COURSE OF SYNTHESIS OF ACTIVE SITE DIRECTED ONCOLYTIC AGENTS

A Thesis

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Dedication

to

My Mother, Asma

and

My Wife, Isaf

ABSTRACT

A unique approach in obtaining new synthetic anticancer compounds involves the chelation of trace metals associated with cancer cells. Several α-(N)-heterocyclic carboxaldehyde thiosemicarbazones (HCT), with the potential to form coordination compounds with certain transition metals inhibit the growth of a number of transplanted rodent neoplasms, spontaneous lymphomas of dogs, and DNA viruses of the Herpes family.

Several researchers have investigated the structure activity relationship of HCT compounds and found the activity was best with 5-amino-1-formyl-thiosemicarbazone isoquinoline.

The objective of this investigation was to explore the principle of active site-directed drug design. This principle has been utilized to develop very potent drugs, for example dilantin and amino sugars like 2-amino-3-β-D-glucose, which have been used as a carrier of nitrogen mustard.

Applying this principle, an attempt has been made to generate a model antitumor agent which would be more potent and be delivered more specifically to the site of action than previously existing antitumor agents. Hence, one of the most active chelators in clinical trail, 5-amino-1-formylisoquinoline thiosemicarbazone was modified by attaching at position five one of the essential amino acids as a carrier, preferably L-phenylalanine. The synthesis of other thiosemicarbazone analogs were also attempted.

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I. INTRODUCTION

Recently, considerable advances have been made in our knowledge of the disease known as cancer. Cancer is characterized by uncontrolled cell growth and current treatment involves surgery, radiation (x-ray) and chemotherapy. Many anticancer agents are now able to increase survival time, prevent or reduce metastasis and reduce the size of the tumors. Some agents are also capable of producing temporary or total remissions of certain neoplasms which are not amenable to surgery or radiation[1]. Unfortunately, most of these agents are nonspecific and produce many toxic and dangerous side effects.

Current directions in the development of new chemotherapeutic agents have centered on chemicals with new or unique actions, on agents which inhibit cell growth via rational procedure, and on analogs of existing agents with lower toxicities. These developments have come about through the trials and errors of past serendipitous synthesis of various chemical agents[2]. Advances in molecular biology coupled with the development of sophisticated instrumentation such as nuclear magnetic resonance (NMR), mass spectroscopy (MS), and electron microscopy (EM), just to name a few, have given tremendous insight into the many processes involved with cancer propagation[3]. Yet the identification of selective inhibition of some of these biosynthetic pathways still remains a matter of trail and error[2].

Screening for potentially active agents from natural sources, structural modification of natural metabolites, and the evaluation of cytotoxic chemicals are still the primary means used for the development of clinically useful antitumor agents[2].

Antitumor agents can be divided into at least six classes that reflect their varying mechanisms of action. These classes include the steroidal hormones, alkylating agents, antimetabolites, antibiotics, specific mitotic inhibitors and chelating agents[4].

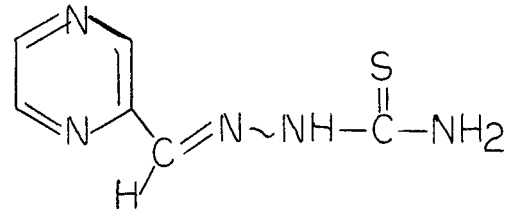
The first evidence of *in vivo* chelation was observed with 2-formylpyrazinethiosemicarbazone (1). This compound possesses antitumor activity, and also causes urinary and fecal excretion of iron, as the ferrous complex, in mice and rats[5,6].

A. Chelating Agents

A unique approach in obtaining new synthetic anticancer compounds involves the chelation of trace metals associated with the cancer cells. Several α-(N)-heterocyclic carboxaldehyde thiosemicarbazones (HCT) (2), with the potential to form coordination compounds with certain transition metals, inhibit the growth of a number of transplanted rodent neoplasms, spontaneous lymphomas of dogs, and deoxynucleic acid (DNA) viruses of the Herpes family[7,8].

It was first reported by Brockman *et al.*[9,10], that only 2-formylpyridine thiosemicarbazone (PT) (3), and not its three or four isomers, was active against L1210, L82T and L4946 leukemias in mice.

It was further established that HCT is the most potent known inhibitor of ribonucleotide diphosphate reductase (RDR), an enzyme critical to the synthesis of DNA[11].



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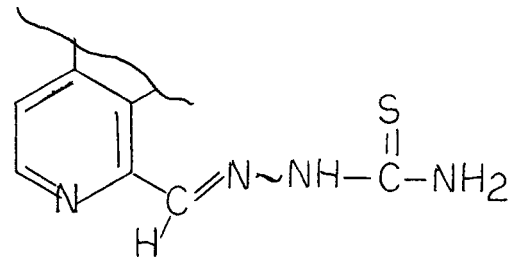

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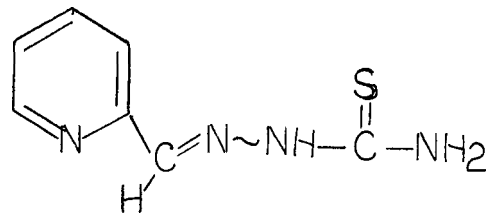

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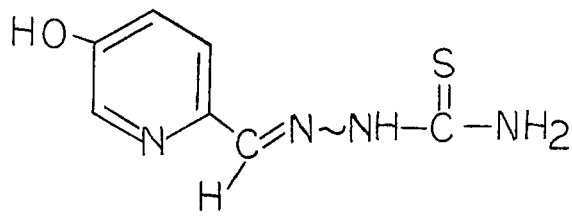
Two other enzymes involved in DNA synthesis, thymidylate synthetase and thymidine kinase, did not demonstrate such a close degree of correlation with tumor growth rate[12].

French and Blanz[11] extended these observations by testing a series of formyl heteroaromatic thiosemicarbazones. Several of these derivatives, especially 2-formyl-5-hydroxy pyridine (5-OH-PT) (4) and 1-formylisoquinoline thiosemicarbazone (IQ-1) (5), showed significant antineoplastic activity and are among the most effective tumor inhibitors in this class.



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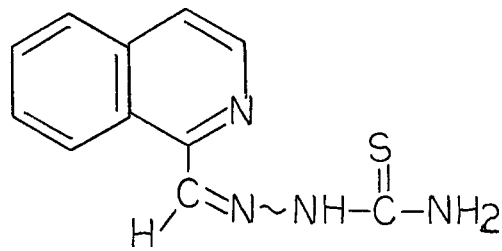

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1. Mechanism of Action

The precise biochemical site of action of IQ-1 is unknown; however, some indications have shown that the biosynthesis of DNA is extremely sensitive to the inhibitory action of IQ-1[8]. The specific site of IQ-1 is suspected to be acting on the enzyme RDR.

Sartorelli et al.[7], in a series of investigations on IQ-1, showed that DNA synthesis was strongly inhibited in L1210 cells and especially Sarcoma 180A cells in mice, while ribonucleic acid (RNA) and protein synthesis were affected less by IQ-1, although inhibition of these processes was seen at higher concentrations.

2. The Function of Ribonucleotide Diphosphate Reductase

Structure activity studies, employing sedimentation of DNA from Sarcoma 180A cells treated with IQ-1 in alkaline sucrose, suggest the presence of single strand breaks[11,13]. The RDR system is responsible for the conversion of the nucleoside diphosphates of cytosine, uracil, adenine and quanine to their respective 2'-deoxynucleoside diphosphates which are inturn incorporated into DNA[14,15]. The highly purified RDR, isolated from Escherichia coli B, is iron dependent. This pure enzyme consists of two nonidentical subunits, B_1 and B_2 proteins, which are required for activity. Protein B_2 contains two iron atoms[15]. Both proteins B_1 and B_2 work in conjuction with the small protein thioredoxin$(SH)_2$ to convert the 2'-CHOH group in the ribosyl moiety to deoxyribose in the products as illustrated in the following equations:

$$\text{XDP} + \text{thioredoxin}(SH)_2 \xrightarrow[Mg^{+2}]{B_1 + B_2} \text{dXDP} + \text{thioredoxin}\begin{array}{l}S\\|\\S\end{array} \qquad \text{(Eq. 1)}$$

$$\text{Thioredoxin}\begin{array}{l}S\\|\\S\end{array} + \text{NADPH} / H^+ \xrightarrow[\text{reductase}]{\text{thioredoxin}} \text{thioredoxin}(SH)_2 + NADP^+ \qquad \text{(Eq. 2)}$$

The mechanism of inhibition of RDR is a complex process whereby, a chelate is formed between IQ-1 and iron which effects the dithiol cofactor in a way to inhibit substrate reduction. Atkin et al.[15] were able to remove the iron from protein B_2. Reconstitution to the active metalloenzyme by addition of excess Fe^{+2} to metal free protein yielded reconstituted, reactivated protein with two to one iron-subunit stoichiometry. It is proposed that the function of iron in protein B_2 is the initial generation of a radical from a protein bound group, and that stability of the radical depends upon some continuing interaction with the iron center. The radical is apparently less stable than is the metalloprotein structure. Also, it is proposed that the free radical participates in the reduction of ribonucleotides by the enzymatically active protein B_1-protein B_2 complex. Kinetic studies on the inhibition of RDR by PT and IQ-1 demonstrated that these agents either bind as tridentate ligands to an iron-charged RDR as illustrated in Figure 1, or a preformed iron chelate of the inhibitor interacts with the target enzyme[11,12] at a site occupied by reduced thioredoxin[7,17]. The iron Fe (II) ligand octahedral coordination complex was established by French et al.[17] (Figure 2). Then Mathew and Palenik[18] confirmed the octahedral structure by precision x-ray diffraction studies.

3. α-Formyl Heteroaromatic Thiosemicarbazone Structure Activity Relationship

The structure-activity relationship (SAR) of the α-formyl heteroaromatic thiosemicarbazones was deduced from the following studies:

a. α-Heterocyclic System

A study by French et al.[10], on different heteroaromatic thiosemicarbazones [e.g. Pyrimidine-4 (6), Pyrazine-2 (7), Pyridazine-3 (8), 6-CH_3-Pyridazine-3 (9), Triazole-4 (10), Isothiazole-4 (11), 1-Methylisatin-3

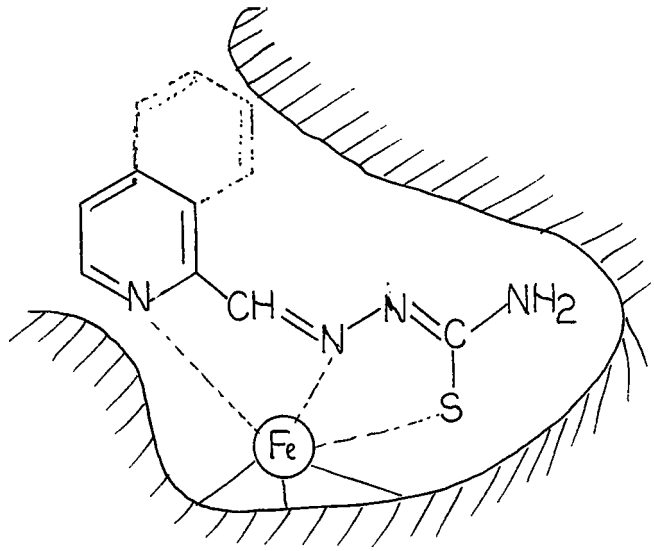


Figure 1

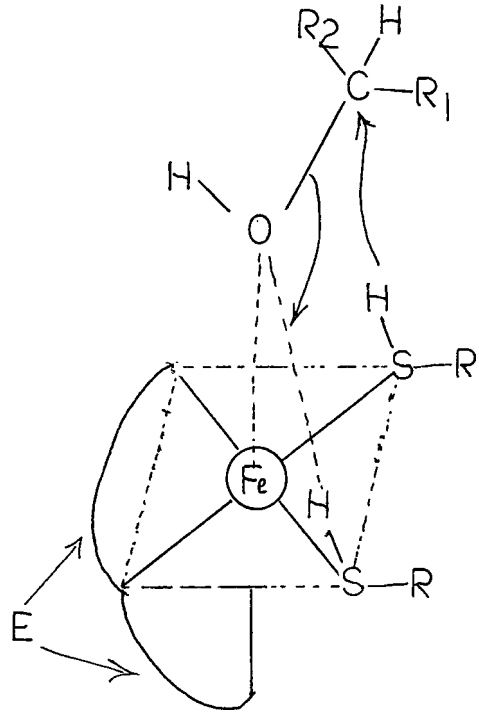


Figure 2

(12), Quinoline-2 (13), Cinnoline-3 (14), Quinoxaline-2 (15), Quinazoline-4 (16), 2-OH-Quinazoline-4 (17), 4-OH-Quinazoline-2 (18), Benzothiazole-2 (19) and Purine-6 (20)], showed that (6), (8) and (9) are active on L1210 and Lewis Lung Carcinoma (LLCa). It was also noted that (7) is more active and less toxic than (3).

Blanz et al.[19] investigated the effect of different substituents (shown in Table I) on the pyridine ring (3). The net results of the study was that activity variation depends on the tumor system. Of the substituents tested, the 5-hydroxy substituent (4) produced the best activity.

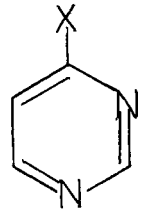
It was noted by Paul et al.[7,11], that the introduction of a methyl group on the pyridine ring of PT (3) at either the three, four, or five positions resulted in derivatives that were better inhibitors of RDR activity than PT (3).

In another study, French et al.[10] reported 97 compounds of which 35 showed activity against L1210, 22 against Sarcoma 180A and 20 against LLCa.

The conclusions drawn from the previous studies indicated the following:

(a) The π-electron density at the point of attachment of the aldehyde moiety should be low, (b) the ring nitrogen should be a reasonably good donor to the transition metals for formation of octahedral coordination compounds (i.e. chelates), and (c) the carbonyl attachment should be in the α position to the heteroaromatic nitrogen atom.

It was concluded that IQ-1 (5) could be visualized as PT (3) with a benzene ring fused at positions three and four of the pyridine ring (see 2), and was approximately a two and one-half fold better inhibitor toward RDR than was PT (3).



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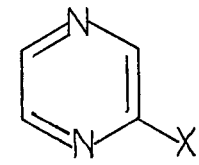

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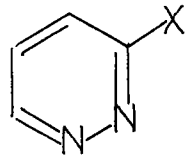

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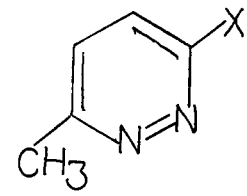

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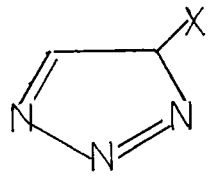

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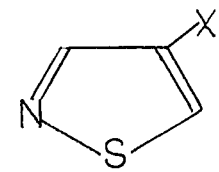

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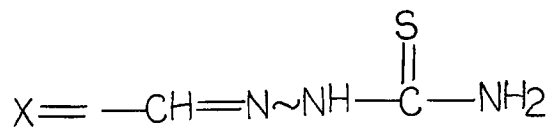

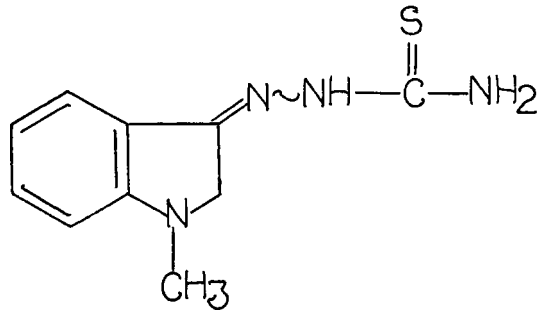

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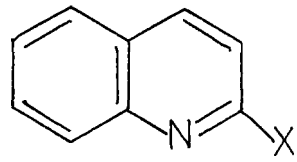

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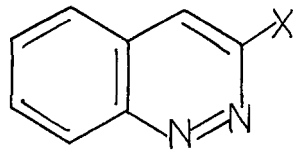

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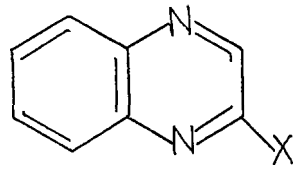

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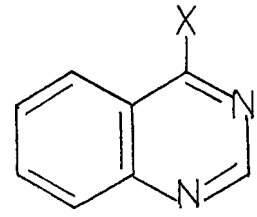

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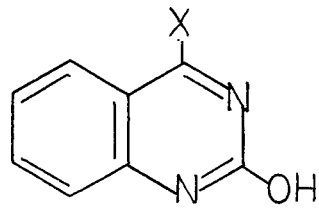

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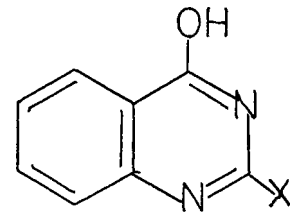

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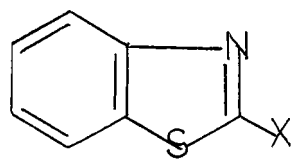

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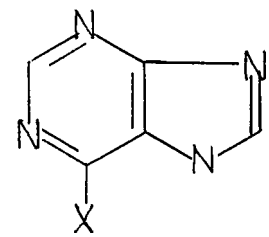

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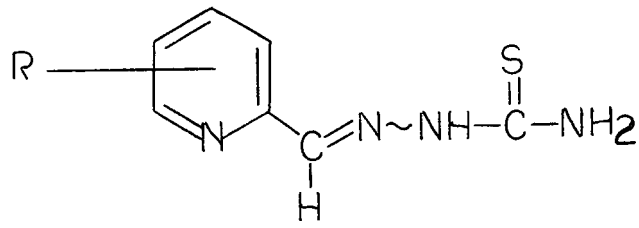
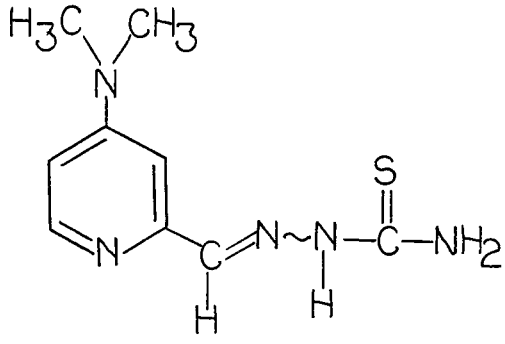
TABLE I

3,5 Substituted pyridinethiosemicarbazone



Compound	Substituent (R)
4	5-OH
21	5-F
22	3-F
23	5-Cl
24	5-Br
25	5-I
26	5-CF_3
27	5-OCH_3
28	5-$N(CH_3)_2$
29	5-SO_2CH_3

Agrawal et al.[20], reported 37 compounds of 4-substituted PT (3) of which $N(CH_3)_2$-PT (30), $N(CH_2\text{-}CH_2)_2O$-PT (31), $N(CH_2\text{-}CH_2)_2N\text{-}CH_3$PT (32), N-$C_5H_{10}$-PT (33), Pyrazole-PT (34), N-C_4H_8-PT (35), $N(CH_2\text{-}CH_2OH)_2$PT (36), and $N(CH_2\text{-}CH_2)_2O$, 3-CH_3-PT (37), showed considerable activity against Sarcoma 180A. Their results, reported as (treated/control) X 100 (% T/C), are shown in Table II.



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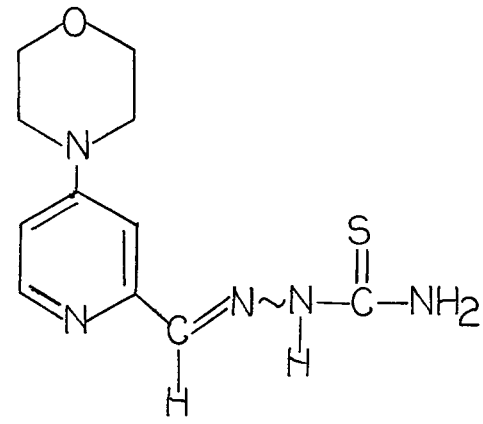

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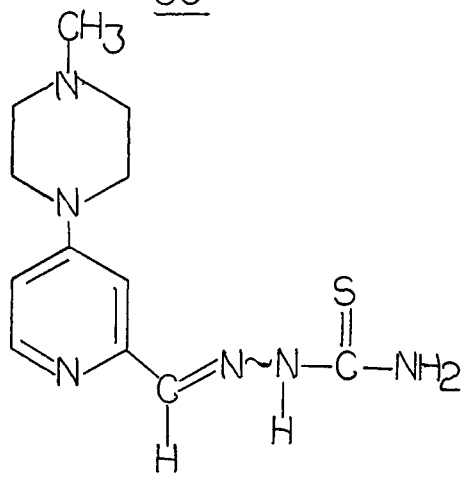

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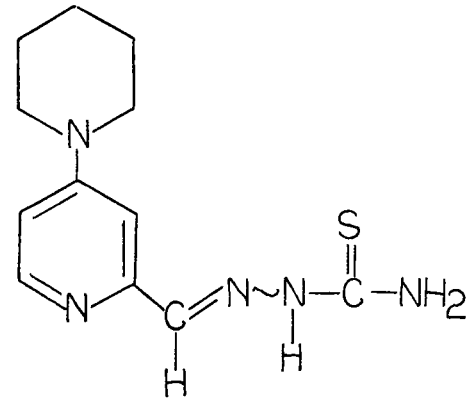

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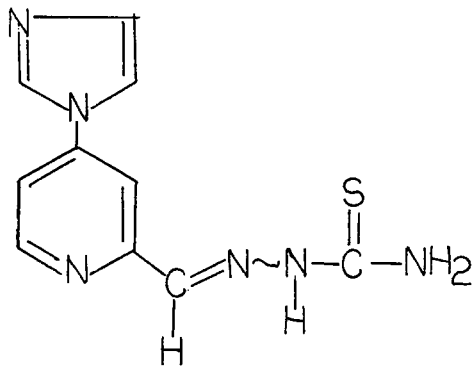

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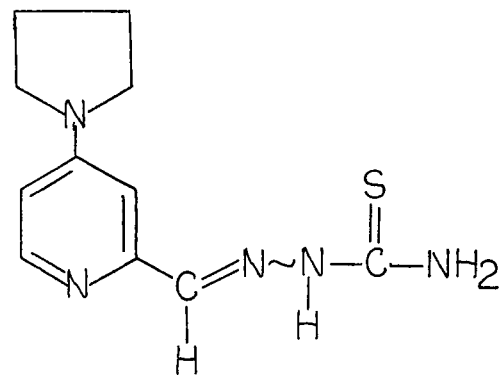

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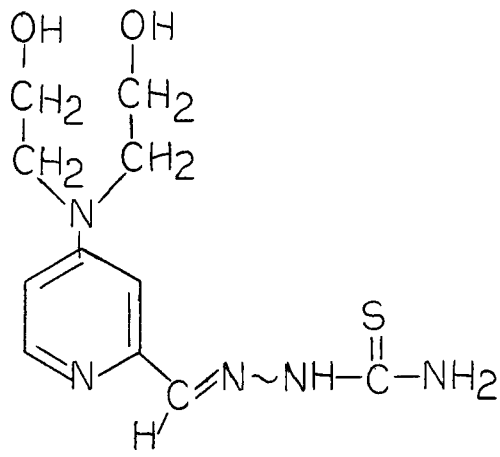

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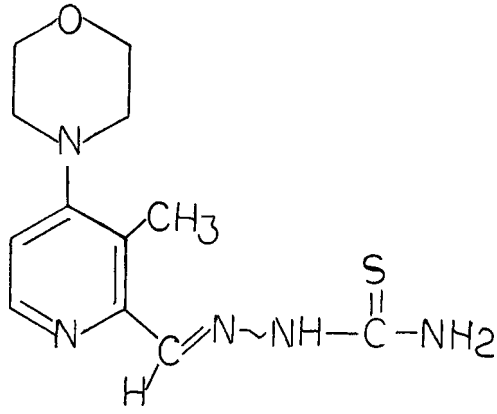
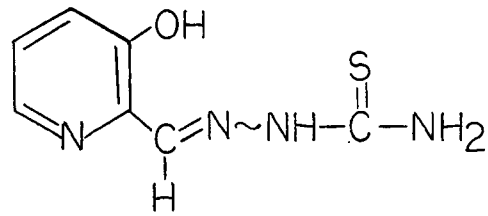


37

A series of 5-substituted derivatives of IQ-1 (5), shown in Table III, were synthesized to determine the various substituent effects on tumor inhibitory potency and host toxicity. The results indicated that subsitution of an additional group at the 5-position reduced carcinostatic activity in the case of (38), (39) and (40), whereas, the insertion of (41), (42), (43), and (44) groups resulted in derivatives possessing tumor-inhibitory activity comparable to the parent compound (5)[21-23].

Other compounds of substituted IQ-1 (5)[5], shown in Table IV, have less activity than IQ-1 (5) or no activity.

When 5-OH-IQ-1 (42) and 3-OH-PT (55) were given as the sodium salt, it was found that activity against L1210 increased markedly[24].



55

The 5-amino-IQ-1 (41) was shown to prolong the life span of tumor bearing animals to essentially the same extent as that produced by the parent compound. In addition, this modification yielded a compound which could be solubilized in water, as the salt of the appropriate acid, an essential property for the ultimate formulation of heterocyclic carboxaldehyde thiosemicarbazones for parenteral administration[25].

b. Side Chain Effect

Agrawal et al.[26], using 5-HP (4) as a model, studied the concentration of substance necessary to cause 50% inhibition of alkaline phosphatase and

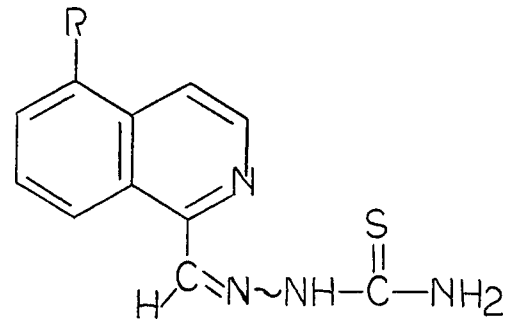
TABLE II

Comparative activity of compounds 30-37 on Sarcoma 180A

Compound	% T/C
30	152
31	279
32	110
33	164
34	100
35	134
36	154
37	146

TABLE III

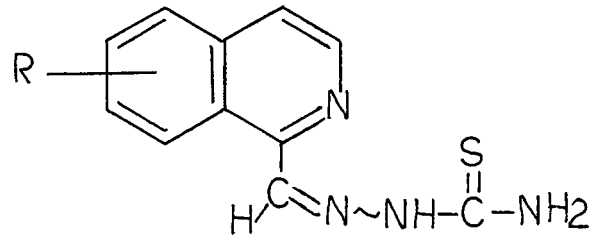
Substituted 1-formylisoquinoline-thiosemicarbazone (IQ-1)



Compound	Substituent (R)
38	$-NO_2$
39	$-NH-\overset{O}{\overset{\|}{C}}-CH_3$
40	$-SO_3H$
41	$-NH_2$
42	-OH
43	$-O-\overset{O}{\overset{\|}{C}}-CH_3$
44	$-O-\overset{O}{\overset{\|}{C}}-Ph$

TABLE IV

Compounds having less activity than IQ-1



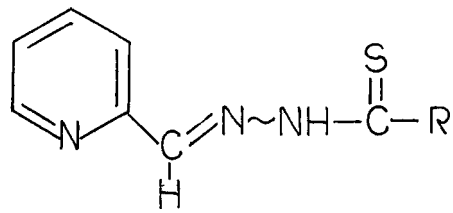
Compound	Substituent (R)
45	5-CN
46	5-COOH
47	5-CF_3
48	5-Cl
49	7-OCH_3
50	7-OH
51	7-NH_2
52	8-NO_3
53	5-Cl, 8-NO_2
54	8-F

RDR. Elevated levels of alkaline phosphatase enzyme have been demonstrated in certain tumors which have developed resistance to chemotherapeutic 6-thiopurines. Alkaline phosphatase hydrolyzes the active nucleoside monophosphate form of these drugs, thus reducing their antitumor effectiveness. Of the few potent alkaline phosphatase inhibitors reported, a series of substituted derivatives of PT have shown good inhibitory activity against alkaline phosphatase from Sarcoma 180 6-thiopurine resistant ascites cells

supposedly by chelation of the zinc ion that is required for alkaline phosphatase activity. The results indicate that RDR was best inhibited by (4), whereas, alkaline phosphatase was best inhibited by (60), as shown in Table V.

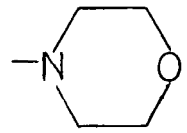
TABLE V

Inhibition of alkaline phosphatase and ribonucleotide diphosphate reductase by pyridine derivatives



(R)	50% Inhibition of Alkaline Phosphatase (μM)	50% Inhibition of RDR (μM)
4	250	3
56	17	69
57	17	42
58	24	72
59	12	82
60	4	66
61	100	74

Lee et al.[27], also explored isosteric substitution of the sulfur atom in 5-HP (4) using alkaline phosphatase as the test system, as shown in Table VI. They noted that when sulfur was replaced by oxygen or NH, complete loss of activity resulted. When sulfur was replaced by selenium, they observed a 55% drop in activity (i.e. 55% more compound necessary to obtain the ID 50). In this series, (66) was the most active.



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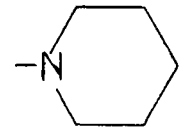

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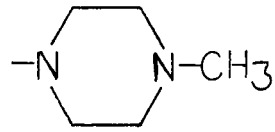

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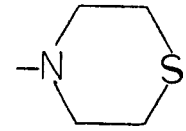

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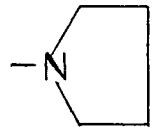

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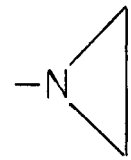

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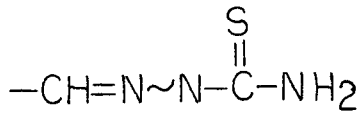
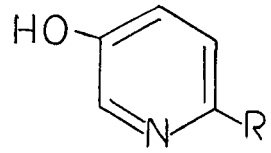


62

In the isoquinoline series shown in Table VII, it is obvious that (71) is the most active.

TABLE VI

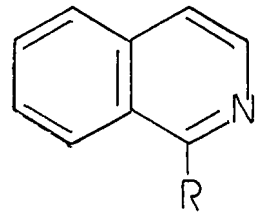
Inhibition of alkaline phosphatase caused by side chain modifications



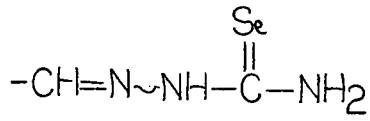
(R)	Alkaline Phosphatase ID 50 (mm)
62	0.25
63	0.39
64	inactive
65	inactive
66	.013

TABLE VII

Inhibition of alkaline phosphatase caused by isoquinoline derivatives



(R)	Alkaline Phosphatase ID 50 (mm)
67	0.058
68	0.032
69	0.05
70	0.009
71	0.008
72	inactive



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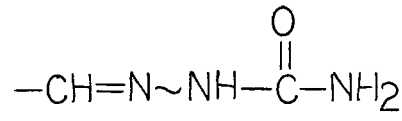

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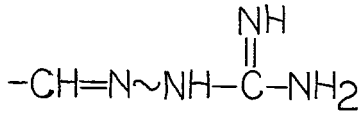

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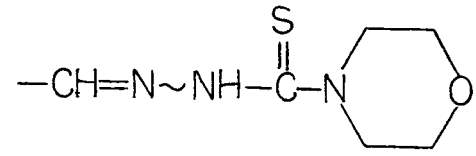

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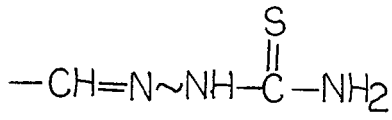

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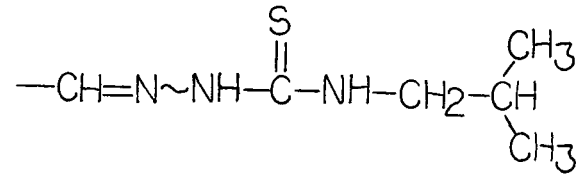

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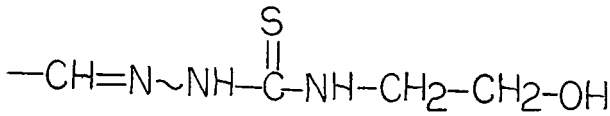

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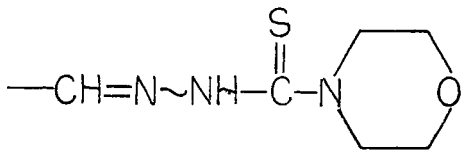

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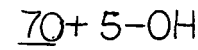

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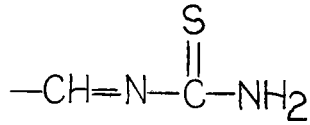
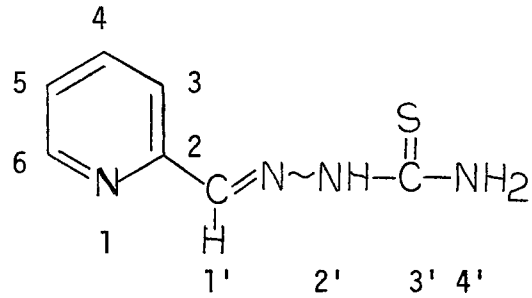


72

In other side chain modifications, shown in Table VIII, activity varied relative to the tumor system. Compounds (73) to (80) were active against L1210 and LLCa, whereas, (81) and (82) were active against Sarcoma 180A, and (83) was active aginst L1210 and Sarcoma 180A[10]. In other publications related to side chain modifications, the compounds tested were less active or inactive against Sarcoma 180A[28,29].

TABLE VIII

Side chain and ring modifications of IQ-1

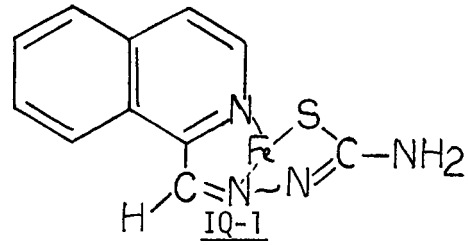


Compound	1'	2'	3'	4'	3	4	5
73	CH_3	CH_3	--	--	--	--	--
74	--	--	--	CH_3	--	CH_3	--
75	--	--	--	CH_3	--	--	--
76	--	--	--	n-Bu	--	--	--
77	--	--	--	Ph	--	--	--
78	--	--	--	2-Pyridyl	--	--	--
79	--	--	CH_3	--	OH	--	--
80	--	--	CH_3	CH_3	OH	--	--
81	--	--	--	n-Bu	--	--	OH
82	--	--	--	2-Pyridyl	--	--	OH
83	--	--	--	Ph	--	--	OH

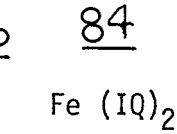
The comparison of inhibition between IQ-1 (5) and Fe $(IQ)_2$ (84)[30], shown in Table IX, was done as a result of studying the mechanism by which HCT analogs inhibit the activity of RDR. It was postulated by Sartorelli et al.[7], that the inhibition is due to the coordination of iron by these analogs, either by a preformed iron complex binding to the enzyme, or by the free ligand complexing with the iron charged enzyme.

TABLE IX

Comparative inhibition between IQ-1 and Fe $(IQ-1)_2$



IQ-1



84

Fe $(IQ)_2$

A - DNA % inhibition	1×10^{-7}M (29%)	4×10^{-8} (60%)
B - RDR ID_{50}	3.5×10^{-8}	2.7×10^{-8}
C - Sarcoma 180A	20 mg/kg	40 mg/kg
Survival days (control 12.4)	37.2	24.2
D - L1210	20 mg/kg	1 mg/kg
Survival days (control 8.1)	11.8	12.8

The results show that DNA synthesis, RDR activity and growth of L1210 were all better inhibited with Fe $(IQ)_2$ (84), whereas, Sarcoma 180A was better inhibited with IQ-1 (5).

Systematic modifications of PT (3) and IQ-1 (5), were made considering (a) hydrophobic interaction between inhibitor and enzyme, (b) bulk tolerance by RDR at both the aldehydic proton and the terminal portion of the side

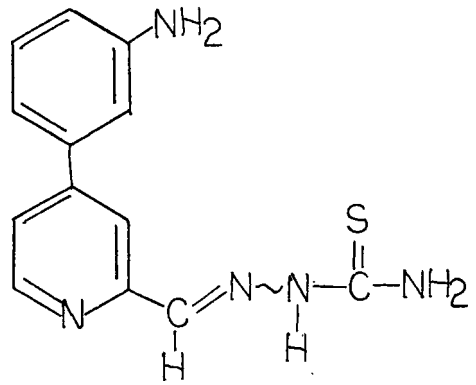
chain, and (c) steric interference with chelation by subsitution at specific positions. These factors coupled with pharmacological investigations led to the selection of 5-amino IQ-1 (41) and 2-formyl-4 (m-amino) phenylpyridine thiosemicarbazone (85) for clinical trial[11].

Recently, it was reported that the insertion of a methyl group at position four of 5-amino IQ-1 (41) resulted in (86) which afforded steric protection of the amino group from in vivo metabolic inactivation. This agent was found to be an effective antineoplastic agent in mice bearing Sarcoma 180A and at the maximum effective dose of 10 mg/kg, increased the average survival of animals three fold over untreated tumor-bearing controls[25].

B. Research Objective

The objective of this investigation was to explore the principle of active site directed drug design. Active site directed drug design is a form of molecular modification which mainly effects the transport properties of the active moiety and having the ultimate potential of delivering the drug more specifically to the site of action, thus increasing the drugs potency. This principle has been utilized to develop a number of very potent drugs, for example, dilantin (87) and amino sugars like 2-amino-β-D-glucose (88), which have been used as carriers of nitrogen mustard (89), (90). Utilization of the drug, dilantin (87), as a carrier, facilitates the attached nitrogen mustard's (89) transport across the blood brain barrier, whereas, the use of 2-amino-β-D-glucose (88), as a carrier, facilitates passage of the nitrogen mustard (90) into bone marrow tissue[31-33].

Applying this principle, we hope to generate a more potent model antitumor agent. It is proposed that the essential amino acid L-phenylalanine be conjugated with the 5-amino group of 5-NH_2-IQ-1 (41) generating a series of amino acid analogs of (91).



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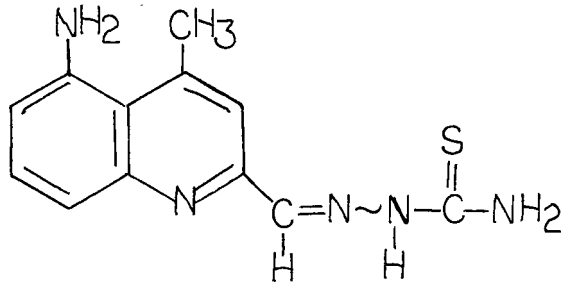

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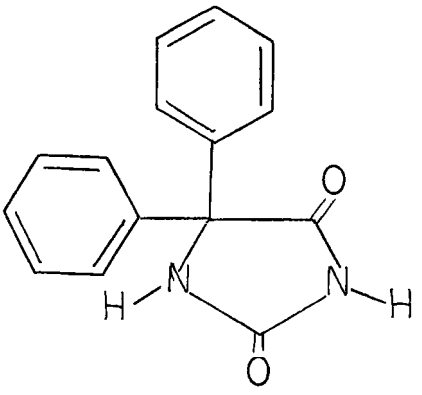

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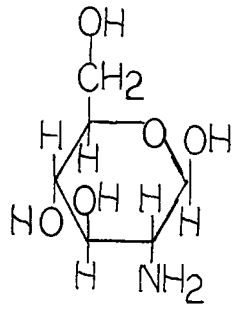

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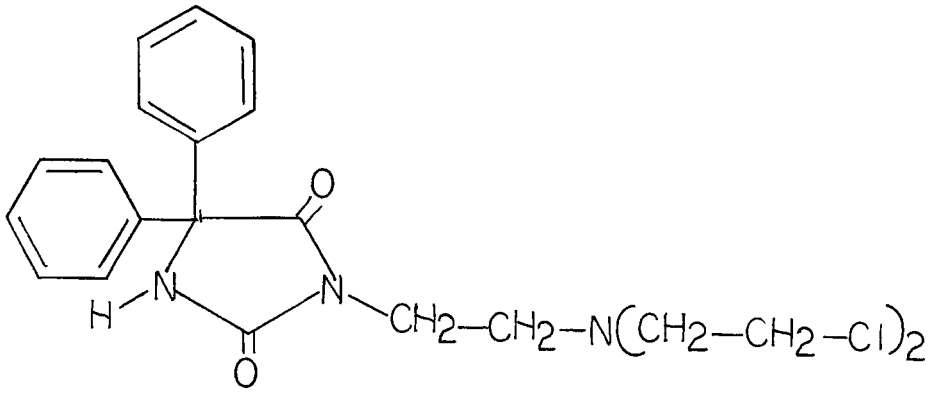

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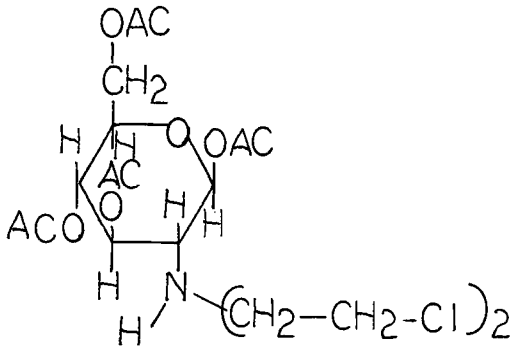

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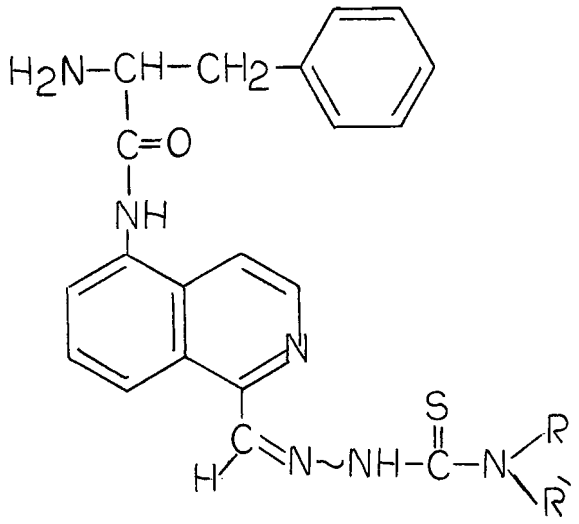


91

II. RESULTS AND DISCUSSION

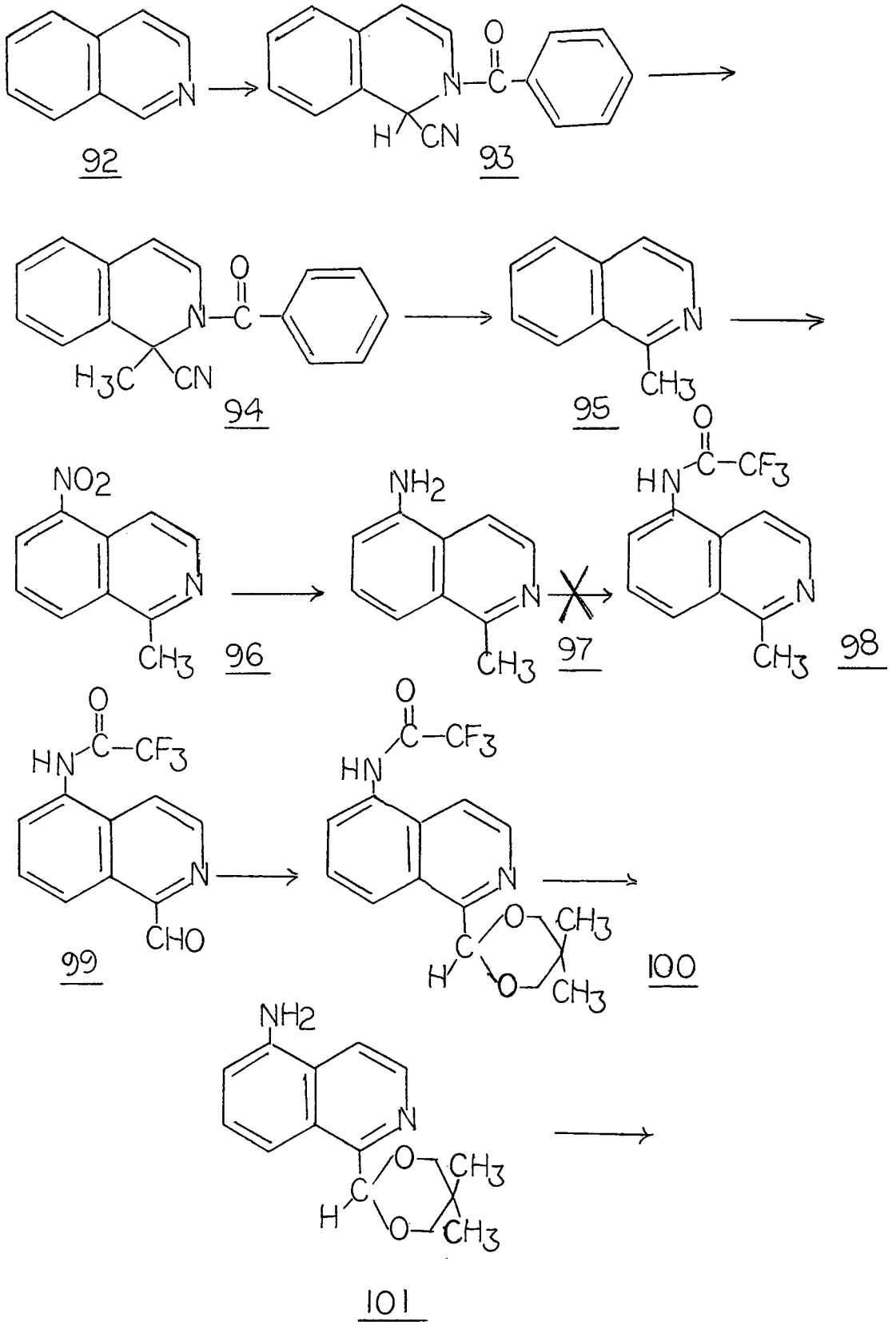
The synthesis of IQ-1 (5) and its derivatives have been reported in many papers, but the synthesis and evaluation of active site directed analogs of IQ-1 have not been investigated.

The use of carriers such as certain drugs (i.e. dilantin), 2-amino glucose, many purine and pyrimidine nucleosides, and amino acid (i.e. phenylalanine mustard sarcolysin) has led to major breakthroughs in chemotherapy[31-36].

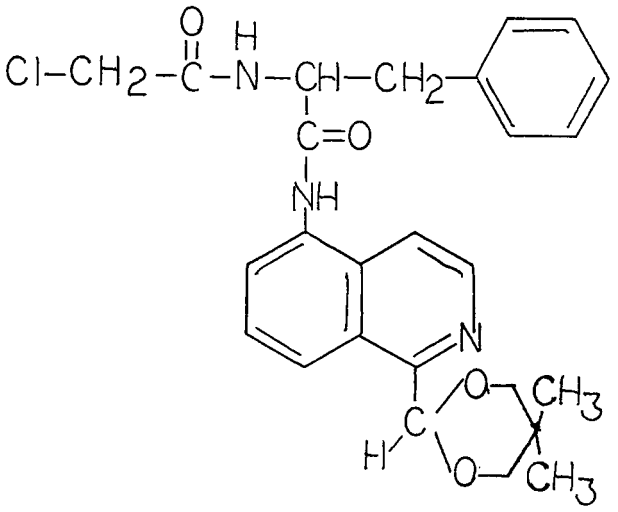
With this in mind and after surveying the literature for the analogs of IQ-1 showing significant activity, 5-amino IQ-1 (41) was selected for exploring the principle of active site direction utilizing an optically active amino acid as carrier. Scheme I represents a general synthetic pathway that was outlined for synthesizing the proposed model system 5-N-L-phenylalanine-IQ-1 (91) were R, R^1=H.

Because 1-methylisoquinoline was not commercially available, the scheme begins with isoquinoline (92) which is converted to (95), as reported by Padbury and Lindwall[37]. It was then proposed that (95) be nitrated to (96) which would then be converted to the corresponding amino analog (97), as reported by Bergstom et al.[38,39]. The amine could then be protected with trifluoro acetic anhydride which would allow for oxidation of the 1-methyl to the corresponding aldehyde. After protecting the aldehyde as in (100), the amine protecting group could be removed and the protected amino acid introduced to the isoquinoline analog. The final step would involve simultaneous removal of protecting groups on the amino acid and the aldehyde followed by subsequent addition of the thiosemicarbazide moiety.

Scheme I



Scheme I
continued



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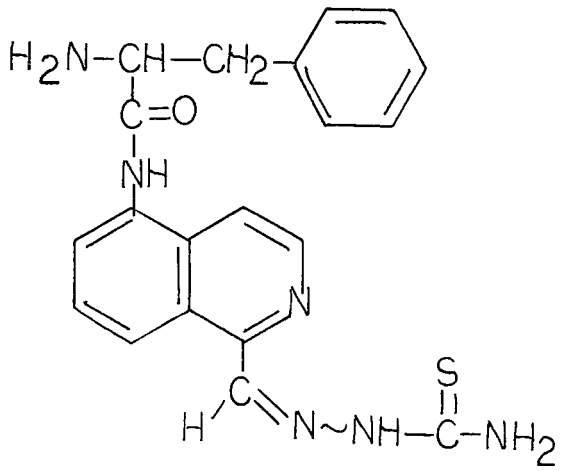


103

One can justify exclusive mononitration occuring at position five of 1-methylisoquinoline by looking at the resonance forms shown in Scheme II. If one examines the resonance forms of 2-methylquinoline, the product formed should be the 8-nitro analog (105 Scheme III). Both 1-methyl-5-nitroisoquinoline (96) and 2-methyl-8-nitroquinoline (105) were synthesized, and the structures were confirmed. The NMR ($CDCl_3$) of (96), Figure 15 showed: δ=3.00 (s,3H), 7.65 (t,1H,J=8Hz), 8.20 (d,1H,J=6Hz), 8.30 (d, 1H,J=6Hz), 8.43 (d,1H,J=6Hz), 8.55 (d,1H,J=6Hz).

The trifluoroacetate was considered as the choice protecting group because of its ease of removal under very mild conditions as compared to acetate itself. But when the protecting reaction was attempted, double addition of trifluoroacetate resulted, as shown in Equation 3.

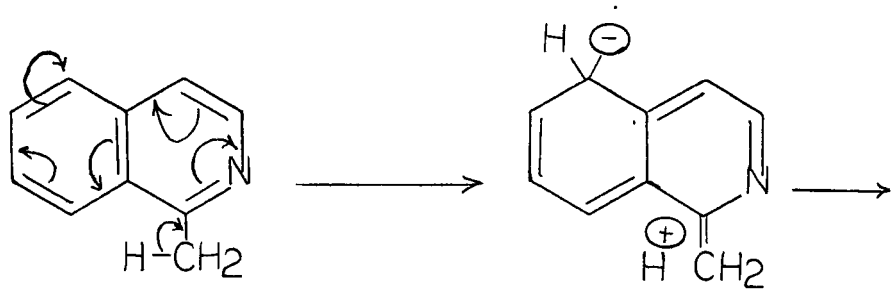
It was felt that this undesirable product resulted largely due to the strong acidity of trifluoroacetic acid. It was then decided that the original protecting reagent used by Sartorelli, acetic anhydride, be substituted for the stronger halogenated acetic acid. Acetic anhydride gave quantitively the amide analog which was converted to the aldehyde with selenium dioxide in dry dioxane (Scheme IV).

Attempts to form the acetal were unsuccessful, primarily due to technical difficulties, and an alternative to Scheme I to obtain the desired product was developed, as shown in Scheme V, starting from 1-methyl-5-nitroisoquinoline (96).

The 1-methyl-5-nitroisoquinoline (96) was oxidized to the corresponding aldehyde (111), which was treated with ethyleneglycol for 24 hours in a Dean-Stark apparatus with para-toluenesulfonic acid as the catalyst.

Reduction of (112) to get (113) was achieved according to the method of Agrawal[21], using hydrogen and carbon over palladium. However, another

Scheme II



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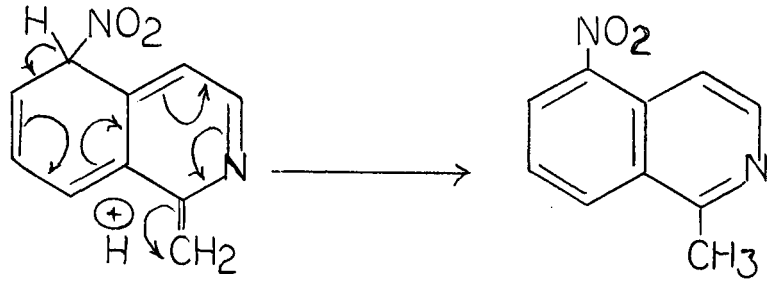

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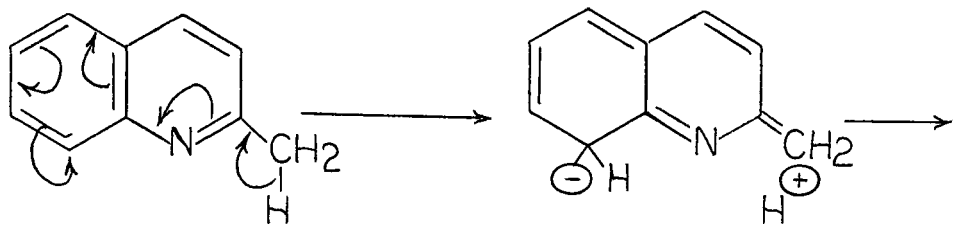
Scheme III



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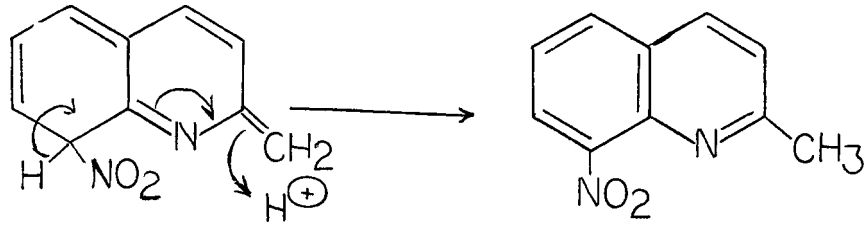

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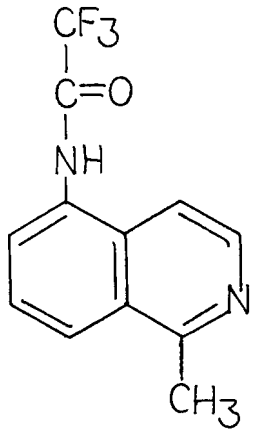

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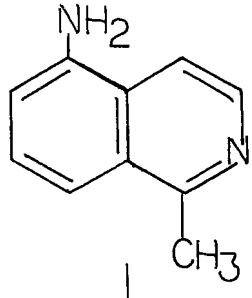
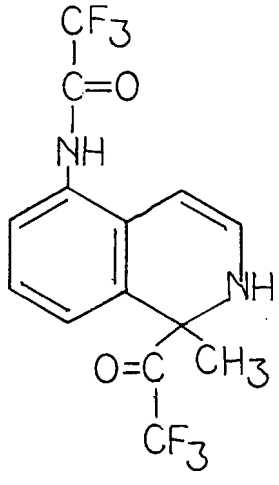


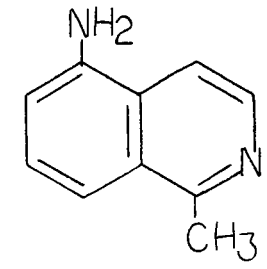
97



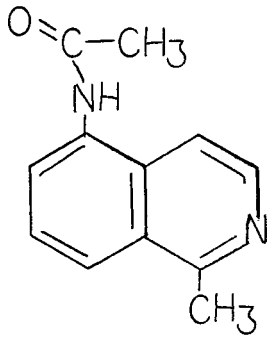
106

(Eq. 3)

Scheme IV



97



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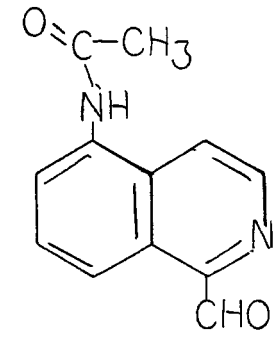
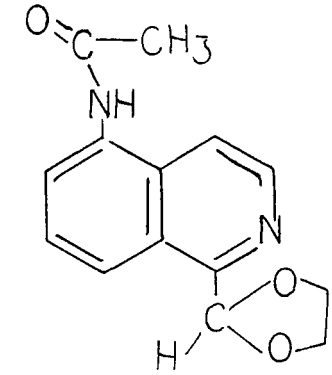
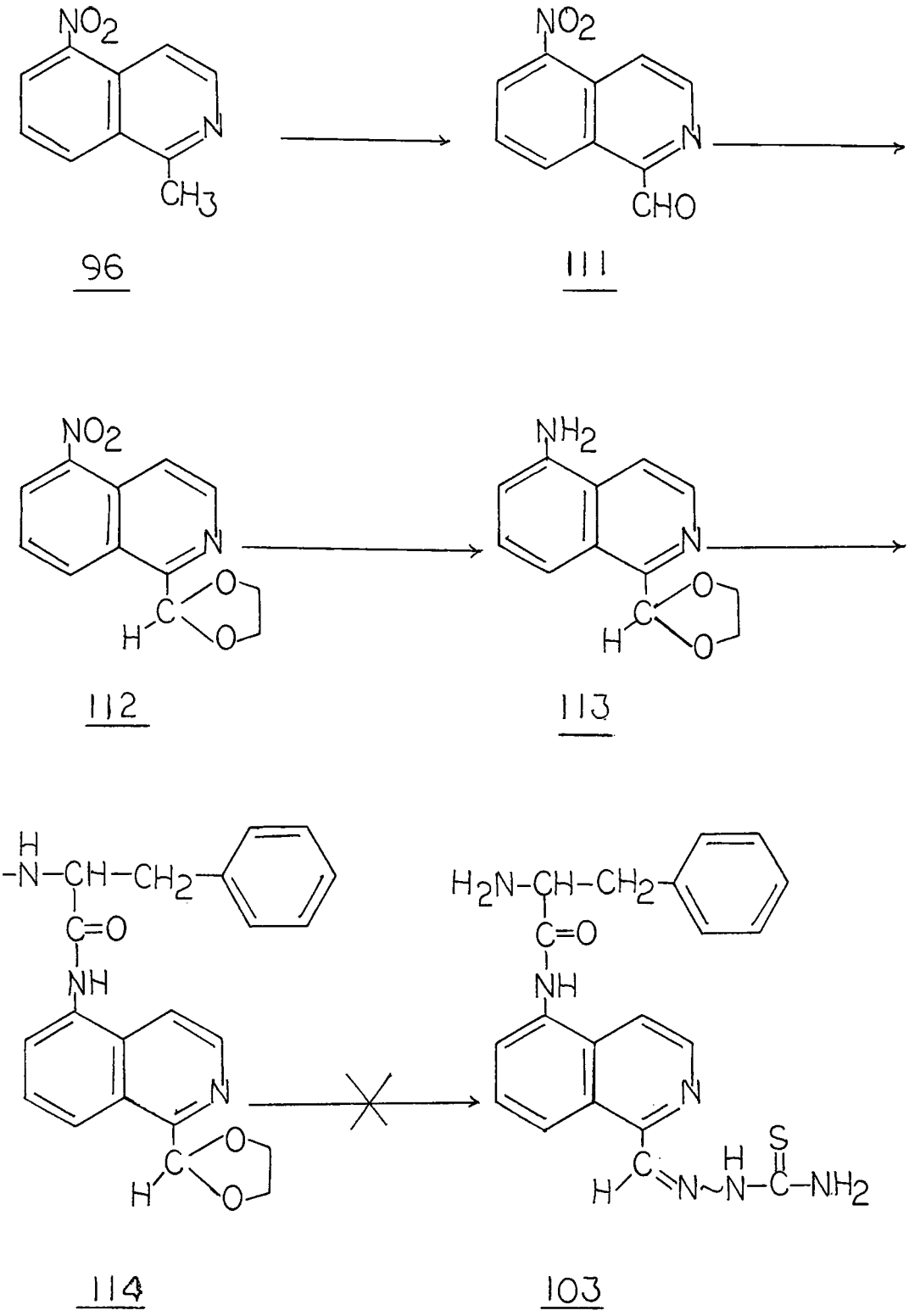


108



109

Scheme V


NO2
CH3
96
NO2
CHO
111
NO2
O
H
O
112
NH2
O
H
O
113
O
H
CH2-C-N-CH-CH2
Cl
C=O
NH
O
H
O
114
H2N-CH-CH2
C=O
NH
S
H
H
C=N~N-C-NH2
103

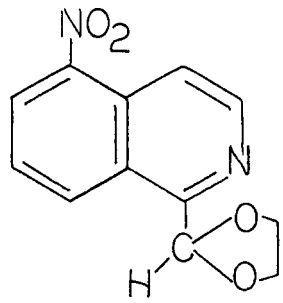
product (115) resulted when heat was applied, Eq. 4. A similar reaction was reported by Adkins and Krsek[40]. Then (113) was reacted with protected amino acid (N-chloroacetyl-phenylalanine) in the presence of dicyclohexylcarbodiimide[41] (DCC) which yielded (114).

The first attempt to synthesize the thiosemicarbazone of (114) in acidic media gave a compound which decomposed at 125-130^{o} to give a black residue, which later melted at 220^{o}. Further analytical data was not consistant with the proposed product mainly due to DCU contamination with compound (114) from the previous reaction.

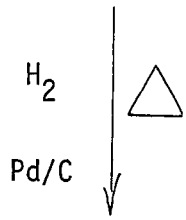
At the same time, compound (112) was used to examine the ease and the yield for the removal of the acetal group and a simultaneous addition of the thiosemicarbazide moiety. As expected, the reaction was easy and the yield was quantitative. The product (116) was confirmed by spectral data (i.e. NMR, IR, MS) (Eq. 5).

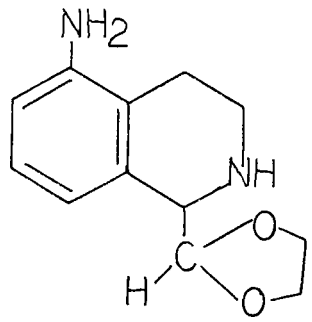
An alternative route to obtain the desired product is shown in Scheme VI. Compound (117) was synthesized easily and quantitively, and was confirmed by spectral data (i.e. NMR, IR, MS). Treatment of (117) with the protected amino acid using DCC as a coupling reagent gave an unidentified product.

The third attempt to form the desired product eliminated the use of DCC as a coupling reagent. The coupling was achieved via the mixed anhydride method with the use of ethyl chloroformate and triethylamine to form the mixed anhydride which was later treated with the amine (113) (Scheme VII). With moderate yield, the product (114) was formed and was confirmed by spectral data (i.e. NMR, IR, MS). This compound was then converted to the final product by reacting with various thiosemicarbazide derivatives. Depending on mass spectral results, it was concluded that the desired products were

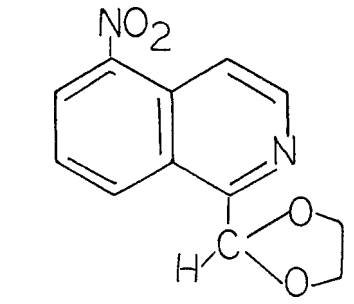


112



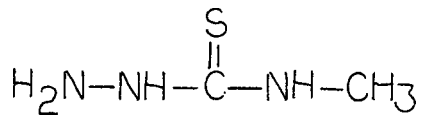


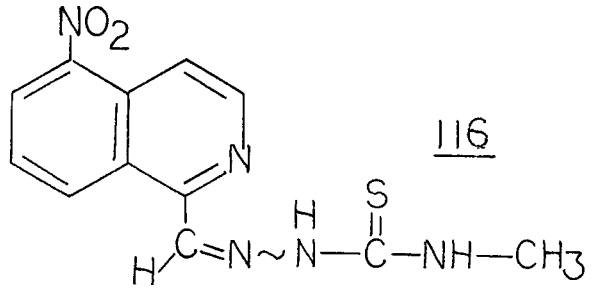
115 (Eq. 4)



112

+

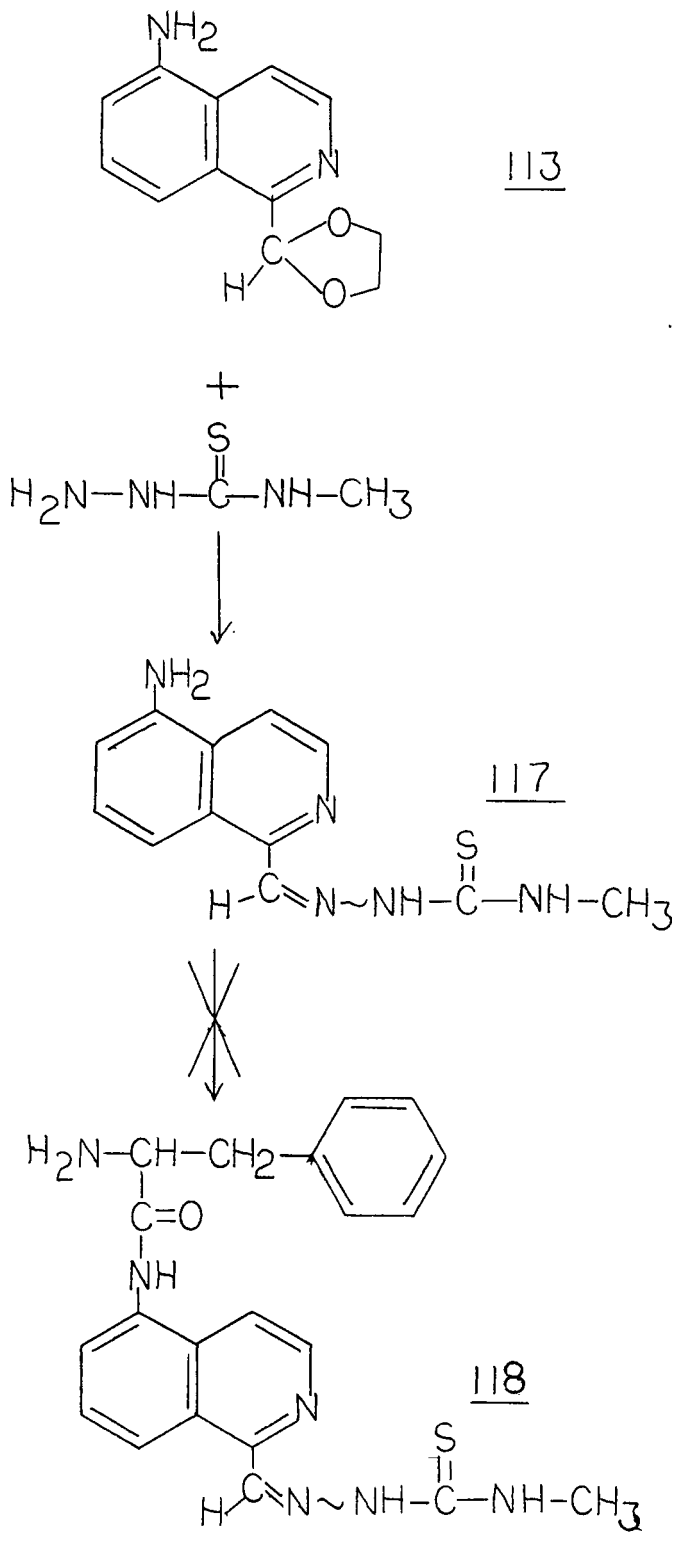




116

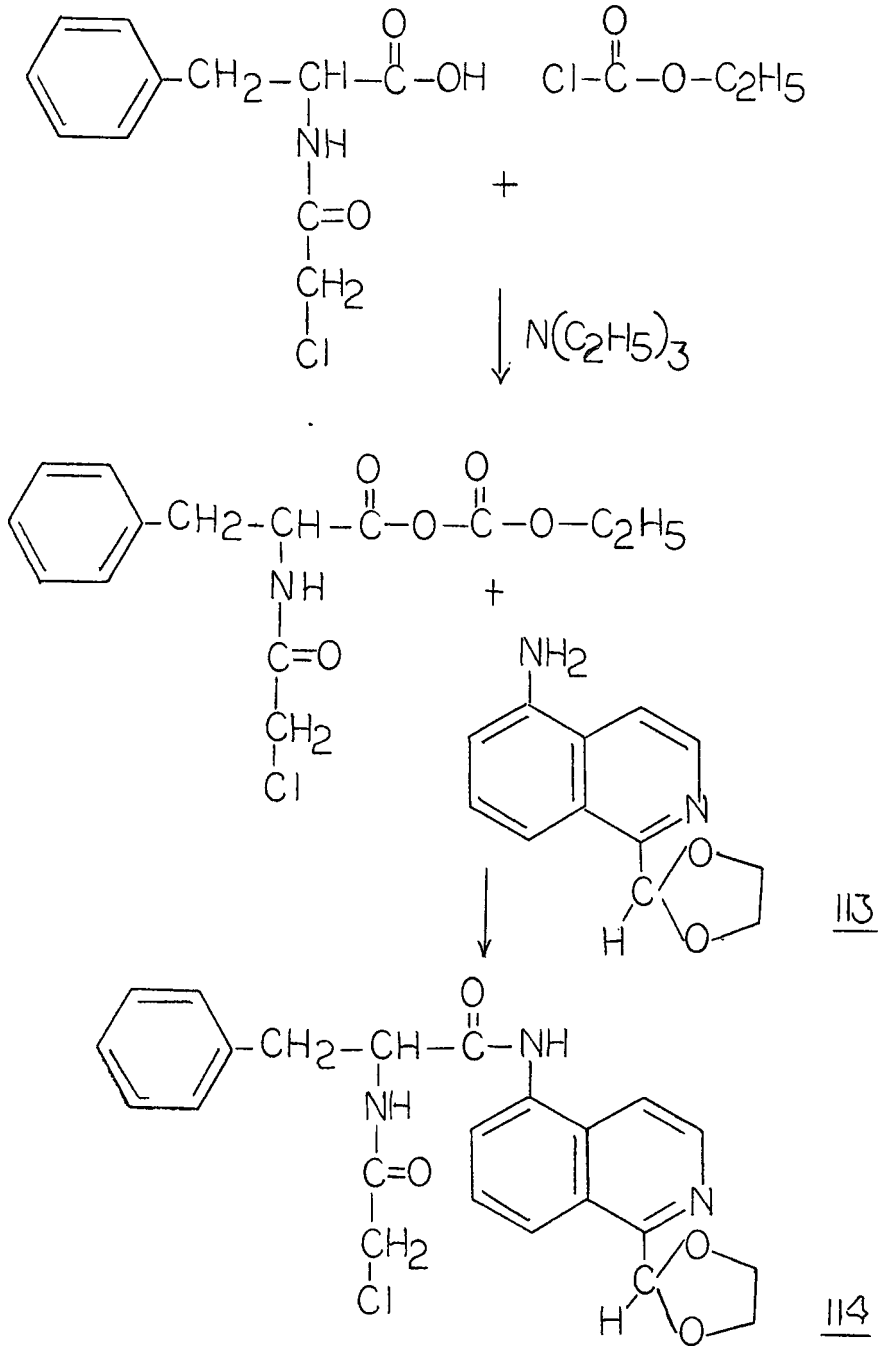
(Eq. 5)

Scheme VI


NH2
N
O
C
H
O
113
+
S
H2N—NH—C—NH—CH3
NH2
N
117
S
H—C=N~NH—C—NH—CH3
H2N—CH—CH2
C=O
NH
N
118
S
H—C=N~NH—C—NH—CH3

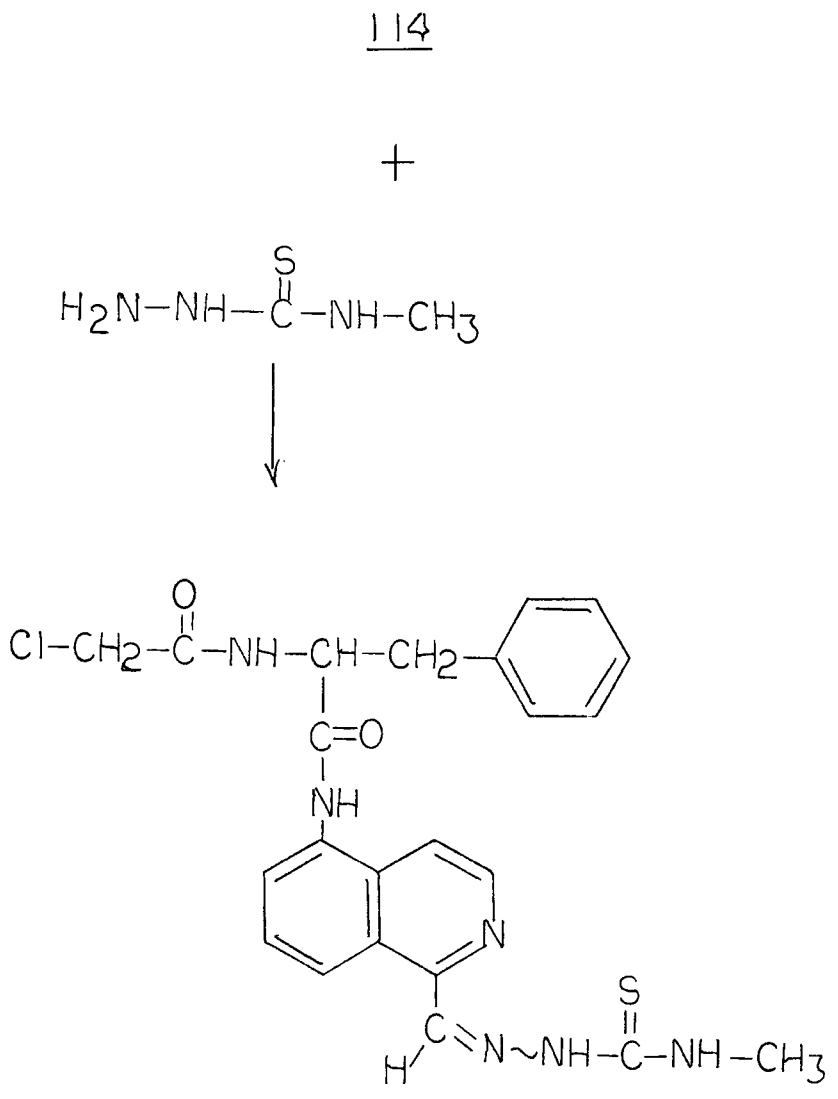
formed with retention of the amino acid protecting group as illustrated in Schemes VII, VIII. Apparently, the acidity of hydrochloric acid was not of sufficient strength to remove the protecting group of the amino acid. As was expected, the products still have the ability to chelate with transition metals. Figure 3 illustrates the two to one complex ratio that exists between compound (112) and cupric chloride (both concentrations are 5×10^{-3} mole).

Scheme VII


CH2-CH-C-OH
NH
C=O
CH2
Cl
O
Cl-C-O-C2H5
+
N(C2H5)3
CH2-CH-C-O-C-O-C2H5
NH
C=O
CH2
Cl
NH2
N
O
C
H
O
113
CH2-CH-C-NH
NH
C=O
CH2
Cl
N
O
C
H
O
114

Scheme VII

Continued 1

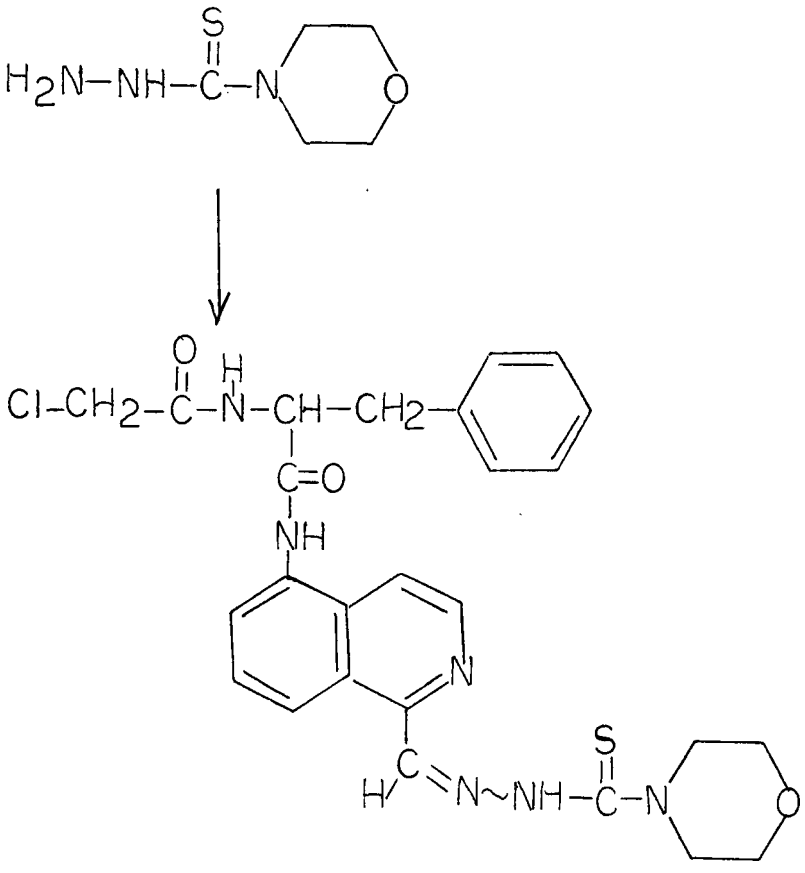


Scheme VII

Continued 2

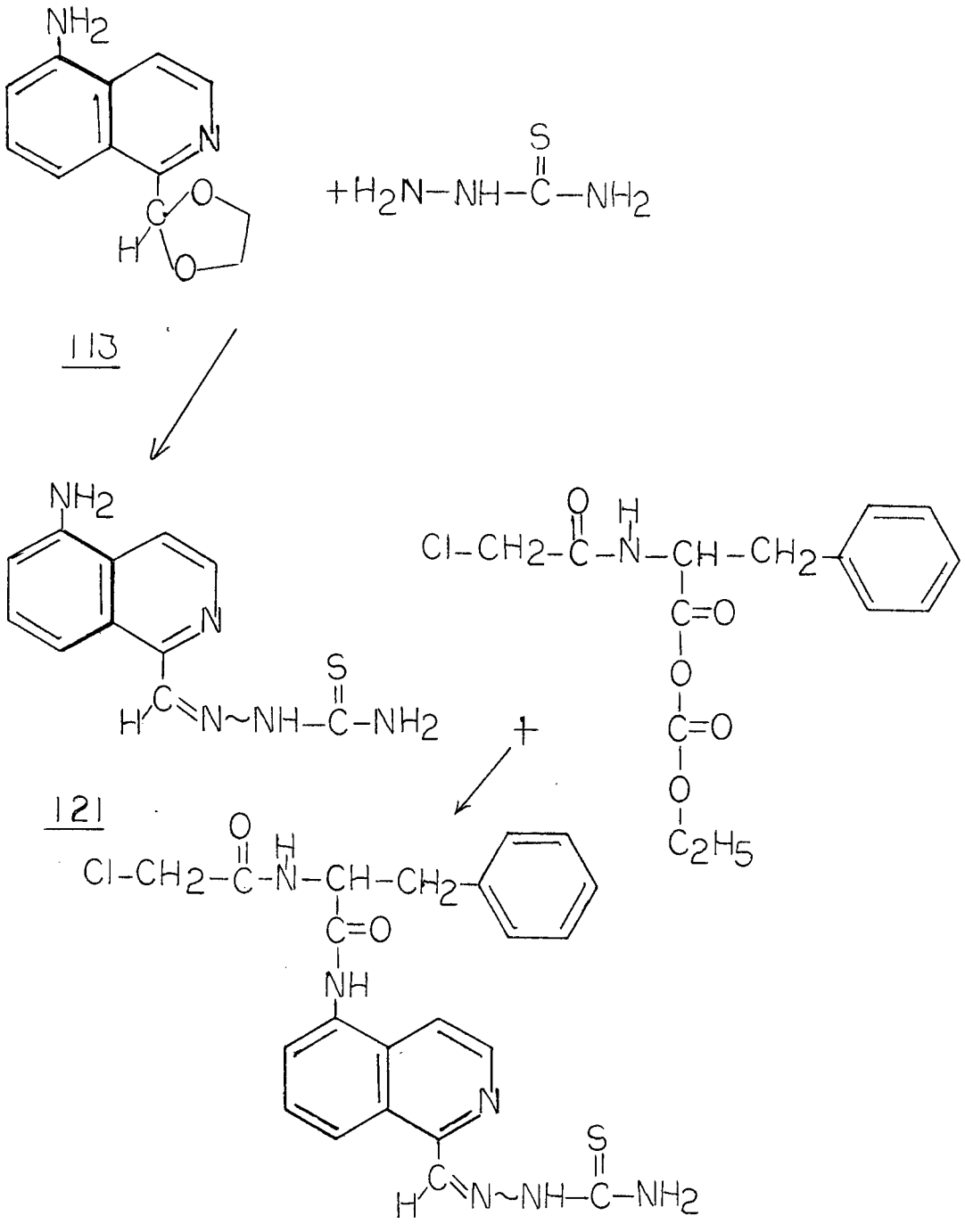
114

+



120

Scheme VIII


NH2
N
O
H
O
+H2N–NH–C(=S)–NH2
113
NH2
N
H
C=N~NH–C(=S)–NH2
Cl–CH2–C(=O)–N(H)–CH–CH2
C=O
O
C=O
O
C2H5
+
121
Cl–CH2–C(=O)–N(H)–CH–CH2
C=O
NH
N
H
C=N~NH–C(=S)–NH2
122

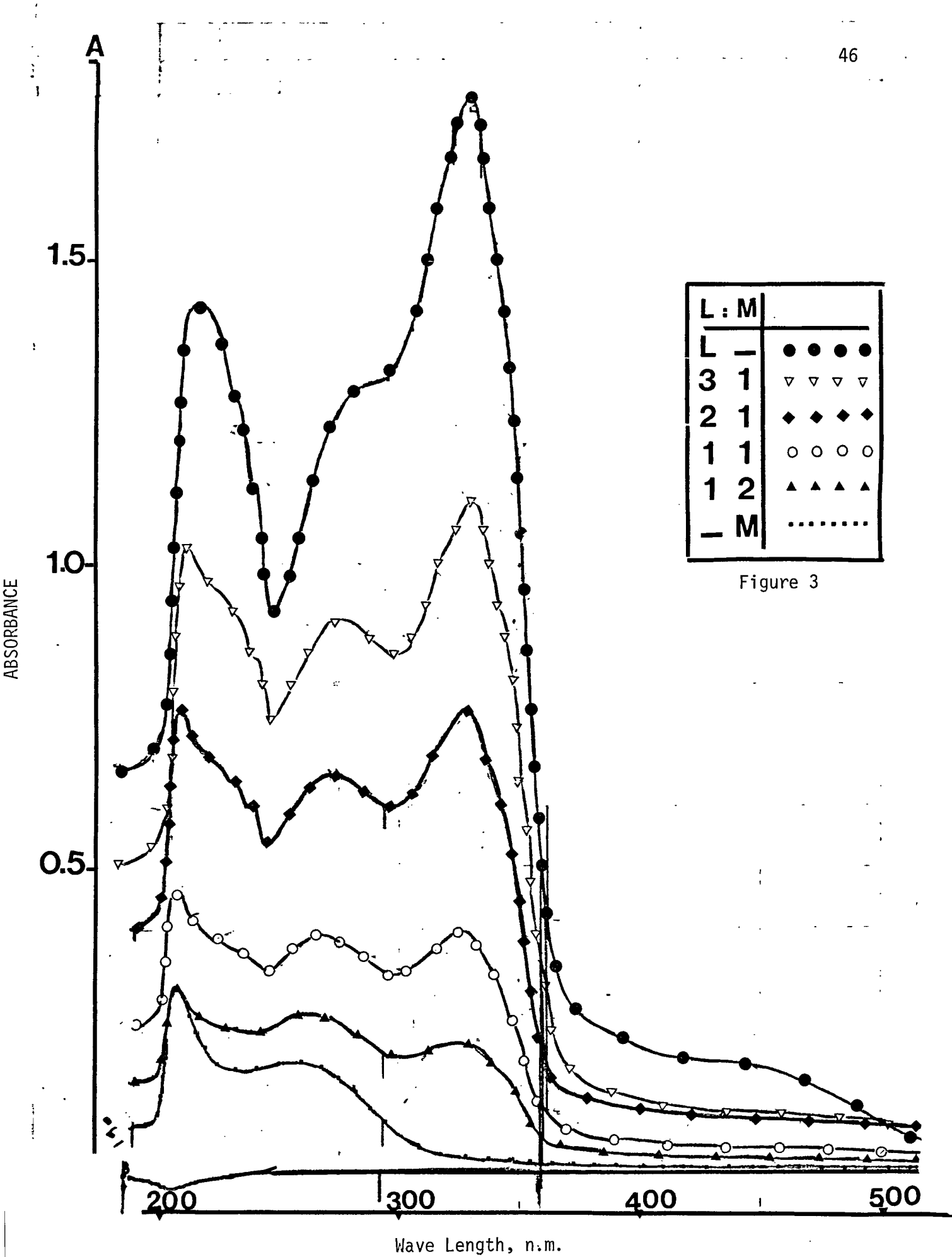

A
1.5
1.0
0.5
ABSORBANCE
L : M
L —
3 1
2 1
1 1
1 2
— M
200
300
400
500
Wave Length, n.m.

Figure 3

III. EXPERIMENTAL

A. Materials and Reagents

Reagent grade starting materials were purchased from different companies and include isoquinoline (Eastman, Kodak), n-butyllithium (Alfa), methyl iodide (Fisher), N,N-dicyclohexylcarbodiimide, N-chloroacetyl-L-phenylalanine, N-benzoyl-DL-phenylalanine (Sigma), and thiosemicarbazide (Aldrich).

All other chemicals and solvents used were reagent grade, and deionized water was used for all aqueous solutions.

B. Instrumentation

Thin layer chromatography (TLC) was used to determine the homogeneity of the compounds synthesized, especially when only a small amount of substance was available. Silica gel was the adsorbent in all experiments. The reported melting points (m.p.) are uncorrected and were taken on a Fisher Jones apparatus. Ultraviolet spectra (UV) were obtained on a Beckman BD-GT dual beam ultraviolet spectrophotometer.

Infrared spectras (IR) were obtained utilizing a Perkin-Elmer Model 700 spectrophotometer. All samples were prepared as KBr (spectrograde-Baker) pellets (concentration, 1mg/300mg KBr).

Proton magnetic resonance ('H-nmr) spectras were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6), both of which were obtained from Aldrich. A Varian Associates Model EM-360 spectrometer was used to obtain the spectral data. Tetramethylsilane (TMS) was used as the internal standard with $CDCl_3$ solvent.

A Hewlett Packard Model 5941A mass marker coupled with a Model 5710A gas chromatograph and a Model 5933A recording system were used for collecting mass spectral data. In some instances, a Hitachi Perkin Elmer Model RMU-6H mass spectrometer was also used.

Elemental analyses were performed by Altantic Microlab Inc., Atlanta, Georgia.

C. Syntheses and Attempted Syntheses of Compounds

Synthesis of 1-Cyano-2-benzoyl-1,2-dihydroisoquinoline (Reissert's compound (93)).

A solution of 195.5 g (3 moles) of potassium cyanide was dissolved in 1250 ml of water, then 129 g (1 mole) of isoquinoline (Figure 4 and Figure 5) was added. The mixture was maintained below 25° by immersion in an ice bath. When the isoquinoline had formed an emulsion upon stirring with the aqueous solution, 281 g (2 moles) of benzoyl chloride was added slowly over three hours. The stirring was continued another hour or until the Reissert's compound had separated as small, hard, tan spheres. The reaction mixture was cooled further in the ice bath and the product collected and washed successively with water, 3N hydrochloric acid and water again. The product was then recrystallized from 150 ml of commercial absolute ethanol using 2.5 g of activated carbon to effect partial decolorization of the solution.

The mixture was heated and filtered to remove the charcoal utilizing a filter aid. The filtrate was cooled and filtered again. The cream-colored crystals which separated were collected, washed with 100 ml of cold 95% ethanol and air dried overnight. The yield of dry 1-cyano-2-benzoyl-1,2-dihydroisoquinoline, sufficiently pure for use in the next

step, was 128.5 g (52%). m.p.=124-126°C (Lit.[40] m.p.=125-127°C). NMR ($CDCl_3$): δ=6.00 (d,1H,J=8Hz), 6.65 (d,2H8Hz), 7.45 (m,9H) (Figure 6). IR (KBr): cm^{-1}=1640(s) (Figure 7).

Synthesis of 1-Cyano-1-methyl-2-benzoyl-1,2-dihydroisoquinoline (94).

The reaction flask used for this step was dried in an oven, then assembled to maintain an inert atmosphere of nitrogen. The apparatus was flushed with dry nitrogen for one hour, and 83.5 g (.32 mole) of (93), 350 ml of dry dioxane and 100 ml of anhydrous ether were then added. When the solid had dissolved completely upon stirring, the flask was immersed in an ice-salt bath at 10°C. Then 175 ml (0.35 mole) of a 2M solution of n-butyl lithium was added dropwise while stirring over 30 minutes. The reaction mixture turned a deep red, and as the addition was continued, a red solid separated. Ten minutes after the addition was completed, 56.2 g (0.40 mole) of methyliodide was added. The reaction mixture was stirred in the cold for two hours, then overnight at room temperature. The reaction mixture was transferred to a separatory funnel and washed with three 50 ml portions of water, the organic solution was filtered and the solvent removed under reduced pressure. If the residue did not crystallize immediately upon evaporation of the solvent, crystallization was induced by scratching the sides of the flask and cooling. The crystals were then transferred to a Buchner funnel, washed with 50 ml of cold 95% ethanol and dried. The yield of dry 1-cyano-1-methyl-2-benzoyl-1,2-dihydroisoquinoline (94) in the form of cream-colored crystals was 58 g (66%). m.p.=112°C (Lit.[40] 120-122°C). NMR ($CDCl_3$): δ=1.25 (s,3H), 7.22 (m,8H), 7.50 (d,1H,J=10Hz), 7.82 (d,H,J=6Hz) (Figure 8). (R (KBr): cm^{-1}=1640(s) (Figure 9).

Synthesis of 1-Methylisoquinoline (95).

In a 500 ml round-bottom flask equipped with a reflux condenser were placed 62.2 g (0.227 mole) of (94), 50 ml of 95% ethanol and a solution of 37.6 g (.57 mole) of 85% potassium hydroxide in 100 ml of water. The mixture was heated at reflux for 1.5 hours, during which time the solid dissolved and the solution became homogeneous. After the solution cooled, it was extracted with ethanol. The extracts were washed with two 25 ml portions of water and dried with anhydrous magnesium sulfate. After removal of the drying agent by filtration, the mixture was concentrated under vacuum, and the residue was distilled under reduced pressure to give 25 g (77%) of colorless 1-methylisoquinoline (95) b.p. 81°/1mm Hg: nD^{25} 1.61 (Lit.[40] b.p. 81°C, 1mm Hg). NMR (Neat): δ=2.00 (s,3H), 6.65 (m,5H), 7.60 (d,1H,J=6Hz) (Figure 10). IR (Neat): cm^{-1}=2990(m) (Figure 11).

Synthesis of 1-Methyl-5-nitroisoquinoline (96).

A solution of 27.5 g of potassium nitrate in 150 ml of concentrated sulfuric acid was added with stirring to a solution of 36 g of (95) in 200 ml of concentrated sulfuric acid. During the addition, the mixture which was chilled in an ice-salt bath such that the temperature of the mixture did not exceed 4°C. The addition required 2.5 hours, after which solution was stirred for an additional two hours during which time the reaction allowed to rise to room temperature. It was then poured into a mixture of two liters of water and 2 kg of crushed ice. After neutralization by cautious addition of ammonium hydroxide, the suspension was cooled and the solid was collected and recrystallized from ethanol yielding 32.5 g (68%). m.p.=148-150 (Lit.[21] 150-151). NMR ($CDCl_3$): δ=3.00 (s,3H), 7.65 (t,1H, J=8Hz), 8.20 (d,1H,J=6Hz), 8.30 (d,1H,J=7Hz), 8.40 (d,1H,J=9Hz), 8.55 (d, 1H,J=7Hz) (Figure 12). IR (KBr): cm^{-1}=1345, 1520(s) (Figure 13).

Synthesis of 1-Methyl-5-aminoisoquinoline (97).

Compound (96) 13.3 g (.85 mole) was dissolved in 60 ml concentrated hydrochloric acid[38,39] and added over a period of about fifteen minutes to a solution of 64 g of stannous chloride in 100 ml of 2N hydrochloric acid at 60°C. The temperature was then increased to 80°C and maintained for one hour. After cooling, the reaction mixture was poured into a solution of 80 g (2 moles) of sodium hydroxide in 2.5 liters of water and allowed to stand about a day before filtering the product, which appeared as white plates weighing 10.95 g (99%). m.p.=213-214°C (Lit.[21] m.p.=213-214). NMR (DMSO): δ=2.8 (s,3H), 5.95 (s,2H), 6.92 (t,1H,J=4Hz), 7.35 (d,2H,J=4Hz), 7.84 (d,1H,J=6Hz), 8.22 (d,1H,J=6Hz) (Figure 14). IR (KBr): cm^{-1}=3300 (Figure 15).

Attempted synthesis of 1-Methyl-5N-trifluoroacetoisoquinoline (98).

Trifluoroacetic anhydride was added dropwise to a 5 g (.03 mole) of (97), until all starting material was dissolved. The compound was air dried overnight. The product was identified to be (106) (Eq. 3), which recrystallized from dioxane as yellow crystals weighing 10.5 g (99%) m.p.= 169°C. NMR (DMSO): δ=3.25 (s,3H), 8.25 (m,7H) (Figure 16). IR (KBr): cm^{-1}=1710(s) (Figure 17). Anal. Calcd. for $C_{14}H_{10}N_2O_2F_6$ (MW 352.24): C, 47.74; H, 2.86; N, 7.96. Found: C, 46.11; H, 2.84; N, 7.81.

Synthesis of 1-Methyl-5N-acetoisoquinoline (107).

By reacting 5 g (0.03 mole) of (97) with acetic anhydride, addition of the aceto group occurred only at position five. The yield was quantitative and the compound was recrystallized from dioxane. m.p.=226°C (Lit.[21] 226-227°C). NMR ($CDCl_3$): δ=2.20 (s,3H), 2.9 (s,3H), 7.8 (m,4H), 8.30

(d,1H,J=Hz), 9.90 (s,1H) (Figure 18). IR (KBr): cm^{-1}=1650(s), 3300(s) (Figure 19).

Synthesis of 1-Formyl-5N-acetoisoquinoline (108) .

A solution of 9.4 g (0.05 mole) of 107 in 200 ml of dioxane was treated with 5.55 g (0.05 mole) of selenium dioxide (freshly resublimed)[21] and the mixture was refluxed for three hours. The precipitated selenium was removed by filtration, and the filtrate was flash evaporated. The residue was dissolved in dilute hydrochloric acid and filtered. The clear solution was then made alkaline with (10%) solution of sodium carbonate. The precipitate was filtered, washed with water, dried and crystallized from benzene to give 6 g (60%). m.p.=206°C (Lit.[21] 206-207°C). NMR (DMSO): δ=2.20 (s,3H), 7.30-9.50 (m,6H), 10.30 (s,1H) (Figure 20). IR (KBr): cm^{-1}=1710(s), 1650(s), 3300 (Figure 21).

Synthesis of 1-Formyl-5-nitroisoquinoline (111)[20,21].

A solution of 9.4 g (0.05 mole) of (96) in 200 ml of dioxane was treated with 5.55 g (0.05 mole) of selenium dioxide (SeO_2) (freshly resublimed) and the mixture was refluxed for three hours. The precipitated selenium was removed by filtration, and the filtrate was flash evaporated. The residue was dissolved in dilute hydrochloric acid, filtered, and the filtrate was made alkaline with solid sodium carbonate. The precipitate was filtered, washed with water, dried and crystallized from benzene to yield 5 g (50%). m.p.=170-171°C (Lit.[21] 175-176°C). NMR ($CDCL_3$): δ=7.20 (t,1H,J=7Hz), 8.95 (d,1H,J=6Hz), 9.70 (d,1H,J=8Hz), 10.35 (s,1H) (Figure 22). IR (KBr): cm^{-1}=1320(s), 1520(s), 1705(s) (Figure 23).

Synthesis of 1-Formyl-ethyleneacetal-5-nitroisoquinoline (112).

The procedure for the synthesis of (112) was adapted from the Agrawal[20] method for the preparation of 1-formyl-ethyleneacetal-4-methyl-5-nitroisoquinoline. To 10.15 g (0.05 mole) of (111) in 300 ml of benzene was added 0.5 g of para-toluenesulfonic acid and 10 ml of ethylene glycol. The mixture was refluxed with stirring for 24 hours using a Dean-Stark trap to remove the water formed during condensation. The mixture was then washed with 25 ml of 10% sodium bicarbonate solution followed by 25 ml of water. The benzene layer was dried with magnesium sulfate and removed under vacuum and the residue was recrystallized from ethanol to yield 8.30 g (67%) m.p.= 124-126°C. NMR ($CDCl_3$): δ=4.25 (s,4H), 6.40 (s,1H), 7.65 (t,1H,J=8Hz), 8.55 (m,3H) (Figure 24). IR (KBr): cm^{-1}=1340(s), 1520(s) (Figure 25). Anal. Calcd. for $C_{12}H_{10}N_2O_4$ (MW 246.21): C, 58.53; H, 4.09; N, 11.38. Found: C, 58.48; H, 4.12; N, 11.39.

Synthesis of 1-Formyl-ethyleneacetal-5-aminoisoquinoline (113).

The synthesis of compound (113) was adapted from the Agrawal[20] method for the preparation of 1-formyl-ethyleneacetal-4-methyl-5-aminoisoquinoline. Compound (112) (2 g, 0.008 mole) was dissolved in 50 ml of ethanol and 0.2 g of Pd/C (10%) was added. The mixture was hydrogenated at 50 psi for one hour, using heat to dissolve the compound, then the product was filtered to remove the catalyst. The ethanol was removed under vacuum and the residue was recrystallized from benzene to yield 1.6 g (90%) of (115) instead of (113). The use of heat was responsible for the reduction of the double bonds at position one and three of (113), as shown in Eq. 4. MW=220.2. m.p.=138-139°C NMR ($CDCl_3$): δ=3.95 (s,4H), 5.25 (d,2H,J=4Hz), 6.8 (m,3H) (Figure 26). IR (KBr): cm^{-1}=3300(m) (Figure 27). Anal. Calcd. for C_{12}

$H_{16}N_2O_2$ (MW 220.2): C, 65.43; H, 7.32; N, 12.72. Found C, 65.37; N, 7.39; N, 12.70. Mass Spectra (M.S.) (M^+=220.2) (Figure 28).

After it was established that the use of heat gave an undesirable product, the same procedure was followed in the absence of heat resulting in a dark oily product. The product could not be crystallized, even after separation by column chromatography. NMR ($CDCl_3$): δ=3.95 (n,4H,J=5Hz), 4.25 (s,2H), 6.20 (s,1H), 6.55 (d,1H,J=7Hz), 7.05 (t,1H,J=8Hz), 7.28 (d,1H J=7Hz), 7.48 (d,1H,J=8Hz), 8.10 (d,1H,J=6Hz) (Figure 29). IR (KBr): cm^{-1}= 1480(s), 1592(s), 1640(s), 3300(s) (Figure 30). M.S. (M^+=216.3) (Figure 31).

Attempted Synthesis of 1-Formyl-ethyleneacetal-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline (114).

A 2.16 g (0.01 mole) portion of compound (113) was dissolved in 100 ml of methylene chloride (CH_2Cl_2), followed by the addition of 2.42 g of N-chloroacetyl-L-phenylalanine. After stirring for five minutes, 2.06 g (0.01 mole) of dicyclohexyl carbodiimide (DCC) was added. After two hours, a white solid was separated by filtration and found to be dicyclohexyl urea (DCU) (Figure 32). The filtrate was flash evaporated, yielding a golden solid upon treatment with ether. Analysis showed that a total separation or removal of DCU was unfeasible even with the use of column chromatography. m.p.=90°C. NMR ($CDCl_3$): δ=3.20 (d,1H,J=7Hz), 4.00 (s,2H), 4.15 (s,4H), 6.00 (m,1H), 6.25 (d,1H,J=5Hz), 6.80 (d,1H,J=8Hz), 7.20 (s,5Hz), 7.55 (m,5H), 8.30 (d,1H,J=6Hz), (Figure 33). IR (KBr): cm^{-1}=1550(s), 1640(s), 1705(s), 3300(s) (Figure 34). Anal. Calcd. for $C_{23}H_{22}N_3O_4Cl$ (MW 439.883): C, 62.796; H, 5.04; N, 9.56. Found: C, 62.43; H, 6.03; N, 10.383. M.S. (M^+=446) (Figure 35).

Attempted Synthesis of 1-Formyl-5N-phenylalanineisoquinoline thiosemicarbazone (103)[20].

A 0.5 g (0.0011 mole) sample of compound (114) in 50 ml of ethanol was added to 2 ml of concentrated hydrochloric acid and 0.091 g (0.01 mole) of the thiosemicarbazide. The mixture was refluxed for 1.5 hours and the precipitate of the hydrochloride salt of the desired compound was collected by filtration, washed with ethanol and dried. The hydrochloride salt was then dissolved in 100 ml of 10% sodium carbonate solution. The greenish precipitate which formed was filtered and washed with water and ethanol. Spectral and elemental analysis showed that the desired compound did not form, and when the reaction was repeated with methyl thiosemicarbazone, still the desired compound did not form. m.p.=214. NMR (DMSO): δ=21 (s,3H), 3.10 (d,2H,J=4Hz), 4.90 (m,2H), 8.7 (m,5H), 12.2 (s,1H) (Figure 36). IR (KBr): cm^{-1}=1400(s), 1650(m), 2390(m) (Figure 37). Anal. Calcd. for $C_{21}H_{22}N_6OH$ (MW=406.39): C, 62.043; H, 5.456; N, 20.676. Found: C, 50.30; H, 5.14; N, 19.86. M.S. (M^+=259.32) (Figure 38). These results indicated that (117) was formed instead of (118).

Synthesis of 1-Formyl-5-nitroisoquinoline-N^4-methylthiosemicarbazone (116).

This reaction was carried out to test the ease of removing the acetal and the addition of methyl thiosemicarbazide, as shown in Equation 5. The same procedure described above was followed. m.p.=214°C. NMR (DMSO): δ= 3.1 (d,3H,J=4Hz), 8.55 (m, 7H), 11.9 (s,1H) (Figure 39). IR (KBr): cm^{-1}= 1345(s), 1520(s) (Figure 40). M.S. (M^+=289.32) (Figure 41).

Synthesis of 1-Formyl-5-aminoisoquinoline-N^4-methylthiosemicarbazone (117).

For the preparation of this compound the same procedure mentioned above was followed. MW=259.32. m.p.=214°C NMR (DMSO): δ=3.10 (d,3H,J=5Hz), 5.90 (s,2H), 6.85 (d,1H,J=8Hz), 7.35 (t,1H,J=8Hz), 7.90 (d,1H,J=6Hz), 8.10 (m,2H), 8.33 (d,1H,J=6Hz), 8.60 (s,1H), 11.70 (s,1H) (Figure 36). IR (KBr): cm^{-1}= 3300(s) (Figure 37). M.S. (M^+=259.32) (Figure 38).

Synthesis of 1-Formyl-ethyleneacetal-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline (114)[41,42].

A solution of 6.05 g (0.025 mole) of N-chloroacetyl-L-phenylalanine and 2.55 g (0.025 mole) of triethylamine in 50 ml of toluene was cooled to 0°C and 2.45 g (0.025 mole) of ethylchloroformate added. After two hours at this temperature, during which time triethylamine hydrochloride separated, 5.40 g (0.025 mole) of 1-formyl-ethyleneacetal-5-aminoisoquinoline (113) was added. The reaction mixture was then stored at 8°C overnight. The desired product crystallized from the reaction mixture and was filtered off together with triethylamine hydrochloride, washed with water, dilute sodium hydroxide solution and dilute hydrochloric acid and dried. The brown crystals were 4 g (37%) MW (439.89). m.p.=168-170°C. Anal Calcd. for $C_{23}H_{22}N_3O_4Cl$ (MW 439.883) + HCl + 1/2 H_2O: C, 56.89; H, 4.98; N, 8.65. Found: C, 57.33; H, 5.68; N, 8.91. M.S. (M^+=439) (Figure 42).

Synthesis of 1-Formyl-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline-N^4-methylthiosemicarbazone (119)[25].

To 0.429 g (0.001 mole) of (114) in 50 ml of ethanol was added five drops of concentrated hydrochloric acid and 0.105 g (0.001 mole) of N^4-methylthiosemicarbazide. The mixture was refluxed for three hours. The

product was collected after removing the solvent. MW=482.5; m.p.=135°C. M.S. (M^+=446) (Figure 43).

Synthesis of 1-Formyl-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline morpholinothiosemicarbazone (120)[25].

To 0.439 g (0.001 mole) of (114) in 50 ml of ethanol was added five drops of concentrated hydrochloric acid and 0.161 g (0.001 mole) of N^4-morpholinothiosemicarbazide. The mixture was refluxed for three hours. The product was collected after removing the solvent. MW=538.5; m.p.=120-122°C. M.S. (M^+=538.4) (Figure 44).

Synthesis of 1-Formyl-5N-(N-choroacetyl-L-phenylalanine)-isoquinoline thiosemicarbazone (121)[25].

A solution of 0.24 g (0.001 mole) of N-chloroacetyl-L-phenylalanine and 0.101 g (0.001 mole) of triethylamine in 50 ml of toluene was cooled to 0°C and 0.108 g (0.001 mole) of ethylchloroformate added. After two hours at this temperature during which time triethylamine hydrochloride separated, 0.259 g (0.001 mole) of 1-formyl-5-aminoisoquinoline (121) was added. The reaction mixture was then stored at 8°C overnight. The desired product crystallized from the reaction mixture and was filtered off together with triethylamine hydrochloride, washed with water, dilute sodium hydroxide solution and dilute hydrochloric acid and dried. The crystals were collected from the filter paper. MW=468. M.S. (M^+=432) (Figure 45).

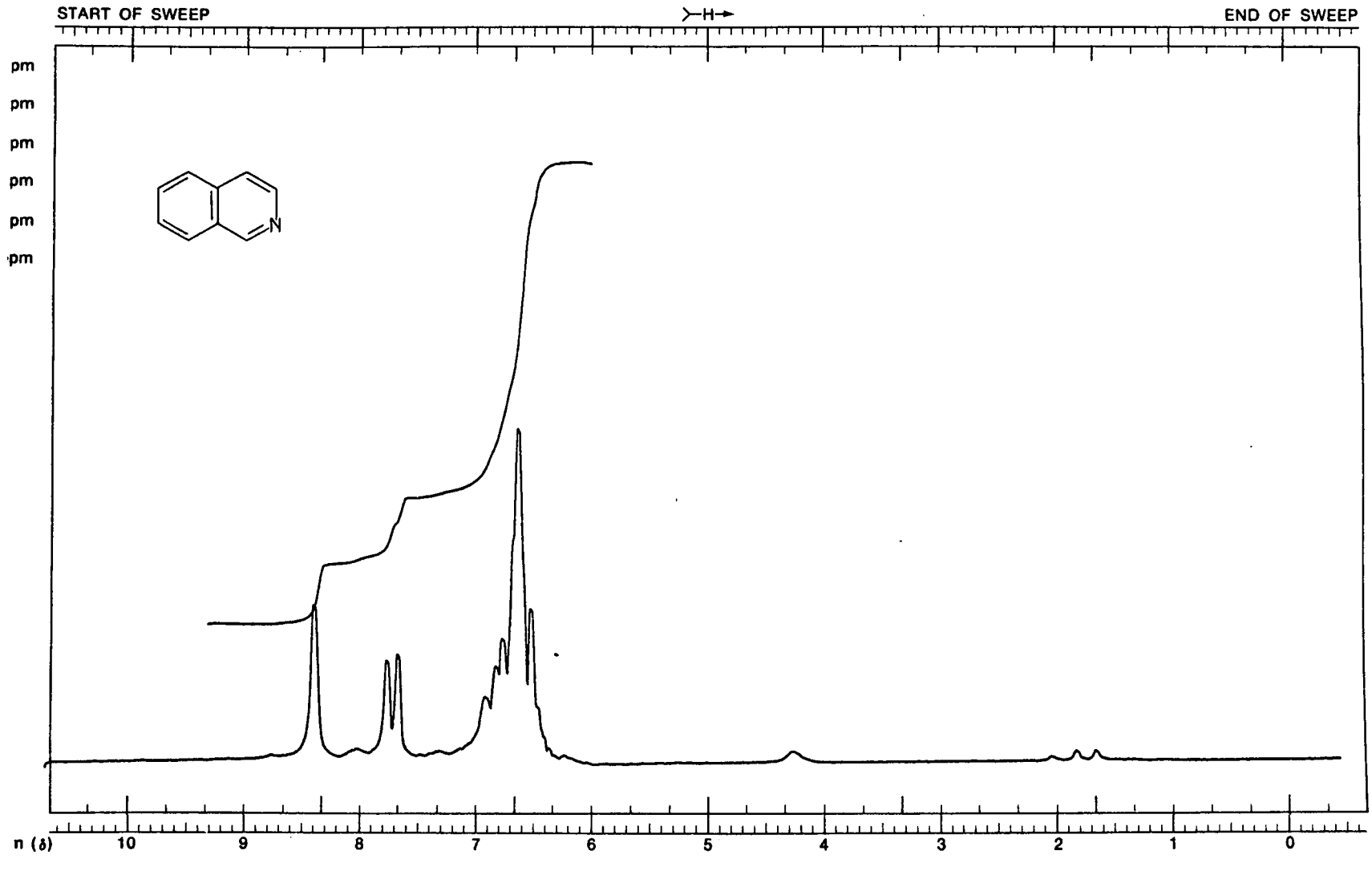


Figure 4: isoquinoline (92).

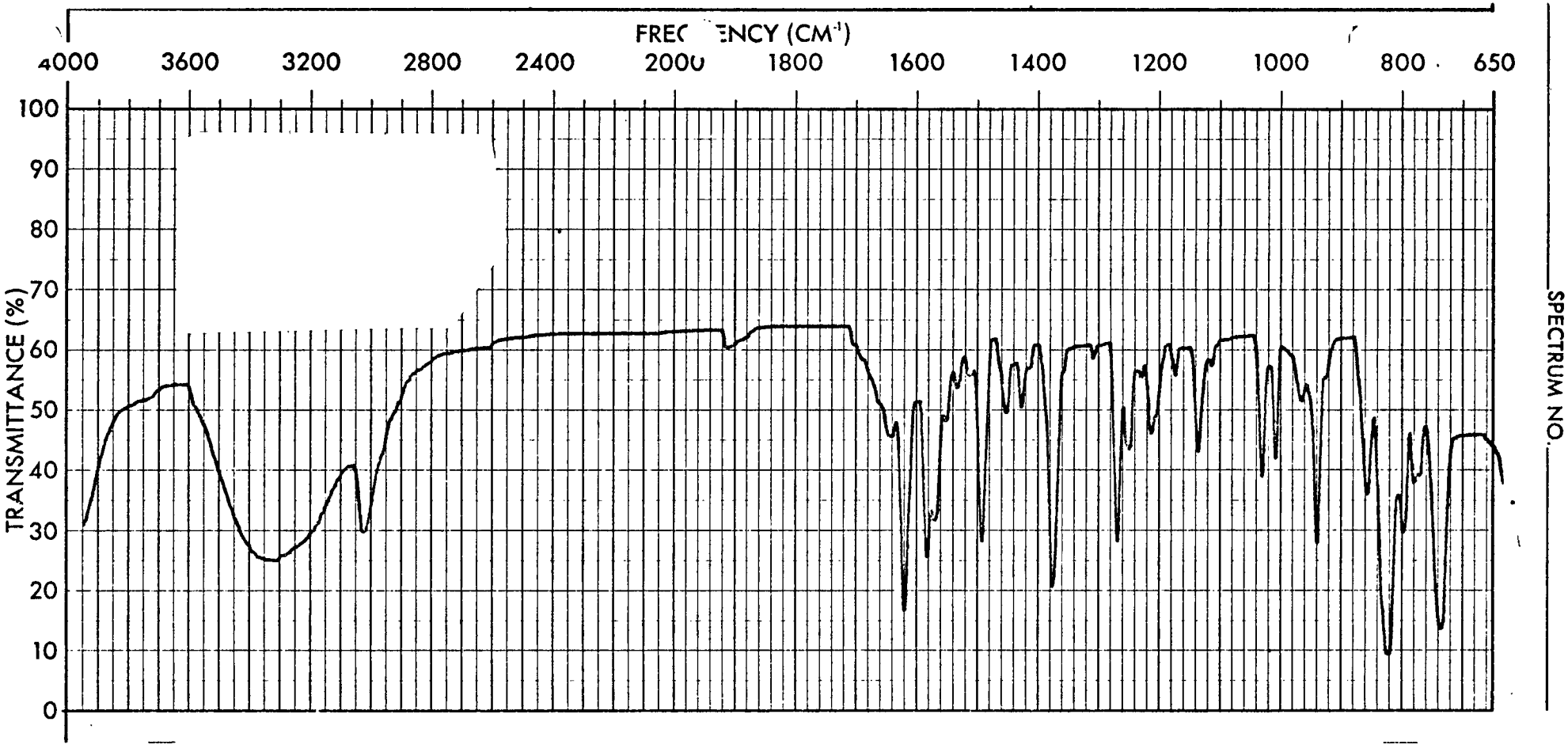
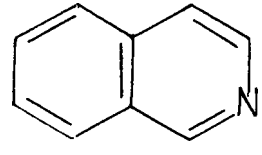


Figure 5: isoquinoline (92).



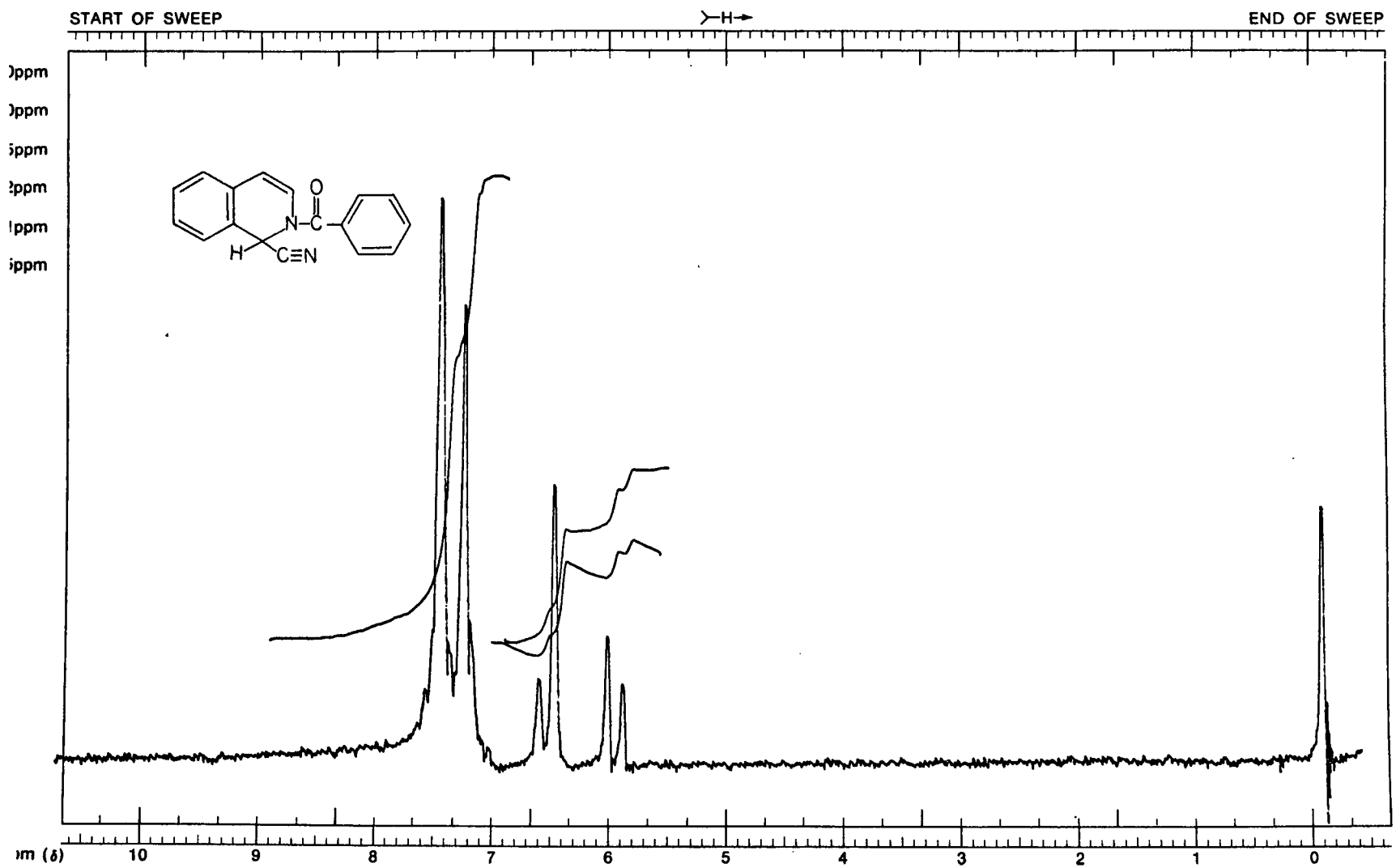
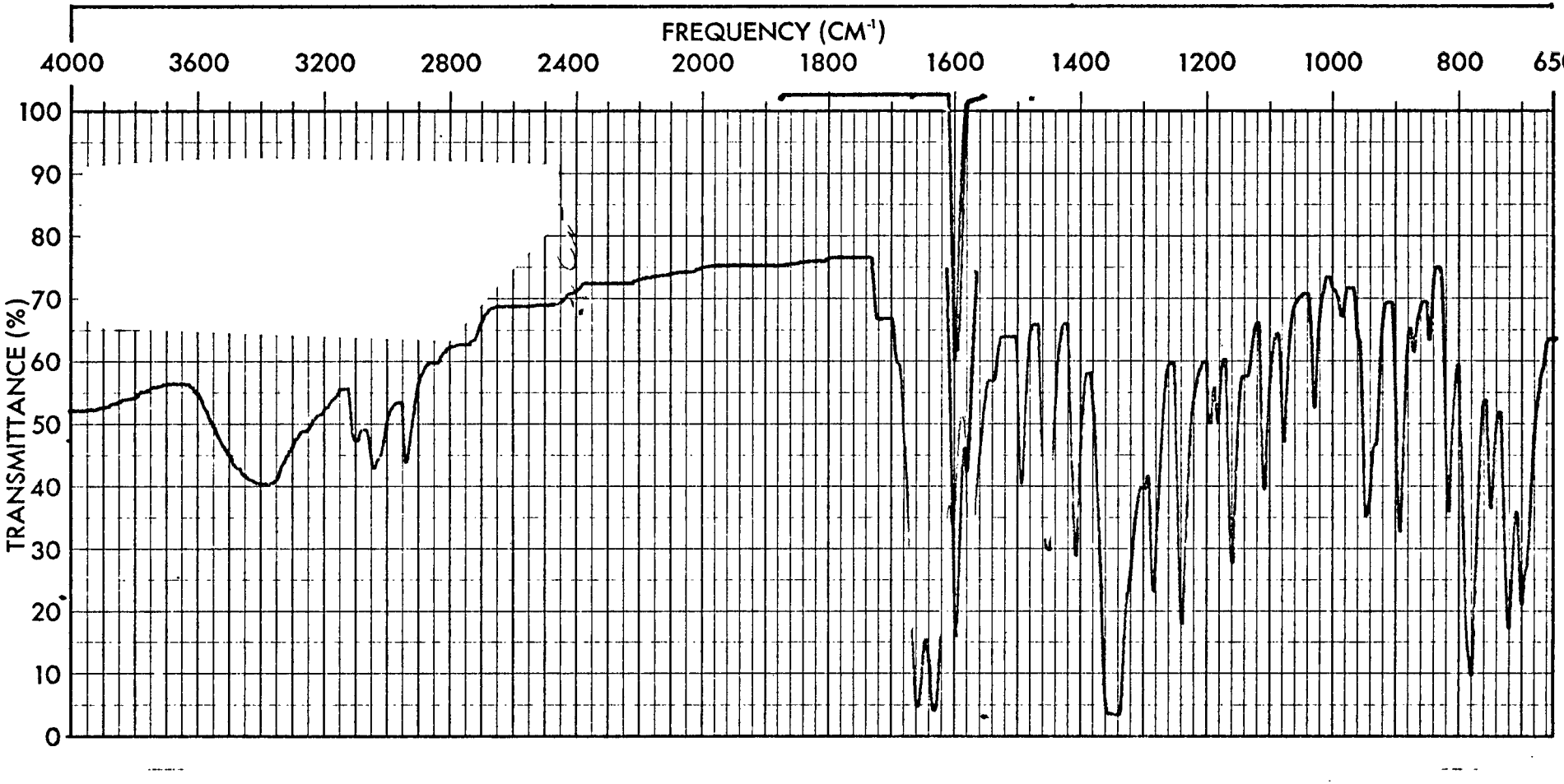
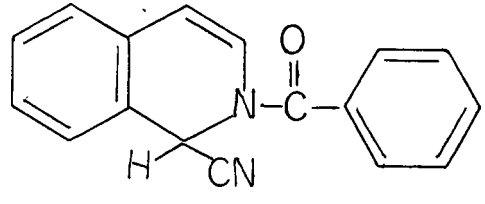


Figure 6: 1-cyano-2-benzoyl-1,2-dihydroisoquinoline (93)



SPECTRUM NO. 1

Figure 7: 1-cyano-2-benzoyl-1,2-dihydroisoquinoline (93)



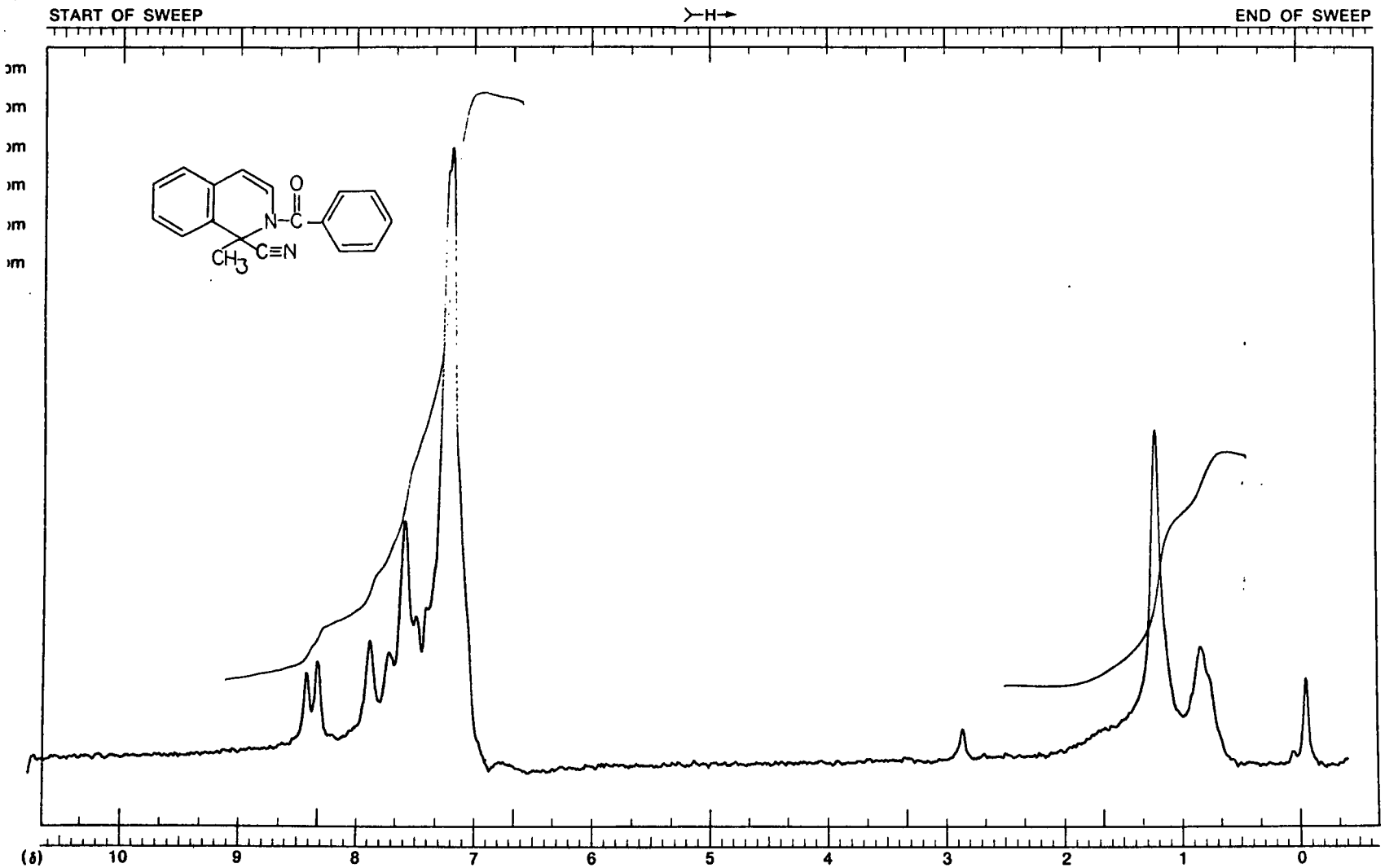


Figure 8: 1-cyano-1-methyl-2-benzoyl-1,2-dihydroisoquinoline (94)

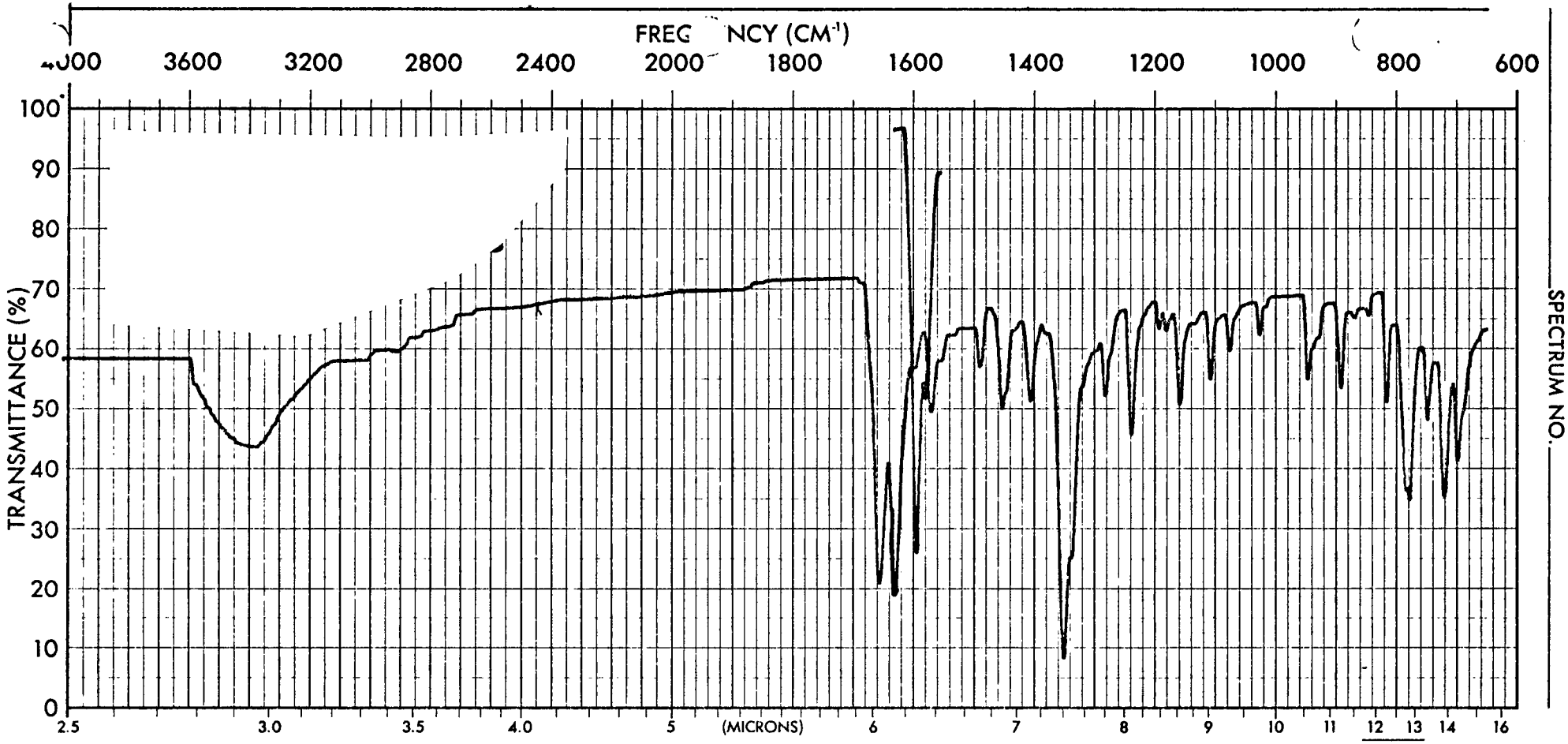
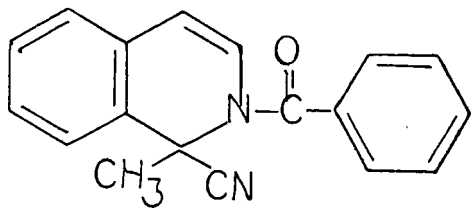


Figure 9: 1-cyano-1-methyl-2-benzoyl-1,2-dihydroisoquinoline (94)



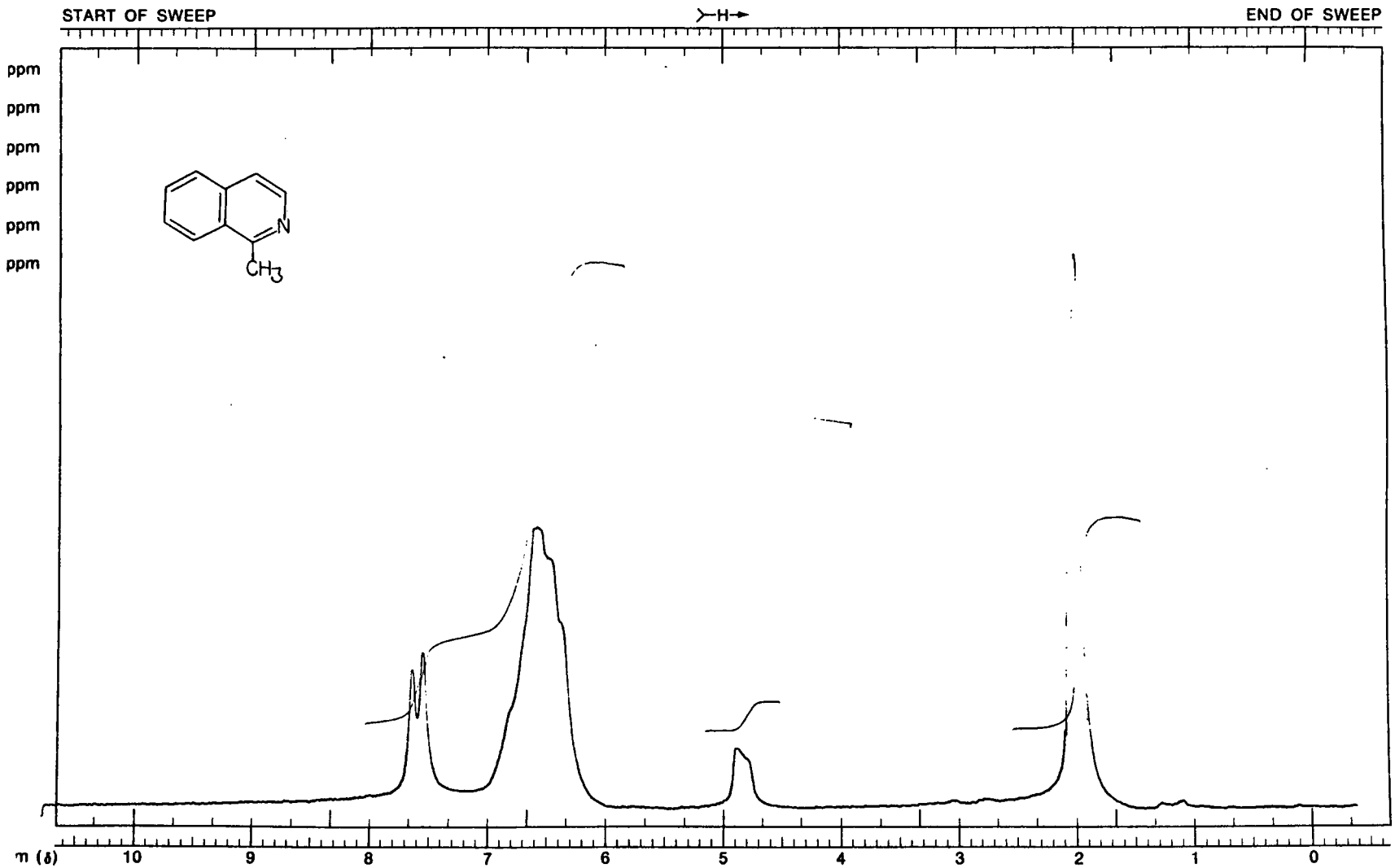


Figure 10: 1-methylisoquinoline (95).

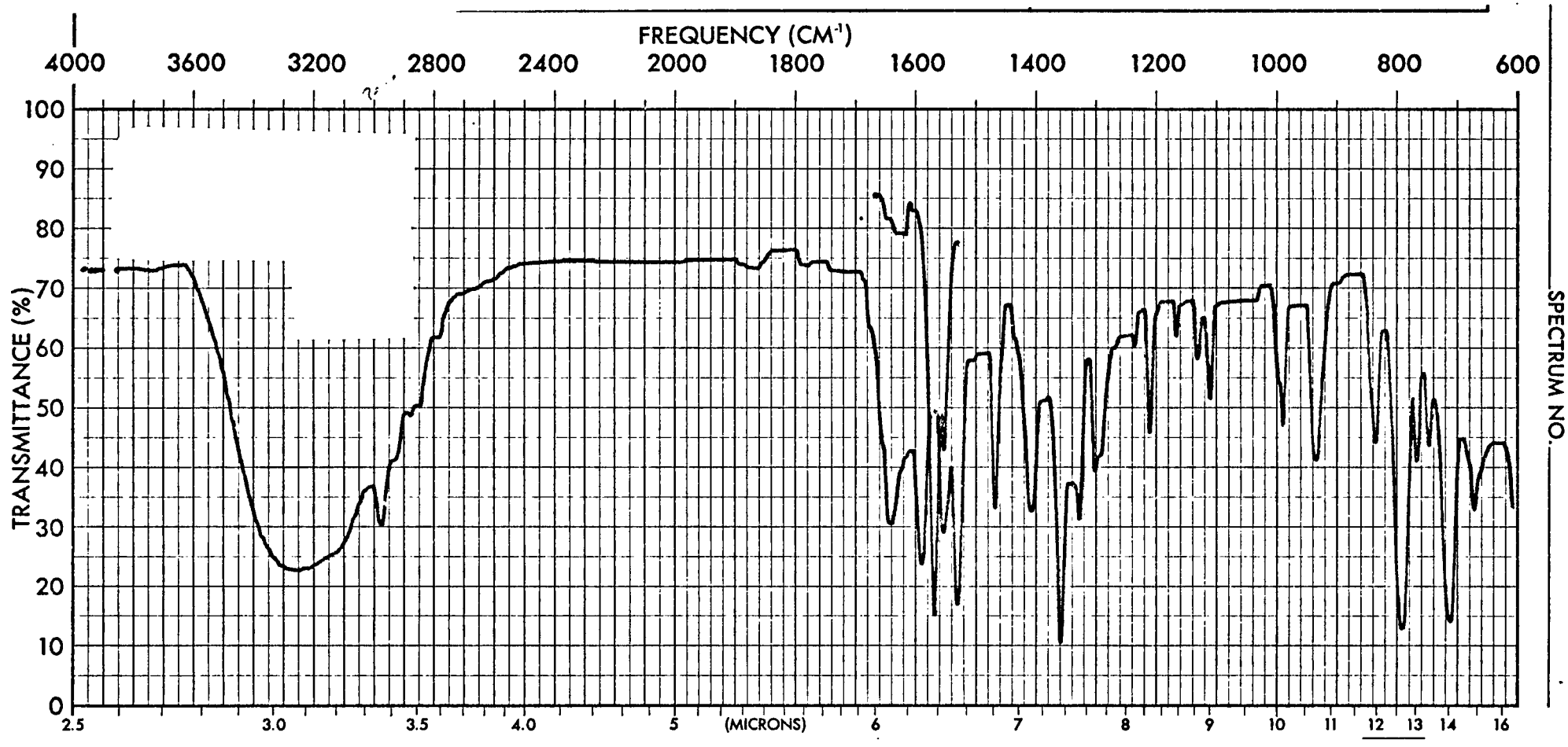
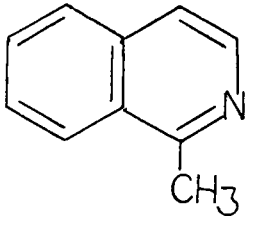


Figure 11: 1-methylisoquinoline (95).



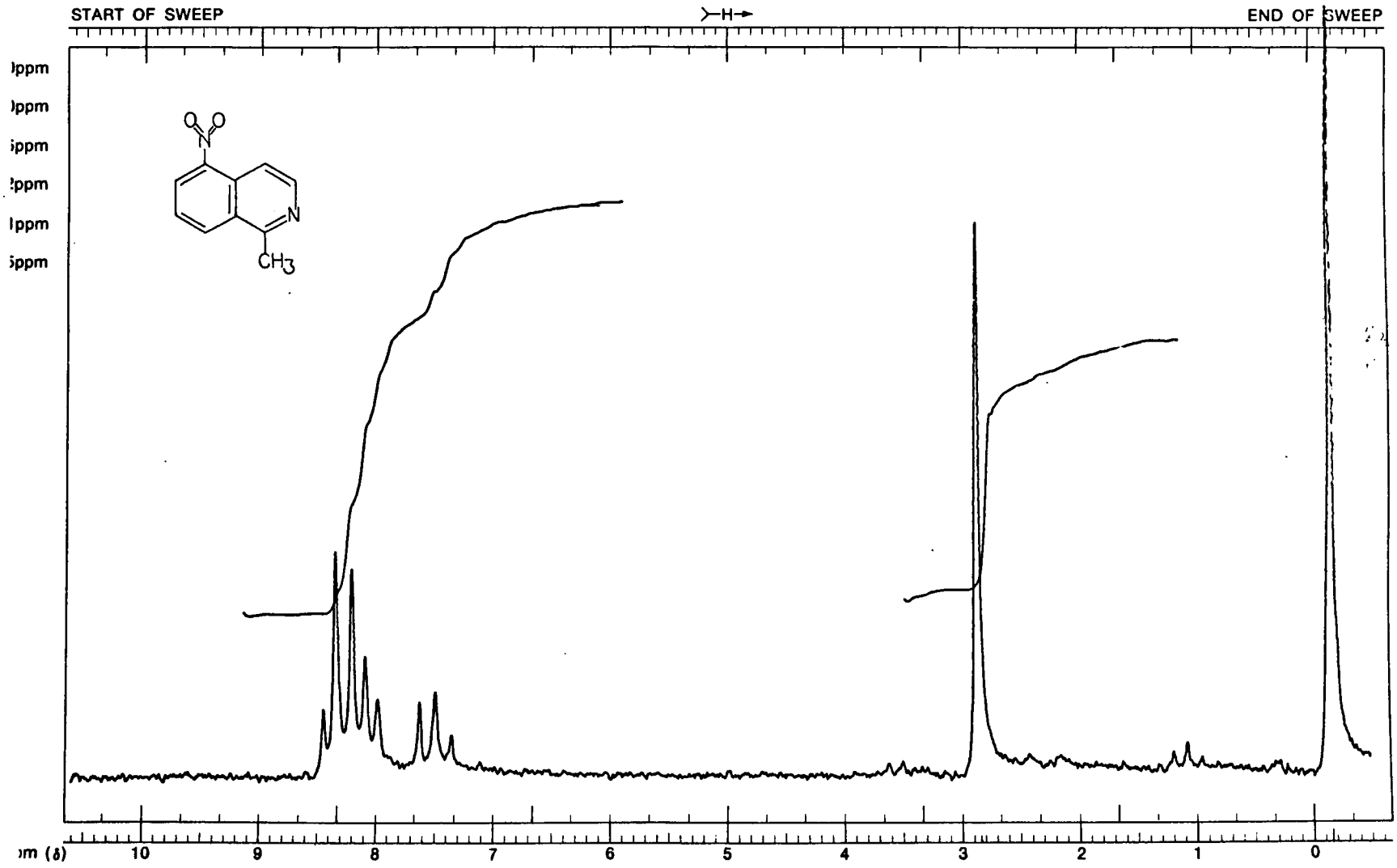


Figure 12: 1-methyl-5-nitroisoquinoline (96).

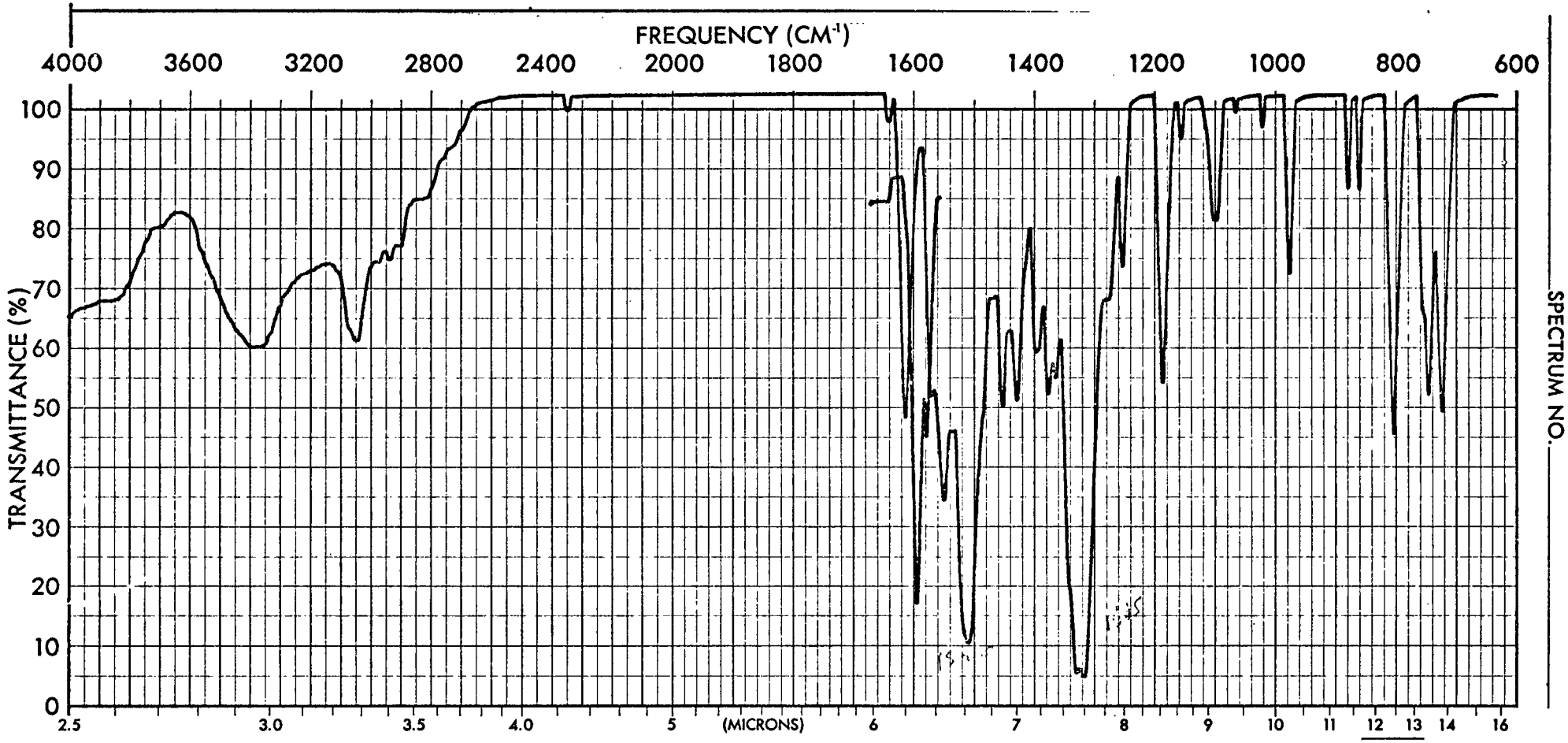
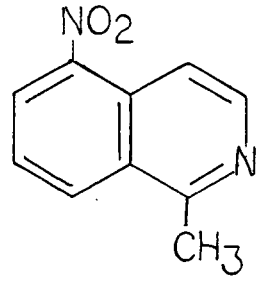


Figure 13: 1-methyl-5-nitroisoquinoline (96).



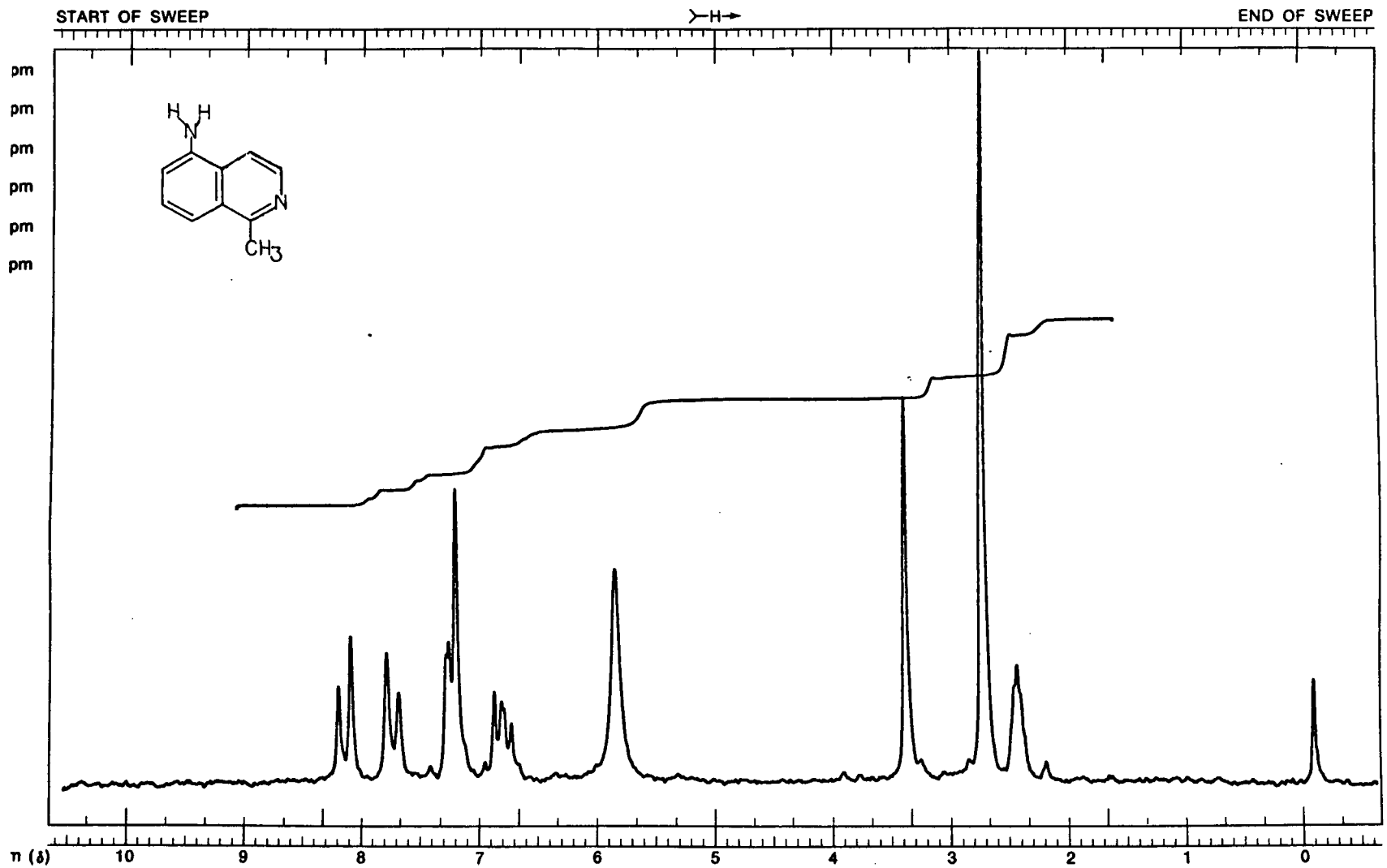
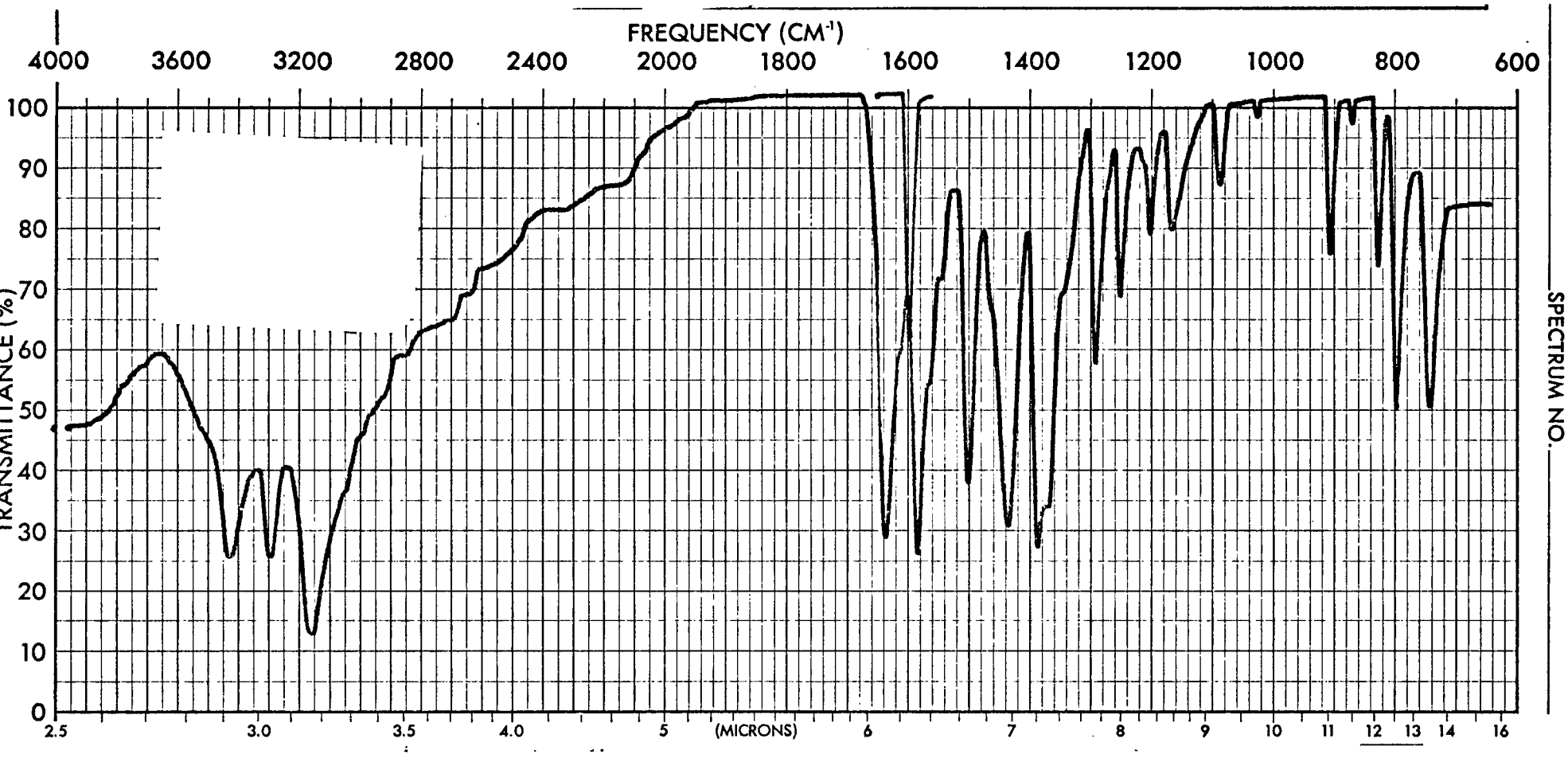


Figure 14: 1-methyl-5-aminoisoquinoline (97).



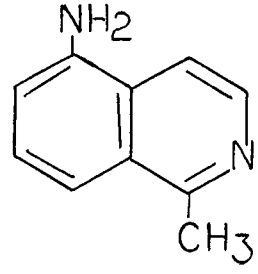


Figure 15: 1-methyl-5-aminoisoquinoline (97).

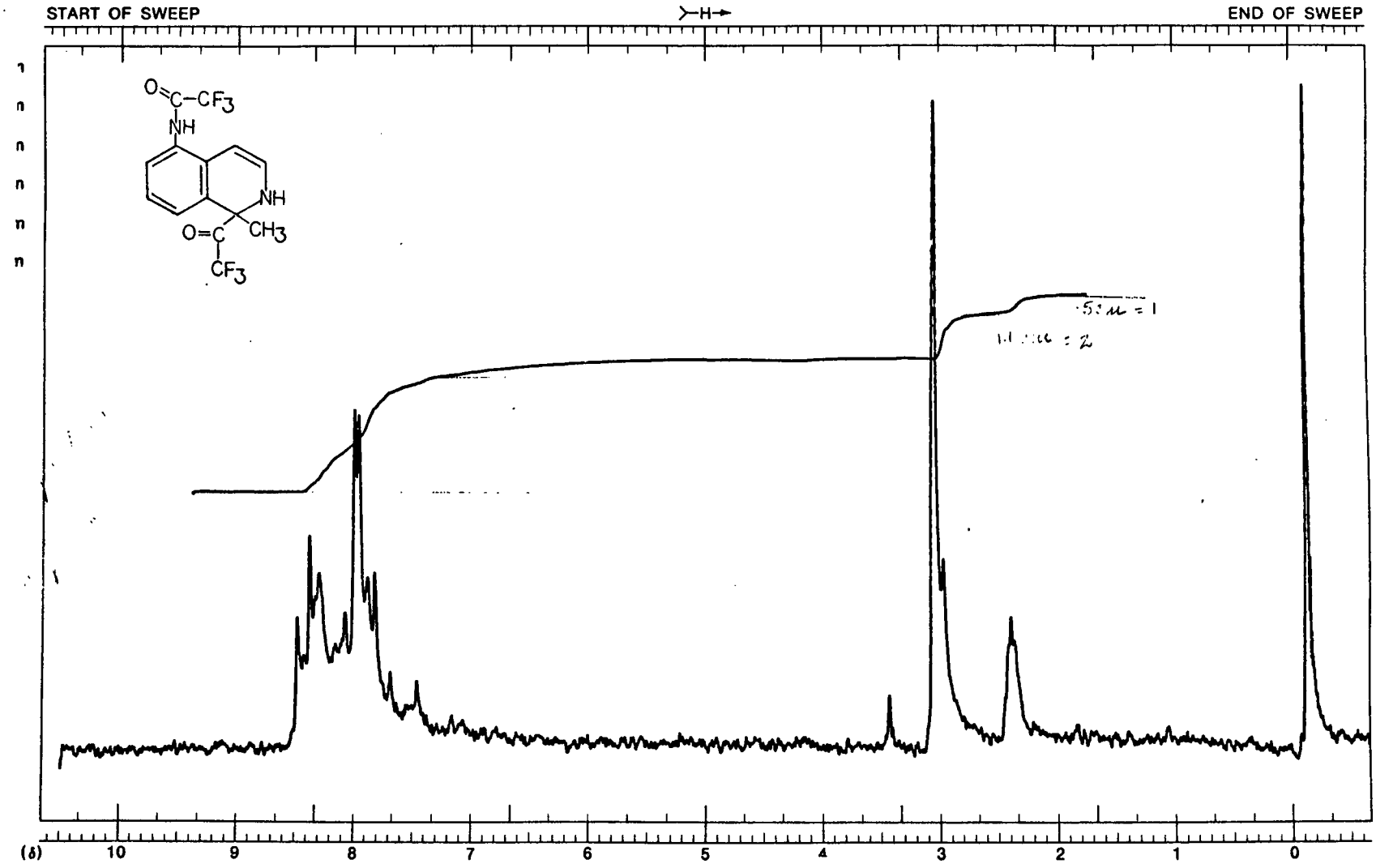


Figure 16: 1-methyl-1,5N-trifluoroacetoisoquinoline (106).

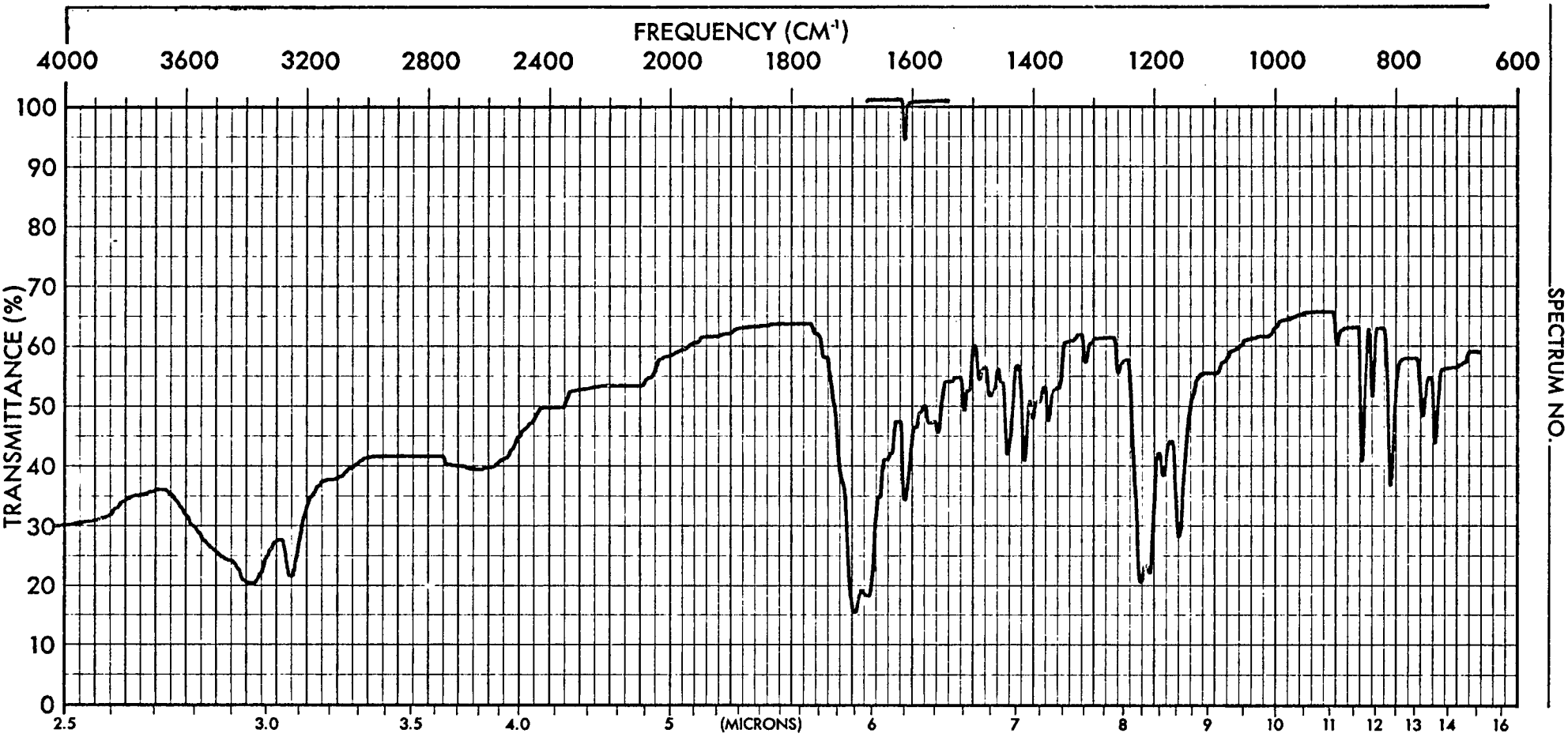
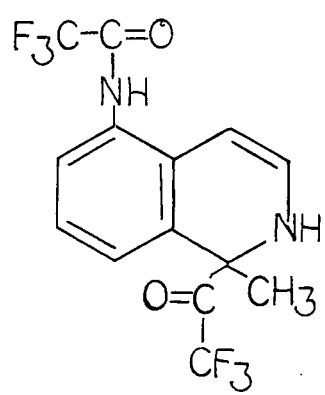


Figure 17: 1-methyl-1,5N -trifluoroacetoisoquinoline (106).



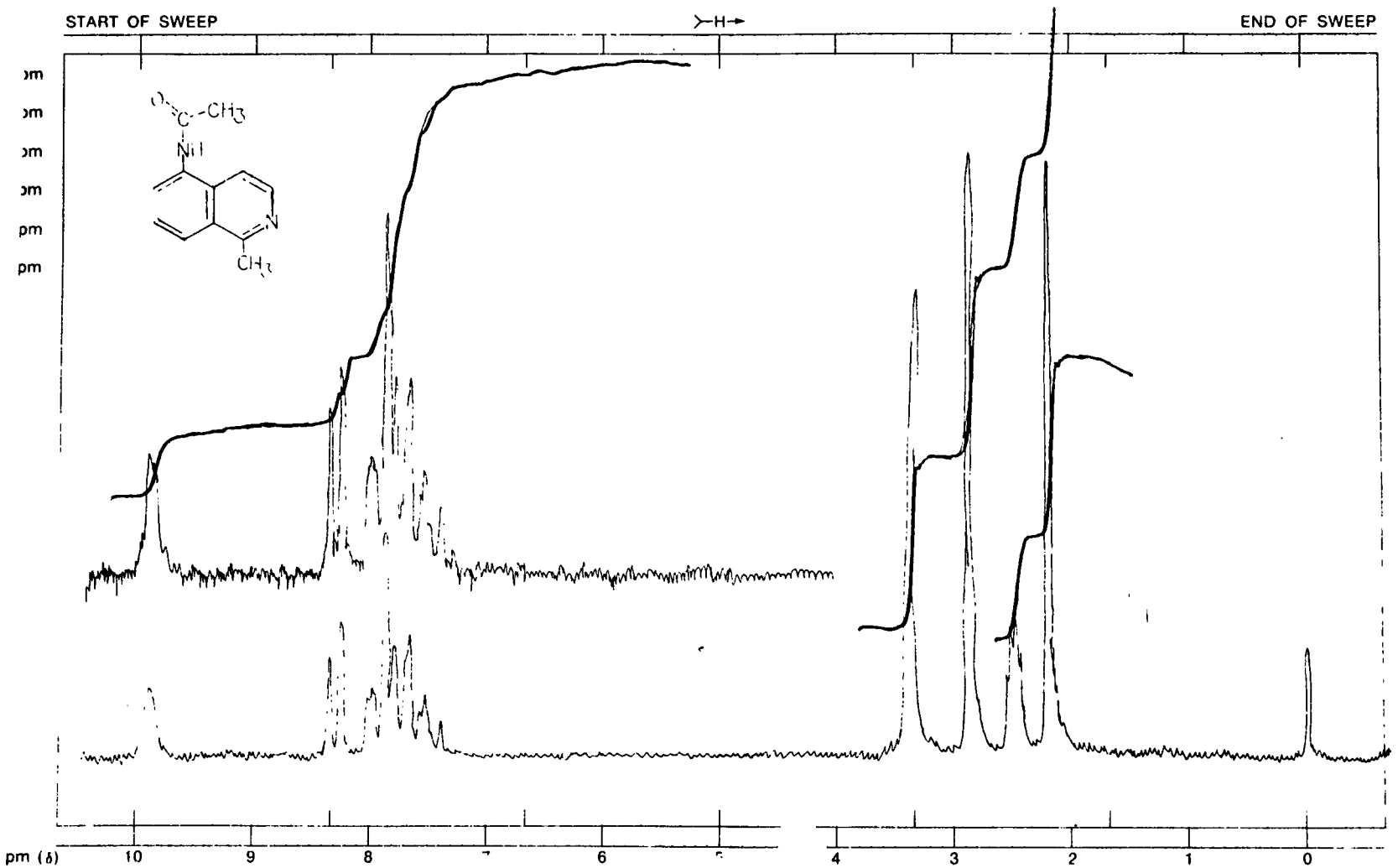
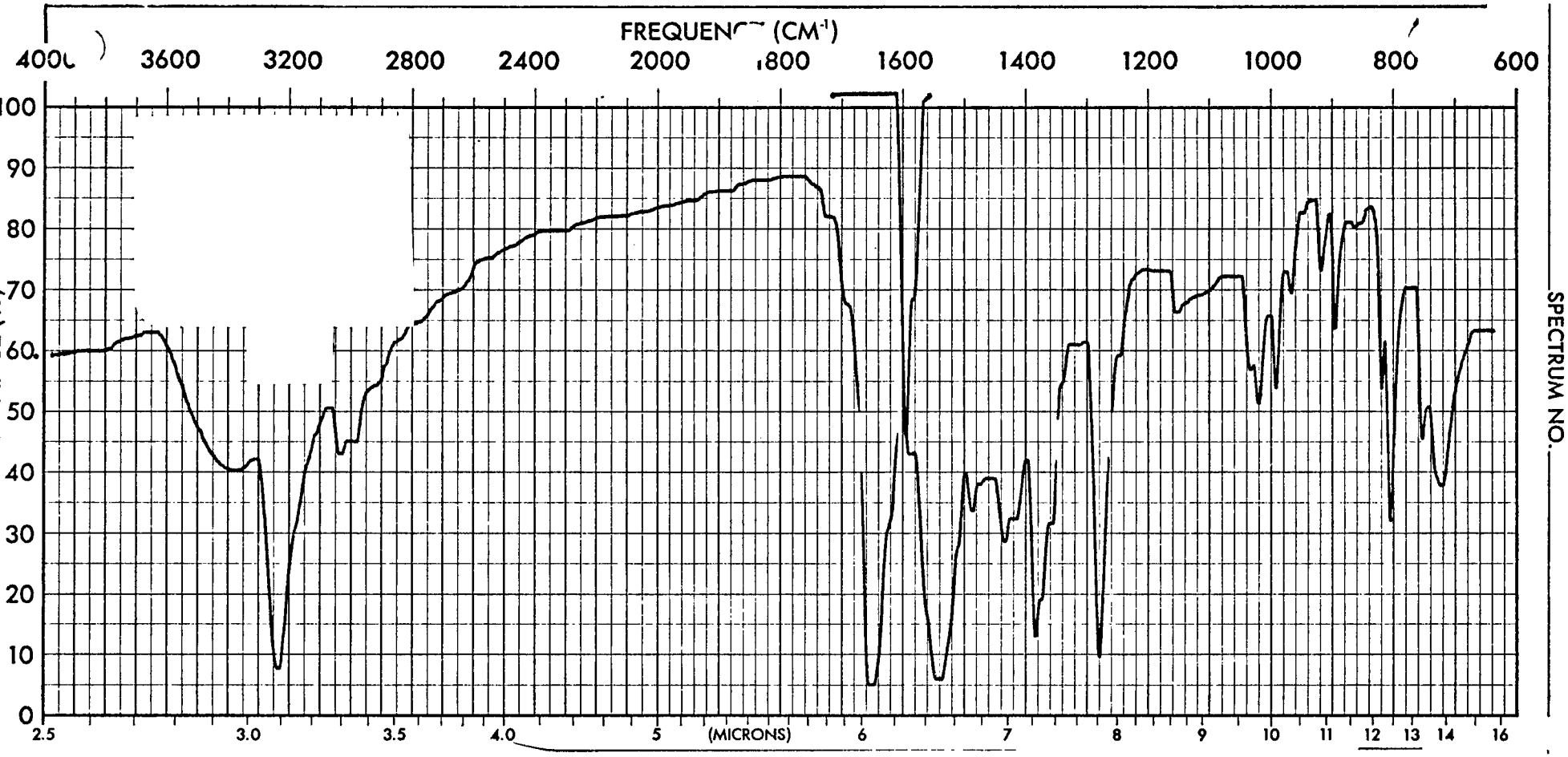


Figure 18: 1-methyl-5-acetoisoquinoline (107).



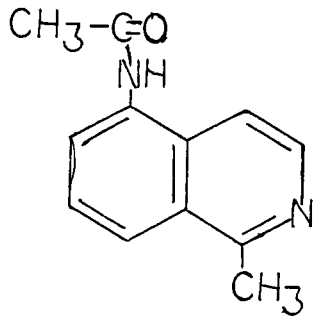


Figure 19: 1-methyl-5N -acetoisoquinoline (107).

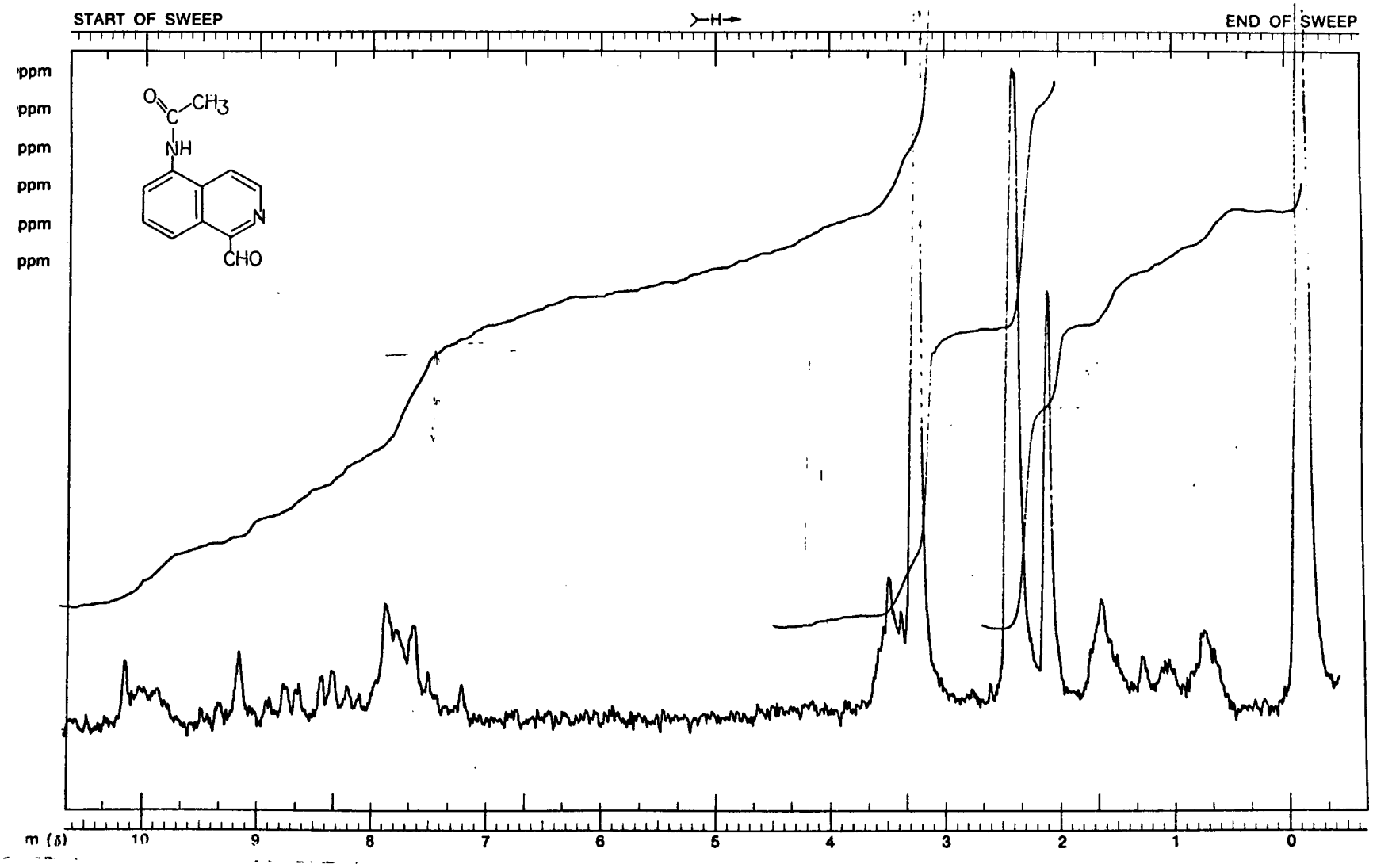
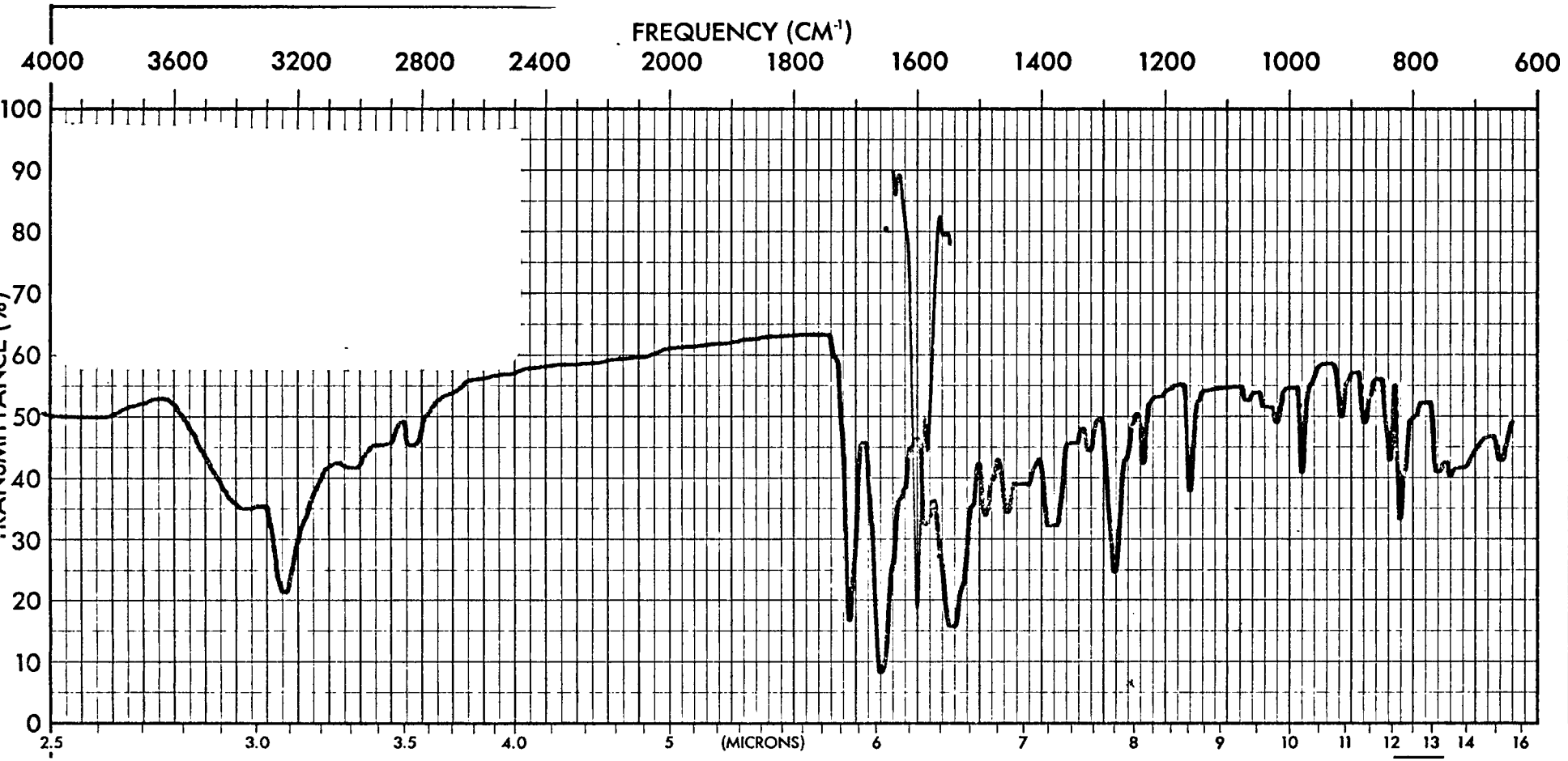


Figure 20: 1-formyl-5N -acetoisoquinoline (108).



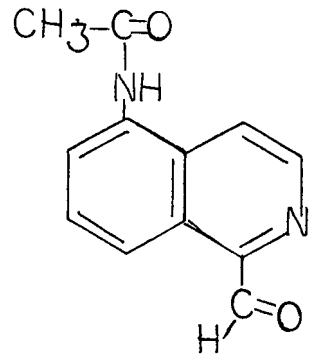


Figure 21: 1-formyl-5N-acetoisoquinoline (108).

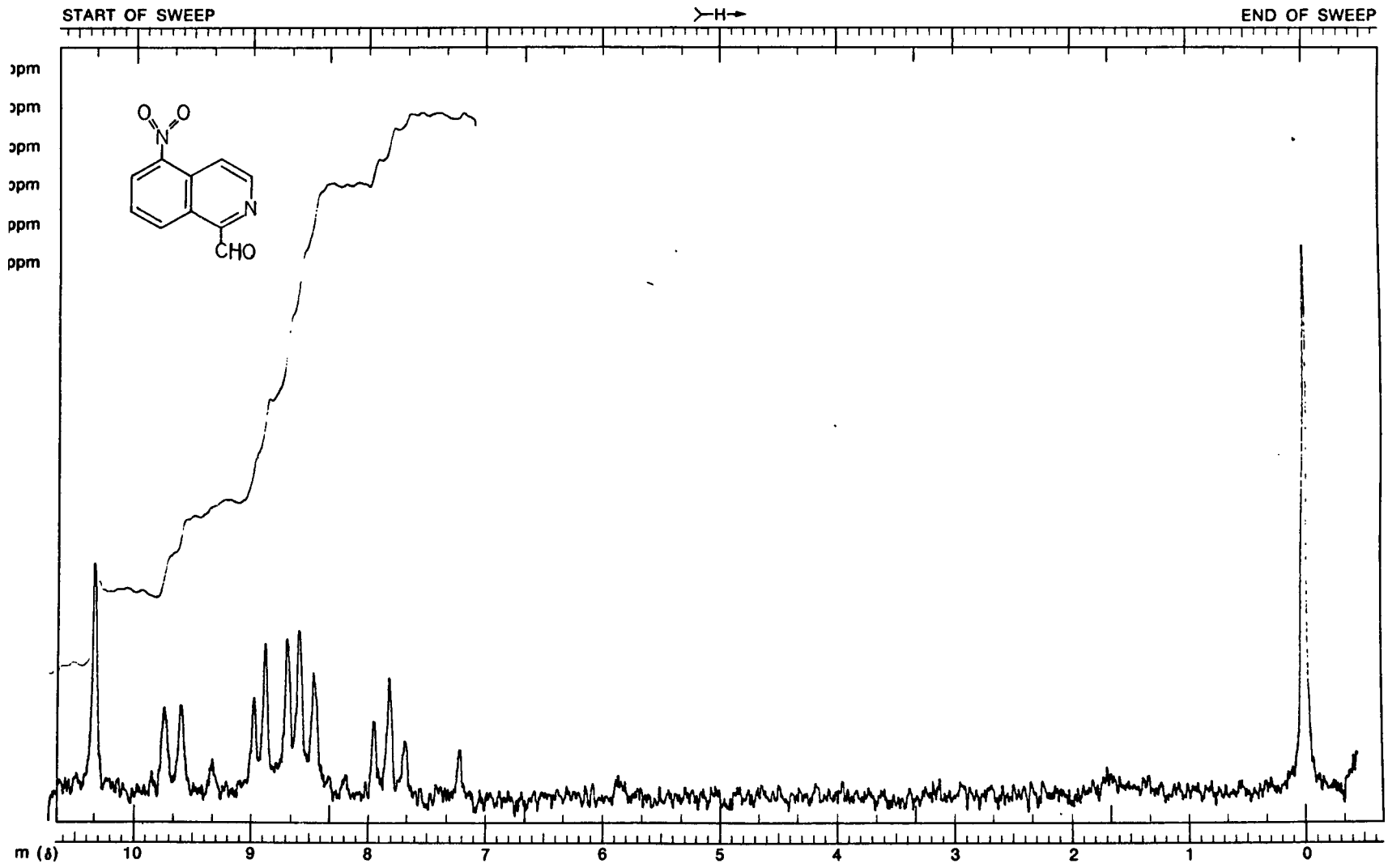


Figure 22: 1-formyl-5-nitroisoquinoline (111).

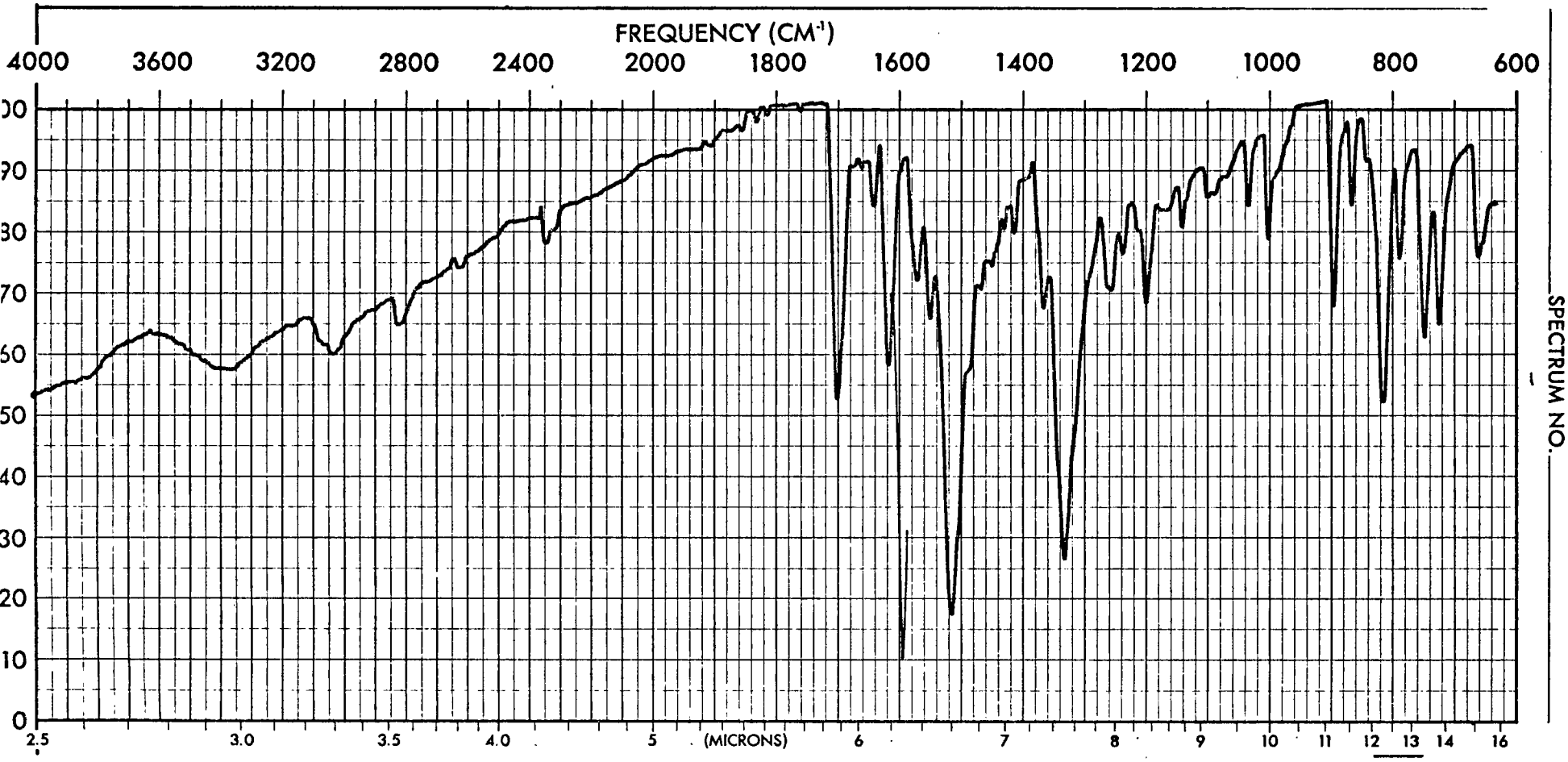
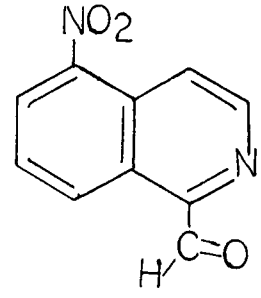


Figure 23: 1-formyl-5-nitroisoquinoline (111).



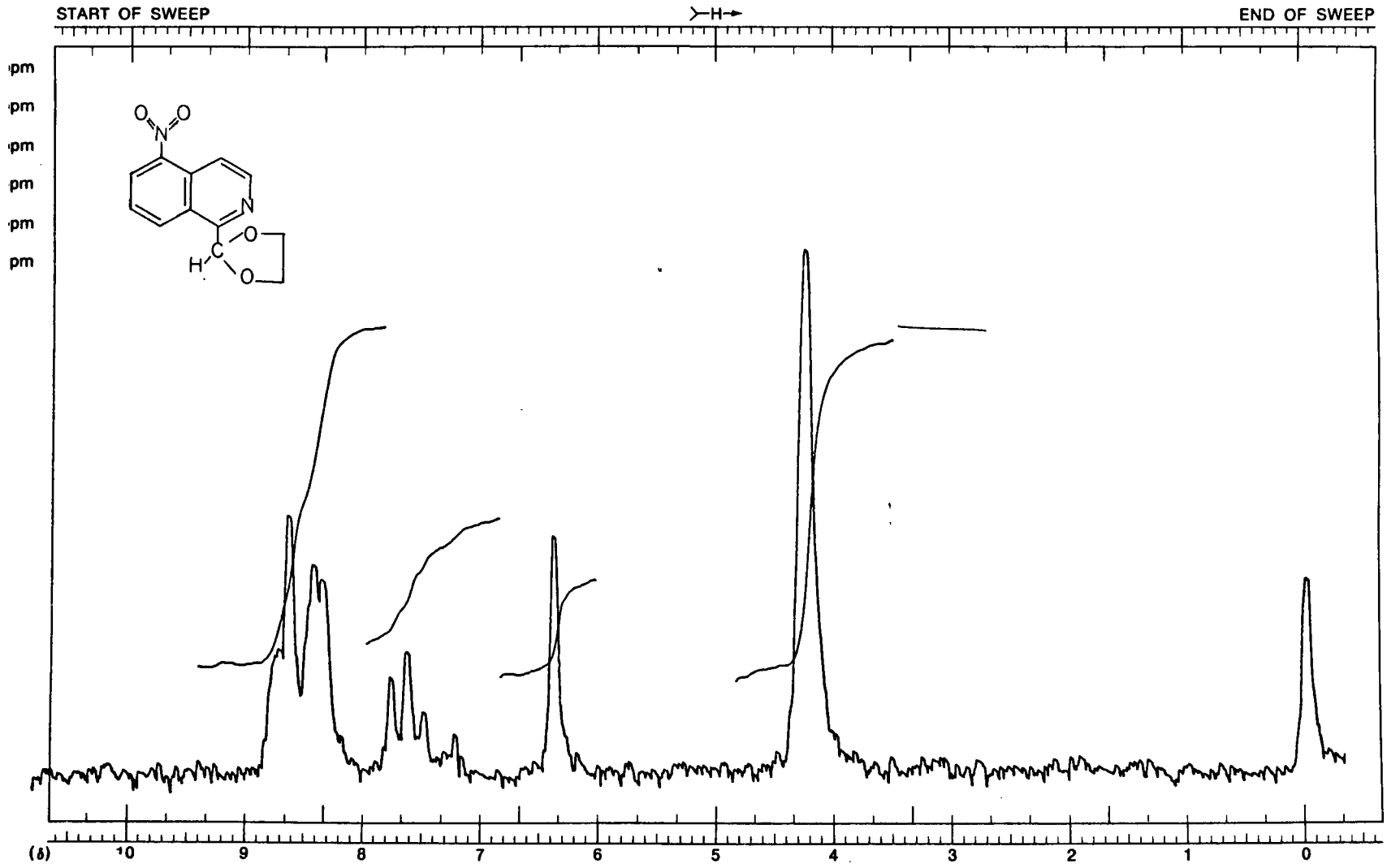


Figure 24: 1-formyl-ethyleneacetal-5-nitroisoquinoline (112).

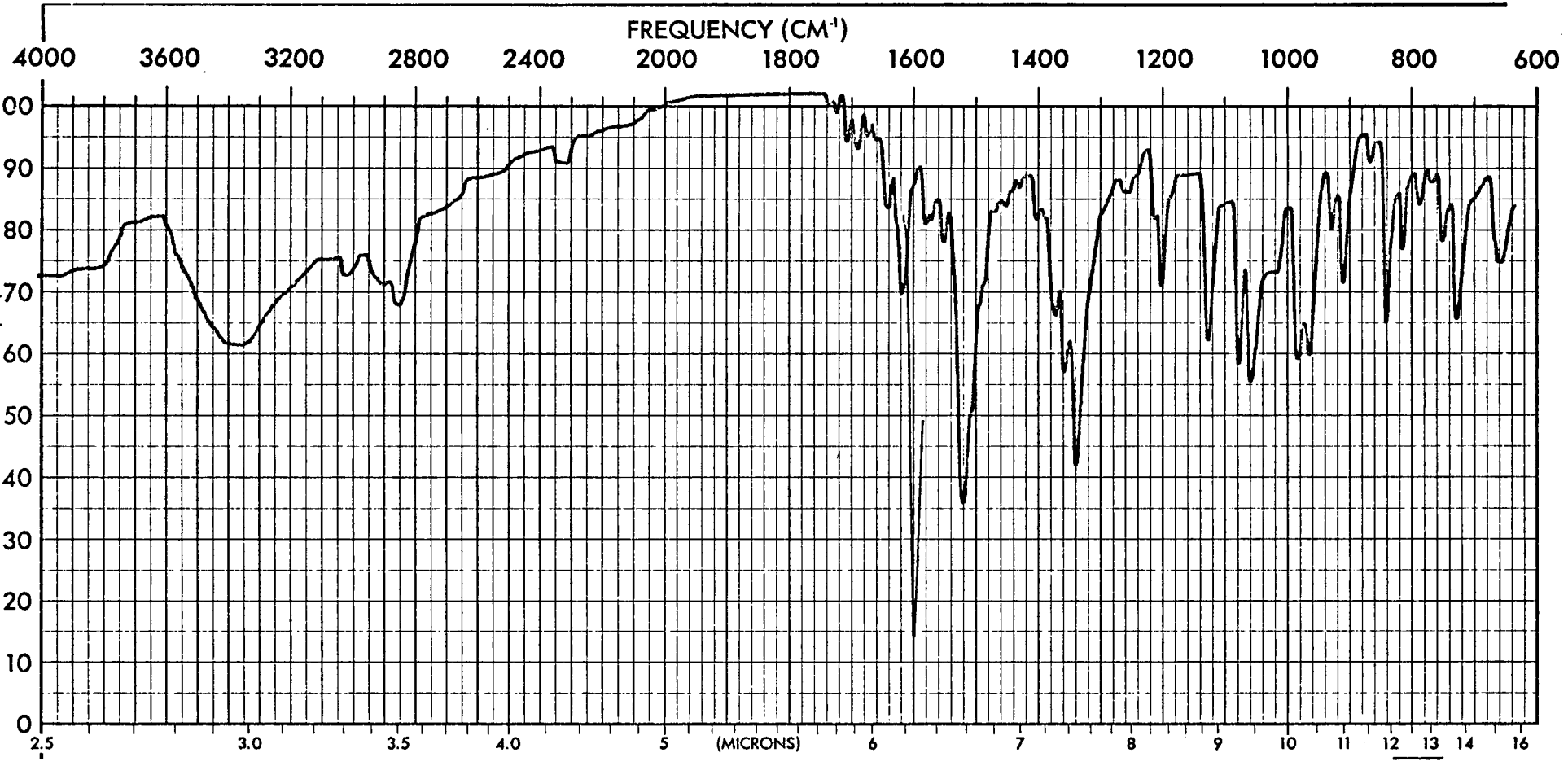
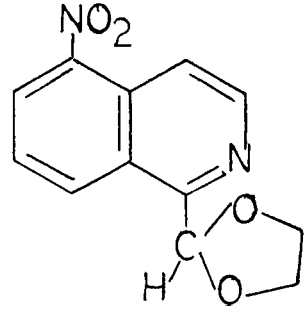


Figure 25: 1-formyl-ethyleneacetal-5-nitroisoquinoline (112).



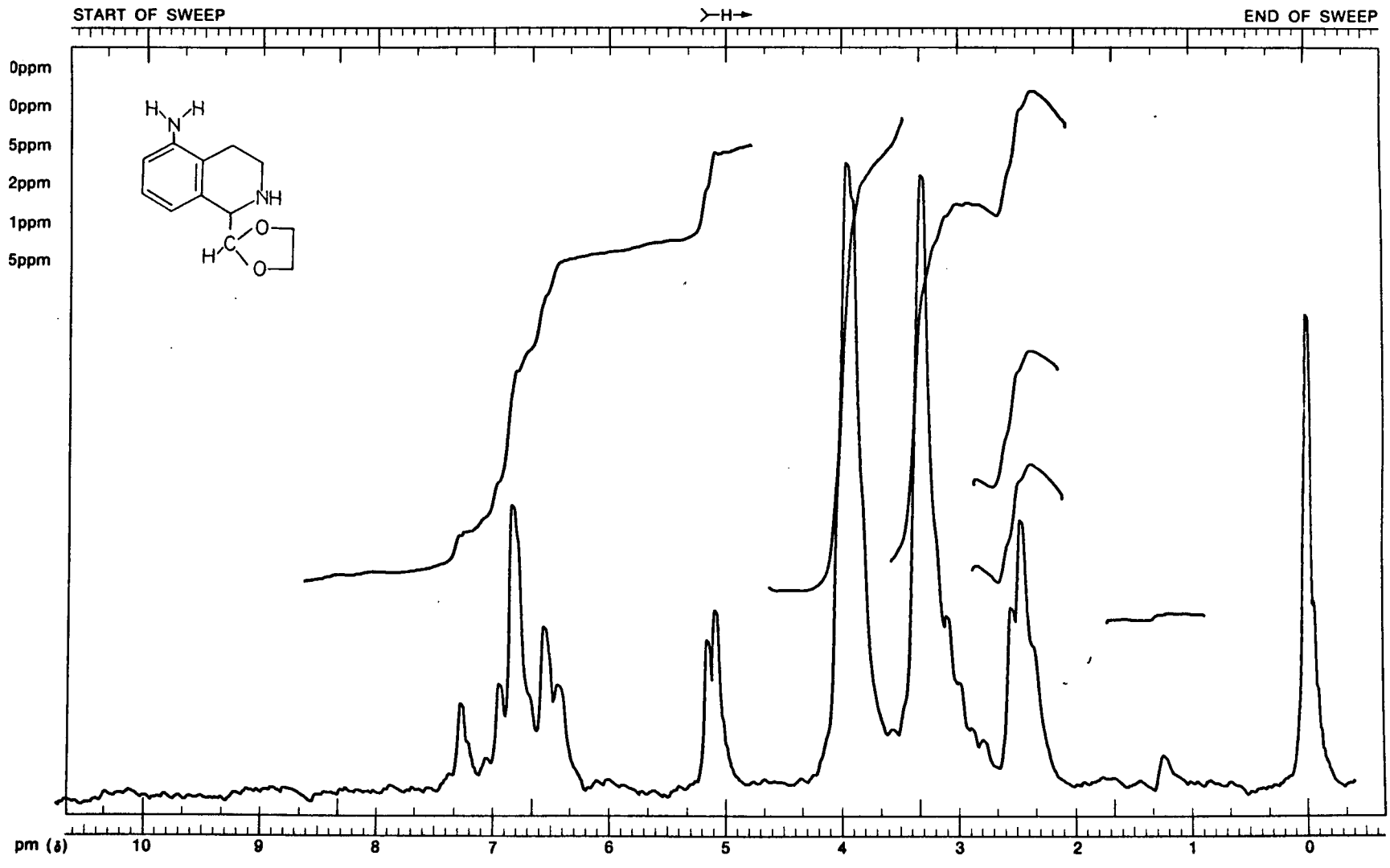


Figure 26: 1-formyl-ethyleneacetal-3,4-dihydro-5-aminoisoquinoline (115).

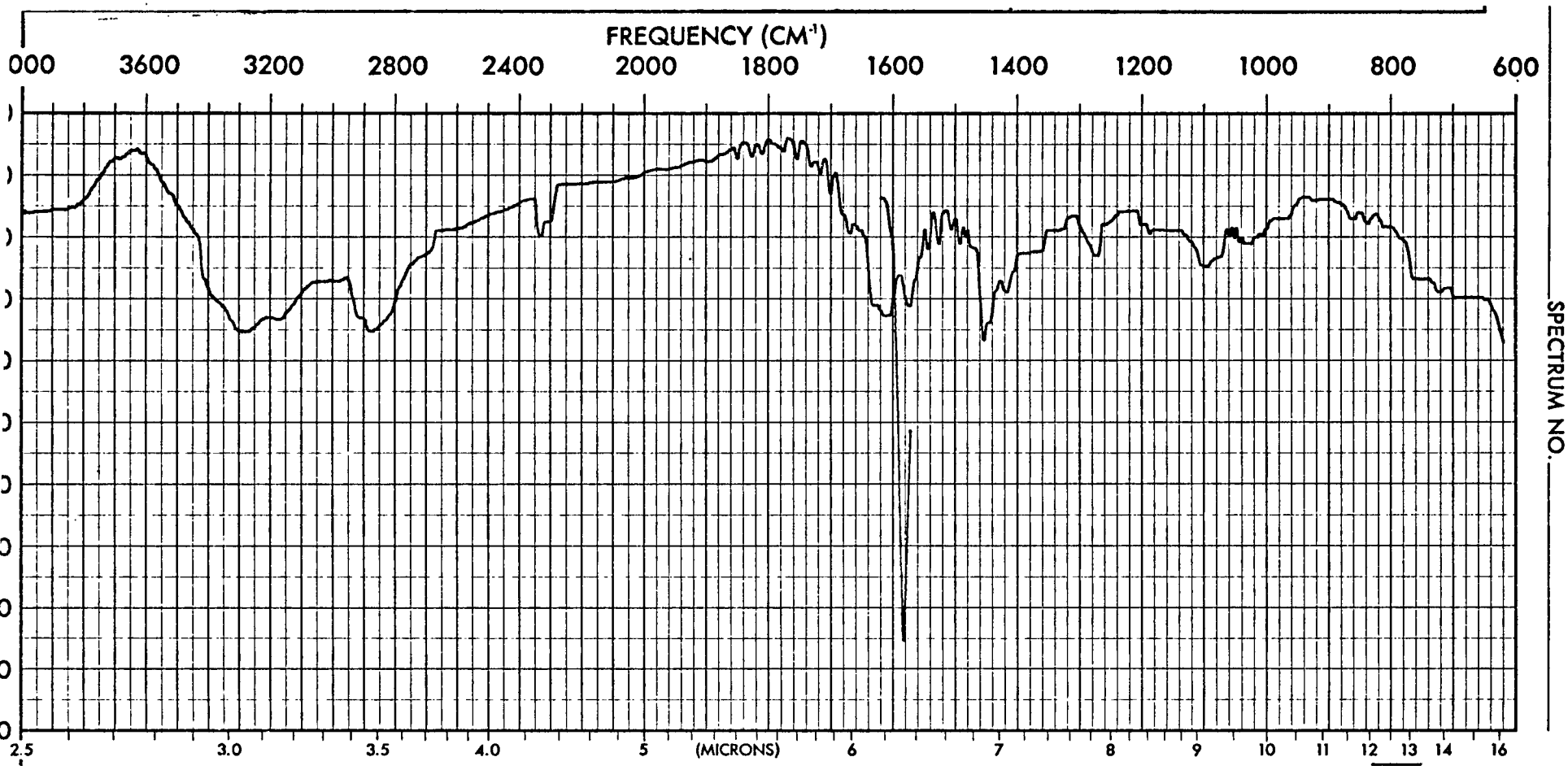


Figure 27: 1-formyl-ethyleneacetal-3,4-dihydro-5-aminoisoquinoline (115).

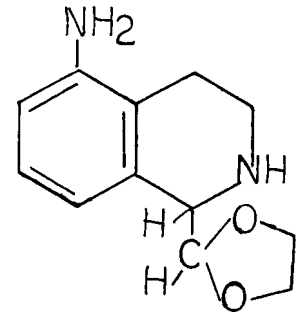
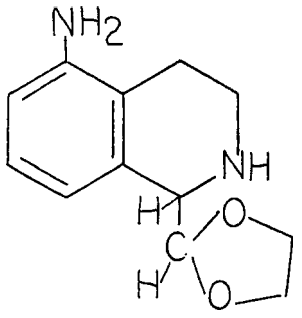
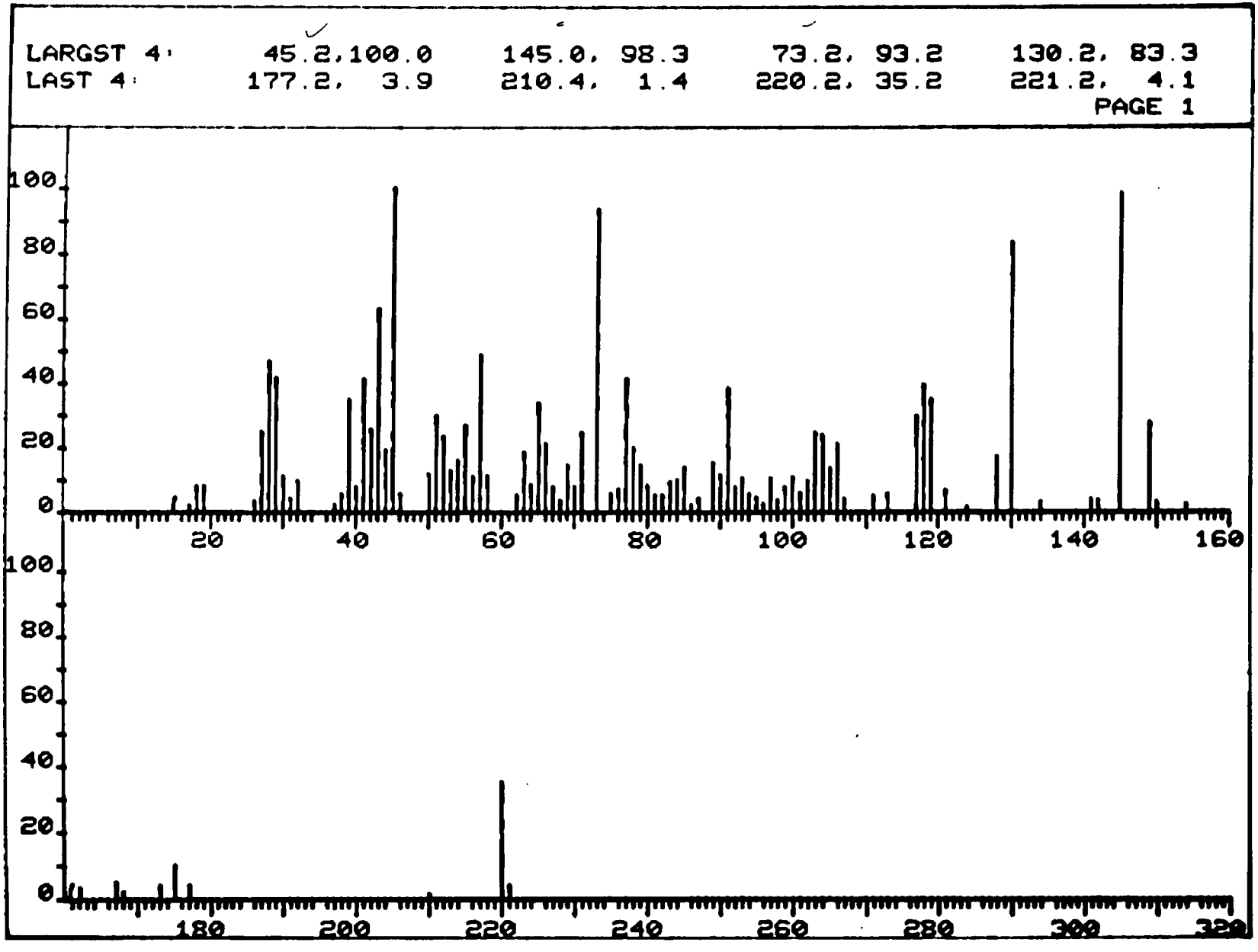


Figure 28: Mass Spectrum of 1-formyl-ethyleneacetal-3,4-dihydro-5-aminoisoquinoline (115).




LARGST 4: 45.2,100.0 145.0, 98.3 73.2, 93.2 130.2, 83.3
LAST 4: 177.2, 3.9 210.4, 1.4 220.2, 35.2 221.2, 4.1
PAGE 1
100
80
60
40
20
0
20
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80
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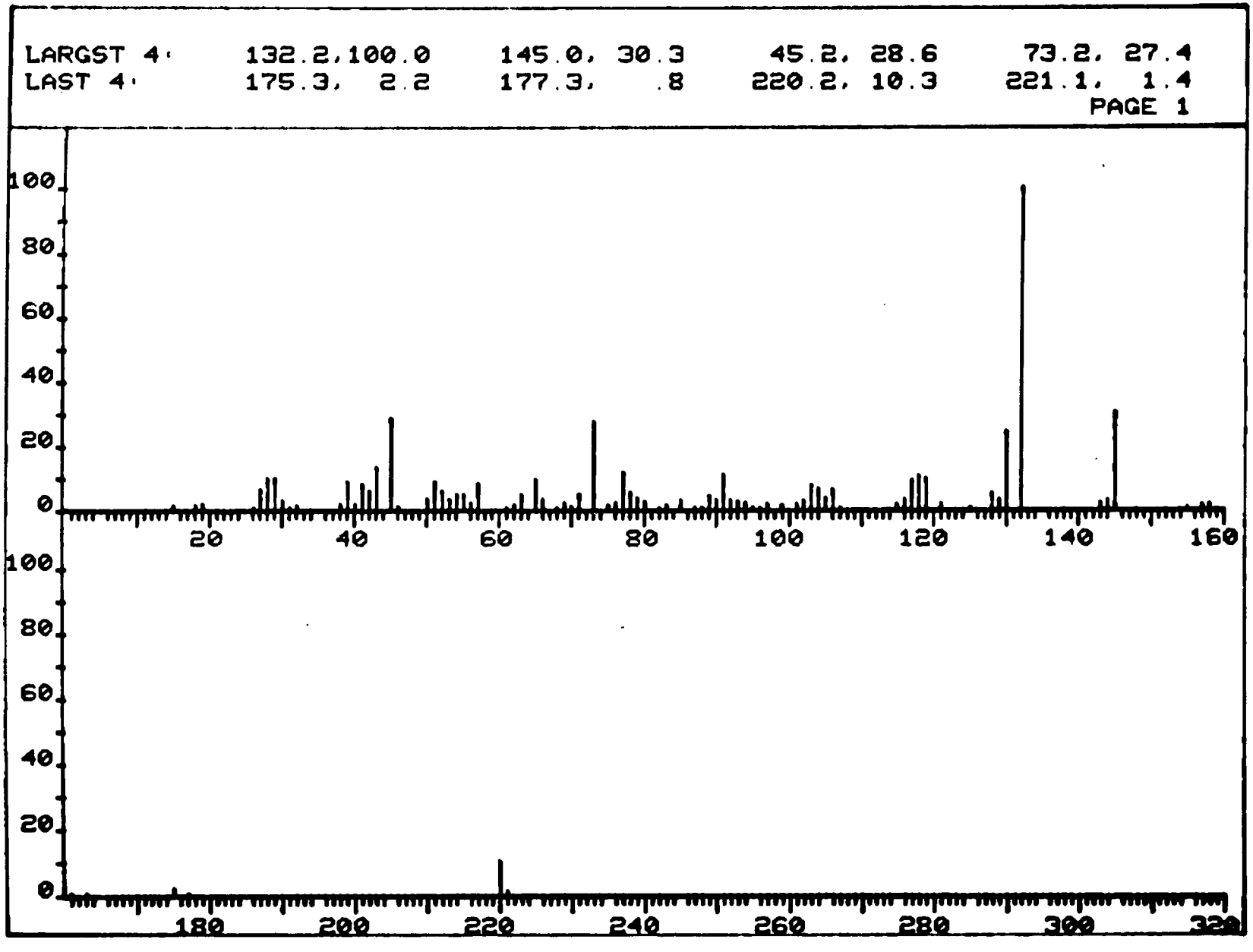
LARGST 4: 132.2,100.0 145.0, 30.3 45.2, 28.6 73.2, 27.4
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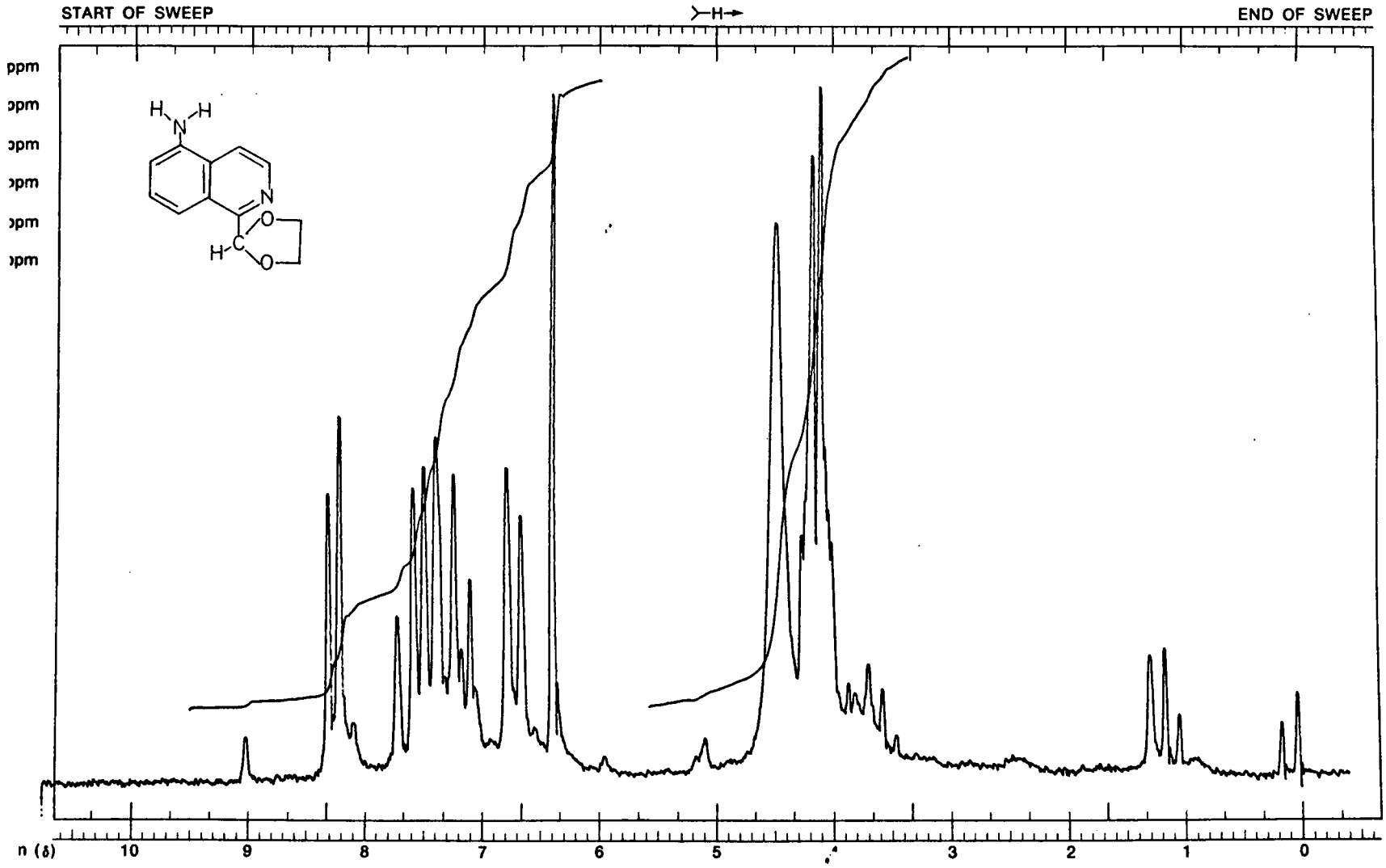


Figure 29: 1-formyl-ethyleneacetal-5-aminoisoquinoline (113).

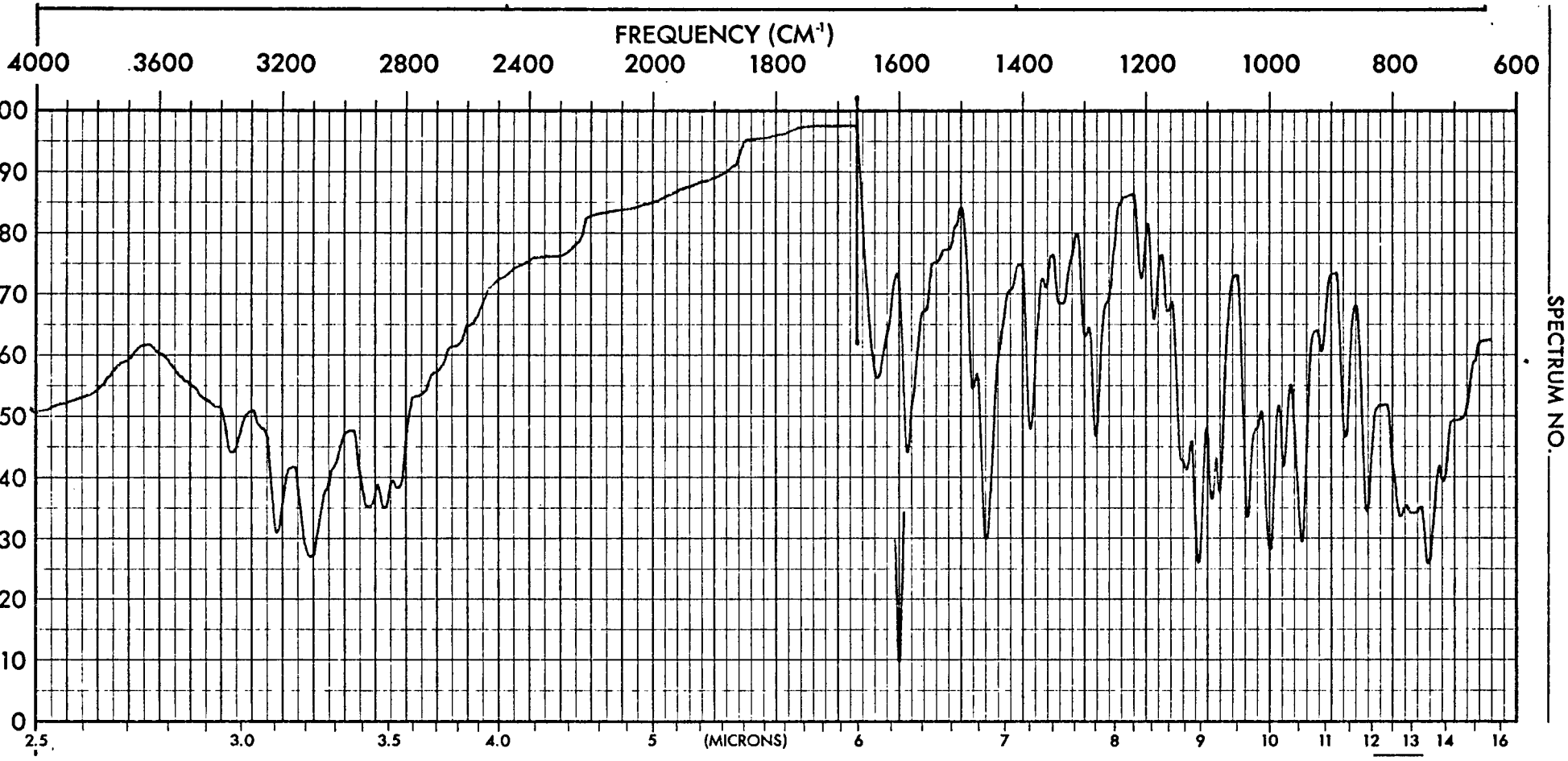


Figure 30: 1-formyl-ethyleneacetal-5-aminoisoquinoline (113).

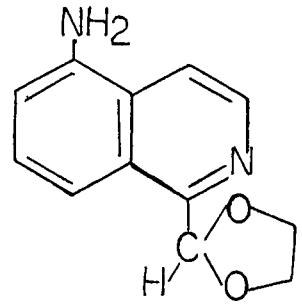
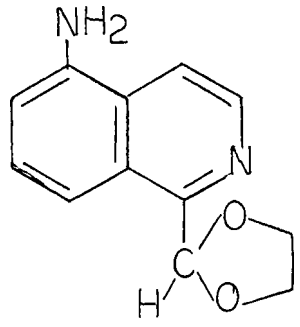
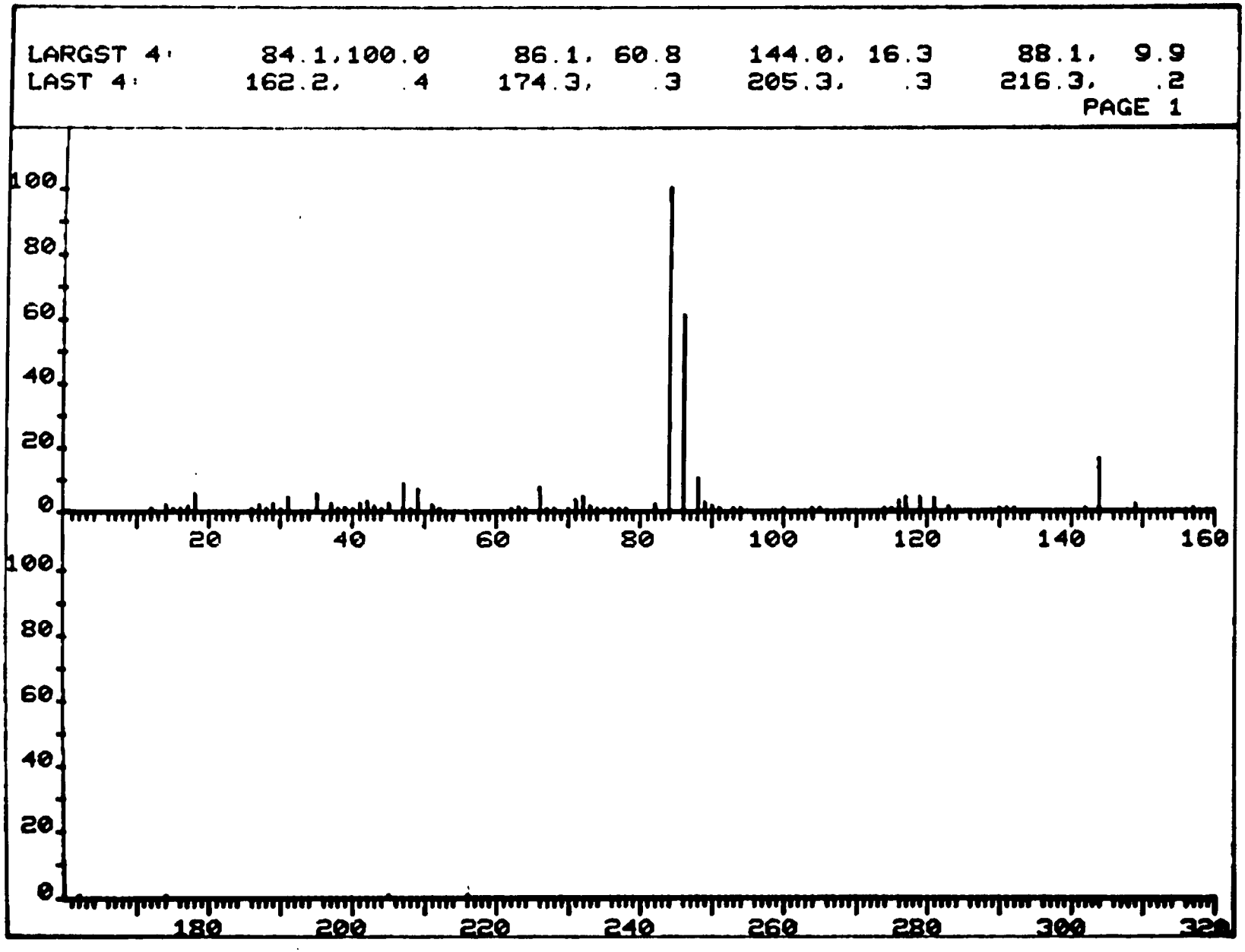


Figure 31: Mass Spectrum of 1-formyl-ethyleneacetal-5-aminoisoquinoline (113).




LARGST 4: 84.1,100.0 86.1, 60.8 144.0, 16.3 88.1, 9.9
LAST 4: 162.2, .4 174.3, .3 205.3, .3 216.3, .2
PAGE 1
100
80
60
40
20
0
20 40 60 80 100 120 140 160
100
80
60
40
20
0
180 200 220 240 260 280 300 320

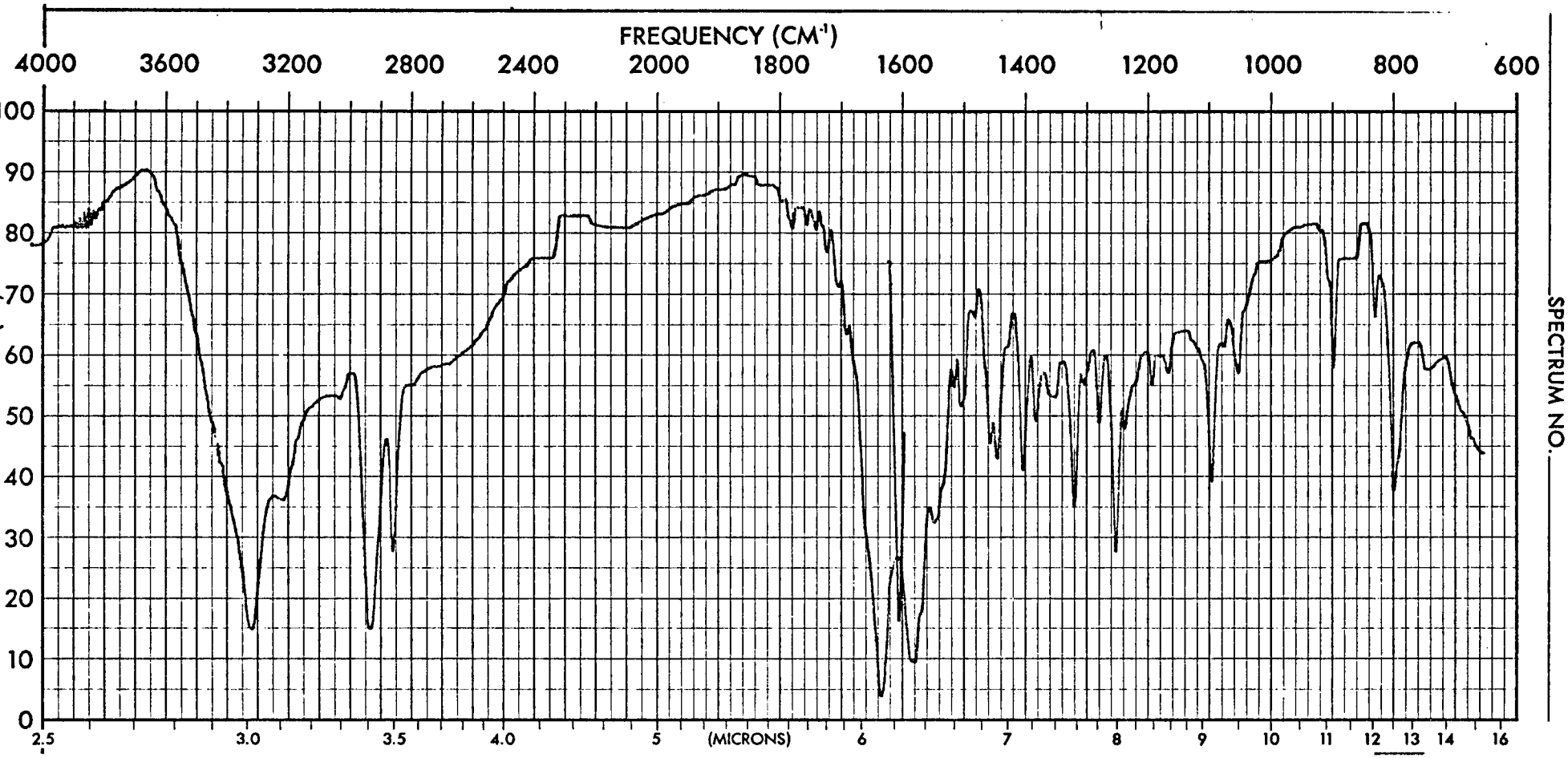
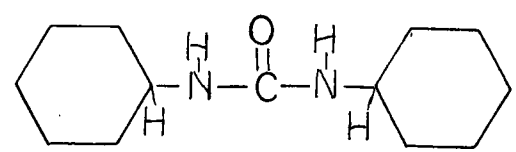


Figure 32: Diclclohexyl Urea



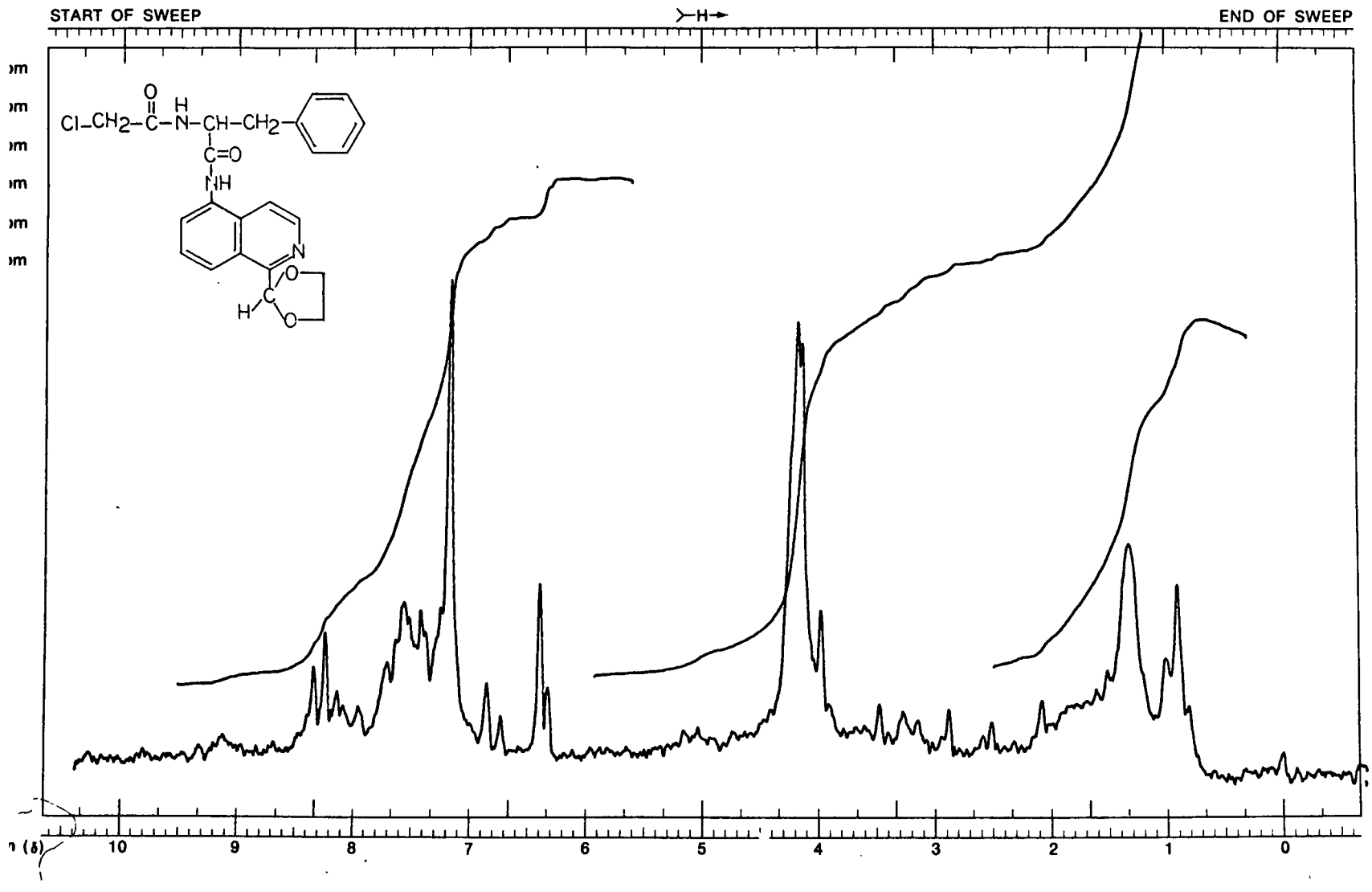
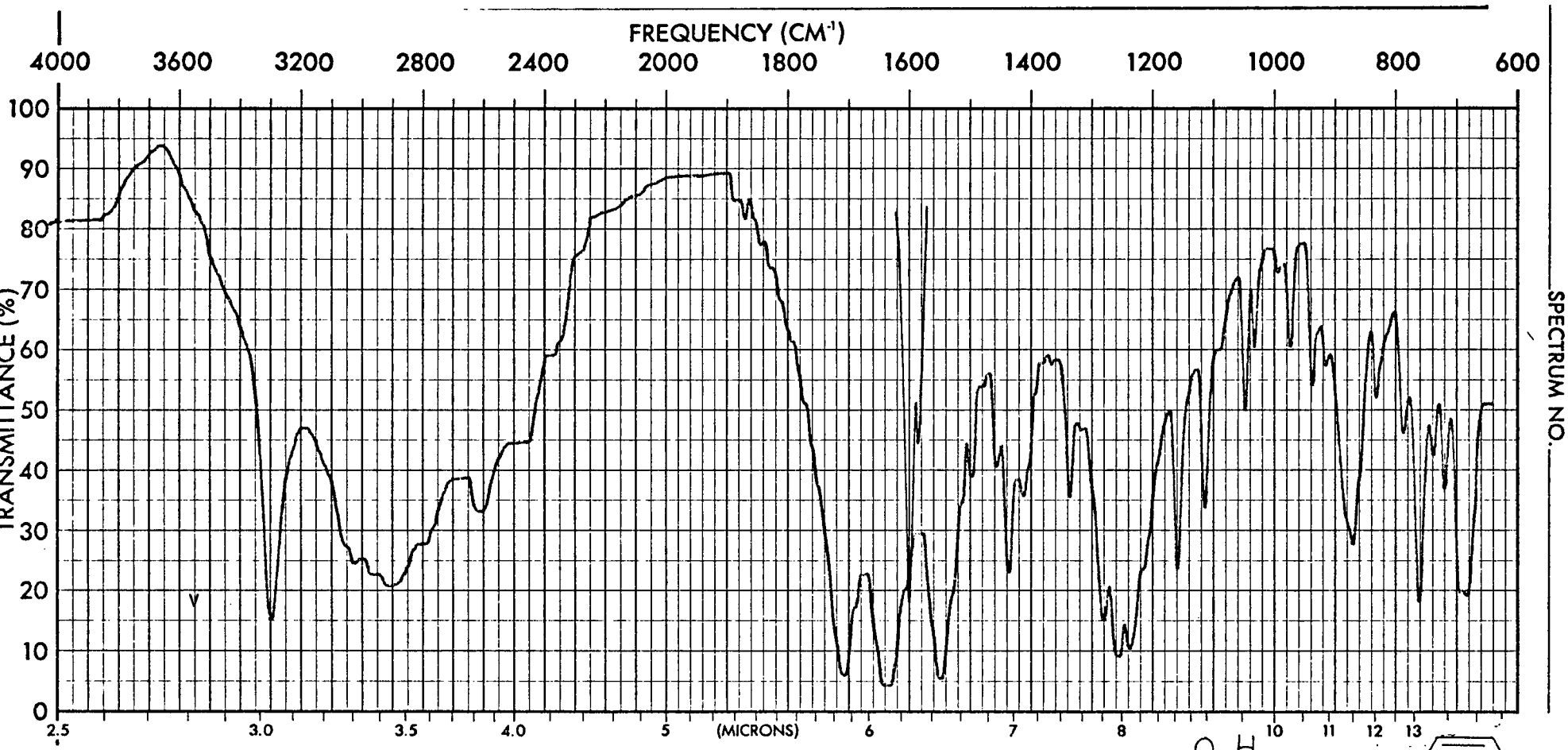
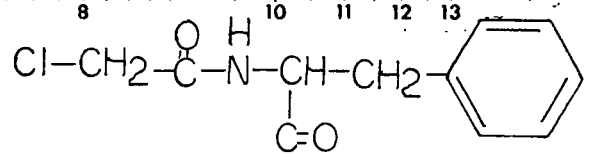


Figure 33: 1-formyl-ethyleneacetal-5N-(N-chloro-acetyl-L-phenylalanine) isoquinoline (114).





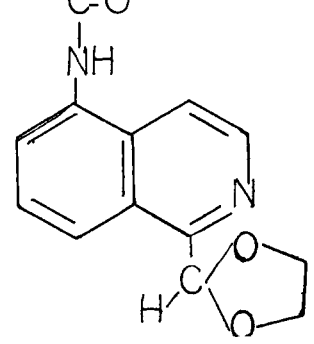
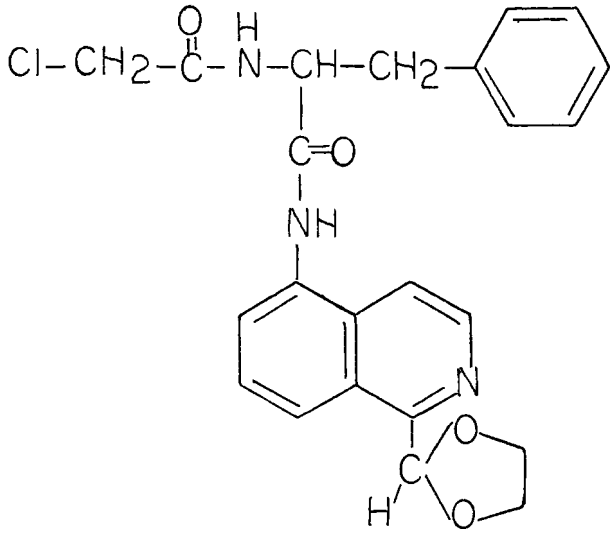


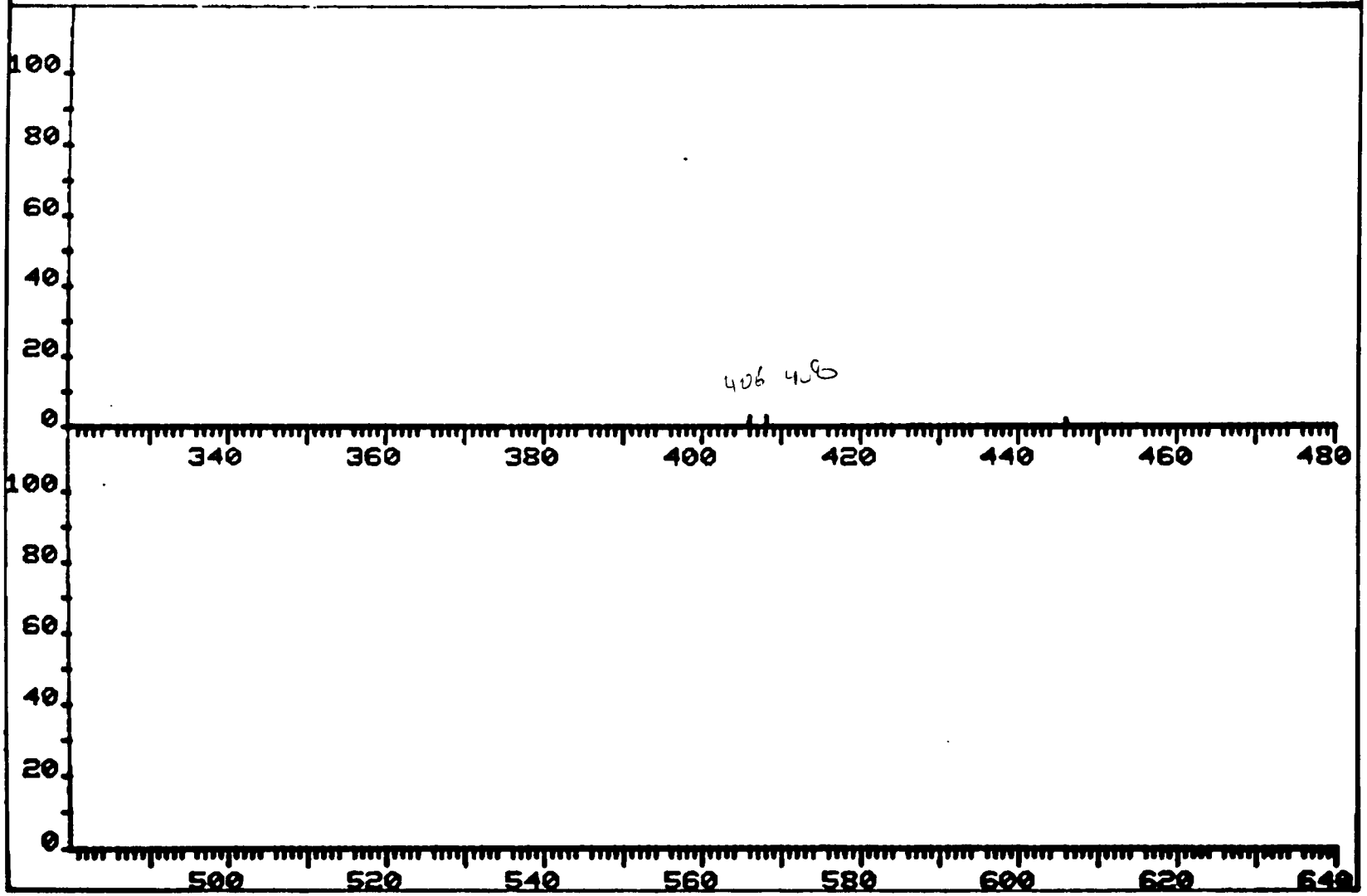
Figure 34: l-formyl-ethyleneacetal-5N-chloro-acetyl-L-phenylalanine) isoquinoline (114)

Figure 35: Mass Spectrum of 1-formyl-ethyleneacetal-5N-(N-chloro-acetyl-L-phenylalanine) isoquinoline (114).



SAMPLE 6011 SPECTRUM 54 RETENTION TIME 6.5

LARGST 4:	57.3,100.0	71.3, 87.1	85.2, 71.3	55.3, 44.4
LAST 4:	225.4, 1.3	406.0, 2.5	407.9, 2.4	446.2, 1.9



SAMPLE 6011 SPECT 54

MASS	ABUND									
446	1.9									
408	2.4	38								
406	2.5	40	2							
225	1.3	221	182	180						
212	2.1	234	196	194	13					
211	7.1	235	196	195	14	1				
210	5.1	236	198	196	15	2	1			
209	6.8	237	199	197	16	3	2	1		
208	4 7	238	200	198	17	4	3	2	1	
200	4.2	246	208	206	25	12	11	10	9	8

>PAUSE

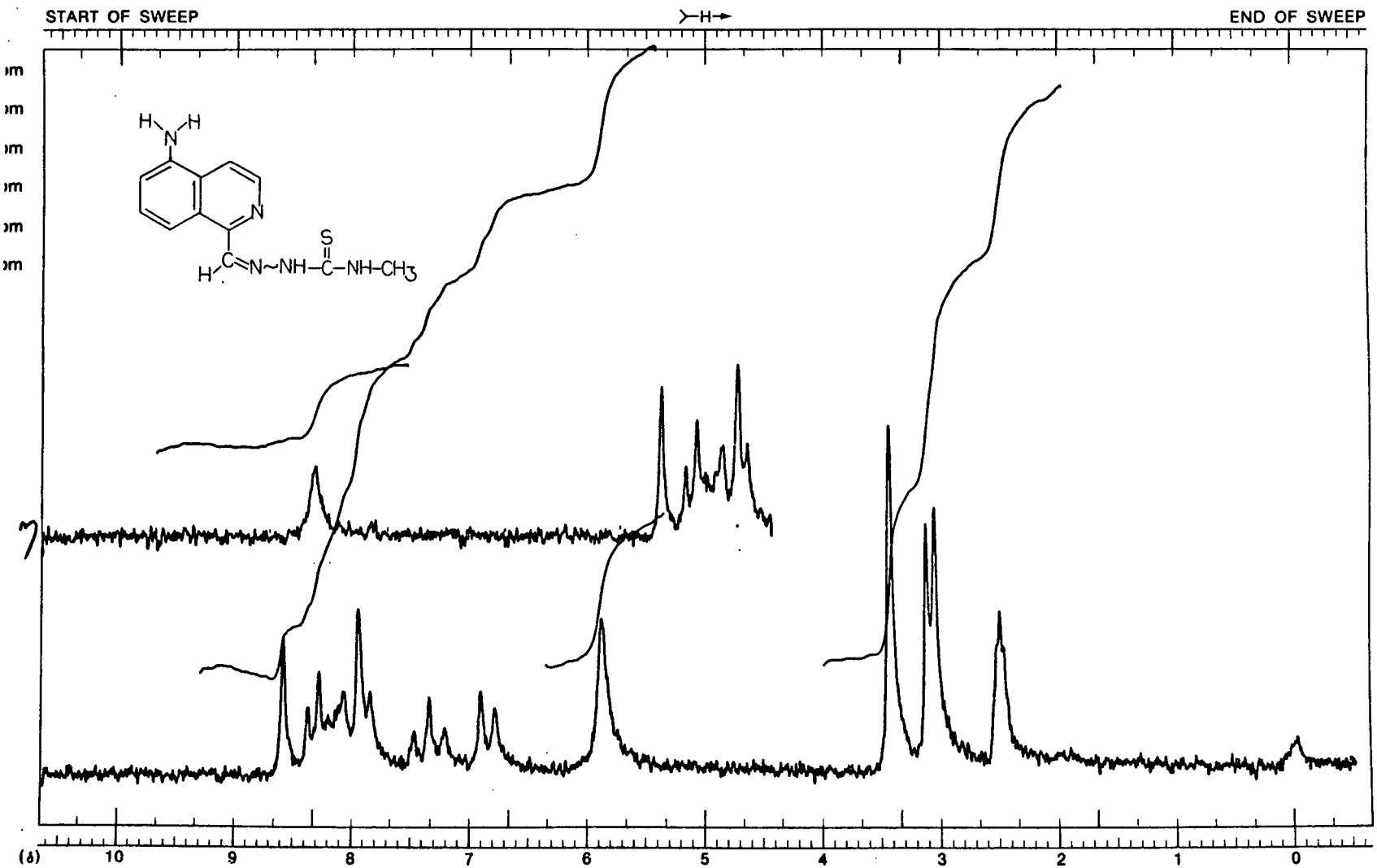


Figure 36: 1-formyl-thiosemicarbazone-5-aminoisoquinoline (117).

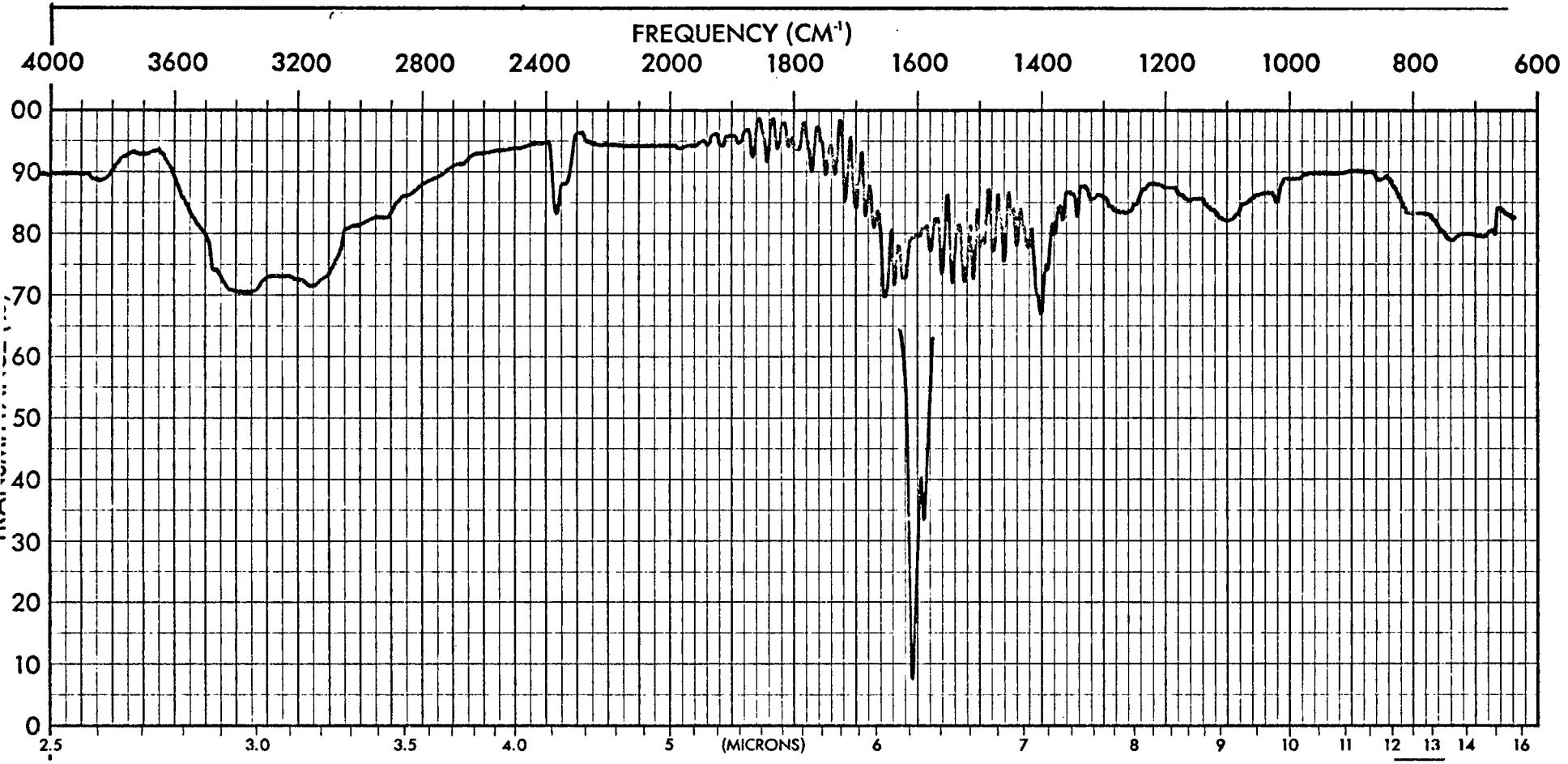


Figure 37: 1-formyl-thiosemicarbazone-5-aminoisoquinoline (117).

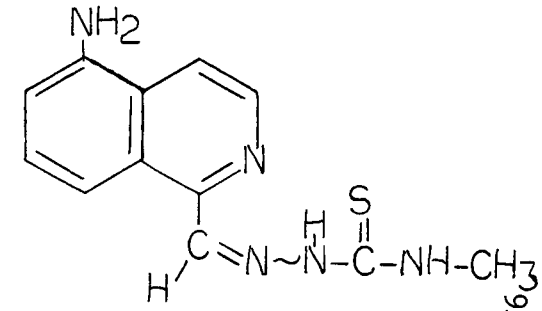
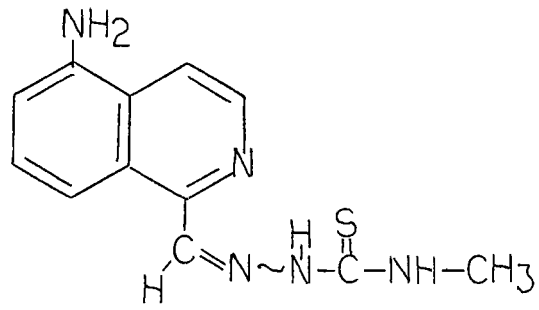
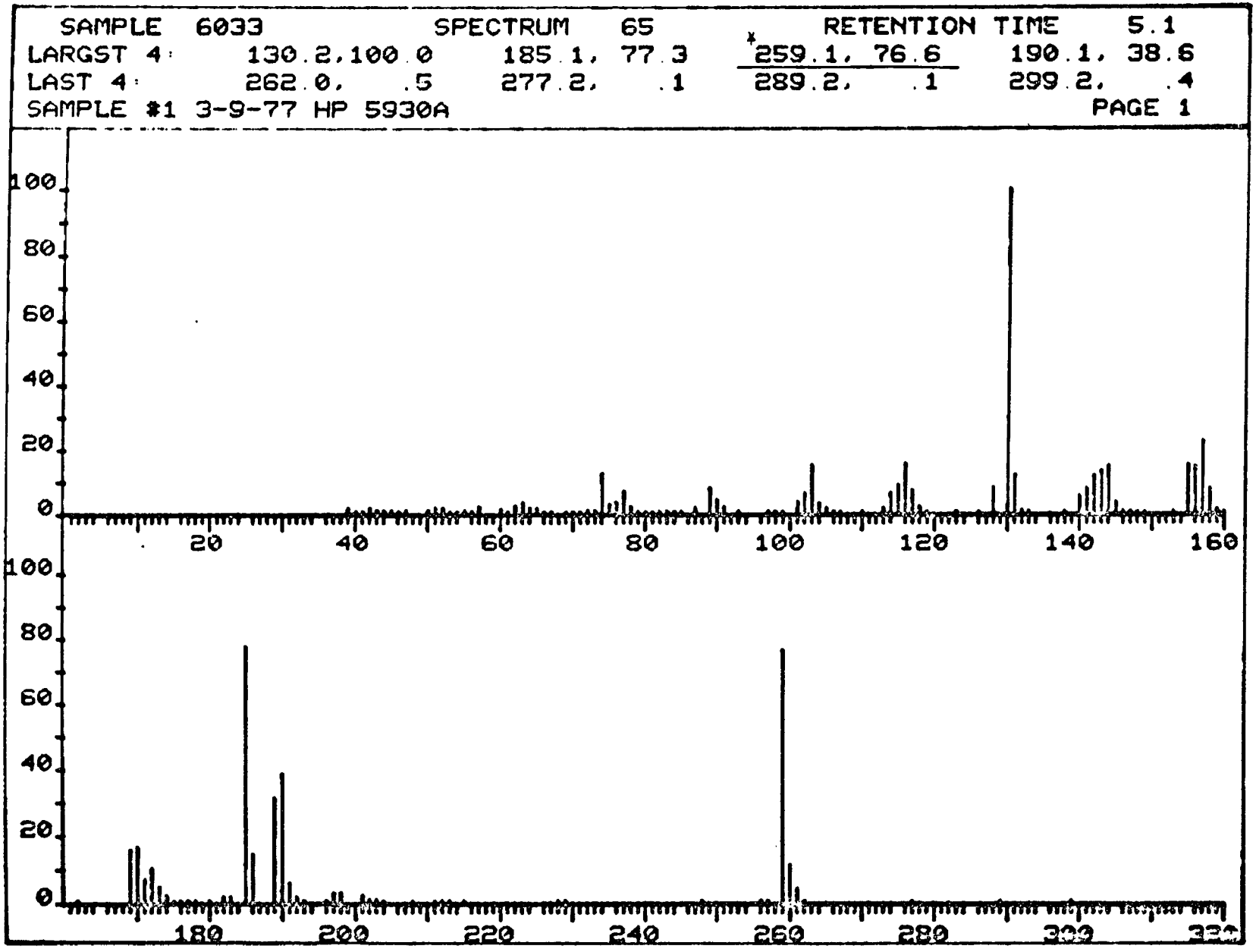


Figure 38: Mass Spectrum of 1-formyl-thiosemicarbazone-5-aminoisoquinoline (117).




SAMPLE 6033 SPECTRUM 65 RETENTION TIME 5.1
LARGST 4: 130.2,100.0 185.1, 77.3 259.1, 76.6 190.1, 38.6
LAST 4: 262.0, .5 277.2, .1 289.2, .1 299.2, .4
SAMPLE #1 3-9-77 HP 5930A PAGE 1
100
80
60
40
20
0
20 40 60 80 100 120 140 160
100
80
60
40
20
0
180 200 220 240 260 280 300 320

34	2.7	TI=	2590.	BP=	43	LV=	17	259=	0	258=	0
35	2.7	TI=	2572.	BP=	43	LV=	.21	259=	0	258=	0
36	2.8	TI=	2656.	BP=	43	LV=	.18	259=	0	258=	0
37	2.9	TI=	2494.	BP=	43	LV=	.18	259=	0	258=	0
38	3.0	TI=	2769.	BP=	43	LV=	.19	259=	0	258=	0
39	3.1	TI=	2791.	BP=	43	LV=	.21	259=	0	258=	0
40	3.1	TI=	2769.	BP=	43	LV=	.22	259=	0	258=	0
41	3.2	TI=	3171.	BP=	43	LV=	.26	259=	0	258=	0
42	3.3	TI=	3937	BP=	43	LV=	.31	259=	0	258=	0
43	3.3	TI=	5823.	BP=	43	LV=	.39	259=	0	258=	0
44	3.4	TI=	8561.	BP=	43	LV=	.54	259=	0	258=	0
45	3.5	TI=	7156.	BP=	43	LV=	.42	259=	0	258=	0
46	3.6	TI=	4318.	BP=	43	LV=	.19	259=	0	258=	0
47	3.7	TI=	3802.	BP=	105	LV=	.16	259=	0	258=	0
48	3.7	TI=	3163.	BP=	105	LV=	.16	259=	0	258=	0
49	3.8	TI=	3094.	BP=	44	LV=	.19	259=	0	258=	0
50	3.9	TI=	2955.	BP=	44	LV=	.17	259=	0	258=	0
51	4.0	TI=	3077.	BP=	44	LV=	.18	259=	0	258=	0
52	4.1	TI=	3295.	BP=	44	LV=	.18	259=	0	258=	0
53	4.1	TI=	3236	BP=	44	LV=	.17	259=	19	258=	0
54	4.2	TI=	3756.	BP=	44	LV=	.23	259=	37	258=	0
55	4.3	TI=	4536.	BP=	44	LV=	.21	259=	99	258=	0
56	4.4	TI=	5834.	BP=	44	LV=	.26	259=	172	258=	0
57	4.4	TI=	7275.	BP=	130	LV=	.32	259=	362	258=	0
58	4.5	TI=	11394.	BP=	130	LV=	.58	259=	721	260=	114
59	4.6	TI=	15974.	BP=	130	LV=	1.04	259=	1101	260=	151
60	4.7	TI=	21701.	BP=	130	LV=	1.44	259=	1687	260=	258
61	4.7	TI=	28961.	BP=	130	LV=	2.17	259=	2427	260=	428
62	4.8	TI=	41772.	BP=	130	LV=	3.00	259=	3497	260=	593
63	4.9	TI=	58679.	BP=	130	LV=	4.17	259=	5267	260=	881
64	5.0	TI=	78993.	BP=	130	LV=	5.53	259=	7962	260=	1269
* 65	5.1	TI=	119496.	BP=	130	LV=	7.88	*259=	12309	260=	1863

/10

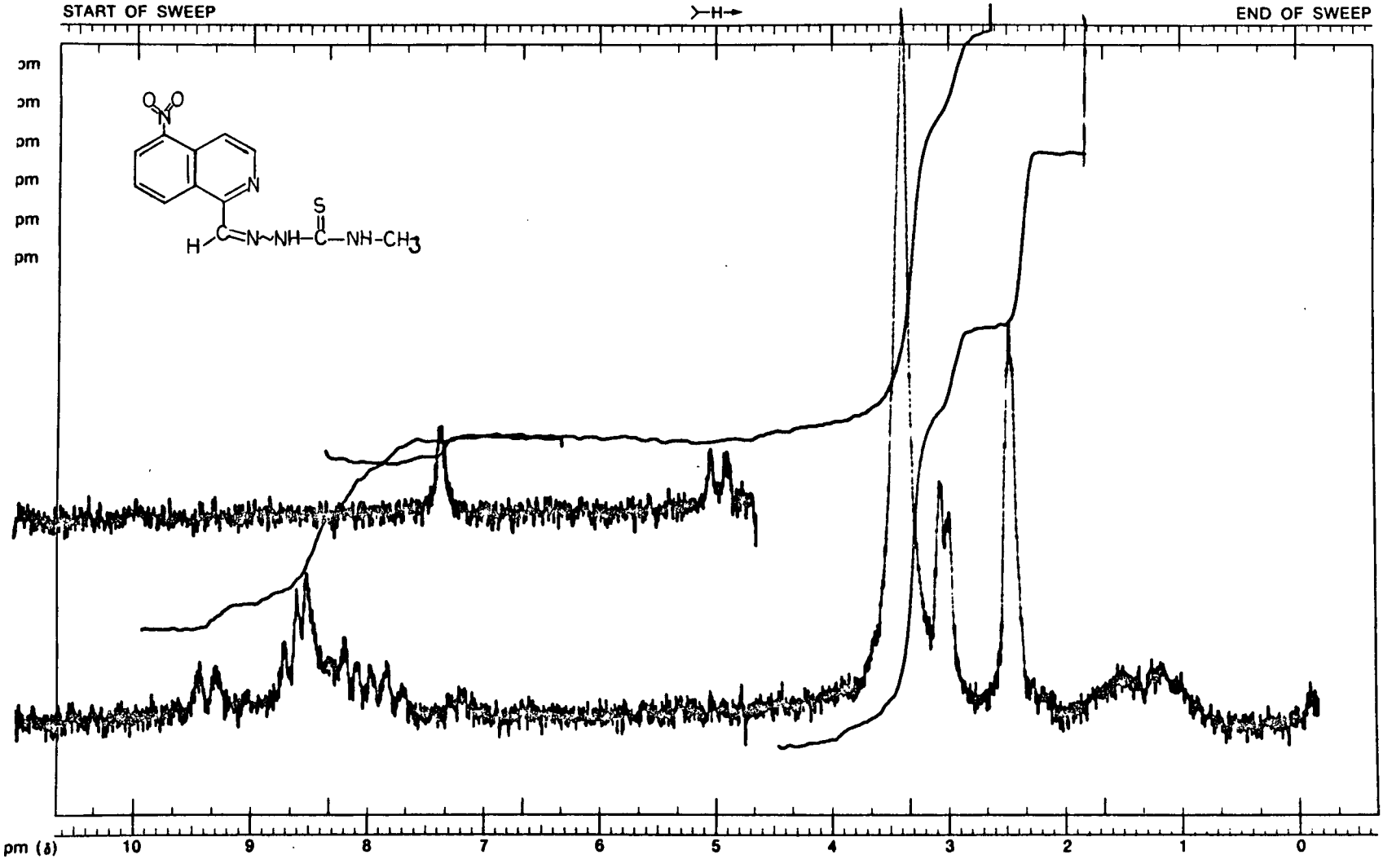


Figure 39: 1-formyl-thiosemicarbazone-5-nitroisoquinoline (116).

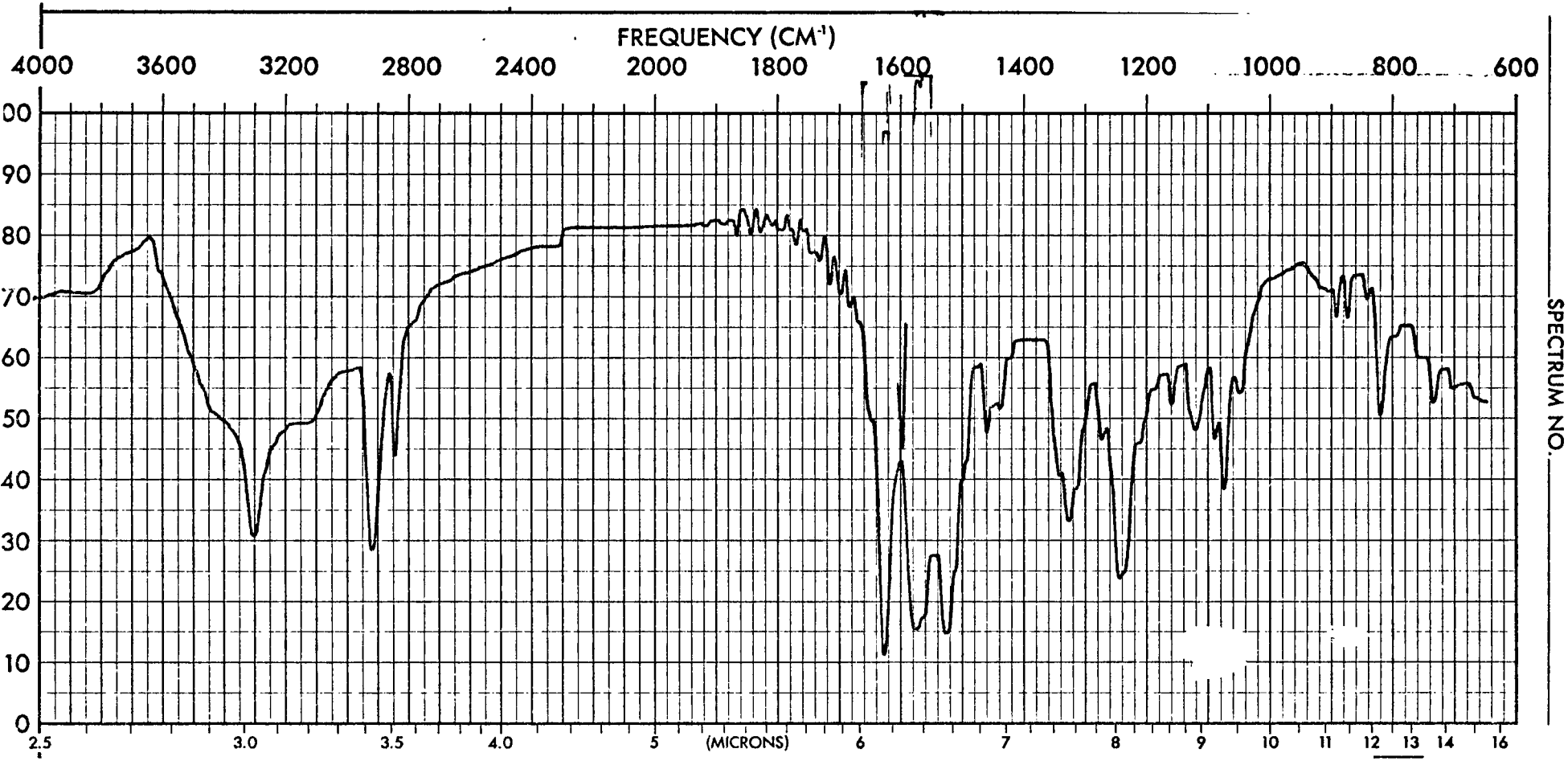


Figure 40: 1-formyl-thiosemicarbazone-5-nitroisoquinoline (116).

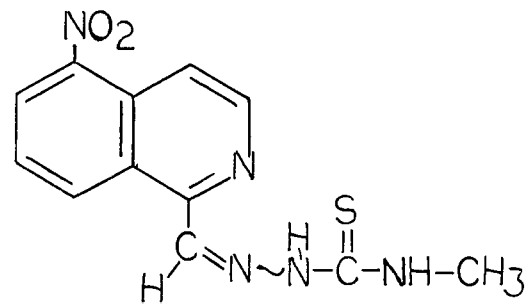
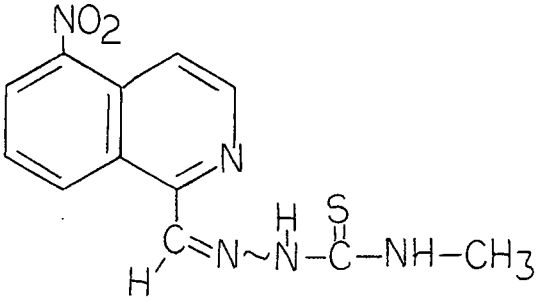
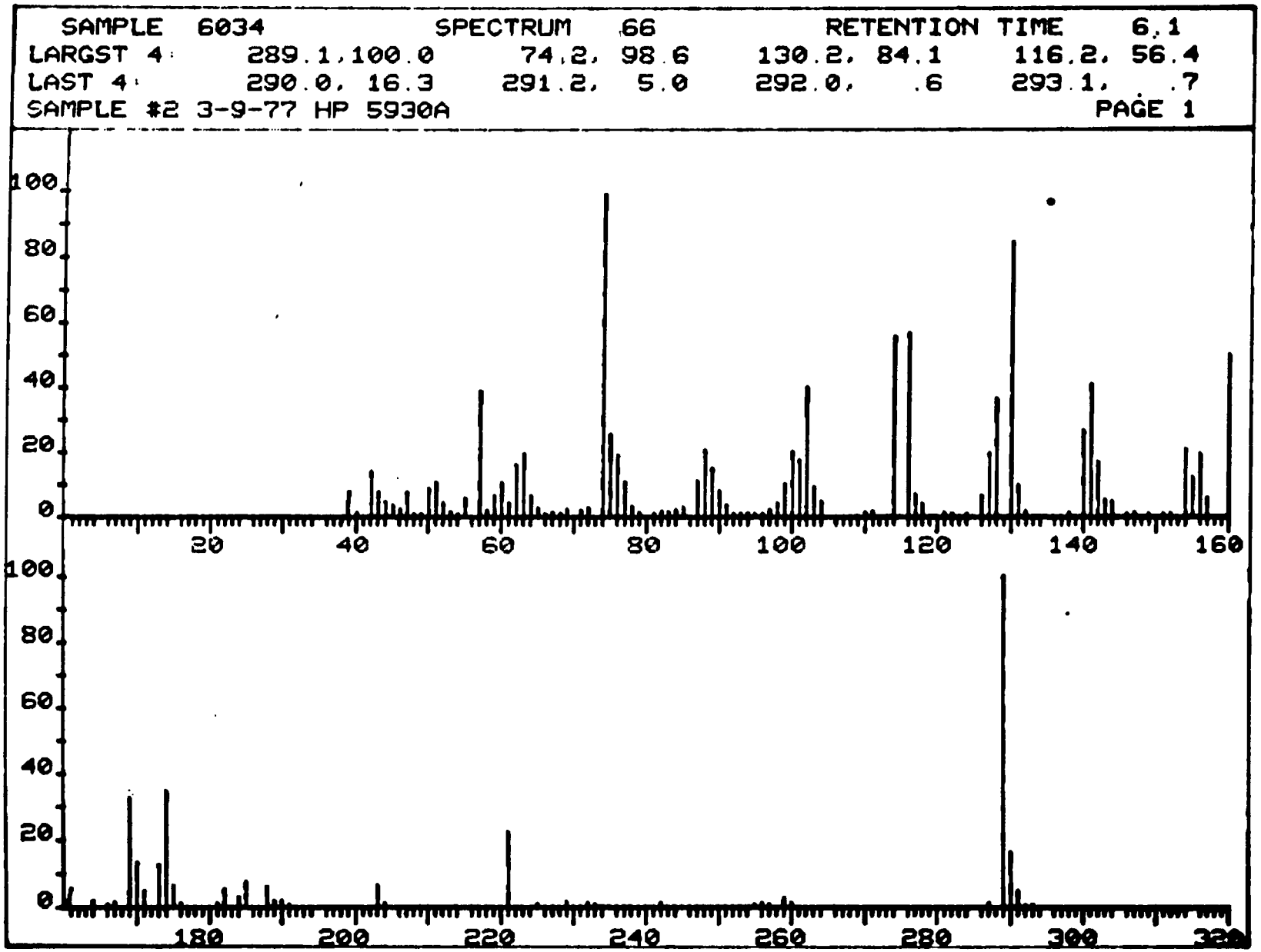


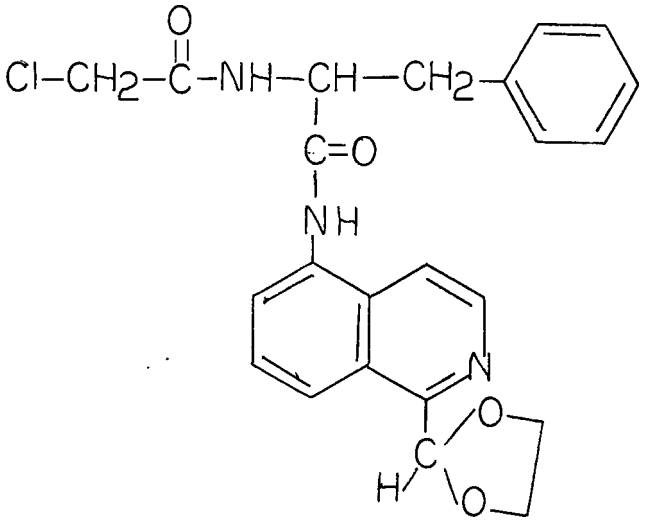
Figure 41: Mass Spectrum of 1-formyl-thiosemicarbazone-5-nitroisoquinoline

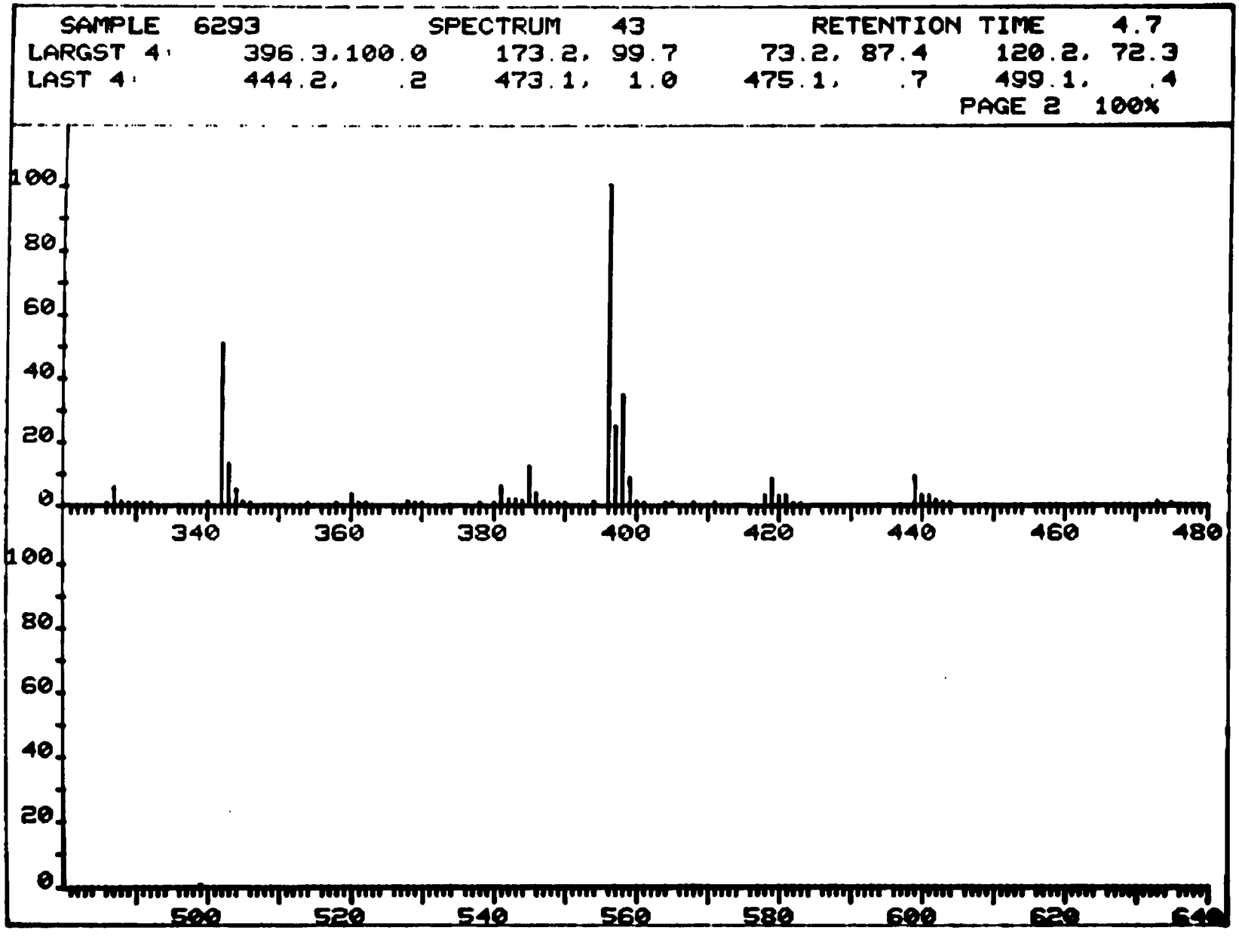


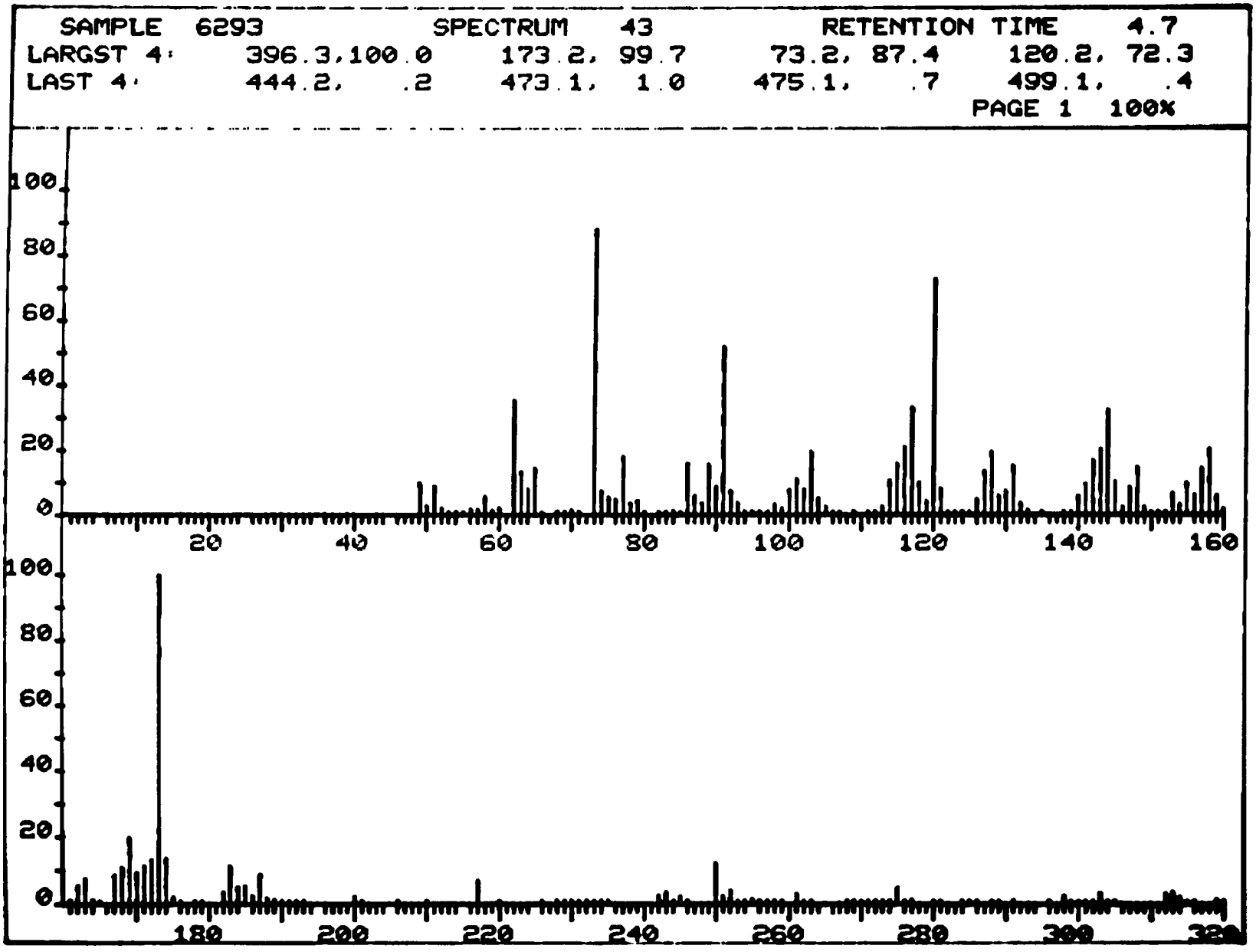

SAMPLE 6034 SPECTRUM 66 RETENTION TIME 6.1
LARGST 4: 289.1,100.0 74.2, 98.6 130.2, 84.1 116.2, 56.4
LAST 4: 290.0, 16.3 291.2, 5.0 292.0, .6 293.1, .7
SAMPLE #2 3-9-77 HP 5930A PAGE 1
100
80
60
40
20
0
20 40 60 80 100 120 140 160
100
80
60
40
20
0
180 200 220 240 260 280 300

34	3.2	TI=	22418.	BP=	56	LV=	2.50	289=	25	290=	0
35	3.2	TI=	22179.	BP=	56	LV=	2.45	289=	33	290=	0
36	3.3	TI=	21822.	BP=	56	LV=	2.44	289=	0	290=	0
37	3.4	TI=	22246.	BP=	56	LV=	2.33	289=	30	290=	0
38	3.5	TI=	21962.	BP=	56	LV=	2.41	289=	0	290=	0
39	3.6	TI=	23422.	BP=	56	LV=	2.61	289=	35	290=	0
40	3.7	TI=	24669.	BP=	56	LV=	2.70	289=	41	290=	0
41	3.8	TI=	26812.	BP=	56	LV=	2.88	289=	54	290=	0
42	3.9	TI=	28433.	BP=	56	LV=	3.16	289=	54	290=	0
43	4.0	TI=	32517.	BP=	56	LV=	3.42	289=	0	290=	0
44	4.1	TI=	36610.	BP=	56	LV=	3.71	289=	62	290=	0
45	4.1	TI=	40457.	BP=	56	LV=	4.07	289=	69	290=	0
46	4.2	TI=	45966.	BP=	56	LV=	4.46	289=	78	290=	0
47	4.3	TI=	49628.	BP=	56	LV=	4.95	289=	71	290=	0
48	4.4	TI=	50433.	BP=	56	LV=	4.97	289=	101	290=	0
49	4.5	TI=	44906.	BP=	56	LV=	4.49	289=	122	290=	0
50	4.6	TI=	33829.	BP=	56	LV=	3.02	289=	135	290=	0
51	4.7	TI=	24405.	BP=	56	LV=	2.02	289=	0	290=	0
52	4.8	TI=	21626.	BP=	56	LV=	1.48	289=	240	290=	33
53	4.9	TI=	17565	BP=	56	LV=	.98	289=	312	290=	55
54	5.0	TI=	12818.	BP=	57	LV=	.48	289=	0	290=	57
55	5.1	TI=	15029.	BP=	57	LV=	.49	289=	590	290=	101
56	5.2	TI=	16345.	BP=	74	LV=	.50	289=	0	290=	0
57	5.2	TI=	22432.	BP=	220	LV=	.65	289=	975	290=	174
58	5.4	TI=	22495.	BP=	74	LV=	.88	289=	1372	290=	182
59	5.4	TI=	28308.	BP=	220	LV=	1.05	289=	0	290=	261
60	5.5	TI=	28529.	BP=	220	LV=	1.12	289=	0	290=	272
61	5.6	TI=	28017.	BP=	74	LV=	1.26	289=	0	290=	297
62	5.7	TI=	31938.	BP=	74	LV=	1.55	289=	0	290=	343
63	5.8	TI=	38384.	BP=	220	LV=	1.76	289=	0	290=	360
64	5.9	TI=	44047.	BP=	220	LV=	1.87	289=	2705	290=	423
65	6.0	TI=	41154.	BP=	74	LV=	2.16	289=	0	290=	527
* 66	6.1	TI=	52635.	BP=	289	LV=	2.63	*289=	3994	290=	651

Figure 42: Mass Spectrum of 1-Formyl-ethyleneacetal-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline (114).




SAMPLE 6293 SPECTRUM 43 RETENTION TIME 4.7
LARGST 4 396.3,100.0 173.2, 99.7 73.2, 87.4 120.2, 72.3
LAST 4 444.2, .2 473.1, 1.0 475.1, .7 499.1, .4
PAGE 2 100%
100
80
60
40
20
0
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620


SAMPLE 6293 SPECTRUM 43 RETENTION TIME 4.7
LARGST 4: 396.3,100.0 173.2, 99.7 73.2, 87.4 120.2, 72.3
LAST 4: 444.2, .2 473.1, 1.0 475.1, .7 499.1, .4
PAGE 1 100%
100
80
60
40
20
0
20
40
60
80
100
120
140
160
100
80
60
40
20
0
180
200
220
240
260
280
300

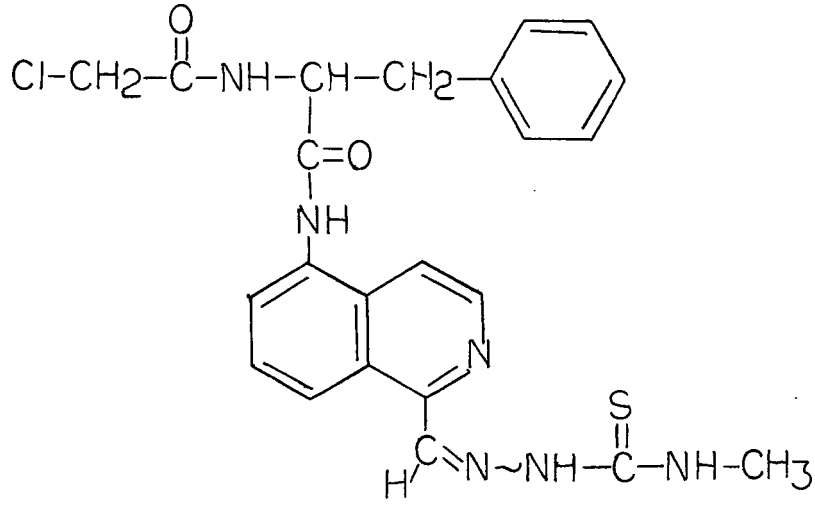
SAMPLE 6293 SPECT 43 M1= 350 M2= 460 TH= 0

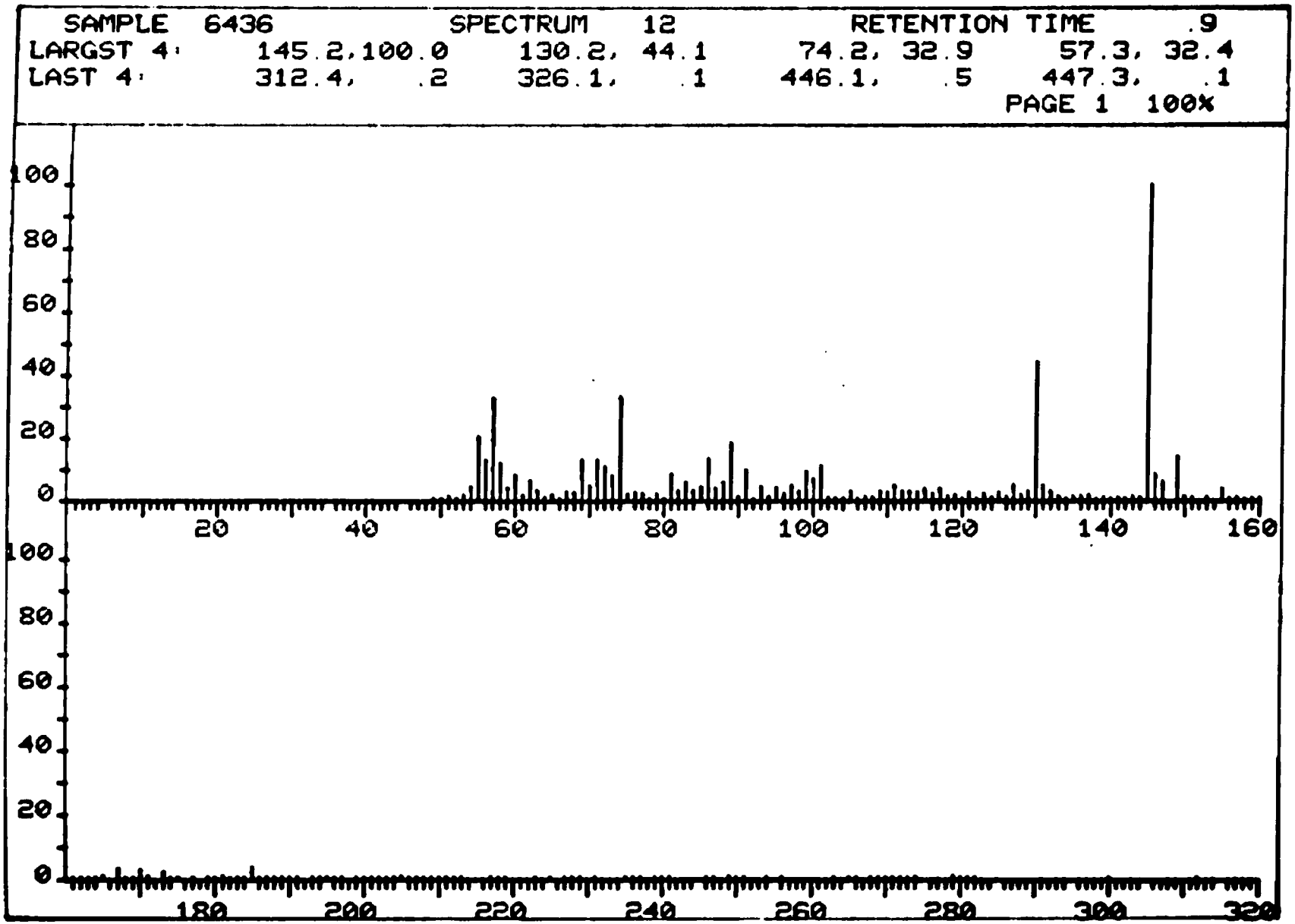
	MASS	ABUND
	354	.2
	358	.2
	360	3.3
	361	.9
	362	.8
	368	.9
	369	.4
	370	.3
	378	.2
	380	.4
	381	6.0
	382	1.7
	383	2.0
	384	1.3
	385	12.1
	386	3.6
	387	1.1
	388	.4
	389	.2
	390	.1
	394	1.1
>>	396	100.0
	397	24.9
	398	34.5
	399	8.4
	400	1.1
	401	.2
	404	.6
	405	.2
	408	.4
	408	.4
	411	.3

```
    418     2.9
    419     8.3
    420     3.1
    421     2.9
    422       7
    423      .4
    439     9.0
    440     3.1
    441     3.1
    442     1.6
    443      .3
    444      .2

>PAUSE
```

```
NUMBER OF CHLORINE ATOMS?1
NUMBER OF BROMINE ATOMS?0

100         **************************************************

32.4        ****************

MORE?
```

Figure 43: Mass Spectrum of 1-Formyl-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline-N^4-methylthiosemicarbazone (119).




SAMPLE 6436 SPECTRUM 12 RETENTION TIME .9
LARGST 4: 145.2,100.0 130.2, 44.1 74.2, 32.9 57.3, 32.4
LAST 4: 312.4, .2 326.1, .1 446.1, .5 447.3, .1
PAGE 1 100%
100
80
60
40
20
0
20 40 60 80 100 120 140 160
100
80
60
40
20
0
180 200 220 240 260 280 300 320

FRN 6436 SPECTRUM 12 RET. TIME = .9

MASS	ABUND
49	7
50	8
51	1.5
52	.7
53	1.7
54	4 3
55	20 5
56	13 0
57	32 4
58	12.0
59	4 2
60	8 2
61	1.7
62	6 4
63	3 3
64	1 0
65	1.8
66	6
67	2.9
68	2.6
69	12.8
70	4.7
71	12.8
72	10.6
73	8.3
74	32.9
75	2.4
76	2.5
77	2.1

78	.6
79	2.2
80	.7
81	8.5
82	3.4
83	5.9
84	3.2
85	4.3
86	13.4
87	3.9
88	5.8
89	18.5
90	1.6
91	9.7
92	.9
93	4.6
94	1.4
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97	4.8
98	2.9
99	9.2
100	7.2
101	11.3
102	1.3
103	.8
104	.8
105	2.8
106	.8
107	1.6
108	1.0
109	2.9
110	2.4

111	4.8
112	3.1
113	3 1
114	2 5
115	3.6
116	2 0
117	3 7
118	1.6
119	2.0
120	6
121	2.4
122	.9
123	2.4
124	1.0
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134	.8
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139	1.1
140	.7
141	1.1
142	.6
143	1.5

144	1.0
145	100.0
146	8.4
147	5.8
149	14.1
150	1.6
151	1.2
153	1.2
155	3.8
156	.7
157	1.1
158	.4
159	.7
160	.4
161	.5
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163	.6
164	.3
165	1.1
167	3.2
168	.5
169	.7
170	3.1
171	1.0
173	2.4
174	.4
175	.4
177	.3
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212	.2
213	.3
217	.7
218	.2
219	.3

223	.5
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227	.3
228	.3
229	.8
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235	.7
236	.4
237	.3
239	.2
240	.4
241	.2
246	.3
247	.2
249	1.1
250	.2
251	.8
254	1
256	.3
256	.4
263	.2
265	.1
266	.3
268	.2
269	.2
270	.2
271	.7
272	.2
273	.2
274	.1
279	.9

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  280          .3
  281          .3
  282          .4

  300          .2
  312          .2

  326          .1

  446          .5
  447          .1

>PAUSE
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FRN = 6439, SAMPLE SPECTRUM
NAME: L.WILLIAMS 6-30-77
MISC DATA: DIP
PERIODIC, GC RUN =100 MIN. 1ST MASS = 50

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7	TI=	28020.	LM=	325.3	BM=	145.3	406=	0	446=	0	447=	0
8	TI=	32743.	LM=	418.2	BM=	145.3	406=	0	446=	0	447=	0
9	TI=	40647.	LM=	309.4	BM=	145.3	406=	0	446=	0	447=	0
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11	TI=	70117.	LM=	450.1	BM=	145.3	406=	0	446=	0	447=	0
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16	TI=	77686.	LM=	446.4	BM=	145.3	406=	0	446=	0	447=	0
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26	TI=	40321.	LM=	467.6	BM=	86.3	406=	0	446=	22	447=	0
27	TI=	36714.	LM=	418.2	BM=	86.3	406=	0	446=	0	447=	0
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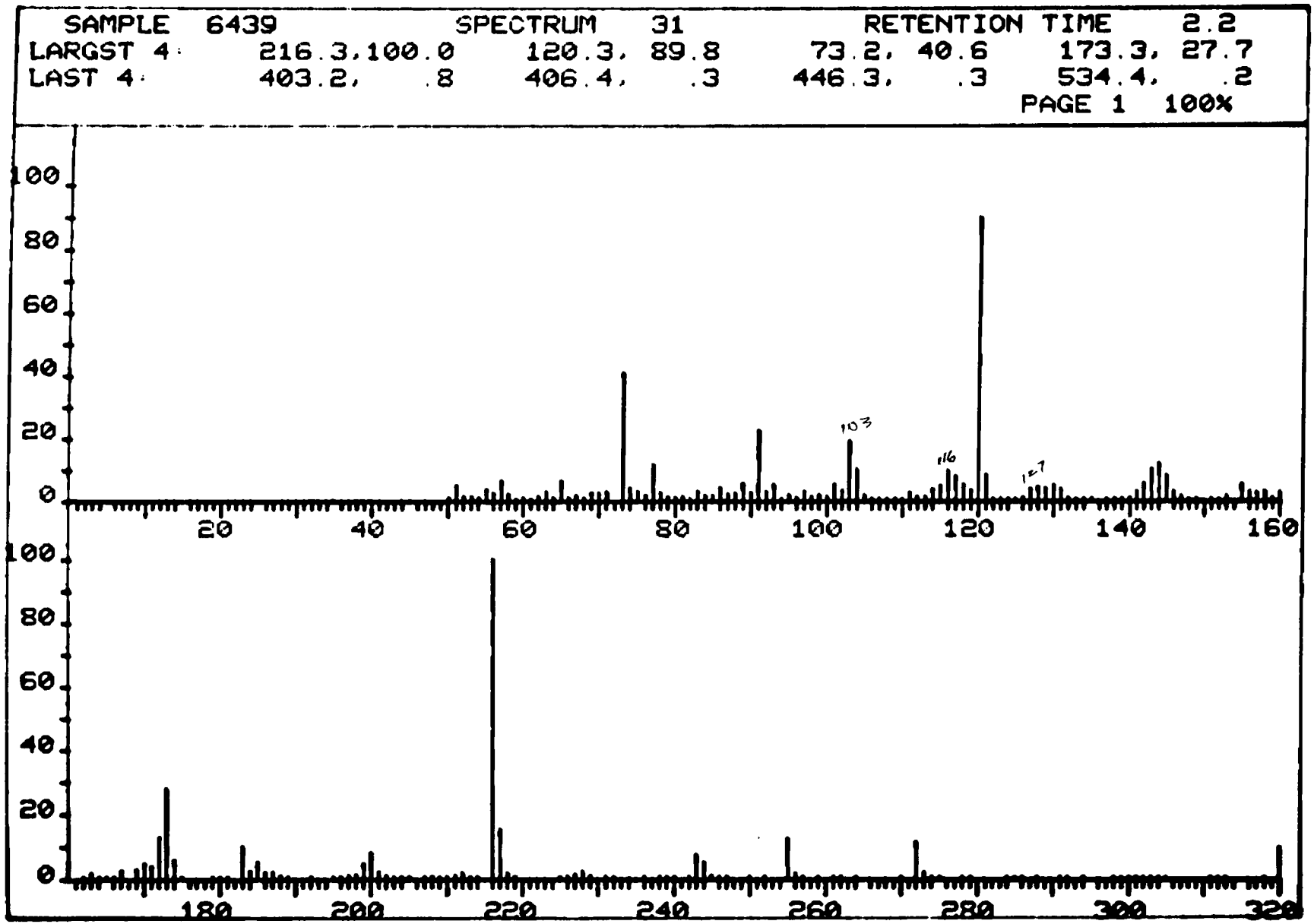
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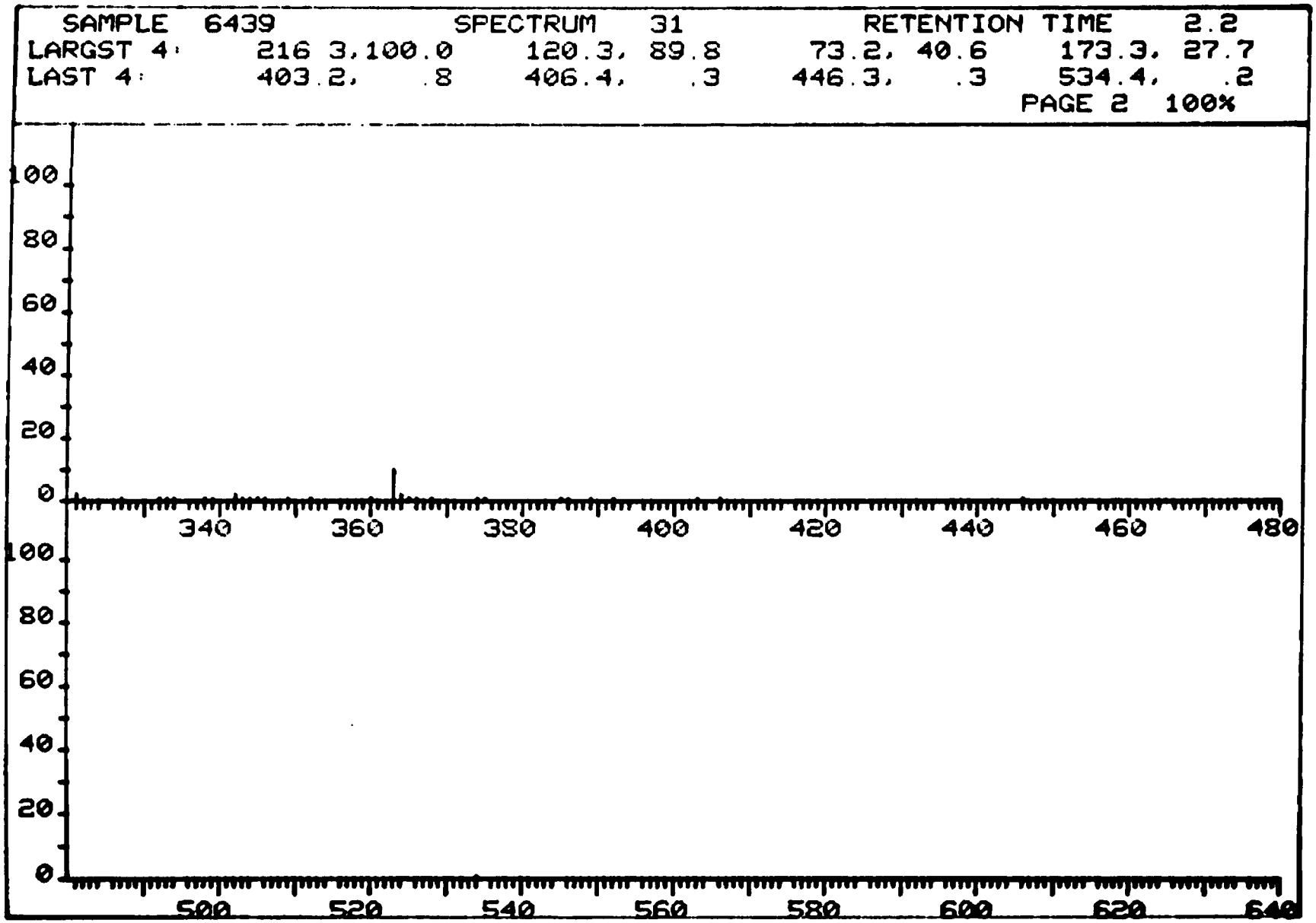
103
116
127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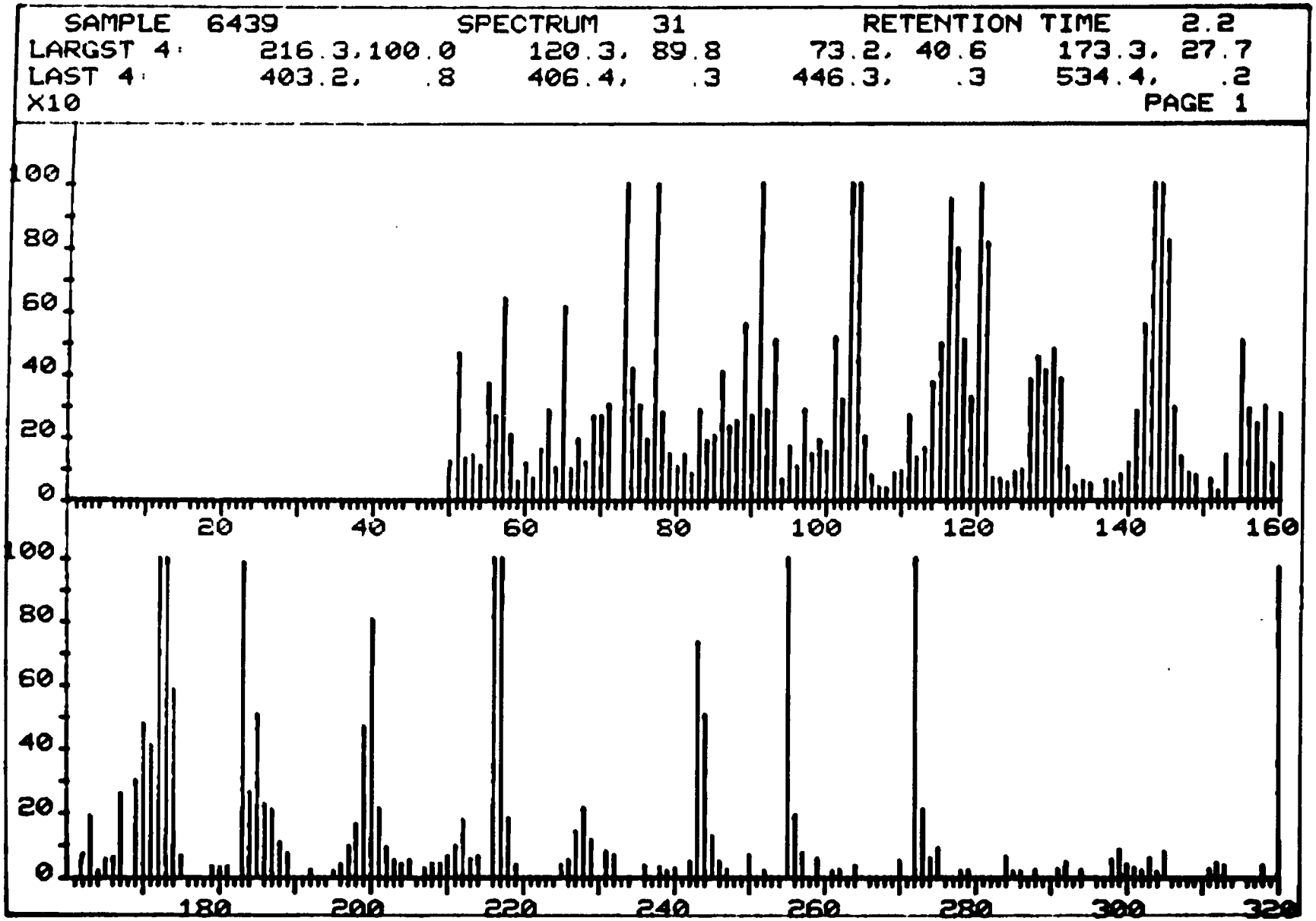
LAST 4: 403.2, .8 406.4, .3 446.3, .3 534.4, .2
PAGE 2 100%
100
80
60
40
20
0
340
360
380
400
420
440
460
480
100
80
60
40
20
0
500
520
540
560
580
600
620
640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LAST 4 403.2, .8 406.4, .3 446.3, .3 534.4, .2
X10
PAGE 1
100
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20 40 60 80 100 120 140 160
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80
60
40
20
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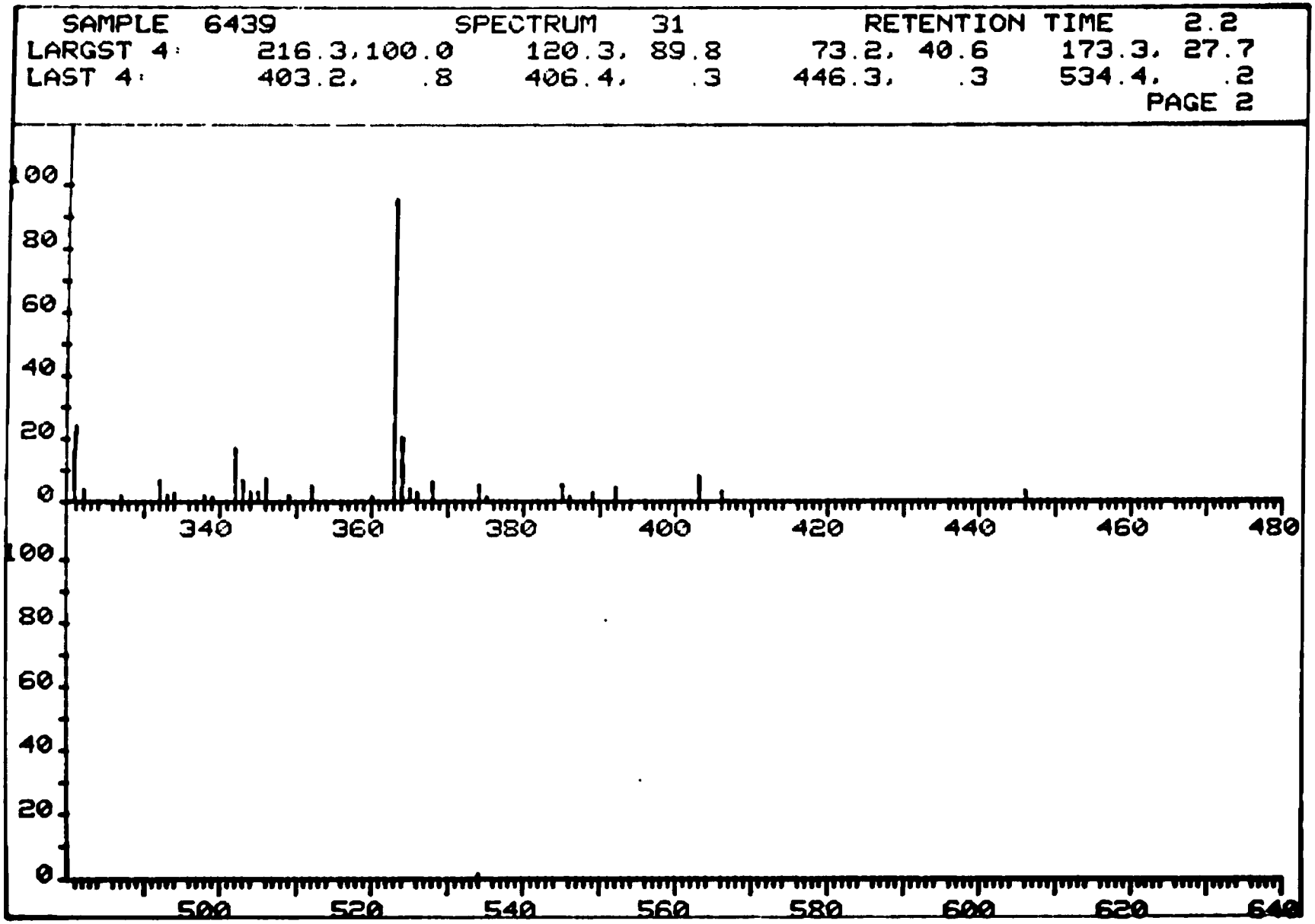
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LAST 4: 403.2, .8 406.4, .3 446.3, .3 534.4, .2
PAGE 2
100
80
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40
20
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340
360
380
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420
440
460
480
100
80
60
40
20
0
500
520
540
560
580
600
620
640

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 29 TI=   39070. LM= 418.1 BM= 216.3  406=    0  446=    0  447=    0
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 58 SPECTRA

EXAMINE SPECTRUM?
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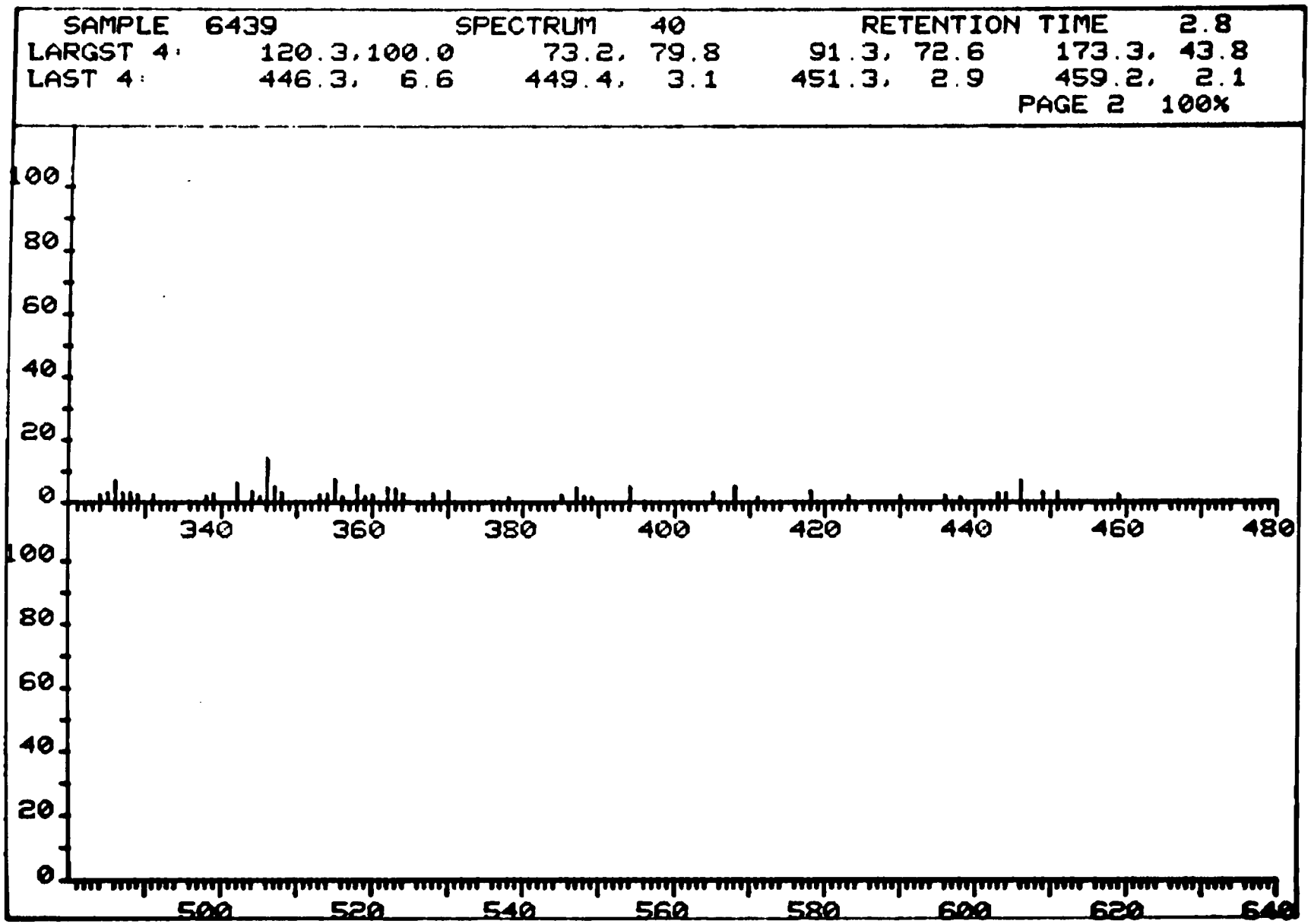
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PAGE 2 100%
100
80
60
40
20
0
340
360
380
400
420
440
460
480
100
80
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40
20
0
500
520
540
560
580
600
620
640

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32	2.5	TI=	47070.	BP=	170	LV=	2.01	407=	0	447=	0
33	2.6	TI=	47496.	BP=	170	LV=	2.05	407=	0	447=	0
34	2.6	TI=	51065.	BP=	170	LV=	1.86	407=	0	447=	15
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36	2.7	TI=	131149.	BP=	170	LV=	5.00	407=	27	447=	20
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43	3.2	TI=	76785.	BP=	170	LV=	3.29	407=	0	447=	0
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65	4 9	TI=	187502.	BP=	170	LV=	6.82	407=	0	447= 15
66	5.0	TI=	178029.	BP=	170	LV=	6.39	407=	0	447= 0
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   3        .3  TI=  44310.  BP= 188  LV=15.48   407=    0   447=    0
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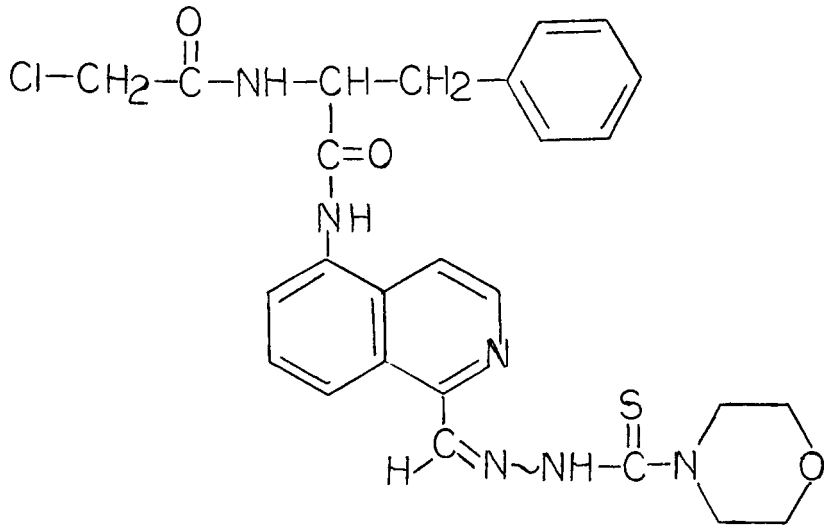
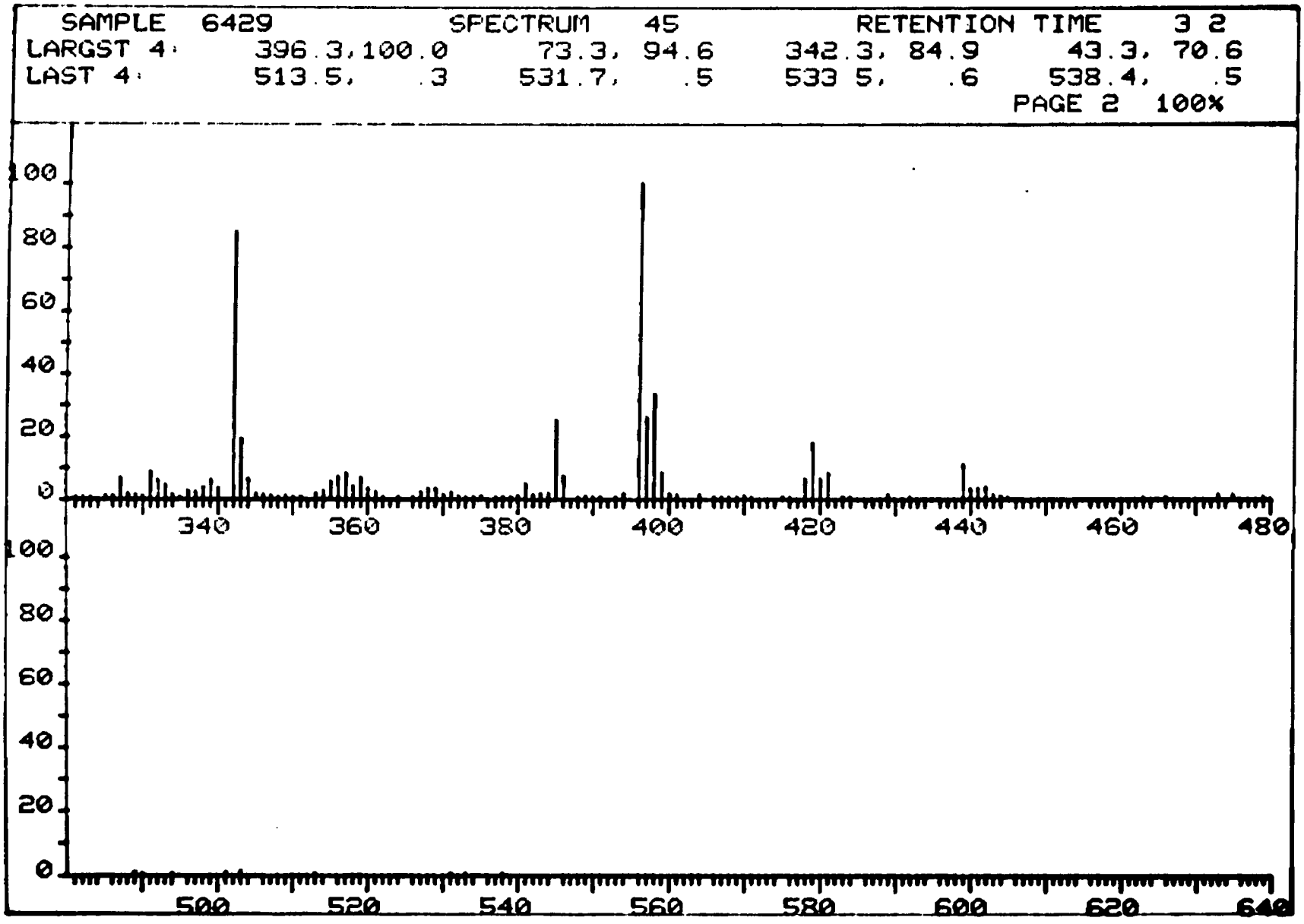
54	3.8	TI=	66328.	BP=	170	LV=	1.84	407=	0	447=	0
55	3.9	TI=	61809.	BP=	170	LV=	1.79	407=	17	447=	0
56	4.0	TI=	59356.	BP=	170	LV=	1.49	407=	0	447=	0
57	4.1	TI=	57003.	BP=	170	LV=	1.44	407=	27	447=	0
58	4.1	TI=	51108.	BP=	170	LV=	1.33	407=	0	447=	0
59	4.2	TI=	49966.	BP=	170	LV=	1.33	407=	0	447=	24
60	4.3	TI=	48055.	BP=	170	LV=	1.38	407=	0	447=	0
61	4.3	TI=	43989.	BP=	170	LV=	1.04	407=	0	447=	0
62	4.4	TI=	46471.	BP=	170	LV=	1.04	407=	0	447=	0
63	4.5	TI=	42641.	BP=	170	LV=	1.20	407=	18	447=	0
64	4.6	TI=	40995.	BP=	170	LV=	1.06	407=	0	447=	0
65	4.6	TI=	40079.	BP=	170	LV=	.94	407=	0	447=	17

Figure 44: 1-Formyl-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline morpholinothiosemicarbazone (120).




SAMPLE 6429 SPECTRUM 45 RETENTION TIME 3 2
LARGST 4: 396.3, 100.0 73.3, 94.6 342.3, 84.9 43.3, 70.6
LAST 4: 513.5, .3 531.7, .5 533 5, .6 538.4, .5
PAGE 2 100%
100
80
60
40
20
0
340
360
380
400
420
440
460
480
100
80
60
40
20
0
500
520
540
560
580
600
620
640

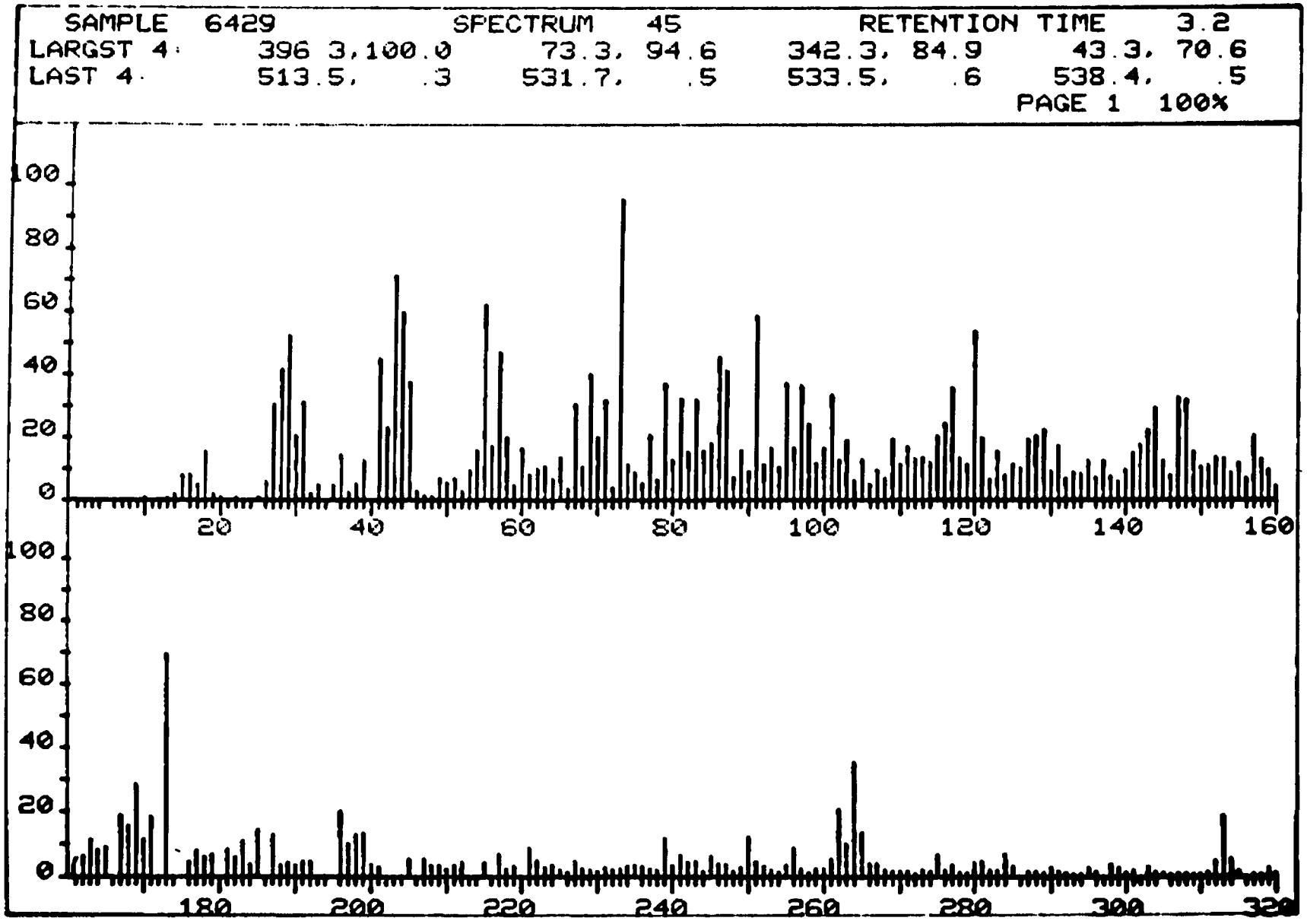
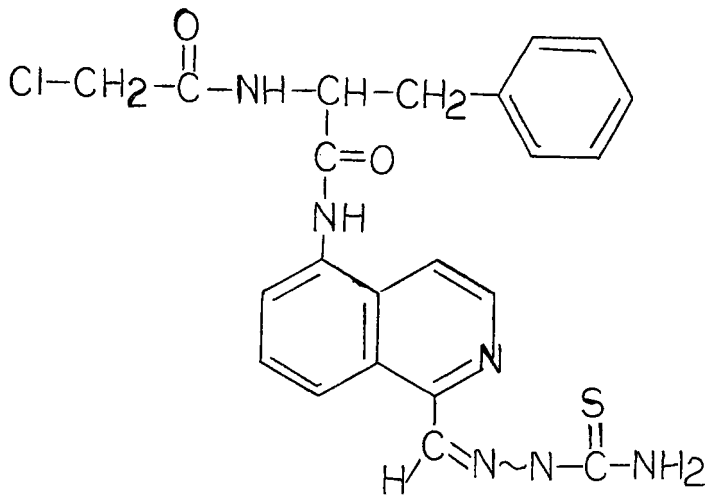
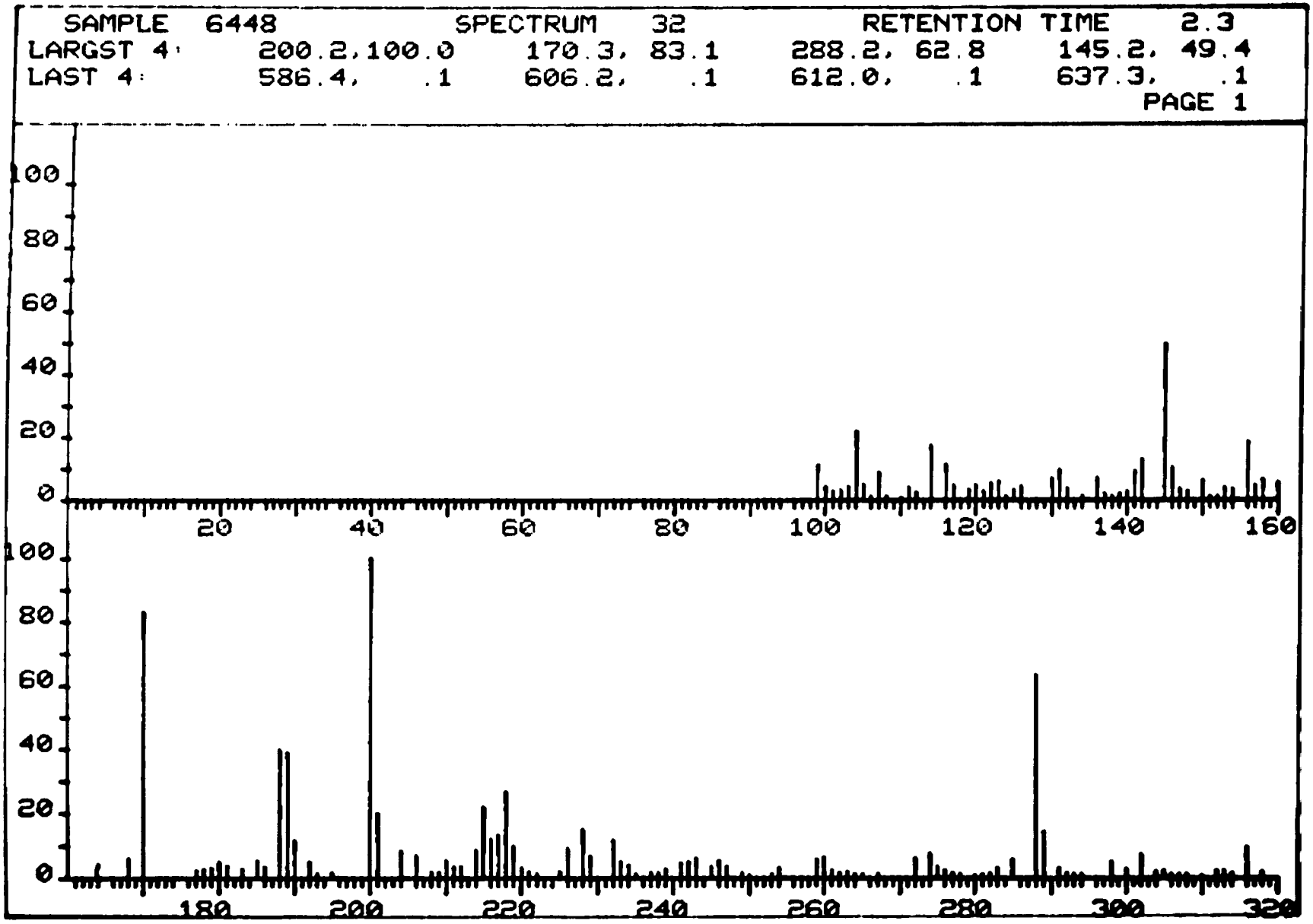

SAMPLE 6429 SPECTRUM 45 RETENTION TIME 3.2
LARGST 4. 396 3,100.0 73.3, 94.6 342.3, 84.9 43.3, 70.6
LAST 4. 513.5, .3 531.7, .5 533.5, .6 538.4, .5
PAGE 1 100%
100
80
60
40
20
0
20
40
60
80
100
120
140
160
100
80
60
40
20
0
180
200
220
240
260
280
300
320

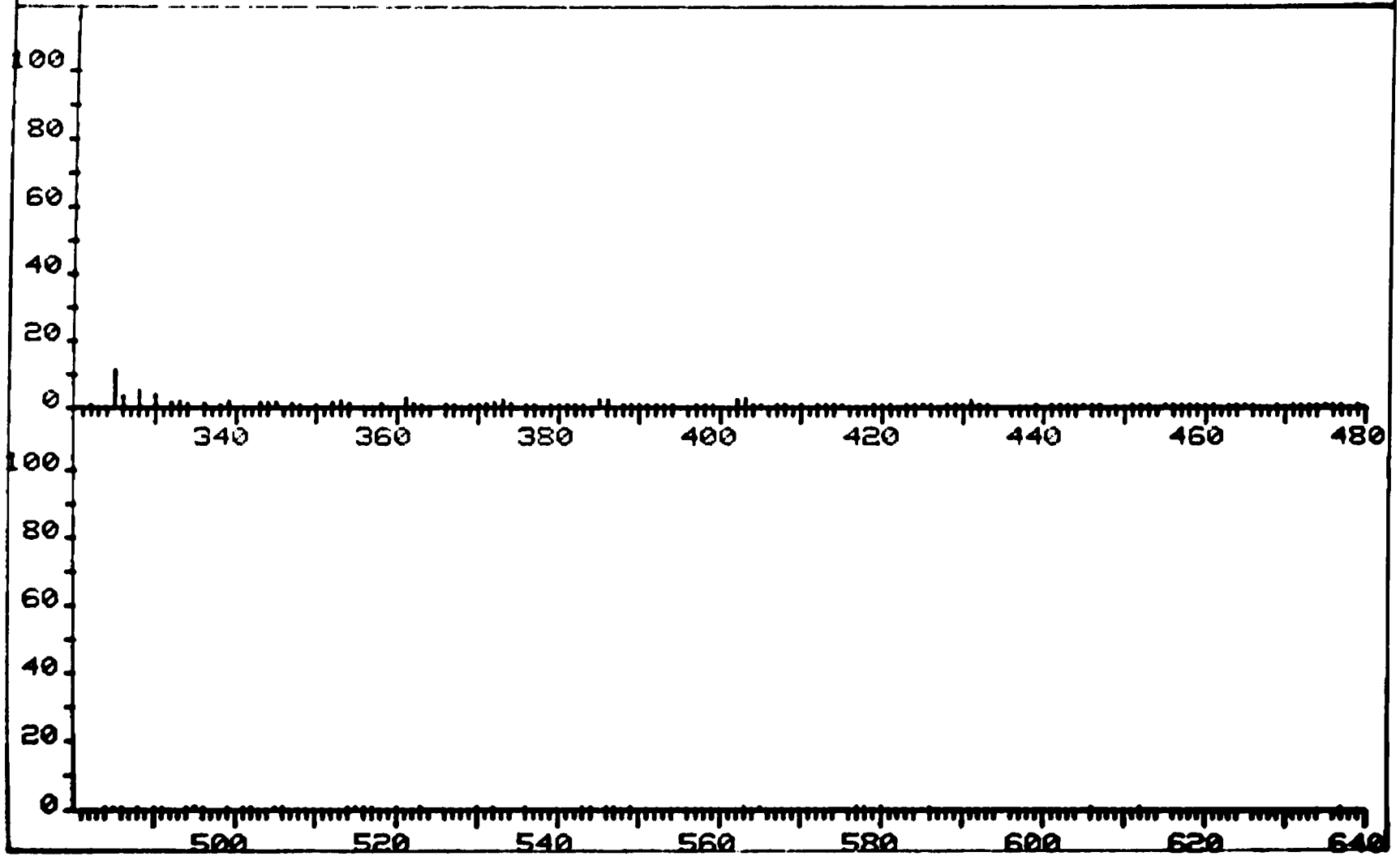
Figure 45: 1-Formyl-5N-(N-chloroacetyl-L-phenylalanine)-isoquinoline thiosemicarbazone (112).




SAMPLE 6448 SPECTRUM 32 RETENTION TIME 2.3
LARGST 4: 200.2, 100.0 170.3, 83.1 288.2, 62.8 145.2, 49.4
LAST 4: 586.4, .1 606.2, .1 612.0, .1 637.3, .1
PAGE 1
100
80
60
40
20
0
20 40 60 80 100 120 140 160
100
80
60
40
20
0
180 200 220 240 260 280 300 320

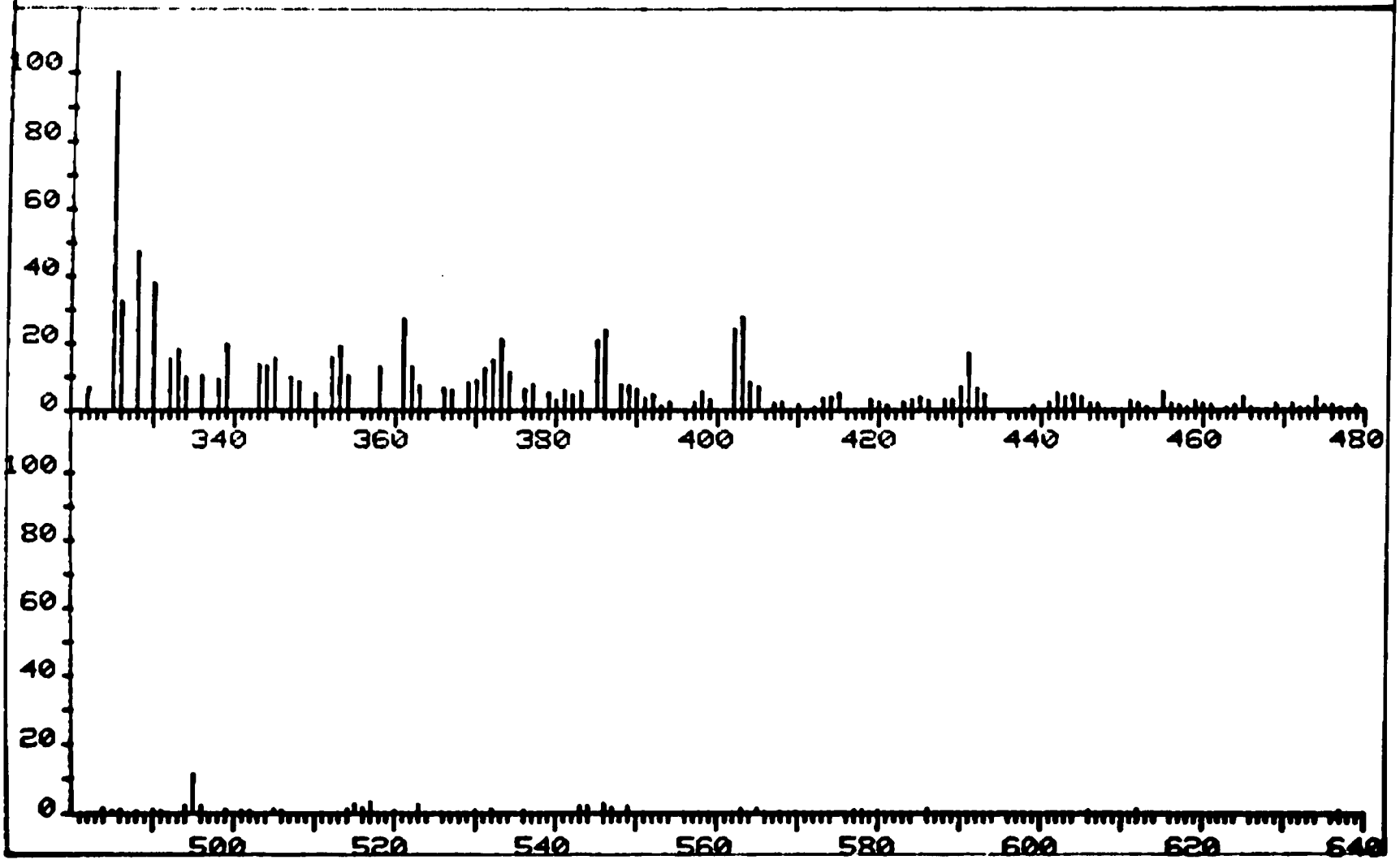
SAMPLE 6448 SPECTRUM 32 RETENTION TIME 2.3

LARGST 4:	200.2, 100.0	170.3, 83.1	288.2, 62.8	145.2, 49.4
LAST 4:	586.4, .1	606.2, .1	612.0, .1	637.3, .1



SAMPLE 6448 SPECTRUM 32 RETENTION TIME 2.3

LARGST 4:	200.2,	100.0	170.3,	83.1	288.2,	62.8	145.2, 49.4
LAST 4:	586.4,	.1	606.2,	.1	612.0,	.1	637.3, .1



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32 TI= 198439. LM= 637.3 BM= 200.2  431= 295  381=  95  447=  31
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34 TI= 162982. LM= 577.7 BM= 128.2  431= 190  381=  30  447=   0
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37 TI= 100715  LM= 577.7 BM= 170.2  431=  96  381=   0  447=   0
38 TI=  80535. LM= 551.5 BM= 170.1  431= 107  381=   0  447=   0
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41 TI=  48073. LM= 594.2 BM= 170.3  431=  20  381=   0  447=   0
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50 TI=  95047. LM= 598.9 BM= 170.1  431=  45  381=   0  447=   0
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62 SPECTRA
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