Saliniketals A and B: Bicyclic Polyketides from the Marine Actinomycete *Salinispora arenicola*

Philip G. Williams[†,§], Ratnakar N. Asolkar[†], Tamara Kondratyuk[‡], John M. Pezzuto[‡,⊥], Paul R. Jensen[†], William Fenical[†,*]

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California-San Diego, La Jolla, California 92093-0204; Purdue University, Department of Medicinal Chemistry and Molecular Pharmacology, West Lafayette, Indiana 47907

[*] To whom correspondence should be addressed: Tel: (858)-534-2133 Fax: (858) 558-3702 Email: wfenical@ucsd.edu

[†] Scripps Institution of Oceanography

[‡] Purdue University

[§] Current address: University of Hawaii at Manoa, Department of Chemistry, Honolulu, Hawaii 96822

[⊥] Current address: University of Hawaii at Hilo, College of Pharmacy, Hilo, Hawaii 96720

Table of Contents

Table 2. Spectral Data for **2** in CD_3OD

C/H no.	δ_C	δ_H (*J* in Hz)	COSY	HMBC
1	173.3, qC			
2	134.6, qC			
3	135.4, CH	6.38, br d (11.1, 1.1)	4, 18	1, 4, 5, 18
4	127.9, CH	6.71, dd (15.2, 11.2)	3, 5	2, 6
5	145.1, CH	5.94, dd (15.2, 8.3)	4, 6	3, 4, 6, 7, 19
6	42.4, CH	2.39, m (9.4, 8.3, 6.7)	5, 7, 19	5, 7, 19
7	75.7, CH	3.74, dd (9.4, 1.7)	6, 8	5, 6, 9, 20
8	35.9, CH	1.86, m (7.4, 4.9, 1.3)	7, 9, 20	9, 20
9	78.1, CH	3.51, dd (8.4, 4.9)	8, 10	7, 21
10	37.0, CH	1.84, br dq (8.4, 7.2)	9, 21	
11	75.0, CH	3.95, br d (10.6)	12	9, 10, 13, 21, 22
12	35.2, CH	2.00, dqd (10.6, 7.3, 6.3)	22	13, 14
13	81.6, CH	4.22, m	14	16
14	24.9, CH_2	1.94, m	13, 15	13, 15a, 16
15_a	35.1, CH_2	2.05, m	15	16
15_b		1.83, m	14	
16	106.4, qC			
17	24.3, CH_3	1.39, s		15a, 16
18	65.1, CH_2	4.22, m	3	1, 2, 3
19	17.0, CH_3	0.96, d (6.8)	6	5, 6,7
20	11.1, CH_3	1.01, d (7.3)	8	8, 9
21	10.3, CH_3	0.88, d (7.2)	10	9, 10, 11
22	12.8, CH_3	0.73, d (7.3)	12	12, 13

Figure S1. ^{1}H NMR Spectrum (500 MHz) of Saliniketal A (**1**) in MeOH-d_4

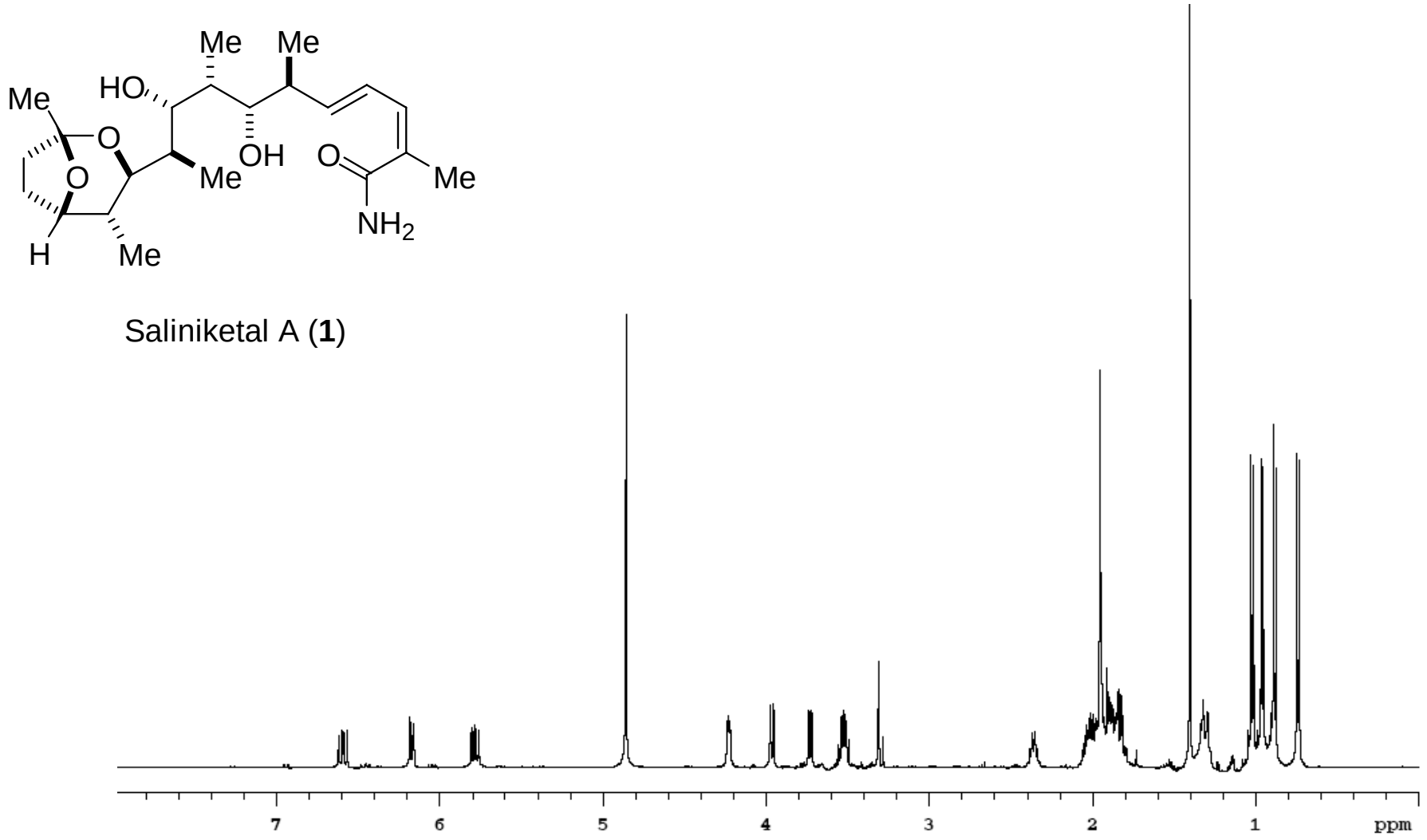


Figure S2. ^{13}C NMR Spectrum (125 MHz) of Saliniketal A (**1**) in MeOH-d_4

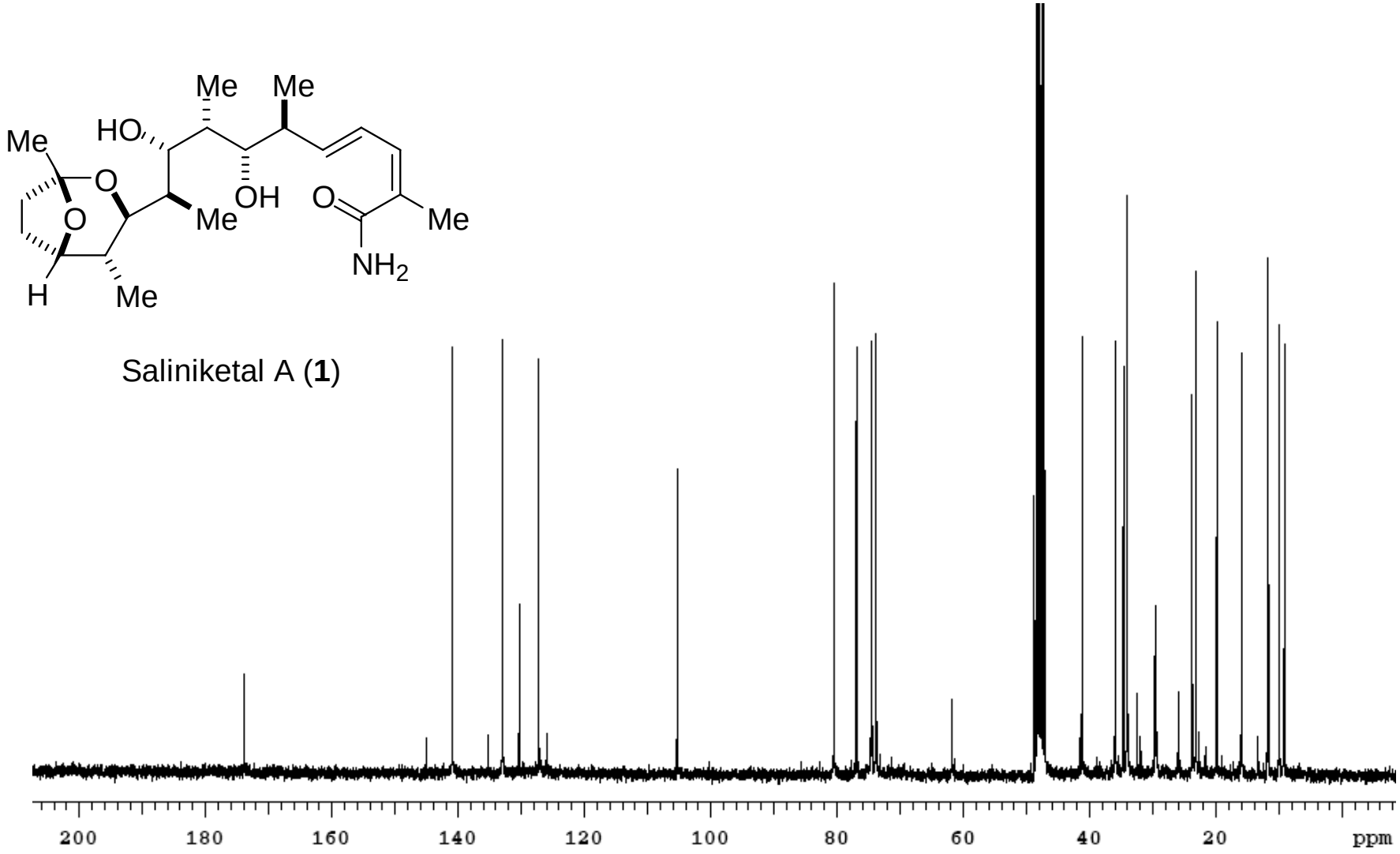
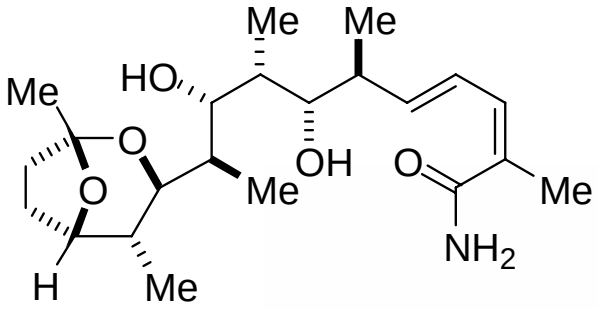


Figure S3. gHSQC NMR Spectrum (500 MHz) of Saliniketal A (**1**) in MeOH-d_4



Saliniketal A (**1**)

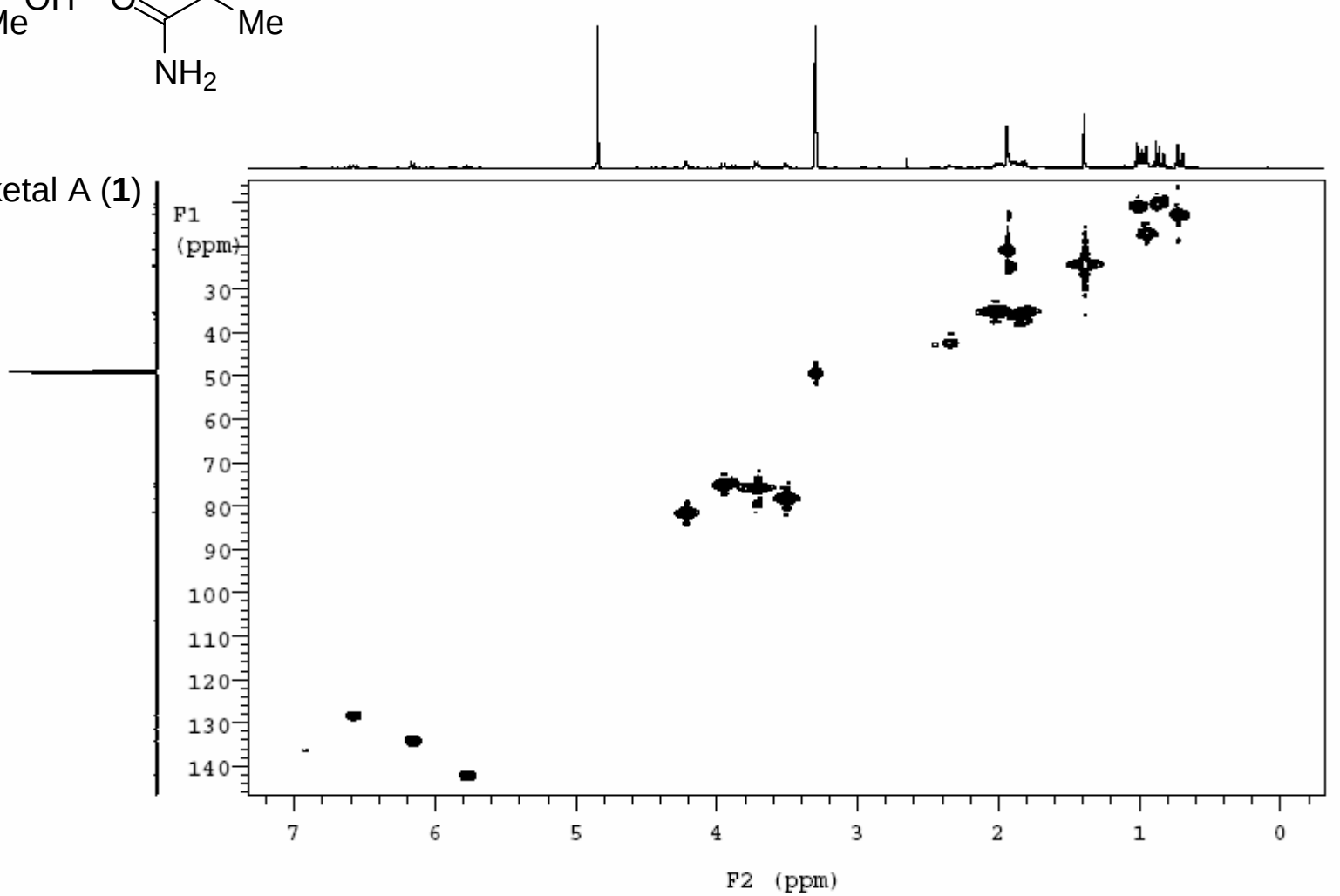
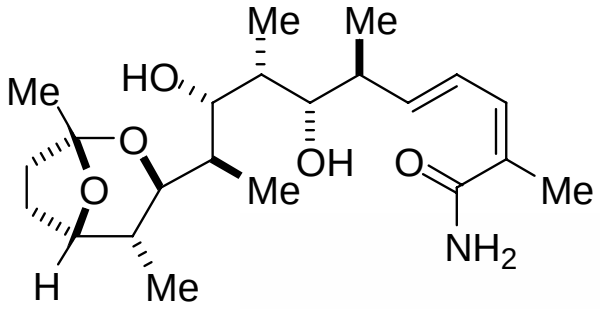


Figure S4. gDQFCOSY NMR Spectrum (500 MHz) of Saliniketal A (**1**) in MeOH-d_4



Saliniketal A (**1**)

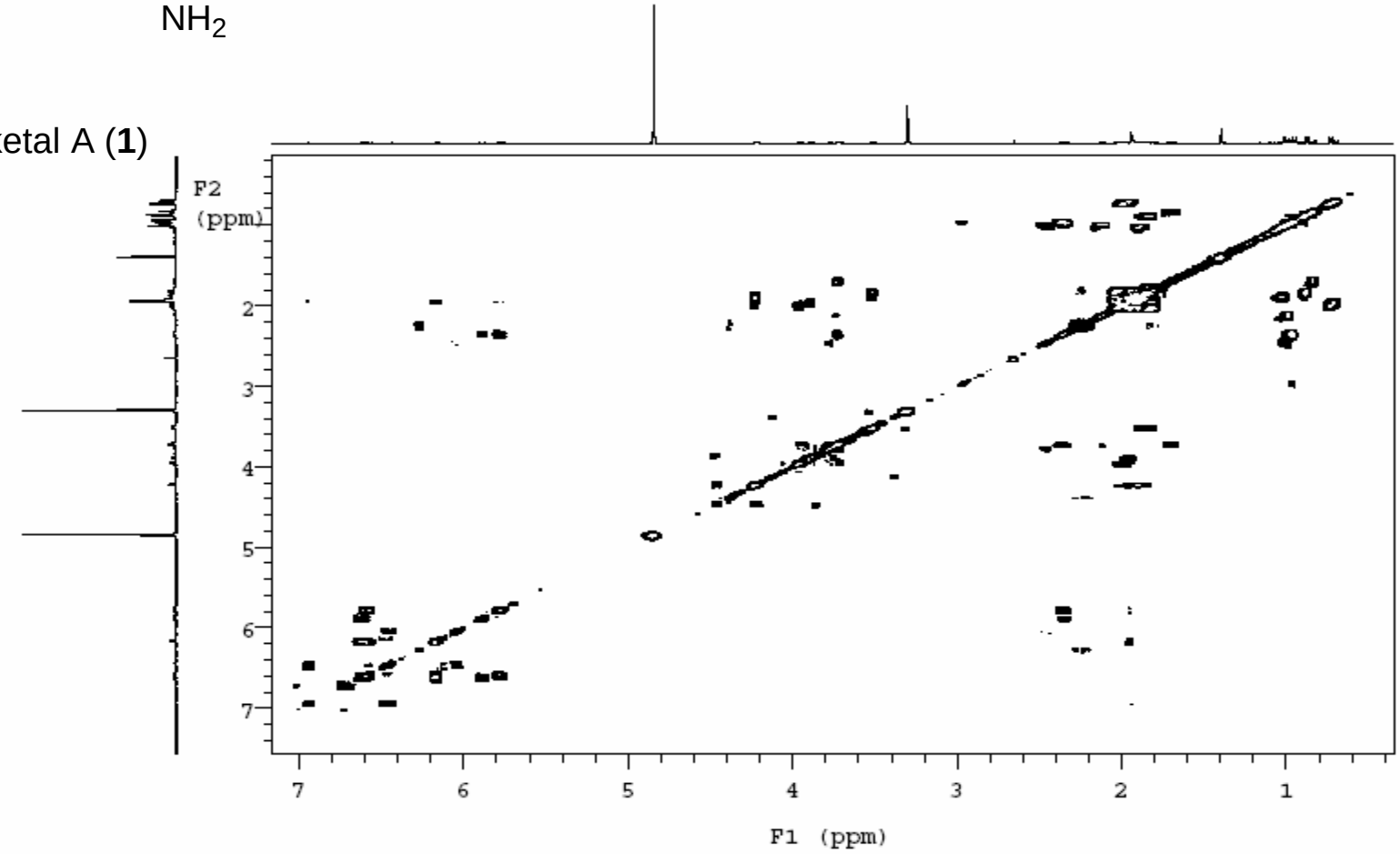


Figure S5. gHMBC NMR Spectrum (500 MHz) of Saliniketal A (**1**) in MeOH-d_4

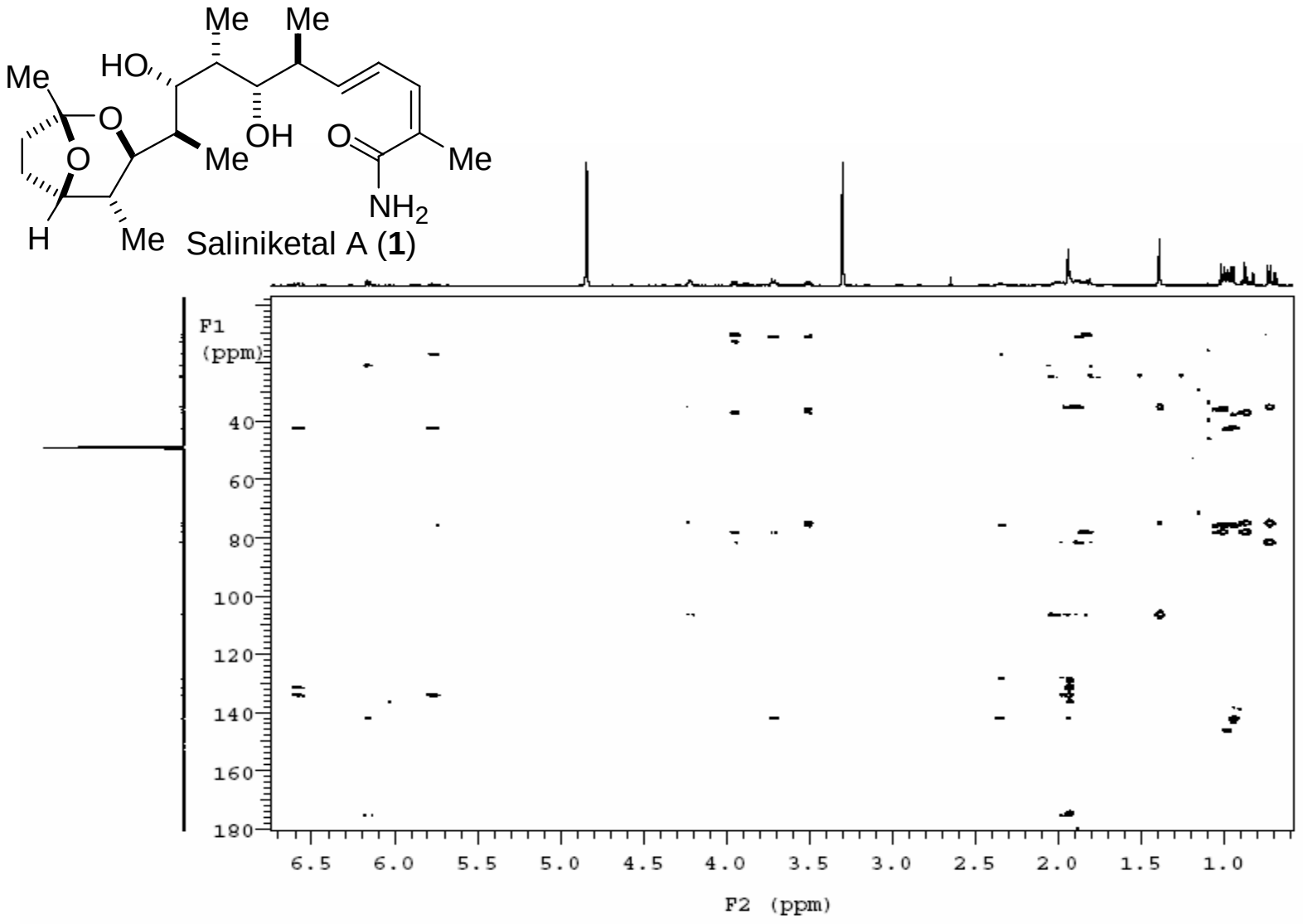


Figure S6 ^{1}H NMR Spectrum (500 MHz) of Saliniketal B (**2**) in MeOH-d_4

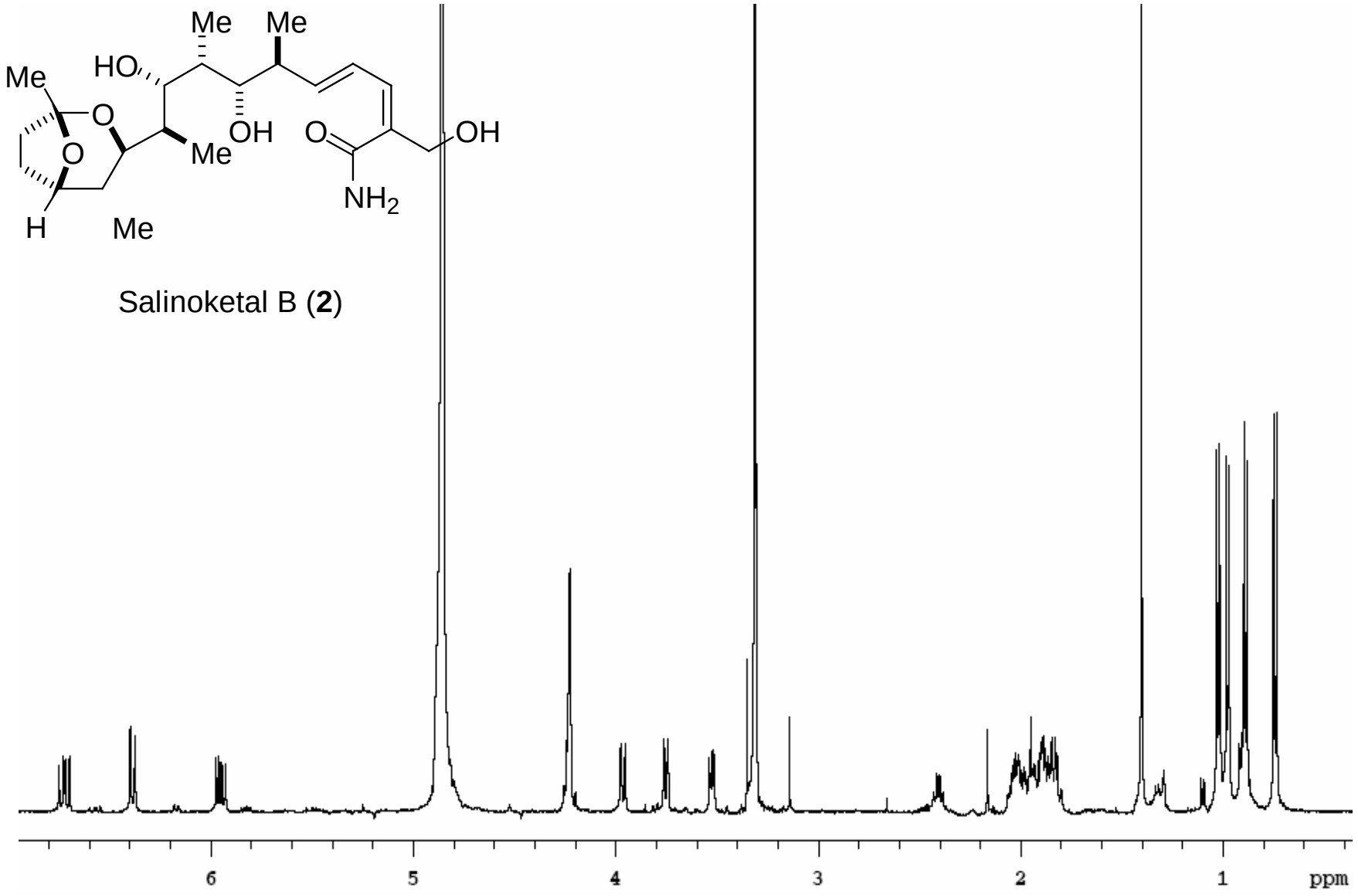


Figure S7. ^{13}C NMR Spectrum (125 MHz) of Saliniketal B (**2**) in MeOH-d_4

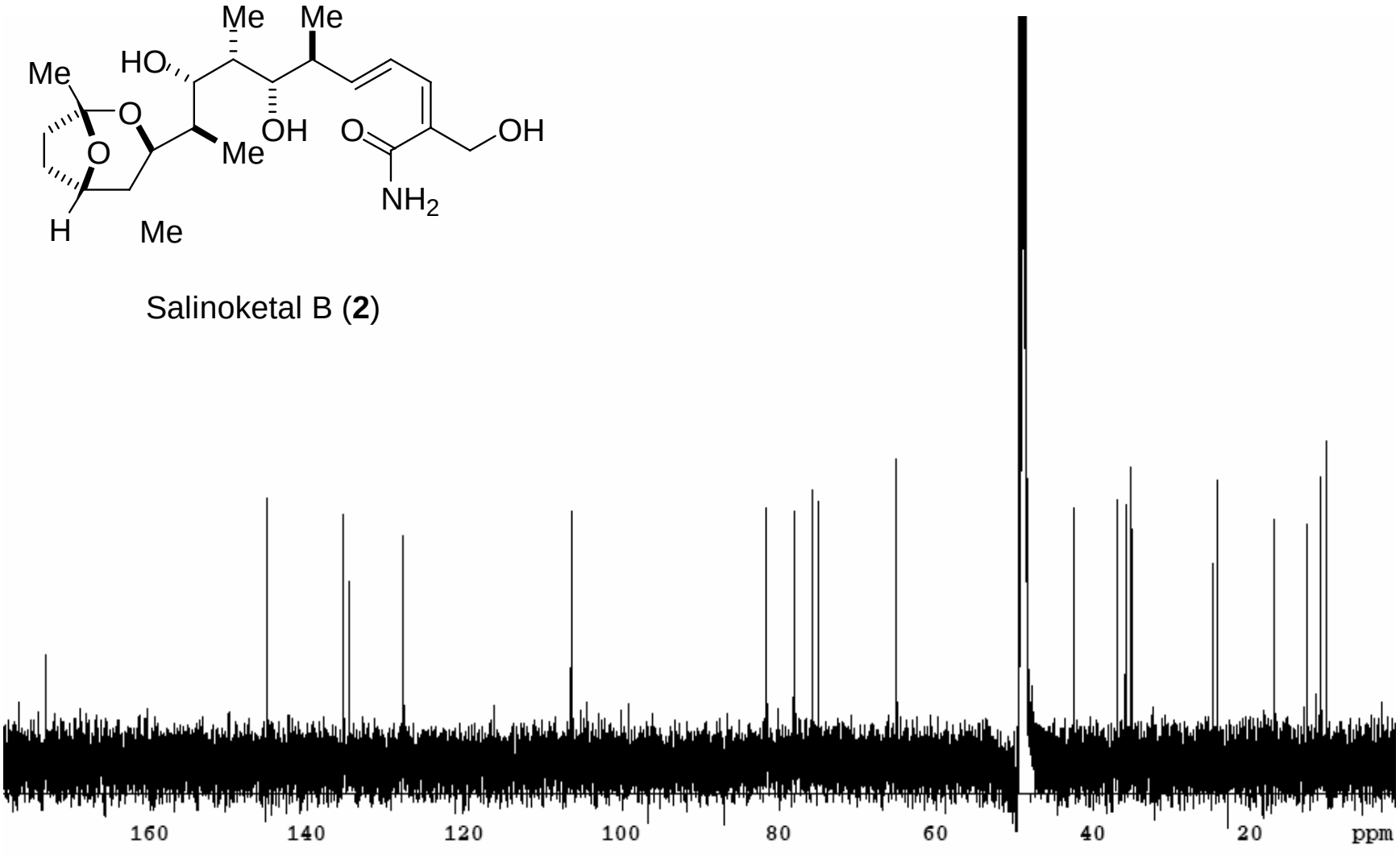


Figure S8. gHSQC NMR Spectrum (500 MHz) of Saliniketal B (**2**) in MeOH-d_4

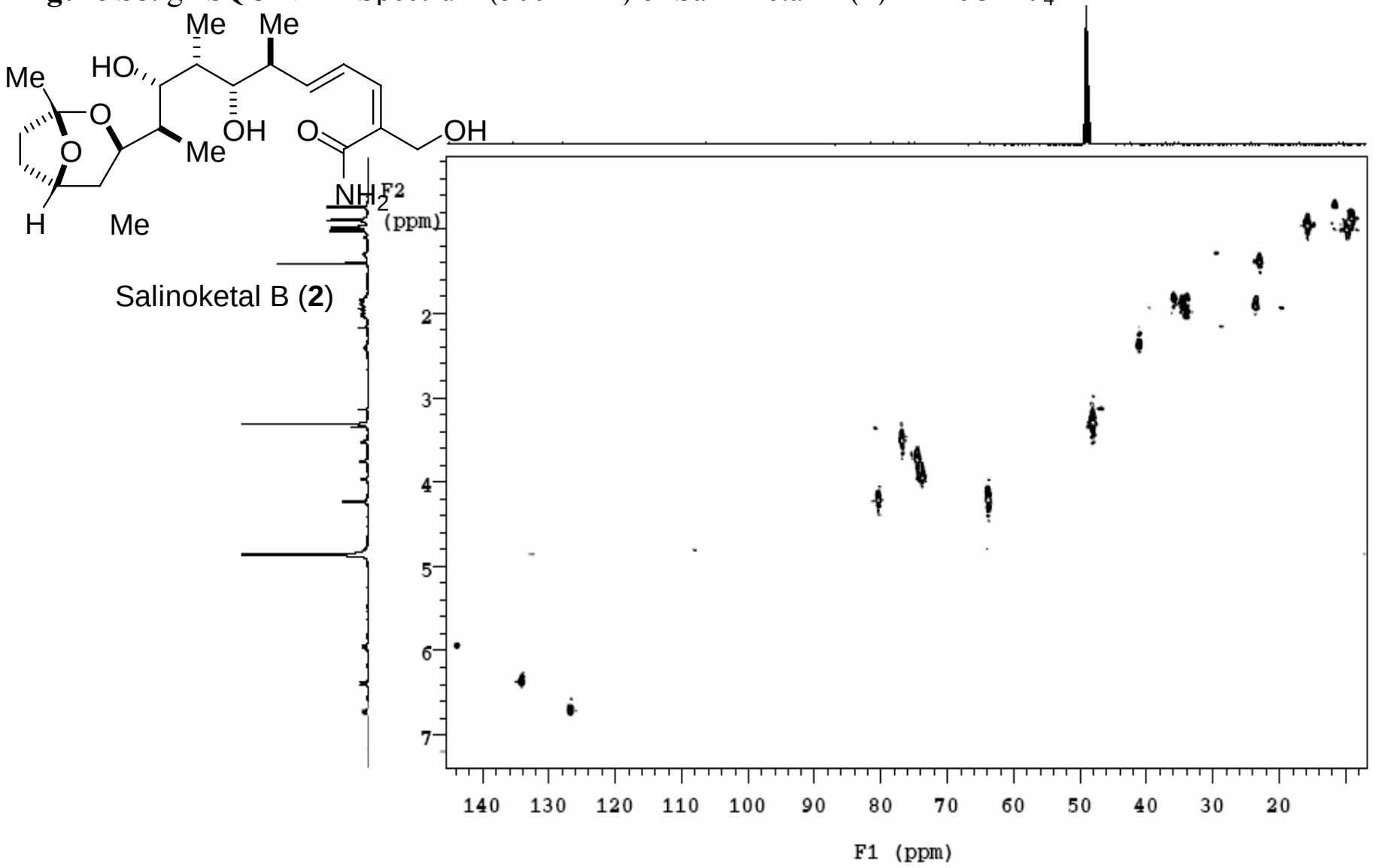


Figure S9. gDQFCOSY NMR Spectrum (500 MHz) of Saliniketal B (**2**) in MeOH-d_4

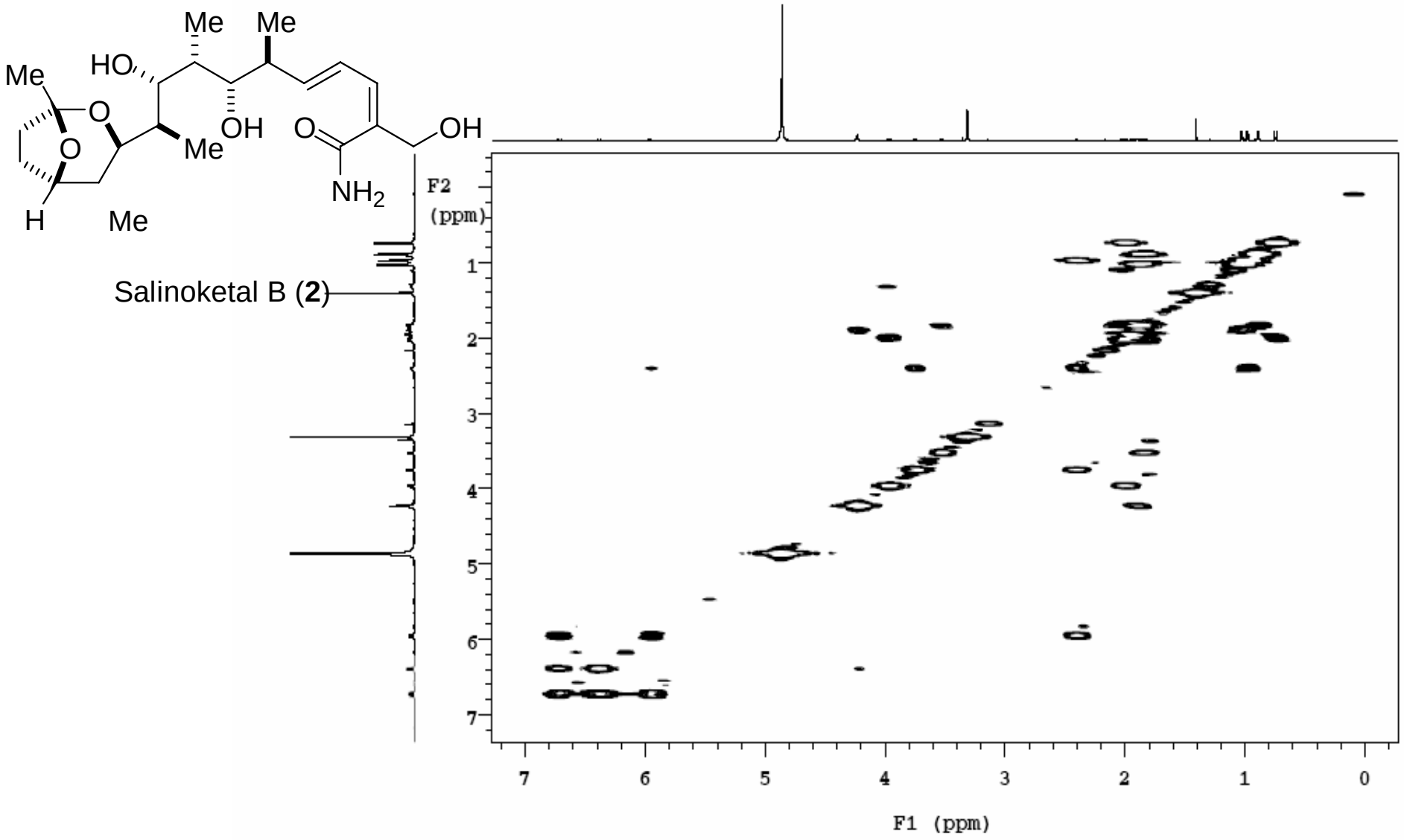
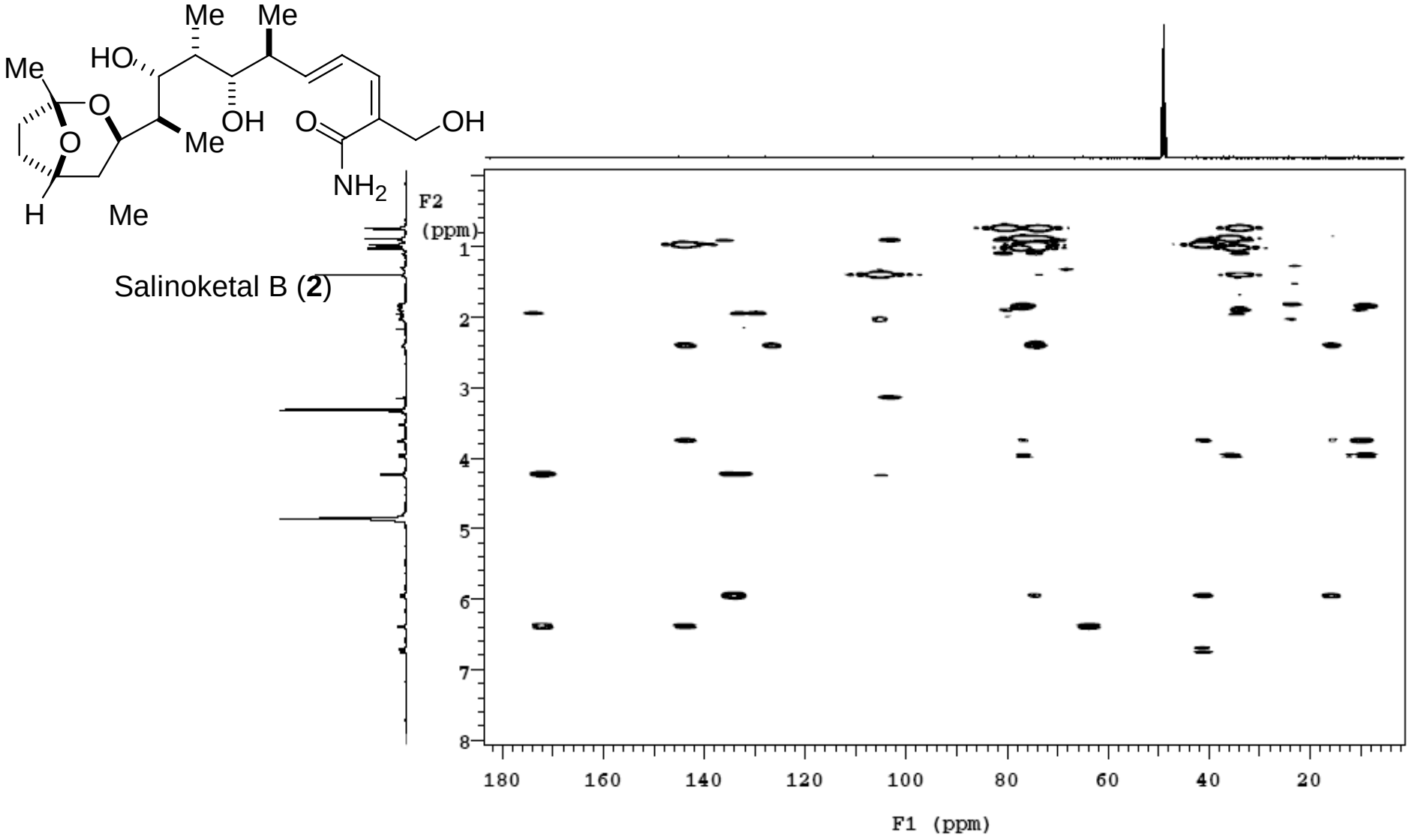


Figure S10. gHMBC NMR Spectrum (500 MHz) of Saliniketal B (**2**) in MeOH-d_4



Additional Experimetnal Procedures

Preparation of the Acetonide Derivative of 1. To 1 mg of saliniketal A (**1**) was added 0.5 mL of acetone, which had been distilled over Drierite, 0.5 mL of dimethoxypropane, and 1 crystal of pyridinium *para*-toluenesulfonic acid. The reaction was stirred for 2 h under N_2 at 23°C. LC-MS analysis of an aliquot indicated **1** had been converted to two products. To the mixture was then added 2 mL of H_2O and the solvent was removed *in vacuo*. The residue was dissolved in MeOH and then filtered. This filtrate was then purified by RP HPLC (Ultracarb C_{18}, 250 x 10 mm; 3 mL/min; 254 nm; 50% CH_3CN for 5 min then a linear gradient up to 80% over 35 more min). This gave two peaks in a ratio of 1:5 with retention times of 23 and 25 min, respectively. The major product (25 min), when analyzed by NMR (^{1}H NMR, gHSQC), had two new methyl singlets assigned to an acetonide that showed a one-bond coupling to carbons at 24.8 and 23.9 ppm. These chemical shifts values indicated an *anti* configuration of the corresponding 1,3-diol.

Mosher Reaction: (*S*)-MTPA-derivative. To saliniketal A (**1**, 0.6 mg) in a dry 2 mL vial was added 1 crystal of DMAP and 1 mL of dry pyridine. This mixture was stirred at 23° C for 30 min before 20 μL of (*R*)-MTPA-Cl was added dropwise. The progress of the reaction was monitored by LC-MS. A 20 μL aliquot was withdrawn and added to 1 mL of CH_3CN, which was then analyzed (C_{18}-Phenomenex Luna, 100 x 4.mm, 0.7 mL/min, 210 and 254 nm detection, 20% CH_3CN for 2 min then a linear gradient to 100% over 20 min then hold at 100%). After 30 min, there was no sign of **1**, instead a new peak (t_R 15.8 min) giving a $[M+Na]^+$ pseudo-molecular ion at 400, was present along with the mono-Mosher product (t_R 20.4 min, $[M+Na]^+$ 616; UVλ_{max} 260 nm). After 4 h the reaction was complete and it was stopped by the addition of 1 mL of H_2O. The solvent was removed *in vacuo* and the residue

dissolved in EtOAc, filtered, and purified by Silica HPLC (Dynamax Si 60A, 250 x 10 mm, detection 282 nm, 3 mL/min, 30% EtOAc in isooctane for 5 min the a linear gradient over the next 30 min to 65% EtOAc in isooctane) giving the (*S*)-MTPA ester (t_R =14.9 min).

(*R*)-MTPA-derivative of 1. Prepare in the same manner as the (*S*)-derivative except the reaction was stirred under N_2 for 16 h, then an additional 20 μL of (*S*)-MTPA-Cl was added and the reaction warmed to 40°C for 8 h. Purification as above yielded the (*R*)-MTPA-derivative (t_R = 19.0 min).

Mosher Reaction. Di-MTPA derivative: To saliniketal A (2.7 mg) in a dry 2 mL vial was added 1 crystal of DMAP and 1 mL of dry pyridine. This mixture was stirred at 23°C for 30 min before 50 μL of (*R*)-MTPA-Cl was added dropwise. The progress of the reaction was monitored by LC-MS in the same manner as above The reaction was allowed to proceed for 16 h before it was worked up as stated above. Purification by silica HPLC (Dynamax Si 60A, 250 x 10 mm, detection at 282 nm; flowrate 3 mL/min, 30% EtOAc in iso-octane for 5 min then a linear gradient over the next 30 min up to 65% EtOAc in isooctane) provided the di-(*S*)-MTPA ester (t_R =12 min) and two mono-derivatives at 15 and 16 min in a ratio of 8:1:2.

TPA-induced ornithine decarboxylase (ODC) activity with cultured T-24 cells. T-24 human epithelial urinary bladder carcinoma cells (ATCC number HTB-4) were propagated in MEM medium with 1.5 mM L-glutamine, 10% FBS, 1 mM sodium pyruvate, non-essential amino acid solution, and 1% antibiotic-antimycotic solution, at 37ºC in a 5% CO_2 atmosphere. When the culture was confluent, cells were washed with PBS, fresh medium was added, and the incubation was continued for 24 h. Cells were

dislodged with trypsin-EDTA and plated at an initial density of $2x10^5$ cells/mL/well in 24-well plates, and incubated for 18 h. Cells were treated with test compounds (5 μL dissolved in DMSO), concentration 20 μg/mL) in duplicate, simultaneously added with 20 μL of TPA solution (final concentration 200 nM), and incubated for 6 h. Cells were washed twice with PBS and frozen immediately by placing the plates at -80°C.

ODC activity was assayed directly in 24-well plate by measuring the release of [^{14}C]CO_2 from [^{14}C]ornithine as described previously with some modifications.[32]

Frozen cells were lysed by briefly thawing the bottom of the culture plates in a warm water bath at 37ºC for 2 min, and activity was initiated by adding a substrate-cofactor mixture with 200 nCi of L-[1-^{14}C]ornithine. The plates were covered with PCR-SP sealing film (Axygen), in which holes were punched. A 1.27 cm diameter paper disk was then placed over each hole and moistened with 30 μL of 1 N NaOH. The released $^{14}CO_2$ gas was captured by the paper disks during incubation of the plates at 37ºC for 1 h and estimated by liquid scintillation counting. The protein content in each well was determined using Quick Start Bradford protein assay (BIO-RAD) with BSA as standard protein. Activity was calculated as pmol of $^{14}CO_2$ release/mg protein/h. The effect of treatment was calculated and expressed as the concentration of test compound required to reduce activity of the TPA-treated control by 50% (IC_{50} value).

Determination of ODC activity with T-24 cell lysate. T-24 cells from confluent monolayers in T75 plates were lysed with 1 mL of ice-cold buffer: 50 mM Tris-HCl, pH 7.5, containing 0.1 mM pyridoxal phosphate and 0.1 mM EDTA. Cells were scraped, centrifuged at 15,000 g for 30 min, protein concentration was determined by the Bradford method, and 100 μg of supernatant per well

was used to determine ODC activity in 24-well plates as described previously.[33] Incubation mixtures were preincubated with test agents for 15 min at 4°C. Assays were conducted 30 min at 37°C. Difluormethylornithine (DFMO) was used as a positive control.