Supporting Information

Coibamide A, a Potent Antiproliferative Cyclic Depsipeptide from the Panamanian Marine Cyanobacterium *Leptolyngbya* sp.

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Experimental Section

General Experimental Procedures. NMR spectra were recorded on Bruker Avance DRX600 (5mm TXI probe) and Bruker Avance DRX300 (5 mm BBO probe) spectrometers operating at ^{1}H frequencies of 600.01 and 300.13 MHz, respectively, and ^{13}C frequencies of 150.90 and 75.47 MHz, respectively. The solvent was used as an internal standard (δ_C 77.23, δ_H 7.26 for $CDCl_3$; δ_C 128.39, δ_H 7.16 for C_6D_6). Low resolution TOF-ESI mass spectrometric data were acquired on a Waters Micromass LCT mass spectrometer. MALDI-TOF MS/MS experiments were carried out on an ABI 4700 Proteomics Analyzer. High resolution mass data were acquired on a Bruker APEXIII-7T FTMS spectrometer with electrospray ionization. LC-MS/MS was carried out using an Applied Biosystems MDS Sciex 4000 Q TRAP spectrometer. HPLC separations were performed using Waters 515 HPLC pumps, a Rheodyne 7725i injector, and a Waters 996 photodiode array detector or Shimadzu LC-6AD HPLC pumps, a Rheodyne 7125 injector, and a SPD-10A dual-wavelength UV detector. *N*-Me-Leu, *N*-Mel-Ala and Ala amino acid standards were purchased from Sigma-Aldrich. Boc-*N*-Me-Ile and *O*-Me-Tyr amino acid standards were obtained from CHEM-IMPEX International, Inc. Amino acid standards of hydroxyisovaleric acid were purchased from Sigma-Aldrich and Acros Organics.

Extraction and Isolation. The marine cyanobacterium *Leptolyngbya* sp. (Family Oscillatoriaceae) was collected by hand using SCUBA from a reef pinnacle in the Coiba National Park. Taxonomical classification of the cyanobacterium is based on morphology. In appearance, this *Leptolynbya* species comprises fine, clustered red filaments several centimeters in length. The thin, fine, immotile filaments (2.5-2.8 μm wide) are coiled into macroscopic clusters (several cm in diameter), with simple, thin colorless sheaths opened at the apical end; sheaths joined to the trichomes or slightly distant from them, enveloping only one, very rarely (in short sections) two trichomes. Trichomes were fine, cylindrical, and not attenuated to the ends, with rounded apical cells; not constricted at the cross walls. Cells are approximately isodiametrical or longer than wide (up to several times), cylindrical, with roughly homogeneous content, without aerotopes, rarely with scarce prominent granules; end cells without thickened cell walls or calyptras. Heterocytes and akinetes are absent.

The collected material was stored immediately in 50% EtOH for transport, and then placed at -20 °C until further work up. The freshly thawed cyanobacterium (1L of wet volume) was extracted repeatedly (5 x 500 mL) with CH_2Cl_2-MeOH (2:1) and the combined organic layers were concentrated to dryness *in vacuo* to give 5.75 g of a dark green oil. This crude extract was fractionated by vacuum liquid chromatography (600 mL sintered glass funnel; Merck Si Gel G for TLC, 10-40 μm) using a

stepwise gradient solvent system of increasing polarity from 100% hexanes to 100% EtOAc to 100% MeOH. In preliminary screening, the 100% EtOAc fraction was cytotoxic (IC_{50} 300 ng/mL) to NCI-460 human lung tumor cells. This fraction was separated further on a Varian Mega Bond Elute C_{18} solid phase extraction cartridge using a stepwise gradient (2g; MeOH-H_2O, 6:4, 7:3, 8:2, 9:1, 100% MeOH and CH_2Cl_2). The fractions that eluted with 9:1 MeOH-H_2O and 100% MeOH were combined and subjected to isocratic reversed-phase HPLC (column, Phenomenex Synergi Fusion 4μ, 250 x 10 mm, 2 mL/min, detection at 216 nm) using 9:1 MeOH-H_2O. Coibamide A **(1)** eluted at t_R 33.7 min (6.3 mg).

Coibamide A (1): colorless oil; $[\alpha]^{23}_D$ -54.1 (*c* 0.02, $CHCl_3$); IR V_{max} (neat) 3377, 2959, 1733, 1645, 1513, 1471, 1406, 1248, 1097, 756 cm^{-1}; NMR data see Table S1; MALDI-TOF MS/MS 1287.9 *m/z* (%) 1202.8 (26), 945.6 (6), 846.5 (8), 567.4 (28), 535.4 (9), 438.3 (24), 343.2 (19), 311.2 (10), 308.2 (4), 198.1 (12), 100.1 (100); TOF MS/MS 1287.56 ESI (positive) *m/z* (%) 1287.8 (42, $[M+H]^+$), 1255.8 (20), 1202.7 (28), 1184.7 (96), 1156.7 (82), 1127.4 (28), 945.6 (14), 880.6 (16), 846.5 (6), 818.5 (19), 814.5 (18), 786.5 (16), 743.4 (25), 721.5 (23), 671.4 (15), 650.4 (55), 618.4 (32), 567.3 (33), 536.2 (64), 438.2 (60), 390.2 (42), 343.2 (68), 326.2 (8), 308.8 (100), 293.2 (31), 264.2 (7), 228.1 (45), 198.1 (15), 183.2 (4), 156.1 (6), 126.1 (8), 100.1 (80); HR FT-MS obsd $[M + Na]^+$ *m/z* 1309.79878 (calcd for $C_{65}H_{110}O_{16}N_{10}Na$, 1309.79990); obsd $[M + H]^+$ *m/z* 1287.8156 (calcd for $C_{65}H_{111}O_{16}N_{10}$, 1287.8180); obsd $[M + 2H]^{2+}$ *m/z* 644.41258 (calcd for $C_{65}H_{112}O_{16}N_{10}$, 644.41295); obsd $[M + H + NH_4]^{2+}$ *m/z* 652.92586 (calcd for $C_{65}H_{115}O_{16}N_{11}$, 652.92620).

Synthesis of L- and D-*N,N*-dimethylvaline. *N,N*-dimethylvaline standards for configuration analysis were synthesized from L- and D-valine as described in the literature (Pettit, G.R. Inventor; Arizona Board of Regents, Assignee. Synthesis of Dolastatin 10. United States Patent U.S. 4, 978,744, 1990.) Formaldehyde (38% solution, 300 μL) and 10% palladium on activated carbon (~100 mg) were added to a solution of L-valine (100 mg) in H_2O (3 mL). The mixture was hydrogenated at room temperature overnight, refluxed (30 min) and filtered to remove the catalyst. The filtrate was concentrated to dryness *in vacuo* and a mixture of H_2O and EtOH (1:7) was added to the residue. After the solvent was evaporated, the preceding process was repeated five times, resulting in a white amorphous powder. ^1H NMR of the filtrate showed the presence of *N,N*-dimethyl-L-valine. The filtrate was purified using chiral HPLC (column, Phenomenex Chirex Phase 3126 (D), 4.6 x 250 mm, 5 μm; 2mM $CuSO_4$ in 95:5 H_2O/MeCN, flow 1.0 mL/min, UV detection at 254 nm). *N,N*-dimethyl-D-valine was synthesized in the same manner starting with D-valine.

Synthesis of *N*-methylthreonine. The four *N*-methylthreonine diastereomers were synthesized using following a literature procedure **(**Ford, P.W. et. al. *J. Am. Chem. Soc.* **1999**, *121*, 5899-5909.)

$SOCl_2$ (910 μL) was added dropwise to dry MeOH (3.5 mL), and the solution cooled to -10 °C. L-Thr (350 mg) was added in three portions to this solution, and the mixture was stirred at room temperature (18 hr). The reaction mixture was evaporated to dryness, and the resulting threonine methyl ester (Thr-OMe) was crystallized from petroleum ether. To a solution of Thr-OMe (100 mg) in CH_2Cl_2 (10 mL) was added 0.1 N aqueous trifluoroacetic acid (TFA, 10 mL), and the mixture was cooled to 0°C. Formaldehyde (37% aq. soln, 62 μL) was added dropwise with vigorous stirring, and stirring was continued at room temperature for 8 hr. The reaction mixture was neutralized with $NaHCO_3$ and the product isolated by extraction with CH_2Cl_2 (25 mL x 4). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to give the oxazolidine derivative which was used without further purification. A solution of the oxazolidine (64.7 mg) in dry CH_2Cl_2 (6 mL) was cooled to 0°C, and TFA (6 mL) was added followed by dropwise addition of triethylsilane (0.6 mL). The reaction mixture was allowed to stir at room temperature for 24 hr and then concentrated *in vacuo*. The pale yellow residue was resuspended in 1 N HCl and washed with petroleum ether. Hydrolysis in 6 N HCl (1 mL) at 110°C for 16 hr yielded *N*-Me-L-Thr (2*S*, 3*R*). The identical method was followed using L-*allo*-Thr, D-Thr, or D-*allo*-Thr as the staring materials which yielded (2*S*,3*S*)-, (2*R*,3*S*)-, or (2*R*,3*R*)-*N*-Me-Thr, respectively.

Assignment of Absolute Configuration. A portion of compound **1** (~0.2 mg) was hydrolyzed in 6 N HCl (1 mL) at 110°C for 24 hr. The hydrolysis product was concentrated and analyzed by chiral HPLC (column, Phenomenex Chirex Phase 3126 (D), 4.6 x 250 mm, 5 μm; mobile phase I: 2mM $CuSO_4$ in 85:15 H_2O/MeCN, mobile phase II: 2mM $CuSO_4$ in 95:5 H_2O/MeCN, and mobile phase III: 2mM $CuSO_4$, flow rate 1.0 mL/min; UV detection at 254 nm). The absolute configurations of the amino acids in the acid hydrolysate were determined by direct comparison with the retention times of authentic standards. Analysis (t_R, min) in mobile phase I established the presence of *O*-Me-L-Tyr (35.0; *O*-Me-D-Tyr, 38.0). *N,O*-dimethyl-L-Ser (10.3; *N,O*-dimethyl-D-Ser, 10.8) and L-Ala (5.0; D-Ala, 5.8). Analysis (t_R, min) in mobile phase III established the presence of *N,N*-dimethyl-L-Val (11.8; *N,N*-dimethyl-D-Val, 12.0).

A portion of the acid hydrolysate was resuspended in ~100 μL MeOH and diazomethane was added dropwise. The resulting solution was stirred until it became clear (10 min), reduced *in vacuo* and resuspended in CH_2Cl_2 (~200 μL). Chiral GC-MS (column Cyclosil B, 30.0 m x 250 μm x 0.25 μm) of this methylated natural product hydrolysate and methylated D- and L-HIV standards (t_R 11.4 and 11.85 min, respectively) led to the assignment of L-HIV in **1**.

Marfey's method was used to determine the absolute configuration of the remaining amino acids. An additional sample of compound **1** (~0.2 mg) was hydrolyzed in 6 N HCl (1 mL, 110°C, 24 hr), evaporated to dryness and resuspended in H_2O (50 µL). A solution of 1% 1-fluoro-2-4-dinitrophenyl-5-L-alanamide in acetone (100 µL), and 50 µL of 1 N $NaHCO_3$, were added to the hydrolysate and the mixture was heated (37°C, 1 hr). The solution was cooled to room temperature, neutralized with 2 N HCl (25 µL), and evaporated to dryness. The residue was resuspended in DMSO-H_2O (1:1, 100 µL) and analyzed by reversed phase HPLC (Waters symmetry shield C_{18}, 3.9 x 150 mm, 5 µm, UV detection at 340 nm) using a linear gradient of 9:1 triethylammonium phosphate (TEAP, 50 mM) buffer (pH 3.1)-CH_3CN to 1:1 TEAP-CH_3CN over 60 min. The remaining amino acid residues (*N*-Me-L-Ile, *N*-Me-L-*allo*-Ile, *N*-Me-D-Ile, *N*-Me-D-*allo*-ILe, *N*-Me-L-Ala, *N*-Me-D-Ala, *N*-Me-L-Leu, *N*-Me-D-Leu, *N*-Me-L-Thr, *N*-Me-L-*allo*-Thr, *N*-Me-D-Thr, *N*-Me-D-*allo*-Thr) were derivatized in the same manner as described for the acid hydrolysate. The retention times (t_R, min) of the derivatized residues in the hydrolysate of **1** matched *N*-Me-L-Leu (43.9; *N*-Me-D-Leu, 45.0), *N*-Me-L-Ala (29.0; *N*-Me-D-Ala, 29.4), and *N*-Me-L-Ile (45.6; *N*-Me-L-*allo*-Ile, 44.6; *N*-Me-D-Ile, 48.0; *N*-Me-D-*allo*-Ile, 47.0). *N*-Me-Thr of the hydrolysate (24.0) eluted close to *N*-Me-L-Thr (24.4) and *N*-Me-L-*allo*-Thr (24.2; *N*-Me-D-Thr, 26.5; *N*-Me-D-*allo*-Thr, 25.7), but could not be assigned with certainty by this method or chiral HPLC.

Computational modeling of Coibamide A (1). Computational modeling was employed to determine whether the *N*-Me-Thr of coibamide A (**1**) could be predicted as either *N*-Me-L-Thr or *N*-Me-L-*allo*-Thr based on ROESY data fitted to energy-minimized structures. NMR spectroscopy of **1** in $CDCl_3$ and C_6D_6 showed only one and two conformers, respectively. Thus, the extremely high degree of *N*-methylation appears to reduce the potential flexibility of the coibamide macrocycle in these solvents. Using Macromodel 9.1 software, each of the two coibamide isomers (model **A** containing *N*-Me-L-Thr; model **B** containing *N*-Me-L-*allo*-Thr) was energy minimized as follows. For each model, starting bond geometries were established *via* a steepest descent (SD) minimization (1000 iterations, $CHCl_3$) using the MM2* force field. This was followed by a Polak-Ribier conjugate gradient (PRCG) minimization (MM2*, 10,000 iterations, $CHCl_3$) using distance constraints based on prominent ROESY correlations observed between γ-CH_3-40 of *N*-Me-Thr and CH_3-4 of *N*-Me-Ala, and α-H-2 of *N*-Me-Ala and *N*-CH_3-48 of the side chain *N*-Me-Leu. Finally, the two minimized isomers **A** and **B**, which were of similar potential energies (829 and 836 $kJmol^{-1}$), were subjected to conformational searching (1,000 step Monte Carlo, MM2*) using the same distance constraints as before to produce the final minimized models shown in Figure S2. Conformational searching applied to model **B** (containing *N*-Me-L-*allo*-Thr) produced lowest energy conformations which did not maintain the designated distance constraints.

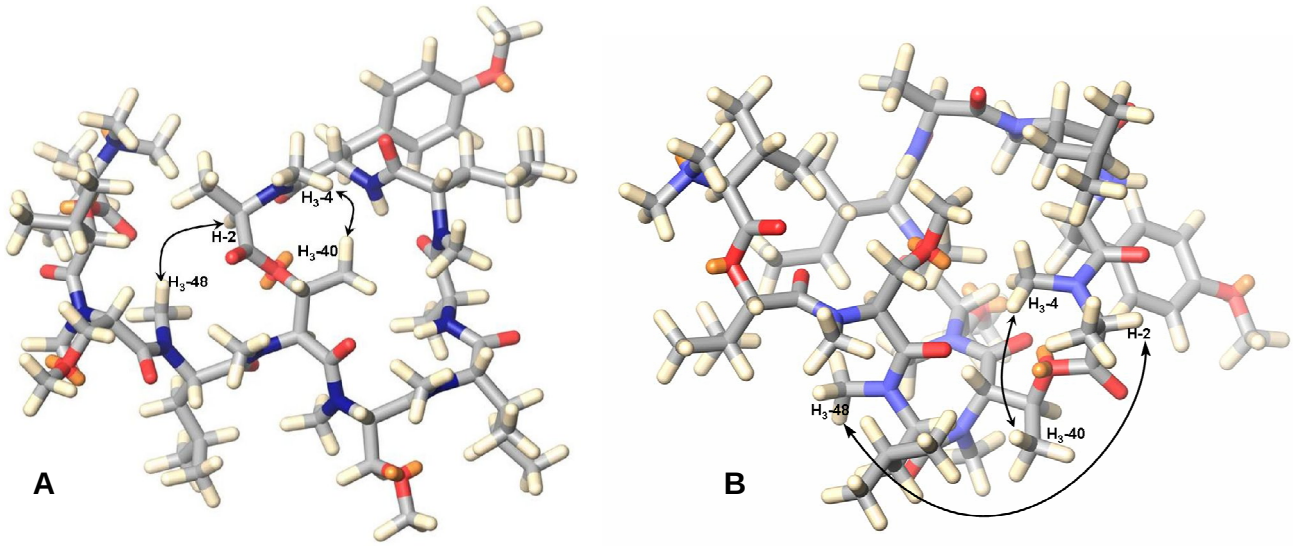


Figure S1. Lowest energy computational models of Coibamide A (**1**) containing *N*-Me-L-Thr (model **A**) and *N*-Me-L-*allo*-Thr (model **B**).

Table S1. ^{1}H and ^{13}C NMR data for Coibamide A (**1**) acquired in $CDCl_3$ and C_6D_6 (600 MHz, 298K)

Unit	Atom #	$CDCl_3$		C_6D_6	
		δ_H	δ_C	δ_H	δ_C
N-Me-Alanine	1	-	170.4[b] C	-	170.9[c] C
	2	5.32 (m, ob)	51.1 CH	5.72 (q, 7.2)	52.1 CH
	3	1.11 (d, 7.2)	12.9 CH_3	1.25 (d, 7.2)	13.7 CH_3
	4	2.35 (s)	30.1 CH_3	2.28 (s)	30.5 CH_3
	*N	-	δ_N 115.1	-	-
O-Me-Tyrosine	5	-	171.4[a] C	-	172.6[d] C
	6	5.11 (m)	50.0 CH	5.39 (m)	50.7 CH
	7	2.99 (m)	38.9 CH_2	3.16 (ob)	39.7 CH_2
		2.85 (ob)		2.95 (ob)	
	8	-	128.4 C	-	129.6 C
	9,13	7.09 (d, 8.3)	130.4 CH	7.14 (d, 8.5)	131.2 CH
	10,12	6.77 (d, 8.3)	113.7 CH	6.76 (d, 8.5)	114.5 CH
	11	-	158.6 C	-	159.6 C
	14	3.77 (s)	55.3 CH_3	3.27 (s)	55.1 CH_3
	NH	6.66 (br s)	δ_N 118.2	6.91 (br s)	-
N-Me-Leucine	15	-	171.2[a] C	-	170.0[e] C
	16	3.62 (m, ob)	63.6 CH	3.34 (m, ob)	64.1 CH
	17	1.50 (m)	36.1 CH_2	1.48 (m)	36.9 CH_2
	18	1.60 (m)	24.9 CH	1.83 (m)	25.7 CH
	19	0.89 (d, 6.5)	21.4 CH_3	0.72 (d, 6.4)	21.6 CH_3
	20	0.94 (d, 6.5)	23.2 CH_3	1.00 (d, 7.1)	23.7 CH_3
	21	3.15 (s)	39.4 CH_3	2.42 (s)	39.1 CH_3
	N	-	δ_N 105.6	-	-
Alanine	22	-	171.5[a] C	-	172.3[d] C
	23	4.73 (br m)	47.0 CH	4.61 (m)	47.9 CH
	24	1.28 (d, 6.5)	18.6 CH_3	1.03 (d, 5.5)	19.0 CH_3
	NH	6.63 (br s)	δ_N 122.4	6.86 (br s)	-
N-Me-Isoleucine	25	-	167.2 C	-	167.9 C
	26	3.75 (m, ob)	64.8 CH	4.12 (m)	65.4 CH
	27	2.05 (m)	32.0 CH	2.33 (m, ob)	33.0 CH
	28	1.35 (m)	24.2 CH_2	1.45 (m, ob)	25.0 CH_2
		1.11 (ob)		1.22 (m, ob)	
	29	0.93 (t, ob)	11.6 CH_3	0.85 (t, 7.2)	12.1 CH_3
	30	0.83 (d, 6.3)	15.7 CH_3	1.13 (d, 6.3)	16.4 CH_3

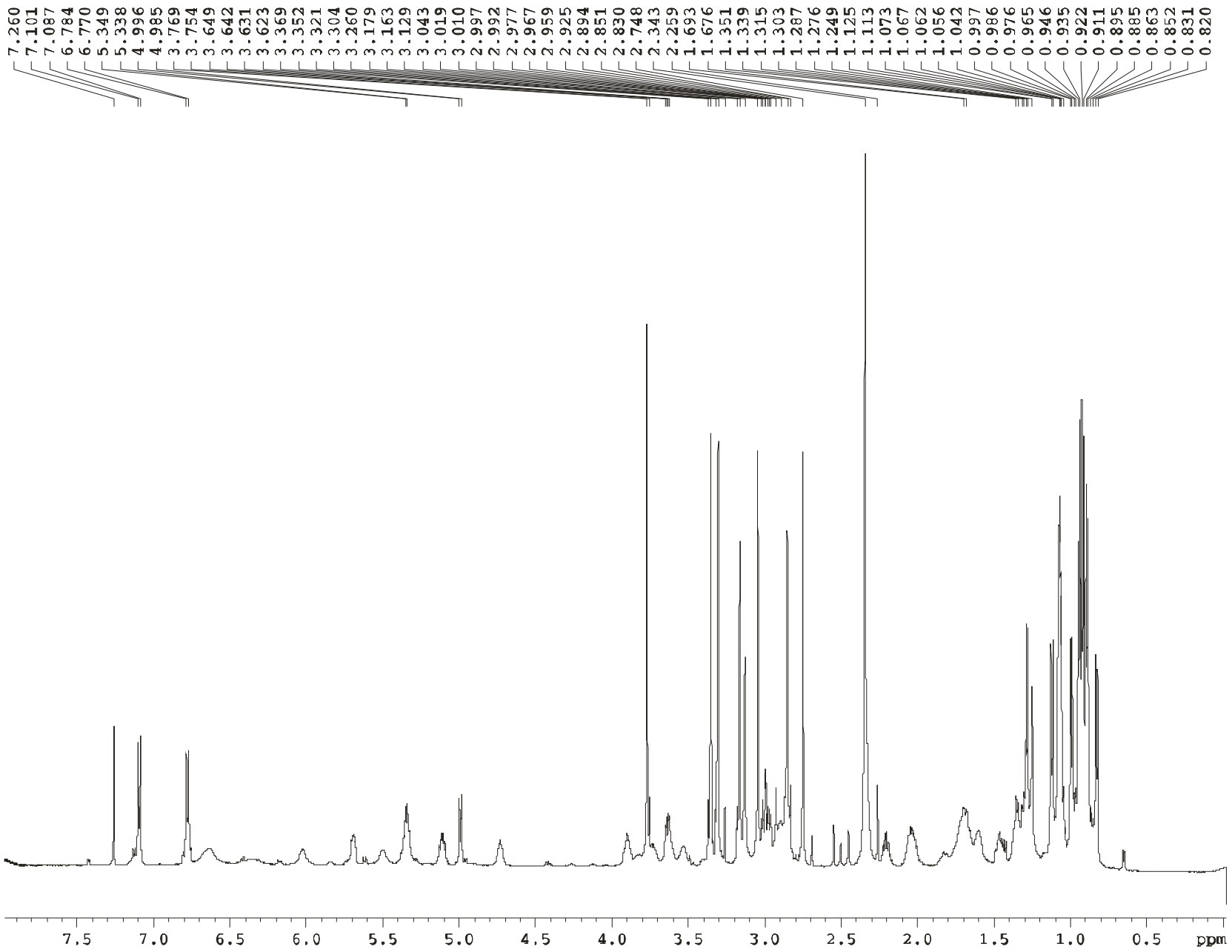
	31	2.75 (s)	28.9 CH_3	2.96 (s)	29.4 CH_3
	N	-	δ_N 120.5	-	-
N,*O*-diMe-Serine	32	-	168.7 C	-	169.1 C
	33	5.69 (m)	52.8 CH	6.07 (dd, 10.2, 4.8)	53.6 CH
	34	3.83 (m)	68.6 CH_2	4.11 (m)	69.7 CH_2
		3.53 (m)		3.35 (ob)	
	35	3.30 (s)	58.8 CH_3	2.99 (s)	58.9 CH_3
	36	2.85 (s)	30.0 CH_3	3.09 (s)	30.1 CH_3
	N	-	δ_N 113.4	-	-
N-Me-Threonine	37	-	169.7 C	-	171.0[c] C
	38	6.35 (br s)	not observed	6.72 (ob)	56.6 CH
	39	5.50 (br s)	68.0 CH	5.96 (m)	68.6 CH
	40	1.07 (ob)	18.4 CH_3	1.36 (d, 6.0)	18.4 CH_3
	41	2.89 (br s, ob)	29.7 CH_3	3.11 (s)	30.2 CH_3
	N	-	δ_N 119.4	-	-
N-Me-Leucine	42	-	170.5[b] C	-	172.2[d] C
	43	5.34 (m)	51.1 CH	5.68 (dd, 11.4, 4.2)	51.6 CH
	44	1.68 (m)	37.9 CH_2	1.92 (m)	38.8 CH_2
		1.31 (m)		1.47 (m)	
	45	1.36 (m)	25.3 CH	1.32 (m)	25.9 CH
	46	0.89 (d, 6.5)	23.1 CH_3	0.97 (d, ob)	23.8 CH_3
	47	0.92 (d, 6.6)	21.2 CH_3	0.96 (d, ob)	21.9 CH_3
	48	3.13 (s)	31.2 CH_3	3.55 (s)	32.1 CH_3
	N	-	δ_N 117.5	-	-
N,*O*-diMe-Serine	49	-	170.5[b] C	-	171.3 C
	50	6.02 (br s)	52.6 CH	6.46 (dd 10.2, 4.3)	53.3 CH
	51	3.90 (m)	69.3 CH_2	4.16 (t, 10.6)	70.4 CH_2
		3.64 (dd, 11.0, 4.0)		4.02 (dd, 11.7, 4.3)	
	52	3.35 (s)	58.6 CH_3	3.43 (s)	59.0 CH_3
	53	3.04 (s)	30.2 CH_3	2.91 (s)	30.4 CH_3
	N	-	δ_N 108.5	-	-
2-Hydroxyisovaleric acid	54	-	170.0 C	-	170.3[e] C
	55	5.00 (d, 6.5)	74.7 CH	4.93 (d, 6.3)	75.5 CH
	56	2.21 (oct, 6.5)	29.9 CH	2.23 (m)	30.6 CH
	57	1.06 (ob)	18.0 CH_3	1.10 (d, 6.8)	18.8 CH_3

	58	1.06 (ob)	18.0 CH_3	1.10 (d, 6.8)	18.5 CH_3
N,*N*-Dimethylvaline	59	-	172.4C	-	172.5[d] C
	60	2.84 (d, ob)	73.8 CH	2.89 (d, ob)	74.6 CH
	61	2.02 (m)	27.6 CH	2.12 (m)	28.3 CH
	62	0.99 (d, 6.6)	19.5 CH_3	1.02 (d, 6.0)	20.3 CH_3
	63	0.92 (d, 6.6)	19.6 CH_3	1.04 (d, ob)	19.9 CH_3
	64	2.34 (s)	41.3 CH_3	2.50 (s)	41.9 CH_3
	65	2.34 (s)	41.3 CH_3	2.50 (s)	41.9 CH_3
	N	-	δ_N 24.6	-	-

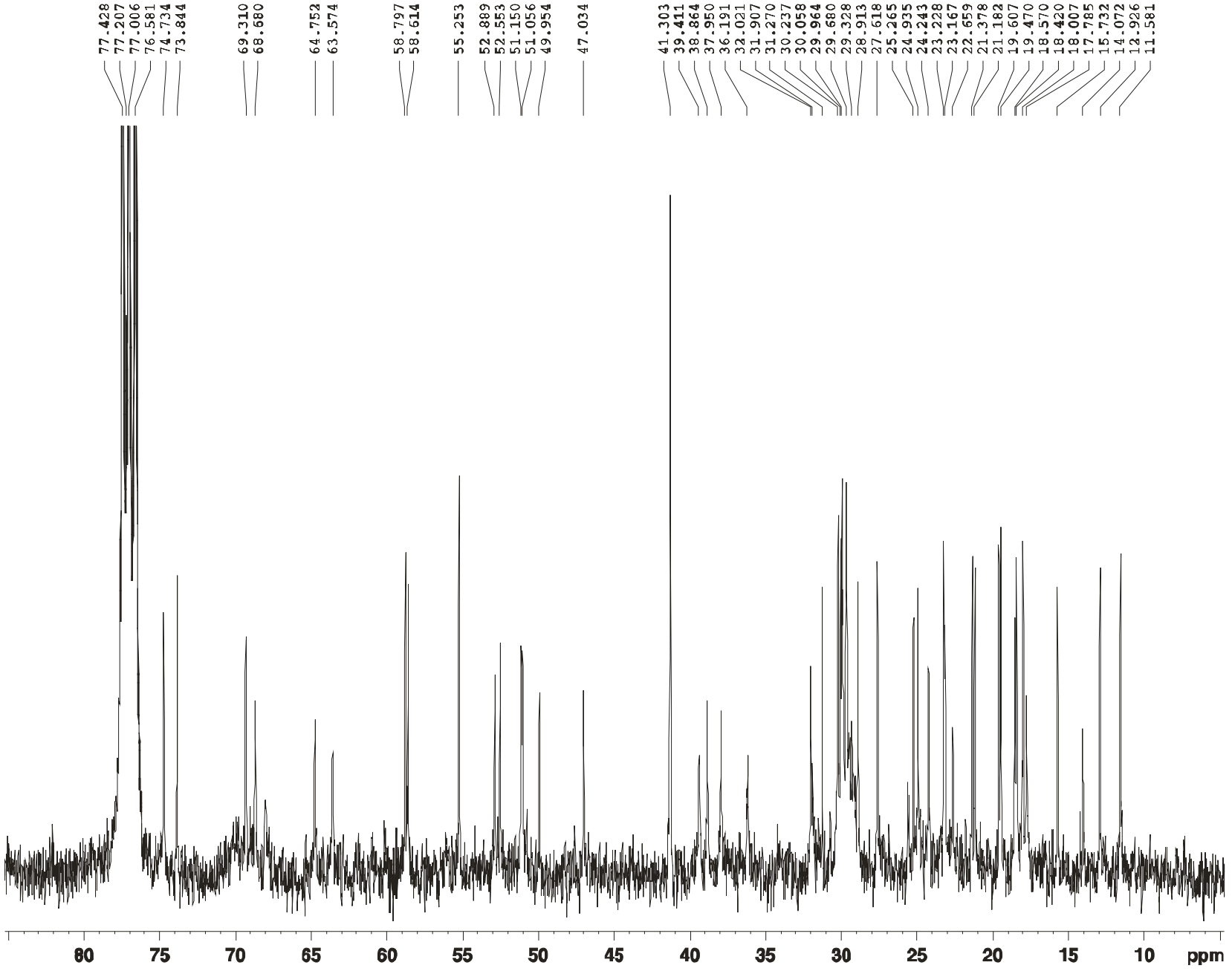
^{15}N shifts referenced to formamide at δ_H 112.0 ppm.

ob = obscured

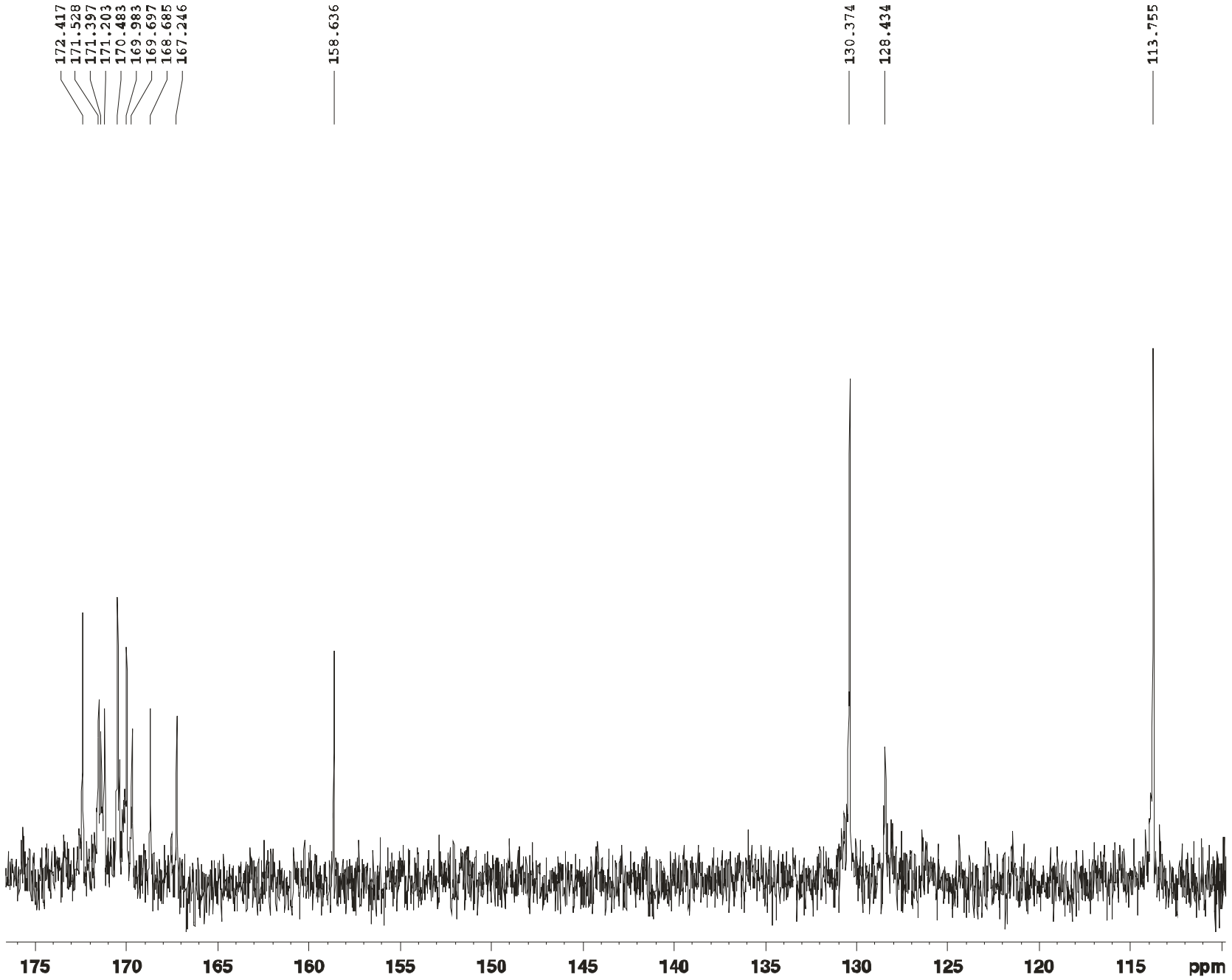
[a, b, c, d, e] carbonyl chemical shifts are exchangeable with others of the same superscript



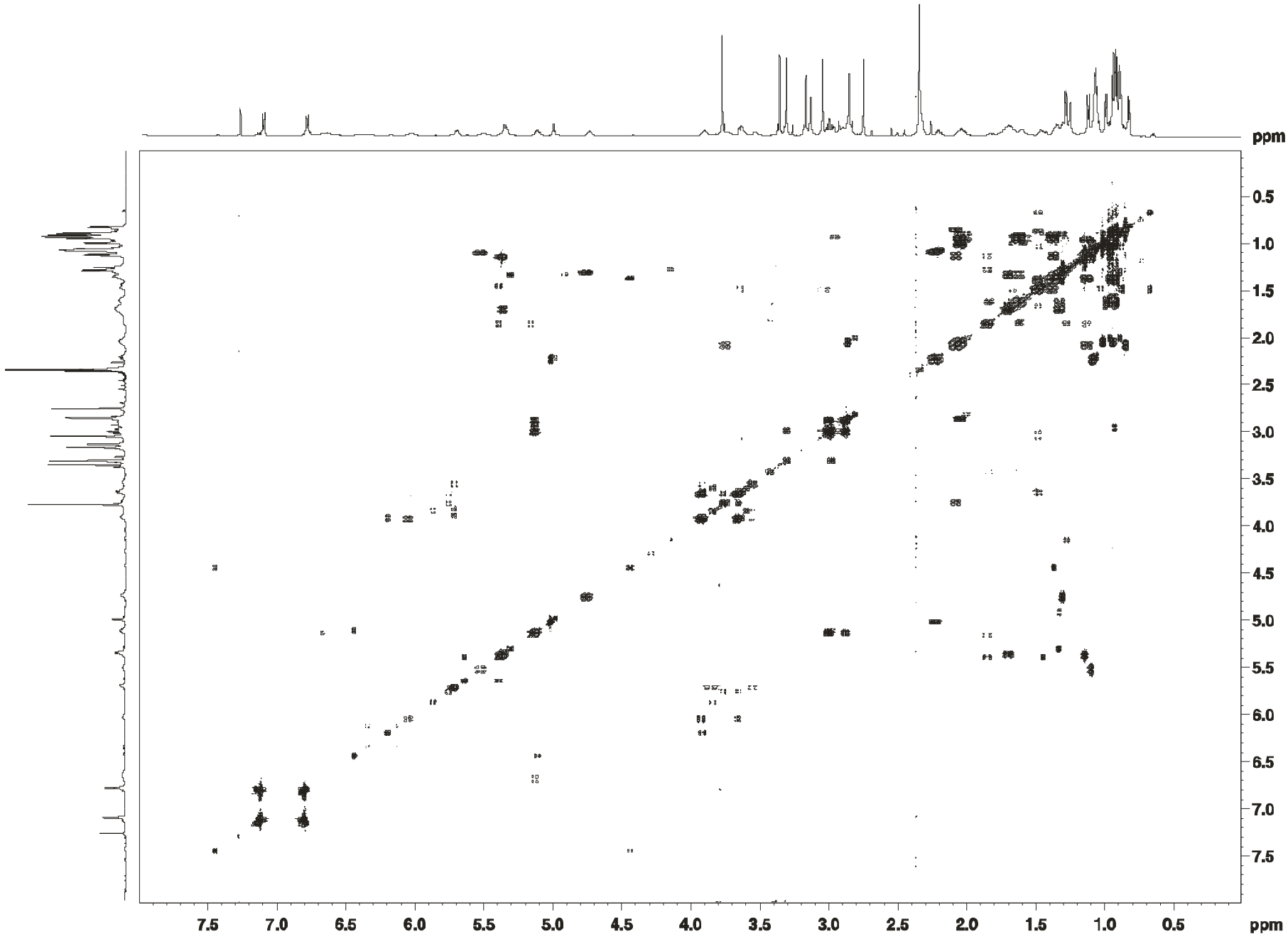
^{1}H NMR Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



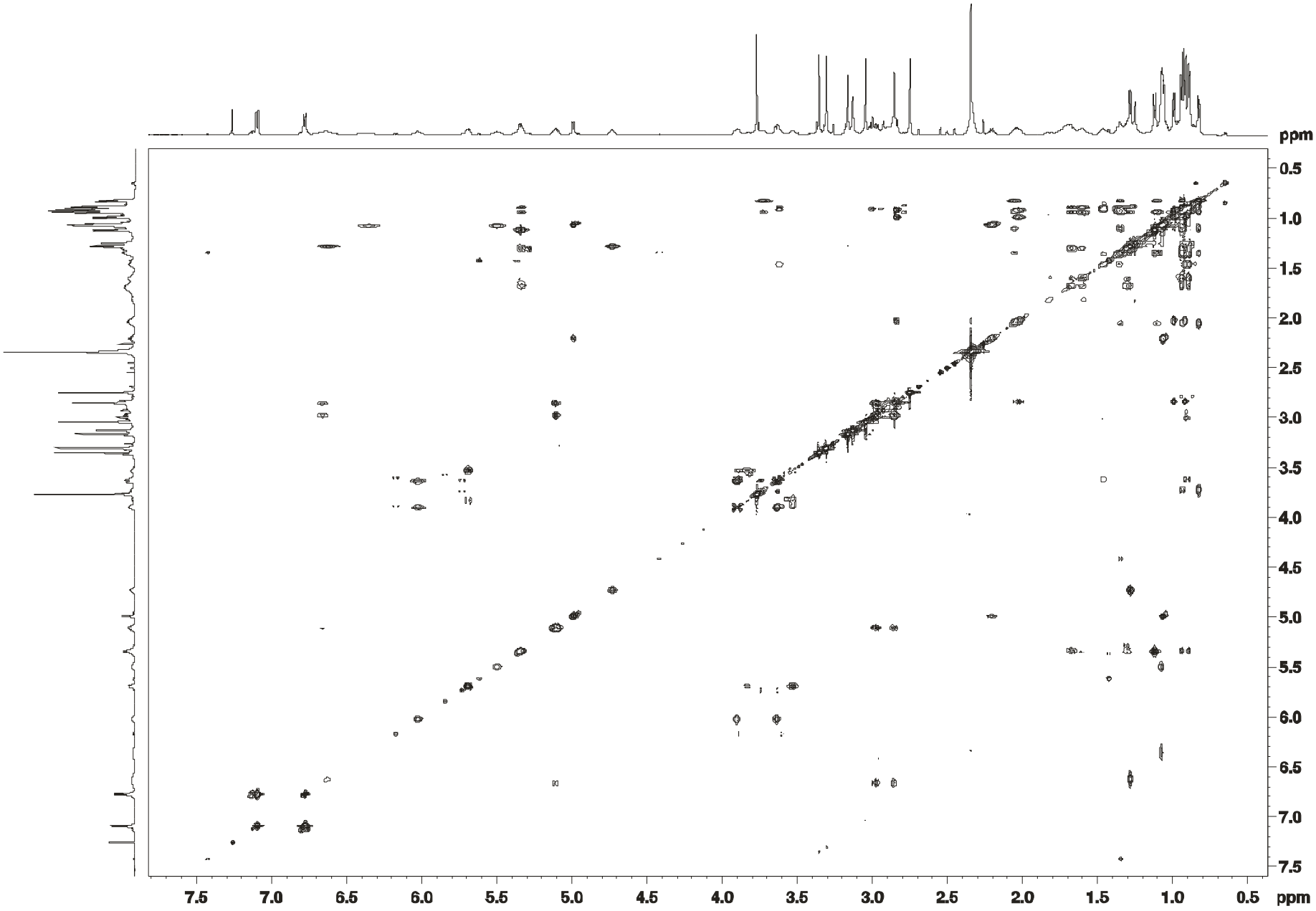
Partial ^{13}C NMR Spectrum for Coibamide A (**1**) in $CDCl_3$ (75 Mhz)



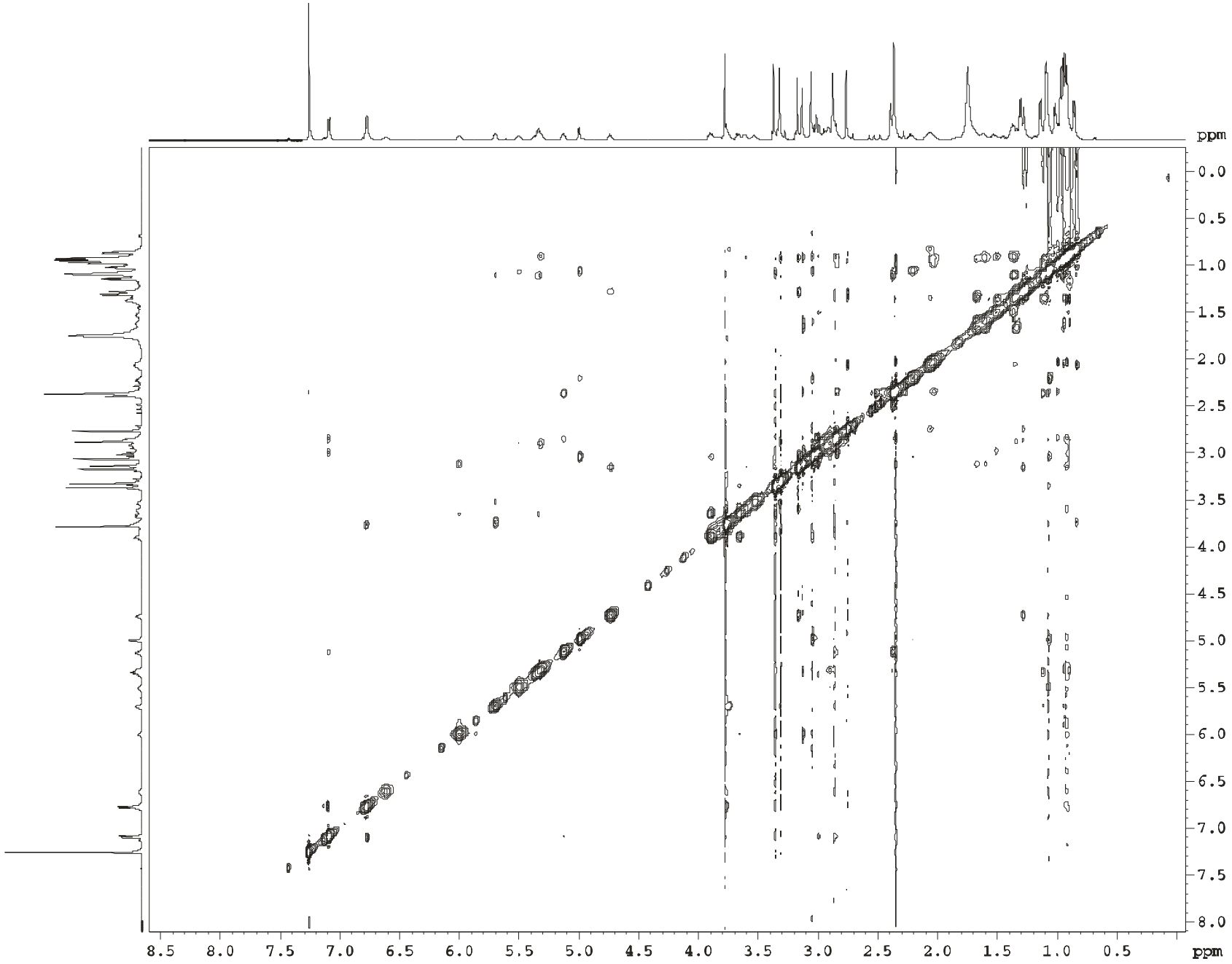
Partial ^{13}C NMR Spectrum for Coibamide A (**1**) in $CDCl_3$ (75 Mhz)



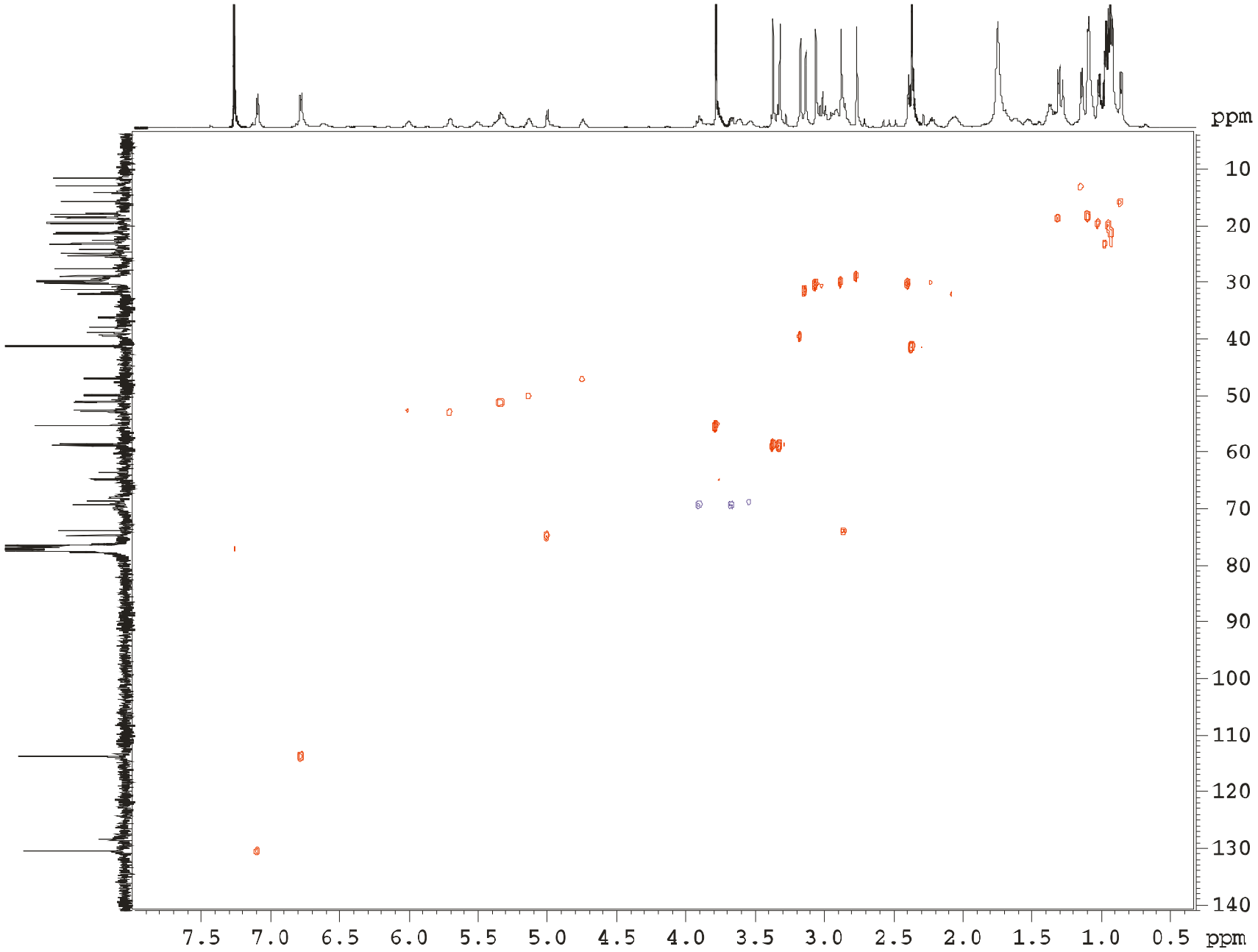
gCOSY Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



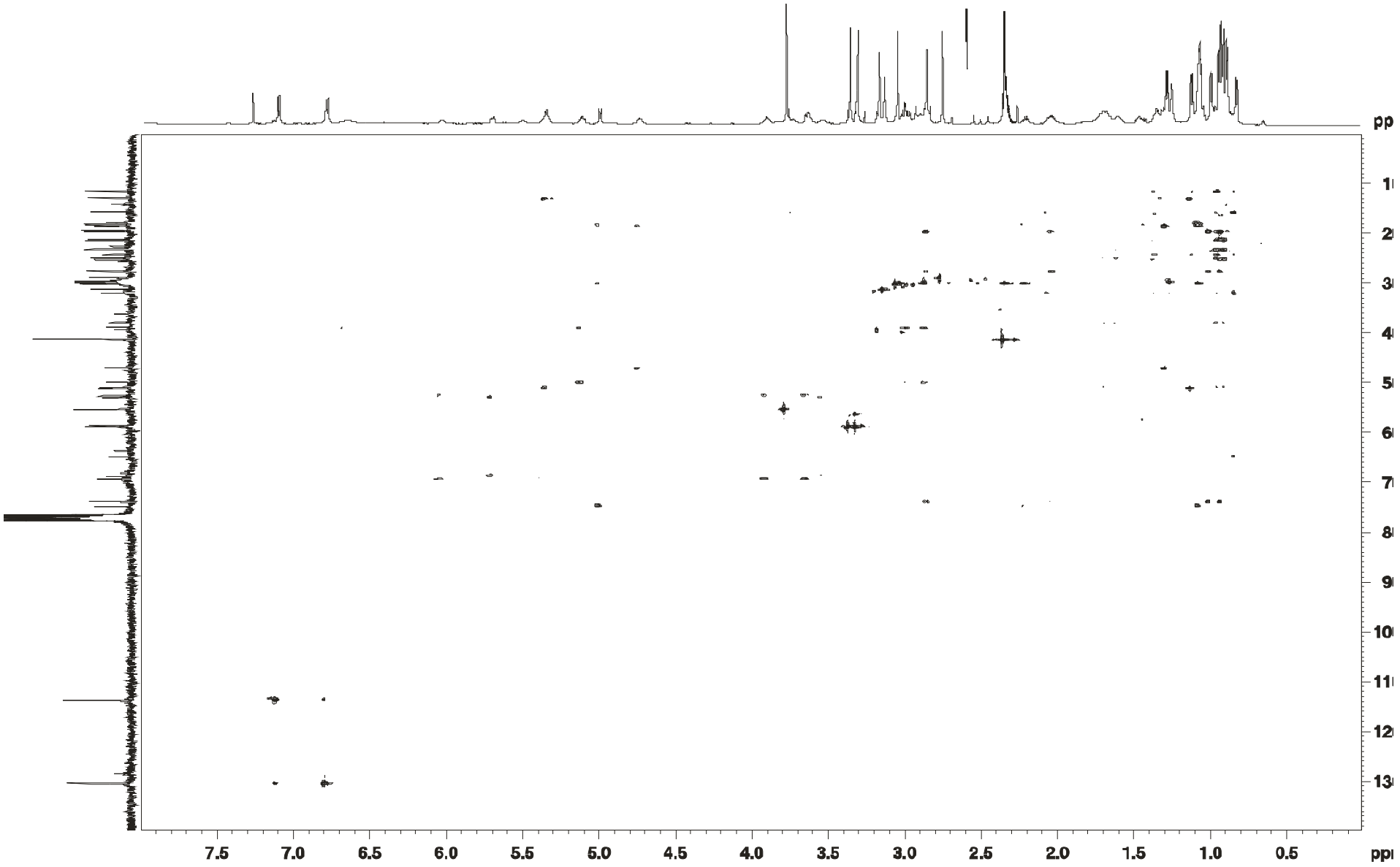
gTOCSY Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



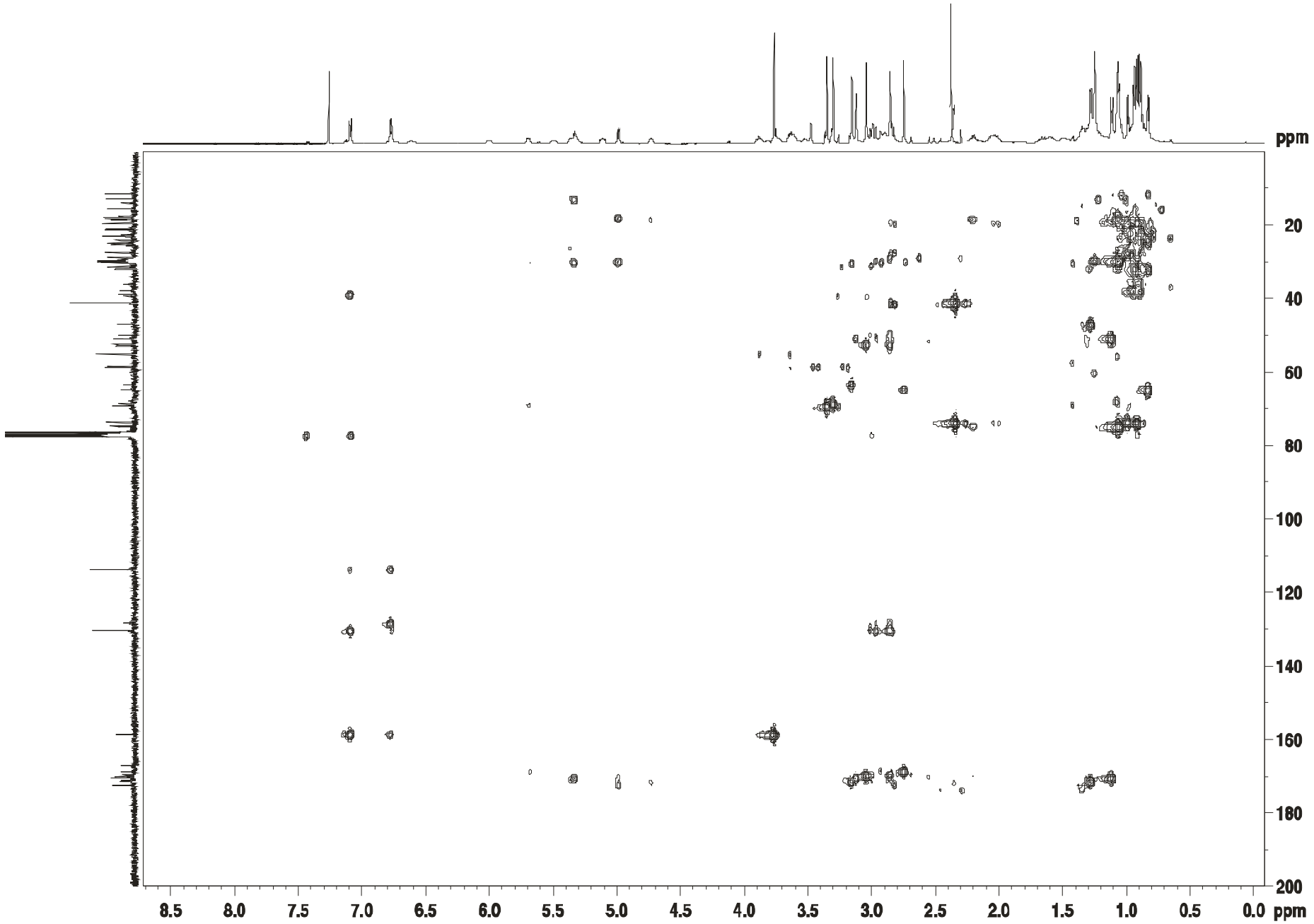
gROESY Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



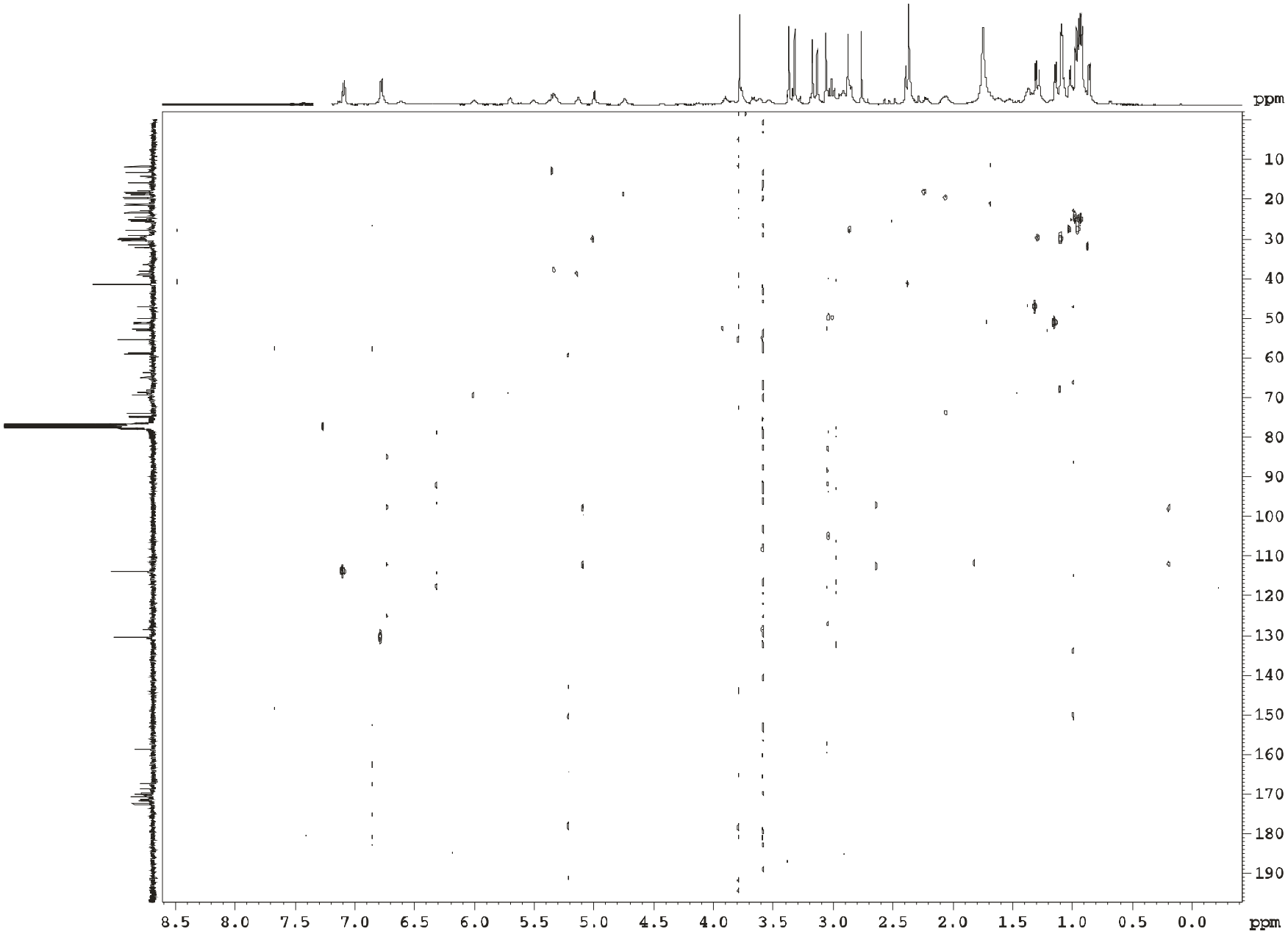
Multiplicity-edited gHSQC Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



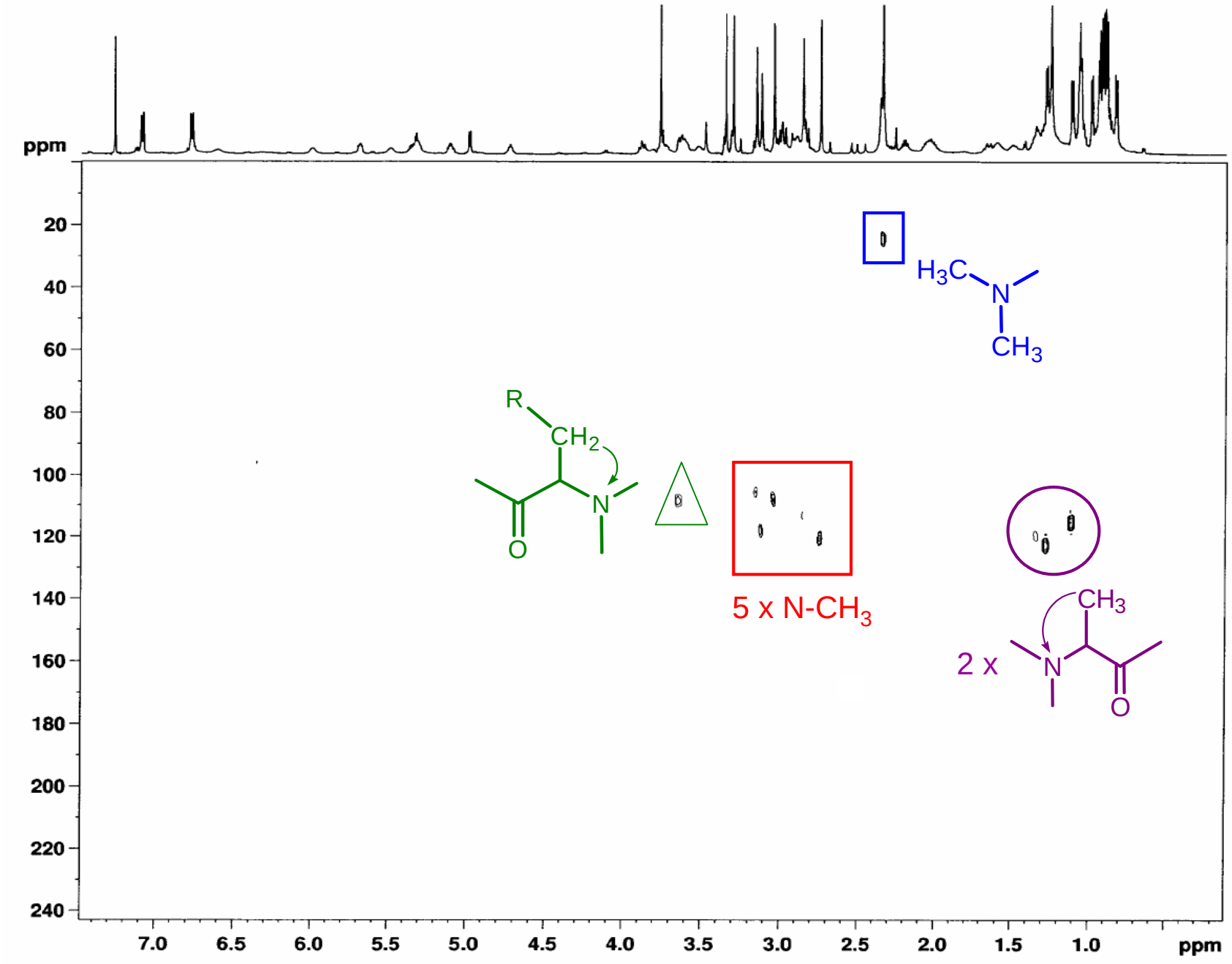
HSQC-TOCSY Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



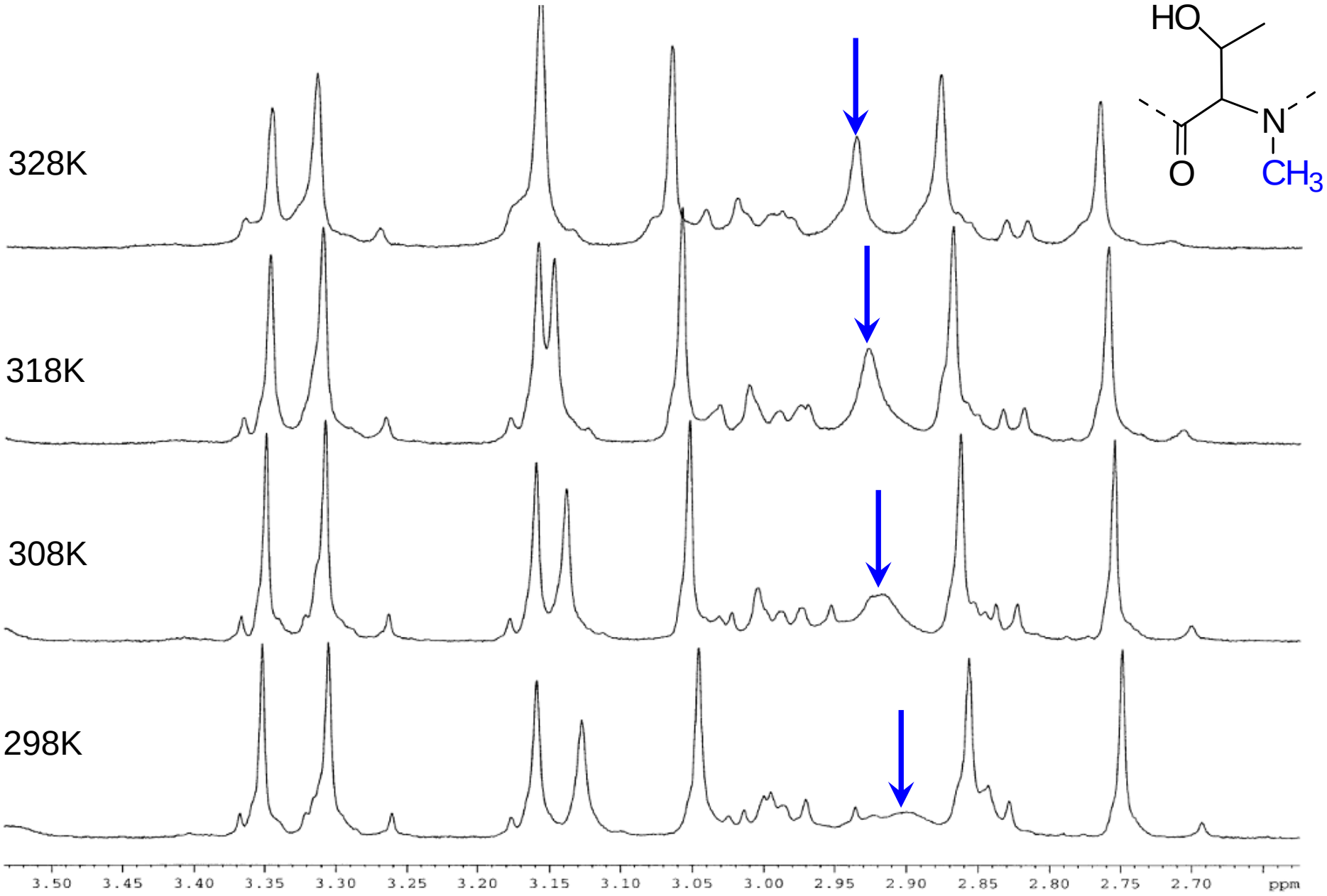
gHMBC Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz, d6 = 65 ms)



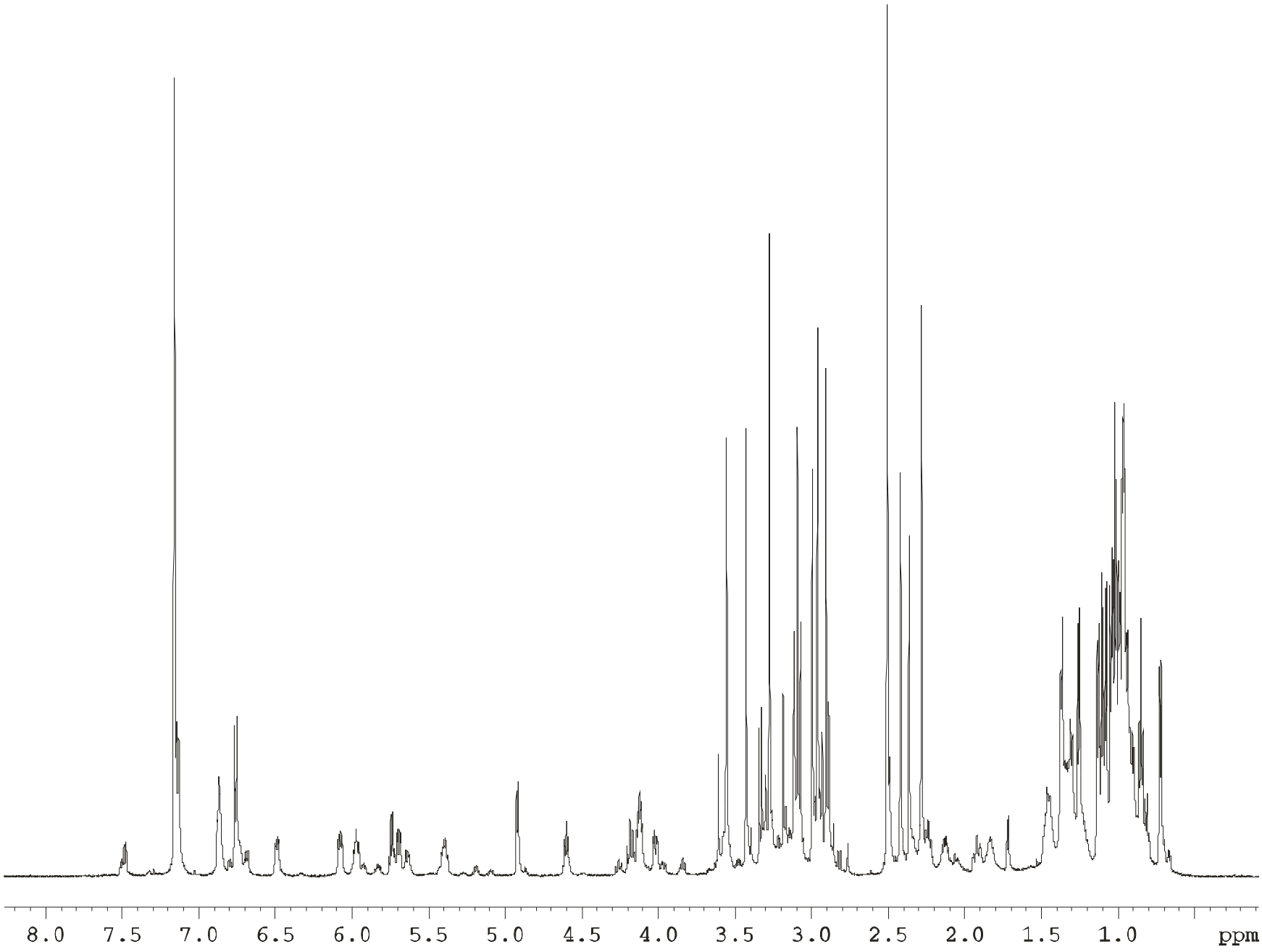
gH2BC Spectrum for Coibamide A (**1**) in $CDCl_3$ (600 Mhz)



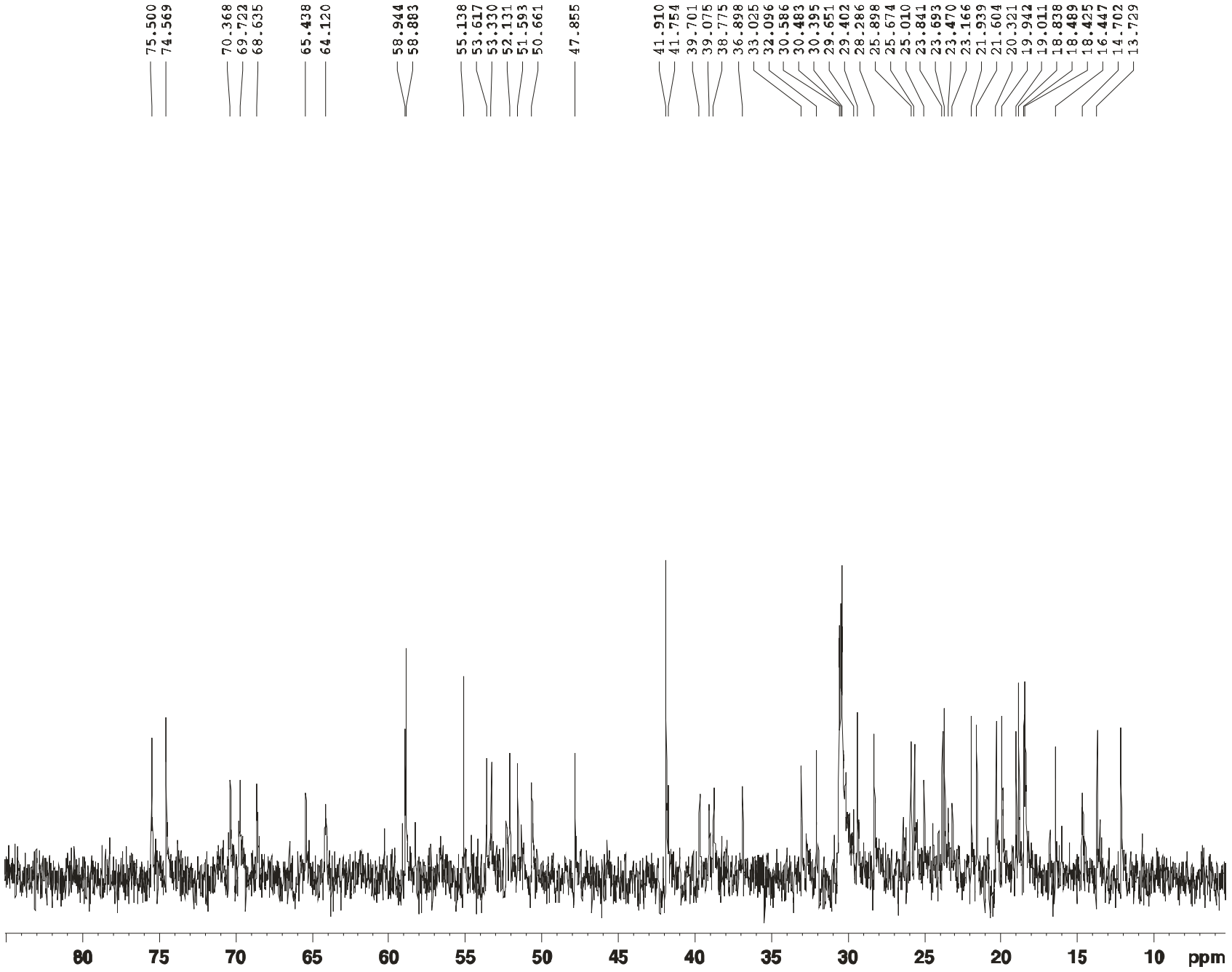
^{1}H-^{15}N gHMBC Spectrum for Coibamide A (**1**) in CDCl3 (600 Mhz)



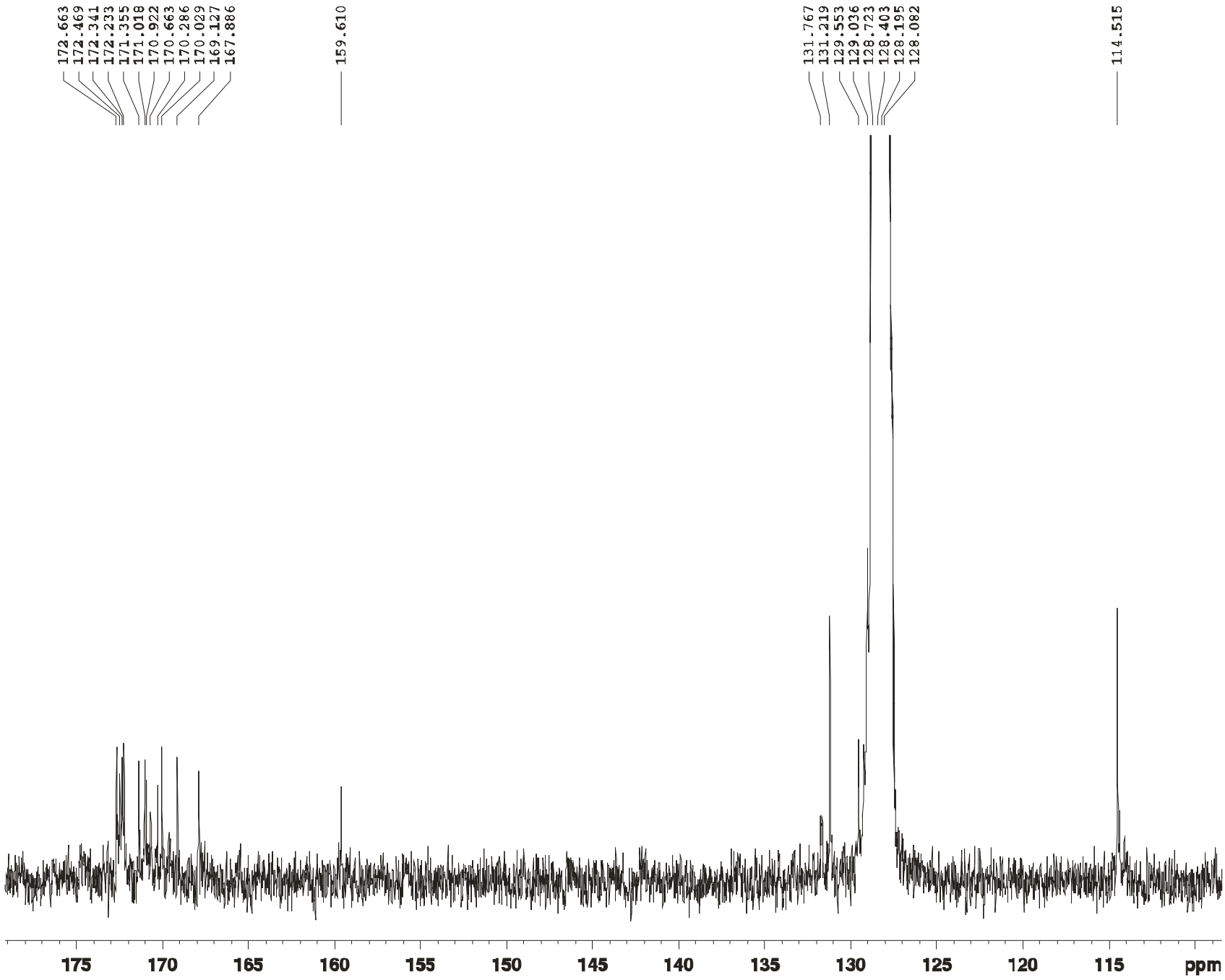
Variable temperature ^{1}H NMR Spectra in $CDCl_3$ for Coibamide A (700 MHz, 1mm cryoprobe)



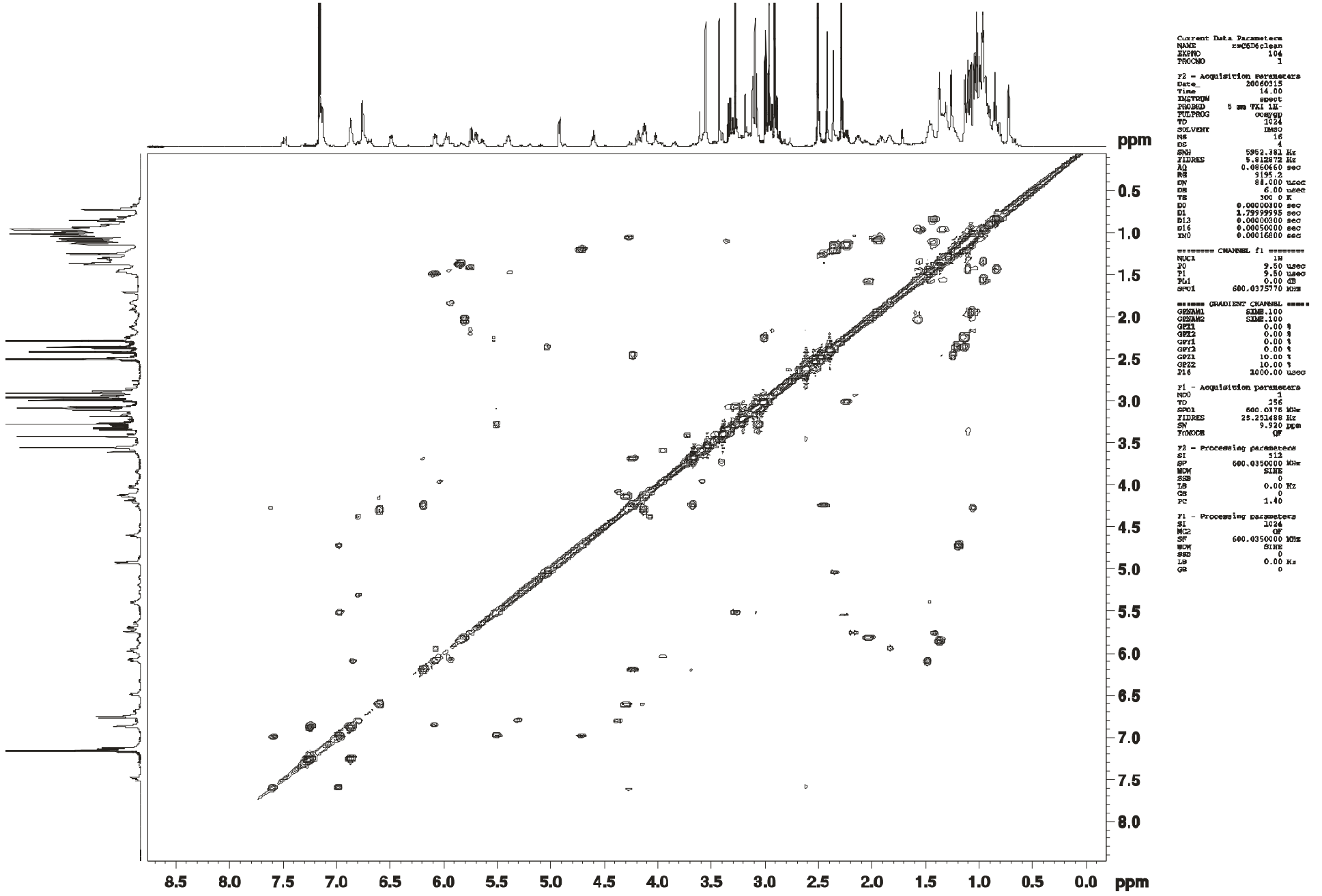
^{1}H NMR Spectrum for Coibamide A (**1**) in C_6D_6 (600 Mhz)



Partial ^{13}C NMR Spectrum for Coibamide A (**1**) in C_6D_6 (75 Mhz)



Partial ^{13}C NMR Spectrum for Coibamide A (**1**) in C_6D_6 (75 Mhz)

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EXPNO               104
PROCNO                1

F2 - Acquisition Parameters
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PULPROG         cosygp
TD                1024
SOLVENT           DMSO
NS                  16
DS                   4
SWH           5952.381 Hz
FIDRES        5.812673 Hz
AQ            0.0860660 sec
RG               9195.2
DW              84.000 usec
DE               6.00 usec
TE              300.0 K
D0           0.00000300 sec
D1           1.79999995 sec
D13          0.00000300 sec
D16          0.00050000 sec
IN0          0.00016800 sec

======== CHANNEL f1 ========
NUC1                1H
P0                9.50 usec
P1                9.50 usec
PL1               0.00 dB
SFO1        600.0375770 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPZ1              0.00 %
GPZ2              0.00 %
GPY1              0.00 %
GPY2              0.00 %
GPZ1             10.00 %
GPZ2             10.00 %
P16            1000.00 usec

F1 - Acquisition Parameters
ND0                  1
TD                 256
SFO1        600.0376 MHz
FIDRES       28.251488 Hz
SW            9.920 ppm
FnMODE             QF

F2 - Processing parameters
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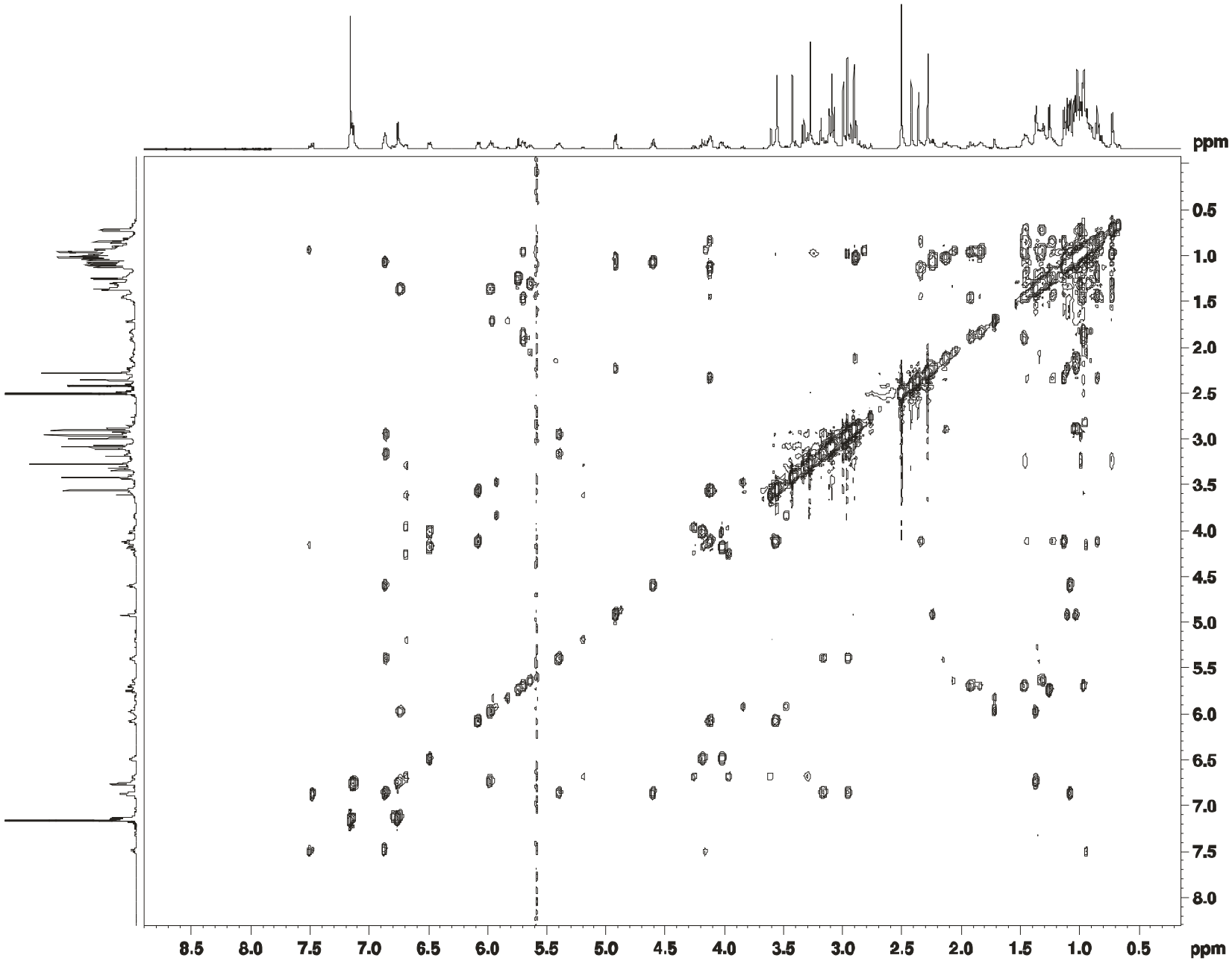
WDW              SINE
SSB                 0
LB               0.00 Hz
GB                  0
PC               1.40

F1 - Processing parameters
SI                1024
MC2               QF
SF          600.0350000 MHz
WDW              SINE
SSB                 0
LB               0.00 Hz
GB                  0
```

gCOSY Spectrum for Coibamide A (**1**) in C_6D_6 (600 Mhz)

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EXPNO                   105
PROCNO                    1

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PULPROG     ekmlevtpA.tr
TD                    2048
SOLVENT              CDCl3
NS                      72
DS                      16
SWH              5952.381 Hz
FIDRES           2.906436 Hz
AQ               0.1720820 sec
RG                    3251
DW                 84.000 usec
DE                  6.00 usec
TE                 300.0 K
d0               0.00000300 sec
D1               2.00000000 sec
D11              0.03000000 sec
d12              0.00002000 sec
D16              0.00030000 sec
d16              0.00030000 sec
IN0              0.00008400 sec
L1                      28

======== CHANNEL f1 ========
NUC1                    1H
P1                   9.30 usec
P2                  18.60 usec
P5                  22.34 usec
P6                  13.50 usec
P7                  67.00 usec
P16               2500.00 usec
PL1                  0.00 dB
PL10                13.00 dB
SFO1          600.0375770 MHz

F1 - Acquisition parameters
ND0                     2
TD                    128
SFO1            600.0376 MHz
FIDRES           46.502975 Hz
SW                9.920 ppm
FnMODE               TPPI

F2 - Processing parameters
SI                    1024
SF            600.0350685 MHz
WDW                 QSINE
SSB                     2
LB                   0.00 Hz
GB                      0
PC                   1.40

F1 - Processing parameters
SI                    1024
MC2                  TPPI
SF            600.0350689 MHz
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SSB                     2
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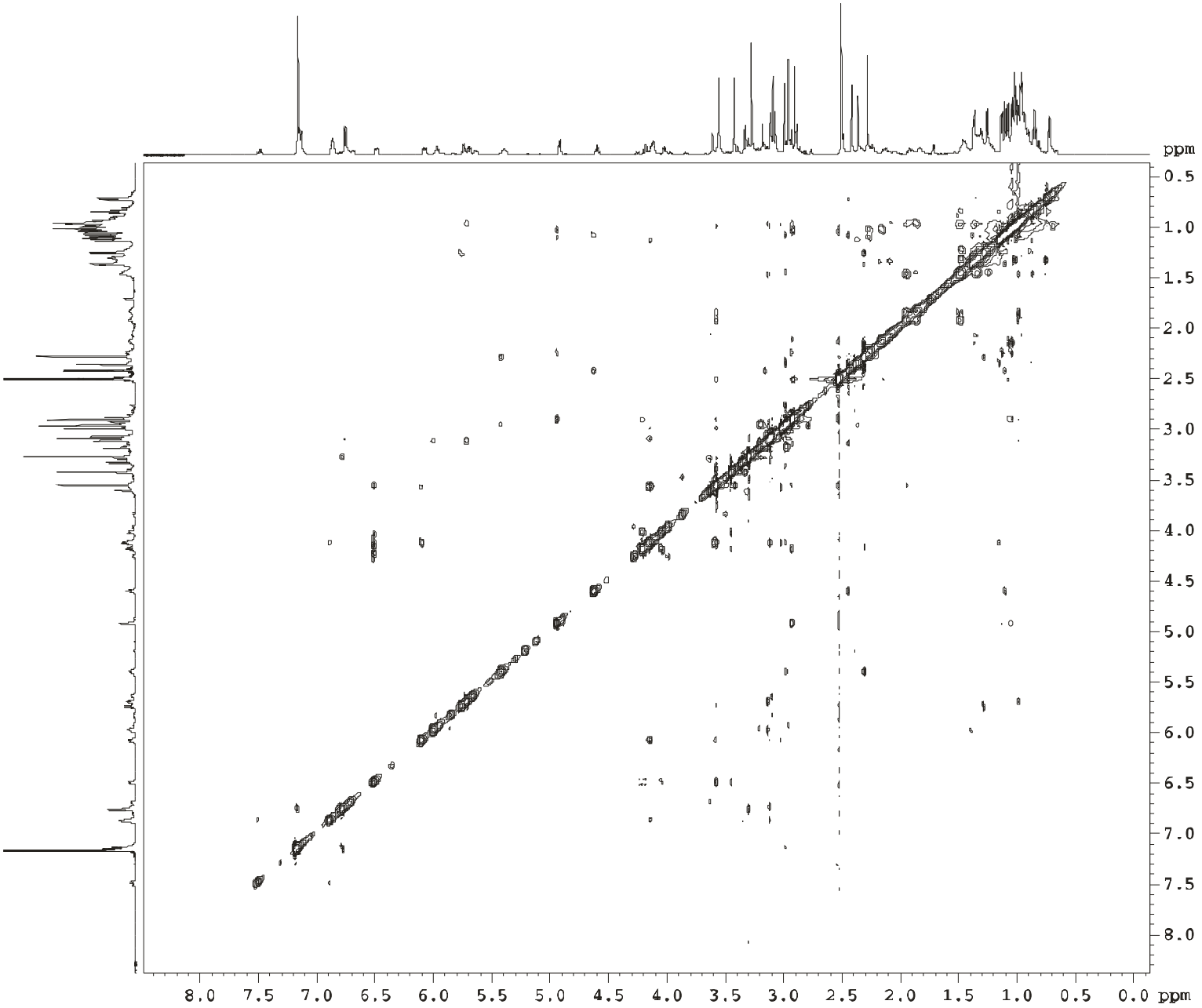
gTOCSY Spectrum for Coibamide A (**1**) in C_6D_6 (600 Mhz)

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    PROCNO                 1

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    PULPROG        roesytp.2
    TD                  2048
    SOLVENT              D2O
    NS                    64
    DS                    32
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  FIDRES          2.906436 Hz
  AQ            0.1720820 sec
    RG                 645.1
  DW               84.000 usec
  DE                4.50 usec
    TE               298.0 K
  D0           0.00000300 sec
  D1           2.00000000 sec
  D12          0.00002000 sec
  IN0          0.00008400 sec
    1d                   71d
P15          250000.00 usec

======== CHANNEL f1 ========
    NUC1                  1H
P1                   9.20 usec
P25                175.00 usec
  PL1                 0.00 dB
  PL11               22.00 dB
  SFO1        600.0375770 MHz

F1 - Acquisition parameters
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    TD                  388
  SFO1          600.0376 MHz
  FIDRES        15.341188 Hz
  SW                9.920 ppm
    FnMODE             TPPI

F2 - Processing parameters
    SI                 1024
  SF          600.0350555 MHz
    WDW               QSINE
    SSB                   2
  LB                 0.00 Hz
    GB                    0
    PC                 1.00

F1 - Processing parameters
    SI                 1024
    MC2                TPPI
  SF          600.0350677 MHz
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```
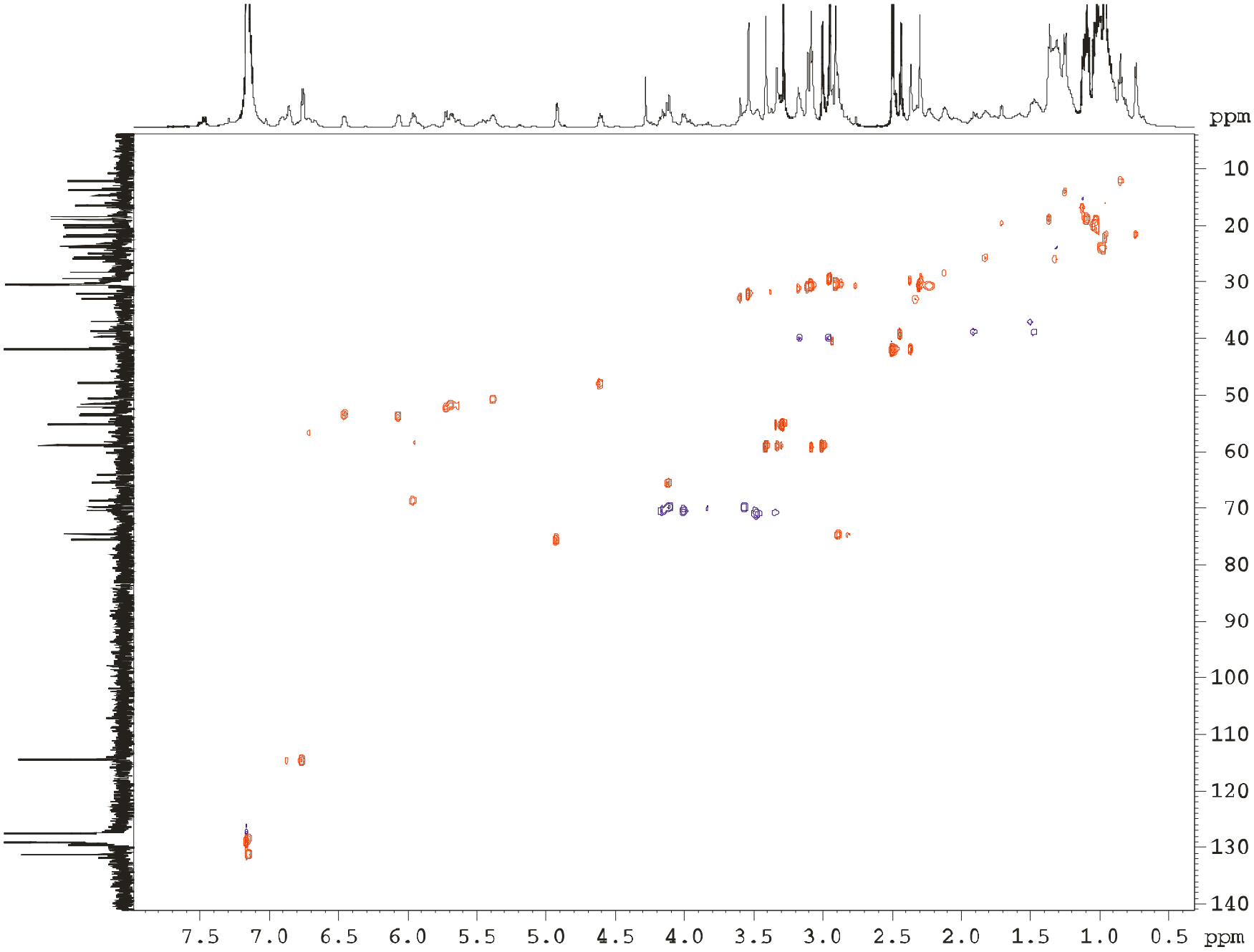
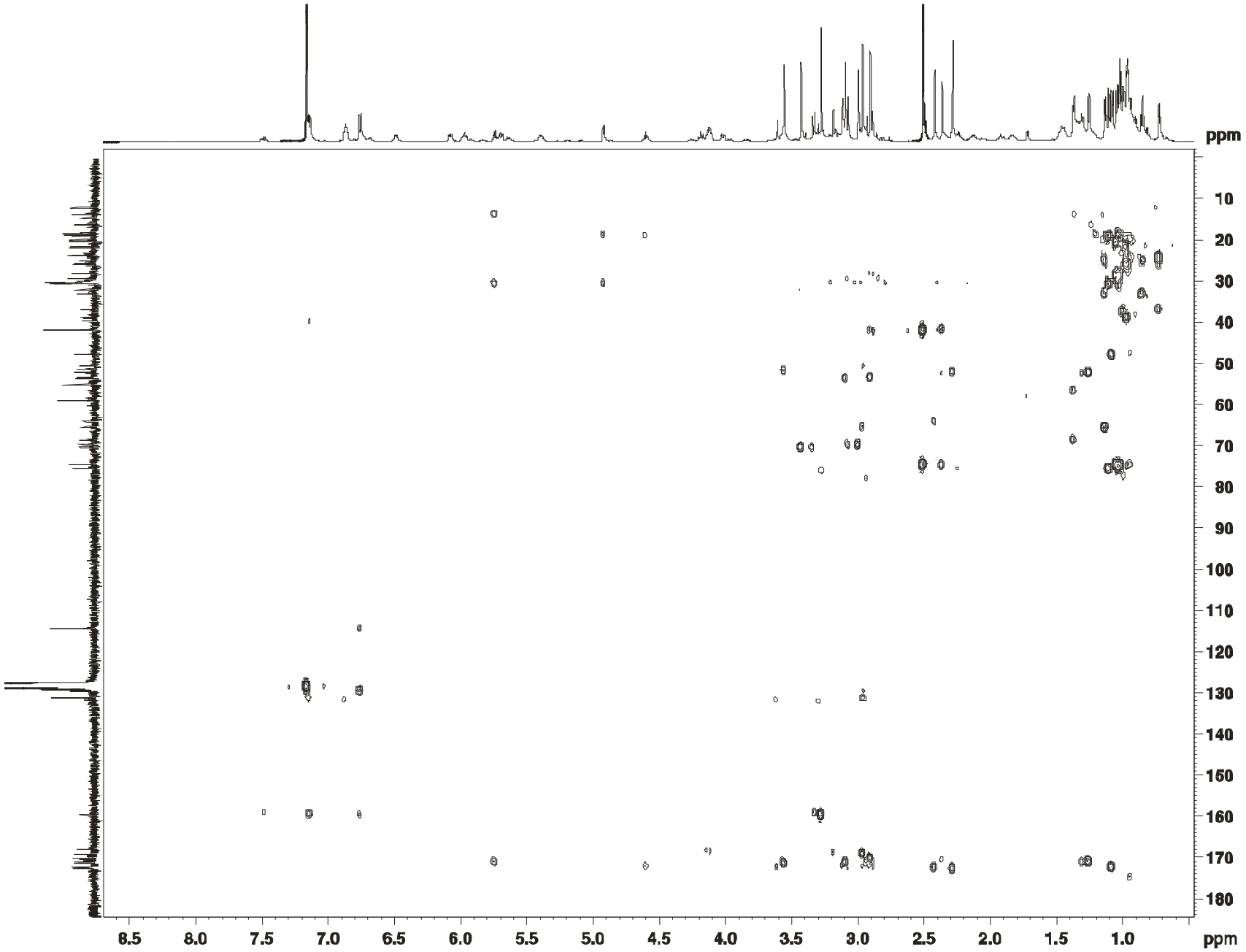

gROESY Spectrum for Coibamide A (**1**) in C_6D_6 (600 Mhz)

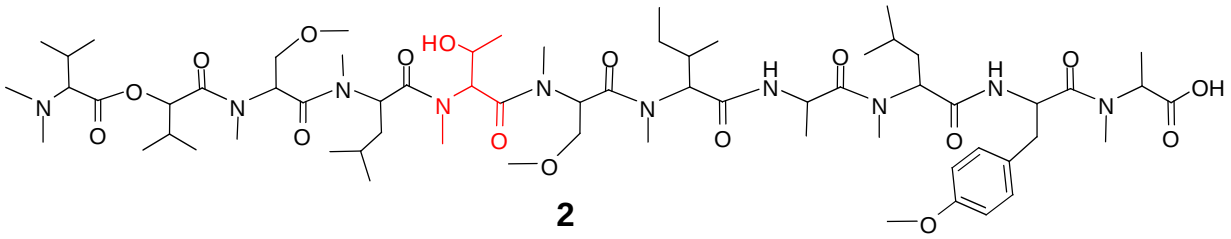


Multiplicity-edited gHSQC Spectrum for Coibamide A (**1**) in C_6D_6 (600 Mhz)

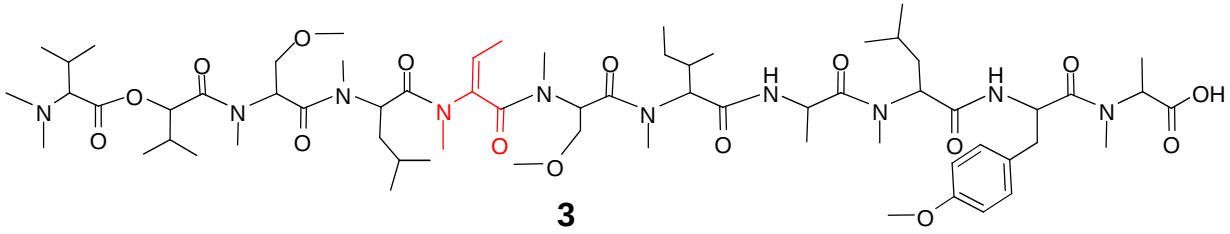


gHMBC Spectrum for Coibamide A (**1**) in C_6D_6 (600 Mhz, d6 = 65 ms)

Base Hydrolysis of Coibamide A (1). A portion of compound **1** (5.0 mg) was hydrolyzed in 1.0 M KOH (1 mL, reflux, overnight). The hydrolysis product was concentrated and analyzed by LC-MS (Zorbax RP-18 2.1 x 10 mm column, 0.1 mL/min, MeCN-H_2O gradient). Four major coibamide derivatives could be distinguished: hydrolyzed lactone only (**2**, t_R 19.0 min, $[M+H]^+$ 1306), dehydrated lactone (**3**, t_R 20 .0 min, $[M+H]^+$ 1288), hydrolyzed lactone and terminal ester (**4**, t_R 20 .6 min, $[M+H]^+$ 1179), dehydrated lactone and hydrolyzed terminal ester (**5**, t_R 21.4 min, $[M+H]^+$ 1161). MS/MS analysis of molecular ions for **2** and **3** gave the fragmentations shown (Figure S2) and these data support the assigned residue sequence of coibamide A (**1**).



2



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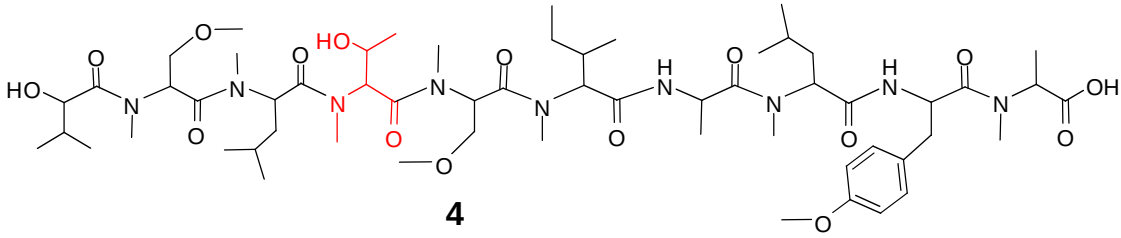

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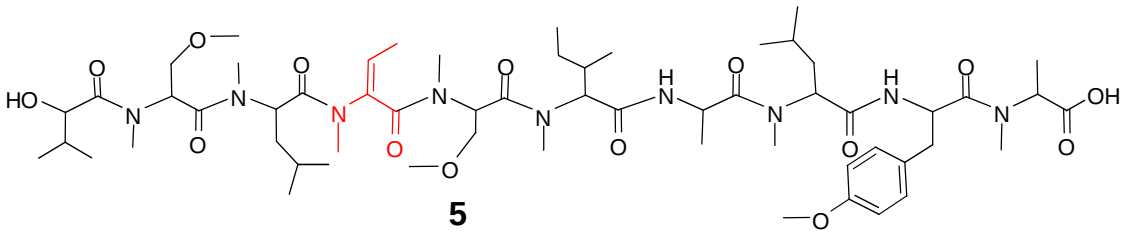

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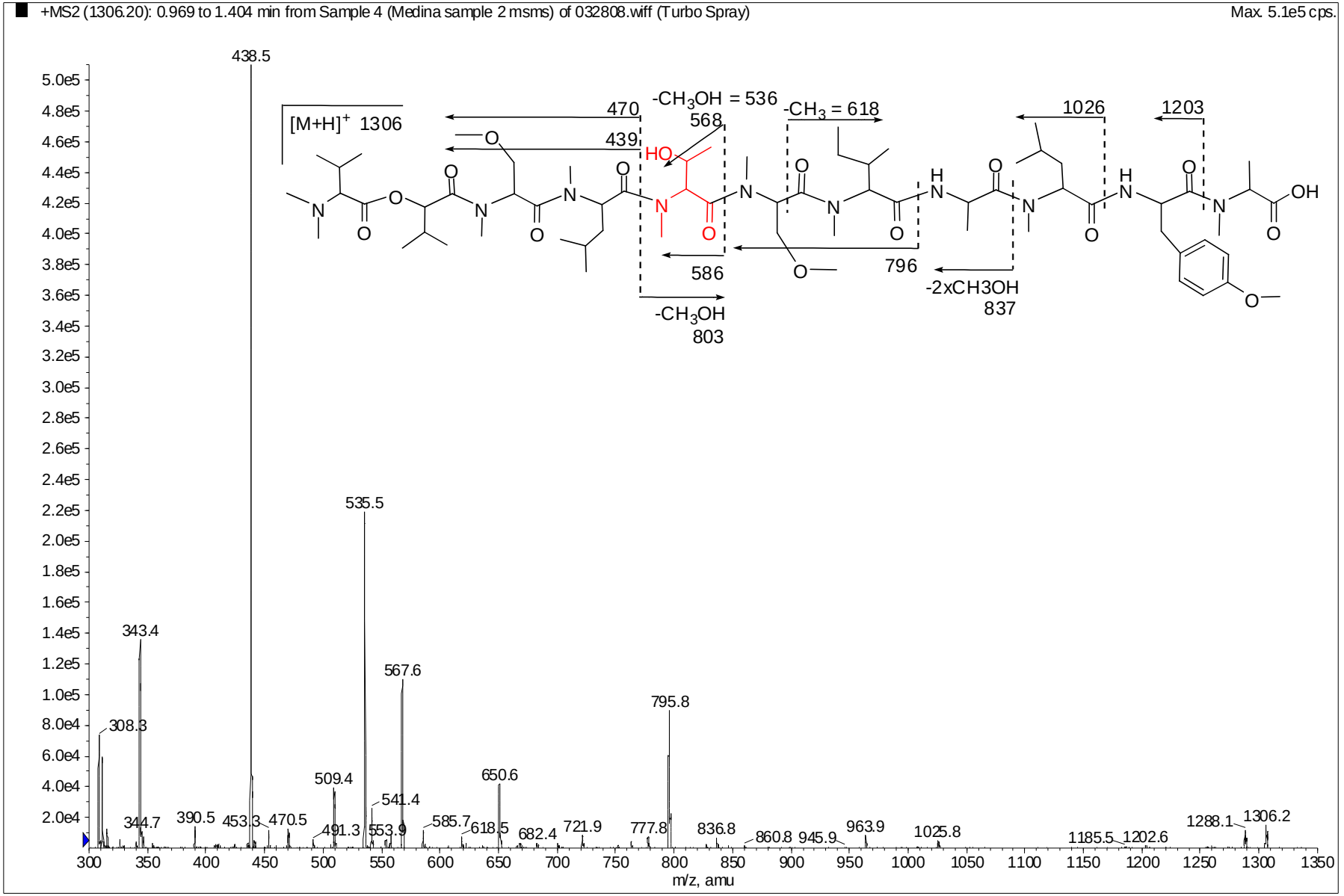


Figure S2. MS^2 of Base Hydrolysis Product **2** (hydrolyzed lactone only).

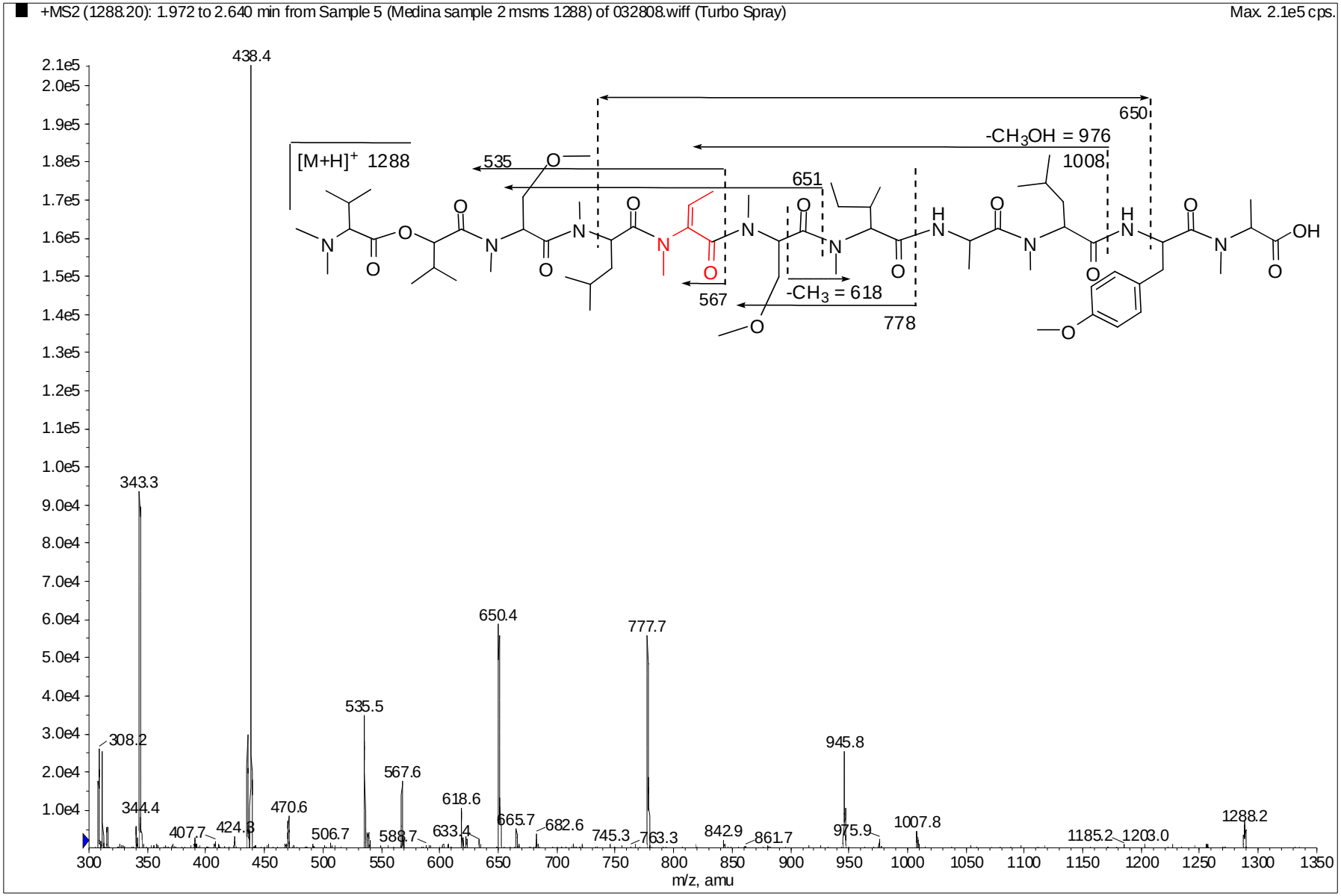
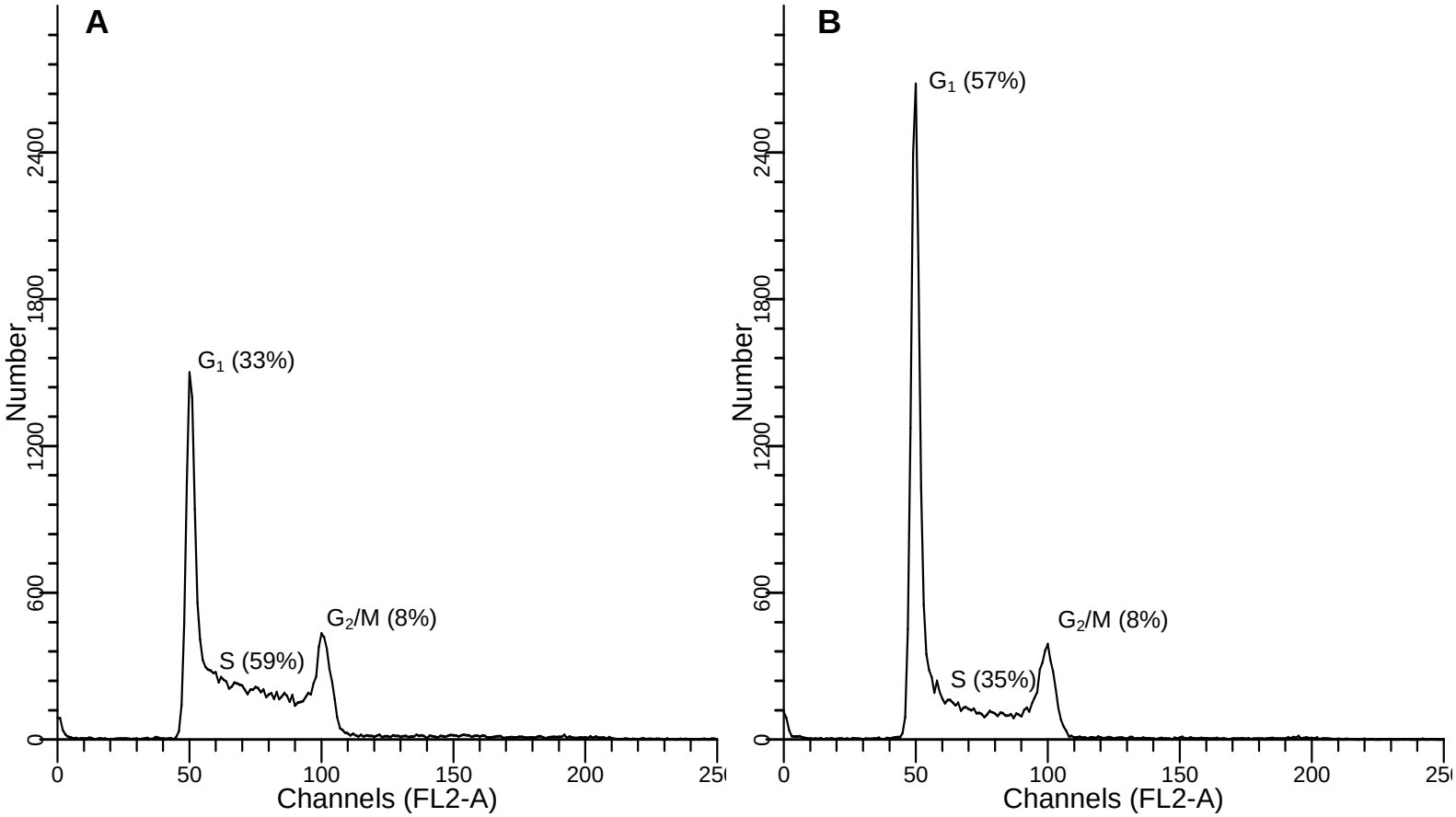
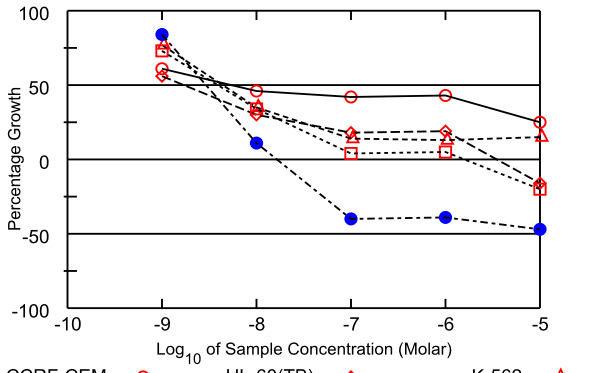


Figure S3. MS^2 of Base Hydrolysis Product **3** (dehydrated lactone, elimination product).



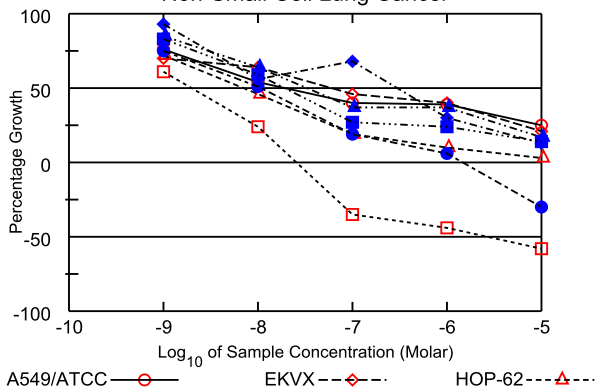
DNA Histograms for Flow Experiments in MDA-MB-435 breast cancer cells: A) control (drug vehicle); B) Coibamide A (25 nM); data obtained using ModFit software.

Leukemia



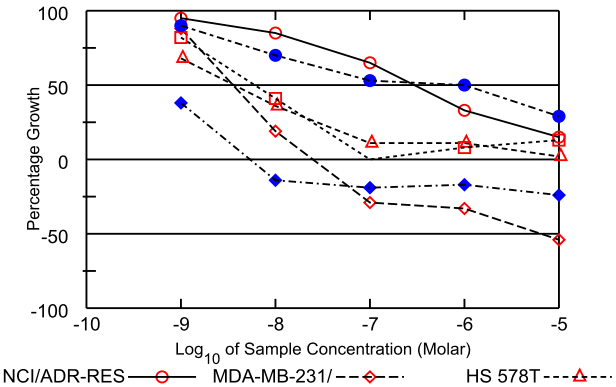
CCRF-CEM, HL-60(TB), K-562, RPMI-8226, SR

Non-Small Cell Lung Cancer



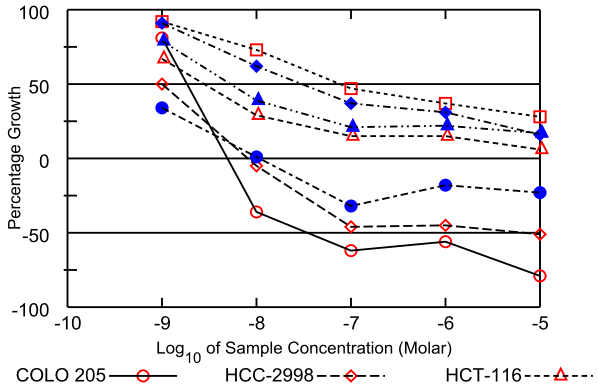
A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H522

Breast Cancer



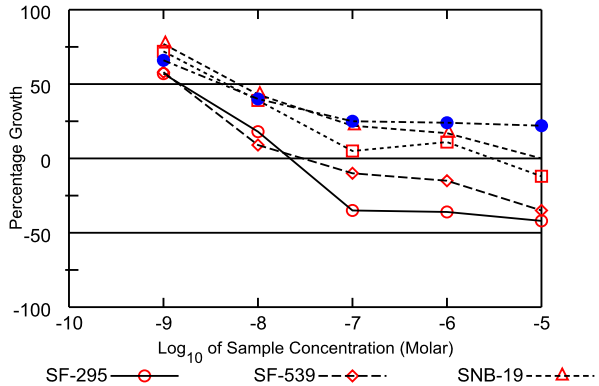
NCI/ADR-RES, MDA-MB-231/, HS 578T, MDA-MB-435, BT-549, T-47D

Colon Cancer



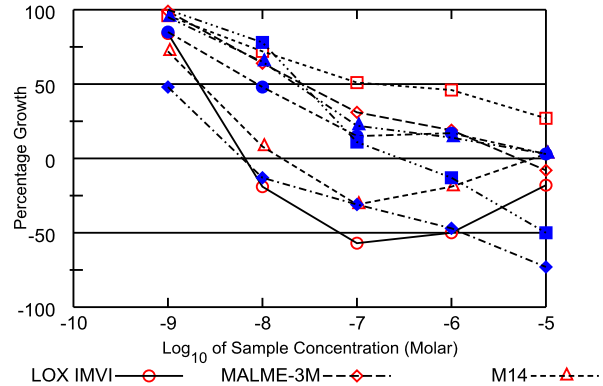
COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620

CNS Cancer



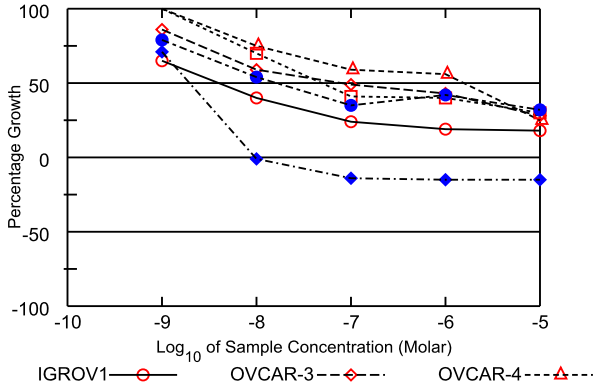
SF-295, SF-539, SNB-19, SNB-75, U251

Melanoma



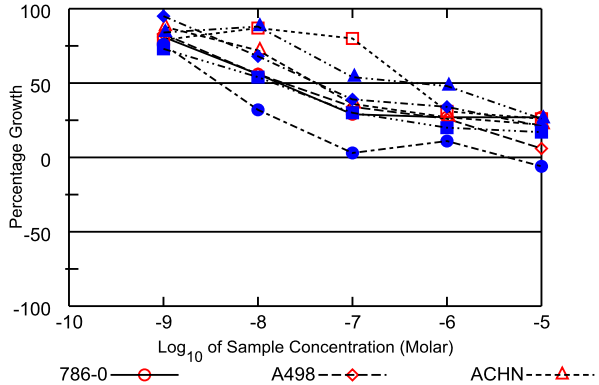
LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62

Ovarian Cancer



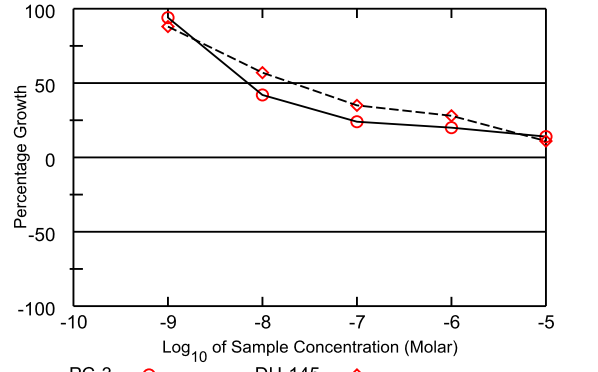
IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3

Renal Cancer



786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31

Prostate Cancer



PC-3, DU-145

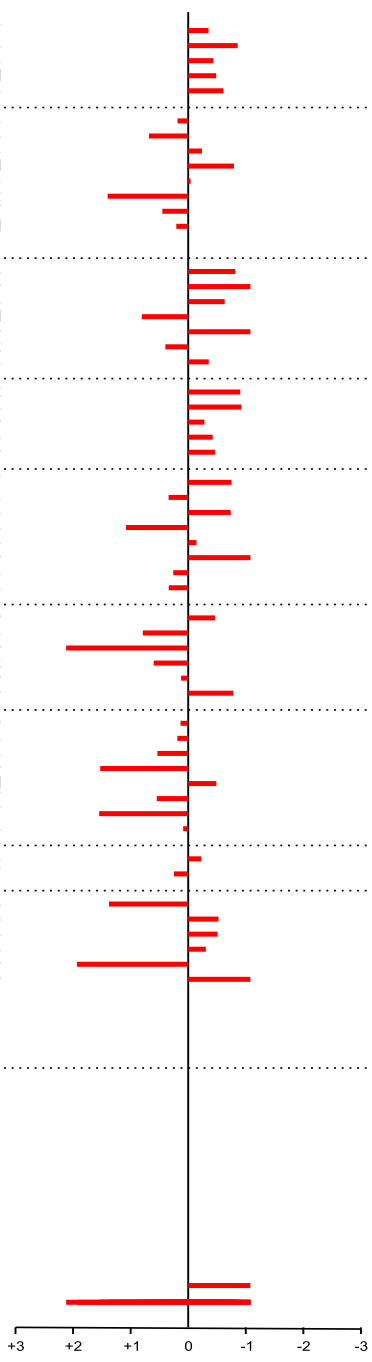
National Cancer Institute Developmental Therapeutics Program

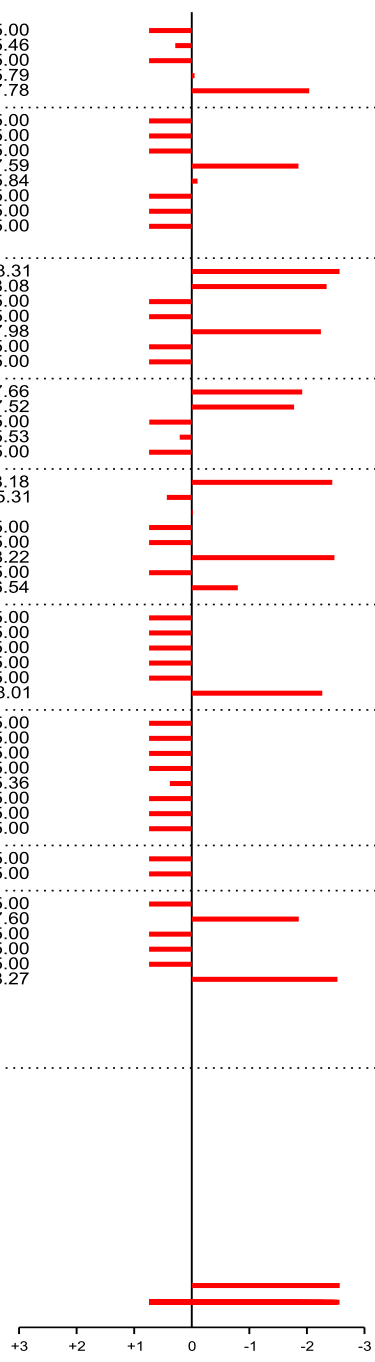
Mean Graphs

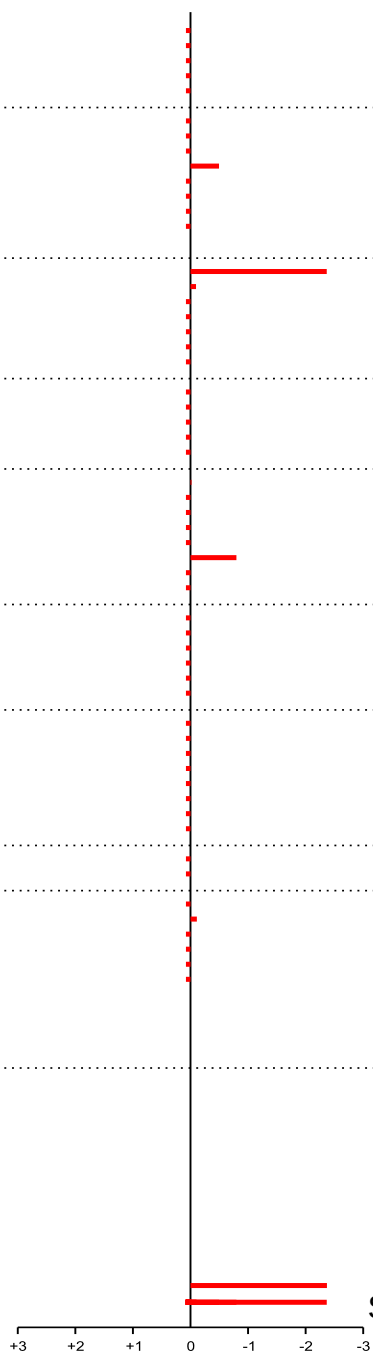
NSC : D - 742997 / 1 | Units : Molar | SSPL : 0WOB | EXP. ID : 0610NS24

Report Date : November 08, 2006 | Test Date : October 16, 2006

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-8.27		> -5.00		> -5.00	
HL-60(TB)	-8.78		-5.46		> -5.00	
K-562	-8.36		> -5.00		> -5.00	
RPMI-8226	-8.41		-5.79		> -5.00	
SR	-8.53		-7.78		> -5.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-7.73		> -5.00		> -5.00	
EKVX	-7.24		> -5.00		> -5.00	
HOP-62	-8.16		> -5.00		> -5.00	
HOP-92	-8.71		-7.59		-5.57	
NCI-H226	-7.96		-5.84		> -5.00	
NCI-H23	-6.52		> -5.00		> -5.00	
NCI-H322M	-7.47		> -5.00		> -5.00	
NCI-H522	-7.71		> -5.00		> -5.00	
Colon Cancer						
COLO 205	-8.74		-8.31		-7.45	
HCC-2998	< -9.00		-8.08		-5.18	
HCT-116	-8.55		> -5.00		> -5.00	
HCT-15	-7.11		> -5.00		> -5.00	
HT29	< -9.00		-7.98		> -5.00	
KM12	-7.52		> -5.00		> -5.00	
SW-620	-8.28		> -5.00		> -5.00	
CNS Cancer						
SF-295	-8.82		-7.66		> -5.00	
SF-539	-8.84		-7.52		> -5.00	
SNB-19	-8.20		-5.00		> -5.00	
SNB-75	-8.34		-5.53		> -5.00	
U251	-8.39		> -5.00		> -5.00	
Melanoma						
LOX IMVI	-8.67		-8.18			
MALME-3M	-7.58		-5.31		> -5.00	
M14	-8.66				> -5.00	
SK-MEL-2	-6.84		> -5.00		> -5.00	
SK-MEL-28	-8.06		> -5.00		> -5.00	
SK-MEL-5	< -9.00		-8.22		-5.88	
UACC-257	-7.66		> -5.00		> -5.00	
UACC-62	-7.58		-6.54		> -5.00	
Ovarian Cancer						
IGROV1	-8.39		> -5.00		> -5.00	
OVCAR-3	-7.13		> -5.00		> -5.00	
OVCAR-4	-5.80		> -5.00		> -5.00	
OVCAR-5	-7.32		> -5.00		> -5.00	
OVCAR-8	-7.80		> -5.00		> -5.00	
SK-OV-3	-8.70		-8.01		> -5.00	
Renal Cancer						
786-0	-7.79		> -5.00		> -5.00	
A498	-7.73		> -5.00		> -5.00	
ACHN	-7.38		> -5.00		> -5.00	
CAKI-1	-6.39		> -5.00		> -5.00	
RXF 393	-8.41		-5.36		> -5.00	
SN12C	-7.37		> -5.00		> -5.00	
TK-10	-6.37		> -5.00		> -5.00	
UO-31	-7.83		> -5.00		> -5.00	
Prostate Cancer						
PC-3	-8.15		> -5.00		> -5.00	
DU-145	-7.67		> -5.00		> -5.00	
Breast Cancer						
NCI/ADR-RES	-6.54		> -5.00		> -5.00	
MDA-MB-231/ATCC	-8.45		-7.60		-5.19	
HS 578T	-8.43		> -5.00		> -5.00	
MDA-MB-435	-8.23		> -5.00		> -5.00	
BT-549	-5.99		> -5.00		> -5.00	
T-47D	< -9.00		-8.27		> -5.00	
MID	-7.92		-5.74		-5.08	
Delta	1.08		2.57		2.37	
Range	3.2		3.31		2.45	

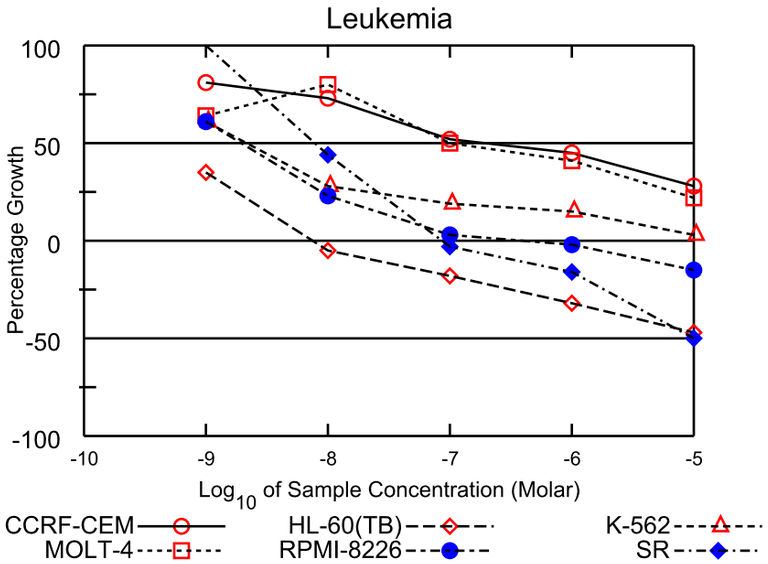


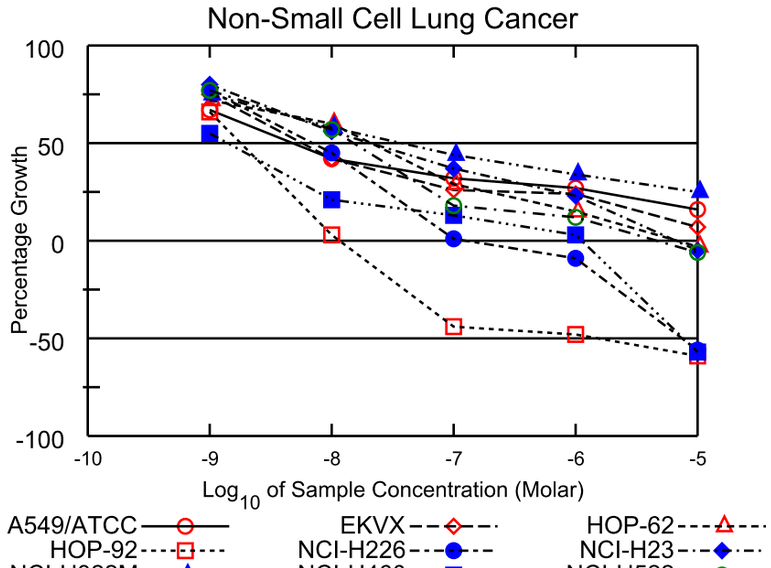


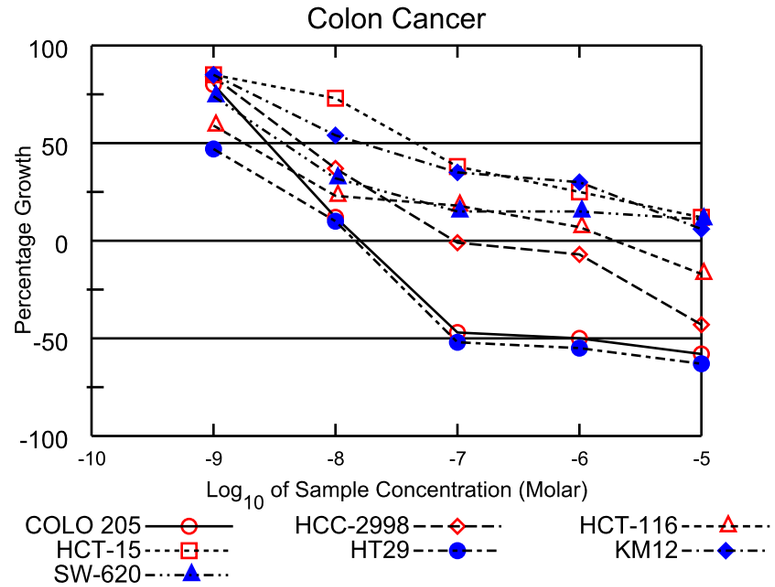


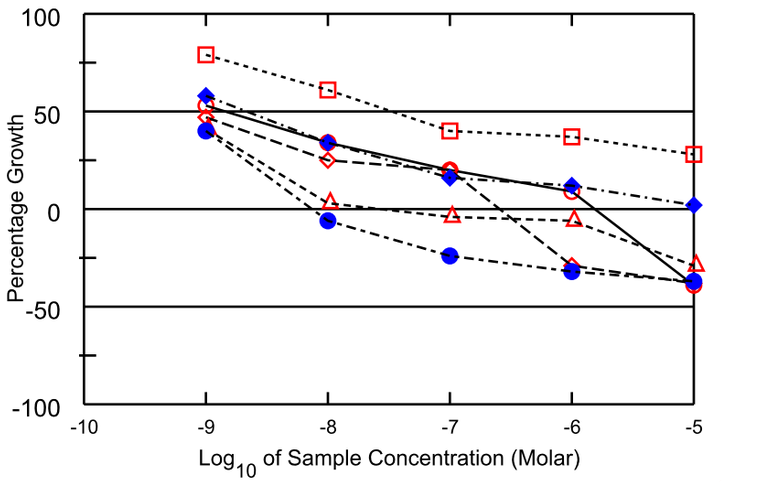
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

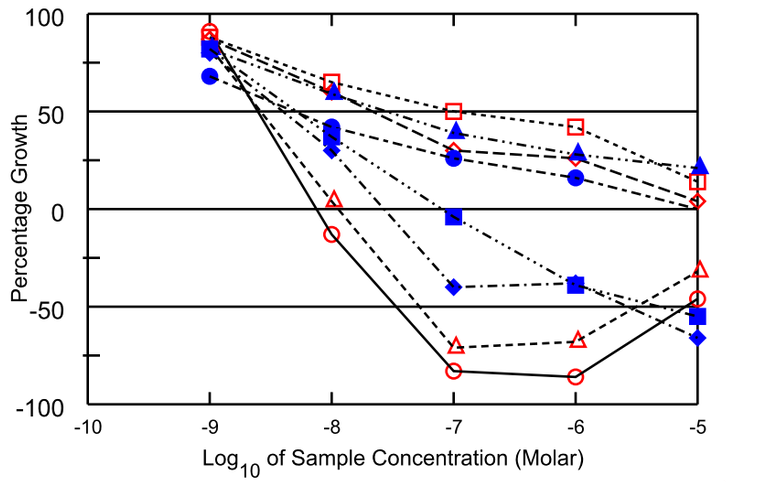
NSC: D - 742997 / 1	SSPL: 0WOB	EXP. ID: 0612RS45
Report Date: April 23, 2007	Test Date: December 04, 2006	

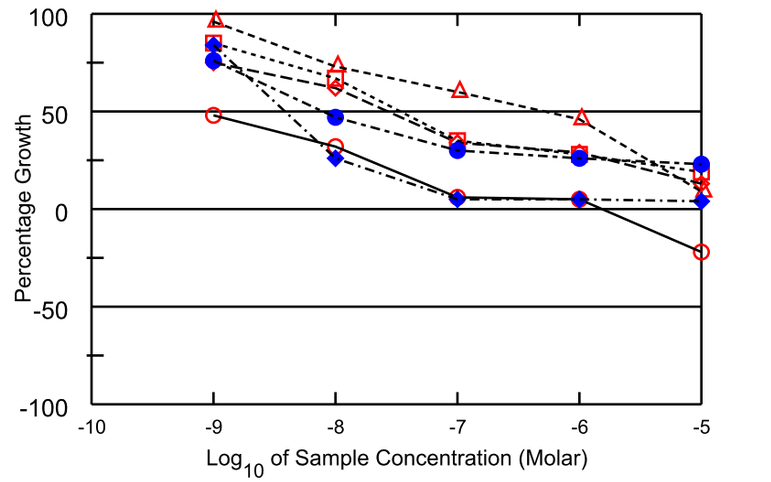


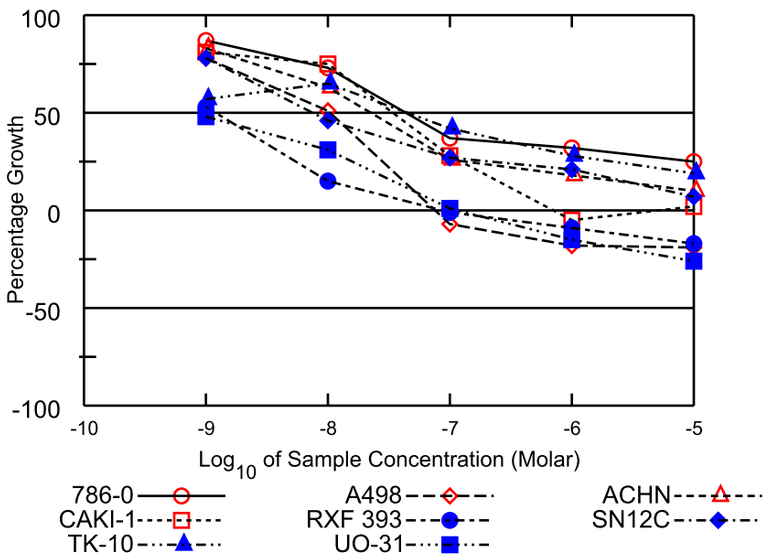


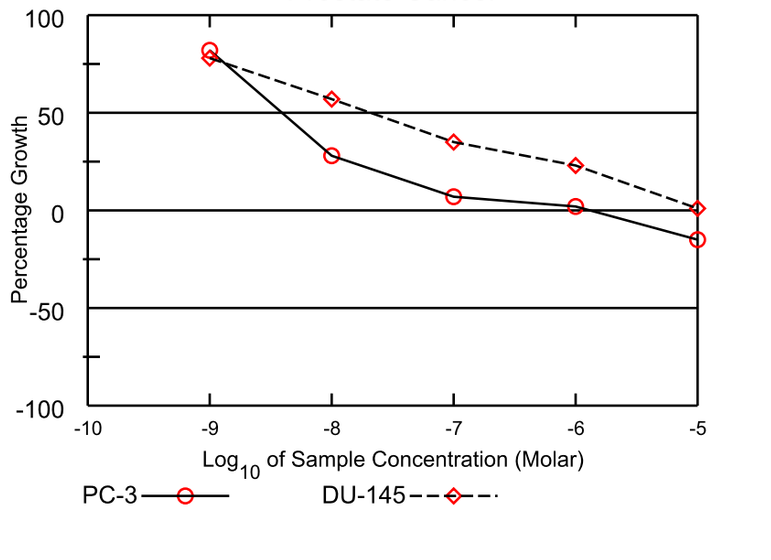


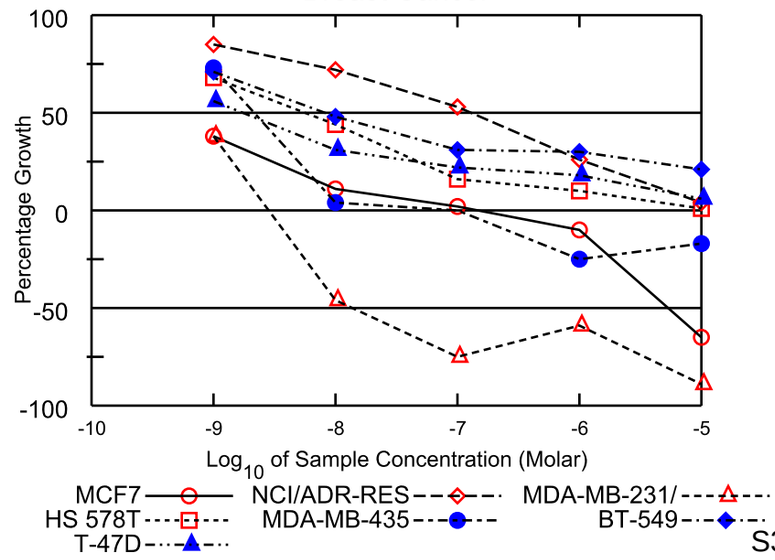












National Cancer Institute Developmental Therapeutics Program

Mean Graphs

NSC : D - 742997 / 1 | Units : Molar | SSPL : 0WOB | EXP. ID : 0612RS45

Report Date : April 23, 2007 | Test Date : December 04, 2006

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-6.71		> -5.00		> -5.00	
HL-60(TB)	< -9.00		-8.13		> -5.00	
K-562	-8.66		> -5.00		> -5.00	
MOLT-4	-6.95		> -5.00		> -5.00	
RPMI-8226	-8.71		-6.42		> -5.00	
SR	-8.08		-7.06		> -5.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-8.31		> -5.00		> -5.00	
EKVX	-8.25		> -5.00		> -5.00	
HOP-62	-7.67		-5.14		> -5.00	
HOP-92	-8.75		-7.94		-5.78	
NCI-H226	-8.16		-6.90		-5.12	
NCI-H23	-7.70		-5.19		> -5.00	
NCI-H322M	-7.43		> -5.00		> -5.00	
NCI-H460	-8.84		-5.95		-5.12	
NCI-H522	-7.83		-5.32		> -5.00	
Colon Cancer						
COLO 205	-8.56		-7.80		-5.94	
HCC-2998	-8.28		-7.03		> -5.00	
HCT-116	-8.76		-5.72		> -5.00	
HCT-15	-7.34		> -5.00		> -5.00	
HT29	< -9.00		-7.83		-7.03	
KM12	-7.78		> -5.00		> -5.00	
SW-620	-8.43		> -5.00		> -5.00	
CNS Cancer						
SF-268	-8.82		-5.82		> -5.00	
SF-295	< -9.00		-6.59		> -5.00	
SF-539	< -9.00		-7.54		> -5.00	
SNB-19	-7.48		> -5.00		> -5.00	
SNB-75	< -9.00		-8.12		> -5.00	
U251	-8.67		> -5.00		> -5.00	
Melanoma						
LOX IMVI	-8.61		-8.13			
MALME-3M	-7.66		> -5.00		> -5.00	
M14	-8.59		-7.95			
SK-MEL-2	-6.96		> -5.00		> -5.00	
SK-MEL-28	-8.30		> -5.00		> -5.00	
SK-MEL-5	-8.40		-7.57		-5.56	
UACC-257	-7.53		> -5.00		> -5.00	
UACC-62	-8.28		-7.09		-5.30	
Ovarian Cancer						
IGROV1	< -9.00		-5.83		> -5.00	
OVCAR-3	-7.58		> -5.00		> -5.00	
OVCAR-4	-6.28		> -5.00		> -5.00	
OVCAR-5	-7.46		> -5.00		> -5.00	
OVCAR-8	-8.09		> -5.00		> -5.00	
SK-OV-3	-8.41		> -5.00		> -5.00	
Renal Cancer						
786-0	-7.36		> -5.00		> -5.00	
A498	-7.99		-7.13		> -5.00	
ACHN	-7.65		> -5.00		> -5.00	
CAKI-1	-7.48				> -5.00	
RXF 393	-8.92		-7.08		> -5.00	
SN12C	-8.14		> -5.00		> -5.00	
TK-10	-7.36		> -5.00		> -5.00	
UO-31	< -9.00		-6.91		> -5.00	
Prostate Cancer						
PC-3	-8.41		-5.90		> -5.00	
DU-145	-7.69		> -5.00		> -5.00	
Breast Cancer						
MCF7	< -9.00		-6.81		-5.27	
NCI/ADR-RES	-6.87		> -5.00		> -5.00	
MDA-MB-231/ATCC	< -9.00		-8.55		-7.87	
HS 578T	-8.24		> -5.00		> -5.00	
MDA-MB-435	-8.67		-7.00		> -5.00	
BT-549	-8.08		> -5.00		> -5.00	
T-47D	-8.76		> -5.00		> -5.00	
_MID	-8.15		-5.96		-5.14	
Delta	0.85		2.59		2.73	
Range	2.72		3.55		2.87	

+3 +2 +1 0 -1 -2 -3