Supporting information

Anti-inflammatory Thiazine Alkaloids Isolated from the New Zealand Ascidian *Aplidium* sp.; Inhibitors of the Neutrophil Respiratory Burst in a Model of Gouty Arthritis

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1. Color in situ photograph of *Aplidium* sp.

2. X-ray crystallographic analysis of the sodium salt of 3.

Purification of a sample of **3** that ultimately yielded X-ray quality crystals was achieved by extracting the freeze-dried sample of ascidian with water followed by repeated C18 flash CC (water) and size exclusion chromatography on Sephadex LH-20 with MeOH. The absence of acid in the purification method yielded **3** as the sodium salt. Recrystallization of **3** from water/MeOH/EtOH (1:2:10) yielding small pink crystals (mp 155 °C (dec)). One of these was subjected to X-ray crystallography confirming the proposed structure (Figure S1). Interestingly **3** acts as a tridentate ligand, coordinating to a Na cation (presumably the natural counterion) through quinonoid O5, N6 and a carboxylate oxygen. The Na is seven-coordinate through these three atoms, three water molecules and a symmetry-related water of a neighboring molecule (Figure S2).

$C_{12}H_{17}N_2NaO_{11}S$, M = 420.33, monoclinic, $P2_1/c$ (No. 14), a = 14.9550 (1), b = 12.8150 (1), c = 8.9773 (1), α = 90, β = 98.535 (1), γ = 90 °, V = 1701.43 (3) $Å^3$, T = 85 (2) K, Z = 4, μ(Mo-K_α) = 0.281 mm^{-1}, 9684 reflections measured, 4292 independent reflections [R(int) = 0.0098], [2782, I > 2σ (I)], R_1 = 0.0237 (observed data), 0.0259 (all data).

CCDC 626466 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

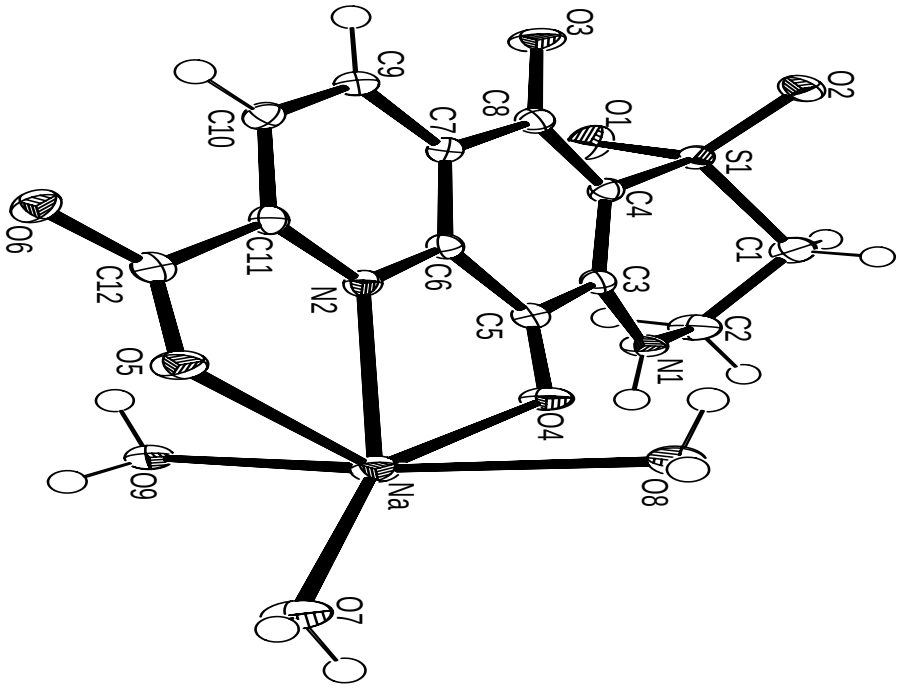


Figure S1. Ortep diagram of sodium salt of **3**. Atoms are represented as 50% probability surfaces.

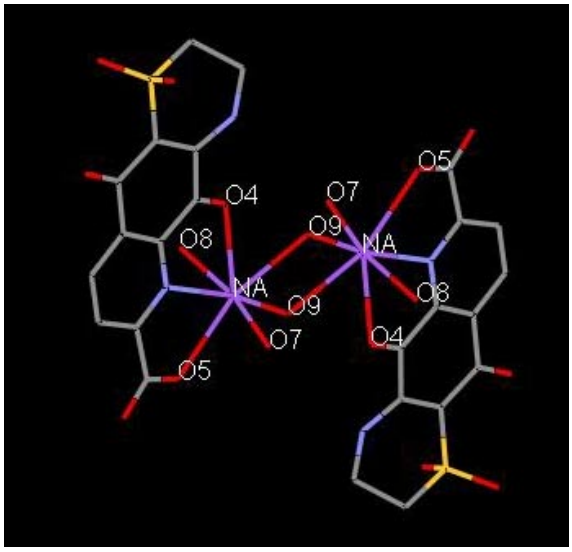


Figure S2. The dimeric structure of **3**.

Table S1. Crystal data and structure refinement for **3**.

Empirical formula	C12 H17 N2 Na O11 S
Formula weight	420.33
Temperature	84(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 14.9550(1) Å alpha = 90 deg.
	b = 12.8150(1) Å beta = 98.535(1) deg.
	c = 8.9773(1) Å gamma = 90 deg.
Volume	1701.43(3) A^3
Z, Calculated density	4, 1.641 Mg/m^3
Absorption coefficient	0.281 mm^{-1}
F(000)	872
Crystal size	0.50 x 0.42 x 0.28 mm
Theta range for data collection	2.10 to 25.64 deg.
Limiting indices	-14<=h<=18, -11<=k<=12, -10<=l<=5
Reflections collected / unique	9684 / 2781 [R(int) = 0.0098]
Completeness to θ = 25.64	86.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9256 and 0.8725
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2781 / 0 / 312
Goodness-of-fit on F^2	1.024
Final R indices [I>2σ (I)]	R_1 = 0.0235, wR2 = 0.0660
R indices (all data)	R_1 = 0.0258, wR2 = 0.0669
Largest diff. peak and hole	0.285 and -0.328 $e.A^{-3}$

Table S2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for **3**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
S(1)	4302(1)	3796(1)	-2601(1)	13(1)
O(1)	4119(1)	3967(1)	-4214(1)	22(1)
N(1)	2487(1)	3266(1)	-1537(1)	15(1)
C(1)	3882(1)	2549(1)	-2221(2)	18(1)
O(2)	5237(1)	3864(1)	-1912(1)	20(1)
N(2)	1997(1)	6550(1)	657(1)	11(1)
C(2)	2856(1)	2539(2)	-2538(2)	19(1)
O(3)	4570(1)	6052(1)	-1867(1)	21(1)
C(3)	2834(1)	4204(1)	-1234(1)	12(1)
O(4)	1708(1)	4461(1)	315(1)	16(1)
C(4)	3620(1)	4611(1)	-1665(1)	12(1)
O(5)	892(1)	7706(1)	2126(1)	16(1)
C(5)	2307(1)	4867(1)	-267(1)	12(1)
O(6)	1574(1)	9183(1)	1526(1)	16(1)
C(6)	2551(1)	5978(1)	-68(1)	12(1)
C(7)	3302(1)	6386(1)	-633(1)	12(1)
C(8)	3896(1)	5690(1)	-1415(1)	14(1)
C(9)	3484(1)	7442(1)	-428(1)	14(1)
C(10)	2908(1)	8045(1)	299(1)	14(1)
C(11)	2165(1)	7564(1)	816(1)	12(1)
C(12)	1484(1)	8208(1)	1557(1)	13(1)
O(7)	-504(1)	5902(1)	2983(1)	21(1)
O(8)	1661(1)	4905(1)	3718(1)	17(1)
O(9)	-10(1)	6080(1)	-941(1)	16(1)
O(10)	6561(1)	9921(1)	1945(1)	21(1)
O(11)	967(1)	12954(1)	4338(1)	19(1)
Na	681(1)	5708(1)	1624(1)	15(1)

Table S3. Bond lengths [Å] and angles [deg] for **3**.

S(1)-O(2)	1.4448(10)
S(1)-O(1)	1.4496(10)
S(1)-C(4)	1.7579(14)
S(1)-C(1)	1.7694(17)
N(1)-C(3)	1.322(2)
N(1)-C(2)	1.4586(19)
N(1)-HN1	0.84(2)
C(1)-C(2)	1.519(2)
C(1)-H(1A)	0.983(18)
C(1)-H(1B)	0.99(2)
N(2)-C(11)	1.328(2)
N(2)-C(6)	1.3437(18)
N(2)-Na	2.5096(12)
C(2)-H(2A)	0.999(18)
C(2)-H(2B)	0.93(2)
O(3)-C(8)	1.2310(17)
C(3)-C(4)	1.3915(18)
C(3)-C(5)	1.5165(19)
O(4)-C(5)	1.2180(17)
O(4)-Na	2.6134(11)
C(4)-C(8)	1.452(2)
O(5)-C(12)	1.2617(18)
O(5)-Na	2.6111(13)
C(5)-C(6)	1.475(2)
O(6)-C(12)	1.257(2)
C(6)-C(7)	1.4006(18)
C(7)-C(9)	1.388(2)
C(7)-C(8)	1.5040(19)
C(9)-C(10)	1.390(2)
C(9)-H(9)	0.954(19)
C(10)-C(11)	1.4086(19)
C(10)-H(10)	0.923(19)
C(11)-C(12)	1.5365(19)
O(7)-Na	2.3095(11)
O(7)-H(7C)	0.87(3)
O(7)-H(7D)	0.85(3)
O(8)-Na	2.4337(11)
O(8)-H(8C)	0.83(2)
O(8)-H(8D)	0.88(2)
O(9)-Na	2.4270(11)
O(9)-Na#1	2.5390(13)
O(9)-H(9D)	0.86(2)
O(9)-H(9C)	0.81(2)
O(10)-H(10C)	0.82(2)
O(10)-H(10D)	0.86(2)
O(11)-H(11C)	0.83(3)
O(11)-H(11D)	0.86(2)

Na-O(9)#1	2.5390(13)
Na-Na#1	3.7641(11)
O(2)-S(1)-O(1)	116.46(6)
O(2)-S(1)-C(4)	110.69(6)
O(1)-S(1)-C(4)	110.53(6)
O(2)-S(1)-C(1)	108.69(7)
O(1)-S(1)-C(1)	108.02(7)
C(4)-S(1)-C(1)	101.31(7)
C(3)-N(1)-C(2)	122.38(12)
C(3)-N(1)-HN1	118.3(14)
C(2)-N(1)-HN1	118.9(14)
C(2)-C(1)-S(1)	110.45(12)
C(2)-C(1)-H(1A)	110.5(10)
S(1)-C(1)-H(1A)	106.7(11)
C(2)-C(1)-H(1B)	112.4(10)
S(1)-C(1)-H(1B)	105.7(11)
H(1A)-C(1)-H(1B)	110.9(15)
C(11)-N(2)-C(6)	117.73(12)
C(11)-N(2)-Na	121.97(9)
C(6)-N(2)-Na	120.30(10)
N(1)-C(2)-C(1)	110.02(12)
N(1)-C(2)-H(2A)	108.7(11)
C(1)-C(2)-H(2A)	110.0(10)
N(1)-C(2)-H(2B)	110.3(10)
C(1)-C(2)-H(2B)	109.8(10)
H(2A)-C(2)-H(2B)	108.0(15)
N(1)-C(3)-C(4)	127.23(13)
N(1)-C(3)-C(5)	113.89(12)
C(4)-C(3)-C(5)	118.85(13)
C(5)-O(4)-Na	116.75(10)
C(3)-C(4)-C(8)	123.10(13)
C(3)-C(4)-S(1)	118.99(12)
C(8)-C(4)-S(1)	117.88(9)
C(12)-O(5)-Na	120.34(9)
O(4)-C(5)-C(6)	123.02(13)
O(4)-C(5)-C(3)	118.98(14)
C(6)-C(5)-C(3)	118.00(12)
N(2)-C(6)-C(7)	123.90(14)
N(2)-C(6)-C(5)	115.21(12)
C(7)-C(6)-C(5)	120.89(12)
C(9)-C(7)-C(6)	117.90(13)
C(9)-C(7)-C(8)	121.45(12)
C(6)-C(7)-C(8)	120.65(14)
O(3)-C(8)-C(4)	122.37(13)
O(3)-C(8)-C(7)	120.02(14)
C(4)-C(8)-C(7)	117.55(12)
C(7)-C(9)-C(10)	118.76(13)
C(7)-C(9)-H(9)	123.0(12)
C(10)-C(9)-H(9)	118.2(12)
C(9)-C(10)-C(11)	119.08(16)

C(9)-C(10)-H(10)	121.1(10)
C(11)-C(10)-H(10)	119.8(10)
N(2)-C(11)-C(10)	122.60(13)
N(2)-C(11)-C(12)	116.46(12)
C(10)-C(11)-C(12)	120.91(15)
O(6)-C(12)-O(5)	126.89(13)
O(6)-C(12)-C(11)	116.35(12)
O(5)-C(12)-C(11)	116.76(15)
Na-O(7)-H(7C)	134.0(15)
Na-O(7)-H(7D)	113.4(16)
H(7C)-O(7)-H(7D)	105(2)
Na-O(8)-H(8C)	113.2(14)
Na-O(8)-H(8D)	104.1(12)
H(8C)-O(8)-H(8D)	108.1(18)
Na-O(9)-Na#1	98.55(4)
Na-O(9)-H(9D)	114.6(13)
Na#1-O(9)-H(9D)	129.3(14)
Na-O(9)-H(9C)	105.2(15)
Na#1-O(9)-H(9C)	106.1(16)
H(9D)-O(9)-H(9C)	101(2)
H(10C)-O(10)-H(10D)	106(2)
H(11C)-O(11)-H(11D)	106(2)
O(7)-Na-O(9)	103.19(4)
O(7)-Na-O(8)	93.37(4)
O(9)-Na-O(8)	159.84(5)
O(7)-Na-N(2)	147.03(5)
O(9)-Na-N(2)	80.37(4)
O(8)-Na-N(2)	92.03(4)
O(7)-Na-O(9)#1	85.31(5)
O(9)-Na-O(9)#1	81.45(4)
O(8)-Na-O(9)#1	88.62(4)
N(2)-Na-O(9)#1	127.34(4)
O(7)-Na-O(5)	83.45(4)
O(9)-Na-O(5)	89.69(4)
O(8)-Na-O(5)	103.73(4)
N(2)-Na-O(5)	63.68(4)
O(9)#1-Na-O(5)	163.73(4)
O(7)-Na-O(4)	148.42(5)
O(9)-Na-O(4)	83.67(4)
O(8)-Na-O(4)	76.23(4)
N(2)-Na-O(4)	64.15(4)
O(9)#1-Na-O(4)	65.00(4)
O(5)-Na-O(4)	127.79(4)
O(7)-Na-Na#1	95.28(4)
O(9)-Na-Na#1	41.84(3)
O(8)-Na-Na#1	126.09(4)
N(2)-Na-Na#1	107.53(3)
O(9)#1-Na-Na#1	39.61(3)
O(5)-Na-Na#1	130.09(3)
O(4)-Na-Na#1	69.13(3)

Symmetry transformations used to generate equivalent atoms: #1 -x,-y+1,-z

Table S4. Anisotropic displacement parameters ($Å^2$ x 10^3) for **3**. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U11 + ... + 2 h k a^* b^* U12]$

	U11	U22	U33	U23	U13	U12
S(1)	13(1)	13(1)	13(1)	1(1)	5(1)	2(1)
O(1)	32(1)	23(1)	13(1)	1(1)	7(1)	5(1)
N(1)	13(1)	13(1)	21(1)	-3(1)	7(1)	-1(1)
C(1)	19(1)	14(1)	23(1)	0(1)	9(1)	2(1)
O(2)	13(1)	20(1)	26(1)	1(1)	5(1)	3(1)
N(2)	12(1)	9(1)	11(1)	-1(1)	1(1)	1(1)
C(2)	19(1)	12(1)	27(1)	-6(1)	9(1)	-1(1)
O(3)	17(1)	17(1)	30(1)	1(1)	13(1)	-2(1)
C(3)	12(1)	13(1)	13(1)	1(1)	1(1)	2(1)
O(4)	14(1)	15(1)	20(1)	-2(1)	7(1)	-3(1)
C(4)	13(1)	11(1)	14(1)	1(1)	4(1)	3(1)
O(5)	16(1)	14(1)	19(1)	-1(1)	7(1)	0(1)
C(5)	12(1)	13(1)	13(1)	0(1)	1(1)	1(1)
O(6)	20(1)	10(1)	19(1)	-1(1)	5(1)	2(1)
C(6)	11(1)	12(1)	11(1)	1(1)	1(1)	1(1)
C(7)	12(1)	11(1)	13(1)	1(1)	1(1)	1(1)
C(8)	13(1)	15(1)	14(1)	2(1)	2(1)	1(1)
C(9)	13(1)	15(1)	17(1)	3(1)	3(1)	-1(1)
C(10)	14(1)	10(1)	16(1)	0(1)	1(1)	0(1)
C(11)	13(1)	13(1)	11(1)	1(1)	0(1)	2(1)
C(12)	13(1)	14(1)	10(1)	-2(1)	0(1)	2(1)
O(7)	24(1)	18(1)	25(1)	-3(1)	12(1)	-5(1)
O(8)	16(1)	19(1)	16(1)	-3(1)	4(1)	-2(1)
O(9)	14(1)	18(1)	18(1)	3(1)	5(1)	-1(1)
O(10)	16(1)	22(1)	24(1)	-6(1)	2(1)	1(1)
O(11)	17(1)	16(1)	23(1)	2(1)	2(1)	-1(1)
Na	14(1)	17(1)	14(1)	1(1)	4(1)	-1(1)

Table S5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for **3**.

	x	y	z	U(eq)
HN1	2005(14)	3114(16)	-1220(20)	33(5)
H(1A)	4093(12)	2404(15)	-1150(20)	25(5)
H(1B)	4157(12)	2056(16)	-2870(20)	27(5)
H(2A)	2646(12)	2753(15)	-3600(20)	26(5)
H(2B)	2642(11)	1865(16)	-2409(18)	17(4)
H(9)	3986(12)	7777(15)	-770(20)	27(5)
H(10)	3006(11)	8751(16)	447(18)	14(4)
H(7C)	-594(15)	6290(19)	3750(30)	46(6)
H(7D)	-777(15)	5330(20)	3110(20)	42(6)
H(8C)	1648(13)	5206(16)	4530(20)	30(5)
H(8D)	2206(14)	4976(16)	3480(20)	34(5)
H(9D)	307(14)	6474(18)	-1440(20)	36(6)
H(9C)	-435(16)	6462(18)	-860(20)	39(6)
H(10C)	6291(15)	9697(19)	1140(30)	47(6)
H(10D)	6168(15)	10263(18)	2350(20)	38(6)
H(11C)	1150(15)	13520(20)	4070(20)	41(7)
H(11D)	434(15)	12871(17)	3830(20)	35(5)

3. Serum toxicity markers.

Serum Toxicity Markers. Blood was collected from the hearts of euthanased mice by cardiac puncture. Whole blood samples were shipped over ice for analysis (Gribbles Analytical Laboratories, Hamilton, New Zealand) to determine the levels of liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase) and renal marker creatinine in the serum.

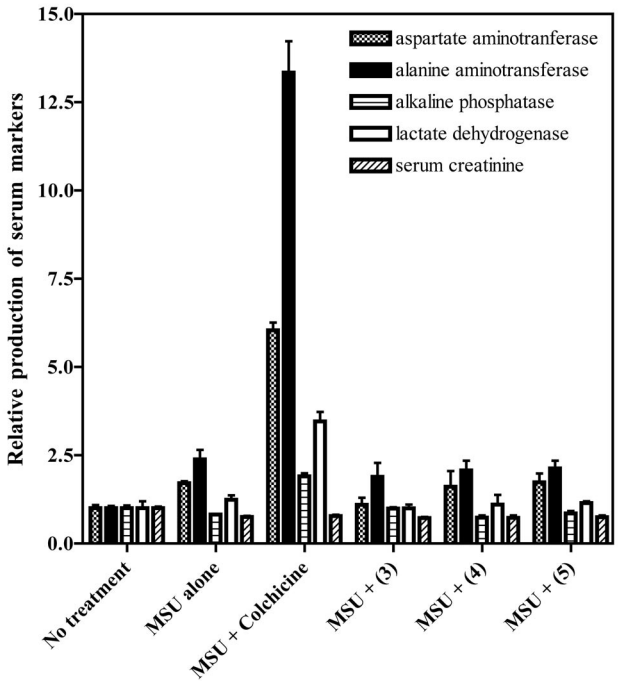
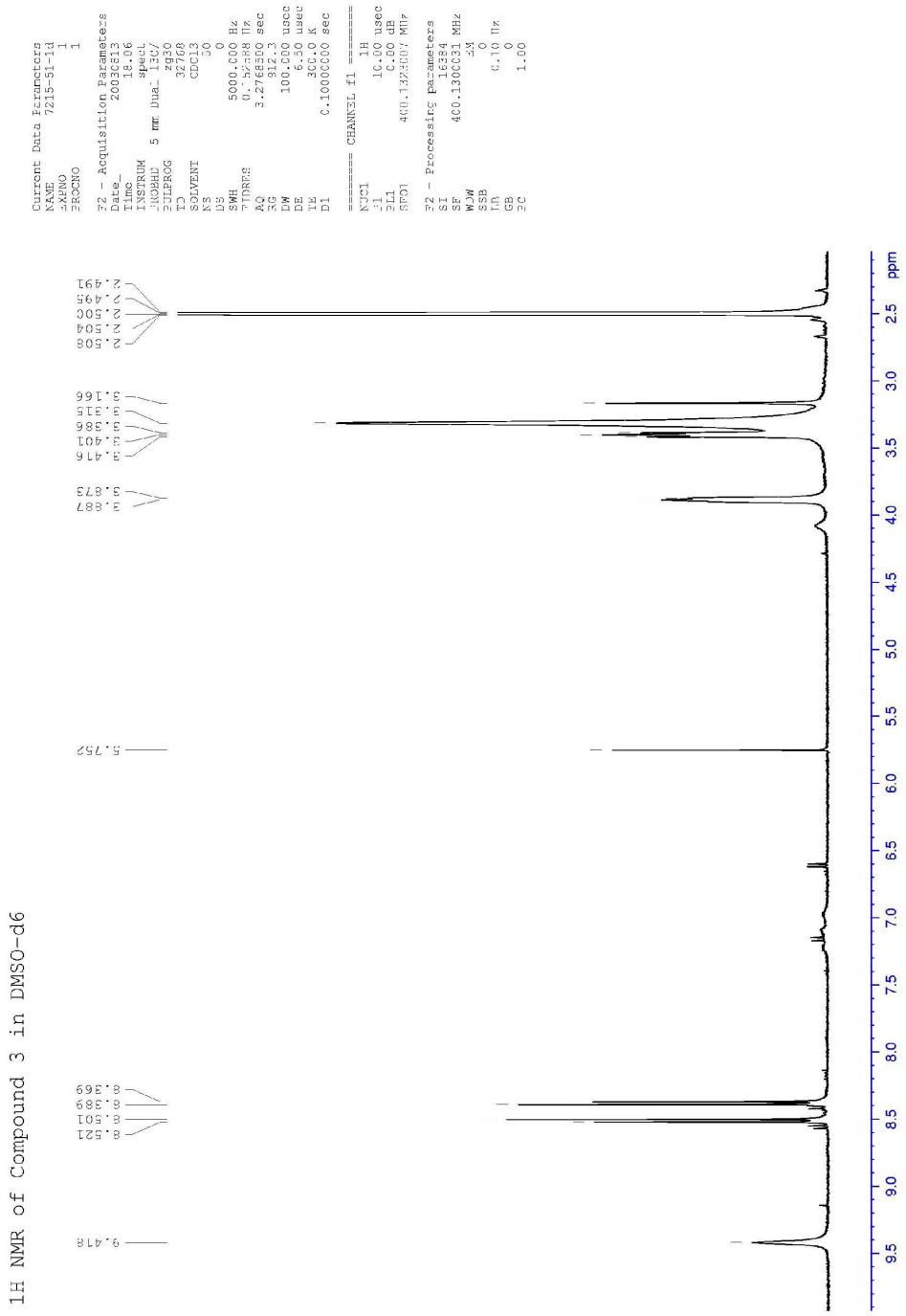


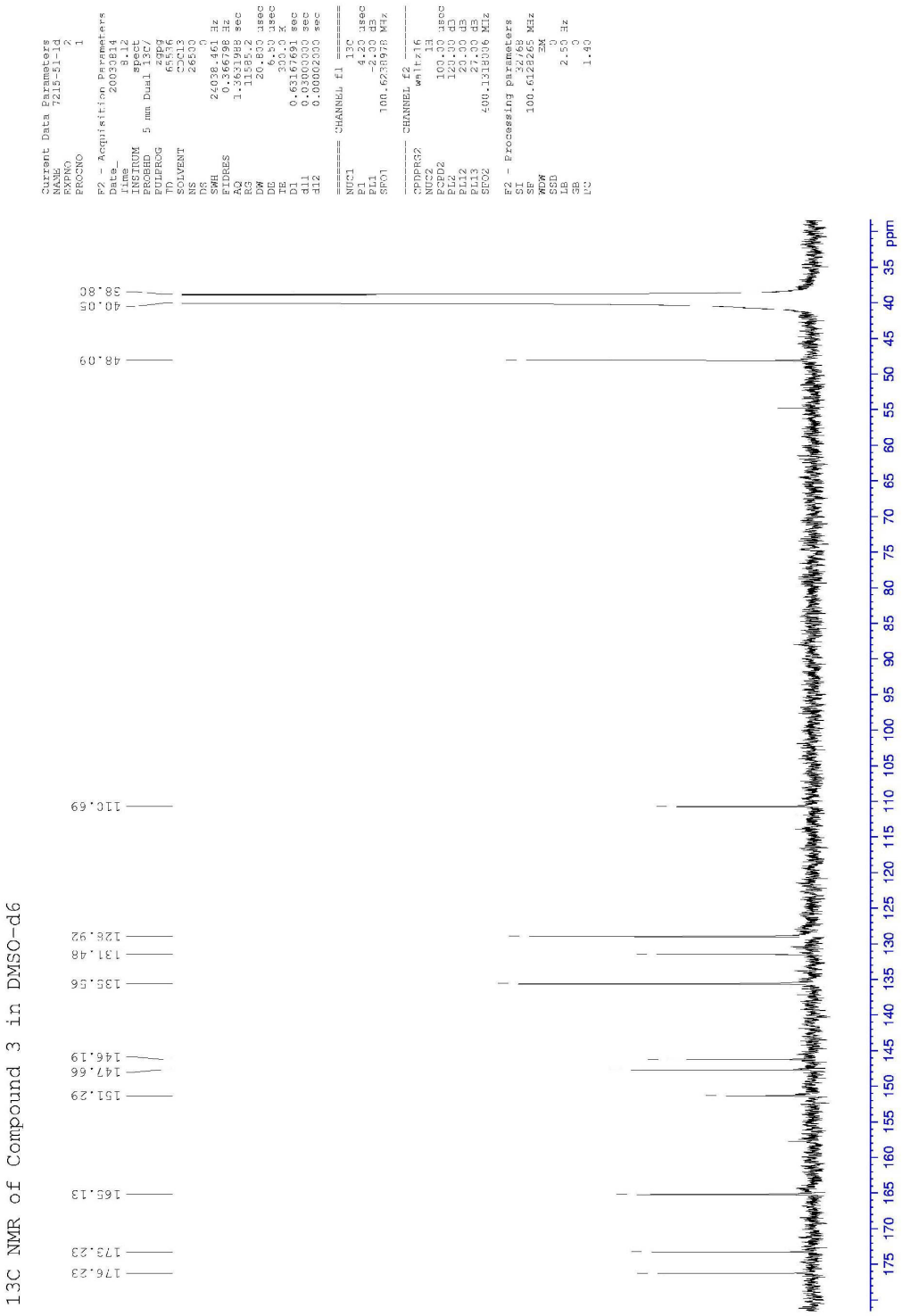
Figure S3. Levels of liver and renal markers in the serum of no treatment naïve control, gouty mice (MSU alone), gouty mice treated with colchicine and gouty mice treated with **3**-**5**. Mice were injected intraperitoneally with MSU crystals (3 mg) in the presence or absence of compounds **3**, **4** or **5**. Serum from the mice was tested for the presence of liver toxicity markers (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase) and the renal toxicity marker creatinine. Results are presented relative to the no treatment control (1.0).

4. Spectroscopic data for compounds.

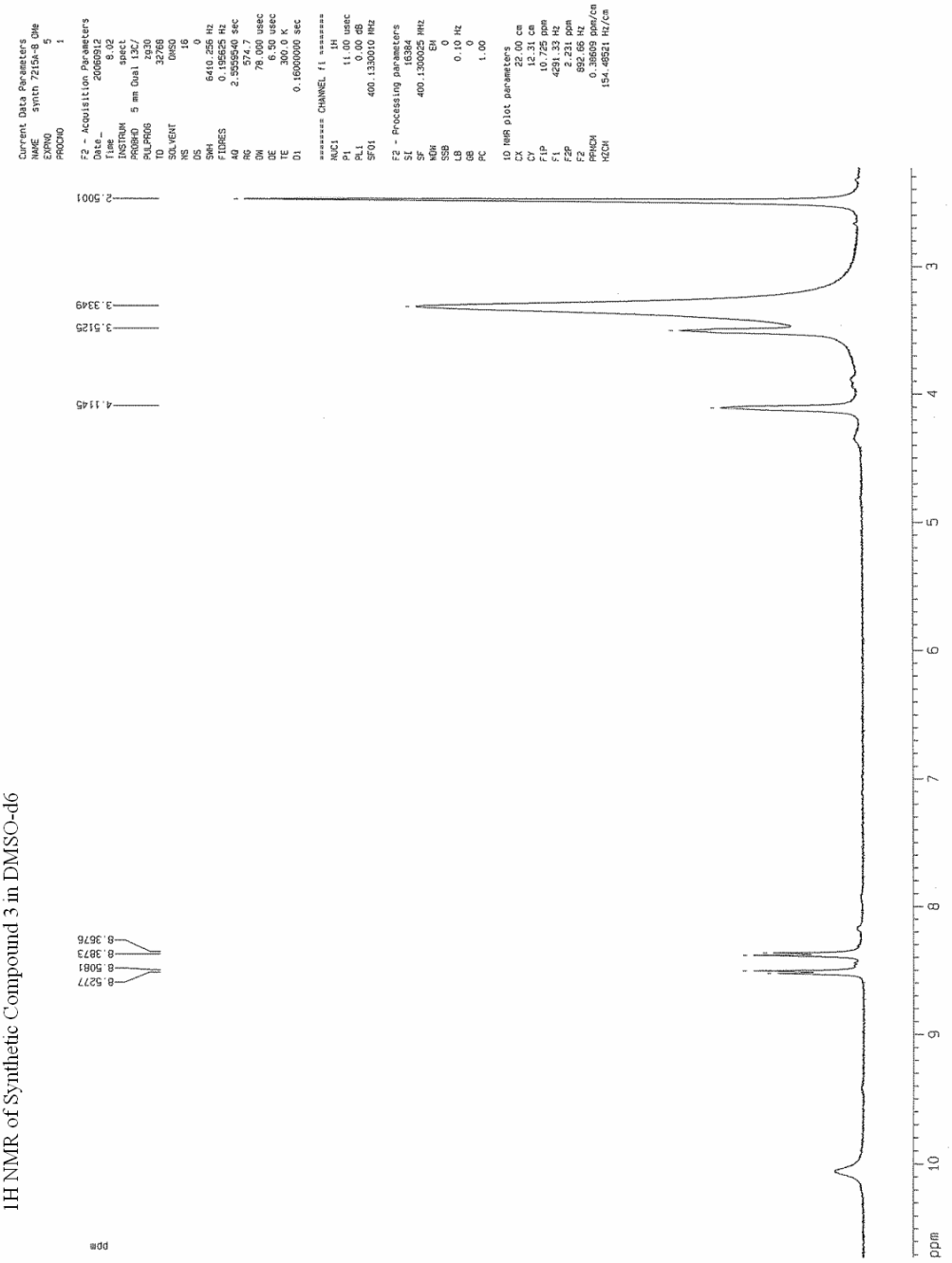
4.1 ^{1}H NMR spectrum of compound 3 in DMSO-d_6.



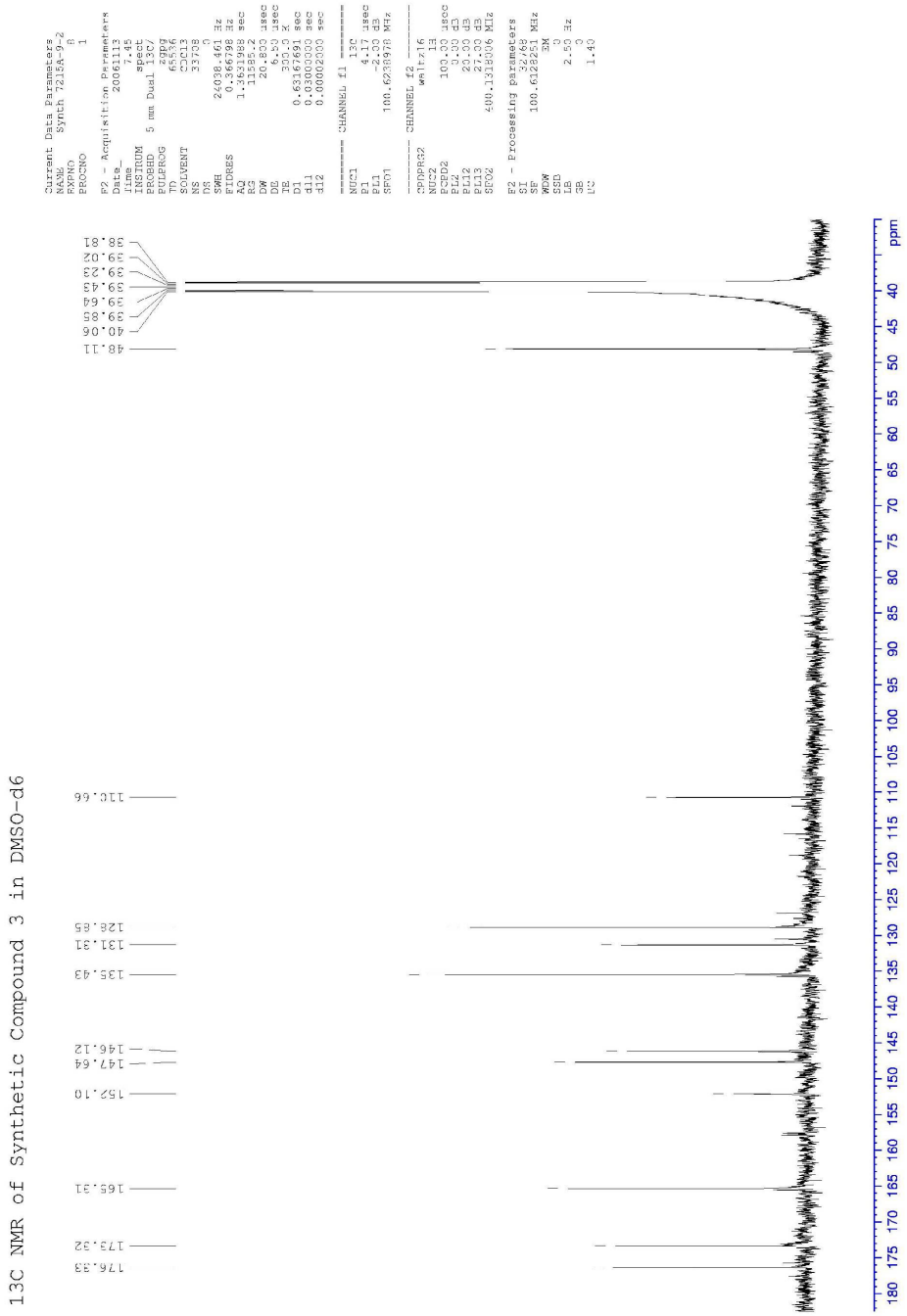
4.2 ^{13}C NMR spectrum of compound 3 in DMSO-d_6.



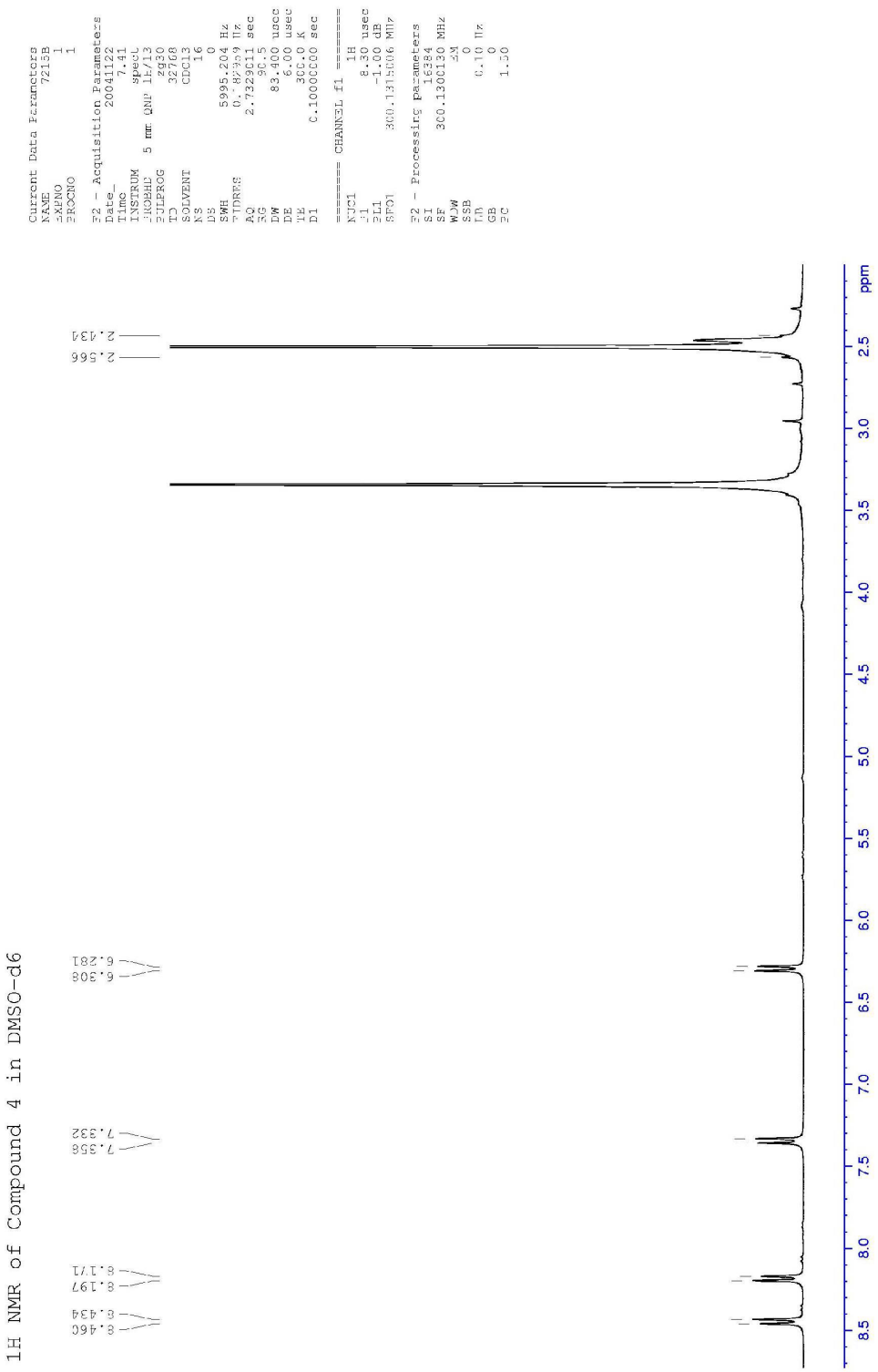
4.3 ^{1}H NMR spectrum of synthetic compound 3 in DMSO-d_6.



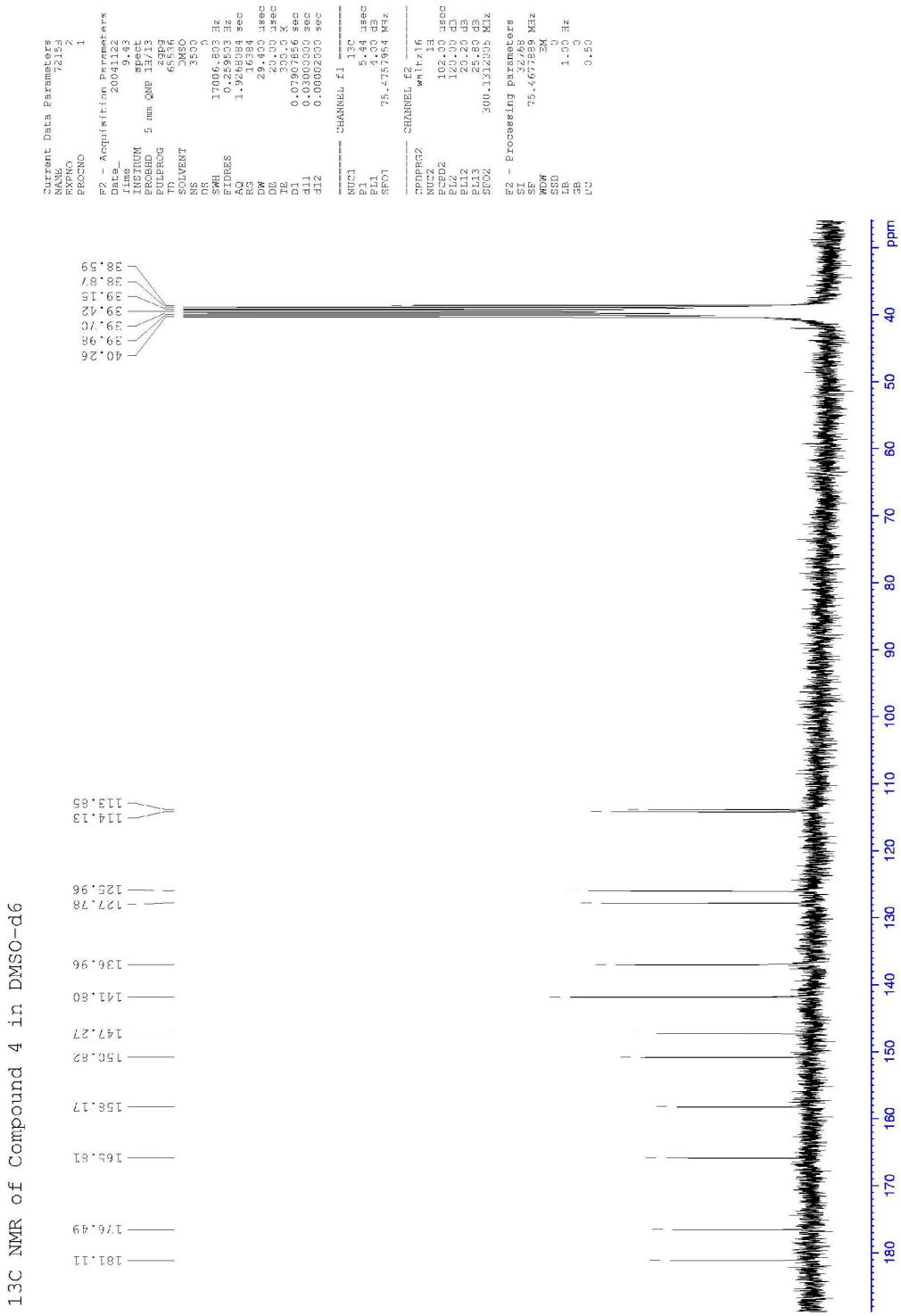
4.4 ^{13}C NMR spectrum of synthetic compound 3 in DMSO-d_6.



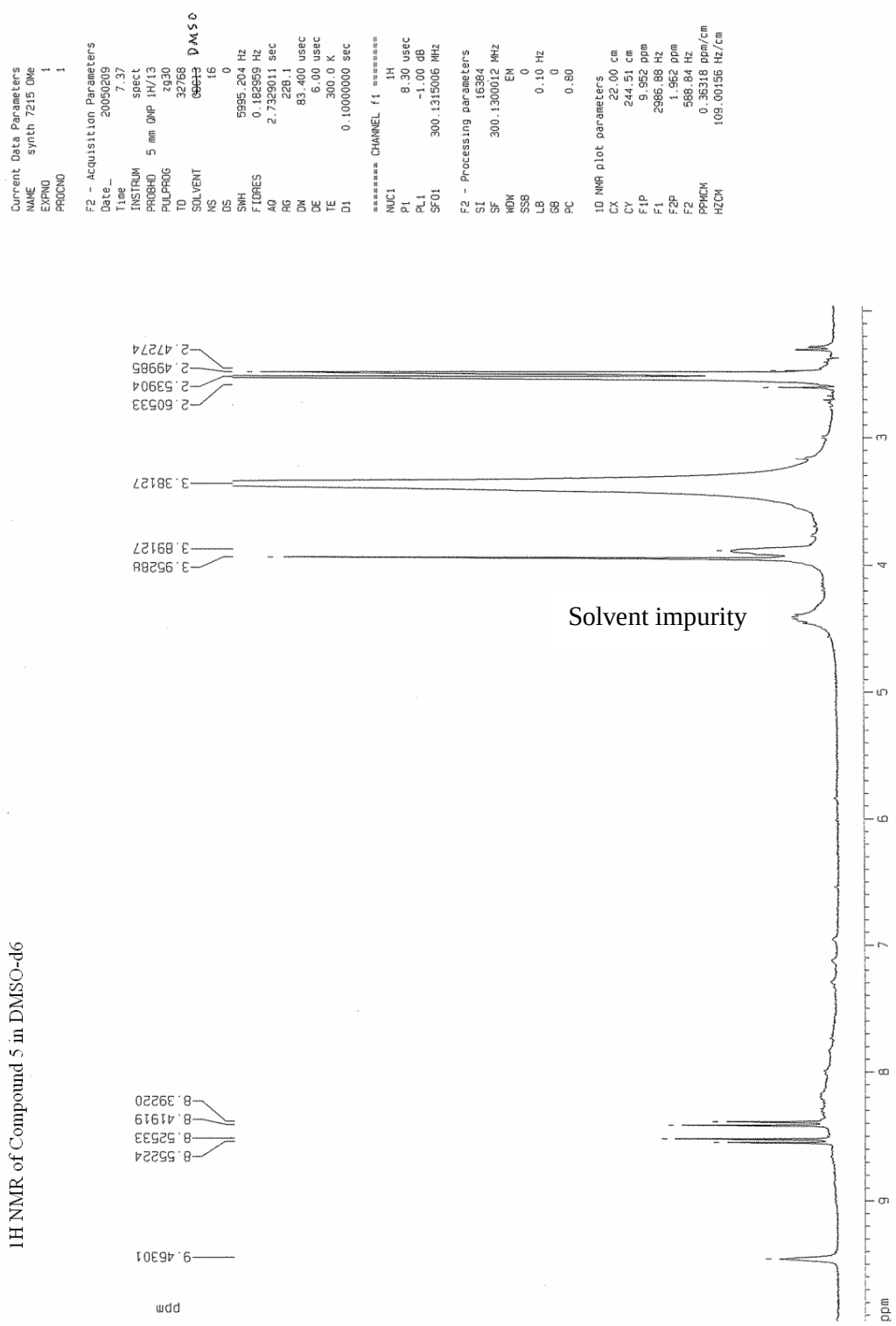
4.5 ^{1}H NMR spectrum of compound 4 in DMSO-d_6.



4.6 ^{13}C NMR spectrum of compound 4 in DMSO-d_6.

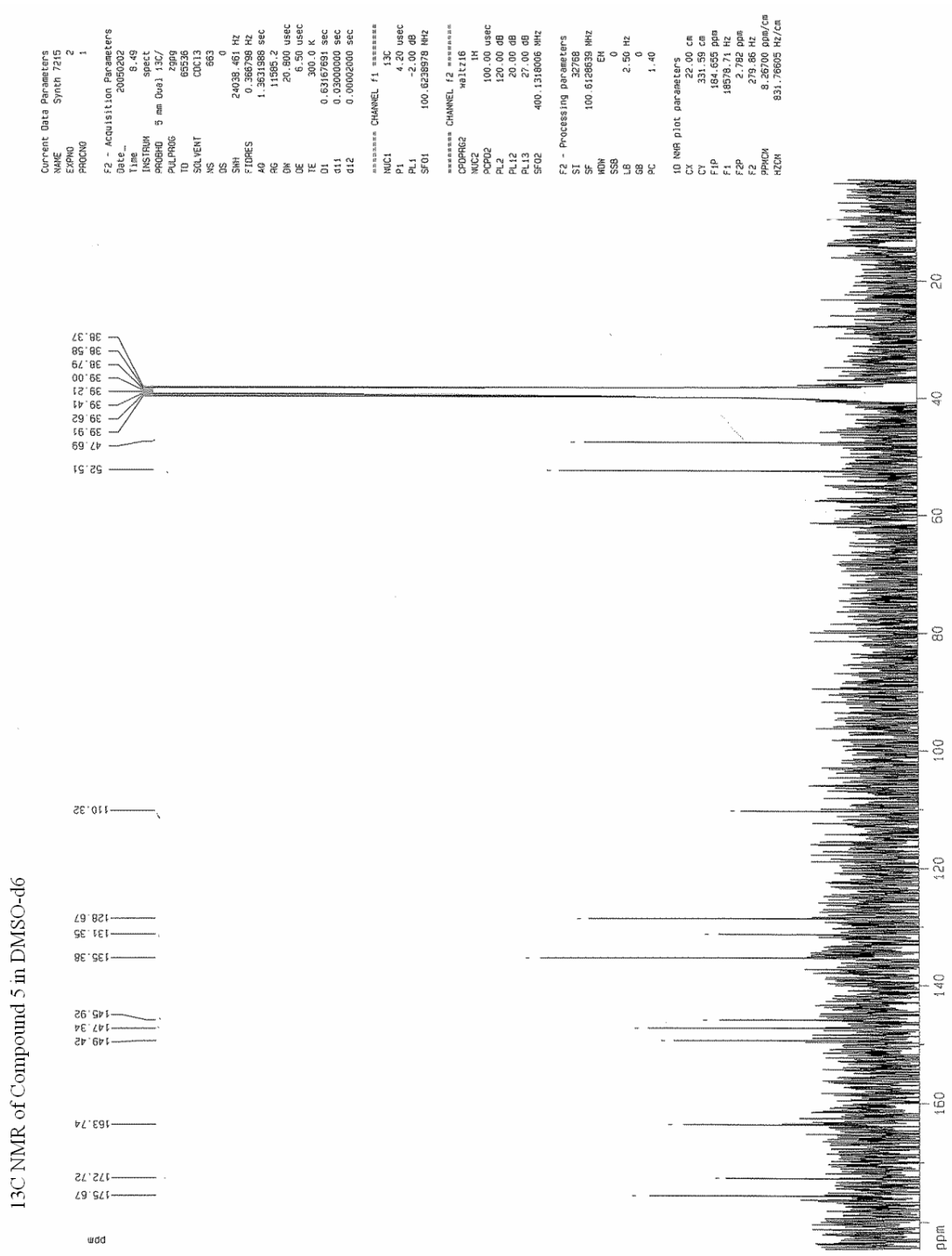


4.7 ^{1}H NMR spectrum of compound 5 in DMSO-d_6.



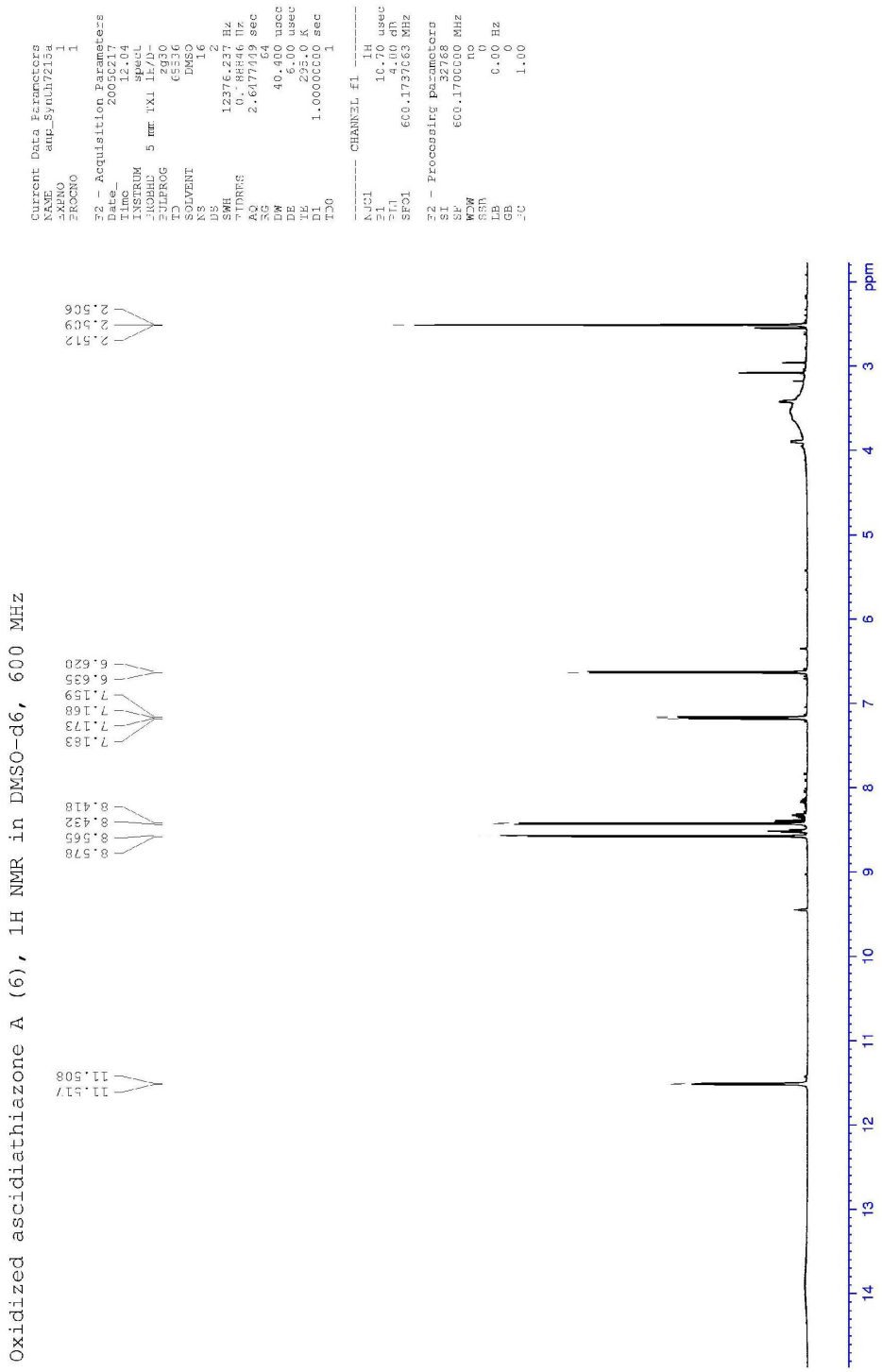
1H NMR of Compound 5 in DMSO-d6

4.8 ^{13}C NMR spectrum of compound 5 in DMSO-d_6.

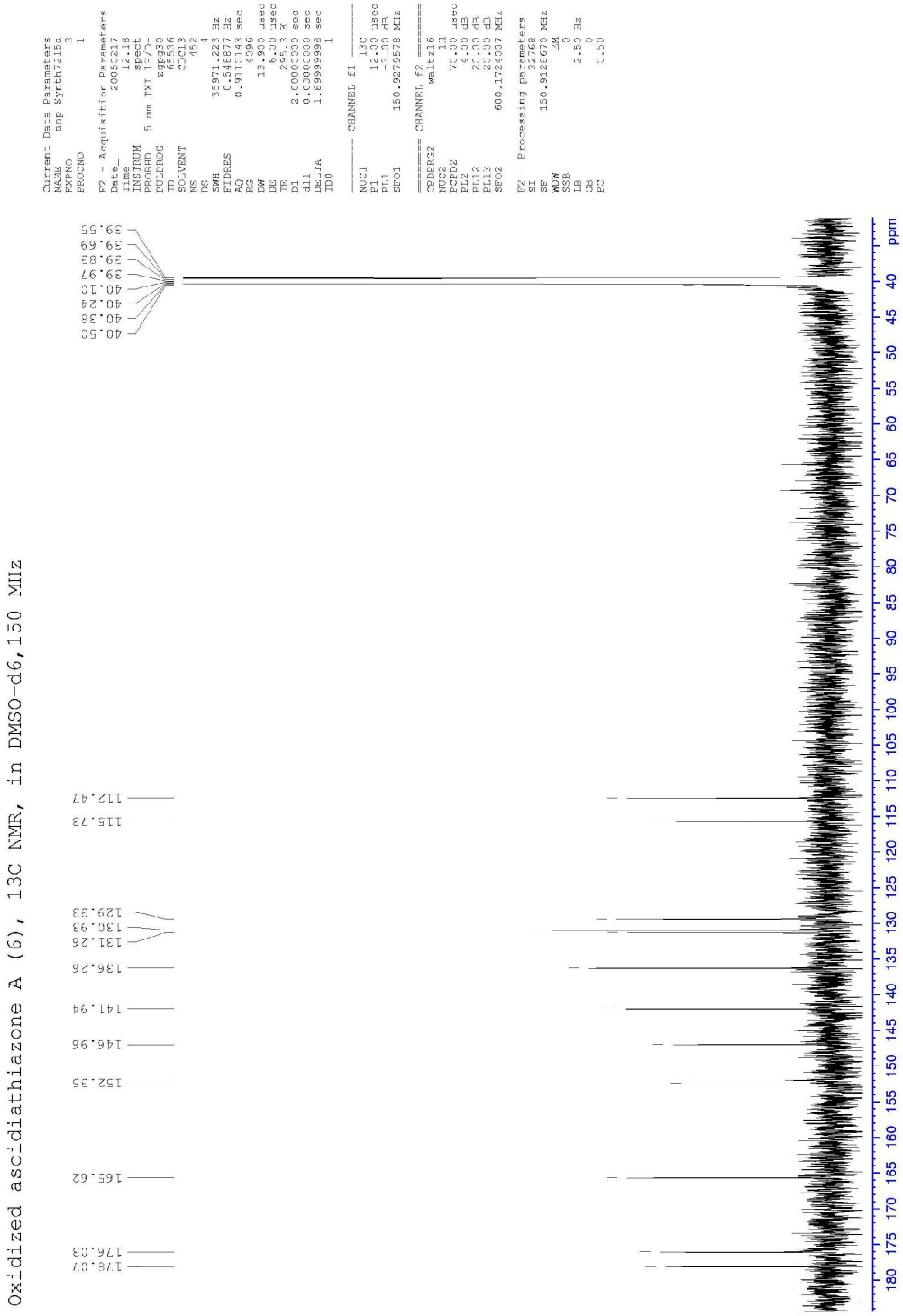


13C NMR of Compound 5 in DMSO-d6

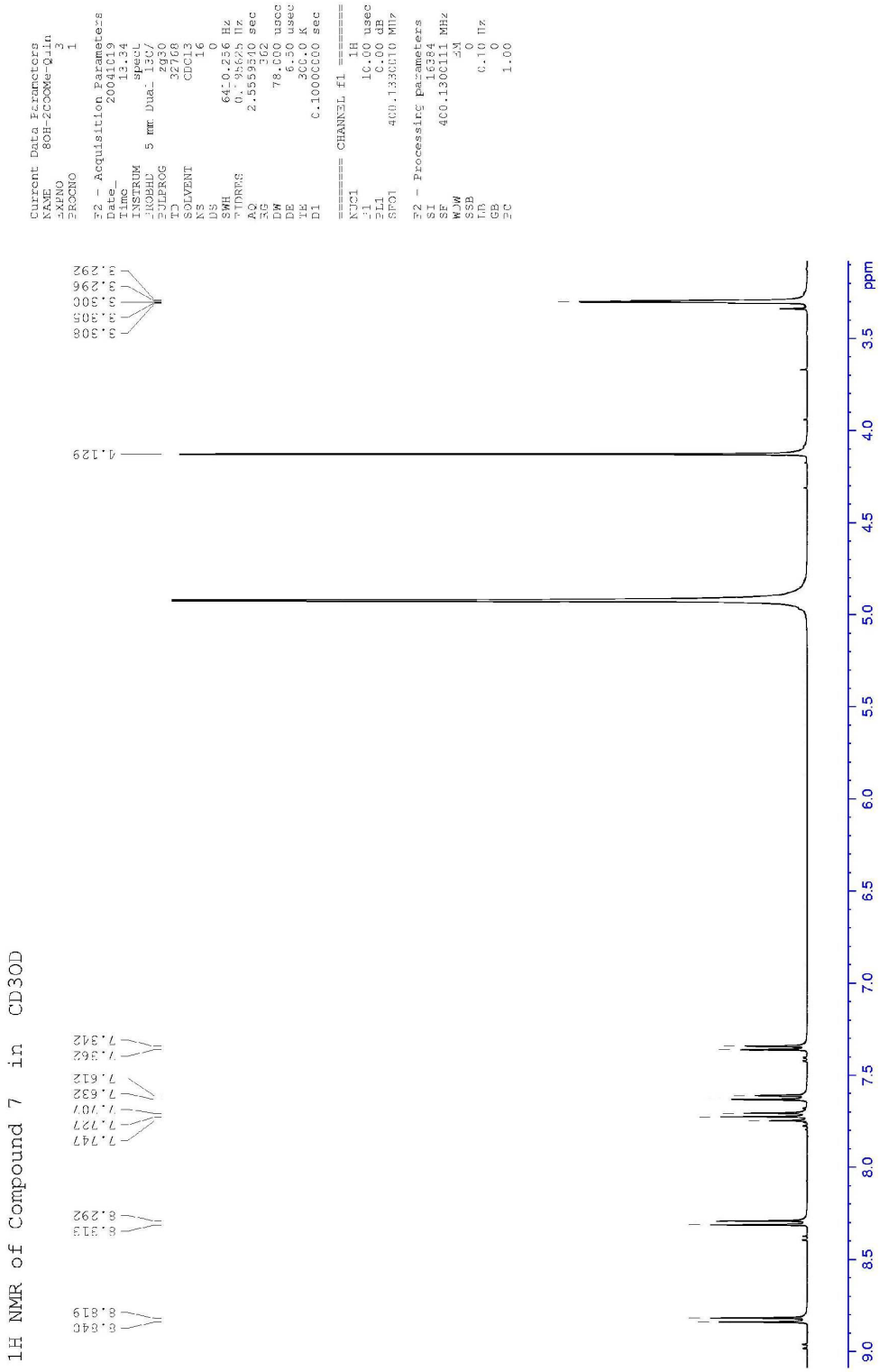
4.9 ^{1}H NMR spectrum of compound 6 in DMSO-d_6.



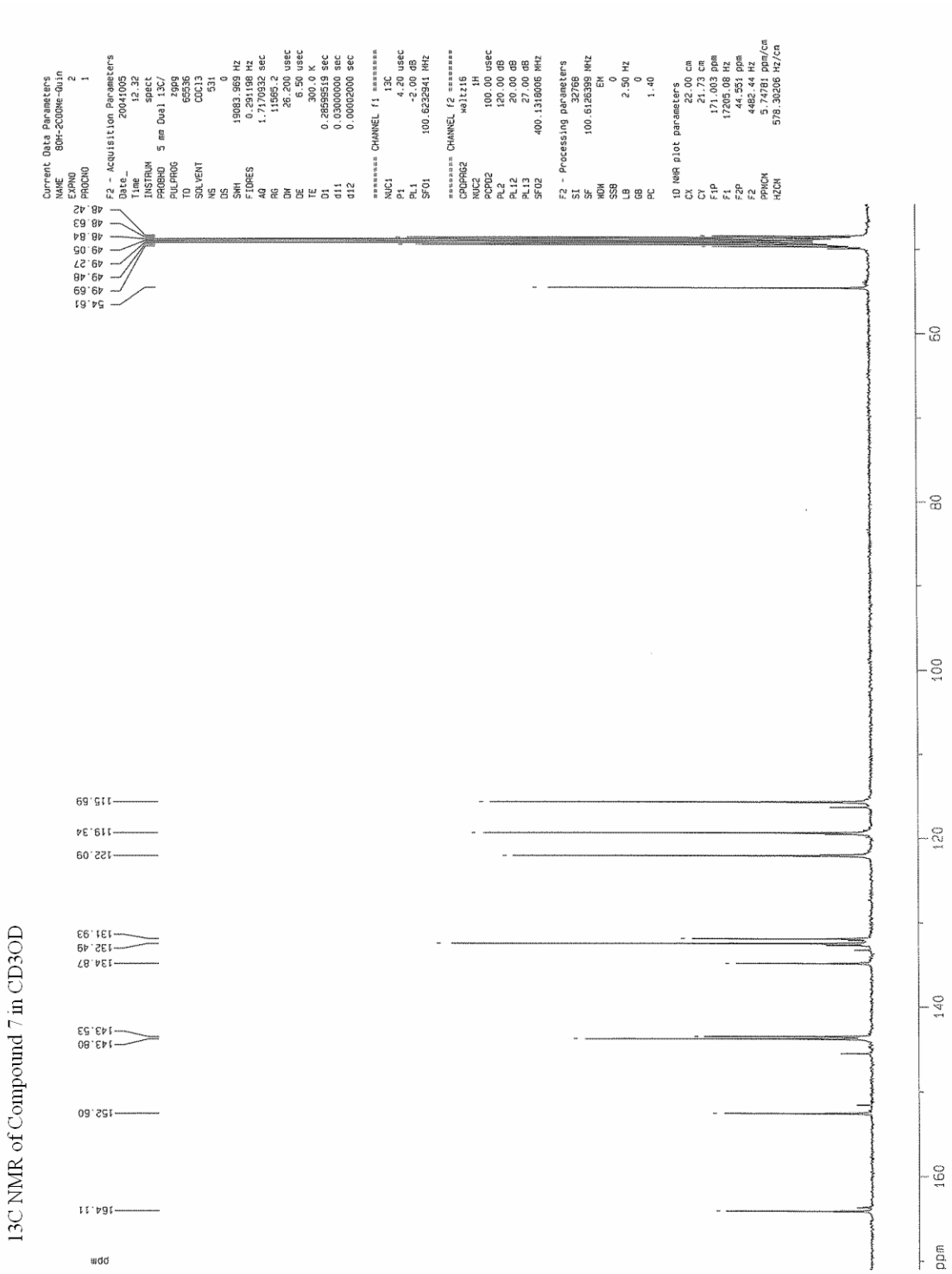
4.10 ^{13}C NMR spectrum of compound 6 in DMSO-d_6.



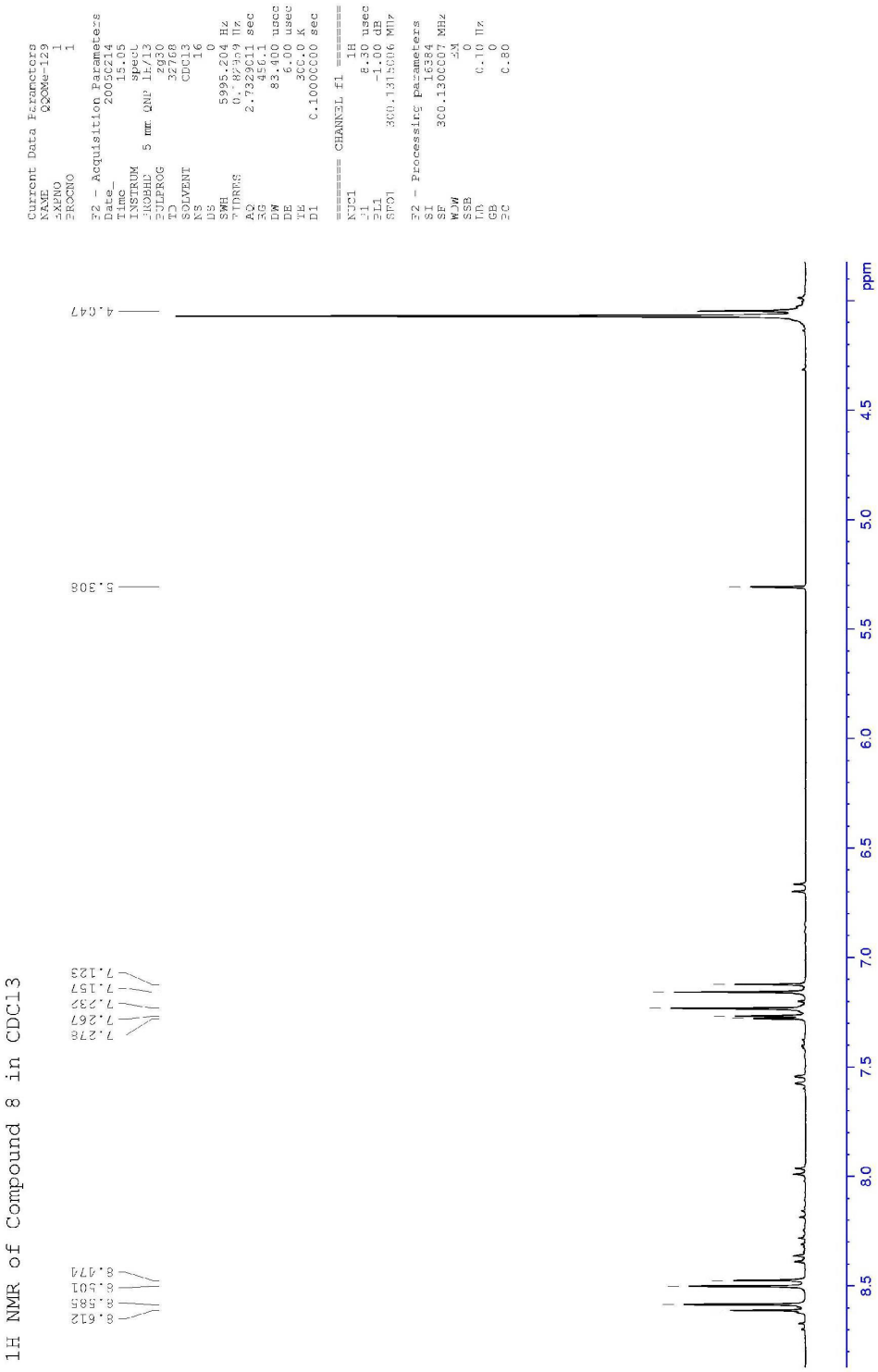
4.11 ^{1}H NMR spectrum of compound 7 in CD_3OD.



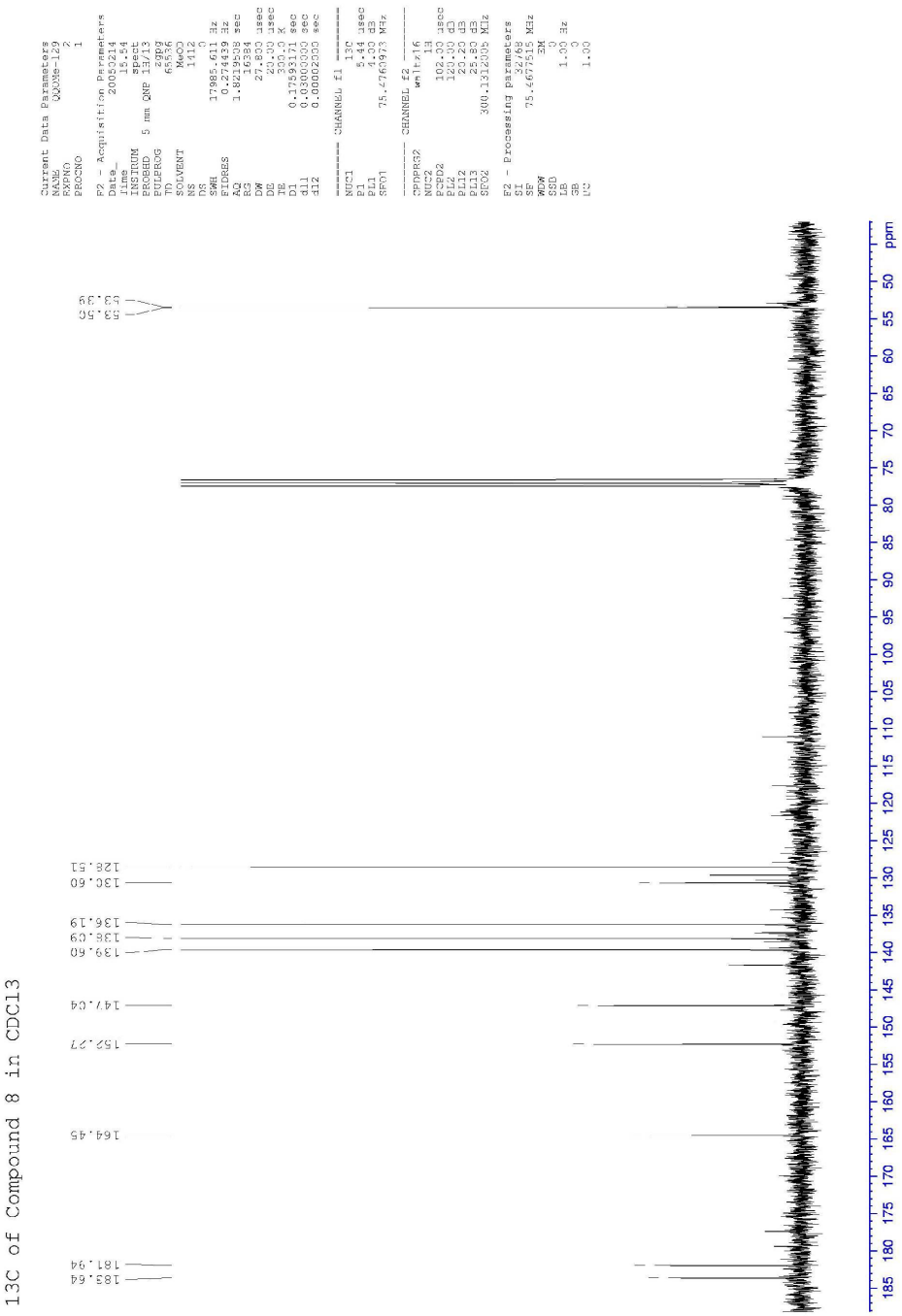
4.12 ^{13}C NMR spectrum of compound 7 in CD_3OD.



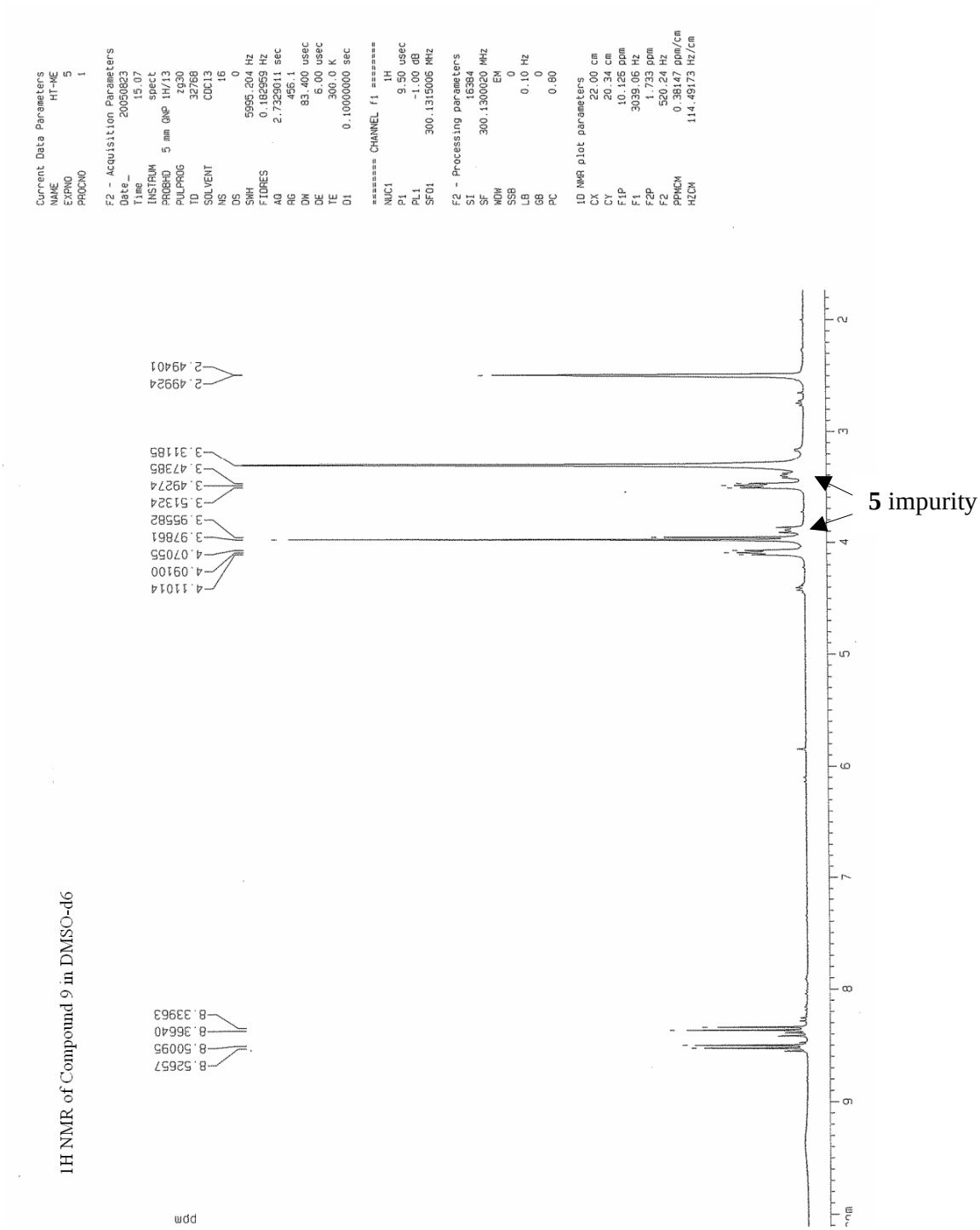
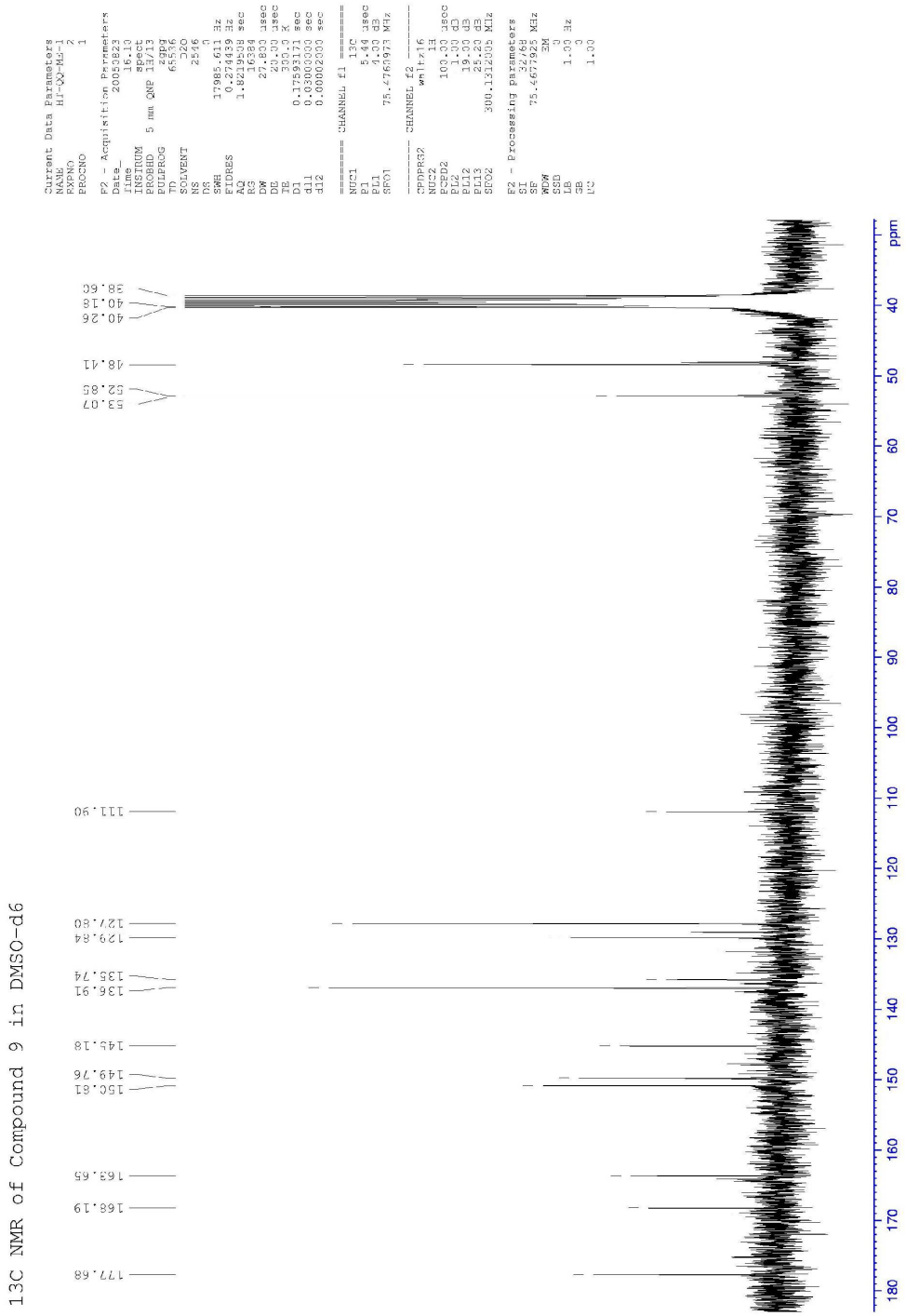
4.13 ^{1}H NMR spectrum of compound 8 in $CDCl_3$.



4.14 ^{13}C NMR spectrum of compound 8 in $CDCl_3$.



4.15 ^{1}H NMR spectrum of compound 9 in DMSO-d_6.



4.16 ^{13}C NMR spectrum of compound 9 in DMSO-d_6.