Ethyl N-bromo-alkylcarbamates as heterocyclic precursors and Extractives from *Oceanapia sp.*

by

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submitted in partial fulfillment of the requirements for the degree of Master of Science in the School of Pure and Applied Chemistry, University of Natal, Durban, 2001.

Preface

The experimental work described in this thesis was carried out in the School of Pure and Applied Chemistry, University of Natal, Durban, from Jan 2000 to June 2001, under the supervision of Dr. JP. Gerber and co-supervision of Prof. D.A. Mulholland.

This study represents original work by the author and has not been submitted in any other form to another university. Where use has been made of the work of others, it has been acknowledged in the text.

signed

M.C. Dovey

signed

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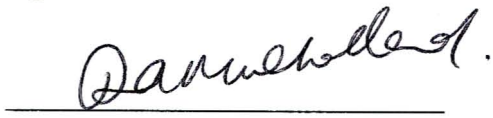
JP Gerber

signed



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Acknowledgments

First, and foremost, I would like to thank my supervisors Dr. JP Gerber and Prof. Dulcie Mulholland. Your support and guidance during the course of this work has been surpassed only by your patience.

I'd like to thank my fellow M.Sc. colleague, Jon Chetty, for ensuring the lab was always full of trivial banter.

To Jen Mackenzie (Honours 2000), it was a pleasure to work in the same lab as you. Also for acetylation of the sterol described in this work.

To the rest of my colleagues in the Natural Products research group, and in other groups in the department, thank you all for your friendship. I wish you all the best in your futures.

I would like to single out Mr. Neil Koorbanally. Your willingness to drop what you were doing to offer me help is greatly appreciated.

To my "third supervisor" Mr. Phil Coombes. Thank you for your assistance, with any problem, which was always over and above the call of duty.

I would, especially, like to thank Mr. Bret Parel for all his work in running the lab, among other things.

I would like to thank Dr. Gert Kruger for introducing me to the world of computational chemistry. I also acknowledge the National Computational Science Allience (USA) under grant number CHE000031N (NCSA SGI/CRAY Origin2000) for computational support of the study conducted and described in this text.

I would like to thank Mr. Dilip Jagjivan for the running of NMR experiments.

I would like to thank Mrs. Zarina Sayed Alley and Mr. Selwyn Petersen for their assistance in the running of UV spectra.

I acknowledge the financial support of the NRF and the NRF/DEA&T research joint venture.

Finally, to my family. Ross thanks for learning how to drink properly. Mom and Dad, thank you for your undying love and support throughout and thanks for asking me what I was doing even though the answer always bored you to tears. You three are my favourite people in the world.

Chapter 1

Ethyl N-bromo-alkylcarbamates as heterocyclic precursors

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List of Abbreviations

GlcNAc – N-acetylglucosamine

MurNAc – N-acetylmuramic acid

ala – alanine

6-APA – 6-aminopenicillanic acid

LDA – lithium diisopropylamide

THF – tetrahydrofuran

TBSCl – *tert*-butyldimethylsilyl chloride

TBS – *tert*-butyldimethylsilyl

ee – enantiomeric excess

DIAD – diisopropyl azodicarboxylate

calcd – calculated value

E – energy

SOMO – singly occupied molecular orbital

HOMO – highest occupied molecular orbital

LUMO – lowest occupied molecular orbital

^{1}H NMR – proton nuclear magnetic resonance spectroscopy

ppm – parts per million

PTC – phase transfer catalyst

NBS – N-bromosuccinimide

LAH – lithium aluminium hydride

AIBN – azo-iso-butyronitrile

BuLi – butyl lithium

HMBC – heteronuclear multiple bond coherence

Hz – Hertz

^{13}C NMR – carbon-13 nuclear magnetic resonance spectroscopy

^{31}P NMR – phosphorus-31 nuclear magnetic resonance spectroscopy

COSY – correlated nuclear magnetic resonance spectroscopy

NOESY – nuclear Overhauser effect spectroscopy

HETCOR – heteronuclear shift correlation nuclear magnetic resonance

IR – infrared spectroscopy

m.p. – melting point

TLC – thin layer chromatography

Et_2O – diethyl ether

Hex – hexane

EtOAc – ethyl acetate

CH_2Cl_2 – dichloromethane

HRMS – high resolution mass spectroscopy

EIMS – electron impact mass spectroscopy

UV – ultraviolet spectroscopy

s – singlet

br s – broad singlet

d – doublet

dd – doublet of doublets

t – triplet

br t – broad triplet

q – quartet

m – multiplet

I – spin quantum number

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Abstract

The synthesis of β-lactams has been of foremost importance since the discovery of penicillin by Sir Alexander Fleming, in 1928, and its susequent structure elucidation in 1945.

Ethyl N-2-bromo-alkylcarbamates show considerable potential as precursors to β-lactams. In the past, β-lactams have been prepared by many methods, none of which have involved 2-3 bond formation. The proposed ring closure using ethyl N-2-bromo-alkylcarbamate involves 2-3 bond formation, making this method of synthesis novel.

This work describes two attempted methods of cyclisation. The first using a Grignard reagent, and the second, using abstraction of an acidic proton α to a phosphonate group.

These methods of intramolecular cyclisation were based on analogous intermolecular additions, which are also described.

The second method was also used to determine the general potential of ethyl N-bromo-alkylcarbamtes as precursors to other heterocyclic systems.

INTRODUCTION

1. β-Lactams

1.1 .General Remarks

The first β-lactam was described in 1907 when Staudinger demonstrated the cycloaddition of ketenes to imines.[1] This was a highly novel method of synthesis and was discovered by Staudinger and his co-workers during their studies of ketenes.[2] Between 1907 and 1943 only two further methods of β-lactam synthesis were described.[2]

With the discovery of penicillin in 1928, and the subsequent structure elucidation of the penicillin nucleus in 1945, the study and synthesis of β-lactams has become a major area of chemical, biochemical and industrial research.

Lactams are defined as cyclic amides,[3] with the prefix indicating the size of the ring. 'α' indicates the smallest of the family, the 3-membered variety, 'β' the 4-membered, and so on.

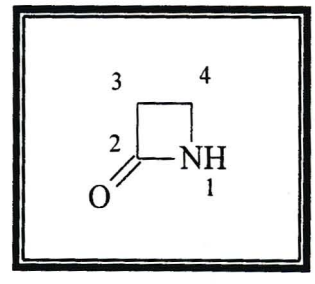


Fig.1: β-Lactam.

As a consequence mainly of ring strain, β-lactams are considerably different, in terms of physical and chemical properties, from acyclic amides and lactams of larger ring size.[2] The most stable conformation for the β-lactam would, presumably, not be a square planar one. The typical geometry of the β-lactam is described by Dexter and van der Veen in their work on the crystal structure determination of two penicillin G salts.[4]

N(4) is 0.40Å from the plane of the remaining three members of the β-lactam ring, and the sum of the bond angles about N(4) is 336.7(7)°. It is presumed that this lack of planarity reduces electron delocalization between N(4) and C(7) which weakens the bond, an observation in keeping with the mechanism of the penicillins' antimicrobial action.

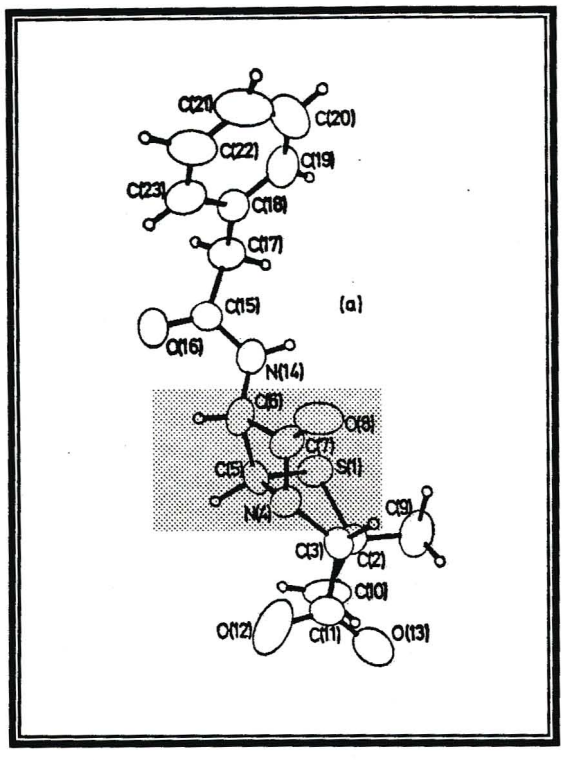


Fig.2: Crystal structure of Penicillin G.

β-Lactam antibiotics derive their antibacterial activity from their ability to inhibit cell wall synthesis.[5] Cell walls of Gram-positive and Gram-negative bacteria differ in certain aspects. However, both comprise a basal peptidoglycan which consists of the amino sugars N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) to which are attached amino acids. Peptidoglycan synthesis involves addition of amino acids to MurNAc followed by formation of a linear polymer by addition to GlcNAc and, ultimately, cross-linking of the linear polymers to give the cell wall rigidity. This cross-linking is carried out by an enzyme, transpeptidase, and occurs when the enzyme binds to the terminus of the amino acid chain attached to MurNAc . This is achieved by cleavage of the peptide bond between the two terminal alanine moieties. One edge of the penicillin nucleus is very similar to the backbone of this amino acid chain in terms of spacial conformation (Fig.3).[6]

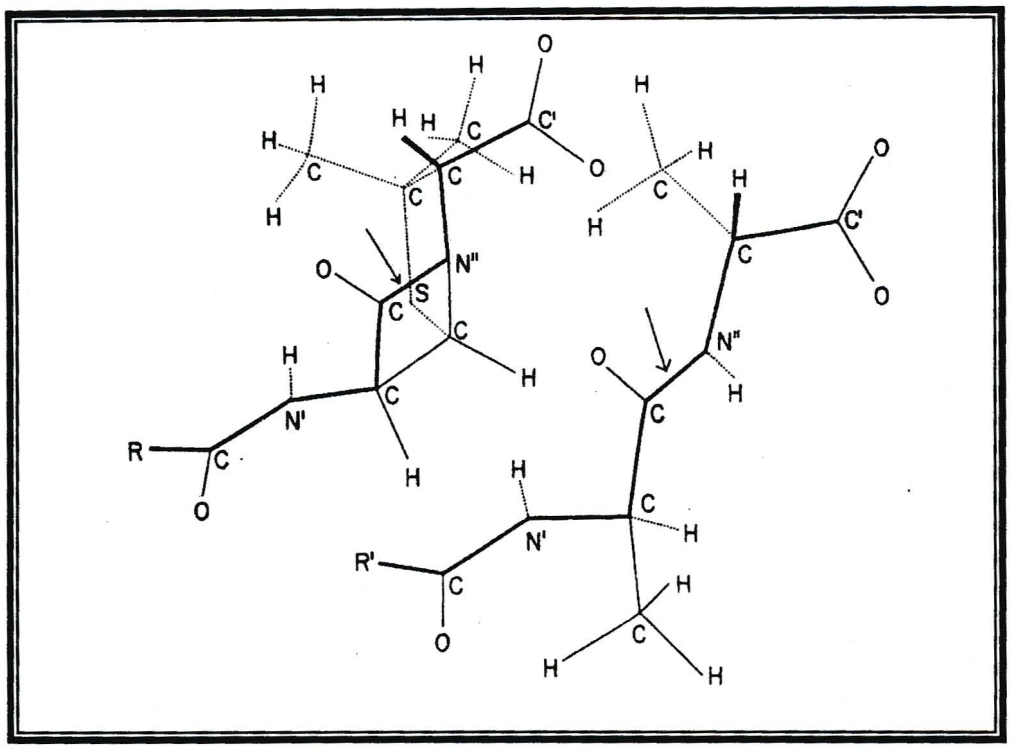


Fig.3: Dreiding stereomodels of a penicillin and acyl-D-ala-D-ala amino acid chain.

However, what is more important is the fact that the bond between the carbonyl carbon and nitrogen of the β-lactam, the most reactive bond in the molecule, lies in exactly the same position as the peptide bond which is broken during transpeptidation. The hypothesis is that the penicillin molecule binds to the active site of the enzyme preferentially which inactivates it and inhibits cross-linking.

1.2. Naturally occurring β-lactams

1.2.1. Antibiotics

The most well known of the naturally occurring β-lactams are the group of antibiotics called the penicillins.

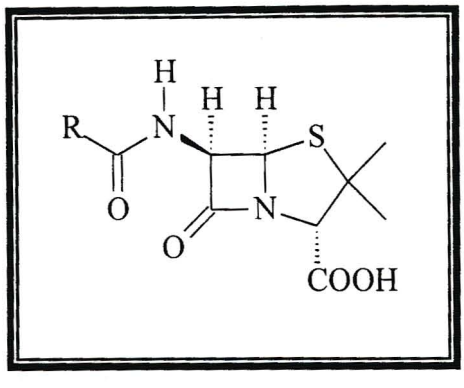


Fig.4: Penicillin nucleus.

The first penicillin, one of the most famous scientific discoveries of all time, was penicillin G (Fig.4 , R = $-CH_2Ph$) obtained from the fungus *Penicillium chrysogenum*, first noticed by Sir Alexander Fleming in 1928. Today, penicillin G is commercially produced by fermentation of high yielding strains of *Penicillium chrysogenum* in the presence of phenylacetic acid.[7] Penicillin G, however, displays a number of clinical flaws. It has a narrow spectrum of activity and is susceptible to β-lactamase enzymes and acidic conditions. It is, therefore, neccesary to modify the penicillin nucleus.[5] Initially the fermentation medium was supplemented with acids other than phenylacetic acid. This approach had limited success, however, as the novel penicillins were restricted to a series of monosubstituted acetic acids. One major improvement was seen in the production of penicillin V (Fig.4, R= $-CH_2OPh$) which was not acid-labile. However, a much wider range of clinically useful penicillins have been produced semi-synthetically. It is now possible to produce 6-aminopenicillanic acid (6-APA) (Fig.5) by a multi-stage chemical procedure.[7]

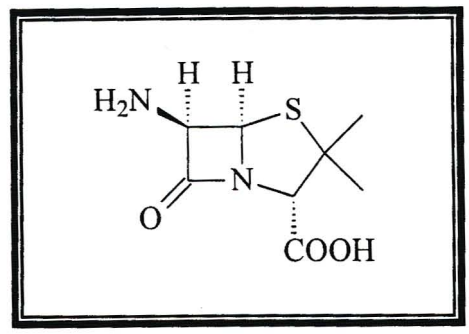


Fig.5: 6-APA.

A much wider range of modifications can now be made to the penicillin nucleus by simple chemical means and a much greater degree of control can be employed in doing so. A host of different clinically useful penicillins have been produced by reactions at the amino group with a smaller number being produced by reaction at the carboxylic acid group.

The penicillins are one member of a larger family of antibiotics simply referred to as the β-lactam antibiotics. Other members of this family include the cephalosporins, oxapenams, carbapenems and the monobactams.

The cephalosporins are closely related to the penicillins with the β-lactam being fused to a six-membered dihydrothiazine ring instead of the five-membered thiazolidine ring system (Fig.6).[7]

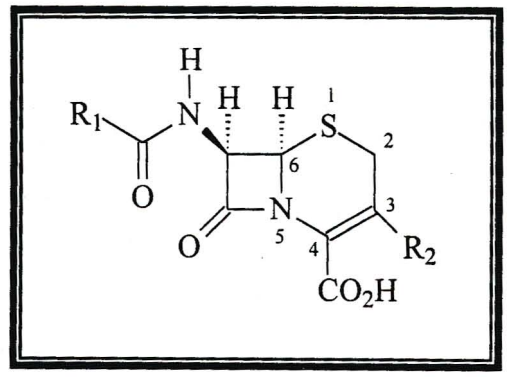


Fig.6: Cephalosporin nucleus.

The position of the double bond is very important. Cephalosporins with the double bond between C_2 and C_3 are not significantly antibacterial, irrespective of the composition of the side-chains.[8] The earliest of the cephalosporins was cephalosporin C (Fig 7), discovered in 1953 by Abraham and Newton.[8] It was isolated from cultures

of *Cephalosporium acremonium* as a minor component, with penicillin N being the major component. Today, cephalosporin C is produced commercially by fermentation using cultures of a high-yielding strain of *Cephalosporium acremonium.*

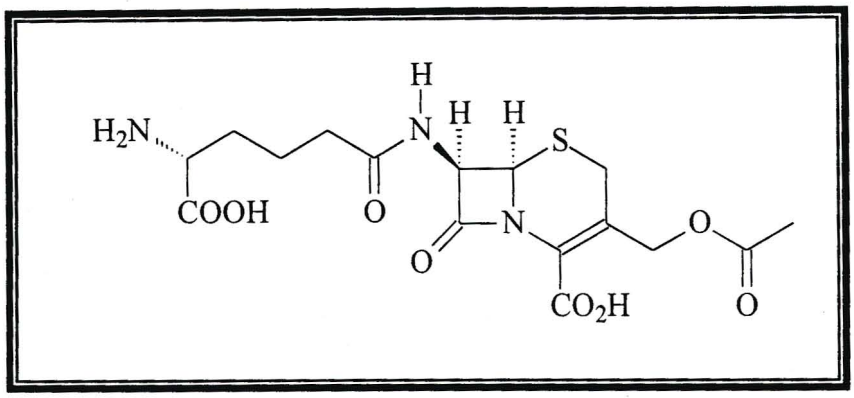


Fig.7: Cephalosporin C.

Although its antibacterial activity is low, cephalosporin C has one major advantage over the early penicillins. It is acid-stable. This property has been attributed to its amino group in the side chain, and this knowledge has led to many semi-synthetic β-lactam antibiotics which are stable in acidic conditions and can, thus, be administered orally. One further property of the cephalosporin nucleus that has made it attractive is the fact that it has two side-chains and, hence, greater potential for modifications. Research in this area is intense, with the result that many of the wide variety of semi-synthetic cephalosporins produced are currently in clinical use.

Other naturally occurring variants on the penicillin nucleus have found clinical use. The oxapenams differ from the penicillins in that the sulphur heteroatom has been replaced by an oxygen atom. The oxapenams are typified by clavulanic acid (Fig.8).

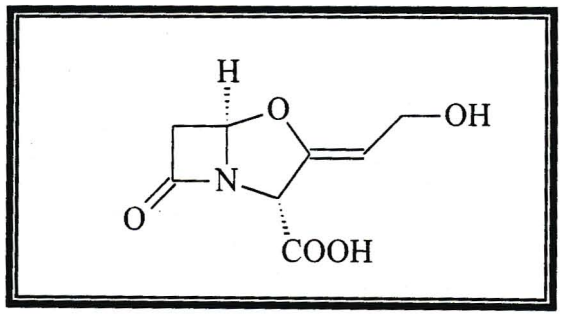


Fig.8: Clavulanic acid.

Clavulanic acid is produced by cultures of *Streptomyces clavuligerus*. It has weak antibacterial activity itself. However, it reacts irreversibly with a wide variety of β-lactamases, rendering them inactive. Clavulanic acid is therefore administered with other standard penicillins, protecting the penicillin and enhancing its activity.[7]

A further variation on the penicillin nucleus is that of the carbapenems in which the sulfur atom has been replaced by a methylene group. The carbapenems also incorporate a property of the cephalosporins. A double bond is observed between C_2 and C_3. The carbapenems are typified by thienamycin (Fig.9).[7]

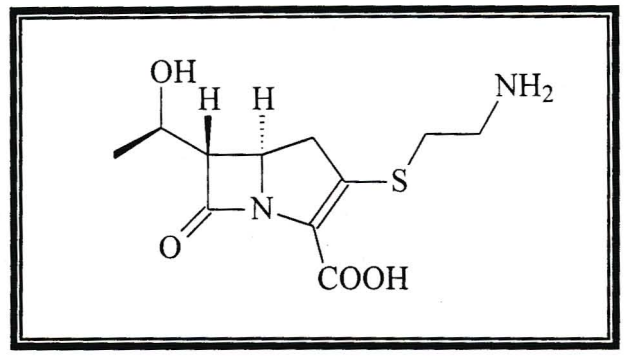


Fig.9: Thienamycin.

Thienamycin is produced by cultures of *Streptomyces cattleya*. However, the amounts produced are insufficient for commercial use and it is, therefore, produced by total synthesis. Thienamycin itself is relatively unstable and, thus, it is administered as its N-formimidoyl derivative imipenem (Fig.10).

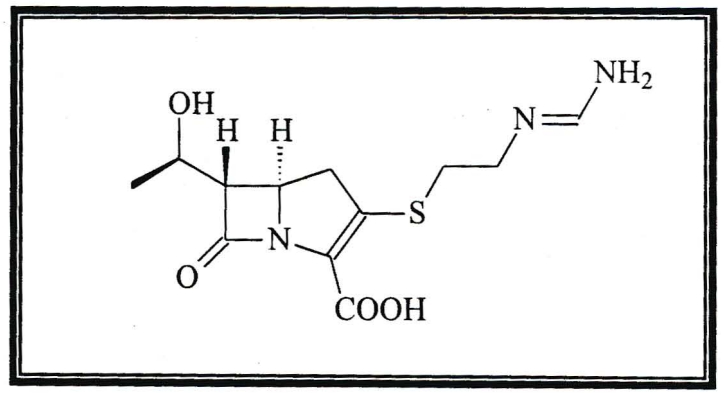


Fig.10: Imipenem.

Imipenem has a broad spectrum of activity, is resistant to hydrolysis by most classes of β-lactamases and also shows inhibitory activity towards β-lactamases.[7]

The simplest of the naturally occurring β-lactam antibiotics are the monobactams. The monobactams are monocyclic β-lactams produced by bacteria. The compound SQ 26,180 (Fig.11) has been isolated from *Chromobacterium violaceum.*[7]

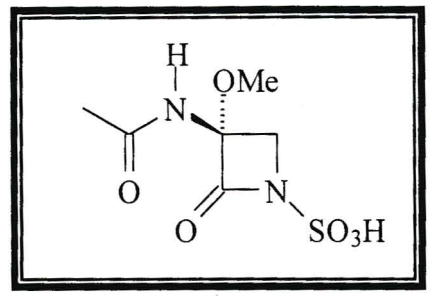


Fig.11: SQ 26,180.

The naturally occurring monobactams show little antibacterial activity. However, side-chain modifications have led to many new highly active compounds. Aztreonam was the first to be used clinically (Fig.12).

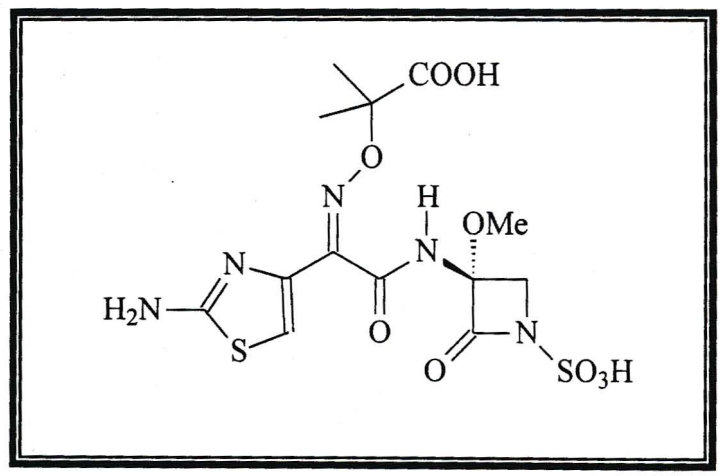


Fig.12: Aztreonam.

Aztreonam combines the side-chain of the cephalosporin, ceftazidine, with the monobactam nucleus and exhibits resistance to hydrolysis by β-lactamases as well as

being very active against Gram-negative bacteria.[5] Gram-negative bacteria have cell walls composed of a thin layer of peptidoglycan and an outer membrane of lipopolysaccharides compared with only a thick layer of peptidoglycan as in Gram-positive bacteria.[9]

1.2.2. Other natural products containing β-lactams

Most of the naturally occurring β-lactams are antibiotics or precursors thereof. It is not surprising, therefore, that toxins and alkaloids are also included in this family of compounds.

Tabtoxin (Fig.13), also known as wildfire toxin, is a well-known toxin which causes a destructive leafspot disease of tabacco plants known as wildfire disease.[10]

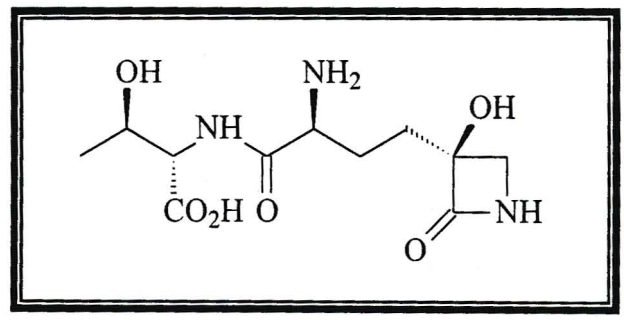


Fig.13: Tabtoxin.

Although the disease was first reported in 1917, wildfire toxin's structure was only solved in 1971, identifying the presence of the β-lactam ring. The toxin is isolated from *Pseudomonas tabaci,* the organism responsible for wildfire disease.

Alkaloids containing β-lactam systems have been isolated from both marine and terrestrial sources. (S)-chartelline A (Fig.14) has been isolated from the marine bryozoan *Chartella papyracea.*[11]

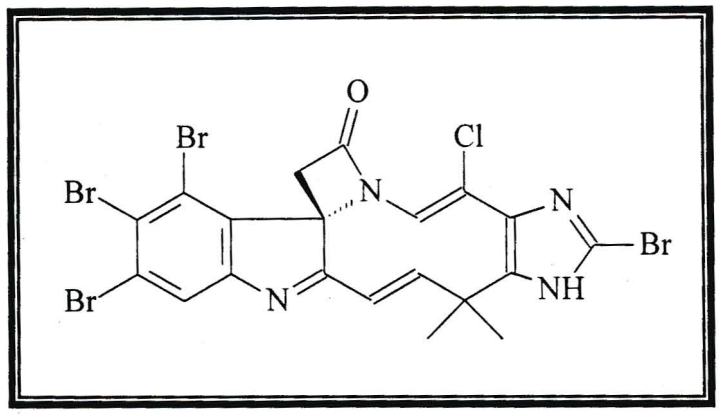


Fig.14: (S)-Chartelline A.

The same group that have reported this unusual alkaloid have subsequently isolated a further two alkaloids form the same organism, chartellamide A and B (Fig.15).[12]

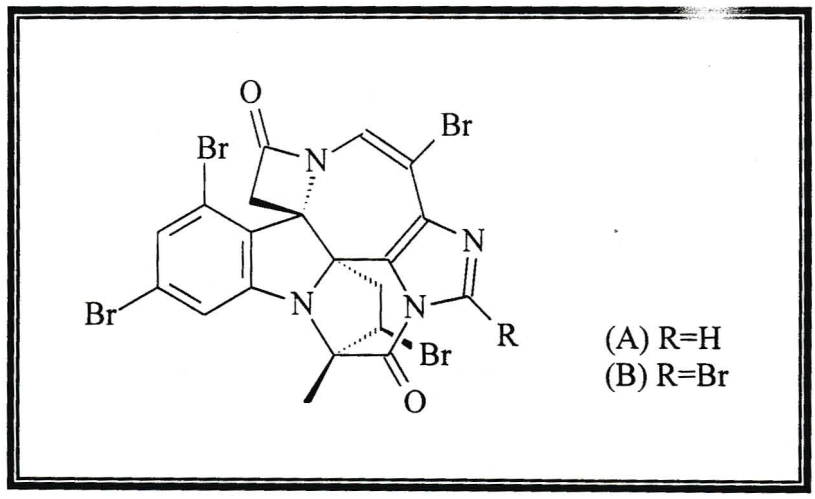


Fig.15: Chartellamide A and B.

In addition to these alkaloids of marine origin, three alkaloids of plant origin containing the β-lactam system have been reported. The steroidal alkaloid pachystermine A (Fig.16) has been isolated as the major alkaloid of *Pachysandra terminalis* (Buxaceae).[13]

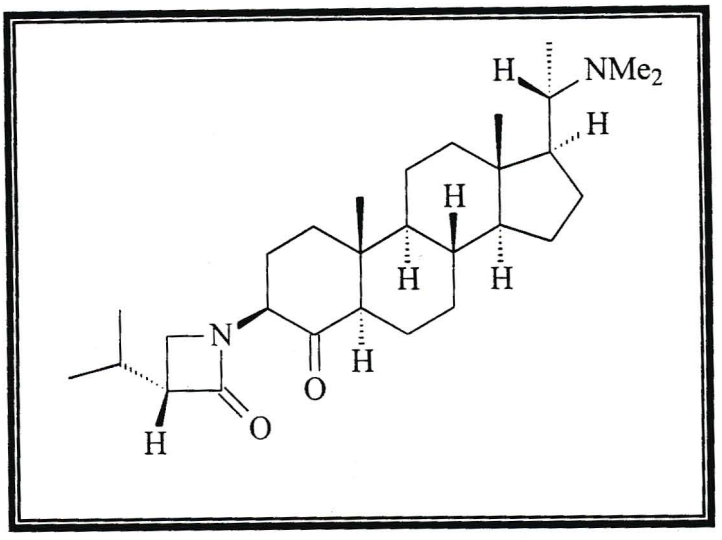


Fig.16: Pachystermine A.

Two further steroidal alkaloids have been isolated from another species of the *Pachysandra* genus, *P. procumbus*. They are (+)-(20S)-20-(dimethylamino)-3-(3'α-isopropyl)-lactam-5α-pregn-2-en-4-one **(1)** and (+)-(20S)-20-(dimethylamino)-16α-hydroxy-3-(3'α-isopropyl)-lactam-5α-pregn-2-en-4-one **(2)** (Fig.17).[14]

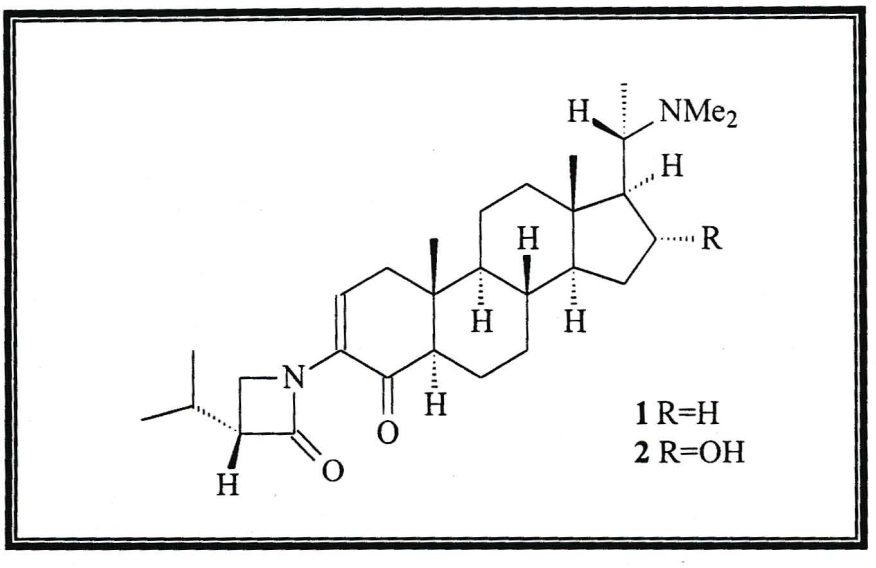
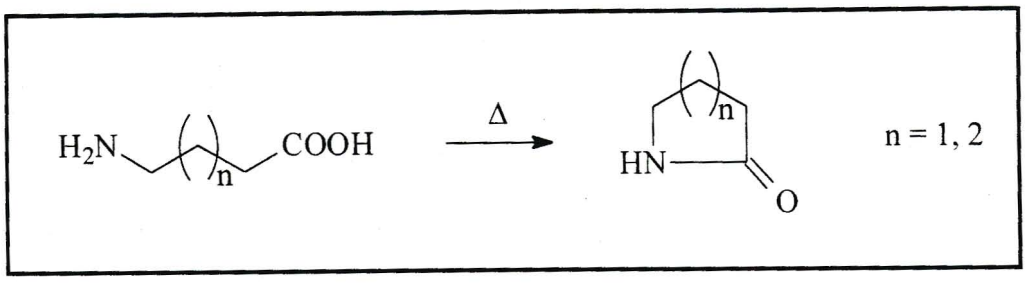


Fig.17: (+)-(20S)-20-(dimethylamino)-3-(3'α-isopropyl)-lactam-5α-pregn-2-en-4-one **(1)**, (+)-(20S)-20-(dimethylamino)-16α-hydroxy-3-(3'α-isopropyl)-lactam-5α-pregn-2-en-4-one **(2)**.

1.3. Synthetic routes to β-lactams

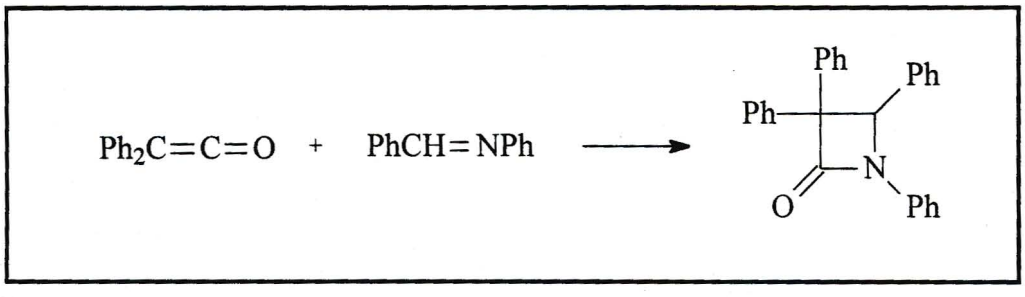
Although other lactams were already known, the first β-lactam was only synthesised at the beginning of the 20th century. This was probably due to the fact that β-lactams could not be formed by the method commonly used to form other lactams, that being the thermal dehydration of an appropriate amino acid (Scheme 1).[2]



Scheme 1: Thermal dehydration of amino acids.

It has been noted earlier that the physical and chemical properties of β-lactams differ greatly from acyclic amides and, indeed, from lactams of greater ring size. This has led to the need to develop new, unique methods of synthesis.

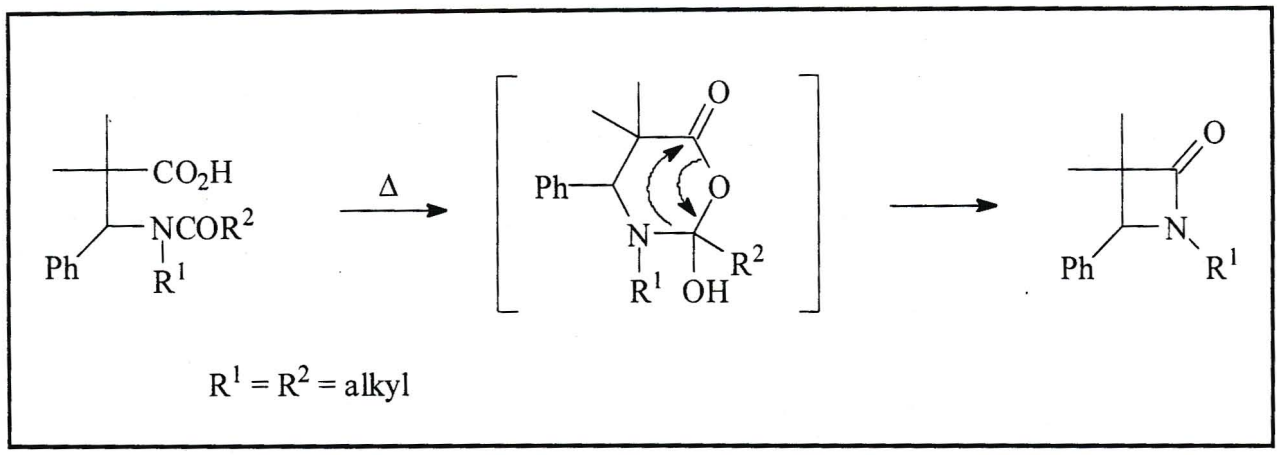
The first β-lactam was prepared by Staudinger and his co-workers during their studies of ketenes.[2] They noticed that diphenylketene reacts readily, at room temperature, with benzylideneaniline to form the crystalline 1,3,3,4-tetraphenyl-2-azetidinone in 72% yield. (Scheme 2).[15]



Scheme 2: Synthesis of β-lactam by direct combination of a ketene with an imine.

Staudinger discovered one further method of β-lactam synthesis during his study of ketenes. Although β-lactams cannot be prepared by thermal decomposition of amino

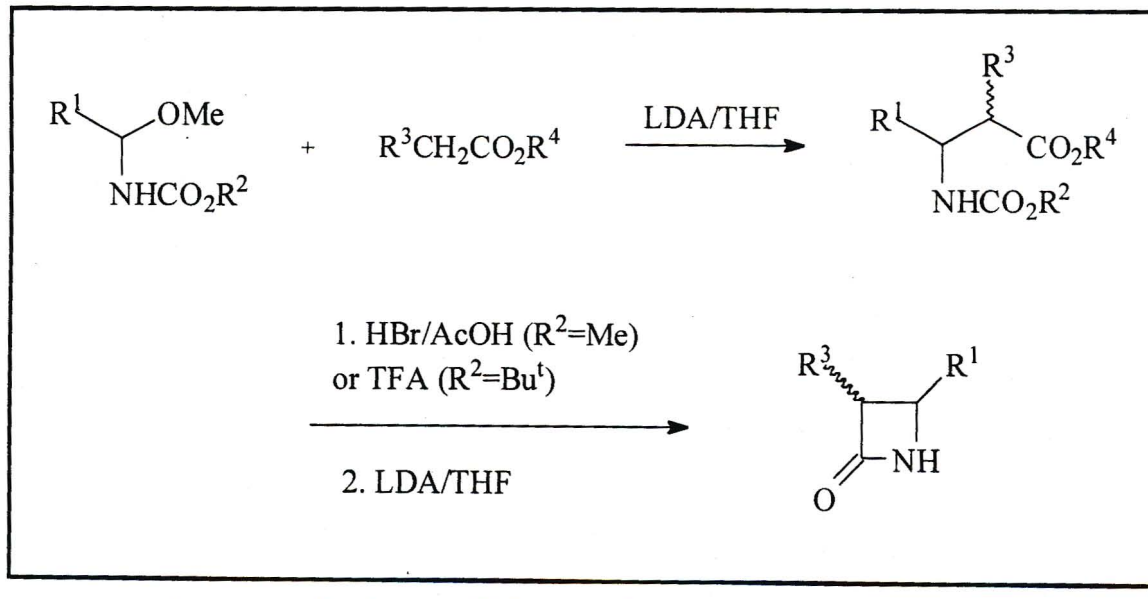
acids, this method can be employed using derivatives of amino acids, specifically acyl derivatives (Scheme 3).[16]



Scheme 3: Synthesis of β-lactams by cyclisation of β-amino acid derivatives.

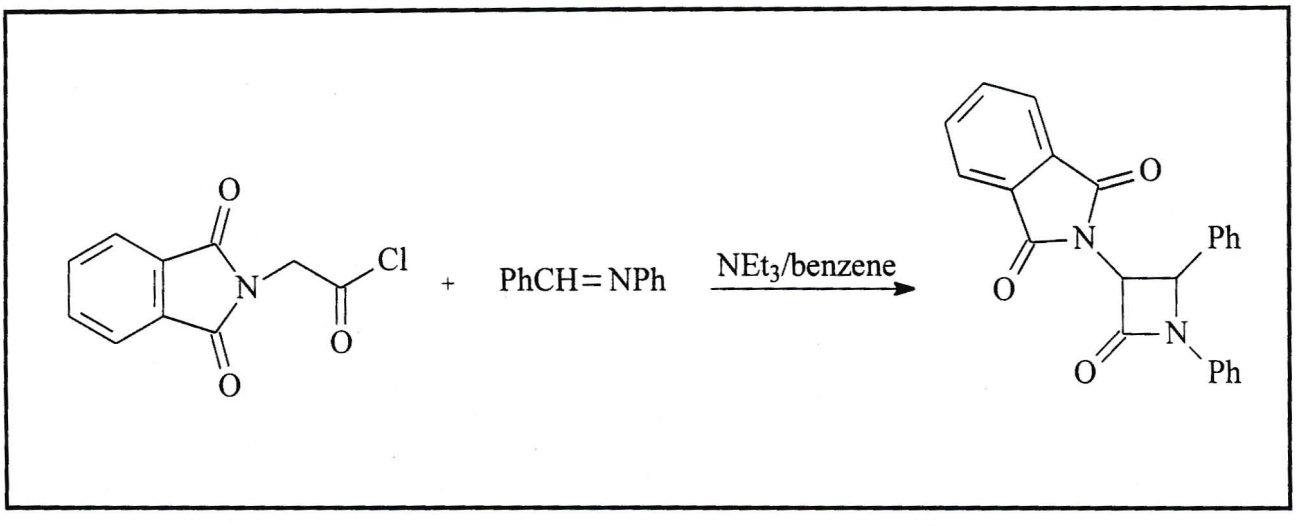
The mechanism by which the acyl group promotes cyclization has been attributed to an O to N acyl rearrangement of an intermediate hydroxylactone.[2]

A recent publication also uses β-amino acids as precursors to β-lactams.[17] The amino acids themselves were prepared by a novel method whereby N-alkoxycarbonyl-1-methoxyamines and an ester are added to LDA (lithium diisopropylamide). The adducts obtained are deprotected using a standard method and subsequently cyclized using LDA. (Scheme 4).



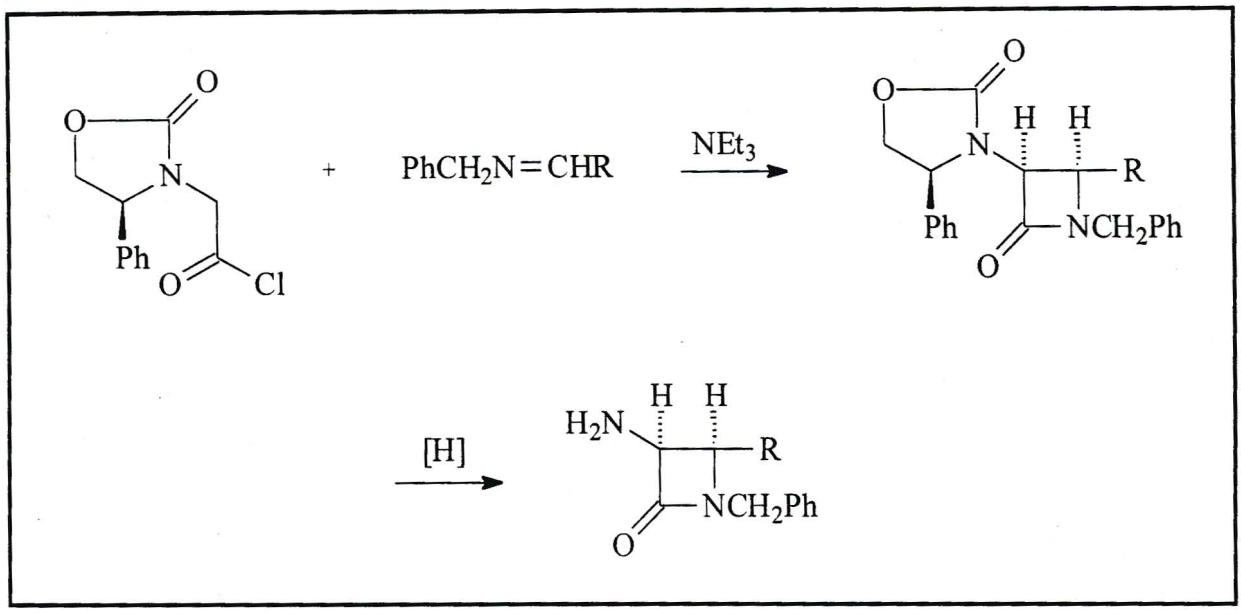
Scheme 4: Synthesis of β-lactams from β-amino acid precursor.

One of the most successful and widely used methods of β-lactam synthesis was first reported in 1951 by J.C. Sheehan and J.J. Ryan.[18] This method is analogous to Staudinger's reaction between ketenes and imines and is often referred to as such. The ketene is replaced by an acid chloride and the reaction is carried out in the presence of a tertiary amine which serves as a base. An example of this method is the reaction between benzylideneaniline and phthaloylglycyl chloride in the presence of triethylamine to give 1,4-diphenyl-3-phthalimido-2-azetidinone in 50% yield. (Scheme 5). The reaction proceeds at room temperature in inert solvents, in this case benzene.



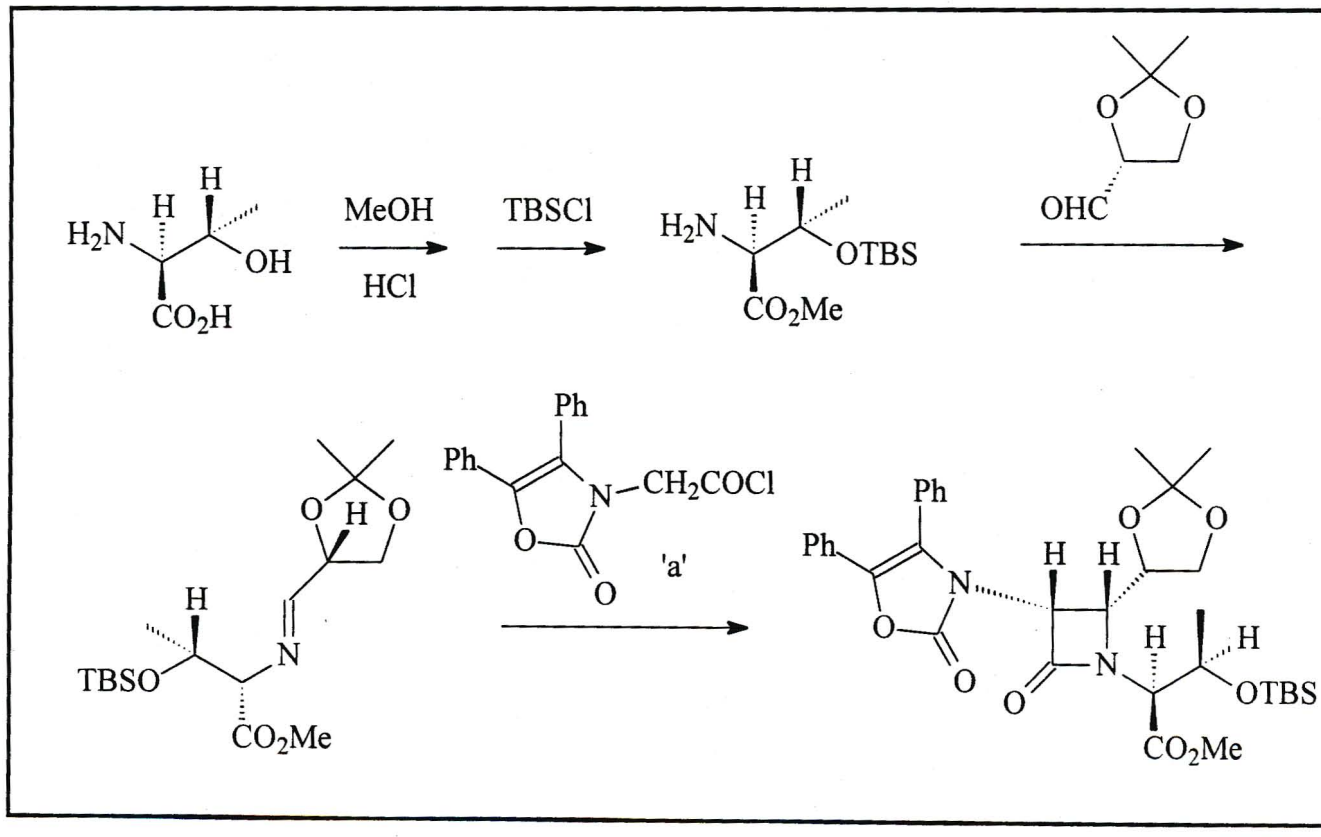
Scheme 5: Synthesis of β-lactams by reaction of an acid chloride and an imine.

Since then, this method has been extensively reported in the literature and several modifications have been made to the procedure, primarily with the aim of greater stereochemical control. Evans and Sjogren have reported an asymmetric synthesis of β-lactams using a homochiral acid chloride, (4S)-phenyloxazolidylacetyl chloride.[19] The method employs N-benzylimines in conjunction with triethylamine and proceeds with excellent stereoselectivity. The chiral oxazolidone auxillary may be reductively removed to yield enantiomerically pure azetidinones. (Scheme 6).



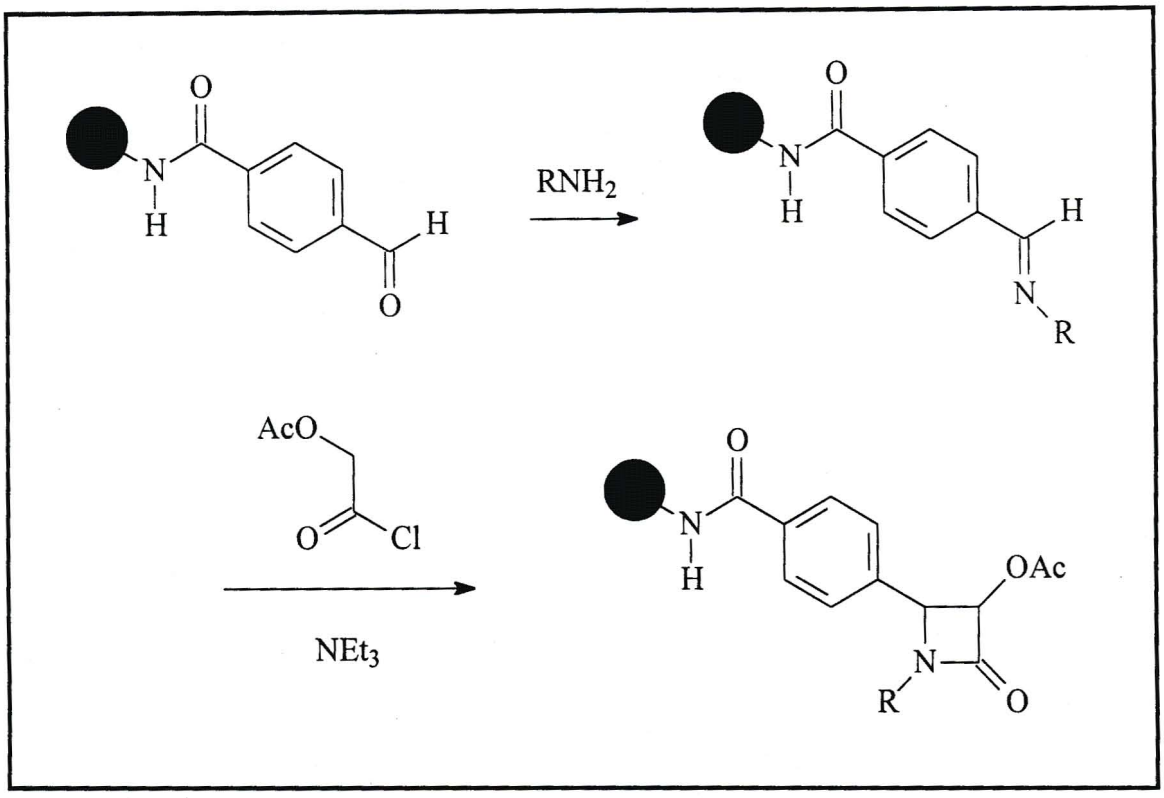
Scheme 6: Synthesis of β-lactams by reaction of an acid chloride and an imine.

One further modification reported uses chiral imines as opposed to chiral acid chlorides.[20] The imines are prepared from D-glyceraldehyde and threonine derivatives. Reaction of these chiral imines with the acid chloride derived from oxglycine (Scheme 7 'a') affords the β-lactam in good yield and with excellent stereochemical control. (Scheme 7).



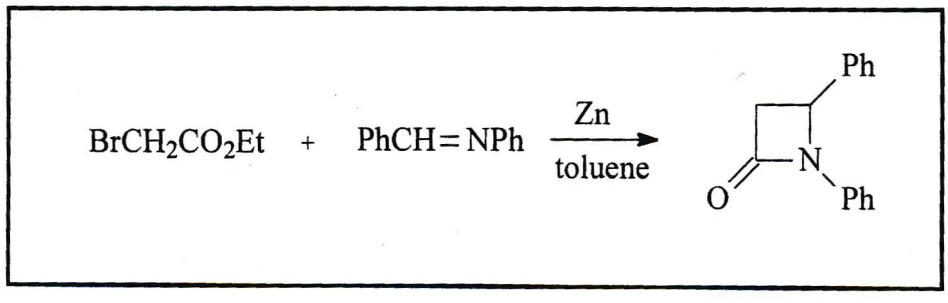
Scheme 7: Synthesis of β-lactams using chiral imines.

In all three of the above examples, yields have been diminished due to unwanted side reactions. It has recently been reported that the acid chloride imine cycloaddition can be carried out on solid support resulting in high yields and simple product purification.[21] The imines are prepared by reaction of primary amines with resin-bound *para*-carboxyaldehyde. Treatment of the prepared imines with acetoxyacetyl chloride in the presence of triethylamine affords the desired β-lactam. (Scheme 8).



Scheme 8: Synthesis of β-lactams using solid support.

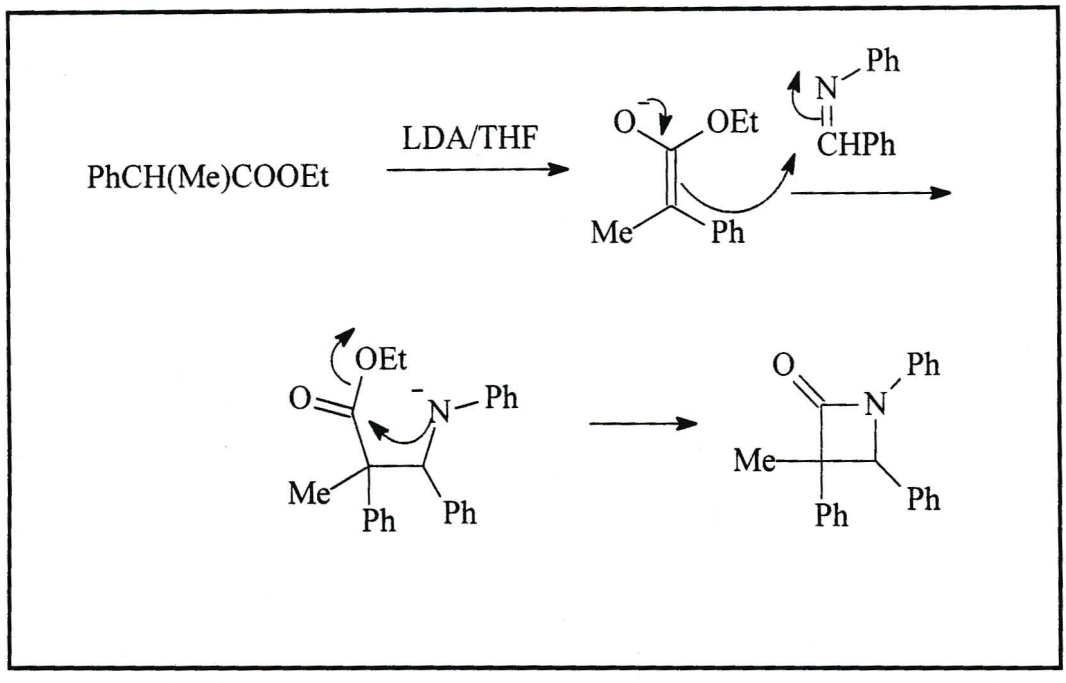
In 1943 it was reported that condensation of the Reformatsky reagent derived from α-bromoacetate with N-phenylbenzaldimine led to formation of the β-lactam, 1,4-diphenyl-2-azetidinone in 56% yield.[22] (Scheme 9).



Scheme 9: Synthesis of β-lactams *via* Reformatsky reagent.

This reaction has become known as the ester enolate imine condensation route to β-lactams. Further work regarding the scope of this synthesis was slow after its discovery. However, in the last twenty years research in this field has increased markedly with the result that this method is now a well-known and widely used path to β-lactams.

In 1980, Bergbreiter and Newcomb reported the reaction of lithium ester enolates with imines.[23] It was shown that enolates formed by treating α,α-disubstituted acetates with LDA would react with non-enolizable N-arylaldimines to produce β-lactams in moderate to high yields (Scheme 10).

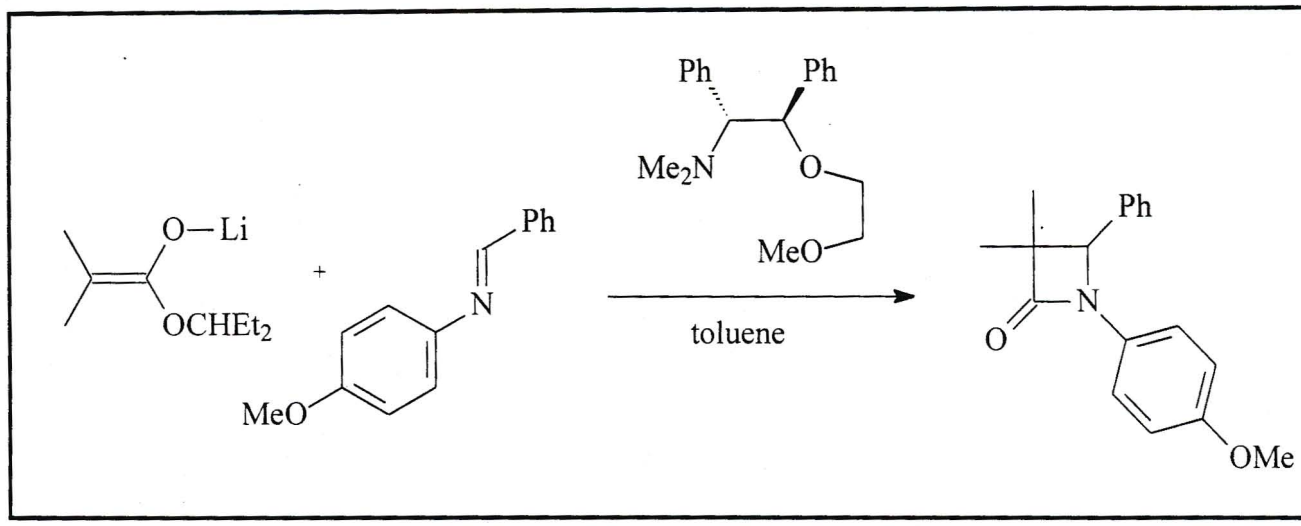


Scheme 10: Synthesis of β-lactams by lithium ester enolate imine condensation.

When chiral ester enolates are used, optically active β-lactams are formed with up to 60% ee. This procedure was an advance on the procedure *via* Reformatsky reagents simply because esters are far more readily available than α-bromo esters. However, this method is limited by the fact that N-alkyl and enolizable aldimines fail to afford β-lactams.

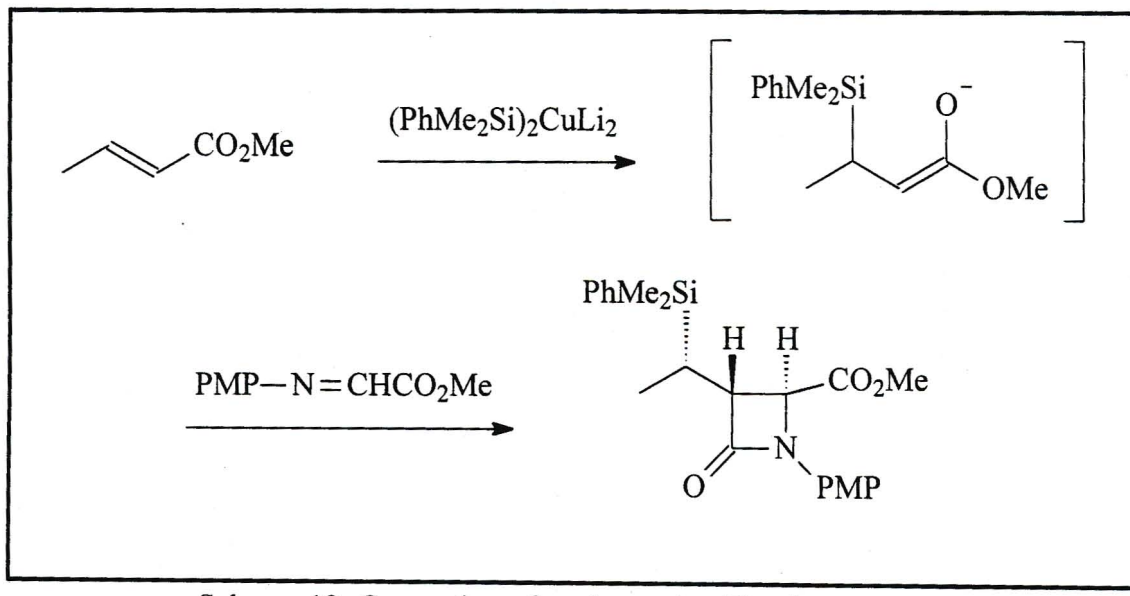
Further advances on this procedure have been very successful. In 1999, a method was reported using an external chiral tridentate amino diether ligand as a catalyst which

resulted in enantiomerically enriched β-lactams in high yield (Scheme 11).[24] As an example of this method, the following reaction is reported to proceed in 99% yield, with 89% ee.



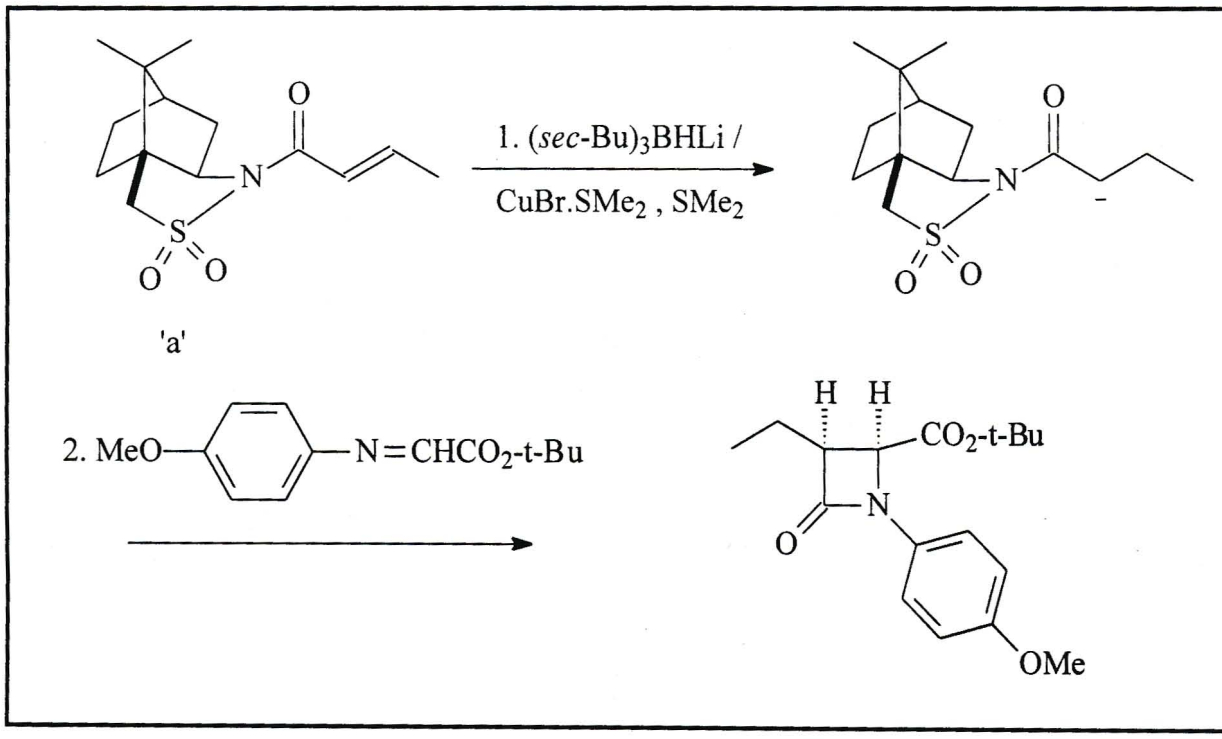
Scheme 11: Lithium ester enolate imine condensation using external chiral ligand.

A further variation of the ester enolate imine condensation employed organometallic reagents in the generation of the enolate.[25] Addition of Fleming's silylcuprate reagent to methyl crotonate, followed by reaction with an iminoester, afforded a β-lactam in 80% yield as the single diastereoisomer (Scheme 12).



Scheme 12: Generation of enolate using Fleming's reagent.

A method that is very similar to that mentioned above involves the use of lithium or potassium tri-*sec*-butylborohydrides in generating the ester enolates.[26] Lithium and potassium tri-*sec*-butylborohydrides are versatile synthetic reagents and are commercially available from Aldrich Chemical Co. as L- and K-Selectride® respectively. The reaction proceeds *via* addition of L- or K-Selectride® to an N-enoylsultam (Scheme 13 'a'). Subsequent reaction with an imine affords a β-lactam in moderate to good yield and excellent enantioselectivity. The reaction is enhanced by the use of copper halide complexes along with dimethyl sulphide as cosolvent. (Scheme 13).

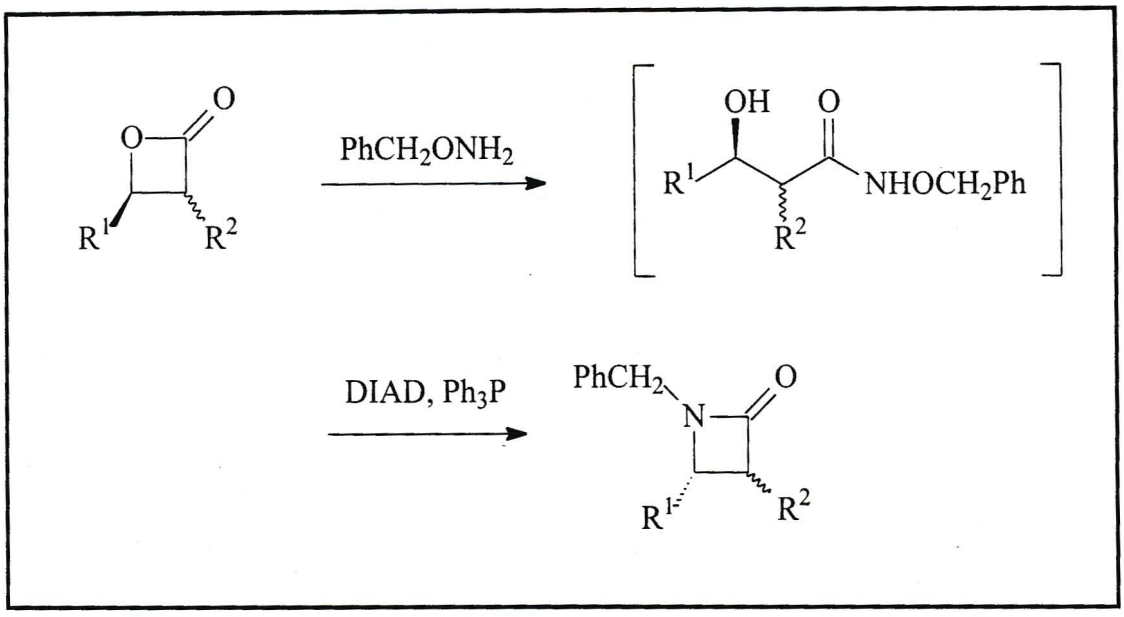


Scheme 13: Generation of enolate using L-Selectride.

As an example of this synthesis, the above reaction afforded the indicated β-lactam in 72% yield and 97% ee.

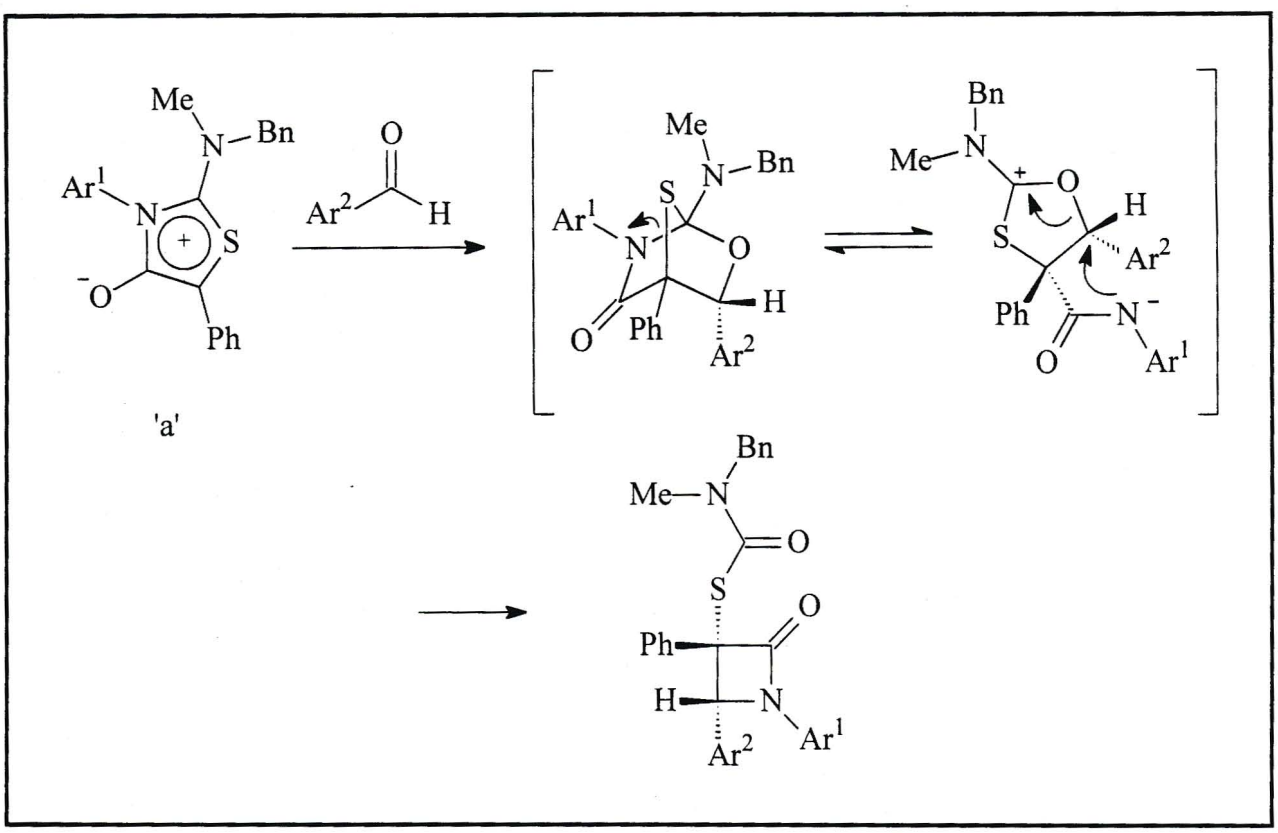
β-Lactams have also recently been prepared from β-lactones *via* a ring-opening ring-closing sequence.[27] The ring-opening is accomplished using *O*-benzyloxyamine as base and the reaction is performed neat, at 25°C. The subsequent ring-closure is analogous to work done by Miller in preparing β-lactams from β-hydroxycarboxylic acids.[28] Ether is added to the reaction mixture followed by triphenylphosphine and

diisopropyl azodicarboxylate (DIAD). The corresponding β-lactams are produced in good overall yield (Scheme 14).



Scheme 14: Conversion of β-lactones to β-lactams.

Very often, organic reactions do not proceed in expected ways. Although all of the above syntheses have been planned and have given the desired products, it occasionally happens that β-lactams are formed by unexpected routes. One such example was reported in 1999, where 1,3-thiazolium-4-olates (Scheme 15 'a') were reacted with aromatic aldehydes and unexpectedly yielded β-lactams.[29] The process has been rationalised by a " [4+2] cycloaddition in which thioisomünchnone plays the role of the dipole to produce a transient cycloadduct which undergoes a spontaneous C-N cleavage, followed by a rearrangement under the reaction conditions" (Scheme 15).[29]



Scheme 15: Unexpected ring opening to afford β-lactams.

2. Kinetic and thermodynamic aspects of ring strain

In 1885, Adolph von Baeyer noted the importance of the deviation of the internal angles in most cycloalkanes from the ideal 109.5°.[30] He predicted that the more the internal angles of the cycloalkanes deviate from the ideal, the more unstable they would be. This led to the idea of "angle strain", a major factor in ring stability which has continued to be used by organic chemists to the present day. However, cycloalkanes need not be planar (with the exception of cyclopropane) and, therefore, a direct parallel cannot be drawn between angle strain and stability.

A quantitative evaluation of strain energy sheds more light on the problem. The term 'strain energy' refers to the difference between the $\Delta H_f°$ of the cycloalkane and the $\Delta H_f°$ of the corresponding straight chain alkane. The bigger the difference, the bigger the strain energy and the less stable the cycloalkane is. Table 1 below shows the heats of formation for some cycloalkanes. Also shown is the $\Delta H_f°$ per methylene group.

Molecule	**$\Delta H_f°$ (kcal/mol)**	**$\Delta H_f°$ per CH_2 (kcal/mol)**
cyclopropane	+12.7	+4.2
cyclobutane	+6.8	+1.7
cyclopentane	-18.7	-3.7
cyclohexane	-29.5	-4.9
cycloheptane	-28.3	-4.0
cyclooctane	-29.7	-3.7
cyclodecane	-36.9	-3.7
cyclododecane	-55.0	-4.6
$(CH_2)_\infty$		-4.9

Table 1: $\Delta H_f°$ for selected cycloalkanes.

An interesting point to note is that the $\Delta H_f°$ per CH_2 for a strain free, straight chain alkane $(CH_2)_\infty$ is identical to that of cyclohexane.

Using these values to calculate strain energies for the cycloalkanes provides an interesting table of results (Table 2). 'Calcd' represents the $\Delta H_f°$ for the strain free, straight chain alkane.

Molecule	$\Delta H_f°$ (kcal/mol)	Calcd $\Delta H_f°$ (kcal/mol)	Strain E (kcal/mol)
cyclopropane	+12.7	-14.7	27.4
cyclobutane	+6.8	-19.6	26.4
cyclopentane	-18.7	-24.5	5.8
cyclohexane	-29.5	-29.4	0.1
cycloheptane	-28.3	-34.3	6.0
cyclooctane	-29.7	-39.2	9.5
cyclodecane	-36.9	-49.0	12.1
cyclododecane	-55.0	-58.8	3.8

Table 2: Strain energies of selected cycloalkanes.

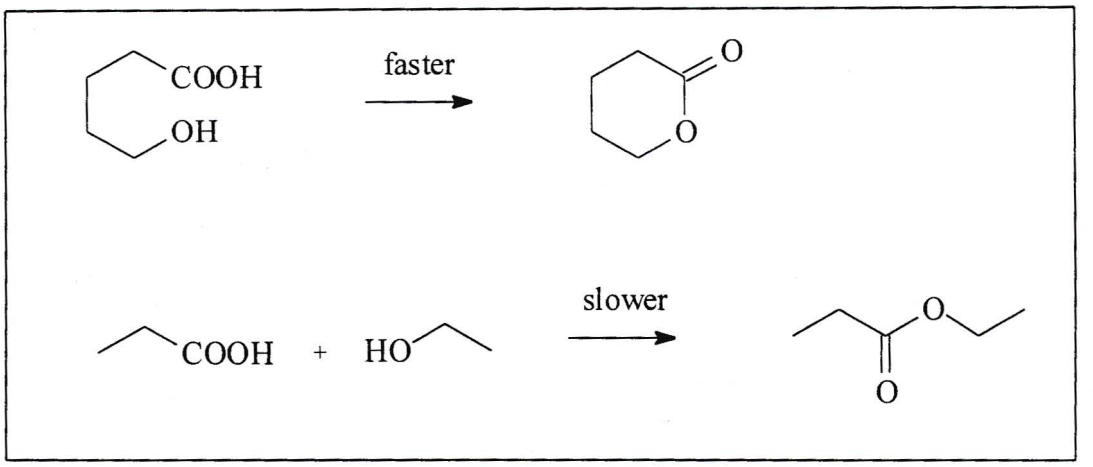
Analysing the data we find that cyclopropane and cyclobutane are similarly strained (although strain per methylene does differ between the two) and are the most unstable of the cycloalkanes. Cyclopentane is only slightly strained while cyclohexane is almost completely strain free, as pointed out earlier. Strain gets worse as we look through the medium sized rings until cyclododecane (12-membered) is reached. Here strain declines until reaching the hypothetical, strain free, infinitely sized ring.

Heats of combustion ($\Delta H_c°$) can also be used to establish strain energies quantitatively. The higher the $\Delta H_c°$ the less stable the compound. $\Delta H_c°$ for each cycloalkane can be measured directly. From this value the $\Delta H_c°$ per methylene group can be calculated. The value of $\Delta H_c°$ per methylene group of a strain free chain is calculated to be 157.5 kcal/mol, and this value subtracted from the measured $\Delta H_c°$ per methylene group for the cycloalkane will give the strain energy per methylene group. This is easily converted to the strain energy of the molecule. Table 3 shows the close correspondence between strain energy calculated from $\Delta H_f°$ and from $\Delta H_c°$.

Molecule	Experimental $\Delta H_c°$ per CH_2 (kcal/mol)	Strain E / CH_2 ($\Delta H_c°$-157.5) (kcal/mol)	Strain E from $\Delta H_c°$ (kcal/mol)	Strain E from $\Delta H_f°$ (kcal/mol)
cyclopropane	166.3	8.8	26.4	27.4
cyclobutane	163.9	6.4	25.6	26.4
cyclopentane	158.7	1.2	6.0	5.8
cyclohexane	157.4	-0.1	-0.6	0.1
cycloheptane	158.3	0.8	5.6	6.0
cyclooctane	158.6	1.1	8.8	9.5
cyclodecane	158.6	1.1	11.0	12.1
cyclododecane	157.8	0.3	3.6	3.8
$(CH_2)_\infty$	157.5	-	-	-

Table 3: Comparison of strain energies for selected cycloalkanes.

A brief look at how entropy of activation $\Delta S^‡$ affects ring closure adds further to our understanding.[31] A ring-closing reaction requires the two ends of a molecule to interact. If these two are the ends of a ten carbon chain, the likelihood of them coming in close enough proximity to interact is small. Therefore, forming the transition state results in a great loss of entropy. The same factor is present in closing rings of five or six members. However, these reactions are more likely to occur as the entropy loss is less than that of bringing two individual molecules together. Take, for example, the reaction between an OH group and a COOH group to form a lactone with a 5- or 6-membered ring which takes place much faster than the corresponding reaction to form an acyclic ester (Scheme 16).



Scheme 16: Comparison of intramolecular and intermolecular reactions.

Although $\Delta H^{\ddagger}$ is similar for both reactions, $\Delta S^{\ddagger}$ is much less for the cyclization. However, if the ring to be closed is a 3- or 4-membered one, angle strain becomes a major contributing factor, and the favourable $\Delta S^{\ddagger}$ may not be enough to overcome the unfavourable $\Delta H^{\ddagger}$.

3. Radical addition reactions

A reaction of this type was used to synthesise ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate, which was to be used as a possible precursor to a β-lactam.

3.1. Production of free radicals *via* photolysis

Absorption of electromagnetic radiation can lead to one of many different processes. One of which is the homolytic fission of a bond.[32] The energy of this radiation is related to its wavelength by the following equation, where 'h' is Plank's constant (6.626×10^{-34} J.s) and 'c' is the speed of light (3.00×10^{8} m/s).

$$E = hc / \lambda \quad \text{or} \quad E \text{ (kcal/mol)} = 28600 / \lambda \text{ (nm)}$$

Scheme 17: Equation relating energy of radiation and its wavelength.

Most bonds encountered in organic compounds have energies of the order of about 50-95 kcal/mol.[33] Radiation of this energy has wavelengths that fall into the UV/visible region of the electromagnetic spectrum.

λ / nm	200	250	300	350	400	450	500
E / kcal/mol	143.0	114.4	95.3	81.7	71.5	63.5	57.2

Table 4: Energy of radiation with associated wavelength.

In theory, radiation of wavelength of 270nm is sufficient to break most bonds in organic compounds. However, in practice this is not the case as not all the energy is usually used in bond breaking and radiation of higher energy is usually needed to effect cleavage.

3.2. Radical addition to olefins

Generally speaking, radical addition to a carbon-carbon double bond is exothermic.[32] The consequence of this exothermicity is that the transition state resembles the reactants which allows us to describe the reactivity in terms of frontier molecular orbital interactions. The orbitals of concern are the SOMO (singly occupied molecular

orbital) of the radical and the HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) of the double bond i.e. the π and π^* orbitals. The HOMO is stabilised by interaction with the SOMO, which is destabilised by interaction with the HOMO and stabilised by interaction with the LUMO. Thus, to a first approximation, the system is stabilised by the drop in energy of the two electrons in the HOMO. The closer the SOMO and the HOMO are in energy, the greater this stabilisation (Fig.18).[32]

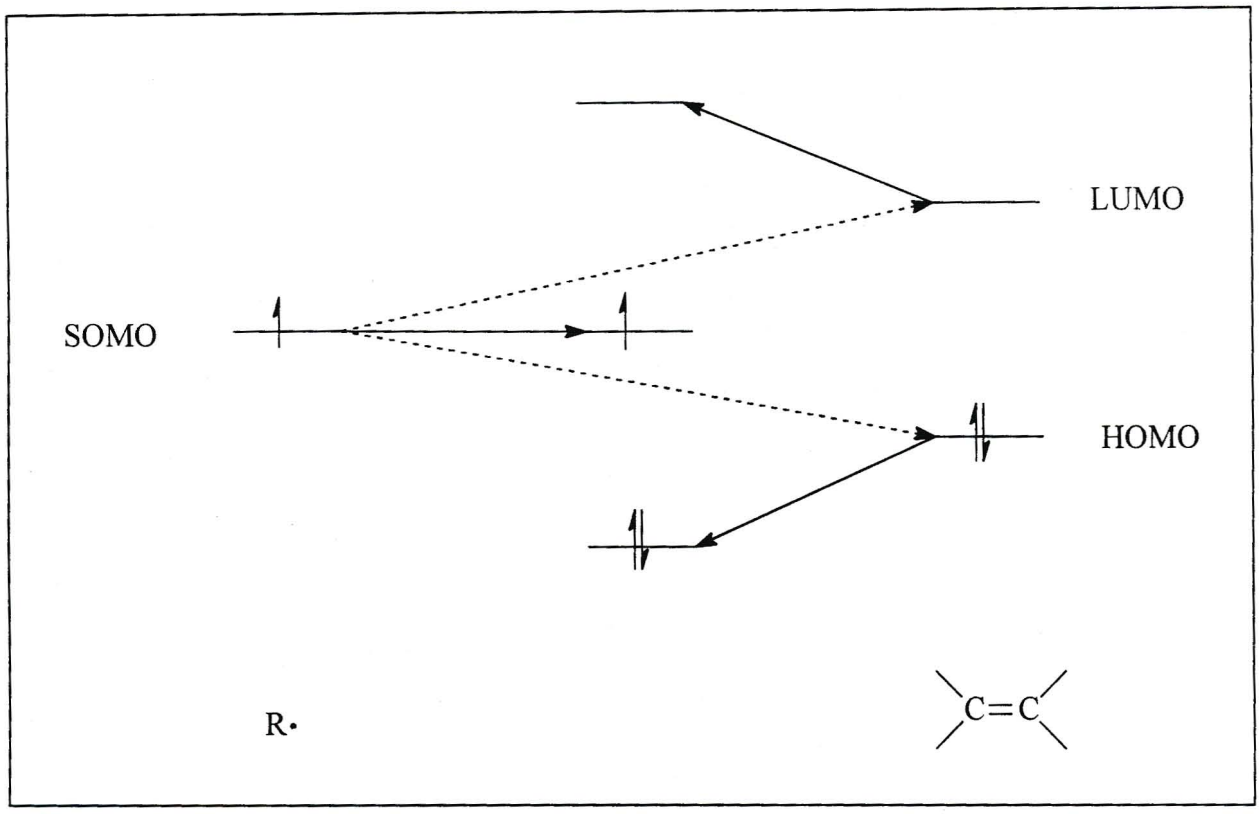


Fig. 18: Frontier molecular orbital interactions.

For radicals with electron donating substituents (high energy SOMO) and an olefin with electron withdrawing substituents (low energy LUMO), the SOMO and LUMO are close in energy and, therefore, reactivity is high. In this case, the radical behaves as a nucleophile. Conversely, the SOMO-HOMO interaction dominates for radicals with electron withdrawing substituents (low energy SOMO) and olefins with electron donating substituents (high energy HOMO). Here the radical behaves as an electrophile.

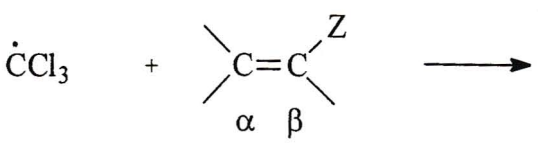
These effects can be illustrated using relative reaction rates.[34] The table below shows the ratio of the addition rates of methyl, fluoromethyl, difluoromethyl and trifluoromethyl radicals to tetrafluoroethylene and ethylene respectively (Table 5).

Radical	$\cdot CH_3$	$\cdot CH_2F$	$\cdot CHF_2$	$\cdot CF_3$
$k(C_2F_4)/ k(C_2H_4)$	9.5	3.4	1.1	0.1

Table 5: Ratio of addition rates to different olefins.

Methyl radicals are electron rich and their rate of addition to an electron poor olefin (C_2F_4) is greatly accelerated compared to their rate of addition to an electron rich olefin (C_2H_4). The converse is the case for the electron deficient trifluoromethyl radical. These polar effects are the major factor in determining the rate of addition.

In addition to this polar effect, the substituents on the olefin also have a steric effect. This effect has a very minor contribution to the rate of reaction, but rather governs the regioselectivity of the addition. The table below shows that the orientation of the addition to an electron rich and an electron poor olefin is almost identical, however, the rate of addition differs very markedly, as discussed in preceding paragraphs. The table shows the relative rates of addition to the olefin and the orientation ratios (α:β) for the addition of trichloromethyl radicals to two olefins (Table 6).

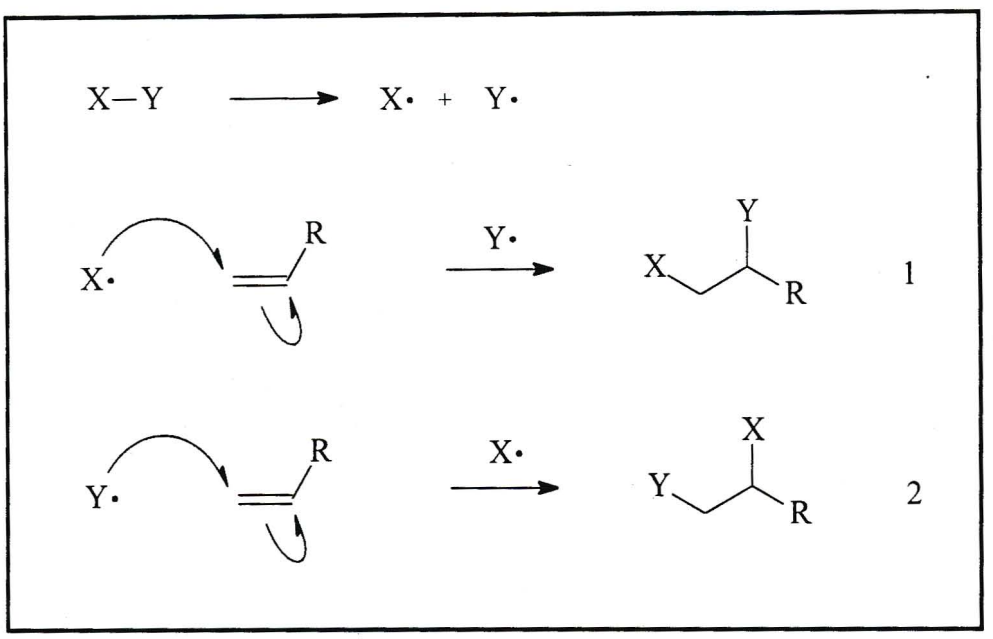


Z	CH_3	CF_3
$\alpha : \beta$	1 : 0.07	1 : 0.08
$2k(\alpha) / k(C_2H_4)$	9	0.7

Table 6: Relative rates of addition and orientation ratios.

Essentially, the radical initiating the reaction will attack the least hindered side of the double bond.[32] In disubstituted olefins the orientation ratios vary with the size of the

substituents, however, in olefins with a methylene group, attack is almost exclusively at the terminal carbon. This makes it possible, in reactions of olefins with terminal methylene groups, to distinguish, which radical is the attacking one by observing which product is formed. In the reaction scheme shown below (Scheme 18) it is possible to distinguish which radical (X or Y) is the attacking radical by observing which product is formed (1 or 2).



Scheme 18: Determination of attacking radical.

4. Footnote to Introduction

Although the aim of this project was to prepare β-lactams by a novel method, this was not successful due to reasons discussed in the following pages. Literature research regarding synthesis and natural occurrence of β-lactams was carried out prior to commencing this work (pages 1-21). This section was included despite the failure to perform the synthesis as it was still deemed relevant to any further work in this area of study.

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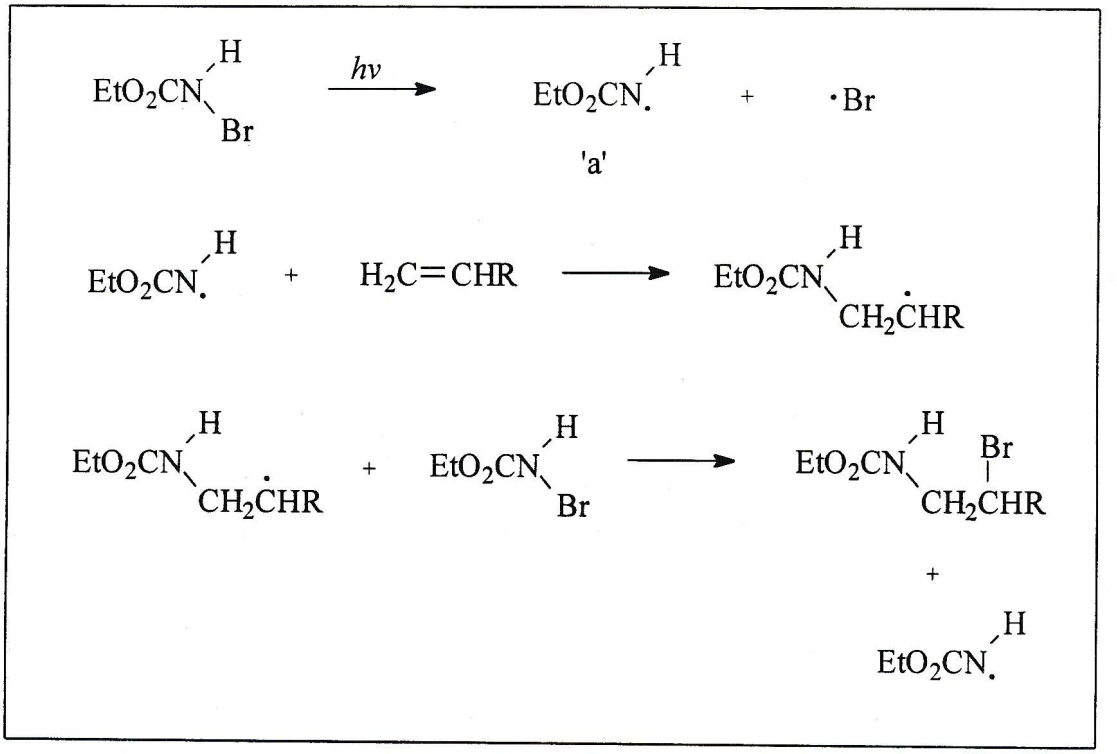
Discussion

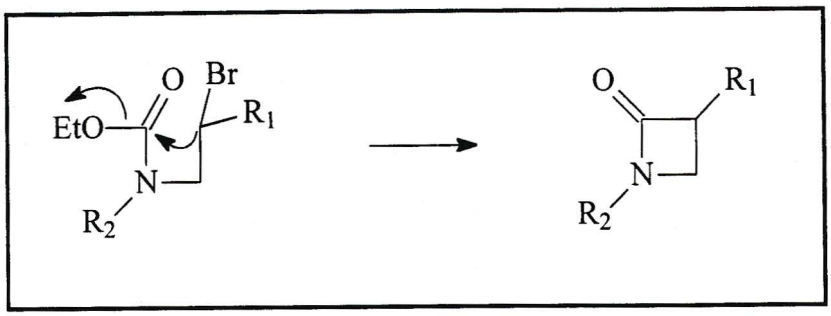
1. Formation of ethyl N-2-bromo-alkylcarbamates *via* photolysis reactions

When N-bromocarbamates are subjected to photolysis, a carbamyl radical (Scheme 19 'a') and a bromine atom are produced.[1] In the presence of an olefin, 1,2-addition can occur. The reaction is initiated by the nitrogen centred carbamyl radical. This is indicated by the fact that the carbamyl group is attached to the terminal carbon of the olefin. This observation is discussed earlier in the text (**Introduction** pages 28-29). Subsequent bromination is achieved either within the solvent cage or *via* an alkyl radical chain process (Scheme 19).



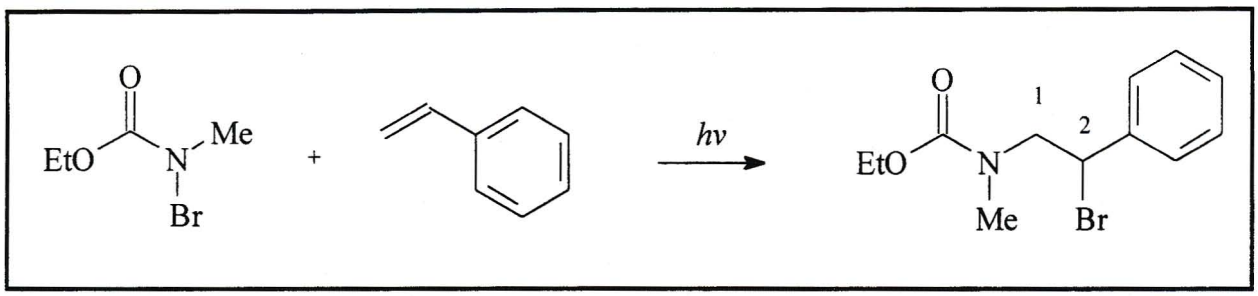
Scheme 19: Radical chain process for addition to olefins.

Reactions of this type form adducts which are generally known as ethyl N-2-bromo-alkylcarbamates. These adducts show considerable potential as precursors to β-lactams.[2] Although, many methods exist for the preparation of β-lactams, as described in the **Introduction** (page 12-21), no practical methods exist for their preparation *via* 2-3 bond formation (Scheme 20).



Scheme 20: Formation of β-lactams *via* 2-3 bond formation.

For the purpose of investigation into the feasibility of these types of reactions, ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate was prepared by photolysing ethyl N-bromo-N-methylcarbamate for 3 hours in the presence of styrene (Scheme 21).



Scheme 21: Formation of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate.

The photolysis was carried out in an inert nitrogen atmosphere at ambient temperature, in a pyrex reaction vessel, using a medium pressure mercury lamp. Temperature was maintained using a circulating water bath (~20-21°C). Ethyl N-bromo-N-methylcarbamate absorbs radiation at 305nm. Workup produced an oil, which was purified by column chromatography (silica gel, ether/hexane). Ethyl N-bromo-N-methylcarbamate was prepared by stirring ethyl N-methylcarbamate, in the presence of bromine, under darkness, for a period of 48 hours.[2] The percent conversion of the starting material was determined by titration against a standard 0.100M sodium thiosulphate solution.[3] This procedure is discussed fully in the 'Foreword to experimental' section (page 60).

A very interesting observation was made in the proton NMR spectrum of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate (Spectrum number 2.1, page 5). H_2 should resonate at approximately 5.4 ppm[4] as a triplet or, if the two adjacent H_1 protons are non-equivalent, as a pair of doublets. What is, in fact, observed is a **pair** of resonances at 5.05 and 5.22 ppm respectively, both of which appear to be triplets and,

combined, integrate to one proton. We observe that the two H_1 protons appear as two separate resonances at 3.75 and 3.90 ppm respectively. This indicates that the two are non-equivalent. We, therefore, expect H_2 to appear as a pair of doublets. However, it was not possible to resolve the resonances sufficiently in order to determine coupling constants and they are, therefore, described as triplets. Why H_2 appears as two separate resonances is not clear. It was suggested that there could be restricted rotation about the C_1-C_2 bond resulting in two fixed rotamers. In order to test this theory, a ^{1}H NMR spectrum was obtained of a sample at room temperature (Spectrum number 2.1, page 6) and then was rerun at 50°C (Spectrum number 2.1, page 7). It appears that the two resonances at 5.05 and 5.22 ppm began to converge at the higher temperature. This observation tends to agree with the theory that there is restricted rotation about the C_1-C_2 bond at room temperature.

In order to substantiate this theory, an *ab initio* computational investigation was conducted. A relaxed scan, using Gaussian '98[5] at the Restricted Hartree-Fock (RHF) level of theory with the 3-21G basis set was used to determine the potential energy about the C_1-C_2 bond as a function of dihedral angle. Note that the basis set is likely to give poor results regarding absolute energies,[6] but since larger basis sets will take too long (this scan job took about 1.5 days) a better basis set could not be afforded. The plot of these values is shown in **Appendix B**, page 1. The dihedral angle in question is the N-C_1-C_2-C dihedral angle shown in **Appendix B**, page 2. During this relaxed scan calculation the only restriction imposed is the dihedral angle. All other atoms in the molecule are free to rearrange (according to the mathematical optimization algorithm of the software) to the lowest possible energy. The scan calculation involves a 15° stepwise increment of the dihedral angle through 360°. At each step the molecule, subject to the dihedral restriction, is allowed to optimize and the energy of the different rotational conformations is plotted as function of the energy. Note that the resulting calculated energies are only a rough estimation of the rotational energy barriers since (a) the basis set (3-21g) used for the scan job is the minimum standard and (b) the optimization code for a normal optimization (which we used for the scan) is mathematically designed to search for a minimum and not designed to determine maxima, which is the case for transition states.

The energy barrier to rotation about a single bond, at room temperature, is 15-20 kcal/mol.[7] The results from the scan calculation determined that the greatest energy barrier to be overcome, from the minimum at about 296° (-64°) to the rotational transition state at about 235°, is approximately 7 kcal/mol. In order to calculate a more accurate value for the maximum rotational energy barrier, the corresponding structures above were taken from the scan output file and resubmitted as (a) a normal optimization for the structure at 296° (-64°) and (b) a transition state optimization for the structure at 235°. An acceptable basis set (6-31+G(d)) was used for these calculations. The optimized coordinates of these structures are given in **Appendix B**, page 3 and 4. Lastly the energies of these optimized structures were calculated (single point calculation) at a better level of theory, namely Density Functional Theory (DFT) with B3LYP.[8,9,10] DFT calculations approximate the effect of electron correlation whereas RHF neglects it completely.

The energies from these B3LYP single point calculations give a maximum rotational energy barrier of about 5.5 kcal/mol. This result indicates that free rotation about the dihedral angle (C_1-C_2 bond) at room temperature should occur and that one should not observe splitting of proton NMR signals due to different stable rotational conformations. This result contradicts the inference, based on the NMR experiment, which seemed to indicate that the splitting of the proton signal at ~5.2 ppm decreases at higher temperature. It is clear, therefore, that the presence of the two resonances in the ^{1}H NMR spectrum is due to factors that have not been considered. Further investigations will have to be conducted in order to establish these.

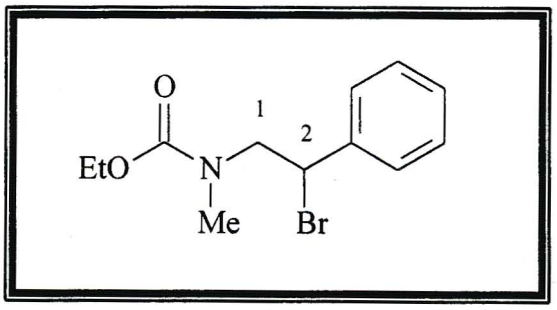
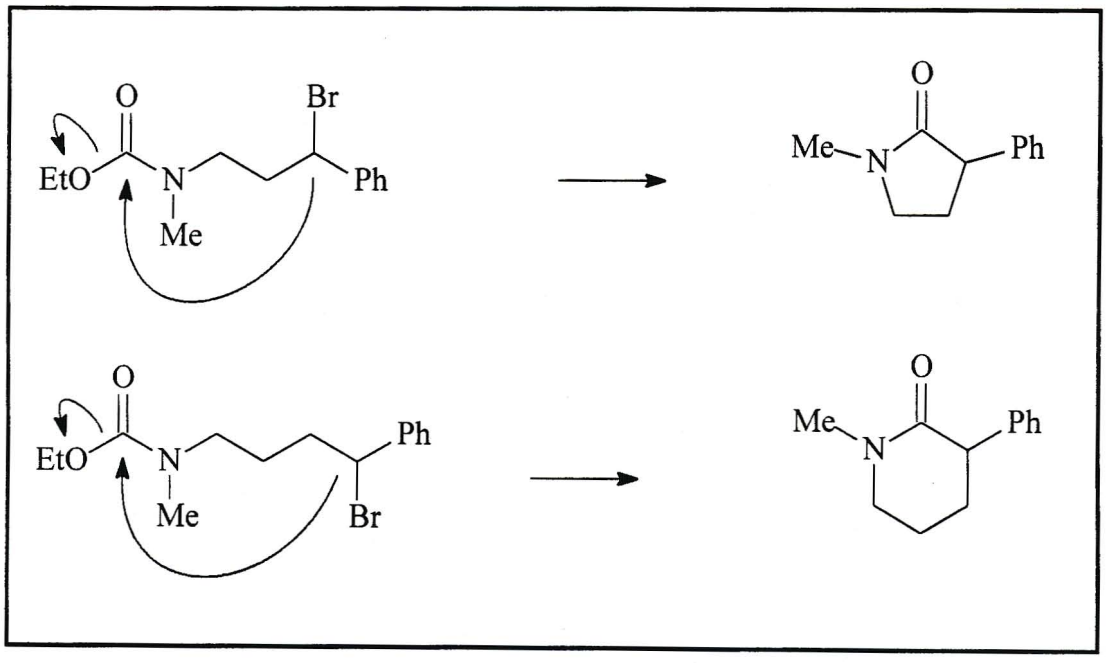


Fig. 19: Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate.

2. Formation of ethyl N-bromo-alkylcarbamates *via* traditional synthetic routes

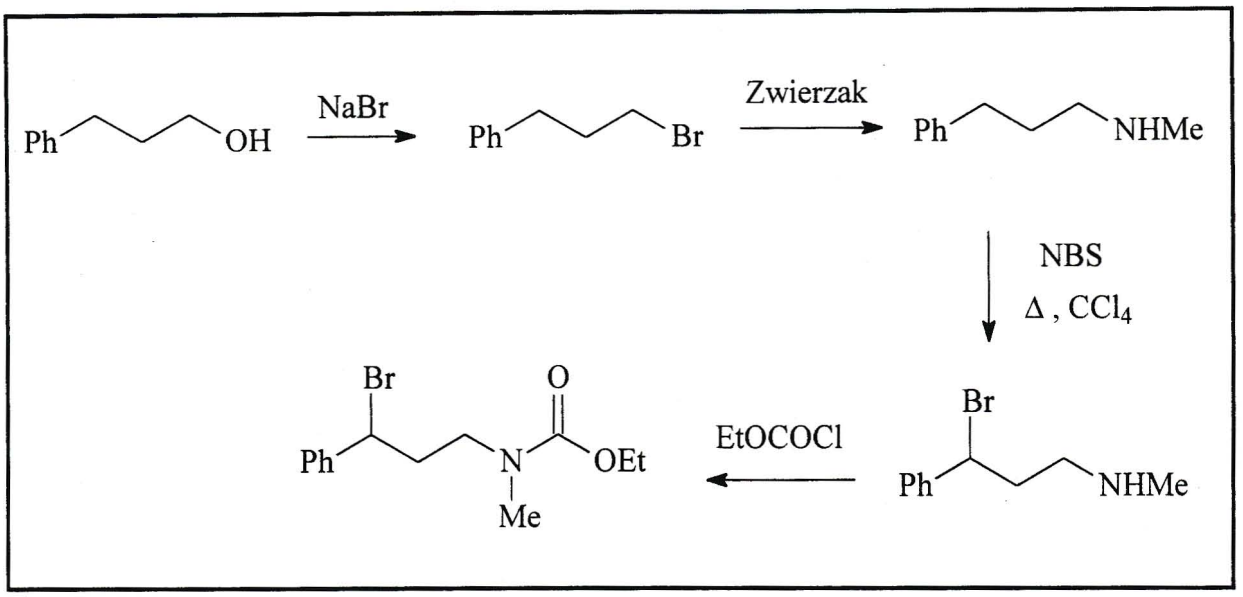
As discussed earlier, 4-membered rings are highly strained and, therefore, quite unstable.[11] Thus, if a reaction to close the 4-membered β-lactam was unsuccessful, it might very well be due to the strained nature of the system and not necessarily to the conditions of the reaction. It was, therefore, deemed necessary to carry out an analogous reaction that eliminates ring strain affects. One possible way to achieve this was by synthesising ethyl N-bromo-alkylcarbamates that, upon cyclisation, would afford 5- and 6-membered lactams, which are almost strain-free. 5- and 6-membered lactams are referred to as γ- and δ-lactams, respectively.



Scheme 22: Formation of γ- and δ-lactams.

Unlike ethyl N-2-bromo-alkylcarbamates, the ethyl N-bromo-alkylcarbamates cannot be prepared *via* photolysis and traditional synthetic methods are needed in order to synthesise them.

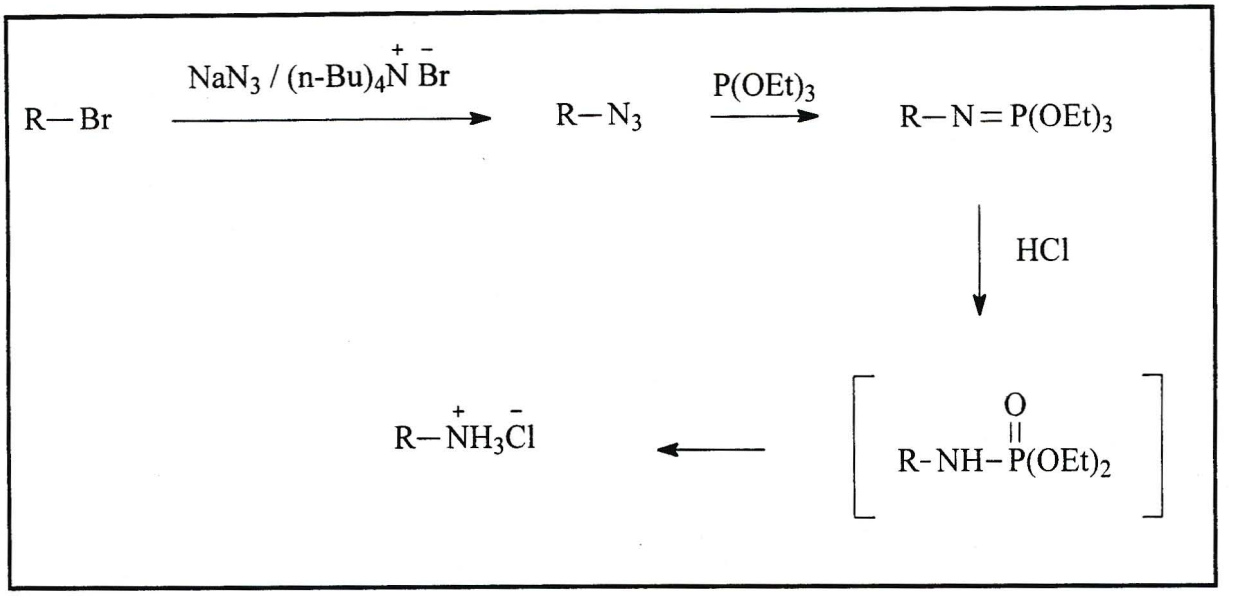
The originally proposed route to the precursor to the γ-lactam is shown below (Scheme 23).



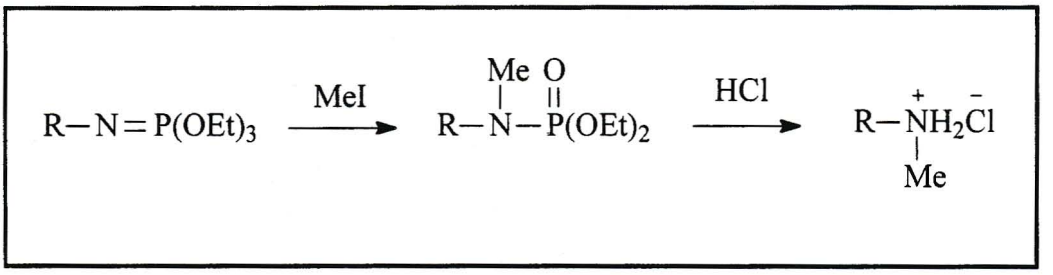
Scheme 23: Originally proposed route to the precursor to γ-lactam.

The first step using NaBr as the brominating agent was abandoned due to poor yields and replaced with the HBr / H_2SO_4 method.[12] A mixture of the alcohol, HBr and H_2SO_4 was heated under reflux for 4 hours. Thereafter, the organic layer was separated and distilled under water vacuum to afford the bromide in very good yield (~80%). This method differs slightly from the literature method, which employs a steam distillation.

The 'Zwierzak' synthesis is a one-pot conversion of alkyl bromides to primary amines (Scheme 24).[13] The preparation involves the azidation of an alkyl bromide under solid-liquid phase transfer catalyst (PTC) conditions. This is followed by the Staudinger reaction of the azide with triethyl phosphite producing an iminophosphorane which is deprotected in two steps by means of hydrogen chloride to afford the amine as the hydrochloride salt. A slight modification is necessary in order to produce a secondary amine as shown below (Scheme 25). The first deprotection step is replaced with a methylation using iodomethane. The product is then treated in the same way as before. The secondary amine is isolated as either the hydrochloride salt or as the free amine.



Scheme 24: Zwierzak synthesis of primary amines.



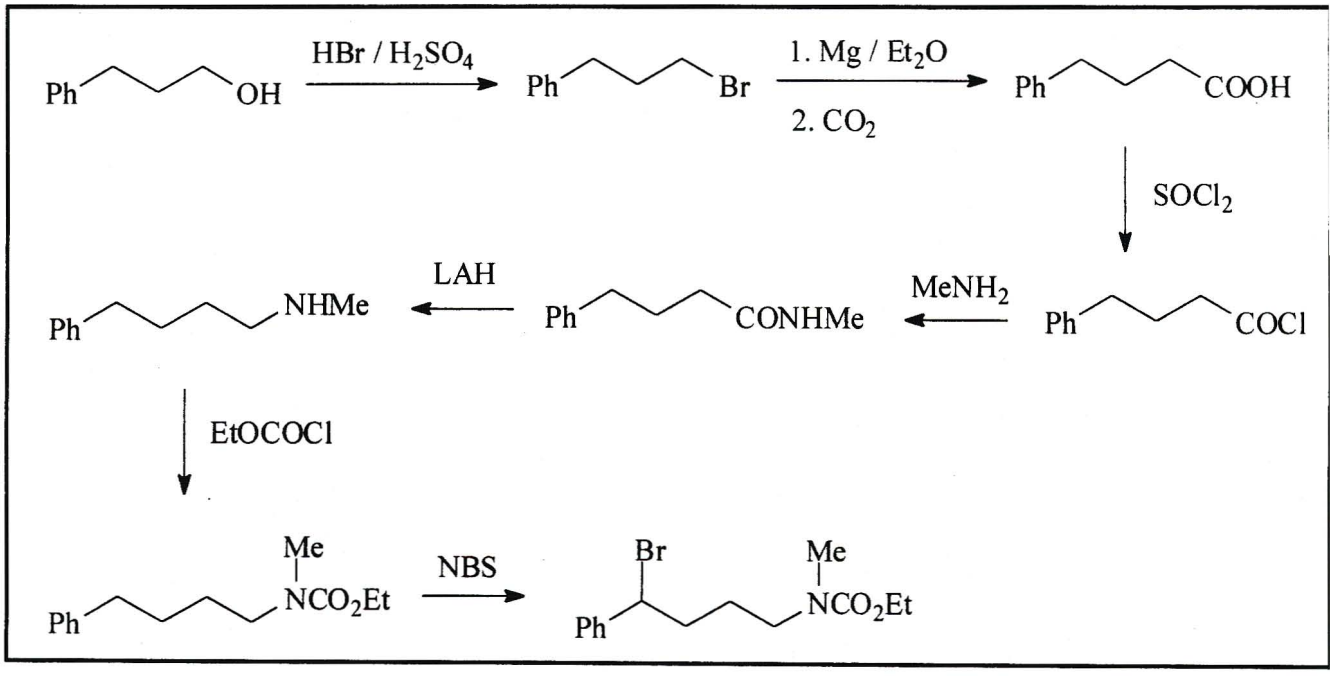
Scheme 25: Modification to Zwierzak method.

The third step of the synthesis using NBS (N-bromosuccinimide) as a brominating agent was unsuccessful. It was thought that the nitrogen proton is too labile and, therefore, it was decided to reverse steps three and four i.e. form the carbamate before attempting the bromination. This strategy was used in the next attempt.

The modified route was attempted using a different starting material, 1-bromo-2-phenylethane. This starting material would produce ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate, the compound produced *via* photolysis described earlier (page 33), and would also eliminate the need to brominate the alcohol. The 'Zwierzak' step was successful. The carbamate was prepared by treating the amine with ethyl chloroformate in the presence of base (NaOH).[14] The reaction proceeded in good yield (~85%).

Subsequent bromination at the benzylic position was attempted by two methods. The carbamate was, first, treated with bromine in the presence of sunlight. However, this was unsuccessful. The carbamate was then treated with NBS in carbon tetrachloride, and heated under reflux overnight. This was unsuccessful at first, however, the NBS was suspected to be impure. A batch of NBS was recrystallized from hot water and the reaction was repeated. This time the method was successful, as was evident from comparison between the proton NMR spectra of the product and that of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate prepared by photolysis. The most striking observation being the presence of the pair of triplets, integrating in total to one proton as discussed earlier.

This proved that this route to the desired ethyl N-bromo-alkylcarbamate was feasible, however, the final product was present in low yield (see **Experimental** page 73) and, thus, a slightly different route was suggested. This is shown below (Scheme 26).



Scheme 26: Second proposed route to ethyl N-bromo-alkylcarbamates.

The first step of the synthesis is identical to the previous route undertaken and was, again, successful in very good yield (~80%). Step two is a standard Grignard reaction with a carbonyl group of carbon dioxide.[15] The reaction proceeded in very good yield (~85%) to afford 4-phenylbutanoic acid.

The carboxylic acid was then treated with a large excess of thionyl chloride.[16] At this stage, the acid was separated into two portions, which were worked with independently. For the first portion, the reaction was spontaneous initially but subsided after the addition of the acid was complete. The mixture was then heated under reflux for one hour. Thereafter, a further portion of thionyl chloride was added and, again, the reaction was spontaneous. The mixture was allowed to stand overnight at room temperature. The mixture was then distilled under high vacuum to afford 4-phenylbutanoyl chloride. The reaction was successful, but in low yield (~20%). Unfortunately, the second portion of the acid afforded no product, which resulted in a very low overall yield for the reaction (~10%).

All the acid chloride was then treated with excess methylamine solution (40%, aqueous). The mixture was stirred overnight at room temperature.[16] Workup afforded N-methyl-4-phenylbutanamide in very good yield (~80%).

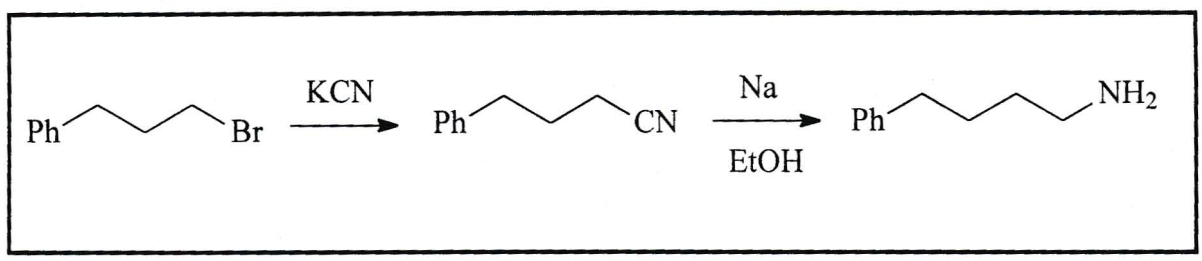
The amide was then subjected to LAH reduction.[16] A modification to the procedure was made in the workup. After heating under reflux for 15 hours, the mixture was carefully treated with water, followed by NaOH solution and finally another three portions of water.[17] This afforded the N-methyl-4-phenylbutylamine in average yield (~40%).

The amine was then treated with ethyl chloroformate in the standard way to produce ethyl N-4-phenylbutyl-N-methylcarbamate.[14] Yield in this case was not as good as on previous occasions but was still adequate (~40%).

The final step in the synthesis was the bromination at the benzylic position using NBS. The NBS was recrystallized from hot water as before. The reaction mixture was heated under reflux for 48 hours and afforded impure ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate. The product was not purified before the next reaction was attempted, as it was assumed that the impurity (parent carbamate) would not affect the outcome of the reaction.

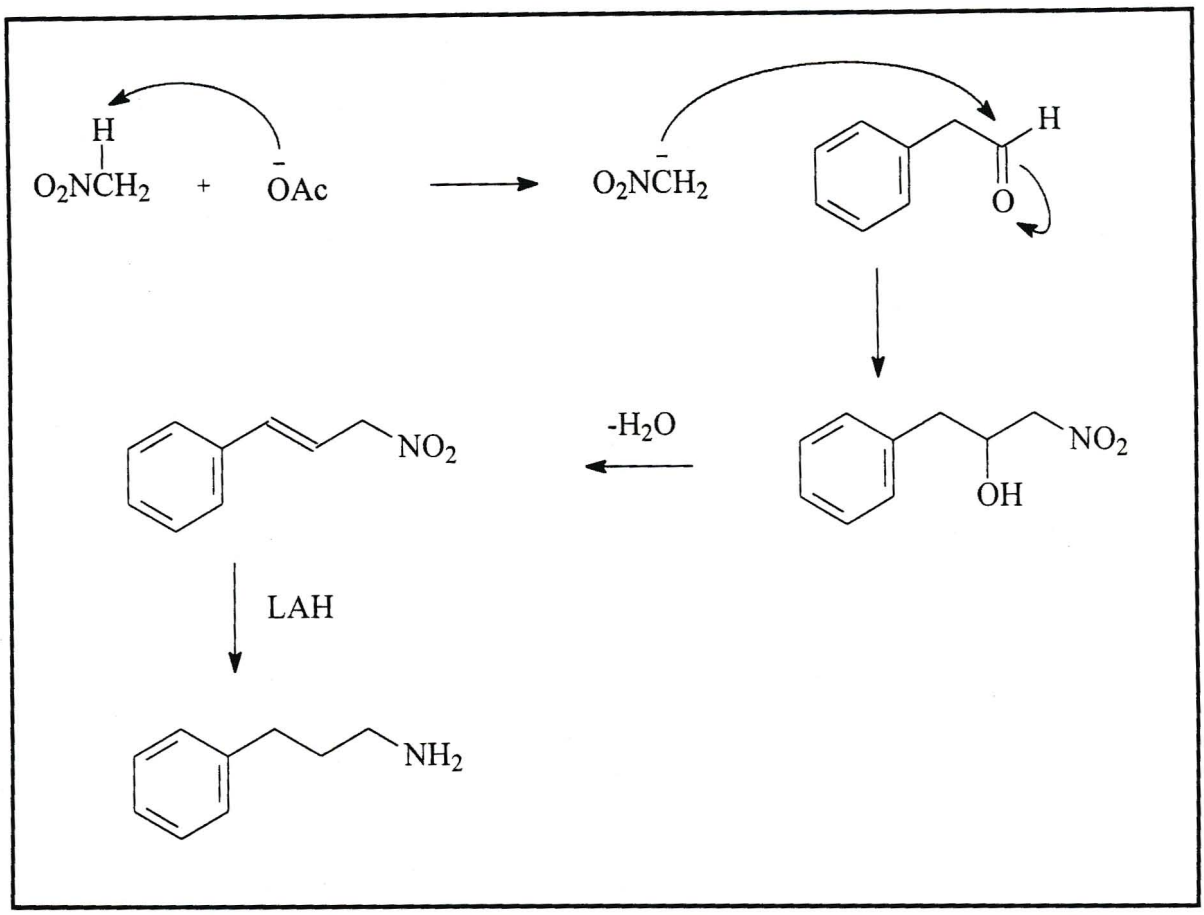
A number of other methods were used in attempts to prepare various amines. These are briefly discussed below.

One proposed method was *via* nitriles (Scheme 27).[12] The nitrile was prepared by treating 3-phenylpropyl bromide with potassium cyanide and heating under reflux for 48 hours. Thereafter, the organic layer was separated and extracted to afford 4-phenylpropyl cyanide. The crude cyanide was added, with ethanol, to an emulsion of sodium and boiling toluene.[12] Workup produced 4-phenylbutylamine. The reaction was successful but not in sufficient yield (14%) to warrant further investigation.



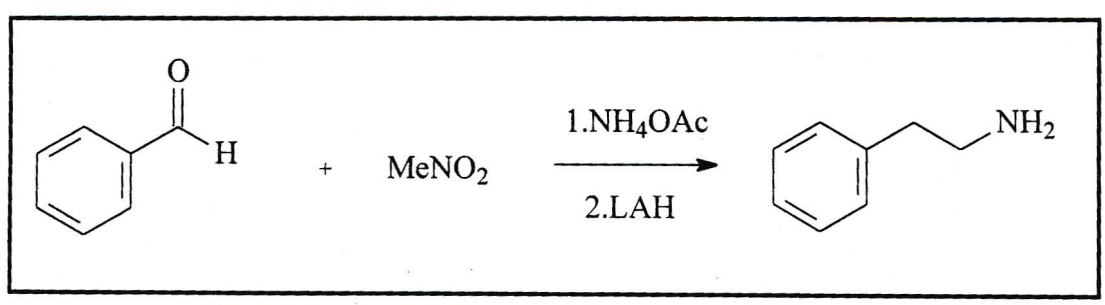
Scheme 27: Preparation of amines *via* nitriles.

Another method that was employed to prepare amines was the Knoevenagel condensation between nitro compounds and aldehydes.[18a] In this case, ammonium acetate was used as a base to abstract an α proton from the nitro compound, which then attacked the carbonyl group of the aldehyde. This led to formation of an alcohol, which spontaneously lost water to form a nitroalkene. This nitroalkene can be reduced, using LAH, to the corresponding amine (Scheme 28).[18b] The reactants used were phenylacetaldehyde and nitromethane. The reaction was carried out by heating a mixture of the aldehyde, nitromethane and ammonium acetate under reflux for 2.5 hours. The excess nitromethane was then removed and the crude material was treated with LAH.



Scheme 28: Mechanism of amine preparation from aldehydes.

NMR studies of the crude intermediate showed no indication that there was a double bond present and NMR spectra of the end product showed no reaction had occurred. This was attributed to the presence of acidic α protons on the aldehyde, which would compete with the nitromethane. In order to test the validity of this statement, the same reaction was carried out using benzaldehyde (Scheme 29). The lack of α protons on benzaldehyde would, presumably, make it a better substrate.



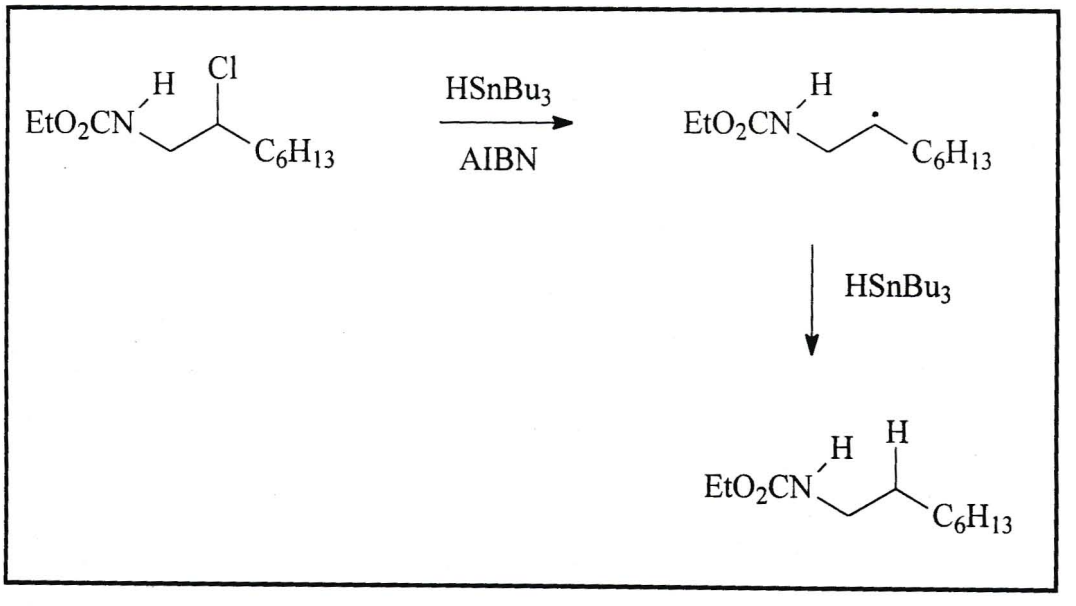
Scheme 29: Amine preparation from benzaldehyde.

In this reaction, the intermediate nitroalkene was successfully isolated, as was evident from a pair of doublets in the ^{1}H NMR spectrum of the crude intermediate. 2-Phenylethylamine was then isolated after reduction with LAH. It appears, therefore, as though the α protons of phenyl acetaldehyde do play a role in the previous reaction. As a result, this method of synthesising amines was abandoned.

Although the original intention of this investigation was to synthesise the precursors to the β-, γ- and δ-lactams, the limited success in the synthesis of the latter two, permitted only the β- and δ-lactam precursors to be prepared. This was not a serious drawback as the ring strain of both the γ- and δ-lactams could be predicted to be very similar.

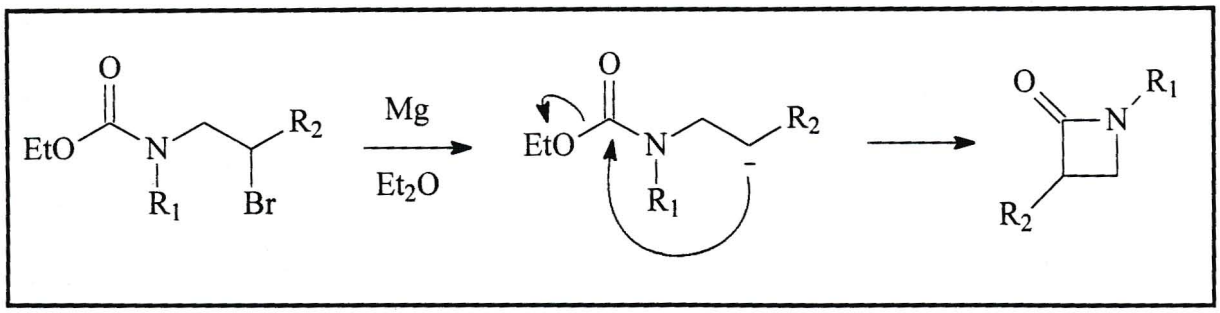
3. Intermolecular additions and intramolecular cyclizations

The ultimate goal in the course of this work was to cyclise ethyl N-bromo-alkylcarbamates. In order to do this, it is necessary to activate the carbon centre α to bromine. In a previous investigation, homolytic cyclisation was attempted by treating ethyl N-2-chloro-octylcarbamate with tributyltin hydride in the presence of azo-iso-butyronitrile (AIBN).[2] In this instance, no cyclisation products were observed. Ethyl N-octylcarbamate and ethyl N-2-chloro-octylcarbamate, the substrate, were the major components of the reaction mixture. The formation of ethyl N-octylcarbamate was attributed to hydrogen abstraction from tributyltin hydride by the intermediate alkyl radical (Scheme 30).



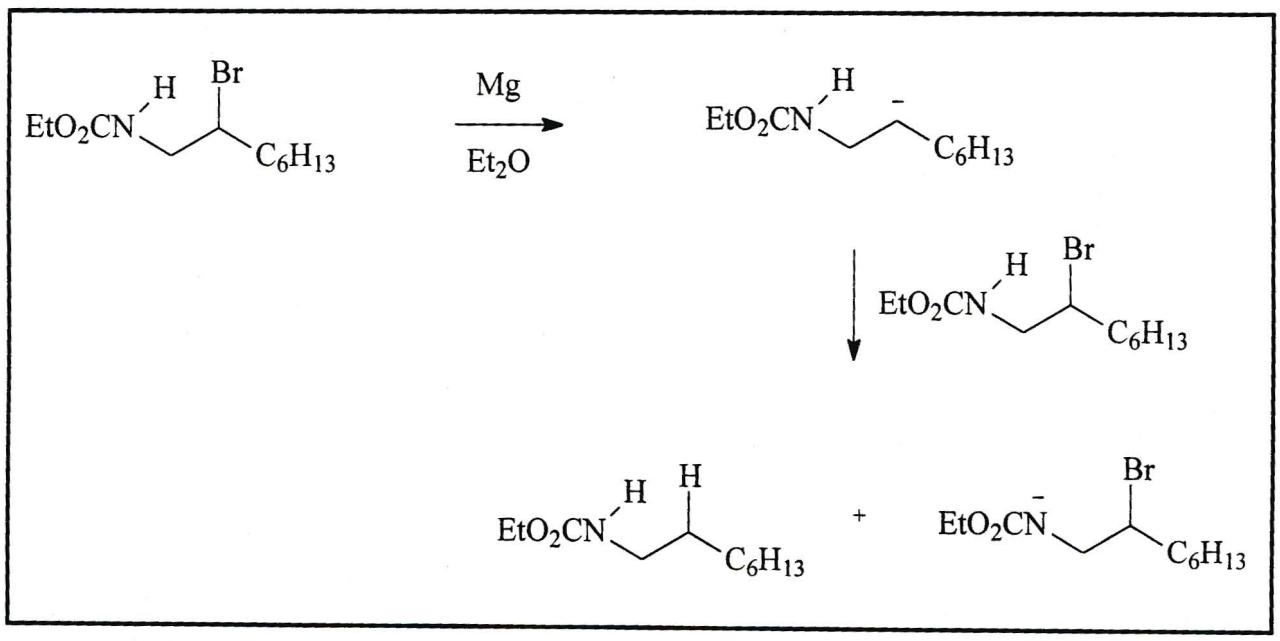
Scheme 30: Formation of ethyl N-octylcarbamate.

The same investigation followed up this homolytic cyclisation attempt with a heterolytic one *via* a Grignard reaction. It was hoped that the carbanion formed would attack the carbonyl carbon, displacing the ethoxy group and forming the β-lactam (Scheme 31).



Scheme 31: Cyclisation *via* Grignard reagent.

Treating ethyl N-2-bromo-octylcarbamate with magnesium produced the Grignard reagent, however, the only compound observed in the final reaction mixture was found to be ethyl N-octylcarbamate. This result was attributed to the lability of the hydrogen atom attached to nitrogen. Abstraction of this hydrogen by the intermediate carbanion is shown below (Scheme 32).

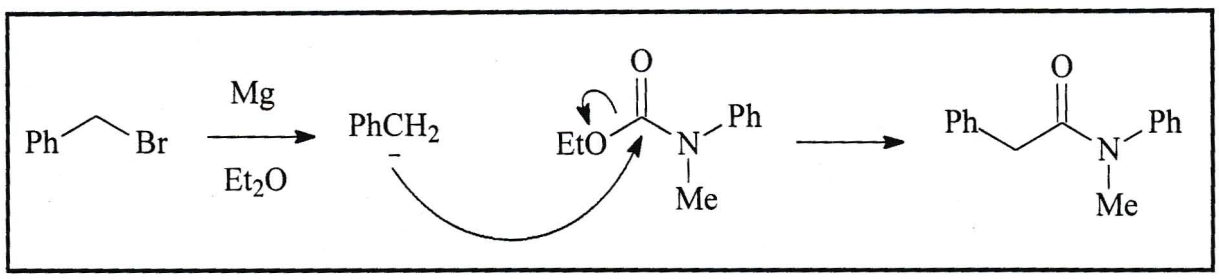


Scheme 32: Formation of ethyl N-octylcarbamate.

The same reaction was then attempted using a substrate not displaying this labile hydrogen. However, treatment of ethyl N-methyl-N-2-bromo-octylcarbamate with magnesium metal did not produce the Grignard reagent.

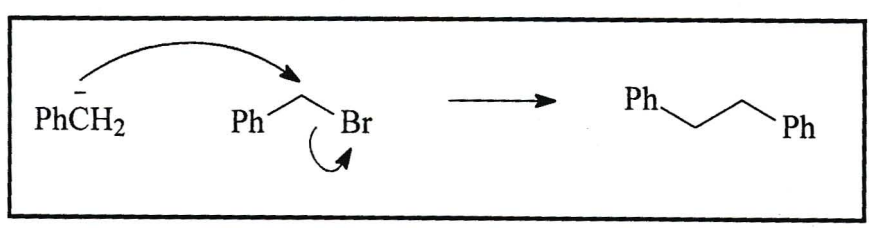
To further this investigation the intermolecular addition of the Grignard reagent of benzyl bromide to another carbamate, ethyl N-methyl-N-phenylcarbamate was

attempted (Scheme 33). It was hoped that this reaction would be analogous to the intramolecular cyclisation.



Scheme 33: Grignard addition to ethyl N-methyl-N-phenylcarbamate.

Treatment of benzyl bromide with magnesium metal gave a spontaneous reaction. The carbamate was then added to the Grignard reagent and the mixture was heated under reflux for 22 hours. Upon workup, the major component of the reaction mixture, apart from unreacted carbamate, was found to be bibenzyl. It was apparent that the Grignard reagent had reacted with unreacted benzyl bromide (Scheme 34).



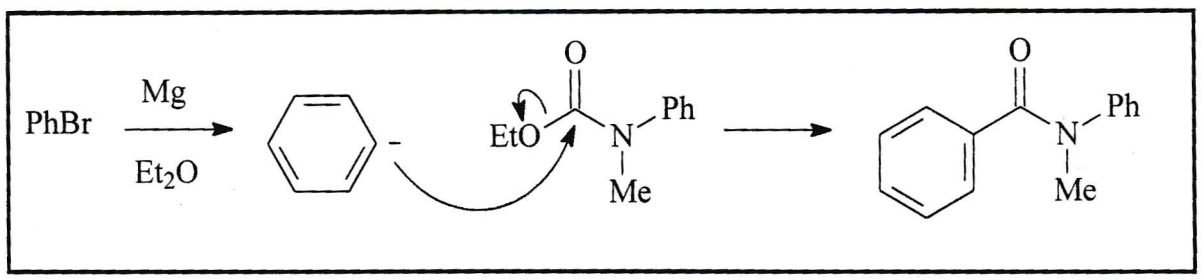
Scheme 34: Formation of bibenzyl.

This could have been due to an insufficient amount of solvent (diethyl ether) which stabilises the Grignard reagent.[7] The reaction was repeated with a greater amount of solvent, however, the result was identical to that observed previously.

It was noted that addition of Grignard reagents to carbonyl compounds is effectively catalysed by copper (I) salts.[19, 20] With this in mind, the above reaction was repeated, with the addition of a catalytic amount of copper (I) iodide. However, the outcome of the reaction was identical to that observed in the first experiment. The yield of bibenzyl was observed to be higher in the experiment using copper (I) iodide though,

which showed that the copper (I) salt did, in fact, enhance the reactivity of the Grignard reagent.

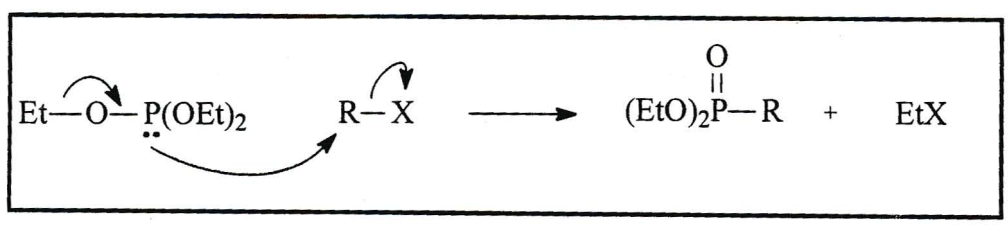
It was decided to employ a bromide, which could not react in the way described above. Bromobenzene was selected as one such reagent. The same procedure was followed and, in this case, the reaction was successful with N-methyl-N-phenylbenzamide being produced (Scheme 35).



Scheme 35: Formation of N-methyl-N-phenylbenzamide.

With the success of this reaction, formation of the Grignard reagent of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate was attempted. Unfortunately, the attempt was a failure as in the case discussed earlier. N-Methyl-5-phenylox-3-azolidin-2-one was observed as a decomposition product. The formation of this compound will be discussed later in the text (pages 49-52).

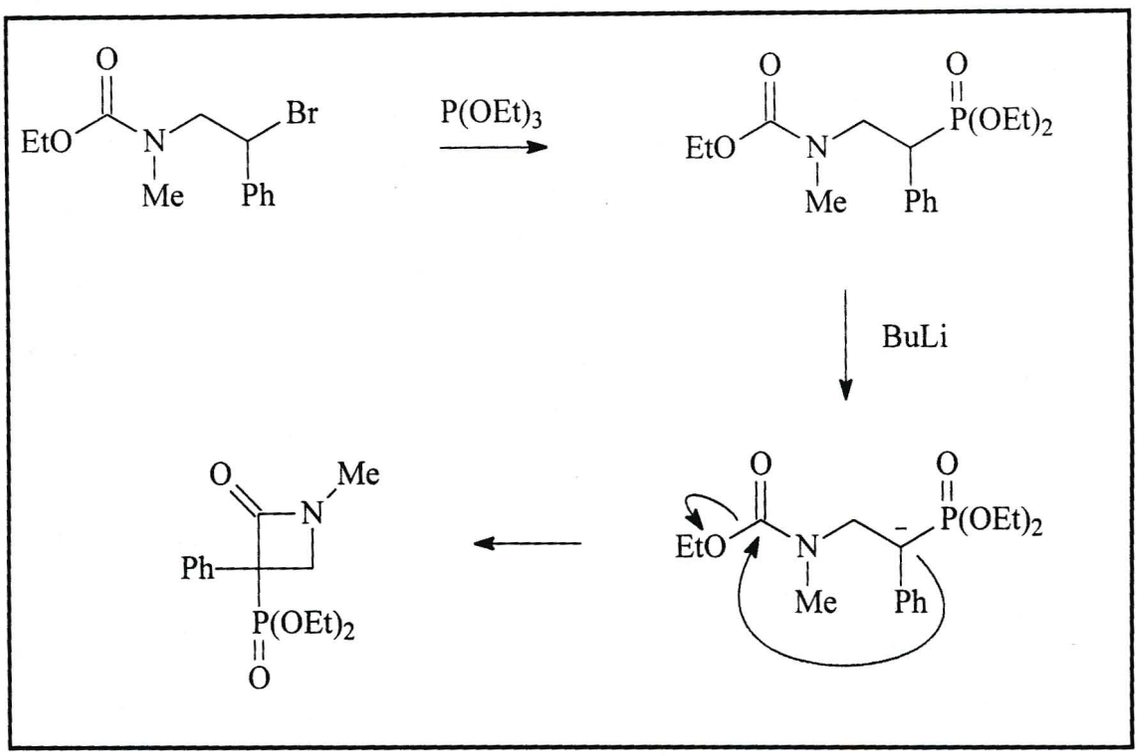
A second strategy was then suggested. Triethyl phosphite reacts with alkyl halides in the following way (Scheme 36).[21]



Scheme 36: Reaction of triethyl phosphite with alkyl halides.

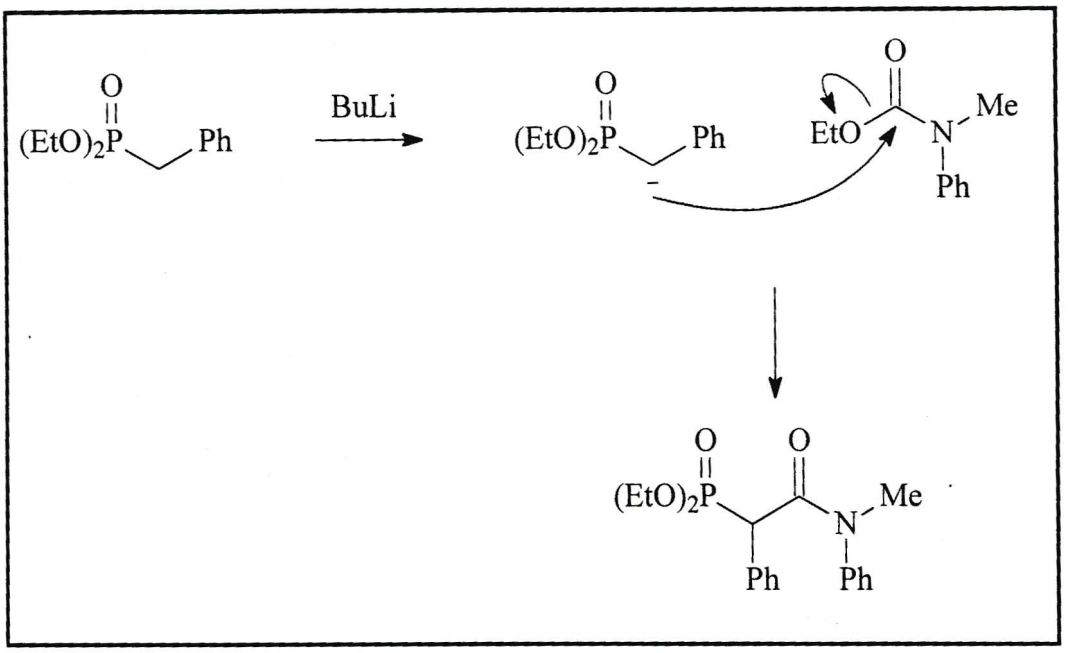
The group now attached to the R group is called a phosphonate group and is very electron withdrawing in character.

It was decided to replace the bromine atom of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate with a phosphonate group. Abstraction of the proton α to the phosphonate group could then be achieved by treatment with base, forming a carbanion, which could attack the carbonyl carbon, displacing the ethoxy group as before (Scheme 37).



Scheme 37: Formation of β-lactam *via* proton abstraction.

Again, an intermolecular addition, analogous to the proposed cyclisation, was employed to test the validity of the method. In this case, diethyl benzylphosphonate was treated with butyl lithium (BuLi) and reacted with ethyl N-methyl-N-phenylcarbamate (Scheme 38). Diethyl benzylphosphonate was prepared by reaction of triethyl phosphite with benzylbromide.

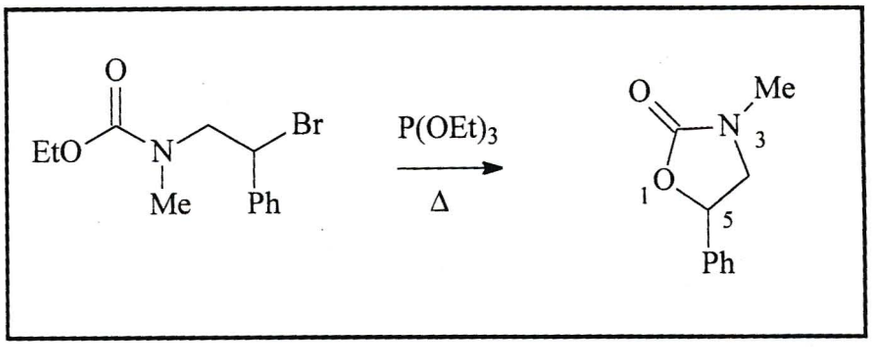


Scheme 38: Intermolecular addition of benzyl phosphonate to a carbamate.

The reaction was successful, with the phosphonate adduct being formed in good yield (~50%).

Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate and ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate were then treated with triethyl phosphite to produce their phosphonate analogues. No complications were expected for these two reactions, as secondary alkyl bromides have been shown to react readily with triethyl phosphite in the manner shown above (Scheme 36).[21]

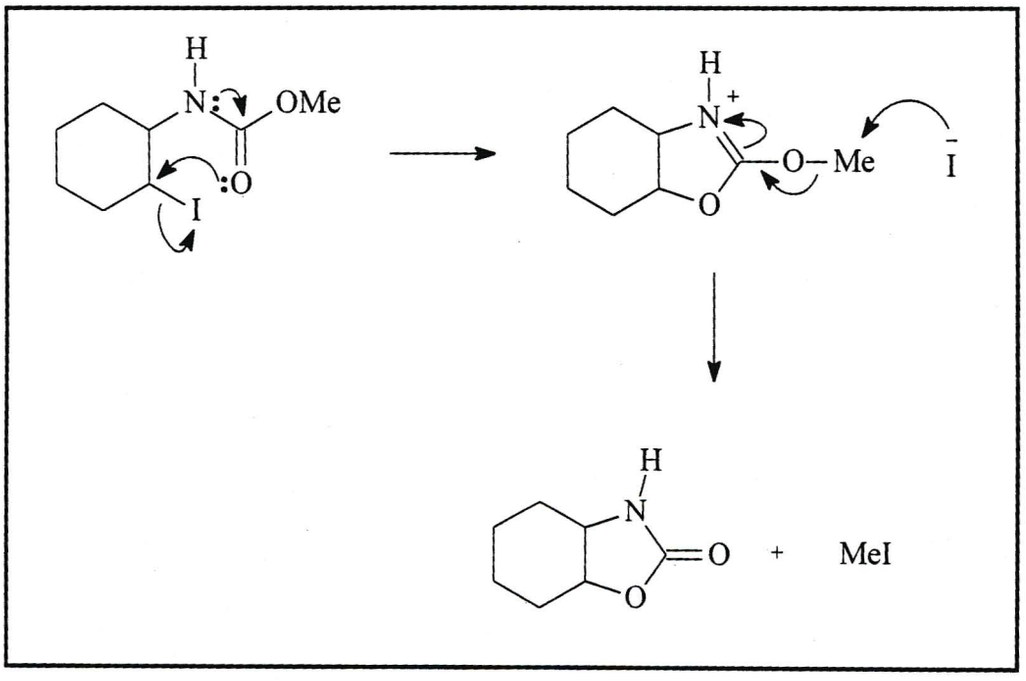
Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate and triethyl phosphite were placed in a reaction flask and placed into an oil-bath preheated to 150°C and maintained at that temperature for one hour. Workup afforded a red oil, which was purified using preparative thin layer chromatography. The major component of the reaction mixture was shown to be N-methyl-5-phenylox-3-azolidin-2-one (Scheme 39).



Scheme 39: Formation of N-methyl-5-phenylox-3-azolidin-2-one.

This structure was confirmed by the HMBC spectrum, which showed a correlation between H_5 and the carbonyl carbon.

It was later noted that it was reported in 1967 that β-iodocarbamates can be converted to ox-3-azolid-2-ones by pyrolysis at 150°C.[22] The reported mechanism is shown below (Scheme 40).



Scheme 40: Mechanism of ox-3-azolid-2-one formation.

However, this mechanism may or may not be operating in the reaction under investigation. Bearing in mind that iodine is a far better leaving group than bromine, it is not immediately clear whether the cyclisation is unimolecular, as in the case of the β-iodocarbamates, or whether it is only possible with triethyl phosphite present,

acting as some form of initiator. The mechanism of the latter can be, at best, speculative.

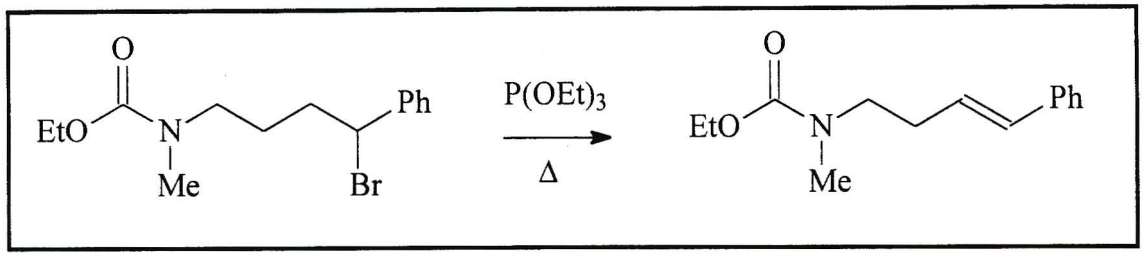
In order to investigate this further, ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate was placed in a reaction flask and placed into a pre-heated oil-bath at 150°, and maintained at that temperature for one hour. Triethyl phosphite was not present at any time during the procedure. The only product observed was 3-methyl-5-phenylox-3-azolidin-2-one, the identical compound observed in the previous reaction in the presence of triethyl phosphite. Thus, it is clear that a unimolecular mechanism is operating and, although it cannot be said, without question, that triethyl phosphite has no role in the reaction, it can be concluded that its presence is not vital for the reaction to take place.

The reason why cyclisation is favoured over nucleophilic substitution is not clear. It has been reported that the substitution product was obtained when 3-bromocyclohexene, a secondary alkyl bromide, was treated with triethyl phosphite.[21] This would suggest that ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate, another secondary alkyl bromide, would also give the substitution product. It has been observed that this is not the case. Both reactions were carried out under identical conditions and, therefore, the reason for the different reaction paths would appear to stem from the different substrates.

It could be argued that the steric hindrance of the phenyl group α to the carbon centre under attack could prevent the bulky triethyl phosphite from coming in close enough proximity to cause substitution. The methyl group attached to nitrogen would enhance this affect.

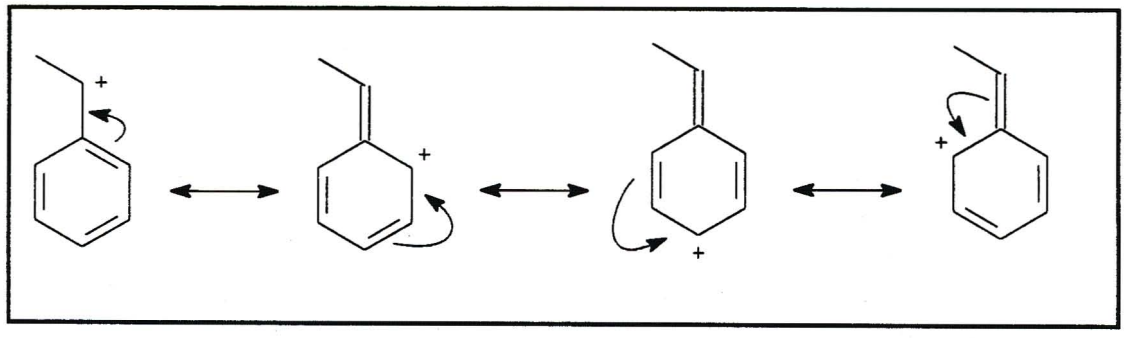
In addition to this steric affect, an entropy affect could also be a factor. The loss of entropy in bringing two separate molecules together is far greater than the loss due to intramolecular cyclisation.[11]
The author speculates that the combination of these two affects has resulted in the cyclisation to be greatly favoured over the substitution to the point that no substitution product has been observed at all.

Ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate and triethyl phosphite, were then placed into a reaction flask and placed into an oil-bath preheated to 150°C and maintained at that temperature for one hour. Workup produced a red oil, which was purified using preparative chromatography. On this occasion, the major component of the reaction mixture was ethyl N-methyl-N-4-phenyl-3-butenylcarbamate (Scheme 41).



Scheme 41: Formation of ethyl N-methyl-N-4-phenyl-3-butenylcarbamate.

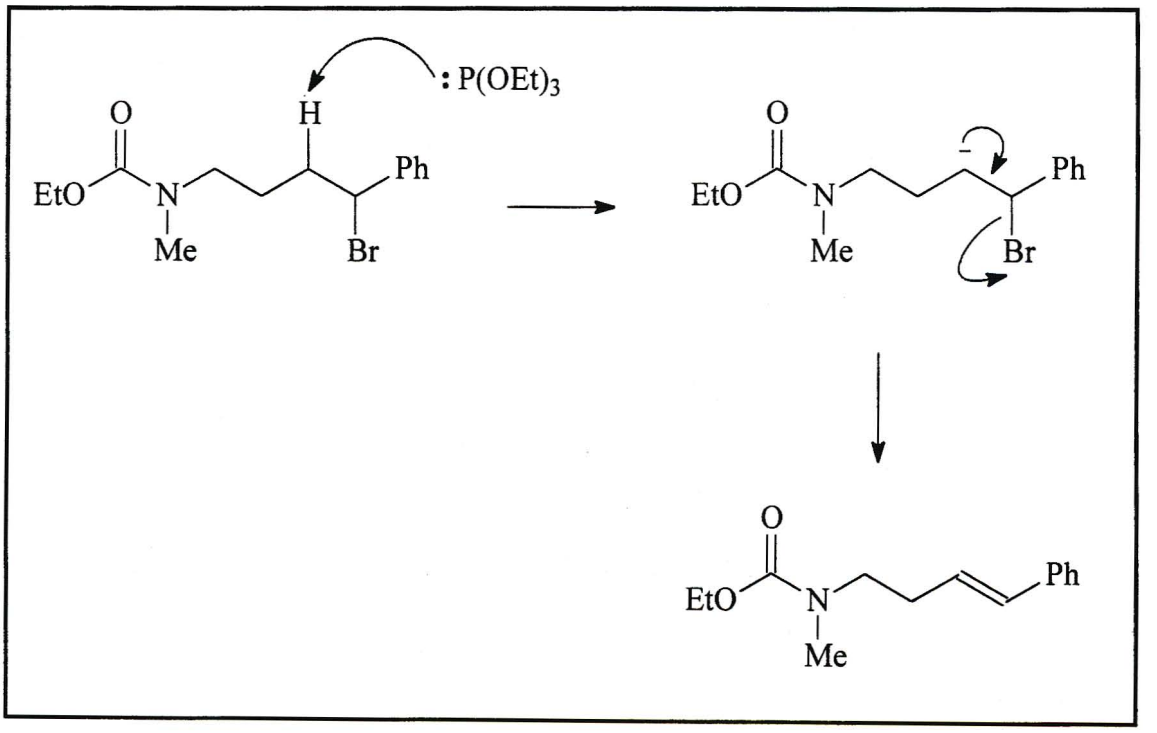
It is clear that the triethyl phosphite has acted as a base instead of as a nucleophile. This has resulted in elimination rather than the desired substitution. It is conceivable that this could have been achieved *via* an E_1 or an E_2 mechanism, as secondary alkyl halides can undergo both methods of elimination.[23] Although both mechanisms are possible, E_1 reaction products are usually found in minor amounts, with the substitution product being the major component. However, this particular substrate contains a phenyl group α to the carbon centre bearing the bromine atom. This results in the intermediate carbocation formed in an E_1 reaction being very stable, and this makes the E_1 path more accessible (Scheme 42).



Scheme 42: Resonance stabilised benzylic carbocation.

In spite of this, an E_2 mechanism is still predicted to be the favoured path. This prediction is confirmed by the stereochemistry of the reaction product. The ^1H NMR spectrum shows the coupling constant between H_3 and H_4 to be approximately 16 Hz, indicating a *trans* orientation.[4] If an E_1 mechanism were in operation, a mixture of isomers would be observed, whereas the E_2 mechanism exclusively yields the *trans* isomer. From this observation the author concludes that an E_2 mechanism is in operation.

Again, rationalising why substitution is not the favoured reaction is difficult. As in the case of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate, the steric bulk of the adjacent phenyl group is a factor. However, in this case cyclisation is not a competing reaction, as formation of a 7-member 1-ox-3-azolidin-2-one ring would result in a very large loss of entropy. With no contribution from the entropy affect, steric hindrance seems to be the major factor in determining the reaction path. Again, it appears as though the bulky triethyl phosphite cannot come in close enough proximity to the carbon centre bearing bromine to affect substitution. Instead, it is far easier to abstract the proton α to the cabon centre, resulting in elimination, and formation of the alkene (Scheme 43).



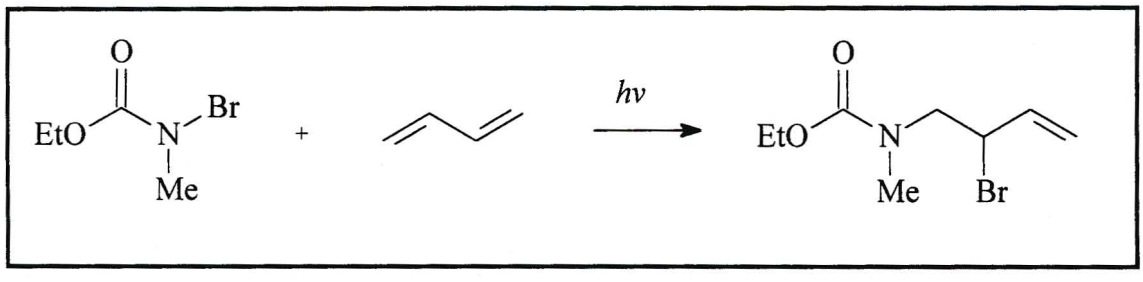
Scheme 43: Formation of ethyl N-4-phenyl-3-butenyl-N-methylcarbamate.

An additional observation is that the product is very stable due to conjugation of the double bond into the phenyl ring. This stability may play some role in the outcome of the reaction, although further experimentation would be needed to prove or disprove this statement.

It may be concludeed that in both cases described above, the phenyl group attached to the carbon centre bearing the bromine atom creates sufficient steric hindrance to prevent triethyl phophite from attacking that carbon centre to cause substitution. In the case of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate, the methyl group attached to nitrogen adds to the steric hindrance around that carbon centre. Also, the entropy affect discussed adds further opposition to the substitution reaction and, in fact, may be the major factor to be considered. In the case of ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate, the methyl group attached to nitrogen plays little, if any, part in steric hindrance around the carbon centre. The stablity of the final product may also play some role in the outcome of the reaction.

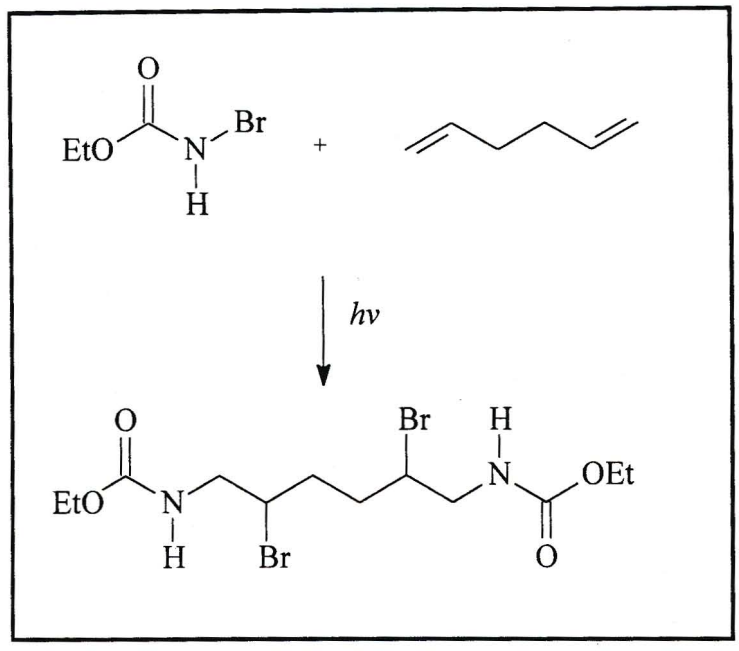
4. Comments

The most obvious change one could make to the above methods would be to replace the phenyl group of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate with a smaller group, negating the steric affects of the bulky phenyl group. Choosing the alternative group must be a careful consideration. The phenyl group was originally chosen for two reasons. Firstly, styrene is a very good substrate for the photolysis reaction used to prepare ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate. The reaction has been known to proceed in yields up to 90-95%. Secondly, preparation of ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate involves bromination at the benzylic position. This would, obviously, not be possible if the phenyl group were not present. With these considerations in mind, the best replacement might be a vinyl group, it is a far smaller group than the phenyl group and bromination at the allylic position is a well known reaction. However, a photolysis reaction to prepare ethyl N-2-bromo-2-vinylethyl-N-methylcarbamate would use 1,3-butadiene as the substrate (Scheme 44).



Scheme 44: Photolysis to prepare ethyl N-2-bromo-2-vinylethyl-N-methylcarbamate.

This creates problems in the form of disubstitution. An analogous reaction using ethyl N-bromocarbamate and 1,5-hexadiene has produced the bisadduct, N,N'-1,6-bis(ethylcarbamyl)-2,5-dibromohexane (Scheme 45) (see also **Experimental** page 85).



Scheme 45: Photolysis to prepare N,N'-1,6-bis(ethylcarbamyl)-2,5-dibromohexane.

However, this reaction employed a 2:1 molar ratio of carbamate to diene, not the case for previous photolysis reactions, which employed a 1:2 molar ratio. The scope of a monosubstitution reaction with a diene is, therefore, open to further investigation. If this type of reaction were successful, the vinyl group would be an ideal replacement for the phenyl group.

Assuming this modification produces the same results described above, the nature of the group in question would be irrelevant. In the case of the oxazolidinone, this would indicate an entropy consideration to be the major factor driving cyclisation. In this case, a temperature study may shed some light on the problem. A possible approach could be to repeat pyrolysis of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate in the absence of triethyl phosphite, decreasing the temperature in order to find the highest temperature at which ox-3-azolid-2-one formation ceases. Treatment of ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate at this temperature may result in substitution. However, these comments are merely speculation and further investigation is required in order to substantiate them.

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Foreword to experimental

Nuclear magnetic resonance spectroscopy (NMR)

Spectra were recorded with a 300 MHz Varian Gemini spectrometer and a Varian Unity Inova 400 MHz spectrometer using deuterated chloroform and deuterated methanol as solvents. Instrument and solvent used for each compound are indicated in the text. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz on the 300 MHz spectrometer. ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR spectra were recorded at 100 MHz and ^{31}P NMR spectra were recorded at 162 MHz on the 400 MHz spectrometer. ^{1}H NMR spectra were referenced against either the deuteriochloroform singlet at δ_H 7.24 ppm or the deuteriomethanol signal at δ_H 3.34 ppm. ^{13}C NMR spectra were referenced against the central line of the deuteriochloroform signal at δ_C 77.0 ppm. ^{31}P NMR spectra were referenced against the triphenyl phosphate singlet at –16.61 ppm.

Infrared spectroscopy (IR)

Infrared spectra were recorded on a Nicolet Impact 400 D spectrometer, which was calibrated against an air background. Spectra for all compounds were recorded as thin films using dichloromethane as solvent.

Melting points (m.p.)

Melting points were determined on a Kofler micro-hot stage melting point apparatus and are uncorrected.

Thin layer chromatography (TLC)

Silica gel (0.2 mm) containing fluorescent indicator (F_{254}) on aluminium backed plates (Merck: Art 5554) was used for thin layer chromatography analysis, to monitor the column chromatographic process. The plates were first examined under UV light and then developed in iodine.

Preparative thin layer chromatography

Pre-coated silica gel 60 (2 mm) containing fluorescent indicator (F_{254}) on 20 × 20 cm glass plates (Merck Art. 5717) was used for preparative chromatography. The plates were first examined under UV light and then developed in iodine. The section of plate of interest was then scraped off using a small spatula and placed in a beaker of methanol. The methanol was filtered and extracted with dichloromethane, which was dried ($MgSO_4$) and concentrated. Solvent systems used are indicated in the text and include diethyl ether (Et_2O), hexane (Hex), ethyl acetate (EtOAc) and dichloromethane (CH_2Cl_2).

Column chromatography

Silica gel (Merck Art: 9385) was used as the solid phase for gravity column chromatography and elution was allowed to proceed by gravity. Solvent systems used are indicated in the text and include diethyl ether (Et_2O), hexane (Hex), ethyl acetate (EtOAc) and dichloromethane (CH_2Cl_2).

Mass spectrometry

GC/MS spectra were recorded using either a Finnigan 1020 GC/MS spectrometer (HRMS) at the Cape Technikon or an Agilent 6890 GC system with a 5973N Mass Selective (EIMS) instrument at the University of Natal-Durban.

Ultraviolet spectroscopy (UV)

All spectra were recorded using a Varian DMS 300 UV-visible spectrometer. Dichloromethane was used as solvent in all cases.

Titration of sodium thiosuphate solution with ethyl N-bromo and N-chlorocarbamates.[1]

This titration was carried out to determine the percent purity of the product. An accurately weighed amount of the carbamate was placed in a flask containing dilute acetic acid (25%, ~20ml) and potassium iodide (~0.5g). The solution was titrated against standard 0.1000M sodium thiosulphate solution to a pale yellow. Starch indicator was added to afford a blue colouration. The titration was continued to a colourless end-point.

Other comments.

Numbers accompanying spectra (1.1 etc.) correlate to the compound number in the **Experimental** section.

Page numbers accompanying spectral data refer to page numbers in Appendix A.

Notes regarding spectral data are shown below data for each compound.

Unless otherwise stated, the term 'ether' refers to diethyl ether.

EXPERIMENTAL

1. Preparation of ethyl N-bromocarbamates

General procedure[2]

The carbamate (25mmol), in dichloromethane (180ml) and saturated sodium bicarbonate solution (135ml), was treated with bromine (25mmol) and stirred under darkness for 24 hours. Thereafter the mixture was treated with a further amount of bromine (25mmol) and stirred for a further 24 hours under darkness. The solution was then cooled (0°C), separated and the organic layer washed with cold saturated sodium bicarbonate solution (2×150ml). The organic layer was then dried ($MgSO_4$) and concentrated (rotary evaporator temperature <30°C) to afford the ethyl N-bromocarbamate.

1.1 **Ethyl N-bromocarbamate** (Spectra number 1.1, page 1 and 2 Appendix A)

Ethylcarbamate was treated with bromine to afford ethyl N-bromocarbamate as an orange solid (88.2%).

m.p. Decomposes.

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.30 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 4.26 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz).

UV (λ_{max} / nm) 230, 284.

Notes: The ^{1}H NMR spectrum is somewhat misleading in that the coupling constants for the triplet appear to be different. This is due to the presence of parent carbamate as an impurity, which has a slightly different chemical shift. The same observation is true for the quartet.

1.2 **Ethyl N-bromo-N-methylcarbamate** (Spectra number 1.2, page 3 and 4)

Ethyl N-methylcarbamate was treated with bromine to afford ethyl N-bromo-N-methylcarbamate as a red oil (90.0%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.25 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 3.36 (N-Me, 3H, s), 4.18 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz).

UV (λ_{max} / nm) 231, 305.

Notes: The same comments apply as for the above compound.

2. Formation of ethyl N-2-bromo-alkylcarbamates *via* photolysis

General procedure[2]

A stirred solution of the ethyl N-bromocarbamate (5mmol) in dichloromethane was placed in a circulating water bath and degassed (nitrogen/10min). The olefin (10mmol) was added dropwise and the solution was degassed for a further 10min. The solution was then photolysed using a medium pressure mercury lamp until no ethyl N-bromocarbamate remained (negative potassium iodide test for positive halogen). The photolysis was then stopped, the solution washed with saturated sodium bicarbonate solution (10ml), aqueous sodium thiosulphate (10ml), dried ($MgSO_4$) and concentrated. The crude product was purified by column chromatography.

2.1 **Ethyl N-2-bromo-2-phenylethylcarbamate** (Spectra number 2.1, page 5-9)

Styrene (9.40g, 90.3mmol) was added to ethyl N-bromo-N-methylcarbamate (8.22g, 45.2mmol) in dichloromethane and photolysed for 3 hours. Purification (Et_2O/Hex) yielded ethyl N-2-bromo-2-phenylethylcarbamate as a colourless oil (2.75g, 9.61mmol, 21%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.21 ($CH_2C\underline{H}_3$, 3H, t, J=7 Hz), 2.74 (N-Me, 3H, s), 3.69-3.80 (H_1, 1H, m), 3.85-3.96 (H_1, 1H, m), 4.09 ($C\underline{H}_2CH_3$, 2H, q, J=7 Hz), 5.05 and 5.22 (2×½ H_2, 2× 1H, 2× br t), 7.25-7.43 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 1703 (s), 702 (w).

HRMS *m/z* (%) 287.0338 (0.68) [M^+] (Calc. 287.0345 $C_{12}H_{16}N^{81}BrO_2$), 285.0360 (0.67) [M^+] (Calc. 285.0383 $C_{12}H_{16}N^{79}BrO_2$), 241.9979 (0.94) [M^+- OEt], 240.0006 (0.95) [M^+- OEt], 206.1175 (3.63) [M^+-Br], 116.0716 (100.00), 104.0628 (19.24), 44.0356 (45.07).

Notes: The two resonances at 5.05 and 5.22 ppm in the ^{1}H NMR spectrum are discussed at length in the **Discussion** (page 34). The HRMS spectrum shows the base peak at *m/z* 116.0716. This corresponds to fragmentation at the benzylic position (i.e. between C_1 and C_2).

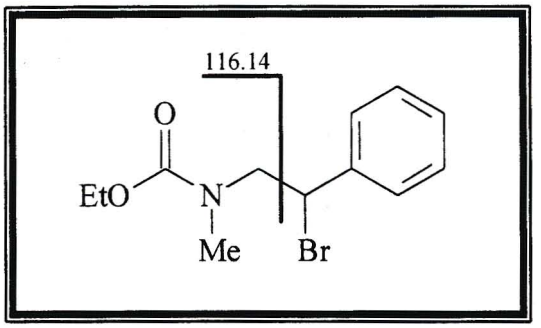

Fig. 20: Fragment corresponding to base peak of HRMS spectrum.

(Spectrum number 2.1, page 9).

3. Formation of ethyl N-bromoalkylcarbamates *via* traditional synthetic methods

3.1 **3-Phenylpropyl bromide**[3] (Spectra number 3.1, page 10 and 11)

To a round-bottomed flask containing 48% hydrobromic acid (75.04g, 0.45mol) and concentrated sulfuric acid (20.04g, 0.20mol) was added 3-phenyl-1-propanol (40.20g, 0.30mol) and a further portion of concentrated sulfuric acid (15g, 0.15mol). The mixture was heated under reflux for 3 hours. Thereafter, the organic layer was separated, washed with water, concentrated hydrochloric acid, water, saturated sodium bicarbonate solution and water. It was dried ($MgSO_4$) and distilled under vacuum to afford 3-phenylpropyl bromide as a clear liquid (48.96g, 0.25mol, 83%).

b.p. 65-66°C, 15 mmHg (lit. 219-220°C).

^1H NMR (300MHz, $CDCl_3$, δ / ppm) 2.11-2.23 (H_2, 2H, m), 2.78 (H_3, 2H, t, J=7 Hz), 3.40 (H_1, 2H, t, J=6 Hz), 7.18-7.33 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 3032 (m), 702 (m).

3.2 Preparation of amines *via* the 'Zwierzak' method

General procedure[4]

A mixture of sodium azide (0.1mol), the alkyl bromide (0.05mol), tetrabutylammonium bromide (5-10mol%) and benzene was heated under reflux for 6-12 hours with efficient stirring. The product was cooled to room temperature and filtered. The precipitate of inorganic salts was washed with benzene. The filtrate was washed with water and dried over $MgSO_4$. Triethyl phosphite (7.65g, 46.0mmol) was added dropwise, with stirring to the organic azide. The solution was stirred overnight at room temperature. The solution was saturated with hydrogen chloride at room temperature for 2 hours and allowed to stir overnight. The benzene was removed under vacuum. Dry ether was added to the resultant residue and refrigerated for 24 hours. The hydrochloride crystals were filtered by suction and dried under vacuum. Recrystalisation from ethanol yielded the pure amine hydrochloride.

3.2.1 **N-Methyl-3-phenylpropylamine hydrochloride** (Spectrum number 3.2.1, page 12)

A mixture of 3-phenylpropyl bromide (48.96g, 0.25mol), sodium azide (32.57g, 0.50mol) and tetrabutylammonium bromide (4.14g, 12.8mmol) in benzene was heated under reflux for 6 hours. The mixture was then filtered and the filtrate treated with triethyl phosphite (41.75g, 0.25mol). Thereafter, the solution was treated with methyl iodide (70.75g, 0.50mol) and stirred at room temperature for 2 hours. It was heated under reflux for a further 2 hours and allowed to stir at room temperature overnight. The solution was saturated with hydrogen chloride for 2.5 hours and stirred overnight. Benzene was evaporated, anhydrous ether was added to the residue and the solution was refrigerated overnight. The precipitate was filtered, recrystalized from absolute ethanol and dried under vacuum to afford N-methyl-3-phenylpropylamine hydrochloride as a white solid (0.78g, 4.20mmol, 1.7%).

m.p. Decomposed.

^{1}H NMR (300MHz, CD_3OD, δ / ppm) 1.97-2.08 (H_2, 2H, m), 2.72 (N-Me, 3H, s), 2.75 (H_3, 2H, t, J=8 Hz), 3.02 (H_1, 2H, t, J=8 Hz), 7.21-7.38 (Ph, 5H, m).

3.2.2 **N-Methyl-2-phenylethylamine hydrochloride** (Spectrum number 3.2.2, page 13)

A mixture of 2-phenylethyl bromide (8.48g, 45.8mmol), sodium azide (6.19g, 95.2mmol) and tetrabutylammonium bromide (0.79g, 2.45mmol) in benzene was heated under reflux for 6 hours. The mixture was then filtered and the filtrate treated with triethyl phosphite (7.65g, 46.0mmol). Thereafter, the solution was treated with methyl iodide (13.23g, 93.2mmol) and stirred at room temperature for 2 hours. It was then heated under reflux for a further 2 hours and then allowed to stir at room temperature overnight. The solution was saturated with hydrogen chloride for 2.5 hours and stirred overnight. Benzene was evaporated, anhydrous ether was added to the residue and refrigerated overnight. The precipitate was filtered, recrystalized from absolute ethanol and dried under vacuum to afford N-methyl-3-phenylethylamine hydrochloride as a white solid (0.70g, 4.08mmol, 8.9%).

m.p. Decomposed.

^{1}H NMR (300MHz, CD_3OD, δ / ppm) 2.72 (N-Me, 3H, s), 3.00 (H_2, 2H, t, J=7 Hz), 3.26 (H_1, 2H, t, J=7 Hz), 7.27-7.41 (Ph, 5H, m).

Notes: The integration of the two sets of methylene protons are distorted due to the presence of impurities.

3.2.3 **3-Phenylpropylamine** (Spectrum number 3.2.3, page 14)

A mixture of 3-phenylpropyl bromide (5.15g, 25.9mmol), sodium azide (3.38g, 52.0mmol) and tetrabutylammonium bromide (0.44g, 1.36mmol) in benzene was heated under reflux for 6 hours. The mixture was filtered and the filtrate treated with triethyl phosphite (4.39g, 26.4mmol). The solution was saturated with hydrogen chloride for 3 hours and stirred overnight. Benzene was evaporated and the residue suspended in water. The suspension was made acidic using 4M hydrochloric acid and washed with dichloromethane. The aqueous layer was made basic using 4M sodium hydroxide and extracted with dichloromethane. The dichloromethane layer was separated, dried ($MgSO_4$) and concentrated to yield a yellow oil which was distilled under high vacuum to afford 3-phenylpropylamine as a pale yellow liquid (1.28g, 9.47mmol, 37%).

b.p. not done, impure (lit. 221.5°C).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.69-1.84 (H_2, 2H, m), 2.50-2.71 (H_1 and H_3, 4H, m), 7.11-7.30 (Ph, 5H, m).

Notes: This method was carried out to determine whether the low yields in the previous two experiments were due to the incorporation of the methylation step. Although the yield for this experiment was much higher than the previous ones, it was not in agreement with the very high yields reported in the literature and, therefore, the low yields observed cannot be, unambiguously, attributed to the incorporation of the methylation step. It is not clear what the impurity in the ^{1}H NMR spectrum is or why the H_2 signal is so sharp. However, the assignments do agree favourably with the standard spectrum of this compound (Ref: The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra, 1st edition, editors Pouchert C.J. and Behnke J., 1993, page 572).

3.3 **4-Phenylbutylamine**[3] (Spectra number 3.3, page 16 and 17)

(Intermediate **4-phenylpropyl cyanide** is also shown, spectrum number 3.3, page 15).

Potassium cyanide (6.96g, 106.9mmol) was dissolved in water. A solution of 3-phenylpropyl bromide in methanol was added dropwise, with stirring and the mixture was heated under reflux for 48 hours. The reaction mixture was then cooled to room

temperature and the layers separated. The upper layer was extracted with dichloromethane, dried ($MgSO_4$) and concentrated to yield crude 4-phenylpropylcyanide (9.39g, 64.7mmol, 60%).

^1H NMR (300MHz, $CDCl_3$, δ / ppm) 1.91-2.01 (H_3, 2H, m), 2.30 (H_2, 2H, t, J=7 Hz), 2.78 (H_4, 2H, t, J=7 Hz), 7.15-7.35 (Ph, 5H, m).

Notes: ^1H NMR spectrum of the crude cyanide was recorded to determine whether the initial reaction had proceeded in the desired fashion. As a result, the spectrum contains a number of impurities but clearly shows that the cyanide is the major component of the mixture. Accompanying the acquired spectrum is a standard spectrum of 4-phenylpropyl cyanide (Ref: The Aldrich Library of ^{13}C and ^1H FT NMR Spectra, 1st edition, editors Pouchert C.J. and Behnke J., 1993, page 1481).

Sodium metal (4.32g, 188mmol) and dry toluene (70ml) were placed in a round-bottomed flask, equipped with a reflux condenser and magnetic stirrer, and heated until the toluene began to boil, whereupon the mixture was stirred vigorously to produce an emulsion. A mixture of 4-phenylpropyl cyanide (4.53g, 31.2mmol) in ethanol (50ml) was run in dropwise, maintaining reflux. Thereafter, ethanol (5g), methanol (5g) and water (sufficient to dissolve NaOH that had formed) were added slowly. The organic layer was separated and the aqueous layer washed with chloroform. The toluene and chloroform layers were combined, dried ($MgSO_4$) and evaporated to give a yellow oil. The oil was suspended in water and made acidic using 4M HCl. The suspension was washed with dichloromethane and then made basic using 4M NaOH. The mixture was then extracted with dichloromethane, dried ($MgSO_4$) and concentrated to yield a red / orange oil which was purified to yield 4-phenylbutylamine as a yellow oil (0.65g, 4.36mmol, 14%).

b.p. not done, impure (lit. 123.-124°C, 17mmHg).

^1H NMR (300MHz, $CDCl_3$, δ / ppm) 1.40-1.51 (H_2, 2H, m), 1.55-1.68 (H_3, 2H, m), 2.60 (H_4, 2H, t, J=6 Hz), 2.67 (H_1, 2H, t, J=6 Hz), 4.73 (N-H, 2H, br s), 7.10-7.29 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 3348 (w), 2940 (m), 702 (m).

Notes: Accompanying the acquired spectrum is a standard spectrum of 4-phenylbutylamine (Ref: The Aldrich Library of ^{13}C and ^1H FT NMR Spectra, 1st edition, editors Pouchert C.J. and Behnke J., 1993, page 573).

3.4 **2-Phenylethylamine**[5] (Spectra number 3.4, page 18 and 19)

A solution of benzaldehyde (20.05g, 0.188mol) in nitromethane (70.03g, 1.15mol) was treated with anhydrous ammonium acetate (3.64g, 47.2mmol) and heated under reflux for 2.5 hours. Excess nitromethane was removed under vacuum to afford crude β-nitrostyrene.

To a stirred solution of lithium aluminium hydride (9.84g, 0.259mol) in anhydrous ether (200ml) was added crude β-nitrostyrene in anhydrous ether (50ml). The mixture was heated under reflux for 24 hours. The mixture was cooled to room temperature and water (10ml) was added very slowly. Thereafter, 10% sodium hydroxide solution (15ml) and a further amount of water (30ml) were added. The mixture was then filtered and the inorganic salts were washed with dichloromethane and water. The filtrate was made acidic using 4M hydrochloric acid and washed with dichloromethane. The aqueous layer was then made basic and extracted with dichloromethane. The dichloromethane layer was separated, dried and concentrated to afford 2-phenylethylamine as a yellow oil (1.45g, 12.0mmol, 6.4%).

b.p. not done, impure (lit. 197-198°C).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 2.72 (H_2, 2H, t, J=7 Hz), 2.92 (H_1, 2H, t, J=7 Hz), 7.17-7.39 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 3282 (w), 3026 (m), 709 (s).

Notes: It is assumed that the large aromatic resonance is the result of impurities as well as a large chloroform peak. The amine was not required for further purposes as this reaction was to test a hypothesis (see **Discussion** p.36 and 37). It was, therefore, not deemed necessary to purify the product further.

3.5 **4-Phenylbutanoic acid**[6] (Spectra number 3.5, page 20 and 21)

3-Phenylpropyl bromide (25.04g, 126mmol) was added dropwise to a mixture of magnesium turnings (6.08g, 250mmol) in anhydrous ether containing a single iodine crystal. Addition of the bromide was at a sufficient rate to maintain reflux. After all the bromide had been added, the solution was stirred at room temperature for 30 min. Thereafter, the solution was decanted into a large beaker leaving excess magnesium behind. A large excess of CO_2 (dry ice) was then added to the solution with manual

stirring using a glass rod. Concentrated hydrochloric acid (120ml) was then added to the mixture with stirring. The mixture was placed in a separating funnel, and the top ether layer was separated, washed with cold water and extracted with ice cold 10% sodium hydroxide solution (125ml). The aqueous layer was separated and made acidic using concentrated hydrochloric acid. The white precipitate which formed was filtered and washed with water to yield 4-phenylbutanoic acid (17.15g, 0.10mol, 83%).

m.p. 49-50°C (lit. 52°C).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.96 (H_3, 2H, m), 2.36 (H_2, 2H, t, J=7 Hz), 2.67 (H_4, 2H, t, J=7 Hz), 7.13-7.30 (Ph, 5H, m).

IR (KBr, ν_{max} / cm^{-1}) 3032 (m), 1709 (s), 702 (m).

3.6 **4-Phenylbutanoyl chloride**[7] (Spectra number 3.6, page 22 and 23)

4-Phenylbutanoic acid (17.15g, 104mmol) was introduced into a round-bottomed flask containing a large excess of thionyl chloride (50ml). The mixture was then heated under reflux for 1 hour. Additional thionyl chloride was then added and the mixture was allowed to stand overnight. The resultant mixture was distilled under high vacuum to yield 4-phenylbutanoyl chloride as a colourless liquid (3.22g, 17.6mmol, 17%).

b.p. 63-65°C, 1 mmHg (lit. 119°C, 9 mmHg).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 2.03 (H_3, 2H, m), 2.69 (H_4, 2H, t, J=7 Hz), 2.89 (H_2, 2H, t, J=6 Hz), 7.13-7.35 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 2947 (w), 1808 (s), 709 (m).

Notes: As would be expected, the three methylene resonances in the ^{1}H NMR spectrum have moved further downfield relative to the 4-phenylbutanoic acid signals. It is also apparent that the carbonyl peak in the IR spectrum has moved to higher wavenumbers (1709 to 1808 cm^{-1}). Both these observations are consistent with the formation of the acid chloride from the carboxylic acid.

3.7 **N-Methyl-4-phenylbutanamide**[7] (Spectrum number 3.7, page 24)

4-Phenylbutanoyl chloride (1.51g, 8.27mmol) was introduced into a round-bottomed flask containing a large excess of methylamine (25ml, 40% aqueous solution) and stirred overnight at room temperature. The mixture was then extracted with

dichloromethane, dried ($MgSO_4$) and concentrated to yield N-methyl-4-butanamide as a red / yellow oil (1.29g, 7.28mmol, 88%).

^1H NMR (300MHz, $CDCl_3$, δ / ppm) 1.94 (H_3, 2H, m), 2.13 (H_2, 2H, t, *J*=7 Hz), 2.60 (H_4, 2H, t, *J*=7 Hz), 2.74 (N-Me, 3H, s), 5.71 (N-H, 1H, br), 7.10-7.29 (Ph, 5H, m).

3.8 **N-Methyl-4-phenylbutylamine**[7] (Spectra number 3.8, page 25 and 26)

A solution of the N-methyl-4-phenylbutanamide (2.98g, 16.8mmol) in anhydrous ether (15ml) was added slowly to a stirred suspension of lithium aluminium hydride (0.62g, 16.3mmol) in anhydrous ether (20ml) in a round bottomed flask equipped with a reflux condenser and drying tubes. The mixture was heated to reflux and maintained for 15 hours. The mixture was then cooled to room temperature and treated very slowly with water (1.5ml), 15% sodium hydroxide solution (1.5ml) and water (3ml). The mixture was filtered and the filtrate made acidic using 4M hydrochloric acid. It was then washed with dichloromethane. The aqueous layer was made basic using 4M sodium hydroxide and extracted with dichloromethane. The dichloromethane layer was separated, dried ($MgSO_4$) and concentrated to yield N-methyl-4-phenylbutylamine as a yellow oil (0.93g, 5.70mmol, 34%).

^1H NMR (300MHz, $CDCl_3$, δ / ppm) 1.47-1.57 (H_2, 2H, m), 1.57-1.68 (H_3, 2H, m), 2.40 (N-Me, 3H, br s), 2.52-2.63 (H_1 and H_4, 4H, m), 7.08-7.24 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 3322 (w), 2940 (s), 709 (m).

3.9 Ethyl N,N-dialkylcarbamates.

General procedure[8]

To a stirred solution of the amine (1mol) in anhydrous ether (150ml) at <5°C, was added ethyl chloroformate (1mol) at such a rate to ensure the temperature did not rise above 5°C. When half of the ethyl chloroformate had been added, sodium hydroxide solution (1mol in 60ml water) was added gradually with the rest of the ethyl chloroformate such that the last portions of each were added simultaneously. The mixture was then allowed to stir for 40 mins. The upper ether layer was separated and the aqueous layer was washed with ether. The ether extracts were combined and evaporated to yield the ethyl N-alkyl-N-methylcarbamate.

3.9.1 **Ethyl N-4-phenylbutyl-N-methylcarbamate** (Spectrum number 3.9.1, page 27)

N-methyl-4-phenylbutylamine (0.93g, 5.70mmol) was treated with ethyl chloroformate (0.62g, 5.71mmol) and sodium hydroxide solution (0.23g/5.75mmol in 10ml water) to yield crude carbamate which was purified by column chromatography (CH_2Cl_2) which afforded ethyl N-4-phenylbutyl-N-methylcarbamate as a yellow oil (0.52g, 2.21mmol, 39%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.21 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 1.50-1.65 (H_2 and H_3, 4H, br m), 2.61 (H_4, 2H, t, *J*=7 Hz), 2.83 (N-Me, 3H, br s), 3.23 (H_1, 2H, br), 4.10 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz), 7.12-7.30 (Ph, 5H, m).

3.9.2 **Ethyl N-2-phenylethyl-N-methylcarbamate** (Spectrum number 3.9.2, page 28)

N-methyl-3-phenylpropylamine hydrochloride (0.66g, 3.84mmol) was treated with ethyl chloroformate (0.41g, 3.78mmol) and sodium hydroxide solution (0.30g/7.50mmol in 10ml water) to yield ethyl N-2-phenylpropyl-N-methylcarbamate as a yellow oil (0.67g, 3.23mmol, 84%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.21 ($CH_2C\underline{H}_3$, 3H, br t), 2.76-2.86 (H_2, 2H, br m), 2.81 (N-Me, 3H, s), 3.45 (H_1, 2H, br t), 4.08 ($C\underline{H}_2CH_3$, 2H, br q), 7.13-7.30 (Ph, 5H, m).

Notes: Poor resolution due to concentrated sample.

3.10 Ethyl N-bromoalkyl-N-alkylcarbamates.

General procedure[9]

N-Bromosuccinimide (1mol) was added to a round-bottomed flask containing the ethyl N-alkyl-N-alkylcarbamate (1mol) in carbon tetrachloride and the mixture was heated under reflux for 24 hours. The carbon tetrachloride was evaporated and the residue taken up in dichloromethane. The solution was washed with saturated sodium bicarbonate solution, sodium thiosulphate solution and water. It was dried and concentrated to yield the ethyl N-bromoalkyl-N-alkylcarbamate.

3.10.1 **Ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate** (Spectrum number 3.10.1, page 29)

Ethyl N-4-phenylbutyl-N-methylcarbamate (0.52g, 2.21mmol) was treated with N-bromosuccinimide (0.41g, 2.30mmol) to afford ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate as a red liquid (0.51g, impure).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.21 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 1.49-1.58 (H_2, 2H, br m), 2.01-2.17 (H_3, 1H, m), 2.17-2.30 (H_3, 1H, m), 2.81 (N-Me, 3H, br s), 3.26 (H_1, 2H, br), 4.09 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz), 4.97 (H_4, 1H, br), 7.13-7.39 (Ph, 5H, m).

Notes: The ^{1}H NMR spectrum shows two components, the desired product and parent carbamate (starting material). The resonances of the ethyl group, the methylene group adjacent to the nitrogen atom and the aromatic region are consistent for both the product and starting material. The evidence that the product had formed is the appearance of the broad resonance at 4.97 ppm, corresponding to the methine proton H_4 and the two multiplets between 2.01 and 2.30, corresponding to the two H_3 protons, which show a downfield shift due to the presence of bromine. The appearance of these peaks is accompanied by a decrease in the size of the peaks at 2.61 ppm, corresponding to H_4 of the parent carbamate and the broad multiplet between 1.50-1.65 ppm, corresponding to H_2 and H_3 of the parent carbamate. It was decided to use the impure product in the next step as it was presumed that the presence of parent carbamate would not affect the outcome of the reaction.

3.10.2 **Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate** (Spectrum number 3.10.2, page 30)

Ethyl N-2-phenylethyl-N-methylcarbamate (0.36g, 1.7mmol) was treated with N-bromosuccinimide (0.26g, 1.5mmol) to afford ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate as a colourless oil (57mg, impure).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.23 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 2.76 (N-Me, 3H, br s), 3.71-3.79 (H_1, 1H, m), 3.86-3.93 (H_1, 1H, m), 4.09 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz), 5.04 and 5.21 (H_2, 1H, m), 7.16-7.42 (Ph, 5H, m).

Notes: The same comments for the above compound applies. The evidence of the presence of the product in this case is, most markedly, the presence of two resonances between 5.04-5.21 ppm which integrate, in total, to one proton. This observation is discussed at length in the text (see **Discussion** p.29-30). Further purification was not

attempted, as this compound was the last in a sequence of steps to test the feasibility of this route (see **Discussion** p.33).

4. Intermolecular additions and intramolecular cyclizations

4.1 Addition of Grignard reagents to carbamates.

General procedure[2]

The alkyl bromide (1mmol) was added, dropwise, to a stirred suspension of magnesium turnings (2mmol) in anhydrous ether. Addition was at a sufficient rate to maintain reflux. Thereafter, the mixture was stirred at room temperature for 30 minutes. Ethyl N-methyl-N-phenylcarbamate (1mmol) was added dropwise to the solution of the Grignard reagent. The mixture was then heated under reflux for 22 hours. After cooling to room temperature, the mixture was washed with dil. HCl, sodium bicarbonate solution and water. Thereafter, it was dried ($MgSO_4$), concentrated and purified using column chromatography (Et_2O / Hex).

4.1.1 **Bibenzyl** (Spectra number 4.1.1, page 31 and 32)

Benzyl bromide (1.15g, 6.14mmol) was added to a stirred suspension of magnesium turnings (0.31g, 12.7mmol) in anhydrous ether. Ethyl N-methyl-N-phenylcarbamate (1.10g, 6.73mmol) was added to the solution and heated under reflux for 22 hours. Subsequent purification afforded bibenzyl as a white solid (12%, from crude ^{1}H NMR spectrum).

m.p. 52-53°C (lit. 52.0-52.5°C).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 2.95 (2 × CH_2, 4H, s), 7.17-7.34 (2 × Ph, 10H, m).

IR (thin film, ν_{max} / cm^{-1}) 3032 (w), 1505 (w), 1459 (w), 702 (s).

4.1.2 **Bibenzyl** (Spectrum number 4.2.1, page 33)

Benzyl bromide (1.23g, 7.19mmol) was added to a stirred suspension of magnesium turnings (0.32g, 13.2mmol) in anhydrous ether. Ethyl N-methyl-N-phenylcarbamate (1.17g, 6.53mmol) was added to the solution, together with a catalytic amount of CuI (40.7mg, 0.21mmol), and heated under reflux for 14 hours. Subsequent purification again afforded bibenzyl as a white solid (26%, from crude ^{1}H NMR spectrum).

m.p. 52-54°C (lit. 52.0-52.5°C).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 2.95 (2 × CH_2, 4H, s), 7.17-7.34 (2 × Ph, 10H, m).

4.1.3 **N-Methyl-N-phenylbenzamide** (Spectra number 4.1.3, page 34-36)

Bromobenzene (1.13g, 7.20mmol) was added to a stirred suspension of magnesium turnings (0.61g, 25.1mmol) in anhydrous ether. Ethyl N-methyl-N-phenylcarbamate (1.11g, 6.19mmol) was added to the solution and heated under reflux for 22 hours. Subsequent purification afforded N-methyl-N-phenylbenzamide as a colourless oil (78.2mg, 0.37mmol, 6.0%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 3.48 (N-Me, 3H, s), 6.99-7.37 (2 × Ph, 10H, m).

IR (thin film, ν_{max} / cm^{-1}) 2927 (w), 1657 (s), 709 (m).

EIMS *m/z* (%) 211.15 [M^+] (Calc. 211.0999 $C_{14}H_{13}ON$), 105.05 (100.00), 77.05 (49.45), 51.05 (12.06).

Notes: Base peak at *m/z* 105.05 corresponds to cleavage of the amide bond.

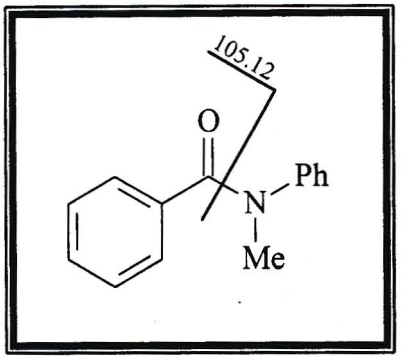

Fig. 21: Fragment corresponding to base peak of EIMS spectrum.

(Spectrum number 4.1.3, page 36).

4.1.4 **Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate** (Spectra number 4.1.4, page 37)

Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate (0.94g, 3.29mmol) was added dropwise to a stirred suspension of magnesium turnings (0.19g, 7.82mmol) in anhydrous ether. CuI (13.8mg, 0.072mmol) was added and the mixture was heated under reflux for 22 hours. Subsequent purification afforded the starting material, ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate, as a colourless oil (0.71g, 2.48mmol, 75%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.21 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 2.74 (N-Me, 3H,s), 3.69-3.80 (H_1, 1H, m), 3.85-3.96 (H_1, 1H, m), 4.09 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz), 5.05 and 5.22 (H_2, 1H, m), 7.25-7.43 (Ph, 5H, m).

Notes: It is clear from the ^{1}H NMR spectrum that no reaction has taken place. The presence of the two resonances between 5.05-5.22 ppm in the ^{1}H NMR spectrum, corresponding to the methine proton adjacent to bromine, indicates this. The impurity marked with × corresponds to the oxazolidone described in the **Discussion** (page 49-52).

4.2 **Diethyl benzylphosphonate**[7] (Spectra number 4.2, page 38-40)

A round-bottomed flask containing benzyl bromide (17.61g, 103mmol) was equipped with a water condenser and a dropping funnel containing triethyl phosphite (10.23g, 62mmol). Reaction began spontaneously on addition of a small portion of phosphite. Phosphite was added at such a rate that reflux was maintained. The mixture was then heated under reflux for 1 hour. The mixture was then cooled to room temperature and the water condenser was replaced with a Vigreux column. The water condenser was set for downward distillation. The crude product was distilled under high vacuum to afford diethyl benzylphosphonate as a colourless liquid (8.17g, 47.8mmol, 77%).

^{1}H NMR (400MHz, $CDCl_3$, δ / ppm) 1.20 (2 × $CH_2\underline{CH}_3$, 6H, t, J=7 Hz), 3.11 (CH_2, 2H, d, J=21 Hz), 3.94-4.00 (2 × $\underline{CH}_2CH_3$, 4H, m), 7.20-7.27 (Ph, 5H, m).

^{31}P NMR (162 MHz, $CDCl_3$, δ / ppm) 27.49.

IR (thin film, ν_{max} / cm^{-1}) 2986 (w), 1249 (m), 1031 (s).

Notes: The ^{1}H NMR spectrum shows the two methylene protons to be split into a doublet. This is due to the adjacent ^{31}P, which has the same splitting pattern as ^{1}H (i.e. ^{31}P has a spin quantum number (I) of $I = ½$).[10]

4.3 **Adduct of addition of diethyl benzylphosphonate to ethyl N-methyl-N-phenylcarbamate**[11] (Spectra number 4.3, page 41-47)

Diethyl benzylphosphonate (1.35g, 5.92mmol), in dry THF (25ml), under an inert nitrogen atmosphere, was cooled to -78°C. Butyl lithium (1.6M) (5.6ml, 8.96mmol) was added dropwise to the stirred solution. The mixture was then stirred at -60°C for 30 minutes. Thereafter, ethyl N-methyl-N-phenylcarbamate (1.16g, 6.47mmol) was added dropwise to the solution and the mixture was stirred at -60°C for a further 30 minutes. It was then allowed to reach room temperature and was stirred overnight. The crude mixture was washed (25ml NH_4Cl), extracted (2 × 20ml CH_2Cl_2) and washed again (2 × 10ml water). The crude mixture was then dried, concentrated and

purified using column chromatography (EtOAc / Hex) to afford the adduct as a colourless oil (0.39g, 1.37mmol, 23%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.04 (H_9, 3H, t, *J*=7 Hz), 1.15 ($H_{9'}$, 3H, t, *J*=7 Hz), 3.17 (N-Me, 3H, s), 3.77-3.86 (H_8, 2H, m), 4.03-4.12 ($H_{8'}$, 2H, m), 4.12 (H_4, 1H, d, *J*=23 Hz), 6.97-7.28 (H_{11}-H_{16} and $H_{11'}$-$H_{16'}$, 10H, m).

^{13}C NMR (75 MHz, $CDCl_3$, δ/ ppm) 16.21 (C_9), 16.40 ($C_{9'}$), 37.75 (N-Me), 50.09 (C_4, d, *J*= 141 Hz), 62.67 (C_8), 63.49 ($C_{8'}$), 127.62 (3CH), 128.37 (2CH), 129.60 (2CH), 129.79 (3CH), 132.03 (CH), 143.43 (CH), 167.16 (C=O).

^{31}P NMR (162 MHz, $CDCl_3$, δ / ppm) 21.08.

IR (thin film, ν_{max} / cm^{-1}) 2979 (w), 1663 (s), 1031 (s), 696 (m).

HRMS *m/z* (%) 361.1445 (14.18) [M^+] (Calc. 361.1443 $C_{19}H_{24}NO_4P$), 316.1104 (32.19) [M^+- OEt], 183.1047 (68.02), 107.0748 (100.00), 91.0486 (47.68).

Notes: The ^{1}H NMR spectrum shows two non-equivalent ethyl groups, with the two methyl triplets at 1.04 and 1.15 ppm respectively and the two methylene multiplets at 3.83-4.01 and 4.11-4.20 ppm respectively. The methylene proton resonances are complex due to splitting by ^{31}P. The methine proton resonates at 4.12 ppm and is split into a doublet, again, by ^{31}P. It is not possible to assign carbon signals from the HETCOR spectrum as the proton signals overlap, thus, the assignments for C_{11}-C_{16} and $C_{11'}$-$C_{16'}$ are interchangeable. In the ^{13}C NMR spectrum, the doublet at 50.09 ppm, *J*= 141 Hz, is due to splitting by the adjacent ^{31}P. One bond coupling constants between carbon and phosphorus vary between *ca.*-50 and +300 Hz.[12] More importantly, one bond coupling constants in **phophonates** vary from 140-160 Hz.[12,13] As a specific example, the carbon-phosphorus one bond coupling constant for dimethyl methylphosphonate is 142.2 Hz.[13]

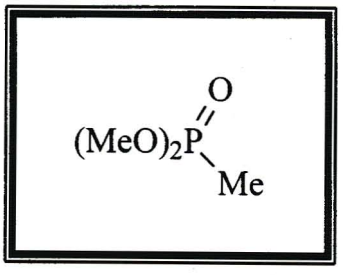

Fig. 22: Dimethyl methyl phosphonate.

In the IR spectrum, the absorbance corresponding to the P=O stretching can be found at 1255 cm^{-1}.[14]

4.4 **N-Methyl-5-phenylox-3-azolidin-2-one**[11] (Spectra number 4.4, page 48-56)

Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate (0.50g, 1.75mmol) and triethyl phosphite (0.32g, 1.93mmol) were placed in a round-bottomed flask and placed in an oil-bath, pre-heated to 150°C. The mixture was maintained at that temperature for 1 hour with the flask equipped for downward distillation. The crude reaction mixture was purified using preparative thin layer chromatography (Et_2O / Hex) and afforded N-methyl-5-phenyl-2-oxazolidinone as a yellow oil (0.11g, 0.62mmol, 35%).

^1H NMR (400MHz, $CDCl_3$, δ / ppm) 2.86 (N-Me, 3H, br s), 3.38 (H_{4b}, 1H, dd, *J*= 8.7, 7.4 Hz), 3.87 (H_{4a}, 1H, dd, *J*= 8.7, 8.7 Hz), 5.42 (H_5, 1H, dd, *J*= 8.7, 7.4 Hz), 7.24-7.37 (Ph, 5H, m).

^{13}C NMR (100 MHz, $CDCl_3$, δ / ppm) 31.27 (N-Me), 54.60 (C_4), 74.37 (C_5), 125.73 (2 CH), 128.98 (CH), 129.07 (2 CH), 138.91 (C), 158.41 (C_2).

IR (thin film, ν_{max} / cm^{-1}) 1762 (s), 1031 (m) 702 (w).

EIMS *m/z* (%) 177.15 [M^+] (Calc. 177.0790 $C_{10}H_{11}O_2N$) 132.05 (100.00), 117.05 (11.90), 105.05 (14.67), 91.05 (33.90), 77.05 (17.41), 65.10 (8.81), 51.10 (12.89), 43.10 (45.10).

Position	COSY	NOESY
1	-	-
2	-	-
3	-	-
4a	4b, 5	4b, 5, 6
4b	4a, 5	4a, 5, 6, 8, 12
5	4a, 4b	4a, 4b, 8, 12
6	-	4a, 4b, 8, 12
7	-	-
8/12*	-	4b, 5
9/11*	-	-
10*	-	-

* resonances superimposed

Table 7: COSY and NOESY correlations for N-methyl-5-phenyl-2-oxazolidinone.

Position	HMBC (C→H)	Position	HMBC (C→H)
1	-	**6**	-
2	4a, 4b, 5, 6	**7**	4a, 4b, 5, 9, 11
3	-	**8/12**	5, 10
4	8, 12	**9,11**	-
5	6	**10**	8, 12

Table 8: HMBC correlations for 3-methyl-5-phenyl-2-oxazolidinone.

Notes: It is apparent from the HMBC and COSY spectra that the triplet at ~1.2 ppm and the multiplets at ~4.1 ppm do not correlate to any of the resonances attributed to the oxazolidone. This impurity, present in all spectra, is triethyl phosphite. In the HMBC, COSY and NOESY spectra the correlations marked with '×' are HETCOR correlations, COSY correlations or impurity.

In the ^{1}H NMR spectrum the two H_4 protons are arbitrarily assigned as H_{4a} (3.87 ppm) and H_{4b} (3.38 ppm). In the NOESY spectrum, a correlation between H_{4b} and H_8, H_{12} indicates that H_{4b} and the phenyl ring are *cis* with respect to the 4-5 bond (Fig. 23).

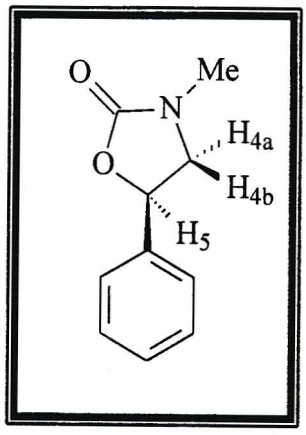

Fig. 23: N-Methyl-5-phenylox-3-azolidin-2-one showing relative stereochemistry.

The correlations between the N-methyl protons and both H_{4a} and H_{4b} could be due to partial double bond character of the amide linkage (Fig. 24).[15] The resonance form containing the C=N double bond would result in Me-N-CH_2 having a planar geometry, with the N-methyl equidistant from both H_4 protons.

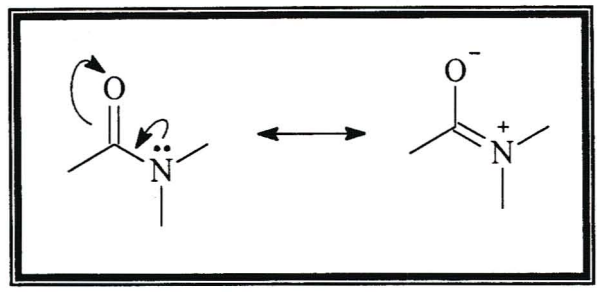

Fig. 24: Resonance forms of an amide linkage.

4.5 **N-Methyl-5-phenylox-3-azolidin-2-one**[11] (Spectra number 4.5, page 57 and 58)

Ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate (0.30g, 1.05mmol) was placed in a round-bottomed flask and placed in an oil bath pre-heated to 150°C. The temperature was maintained for 1 hour and afforded N-methyl-5-phenylox-3-azolidin-2-one as a colourless oil (0.14g, 0.79mmol, 75%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 2.88 (N-Me, 3H, s), 3.41 (H_{4b}, 1H, m), 3.89 (H_{4a}, 1H, m), 5.44 (H_5, 1H, m), 7.30-7.39 (Ph, 5H, m).

IR (thin film, ν_{max} / cm^{-1}) 2933 (w), 1762 (s), 1031 (s), 702 (m).

4.6 **Ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**[11] (Spectra number 4.6, page 59-65)

Ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate (0.43g, 1.37mmol) and excess triethyl phosphite were placed in a round-bottomed flask and placed in an oil-bath, pre-heated to 150°C. The mixture was maintained at that temperature for 1 hour with the flask equipped for downward distillation. The crude reaction mixture was purified using preparative thin layer chromatography (Et_2O / Hex) and afforded ethyl N-methyl-N-4-phenyl-3-butenylcarbamate as a colourless oil (33.1mg, 0.14mmol, 35%).

^{1}H NMR (300MHz, $CDCl_3$, δ / ppm) 1.22 ($CH_2C\underline{H}_3$, 3H, t, *J*=7 Hz), 2.41 (H_2, 2H, br m), 2.90 (N-Me, 3H, br s), 3.36 (H_1, 2H, br t), 4.10 ($C\underline{H}_2CH_3$, 2H, q, *J*=7 Hz), 6.11-6.19 (H_3, 1H, m), 6.41 (H_4, 1H, d, *J*=16 Hz), 7.14-7.33 (Ph, 5H, m).

^{13}C NMR (75 MHz, $CDCl_3$, δ / ppm) 14.76 ($CH_2\underline{C}H_3$), 31.69 (C_2), 34.21 (N-Me), 48.70 (C_1), 61.21 ($\underline{C}H_2CH_3$), 126.02 (2 CH), 127.14 (C_3), 128.51 (3 CH), 131.88 (C_4), 156.55 (C=O).

IR (thin film, ν_{max} / cm^{-1}) 2940 (w), 1716 (s), 1209 (m), 702 (w).

EIMS *m/z* (%) 233.15 [M^+] (Calc. 233.1415 $C_{14}H_{19}O_2N$), 116.15 (100.00), 91.10 (10.19), 72.15 (7.62), 44.1 (37.82).

Position	COSY
1	2
2	1, 3
3	2, 4
4	3

Table 9: COSY correlations for ethyl N-methyl-N-4-phenyl-3-butenylcarbamate.

Notes: The base peak shown in the EIMS spectrum at *m/z* 116.15 corresponds to the fragmentation shown below (Fig. 25).

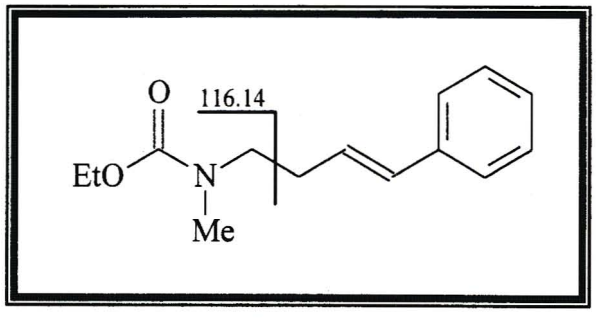

Fig. 25: Fragment corresponding to base peak of EIMS spectrum.

(Spectrum number 4.6, page 65).

5 Miscellaneous reactions

5.1 **N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane**[2] (Spectra number 5.1, page 66-70)

Ethyl N-bromocarbamate (2.47g, 14.7mmol) in dichloromethane was degassed for 10 minutes. Thereafter, 1,5-hexadiene (0.62g, 7.55mmol) was added to the stirred solution and the mixture was degassed for a further 10 minutes. The mixture was then photolised, using a medium pressure mercury lamp, for 2.5 hours. The mixture was washed with sodium thiosulphate solution and sodium bicarbonate solution, dried ($MgSO_4$) and concentrated to yield a yellow oil. The crude product was purified using column chromatography (Et_2O / Hex) to yield N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane as a white solid (0.57g, 1.36mmol, 9.3%).

m.p. 134-136°C.

^1H NMR (300MHz, $CDCl_3$, δ / ppm) 1.23 (2 × $CH_2C\underline{H}_3$, 6H, t, J=7 Hz), 1.85-1.89 (H_3 and H_4, 2H, m), 2.12-2.19 (H_3 and H_4, 2H, m), 3.37-3.46 (H_1 and H_6, 2H, m), 3.60-3.65 ($H_1$and H_6, 2H, m), 4.05-4.14 (N-H, 2H, br), 4.11 (2 × $C\underline{H}_2CH_3$, 4H, q, J=7 Hz), 5.11-5.15 (H_2 and H_5, 2H, br).

^{13}C NMR (75 MHz, $CDCl_3$, δ / ppm) 14.60 (2 × $CH_2\underline{C}H_3$), 33.85 (C_3 and C_4), 47.47 (C_1 and C_6), 55.49 (C_2 and C_5), 61.24 (2 × $\underline{C}H_2CH_3$), 156.57 (C=O).

IR (thin film, ν_{max} / cm^{-1}) 3315 (m), 1696 (s), 1538 (m), 1262 (m).

EIMS *m/z* (%) 418.4 [M^+] (Calc. 418.3982 $C_{12}H_{22}O_4N_2Br_2$), 122.1 (46.08), 109.1 (95.18), 108.1 (43.39), 107.1 (65.91), 69.1 (67.55), 43.1 (100.00), 41.05 (61.87).

Notes: Molecule is symmetrical.

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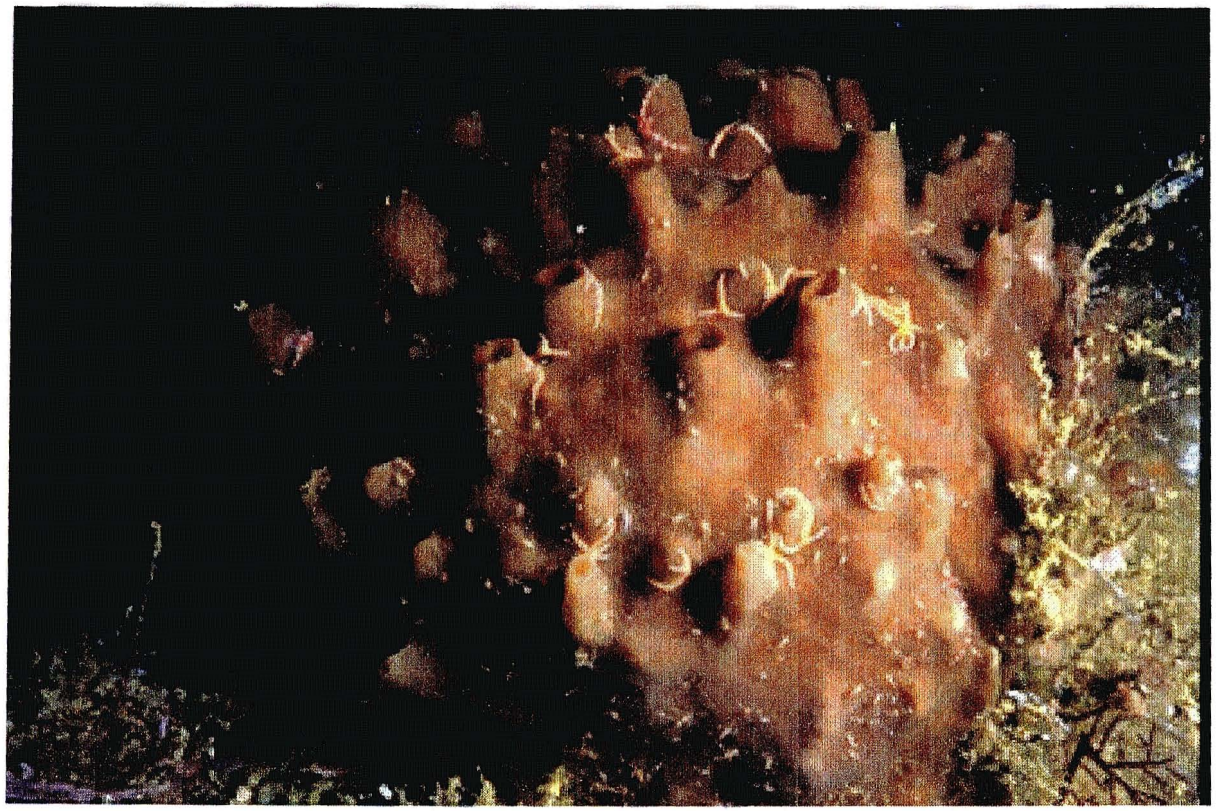
Chapter 2

Extractives from *Oceanapia sp.*



Oceanapia sp.

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List of Abbreviations

SAM – *S*-adenosylmethionine

m.p. – melting point

HRMS – high resolution mass spectroscopy

^{1}H NMR – proton nuclear magnetic resonance spectroscopy

ppm – parts per million

HETCOR – heteronuclear shift correlation nuclear magnetic resonance

NOESY – nuclear Overhauser effect spectroscopy

HMBC – heteronuclear multiple bond coherence

Hz – Hertz

^{13}C NMR – carbon-13 nuclear magnetic resonance spectroscopy

COSY – correlated nuclear magnetic resonance spectroscopy

IR – infrared spectroscopy

ORI – Oceanographic Research Institute

TLC – thin layer chromatography

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Abstract

Oceanapia sp. is a sponge (Porifera) which occurs off the KwaZulu Natal coast.

Sponges are typically rich in sterols and it has become clear in the past 10 years that sponges have the most diverse array of novel sterols of all organisms. Due to their relatively advanced biochemistry, they provide the largest number of biologically active marine natural products.

Studies of *Oceanapia* have not previously been reported, therefore, a chemical investigation of this organism was carried out with the hope of learning about its chemistry and isolating novel or active compounds.

Introduction

1. Porifera (The sponges)[1]

Sponges are the simplest of the metazoan (multi-cellular) animals. They do not have muscles or nerves and are, therefore, not able to move about. In spite of this handicap, sponges serve an extremely important ecological function, that being the circulation of water, a mechanism they use to capture small, suspended particles to use as food.

A sponge's body resembles a tall vase with a single large opening at the top (the osculum) and numerous tiny pores, which penetrate the body walls. Sponges grow throughout their lives and spread across the sea floor and may give rise to many vase-like outgrowths. Although, to many observers, these may appear as separate individuals, they are interconnected *via* a common base and should be regarded as a single colony. The body wall is composed of three layers; an outer layer of flat cells, a middle, jelly-like mesenchyme, and an inner layer lining the central cavity. The figure below (Fig. 1) shows a cross section of a simple sponge with accompanying detail of a body wall.

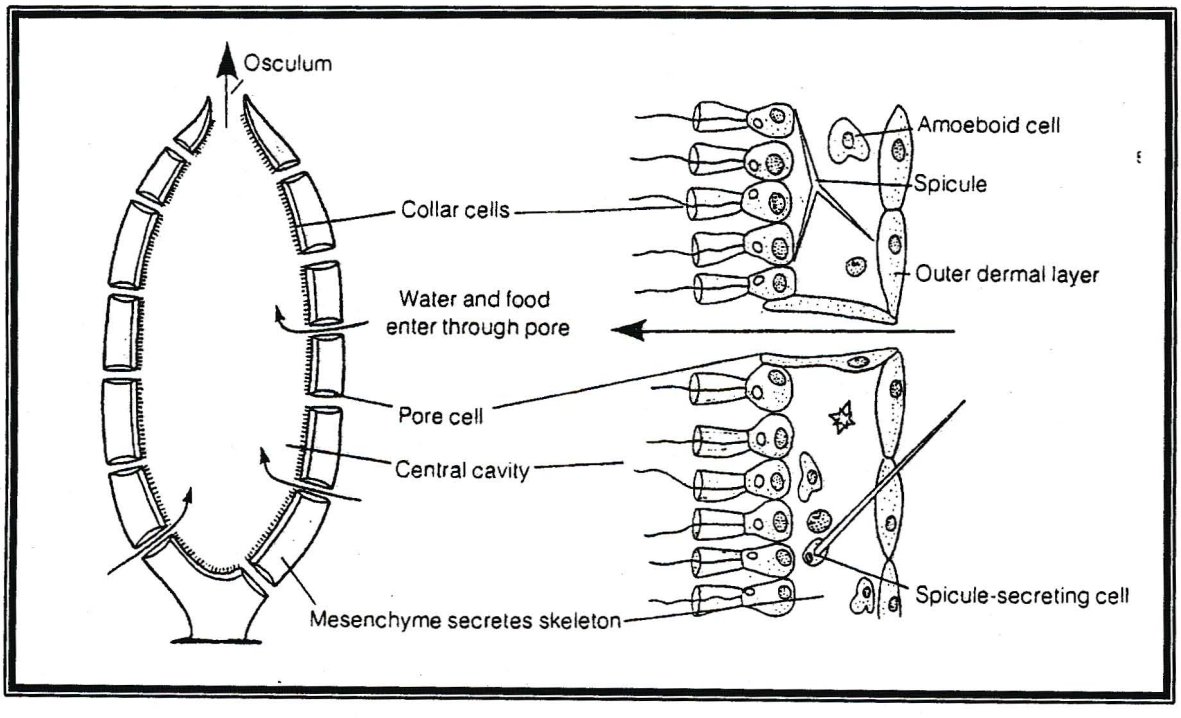


Fig. 1: Cross section of a simple sponge and detail of body wall.[1]

The cells of the central mesenchyme form a skeleton which provides support for the sponge. In addition to this organic skeleton, the mesenchyme comprises spiky silicon spicules, which give the sponge a cactus-like appearance under a microscope. These spicules are often fiercely irritant and serve as a defence mechanism for the sponge.

The inner layer of the body wall consists of flagellated cells, also called collar cells, whose whipping action drives water through the sponge and out the osculum. This water is replaced by sucking more from the surroundings through the pores of the body wall. The flagellated cells each have a collar, which resembles a funnel, surrounding the flagellum, giving these cells the name 'choanocytes' (Greek for 'funnel-cells'). The collars are made up of tiny threads called microvilli closely applied and connected by strands. This forms an extremely fine net, which, when water flows through the sponge, traps small particles. These particles are then absorbed by the cells of the sponge. The figure below shows the structure of a collar cell (Fig. 2).

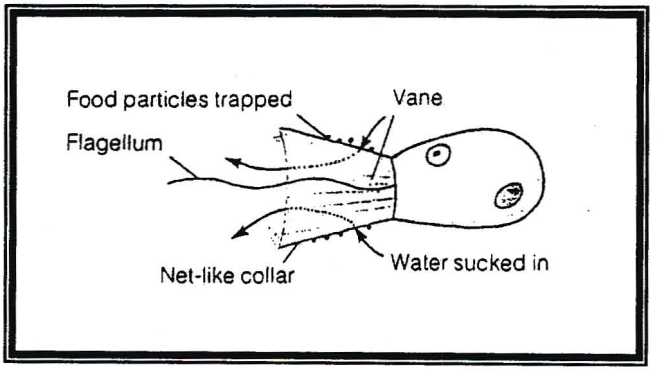


Fig. 2: Structure of a collar cell.[1]

Many sponges have optimised this process by evolving complex body walls that are folded to increase the surface area of collar cells. The figure below shows the structure of a complex sponge (Fig. 3).

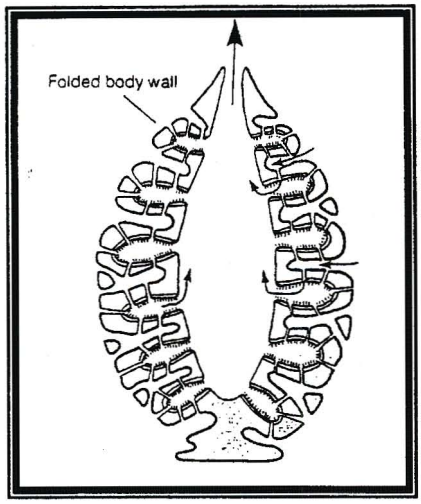


Fig. 3: Cross-section of a complex sponge.[1]

The efficiency of their filtration makes this function an important ecological one, as mentioned earlier in the text. In calm, shallow waters for instance, sponges are able to completely clear the water of all suspended particles, even down to one micron in size.

2. Introduction to sterols

2.1. General Remarks

The most commonly accepted numbering system for sterols is that of the IUPAC-IUB Recommendations for Sterol Nomenclature of 1989. This numbering system is shown below (Fig. 4).[2]

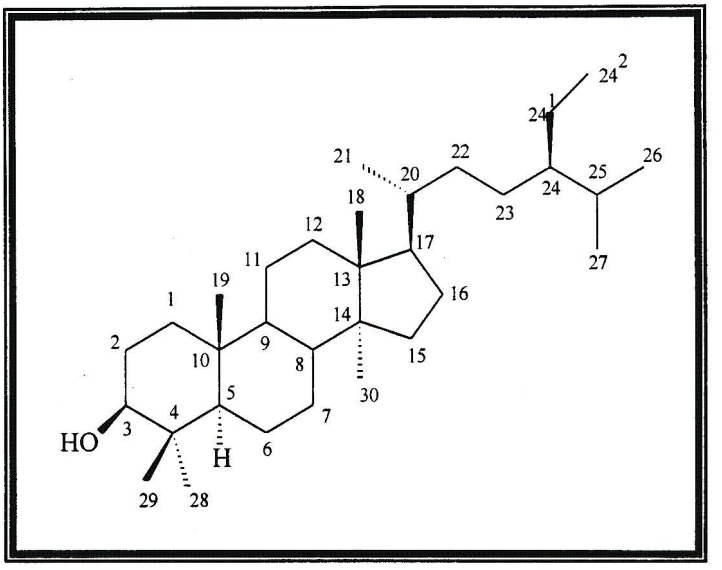


Fig. 4: Numbering system for sterols.

The perhydrocyclopentanophenanthrene ring system shown below (Fig. 5) is the system from which all steroids are derived.[3] 'Sterol' was the term originally used to describe the 3β-monohydroxy derivatives of this system.[2]

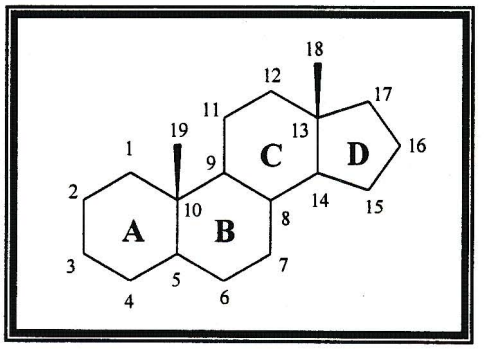


Fig. 5: Perhydrocyclopentanophenanthrene ring system.

The four rings are designated with letters. Most often, the B-C and C-D ring junctions are *trans*, however, the A-B junction may be either *cis* or *trans* and this leads to two general 3-dimensional systems (Fig. 6).[3] They are referred to as the 5α and 5β series.

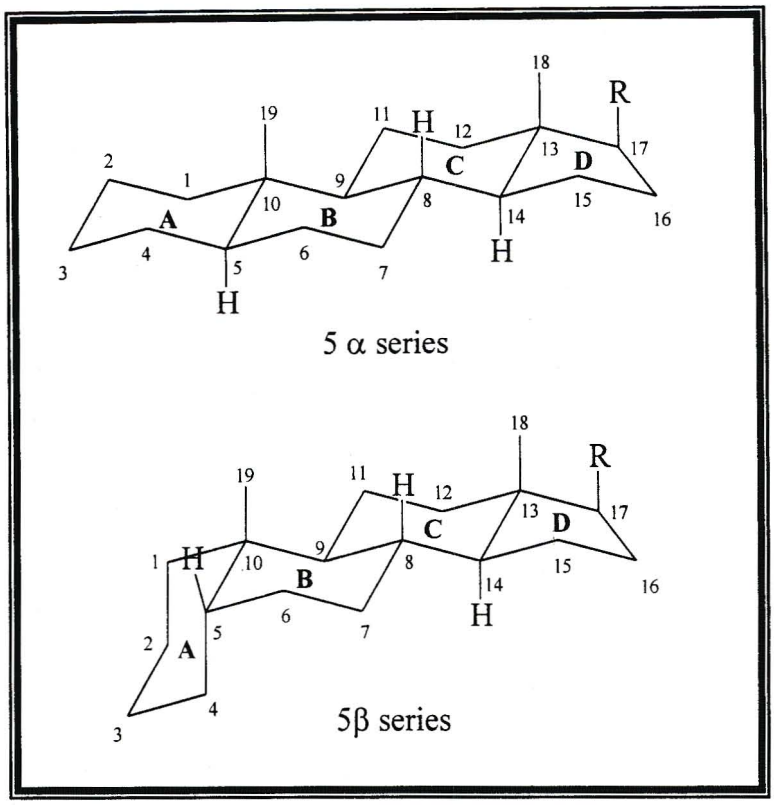


Fig. 6: The two basic 3-dimensional ring systems of sterols.

The methyl groups at ring junctions C_{10} and C_{13} are known as angular methyl groups and serve as important reference points for stereochemical assignments. These methyl groups protrude above the general plane of the ring system as it is drawn above. Groups that lie on the same side as the angular methyl groups are, by convention, assigned as β substituents. Groups on the opposite side are assigned as α substituents. This accounts for the names given to the two 3-dimensional ring systems, referring to the assignments of H_5 in each system.

Modifications to this general structure, particularly to the side-chain at C_{17}, create a wide range of biologically important natural products.[4] In addition to the sterols, these include steroidal saponins, cardioactive glycosides, bile acids, corticosteroids and mammalian sex hormones.[4]

2.2. Marine Sterols

According to Goad[5], there are four possible sources of sterols in marine invertebrates, and the balance between them depends on the organism. These are,

- *de novo* biosynthesis (biosynthesis by the organism itself)
- assimilation of sterols produced by micro-organisms living symbiotically with the organism
- assimilation of dietary sterols
- modification of dietary sterols

Sterols are required compounds in all eukaryotes, however, the nature of sterols found in different organisms can vary markedly.[6] Their basic role is to optimise the mechanical properties of cell membranes. Generally speaking, the less advanced eukaryotes contain more complex sterol mixtures and more unconventional structures than their more advanced counterparts. Prokaryotic organisms are normally devoid of sterols, but rather contain polyterpenoid alcohols, which are believed to be functional equivalents of sterols. This would seem to indicate that sterol evolution could be associated with the progression from prokaryotic to eukaryotic life. Marine sterols are a large and diverse group of compounds, many of which have no counterparts in the terrestrial kingdom.

Sponges were the first invertebrates shown to have sterols other than cholesterol.[7] Generally, sponges contain between ten and twenty monohydroxylated sterols, although this number has been seen to vary between one and seventy-four.[8] The latter case being a report made in 1983 of the isolation of seventy-four minor and trace sterols from *Axinella cannabina*, twenty-four of which were novel. Taking into account the rich diversity of sponge sterols, it's not surprising that they were the first class of compounds to be used in chemotaxonomic studies of sponges.[6] The first such investigation, in 1949, showed that all sponges contain sterols and that each sponge has a unique sterol mixture.[7] Some of these results were inaccurate due to the lack of adequate analytical instruments at that time. However, these ideas led to further work.

In 1980 and 1986, Bergquist and co-workers reported two separate studies on sterols from 82 species of marine sponges.[9,10] Their results showed that sterol composition

was qualitatively consistent with season and location and the occurrence of particular sterols was found to be of some chemotaxanomic value.

The most striking observation relating to sponge sterols is the number of unconventional side-chains that are unique to the phylum. The most curious of these side-chains are those containing cyclopropane rings. There are several examples of these. In the few examples that are described below the focus is firmly on the structure of the side-chain. The figure below (Fig. 7) shows the two sterol nulcei to which the side-chains (R) are attached, labeled **A** and **B.**

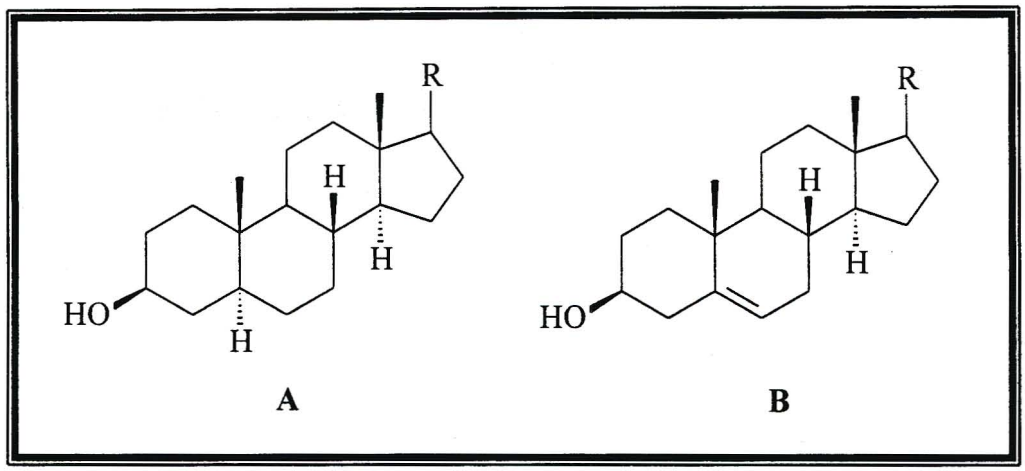


Fig. 7: Sterols nuclei referred to in text.

In April 1970 Hale *et al.* reported chemical evidence that supported the discovery of a biogenetically unprecedented sterol side-chain in the sterol gorgosterol.[11] However, they could not locate the cyclopropane ring unambiguously. In August of that same year, Ling *et al.* reported a confirmed structure of gorgosterol (Fig. 8a).[12] They did this using X-ray diffraction analysis of 3β-bromogorgosterol.

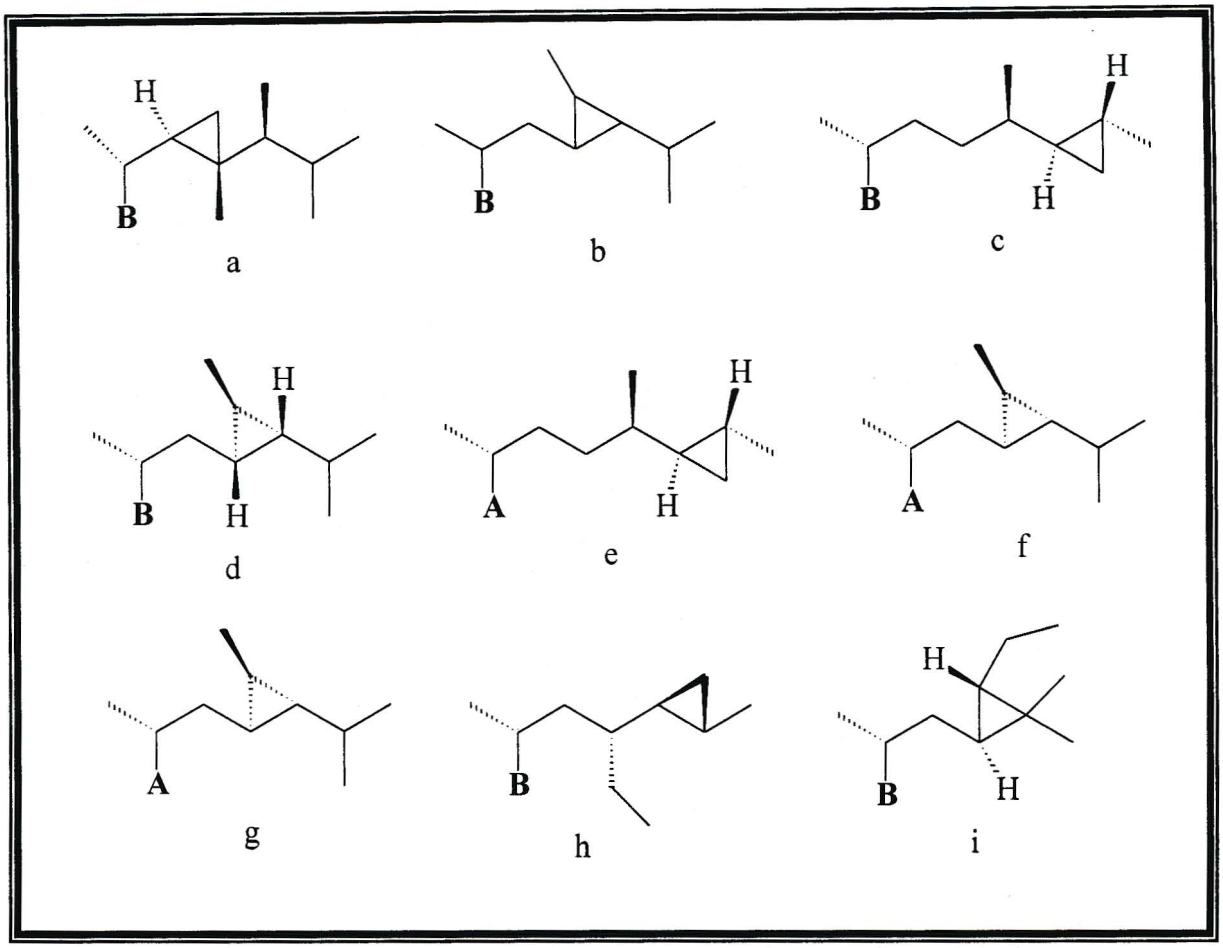


Fig. 8: Examples of sterol side-chains containing cyclopropane rings.

In 1978, Sica *et al.* reported the isolation and structure elucidation of petrosterol, another sterol containing a cyclopropane ring in its side-chain (Fig. 8b).[13] This sterol was present as the major sterol from the sponge *Petrosia ficiformis*. Later that year Mattia *et al.*, again using X-ray diffraction analysis, found the reported structure to be incorrect. The revised the structure of petrosterol is shown above (Fig. 8c).[14] The *p*-bromobenzoate derivative was used in this X-ray diffraction analysis.

In 1982 Li *et al.* isolated yet another sterol containing a cyclopropane ring in its side-chain, 23,24 dihydrocalysterol (Fig. 8d).[15] The organism under investigation in this case was the sponge *Calyx niceaensis*. This was not merely another occurrence of an unconventional side-chain. It provided added weight to the proposed biosynthetic sequence to calysterol, the only naturally occurring steroidal cyclopropene known at the time.[15] Side-chain stereochemistry was reported as being (23S, 24S, 28R). This stereochemistry was determined using catalytic hydrogenation techniques.

Three novel sterols containing cyclopropane rings from the New Zealand sponge *Petrosia hebes* were reported in 1987 by Cho *et al.*.[16] The principal sterol in this case was again petrosterol (Fig. 8c) constituting over 50% of the sterol mixture, which totaled twenty-five sterols. The saturated analogue of petrosterol, petrostanol (Fig. 8e) was one of the novel sterols isolated. Also isolated was 23,24-dihydrocalysterol (Fig. 8d), as well as its saturated analogue 23,24-dihydrocalystanol (Fig. 8f), the second novel sterol. The third novel sterol containing a cyclopropane ring was hebesterol (Fig. 8h). The isolation of hebesterol provided what was described as "the key 'missing link'" in a proposed biosynthetic sequence leading to 23,24 dihydrocalysterol (Fig. 8d) and petrosterol (Fig. 8c).

Nicasterol (Fig. 8i) was isolated as a trace sterol from *Calyx nicaensis* in 1985 by Proudfoot *et al.*.[17] The structure and absolute stereochemistry of nicasterol was determined using partial synthesis. This showed that the two cyclopropane ring protons were *trans* to one another across the 23-24 bond as shown (Fig. 8i).

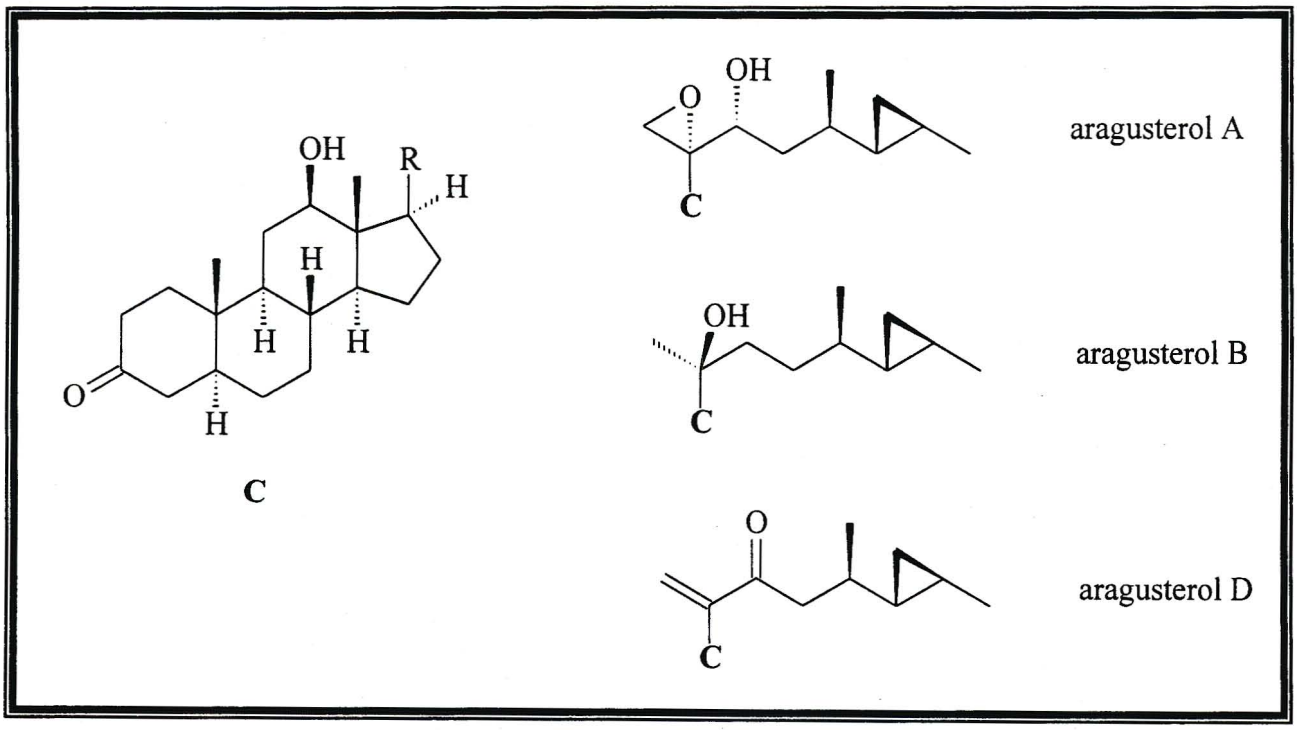


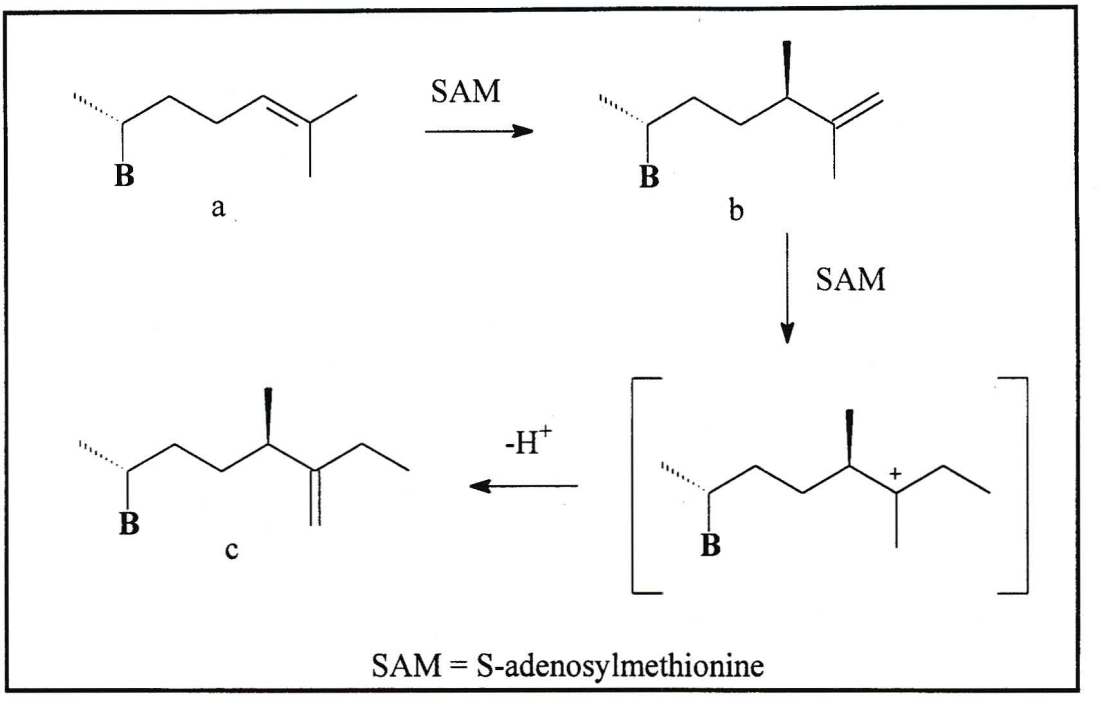
Fig. 9: Aragusterol A, B and D.

The three sterols shown above (Fig. 9) were reported between 1992 and 1994 by Iguchi *et al.*.[18,19] All three compounds were isolated from the same sponge from the

genus *Xestospongia*. They are not typical sterols in the sense that they do not have a 3β-hydroxy group, but a 3-keto and a 12β-hydroxy group. This aside, they still contain very unusual side-chains. Most importantly, aragusterol A possesses potent antitumor activity. Unfortunately, aragusterol B and D show only moderate activity and no activity respectively.

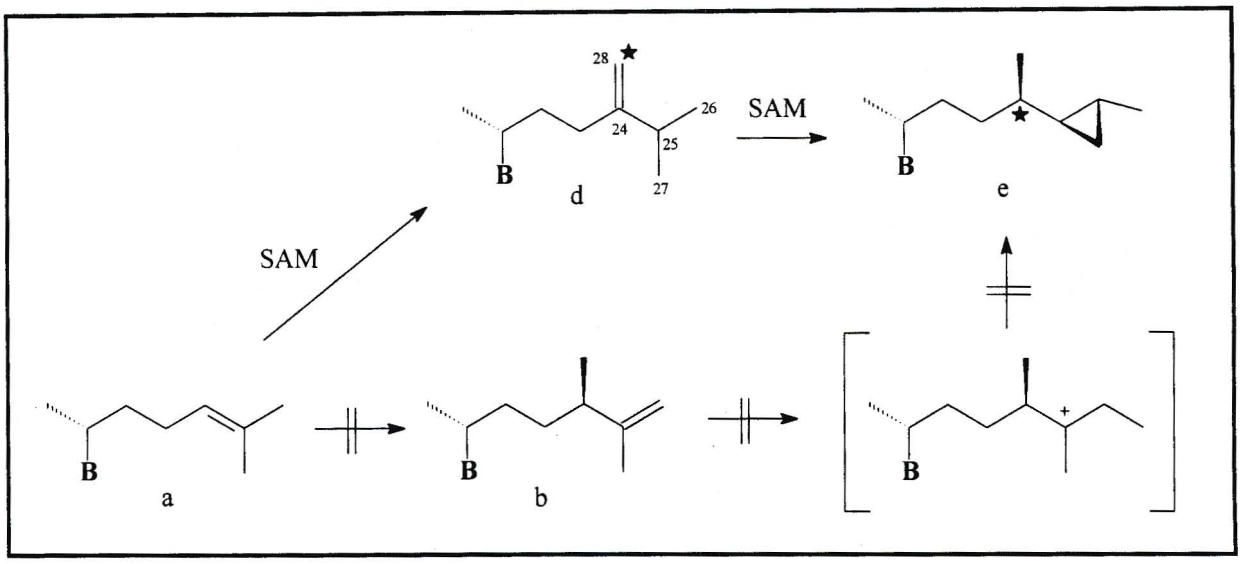
3. Biosynthesis of cyclopropane-containing sterols

The biosynthesis of the multiply alkylated side-chain of the sterol 25,27-dehydroaplysterol (Scheme 1c) was the first of its kind to be studied (Scheme 1).[20] It was found, in the sponge *Aplysina fistularis*, to be biosynthesised by the biomethylation of epicodisterol (Scheme 1b).



Scheme 1: Biosynthesis of 25,27-dehydroaplysterol (c).[21]

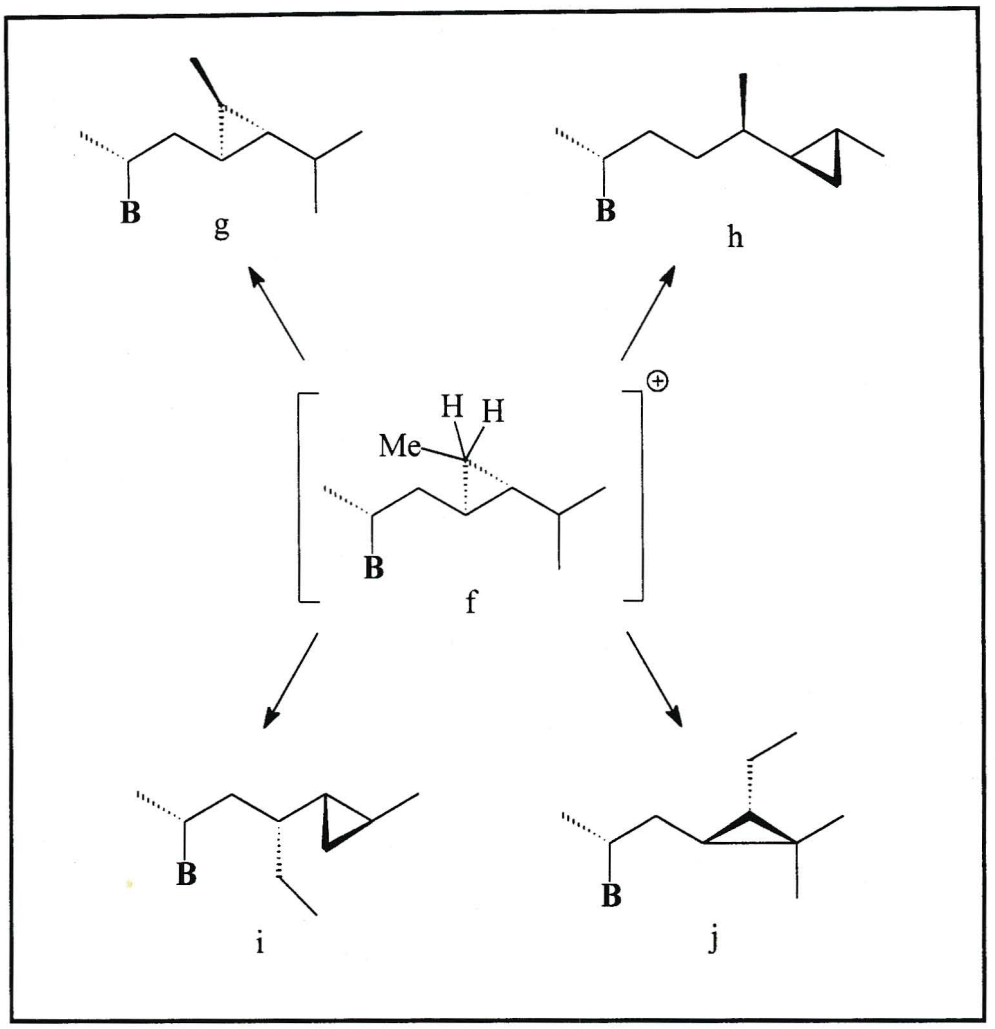
The structure of one of the most abundant cyclopropane-containing sterols, petrosterol (Scheme 2e), suggests an iterative biosynthesis from epicodisterol (Scheme 2b) in an analogous way (Scheme 2).[21]



Scheme 2: Results of feeding epicodisterol (b) and 24-methylenecholesterol (d) to the sponge *Petrosia ficiformis.*

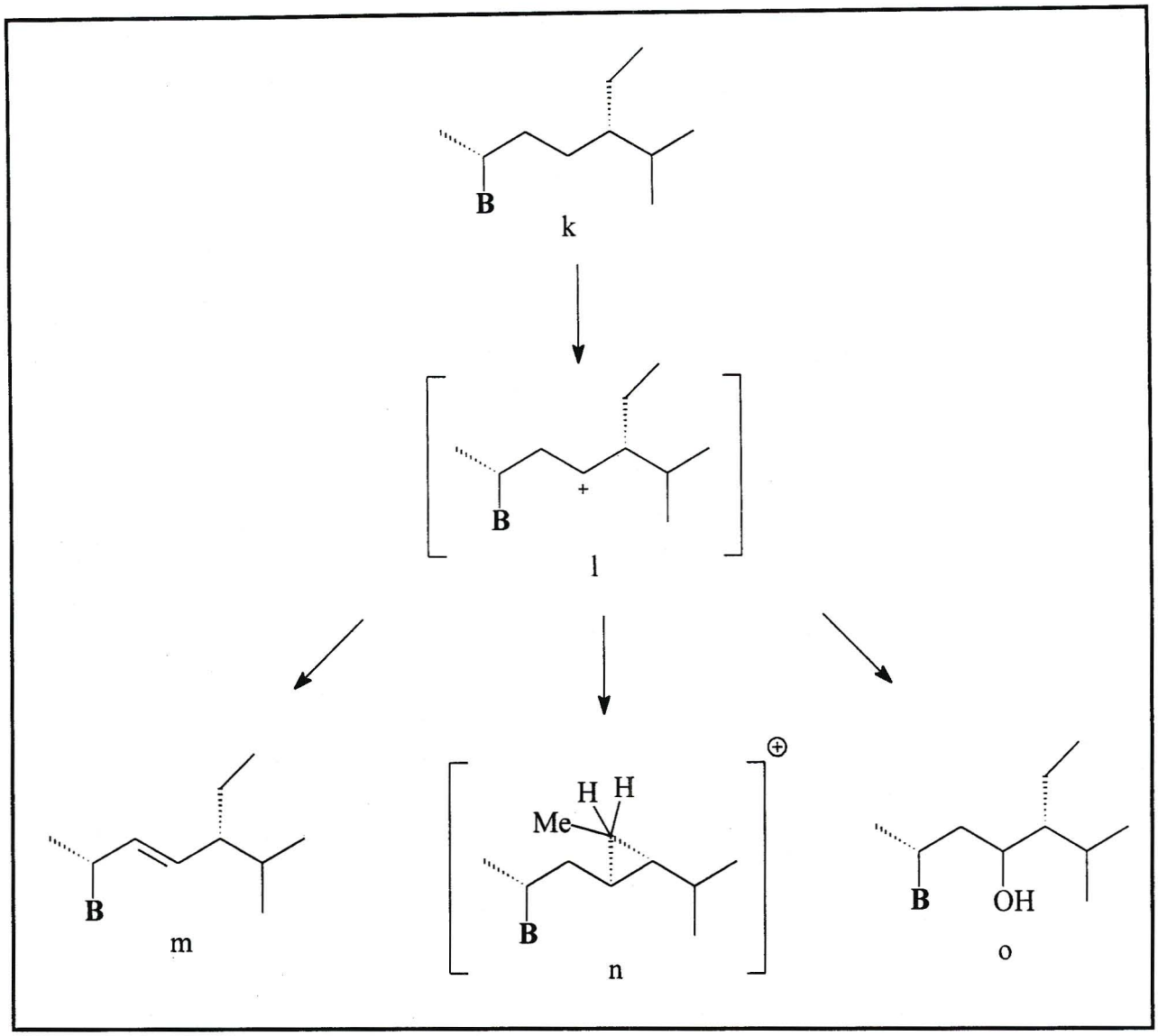
However, when 28-^{14}C-radiolabeled epicodisterol was fed to the sponge *Petrosia ficiformis,* no radioactivity was noticed in petrosterol. This led researchers to conclude that petrosterol was not the product of an iterative biomethylation, but rather a rearrangement.[22] This was confirmed by feeding [28-^{14}C]-24-methylenecholesterol (Scheme 2d) to the sponge *Cribrocalina vasculum.* It was noticed that the ^{14}C label had migrated from C_{28} of 24-methylenecholesterol to C_{24} of petrosterol (Scheme 2).[22] These results and observations, however, did not identify the immediate biosynthetic precursor.

In 1987, Proudfoot and Djerassi proposed a unified biosynthetic pathway for the synthesis of a number of unusual marine sterols, among them the cyclopropane-containing sterols (Scheme 3 g-j).[23] The scheme is based on the rearrangement of a protonated dihydrocalysterol species (Scheme 3f).



Scheme 3: Unified biosynthetic pathway to cyclopropane-containing sterols.[23]

At the time, the origin of the protonated cyclopropane intermediate (Scheme 3f) was not known. Direct protonation of dihydrocalysterol (Scheme 3g) was ruled out when no conversion to petrosterol was noticed in feeding experiments.[24] Further investigations[25] suggested that the precursors to these sterols were 24 (S)-ethylcholesterol (Scheme 4k, also called clionasterol) and 24 (S)-methylcholesterol. When radiolabeled 24 (S)-ethylcholesterol was fed to *Petrosia ficiformis*, the novel sterols g-j (Scheme 3) were observed.[24]



Scheme 4: Products of the Δ^{22}-desaturase.[24]

The mechanism by which the protonated cyclopropane intermediate is formed *via* 24 (S)-ethylcholesterol is shown above (Scheme 4).[24] A sterol bearing a 23-hydroxy group (Scheme 4o) was isolated from a yeast mutant incapable of desaturation at C_{22}. Desaturation in this yeast is brought about by cyctochrome P-450 Δ^{22}-desaturase which initiates deprotonation at C_{23}, forming a secondary carbonium ion (Scheme 4l).[24] This carbonium ion can then eliminate a proton directly from C_{22} producing the C_{22}-C_{23} double bond (Scheme 4m), capture a hydroxy group to produce the 23-hydroxy sterol (Scheme 4o), or rearrange *via* the protonated dihydrocalysterol (Scheme 4n) to produce cyclopropane-containing sterols (Scheme 3).[24]

Discussion

One compound, (24R)-24,25-methylene-5α-cholestan-3-ol, was isolated from the hexane extract of the sponge *Oceanapia sp.* This compound has been reported only once previously, by Makarieva *et al.* in 1996,[26] as the principal sterol of *Rhizochalina incrustata.* This is, therefore, the first time this compound has been isolated from the genus *Oceanapia.* The optical rotations of the reported compound and the one isolated have the same sign. Therefore, the isolated compound is assigned the 24R configuration (see **Experimental** page 22).

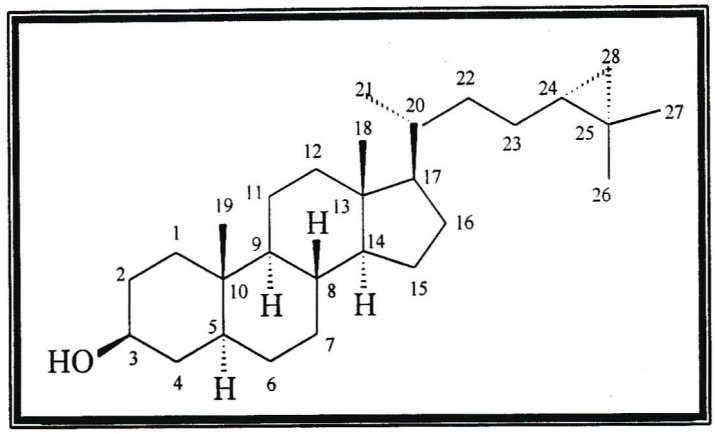


Fig. 10: Compound 1.

1. Structure elucidation of compound 1

(All spectra described in the following text can be found in Appendix C, Volume 2, of this work. The impurity present, marked with '×' is ethyl acetate.)

This compound was isolated as a colourless crystalline solid (m.p. 116-118°C). The HRMS spectrum showed a molecular ion peak at *m/z* 400.37159 corresponding to a molecular formula of $C_{28}H_{48}O$, consistent with the proposed structure.

In the ^1H NMR spectrum there are three important observations to be made. First and most noticeably, is the presence of two very high-field resonances at 0.36 and –0.18 ppm. This is indicative of a cyclopropane ring. The integration of 2:1 indicates a tri-substituted system. From the HETCOR spectrum it can be seen that the two protons at 0.36 ppm are attached to different carbons and, therefore, the broad resonance at 0.36 ppm is actually two signals at 0.36 and 0.38 ppm. The resonances at 0.36 and –0.18

ppm **do** correlate to the same carbon and, hence, are assigned as the two protons attached to C_{28}. The signal at 0.38 ppm is assigned as H_{24}. In the NOESY spectrum a correlation is observed between H_{24} and one of the terminal methyl groups. The proton at 0.36 ppm correlates to the same methyl group. Due to the compound having the 24R configuration, H_{24} is assigned as β. Therefore, the proton resonating at 0.36 ppm is also assigned as β. In summary, the downfield resonance at 0.36 ppm is assigned as $H_{28\beta}$ and the upfield resonance at –0.18 ppm is assigned as $H_{28\alpha}$. The methyl group with which H_{24} and $H_{28\beta}$ correlate in the NOESY spectrum is assigned, by convention, as C_{27}. In the HMBC spectrum a correlation is observed between methyl groups C_{26} and C_{27}, and H_{24}. This locates the cyclopropane ring, unambiguously at the 24,25 position.

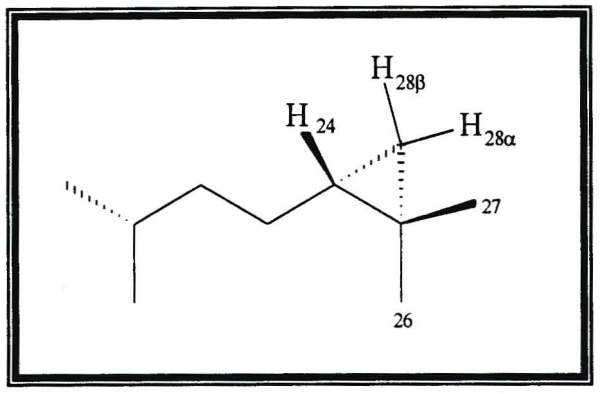


Fig. 11: Absolute stereochemistry of cyclopropane ring.

Also in the ^{1}H NMR spectrum, five methyl groups are observed at 0.62, 0.77, 0.98 and 0.99 ppm as singlets, and at 0.87 ppm as a doublet (J = 6.5 Hz). The singlets correspond to methyl protons H_{18}, H_{19}, H_{27} and H_{26} respectively. The doublet corresponds to methyl protons H_{21} split into a doublet by the single proton H_{20}.

A final observation in the ^{1}H NMR spectrum is the broad resonance at 3.56 ppm. This was attributed to H_3 and was assigned as α due to a correlation with H_5 in the NOESY spectrum. On this basis the hydroxy group at C_3 was assigned as β.

In the ^{13}C NMR spectrum the signals at 12.05 and 12.32 ppm are attributed to C_{18} and C_{19} respectively, C_{21} resonates at 18.72 ppm, and C_{26} and C_{27} resonate at 27.71 and 19.90 ppm respectively. C_3 resonates at 71.38 ppm.

The three carbons of the cyclopropane ring resonate at 19.82 ppm (C_{28}), 25.04 ppm (C_{24}) and 15.18 ppm (C_{25}). It is very unusual to see such a highfield methine resonance. This is due to the very strained nature of the cyclopropane ring.

The remaining two quaternary carbons resonate at 42.61 (C_{13}) and 35.46 (C_{10}) ppm. Also of interest is C_5, which resonates at 44.87 ppm. The remaining carbon assignments appear below (Table 1).

The COSY spectrum shows coupling between the two methylene protons (H_{28}) and H_{24}.

In the IR spectrum a broad band is observed at ν_{max} 3360 cm^{-1} (OH stretching). Strong bands are observed at 2944 cm^{-1} (C-H asymmetric stretching) and 2851 cm^{-1} (C-H asymmetric stretching). Weak bands are observed at 1457 cm^{-1} (C-H asymmetric bending) and 1373 cm^{-1} (C-H symmetric bending). A weak absorbance at 1040 cm^{-1} is characteristic of a deformation mode due to a cyclopropane ring.[27]

Position	Compound 1
3	3.56
18	0.62
19	0.77
21	0.87
24	0.38
26	0.99
27	0.98
28β	0.36
28α	-0.18

Table 1: ^{1}H NMR data for compound 1.

Position	Cpd 1	Position	Cpd 1	Position	Cpd 1	Position	Cpd 1
1	37.01	**8**	35.52	**15**	24.23	**22**	36.48
2	31.51	**9**	54.37	**16**	28.27	**23**	26.49
3	71.38	**10**	35.46	**17**	56.26	**24**	25.04
4	38.20	**11**	21.26	**18**	12.05	**25**	15.18
5	44.87	**12**	40.04	**19**	12.32	**26**	19.90
6	28.74	**13**	42.61	**20**	35.70	**27**	27.71
7	32.09	**14**	56.51	**21**	18.72	**28**	19.82

Table 2: ^{13}C NMR data for compound 1.

Compound 1 was acetylated using acetic anhydride (see **Experimental** page 23). In the ^{1}H NMR spectrum of the product it was clear that the signal attributed to H_3 had moved downfield, from its position at 3.56 ppm, to 4.66 ppm due to now being near the electron-withdrawing acetyl group. In the ^{13}C NMR spectrum two observations were made. C_3 had moved slightly downfield from 71.38 ppm to 73.80 ppm and a carbonyl signal was clearly evident at 170.74 ppm due to the carbonyl of acetyl group. In the adept spectrum it was also clear that an extra methyl group was now present at 21.49 ppm, due to the methyl group carbon of the acetyl group.

Foreword to experimental

Nuclear magnetic resonance spectroscopy (NMR)

Spectra were recorded with a 300 MHz Varian Gemini spectrometer and a Varian Unity Inova 400 MHz spectrometer using deuterated chloroform as solvent. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz on the 300 MHz spectrometer. The instrument used for each spectrum is indicated in the text. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz on the 400 MHz spectrometer. ^{1}H NMR spectra were referenced against the deuteriochloroform singlet at δ_H 7.24 ppm. ^{13}C NMR spectra were referenced against the central line of the deuteriochloroform signal at δ_C 77.0 ppm.

Infrared spectroscopy (IR)

Infrared spectra were recorded on a Nicolet Impact 400 D spectrometer, which was calibrated against an air background. Spectra for all compounds were recorded as thin films using dichloromethane as solvent.

Melting points (m.p.)

Melting points were determined on a Kofler micro-hot stage melting point apparatus and are uncorrected.

Thin layer chromatography (TLC)

Silica gel (0.2 mm) containing fluorescent indicator (F_{254}) on aluminium backed plates (Merck: Art 5554) was used for thin layer chromatography analysis, to monitor the column chromatographic process. The plates were first examined under UV light and then developed either in iodine or using anisaldehyde spray reagent (anisaldehyde : conc. sulphuric acid : methanol [1:2:97]) followed by heating.

Column chromatography (normal phase)

Silica gel (Merck Art: 9385) was used as the solid phase for gravity column chromatography and elution was allowed to proceed by gravity. Solvent systems used are indicated in the text.

Reverse phase chromatography

C_{18} reverse phase cartridges (Isolute SPE) were used. Elution was allowed to proceed under gravity, but was often too slow and a pump had to be used.

Mass spectrometry

The GC/MS spectrum was recorded using a Finnigan 1020 GC/MS spectrometer (HRMS) at the Cape Technikon.

Optical rotations

Optical rotation was measured at room temperature in dichloromethane, using an Optical Activity AA-5 polarimeter together with a series A2 stainless steel (4×200 mm) unjacketed flow tube. Concentrations are expressed in g/100 ml.

Experimental

The sponge specimen, *Oceanapia sp.*, was collected from Aliwal Shoal off the South Coast of KwaZulu Natal by Dr. Michael Schleyer of the Oceanographic Research Institute (ORI) in Durban. The species is unnamed, although the genus has been assigned. A voucher specimen is held by Dr. Schleyer.

The sponge was stored frozen until used. Thereafter, a portion of the organism (585g) was cut into small pieces and subjected to a methanol extraction on a shaker for 24 hours. The methanol was filtered and evaporated to yield the first extract. The sponge was subjected to a second methanol extraction for 24 hours. Thereafter, the methanol was filtered and evaporated to yield a second extract. ^{1}H NMR spectra of both extracts were obtained and compared. Being very similar, they were combined. The combined extract was washed, successively, with hexane and ethyl acetate.

The hexane and ethyl acetate extracts were subjected to normal phase column chromatography (see **Foreword to Experimental** page 19) using a variety of solvent systems. These included combinations of hexane, ethyl acetate, dichloromethane and methanol. One compound was isolated from the hexane extract, (24R)-24,25-methylene-5α-cholestan-3-ol.

The aqueous/methanol extract was subjected to reverse phase chromatography (see **Foreword to Experimental** page 20). The solvent system used was a water: methanol step gradient (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) and six crude fractions were collected. A number of interesting signals were present in the crude spectra of these extracts. However, their quantities were not sufficient to allow for purification.

Physical data for compound 1

Compound name: (24R)-24,25-methylene-5α-cholestan-3β-ol

Yield: 235 mg

Melting point: 116-118°C (lit. 117-119°C)

Optical rotation: $[\alpha]_D$ +10° (c = 0.207, CH_2Cl_2)

HRMS *m/z* (%) : 400.37159 [M^+] (Calc. 400.37050 $C_{28}H_{48}O$), 385.34497 (14.08), 316.27938 (17.50), 302.26091 (15.32), 301.25376 (16.17), 273.22146 (100.00), 233.19012 (17.39), 215.17934 (24.74), 147.11677 (18.09), 121.10217 (25.63), 107.08638 (43.49), 95.08529 (49.84), 55.05461 (59.95).

^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm) : 3.56 (1H, m, H_3), 0.99 (3H, s, H_{26}), 0.98 (3H, s, H_{27}), 0.87 (3H, d, *J*= 6.5 Hz, H_{21}), 0.77 (3H, s, H_{19}), 0.62 (3H, s, H_{18}), 0.38 (1H, m, H_{24}), 0.36 (1H, m, $H_{28\beta}$), -0.18 (1H, m, $H_{28\alpha}$).

^{13}C NMR (100 MHz, $CDCl_3$, δ/ppm) : 71.38 (CH_2, C_3), 56.51 (CH, C_{14}), 56.26 (CH, C_{17}), 54.37 (CH, C_9), 44.87 (CH, C_5), 42.61 (C, C_{13}), 40.04 (CH_2, C_{12}), 38.20 (CH_2, C_4), 37.01 (CH_2, C_1), 36.48 (CH_2, C_{22}), 35.70 (CH, C_{20}), 35.52 (CH, C_8), 35.46 (C, C_{10}), 32.09 (CH_2, C_7), 31.51 (CH_2, C_2), 28.74 (CH_2, C_6), 28.27 (CH_2, C_{16}), 27.71 (CH_3, C_{27}), 26.49 (CH_2, C_{23}), 25.04 (CH, C_{24}), 24.23 (CH_2, C_{15}), 21.26 (CH_2, C_{11}), 19.90 (CH_3, C_{26}), 19.82 (CH_2, C_{28}), 18.72 (CH_3, C_{21}), 15.18 (C, C_{25}), 12.32 (CH_3, C_{19}), 12.05 (CH_3, C_{18}).

IR (CH_2Cl_2, ν_{max} /cm^{-1}) : 3360 (w), 2944 (s), 2851 (w), 1457 (s), 1373 (w), 1040 (w).

Acetylation of compound 1 (compound 2)

To compound 1 (35 mg) was added distilled acetic anhydride ($1cm^3$) and pyridine ($2cm^3$) (dried over sodium hydroxide). The vial was sealed and left to stand on top of an oven (approximately 60°C) overnight. Methanol ($10cm^3$) was added, followed by toluene ($5 \times 20\ cm^3$) to remove the pyridine. After each addition, the solvent was removed under vacuum. A further portion of methanol ($10cm^3$) was added to remove traces of toluene.

Physical data for compound 2

Name: (24R)-24,25-methylene-5α-cholestanyl 3-acetate

Yield: 28 mg (80%)

^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm) : 4.66 (1H, m, H_3), 1.99 (3H, $COCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$, δ/ppm) : 170.74 ($COCH_3$), 21.49 ($COCH_3$).

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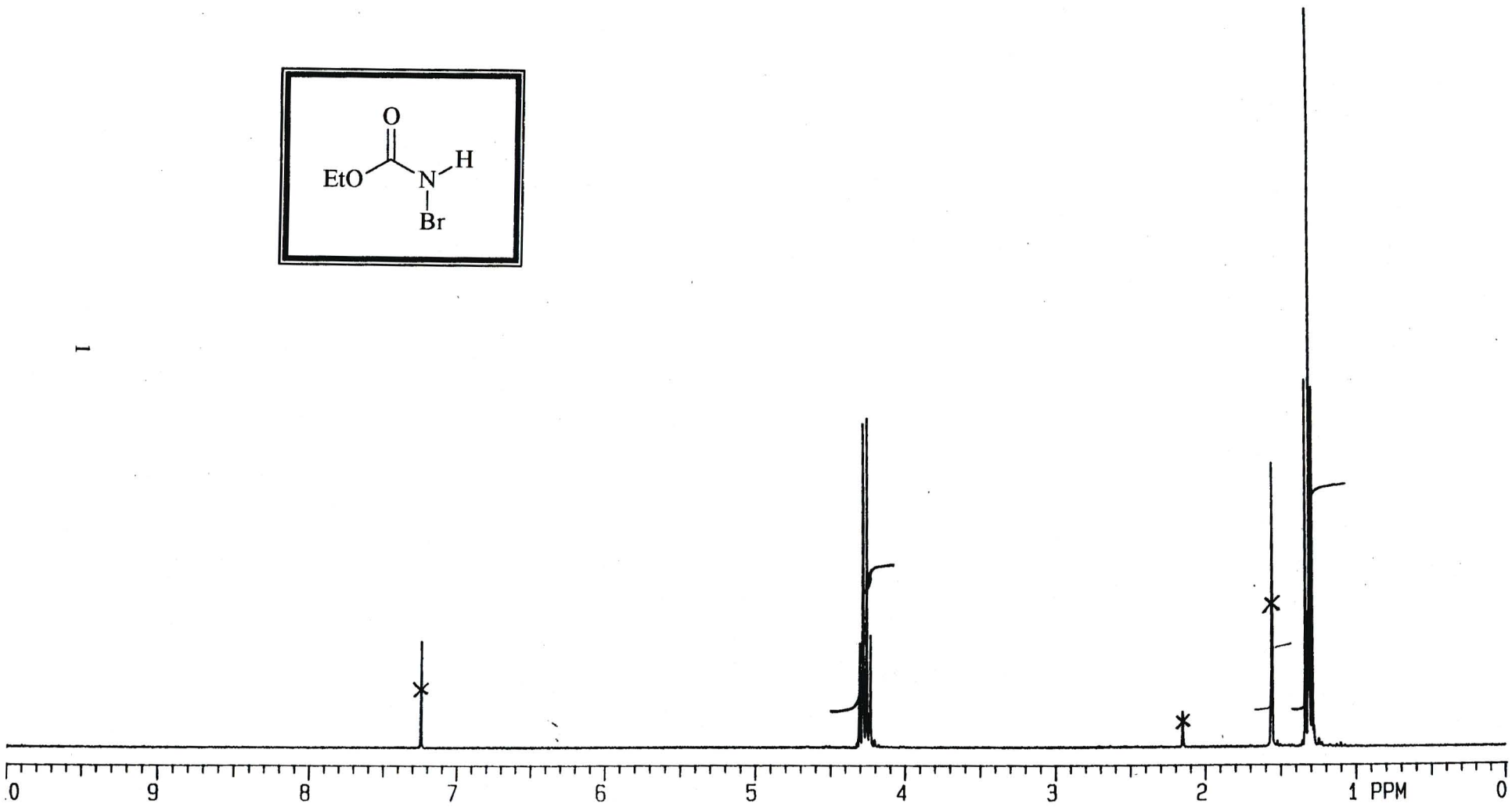
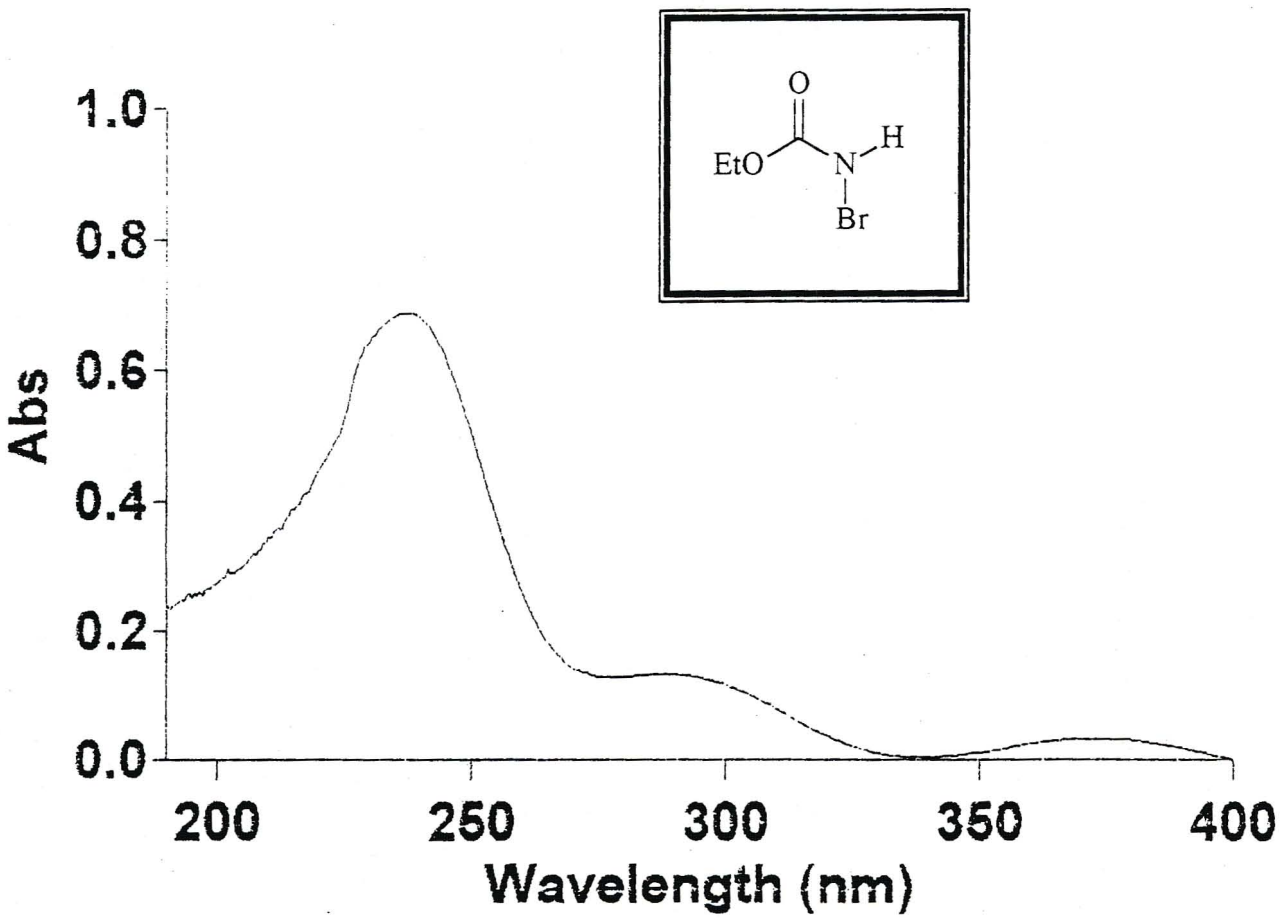
Appendices

Appendix A

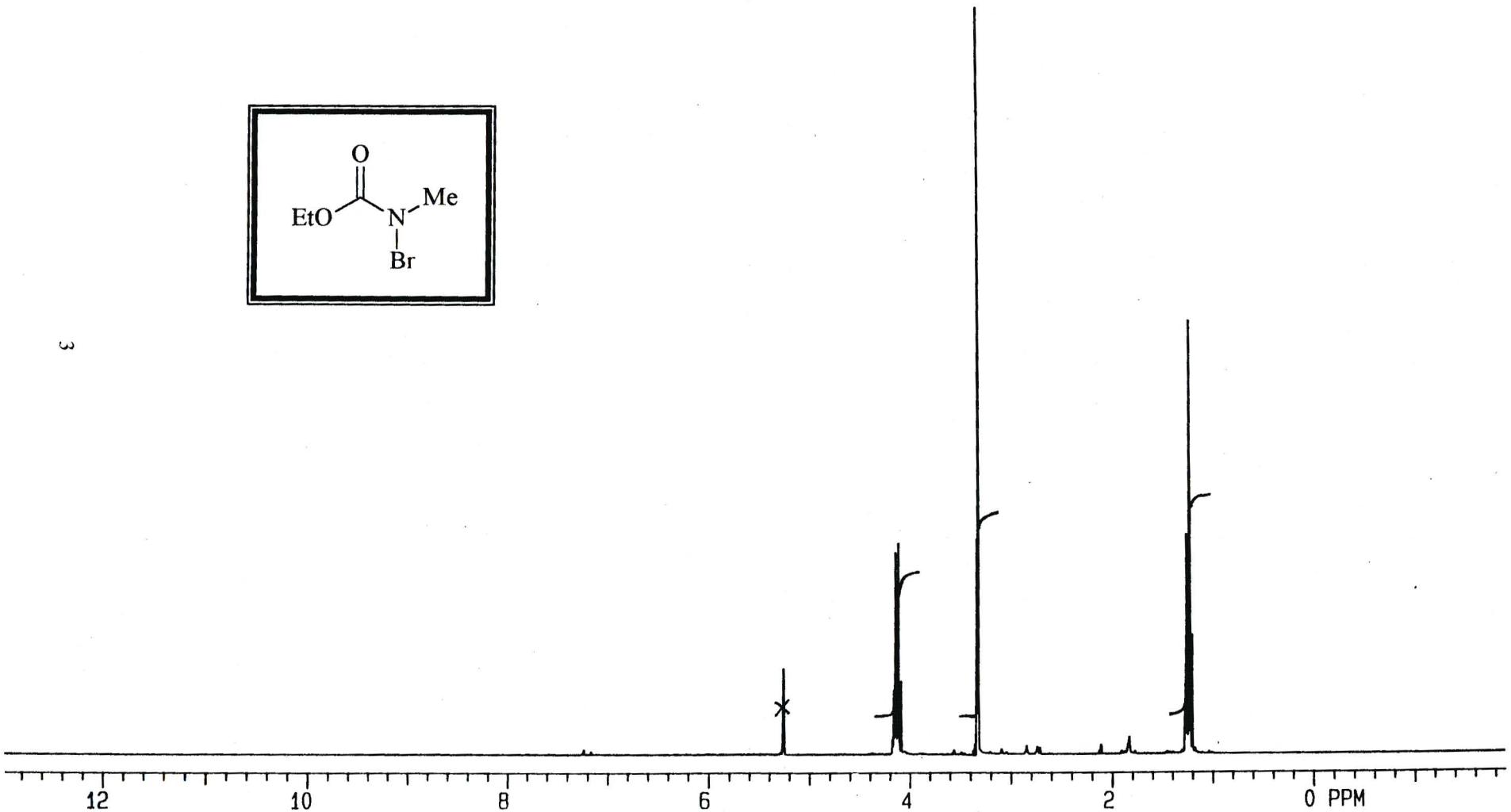
List of spectra (chapter 1)



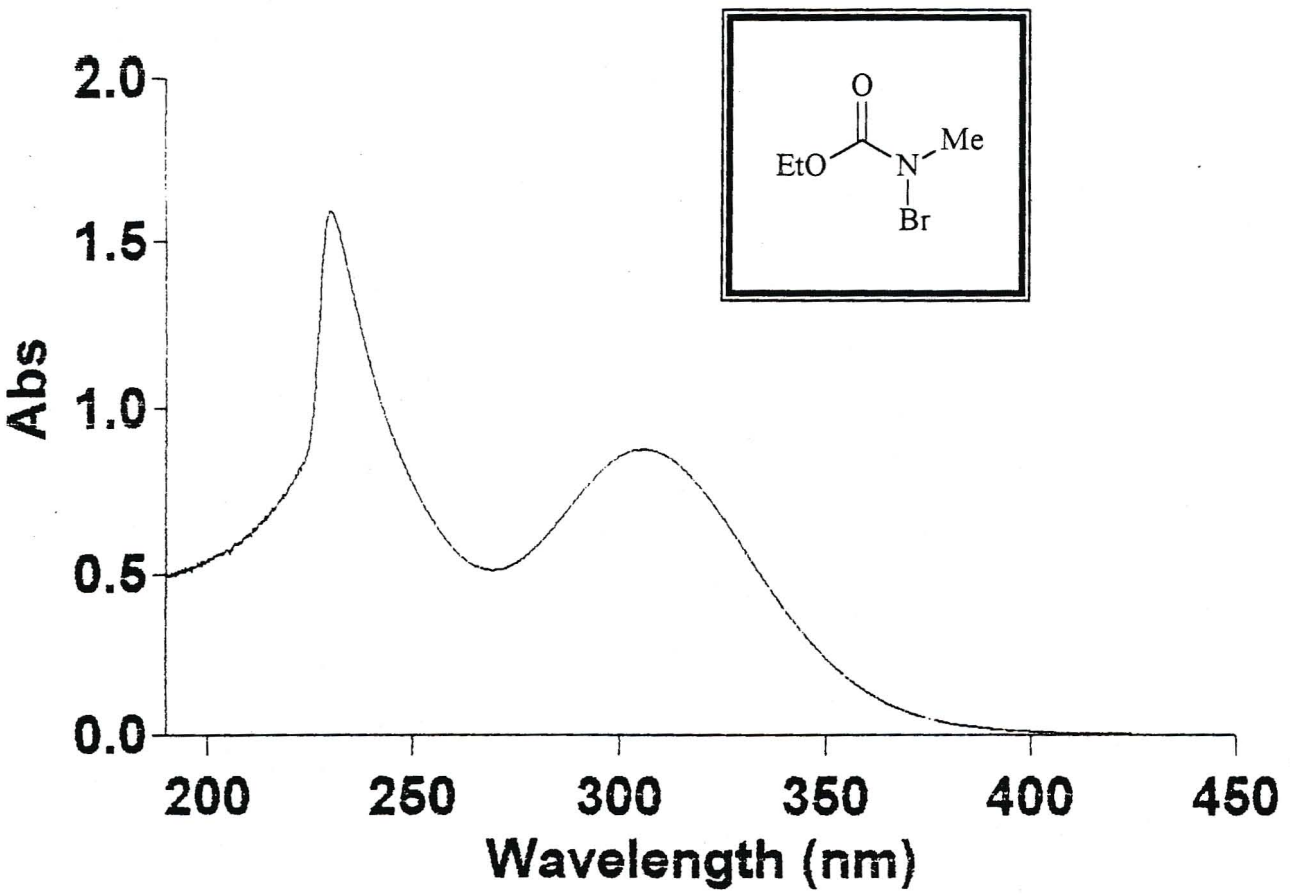
1.1 ^{1}H NMR spectrum of **ethyl N-bromocarbamate**



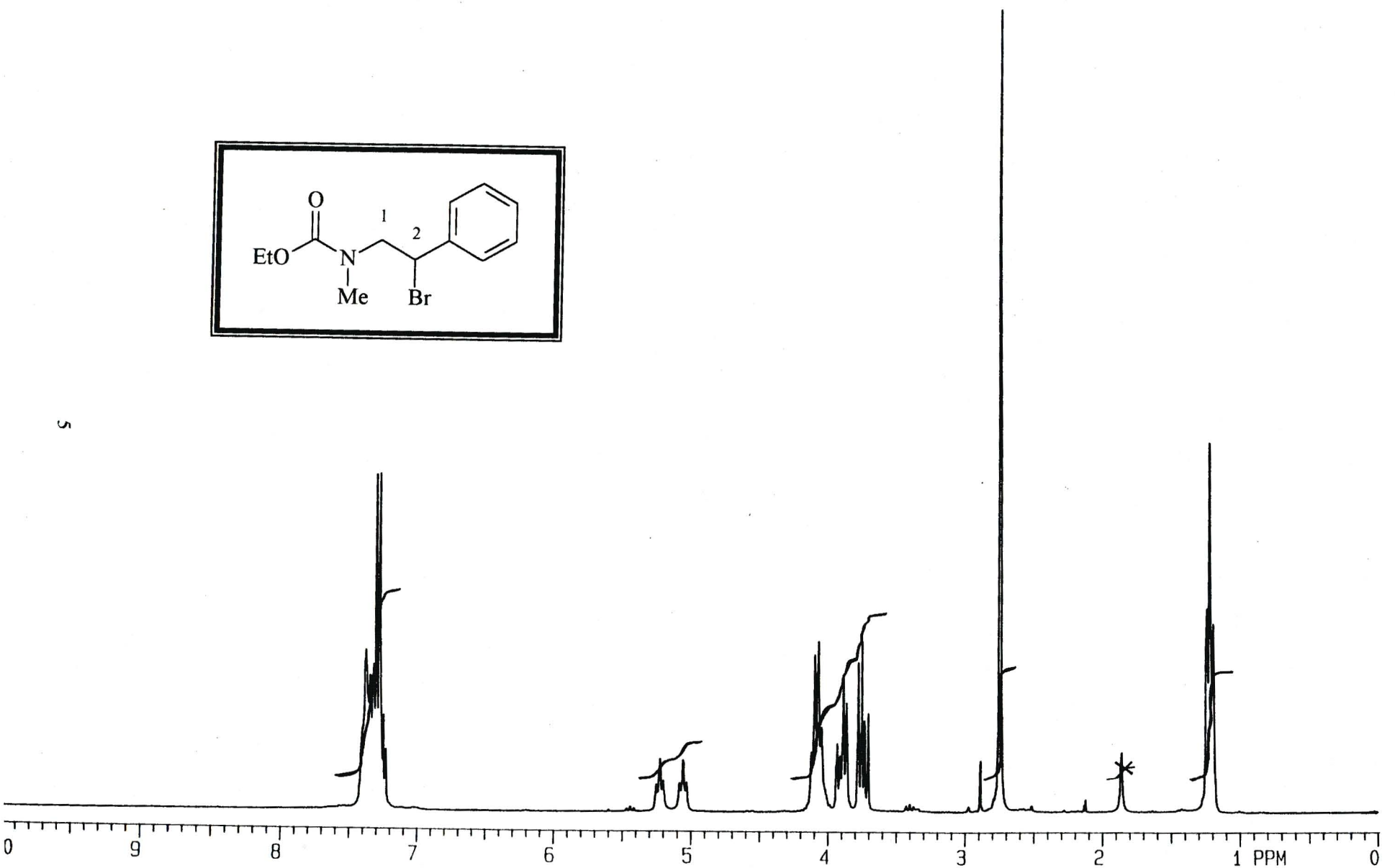
1.1 Ultraviolet spectrum of **ethyl N-bromocarbamate**



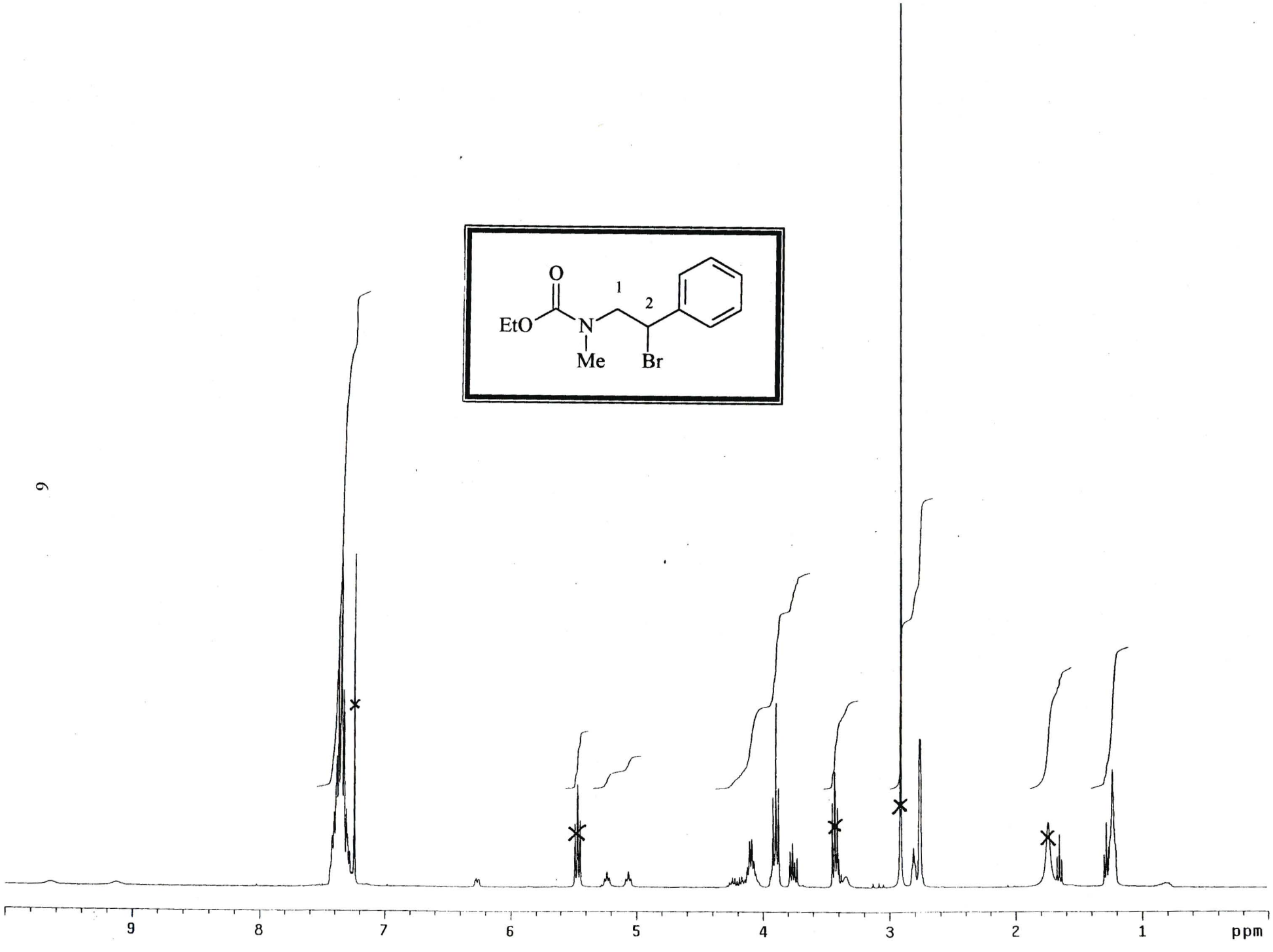
1.2 ^{1}H NMR spectrum of **ethyl N-bromo-N-methylcarbamate**



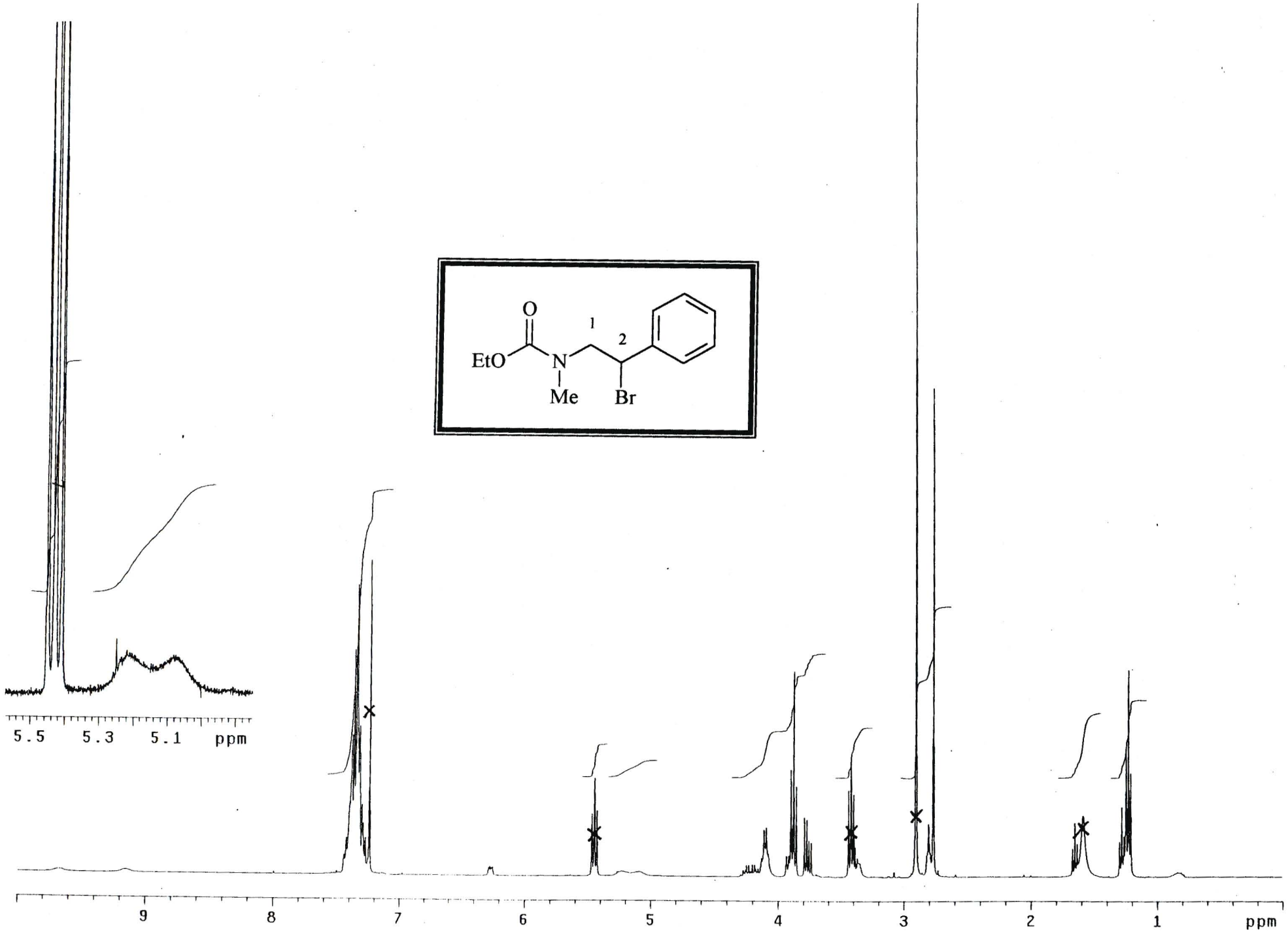
1.2 Ultraviolet spectrum of **ethyl N-bromo-N-methylcarbamate**



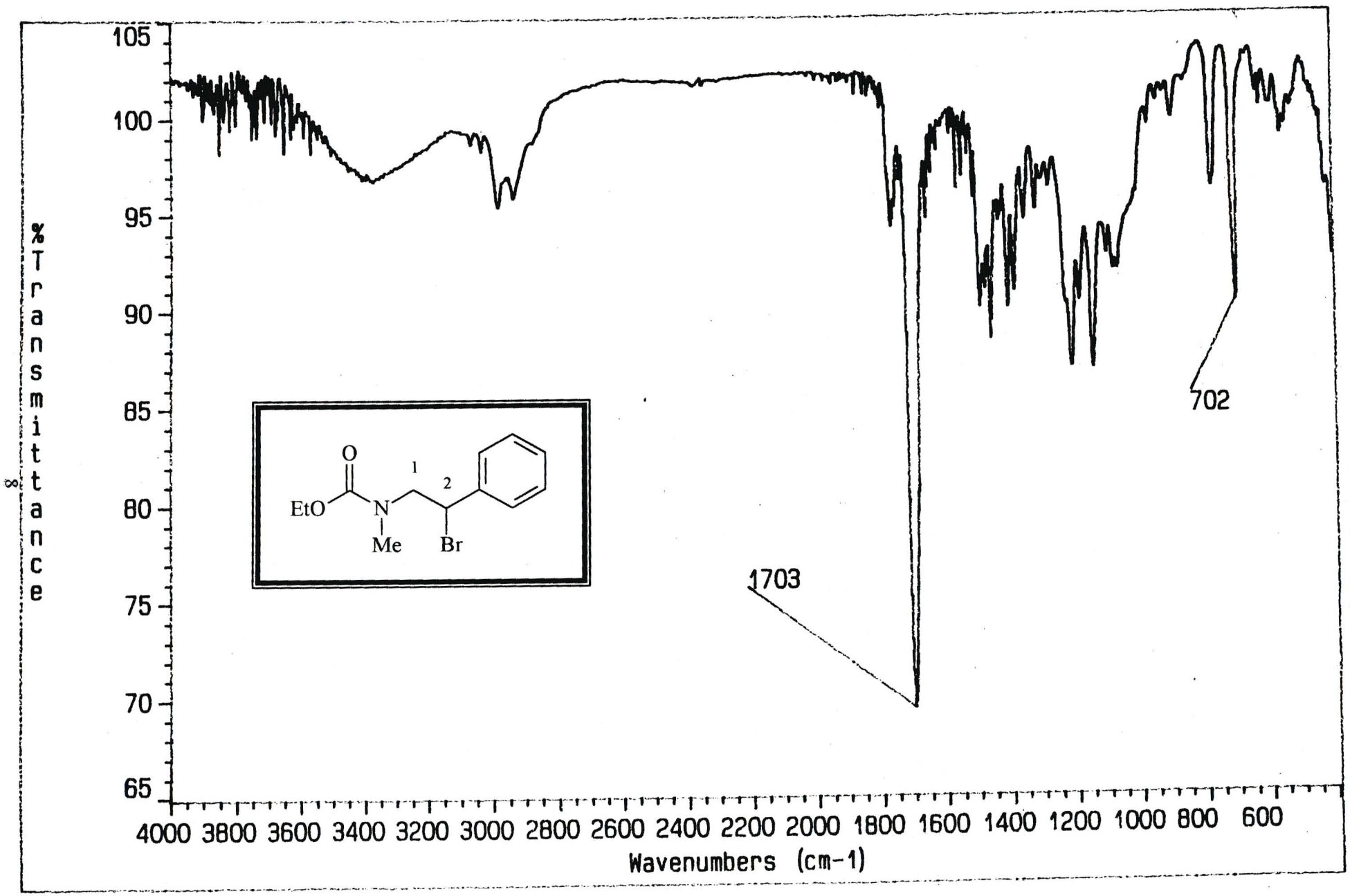
2.1 ^{1}H NMR spectrum of **ethyl N-2-bromo-2-phenylethylcarbamate**



2.1 ^{1}H NMR spectrum of **ethyl N-2-bromo-2-phenylethylcarbamate (P**



2.1 ^{1}H NMR spectrum of **ethyl N-2-bromo-2-phenylethylcarbamate** (50°C)



2.1 Infrared spectrum of **ethyl N-2-bromo-2-phenylethylcarbamate**

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File Source : Acquired on MASPEC II system [II32/A002]
File Title : STYRENE ADDUCT
Operator : Dr. P. Boshoff
Instrument : VG70-SEQ

SCAN GRAPH. Flagging=High Resolution M/z. Filter=[Excl: Ref/Ex.].Highlighting=Base Peak.
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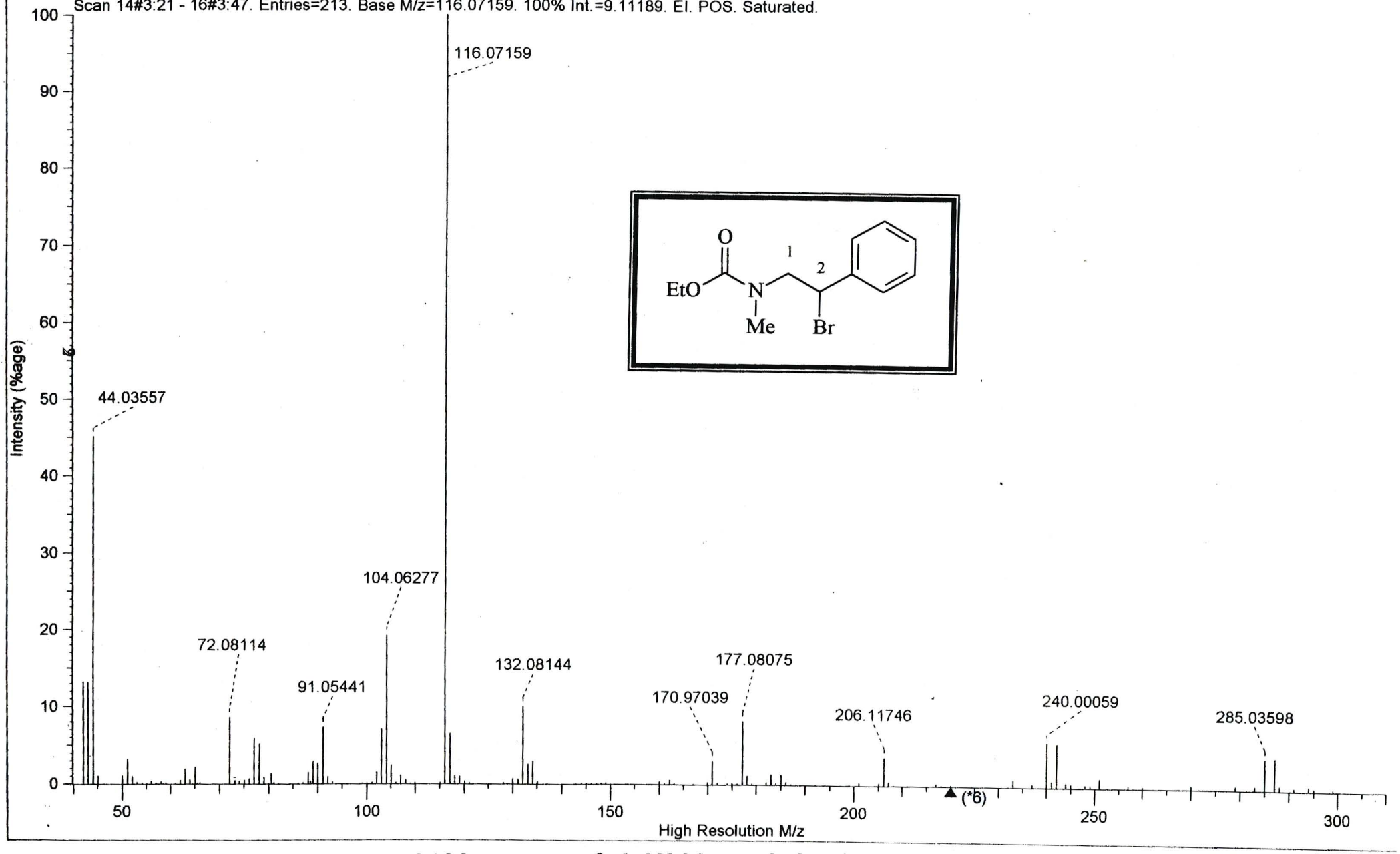
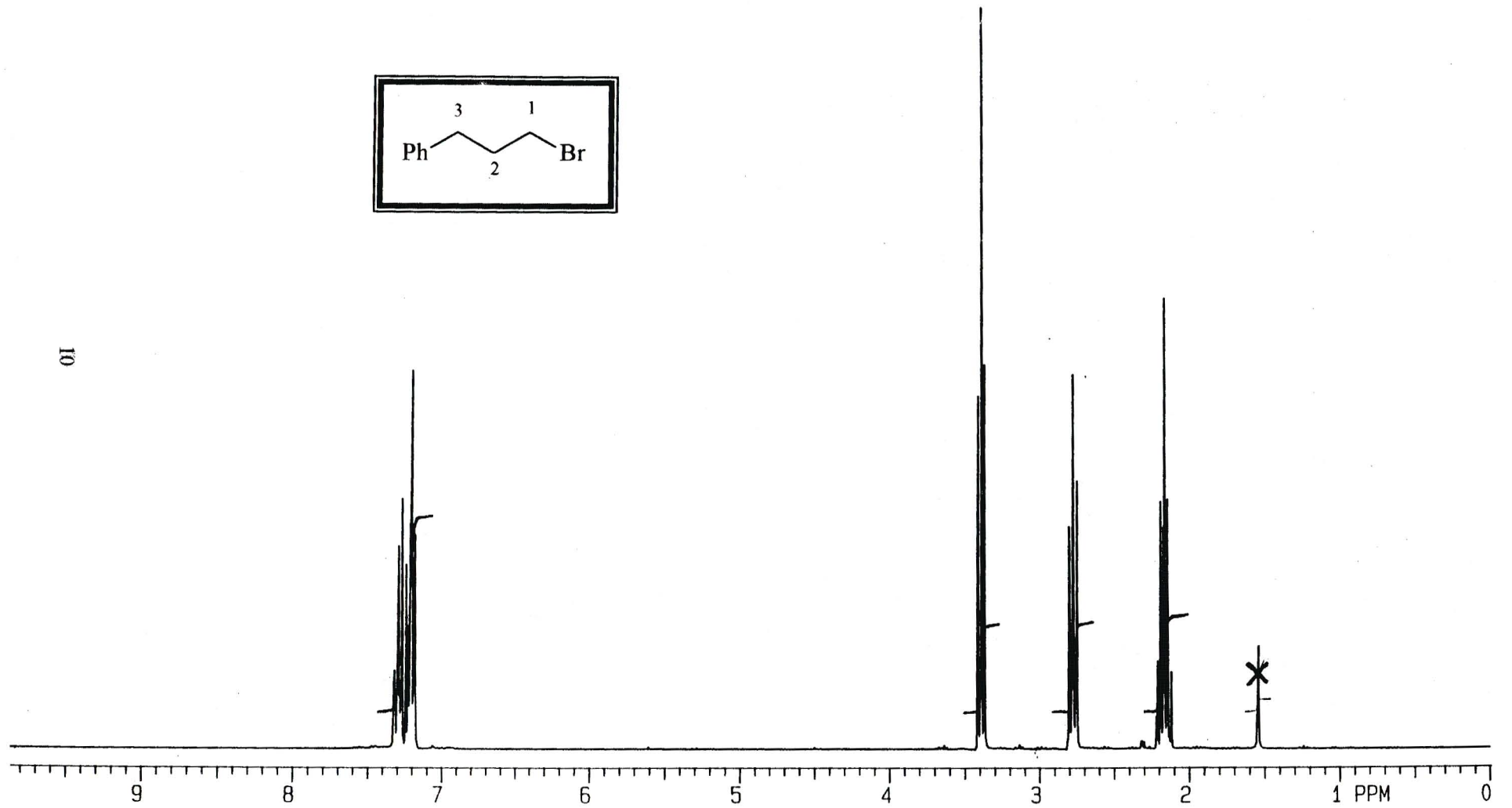
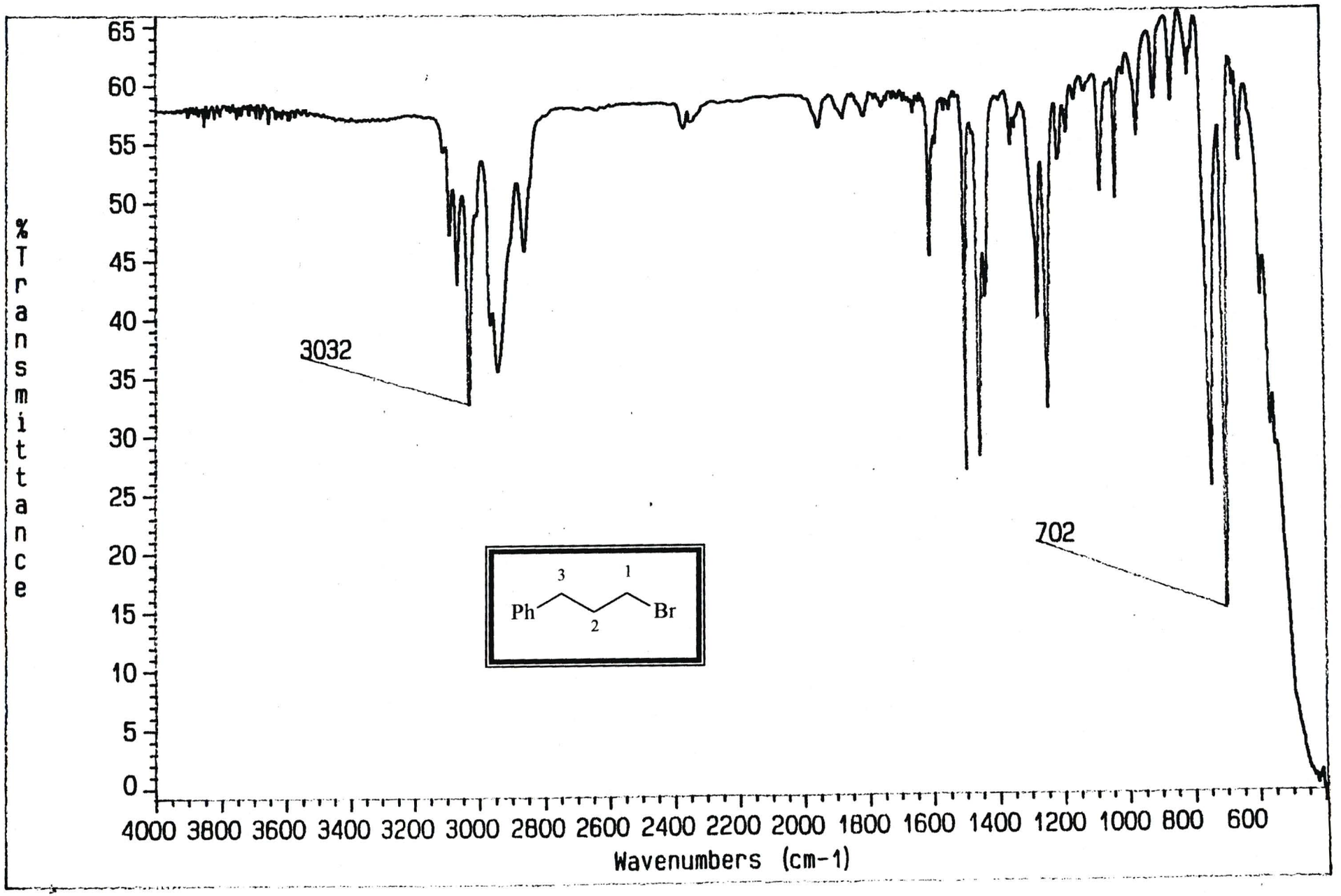


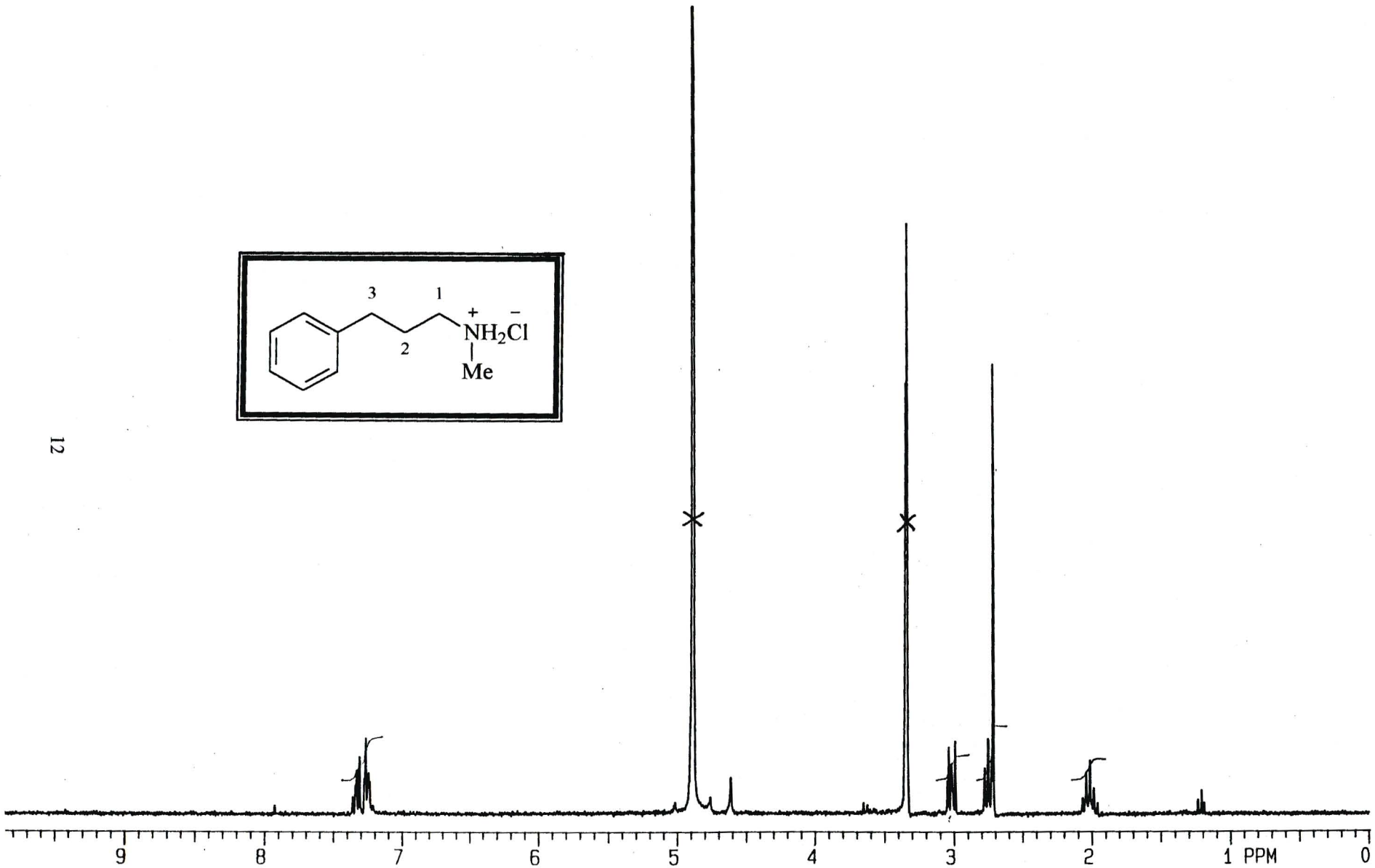
2.1 Mass spectrum of **ethyl N-2-bromo-2-phenylethylcarbamate**



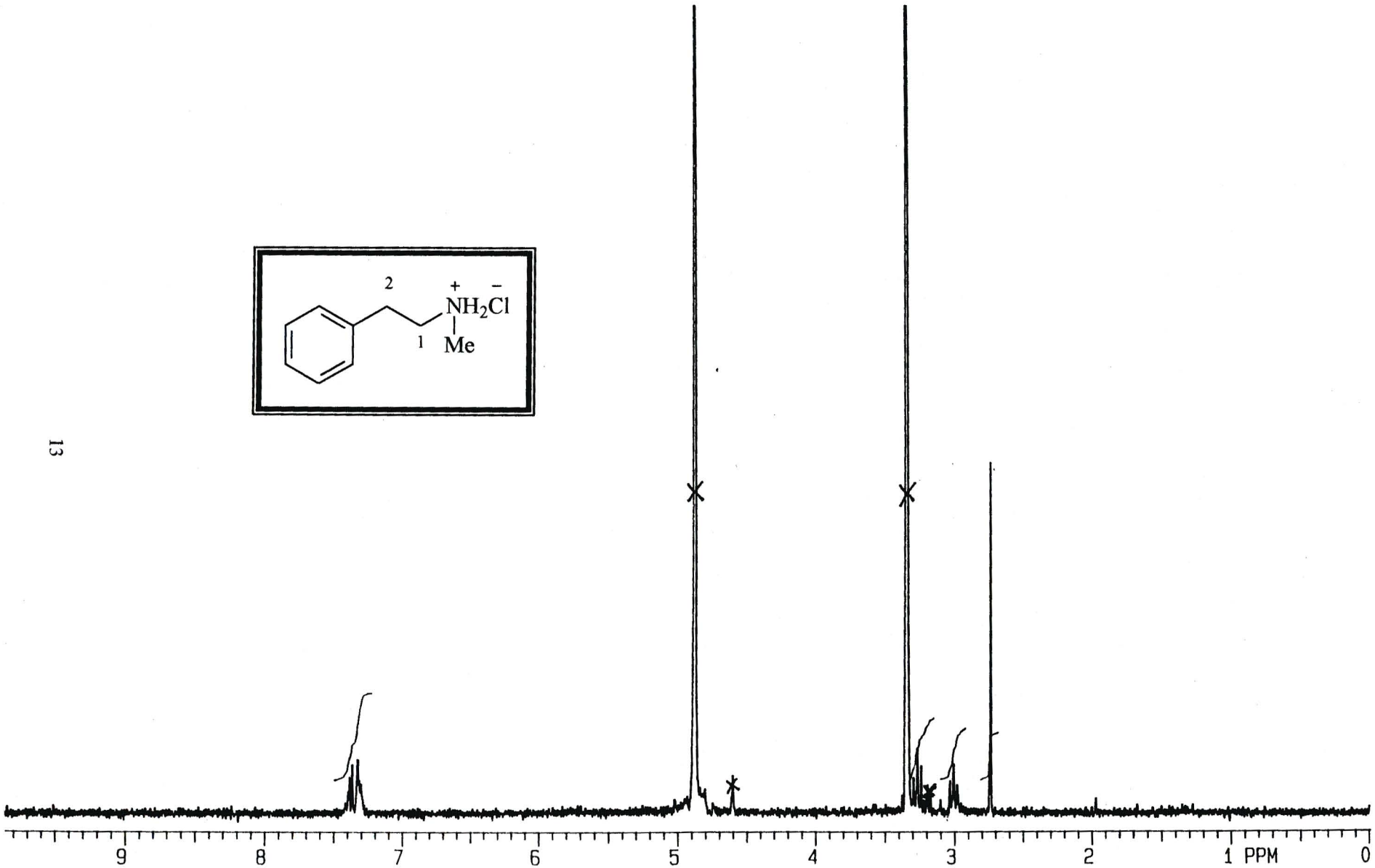
3.1 ^{1}H NMR spectrum of **3-phenylpropyl bromide**



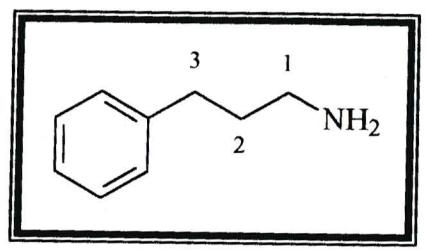
3.1 Infrared spectrum of **3-phenylpropyl bromide**

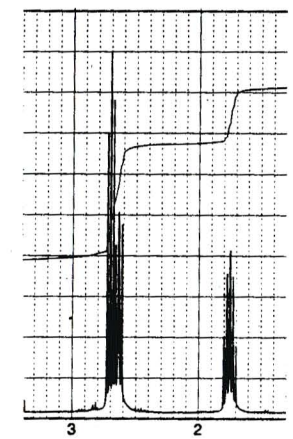


3.2.1 ^{1}H NMR spectrum of **N-methyl-3-phenylpropylamine hydrochloride**



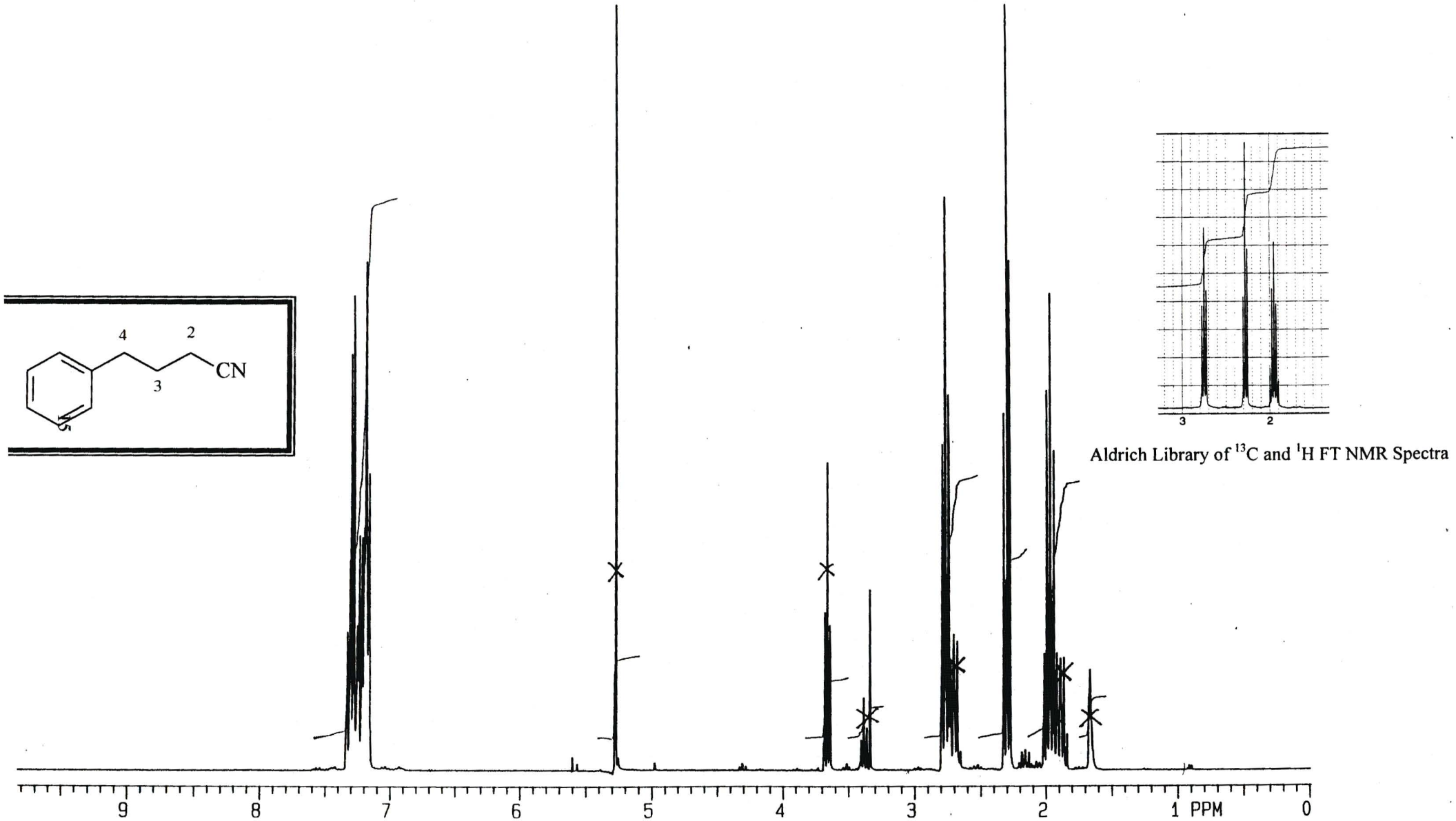
3.2.2 ^{1}H NMR spectrum of **N-methyl-2-phenylethylamine hydrochloride**





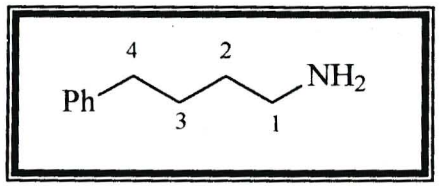
Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra

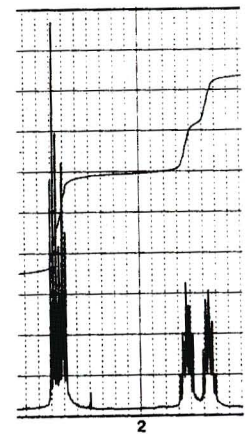
3.2.3 ^{1}H NMR spectrum of **3-phenylpropylamine**



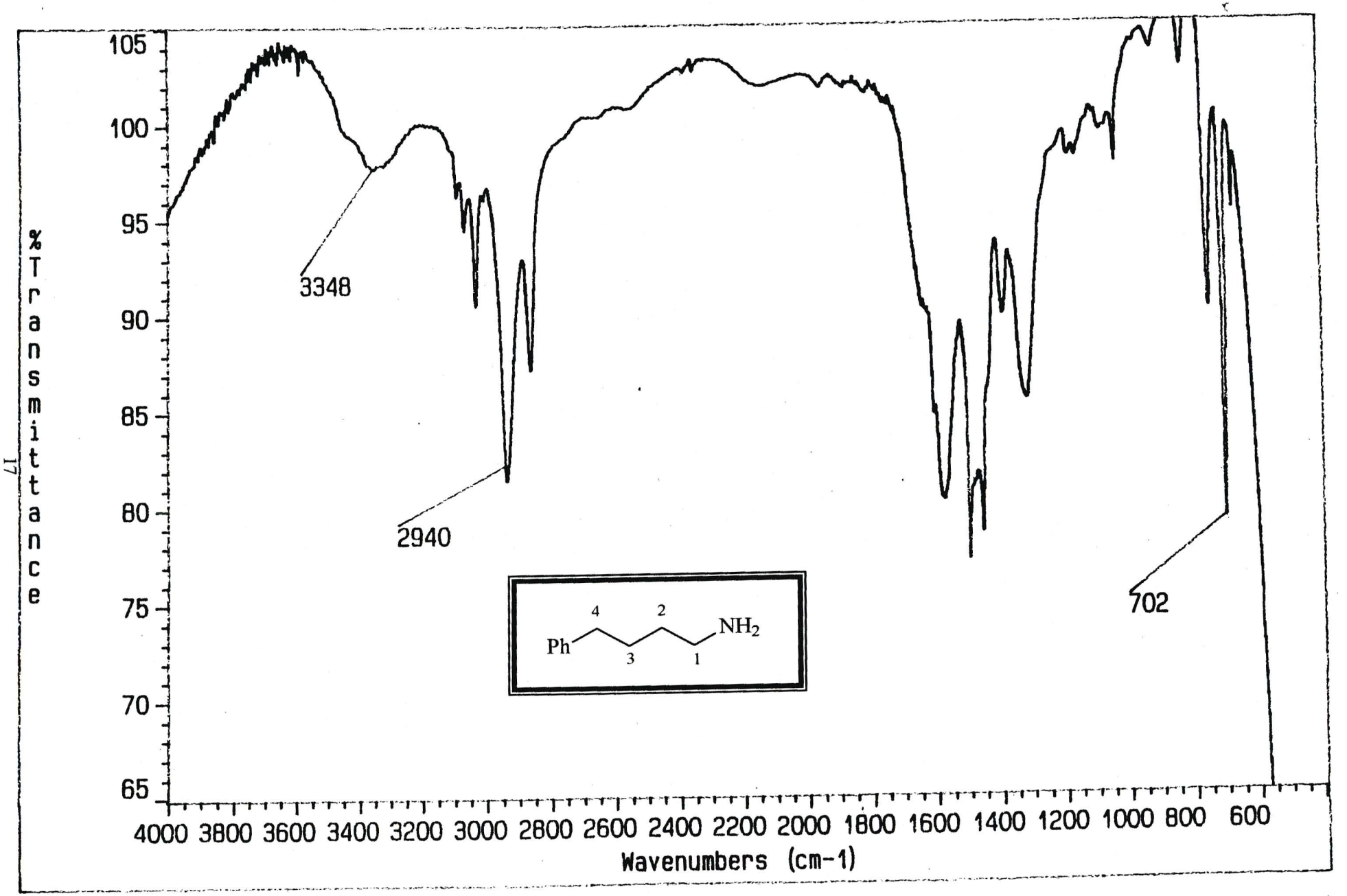
Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra

3.3 ^{1}H NMR spectrum of **4-phenylpropyl cyanide**

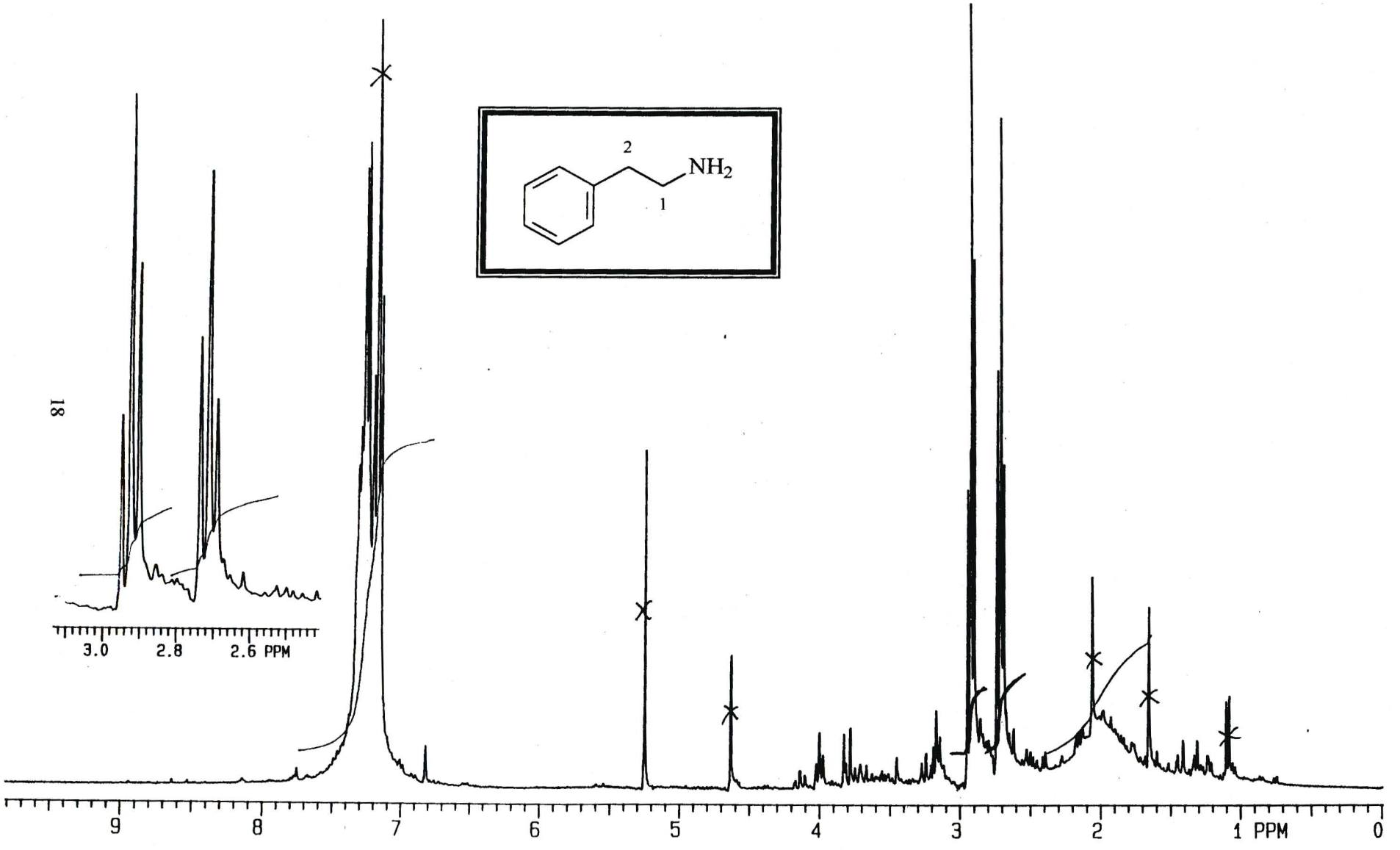




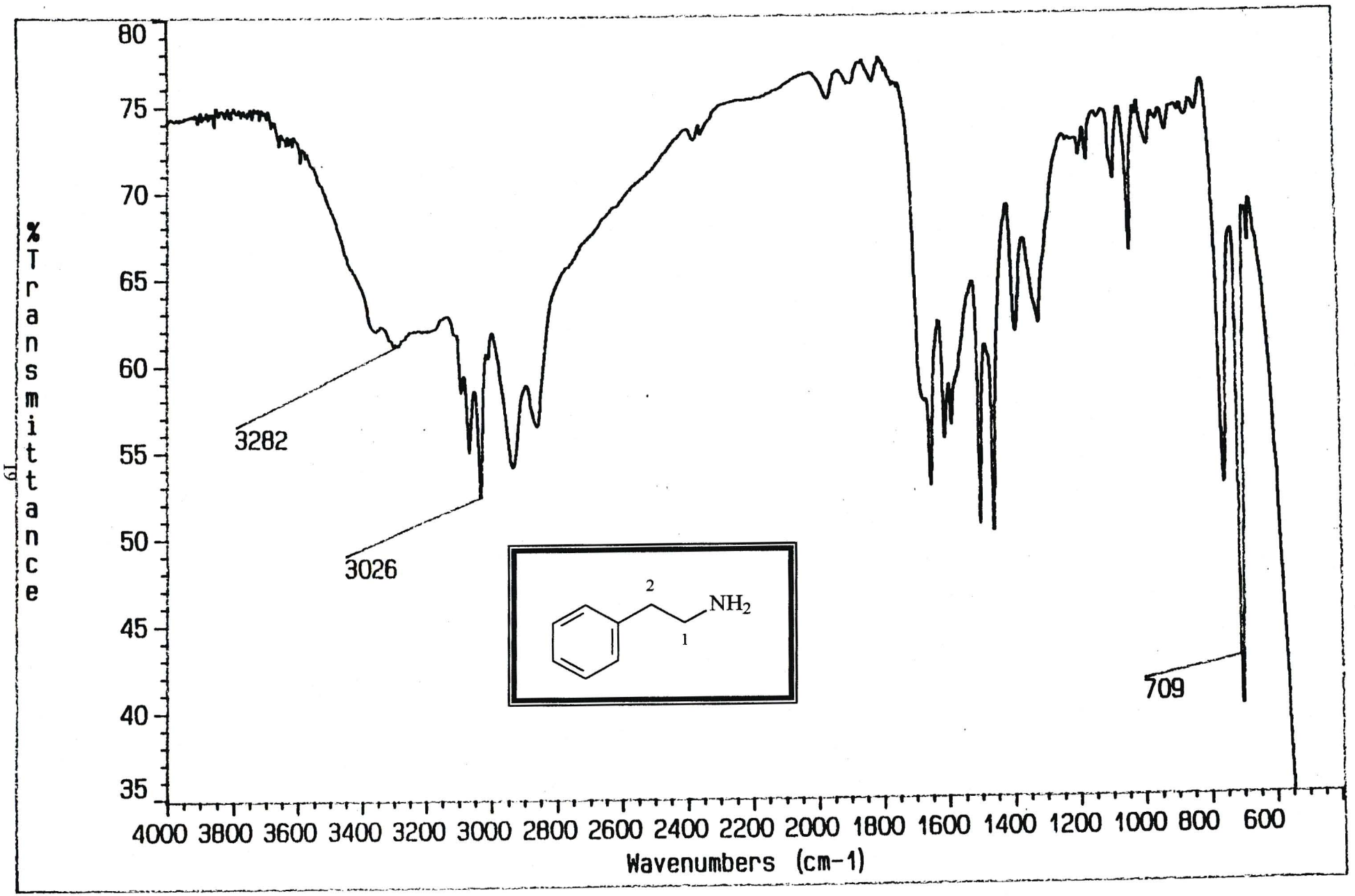
3.3 ^{1}H NMR spectrum of **4-phenylbutylamine**



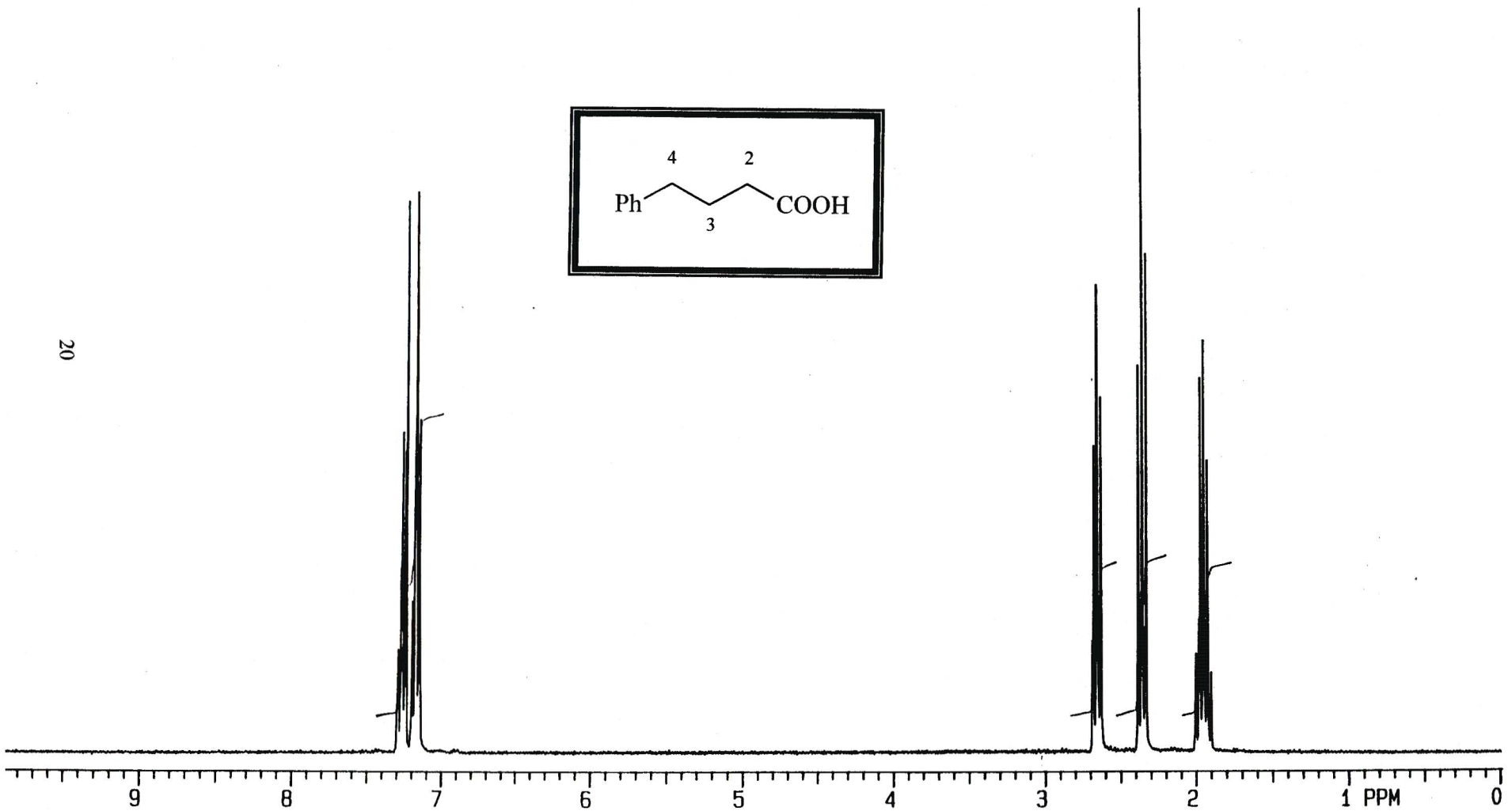
3.3 Infrared spectrum of **4-phenylbutylamine**



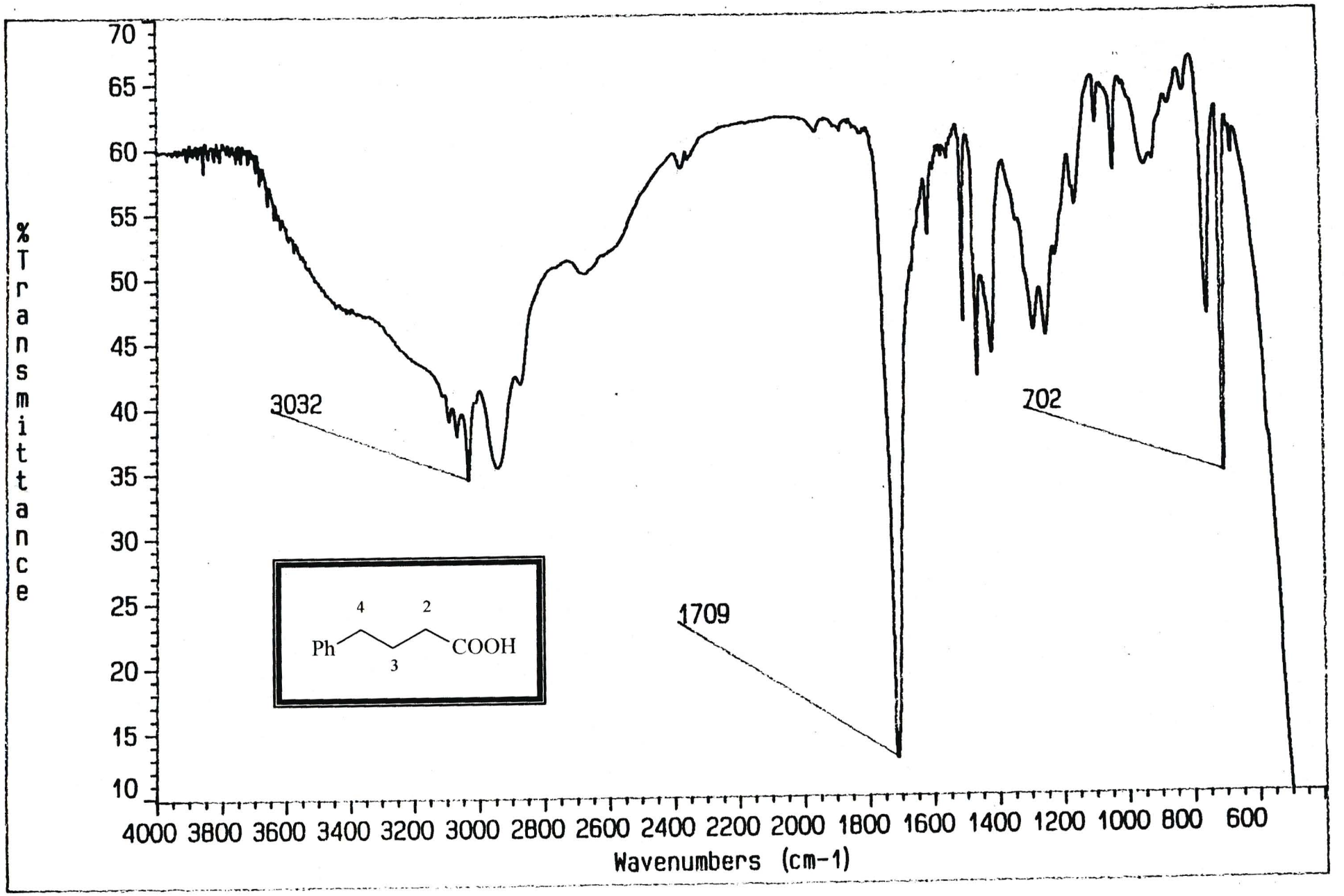
3.4 ^{1}H NMR spectrum of **2-phenylethylamine**



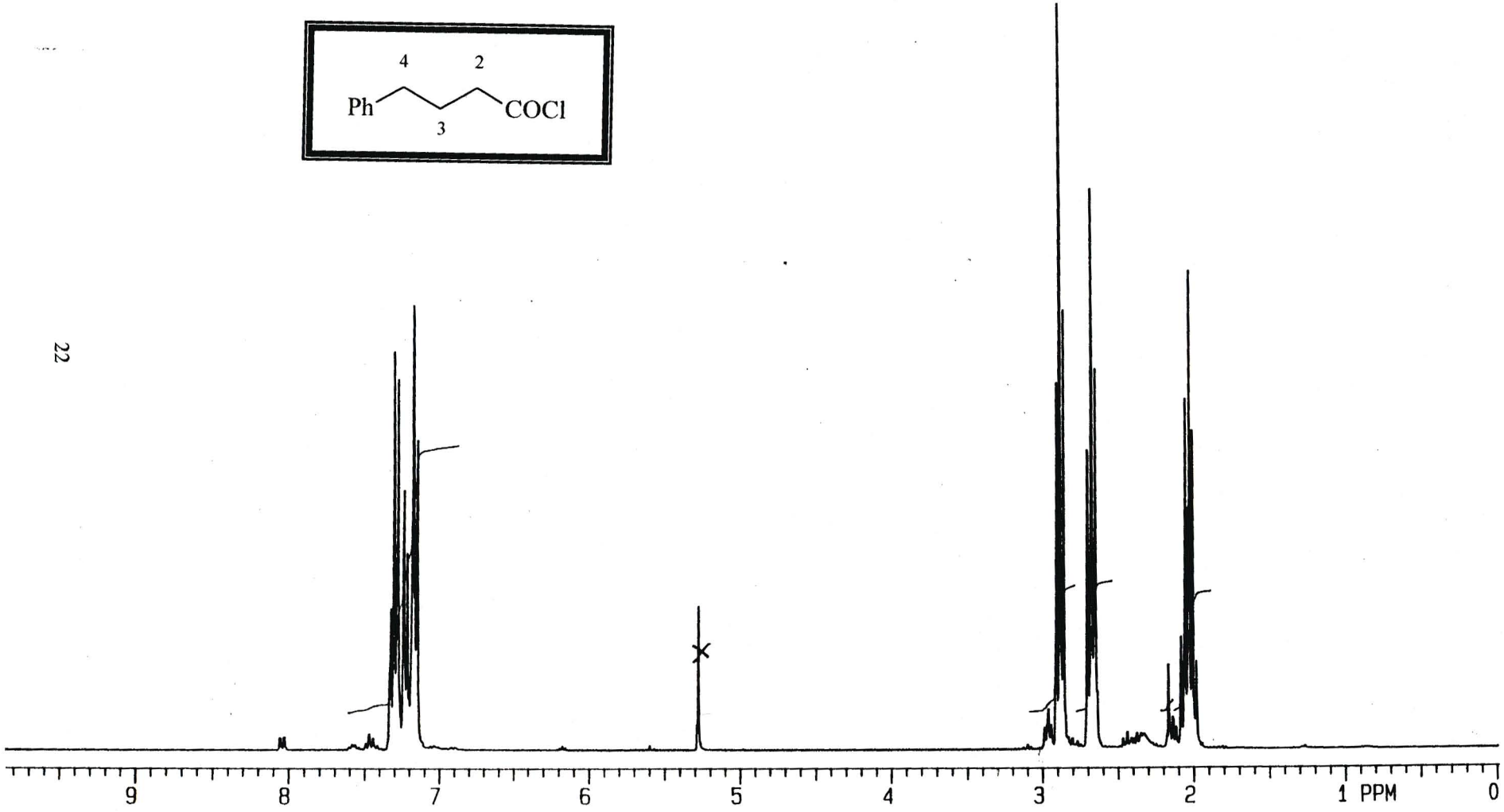
3.4 Infrared spectrum of **2-phenylethylamine**



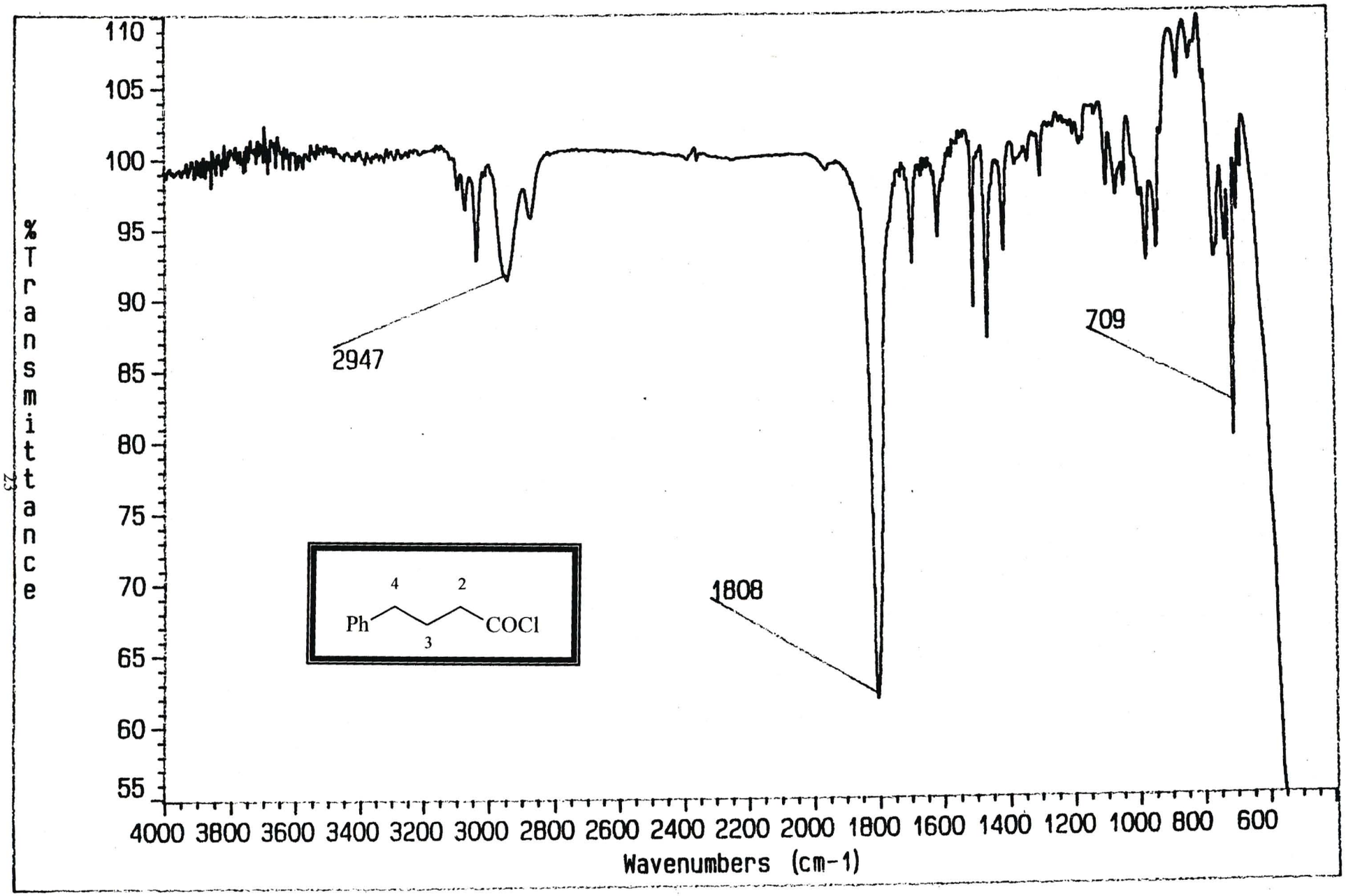
3.5 ^{1}H NMR spectrum of **4-phenylbutanoic acid**



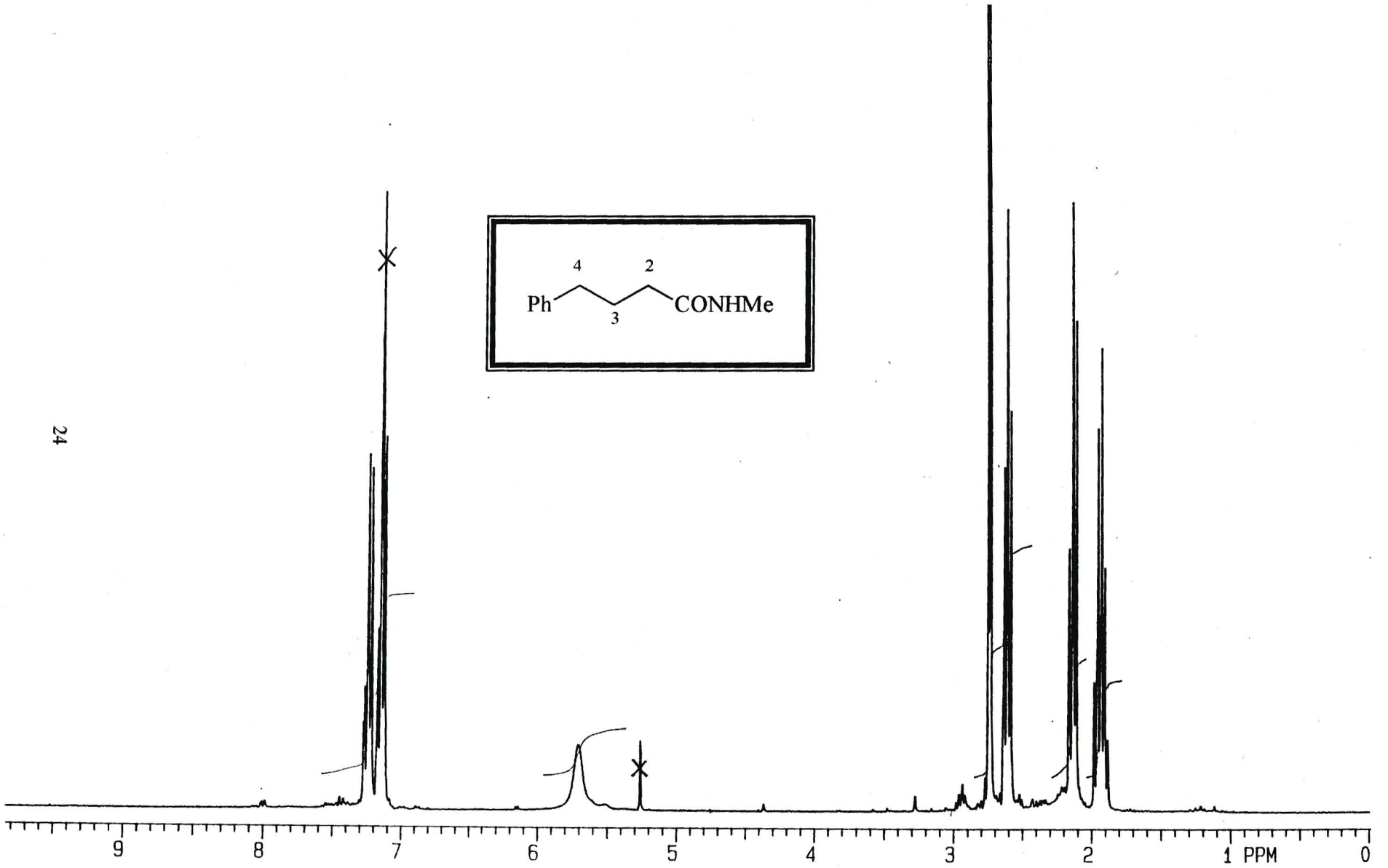
3.5 Infrared spectrum of **4-phenylbutanoic acid**



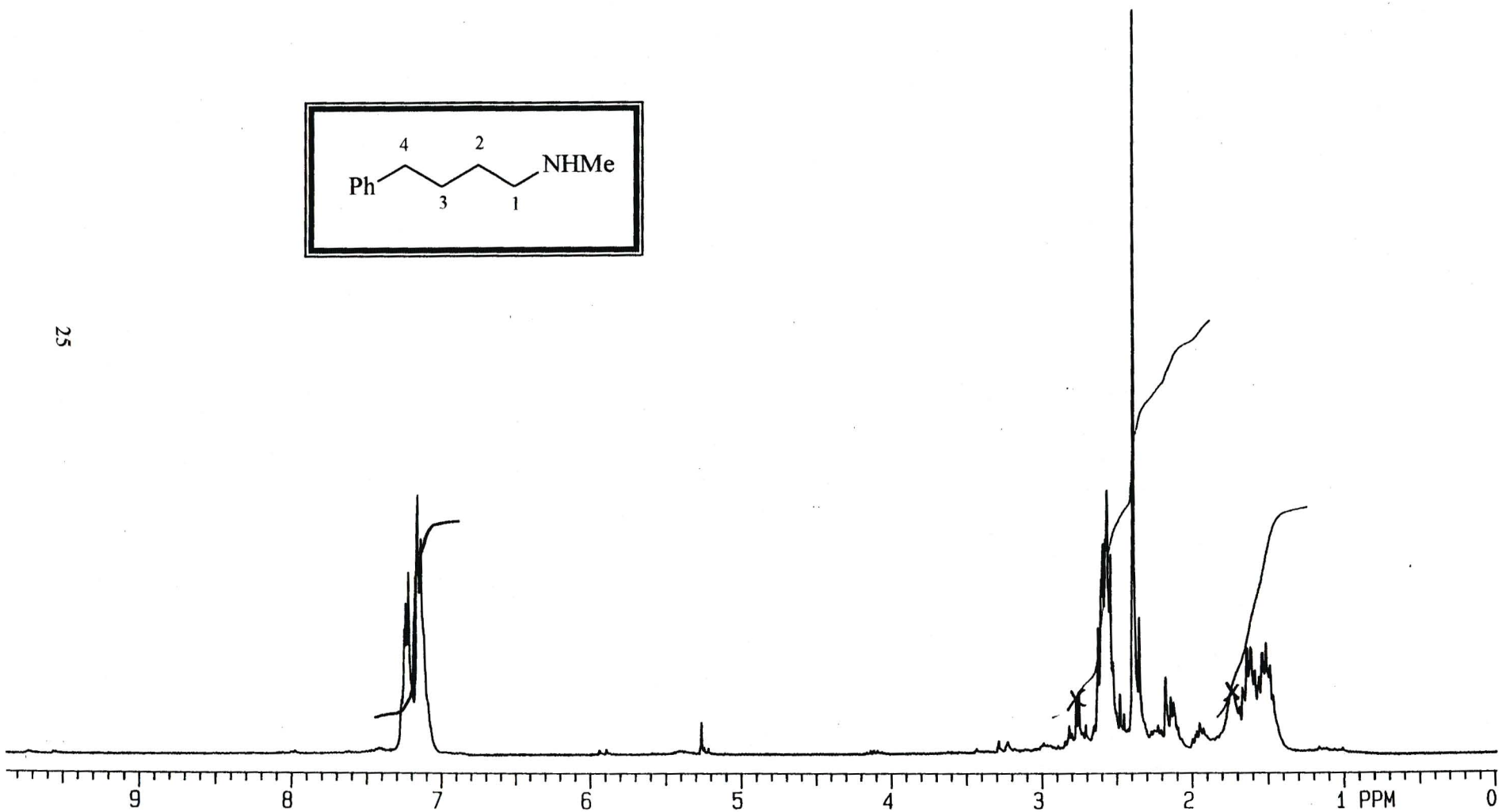
3.6 ^{1}H NMR spectrum of **4-phenylbutanoyl chloride**



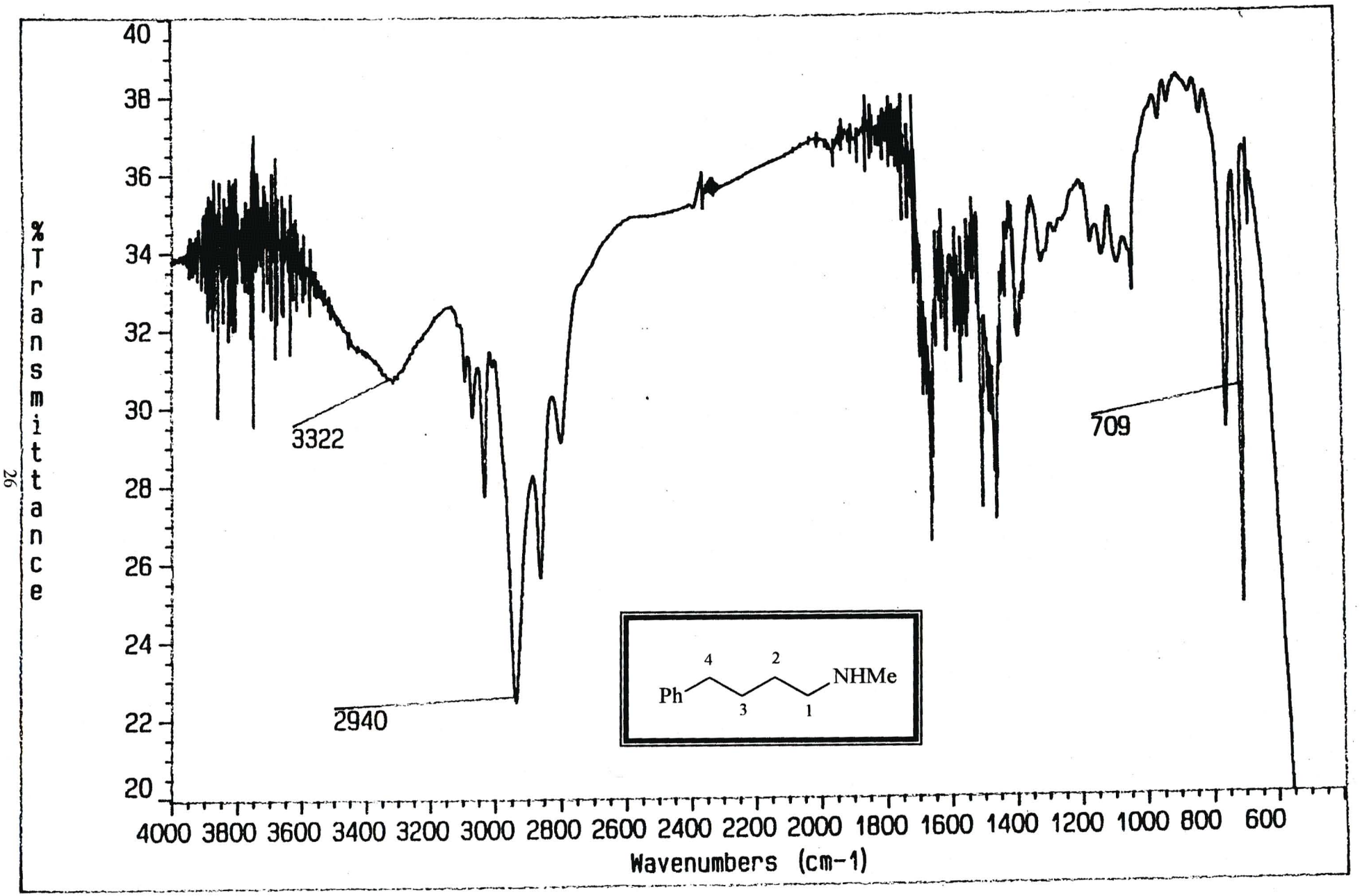
3.6 Infrared spectrum of **4-phenylbutanoyl chloride**



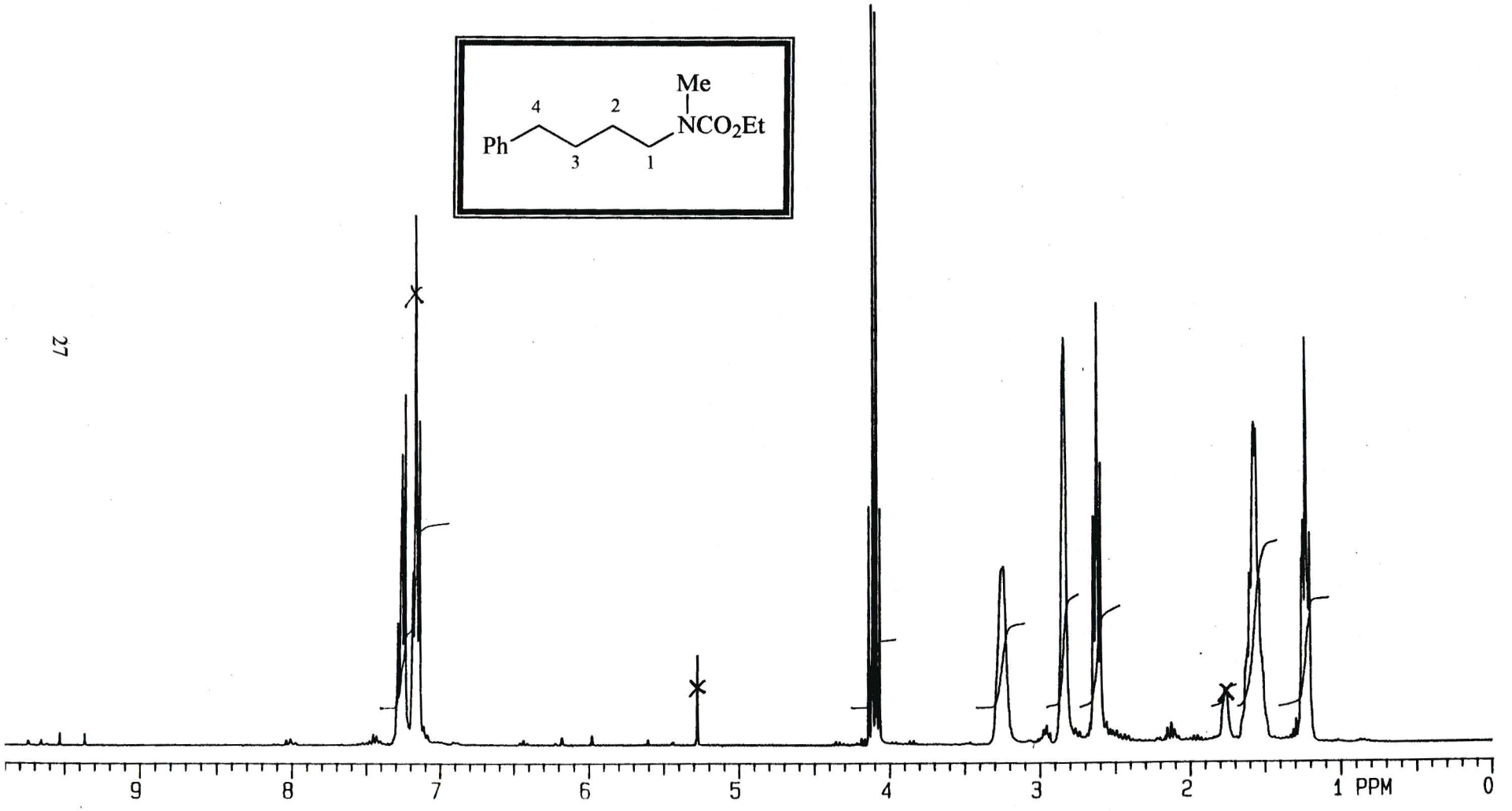
3.7 ^{1}H NMR spectrum of **N-methyl-4-phenylbutanamide**



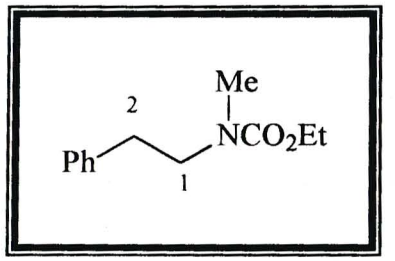
3.8 ^{1}H NMR spectrum of **N-methyl-4-phenylbutylamine**

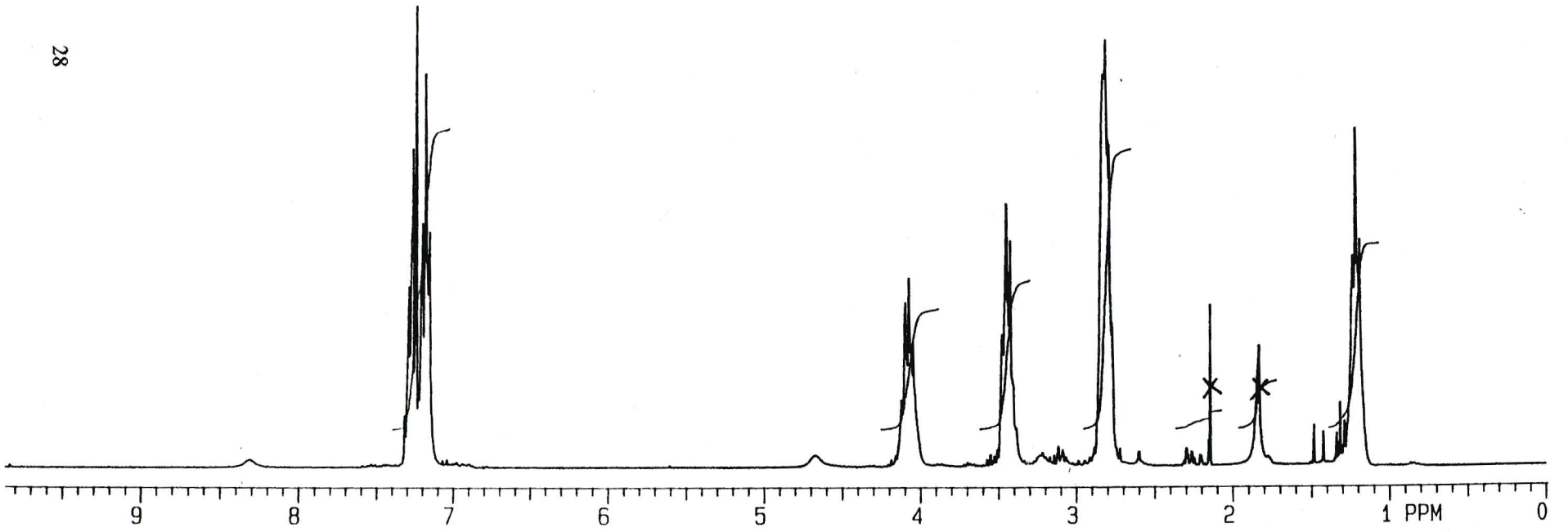


3.8 Infrared spectrum of **N-methyl-4-phenylbutylamine**

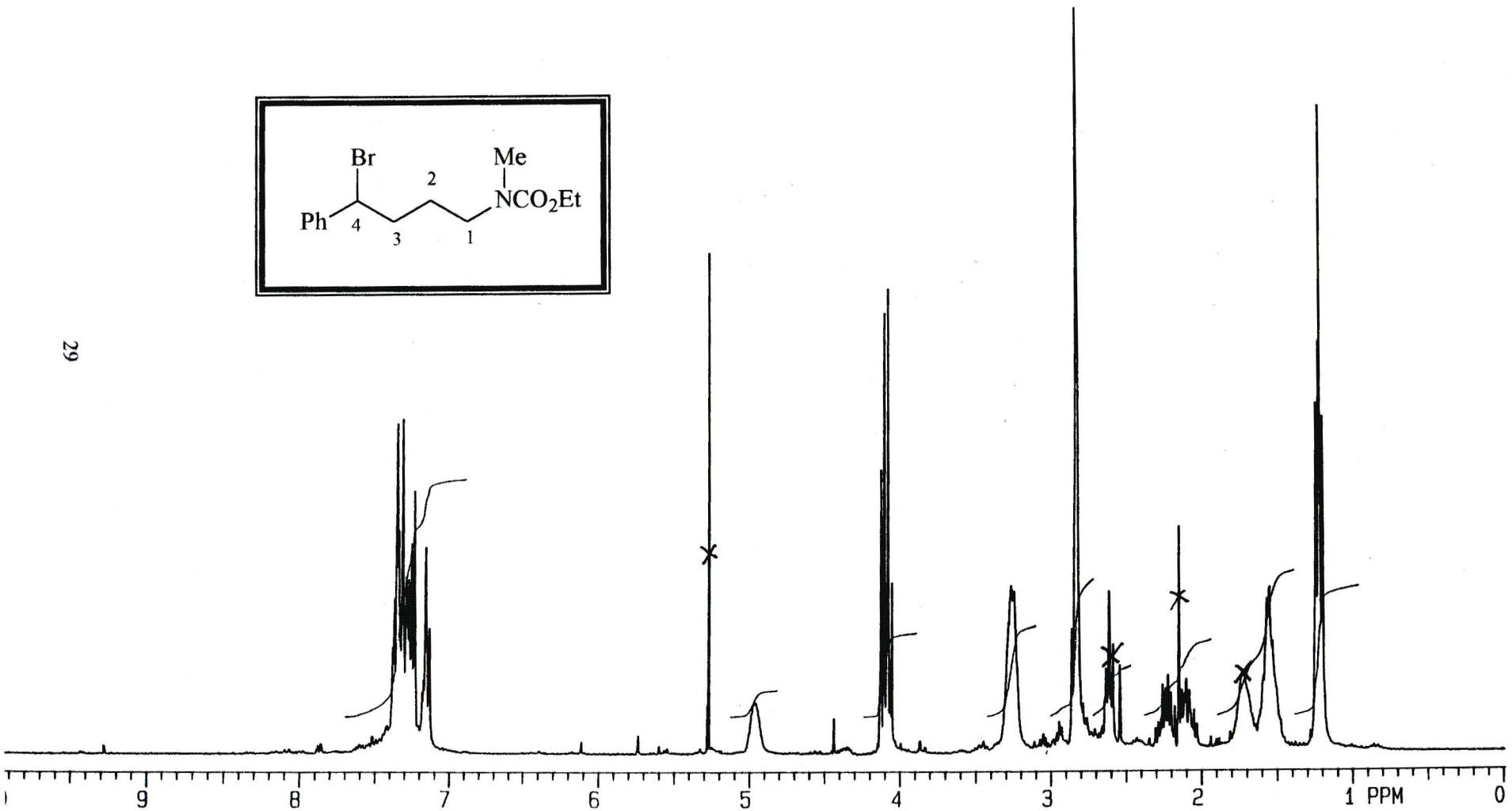


3.9.1 ^{1}H NMR spectrum of **ethyl N-4-phenylbutyl-N-methylcarbamate**

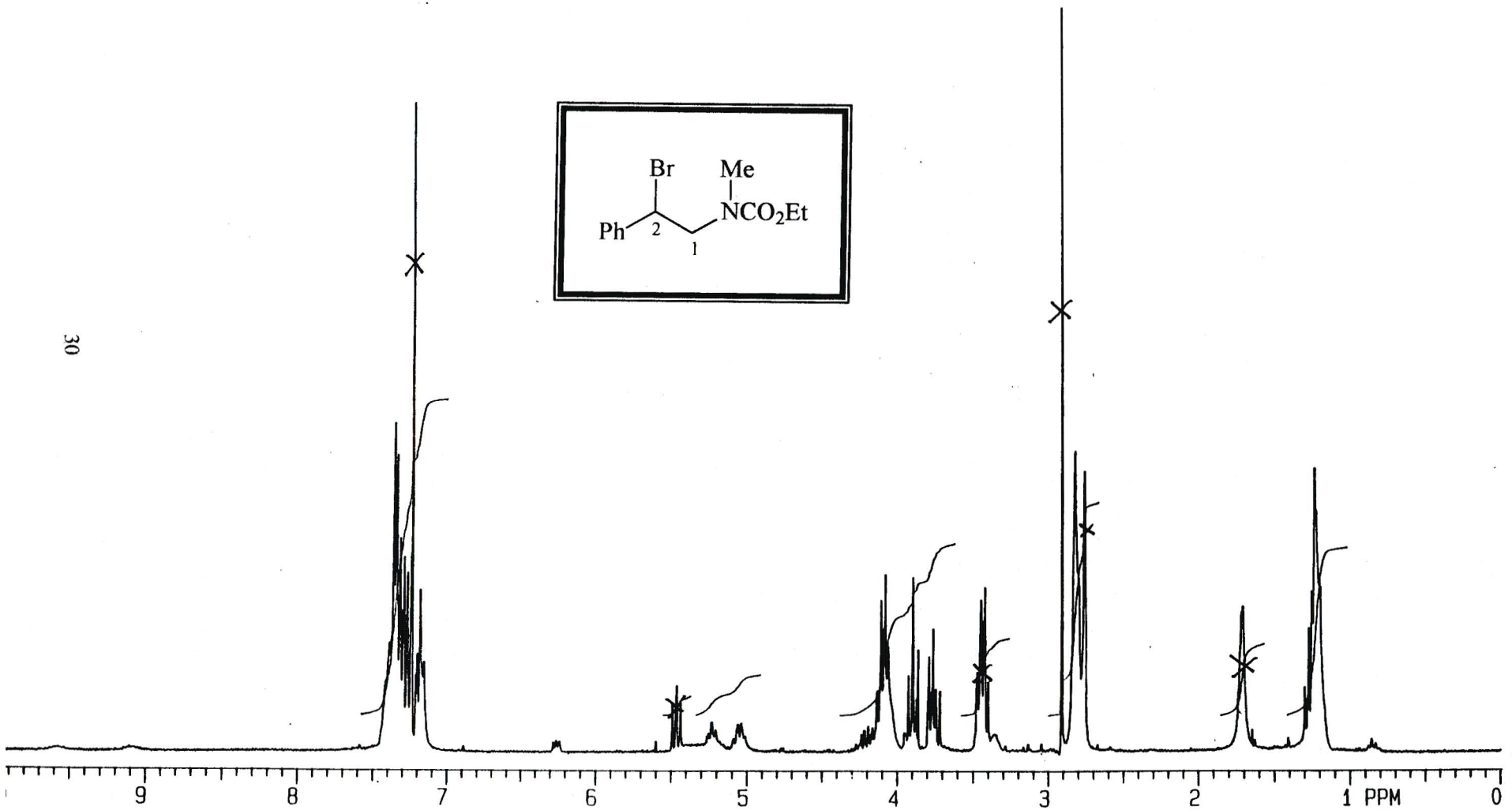




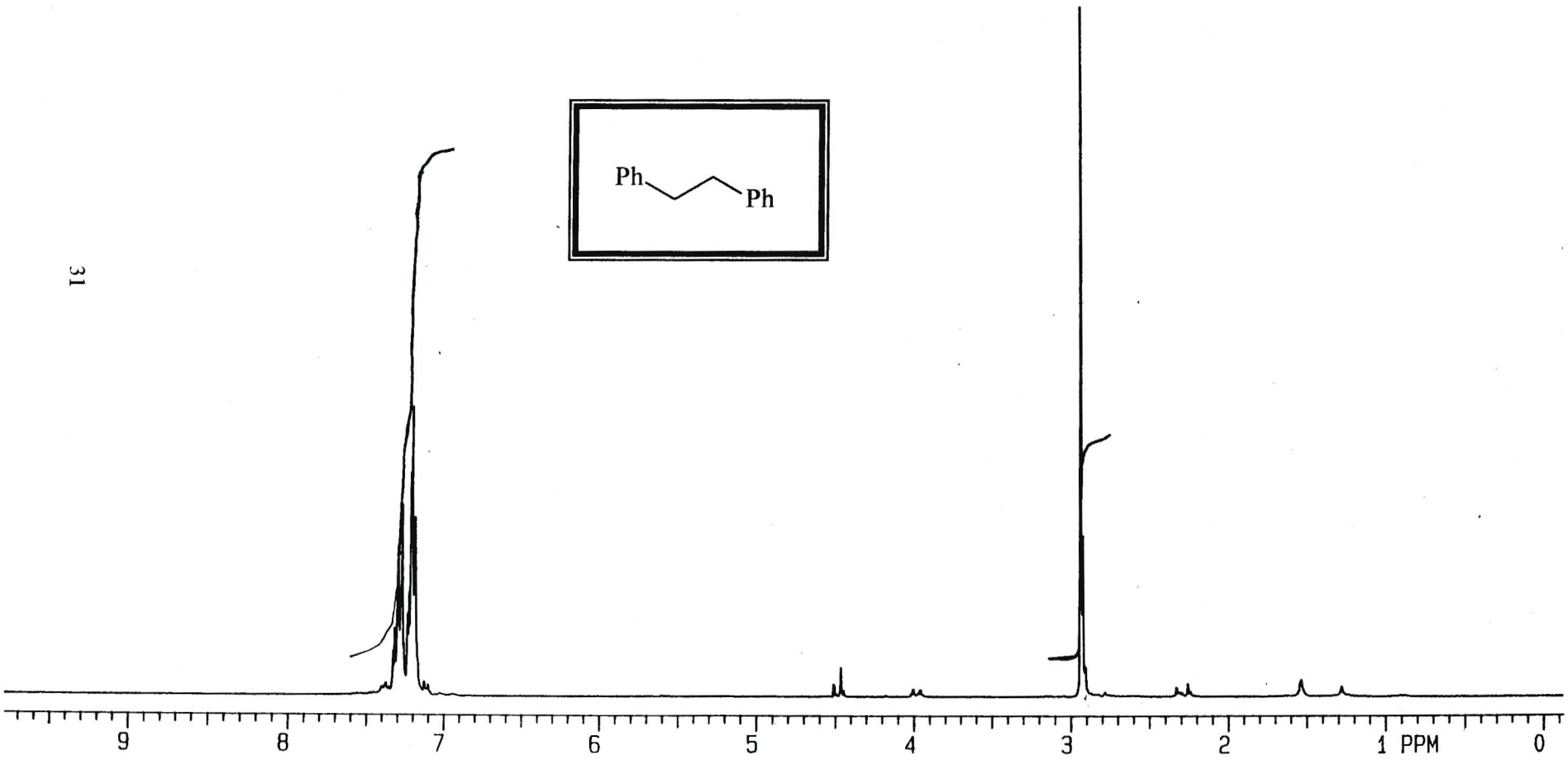
3.9.2 ^{1}H NMR spectrum of **ethyl N-2-phenylethyl-N-methylcarbamate**



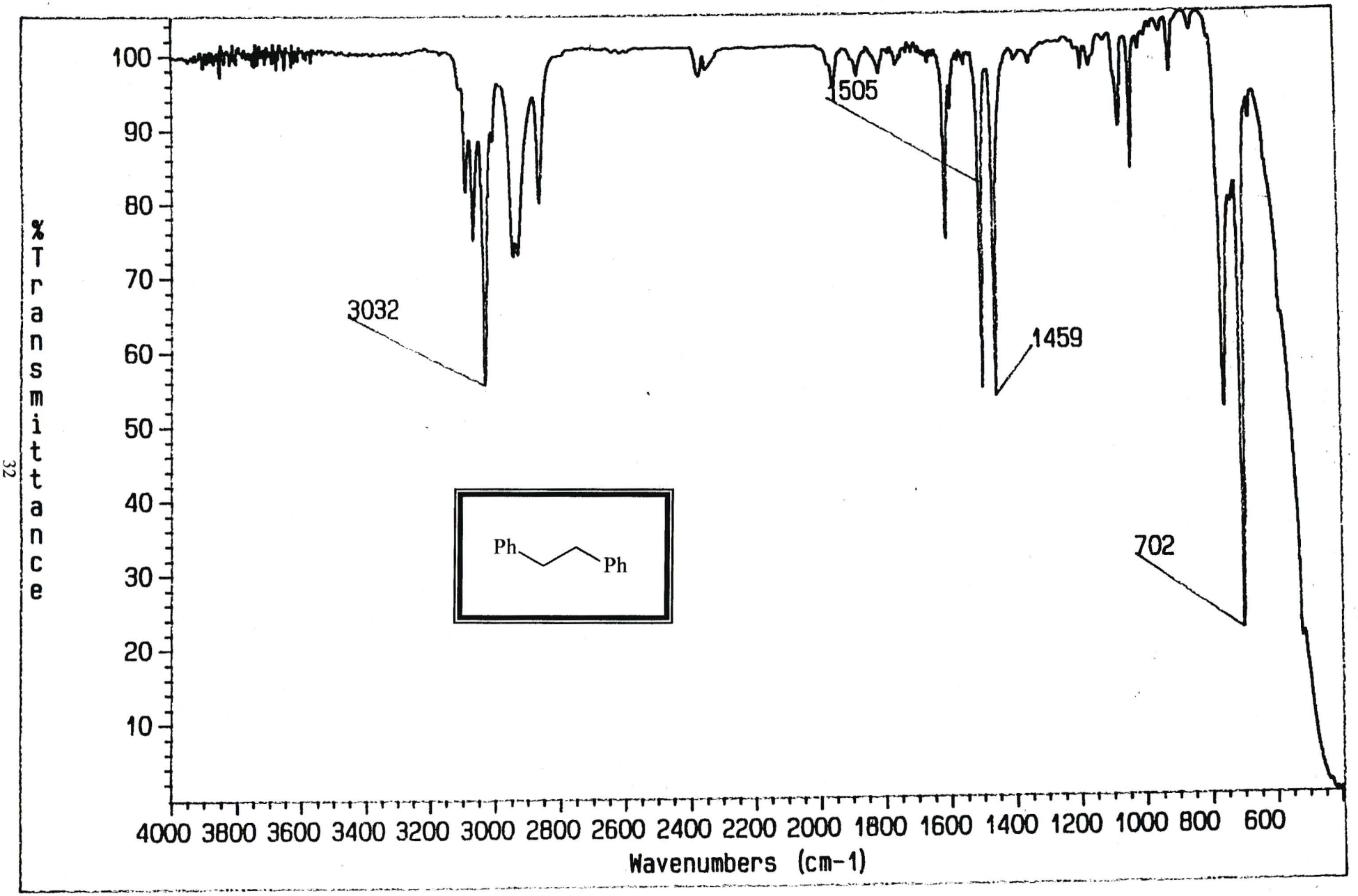
3.10.1 ^{1}H NMR spectrum of **ethyl N-4-bromo-4-phenylbutyl-N-methylcarbamate**



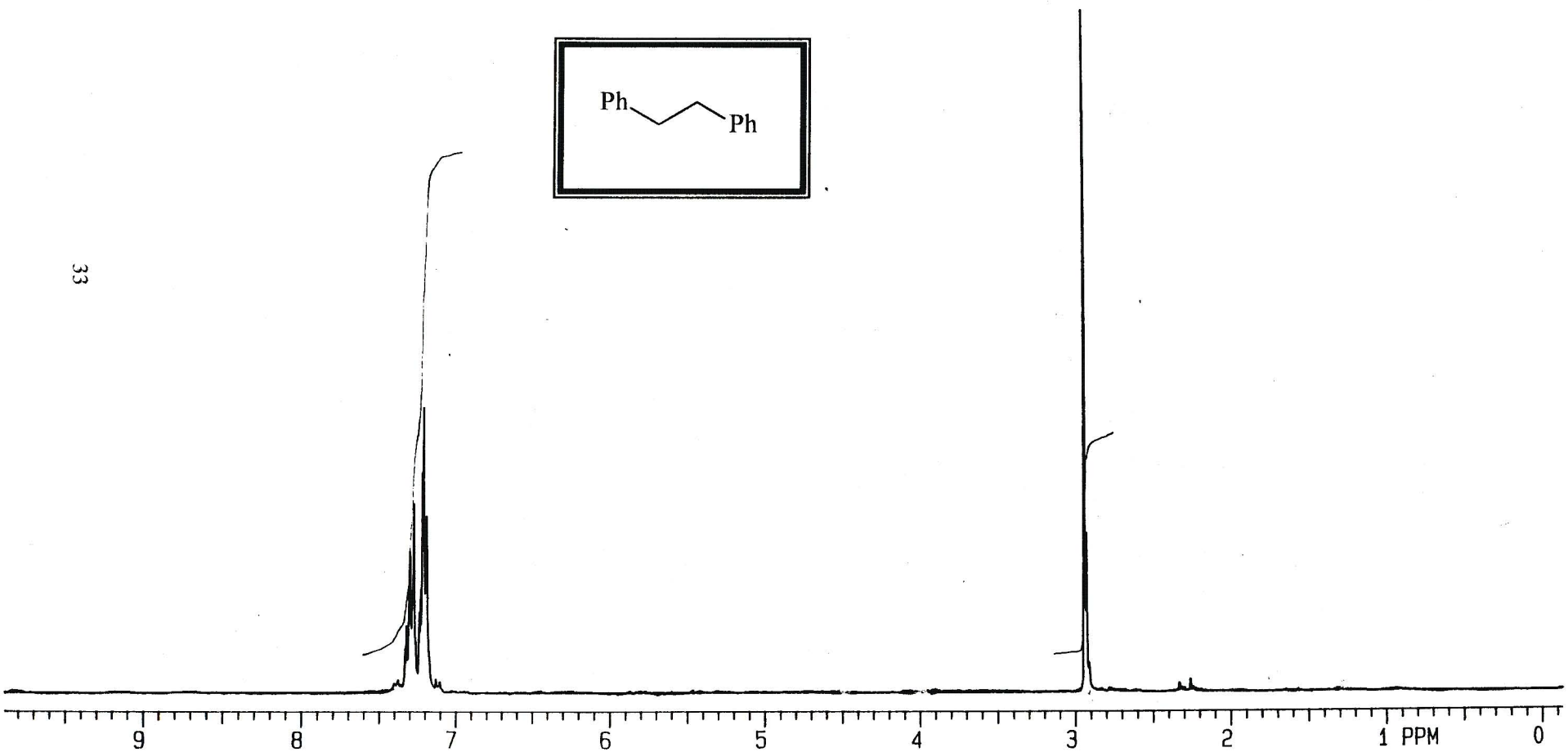
3.10.2 ^{1}H NMR spectrum of **ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate**



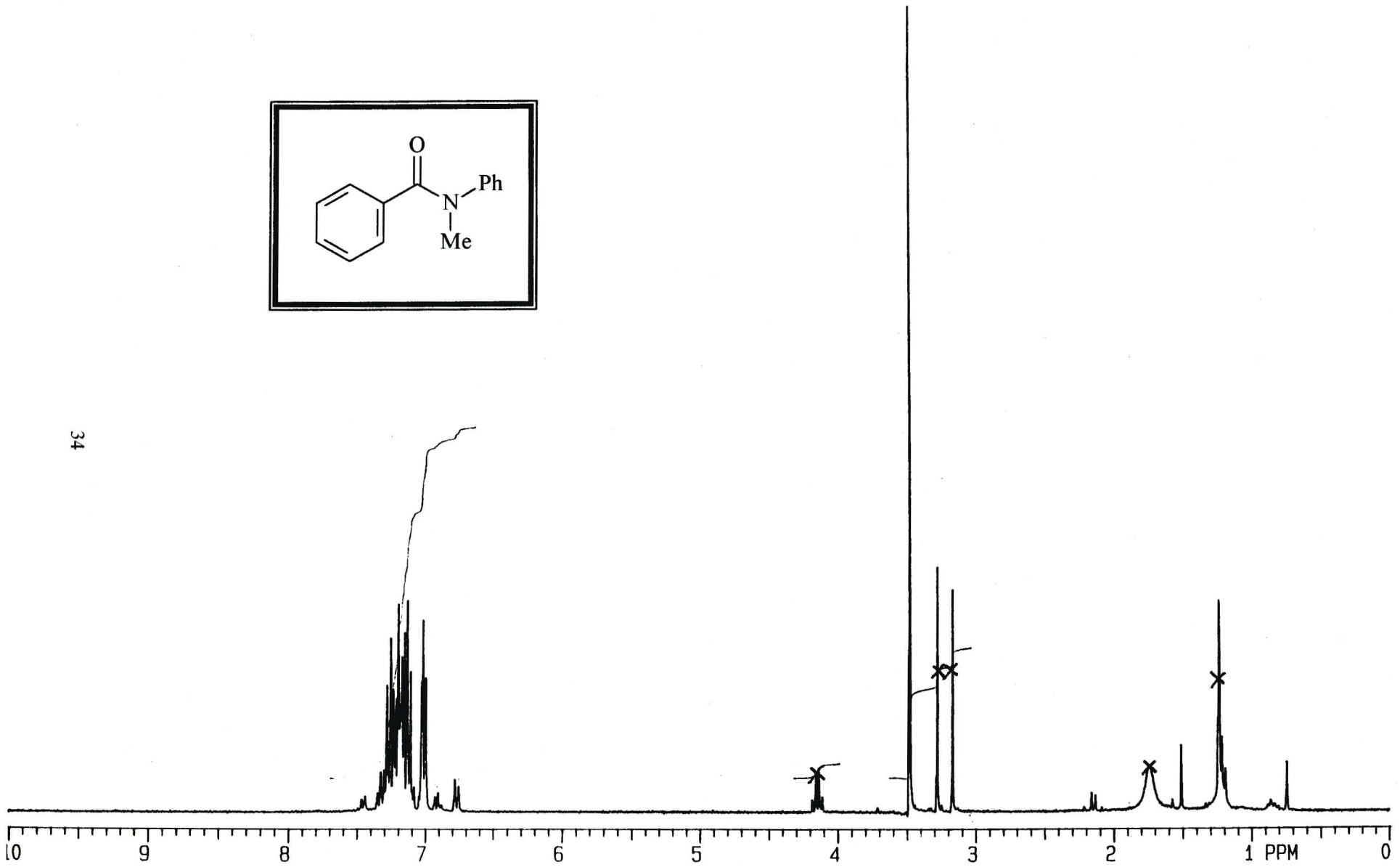
4.1.1 ^{1}H NMR spectrum of **bibenzyl**



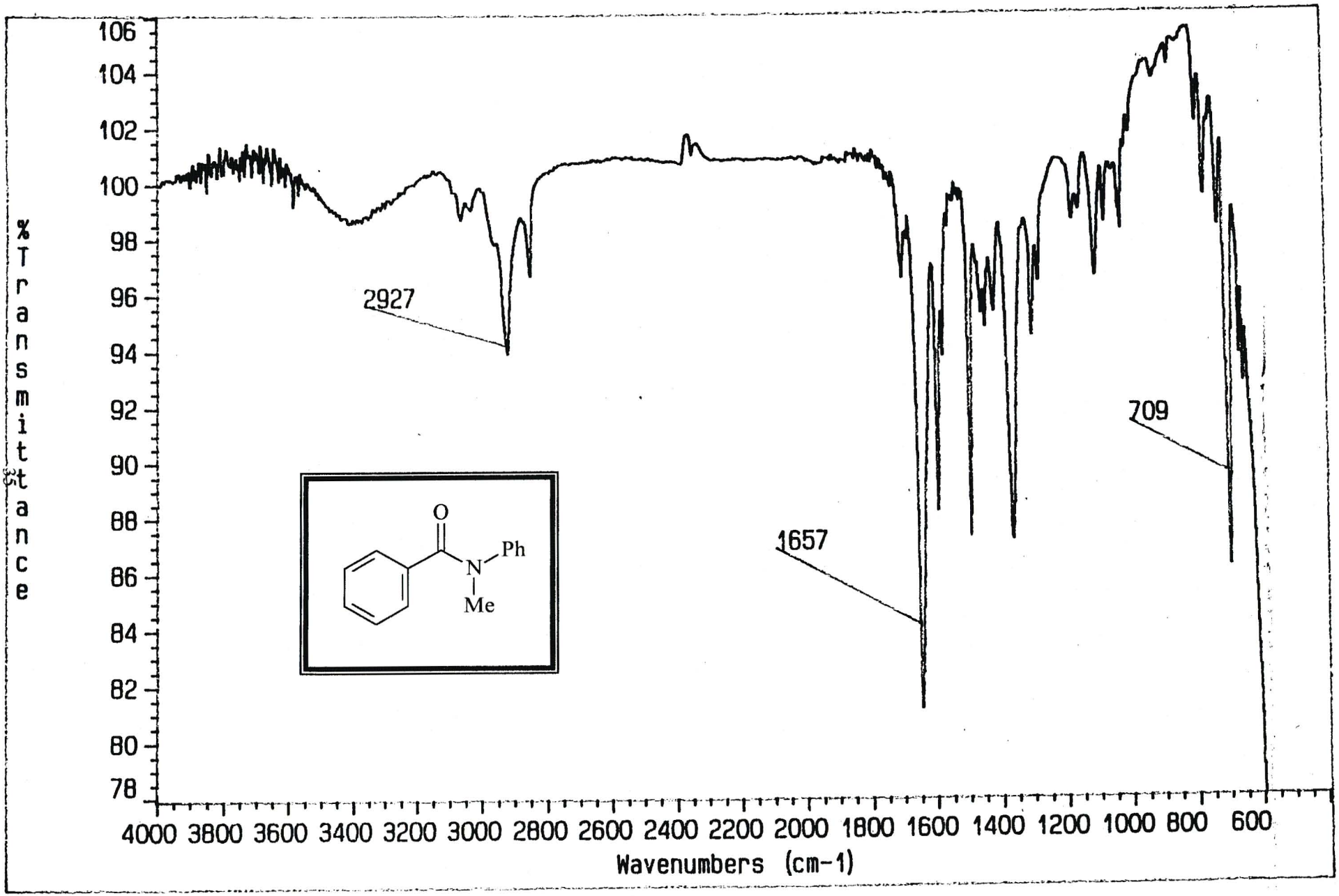
4.1.1 Infrared spectrum of **bibenzyl**



4.1.2 ^{1}H NMR spectrum of **bibenzyl**

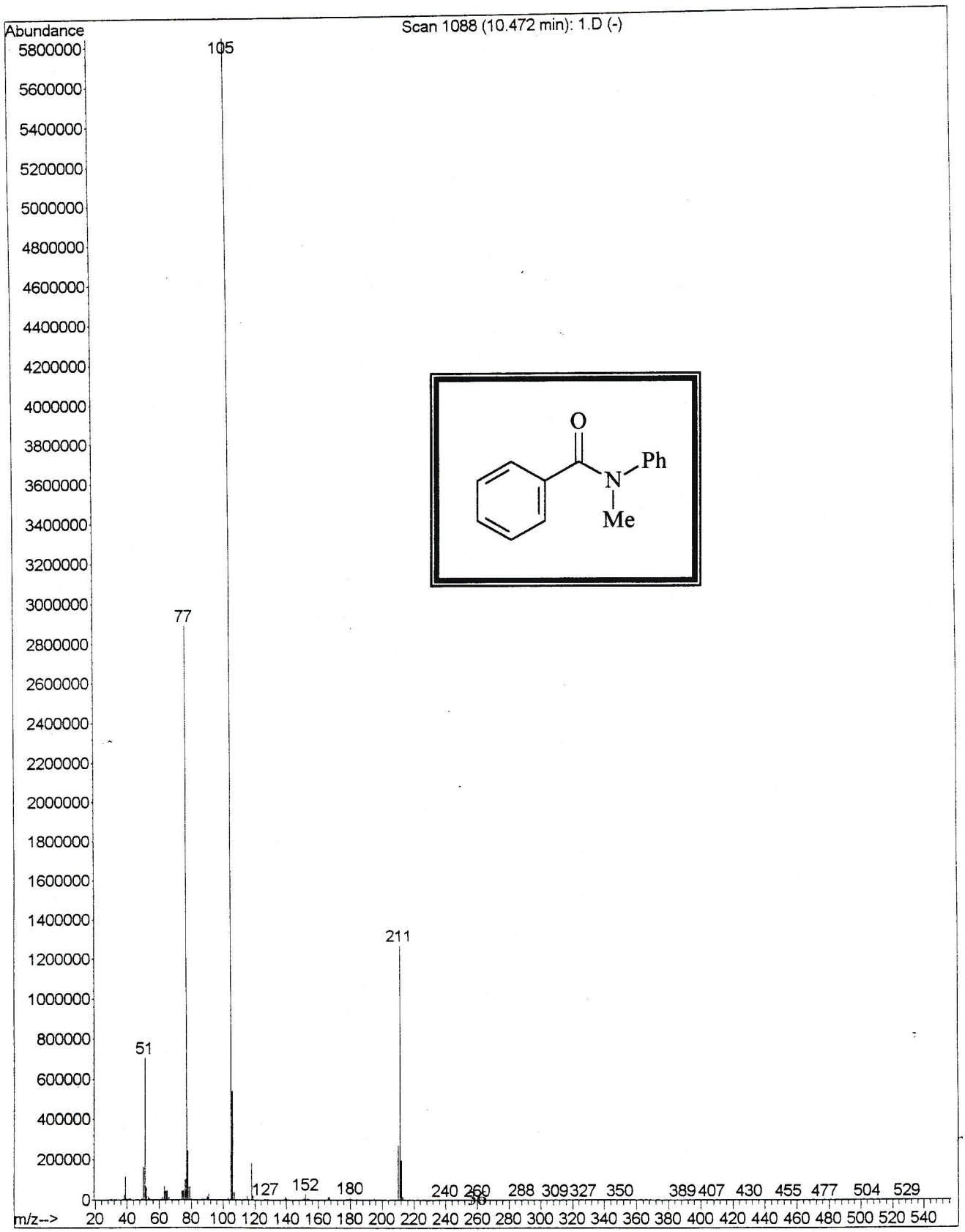


4.1.3 ^{1}H NMR spectrum of **N-methyl-N-phenylbenzamide**

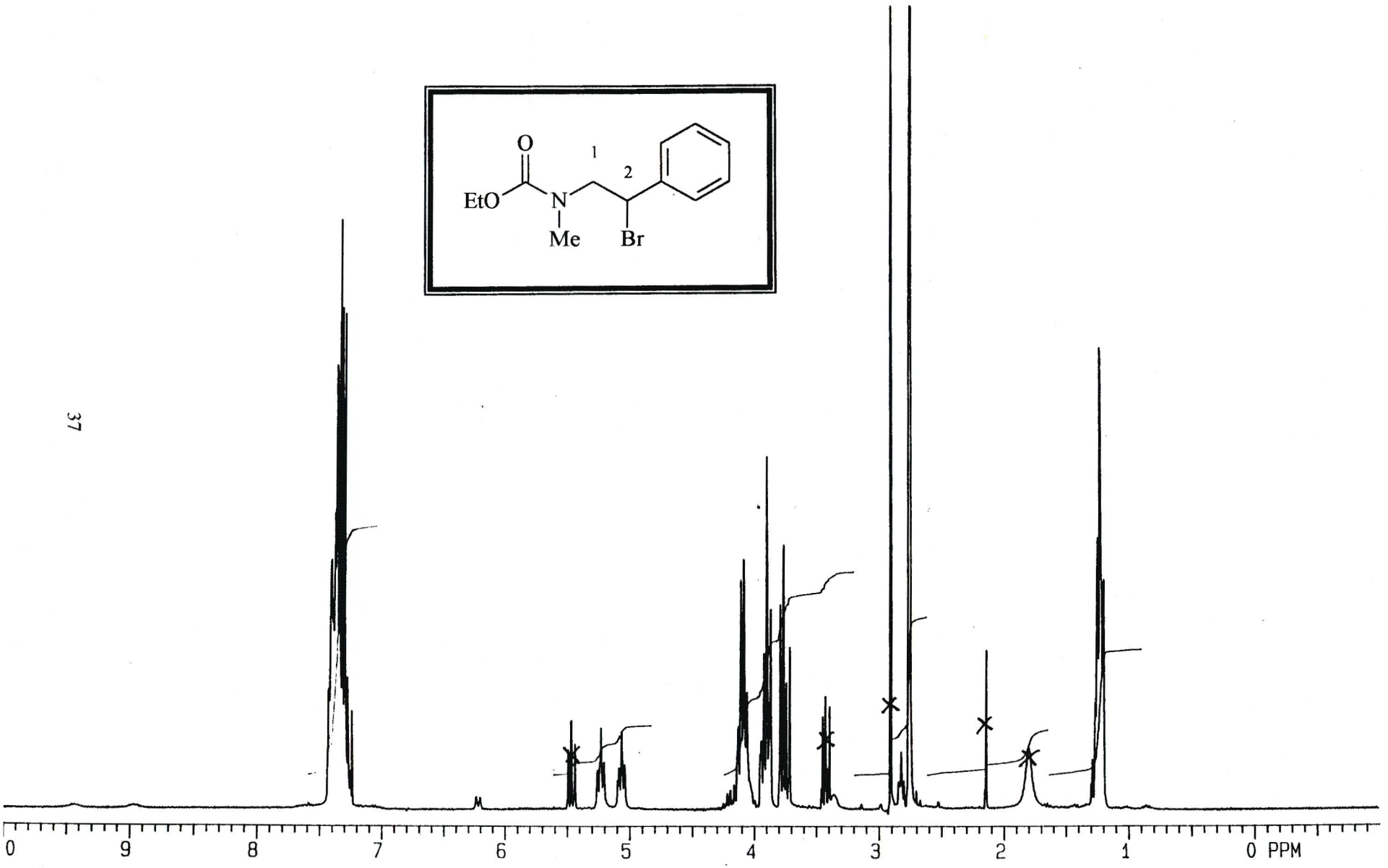


4.1.3 Infrared spectrum of **N-methyl-N-phenylbenzamide**

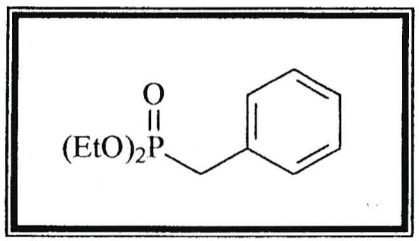
File : D:\MARTIN\1.D
Operator : Bret
Acquired : 23 Apr 2001 12:36 using AcqMethod NEW
Instrument : Instrumen
Sample Name: 1
Misc Info : 1ul inject, MeCl2, 1:75 split, 20dpm
Vial Number: 89

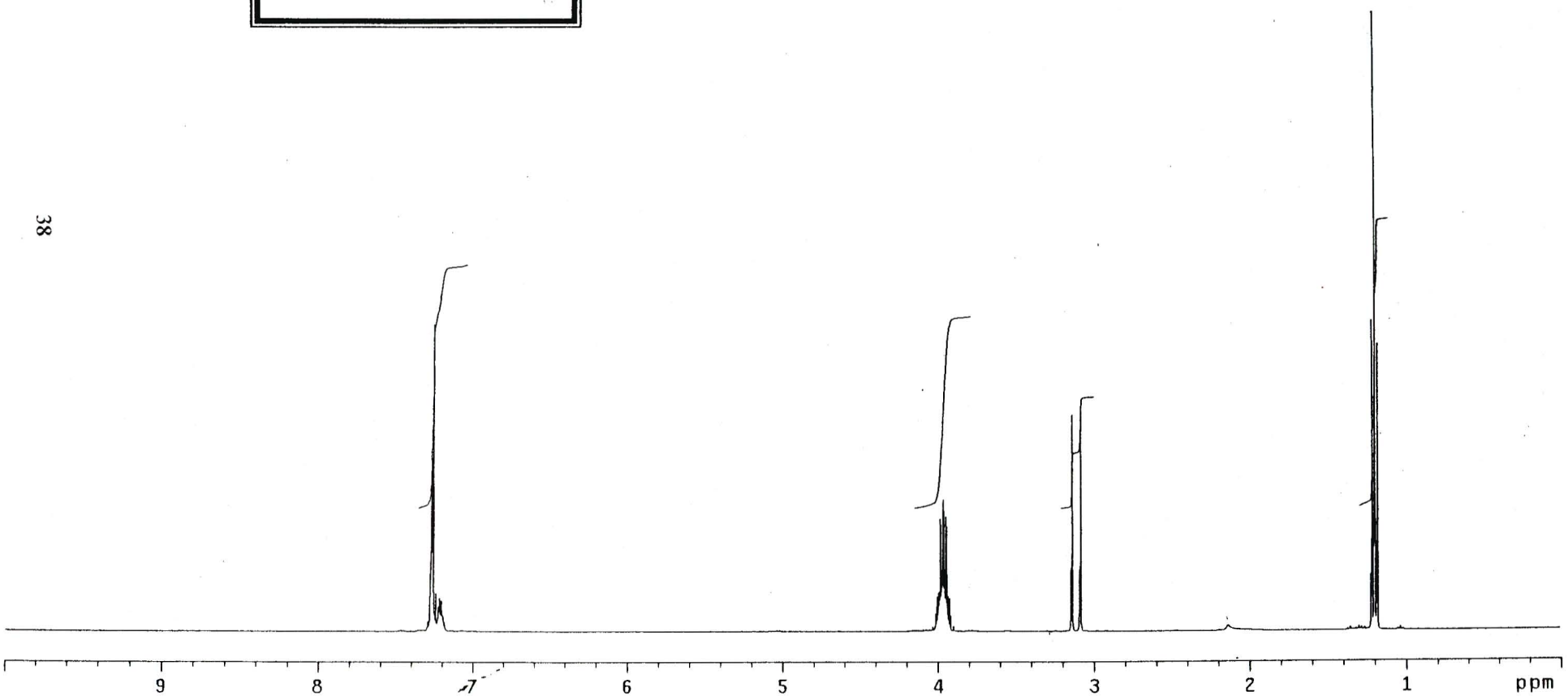


4.1.3 Mass spectrum of **N-methyl-N-phenylbenzamide**

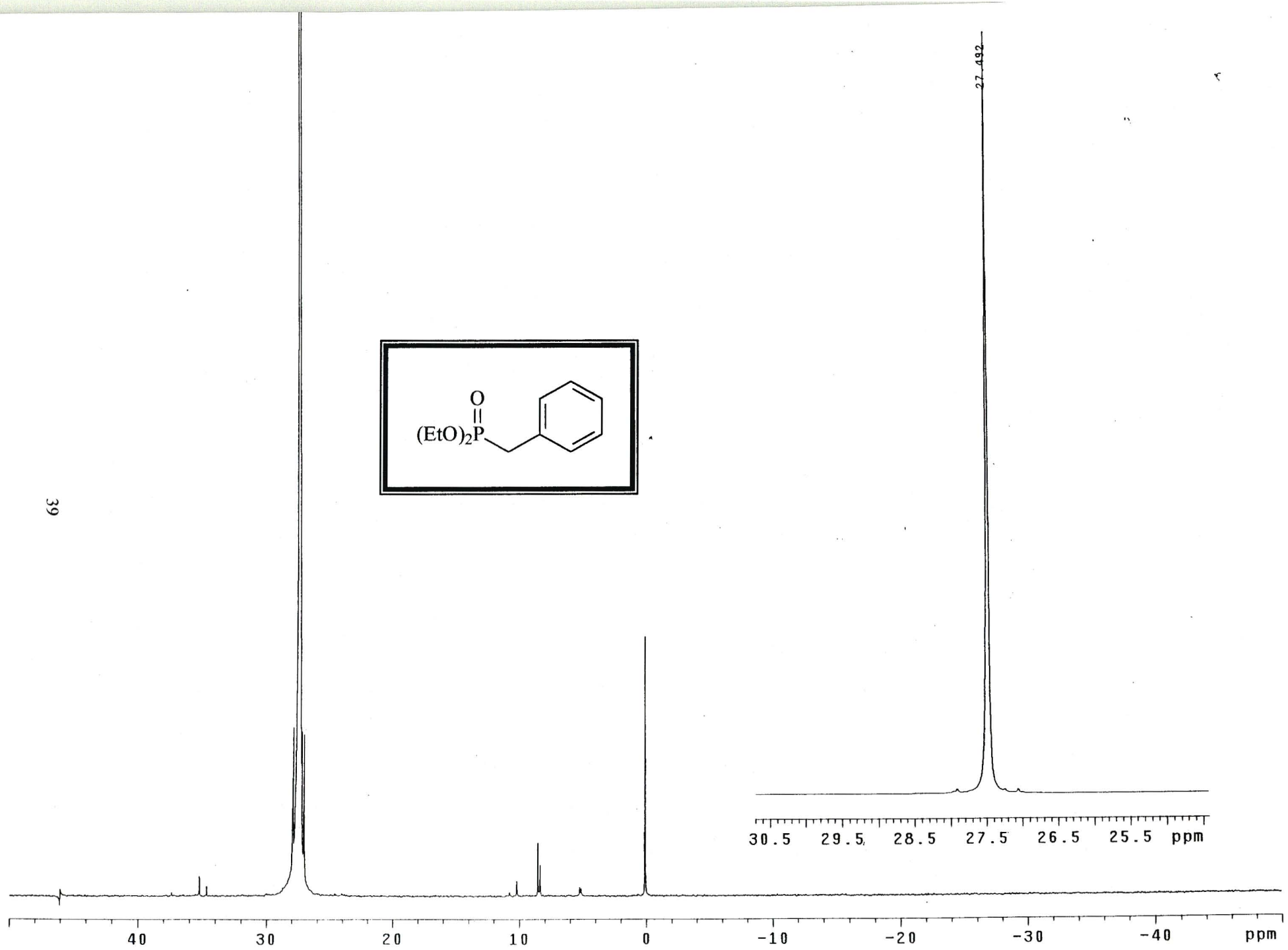


4.1.4 ^{1}H NMR spectrum of **ethyl N-2-bromo-2-phenylethyl-N-methylcarbamate**

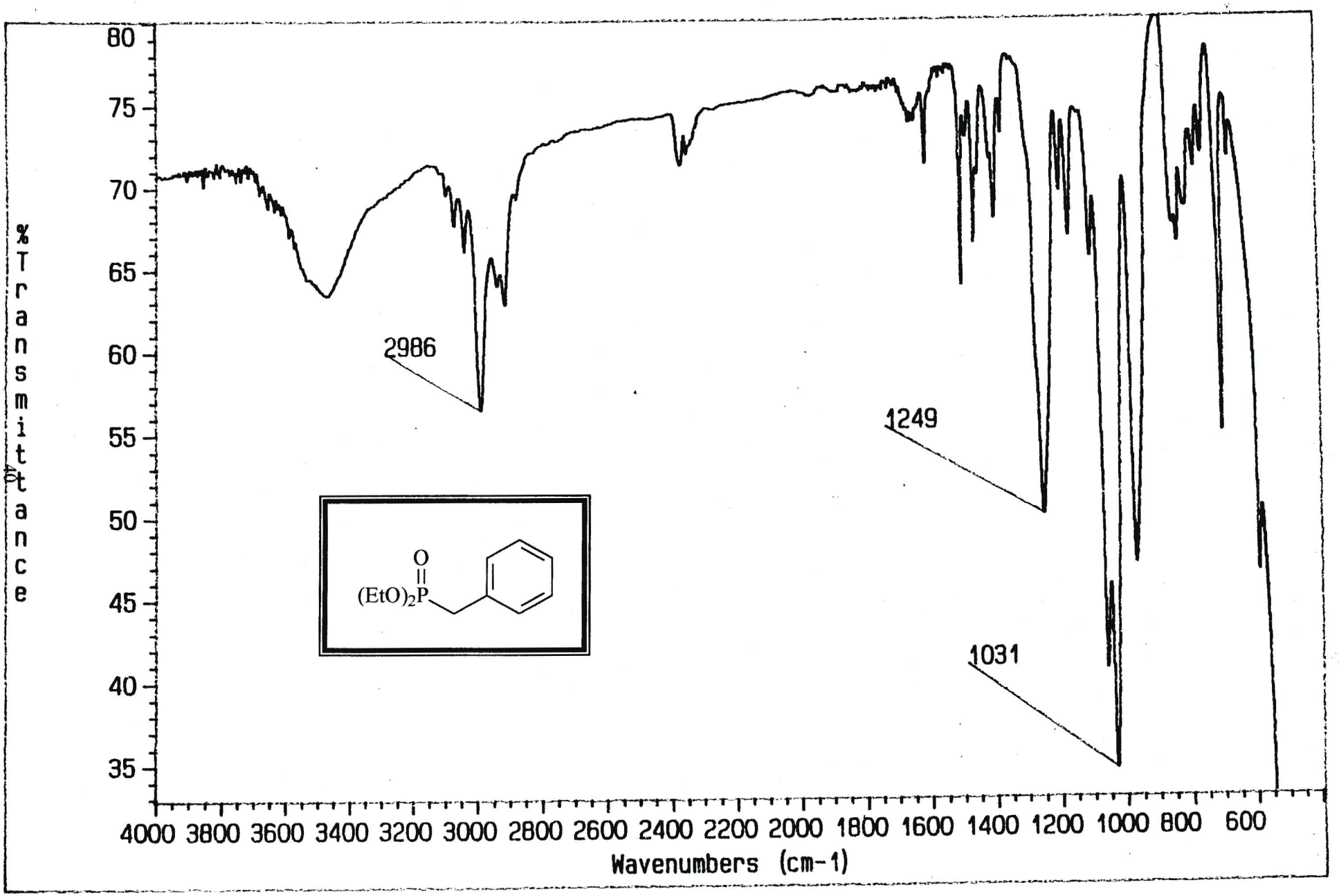




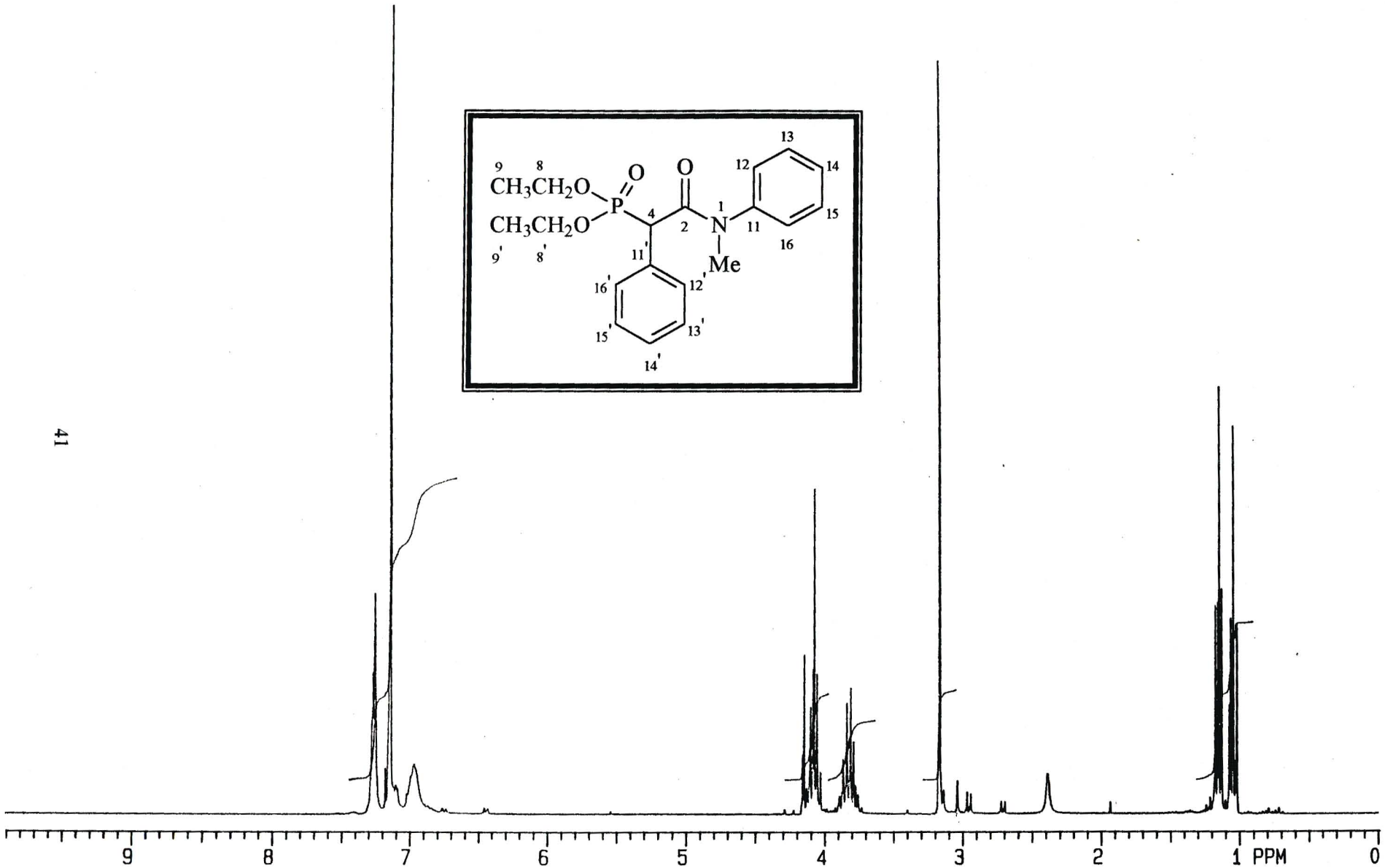
4.2 ^{1}H NMR spectrum of **diethyl benzylphosphonate**



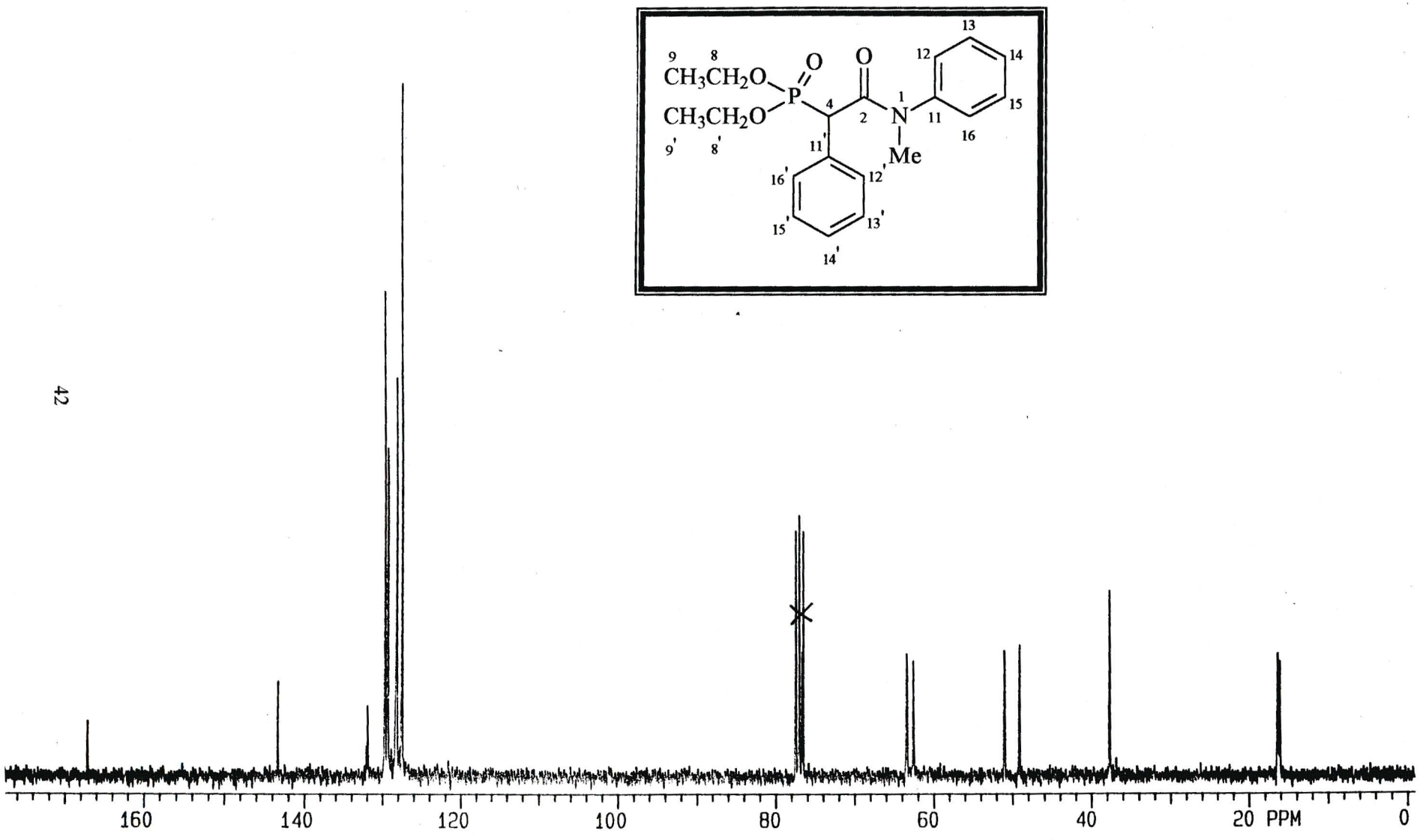
4.2 ^{31}P NMR spectrum of **diethyl benzylphosphonate**



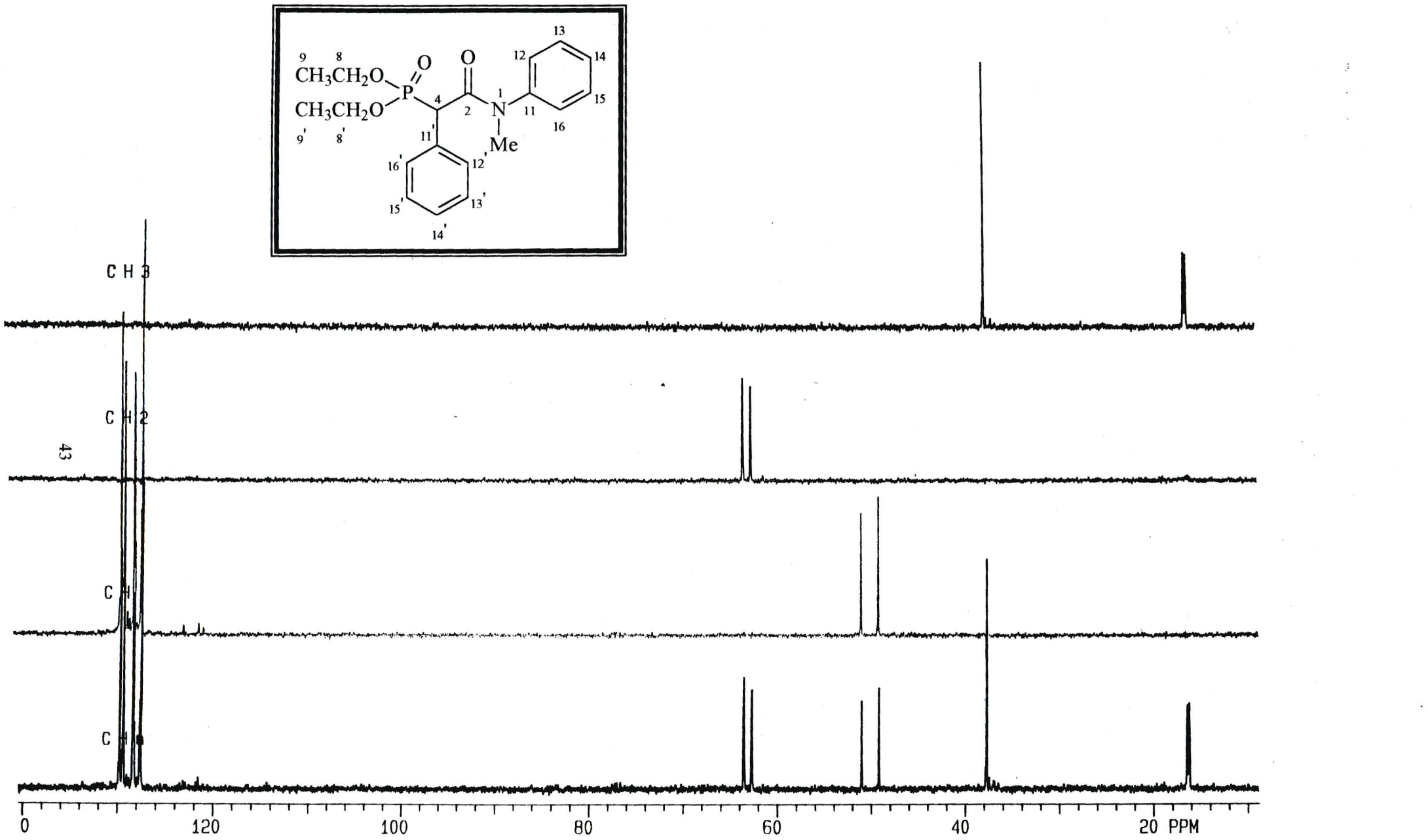
4.2 Infrared spectrum of **diethyl benzylphosphonate**



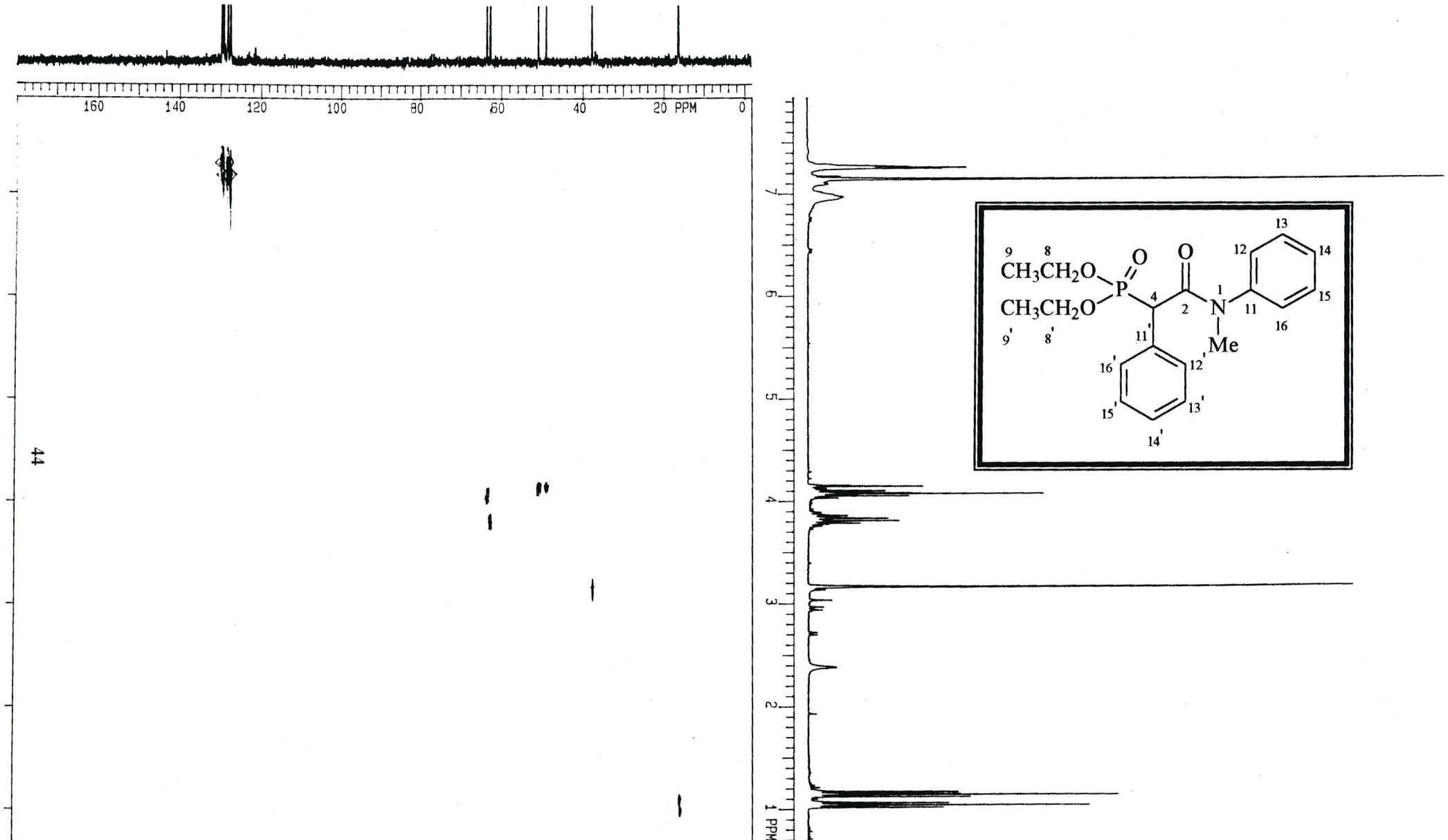
4.3 ^{1}H NMR spectrum of **phosphonate adduct**



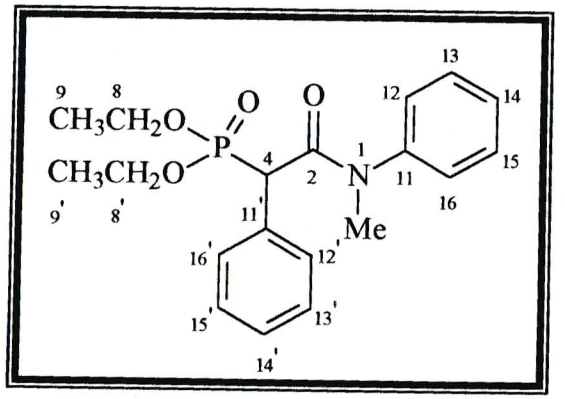
4.3 ^{13}C NMR spectrum of **phosphonate adduct**



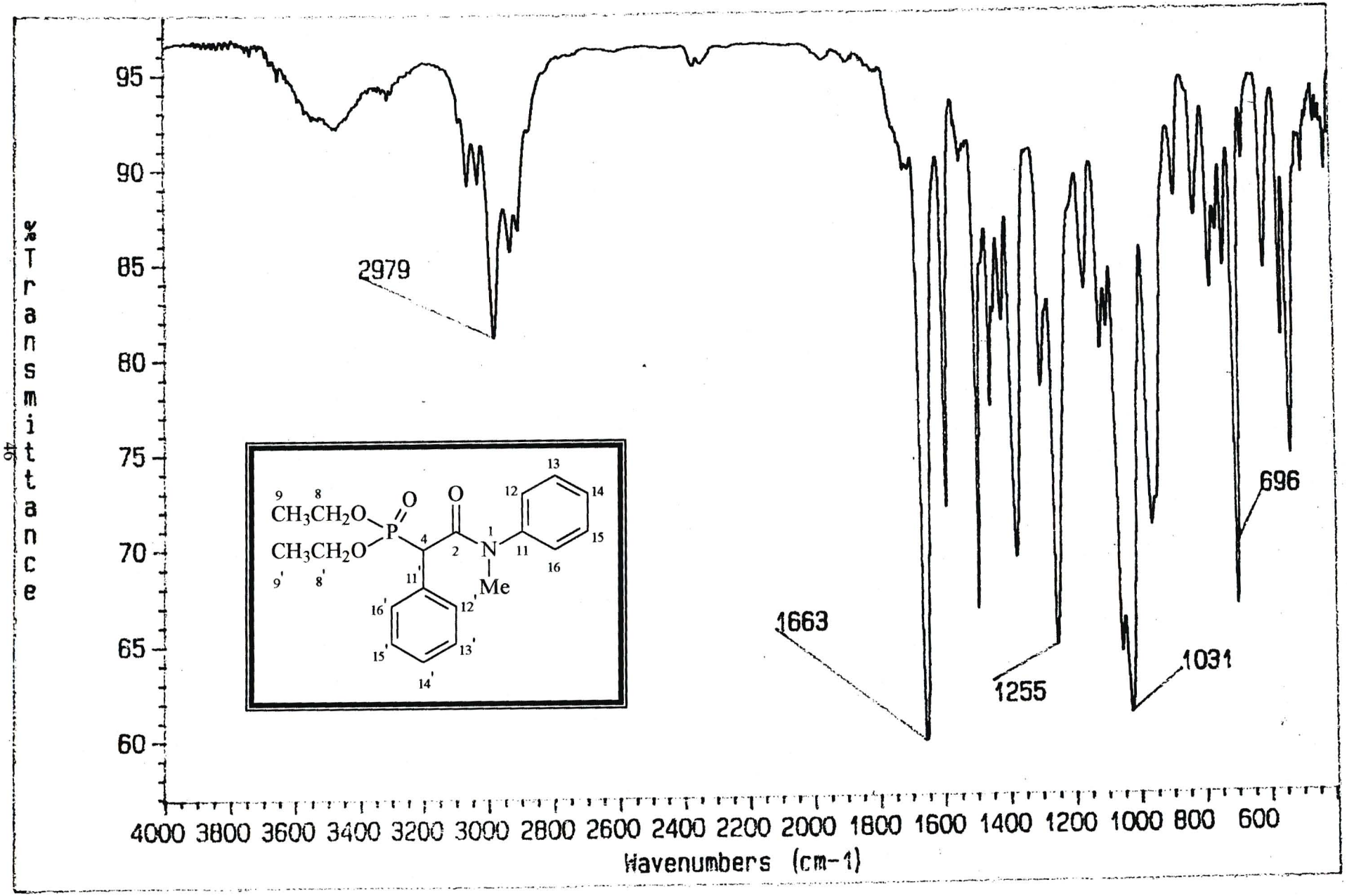
4.3 Adept spectrum of **phosphonate adduct**



4.3 HETCOR spectrum of **phosphonate adduct**



4.3 ^{31}P NMR spectrum of **phosphonate adduct**



4.3 Infrared spectrum of **phosphonate adduct**

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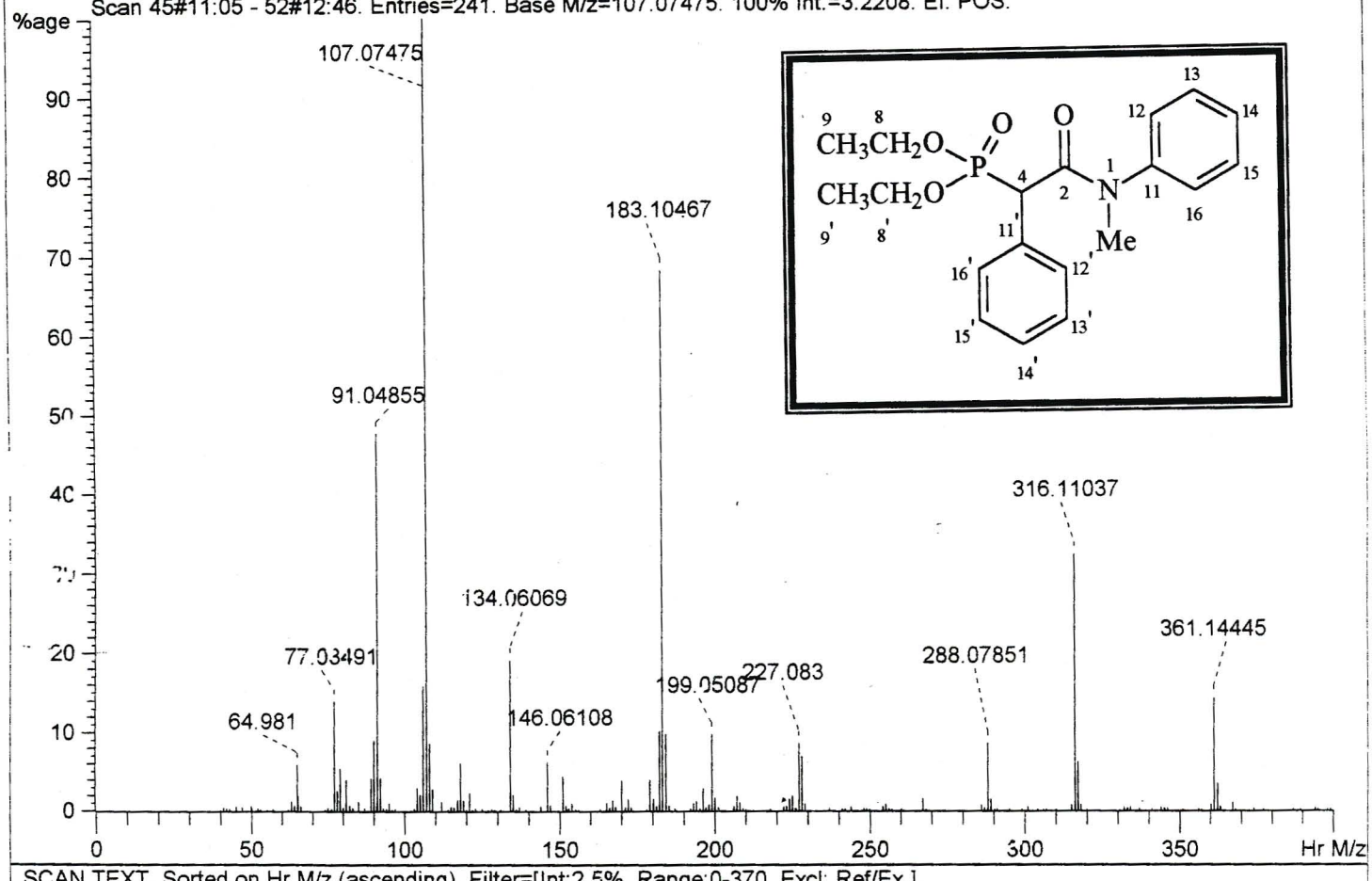
File Source : Acquired on MASPEC II system [II32/9913]
File Title : JPG 51>>>>>>>>>>
Operator : Dr. P. Boshoff
Instrument : VG70-SEQ

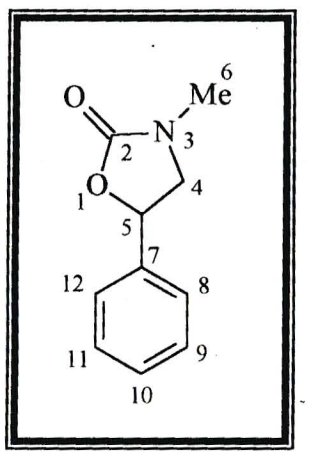
SCAN GRAPH. Flagging=Hr M/z. Filter={Excl: Ref/Ex.].
Scan 45#11:05 - 52#12:46. Entries=241. Base M/z=107.07475. 100% Int.=3.2208. EI. POS.



SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:2.5%. Range:0-370. Excl: Ref/Ex.].
Scan 45#11:05 - 52#12:46. Entries=32. Base M/z=107.07475. 100% Int.=3.2208. EI. POS.

Hr M/z	%age	Width
64.98100	5.90	58
77.03491	13.90	59
78.04026	2.53	63
79.04953	5.40	59
80.96849	3.95	70
89.03217	4.09	57
90.04020	8.94	59
91.04855	47.68	58
92.05205	4.17	59
104.05263	2.93	65
106.06678	15.89	59
107.07475	100.00	60
108.07818	8.56	60
109.00738	2.77	61
118.04302	6.05	87
134.06069	19.10	57
146.06108	6.19	56
150.99987	4.38	62
170.03624	3.84	77
179.04810	3.91	63
182.09636	10.04	75
183.10467	68.02	56
184.10812	9.73	56
196.11185	2.83	55
199.05087	9.70	65
227.08300	8.53	59
228.09078	6.89	57
288.07851	8.50	56
316.11037	32.19	55
317.11317	6.08	55
361.14445	14.18	55
362.14764	3.40	54

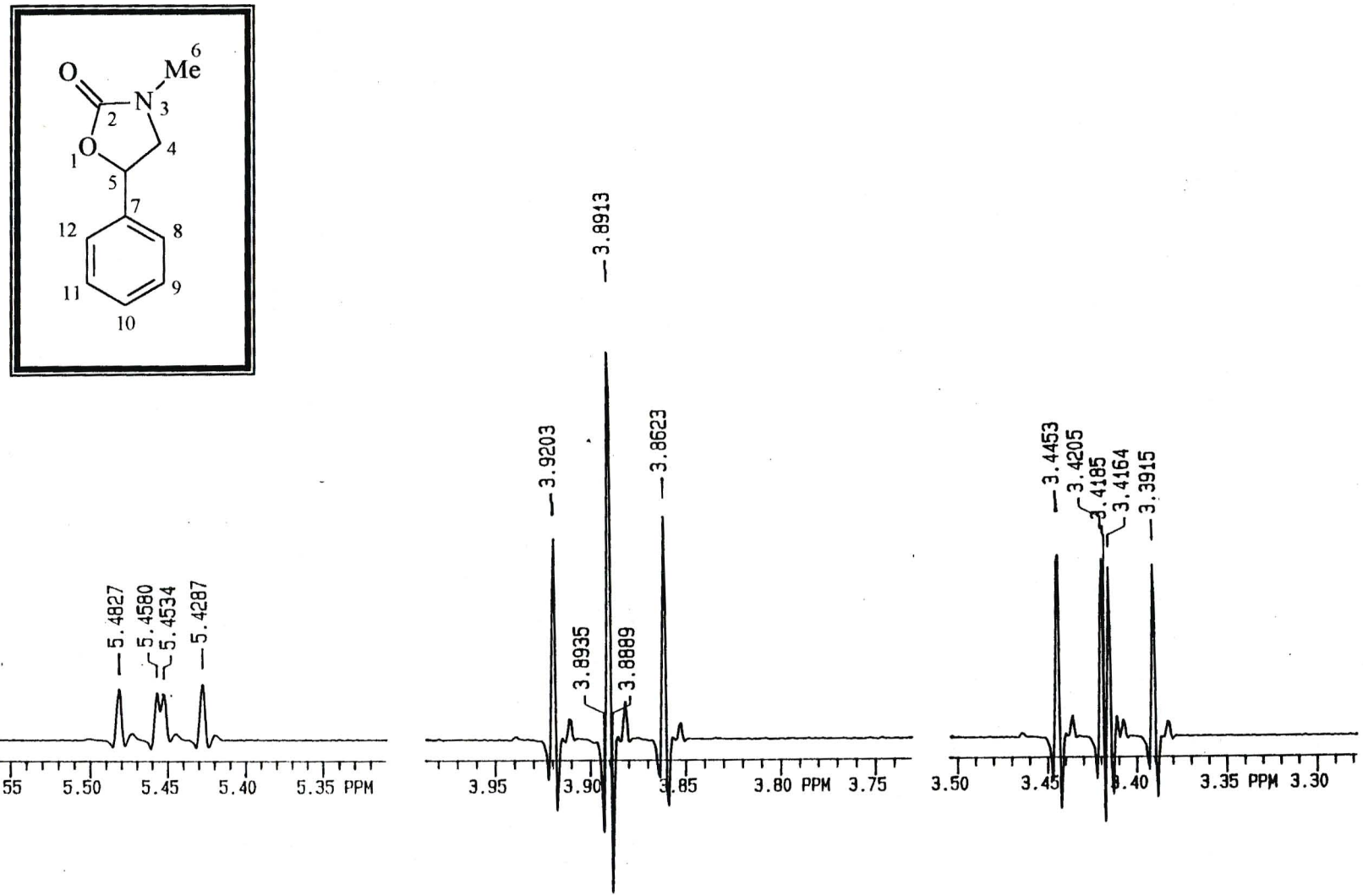
4 3 Mass spectrum of phenol



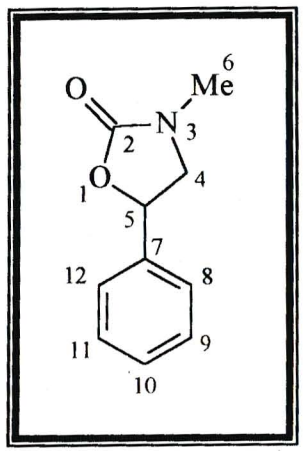
X = P(OEt)$_3$

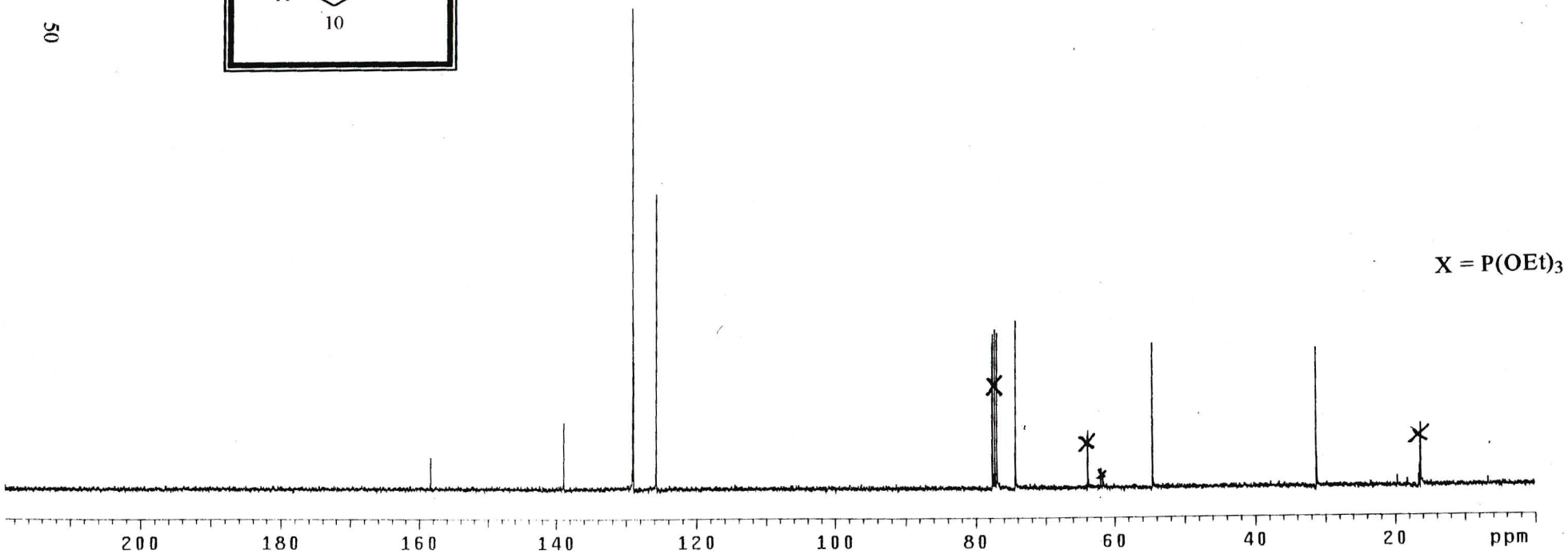
9 8 7 6 5 4 3 2 1 ppm

4.4 ^{1}H NMR spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**

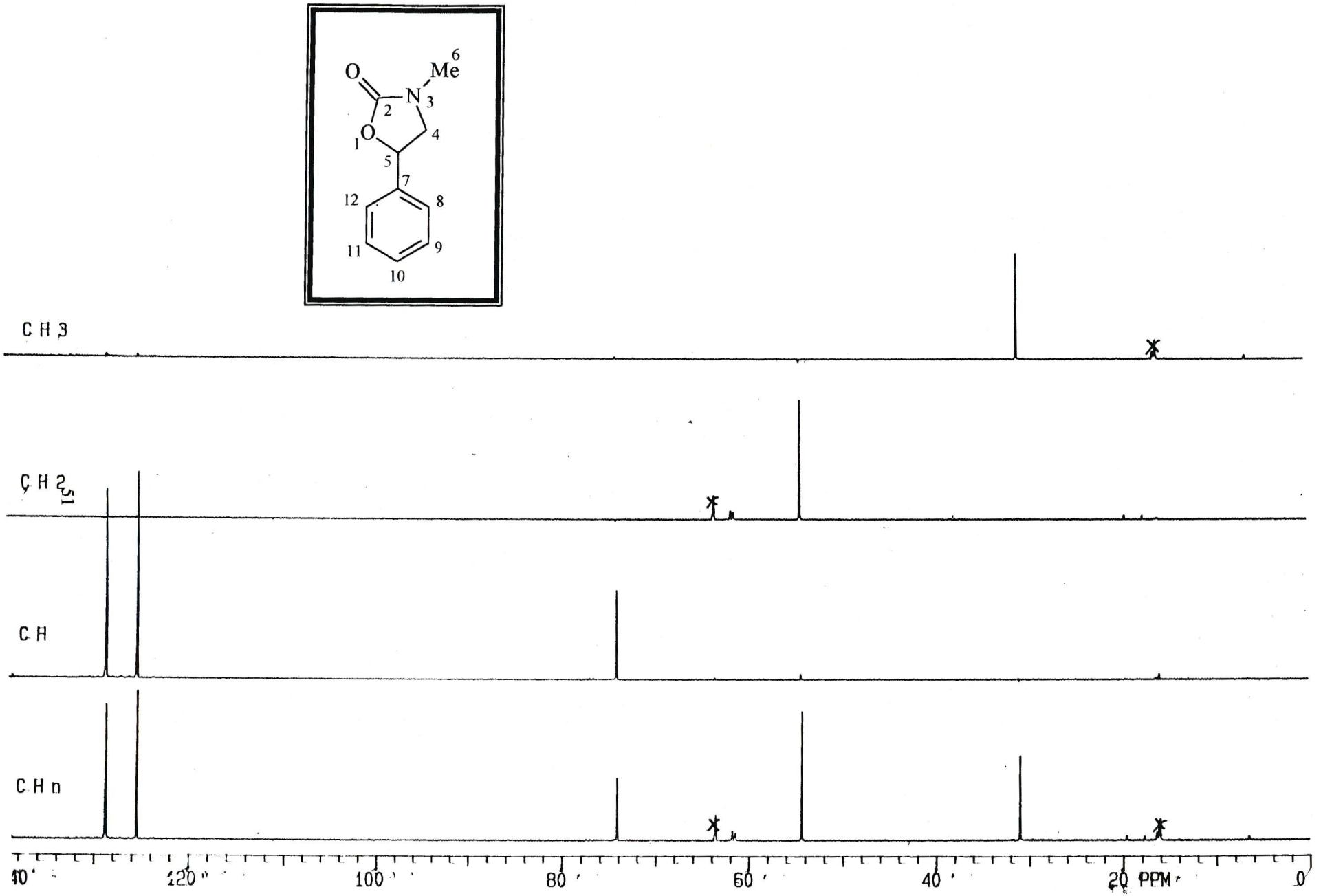


4.4 Resolution enhanced ^{1}H NMR spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**

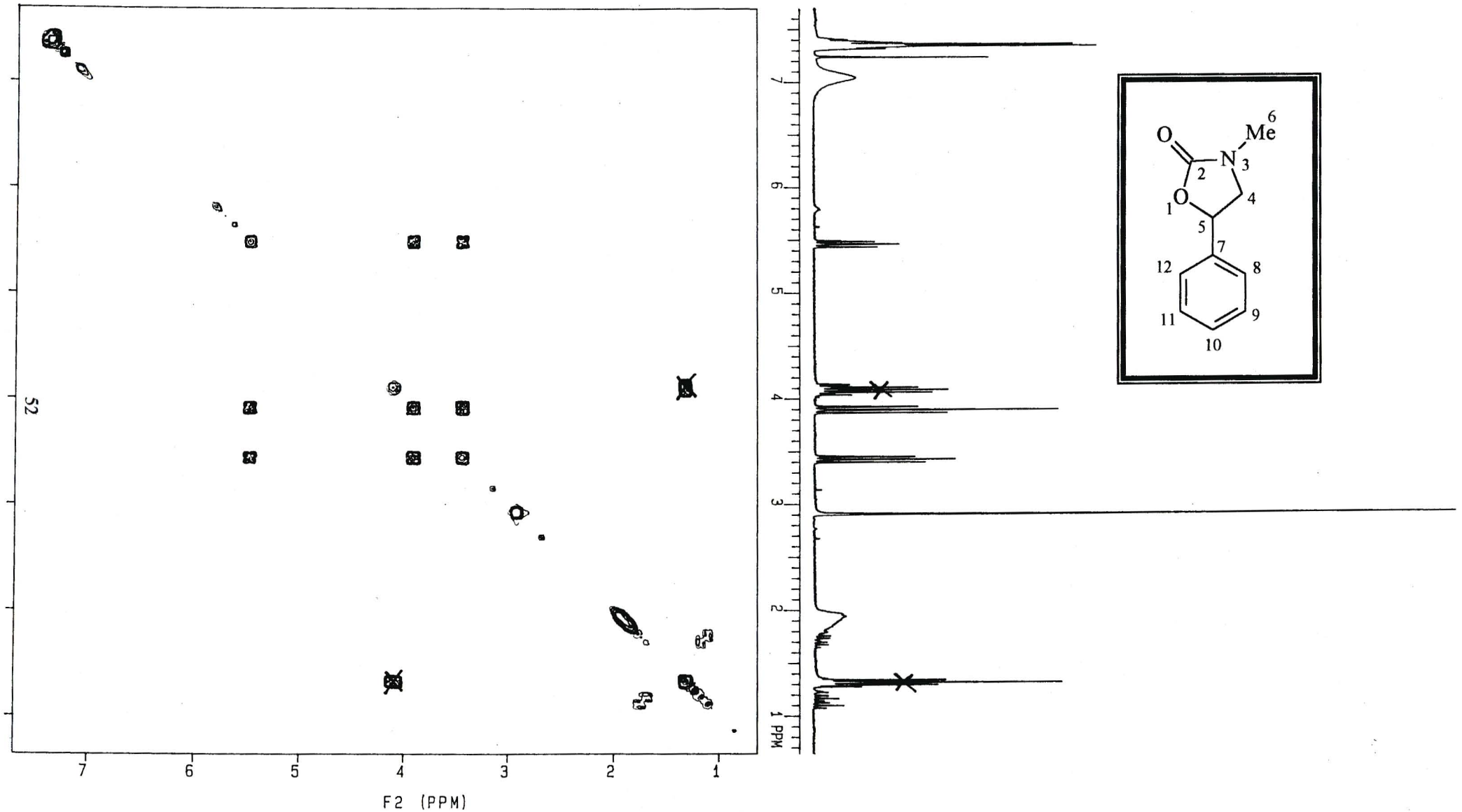




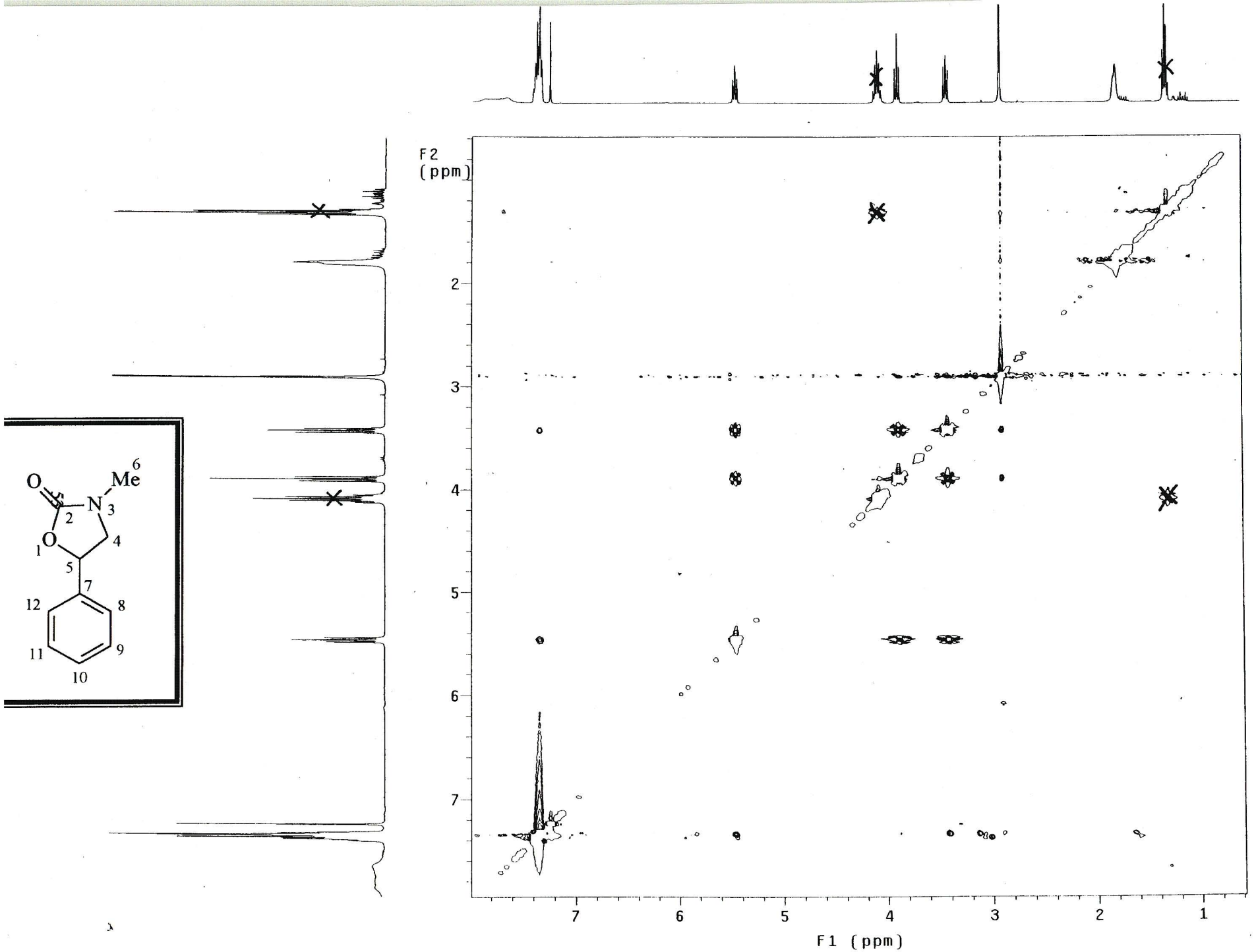
4.4 ^{13}C NMR spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**



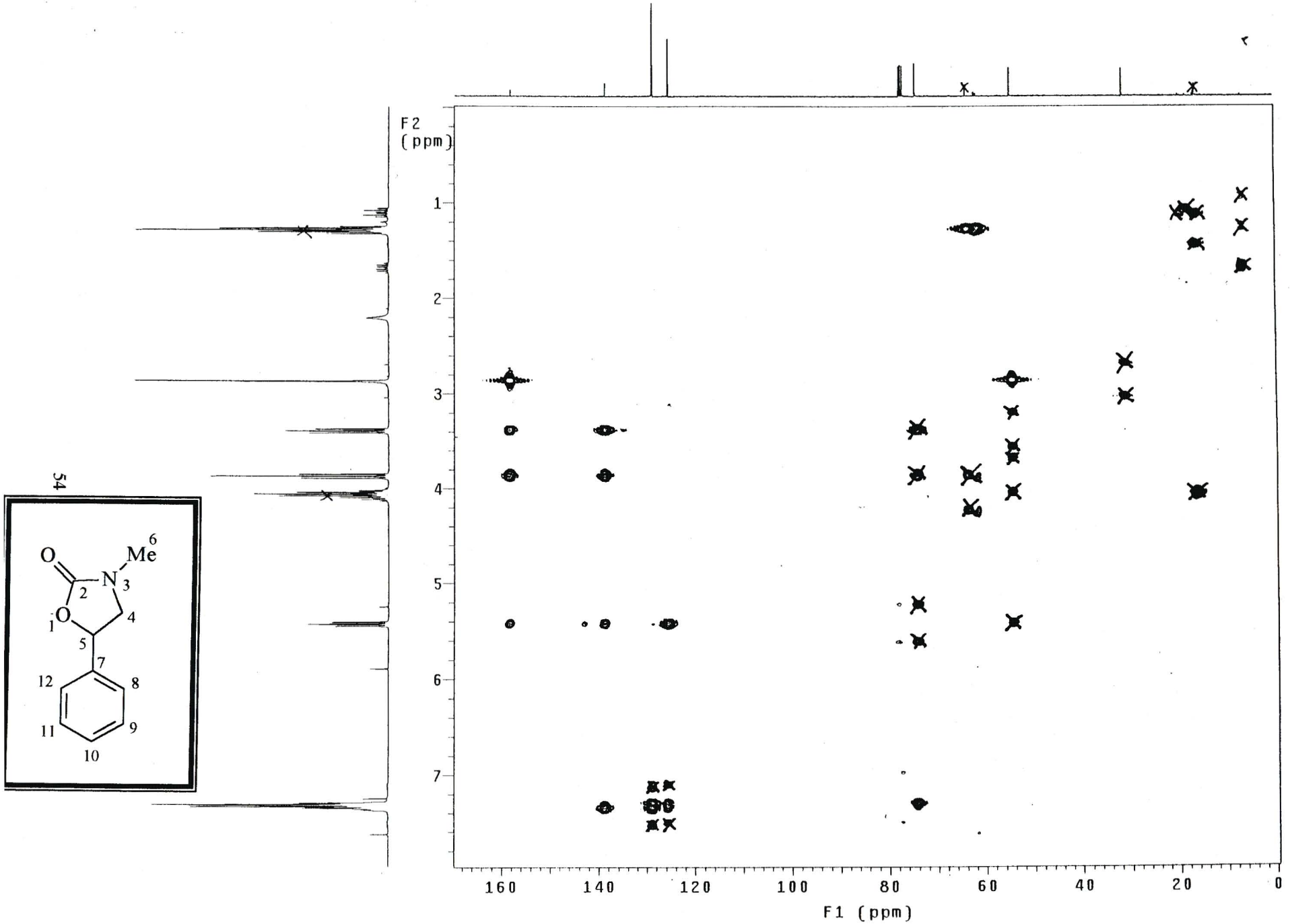
4.4 Adept spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**



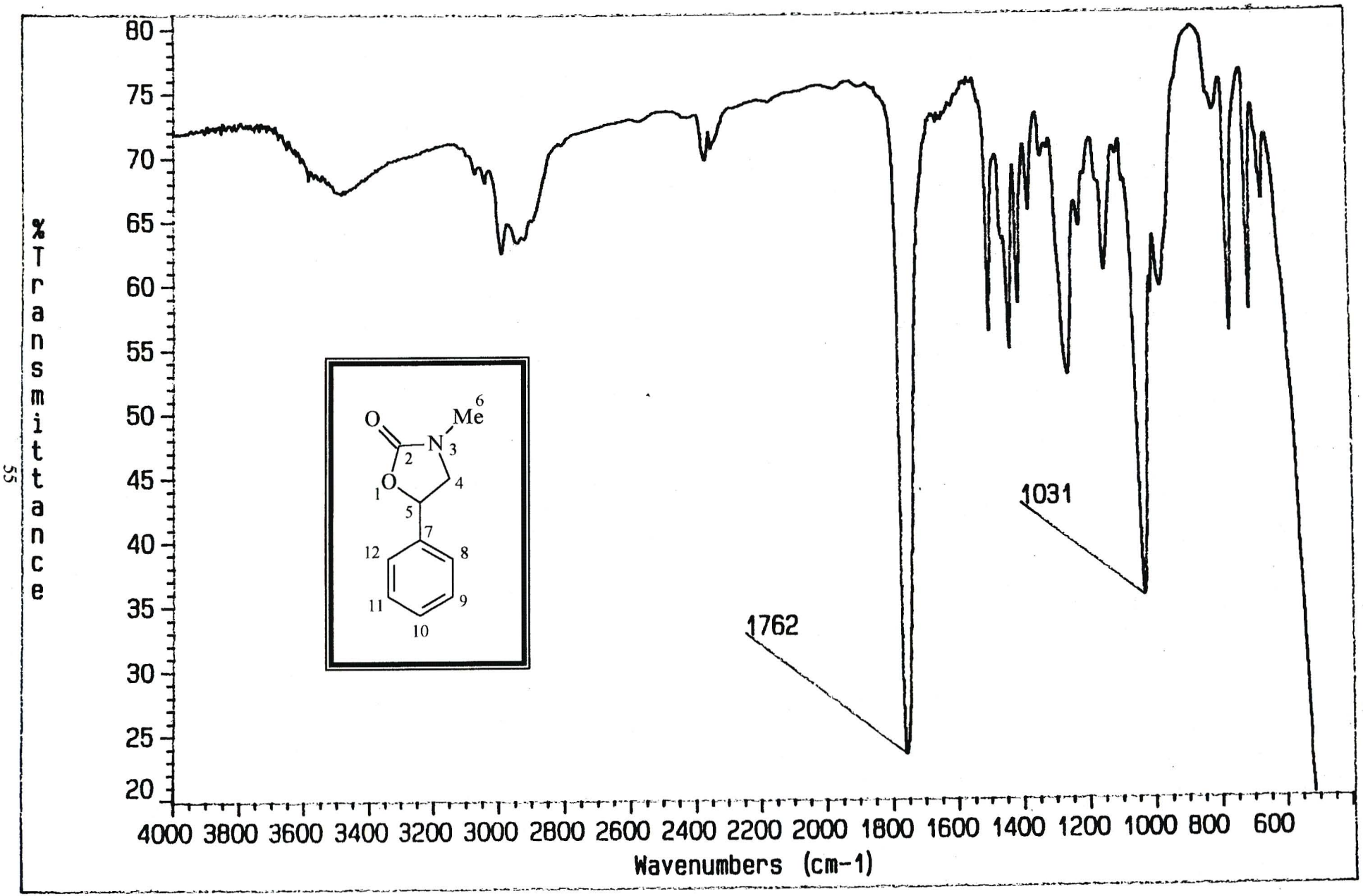
4.4 COSY spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**



4.4 NOESY spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**

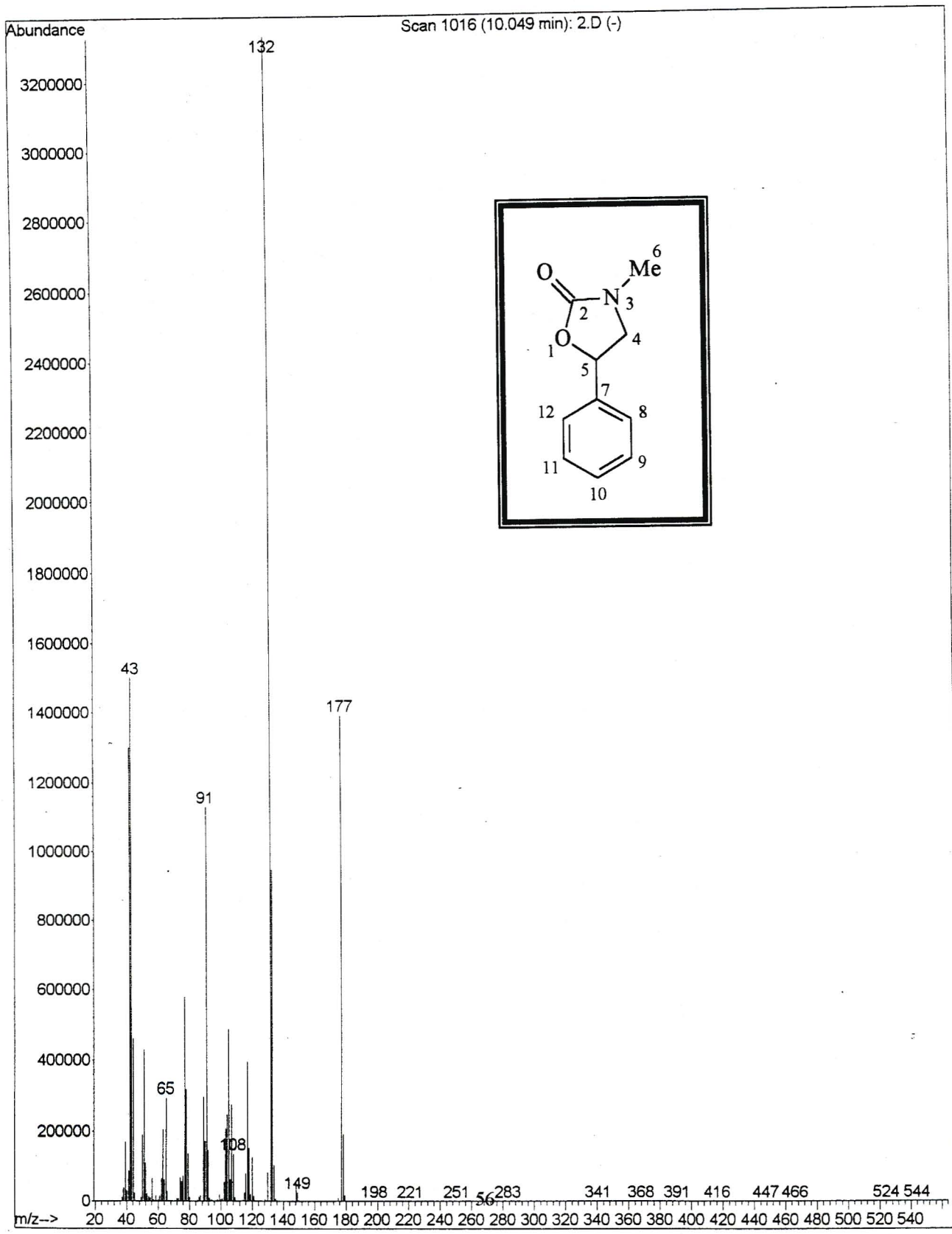


4.4 HMBC spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**

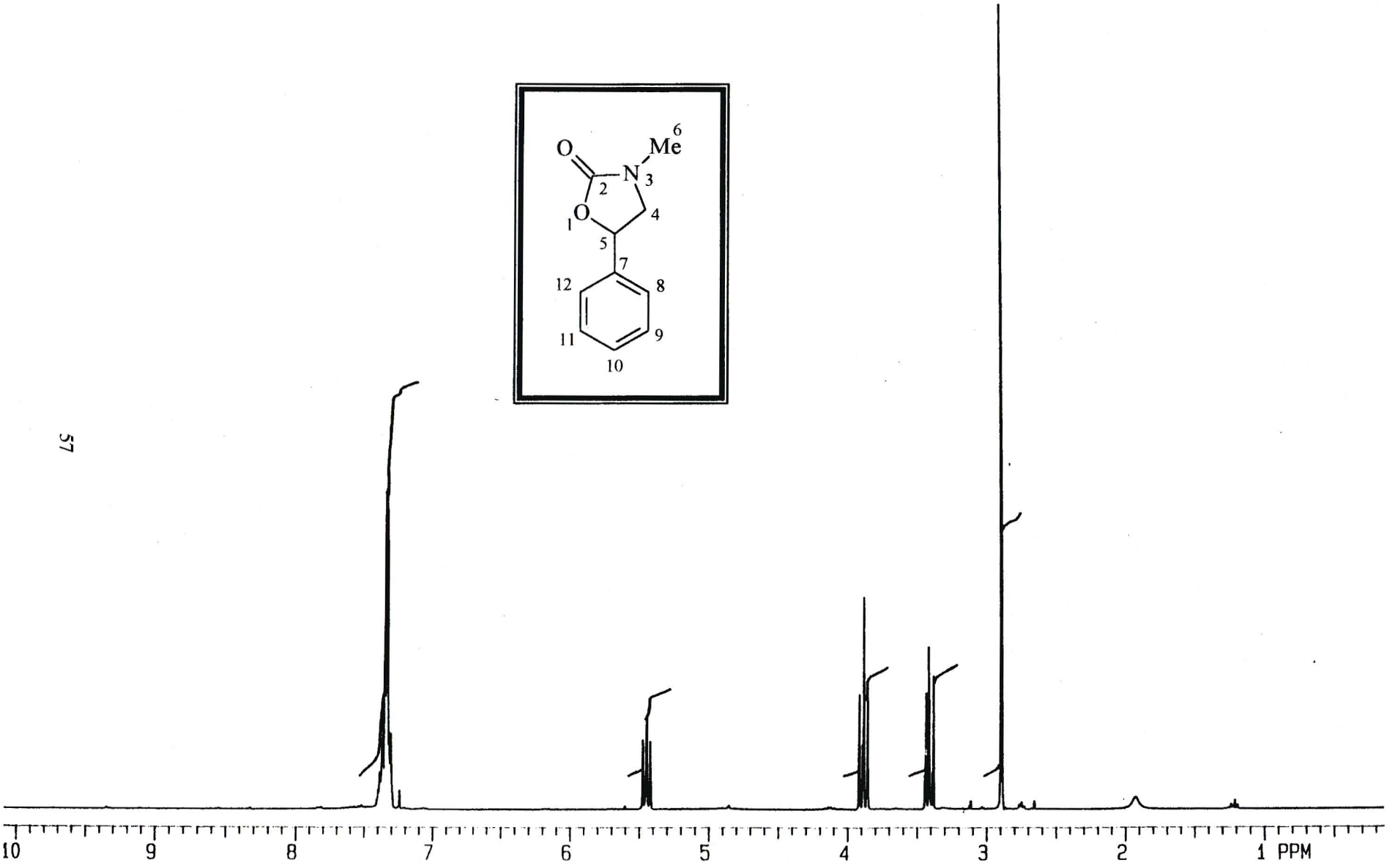


4.4 Infrared spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**

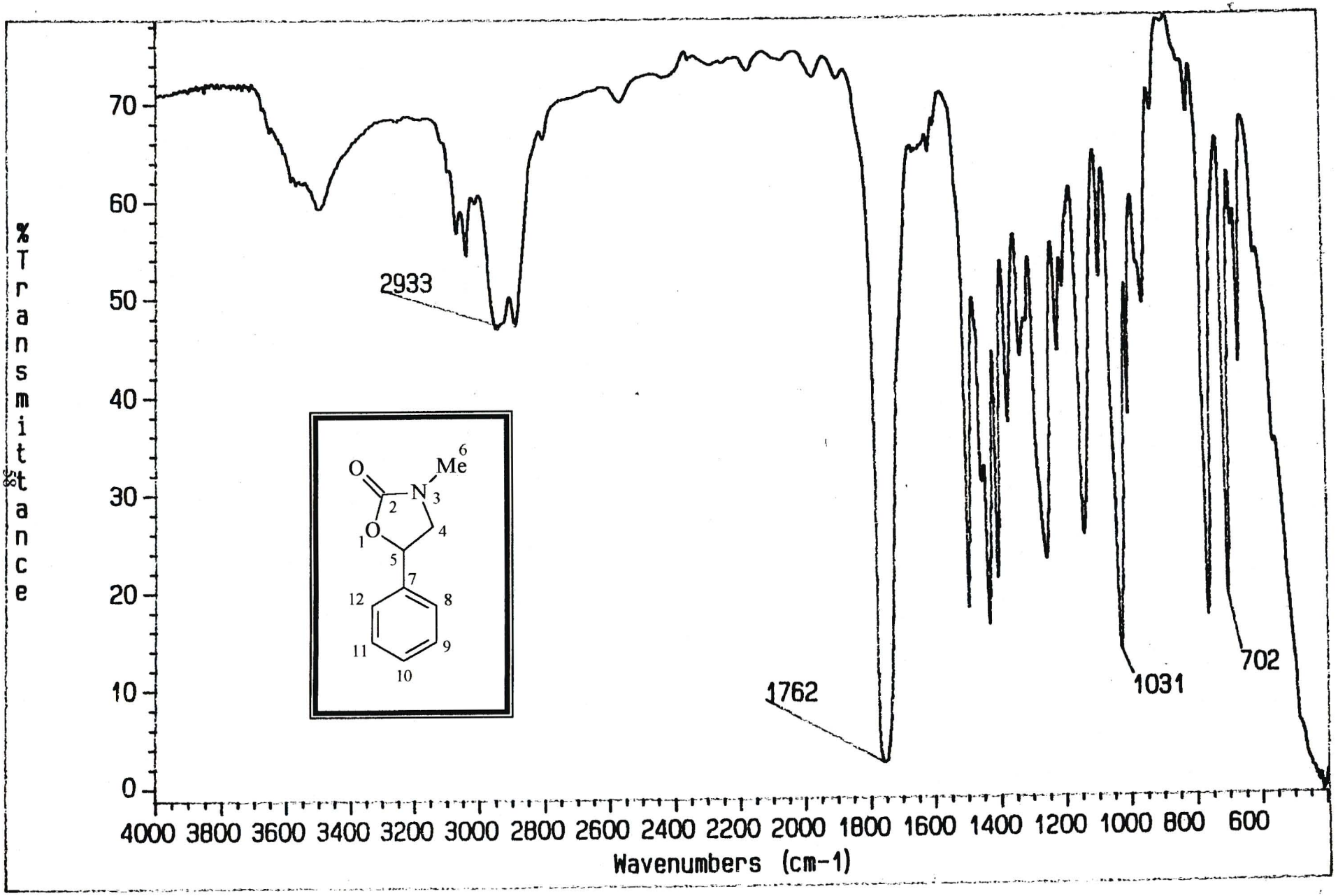
File : D:\MARTIN\2.D
Operator : Bret
Acquired : 23 Apr 2001 13:30 using AcqMethod NEW
Instrument : Instrumen
Sample Name: 2
Misc Info : 1ul inject, MeCl2, 1:75 split, 20dpm
Vial Number: 88



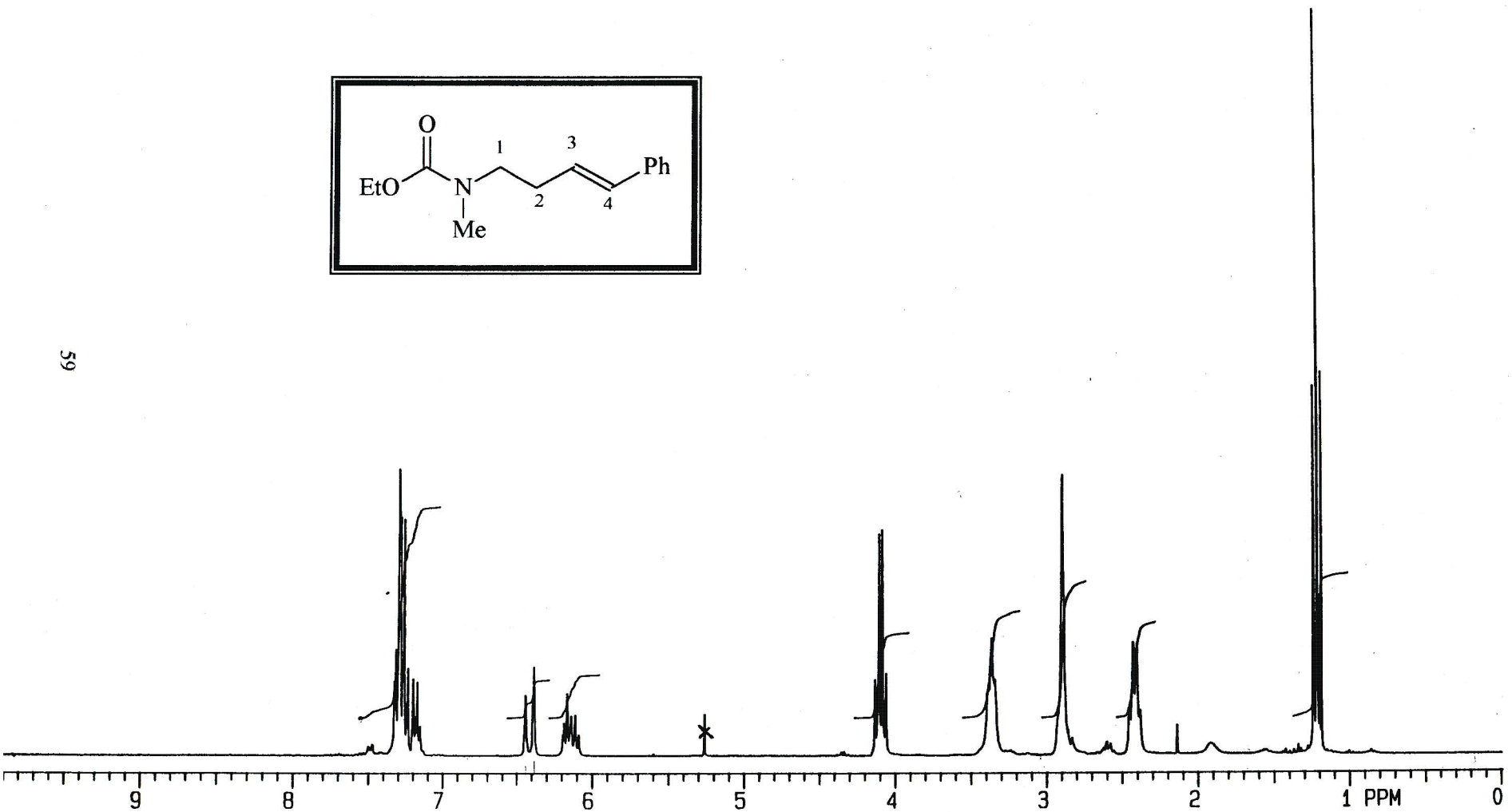
4.4 Mass spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**



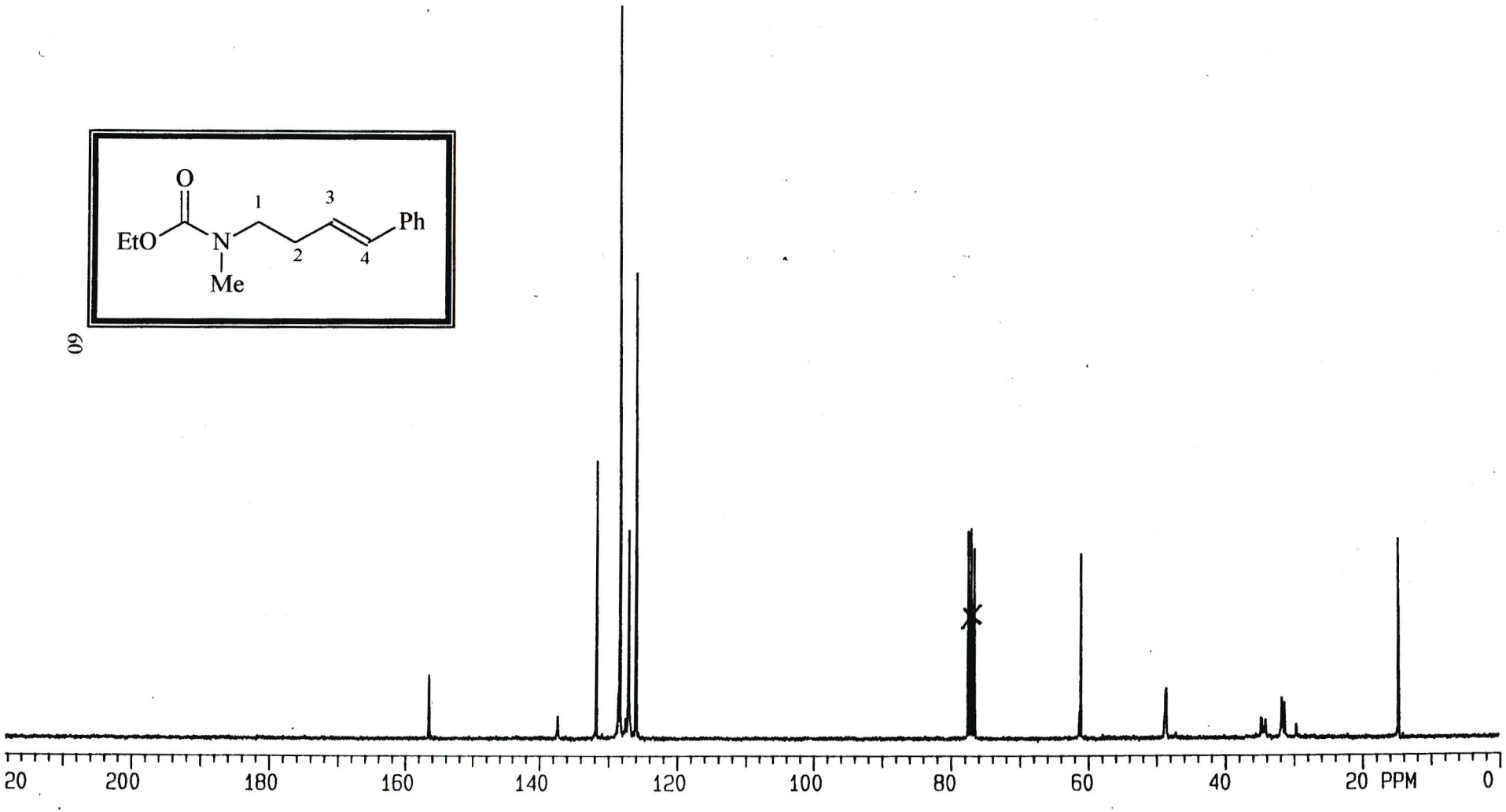
4.5 ^{1}H NMR spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**



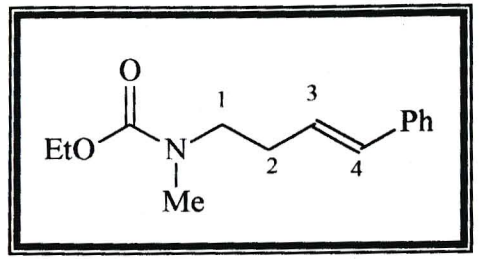
4.5 Infrared spectrum of **N-methyl-5-phenylox-3-azolidin-2-one**

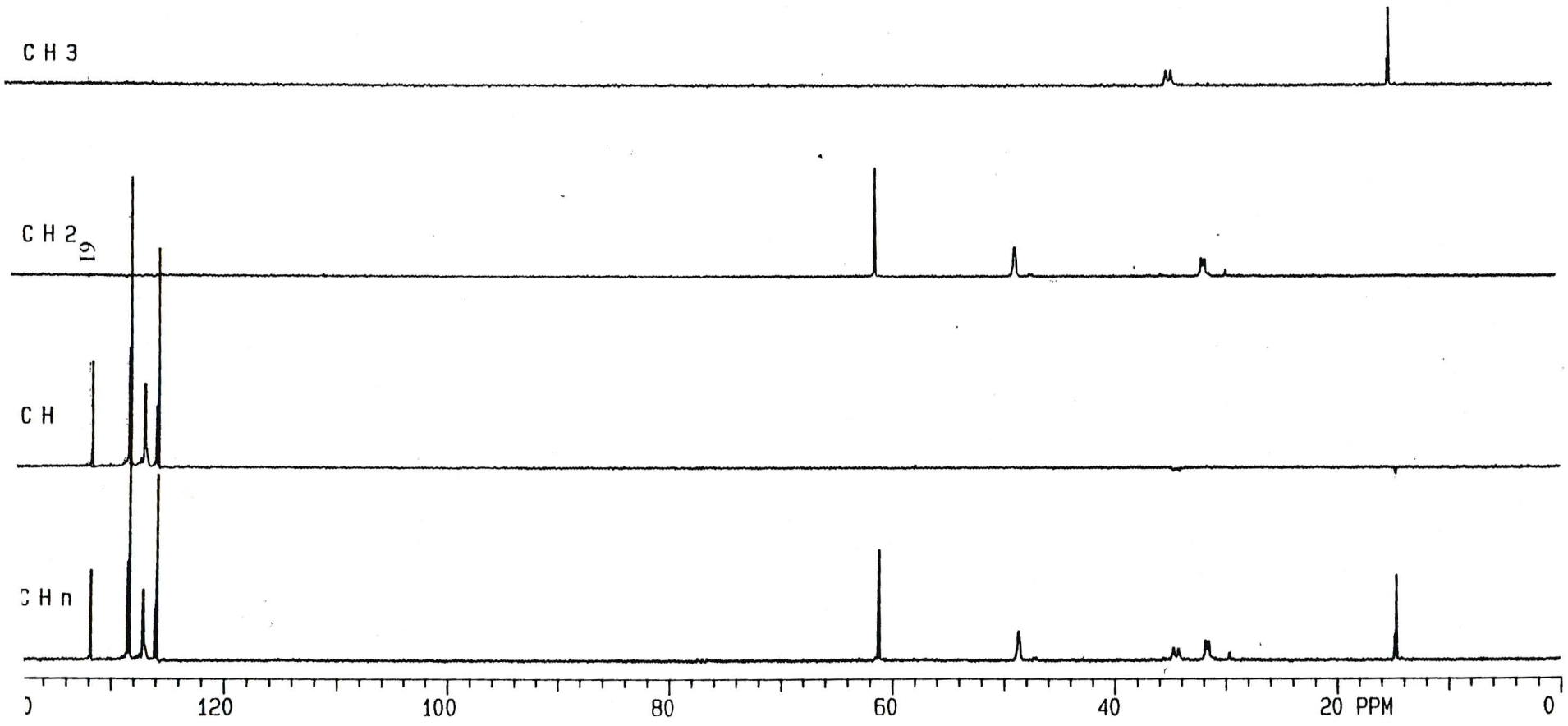


4.6 ^{1}H NMR spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**

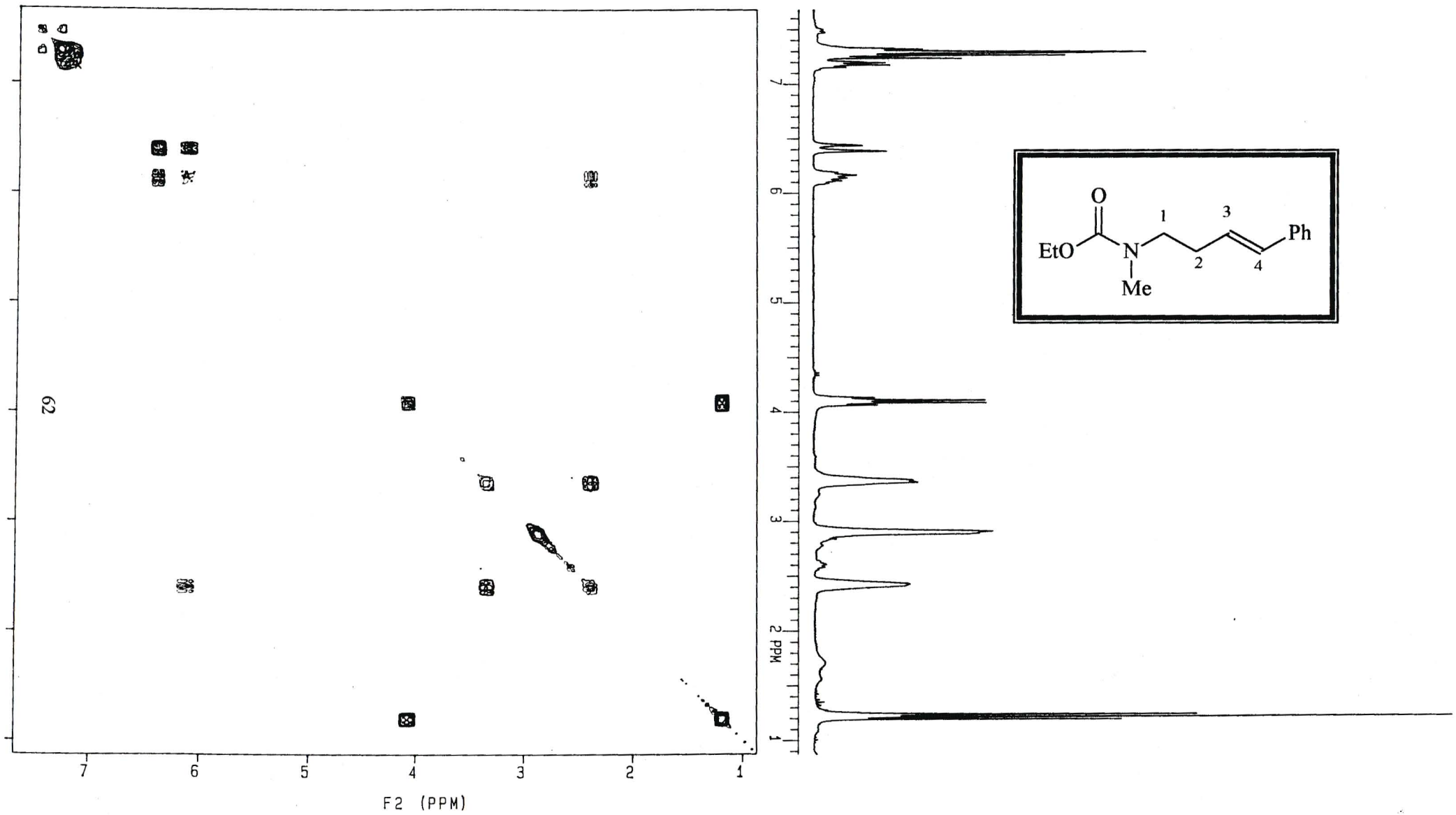


4.6 ^{13}C NMR spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**

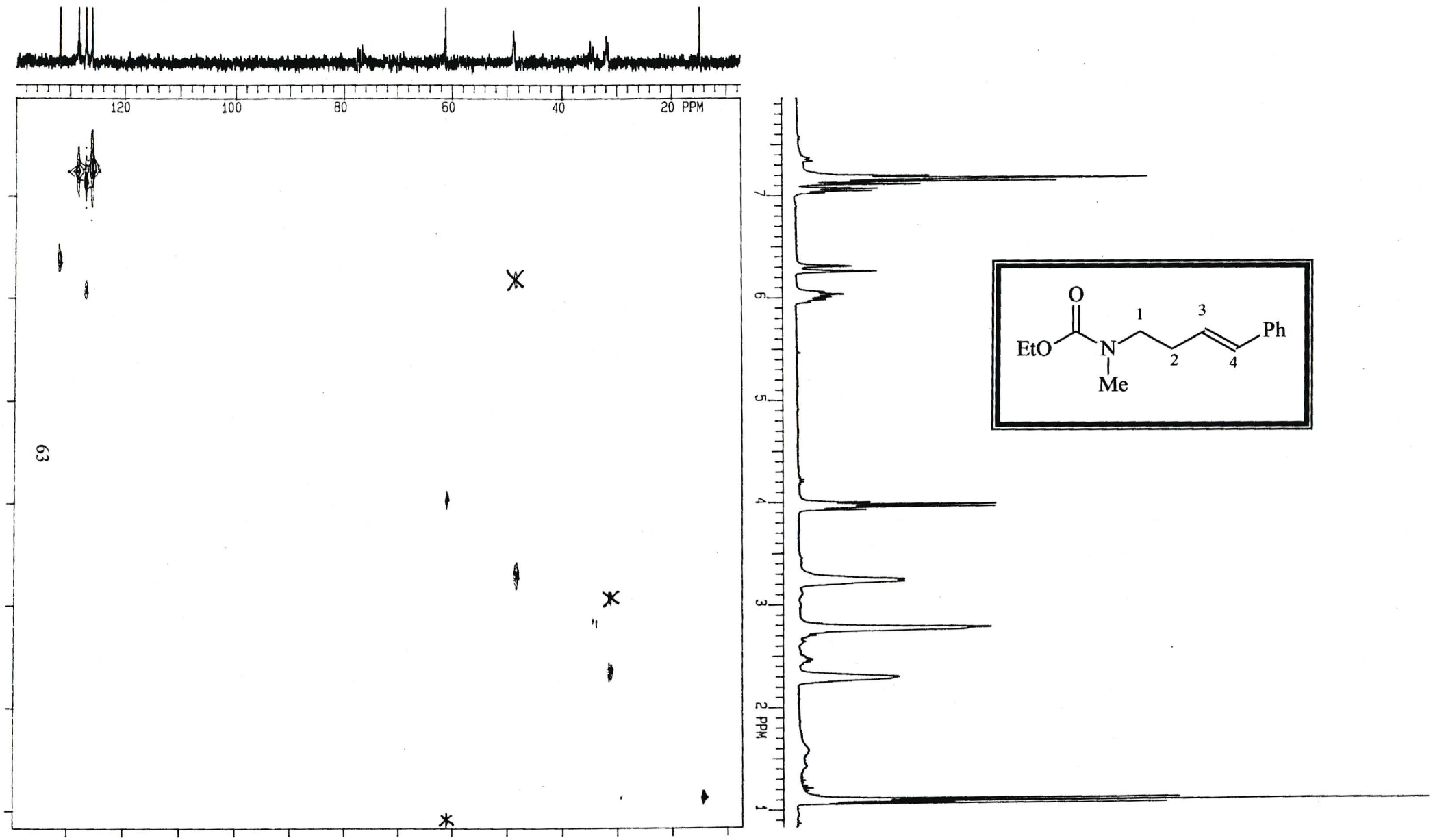




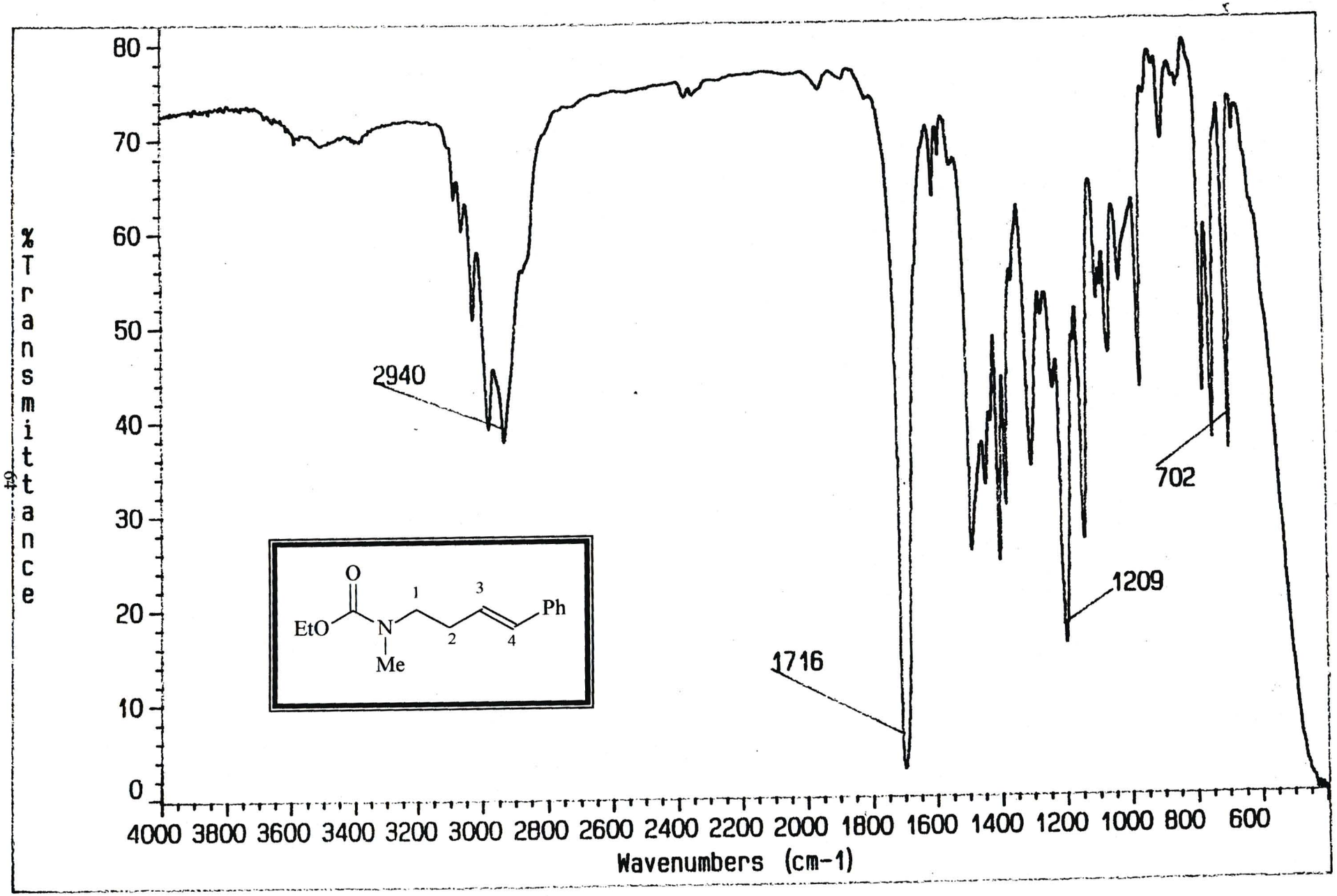
4.6 Adept spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**



4.6 COSY spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**

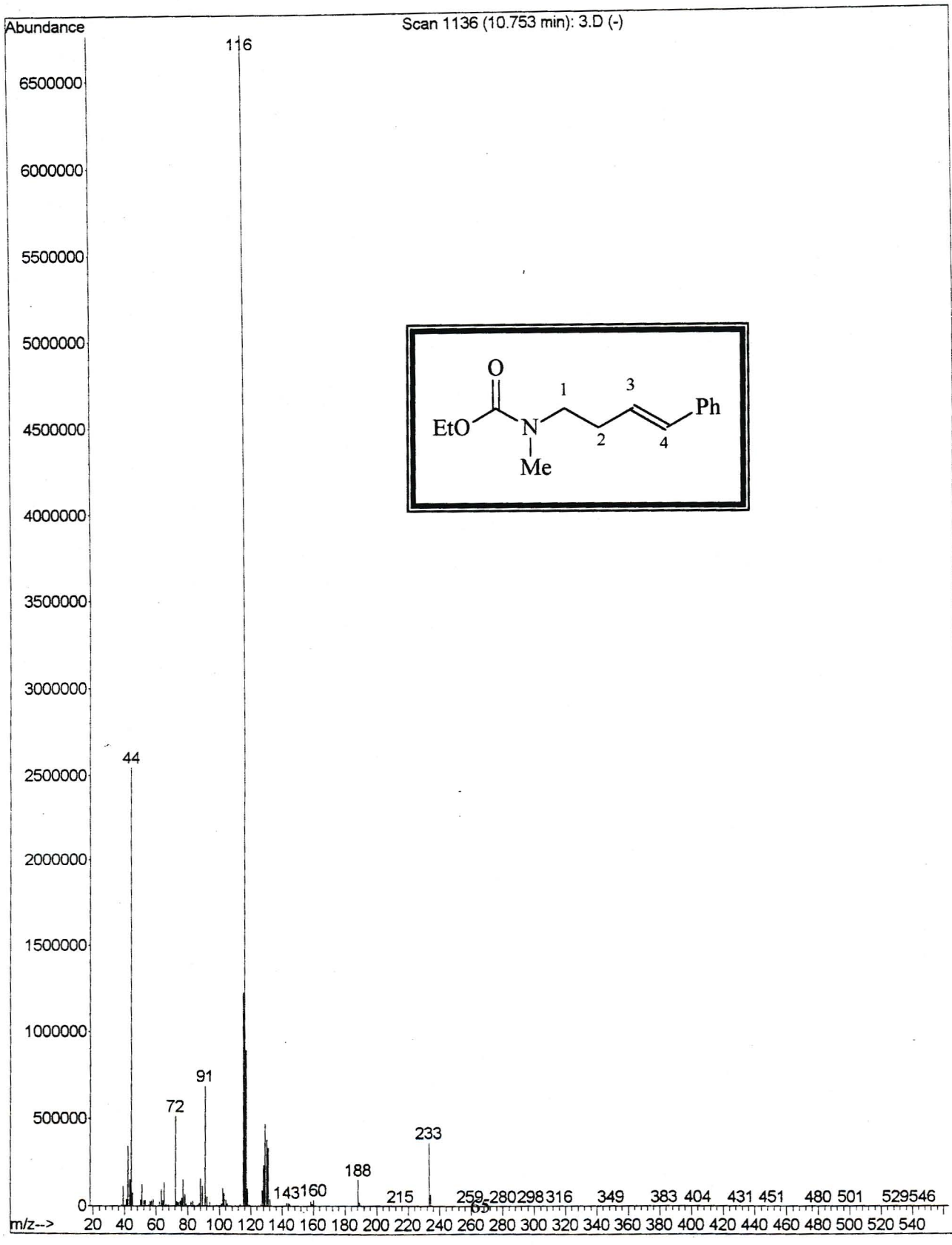


4.6 HETCOR spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**

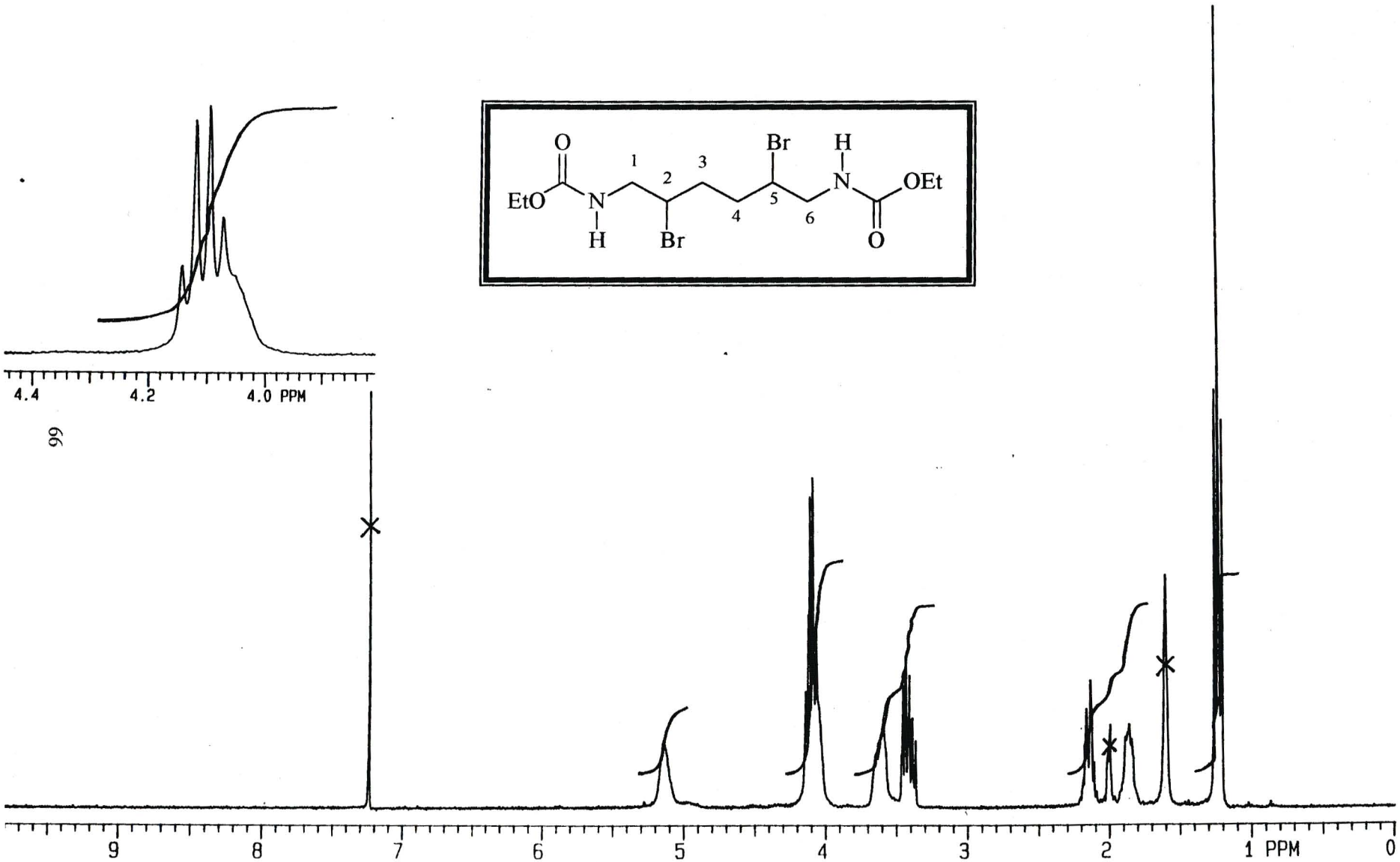


4.6 Infrared spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**

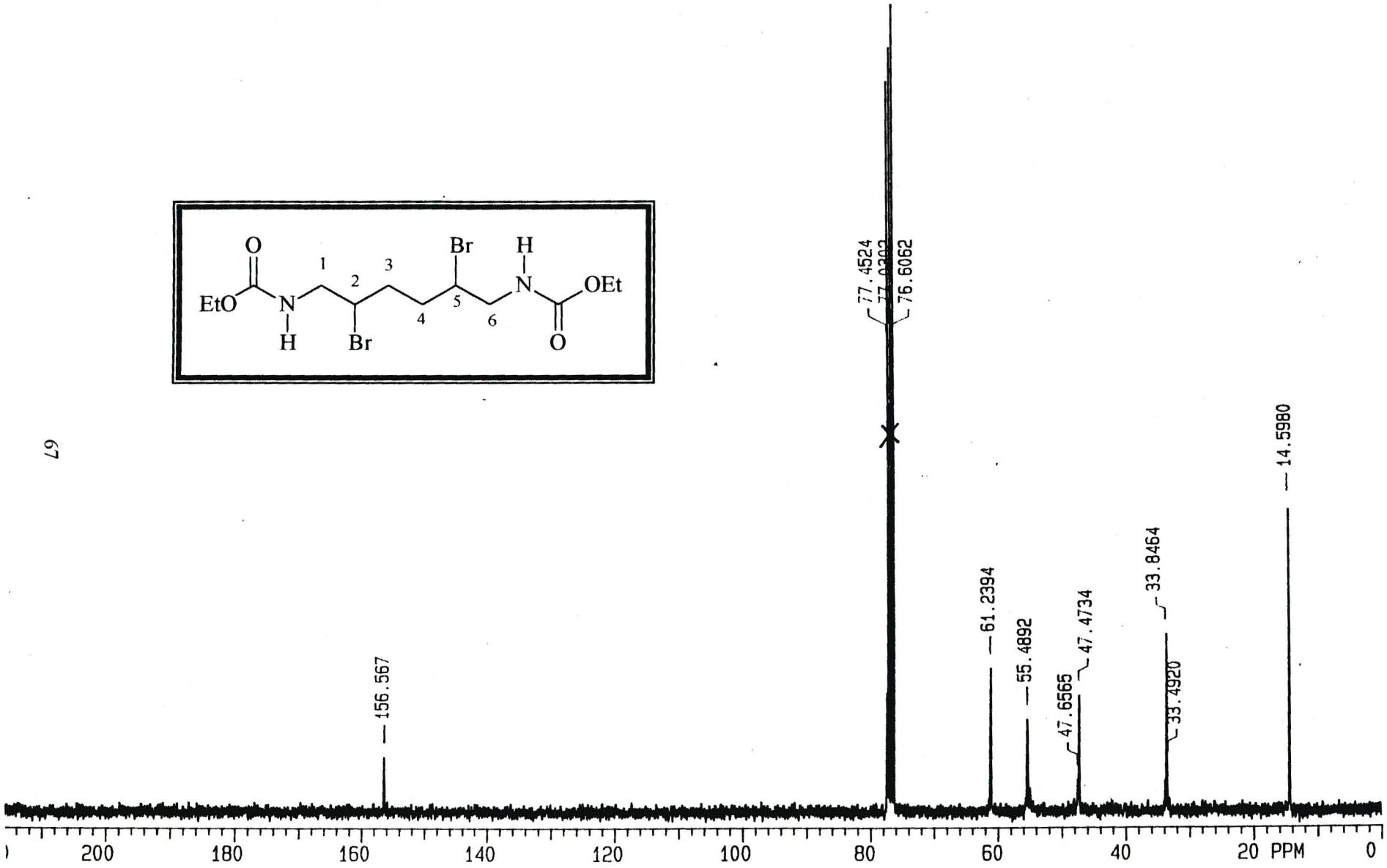
File : D:\MARTIN\3.D
Operator : Bret
Acquired : 23 Apr 2001 10:48 using AcqMethod NEW
Instrument : Instrumen
Sample Name: 3
Misc Info : 1ul inject, MeCl2, 1:75 split, 20dpm
Vial Number: 90



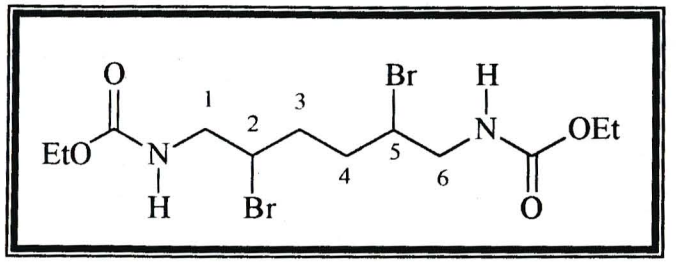
4.6 Mass spectrum of **ethyl N-methyl-N-4-phenyl-3-butenylcarbamate**

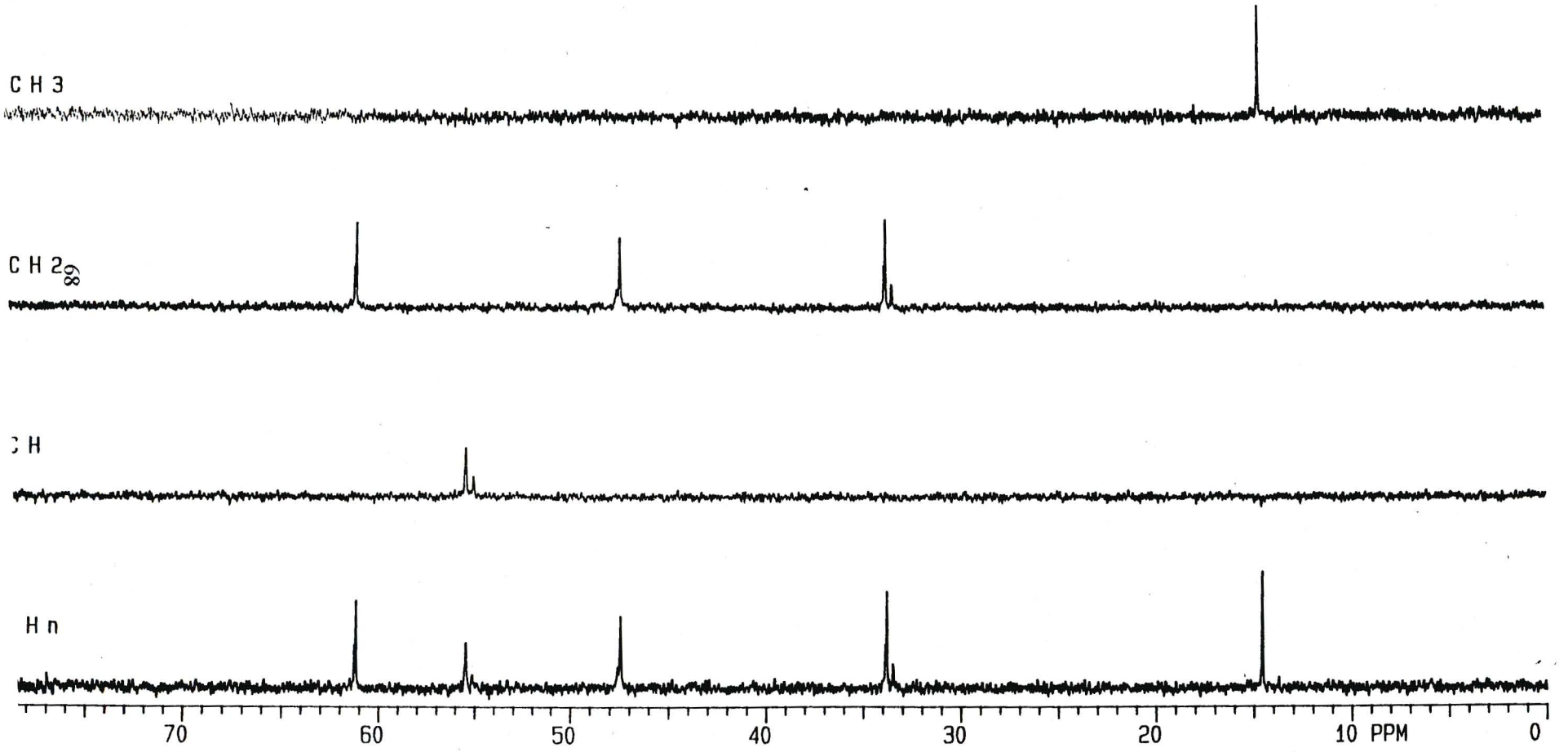


5.1 ^{1}H NMR spectrum of **N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane**

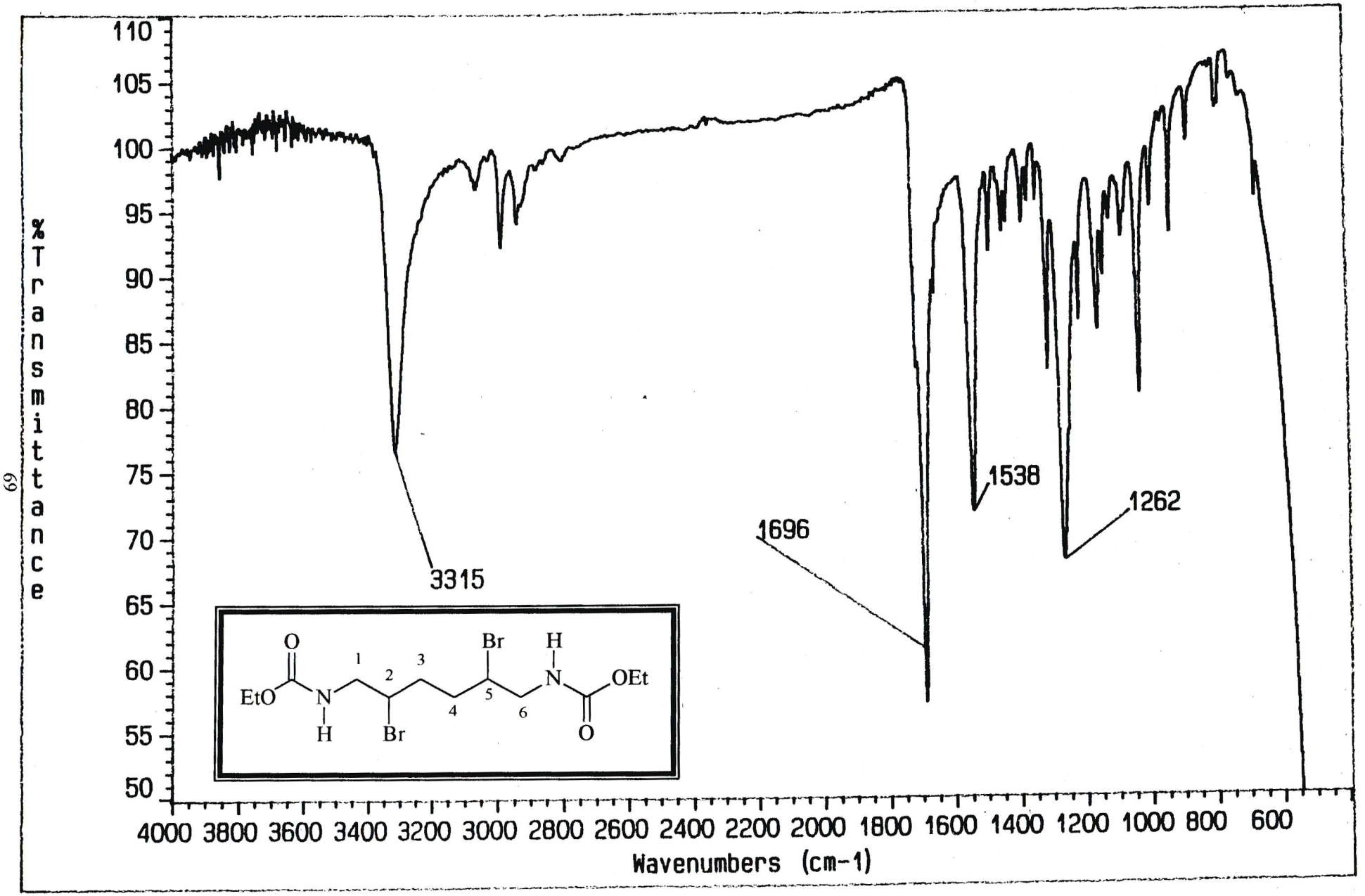


5.1 ^{13}C NMR spectrum of **N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane**



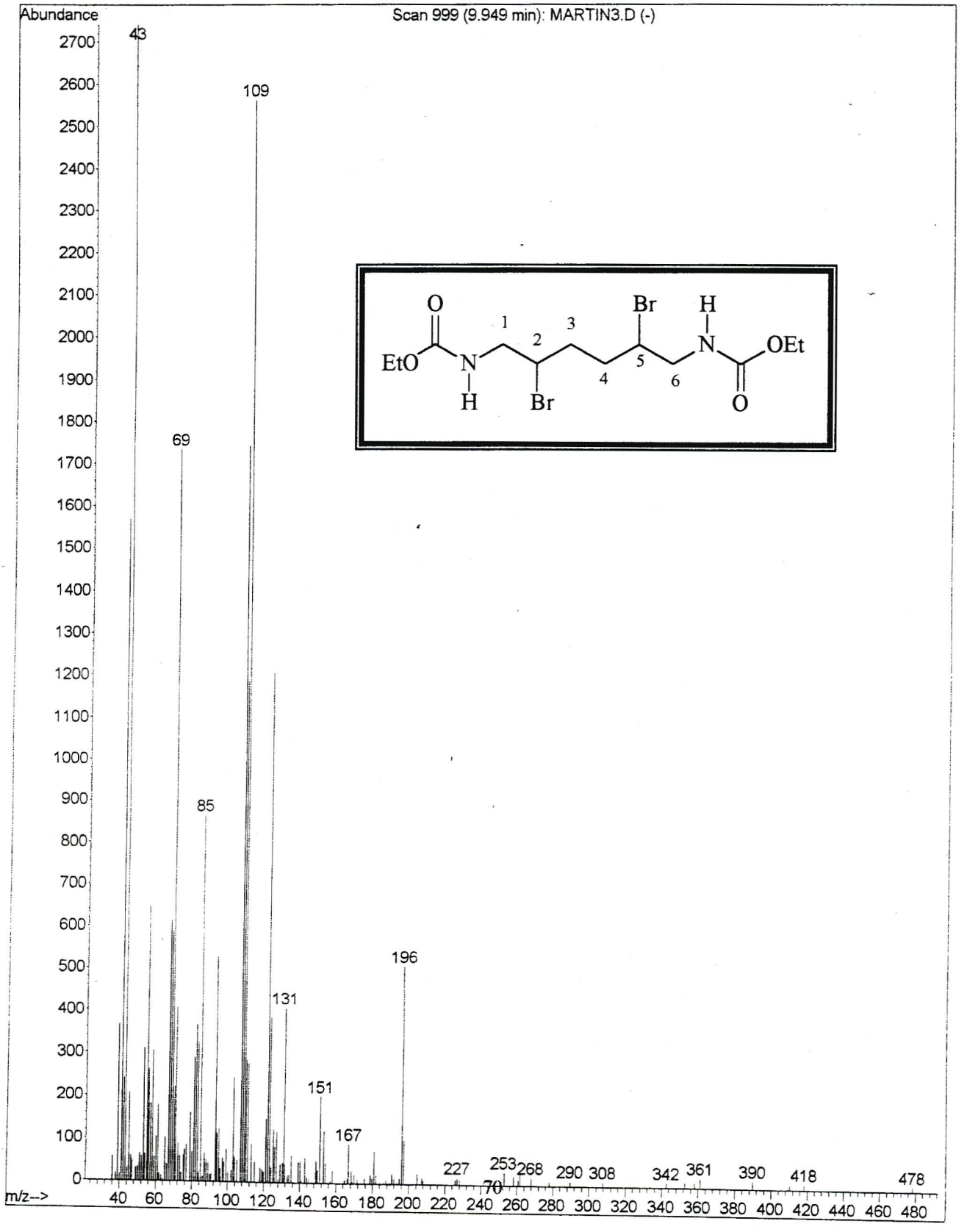


5.1 Adept spectrum of **N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane**



5.1 Infrared spectrum of **N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane**

```
File        : C:\MSDCHEM\1\DATA\MARTIN\MARTIN3.D
Operator    : Bret
Acquired    : 27 Oct 2000    9:44      using AcqMethod MARTIN
Instrument :    Instrumen
Sample Name: E6-17 pure
Misc Info   : splitless 2ul injection in dichloromethane
Vial Number: 6
```



5.1 Mass spectrum of **N,N'-1,6-bis(ethyl carbamyl)-2,5-dibromohexane**

Appendix B

(Explanation of pages found in Appendix B can be found on page 34 of chapter 1.)

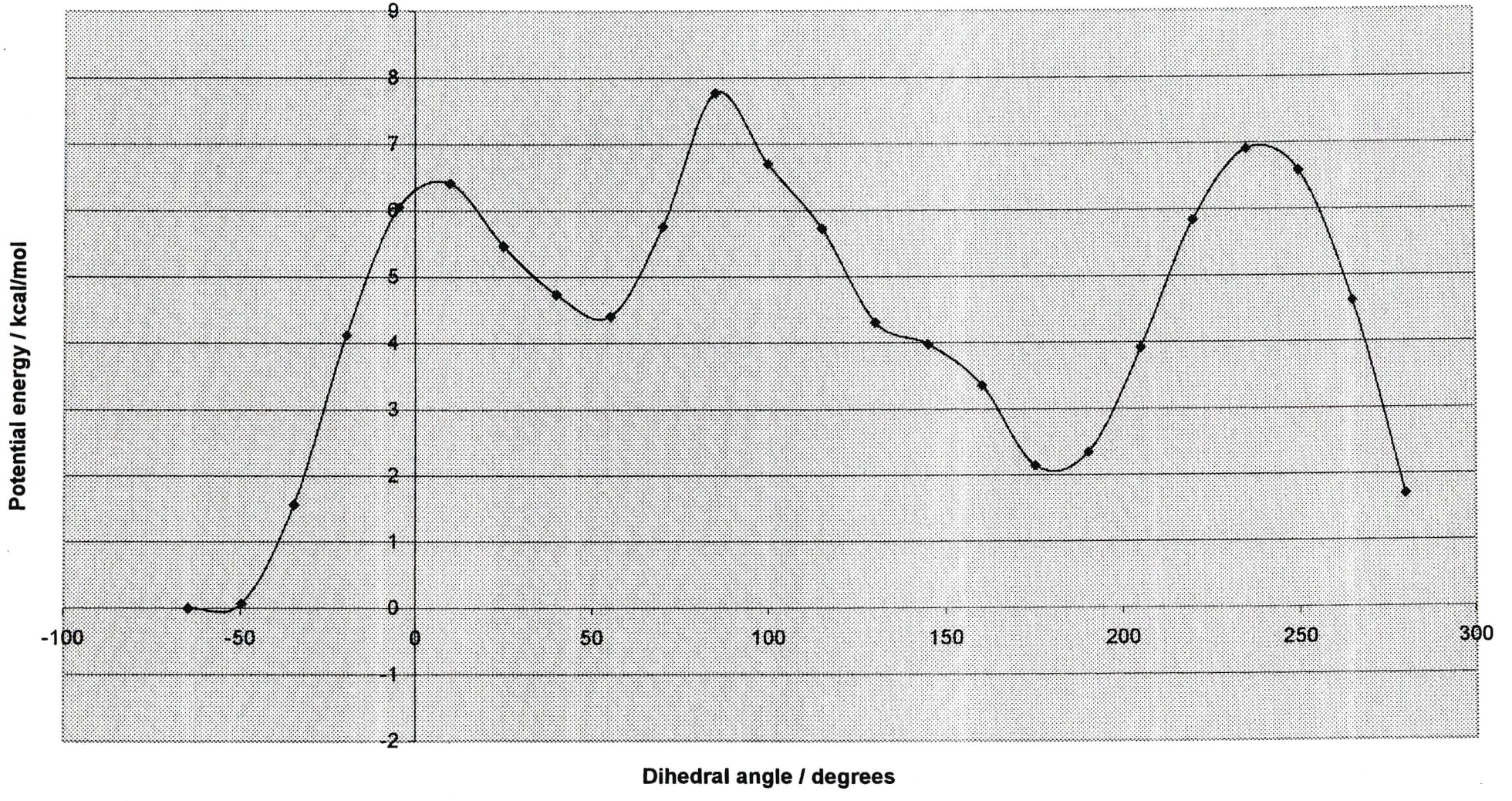
(p 1) Plot of potential energy versus dihedral angle.

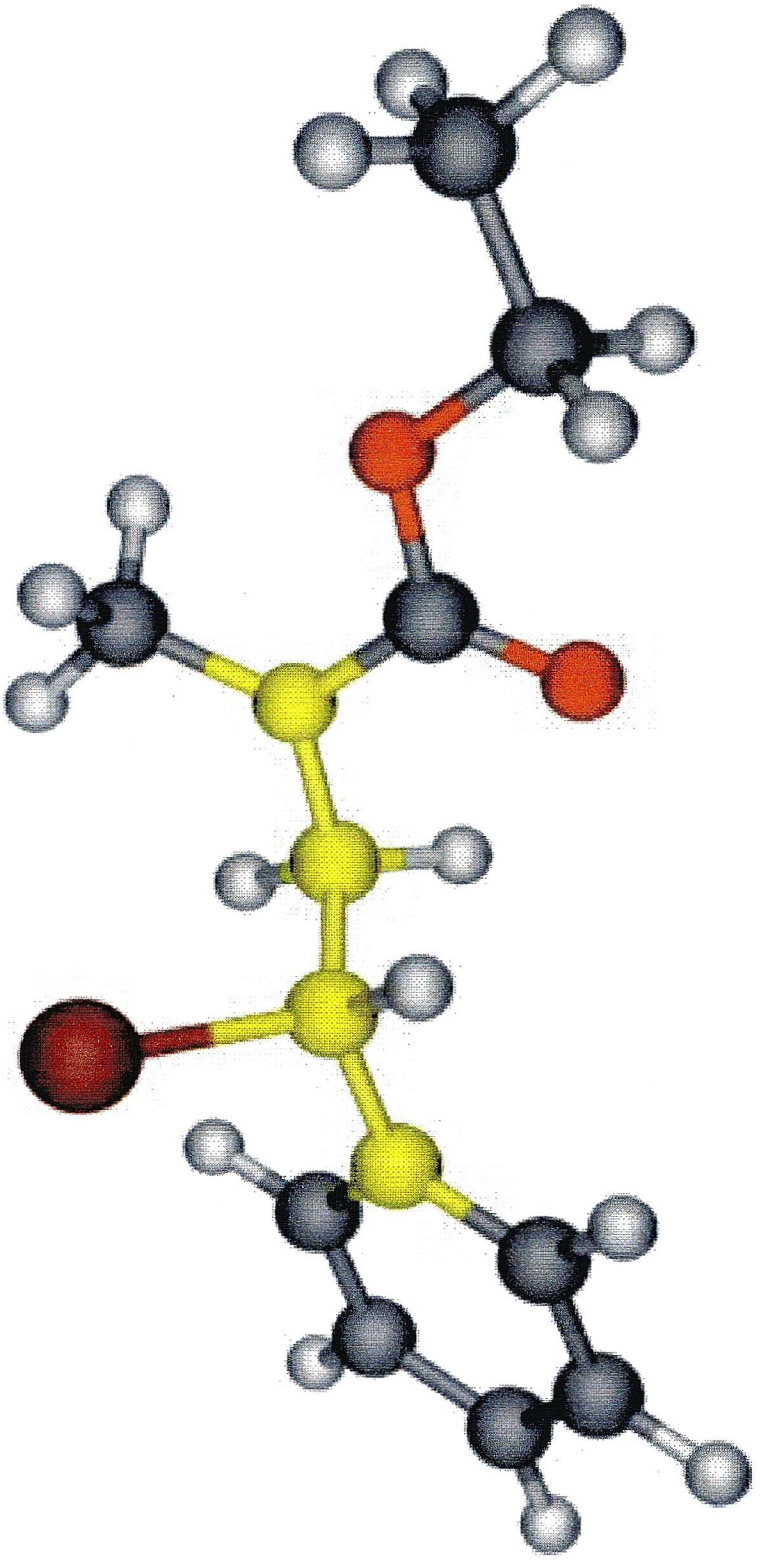
(p 2) Dihedral angle under investigation.

(p 3) Coordinates for transition state at 235°.

(p 4) Coordinates for transition state at 296° (-64°).

Plot of potential energy vs. dihedral angle





Coordinates for transition state at 235°.

%nproc=2
#p rhf/6-31+g(d) opt=(ts,noeigentest,gdiis) optcyc=100

Styrene adduct optimized at rhf/6-31+g(d)

0 1
C,-5.9201469212,-1.2676258057,-0.364266508
C,-4.9528954428,-0.2158600781,0.1349601849
O,-3.6388047983,-0.7584110658,0.0217537976
C,-2.6313669473,0.0101814768,0.4112734436
O,-2.7827451796,1.1061908235,0.8726183257
N,-1.4281281194,-0.5901357849,0.2129614444
C,-1.3384429224,-1.9994988422,-0.1390388637
C,-0.2441557816,0.0382770976,0.7700009614
C,0.8302717126,0.3018401315,-0.3153031255
Br,1.1721297706,2.2468548916,-0.426904438
C,2.1368401818,-0.4233631155,-0.1173984606
C,2.8311142861,-0.3417009847,1.0885880185
C,3.9993866098,-1.0562431706,1.2775192206
C,4.5042674104,-1.8556201867,0.2578035444
C,3.8263877836,-1.9344665709,-0.9457872254
C,2.6450157797,-1.2261507525,-1.1295371883
H,-5.7210999589,-1.5183550746,-1.4003965165
H,-6.9365959712,-0.8924972344,-0.2932417802
H,-5.8487447952,-2.1733106965,0.2279483166
H,-5.1372263803,0.0398701469,1.1686642008
H,-5.0076721362,0.6902260934,-0.4517819804
H,-0.3267682926,-2.2130363445,-0.4550417513
H,-2.0082011161,-2.2357270084,-0.9505904654
H,-1.573069091,-2.6404257485,0.7065638945
H,0.1717604436,-0.6130112157,1.5312165345
H,-0.5483904649,0.9531037862,1.2434132477
H,0.4220221565,0.0718404227,-1.2836468866
H,2.4645104201,0.2927031037,1.8759806518
H,4.5233617557,-0.9837646806,2.2139014145
H,5.4165421843,-2.4058566236,0.4034640456
H,4.2095949666,-2.5465117685,-1.7428168133
H,2.1270764756,-1.2950711244,-2.0705718902

Coordinates for lowest energy state at 296° (-64°).

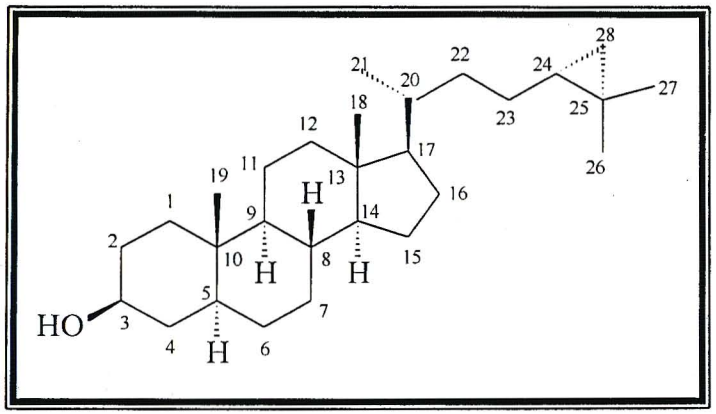
%nproc=2
#p rhf/6-31+g(d) opt optcyc=200

Styrene adduct optimized at rhf/6-31+g(d)

0 1
C,5.6308813681,0.3906319569,-0.1997334847
C,4.4218271004,-0.4168080444,-0.6204412487
O,3.3485673605,-0.0608982508,0.248419114
C,2.1918308208,-0.6798658581,0.0674389712
O,2.0247365771,-1.5344716605,-0.761763623
N,1.2207390225,-0.2452903259,0.9027632515
C,1.4608455568,0.7387148121,1.9471977943
C,-0.023135154,-0.9978002222,0.9164835806
C,-0.9349137166,-0.6386878234,-0.2604738132
Br,-2.4676156478,-1.8808226188,-0.1637317525
C,-1.3931680881,0.7960266826,-0.2952621908
C,-2.1581562582,1.3533669018,0.7277180724
C,-2.497710597,2.6943822191,0.7000199467
C,-2.0853681763,3.5010661832,-0.3543957817
C,-1.3257314364,2.9539497713,-1.3735901408
C,-0.9763485626,1.6105801428,-1.3402465525
H,5.4366689939,1.4543579832,-0.2824021593
H,6.4736002299,0.1467274669,-0.8394368401
H,5.9060925021,0.1707156856,0.8258276087
H,4.6012613553,-1.4791024215,-0.5377422652
H,4.1315006127,-0.2020230279,-1.6395362874
H,0.5470617445,1.2867142707,2.1270646169
H,2.220808877,1.4355682387,1.6360164544
H,1.7765474502,0.2655679518,2.8735009408
H,-0.5291382204,-0.7960330206,1.8497340993
H,0.1914048115,-2.0553328274,0.8762402389
H,-0.4456784598,-0.9019622952,-1.1805982896
H,-2.5043858667,0.7355063012,1.5370333687
H,-3.0932081333,3.1097103622,1.4936755538
H,-2.3574840844,4.5411312673,-0.378271906
H,-1.0021123189,3.5680995584,-2.1951617047
H,-0.3831136205,1.1957974049,-2.1362096962

Appendix C

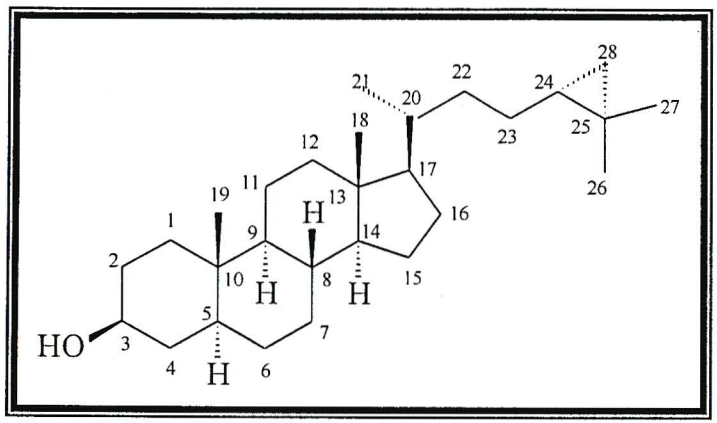
List of spectra (chapter 2)

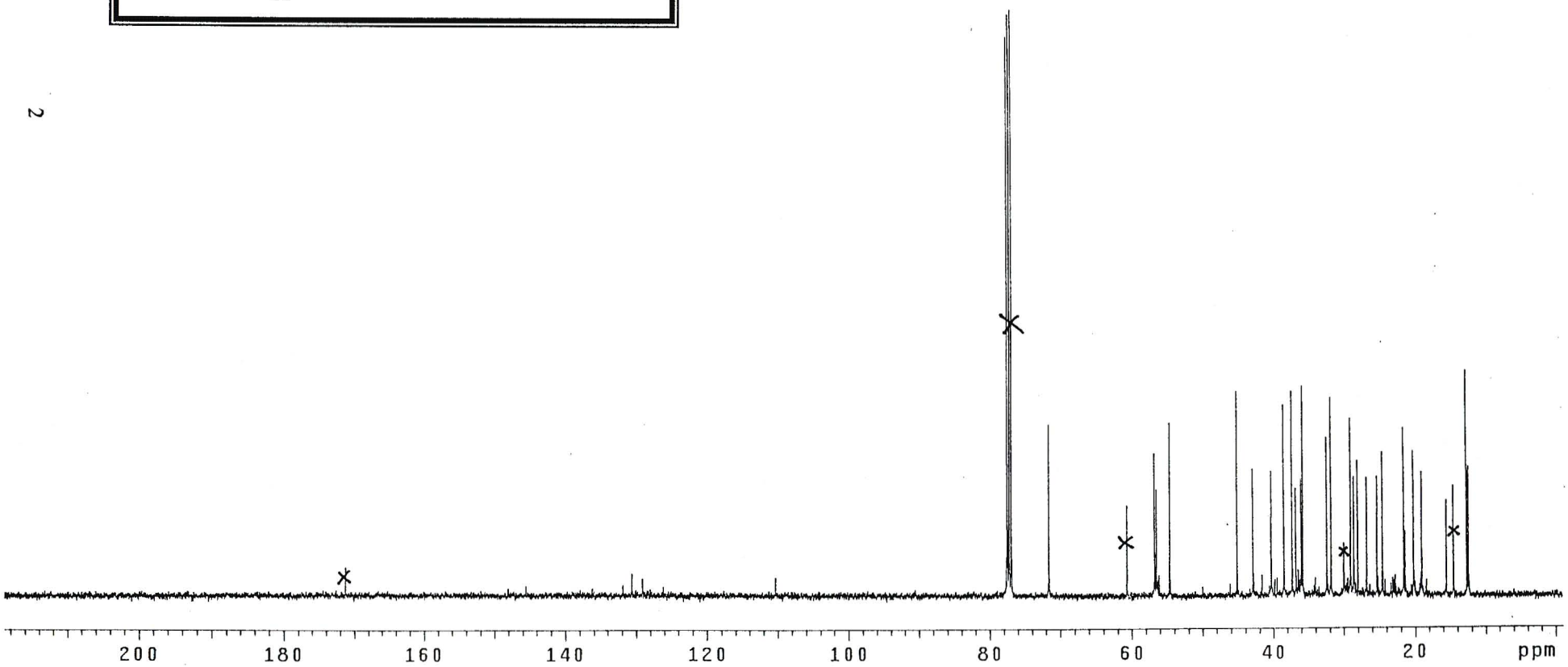


X = ethyl acetate

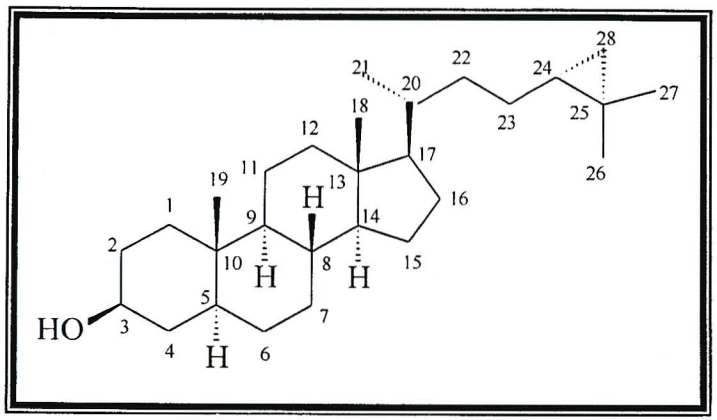
ppm

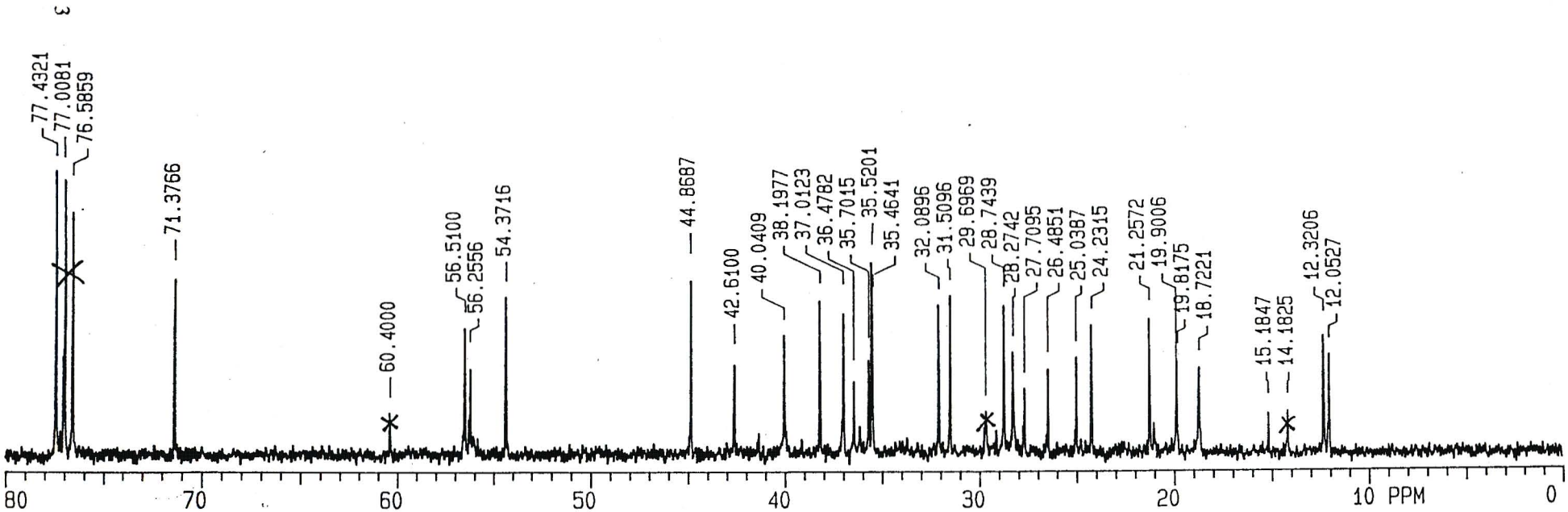
^{1}H NMR spectrum of **Compound 1**



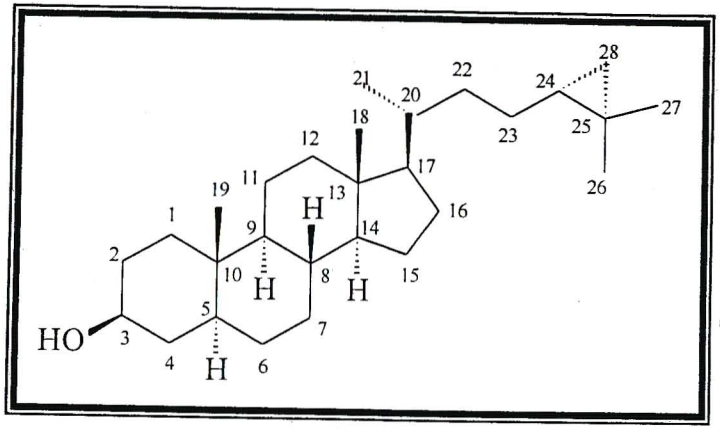


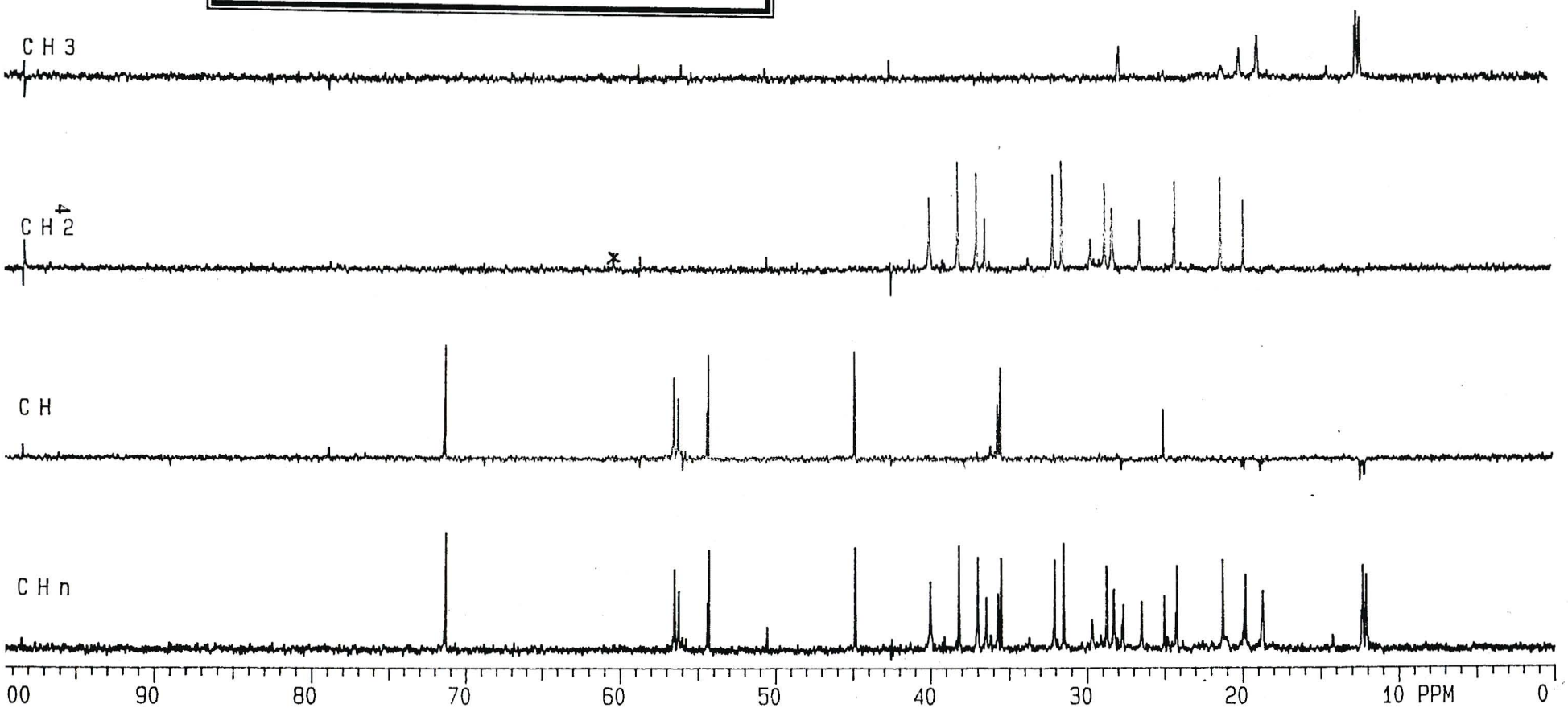
^{13}C NMR spectrum of **Compound 1**



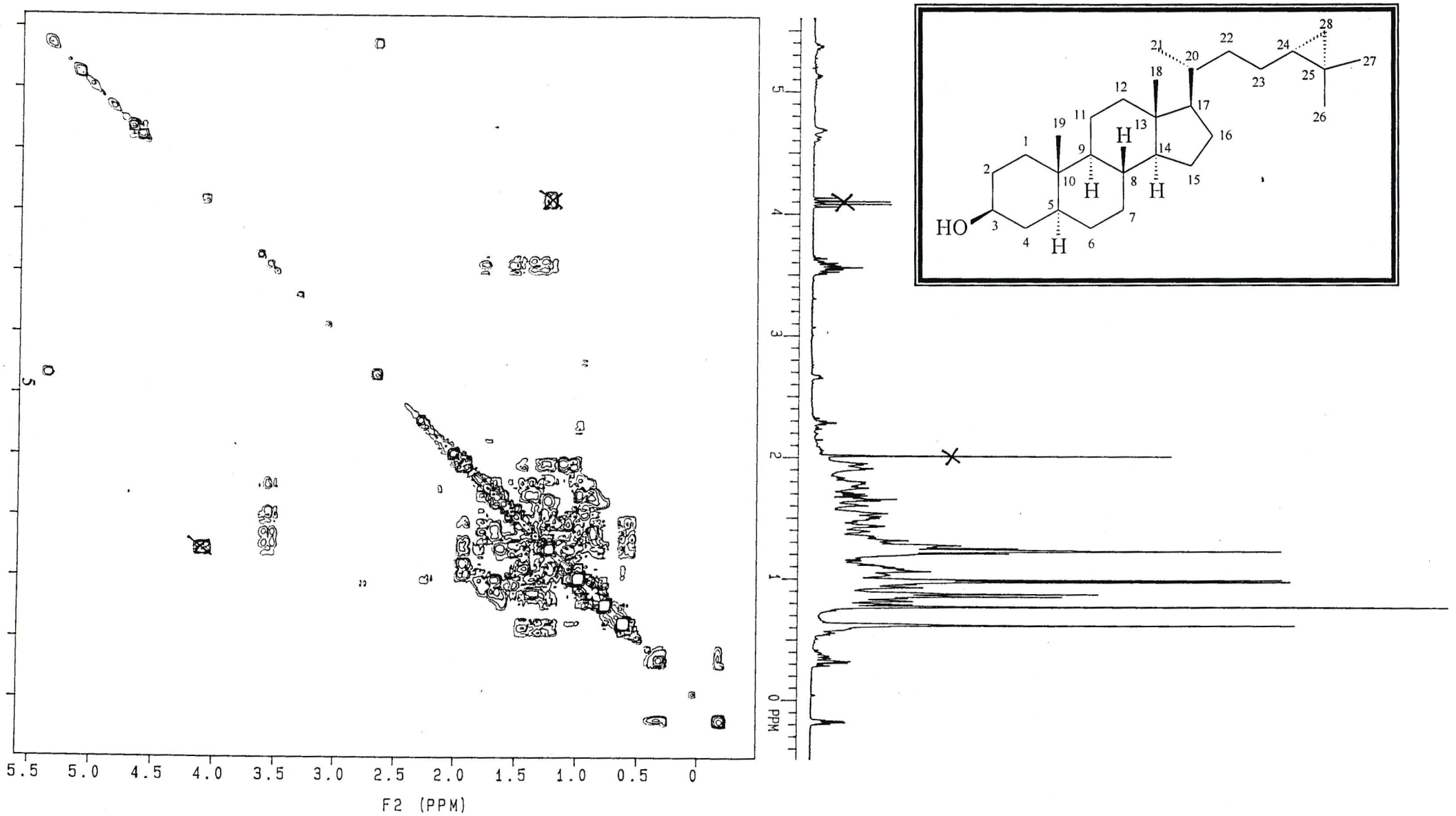


Expanded ^{13}C NMR spectrum of **Compound 1**

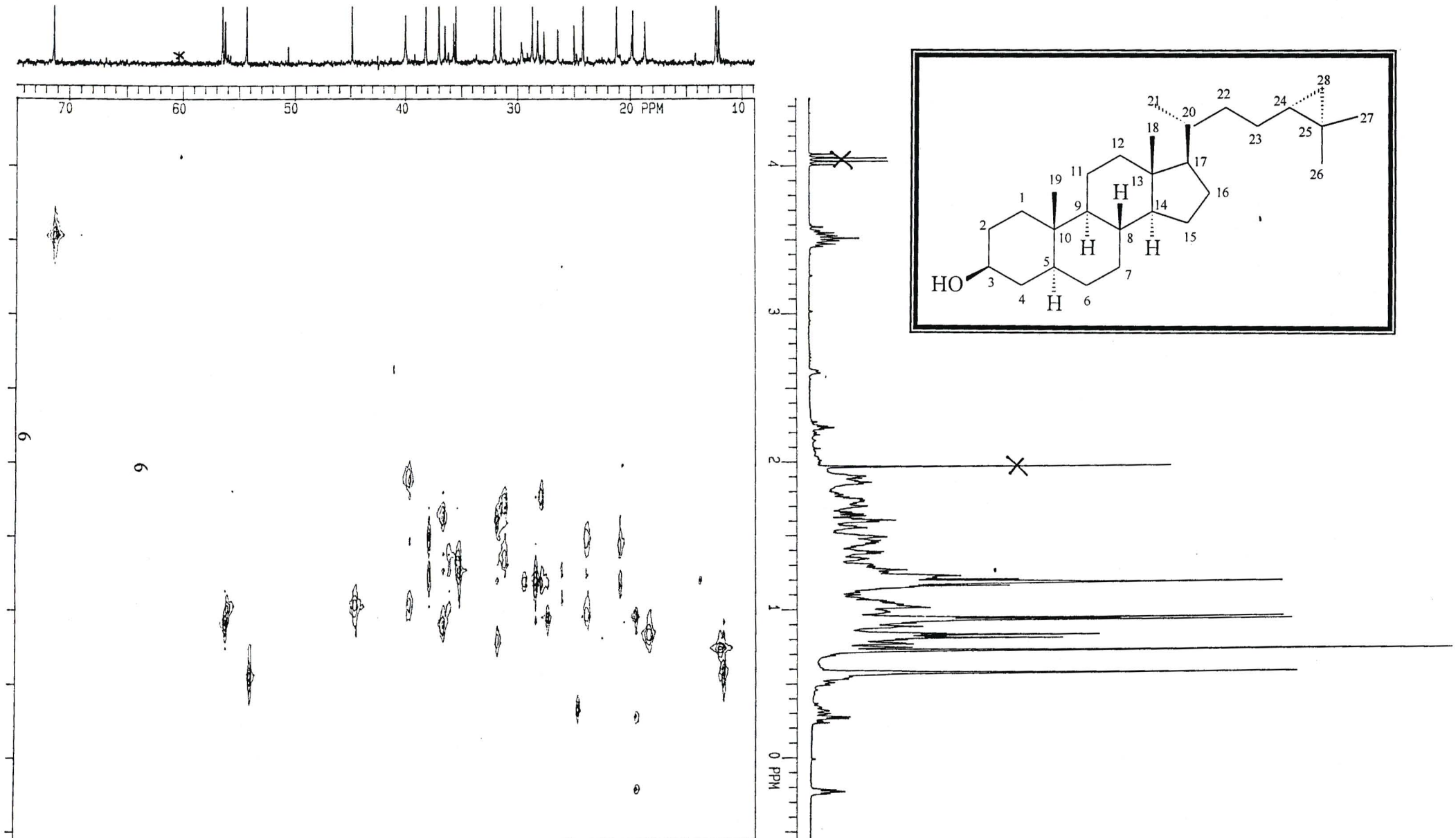




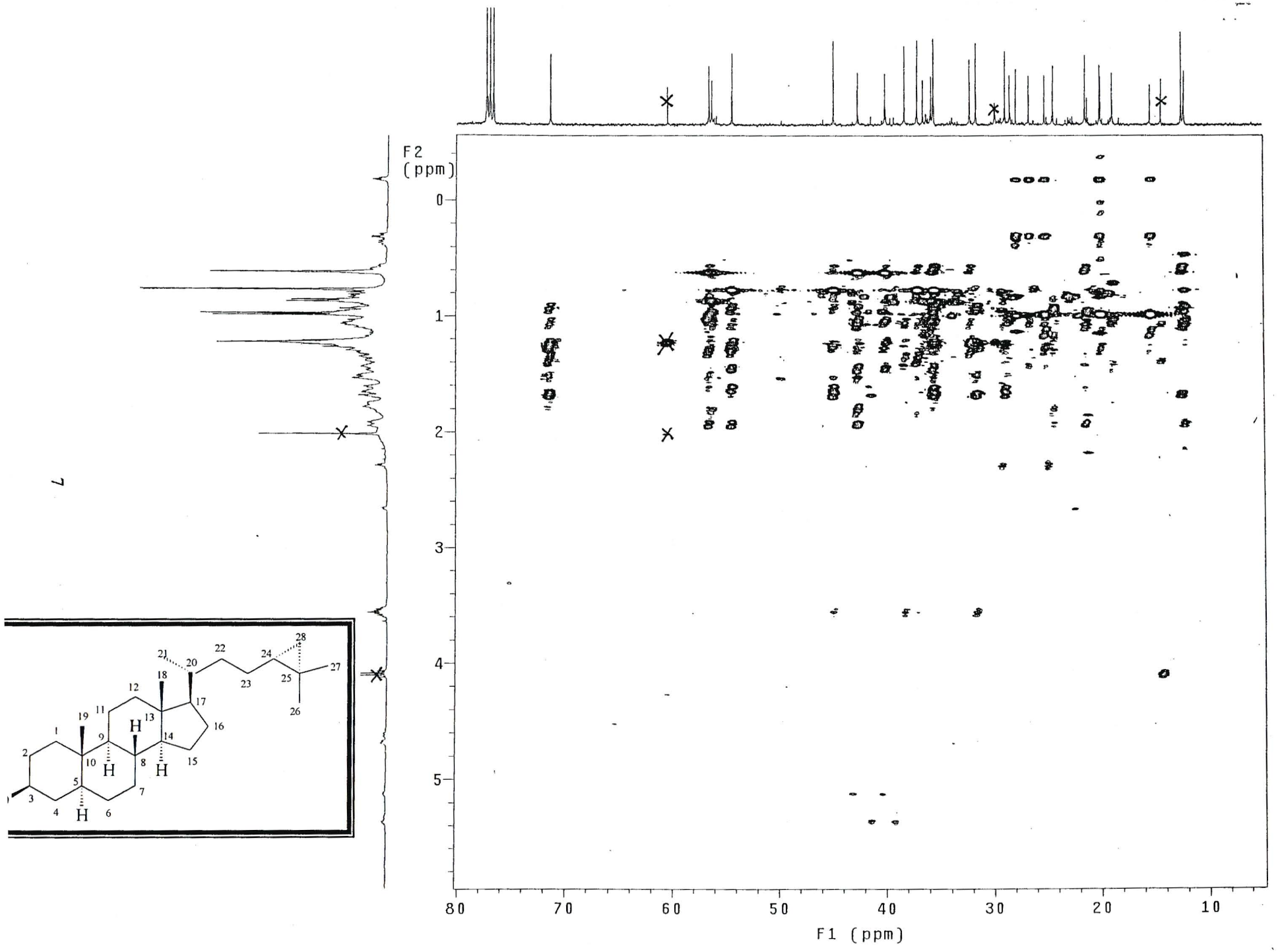
Adept spectrum of **Compound 1**



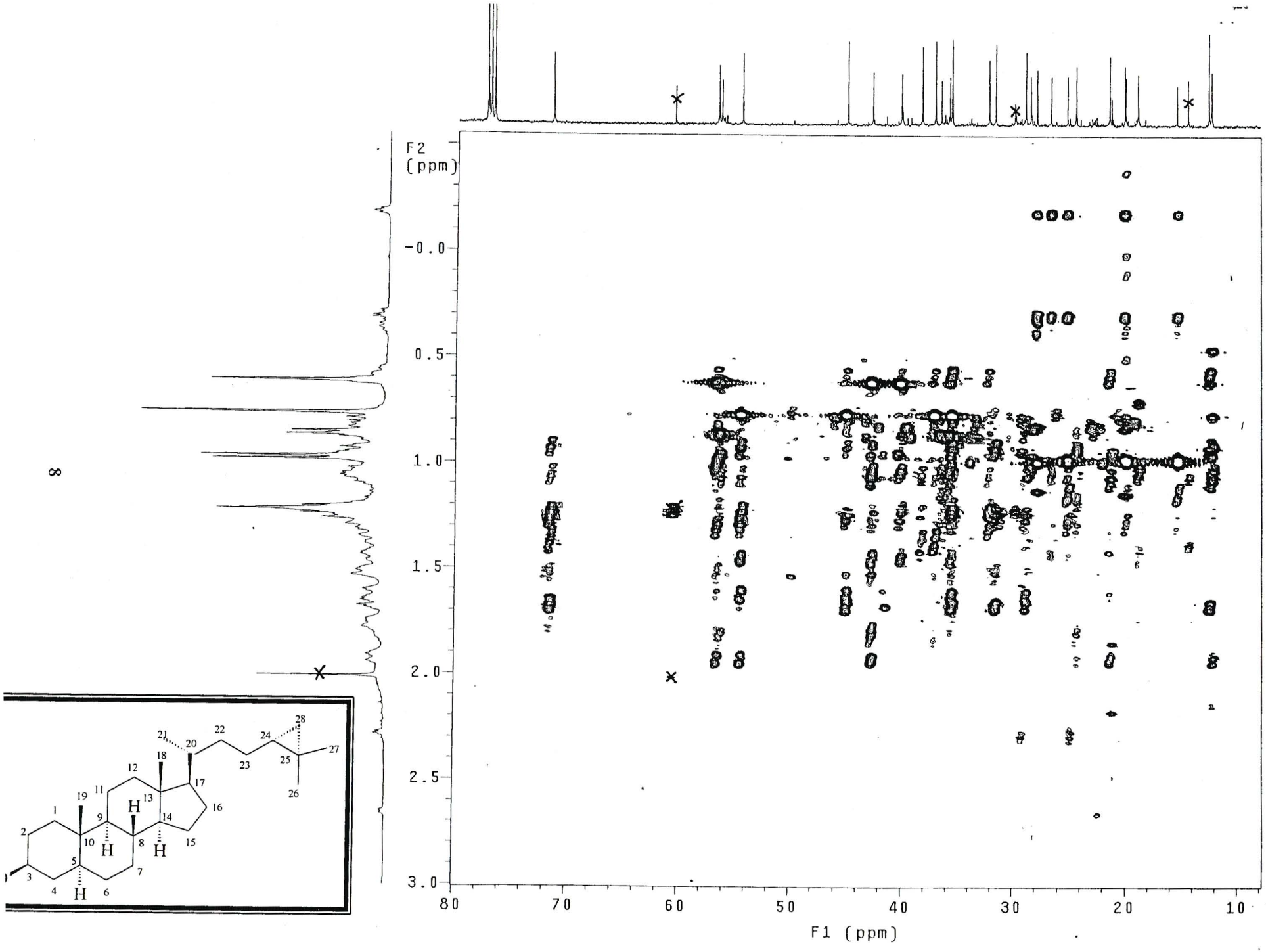
COSY spectrum of **Compound 1**



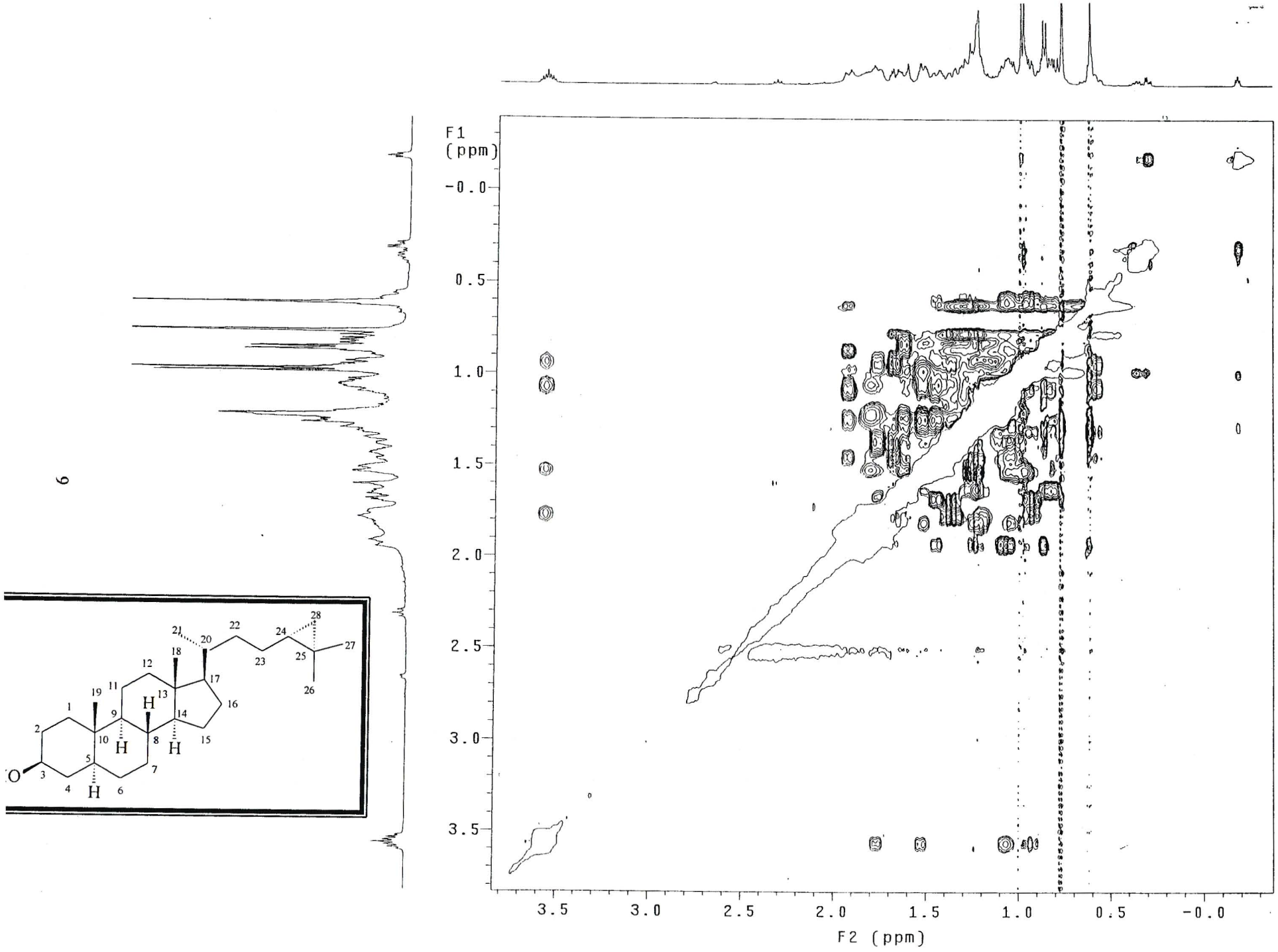
HETCOR spectrum of **Compound 1**



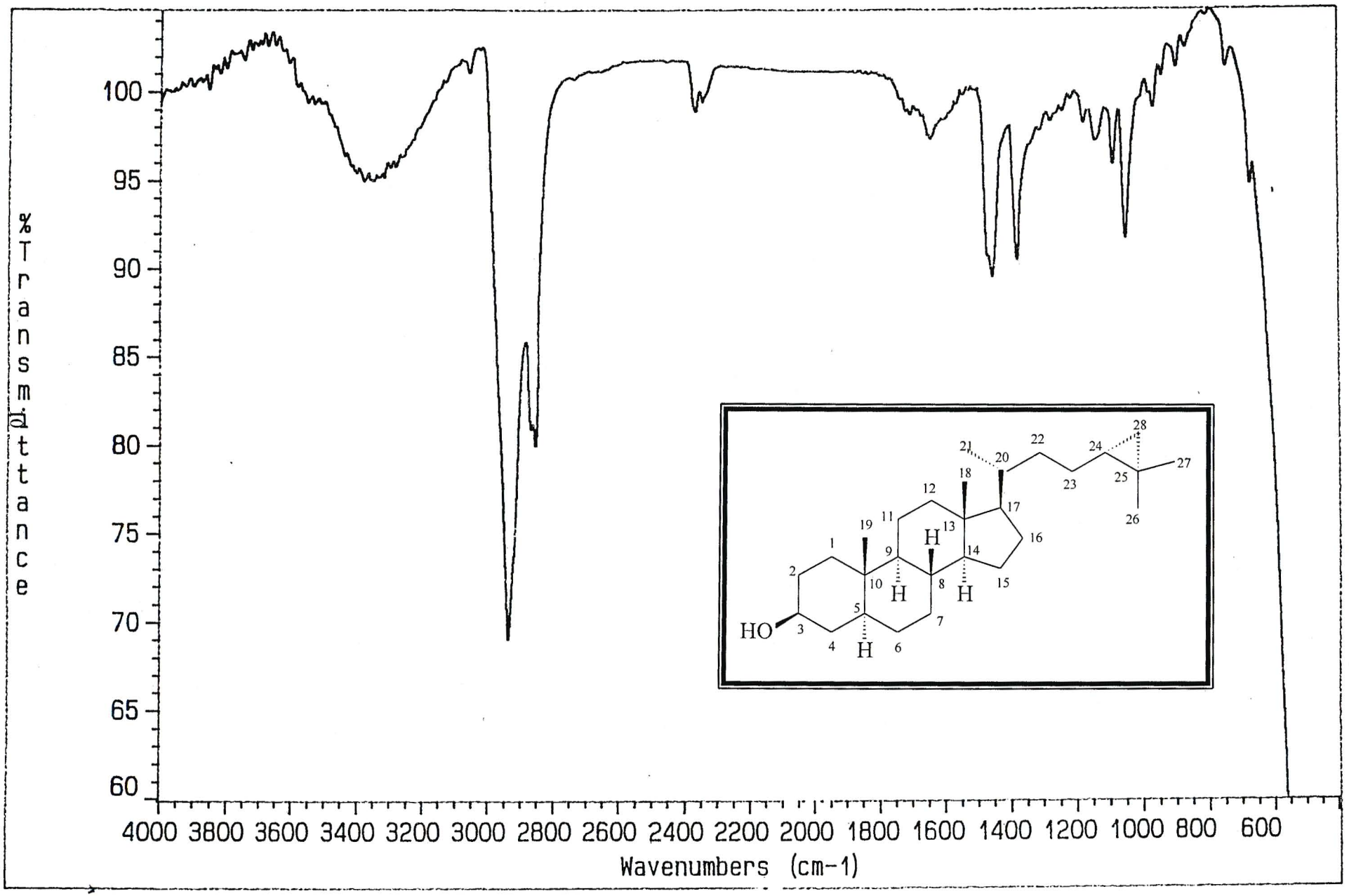
HMBC spectrum of **Compound 1**



Expanded HMBC spectrum of **Compound 1**



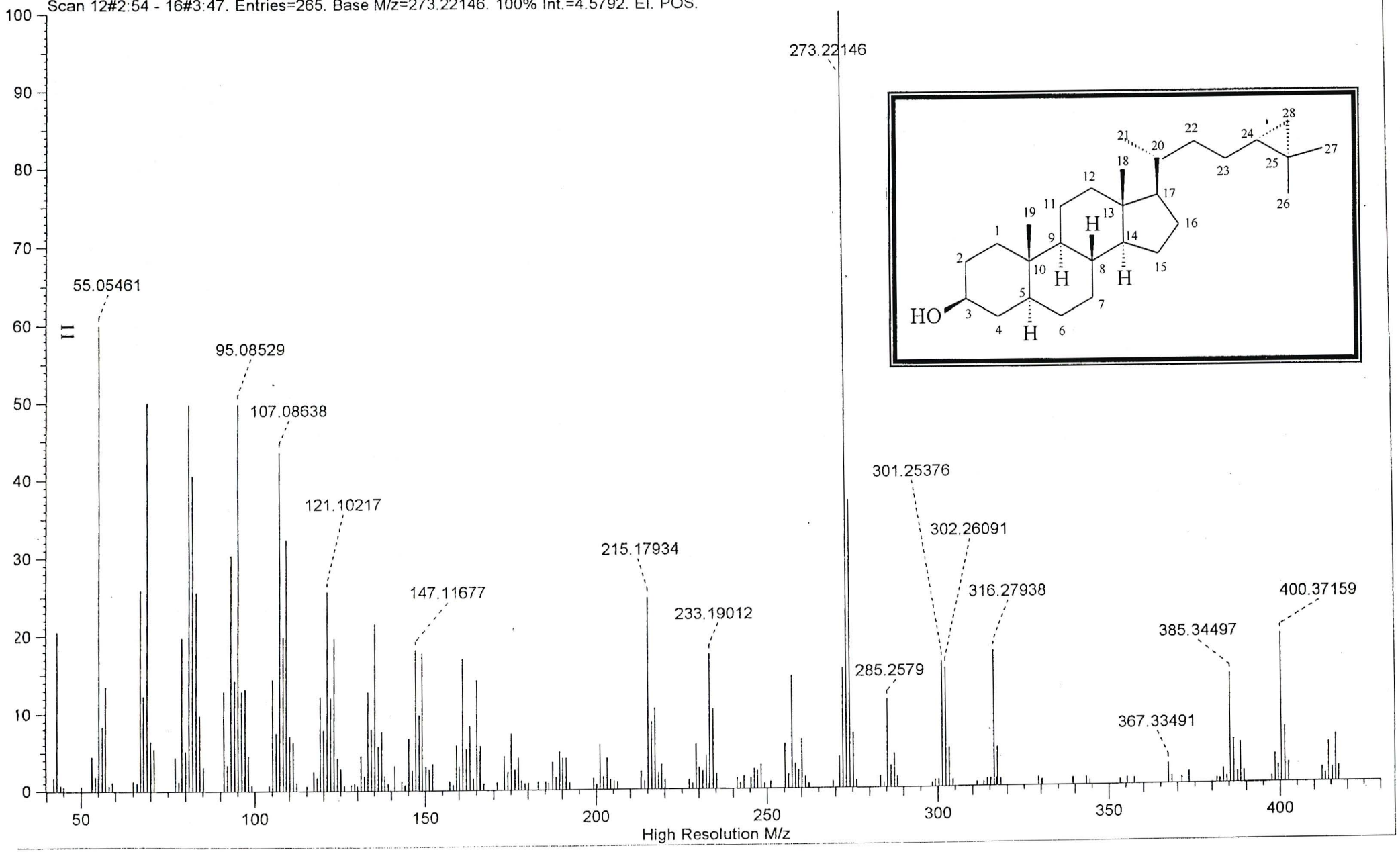
NOESY spectrum of **Compound 1**

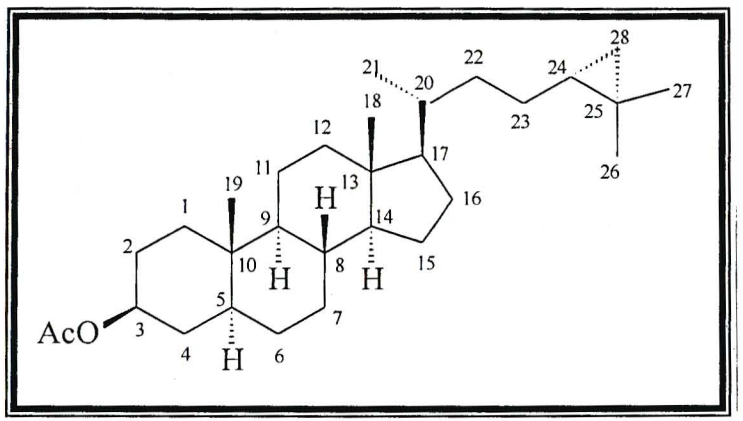


Infrared spectrum of **Compound 1**

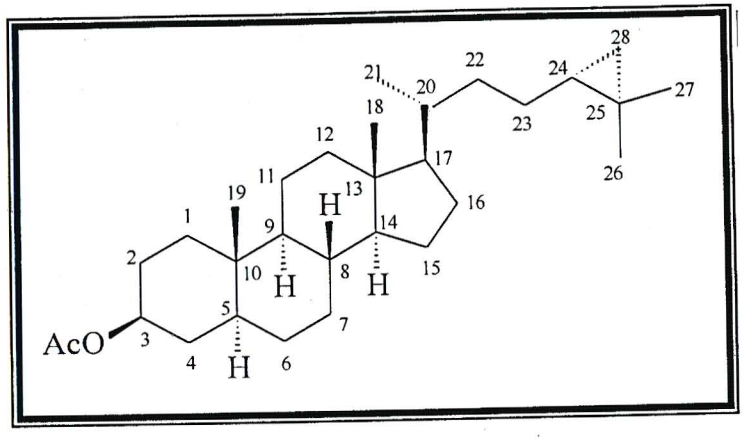
le Name	:	C:\MASPEC\data\hc050209.ms2
le Source	:	Acquired on MASPEC II system [II32/A002]
le Title	:	BR4/H/9-12/3
perator	:	Dr. P. Boshoff
strument	:	VG70-SEQ

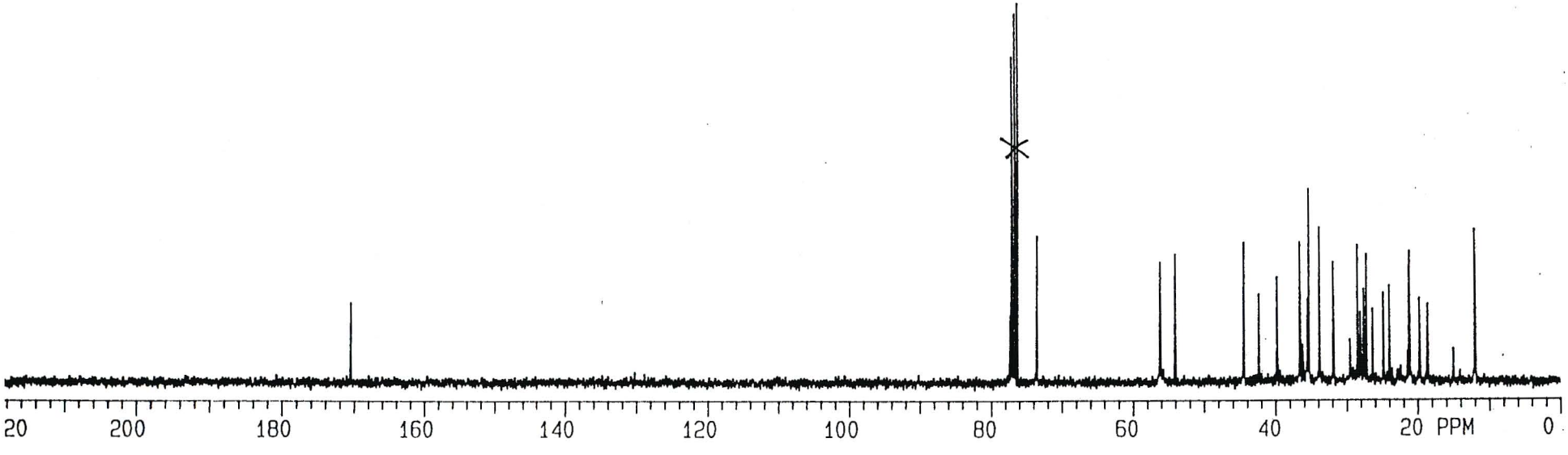
CAN GRAPH. Flagging=High Resolution M/z. Filter=[Int:0.5%. Range:0-420. Excl: Ref/Ex.].Highlighting=Base Peak.
Scan 12#2:54 - 16#3:47. Entries=265. Base M/z=273.22146. 100% Int.=4.5792. EI. POS.



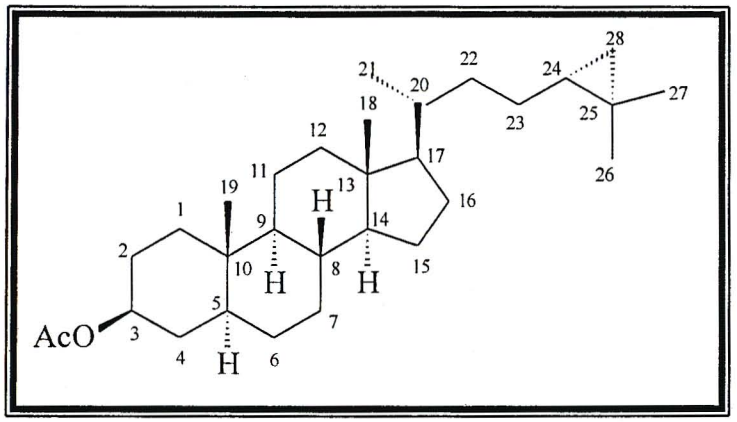


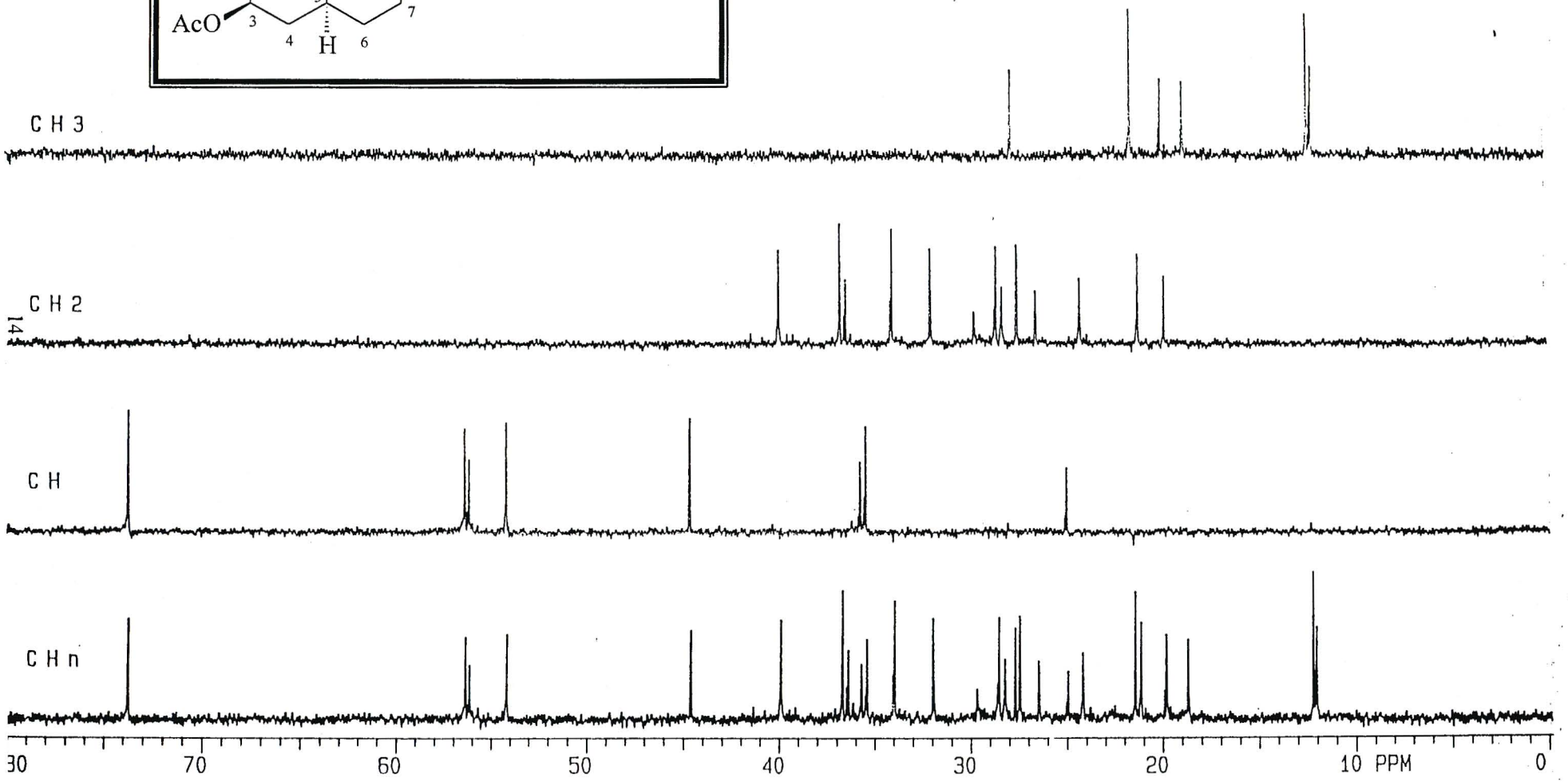
^{1}H NMR spectrum of **Compound 2**



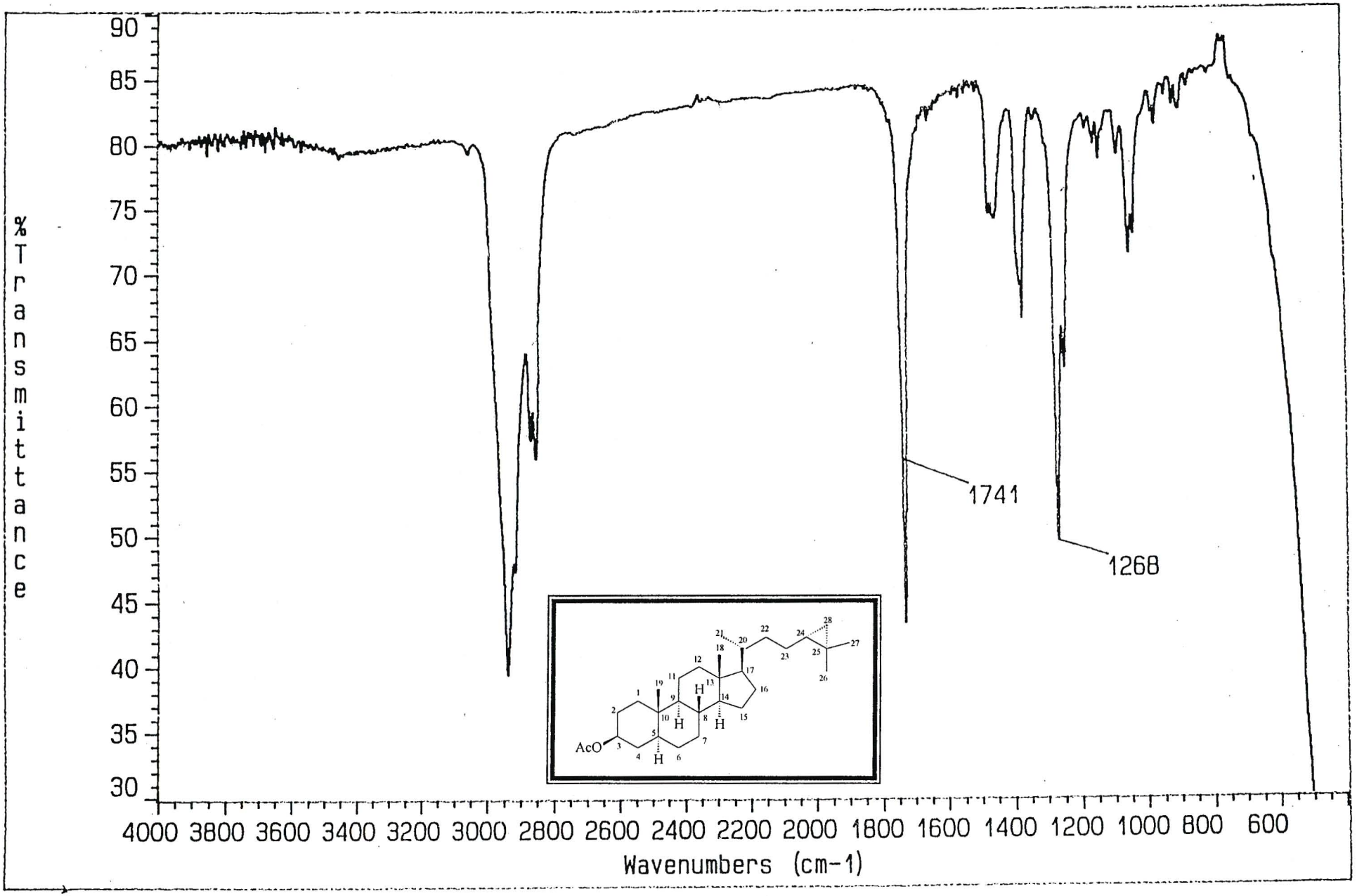


^{13}C NMR spectrum of **Compound 2**





Adept spectrum of **Compound 2**



Infrared spectrum of **Compound 2**