Spring 1975

PART I THE SYNTHESIS OF PROLINE-CONTAINING POLYPEPTIDES PART II CHEMICAL SHIFT NONEQUIVALENCE IN 2,4-DINITROPHENYL SULFENAMIDES

THOMAS DAVID HARRIS

INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

75-19,874

HARRIS, Thomas David, 1948-
PART I. THE SYNTHESIS OF PROLINE-CONTAINING
POLYPEPTIDES. PART II. CHEMICAL SHIFT
NONEQUIVALENCE IN 2,4-DINITROPHENYLSULFENAMIDES.

University of New Hampshire, Ph.D., 1975
Chemistry, organic

Xerox University Microfilms, Ann Arbor, Michigan 48106

PART I.

THE SYNTHESIS OF PROLINE-CONTAINING

POLYPEPTIDES

PART II.

CHEMICAL SHIFT NONEQUIVALENCE IN

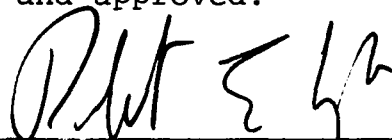2,4-DINITROPHENYLSULFENAMIDES

by

THOMAS D. HARRIS

B. S., Saginaw Valley College, 1971

A THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Doctor of Philosophy
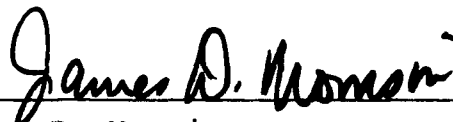
Graduate School

Department of Chemistry

May, 1975
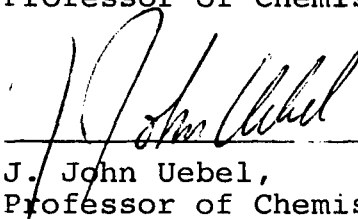
This thesis has been examined and approved.

Thesis director, Robert E. Lyle
Professor of Chemistry

James D. Morrison
Professor of Chemistry

J. John Uebel,
Professor of Chemistry

N. Dennis Chasteen, Associate
Professor of Chemistry

Gerald L. Klippenstein
Associate Professor of Biochemistry

Date

This thesis is dedicated to my wife, Susan, and to my parents.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

x

xii

LIST OF TABLES

LIST OF FIGURES
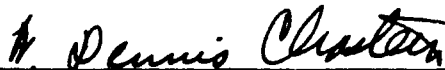
ABSTRACT

PART I.

THE SYNTHESIS OF PROLINE-CONTAINING

POLYPEPTIDES

PART II.

CHEMICAL SHIFT NONEQUIVALENCE IN

2,4-DINITROPHENYLSULFENAMIDES

by

THOMAS D. HARRIS

Part I

Structural comparison of several biologically active
polypeptides with the natural prostaglandins and with the
steroids suggests that small polypeptides containing proline
as the central residue of the chain should be biologically
active in the reproductive cycle. The synthesis and biolog-
ical screening of several peptides of this type is reported.

The syntheses closely followed the standard methods
of peptide synthesis. The coupling reactions were accomplished
by the active ester, mixed anhydride, and carbodiimide methods.
Use of the active esters proved to be the superior method,
while N,N'-dicyclohexylcarbodiimide generally gave the

poorest yields. The benzyloxycarbonyl group was used for nitrogen protection while the t-butyl ester was shown to be superior to the methyl or ethyl esters for carboxyl protection.

Two of the peptides showed slight activity in an anti-fertility screen and a third showed a slight inhibition of luteinizing hormone-follicle stimulating hormone release. None of the compounds tested showed any activity in an anti-biotic screen.

Part II

The synthesis and variable-temperature nuclear magnetic resonance studies of 2,4-dinitrophenylsulfenamides of substituted piperidines and pyrrolidines is described. These sulfenamides were shown to exhibit chemical shift non-equivalence of both aromatic and aliphatic protons due to restricted rotation around the S-N bond. The magnitude of this nonequivalence was shown to be dependent on the nature of the substituent on the piperidine or pyrrolidine ring. Only substituents containing a carbonyl group exhibited chemical shift nonequivalence. A comparison of the sulfen-amides of disubstituted pyrrolidines suggests that the pre-ferred ground state conformation is one in which the plane of the C-S-N atoms bisects the angle formed by the other two ligands on nitrogen.

PART I.

THE SYNTHESIS OF PROLINE-CONTAINING

POLYPEPTIDES

SECTION I

INTRODUCTION

The world is becoming increasingly aware that
problems such as hunger and malnutrition, disease, and
shortages of natural resources can be attributed, at least
in part, to overpopulation. It is clear that a convenient,
harmless, and inexpensive means of birth control is needed.
Thus, several nations of the world have set as their goal
the achievement of zero population growth in the near future.
The attainment of this goal will almost certainly require
the use of chemical contraceptives. A number of excellent
reviews of this topic have been published.[1,2,3,4,5]

The chemical contraceptives in use today are very
effective birth control agents, but they suffer from a
number of drawbacks. Primarily, they cause a wide range
of undesirable physiological responses outside of the re-
productive cycle.[1,3,6] Areas that are affected include the
adrenal gland, thyroid gland, skin, skeletal growth and
calcium metabolism, the thymus gland, and the liver. Other
adverse effects include depression, hypertension, electrolyte
imbalance, and changes in the blood elements and circulatory
system. Most of these effects are reversible on withdrawal
of medication, but information is lacking on the effects of
long term continuous use. The fact that chemical contracep-
tives presently in use are able to cause responses in a
wide variety of tissues, indicates that the use of such

agents requires caution. Thus, a chemical contraceptive
that is both effective and free from adverse side effects
is clearly needed. In addition, areas which are likely to
receive increasing attention include the "30-day" pill and
male contraceptives. Taking a pill once a month is more
convenient, and probably would be safer, than daily admin-
istration, and an agent that would safely and reversibly
inhibit fertility in the male would find practical appli-
cation in fertility regulation.

The hormones involved in the reproductive cycle are
summarized in Figure 1. Control resides in the hypothal-
amus which, in turn, is influenced by the cerebral cortex.
The hypothalamus produces follicle stimulating hormone- and
luteinizing hormone-releasing factor (FSH-RF and LH-RF,
respectively), which cause the pituitary to release
follicle stimulating hormone (FSH) and luteinizing hormone
(LH), respectively. These gonadotrophins, in turn, cause
the production of hormones by the gonads. At each of the
hormone target sites, and in particular at the hypothalamus,
pituitary, ovary, fallopian tubes, and uterus, there is an
opportunity to influence fertility. As indicated in Figure
1, the production of hormones such as estradiol, testosterone,
and progesterone is dependent, either directly or indirectly,
upon the release of FSH and LH by the pituitary gland. These
steroidal hormones act by a feedback mechanism to inhibit
the production of FSH and LH either by direct action upon
the ovary or pituitary, or through the hypothalamus. These

CEREBRAL CORTEX

HYPOTHALAMUS

FSH* & LH*   Releasing Factors

PITUITARY

FSH   and LH

GONAD

OVARY   TESTIS

Follicle $\xrightarrow{\text{FSH (LH)}}$ Estradiol $\xleftarrow{\text{FSH (LH)}}$ Sertoli Cells

19-Nortestosterone

Corpus
Luteus $\xrightarrow{\text{LH (FSH)}}$ Testosterone $\xleftarrow{\text{LH (FSH)}}$ Leydig Cells

17α-Hydroxyprogesterone

Progesterone   Progesterone

Stroma $\xrightarrow{\text{FSH/LH}}$ Androgens $\xleftarrow{\text{FSH/LH}}$ Stroma
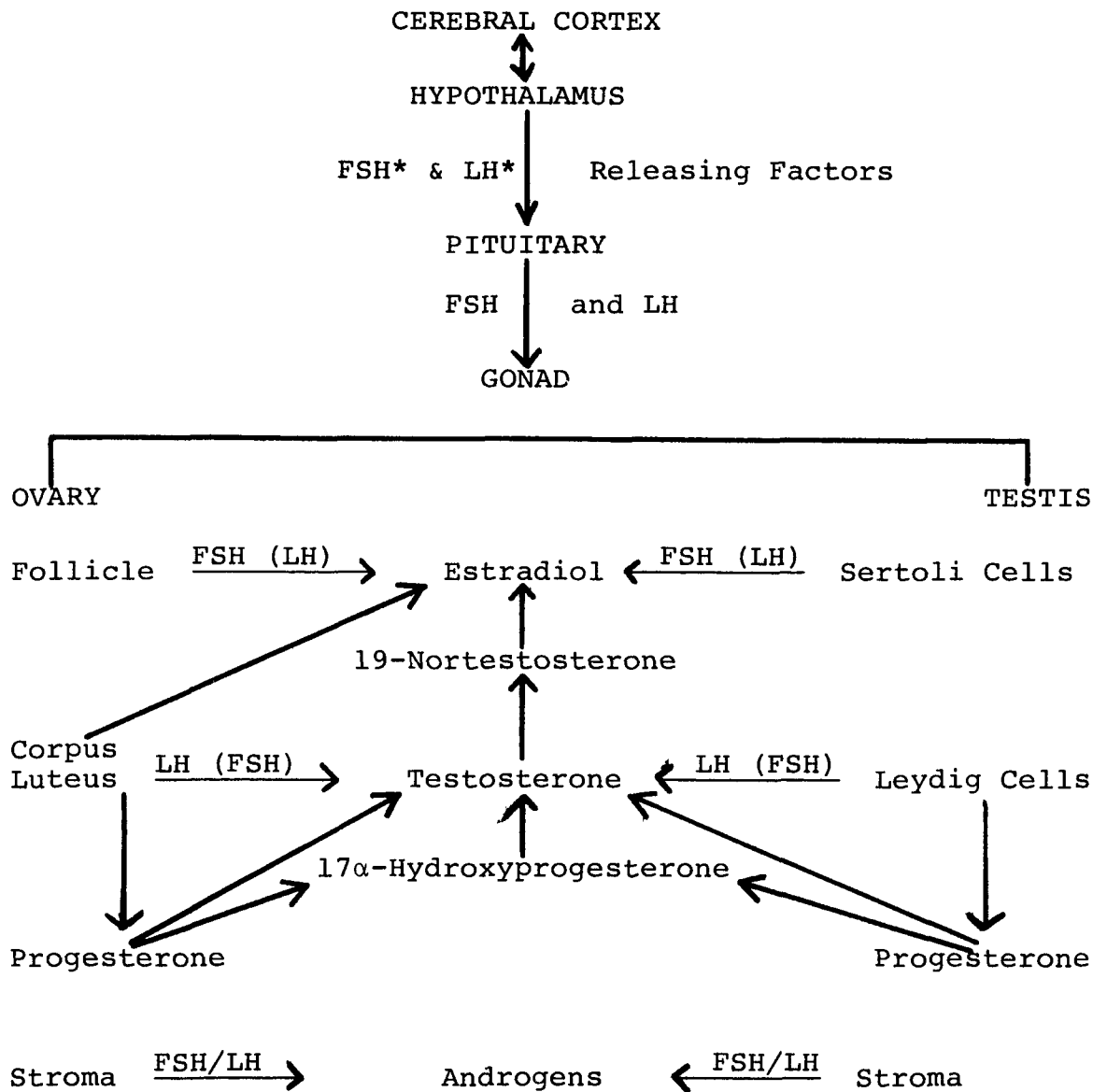
Figure 1

Hormones of the Human Reproductive Cycle

*See page

steroids were thus a natural starting point for the control of fertility, and when effective contraceptives were found, they were derivatives of estradiol, progesterone, and testosterone.

In both men and women there is a continuous production of hypothalamic releasing factors so that there is a continuous production of gonadal hormones. However, in females alone, there is an explosive cyclical release of FSH-RF and LH-RF which are essential for the ovulatory process. The principle mode of action of present-day contraceptives is the inhibition of pituitary gonadotrophins, primarily the inhibition of the mid-cycle "burst" of luteinizing hormone and thus the inhibition of ovulation. Figure 1 also suggests that chemical control of fertility is possible in the male. Since normal male spermatogenesis appears to depend upon gonadotrophins and testicular androgens, it is possible that a specific anti-androgen may disrupt sperm formation sufficiently to render an individual infertile. And finally, the use of prostaglandins[7] to cause spontaneous abortion is a very practical possibility. However, prostaglandins, like steroids, are active in a wide range of tissues outside the reproductive cycle and must be used with caution. Thus agents more specific than either the prostaglandins or the steroids in use today are desired.

It has been shown that a structural similarity exists among a variety of compounds which show activity in the re-

productive cycle. A few of these compounds are shown in Figure 2 and include the prostaglandins (<u>1</u>)[7,8], the estrogenic antibiotic zearalenone (<u>2</u>)[1], and the steroid estrone (<u>3</u>).[1] In addition, a number of proline-containing polypeptides are active in the reproductive cycle. These include actinomycin D (<u>4</u>)[9], oxytocin[10], the follicle stimulating-luteinizing hormone-releasing factor (FSH-RF/LH-RF)[11,12], and analogues of the FSH-LH releasing factor which either inhibit or stimulate production of FSH-LH to varying degrees.[11,13,14] In addition, even simple amines have been reported[15] to partially duplicate the activity of the releasing factor. Very recently, a proline-containing tetrapeptide[16], isolated from pregnant hamsters, has been shown to prevent ovulation in mammals.

These molecules have several structural features in common. A ring is central to all the structures and there are two chains on vicinal atoms of the ring. Each system also has an oxygen atom on one chain seven atoms from the ring. The chains are held together in a steroid-like conformation by a lactone function in the case of zearalenone, by the cyclic structure in the case of actinomycin D, and by hydrogen bonding in the prostaglandins. The steroids and prostaglandins were shown to have similar conformations by the theoretical calculations of Kier.[17]
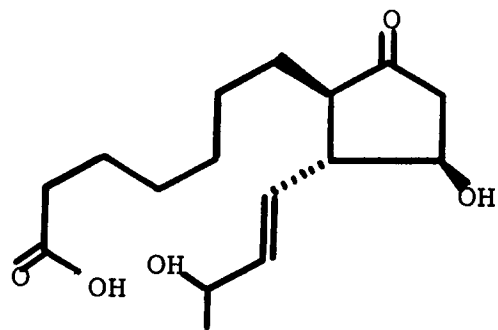
PGE$_1$ (1)
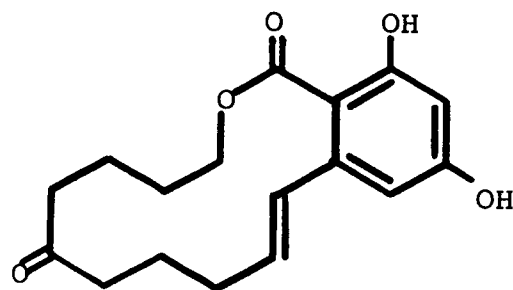
Zearalenone (2)
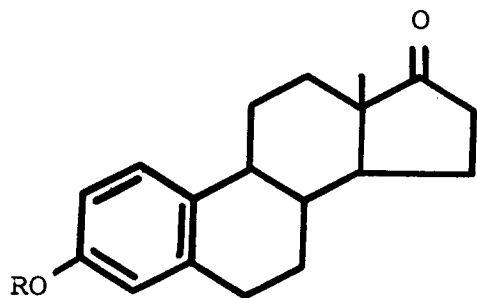
Esterone (3)
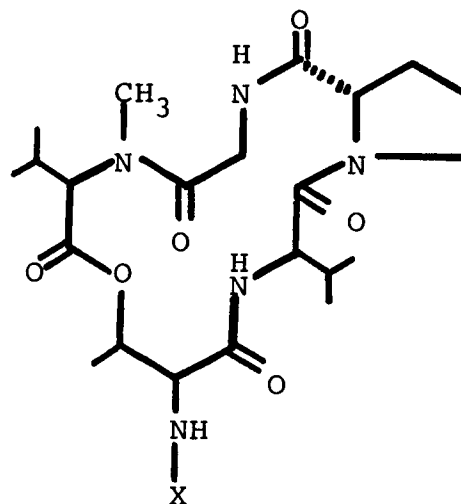
Actinomycin D (4)

X = Heterocycle
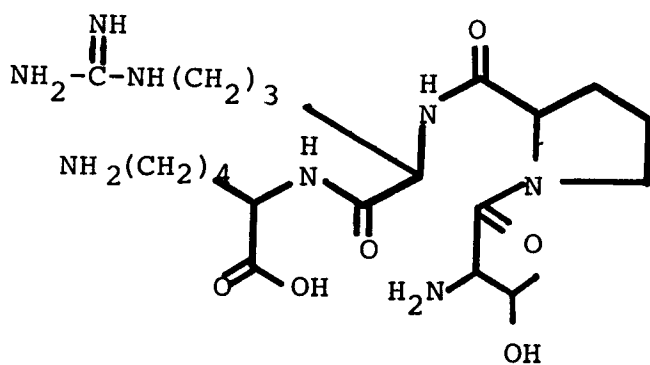
5

Figure 2

Structural Relationships Among Biologically Active Compounds
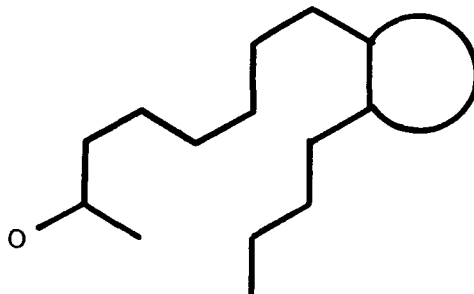
These structural simularities suggest that derivatives
of proline should show activity in the reproductive cycle.
This thesis describes the synthesis of several tripeptides
(6) and tetrapeptides (7) which contain a central prolyl
residue and either a five-carbon or six-carbon aliphatic acid
on the N-terminal end of the chain. These peptides should
function in a manner similar to the prostaglandins or as
prostaglandin antagonists or could act as inhibitors of
FSH-LH releasing factor. Since these compounds would be
simple polypeptides, their degradation products would be
easily metabolized and they should show more specificity
of action and avoid the undesirable side effects of con-
traceptives currently in use.
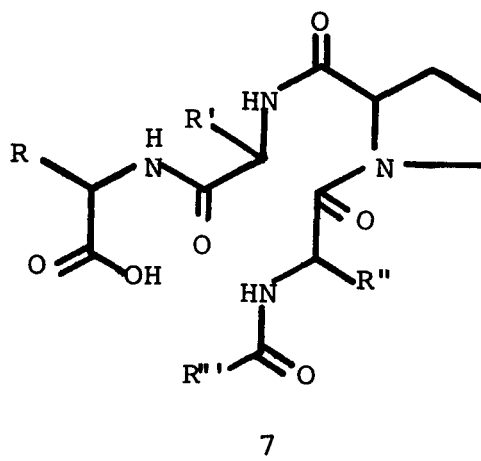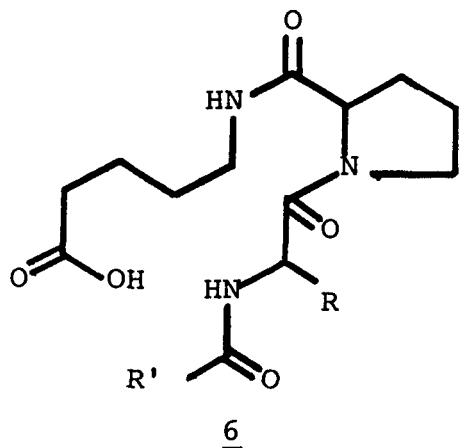
The structural similarities of the compounds in Figure 2 also suggest that derivatives of proline which contain an oxygen atom on carbon three or five of the proline ring should show antifertility activity. Compounds of this type (8 and 9) were prepared by Dr. Richard Davenport[18] of these laboratories and will not be discussed in this thesis.
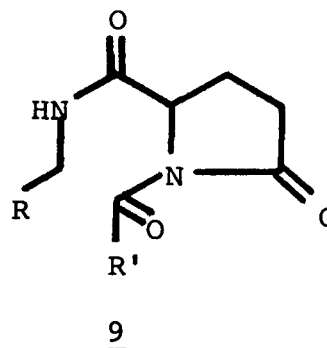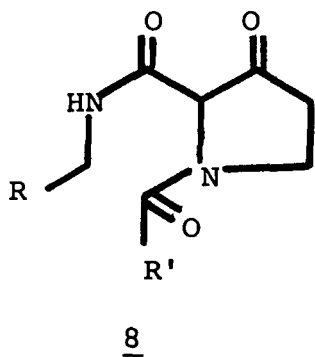
SECTION II

RESULTS AND DISCUSSION

A. Underline General Considerations of Peptide Synthesis

This thesis is not intended as a detailed review of
the chemistry of peptide synthesis but as a description of
the synthesis of a number of proline-containing peptides. A
brief discussion of the approach to peptide synthesis is
offered below and the experimental methods employed in this
research will be covered individually at the appropriate
place in the discussion. For a more extensive account of
peptide synthesis, the two-volume treatise by Schröder and
Lübke[19] remains the most useful general work, and it is
complimented by a less detailed but very readable book by
Bodanszky and Ondetti.[20]

Individual amino acids in the text are always men-
tioned by their full names. Amino acid abbreviations are
used only for the presentation of peptides in tables and
reaction schemes. The abbreviations of all the amino acids
and alkanoic acids used in this work consist of the first
three letters of the trivial names and are illustrated in
Table I.

Abbreviations of protecting groups and commonly used
reagents are used only in reaction schemes. These abbrevia-
tions, along with the structures and full names, are listed
in Table II.

Table I

Abbreviations of Amino Acid

Residues and Alkanoic Acids

Full Name	Abbreviation
Aminovaleric acid	Amv
Glycine	Gly
Hexanoic acid	Hex
Leucine	Leu
Octanoic acid	Oct
Pentanoic acid	Pen
Phenylalanine	Phe
Proline	Pro
Tyrosine	Tyr

Table II. Abbreviations of Protecting Groups and Reagents
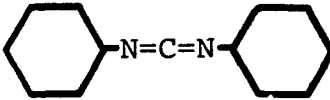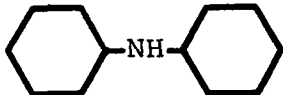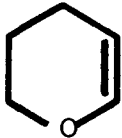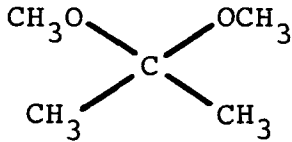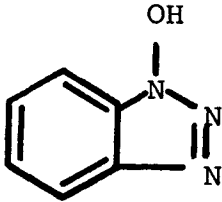
Full Name	Structure	Abbreviation
Amide	$-NH_2$	$-NH_2$
Benzyl ester	$-CH_2-\phi$	$-Bzl$
Benzyloxycarbonyl	$\phi-CH_2-O-CO-$	Cbo-
t-Butyl ester	$-O-C(CH_3)_3$	$-O-\underline{t}-Bu$
Ethyl ester	$-OCH_2CH_3$	$-OEt$
Methyl ester	$-OCH_3$	$-OMe$
p-Nitrophenyl ester	$-O-\phi-NO_2$	$-OPhNO_2$
N,N'-Dicyclohexyl-carbodiimide	$\text{cyclohexyl}-N=C=N-\text{cyclohexyl}$	DCC

Table II. (Continued)

Full Name	Structure	Abbreviation
Dicyclohexylamine		DCHA
Dihydropyran		DHP
2,2-Dimethoxypropane		2,2-DMP
1-Hydroxybenzotriazole		HOBT
Mixed anhydride*		MA
Trifluoroacetic acid	CF_3CO_2H	TFA

*This does not represent a specific reagent but rather the process of forming a peptide bond by the mixed anhydride method.

In principle, peptide synthesis is a simple, straight-forward process. It involves the formation of an amide bond between the carboxyl group of one amino acid and the amino group of a second amino acid. This reaction formally represents the elimination of water and requires activation of the carboxyl group by introduction of a negative group, X, which will increase the electrophilic character of the carbonyl carbon atom. This facilitates the attack of a nucleophilic amino group to form the peptide bond. Since the carbonyl group that is being activated also contains an amino group, a peptide synthesis utilizing free amino acids will give un-controlled polycondensation. The synthesis of pure peptides requires that reactive groups of the amino acids or peptides which are not to participate in the coupling reaction be protected. The residues used for this blocking are called amino- or N-protecting groups (P) and carboxyl- or C-protecting groups (Y), respectively. After the coupling reaction, the appropriate protecting group is selectively removed and another partially protected amino acid or peptide is coupled. When the desired peptide chain is reached, all protecting groups are removed. In addition to the amino and carboxyl functions, many amino acids contain reactive side chains, generally called ω-groups, which may give rise to side reactions and must be blocked.

$$\underset{\text{H}_2\text{NCHCO}_2\text{H}}{\overset{\text{R}}{|}} + \underset{\text{H}_2\text{NCHCO}_2\text{H}}{\overset{\text{R'}}{|}} \xrightarrow{-\text{H}_2\text{O}} \underset{\text{H}_2\text{NCHCO-NH-CHCOOH}}{\overset{\text{R}\qquad\text{R'}}{|\qquad\quad|}}$$

$$\underset{\underset{\text{X}\delta^-}{|}}{\overset{\overset{\text{O}}{\|}}{\underset{}{\text{R-C}\delta^+}}} + \underset{\overset{\text{H}}{|}}{\underset{\overset{}{\underset{\text{H}}{|}}}{:\text{N-R'}}} \longrightarrow \text{R-CO-NH-R'} + \text{HX}$$

$$\underset{\text{P-NHCHCO-X}}{\overset{\text{R}}{|}} + \underset{\text{H}_2\text{NCHCO-Y}}{\overset{\text{R'}}{|}} \xrightarrow{-\text{HX}} \underset{\text{P-NHCHCO-NHCHCO-Y}}{\overset{\text{R}\qquad\quad\text{R'}}{|\qquad\qquad|}}$$

$$\xrightarrow{-\text{P}} \underset{\text{H}_2\text{NCHCO-NHCHCO-Y}}{\overset{\text{R}\qquad\quad\text{R'}}{|\qquad\quad|}} + \underset{\text{P-NHCHCO-X}}{\overset{\text{R''}}{|}} \xrightarrow{-\text{HX}}$$

$$\underset{\text{P-NHCHCO-NHCHCO-NHCHCO-Y}}{\overset{\text{R''}\qquad\quad\text{R}\qquad\quad\text{R'}}{|\qquad\qquad|\qquad\qquad|}}$$
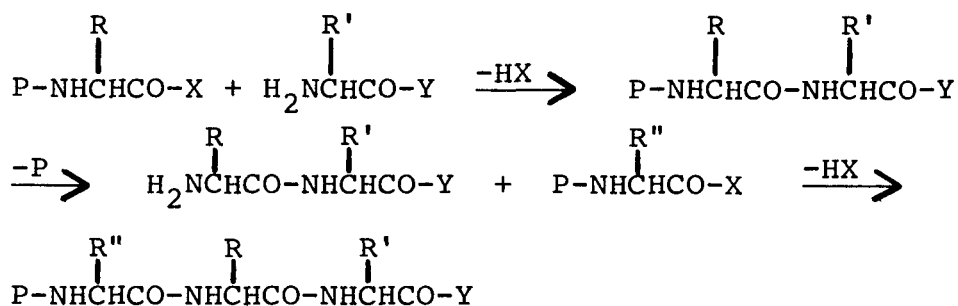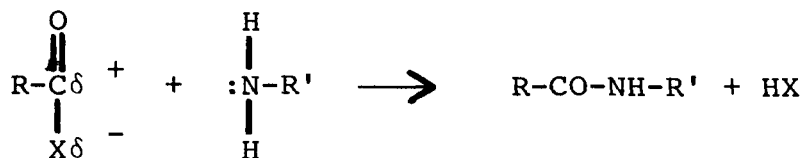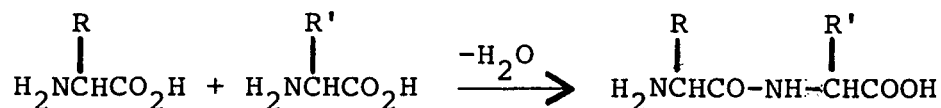
Whether a group is used for the protection of α-amino, α-carboxyl, or ω-groups, it must fulfill certain requirements. It must be possible to obtain the protected amino acid easily and in high yield. The protecting groups must not be removed or undergo unwanted side reactions during coupling reactions. The blocking groups must be selectively removable without affecting other protecting groups or functional groups in the growing peptide chain. For all practical purposes these conditions are not completely filled. Thus while the basic scheme of peptide synthesis is simple, the actual synthesis of peptides is far from straightforward.

One of the major problems which must be considered during a peptide synthesis is the possibility of racemization. The tendency of amino acids to racemize varies considerably

with the reaction conditions. Factors such as the presence
of base or salts, the polarity of the solvent system, the
temperature of the reaction, the substituents on nitrogen,
and the coupling method used will affect the amount of race-
mization. The racemization caused by the more important
coupling methods has been systematically investigated.[21,22,23,67]

Racemization under alkaline conditions is believed
to be the result of withdrawal of a proton from the chiral
carbon atom. The stability of the C--H bond is influenced
by the substituent on nitrogen. Acyl residues increase the
acidity of the proton via possible tautomeric forms. Urethan-
type protecting groups, however, stabilize the hydrogen atom
by resonance forms within the protecting groups.

$$
\begin{array}{ccc}
& R & \\
& | & \\
P\text{-}NH\text{-}C\text{-}CO\text{-}Y & \rightleftharpoons & P\text{-}NH\text{-}\overset{\displaystyle R}{\underset{\displaystyle -}{C}}\text{-}CO\text{-}Y \\
& | & \\
& H &
\end{array}
$$

$$
\begin{array}{ccc}
& R' & OH \quad R' \\
& | & | \quad\;\; | \\
R\text{-}CO\text{-}NH\text{-}C\text{-}CO\text{-}R'' & \rightleftharpoons & R\text{-}C\text{=}N\text{-}C\text{-}CO\text{-}R'' \\
& | & | \\
& H & H
\end{array}
$$

$$
\begin{array}{ccc}
O \quad R' & & O^- \quad R' \\
\| \quad\, | & & \;| \quad\;\; | \\
R\text{-}O\text{-}C\text{-}NH\text{-}C\text{-}CO\text{-}R'' & \rightleftharpoons & \overset{+}{R\text{-}O\text{=}C}\text{-}NH\text{-}C\text{-}CO\text{-}R'' \\
| & & | \\
H & & H
\end{array}
$$
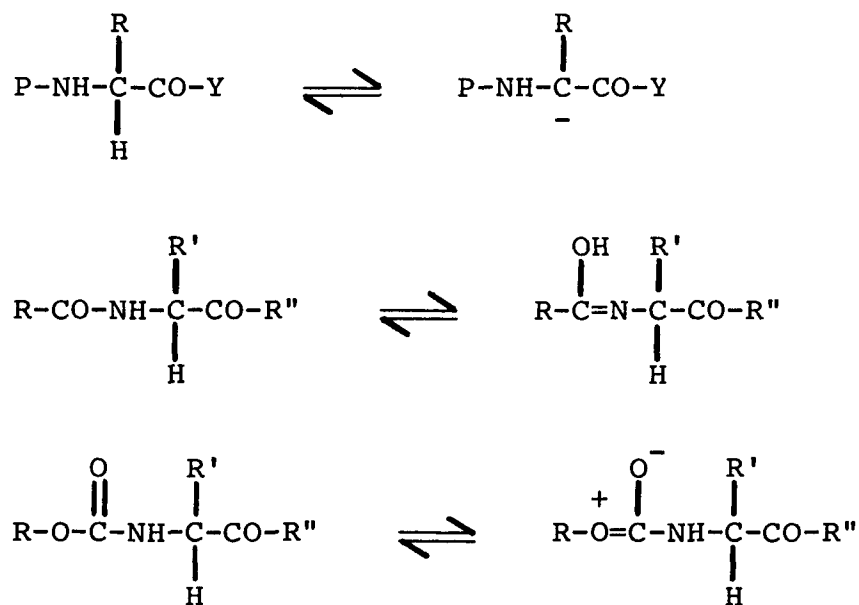
A second mechanism of racemization is the formation
of azlactones. This mechanism is possible with any acyl
amino acid or peptide. Urethan type groups inhibit azlactone

formation. From this discussion it is evident that the best method of synthesizing a peptide is the stepwise addition of amino acids starting with the protected C-terminal residue.

$$
\begin{array}{ccc}
\text{R} & \text{O} & \text{R}' \\
| & || & | \\
-\text{NH-CH-C-NH-CH-CO-X}
\end{array}
\quad \longrightarrow \quad
\begin{array}{c}
\text{R} \quad \text{N---CH-R}' \\
| \quad || \quad | \\
-\text{NH-CH-C} \quad \text{C=O} \\
\backslash \ / \\
\text{O}
\end{array}
$$
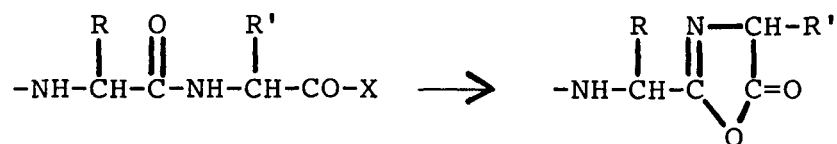
B. Preparation of N-Hexanoylglycyl-L-prolyl-5-aminovaleric Acid (19)

The initial preparation of 19 utilized the unorthodox method of constructing the chain by the stepwise addition of amino acids starting with the N-terminal residue. This approach could be successfully applied to the synthesis of this simple peptide (19) because racemization was not considered a problem with a system containing only one chiral atom. The only optically active amino acid in the chain is proline, and the chiral carbon of this amino acid is very resistant to racemization. Where large polypeptides are synthesized by the fragment method, the synthesis is frequently designed so that either proline or glycine is the C-terminal residue of as many fragments as possible.

Nitrogen protection was provided by the hexanoyl group on glycine. This gave the final peptide with the desired substituent on the N-terminal end of the chain and avoided the additional reactions which would have been required if one of the standard protecting groups had been used.

Three methods of preparing N-hexanoylglycine were investigated. A Schotten-Baumann reaction between hexanoyl chloride (10) and glycine (11) in aqueous base lead to the formation of N-hexanoylglycine (12) in 51.5% yield. The second approach involved hydrolysis of N-hexanoylglycine ethyl ester (15) by heating under reflux in a solution of 2N HCl and acetone. The low yield for this reaction (35%) probably reflects the poor choice of reaction conditions. Basic hydrolysis at ambient temperatures probably would have been a better choice. The ethyl ester was prepared in 61.1% yield from hexanoic acid (13) and ethyl glycinate hydrochloride (14) using phosphorous trichloride. This is the phosphazo method[24] of peptide synthesis. It is little used because it causes extensive racemization.

$$\text{Hex-Cl} \quad + \quad \text{Glycine} \quad \xrightarrow{\text{NaOH}} \quad \text{Hex-Gly-OH}$$
$$\underline{10} \qquad\qquad \underline{11} \qquad\qquad\qquad\qquad \underline{12}$$

$$\text{Hex-OH} \quad + \quad \text{H-Gly-OEt·HCl} \quad \xrightarrow{\text{PCl}_3} \quad \text{Hex-Gly-OEt} \quad \xrightarrow{\text{HCl}} \quad \underline{12}$$
$$\underline{13} \qquad\qquad \underline{14} \qquad\qquad\qquad\qquad \underline{15}$$
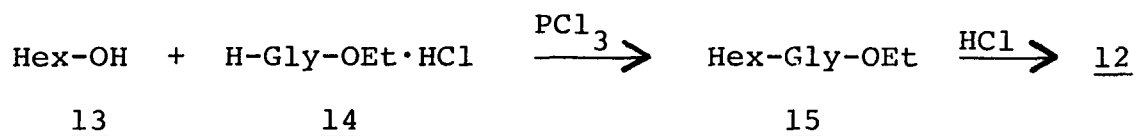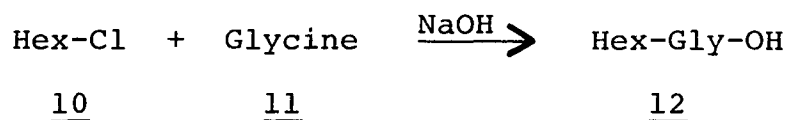
The third method of preparing compound 12 involved activation of the carboxyl group of 13 by formation of the mixed anhydride with ethyl chloroformate. The mixed anhydride method was reported independently by three groups of workers [25,26,27] in 1951. It involves reaction of the

carboxyl function with an alkyl chloroformate in an anhydrous organic solvent containing one equivalent of a tertiary base such as N-ethylmorpholine. The free amine is then added to the anhydride to give the peptide and carbon dioxide and alcohol as by-products (Scheme 1). Scheme 1 also indicates that the anhydride could be opened in a different manner to give urethanes, but this side reaction has only rarely been observed. The amount of racemization which occurs during a reaction using this method can be kept low by optimizing the reaction conditions, but even under the best conditions the complete absence of racemization cannot be guaranteed.

Protection of the carboxyl group of glycine was provided by salt formation. This method of carboxyl protection is the least elaborate approach, and is facile, for the salt need not be isolated. The free acid can be obtained by addition of mineral acid at the completion of the peptide synthesis. Application of this method for the preparation of N-hexanoylglycine (12) resulted in a 52.5% yield of the desired product. This method was clearly superior to the Schotten-Bauman or hydrolysis methods in terms of overall yield and convenience.

Hex-OH + Glycine $\xrightarrow{MA}$ Hex-Gly-OH

13   11   12
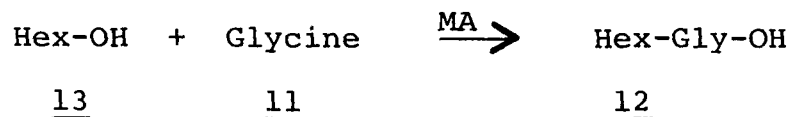
Activation by mixed anhydride formation and carboxyl protection by salt formation was also used for the condensation reactions of L-proline (16) and 5-aminovaleric acid (18).

$$\underset{\displaystyle P-NH-\overset{\displaystyle \overset{R}{|}}{C}H-CO_2H}{} \quad + \quad \underset{\displaystyle R'O-\overset{\displaystyle \overset{O}{\|}}{C}-Cl}{} \quad \longrightarrow$$

$$P-NH-\overset{R}{\underset{|}{C}}H-\overset{O}{\underset{\underset{a}{\|}}{C}}-O-\overset{O}{\underset{\underset{b}{\|}}{C}}-OR' \quad + \quad H_2N-\overset{R''}{\underset{|}{C}}H-CO-Y$$

Attack at a   Attack at b

$$P-NH-\overset{R}{\underset{|}{C}}H-CO-NH-\overset{R''}{\underset{|}{C}}H-CO-Y \qquad R'O-CO-NH-\overset{R''}{\underset{|}{C}}H-CO-Y$$

$$+$$

$$P-NH-\overset{R}{\underset{|}{C}}H-CO_2H$$

$$+ \quad CO_2 \quad + \quad R'OH$$
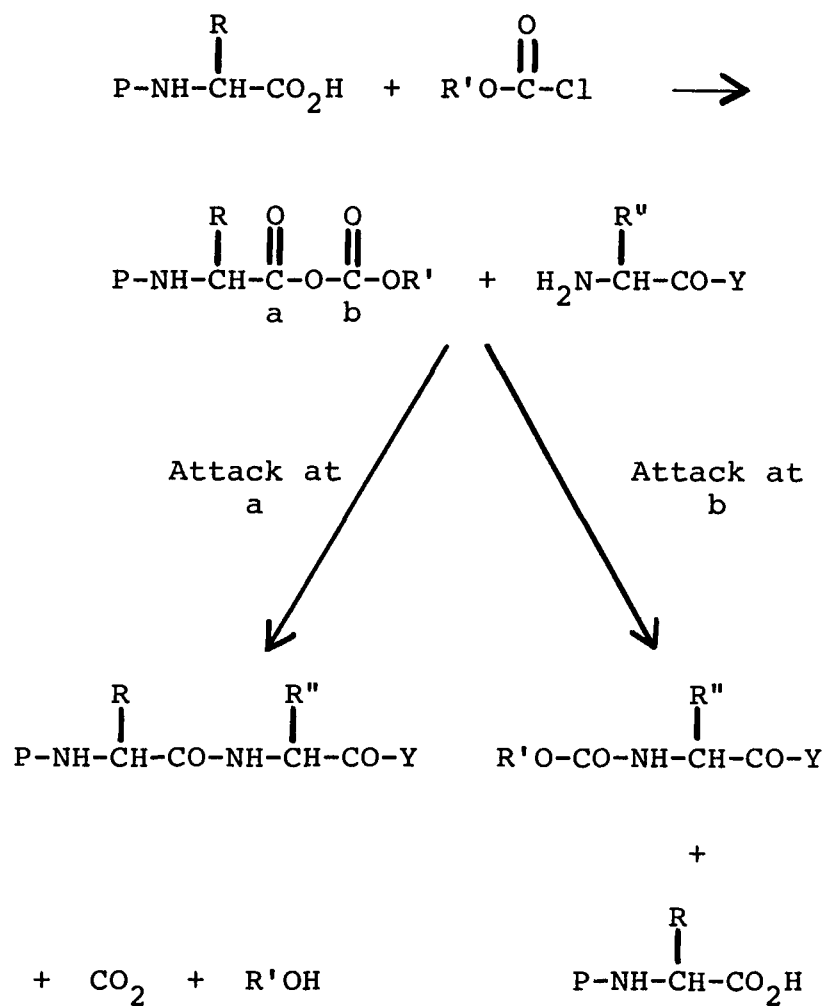
SCHEME 1

The anhydride of 12 was prepared by reaction with ethyl chloroformate and triethylamine in anhydrous benzene at -5°. L-Proline (16) was added as a solution in 2N NaOH. Following the workup, crude N-hexanoylglycyl-L-proline (17) was obtained as a waxy solid. This material could not be purified by recrystallization and it was used in this form in the next coupling reaction. The mixed anhydride of 17 was prepared by the procedure outlined above and 5-aminovaleric acid (18) was added as a solution in aqueous base. Workup afforded a 29.6% yield of N-hexanoylglycyl-L-prolyl-5-aminovaleric acid (19), identified by ir, nmr, and elemental analysis. This represents a 15.5% overall yield from hexanoic acid.

$$\text{Hex-Gly-OH} \quad + \quad \text{L-Proline} \quad \xrightarrow{\text{MA}} \quad \text{Hex-Gly-L-Pro-OH}$$
$$\underline{12} \qquad\qquad\qquad \underline{16} \qquad\qquad\qquad\qquad \underline{17}$$

$$\underline{17} + \text{5-Aminovaleric acid} \quad \xrightarrow{\text{MA}} \quad \text{Hex-Gly-L-Pro-5-Amv-OH}$$
$$\underline{18} \qquad\qquad\qquad\qquad\qquad \underline{19}$$
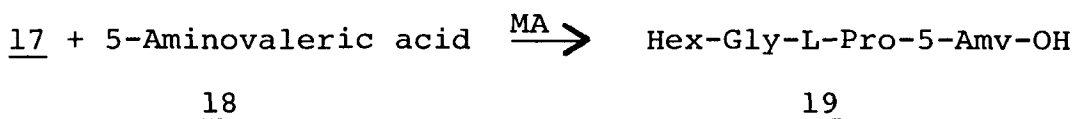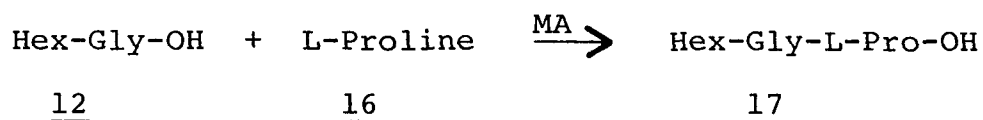
The fact that 17 could not be obtained in pure form represents the major drawback of using salt formation for carboxyl protection. Unless all reactions are quantitative the product will consist of a mixture of two acids. Generally, increasing the peptide chain by one amino acid will not change the solubility sufficiently to allow separation. In this case, however, addition of the 5-aminovaleric acid (18) did change the solubility enough to allow separation by recrystallization. When a mixture of two acids cannot be

separated, the matter is further complicated in the next
coupling reaction, for now two mixed anhydrides will be
formed, resulting in a myriad of products. This was aptly
demonstrated when the above reaction sequence was repeated
a second time. N-Hexanoylglycine (12) was activated as be-
fore and condensed with L-proline (16) in aqueous base. The
resulting crude product was activated and condensed with 5-
aminovaleric acid (18) in aqueous base, but did not give 19
as expected. Instead a solid identified as N-hexanoylglycyl-
5-aminovaleric acid (20) was isolated in 7.6% yield. There
was no evidence for the formation of 19 and crude N-hexanoyl-
glycyl-L-proline (17) was recovered.

Hex-Gly-OH + L-Proline $\xrightarrow{\text{MA}}$ Hex-Gly-L-Pro-OH

  12   16   17

                                        +

                                  Hex-Gly-OH

                                     12

17 + 12 $\xrightarrow[\text{5-Aminovaleric Acid}]{\text{MA}}$ Hex-Gly-5-Amv-OH

                                     20
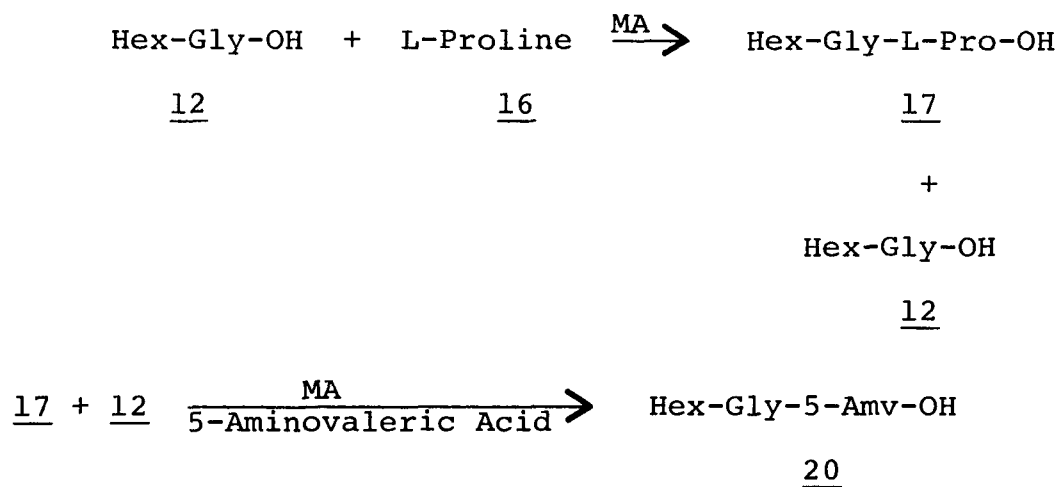
In the above reactions the amount of ethyl chloro-
formate to be used was determined by volume, not by weight.
The density of ethyl chloroformate found in the CRC Handbook[28]
was used to calculate the volume to be used. It was later
discovered that this density is incorrect, being high by
about 20%. Thus an insufficient amount of ethyl chloroformate

was being used, which means that there will be a large amount of unreacted N-hexanoylglycine (12) mixed with N-hexanoyl-glycyl-L-proline (17). When the mixture is activated for the next coupling reaction, two mixed anhydrides are formed. Condensation of the mixed anhydride of 12 with 5-aminovaleric acid (18) will then result in the formation of 20. Although the use of an insufficient amount of ethyl chloroformate was one reason for the formation of 20, it was felt that such problems would have been encountered anyway.

C. An Alternate Approach to N-Hexanoylglycyl-L-prolyl-5-aminovaleric Acid (19)

The results in the synthesis of 20 suggested that carboxyl protection by salt formation could not be used as a general method for preparing these peptides. The preparation of N-hexanoylglycyl-L-prolyl-5-aminovaleric acid (19) by the stepwise addition of amino acids to the ester of 5-amino-valeric acid, was next studied. Esterification of 5-amino-valeric acid was performed by using 2,2-dimethoxypropane and 12N HCl to give the hydrochloride (21) in 71% yield.

$$\text{5-Aminovaleric acid} \xrightarrow[\text{HCl}]{\text{2,2-DMP}} \text{H-5-Amv-OMe·HCl}$$

18   21
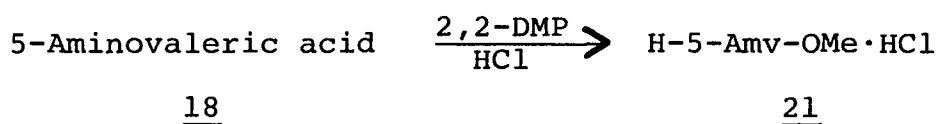
The condensation of 21 with L-proline (16) required the use of an amino protecting group for 16, and the benzyloxy-carbonyl group was chosen. The application of the benzyloxy-

carboxyl group for amine protection was introduced by Bergmann and Zervas[29] in 1932, marking the beginning of modern peptide chemistry. This is still the most important amino-protecting group because of the ease of synthesis of the N-protected amino acids, the nearly universal application in peptide synthesis, and the numerous, convenient ways for removing the protecting group.

The benzyloxycarbonylamino acids are obtained almost exclusively from benzyl chloroformate and the corresponding amino acid in aqueous alkaline solution[29] using Schotten-Baumann type conditions. Yields are generally high and the reaction proceeds without complicating side reactions. This group can also be used to protect certain amino acid side chains.[30] The only coupling method that may give rise to side reactions with benzyloxycarbonylamino acids is activation of the carboxyl by acid chloride formation, for this may give formation of N-carboxylic anhydrides. The benzyl-oxycarbonyl group can be removed by mild hydrogenation using palladium on carbon. Alternatively the group can be removed using a variety of acid and solvent combinations. The most important of these is hydrogen bromide in glacial acetic acid.

The reaction of benzyl chloroformate with L-proline (16) in aqueous NaOH gave a high yield of N-benzyloxycarbonyl-L-proline (22) as a viscous oil. This oil could not be crystallized, so a portion was converted to the dicyclohexyl-amine salt (23) for characterization. Both the oil and the salt were used for reactions but, unfortunately, no compara-

tive study was made. Usually the salt was the preferred
form because of the convenience in handling the material.

Cbo-Cl + L-Proline $\xrightarrow{\text{NaOH}}$ Cbo-L-Pro-OH $\xrightarrow{\text{DCHA}}$
                 16   22

Cbo-L-Pro-OH·DCHA

23
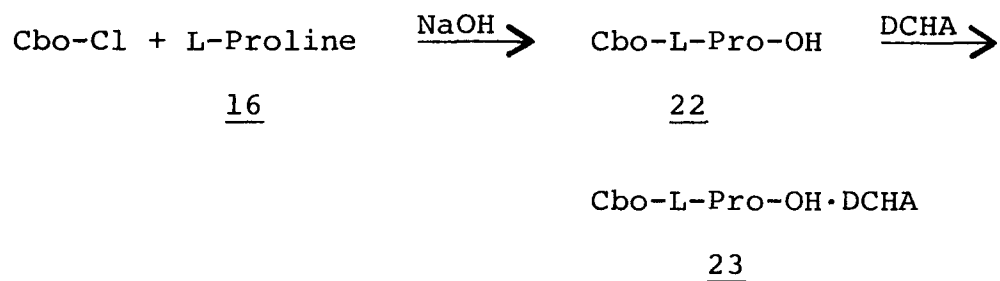
    Reactions leading to the preparation of 19 are repre-
sented in Scheme 2. Methyl 5-aminovalerate hydrochloride
(21) was coupled with 22 using the mixed anhydride method to
give a 79.5% yield of N-benzyloxycarbonyl-L-prolyl-5-amino-
valeric acid methyl ester (24) as a viscous oil. Identifi-
cation of 24 was made by the infrared (ir) and nuclear
magnetic resonance (nmr) spectra and TLC, which showed the
material to be homogeneous in three solvent systems. Ele-
mental analyses were not obtained and the material was used
directly in the following reactions.

    The benzyloxycarbonyl group was removed by the hydrog-
enation of 24 over 10% palladium on carbon in methanol. The
reduction was carried out at atmospheric pressure and was
monitored by the evolution of carbon dioxide using a saturated
solution of barium hydroxide. Workup gave a 90.5% yield of
L-prolyl-5-aminovaleric acid methyl ester (25) as an oil.
A sample of the oil was converted to the hydrochloride (26),
also giving an oil. Both 25 and 26 were identified by their
ir and nmr spectra and used without obtaining elemental
analyses.

Cbo-L-Pro-OH + H-5-Amv-OMe·HCl $\xrightarrow{\text{MA}}$

$\underline{22}$ $\underline{21}$

Cbo-L-Pro-5-Amv-OMe $\xrightarrow{\text{H}_2\text{-Pd/C}}$ H-L-Pro-5-Amv-OMe

$\underline{24}$ $\underline{25}$

$\underline{25}$ + Hex-Gly-OH $\xrightarrow{\text{MA}}$ Hex-Gly-L-Pro-5-Amv-OMe

      $\underline{12}$ $\underline{27}$

$\xrightarrow{\text{HCl}}$ Hex-Gly-L-Pro-5-Amv-OH

                      $\underline{19}$
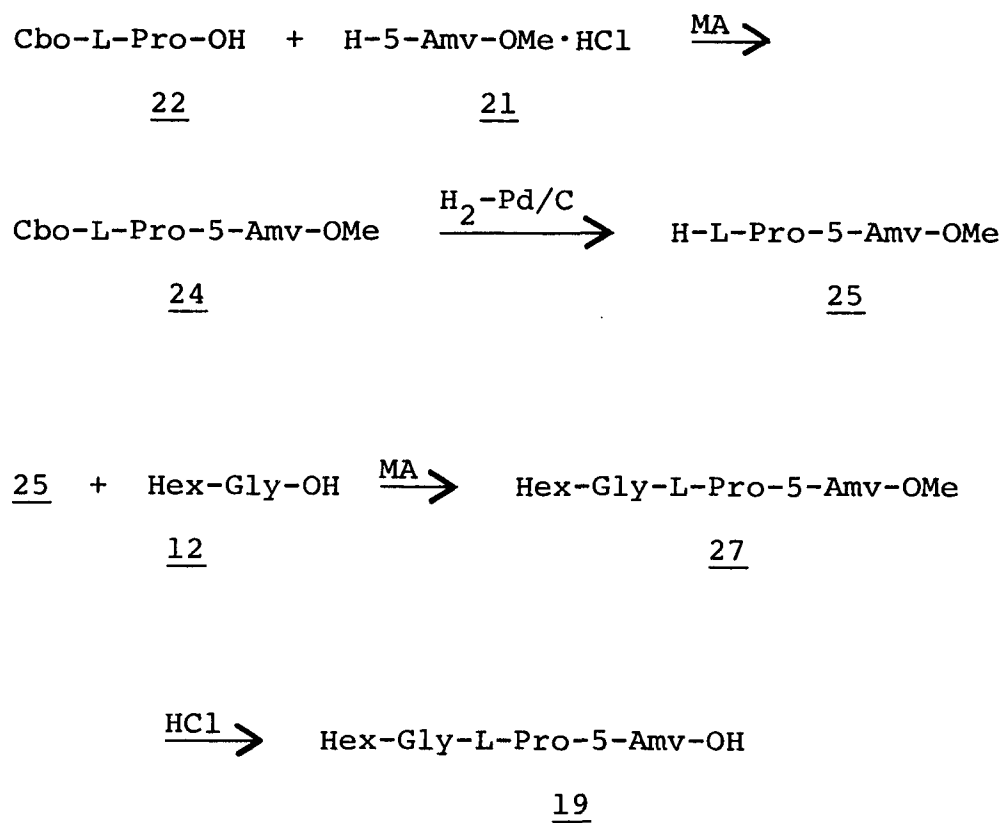
SCHEME 2

Condensation of 25 with N-hexanoylglycine (12) using the mixed anhydride method proceeded without difficulty to provide N-hexanoylglycyl-L-prolyl-5-aminovaleric acid methyl ester (27) in 53% yield. This yield was lower than expected and may result from the use of crude 25 which was prepared from crude N-benzyloxycarbonyl-L-prolyl-5-aminovaleric acid methyl ester (24). Compound 27 was obtained as a crystalline solid which was readily purified by recrystallization from hexane-cyclohexane (50:50).

Completion of the synthesis was accomplished by hydrolysis of the ester in a refluxing solution of acetone and 2N HCl. This provided N-hexanoylglycyl-L-prolyl-5-amino-valeric acid (19) in only 24% yield. This low yield may be the result of the choice of incorrect reaction conditions. The hydrolysis of methyl and ethyl peptide esters normally is performed by alkaline hydrolysis using a slight excess of base at room temperatures. Where acid hydrolysis is used the reaction conditions usually require reaction times of 0.5-1.5 hr at room temperatures. It is reasonable to assume that some cleavage of the amide bonds occurred under the conditions used.

This approach for the synthesis of compound 19 represents a 9.2% overall yield from N-benzyloxycarbonyl-L-proline (22). In terms of yield this method is inferior to the method using salt formation as carboxyl protection (9.2% vs. 15.5%). However, the ease of isolation and purification of the inter-mediates by simply washing and extraction gave the final

product in a higher state of purity as evidenced by the
melting points (122.5-124.5° vs. 111-114°).

D. **Preparation of N-Pentanoylglycyl-L-prolyl-5-aminovaleric**
   Acid Methyl Ester (30)

The preparation of the peptide described in the pre-
ceeding sections represents an analogue of a prostaglandin
in which the N-terminal chain contains one carbon more than
the corresponding chain on the central ring of the natural
prostaglandins. Thus, compounds containing a pentanoic acid
on the N-terminal end of the peptide chain were also synthe-
sized.

N-Pentanoylglycine (29) was prepared by the two
methods analogous to those used to prepare N-hexanoylglycine
(12). The first approach was a Schotten-Baumann reaction
between pentanoyl chloride (28) and glycine in aqueous base.
The yield for this reaction was only 12%. The low yield of
recovery resulted from the high water solubility of 29 and a
large portion of the material was lost in the aqueous layer.
This was totally unexpected since compound 12 is water insolu-
ble. This observation made the synthesis of a pentanoic acid
series all the more interesting. If the difference of one
methylene group significantly changes the solubility of the
final product, the biological activity might also be signifi-
cantly affected.

$$\text{Pen-Cl} + \text{Glycine} \xrightarrow{\text{NaOH}} \text{Pen-Gly-OH}$$
$$\underline{28} \qquad\qquad \underline{11} \qquad\qquad\qquad \underline{29}$$
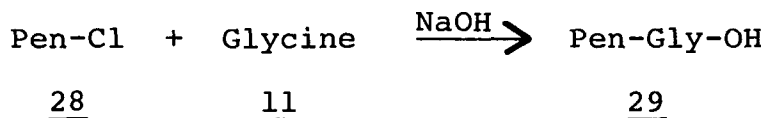
The second approach to the synthesis of 29 involved activation of the carboxyl group by formation of the mixed anhydride and coupling with glycine (11) protected as it's sodium salt. Extraction of the aqueous layer on a liquid-liquid extractor for 48 hr gave a 64% yield of 29.

$$\text{Pen-OH} + \text{H-Gly-OH} \xrightarrow{\text{MA}} \text{Pen-Gly-OH}$$
$$\underline{11} \qquad\qquad \underline{29}$$
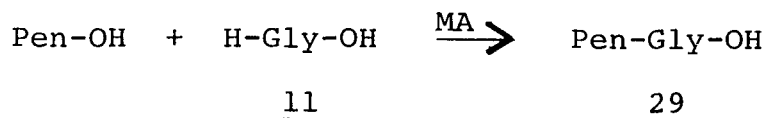
Compound 29 was then condensed with crude L-prolyl-5-aminovaleric acid methyl ester (25) via it's mixed anhydride to provide N-pentanoylglycyl-L-prolyl-5-aminovaleric acid methyl ester (30) in the very low yield of 6%. The reasons for this low yield are not clear. The water solubility of 30 was checked and, although it was shown to be slightly water soluble, the amount was not great enough to cause such a large loss of material.

$$\text{Pen-Gly-OH} + \text{H-L-Pro-5-Amv-OMe} \xrightarrow{\text{MA}} \text{Pen-Gly-L-Pro-5-Amv-OMe}$$
$$\underline{29} \qquad\qquad \underline{25} \qquad\qquad\qquad\qquad \underline{30}$$
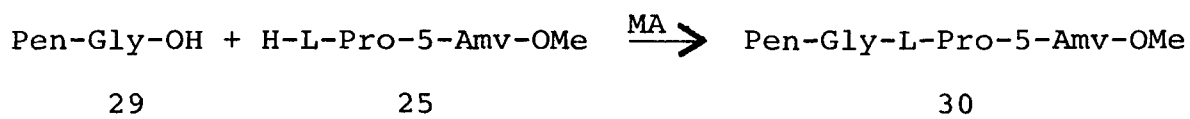
Because of the low yield obtained, the condensation of 29 with 25 was repeated using N,N'-dicyclohexylcarbodiimide for carboxyl activation. This method of peptide synthesis was introduced in 1955 by Sheehan and Hess[31] and in a short time it became one of the most important coupling methods. The activation is initiated by addition of the carboxyl group to the carbodiimide. The course of the mechanism

from this point is still under debate. The initial addition product can react directly with the amine component to form the peptide bond and dicyclohexylurea, or the addition product can react with a second molecule of acid to form the symmetrical anhydride which can then react with the amine component to product the peptide.

$$R\text{-}CO_2H \ + \ \underset{\underset{N\text{-}R'}{\|}}{\overset{\overset{N\text{-}R'}{\|}}{C}} \ \longrightarrow \ R\text{-}CO\text{-}O\text{-}\underset{\underset{NH\text{-}R'}{|}}{\overset{\overset{N\text{-}R'}{\|}}{C}}$$

$$R\text{-}CO\text{-}O\text{-}\underset{\underset{NH\text{-}R'}{|}}{\overset{\overset{N\text{-}R'}{\|}}{C}} \ + \ H_2N\text{-}R'' \ \longrightarrow \ R\text{-}CO\text{-}NH\text{-}R'' \ + \ R'NH\overset{\overset{O}{\|}}{C}NH\text{-}R'$$

$$R\text{-}CO\text{-}O\text{-}\underset{\underset{NH\text{-}R'}{|}}{\overset{\overset{N\text{-}R'}{\|}}{C}} \ + \ R\text{-}CO_2H \ \longrightarrow \ R\text{-}CO\text{-}O\text{-}CO\text{-}R \ + \ R'NH\overset{\overset{O}{\|}}{C}NH\text{-}R'$$

$$R\text{-}CO\text{-}O\text{-}CO\text{-}R \ + \ H_2NR'' \ \longrightarrow \ R\text{-}CO\text{-}NH\text{-}R'' \ + \ R\text{-}CO_2H$$
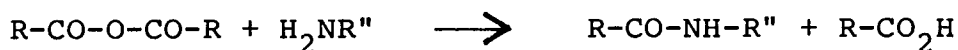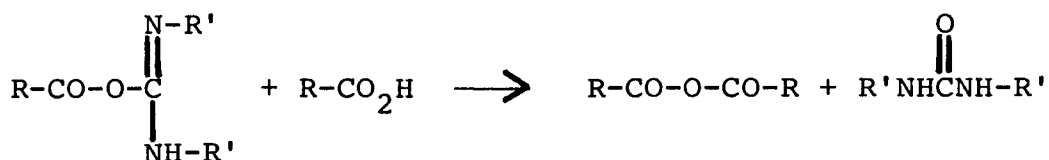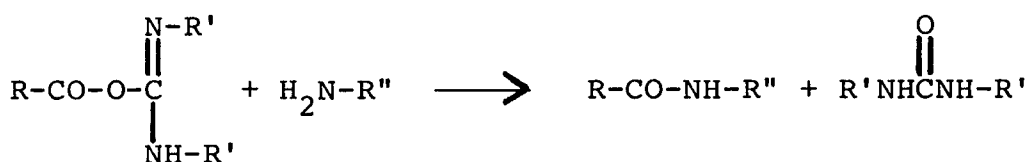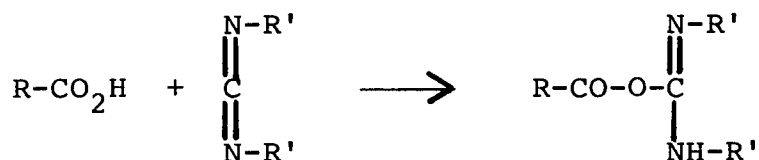
The great advantage of this method is the simplicity of the preparative procedure and the almost complete absence of racemization when the reaction is carried out under optimum conditions. The by-product of the reaction is di-cyclohexylurea, which is very insoluble in most organic solvents, and therefore can be easily removed by filtration.

When this method was applied to the synthesis of N-pentanoyl-glycyl-L-prolyl-5-aminovaleric acid methyl ester (30), the product was obtained in the moderate yield of 44.5%. This represents a 32% overall yield from N-benzyloxycarbonyl-L-proline (22).

Pen-Gly-OH + H-L-Pro-5-Amv-OMe $\xrightarrow{DCC}$ Pen-Gly-L-Pro-5-Amv-OMe

29   25   30
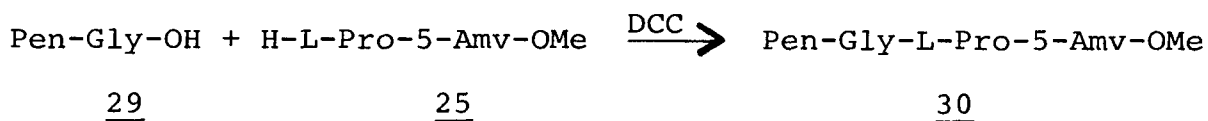
It has been observed[32] that prostaglandin methyl esters show nearly the same activity as the acids. Therefore, compound 30 was not hydrolyzed to the acid before being submitted for biological testing.

E. Preparation of N-Hexanoylglycyl-L-prolylglycylglycine (36)

Early in this research it appeared probable that additional functional groups on the two chains attached to proline would be necessary to provide a rigid conformation and supply additional possibilities for hydrogen bonding between the two chains. Thus, a series of tetrapeptides, in which the 5-aminovaleric acid residue was replaced by a dipeptide, were prepared. The initial system was kept as simple as possible, and glycylglycine was the dipeptide chosen.

The reactions to be discussed in this section are illustrated in Scheme 3. The approach taken in this synthesis was the condensation of N-hexanoylglycyl-L-proline

L-Proline $\xrightarrow[\text{HCl}]{\text{2,2-DMP}}$ H-L-Pro-OMe

16   31

31 + Hex-Gly-OH $\xrightarrow{\text{MA}}$ Hex-Gly-L-Pro-OMe

12   32

32 $\xrightarrow[\text{or NaOH}]{\text{HCl}}$ Hex-Gly-L-Pro-OH

33

33 + H-Gly-Gly-OBzl·p-TsOH $\xrightarrow[\text{DCC}]{\text{MA or}}$ Hex-Gly-L-Pro-Gly-Gly-OBzl

34   35

35 $\xrightarrow{\text{H}_2\text{-Pd/C}}$ Hex-Gly-L-Pro-Gly-Gly-OH

36
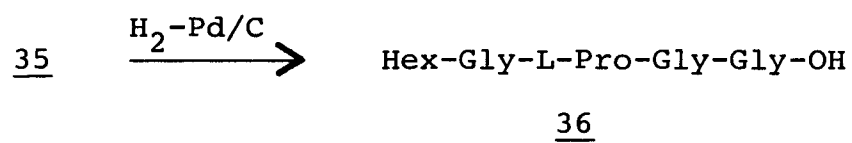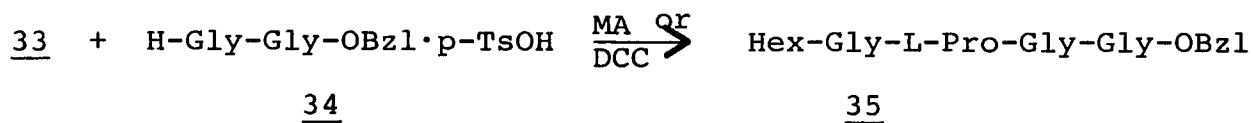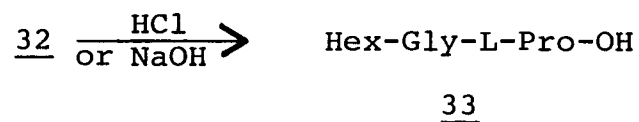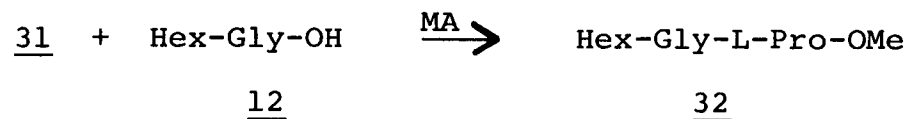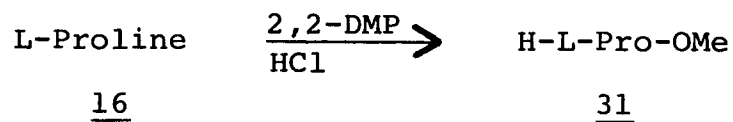
SCHEME 3

(33) with glycylglycine benzyl ester p-toluenesulfonate (34).
Glycylglycine is commercially available and was converted to
the benzyl ester p-toluenesulfonate by Dr. Richard Davenport
of this laboratory.[18]

Synthesis of 33 required the use of L-proline methyl
ester (31). This was obtained as an oil using 2,2-dimethoxy-
propane as in the preparation of methyl 5-aminovalerate
hydrochloride (21). The yield was only moderate (44.5%).
This material was not used as the hydrochloride but was con-
verted to the free ester. If stored for long periods at
room temperatures, a crystalline precipitate (diketopiper-
azine) formed. The oil was, however, unchanged after several
months storage in the freezer.

The carboxyl group of N-hexanoylglycine (12) was
activated via mixed anhydride formation and condensed with
31 to provide a 69% yield of N-hexanoylglycyl-L-proline
methyl ester (32) as a viscous red oil. Identification
was made on the basis of the ir and nmr spectra. Hydrolysis
of 32 to the acid was accomplished by two different methods.
Acidic hydrolysis by refluxing in a solution of 2N HCl and
acetone gave a 22% yield of 33. This is comparable to the
yields observed for the acidic hydrolysis of N-hexanoylglycine
ethyl ester (15) and N-hexanoylglycyl-L-prolyl-5-aminovaleric
acid methyl ester (27) to N-hexanoylglycine (12) and N-hexa-
noylglycyl-L-prolyl-5-aminovaleric acid (19), respectively.

Basic hydrolysis in 1N NaOH for 5 min at 20° gave 33 in 58%
yield. These findings support the suggestion made earlier

that acid hydrolysis results in cleavage of amide bonds.

The coupling of 33 with glycylglycine benzyl ester p-toluenesulfonate (34) was accomplished both with the mixed anhydride procedure and with N,N'-dicyclohexylcarbodiimide. The reaction proceeded without difficulty in each case to give N-hexanoylglycyl-L-prolylglycylglycine benzyl ester (35) in high yield and purity. The mixed anhydride method gave a 90% yield while the N,N'-dicyclohexylcarbodiimide method gave an 80% yield.

Removal of the benzyl ester was accomplished in 63.5% yield by hydrogenation over 10% palladium on carbon at 60 psi to give N-hexanoylglycyl-L-prolylglycylglycine (36) in a 23% overall yield from L-proline methyl ester (31).

F. Synthesis of A FSH/LH-RF Analog

The natural follicle stimulating hormone/luteinizing hormone releasing factor (FSH/LH-RF) is known[12] to have a terminal carboxamide function. To test this series for in-hibition of FSH and LH release the terminal carboxyl group of 36 was converted to the amide. Ammonolysis of the benzyl ester (35) in methanol saturated with ammonia gave N-hexanoyl-glycyl-L-prolylglycylglycinamide (37) in 68.4% yield or 24.5% overall yield from L-proline methyl ester (31).

$$\text{Hex-Gly-L-Pro-Gly-Gly-OBzl} \xrightarrow{\text{NH}_3} \text{Hex-Gly-L-Pro-Gly-Gly-NH}_2$$

<div align="center">
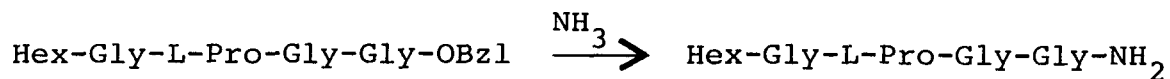
35   37

</div>

G. Preparation of N-Hexanoylglycyl-L-prolyl-L-leucyl-L-
    leucine (56)

   The conformation of proteins is determined to a large

extent by the constituent amino acids. It was felt that

tetrapeptides containing residues other than glycine would

provide a more rigid conformation because of the increased

bulk of the side chains. In addition, the types of residues

in the peptide will determine the properties (i.e., the

acidic or basic, hydrophilic or hydrophobic, aromatic or

aliphatic nature of the amino acids).

   The decision to incorporate leucine residues into

the proposed tetrapeptides was based on an examination of

the structures of a number of biologically active cyclic

peptides. Many cyclic proline-containing peptides also

contain hydrophobic amino acid residues such as leucine and

valine. Hassall and Thomas have published a short review on

the conformation of cyclic peptides.[33] Leucine and valine

have a large steric bulk and may be important in holding the

peptide in a rigid conformation. Based on these observations

the synthesis of N-hexanoylglycyl-L-prolyl-L-leucyl-L-leucine

(56) was undertaken.

   The initial synthetic approach was to protect the

carboxyl group as the methyl ester and use the benzyloxy-

carbonyl group for nitrogen protection. Condensation of

N-benzyloxycarbonyl-L-leucine (38) with L-leucine methyl

ester hydrochloride (39) using N,N'-dicyclohexylcarbodiimide

gave a 42.5% yield of N-benzyloxycarbonyl-L-leucyl-L-leucine

methyl ester (<u>40</u>). The yield could be improved by coupling
using the mixed anhydride to provide a 73.5% yield of <u>40</u>.

$$Cbo-L-Leu-OH \ + \ H-L-Leu-OMe \cdot HCl \ \xrightarrow[\text{or MA}]{\text{DCC}} \ Cbo-L-Leu-L-Leu-OMe$$

<u>38</u>   <u>39</u>   <u>40</u>
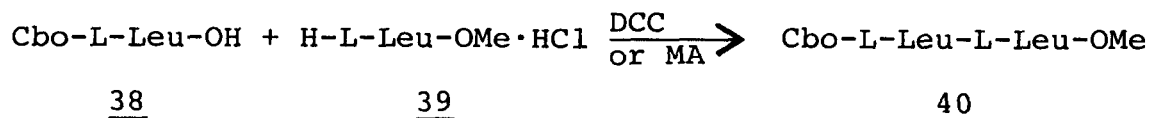
    Attempted removal of the benzyloxycarbonyl group met
with failure. Hydrolysis using 30% HBr in glacial acetic
acid resulted in cleavage of the methyl ester as shown by the
nmr spectrum. Apparently traces of water present in the
acid caused hydrolysis. Attempted removal of the benzyloxy-
carbonyl group by hydrogenation over 10% palladium on carbon
gave the diketopiperazine <u>42</u> rather than L-leucyl-L-leucine
methyl ester (<u>41</u>). The ease with which <u>41</u> cyclized to <u>42</u> is
surprising in view of the steric bulk of the leucine resi-
dues.

$$Cbo-L-Leu-L-Leu-OMe \ \xrightarrow{\text{H}_2\text{-Pd/C}}$$

<u>40</u>

[H-L-Leu-L-Leu-OMe] $\longrightarrow$

<u>41</u>

<u>42</u>
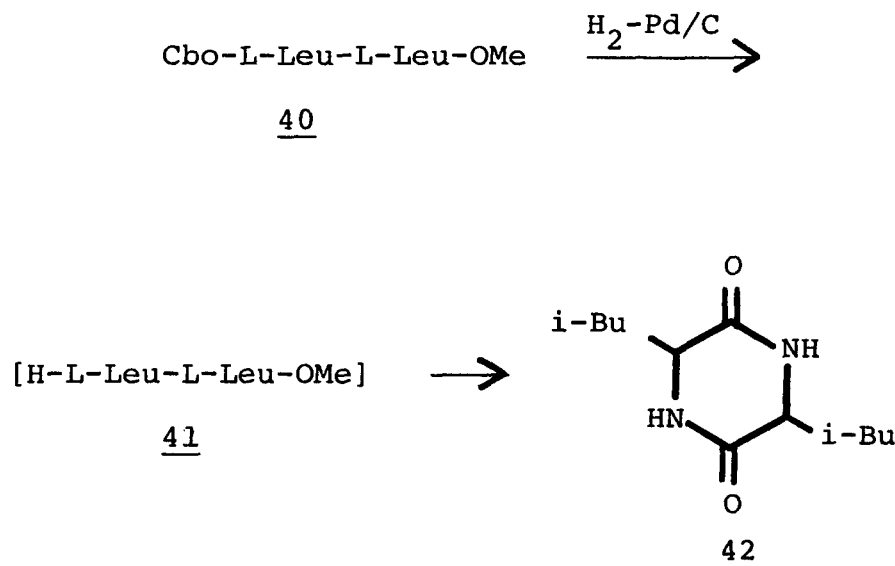
It became apparent that a carboxyl protecting group having a high degree of steric hindrance was necessary to prevent cyclization of the dipeptide ester to diketopiperazine. The introduction of t-butyl esters to peptide synthesis was an important breakthrough[34] because they prevent this undesirable side reaction and because they are selectively removable in the presence of most protecting groups. These esters are very readily hydrolyzed under mildly acidic conditions which leave the methyl and ethyl esters and the benzyloxycarbonyl group intact. The benzyloxycarbonyl group can be removed by catalytic hydrogenation without affecting the t-butyl ester. The free t-butyl esters of most amino acids are stable and are not subject to self-condensation.

The esterification of L-leucine (43) with isobutylene was carried out according to the procedure of Roeske[35] to give a 74% yield of L-leucine t-butyl ester as the phosphite salt (44).

$$\text{L-Leucine} + CH_2=C(CH_3)_2 \xrightarrow{H^+} \text{H-L-Leu-O-t-Bu} \cdot H_3PO_3$$

43   44
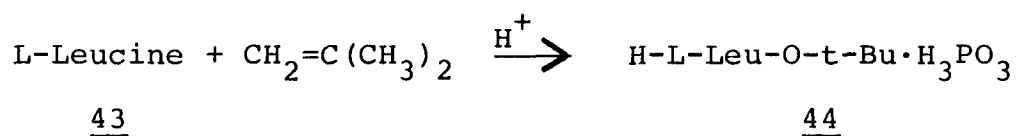
Condensation of 44 with N-benzyloxycarbonyl-L-leucine (38) was accomplished by two different methods. Use of the mixed anhydride method provided a 62.5% yield of N-benzyloxycarbonyl-L-leucyl-L-leucine t-butyl ester (45). The second coupling method involved the use of 1-hydroxybenzotriazole as a cocatalyst[45] to the N,N'-dicyclohexylcarbodiimide

reaction. The effectiveness of this cocatalyst was studied extensively by König and Geiger[36] and it was found to reduce racemization and increase the yields of N,N'-dicyclohexyl-carbodiimide coupling reactions. Additives to the DCC reaction are usually nucleophilic hydroxy compounds which react with the activated carboxyl group forming highly active esters. These esters, in turn, react rapidly with the amino component. Because of the fast formation and consumption of the active esters, racemization is reduced and yields are improved. However, the use of this method for the preparation of 45 gave only a 51% yield as compared to the 62.5% yield of 45 obtained using the mixed anhydride.

$$\text{Cbo-L-Leu-OH} + \text{H-L-Leu-O-t-Bu} \cdot \text{H}_3\text{PO}_3 \xrightarrow[\text{or DCC}]{\text{MA}}$$

38   44

Cbo-L-Leu-L-Leu-O-t-Bu

45

$$\text{R-CO}_2\text{H} + \begin{array}{c} \text{N-R'} \\ \| \\ \text{C} \\ \| \\ \text{N-R'} \end{array} \longrightarrow \begin{array}{c} \text{N-R'} \\ \| \\ \text{R-CO-O-C} \\ | \\ \text{NH-R'} \end{array} \longrightarrow$$

$$+ \text{H}_2\text{N-R''} \longrightarrow \text{R-CO-NH-R''}$$
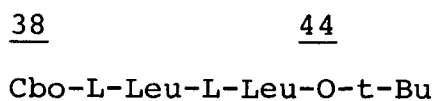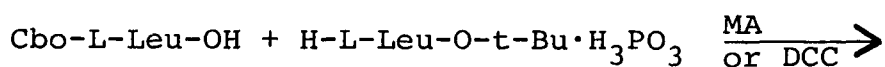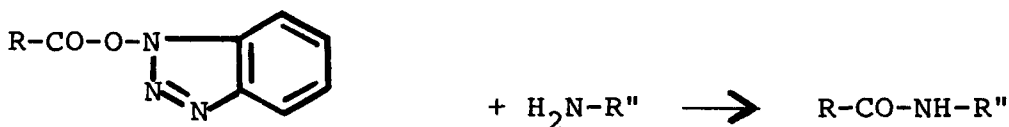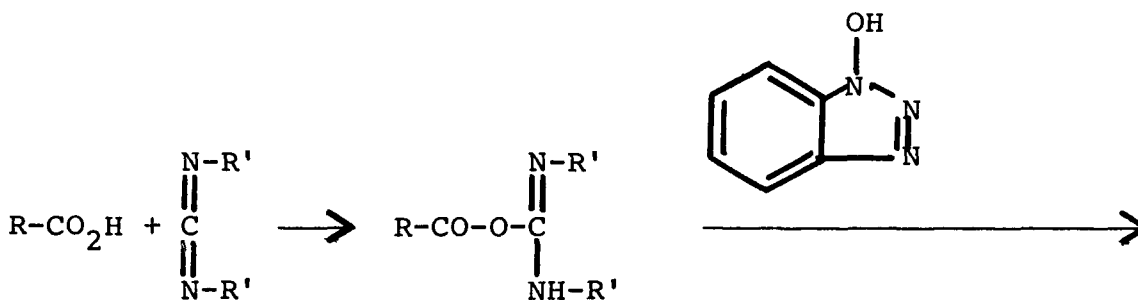
Removal of the benzyloxycarbonyl group from <u>45</u> by

catalytic hydrogenation over 10% palladium on carbon gave L-

leucyl-L-leucine t-butyl ester (<u>46</u>) in the high yield of 89%.

An attempt was then made to couple <u>46</u> with N-benzyloxycarbonyl-

L-proline as the dicyclohexylamine salt (<u>23</u>). It has been

shown by Schröder and coworkers[37] that dicyclohexylamine

salts of protected amino acids can be used directly in

coupling reactions without prior conversion to the free acid.

This method was reported to work with both N,N'-dicyclohexyl-

carbodiimide and the mixed anhydride procedures to give high

yields of products. They did not report the formation of

products resulting from condensation with dicyclohexylamine,

even though it is a secondary amine. Presumably this is due

to the steric hindrance of the amino nitrogen.

$$\text{Cbo-L-Leu-L-Leu-O-t-Bu} \xrightarrow{\text{H}_2\text{-Pd/C}} \text{H-L-Leu-L-Leu-O-t-Bu}$$

<u>45</u>   <u>46</u>
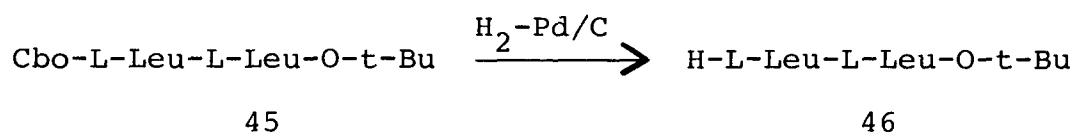
When this procedure was applied to the preparation

of N-benzyloxycarbonyl-L-prolyl-L-leucyl-L-leucine t-butyl

ester (<u>47</u>) only N-ethoxycarbonyl-L-leucyl-L-leucine t-butyl

ester (<u>48</u>) was isolated, in 66% yield. Formation of this

product can be explained in two ways. First, it can be

formed from the intermediate mixed anhydride (<u>49</u>) by reaction

of the free amine at the wrong carbonyl carbon. This reac-

tion was discussed earlier in this section as an uncommon

side reaction of the mixed anhydride method and is shown in

Scheme 1. The second explanation is that the ethyl chloro-
formate failed to react with N-benzyloxycarbonyl-L-proline
dicyclohexylamine salt (23). Normally, the reaction of
alkyl chloroformates with the carboxyl function goes to
completion, but the dicyclohexylamine salts are very insol-
uble in most solvents. This insolubility could prevent the
formation of the mixed anhydride. If this were the case the
unreacted ethyl chloroformate would be able to react with
L-leucyl-L-leucine t-butyl ester (46) to form the urethan 48.

Cbo-L-Pro-OH·DCHA + H-L-Leu-L-Leu-O-t-Bu $\xrightarrow{\text{MA}}$

23   46

$$\downarrow \text{EtO-}\overset{\overset{\displaystyle O}{\|}}{\text{C}}\text{-Cl}$$

Cbo-L-Pro-L-Leu-L-Leu-O-t-Bu

47

Cbo-L-Pro-O-$\overset{\overset{\displaystyle O}{\|}}{\text{C}}$-OEt $\xrightarrow{\text{:46}}$ EtO-$\overset{\overset{\displaystyle O}{\|}}{\text{C}}$-L-Leu-L-Leu-O-t-Bu

49   48

EtO-$\overset{\overset{\displaystyle O}{\|}}{\text{C}}$-Cl + H-L-Leu-L-Leu-O-t-Bu $\rightarrow$ EtO-$\overset{\overset{\displaystyle O}{\|}}{\text{C}}$-L-Leu-L-Leu-O-t-Bu

46   48
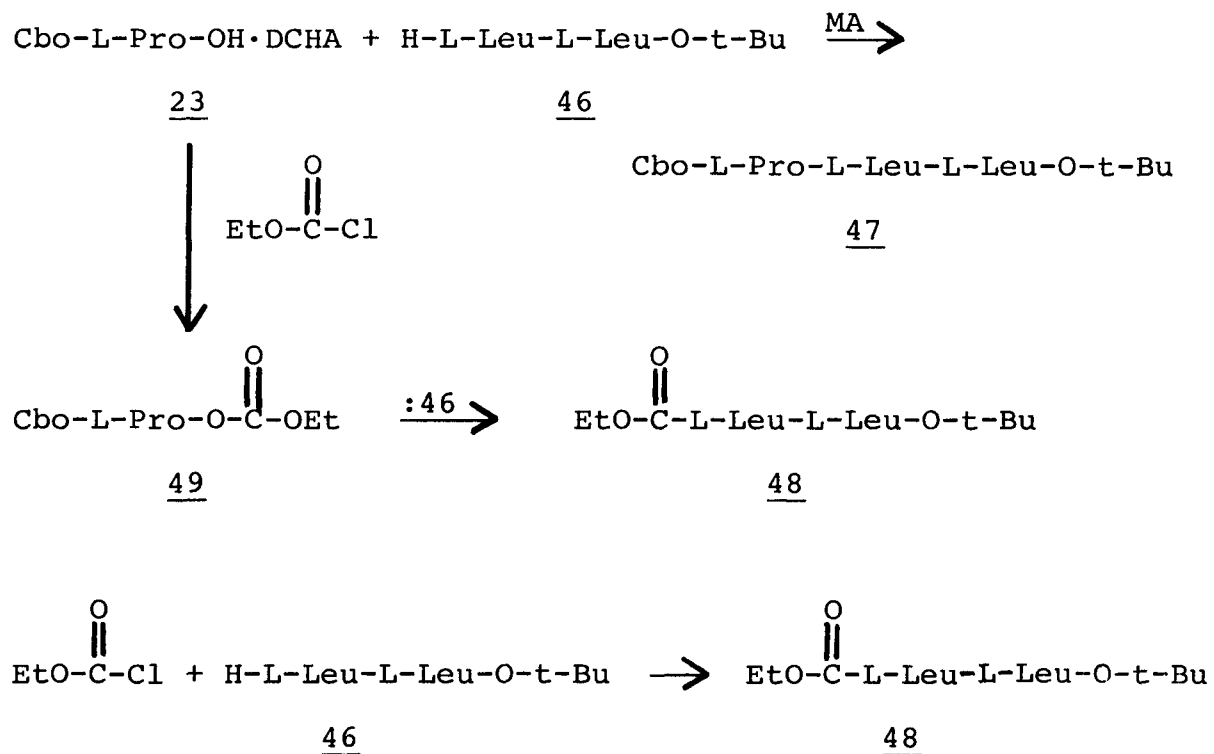
In order to determine the mechanism whereby 48 is
formed, a second experiment was performed in which N-benzyl-
oxycarbonyl-L-proline (22) was prepared from the dicyclohexyl-
amine salt (23) and then coupled with 46 via the mixed

anhydride. Two compounds were obtained and were separated by recrystallization and identified by TLC comparison with authentic samples. N-Ethoxycarbonyl-L-leucyl-leucine t-butyl ester (48) was isolated in 29% yield while a 54.2% yield of N-benzyloxycarbonyl-L-prolyl-L-leucyl-L-leucine t-butyl ester (47) was observed. Although the yield of the urethan (38) was much lower in this experiment, the yield was sufficient to show that the insolubility of the dicyclohexylamine salt was not the only factor contributing to urethan formation. The steric interaction of the crowded amine of leucine with the carboxyl of the secondary amino acid, proline, is so severe that the mixed anhydride 49 undergoes reaction at the carbonate carbonyl to give the urethan (48) rather than the tripeptide, 47.

It became apparent that an alternate coupling method was needed. The p-nitrophenyl ester method[38] was chosen because this procedure gives few side reactions, the high yield, and a minimum of racemization when the reaction is run under optimum conditions. It has been found[39] that the aminolysis is catalyzed by the addition of imidazole and this technique was used in this work in all reactions with p-nitrophenyl esters.

The condensation of N-benzyloxycarbonyl-L-proline (22) with p-nitrophenol using N,N'-dicyclohexylcarbodiimide in tetrahydrofuran as the solvent gave a 62% yield of N-benzyloxycarbonyl-L-proline p-nitrophenyl ester (50). It has been claimed[40] that the use of pyridine as solvent in-

creases the yield for this reaction. When the reaction was
repeated using pyridine as solvent the yield of 50 was 63%.
Solvent, therefore, made very little difference in this case.

Cbo-L-Pro-OH + HO—⟨benzene ring⟩—NO$_2$ —DCC→ Cbo-L-Pro-OPhNO$_2$

22   50
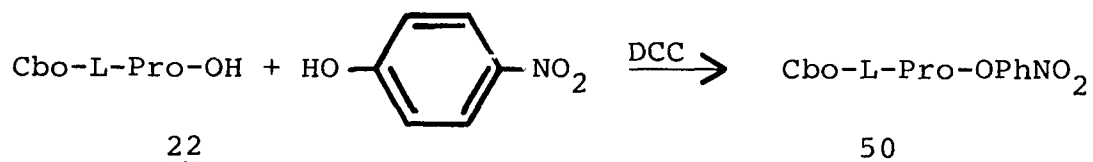
Compound 50 was condensed with L-leucyl-L-leucine t-
butyl ester (46) to provide N-benzyloxycarbonyl-L-prolyl-L-
leucyl-L-leucine t-butyl ester (47). Peptide synthesis with
p-nitrophenyl esters can be carried out under a wide variety
of reaction conditions. A short series of experiments were
performed in order to determine the reaction conditions
which would maximize the yields. Tetrahydrofuran was the
solvent in all cases and free esters were always used to
avoid the presence of salts. Two equivalents of imidazole
were added in each case. The variables in these experiments
were reaction time and reaction temperature. In the first
experiment the reaction was allowed to proceed for 24 hr at
20° to give a 76% yield of 47. In the second experiment
the temperature was maintained at 20° but the reaction time
was extended to 72 hr to increase the yield to 86.5%. Finally,
the temperature of the reaction was increased to 50° for a
24 hr period, giving a 90.5% yield of 47. Thus, it appears
that temperatures slightly above room temperatures provide
maximum yields. Therefore, in all future syntheses with p-
nitrophenyl esters, the reaction conditions were 50-55° for

at least 24 hr.

Cbo-L-Pro-OPhNO$_2$ +   H-L-Leu-L-Leu-O-t-Bu

<u>50</u>   <u>46</u>

———>   Cbo-L-Pro-L-Leu-L-Leu-O-t-Bu

<u>47</u>
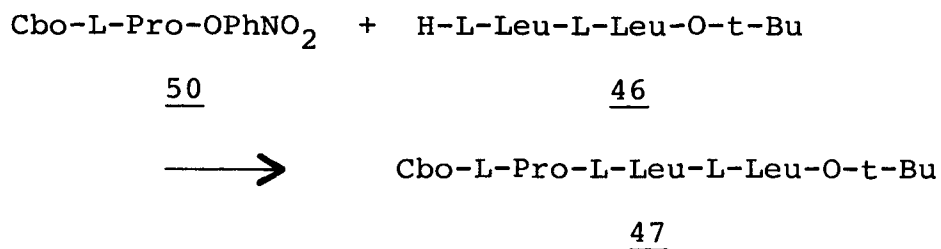
Removal of the benzyloxycarbonyl group from <u>47</u> was
readily achieved by hydrogenation over 10% palladium on
carbon to provide a 77.5% yield of L-prolyl-L-leucyl-L-
luecine <u>t</u>-butyl ester (<u>51</u>). It is interesting to note that
this material is a high melting crystalline solid. This is
in contrast to all the free amines in the previous series,
which were oils. It seems that the replacement of the two
adjacent glycine residues with larger amino acids has in-
creased the conformational rigidity of the molecule.

Cbo-L-Pro-L-Leu-L-Leu-O-t-Bu $\xrightarrow{\text{H}_2\text{-Pd/C}}$

<u>47</u>

H-L-Pro-L-Leu-L-Leu-O-t-Bu

<u>51</u>
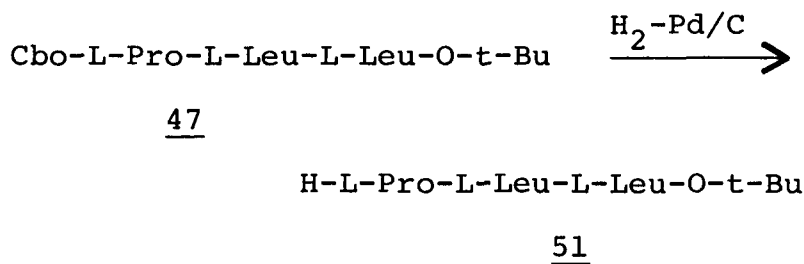
It was expected that the condensation of <u>51</u> with
N-hexanoylglycine (<u>12</u>) via the mixed anhydride would proceed
without difficulty to give N-hexanoylglycyl-L-prolyl-L-

leucyl-L-leucine t-butyl ester (52) in moderate yield. When
this reaction was performed, however, a mixture of two major
products was obtained. These could be separated by recrys-
tallization and column chromatography over silica gel to give
only an 11% yield of 52. This was identified by the nmr
spectrum and mixed melting point with an authentic sample
prepared by the method described below. The second compound
was identified by the nmr spectrum as N-ethoxycarbonyl-L-
prolyl-L-leucyl-L-leucine t-butyl ester (53), which was
isolated in 24% yield. This compound was formed by attack
of the nitrogen at the wrong carbonyl carbon of the mixed
anhydride 54, as was the case in the formation of N-ethoxy-
carbonyl-L-leucyl-L-leucine t-butyl ester (48). Again, the
reason for this is probably steric hindrance which prevents
the amine from approaching the carbonyl carbon of the amino
acid. This is very surprising since glycine is not consid-
ered to offer much steric hindrance. It was apparent that
the p-nitrophenyl ester method would be required in this
synthesis also.

Hex-Gly-OH + H-L-Pro-L-Leu-L-Leu-O-t-Bu $\xrightarrow{\text{MA}}$

<u>12</u>   <u>51</u>

        O
        ||
    EtO-C-Cl   Hex-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu

                                       <u>52</u>

        O   O   +
        || :51 ||
Hex-Gly-O-C-OEt $\xrightarrow{}$ EtO-C-L-Pro-L-Leu-L-Leu-O-t-Bu

    <u>54</u>   <u>53</u>
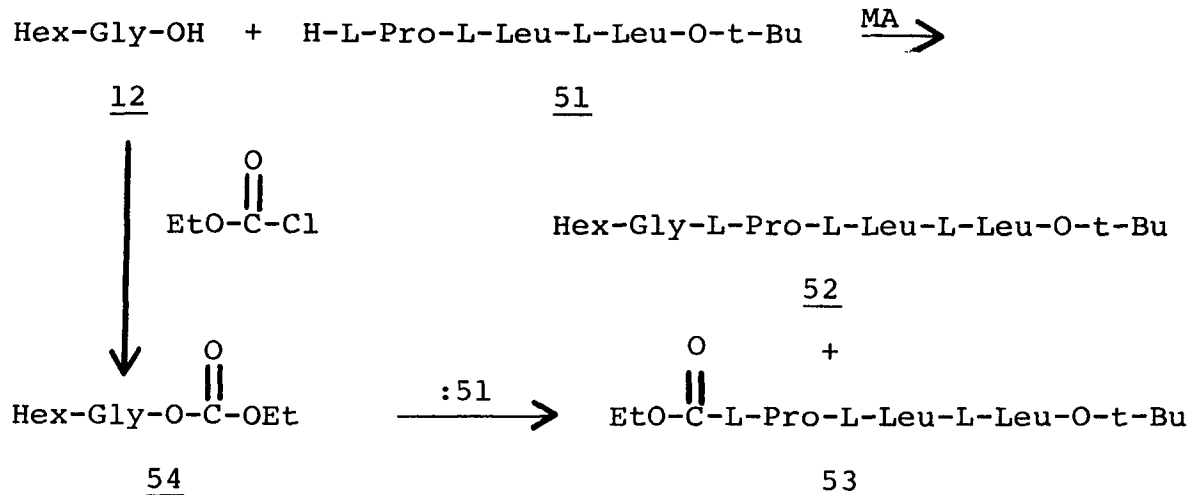
N-Hexanoylglycine <u>p</u>-nitrophenyl ester (<u>55</u>) was pre-
pared in 66.5% yield from N-hexanoylglycine (<u>12</u>) and <u>p</u>-nitro-
phenol using N,N'-dicyclohexylcarbodiimide. Reaction of <u>55</u>
with L-prolyl-L-leucyl-L-leucine <u>t</u>-butyl ester (<u>51</u>) in the
presence of two equivalents of imidazole gave a 79% yield of
<u>52</u>. Completion of the synthesis required only the hydrolysis
of the <u>t</u>-butyl ester. This was accomplished by the use of
trifluoroacetic acid[41] to provide N-hexanoylglycyl-L-prolyl-
L-leucyl-L-leucine (<u>56</u>) in 71% yield for an overall yield of
22% from L-leucine <u>t</u>-butyl ester phosphite salt (<u>44</u>). It
was found that the crude <u>t</u>-butyl ester could be hydrolyzed
directly to the acid without first purifying it by recrys-
tallization. This gave an 86% yield of <u>56</u> from L-prolyl-L-
leucyl-L-leucine <u>t</u>-butyl ester (<u>51</u>) and increased the over-
all yield to 33.5%.

Hex-Gly-OH $\overset{+}{\text{HO}}$—⟨benzene ring⟩—NO$_2$ $\xrightarrow{\text{DCC}}$ Hex-Gly-OPhNO$_2$

<u>12</u>   <u>55</u>

<u>55</u> + H-L-Pro-L-Leu-L-Leu-O-t-Bu $\longrightarrow$

        <u>51</u>

                    Hex-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu

                                 <u>52</u>

Hex-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu $\xrightarrow{\text{TFA}}$

        <u>52</u>

                    Hex-Gly-L-Pro-L-Leu-L-Leu-OH

                           <u>56</u>
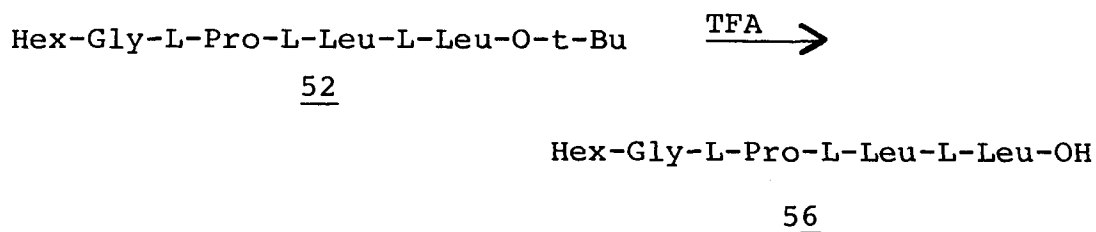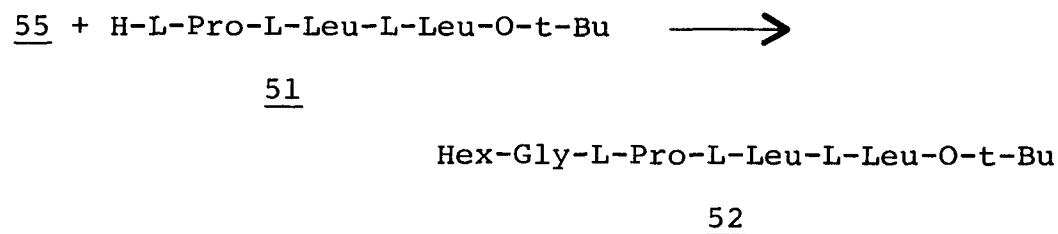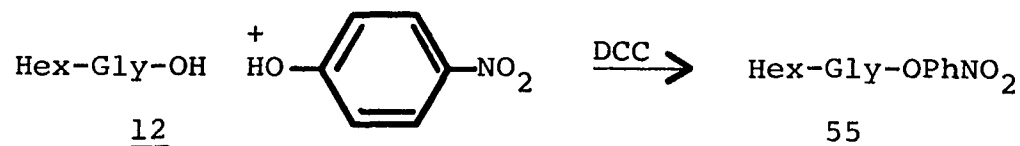
H.   <u>Preparation of N-Pentanoylglycyl-L-prolyl-L-leucyl-L-</u>
     <u>leucine</u> (<u>59</u>)

The reasons for preparing peptides containing a five carbon, in addition to a six carbon, aliphatic acid on the N-terminal end of the chain were discussed earlier. The observations reported in the previous section indicated that the p-nitrophenyl ester method is superior to the mixed anhydride method for the synthesis of these particular peptides, and this approach was utilized here also.

The preparation of N-pentanoylglycine p-nitrophenyl ester (<u>57</u>) from N-pentanoylglycine (<u>29</u>) and p-nitrophenyl was achieved in 51% yield using N,N'-dicyclohexylcarbodiimide.

Reaction of this active ester with L-prolyl-L-leucyl-L-leucine

t-butyl ester (51) gave a 71% yield of N-pentanoylglycyl-L-

prolyl-L-leucyl-L-leucine t-butyl ester (58). Hydrolysis of

this t-butyl ester with trifluoroacetic acid for 2 hr at room

temperatures gave a 57.5% yield of N-pentanoylglycyl-L-prolyl-

L-leucyl-L-leucine (59) for an overall yield of 16% from L-

leucine t-butyl ester phosphite salt (44). The reason for

the lower-than-expected yield for the hydrolysis is unknown,

for the reaction was not repeated. This acid was tested for

solubility in water and was found to be very water insoluble.

Apparently the hydrophobic side chains on the leucine resi-

dues negate the water solubility of the N-pentanoylglycine

portion of the molecule.

Pen-Gly-OH + HO $-\langle\!\bigcirc\!\rangle-$ NO$_2$ $\xrightarrow{\text{DCC}}$ Pen-Gly-OPhNO$_2$

<u>29</u>   <u>57</u>

<u>57</u> + H-L-Pro-L-Leu-L-Leu-O-t-Bu $\longrightarrow$

<u>51</u>

Pen-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu

<u>58</u>

<u>58</u> $\xrightarrow{\text{TFA}}$ Pen-Gly-L-Pro-L-Leu-L-Leu-OH

<u>59</u>
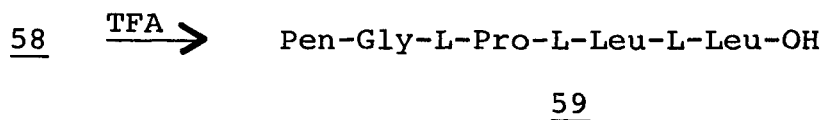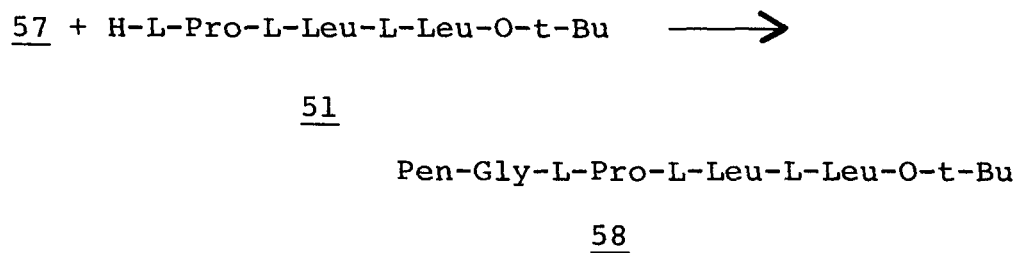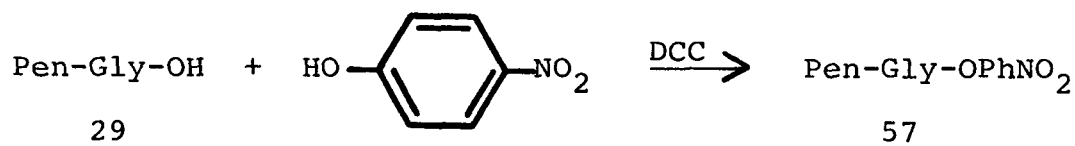
I. Preparation of N-Pentanoylglycyl-D-prolyl-L-phenylalanyl-
    L-leucine (79)

A comparison of the structures of PGE_1 (1) and actino-
mycin D (4) shown in Figure 2 reveals that the configuration
at the alpha carbon of the proline residue in 4 is the
opposite of the configuration at the corresponding carbon of
the prostaglandins. This suggested that peptides containing
a D-proline residue should be synthesized and tested for
biological activity. Extrapolation from the structures of
other naturally occurring, proline-containing cyclic peptides
also suggests that the presence of a residue having the D
configuration is important in determining the conformation
of the peptide.[33,42,43] Empirical estimates of conformational
energies indicate that the sequence D-residue-L-residue, or
its' enantiomer, is a particularly favorable one for a sharp
reversal of peptide chain direction. Experimental evidence
also shows that the synthesis of cyclic peptides is more
readily achieved when there is the sequence D-residue-L-
residue, or its' enantiomer. In a large proportion of these
cases, one member of the DL pair is proline. Another reason
for preparing peptides containing D-amino acids is that the
presence of amino acids of unusual structure or of the un-
natural D-configuration can cause the peptide to be resistant
to enzymatic hydrolysis and thus result in longer biological
activity.

L-Proline (16) was easily racemized following a known procedure[44] and converted directly to N-benzyloxycarbonyl-DL-proline (61). This material was obtained as an oil and was converted to the dicyclohexylamine salt (62) for purification and characterization in 70% yield from L-proline.

L-Proline $\longrightarrow$ DL-Proline $\xrightarrow{\text{Cbo-Cl}}$ Cbo-DL-Pro-OH

16   60   61

$\xrightarrow{\text{DCHA}}$ Cbo-DL-Pro-OH·DCHA

62
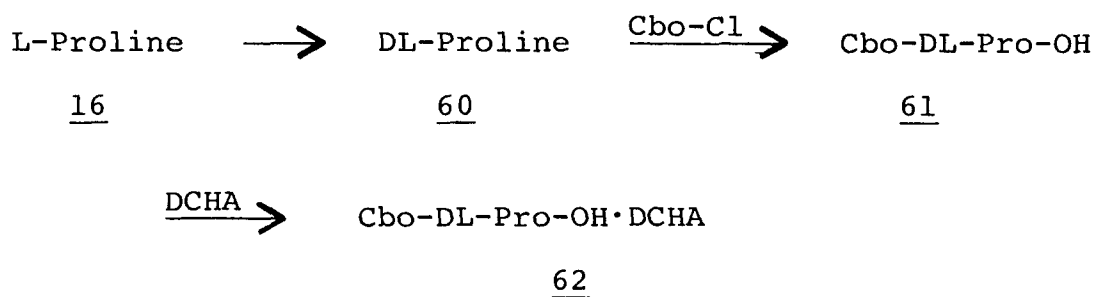
Resolution of N-benzyloxycarbonyl-DL-proline (Scheme 4) was accomplished by the method of Vogler and Lanz[45] which involves the formation of diastereomeric salts with L-tyrosine hydrazide (64) and separation of these salts by recrystallization. The hydrazide was prepared in 74% yield from L-tyrosine methyl ester (63) and hydrazine hydrate. Reaction of 64 with N-benzyloxycarbonyl-DL-proline (61) gave diastereomeric salts (65 & 66) which could be separated by recrystallization from methanol. The salts were decomposed by addition of acid to provide N-benzyloxycarbonyl-D-proline (67) and N-benzyloxycarbonyl-L-proline (22) which were converted to their respective dicyclohexylamine salts for purification and characterization. N-Benzyloxycarbonyl-D-proline dicyclohexylamine salt (68) was obtained 100% optically pure in 63% yield, while the L-isomer was obtained 96% optically pure in the chemical

Cbo-DL-Pro-OH + H-L-Tyr-NHNH$_2$ $\longrightarrow$

61   64

Cbo-D-Pro-OH·H-L-Tyr-NHNH$_2$ + Cbo-L-Pro-OH·H-L-Tyr-NHNH$_2$

65   66

H$^+$ H$^+$

Cbo-D-Pro-OH   Cbo-L-Pro-OH

67   22

DCHA   DCHA

Cbo-D-Pro-OH·DCHA   Cbo-L-Pro-OH·DCHA

68   23
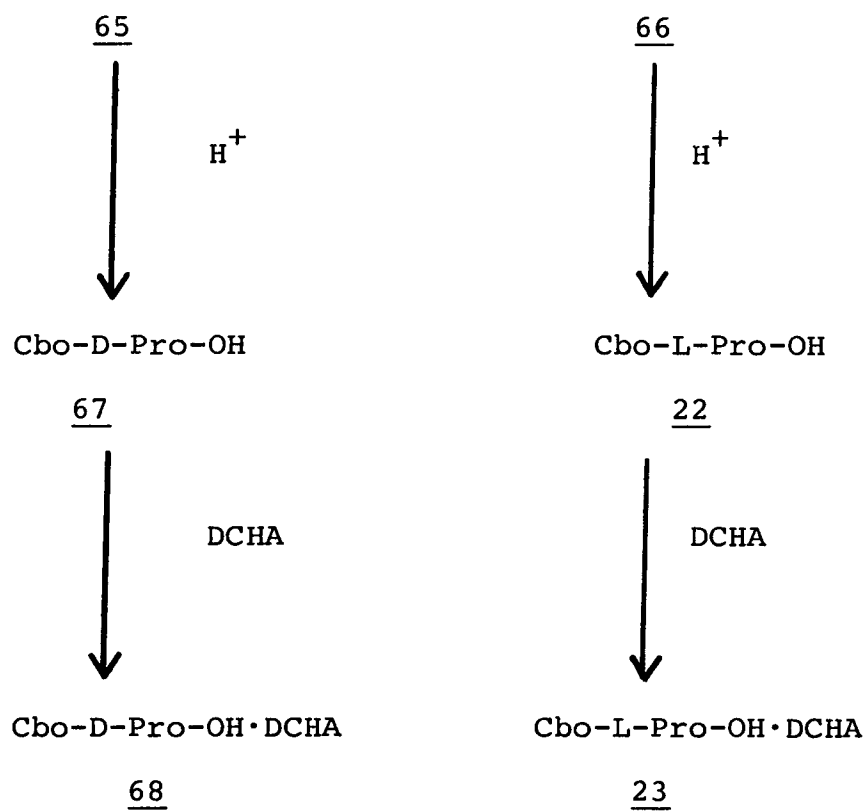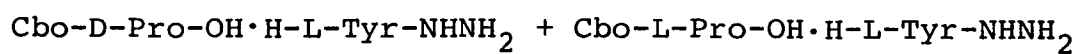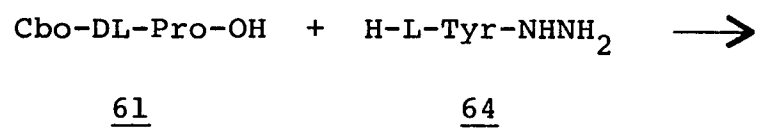
SCHEME 4

yield of 51%. The D-isomer was converted to the p-nitrophenyl ester (69) in 66.5% yield using the procedure discussed earlier.

$$\text{H-L-Tyr-OMe} \quad \xrightarrow{\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}} \quad \text{H-L-Tyr-NHNH}_2$$

$$\underline{63} \qquad\qquad\qquad\qquad\qquad\qquad \underline{64}$$

Cbo-D-Pro-OH + HO—⟨ ⟩—NO₂ $\xrightarrow{\text{DCC}}$ Cbo-D-Pro-OPhNO₂

$$\underline{67} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \underline{69}$$
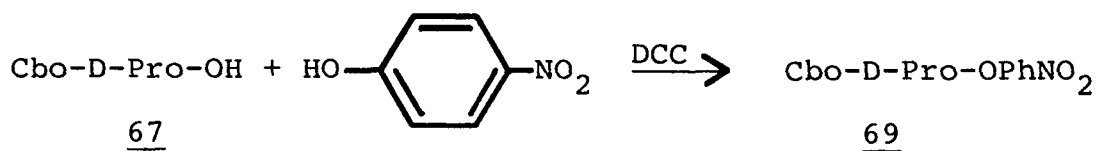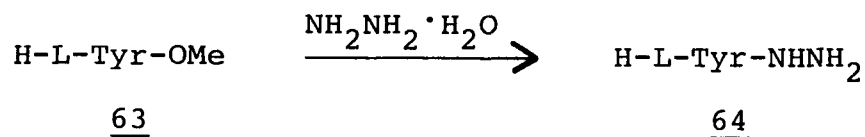
The remaining starting material, N-benzyloxycarbonyl-L-phenylalanine (71), was prepared in 71% yield by a Schotten-Baumann type reaction between benzyl chloroformate and L-phenylalanine (70) and was isolated as the dicyclohexylamine salt (72).

$$\text{L-Phenylalanine} + \text{Cbo-Cl} \xrightarrow{\text{NaOH}} \text{Cbo-L-Phe-OH} \xrightarrow{\text{DCHA}}$$

$$\underline{70} \qquad\qquad\qquad\qquad\qquad\qquad \underline{71}$$

Cbo-L-Phe-OH·DCHA

$$\underline{72}$$
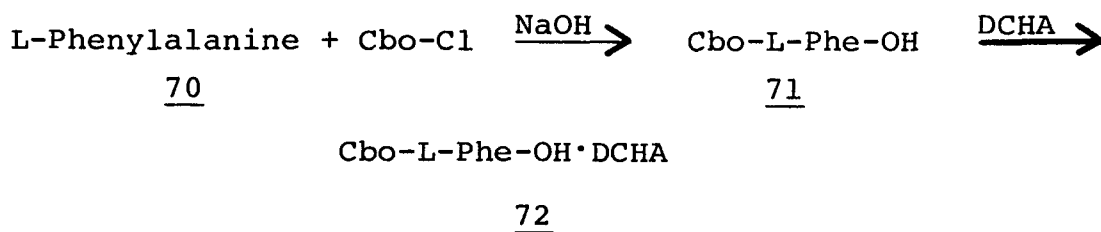
The preparation of the p-nitrophenyl ester (73) of 71 using N,N'-dicyclohexylcarbodiimide in tetrahydrofuran gave a 49% chemical yield but was accompanied by racemization to give a product of only 76% optical purity.

Cbo-L-Phe-OH + HO—⟨benzene ring⟩—NO$_2$ $\xrightarrow{\text{DCC}}$ Cbo-L-Phe-OPhNO$_2$

<u>71</u>   <u>73</u>
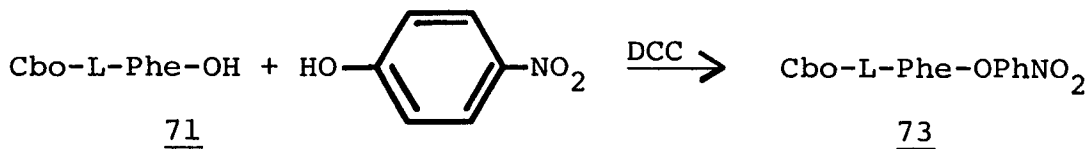
The reactions leading to the preparation of N-penta-
noylglycyl-D-prolyl-L-phenylalanyl-L-leucine (<u>79</u>) are
illustrated in Scheme 5. The preparation of N-benzyloxy-
carbonyl-L-phenylalanyl-L-leucine <u>t</u>-butyl ester (<u>74</u>) was
accomplished by three different methods. Activation of the
carbonyl group of <u>71</u> by mixed anhydride formation and con-
densation with the phosphite salt of L-leucine <u>t</u>-butyl ester
(<u>44</u>) gave a 62.5% yield of <u>74</u>. Alternatively, <u>74</u> could be
prepared in 54% yield using N,N'-dicyclohexylcarbodiimide.
For comparison the <u>p</u>-nitrophenyl ester, <u>73</u>, was condensed
with <u>44</u> to provide a 55.5% yield of <u>74</u>. Since the prepara-
tion of the <u>p</u>-nitrophenyl ester was accompanied by racemiza-
tion, the material prepared by this third method was not
used in any further reactions.

The benzyloxycarbonyl group was easily removed from
<u>74</u> by hydrogenation over 10% palladium on carbon to give a
94% yield of L-phenylalanyl-L-leucine <u>t</u>-butyl ester (<u>75</u>) as
a viscous oil. The condensation reaction between <u>75</u> and N-
benzyloxycarbonyl-D-proline <u>p</u>-nitrophenyl ester (<u>69</u>) pro-
ceeded smoothly and cleanly to provide an 81.5% yield of
N-benzyloxycarbonyl-D-prolyl-L-phenylalanyl-L-leucine <u>t</u>-butyl
ester (<u>76</u>). Hydrogenation over 10% palladium on carbon

Cbo-L-Phe-OH + H-L-Leu-O-t-Bu·H$_3$PO$_3$ $\xrightarrow[\text{or DCC}]{\text{MA}}$

     71   44

Cbo-L-Phe-L-Leu-O-t-Bu $\xrightarrow{\text{H}_2\text{Pd/C}}$ H-L-Phe-L-Leu-O-t-Bu

     74   75

75 + Cbo-D-Pro-OPhNO$_2$ $\longrightarrow$ Cbo-D-Pro-L-Phe-L-Leu-O-t-Bu

          69   76

76 $\xrightarrow{\text{H}_2\text{Pd/C}}$ H-D-Pro-L-Phe-L-Leu-O-t-Bu

                         77

77 + Pen-Gly-OPhNO$_2$ $\longrightarrow$ Pen-Gly-O-Pro-L-Phe-L-Leu-O-t-Bu

         57   78

78 $\xrightarrow{\text{TFA}}$ Pen-Gly-D-Pro-L-Phe-L-Leu-OH

                         79
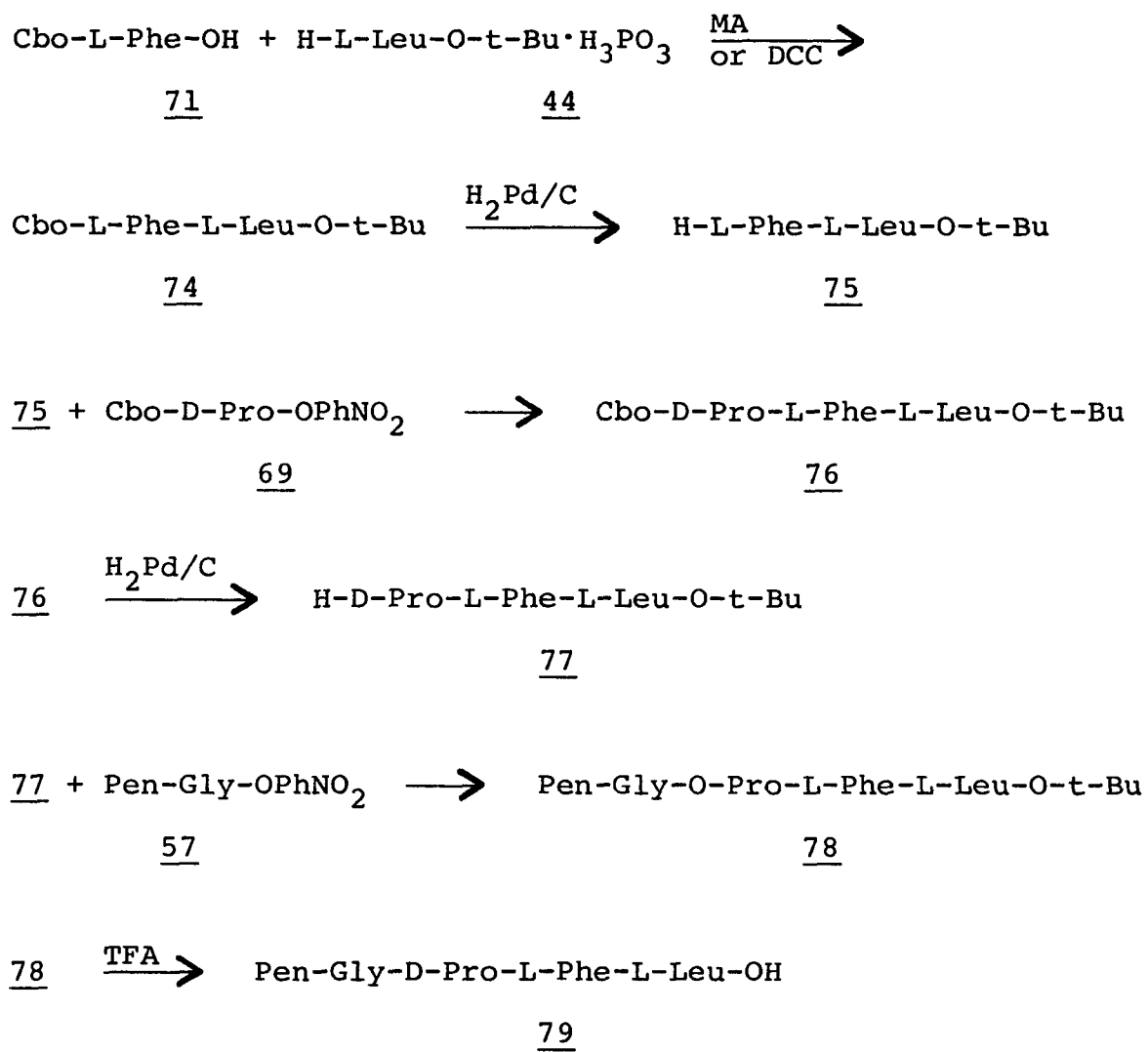
SCHEME 5

removed the nitrogen protecting group to provide a 76.5% yield of D-prolyl-L-phenylalanyl-L-leucine t-butyl ester (77) as a crystalline solid.

The synthesis was completed by condensation of 77 with N-pentanoylglycine p-nitrophenyl ester (57) and hydrolysis of the t-butyl ester to N-pentanoylglycyl-D-prolyl-L-phenyl-alanyl L-leucine (79) with trifluoroacetic acid. The ester was not purified but was hydrolyzed directly to the acid to provide an 85% yield of 79 for a 31% overall yield from L-leucine t-butyl ester phosphite salt (44).

J. Attempted Preparation of Derivatives of 5-Hydroxyproline

A comparison of the structures of PGE_1 (1) and zearalenone (2) suggests that proline derivatives containing an hydroxy group on carbon 5 of the ring would be closely related analoges. Substituted 5-hydroxyprolines have been reported by Cox and coworkers[46] and by Magerlein[47] to be intermediates in the synthesis of various substituted prolines. They found that the Michael addition of diethyl N-acylaminomalonates to α,β-unsaturated aldehydes gave inter-mediate products which tend to exist largely in the cyclic form. The cyclic nature of the intermediates was confirmed[46] by the nmr and ir spectra. In the ir spectrum the amide II band present in the diethyl N-acylaminomalonates disappeared upon condensation with the aldehyde. In the nmr spectrum the hydroxyl proton appeared as a singlet and the adjacent C-5 proton appeared as a doublet. The hydroxyl signal was absent

in the spectrum of the corresponding phenylurethane and the doublet corresponding to the C-5 proton was shifted downfield. However, the intermediates are also reported to form semi-carbazones[46] and 2,4-dinitrophenylhydrazones[48], suggesting that a small amount of the keto-amide is in equilibrium with the cyclic hydroxyproline.

$$R-CO-NH-CH(CO_2Et)_2 \ + \ R'-CH=CH-CHO \ \longrightarrow$$
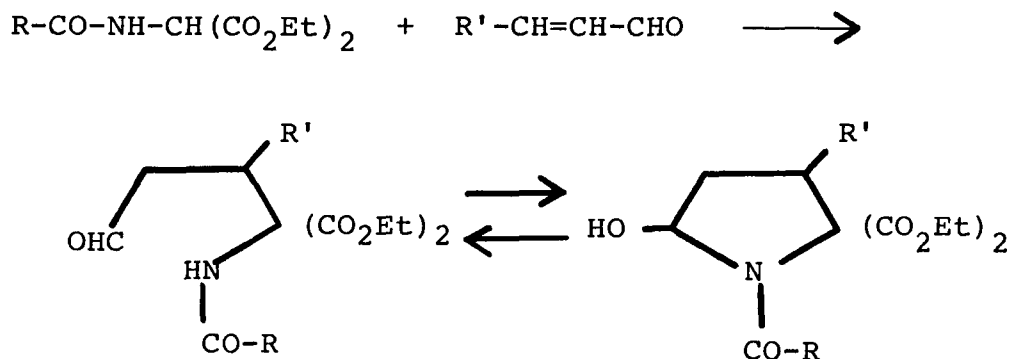
Diethyl aminomalonate hydrochloride (80) was easily prepared using the Organic Syntheses method.[49] Condensation of 80 with octanoic acid (81) was achieved by two different methods. Condensation using the mixed anhydride gave N-octanoylaminomalonic acid diethyl ester (82) in 70% yield while coupling with N,N'-dicyclohexylcarbodiimide gave 82 in 56% yield.

$$H_2NCH(CO_2Et)_2 \cdot HCl \ + \ Oct-OH \ \xrightarrow[\text{or DCC}]{\text{MA}} \ Oct-NHCH(CO_2Et)_2$$

| 80 | 81 | 82 |
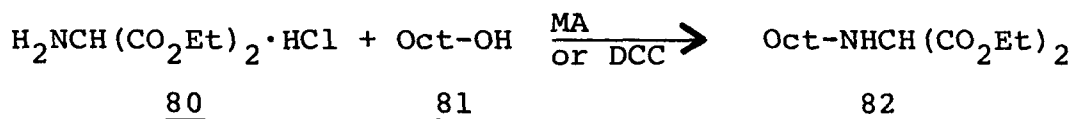
The condensation of <u>82</u> with acrolein was carried out in benzene solution using a small amount of sodium methoxide as base. On workup a viscous oil was obtained. TLC showed the complete absence of starting material. The amide II band present at 1525 cm^{-1} in <u>82</u> was now absent. While the ir spectrum supported structure <u>83</u> the nmr data were confusing. The spectrum contained a singlet at δ1.98, which is the region of the hydroxyl singlet reported by Cox and coworkers, and also a broad singlet at δ6.40. This latter signal corresponds roughly to the C-5 proton reported by Cox, except that it disappeared on deuterium exchange with D_2O, suggesting an amide proton. The singlet at δ1.98 did not disappear on deuterium exchange, although the intensity was reduced and a second peak appeared at δ1.78. Despite these confusing spectral data, the later steps in the synthesis were attempted.

Oct-NHCH(CO$_2$Et)$_2$ + CH$_2$=CHCHO $\xrightarrow{\text{NaOMe}}$

<u>82</u>

HO— (ring with N) (CO$_2$Et)$_2$

N
|
Oct

<u>83</u>
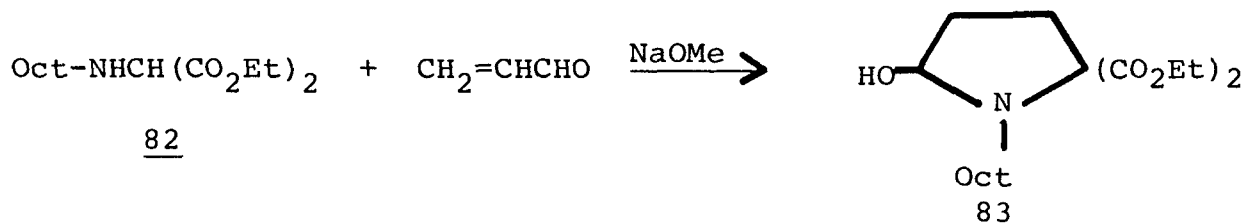
The experimental work of Cox and coworkers and Magerlein indicated that the 5-hydroxy group is very labile, undergoing removal hydrogenolytically over palladium on carbon. The material is also dehydrated by heating under aqueous acidic conditions. For these reasons it was considered necessary to protect the hydroxy function with a very easily removable protecting group to allow hydrolysis of the two ethoxycarbonyl groups and decarboxylation to give the 5-hydroxyproline. Di-

hydropyran was chosen initially. This protecting group is easily removed by acid but is stable to base. This allowed basic hydrolysis and decarboxylation to be used.

Reaction of 5,5-bis-ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (83) with dihydropyran containing a trace of acid gave a yellow viscous oil which was presumed to be the dihydropyran derivative (84) based on spectral evidence. In the ir spectrum the amide II band which was present in 82 was absent and in the nmr spectrum the broad singlet at $\delta 6.40$ was gone. This suggested that the signal at $\delta 6.40$ was due to the hydroxyl proton. The signals for the ethyl ester groups appeared as two overlapping triplets and two overlapping quartets, which could represent the different environments of the ethoxycarbonyl groups cis and trans to the protected hydroxyl group. This material could not be crystallized and was used without further purification.
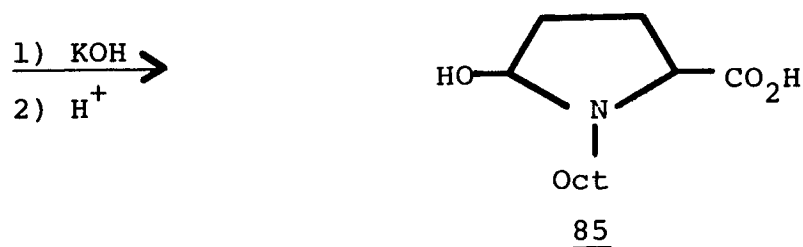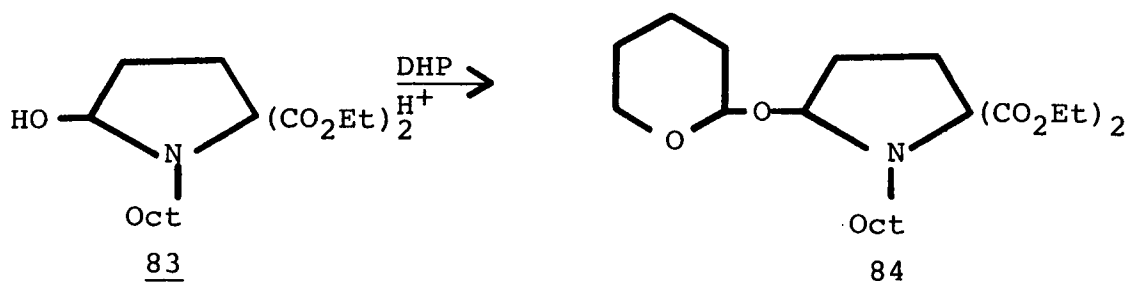
Hydrolysis of the ethoxycarbonyl groups was accomplished by refluxing in a solution of aqueous KOH and ethanol. Workup gave a yellow oil which failed to crystallize. There was no evidence in the nmr spectrum for the presence of the ethyl ester groups, while a broad singlet at $\delta 10.28$ (2 protons) suggested the presence of a hydroxy group and a carboxyl group and thus the formation of N-octanoyl-5-hydroxyproline (85). The ir spectrum of 85 showed a band at 1525 cm^{-1} indicating possible ring opening. The oil was dissolved in ether and treated with dicyclohexylamine in order to obtain a crystalline salt. A precipitate failed to form, however, and no further workup was attempted.

A second attempted preparation of 85 involved basic hydrolysis of 5,5-bis-ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (83) directly without protection of the hydroxy group. A solution of 83 in aqueous KOH and ethanol was warmed on a steam bath for 10 min. Workup gave a colorless solid having a very broad melting range. Further recrystallization failed to improve the melting point and the material was used directly without further purification. It was felt that coupling this crude material with a dipeptide ester might give a crystalline solid, which would facilitate purification. The crude solid was condensed with glycylglycine benzyl ester p-toluenesulfonate (34 using N,N'-dicyclohexylcarbodiimide to prepare N-octanoyl-5-hydroxyprolylglycylglycine benzyl ester (86). The reaction afforded a yellow oil which appeared very complex by TLC. The nmr spectrum failed to support the structure of 86 and no further workup was attempted.

HO⟍⟍⟋⟍—CO₂H   +   H-Gly-Gly-OBzl·p-TsOH

34

85

DCC →

HO—⟍⟋⟍—Co-Gly-Gly-OBzl

Oct

86
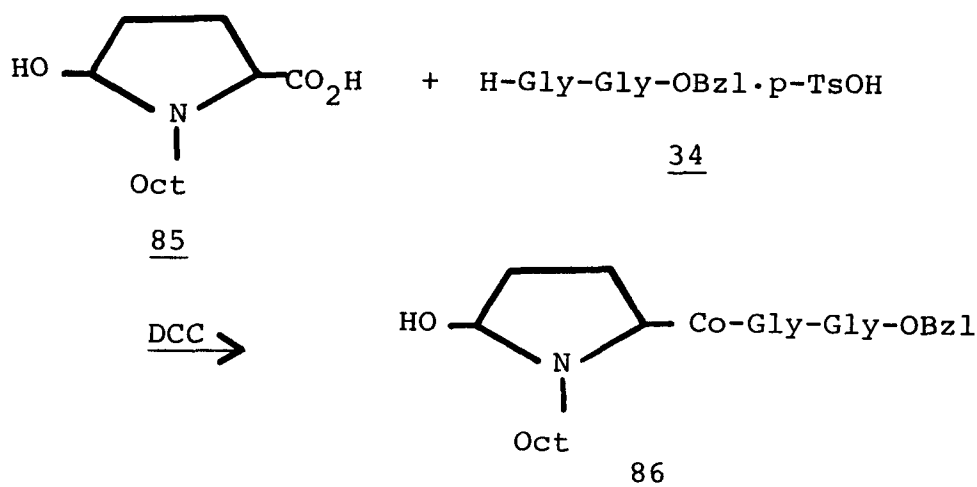
The failure of the above reactions prompted another search for suitable hydroxyl protecting groups. The benzyloxycarbonyl group has been used[50] for hydroxyl protection in serine and tyrosine. Reaction of 5,5-bis-ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (83) with benzyl chloroformate and triethylamine failed to produce 5,5-bis-ethoxycarbonyl-1-octanoyl-2-benzyloxycarbonylpyrrolidinol (87) but gave unreacted starting material. Attempts to prepare the p-nitro-phenylhydrazone (88) also met with failure, giving unreacted starting material.

HO—⟍⟋⟍(CO₂Et)₂ $\xrightarrow[\text{Et}_3\text{N}]{\text{Cbo-Cl}}$ Cbo-O—⟍⟋⟍(CO₂Et)₂

Oct   Oct

83   87

83 + (p-nitrophenylhydrazine) → O_2N—⟨⟩—NHN=⟨...⟩(CO₂Et)₂

Oct   88
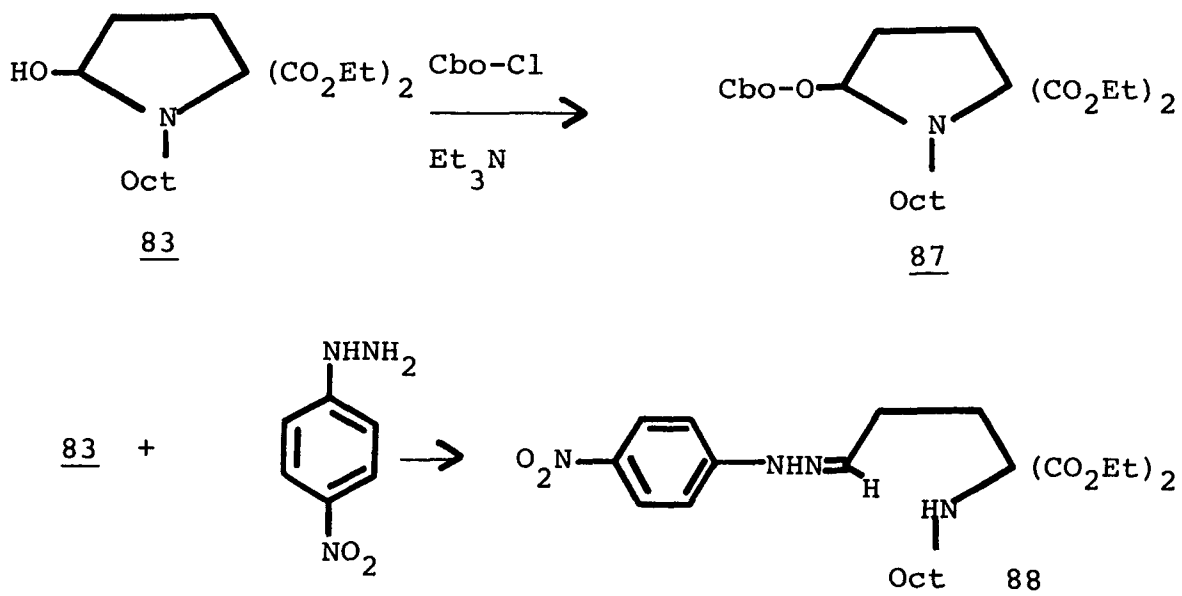
K.   Biological Results

The testing results for interruption of pregnancy
and inhibition of FSH-LH release were provided by the
National Institutes of Health in Bethesda, Md. The four
compounds shown in Table III were tested for interruption
of pregnancy in hamsters. The compounds were dissolved in
an organic solvent such as acetone or ethanol and neutralized
with 0.02% sodium carbonate. A single dose of 10 mg of
compound was given subcutaneously three days after the
hamsters had mated. As shown in the table, two of the com-
pounds showed only slight activity, while the other two were
inactive. Three other compounds, N-hexanoylglycyl-L-prolyl-
L-leucyl-L-leucine (56), N-pentanoyl-glycyl-L-prolyl-L-leucyl-
L-leucine (59), and N-pentanoylglycyl-D-prolyl-L-phenylalanyl-
L-leucine (79), have not yet been tested.

N-Hexanoylglycyl-L-prolylglycylglycinamide (37) was
screened for inhibition of LH-FSH release (Table IV) from
pituitaries of 20-day old, female rats. This compound showed
a slight inhibition of LH release stimulated by LH-RF, but
the effect did not seem to be dose-related.

The fact that actinomycin D (4) and zearalenone (2)
are antibiotic suggested that the peptides described in this
Thesis should be tested for antibacterial activity. To date,
four proline derivatives have been tested. They are N-
hexanoylglycyl-L-proline (33), N-hexanoylglycyl-L-prolyl-5-
aminovaleric acid (19), N-hexanoylglycyl-L-prolyl-5-amino-
valeric acid methyl ester (27), and N-pentanoylglycyl-L-

TABLE III

Interruption of Pregnancy Screening Results

on Hamster at 10 mg Dose Level

Compound No.	Structure	Activity
19	Hex-Gly-L-Pro-5-Amv-OH	inactive
27	Hex-Gly-L-Pro-5-Amv-OCH$_3$	slight[a] activity
30	Pen-Gly-L-Pro-5-Amv-OCH$_3$	inactive
36	Hex-Gly-L-Pro-Gly-Gly-OH	slight activity

[a]This activity represents a 30% reduction in the number of pregnancies.

TABLE IV

LH-FSH Release Inhibition Assay of Hex-Gly-L-Pro-Gly-Gly-NH$_2$ (37)

After Treatment of Rat Pituitaries with 0.3 mug/ml LH-RF

Concentration of 37 ug/ml		Concentration of LH (mug/ml medium)		
		3	4	5
1		265 ± 33	373 ± 27	518 ± 80
	Inhibition	ns[a]	sa[b]	ns
10		207 ± 31	350 ± 27	591 ± 120
		sa	sa	ns
100		252 ± 11	391 ± 46	212 ± 42
		sa	sa	sa
Control (O)		393 ± 54	615 ± 73	714 ± 0

[a] not significant

[b] slight activity

prolyl-5-aminovaleric acid methyl ester (30). The testing was performed by Rita Furman of the Department of Micro-biology, The University of New Hampshire using a cylinder-plate method. Concentrations of the chemicals were between 1000 ug/ml and 31.25 ug/ml. Test organisms were Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. No inhibition of bacterial growth due to any of the chemicals was observed.

SECTION III

EXPERIMENTAL

General

Melting Points.   Melting points were determined with a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected.

Boiling Points.   Boiling points were measured at the pressure indicated and are uncorrected.

Elemental Analyses.   Elemental analyses were determined at the University of New Hampshire using an F & M Model 185 carbon, hydrogen and nitrogen analyzer.

Infrared Spectra.   Infrared spectra (ir) were recorded on a Perkin-Elmer Model 137 Infracord prism spectrometer and were calibrated with polystyrene at 1944 cm^{-1}. Solid samples were recorded as KBr discs while liquid samples were recorded either as neat films between sodium chloride plates, or as Nujol mulls.

Nuclear Magnetic Resonance Spectra.   Nuclear magnetic resonance (nmr) spectra were recorded on a Varian Model A-60 Spectrometer and on a Jeol MN 100 Spectrometer and are reported in parts per million (δ) from TMS.   Samples recorded in $CDCl_3$ contained 1% TMS as an internal standard and samples recorded in D_2O, TFA, D_6-DMSO, and D_6-acetone were calibrated with TMS as an external standard.   For all new compounds reported, the nmr spectra are reproduced with assignments in Appendix B and are given in the Experimental Section as follows:

nmr (solvent); in ppm (multiplicity, number of hydrogens, coupling constant in Hz). The description of multiplicity is s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Materials and Methods. A. Reagents. L-Proline, methyl glycinate hydrochloride, methyl L-leucinate hydrochloride, L-leucine, L-phenylalanine, methyl L-tyrosinate, ethyl chloroformate, benzyl chloroformate, octanoic acid, N,N'-dicyclohexylcarbodiimide, dihydropyran, acrolein, citric acid, o-chloronitrobenzene, dicyclohexylamine, and trifluoroacetic acid were purchased from Aldrich Chemical Company. Ethyl glycinate hydrochloride, pentanoic acid, 2,2-dimethoxypropane, imidazole, N-ethylmorpholine, p-nitrophenol, and p-nitrophenylhydrazine were purchased from Eastman Kodak Company. Triethylamine, phosphorous acid, basic alumina, and silica gel were purchased from J. T. Baker Company. Hexanoic acid, thionyl chloride, and acetic anhydride were purchased from Fisher Scientific Company. Glycine, hydrazine, hydrate, and phosphorous trichloride were purchased from Matheson Scientific. N-Benzyloxycarbonyl-L-leucine was purchased from Nutritional Biochemicals Corporation. The catalyst, 10% palladium on carbon, was purchased from Engelhard Industries. Hydrogen and ammonia were purchased from Airco Gas Products, while anhydrous hydrogen chloride, hydrogen bromide, and isobutylene were purchased from Scientific Gas Products. Glycylglycine benzyl ester p-toluenesulfonate and 5-aminovaleric acid were prepared by

Richard Davenport[18] of these laboratories.

B. Solvents. The following solvents were used without further purification: methylene chloride, chloroform, ethyl acetate, carbon tetrachloride, benzene, chlorobenzene, acetone, ether, hexane, cyclohexane, petroleum ether, dioxane, tetrahydrofuran, methanol, and ethanol. Benzene was dried over sodium wire. Methylene chloride, acetonitrile, and N,N-dimethylformamide were stored over calcium hydride and filtered before use. Pyridine was dried and stored over barium oxide. Tetrahydrofuran was distilled from calcium hydride immediately before use.

C. Products. Yields of the products are reported on the purified material unless stated otherwise. In several cases yields are reported for both crude and purified material. Where an analytical sample was obtained, the ir and nmr spectra were recorded on the analytical sample.

D. Experimental Methods. Reagents were weighed to the number of significant figures shown and this number was converted to moles (mol). Following extraction, the normal isolation procedure was to dry the extract over $MgSO_4$ for at least 0.5 hr, remove the drying agent by filtration, and wash the residual drying agent thoroughly with solvent. The filtrate was then concentrated under reduced pressure on a rotatory evaporator. Hydrogenation of N-benzyloxycarbonyl groups at pressures other than atmospheric were performed on a Parr apparatus. The flask was emptied and refilled several times during the hydrogenation to remove CO_2.

E. <u>Thin Layer Chromatography</u>.   TLC was performed on silica gel-coated microscope slides.

Preparation of Hexanoyl Chloride (10). In a 100 ml

flask fitted with a reflux condenser and drying tube was

placed 34.80 g (0.30 mol) of hexanoic acid (13) and 43.60 g

(0.38 mol) of thionyl chloride. Hydrogen chloride gas was

immediately given off. The evolution of gas ceased after 1

hr and the solution was heated under reflux for 30 min. Dis-

tillation gave 34.80 g (86%) of 10, bp 150-153° at 760 mm

(lit.[51] bp 153° at 760 mm).

Preparation of N-Hexanoylglycine Ethyl Ester (15).

A suspension of 2.50 g (0.018 mol) of ethyl glycinate hydro-

chloride (14) in 50 ml of anhydrous pyridine was cooled to

5° and treated with 0.8 ml (0.009 mol) of phosphorous tri-

chloride in 20 ml of anhydrous pyridine. The suspension was

stirred 30 min at 20°, 2.10 g (0.018 mol) of hexanoic acid

(13) was added, and the resulting solution was heated 3 hr

on a steam bath. The solvent was removed under reduced

pressure, and the residue was dissolved in 50 ml of water.

The aqueous solution was extracted with five 50-ml portions

of ethyl acetate. The combined organic extracts were washed

with two 75 ml portions of 2N HCl, 75 ml of water, two 75 ml

portions of 5% $NaHCO_3$, and 75 ml of water. The organic solu-

tion was dried ($MgSO_4$) and concentrated under reduced pressure

to give a brown liquid. Distillation gave 2.19 g (61.1%)

of 15, bp 157° at 2.6 mm (lit.[52] bp 151° at 3 mm).

Preparation of N-Hexanoylglycine (12). Method A.

A solution of 9.00 g (0.12 mol) of glycine (11) in 300 ml of

2N NaOH was cooled to -10° and treated with 13.4 g (0.10 mol)

of hexanoyl chloride (10). The mixture was stirred rapidly for 3 hr and extracted with three 75 ml portions of chloroform. The aqueous solution was acidified to Congo Red with 6N HCl and extracted with three 100 ml portions of chloroform. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow solid. Recrystallization from carbon tetrachloride gave 9.05 g (52.3%) of 12 as colorless needles, mp 89.5-93.5° (lit.[53] mp 91-92°). A second recrystallization from benzene gave 8.90 g (51.5%) of colorless needles, mp 93-94°.

Method B. A solution of 2.00 g (0.01 mol) of N-hexanoylglycine ethyl ester (15) in 20 ml of acetone and 10 ml of 2N HCl was heated under reflux for 4 hr. The acetone was removed under reduced pressure and the aqueous layer was extracted with two 50 ml portions of ether. The combined ether extracts were extracted with two 50 ml portions of 5% NaHCO$_3$. The combined aqueous extracts were acidified to Congo Red with 6N HCl, and extracted with two 50 ml portions of ether. The combined ether extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a colorless liquid which solidified upon trituration with hexane. Recrystallization from ether-hexane gave 0.60 g (35%) of 12 as colorless needles, mp 92-93° (lit.[53] mp 91-92°).

Method C. A solution of 12.60 ml (0.10 mol) of hexanoic acid (13) and 15.80 ml (0.11 mol) of triethylamine in 250 ml of anhydrous N,N-dimethylformamide was cooled to 0°, and treated with 8.80 ml (0.088 mol) of ethyl chlorofor-

mate $(D_4^4$ 1.1596).[54]   After 5 min a solution of 7.50 g

(0.10 mol) of glycine (11) in 100 ml of 1N NaOH was added,

and the solution was stirred for 1 hr at 20°. The solution

was concentrated under reduced pressure, and the residue was

dissolved in a mixture of 100 ml of water and 150 ml of

chloroform. The layers were separated and the chloroform

solution was extracted with several portions of 6.5% $NaHCO_3$.

The combined aqueous extracts were acidified to Congo Red

with 6N HCl and extracted with two 50 ml portions of chloro-

form. The combined organic extracts were dried ($MgSO_4$) and

concentrated under reduced pressure to give a yellow solid.

Recrystallization of the solid from benzene gave 8.02 g

(52.5%) of 12 as colorless needles, mp 94-95° (lit.[53] mp

91-92°).

Preparation of N-Hexanoylglycyl-L-prolyl-5-amino-

valeric Acid (19). Method A.   A solution of 3.46 g (0.02

mol) of N-hexanoylglycine (12) and 2.83 ml (0.02 mol) of

triethylamine in 50 ml of anhydrous benzene was cooled to

-5° and treated with 1.60 ml (0.016 mol) of ethyl chlorofor-

mate. After 25 min a solution of 2.30 g (0.02 mol) of L-

proline (16) in 20 ml of 2N NaOH was added and the mixture

was stirred rapidly for 3 hr at 20°. The layers were sep-

arated and the aqueous solution was washed with two 50 ml

portions of ether. The aqueous solution was acidified to

Congo Red with 6N HCl and extracted with three 50 ml portions

of chloroform. The combined organic extracts were dried

($MgSO_4$) and concentrated under reduced pressure to give a

viscous oil. Trituration with hexane produced a waxy solid. The solid was removed by filtration, washed with hexane, and dried to give 3.57 g (82.5%) of crude N-hexanoylglycyl-L-proline (17).

A solution of 2.70 g (0.01 mol) of crude 17 and 1.42 ml (0.01 mol) of triethylamine in 50 ml of anhydrous benzene was cooled to -5° and treated with 0.80 ml (0.008 mol) of ethyl chloroformate. After 25 min a solution of 1.17 g (0.01 mol) of 5-aminovaleric acid (18) in 20 ml of 1N NaOH was added and the mixture was stirred rapidly for 3 hr at 20°. The layers were separated and the aqueous solution was washed with two 50 ml portions of ether. The aqueous solution was acidified to Congo Red with 6N HCl and extracted with three 50 ml portions of chloroform. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a viscous oil. Trituration with ether caused the formation of a gummy solid. Recrystallization from ether-tetrahydrofuran gave 0.85 g (29.6%) of 19 as colorless crystals: mp 111-113°; ir (Appendix A, Figure 1, KBr) 3210 (NH), 1695 (acid C=O), and 1630 (amide C=O), with shoulders at 1670 (amide C=O), and 1650 cm^{-1} (amide C=O); nmr (Appendix B, Figure 1, TFA) δ8.30 (d, 1, J = 16 Hz), 7.64 (s, 1), 4.94-4.58 (m, 1), 4.58-4.13 (m, 2), 4.05-3.65 (m, 2), 3.65-3.30 (m, 2), 2.90-1.08 (m, 19), 0.94 (t, 3, J = 5 Hz).

An analytical sample was prepared by recrystallization of the solid from ether-tetrahydrofuran, mp 111-114°.

Anal. Calcd for $C_{18}H_{31}N_3O_5$: C, 58.52; H, 8.46; N, 11.37. Found: C, 58.44; H, 8.66; N, 11.06.

Attempted Preparation of N-Hexanoylglycyl-L-prolyl-5-aminovaleric Acid (19). A solution of 6.92 g (0.04 mol) of N-hexanoylglycine (12) and 5.66 ml (0.04 mol) of triethyl-amine in 150 ml of anhydrous benzene was cooled to -14° and treated with 3.2 ml (0.032 mol) of ethyl chloroformate. After 25 min a solution of 4.60 g (0.04 mol) of L-proline (16) in 40 ml of 2N NaOH was added, and the mixture was stirred rapidly for 3 hr at 20°. The layers were separated, and the aqueous solution was washed with three 50 ml por-tions of ether. The aqueous solution was acidified to Congo Red with 6N HCl and extracted with three 75 ml por-tions of chloroform. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a viscous oil. Trituration with hexane gave 6.78 g (78.5%) of crude N-hexanoylglycyl-L-proline (17) as a waxy solid.

A solution of 5.40 g (0.02 mol) of crude 17 and 3.12 ml (0.022 mol) of triethylamine in 75 ml of anhydrous methylene chloride was cooled to -9° and treated with 1.76 ml (0.018 mol) of ethyl chloroformate. After 25 min a solution of 2.57 g (0.022 mol) of 5-aminovaleric acid (18) in 40 ml of 2N NaOH was added and the mixture was stirred rapidly for 5 hr at 20°. The layers were separated and the aqueous solution was washed with two 50 ml portions of ether. The aqueous solution was acidified to Congo Red with

6N HCl and extracted with three 75 ml portions of chloro-
form. The combined organic extracts were dried (MgSO$_4$) and
concentrated under reduced pressure to give a yellow oil.
Crystallization of this oil from ether-tetrahydrofuran gave
370 mg (7.55%) of a solid identified by nmr as N-hexanoyl-
glycyl-5-aminovaleric acid (20), mp 141-144°. Concentration
of the mother liquor gave a mixture of N-hexanoylglycine
(12) and N-hexanoylglycyl-L-proline (17). Recrystallization
of 20 from ether-tetrahydrofuran gave 240 mg (4.9%) of
colorless solid: mp 144-145.5°; ir (Appendix A, Figure 2,
KBr) 3250 (NH), 1685 (acid C=O), and 1630 cm^{-1} (amide C=O);
nmr (Appendix B, Figure 2, TFA) δ8.40 (s, 1), 7.86 (s, 1),
4.45 (s, 2), 4.10-3.32 (m, 2), 3.20-2.40 (m, 4), 2.40-1.24
(m, 10), 1.00 (t, 3, J = 6 Hz).

Anal. Calcd for C$_{13}$H$_{24}$N$_2$O$_4$: C, 57.33; H, 8.88;
N, 10.29. Found: C, 57.41; H, 8.91; N, 10.30.

Preparation of N-Benzyloxycarbonyl-L-proline (22).
A solution of 2.30 g (0.02 mol) of L-proline (16) in 100 ml
of 2N NaOH was cooled to 5° and treated with 3.48 g (0.022
mol) of benzyl chloroformate. The mixture was stirred
vigorously for 2 hr at 5° and washed with two 75 ml portions
of ether. The aqueous solution was acidified to Congo Red
with 6N HCl and extracted with four 50 ml portions of chloro-
form. The combined organic extracts were dried (MgSO$_4$) and
concentrated under reduced pressure to give 7.50 g of yellow
oil. This oil could not be crystallized and a sample was
converted to the dicyclohexylamine salt (23) for character-

ization: mp 176-177°; $[\alpha]_D^{26}$ -24.0° (c 2, MeOH) (lit.[37] mp

179-180°, $[\alpha]_D$ -25.5° (c 2, MeOH)).

Preparation of Methyl 5-Aminovalerate Hydrochloride

(21). A solution of 3.51 g (0.03 mol) of 5-aminovaleric

acid (18), 30 ml of 2,2-dimethoxypropane, and 3 ml of 12N

HCl was allowed to stand 25 hr at 20°. The solution was

concentrated under reduced pressure and the residue was dis-

solved in 75 ml of methanol. The solution was diluted with

200 ml of ether, causing the formation of a crystalline

precipitate. The precipitate was removed by filtration,

washed with ether, and dried to give 3.56 g (71%) of 21 as

colorless crystals, mp 145-147° (lit.[55] mp 145-147°).

Preparation of L-Prolyl-5-aminovaleric Acid Methyl

Ester (25). A solution of 12.50 g (0.05 mol) of crude N-

benzyloxycarbonyl-L-proline (22) and 7.0 ml (0.05 mol) of

triethylamine in 100 ml of anhydrous benzene was cooled to

5° and treated with 4.0 ml (0.04 mol) of ethyl chloroformate.

After 20 min a solution of 8.35 g (0.05 mol) of methyl 5-

aminovalerate hydrochloride (21) and 14.0 ml (0.10 mol) of

triethylamine in 150 ml of anhydrous benzene was added.

After 18 hr at 20° the solution was washed successively with

200 ml of water, two 100 ml portions of 2N HCl, 100 ml of

water, two 100 ml portions of 2N NaOH, and 100 ml of water.

The organic solution was dried ($MgSO_4$) and concentrated

under reduced pressure to give 11.50 g (79.5%) of N-benzyl-

oxycarbonyl-L-prolyl-5-aminovaleric acid methyl ester (24)

as a viscous oil: ir (Appendix A, Figure 3, neat) 3320 (NH),

1700 (C=O), with shoulders at 1735 (C=O), 1660 (C=O), and 1680 cm^{-1} (C=O); nmr (Appendix B, Figure 3, CDCl$_3$) δ7.90 (s, 5), 7.50 (s, 2), 4.80-4.30 (m, 1), 3.95-3.20 (m, 7), 2.60-1.30 (m, 10). TLC in three solvent systems indicated the product was homogeneous and it was used without further purification in the next reaction.

A solution of crude 24 in 200 ml of methanol containing 0.5 ml of glacial acetic acid was hydrogenated over 2.00 g of 10% palladium on carbon for 24 hr at one atmosphere pressure and 20°. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a viscous oil. This oil was dissolved in 150 ml of ether and extracted with dilute acetic acid. The combined aqueous extracts were made basic with K$_2$CO$_3$ and extracted with four 50 ml portions of chloroform. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give 8.00 g (90.5%) of 25 as a viscous oil: ir (Appendix A, Figure 4, neat) 3300 (NH), 1730 (ester C=O), 1650 cm^{-1} (amide C=O); nmr (Appendix B, Figure 4, CDCl$_3$) δ7.80 (s, 1), 3.61 (s, 3), 3.42-1.30 (m, 16). TLC in three solvent systems showed the product to be free of starting material. The product was used without further purification. A sample was converted to the hydrochloride 26: ir (Appendix A, Figure 5, neat); nmr (Appendix B, Figure 5, TFA). This salt could not be crystallized and it was used without further purification.

Preparation of N-Hexanoylglycly-L-prolyl-5-amino-
valeric Acid Methyl Ester (27).   A solution of 5.19 g (0.03
mol) of N-hexanoylglycine (12) and 4.45 ml (0.032 mol) of
triethylamine in 150 ml of anhydrous tetrahydrofuran was
cooled to -5° and treated with 2.27 ml (0.023 mol) of ethyl
chloroformate. After 25 min a solution of 7.55 g (0.028 mol)
of L-prolyl-5-aminovaleric acid methyl ester hydrochloride
(26) and 4.0 ml (0.028 mol) of triethylamine in 75 ml of an-
hydrous tetrahydrofuran was added. After 6.5 hr at 20° the
solution was concentrated under reduced pressure. The
residue was dissolved in 100 ml of methylene chloride and
washed successively with 75 ml of water, two 75 ml portions
of 2N HCl, 75 ml of water, three 75 ml portions of 2N NaOH,
and 75 ml of water. The organic solution was dried ($MgSO_4$)
and concentrated under reduced pressure to give a mushy
solid. Recrystallization from hexane-benzene gave 4.69 g
(53%) of colorless solid, mp 92-93.5°. A second recrystal-
lization from hexane-cyclohexane (50:50) gave 3.29 g (37%)
of 27 as a colorless solid: mp 92.5-93.5°; $[\alpha]_D^{25}$ -68.3° (c
2, EtOH); ir (Appendix A, Figure 6, KBr) 3245 (NH), 1740
(ester C=O), 1625 (amide C=O), with shoulders at 1650
(amide C=O), and 1665 cm^{-1} (amide C=O); nmr (Appendix B,
Figure 6, $CDCl_3$) δ7.45-6.96 (m, 2), 4.68-4.36 (m, 1), 4.08
(d, 2, J = 5 Hz), 3.70 (s, 5), 3.45-3.12 (m, 2), 2.56-1.88
(m, 8), 1.88-1.18 (m, 10), 0.92 (t, 3, J = 6 Hz).

Anal. Calcd for $C_{19}H_{33}N_3O_5$: C, 59.51; H, 8.67; N,
10.96. Found: C, 59.54; H, 8.99; N, 10.88.

Preparation of N-Hexanoylglycyl-L-prolyl-5-amino-

valeric Acid (19). Method B. A solution of 1.40 g (0.0036

mol) of N-hexanoylglycyl-L-prolyl-5-aminovaleric acid methyl

ester (27) in 20 ml of acetone and 10 ml of 2N HCl was heated

under reflux for 2 hr. The acetone was removed under reduced

pressure and the aqueous solution was extracted with three

75 ml portions of chloroform. The combined organic extracts

were extracted with two 50 ml portions of 6.5% $NaHCO_3$. The

combined aqueous extracts were acidified to Congo Red with

6N HCl and extracted with four 25 ml portions of chloroform.

The combined organic extracts were dried ($MgSO_4$) and concen-

trated under reduced pressure to give a viscous oil. Crys-

tallization from benzene gave 320 mg (24%) of 19 as colorless

crystals: mp 122.5-124.5°; $[\alpha]_D^{25}$ -70.6° ($\underline{c}$ 2, EtOH). Nmr

and ir spectra showed this sample to be identical to the

material prepared by method A.

Preparation of Pentanoyl Chloride (28). A solution

of 102.0 g (1.0 mol) of pentanoic acid and 142.8 g (1.2 mol)

of thionyl chloride was allowed to react for 3.5 hr at 20°

and warmed on a steam bath for 1 hr. Fractional distillation

gave 93.9 g (78.3%) of 28 as a yellow liquid, bp 126-126.5°

at 760 mm (lit.[56]bp 127° at 760 mm).

Preparation of N-Pentanoylglycine (29). Method A.

A solution of 15.0 g (0.2 mol) of glycine (11) in 300 ml of

2N NaOH was cooled to -5° and treated with 24.0 g (0.2 mol)

of pentanoyl chloride (28). The mixture was stirred vigor-

ously for 1 hr and washed with three 50 ml portions of

chloroform. The aqueous solution was acidified to Congo Red

with 6N HCl and extracted with three 50 ml portions of methylene chloride. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow oil. Trituration with hexane gave a colorless solid. Recrystallization from chlorobenzene gave 3.80 g (12%) of 29 as colorless needles, mp 83-84° (lit.[57] mp 80.5-82°).

Method B. A solution of 20.4 g (0.2 mol) of pentanoic acid and 26.0 ml (0.2 mol) of N-ethylmorpholine in 400 ml of anhydrous tetrahydrofuran was cooled to 0° and treated with 20.0 ml (0.2 mol) of ethyl chloroformate. After 10 min a solution of 15.0 g (0.2 mol) of glycine (11) in 200 ml of cold 1N NaOH was added and the mixture was stirred vigorously for 30 min at 0° and 3.5 hr at 20°. The tetrahydrofuran was removed under reduced pressure and the aqueous solution was washed with two 100 ml portions of methylene chloride. The aqueous solution was acidified to Congo Red with 6N HCl and extracted with methylene chloride for 48 hr on a liquid-liquid extractor. The organic solution was dried (MgSO$_4$) and concentrated under reduced pressure to give an oily solid. Recrystallization from benzene-chloroform (50:50) gave 20.3 g (64%) of 29 as colorless crystals, mp 81.5-83° (lit.[57] mp 80.5-82°).

Preparation of N-Pentanoylglycyl-L-prolyl-5-amino-valeric Acid Methyl Ester (30). Method A. A solution of 1.59 g (0.01 mol) of N-pentanoylglycine (29) and 1.4 ml (0.01 mol) of triethylamine in 20 ml of anhydrous tetrahydrofuran was cooled to 0° and treated with 0.9 ml (0.009 mol)

of ethyl chloroformate. After 5 min a cold solution of 2.28

g (0.01 mol) of L-prolyl-5-aminovaleric acid methyl ester (25)

in 20 ml of anhydrous tetrahydrofuran was added. The solution

was stirred for 30 min at 0° and for 7 hr at 20°. The solution

was concentrated under reduced pressure and the residue was

dissolved in 75 ml of ethyl acetate. The organic solution

was washed with two 50 ml portions of 1N HCl and two 50 ml

portions of 6.5% NaHCO$_3$. The organic solution was dried

(MgSO$_4$) and concentrated under reduced pressure to give a

solid. Recrystallization from cyclohexane-benzene (90:10)

gave 0.2 g (6%) of 30 as colorless crystals: mp 98-99.5°;

$[\alpha]_D^{20}$ -75.0° (c 2, EtOH); ir (Appendix A, Figure 7, nujol)

3250 (NH), 1735 (ester C=O), 1630 (amide C=O), with a

shoulder at 1665 cm^{-1} (amide C=O); nmr (Appendix B, Figure

7, CDCl$_3$) δ7.40-6.80 (m, 2), 4.72-4.35 (m, 1), 4.05 (d, 2,

J = 4 Hz), 3.68 (s, 3), 3.60-3.00 (m, 4), 2.50-1.10 (m, 16),

0.91 (t, 3, J = 6 Hz).

Anal. Calcd for C$_{18}$H$_{31}$N$_3$O$_5$: C, 58.52; H, 8.46;

N, 11.37. Found: C, 58.72; H, 8.68; N, 11.44.

Method B. A solution of 4.56 g (0.02 mol) of L-

prolyl-5-aminovaleric acid methyl ester (25) and 3.18 g (0.02

mol) of N-pentanoylglycine (29) in 55 ml of ethyl acetate

and 10 ml of methylene chloride was treated with 4.53 g

(0.022 mol) of N,N'-dicyclohexylcarbodiimide. After standing

48 hr at 20° the N,N'-dicyclohexylurea was removed by filtra-

tion and washed with ethyl acetate. The filtrate was washed

successively with 25 ml of 1N HCl, 25 ml of water, 25 ml of

6.5% NaHCO$_3$, and 25 ml of water. The organic solution was

dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow oil. Crystallization from cyclohexane-benzene (90:10) gave 3.28 g (44.5%) of 30 as colorless crystals: mp 98.5-99.5°; [α]$_D^{20}$ -69.1° (c 2, EtOH). This material was shown to be identical to that prepared by Method A by melting point and optical rotation.

Preparation of L-Proline Methyl Ester (31). A solution of 4.60 g (0.04 mol) of L-proline (16) and 5 ml of 12N HCl in 50 ml of 2,2-dimethoxypropane was allowed to stand for 19 hr at 20°. The solution was concentrated under reduced pressure and the residue was treated with a slurry of K$_2$CO$_3$ in water and 75 ml of ether. This mixture was stirred thoroughly and the ether was removed by decantation. The residue was triturated with a second 75 ml portion of ether. The combined ether extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow oil. Fractional distillation gave 2.54 g (49.2%) of 31 as a colorless liquid, bp 58-58.5° at 4.1 mm (lit.[58] bp 70° at 10 mm).

Preparation of N-Hexanoylglycyl-L-proline Methyl Ester (32). A solution of 8.65 g (0.05 mol) of N-hexanoylglycine (12) and 7.0 ml (0.05 mol) of triethylamine in 200 ml of anhydrous methylene chloride was cooled to -10° and treated with 4.0 ml (0.04 mol) of ethyl chloroformate. After 5 min a cold solution of 8.28 g (0.05 mol) of L-proline methyl ester hydrochloride and 7.0 ml (0.05 mol) of triethyl-amine in 100 ml of anhydrous methylene chloride was added. The solution was stirred for 30 min at -10° and for 21 hr at

20°. The solution was washed successively with two 100 ml

portions of 2N HCl, 100 ml of water, two 150 ml portions of

6.5% NaHCO$_3$, and 200 ml of water. The organic solution was

dried (MgSO$_4$) and concentrated under reduced pressure to

give 7.85 g (69.0%) of crude 32 as a viscous red oil: ir

(Appendix A, Figure 8, neat) 3300 (NH), 1765 (ester C=O), 1660

(amide C=O), with a shoulder at 1725 cm^{-1} (amide C=O); nmr

(Appendix B, Figure 8, CDCl$_3$) δ6.90 (s, 1), 4.70-3.90 (m, 3),

3.72 (s, 3), 3.65-3.16 (m, 2), 2.45-1.05 (m, 12), 0.88 (t, 3,

J = 5 Hz). This crude material was used without further

purification.

Preparation of N-Hexanoylglycyl-L-proline (33).

Method A. A 10.20 g (0.036 mol) sample of N-hexanoylglycyl-

L-proline methyl ester (32) was hydrolyzed with 40 ml of 1N

NaOH for 5 min at 20°. The mixture was washed with three 50

ml portions of chloroform and acidified to Congo Red with 6N

HCl. The oil which formed was extracted into three 50 ml

portions of chloroform. The combined organic extracts were

dried (MgSO$_4$) and concentrated under reduced pressure to give

a yellow solid. Recrystallization from cyclohexane-dioxane

(65:35) and then from cyclohexane-benzene (50:50) gave 5.65 g

(58%) of 33 as colorless crystals: mp 122-123.5°; ir

(Appendix A, Figure 9, KBr) 3250 (NH), 1725 (acid C=O), 1645

(amide C=O), 1615 cm^{-1} (amide C=O); nmr (Appendix B, Figure

9, TFA) δ7.88 (s, 1), 4.70-3.80 (m, 3), 3.70-3.20 (m, 2),

2.45-0.98 (m, 10), 0.98-0.40 (m, 3).

Anal. Calcd for $C_{13}H_{22}N_2O_4$: C, 57.75; H, 8.22;
N, 10.36. Found: C, 57.67; H, 8.23; N, 10.35.

Method B. A solution of 7.85 g (0.028 mol) of N-
hexanoylglycyl-L-proline methyl ester (32) in 300 ml of
acetone and 150 ml of 2N HCl was heated under reflux for 5
hr. The acetone was removed under reduced pressure and the
product was extracted into three 50 ml portions of chloroform.
The combined organic extracts were extracted with four 75 ml
portions of 6.5% $NaHCO_3$. The combined aqueous extracts were
acidified to Congo Red with 6N HCl and extracted with four
75 ml portions of chloroform. The combined organic extracts
were dried ($MgSO_4$) and concentrated under reduced pressure
to give 2.00 g (25%) of yellow solid. Recrystallization
from cyclohexane-dioxane (65:35) gave 1.67 g (22%) of 33 as
colorless crystals, mp 124-125°.

Preparation of N-Hexanoylglycyl-L-prolylglycylglycine
Benzyl Ester (35). Method A. A solution of 2.70 g (0.01
mol) of N-hexanoylglycyl-L-proline (33) and 1.40 ml of tri-
ethylamine in 40 ml of anhydrous methylene chloride was cooled
to 0° and treated with 0.88 ml (0.009 mol) of ethyl chloro-
formate. After 20 min a cold solution of 3.95 g (0.01 mol)
of glycylglycine benzyl ester p-toluenesulfonate (34) and
1.40 ml of triethylamine in 40 ml of anhydrous methylene
chloride was added. The solution was stirred for 30 min at
0° and for 8 hr at 20°. The solution was washed with two
50 ml portions of 1N HCl and two 50 ml portions of 6.5%

NaHCO$_3$. The organic solution was dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow solid. Recrystallization from benzene gave 3.85 g (90%) of 35 as colorless crystals: mp 149.5-151.5°; ir (Appendix A, Figure 10, nujol) 3300 (NH), 1720 (ester C=O), 1650 (amide C=O), with shoulders at 1665 (amide C=O), and 1680 (amide C=O), 1625 cm^{-1} (amide C=O); nmr (Appendix B, Figure 10, CDCl$_3$) δ8.10-7.56 (m, 2), 7.38 (s, 5), 6.99 (s, 1), 5.18 (s, 2), 4.75-3.00 (m, 9), 2.50-1.00 (m, 12), 1.00-0.60 (m, 3).

Anal. Calcd for C$_{24}$H$_{34}$N$_4$O$_6$: C, 60.74; H, 7.22; N, 11.81. Found: C, 60.75; H, 7.25; N, 11.63.

Method B. A solution of 3.95 g (0.01 mol) of glycylglycine benzyl ester p-toluenesulfonate (34), 1.40 ml of triethylamine, and 2.70 g (0.01 mol) of N-hexanoylglycyl-L-proline (33) in 40 ml of methylene chloride was treated with 2.27 g (0.011 mol) of N,N'-dicyclohexylcarbodiimide. After 48 hr at 20° the N,N'-dicyclohexylurea was removed by filtration and washed with methylene chloride. The filtrate was washed successively with 25 ml of 1N HCl, 25 ml of water, 25 ml of 6.5% NaHCO$_3$, and 25 ml of water. The organic solution was dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow oil. Crystallization from benzene gave 3.85 g (80%) of 35 as colorless crystals, mp 147.5-151.5°.

Preparation of N-Hexanoylglycyl-L-prolylglycylglycine (36). A solution of 3.85 g (0.008 mol) of N-hexanoylglycyl-L-prolylglycylglycine benzyl ester (35) in 150 ml of ethanol

was hydrogenated over 1.00 g of 10% palladium on carbon for 8 hr at 60 psi and 20°. The catalyst was removed by filtration through Celite and washed with ethanol. The filtrate was concentrated under reduced pressure to give a viscous oil, which was crystallized from tetrahydrofuran to give 1.95 g (63.5%) of 36 as a tan solid: mp 140-142°; ir (Appendix A, Figure 11, KBr) 3290 (NH), 1720 (acid C=O), 1640 (amide C=O), with shoulders at 1655 (amide C=O), and 1625 cm^{-1} (amide C=O); nmr (Appendix B, Figure 11, TFA) δ8.44 (s, 1), 8.10 (s, 1), 7.80 (s, 1), 4.95-4.64 (m, 2), 4.64-4.20 (m, 5), 3.95-3.72 (m, 2), 2.83-2.10 (m, 6), 2.03-1.20 (m, 6), 1.00 (t, 3, J = 6 Hz).

An analytical sample was prepared by recrystallization from dioxane, mp 140-142°.

Anal. Calcd for $C_{17}H_{28}N_4O_6$: C, 53.11; H, 7.34; N, 14.57. Found: C, 52.85; H, 7.58; N, 14.72.

Preparation of N-Hexanoylglycyl-L-prolylglycylglycin-amide (37). A cold solution of 0.80 g (0.00168 mol) of N-hexanoylglycyl-L-prolylglycylglycine benzyl ester (35) in 150 ml of methanol was saturated with ammonia. After 3 hr at 0° the solution was concentrated under reduced pressure to give a yellow solid. Recrystallization from chloroform-methanol (95:5) gave 0.44 g (68.4%) of 37 as colorless crystals: mp 186-186.5°; $[\alpha]_D^{20}$ -47.6°; ir (Appendix A, Figure 12, KBr) 3250 (NH), 1630 (amide C=O), with shoulders at 1666 (amide C=O), 1645 (C=O), and 1625 cm^{-1} (amide C=O); nmr (Appendix B, Figure 12, TFA) δ8.50-7.25 (m, 5), 4.95-4.64 (m, 1), 4.64-

4.00 (m, 6), 4.00-3.56 (m, 2), 2.80-2.00 (m, 6), 2.00-1.60
(m, 4), 0.96 (t, 3, $\underline{J}$ = 6 Hz).

Anal. Calcd for $C_{17}H_{29}N_5O_5 \cdot H_2O$: C, 50.86; H, 7.78;
N, 17.44. Found: C, 51.13; H, 7.38; N, 17.69.

Preparation of N-Benzyloxycarbonyl-L-leucyl-L-leucine

Methyl Ester (40). Method A. A solution of 2.65 g (0.01 mol)
of N-benzyloxycarbonyl-L-leucine (38), 1.30 ml (0.01 mol) of
N-ethylmorpholine, and 1.82 g (0.01 mol) of L-leucine methyl
ester hydrochloride (39) in 50 ml of methylene chloride was
cooled to -1° and treated with 2.27 g (0.011 mol) of N,N'-
dicyclohexylcarbodiimide. The solution was stirred 1 hr at
-1° and for 4 hr at 20°. The N,N'-dicyclohexylurea was re-
moved by filtration and washed with methylene chloride. The
filtrate was washed with two 25 ml portions of 1N HCl and
two 50 ml portions of 5% NaHCO$_3$. The organic solution was
dried (MgSO$_4$) and concentrated under reduced pressure to
give a viscous oil. Crystallization from medium boiling
petroleum ether-ethyl acetate (75:5) gave 1.66 g (42.5%) of
40 as colorless crystals, mp 86-87° (lit.[59] mp 93-95°). Con-
centration of the mother liquor gave 2.26 g of a yellow oil
that was shown by nmr to be mainly N-acylurea.

Method B. A solution of 2.65 g (0.01 mol) of N-
benzyloxycarbonyl-L-leucine (38) and 1.3 ml (0.01 mol) of
N-ethylmorpholine in 50 ml of anhydrous tetrahydrofuran was
cooled to -13° and treated with 0.8 ml (0.008 mol) of ethyl
chloroformate. After 7 min a cold solution of 1.82 g (0.01
mol) of L-leucine methyl ester hydrochloride (39) and 1.3 ml

of N-ethylmorpholine in 25 ml of anhydrous tetrahydrofuran
was added. The solution was stirred for 2 hr at -13° and for
16 hr at 20°. The solution was concentrated under reduced
pressure and the residue was dissolved in a mixture of 50 ml
of ethyl acetate and 50 ml of water. The layers were sep-
arated and the organic solution was washed with 50 ml of 1N
HCl and two 50 ml portions of 5% NaHCO$_3$. The organic solution
was dried (MgSO$_4$) and concentrated under reduced pressure to
give an oily solid. Recrystallization from hexane-cyclohexane
(50:50) gave 2.30 g (73.5%) of 40 as colorless needles: mp
93-96°; $[\alpha]_D^{26}$ -35.2° (c 2, EtOH) (lit.[59] mp 93-95°, $[\alpha]_D^{26}$
-40°).

Attempted Preparation of L-Leucyl-L-leucine Methyl
Ester Hydrobromide (41). Method A. A solution of 1.26 g
of N-benzyloxycarbonyl-L-leucyl-L-leucine methyl ester (40)
in 10 ml of glacial acetic acid was treated with 10 ml of
30% HBr in acetic acid. The solution was allowed to stand
1 hr at 20° and was concentrated under reduced pressure to
give an oily residue. The nmr spectrum of this oil showed
the absence of a signal due to a methyl ester and no further
workup was attempted.

Method B. A solution of 1.66 g of N-benzyloxycar-
bonyl-L-leucyl-L-leucine methyl ester (40) and 1.0 ml of
glacial acetic acid in 50 ml of methanol was hydrogenated
over 0.2 g of 5% palladium on carbon for 16 hr at 20°. The
catalyst was removed by filtration through Celite and washed
with methanol. The filtrate was concentrated under reduced

pressure and the residue was dissolved in 50 ml of ethyl acetate. The organic solution was extracted with three 20 ml portions of 1N HCl. The combined aqueous extracts were made basic with K_2CO_3 and extracted with three 30 ml portions of ether. The combined extracts were dried ($MgSO_4$) and hydrogen bromide gas was bubbled into the solution, causing the formation of a brown oil. The oil was removed by decantation and dried under reduced pressure. An nmr of this oil suggested it was the hydrobromide salt of the diketopiperazine of L-leucyl-L-leucine, 42.

Preparation of L-Leucine t-Butyl Ester Phosphite Salt (44). The method of Roeske[35] was followed to give a 74% yield of 33: mp 167-168°; $[\alpha]_D^{26}$ 6.2° (c 5, H_2O) (lit.[60] mp 163-164°, $[\alpha]_D^{25}$ 5.0° (c 5, H_2O)).

Preparation of 1-Hydroxybenzotriazole. The procedure of Konig and Geiger[36] was followed to give a 49.7% yield of 1-hydroxybenzotriazole, mp 154-157.5° (lit.[61] mp 157°).

Preparation of N-Benzyloxycarbonyl-L-leucyl-L-leucine t-Butyl Ester (45). Method A. A solution of 5.30 g (0.02 mol) of N-benzyloxycarbonyl-L-leucine (38) and 2.6 ml (0.02 mol) of N-ethylmorpholine in 75 ml of anhydrous tetrahydrofuran was cooled to -15° and treated with 2.0 ml (0.02 mol) of ethyl chloroformate. After 5 min a cold mixture of 5.38 g (0.02 mol) of L-leucine t-butyl ester phosphite salt (44) and 2.6 ml of N-ethylmorpholine in 100 ml of anhydrous tetrahydrofuran was added. The mixture was

stirred for 1.5 hr at -15° and for 24 hr at 20°. The solvent was removed under reduced pressure, and the residue was dissolved in a mixture of 75 ml of water and 75 ml of ethyl acetate. The layers were separated and the organic solution was washed with 75 ml of 5% citric acid and 75 ml of 5% $NaHCO_3$. The organic solution was dried $(MgSO_4)$ and concentrated under reduced pressure to give a colorless solid. Recrystallization from hexane-cyclohexane (50:50) gave 5.43 g (62.5%) of <u>45</u> as colorless needles: mp 130.5-131.5°; $[\alpha]_D^{27}$ -42.4° (<u>c</u> 4, EtOH); ir (Appendix A, Figure 13, KBr) 3300 (NH), 1740 (ester C=O), 1680 (carbonate C=O), 1645 cm^{-1} (amide C=O); nmr (Appendix B, Figure 13, $CDCl_3$) δ7.08 (s, 5), 6.73 (s, 1), 5.73 (s, 1), 4.93 (s, 2), 4.46-3.96 (m, 2), 1.78-1.20 (m, 15), 0.87 (d, 12, <u>J</u> = 5 Hz).

Recrystallization of the solid from hexane gave an analytical sample, mp 131-132°.

<u>Anal</u>. Calcd for $C_{24}H_{38}N_2O_5$: C, 66.33; H, 8.81; N, 6.45. Found: C, 66.49; H, 8.60; N, 6.61.

<u>Method B</u>. A solution of 2.65 g (0.01 mol) of N-benzyloxycarbonyl-L-leucine (<u>38</u>) 2.69 g (0.01 mol) of L-leucine <u>t</u>-butyl ester phosphite salt (<u>44</u>), 1.3 ml (0.01 mol) of N-ethylmorpholine, and 3.06 g (0.02 mol) of 1-hydroxybenzotriazole in 75 ml of tetrahydrofuran was cooled to -14° and treated with 2.06 g (0.01 mol) of N,N'-dicyclohexylcarbodiimide. The solution was stirred for 1 hr at -14° and for 4 hr at 20°. The N,N'-dicyclohexylurea was removed by filtration and washed with tetrahydrofuran. The filtrate

was concentrated under reduced pressure and the residue was
dissolved in 100 ml of ethyl acetate. The organic solution
was washed with 50 ml of water, 50 ml of 5% citric acid, and
50 ml of 5% NaHCO$_3$. The organic solution was dried (MgSO$_4$)
and concentrated under reduced pressure. The residue was
dissolved in 10 ml of ethyl acetate and filtered through a
10 cm column of basic alumina. Concentration of the filtrate
gave a yellow solid. Recrystallization from hexane-cyclohex-
ane (50:50) gave 2.20 g (51%) of 45 as colorless needles,
mp 130-130.5°.

Preparation of L-Leucyl-L-leucine t-Butyl Ester (46).
A solution of 11.20 g (0.026 mol) of N-benzyloxycarbonyl-L-
leucyl-L-leucine t-butyl ester (45) in 200 ml of ethanol
containing 0.2 ml of glacial acetic acid was hydrogenated
for 48 hr over 1.0 g of 10% palladium on carbon at 60 psi
and 20°. The catalyst was removed by filtration through
Celite and washed with ethanol. The filtrate was concentra-
ted under reduced pressure and the residue was dissolved in
150 ml of ether. The organic solution was extracted with
four 75 ml portions of 5% citric acid. The combined aqueous
extracts were made basic with K$_2$CO$_3$ and extracted with four
50 ml portions of ether. The combined organic extracts were
dried (MgSO$_4$) and concentrated under reduced pressure to
give 6.99 g (89%) of 46 as a viscous oil: ir (Appendix A,
Figure 14, neat) 3330 (NH), 1730 (ester C=O), 1650 cm^{-1}
(amide C=O); nmr (Appendix B, Figure 14, CDCl$_3$) δ7.68 (s, 1),
4.64-4.33 (s, 2), 3.52-3.24 (m, 2), 1.95-1.20 (m, 15), 0.98

(d, 12, $\underline{J}$ = 5 Hz).

$\underline{\text{Anal}}$. Calcd for $C_{16}H_{32}N_2O_3$: C, 63.96; H, 10.74; N, 9.32. Found: C, 63.90; H, 10.91; N, 9.23.

Attempted Preparation of N-Benzyloxycarbonyl-L-prolyl-L-leucyl-L-leucine $\underline{t}$-Butyl Ester (47). A mixture of 3.08 g (0.0072 mol) of N-benzyloxycarbonyl-L-proline dicyclohexylamine salt (23) in 50 ml of anhydrous tetrahydrofuran was cooled to -14° and treated with 0.71 ml (0.0072 mol) of ethyl chloroformate. After 15 min a cold solution of 2.14 g (0.0072 mol) of L-leucyl-L-leucine $\underline{t}$-butyl ester (46) in 25 ml of anhydrous tetrahydrofuran was added. The mixture was stirred for 2 hr at -14° and for 20 hr at 20°. The solvent was removed under reduced pressure and the residue was dissolved in a mixture of 50 ml of ethyl acetate and 50 ml of water. The layers were separated and the organic solution was washed with two 50 ml portions of 5% citric acid and two 50 ml portions of 5% NaHCO_3. The organic solution was dried (MgSO_4) and concentrated under reduced pressure to give a yellow solid. Recrystallization from hexane gave 1.76 g (66%) of colorless needles, mp 106-109°, which was shown to be N-ethoxycarbonyl-L-leucyl-L-leucine $\underline{t}$-butyl ester (48). Two additional recrystallizations from hexane-cyclohexane (50:50) gave 48 as colorless needles: mp 114-115.5°; ir (Appendix A, Figure 15, KBr) 3335 (NH), 3220 (NH), 1745 (ester C=O), 1680 (carbamate C=O), 1645 cm^{-1} (amide C=O); nmr (Appendix B, Figure 15, CDCl_3)

$\delta 6.65-6.36$ (m, 1), 5.46-5.14 (m, 1), 4.62-4.30 (m, 2), 4.13
(q, 2, $\underline{J}$ = 7 Hz), 1.90-1.34 (m, 15), 1.24 (t, 3, $\underline{J}$ = 7 Hz),
0.94 (d, 12, $\underline{J}$ = 6 Hz).

$\underline{Anal}$. Calcd for $C_{19}H_{36}N_2O_5$: C, 61.26; H, 9.74;
N, 7.52. Found: C, 61.19; H, 9.91; N, 7.54.

$\underline{Preparation\ of\ N-Benzyloxycarbonyl-L-prolyl-L-leucyl-}$
$\underline{L-leucine\ \underline{t}-Butyl\ Ester\ (\underline{47})}$. Method A. A mixture of 1.11
g (0.0026 mol) of N-benzyloxycarbonyl-L-proline dicyclohexyl-
amine salt ($\underline{23}$), 50 ml of ether, and 50 ml of 0.5N H_2SO_4 was
shaken in a separatory funnel until the solid had dissolved.
The layers were separated and the aqueous layer was extracted
with 50 ml of ether. The combined ether extracts were dried
($MgSO_4$) and concentrated under reduced pressure to give a
viscous oil. This oil and 0.35 ml (0.0026 mol) of N-ethyl-
morpholine were dissolved in 40 ml of dry tetrahydrofuran,
cooled to -14°, and treated with 0.25 ml (0.026 mol) of
ethyl chloroformate. After 10 min a solution of 0.77 g
(0.0026 mol) of L-leucyl-L-leucine $\underline{t}$-butyl ester ($\underline{46}$) in 40
ml of dry tetrahydrofuran was added. The solution was
stirred for 2 hr at -14° and for 21 hr at 20°. The solvent
was removed under reduced pressure and the residue was dis-
solved in 50 ml of ethyl acetate. The solution was washed
with 50 ml of 5% citric acid and 50 ml of 5% $NaHCO_3$. The
organic solution was dried ($MgSO_4$) and concentrated under
reduced pressure to give a viscous oil which was crystallized
from hexane-cyclohexane (50:50) to give 0.66 g of a colorless
solid, A, mp 108-118°. Concentration of the mother liquor
gave an oil which was purified by chromatography on a

3.5 x 39 cm column of silica gel using chloroform-methanol
(95:5) as the eluent. Separation of this material gave an
additional 0.08 g of A and 0.28 g of a second solid, B.
Compounds A and B were identified by TLC comparisons with
authentic samples. Compound A was shown to be N-benzyloxy-
carbonyl-L-prolyl-L-leucyl-L-leucine t-butyl ester (47).
The 0.74 g isolated represents a 54.2% yield. Compound B
was shown to be N-ethoxycarbonyl-L-leucyl-L-leucine t-butyl
ester (48) and the 0.28 g isolated represents a 29% yield.

Preparation of N-Benzyloxycarbonyl-L-prolyl-L-leucyl-
L-leucine t-Butyl Ester (47). Method B. A solution of
0.93 g (0.0026 mol) of N-benzyloxycarbonyl-L-proline p-
nitrophenyl ester (50), 0.77 g (0.0026 mol) of L-leucyl-L-
leucine t-butyl ester (46) and 0.36 g (0.0052 mol) of imid-
azole in 50 ml of anhydrous tetrahydrofuran was allowed to
stand for 24 hr at 20°. The solution was concentrated under
reduced pressure and the residue was dissolved in 75 ml of
ether. The organic solution was washed with two 50 ml por-
tions of 5% citric acid and two 50 ml portions of cold 1N
NaOH. The organic solution was dried ($MgSO_4$) and concen-
trated under reduced pressure to give a colorless solid.
Recrystallization from hexane-cyclohexane (50:50) gave 1.04
g (76%) of 47 as colorless solid: mp 121-122°; $[\alpha]_D^{21}$
-91.8° (c 2, EtOH); ir (Appendix A, Figure 16, KBr) 3210
(NH), 1720 (C=O), with shoulders at 1730 (C=O), and 1690
(C=O), 1640 cm^{-1} (amide C=O); nmr (Appendix B, Figure 16,
$CDCl_3$) δ7.42 (s, 5), 6.98-6.24 (m, 2), 5.20 (s, 2), 4.52-

4.30 (m, 3), 3.61-3.46 (m, 2), 2.30-1.48 (m, 19), 0.93 (d, 12, $\underline{J}$ = 3 Hz).

Recrystallization of the solid from hexane-cyclo-hexane (50:50) gave an analytical sample, mp 121-122°.

$\underline{Anal}$. Calcd for $C_{29}H_{45}N_3O_6$: C, 65.51; H, 8.53; N, 7.90. Found: C, 65.62; H, 8.71; N, 7.83.

Method C. The above procedure was followed except that the reaction time was extended to 72 hr at 20° to give an 86.5% yield of $\underline{47}$ as colorless needles, mp 121-122.5°.

Method D. The procedure in method B was followed except that the reaction conditions were 24 hr at 50° to give a 90.5% yield of $\underline{47}$ as colorless needles, mp 121-122.5°.

Preparation of N-Benzyloxycarbonyl-L-proline $\underline{p}$-Nitrophenyl Ester ($\underline{50}$). Method A. A mixture of 23.91 g (0.056 mol) of N-benzyloxycarbonyl-L-proline dicyclohexyl-amine salt ($\underline{23}$), 200 ml of ether, and 200 ml of 0.5N H_2SO_4 was shaken in a separatory funnel until the solid dissolved. The layers were separated and the aqueous solution was ex-tracted with 100 ml of ether. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure) to give a viscous oil. This oil, and 8.53 g (0.061 mol) of $\underline{p}$-nitrophenol was dissolved in 200 ml of anhydrous tetrahydro-furan,cooled to -5°, and treated with 11.45 g (0.056 mol) of N,N'-dicyclohexylcarbodiimide. The solution was stirred for 0.5 hr at -5° and for 2.5 hr at 20°. The N,N'-dicyclohexyl-urea was removed by filtration and washed with tetrahydro-

furan. The filtrate was concentrated under reduced pressure
to give a yellow solid. Two recrystallizations from ethanol
gave 12.70 g (62%) of 50 as colorless needles, mp 94-96°
(lit.[62] mp 94-96°).

Method B. The above procedure was followed exactly
with the exception that anhydrous pyridine was used as solvent
in place of tetrahydrofuran. This method gave a 63% yield of
50 as colorless needles, mp 94.5-97° (lit.[62] mp 94-96°).

Preparation of L-Prolyl-L-leucyl-L-leucine t-Butyl
Ester (51). A solution of 2.12 g (0.004 mol) of N-benzyloxy-
carbonyl-L-prolyl-L-leucyl-L-leucine t-butyl ester (47) in
100 ml of methanol containing 0.2 ml of glacial acetic acid
was hydrogenated over 0.2 g of 10% palladium on carbon for 8
hr at 60 psi and 20°. The catalyst was removed by filtration
through Celite and washed with methanol. The filtrate was
concentrated under reduced pressure and the residue was dis-
solved in 100 ml of ether. The organic solution was extrac-
ted with three 50 ml portions of 5% citric acid. The combined
aqueous extracts were made basic with K_2CO_3 and extracted with
three 75 ml portions of methylene chloride. The combined
organic extracts were dried ($MgSO_4$) and concentrated under
reduced pressure to give a yellow solid. Recrystallization
from hexane-cyclohexane (50:50) gave 1.23 g (77.5%) of 51
as colorless crystals: mp 125-125.5°; $[\alpha]_D^{22}$ -79.1° (c 1,
EtOH); ir (Appendix A, Figure 17, KBr) 3290 (NH), 1735
(ester C=O), 1650 (amide C=O), with shoulder at 1665 cm^{-1}
(amide C=O); nmr (Appendix B, Figure 17, $CDCl_3$) δ7.96 (d, 1,
J = 9 Hz), 6.72 (d, 1, J = 8 Hz), 4.52-4.24 (m, 2), 3.80-

3.64 (m, 1), 3.04-2.80 (m, 2), 2.18-1.16 (m, 20), 1.00-0.70
(m, 12). A second crop of crystals, mp 118-120° was obtained
from the mother liquor to raise the yield to 85%.

Anal. Calcd for $C_{21}H_{39}N_3O_4$: C, 63.44; H, 9.89;
N, 10.60. Found: C, 63.61; H, 9.62; N, 10.58.

Preparation of N-Hexanoylglycine p-Nitrophenyl Ester
(55). A solution of 12.00 g (0.007 mol) of N-hexanoylglycine
(12) and 10.70 g (0.077 mol) of p-nitrophenol in 50 ml of
tetrahydrofuran was cooled to -5° and treated with 15.15 g
(0.074 mol) of N,N'-dicyclohexylcarbodiimide. The solution
was stirred for 30 min at -5° and for 16 hr at 20°. The N,N'-
dicyclohexylurea was removed by filtration and washed with
tetrahydrofuran. The filtrate was concentrated under reduced
pressure to give a yellow solid. Recrystallization from
ethanol gave 11.72 g of yellow solid. The mother liquor gave
a second crop of 2.43 g of yellow solid for a total crude
yield of 68.7%. A second recrystallization from carbon tetra-
chloride gave 13.68 g (66.5%) of 55 as colorless crystals,
mp 100.5-101° (lit.[63] mp 97-98°).

Preparation of N-Hexanoylglycyl-L-prolyl-L-leucyl-L-
leucine t-Butyl Ester (52). Method A. A solution of 0.7 g
(0.004 mol) of N-hexanoylglycine (12) and 0.52 ml (0.004 mol)
of N-ethylmorpholine in 40 ml of anhydrous tetrahydrofuran
was cooled to -15° and treated with 0.4 ml (0.004 mol) of
ethyl chloroformate. After 5 min a cold solution of 1.60 g
(0.004 mol) of L-prolyl-L-leucyl-L-leucine t-butyl ester
(51) in 20 ml of anhydrous tetrahydrofuran was added. The

solution was stirred for 2 hr at -15° and for 16 hr at 20°.
The solvent was removed under reduced pressure, and the
residue was dissolved in a mixture of 50 ml of ethyl acetate
and 50 ml of water. The layers were separated and the
organic solution was washed with 50 ml of 5% citric acid
and 50 ml of 5% NaHCO$_3$. The organic solution was dried
(MgSO$_4$) and concentrated under reduced pressure to give a
viscous oil, which solidified after standing several days.
This solid was recrystallized by dissolving it in 100 ml of
cyclohexane-carbon tetrachloride (50:50), diluting with 900
ml of low boiling petroleum ether, and cooling for 48 hr to
give 0.67 g of colorless solid, A. The mother liquor was
concentrated to give 0.81 g of a yellow solid, B. Compound
A was recrystallized from ether-medium boiling petroleum
ether to give 0.24 g (11%) of 52 as colorless needles, mp
157.5-158°. Mixed melting point with an authentic sample
of 52 prepared by method B, below, gave an mp of 159-161°;
nmr (CDCl$_3$, Appendix B, Figure 18).

Anal. Calcd for C$_{29}$H$_{52}$N$_4$O$_6$: C, 63.01; H, 9.48;
N, 10.14. Found: C, 62.71; H, 9.29; N, 10.16.

Compound B was purified by chromatography on a 22 X 300
mm column of silica gel using chloroform-methanol (98:2) as
the eluent to give 0.45 g (24%) of a clear amorphous solid
identified as N-ethoxycarbonyl-L-prolyl-L-leucyl-L-leucine
t-butyl ester (53) by nmr (CDCl$_3$, Appendix B, Figure 19).

Method B.   A solution of 0.64 g (0.0022 mol) of N-hexanoylglycine p-nitrophenyl ester (55), 0.79 g (0.002 mol) of L-prolyl-L-leucyl-L-leucine t-butyl ester (51), and 0.14 g (0.004 mol) of imidazole in 40 ml of anhydrous tetrahydrofuran was allowed to react for 48 hr at 50°. The solvent was removed under reduced pressure and the residue was dissolved in 50 ml of ether. The organic solution was washed with two 50 ml portions of 5% citric acid and two 50 ml portions of cold 1N NaOH. The organic solution was dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow solid. Recrystallization from hexane-tetrahydrofuran gave 0.87 g (79%) of 52 as a colorless solid: mp 160-161.5°; $[\alpha]_D^{26}$ -96.6° (c 2, EtOH);   ir (Appendix A, Figure 18, KBr);   3310 (NH), 1735 (ester C=O), 1625 (amide C=O), with shoulders at 1680 (amide C=O), and 1650 cm^{-1} (amide C=O);   nmr (Appendix B, Figure 20, CDCl$_3$) δ7.11 (d, 1, J = 8 Hz), 6.71-6.50 (m, 2), 4.70-4.28 (m, 3), 4.10 (d, 2, J = 4 Hz), 3.72-3.35 (m, 2), 2.44-1.18 (m, 30), 0.95 (d, 12, J = 6 Hz).

Preparation of N-Hexanoylglycyl-L-prolyl-L-leucyl-L-leucine (56).   Method A.   A solution of 0.41 g (0.00074 mol) of N—hexanoylglycyl-L-prolyl-L-leucyl-L-leucine t-butyl ester (52) in 10 ml of trifluoroacetic acid was allowed to stand for 2 hr at 20°. The solution was concentrated under reduced pressure and the residue was dissolved in 50 ml of ethyl acetate. The organic solution was extracted with two 35 ml portions of 5% NaHCO$_3$. The combined aqueous extracts were acidified to Congo Red with 6N HCl and extracted with two 40

ml portions of chloroform. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a colorless solid. Recrystallization from benzene gave 0.26 g (71%) of a colorless solid. A second recrystallization from benzene gave 56 as a colorless solid: mp 120-121°; [α]$_D^{20}$ -96.0° (c 1, EtOH); ir (Appendix A, Figure 19, KBr) 3290 (NH), 1715 (acid C=O), 1610 cm^{-1} (amide C=O); nmr (Appendix B, Figure 21, CDCl$_3$) δ8.90 (s, 1), 7.85-7.05 (m, 3), 4.90-3.40 (m, 7), 2.55-1.20 (m, 21), 1.20-0.50 (m, 12).

Anal. Calcd for C$_{25}$H$_{44}$N$_4$O$_6$: C, 60.46; H, 8.93; N, 11.28. Found: C, 60.28; H, 9.01; N, 11.14.

Method B. A solution of 1.28 g (0.0044 mol) of N-hexanoylglycine p-nitrophenyl ester (55), 1.58 g (0.004 mol) of L-prolyl-L-leucyl-L-leucine t-butyl ester (51), and 0.28 g (0.008 mol) of imidazole in 70 ml of anhydrous tetrahydrofuran was allowed to react for 48 hr at 55°. The solution was concentrated under reduced pressure and the residue was dissolved in 75 ml of ether. The organic solution was washed with 50 ml of 5% citric acid and 50 ml of cold 1N NaOH, dried (MgSO$_4$), and concentrated under reduced pressure. The residue was dissolved in 25 ml of trifluoroacetic acid and allowed to stand for 2 hr at 20°. The solution was concentrated under reduced pressure and the residue was dissolved in 50 ml of ethyl acetate. The organic solution was extracted with three 50 ml portions of 5% NaHCO$_3$. The combined aqueous extracts were acidified to Congo Red with 6N HCl and extracted with three 50 ml portions of chloroform. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced

pressure to give a colorless solid. Recrystallization from
benzene gave 1.71 g (86%) of 56 as a colorless solid, mp
119.5-121°. This material was shown to be identical to that
prepared in Method A by the ir spectrum.

Preparation of N-Pentanoylglycine p-Nitrophenyl Ester
(57). A solution of 11.10 g (0.07 mol) of N-pentanoylgly-
cine (29) and 10.70 g (0.077 mol) of p-nitrophenol in 200 ml
of anhydrous pyridine was cooled to -15° and treated with
15.15 g (0.074 mol) of N,N'-dicyclohexylcarbodiimide. The
solution was stirred for 30 min at -15° and for 16 hr at 20°.
The N,N'-dicyclohexylurea was removed by filtration and
washed with chloroform. The filtrate was concentrated under
reduced pressure to give a brown solid. Recrystallization
from ethanol gave 12.80 g (65.5%) of yellow solid, mp 128-
129.5°. A second recrystallization from carbon tetrachloride
gave 9.95 g (51%) of 57 as a colorless solid: mp 128.5-
129.5°; ir (Appendix A, Figure 20, KBr) 3300 (NH), 1760
(ester C=O), 1640 cm^{-1} (amide C=O); nmr (Appendix B, Figure
22, CDCl$_3$) δ8.48 (d, 2, J = 9 Hz), 7.50 (d, 2, J = 9 Hz),
6.54 (s, 1), 4.42 (d, 2, J = 7 Hz), 2.37 (t, 2, J = 6 Hz),
1.84-1.22 (m, 4) 0.94 (t, 3, J = 7 Hz).

Anal. Calcd for C$_{13}$H$_{16}$N$_2$O$_5$: C, 55.71; H, 5.75;
N, 10.00. Found: C, 56.05; H, 6.06; N, 10.01.

Preparation of N-Pentanoylglycyl-L-prolyl-L-leucyl-
L-leucine t-Butyl Ester (58). A solution of 1.60 g (0.004
mol) of L-prolyl-L-leucyl-L-leucine t-butyl ester (51), 1.23
g (0.0044 mol) of N-pentanoylglycine p-nitrophenyl ester
(57), and 0.54 g (0.008 mol) of imidazole in 40 ml of an-

hydrous tetrahydrofuran was allowed to react for 80 hr at 50°. The solution was concentrated under reduced pressure and the residue was dissolved in 60 ml of ether. The organic solution was washed with 50 ml of 5% citric acid and two 50 ml portions of cold 1N NaOH. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give 2.25 g of yellow solid. Recrystallization from hexane-carbon tetrachloride (50:50) gave 1.53 g (71%) of <u>58</u> as colorless crystals: mp 169.5-170.5°; $[\alpha]_D^{20}$ -101.9° (<u>c</u> 1, EtOH); ir (Appendix A, Figure 21, KBr) 3325 (NH), 1730 (ester C=O), 1625 (amide C=O), with shoulders at 1680 (amide C=O), and 1650 cm^{-1} (amide C=O); nmr (Appendix B, Figure 23, $CDCl_3$) δ7.36 (d, 1, <u>J</u> = 9 Hz), 7.04-6.68 (m, 2), 5.00-4.40 (m, 3), 4.27-4.04 (m, 2), 3.84-3.42 (m, 2), 2.56-1.26 (m, 28), 0.96 (d, 12, <u>J</u> = 4 Hz).

<u>Anal</u>. Calcd for $C_{28}H_{50}N_4O_6$: C, 62.42; H, 9.36; N, 10.40. Found: C, 62.63; H, 9.09; N, 10.51.

<u>Preparation of N-Pentanoylglycyl-L-prolyl-L-leucyl-L-leucine (59)</u>. A solution of 1.35 g (0.0025 mol) of N-pentanoylglycyl-L-prolyl-L-leucyl-L-leucine <u>t</u>-butyl ester (<u>58</u>) in 15 ml of trifluoroacetic acid was allowed to react for 2 hr at 20°. The solution was concentrated under reduced pressure and the residue was dissolved in 50 ml of ethyl acetate. The organic solution was extracted with two 25 ml portions of 5% $NaHCO_3$. The combined aqueous extracts were acidified to Congo Red with 6N HCl and extracted with two 35 ml portions of chloroform. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure

to give a colorless solid. Recrystallization from benzene-ether (50:50) gave 0.81 g (67%) of colorless solid, mp 144-146°. A second recrystallization gave 0.55 g of 59 as a colorless solid, mp 148.5-151°. A second crop gave an additional 0.14 g of solid, mp 147.5-149° for a total yield of 57.5%: $[\alpha]_D^{19}$ -102.4° (c 1, EtOH); ir (Appendix A, Figure 22, KBr) 3325 (NH), 1725 (acid C=O), 1635 cm^{-1} (amide C=O); nmr (Appendix B, Figure 24, $CDCl_3$) δ9.96 (s, 1), 7.33-6.84 (m, 3), 4.64-4.25 (m, 3), 4.08-3.28 (m, 4), 2.40-1.12 (m, 19), 0.95 (d, 12, J = 5 Hz).

Anal. Calcd for $C_{24}H_{42}N_4O_6$: C, 59.75; H, 8.77; N, 11.61. Found: C, 59.68; H, 8.81; N, 11.79.

Preparation of N-Benzyloxycarbonyl-L-phenylalanine Dicyclohexylamine Salt (72). A vigorously stirred suspension of 24.22 g (0.147 mol) of phenylalanine (70) and 42.0 g (0.5 mol) of $NaHCO_3$ in 400 ml of water was treated with 27.5 g (0.162 mol) of benzyl chloroformate in four equal portions over a 3 hr period. The mixture was stirred an additional 2 hr and washed with two 100 ml portions of ether. The aqueous solution was acidified to Congo Red with 6N HCl and extracted with two 300 ml portions of ether. The combined organic extracts were dried ($MgSO_4$) and treated with 29.0 ml (0.147 mol) of dicyclohexylamine. After cooling the mixture for several hours the salt which precipitated was removed by filtration, washed with ether, and dried to give 49.9 g (71%) of colorless solid, mp 152-157°. Recrystallization from ether-methanol gave 41.8 g (59%) of 72 as a colorless solid:

mp 155.5-157°; $[\alpha]_D^{27}$ 28.8° (c 3, EtOH) (lit.[37] mp 155-156°, $[\alpha]_D^{20}$ -34.2° (c 1, EtOH)).

Preparation of N-Benzyloxycarbonyl-L-phenylalanine p-Nitrophenyl Ester (73).

A mixture of 24.0 g (0.05 mol) of N-benzyloxycarbonyl-L-phenylalanine dicyclohexylamine salt (72), 200 ml of ether, and 200 ml of 0.5N H_2SO_4 was shaken in a separatory funnel until the solid dissolved. The layers were separated and the aqueous solution was extracted with 100 ml of ether. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give an oil. This oil and 7.65 g (0.055 mol) of p-nitrophenol was dissolved in 200 ml of anhydrous tetrahydrofuran. The solution was cooled to -5° and treated with 10.3 g (0.05 mol) of N,N'-dicyclohexylcarbodiimide. The solution was stirred for 30 min at -5° and for 2.5 hr at 20°. The N,N'-dicyclohexylurea was removed by filtration and washed with tetrahydrofuran. The filtrate was concentrated under reduced pressure and the residue was recrystallized from ethanol to give 11.77 g (56%) of yellow crystals, mp 120-125°. A second recrystallization from ethanol gave 10.30 g (49%) of 73 as colorless needles: mp 126-127°; $[\alpha]_D^{20}$ -18.7° (c 2, DMF) (lit.[64] mp 126-126.5°, $[\alpha]_D^{20}$ -24.7° (c 2, DMF)).

Preparation of N-Benzyloxycarbonyl-L-phenylalanyl-L-leucine t-Butyl Ester (74). Method A.

A mixture of 9.61 g (0.02 mol) of N-benzyloxycarbonyl-L-phenylalanine dicyclohexylamine salt (72), 150 ml of 0.5 N H_2SO_4, and 75 ml of ether was shaken in a separatory funnel until the solid dis-

solved. The layers were separated and the aqueous solution
was extracted with 75 ml of ether. The combined organic ex-
tracts were dried (MgSO$_4$) and concentrated under reduced
pressure to give an oil. A solution of this oil and 2.6 ml
(0.02 mol) of N-ethylmorpholine in 100 ml of anhydrous tetra-
hydrofuran was cooled to -15° and treated with 2.0 ml (0.02
mol) of ethyl chloroformate. After 5 min a cold mixture of
5.38 g (0.02 mol) of L-leucine t-butyl ester phosphite salt
(44) and 2.6 ml of N-ethylmorpholine in 100 ml of anhydrous
tetrahydrofuran was added. The mixture was stirred for 2 hr
at -15° and for 16 hr at 20°. The solvent was removed under
reduced pressure and the residue was dissolved in 100 ml of
ethyl acetate. This organic solution was washed with two
50 ml portions of 5% citric acid and two 50 ml portions of
5% NaHCO$_3$. The organic solution was dried (MgSO$_4$) and con-
centrated under reduced pressure to give a colorless solid
which on recrystallization from low boiling petroleum ether
gave 5.87 g (62.5%) of 74 as colorless crystals: mp 93-95°;
$[\alpha]_D^{26}$ -26.0° (c 2, EtOH) (lit.[65] mp 94-95°, $[\alpha]_D^{25}$ -27.0° (c
1, MeOH)).

Method B.   A solution of 2.10 g (0.005 mol) of N-
benzyloxycarbonyl-L-phenylalanine p-nitrophenyl ester (73),
1.35 g (0.005 mol) of L-leucine t-butyl ester phosphite salt
(44), 0.68 g (0.01 mol) of imidazole, and 0.65 g (0.005 mol)
of N-ethylmorpholine in 50 ml of tetrahydrofuran was allowed
to react for 48 hr at 50°. The solution was concentrated
under reduced pressure and the residue was dissolved in 100

ml of ether. This organic solution was washed with 75 ml
of 5% citric acid and two 75 ml portions of cold 1N NaOH.
The organic solution was dried ($MgSO_4$) and concentrated
under reduced pressure to give a crystalline solid. Re-
crystallization from medium boiling petroleum ether gave
1.30 g (55.5%) of 75 as colorless crystals, mp 90-92.5°
(lit.[65] mp 94-95°).

Method C. A mixture of 3.84 g (0.008 mol) of N-
benzyloxycarbonyl-L-phenylalanine dicyclohexylamine salt
(74), 75 ml of ether, and 75 ml of 0.5N H_2SO_4 was shaken in
a separatory funnel until the solid dissolved. The layers
were separated and the aqueous solution was extracted with
75 ml of ether. The combined organic extracts were dried
($MgSO_4$) and concentrated under reduced pressure to give an
oil. A solution of this oil, 2.12 g (0.008 mol) of L-leucine
t-butyl ester phosphite salt (44), 1.0 ml (0.008 mol) of N-
ethylmorpholine, and 2.42 g (0.016 mol) of 1-hydroxybenzo-
triazole in 80 ml of anhydrous tetrahydrofuran was cooled to
-15° and treated with 1.63 g (0.008 mol) of N,N'-dicyclo-
hexylcarbodiimide. The solution was stirred for 1 hr at -15°
and for 110 hr at 20°. The precipitated N,N'-dicyclohexyl-
urea was removed by filtration and washed with tetrahydro-
furan. The filtrate was concentrated under reduced pressure
to give a yellow oil. This oil was dissolved in ethyl
acetate, filtered through a column of basic alumina, and
washed with 50 ml of 5% citric acid and 50 ml of 5% $NaHCO_3$.
The organic solution was dried ($MgSO_4$) and concentrated under

reduced pressure to give a crystalline solid which on re-
crystallization from medium boiling petroleum ether gave
1.91 g of slightly yellow crystals, mp 90-95°. The mother
liquor gave a second crop of 0.46 g of slightly yellow
crystals, mp 85-92° for a total crude yield of 64%. These
two crops were combined and recrystallized from low boiling
petroleum ether to give 2.00 g (54%) of 74 as colorless
crystals, mp 92.5-96° (lit.[65] mp 94-95°).

Preparation of L-Phenylalanyl-L-leucine t-Butyl Ester
(75). A solution of 7.53 g (0.016 mol) of N-benzyloxycar-
bonyl-L-phenylalanyl-L-leucine t-butyl ester (74) and 0.2 ml
of glacial acetic acid in 200 ml of ethanol was hydrogenated
over 0.75 g of 10% palladium on carbon for 24 hr at 60 psi
and 20°. The catalyst was removed by filtration through
Celite and washed with ethanol. The filtrate was concentrated
under reduced pressure and the residue was dissolved in 200
ml of ether. The ether solution was extracted with two 75 ml
portions of 5% citric acid. The combined aqueous extracts
were made basic with K_2CO_3 and extracted with two 100 ml
portions of ether. The combined organic extracts were dried
($MgSO_4$) and concentrated under reduced pressure to give 5.05
g (94%) of 75 as a viscous oil: ir (Appendix A, Figure 23,
neat) 3320 (NH), 1725 (ester C=O), 1645 cm^{-1} (amide C=O);
nmr (Appendix B, Figure 25, $CDCl_3$) δ8.08-7.76 (m, 1), 7.51
(s, 5), 4.80-4.44 (m, 1), 3.88-3.64 (m, 1), 3.48-3.21 (m, 1),
2.96-2.64 (m, 1), 1.80-1.22 (m, 14), 0.98 (d, 6, $\underline{J}$ = 6 Hz).

Anal. Calcd for $C_{19}H_{30}N_2O_3$: C, 68.23; H, 9.04; N, 8.38. Found: C, 68.27; H, 9.10; N, 8.37.

Preparation of L-Tyrosine Hydrazide (64). A solution of 19.9 g (0.1 mol) of L-tyrosine methyl ester (63) and 50 ml of hydrazine hydrate in 300 ml of methanol was allowed to stand 72 hr at 20°. The solution was concentrated under reduced pressure and the residue was recrystallized from ethanol to give 14.10 g (74%) of L-tyrosine hydrazide (64) as colorless needles: mp 199-199.5° (lit.[66] mp 195.5°); $[\alpha]_D^{25}$ 82.3° (c 2, HOAc) (lit.[45] $[\alpha]_D^{23}$ 70° (c 2, 3N HCl)).

Preparation of N-Benzyloxycarbonyl-DL-proline Dicyclohexylamine Salt (62). L-Proline (16) was racemized according to a known procedure[44] and converted to N-benzyloxycarbonyl-DL-proline (61) by the same method outlined for the preparation of N-benzyloxycarbonyl-L-proline (22). The material could not be crystallized and was converted to the dicyclohexylamine salt (62) in 70% yield: mp 160-161° (lit.[45] mp 160°); $[\alpha]_D^{26}$ 2.8° (c 2, MeOH) (lit.[37] $[\alpha]_D$ of L-isomer -25.3° (c 2, MeOH)).

Resolution of N-Benzyloxycarbonyl-DL-proline (61). The method of Vogler and Lanz[45] was followed to prepare N-benzyloxycarbonyl-D-proline dicyclohexylamine salt (68) in 63% yield: mp 176-177.5°; $[\alpha]_D^{20}$ 25.1° (c 2, MeOH) (lit.[45] mp of L-isomer 178-180°, $[\alpha]$ of L-isomer -25° (c 2, MeOH)). The L-isomer (23) was obtained in 51% yield, mp 174-176°, $[\alpha]_D^{20}$ -23.9° (c 2, MeOH).

Preparation of N-Benzyloxycarbonyl-D-proline p-
Nitrophenyl Ester (69). A mixture of 23.91 g (0.056 mol)
of N-benzyloxycarbonyl-D-proline dicyclohexylamine salt (68),
200 ml of ether, and 200 ml of 0.5N H_2SO_4 was shaken in a
separatory funnel until the solid dissolved. The layers
were separated and the aqueous solution was extracted with
100 ml of ether. The combined organic extracts were dried
($MgSO_4$) and concentrated under reduced pressure to give a
viscous oil. A solution of this oil and 8.53 g (0.061 mol)
of p-nitrophenol in 200 ml of anhydrous pyridine was cooled
to -5° and treated with 11.45 g (0.056 mol) of N,N'-dicyclo-
hexylcarbodiimide. The solution was stirred for 30 min at
-5° and for 2.5 hr at 20°. The precipitated N,N'-dicyclo-
hexylurea was removed by filtration and washed with chloro-
form. The filtrate was concentrated under reduced pressure
and the residue was recrystallized from ethanol to give
13.65 g (66.5%) of slightly yellow needles, mp 94.5-96°. A
second recrystallization from ethanol gave 12.56 g (61%) of
69 as colorless needles: mp 94.5-97°; $[\alpha]_D^{20}$ 71.4° (c 2,
DMF) (lit.[62] mp of L-isomer 94-96°, $[\alpha]_D^{20}$ of L-isomer -68°
(c 2, DMF)).

Preparation of N-Benzyloxycarbonyl-D-prolyl-L-phenyl-
alanyl-L-leucine t-Butyl Ester (76). A solution of 5.05 g
(0.015 mol) of L-phenylalanyl-L-leucine t-butyl ester (75),
5.55 g (0.015 mol) of N-benzyloxycarbonyl-D-proline p-nitro-
phenyl ester (69), and 1.02 g (0.03 mol) of imidazole in 200
ml of anhydrous tetrahydrofuran was allowed to react for 48

hr at 50°. The solution was concentrated under reduced pressure and the residue was dissolved in 200 ml of ether. This ether solution was washed with 100 ml of 5% citric acid and two 100 ml portions of cold 1N NaOH. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give 7.90 g of a slightly yellow solid. Recrystallization from hexane-cyclohexane (50:50) gave 6.90 g (81.5%) of colorless solid, mp 92-93°. A second recrystallization from hexane-cyclohexane (50:50) gave 6.60 g (78%) of 76 as colorless crystals: mp 90-91.5°; $[\alpha]_D^{29}$ 0.0° (c 1, EtOH); ir (Appendix A, Figure 24, KBr) 3300 (NH), 1740 (ester C=O), 1705 (amide C=O), 1640 cm^{-1} (amide C=O); nmr (Appendix B, Figure 26, $CDCl_3$) δ7.78-7.32 (m, 10), 7.15-6.68 (m, 2), 5.30 (s, 2), 4.98-4.20 (m, 3), 3.80-3.51 (m, 2), 3.32-3.00 (m, 2), 2.36-1.40 (m, 16), 0.92 (d, 6, J = 6 Hz).

Anal. Calcd for $C_{32}H_{43}N_3O_6$: C, 67.94; H, 7.66; N, 7.43. Found: C, 67.90; H, 7.50; N, 7.48.

Preparation of D-Prolyl-L-phenylalanyl-L-leucine t-Butyl Ester (77). A solution of 6.50 g (0.0115 mol) of N-benzyloxycarbonyl-D-prolyl-L-phenylalanyl-L-leucine t-butyl ester (76) in 200 ml of ethanol containing 0.2 ml of glacial acetic acid was hydrogenated over 0.65 g of 10% palladium on carbon for 24 hr at 60 psi and 20°. The catalyst was removed by filtration through Celite and washed with ethanol. The filtrate was concentrated under reduced pressure and the residue was dissolved in 75 ml of ethyl acetate. This organic solution was extracted with three 75 ml portions of 5% citric

acid. Concentration of the ether solution gave 1.00 g of crude unreacted starting material. The combined aqueous extracts were made basic with K_2CO_3 and extracted with three 50 ml portions of chloroform. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a crystalline solid. Recrystallization from hexane-cyclohexane (50:50) gave 3.80 g (76.5%) of 77 as colorless crystals: mp 121-121.5°; $[\alpha]_D^{19}$ -0.6° (c 1, EtOH); ir (Appendix A, Figure 25, KBr) 3340 (NH), 1740 (ester C=O), 1645 cm^{-1} (amide C=O); nmr (Appendix B, Figure 27, $CDCl_3$) δ7.96-7.74 (m, 1), 7.12 (s, 5), 6.60-6.34 (m, 1), 4.64-4.16 (m, 2), 3.72-3.52 (m, 1), 3.16-2.68 (m, 5), 2.06-1.25 (m, 16), 0.87 (d, 6, J = 6 Hz).

Anal. Calcd for $C_{24}H_{37}N_3O_4$: C, 66.79; H, 8.64; N, 9.74. Found: C, 66.71; H, 8.61; N, 9.95.

Preparation of N-Pentanoylglycyl-D-prolyl-L-phenyl-alanyl-L-leucine (79). A solution of 1.23 g (0.0044 mol) of N-pentanoylglycine p-nitrophenyl ester (57), 1.72 g (0.004 mol) of D-prolyl-L-phenylalanyl-L-leucine t-butyl ester (77), and 0.55 g (0.008 mol) of imidazole in 50 ml of anhydrous tetrahydrofuran was allowed to react for 68 hr at 20°. The tetrahydrofuran was removed under reduced pressure and the residue was dissolved in 50 ml of ether. This ether solution was washed with 75 ml of 5% citric acid and 75 ml of cold 1N NaOH. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give 2.41 g of crude N-pentanoylglycyl-D-prolyl-L-phenylalanyl-L-leucine t-butyl

ester (78) as a yellow oil.

The crude ester was dissolved in 25 ml of trifluoro-
acetic acid and allowed to react for 2 hr at 20°. The solu-
tion was concentrated under reduced pressure and the residue
was dissolved in 75 ml of ethyl acetate. This solution was
extracted with three 50 ml portions of 5% NaHCO$_3$. The com-
bined aqueous extracts were acidified to Congo Red with 6N
HCl and extracted with three 50 ml portions of chloroform.
The combined organic extracts were dried (MgSO$_4$) and concen-
trated under reduced pressure to give a slightly yellow
solid. Recrystallization from benzene-ether (50:50) gave
1.51 g of 79 as a colorless solid, mp 162-162.5°. A second
crop from the mother liquor gave 0.24 g of colorless solid,
mp 161.5-162.5° for a total yield of 85%. $[\alpha]_D^{22}$ 16.1° (c
1, EtOH); ir (Appendix A, Figure 26, KBr) 3300 (NH), 1625
(amide C=O), with a shoulder at 1705 cm^{-1} (acid C=O); nmr
(Appendix B, Figure 28, CDCl$_3$) δ10.30 (s, 1), 7.80-6.65 (m,
8), 4.90-3.05 (m, 9), 2.50-0.63 (m, 22).

An analytical sample was prepared by recrystalliza-
tion from ether-benzene (50:50), mp 161-162.5°.

Anal. Calcd for C$_{27}$H$_{40}$N$_4$O$_6$: C, 62.77; H, 7.80;
N, 10.84. Found: C, 62.82; H, 7.90; N, 10.97.

Preparation of Diethyl Aminomalonate Hydrochloride
(80). The standard method[49] was followed to prepare 80 in
41.7% yield, mp 166-167.5° (lit.[49] mp 164-165°).

Preparation of N-Octanoylaminomalonic Acid Diethyl

Ester (82). Method A. A solution of 2.88 g (0.02 mol) of octanoic acid (81) and 2.8 ml (0.02 mol) of triethylamine in 50 ml of anhydrous methylene chloride was cooled to 0° and treated with 1.55 ml (0.016 mol) of ethyl chloroformate. After 5 min a cold solution of 4.23 g (0.02 mol) of diethyl aminomalonate hydrochloride (80) and 2.8 ml of triethylamine in 75 ml of anhydrous methylene chloride was added. The solution was allowed to stand 16 hr at 20° and washed successively with three 50 ml portions of 2N HCl, 50 ml of water, two 50 ml portions of 6.5% NaHCO$_3$, and 50 ml of water. The organic solution was dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow solid. Recrystallization from hexane gave 3.40 g (70%) of 82 as colorless crystals: mp 72.5-76.5°; ir (Appendix A, Figure 27, KBr) 3310 (NH), 1745 (ester C=O), 1645 cm^{-1} (amide C=O); nmr (Appendix B, Figure 29, CDCl$_3$) δ7.10 (d, 1, J = 8 Hz), 5.28 (d, 1, J = 8 Hz), 4.28 (q, 4, J = 7 Hz), 2.50-2.15 (m, 2), 1.90-1.18 (m, 16), 1.18-0.67 (m, 3).

Anal. Calcd for C$_{15}$H$_{27}$N$_1$O$_5$: C, 59.78; H, 9.03; N, 4.65. Found: C, 59.65; H, 8.90; N, 5.01.

Method B. A solution of 8.46 g (0.04 mol) of diethyl aminomalonate hydrochloride (80), 5.6 ml (0.04 mol) of triethylamine, and 5.76 ml (0.04 mol) of octanoic acid (81) in 300 ml of methylene chloride was treated with 9.06 g (0.044 mol) of N,N'-dicyclohexylcarbodiimide. After standing

for 48 hr at 20°, the N,N'-dicyclohexylurea was removed by filtration. The filtrate was washed with two 25 ml portions of 1N HCl and two 25 ml portions of 6.5% $NaHCO_3$. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow solid. Recrystallization from hexane gave 6.70 g (56%) of <u>82</u> as colorless needles, mp 74-75°.

Preparation of 5,5-<u>bis</u>-Ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (<u>83</u>). A solution of 1.00 g (0.0033 mol) of N-octanoylaminomalonic acid diethyl ester (<u>82</u>) and 0.20 g (0.0036 mol) of acrolein in 20 ml of anhydrous benzene was treated with 0.5 ml of 2N NaOMe in methanol. After standing for 18 hr at 20° TLC showed the complete absence of starting material. The solution was acidified with glacial acetic acid and was concentrated under reduced pressure to give a viscous oil: ir (Appendix A, Figure 28, neat) 3300 (NH, OH), 1745 (ester C=O), 1645 (amide C=O); nmr (Appendix B, Figure 30, $CDCl_3$) δ6.40 (s, 1), 4.60-3.90 (m, 1), 3.90-3.50 (m, 4), 3.00-1.10 (m, 22), 0.90 (t, 3, $\underline{J}$ = 5 Hz). The peak at δ6.40 disappeared upon deuterium exchange with D_2O. The above material was used without further purification.

Attempted Preparation of N-Octanoyl-5-hydroxyproline (<u>85</u>). A solution of 1.00 g of 5,5-<u>bis</u>-ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (<u>83</u>) in 10 ml of dihydropyran was acidified with 0.5 ml of concentrated HCl and allowed to stand at 20° for 10 hr. The solution was diluted with 100 ml of ether and washed with two 25 ml portions of 6.5% $NaHCO_3$.

The organic solution was concentrated under reduced pressure
to give a yellow oil: ir (Appendix A, Figure 29, neat)
3400 (OH), 1740 (ester C=O), 1650 cm^{-1} (amide C=O); nmr
(Appendix B, Figure 31, CDCl$_3$) δ5.60 (s, 1), 5.10-4.50 (m,
1), 4.29 (two overlapping q, 4, J = 7 Hz), 3.94-3.17 (m, 4),
2.89-1.02 (m), 0.88 (t, 3, J = 5 Hz). The oil was dissolved
in a solution of 20 ml of ethanol and 20 ml of 2N KOH and
the solution was heated under reflux for 1 hr. The ethanol
was removed under reduced pressure and the aqueous solution
was washed with two 15 ml portions of chloroform. The
aqueous solution was acidified with 6N HCl and extracted
with three 50 ml portions of ethyl acetate. The combined
organic extracts were concentrated under reduced pressure
to give a yellow oil: ir (Appendix A, Figure 30, neat), 3300
(NH), 1730 (C=O), 1640 cm^{-1} (C=O); nmr (Appendix B, Figure
32, CDCl$_3$). The oil was dissolved in ether and treated with
1.0 ml of dicyclohexylamine. The solution became slightly
cloudy, but no precipitate formed. No further workup was
attempted.

Attempted Preparation of N-Octanoyl-5-hydroxyprolyl-
glycylglycine Benzyl Ester (86). A solution of 1.50 g of
5,5-bis-ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (83) in 20
ml of ethanol and 20 ml of 2N KOH was warmed on a steam bath
for 10 min. The ethanol was removed under reduced pressure
and the aqueous solution was washed with two 15 ml portions
of chloroform. The aqueous solution was acidified with 6N
HCl and extracted with two 25 ml portions of ethyl acetate.

The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow solid. Recrystallization from ether-hexane gave a colorless solid having a broad melting range centered around 92°: ir (Appendix A, Figure 31, KBr) 3300, 1730 (C=O), 1625 cm^{-1} (C=O); nmr (Appendix B, Figure 33, CDCl$_3$). The crude solid could not be purified or characterized further and was used in the following reaction.

A solution of this crude solid, 1.30 g of glycyl-glycine benzyl ester p-toluenesulfonate (34), and 0.5 ml of triethylamine in 40 ml of ethyl acetate was treated with 0.75 g of N,N'-dicyclohexylcarbodiimide and allowed to stand for 30 hr at 20°. The precipitated N,N'-dicyclohexylurea was removed by filtration and washed with ethyl acetate. The filtrate was washed with two 25 ml portions of 1N HCl and two 25 ml portions of 6.5% NaHCO$_3$. The organic solution was dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow oil. TLC in five different solvent systems showed the oil consisted of at least six components. The nmr spectrum showed the presence of an aromatic group, but an insufficient number of aliphatic protons. The spectrum was unchanged after deuterium exchange with D$_2$O. Ir (Appendix A, Figure 32, neat); nmr (Appendix B, Figure 34, CDCl$_3$). No further workup was attempted.

Attempted Preparation of 5,5-bis-Ethoxycarbonyl-1-octanoyl-2-benzyloxycarbonylpyrrolidinol (87). A solution of 1.50 g of 5,5-bis-ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (83) and 1.4 ml of triethylamine in 20 ml of anhydrous

methylene chloride was cooled to 0° and treated with 0.63 g

of benzyl chloroformate. The solution was allowed to stand

for 1 hr at 20°. The solvent was removed under reduced

pressure and the residue was dissolved in ether. The solu-

tion was filtered to remove triethylamine hydrochloride, and

the filtrate was concentrated under reduced pressure to give

a yellow oil. TLC and the ir spectrum showed this oil to be

unreacted starting material.

Attempted Preparation of the p-Nitrophenylhydrazone

of 5,5-bis-Ethoxycarbonyl-1-octanoyl-2-pyrrolidinol (88).

A solution of 1.50 g of 5,5-bis-ethoxycarbonyl-1-octanoyl-2-

pyrrolidinol (83) and 0.50 g of p-nitrophenylhydrazine in

15 ml of ethanol was heated to reflux and treated with one

drop of glatial acetic acid. Heating was continued 5 min

and the solution was allowed to stand 70 hr at 20°. The

solution was diluted to a large volume with water, causing

the formation of an orange precipitate. This precipitate

was removed by filtration and dried to give a nearly quant-

itative recovery of p-nitrophenylhydrazine.

BIBLIOGRAPHY

1. D. Lednicer, Ed., "Contraception: The Chemical Control of Fertility," Marcel Dekker, Inc., New York, N. Y., 1969.

2. C. Djerassi, Science, 169, 941 (1970).

3. V. Petrow in "Progress in Medicinal Chemistry", Vol. 8, G. P. Ellis and G. B. West, Eds., Butterworth & Co., Ltd., 1971, pp 171-229.

4. H. Jackson, Fortschritte der Arzneimittelforschung, 7, 133 (1964).

5. M. J. K. Harper, ibid., 12, 47 (1968).

6. R. L. Vande Wiele, Ed., "Metabolic Effects of Gonadal Hormones and Contraceptive Steroids", Plenum Press, New York, N.Y., 1969.

7. M. P. L. Caton in "Progress in Medicinal Chemistry", Vol. 8, G. P. Ellis and G. B. West, Eds., Butterworth & Co., Ltd., 1971, pp 317-376.

8. P. Ramwell and J. E. Shaw, Eds., "Prostaglandins", Ann. N. Y. Acad. Sci., 180, 1-568 (1971).

9. Ref. 1, pp 172-189, 219-230.

10. Ref. 8, pp 499-512.

11. A. V. Schally et al., Fertility and Sterility, 22, 703 (1971).

12. K. Folkers et al., Angew. Chem. Internat. Edit., 12, 255 (1973).

13. W. Vale et al., Science, 176, 933 (1972).

14. H. C. Beyermann et al., Rec. Trav. Chim. Pay-Bas, 91, 1239 (1972).

15. Ref. 1, p 163.

16. H. A. Kent, Jr., Research and Inventions, 3, 1 (winter 1974-1974).

17. L. B. Kier and J. R. Hoyland, J. Med. Chem., 15, 84 (1972).

18. R. E. Lyle et al., "Synthesis of Nitrogen Containing Analogs of Prostaglandins", Final Report NIH-NICHD-72-2715, September, 1973.

19. E. Schroder and K. Lubke, "The Peptides", Vol. I, Academic Press, New York, N. Y., 1965.

20. M. Bodanszky and M. A. Ondetti, "Peptide Synthesis", Interscience, New York, N. Y., 1965.

21. N. A. Smart et al., J. Chem. Soc., 3902 (1960).

22. M. W. Williams and G. T. Young, J. Chem. Soc., 881 (1963).

23. F. Weygand et al., Angew. Chem., 75, 282 (1963).

24. Ref. 19, pp 130-132 and references cited therein.

25. R. A. Boissonnas, Helv. Chim. Acta, 34, 874 (1951).

26. J. R. Vaughan, Jr., J. Amer. Chem. Soc., 73, 3547 (1951).

27. T. Wieland and H. Bernhard, Justus Liebigs Ann. Chem., 572, 190 (1951).

28. R. C. Weast, Ed., "Handbook of Chemistry and Physics", 49th ed., The Chemical Rubber Co., Cleveland, Ohio, 1968, p C-328.

29. M. Bergmann and L. Zervas, Chem. Ber., 65, 1192 (1932).

30. Ref. 19, pp 23-24 and references cited therein.

31. J. C. Sheehan and G. P. Hess, J. Amer. Chem. Soc., 77, 1067 (1955).

32. Marvin Karten, NICHD-NIH, Bethesda, Md. 20014, private communication.

33. C. H. Hassall and W. A. Thomas, Chem. in Britain, 7, 145 (1971).

34. Ref. 19, pp 57-59 and references cited therein.

35. R. Roeske, J. Org. Chem., 28, 1251 (1963).

36. W. Konig and R. Geiger, Chem. Ber., 103, 788 (1970).

37. E. Schroder et al., Justus Liebigs Ann. Chem., 640, 157 (1961).

38. Ref. 19, pp 98-103.

39. R. M. Mazur, J. Org. Chem., 28, 2498 (1963).

40. A. Buzas et al., Compt. Rend., 255, 945 (1962).

41. H. Kappeler and R. Schwyzer, Helv. Chim. Acta, 44, 1136 (1961).

42. K. D. Kopple et al., J. Amer. Chem. Soc., 95, 6091 (1973).

43. K. D. Kopple, J. Pharm. Sci., 61, 1345 (1972).

44. J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. III, John Wiley & Sons, Inc., New York, N. Y., 1961, p 2195.

45. K. Vogler and P. Lanz, Helv. Chim. Acta, 49, 1348 (1966).

46. D. A. Cox et al., J. Chem. Soc., 5024 (1964).

47. B. J. Magerlein, J. Med. Chem., 15, 1255 (1972).

48. O. A. Moe and D. T. Warner, J. Amer. Chem. Soc., 70, 2763 (1948).

49. M. Newmann, Ed., "Organic Syntheses", Vol. 40, John Wiley & Sons, Inc., New York, N. Y., 1960, pp 21-26.

50. Ref. 19, pp 211-224 and references cited therein.

51. G. Y. Kondrat'eva and C. H. Huang, Chem. Abstr., 58, 7919g (1963).

52. F. Ueda et al., Yakugaku Zasshi, 79, 925 (1959); Chem. Abstr., 53, 21889e (1959).

53. I. R. Neher et al., Helv. Chim. Acta, 29, 1815 (1946); Chem. Abstr., 41, 2036h (1947).

54. G. Harris, Ed., "Dictionary of Organic Compounds", Vol. III, Oxford University Press, New York, N. Y., 1965, p 1373.

55. W. Oelofsen and C. H. Li, J. Org. Chem., 33, 1581 (1968).

56. Ref. 28, p C-454.

57. J. Katz et al., J. Biol. Chem., 200, 431 (1953).

58. G. Harris, Ed., "Dictionary of Organic Compounds", Vol. V, 4th Ed., Oxford University Press, New York, N. Y., 1965, p 2779.

59. R. H. Mazur and J. M. Schlatter, J. Org. Chem., 29, 3212 (1964).

60. G. W. Anderson and F. M. Callahan, J. Amer. Chem. Soc., 82, 3359 (1960).

61. E. Nietzki and E. Braunschweig, Chem. Ber., 27, 3381 (1894).

62. M. Bodanszky and V. Du Vigneaud, J. Amer. Chem. Soc., 81, 5688 (1959).

63. J. H. C. Nayler et al., J. Chem. Soc., C, 242 (1968).

64. M. Bodanszky and V. Du Vigneaud, J. Amer. Chem. Soc., 81, 6072 (1959).

65. K. T. Wang and C. H. Li, J. Org. Chem., 36, 2419 (1971).

66. Ref. 58, p 3218.

67. D. S. Kemp et al., J. Amer. Chem. Soc., 92, 1043 (1970).

PART I

APPENDIX A

INFRARED SPECTRA

A-Figure 1.   (KBr).   Compound 19.

WAVELENGTH (MICRONS)

Hex-Gly-5-Amy-OH

CM-1

PR 1112 (137-1254)

A-Figure 2. (KBr). Compound 20.

WAVELENGTH (MICRONS)

Cbo-L-Pro-5-Amv-OMe

PR 1112 (137-1254)

CM-1
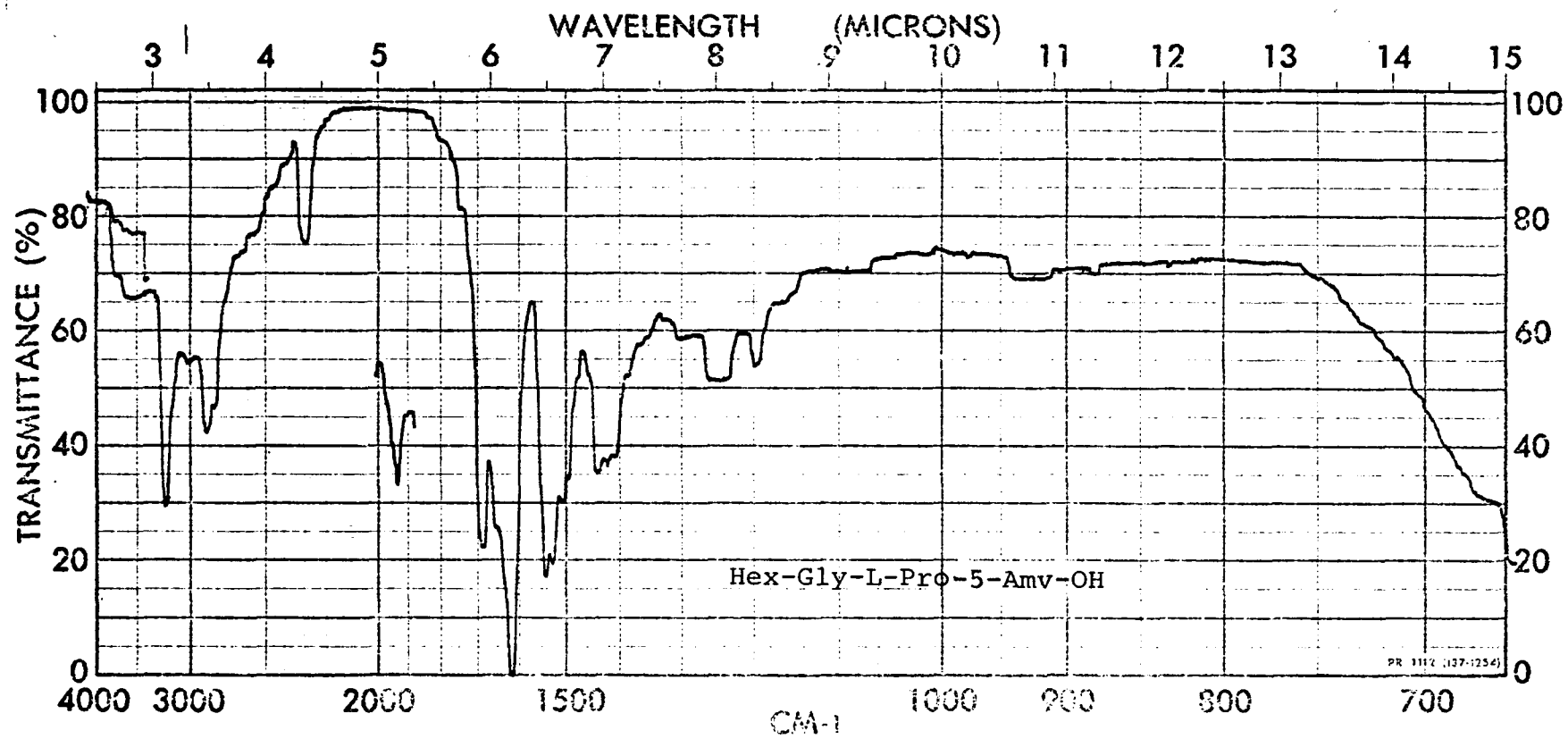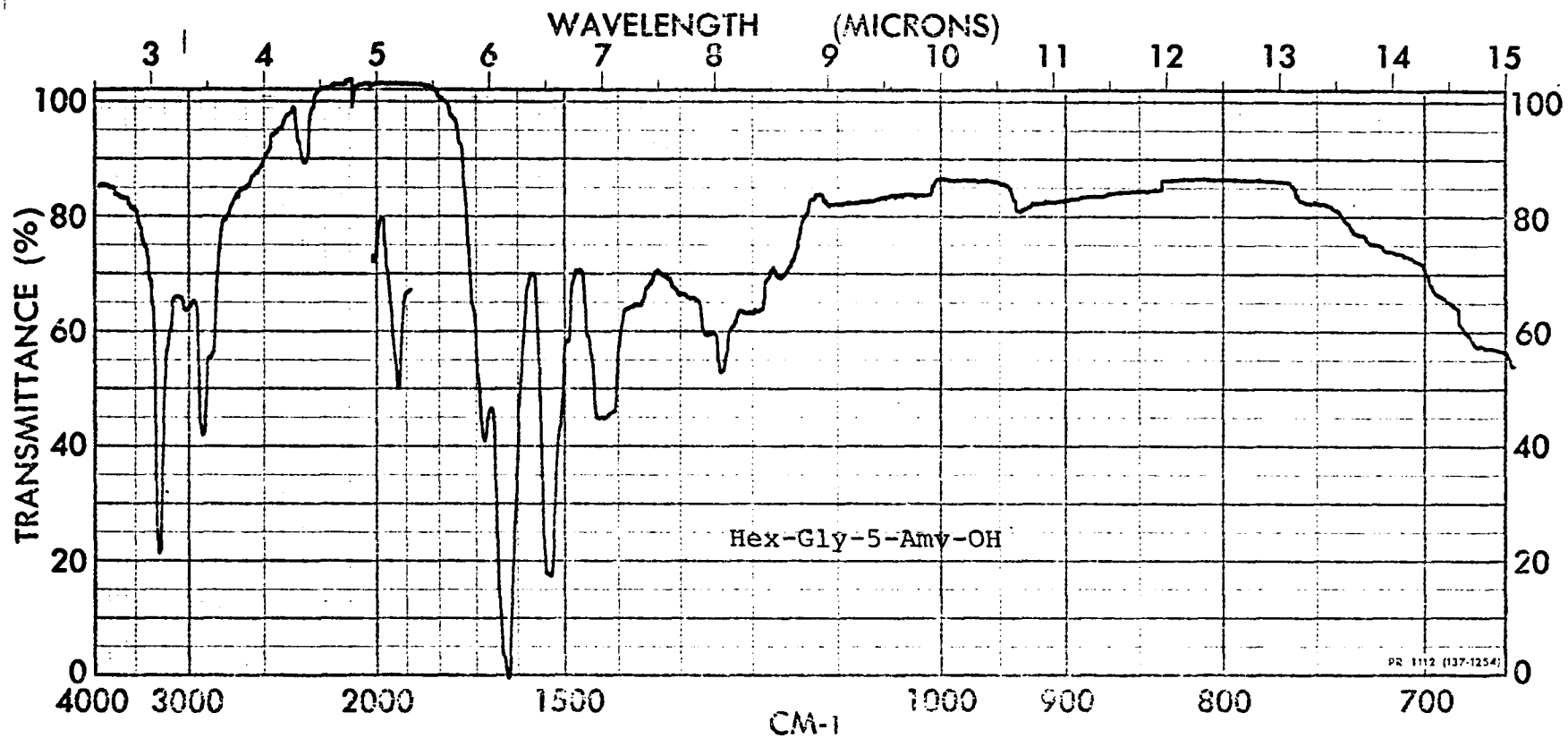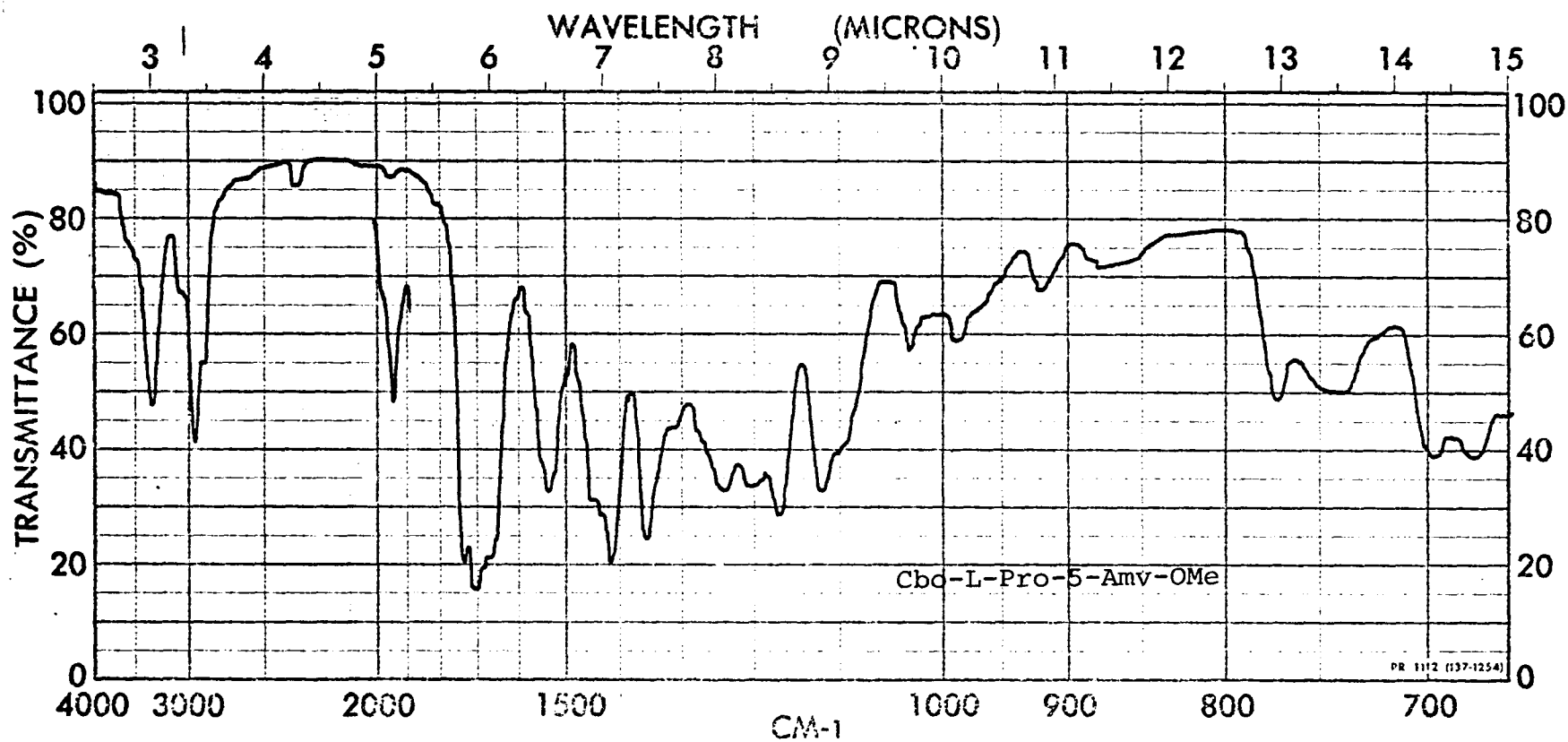
A-Figure 3. (neat). Compound 24.
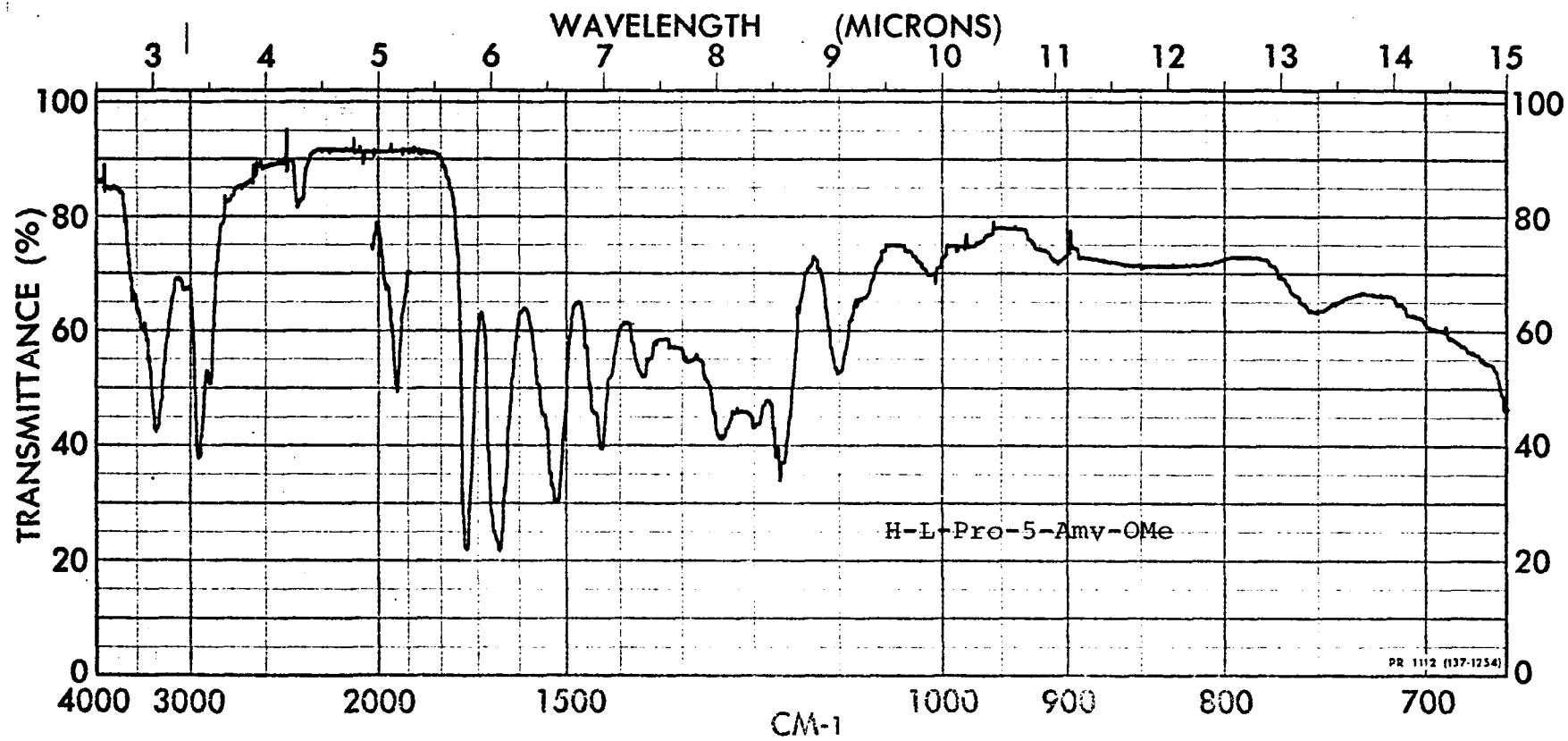
A-Figure 4. (neat). Compound 25.
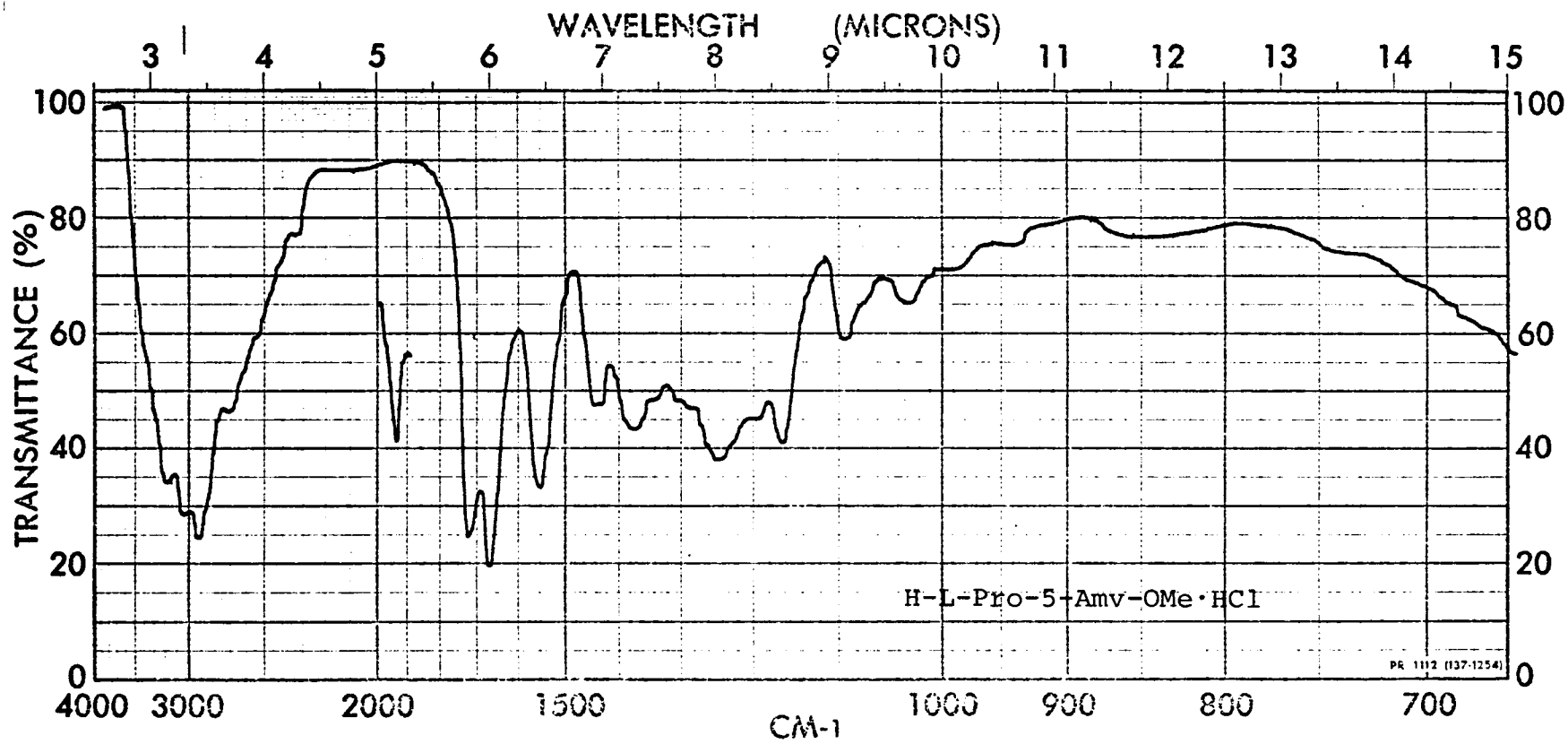
A-Figure 5.   (neat).   Compound 26.

A-Figure 6. (KBr). Compound 27.

The spectrum is labeled: Hex-Gly-L-Pro-5-Amv-OMe

Figure: IR spectrum (nujol), labeled "Pen-Gly-L-Pro-5-Amv-OMe". Axes: WAVELENGTH (MICRONS) across top (3–15), TRANSMITTANCE (%) on vertical (0–100), CM-1 across bottom (4000–700).
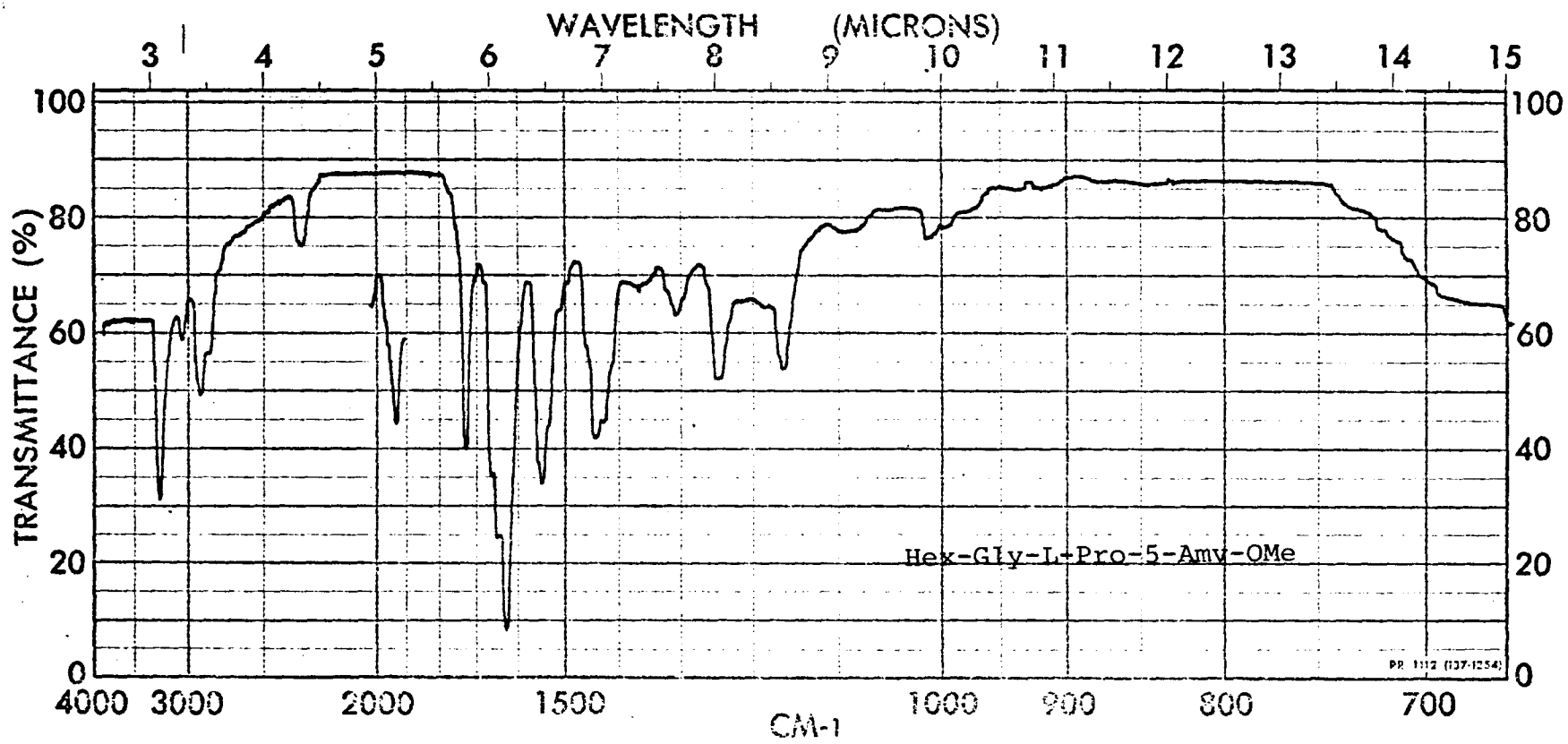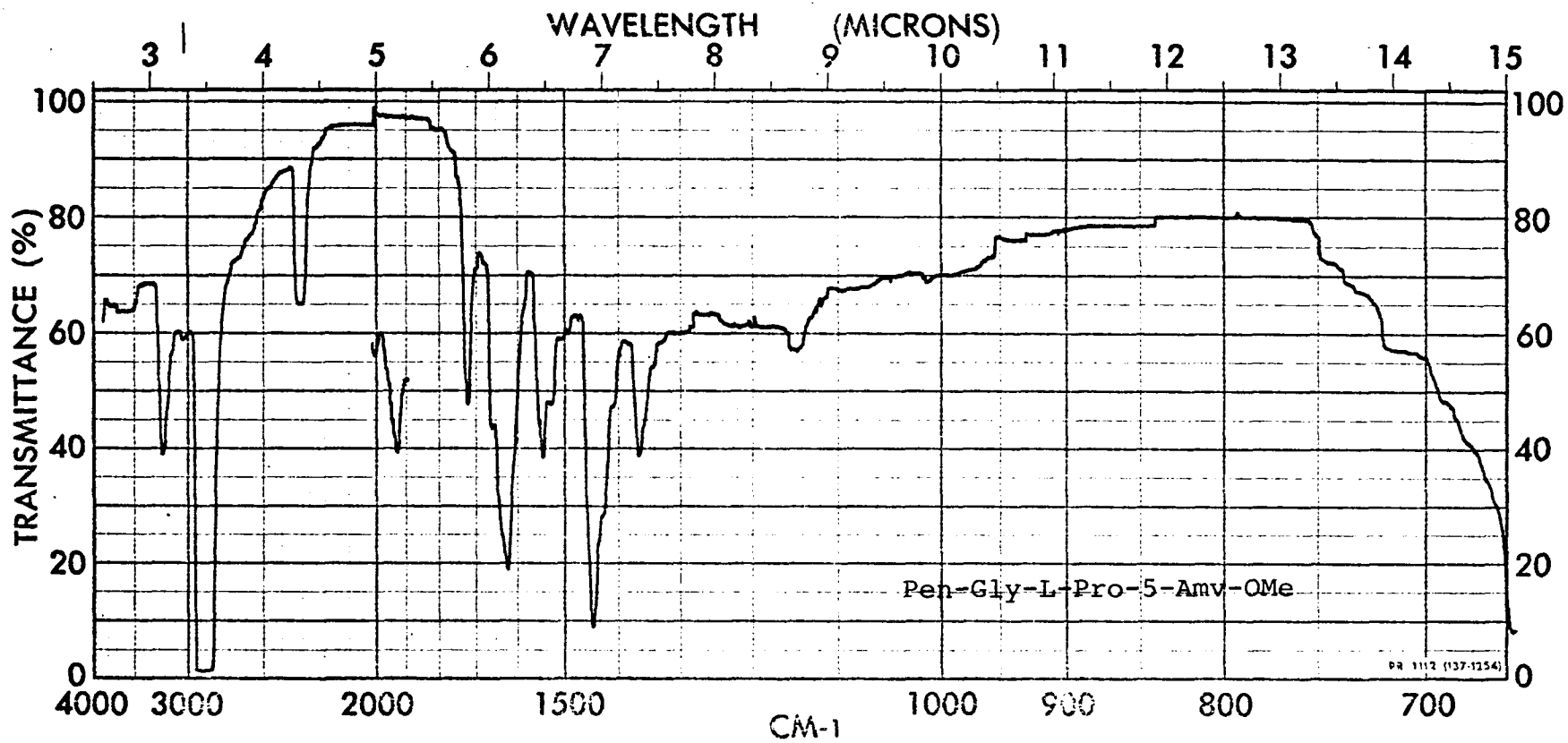
A-Figure 7.   (nujol).   Compound 30.
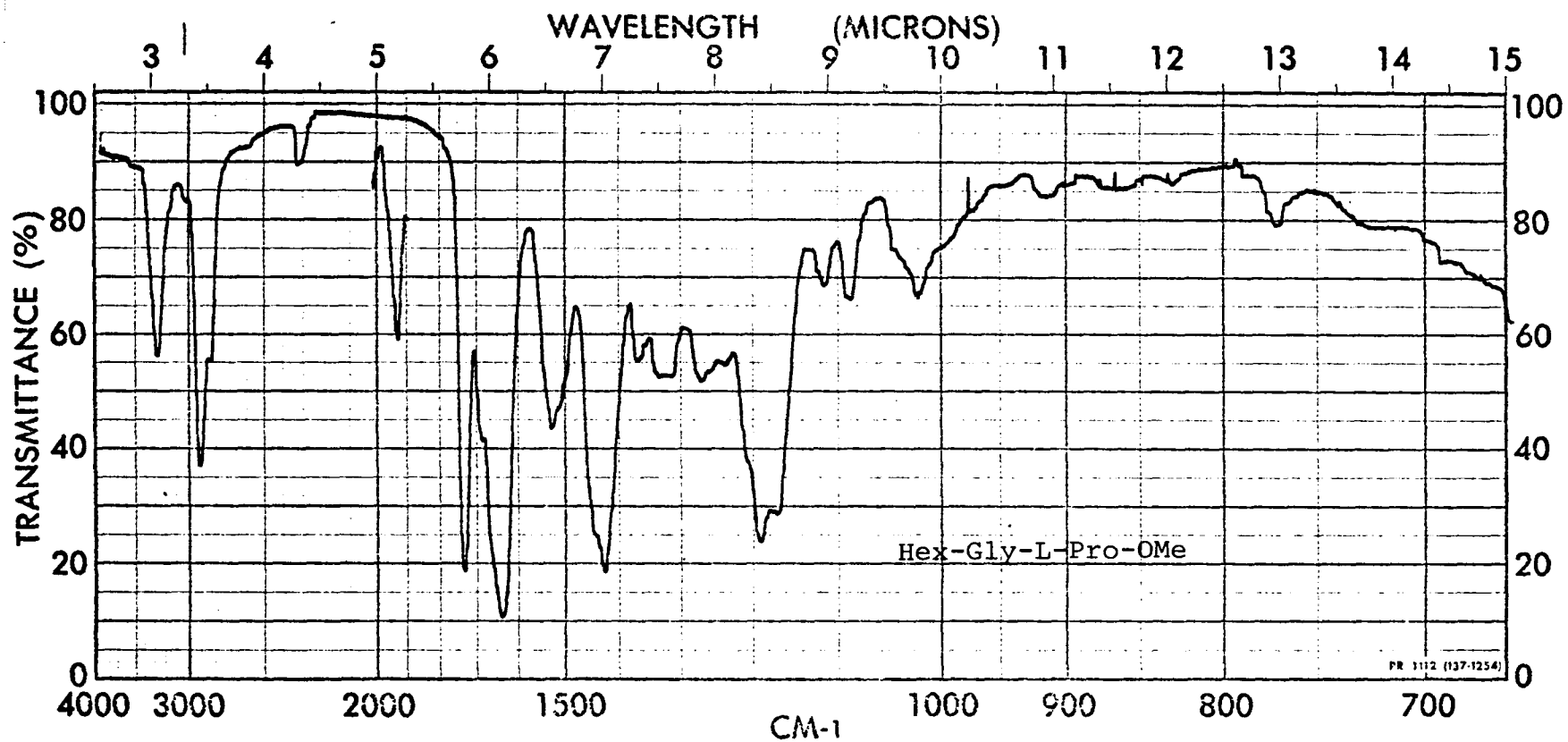
A-Figure 8. (neat). Compound 32.

A-Figure 9. (KBr). Compound 33.

The spectrum is labeled "Hex-Gly-L-Pro-OH"
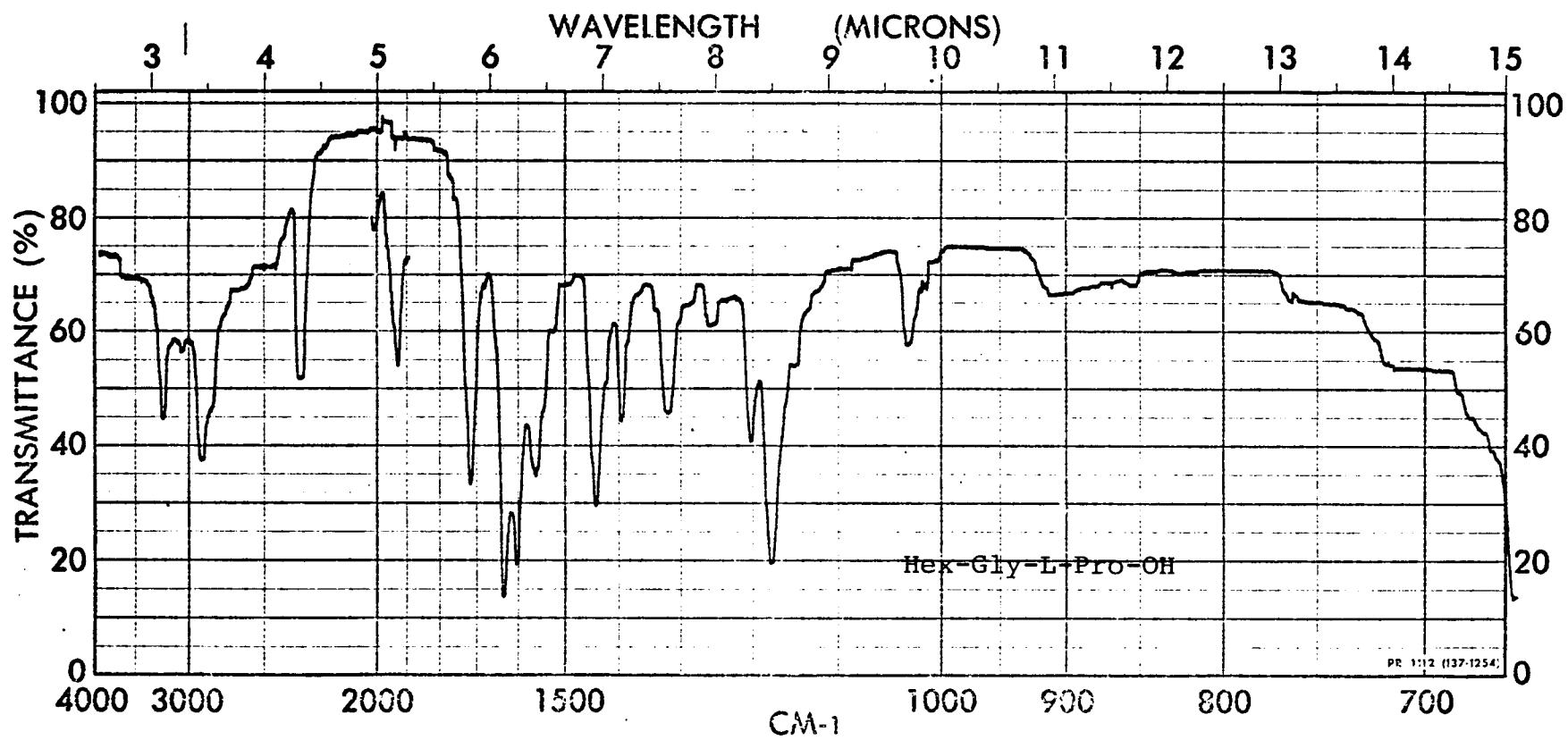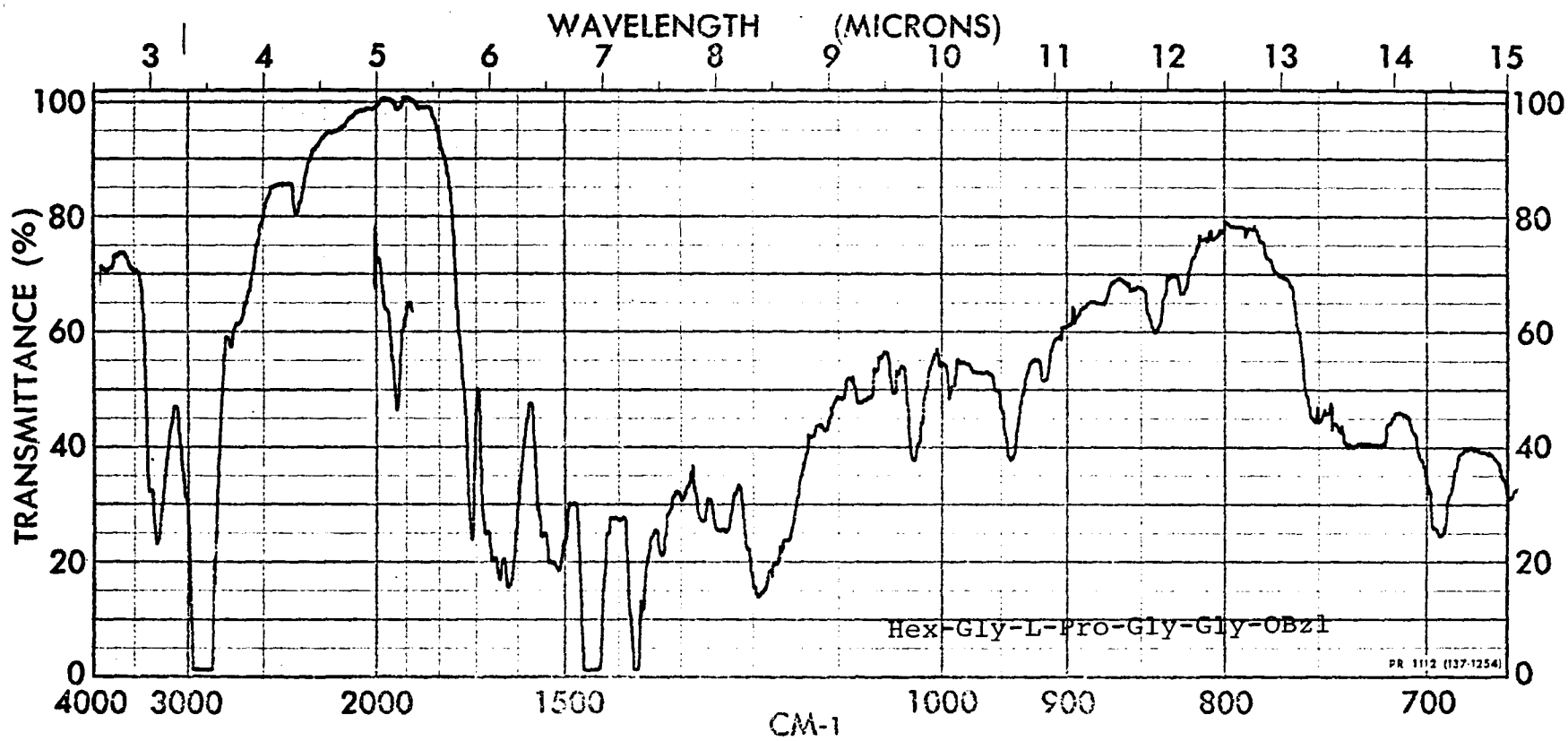
A-Figure 10. (nujol). Compound 35.
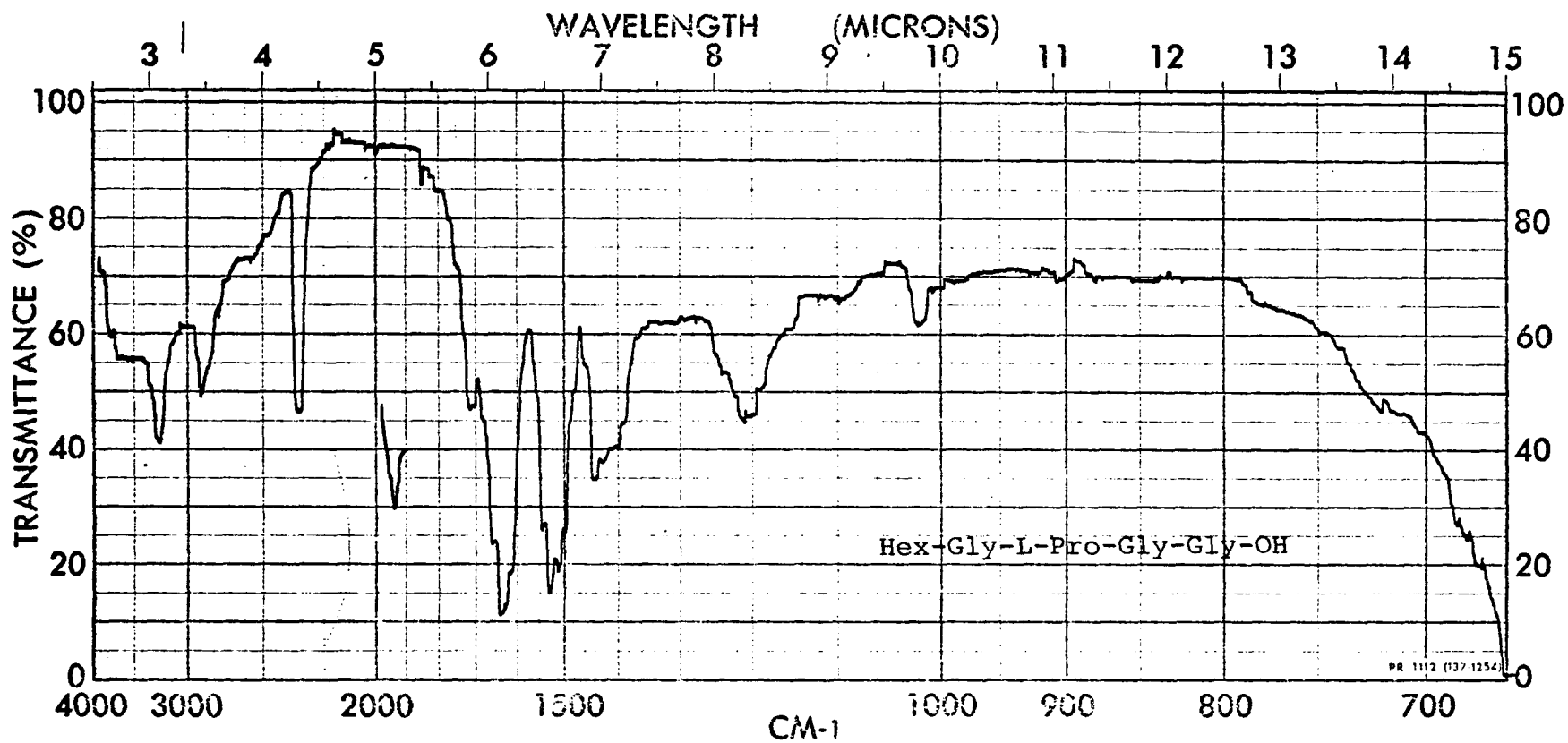
A-Figure 11. (KBr). Compound 36.
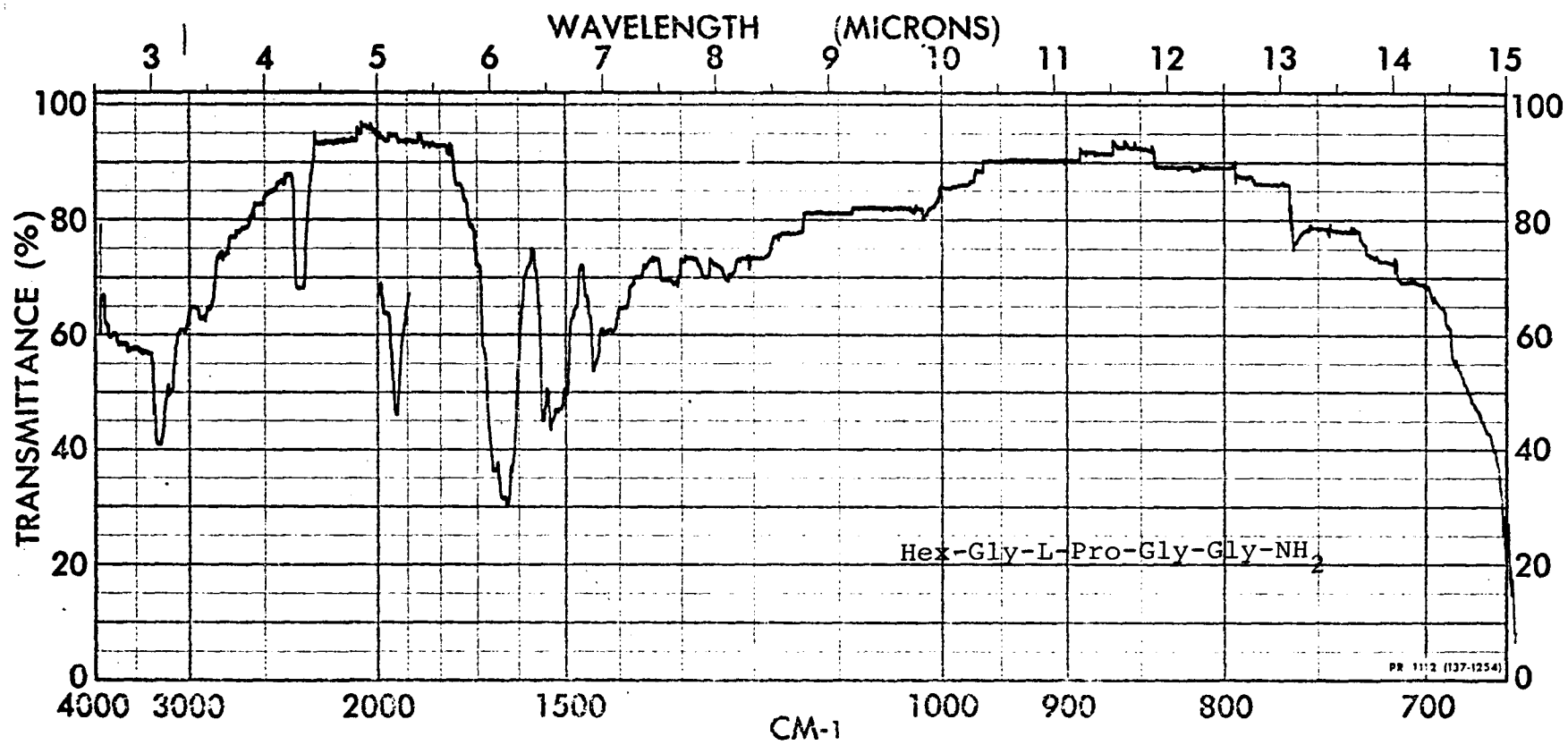
A-Figure 12. (KBr). Compound 37.

Hex-Gly-L-Pro-Gly-Gly-NH$_2$
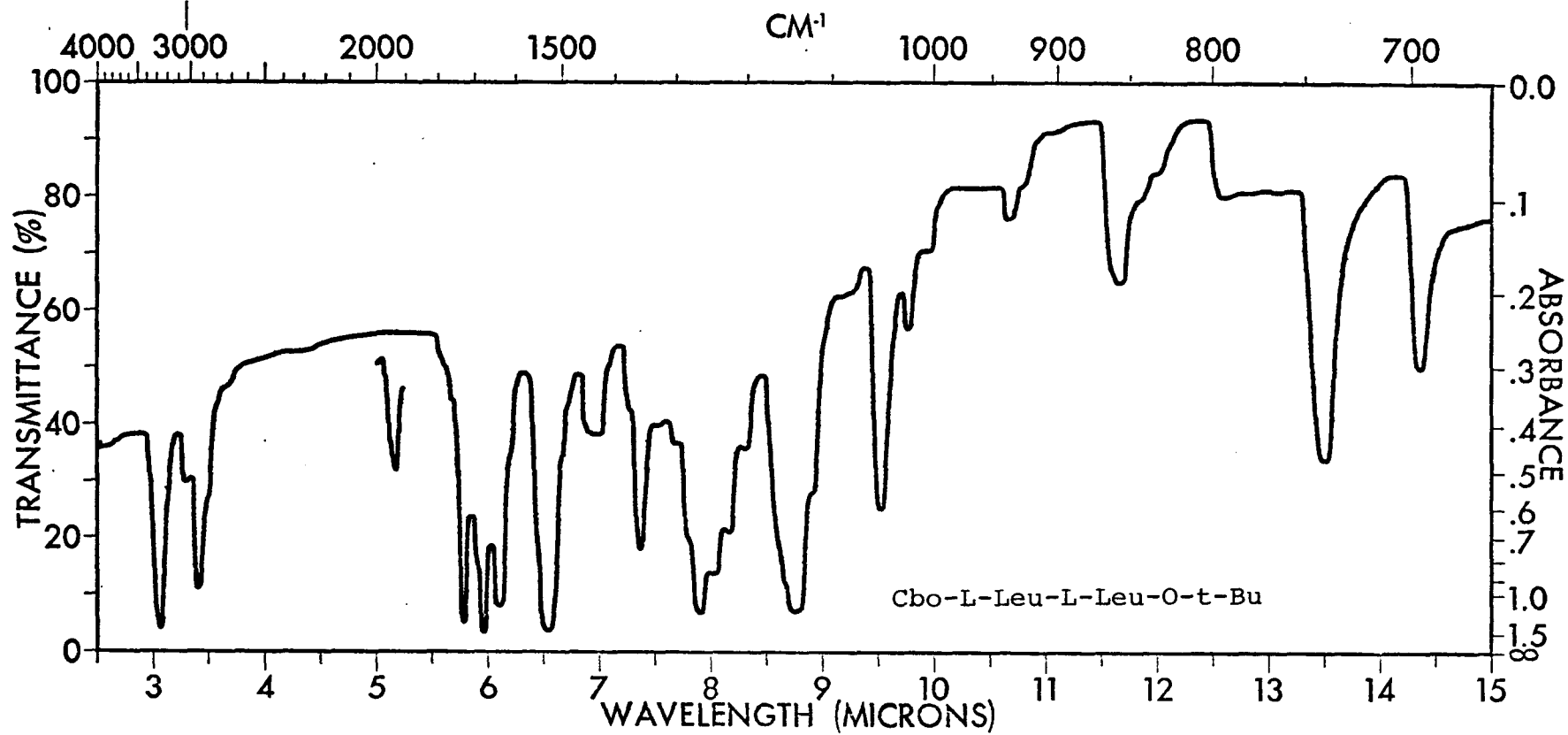
A-Figure 13. (KBr). Compound <u>45</u>.
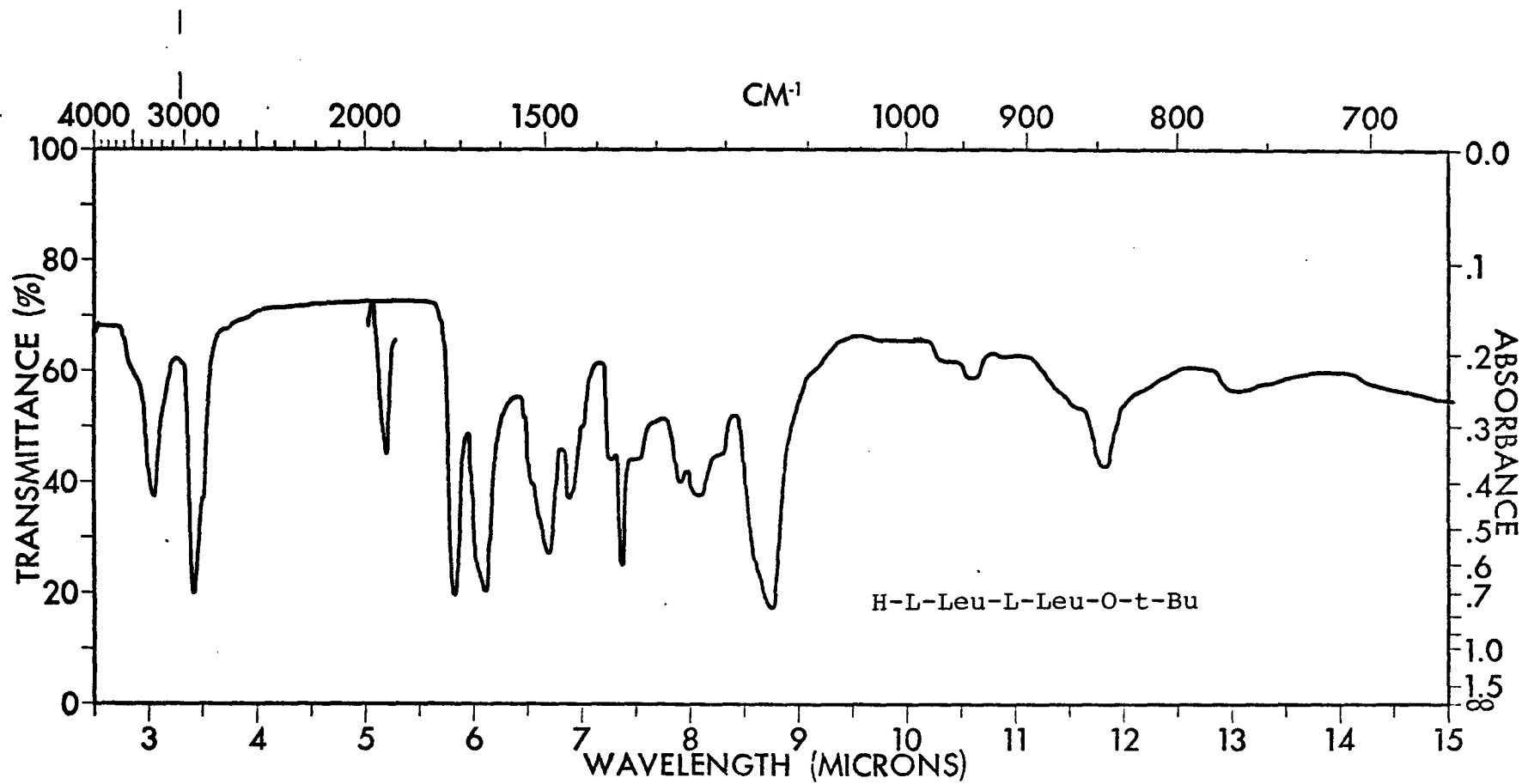
A-Figure 14. (neat). Compound 46.

EtO-CO-L-Leu-L-Leu-O-t-Bu
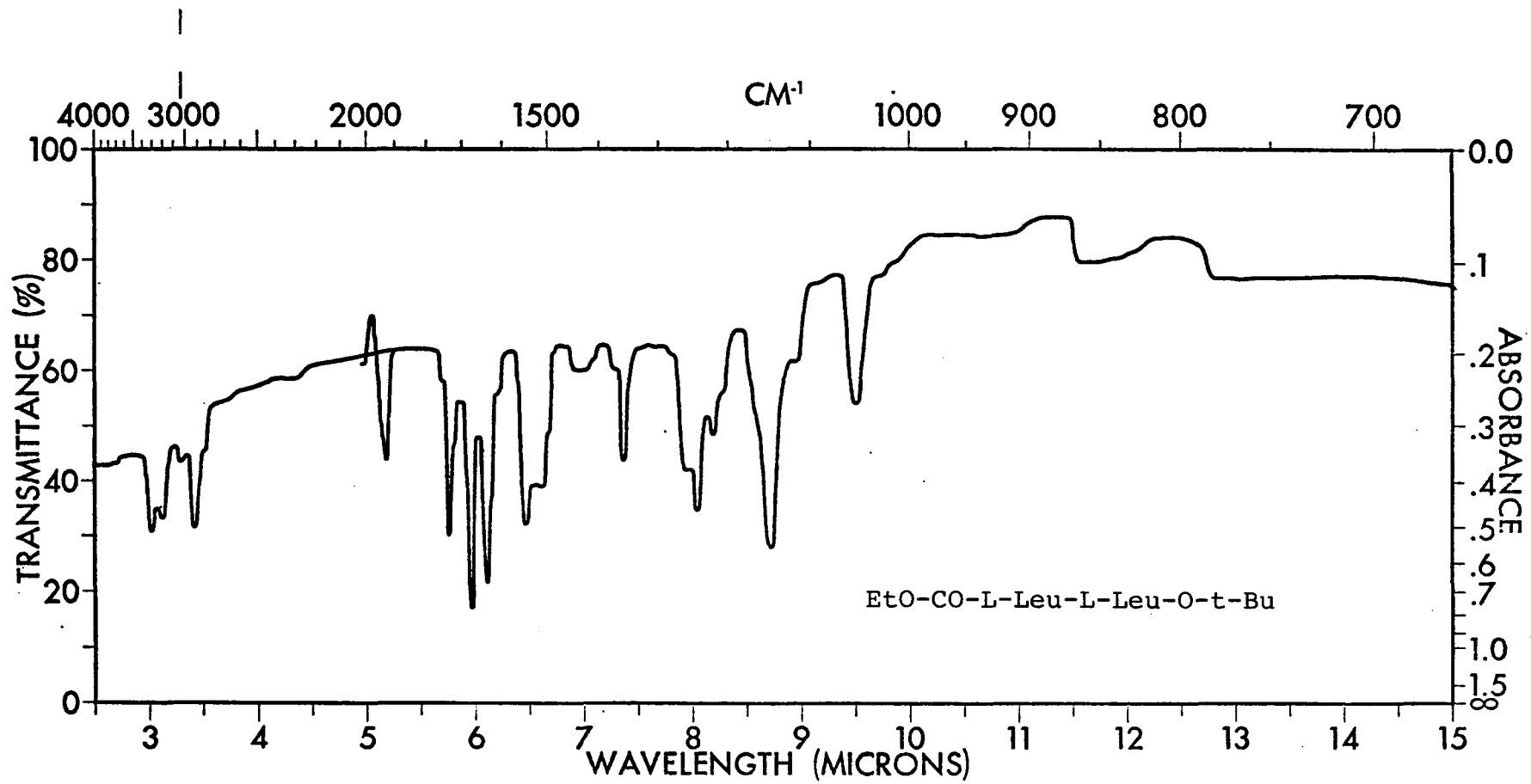
A-Figure 15.   (KBr).   Compound 48.

A-Figure 16.   (KBr).   Compound 47.

Cbo-L-Pro-L-Leu-L-Leu-O-t-Bu

A-Figure 17.   (KBr).   Compound 51.

The spectrum is labeled: H-L-Pro-L-Leu-L-Leu-O-t-Bu

Hex-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu
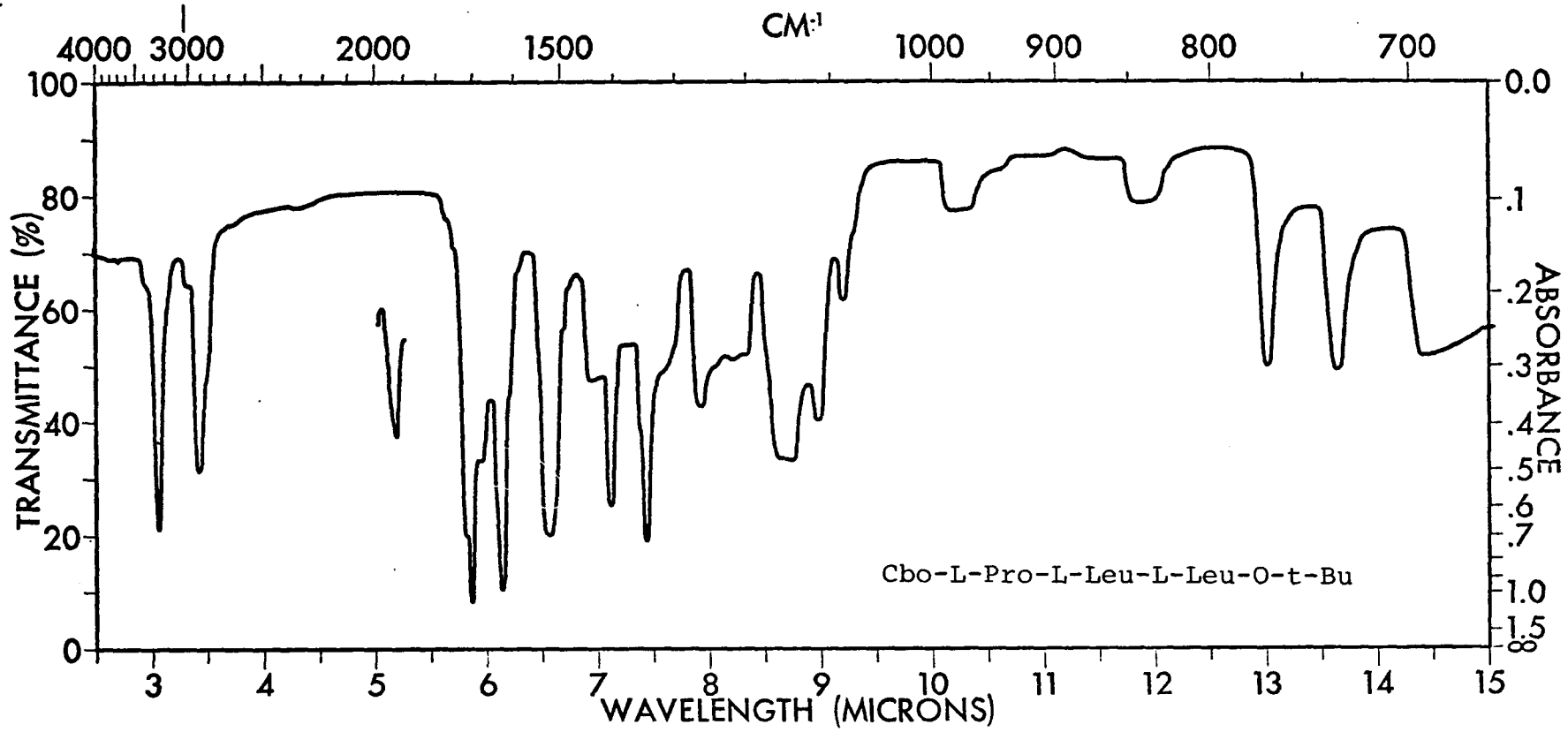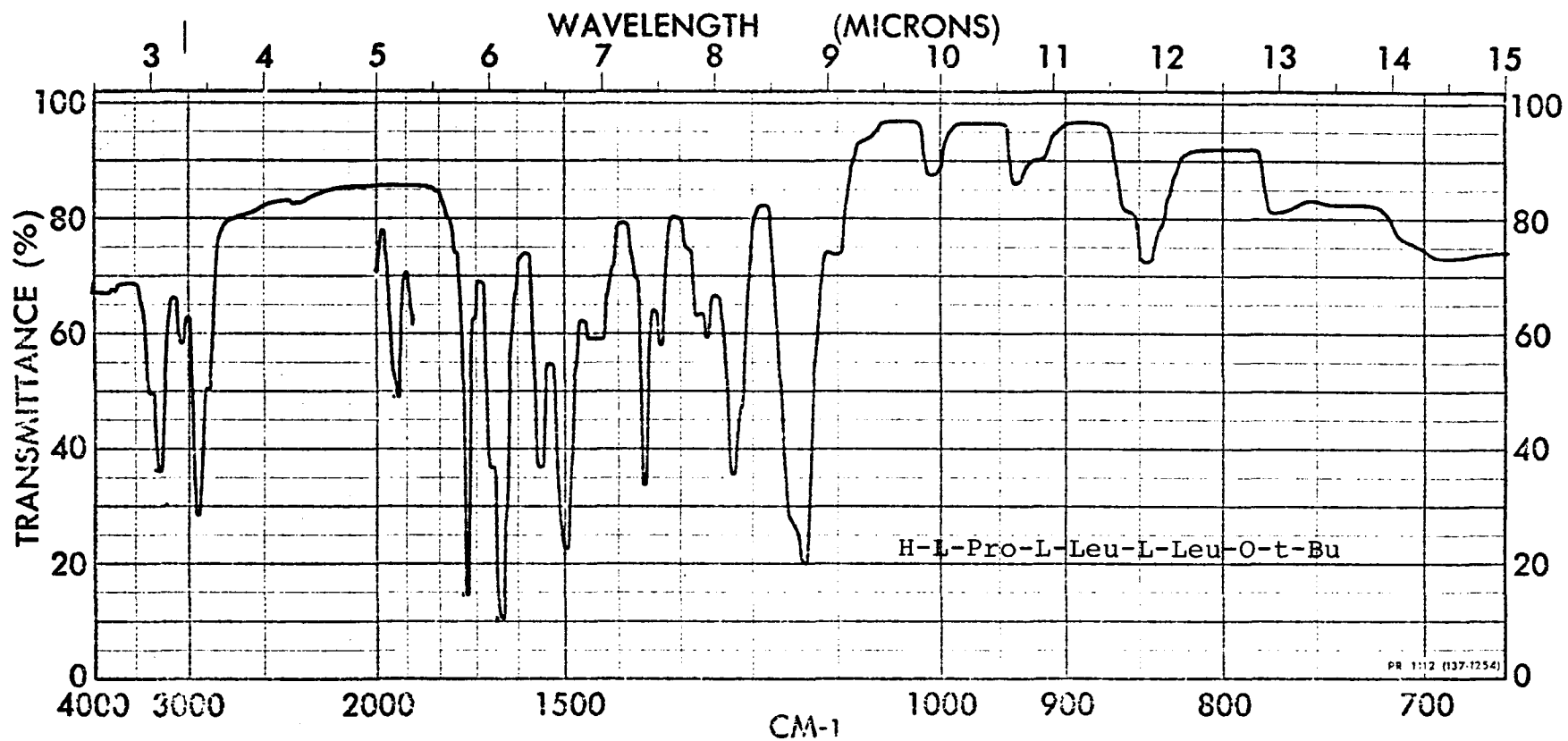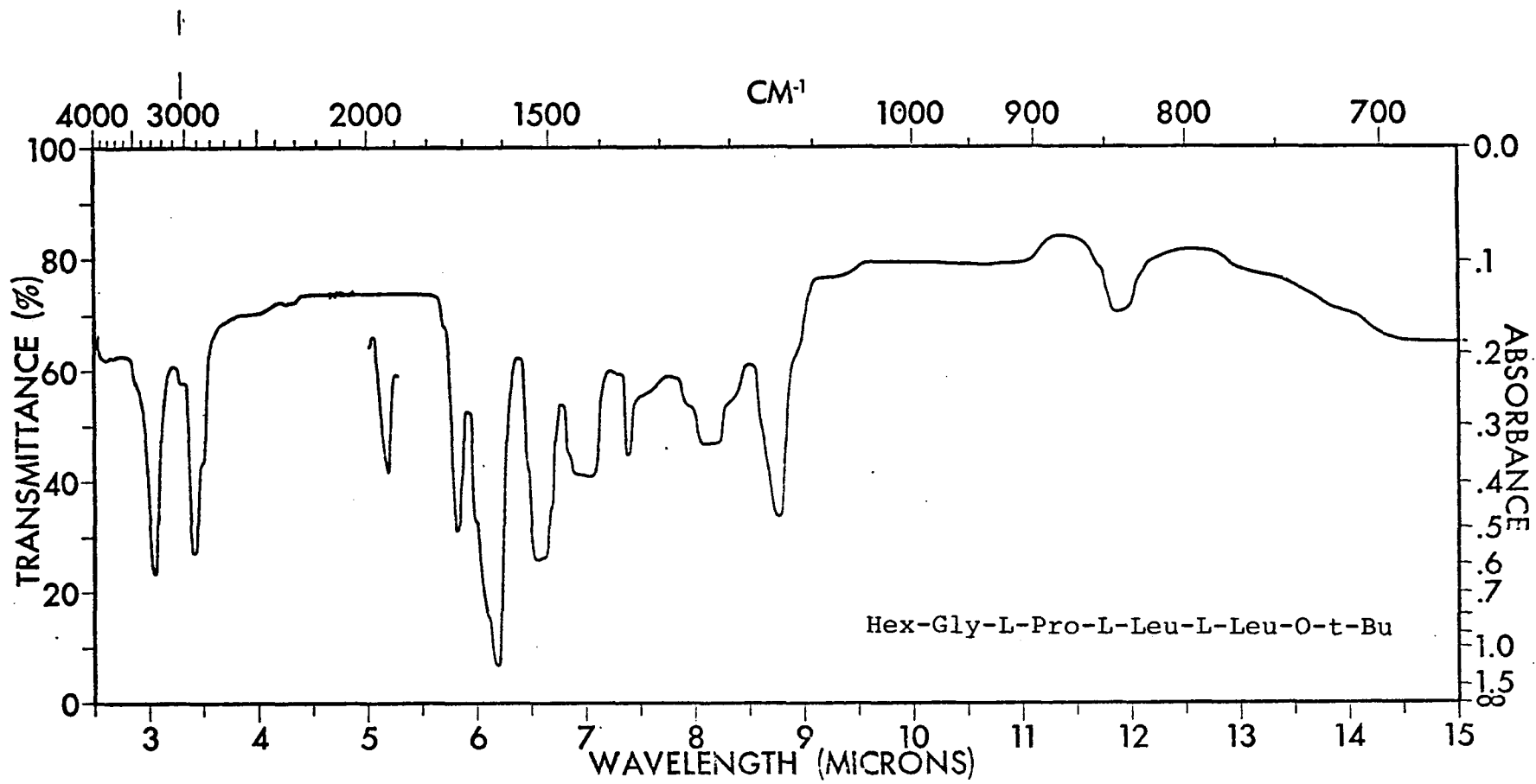
A-Figure 18. (KBr). Compound 52.
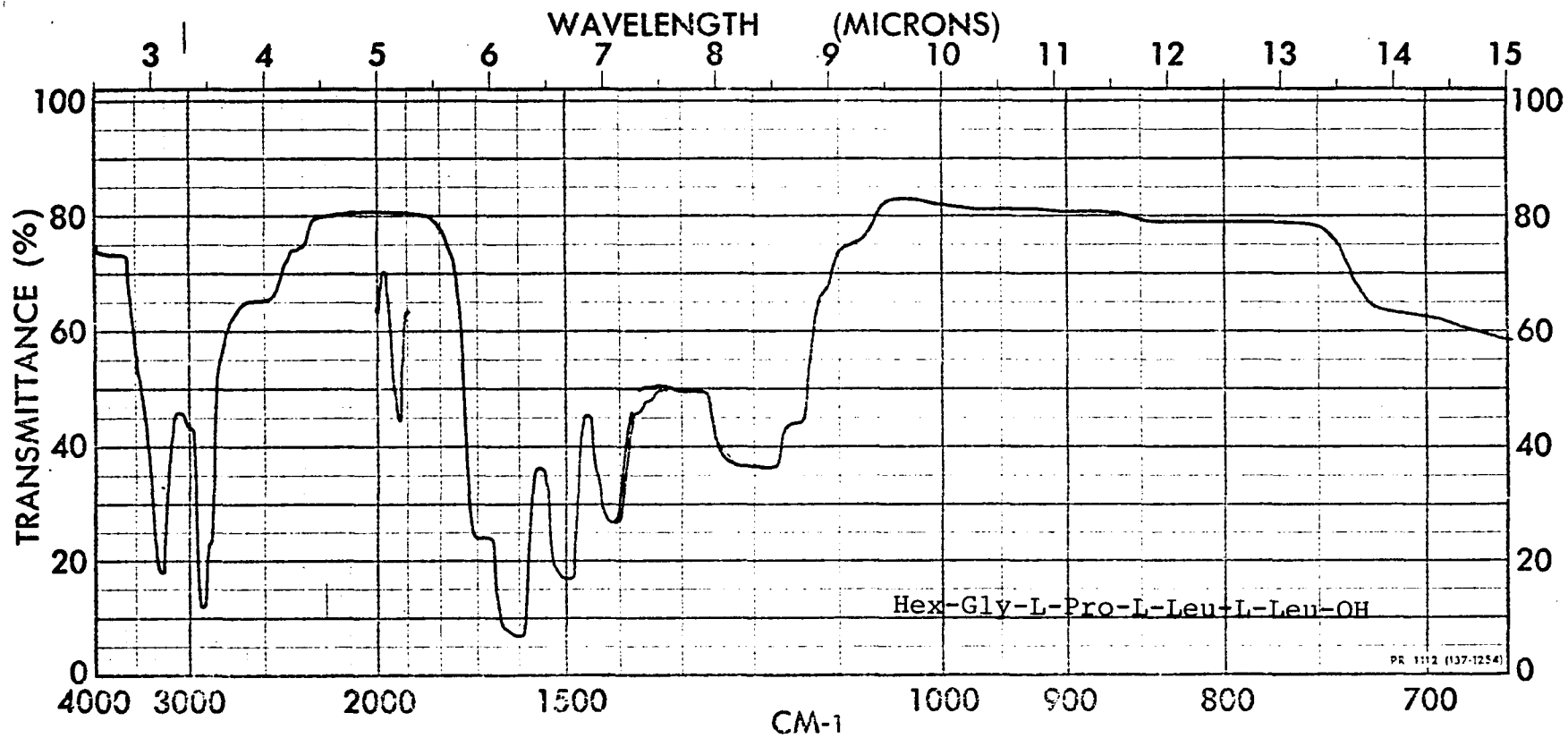
A-Figure 19.   (KBr).   Compound 56.

CM⁻¹

Pen-Gly-OPhNO₂
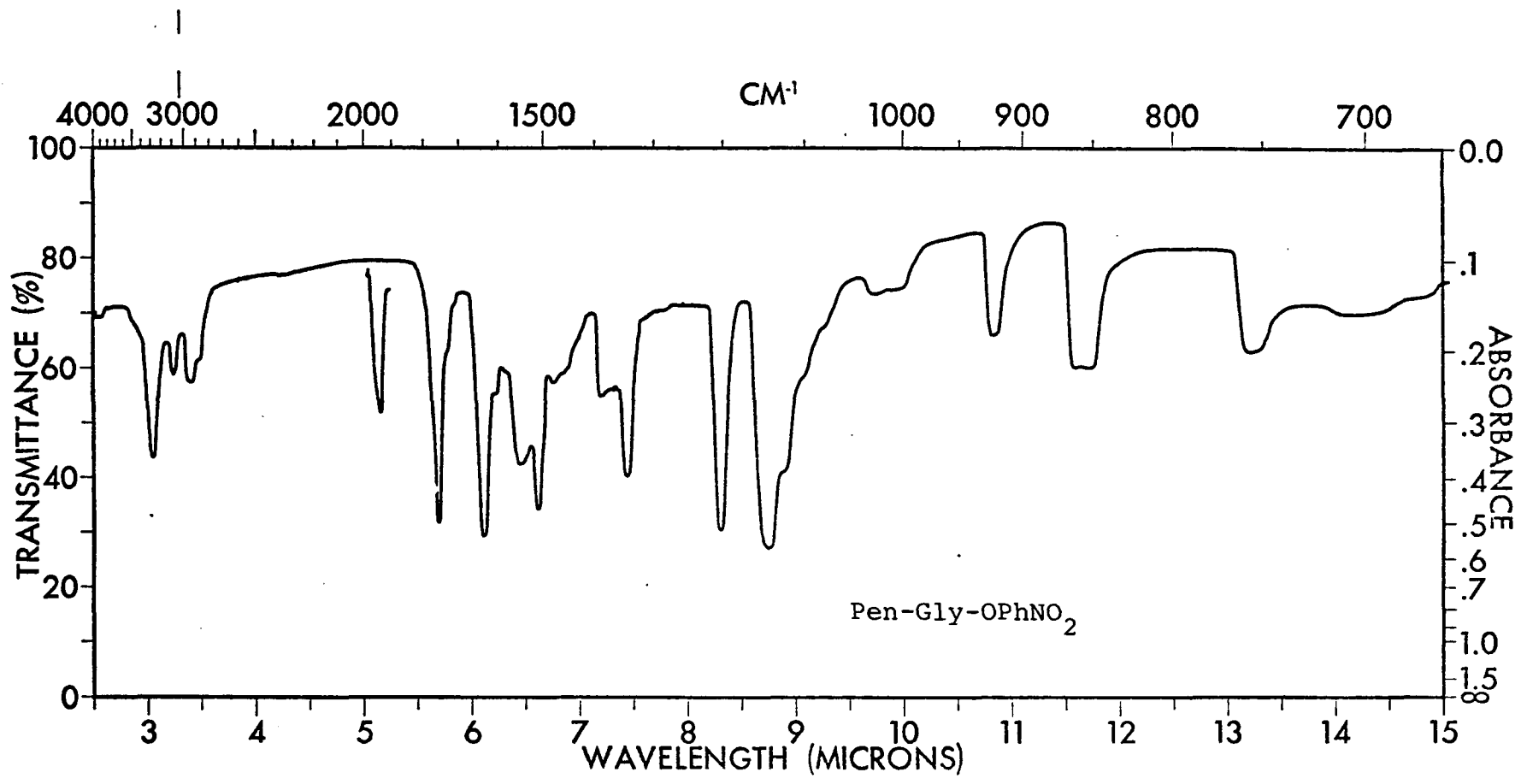
A-Figure 20.   (KBr).   Compound 57.

140
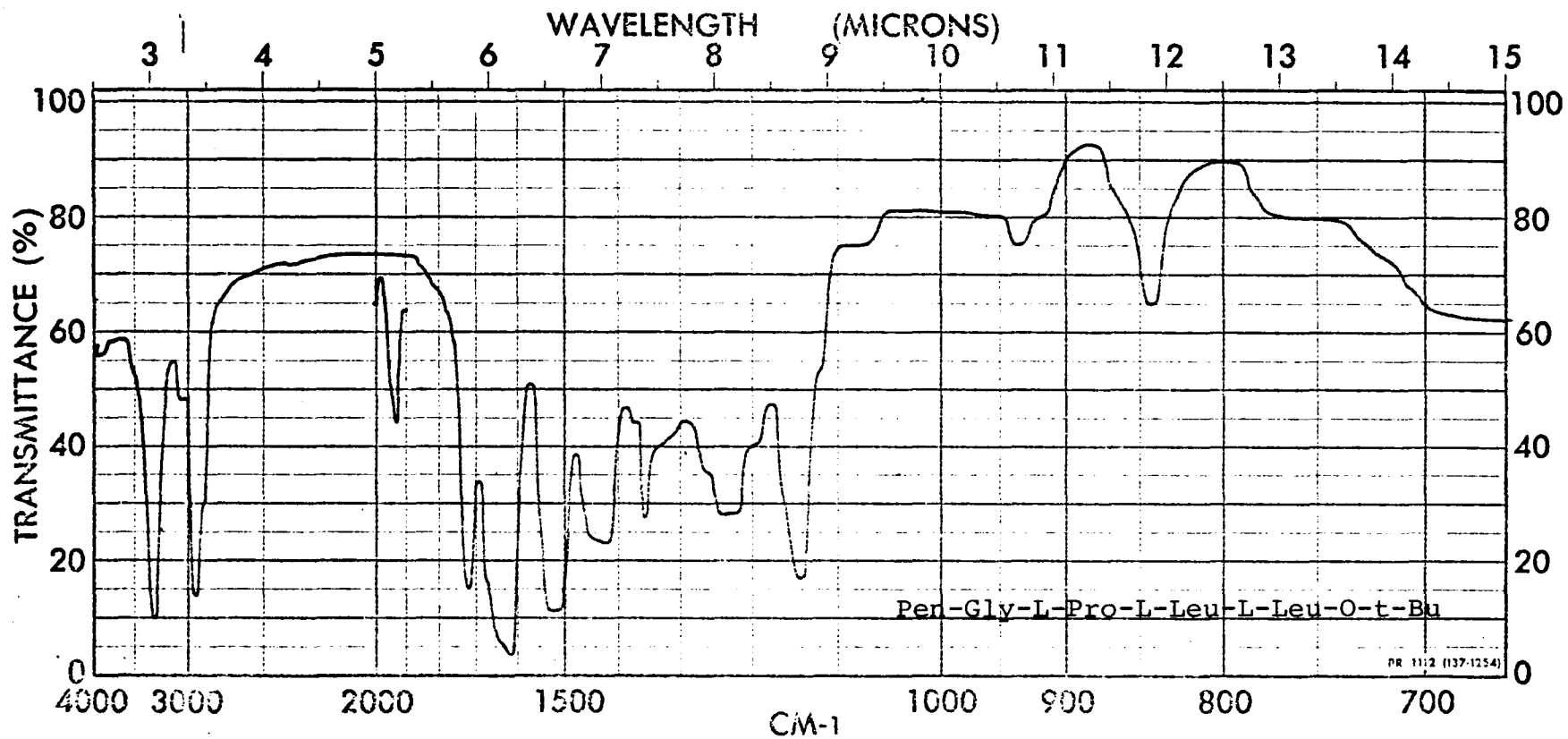
A-Figure 21.   (KBr).   Compound 58.
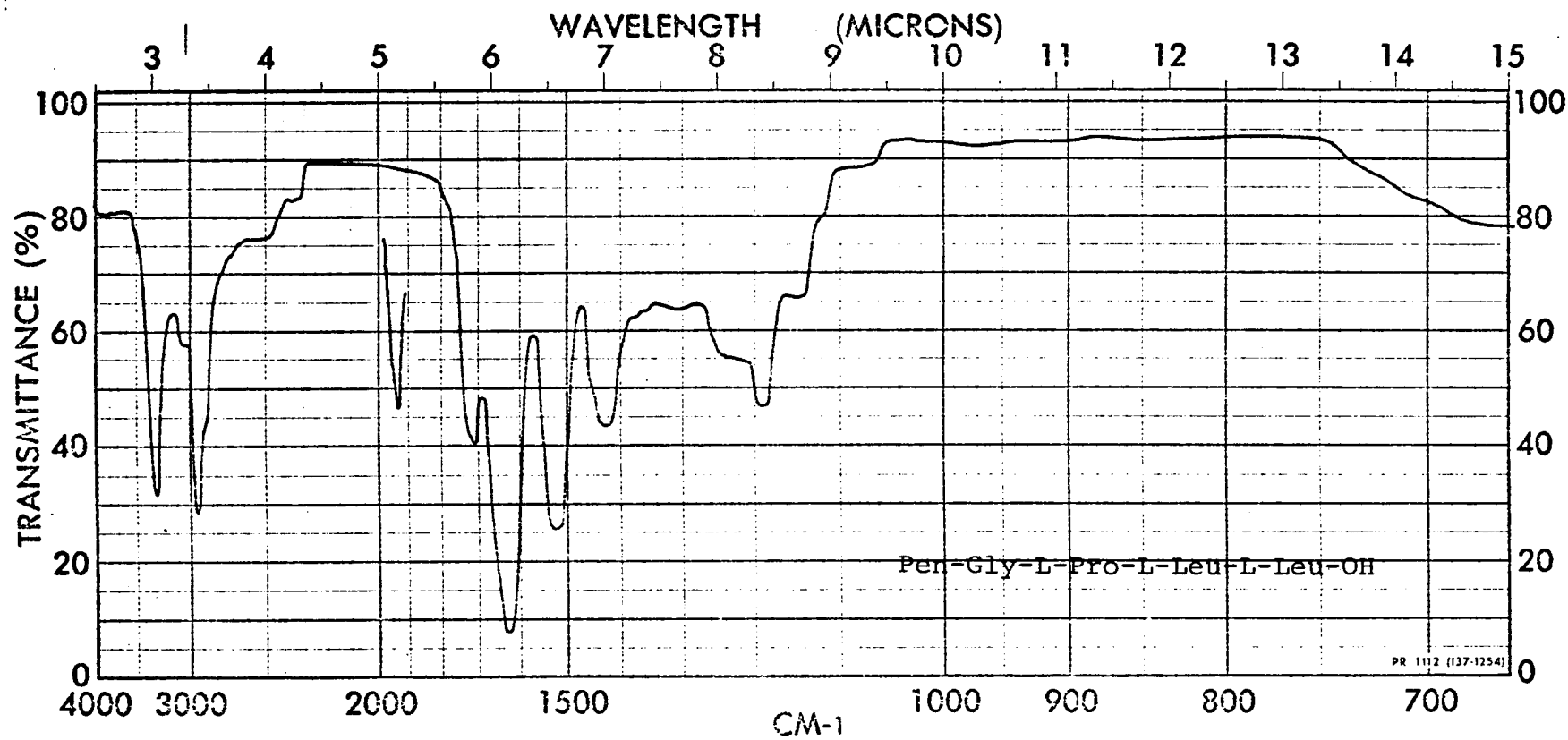
A-Figure 22.　(KBr).　Compound <u>59</u>.

Pen-Gly-L-Pro-L-Leu-L-Leu-OH
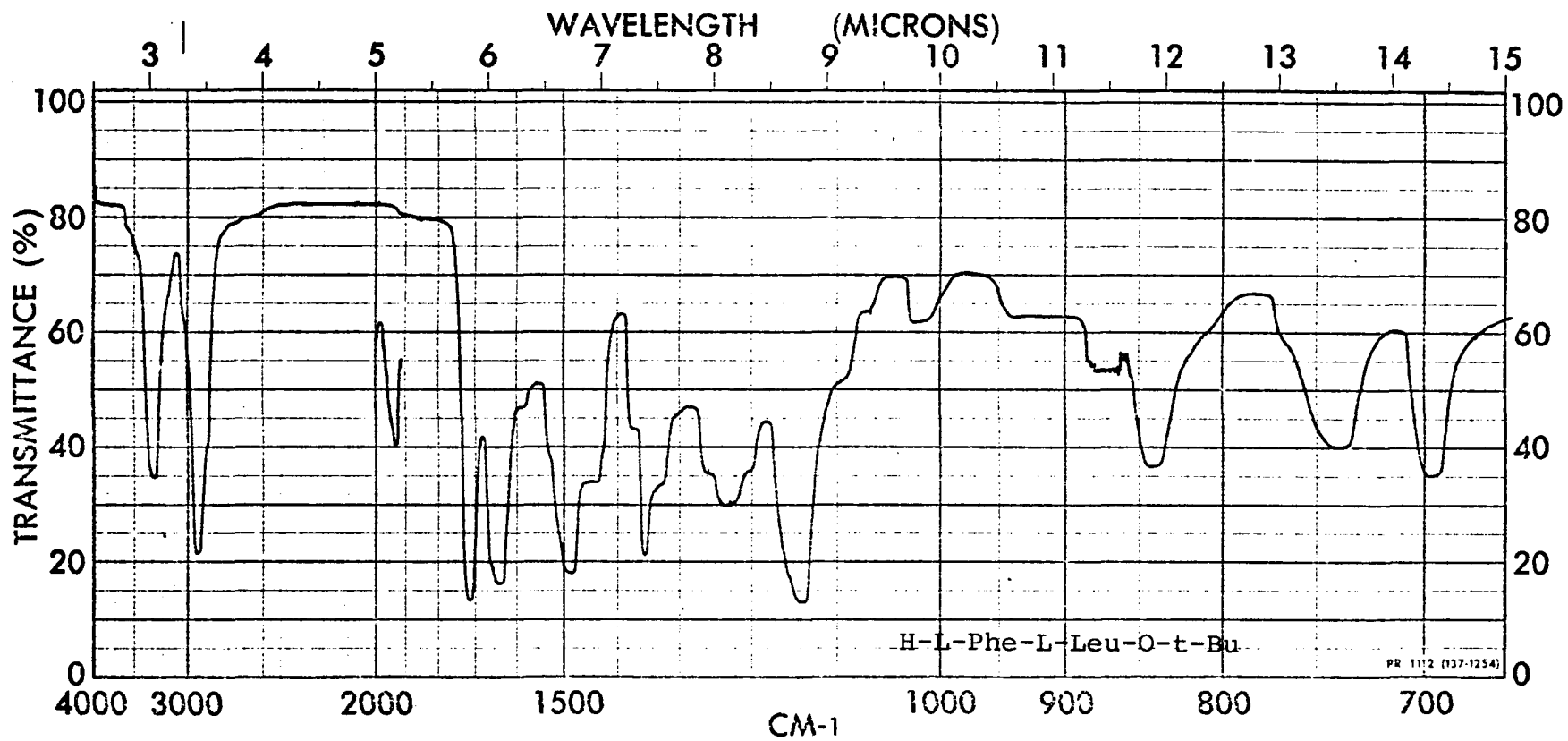
A-Figure 23. (neat). Compound 75.
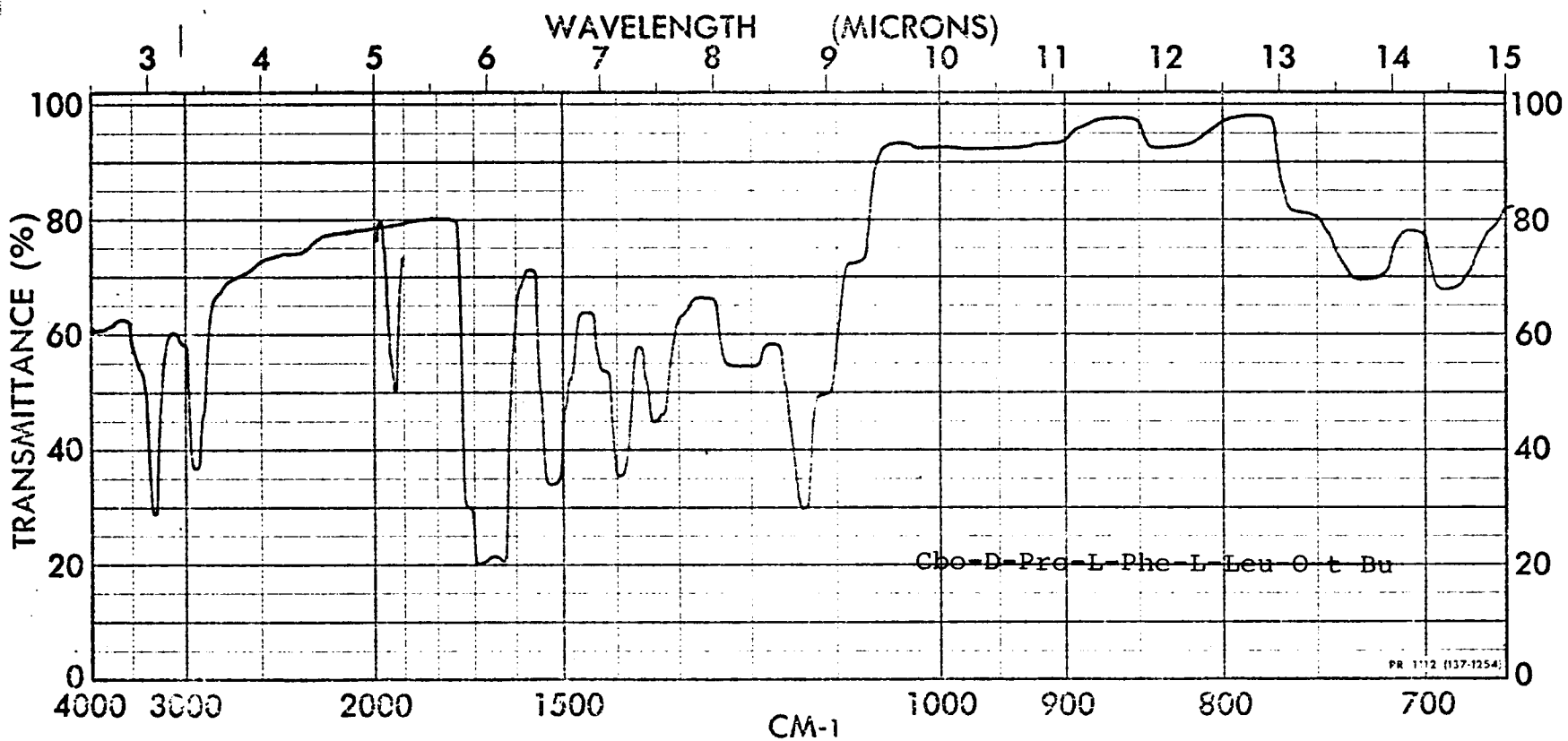
A-Figure 24. (KBr). Compound 76.
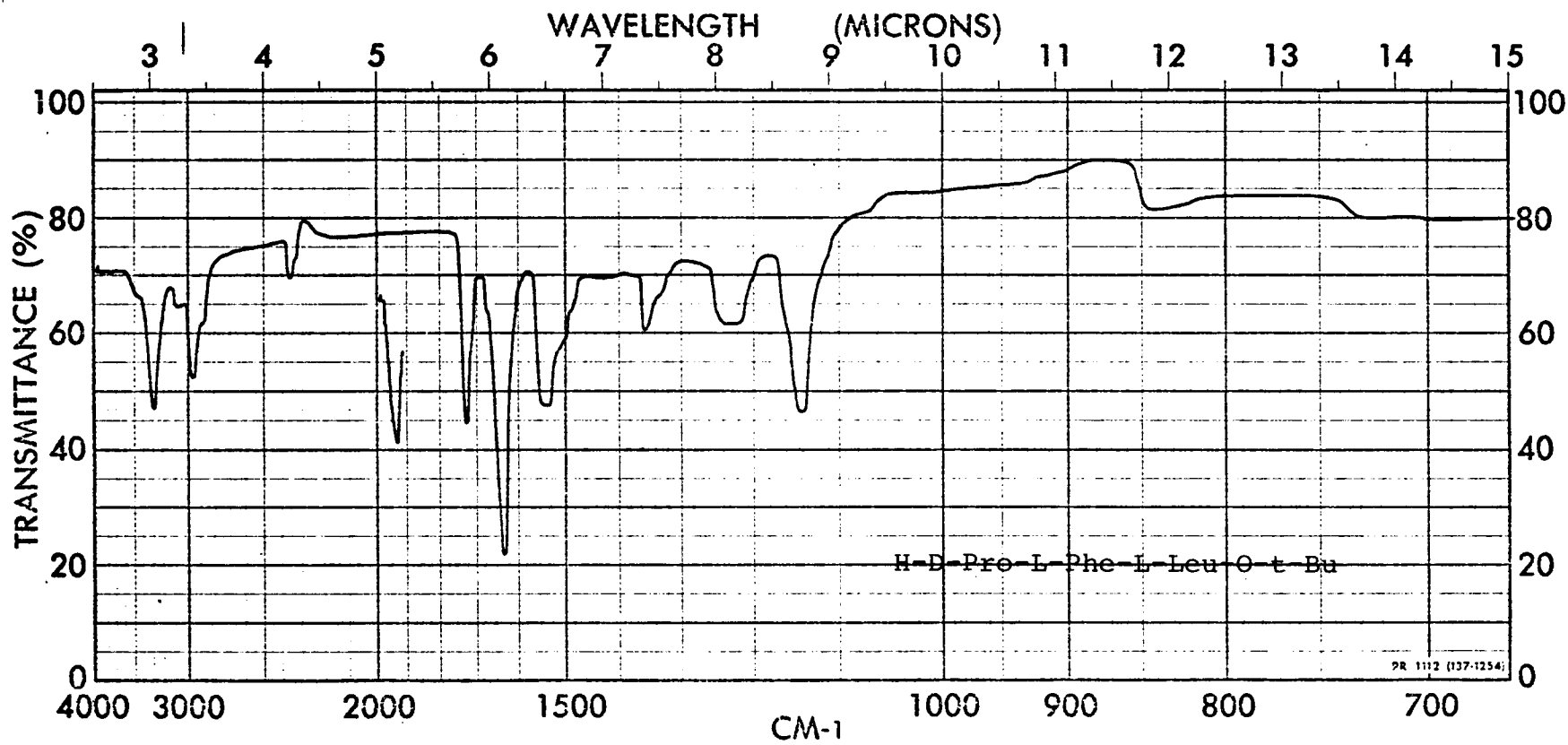
A-Figure 25.   (KBr).   Compound 77.
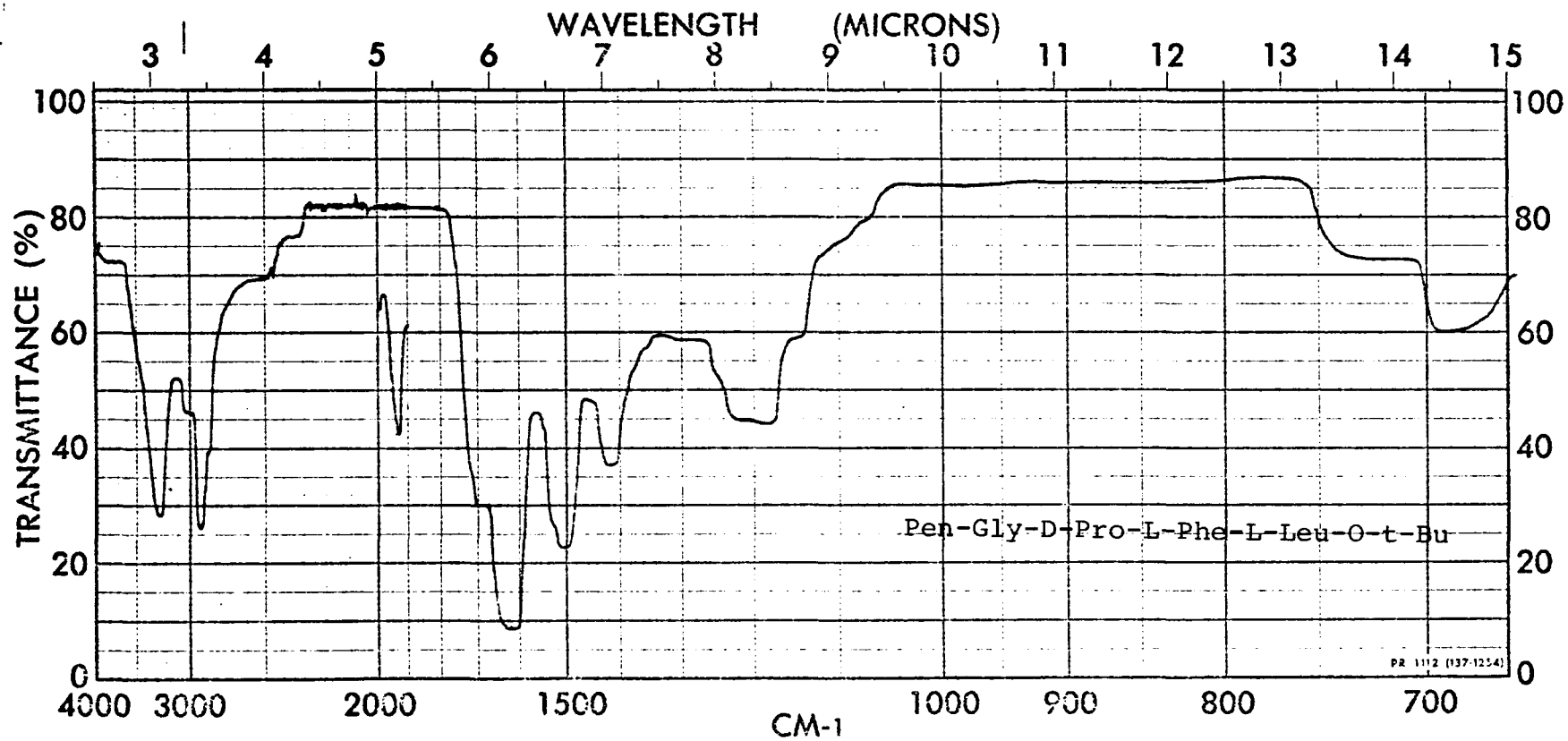
A-Figure 26.　(KBr).　Compound 79.

Pen-Gly-D-Pro-L-Phe-L-Leu-O-t-Bu
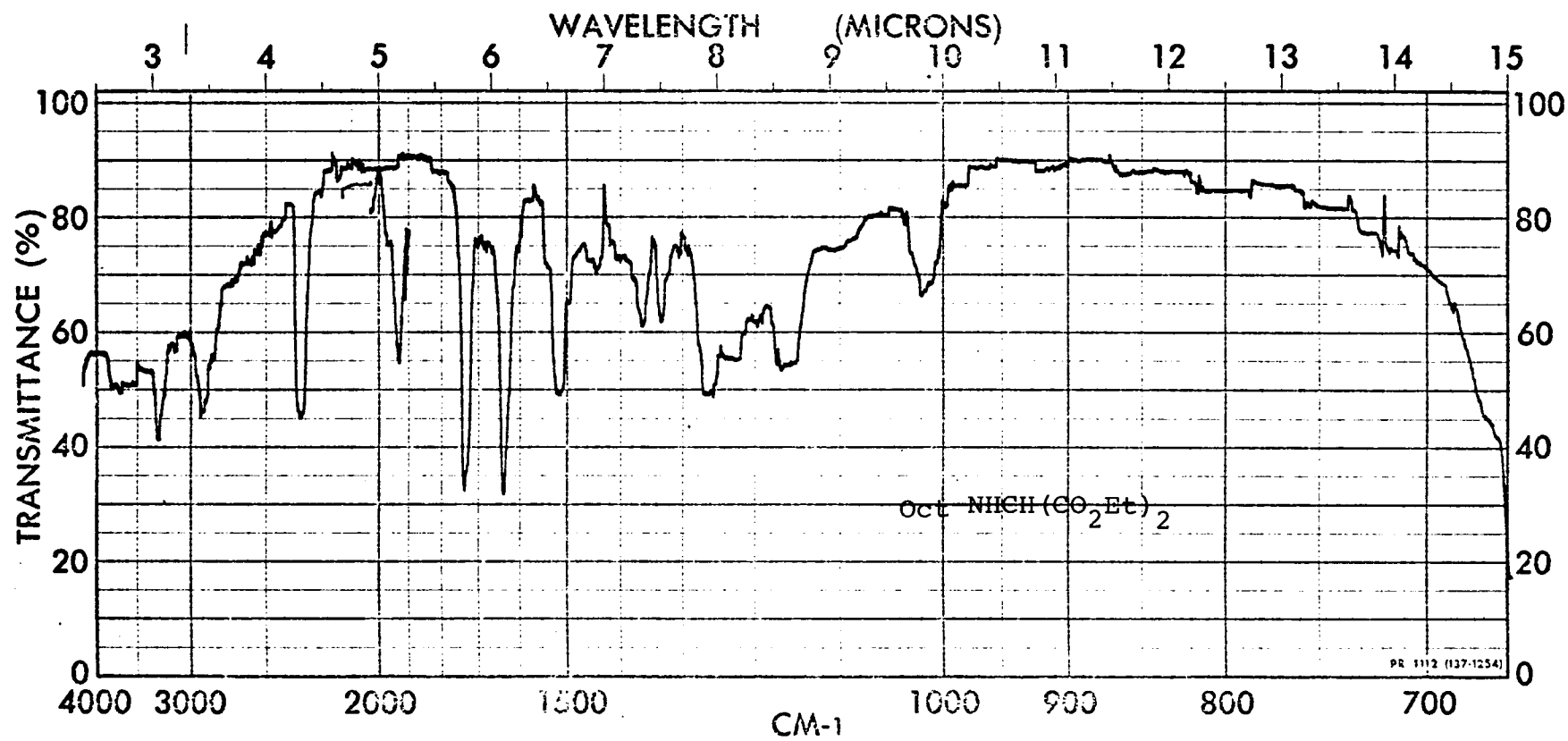
A-Figure 27.   (KBr).   Compound 82.
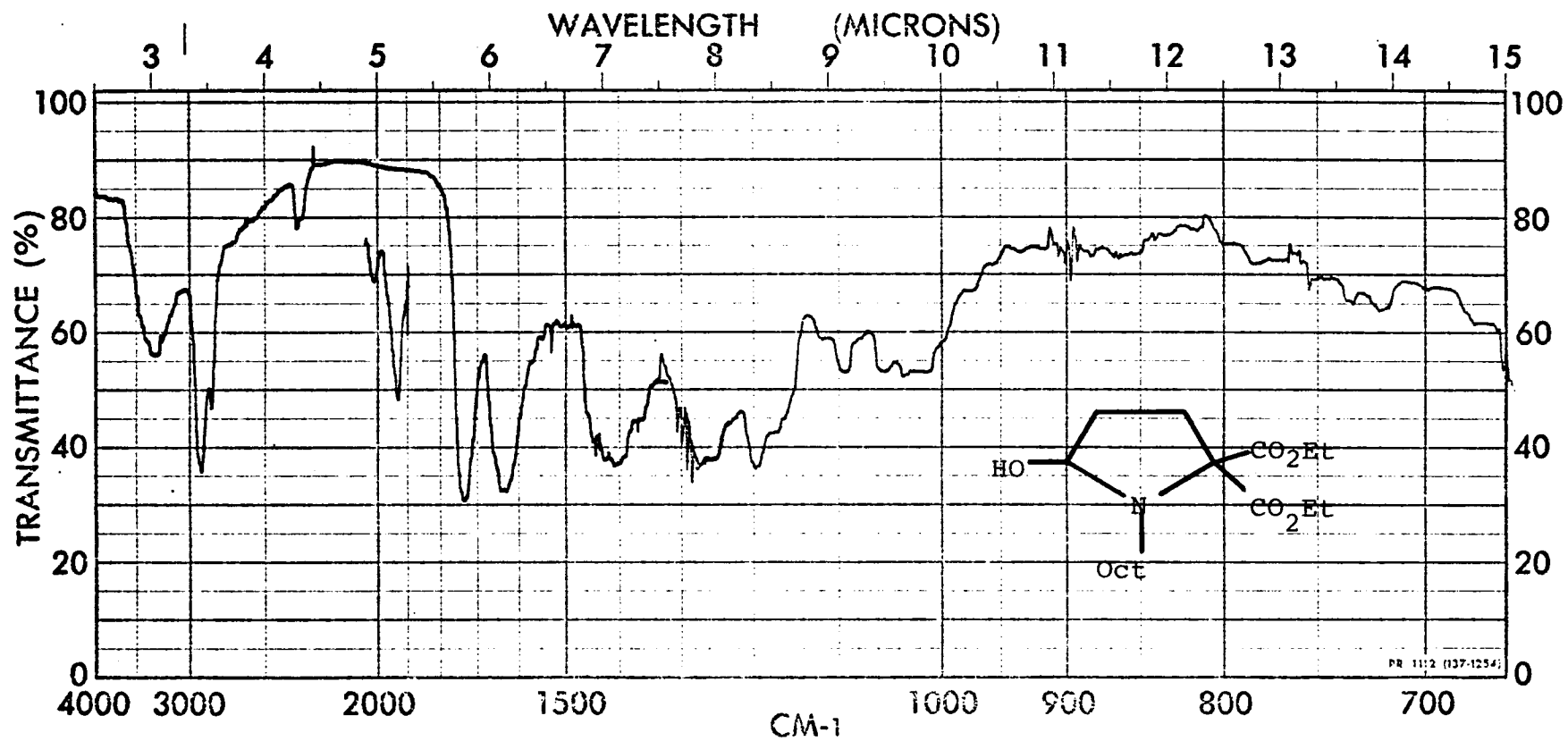
A-Figure 28.   (neat).   Compound 83.
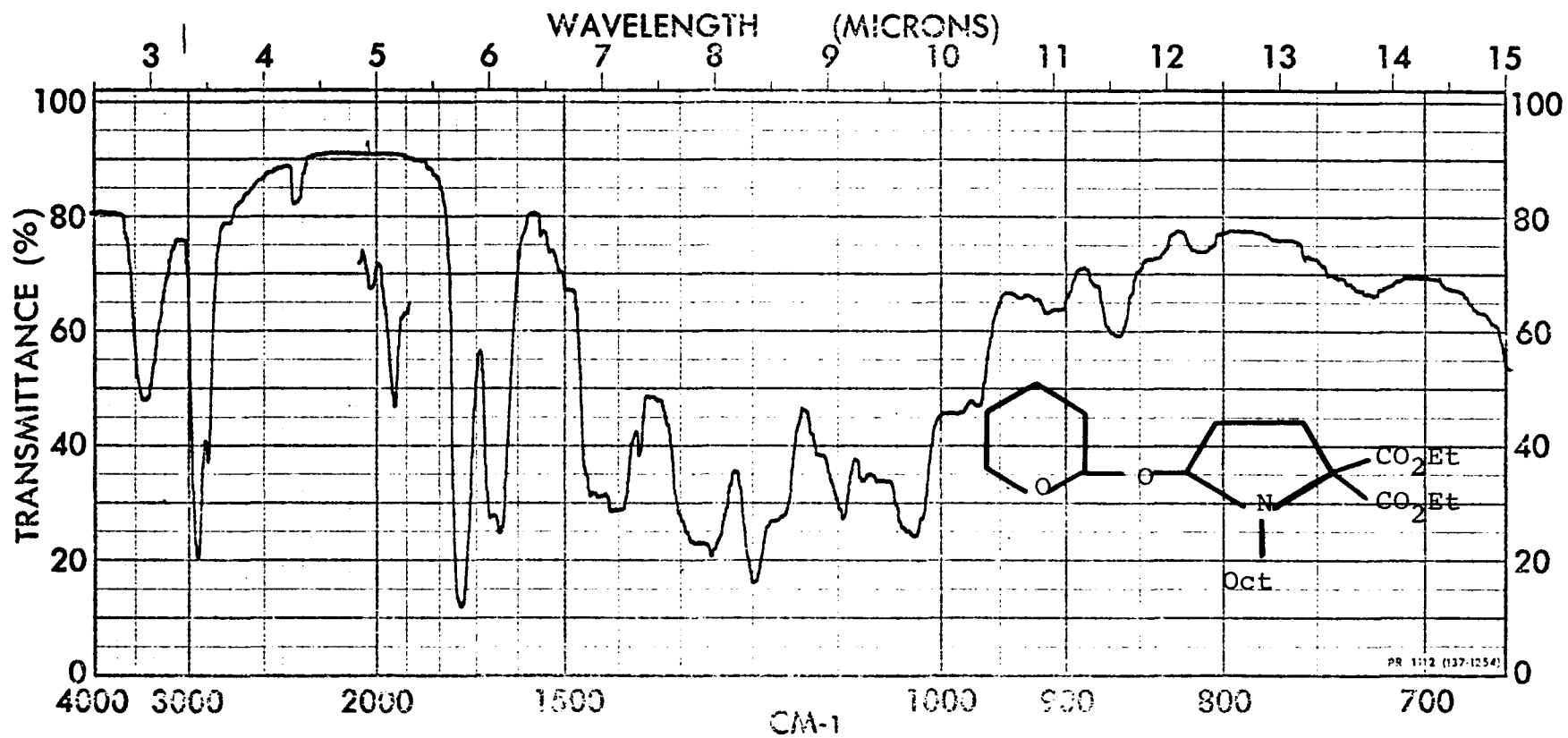
A-Figure 29. (neat). Compound 84.
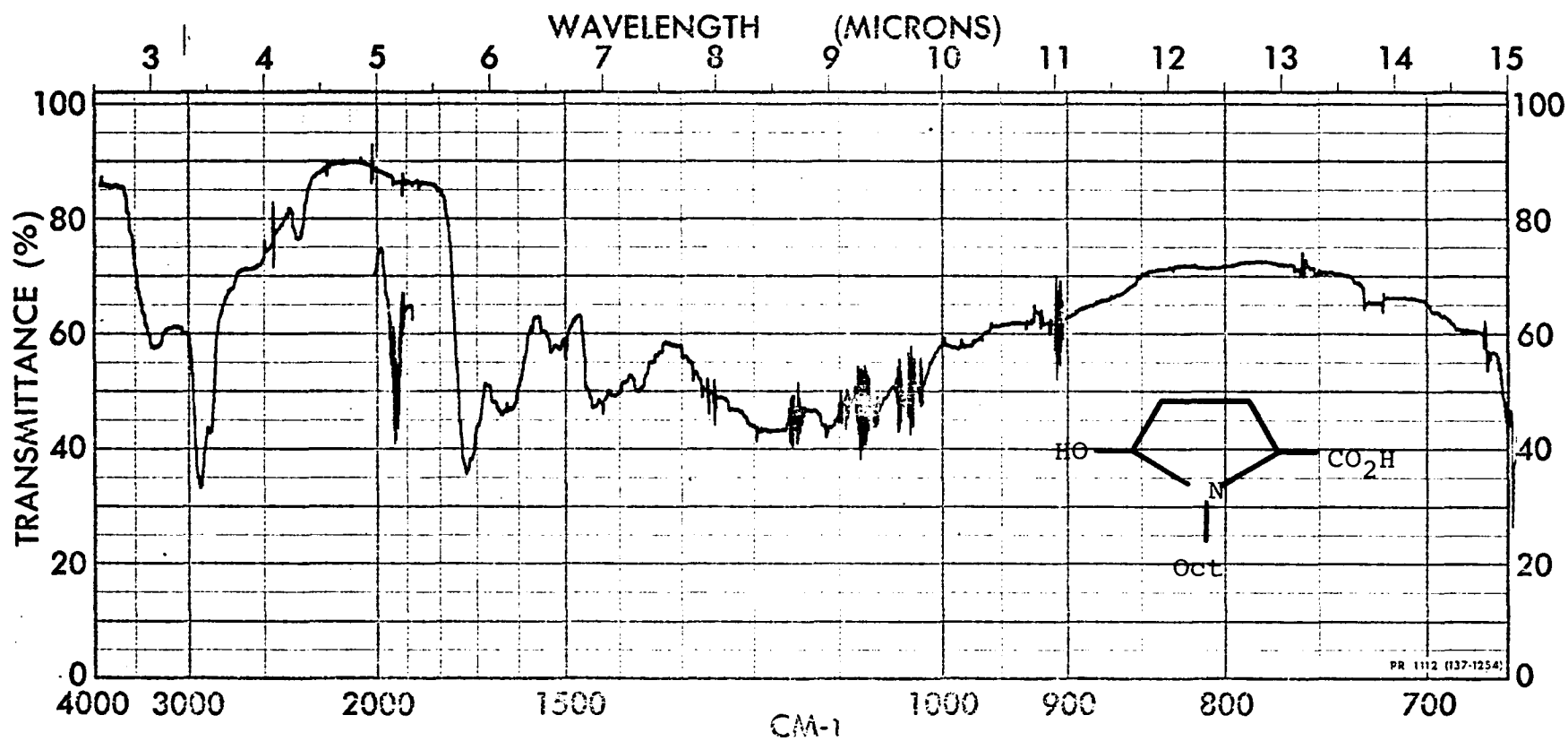
A-Figure 30.   (neat).   Compound 85.
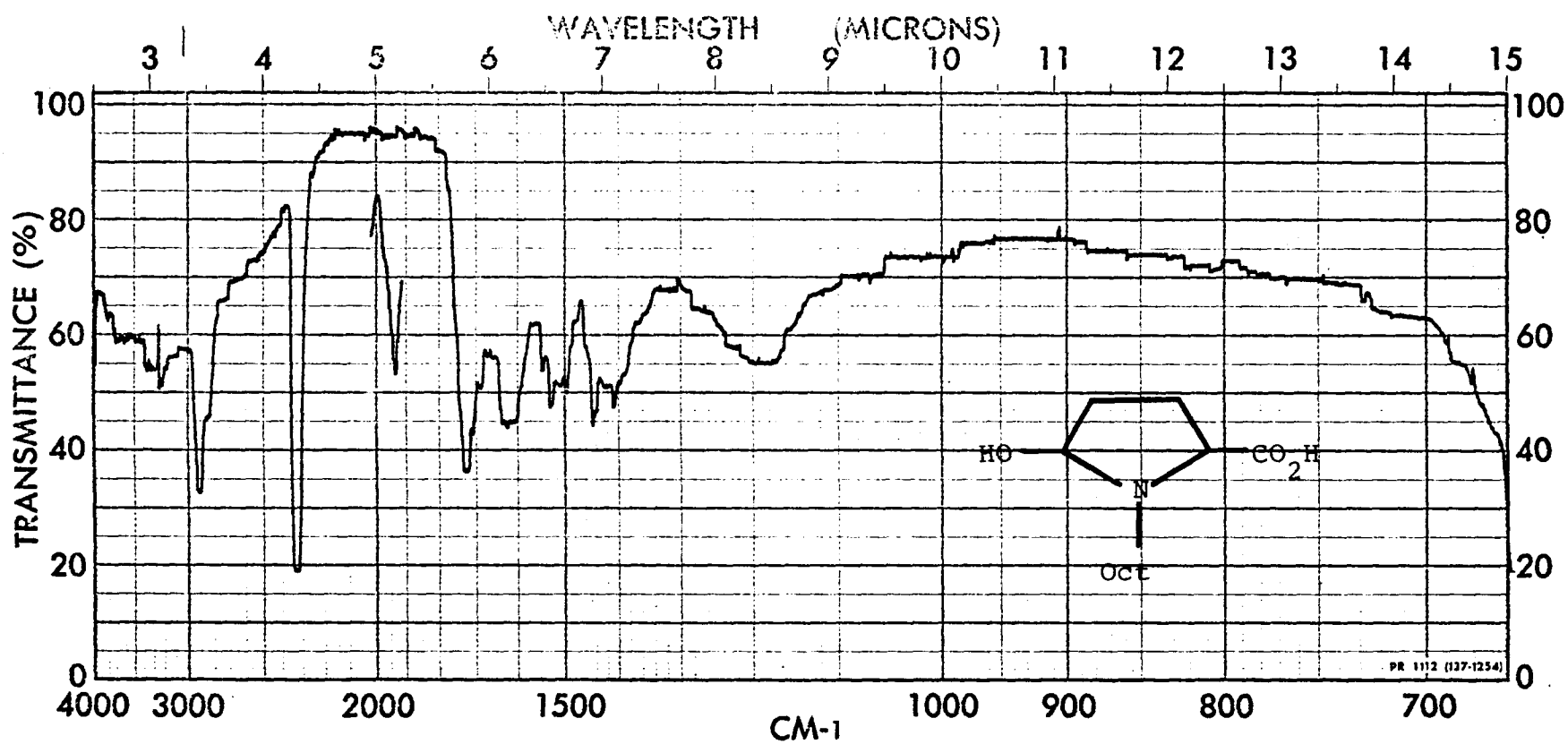
A-Figure 31.   (KBr).   Compound 85.
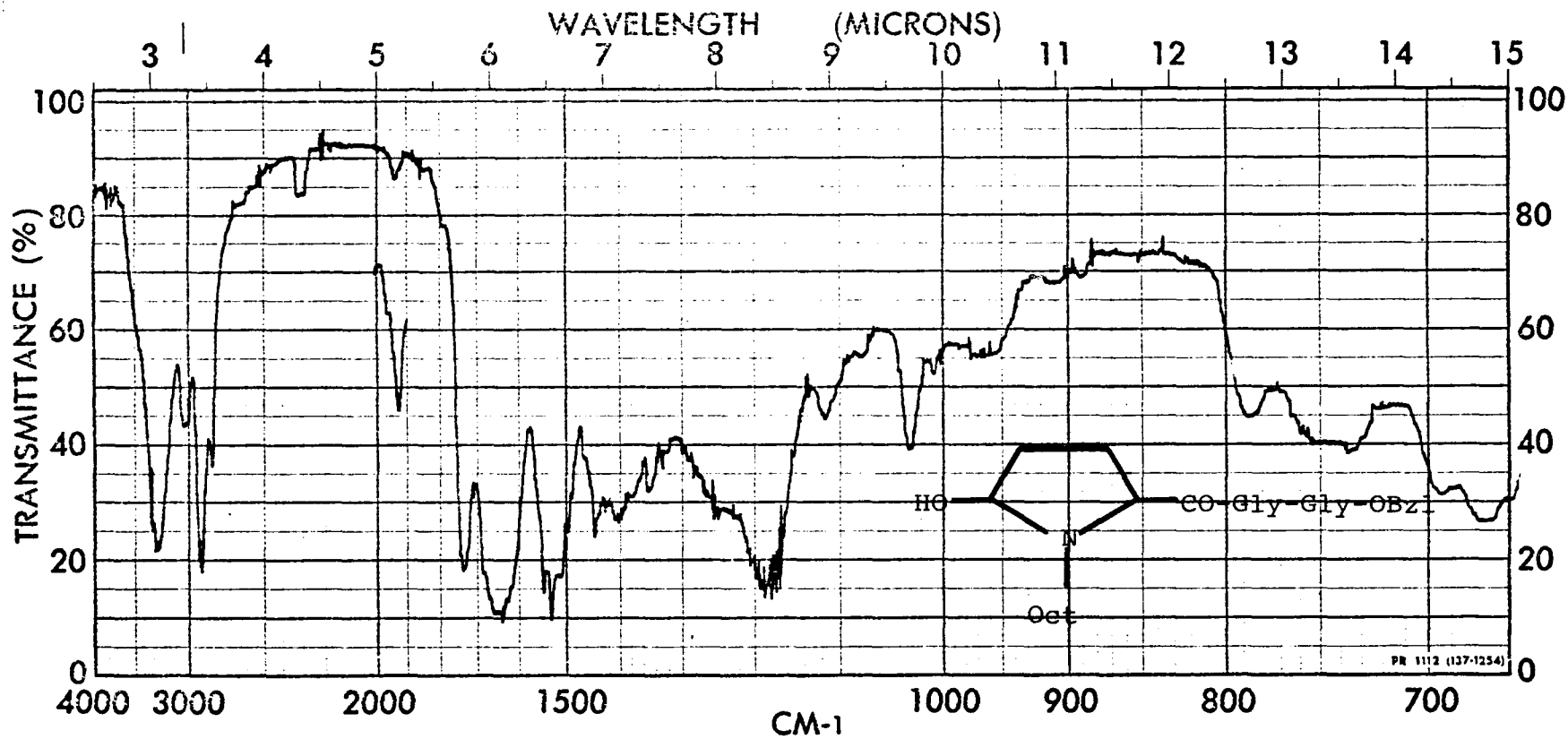
A-Figure 32. (neat). Compound 86.

PART I

APPENDIX B

NUCLEAR MAGNETIC RESONANCE SPECTRA

Hex-Gly-L-Pro-5-Amv-OH
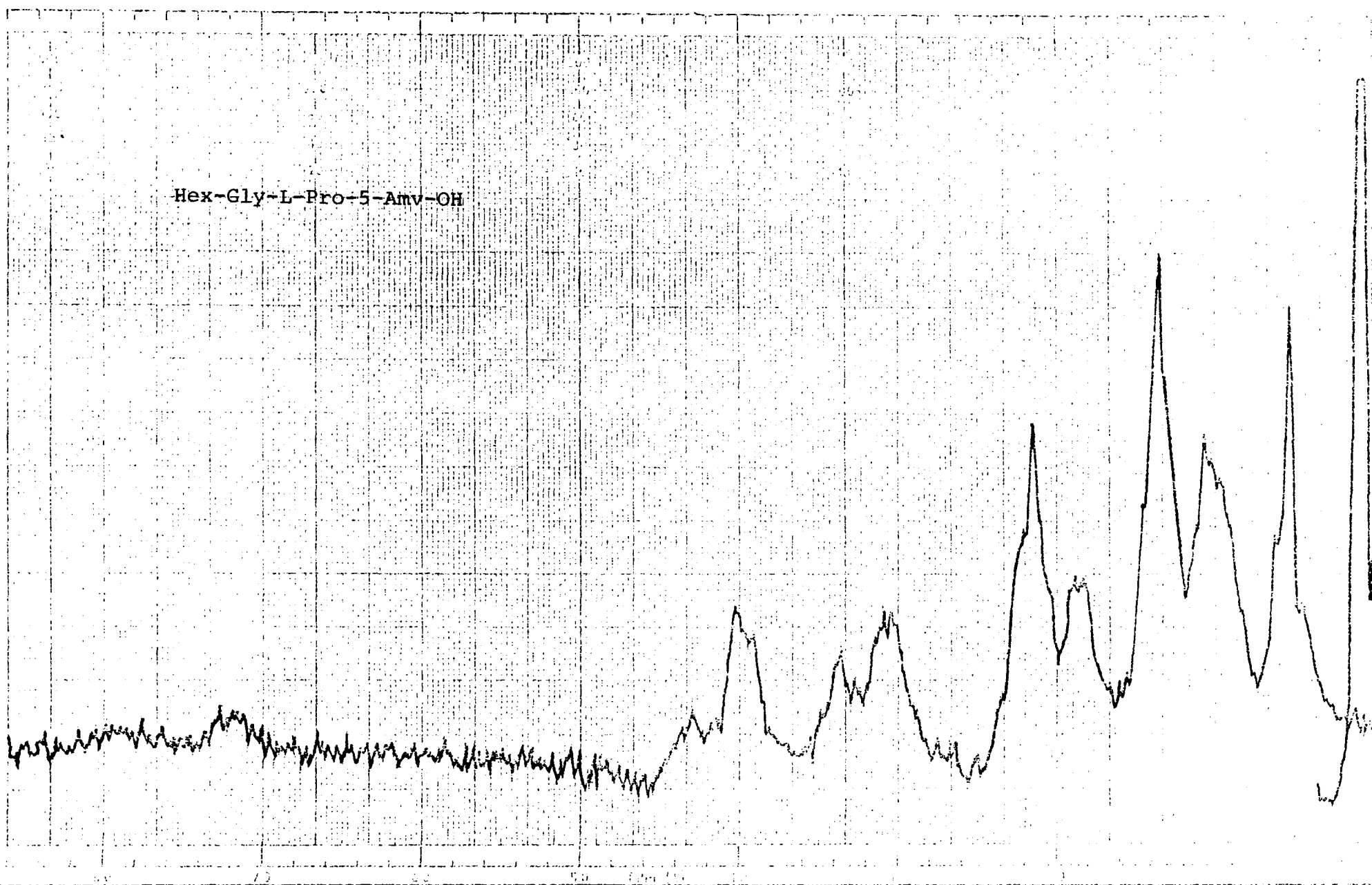
B-Figure 1. (TFA). Compound 19.

Hex-Gly-5-Amv-OH

B-Figure 2.   (TFA).   Compound 20.

Cbo-L-Pro-5-Amv-OMe

B-Figure 3. (CDCl$_3$). Compound 24.

H-L-Pro-5-Amv-OMe
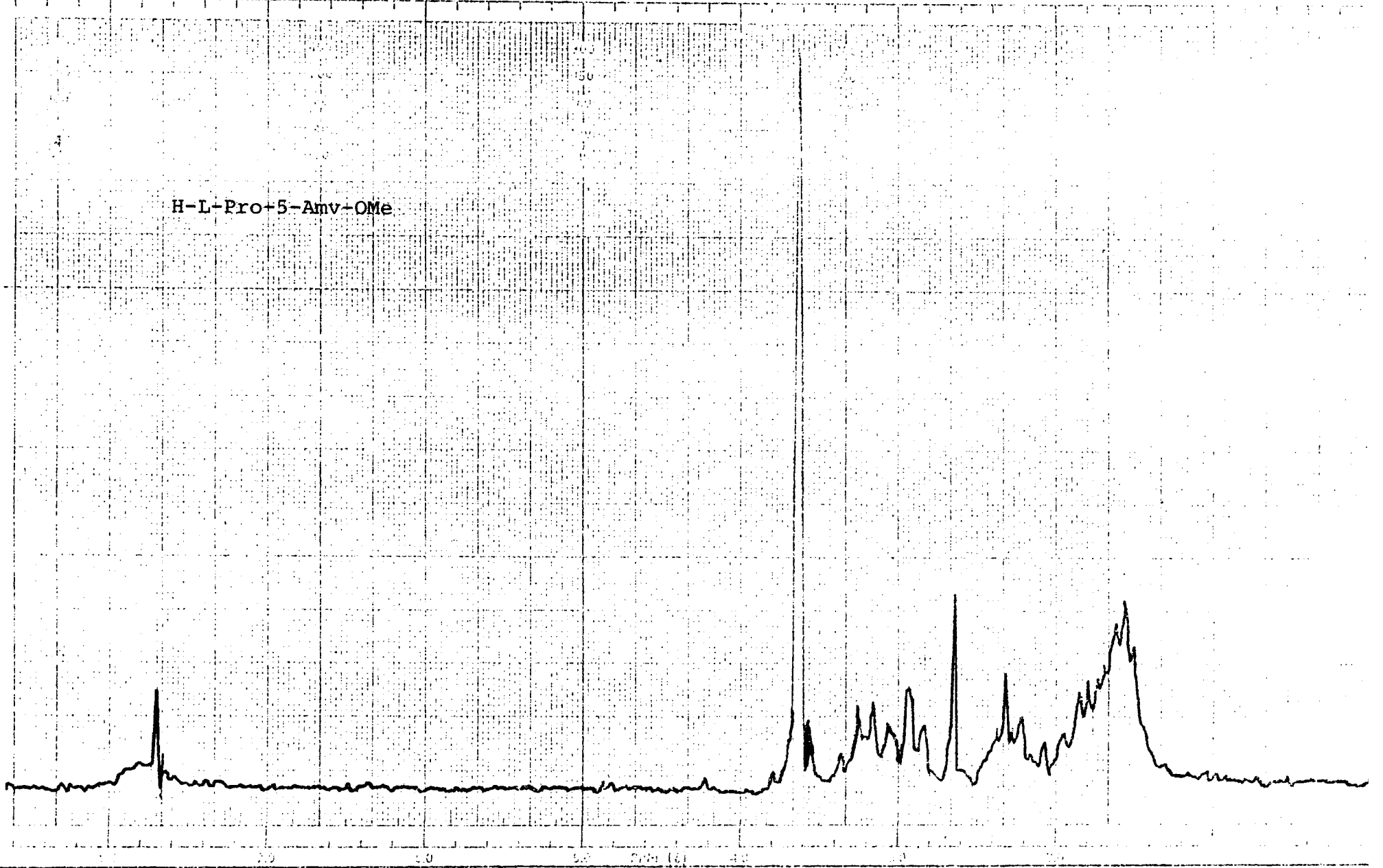
B-Figure 4. (CDCl$_3$). Compound 25.

H-L-Pro-5-Amv-OMe·HCl
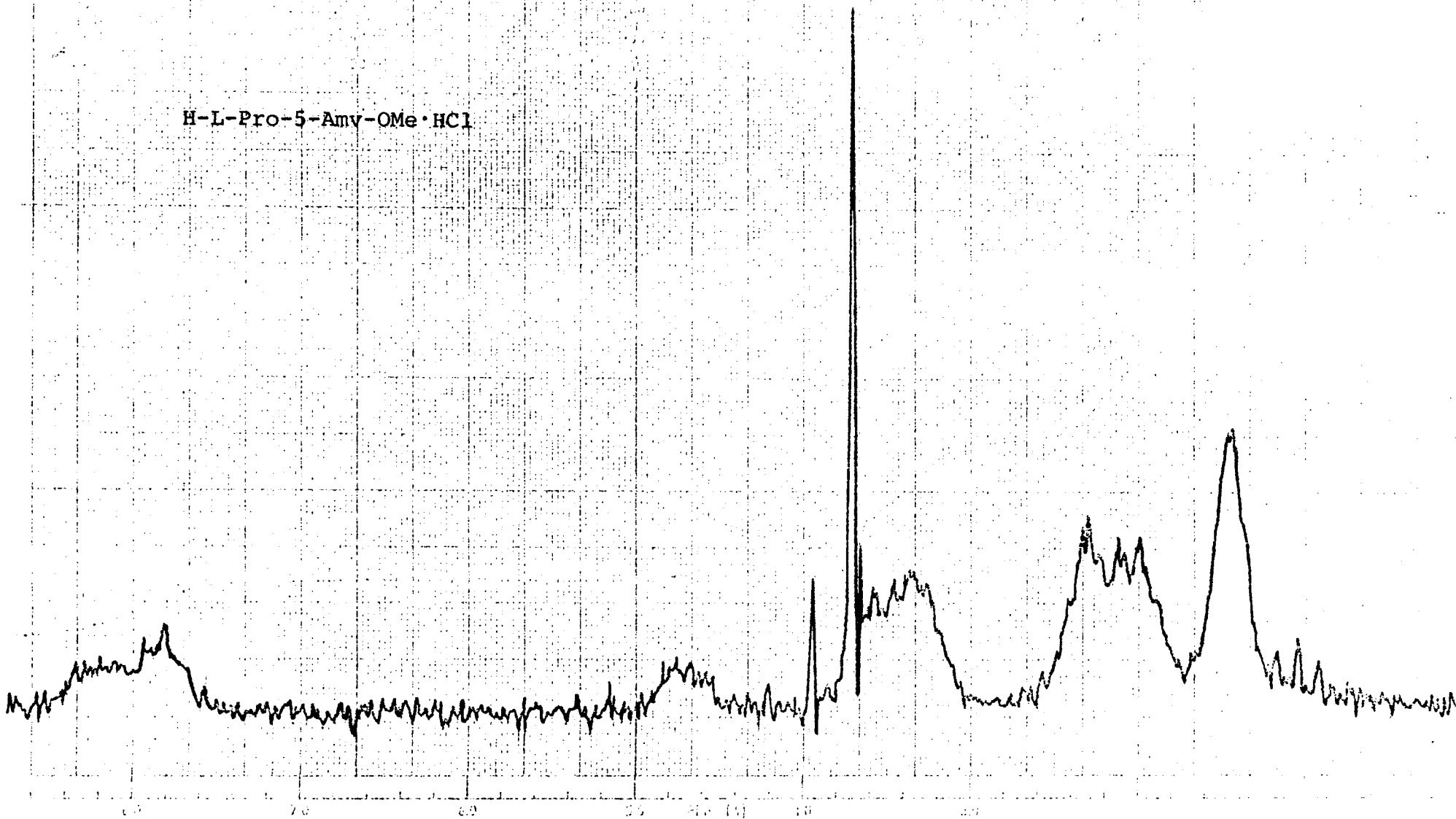
B-Figure 5. (TFA). Compound 26.

Hex-Gly-L-Pro-5-Amv-OMe

JAPAN ELECTRON OPTICS LAB.   TOKYO JAPAN

B-Figure 6.   (CDCl$_3$).   Compound 27.

Pen-Gly-L-Pro-5-Amv-OMe
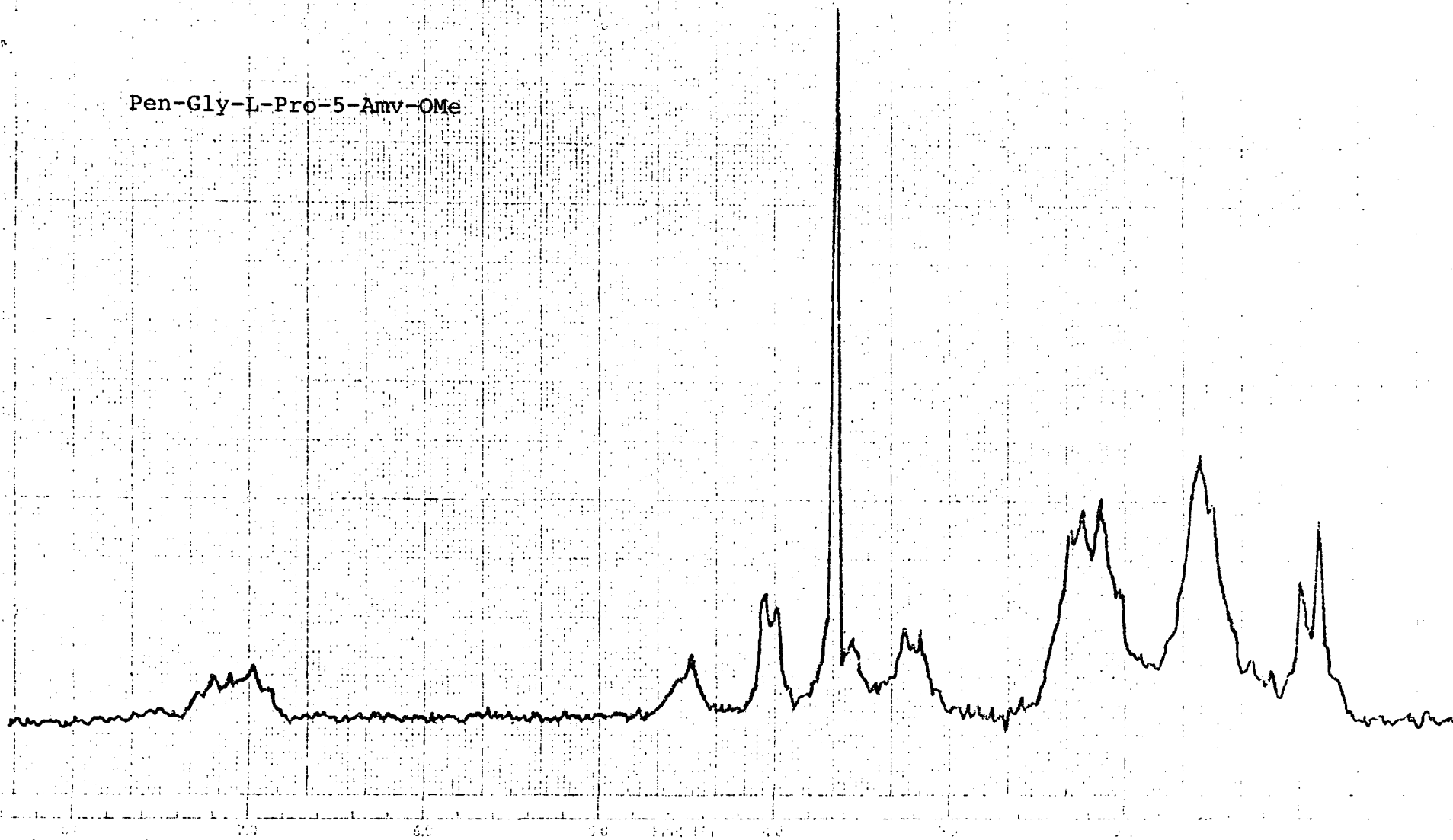
B-Figure 7. (CDCl$_3$). Compound $\underline{30}$.

Hex-Gly-L-Pro-OMe
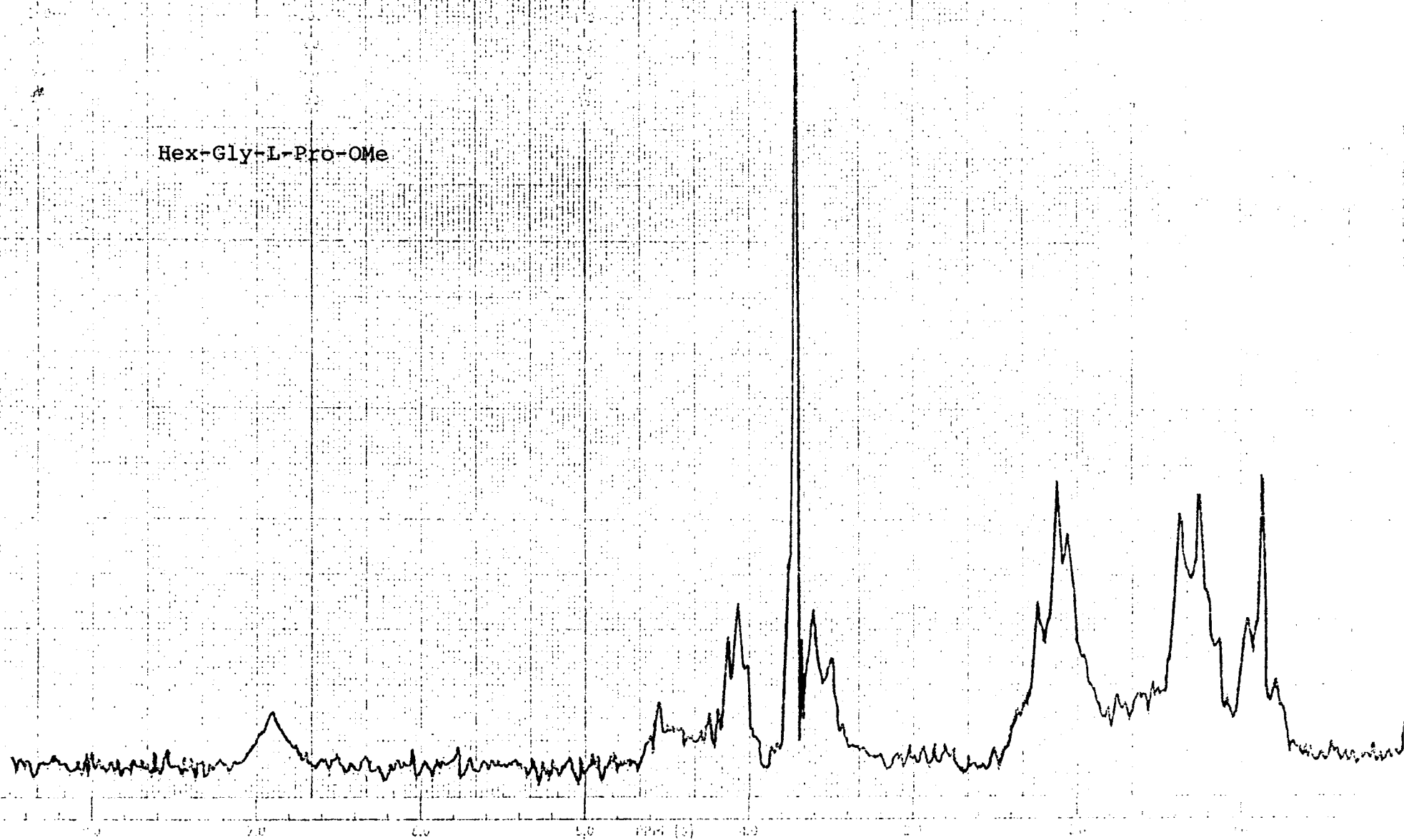
B-Figure 8. (CDCl$_3$). Compound 32.

Hex-Gly-L-Pro-OH
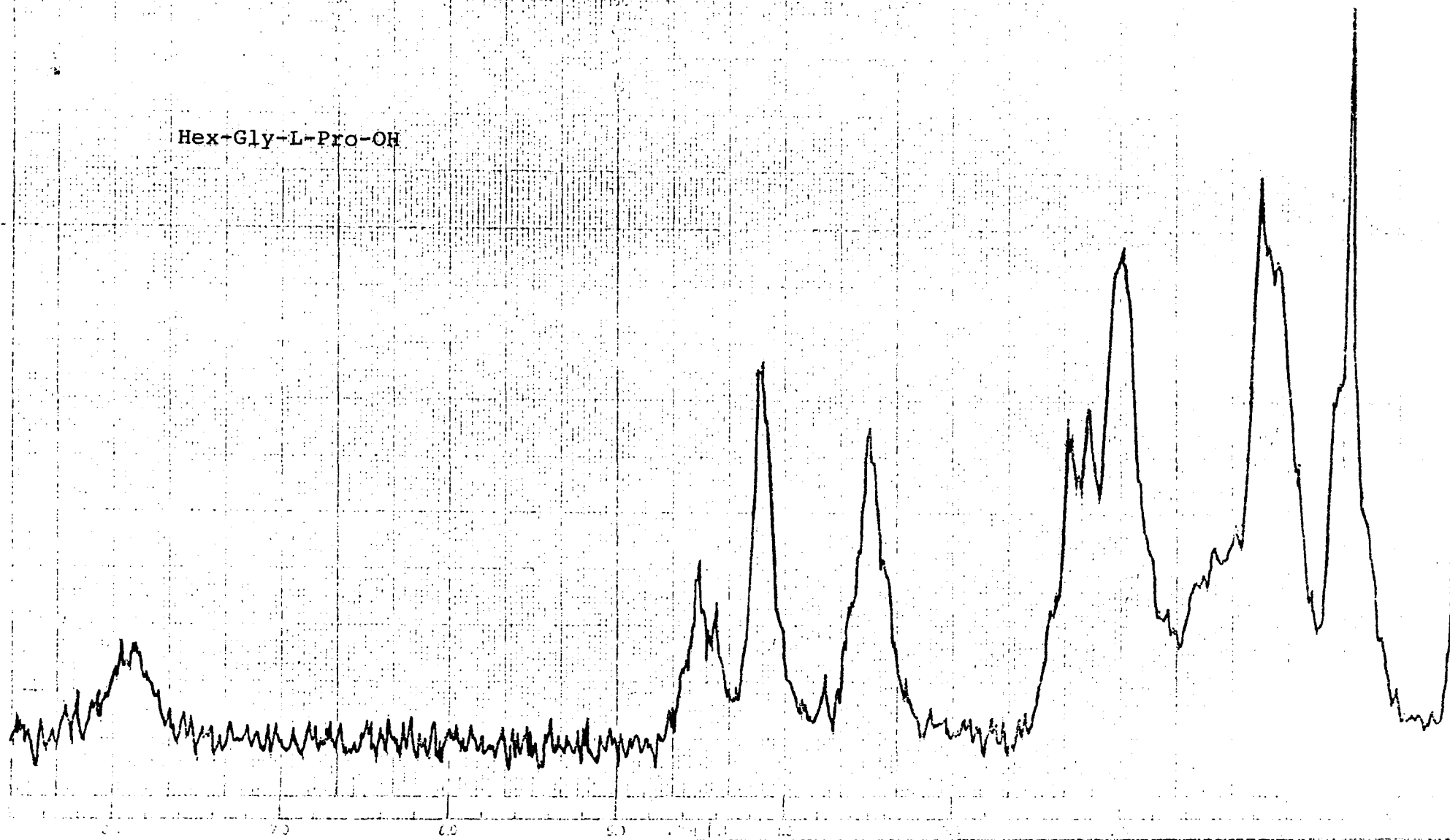
B-Figure 9. (TFA). Compound 33.

Hex-Gly-L-Pro-Gly-Gly-OBzl
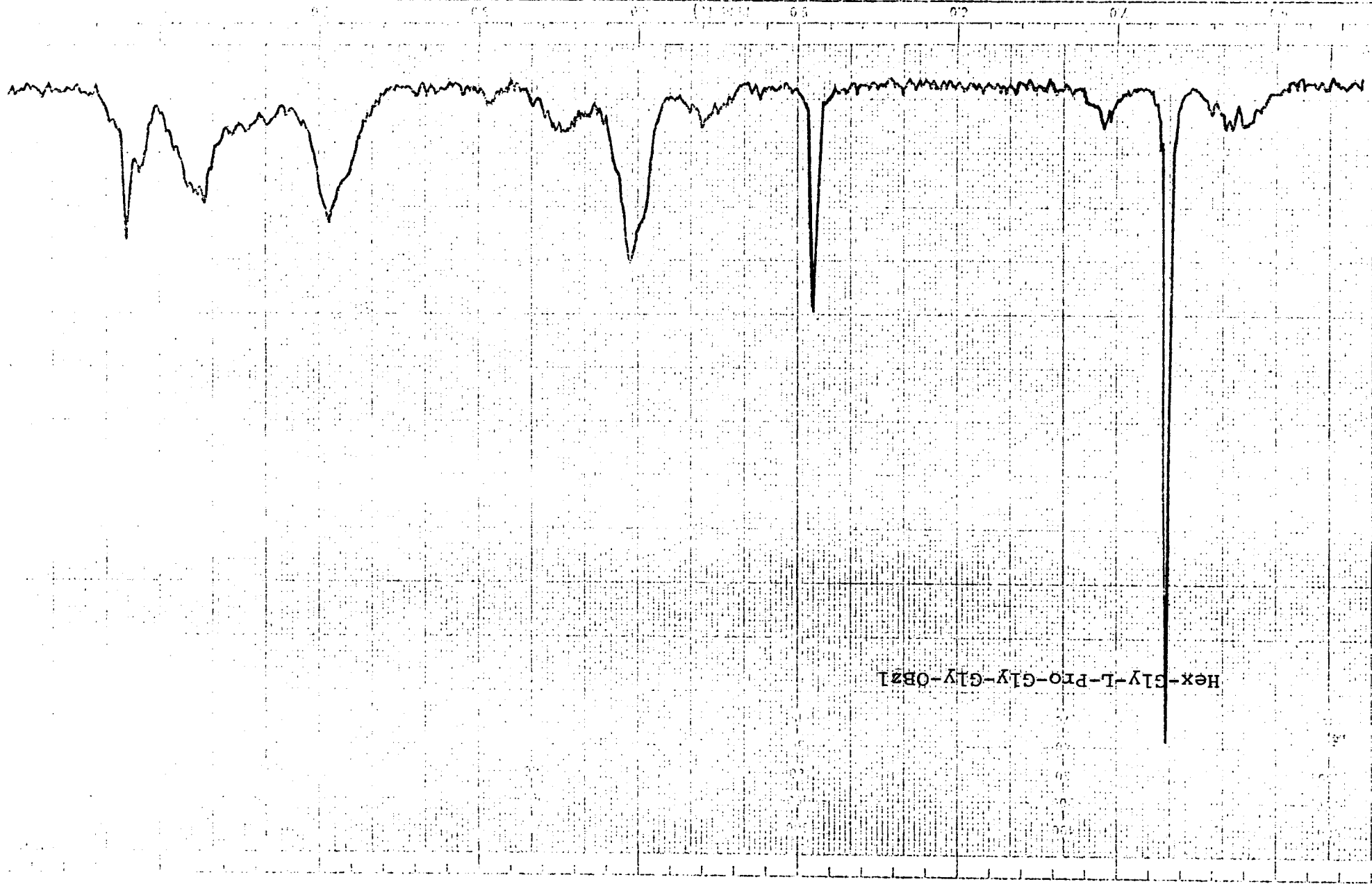
Hex-Gly-L-Pro-Gly-Gly-OH
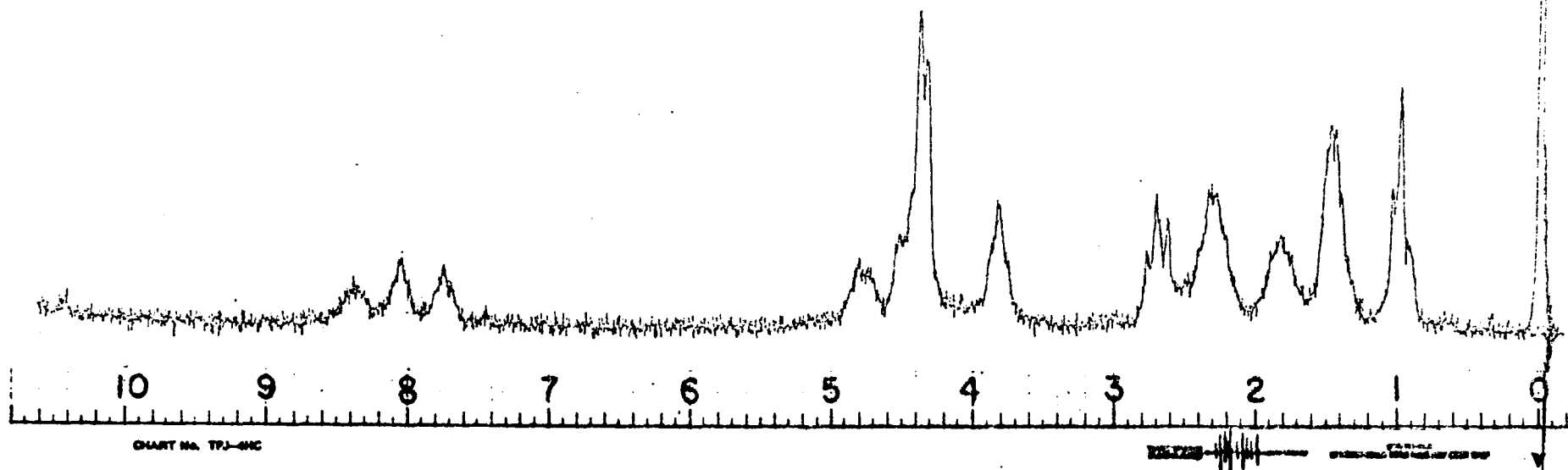
B-Figure 11. (CDCl$_3$). Compound 36.

Hex-Gly-L-Pro-Gly-Gly-NH$_2$
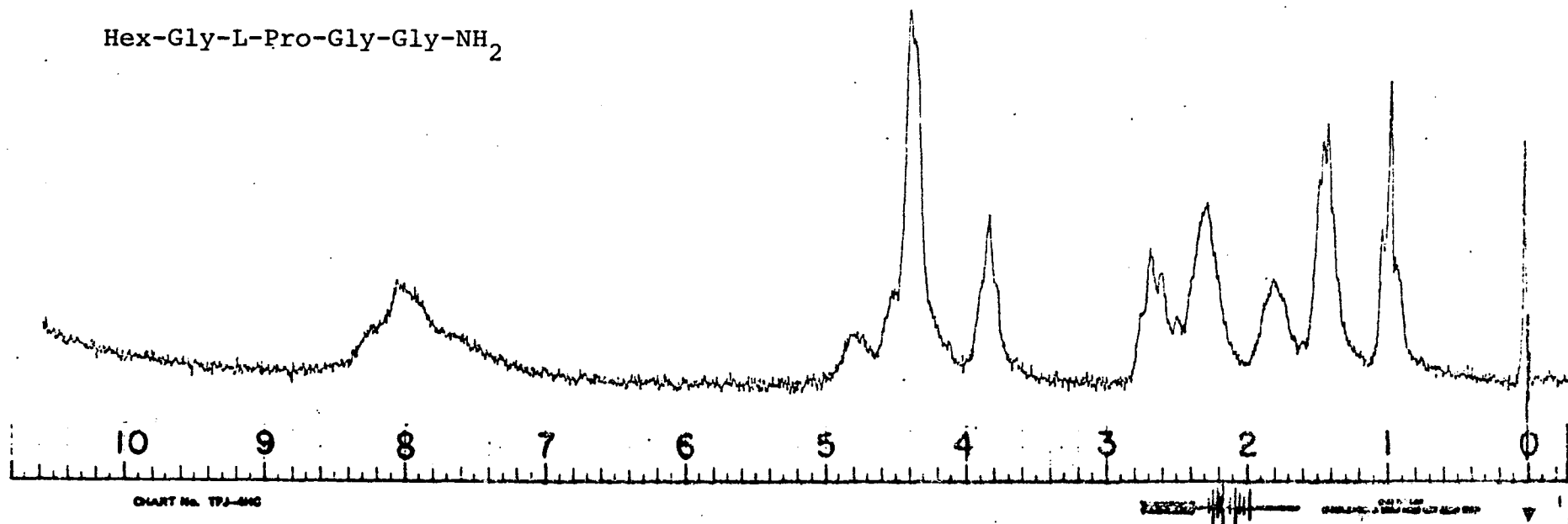
B-Figure 12.   (TFA).   Compound 37.

Cbo-L-Leu-L-Leu-O-t-Bu
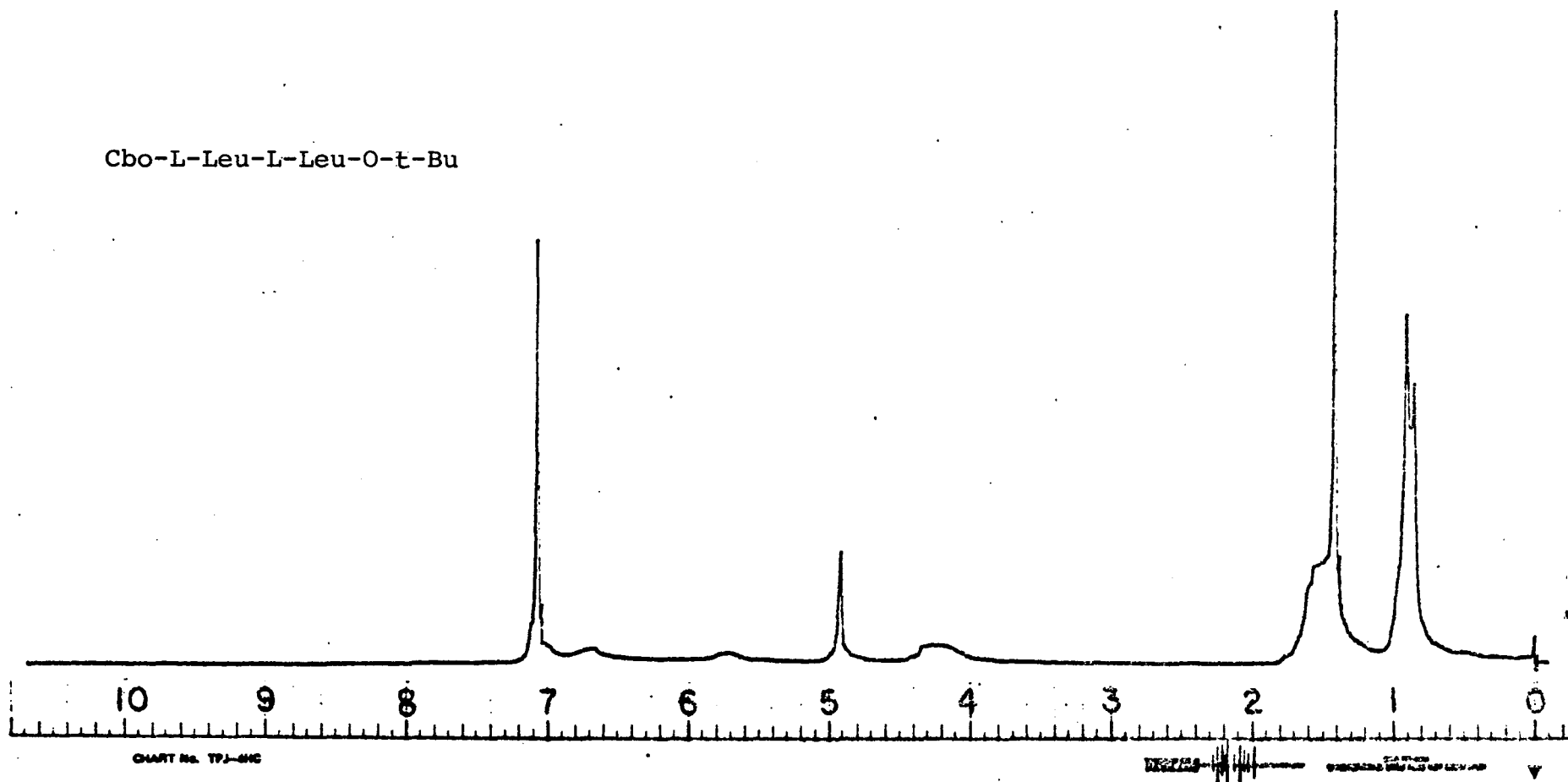
B-Figure 13.   (CDCl$_3$).   Compound 45.

H-L-Leu-L-Leu-O-t-Bu
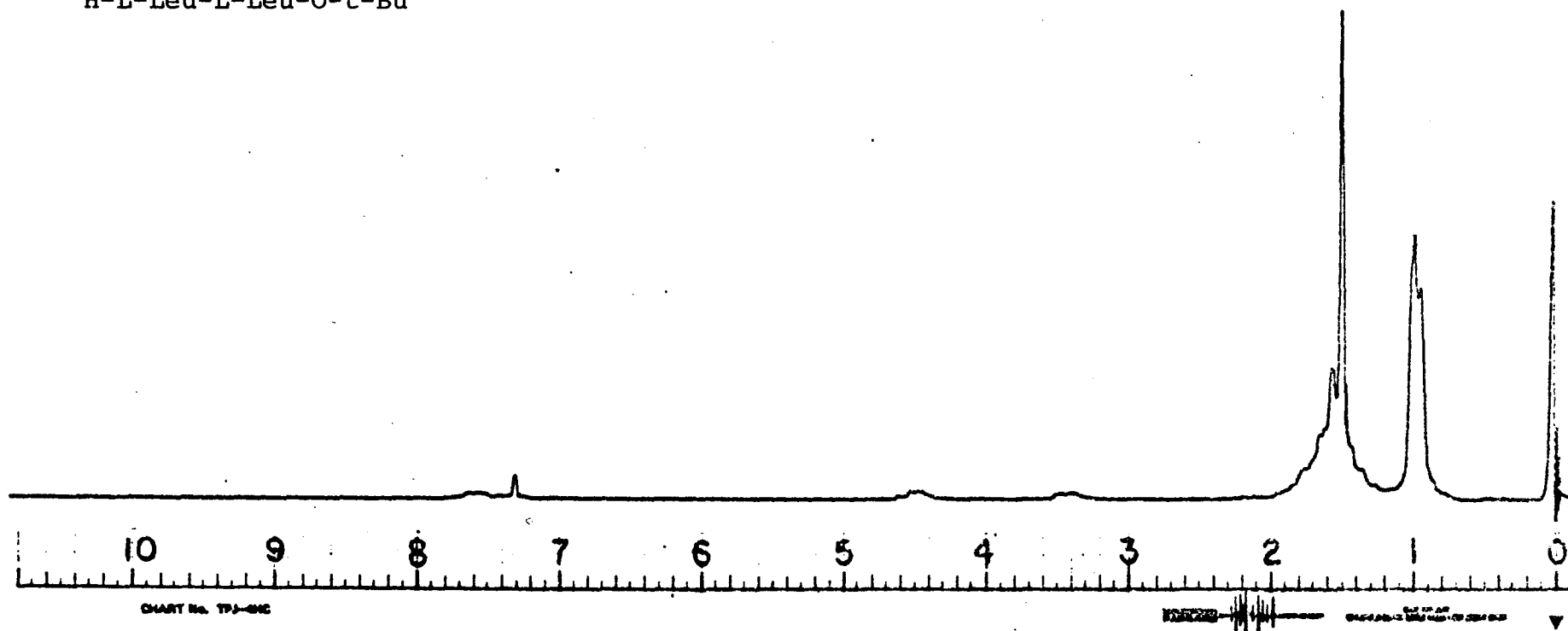
B-Figure 14.   (CDCl$_3$).   Compound $\underline{46}$.
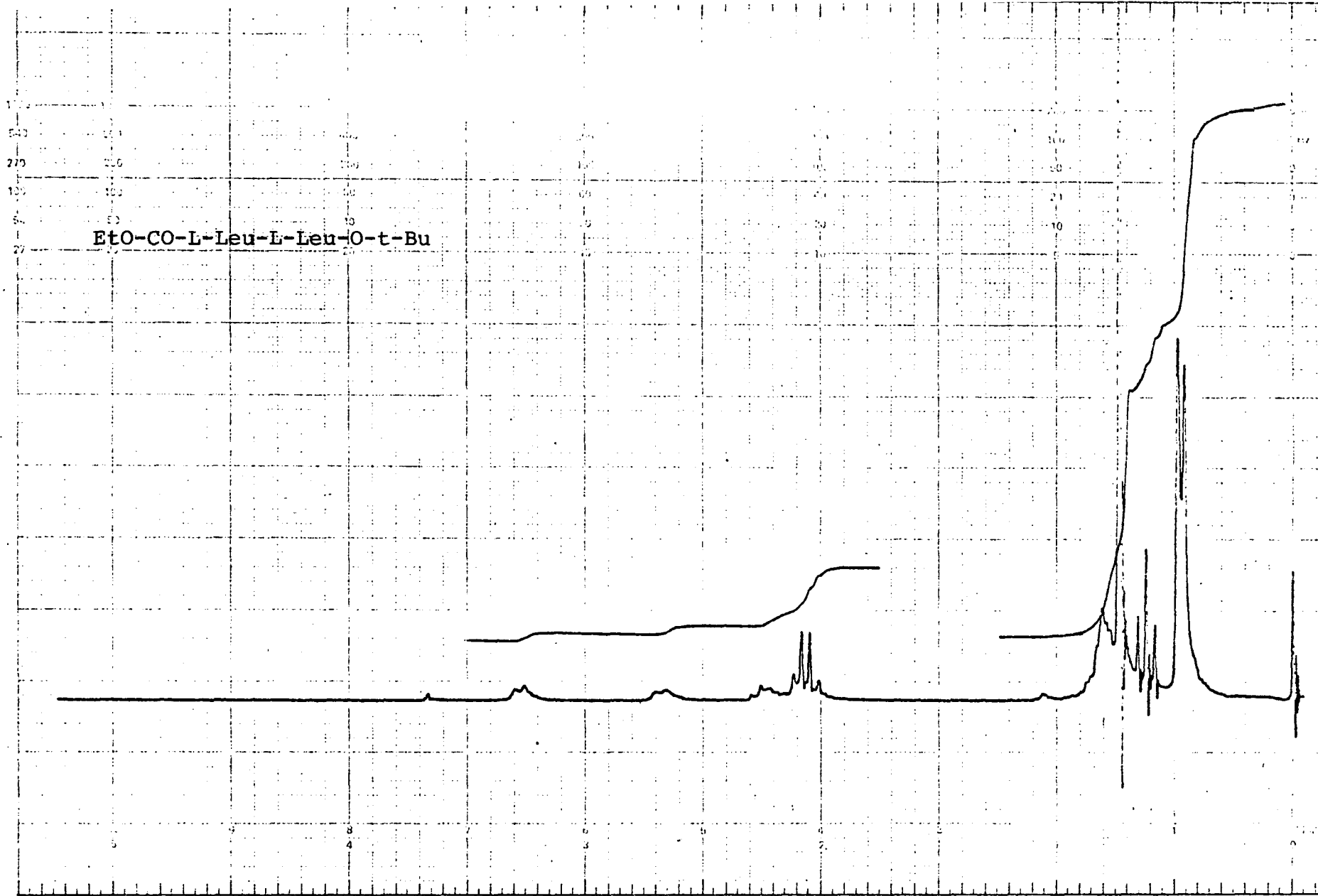
EtO-CO-L-Leu-L-Leu-O-t-Bu

B-Figure 15. (CDCl$_3$). Compound 48.

Cbo-L-Pro-L-Leu-L-Leu-O-t-Bu
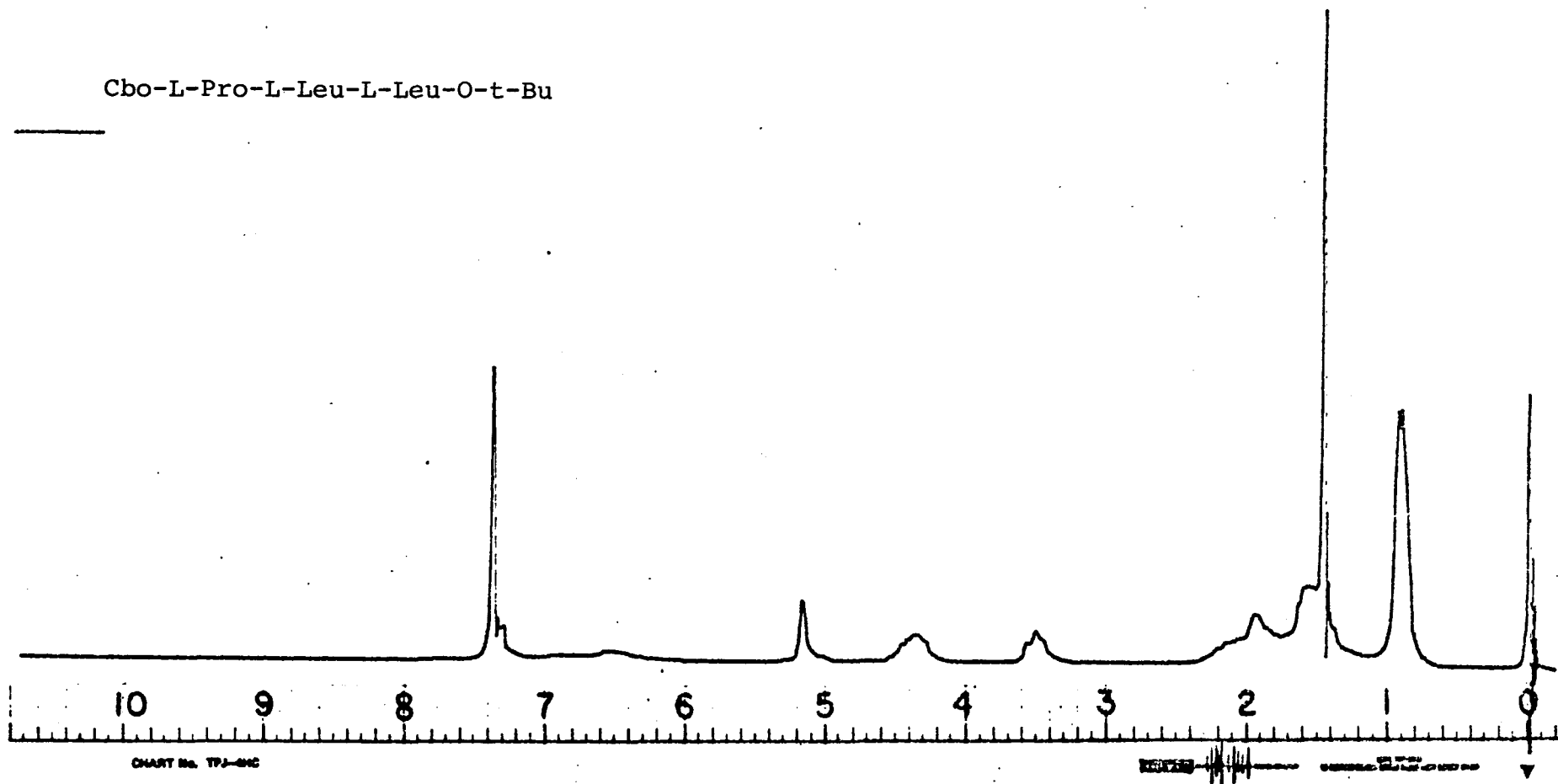
B-Figure 16.   (CDCl$_3$).   Compound 47.

H-L-Pro-L-Leu-L-Leu-O-t-Bu

B-Figure 17.   (CDCl$_3$).   Compound 51.
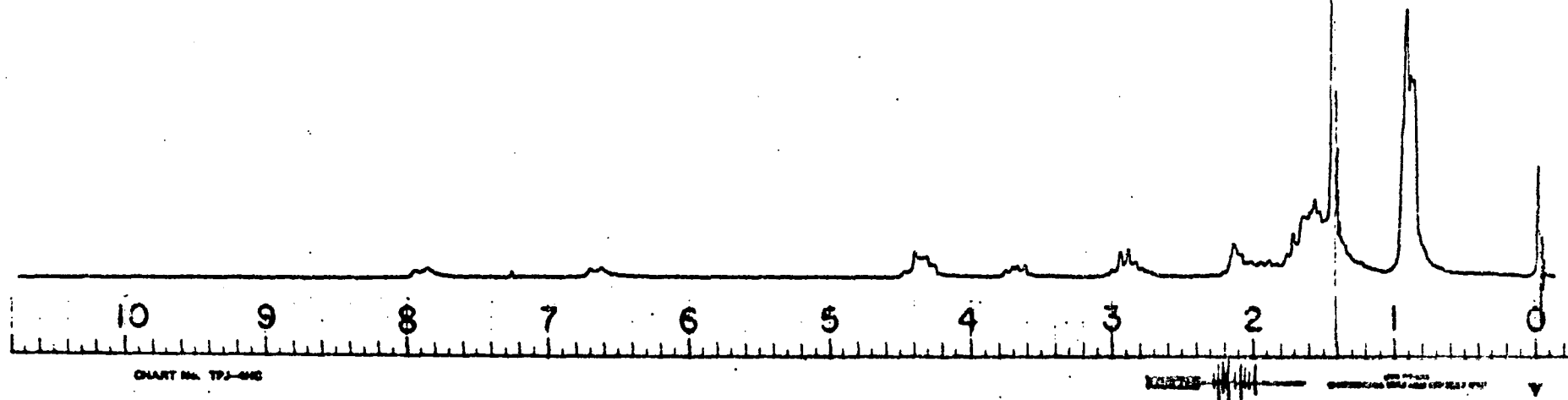
Hex-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu
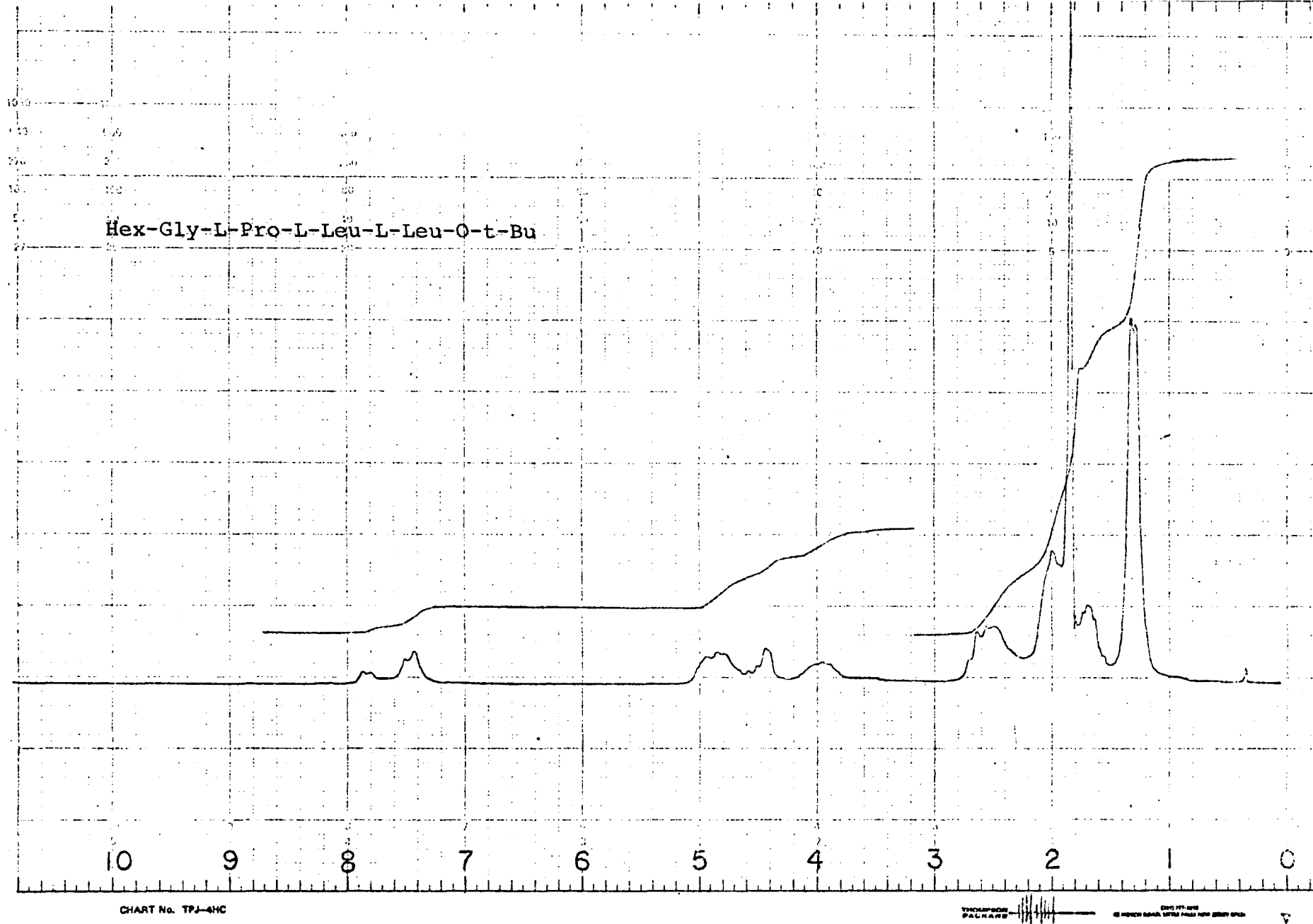
CHART No. TPJ-4HC

B-Figure 18. (CDCl$_3$). Compound 52.

EtO-CO-L-Pro-L-Leu-L-Leu-O-t-Bu

B-Figure 19. (CDCl₃). Compound 53.

Hex-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu
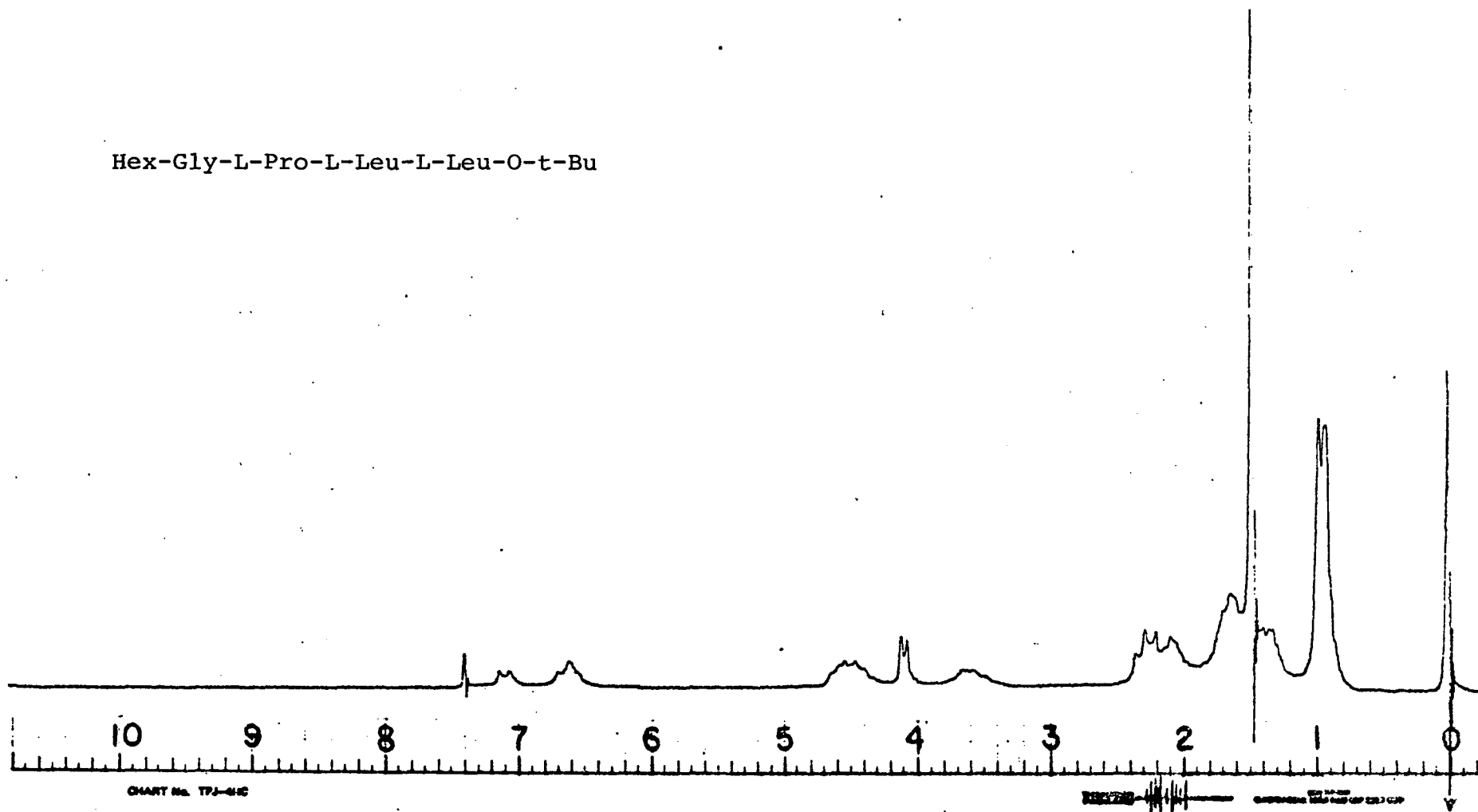
B-Figure 20.   (CDCl$_3$).   Compound 52.

Hex-Gly-L-Pro-L-Leu-L-Leu-OH
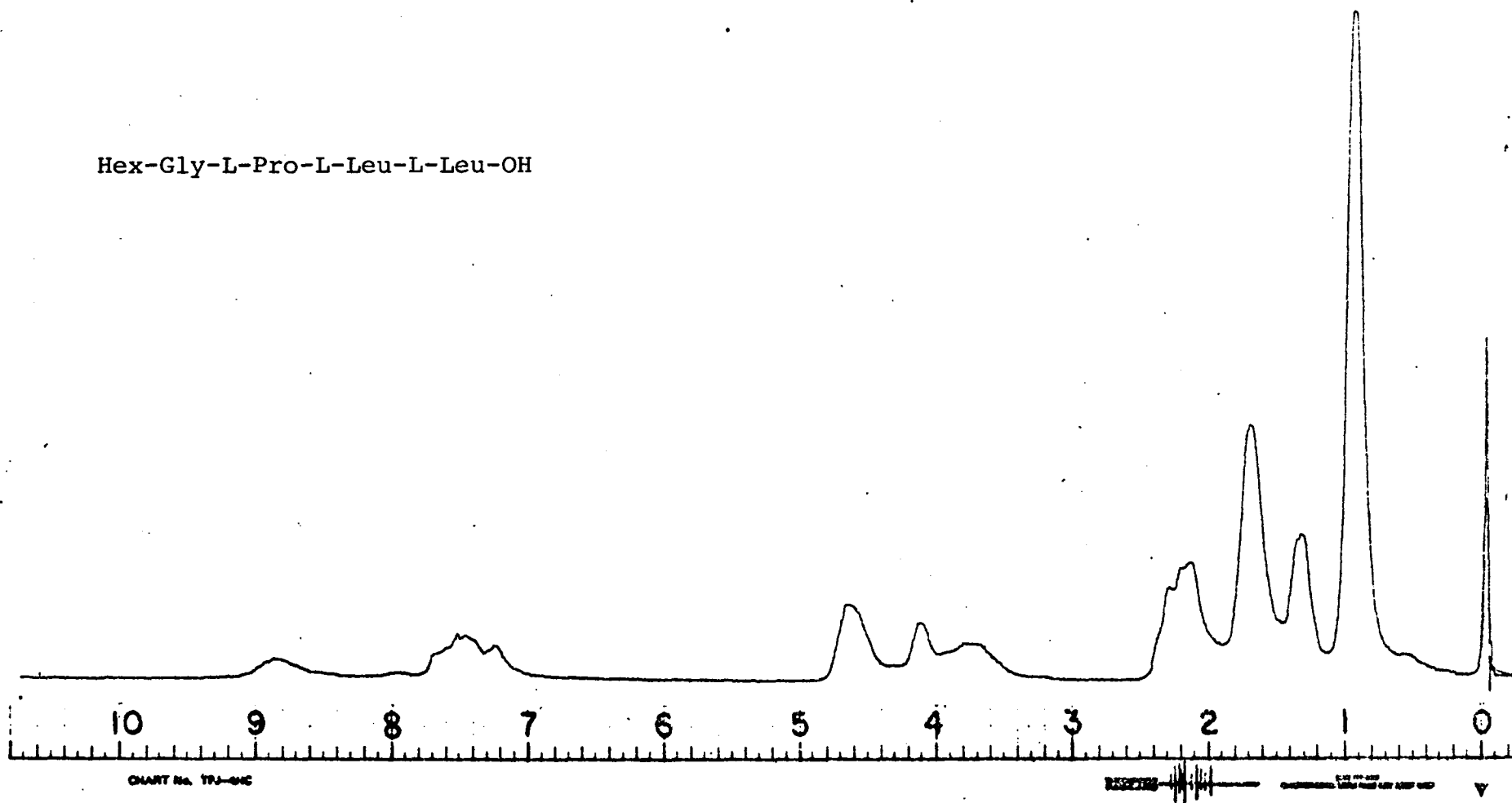
B-Figure 21.   (CDCl$_3$).   Compound 56.

Pen-Gly-OPhNO$_2$
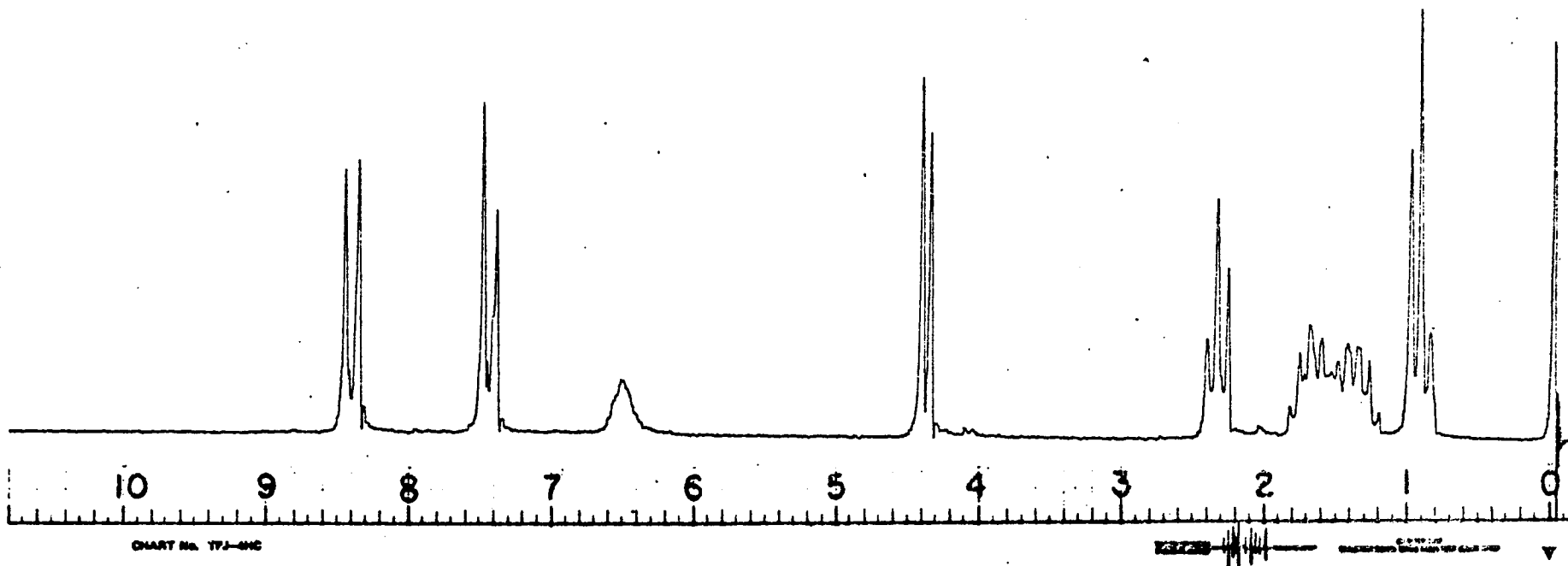
B-Figure 22. (CDCl$_3$). Compound 57.

Pen-Gly-L-Pro-L-Leu-L-Leu-O-t-Bu
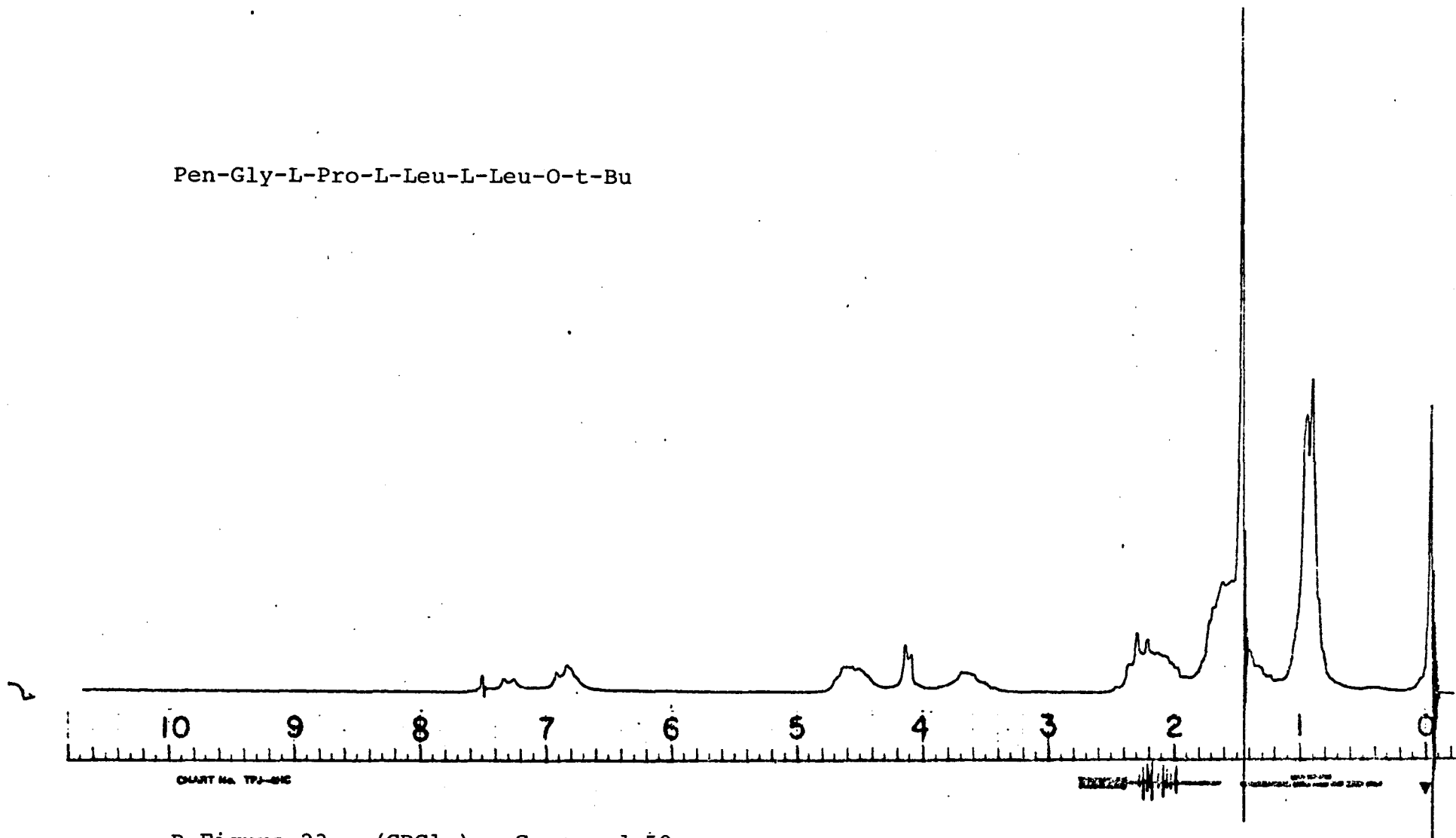
B-Figure 23. (CDCl$_3$). Compound 58.

Pen-Gly-L-Pro-L-Leu-L-Leu-OH
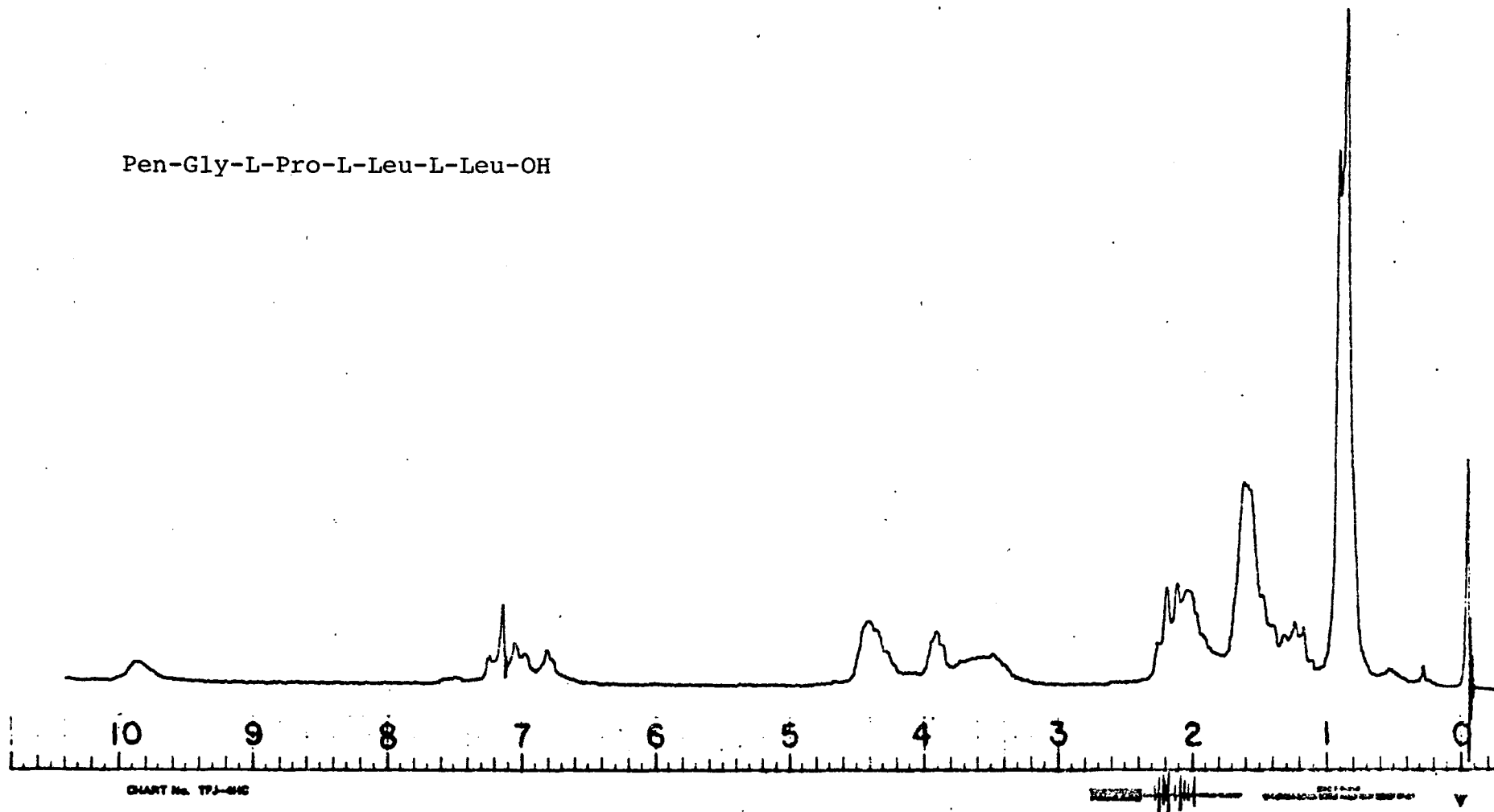
B-Figure 24.   (CDCl$_3$).   Compound $\underline{59}$.

H-L-Phe-L-Leu-O-t-Bu
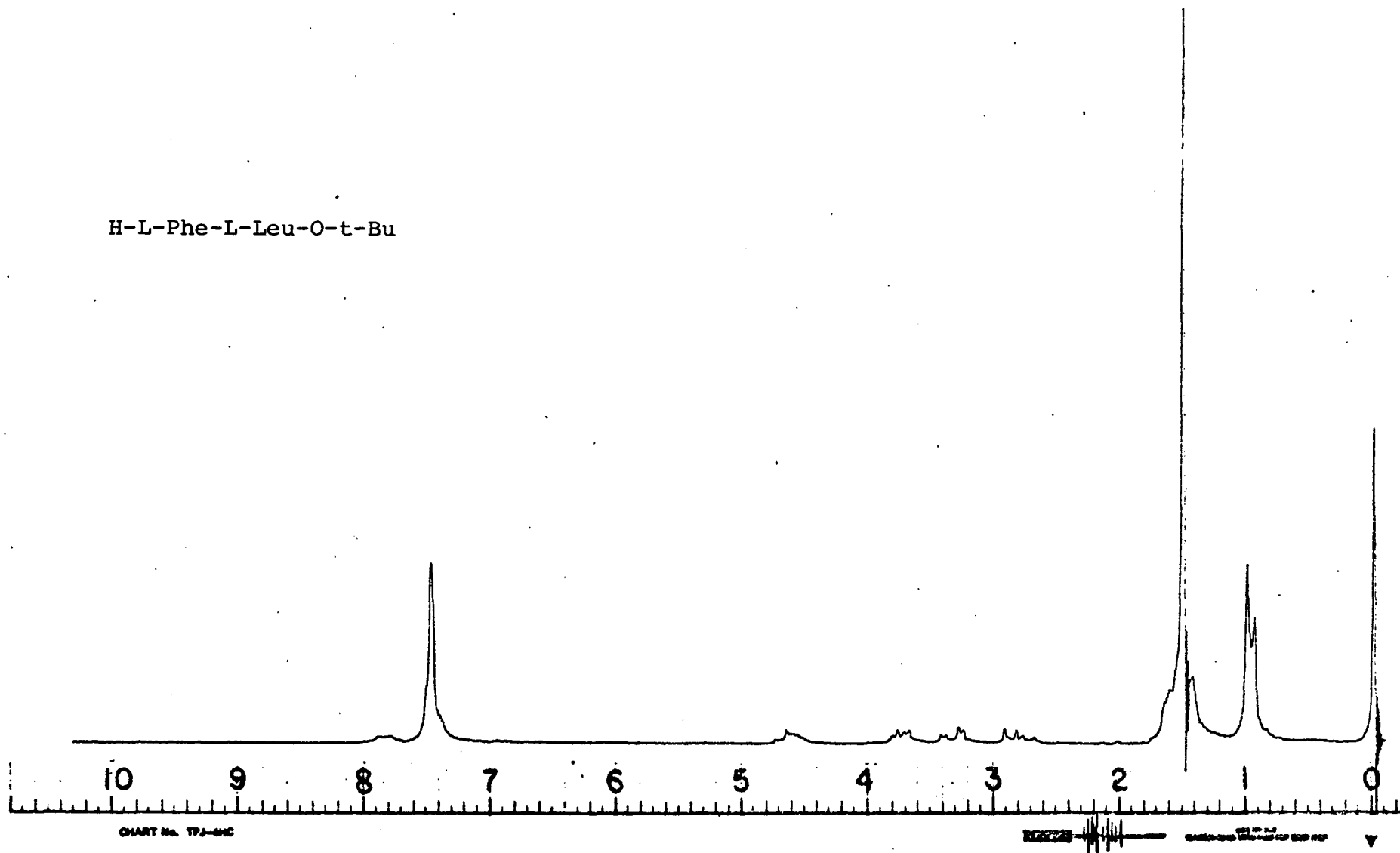
B-Figure 25.   (CDCl$_3$).   Compound $\underline{75}$.

Cbo-D-Pro-L-Phe-L-Leu-O-t-Bu
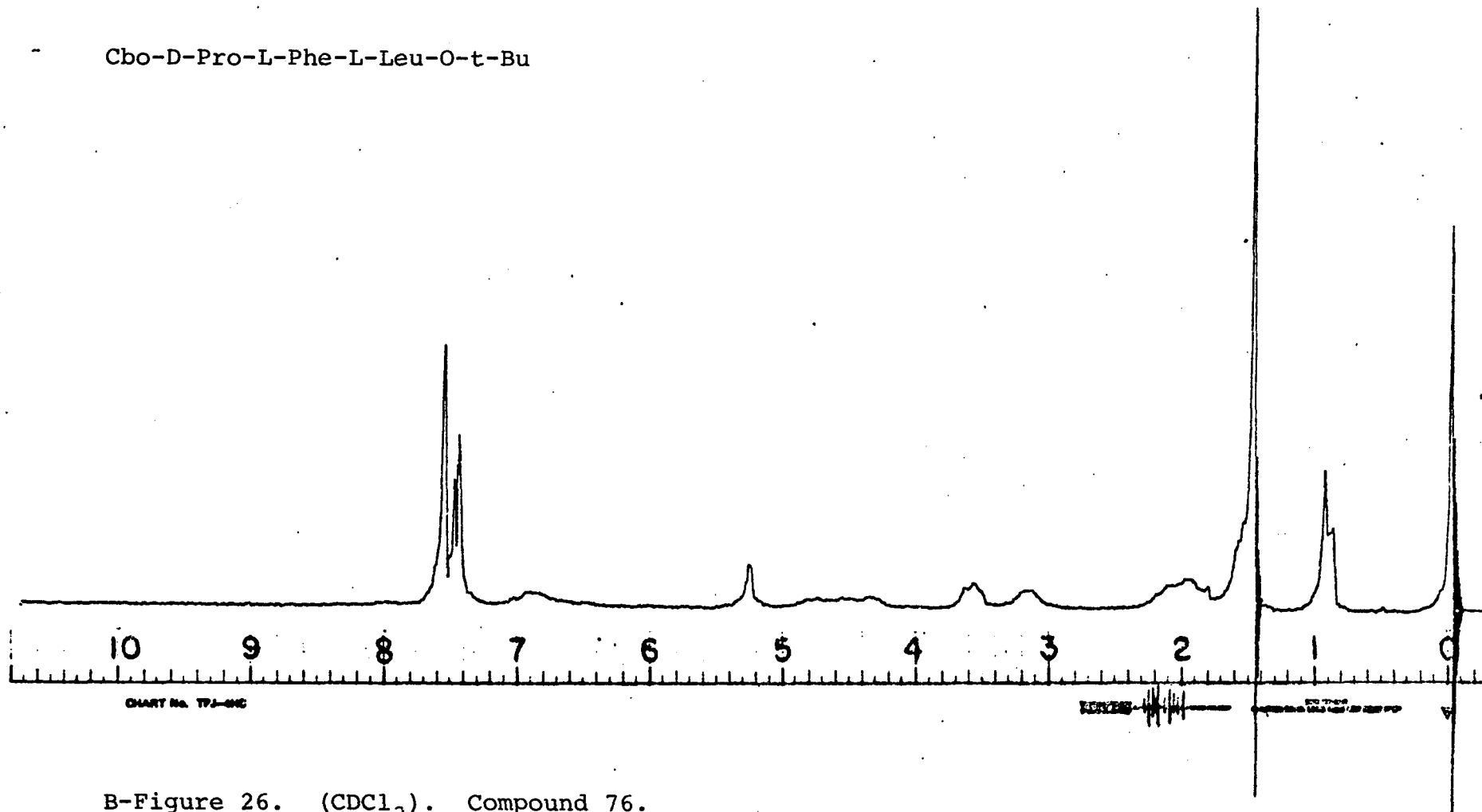
B-Figure 26. (CDCl$_3$). Compound 76.

H-D-Pro-L-Phe-L-Leu-O-t-Bu
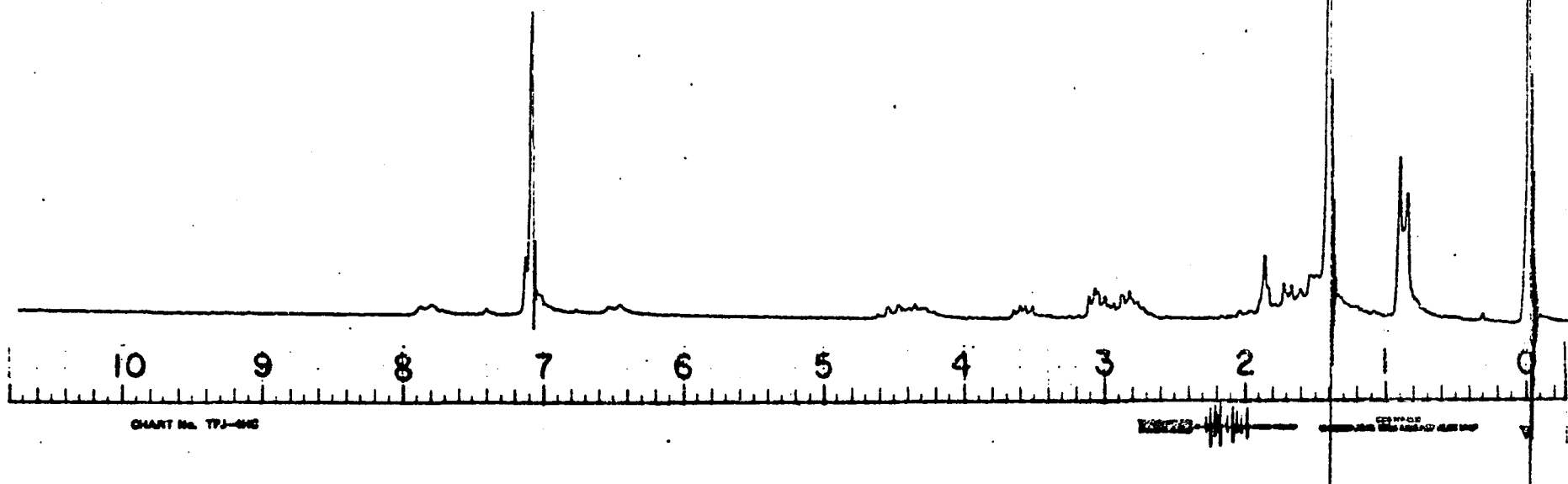
B-Figure 27. (CDCl$_3$). Compound 77.

Pen-Gly-D-Pro-L-Phe-L-Leu-OH
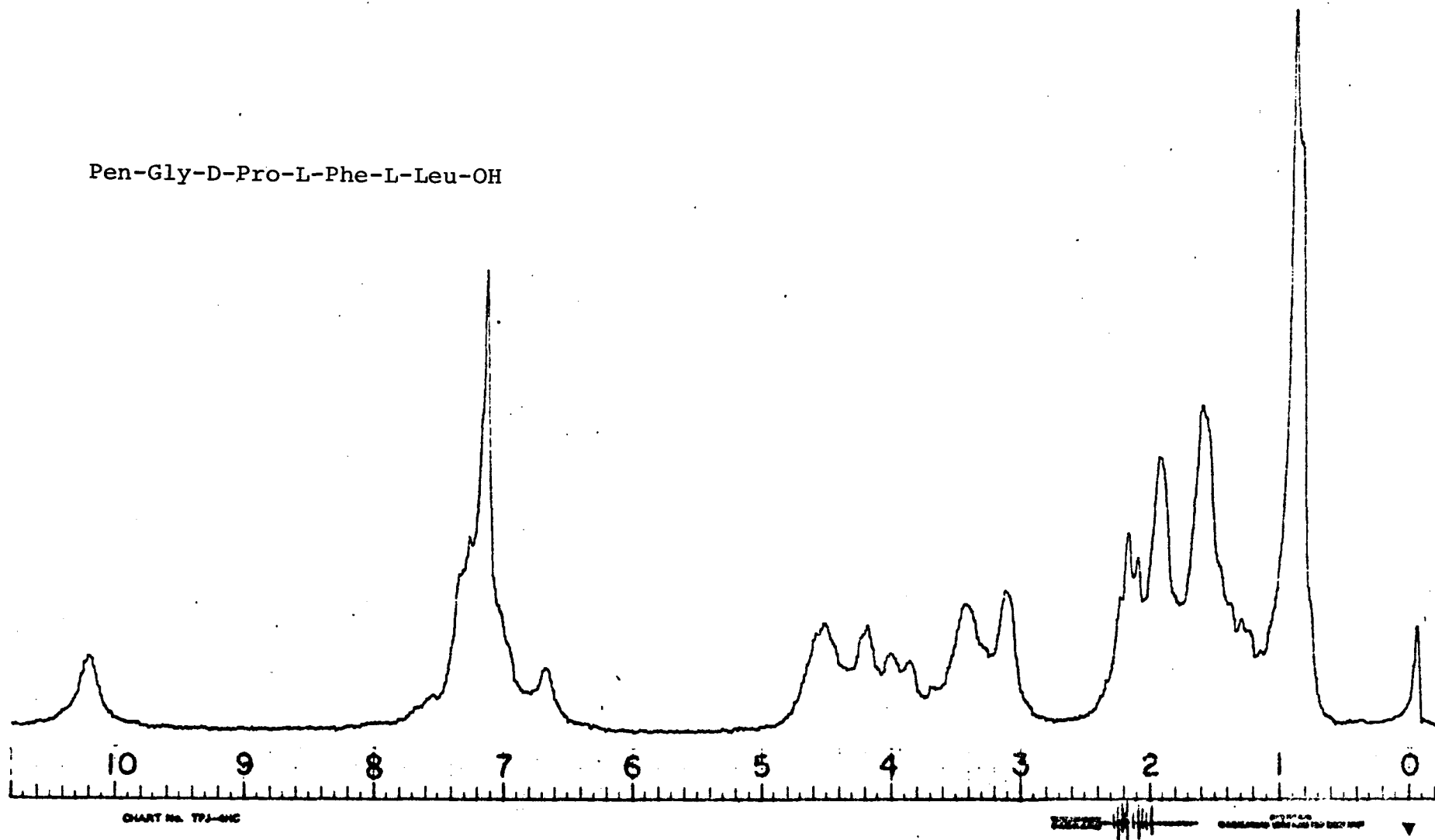
B-Figure 28. (CDCl$_3$). Compound 79.

Oct-NHCH(CO$_2$Et)$_2$
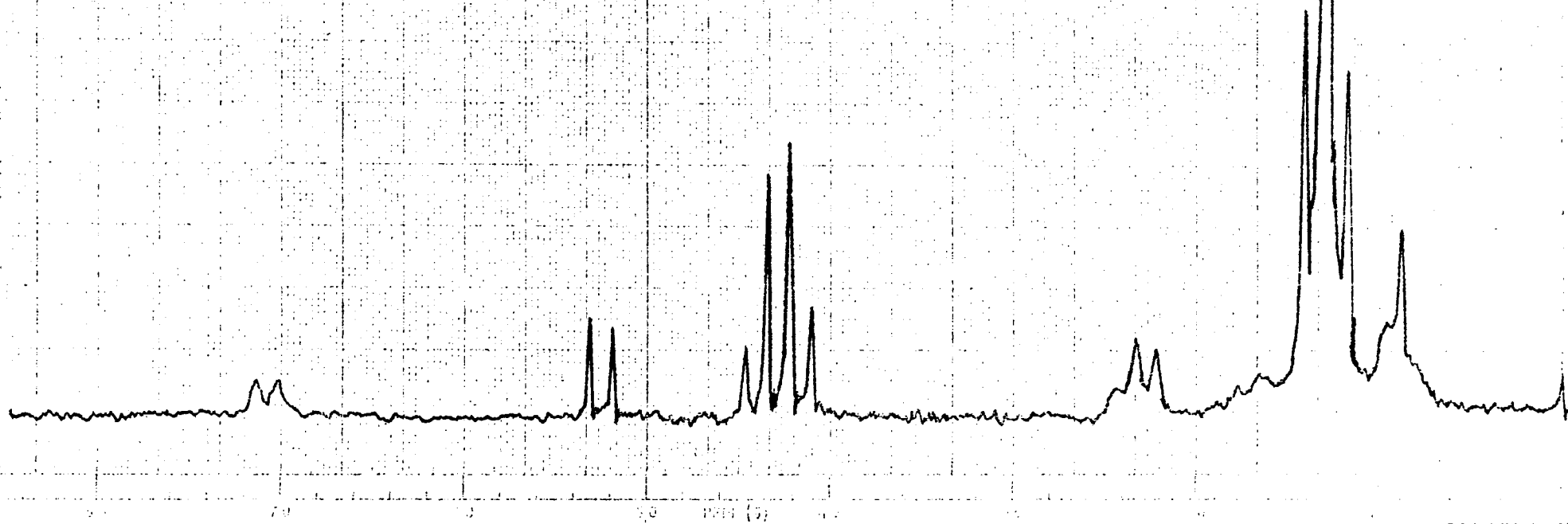
B-Figure 29. (CDCl$_3$). Compound 82.
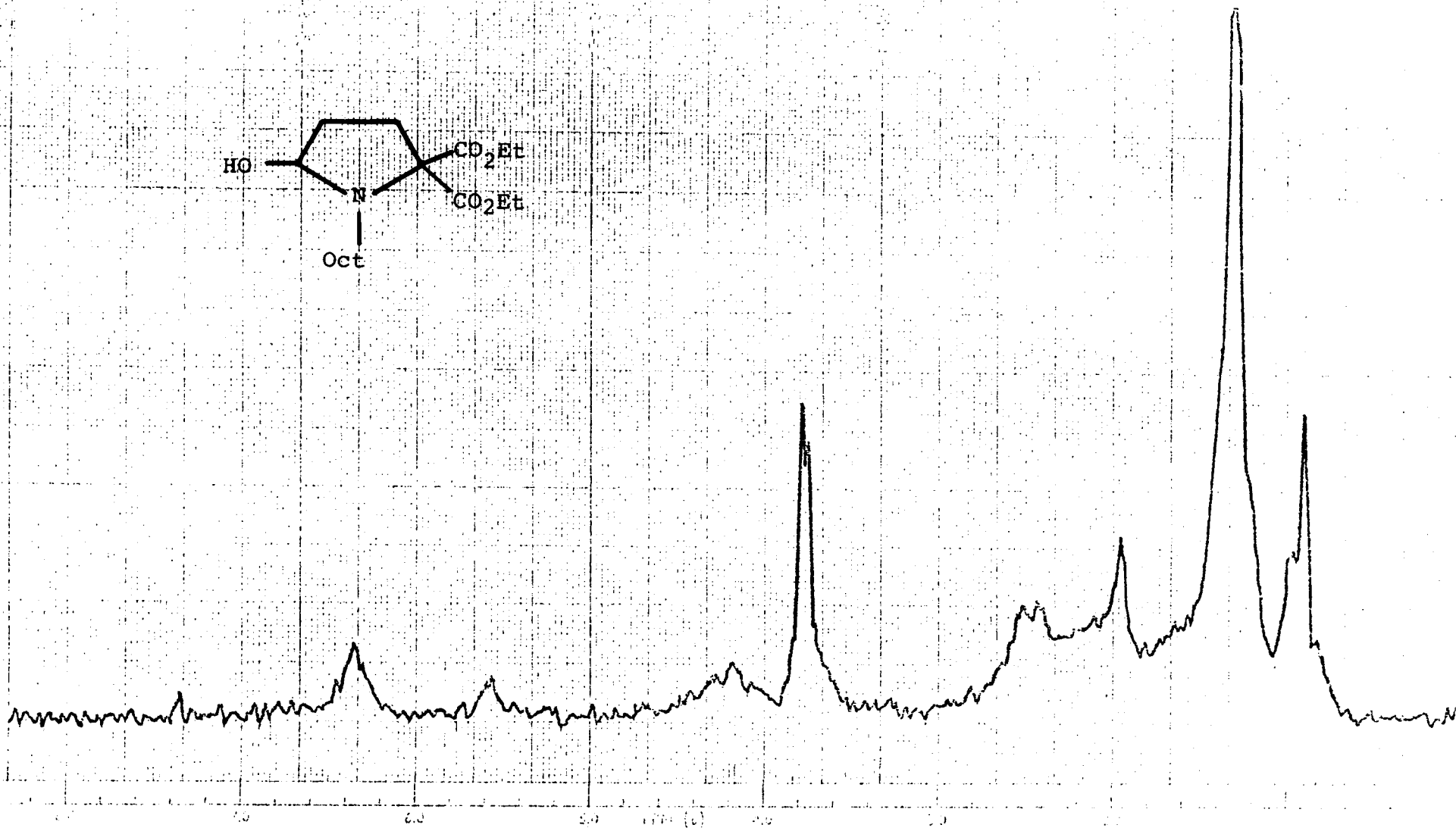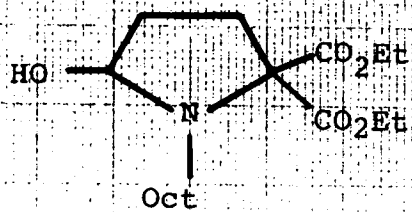
B-Figure 30. (CDCl$_3$). Compound 83.
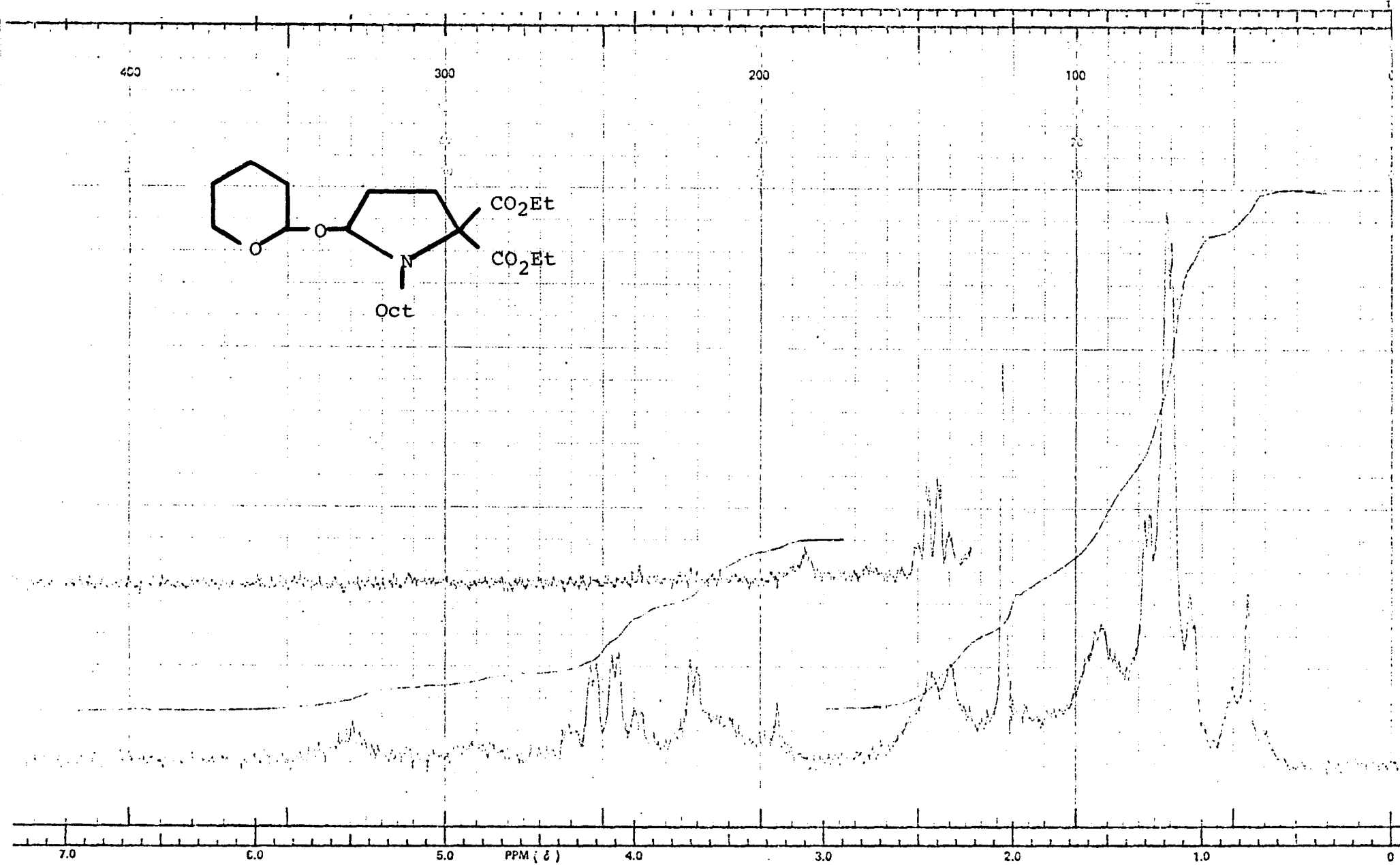
B-Figure 31.   (CDCl$_3$).   Compound 84.
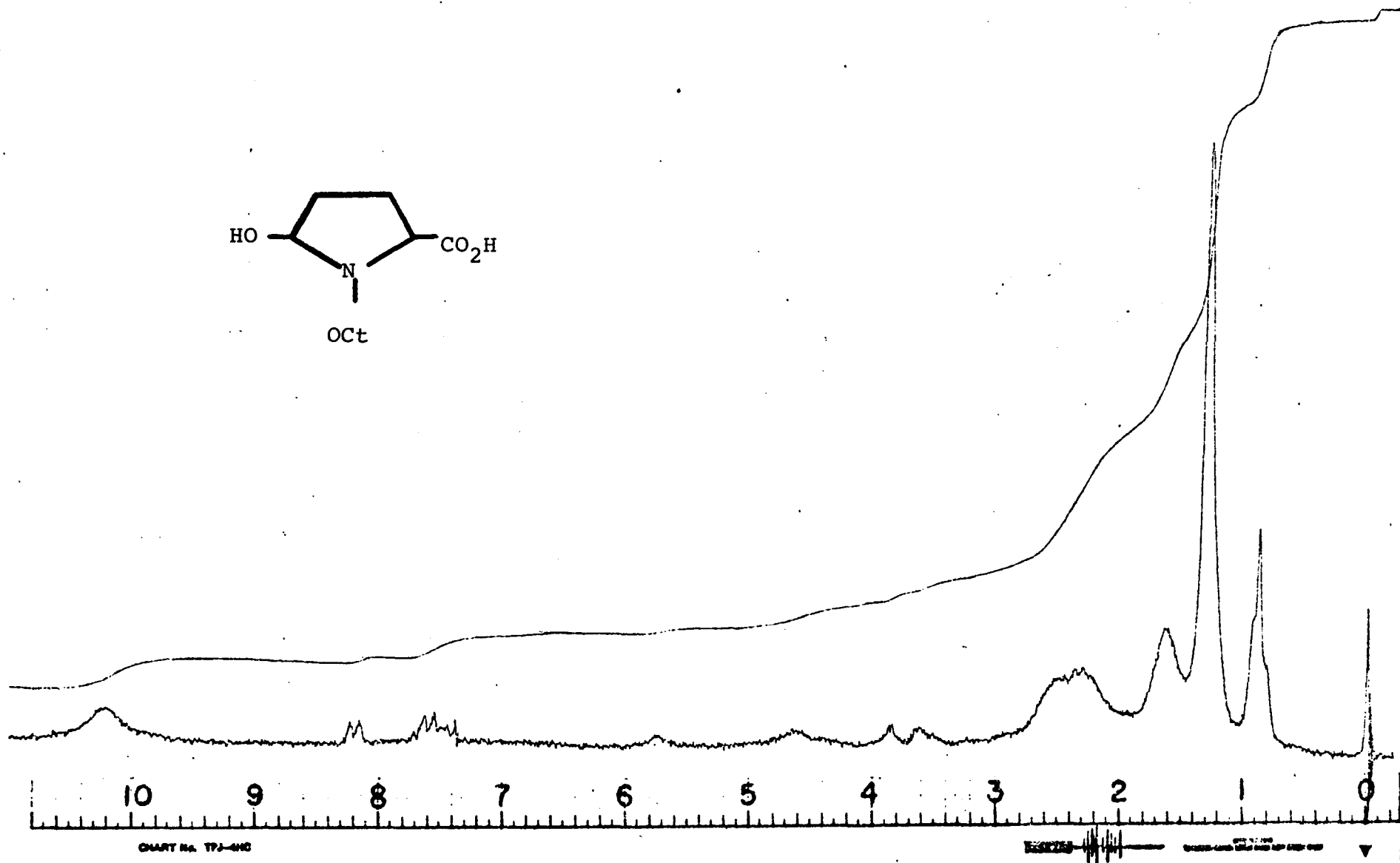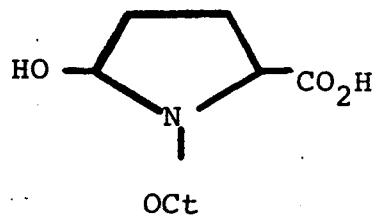
B-Figure 32. (CDCl$_3$). Compound 85.
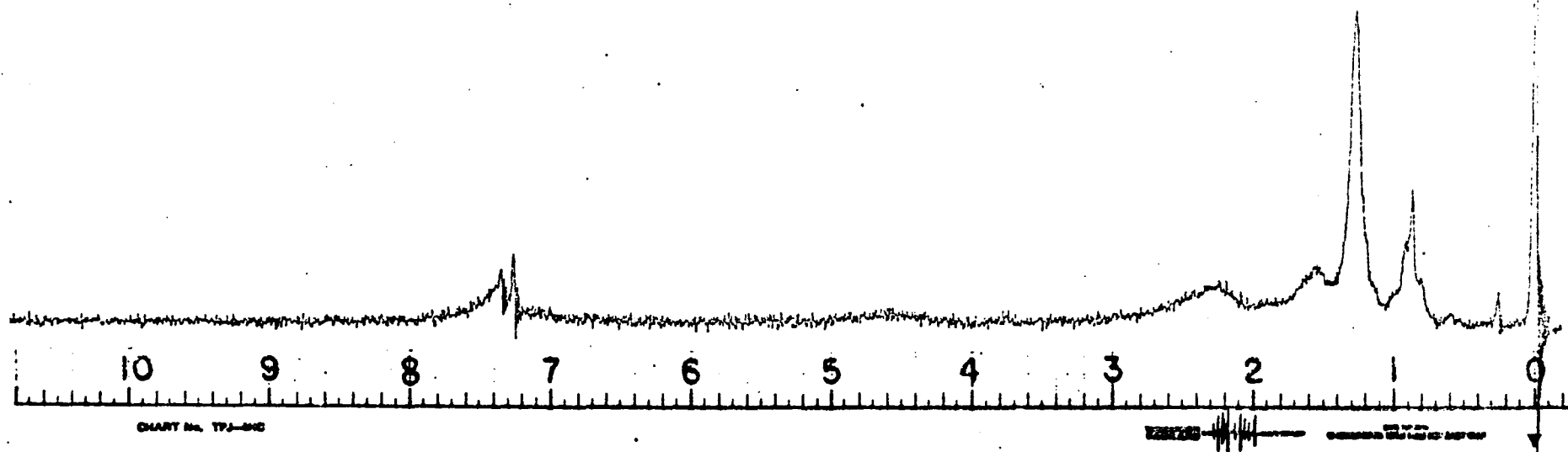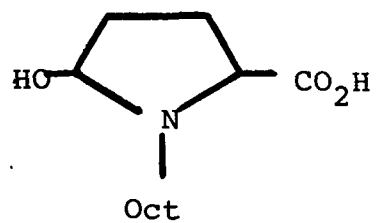
B-Figure 33., (CDCl$_3$). Compound 85.
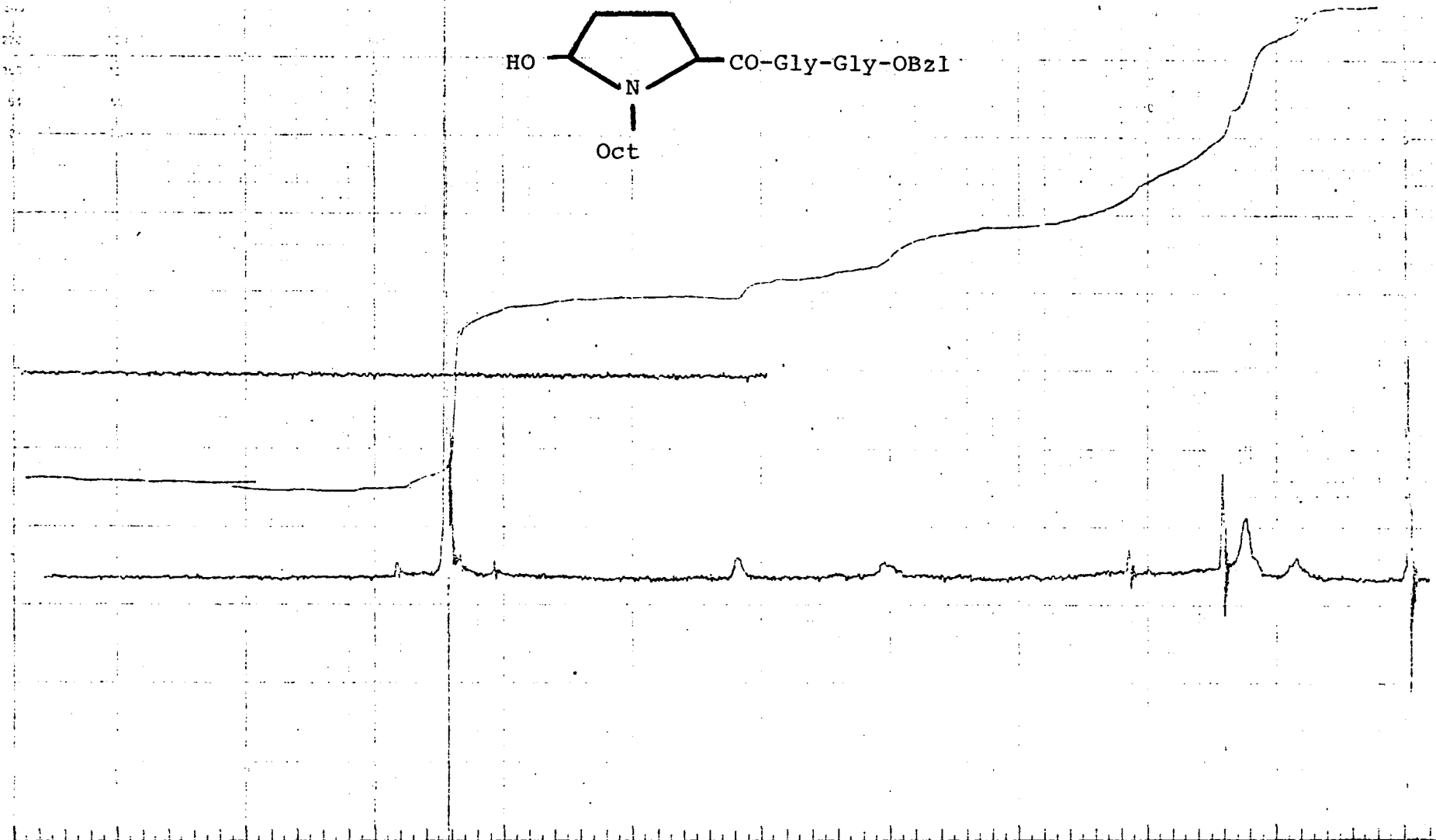
B-Figure 34. (CDCl$_3$). Compound 86.

PART II.

CHEMICAL SHIFT NONEQUIVALENCE IN

2,4-DINITROPHENYLSULFENAMIDES

SECTION I

INTRODUCTION

If we ignore the barriers to rotation derived solely from steric hindrance, we can make the statement that the barriers to rotation about C-C sigma bonds are low. Evidence has accumulated recently that compounds containing trivalent nitrogen bonded to atoms which, like nitrogen, contain nonbonded valence electrons and/or vacant d orbitals exhibit substantial torsional barriers. Systems studied to date include aminophosphines[1], hydroxylamines[2,3], hydrazines[4], and sulfenamides.[5] An excellent review of this subject has recently been published by Kessler.[6]

The barrier to rotation about the S-N bond in sulfenamides imparts to the sulfenamide moiety an element of axial chirality, analogous to the axial chirality of 1,3-disubstituted allenes, as shown by the Newman projections (R)-$\underline{1}$ and (S)-$\underline{1}$. The processes involved in the interconversion of two such enantiomers has come to be known as degenerate racemization. Nuclear magnetic resonance (nmr) spectroscopy has proved to be a valuable tool for the study of the stereochemistry of sulfenamides and their degenerate racemization. If a solution of such a sulfenamide is cooled sufficiently the rotation around the S-N bond becomes slow on the nmr time scale. If a group containing diastereotopic protons is present in the sulfenamide, we have the possibility of observ-

ing chemical shift nonequivalence of the diastereotopic protons due to the absence of a plane of symmetry in the molecule. This effect is usually displayed by the appearance of an AB quartet. Such experiments provide information on the rates of rotation about the S-N bond in the temperature range in which the AB quartet coalesces into a singlet. Complete line shape analysis (CLSA) over this temperature range will provide values of the free energy of activation (ΔG^*) for the barrier to rotation.

(R)-1   (S)-1
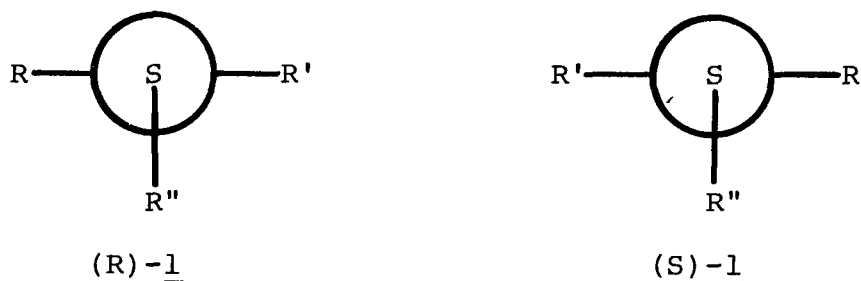
The presence of a group containing an asymmetric carbon atom introduces the possibility of diastereomerism due to the presence of the additional chiral center. The low temperature nmr spectra of such compounds exhibit non-equivalent signals for corresponding groups in the two diastereomers. The ratios of intensities of such signals provide a means of measuring the equilibrium for rotational epimerization.

All of the nmr studies of sulfenamides studied to date have involved the observation of chemical shift non-equivalence of protons of the groups attached to nitrogen, such as the benzyl protons of structure 2. No systematic

study of chemical shift nonequivalence of protons of groups

attached to sulfur (e.g., structure 3) has been conducted

and in only one instance has such nonequivalence been reported.

Raban and coworkers[7] have reported that the nmr spectrum of

N-(p-toluenesulfenyl)-N-(1-phenylethyl)benzenesulfonamide

(4) features a doublet for the p-tolyl methyl group when

cooled to -60°. No mention was made of nonequivalence of the

aromatic protons of the p-tolyl group.

$$\underset{2}{\text{R-S-N}\begin{smallmatrix}R'\\CH_2-\phi\end{smallmatrix}} \qquad \underset{3}{CH_3-\phi-S-N\begin{smallmatrix}R\\R'\end{smallmatrix}}$$

$$\underset{4}{CH_3-\phi-S-N\begin{smallmatrix}CH(CH_3)-\phi\\SO_2-\phi\end{smallmatrix}}$$

$$\underset{5}{O_2N-\phi(NO_2)-S-N-\phi-R}$$
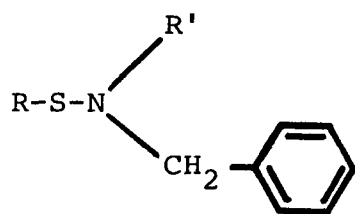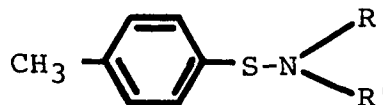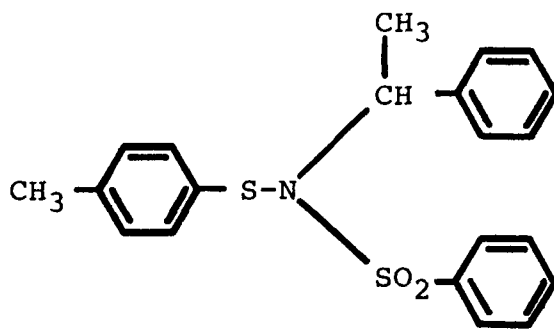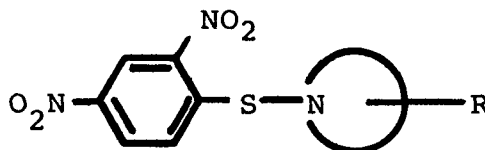
We have observed that 2,4-dinitrophenylsulfenamides of substituted piperidines and pyrrolidines (5) exhibit chemical shift nonequivalence of the ortho proton of the 2,4-dinitrophenyl group. In order to determine which structural features are necessary to observe this effect, a series of sulfenamides of piperidines and pyrrolidines were prepared and their nmr spectra have been studied over a wide temperature range in order to determine the coalescence temperature.

SECTION II

HISTORICAL

In his original papers on the subject, Raban[7,8] proposed that the chemical shift nonequivalence of diastereotopic protons of sulfenamides was due to restricted pyramidal inversion about nitrogen (Reaction 1). This was based on the fact that nitrogen inversion has been shown to be dramatically slowed by the presence of an adjacent heteroatom in three and four membered rings. He later firmly established that the nonequivalence is due to hindered rotation around the S-N bond and not to restricted pyramidal inversion.

Reaction 1
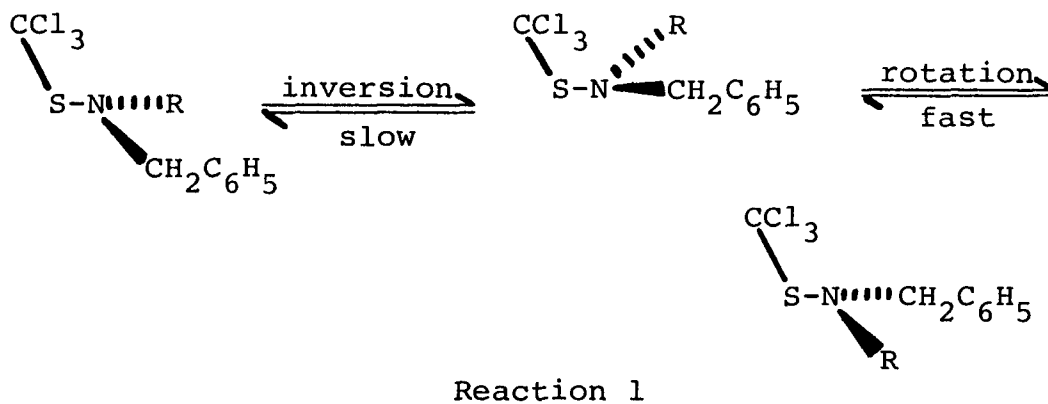
The effect of amide resonance in lowering the barrier to nitrogen inversion is a well-known phenomenon. It is reasonable to assume that the geometry of the nitrogen in 2,2-dimethyl-N-(trichloromethanesulfenyl)succinimide (6) is very nearly planar and that any barrier to inversion would be very small. The nmr spectrum of 6, however, does

exhibit chemical shift nonequivalence of diastereotopic methyl and methylene signals. Since slow pyramidal inversion can be eliminated as a source of this nonequivalence, it is clear that there exists a substantial rotational barrier about the S-N bond.

6
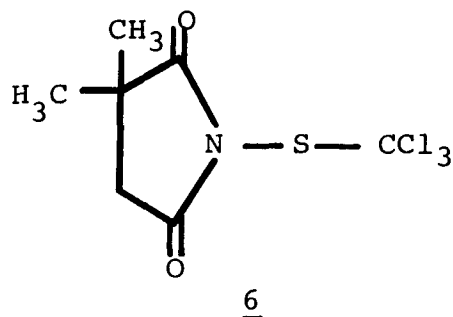
The above example is a specialized case, in which carbonyl groups are attached to nitrogen. In the same paper Raban presented evidence that the rate-determining step in the degenerate racemization of dialkyl sulfenamides was also due to hindered S-N rotation. The steric effect on pyramidal nitrogen inversion in aziridines has been well studied.[9] It has been found that an increase in the steric bulk of the substituent on nitrogen results in a decrease in the barrier to nitrogen inversion. This phenomenon can be explained by considering the transition state for pyramidal inversion. At the transition state for nitrogen inversion the planar nitrogen is sp^2-hybridized, so that the bond angle must increase to about 120° from about 109° in the ground state. This expansion reduces the congestion which occurs in the ground state when bulky groups are present. By

contrast, rotation about the S-N bond of sulfenamides involves passage through a transition state in which the C-S-N-C dihedral angle is smaller than in the ground state. Consequently there should be more steric hindrance in the transition state if bulky groups are present.

A series of trichloromethanesulfenamides (7) with ligands of increasing bulk were prepared and examined by nmr spectroscopy. It was found that as the size of the alkyl group increased the racemization was slowed down as evidenced by an increase in both the coalescence temperature and the free energies of activation. This indicates a greater congestion in the transition state, which is more compatible with a rotational barrier than with an inversion barrier. Raban's findings are supported by the work of Lehn and Wagner[10], who studied the degenerate racemization of aziridines, azetidines, and piperidines. They found that the rate-determining step for degenerate racemization in aziridines is pyramidal inversion while for azetidines and piperidines the rate-determining step is hindered rotation about the S-N bond.

$$CCl_3-S-N \overset{\textstyle CH_2C_6H_5}{\underset{\textstyle R}{<}}$$
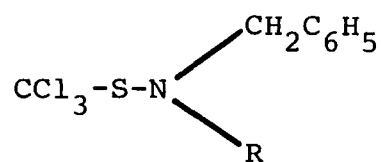
7

The above experiments indicate that steric hindrance makes an important contribution to the torsional barrier about the S-N bond. However, other factors must be contrib-

uting to the torsional barrier, since even the least hindered compound studied by Raban (7, R = Me) showed a substantial barrier to rotation (T_c = 17°, ΔG^* = 14.4 kcal/mol).

In order to determine what these factors were, Raban and coworkers[11] studied the dependence of the sulfenamide torsional barrier on the electronegativity of the substituent on the sulfur atom and on the electronegativity of the substituent on the nitrogen atom. They prepared a series of N-benzenesulfonylarenesulfenamides (8, 9, 10, and 11) and studied the rates of degenerate racemization by observing the coalescence of the signals of the diastereotopic methyl groups in the nmr spectrum. Hammett linear free-energy relationships were used to study the importance of polar substituents on the torsional barrier. The Hammett plots for series 8, 9, 10, and 11 are shown in Figure 1.

One factor which has been implicated in barriers to nitrogen inversion as well as to rotation around nitrogen-heteroatom bonds is the coulombic repulsion between vicinal pairs of nonbonding valence electrons.[12] If this electron repulsion were the only contributor to the rotational barrier, one would predict that electronegative substituents attached to the sulfur atom would decrease this repulsion and lower the rotational barrier. However, the Hammett reaction constants (ρ) for series 8 and 9 (Figure 1) indicates that an increase in the electronegativity of the substituent on sulfur increases the barrier to rotation about the S-N bond.
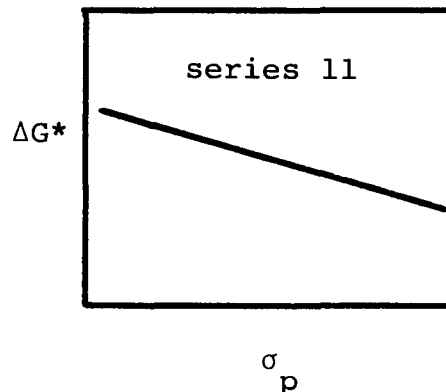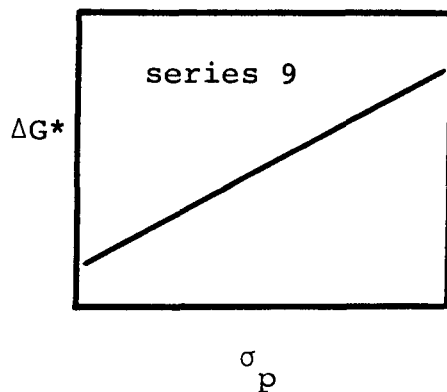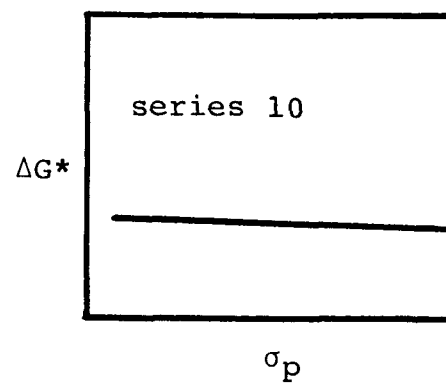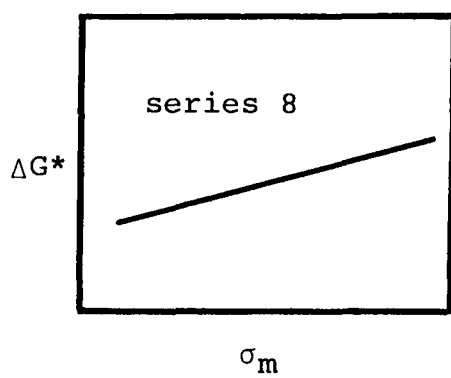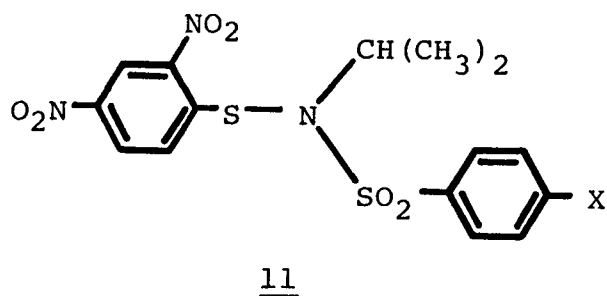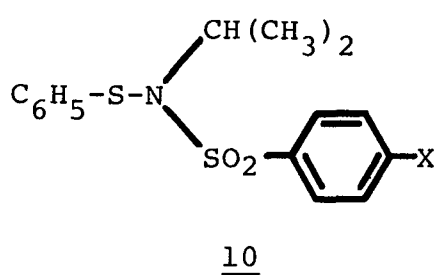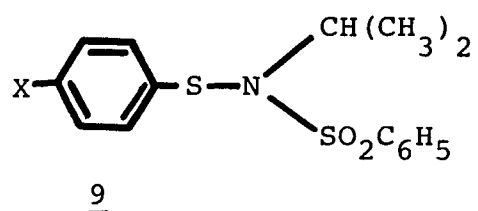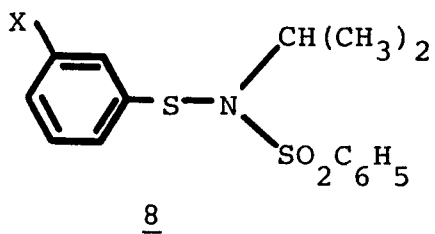
Figure 1.   Hammett Plots of N-Benzenesulfonylarenesulfenamides.

198

In order to explain these results, Raban has proposed
that partial S-N double bond formation between the lone pair
of electrons on nitrogen and an orbital on sulfur contributes
to the rotational barrier. The proposed mechanism of this
bond formation is p-d π bonding in which the lone pair of
electrons on nitrogen overlaps with an empty d orbital on
sulfur. In this way the sulfur is able to transmit the effect
of electronegative substituents on the phenyl ring to the
lone pair on nitrogen. If this overlap is to lead to an
increase in the torsional barrier it is a necessary require-
ment that the overlap occur in the torsional ground state but
not in the transition state.

The Hammett plots of series 8, 9, 10, and 11 are
understandable in terms of this mechanism. A strongly elec-
tronegative substituent on the sulfenyl phenyl ring (series
8 and 9) lowers the energy of the empty d orbitals on sulfur
and permits a greater degree of overlap of the lone pair of
electrons on nitrogen. When the electronegativity of the
sulfonyl phenyl ring is increased, the sulfonyl sulfur atom
is able to compete with the sulfenyl sulfur atom in partial
double bonding with the nitrogen atom. This explains the
reversal of the Hammett reaction constant in going from series
9 to 11. The independence of the rotational barrier in series
10 with respect to substituents in the sulfonyl ring ($\rho \approx 0$)
was taken as an indication that partial double bonding makes
a negligible contribution in this series. The rotational
barrier in series 10 is derived mainly from lone pair-lone

pair interaction and steric hindrance. Apparently, partial double bonding makes an important contribution to the rotational barrier only when there is a strongly electronegative group attached to the sulfenyl sulfur atom. From a comparison of the free energy values of the compounds in these four series, Raban was able to conclude that in series 11 partial double bond formation is as important as repulsion between pairs of nonbonded valence electrons.

SECTION III

RESULTS AND DISCUSSION

A. Preparation of 2,4-Dinitrophenylsulfenamides

The synthesis of the 2,4-dinitrophenylsulfenamides
(5) used in this study was accomplished by condensation of
the appropriate piperidine or pyrrolidine with 2,4-dinitro-
phenylsulfenyl chloride (12) in methylene chloride containing
a base to neutralize the hydrogen chloride produced in the
reaction. Generally, the yields were moderate to good and
the sulfenamides were obtained as crystalline solids which
could be easily recrystallized from ethanol.
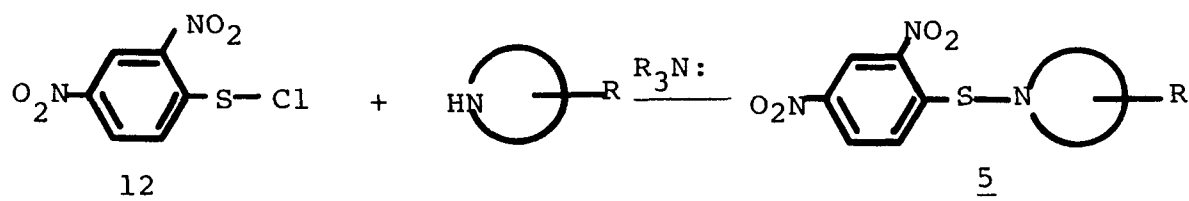
Because 2,4-dinitrophenylsulfenamides are generally
crystalline, they can be used for the identification of
amines. In 1941 Billman and coworkers[13] characterized 14
amines in this manner and more recently Kornet et al.[14]
have prepared the 2,4-dinitrophenylsulfenamides of 72 amines.
Kharasch has shown[15] that 12 can also be used for the prepa-
ration of derivatives of many other functional groups and
tables containing the physical properties of such derivatives
have been published.[16] The 2,4-dinitrophenylsulfenyl group

has also been advocated[17] as a nitrogen protecting group for peptide synthesis.

Three different methods were used for the preparation of the sulfenamides. These three methods differed only in the base used. Where the piperidine or pyrrolidine was inexpensive and readily available a two-fold excess was used and the addition of a second amine was therefore unnecessary. By this method (Method A) the reaction of 2,4-dinitrophenylsulfenyl chloride (12) with pyrrolidine (13), piperidine (14), 2-methylpiperidine (15), and 2-n-propylpiperidine (16) gave the corresponding sulfenamides 17, 18, 19, and 20 in yields ranging from 67-78%. These four derivatives were crystalline solids, easily recrystallized from ethanol.

The second method (Method B) of preparing these sulfenamides involved the use of one molar equivalent of the piperidine or pyrrolidine and one molar equivalent of triethylamine as the hydrogen chloride scavenger. The 2,4-dinitrophenylsulfenamides (24, 25, and 26) of 2-cyclohexylmethylpiperidine (21), 2-phenylpiperidine (22), and t-butyl prolinate (23), respectively, were prepared in this manner in 37-57% yields. Again, these sulfenamides were high melting crystalline solids.

Two additional sulfenamides prepared by Method B were obtained as viscous oils. Neither ethyl N-(2,4-dinitrophenylsulfenyl)pipecolinate (29) or N-(2,4-dinitrophenylsulfenyl)-2,2-bis-ethoxycarbonylpyrrolidine (30) could be crystallized and they were purified by molecular distillation[43]

in a microsublimation apparatus. Compound 30 was isolated in the very low yield of 12%. This may result from the use of crude 2,2-bis-ethoxycarbonylpyrrolidine (28).

The use of triethylamine was found to lead to undesirable side products. Traynelis and Rieck[18] studied the reaction of 2,4-dinitrophenylsulfenyl chloride (12) with tertiary aliphatic amines containing an ethyl group and reported the formation of bis-arylthioenamines (31) and 2,2', 4,4'-tetranitrodiphenyldisulfide (32). The yield of the enamine (31) was 50% as was the yield for the disulfide. Amines which were found to give this reaction always contained an ethyl group. Amines which failed to produce enamines included tri-n-propylamine, tri-n-butylamine, and N-methylpiperidine. In view of this possible reaction with triethylamine, all further sulfenamides were prepared using N-methylpiperidine as base in place of triethylamine. This modification represents Method C.
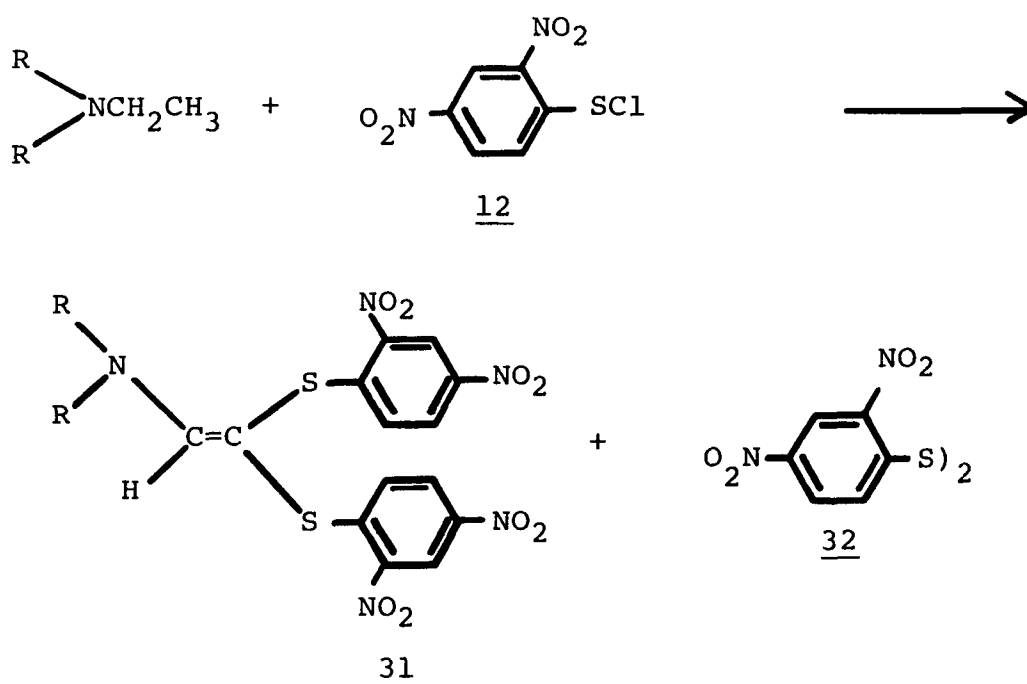
The reaction of 2,4-dinitrophenylsulfenyl chloride
(12) with 2-propionylpiperidine (33), ethyl 2-piperidylacetate
(34), ethyl 2-pyrrolidinylacetate (35), and cis-2,5-bis-
ethocycarbonylpyrrolidine (36) gave the corresponsing sulfen-
amides 37, 38, 39, and 40 in yields ranging from 53% to 82%.
This is approximately 15% higher than the range of yields
observed when triethylamine was used as base, and is only
slightly lower than the yields reported for Method A. Al-
though enamines of the type reported by Traynelis were not
isolated in these experiments, the lower yields of sulfen-
amides prepared by Method B suggests the existence of some
side reactions.

A fifth sulfenamide, N-(2,4-dinitrophenylsulfenyl)-2-
benzoylpiperidine (42), was prepared by Method C in only
28.8% yield. This low yield may be due to the fact that 2-
benzoylpiperidine (41) is unstable, giving rise to dark
resinous material after standing several days at room temp-
eratures.

B. Preparation of o-Nitrophenylsulfenamides and p-Nitro-
    phenylsulfenamides

These sulfenamides were prepared by a method similar
to Method B, which was described above. The reaction of o-
nitrophenylsulfenyl chloride (43) with t-butyl prolinate
(23) gave t-butyl N-(o-nitrophenylsulfenyl)prolinate (44) as
a viscous oil in 65% yield. An analytical sample was pre-
pared by molecular distillation.[43] In a similar manner, 43

was condensed with ethyl pipecolinate hydrochloride (27) to give a 23.3% yield of ethyl N-(o-nitrophenylsulfenyl)pipeco-linate (45) as a yellow solid. Purification by recrystallization and sublimation gave an analytical sample.

Compounds 44 and 45 proved to be reasonably stable materials. In contrast, the p-nitrophenylsulfenamides were very unstable and difficult to purify. Reaction of p-nitro-phenylsulfenyl chloride (46) with t-butyl prolinate (23) gave t-butyl N-(p-nitrophenylsulfenyl)prolinate (47) as a viscous oil. TLC revealed the presence of several impurities and the oil was separated into its components by preparative layer chromatography (PLC), to provide a very low yield of 47. This sulfenamide was the more stable of the two p-nitro-phenylsulfenamides prepared, but it slowly decomposed at room temperatures, as evidenced by TLC and the formation of a yellow precipitate (probably 4,4'-dinitrodiphenyldisulfide). It was sufficiently stable, however, to obtain correct elemental analyses.

Ethyl N-(p-nitrophenylsulfenyl)pipecolinate (48) proved to be very unstable and could not be obtained analytically pure even after purification by PLC and repeated sublimation. The compound gave rise to disulfide very rapidly.

C. Preparation of Piperidines and Pyrrolidines

The preparation of the sulfenamides described above required a series of piperidines and pyrrolidines. Several of the simpler members of the series were commercially

available, while most of the remaining compounds were pre-
pared following standard procedures.

t-Butyl prolinate (23) was prepared in 50% yield from
N-benzyloxycarbonyl-L-proline (49) (see experimental section
of Part I) by the procedure of Anderson and Callahan.[19]
Esterification of 49 was accomplished in a solution of iso-
butylene and dioxane containing a catalytic amount of sulfuric
acid.   The N-benzyloxycarbonyl-L-proline t-butyl ester (50)
was not purified but hydrogenated directly to 23 using 10%
palladium on carbon.   The free ester has very good storage
properties and is not subject to self-condensation.

$$Cbo-L-Pro-OH \xrightarrow[\text{H}^+]{\text{CH}_2=\text{C(CH}_3)_2} Cbo-L-Pro-O-t-Bu \xrightarrow{\text{H}_2-Pd/C}$$

49   50

23
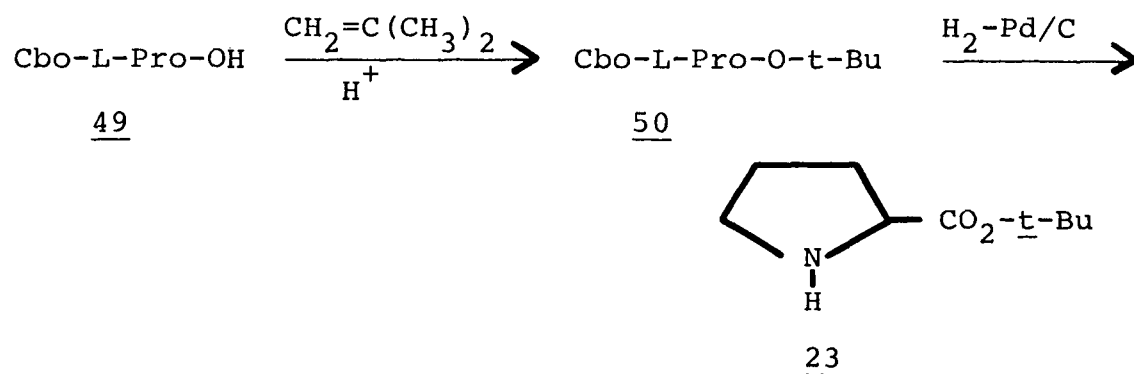
Many of the substituted piperidines were prepared
from the corresponding pyridines by reduction over Adams
catalyst.[20]   Reduction of ethyl picolinate (51) and ethyl
2-pyridylacetate (52) gave ethyl pipecolinate hydrochloride
(27) and ethyl 2-piperidylacetate (34), in 85% and 77.5%
yields, respectively.   Reduction of 2-propionylpyridine (53)
and 2-benzoylpyridine (54) over Adams catalyst reduced the
aromatic ring to the piperidine, but also caused the reduc-
tion of the ketone to the alcohol.   The mixture of diastereo-

meric alcohols formed in each case were not separated, but oxidized directly to the ketone using chromium trioxide in acetic acid according to the procedure of Heer and co-workers.[21] Both 2-propionylpiperidine (33) and 2-benzoyl-piperidine (41) were unstable materials and gave rise to colored, resinous impurities on standing at room temperatures. An analytical sample of 33 was never obtained. The 2,4-dinitrophenylsulfenamides of both 33 and 41 were stable, however, and analytical samples were obtained without difficulty. The ir spectrum of 2-benzoylpiperidine (41) showed no band in the carbonyl region of the spectrum. The ir spectrum of the 2,4-dinitrophenylsulfenamide of 41 did, however, contain a strong carbonyl absorption band at 1680 cm^{-1}. The absence of a carbonyl band in 41 was probably due to addition of the secondary amine to the carbonyl carbon of a second molecule to form a geminal amino-alcohol. When the sulfenamide is formed the nitrogen is no longer able to add to the carbonyl and thus we see an absorption band.

Ethyl 2-pyrrolidinylacetate (35) was prepared by reduction of the corresponding pyrrole, ethyl 2-pyrrolyl-acetate (55), over 5% rhodium on carbon. The substituted pyrrole was prepared in 39.3% yield from pyrrole (56) and ethyl diazoacetate (57) using an activated copper catalyst according to the procedure of Mandell and Roberts.[22]
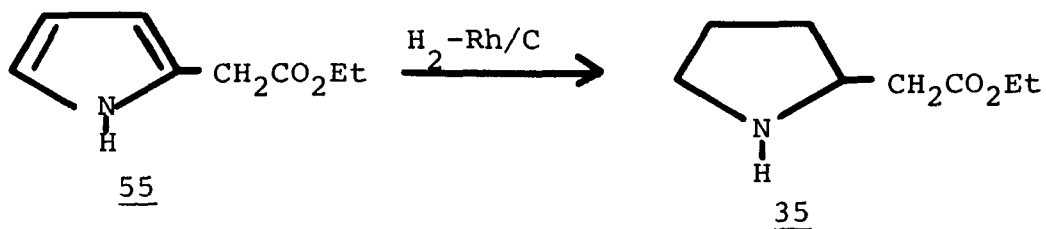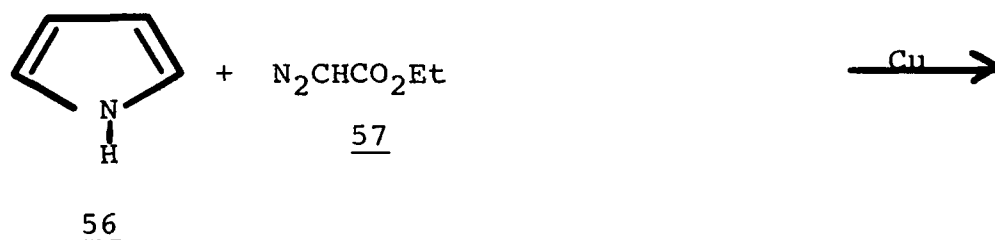
The two remaining substituted pyrrolidines were prepared by two different multistep approaches. Part I of this thesis describes the synthesis, by Cox[23] and Magerlein[24], of substituted prolines by the Michael addition of diethyl N-acylaminomalonates to α,β-unsaturated aldehydes and ketones. Following their procedure, diethyl N-benzyloxycarbonylamino-malonate (58) was condensed with acrolein to give crude N-benzyloxycarbonyl-2,2-bis-ethoxycarbonyl-5-pyrrolidinol (59). Hydrogenation of 59 over 10% palladium on carbon gave crude 2,2-bis-ethoxycarbonylpyrrolidine (28) in 34% yield.
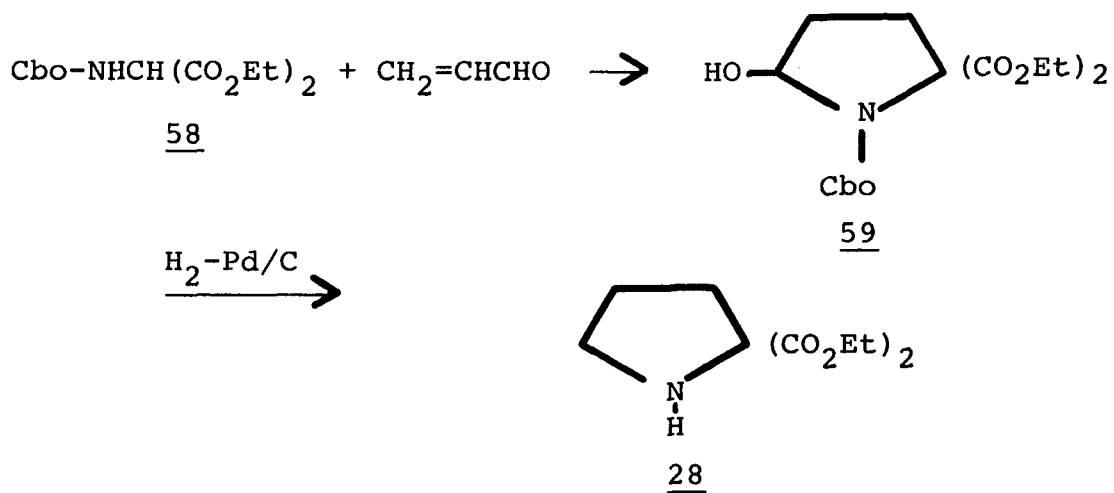
The preparation of cis-2,5-bis-ethoxycarbonylpyrrol-
idine (36) followed the procedure outlined by Cignarella and
Nathansohn.[25] Condensation of meso diethyl 2,2'-dibromo-
adipate (60) with benzylamine (61) gave N-benzyl-cis-2,5-
bis-ethoxycarbonylpyrrolidine (62) in 84.5% yield. Hydrog-
enation of 62 over 10% palladium on carbon gave a 67.5%
yield of 36 as a colorless liquid.

$$EtO_2CCHCH_2CH_2CHCO_2Et \quad + \quad C_6H_5CH_2NH_2 \quad \longrightarrow$$

Br   Br

meso-60   61

EtO_2C —[pyrrolidine ring, N-Bzl]— CO_2Et

Bzl

62

$\xrightarrow{H_2-Pd/C}$ EtO_2C —[pyrrolidine ring, N-H]— CO_2Et

N
H

36
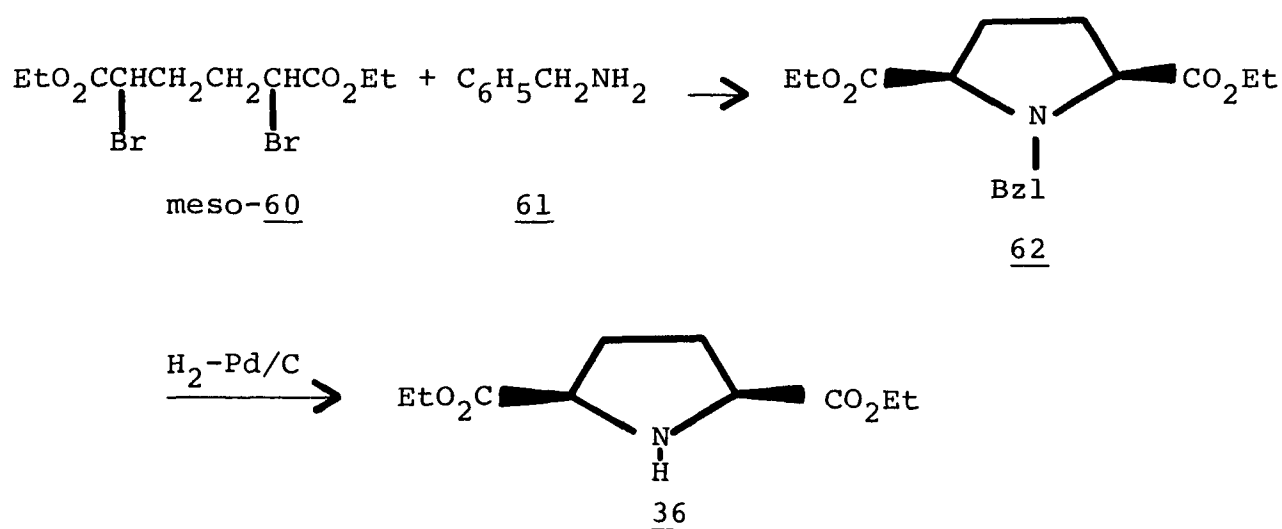
It was also considered desirable to prepare trans-
2,5-bis-ethoxycarbonylpyrrolidine (63) in order to compare
the nmr spectrum of its 2,4-dinitrophenylsulfenamide with
that of the sulfenamide of 36. Blackman and Baltzky[26] have
reported the preparation of the cis and trans isomers of
N-phenyl-2,5-bis-methoxycarbonylpyrrolidine (65) from the
meso and racemic forms of dimethyl α,α'-dibromoadipate (64),
respectively. They reported a 70% yield for the cis isomer
but only a 10% yield for the trans isomer. They attributed

this difference to a much greater steric hindrance required for the cyclization of racemic 64 to trans 65. They reported the formation of a large amount of polymeric material in both cases. They also prepared the N-benzyl derivative, but were unable to isolate it in crystalline form and, therefore, were unable to determine the amount, if any, of trans isomer present. In this case also, the reaction produced a large amount of polymeric material.

$$MeO_2CCHCH_2CH_2CHCO_2Me + C_6H_5NH_2 \longrightarrow$$

(with Br, Br substituents below)

64

(pyrrolidine product) MeO_2C~⟨N⟩~CO_2Me, N-substituent C_6H_5

65
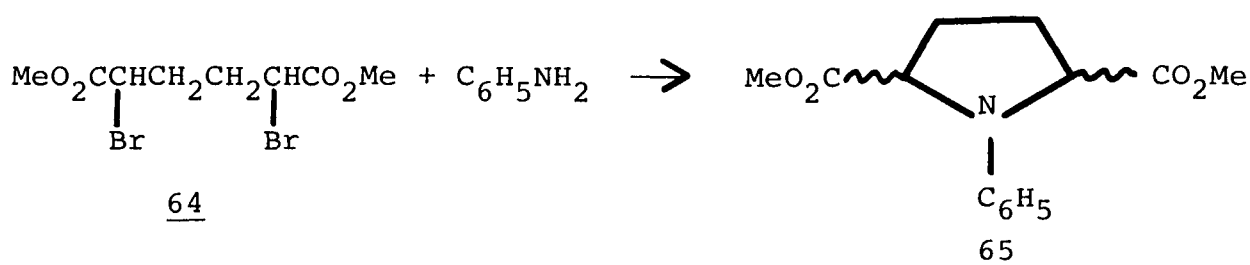
The attempted preparation of trans-2,5-bis-ethoxy-carbonylpyrrolidine (63) involved a high-dilution reaction between benzylamine (61) and racemic α,α'-dibromoadipate (60). It was reasoned that running the reaction under high-dilution conditions would reduce the amount of polymeric material produced by intermolecular condensations. The reaction produced only polymeric materials, however.

$$EtOCCHCH_2CH_2CHCOEt +$$ (with O double bonds and Br, Br)

(benzene ring)-CH_2NH_2 ⟶

racemic 60

61

(pyrrolidine) EtO_2C~⟨N⟩~CO_2Et, N-substituent Bzl

63
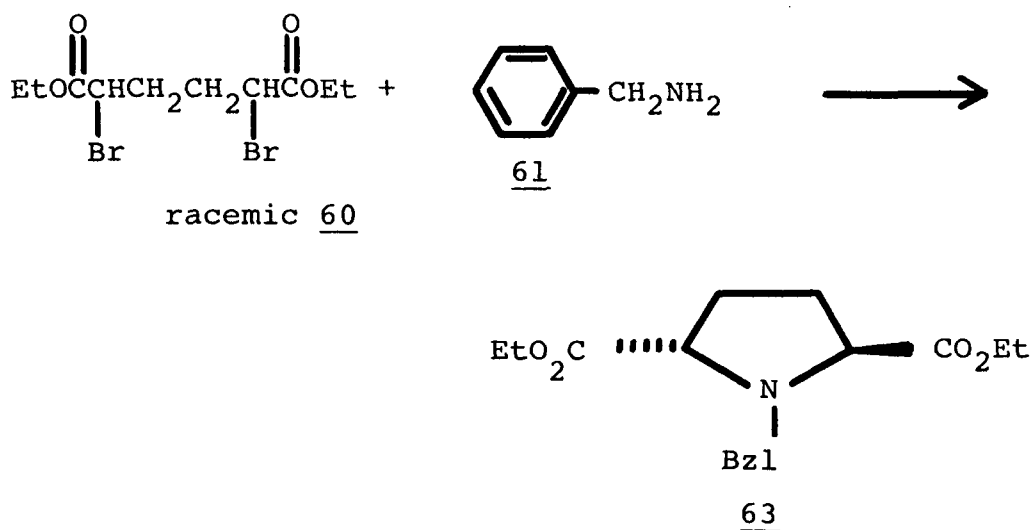
The second approach to the preparation of trans-2,5-disubstituted pyrrolidine involved attempted isomerization of the cis isomer. Part I of this thesis described a method for the racemization of proline by heating a solution of proline in glacial acetic acid and acetic anhydride. The N-acetyl-proline produced was hydrolyzed to give DL-proline in good yield. This method was used with cis-pyrrolidine-2,5-dicarboxylic acid (66) to give a moderate yield of N-acetyl-pyrrolidine-2,5-dicarboxylic acid (67) of unknown stereochemistry. Acidic hydrolysis of 67 followed by esterification with 2,2-dimethoxypropane gave a solid identified as cis-2,5-bis-methoxycarbonylpyrrolidine hydrochloride (68) by comparison with an authentic sample. Apparently, 66 was not isomerized to the trans isomer, possibly because of formation of an internal anhydride (69) which could only be cis.
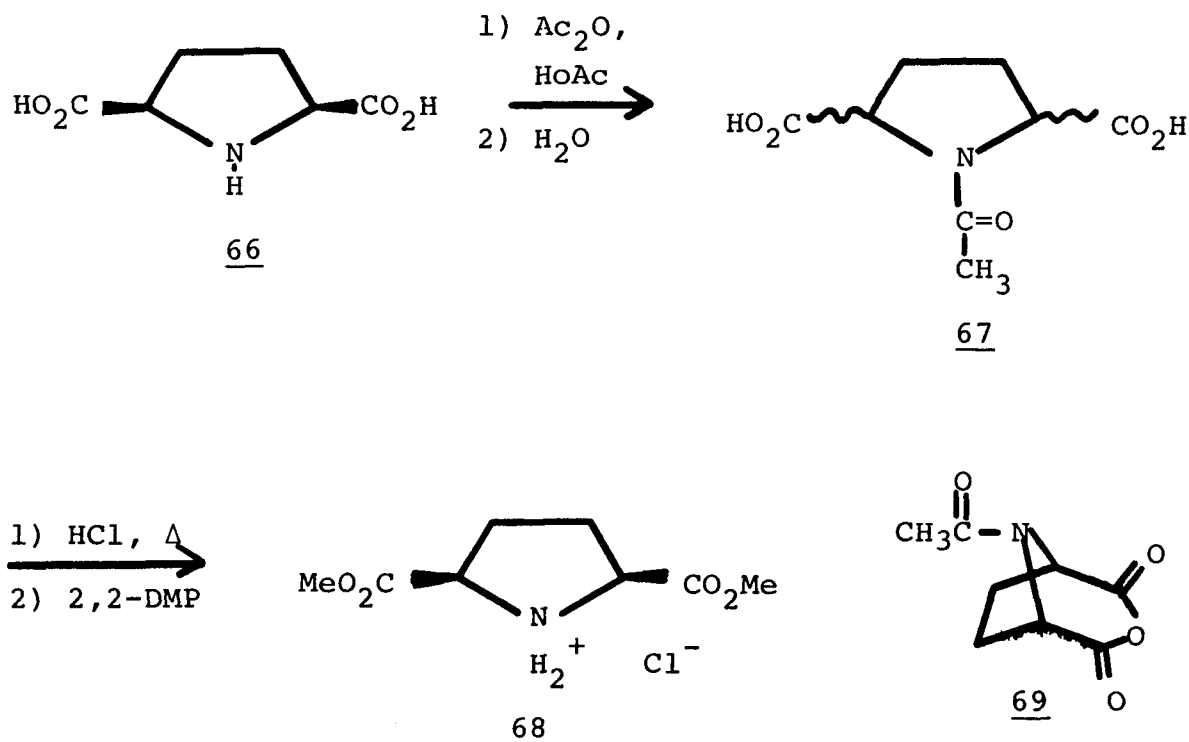
A third approach involved attempted isomerization of cis-2,5-bis-methoxycarbonylpyrrolidine hydrochloride (68) by the abstraction of an alpha proton using sodium hydride. This method appeared to result in destruction of the ring, for neither trans-2,5-bis-methoxycarbonylpyrrolidine (70) nor unreacted starting material could be isolated. Cignarella and Nathansohn[25] have claimed that pyrrolidine-2,5-dicarboxylic acid is very susceptible to both acidic and basic degradation.

It was felt that the di-t-butyl ester of 66 might be stable to strongly basic conditions and allow isomerization of the cis to the trans isomer by the above procedure. Attempted preparation of the di-t-butyl ester of N-acetyl-pyrrolidine-cis-2,5-dicarboxylic acid (67) with liquid iso-butylene gave a very low yield of an oil which could have been N-acetyl-cis-2,5-bis-t-butyloxycarbonylpyrrolidine (71). Basic hydrolysis of this oil gave a material lacking carbonyl adsorption bands in the ir spectrum. No further workup was attempted.
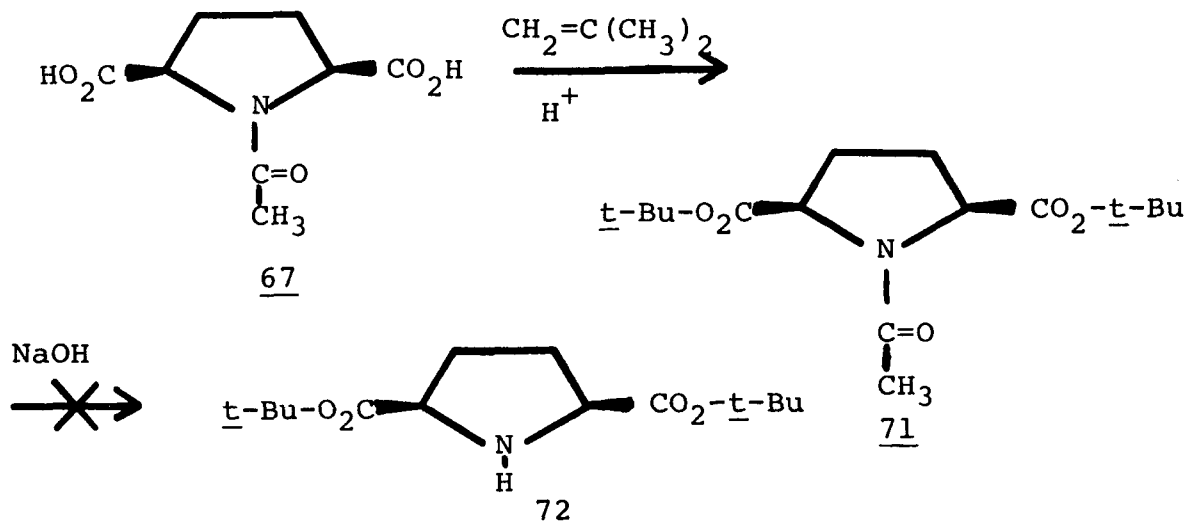
D. Nuclear Magnetic Resonance Studies of Sulfenamides

It has been observed that 2,4-dinitrophenylsulfen-
amides of piperidines and pyrrolidines (5) exhibit chemical
shift nonequivalence of the ortho proton of the 2,4-dinitro-
phenyl group. This is believed to be the first reported
instance in which the aromatic proton of a group attached
to the sulfur atom of a sulfenamide exhibited such non-
equivalence. In the nmr spectra of these sulfenamides at
room temperatures the ortho protons appeared as a doublet
of doublets. As the temperature was increased these two
doublets broadened and coalesced into a singlet which
sharpened into a doublet as the temperature was increased
above the coalescence temperature. This nonequivalence was
assumed to result from hindered rotation about the S-N bond.
The factors responsible for this hindered rotation are
probably a combination of steric factors, lone pair-lone
pair interactions, and p-d π, partial double bonding as was
suggested by Raban.[11] In many of the sulfenamides studied

the substituent on the piperidine or pyrrolidine ring also exhibited chemical shift nonequivalence at room temperature. As the temperature was increased the coalescence of these signals was observed. This provided an opportunity to calculate the free energy of activation based on two different sets of coalescing signals in the same molecule. These two values were in close agreement in all cases.

It was also observed that o-nitrophenylsulfenamides of piperidines and pyrrolidines exhibited chemical shift nonequivalence of the ortho proton of the aromatic ring. The coalescence temperatures (T_c) were lower than for the 2,4-dinitrophenylsulfenamides, requiring cooling below room temperatures to observe coalescence. As cooling was continued below T_c the broad singlet split into two doublets. The substituents on the piperidine or pyrrolidine ring also exhibited nonequivalence. The free energies of activation were calculated using data for coalescence of both the aromatic proton and aliphatic protons of the substituent on piperidine or pyrrolidine. As was the case for the 2,4-dinitrophenylsulfenamides, the two values obtained from these two sets of signals were in close agreement.

The two p-nitrophenylsulfenamides studied did not exhibit chemical shift nonequivalence in any part of the molecule down to -57°.

The coalescence temperature (T_c) and chemical shift difference ($\Delta\nu$) were measured directly from the nmr spectra. The coalescence rate constant (k_c) was calculated using

equation (1). The free energy of activation (ΔG^*) at the coalescence temperature was calculated using the Eyring equation (2).[27]

$$k_c = \pi \Delta \nu / \sqrt{2} = 2.2 \, \Delta \nu \tag{1}$$

$$k_c = \frac{k_b T}{h} C \exp \left(- \frac{\Delta G^*}{RT_c}\right) \text{ or } \Delta G^* = 4.57 T_c (10.32 + \log \frac{T_c}{k_c}) \tag{2}$$

k_b = Boltzmann constant

These values of ΔG^* and k_c are approximate because a number of assumptions must be made. First, the value of the chemical shift difference at the coalescence temperature must be extrapolated from low temperature measurements. Since $\Delta \nu$ is sometimes strongly dependent on temperature, a possible error in k_c is introduced by using $\Delta \nu$ as an experimental parameter. We are therefore making the assumption that the chemical shift differences obtained at temperatures well below the region of line broadening are approximately equal to $\Delta \nu$ at T_c. Another assumption which must be made when using equation (1) is that the two conformers are equally popu-lated. This is clearly not the case with the sulfenamides studied here. Visual inspection of the nmr spectra shows that one conformer is in slight excess in most cases. It is assumed, however, that the conformer populations are sufficiently near to equal to permit the use of equation (1).

The use of these approximate equations has been criticized recently as an unreliable procedure.[28] However, it has been shown[29,30] that, within certain limits, the

free energies of activation obtained by the use of approximate
equations are in good agreement with the results obtained
using complete line shape analysis (CLSA). Raban[30] has
carried out a systematic comparison of the rate constants
obtained using these approximate equations with those obtained
by CLSA. Equation (1) is derived from the complete line
shape expression for equally intense coalescing singlets.
However, Raban has found that the values of k_c obtained using
equation (1) for coalescing singlets or doublets (A and B
not coupled) are in general agreement with the rates obtained
using CLSA when $\Delta\nu$ is greater than 3 Hz, and when $\Delta\nu > 3$ J.
Raban also found that the approximate values of k_c obtained
for unequally intense (K = 2) coalescing singlets and doublets
were in general agreement with values of k_c obtained by CLSA.
Thus, although the equation ($k_c = \pi\Delta\nu/\sqrt{2}$) is based on equally
populated, two-site, uncoupled spin systems, it can be used,
within certain limitations, in cases where the assumptions
made in its derivation are not valid.

The sulfenamides studied are presented in Tables I,
II, and III along with relevant data, which includes the
coalescence temperature (T_c), the coalescence rate constant
(k_c), chemical shift difference ($\Delta\nu$), coupling constant (J),
and free energy of activation (ΔG^*). The data for the
ortho proton of the aromatic ring as well as the data for
the substituent on either piperidine or pyrrolidine is in-
cluded in these tables. Table I lists the N-(2,4-dinitro-
phenylsulfenyl)piperidines, while Table II lists the N-(2,4-

TABLE I. NMR Parameters and Free Energies of Activation for Conformational Change in 2,4-Dinitrophenyl-sulfenamides of Piperidines.
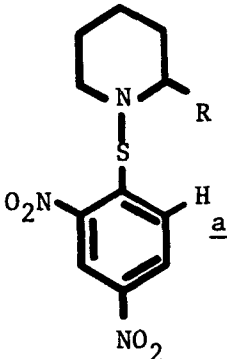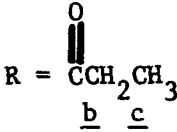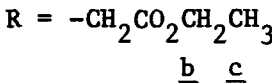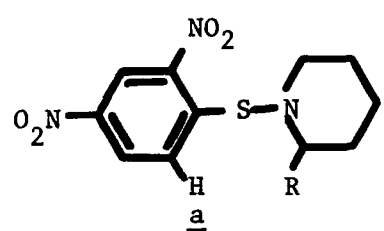
Compound	R	T_c °C ±2	Δν,Hz (±1)	k_c x 10	J,Hz	ΔG*, kcal/mol	Appendix C, Figure
19	-CH$_3$	*	--	--	--	--	
20	-CH$_2$CH$_2$CH$_3$	*	--	--	--	--	
25	-C$_6$H$_5$	*	--	--	--	--	
24	-CH$_2$C$_6$H$_{11}$	*	--	--	--	--	
	O						
37	-CCH$_2$CH$_3$	a) 43	48	11	9	16	1
	b c	b) 38	16	3.6	8	16	2
		c) **	15	--	8	--	2
	O						
42	CC$_6$H$_5$	a) 43	45	10	10	16	3
29	CO$_2$CH$_2$CH$_3$	a) 59	47	10	10	16	4
	b c	b) 52	18	4.0	7	17	5
		c) 52	22	4.9	7	16	5

O
‖
R = CCH$_2$CH$_3$
b c

R = CO$_2$CH$_2$CH$_3$
b c

R = -CH$_2$CO$_2$CH$_2$CH$_3$
b c

Table I. (Continued)

Compound	R	$T_c, °C$ ± 2	$\Delta\nu$, Hz ± 1	k_c x 10	J,Hz	ΔG^*, kcal/mol	Appendix C Figure
<u>38</u>	$CH_2CO_2CH_2CH_3$	a) **	--	--	--	--	6
	<u>b</u> <u>a</u>	b) 31	13	2.9	7	16	7
		c) 38	19	4.2	7	16	7

$R = CH_2CO_2CH_2CH_3$

<u>b</u> <u>c</u>

*Chemical shift equivalence was observed down to $-57°$..

**Although this group did exhibit a chemical shift nonequivalence, insufficient data was obtained to permit an accurate measurement of T_c.

TABLE II. NMR Parameters and Free Energies of Activation for Conformational Change in 2,4-Dinitrophenyl-sulfenamides of Pyrrolidines.

R = -CO₂C(CH₃)₃
b

R = CH₂CO₂CH₂CH₃
b c

Compound	R	T_c,°C ±2	$\Delta\nu$,Hz ±1	k_cx10	J,Hz	ΔG^* kcal/mol	Appendix C, Figure
26	CO_2-t-Bu	a) 72	83	18	10	17	8
	b	b) 38	13	2.9	--	16	9
39	$CH_2CO_2CH_2CH_3$	a) 52	65	14	9	16	10
	b c	b) *	3.5	--	7	--	11
		c) *	4	--	7	--	11
40		a) 72	60	13	8	17	12
		b) **	--	--	--	--	
		c) **	--	--	--	--	

Table II. (Continued)

	Compound	R	$T_c, °C$ ± 2	$\Delta\nu, Hz$ ± 1	$k_c \times 10$	J, Hz	ΔG^* kcal/mol	Appendix C, Figure
	30		a) ***	--	--	--	--	
			b) 66	35	7.8	7	17	13
			c) 66	35	7.8	7	17	14

Structure (left of table): a nitro-substituted benzene ring —
NO_2, O_2N—ring—S—N (pyrrolidine), with H and $(CO_2CH_2CH_3)_2$; labeled a, b, c.

*Although the group did exhibit chemical shift nonequivalence, insufficient data was obtained to permit an accurate measurement of T_c.

**Equivalent at $-35°$.

***Equivalent at $-57°$.

219

Table III. NMR Parameters and Free Energies of Activation for Conformational Change in o-Nitrophenyl-
sulfenamides and p-Nitrophenylsulfenamides.
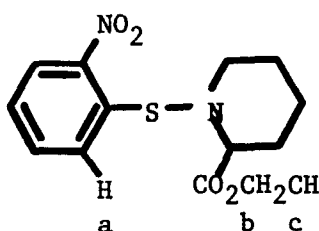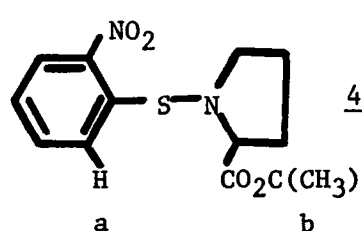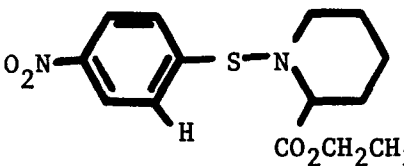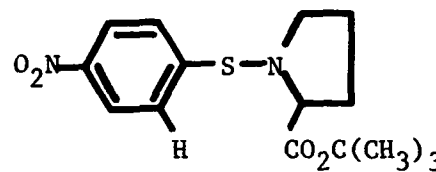
	Compound	T_c, °C ±2	Δν,Hz ±1	k_c x10	J,Hz	ΔG* kcal/mol	Appendix C Figure
	45	a) 5	42	9.3	8	14	15
		b) 6	23	5.1	7	14	16
		c) 6	21	4.7	7	14	16
	44	a) -3	68	15	8	13	17
		b) 1	15	3.3	--	14	18
	48	*					

Table III. (Continued)

Compound	T_c, ^oC	$\Delta\nu, H_z$	k_c	J,Hz	ΔG^*, kcal/mol	Appendix C, Figure
47	*					

---

*Equivalent at -57°.

dinitrophenylsulfenyl)pyrrolidines.   The o-nitrophenylsul-

fenamides and p-nitrophenylsulfenamides are listed together

in Table III.   The variable temperature spectra of all

compounds which exhibit chemical shift nonequivalence are

reproduced in Appendix C.   The figure numbers of these

spectra are listed in the last columns of Tables I, II, and

III.   The spectra which are reproduced include the coales-

cence temperature spectra and at least one spectra on either

side of the coalescence temperature.

It is readily apparent from the data in Table I that

the nature of the substituent on the piperidine ring is very

important in determing whether chemical shift nonequivalence

will be observed.   Chemical shift equivalence down to -57°

was observed in compounds 19, 20, and 24, which all contain

an alkyl substituent in the 2-position.   This equivalence

was observed for the ortho proton of the aromatic ring as

well as for the substituent on the piperidine ring.   An

aromatic ring in the 2-position (e.g., 25) also exhibited

equivalence down to -57°.   The remaining sulfenamides in

Table I (37, 42, 29, and 38) exhibited chemical shift non-

equivalence at room temperatures and warming was necessary

in order to observe the coalescence temperature.   It appears

that a carbonyl-containing substituent on the piperidine

ring is a necessary requirement for observation of nonequiv-

alence of the aromatic proton of the 2,4-dinitrophenyl group.

Although diastereotopic groups must, in principle,

have different chemical shifts, this difference is sometimes

too small to be observable.   We then speak of the groups as

being accidentally equivalent. This appears to be the reason for the apparent equivalence of the first four compounds of Table I. Steric effects can be ruled out by a comparison of compound 25 with 37. The phenyl group of 25 has a larger steric bulk than the propionyl group of 37 and compound 25 would therefore be expected to exhibit nonequivalence also. It seems highly unlikely that the barrier to rotation in these first four compounds is so low that rotation about the S-N bond is fast on the nmr time scale at -57°, because this would imply that the carbonyl group is affecting the S-N bond either by through-bond or through-space effects. Through-bond effects can be ruled out by a comparison of compounds 38 and 29. In 38 the carbonyl carbon is separated from the ring nitrogen by one carbon more than in compound 29, and yet the free energy of activation for 38 is almost identical with 29. If through-bond effects were responsible for the torsional barrier, 29 would show a considerably larger ΔG* value than 38.

Through-space effects have been proposed to explain invertomer ratios about the heterocyclic nitrogen of aziridines. Atkinson[31] has shown that aziridines such as 73 show a preference for the cis conformation and proposed a dipolar attraction between the ester carbonyl and the electrophilic carbons of the heterocyclic substituent as a possible explanation. It should be noted, however, that many workers have observed chemical shift nonequivalence in sulfenamides containing only simple alkyl and aryl groups.

The presence of a carbonyl group, therefore, is not a
necessary requirement for the existence of a rotational
barrier.

The best explanation, then, appears to be that the
carbonyl group increases the chemical shift differences of
the diastereotopic groups by creating sufficiently differ-
ent environments in the two diastereomeric conformations.
Most of the conformational work performed with sulfenamides
has used aliphatic and aromatic groups which do not contain
carbonyl groups, and this helps explain why chemical shift
nonequivalence has never been reported for the aromatic
proton of the 2,4-dinitrophenyl group. However, it is hard
to understand why the aliphatic and aromatic substituents of
the piperidine ring in compounds 19, 20, 24, and 25 do not
exhibit nonequivalence as do the substituents of the last
four compounds of Table I, because all of these groups are
being affected by the 2,4-dinitrophenyl ring.

73
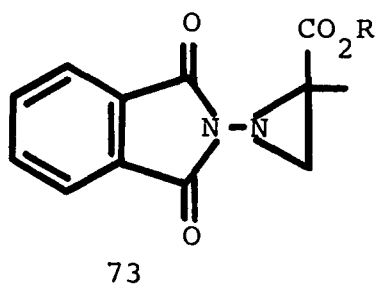
Chemical shift nonequivalence was also observed for
two monosubstituted pyrrolidine sulfenamides, t-butyl N-
(2,4-dinitrophenylsulfenyl)prolinate (26) and ethyl N-(2,4-
dinitrophenylsulfenyl)pyrrolidinylacetate (39), and for two

disubstituted pyrrolidine sulfenamides, N-(2,4-dinitrophenyl-sulfenyl)-cis-2,5-bis-ethoxycarbonylpyrrolidine (40) and N-(2,4-dinitrophenylsulfenyl)-2,2-bis-ethoxycarbonylpyrrolidine (30). The nmr data for these four compounds is presented in Table II. All of these sulfenamides contain a carbonyl-bearing substituent on the pyrrolidine ring.

Compounds 30 and 40 were prepared to determine the ground state conformation of the S-N bond. The suggestion[11] that partial S-N double bonding makes a contribution to the S-N torsional barrier requires that the bond strength be a function of the C-S-N-C dihedral angle in order for partial double bonding to result in a torsional barrier. Raban has suggested[11] that the p-d π overlap which gives rise to this partial double bonding is possible only in the torsional ground state when the C-S-N plane bisects the angle formed by the two other ligands at nitrogen (e.g., 74). Based on this model the ground state conformations of compounds 40 and 30 would be those represented in Figure 2.

74
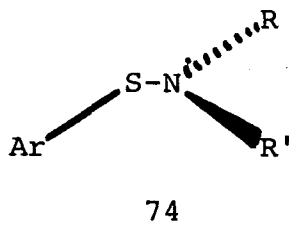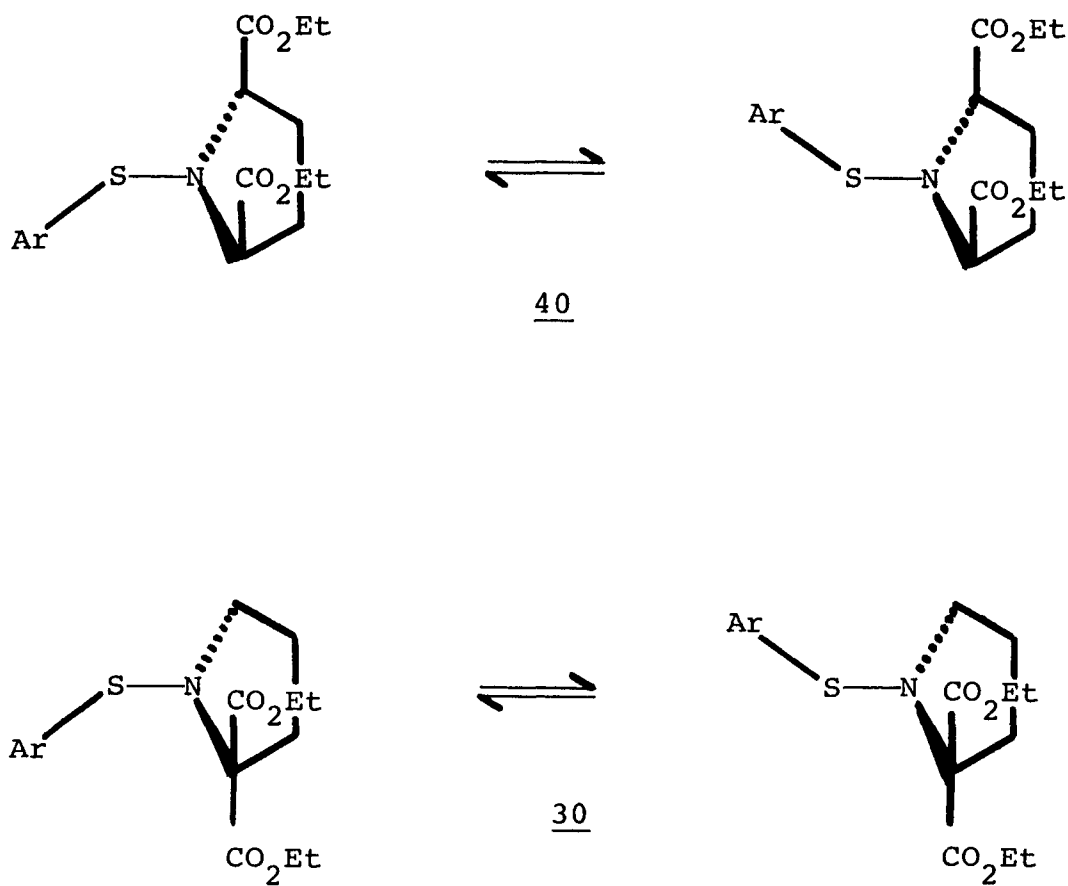
Figure 2. Ground State Conformations of N-(2,4-Dinitro-phenylsulfenyl)-cis-2,5-bis-ethoxycarbonyl-pyrrolidine (40) and N-(2,4-Dinitrophenylsulfenyl)-2,2-bis-ethoxycarbonylpyrrolidine (30).

In 30 the two conformations are of equal energy and there will be a 50-50 mixture of these two conformations. The environments of the 2,4-dinitrophenyl group are enantiomeric in the two conformers and we would expect to see only one signal for the ortho proton of the aromatic ring. However, the two ester groups are in diastereomeric environments in either conformer and thus should exhibit chemical shift nonequivalence. The experimental data of Table II bears out these expectations. The aromatic proton shows chemical shift equivalence down to -57°, while the methylene and methyl groups of the esters show nonequivalence at room temperatures. In compound 40 the two conformations are of unequal energy. Both the aromatic group and the ester groups are in diastereomeric environments in the two conformations and we would expect both of these groups to exhibit chemical shift nonequivalence. However, only the aromatic proton shows this nonequivalence, with the ester groups being equivalent at -35°. This does not disprove the ground state conformations proposed by Raban, for no pair of conformers which can be drawn for 40 will provide identical environments for the ethyl groups. The two ester groups must therefore be accidentally equivalent. These results must be viewed with caution for accidental equivalence may also be the reason that a single signal was observed for the aromatic proton of compound 30.

The above conclusions were made on the assumption that their is either rapid rotation about the aryl-S

bond or that the plane of the aromatic ring is perpendicular
to the plane of the nitrogen heterocycle or both. Unfor-
tunately, the data obtained in this study was insufficient
to determine the geometry of the aromatic ring relative to
the nitrogen heterocycle. It can be said, however, that
hindered rotation about the aryl-S bond and coplanarity be-
tween the aromatic ring and the nitrogen heterocycle are not
both occurring. If this were the case, compound 30 would be
expected to exhibit chemical shift nonequivalence of the
aromatic proton.

Table III lists the nmr data for two o-nitrophenyl-
sulfenamides, ethyl N-(o-nitrophenylsulfenyl)pipecolinate
(45) and t-butyl N-(o-nitrophenylsulfenyl)prolinate (44),
and two p-nitrophenylsulfenamides, ethyl N-(p-nitrophenyl-
sulfenyl)pipecolinate (48) and t-butyl N-(p-nitrophenyl-
sulfenyl)prolinate (47). It is readily apparent from the
data that 45 and 44 exhibited chemical shift nonequivalence
in both the aromatic ring and in the ester groups, while 48
and 47 were equivalent in all parts of the molecule at -57°.
It should also be noted that the free energy of activation
(ΔG*) for 45 and 44 is approximately 2 kcal/mol less than
the free energy of activation for the 2,4-dinitrophenylsul-
fenamides. This effect of electronegativity on the ΔG*
value and T_c was also noted by Raban[32] for N-1-phenylethyl-
N-(p-toluenesulfonyl)-2,4-dinitrophenylsulfenamide (75) and
N-1-phenylethyl-N-(p-toluenesulfonyl)-o-nitrophenylsulfen-
amide (76). The free energy of activation of 75 was found

to be larger than that of 76 by 1.3 kcal/mol. Presumably this is because of increased partial double bonding of the S-N bond in 75.
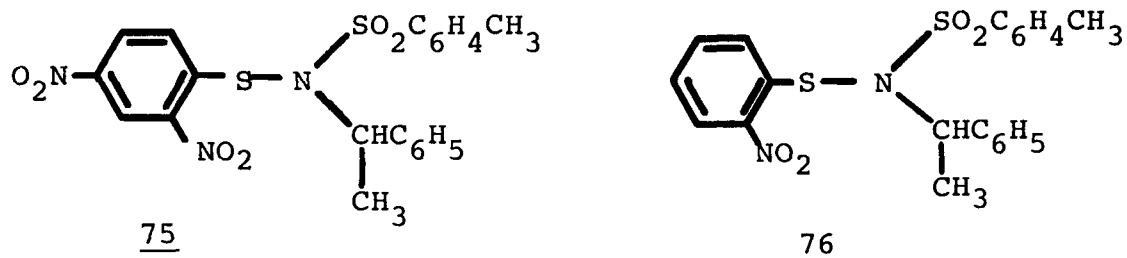
Harder to explain is the chemical shift equivalence of compounds 47 and 48. This could be due to the fact that the groups are accidentally equivalent or it might reflect a lower torsional barrier. If the latter were the case, it would probably result from a combination of less steric hindrance in the transition state and a decrease in the amount of partial double bonding because of the smaller inductive effect of the nitro group in the para position.

SECTION IV

EXPERIMENTAL

General

Melting Points.   Melting points were determined
with a Thomas Hoover Capillary Melting Point Apparatus and
are uncorrected.

Boiling Points.   Boiling points were measured at
the pressure indicated and are uncorrected.

Elemental analyses.   Elemental analyses were deter-
mined at the University of New Hampshire using an F & M
Model 185 carbon, hydrogen, and nitrogen analyzer.

Infrared Spectra.   Infrared spectra were recorded
on a Perkin-Elmer Model 137 Infracord prism spectrometer
and were calibrated with polystyrene at 1944 cm^{-1}. Solid
samples were recorded as KBr discs while liquid samples were
recorded as neat films between sodium chloride plates.

Nuclear Magnetic Resonance Spectra.   Nuclear magnetic
resonance spectra were recorded on a Jeol MH-100 Spectrometer
and are reported in parts per million (δ) from TMS. Samples
recorded in $CDCl_3$ contained 1% TMS as an internal standard,
while samples recorded in TFA or D_6-DMSO are calibrated with
TMS as an external standard. For all new compounds reported,
the nmr spectra are reproduced in Appendix B and are given
in the experimental section as follows: nmr (solvent); in
ppm (multiplicity, number of hydrogens, coupling constant

in Hz). The description of multiplicity is s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Variable temperature spectra were obtained using a JES-VT-3B variable-temperature controller. Low temperature spectra were run as 10% solution in $CDCl_3$, while high temperature spectra wer run as 10% solutions in 1,1,2,2-tetrachloroethane. Temperatures were checked before and after each spectrum by measuring the chemical shift difference in the absorption peaks of methanol at low temperature, and ethylene glycol at high temperature. Temperature-dependent spectra are reproducibly reversible in all cases.

Materials and Methods

A. Reagents: Piperidine, pyrrolidine, N-methyl-piperidine, 2-n-propylpiperidine, ethyl picolinate, ethyl 2-pyridylacetate, benzylamine, benzyl chloroformate, o-nitrophenylsulfenyl chloride, 2,4-dinitrophenylsulfenyl chloride, 4,4'-dinitrodiphenyldisulfide, citric acid, tri-fluoroacetic acid, and acrolein were purchased from Aldrich Chemical Company. Pyrrole, 2-methylpiperidine, ethyl glycinate hydrochloride, 2,2-dimethoxypropane, and sodium nitrite were purchased from Eastman Kodak Company. Tri-ethylamine, 2-benzoylpyridine, chromium trioxide, and silica gel were purchased from J. T. Baker Company. Acetic anhy-dride was purchased from Fisher Scientific Company. Iso-butylene and anhydrous hydrogen chloride were purchased from Scientific Gas Products. Platinum oxide, 5% rhodium

on carbon, and 10% palladium on carbon were purchased from Englehard Industries. Sodium hydride was purchased from Ventron Corp. 2-Cyclohexylmethylpiperidine and 2-phenylpiperidine were prepared by James J. Kaminski[33] while 2-propionylpyridine was prepared by Michael J. Kane[34], both of this laboratory.

B. Solvents: The following solvents were used without further purification: methylene chloride, chloroform, benzene, hexane, cyclohexane, ether, ethyl acetate, dioxane, methanol, ethanol, absolute ethanol, and glacial acetic acid. Dimethoxyethane was dried over sodium hydride immediately before use. The sodium hydride was washed with petroleum ether before use.

C. Products: Yields of the products are reported on the purified material unless stated otherwise. Where an analytical sample was obtained, the ir and nmr spectra were recorded on the analytical sample.

D. Experimental Methods: Reagents were weighed to the number of significant figures shown and this number was converted to moles (mol). Following extraction, the normal procedure was to dry the extract over $MgSO_4$ for at least 0.5 hr, remove the drying agent by filtration and wash the residual drying agent with solvent. The filtrate was then concentrated under reduced pressure on a rotatory evaporator.

E. Thin Layer Chromatography: TLC was performed on silica gel-coated microscope slides. The chromatograms were detected using a UV source or an iodine chamber.

Preparation of 2,4-Dinitrophenylsulfenamides

General. Three methods were used for the preparation of the sulfenamides. These methods differed mainly in the base used in the reaction. Where the hydrochloride of the piperidine or pyrrolidine was used, an additional molar equivalent of base was added. All of the reactions used the same solvent (methylene chloride) and the same workup procedure.

Method A. A 0.05-0.2 molar solution of 2,4-dinitrophenylsulfenyl chloride (12) in 50 ml of methylene chloride was treated with two molar equivalents of the piperidine or pyrrolidine and was allowed to react for 48 hr at 20°. The precipitate which formed was removed by filtration, and the filtrate was washed with 50 ml of 5% acetic acid, 50 ml of water, and two 50 ml portions of 5% $NaHCO_3$. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give a viscous oil which slowly solidified on standing. Recrystallization from ethanol gave an analytical sample.

Method B. A 0.05-0.2 molar solution of the piperidine or pyrrolidine and a molar equivalent of triethylamine in 50 ml of methylene chloride was treated with a molar equivalent of 2,4-dinitrophenylsulfenyl chloride (12) and was allowed to react for 24 hr at 20°. The precipitate which formed was removed by filtration and the filtrate was washed with 50 ml of 5% acetic acid, 50 ml of water, and two 50 ml portions of 5% $NaHCO_3$. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure. If the oil crystallized,

the solid was purified by recrystallization from ethanol. Oils which failed to crystallize were purified by molecular distillation[43] at 100° at 0.1 mm, and by preparative layer chromatography (PLC).

Method C. Method C is identical to Method B except that N-methylpiperidine was used as the base instead of triethylamine. Crystalline products were purified by recrystallization from ethanol, while oils were either used without further purification or purified by molecular distillation[43] at 100° and 0.1 mm.

The following sulfenamides were prepared by Method A.

N-(2,4-Dinitrophenylsulfenyl)pyrrolidine (17). Reaction of 1.18 g of 2,4-dinitrophenylsulfenyl chloride (12) with 1.42 g of pyrrolidine (13) gave 0.97 g (72%) of 17 as red needles, mp 146-147.5°: ir (Appendix A, Figure 1, KBr); nmr (Appendix B, Figure 1, $CDCl_3$) $\delta9.03$ (d, 1, J = 2 Hz), 8.48 (dd, 1, J_{BC} = 9 Hz, J_{AB} = 2 Hz, by 1st order analysis of ABC system), 8.10 (d, 1, J_{BC} = 9 Hz), 3.52-3.02 (m, 4), 2.16-1.92 (m, 4).

Anal. Calcd for $C_{10}H_{11}N_3O_4S_1$: C, 44.60; H, 4.12; N, 15.60. Found: C, 44.97; H, 4.49; N, 15.39.

N-(2,4-Dinitrophenylsulfenyl)piperidine (18). Reaction of 2.36 g of 2,4-dinitrophenylsulfenyl chloride (12) with 1.70 g of piperidine (14) gave 2.20 g (78%) of 18 as flocculent orange needles, mp 152-153.5° (lit.[14] mp 151-152.5°).

N-(2,4-Dinitrophenylsulfenyl)-2-methylpiperidine (19).

Reaction of 2.36 g of 2,4-dinitrophenylsulfenyl chloride (12) with 1.98 g of 2-methylpiperidine (15) gave 1.99 g (67%) of 19 as orange needles, mp 76.5-79°; ir (Appendix A, Figure 2, KBr); nmr (Apppendix B, Figure 2, $CDCl_3$) δ9.12 (s, 1), 8.54-8.20 (m, 2), 3.48-2.64 (m, 3), 2.08-1.28 (m, 6), 1.20 (d, 3, J = 6 Hz).

Anal. Calcd for $C_{12}H_{15}N_3O_4S_1$: C, 48.47; H, 5.08; N, 14.13. Found: C, 48.68; H, 5.08; N, 14.35.

N-(2,4-Dinitrophenylsulfenyl)-2-n-propylpiperidine (20). Reaction of 2.36 g of 2,4-dinitrophenylsulfenyl chloride (12) with 2.54 g of 2-n-propylpiperidine (16) gave 2.50 g (77%) of 20 as orange crystals, mp 95-97°; ir (Appendix A, Figure 3, KBr); nmr (Appendix B, Figure 3, $CDCl_3$) δ9.12 (s, 1), 8.54-8.28 (m, 2), 3.40-2.60 (m, 3), 2.16-1.04 (m, 10), 0.87 (t, 3, J = 6 Hz).

Anal. Calcd for $C_{14}H_{19}N_3O_4S_1$: C, 51.68; H, 5.89; N, 12.91. Found: C, 51.79; H, 5.87; N, 12.87.

The following sulfenamides were prepared by Method B.

N-(2,4-Dinitrophenylsulfenyl)-2-cyclohexylmethyl-piperidine (24). Reaction of 1.75 g of 2-cyclohexylmethyl-piperidine (21) with 2.36 g of 2,4-dinitrophenylsulfenyl chloride (12) gave 1.65 g (44.2%) of 24 as yellow needles, mp 90.5-92°; ir (Appendix A, Figure 4, KBr); nmr (Appendix B, Figure 4, $CDCl_3$) δ9.02 (s, 1), 8.44-8.08 (m, 2), 3.40-2.72 (m, 3), 2.22-0.52 (m, 19).

Anal. Calcd for $C_{18}H_{25}N_3O_4S_1$: C, 56.97; H, 6.64;

N, 11.07. Found: C, 56.84; H, 6.49; N, 11.00.

N-(2,4-Dinitrophenylsulfenyl)-2-phenylpiperidine (25).

Reaction of 1.61 g of 2-phenylpiperidine (22) with 2.36 g of

2,4-dinitrophenylsulfenyl chloride (12) gave 1.33 g (37%) of

25 as yellow crystals, mp 125-126°; ir (Appendix A, Figure 5,

KBr); nmr (Appendix B, Figure 5, $CDCl_3$) δ8.88 (s, 1), 8.52-

8.20 (m, 2), 7.30-6.92 (m, 5), 4.02-3.70 (m, 1), 3.40-2.90

(m, 2), 2.16-1.30 (m, 6).

Anal. Calcd for $C_{17}H_{17}N_3O_4S_1$: C, 56.81; H, 4.77;

N, 11.69. Found: C, 56.79; H, 4.64; N, 11.61.

t-Butyl N-(2-Dinitrophenylsulfenyl)prolinate (26).

Reaction of 1.70 g of t-butyl prolinate (23) with 2.36 g of

2,4-dinitrophenylsulfenyl chloride (12) gave 2.10 g (57%) of

26 as yellow needles, mp 128-129.5°; ir (Appendix A, Figure

6, KBr); nmr (Appendix B, Figure 6, $CDCl_3$) δ9.24 (s, 1),

8.86 (d, 0.5, J_{BC} = 9 Hz), 8.56 (dd, 1, J_{BC} = 9 Hz, J_{AB} = 2

Hz, by 1st order analysis of ABC system), 8.04 (d, 0.5, J_{BC}

= 9 Hz), 4.10-3.05 (m, 3), 2.50-1.90 (m, 4), 1.52 (d, 9, J

= 13 Hz).

Anal. Calcd for $C_{15}H_{19}N_3O_6S_1$: C, 48.77; H, 5.18;

N, 11.38. Found: C, 48.61; H, 5.34; N, 11.33.

Ethyl N-(2,4-Dinitrophenylsulfenyl)pipecolinate

(29). Reaction of 1.57 g of ethyl pipecolinate hydrochloride

(27) with 2.36 g of 2,4-dinitrophenylsulfenyl chloride (12)

gave 29 as a viscous yellow oil, which failed to crystallize:

ir (Appendix A, Figure 7, neat); nmr (Appendix B, Figure 7,

CDCl$_3$) δ8.92 (d, 1, J_{AB} = 2 Hz), 8.78-8.08 (m, 2), 4.40-3.90 (m, 2), 3.90-2.72 (m, 3), 2.50-1.52 (m, 6), 1.52-1.00 (m, 3).

Molecular distillation gave an analytical sample of 29 as a viscous yellow oil.

Anal. Calcd for C$_{17}$H$_{17}$N$_3$O$_6$S$_1$: C, 47.32; H, 4.82; N, 11.82. Found: C, 47.53; H, 4.85; N, 11.86.

N-(2,4-Dinitrophenylsulfenyl)-2,2-bis-ethoxycarbonyl-pyrrolidine (30). Reaction of 0.56 g of 2,2-bis-ethoxy-carbonylpyrrolidine (28) with 0.62 g of 2,4-dinitrophenyl-sulfenyl chloride (12) gave 30 as a viscous red oil. TLC indicated that the oil consisted of two major components. Separation of these compounds was accomplished by PLC on a 20 x 80 cm plate of PF-254 silica gel using benzene as the developer. The material was removed from the silica gel by extraction with chloroform in a Soxlet extractor. Concentration of the chloroform gave 0.13 g (12%) of 30 as a yellow oil: ir(Appendix A, Figure 8, neat), nmr (Appendix B, Figure 8, CDCl$_3$) δ8.94 (d, 1, J_{AB} = 2 Hz), 8.30 (dd, 1, J_{BC} = 9 Hz, J_{AB} = 2 Hz, by 1st order analysis of ABC system), 7.98 (d, 1, J_{BC} = 9 Hz), 4.44-3.16 (m, 6), 2.92-1.80 (m, 4), 1.34 (t, 3, J = 7 Hz), 1.00 (t, 3, J = 7 Hz).

Molecular distillation gave an analytical sample of 30 as a viscous yellow oil.

Anal. Calcd for C$_{16}$H$_{19}$N$_3$O$_8$S$_1$: C, 46.48; H, 4.63; N, 10.16. Found: C, 46.75; H, 4.91; N, 10.05.

The following sulfenamides were prepared by Method C.

N-(2,4-Dinitrophenylsulfenyl)-2-propionylpiperidine

(37). Reaction of 0.81 g of 2-propionylpiperidine (33) and 1.45 g of 2,4-dinitrophenylsulfenyl chloride (12) gave 1.25 g (64%) of 37 as yellow needles, mp 142.5-143°; ir (Appendix A, Figure 9, KBr); nmr (Appendix B, Figure 9, $CDCl_3$) δ8.90 (d, 1, J = 2 Hz), 8.65-8.00 (m, 2), 3.90-2.60 (m, 3), 2.60-1.40 (m, 8), 1.20-0.80 (m, 3).

Anal. Calcd for $C_{14}H_{17}N_3O_5S_1$: C, 49.55; H, 5.05; N, 12.38. Found: C, 49.55; H, 5.27; N, 12.30.

N-(2,4-Dinitrophe⬛⬛⬛⬛yl)-2-benzoylpiperidine

(42). Reaction of ⬛⬛⬛⬛⬛piperidine (41) with 0.59 g of 2,4-dini⬛⬛⬛⬛⬛de (12) gave 0.28 g (28.8%) of 42 a⬛⬛⬛⬛57.5°; ir (Appendix A, Figu⬛⬛⬛⬛x B, Figure 10, $CDCl_3$) δ8.88 (s, 1⬛⬛⬛4-7.20 (m, 5), 4.90-4.56 (m, 1), 4.⬛⬛⬛-1.10 (m, 6).

Anal. Calcd for ⬛⬛⬛⬛: C, 55.80; H, 4.42; N, 10.85. Found: C, 56.04; H, 4.65; N, 10.86.

Ethyl N-(2,4-Dinitrophenylsulfenyl)-2-piperidyl-acetate (38).

Reaction of 0.86 g of ethyl 2-piperidyl-acetate (34) with 1.18 g of 2,4-dinitrophenylsulfenyl chloride (12) gave 38 as a viscous oil. Molecular distillation of this oil gave 0.98 g (53%) of 38 as an orange oil, ir (Appendix A, Figure 11, neat); nmr (Appendix B, Figure 11, $CDCl_3$) δ8.94 (s, 1), 8.48-8.00 (m, 2), 4.24-1.34 (m, 11), 1.16 (t, 3, J = 8 Hz).

Anal. Calcd for $C_{15}H_{19}N_3O_6S_1$: C, 48.77; H, 5.18; N, 11.38. Found: C, 48.92; H, 5.02; N, 11.37.

N-(2,4-Dinitrophenylsulfenyl)-2-propionylpiperidine

(37). Reaction of 0.81 g of 2-propionylpiperidine (33) and 1.45 g of 2,4-dinitrophenylsulfenyl chloride (12) gave 1.25 g (64%) of 37 as yellow needles, mp 142.5-143°; ir (Appendix A, Figure 9, KBr); nmr (Appendix B, Figure 9, $CDCl_3$) $\delta 8.90$ (d, 1, J = 2 Hz), 8.65-8.00 (m, 2), 3.90-2.60 (m, 3), 2.60-1.40 (m, 8), 1.20-0.80 (m, 3).

Anal. Calcd for $C_{14}H_{17}N_3O_5S_1$: C, 49.55; H, 5.05; N, 12.38. Found: C, 49.55; H, 5.27; N, 12.30.

N-(2,4-Dinitrophenylsulfenyl)-2-benzoylpiperidine

(42). Reaction of 0.47 g of 2-benzoylpiperidine (41) with 0.59 g of 2,4-dinitrophenylsulfenyl chloride (12) gave 0.28 g (28.8%) of 42 as yellow needles, mp 157-157.5°; ir (Appendix A, Figure 10, KBr); nmr (Appendix B, Figure 10, $CDCl_3$) $\delta 8.88$ (s, 1), 8.80-8.04 (m, 2), 7.94-7.20 (m, 5), 4.90-4.56 (m, 1), 4.04-2.84 (m, 2), 2.50-1.10 (m, 6).

Anal. Calcd for $C_{18}H_{17}N_3O_5S_1$: C, 55.80; H, 4.42; N, 10.85. Found: C, 56.04; H, 4.65; N, 10.86.

Ethyl N-(2,4-Dinitrophenylsulfenyl)-2-piperidyl-acetate (38).

Reaction of 0.86 g of ethyl 2-piperidyl-acetate (34) with 1.18 g of 2,4-dinitrophenylsulfenyl chloride (12) gave 38 as a viscous oil. Molecular distillation of this oil gave 0.98 g (53%) of 38 as an orange oil, ir (Appendix A, Figure 11, neat); nmr (Appendix B, Figure 11, $CDCl_3$) $\delta 8.94$ (s, 1), 8.48-8.00 (m, 2), 4.24-1.34 (m, 11), 1.16 (t, 3, J = 8 Hz).

Anal. Calcd for $C_{15}H_{19}N_3O_6S_1$: C, 48.77; H, 5.18; N, 11.38. Found: C, 48.92; H, 5.02; N, 11.37.

Ethyl N-(2,4-Dinitrophenylsulfenyl)-2-pyrrolidinyl-
acetate (39).   Reaction of 0.80 g of ethyl 2-pyrrolidinyl-
acetate (35) with 1.18 g of 2,4-dinitrophenylsulfenyl
chloride (12) gave 39 as a viscous oil. Molecular distilla-
tion gave 1.02 g (57.5%) of 39 as an orange oil: ir (Appendix
A, Figure 12, neat); nmr (Appendix B, Figure 12, $CDCl_3$) δ8.91
(d, 1, J_{AB} = 2 Hz), 8.40-7.40 (m, 2), 4.18-1.35 (m, 11), 1.20
(t, 3, J = 7 Hz).

Anal. Calcd for $C_{14}H_{17}N_3O_6S_1$:   C, 47.32; H, 4.82;
N, 11.82. Found: C, 47.48; H, 4.90; N, 11.97.

N-(2,4-Dinitrophenylsulfenyl)-cis-2,5-bis-ethoxy-
carbonylpyrrolidine (40).   Reaction of 2.15 g of cis-2,5-
bis-ethoxycarbonylpyrrolidine (36) with 2.36 g of 2,4-dinitro-
phenylsulfenyl chloride (12) gave 3.40 g (82%) of 40 as a
viscous oil: ir (Appendix A, Figure 13, neat); nmr (Appendix
B, Figure 13, $CDCl_3$) δ9.18-9.08 (m, 2), 8.52-8.40 (m, 1),
4.40-3.80 (m, 6), 2.80-1.84 (m, 4), 1.40-0.96 (m, 6).

Anal. Calcd for $C_{16}H_{19}N_3O_8S_1$:   C, 46.48; H, 4.63;
N, 10.16. Found: C, 46.41; H, 4.62; N, 10.09.
Preparation of o-Nitrophenylsulfenamides and p-Nitrophenyl-
sulfenamides

General. A 0.1 molar solution of the piperidine or
pyrrolidine and a molar equivalent of triethylamine (where the
hydrochloride of the piperidine or pyrrolidine was used an
additional molar equivalent of base was added) in 50 ml of
methylene chloride was treated with a molar equivalent of

either o-nitrophenylsulfenyl chloride (43) or p-nitrophenyl-sulfenyl chloride (46)[42] and was allowed to react for 72 hr at 20°. The solution was washed with 50 ml of 5% acetic acid, 50 ml of water, and two 50 ml portions of 5% $NaHCO_3$. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the crude products is described individually below.

t-Butyl N-(o-Nitrophenylsulfenyl)prolinate (44).
Reaction of 0.85 g of t-butyl prolinate (23) with 0.95 g of o-nitrophenylsulfenyl chloride (43) gave 1.05 g (65%) of 44 as a viscous orange oil. Molecular distillation at 100° and 0.1 mm gave an analytical sample of 44 as a viscous orange oil: ir (Appendix A, Figure 14, neat); nmr (Appendix B, Figure 14, $CDCl_3$) $\delta 8.14$ (d, 1, J = 8 Hz), 8.05-7.65 (m, 1), 7.52 (t, 1, J = 8 Hz), 7.13 (t, 1, J = 8 Hz), 3.92-3.00 (m, 3), 2.50-1.80 (m, 4), 1.44 (s, 9).

Anal. Calcd for $C_{15}H_{20}N_2O_4S_1$: C, 55.54; H, 6.21; N, 8.64. Found: C, 55.66; H, 6.08; N, 8.56.

Ethyl N-(o-Nitrophenylsulfenyl)pipecolinate (45).
Reaction of 0.78 g of ethyl pipecolinate hydrochloride (27) with 0.95 g of o-nitrophenylsulfenyl chloride (43) gave a yellow solid. Recrystallization of the solid from hexane followed by sublimation gave 0.36 g (23.3%) of 45 as a yellow solid, mp 77-79°; ir (Appendix A, Figure 15, KBr); nmr (Appendix B, Figure 15, $CDCl_3$) $\delta 8.80-8.20$ (m, 2), 7.92 (t, 1, J = 8 Hz), 7.68-7.40 (m, 1), 4.60-4.10 (m, 2), 4.10-2.88 (m, 3), 2.50-0.90 (m, 9).

Anal. Calcd for $C_{14}H_{18}N_2O_4S_1$: C, 54.18; H, 5.85; N, 9.03. Found: C, 54.04; H, 5.64; N, 9.01.

t-Butyl N-(p-Nitrophenylsulfenyl)prolinate (47).

Reaction of 0.85 g of t-butyl prolinate (23) with 0.95 g of p-nitrophenylsulfenyl chloride (46) gave 47 as a viscous orange oil. This mixture was separated into its components by PLC on a 25 x 80 cm plate of PF-254 silica gel. The plate was developed twice using cyclohexane-benzene (50:50) and once using cyclohexane-benzene (25:75) as the solvent system. The material was removed from the silica gel by extraction with chloroform in a Soxlet extractor. Concentration of the chloroform solution gave 47 as a viscous brown oil: ir (Appendix A, Figure 16, neat); nmr (Appendix B, Figure 16, CDCl$_3$) δ8.14 (d, 2, $J_{AX + AX'}$ = 8 Hz, by 1st order analysis of AA'XX' system), 7.38 (d, 2, $J_{A'X' + A'X}$ = 8 Hz, by 1st order analysis of AA'XX' system), 3.92-3.80 (m, 1), 3.60-3.14 (m, 2), 2.40-1.78 (m, 4), 1.46 (s, 9).

Anal. Calcd for $C_{15}H_{20}N_2O_4S_1$: C, 55.54; H, 6.21; N, 8.64. Found: C, 55.47; H, 6.22; N, 8.46.

Ethyl N-(p-Nitrophenylsulfenyl)pipecolinate (48).

Reaction of 0.78 g of ethyl pipecolinate hydrochloride (27) with 0.95 g of p-nitrophenylsulfenyl chloride (46) gave 48 as an orange oil. This oil was separated into its components by PLC on a 25 x 80 cm plate of PF-254 silica gel. The plate was developed twice in cyclohexane-benzene (50:50) and once in cyclohexane-benzene (25:75). The material was removed from the silica gel by extraction with chloroform in a

Soxlet extractor. Concentration of the chloroform solution gave 48 as a yellow solid. Repeated sublimation failed to give an analytical sample, nmr (Appendix B, Figure 17, $CDCl_3$) $\delta 8.40$ (d, 2, $\underline{J}_{AX + AX'}$ = 8 Hz, by 1st order analysis of AA'XX' system), 7.64 (d, 2, $\underline{J}_{A'X' + A'X}$ = 8 Hz, by 1st order analysis of AA'XX' system), 4.34 (q, 2, $\underline{J}$ = 7 Hz), 3.98-2.86 (m, 3), 2.34-1.02 (m, 9).

Preparation of Piperidines and Pyrrolidines

Preparation of $\underline{t}$-Butyl Prolinate (23). A solution of 35.0 g (0.14 mol) of N-benzyloxycarbonyl-L-proline (49) (see experimental section of Part I) and 1.5 ml of concentrated H_2SO_4 in 300 ml of methylene chloride was mixed with 200 ml of liquid isobutylene and sealed in a hydrogenation flask for 100 hr at 20°. The solution was poured with vigorous stirring into 350 ml of 5% $NaHCO_3$. The layers were separated, and the organic solution was washed with two 150 ml portions of water, dried ($MgSO_4$), and concentrated under reduced pressure to give N-benzyloxycarbonyl-L-proline $\underline{t}$-butyl ester (50) as a tan solid.

A solution of crude 50 in 250 ml of methanol was hydrogenated over 2.00 g of 10% palladium on carbon for 7 hr at 20° and 60 psi. The catalyst was removed by filtration through Celite and washed with methanol. The filtrate was concentrated under reduced pressure to give a dark oil. Distillation of the oil afforded 12.06 g (50%) of 23, bp 70-72.5° at 3.5 mm (lit[19] bp 57° at 1.5 mm).

Preparation of Ethyl Pipecolinate Hydrochloride (27).

A stream of dry hydrogen chloride gas was bubbled into a solution of 7.55 g (0.05 mol) of ethyl pipecolinate (51) in 200 ml of ether, causing the formation of a large amount of precipitate. This precipitate was removed by filtration and washed thoroughly with ether. A solution of this salt in 200 ml of absolute ethanol was hydrogenated over 0.2 g of platinum oxide at 60 psi and 20°. The stoichiometric amount of hydrogen was consumed after 8 hr. The catalyst was removed by filtration through Celite and washed with ethanol. Concentration of the filtrate under reduced pressure gave a colorless solid, which was recrystallized from ether-ethanol to give 8.43 g (87%) of 27 as colorless crystals, mp 215-216° (lit.[35] mp 202-203°).

Preparation of Diethyl N-Benzyloxycarbonylamino-malonate (58).

A suspension of 4.22 g (0.02 mol) of diethyl aminomalonate hydrochloride (77) (see experimental section, Part I) in 100 ml of anhydrous methylene chloride was cooled to 0° and treated with 3.5 ml (0.025 mol) of triethylamine followed immediately by 3.50 g (0.02 mol) of benzyl chloroformate. After stirring for 3 hr the solution was washed with 100 ml of water, two 75 ml portions of 1N HCl, and two 75 ml portions of 5% $NaHCO_3$. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give a mushy solid. This material was washed with benzene and the solid was removed by filtration to give 1.07 g of material which was shown by ir and nmr spectra to be 3,6-bis-ethoxy-

carbonyl-2,5-piperazinedione (78): ir (KBr) 3350 (NH), 1745 (ester C=O), 1640 cm^{-1} (amide C=O); nmr (TFA) δ6.80-4.60 (m, 2), 3.88 (q, 4, $\underline{J}$ = 7 Hz), 0.84 (t, 6, $\underline{J}$ = 7 Hz).

The filtrate was concentrated under reduced pressure to give a yellow oil. Distillation gave 2.36 g (61%) of 58 as a slightly yellow oil, bp 155-158° at 0.1 mm (lit.[23] bp 187-190° at 0.45 mm).

Preparation of 2,2-bis-ethoxycarbonylpyrrolidine (28). A solution of 2.36 g (0.0076 mol) of diethyl N-benzyloxycarbonylaminomalonate (58) and 0.6 ml of acrolein in 75 ml of ethanol was treated with a catalytic amount of sodium ethoxide and allowed to react for 1.5 hr at 20°. The solution was acidified with 1.0 ml of glacial acetic acid and hydrogenated over 0.25 g of 10% palladium on carbon for 18 hr at 60 psi and 20°. The catalyst was removed by filtration through Celite and washed with ethanol. The filtrate was concentrated under reduced pressure and the residue was dissolved in 50 ml of ether. The ethereal solution was extracted with two 20 ml portions of 1N HCl. The combined aqueous extracts were made basic with NaHCO$_3$ and extracted with three 25 ml portions of ether. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give 0.56 g (34%) of 28 as a yellow oil. This material was not purified further but was converted directly to the sulfenamide.

Preparation of 1-(2-Piperidyl)propanol (79). A solution of 13.5 g (0.1 mol) of 2-propionylpiperidine (53) in 200 ml of methanol and 10 ml of concentrated HCl was

hydrogenated over 0.4 g of platinum oxide for 44 hr at 20°.
The catalyst was removed by filtration through Celite and
washed with methanol. The filtrate was concentrated under
reduced pressure and the residue was dissolved in 200 ml of
methylene chloride. This solution was dried ($MgSO_4$) and
concentrated under reduced pressure to give an oil. Tri-
turation of the oil with ether caused crystallization and
the solid was dissolved in a mixture of 20 ml of 6N NaOH
and 100 ml of chloroform. The layers were separated and the
aqueous layer was extracted with two 100 ml portions of
chloroform. The combined organic extracts were dried ($MgSO_4$)
and concentrated under reduced pressure to give a gummy
solid. Recrystallization from hexane gave 4.50 g of a tan
solid, mp 98-99.5°. An additional 3.07 g of solid, mp 70-
75°, was obtained from the mother liquor. Repeated recrys-
tallization and sublimation failed to increase the melting
point significantly, but this material was shown to be
identical to the higher melting solid by ir and nmr spectra.
The difference in melting points was probably the result of
the formation of diastereomers during reduction. The com-
bined weights represent a 52.5% yield of 79. An analytical
sample was prepared by recrystallization of the higher
melting solid from hexane to give 79 as a colorless solid
mp 98-99°; ir (Appendix A, Figure 17, KBr) 3300 (free NH
and OH), 3100 cm^{-1} (bonded NH and OH); nmr (Appendix B,
Figure 18, $CDCl_3$) $\delta3.50-3.28$ (m, 1), 3.20-2.96 (m, 1),
2.80-2.25 (m, 4), 1.92-1.08 (m, 8), 0.96 (t, 3, $\underline{J}$ = 7 Hz).

Anal. Calcd for $C_8H_{17}N_1O_1$: C, 67.09; H, 11.96;
N, 9.78. Found: C, 66.83; H, 12.02; N, 9.69.

Preparation of 2-Propionylpiperidine (33). A
solution of 1.32 g of chromium trioxide in 9 ml of 85% acetic
acid was added carefully and with stirring to a solution of
2.40 g (0.0168 mol) of 1-(2-piperidyl)propanol (79) in 20 ml
of glacial acetic acid. The solution was allowed to stand
for 18 hr at 20° and concentrated under reduced pressure.
The residue was dissolved in 50 ml of concentrated ammonium
hydroxide and extracted into four 40 ml portions of ether.
The combined organic extracts were dried ($MgSO_4$) and concen-
trated under reduced pressure to give a yellow oil. Distil-
lation gave 0.87 g (36.8%) of 33 as a colorless liquid, bp
37-42° at 0.3 mm; nmr (Appendix B, Figure 19, $CDCl_3$). This
oil proved to be unstable and correct elemental analyses
could not be obtained.

Preparation of 2-Benzoylpiperidine (41). A stream
of dry hydrogen chloride was bubbled into a solution of 18.33
g (0.1 mol) of 2-benzoylpyridine (54) in 200 ml of ether,
causing the formation of a gummy precipitate. The ether
was removed by decantation and the salt was washed with
three 100 ml portions of ether. The salt was dissolved in
200 ml of methanol and hydrogenated over 0.6 g of platinum
oxide for 72 hr at 60 psi and 20°. The catalyst was removed
by filtration through Celite and washed with methanol. The
filtrate was concentrated under reduced pressure to give
the alcohol 80 as a colorless oil.

A solution of this oil in 80 ml of glacial acetic

acid was carefully treated with a solution of 7.5 g of

chromium trioxide in 45 ml of 85% acetic acid and allowed

to stand for 18 hr at 20°. The solution was concentrated

under reduced pressure and the residue was dissolved in 200

ml of concentrated ammonium hydroxide. The aqueous solution

was extracted with three 100 ml portions of ethyl acetate.

The combined organic extracts were dried ($MgSO_4$) and con-

centrated under reduced pressure to give a mushy solid,

which was recrystallized from ether to give 7.30 g (38.5%)

of 41 as a tan solid, mp ___ second recrystalli-

zation from ether gave ___ als, mp 93-94.5°

(lit.[21] mp 90-92°); ___ , KBr) 3550

cm^{-1} (NH); nmr (Appe ___ 7.88-7.02

(m, 5), 4.50-4.04 (m

Preparation o ___ te (34). A

stream of dry hydrogen c ___ bbled into a

solution of 8.25 g (0.05 mol) or ethyl 2-pyridylacetate (52)

in 200 ml of ether, causing the formation of a precipitate.

The precipitated salt was removed by filtration and washed

with ether. The salt was dissolved in 100 ml of methanol

and hydrogenated over 0.3 g of platinum oxide at 60 psi.

The stoichiometric amount of hydrogen was taken up in 4 hr

and the catalyst was removed by filtration through Celite.

The filtrate was concentrated under reduced pressure and

the residue was dissolved in a mixture of 50 ml of methylene

chloride and 50 ml of 2N KOH. The layers were separated

A solution of this oil in 80 ml of glacial acetic acid was carefully treated with a solution of 7.5 g of chromium trioxide in 45 ml of 85% acetic acid and allowed to stand for 18 hr at 20°. The solution was concentrated under reduced pressure and the residue was dissolved in 200 ml of concentrated ammonium hydroxide. The aqueous solution was extracted with three 100 ml portions of ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a mushy solid, which was recrystallized from ether to give 7.30 g (38.5%) of 41 as a tan solid, mp 90.5-93.5°. A second recrystallization from ether gave 41 as colorless crystals, mp 93-94.5° (lit.[21] mp 90-92°); ir (Appendix A, Figure 18, KBr) 3550 cm^{-1} (NH); nmr (Appendix B, Figure 20, $CDCl_3$) δ7.88-7.02 (m, 5), 4.50-4.04 (m, 1), 3.04-0.80 (m, 9).

Preparation of Ethyl 2-Piperidylacetate (34). A stream of dry hydrogen chloride gas was bubbled into a solution of 8.25 g (0.05 mol) of ethyl 2-pyridylacetate (52) in 200 ml of ether, causing the formation of a precipitate. The precipitated salt was removed by filtration and washed with ether. The salt was dissolved in 100 ml of methanol and hydrogenated over 0.3 g of platinum oxide at 60 psi. The stoichiometric amount of hydrogen was taken up in 4 hr and the catalyst was removed by filtration through Celite. The filtrate was concentrated under reduced pressure and the residue was dissolved in a mixture of 50 ml of methylene chloride and 50 ml of 2N KOH. The layers were separated

and the aqueous solution was extracted with two 50 ml por-
tions of methylene chloride. The combined organic extracts
were dried (MgSO$_4$) and concentrated under reduced pressure
to give 9.21 g of a yellow oil. Distillation of the oil
gave 6.62 g (77.5%) of <u>34</u> as a colorless oil, bp 50° at 0.4
mm (lit.[36] 105° at 14 mm).

Preparation of Ethyl Diazoacetate (<u>57</u>). Ethyl diazo-
acetate (<u>57</u>) was prepared in 90% yield according to the
Organic Syntheses[37] procedure.

Preparation of Ethyl 2-Pyrrolylacetate (<u>55</u>). A
mixture of 32.00 g (0.475 mol) of practical grade pyrrole
(<u>56</u>) and 2.0 g of freshly prepared activated copper catalyst[38]
was warmed to 85°. To this mixture was added 25.6 g (0.224
mol) of freshly prepared ethyl diazoacetate (<u>57</u>) at a rate
sufficient to maintain the temperature between 90° and 100°.
After the addition was complete, the temperature of the
reaction mixture was held at 100-110° for 2 hr. The copper
catalyst was removed by filtration and the filtrate was
distilled to give 13.48 g (39.3%) of <u>55</u> as a pale yellow
liquid, bp 82.5-85° at 0.2 mm (lit.[22] bp 76° at 0.2 mm).

Preparation of Ethyl 2-Pyrrolidinylacetate (<u>35</u>). A
solution of 5.30 g (0.0346 mol) of ethyl 2-pyrrolylacetate
(<u>55</u>) in 35 ml of ethanol and 70 ml of glacial acetic acid
was hydrogenated over 0.5 g of 5% rhodium on carbon for 24
hr at 80 psi and 20°. The catalyst was removed by filtra-
tion through Celite and washed with ethanol. The filtrate
was concentrated under reduced pressure and the residue was

dissolved in 50 ml of 1N HCl. The aqueous solution was washed with two 25 ml portions of ether, made basic with K_2CO_3, and extracted with ether for 24 hr in a liquid-liquid extractor. The organic solution was dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow liquid. Distillation gave 3.00 g (55.4%) of 35 as a colorless liquid, bp 51-53° at 0.3 mm (lit.[39] bp 110° at 27 mm).

Preparation of Diethyl α,α'-Dibromoadipate (60).
The Organic Syntheses procedure[40] was followed to give a 14.5% yield of the meso isomer of 60 as colorless crystals, mp 67-69° (lit.[41] mp 66°), and a 40% yield of the racemate as a dark brown oil. On standing undisturbed at room temperatures the racemate was slowly converted to the meso isomer.

Preparation of N-Benzyl-cis-2,5-bis-ethoxycarbonyl-pyrrolidine (62). A solution of 25 g (0.07 mol) of meso diethyl α,α'-dibromoadipate (60) in 75 ml of benzene was brought to reflux. Heating was discontinued and 25 ml (0.23 mol) of benzylamine (61) was added dropwise during 1 hr and the solution was heated under reflux for 24 hr. The solution was cooled and the precipitated salt was removed by filtration. The filtrate was concentrated under reduced pressure to give a brown oil. Distillation gave 18.00 g (84.5%) of 62 as a colorless oil, bp 134-142° at 0.18 mm (lit.[25] bp 145-148° at 0.3 mm).

Preparation of <u>cis</u>-2,5-Ethoxycarbonylpyrrolidine (<u>36</u>).

A solution of 18.00 g (0.059 mol) of N-benzyl-<u>cis</u>-2,5-bis-ethoxycarbonylpyrrolidine (<u>62</u>) in 100 ml of ethanol was hydrogenated over 1.00 g of 10% palladium on carbon for 18 hr at 80 psi and 50°. The catalyst was removed by filtration through Celite and washed with ethanol. The filtrate was concentrated under reduced pressure to give a brown oil. Distillation gave 8.55 g (67.5%) of <u>36</u> as a colorless liquid, bp 78-81° at 0.09 mm (lit.[25] bp 95-96° at 0.3 mm).

Attempted Preparation of N-Benzyl-<u>trans</u>-2,5-bis-ethoxycarbonylpyrrolidine (<u>63</u>). Into a 5 ℓ three-neck flask fitted with two addition funnels and a reflux condenser was placed 4 ℓ of benzene. Into one addition funnel was placed a solution of 18.00 g (0.05 mol) of crude <u>racemic</u> diethyl α,α'-dibromoadipate (<u>60</u>) in 500 ml of benzene. Into the other addition funnel was placed a solution of 17.66 g (0.0165 mol) of benzylamine (<u>61</u>) in 500 ml of benzene. The contents of the flask was brought to a gentle boil and the two solutions were added simultaneously at a rate of about 10 ml/hr. After the addition was complete the solution was heated under reflux for an additional 18 hr. The solution was concentrated under reduced pressure and the residue was triturated with ether, causing the formation of the hydro-bromide. The salt was removed by filtration and washed with ether. The filtrate was extracted with three 100 ml portions of 1N HCl. The combined aqueous extracts were made basic with K_2CO_3 and extracted with three 100 ml portions of

chloroform. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give a brown oil. Distillation gave 1.36 g of a single fraction having a broad boiling range of 122-145° at 0.3 mm. This oil was converted to its hydrochloride and shown to be benzylamine (61) by ir, nmr, and melting point. The large amount of material remaining in the distillation pot would not distil at an oil bath temperature of 210° and it was presumed to be polymeric material. No further workup was attempted.

Preparation of cis-Pyrrolidine-2,5-dicarboxylic Acid (66). A suspension of 5.06 g (0.0236 mol) of cis-2,5-bis-ethoxycarbonylpyrrolidine (36) in 150 ml of water was heated under reflux for 26 hr. Concentration of the solution under reduced pressure gave a quantitative yield of 66 as a colorless solid, mp 260-262° (lit.[25] mp 260-261°).

Attempted Preparation of trans-2,5-bis-Methoxycarbonyl-pyrrolidine (70). A suspension of 3.75 g (0.0236 mol) of cis-pyrrolidine-2,5-dicarboxylic acid (66) in 150 ml of glacial acetic acid was heated to a reflux and treated carefully with 25 ml of acetic anhydride. Heating was continued until all the solid had gone into solution. The solution was allowed to cool at room temperature and concentrated under reduced pressure. The residue was dissolved in water and this solution was concentrated under reduced pressure to give 2.78 g of crude N-acetylpyrrolidine-2,5-dicarboxylic acid (67) as a viscous oil. The ir spectrum contained a broad band centered at 1700 cm^{-1}, suggesting the presence

of amide and acid carbonyl groups. No evidence was found for the presence of an anhydride.

A solution of 0.61 g of $\underline{67}$ in 50 ml of 1N HCl was heated under reflux for 2 hr. The solution was concentrated under reduced pressure to give a gummy solid. This solid was dissolved in 50 ml of 2,2-dimethoxypropane and treated with 2 ml of concentrated HCl. A precipitate began to form within a few minutes. After 3 hr this precipitate was removed by filtration to give 0.17 g of colorless solid, mp 214-218°, identified by the ir and nmr spectra as the hydrochloride of cis-pyrrolidine-2,5-dicarboxylic acid $(\underline{66})$, nmr $(D_6$-DMSO) $\delta 11.30$ (s, 4), 4.72-4.20 (m, 2), 2.80-1.86 (m, 4). The filtrate was concentrated under reduced pressure to give 0.22 g of a brown solid, which was recrystallized from methanol-ether to give tan needles, mp 177-177.5°. This solid was shown to be the hydrochloride of cis-2,5-bis-methoxycarbonylpyrrolidine $(\underline{68})$ by ir and nmr spectra, and by mixed melting point comparison with an authentic sample prepared by the method described below, mp 176-178°.

Preparation of cis-2,5-bis-Methoxycarbonylpyrrolidine
Hydrochloride $(\underline{68})$. A solution of cis-2,5-bis-ethoxycarbonyl-pyrrolidine $(\underline{36})$ in 75 ml of water was heated under reflux for 48 hr and concentrated under reduced pressure. The residue was dissolved in 50 ml of 2,2-dimethoxypropane and treated with 4 ml of concentrated HCl. After standing 24 hr at 20° the precipitate was removed by filtration and the filtrate was concentrated under reduced pressure. The residue

was recrystallized from ether-methanol to give 68 as tan

crystals: mp 178.5-179.5°; ir (KBr) 1740 cm^{-1} (ester C=O);

nmr (D$_6$-DMSO) δ4.72-4.40 (m, 2), 3.86 (s, 6), 2.72-1.90 (m,

6).

Attempted Preparation of trans-2,5-bis-Methoxycarbonyl-

pyrrolidine (70). A suspension of 0.58 g (0.003 mol) of

cis-2,5-bis-methoxycarbonylpyrrolidine (68) and 1.0 g of

sodium hydride in 75 ml of anhydrous DME was stirred for 30

min at 20°. Water was added dropwise until the evolution of

hydrogen ceased and the solution was concentrated under re-

duced pressure. The residue was triturated with ether and

filtered through Celite. The filtrate was concentrated under

reduced pressure to give a yellow solid. Recrystallization

from ether-ethanol gave 0.74 g of yellow solid. This material

did not melt at 250° and there was no carbonyl band in the

ir spectrum, suggesting it was inorganic. No further workup

was attempted.

Attempted Preparation of cis-2,5-bis-t-Butyloxycar-

bonylpyrrolidine (72). A mixture of 2.95 g (0.0147 mol) of

N-acetylpyrrolidine-cis-2,5-dicarboxylic acid (67) in a

solution of 100 ml of dioxane, 100 ml of liquid isobutylene,

and 1 ml of concentrated H$_2$SO$_4$ was placed in a hydrogenation

flask and shaken mechanically for 20 hr at 20°. The result-

ing solution was poured into a mixture of 100 ml of 1N NaOH

and 100 ml of ether and mixed thoroughly. The layers were

separated and the aqueous layer was extracted with two 200

ml portions of ether. The combined organic extracts were

dried (MgSO$_4$) and concentrated under reduced pressure to give 0.21 g of a brown oil. Extraction of the aqueous layer in a liquid-liquid extractor gave no additional product.

A solution of this oil in 10 ml of 1N NaOH was warmed on a steam bath for 1.5 hr. The solution was acidified with 5% citric acid and extracted with two 25 ml portions of ethyl acetate. The combined organic extracts were dried (MgSO$_4$) and concentrated under reduced pressure to give 0.13 g of a yellow oil. The ir spectrum showed the complete absence of carbonyl bands and no further workup was attempted.

BIBLIOGRAPHY

1. A. H. Cowley et al., J. Amer. Chem. Soc., 92, 1085, 5206 (1970).

2. M. Raban and G. W. J. Kennedy, Jr., Tetrahedron Lett., 1295 (1969).

3. W. Walter and M. E. Schauman, Justus Liebigs Ann. Chem., 747, 191 (1971).

4. G. J. Bishop et al., Chem. Commun., 672 (1967).

5. M. Raban et al., J. Amer. Chem. Soc., 91, 6677 (1969).

6. H. Kessler, Angew. Chem. Int. Ed. Engl., 9, 219 (1970).

7. M. Raban et al., J. Amer. Chem. Soc., 90, 2985 (1968).

8. M. Raban, Chem. Commun., 1017 (1967).

9. S. J. Brois, J. Amer. Chem. Soc., 89, 4242 (1967).

10. J. M. Lehn and J. Wagner, Chem. Commun., 1298 (1968).

11. M. Raban and F. B. Jones, Jr., J. Amer. Chem. Soc., 93, 2692 (1971).

12. L. Pauling, Proc. Natl. Acad. Sci. U.S.A., 35, 495 (1949).

13. J. H. Billman et al., J. Amer. Chem. Soc., 63, 1920 (1941).

14. M. J. Kornet et al., J. Pharm. Sci., 60, 803 (1971).

15. N. Kharasch, J. Chem. Ed., 33, 585 (1956).

16. R. B. Langford and D. D. Lanson, ibid., 34, 510 (1957).

17. A. Fontana et al., Rec. Sci., 36, 201 (1966); Chem. Abstr., 65, 3958h (1966).

18. V. J. Traynelis and J. N. Rieck, J. Org. Chem., 38, 4339 (1973).

19. G. W. Anderson and F. M. Callahan, J. Amer. Chem. Soc., 82, 3359 (1960).

20. E. N. Shaw in "Pyridine and Its Derivatives, Part III," E. Klingsberg, Ed., Interscience Publishers, New York, N. Y., 1961, p 46.

21. J. Heer et al., Helv. Chim. Acta, 38, 134 (1955).

22. L. Mendell and E. C. Roberts, J. Heterocycl. Chem., 2, 479 (1965).

23. D. A. Cox et al., J. Chem. Soc., 5024 (1964).

24. B. J. Magerlein, J. Med. Chem., 15, 1255 (1972).

25. G. Cignarella and G. Nathansohn, J. Org. Chem., 26, 1500 (1961).

26. S. W. Blackman and R. Baltzky, J. Org. Chem., 26, 2750 (1961).

27. A. Frost and R. Pearson, "Kinetik und Mechanismus homogener chemischer Reaktionen," Verlag Chemie, Weinheim/Bergstrasse, 1967.

28. G. Binsch in "Topics in Stereochemistry," Vol. 3, E. L. Eliel and N. L. Allinger, Eds., John Wiley & Sons, Inc., New York, N.Y., 1968, p 97.

29. M. Raban et al., J. Org. Chem., 35, 1496 (1970).

30. M. Raban et al., Chem. Commun., 656 (1971).

31. R. S. Atkinson, J. Chem. Soc., D, 1189 (1969).

32. M. Raban et al., J. Amer. Chem. Soc., 94, 2738 (1972).

33. J. J. Kaminski, Ph.D. Thesis, The University of New Hampshire, Durham, N. H., 1972.

34. M. J. Kane, Ph.D. Thesis, The University of New Hampshire, Durham, N. H., 1972.

35. G. Harris, Ed., "Dictionary of Organic Compounds," Vol. 5, 4th ed., Oxford University Press, New York, N. Y., 1965, p 2761.

36. G. R. Cleno et al., J. Chem. Soc., 1743 (1935).

37. N. E. Searle in "Organic Synthesis," Coll. Vol. 4, N. Rabjohn, Ed., John Wiley & Sons, Inc., New York, N. Y., 1963, pp 424-426.

38. R. Q. Brewster and R. Groening in "Organic Syntheses", Coll. Vol. 2, A. H. Blatt, Ed., John Wiley & Sons, Inc., New York, N. Y., 1943, p 446.

39. G. R. Cleno and T. A. Melrose, J. Chem. Soc., 424 (1942).

40. P. C. Guha and D. K. Sankaran in "Organic Syntheses," Coll. Vol. 3, E. C. Horning, Ed., John Wiley & Son, Inc., New York, N. Y., 1955, pp 623-625.

41. C. K. Ingold, J. Chem. Soc., 119, 951 (1921).

42. M. H. Hubacher, Organic Syntheses, 15, 45 (1935).

43. A. Weissberger, Ed., "Technique of Organic Chemistry", Vol. IV, Interscience Publishers, Inc., New York, N. Y., 1951, pp 496-499, 509-511.
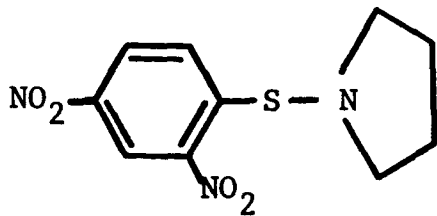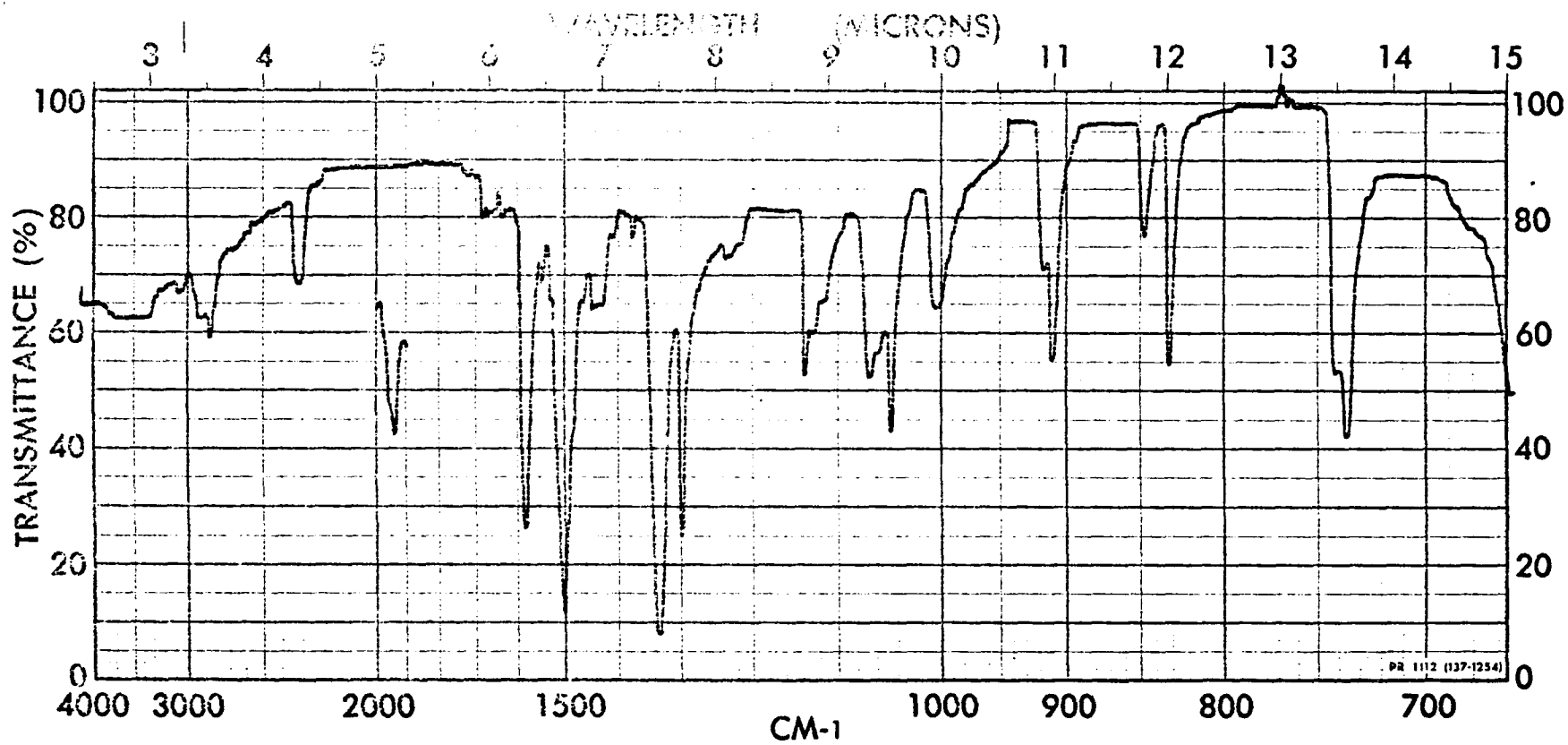
A - Figure 1 (KBr) Compound 17
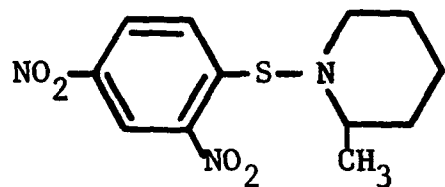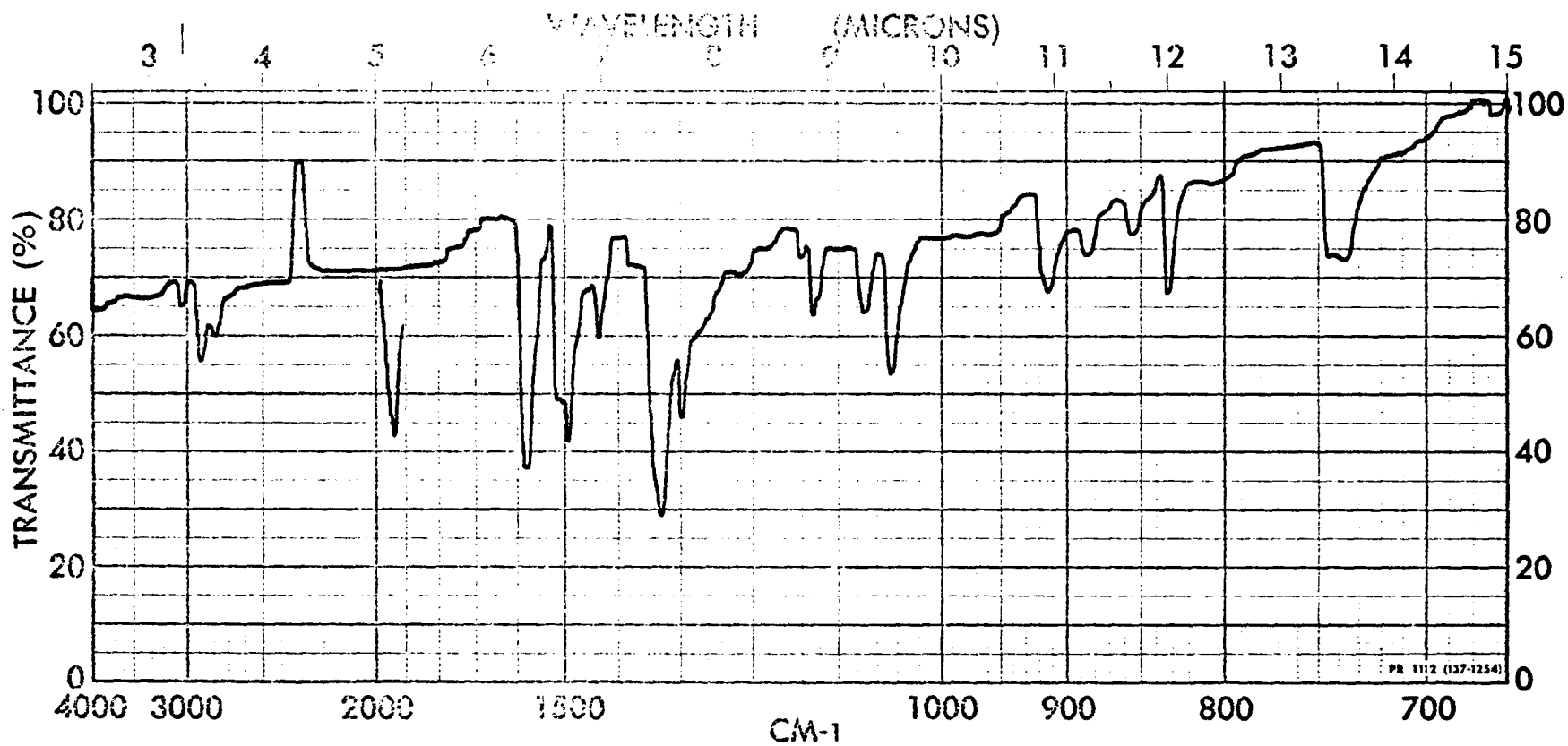
A - Figure 2 (KBr) Compound 19
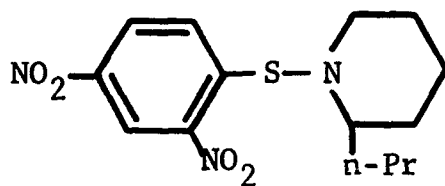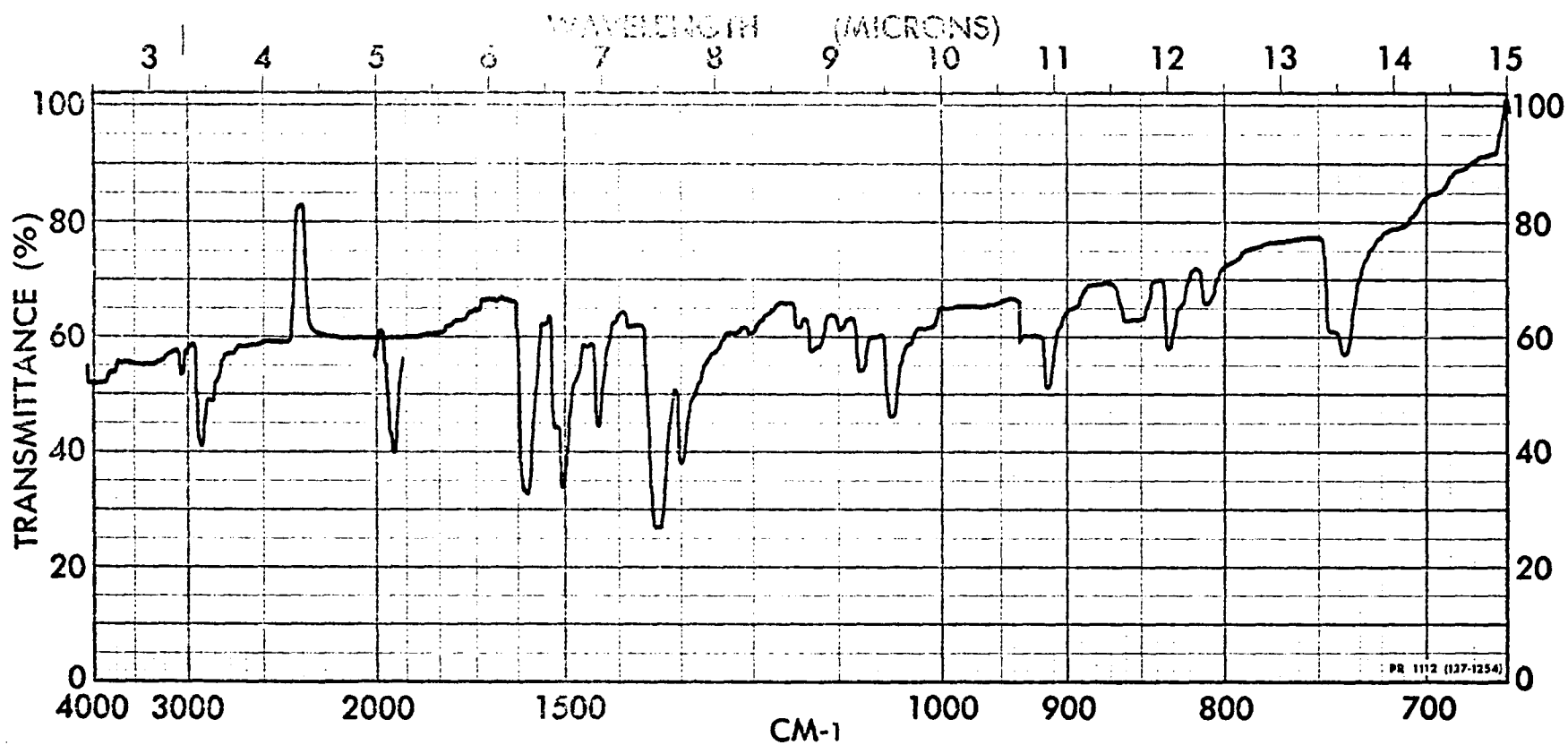
A - Figure 3   (KBr)   Compound 20
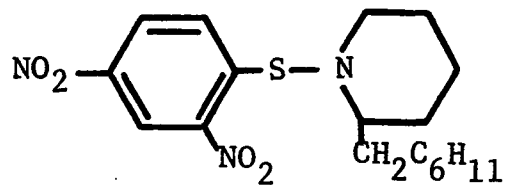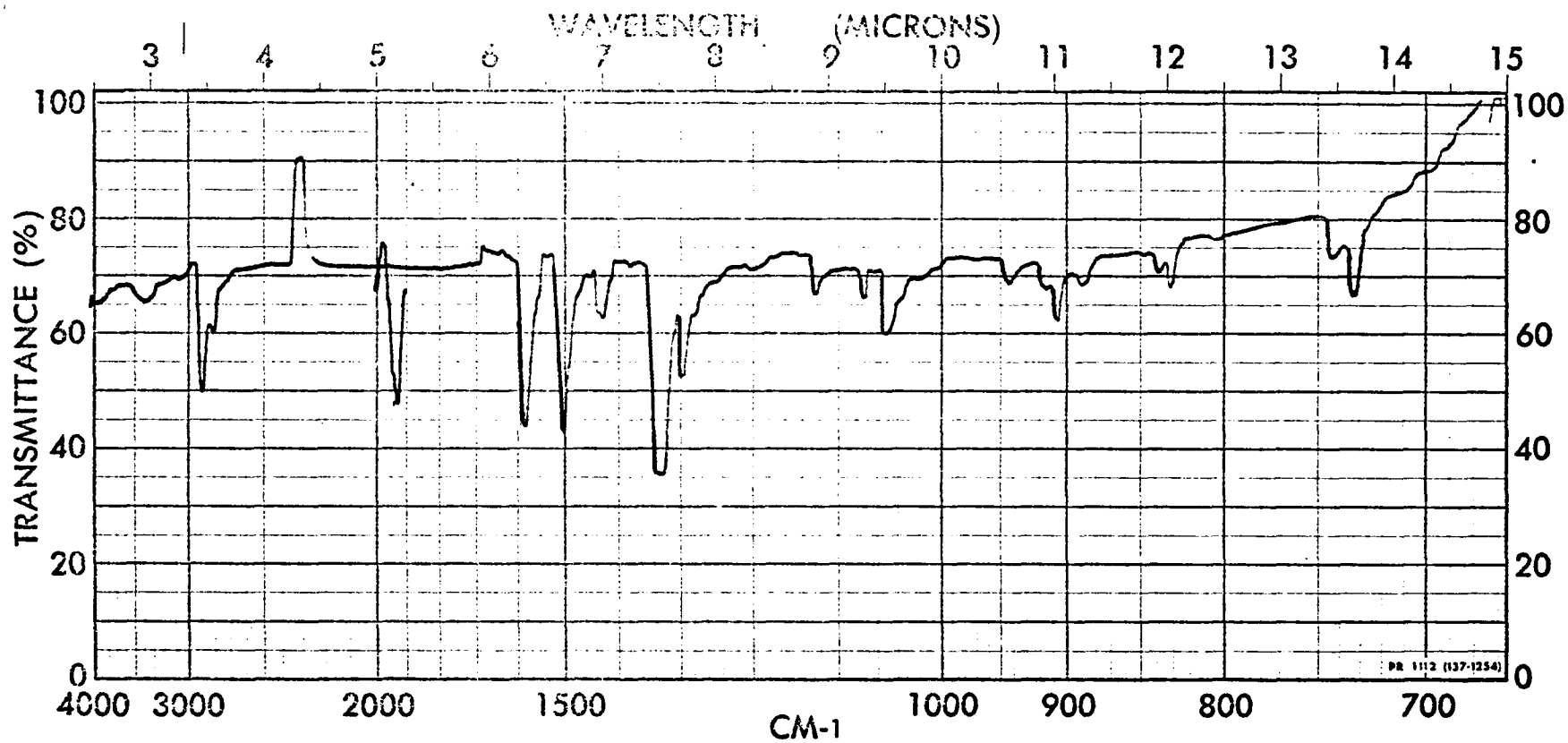
A - Figure 4   (KBr)   Compound 24

WAVELENGTH (MICRONS)
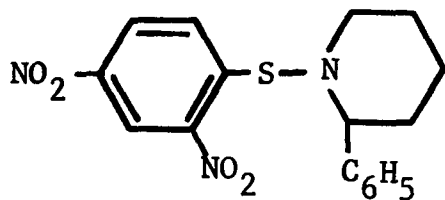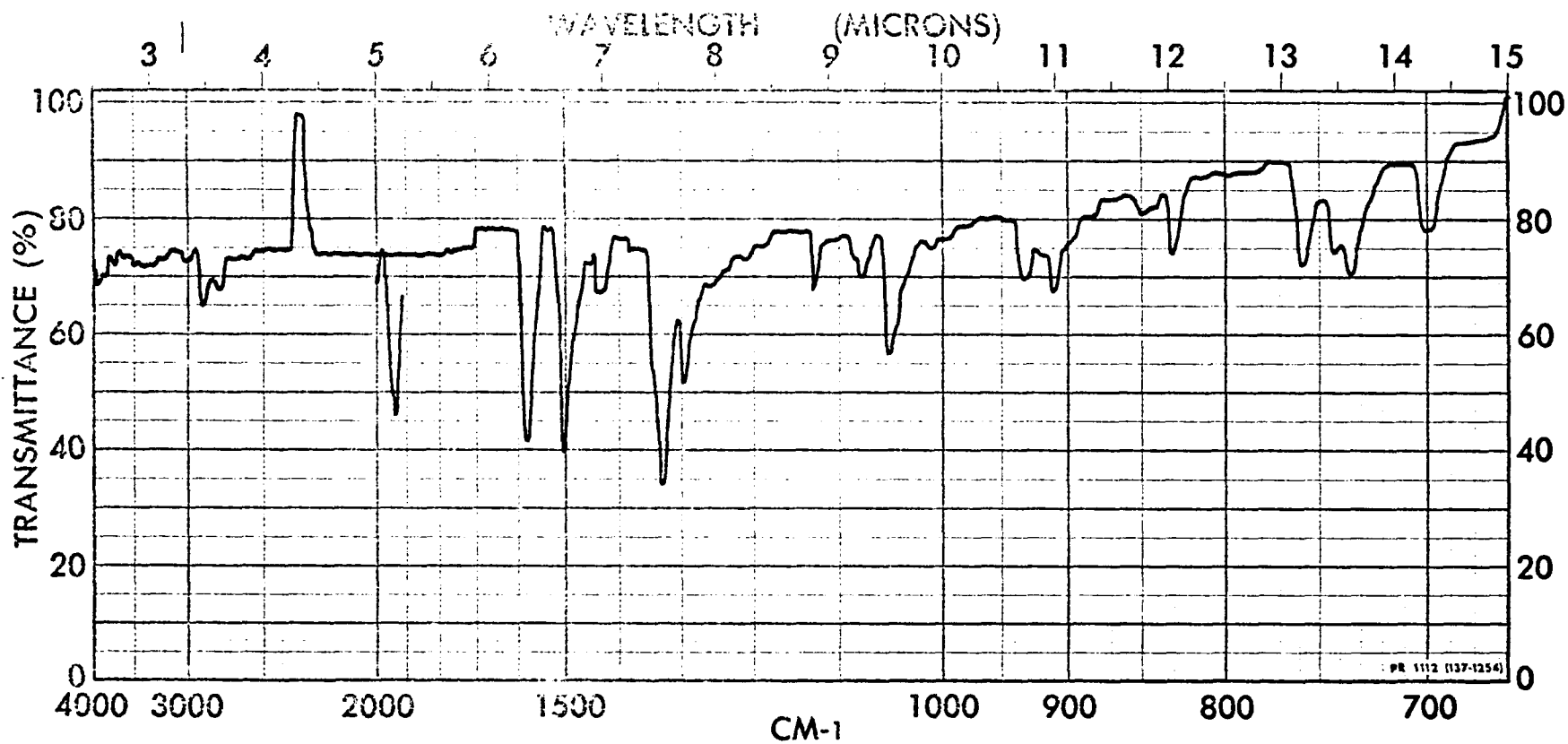
A - Figure 5 (KBr) Compound 25
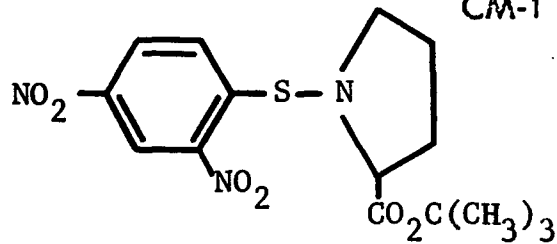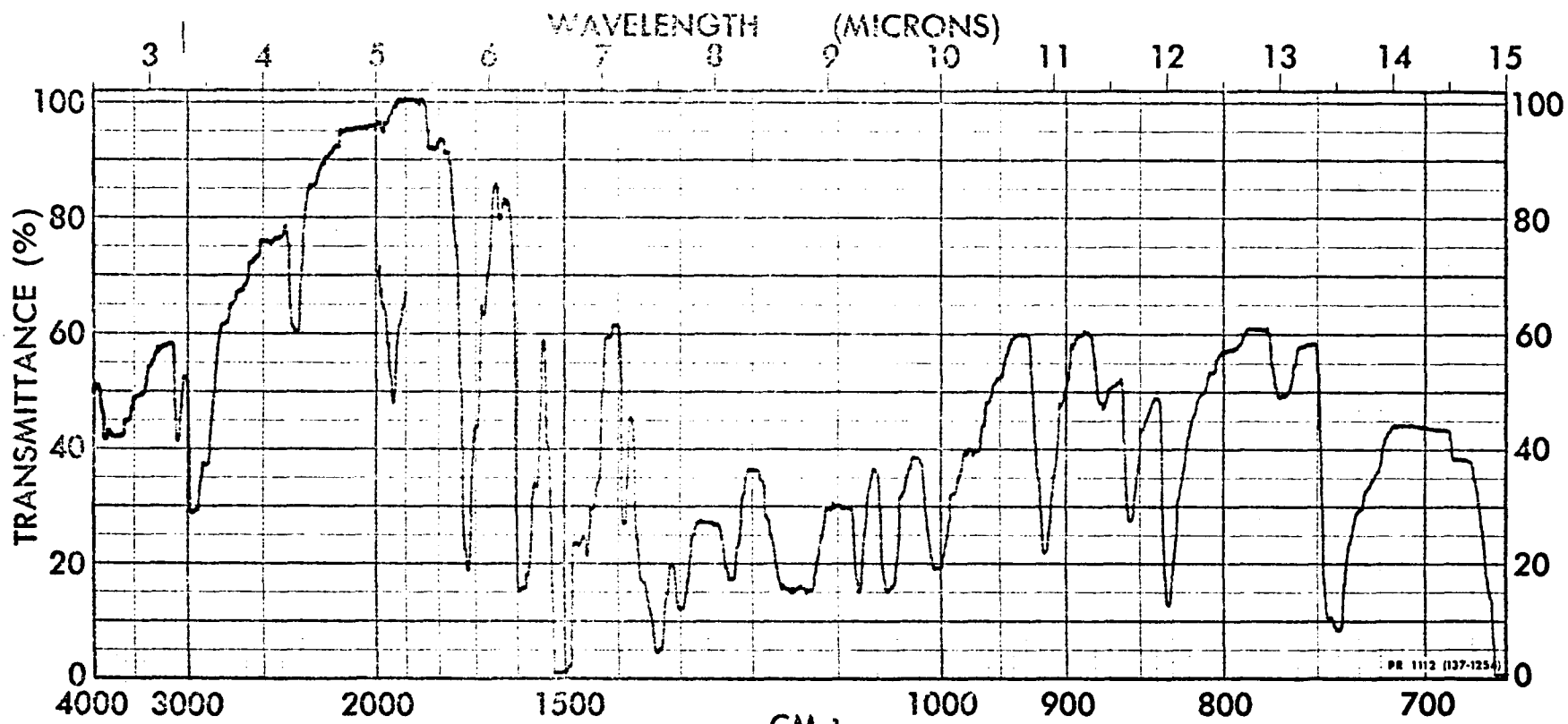
A - Figure 6 (KBr) Compound **26**
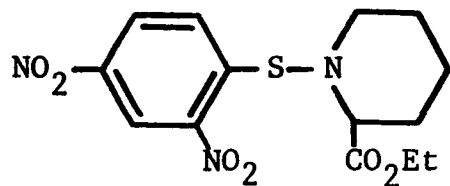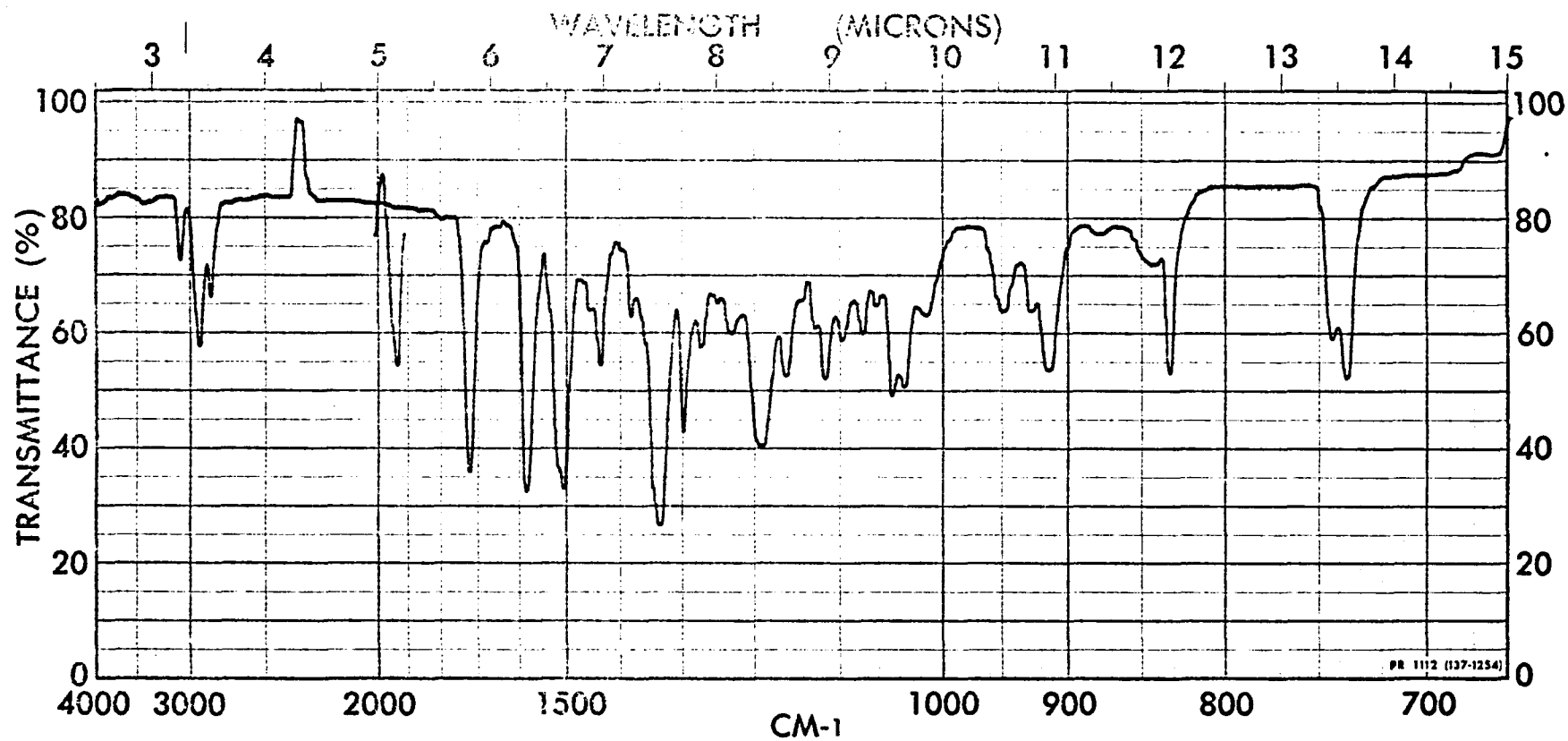
A - Figure 7 (neat) Compound 29
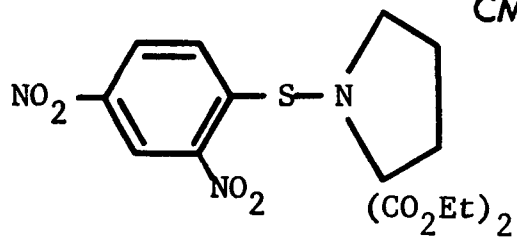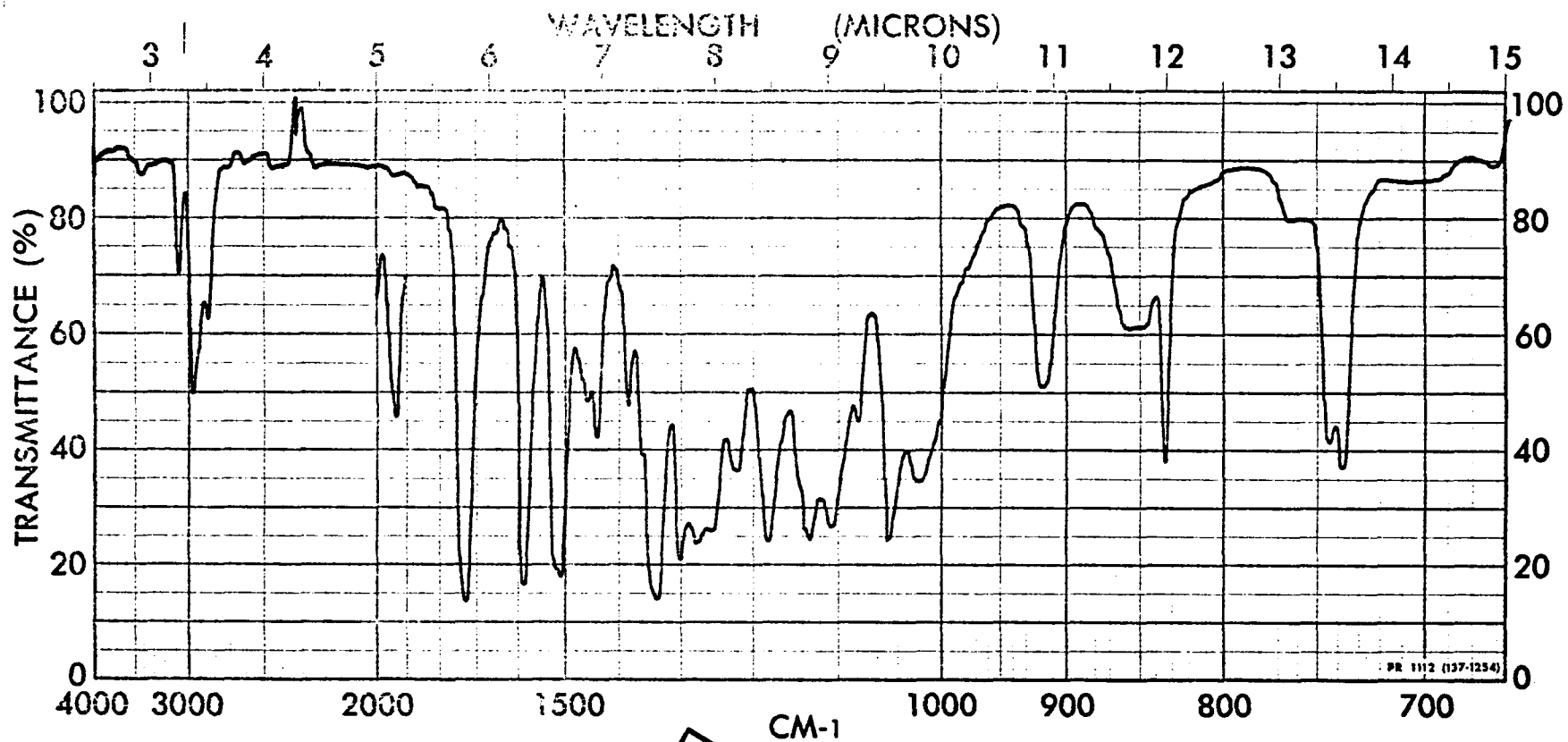
A - Figure 8 (neat) Compound 30
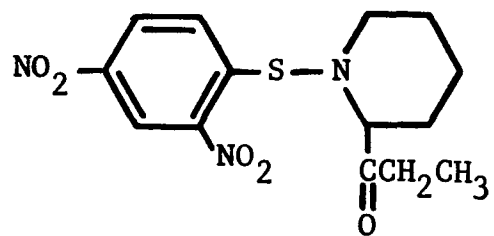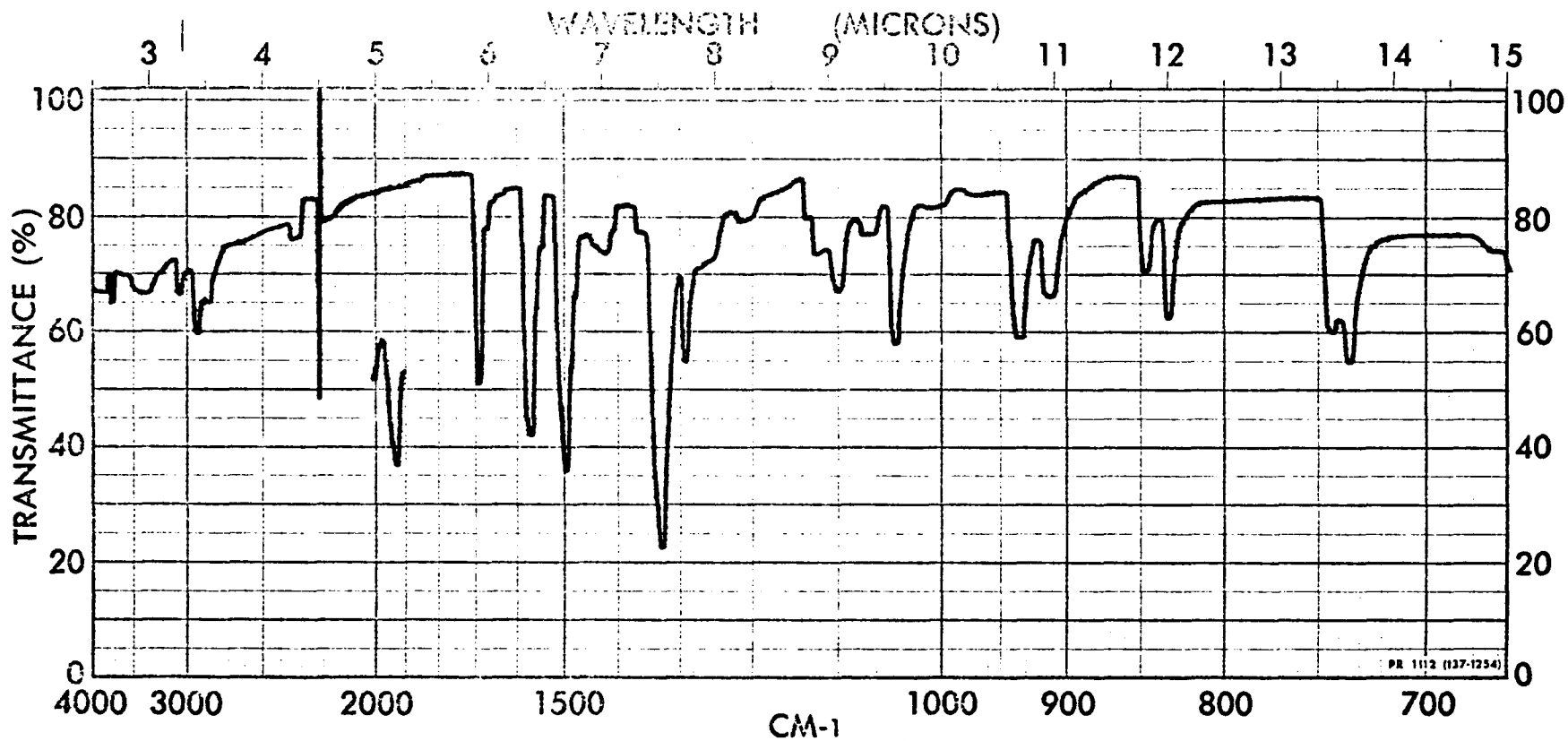
A - Figure 9   (KBr)   Compound 37
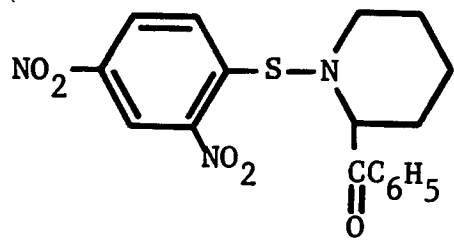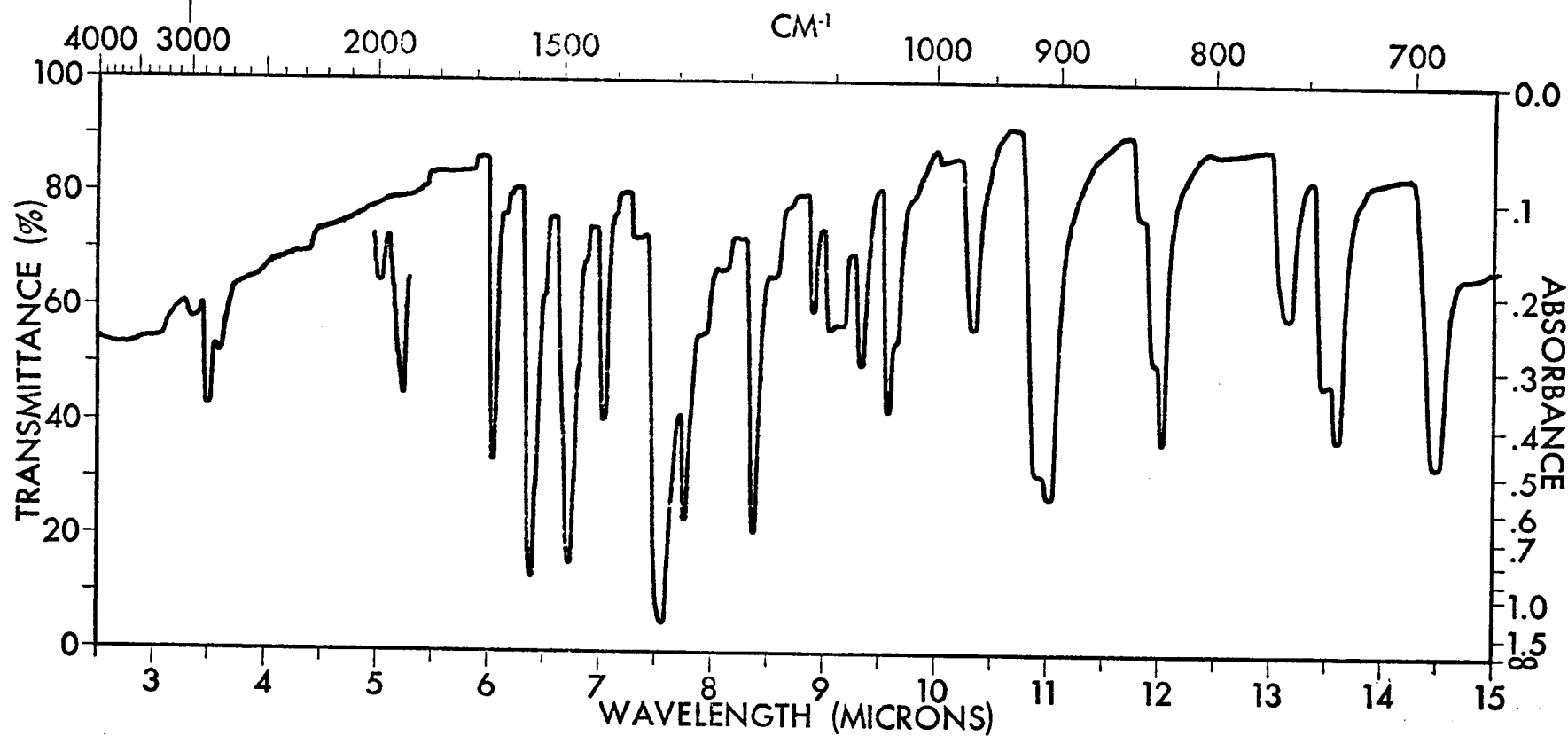
A - Figure 10   (KBr)   Compound 42
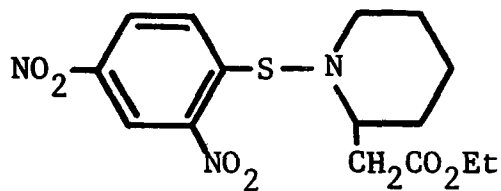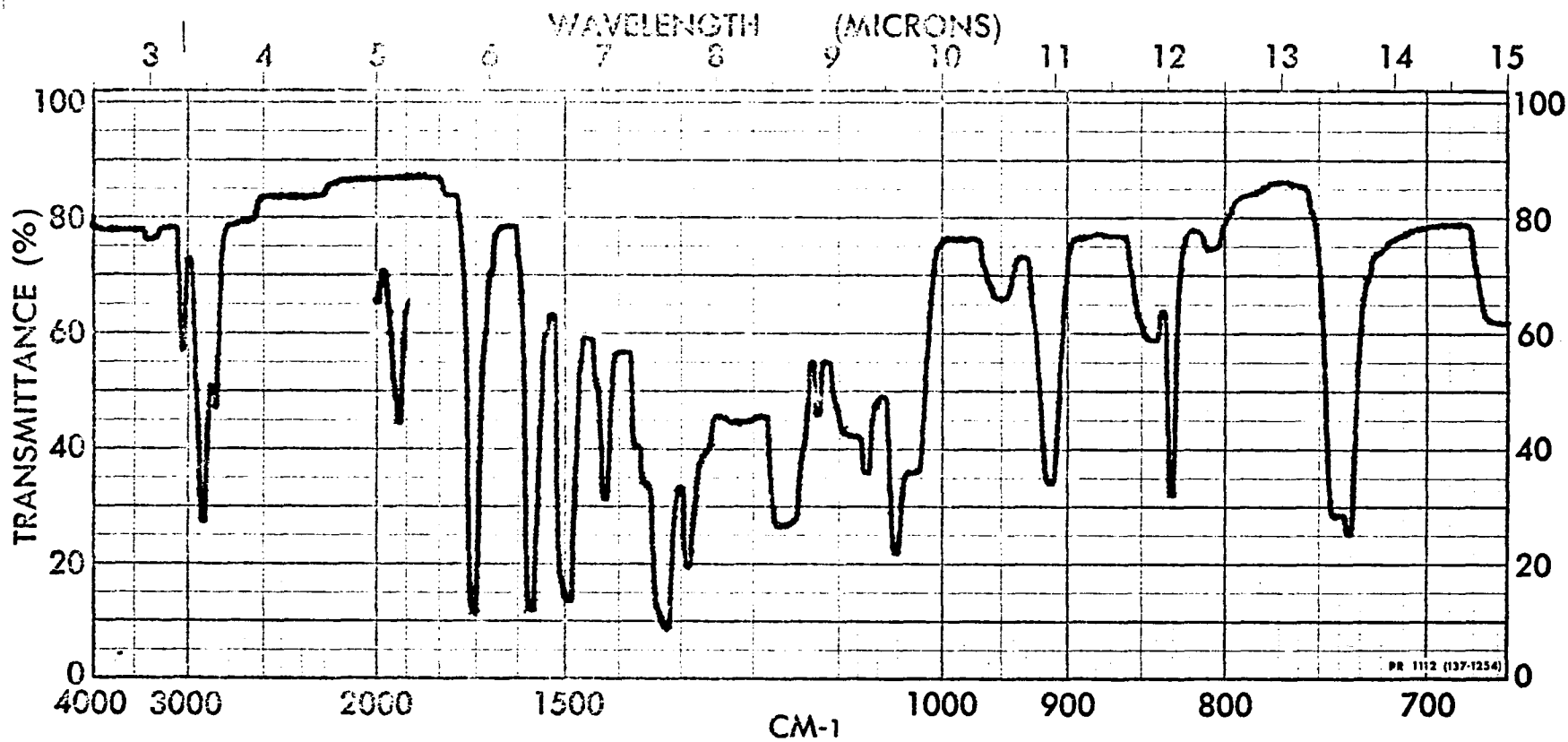
A - Figure 11 (neat) Compound 38
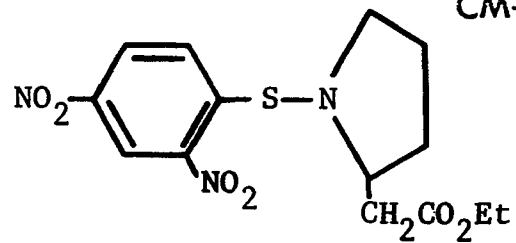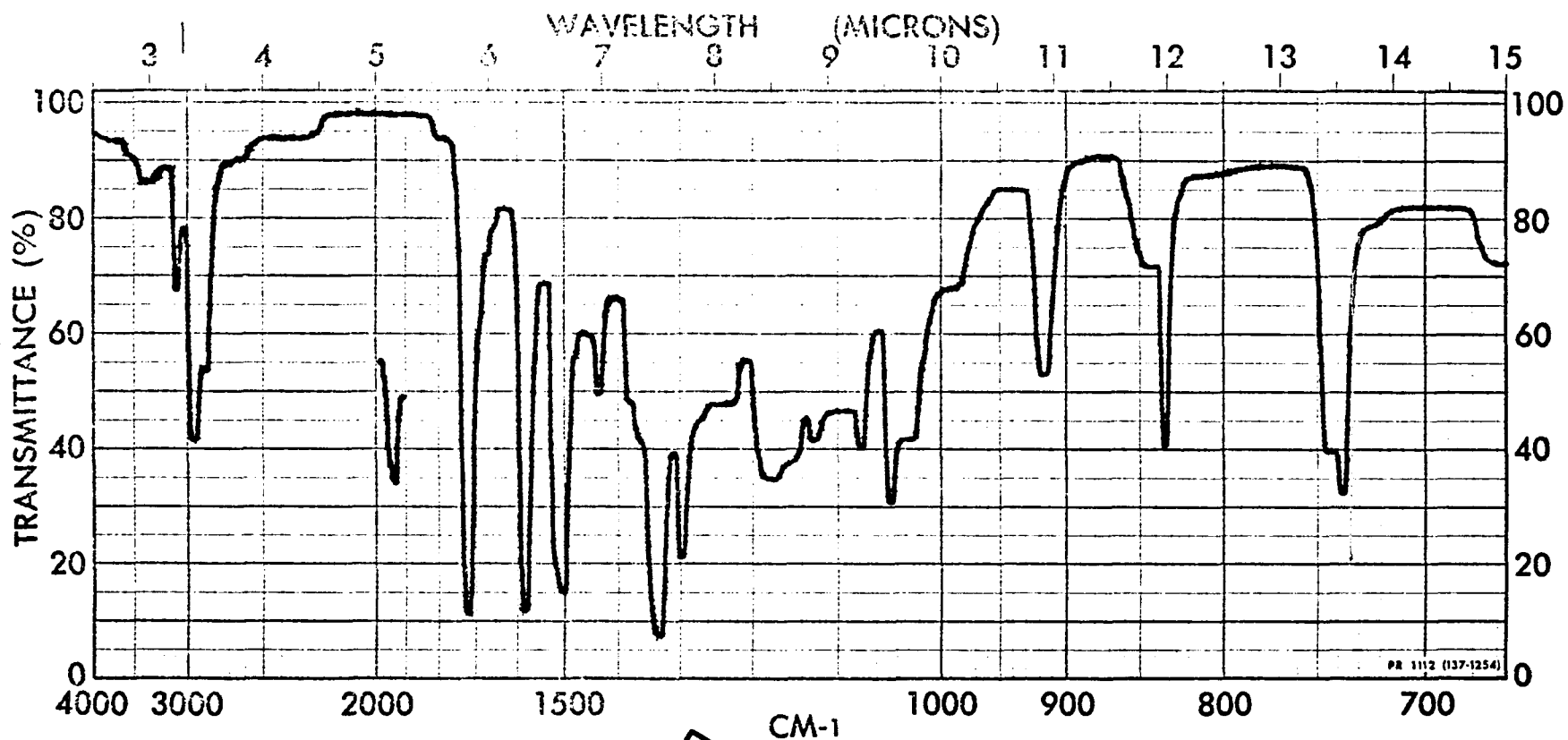
A - Figure 12 (neat)   Compound 39
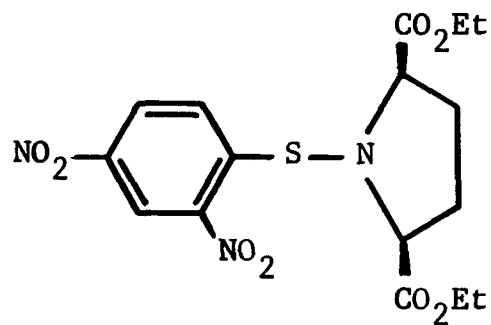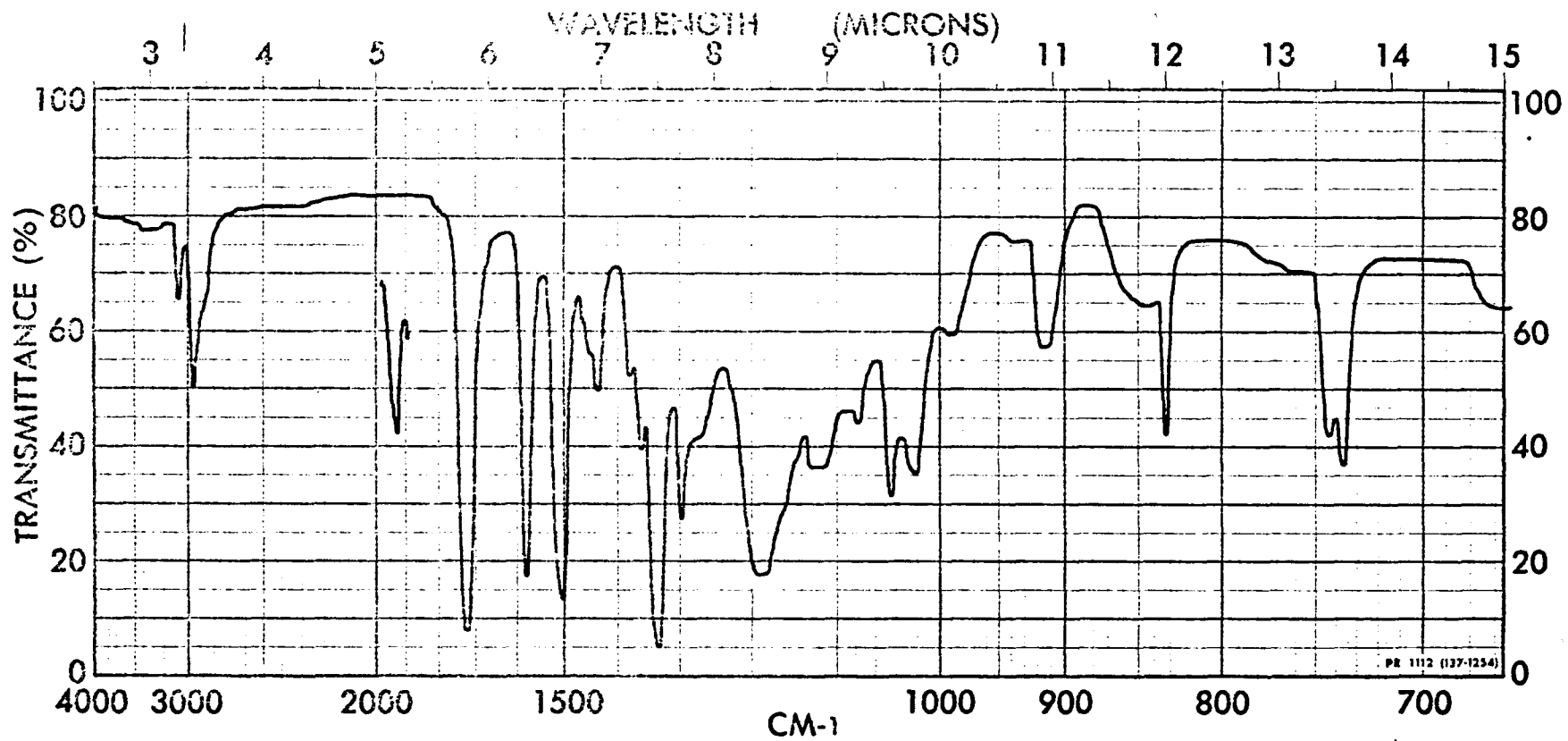
A - Figure 13 (neat) Compound <u>40</u>
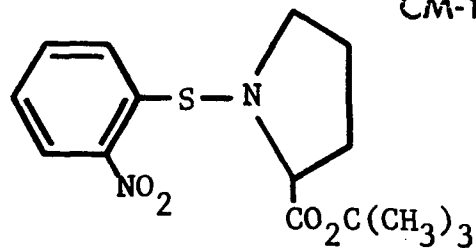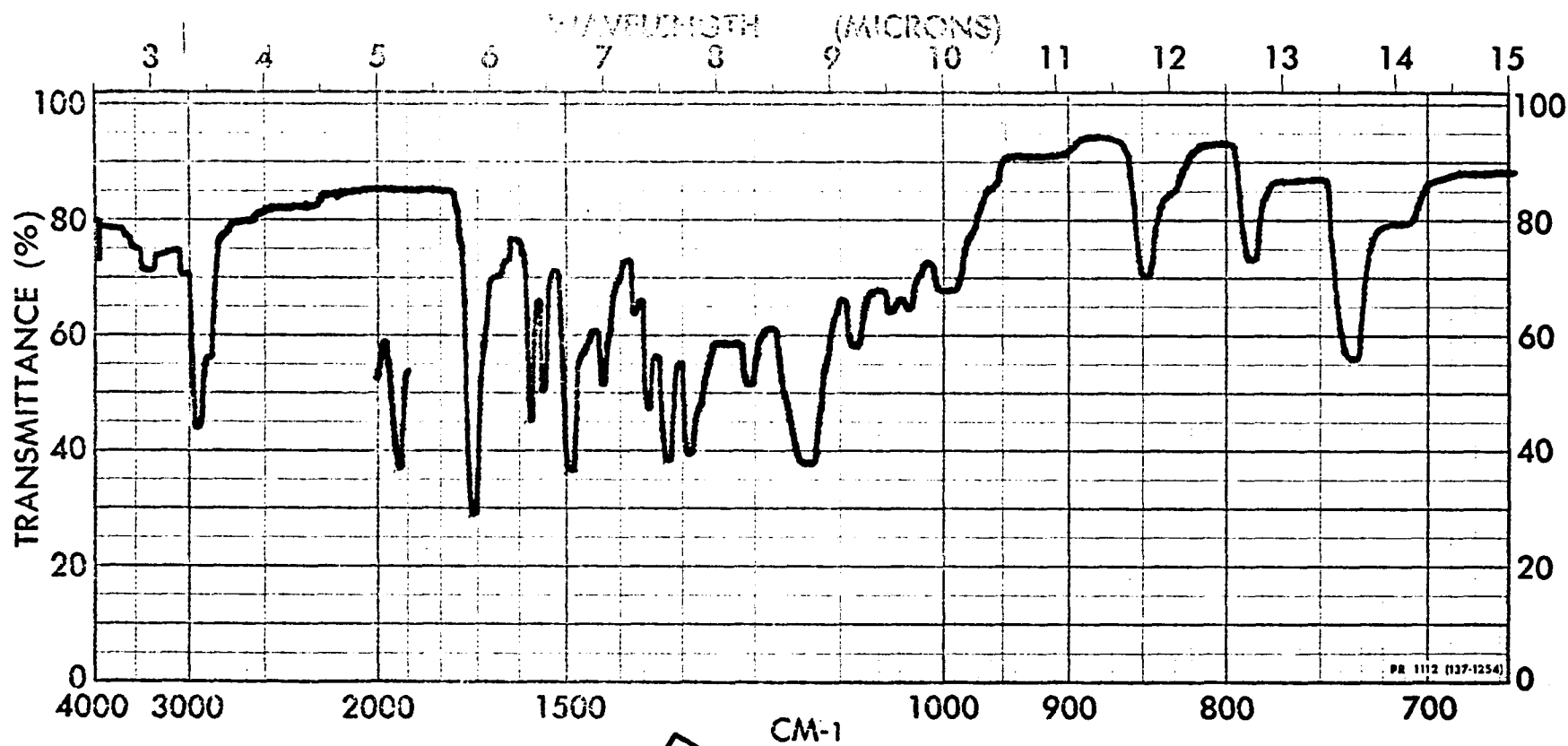
A - Figure 14 (neat) Compound 44

WAVELENGTH (MICRONS)

A - Figure 15 (KBr) Compound 45

WAVELENGTH (MICRONS)

A - Figure 16   (KBr)   Compound 47

WAVELENGTH (MICRONS)
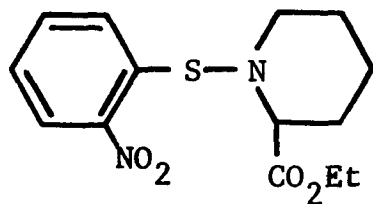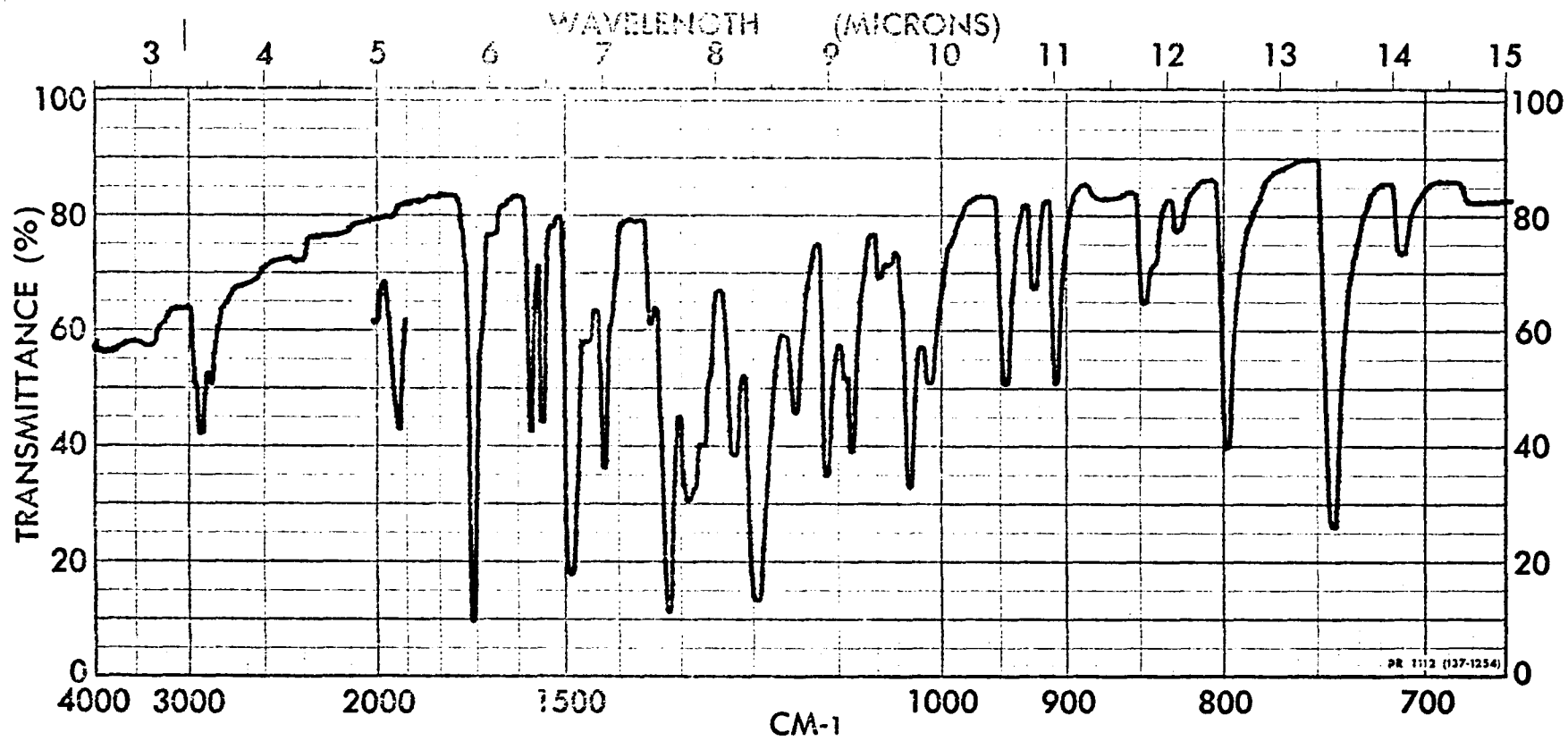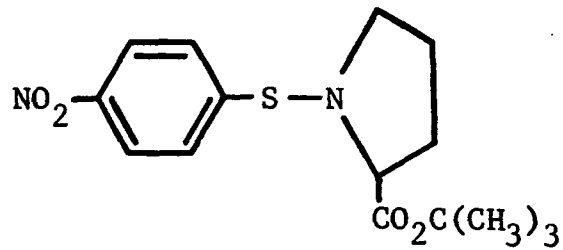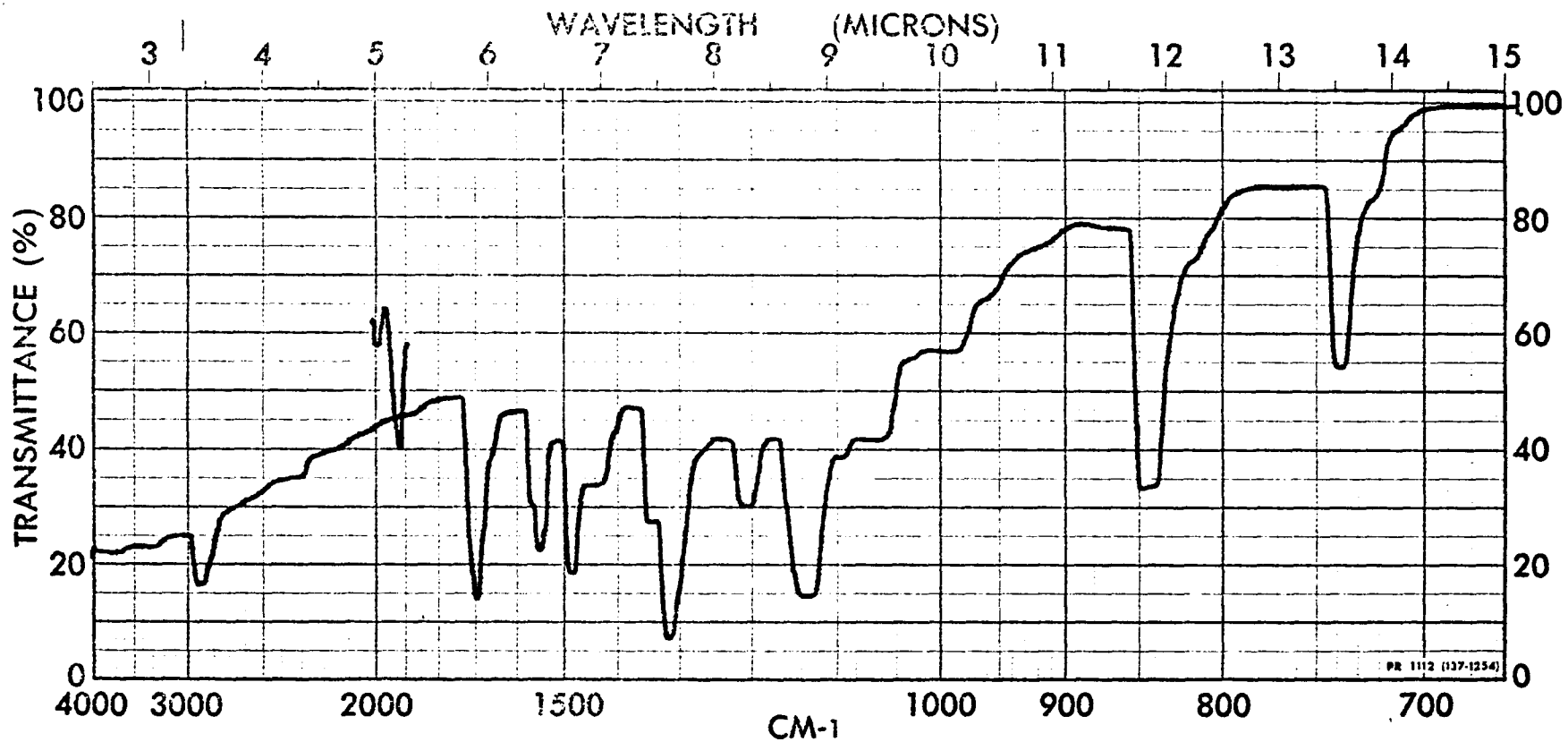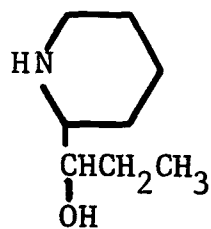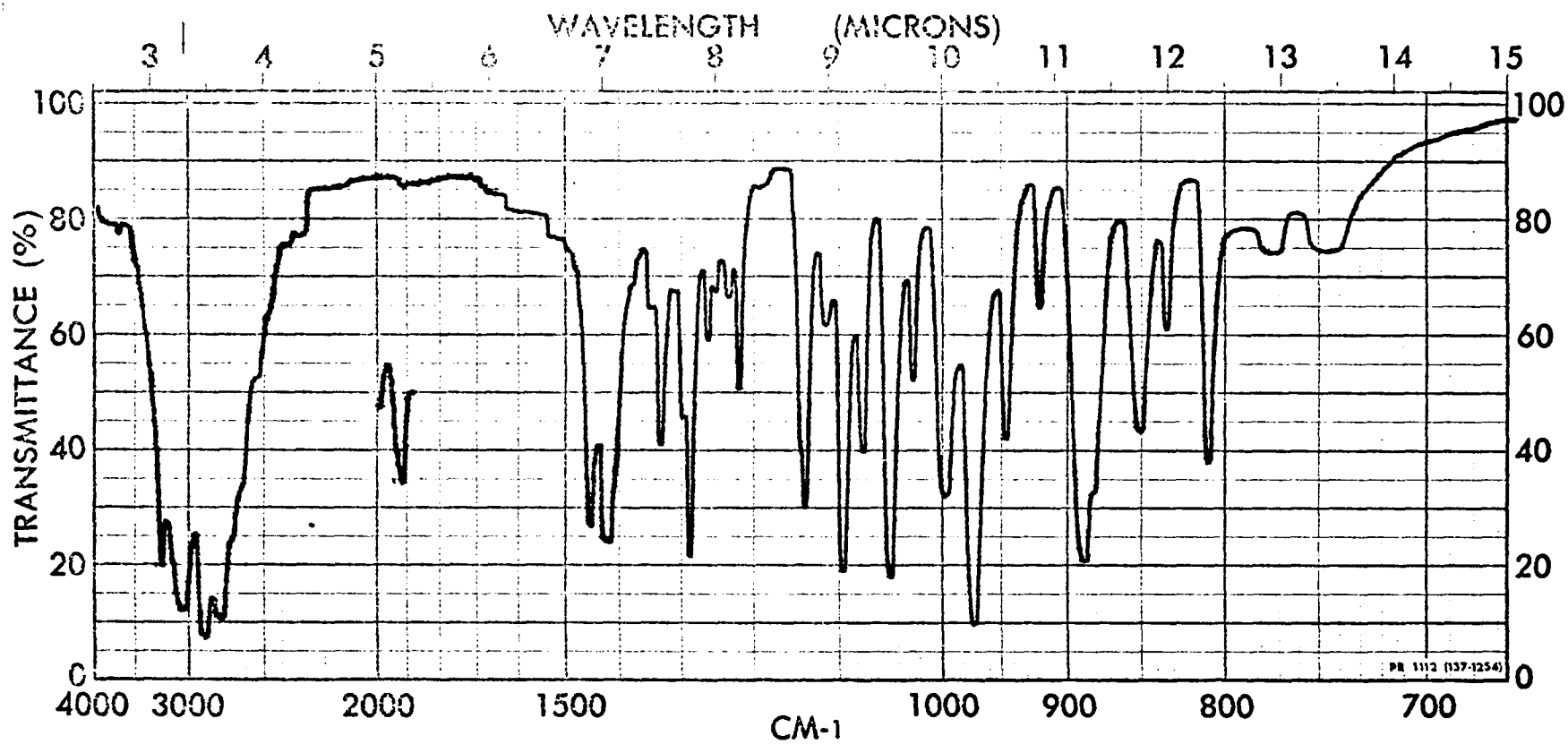
A - Figure 17 (KBr) Compound 79
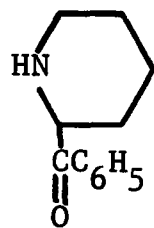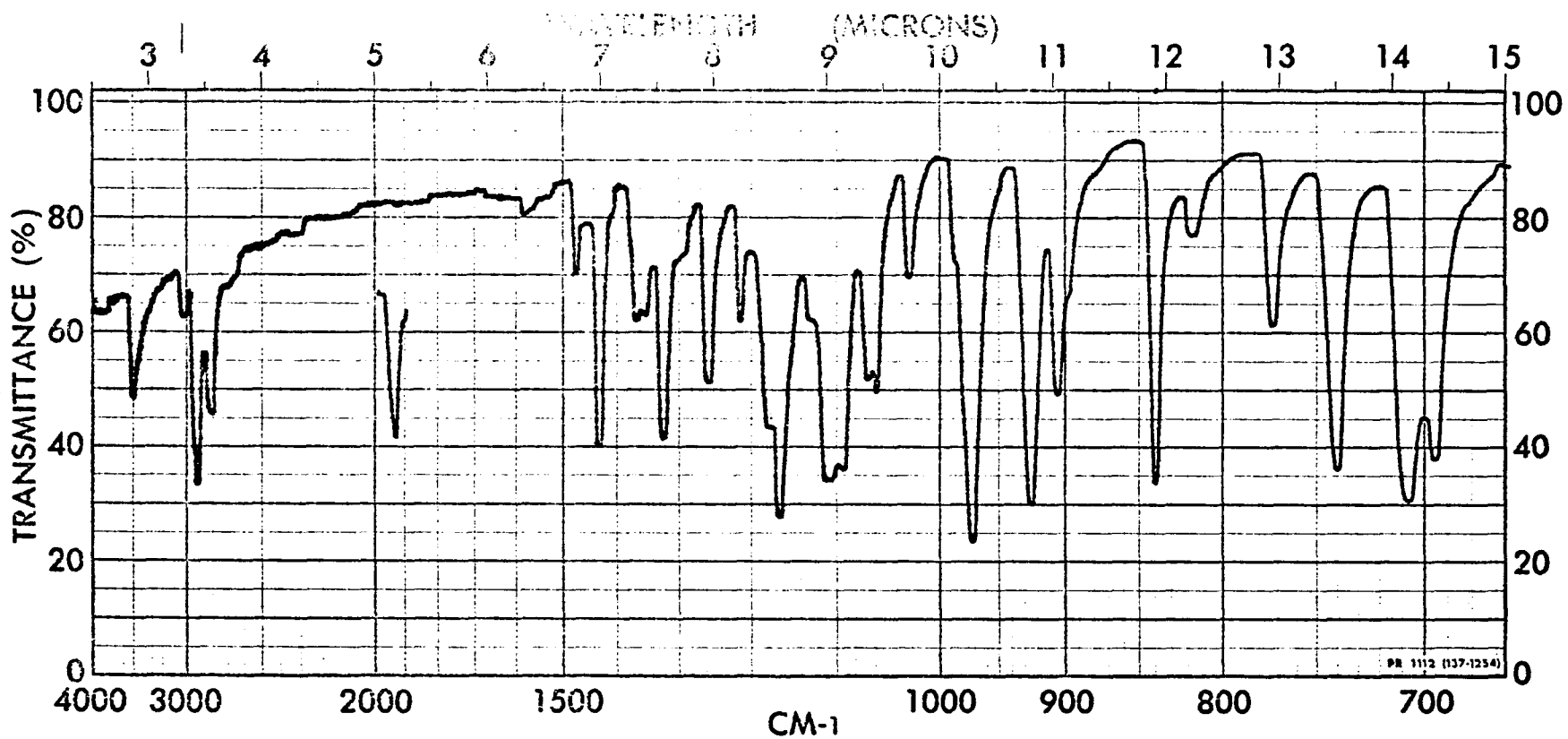
A - Figure 18 (KBr) Compound 41
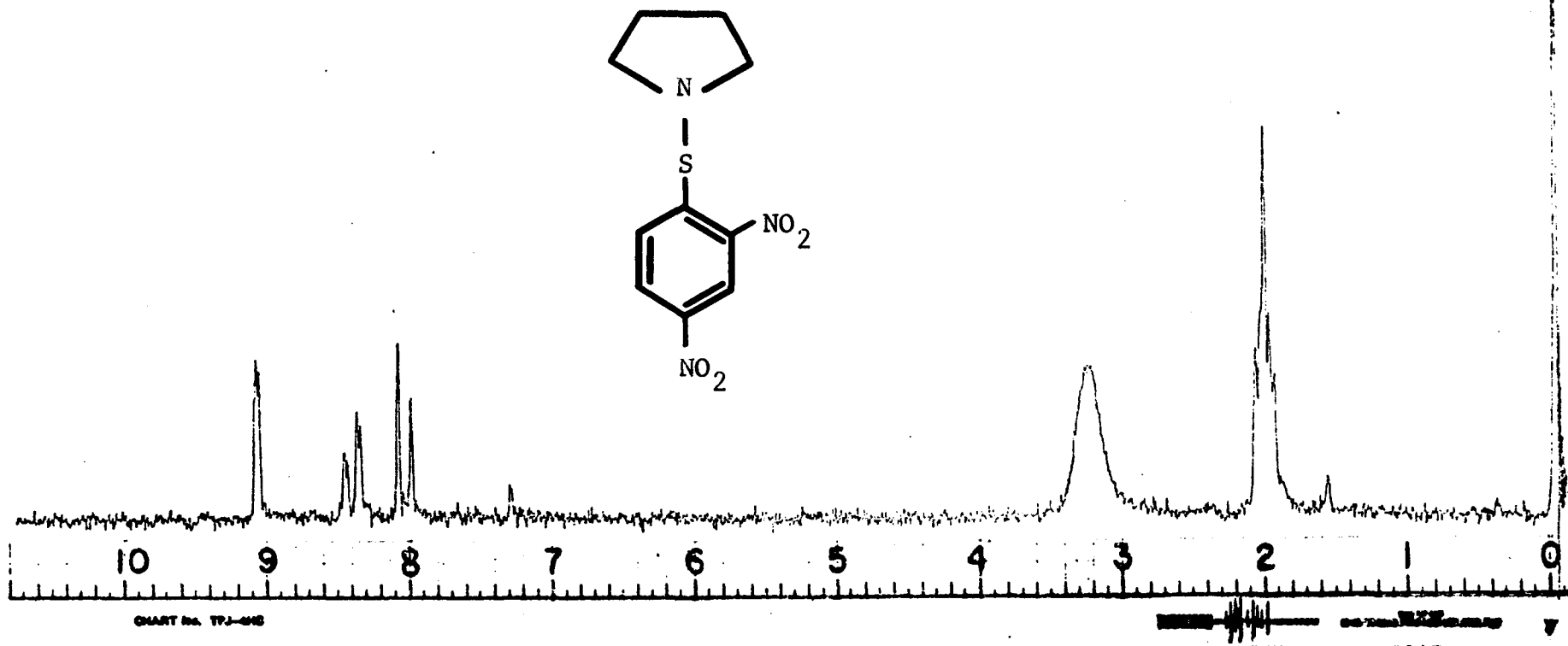
B - Figure 1   (CDCl$_3$)   Compound 17
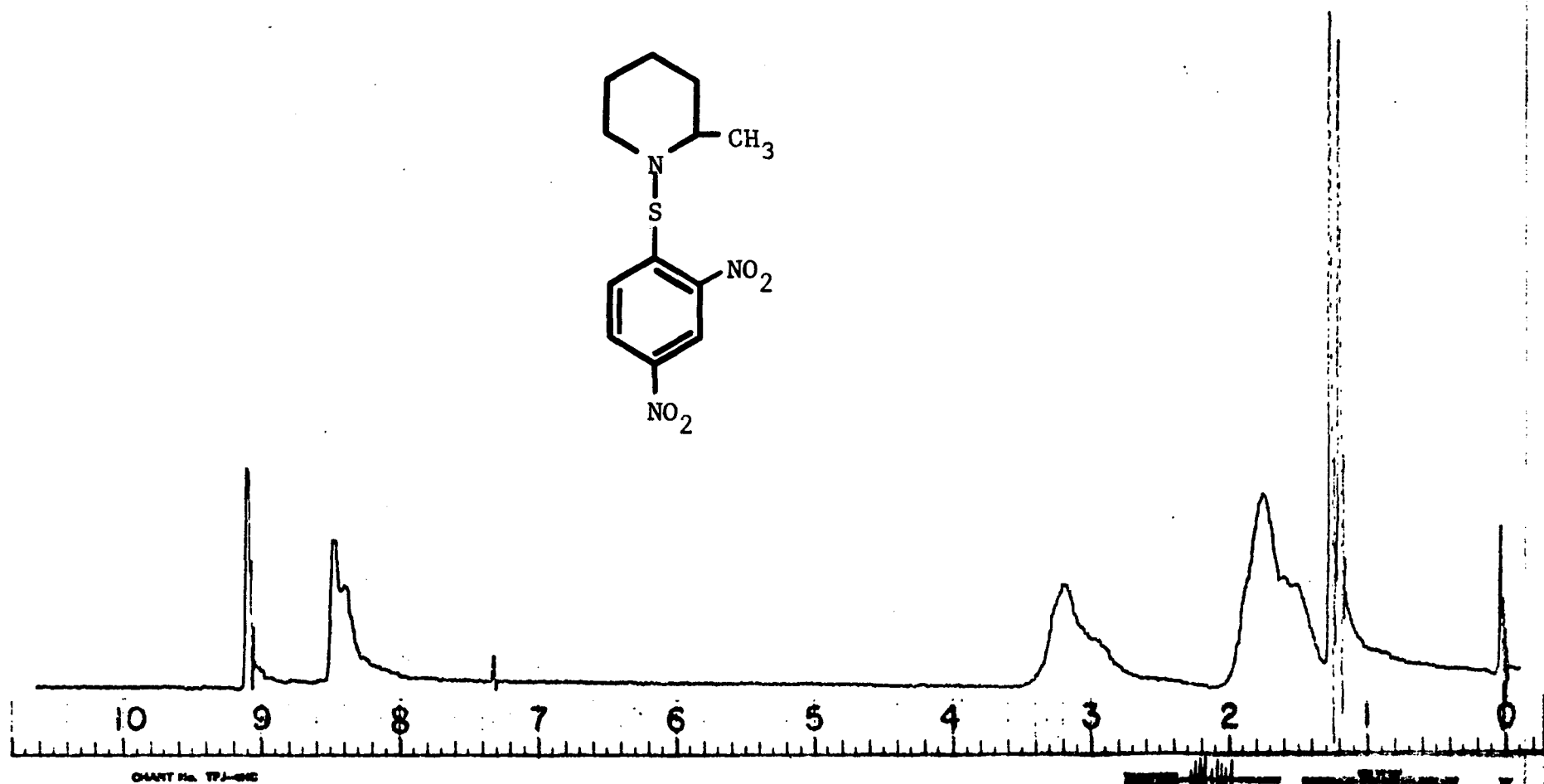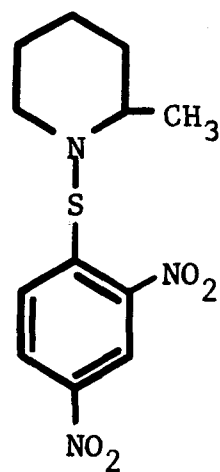
B - Figure 2 (CDCl$_3$) Compound 19

277
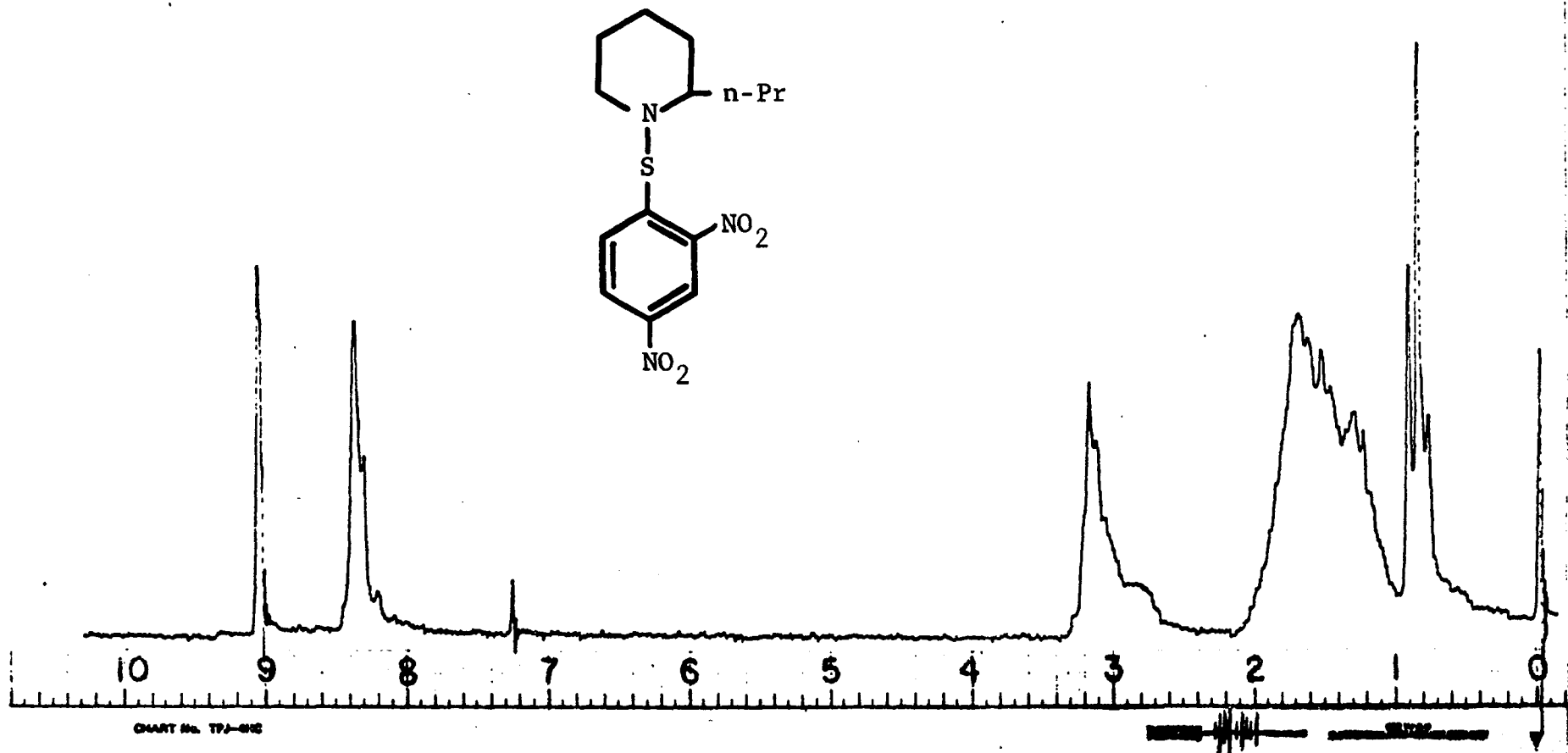
B - Figure 3   (CDCl$_3$)   Compound 20
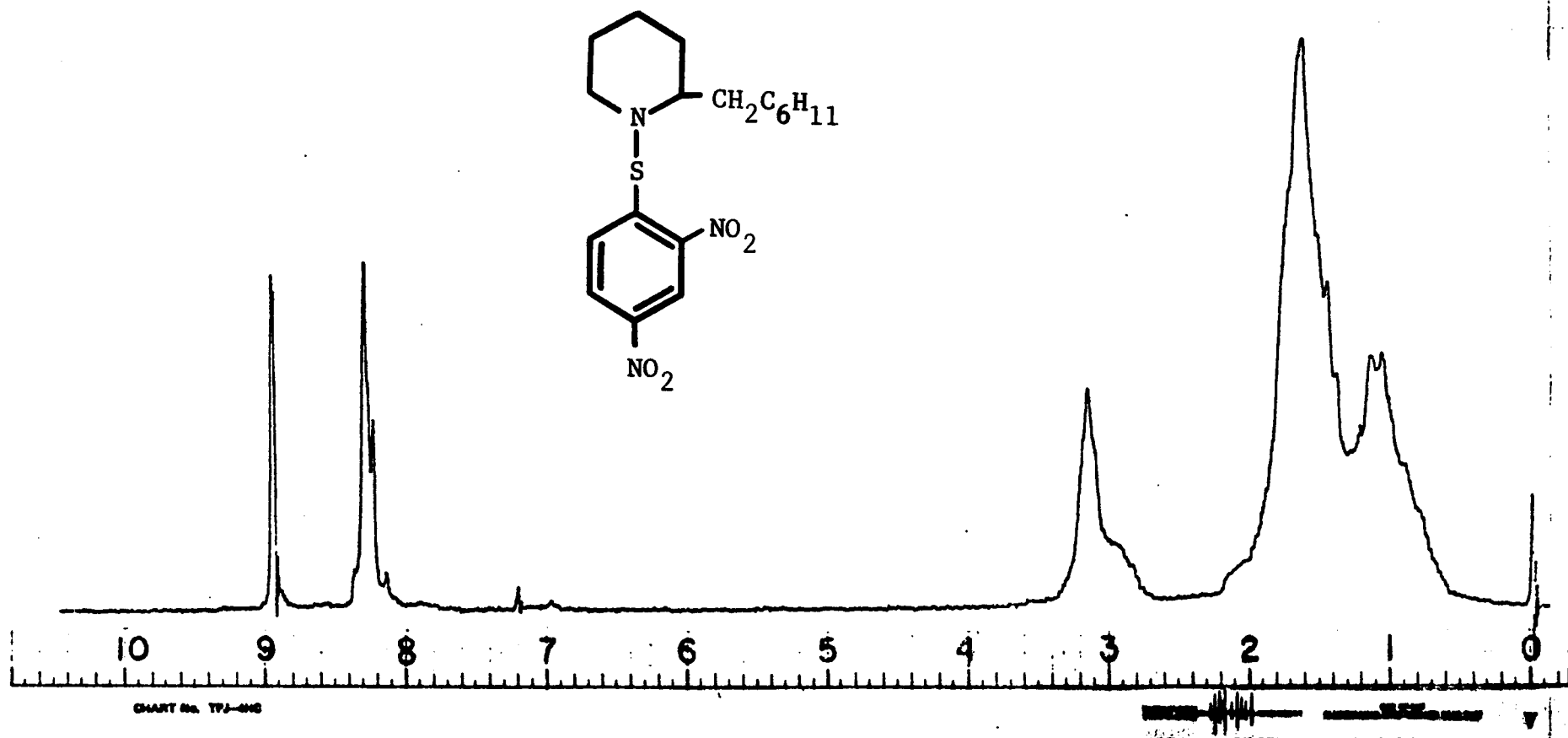
B - Figure 4   (CDCl₃)   Compound 24
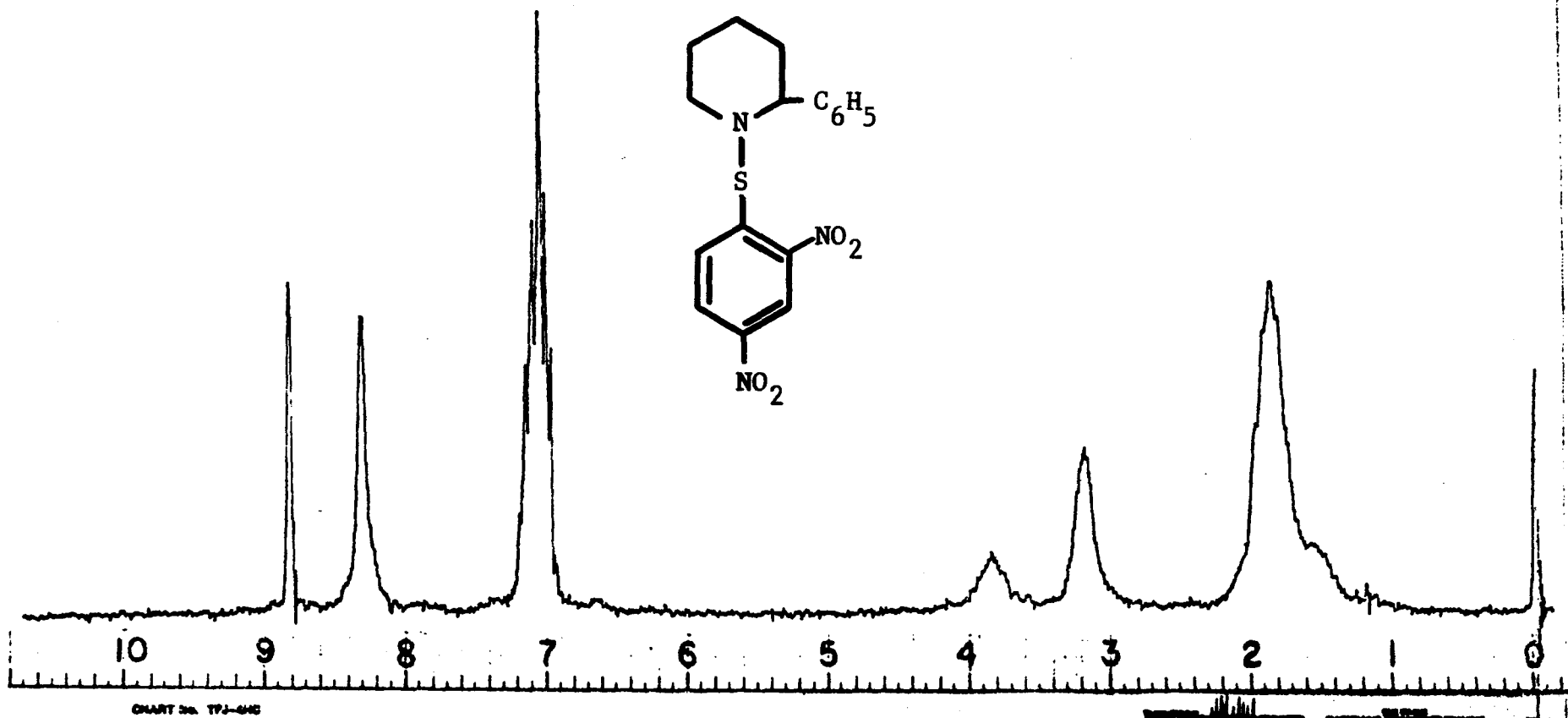
B - Figure 5 (CDCl$_3$) Compound 25
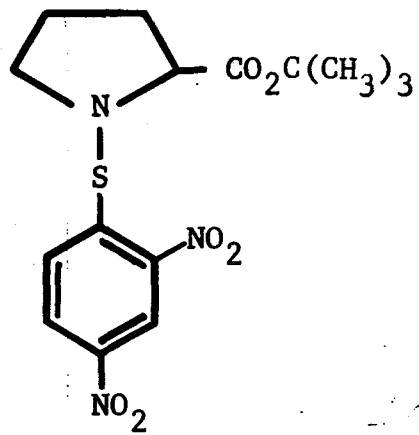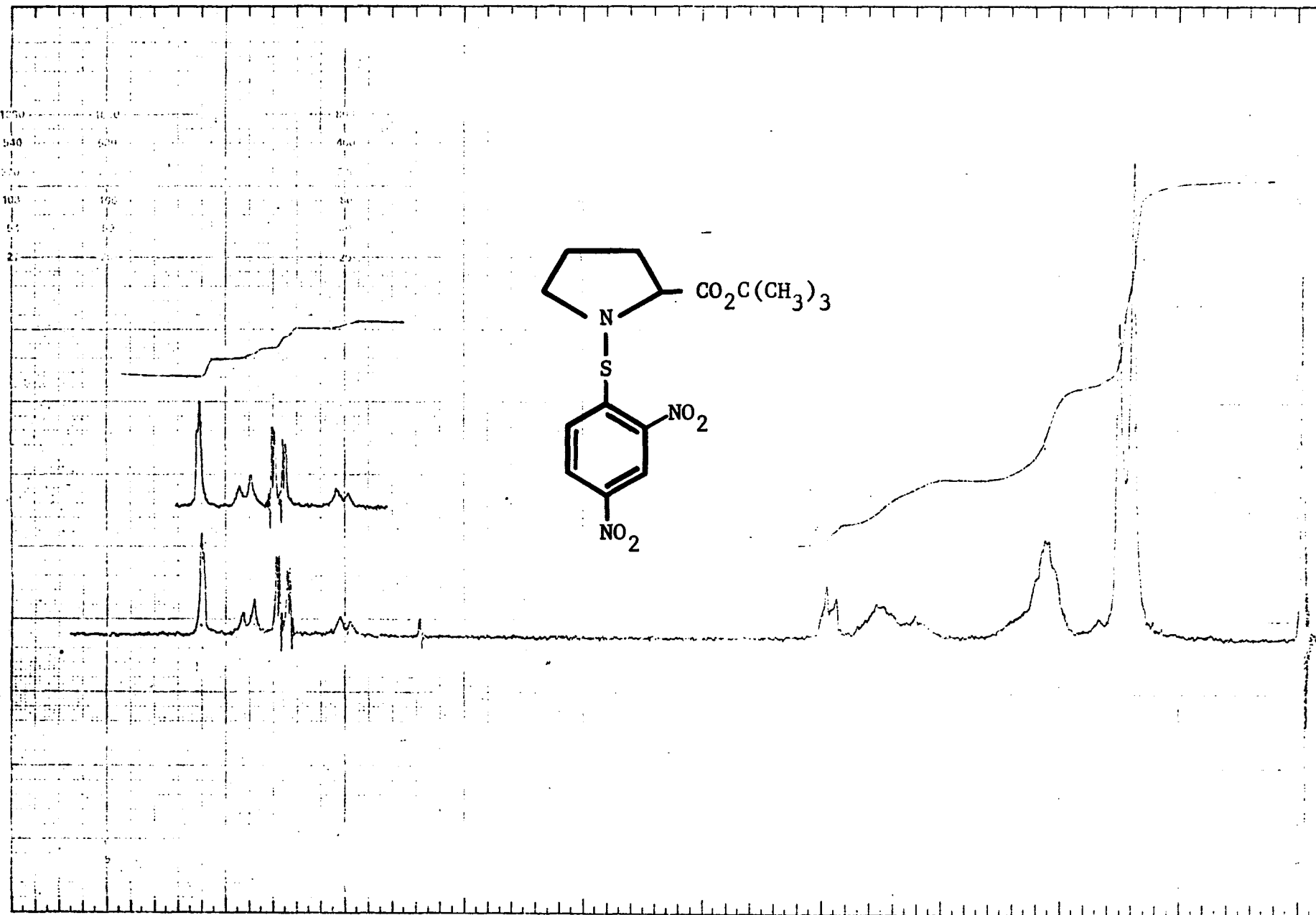
B - Figure 6   (CDCl$_3$)   Compound 26
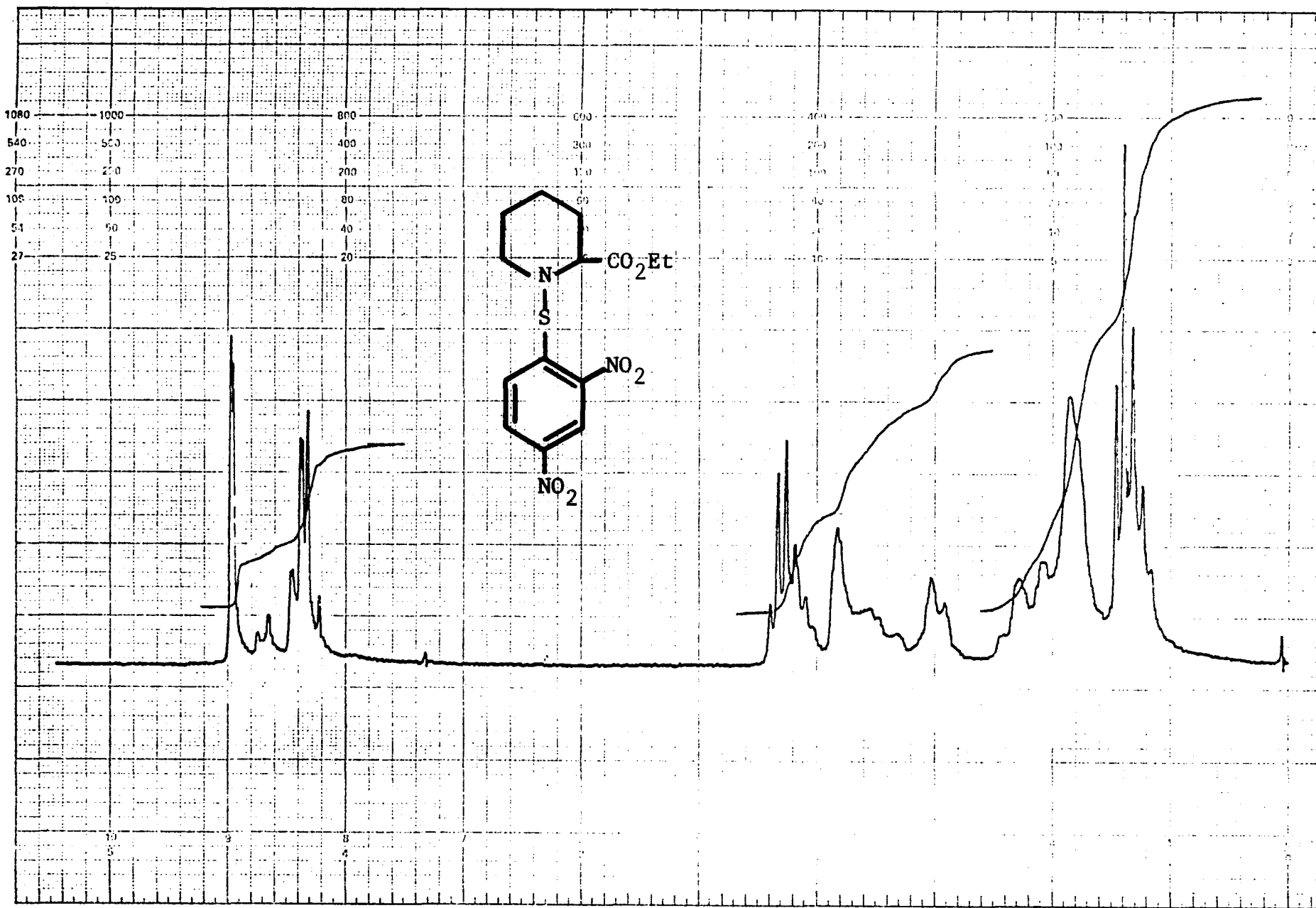
B - Figure 7 (CDCl₃) Compound 29
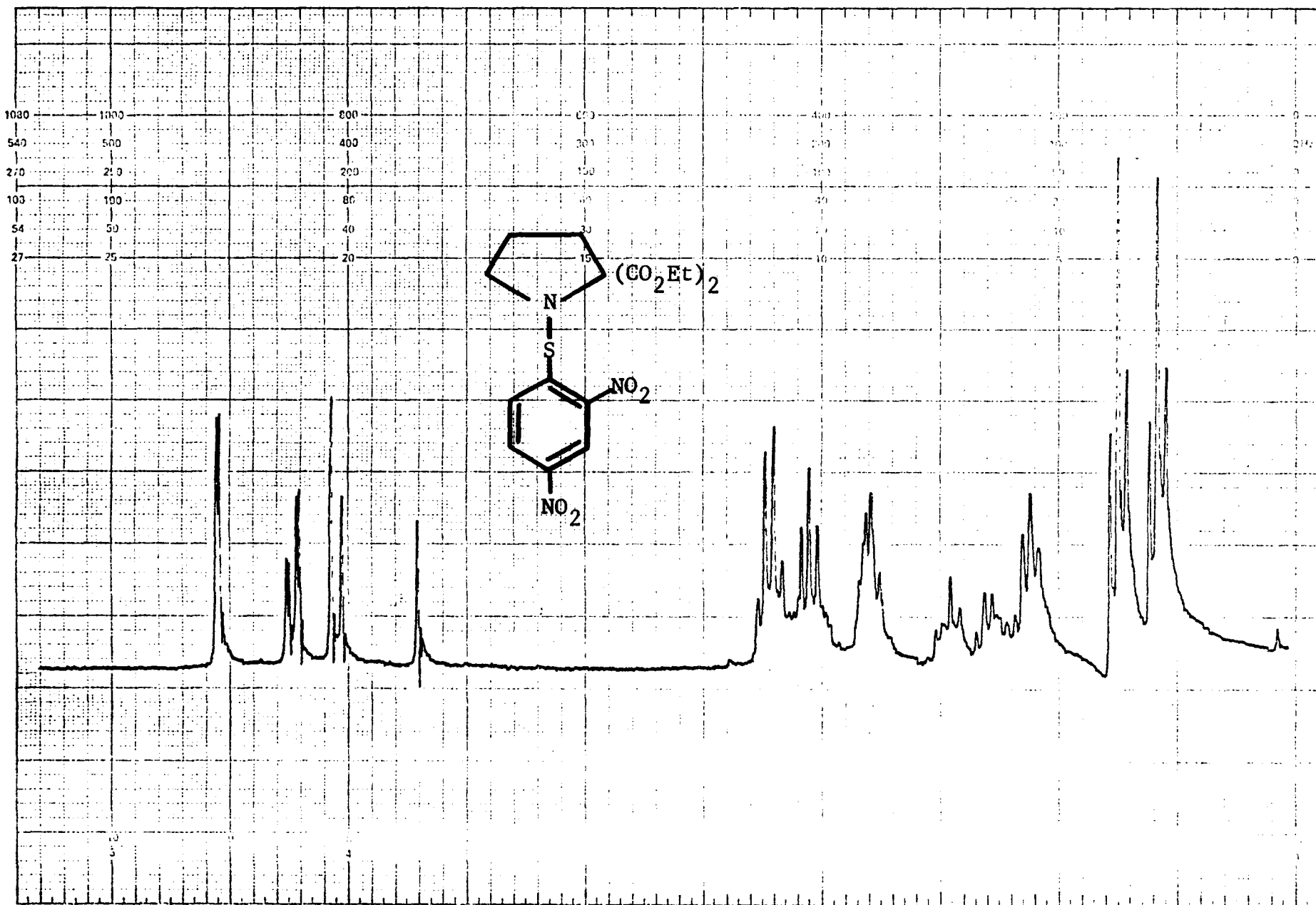
CHART No. TPJ-4HC

B - Figure 8 (CDCl$_3$) Compound 30

A

CHART No. TPJ-4HC
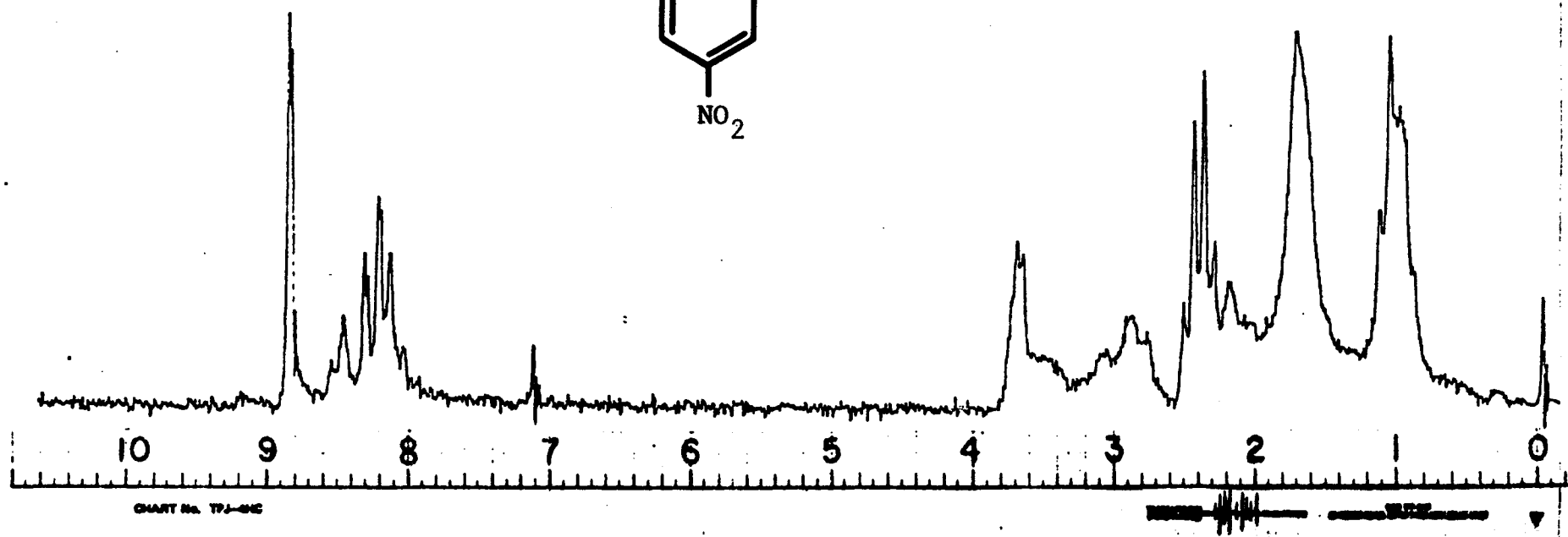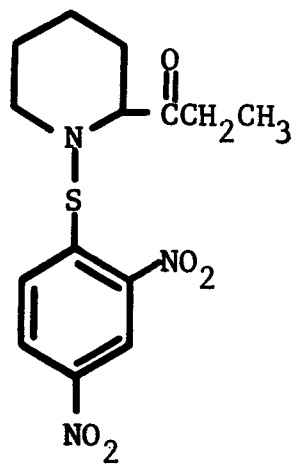
THOMPSON PACKARD

B - Figure 9 (CDCl$_3$) Compound 37
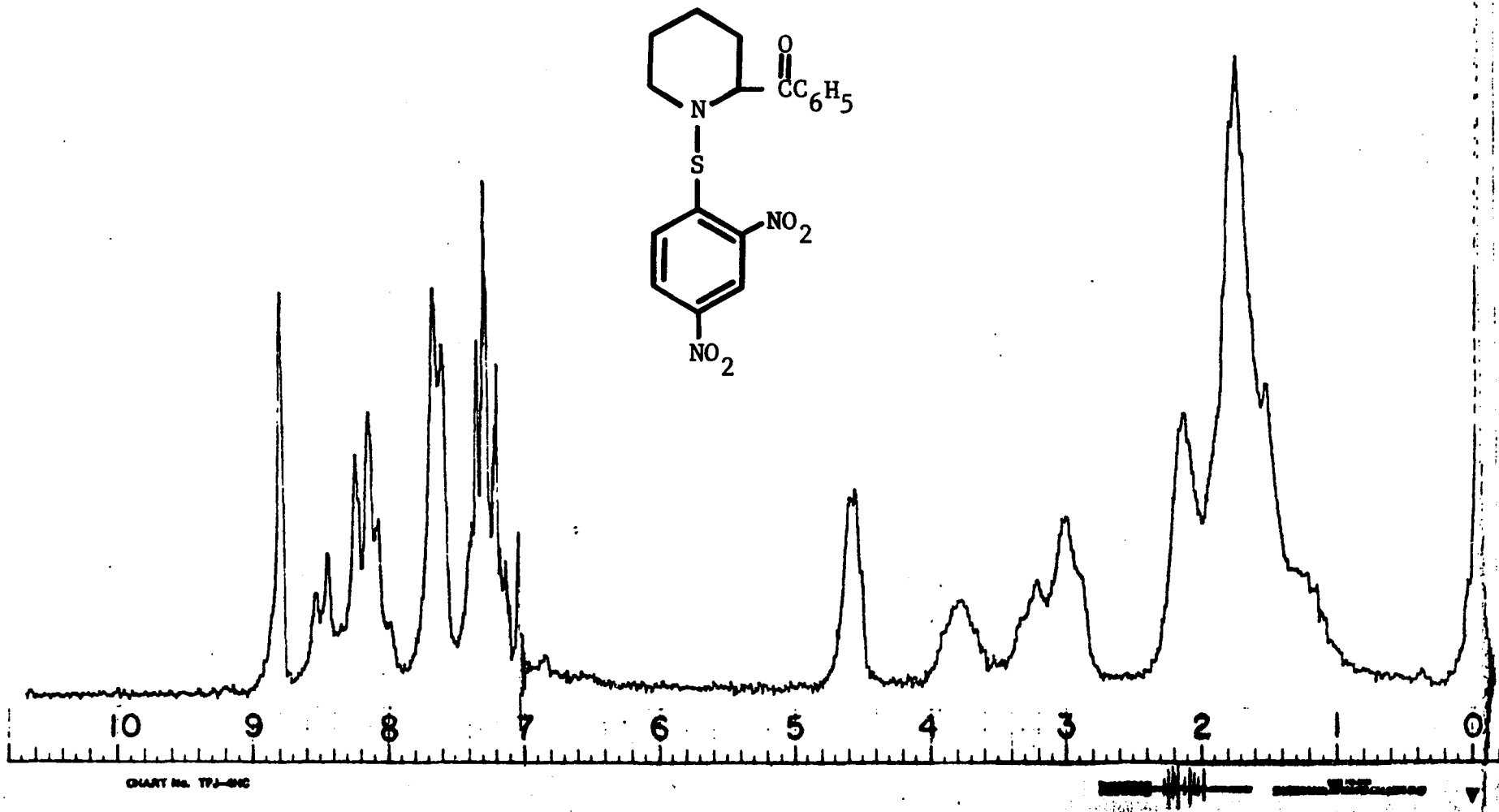
B - Figure 10   (CDCl$_3$)   Compound 42
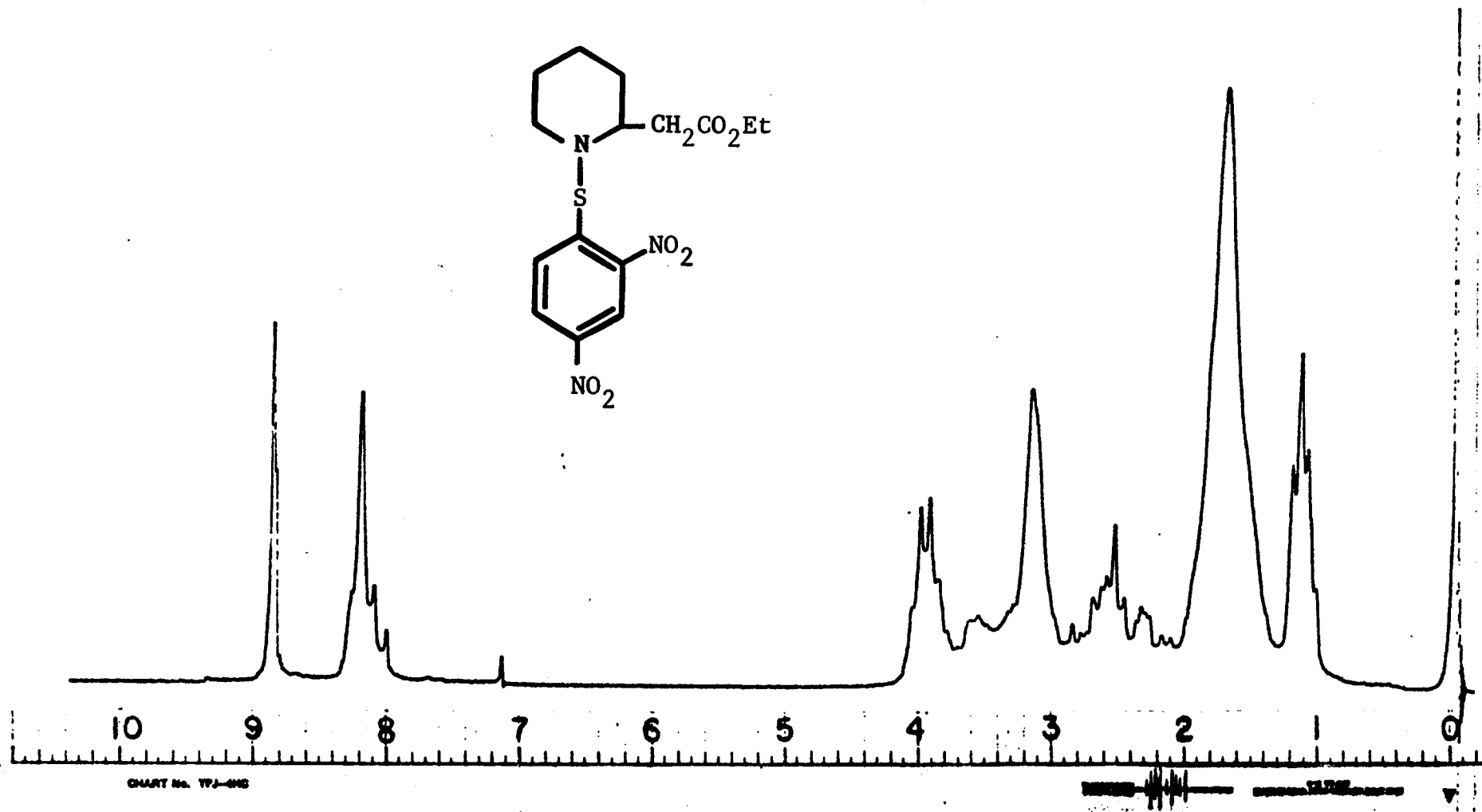
B – Figure 11 (CDCl$_3$) Compound 38
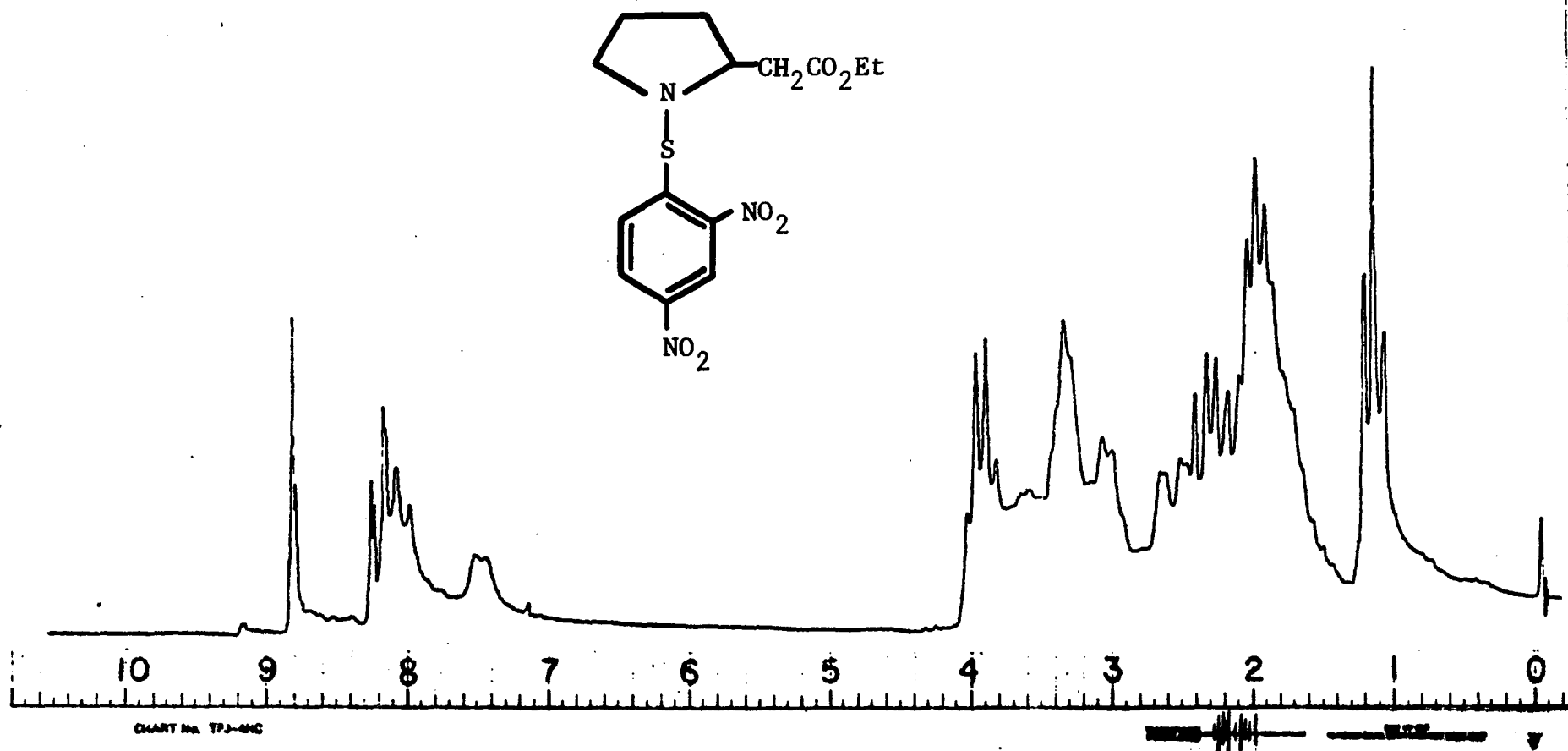
B - Figure 12   (CDCl$_3$)   Compound 39
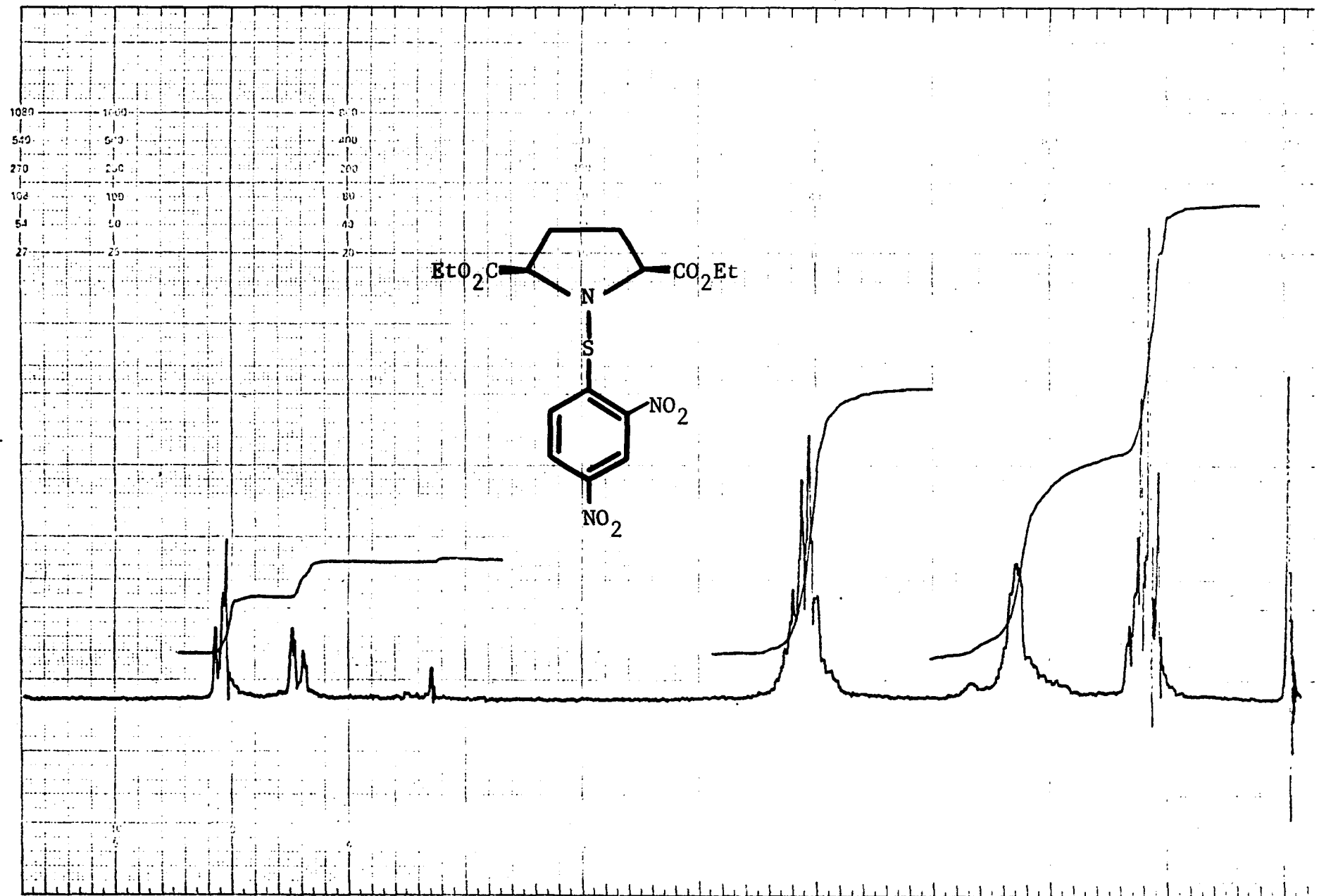
B - Figure 13 (CDCl$_3$) Compound <u>40</u>
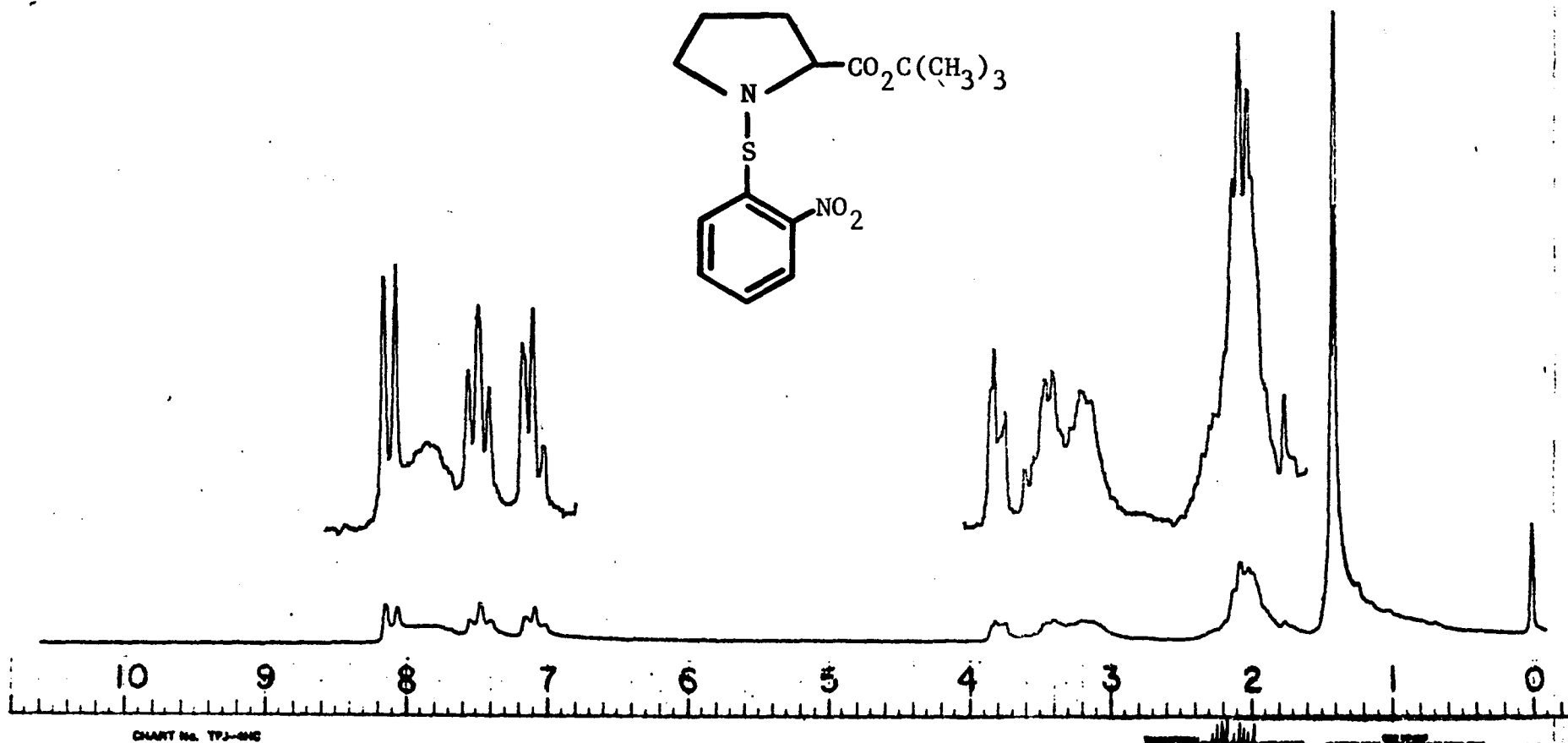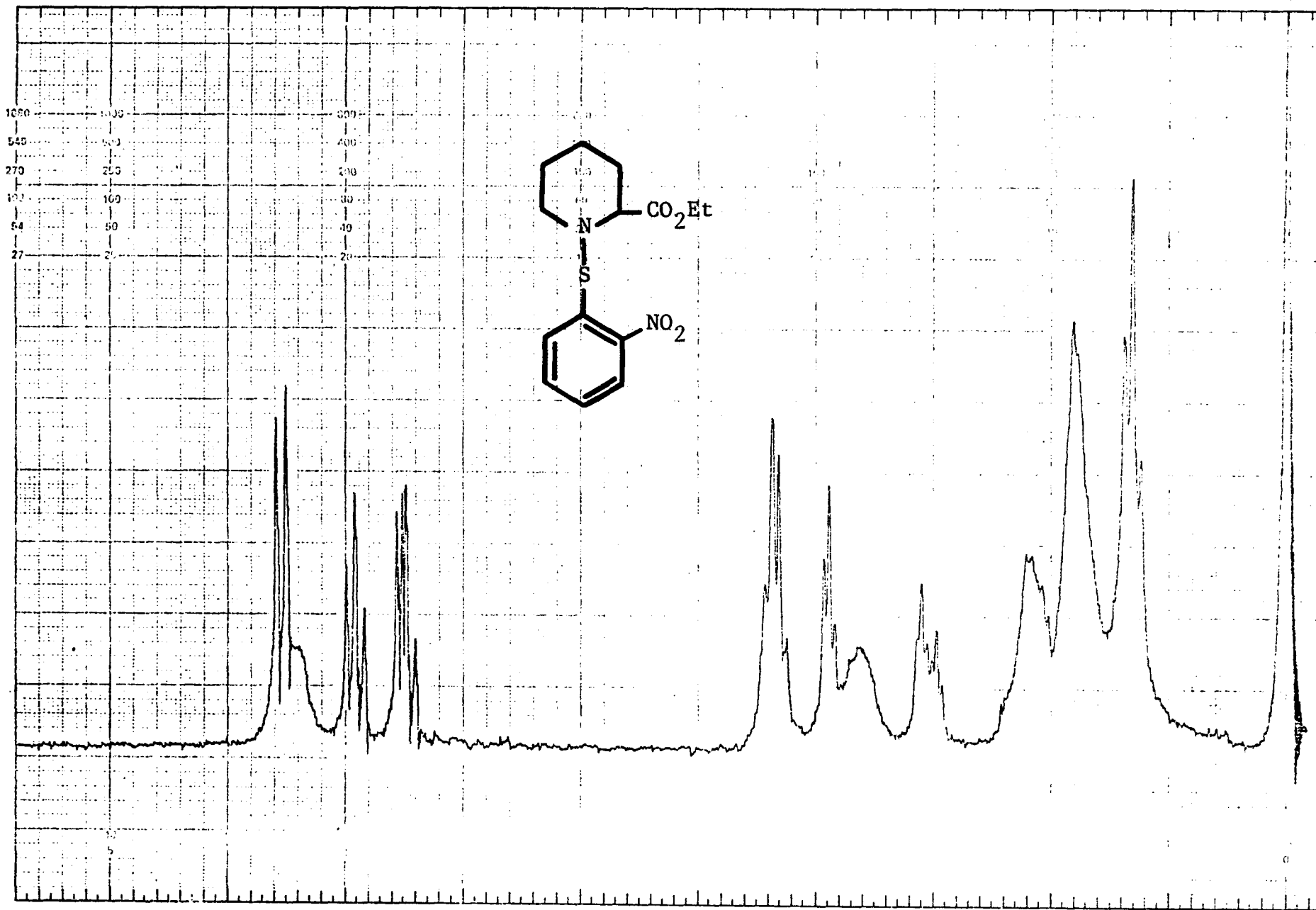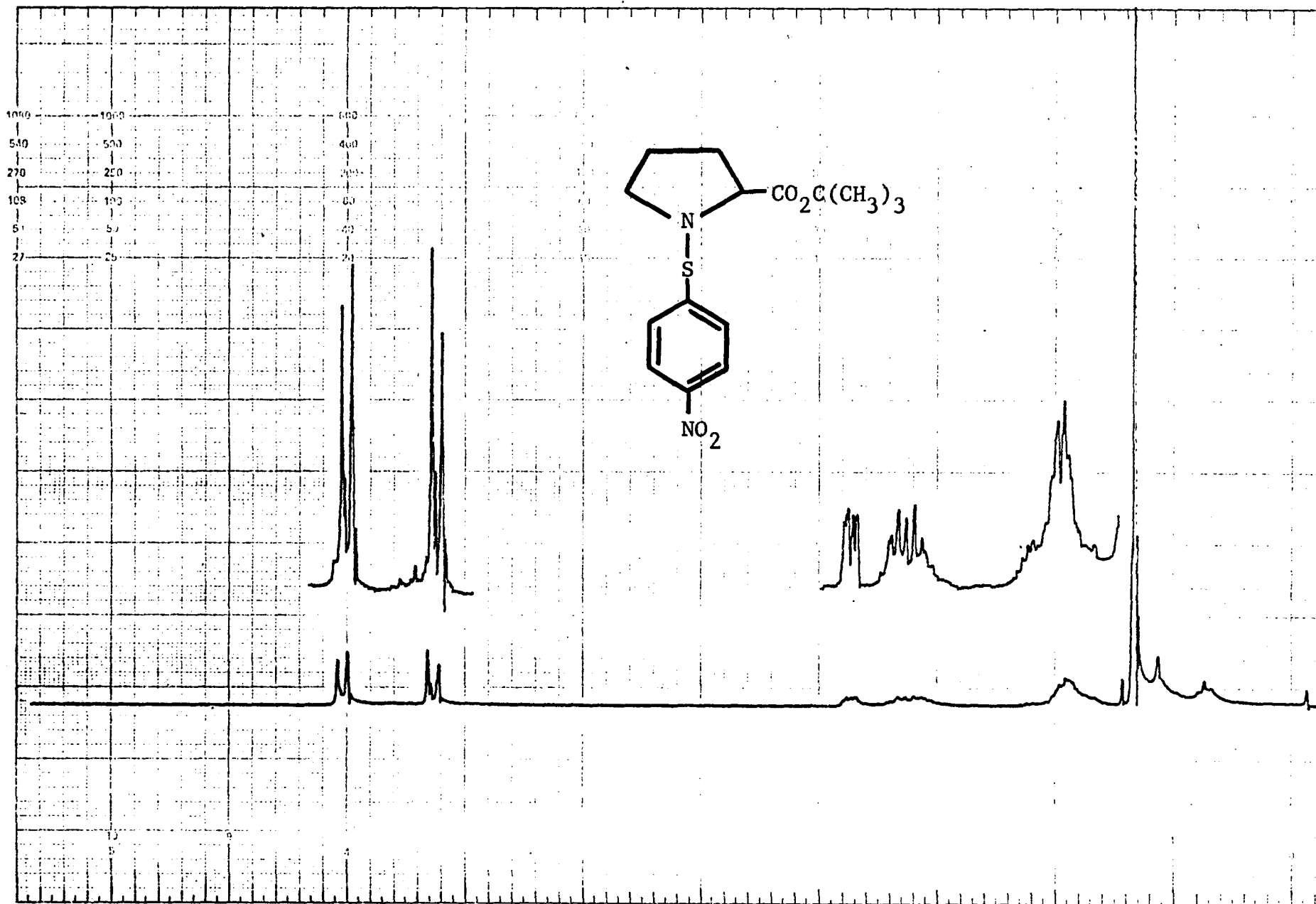
B - Figure 14   (CDCl₃)   Compound 44
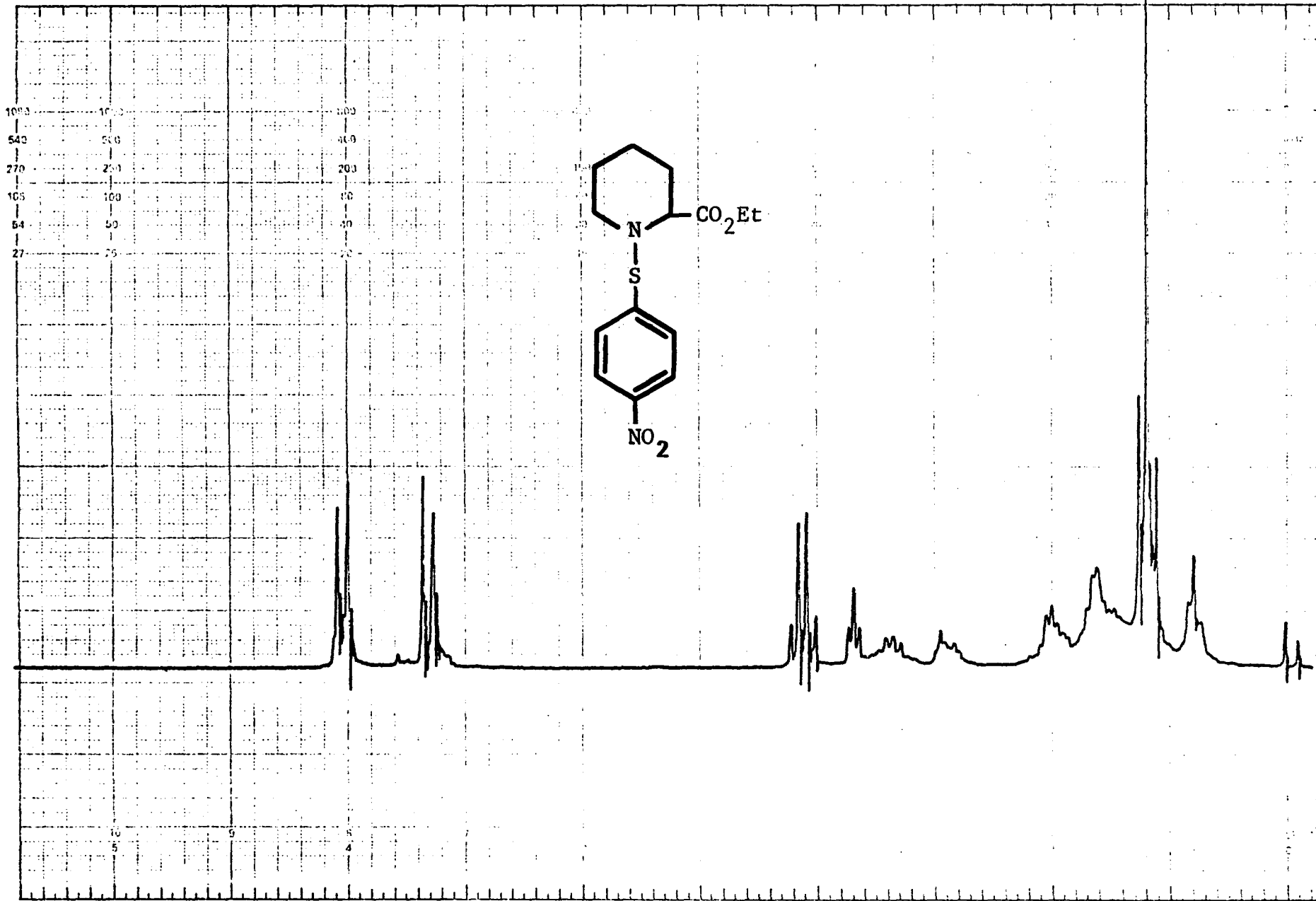
B - Figure 15   (CDCl$_3$)   Compound 45

B - Figure 16 (CDCl$_3$) Compound 47

B - **Figure 17** (CDCl$_3$) Compound $\underline{48}$
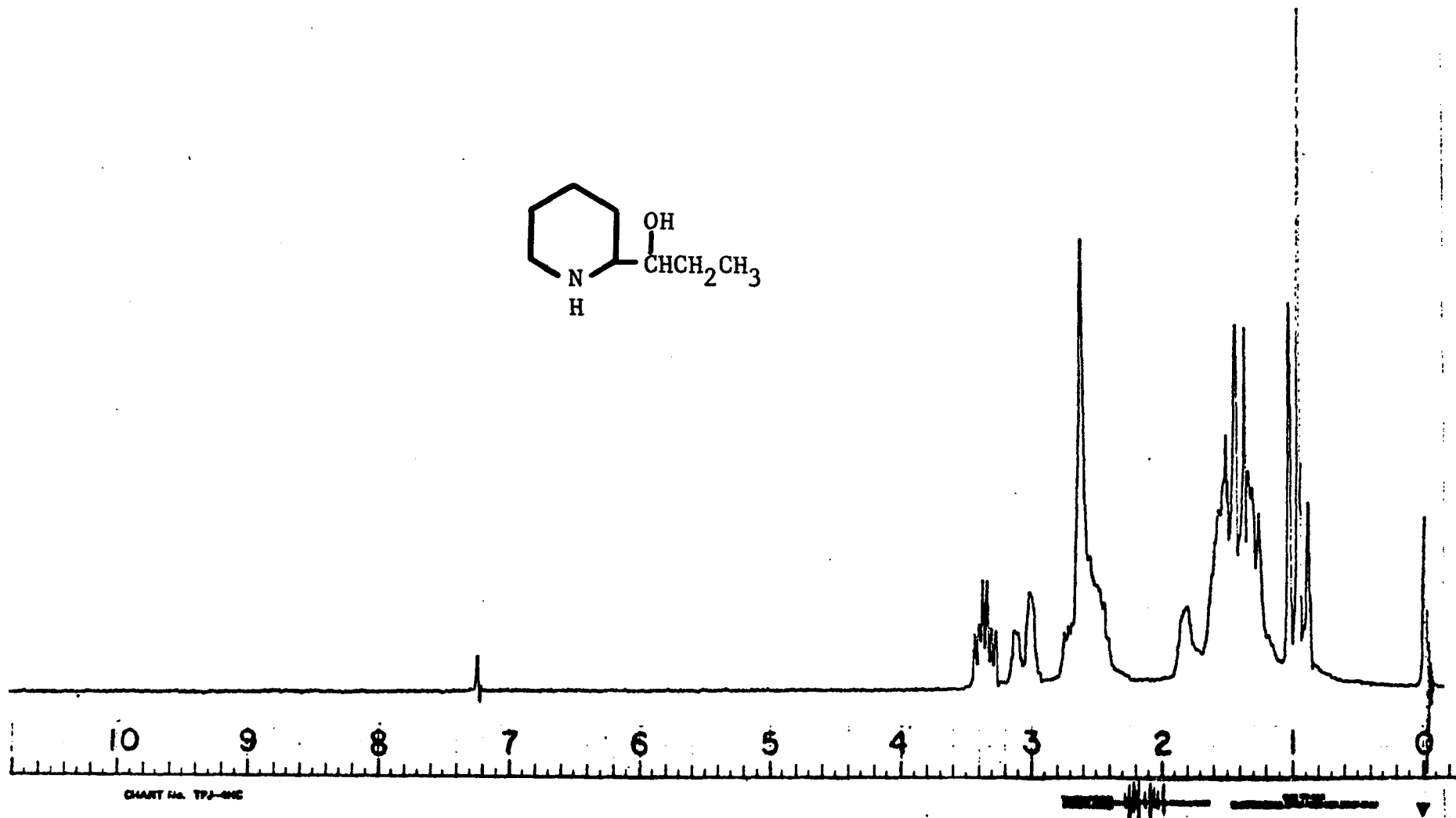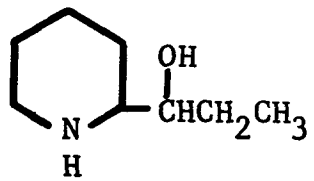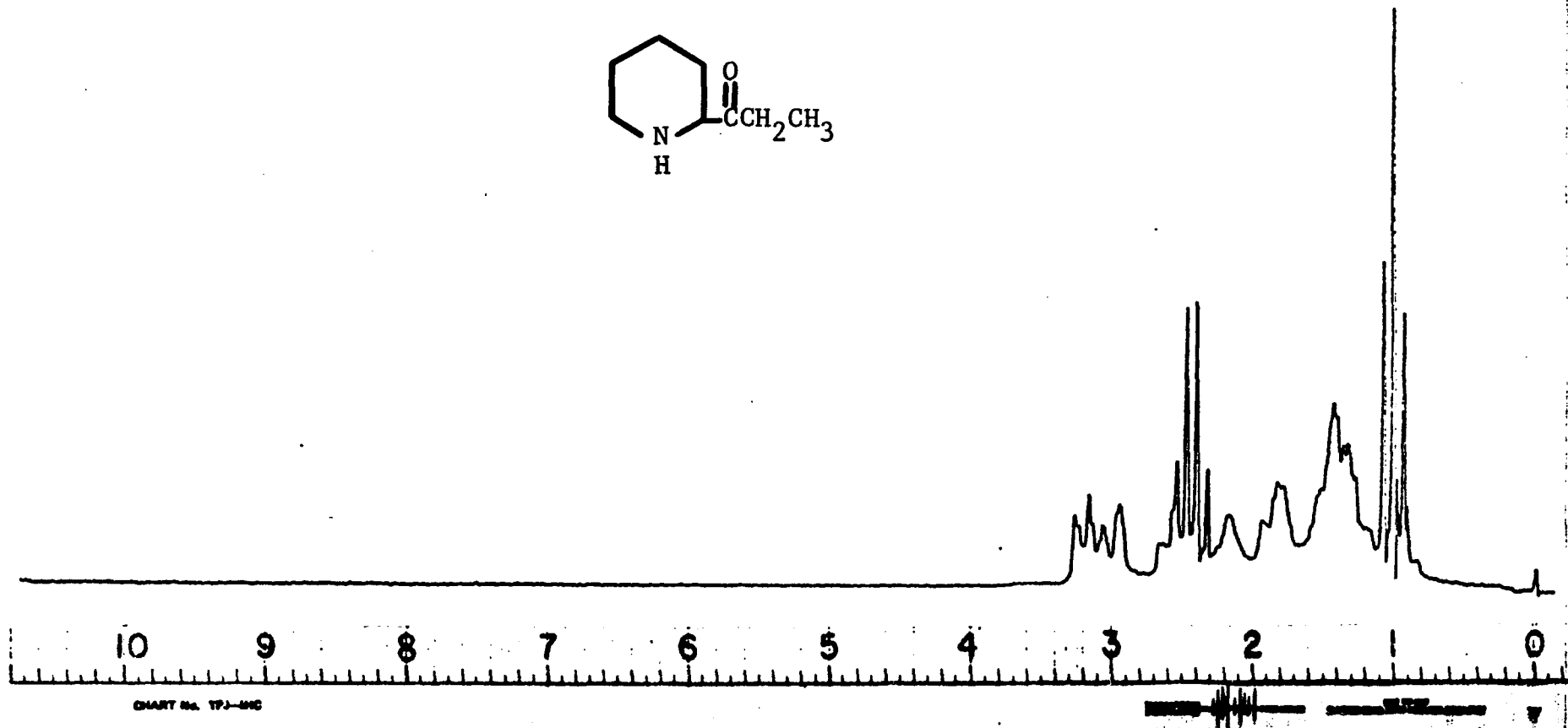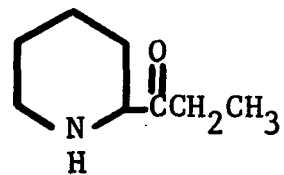
B - Figure 18   (CDCl$_3$)   Compound 79

B - Figure 19   (CDCl$_3$)   Compound $\underline{33}$
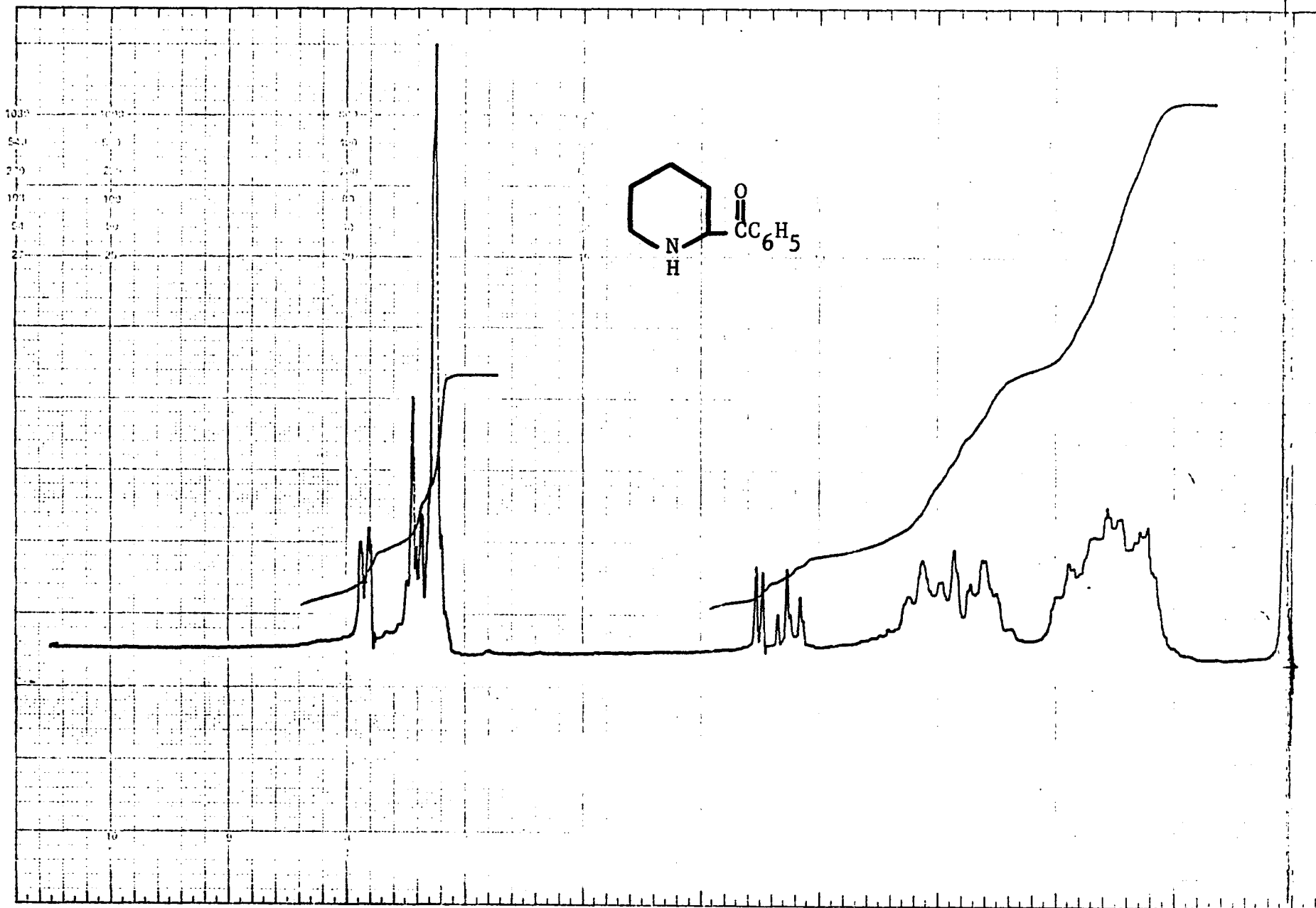
B - Figure 20 (CDCl$_3$) Compound 41
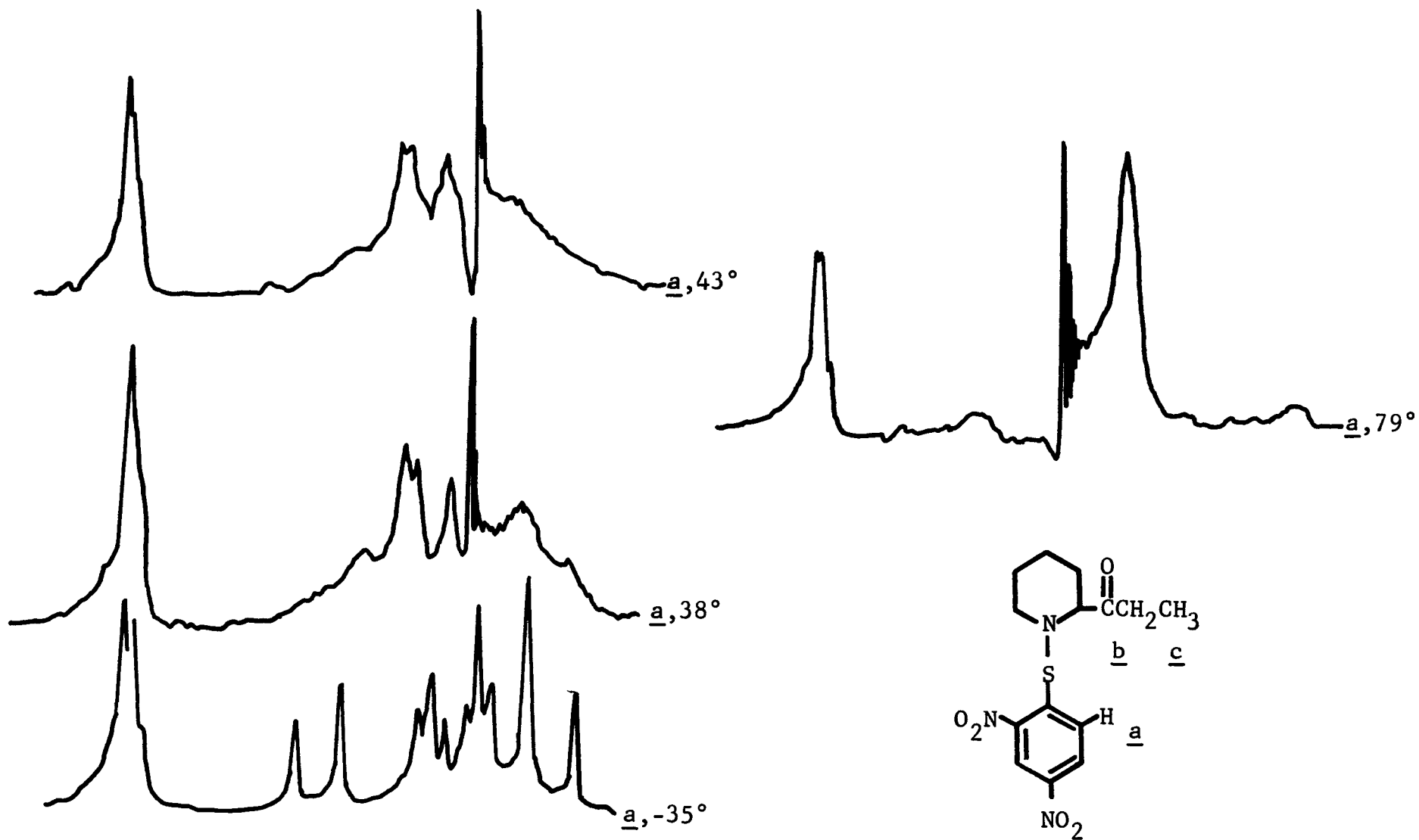
C – Figure 1. Compound 37
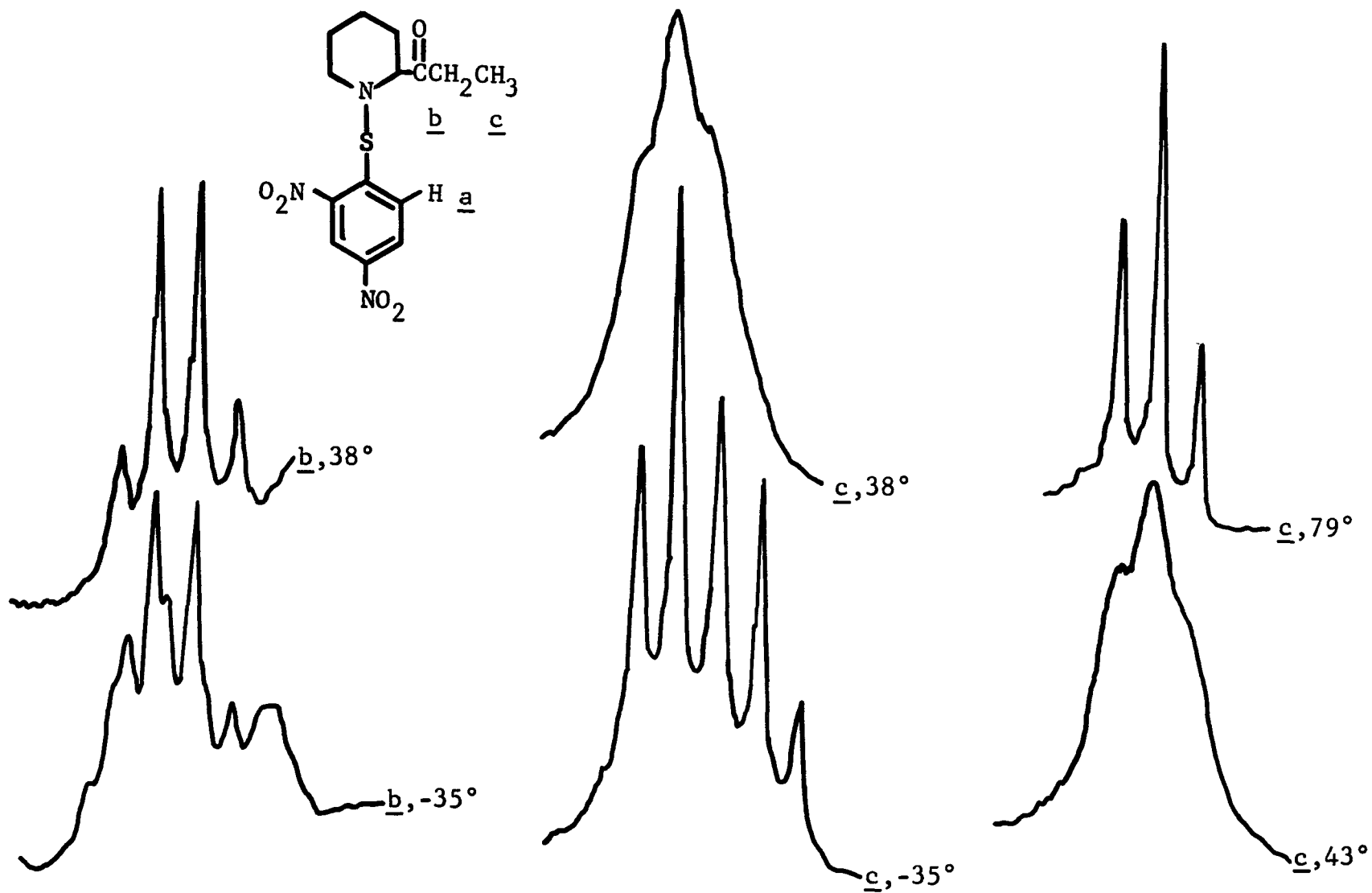
C - Figure 2. Compound 37

a,119.5°

a,43°

a,31°
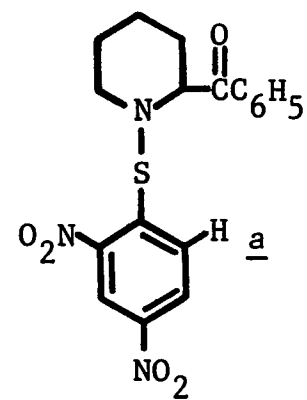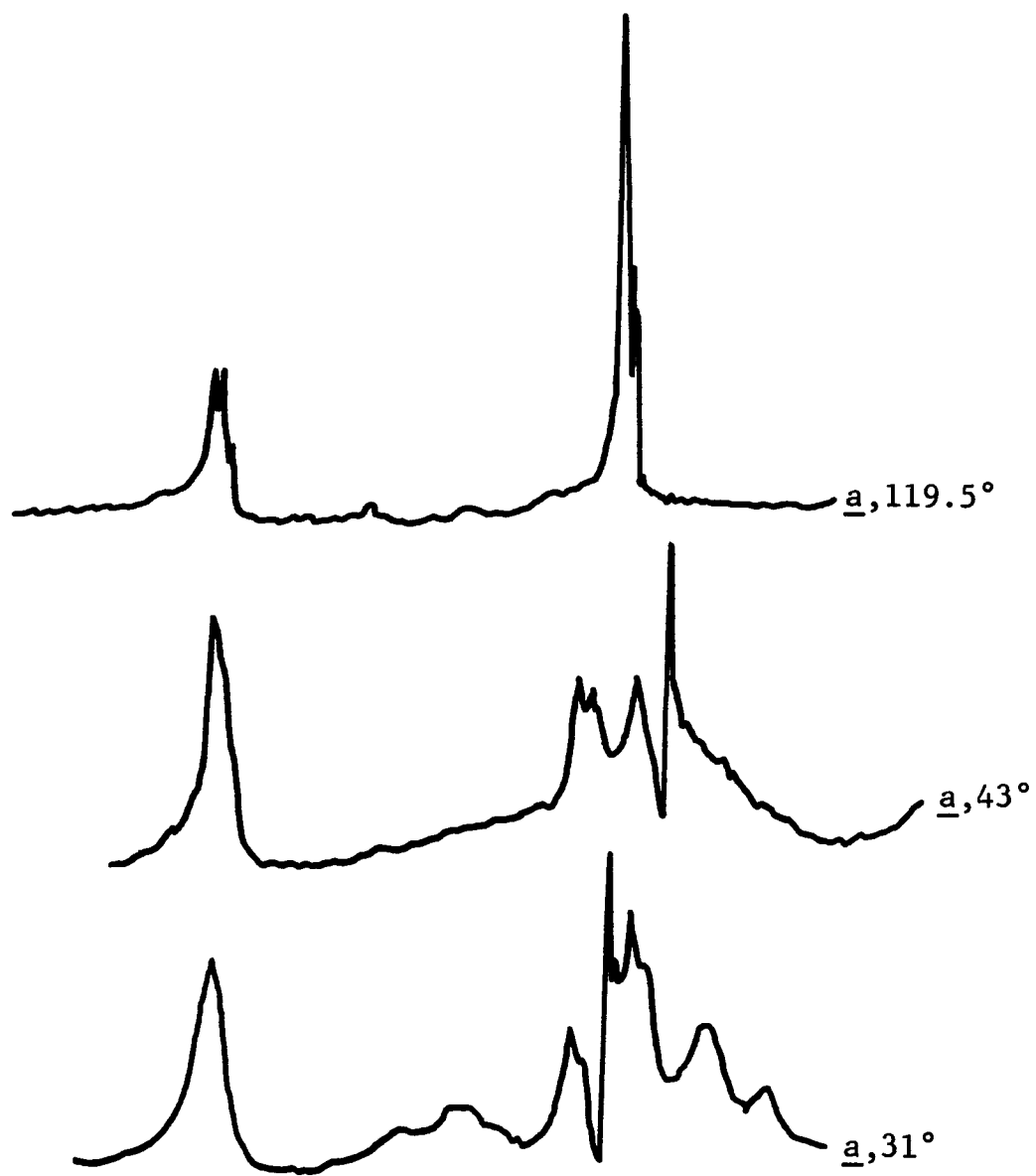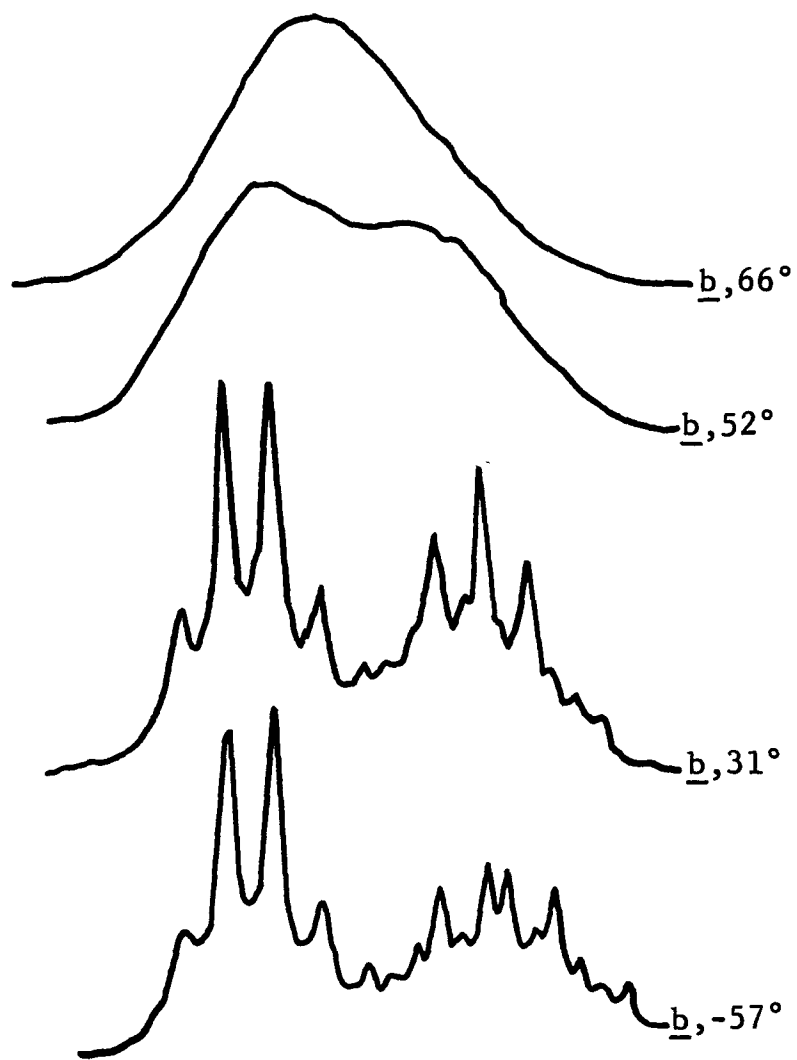
C - Figure 3. Compound 42

a,79°

a,59°

a,52°

a,0°
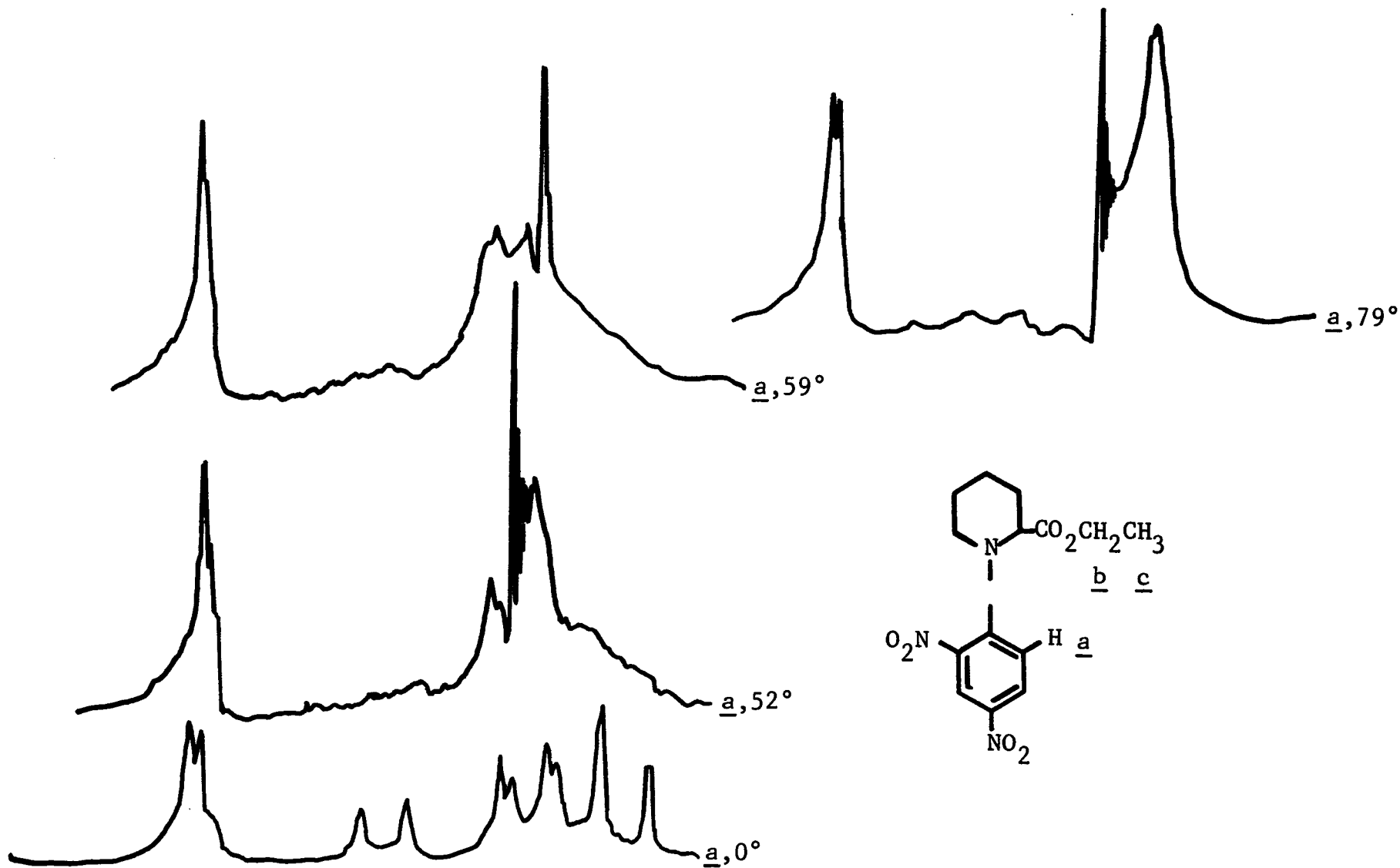
C - Figure 4.   Compound 29
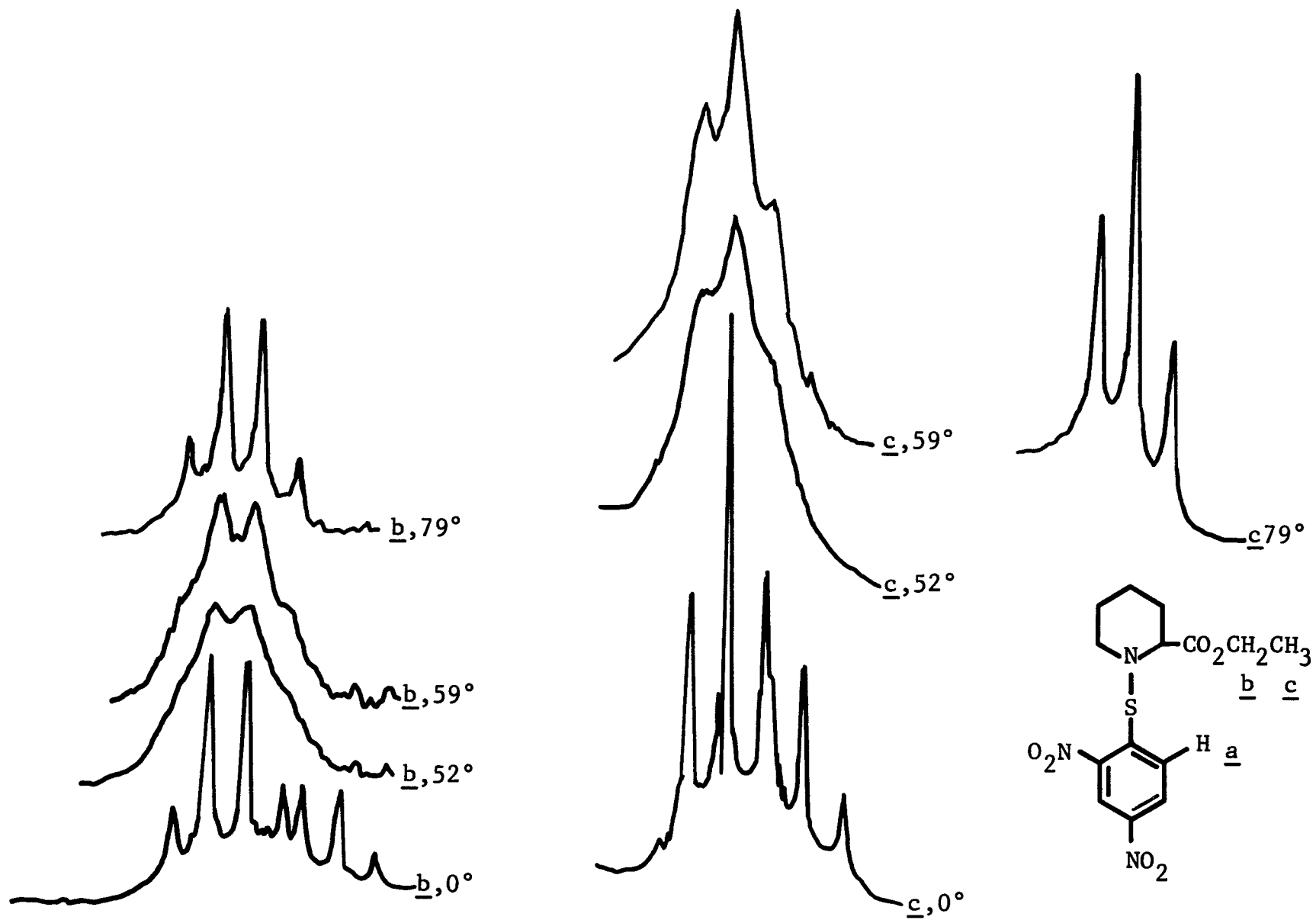
C - Figure 5. Compound 29
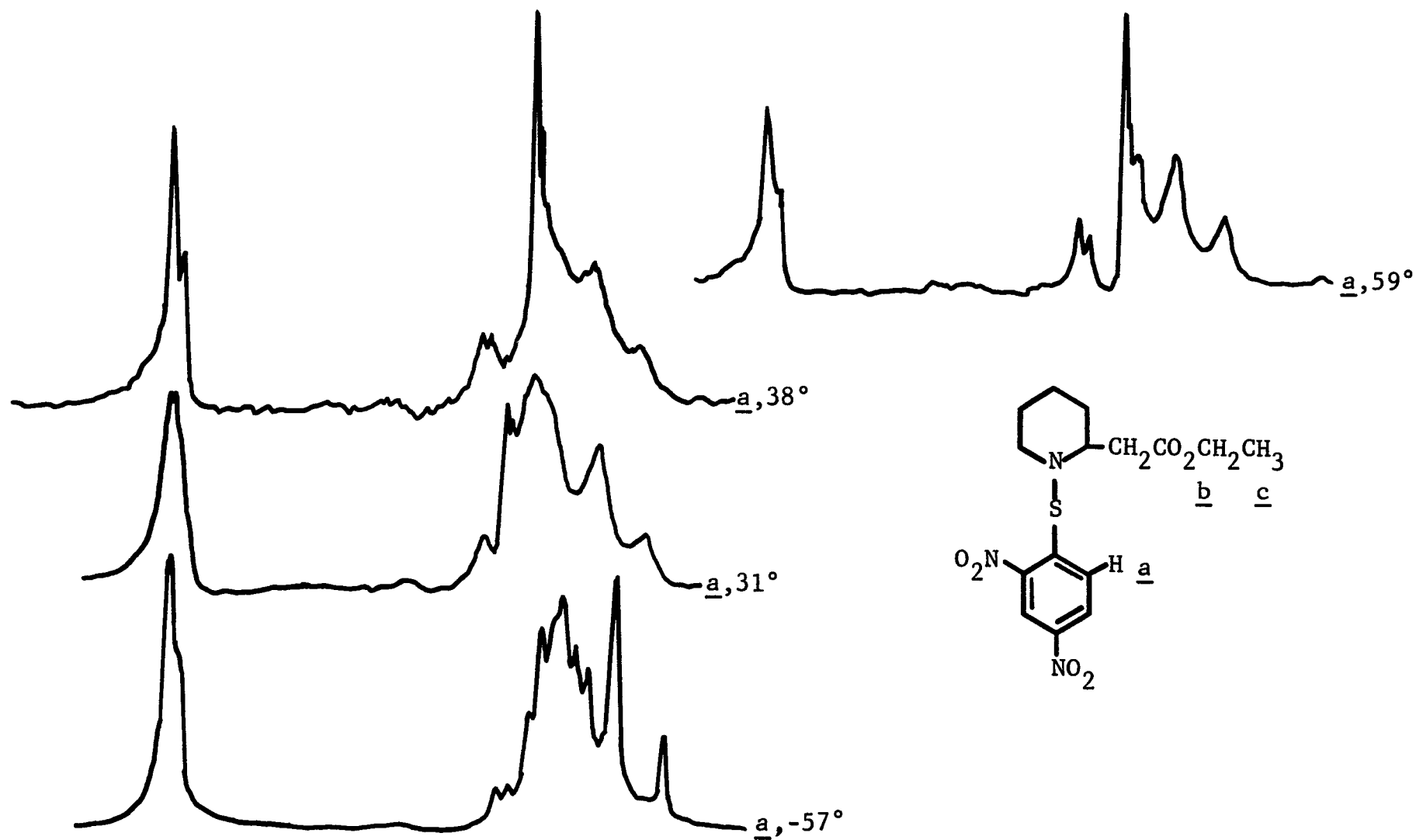
C - Figure 6. Compound <u>38</u>
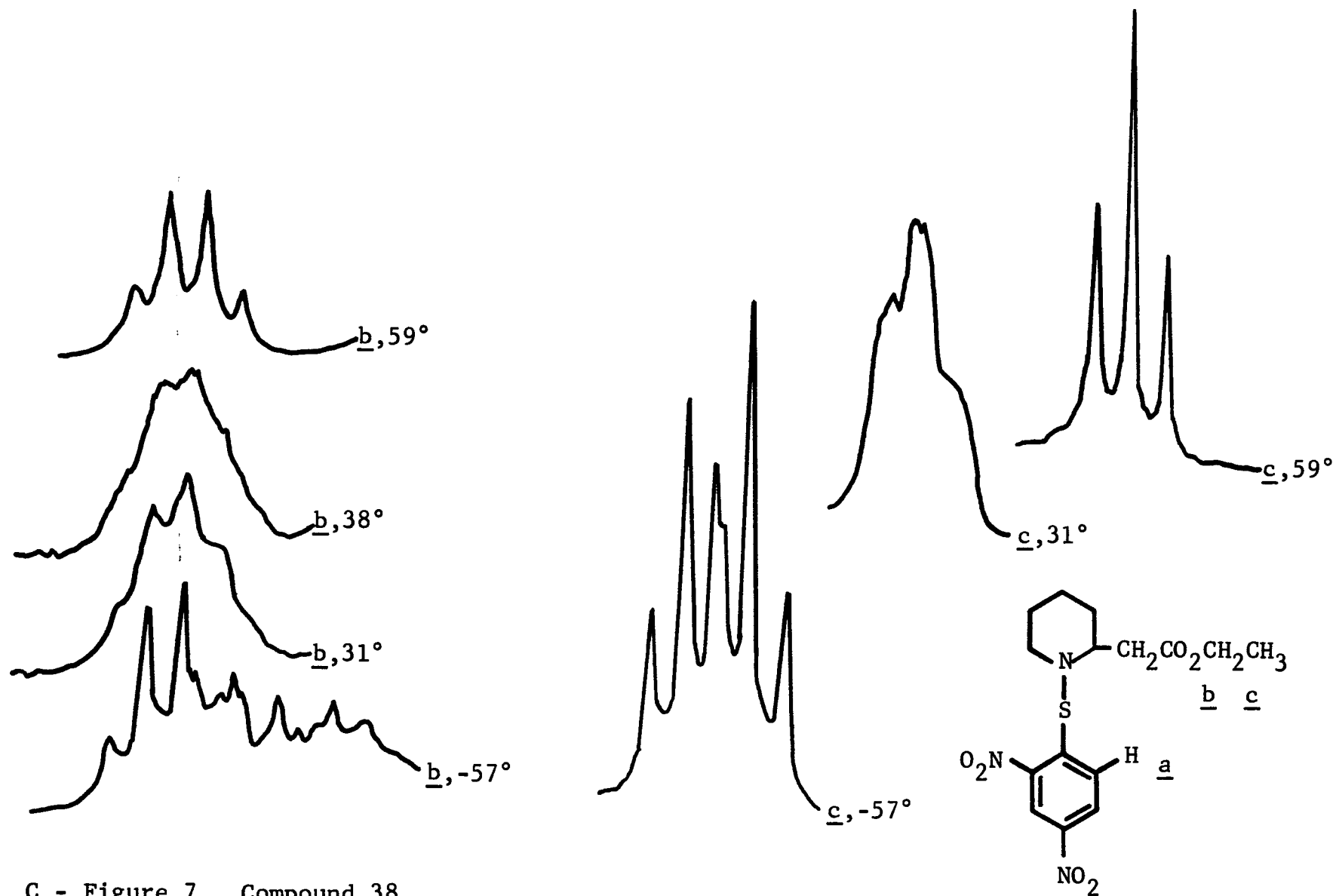
C - Figure 7. Compound 38
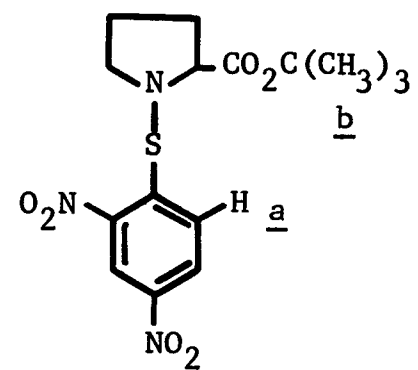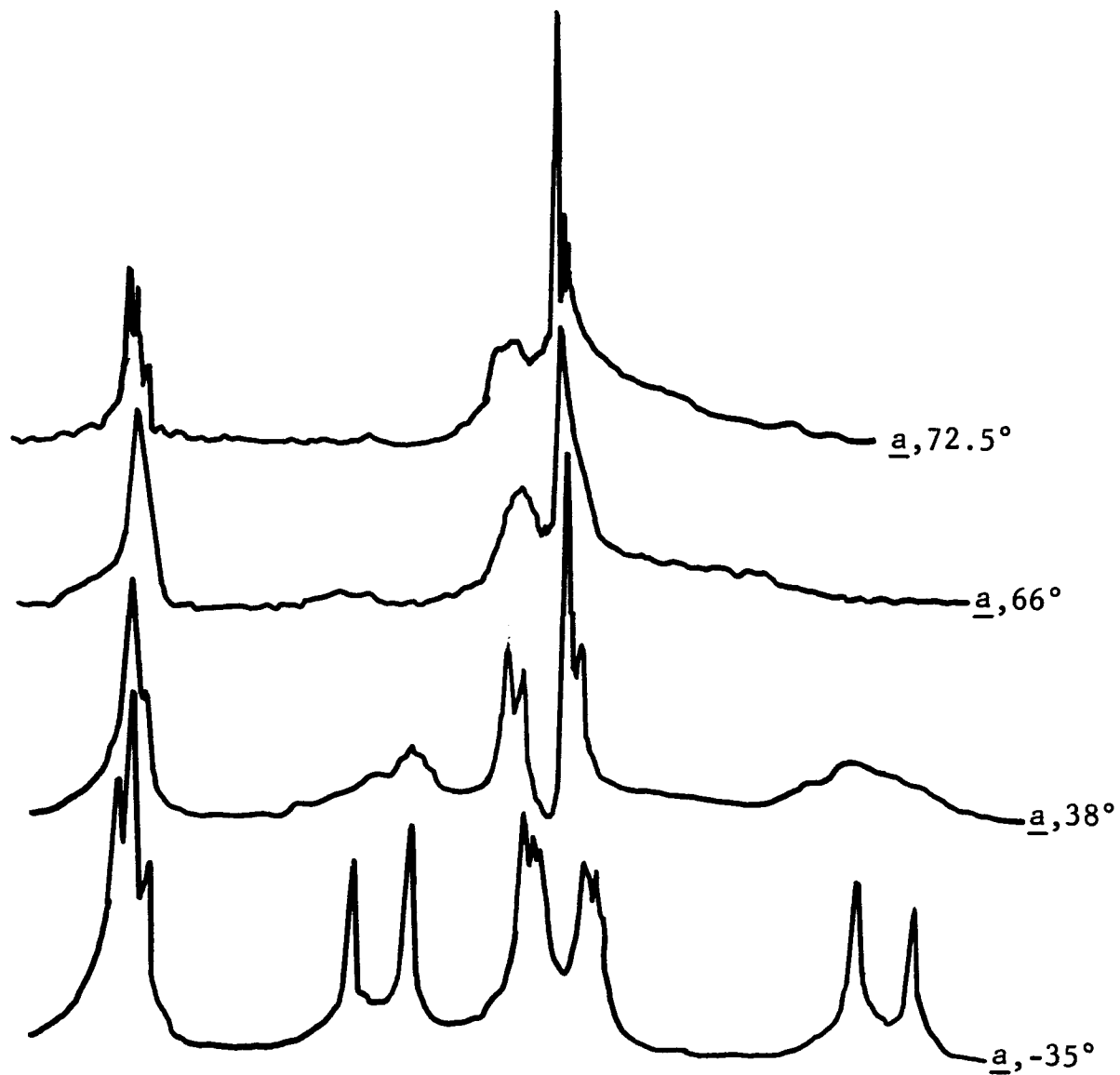
C - Figure 8. Compound 26
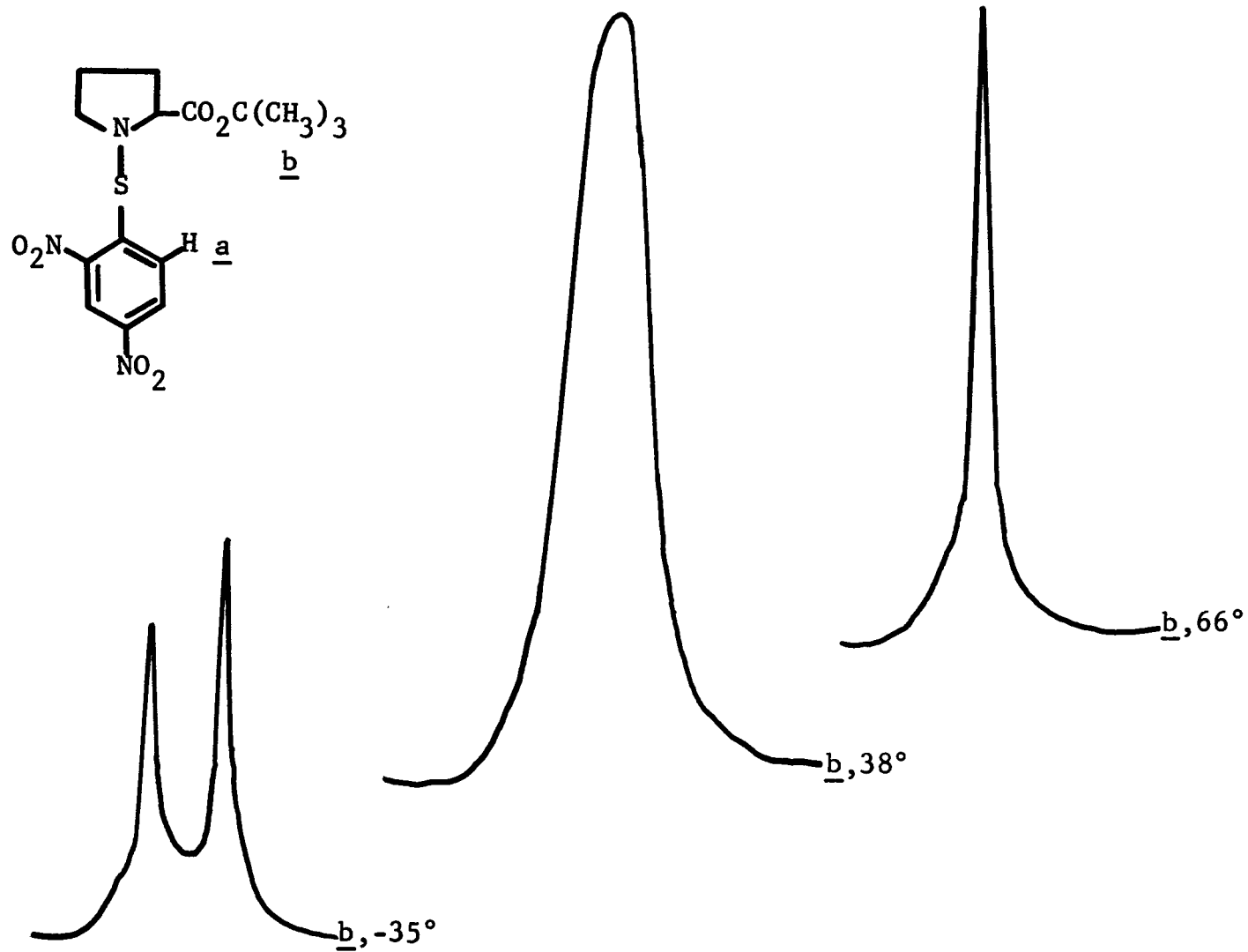
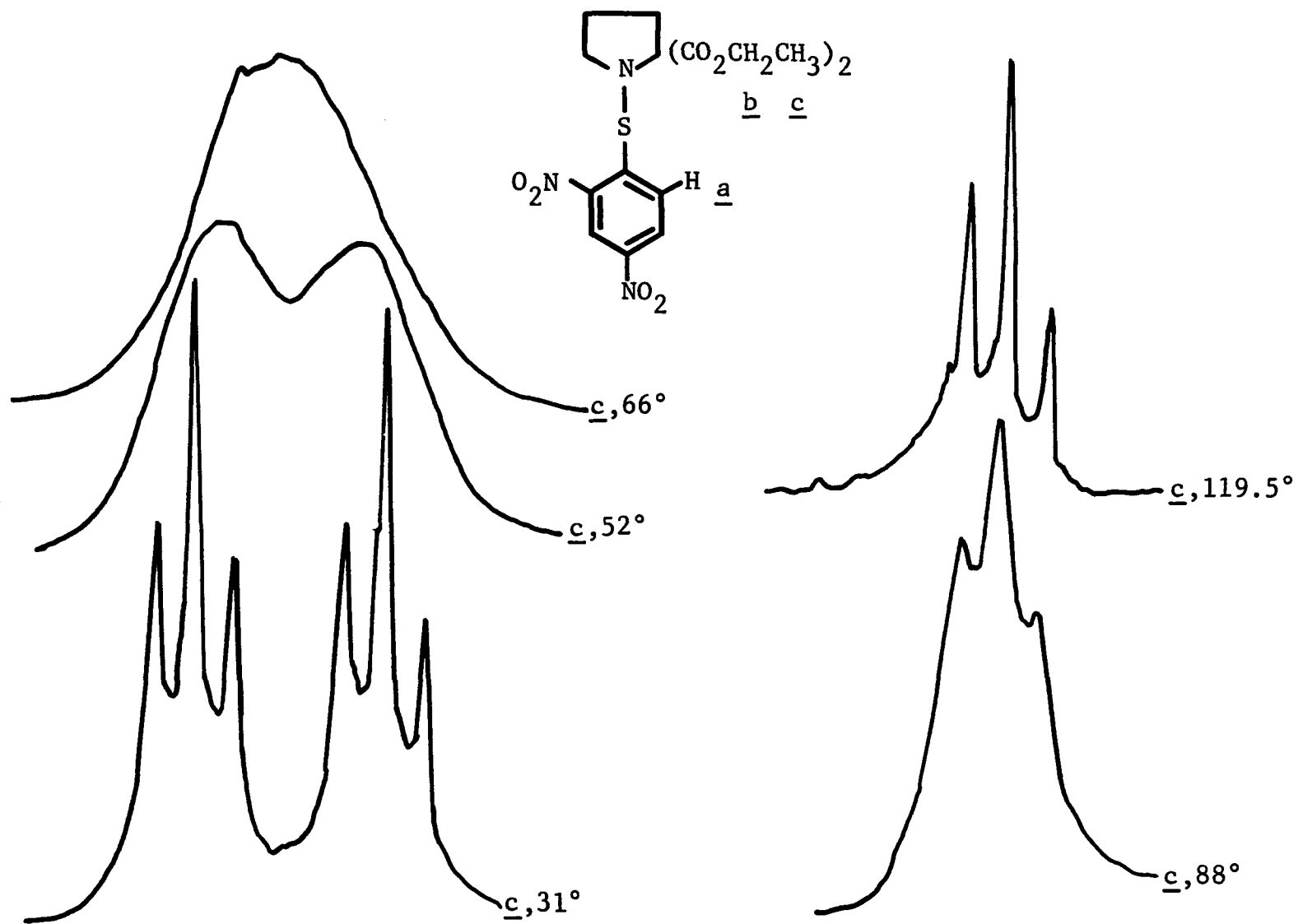C – Figure 9.   Compound 26
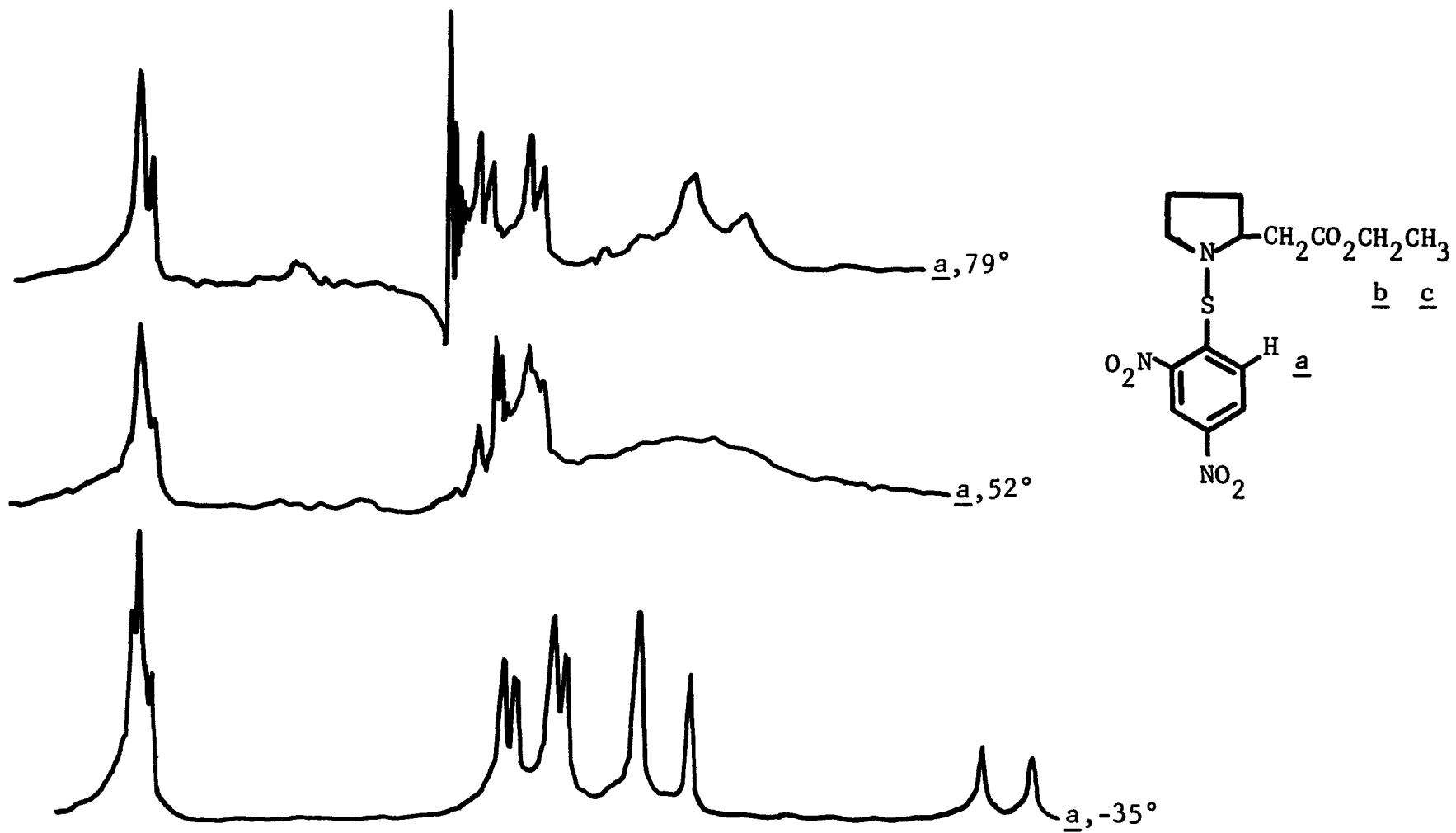
C - Figure 10.   Compound 39
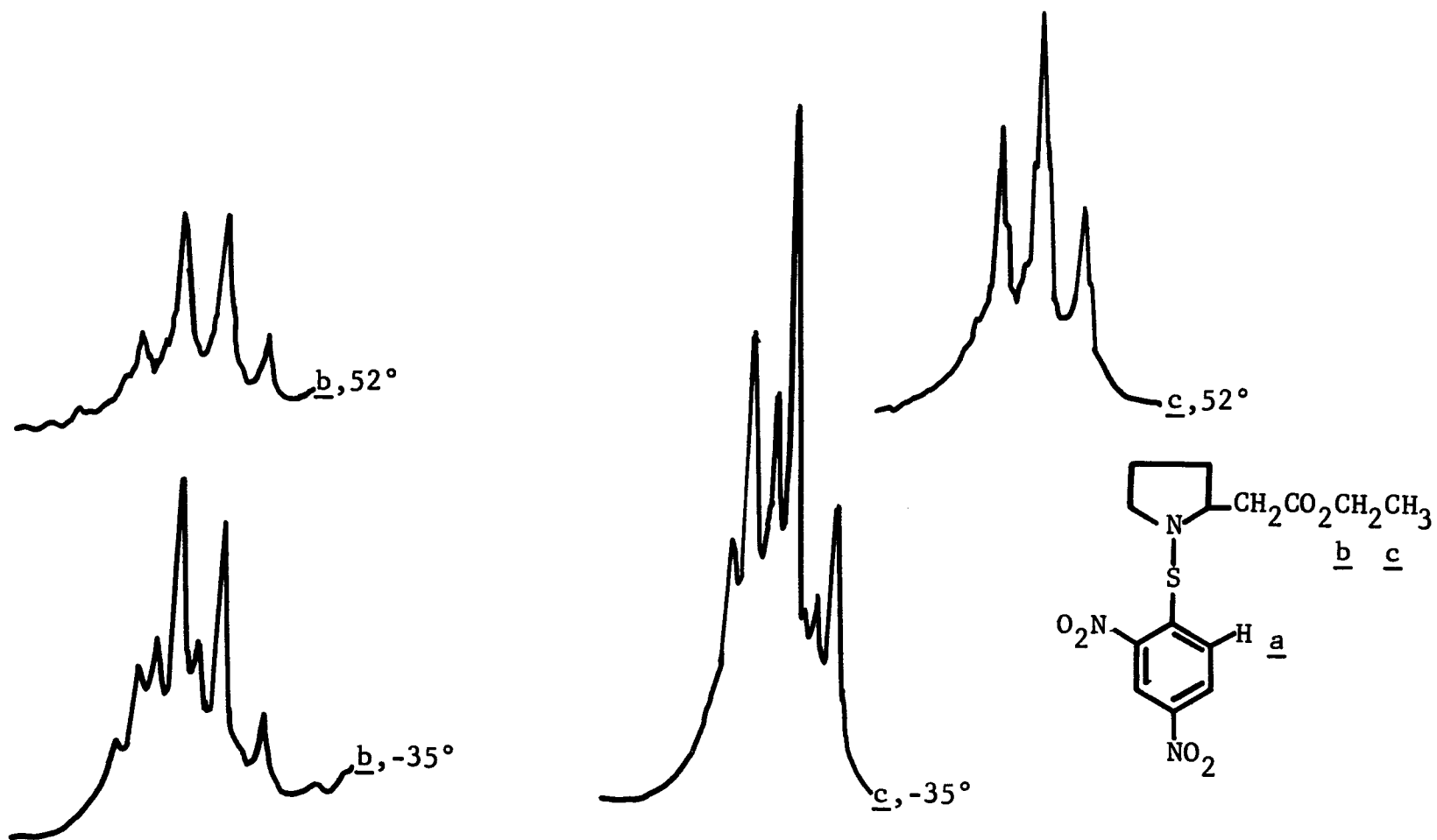
C - Figure 11.   Compound 39
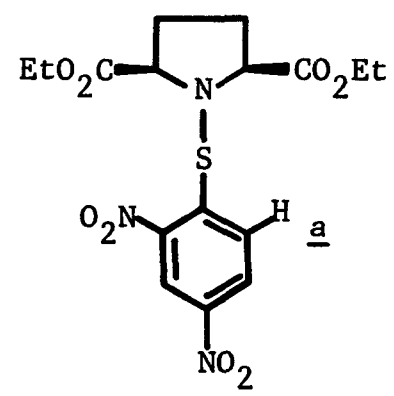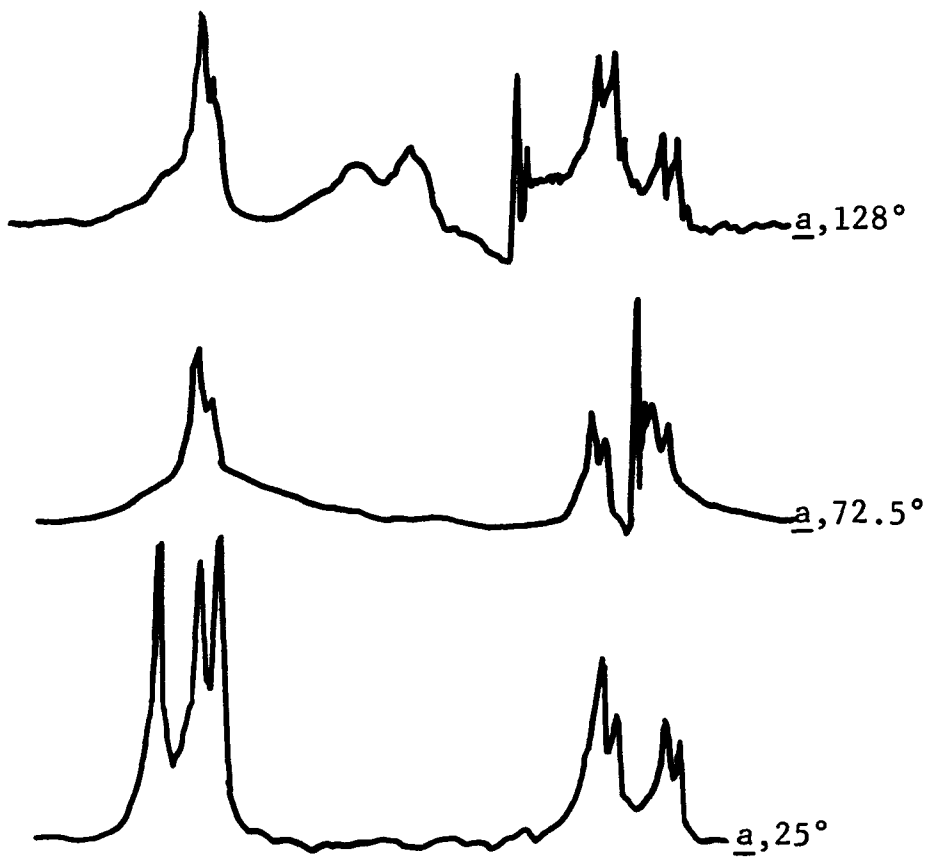
C - Figure 12. Compound 40

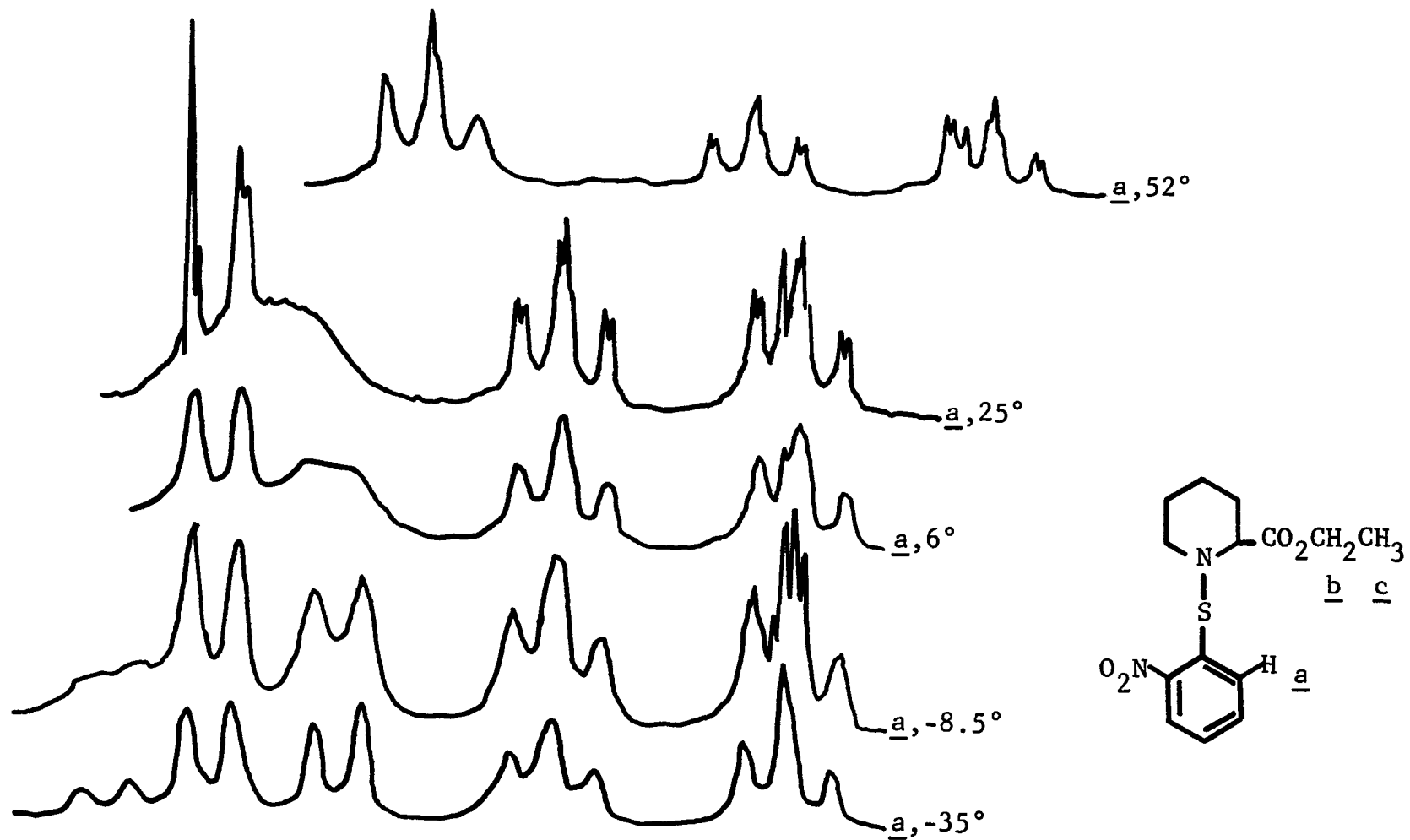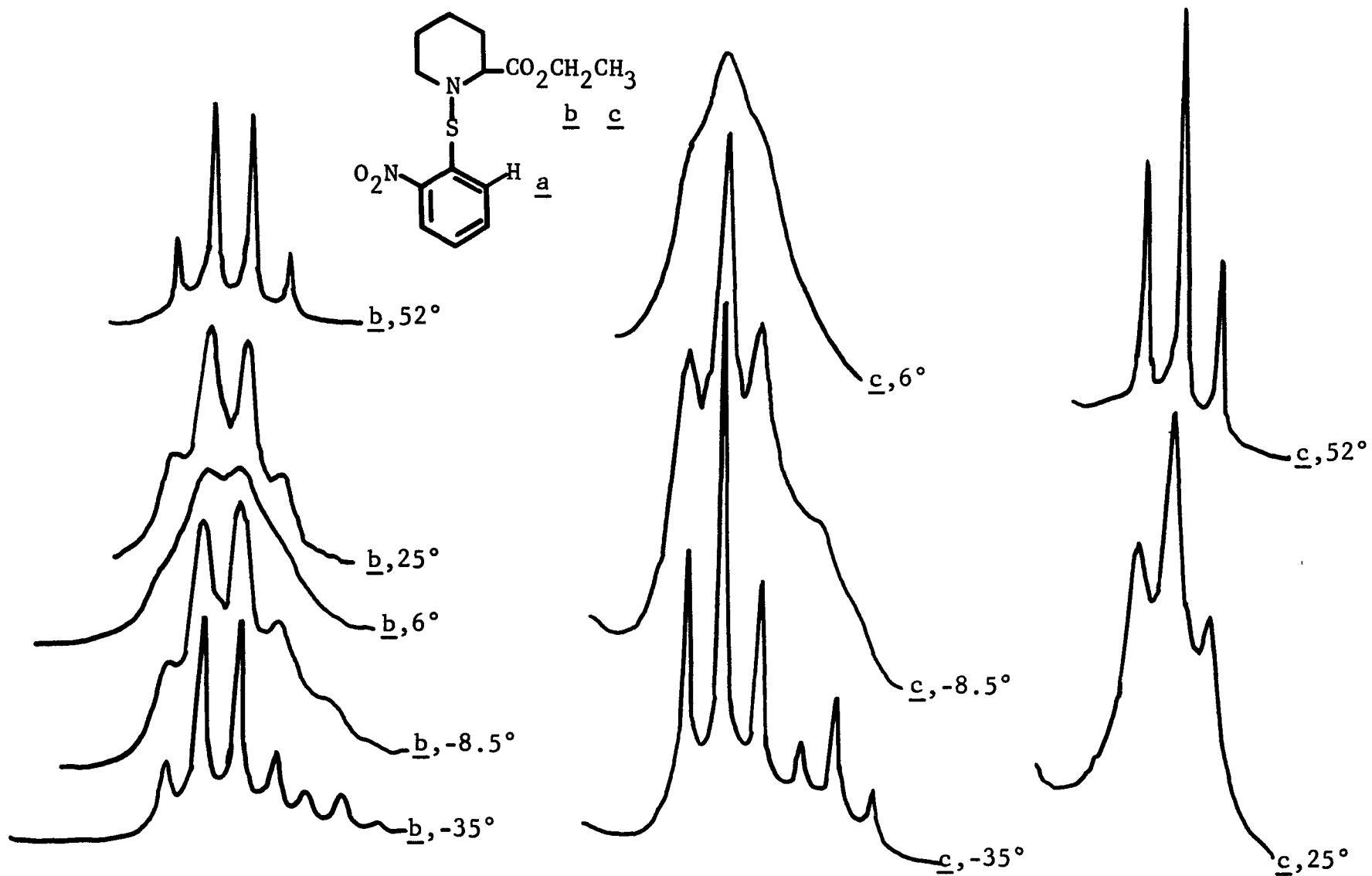b,66°

b,52°

b,31°

b,-57°

b,119.5°

b,88°
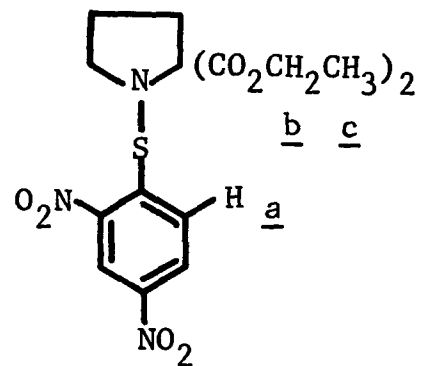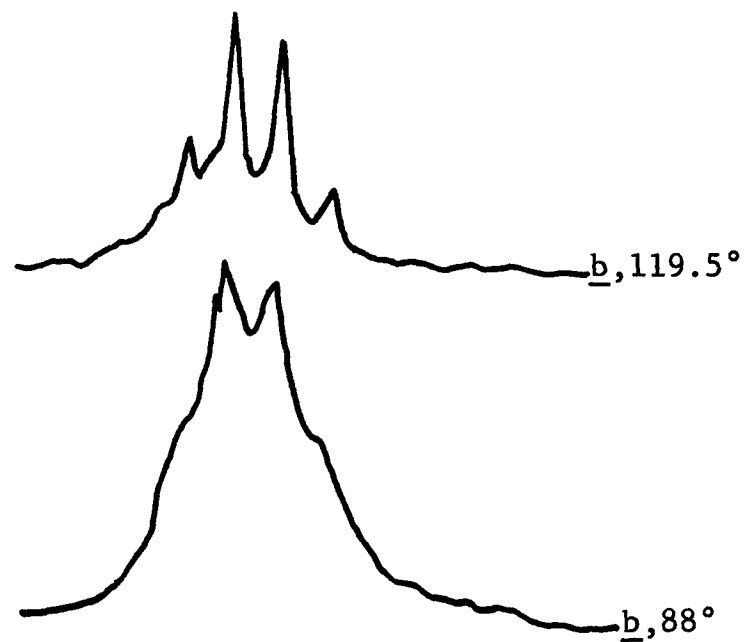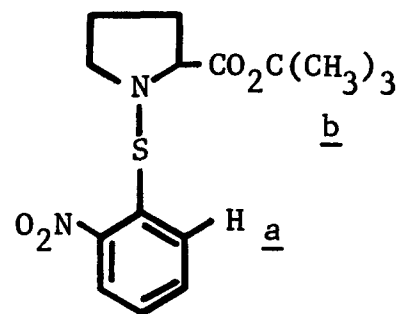
C - Figure 13.   Compound 30

C - Figure 14.   Compound 30

C - Figure 15.   Compound 45
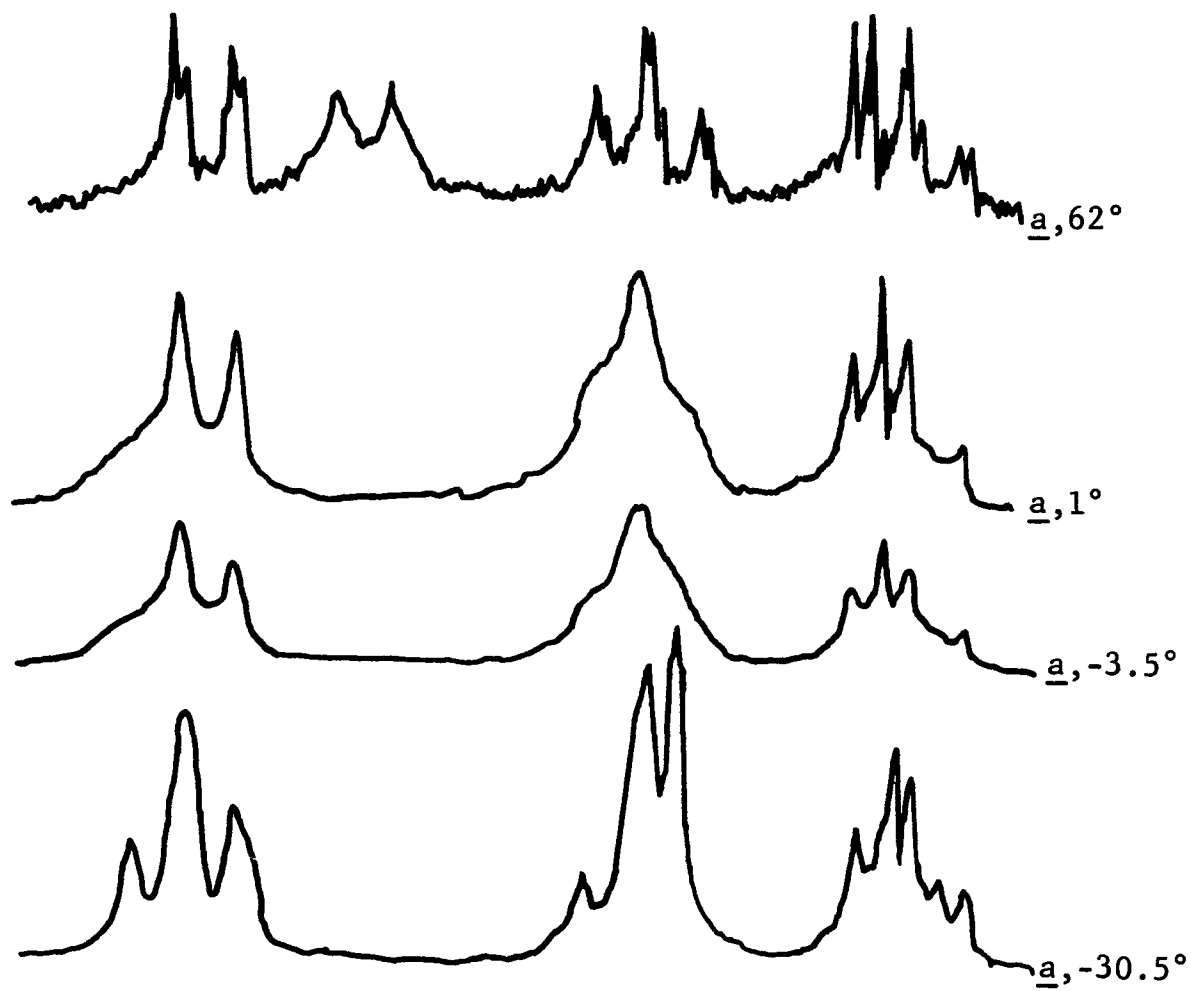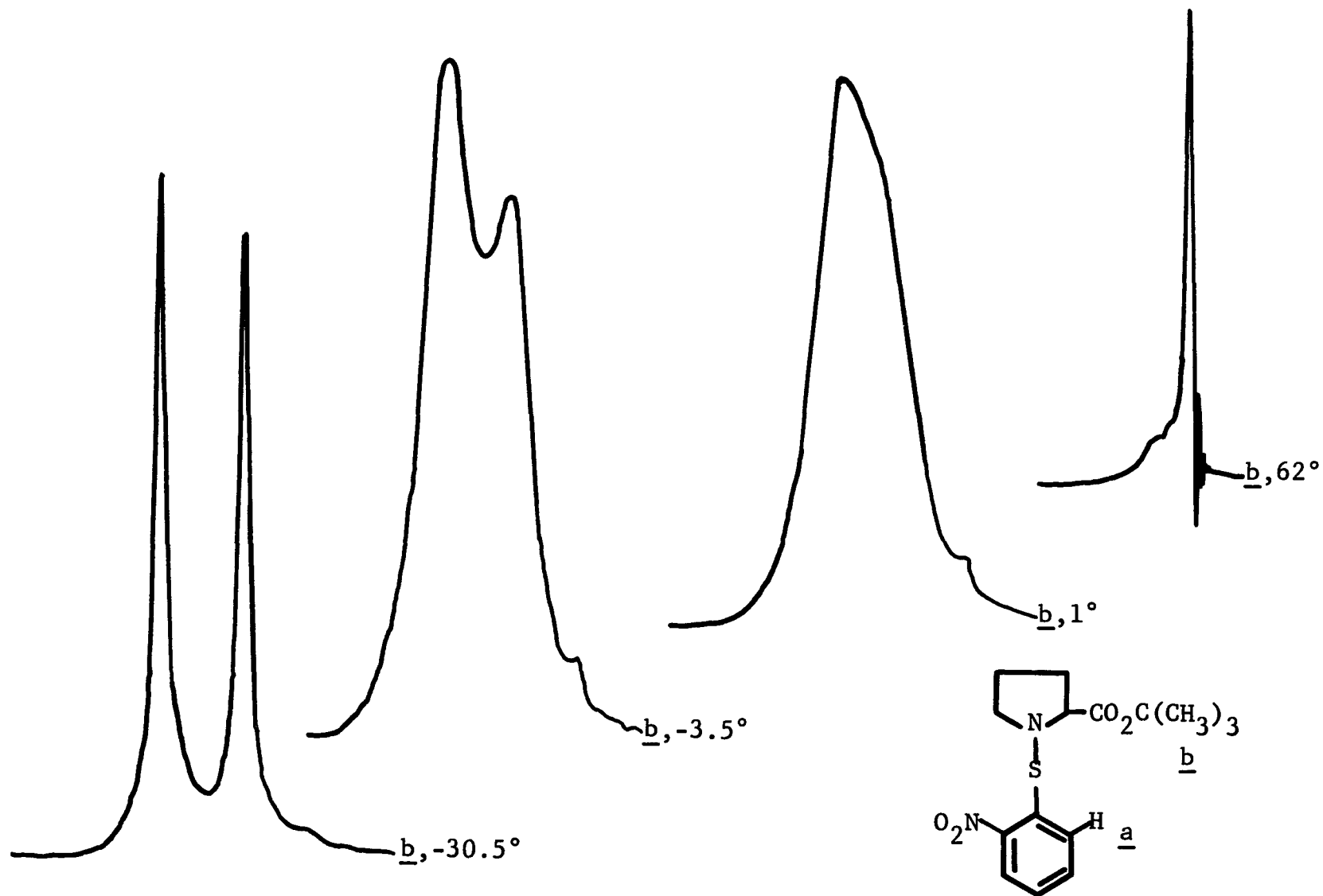
C - Figure 16. Compound 45

C - Figure 17. Compound 44

b,62°

b,1°

b,-3.5°

b,-30.5°

CO₂C(CH₃)₃

$CO_2C(CH_3)_3$

b

O_2N

H   a

C - Figure 18.   Compound 44

BIOGRAPHICAL DATA

NAME: Thomas David Harris

DATE OF BIRTH: December 29, 1948

PLACE OF BIRTH: Saginaw, Michigan

SECONDARY EDUCATION: Arthur Hill High School
                      Saginaw, Michigan

COLLEGIATE EDUCATION:	DATES	DEGREE
Saginaw Valley College	1967=71	B.S.
Saginaw, Michigan		

HONORS: Outstanding Senior Chemistry Award, 1971
         UNH Summer Fellowships, 1972-74

PUBLICATIONS:

1. "Photochemistry of 2-Phenyloxazolo(4,5-C)
    Pyridines. Photoalkylation by Diethyl Ether",
    P. L. Kumber, T. D. Harris, J. Org. Chem. $\underline{37}$,
    1830 (1972).

2. "1,3-Disubstituted Tetraphenyldisilthianes.
    Preparation and Properties", M. Gebler, T. D.
    Harris, P. J. Moehs. Presented at the 7th Great
    Lakes Regional Meeting of the A.C.S.; Kalamazoo,
    Michigan, June, 1973.

3. "1,3-Disubstituted Tetraphenyldisilthianes",
    M. Gebler, T. D. Harris, P. J. Moehs. Presented
    at the 30th Fall Scientific Meeting of the Midland
    Section of the A.C.S., November, 1973.

4. "Synthesis of Proline Derivatives", R. E. Lyle,
    R. W. Davenport, T. D. Harris, P. Herlihy.
    Presented to IV Northeast Regional Meeting of the
    A.C.S., Hartford, Ct., October, 1972.

5. "Proline Derivatives as Prostaglandin Analogs",
    R. E. Lyle, P. Herlihy, T. D. Harris, R. W.
    Davenport. Presented to National A.C.S. Meeting
    in Los Angeles, March, 1974.

6. "Synthesis of Nitrogen Containing Analogs of
    Prostaglandins:, R. E. Lyle, R. W. Davenport, P.
    Herlihy, T. D. Harris. Final Report NIH-NICHD-
    72-2715, September, 1973.