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Supporting Information

Efficient synthesis of some new antiproliferative *N*-fused indoles and isoquinolines via 1,3-dipolar cycloaddition reaction in ionic liquid

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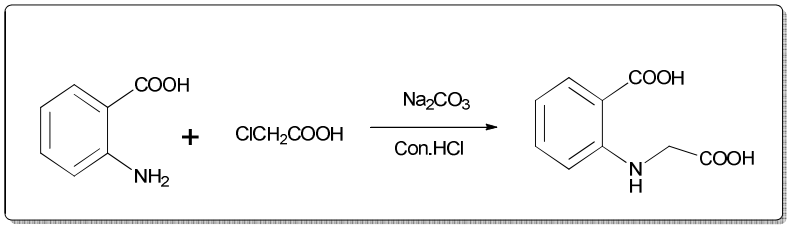
1.1 General method:

All solvents and reagents were used as supplied from commercial sources. The recorded melting points are uncorrected. IR spectra were recorded in KBr on Shimadzu FT-IR 8401 spectrometer and are reported in wave numbers (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR as solutions in $CDCl_3$, unless otherwise indicated. Chemical shifts are reported as parts per million (ppm) and referenced to the residual protic solvent. Coupling constants are reported in Hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet. The degree of substitution (C, CH, CH_2, and CH_3) was determined by the DEPT-135 method. UV spectra were record on Shimadzu Type 160-A spectrometer. The ESI mass spectra were measured on Shimadzu LCMS-2010 spectrometer. TLC was performed on Merck 60 F254 Precoated silica plates, spots were detected either by UV (254 nm, 366 nm) or dipping into a permanganate [$KMnO_4$ (3 g), K_2CO_3 (20 g), NaOH (5 mL, 5% in H_2O), H_2O (300 mL)] or an anisaldehyde solution [3% p-methoxybenzaldehyde and 1% H_2SO_4 in MeOH] or 2,4 Dinitro phenyl hydrazine solution [2,4-DNP (12 g), Conc. H_2SO_4 (6 mL), Water (8 mL), EtOH (20 mL)] followed by heating.

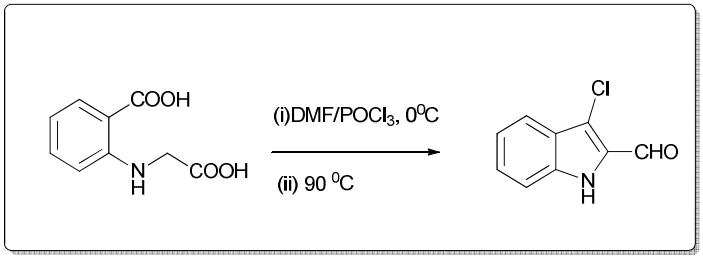
1.2 General Procedure for Synthesis of Aldehydes

1.2.1 Preparation of (phenylglycine)-*o*-carboxylic acid



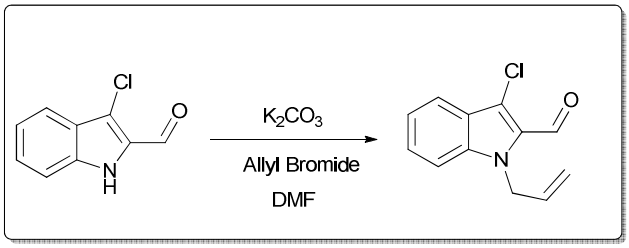
Procedure: A mixture of Anthranilic acid (0.1 mol; 14 g) with Chloroacetic acid (0.1 mol; 10 g) dissolved in 100 mL water was refluxed after adding 100 mL of aqueous sodium carbonate (0.19 mol; 20 g), in a three–necked round–bottom flask fitted with a reflux condenser, for 3 h. After cooling, acidifying slightly with concentrated HCl, the resulted reaction mass was then allowed standing overnight at rt. The solid crude acid was filtered off and washed with water. Finally the acid was re–crystallized from hot water using a little decolorizing carbon and dried at 100 °C. It gave 70 % yields, mp. 208-210℃

1.2.2 Preparation of 3-chloroindole-2-carbaldehyde



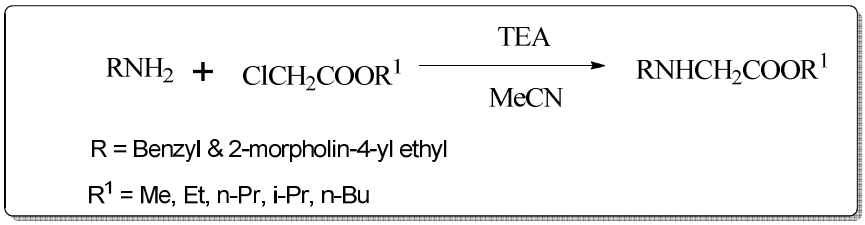
Procedure: A three–necked round–bottom flask equipped with a thermometer, a drying tube and a mechanical stirrer, was charged with DMF (0.26 mol; 20 mL). It was added $POCl_3$ (0.06 mol; 5.6 mL) drop–wise after cooling to 0 ℃ with constant stirring followed by addition of (phenylglycine)-*o*-carboxylic acid (0.01 mol, 1.9 g). The resulting mass was heated at 90 ℃ for 4–6 h, cooled to rt, and then poured into crushed ice (300 g) with mechanical stirring. The solid products were filtered and washed with water till the washings got neutralized. The yields were 75 % and mp, 172–174 ℃.

1.2.3 Preparation of N-allyl 3-chloroindole-2-carbaldehyde.



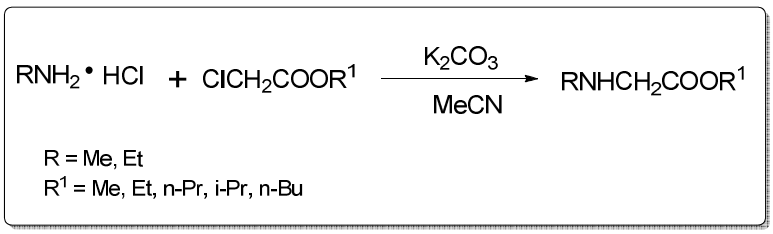
Procedure: A stirred solution of 3-chloroindole-2-carbaldehyde (10.0 mmol; 1.79 g), in anhydrous potassium carbonate (15.0 mmol; 2.06 g) suspended in DMF (25 mL), was added drop-wise allyl bromide (15.0 mmol; 1.79 g) in DMF (10 mL). It was stirred further at rt till the starting components were disappeared. On completion of the reaction as monitored by TLC (10–12 h), the reaction mass was poured into ice with constant stirring. Resulting mass will extract with diethyl ether (10ml x 3), after that ether layer dry with sodium sulphate and evaporate solvent to get product. Product yield was in the 90–92 % range

1.3.1 General procedure for the synthesis of secondary amine (4a-e and 5a-e):



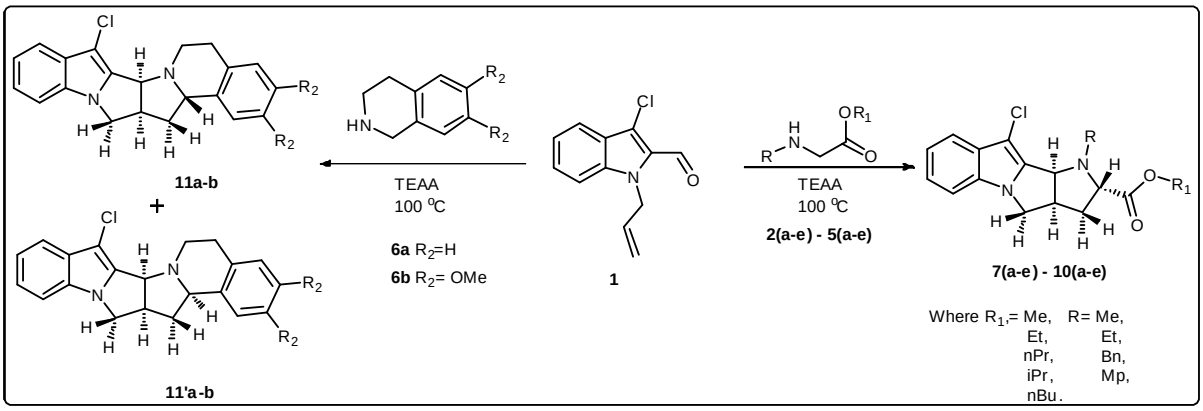
To a stirred solution of amine (0.047 mole) and TEA (triethyl amine) (0.070 mole) in acetonitrile (20ml), was added corresponding chloroacetic acid ester $ClCH_2COOR^1$ (0.047 mole) in acetonitrile (10ml) drop wise at room temperature with continue stirring for further 4 h. Inorganic precipitates TEA·HCl salt thus formed was then filtered, washed with acetonitrile. Finally, the acetonitrile washing, along with the filtrate left were combined and evaporated under vacuum to give colourless liquid product which was then used in the next step without further purification.

1.3.2. General procedure for the synthesis of secondary amines (2a-e & 3a-e):



To a stirred suspension of RNH_2·HCl (0.075 mole) at 15˚C with incrementally added K_2CO_3 (0.15 mole) in acetonitrile (50ml) was over a period of 1.5 h, was added drop wise a solution of corresponding ester $ClCH_2COOR_1$ (0.075mole) in acetonitrile (10ml) at room temperature with vigorous stirring for 10 h. Precipitates of inorganic salts formed was then filtered off, washed with acetonitrile. Finally, the solvent was evaporated under vacuum to give colorless liquid product which was then freshly used in the next step without further purification**.**

1.4. Preparation of pyrrolo-fused indoles and isoquinolines.



In a round-bottom flask, a mixture of an aldehyde (1 equiv) and an amine (1 equiv) was taken in 2 mL ionic liquid TEAA was heated at 100˚C. After confirming the completion of reaction by TLC, reaction mass was pour into ice, it gave quantitative desired products which purified by column chromatography using a mixture containing ethyl acetate and n-hexane in a ratio of 10:90 as an eluent. The overall yields were in the 75-85 % range.

1.5 Biological activities

Antimicrobial activity

The in vitro antimicrobial activity of all the compounds were determined against three Gram-positive Streptococcus pneumoniae (MTCC 1936), Clostridium tetani (MTCC 449), Bacillus subtilis (MTCC 441), three Gram-negative Salmonella typhi (MTCC 98), Vibrio cholerae (MTCC 3906), Escherichia coli (MTCC 443) bacteria and two Aspergillus fumigatus (MTCC 3008), Candida albicans (MTCC 227) fungi by the broth microdilution MIC (minimum inhibitory concentration) method according to NCCLS (National Committee for Clinical Laboratory Standards).[1] Strains employed for the activity were procured from [MTCC (Micro Type Culture Collection)] Institute of Microbial Technology, Chandigarh. Mueller–Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose Broth used for fungal nutrition. Bacterial strains were primarily inoculated into Mueller–Hinton agar and, after overnight growth, a number of colonies were directly suspended in saline solution until the turbidity matched the turbidity of the McFarland standard (approximately 108 CFU/mL), i.e., inoculum size for test strain was adjusted to 108 CFU/mL (colony forming unit) per milliliter well by comparing the turbidity (turbidimetric method). Similarly, fungi were inoculated on Sabouraud Dextrose Broth; the procedures of inoculum standardization were also similar. DMSO was used as diluents/vehicle to get desired concentration of compounds to test upon standard microbial

strains, i.e., the compounds were dissolved in DMSO and the solutions were diluted with a culture medium. Each compound and standard drugs were diluted obtaining 2,000 lg/mL concentration, as a stock solution. By further progressive dilutions with the test medium, the required concentrations were obtained for primary and secondary screening. In primary screening 1,000, 500, and 250 µg/mL concentrations of the compounds were taken. The active compounds found in this primary screening were further diluted to obtain 200, 100, 62.5 lg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Briefly, the control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism. The tubes are then put for incubation at 37 ℃ for 24 h for bacteria and 48 h for fungi. Growth or a lack of growth in the tubes containing the antimicrobial agent was determined by comparison with the growth control, indicated by turbidity. The lowest concentration that completely inhibited visible growth of the organism was recorded as the minimum inhibitory concentration (MIC, µg/mL), i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. All bioassays were repeated at least three times and the calculated error margin value was ±2 µg/mL. Protocols are summarized in Table 2 as the minimum inhibitory concentration (MIC, µg/mL).

Antitubercular activity

Screening was conducted at 250 µg/ml against M. tuberculosis H37Rv following a Lowenstein–Jensen (L-J) MIC method. Compounds were added to liquid L-J medium and then media were sterilized by inspissations method.[2] A culture of M. tuberculosis H37Rv grown on L-J medium was harvested in 0.85 % saline in bijou bottles. DMSO was used as vehicle to get a desired concentration. These tubes were then incubated at 37 ℃ for 24 h followed by streaking of M. tuberculosis H37Rv (5 9 104 bacilli per tube). These tubes were then incubated at 37 ℃. Growth of bacilli was seen after 12, 22, and finally 28 days incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with M. tuberculosis H37Rv. The standard strain M. tuberculosis H37Rv was tested with known drugs; isoniazide, fluconazole and miconazole. The creening test results are summarized as % inhibition relative to standard drugs.

Antioxidant activity[3]

Reagents:

1) Acetate buffer, pH 3.6, 300 mmol/L; (3.1g sodium acetate trihydrate and 16 mL glacial acetic acid per liter of buffer solution).
2) 10 mmol/L, 2,4,6-tripyridyl-s-triazine (TPTZ) (MW 312.34) solution in 40 mmol/L HCl.
3) 20 mmol/L, $FeCl3 \cdot 6H2O$ (MW 270.30) in distilled water (DW).

FRAP working solution: It can be prepared by mixing of 25 mL acetate buffer (1), 2.5 mL TPTZ solution and 2.5 mL $FeCl3 \cdot 6H2O$ solution. The working solution must always be a freshly prepared.

Sample solution: 0.005 g dissolved in 25 mL DMF.

Standard solution: 1mmol of ascorbic acid (MW 176.13 g/mol) in 100 mL DW. Procedure: 400 μL sample or 200 μL standard was mixed with 3.0 mL FRAP working solution and incubated the resulted mixture at 37°C for 10 min. Its absorbance was measured at 593 nm using respective blank solutions. The antioxidant activity is expressed as ascorbic acid equivalent (mM/100 g of dried compound).

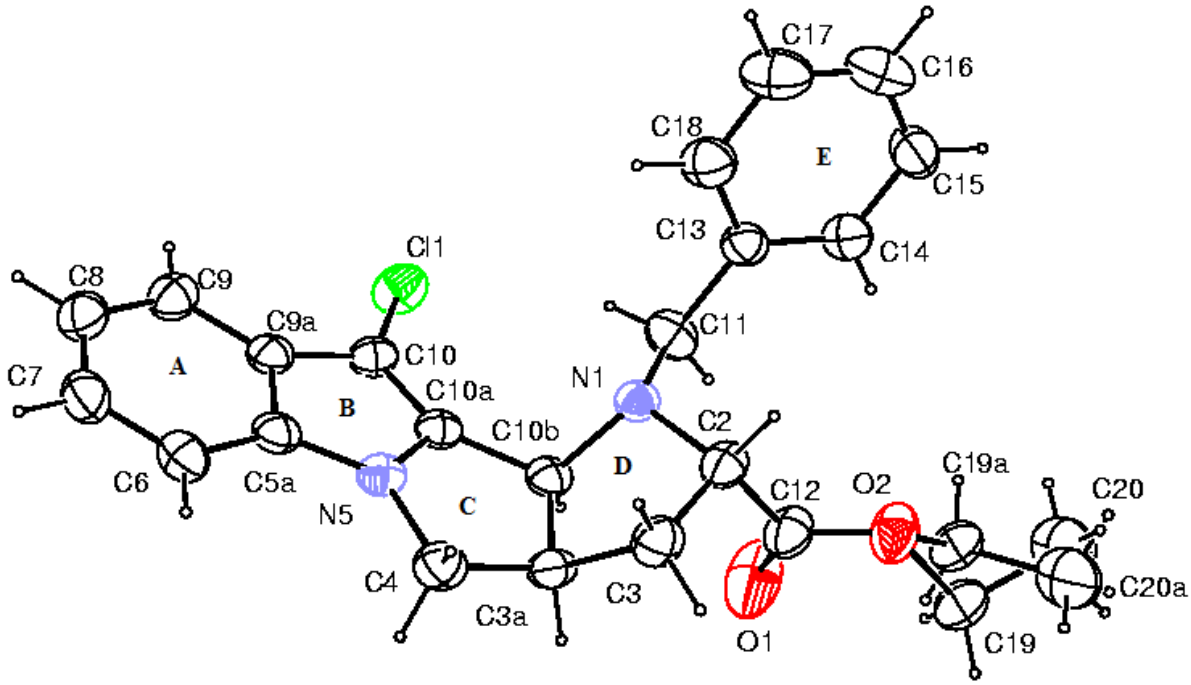
Calculation: FRAP value can be calculated by following equation:

$$\text{Ascorbic acid concentration (mM/100 g of sample)} = \frac{\triangle A_{593nm} \text{ of test sample}}{\triangle A_{593nm} \text{ of standard}} \text{ X } \frac{\text{Standard (mM)}}{\text{Sample (mg)}} \text{ X } 10^5$$

Antiproliferative activity

Antiproliferative assays for antitumor activity were performed in 96-well plates using the National Cancer Institute (NCI) protocol.46 As a model to study the anticancer activity of the synthetic compounds, we used six human solid tumor cell lines A549 (non-small cell lung), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon). The in vitro antiproliferative activity was evaluated after 48 h of drug exposure using the sulforhodamine B (SRB) assay.45 According to NCI protocol; only compounds soluble in DMSO at 40 mM were tested. The results expressed as GI50 (50% growth inhibition)

1.6.1 Single crystal X-Ray data of 9b



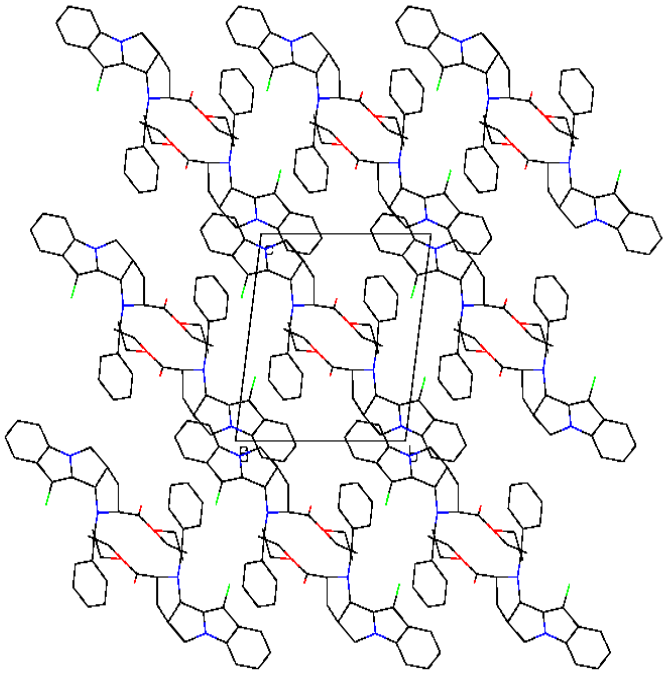
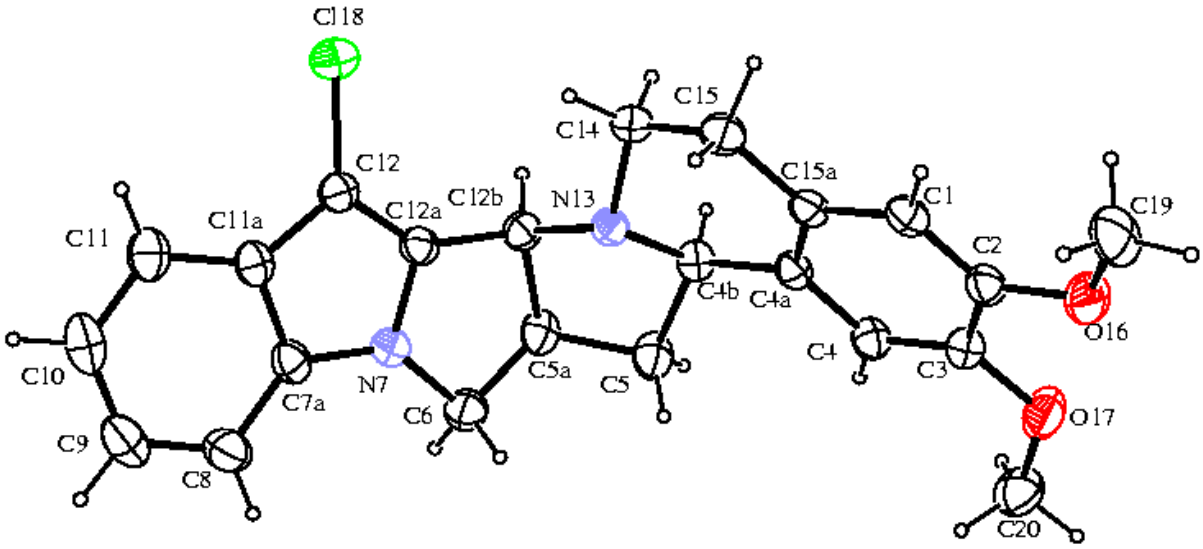


Table 1 Crystal and experimental data of 9b

CCDC Number	960921
Crystal description	White block
Crystal size	0.30 x 0.20 x 0.20 mm
Empirical formula	$C_{23}H_{23}ClN_2O_2$
Formula weight	394.9
Radiation, Wavelength	Mo *K*α, 0.71073 Å
Unit cell dimensions	a=9.2721(7), b= 10.3272(6), c=10.7817 Å,
	α=81.335(6), β= 81.614(5), γ = 89.456(5) º
Crystal system, Space group	Triclinic, P-1
Unit cell volume	1009.62(12) $Å^3$
No. of molecules per unit cell, Z	2
Absorption coefficient	0.210 mm^{-1}
F(000)	415.9
θ range for entire data collection	$3.5 < \theta < 26.00$
Reflections collected / unique	7318 / 3954
Reflections observed ($I > 2\sigma(I)$)	1914
No. of parameters refined	262
Final R-factor	0.0569
$wR(F^2)$	0.1606
Goodness-of-fit	0.996
$(\Delta/\sigma)_{max}$	0.001
Final residual electron density	$-0.256 < \Delta\rho < 0.257$ $eÅ^{-3}$

1.6.2 Single crystal X-Ray data of 11'b



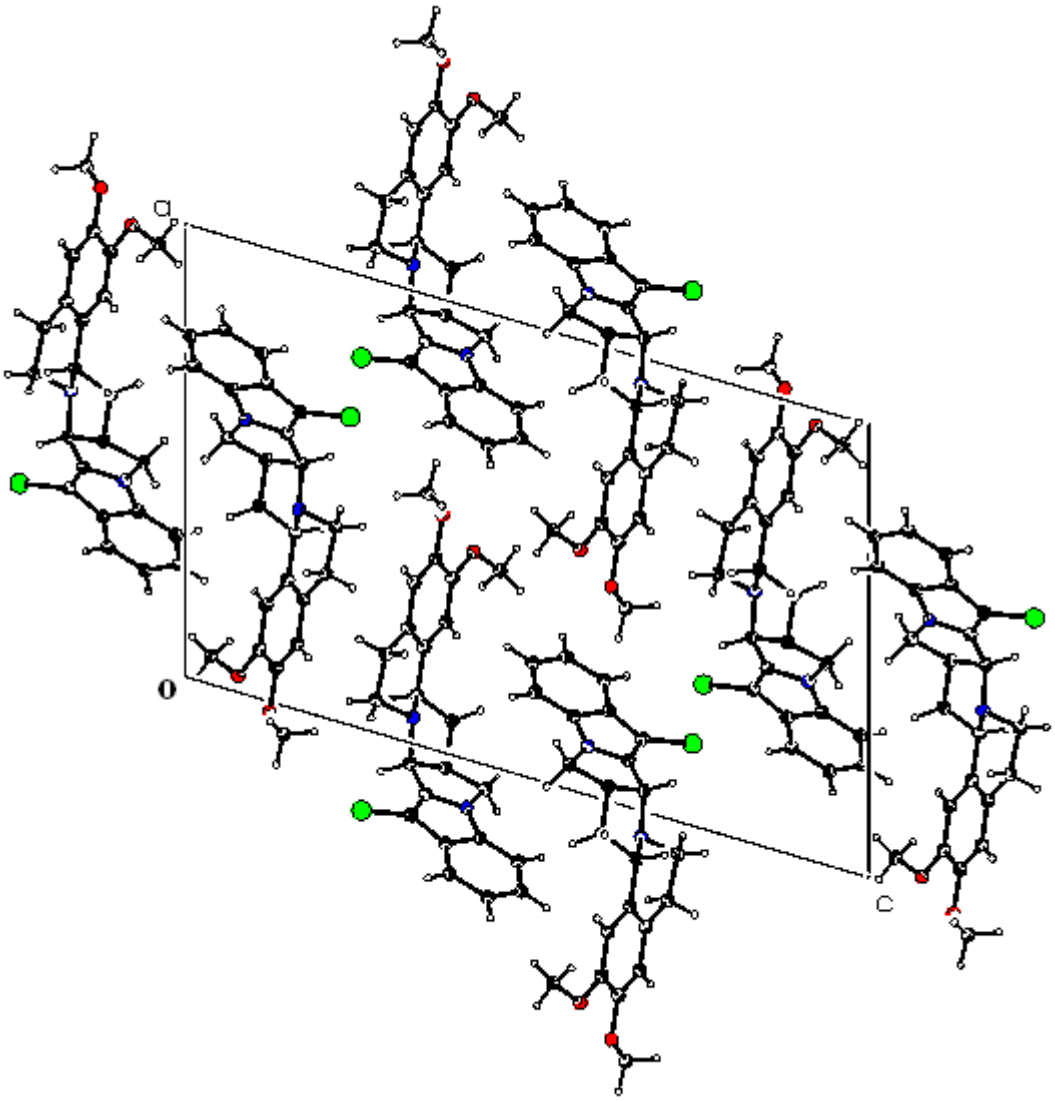
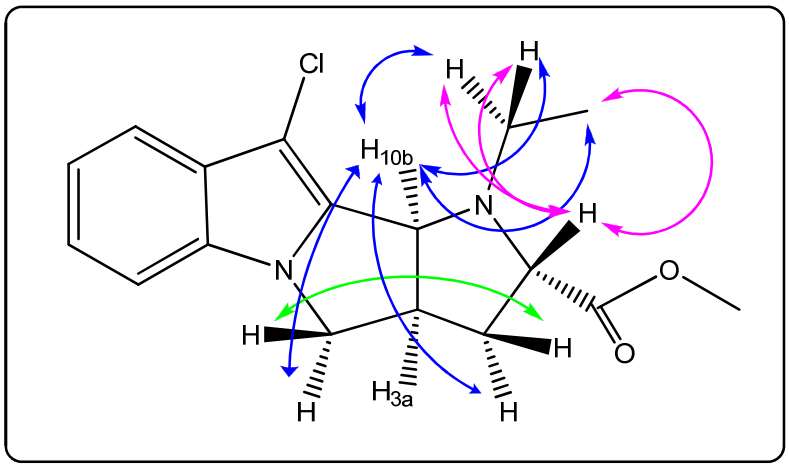


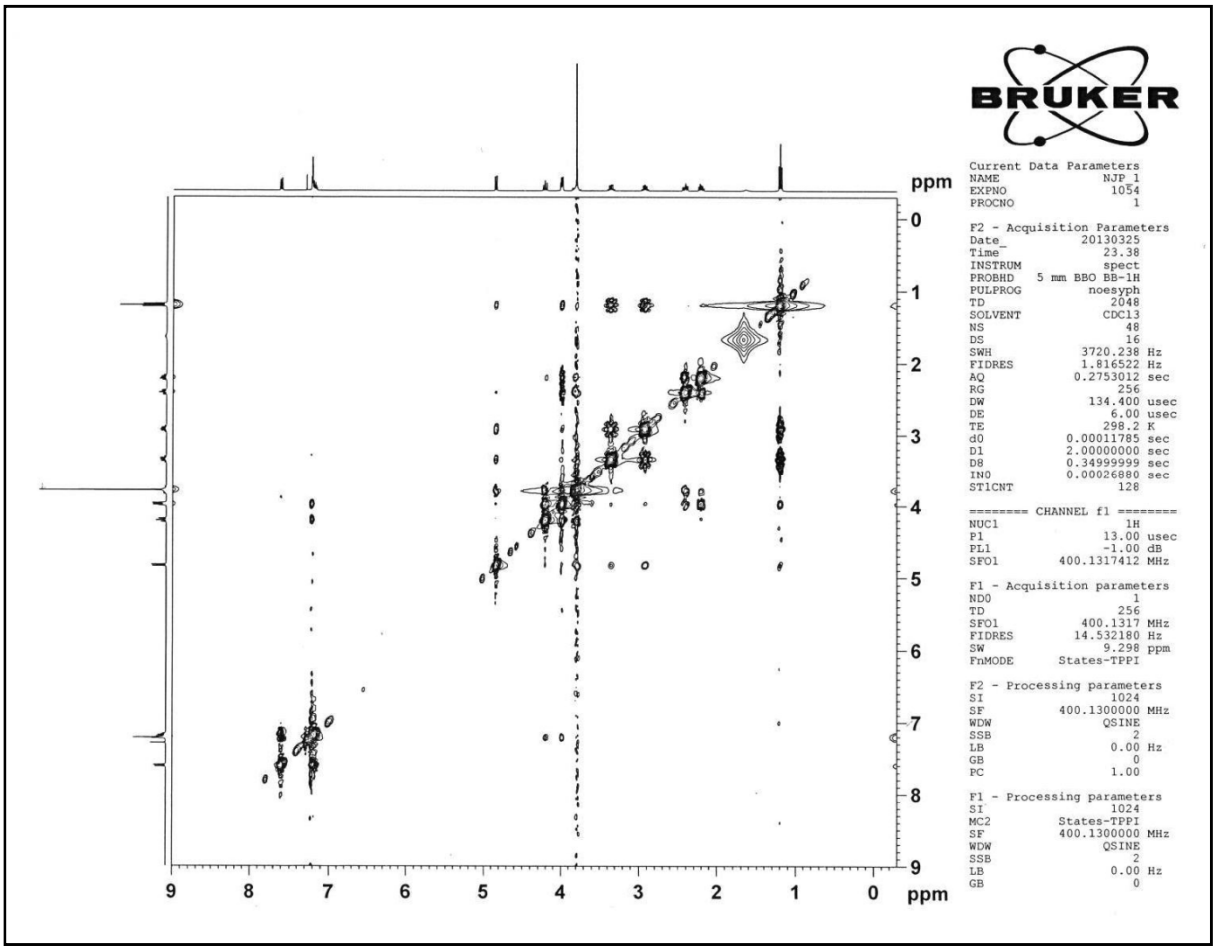
Table 1 Crystal and experimental data of 11'b

Crystal description	Brown block
Crystal size	0.3 X 0.2 X 0.2 mm
Empirical formula	$C_{23}H_{23}ClN_2O_2$
Formula weight	394.88
Radiation, Wavelength	Mo *K*α, 0.71073 Å
Unit cell dimensions	a= 13.9521(10), b= 6.6102(4),
	c= 21.9770(13) Å, β = 106.352(7)^o
Crystal system	monoclinic
Space group	P 2_1/n
Unit cell volume	1944.9(2)
No. of molecules per unit cell, Z	4
Temperature	293(2) K
Absorption coefficient	0.218 mm^{-1}
F(000)	832
Scan mode	ω scan
θ range for entire data collection	3.63 <θ< 26.00^o
Range of indices	h = -12 to 17, k = -8 to 7, l = -26 to 27
Reflections collected / unique	7404 / 3804
Reflections observed (I > 2□(I))	2803
R_{int}	0.0246
R_{sigma}	0.0432
Structure determination	Direct methods
Refinement	Full-matrix least-squares on F^2
No. of parameters refined	255
Final R	0.0441
$wR(F^2)$	0.1118
Goodness-of-fit	1.019
Final residual electron density	-0.278 <□□□ < 0.187 $Å^{-3}$

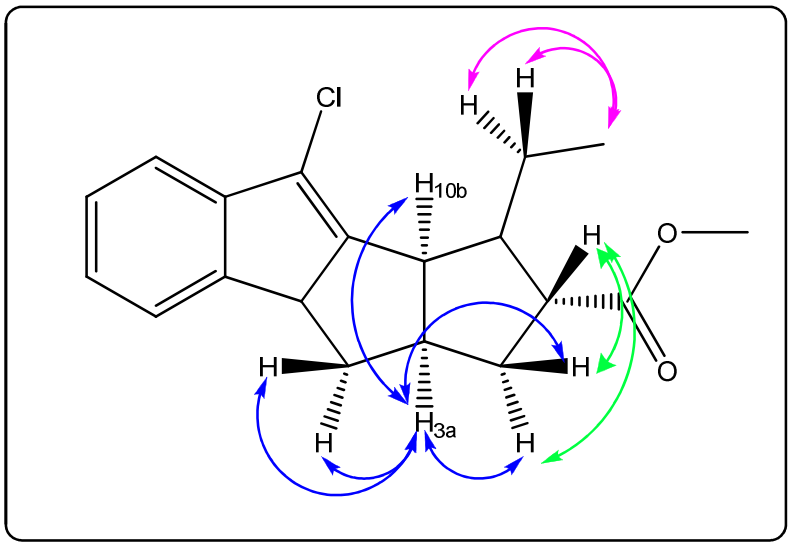
1.7 2D NMR experiments: NOESY and COSY of 8a;

NOESY





COSY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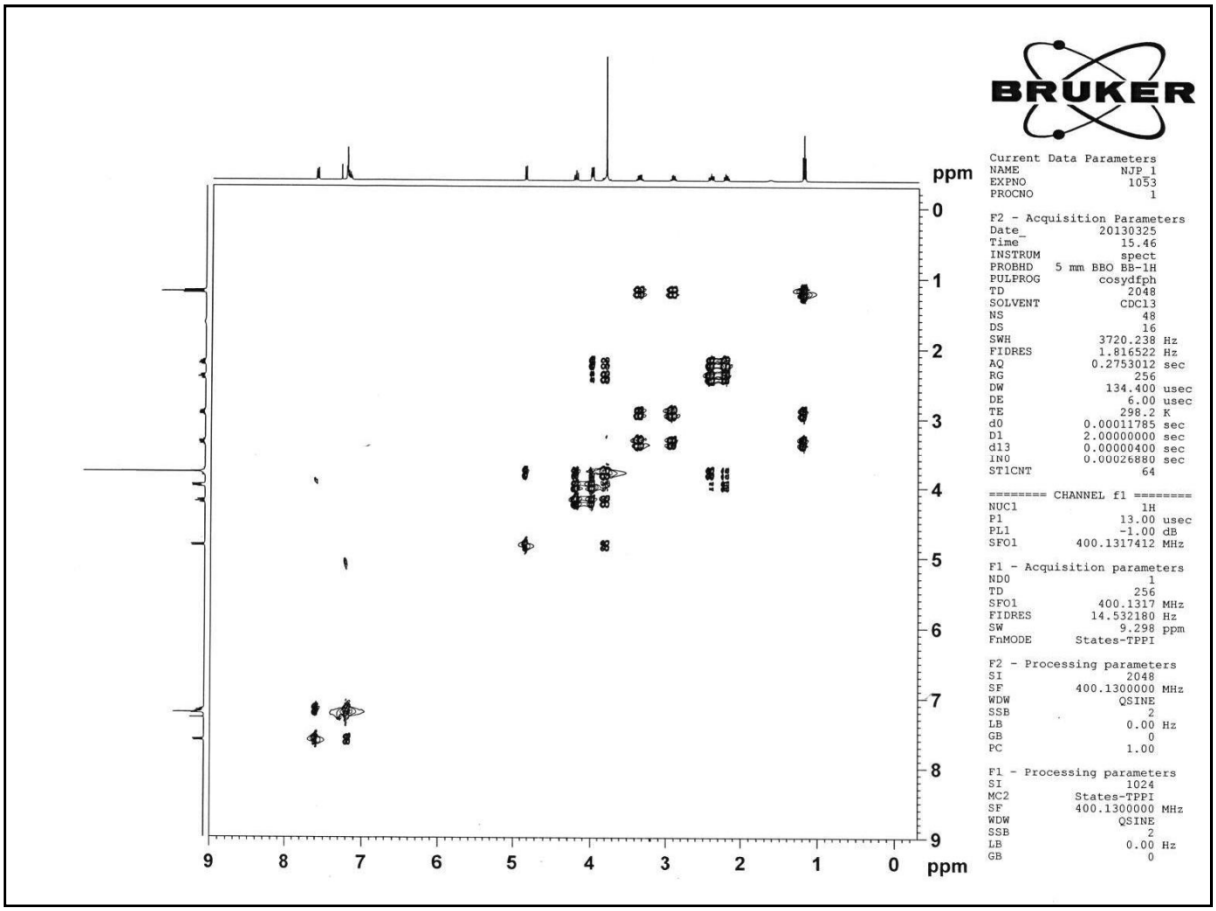
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BRUKER
Current Data Parameters
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EXPNO 1053
PROCNO 1
F2 - Acquisition Parameters
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Time 15.46
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PULPROG cosydfph
TD 2048
SOLVENT CDCl3
NS 48
DS 16
SWH 3720.238 Hz
FIDRES 1.816522 Hz
AQ 0.2753012 sec
RG 256
DW 134.400 usec
DE 6.00 usec
TE 298.2 K
d0 0.00011785 sec
D1 2.00000000 sec
d13 0.00000400 sec
IN0 0.00026880 sec
ST1CNT 64
======== CHANNEL f1 ========
NUC1 1H
P1 13.00 usec
PL1 -1.00 dB
SFO1 400.1317412 MHz
F1 - Acquisition parameters
ND0 1
TD 256
SFO1 400.1317 MHz
FIDRES 14.532180 Hz
SW 9.298 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 2048
SF 400.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.00
F1 - Processing parameters
SI 1024
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SF 400.1300000 MHz
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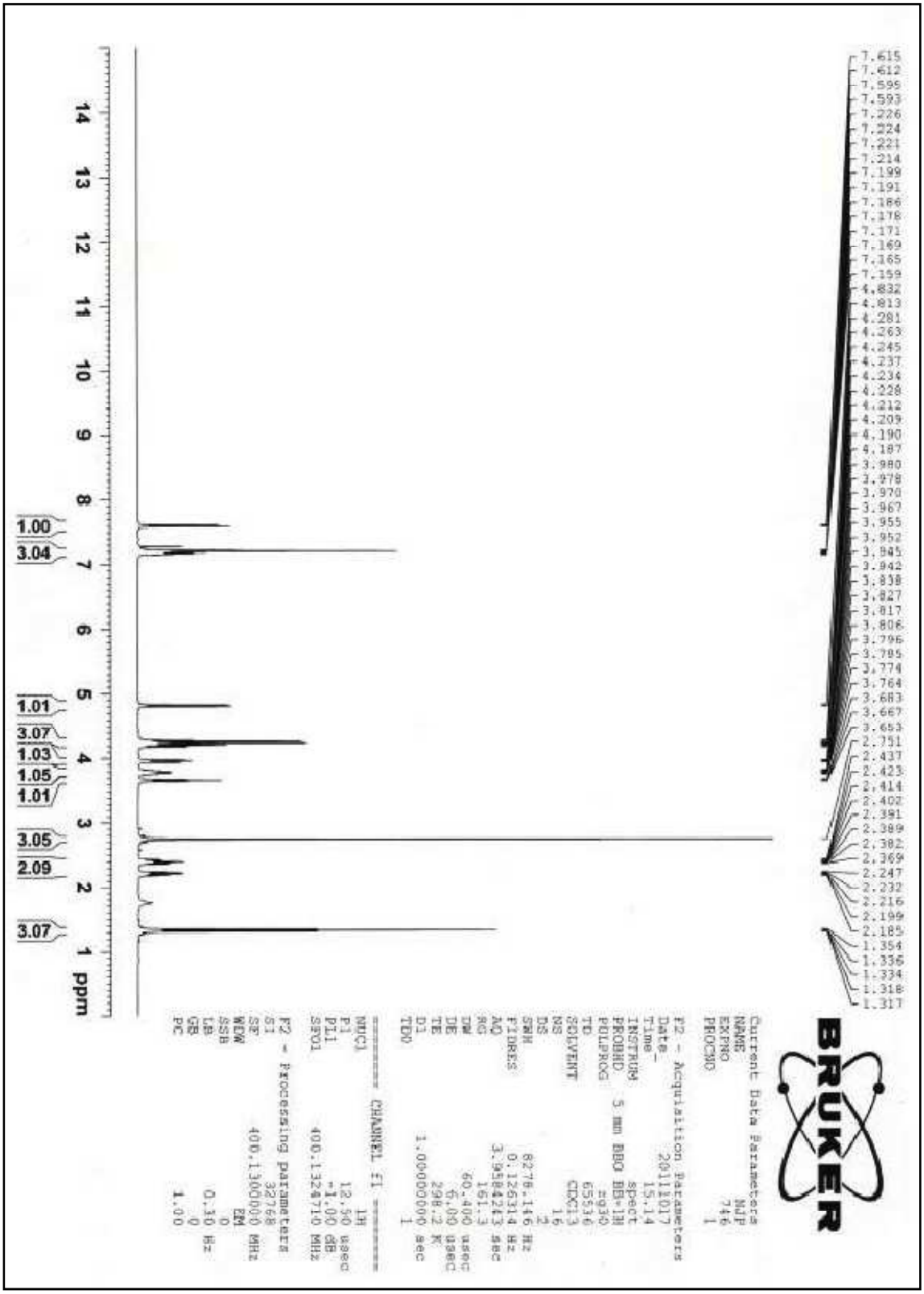
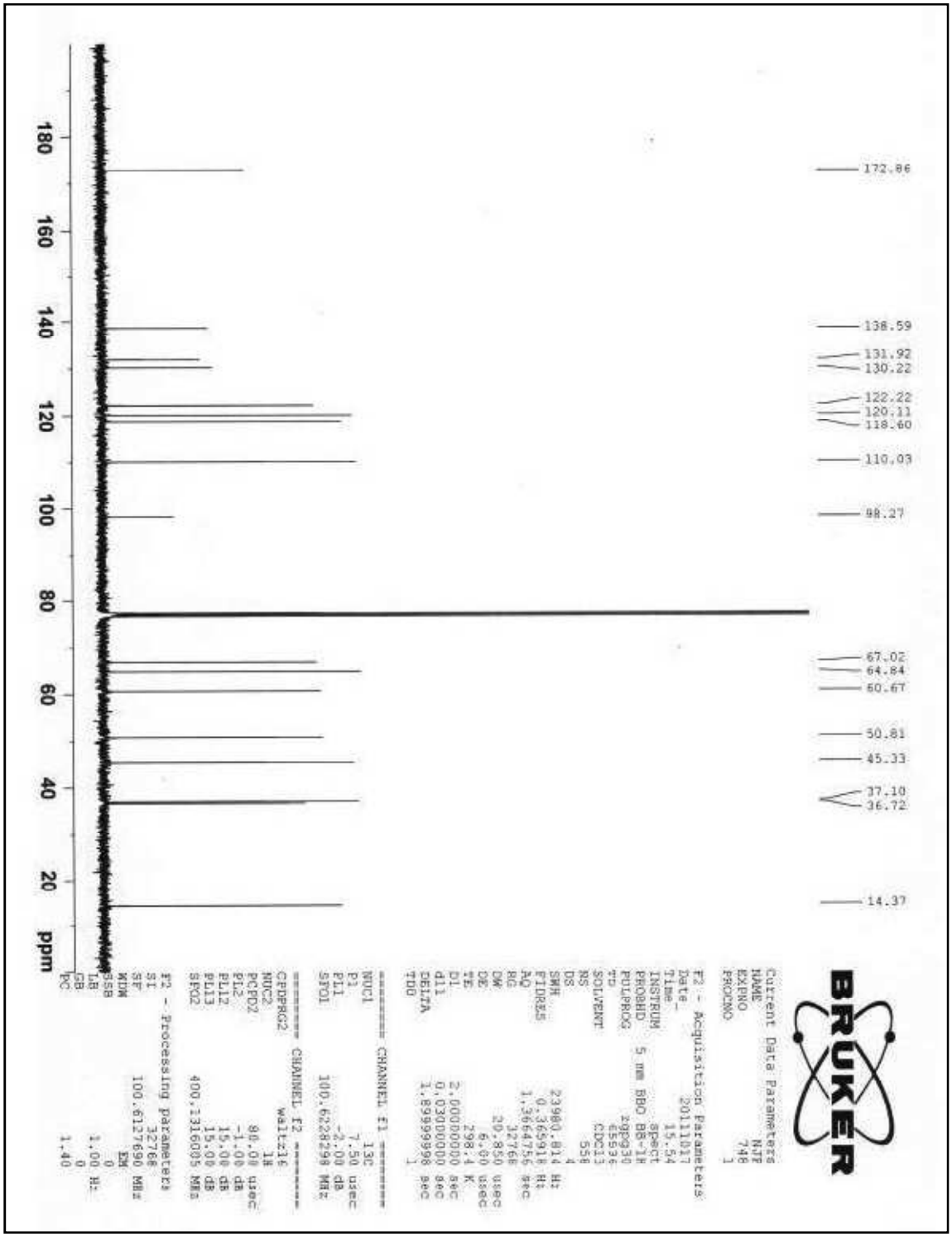
1.8 ^{1}H NMR, ^{13}C NMR and Mass Spectral Data

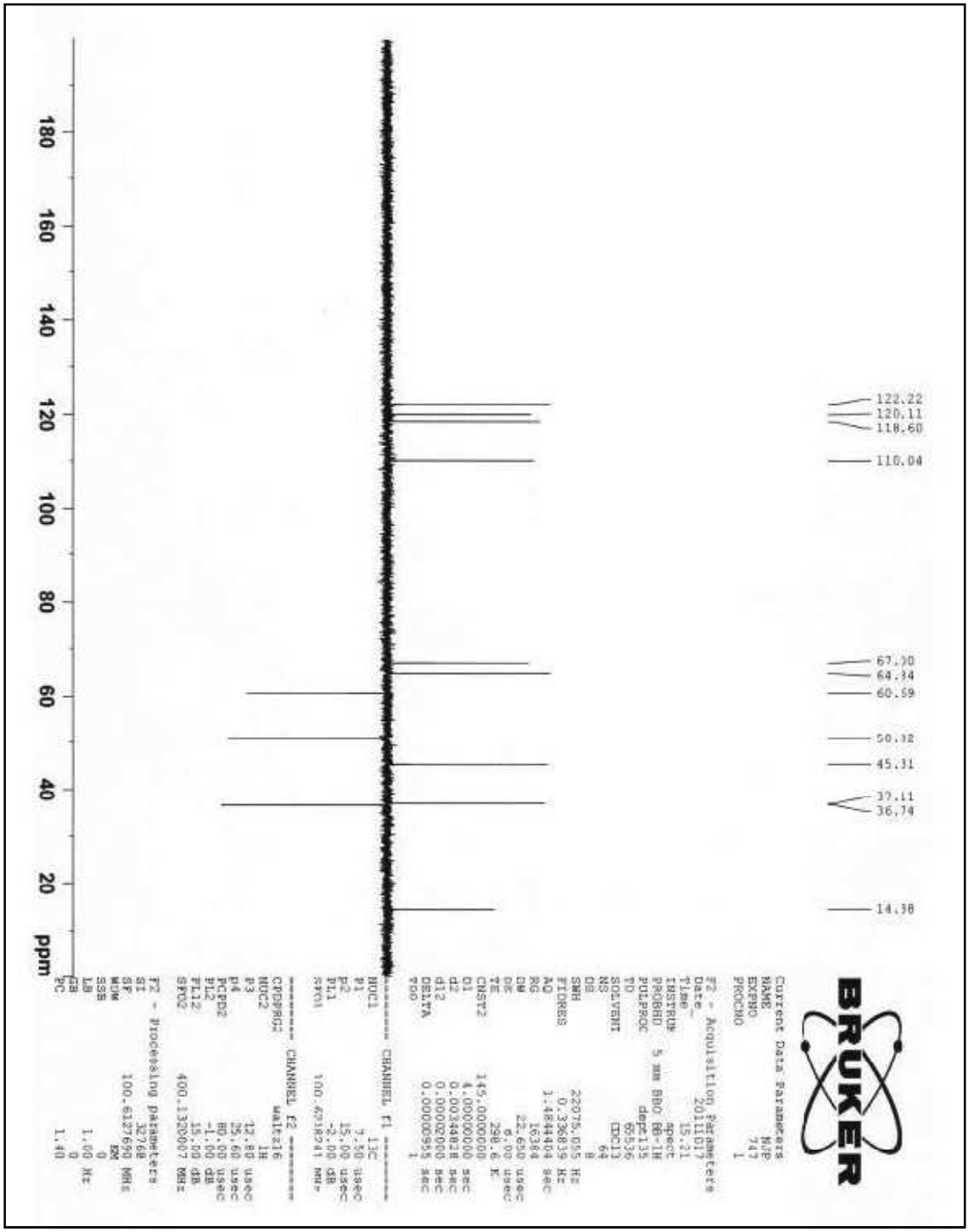
^{1}H NMR spectrum of compound (7b)



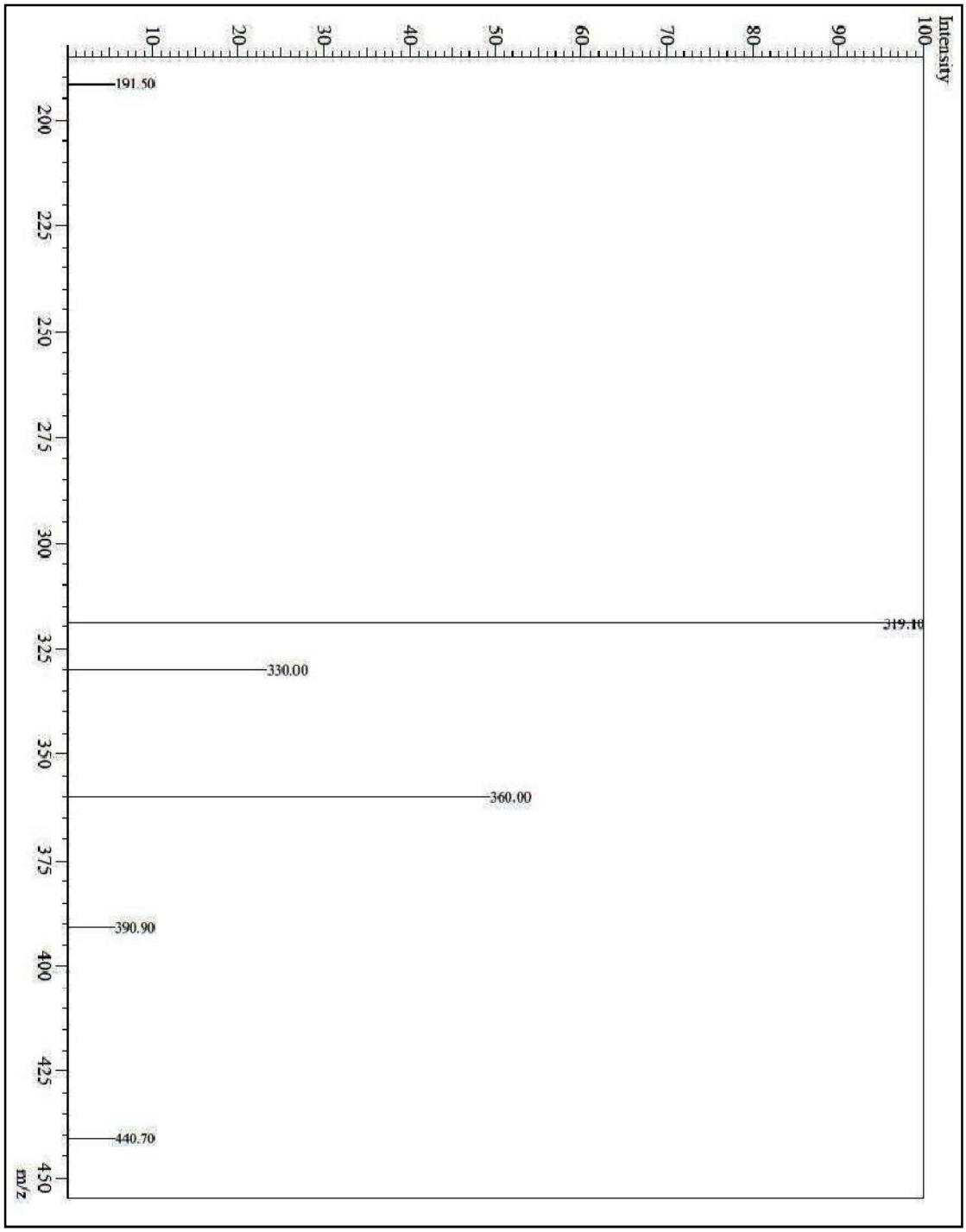
CMR spectrum of compound (7b)



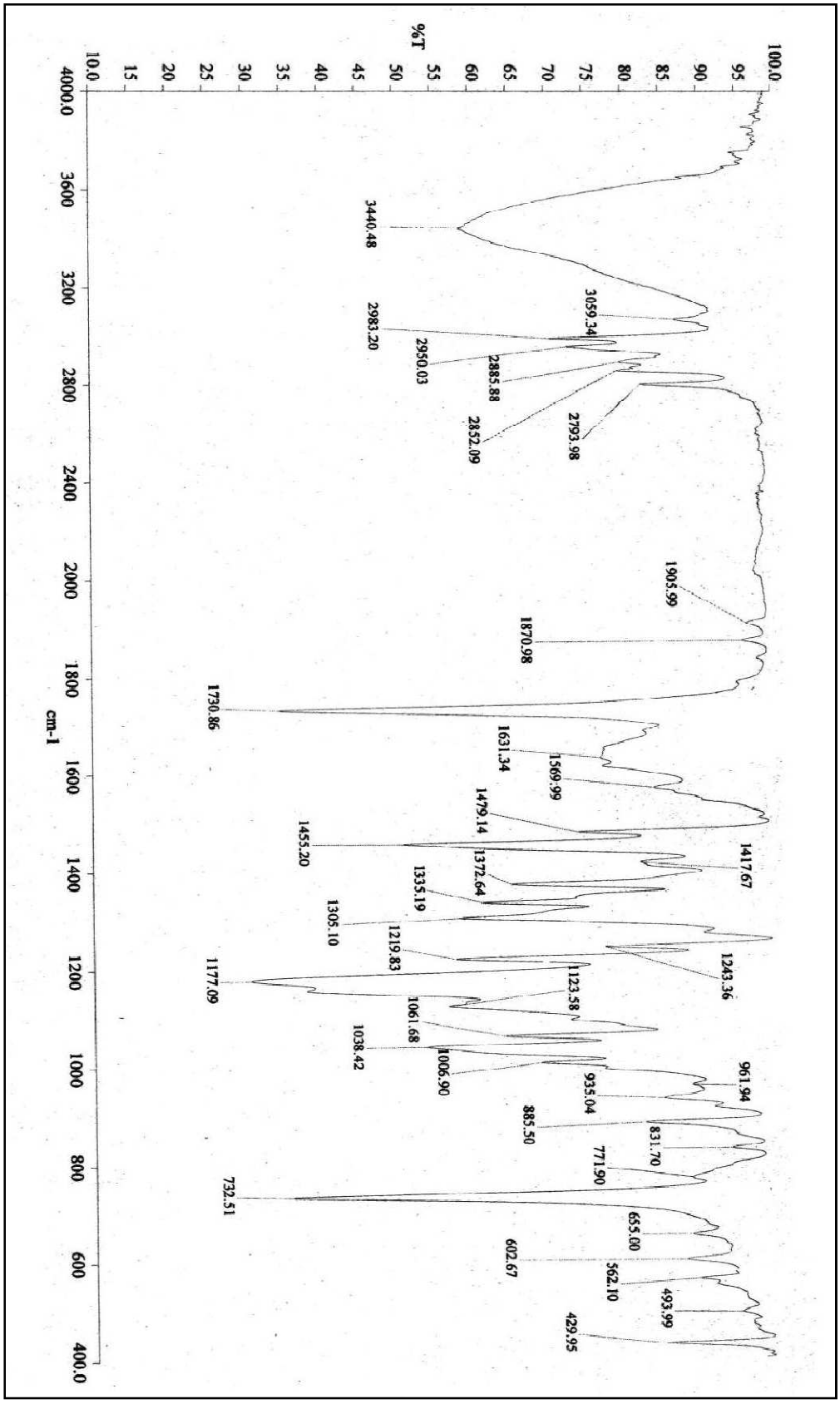
DEPT–135 spectrum of compound (7b)



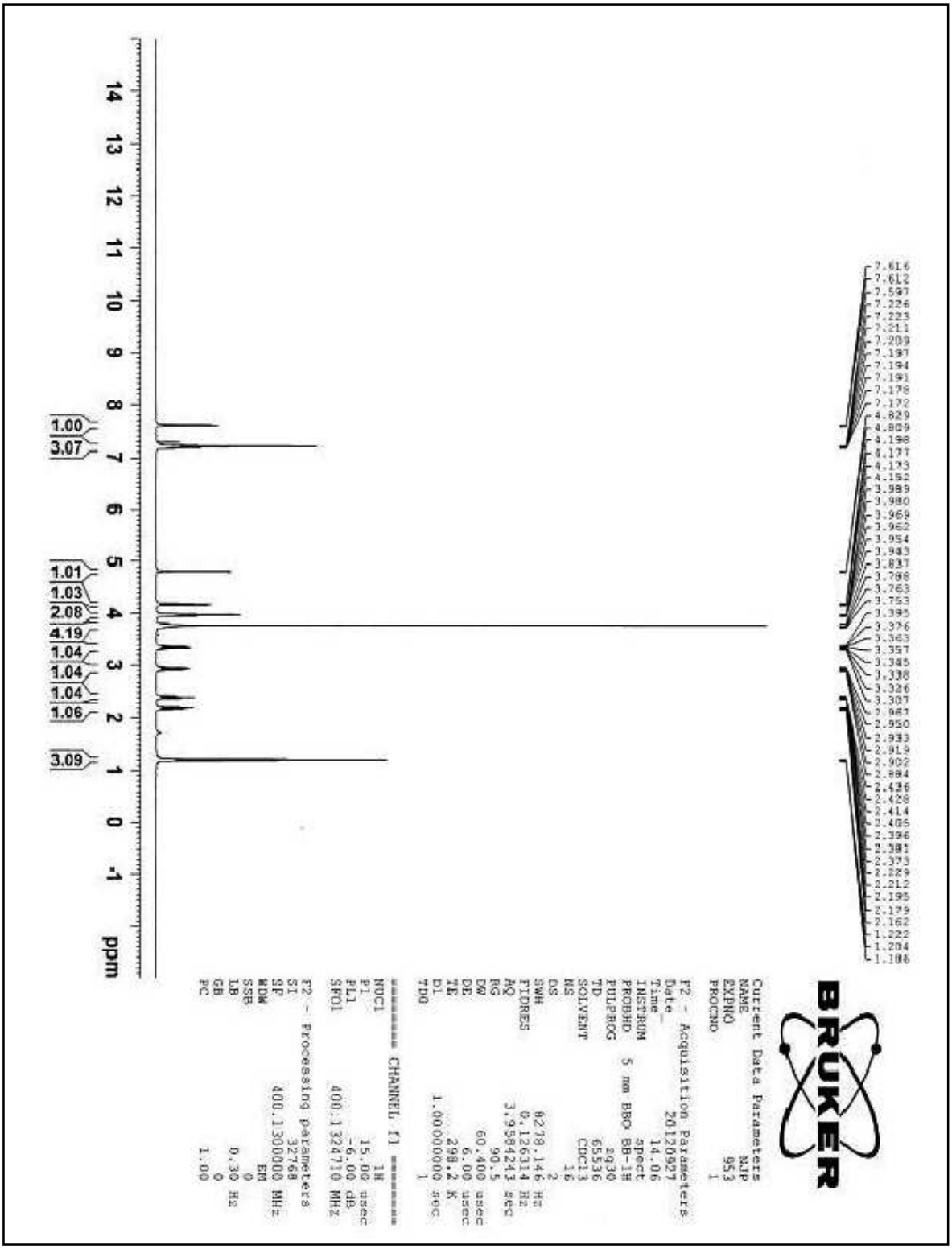
ESI–MS of compound (7b)



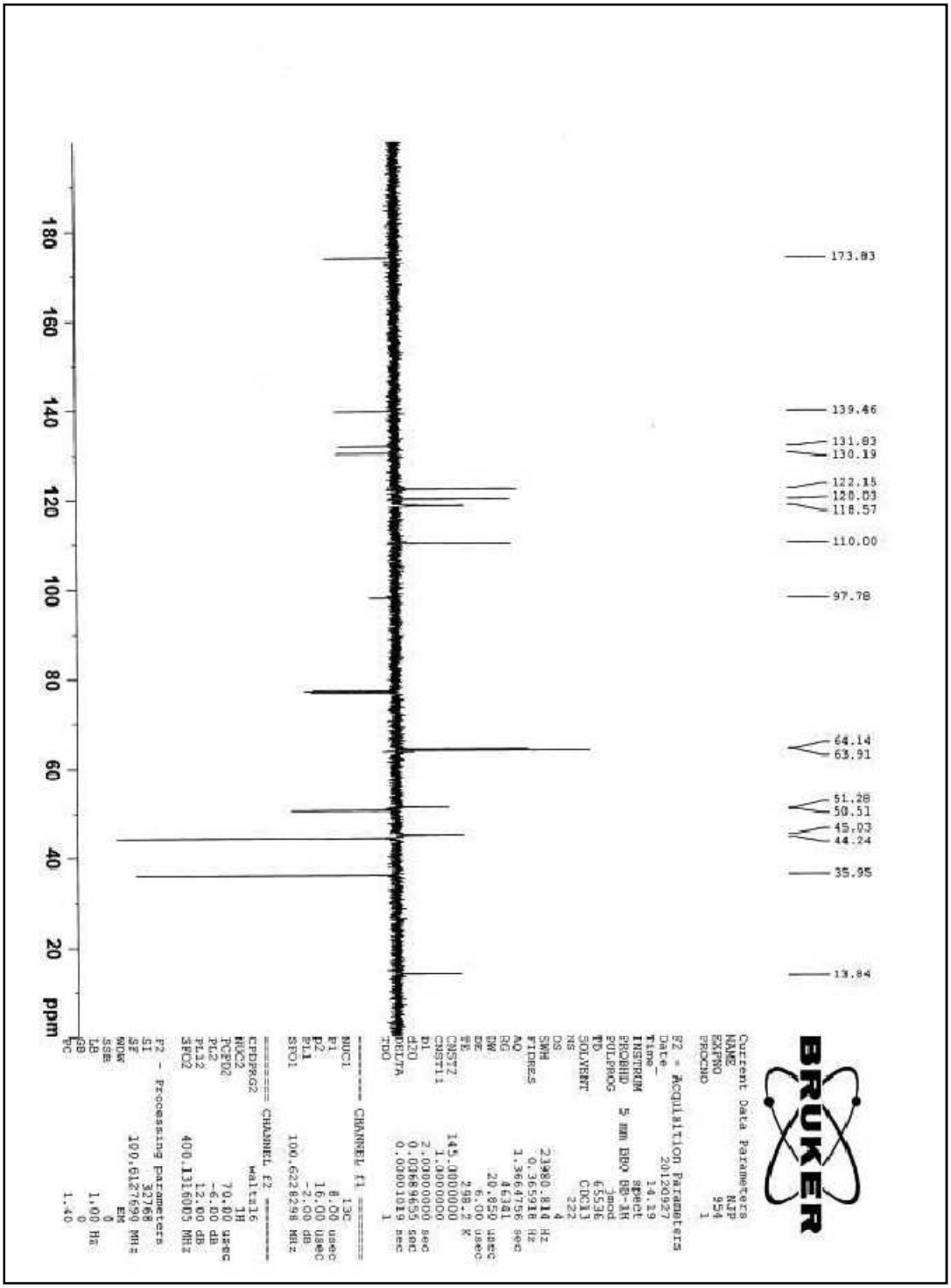
IR of compound (7b)



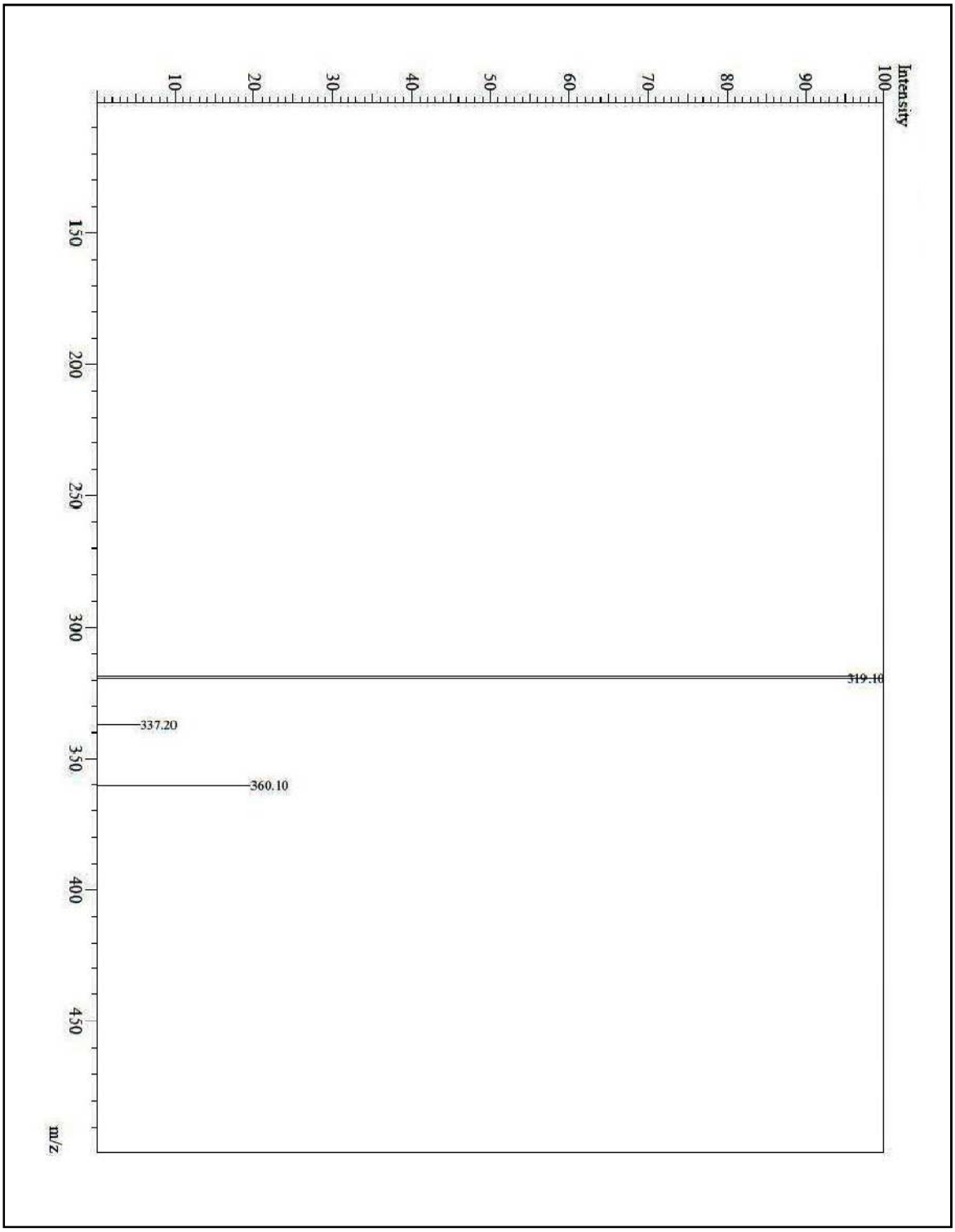
^{1}H NMR spectrum of compound (8a)



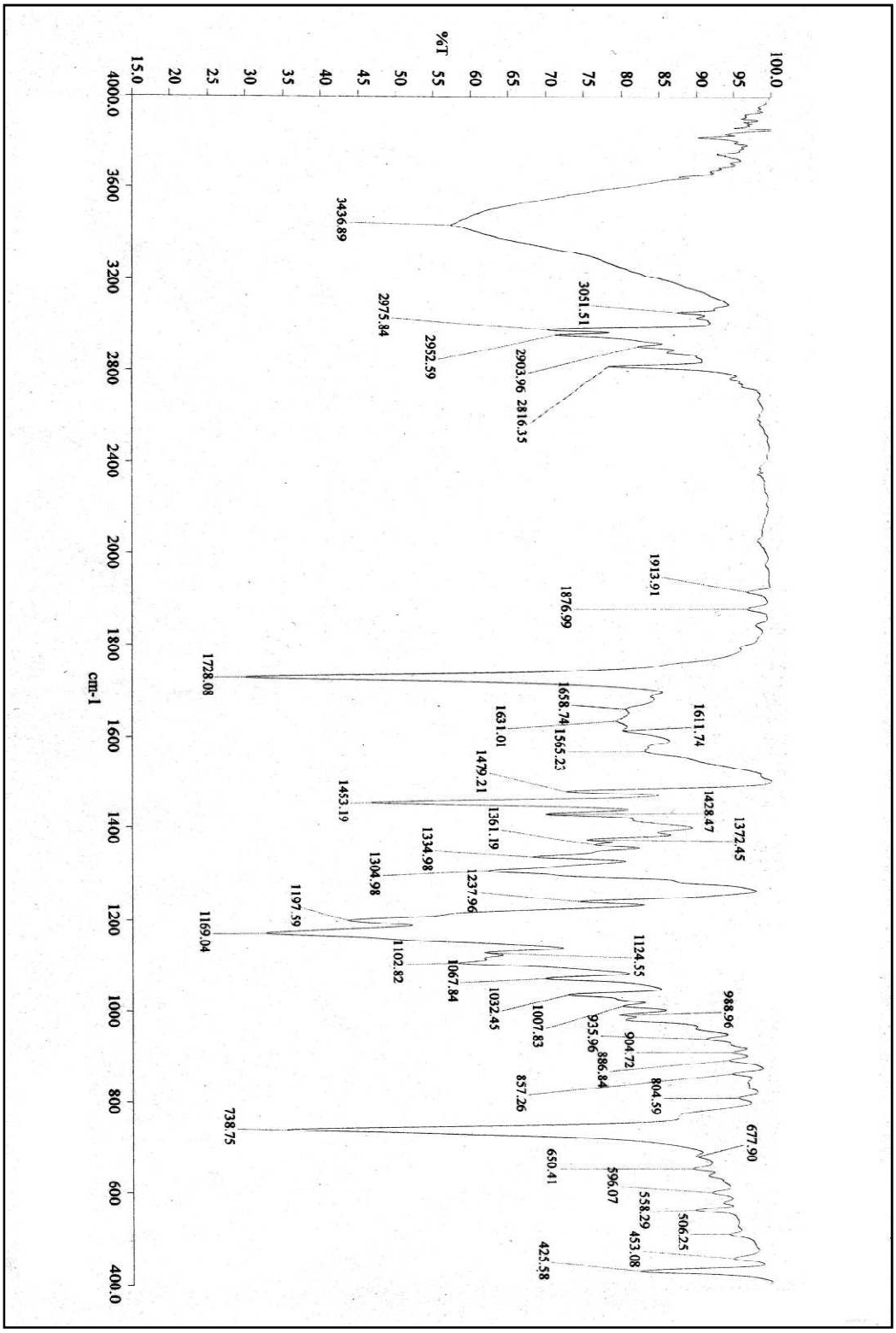
APT spectrum of compound (8a)



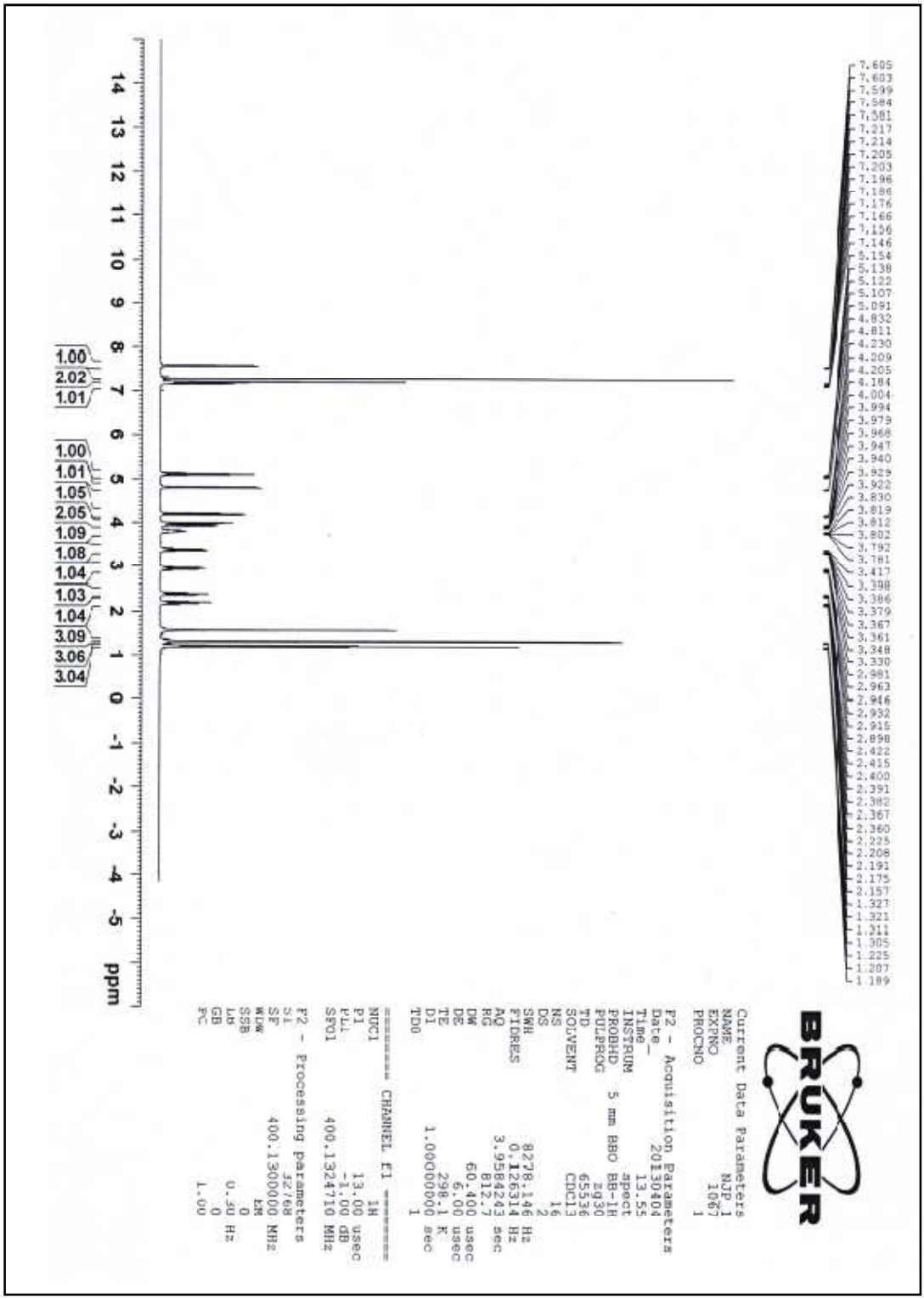
ESI–MS of compound (8a)



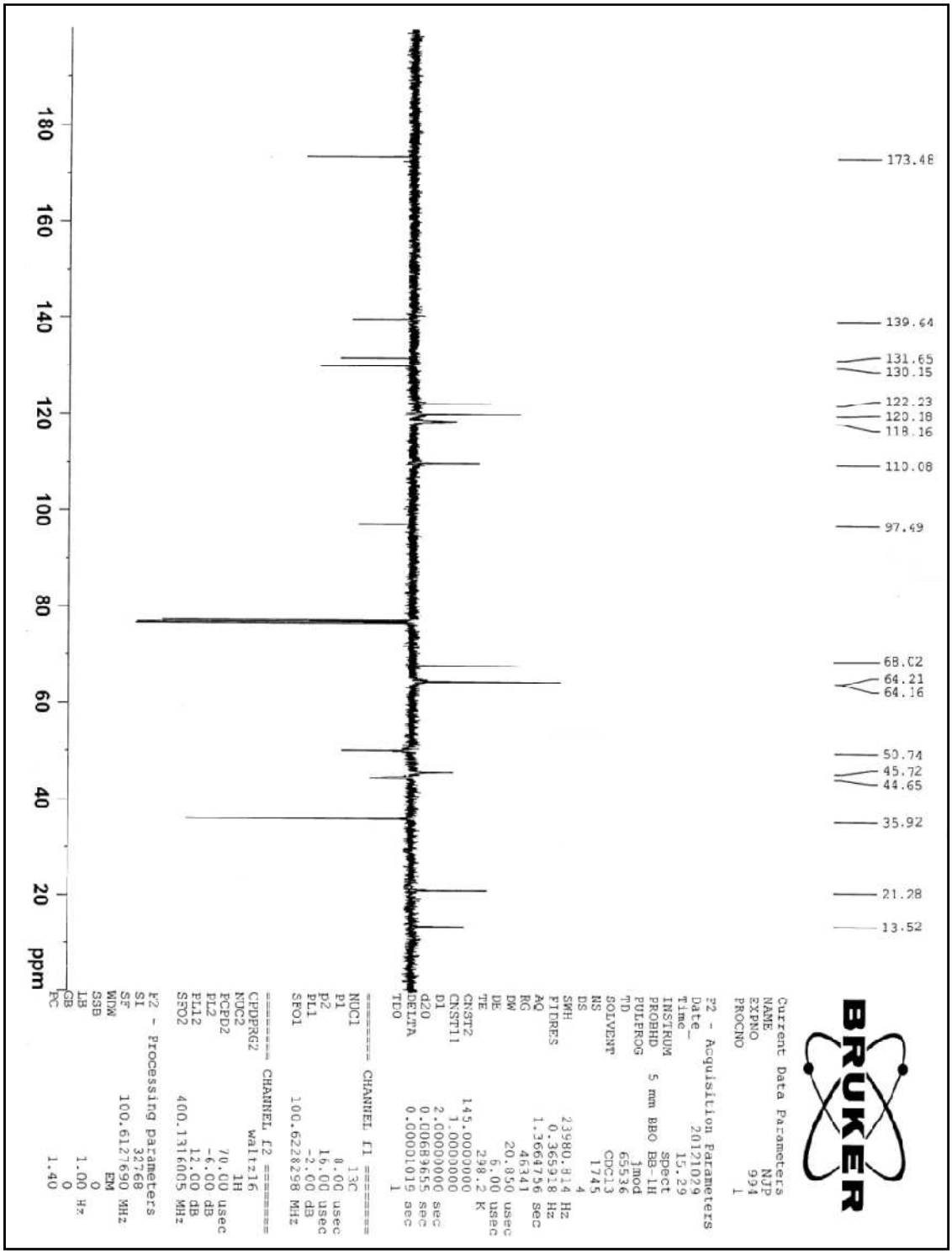
IR of compound (8a)



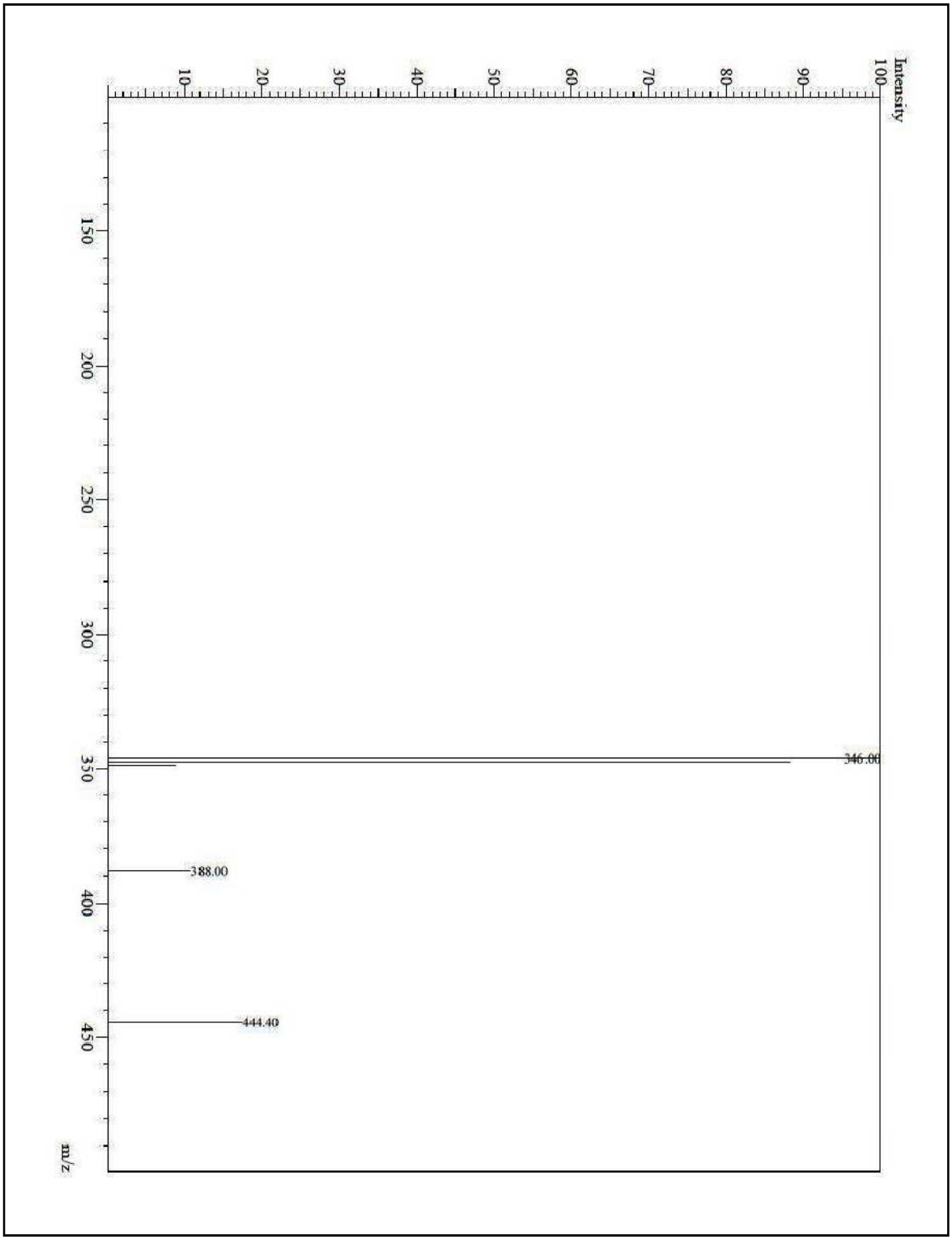
^{1}H NMR spectrum of compound (8d)



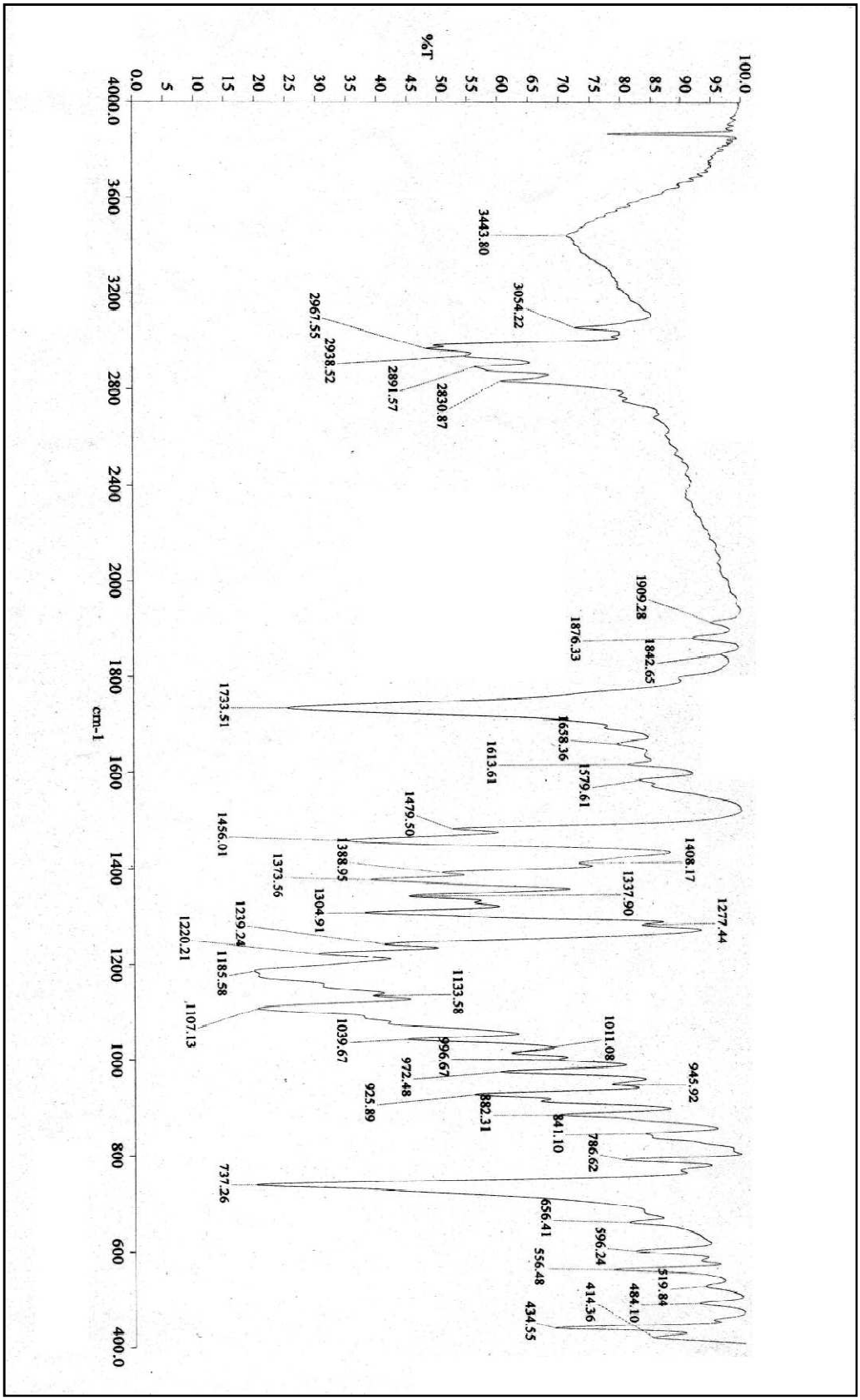
APT spectrum of compound (8d)



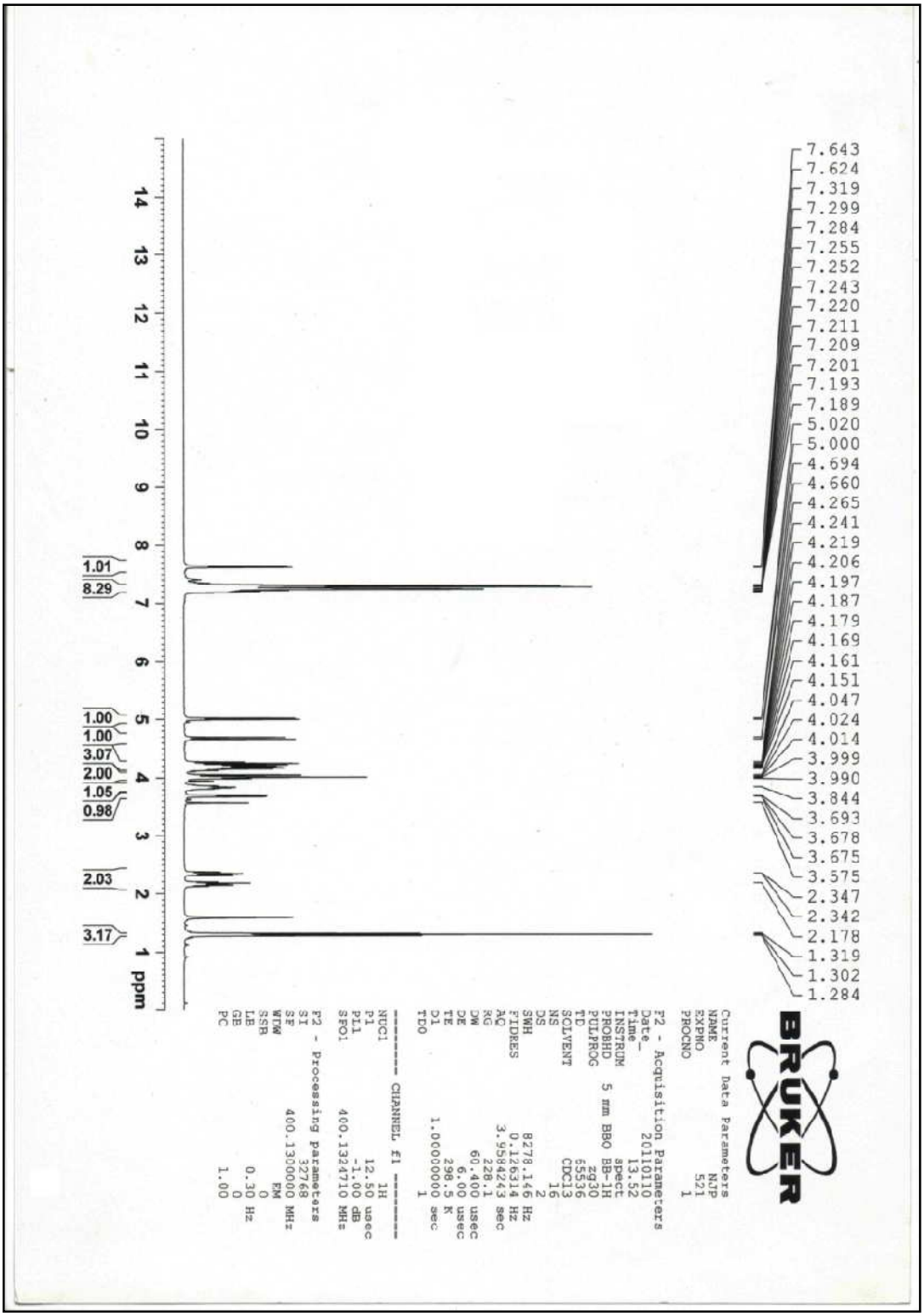
ESI–MS of compound (8d)



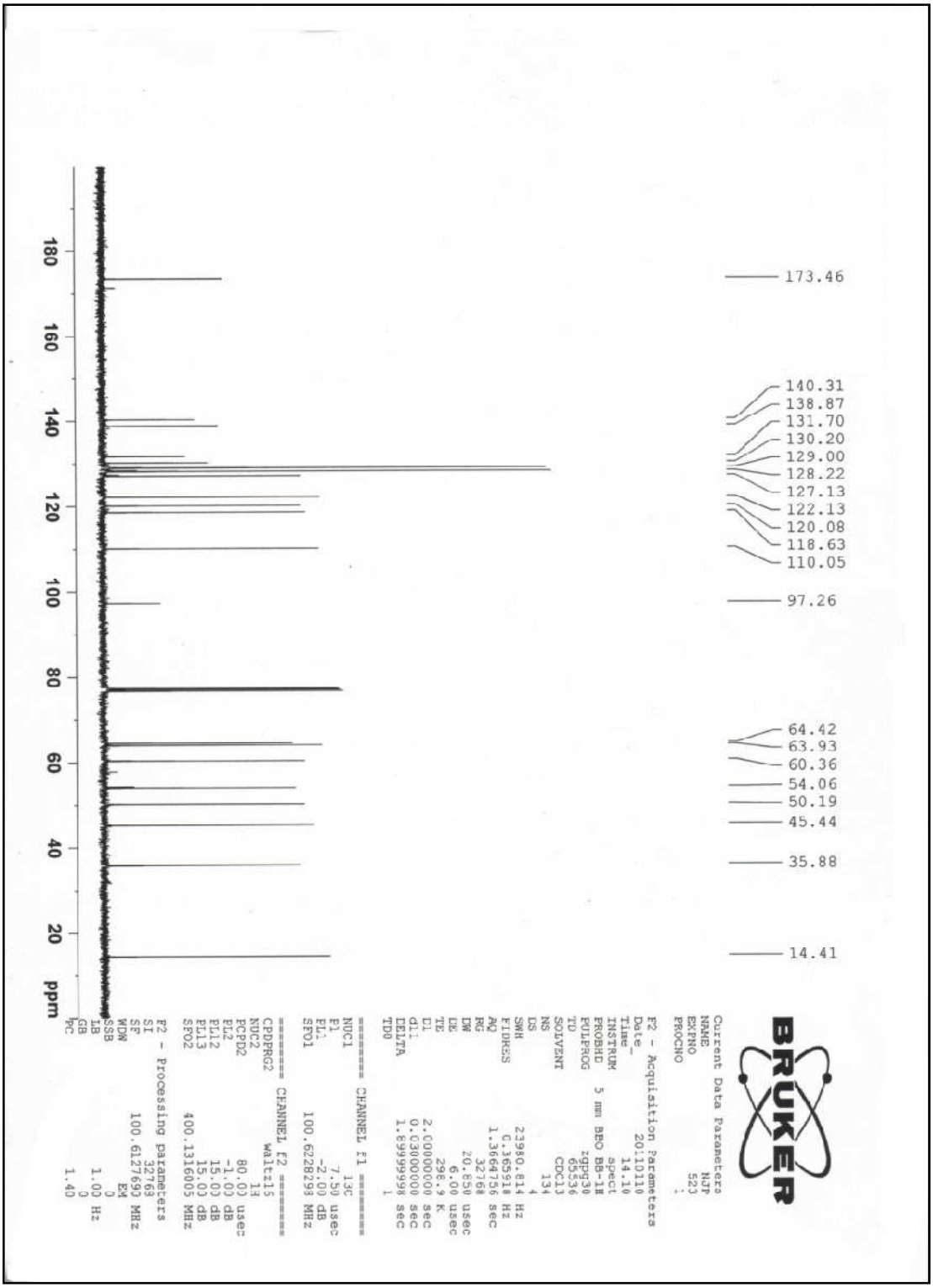
IR of compound (8d)



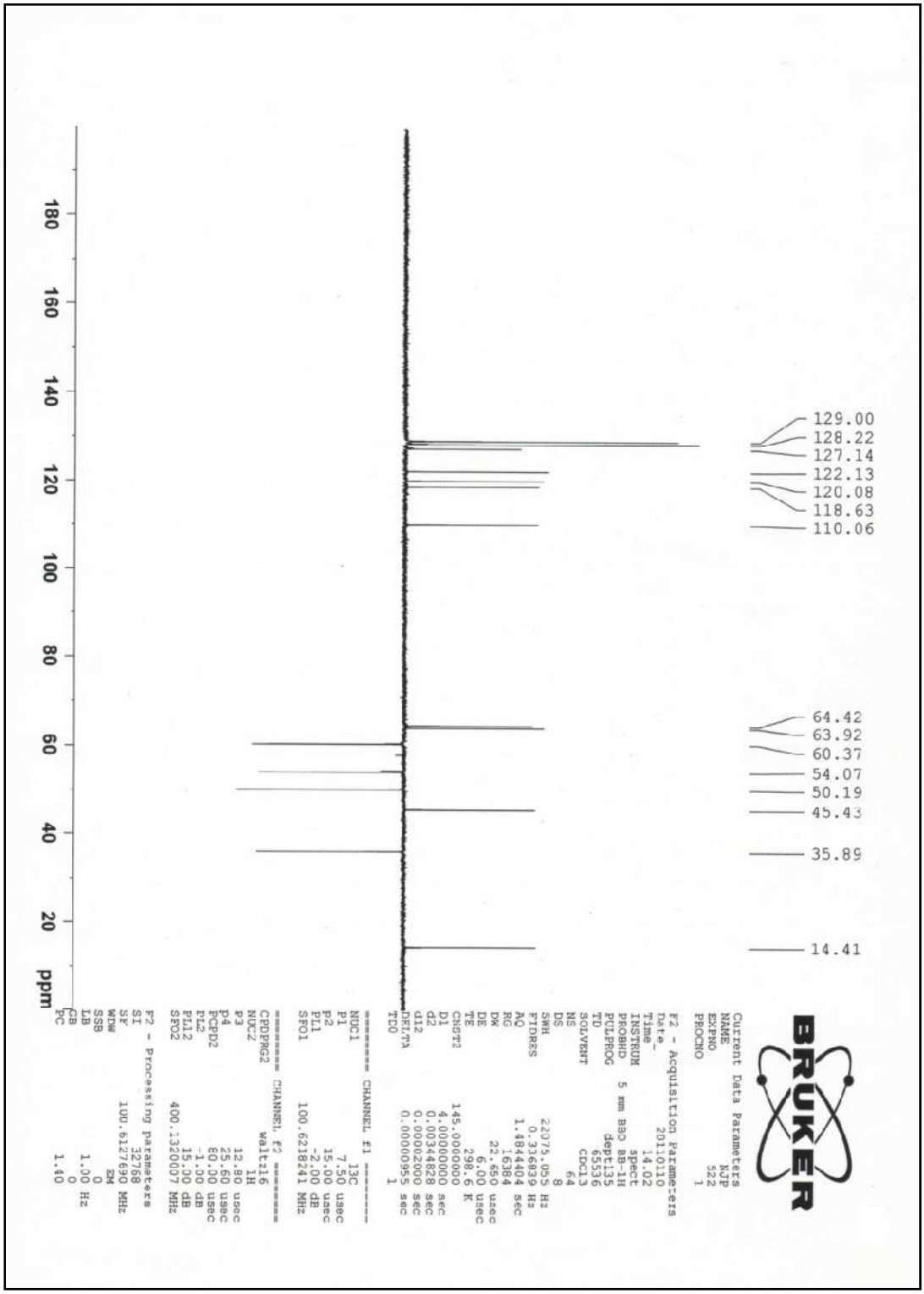
^{1}H NMR spectrum of compound (9b)



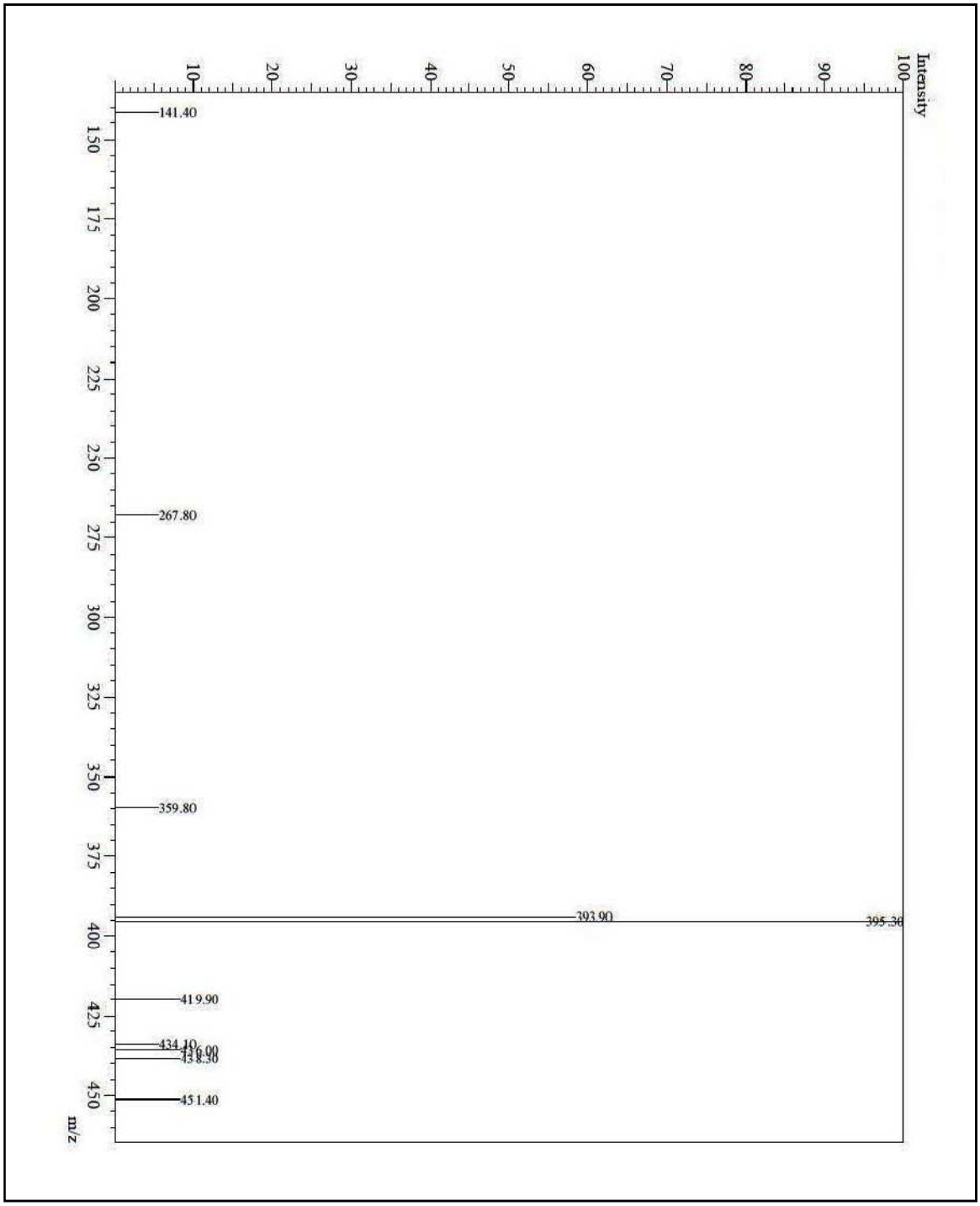
^{13}C NMR spectrum of compound (9b)



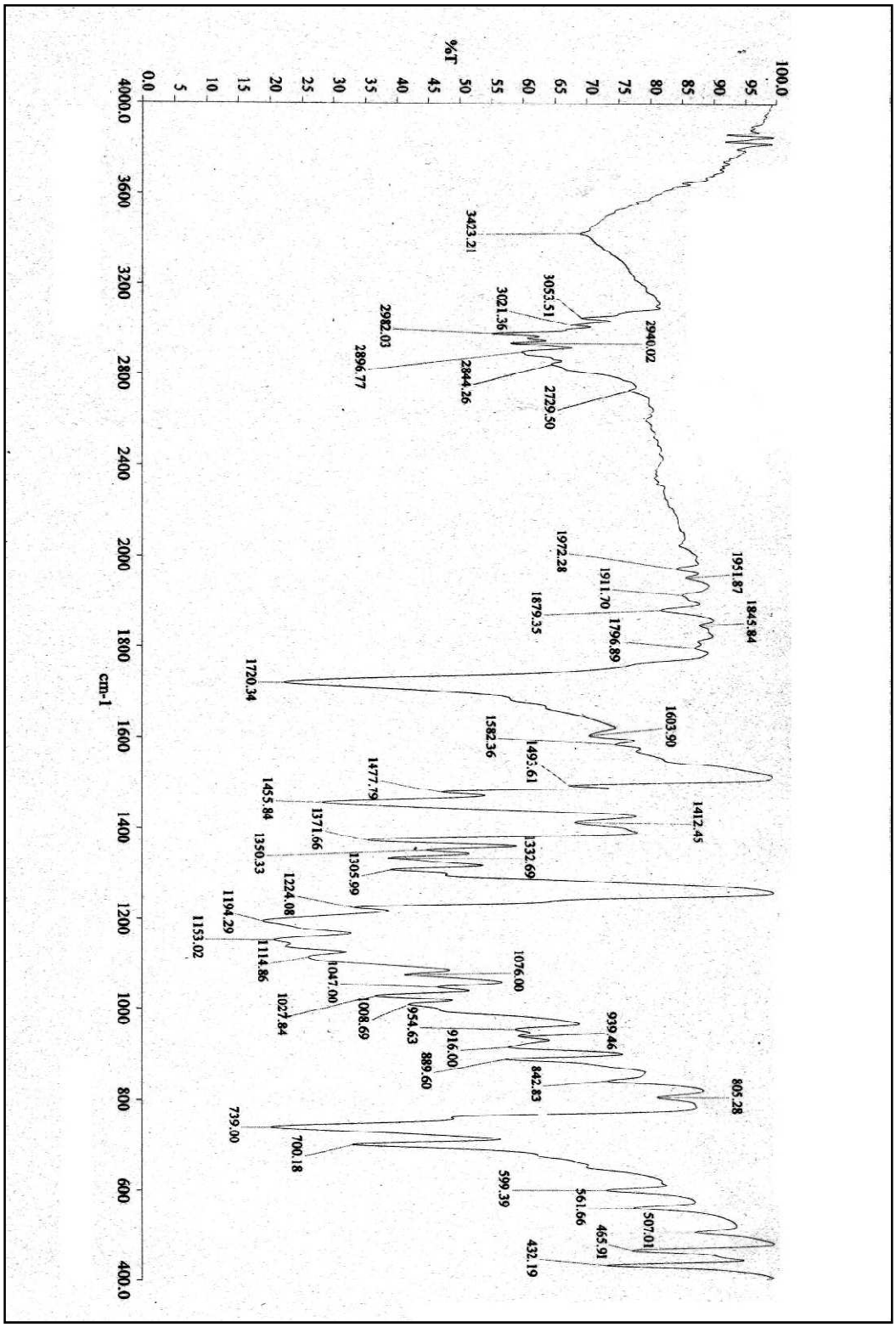
DEPT–135 spectrum of compound (9b)



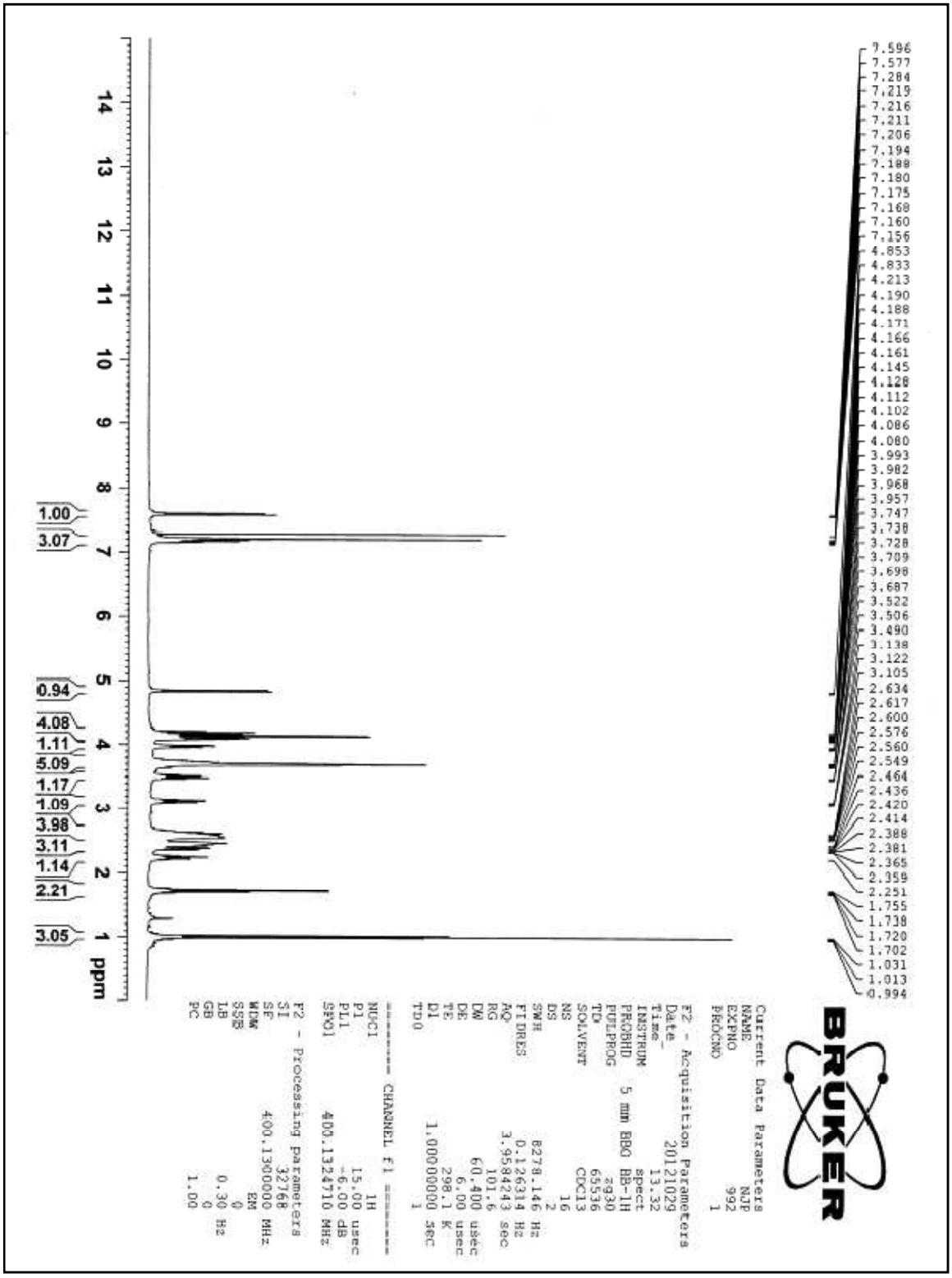
ESI–MS of compound (9b)



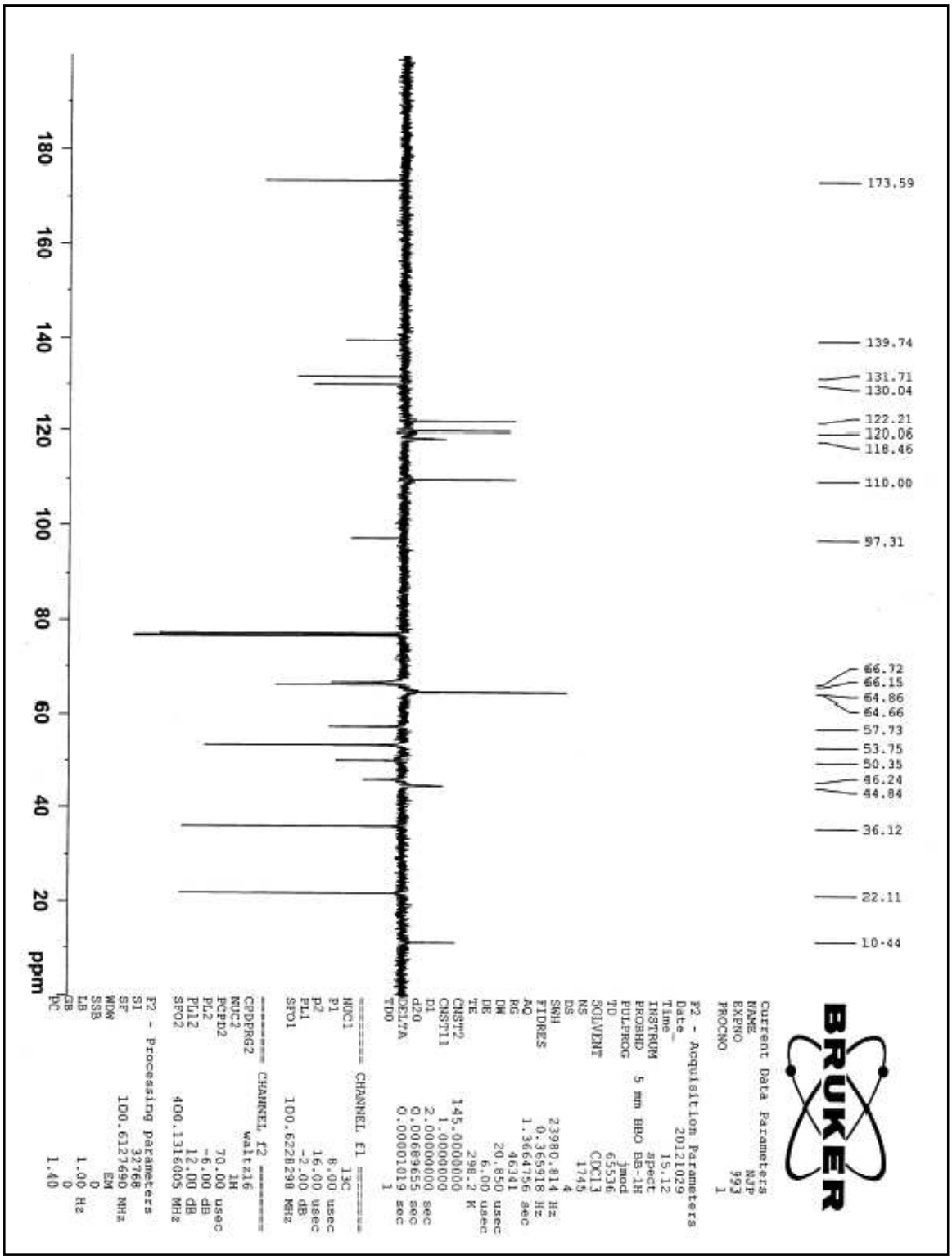
IR of compound (9b)



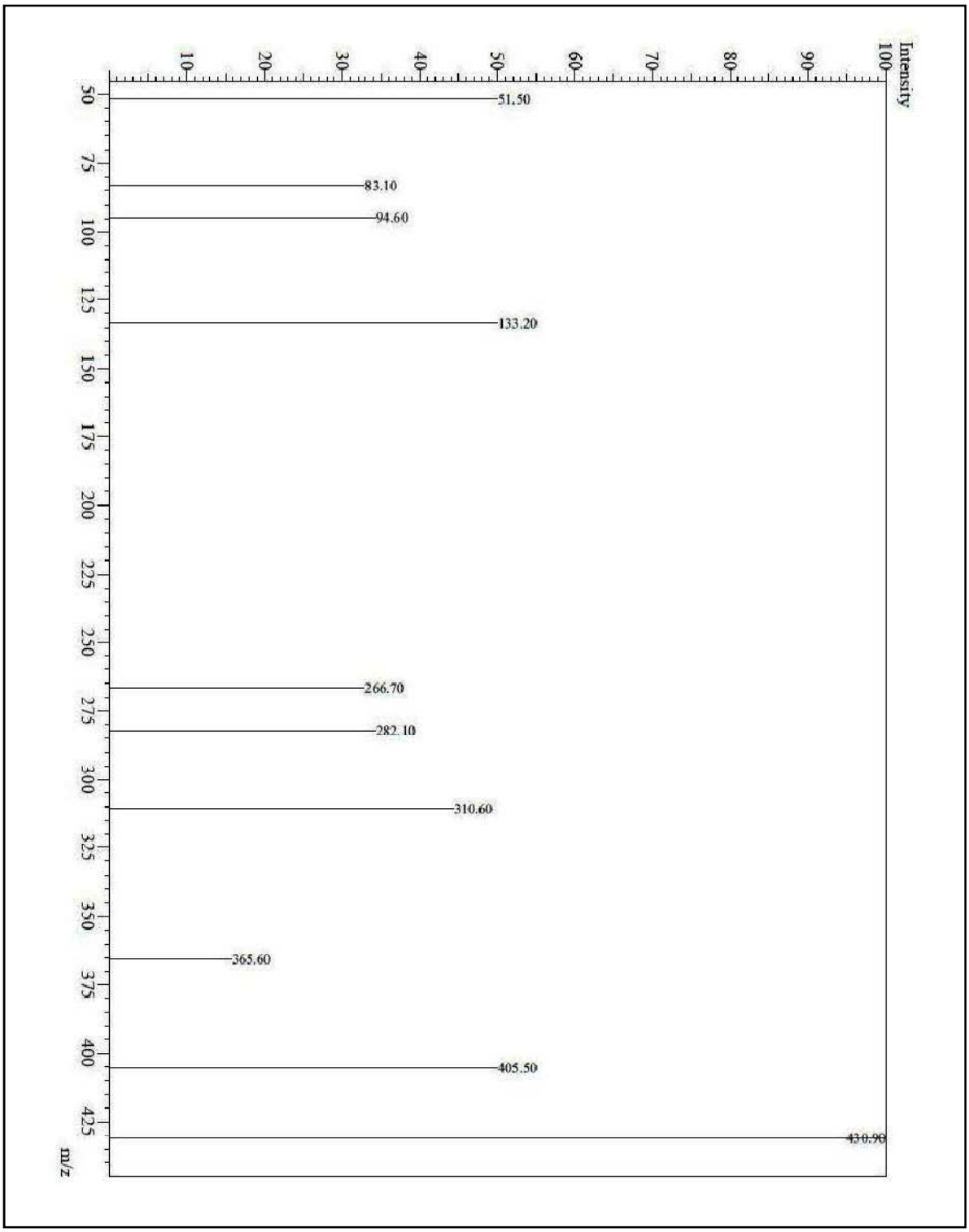
^{1}H NMR spectrum of compound (10c)



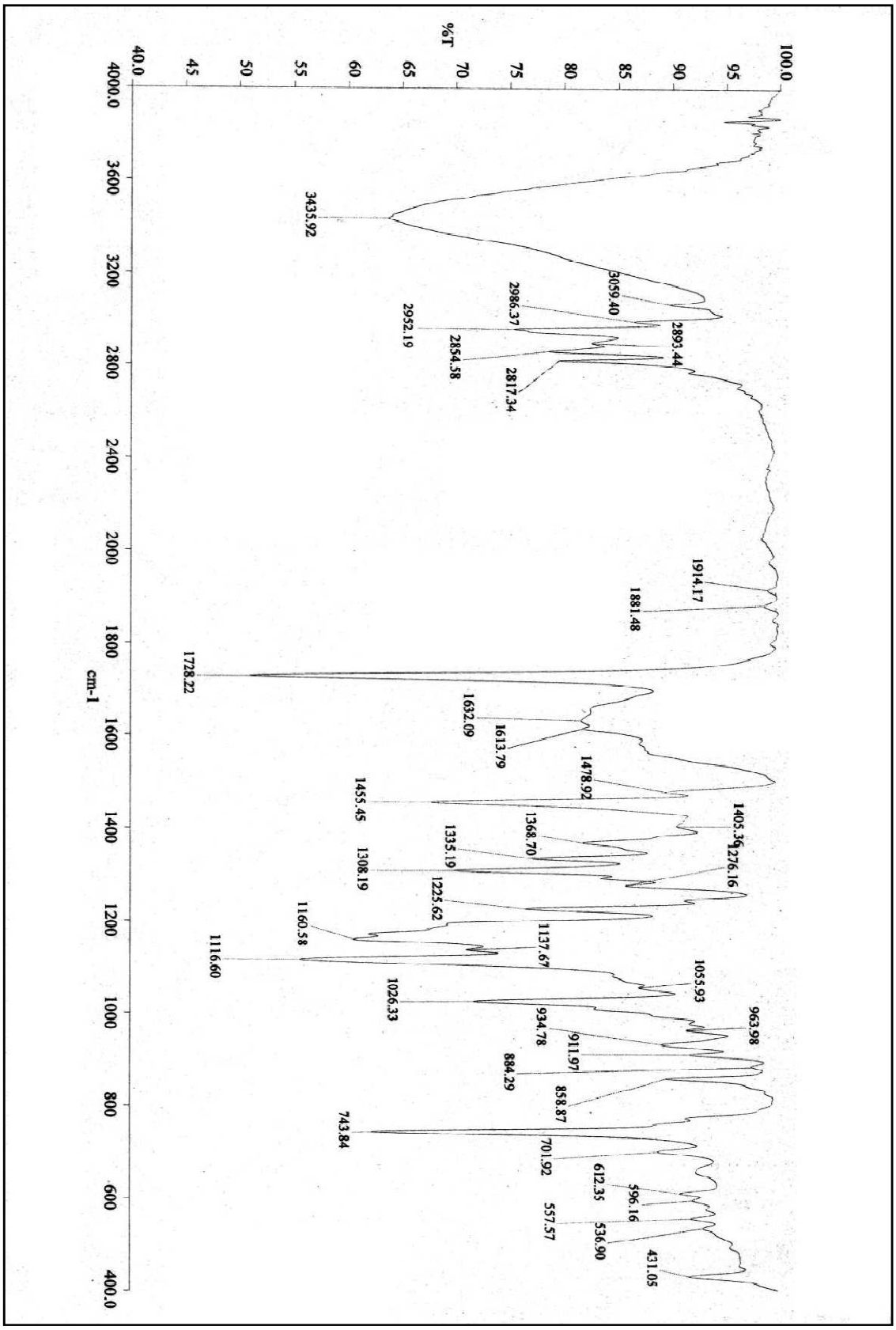
APT spectrum of compound (10c)



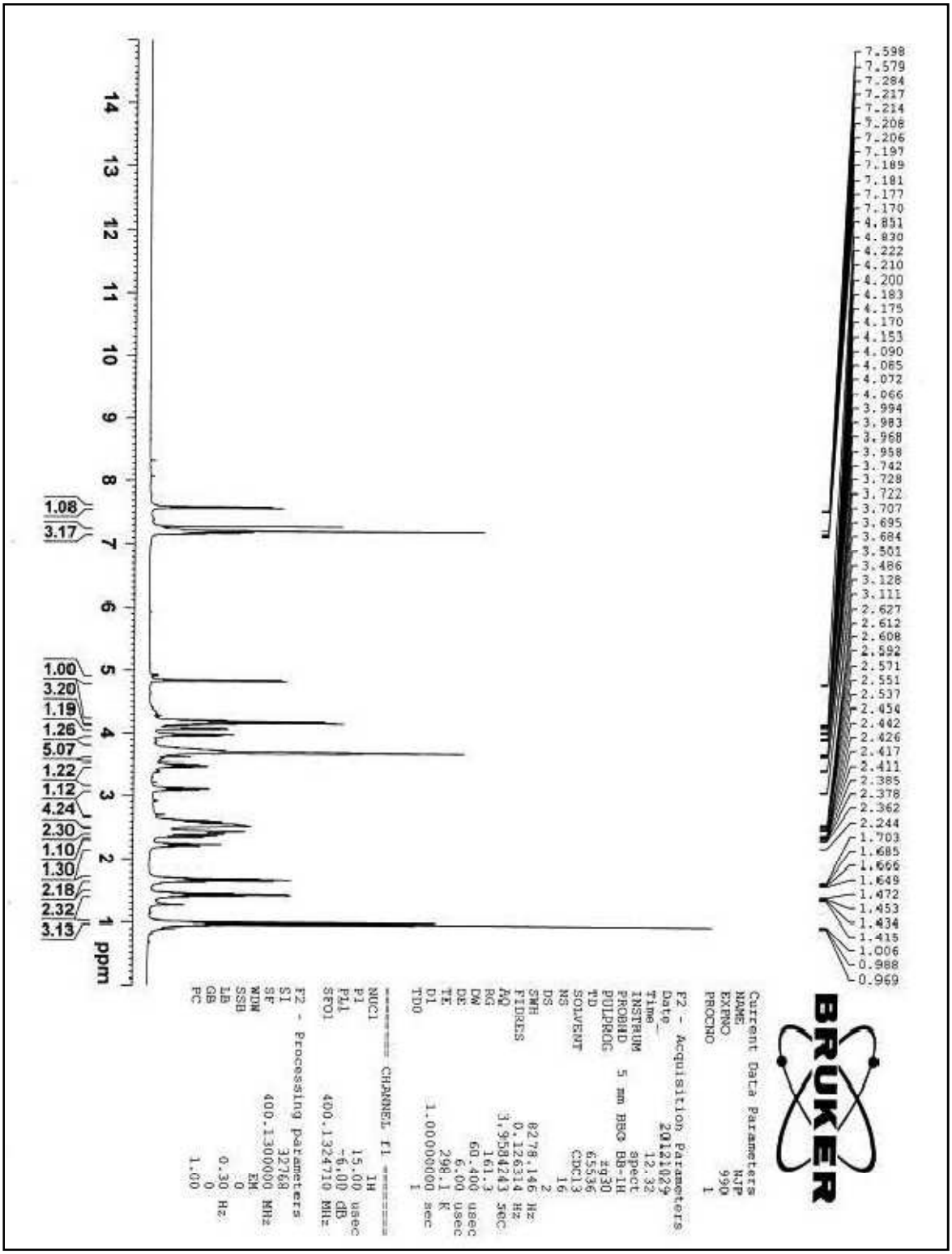
ESI–Ms of compound (10c)



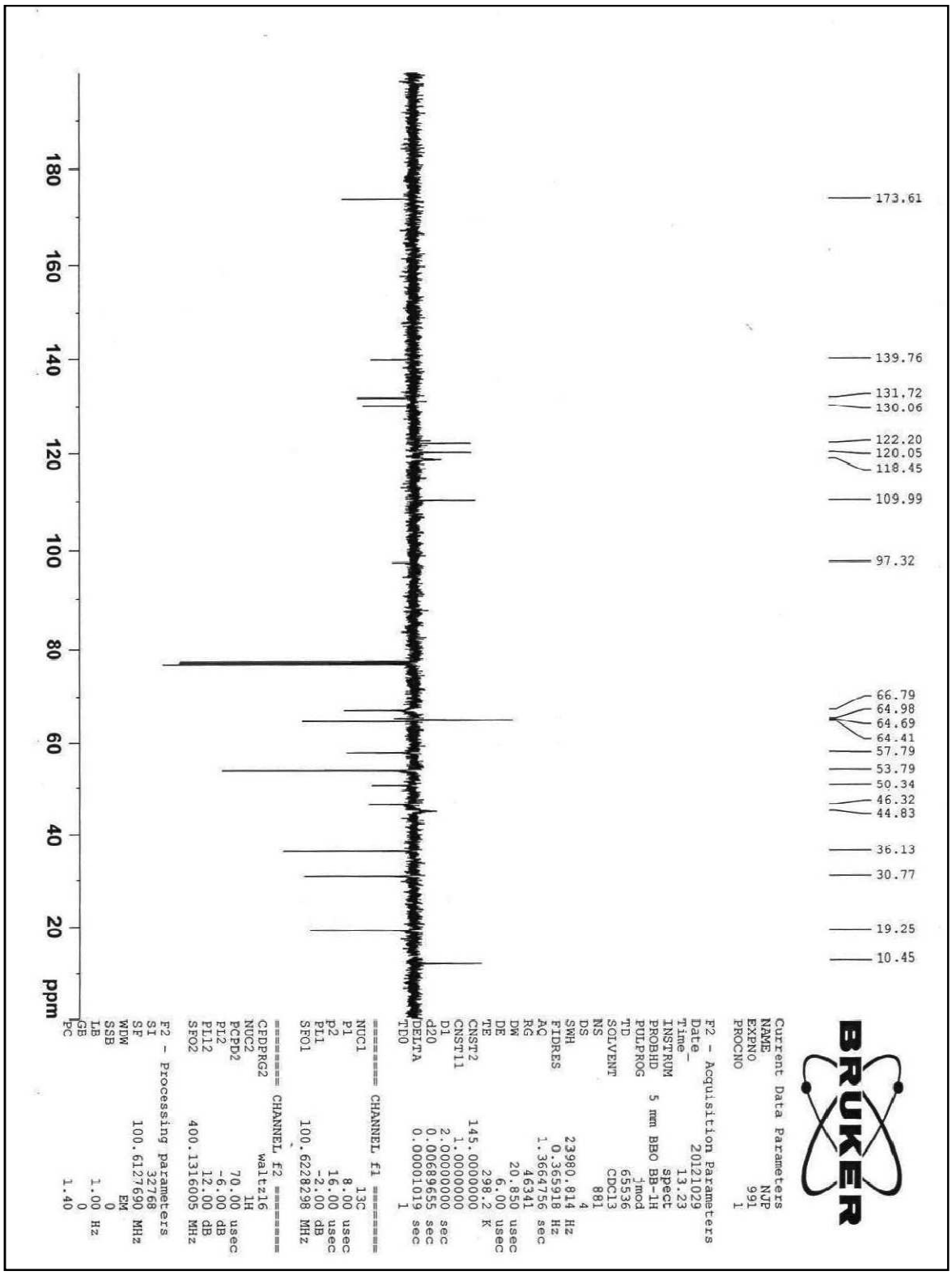
IR of compound (10c)



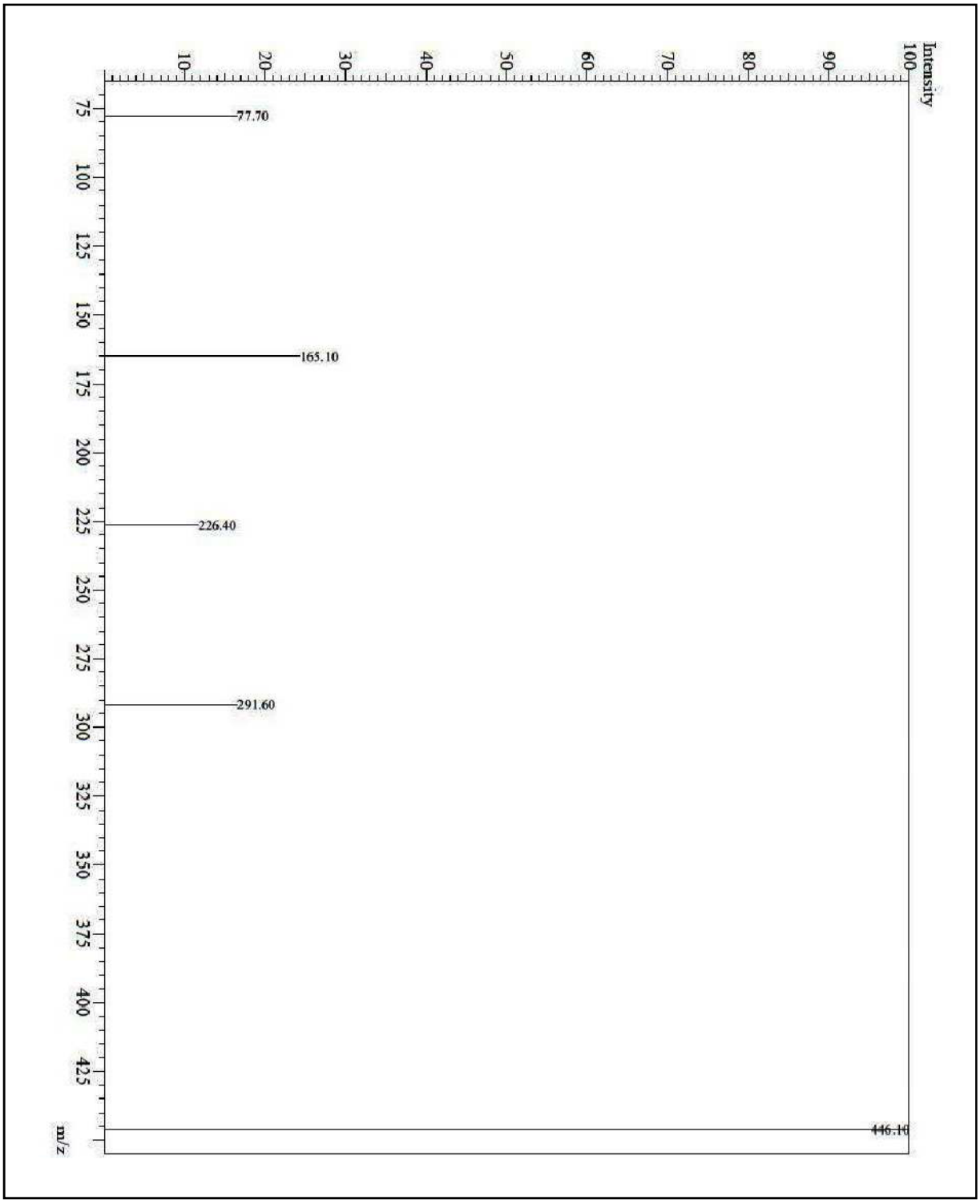
^{1}H NMR spectrum of compound (10e)



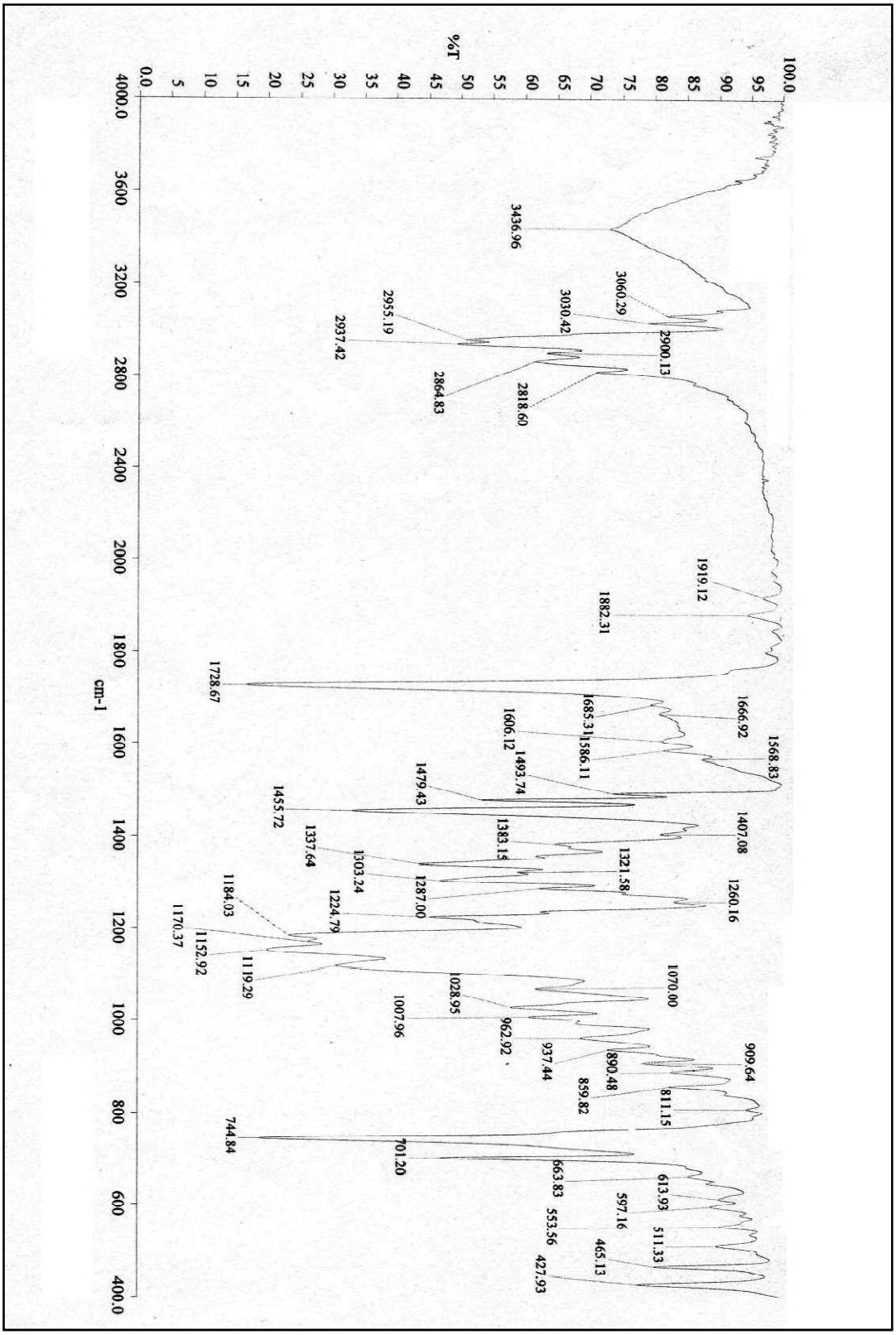
APT spectrum of compound (10e)



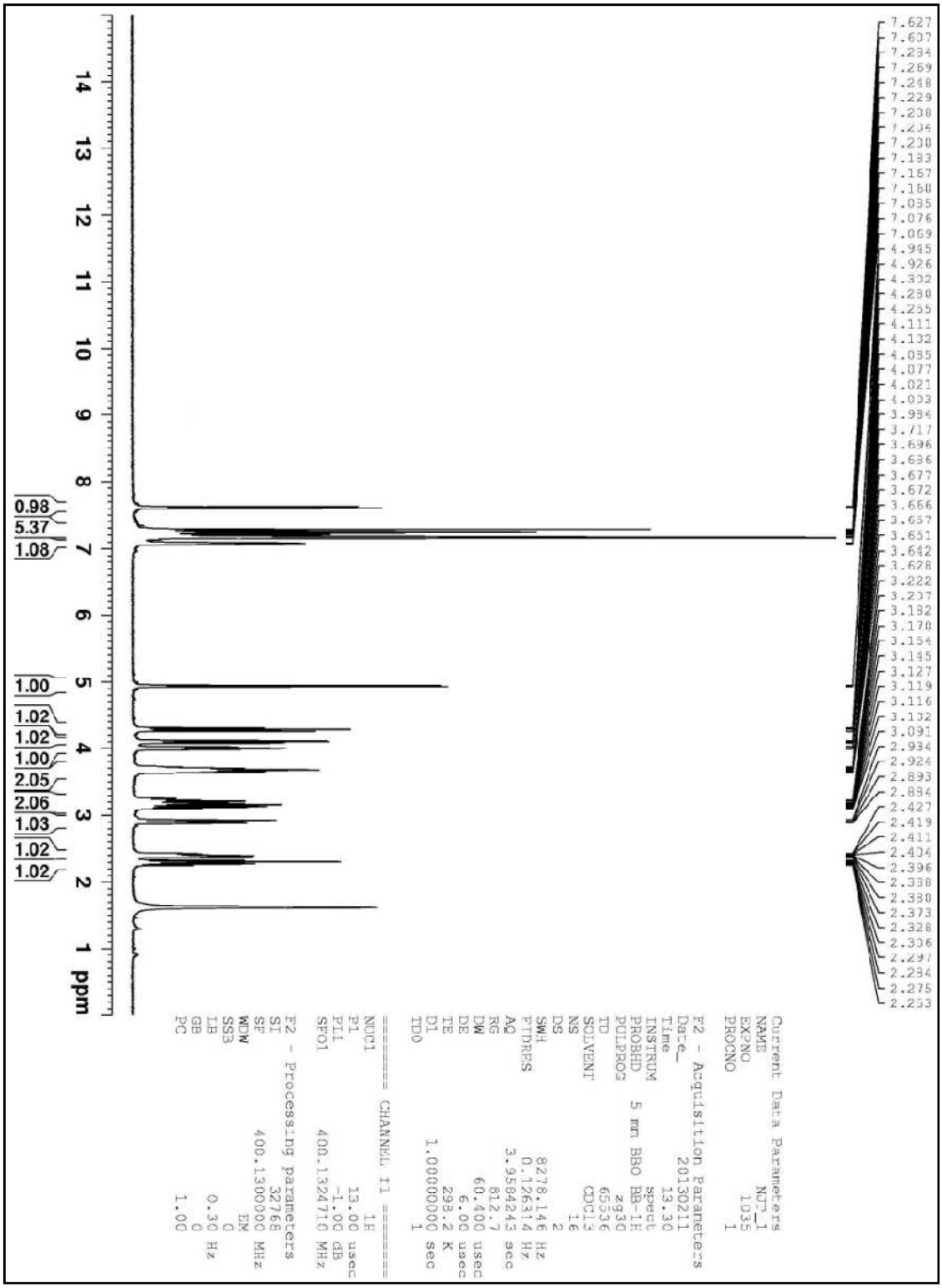
ESI–MS of compound (10e)



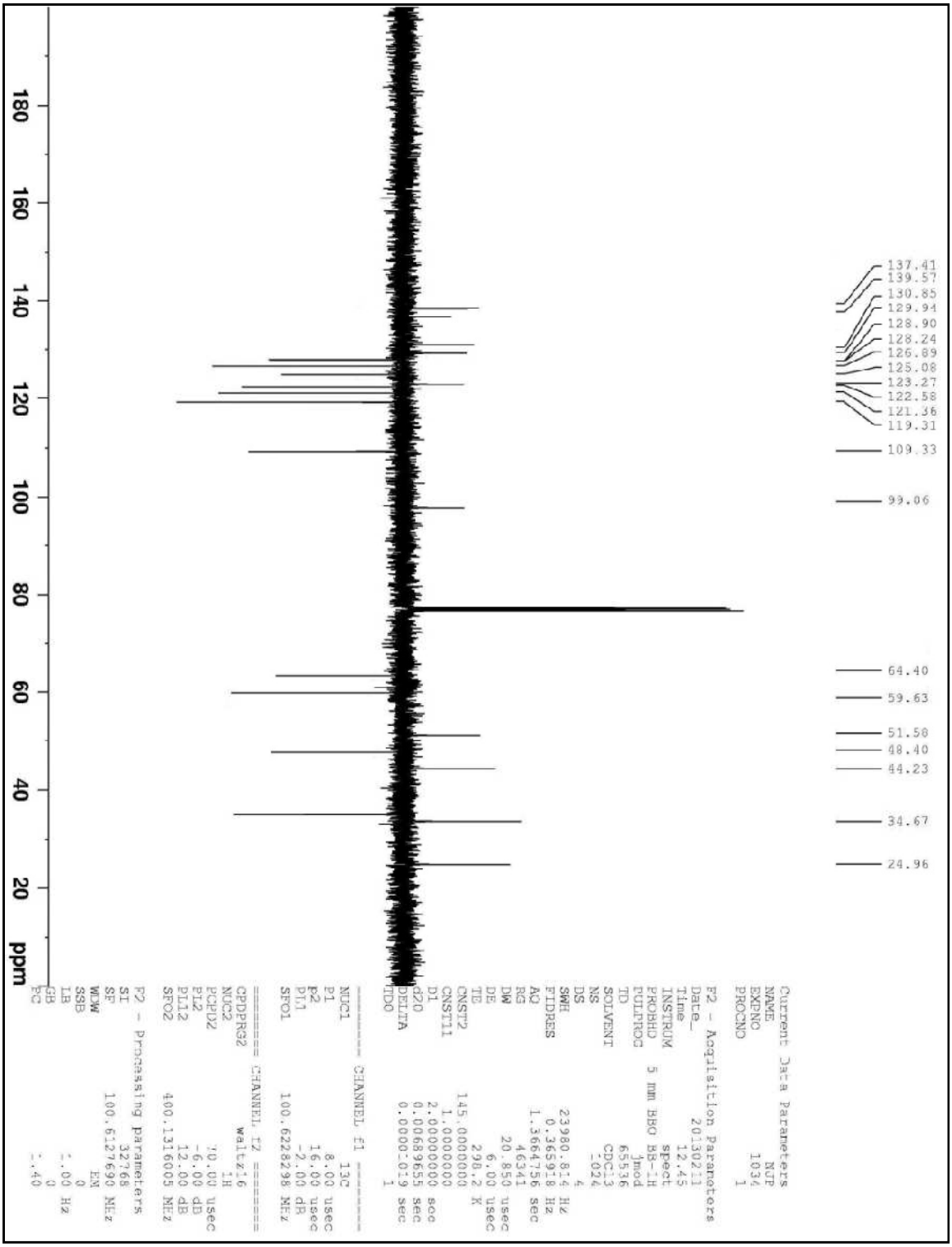
IR of compound (10e)



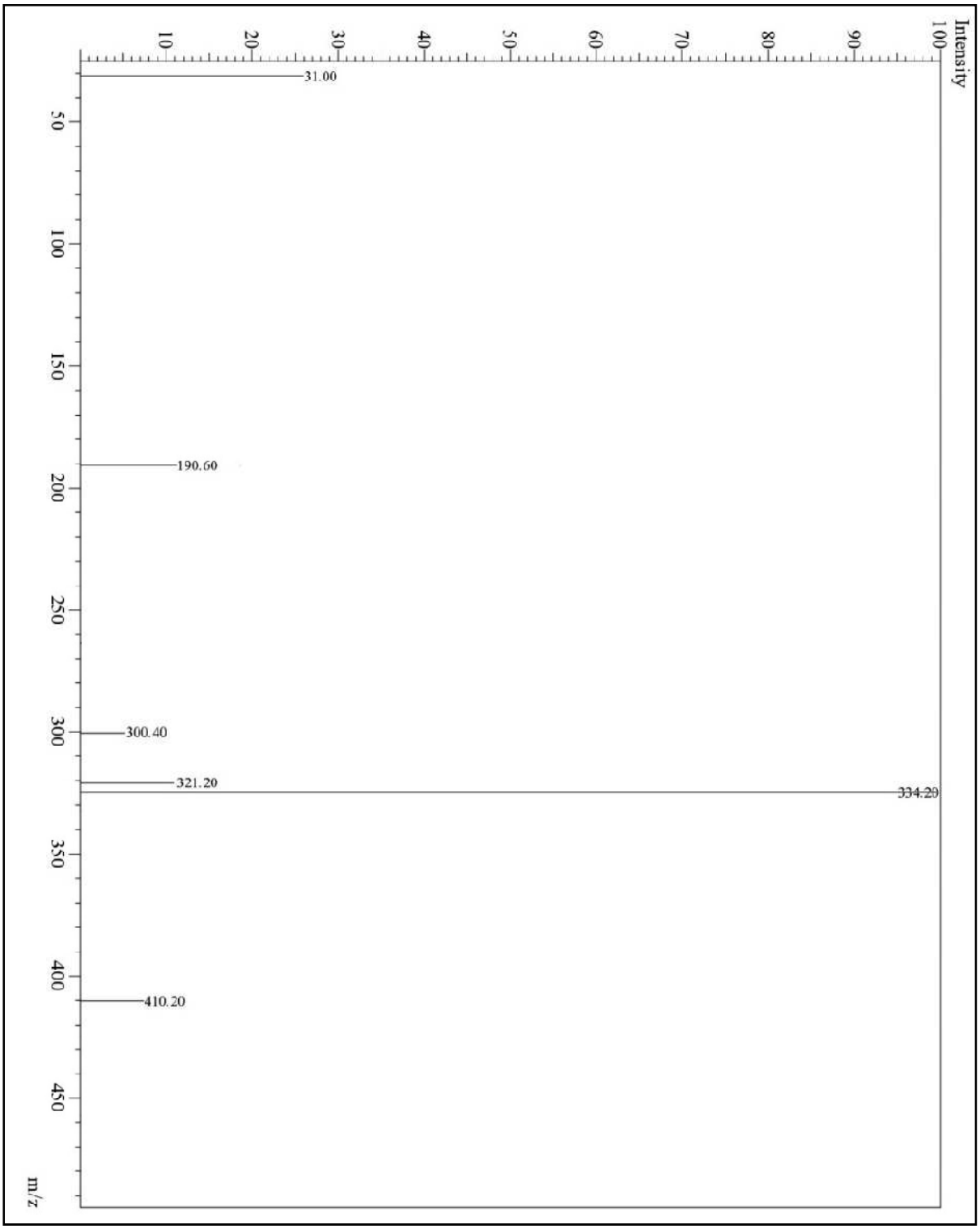
^{1}H NMR spectrum of compound (11a)



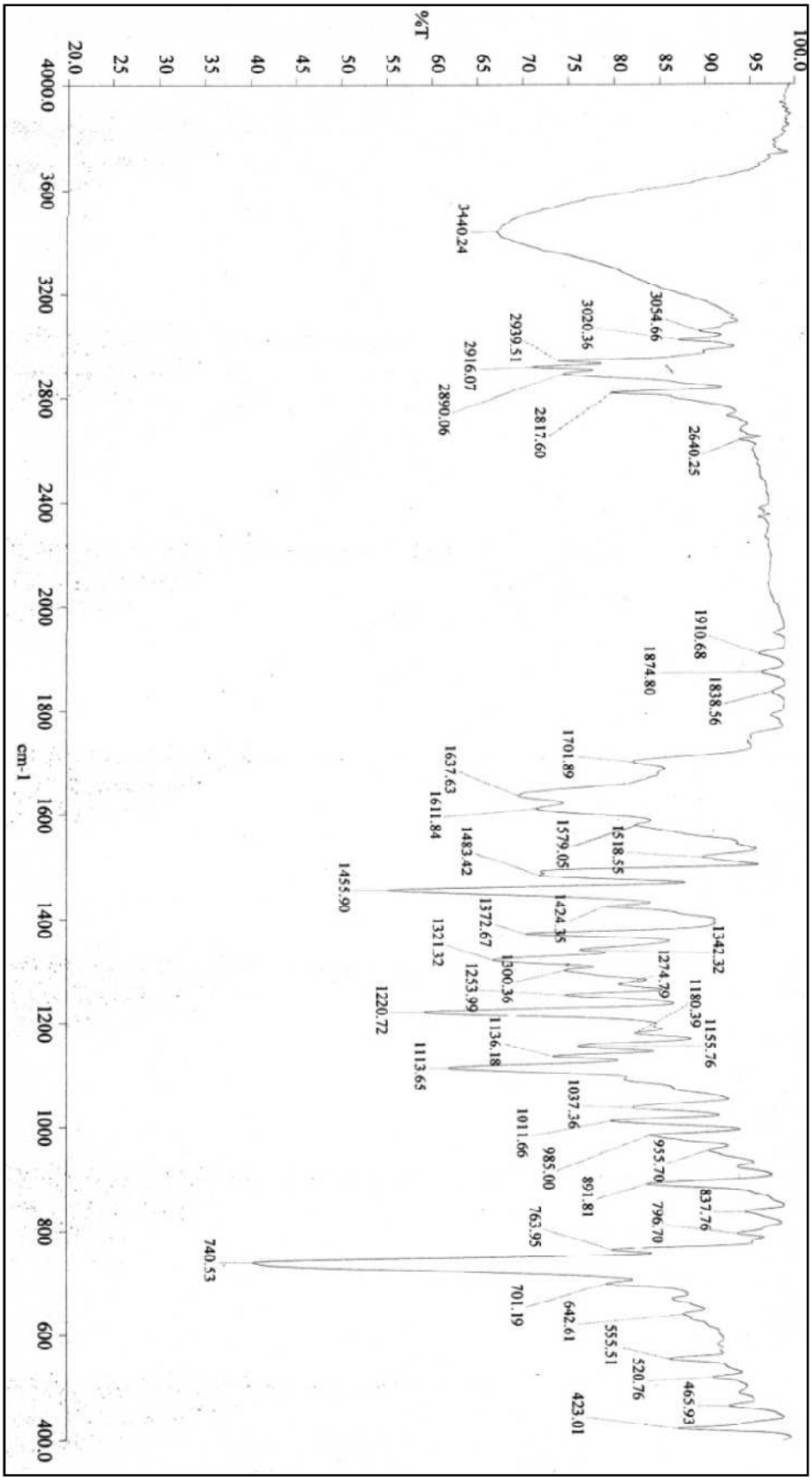
APT spectrum of compound (11a)



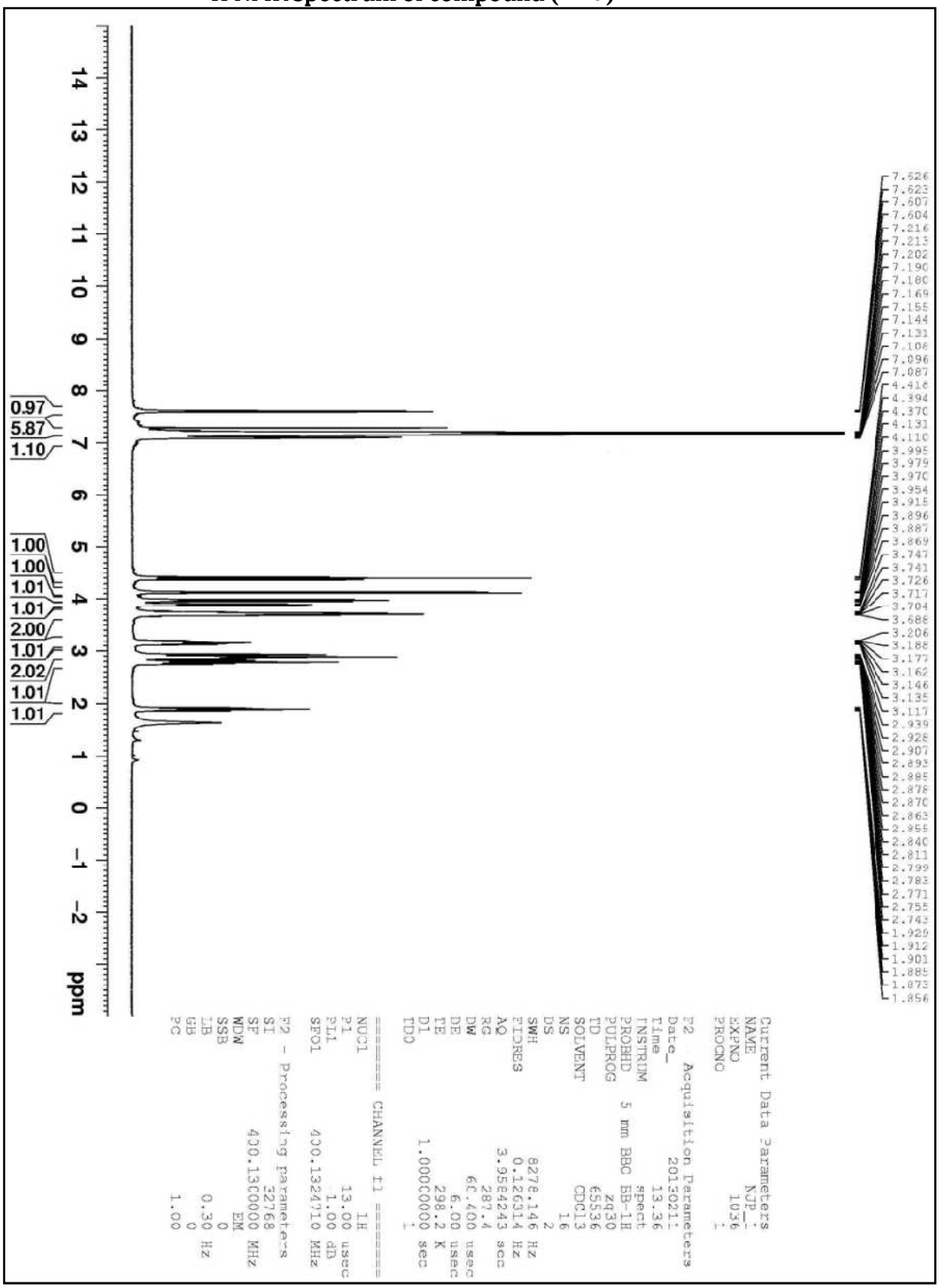
ESI–MS of compound (11a)



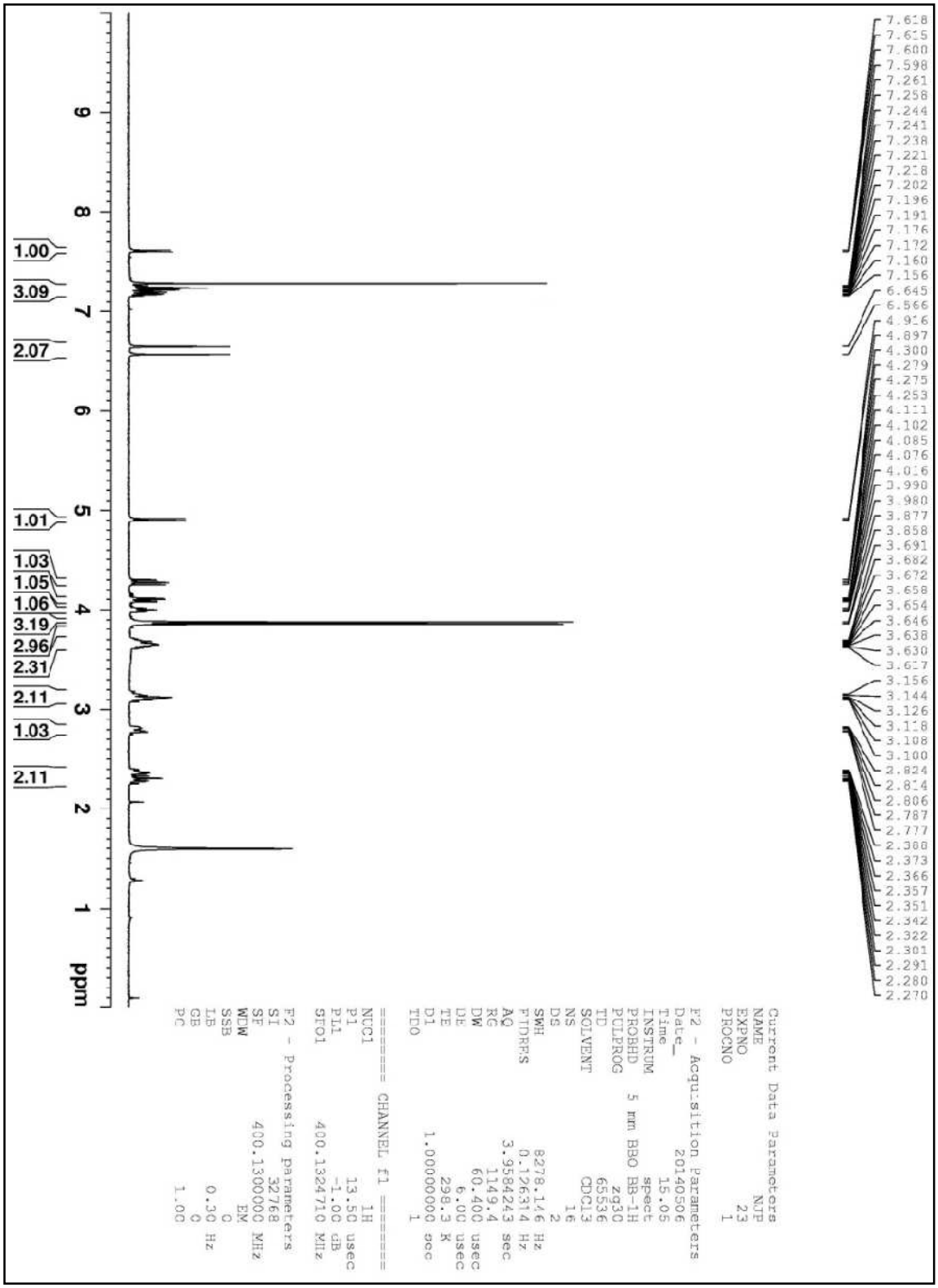
IR of compound (11a)



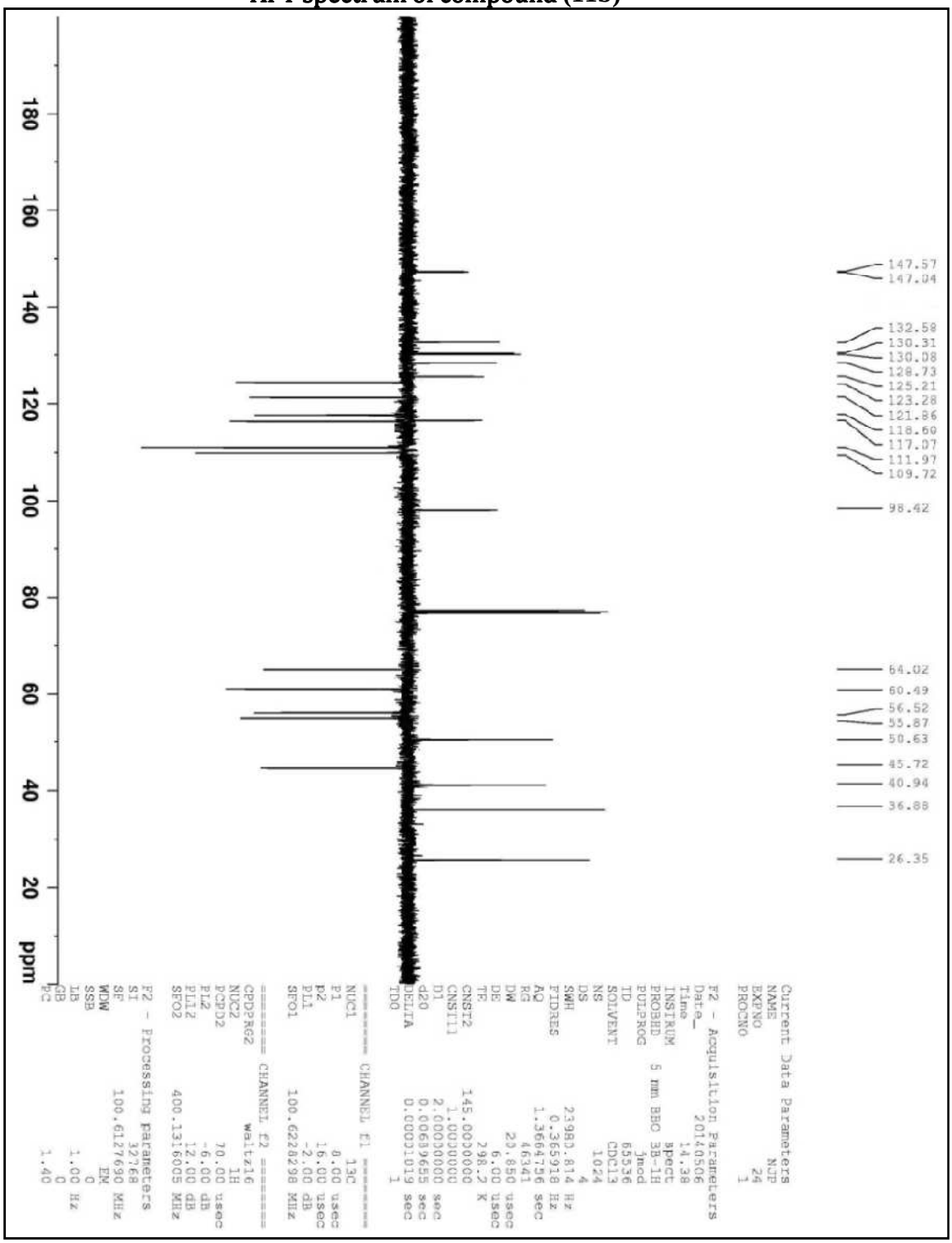
^{1}H NMR spectrum of compound (11'a)



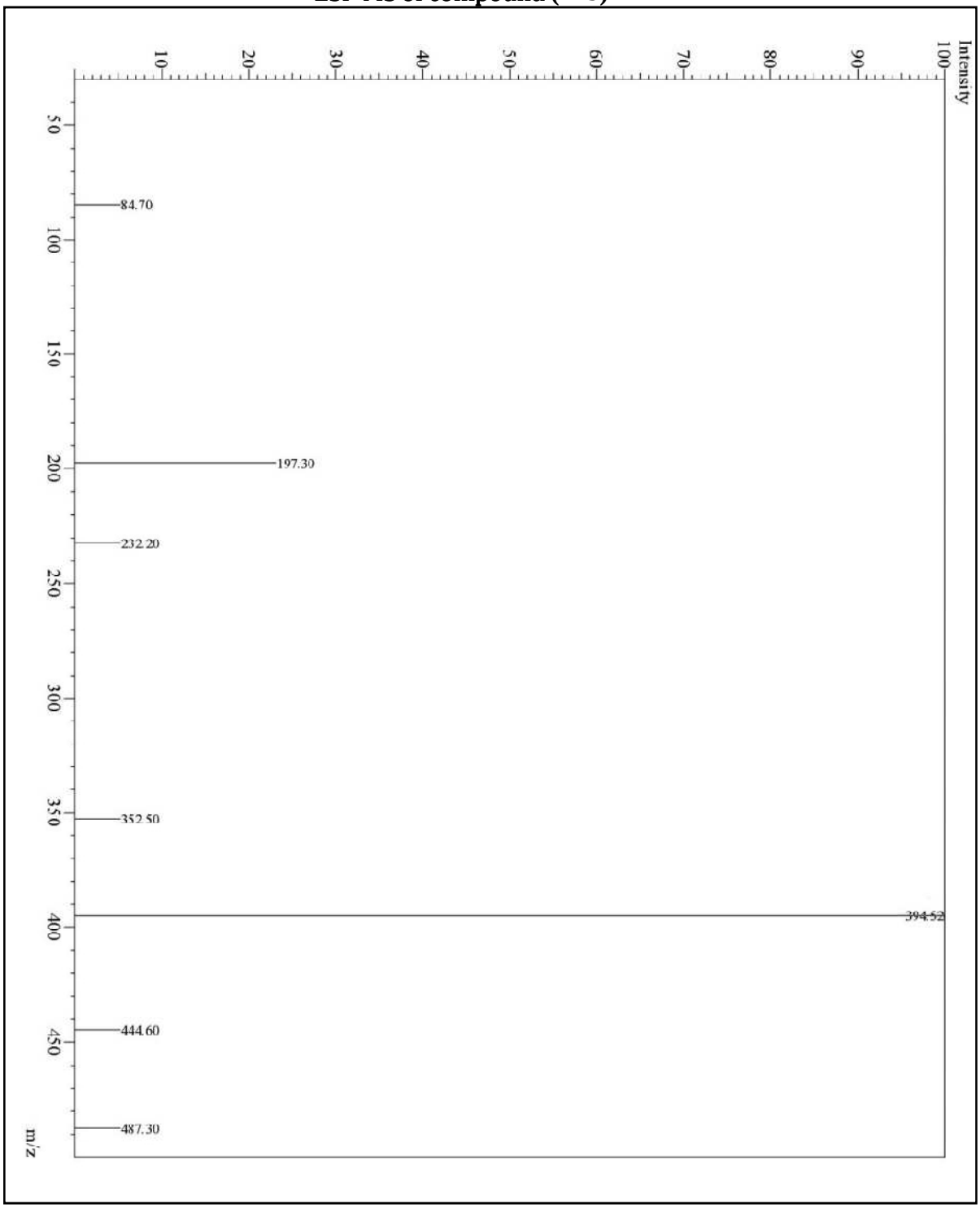
^{1}H NMR spectrum of compound (11b)



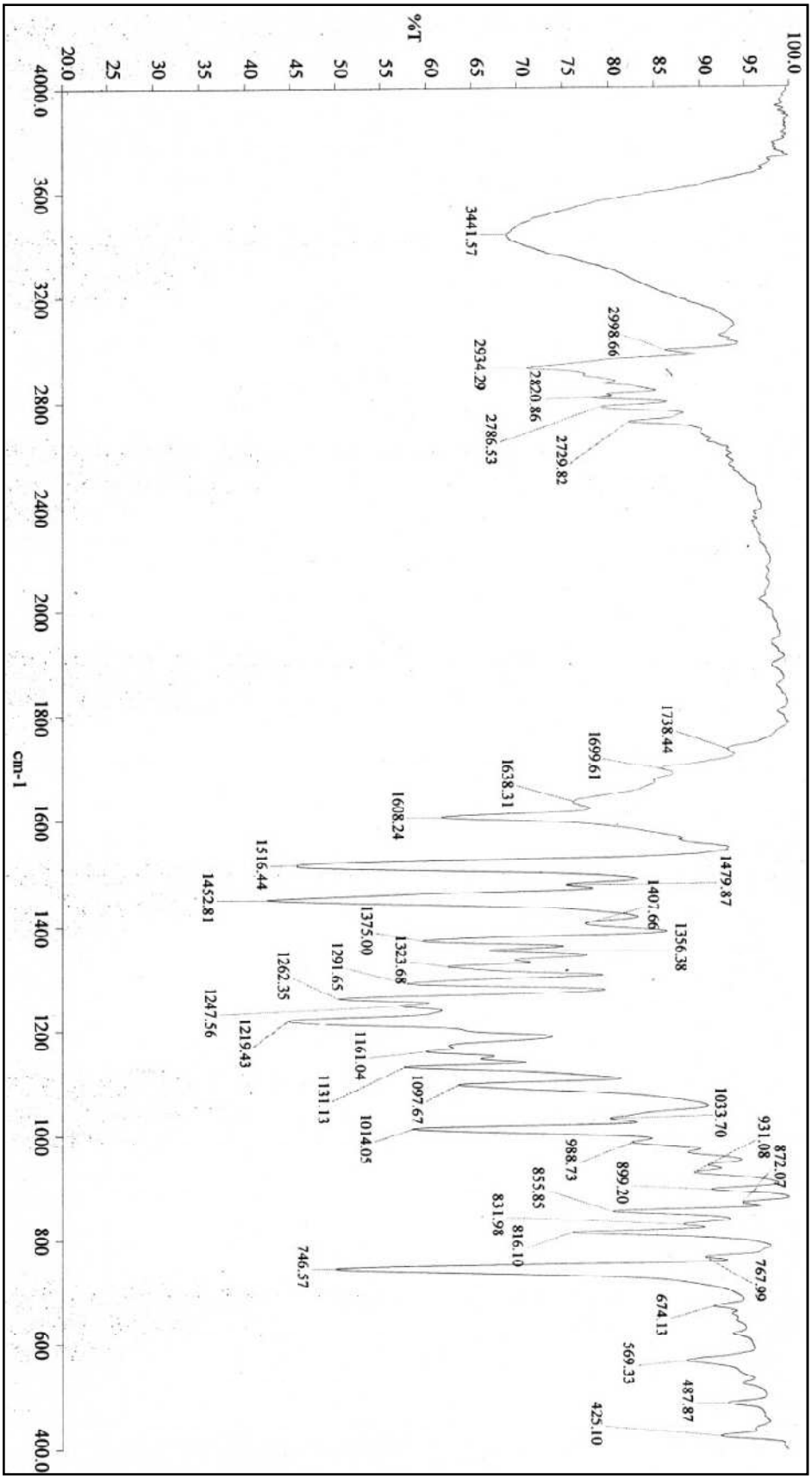
APT spectrum of compound (11b)



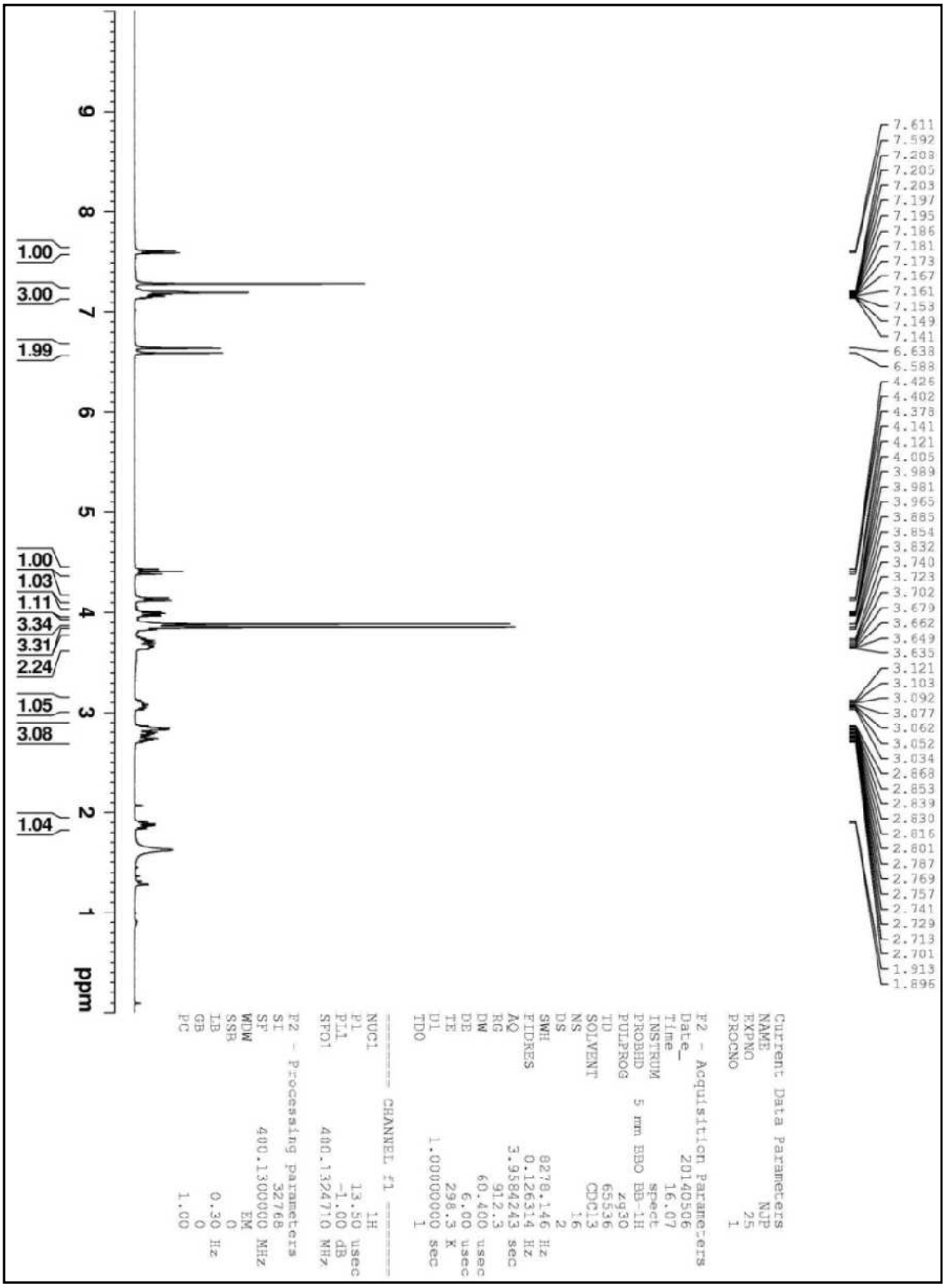
ESI–MS of compound (11b)



IR of compound (11b)



^{1}H NMR spectrum of compound (11’b)



1 NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement (2002), ISBN 1-56238-454-6 M100-S12 (M7).
2 A. Rattan, in Antimicrobials in Laboratory Medicine, ed. B. I. Churchill, Livingstone, New Delhi, 2000, pp. 85–108.
3 (a) Benzie, I. F. F.; Strain, J. J. *Redox* Rep. 1997, 3, 233; (b) Benzie, I. F. F.; Strain, J. J. Methods in Enzymology: Oxidants and Antioxidants Part A. 1st Ed.; London: Academic Press Limited, 1999; Vol. 299, p 15; (c) Choy, C. K. M.; Benzie, I. F. F.; Cho. P. IOVS, 2000, 41, 3293.