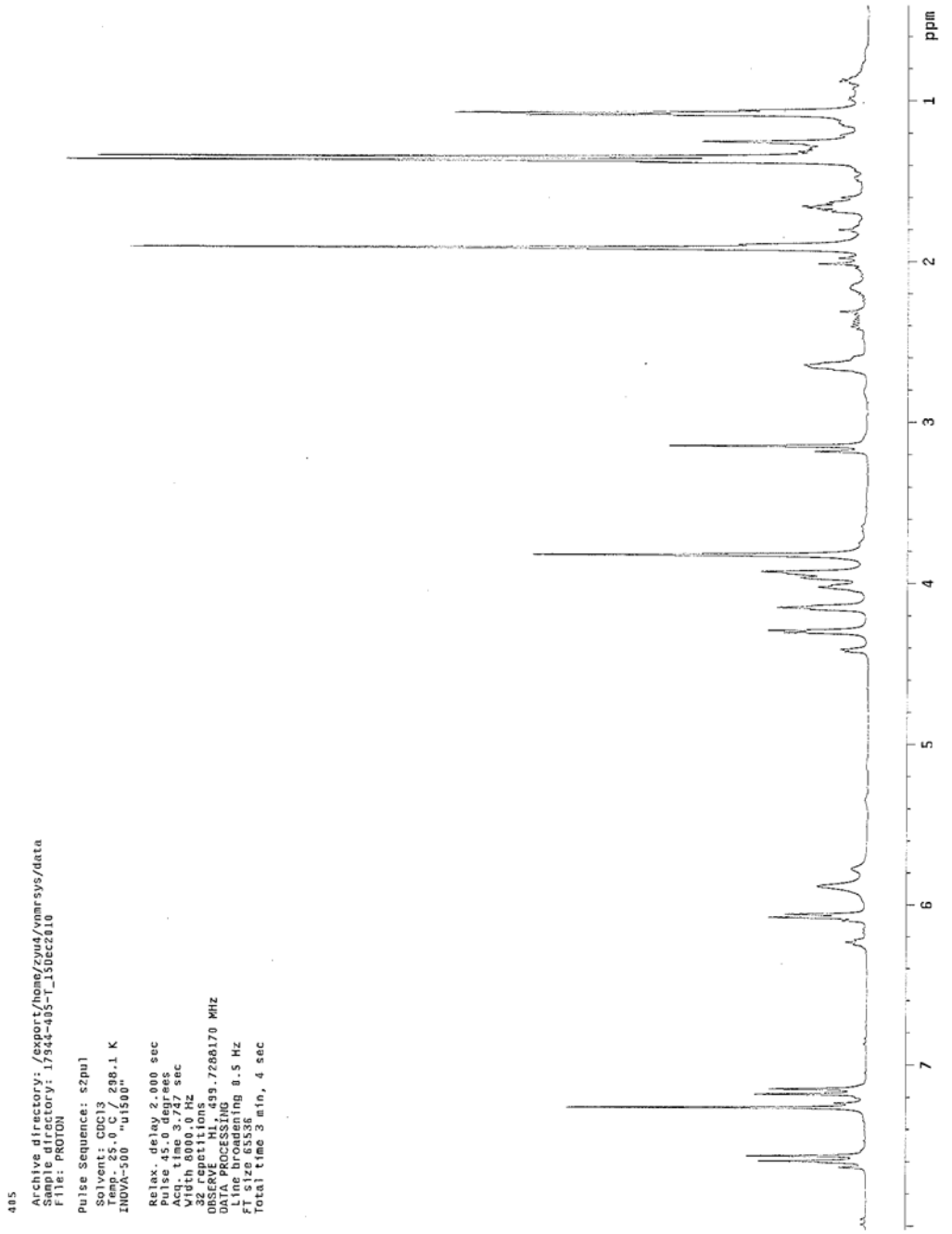
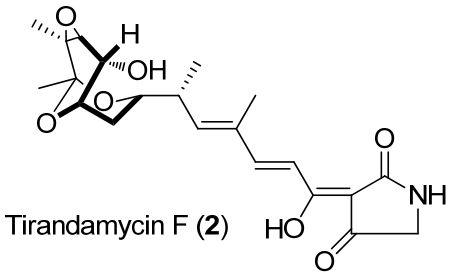


Figure S8. ^{13}C NMR spectrum of tirandamycin F (**2**) in $CDCl_3$



Tirandamycin F (**2**)

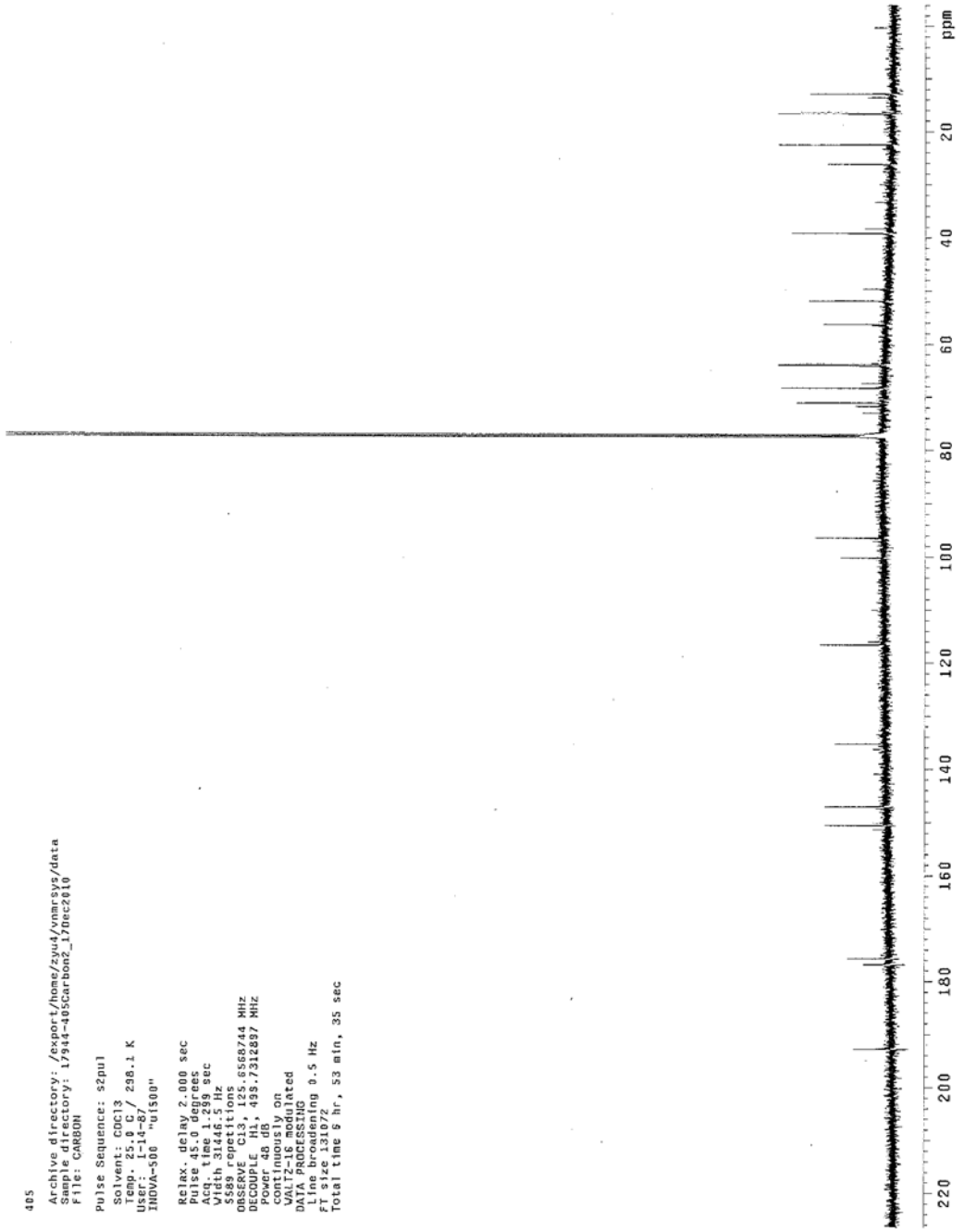


Figure S9. gHSQC spectrum of tirandamycin F (**2**) in $CDCl_3$

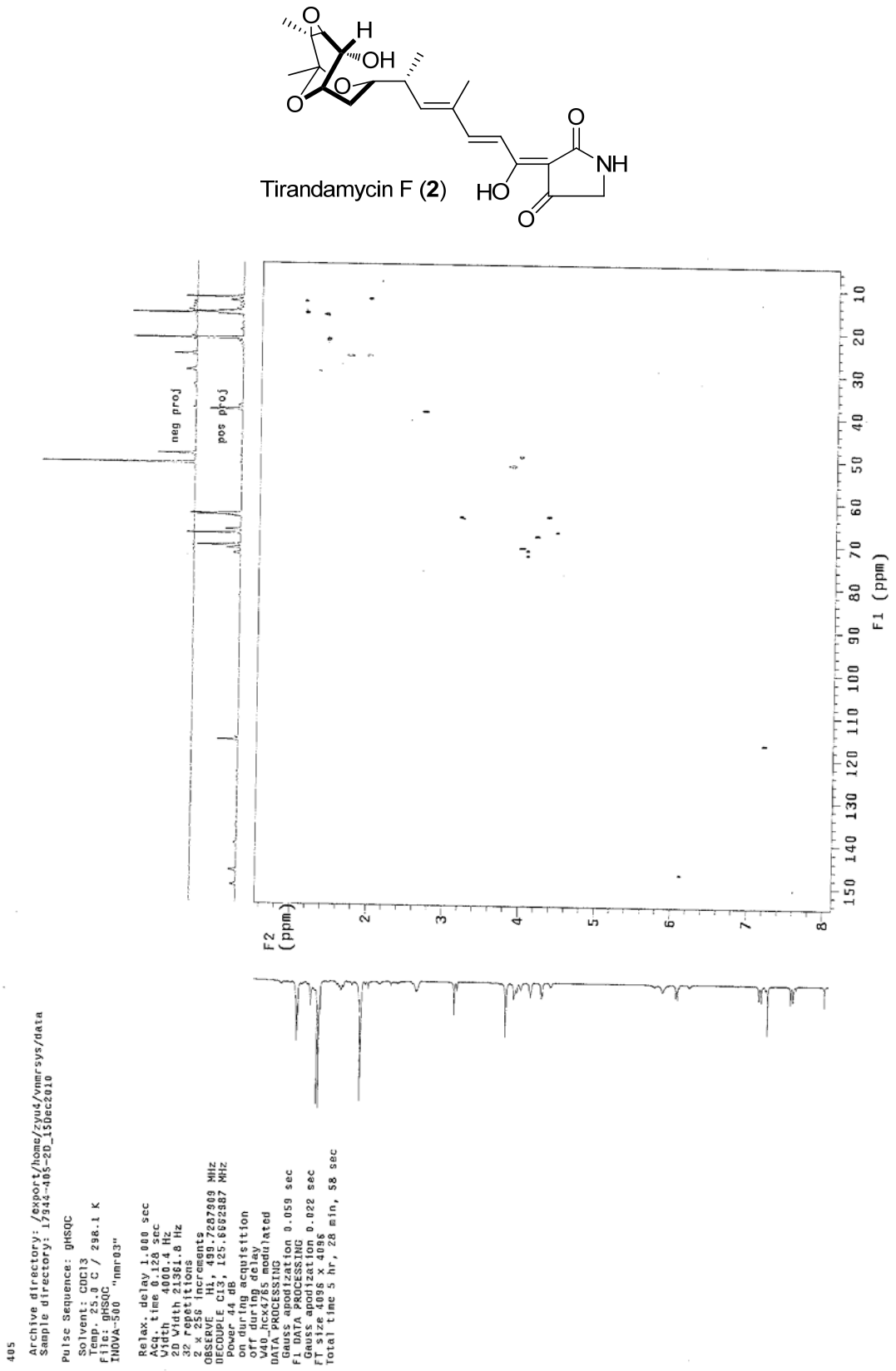
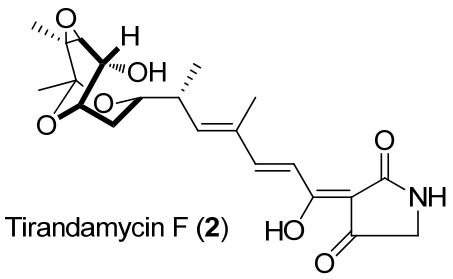


Figure S10. gHMBC spectrum of tirandamycin F (2) in $CDCl_3$



Tirandamycin F (**2**)

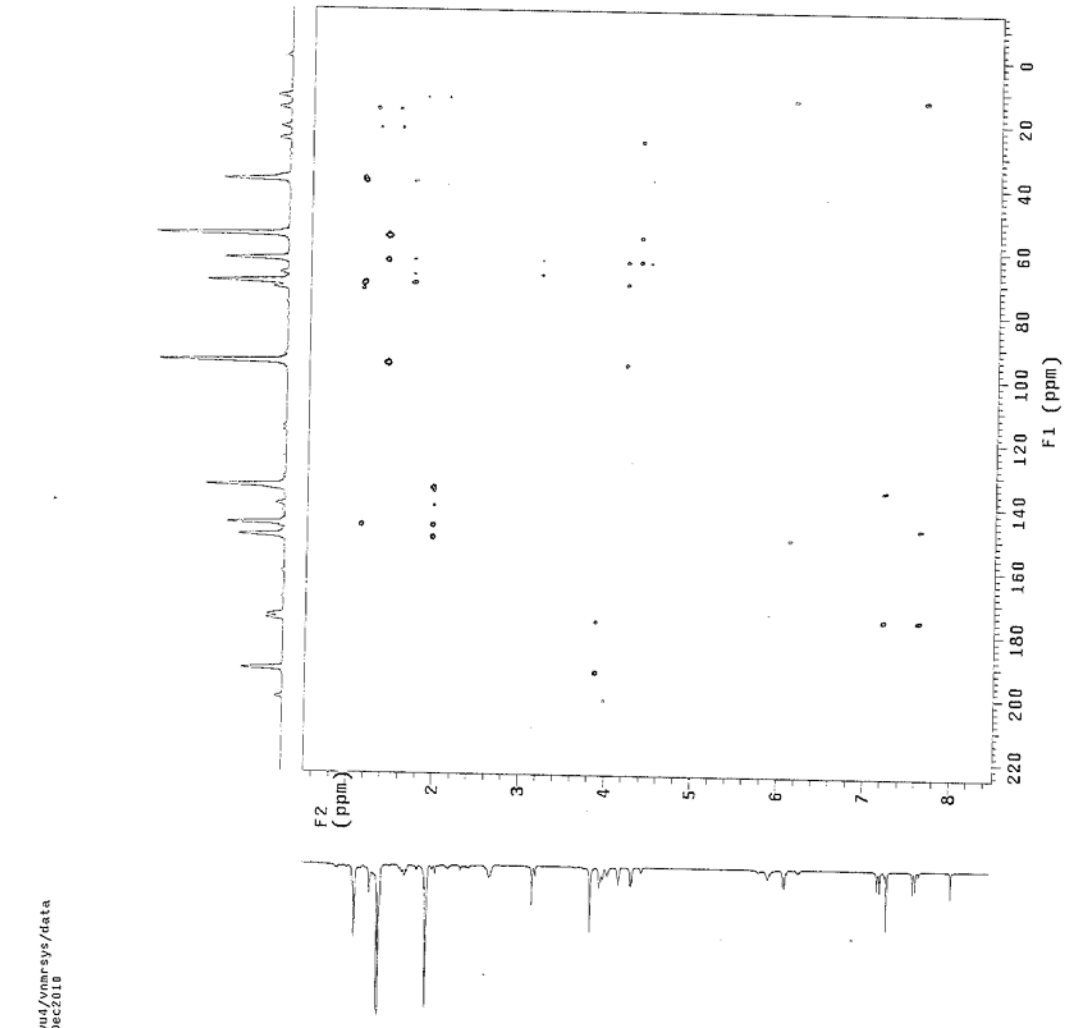
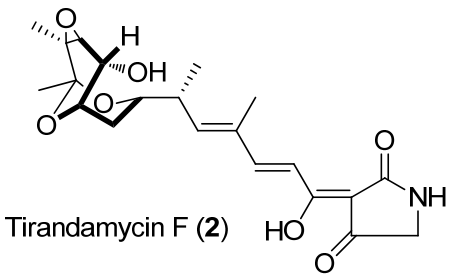
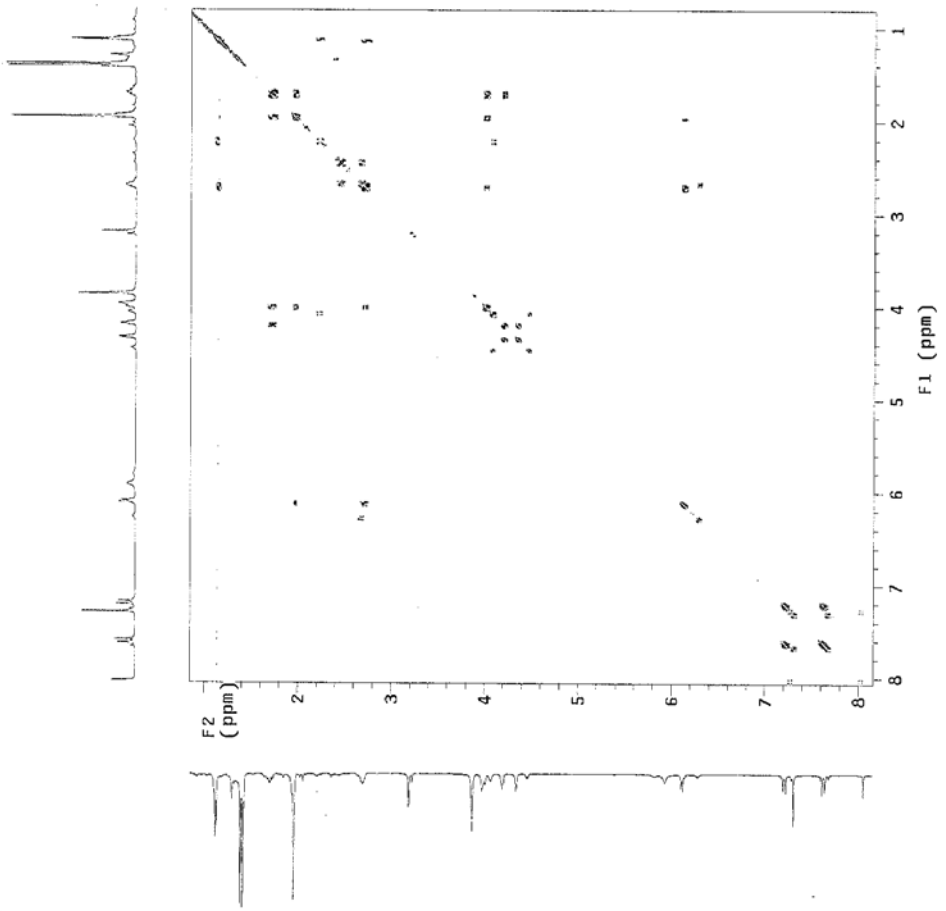


Figure S11. ^{1}H-^{1}H COSY spectrum of tirandamycin F (**2**) in $CDCl_3$



Tirandamycin F (**2**)



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2D Width 4001.6 Hz
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Shifted by -0.064 sec
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Figure S12. NOESY spectrum of tirandamycin F (**2**) in $CDCl_3$

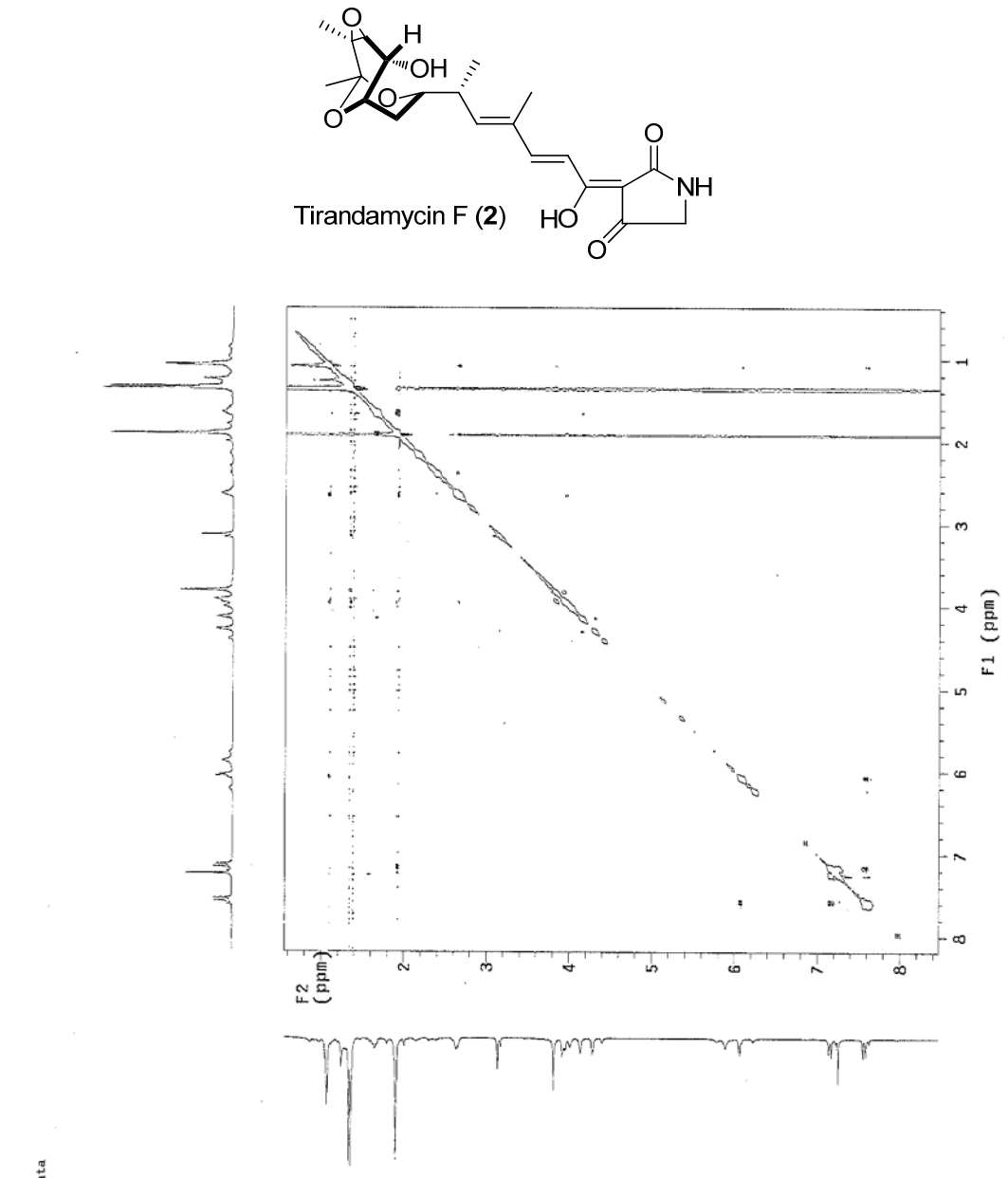
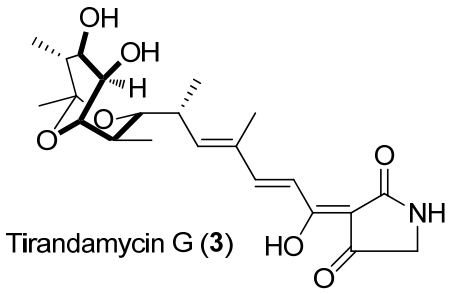


Figure S13. ^{1}H NMR spectrum of tirandamycin G (**3**) in $CDCl_3$



Tirandamycin G (**3**)

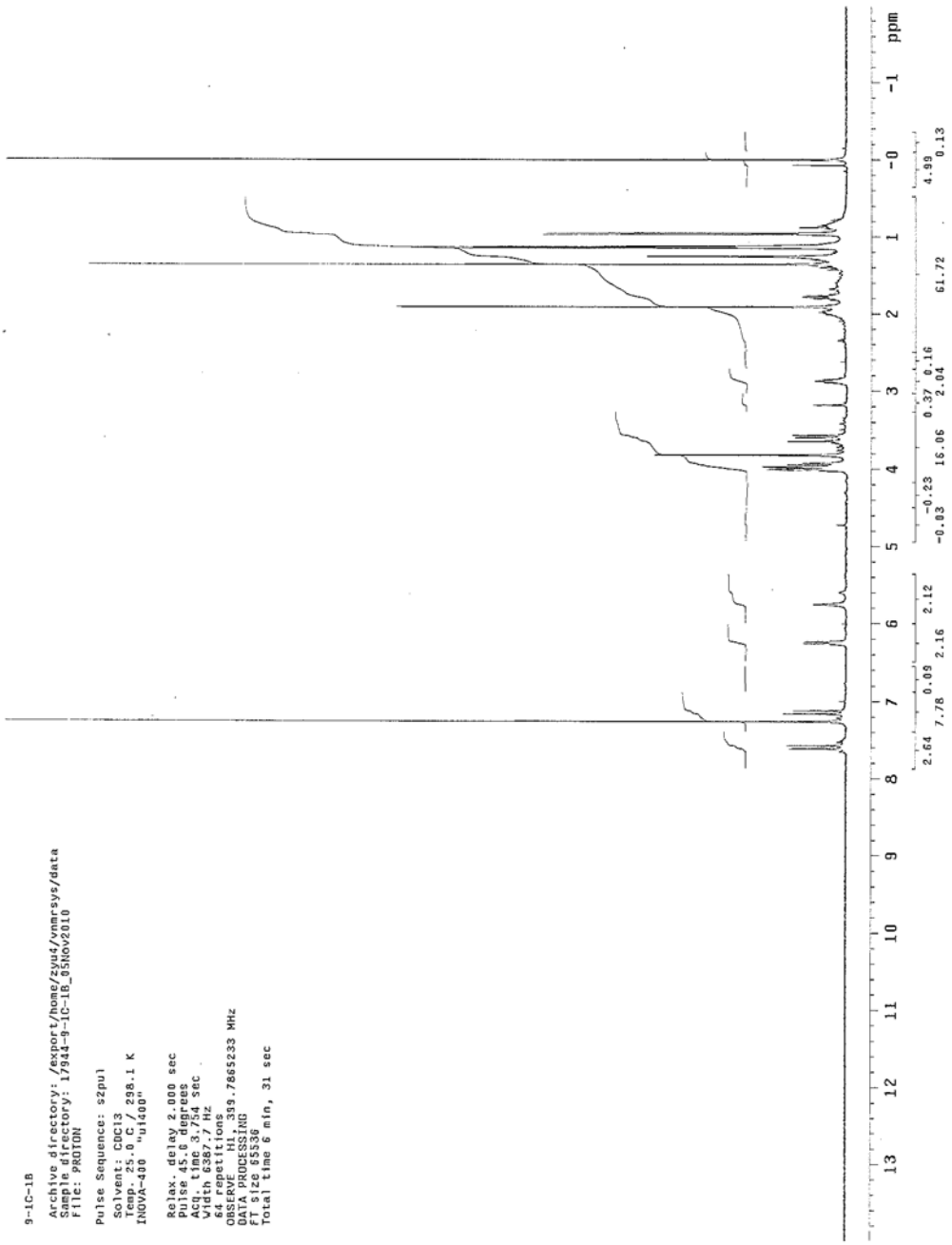
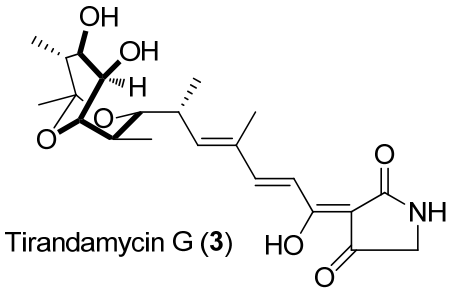


Figure S14. ^{13}C NMR spectrum of tirandamycin G (**3**) in $CDCl_3$



Tirandamycin G (**3**)

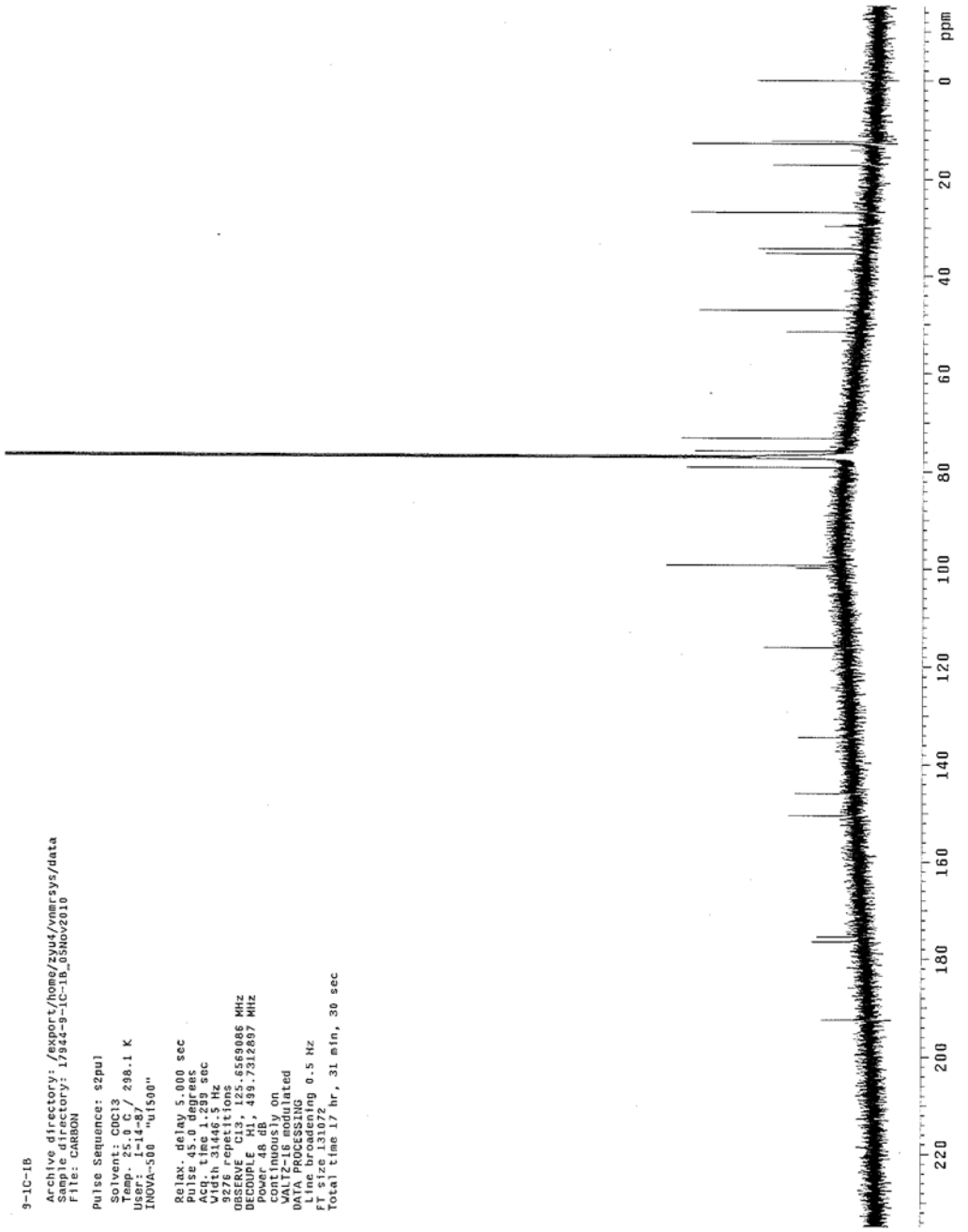


Figure S15. gHSQC spectrum of tirandamycin G (**3**) in $CDCl_3$

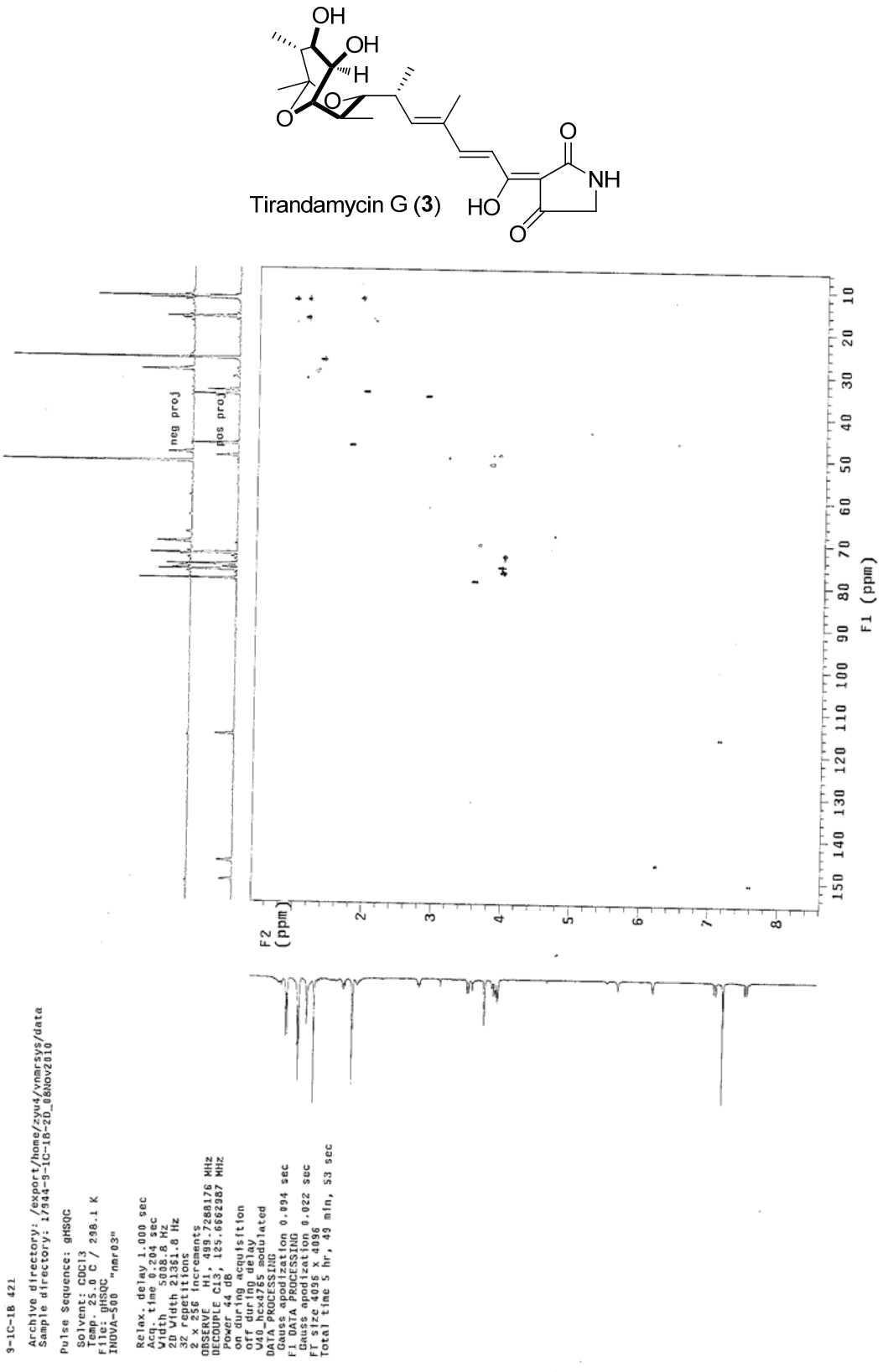
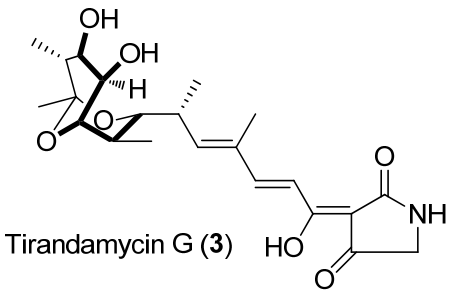


Figure S16. gHMBC spectrum of tirandamycin G (**3**) in $CDCl_3$



Tirandamycin G (**3**)

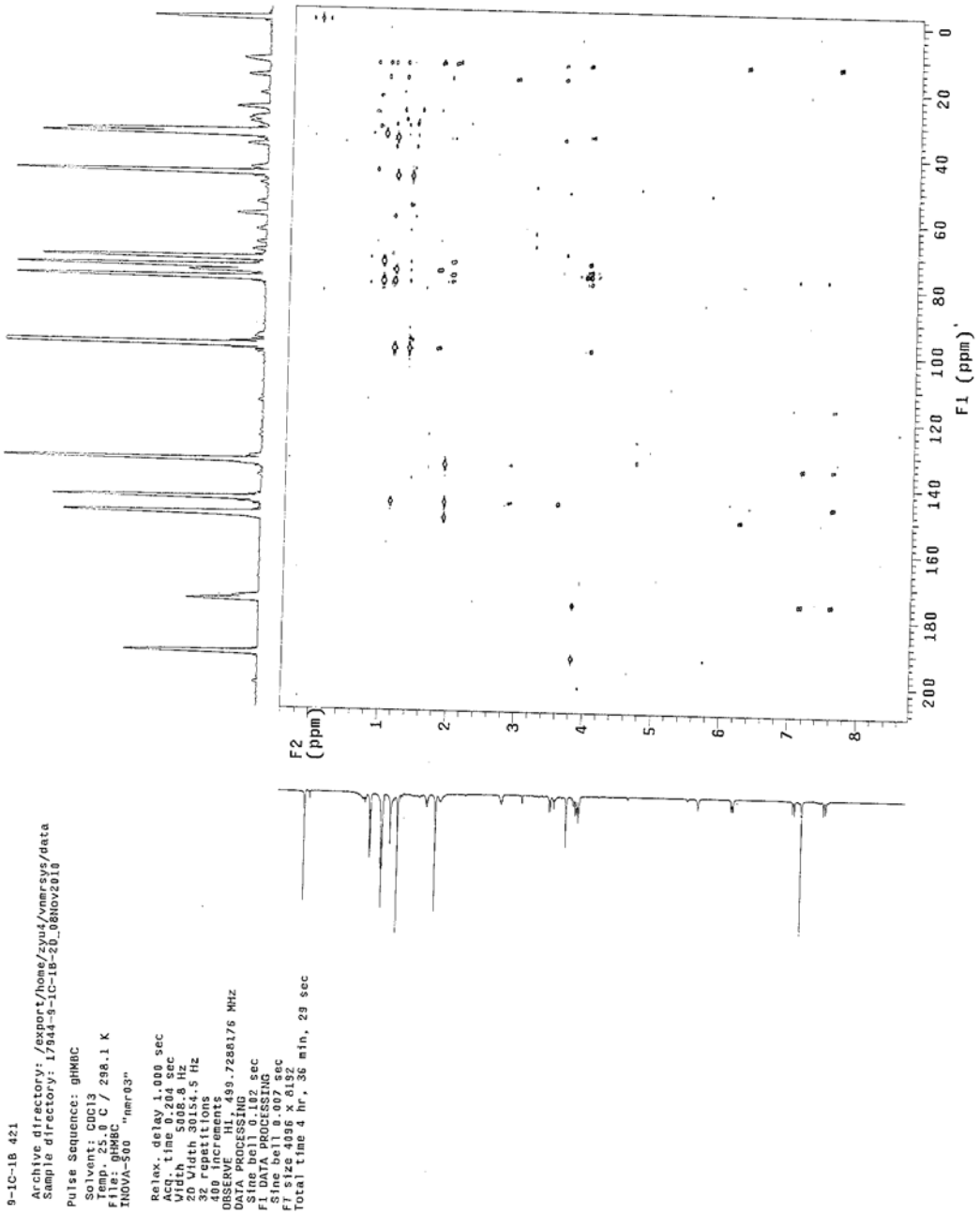
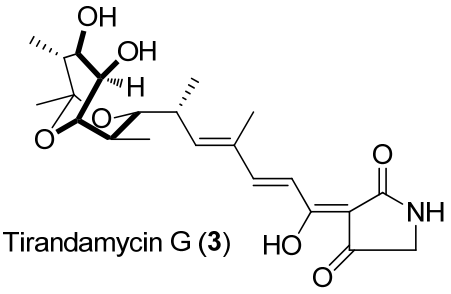
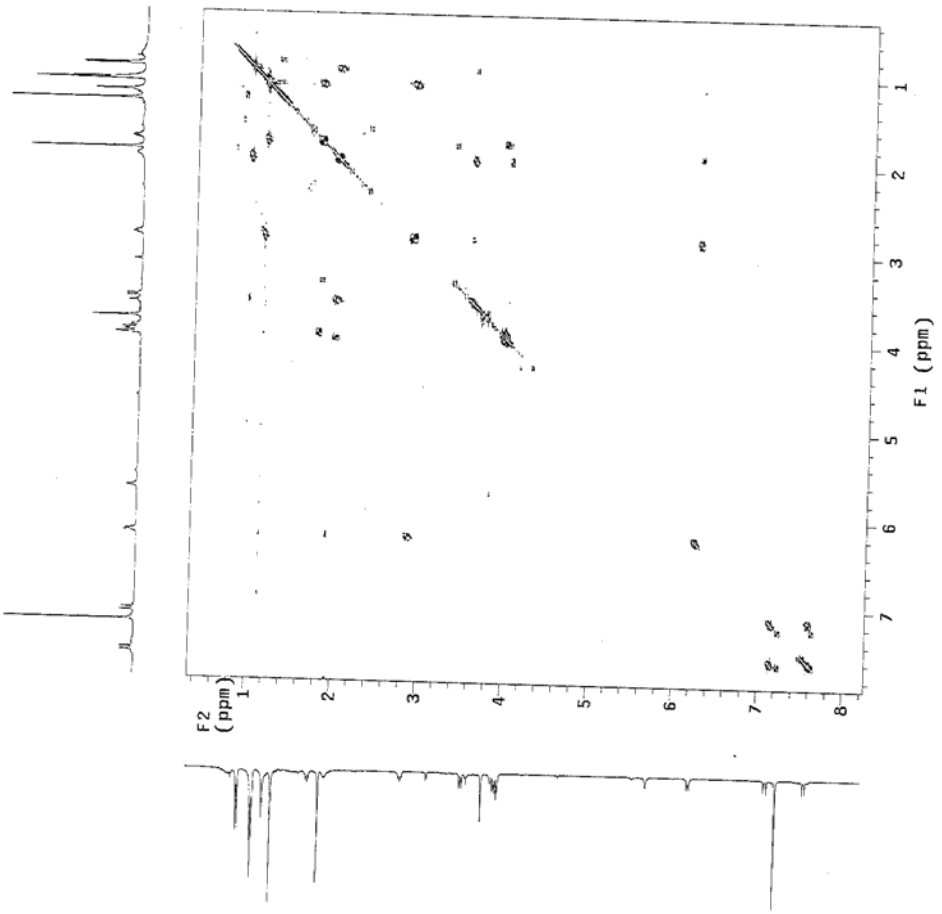


Figure S17. ^{1}H-^{1}H COSY spectrum of tirandamycin G (**3**) in $CDCl_3$



Tirandamycin G (**3**)

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  2D Width 5008.3 Hz
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  256 increments
  2 X 256 increments
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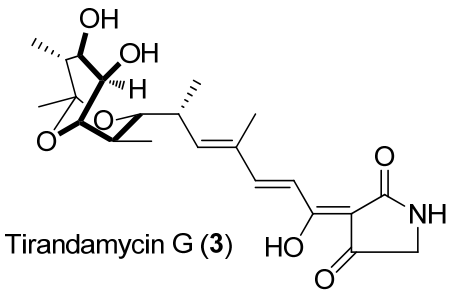
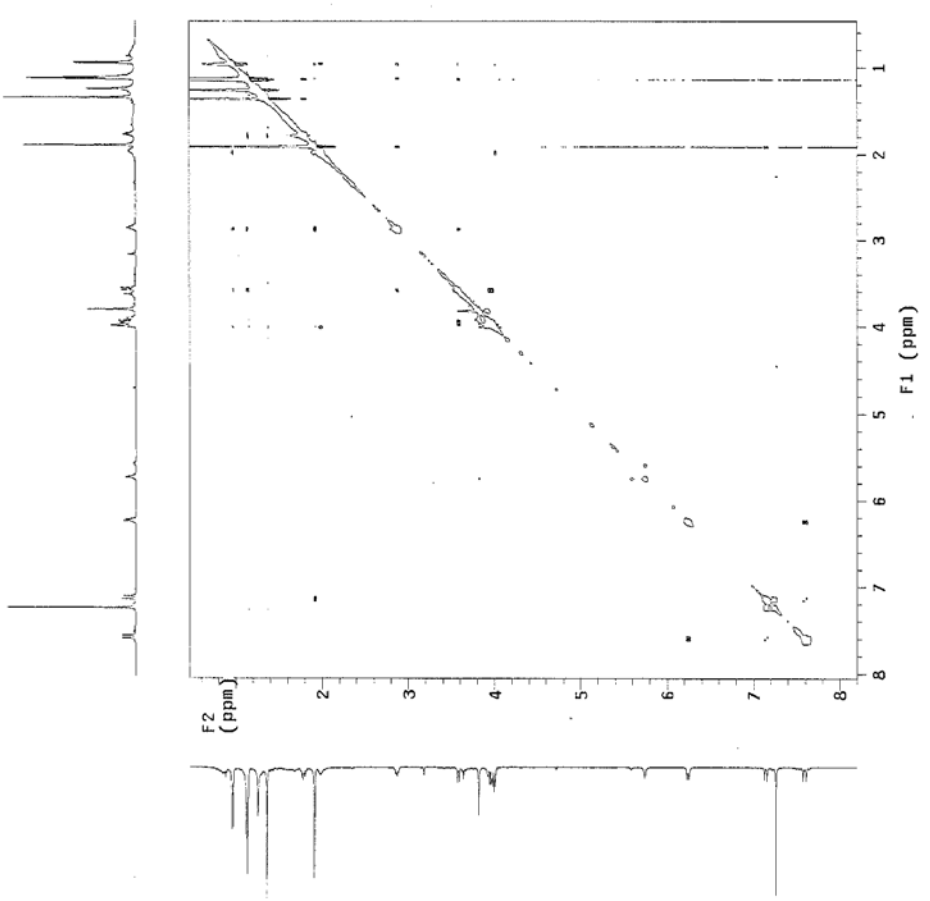
  DATA PROCESSING
  Sq. sine bell 0.153 sec
    Shifted by -0.102 sec
  F1 DATA PROCESSING
  Sq. sine bell 0.077 sec
    Shifted by -0.051 sec
  FT size 4096 x 4096
  Total time 22 min, 2 sec
```

Figure S18. NOESY spectrum of tirandamycin G (**3**) in $CDCl_3$



Tirandamycin G (**3**)




```
9-IC-18 421

  Archive directory: /export/home/zyu4/vnmrsys/data
  Sample directory: 17944-9-IC-18-2D_08Nov2010

Pulse Sequence: NOESY

  Solvent: CDCl3
  Temp. 25.0 C / 298.1 K
File: NOESY
INOVA-500  "nmr03"

  Relax. delay 1.500 sec
  Mixing 0.800 sec
  Acq. time 0.284 sec
  Width    5008.8 Hz
  2D Width 5006.3 Hz
  8 repetitions
  2 x 512 increments
OBSERVE    H1, 499.7288176 MHz
DATA PROCESSING
  Gauss apodization 0.094 sec
F1 DATA PROCESSING
  Gauss apodization 0.094 sec
FT size 4096 x 8192
Total time 5 hr, 52 min, 7 sec
```