An-Najah National University
Faculty of Graduate Studies
Chemistry Department

SYNTHESIS OF A NEW SERIES OF HETEROCYCLIC SCAFFOLDS FOR MEDICINAL PURPOSES

By
Randa Yaser Arafat

Supervised by

Dr. Taleb H. AL-Tel

&

Prof. Hikmat S. Hilal

Submitted in Partial Fulfillment of the Requirement for the Degree of Master of Science in Chemistry, Faculty of Graduate Studies

An-Najah National University

Nablus, Palestine

January 2001

Synthesis of a New Series of hetercyclic Scaffolds for Medicinal Purposes

By

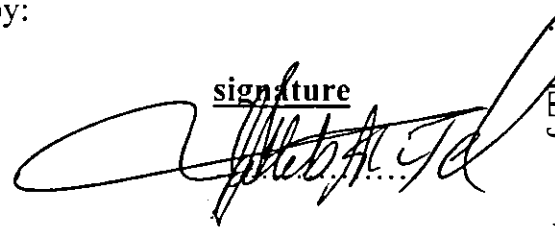
Rnada Yaser Arafat

This thesis was successfully defended and approved by:

Committee members **signature**

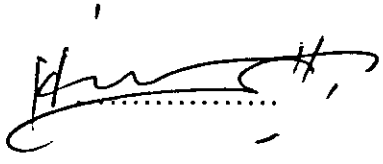
1 – Dr. Taleb H. Al-Tel

An-Najah National University



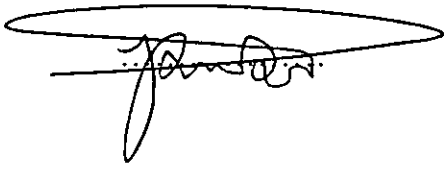
2 – Prof. Hikmat S. Hilal

An-Najah National University



3 - Prof. Mohammed J. Musmar

An-Najah National University



4 – Dr. Imad Oudeh

Al Quds University

Dedication

To my father, my mother, my brother, my sisters

To my husband

And
To all my friends

Acknowledgement

I would like to express my deep appreciation to Dr. Taleb H. Al-Tel and Prof. Hikmat S. Hilal for their direct supervision, guidance and assistance during the course of this work.

I would also like to thank Dr. Nidal A. Zatar for his assistance in obtaining the GC/MS spectra, and Amjad Iz El-Deen for elemental analysis data. Thanks also for Prof. Mohammad Abu Zarga and Keyan Bargathi at the Jordan University for obtaining the NMR spectra, Prof. Mohammad S. Eshtieh and Rabie Zaid for measuring the biological activity of the compounds, Prof. W. Voelter and Angelica R. Matinez for measuring the anticancer activity.

Finally, especial thanks for Omir Al-Nabulsi, Issa Abu al-Izz, Mahmoud Al-Shamali and Ashraf Salman in the chemistry labs for their support and assistance.

List of contents

List of tables

List of figures

Abstract

A new series of substituted 8-fluoro-4H-pyrimido[2, 1-b][1,3] benzo-thiazole-4-ones (**24**) and (**25**), substituted 7-methyl-4H-isoxazolo[2, 3-a] pyrimidin-4-ones (**26-28**), and substituted 2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo[2, 3-a] pyridopyrimidin-9-ones (**29**) and (**30**), have been prepared via condensation of β- keto esters with 2-aminopyridine derivatives. Furthermore diazipine Compounds (**31-33**) have been prepared via condensation of a γ-keto ester with 2-aminopyridine derivatives. The reactions were conducted in the presence of poly phosphoric acid (PPA). The details of the synthesis procedures are described.

The new compounds have been characterized using elemental analysis, GC MS, FT IR and NMR spectrometry.

Compounds **24**, **27** and **28** were tested against *Staphylococcus aureus*, *proteus vulgaris*, and *candida albicans* bacteria. None of the three compounds showed antibacterial activity. The same compounds were tested against *Microsporum canis, fusiarium tricincutum, pythium ultimum, pythium aphanidermatum,* and *pythium middletonii* fungi types. All of them showed antifungal activity.

Compounds **27**, **32** and **33** were tested for anticancer activity by the MTT assay. They all showed significant anticancer activity.

Chapter One

INTRODUCTION

1.1 SOME MEDICALLY VALUABLE ORGANIC COMPOUNDS:

Many heterocyclic compounds are medically valuable, such as anticancer, antiallergic, and antimalarial agents. Therefore, we are looking for an efficient method for the synthesis of new polycyclic hetero frameworks that might have important bioactivities.

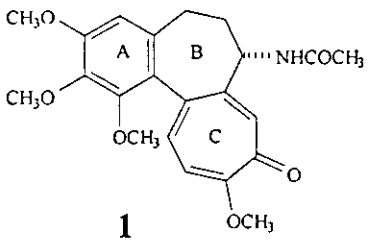
1.1.1 Antitumor agents:

Microtubules are cylindrical organelles found in almost all cell types in *eukaryotes*. They are involved in many cellular processes, including mitosis, cell signaling, and motility and consequently are an important target for development of compounds potentially useful as anticancer chemo-therapeutics.

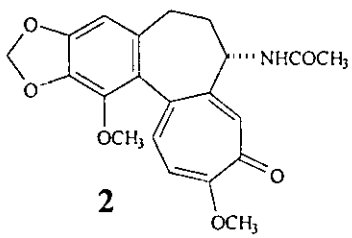
Microtubule dynamics play an important role in cell proliferation. Inhibition of microtubule dynamics now appears to be the mechanistic basis underlying the antitumor effects of most antimitotic compounds.[1]

1.1.1.1 Natural products as antitumor agents:

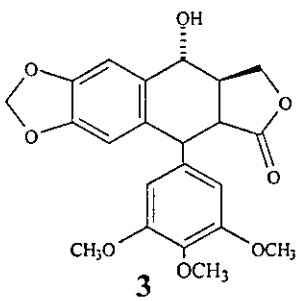
Numerous chemically diverse antimitotic agents, many of which are derived from natural products, have been found to interact specifically with tubulin rather than with other components of microtubules or other proteins involved in mitosis.[2] An example of antimitotic agents is colchicine **(1)**. Other natural products that play the role of colchicin are cornigerine **(2)**, podophyllotoxin **(3)**, steganacin **(4)** and combretastatin A-4 **(5)**. These compounds share homology with A and C rings of colchicin.[1]



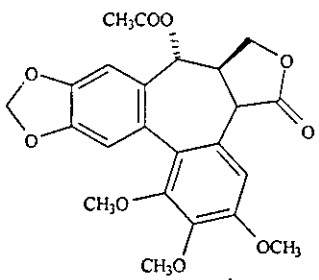
1



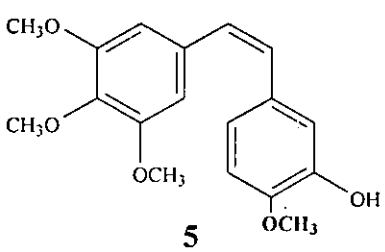
2



3



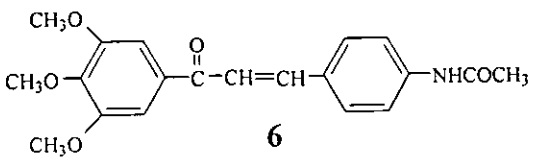
4



5

1.1.1.2 Chalcones as antimitotic agents:

Appropriately substituted chalcones, as typified by compound (**6**), were found to be potent antimitotic agents.[3]



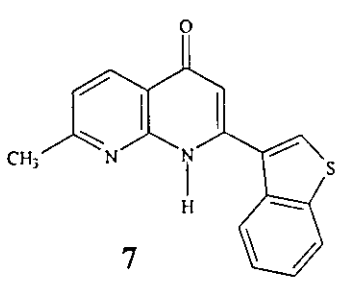
6

1.1.1.3 2-Aryl-1,8-naphthyridin-4-ones :

A series of substituted 2-aryl-1,8-naphthyridin-4-one compounds were synthesized as potential anticancer drug candidates .[2]

Most compounds showed significant cytotoxic effect. It was found that: log CI_{50} <-4. CI_{50}: molar drug concentration required to cause 50% growth inhibition, against a variety of human tumor cell lines including cells derived from solid tumors such as non-small-cell lung, colon, central nervous system, melanoma, ovarian, prostate and breast cancers.

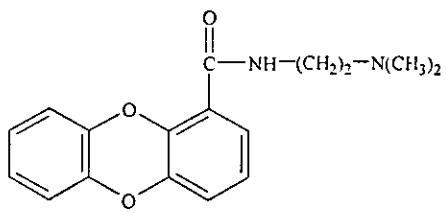
The most potent inhibitor of polymerization is 3′-benzo [b] thienyl-7-methyl-1,8-naphthyridin-4-one (**7**), which has effects comparable to those of podophyllotoxin and combrestatin A-4.[1]



7

1.1.1.4 Dibenzo[1,4]dioxin-1-carboxamides :

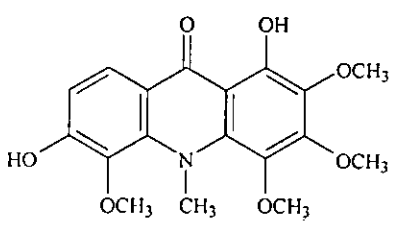
In the general study of the antitumor properties of linear tricyclic carboxamides, it was noted that dibenzo[1,4]dioxin-1-carboxamide **(8)** is a DNA intercalating agent.[4]



8

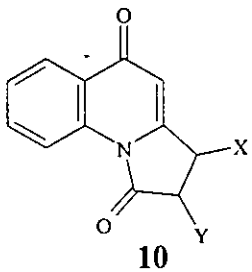
1.1.1.5 Acridone alkaloids:

The anticancer properties of glyfoline **(9)** focused attention on the acridone alkaloids.[5]



9

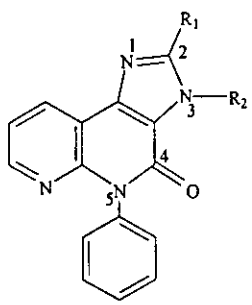
Other members of the class; most highly oxygenated derivatives of the acridone skeleton, have shown a range of physiological activities including antimalarial, antiviral, and antibiotic activities.[6] Pyrrolo[1,2-a]quinoline-1,5-diones **(10)** are intercalating heterocycles [7].



10

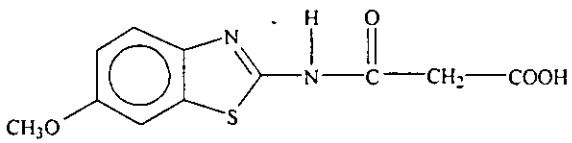
1.1.2 Anti-inflammatory agents:

A series of substituted 5-phenylimidazo[4,5-c][1,8]naphthyridin-4(5H)-ones (**11**) were synthesized as anti-inflammatory agents.



11

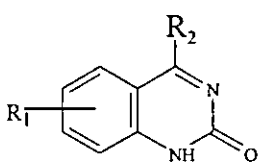
In this series of compounds, anti-inflammatory activities were greatly influenced by the position and nature of substituents on imidazole. 3-Alkyl or 3-benzyl substitution resulted in potent activity, but the substitution at N-1 did not.[8]Malonamic acid, malonamate, and malonamide derivatives of some heterocyclic compounds showed anti-inflammatory activity, an example is N-[2-(6-methoxy)benzothiazolyl]malonamic acid [9] (**12**).



12

1.1.3 Cardiotonic activity of some heterocyclic compounds:

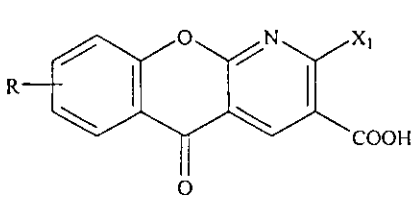
A series of substituted 4-alkyl-2-(1H)-quinazolinones (**13**) were observed to have cardiotonic activity.[10]



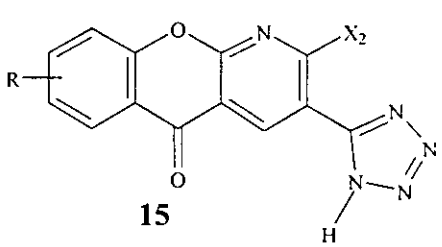
13

1.1.4 Anti-allergic agents:

5-Oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carboxylic acids (**14**) and their tetrazole analogues **15** exhibit anti-allergic activity .In the carboxylic acid series, the activity was influenced by the substituents at the 2- position (X_1), and increased substantially in the following order: Me, OMe < NH_2 < OH, H <NHOMe. On the other hand, in the tetrazole series the 2-unsubstituted derivative (X_2=H) showed the highest activity.[11]



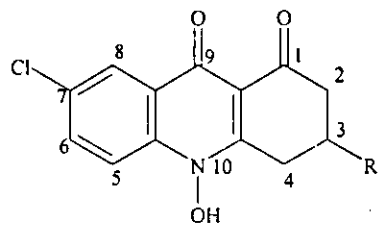
14



15

1.1.5 Antimalarial agents:

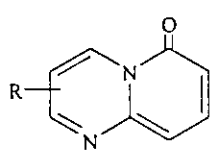
A series of 1-amino derivatives of 7-chloro-3-substituted-3,4-dihydro-1,9(2H,10H)-acridinediones **(16)** showed antimalarial properties.[12]



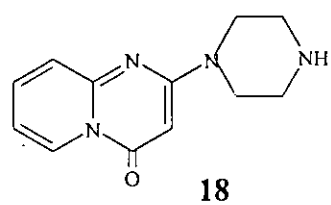
16

1.1.6 2-Pyridones as potent DNA Gyrase inhibitors and antibacterial agents :

2-Pyridones **(17)** are active against *Staphylococcus aureus* which is resistant to the *Ciprofloxacin*, and are active against *Streptococcus Pneumonia* which is resistant to *Penicillin* and it is a DNA gyrase inhibitor.[13]



17



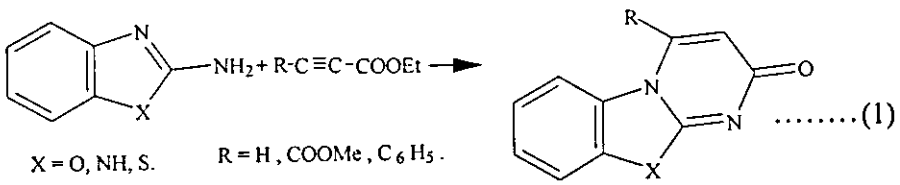
18

2-(1-Piperazinyl)-4H-pyrido[1, 2a] pyrimidine-4-one **(18)** is a recently described *in vitro* inhibition of human platelet aggregation.[14,15,16]

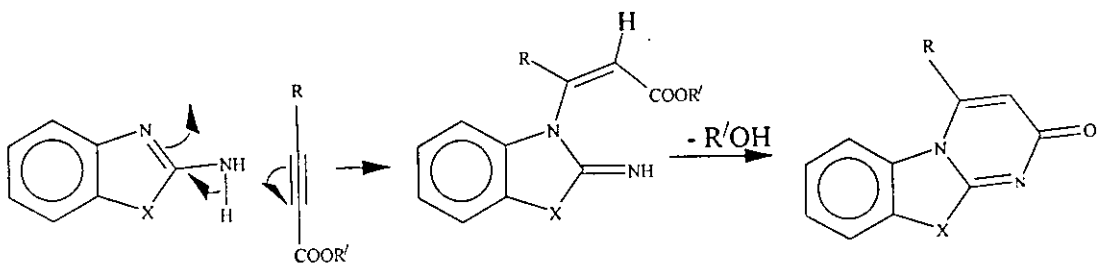
1.2 SOME SYNTHETIC METHODS FOR SOME RELATED HETEROCYCLIC COMPOUNDS:

1.2.1 Cyclization through the reaction of amino-heterocycles with acetylenic compounds:

In this reaction the nucleophilic addition to the carbon-carbon triple bond is used. Synthesis of the tricyclic compounds is shown in equation (1).

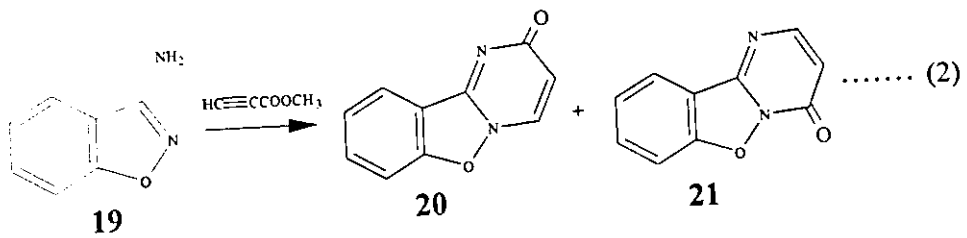


In these cases only 2-oxo derivatives were obtained, without any other isomeric 4-oxo compounds.A mechanism has been proposed for this reaction as shown in Scheme (1).



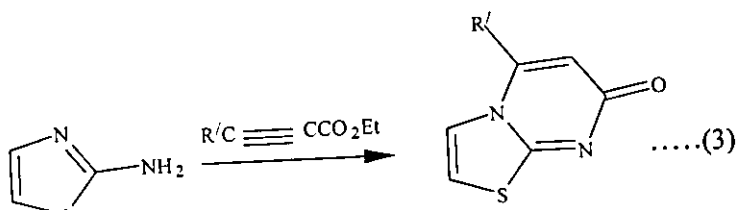
Scheme (1)

In the reaction of methyl propiolate with 3-aminobenzisoxazole **(19)**, 15% of 2H-pyrimido[1,2-b]benzisoxazole-2-one **(20)** and 8 % of the alternative isomer 4-oxo compound **(21)** were obtained, equation(2).[17]



19 **20** **21** ..... (2)

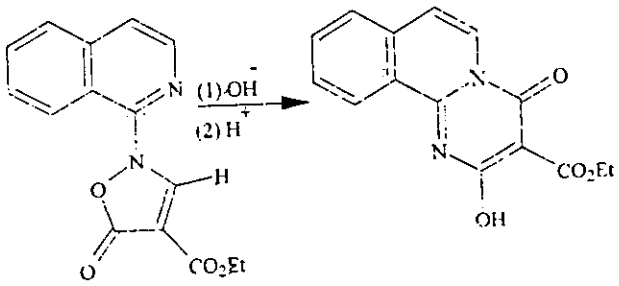
The reaction of 2-aminothiazole **(22)** with propiolic acid and its esters gives mainly 7H-thiazolo[3,2-a] pyrimidine-7-one **(23)**, equation (3).[18]



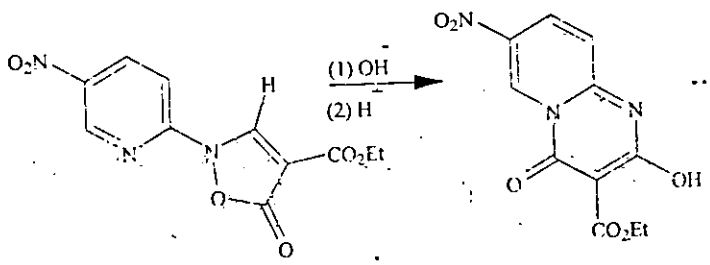
22 **23** .....(3)

1.2.2 Base-catalyzed rearrangement of isoxazolinyl heterocycles:

The rearrangement of a number of ethyl 2-(heterocyclic)-5-oxo-2,5-dihydroisoxazole-4-carboxylates by mild base was reported. Equations (4) and (5) illustrate two examples of such rearrangement.[19]



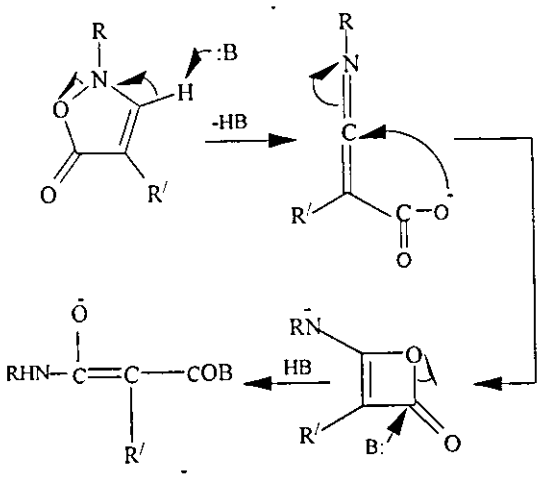
..... (4)



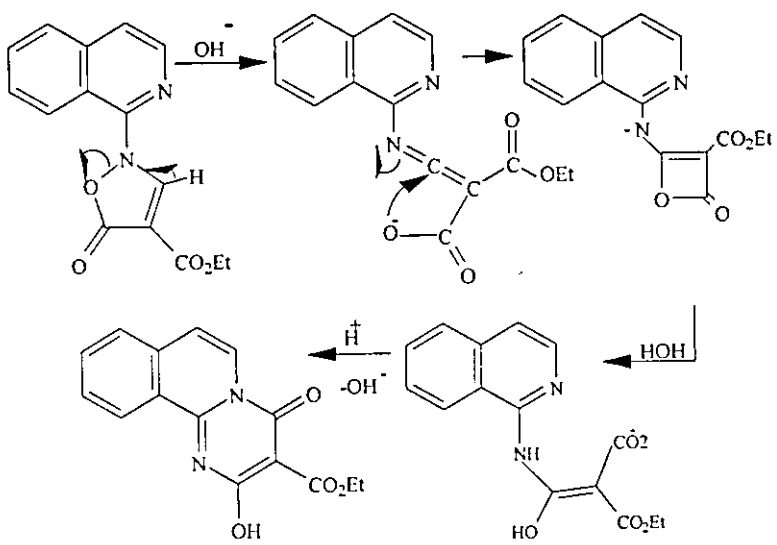
..... (5)

The synthesis of the starting materials has generally involved the nucleophilic displacement of an activated halide in a heterocyclic system by the 2H-isoxazoline.[20]

Schemes (2) and (3) illustrate the proposed mechanism for this rearrangement.[21]



Scheme (2)



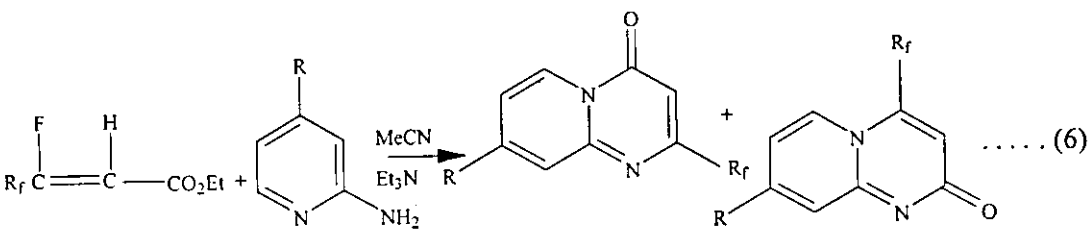
Scheme (3)

1.2.3 Intramolecular nucleophilic acyl substitution:

This is the most common method for the synthesis of heterocyclic compounds, and is achieved by several different routes.

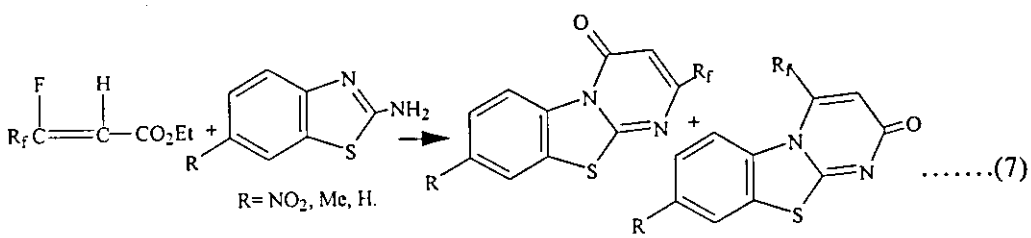
1.2.3.1 The reaction of amino heterocycles with ethyl-2-polyhalogen alkyl-2-enoate:

The reaction is illustrated in equations (6), (7) and (8).

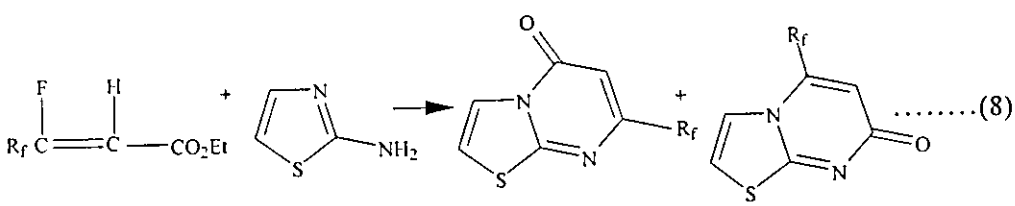


.....(6)

R_f = $CF_2Cl(CF_2)_2$, $CF_3(CF_2)_2$, $CF_2Cl(CF_2)_4$, $CF_3(CF_2)_4$, R = Me, H.

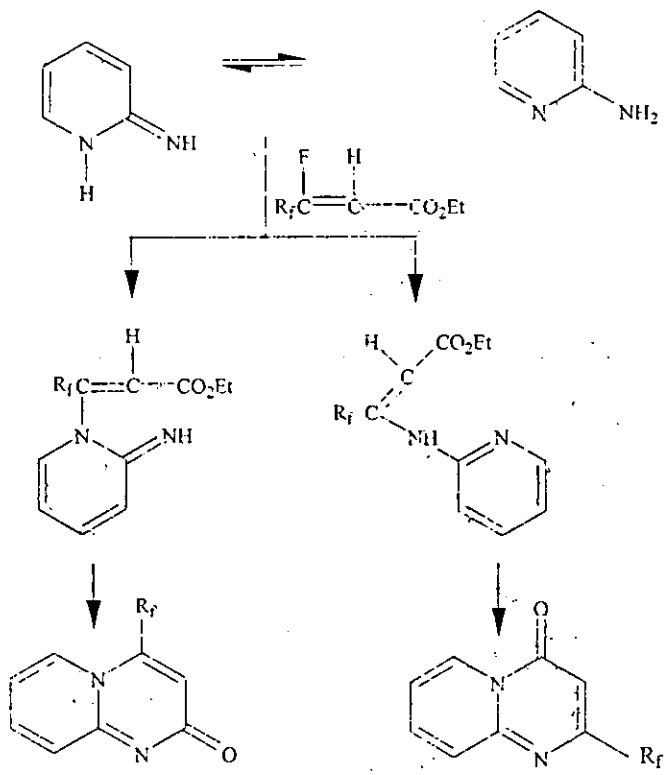


.....(7)



.....(8)

Formation of the isomeric 2- and 4-oxo compounds was the result of Michael addition of the ring nitrogen atom or the amine nitrogen atom on the fluorinated ester, followed by intramolecular cyclization, as shown in Scheme (4). [22]

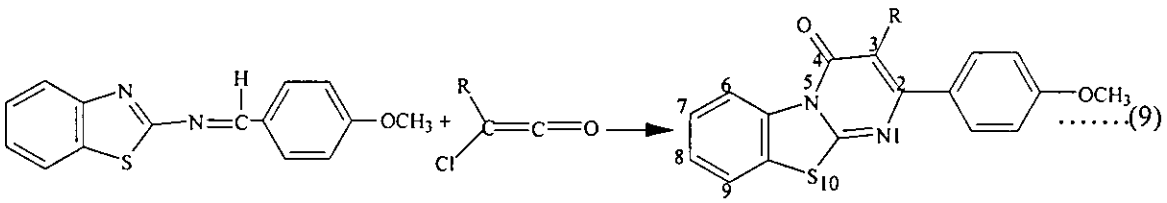


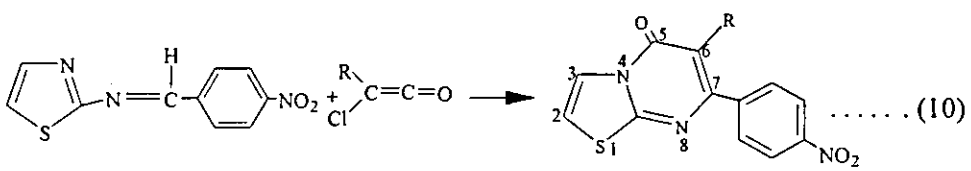
Scheme (4)

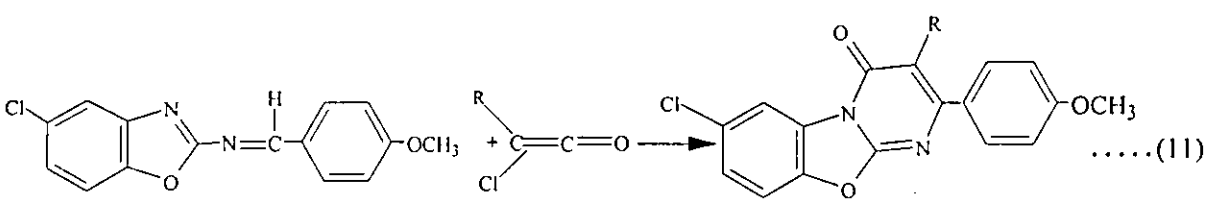
1.2.3.2 Reaction of haloketenes with 1,3-diaza-1,3-butadienes:

Hetero-Diels-Alder reaction of some 1,3-diaza-1,3-butadienes with ketenes leads to the synthesis of some [2+4] cycloadducts in the form of fused pyrimidones, equations (9), (10) and (11).[23,24]

The starting material is prepared by condensation of 2-amino-heterocycles with aromatic aldehydes.[25]

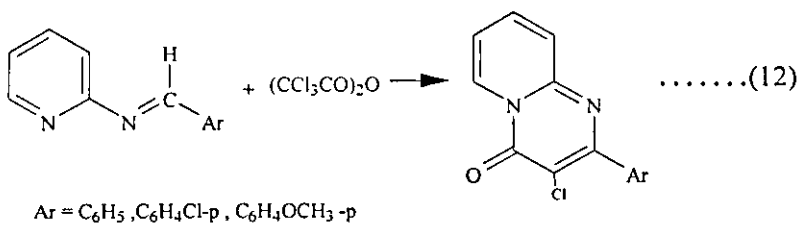






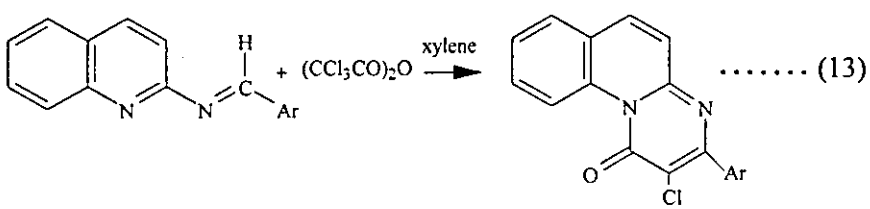
1.2.3.3 Reaction of trichloroacetic anhydride with 2-arylidine amino-heterocycles:

Trichloroacetic anhydride reacts with 2-arylidine amino-heterocycle to give fused pyrimidones, equations (12), (13) and (14).

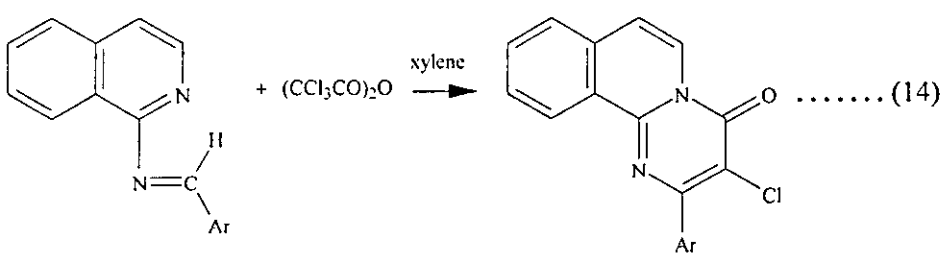


Ar = C_6H_5 , C_6H_4Cl-p , $C_6H_4OCH_3$ -p

.....(12)

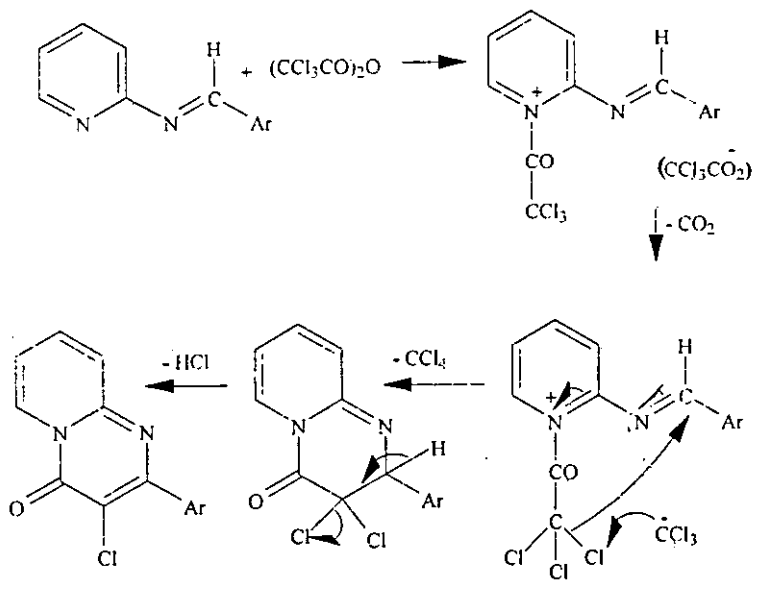


..... (13)



..... (14)

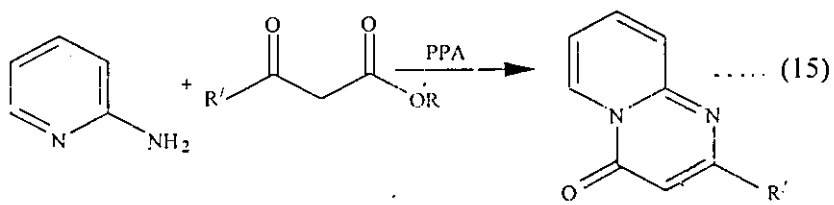
A mechanism has been proposed for this reaction, as shown in Scheme (5).[26]



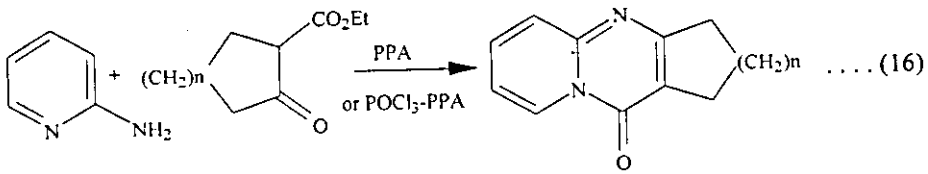
Scheme (5)

1.2.3.4 Reaction of 2-aminopyridine with β-ketoesters:

2-Aminopyridine reacts with β-ketoesters in the presence of poly phosphoric acid (PPA) to give a good yield of fused pyrimidones in only one step, equations (15)[27] and (16).[28,29]



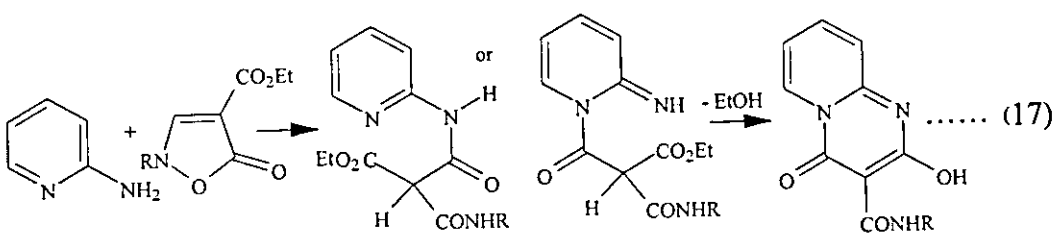
..... (15)



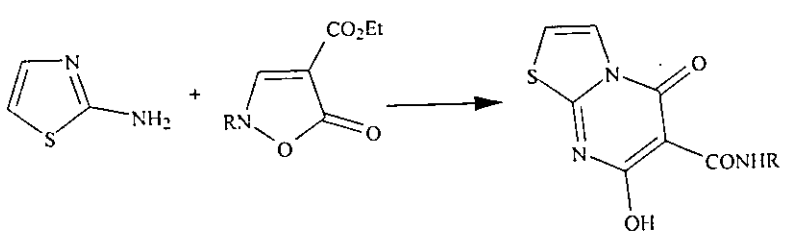
.... (16)

1.2.3.5 Ring cleavage of isoxazoles with heterocyclic amines:

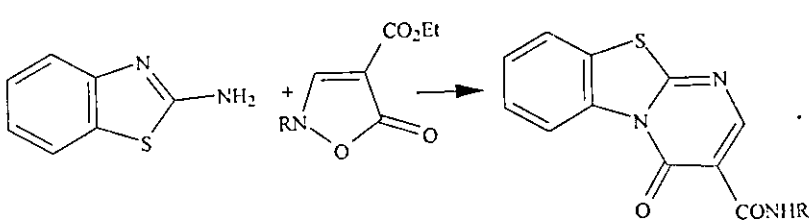
The reactions were performed very easily by heating the two reactants in the absence of a solvent to give the reaction products in fair yields, equations (17),(18) and (19).[30]



..... (17)



.....(18)

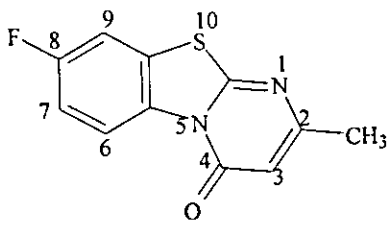


..... (19)

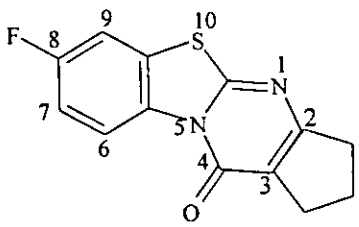
1.3 PURPOSE OF THE PRESENT WORK :

As discussed in the previous sections, we are planning to synthesize a new series of polycyclic heterocycles that may mimic the biological activity of the previously mentioned heterocycles. The present work aims at the synthesis and characterization of the following classes of fused heterocyclic compounds:

I- 2-Substituted and 2,3-disubstituted 8-fluoro-4H-pyrimido[2, 1-b] [1,3]benzothiazol-4-ones:

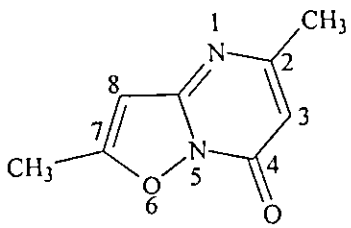


8-Fluoro-2-methyl-4H-pyrimido[2, 1b][1,3] benzothiazol-4-one **(24)**

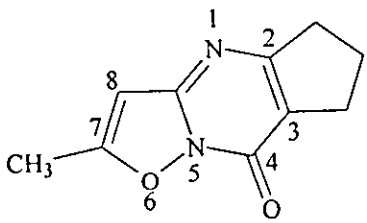


8-Fluoro-2,3-trimethylene-4H-pyrimido [2, 1b][1,3]benzothiazol-4-one **(25)**

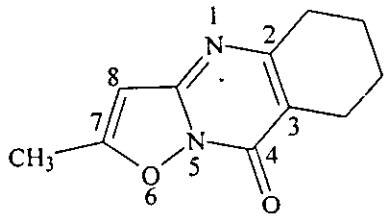
II- 2-Substituted and 2,3- disubstituted 7-methyl-4H-isoxazolo[2, 3-a] pyrimidin-4-ones:



2,7-Dimethyl-4H-isoxazolo- [2, 3-a]pyrimidin-4-one. **(26)**

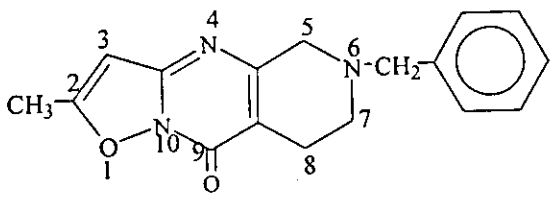


7-Methyl-2,3-trimethylene-4H-isoxazolo- [2, 3-a]pyrimidin-4- one. **(27)**

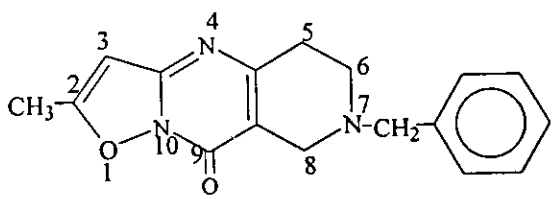


7-Methyl-2,3-tetramethylene-4H-isoxazolo-
[2, 3-a]pyrimidin-4- one. **(28)**

III- 6- And 7- substituted 2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo-[2, 3-a] pyridopyrimidin-9-ones:

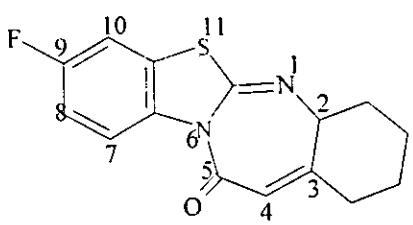


6-Benzyl-2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo
[2, 3-a]pyrido[3, 4-d]pyrimidin-9-one.(**29**)

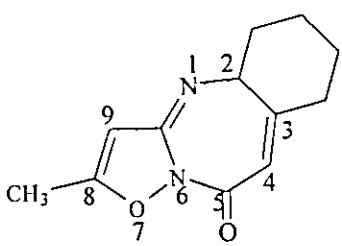


7-Benzyl-2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo
[2, 3-a]pyrido[4, 3-d]pyrimidin-9-one. **30**

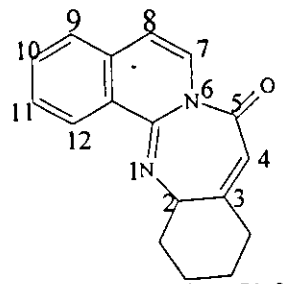
IV- Diazipine compounds:



9-Fluoro-2,3-tetramethylene[1,3]diazipino
[2, 1-b]benzothiazol-5(2H)-one. **31**



8-Methyl-2,3-tetramethyleneisoxazolo
[2, 3-a][1,3]diazipin-5(2H)-one. **32**



2,3-Tetramethylene[1,3]diazipino
[1, 2-a]isoquinolin-5(2H)-one. **33**

The biological activities of some of these compounds against some kinds of bacteria and fungi will be studied. The anti-cancer activities of some of these compounds will also be investigated.

Chapter Two

RESULTS AND DISCUSSION

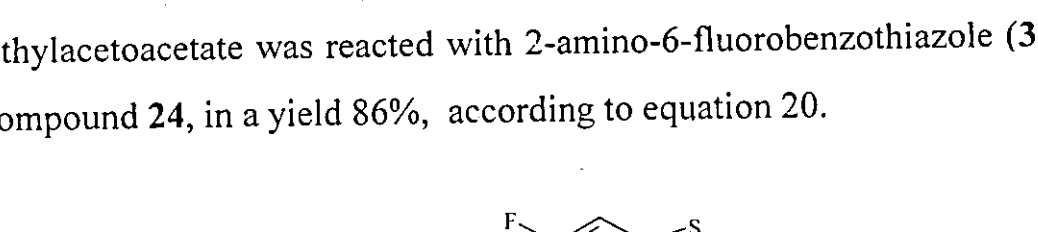
2.1 SYNTHESIS AND CHARACTERIZATION OF THE PREPARED COMPOUNDS:

The compounds **24-30** were prepared by reaction of the corresponding amino-heterocycle derivatives with β-keto ester in the presence of PPA, with continuously stirring for 2 hours at 120 °C.[27]

The diazipine compounds, **31-33**, were prepared by the reaction of the amino-heterocycle with γ-keto ester, ethyl-2-cyclohexanone acetate, in the presence of PPA, with continuous stirring for 1 hour at 70°C, then for 2 hours at 120°C.[31]

2.1.1 8-Fluoro-2-methyl-4H-pyrimido[2, 1-b][1,3] benzo-thiazole-4-one (24):

Ethylacetoacetate was reacted with 2-amino-6-fluorobenzothiazole (**34**) to give compound **24**, in a yield 86%, according to equation 20.



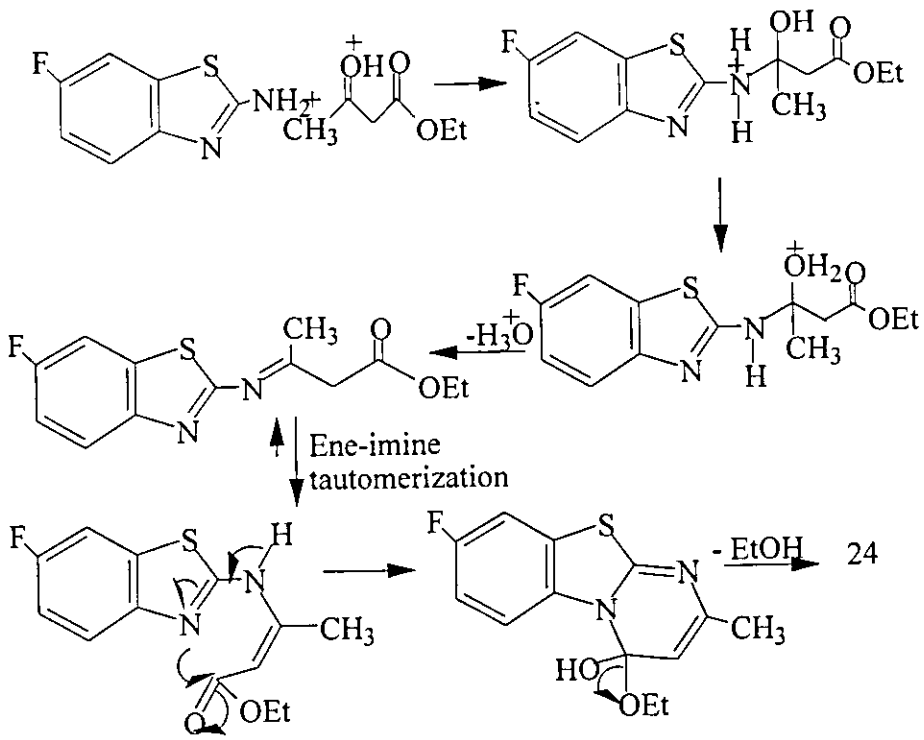
34 24 .....(20)

The pale yellow crystals melted in the range 193-195°C. The elemental analysis data are shown in Table (1).

Table (1): elemental analysis data of compounds **(24-32)**.

Compound number	**Elemental analysis data (%w/w) Found (calculated) values**		
	%C	%H	%N
24	56.56 (56.40)	3.54 (3.02)	11.66 (11.96)
25	60.42 (59.98)	4.63 (3,49)	11.22 (10.77)
26	57.54 (58.52)	5.12 (4.88)	17.35 (17.07)
27	51.01 (63.14)	7.08 (5.31)	12.51 (14.73)
28	61.71 (64.68)	6.39 (5.93)	15.17 (13.72)
29	68.01 (69.12)	6.44 (5.81)	16.62 (14.23)
30	68.55 (69.12)	6.46 (5.81)	15.87 (14.23)
31	53.89 (62.47)	3.81 (4.55)	14.52 (9.72)
32	65.42 (66.03)	6.83 (6.48)	14.54 (12.84)

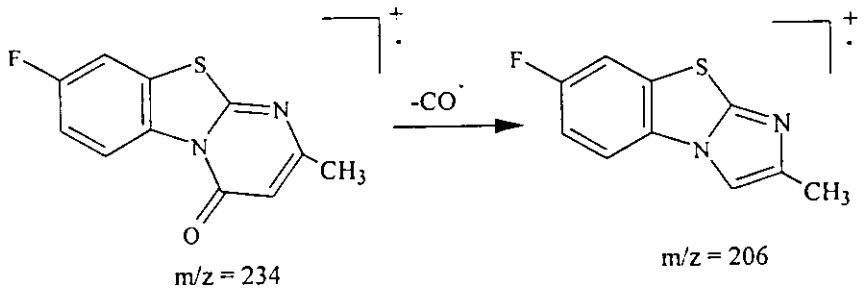
A plausible mechanism has been proposed for the reaction, as shown in Scheme (6).



Scheme (6)

The mechanism involves a condensation of the free amine with the protonated keto group. This is followed by a protonation of the hydroxyl group and hydronium ion elimination. Ene-imine tautomerization, followed by a nucleophilic acyl substitution leads to **24**.

Exact structure elucidation was achieved spectroscopically. The mass spectrum of **24,** (Fig. 1), has a parent molecular ion at m/z 234 and a base peak at m/z 28. The fragment at m/z 206 is due to the expulsion of the carbonyl group that could be best described as shown in Scheme (7).

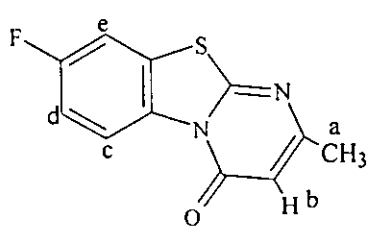


Scheme (7)

The IR spectrum, (Fig. 2), showed a strong absorption at 1681.8 cm^{-1}, due to the (C=O) stretching vibration. The band at 1365.5 cm^{-1} is characteristic for the CH_3. The bands at 1600.8, 1577.7 and 1506.3 cm^{-1} are for the (C=C) stretching frequency.

The reacted ethylacetoacetate is known to have two (C=O) stretching bands. The first at 1725 cm^{-1} for the keto group, and the second at 1800 cm^{-1} for the ester group[32]. The formation of **24** was accompanied by the disappearance of the two stretching bands. These observations are consistent with the proposed structure for **24**.

The 300 MHz ^{1}H-NMR spectrum of **24,** (Fig. 3), indicated the presence of five different types of protons, *a-e*.



24

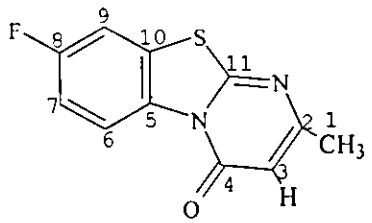
Type (a) includes three protons at 2.38 ppm (s). The signal at 2.62 ppm could not be explained. *Type (b)* includes one proton at 6.26 ppm (s). *Type (c)* includes one proton at 7.22 ppm shielded with respect to the other two aromatic protons. This is due to the presence of the nitrogen atom in the ortho- position, which donates electrons. The splitting of this proton is (dd, J_{Hc-Hd} =8.3 Hz, J_{Hc-F} =7.9 Hz), as shown in Figure (4). The high value of J_{Hc-F} is due to the meta-position of F with respect to H_c, which has special high J value.[33] *Type (d)* includes one proton at 9.05 ppm which is highly deshielded due to the presence of the F atom in the ortho- position. It is also away from S and N atoms.

The splitting of this proton is (dd, J_{Hd-Hc} =8.8 Hz, J_{Hd-F} =4.7 Hz). *Type (e)* includes one proton at 7.39 ppm that is shielded with respect to proton (*d*). This is due to the presence of the S atom, which donates electrons. The *type (e)* proton is deshielded with respect to proton (*c*) due to its ortho- position to the F atom. The splitting of this proton is (d, J_{He-F} =5.8 Hz). The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **24**.

The ^{13}C-NMR spectrum, (Fig. 5), reflected the presence of eleven carbon atoms. The observed chemical shift values are listed in Table (2) together with the calculated values.[34]

Table (2): ^{13}C-NMR observed and calculated chemical shift values of compound **24.**

Carbon-number	1	2	3	4	5	6	7	8	9	10	11
Chemical Shift(ppm)	23.7	159.1	107.3	162.9	125.7	121.4	108.9	161.7	114.5	132.4	160.8
Calculated value(ppm)	22.4	153.2	114.1	163.0	127.0	122.3	112.5	158.0	116.0	137.0	163.0



24

Coupling between C and F atoms was not completely eliminated. Carbons 6, 7, 8, and 9 appeared as doublets. The $J_{^{13}C-F}$ coupling constant values for these carbons are listed in Table (3). For C atoms closer to F atom, the $J_{^{13}C-F}$ value is considerably higher.

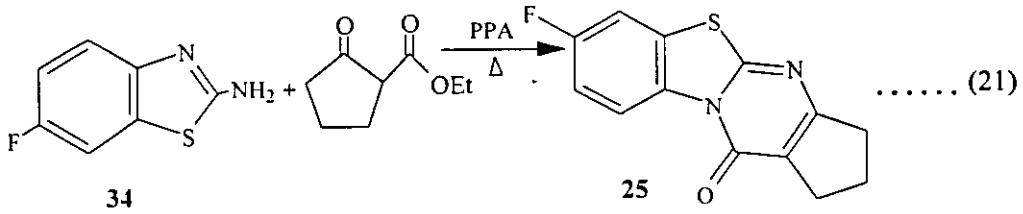
Table (3): The $J_{^{13}C-F}$ coupling constant value for carbons 6-9 in compound **24**.

Carbon number	6	7	8	9
Position with F	meta	ortho	epso	ortho
$J_{^{13}C-F}$ (Hz)	8.6	27.3	106.2	23.3

DEPT spectra of compound **24**, (Figs. 6 and 7), show one methyl carbon and four methine carbons. These data are another evidence for the proposed structure of **24**.

2.1.2 8-Fluoro-2,3-trimethylene-4H-pyrimido [2, 1-b] [1,3]benzothiazole-4-one (25):

Ethyl-2-cyclopentanone carboxylate was reacted with compound **34** to give compound **25**, in a yield 52%, according to equation 21.

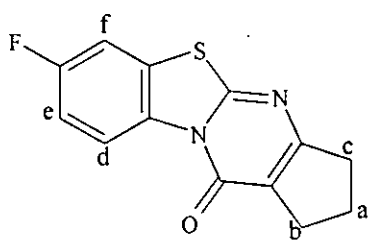


34 25 ..... (21)

The brown crystals melt in the range 150-152°C. The elemental analysis data are shown in Table (1). The proposed mechanism is similar to that shown in Scheme (6). The mass spectrum of compound **25,** (Fig. 8), has a parent molecular ion at m/z 260 and a base peak at m/z 28. The fragment at m/z 232 is due to the expulsion of the carbonyl group.

The IR spectrum, (Fig. 9), showed a strong absorption at 1678.0 cm^{-1}, due to the (C=O) stretching vibration. The band at 1463.4 cm^{-1} is for the methylene groups (C-H bending). The bands at 1579.1 and 1501.5 cm^{-1} are for the (C=C) stretching of the benzene ring. These observations are consistent with the proposed structure for compound **25**.

The 300 MHz ^{1}H-NMR spectrum of **25,** (Fig. 10), indicated the presence of six different types of protons, *a-f*.

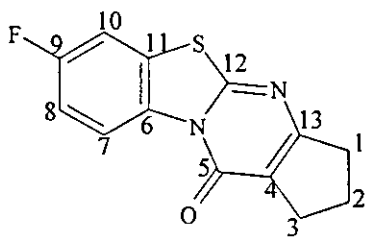


25

Type (a) includes two protons at 2.17 ppm (tt, $J_{Ha-Hb} = J_{Ha-Hc} = 7.4$ Hz), the splitting appears as quintet, (Fig. 11).

Type (b) includes two protons at 2.93 ppm (t, J_{Hb-Ha} = 7.2 Hz). *Type (c)* includes two protons at 2.96 ppm (t, J_{Hc-Ha} = 7.0 Hz). The two triplets overlap together. *Type (d)* includes one proton at 7.00 ppm (dd, J_{Hd-He} = 8.5 Hz, J_{Hd-F} = 7.8 Hz). *Type (e)* includes one proton at 9.12 ppm (dd, J_{He-Hd} = 9.0 Hz, J_{He-F} = 4.7 Hz). *Type (f)* includes one proton at 7.36 ppm (d, J_{Hf-F} = 5.6 Hz).

The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **25**. The 13C-NMR spectrum, (Fig. 12), reflected the presence of thirteen carbon atoms. The observed chemical shift values are listed in Table (4) together with the calculated values.



25

Table (4): ^{13}C-NMR observed and calculated chemical shift values of compound **25.**

Carbon number	1	2	3	4	5	6	7	8	9	10	11	12	13
Chemical Shift(ppm)	34.8	21.7	27.3	132.6	168.1	132.6	121.4	108.9	161.7	114.4	126.1	159.3	159.0
Calculated value(ppm)	35.5	15.0	29.5	127.4	163.0	134.5	122.3	112.5	158.0	116.4	126.0	163.0	149.7

Coupling between C and F atoms was not completely eliminated. Carbons 6, 7, 8, 9,10 and 11 appeared as doublets. The $J_{^{13}C-F}$ coupling constant values for these carbons are listed in Table (5). For C atoms closer to F atom, the $J_{^{13}C-F}$ value is considerably higher.

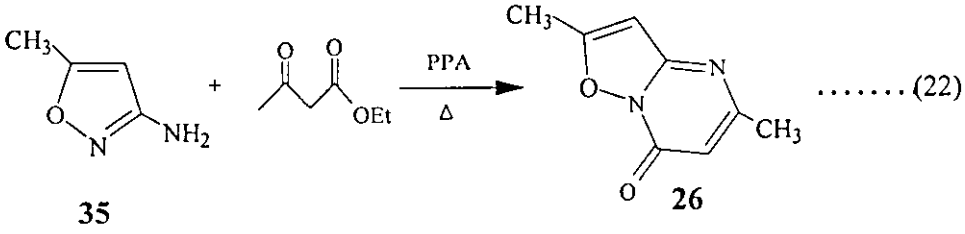
Table (5):The $J_{^{13}C-F}$ coupling constant values for carbons 6-11 in compound **25**.

Carbon-number	6	7	8	9	10	11
Position with F	para	meta	ortho	epso	ortho	meta
$J_{^{13}C-F}$ (Hz)	2.3	8.6	27.3	96.5	23.3	10.2

DEPT spectra of compound **25**, (Figs. 13 and 14), show three methine carbons and three methylene carbons. These data are another evidence for the proposed spectrum of **25**.

2.1.3 2,7-Dimethyl-4H-isoxazolo[2, 3-a] pyrimidin-4-one (26):

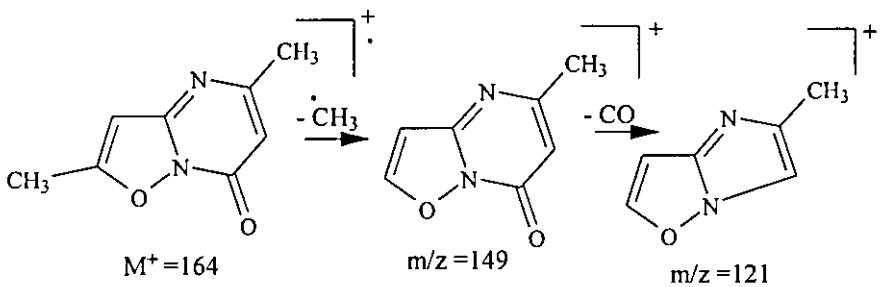
Ethylacetoacetate was reacted with 3-amino-5-methylisoxazole (**35**) to give compound **26**, in a yield 36%, according to equation 22.



35 26 .....(22)

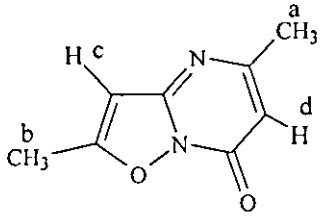
The white crystals melt in the range 176-178°C. The elemental analysis data are shown in Table (1). The proposed mechanism is similar to that shown in Scheme (6).

The mass spectrum of compound **26,** (Fig. 15), has a parent molecular ion at m/z 164 and a base peak at m/z 28. The fragment at m/z 121 is due to the expulsion of the carbonyl and the methyl groups as shown in Scheme (8).



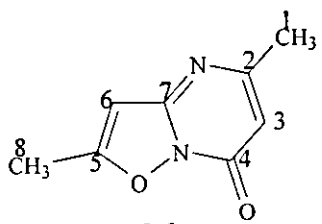
Scheme (8)

The IR spectrum, (Fig. 16), showed a strong absorption at 1689.5 cm^{-1}, due to the (C=O) stretching vibration. The band at 1371.3 cm^{-1} is characteristic for the methyl groups. The band at 1625.9 cm^{-1} is for the (C=C) stretching vibration. These observations are consistent with the proposed structure for **26.** The 300 MHz ^{1}H-NMR spectrum of **26,** (Fig.17), indicated the presence of four different types of protons, *a-d.*



26

Type (a) includes three protons at 2.38 (s). *Type (b)* includes three protons at 2.57 ppm (s). *Type (c)* includes one proton at 6.15 ppm (s). *Type (d)* includes one proton at 6.29 ppm (s). The ^{1}H-NMR spectral data were thus conclusive in determining the structure of compound **26**. The ^{13}C-NMR spectrum, (Fig. 18), reflected the presence of eight carbon atoms. The observed chemical shift values are listed in Table (6) together with the calculated values.



26

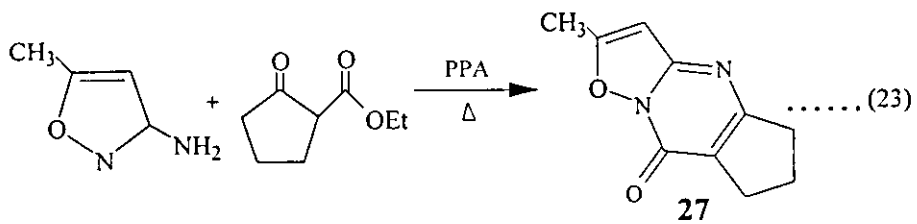
Table (6): ^{13}C-NMR observed and calculated chemical shift values of compound **26.**

Carbon number	1	2	3	4	5	6	7	8
Chemical Shift(ppm)	24.2	153.4	105.3	165.5	153.6	99.5	166.5	12.8
Calculated value(ppm)	23.2	153.2	114.1	161.0	160.0	81.0	164.0	20.7

DEPT spectrum, (Fig. 19), shows two methyl carbons and two methine carbons. These data are another evidence for the proposed spectrum of compound **26**.

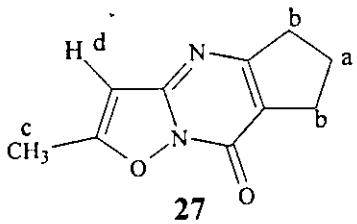
2.1.4 7-Methyl-2,3-trimethylene-4H-isoxazolo[2, 3-a] pyrimidin-4-one (27):

Ethyl-2-cyclopentanone carboxylate was reacted with compound **35** to give compound **27**, in a yield 87%, according to equation 23.



The brown crystals melt in the range 147-150°C. The elemental analysis data are shown in Table (1). The proposed mechanism is similar to that shown in Scheme (6). The mass spectrum of **27,** (Fig. 20), has a parent molecular ion at m/z 190 and a base peak at m/z 28. The fragment at m/z 147 is due to the expulsion of the carbonyl and the methyl groups.

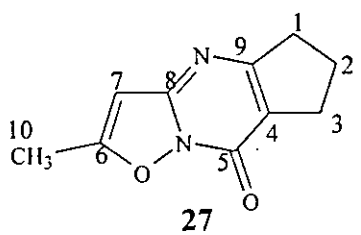
The IR spectrum (Fig. 21), showed a strong absorption at 1676.0 cm^{-1}, due to the (C=O) stretching vibration. The band at 1355.9 cm^{-1} is for the methyl group (C-H bending). The bands at 1421.4 and 1438.8 cm^{-1} are for the methylene groups (C-H bending). The band at 1622.0 cm^{-1} is for the (C=C) stretching. These observations are consistent with the proposed structure for compound **27**. The 300 MHz ^{1}H-NMR spectrum of **27,** (Fig.22), indicated the presence of four different types of protons, *a-d*.



27

Type (a) includes two protons at 2.16 (quintet, $J_{Ha-Hb} = 7.5$ Hz). *Type (b)* includes four protons at 2.92 (t, $J_{Hb-Ha} = 7.5$ Hz). *Type (c)* includes three protons at 2.57 ppm (s). *Type (d)* includes one proton at 6.31ppm (s). The ^{1}H-NMR spectral data were thus conclusive in determining the structure of compound **27**.

The ^{13}C-NMR spectrum, (Fig. 23), reflected the presence of ten carbon atoms. The observed chemical shift values are listed in Table (7) together with the calculated values.



27

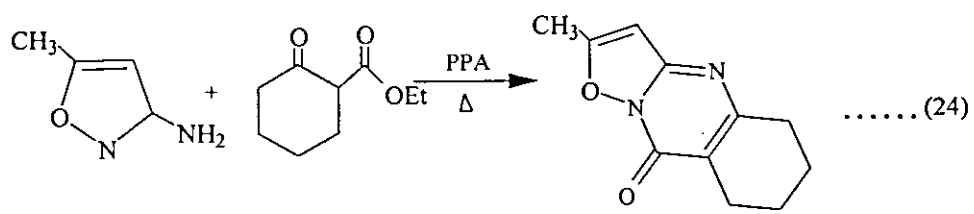
Table (7): ^{13}C-NMR observed and calculated chemical shift values of compound **27.**

Carbon number	1	2	3	4	5	6	7	8	9	10
Chemical Shift(ppm)	35.0	22.4	27.4	117.6	154.0	166.1	99.4	170.4	152.1	12.9
Calculated value(ppm)	36.3	15.0	29.9	127.4	161.0	160.0	81.0	164.0	149.7	20.7

DEPT spectra, (Figs. 24 and 25), show one methyl carbon, one methine carbon and three methylene carbons. These data are another evidence for the proposed spectrum of **27**.

2.1.5 7-Methyl-2,3-tetramethylene-4H-isoxazolo[2, 3-a] pyrimidin-4-one (28):

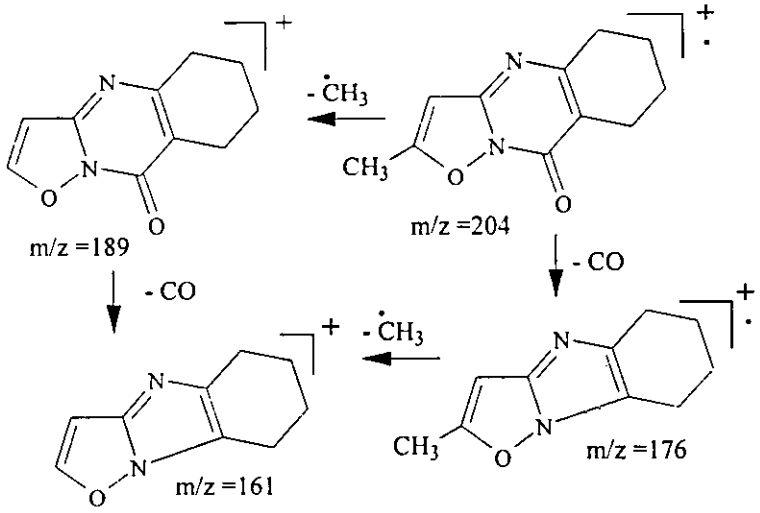
Ethyl-2-cyclohexanone carboxylate was reacted with compound **35** to give compound **28**, in a yield 83%, according to equation 24.



35 28 .....(24)

The pale yellow crystals melt in the range 98-100°C. The elemental analysis data are shown in Table (1). The proposed mechanism is similar to that shown in Scheme (6).

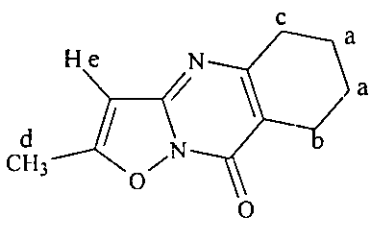
The mass spectrum of **28,** (Fig. 26), has a parent molecular ion at m/z 204 and a base peak at m/z 28. The fragment at m/z 189 is due to the expulsion of the methyl group. The fragment at m/z 176 is due to the expulsion of the carbonyl group, and that at m/z 161 is due to the expulsion of the methyl group from the 176 fragment, as shown in Scheme (9).



Scheme (9)

The IR spectrum, (Fig. 27), showed a strong absorption at 1670.2 cm^{-1}, due to the (C=O) stretching vibration. The band at 1384.8 cm^{-1} is characteristic for the CH_3. The band at 1637.5 cm^{-1} is for the (C=C) stretching. These observations are consistent with the proposed structure of **27**.

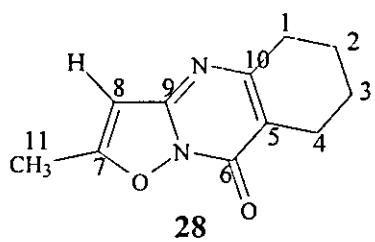
The 300 MHz ^{1}H-NMR spectrum of **28,** (Fig.28), indicated the presence of five different types of protons, *a-e*.



28

Type (a) includes four protons at 1.81 ppm (multiplet, which is not clear). *Type (b)* includes two protons at 2.65 ppm (distorted triplet, J = 5.8). *Type (c)* includes two protons at 2.71 ppm (distorted triplet, J = 5.9). *Type (d)* includes three proton at 2.54 ppm (br. s). *Type (e)* includes one proton at 6.23 ppm (s).

The ^{1}H-NMR spectral data were thus conclusive in determining the structure of compound **28**. The ^{13}C-NMR spectrum, (Fig. 29), reflected the presence of eleven carbon atoms. The observed chemical shift values are listed in Table (8) together with the calculated values.



28

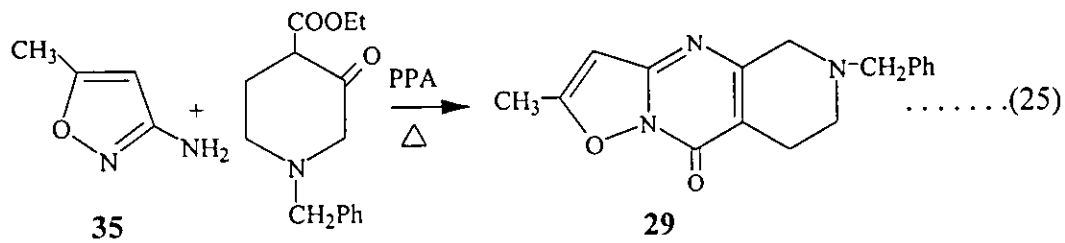
Table (8): ^{13}C-NMR observed and calculated chemical shift values of compound **28.**

Carbon number	1	2	3	4	5	6	7	8	9	10	11
Chemical Shift(ppm)	23.2	22.5	21.8	22.3	115.1	161.3	153.8	99.2	165.3	150.8	12.7
Calculated value(ppm)	33.8	27.2	29.0	27.0	129.9	161.0	160.0	81.0	164.0	152.2	20.7

DEPT spectra, (Figs. 30 and 31), show one methyl carbon, one methine carbon, and four methylene carbons. These data are another evidence for the proposed spectrum of **28**.

2.1.6 6-Benzyl-2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo [2, 3-a] pyrido[3, 4-d] pyrimidin-9-one (29):

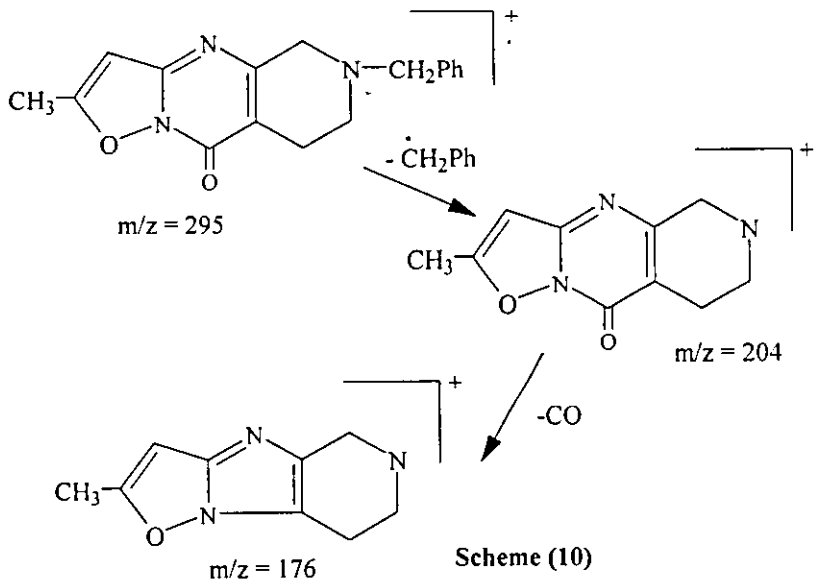
Ethyl-1-benzyl-3-oxo-4-pipyridinecarboxylate was reacted with compound **35** to give compound **29**, in a yield 53%, according to equation 25.



35 **29** .....(25)

The brown crystals melt in the range 142-144°C. The elemental analysis data are shown in Table (1). The proposed mechanism is similar to that shown in Scheme (6).

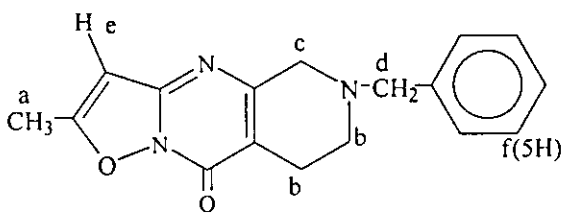
The mass spectrum of **29,** (Fig. 32), has a parent molecular ion at m/z 295 and a base peak at m/z 28. The fragment at m/z 176 is due to the expulsion of the benzyl and the carbonyl groups. The fragment at m/z 91 is the benzyl ion, The fragmentation pattern is shown in Scheme (10).



Scheme (10)

The IR spectrum, (Fig. 33), showed a strong absorption at 1679.9 cm^{-1}, due to the (C=O) stretching vibration. The band at 1382.9 cm^{-1} is characteristic for the CH_3. The band at 1624.0 cm^{-1} is for the (C=C) stretching. The bands at 1587.3 and 1541.0 cm^{-1} are for the (C=C) stretching of the phenyl group.These observations are consistent with the proposed structure of **29**.

The 300 MHz ^{1}H-NMR spectrum of **29,** (Fig.34), indicated the presence of six different types of protons, *a-f.*



29

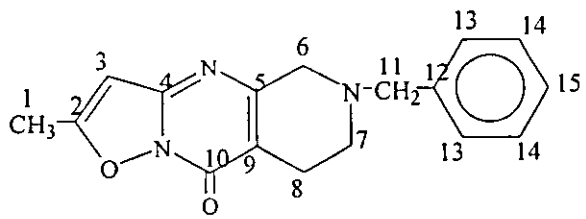
Type (a) includes three protons at 2.5 ppm (s). *Type (b)* includes four protons at 2.75 ppm (br.s). *Type (c)* includes two protons at 3.50 ppm (s). *Type (d)* includes two proton at 3.70 ppm (s). *Type (e)* includes one proton at 6.19 ppm (br.s). *Type (f)* includes five aromatic protons at 7.23 ppm (m). The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **29**.

The ^{13}C-NMR spectrum, (Fig. 35), reflected the presence of seventeen carbon atoms. The observed chemical shift values are listed in Table (9) together with the calculated values.

Table (9): ^{13}C-NMR observed and calculated chemical shift values of compound **29.**

Carbon number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chemical Shift(ppm)	12.7	153.4	99.2	165.6	151.5	57.2	49.3	22.7	112.9	159.0	62.2	137.5	129.1	128.3	127.3
Calculated value(ppm)	20.7	160.0	81.0	164.0	146.6	57.3	52.5	26.7	127.1	161.0	59.5	136.3	129.0	128.2	127.0

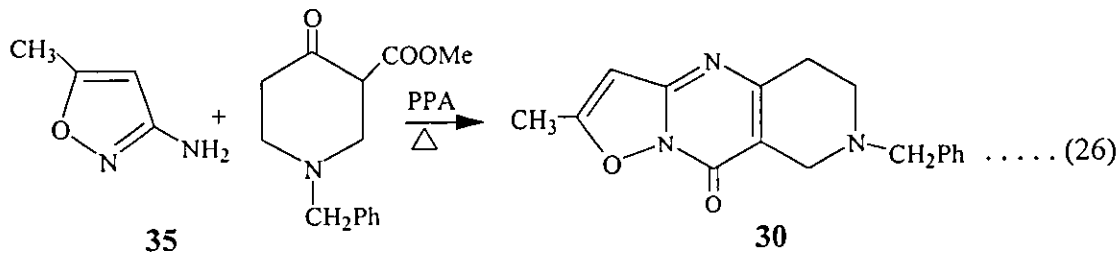
DEPT spectra of compound **29**, (Figs. 36 and 37), show one methyl carbon, four methine carbons and four methylene carbons. These data are another evidence for the proposed spectrum of **29**.



29

2.1.7 7-Benzyl-2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo [2, 3-a] pyrido[4, 3-d] pyrimidin-9-one (30):

Methyl-1-benzyl-4-oxo-3-pipyridinecarboxylate was reacted with compound **35** to give compound **30**, in a yield 35%, according to equation 26.

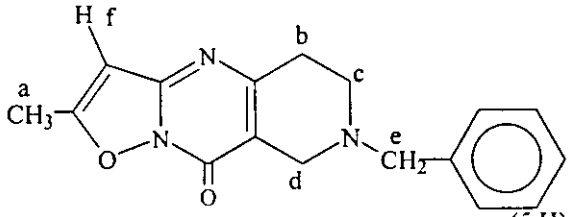


35 30 ..... (26)

The yellow crystals melt at 176-177°C, the elemental analysis data are shown in Table (1). The proposed mechanism is similar to that shown in Scheme (6). The mass spectrum of **30,** (Fig. 38), has a parent molecular ion at m/z 295 and a base peak at m/z 28. The same fragmentation pattern as of compound **29** with one exception that the fragment at m/z 204 is more intense in compound **30**.

The IR spectrum, (Fig. 39), showed a strong absorption at 1666.4 cm^{1}, due to the (C=O) stretching vibration. The band at 1359.7 cm^{-1} is characteristic for the CH_3. The band at 1620.1 cm^{-1} is for the (C=C) stretching. The bands at 1581.5 and 1529.4 cm^{-1} are for the (C=C) stretching of the phenyl group.These observations are consistent with the proposed structure of **30**.

The 300 MHz ^{1}H-NMR spectrum of **30,** (Fig.40), indicated the presence of seven different types of protons, *a-g*.



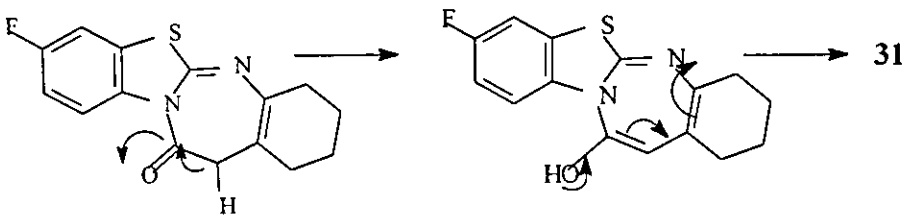
30

Type (a) includes three protons at 2.52 ppm (s). *Type (b)* includes two protons at 2.75 ppm (t, J_{Hb-Hc} = 5.2 Hz). *Type (c)* includes two protons at 2.82 ppm (t, J_{Hc-Hb} = 5.0 Hz). *Type (d)* includes two proton at 3.61 ppm (s). *Type (e)* includes two protons at 3.74 ppm (s). *Type (f)* includes one proton at 6.22 ppm (s). *Type (g)* includes five aromatic protons at 7.35 ppm (m).

The four protons that appeared as singlet in compound **29**, split into two peaks in compound **30**. The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **30**.

The ^{13}C-NMR spectrum, (Fig. 41), reflected the presence of seventeen carbon atoms. The observed chemical shift values are listed in Table (10) together with the calculated values.

The pale yellow crystals melt in the range 158-160°C. The elemental analysis data are shown in Table (1). Scheme (6) explains the proposed mechanism of the formation of compound (**36**), which rearranges to compound (**31**) as shown in Scheme (11).



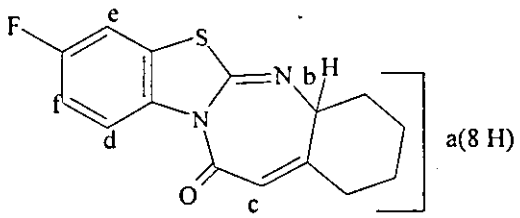
36

Scheme(11)

The mass spectrum of **31,** (Fig. 44), has a parent molecular ion at m/z 288 and a base peak at m/z 28. The fragment at m/z 260 is due to the expulsion of the carbonyl group.

The IR spectrum, (Fig. 45), showed a strong absorption at 1687.6 cm^{-1}, due to the (C=O) stretching vibration. The band at 1458.1 cm^{-1} is for the methylene groups (C-H bending). The band at 1635.5 cm^{-1} is for the (C=C) stretching of the seven membered ring. The bands at 1606.6 and 1541.0 cm^{-1} are for the (C=C) stretching of the benzene ring. These observations are consistent with the proposed structure of **31**.

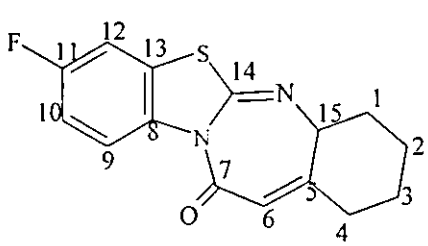
The 300 MHz ^{1}H-NMR spectrum of **31,** (Fig.46), indicated the presence of six different kinds of protons, *a-f*.



31

Type (a) includes eight protons in the range 1.15-3.35 ppm (m). *Type (b)* includes one proton at 4.70 ppm (dd, J = 5.70, 11.0 Hz). *Type (c)* includes one proton at 5.92 ppm (br.s). *Type (d)* includes one proton at 7.13 ppm (dd, J_{Hd-Hf} = 8.2 Hz, J_{Hd-F} = 8.9 Hz). *Type (e)* includes one proton at 7.49 ppm (d, J_{Hf-F} = 5.8 Hz). *Type (f)* includes one proton at 7.72 ppm (dd, J_{Hf-Hd} = 8.6 Hz, J_{Hf-F} = 4.6 Hz).

The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **31**. The ^{13}C-NMR spectrum, (Fig. 47), reflected the presence of fifteen carbon atoms. The observed chemical shift values are listed in Table (11) together with the calculated values.



31

Table (11): ^{13}C-NMR observed and calculated chemical shift values of compound **31.**

Carbon number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chemical Shift(ppm)	34.1	23.1	28.0	29.1	145.6	116.9	169.7	133.0	121.7	107.5	156.9	114.1	119.7	166.9	63.9
Calculated value(ppm)	35.1	28.0	29.7	34.8	162.6	119.8	169.5	137.2	114.1	112.7	150.7	114.4	121.0	163.7	56.0

Coupling between C and F atoms was not completely eliminated. Carbons 8, 9, 10, 11, 12 and 13 appeared as doublets. The $J_{^{13}C-F}$ coupling constant values for these carbons are listed in Table (12). For C atoms closer to F atom, the $J_{^{13}C-F}$ value is considerably higher, carbon (13) being an exception.

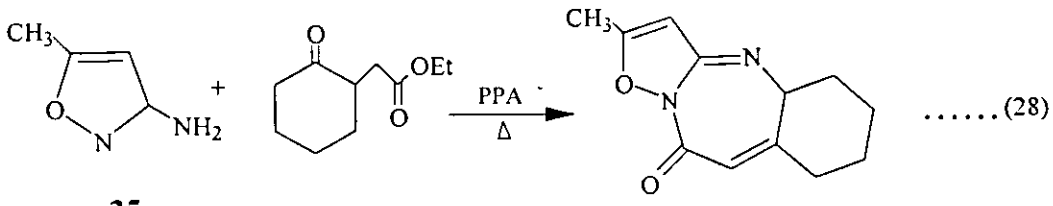
Table (12): The $J_{^{13}C-F}$ coupling constant values for carbons 8-13 in compound **31.**

Carbon-number	8	9	10	11	12	13
Position with F	para	meta	ortho	epso	ortho	meta
$J_{^{13}C-F}$ (Hz)	10.9	8.3	26.6	135.7	24.5	7.5

DEPT spectrum, (Fig. 48), shows five methine carbons and four methylene carbons. These data are another evidence for the proposed spectrum of compound **31.**

2.1.9 8-Methyl-2,3- tetramethyleneisoxazolo[2, 3-a][1,3] diazipin-5(2H)-one (**32**):

Ethyl-2-cyclohexanone acetate was reacted with compound **35** to give compound **32**, in a yield 54.5%, according to equation (28).



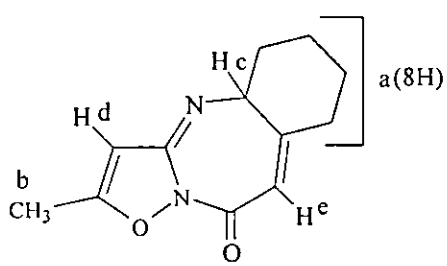
35 32 .....(28)

The brown crystals melt in the range 87-90°C. The elemental analysis data are shown in Table (1). Scheme (6) and (11) above illustrate the proposed mechanism of the reaction.

The mass spectrum of **32,** (Fig. 49), has a parent molecular ion at m/z 218 and a base peak at m/z 28. The fragment at m/z 190 is due to the expulsion of the carbonyl group. The fragment at m/z 175 is due to the expulsion of the methyl group from the 190 fragment.

The IR spectrum, (Fig. 50), showed a strong absorption at 1697.2 cm^{-1}, due to the (C=O) stretching vibration. The band at 1456.2 cm^{-1} is for the methylene groups (C-H bending). The bands at 1654.8 and 1635.5 cm^{-1} are for the (C=C) stretching. These observations are consistent with the proposed structure of **32**.

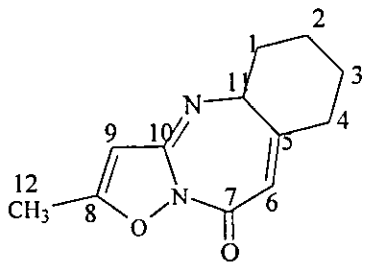
The 300 MHz ^{1}H-NMR spectrum of **32,** (Fig.51), indicated the presence of five different types of protons, *a-e*.



32

Type (a) includes eight protons in the range 1.12-3.25 ppm (m). *Type (b)* includes three protons at 2.40 ppm (s). *Type (c)* includes one proton at 4.39 ppm (dd, J = 3.4, 10.7 Hz). *Type (d)* includes one proton at 5.83 ppm (s). *Type (e)* includes one proton at 6.84 ppm (s).

The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **32**. The ^{13}C-NMR spectrum, (Fig. 52), reflected the presence of twelve carbon atoms. The observed chemical shift values are listed in Table (13) together with the calculated values.



32

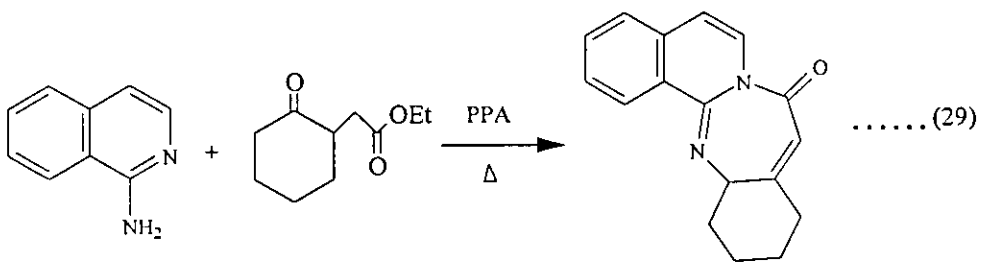
Table (13): ^{13}C-NMR observed and calculated chemical shift values of compound **32.**

Carbon number	1	2	3	4	5	6	7	8	9	10	11	12
Chemical Shift(ppm)	33.8	28.0	23.1	28.8	157.4	117.6	165.5	169.5	95.2	169.8	62.2	12.6
Calculated value(ppm)	35.1	28.0	29.3	34.3	163.7	113.0	160.0	160.0	81.0	164.0	55.7	20.7

DEPT spectra of compound **32**, (Figs. 53 and 54), show one methyl carbon, three methine carbons and four methylene carbons.These data are another evidence for the proposed spectrum of **32**.

2.1.10 2,3-Tetramethylene[1,3]diazipino[1,2-a]isoquinolin – 5(2H)-one (33):

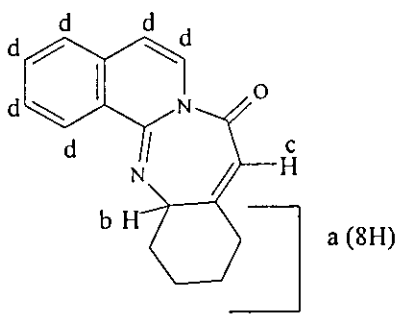
Ethyl-2-cyclohexanone acetate was reacted with 1-aminoisoquinoline (**37**) to give compound **33**, in a yield 97.8%, according to equation (29).



37 **33** .....(29)

The brown crystals melt in the range 190-192°C. The proposed mechanism of the reaction is similar to that shown in Schemes (6) and (11) above. No sufficient elemental analysis data, GC MS, or FT IR spectra were obtained.

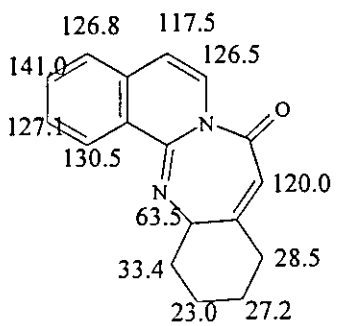
The 250 MHz ^{1}H-NMR spectrum of **33,** (Fig.55), indicated the presence of four different types of protons, *a-d.*



33

Type (a) includes eight protons in the range 0.78-2.31 ppm (m). *Type (b)* includes one proton at 4.98 ppm (dd, J = 5.0, 10.0 Hz). *Type (c)* includes one proton at 5.82 ppm (s). *Type (d)* includes six protons in the range 7.19-8.38 ppm (m). The ^{1}H-NMR spectral data were thus conclusive in determining the structure of **33**.

GASPE spectrum, (Fig. 56), shows eight methine carbons and four methylene carbons. The observed chemical shift values for ^{13}C-NMR spectrum are shown below.



33

These data are another evidence for the proposed spectrum of compound **33**.

2.2 BIOLOGICAL ACTIVITY

Some of the prepared compounds were tested as antibacterial, antifungal and anticancer compounds.

2.2.1 Antibacterial and antifungal activity:

Three of the prepared compounds were tested against some types of bacteria and fungi.[35] The compounds tested were : 8-fluoro-2-methyl-4H-pyrimido[2, 1-b] benzothiazol-4-one **(24)**, 7-methyl-2,3-trimethylene-4H-isoxazolo[2, 3-a] pyrimidin-4-one **(27)** and 7-methyl-2,3-tetramethylene-4H-isoxazolo- [2,3a] pyrimidin-4-one **(28)**.

The antibacterial activity was evaluated by the Disk Diffusion Method [36], the compounds were tested against *Staphylococcus aureus*, *proteus vulgaris*, and *candida albicans*. None of the tested compounds showed antibacterial activity.

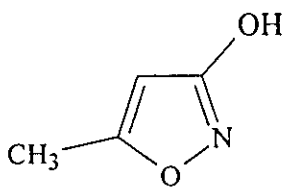
The antifungal activity of the same three compounds was evaluated by the Poisoned Food Technique [37]; the compounds were tested against *Microsporum canis, Fusiarium tricincutum, Pythium ultimum, Pythium aphanidermatum,* and *Pythium middletonii.* The results in the form of % inhibition are listed in Table (14).

Table (14): Antifungal activity results of compounds **24**, **27** and **28** against different fungi types.

Compound number	% Inhibition against different fungi types.				
	M. canis [a]	F. tricincutum [b]	P. ultimum [c]	P. aphanider-matum [d]	P. middletonii [e]
24	100	67	81	95	45
27	92	42	100	100	80
28	100	50	92	100	80

The % inhibition for different reference antibiotics used for different fungi types were:
a) griseofulvin 77%, b) nystatin 67%, c) hymexazole 81%, d) hymexazole 70 %,
e) hymexazole 25%.

Hymexazole antibiotic [38], is the compound 3-hydroxy-5-methylisoxazole **38**. Its structure resembles the structure of the starting material of compounds **27** and **28**.



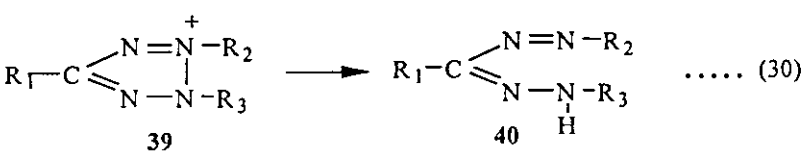
38

Unfortunately, we could not find any relationship between the % inhibition of the compound and its structure.

2.2.2 Anticancer activity:

Three of the prepared compounds were tested as anticancer compounds by using the MTT assay.[39] The MTT is a tetrazolium salt; for which the general formula is (**39**), which can be transformed into the blue colored formazan (**40**) through the mitochondria enzyme dehydrogenase[40].

The greater the number of mitochondria the more MTT will be transformed into formazan, according to equation (30).



39 40 ..... (30)

The formazans are blue crystals that are difficult to be dissolved. Prior to measurement, they are dissolved in isopropanol. The solubilized formazan reagents are measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The compounds tested are: 7-methyl-2,3-trimethylene-4H-isoxazolo[2,3-a] pyrimidin-4-one (**27**), 8-methyl-2,3-tetramethyleneisoxazolo[2, 3-a][1,3] diazipin-5(2H)-one (**32**) and 2,3- tetramethylene [1,3] diazipino[1, 2-a] isoquinolin–5(2H)-one (**33**).

The results of the MTT experiments are shown in Table (15). From the Table below, it is found that compound **33** has the most anticancer effect (96%) at concentration 3.00×10^{-3} M.

Compound **27** has the most anticancer effect (41.2%) at concentration 2.90×10^{-3} M. Finally, compound **32** has the most anticancer effect (39.2%) at concentrations 3.13×10^{-4} and 6.25×10^{-4} M.

Table (15): MTT assay results of compounds **27**, **32** and **33**.

Compound number	Molarity of stock solution	Final concentration (M)	Absorbance at λ_{max} (630 nm)	% Mortality of cells
27	6.10×10^{-2}	2.90×10^{-3}	0.030	41.20
		1.50×10^{-3}	0.039	23.50
		7.30×10^{-4}	0.043	15.70
		3.60×10^{-4}	0.050	1.96
32	5.25×10^{-2}	1.25×10^{-3}	0.040	12.60
		6.25×10^{-4}	0.031	39.20
		3.13×10^{-4}	0.031	39.20
33	6.30×10^{-2}	3.00×10^{-3}	0.002	96.00
		1.50×10^{-3}	0.003	94.10
		7.50×10^{-4}	0.009	82.40
		3.70×10^{-4}	0.028	45.10
		1.90×10^{-4}	0.039	22.90

Absorbance for the reference was 0.051 at 550 nm.

2.3 CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

1- Compounds **24-33** have been prepared. Compounds **26** and **30** have been synthesized with low yield (36 and 35% respectively). The other eight compounds have been prepared with a yield reaching 97.8% for compound **33**.

2- The new compounds have been characterized using elemental analysis, GC MS, FT IR and NMR spectrometry. The spectral data confirm the proposed structures of the compounds.

3- Compounds **24, 27** and **28** were tested against *Staphylococcus aureus, proteus vulgaris,* and *candida albicans* bacteria. None of the three compounds showed antibacterial activity. The same compounds were tested against *Microsporum canis, Fusiarium tricincutum, Pythium ultimum, Pythium aphanidermatum,* and *Pythium middletonii fungi* types. All of them showed antifungal activity.

4- Compounds **27, 32** and **33** were tested for anticancer activity by the MTT assay. They all showed significant anticancer activity. Compound **33** being the most efficient (96%).

5- Testing the other compounds for antibacterial, antifungal and anticancer activities is suggested for further work.

Chapter Three

EXPERIMENTAL

3.1 INSTRUMENTS:

3.1.1 Electrothermal Melting Temperature Apparatus was used for measuring the melting points.

3.1.2 Elemental analysis data were measured on Perkin Elmer PE 2400 series II , CHNS/O analyzer.

3.1.3 FT-IR spectra were measured on a Shimadzu 820 PC FT-IR Spectrometer.

3.1.4 Mass spectra and gas chromatography results were recorded on a Shimadzu GC MS-QP5000, using chloroform and methanol as solvents.

The GC runs were conducted using a J&W Scientific DB-5MS (5% phenyl methyl polysilane, stationary phase) 30.00 m long and was 0.25 mm in diameter, capillary column. Helium carrier gas was used at flow rate of 28.2 ml/min.

The injector was kept at 250°C, and the interface temperature was 230°C. The oven temperature was initially set at 120 °C, the ram rate was 5°C/min reaching a final temperature of 300°C, with a 0.5 min waiting time interval at each stage.

3.1.5 ^{13}C-NMR spectra were recorded on a Bruker 75.48 MHz, using $CDCl_3$ as a solvent. Its characteristic signal was observed as triplet at 77.1 ppm.

3.1.6 ^{1}H-NMR spectra were recorded on a Bruker 300 MHz, using $CDCl_3$ as a solvent.

3.2 CHEMICALS:

Starting materials, 2-amino-6-fluorobenzothiazole, 1-aminoisoquinoline, 3-amino-5-methylisoxazole, ethylacetoacetate, ethyl-2-oxocyclopentane carboxylate, ethyl-2-oxocyclohexanecarboxylate, ethyl-2-cyclohexanone acetate, methyl-1-benzyl-4-oxo-3-pipyridinecarboxylate hydrochloride, ethyl-1-benzyl-3-oxo-4-pipyridine carboxylate hydrochloride, and PPA were purchased from Aldrich Chemical Company Ltd., and were used as received.

Solvents, such as chloroform, petroleum ether, diethylether, ethylacetate and ethanol, were purchased from Aldrich Chemical Company Ltd., and were used as received.

3.3 EXPERIMENTS:

3.3.1 Preparation of compounds 24-33:

The general experimental procedure for the preparation of compounds **24** to **33** was as follows: Polyphosphoric acid (PPA), 3-6 gm, was weighed in a 50-mL conical flask. The aminopyridine derivative (0.5 gm) was added. The β-, or γ- keto ester was then added as excess (the molar ratio of the keto ester / amino heterocycle was 1.1 /1 respectively). The mixture was heated, while manually mixing, in an oil bath for 20 minutes until a 120°C final temperature was reached. The mixture was kept at this temperature for 2 more hours while mixing. The reaction was monitored periodically by thin layer chromatography (TLC) using 5% ethylacetate chloroform developing solvent. After completion,

the reaction was cooled in an ice bath. Ice water was added to the mixture. The solution was then neutralized by NaOH (4N) solution. The precipitate product was filtered on a sintered funnel and washed with distilled water. Non-precipitate products were alternatively extracted.

3.3.1.1 Preparation of 8-fluoro-2-methyl-4H-pyrimido-[2, 1-b][1,3]benzothiazol-4-one, (24):

As described in the general procedure, 2-amino-6-fluorobenzothiazole (0.5 gm, 2.97 mmole) was mixed with ethylacetoacetate (0.4 gm, 3.08 mmole) and 3 gm PPA. The yellow precipitate was recrystalized from ethanol (m.p. range was 193-195).The yield was 0.6 gm, 86%.

3.3.1.2 Preparation of 8-fluoro-2,3-trimethylene-4H-pyrimido-[2, 1- b][1,3]benzothiazol-4-one, (25):

As described in the general procedure, 2-amino-6-fluorobenzothiazole (0.5 gm, 2.97 mmole) was mixed with ethyl-2-oxocyclopentane carboxylate (0.51 gm, 3.27 mmole) and 3 gm PPA, the brown precipitate was recrystalized from ethanol (m.p. range was 150-152). The yield was 0.4 gm, 52%.

3.3.1.3 Preparation of 2,7-dimethyl-4H-isoxazolo[2, 3-a] pyrimidin-4-one, (26):

As described in the general procedure, 3-amino-5-methylisoxazole (0.5 gm, 5.10 mmole) was mixed with ethylacetoacetate (0.73 gm, 5.6 mmole) and 3

gm PPA. After neutralization, the product did not precipitated. It was isolated by extraction with chloroform.

The solvent was then evaporated off by a rotary evaporator. The brown precipitate was collected by washing with diethyl ether and petroleum ether. The suspension was filtered and the solid product was recrystalized from ethanol (m.p. range was 176-178). The yield was 0.3 gm, 36%.

3.3.1.4 Preparation of 7-methyl-2,3-trimethylene-4H-isoxazolo[2, 3- a] pyrimidin-4-one, (27):

As described in the general procedure, 3-amino-5-methylisoxazole (0.5 gm, 5.10 mmole) was mixed with ethyl-2-oxocyclopentane carboxylate (0.87 gm, 5.6 mmole) and 3 gm PPA. The brown precipitate was recrystalized from ethanol (m.p. range was 1147-150). The yield was 0.84 gm, 87%.

3.3.1.5 Preparation of 7-methyl-2,3-tetramethylene-4H-isoxazolo[2, 3- a] pyrimidin-4-one, (28):

As described in the general procedure, 3-amino-5-methylisoxazole (0.5 gm, 5.10 mmole) was mixed with ethyl-2-oxocyclohexane carboxylate (0.95 gm, 5.6 mmole) and 3 gm PPA. The pale yellow precipitate was recrystalized from ethanol (m.p. range was 98-100). The yield was 0.86 gm, 83%.

3.3.1.6 Preparation of 6-Benzyl-2-methyl-5,6,7,8-tetrahydro-9H-isoxazolo[2, 3-a]pyrido[3, 4-d] pyrimidin-9-one, (29):

As described in the general procedure, 3-amino-5-methylisoxazole (0.5 gm, 5.1 mmole) was mixed with ethyl-1-benzyl-3-oxo-4-pipyridine carboxylate hydrochloride (1.67 gm, 5.6 mmole) and 6 gm PPA.

After neutralization, the product did not precipitated. It was isolated by extraction with ethyl acetate. The solvent was then evaporated off by a rotary evaporator. The brown precipitate was collected by washing with diethyl ether and petroleum ether. The suspension was filtered and the solid product was recrystalized from ethanol (m.p. range was 142-144). The yield was 0.8 gm, 53%.

3.3.1.7 Preparation of 7-Benzyl-2-methyl-5,6,7,8-tetra-hydro-9H-isoxazolo [2, 3-a] pyrido [4, 3-d] pyrimidin-9-one, (30):

As described in the general procedure, 3-amino-5-methylisoxazole (0.5 gm, 5.1 mmole) was mixed with methyl-1-benzyl-4-oxo-3-pipyridine carboxylate hydrochloride (1.67 gm, 5.6 mmole) and 6 gm PPA. After neutralization, the product did not precipitated. It was isolated by extraction with ethyl acetate. The solvent was then evaporated off by a rotary evaporator. The yellow precipitate was collected by washing with diethyl ether and petroleum ether. The suspension was filtered and the solid product was recrystalized from ethanol (m.p. range was 176-177). The yield was 0.53 gm, 35%.

3.3.1.8 Preparation of 9-fluoro-2,3-tetramethylene [1,3] diazipino[2, 1- b][1,3] benzothiazol-5(2H)-one, (31):

As described in the general procedure, 2-amino-6-fluorobenzothiazole (0.5 gm, 2.97 mmole) was mixed with ethyl-2-cyclohexanone acetate (0.6 gm, 3.27 mmole) and 3 gm PPA. The flask contents were mixed for one hour at 60°C, then at 120°C for two hours. The pale yellow precipitate was recrystalized from ethanol (m.p. range was 158-160). The yield was 0.68 gm, 79%.

3.3.1.9 Preparation of 8-methyl-2,3-tetramethylene-isoxazolo[2, 3- a] [1,3] diazipin-5(2H)-one, (32):

As described in the general procedure, 3-amino-5-methylisoxazole (0.5 gm, 5.1 mmole) was mixed with ethyl-2-cyclohexanone acetate (1.03 gm,5.6 mmole) and 3 gm PPA. The flask contents were mixed for one hour at 60°C, then at 120° for two hours. The brown precipitate was recrystalized from ethanol (m.p. range was 87-90). The yield was 0.6 gm, 54.5%.

3.3.1.10 Preparation of 2,3-tetramethylene[1,3]diazipino-[1, 2- a]isoquinolin-5(2H)-one, (33):

As described in the general procedure, 1-aminoisoquinoline (0.5 gm, 3.47 mmole) was mixed with ethyl-2-cyclohexanone acetate (0.7 gm, 5.6 mmole) and 3 gm PPA. The flask contents were mixed for one hour at 60°C, then at 120°C

for two hours.The brown precipitate was recrystalized from ethanol (m.p. range was 190-192).The yield was 0.9 gm,.97.8 %.

3.3.2 MTT Assay: [39]

3.3.2.1 Culture of neuroblastoma cells:

Adherently growing SK-N-SH were detached from the culture flasks as follows:

1- the cell culture medium was removed completely by adding trypsin (~2.5 mL) of were added to each culture flask (150 cm^2). The cells were incubated with trypsin at room temperature for 5 minutes.

2- the cells were detached from the bottom of the flask by shaking.

3- the effect of trypsin was blocked by the addition of 10 mL of cell culture medium.

4- the cell suspension was then centrifuged (10 minutes, 400g X). Supernatant was removed and the cells were resuspended in 10 mL of cell culture medium. The cell count was determined with a Neubauer cell chamber in the presence of trypan blue. Fore this purpose 20 µL of cell suspension were mixed with 20 µL of trypan blue. The cells were counted in 4 large squares of cell chamber, whereby only the nonstained cells were used to adjust the cell concentration. A definite number of cells was used for further culturing of the cells, whereas the rest of the cells was used for the experiments.

3.3.2.2 Preparation of MTT solution:

The MTT solution was prepared by dissolving 5 mg of MTT in 1 mL of PBS (Phosphate Buffered Saline). The solution was filtered through a 0.2 µm

filter, wrapped in an aluminum foil (MTT is a light sensitive substance) and stored at 4°C.

3.3.2.3 Preparation of isopropanol-triton X-100 solution:

In order to get this solution 0.1 M HCl was mixed with Isopropanol and 10 % triton X-100. This solution was stored at room temperature.

3.3.2.4 MTT Assay with neuroblastoma cells:

1- Cell suspension (200 µL, 2 $\times 10^5$ cells/mL of cell culture medium) were placed with a dispenser multipipette (Eppendorf) into each well microtiter plate. Three wells were filled with cell culture medium only and were used as BLANK. The plate was incubated overnight at 37°C, 5% CO_2.

2- After 24 hours, the compounds **27, 32** and **33** were separately added to the plate. Each compound was added in the form of solution of known concentration in ethanol. The concentrations are listed earlier in Table (15). 10 µL aliquot of each solution concentration for each compound was added to a different well. In the control wells the same volumes of PBS were added instead of the compounds. The 96 wells microtiter plate is further incubated for 48 hours at 37°C, 5% CO_2.

3- After 48 hours of incubation, the cell culture medium was removed completely by a Pasteur pipette that is installed on a vacuum pump. Further, 100 µL of MTT solution are added to each well.

4- The microtiter plate was further incubated for 3 hours. During this time MTT was changed into formazan. The formazan microscopically appeared as blue crystals between the cells at the bottom of the wells.

5- Isopropanol solution (100 μL) was then added into each well. The plate was shaken horizontally overnight at room temperature. During this time, formazan crystals were dissolved.

Finally the absorbance of the color in each well was measured by a Microplate reader (MR 700) in a dual mode with a reference wavelength of 550 nm (filter number 5) and a test wavelength of 630 nm (filter number 4).

References

1- S.Zhang and K. Bastow, " Substituted 2-thienyl-1,8-naphthyidin-4-ones: their synthysis, cytotoxity, and inhibiton of tubulin polymerization", *J. Med. Chem.*, **42,** (1999), 4081-4087.

2- K. Chem and S. Kuo, " 2,3,4,5,6,7-Substituted 2-phenyl-1,8-naphthyridin-4-ones: their synthysis, cytotoxity, and inhibiton of tubulin polymerization", *J. Med. Chem.*, **40,** (1997), 2266-2275.

3- M.L. Edwards, D.M. Stemenick and P.S. Sunkama, " Chalcones: a new class of antimiotic agents", *J. Med. Chem.*, **33,** (1990), 1948-1954.

4- H. Plamer, M. Boyd, B. Baguley and W. Deny, "Synthesis and antitumor evaluation of dibenzo[1,4] dioxin-1-carboxamides: a new class of weakly binding DNA-interchalating agents", *J. Med. Chem.*, **35,** (1992), 258-266.

5- J.J.Chen, L.W. Deady and M.F. Mackay, " Synthesis of some acridone alkaloids and related compounds", *Tetrahedron*, **53,** (1997), 12717-12728.

6- J.J.Chen and L.W. Deady," A versatile synthesis of acridine –1,9-diones", *Synthetic Communications* , **27,** (1997), 95-106.

7- L.W. Deady and D.M. Werden," New synthesis of acridone-9-ones, pyrrolo [1, 2-a] quinoline-1,5-diones, and some related tetracyclic compounds", *J. Org. Chem.*, **52,** (1987), 3930-3932.

8- F. Suzuki, T. Kuroda, T. Tamurs, S. Sato, K. Ohmori and S. Ichikaws, "5-Phenyl-3H-imidazo[4,5-c][1,8]naphthyridin–4(5H)-ones: a new class of nonsteroidal antiinflammatory agents with potent activity like glucocorticoids", *J. Med. Chem.,* **35,** (1992), 2863-2870.

9- T. Katagi, M. Aoki and M. Kashwagi, "Synthesis and anti-inflammatory activity of malonic acids, malonamate and malonamide derivatives of some heterocyclic compounds", *Chem. Pherm. Bull.,* **33,** (1985), 4878- 4888.

10- V. Bundurco and C. Schwender, " Synthesis and cardiotonic activity of a series of substituted 4-alkyl-2(1H)-quinazolinones", *J. Med. Chem.*, **30,** (1987), 1421-1426.

11- A. Nohara, J. Ishiguro and K. Ukawa, " Synthesis of antiallergic-5-oxo-5H [1] benzopyrano [2,3-b] pyridones", *J. Med. Chem.*, **28,** (1985), 559-568.

12- S. Kester, M. Dengnan and J. Hang, " Synthesis and antimalarial properties of 1-imino derivatives of 7-chloro-3-substituted –3,4-dihydro-1,9 (2H,10H) acridine diones and related structures", *J. Med. Chem.*, **35,** (1992), 3428-3441.

13- L. Qun, T.W. Danial and A. Claiborne, "Synthesis and structure activity relationships of 2-pyridones: a novel series of potent DNA gyrase inhibitors and antibacterial agents", ***J. Med. Chem.*, 39,** (1996), 3070-3088.

14- G. Leoncini, M. Signorello, G. Roma and M. Braccio, " Effect of 2- (1-piperazinyl)-4H-Pyido[1,2-a]pyrimidin-4-one (APISS) on human platelets in vitro", *Biochemical Pharmacology*, **53,** (1997), 1667-1672.

15- G. Romai, N. Cinone, M.Braccio, G. Grossi, G. Leoncini, M. Signorello, and A. Carott, " Synthesis, antiplatelet activity and molecular field analysis of substituted 2-amino-4H-pyrido[1,2-a]pyrimidin-4-ones; their congeners and isosteric analogues", *Bioorganic & Medical chemistry*, **8,** (2000), 751-768.

16- D. Fry, J. Nelson, V. Slintak, P. Keller, G. Rewcastle, W.Denny , H. Zhous and A. Bridges, "Biochemical and antiproliferative properties of 4-[A-alkylamino] pyridopyrimidines; a new chemical class of potent and specific epidermal growth factor receptor tyrosine kinase inhibitor", *Biochemical Pharmacology*, **54,** (1997), 877-887.

17- H. Ogura, M. Kawaro and T. Itoh, " Reaction of 2-amino-benzothiazoles with acetylinic compounds", *Chem. Pharm. Bull.*, **21,** (1973) 2019-2025.

18- D. Dunwell and D. Evans, " The reaction of 2-amino thiazoles and 2-aminobenzothiazoles with propiolic acid and its esters", *J. Chem. Soc.*, **C,** (1971), 2094-2097.

19- R. Prager, T. Rosenzweig and Y.Singh, " The chemistry of 5-oxodihydroisoxazoles III; synthesis of further annelated pyrimidones", *Ast. J. Chem.*, **45,** (1992), 1825-1832.

20- J. Singh and R. Prager, "The chemistry of 5-oxodihydroisoxazoles II; Photolysis and pyrolysis of 2-(isoquinolin-1-yl)-3-methyl-5-oxo-2,5-dihydroisoxazoles-4- carboxylate", *Ast. J. Chem.*, **45,** (1992), 1811-1823.

21- C. Donati, W. Janowski and R. Prager, " Base catalyzed rearrangement of isoxazolinyl heterocycles; synthesis of annelated pyrimidines", *Aust. J. Chem.*, **42,** (1989) 2161-2169.

22- Y. Liu and W.Huang, "polyfluoro alkylated bicyclic and tricyclic heterocyclic compounds", *J. chem. Soc. Perkin 1*, (1996), 481-487.

23- R. Aiwa, N. Katagiri and T. Kato, " Reaction of haloketenes with 1,3-Diazo-1,3-diene compounds", *Chem. Pharm. Bull.*, **32,** (1984), 4194-4153.

24- K. Szwed and M. Krasodomska, " Hetero Diels Alder reaction of some 1,3-diazo-1,3-butadienes with ketenes; Synthesis of functionalized pyrimido[1, 2-b]benzothiazoles and 1,3,4-thiadiazole[3, 2-a]pyrimidines", *Monatshefte Fùr chemie*, **127**, (1996), 1273-1281.

25- N. Katagiri and T. Kato, " Reaction of haloketenes with 2-arylidine amino pyridines", *J. Heterocyclic chem.*, **21,** (1984), 407-412.

26- J. Morimoto and M. Sekiya, "Reaction of α-arylmethylene amino substituted derivatives of pyridine, quinoline and isoquinoline with trichloroacetic anhydride", *Chem. Pharm. Bull.*, **25,** (1977) 1607-1609.

27- M. Shur and S. Israelstam, " The reaction of aminohetrocycles with reactive esters", *J. Org. Chem.*, **33,** (1968), 3015-3020.

28- G. Fulop, J. Moszaros and G. Joth, "Synthesis and reactions of 2,3-cycloalkene-4H-pyrido[1, 2-a]pyrimidin-4-ones", *J. Heterocyclic. Chem.*, **16,** (1979), 137-144.

29- R.J. Brown, F. Carver and B. Hollingsworth, "The reaction of ethyl-2-oxocyclo pentane carboxlate with aryl amines. Part II", *J. Heterocyclic chem.*, (1962), 2624-2626.

30- R. Bossio, S. Marcaccini and R. Pepino, " Ring cleavage of isoxazoles with hetrocyclic amines; synthesis of fused pyrimidines", *J. Heterocyclic chem.*, **30,** (1993), 33-36.

31- R. Yasin, "M. Sc. Thesis: Syntheris of a new series of heterocyclic compounds for medicinal purposes", An-Najah N. University, (1999), pp. 22-23.

32- C.J. Pouchert, ed., "The Aldrich Library of Infrared Spectra", 3rd ed., Aldrich Chemical Co. Inc., Milwaukee, (1981), p.402.

33- B.K. Harris and B.E. Mann, "NMR and the Periodic Table", Academic Press, London, (1978), p. 100.

34- ACD-lab software.

35- R. Ziad, (Proff. Ishtaia M.S. group), An-najah National University.

36- P.R. Murray, E.J. Baron , M.A. Pfaller , F.C. Tenover and R.H. Yolke, "Manual of clinical Microbiology", 6th ed., USA: ASM press (1995).

37- A. Dikshat and A.husain, "Antifungal action of some essential oils against animal pathogens", *Fitoterapia LV*, **3,** (1984), 171-176.

38- S. Kamimura, M. Nishikawa, H. Saeki and Y. Takahi, "Absorption and metabolism of 3-hydroxy-5-methylisoxazole in plants and the biological activities of its metabobites", *phytopathology*, **64,** (1974), 1273-1281.

39- A.R. Martinez, post doctorate, (Prof. Voelter group), Tuebingen University hospital,.

40- F.P. Altman and R.G. Butcher "Studies on the reduction of tetrazollium salts", *Histochemie,* **37**, (1973), 333-350.

Appendix of Spectral Data

(Figures Showing Mass, FT-IR, ^{1}H-NMR and ^{13}C-NMR Spectra)

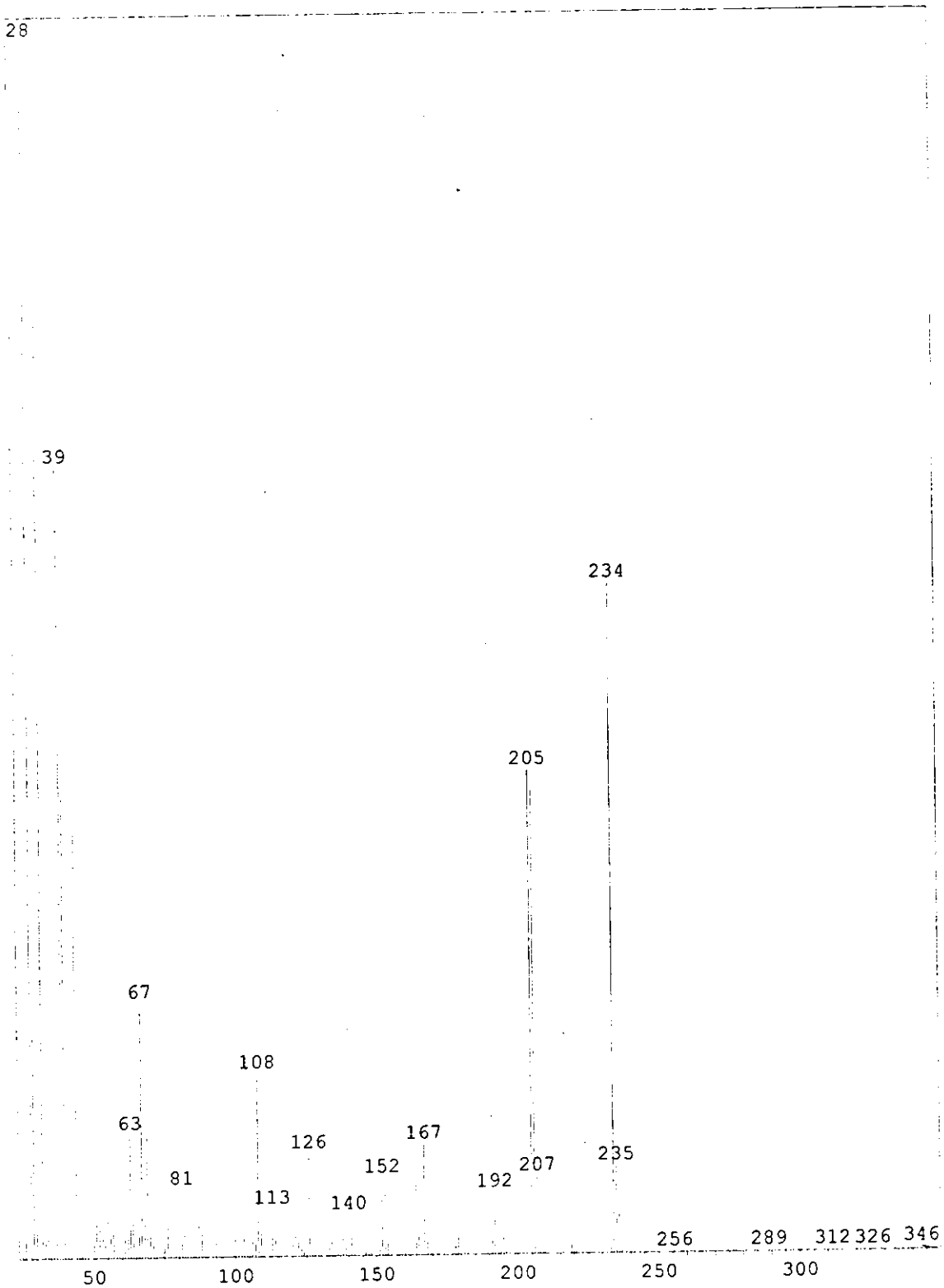


Figure (1): Mass spectrum of compound **24**.

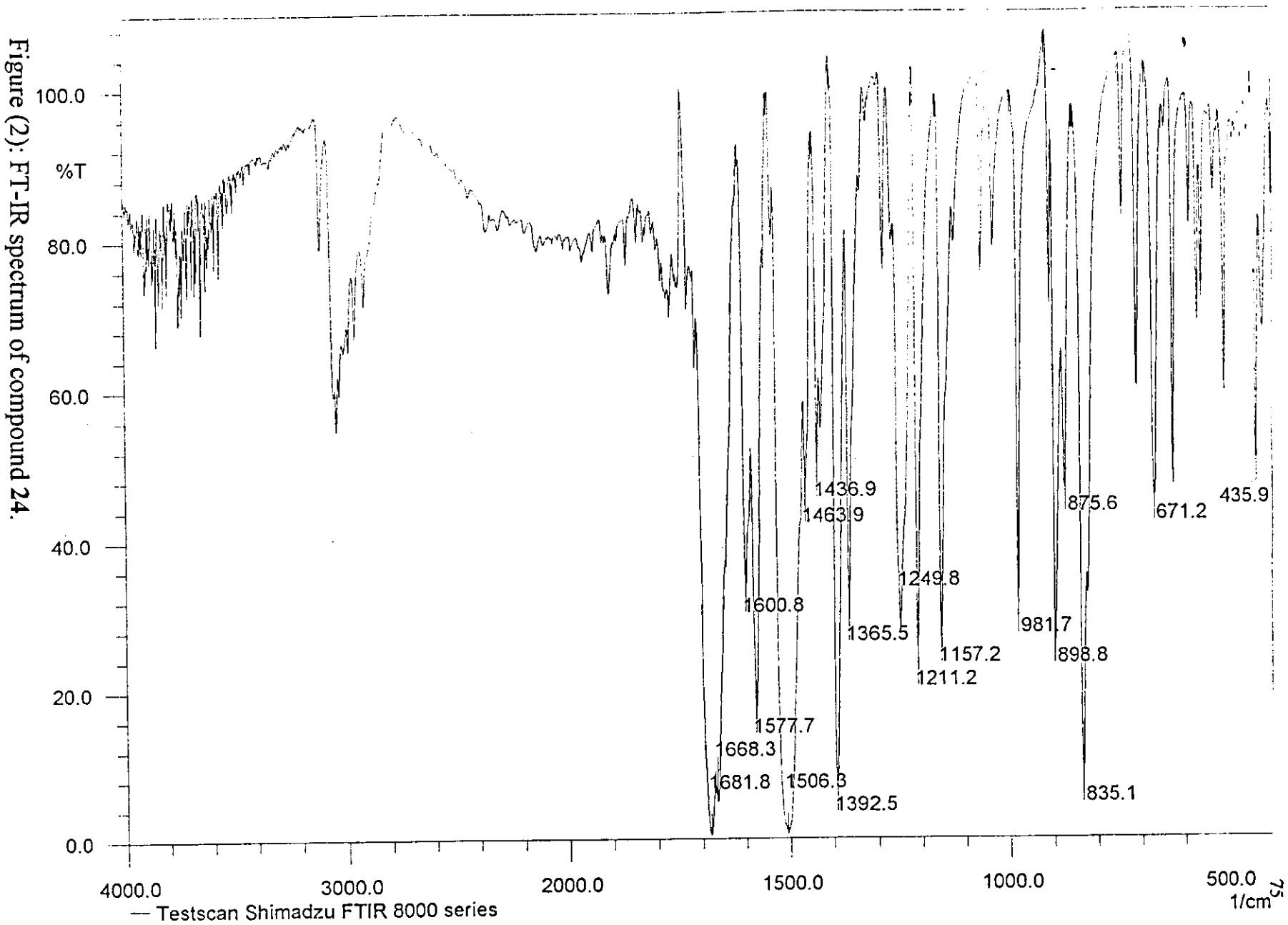


Figure (2): FT-IR spectrum of compound **24**.

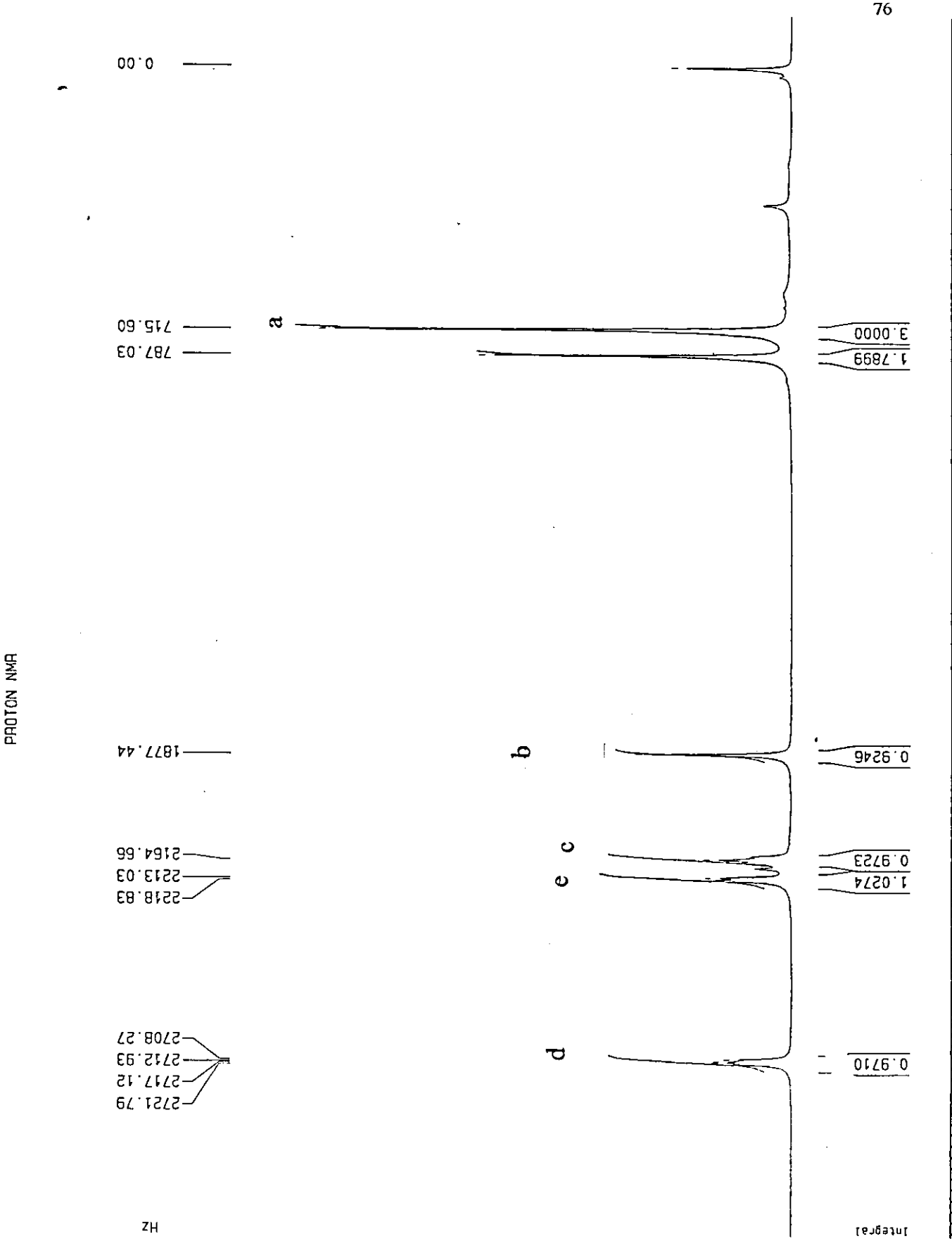


Figure (3): ^{1}H-NMR (1) spectrum of compound **24**.

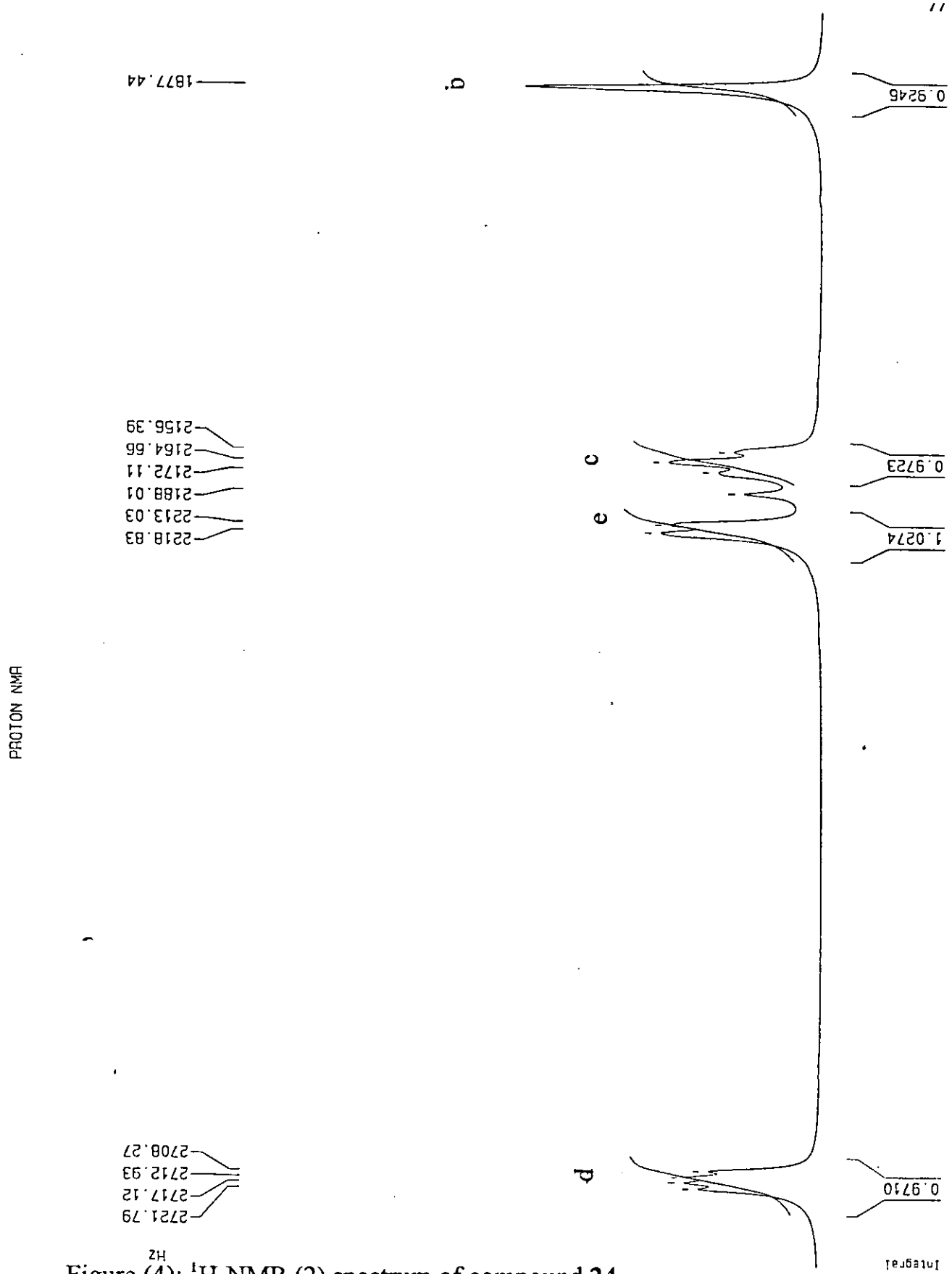


Figure (4): ^{1}H-NMR (2) spectrum of compound **24**.

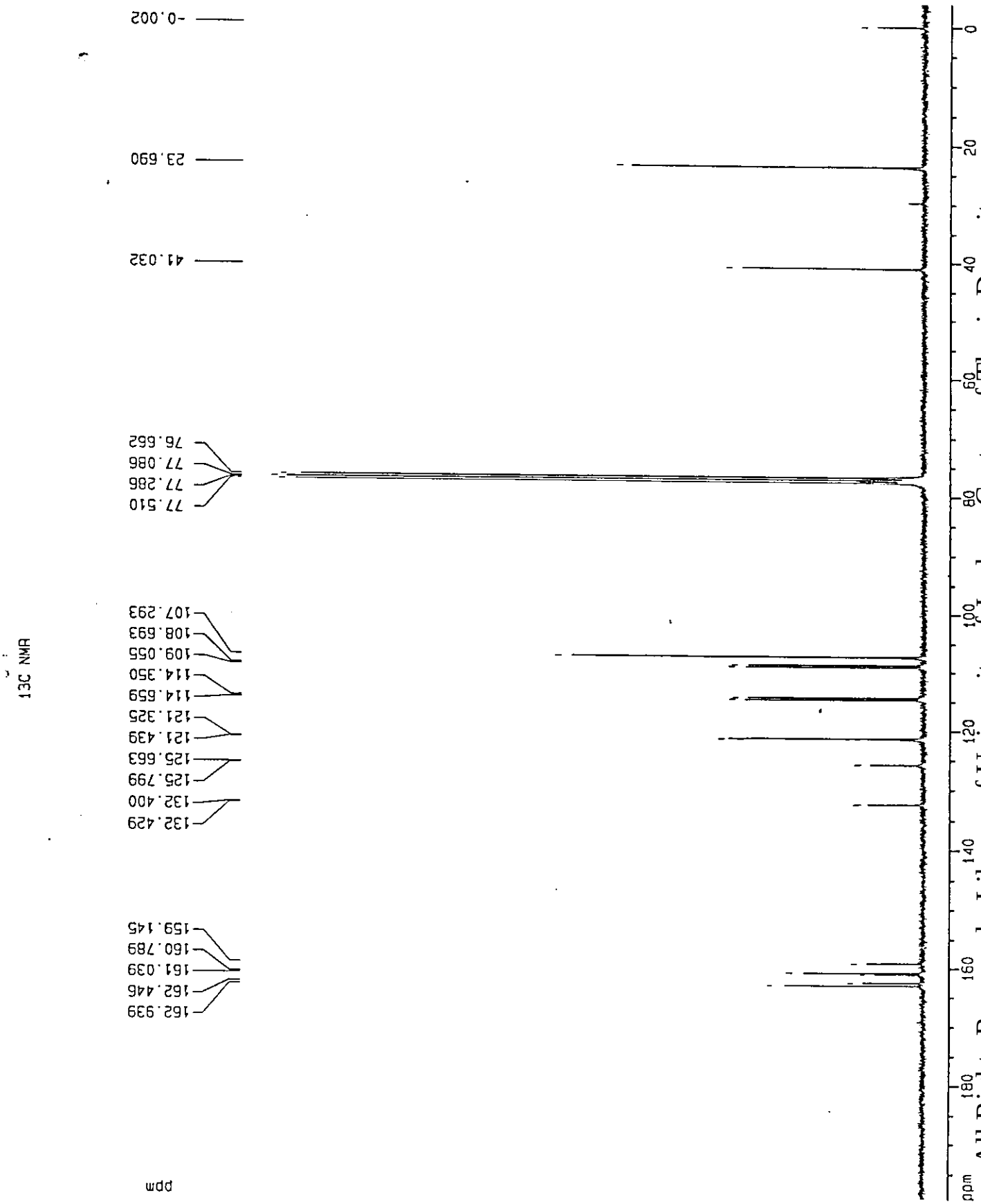


Figure (5): ^{13}C-NMR spectrum of compound **24**.

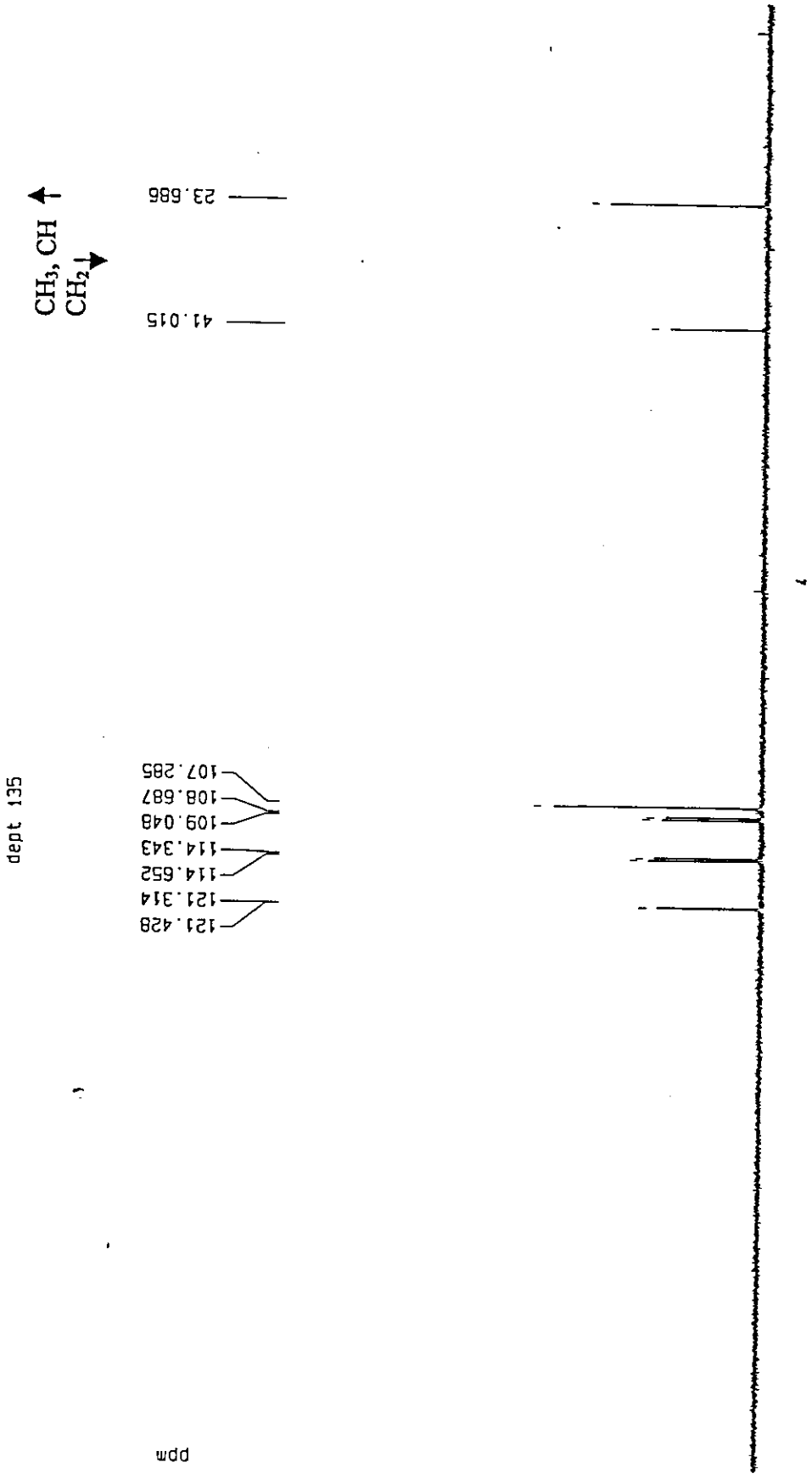


Figure (6): DEPT 135 spectrum of compound **24**.

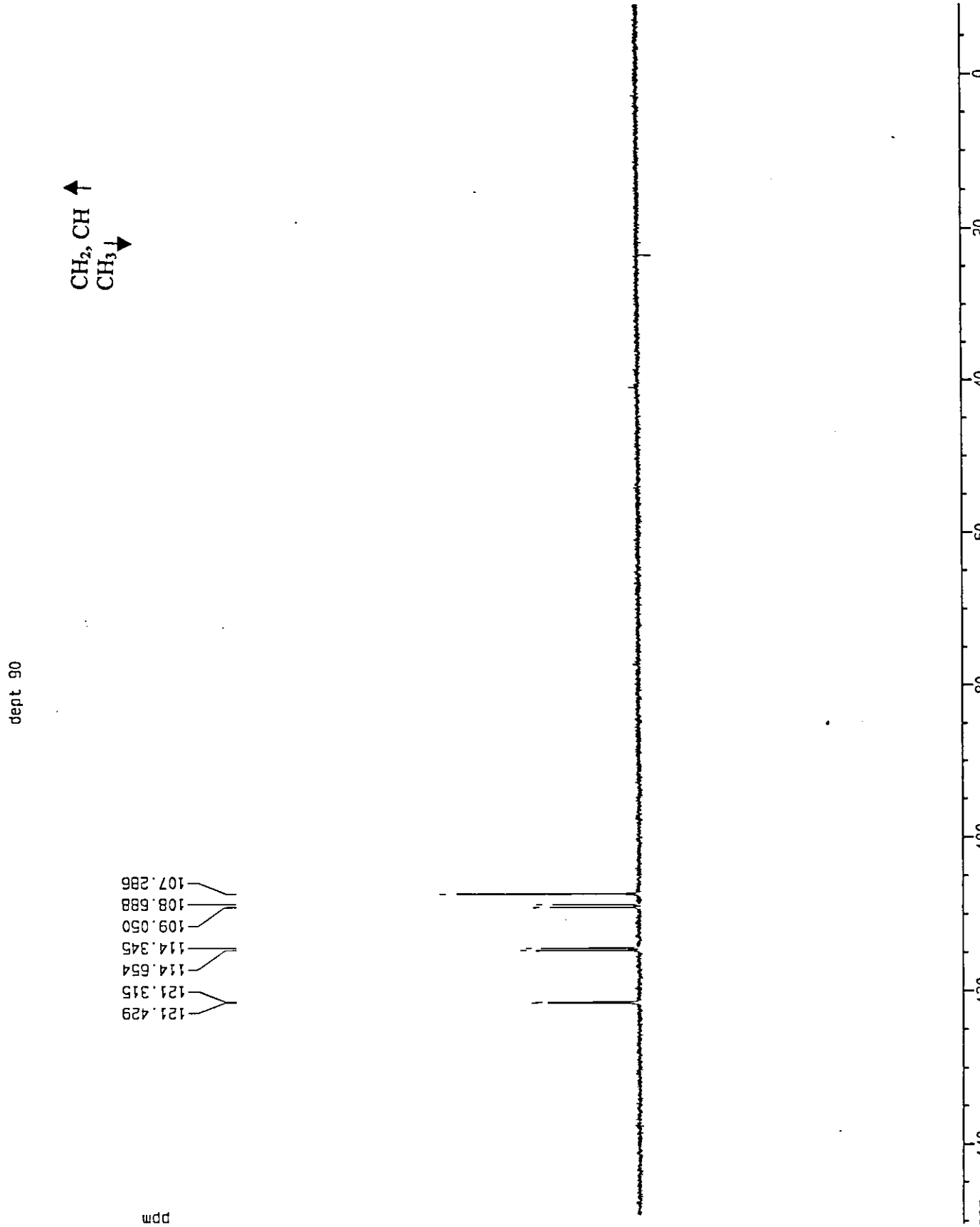


Figure (7): DEPT 90 spectrum of compound **24**.

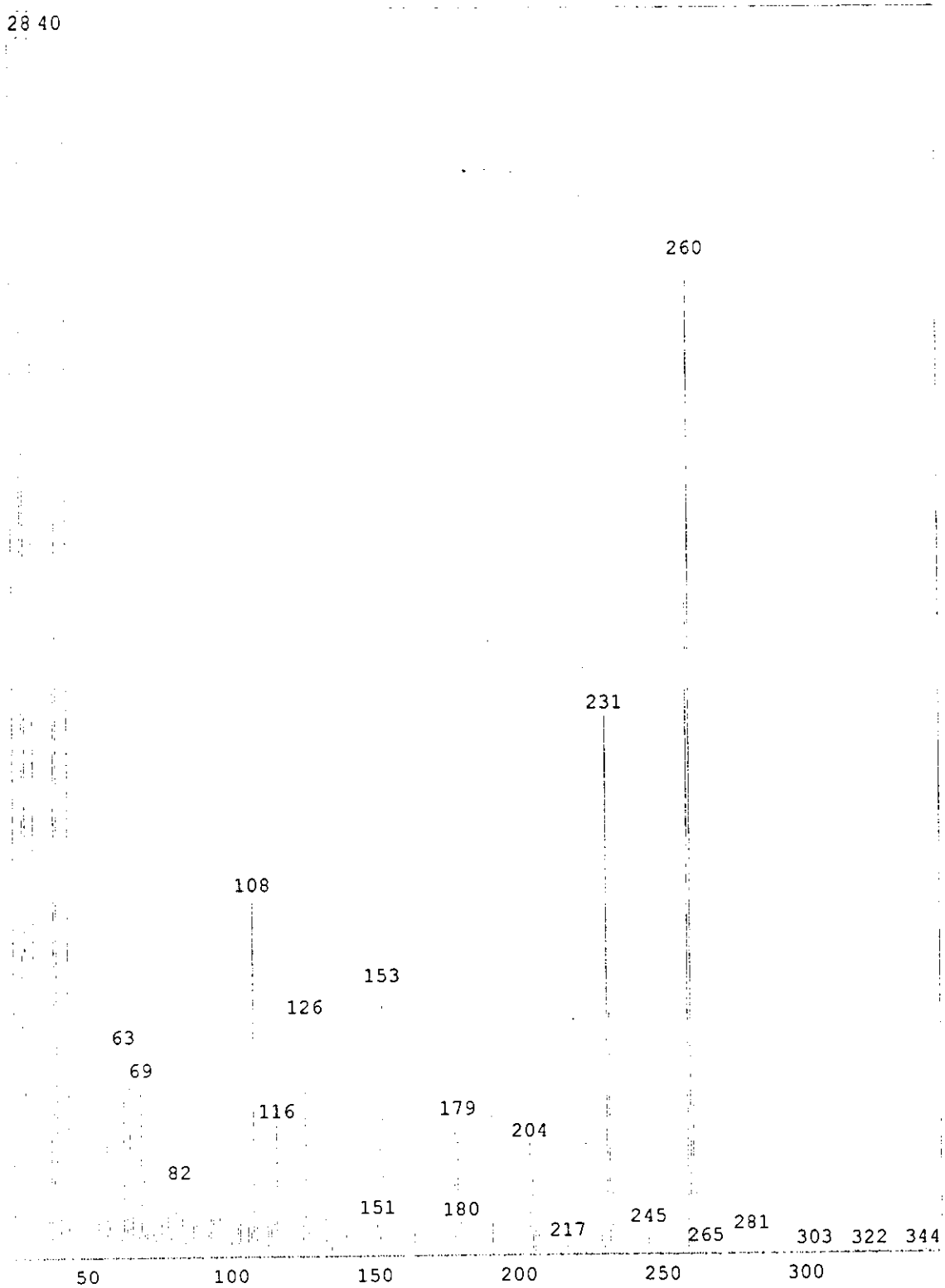


Figure (8): Mass spectrum of compound **25**.

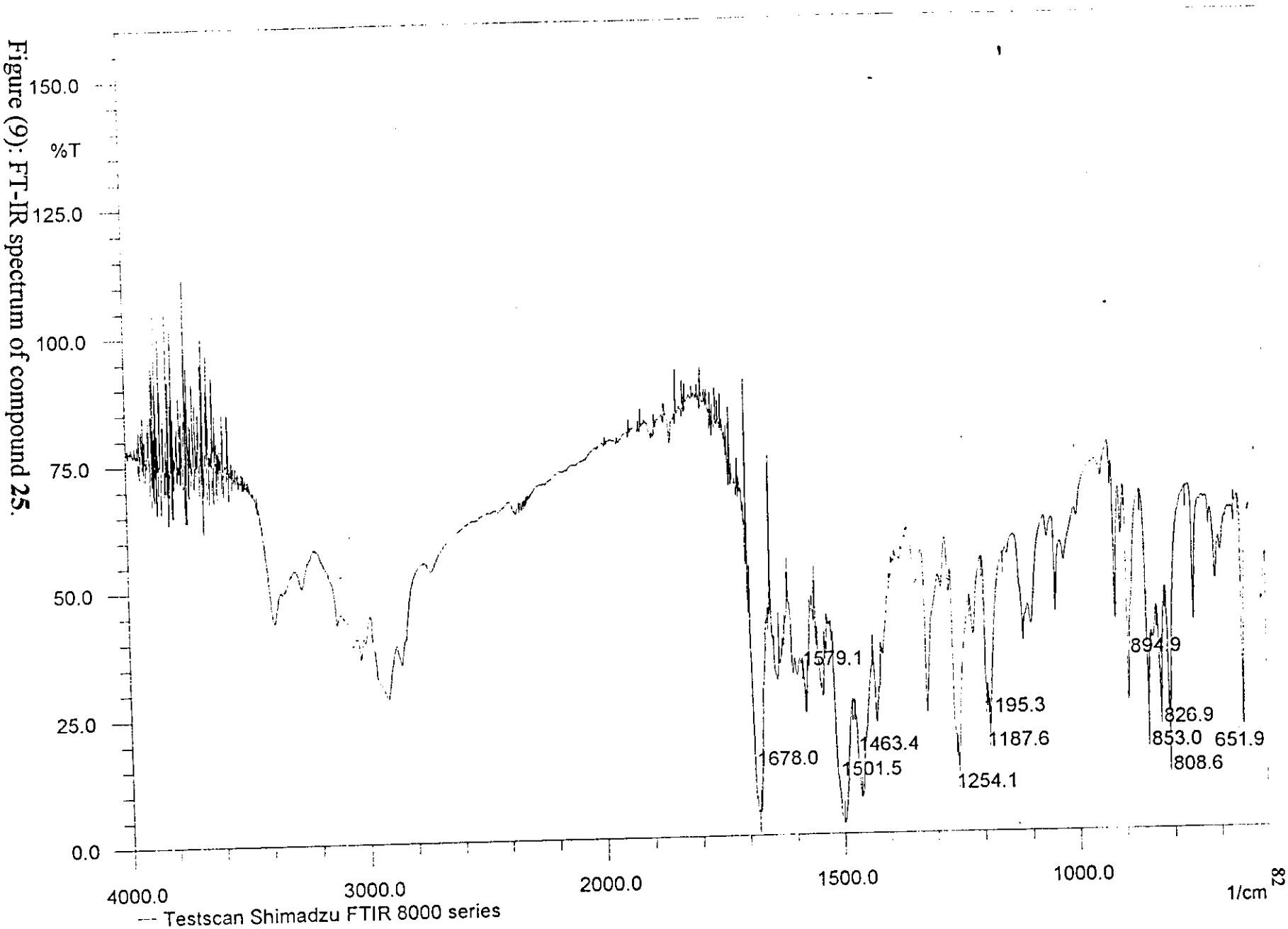


Figure (9): FT-IR spectrum of compound **25**.

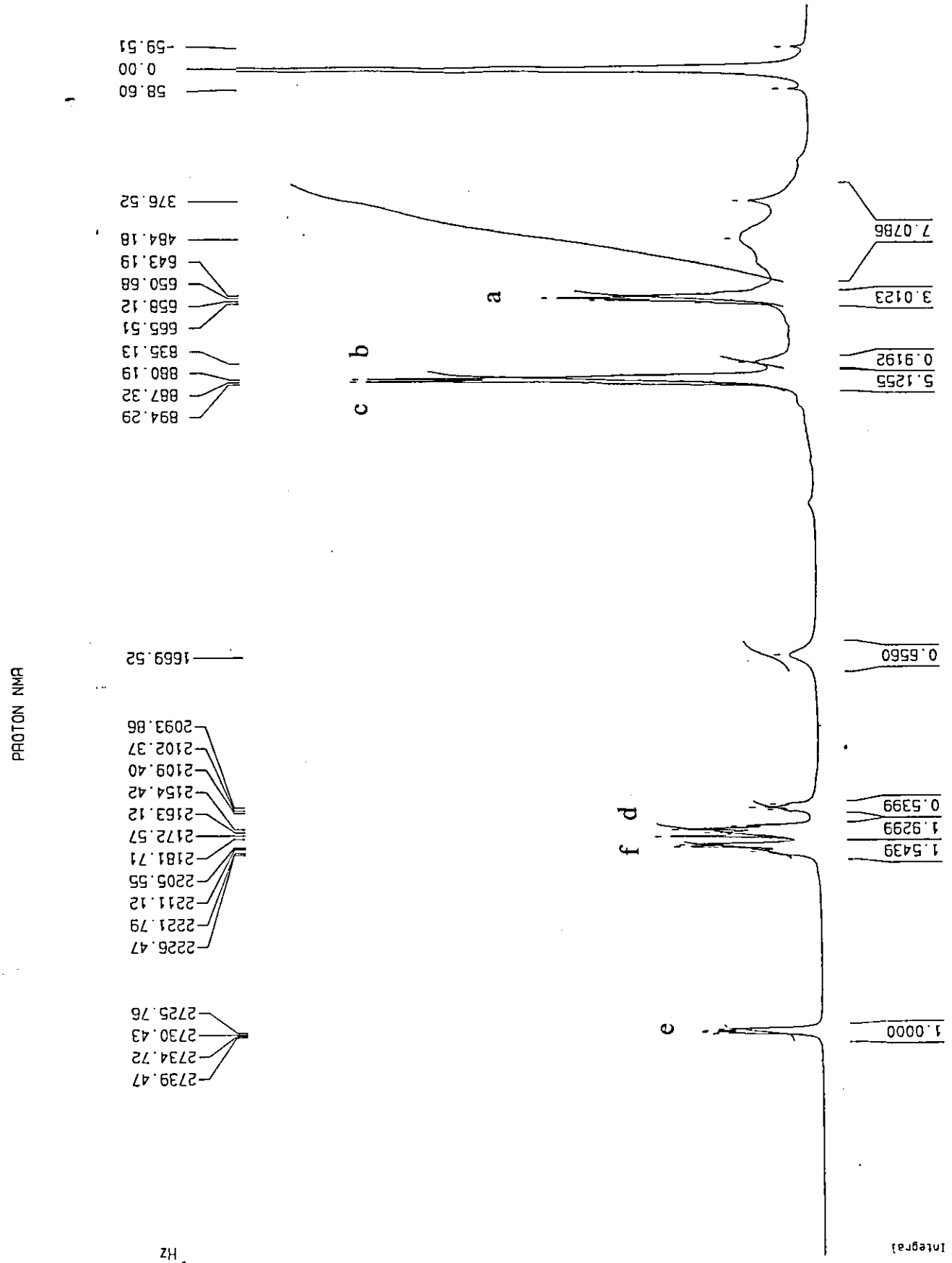


Figure (10): ^{1}H-NMR (1) spectrum of compound **25**.

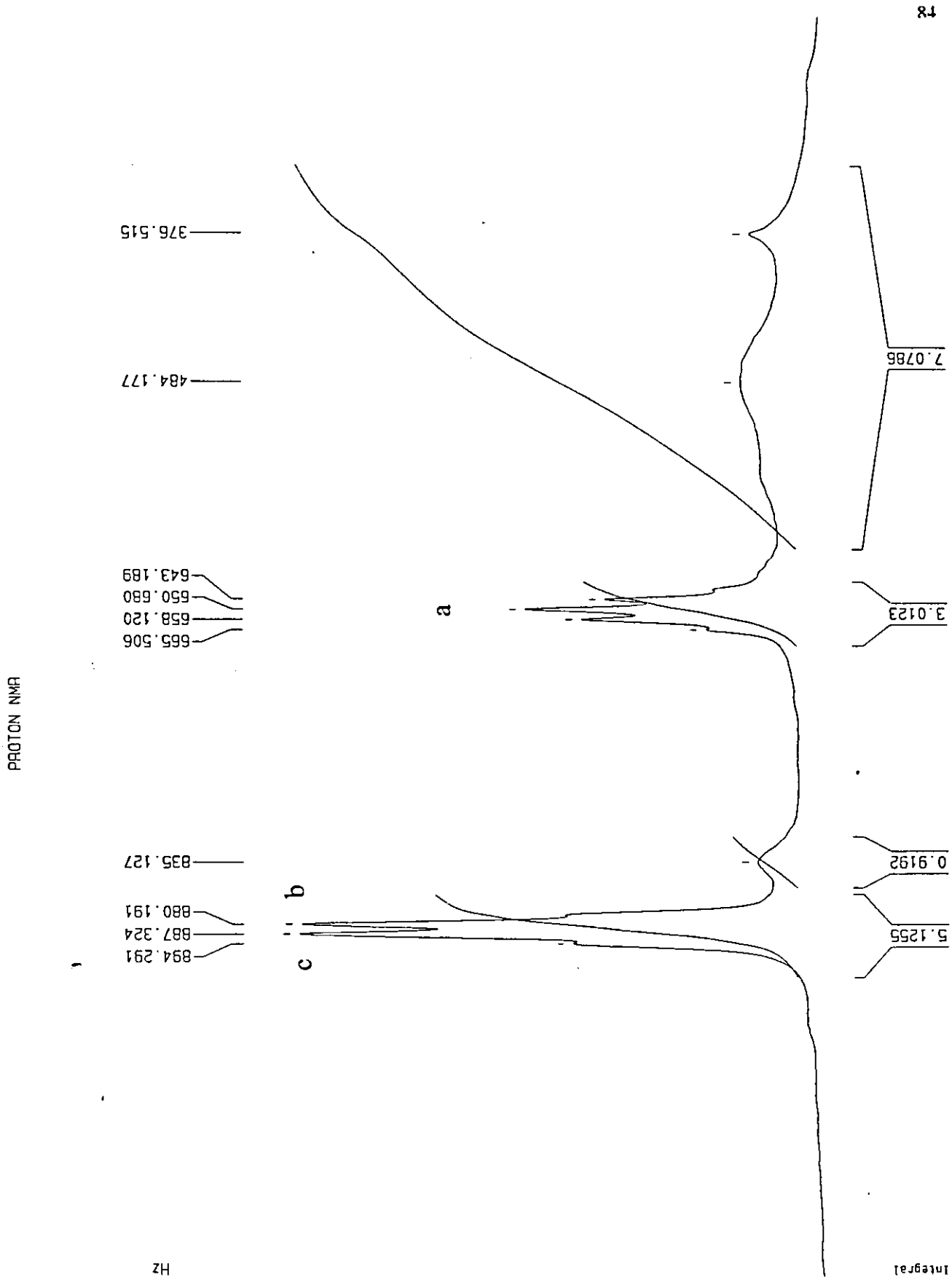


Figure (11): ^{1}H-NMR (2) spectrum of compound **25**.

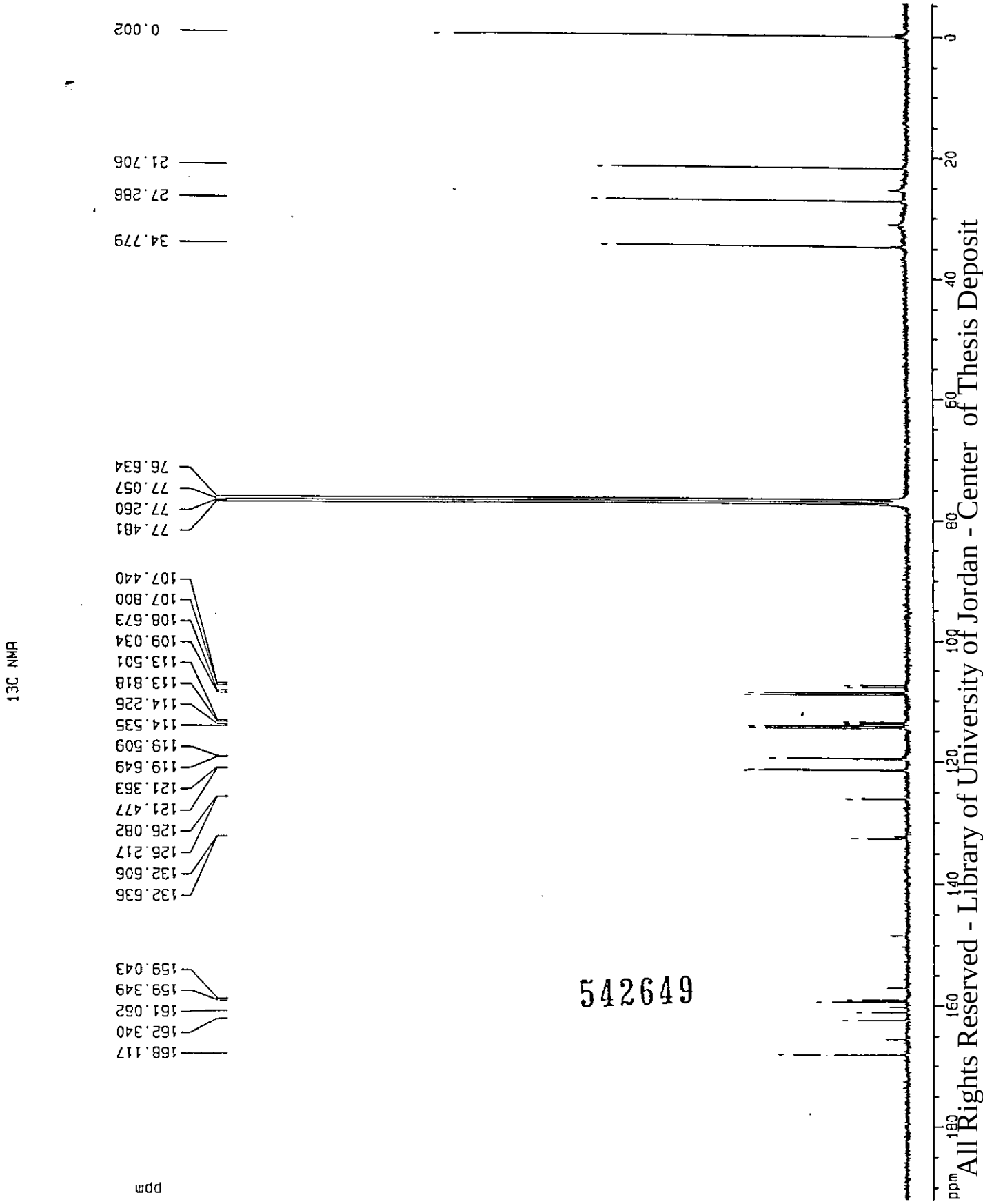


Figure (12): ^{13}C-NMR spectrum of compound **25**.

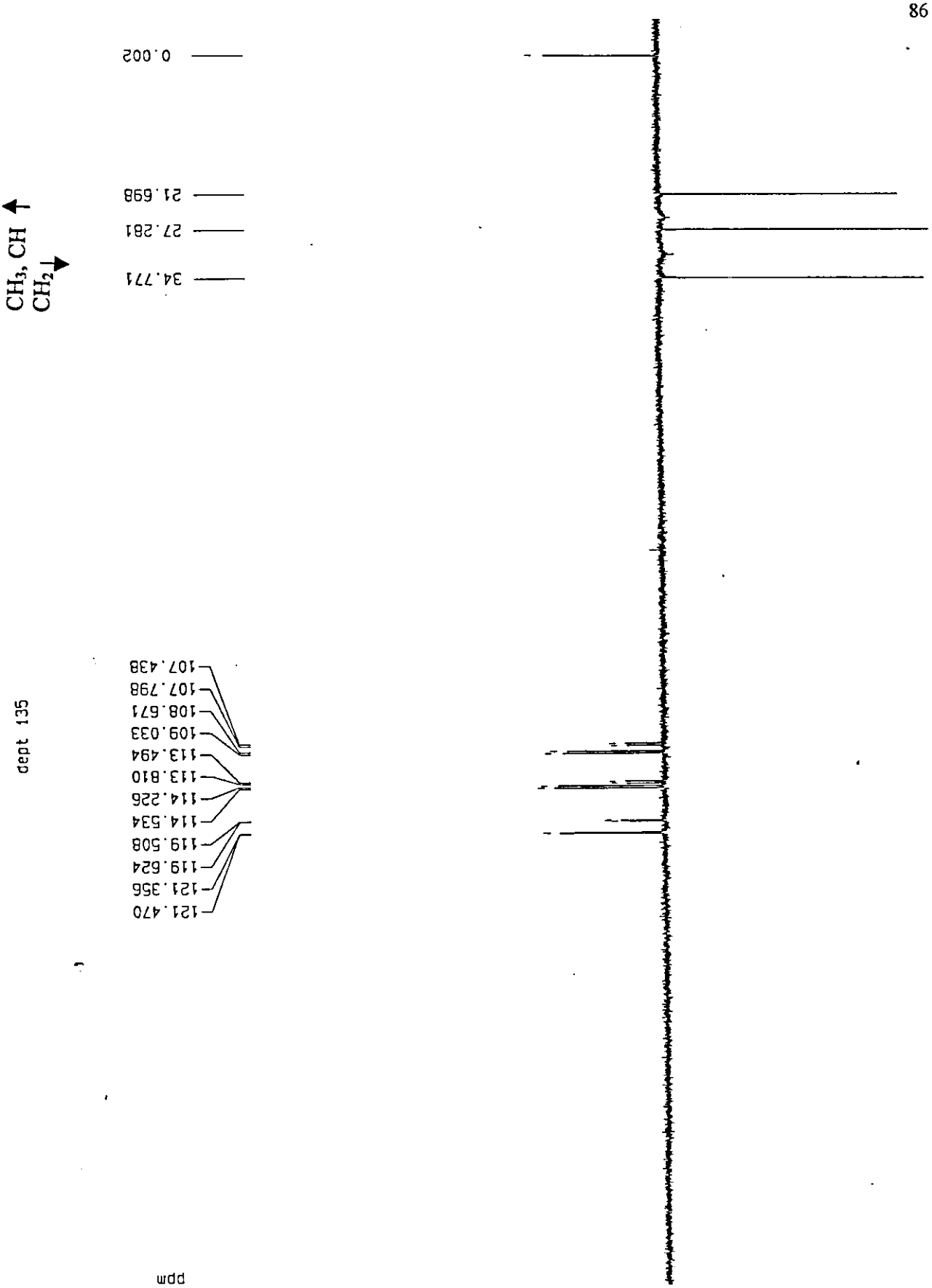


Figure (13): DEPT 135 spectrum of compound **25**.

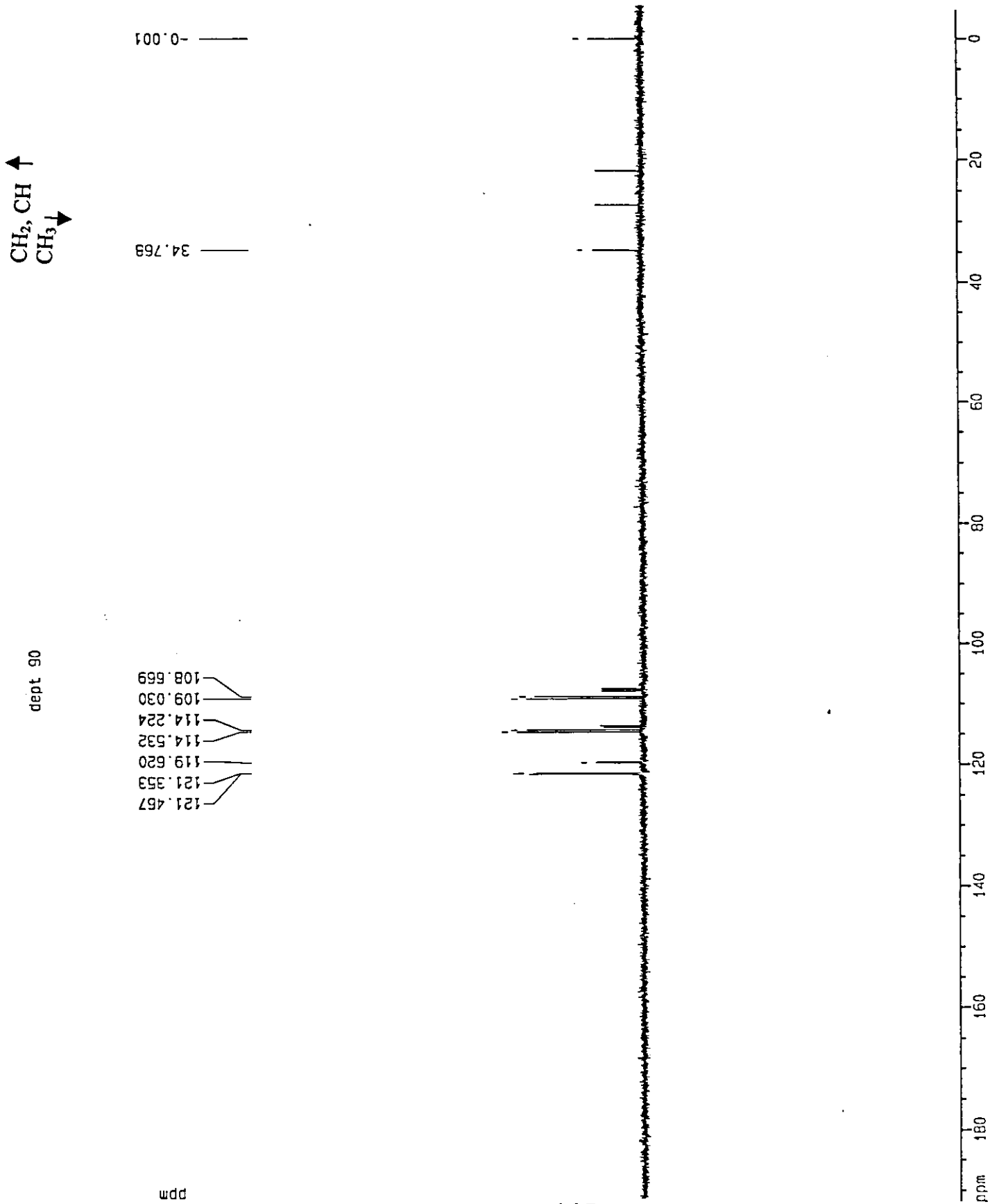


Figure (14): DEPT 90 spectrum of compound **25**.

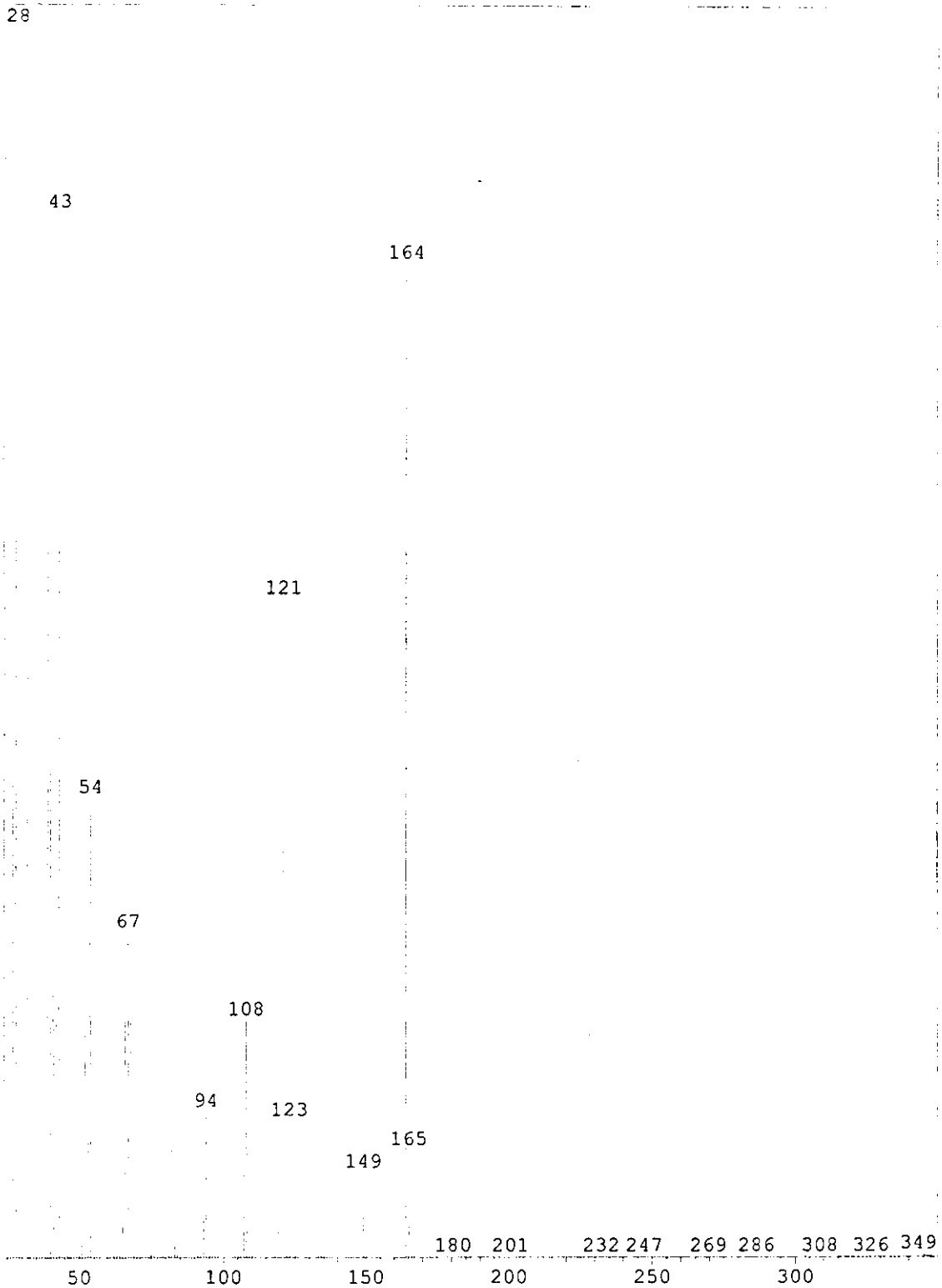


Figure (15): Mass spectrum of compound **26**.

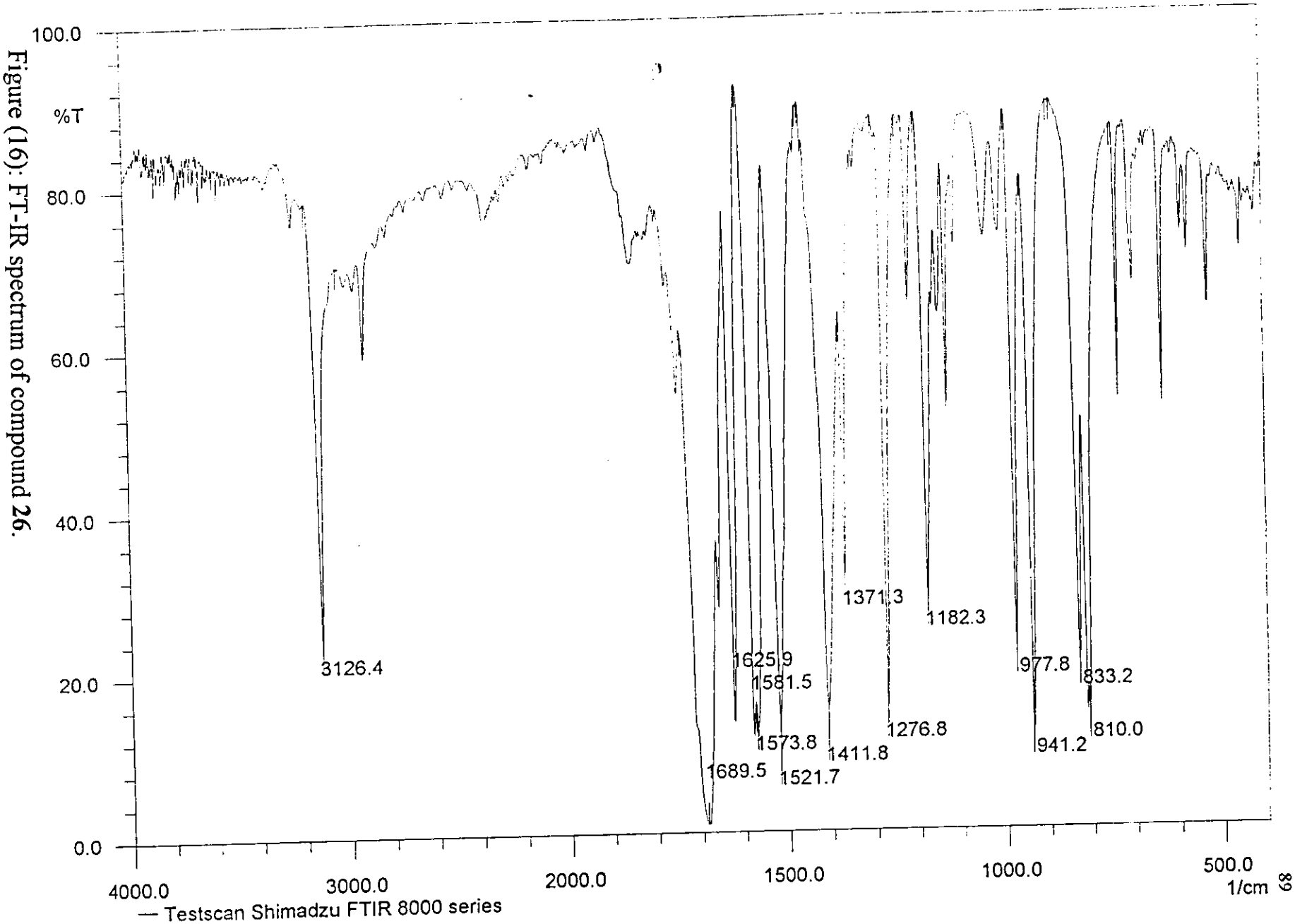


Figure (16): FT-IR spectrum of compound **26**.

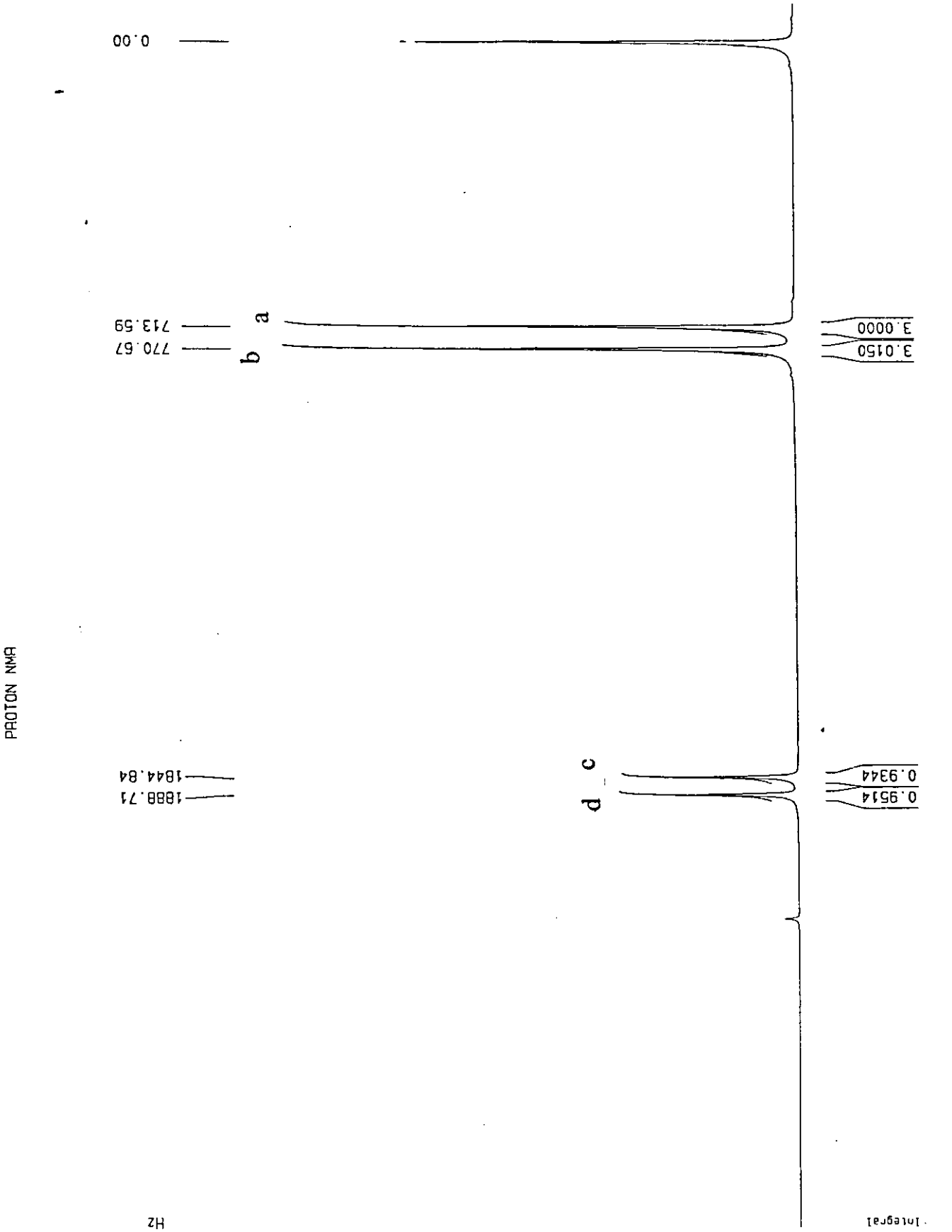


Figure (17): ^{1}H-NMR spectrum of compound **26**.

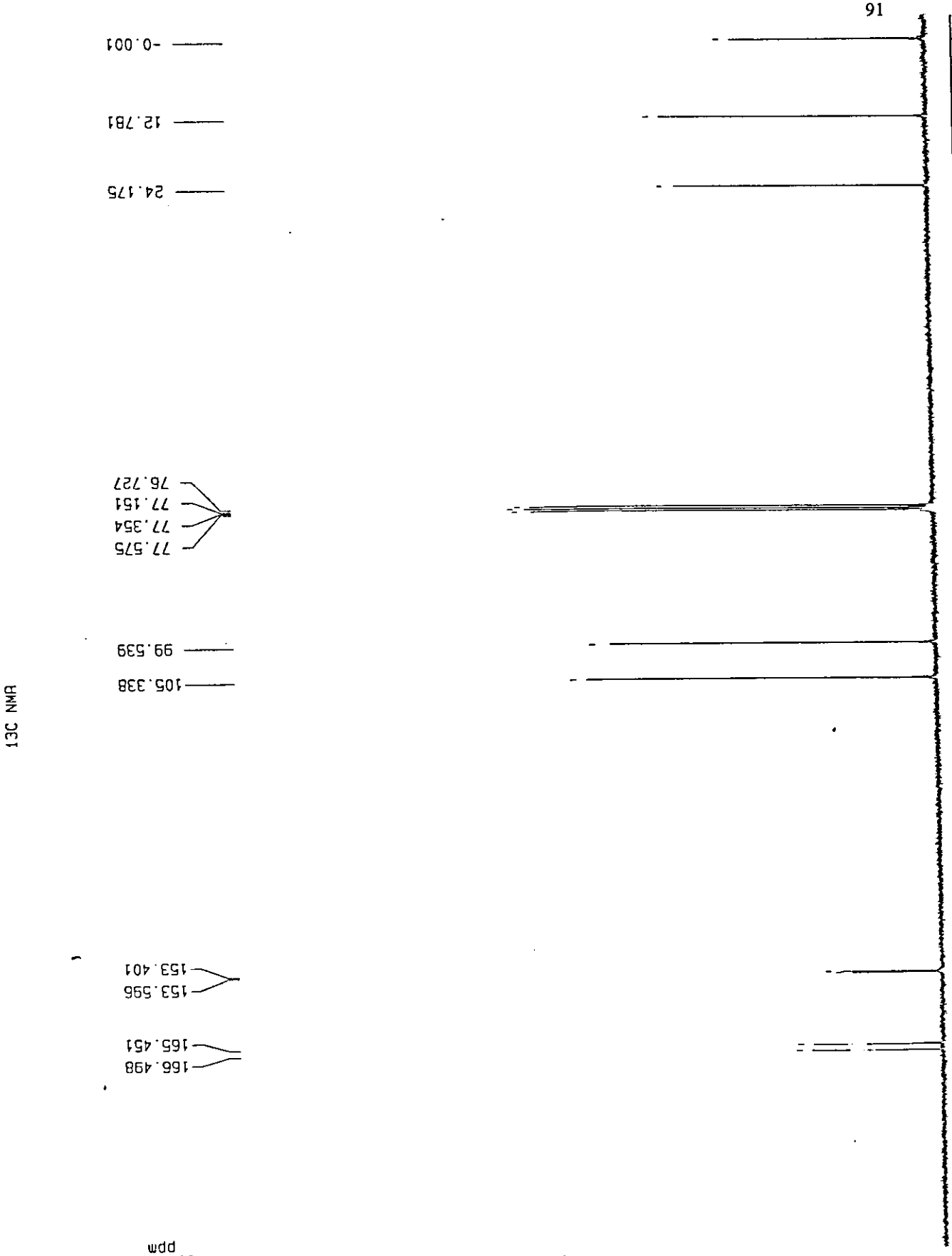


Figure (18): ^{13}C-NMR spectrum of compound **26**.

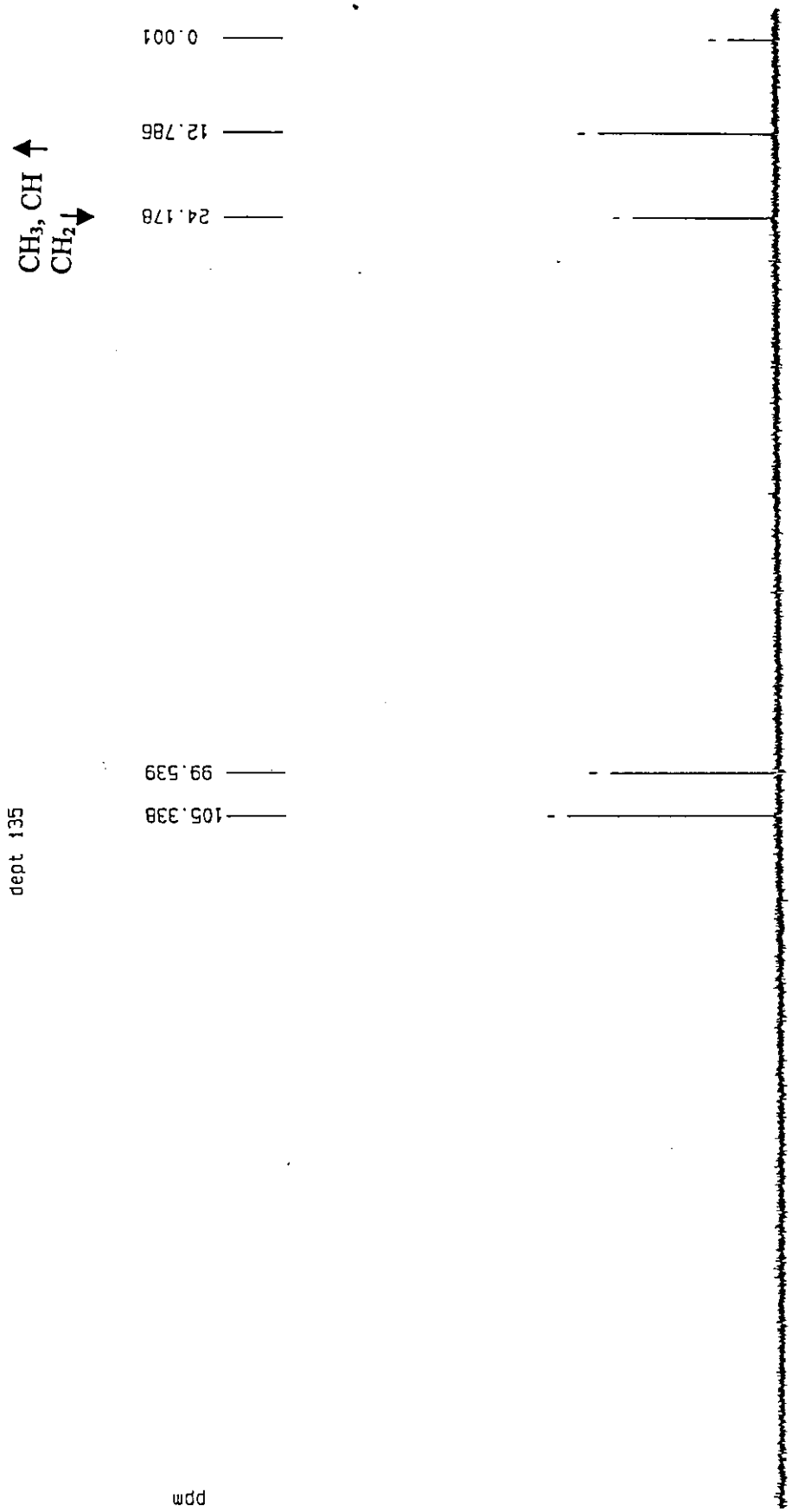


Figure (19): DEPT 135spectrum of compound **26**.

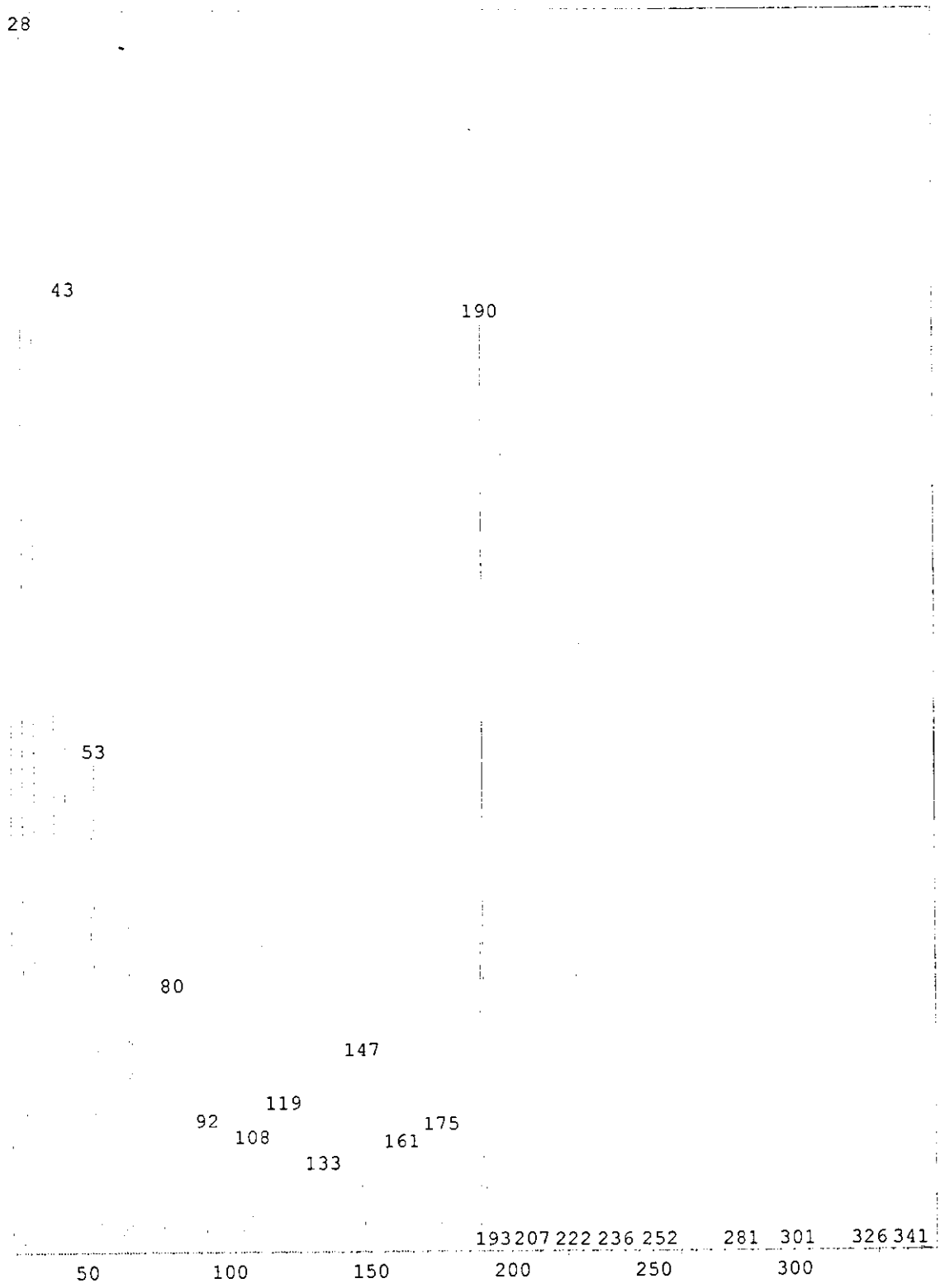


Figure (20): Mass spectrum of compound **27**.

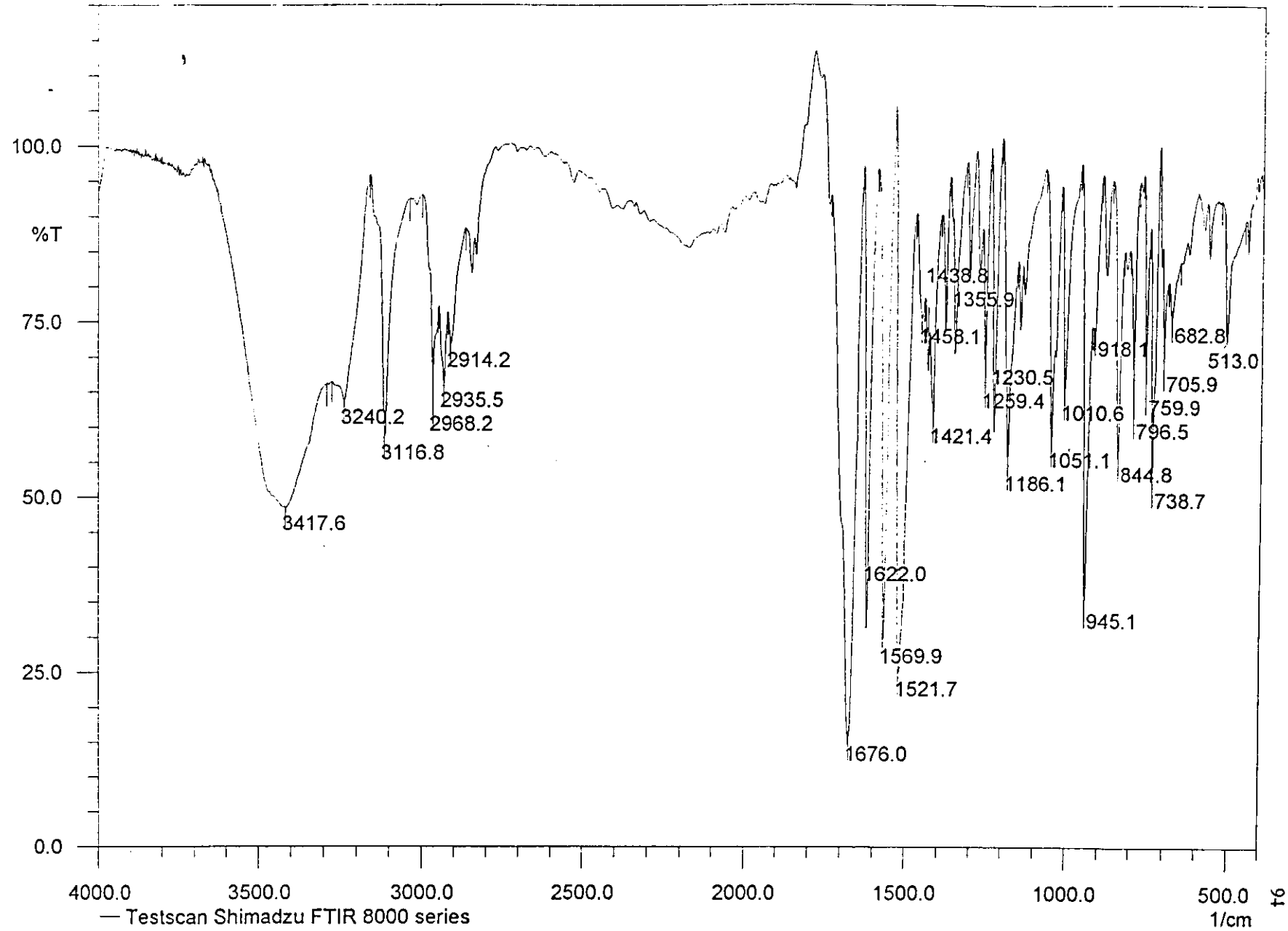


Figure (21): FT-IR spectrum of compound **27**.

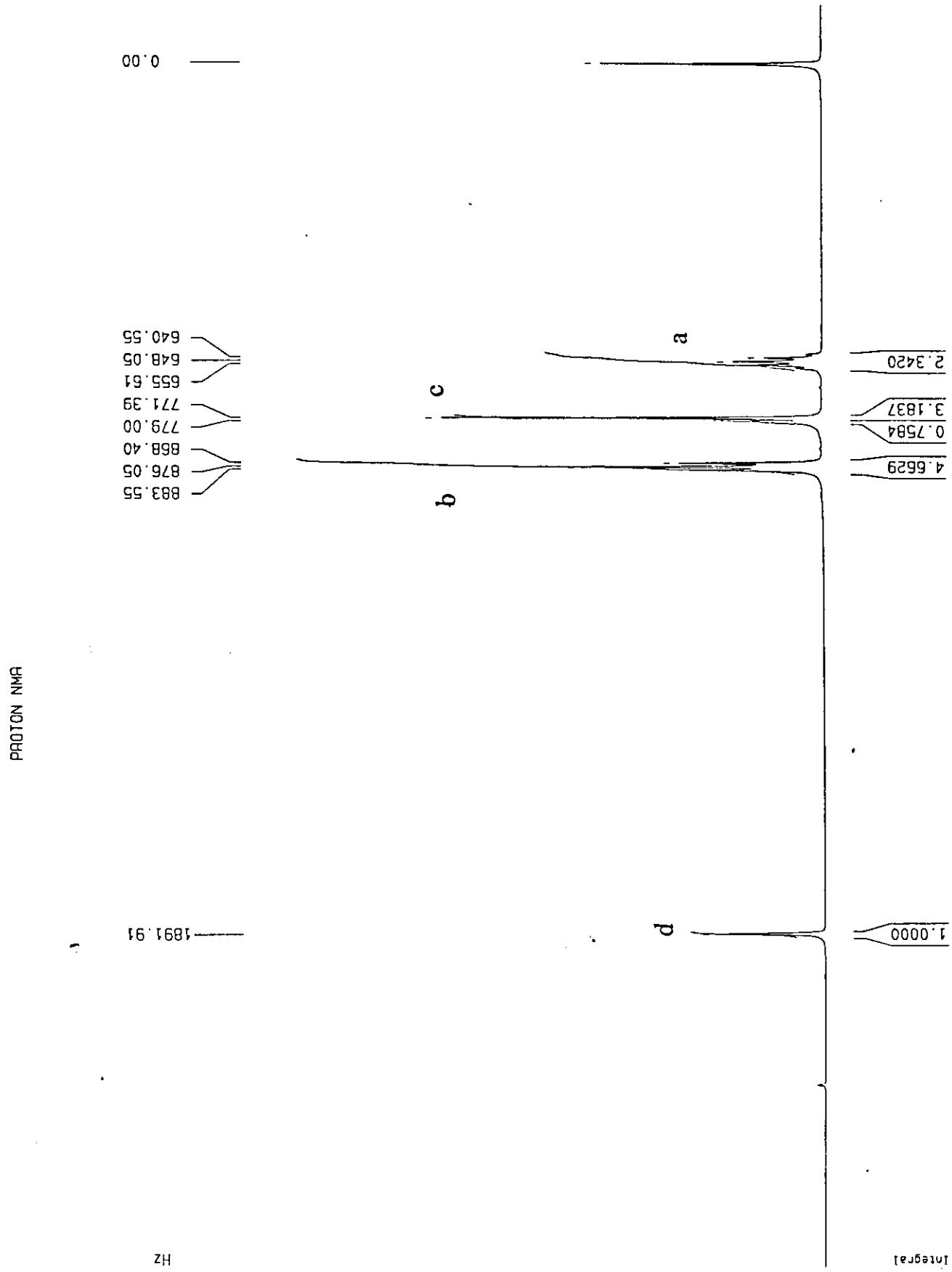


Figure (22): ^{1}H-NMR spectrum of compound **27**.

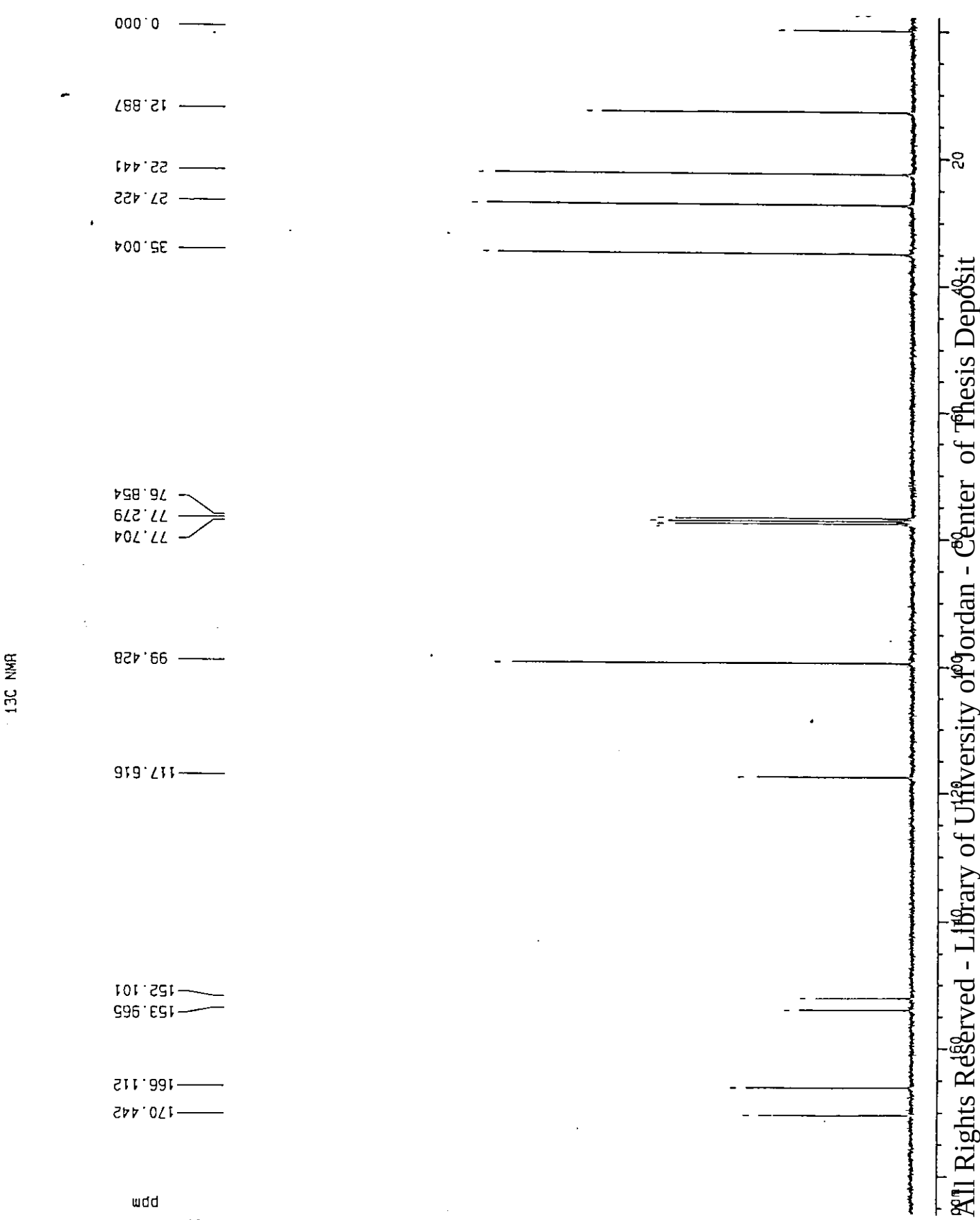


Figure (23): ^{13}C-NMR spectrum of compound **27**.

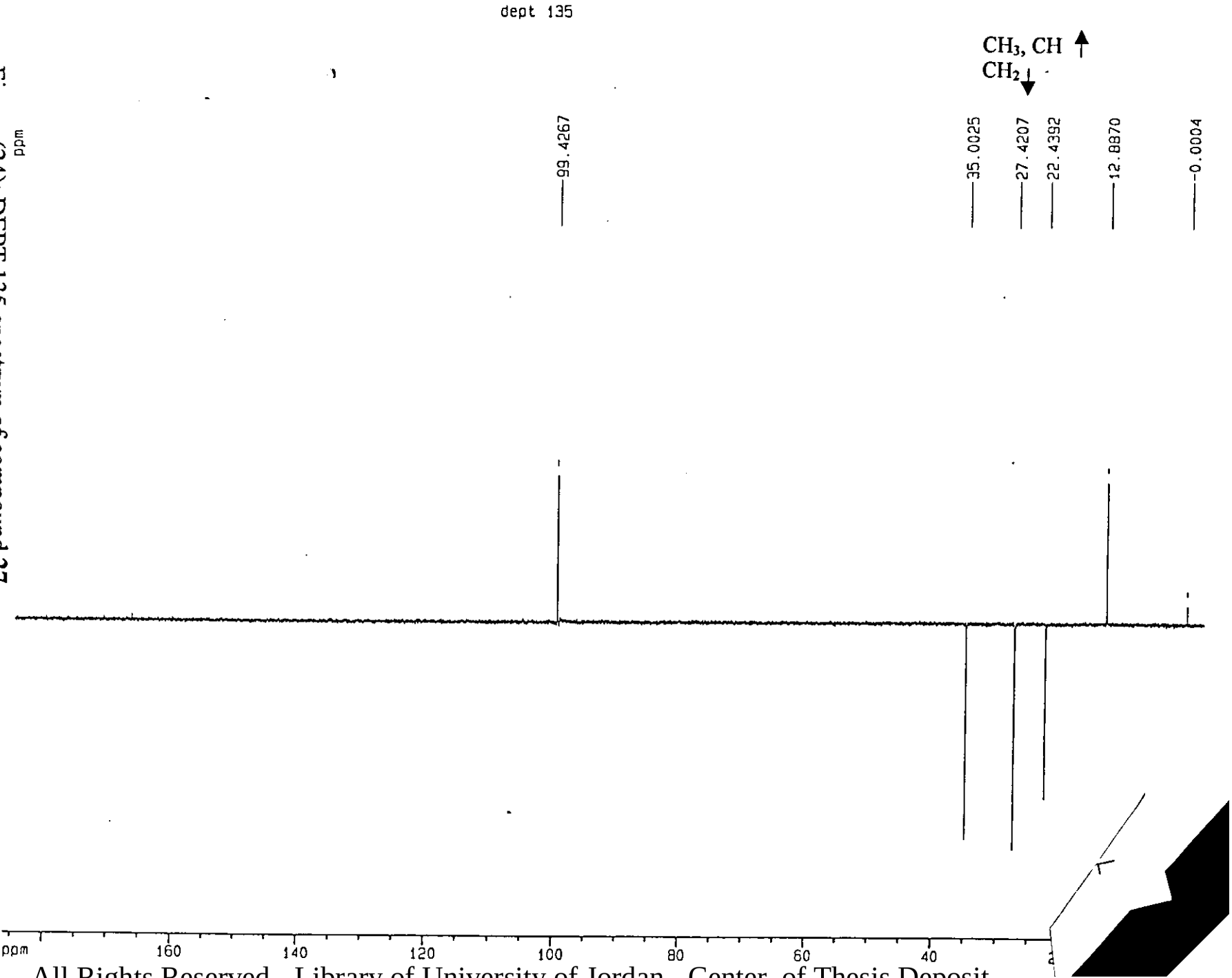


Figure (24): DEPT 135 spectrum of compound **27**.

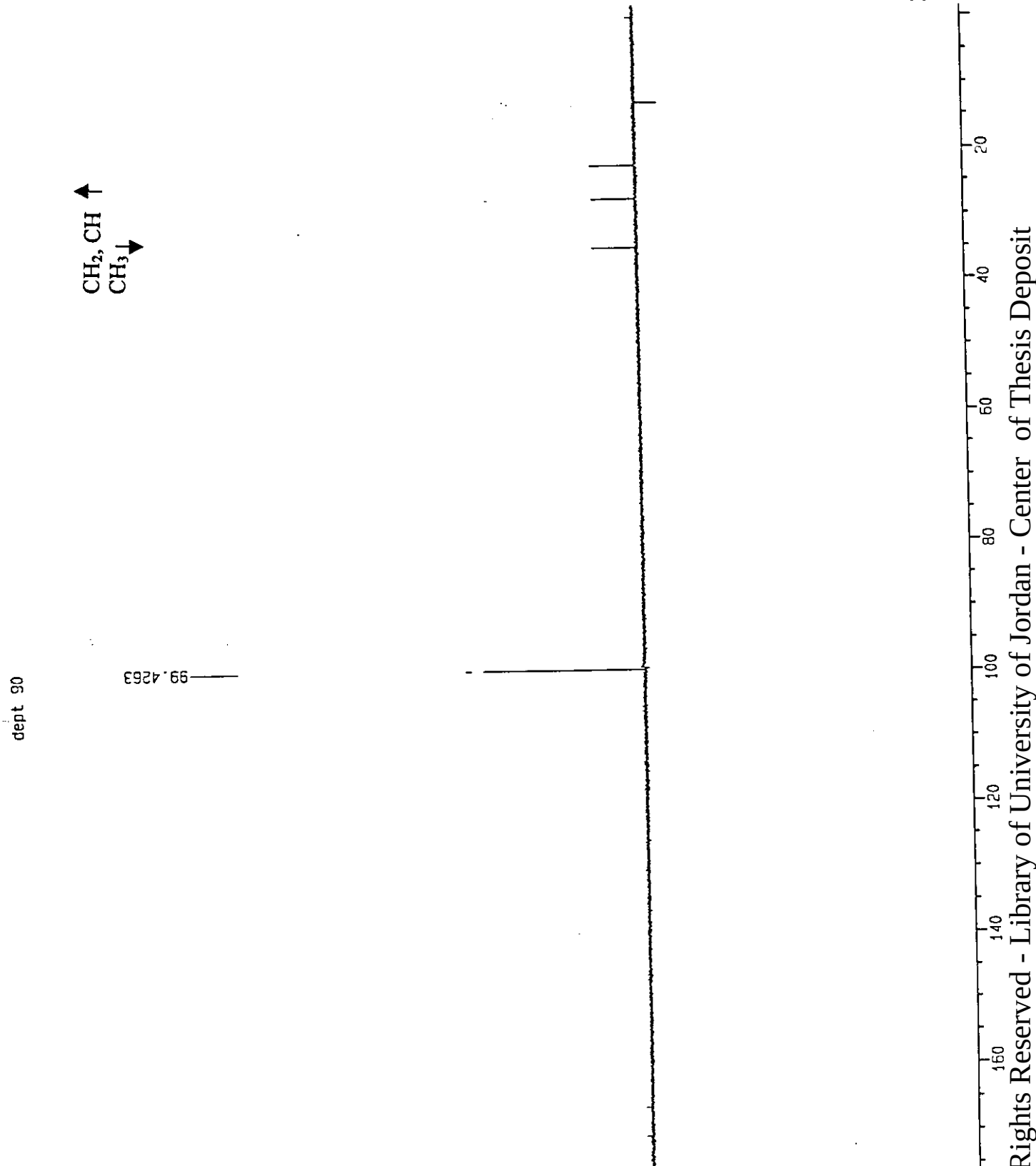


Figure (25): DEPT 90 spectrum of compound **27**.

Figure (26): Mass spectrum of compound **28**.

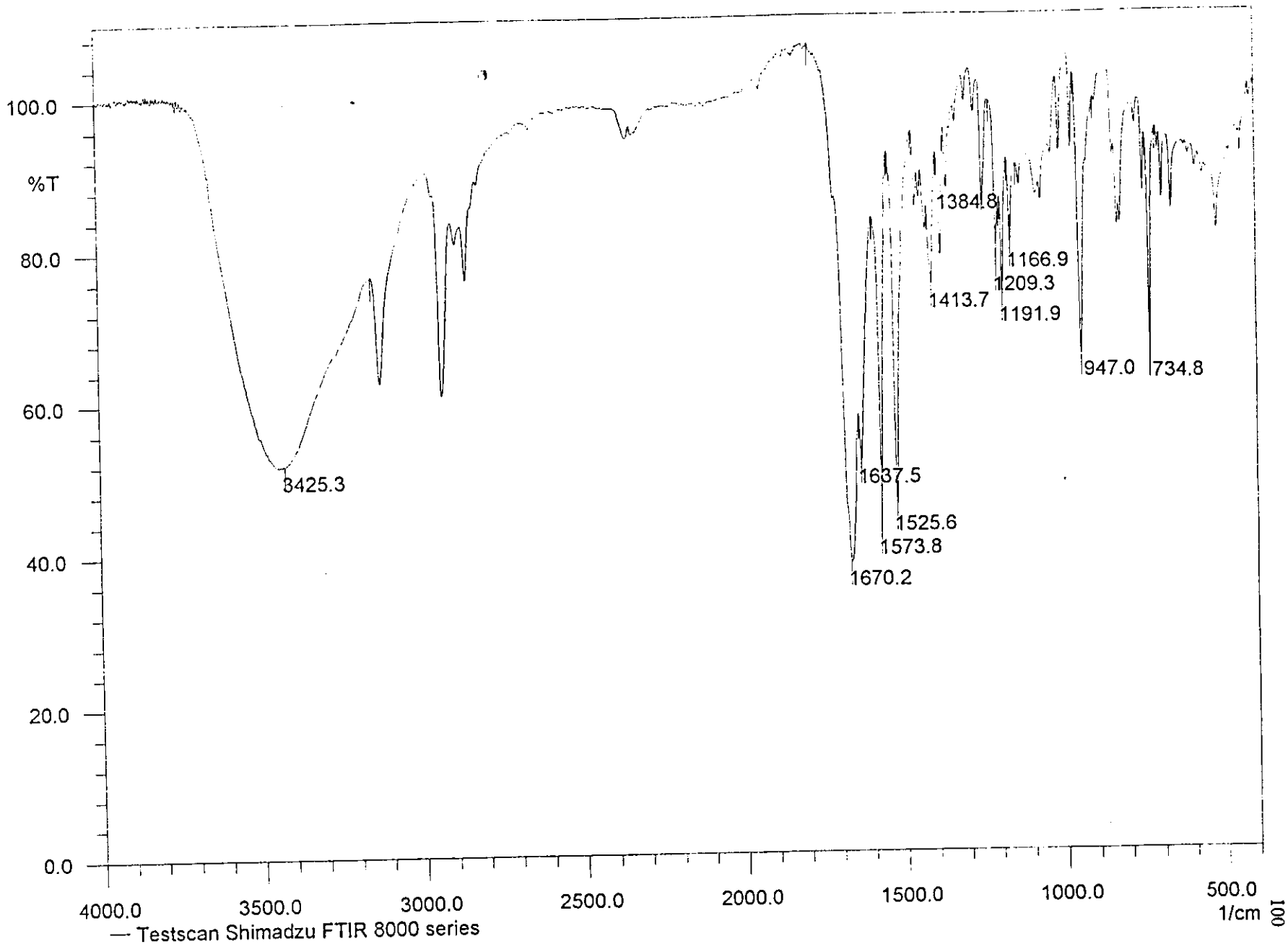


Figure (27): FT-IR spectrum of compound **28**.

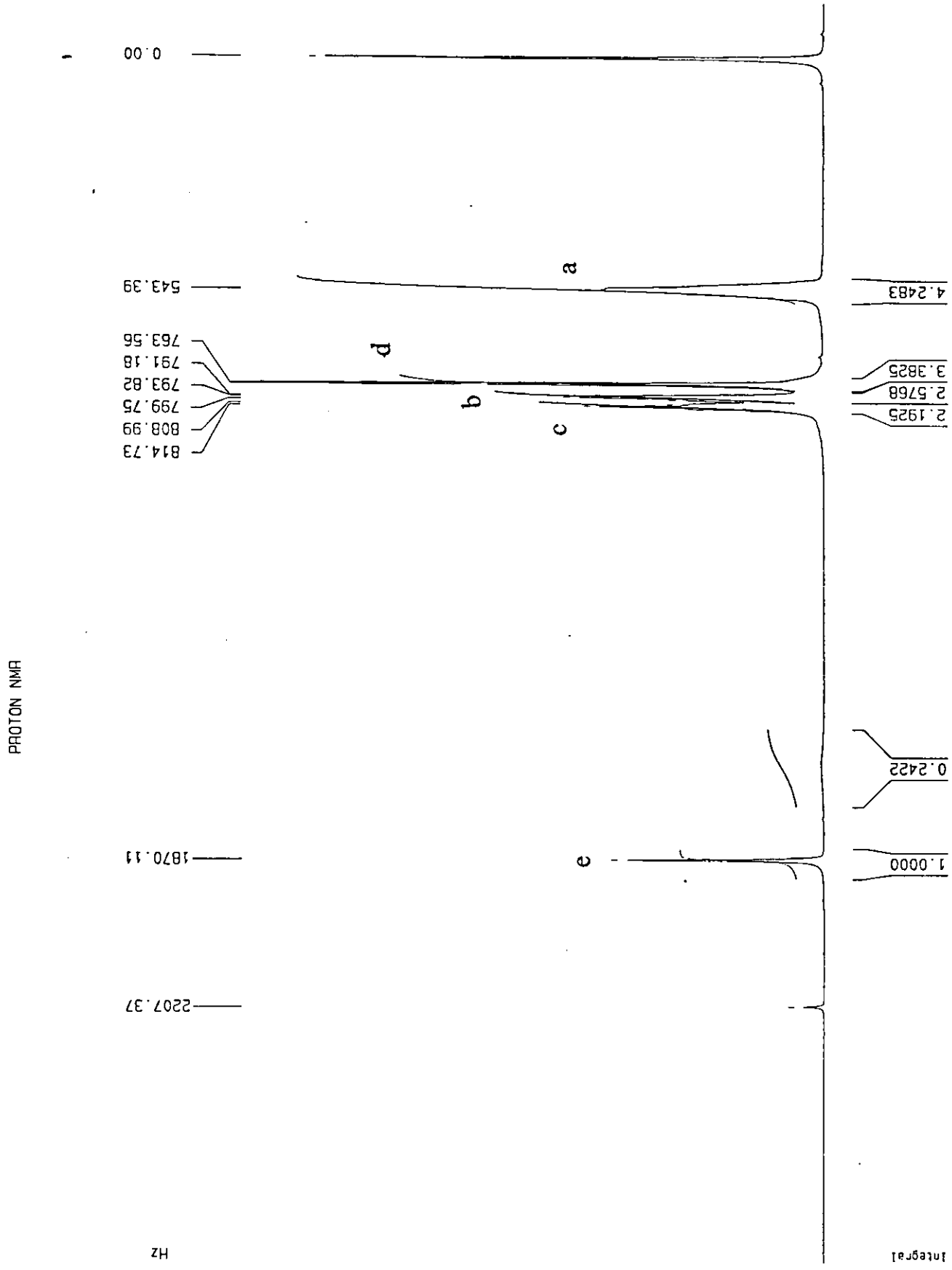


Figure (28): ^{1}H-NMR spectrum of compound **28**.

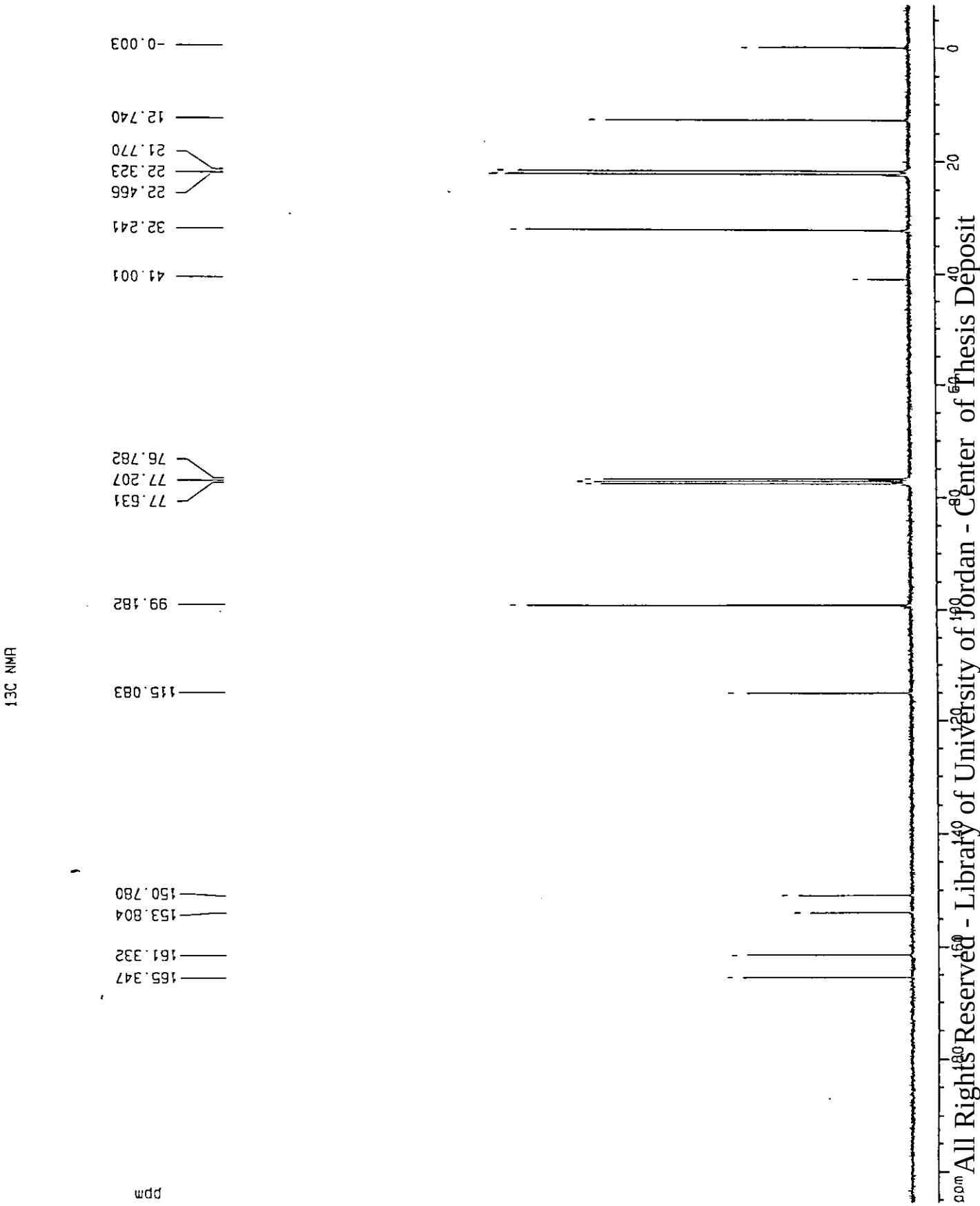


Figure (29): ^{13}C-NMR spectrum of compound **28**.

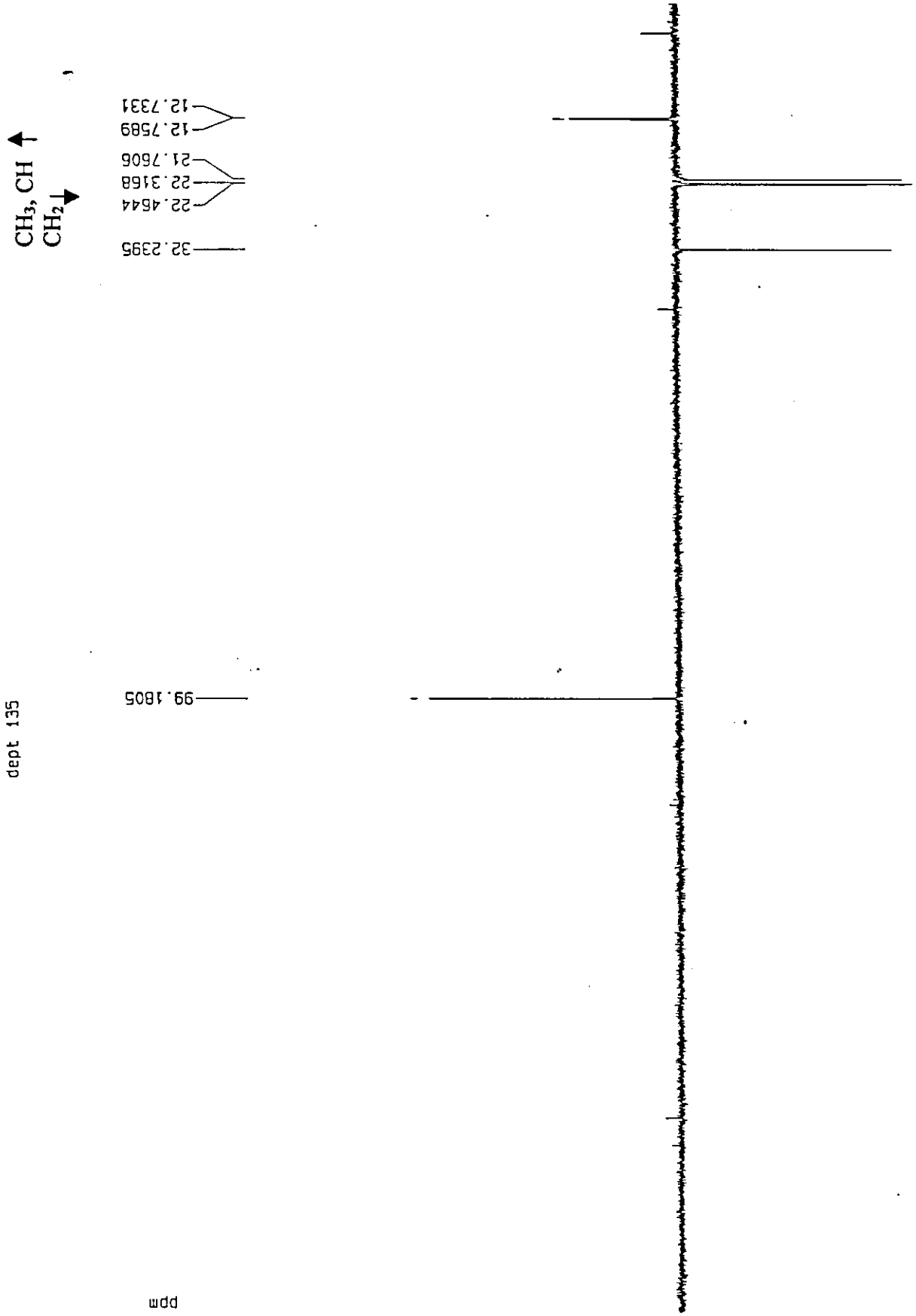


Figure (30): DEPT 135 spectrum of compound **28**.

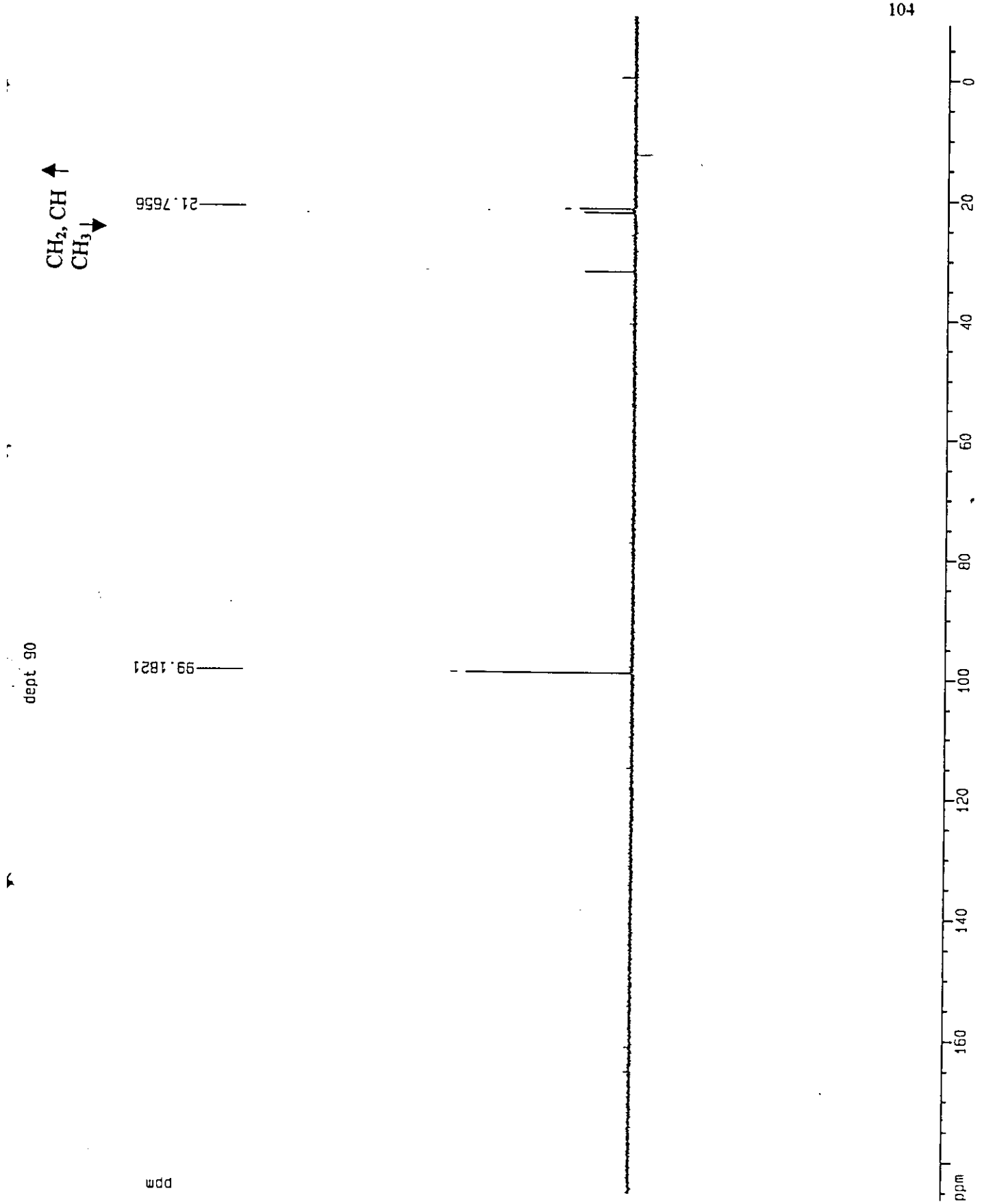


Figure (31): DEPT 90 spectrum of compound **28**.

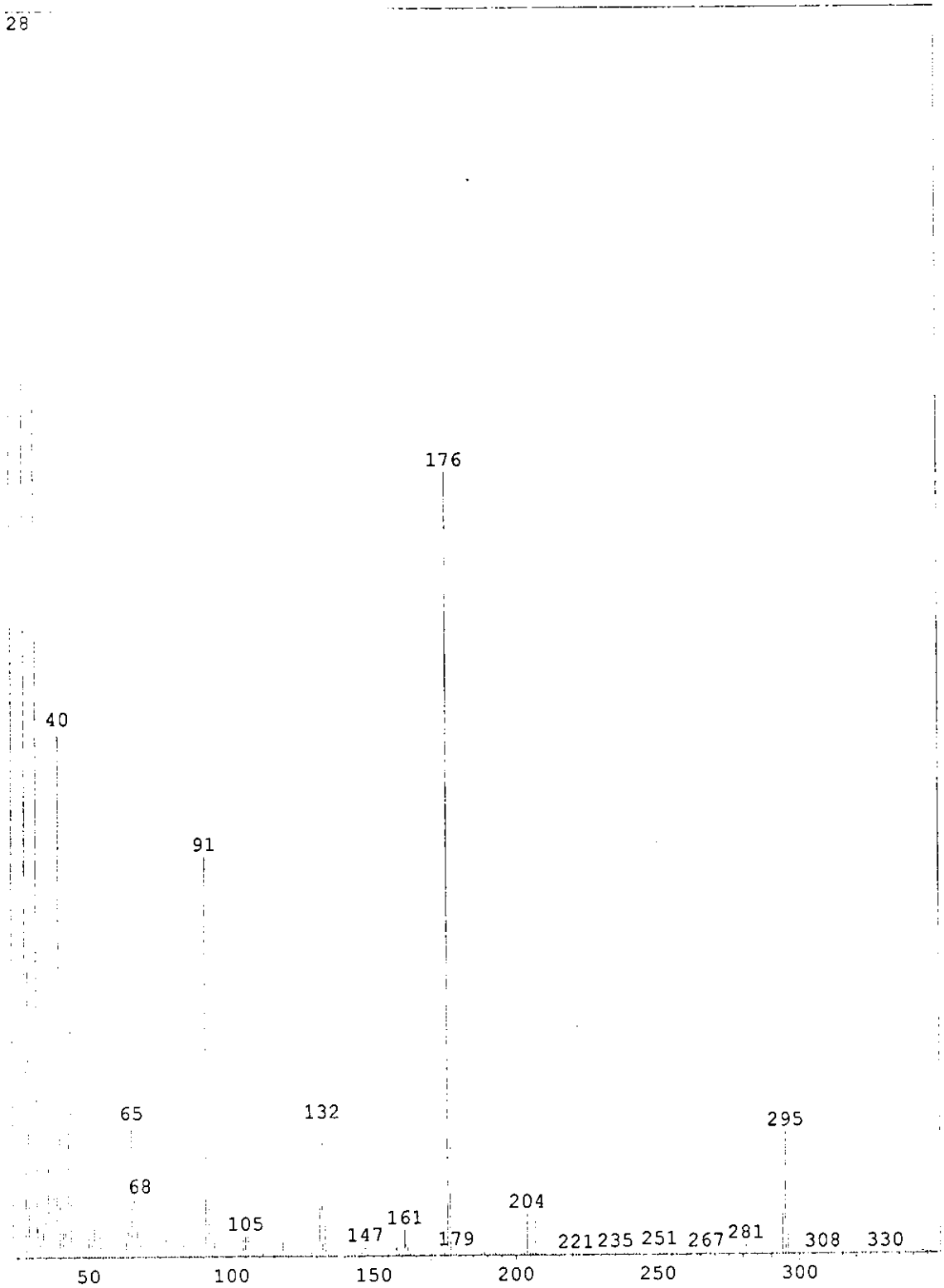


Figure (32): Mass spectrum of compound **29**.

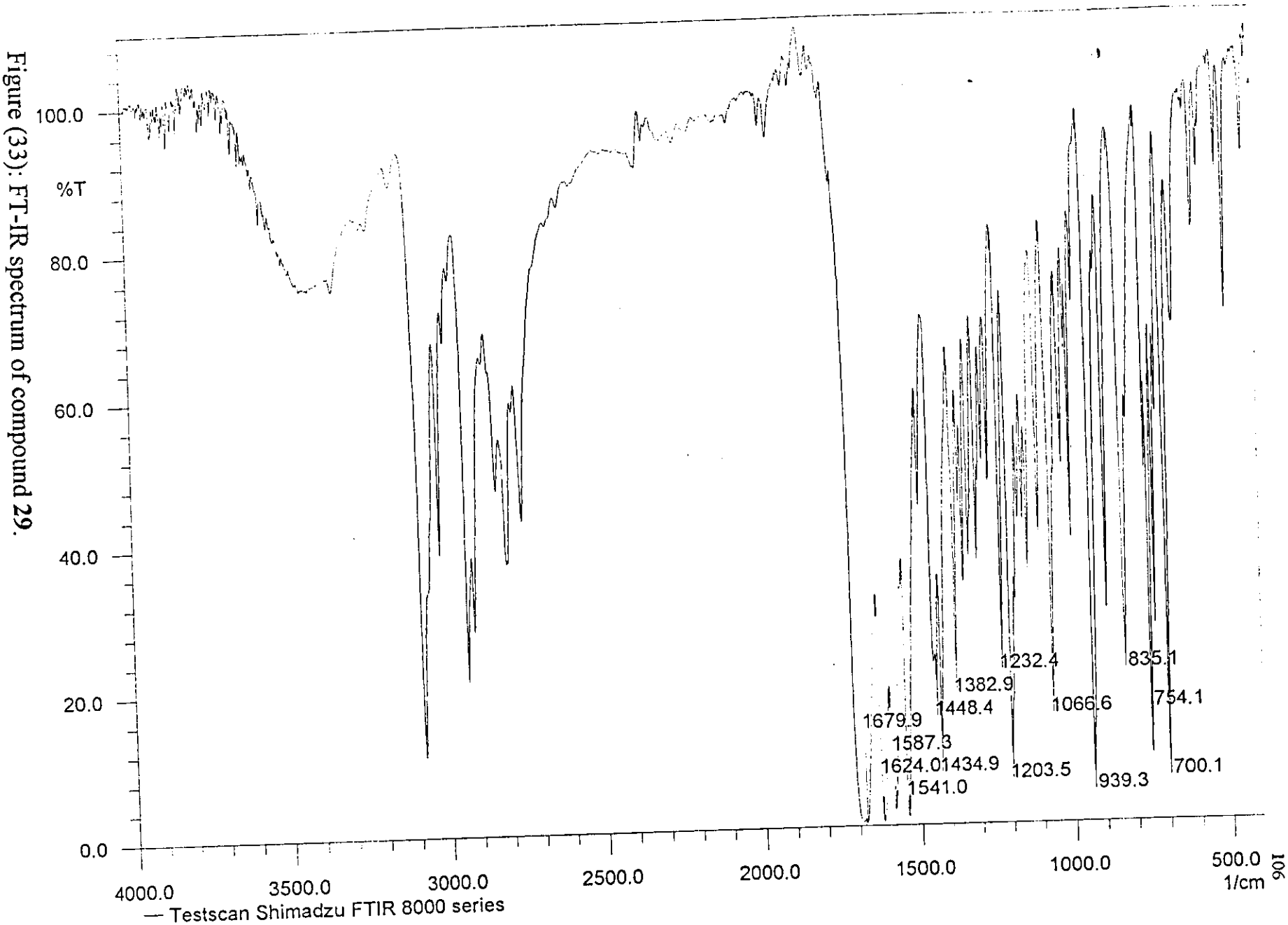


Figure (33): FT-IR spectrum of compound **29**.

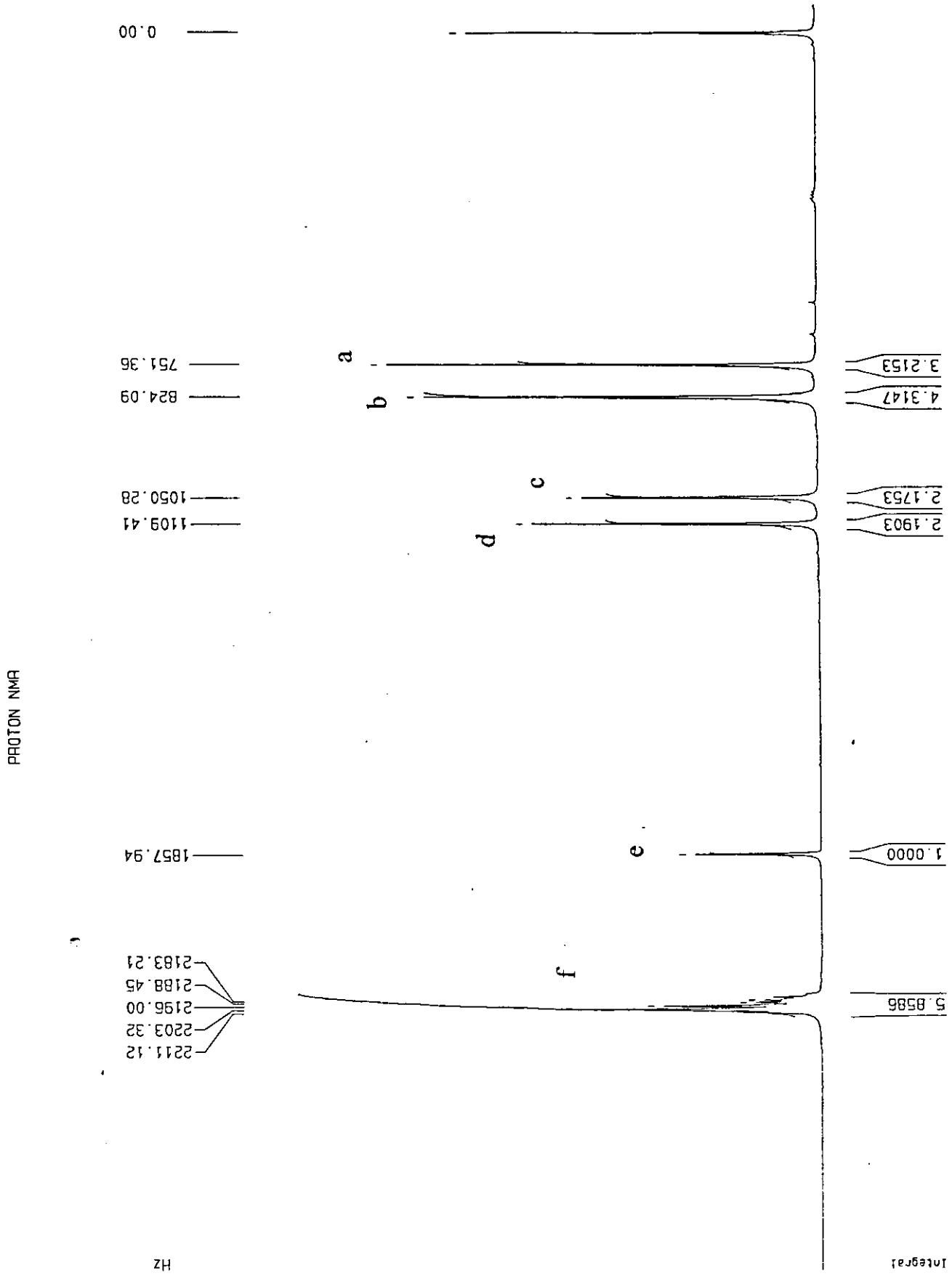


Figure (34): ^{1}H-NMR spectrum of compound **29**.

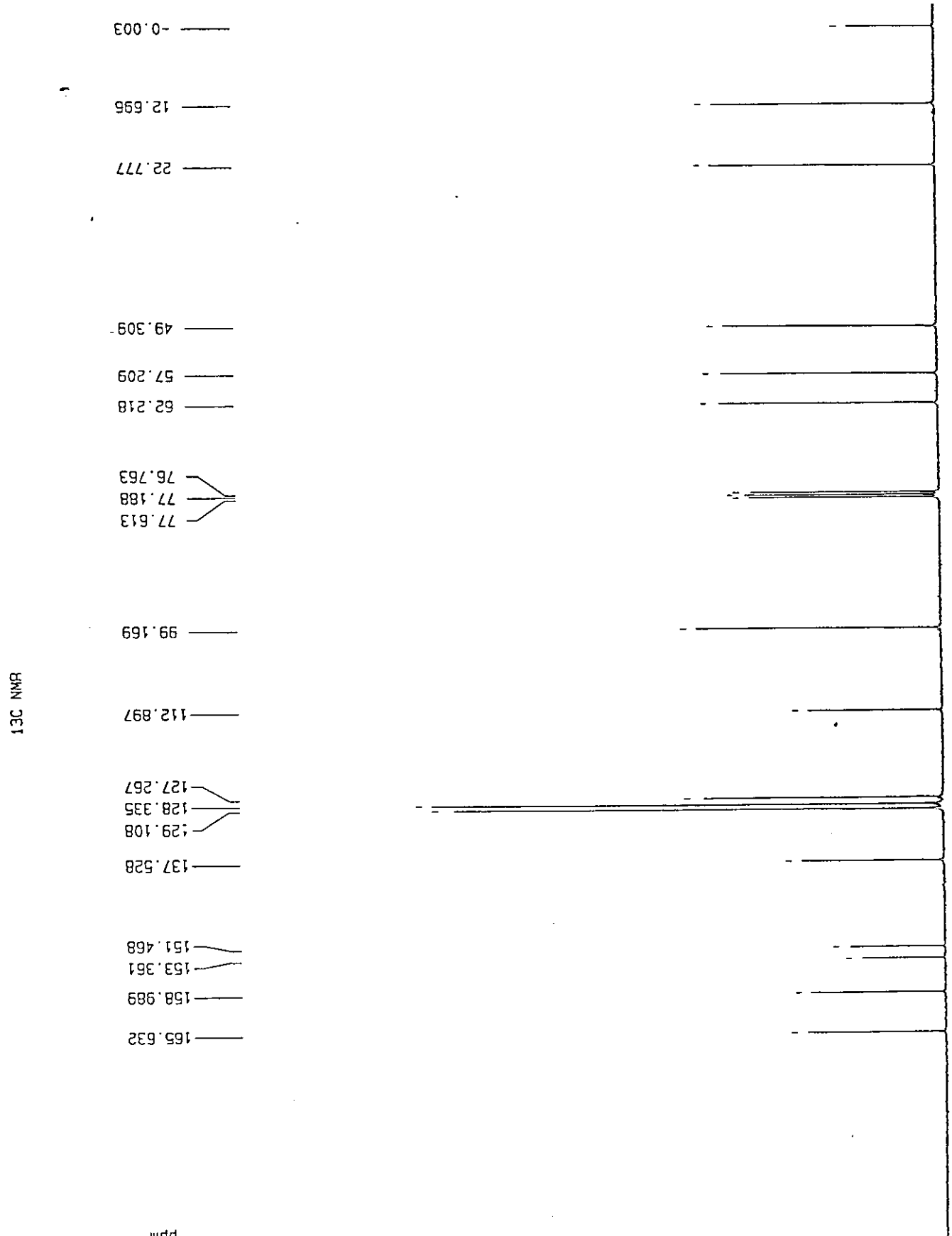


Figure (35): ^{13}C-NMR spectrum of compound **29**.

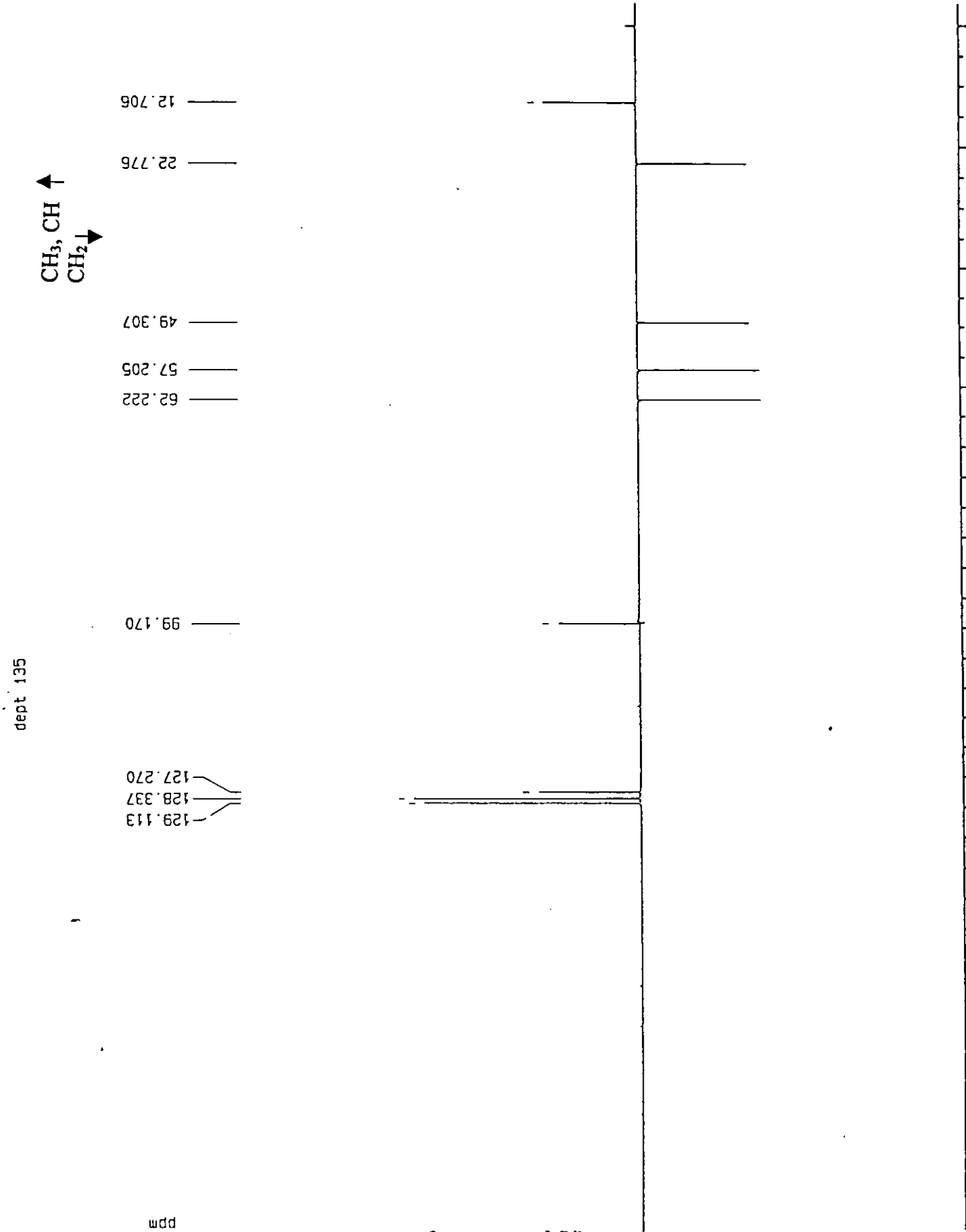


Figure (36): DEPT 135 spectrum of compound **29**.

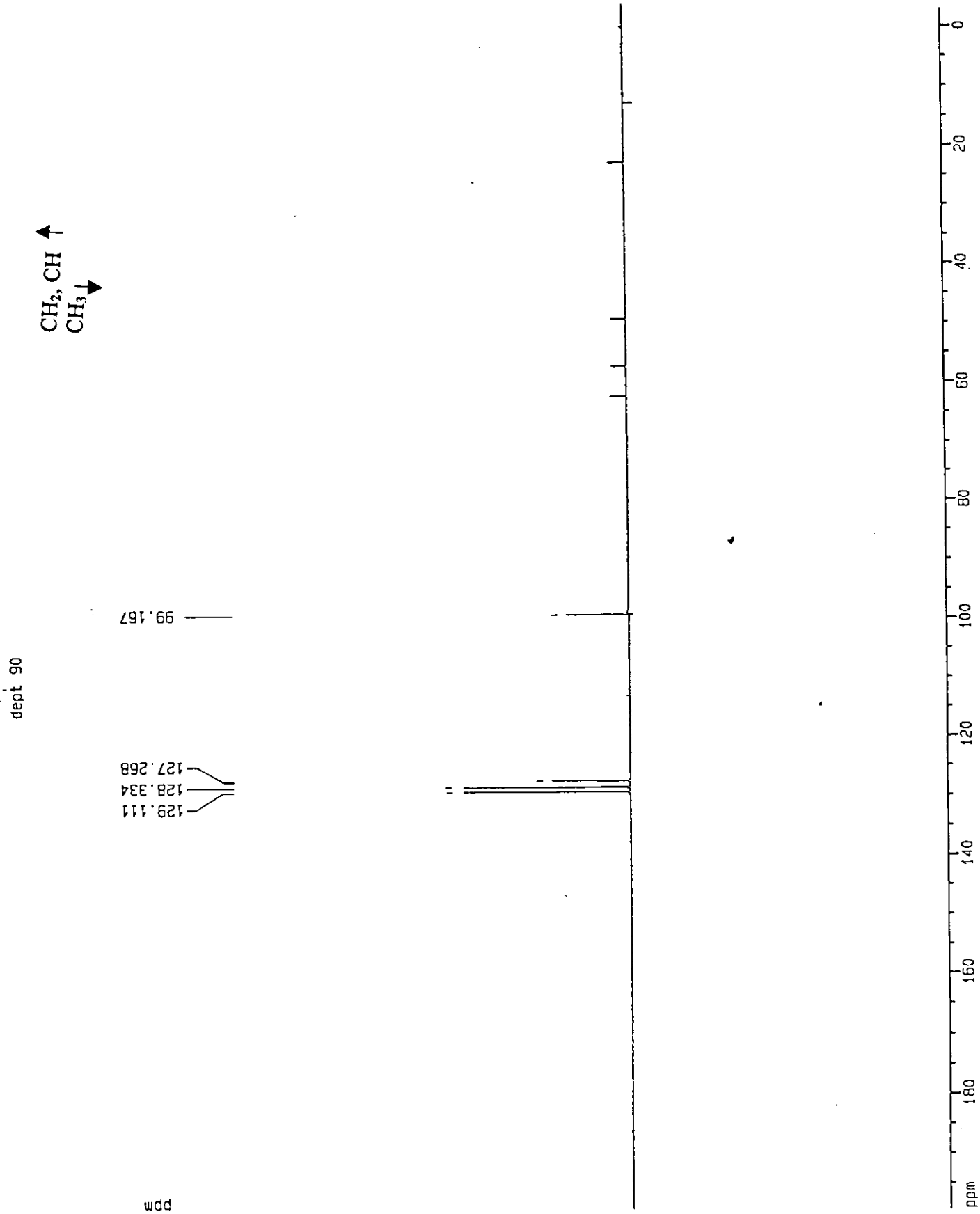


Figure (37): DEPT 90 spectrum of compound **29**.

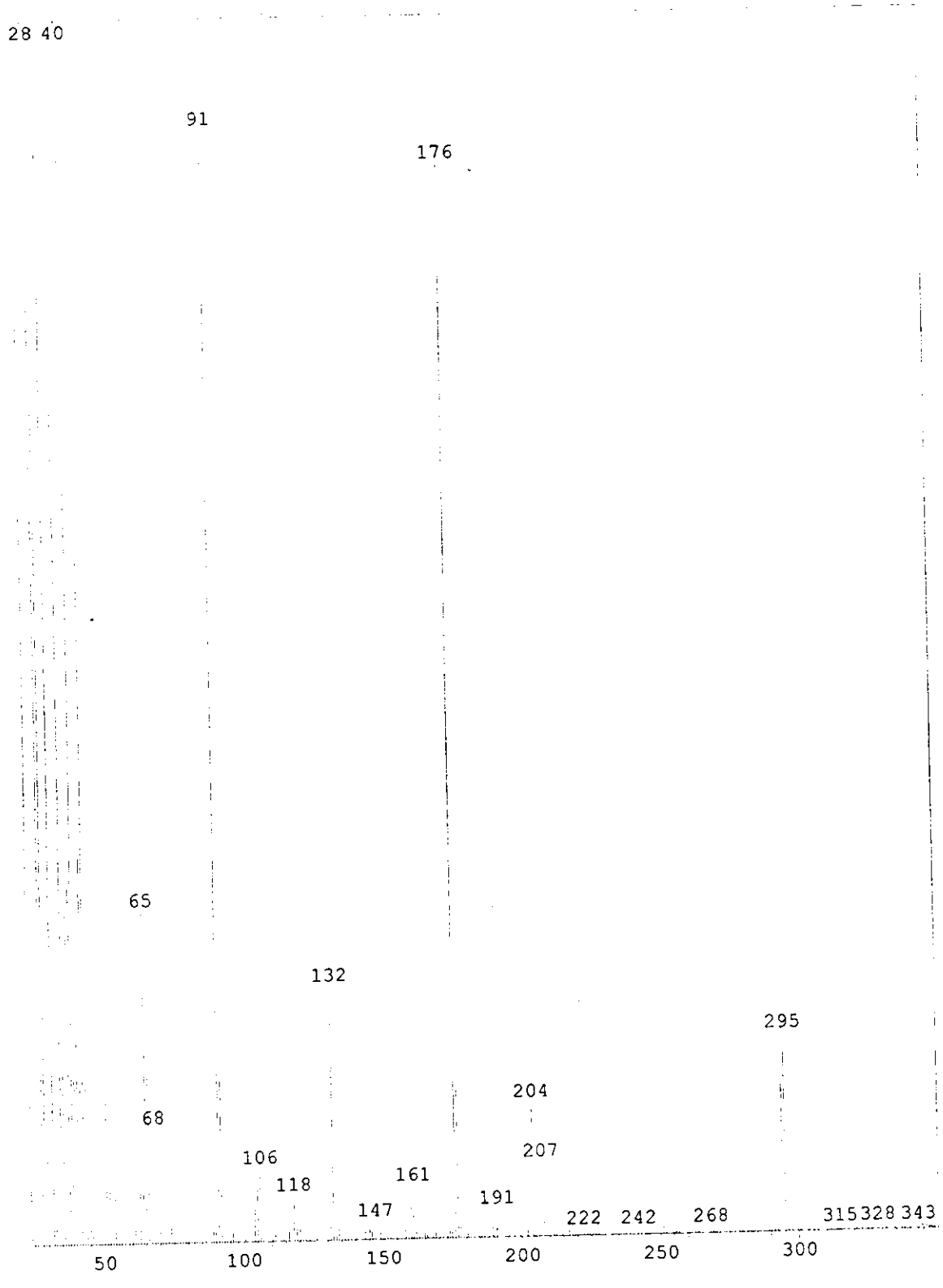


Figure (38): Mass spectrum of compound **30**.

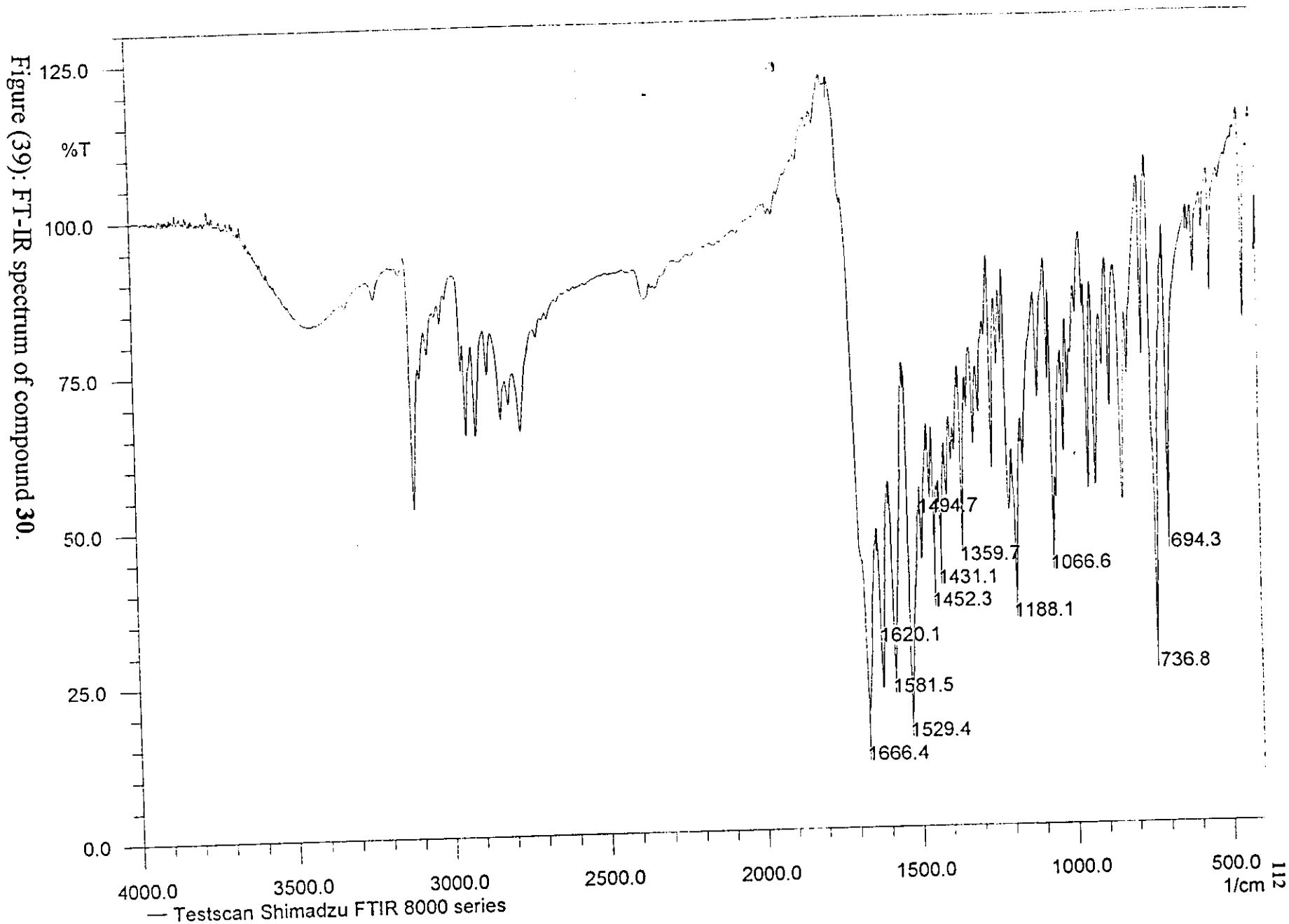


Figure (39): FT-IR spectrum of compound **30**.

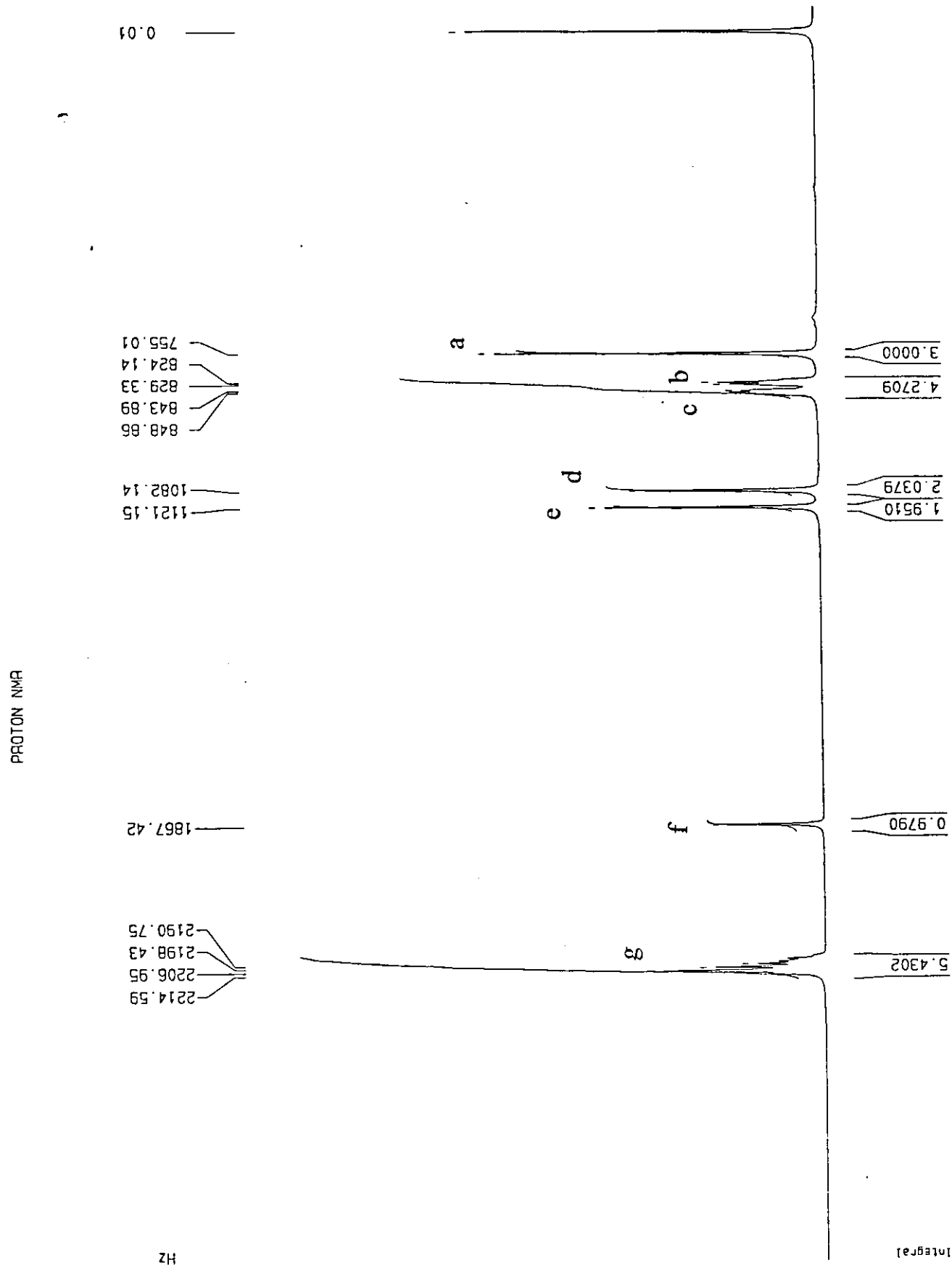


Figure (40): ^{1}H-NMR spectrum of compound **30**.

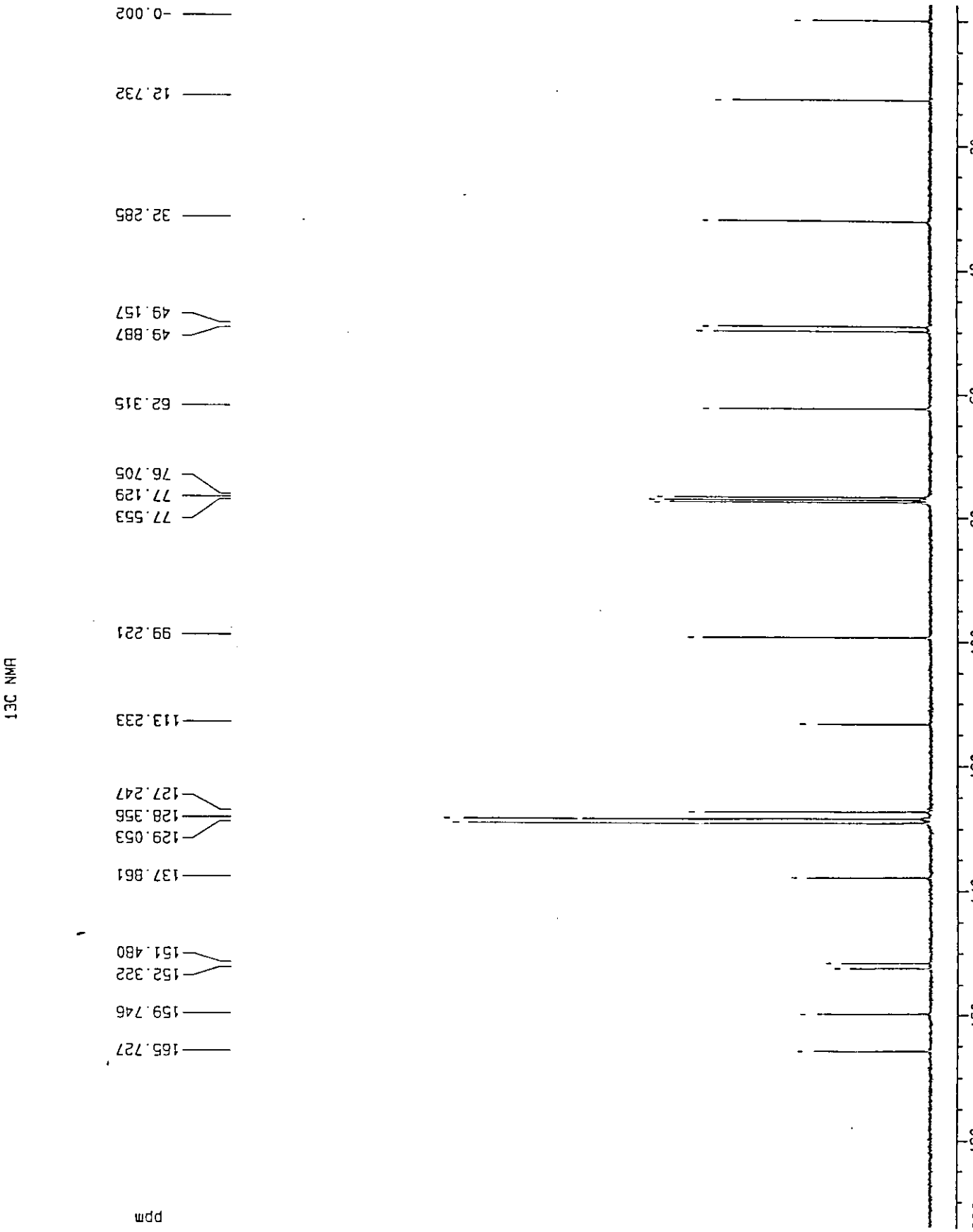


Figure (41): ^{13}C-NMR spectrum of compound **30**.

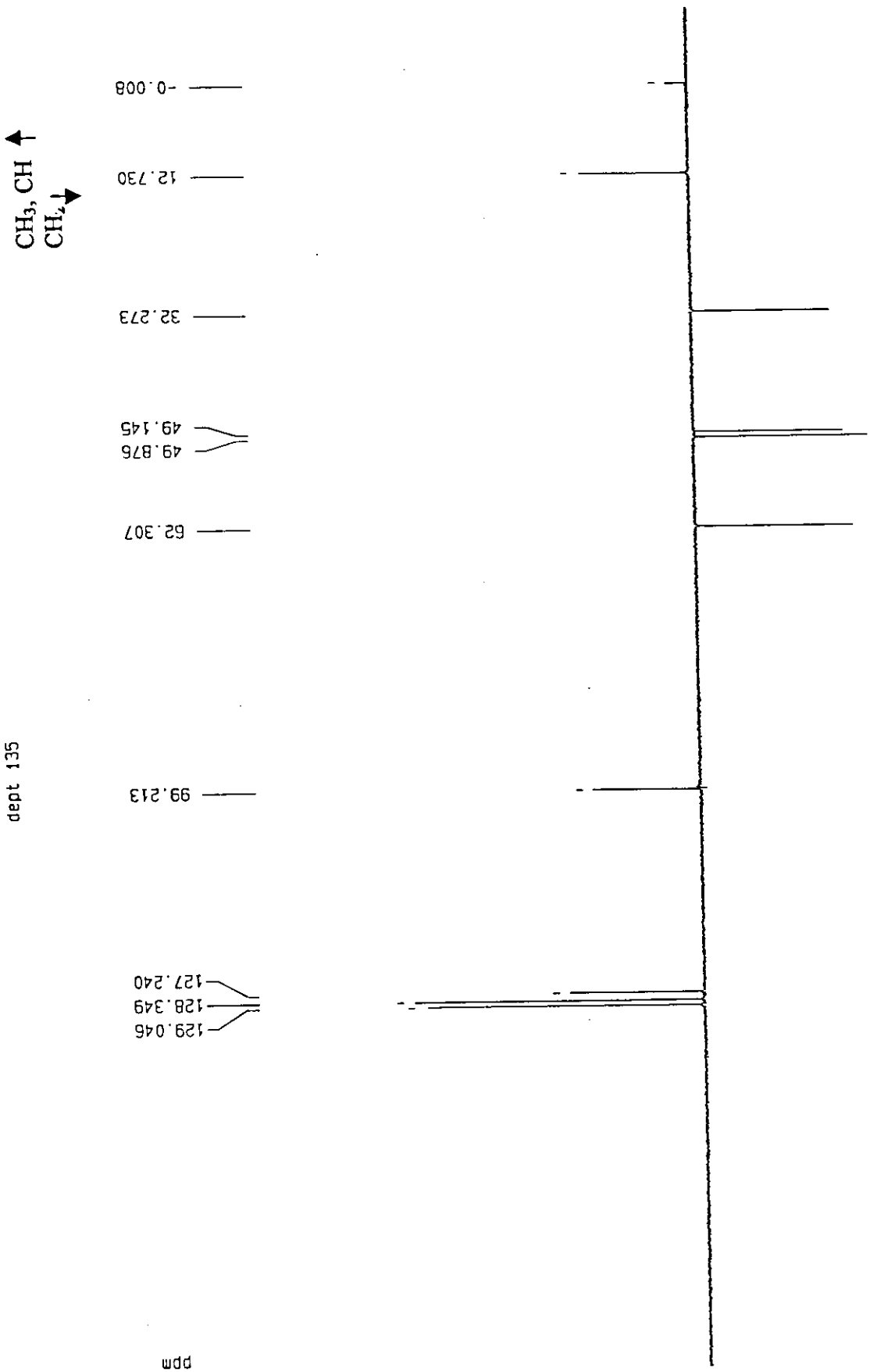


Figure (42): DEPT 135 spectrum of compound **30**.

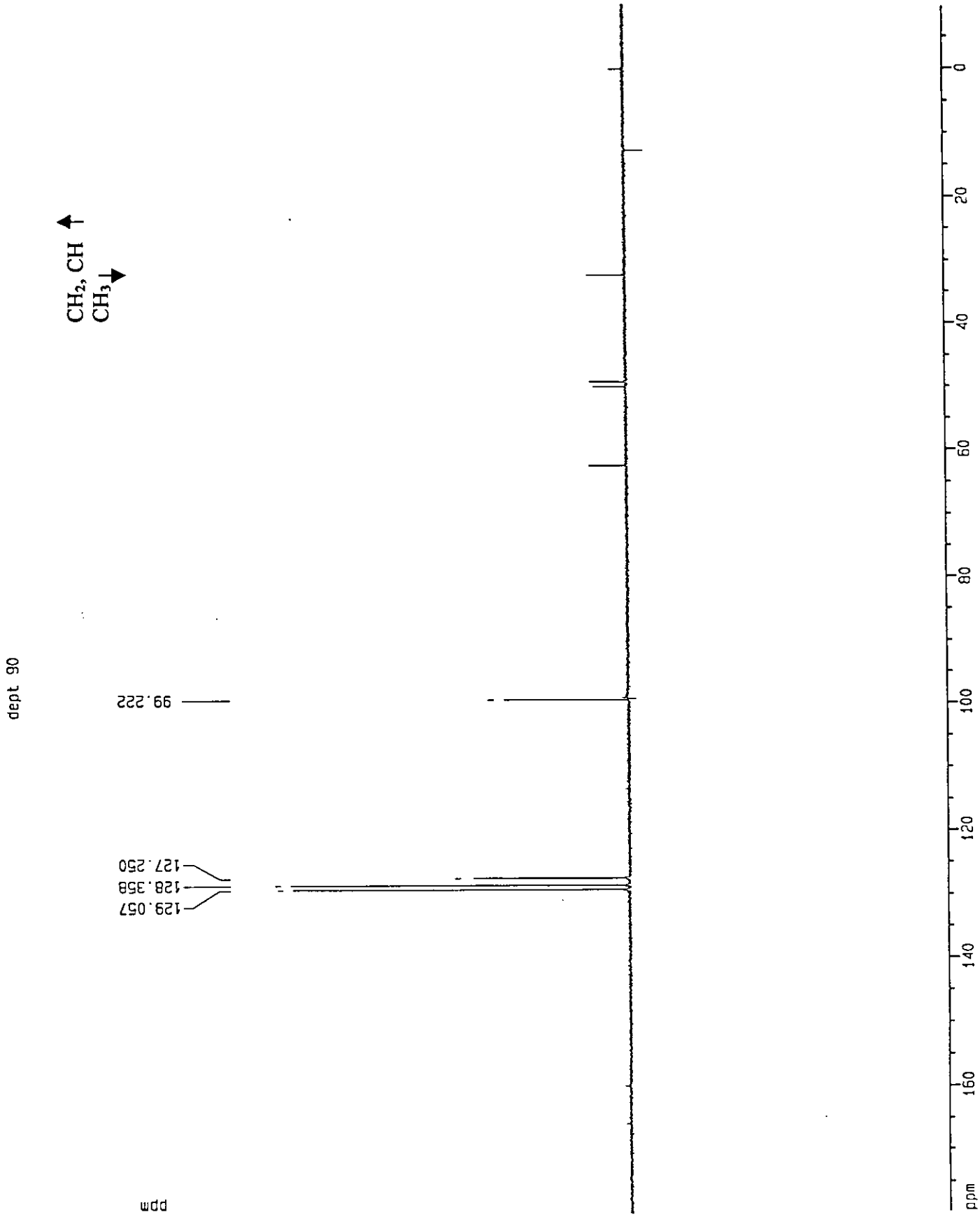


Figure (43): DEPT 90 spectrum of compound **30**.

Figure (44): Mass spectrum of compound **31**.

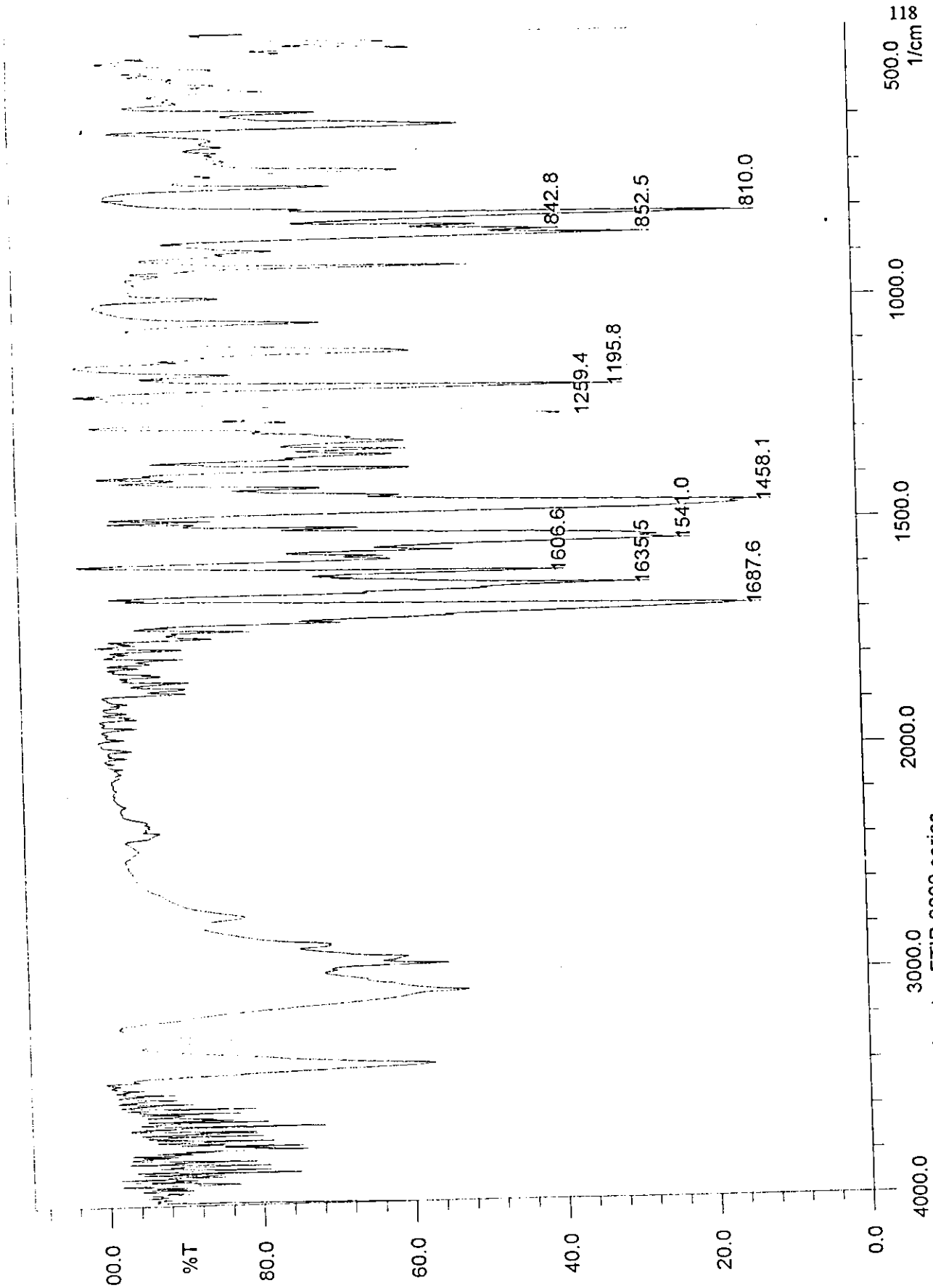


Figure (45): FT-IR spectrum of compound **31**.

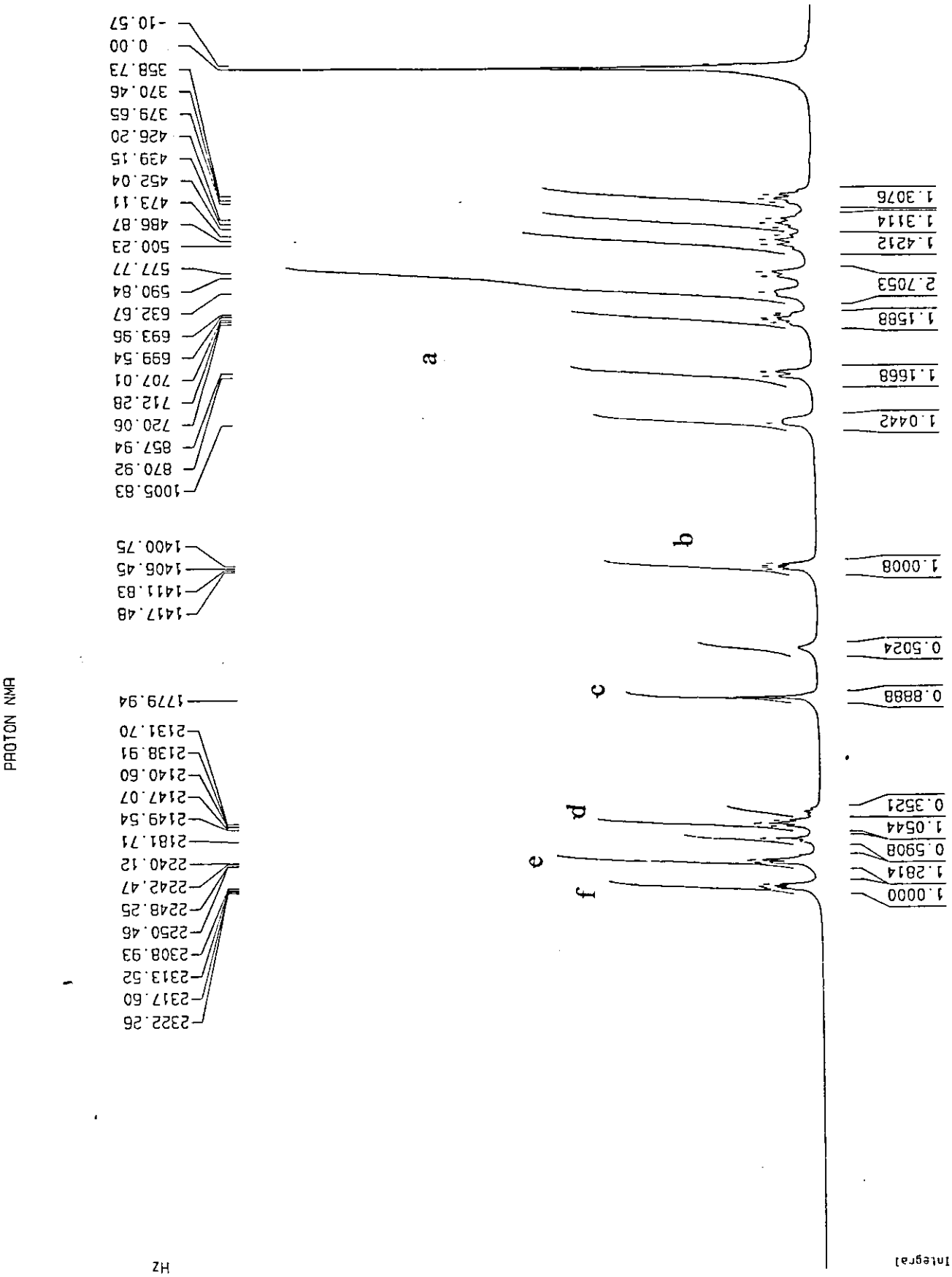


Figure (46): ^{1}H-NMR spectrum of compound **31**.

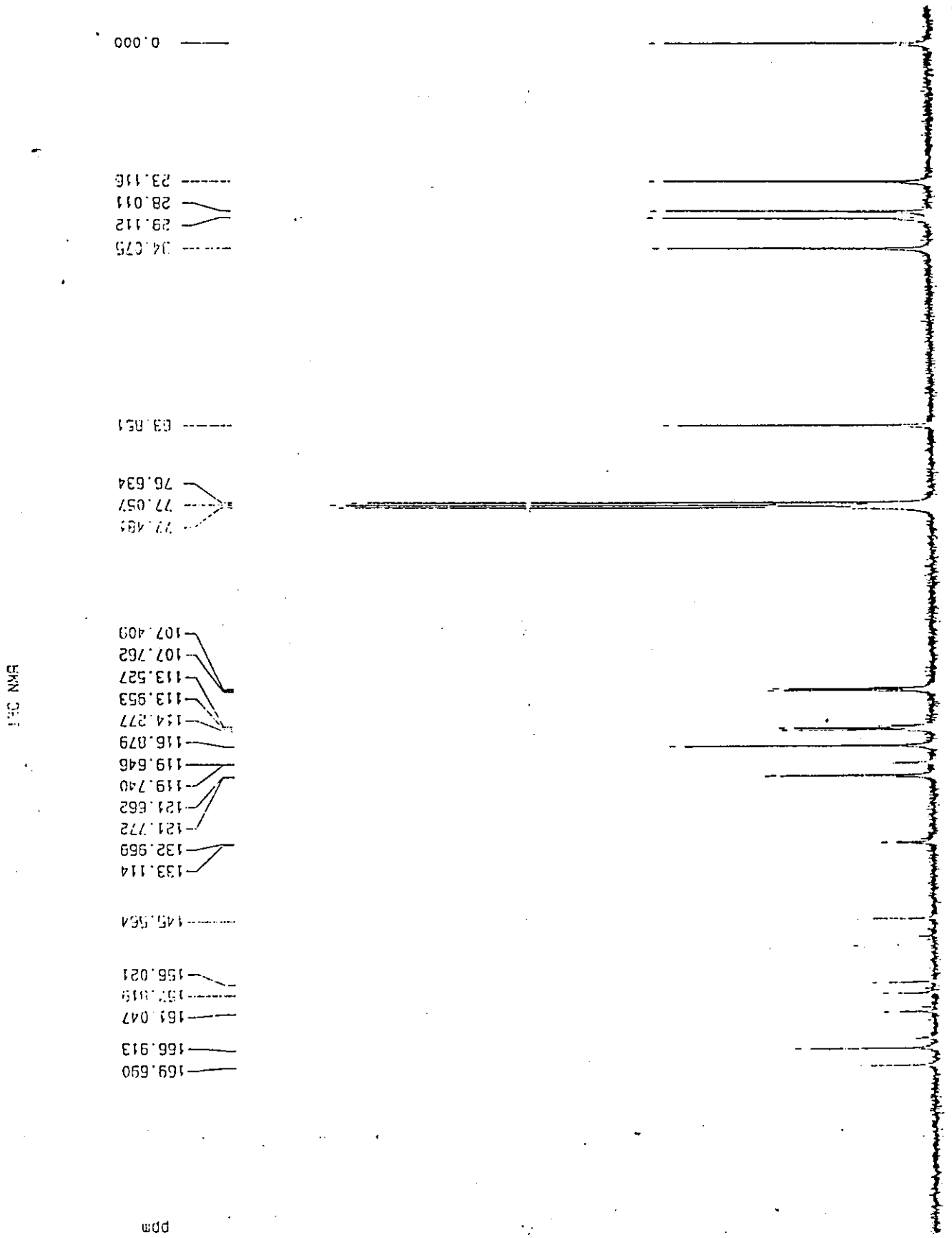


Figure (47): ^{13}C-NMR spectrum of compound **31**.

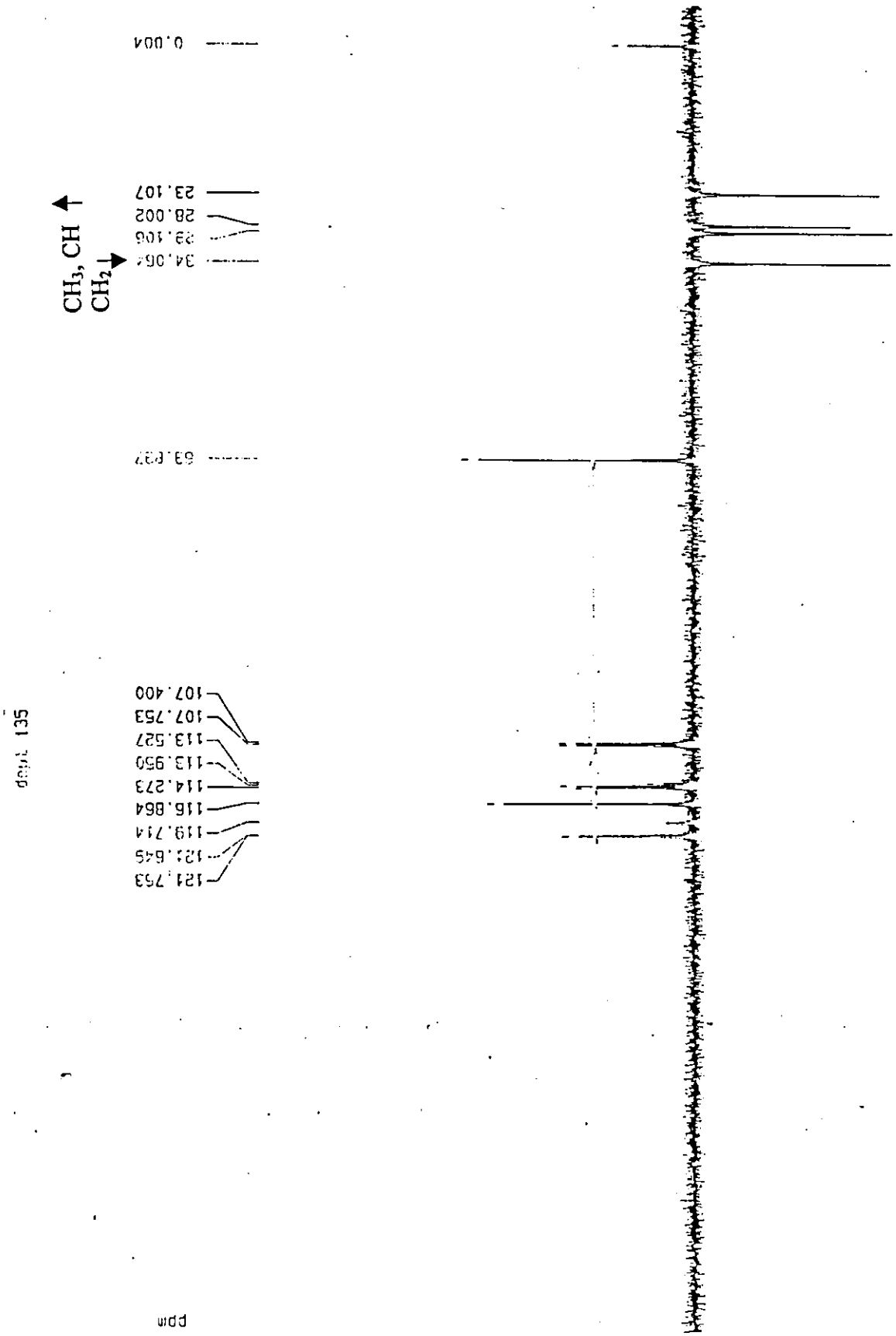


Figure (48): DEPT 135 spectrum of compound **31**.

Figure (49): Mass spectrum of compound **32**.

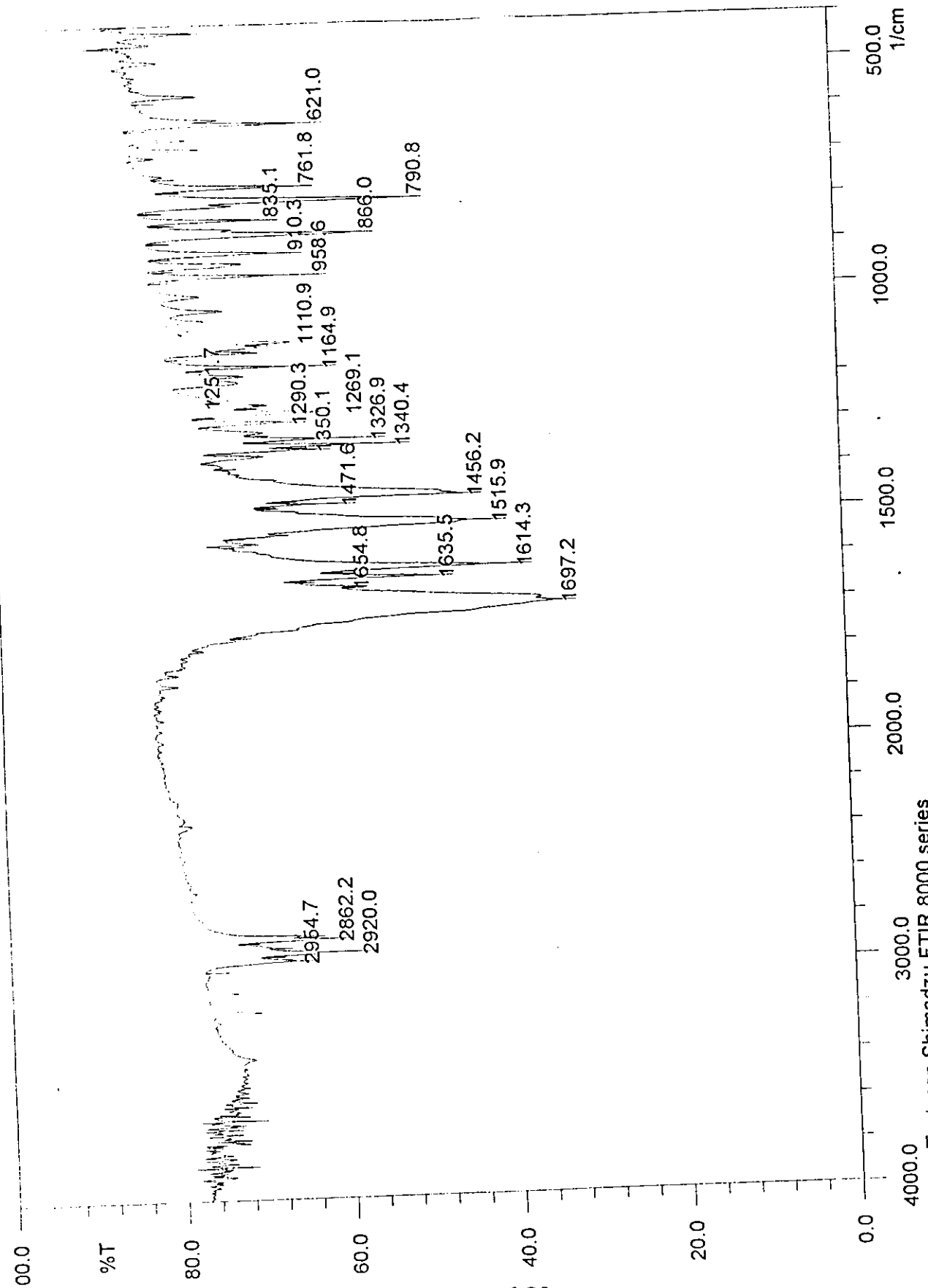


Figure (50): FT-IR spectrum of compound **32**.

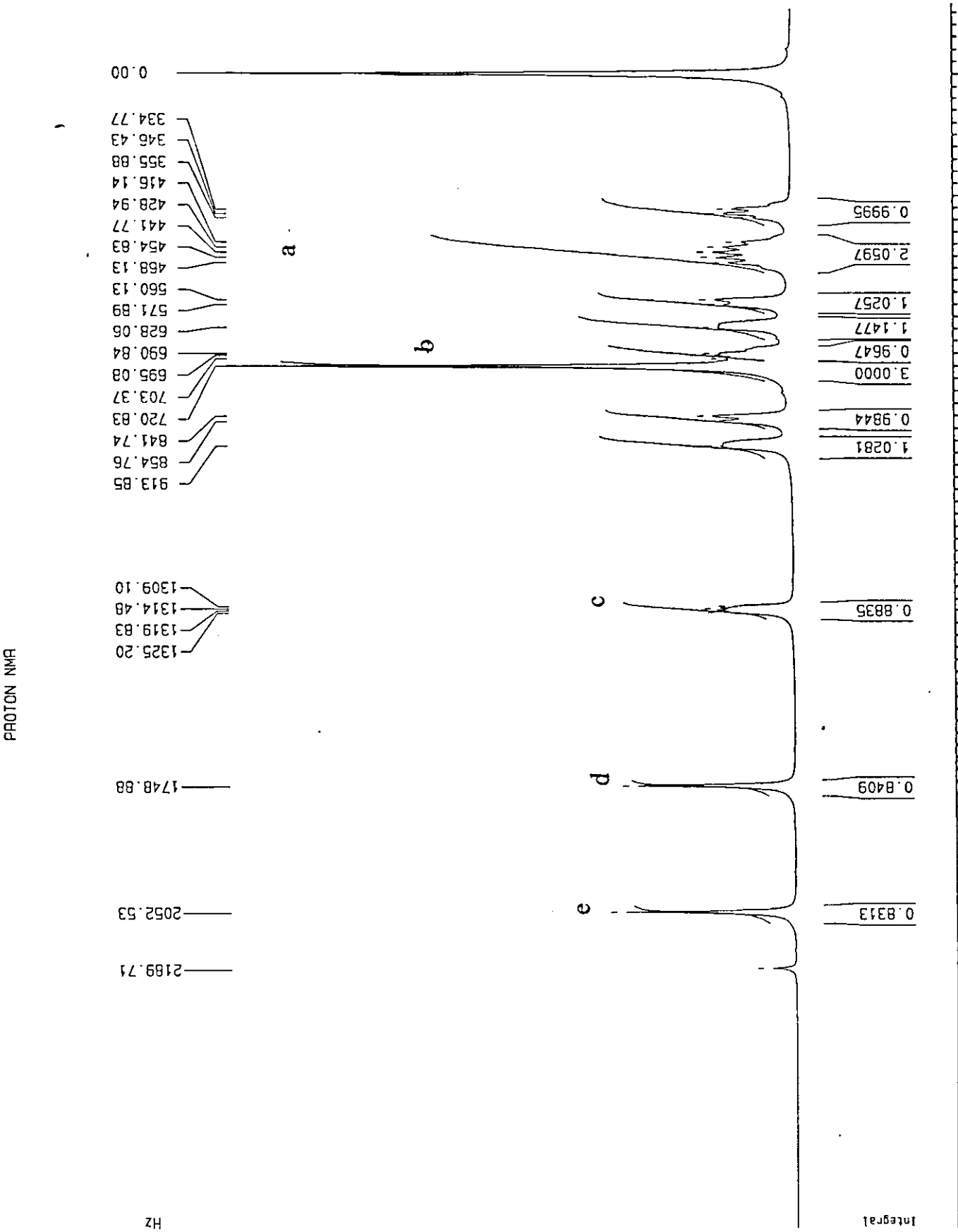


Figure (51): ^{1}H-NMR spectrum of compound **32**.

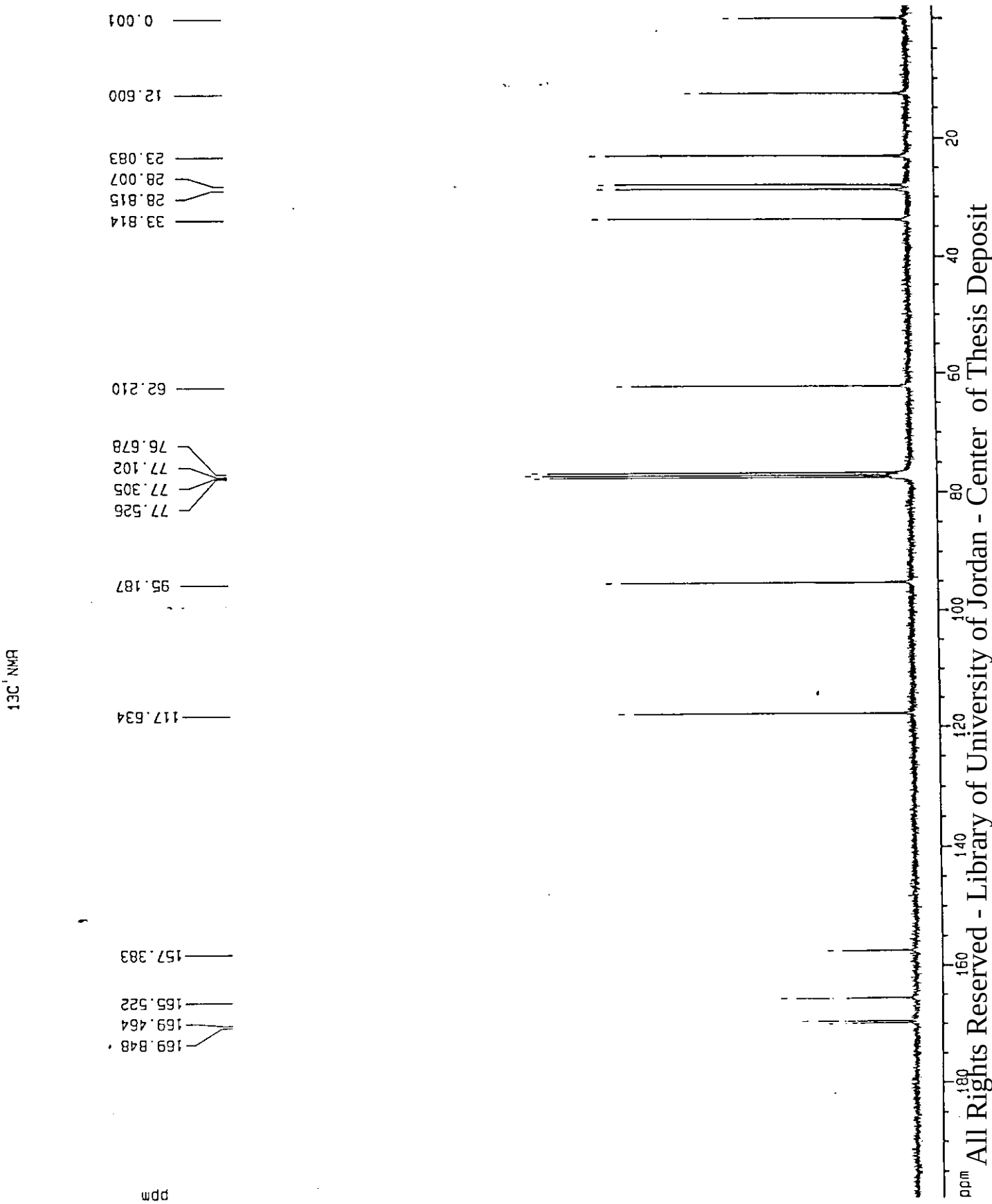


Figure (52): ^{13}C-NMR spectrum of compound **32**.

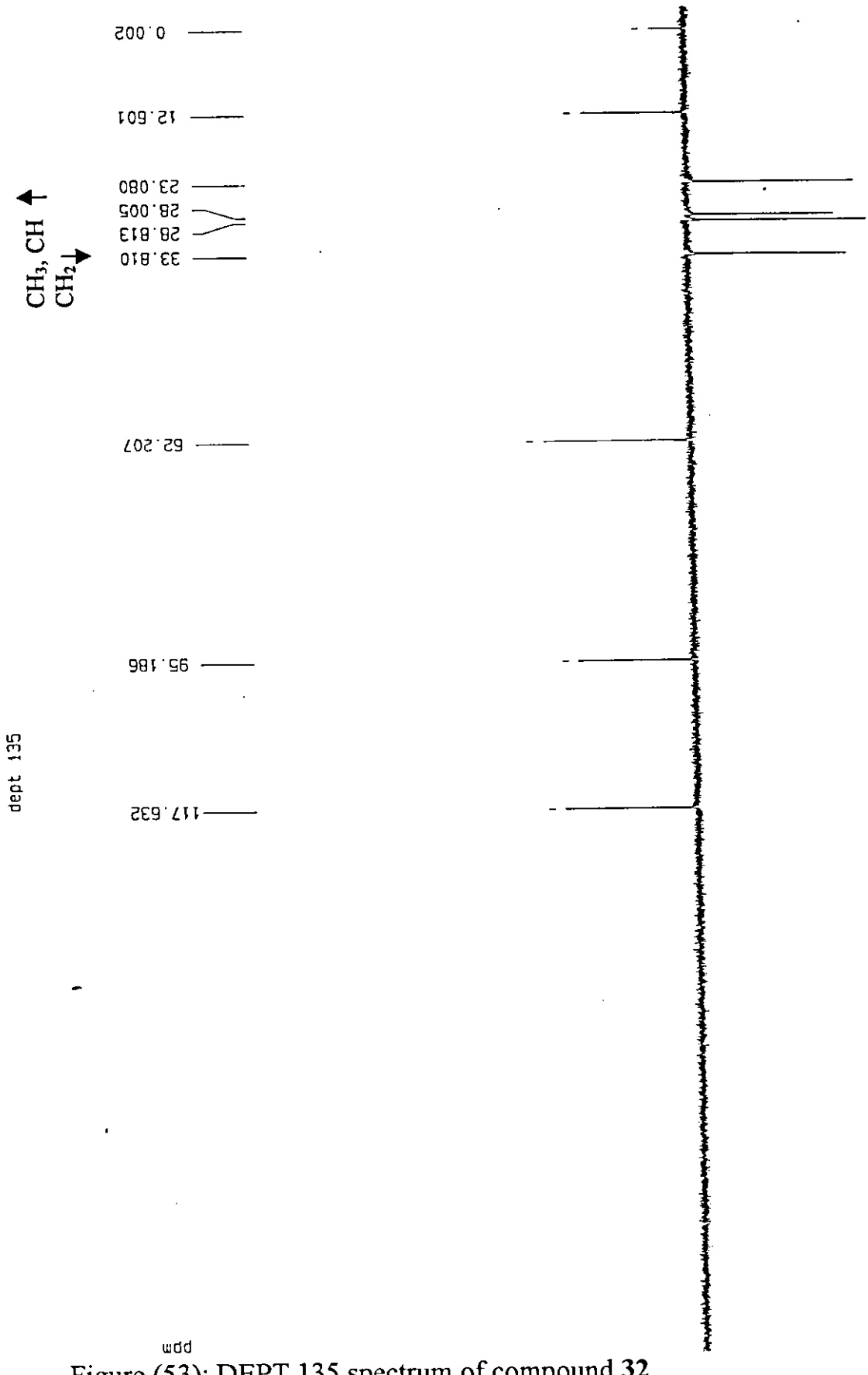


Figure (53): DEPT 135 spectrum of compound **32**.

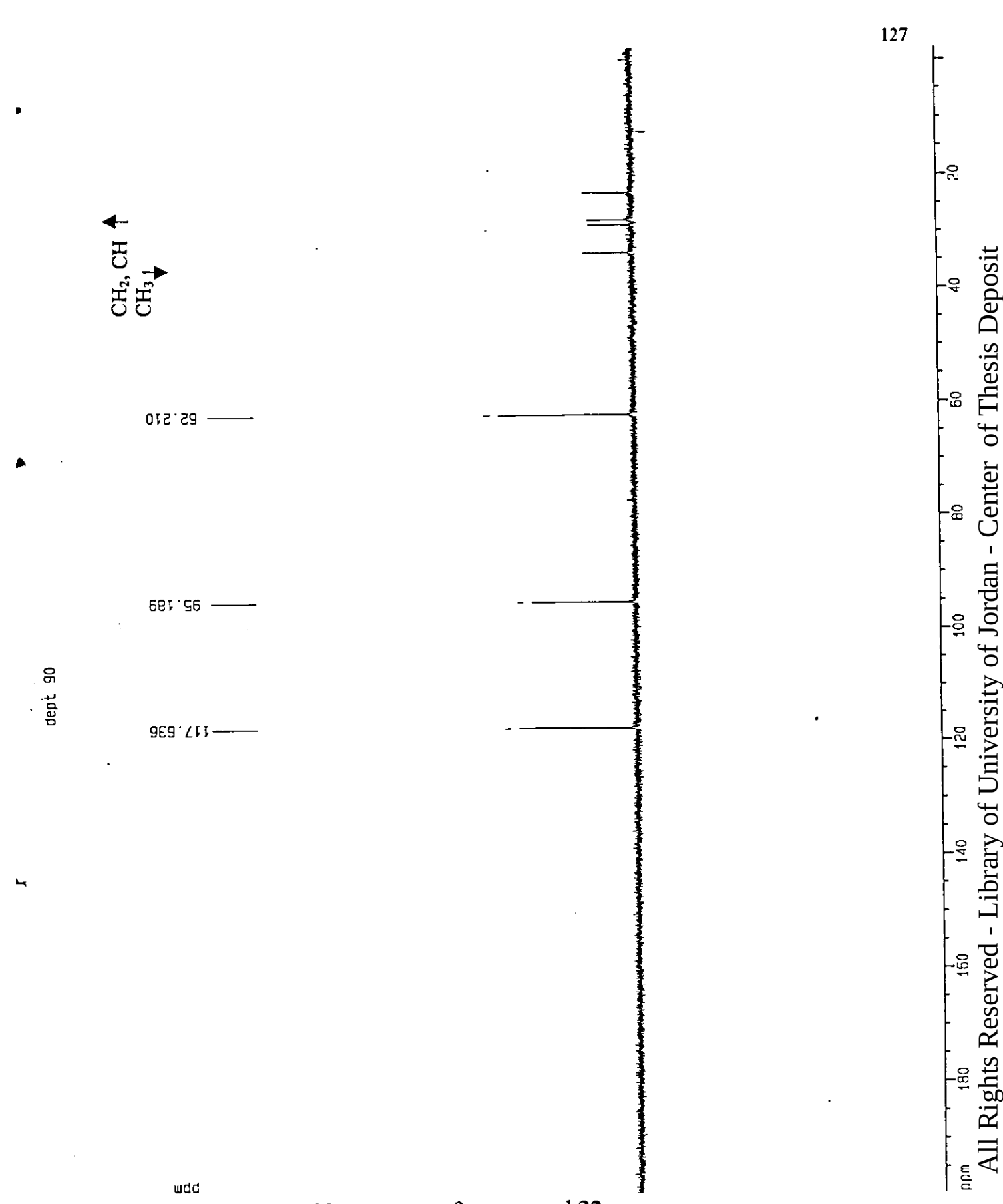


Figure (54): DEPT 90 spectrum of compound **32**.

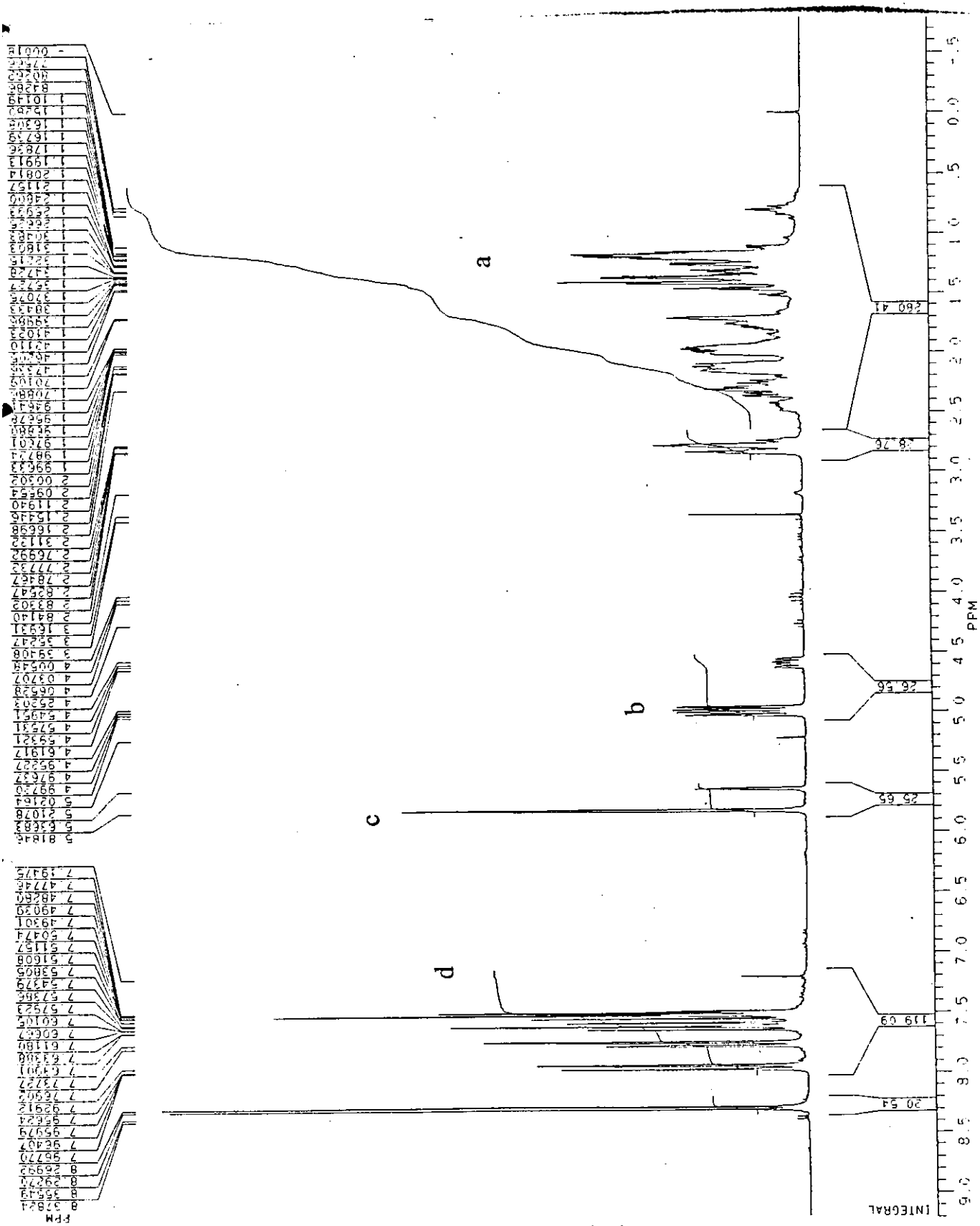


Figure (55): ^{1}H-NMR spectrum of compound **33**.

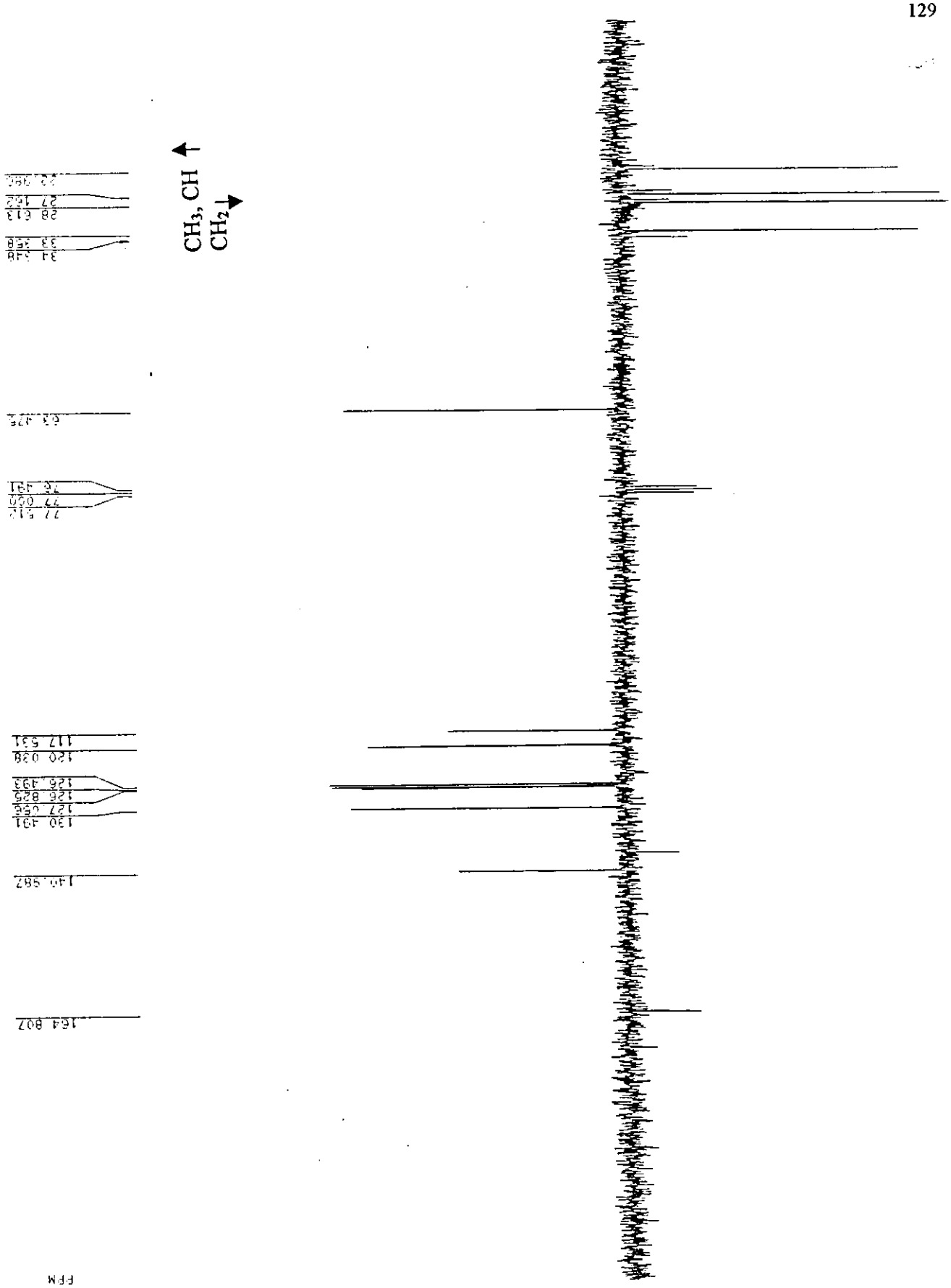


Figure (56): GASPE spectrum of compound **33**.

ملخص

إنتاج سلسلة جديدة من المركبات الحلقية غير المتجانسة للاستخدامات الطبية

تم تحضير سلسلة جديدة من المركبات الحلقية غير المتجانسة وهي المركبات من (٢٤-٣٠) من خلال تفاعل مشتقات أل ٢- أمينو بريدين مع مركبات بيتا كيتوإيستر بوجود البولي فوسفوريك أسد.

كما و تم تحضير مركبات الديازبين (٣١-٣٣)من خلال تفاعل مشتقات ال٢-امينوبريدين مع مركب أل جاما كيتو ايستر بوجود البولي فسفوريك أسد.

ومن خلال هذا البحث تم التأكد من أشكال المركبات الجديدة وذلك باستخدام عدة طرق وهي التحليل الدقيق للعناصر ، أطياف الكتلة ، التحليل بواسطة الأشعة تحت الحمراء وأخيرا أطياف الرنين المغناطيسي.

وبعد ذلك تم فحص المركبات ٢٤، ٢٧، ٢٨ لفعاليتها ضد أنواع البكتيريا التالية :ستافيلوكوكس أريس ، بروتيس فولجاريس و خميرة البيكانز،وقد لوحظ أنه لم تكن هناك أية فعالية للمركبات السابقة ضد هذه الأنواع من البكتيريا .

كما و لوحظ أن نفس المركبات لها تأثير فعال ضد بعض أنواع الفطريات حيث تم فحصها ضد الأنواع التالية من الفطر: مايكروسبورم كانز ، فيوسياريوم تراي سينكتم ،بيثيوم ألتمام ،بيثيوم أفانيديرماتم، بيثيوم ميدليتوني .

وفي النهاية تم فحص المركبات ٢٧، ٣٢، ٣٣ كمواد فعالة ضد السرطان وذلك باستخدام طريقة أل . (MTT) والتي أظهرت أن لجميع هذه المركبات فعالية جيدة.