Supporting Information for:

Biosynthetic studies on the azinomycins: The pathway to the naphthoate fragment

Christophe Corre#, Cyrille A.S. Landreau#, Michael Shipman#, Philip A.S. Lowden‡

School of Biological and Chemical Sciences
University of Exeter
Stocker Road
Exeter
EX4 4QD
U.K.

‡ Current Address:
School of Biological and Chemical Sciences
Birkbeck College, University of London
Malet Street
London
WC1E 7HX
U.K.
Tel. +44 (0)20 70790789
Fax. +44 (0)20 76316246
email: p.lowden@bbk.ac.uk

#Current Address
Department of Chemistry
University of Warwick
Gibbet Hill Road
Coventry
CV4 7AL
U.K.
Tel +44 (0)24 76523186
Fax +44 (0)24 76524429
Email m.shipman@warwick.ac.uk

Contents:

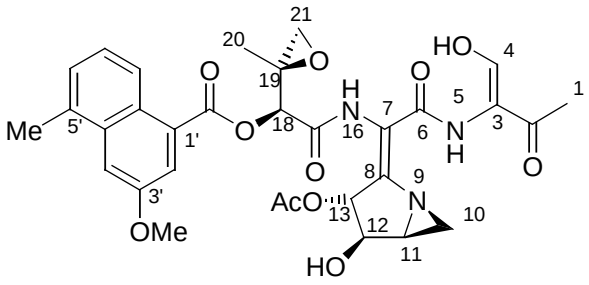
Experimental procedures for feeding studies: A 100 ml seed culture of *S. sahachiroi* (NRRL 2485), maintained on GYM agar plates (glucose monohydrate, 4 g/L; yeast extract, 4 g/L; malt extract, 10 g/L; $CaCO_3$, 2 g/L; agar, 12 g/L; tap water to balance; adjusted to pH 6.8 with NaOH, 1 M before sterilisation) at 28 °C, was grown in PS5 medium (Pharmamedia, 5 g/L; starch, 5 g/L; tap water to balance; adjusted to pH 6.0) at 30 °C, 200 rpm for 24 hours then 25 ml was used to inoculate 500 ml of PS5 medium, which was grown at 30 °C, 200 rpm for 72 hours.

Aqueous solutions of labeled precursors as their sodium salts were added through a 0.2 μm filter at concentrations as outlined in the text.

After centrifugation of the cultures, azinomycin B was isolated by extraction of the supernatant (pH 8.0) with an equal volume of chloroform at 4°C, concentration and then a series of precipitations. For each 100 ml of culture, the residue was precipitated from 600 μl chloroform/hexane (1:29), centrifuged at 2000 rpm and the supernatant discarded. This was repeated and then the residue dissolved in 600 μl chloroform/hexane (2:1), centrifuged and the supernatant retained. This residue was then dissolved in 600 μl chloroform/diethyl ether (1:4), centrifuged and the supernatant concentrated to give pure azinomycin B (~1.5 mg per 100 ml).

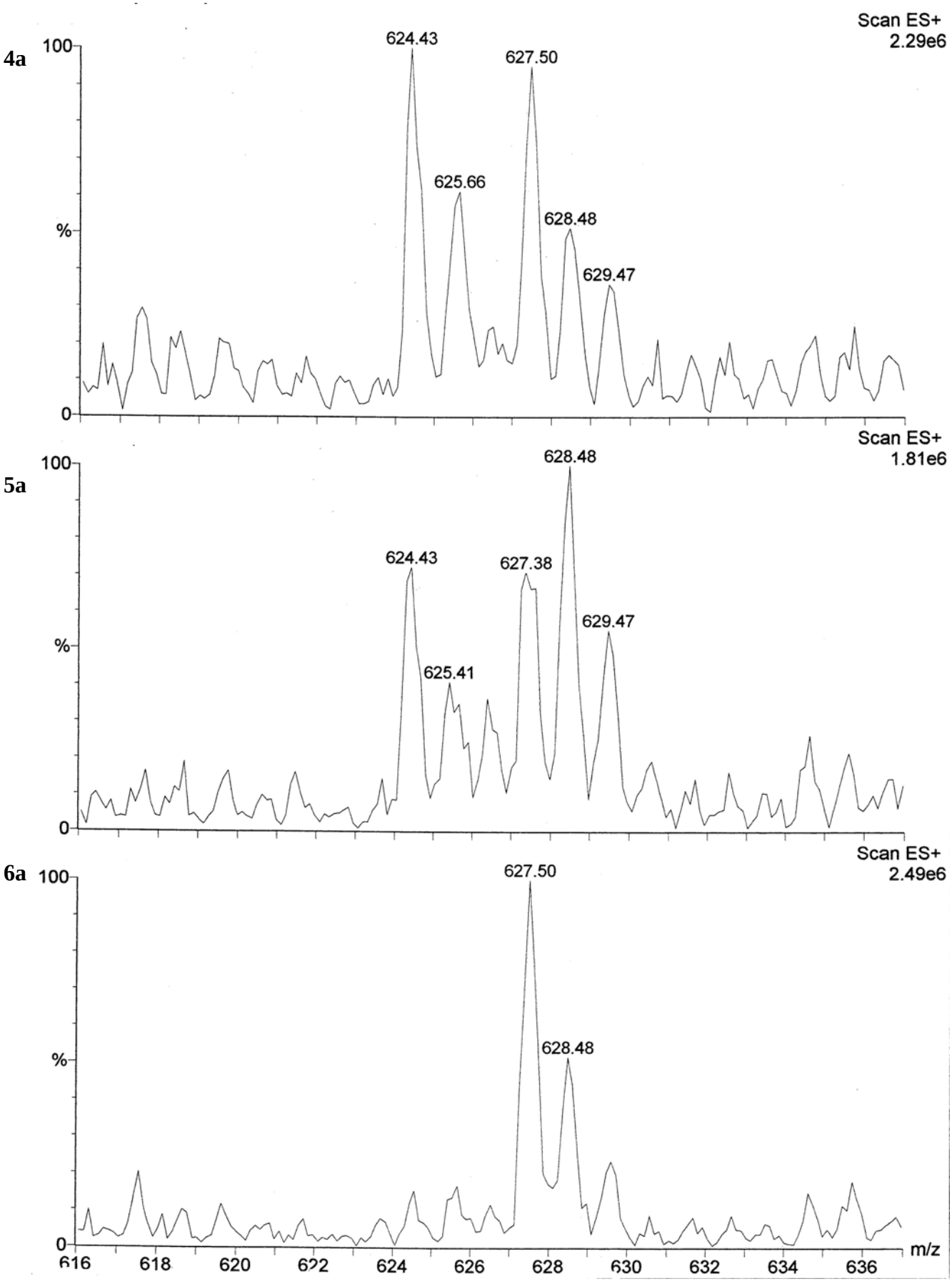
^{1}H NMR spectra were obtained on a Bruker Advance 400 in CDCl3, d6-acetone or d6-DMSO, referenced to solvent. ^{2}H NMR spectra were obtained on a Bruker Advance 400 in $CHCl_3$, referenced to d_6-acetone. Electrospray ionisation mass spectrometry (ESI-MS) was performed on a MicroMass Platform LC. Samples were prepared in a solution generating positive ions (acetonitrile/H_2O 50:50 + 1% formic acid).

^{1}H NMR assignments for azinomycin B.

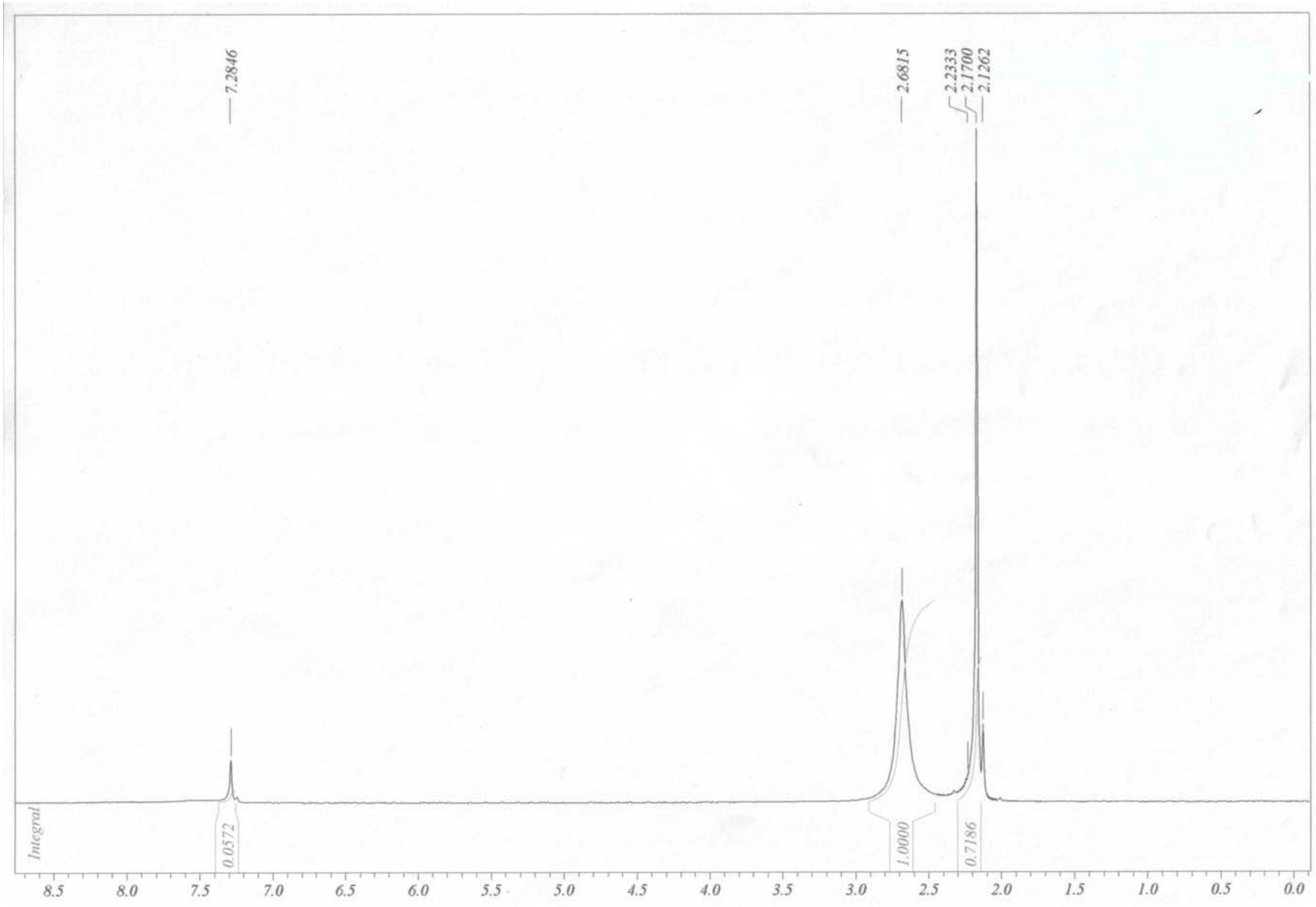


δ_H (400 MHz; $CDCl_3$) 12.47 (1 H, br s, OH-4), 12.47 (1 H, s, H-5), 8.55 (1 H, dd, *J* 7.0, 3.6, H-8'), 8.20 (1 H, br s, H-16), 7.94 (1 H, d, *J* 2.9, H-2'), 7.48 (1H, d, *J* 2.5 Hz, H-4'), 7.32 (1 H, m, H-7'), 7.32 (1 H, m, H-6'), 5.50 (1 H, d, *J* 4.0, H-13), 5.12 (1 H, s, H-18), 4.64 (1 H, dd, *J* 4.8, 4.0, H-12), 3.96 (3 H, s, OCH_3-3'), 3.96 (1 H, OH-12), 3.36 (1 H, m, H-11), 2.98 (1 H, d, *J* 4.3, H-21b), 2.80 (1 H, d, *J* 4.3, H-21a), 2.70 (1 H, H-10b), 2.68 (3 H, s, CH_3-5'), 2.30 (1 H, H-10a), 2.30 (3 H, s, CH_3-1), 2.20 (3 H, s, CH_3-15), and 1.53 (3 H, s, CH_3-20).

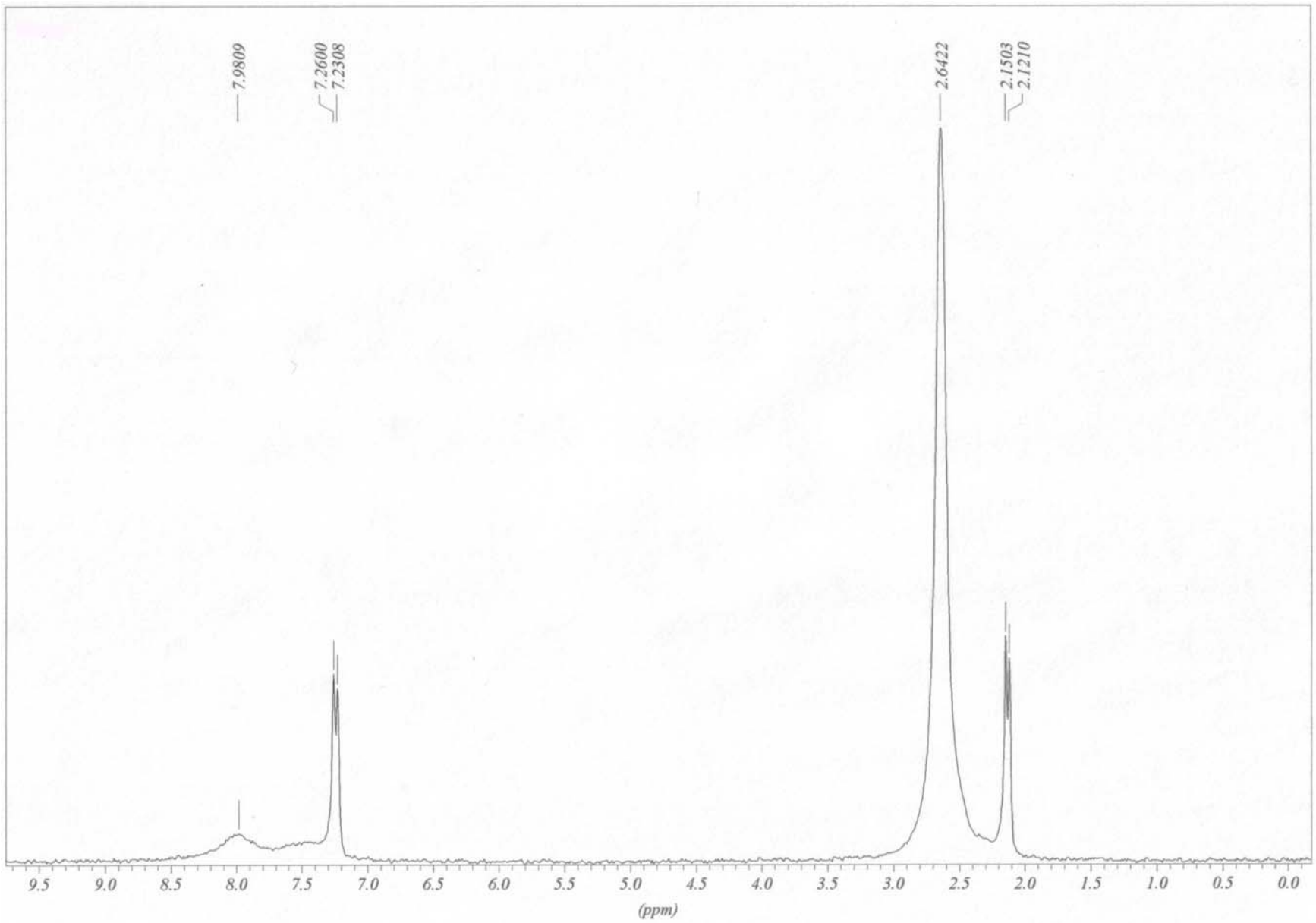
ESMS of azinomycin B after feeding of **4a**, **5a** and **6a**.



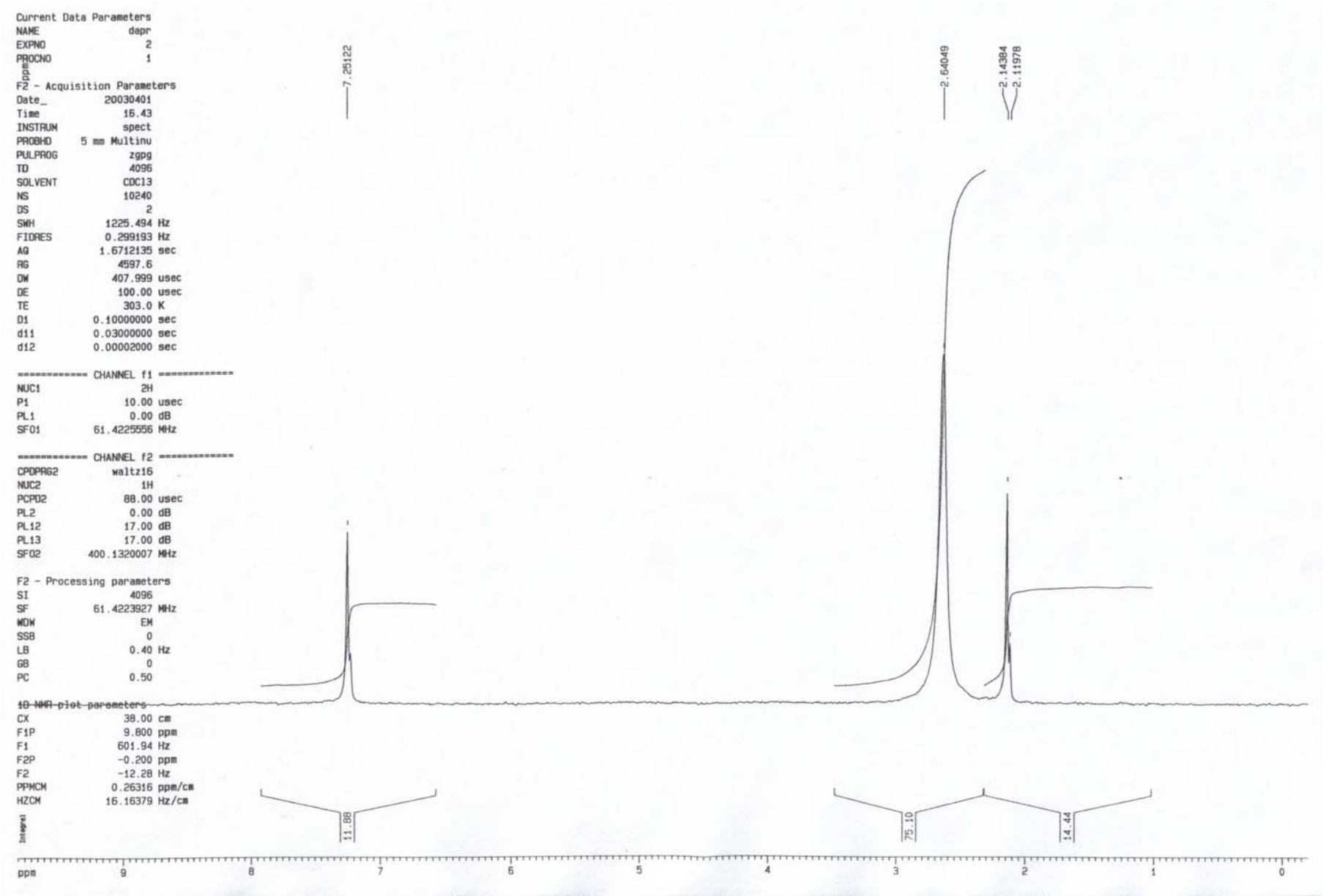
^{2}H NMR of azinomycin B after feeding of **4a**.



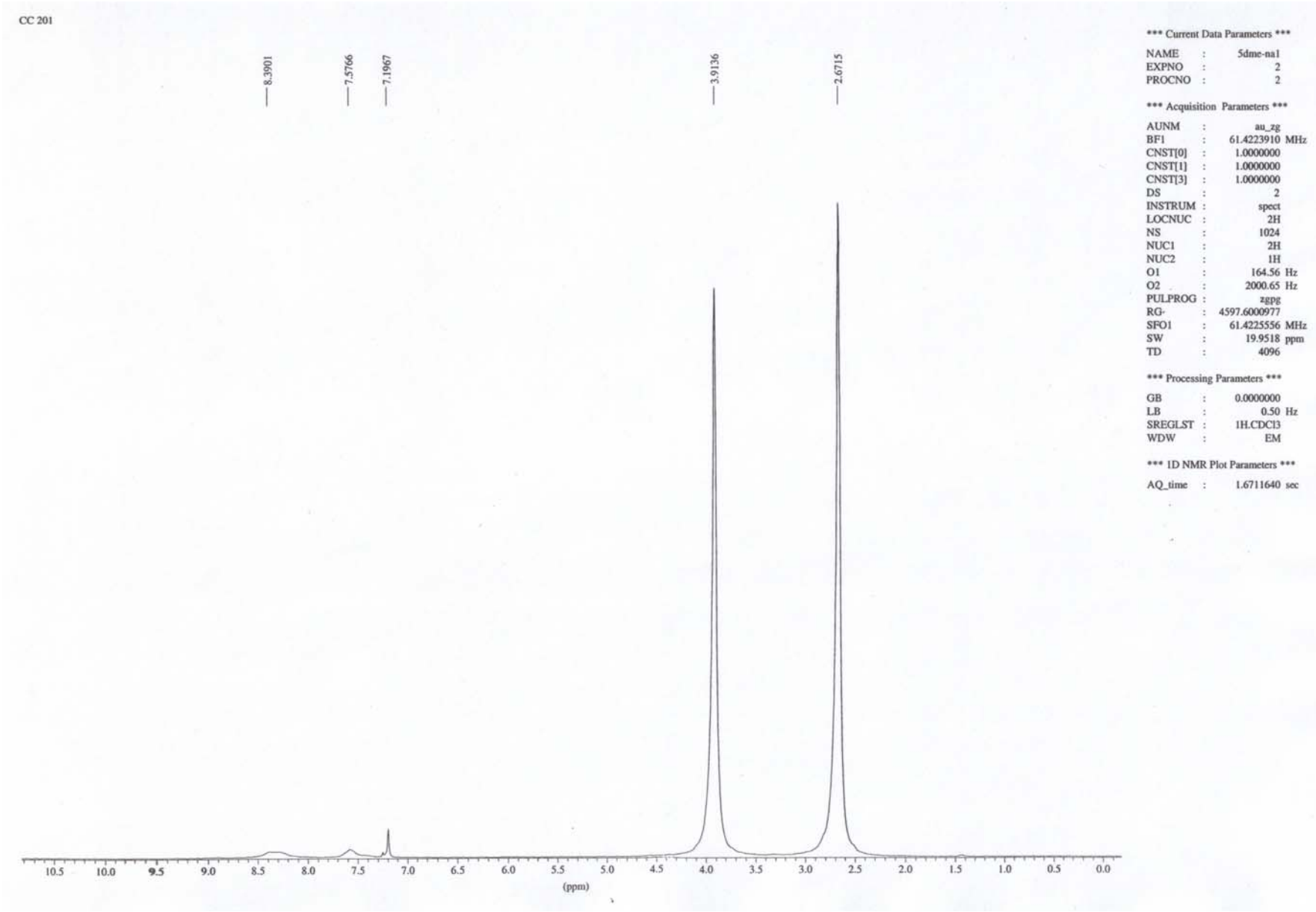
^{2}H NMR of azinomycin B after feeding of **5a**.



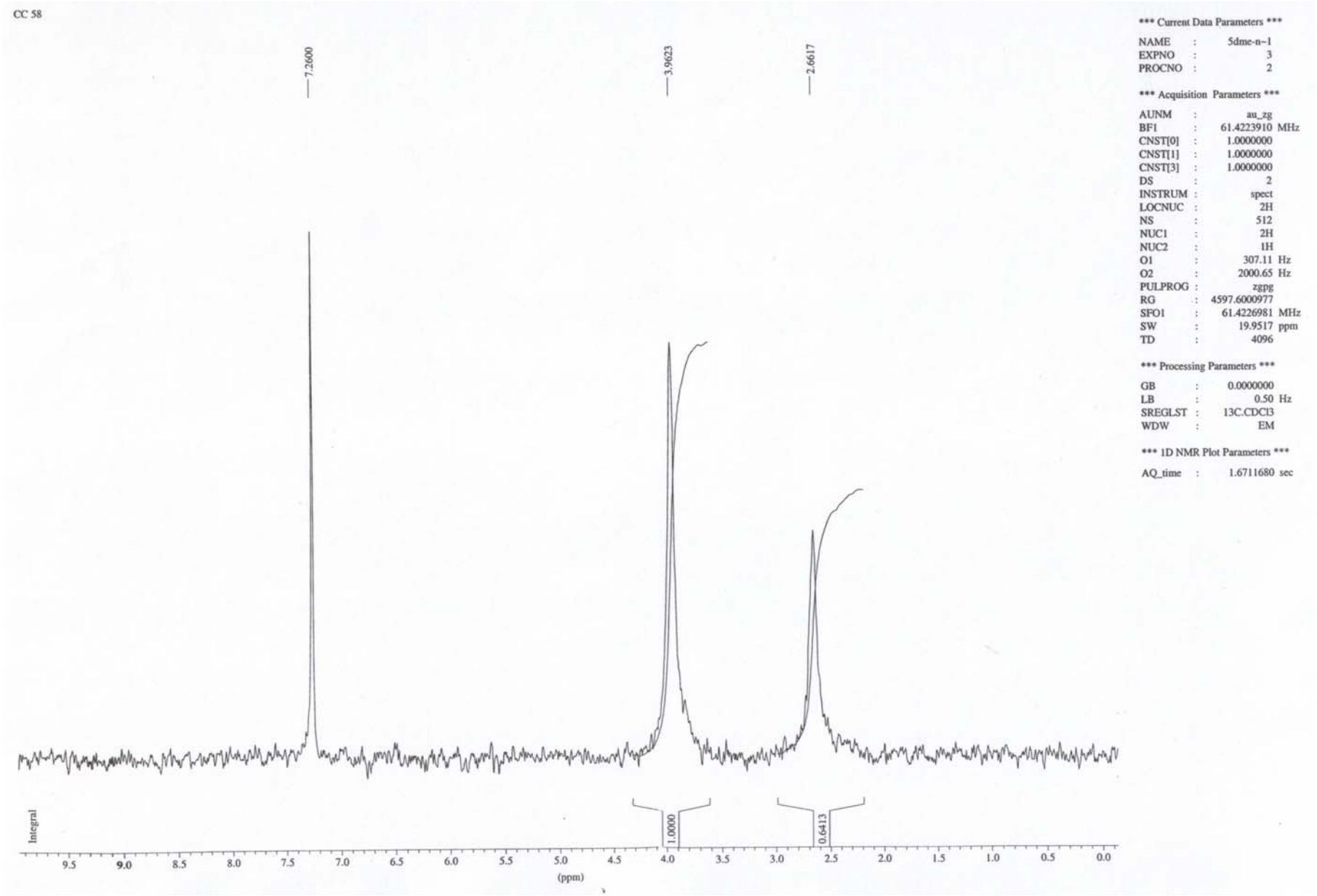
^{2}H NMR of azinomycin B after feeding of **6a**.



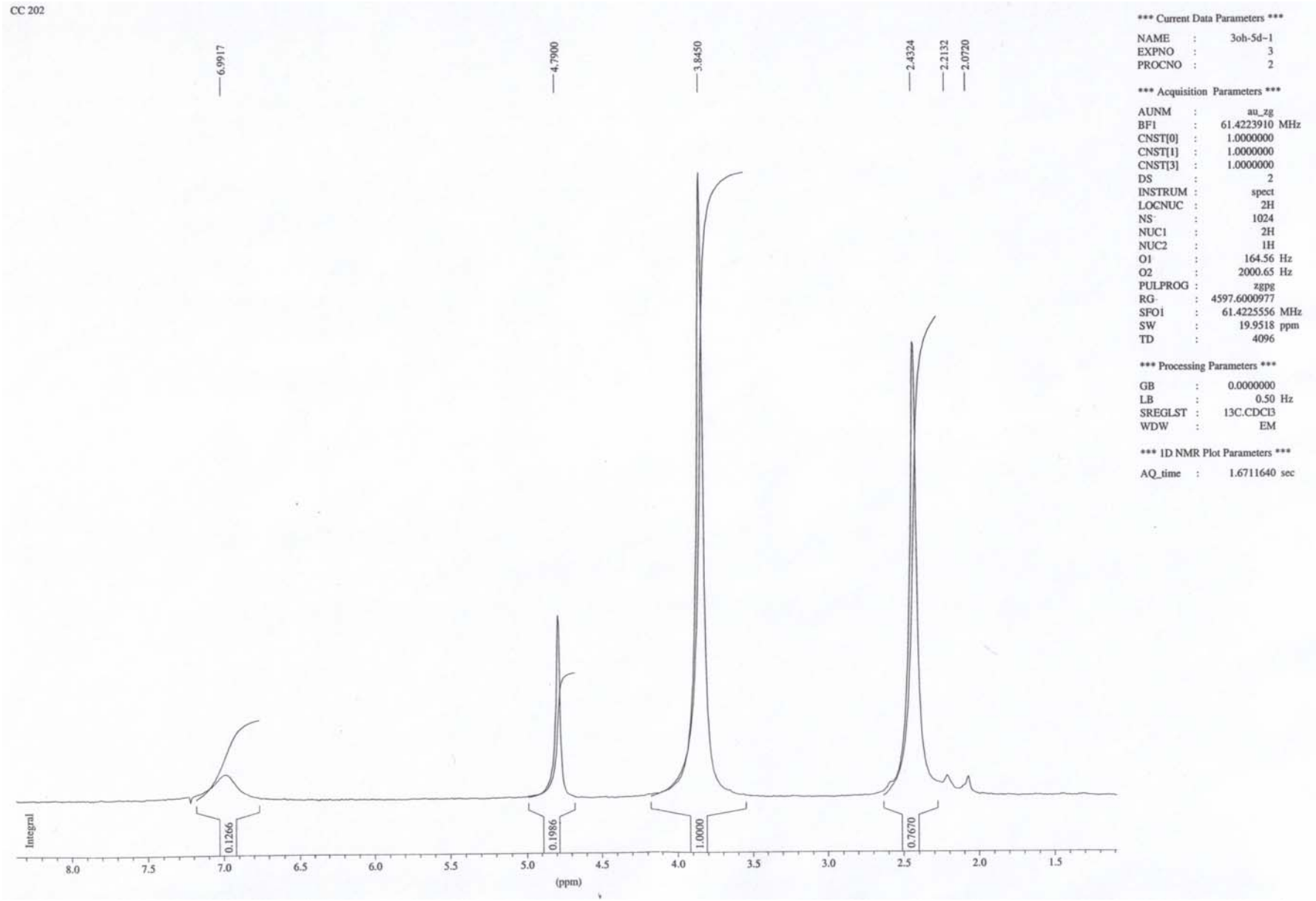
^{2}H NMR of the mixture of **4a** and **6b** used for competition feeding.



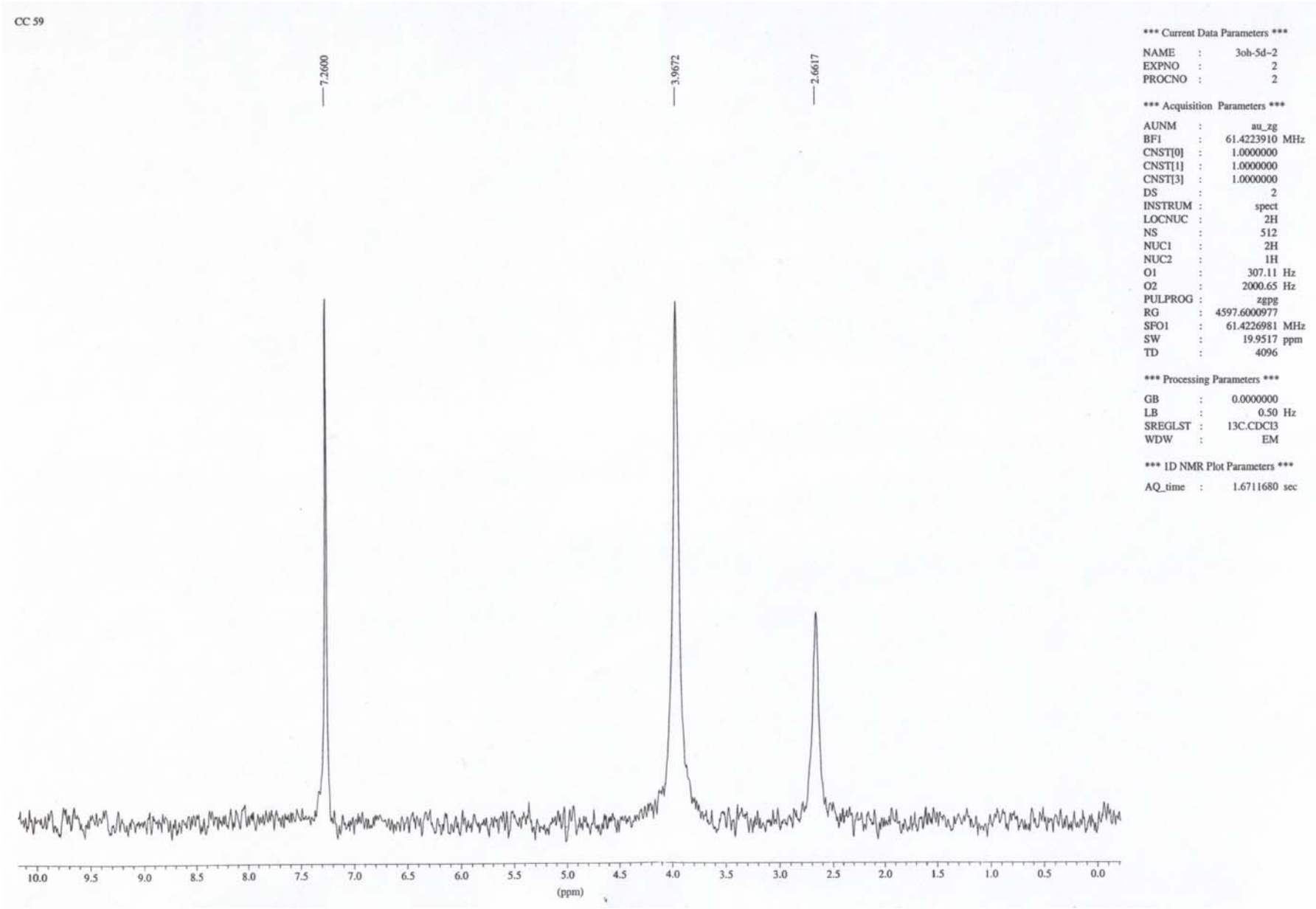
^{2}H NMR of azinomycin B after feeding of **4a** and **6b**.



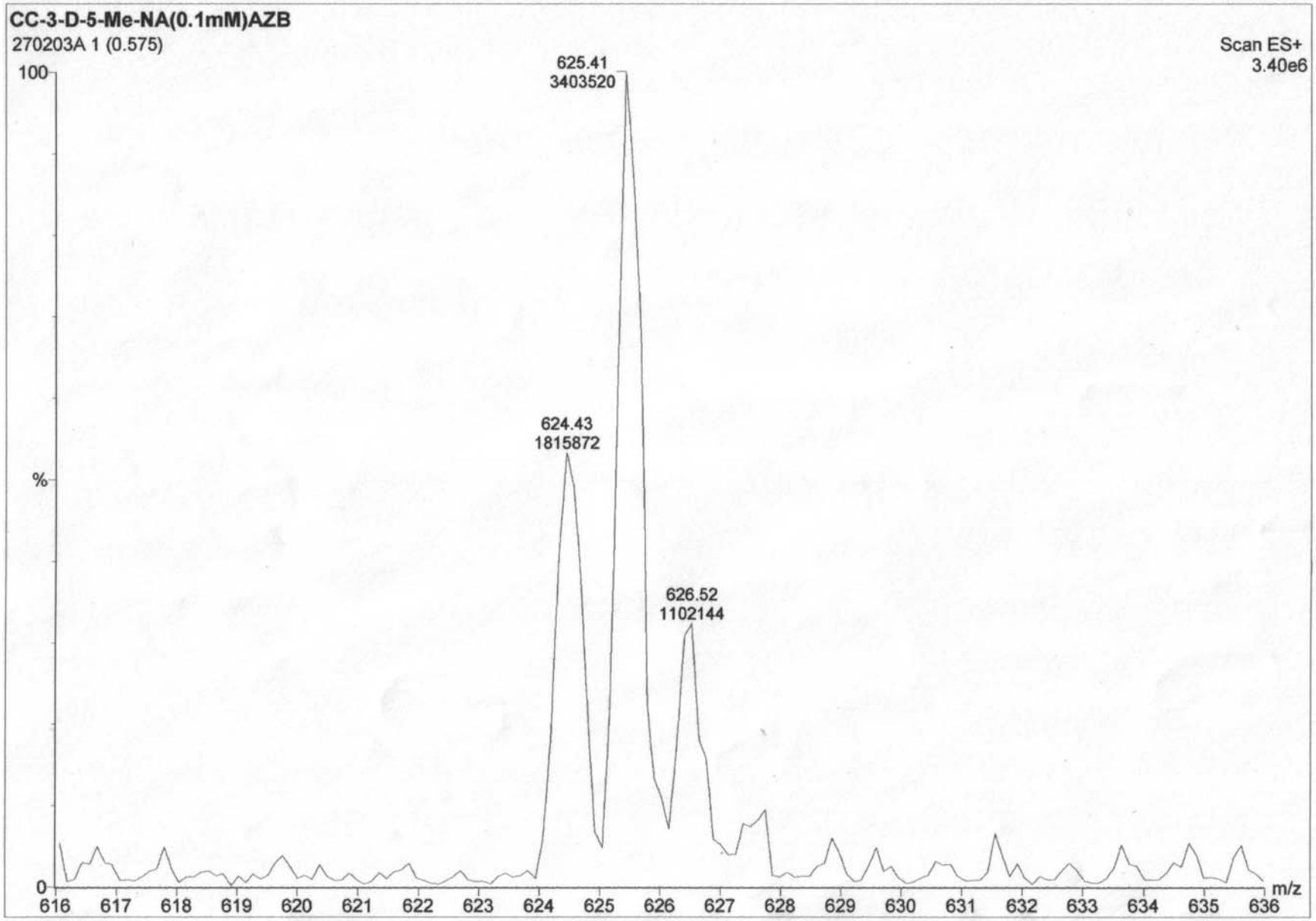
^{2}H NMR of the mixture of **5a** and **6b** used for competition feeding.



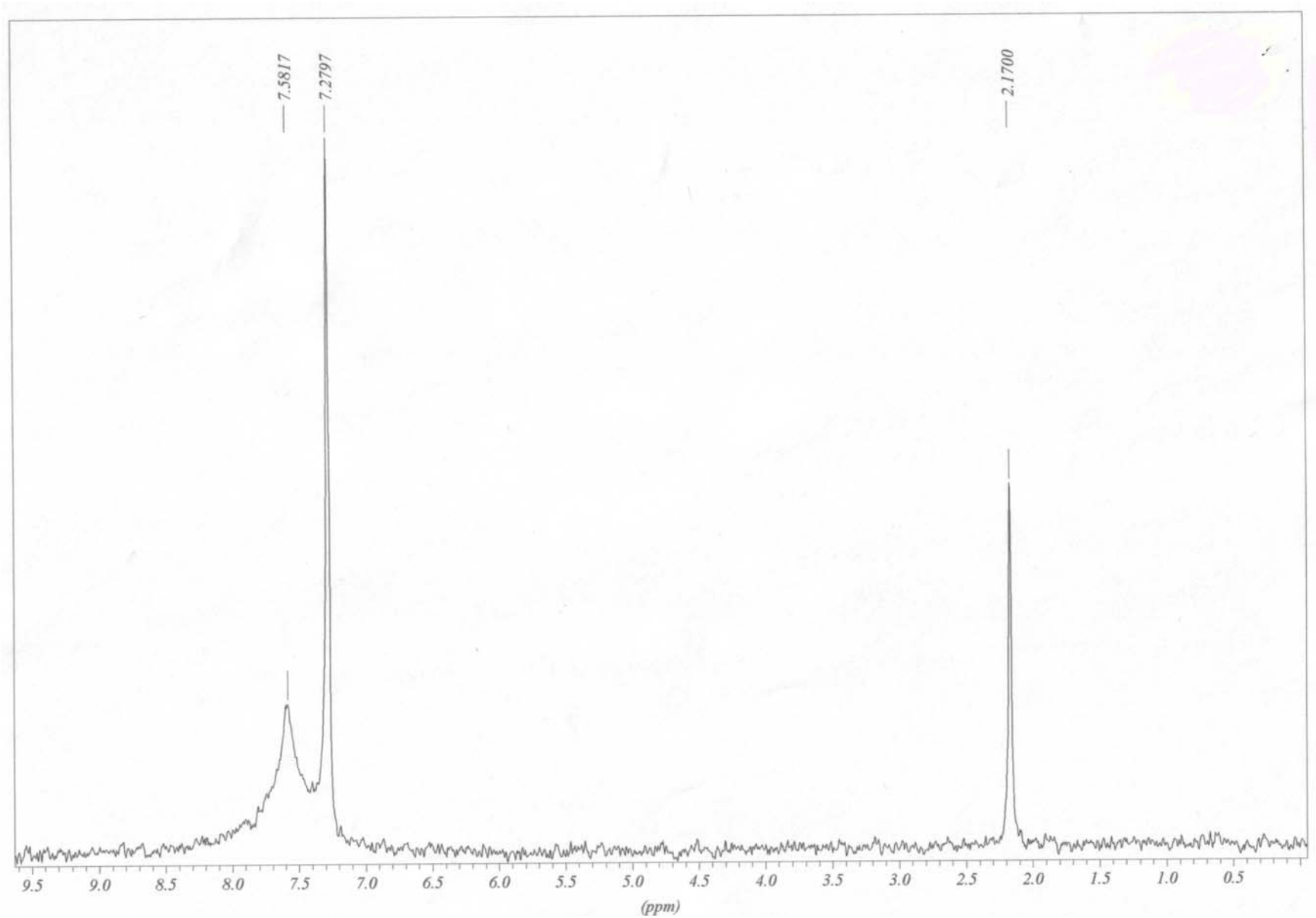
^{2}H NMR of azinomycin B after feeding of **5a** and **6b**.



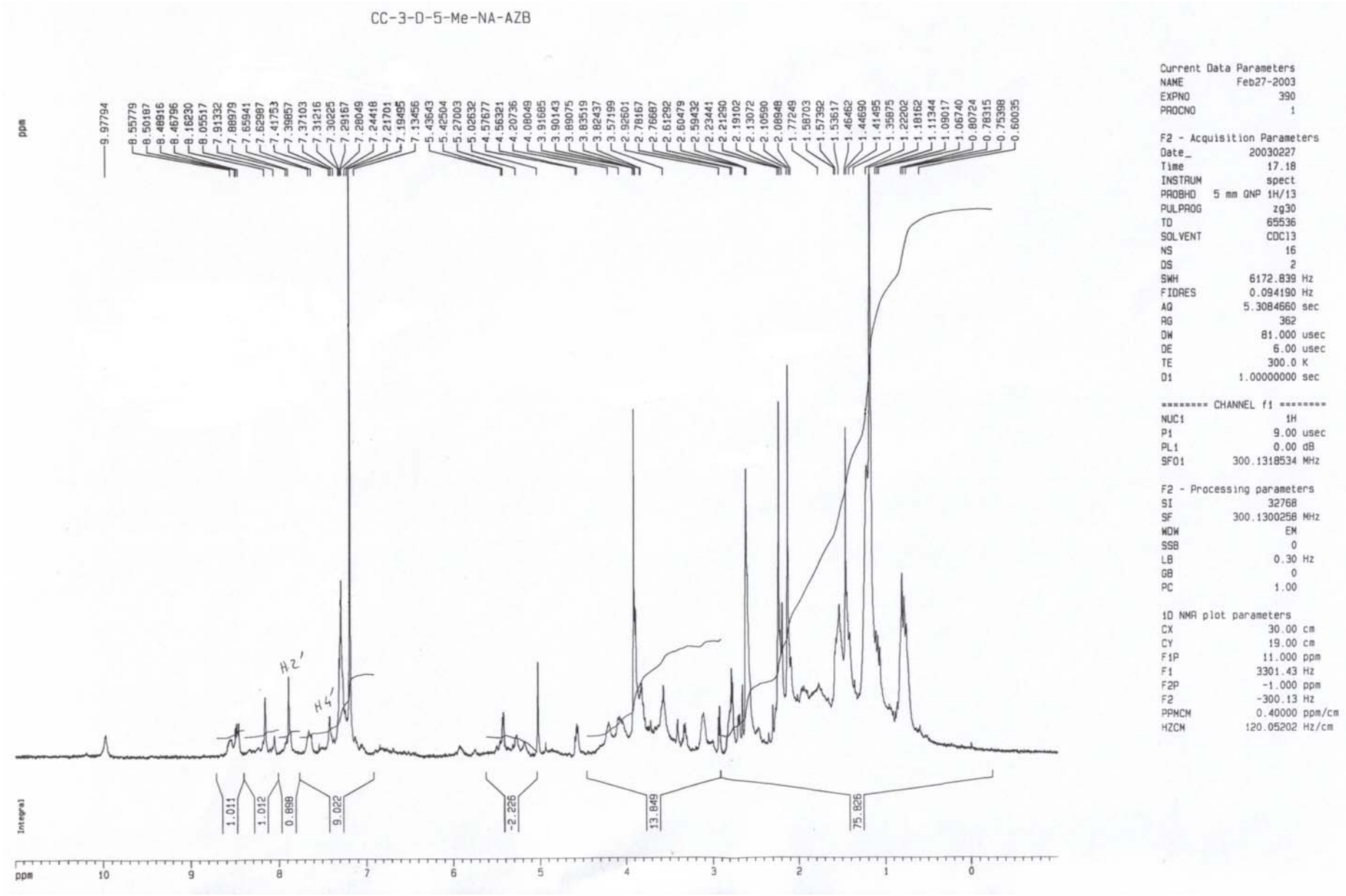
ESMS of azinomycin B after feeding of **4b**.



^{2}H NMR of azinomycin B after feeding of **4b**.



^{1}H NMR of azinomycin B after feeding of **4b**.



Current Data Parameters
NAME Feb27-2003
EXPNO 390
PROCNO 1

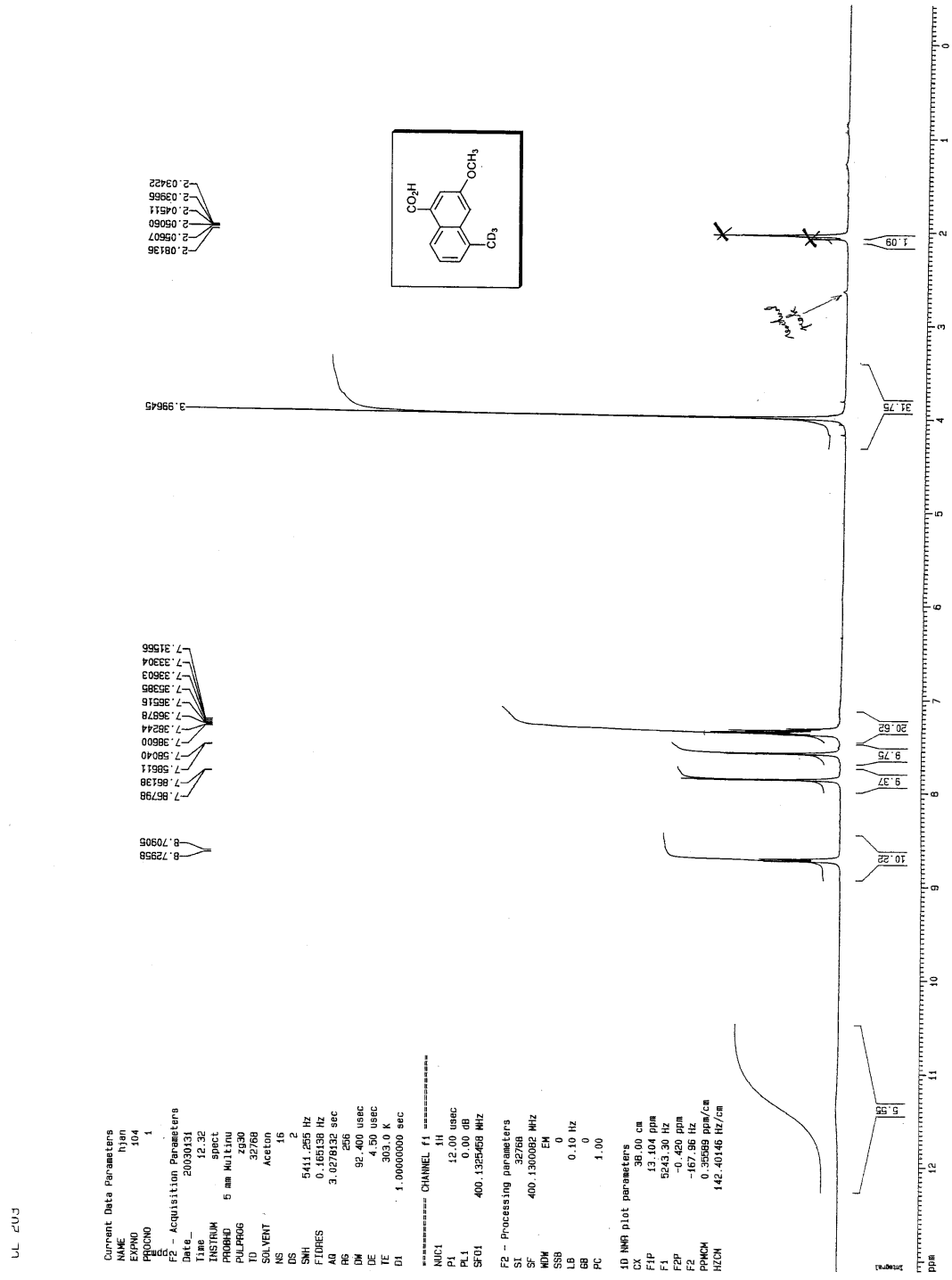
F2 - Acquisition Parameters
Date_ 20030227
Time 17.18
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 362
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 0.00 dB
SFO1 300.1318534 MHz

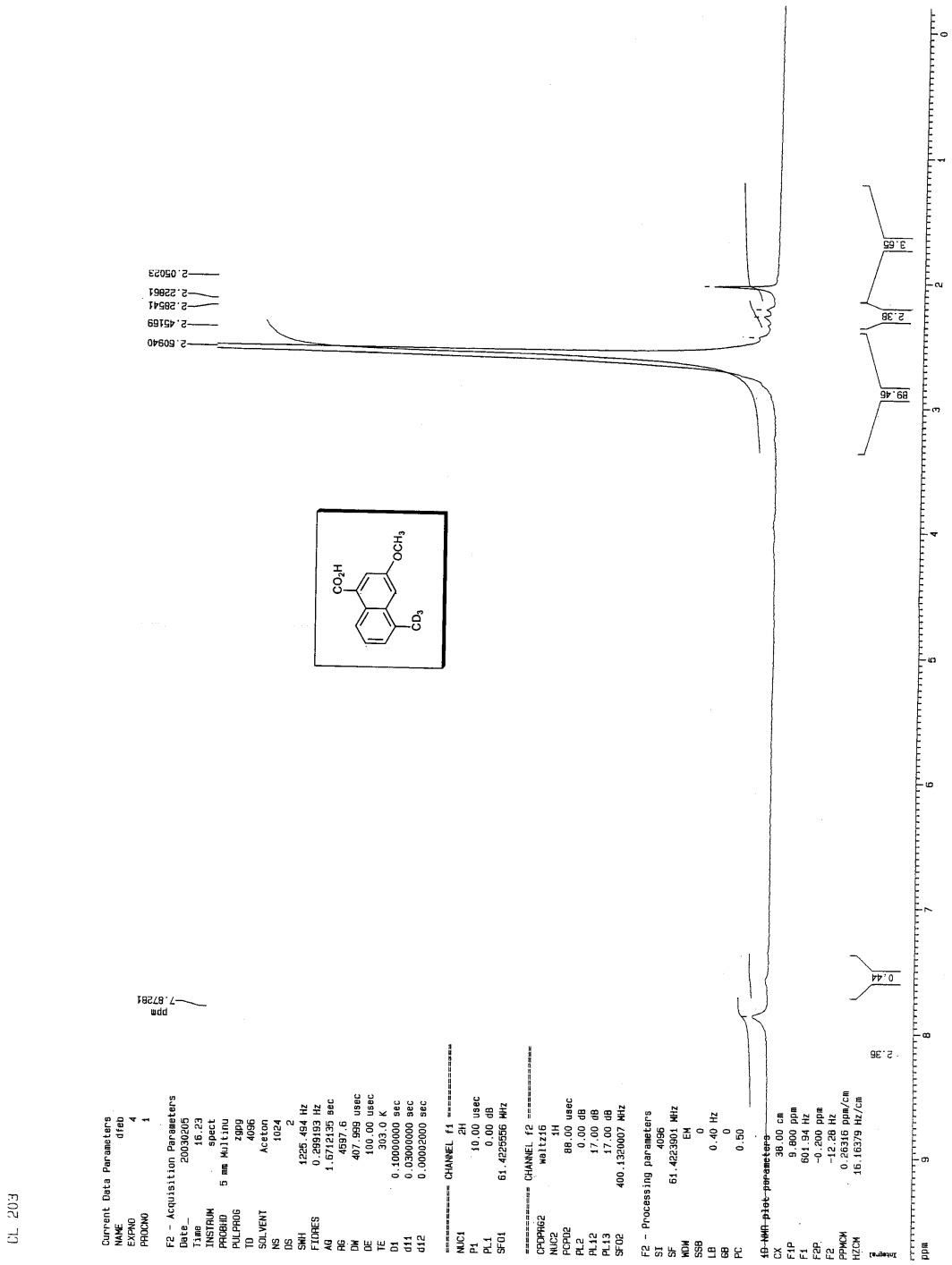
F2 - Processing parameters
SI 32768
SF 300.1300258 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 30.00 cm
CY 19.00 cm
F1P 11.000 ppm
F1 3301.43 Hz
F2P -1.000 ppm
F2 -300.13 Hz
PPMCM 0.40000 ppm/cm
HZCM 120.05202 Hz/cm

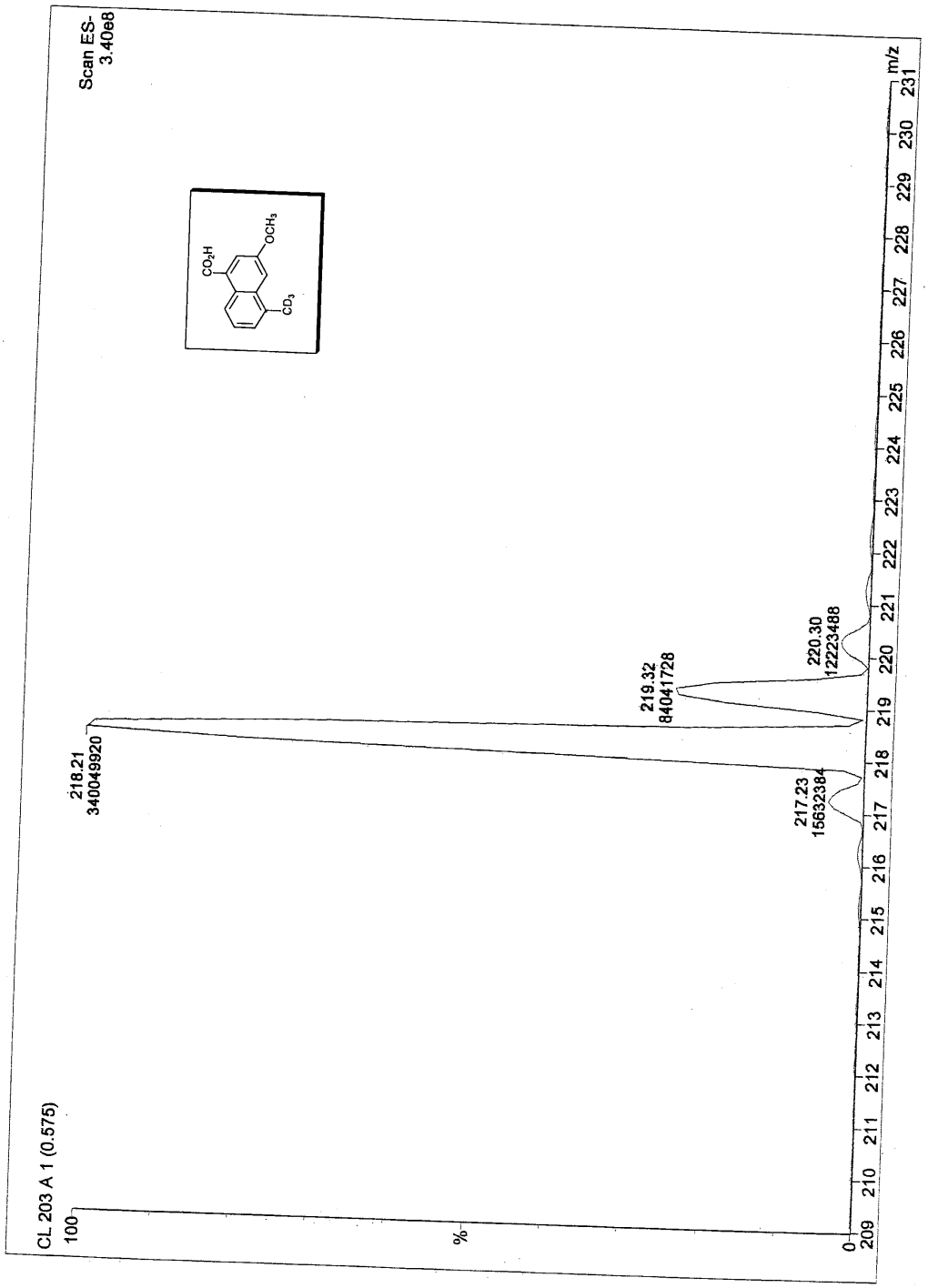
^{1}H NMR spectrum of **6a**.



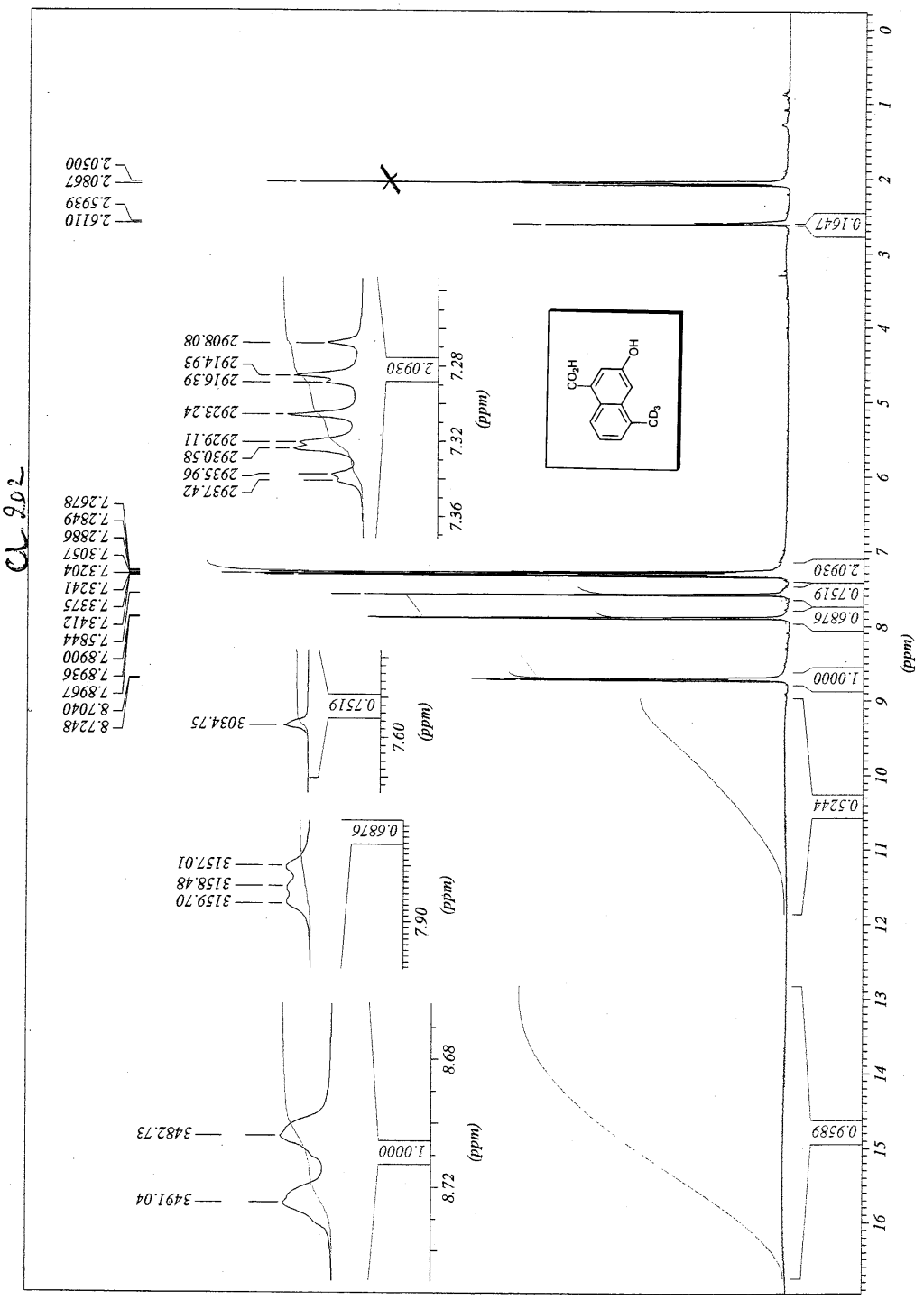
^{2}H NMR spectrum of **6a**.



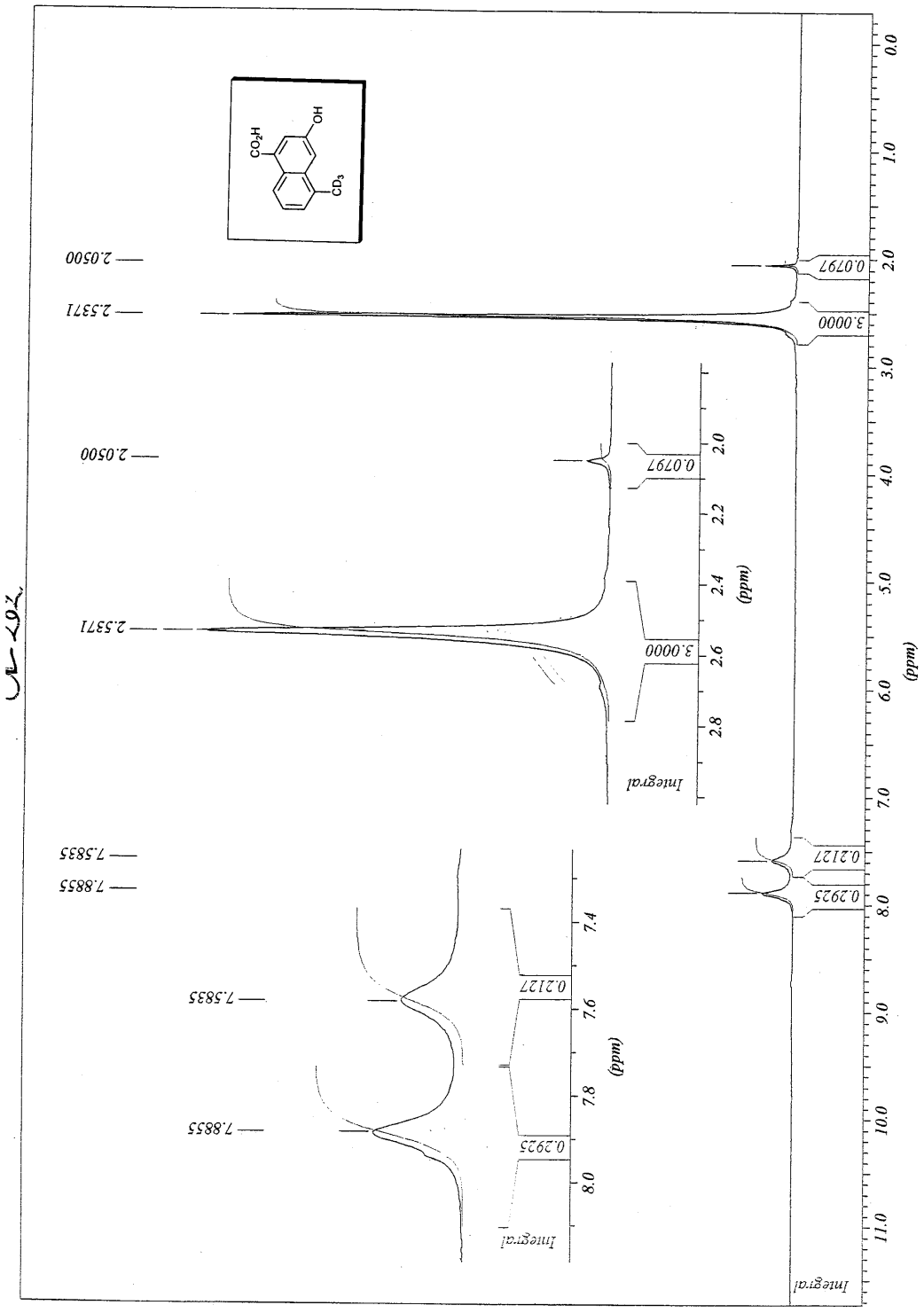
ESMS spectrum of **6a**.



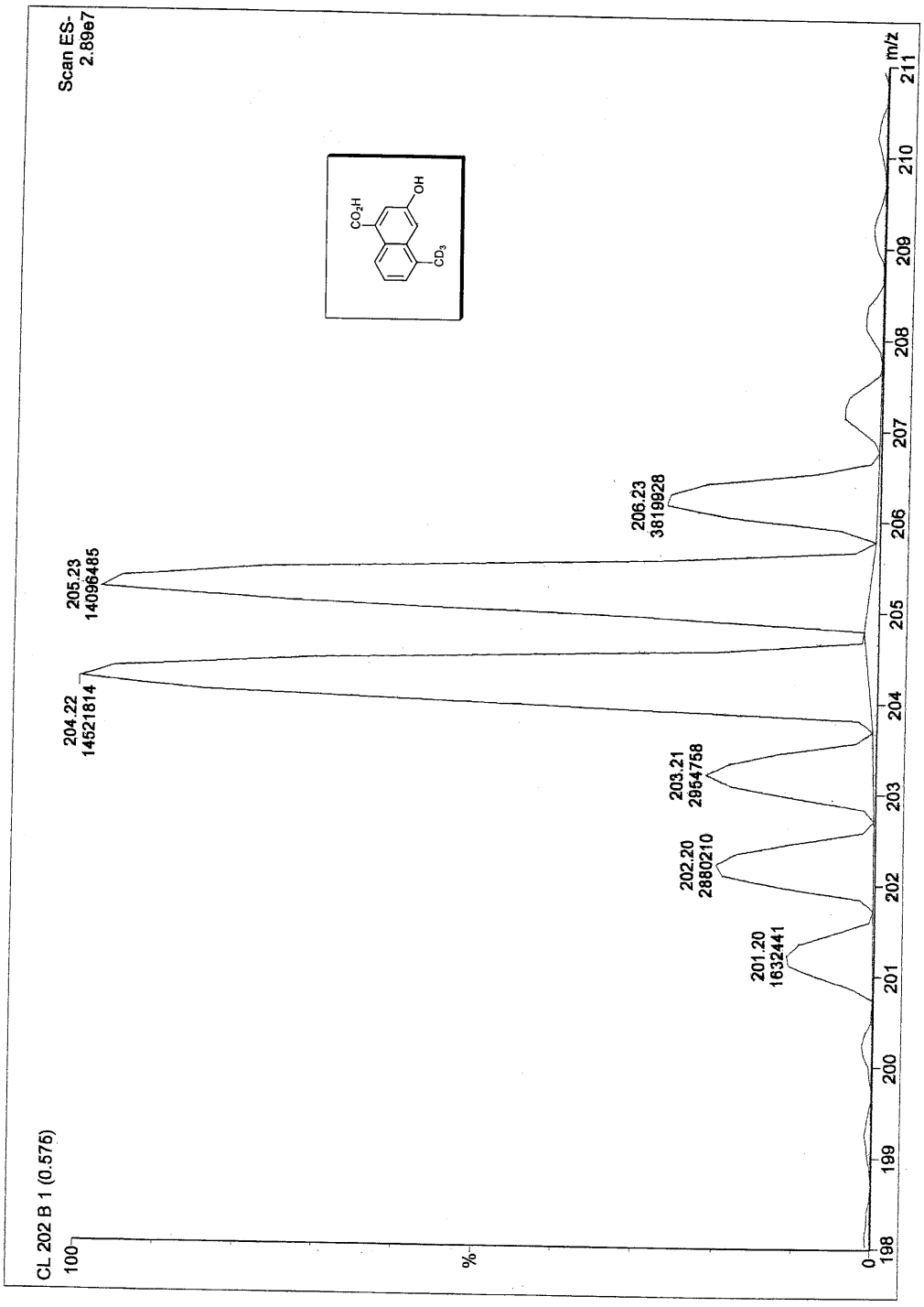
^{1}H NMR spectrum of **5a**.



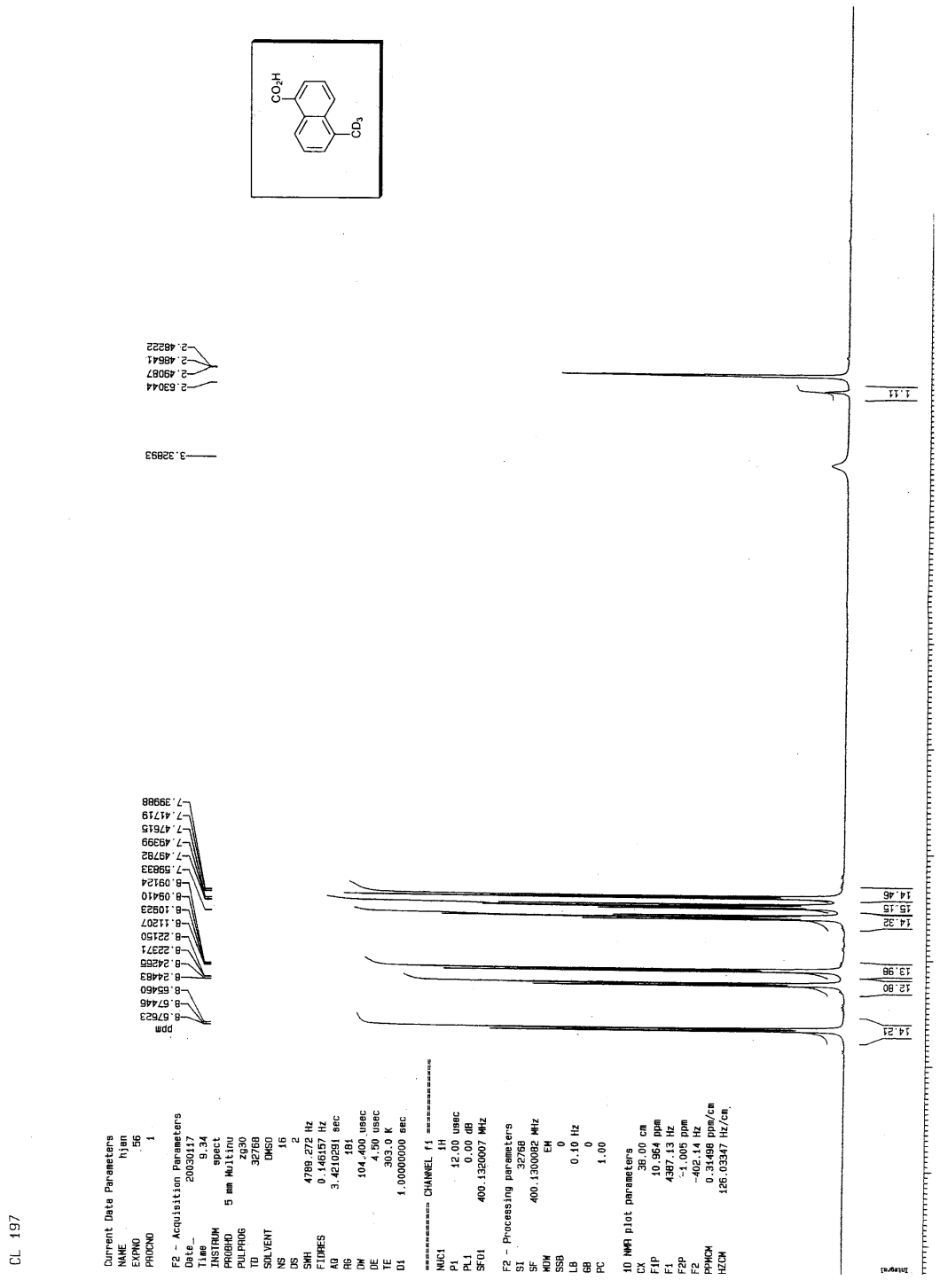
^{2}H NMR spectrum of **5a**.



ESMS spectrum of **5a**.

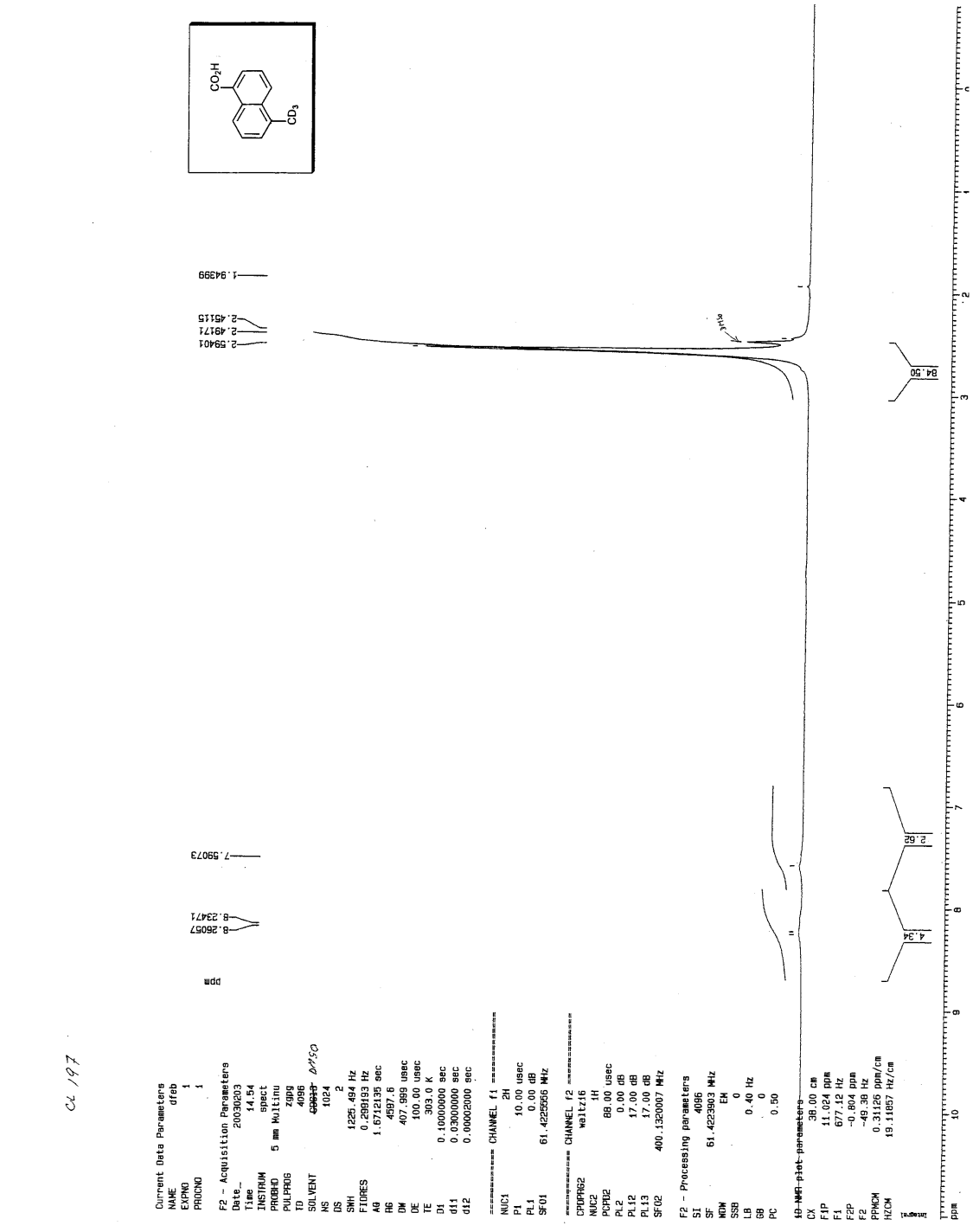


^{1}H NMR spectrum of **4a**.

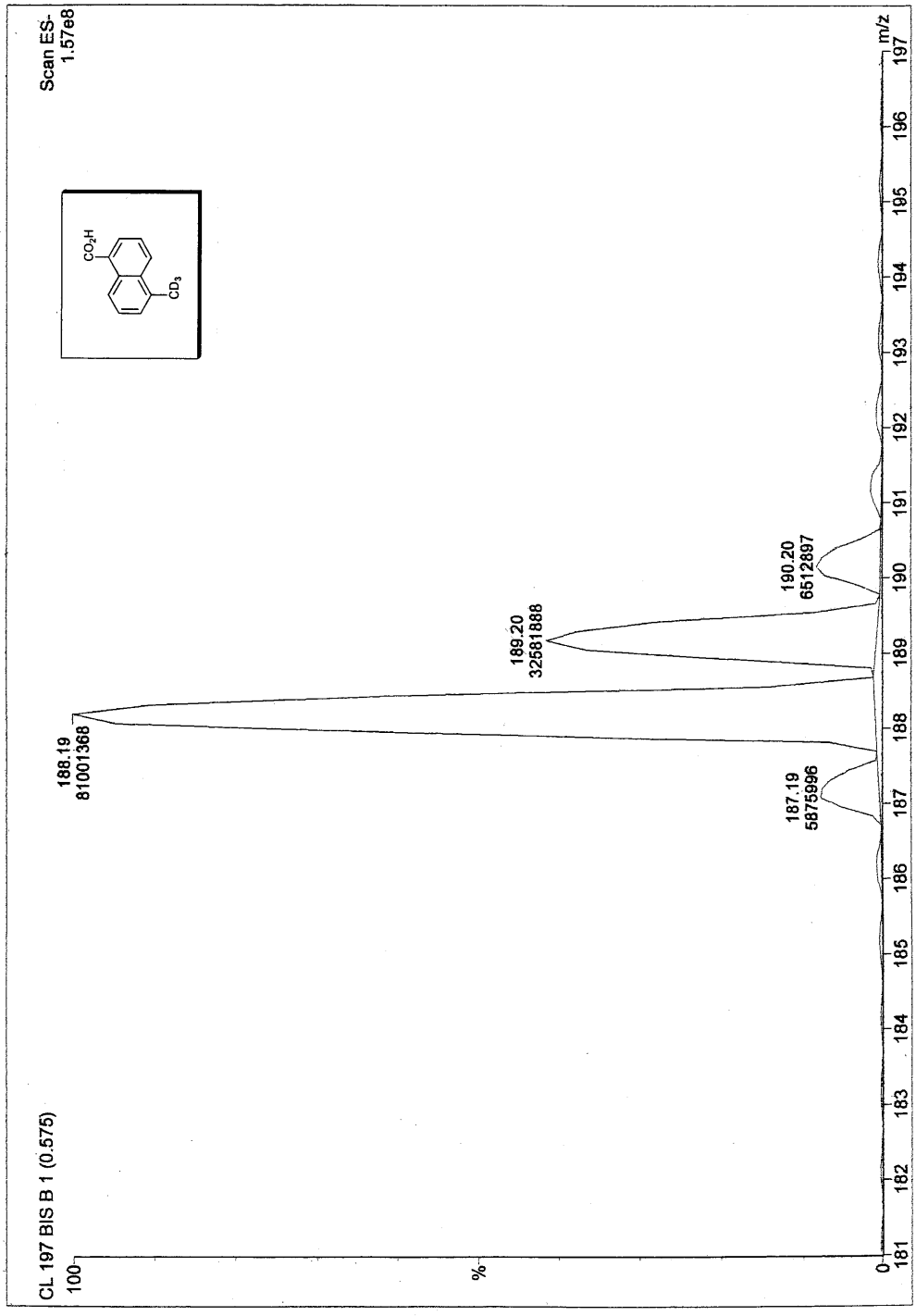


CL 197

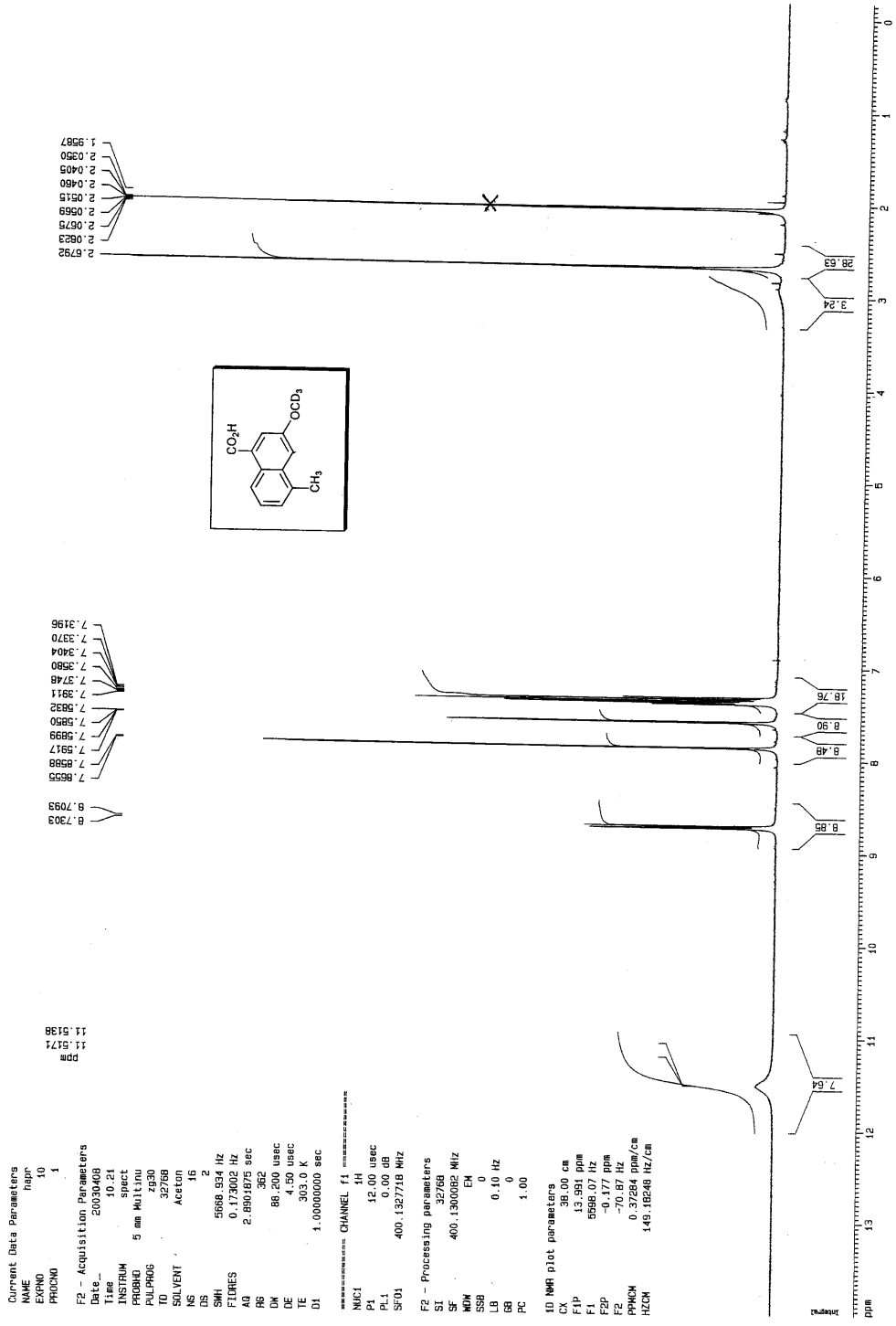
^{2}H NMR spectrum of **4a**.



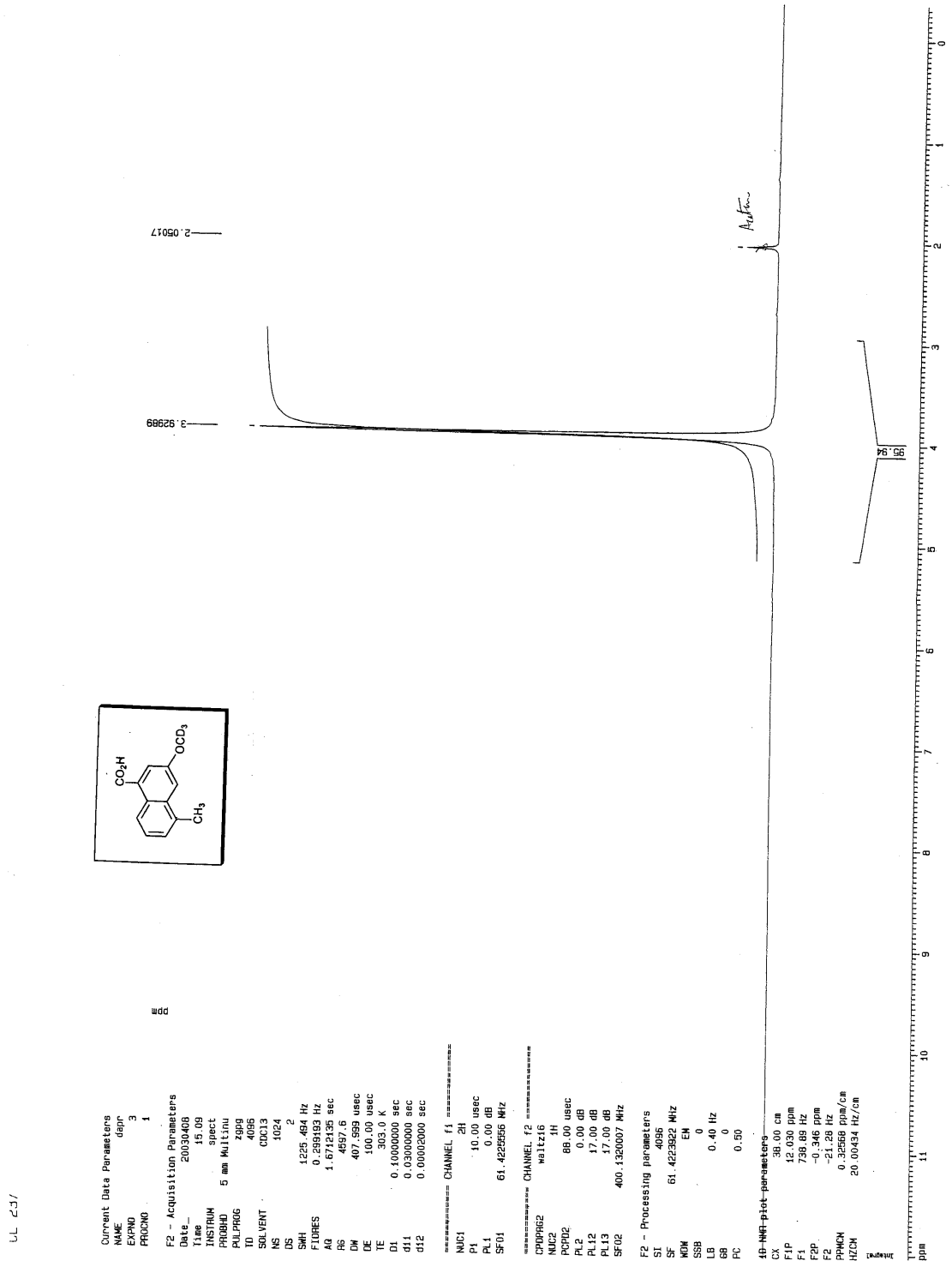
ESMS spectrum of **4a**.



^{1}H NMR spectrum of **6b**.



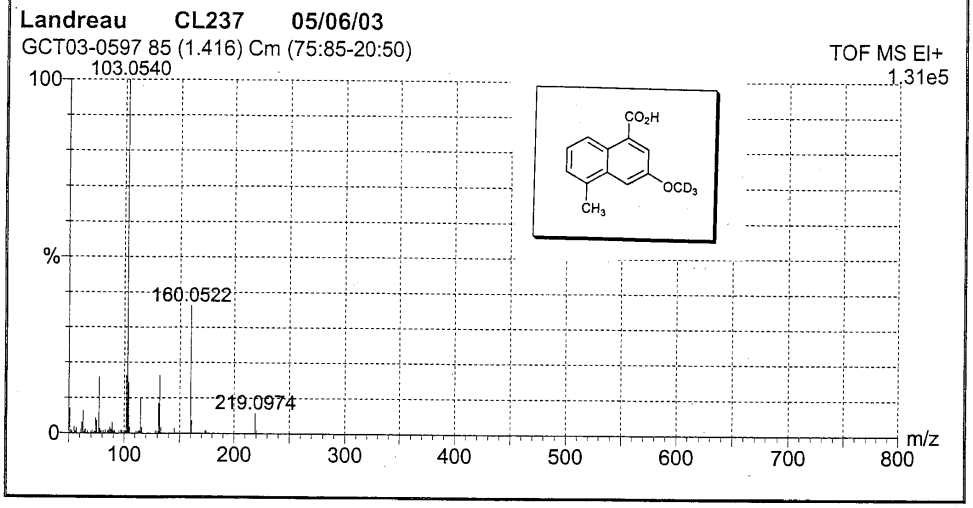
^{2}H NMR spectrum of **6b**.



EIMS spectrum of **6b**.

University of Exeter **School of Chemistry** **Mass Spectrometry Service**

Submitted By	Landreau	**Sample Reference**	CL237
Service Reference	GCT03-0597	**Predicted Mol. Formula**	C13H9D3O3
Ionisation	EI^+	**Calculated Exact Mass**	219.0975
Inlet	Solids probe	**M/Z Found**	219.0974



Elemental Composition Report

Single Mass Analysis (displaying only valid results)
Tolerance = 3.0 mDa / DBE: min = 0.0, max = 50.0

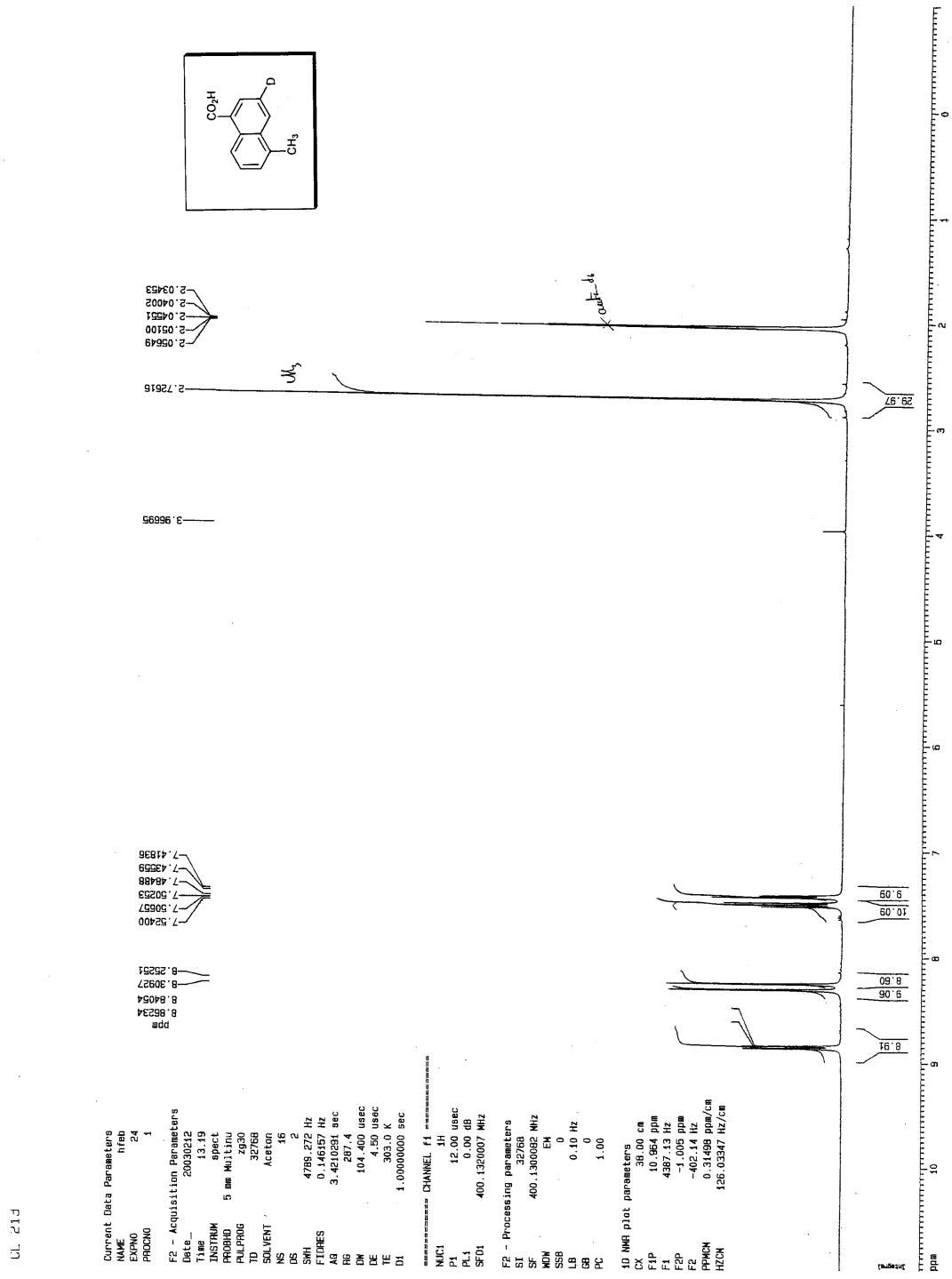
Monoisotopic Mass, Odd and Even Electron Ions
4029 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Minimum:			0.0		
Maximum:		3.0	5.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
219.0974	219.0975	0.0	-0.1	8.0	12C13 1H9 2H3 16O3
	219.0990	-1.6	-7.2	7.5	12C13 1H11 2H2 16O3

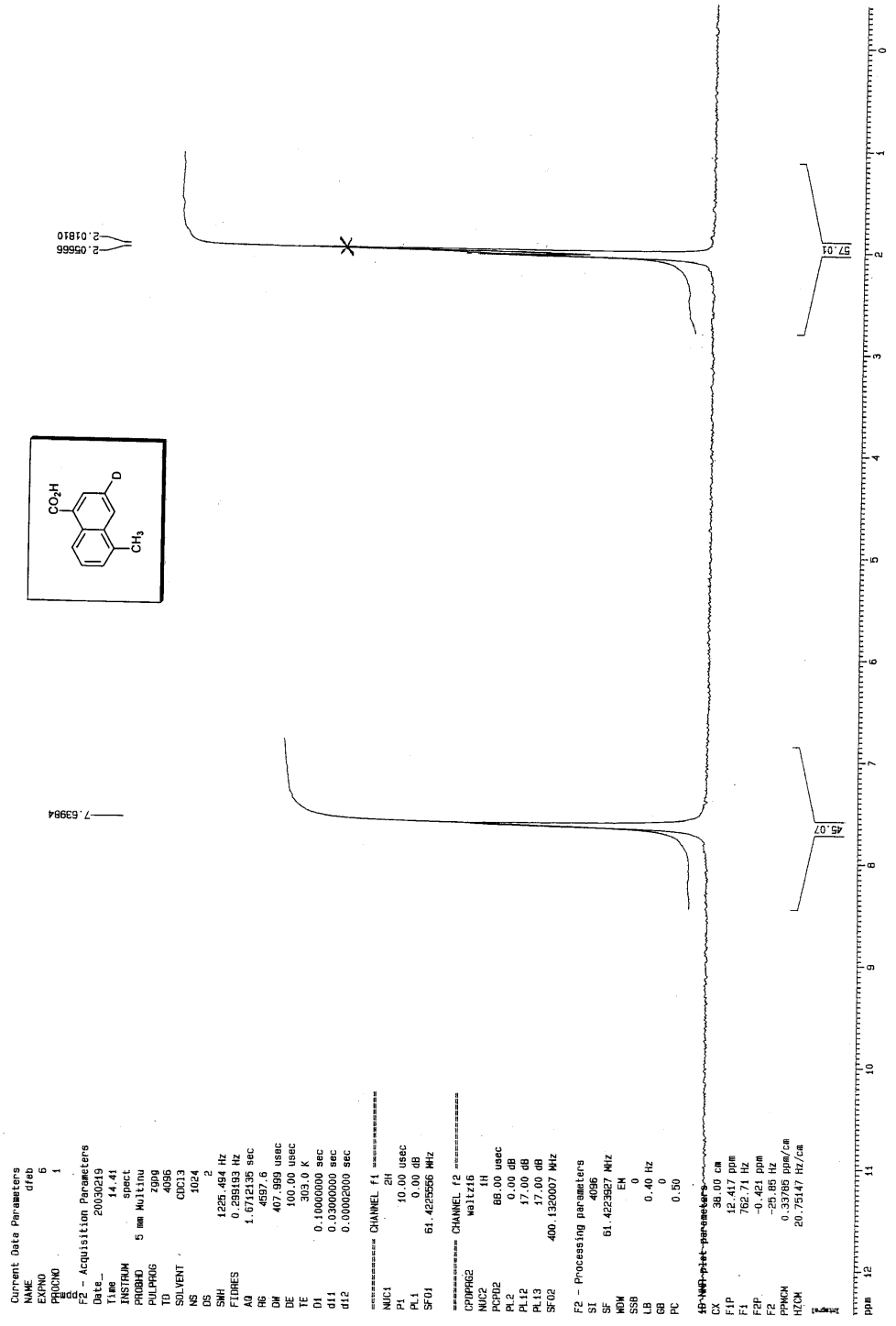
Operator's Comments

EI+
Probe at 100 deg C

^{1}H NMR spectrum of **4b**.



^{2}H NMR spectrum of **4b**.



ESMS spectrum of **4b**.