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Extractives from marine organisms

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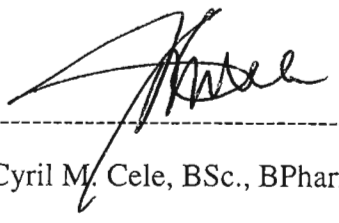
PREFACE

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban, from February 1998 to February 2000 under the supervision of Dr J.P Gerber.

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

Signed 

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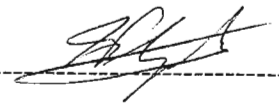
I hereby certify that the above statement is correct.

Signed 

Dr. J.P Gerber, BSc., MSc., PhD

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8. NRF for financial assistance.

Abbreviations

s - singlet

d - doublet

dd - double of doublets

t - triplet

m - multiplet

q - quartet

t.l.c. - Thin layer chromatography

COSY - Correlated spectroscopy

DEPT – Distortionless Enhancement by Polarization Transfer

HETCOR – Heteronuclear chemical shift correlation

^{1}H NMR – Proton Nuclear Magnetic resonance

^{13}C NMR – Carbon-13-Nuclear Magnetic resonance

NOE – Nuclear overhauser effect

KZN – Kwazulu-Natal

HPLC – High Performance Liquid Chromatography

HMBC – Heteronuclear multiple bond coherence

HMQC (HSQC) – Heteronuclear multiple quantum coherence

IR – Infra red

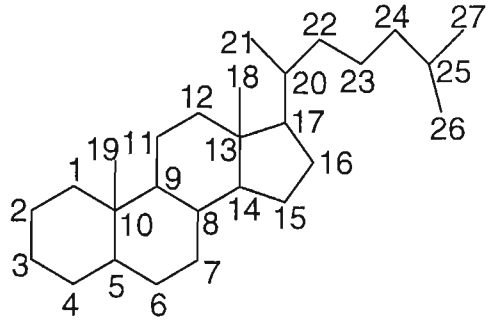
HRMS – High resolution mass spectroscopy

Hz – Hertz

UND – University of Natal, Durban

Nomenclature and numbering

The nomenclature that will be used when referring to sterols in the text will be based on the numbering system illustrated below. The exact stereochemistry is shown under specific compounds.



Abstract

The study involves the investigation of the chemical composition of some marine organisms. This entails collecting the organism, extracting compounds from it and separation, characterization and identification of these compounds.

Marine chemistry has been ignored by many scientists in the past and it is for this reason that these organisms have been investigated, with the aim of discovering their chemistry and also finding new compounds which might be of value in our society. Such value may be the medicinal benefit and/or the understanding of some toxicological effect of some species.

This study was conducted on *Codium extricatum*, *Palythoa natalensis*, *Zoanthus sansibaricus* and *Zoanthus durbanensis* all of which were collected from reefs situated at the southern part of Durban in KwaZulu-Natal (KZN). *Carpobrotus edulis* was another organism that was used in this work. The plant normally occurs on sand dunes. This, however, was collected from the terrestrial environment within the premises of University of Natal (Durban). Sterols, i.e., compound 1, 2 and compound 4 were obtained from both the *Codium* and *Zoanthus* genera. *Zoanthus* also gave compounds which are derivatives of genetic material and these include inosine nucleoside, adenine nucleoside and guanine nucleoside. *Zoanthus* further gave a compound which is aromatic in nature i.e., compound 5 and this belongs to a class of compounds known as the tyramines. Cinnamic acid was found from *Carpobrotus edulis*.

The structures of all compounds were elucidated by conducting a number of experiments using spectroscopic methods. These included nuclear magnetic resonance spectroscopy (n.m.r), mass and infrared spectroscopy.

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CHAPTER 1: INTRODUCTION

CHAPTER 2: RESULTS AND DISCUSSION

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Five organisms were studied in this project. A marine alga, *Codium extricatum,* was investigated as it is common and experimental techniques could be practised without losing valuable material.

Zoanthus durbanensis, Palythoa natalensis and Zoanthus sansibaricus were extensively studied. These organisms inhabit large areas of intertidal and subtidal reefs. Accordingly, they were thought to contain metabolites which act as antifeedants or otherwise to prevent the settlement of competitors' larvae on rocks.

Due to problems obtaining sufficient quantities of marine extracts, a terrestrial plant was investigated. *Carpobrotus edulis*, was obtained from the University of Natal premises. It is known as a folk medicine for the treatment of various infections. Hence, it was deemed interesting to investigate.

1.1 ZOANTHIDS

Zoanthids are marine animals normally found on rocky surfaces in both the intertidal and subtidal zones. Some are shown in figure 1.1 a and b below.

Fig 1.1(a) *Palythoa natalensis* in its extended state

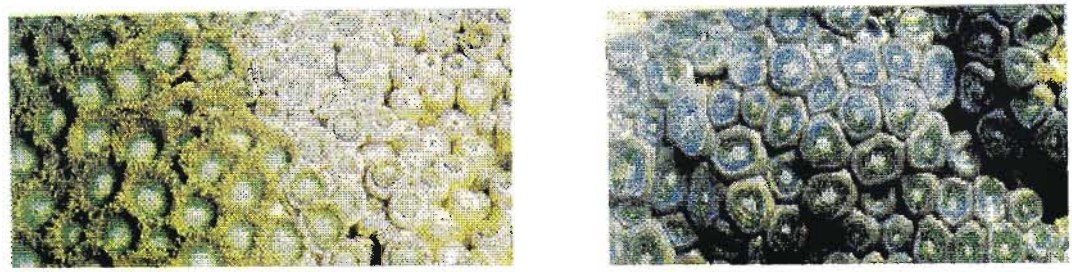


***Fig 1.1*(b)** *Zoanthus sansibaricus* (left) and *Z. durbanensis* (right) in their partially contracted state

Zoanthids fall under the Phylum Cnidaria. Of the four classes of Cnidarians, the Zoanthids belong to the Anthozoa. There are two subclasses of Anthozoa. Zoanthids belong to Zoantharia which is made up of four orders. Zoanthidae is found in the order Zoanthidea. Both *Zoanthus* and *Palythoa* can be found in family of Zoanthidae. A complete classification of Zoanthids is shown in the table below[1] (Table 1.1).

Table 1.1 The classification system of Zoanthids

KINGDOM	-----	ANIMALIA
PHYLUM	-----	CNIDARIA
CLASS	-----	ANTHOZOA
SUBCLASS	-----	ZOANTHARIA
ORDER	-----	ZOANTHIDEA
FAMILY	-----	**ZOANTHIDAE**
GENUS	-----	*Zoanthus, Palythoa*
SPECIES	---------	13 Species

Zoanthids are known to have a symbiotic relationship with the algae called zooxanthellae which are found inside these organisms.[2] In such a situation it is very difficult to determine the origin of the extracted compounds isolated from one of the two organisms, because they could come either from the organism itself, its symbionts, or from the symbiotic association considered as a whole.[2]

Classes of compounds known to be present in zoanthids include:

A. Sterols

B. Nitrogen containing compounds

(a) Amino acid derivatives

(b) Alkaloids – (i) Xanthins

- (ii) Zoanthaminones

C. Fatty acids

D. Palytoxins

A. Sterols

A variety of sterols are produced by Zoanthids. The genus *Zoanthus* produces a particular set of sterols depending on the species involved i.e., the type of sterols differs from one Zoanthid to the next. The genus *Palythoa* is known to produce a constant set of sterols known as "palysterols." This was initially thought to be a fingerprint of the genus *Palythoa*. However, this was found to be erroneous as a new set of sterols was found from another species of *Palythoa*. The conclusion was that *Palythoa* could be divided into two subgenera.[3] The most important consideration was that, depending on the type of zooxanthellae that is associated with a particular *Palythoa*, they will produce a particular set of sterols.

A complete set of sterols produced by Zoanthidea is shown below [fig 1.2 (a) and (b)].

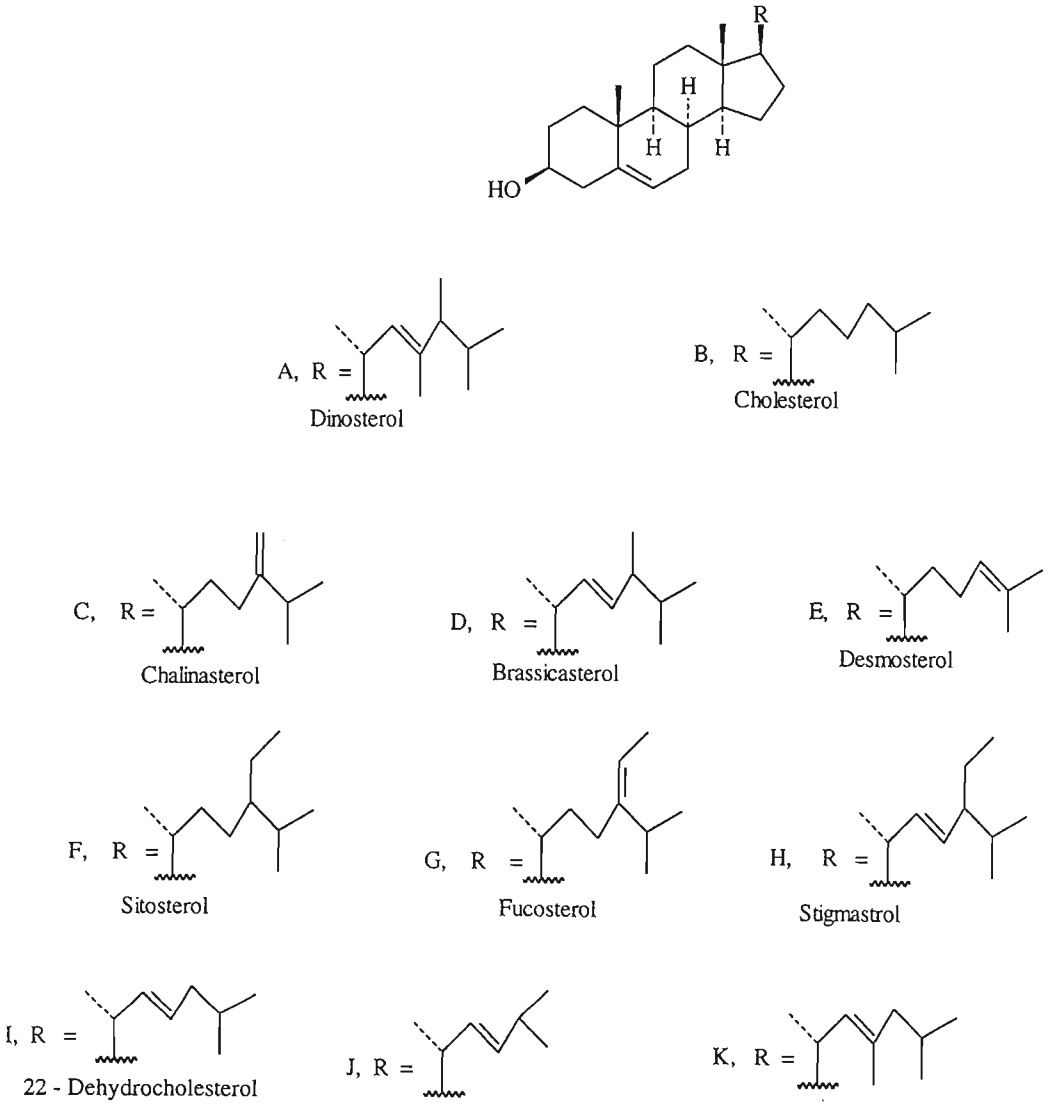


Fig 1.2 (a)

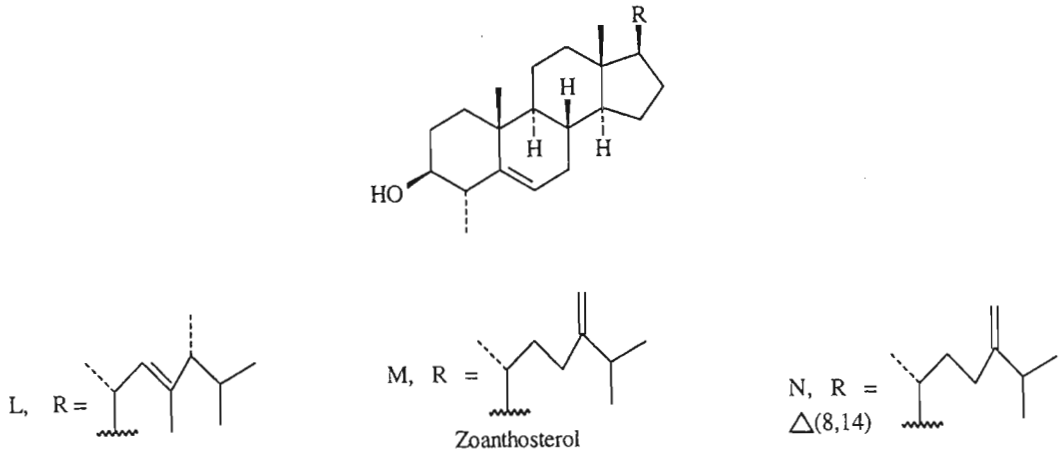
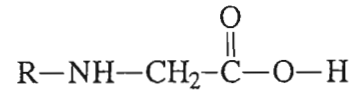


Fig 1.2 (b)

B. Nitrogen containing compounds

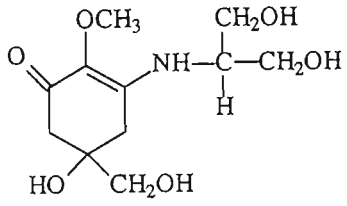
(a) Amino acid derivatives

Zoanthids produce a group of compounds referred to as amino acid derivatives due to their structures. These compounds are basically N-substituted glycine amino acids.

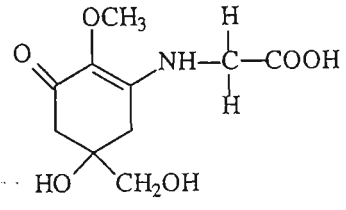


R varies depending on the compound in question as shown in figure 1.3. This type of compounds was first identified from fungi. It is known that near-ultraviolet radiation stimulates reproduction in many fungi, which is accompanied by the formation of water-soluble compounds having a sharp absorption maximum at 310 nm[3].

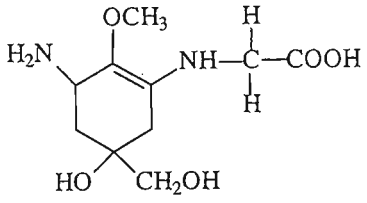
It was reported that Favre-Bonvin, *et al.*, isolated one of these compounds and identified it as mycosporin[4] (fig 1.3). The latter is not a true amino acid.



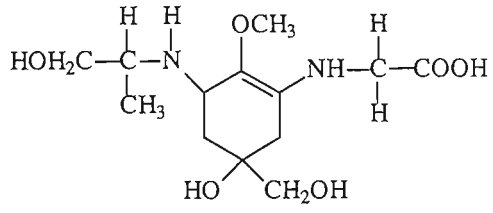
Mycosporin



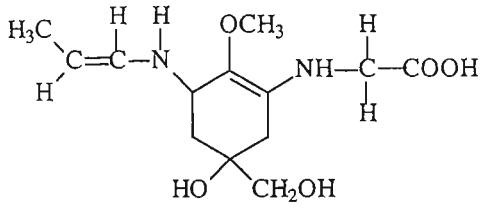
Mycosporin-glycine



Palythine



Palythinol



Palythene

Fig. 1.3

Palythoa tuberculosa also produces compounds which show absorption properties of similar nature to compounds produced by fungi. These absorb in the region of 310 – 360 nm. The first compound to be identified from *Palythoa* was mycosporin-glycine (fig 1.3), with a maximum absorption at 310 nm[4]. It is well known that a compound with an absorption maximum at 320 nm is present in many marine plants and animals[5], however, the structure and the role *in vivo* are still uncertain.

A compound with the same absorption maximum is also found in *Palythoa tuberculosa*. It has been assigned a structure and named palythine (fig 1.3). Further investigations on the UV absorbing compounds from *Palythoa tuberculosa*, yielded two new compounds with an absorption maximum at 332

and 360 nm, respectively, which have been assigned structures and named palythinol and palythene[6] (fig 1.3).

All these compounds i.e. mycosporin, mycosporin-glycine, palythine, palythinol, palythene are widely distributed in nature, but their biological roles are obscure. The proposed hypothesis is that UV absorbing compounds may play a protective role against strong ultraviolet irradiation,[4,7] and they may be precursors of the various pigments found in these organisms.[7] Alternatively they may play an important role in the photosynthetic system or reproduction in many fungi as it has been noted that the production of mycosporine occurs during the UV stimulated reproduction stage of many fungi.[7]

Amongst the amino acid derivatives are the compounds referred to as nucleic acid nitrogen bases and include adenine, guanine, cytosine, thymine, and uracil. They are grouped into two major groups i.e., **purines** and **pyrimidines**. The grouping is based on the chemical structures of these compounds. The purine group consists of adenine and guanine while the pyrimidine group has cytosine, thymine and uracil. The compounds are known to form part of the building blocks of nucleic acids i.e., ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of any living organism. The chemical structures of these compounds are shown in the figure below (fig 1.4)

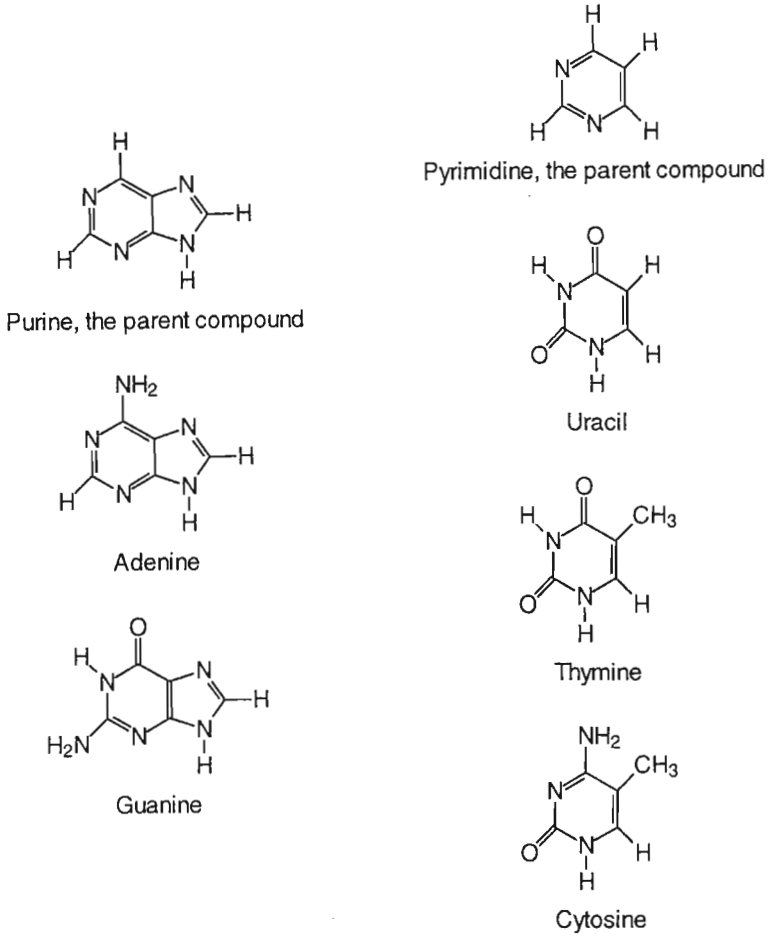


Fig 1.4

The nitrogen bases occur in nucleic acids in the form of nucleotides i.e., the base is linked to a sugar which in turn is bonded to a phosphate. An example of a nucleotide is shown below:

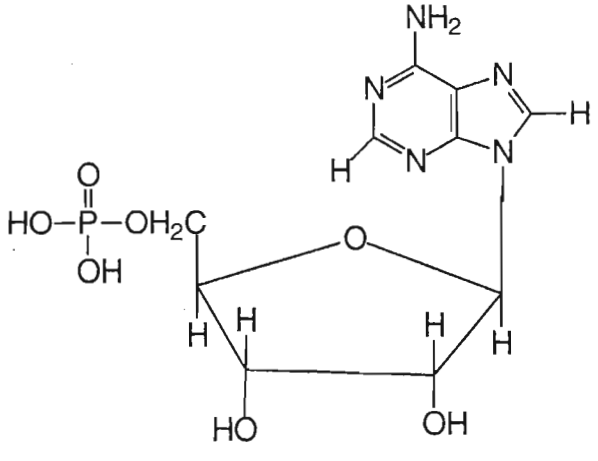


Fig 1.5: Adenylic acid

(i) Biosynthesis of purine nucleotides

The origin of each and every atom in purine nucleus can be accounted for, as shown in figure 1.6 below[8].

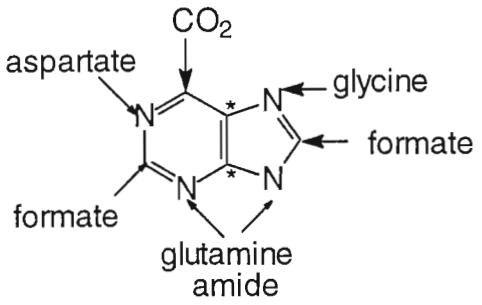
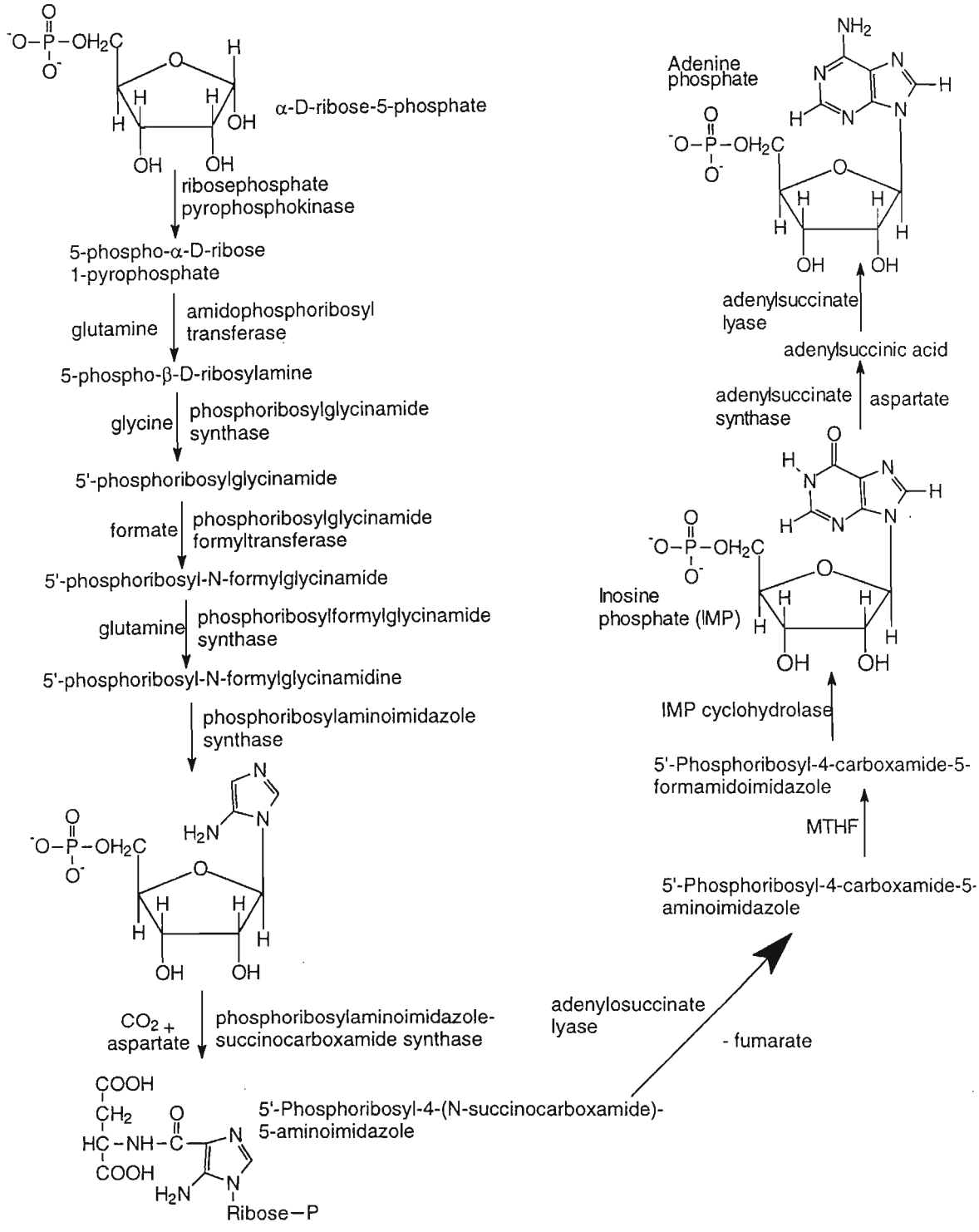


Fig 1.6

(*the two carbons come from the same glycine molecule as the labelled nitrogen)

The biosynthesis begins with α-D-ribose-5-phosphate. This molecule undergoes a number of biochemical transformations with reactants such as adenosine triphosphate, a number of amino acids, different enzymes and coenzymes.

The pathway is shown in scheme 1.1.[8]



Scheme 1.1

The starting material is an activated form of D-ribose-5-phosphate, on which a purine ring is built, resulting directly in the formation of a nucleotide. In the next step (Scheme 1.1) the pyrophosphate reacts with glutamine so that the amino

group from the amide portion of glutamine displaces the pyrophosphate group from the 1-position in the pentose to yield 5-phospho-β-D-ribosylamine. In this step, catalysed by an iron-containing enzyme, the anomeric carbon of the D-ribose undergoes inversion from the α to the β configuration and this configuration is retained in the final purine nucleotide products.

In the third step, the carboxyl group of glycine reacts with the amino group of 5-phosphoribosylamine to form 5'-phospho-β-D-ribosylglycinamide, together with ADP and phosphate. The next step is the addition of one formyl group to the free α amino group of of 5'-phosphoribosylglycinamide. The formyl group is donated by the formyl group carrier N^5,N^{10}-methylenetetrahydrofolate (MTHF) which is shown in figure 1.7 below.

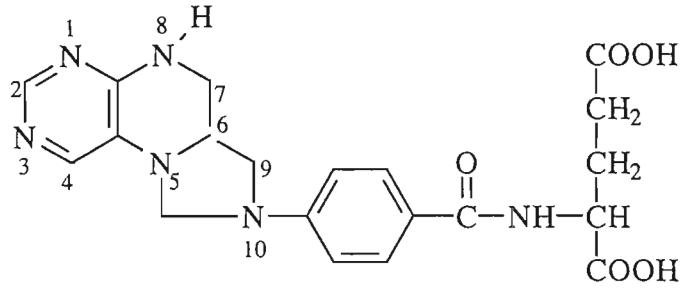


Fig 1.7: N^5,N^{10}-methylenetetrahydrofolate

The subsequent step in purine nucleotide biosynthesis involves the oxidation of the amidyl group from glutamine, resulting in the formation of 5'-phosphoribosyl-N-formylglycinamadine. The imidazole ring is then closed by elimination of water (H_2O) to yield 5'-phosphoribosyl-5-aminoimidazole. The latter is carboxylated and then followed by incorporation of aspartic acid, forming 5'-phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole and a free fumaric acid residue, which is eliminated.

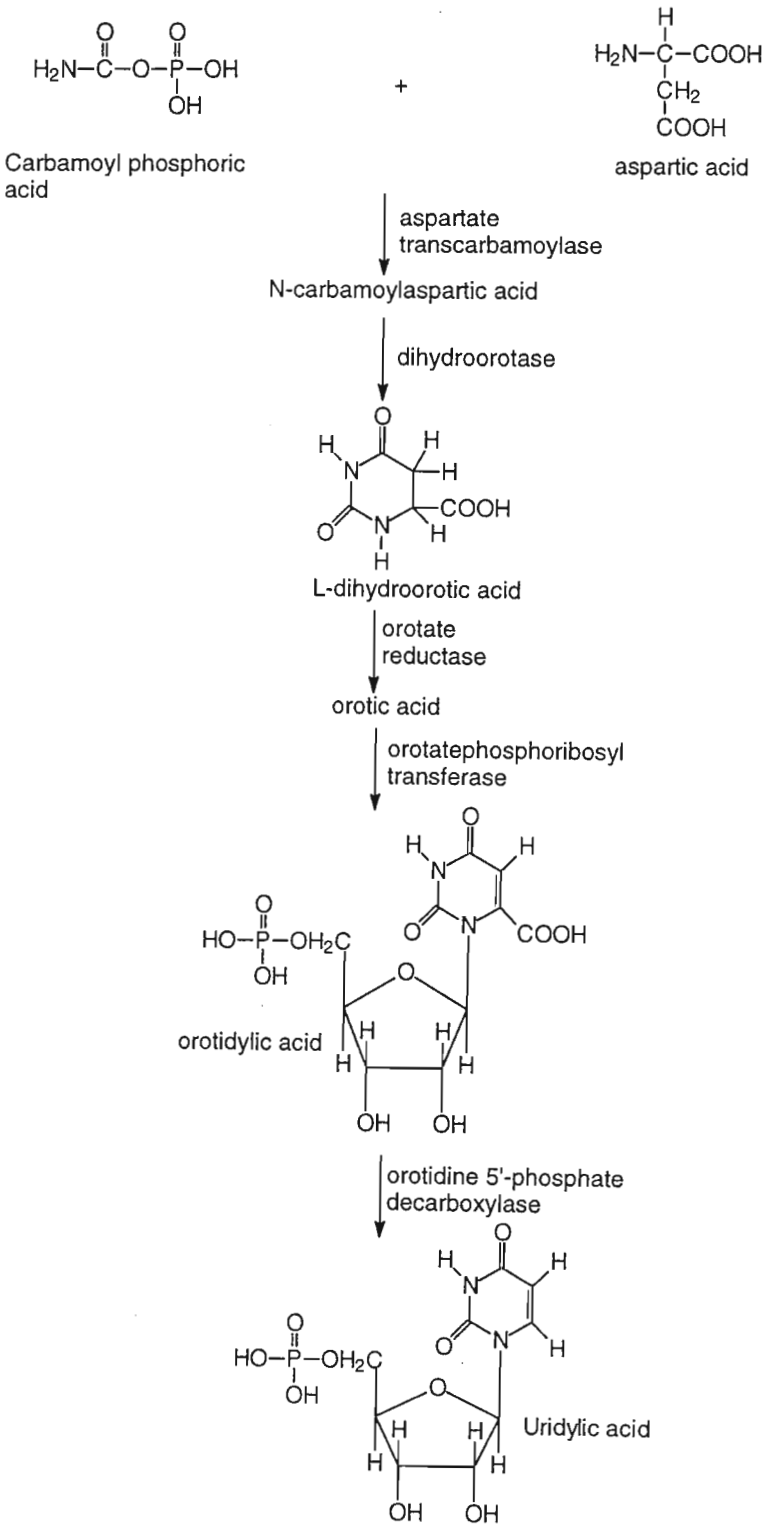
The last carbon atom of the purine ring is introduced by transfer of the formyl group from N^{10}-formyltetrahydrofolate to the amino group of the almost completed ribonucleotide, 5'-phosphoribosyl-4-carboxamide-5-

formamidoimidazole. The pyrimidine portion of the purine ring system is then closed by elimination of water (H_2O) to form the ribonucleotide, inosinic acid, the first product in this biosynthetic pathway to possess a complete purine ring system, which then acts as a precursor for adenylic and guanylic acid.

(ii) Biosynthesis of pyrimidine nucleotides[8]

As opposed to purine biosynthesis, where the starting material is a sugar unit, a non-sugar unit serves as a starting material and the D-ribose-5-phosphate moiety is attached after the pyrimidine ring has been formed from its open chain precursors. The scheme is shown below (Scheme 1.2).

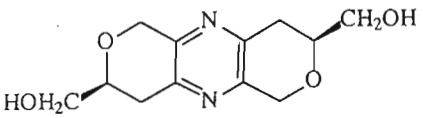
Aspartic acid and carbamoyl phosphoric acid are involved in the first step of the biosynthesis where they condense to form N-carbamoylaspartic acid. This is followed by ring closure by removal of water (H_2O) from N-carbamoylaspartic acid by the action of dihydroorotase to yield L-dihydroorotic acid. Oxidation follows to give orotic acid. At this point the D-ribose-5-phosphate side chain is attached to orotic acid to form orotidine -5'-phosphoric acid (orotidylic acid). The latter is decarboxylated to yield uridylic acid (Scheme 1.2). Uridylic acid serves as a precursor for other pyrimidine bases, i.e., thymine and cytosine.


Carbamoyl phosphoric acid
+
aspartic acid
aspartate transcarbamoylase
N-carbamoylaspartic acid
dihydroorotase
L-dihydroorotic acid
orotate reductase
orotic acid
orotatephosphoribosyl transferase
orotidylic acid
orotidine 5'-phosphate decarboxylase
Uridylic acid

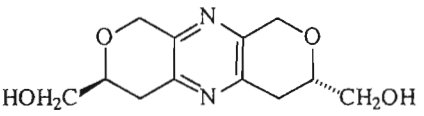
Scheme 1.2

(b) Alkaloids

In the course of a continuous investigation on the UV-absorbing substances at 310 – 360 nm from *Palythoa tuberculosa*, two new alkaloids have been found and isolated.[9] The compounds belong to a class called the pyrazines and they include palythazine and isopalythazine (fig 1.8).



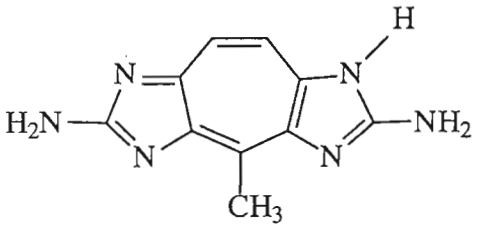
Palythazine



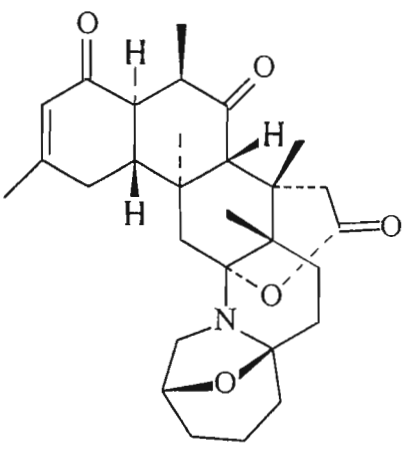
Isopalythazine

Fig 1.8

Alkaloids are naturally occurring compounds containing nitrogen in a form of an amine or imine. The amino group can be primary, but alkaloids usually appear as secondary or tertiary amines. The alkaloids found in zoanthids are of three structural types i.e., pyrazines (fig 1.8), xanthin types and triterpenoid derivatives (fig 1.9).



Xanthin



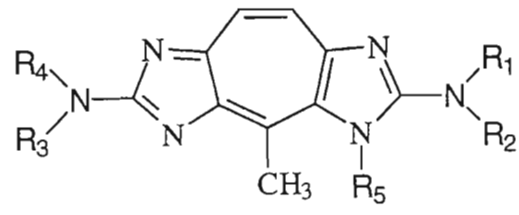
Triterpenoid

Fig 1.9

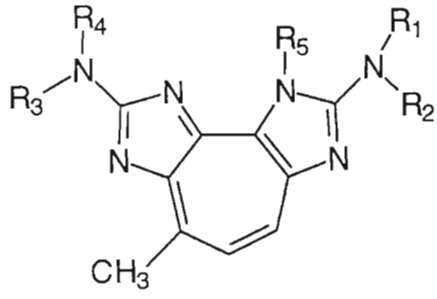
(i) Xanthins

The specific name given to xanthins produced by zoanthids is zoanthoxanthins. These compounds are non-benzenoid aromatic alkaloids.[10] Most of the brilliant yellow or red colouration of marine coelenterates, as seen for example from sea anemones and stone corals, is due to carotenoid pigments either as such or as carotenoproteins.[11] Among the few exceptions that are known are some colonial anthozoans of the order Zoantharia, e.g *Parazoanthus axinellae,* which contain a remarkable group of yellow nitrogeneous pigments, named zoanthoxanthin, characterized by intense blue or yellowish-green fluorescence in UV light and to some extent in daylight.[11] A recent survey of more than 80 different invertebrate species showed that these yellow fluorescent substances emanate solely from colonial anthozoans in both major families Epizoanthidae and Zoanthidae of the order Zoanthidea.[10]

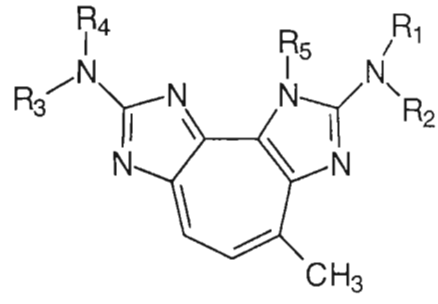
Extensive chemical studies have shown that these pigments are tetrazacyclopentazulenes that have either the zoanthoxanthin or the pseudozoanthoxanthin skeleton[11] (fig1.10). They undoubtedly arise by a dimerization-rearrangement of arginine-derived C_5N_3 subunits, because three skeletons are known : 3H –zoanthoxanthin [**A**], 4H –pseudozoanthoxanthin [**B**], and 3H –pseudozoanthoxanthin [**C**].[10]



A



B



C

$R_{1\text{-}5}$ = H, CH_3

Fig 1.10

Distinction among these isomeric framework possibilities can be tricky. Structural types **A – C** exist as equilibrating tautomeric pairs when the annular N is substituted with R_5 = H. Disymmetrical tautomers are possible for **A** when any one of the four substituents R_1-R_4 are identical. Examples of **A – C** (each having an R_5 = Me) have been characterized by X-ray crystallography.

Other derivatives are usually identified by comparing ^{1}H vinyl and methyl shifts to those which co-occur with analogues that have been analysed by X-ray crystallography. Alternatively, a distinction among families **A-C** is sometimes made by assuming that these compounds are genus-specific.[10]

Surprisingly, no definitive ^{13}C-NMR assignments can be found for any members of this group, as none have been examined by 2D NMR techniques.[10] Previous information about the bioactivity of zoanthoxanthins includes histamine-like

action on the guinea-pig ileum and papaverine-like activity.[10]

Efforts to evaluate the bioactive potential of zoanthoxanthins were stimulated by prior observations that polyaromatic zoochromic alkaloids such as dercitin have *in vivo* activity against both P-388 (leukemia) and B-16 melanoma tumours in mice. Compound **C** (fig. 3) where $R_1 = R_3 = R_4 = R_5 =$ Me and $R_2 =$ H was active in cytotoxicity assays *(in vitro)* and exhibited the following IC_{50}'s (ug/ml): HCT8 (human colon adenocarcinoma) = 1.61, A549 (human lung carcinoma) = 2.38, HT29 (human colon adenocarcinoma) = 0.824, and P-388 (mouse lymphocytic leukemia) = 1.77.[10]

(ii) Triterpenoid derivatives

This group of compounds is believed to result from the rearrangement of a triterpenoid . The very first compound that was isolated among this series of alkaloids was zoanthamine.[12] Later other compounds like zoanthamide, zoanthaminone and zoanthenamine were isolated as well.[13,14] The structures of these compounds are shown below in figure 1.11.

Although the structures contain isoprenoid elements, there is no clear sequence of head-to-tail linkages that could suggest an obvious triterpenoid origin. However, the latter is generally accepted.[13]

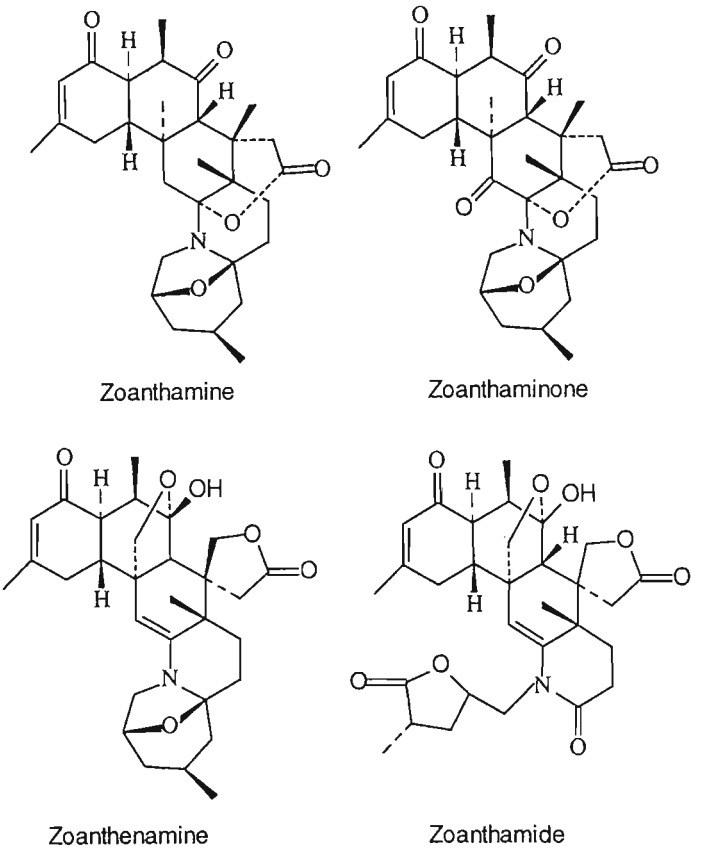


Fig 1.11

C. Fatty acids

The literature on fatty acid composition of the *Palythoa* genus is very limited. From the previous studies it has been shown that *Palythoa* contain a number of fatty acids ranging from saturated fatty acids (SATFAs) to monounsubstituted fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).[15] Depending on the species of Palythoa in question, the amount of each kind of fatty acids differs. Based on these different kinds of fatty acids, three groups of *Palythoa* can be identified. The first group are those which can be characterized by the global distribution of SATFAs > MUFAs > PUFAs. The second group shows a distribution of SATFAs > PUFAs > MUFAs. A third group also has a global distribution of SATFAs > PUFAs > MUFAs, like the second group, but with more similar quantities of each fraction.[15] Furthermore, one can use each type of fatty

acid, e.g. SATFAs, to distinguish one species of *Palythoa* from the next. Thus, fatty acids might be a good chemotaxanomic criterion for the *Palythoa* genus. Recently, a new fatty acid has been identified from *Palythoa cabaeorum* which has a brominated chain.[16] This is shown in figure 1.12 below.

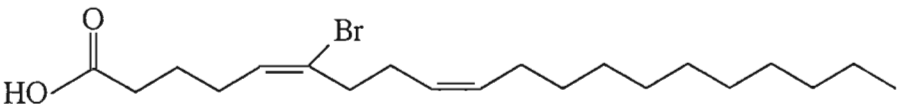
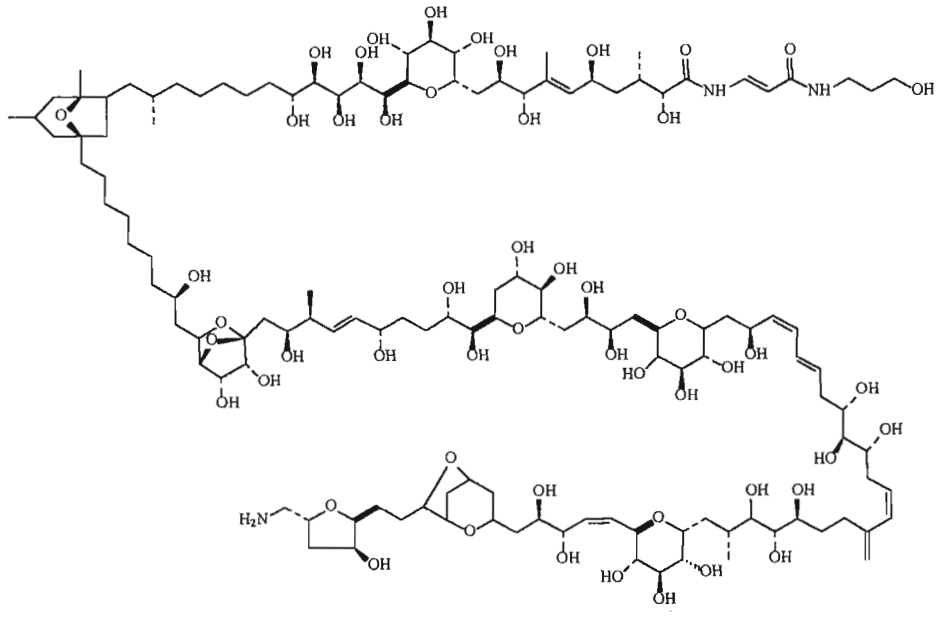


Fig 1.12: 6-Bromo-5,9-eicosadienoic acid

D. Palytoxins

Palytoxins are a group of the most poisonous substances known. The first palytoxin to be isolated was from a marine coelenterate known to the Hawaiians as the "deadly seaweed of Hana", and now designated *Palythoa toxica.*[17] Since then, seemingly identical toxins have been isolated from several other species of palythoans of the genus *Palythoa.* These palytoxins possess identical lethal and anticancer activities and exhibit the same UV- absorption (233 and 263 nm). Palytoxins have very large molecular weights, and these have been determined with great difficulty.[17]

A number of methods have been tried to elucidate the structure,[18] the major one being the oxidation of a whole molecule by sodium periodate, resulting in fragments which can be connected so as to give the original palytoxin molecule.[18-20] Although different palytoxins are not named individually, they all consist of highly functionalized polyhydroxyl cyclic ethers. The suggested structure of one such palytoxin is shown in fig 1.13.



Palytoxin

Fig 1.13

1.2 *CODIUM EXTRICATUM*

Codium extricatum, a marine green alga, occurs on rocky surfaces (fig 1.14).

Fig 1.14

It has been studied to some extent and a number of compounds have been described with some activity. The types of compounds include taurines,[21] siphonaxathin and siphanein[22] and common amino acids.[23] A number of fatty acids and sterols have also been isolated, these include oxygenated clerosterols[24] as shown in figure 1.15.

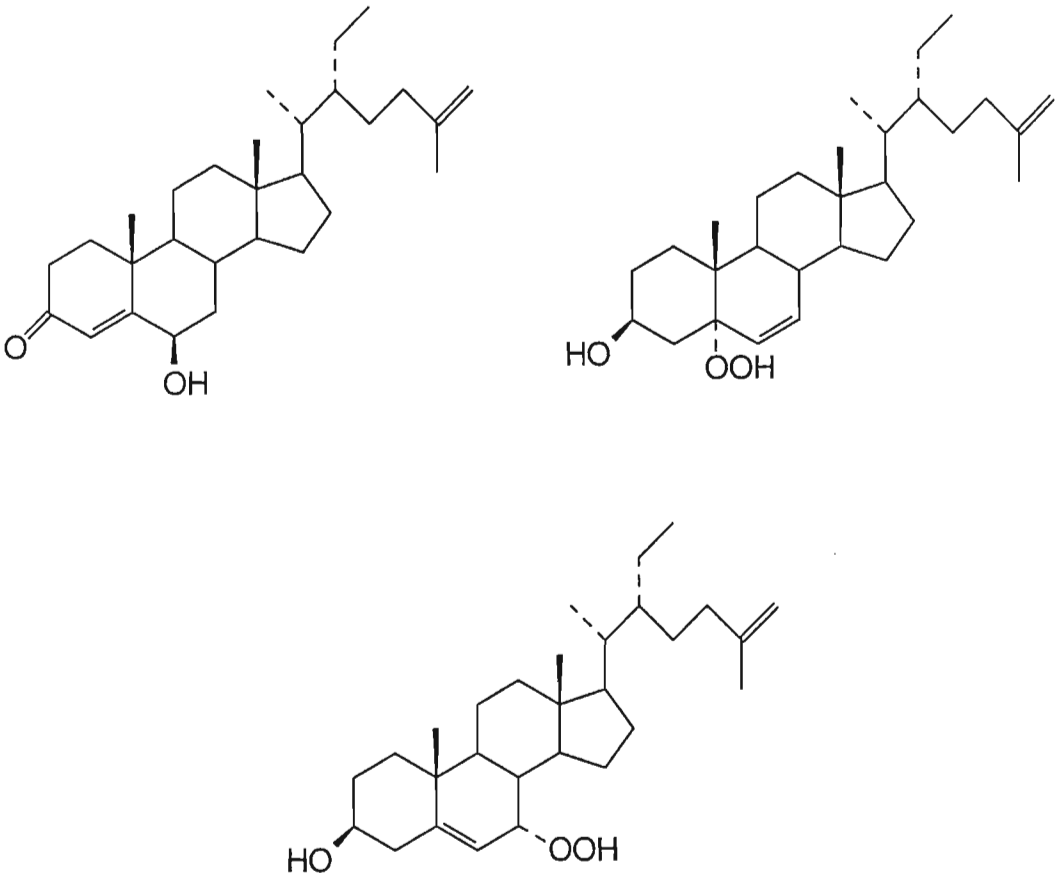


Fig 1.15

Some extracts and compounds from *Codium* have significant application. The ethanol and water extract exhibit prostaglandin synthesis inhibition by blocking an enzyme called cyclooxygenase.[25] Sulphonated protoglycan from *Codium fragile* was shown to have anticoagulent activity.[26] The lipophilic extract has antibiotic activity against some bacteria.[27] The ethyl acetate extract of *Codium* yielded diterpenes and these were found to have a strong toxicity effect.[28] Such diterpenes include 3,7,11,15-tetramethylhexadecan-1,2-diol and similar compounds. Polysaccharides from marine algae show strong antiviral (HIV) activity.[29]

to have taxonomic significance since they have been found to be genus specific.

1.3 *CARPOBROTUS EDULIS*

Carpobrotus edulis is a terrestrial plant, commonly referred to as the *sour fig* (fig 1.16).

Fig 1.16

This pant originally occurred in sandy areas of the Western Cape and along the southern Cape Coast to the Eastern Cape. The species is now grown in most parts of the world. Another species, *Carpobotus acinaciformis,* which is very similar to *Carpobrotus edulis,* is also called the *sour fig.*

The leaf juice or leaf pulp is used medicinally. It is indicated for infections like mouth/throat infections, dysentry and tuberculosis. It can also act as a diuretic and has strong activity in causing the blood vessels and body tissue to contract. Externally it is used to treat eczema, wounds and burns while it can be used treat oral and vaginal fungal infections.[31]

The pressed extract of *Carpobrotus edulis* has pesticidal activity.[32] The plant has the ability to vary its metabolism as it has been observed that it can be induced to undergo crassulaean acid metabolism (CAM) by growing it in sodium chloride solution while it was a non-CAM plant originally.[33]

solution while it was a non-CAM plant originally.[33]

Carpobrotus contains pigments which include betanin, isobetanin, betanidin, isobetanidin, lamprathin II, isolamprathin II and a minor pigment, 2-decarboxybetanidin[34] which is shown in figure 1.17 below:

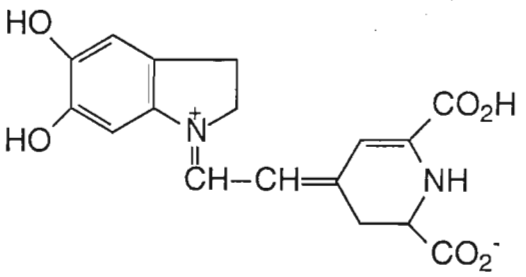


Fig 1.17: 2-Decarboxybetanidin

CHAPTER 2

RESULTS AND DISCUSSION

2.1 Investigation of *Codium extricatum*

Codium extricatum, a marine algae, was investigated as it is a higher organism growing abundantly along the KZN coast. Marine algae are not known for their diverse chemical composition. Thus, the techniques required for the successful completion of the project could be practiced without there being very complex mixtures. Only a single sterol was isolated from the hexane extract.

2.1.A Structural elucidation of compound 1

A methanolic extract of the blended organism was washed with hexane. Column chromatography of the latter gave compound 1 as an amorphous solid.

The ^{1}H NMR (spectrum 1.1) had a number of peaks between 0.60 ppm and 2.50 ppm. This suggested the presence of the hydrocarbon groups. Large singlets were observed in this region and these were suggested to be the CH_3 group protons while a number of small peaks in between were thought to be either CH or CH_2 group protons. A broad multiplet at 3.50 ppm was indicative of methine proton adjacent to a 8-hydroxyl group[35] (as opposed to a narrow multiplet). This in turn was attached to other methylenes, hence, giving such a multiplet. The peaks between 4.50 ppm and 5.50 ppm indicated the presence of a double bonded system. The two signals at 4.64 ppm and 4.72 ppm seemed to be a doublet but the coupling constant between them was 24.00 Hz which ruled out a double bond and this implied that they were two separate singlets (fig. 2.1 C). This type of resonance is a feature of a terminal unsaturated methylene group bonded to a tertiary carbon.[36] The peak at 5.35 ppm was observed as a broadened doublet with a coupling constant of 5.19 Hz, typical of a vinylic hydrogen split by allylic hydrogens. The methyl protons mentioned above include a signal at 0.77 ppm which was observed as a triplet, suggesting that this methyl group was bonded to a CH_2 group. Another signal at 0.88 ppm was a doublet which suggested that this methyl group was linked to a CH group. The signals at 0.62 ppm, 0.98 pm and 1.51 ppm were all singlets and were assigned to

protons of the methyl groups which were bonded to tertiary carbons. The resonance at 1.51 ppm is typical of the protons of an allylic CH_3 group which usually resonate at 1.59 ppm. In addition, the chemical shift is characteristic of a methyl on a terminal double bond (fig 2.1 D). The information derived from the ^1H NMR spectrum, led to the conclusion that the following fragments were present in compound 1 (fig. 2.1).

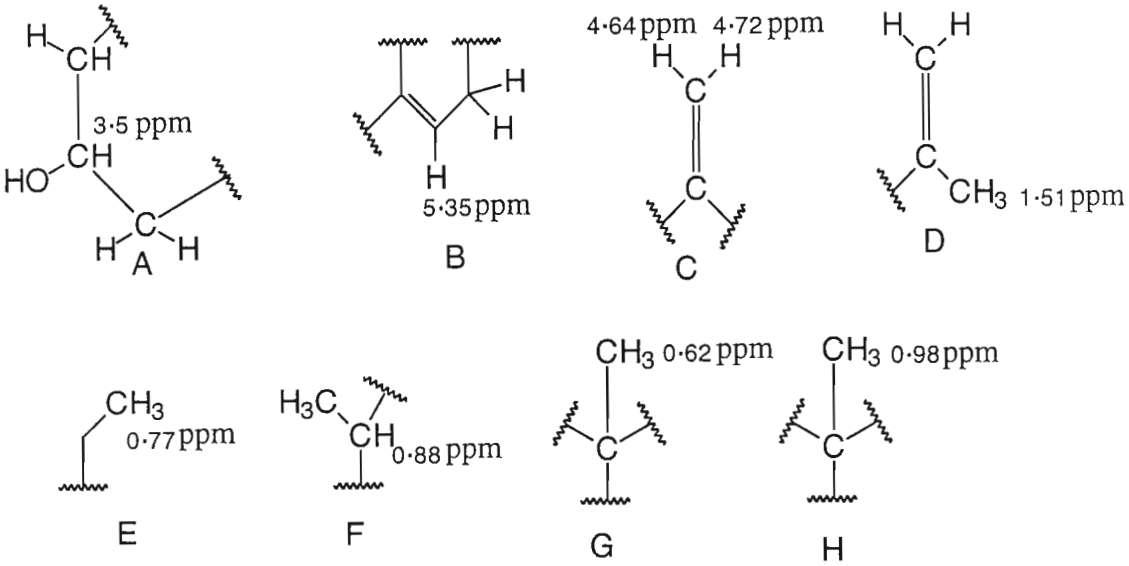


Fig 2.1

The nature of the functional groups proposed to be present in compound 1 and the nature of resonances in the ^1H NMR spectrum suggested that compound 1 was either a hydroxylated fatty acid with double bonded systems or some sort of a sterol. Compound 1 was thought to be a sterol as opposed to a fatty acid due to the presence of a number of tertiary carbons which are very rare for fatty acids but common in sterols. The presence of a multiplet at 3.50 ppm also supported the former statement as this signal is typical for a H-3 proton where C-3 has OR (R = H, alkyl) attached in sterols.

The ^{13}C NMR spectrum (spectrum 1.2) had four peaks between 110 ppm and 150 ppm which meant that there were four vinylic carbons in the compound. The signal

at 72.00 ppm suggested that a -CH-O group was present which agreed with the results from ^{1}H NMR spectrum. A number of signals at 12 ppm - 58 ppm confirmed the previously proposed statements since only protonated carbon atoms were present in this region.

The DEPT spectrum (spectrum 1.3) had five CH_3 groups and a number of CH_2 and CH groups, confirming the results from the ^{1}H NMR spectrum.

The HETCOR spectrum (spectrum 1.4) showed a doublet at 5.35 ppm to be correlating to a carbon resonating at 122 ppm. The two singlets at 4.64 ppm and 4.72 ppm, both correlating to the carbon which resonates at 110 ppm, implied that these two protons were bonded to the same carbon. The HETCOR results further showed that the proton signals between 4.50 ppm and 5.50 ppm correlated to carbons resonating in the double bond region. The signal at 3.50 ppm correlated to a carbon at 72.00 ppm which is characteristic of the –CH-O region. These results confirmed previous assignments.

Comparing the spectra of compound 1 against those of known sterols[37,38,39] and considering the facts derived from the data of this compound, it was concluded that the compound in question was 24-ethylcholesta-5,25 diene-3β-ol (fig 2.2). The latter has been isolated from *Codium fragile* before.[39]

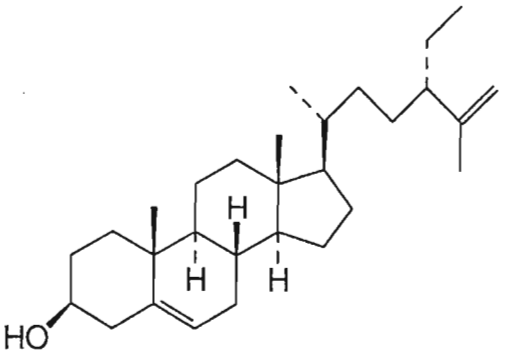


Fig 2.2: 24-ethylcholesta-5,25 diene-3β-ol

2.2 Investigation of *Palythoa natalensis*

The Oceanographic Research Institute (ORI) supplied the specimen which was collected on Aliwal shoal, off the south coast of KZN. These colonial cnidarians occur very freely along the coast and on reefs and, therefore, are thought to compete efficiently for limited space. The zoanthid was thus investigated in order to find compounds which may have antifeedant properties. However, owing to the small amount of organism, only the ethyl acetate extract was investigated. This extract gave two isomers of a sterol upon column chromatography.

2.2.A Structural elucidation of compound 2

The ^{1}H NMR (spectrum 2.1) had peaks characteristic of a sterol. These included a multiplet at 3.50 ppm which is typical for a 3-hydroxylated methine in many sterols. Another characteristic peak was a doublet at 5.35 ppm which is typical for an endocyclic double bond. The proton NMR also had five distinct absorptions at 0.60 ppm – 1.00 ppm which were indicative of five methyl groups being present in compound 2. These five peaks consisted of three doublets and two singlets. Between 1.00 ppm and 2.50 ppm appeared a number of multiplet peaks which represented the balance of hydrocarbon groups i.e., methines and methylenes.

From the proton spectrum, it was apparent that compound 2 was a sterol with a hydroxyl group at position 3, one double bond (endocyclic) and five methyl groups. Close inspection of the ^{1}H NMR spectrum showed doubling of some of the signals, indicating the possible presence of more than one isomer.

The ^{13}C NMR spectrum (spectrum 2.2) had two peaks at 121.00 and 140.05 ppm, indicative of a double bond. The signal at 72.00 ppm confirmed the presence of a carbon bonded to an oxygen atom. A number of peaks between 10.00 ppm and 41.50 ppm represented the hydrocarbon group carbons.

The DEPT spectrum (spectrum 2.3) showed the presence of six methyl groups as opposed to five observed in the proton NMR spectrum. This meant that there were two carbons bearing hydrogens of almost the same chemical shift, hence, one broadened signal in the proton NMR. Such phenomena can be produced by the following system, which was proposed to be present in compound 2:

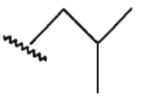


Fig 2.3

A mixture of diastereomers will also cause this effect and give more peaks than expected. The DEPT also showed a number of methylenes and methine groups as expected. The HETCOR spectrum (spectrum 2.4) had a doublet at 5.35 ppm which correlated to a carbon signal at 120.00 ppm. It also showed that a multiplet at 3.35 ppm correlated to a carbon signal at 72.00 ppm. These results agreed with the previous findings from proton NMR and ^{13}C NMR spectra.

The COSY spectrum (spectrum 2.5) gave a multiplet at 3.50 ppm which correlated to two sets of methylene protons. Thus, the signal at 3.50 ppm appeared as a multiplet. The doublet at 5.35 ppm correlated to a methylene group as well. Since the carbon bearing the hydroxyl group was found to link to two methylenes on either side, it meant that the endocyclic double bond is at the 5,6-position as opposed to the 4,5-position.

Confirmation of the presence of a 5,6-double bond, was the observation that the C-4 protons were deshielded more than what would be expected if only one electron withdrawing group (β-OH)[35] was adjacent to it. This downfield shift can only be caused if C-4 was bridging the double bond and C-3 hydroxyl. These results implied that the following structural system existed:

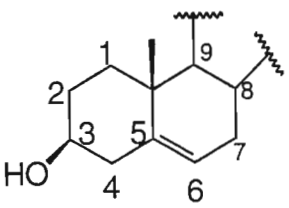


Fig 2.4

Considering the fragments proposed and bearing a sterol structure in mind, the following skeleton was suggested to be the compound in question (figure 2.5).

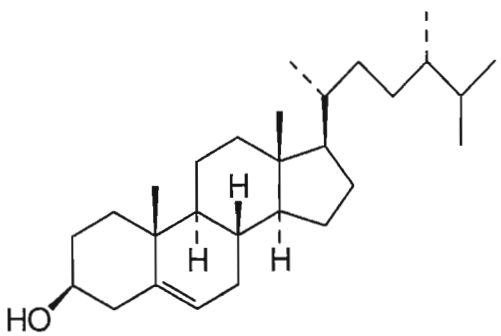
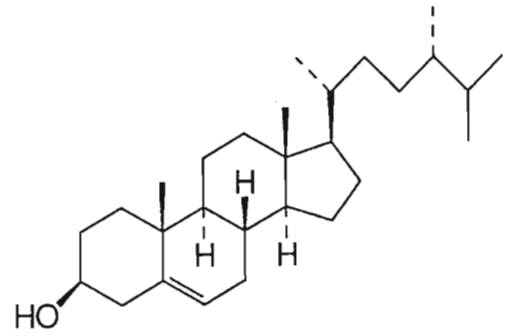
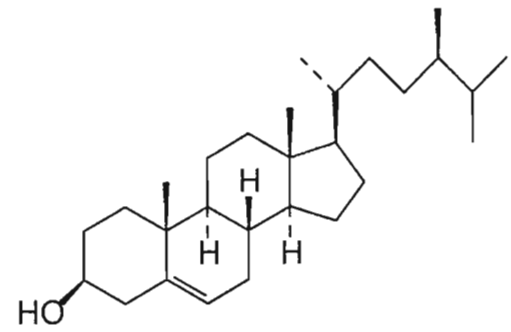


Fig 2.5: 24-methylcholesterol

It is apparent from figure 2.5 that C-24 is chiral. This was thought to be the reason for the observed doubling up on signals in the proton spectrum and the extra methyl group proton resonance in the DEPT spectrum. Inspection of the HETCOR showed the correlation of two carbons resonating at 17.50 and 15.20 ppm to the methyl proton doublet at 0.68 ppm. Comparison with data from literature[40], showed that the mixture of sterols is in fact common, being known as 22-dihydrobrassicasterol and campesterol.[41,42] In the table below (Table 2.1) the ^{1}H peaks for compound 2 are compared to the ^{1}H peaks of 22-dihydrobrassicasterol and those of campesterol (fig 2.5 a).



Campesterol



22-dihydrobrassicasterol

Fig 2.5 (a):structural comparison of campesterol and 22-dihydobrassicasterol

Table 2.1: Comparison table for selected ^{1}H NMR peaks of compound 2, 22-dihydrobrassicasterol and campesterol.

Proton No.	Compound 2 (ppm)	22-dihydrobrassicasterol (ppm)	Campesterol (ppm)
H-18	0.65	0.68	0.68
H-19	0.98	1.02	1.02
H-21	0.89	0.91	0.91
H-26	0.83	0.85	0.85
H-27	0.76	0.78	0.80
H-28	0.75	0.77	0.77

2.3 Investigation of *Carpobrotus edulis*

Due to the dependence on ORI (Oceanographic Research Institute) for the supply of Marine organisms, and in order to obtain larger quantities of material, a terrestrial plant was considered.

Carpobrotus edulis is known to have anti-inflammatory action and the aim was to isolate compounds with such effects. Mr. K. Crampton, the University of Natal,Durban (UND) chief horticulturist, identified the specimen which was collected on the UND premises. The methanol extract of the organism gave *para*-methoxycinnamic acid.

2.3A Structural elucidation of compound 3

Inspection of the ^{1}H NMR made it clear that a trans-substituted double bond and a para-disubstuted aromatic ring were present (fig. 2.6).

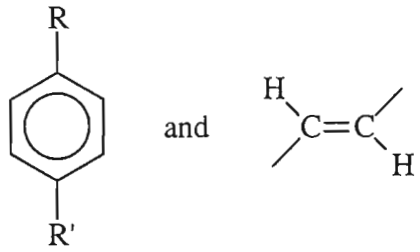


Fig 2.6

The COSY spectrum (spectrum 3.2) showed that a doublet at 6.30 ppm is coupled to a doublet at 7.65 ppm and these split each other with a coupling constant of 16.00 Hz, typical for a *trans* double bonded system.[36] The doublet at 6.85 ppm correlated to a doublet at 7.40 ppm and these had a coupling constant of 9.00 Hz, typical for a *para*-substituted benzene system.[32] These results are shown in the table below (Table 2.2).

Table 2.2: Results from the COSY spectrum (spectrum 2)

Proton resonating at	Correlates to proton resonating at
6.30	7.65
6.85	7.40

A signal at 3.80 ppm (spectrum 3.1) is characteristic of an aromatic methoxy substituent. From the ^{1}H and COSY spectra, it was suggested that the following systems existed in the compound (fig 2.7).

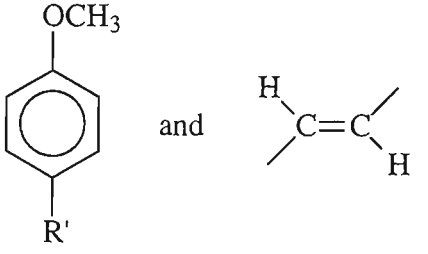


Fig 2.7

From the results obtained so far i.e., ^{1}H and COSY spectra, it was proposed that R was a methoxyl and R' was a *trans* disubstituted ethylene group (fig 2.7) which led to the following skeleton (fig 2.8).

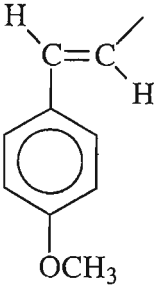


Fig 2.8

From the ^{13}C NMR spectrum (spectrum 3.3), a signal at 168.00 ppm was observed and this indicated the presence of the carbon atom of a carboxyl group. In cinnamic acid, this carboxyl group carbon resonates at 172.00 ppm. This suggested that

compound 3 was a cinnamic acid derivative with the carboxyl resonance shifted upfield i.e., to 168.00 ppm, due to the presence of the methoxyl group at the *para*-position. The methoxyl group has a positive inductive effect, which causes the shielding of the carbonyl group and, hence, resonating upfield.

Combining results from the ^{1}H, COSY, and ^{13}C NMR spectra led to the conclusion that the compound in question was *trans*-4-methoxycinnamic acid.

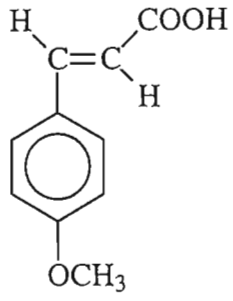


Fig 2.9

The results from the DEPT and HETCOR experiments (spectra 3.4 and 3.5) confirmed the above proposed structure, i.e., there is one methyl group present which was assigned to the methoxyl group (Table 2.3). No methylene group was observed. Four unsaturated methines i.e., two pairs from the benzene ring and two olefenic carbons were present.

Table 2.3: Results from the HETCOR spectrum (spectrum 3.5)

Proton resonating at (ppm)	Correlates to carbon resonating at (ppm)
3.78 (s)	52.00
6.30 (d, *J* 16 Hz)	114.50
6.85 (d, *J* 9 Hz)	116.00
7.40 (d, *J* 9 Hz)	130.00
7.65 (d, *J* 16 Hz)	144.00

2.4 Investigation of *Zoanthus durbanensis*

Like *Palythoa natalensis*, *Zoanthus durbenensis* is another organism that belongs to the family of the zoanthids. It was collected off Treasure Beach, KZN, and has not been formally classified. *Zoanthus durbenensis* yielded only a single sterol from the hexane soluble fraction of the methanol extract. The polar compounds were not investigated, since another closely related species, i.e., *Zoanthus sansibaricus,* was used for polar compound studies.

2.4.A Structural elucidation of compound 4

The ^{1}H NMR spectrum (spectrum 4.1) had characteristic signals i.e., a multiplet at 3.45 ppm, two singlets at 4.60 ppm and 4.61 ppm and a doublet at 5.26 ppm. The signal at 3.45 ppm is typical for a proton on a carbon to which a hydroxyl group is attached in many sterols and their derivatives. This peak was broad in nature and from that it was deduced that the hydroxyl group was in the β position.[35] The two singlets at 4.60 ppm and 4.61 ppm are characteristic of the methylene protons of a terminal double bond. Since these were singlets, it meant that a carbon β to the hydrogens did not have protons attached. The doublet at 5.26 ppm was observed to be broadened with a coupling constant of 5.25 Hz which was within the typical range of a vinylic hydrogen, split by allylic hydrogens. The peaks at 0.59 ppm – 2.25 ppm suggested the presence of hydrocarbon groups i.e., CH_3, CH_2 and CH. Compound 4 was thought to be a sterol bearing methyl groups, a hydroxyl group at position 3, an endocyclic double bond and a terminal double bond.

The DEPT spectrum (spectrum 4.2) had a signal at 12.00 ppm which appeared as a CH_3. From the HETCOR (spectrum 4.3), this carbon correlated to a singlet at 0.60 ppm which meant that this methyl was attached to a tertiary carbon. The DEPT spectrum indicated the presence of the four further methyl groups. Combining

DEPT, HETCOR and ^1H spectra, it was deduced that two CH_3's were singlets, while three of them were doublets.

From the above information, the following skeleton was proposed:

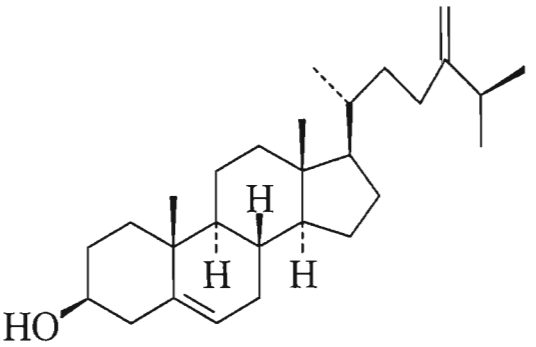


Fig 2.10: Chalinasterol

The spectra (4.1 – 4.5) obtained from NMR experiments i.e., ^1H, ^{13}C, COSY, DEPT and HETCOR spectra, were studied further in order to confirm the above structure. The substance is known as chalinasterol and has been isolated from *Palythoa caribaeorum* before.[3]

The position of the endocyclic double bond was verified using ^1H NMR chemical shifts and HMBC correlations. The two protons at position 4 had a down-field chemical shift due to the influence of both the double bond and the hydroxyl group at position 3. Had the double bond been at position 4, the two protons at position 6 would be equivalent to the two protons mentioned above (at position 4), but influenced by the double bond only. From the chemical shifts of the ^1H NMR spectrum the two protons were shifted in such a way as they were influenced by both the double bond and the hydroxyl group, hence, it was concluded that the double bond was located at position 5.

2.5 Investigation of *Zoanthus sansibaricus*

A third type of zoanthid, *Zoanthus sansibaricus* was investigated in order to compare the chemotaxonomy of the genus. The crude extract appeared identical to those of *Palythoa natalensis* and *Zoanthus durbanensis*. Therefore, only *Zoanthus*

sansibaricus was studied in detail. Since it was evident from the spectra of the hexane fraction that the same sterols already described were present, only the polar fractions were considered. As *Zoanthus sansibaricus* is significantly more coloured than the others, it was thought that xanthins (fig 1.7) would be isolated. This was indeed the case as many fractions showed the characteristic fluorescence associated with these xanthins. However, only trace amounts were present and so were not investigated further. A literature survey[11] revealed that research groups which did isolate xanthins from zoanthids, used large quantities of organism (> 40 kg) which could not be justified in this project.

2.5.A Structural elucidation of compound 5

The first compound to be isolated from *Zoanthus sansibaricus* was obtained from the methanolic extract. Repeated chromatography gave compound 5 as a solid.

The ^{1}H NMR spectrum (spectrum 5.1) had signals which were a pair of doublets in the aromatic region. These doublets occurred at 6.80 ppm and 7.12 ppm and each was split with a frequency of 8.54 Hz. These characteristics are a typical feature of *para*-disubstituted benzene rings. Furthermore, the ^{1}H NMR spectrum had two triplet signals occurring at 2.86 ppm and 3.30 ppm and these were observed to be coupled to each other in the COSY spectrum (spectrum 5.2). The COSY spectrum also showed the coupling of the two doublets occurring in the aromatic region which agreed with the results from the ^{1}H NMR spectrum, i.e., that these doublets were coupled.

The HETCOR spectrum (spectrum 5.3) showed that the triplets at 2.86 ppm and 3.30 ppm were correlated to carbon resonances at 34.00 ppm and 42.50 ppm, respectively. These carbon signals were present in the DEPT spectrum (spectrum 5.4) as methylene carbons.

From the information collected so far, it was proposed that the following groups were present in compound 5.

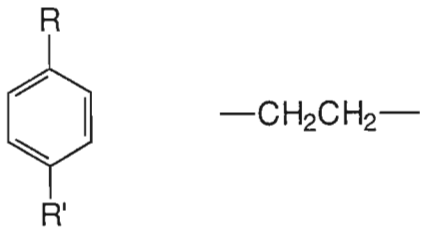


Fig 2.11

In order to establish the connectivity of these groups, further experiments were conducted.

The HETCOR spectrum showed that the doublets at 6.80 ppm and 7.12 ppm in the aromatic region were correlated to carbon resonances at 117,00 ppm and 131 ,00 ppm, respectively, which were also in the aromatic region of the carbon spectrum.

The DEPT spectrum showed the presence of two methylene carbons, two methine carbons and no methyl carbons. All these results agreed with the previous findings.

The ^{13}C NMR spectrum (spectrum 5.5) had, amongst other signals, a signal at 157.80 ppm which is typical for an *ipso* carbon linked to an OH group. This suggested that there was a hydroxyl group linked to a benzene ring.

Combining the fragments proposed previously, the following skeleton could further be proposed to be part of compound 5:

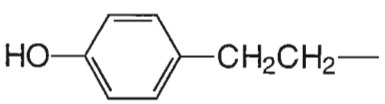


Fig 2.12

When all spectra were further studied in detail, there was no evidence of an extra carbon in the above skeleton. It, therefore, meant that the ethylene group shown

above had a terminal group containing no carbon. Such a terminal group could be a hydroxyl, amino or a halo group. The halogens were excluded because the chemical shits of protons and carbon linked to a halogen did not match. The hydroxyl group was excluded on the grounds that the ^{13}C NMR spectrum (spectrum 5.5) had no signal in the region of 50.00 – 80.00 ppm, which is a CH-O region. The group that was likely to be present was the amino group, which also agreed with the chemical shifts observed.

It could, therefore, be concluded that compound 5 had the following structure (fig 2.13):

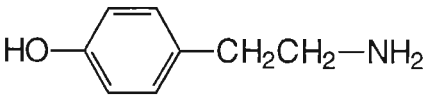


Fig 2.13

It has been isolated from several plant species and putrefied animal tissues before.[47] It results from the metabolism of tyrosine leading to decarboxylation.

2.5.B Structural elucidation of compound 6

Compound 6 was found to be a nucleoside consisting of a ribose sugar and cytosine (figure 2.14).

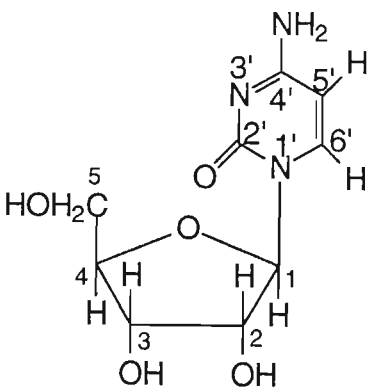


Fig 2.14: Compound 6

The ^{1}H NMR spectrum (spectrum 6.1) had a doublet at 7.89 ppm. This signal occurred at the same position as that of cytosine nucleoside.[43] The latter also has a doublet at approximately 6.00 ppm, while compound 6 had a number of doublets between 5.00 - 6.00 ppm, of which one was believed to belong to cytosine. Four peaks appeared in the region of 3.50 – 4.20 ppm, i.e., three quartets and one multiplet (spectrum 6.1). Such peaks were indicative of a sugar moiety being possibly present. Treatment of the NMR sample with D_2O led to the disappearance of the resonances at 5.10 and 5.40 ppm (spectrum 6.2). These doublets were thought to be either hydroxyl or aminyl groups, but since the peaks were narrow, it was thought more likely that hydroxyls were present. The integral showed that the doublet at 5.10 ppm (spectrum 6.1) was due to two hydrogens which meant that there were two hydroxyl groups producing signals that were overlapping with each other. The doublet at 5.40 ppm integrated to one proton.

The COSY spectrum (spectrum 6.3) showed that a doublet at 7.89 ppm was coupled to another doublet at 5.65 ppm. Such a doublet to doublet correlation was expected upon considering the structure of the proposed fragment, cytosine. The absence of the amino resonance in the ^{1}H spectrum could not be accounted for. However, these are often broadened and it suggested that the sensitivity of the instrument was not sufficient to distinguish the band from the baseline.

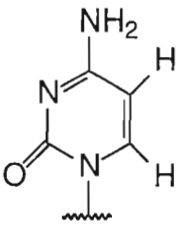


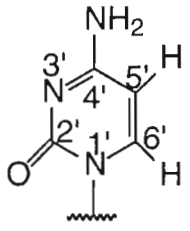
Fig 2.15: Cytosine

The other doublets between 5.00 ppm and 5.90 ppm all correlated to the signals between 3.50 and 4.20 ppm, which were proposed to be due to the sugar that was suggested to be present. The complete set of COSY results are shown in a tabular form below (Table 2.4).

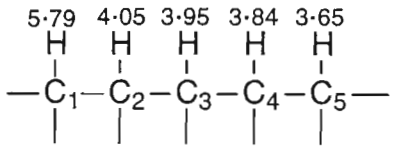
Table 2.4: Results from the COSY spectrum (spectrum 6.3)

Proton/s resonating at (ppm)	Correlate/s to proton/s resonating at (ppm)
7.89 (d, *J* 8.06 Hz)	5.65 (d, *J* 8.06 Hz)
5.79 (d, *J* 8.06 Hz)	4.05 (q, *J* 5.22 Hz)
4.05 (q, *J* 5.22 Hz)	3.95 (q, *J* 4.25 Hz)
3.95 (q, *J* 4.25 Hz)	3.84 (q, *J* 3.69 Hz)
3.84 (q, *J* 3.69 Hz)	3.60 (m, *J* 3.81 Hz)
5.40 (d, *J* 4.98 Hz) OH	4.05 (q, *J* 5.22 Hz)
5.10 (d, *J* 4.40 Hz) OH	3.95 (q, *J* 4.25 Hz)
5.10 (d, *J* 3.62 Hz) OH	3.60 (m, *J* 3.81 Hz)

From the ^{1}H and COSY results it could be deduced that a cytosine moiety was present in compound 6 as shown by the first entry in table 2.4. The second entry onwards showed a chain being present as demonstrated by one set of protons correlating to the adjacent set and this set in turn correlated to the next, etc. Such a chain and cytosine moiety are shown in the figure below (fig. 2.16).



Cytosine



A carbon chain

Fig 2.16

Considering the presence of hydroxyl groups, together with their positions according to the COSY results (Table 2.4), the chain in figure 2.16 above could be drawn as follows:

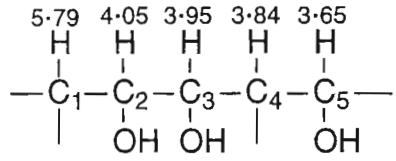


Fig 2.17: A hydroxylated carbon chain

The ^{13}C NMR spectrum (spectrum 6.4) showed the presence of nine carbons and this corresponded exactly to the total number of carbons of the two subunits proposed previously i.e., cytosine and a carbon chain. It was observed from the ^{13}C NMR spectrum that five carbons appeared in the region of 60.05 – 88.00 ppm which is the CH-O bonded region. This supported the previous findings that some of the carbons were hydroxylated, thus indicating the presence of a sugar as was previously proposed from the ^{1}H NMR spectrum (spectrum 6.1). A set of ^{13}C NMR signals (four) appeared at 102.00 ppm – 163.00 ppm, of which all four were assigned to the cytosine moiety. Comparison with literature values confirmed this unit to be identical to cytosine (Table 2.5).[43] Differences were ascribed to the different solvents used.

Table 2.5: ^{13}C NMR signals of compound 6 compared to those of cytosine

Carbon No.	^{13}C NMR signals of compound 6 (DMSO)	^{13}C NMR signals of cytosine (D_20/NaOH)
C-5'	102.00 ppm	99.00 ppm
C-6'	140.50 ppm	144.00 ppm
C-4'	151.00 ppm	160.00 ppm
C-2'	163.00 ppm	169.00 ppm

The DEPT spectrum (spectrum 6.5) showed that a signal at 60.05 ppm was a methylene carbon while the rest of the carbons in the CH-O region were all methines. Out of the four signals belonging to cytosine, the signals at 102.00 and 140.05 ppm appeared as methines and were assigned to carbons 5' and 6'.

The HSQC spectrum (spectrum 6.6) showed the multiplet at 3.60 ppm to be the methylene protons and it correlated to the carbon resonance at 60.05 ppm. The rest of the carbons were all methines and their correlation to protons are shown in a tabular form below (Table 2.6).

Table 2.6: Results from the HSQC spectrum (spectrum 6.6)

Proton/s resonating at (ppm)	Correlate/s to carbon resonance at (ppm)
5.79 (d)	87.80 (C-1)
4.05 (q)	70.00 (C-2)
3.95 (q)	74.00 (C-3)
3.84 (q)	85.00 (C-4)
3.60 (m)	60.05 (C-5)
5.65 (d)	102.00 (C-5' or C-6')
7.89 (d)	140.00 (C-5' or C-6')

The HMBC spectrum (spectrum 6.7) showed that the proton at C-1 (a doublet at 5.79) was two to three bonds away from C-4 (75.00 ppm). This suggested that, since these two carbons are far away in the carbon chain, they have been brought to close proximity by some sort of a bridge and an oxygen bridge was suggested. Such an oxygen bridge was proposed because carbons 1 and 4 resonanated in the CH-O bonded region in the ^{13}C NMR spectrum (spectrum 6.4).
The existence of a bridge suggested a cyclic structure of a sugar as shown below:

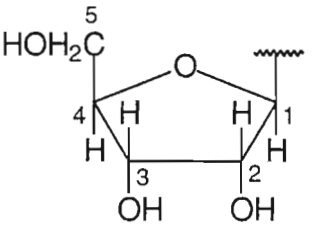


Fig 2.18

The assignment of C-5 as a methylene carbon was derived from the HMQC experiment (spectrum 6.6) and DEPT experiment (spectrum 6.5).

Furthermore, a proton at C-1 correlated to carbons resonating at 140.00 ppm and 150.00 ppm, which belong to the cytosine moiety. This meant that the sugar was linked to cytosine through C-1. These results led to the proposal of the following structure:

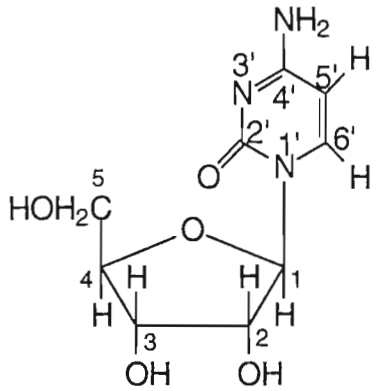


Fig 2.19

The HMBC spectrum also showed that a doublet at 5.65 ppm for cytosine showed no correlation to the sugar unit and, hence, was far removed from it. The doublet at 7.89 ppm showed correlation to C-1of the sugar. The proton resonating at 7.89 ppm would therefore be assigned to C-6' while the proton at 5.65 ppm belong to C-5' (Table 2.6). The rest of the HMBC results are shown in the table below (Table 2.7).

Table 2.7: Results from the HMBC spectrum (spectrum 6.7)

Proton/s	Correlate to
H-1	C-4, C-2', C-6'
H-2	C-1, C-4
H-3	C-1, C-5
H-4	C-1, C-2, C-5
H-5	C-4
H-5'	C-6'
H-6'	C-1, C-2', C-4', C-5'

The HMBC results confirmed the structure proposed.

The NOE results (spectra 6.8 – 6.13) indicated that when irradiating some protons of the molecule attached to a carbon, certain proton signals were enhanced. These NOE effects clearly demonstrated that the saccharide ring is flexible so that correlations between *trans*-situated protons could be observed.

The NOE results are shown in the table below (Table 2.8).

Table 2.8: Results from the NOE spectra (spectra 6.8 – 6.13)

Protons irradiated	**Signals enhanced**	**Reference**
Doublet at 7.89 ppm (C-6')	Doublet at 5.79 ppm (C-1), Quartet at 4.05 ppm (C-2), Quartet at 3.95 ppm (C-3), Doublet at 5.65 ppm (C-5').	Spectrum 6.8
Doublet at 5.79 ppm (C-1)	Doublet at 7.89 ppm (C-6'), Quartet at 4.05 ppm (C-2), Quartet at 3.95 ppm (C-3), Quartet at 3.84 ppm (C-4).	Spectrum 6.9
Doublet at 5.65 ppm (C-5')	Doublet at 7.89 ppm (C-6').	Spectrum 6.10
Quartet at 4.05 ppm (C-2)	Doublet at 7.89 ppm (C-6'), Quartet at 3.95 ppm (C-3).	Spectrum 6.11
Quartet at 3.95 ppm (C-3)	Doublet at 7.89 ppm (C-6').	Spectrum 6.12
Quartet at 3.84 ppm (C-4)	Doublet at 5.79 ppm (C-1), Multiplet at 3.60 ppm (C-5).	Spectrum 6.13

The results from ^{1}H NMR, COSY, ^{13}C, DEPT, HSQC, HMBC and NOE experiments all confirmed that the compound in question i.e., compound 6 had the structure previously proposed and shown below (compound 6). This is identical to the known ribonucleic acid nucleoside called cytosine nucleoside.

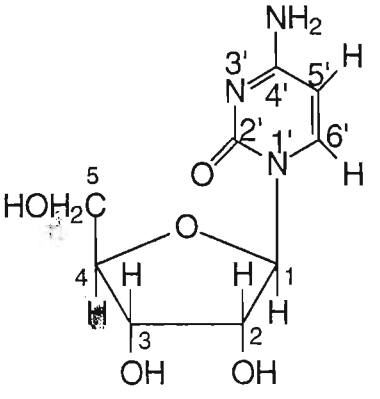


Fig 2.20: **Compound 6**

In order to establish whether or not the compound was a nucleoside or nucleotide, a ^{31}P NMR spectrum was acquired. However no signal was obtained which implied that the hydroxyls were indeed free, as concluded from the proton NMR.

2.5.C Structural elucidation of compound 7

The ^{1}H NMR spectrum (spectrum 7.1) had two singlets at 8.10 ppm and 8.40 ppm which were indicative of the proton peaks of the inosine nucleic acid base.[43] The presence of a number of peaks between 3,50 ppm and 5.00 ppm suggested the presence of a sugar. Therefore, compound 7 was again thought to be a nucleoside (Figure 2.21).

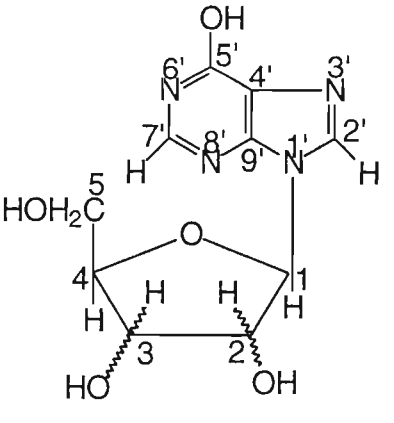


Fig 2.21

From the COSY NMR experiment (spectrum 7.2), it was observed that a doublet at 6.06 ppm was coupled to a triplet at 4.65 ppm which in turn was coupled to a quartet at 4.35 ppm. The protons resonating at 4.35 ppm correlated further to a signal at 4.25 ppm. The latter was coupled to a set of doublets at 3.75 ppm – 3.95ppm.

The HMQC spectrum (spectrum 7.3) had a doublet at 6.05 ppm which correlated to a carbon resonance at 90.50 ppm which was assigned to C-1. A triplet at 4.65 ppm correlated to a carbon at 76.10 ppm (C-2), while the quartet at 4.35 ppm correlated to a carbon at 72.10 (C-3). The resonance at 4.25 ppm appeared as a quartet and correlated to a carbon at 87.50 (C-4). The protons on carbon-5 which resonated at 63.00 ppm, were non-equivalent and appeared as a set of doublets between 3.75 ppm and 3.95 ppm. In the aromatic region, two singlets at 8.40 ppm and 8.10 ppm correlated to carbons resonating at 141.00 ppm and 147.00 ppm, respectively.

The DEPT experiment (spectrum 7.4) indicated that the carbon atoms at 90.50 ppm (C-1), 76.10 ppm (C-2), 72.10 ppm (C-3) and 87.50 ppm (C-4) all had one proton attached while the carbon atom at 63.00 ppm (C-5) had two protons attached to it. From the ^{13}C NMR chemical shifts for carbons 1-5 it was apparent that these atoms were each bonded to an oxygen, therefore, confirming the proposal that a sugar was present in compound 7. Combining the results from the ^{13}C, COSY, HMQC and DEPT NMR experiments led to the conclusion that the following chain existed.

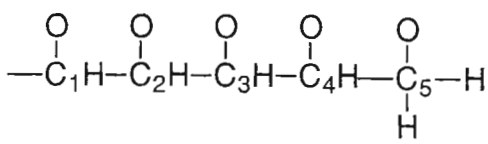


Fig 2.22

The results from the HMBC experiment confirmed the existence of the chain shown above, i.e., the proton at C-1 correlated to C-3 which meant that the proton was 2 to 3 bonds away from C-3 (spectrum 7.5). The other results are shown in a tabular form below (Table 2.9).

Table 2.9: Results from the HMBC spectrum (spectrum 7.5)

Proton/s	Correlates to
H-1	C-3
H-2	C-1 and C-4
H-3	C-1 , C-4 and C-5
H-4	C-1 and C-3
H-5	C-3 and C-4

In addition, the HMBC (spectrum 7.5) also showed that the proton at C-4 was 2 to 3 bonds away from C-1. This meant that C-1 and C-4 were in close proximity and an oxygen bridge was suggested between these two carbons since they appeared in the C-O region as concluded from ^{13}C results (spectrum 7.6). Hence, it was suggested that the sugar was a pentose present in its furanose form. Comparison of the proton and ^{13}C NMR spectra of compound 7 with literature,[43] suggested that the unknown compound might have an inosine moiety attached to a sugar.

The carbon resonances at 149.50 ppm and 141.00 ppm matched C-2 and C-5 of inosine while the proton resonances at 8.40 ppm and 8.10 ppm corresponded to C-2 (H) and C-7 (H).

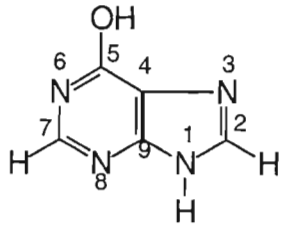


Fig 2.23: inosine

The rest of the comparison is shown in Table 2.10 (^{1}H NMR signals) and 2.11 (^{13}C NMR signals).

Table 2.10: ^{1}H NMR signals of compound 7 compared to those of inosine riboside[43]

Proton No.	^{1}H NMR signals of compound 7	^{1}H NMR signals of cytosine
H-5	dd at 3.85 ppm	dd at 3.65 ppm
H-4	q at 4.15 ppm	q at 3.98 ppm
H-3	q at 4.35 ppm	q at 4.15 ppm
H-2	t at 4.65 ppm	t at 4.50 ppm
H-1	d at 6.05 ppm	d at 5.90 ppm

Table 2.11: ^{13}C NMR signals of compound 7 compared to those of inosine riboside[43]

Carbon No.	^{13}C NMR signals of compound 7 (MeOH)	^{13}C NMR signals of inosine (DMSO)
C-5	62.98 ppm	61.00 ppm
C-3	72.13 ppm	70.00 ppm
C-2	76.11 ppm	74.00 ppm
C-4	87.54 ppm	85.00 ppm
C-1	90.58 ppm	87.00 ppm
C-4'	126.28 ppm	124.00 ppm
C-2'	143.18 ppm	138.00 ppm
C-7'	146.85 ppm	146.00 ppm
C-5'	150.00 ppm	148.00 ppm
C-9'	158.00 ppm	156.20 ppm

HMBC results (spectrum 7.7) also indicated that the protons at C-1 were 2 to 3 bonds removed from the carbons at 149.50 ppm and 141.00 ppm. These two carbons were part of inosine nucleus, which meant that the sugar was linked to inosine through C-1.

The results obtained from the NOE experiments (spectrum 7.8) demonstrated that when the singlet at 8.40 ppm was irradiated, the doublet at 6.05 ppm was enhanced,

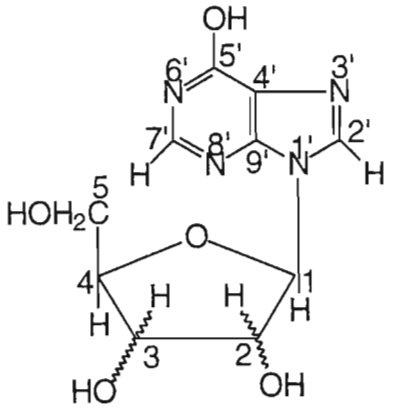
which meant that these protons must be close in space with respect to each other. This implied that C-1 in the sugar was linked to a nitrogen of the imidazole portion of inosine, and that this nitrogen must be between the two carbons resonating at 141.00 ppm and 149.50 ppm, in support of the conclusions derived from HMBC results (spectrum 7.7). The other NOE results (spectra 7.9 – 7.13) confirmed those already suggested above, i.e., when a quartet at 4.25 ppm was irradiated, the doublet of doublets at 3.75 ppm – 3.95 ppm, a quartet at 4.35 ppm and a doublet at 6.06 ppm were all enhanced (spectrum 7.9). Other results are shown in the form of a table below (Table 2.12).

Table 2.12: Results from the NOE spectrum (spectra 7.9 – 7.13)

Proton/s irradiated*	**Signal/s enhanced***	**Reference:**
Quartet at 4.25 ppm (C-4)	Double of doublets at 3.75-3.95 (C-5) Quartet at 4.35 ppm (C-3) Doublet at 6.06 ppm (C-1)	(spectrum 7.9)
Quartet at 4.35 ppm (C-3)	Quartet at 4.25 ppm (C-4) Double of doublets at 3.75-3.95 (C-5) Doublet at 6.06 ppm (C-1)	(spectrum 7.10)
Triplet at 4.65 ppm (C-2)	Quartet at 4.35 ppm (C-3) Doublet at 6.06 ppm(C-1), Singlets at 8.10 ppm and 8.40 ppm (weakly) (C-7' and C-2', respectively).	(spectrum 7.11)
Doublet at 6.06 ppm (C-1)	Triplet at 4.65 ppm (C-2), Quartet at 4.35 ppm (C-3), Quartet at 4.25 ppm (C-4), Singlet at 8.10 ppm (C-7').	(spectrum 7.12)
Singlet at 8.1 (C-7')	Quartet at 4.25 ppm (C-4)	(spectrum 7.13)
Singlet at 8.40 ppm (C-2')	Doublet at 6.06 ppm (C-1), Triplet at 4.65 ppm (C-2).	(spectrum 7.8)

*signals arise due to protons on the indicated carbons.

From the above results the following skeleton was proposed:

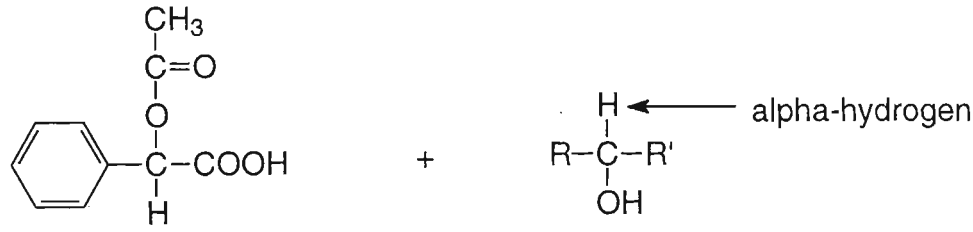


Compound 7 (Fig 2.21)

It is well known that monomers present in the genetic material, i.e., RNA and DNA, contain a nitrogen base and a ribose unit. Inspection of the NOE spectra demonstrated each pair of vicinal hydrogens had a spatial interaction. This led to the conclusion that all the hydrogens in the sugar moiety were on the same face of the molecule. As the pentose with such a configuration is lyxose, of which no example is known to be present in genetic material, a comprehensive literature review was conducted. Apart from ribose, only one example of a sulphur containing xylose nucleoside was found.[44] Upon constructing a model of ribose, it was apparent that the five membered ring is very flexible and that even vicinal hydrogens which are *trans* with respect to each other can be in close proximity and show a spatial interaction when conducting NOE experiments.

A ^{31}P NMR was recorded to confirm the absence of a phosphate moiety in the furanoside. No signal was obtained which meant that the molecule was indeed a nucleoside and not a nucleotide. In order to establish the absolute configuration of the free hydroxyls on the sugar, an attempt was made to prepare the mandelic acid ester derivative of compound 9.

According to Mosher,[45] if a hydroxyl group on a chiral centre is reacted with a single enantiomer of a chiral acid, e.g. O-acetylmandelic acid, the upfield or downfield shift of the α-hydrogen will indicate the absolute configuration of that centre.



O-acetyl mandelic acid

Fig 2.24

However, possibly due to steric congestion, the mandelic ester of compound 7 could not be formed in sufficient quantities to give meaningful NMR results. As at least two of the hydroxyls on the sugar are tertiary, the esterification using a bulky reagent such as O-acetyl mandelic acid chloride was not unexpected.

Comparison of the proton and carbon chemical shifts of the sugar portion of inosine riboside[43] with that of compound 7, clearly showed that the signals were identical. Hence, it was concluded that the saccharide moiety of compound 7 was ribose and the compound inosine riboside.

2.5.D Structural elucidation of compound 8

The ^{1}H NMR spectrum of compound 8 (spectrum 8.1) had a singlet at 7.88 ppm which was similar in chemical shift and shape to the one of thymine, a nucleic acid base.[43] The peaks between 3.50 ppm and 4.50 ppm suggested that a sugar might be present in compound 8 (figure 2.25).

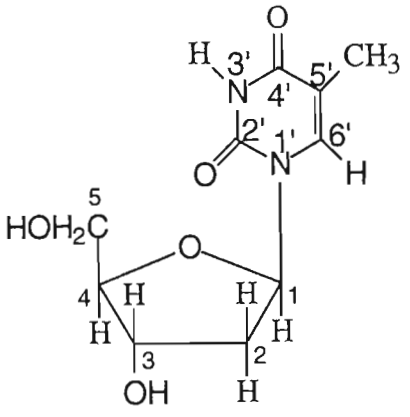


Figure 2.25

The spectrum from the COSY experiment (spectrum 8.2) showed that a triplet at 6.30 ppm was coupled to a multiplet at 2.25 ppm which in turn correlated to a quintet at 4.45 ppm. The latter correlated to a quartet at 3.95 ppm which further coupled to a set of doublet of doublets appearing at 3.71 ppm - 3.85 ppm. A singlet at 7.85 ppm was weakly coupled to a singlet at 1.90 ppm. These results are tabulated below (Table 2.13).

Table 2.13: Results from the COSY spectrum (Spectrum 8.2)

Proton/s resonating at	Correlate/s to protons resonating at
6.30 (t, *J* 6.78 Hz)	2.25 (m, *J* 5.86 Hz)
2.25 (m, *J* 5.86 Hz)	4.45 (p, *J* 3.29 Hz)
4.45 (p, *J* 3.29 Hz)	3.95 (q, *J* 6.96 Hz)
3.95 (q, *J* 6.96 Hz)	3.71 – 3.85 (dd, *J* 27.75 Hz)
7.85 (s)	1.90 (s)

The HSQC spectrum (spectrum 8.3) had a triplet at 6.30 ppm which correlated to a carbon resonating at 90.00 ppm and this carbon was assigned to C-1 of the sugar moiety. The correlations due to the saccharide are tabulated below (Table 2.14).

Table 2.14: Results from the HSQC spectrum (spectrum 8.3)

Proton/s resonating at (ppm)	Correlate/s to carbon resonating at (ppm)
6.30	90.00 (C-1)
2.25	44.00 (C-2)
4.45	96.00 (C-3)
3.95	93.00 (C-4)
2.85 – 3.71	65.00 (C-5)

Other results from HSQC spectrum indicated that the protons resonating at 1.90 ppm and 7.85 ppm correlated to carbons resonating at 12.00 ppm and 145.00 ppm, respectively (spectrum 8.3).

The spectrum from the DEPT experiment (spectrum 8.4) indicated that the carbon atoms at 90.00 ppm (C-1), 76.00 ppm (C-3) and 93.00 ppm (C-4) all had one proton attached while the carbon atoms at 44.00 ppm (C-2) and 65.00 ppm (C-5) both had two protons attached to them.

The ^{13}C NMR spectrum (spectrum 8.5) showed chemical shifts for C-1, C-3, C-4 and C-5 to be in the CH-O bonded region which suggested that these carbons were linked to an oxygen atom, while C-2 had a chemical shift upfield from the CH-O region. These results from ^{13}C chemical shifts confirmed the proposal that a sugar was present in compound 8. However, C-2 has been deoxygenated.

Combining the results from the ^{13}C, COSY, HSQC and DEPT experiments led to the conclusion that the following chain was present in compound 8:

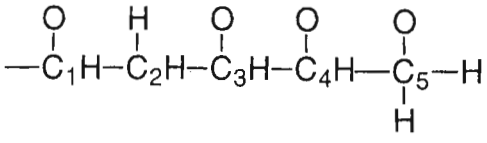


Fig 2.26

The results from the HMBC experiment (spectrum 8.6) confirmed the presence of the chain shown above i.e., the protons at C-2 were coupled to C-1, C-3, and C-4 which meant that these protons are 2 to 3 bonds removed from each of the carbons mentioned above (spectrum 8.6). Some results are shown in a tabular form below (Table 2.15).

Table 2.15: Results from the HMBC spectrum (spectrum 8.6)

Proton/s	Correlate/s to
H-2	C-1, C-3, C-4
H-3	C-1, C-5
H-4	C-5
H-5	C-3, C-4
H-6'	C-1, C-2', C-4', C-5', C-5'-CH_3

When spectra of compound 8 were compared to those of compound 7, it was observed that they both belong to the same class of compounds i.e., nucleic acid bases containing a sugar in its furanose form. This implied that a sugar in compound 8 has C-1 linked to C-4 through an oxygen bridge. This could not be confirmed for the HMBC spectrum, but the similar profile of the ^{1}H and ^{13}C NMR spectra compared to that of compound 7 suggested that this was a valid conclusion.

Comparison of the proton and ^{13}C spectra of compound 8 with literature,[43] suggested that the unknown compound might have a thymine moiety attached to a sugar. Such comparisons are shown in Table 2.16 (^{13}C signals) and Table 2.17 (^{1}H signals).

HMBC results also demonstrated that the proton situated on the anomeric centre of the sugar (C-1) was correlated to carbons resonating at 154.00 ppm and 140.00 ppm (spectrum 8.6). This implied that the sugar was linked to the thymine base through its C-1 with the linkage *via* the nitrogen atom (N_1') situated between carbons C-2' and C-6'.

Table 2.16: ^{13}C NMR signals of compound 8 compared to those of thymine[43]

Carbon No.	^{13}C NMR signals of compound 8 (MeOH)*	^{13}C NMR signals of thymidine (DMSO)*
C-6'	186.00 ppm	163.90 ppm
C-6'	169.00 ppm	150.00 ppm
C-6'	138.00 ppm	136.00 ppm
C-6'	111.50 ppm	109.00 ppm
C-1	89.00 ppm	87.00 ppm
C-3	86.00 ppm	83.00 ppm
C-4	72.00 ppm	70.00 ppm
C-5	62.00 ppm	61.50 ppm
C-2	41.00 ppm	39.40 ppm
$C_{5'}$-CH_3	12.10 ppm	12.00 ppm

*in enol (aromatic) form *in keto form

Table 2.17: ^{1}H NMR signals of compound 8 compared to those of thymidine[43]

Proton No.	^{1}H NMR signals of compound 8	^{1}H NMR signals of thymine
H-2'	s at 7.85 ppm	s at 7.70 ppm
H-1	t at 6.30 ppm	t at 6.19 ppm
H-3	p at 4.45 ppm	p at 4.25 ppm
H-4	q at 3.95 ppm	q at 3.79 ppm
H-5	dd at 3.80 ppm	dd at 3.60 ppm
H-2	m at 2.25 ppm	m at 2.10 ppm
H-($C_{5'}$-CH_3)	s at 1.90 ppm	s at 1.80 ppm

In support of the proposed structure, results from the NOE experiment (spectrum 8.7) showed that when the singlet at 7.85 ppm was irradiated, a triplet at 6.30 ppm (proton for C-1) was enhanced. This meant that the protons must be in close

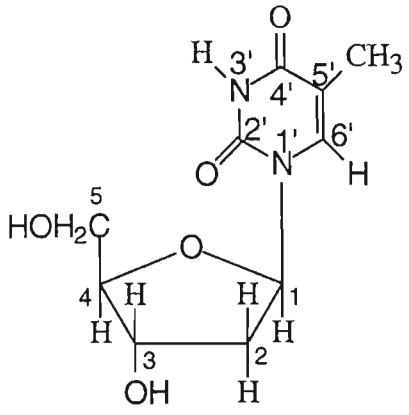
proximity. Other NOE correlations (spectrum 8.8 – 8.13) confirmed the proposals made and are tabulated below (Table 2.18).

Table 2.18: Results from the NOE spectra (spectrum 8.7 – 8.13)

Proton/s irradiated	Signal/s enhanced	Reference
Singlet at 7.85 ppm (C-2')	Triplet at 6.30 ppm (C-1), Multiplet at 2.25 ppm (C-2), Singlet at 1.90 ppm (N-5'-CH_3).	Spectrum 8.7
Triplet at 6.30 ppm (C-1)	Singlet at 7.85 ppm (C-2'), Quartet at 3.95 ppm (C-4), Multiplet at 2.25 ppm (C-2).	Spectrum 8.8
Quintet at 4.45 ppm (C-3)	Singlet at 7.85 ppm (C-2'), Quartet at 3.95 ppm (C-4), Doublet of doublets At 3.71-3.85 ppm (C-5), Multiplet at 2.25 (C-2).	Spectrum 8.9
Quartet at 3.95 ppm (C-4)	Doublet of doublets At 3.71-3.85 ppm (C-5), Pentet at 4.45 ppm (C-3), Triplet at 6.30 ppm (C-1)	Spectrum 8.10
Doublet of doublets at 3.71 ppm-3.85 ppm(C-5)	Quartet at 3.95 ppm (C-4)	Spectrum 8.11
Multiplet at 2.25 ppm (C-2)	Pentet at 4.45 ppm (C-3), Triplet at 6.30 ppm (C-1), Singlet at 7.85 ppm (C-2')	Spectrum 8.12
Singlet at 1.90 ppm (C-5'-CH_3)	Singlet at 7.85 ppm (C-2')	Spectrum 8.13

The proposed structure only had two free hydoxyl groups. This was confirmed by acetylating compound 8. The ^1H NMR spectrum of the acetylated compound clearly showed the presence of two acetyl moieties (spectrum 8A) and, hence, that

the 2-position of compound 8 was deoxygenated. Combining all the results presented above, the following skeleton was proposed:



Compound 8 (Fig 2.25)

2.5.E Resolution of fraction P3-10

A fraction labelled P3-10 had a distinctly higher R_f value than compound 5. This fraction (NMR spectrum A) was thought to be a pure compound, since repeated chromatography gave a single spot despite using different stationery phases (C18, Diol™, SiO_2). After each separation, identical ^1H spectra were obtained. However, the peak areas upon integration had contradicting results in terms of the number of hydrogens in some cases. Therefore, it was concluded that this fraction was a mixture. The mixture was then acetylated and purified resulting in two pure compounds, 9 and 10 (NMR spectrum 9.1 and spectrum 10.1), being obtained.

2.9.E (a) Structural elucidation of compound 9

The ^1H NMR spectrum (spectrum 9.1) had two doublets at 6.68 ppm and 7.01 ppm with coupling constants of 8.42 Hz and 8.61 Hz, respectively, typical for a *para*-disubstituted benzene. The peak at 1.97 ppm was thought to be due to protons of a methyl of the acetyl group resulting from acetylation.

The COSY spectrum (spectrum 9.2) had two doublets at 6.68 ppm and 7.01 ppm coupled to each other of which confirmed the results proposed from the ^{1}H NMR spectrum. The triplet at 2.71ppm was observed to be coupled to another triplet at 3.35 ppm. These results are shown in tabular form below (table 2.19):

Table 2.19: Results from the COSY spectrum (spectrum 9.2).

Proton/s resonating at (ppm)	Correlate/s to proton/s resonating at (ppm)
2.71 (t, *J* 7.40 Hz)	3.35 (t, *J* 7.14 Hz)
6.68 (d, *J* 8.82 Hz)	7.01 (d, *J* 8.61 Hz)

From the above information, the following groups were proposed to be present in compound 9 (fig 2.27).

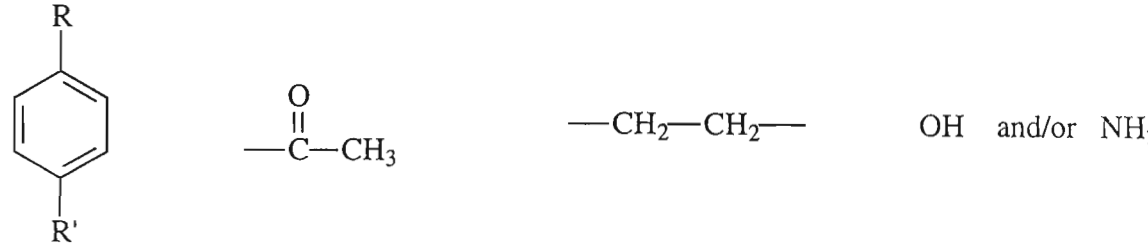


Fig 2.27

The ^{13}C NMR spectrum (spectrum 9.3) had some signals resonating between 20.00 ppm - 42.00 ppm which indicated the presence of hydrocarbon carbons, but no signals at 50.00 ppm - 80.00 ppm which proved that there was no CH-O link in the compound. The peaks at 114.00 ppm – 158.00 ppm appeared as two intense peaks which were assigned to the CH's of the aromatic ring and two small peaks which were assigned to the *ipso* carbons. The signal at 173.00 ppm was due to the carbonyl carbon of the acetyl group.

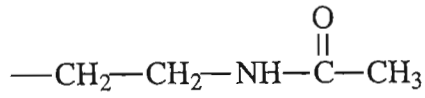
The DEPT spectrum (spectrum 9.4) showed one CH_3 group to be present, two CH_2 and two CH groups in the aromatic region. All these results agreed with the previous findings.

The HSQC spectrum (spectrum 9.5) gave a correlation between the methylene triplet at 2.71 ppm and a carbon resonating at 34.50 ppm. The experiment was set up in such a fashion that it differentiated between methylenes and methyls/methines. The HSQC spectrum also showed a triplet at 3.35 ppm to be a CH_2 which coupled to a carbon resonance at 40.50 ppm. The two doublets in the aromatic region of the ^1H NMR spectrum correlated to the two intense carbon resonances confirming the presence of a *para*-disubstituted benzene ring. These results are shown in tabular form below (Table 2.20):

Table 2.20: Results from the HSQC spectrum (spectrum 9.5)

Proton/s resonating at (ppm)	Correlate/s to carbon resonating at (ppm)
2.71 (t, *J* 7.40 Hz)	34.50
3.35 (t, *J* 7.14 Hz)	40.50
6.68 (d, *J* 8.82 Hz)	114.50
7.01 (d, *J* 8.61 Hz)	132.00

The HMBC spectrum (spectrum 9.6) had a singlet at 1.97 ppm coupled to the carbon resonance at 173.00 ppm which was previously assigned to the carbonyl carbon of the acetyl moiety. The triplet at 3.35 ppm correlated to the same carbonyl carbon which meant that the acetyl group was situated on the ethyl group which was previously proposed to be present in the compound (fig 2.10). The success of the acetylation reaction indicated the presence of a heteroatom. Since no CH-O link was present in the ^{13}C NMR spectrum, a nitrogen was suggested. This led to the conclusion that the following fragment was part of compound 9 (figure 2.28).



Fragment X (*Fig 2.28*)

The tabular form of the HMBC results (spectrum 9.6) is presented below (Table 2.21).

Table 2.21: Results from the HMBC spectrum (spectrum 9.6)

Proton/s of	Correlate/s to
CH_3 (1.97) s	C=O (173.00)
CH_2 (2.71) t	CH (132.00)
CH_2 (3.35) t	C=O (173.00)
CH_2 (3.35) t	CH_2 (34.50)
CH (6.68) d	CH (132.00)
CH (7.01) d	*Ipso* C (157.00)
CH (7.01) d	CH (132.00)

The carbon resonance at 157.00 ppm is typical for an *ipso* carbon of phenol as opposed to the one of aniline, which meant that on the previously proposed *para*-substituted benzene ring, one substituent is OH as opposed to NH_2, while the other substituent is fragment X (fig 2.28). Combining all the above information led to the conclusion that the compound in question had the following skeleton:

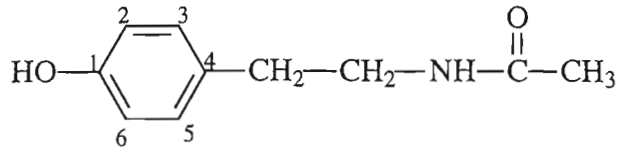


Fig 2.29

The results from the MS spectrum (spectrum 9.7) indicated a molecular ion of 179 m/z. This was compared with the calculated value of 179 for compound 9, and the values were in agreement.

2.5.E (a) Structural elucidation of compound 10

The ^{1}H NMR spectrum of 10 (spectrum 10.1) had the same profile as that of compound 9. The difference was that there was a slight change in the chemical shifts. The two compounds were suspected to be very similar, having the same groups but arranged differently. The following table (Table 2.22) shows the comparison of the proton NMR peaks for compound 9 and compound 10.

Table 2.22: Proton peaks for compound 9 and compound 10.
(spectrum 9.1 of compound 9 *vs* spectrum 10.1 of compound 10)

Proton No.	Compound 9 ^{1}H peaks in ppm	Compound 10 ^{1}H peaks in ppm
H-(CH_3)	1.97 (s)	2.21 (s)
H-(CH_2)	2.71 (t)	2.78 (t)
H-(CH_2-N)	3.35 (t)	3.38 (t)
H-3	6.68 (d)	7.21 (d)
H-2	7.01 (d)	7.01 (d)

The expected ^{1}H - ^{1}H correlations were observed for compound 10 (spectrum 10.2) as shown in the table below (Table 2.23):

Table 2.23: Results from the COSY spectrum (spectrum 10.2)

Proton/s resonating at (ppm)	Correlate/s to proton/s resonating at (ppm)
2.78 (t, *J* 7.33 Hz)	3.38 (t, *J* 7.33 Hz)
7.21 (d, *J* 8.61 Hz)	7.01 (d, *J* 8.61 Hz)

The other experiments, i.e., ^{13}C, DEPT, HSQC gave results which were similar to those of compound 9, with only the differences in the chemical shifts. The experiment which produced results of striking difference was the HMBC spectrum (spectrum 10.3). The carbon resonance at 169.00 ppm, assigned to the carbonyl carbon, correlated to the proton resonance at 2.25 ppm, previously assigned to the methyl of the acetyl group. However, this carbon resonance showed no correlation to proton resonances in the region of 2.50 ppm – 3.50 ppm, the region containing the methylenes of the compound. Thus, the acetyl group was not close to the methylene groups of the compound and, hence, it was attached to the other substituent of the benzene ring. Combining all the results from above led to the proposal that compound 10 had the following structure (fig 2.30):

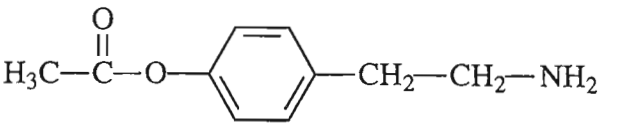


Fig 2.30

Therefore, the fraction that gave rise to compound 9 and 10 upon acetylation, was tyramine (compound 5). It is unclear why compound 5 and this fraction had significantly different R_f values. The conclusion is that the fraction leading to compound 9 and 10 was complexed to a molecule, which was lost upon acetylation.

Conclusion and Recommendations

Part of the aim of the project, as stated in the abstract, was to discover or explore the chemistry of marine organisms. A number of compounds have been isolated from *Codium extraticum*, zoanthids and *carpobrotus edulis* as discussed. Most compounds isolated are known to exist in biological systems. These compounds include nucleic acids i.e., thymine, inosine and cytosine nucleotides. Other compounds are the tyramines which are known to occur in large amounts in the human brain. Two sterols were also isolated. The nature of these compounds shows that zoanthids also have the basic type of metabolites which are found in the terrestrial systems e.g., plants and animals.

Since the environment for marine organisms is different from that of terrestrial life, some differences in compounds isolated would be expected. The literature survey shows compounds unique to marine organisms which have never been found from plants and/or animals. During this research project, a number of compounds could be isolated, but due to small quantities, could not be identified. Other spectroscopic methods could not be done on these compounds so as to identify them.

The crude organic extracts of *zoanthids* were sent for bioactivity studies at the University of Durban Westville. Some results were very promising in terms of antibacterial and antifungal activities. This leads to the conclusion that if the individual compounds producing such effects, were isolated and identified, the results would even be of more value and this may be the basis for future studies.

Further studies linked to this project can be performed where large amounts of the organisms could be used. The aim would be to get all compounds in sufficient quantities so that identification could be possible. Furthermore, the biological testing of such compounds can be performed with the aim of screening the biological active and cytotoxic compounds. The use of very sensitive instruments, which detect small quantities, would be highly recommended.

The zoanthids are worthwhile to pursue, since their chemistry has not been extensively explored. This gives a hope of finding new compounds from these organisms. Furthermore, the previous work which has been done on these organisms shows that they are rich in secondary metabolites. In this project, few such compounds were isolated. However, this was a function of small quantities of material available. Wherever these organisms are in abundance, it would be very interesting to explore its defensive mechanism against predators, which is thought to be at a chemical level since no physical protection exists. Further investigations of this organism will lead to the discovery of new chemistry.

A terrestrial organism, *Carpobrotus edulis*, was also investigated in this project. This plant normally occurs on sand dunes and it was investigated so as to find any possible relationship to marine chemistry. The plant is also known to exhibit anaesthetic effects. Therefore, another part of the aim was to identify compounds which possible could be responsible for such an effect. From this plant, a cinnamic acid derivative, 4-*trans*-methoxycinnamic acid was isolated from the hexane extract. The other fractions had spectra which could not be assigned to specific compounds. The target compounds could be in these fractions. Therefore, further studies can be done on this plant by collecting large amounts of material and analyzing these fractions and more other extracts.

CHAPTER 3

EXPERIMENTAL

3.1 General

Melting points were determined on an Ernst Leitz Weltzlar melting point apparatus.

The samples were run on sodium chloride discs and spectra were recorded on a Mattison FTIR spectrophotometer.

High resolution mass spectroscopy were acquired by Dr. P. Boschoff at the Cape Technikon on a Kratos High Resolution mass spectrometer.

NMR spectra were recorded on Varian Gemini 300 MHz and 400 MHz spectrometers. The solvents used were deuteromethanol (CD_3OD), deuterodimethylsulfoxide (DMSO) and deuterochloroform ($CDCl_3$)

For chromatographic columns, glass columns were used ranging from pasteur pipette size to about 5 cm columns. Silica gel used had particle size ranging from 0.04 mm to 0.20 mm.

Thin layer chromatographic plates were used to monitor the level of separation/purity and to detect the composition of different fractions.

3.2 Preparation and extraction of organism

3.2.A General extraction procedure:

The organisms for this project were collected from reefs along the coast of southern KwaZulu-Natal, except for *Carpobrotus edulis* which was collected from the premises of UND. The whole organism of either *Palythoa natalensis, Carpobrotus edulis, Zoanthus durbanensis, Zoanthus sansibaricus or Codium extricatum* was collected fresh and frozen immediately or soaked in solvent. This was followed by freeze drying, where appropriate. The dried organism was soaked in either ethyl acetate or methanol and agitated on a mechanical shaker at 157 rpm for 24 hours. This was repeated twice, using fresh methanol each time. The methanol extract was then washed with hexane and the methanol evaporated. The residue was taken up in water and extracted with ethyl acetate. Each extract i.e. aqueous/methanol, hexane and ethyl acetate extract, was put onto a chromatography column for further separation.

3.2.B General column separation procedures

Each extract was introduced onto a column and fractions of a particular volume were collected. These fractions were applied as spots on t.l.c plates to check the purity. The fractions were then concentrated and weighed. Impure fractions were reintroduced onto smaller columns of varying sizes, depending on the amount of substance. This was repeated until a single spot could be seen on a t.l.c plate. The stationary phase used for non polar compounds was silica gel while Diol™ and C18 Sep Pak™ cartridges were used for polar compounds. The solvent systems were changed as appropriate. Semi-preparative HPLC was also employed for the purification of some smaller fractions. Exact conditions are listed under the individual compounds.

3.3 Extractives from *Codium extricatum*

Fresh organism (144.64 g) was collected from Treasure Beach in Durban. A specimen was retained for identification purposes. The rest of the material was then soaked in methanol (approximately 500 ml) for 24 hours at room temperature. The aqueous/methanol extract was washed with hexane, followed by dichloromethane and finally by ethyl acetate. The masses of different extracts obtained after evaporation of the solvent were:

Hexane – 0.21 g
Dichloromethane – 0.04 g
Ethyl acetate – 0.01 g

The hexane extract, after concentration, was redissolved in hexane and was introduced onto a silica column for further separation. The silica column was prepared using hexane as solvent. The eluent was a solvent system of hexane and ethyl acetate. The starting system was 100% hexane to which increasing proportions of ethyl acetate were added. Using a solvent system of 10% ethyl acetate in hexane and by continuous collection of fractions, the hexane extract yielded compound 1.

3.3.A Physical data of compound 1

Name: 24-ethylcholesta-5,25 diene-3β-ol

Yield: 10 mg

Physical description: amorphous brown solid

^{1}H NMR ($CDCl_3$) (300 MHz) 0.65 (s, 3H) 0.80 (t. 3H, *J* 6.75) 0.90 (d, 3H, *J* 7.01) 1.00 (s, 3H) 1.18 (s, 3H) 3.50 (m, 1H, *J* 4.25 Hz) 4.60 (s, 1H) 4.70 (s, 1H) 5.35 (d, 1H, *J* 6.20) [spectrum 1.1]

Proton signals (δ ppm)	**Proton No.**
0.65	H-18
0.80	H-29
0.90	H-21
1.00	H-19
1.00 – 2.30	*
1.18	H-27
3.50	H-3
4.60	H-26
4.70	H-26'
5.35	H-4

* Some protons could not be unambiguously assigned and these represent the rest of the molecule.

^{13}C NMR ($CDCl_3$) (300 MHz) δ (ppm) 12.00 (CH_3) 17.90 (CH_3) 18.20 (CH_3) 18.70 (CH_3) 21.00 (CH_2) 24.10 (CH_2) 26.80 (CH_2) 28.20 (CH_2) 29.40 (CH_2) 32.00 (CH_2) 32.01 (CH) 32.90 CH_2) 34.80 (CH) 37.40 (CH_2) 39.90 (CH_2) 42.50 (CH_2) 49.60 (CH) 50.10 (CH) 56.00 (CH) 57.00 (CH) 72.00 (CH) 110.50 (CH) 122:00 (CH) 140.50 (C) 148.00 (C) [spectrum 1.2]

Carbon signals (δ ppm)	Carbon No.
12.00	C-18
17.90	C-29
18.20	C-20
18.70	C-19
21.00 – 57.00	*
72.00	C-3
110.00	C-26
122.00	C-6
140.00	C-25
148.00	C-5

* Some carbons could not be unambiguously assigned and these represent the rest of the molecule.

Mass spectrum: HRMS *m/z*: 412.3716 [M^+] ($C_{29}H_{48}O$ requires 412.3703), EIMS *m/z* (relative intensities): 412 [M^+] (86), 379 (12), 314 (21), 279 (22), 271(36), 213 (18), 175 (11), 149 (100), 107 (35), 84 (56), 55 (92) [spectrum 1.6]

I.R data: ν_{max} [NaCl] (cm^{-1}): 3450 (O-H stretching) 2950 (C-H aliphatic stretching) 1700 (C=C stretching) 1490, 1400, 1200 (CH_2 and CH_3 bending) [spectrum 1.7]

3.4 Extractives from *Palythoa natalensis*

A voucher specimen, nr 3, has been retained by ORI.

The organism (68 g) was collected from Aliwal shoal off Umkomaas in the far south of KwaZulu-Natal. It was immediately frozen and this was followed by a freeze-drying process. The material was extracted with ethyl acetate which was evaporated to give a

crude extract. This extract was introduced onto a silica gel column and a solvent system of 20% ethyl acetate in hexane was used as the eluent. This ethyl acetate fraction yielded a compound (compound 2) in its two isomeric forms i.e., 24-α-methylcholesterol (campesterol) and 24-β-methylcholesterol (22-dihydrobrassicasterol).

3.4.A Physical data of compound 2

Name: 24-methylcholesterol

Yield: 15 mg

Physical description: colourless powder

^{1}H NMR ($CDCl_3$) (300 MHz) δ (ppm) 0.65 (s, 3H) 0.75 (d, 3H, *J* 6.69 Hz) 0.76 (d, 3H, *J* 6.78 Hz) 0.83 (d, 3H, *J* 6.9 Hz) 0.89 (d, 3H, *J* 8.49 Hz) 0.98 (s, 3H) 1.00 – 1.80 (m, 20H) 1.95 (m, 2H) 2.45 (m, 2H) 3.50 (m, 1H) 5.65 (d,1H, *J* 6.00 Hz) [spectrum 2.1]

Proton signals (δ ppm)	**Proton No.**
0.65	H-18
0.75	H-28
0.76	H-27
0.83	H-26
0.89	H-21
0.98	H-19
1.00 – 2.45	*
3.50	H-3
5.65	H-6

* Some protons could not be unambiguously assigned and these represent the rest of the molecule.

^{13}C NMR ($CDCl_3$) (300 MHz) δ (ppm) 14.00 (CH_3) 15.00 (CH_3) 18.00 (CH_3) 19.00 (CH_3) 19.50 (CH_3) 20.50(CH_3) 21.00 (CH_2) 24.00 (CH_2) 28.00 (CH_2) 30.20 (CH_2) 32.00 (CH_2) 32.20 (CH) 32.25 (CH_2) 34.00 (CH_2) 36.00 (CH) 36.50 (C) 37.00 (CH_2) 39.50 (CH) 39.80 (CH_2) 42.00 (CH_2) 50.00 (CH) 56.00 (CH) 56.50 (CH) 57.00 (C) 72.00 (CH) 141.00 (C) 122.00 (CH) [spectrum 2.2]

Carbon signals (δ ppm)	Carbon No.
14.00	C-18
15.00	C-28
18.00	C-27
19.00	C-26
19.50	C-21
20.50	C-19
21.00 – 57.00	*
72.00	C-3
122.00	C-6
141.00	C-5

* Some carbons could not be unambiguously assigned and these represent the rest of the molecule.

Mass spectrum: $C_{28}H_{47}O$ requires 399, EIMS *m/z* (relative intensities): 382 (98), 368 (75), 279 (24), 255 (23), 219 (34), 167 (39), 149 (98), 81 (61), 57 (100) [spectrum 2.6]

3.5 Extractives from *Carpobrotus edulis*

Fresh organism (approximately1000 g) was collected from the premises of Natal University, Durban. Mr. K. Crampton, the University of Natal,Durban (UND) chief horticulturist, identified the specimen. The leaves were detached from the stems and then

soaked in methanol to yield a methanol extract. The extract was concentrated *in vacuo* after which it was taken up in ether, acidified to pH 4 using 4 M HCl, followed by a chloroform wash. The aqueous phase was subsequently basified to pH 10 using ammonium hydroxide, followed by another chloroform wash and then washed with a mixture of 50% methanol 50% chloroform. This procedure gave rise to four extracts.

Ether extract – fraction 1 – 60 g
Acidic chloroform extract – fraction 2 – 10 g
Basic chloroform extract – fraction 3 – 3 g
50/50 methanol/chloroform extract – fraction 4 – 0.170 g

Fraction 4 was adsorbed onto Kieselguhr and introduced onto a Diol™ column, and was eluted using different ratios of ethyl acetate and hexane, starting from non polar to polar. A solvent system of 6% ethyl acetate in hexane yielded a fraction containing compound 3 (4-methoxycinnamic acid).

3.5.A Physical data of compound 3

Name: 4-methoxycinnamic acid

Yield: 20 mg

Physical description: Needle-like colourless crystals

Melting point: 125°C – 128°C (literature 129°C – 131°C)[46]

^{1}H NMR ($CDCl_3$) (300 MHz) δ (ppm) 3.78 (s, 3H) 6.30 (d, 1H, *J* 16.00 Hz) 6.85(d, 2H, *J* 9.00 Hz) 7.40 (d, 2H, *J* 9.00 Hz) 7.65 (d, 1H, *J* 16.00 Hz) [spectrum 3.1]

Proton signals (δ ppm)	Proton No.
3.78	$OC\underline{H}_3$
6.30	C<u>H</u>-Ar
6.85	H-3
7.40	H-2
7.65	C<u>H</u>-COOH

^{13}C NMR ($CDCl_3$) (300 MHz) δ (ppm) 52.00 (CH_3) 114.50 (CH) 116.00(CH) 130.00 (CH) 144.00 (CH) 168.00 (C) [spectrum3.3]

Carbon signals (δ ppm)	Carbon No.
52.00	$O\underline{C}H_3$
114.50	<u>C</u>H-Ar
116.00	C-3
130.00	C-2
144.00	<u>C</u>H-COOH
168.00	<u>C</u>OOH

Mass spectrum: $C_{10}H_{10}O_3$ requires 178, EIMS *m/z* (relative intensities): 178 (70), 147 (100), 123 (14), 119 (26), 91 (18), 65 (14) [spectrum 3.6]

I.R data: ν_{max} [NaCl] (cm^{-1}): 3350 (O-H of COOH stretching) 2900 (C-H aromatic streching) 1700, 1600 (C=C stretching) 1500 (CH_3 bending) 1175 (C-O stretching) [spectrum 3.7]

3.6 Extractives from *Zoanthus durbanensis*

Fresh organism (approximately 500 g) was collected from Treasure beach in Durban. A voucher specimen, CEL1, has been retained and is awaiting final identification. It was immediately soaked in methanol. The methanol extract was washed with hexane followed by an ethyl acetate wash. This procedure yielded three extracts with the following yields obtained from each:

Hexane – 8.00 g
Ethyl acetate – 11.54 g
Methanol - 85.64 g

The hexane extract was dissolved in dichloromethane and introduced onto a silica column. The solvent system of 1% methanol in dichloromethane was used to elute and prepare the column. All the fractions were collected using the same solvent system. This hexane extract yielded compound 4.

3.6.A Physical data of compound 4

Name: Chalinasterol

Yield: 10 mg

Physical description: amorphous solid (brown)

^{1}H NMR ($CDCl_3$) (300 MHz) δ (ppm) 0.60 (s, 3H) 0.80 (d, 3H, *J* 7.28) 0.86 (d, 3H, *J* 6.61 Hz) 0.95 (d, 3H, *J* 9.20) 1.15 (s, 3H) 3.43 (m, 1H, *J* 6.50 Hz) 4.58 (s, 1H) 4.62 (s, 1H) 5.25 (d, 1H, *J* 7.95) [spectrum 4.1]

Proton signals (δ ppm)	Proton No.
0.60	H-18
0.80	H-26
0.86	H-27
0.95	H-21
1.15	H-19
1.00 – 2.21	*
3.43	H-3
4.58	H-28
4.62	H-28'
5.25	H-6

* Some protons could not be unambiguously assigned and these represent the rest of the molecule.

^{13}C NMR ($CDCl_3$) (300MHz) δ (ppm) 12.00 (CH_3) 19.00 (CH_3) 19.50 (CH_3) 21.00 (CH_2) 21.99 (CH_3) 22.01 (CH_3) 24.30 (CH_2) 28.00 (CH_2) 30.00 (CH_2) 31.00 (CH_2) 31.90 (CH_2) 32.00 (CH_2) 32.90 (CH) 35.00 (CH_2) 36.00 (CH) 37.20 (CH_2) 39.80 (CH_2) 42.30 (CH_2) 50.00 (CH) 55.90 (CH) 56.30 (CH) 72.00 (CH) 113.00 (CH) 121.00 (CH) 140.50 (C) 156.50 (C) [spectrum 4.5]

Carbon signals (δ ppm)	Carbon No.
12.00	C-18
19.00	C-26
19.50	C-27
21.99	C-21
22.01	C-19
24.30 – 56.30	*
72.00	C-3
113.00	C-28
121.00	C-6
140.50	C-5
156.50	C-24

* Some carbons could not be unambiguously assigned and these represent the rest of the molecule.

Mass spectrum: $C_{28}H_{46}O$ requires 398, EIMS *m/z* (relative intensities): 412 (2), 400 (16), 396 (6), 384 (10), 330 (11), 316 (8), 287 (8), 273 (4), 215 (6), 192 (4), 135 (11), 83 (59), 55 (100) [spectrum 4.6]

I.R data: ν_{max} [NaCl] (cm^{-1}): 3450 (O-H stretching) 2950 (C-H aliphatic stretching) 1700 (C=C stretching) 1200, 1400, 1490 (CH_2 and CH_3 bending) [spectrum 4.7]

3.7 Extractives from *Zoanthus sansibaricus*

Fresh organism (approximately 500 g) was collected from Treasure beach in Durban. A voucher specimen, CEL2, has been retained and is awaiting final identification. It was freeze dried and then soaked in methanol for 24 hours. The methanol extract was washed with hexane. Both hexane and methanol extracts were concentrated *in vacuo*. The

methanol extract was washed with ethyl acetate, where part of it dissolved. The dissolved portion was transferred to a round bottom flask, concentrated and it was considered as an ethyl acetate extract. The masses of extracts obtained were as follows:

Hexane – 6.50 g
Ethyl acetate – 9.45 g
Methanol - 60.00 g

The methanol extract consisted mainly of salt and this was introduced into a normal phase silica gel column to remove salt and afford some partial separation. The fractions obtained were adsorbed onto Kieselguhr and put onto a Diol™ Sep Pak cartridge (0.5 g). A solvent system of methanol and ethyl acetate was used to elute the column. Compounds of interest came out during the use of solvent system ranging from 10% - 15% methanol in ethyl acetate. This methanol extract yielded compound 7.

Some of the methanol extract was washed with ethyl acetate (3 x 200 ml), followed by an ether wash (3 x 150 ml) and lastly with dichloromethane (3 x 100ml). The remaining methanol extract was introduced into the HPLC for separation. A Supelcosil LC-18. 25 cm x 4.6 mm, 5um column was used with a flow rate of 1.5 $mlmin^{-1}$, using 100% trifluoroacetic acid (TFA) as an eluent. The wavelength was set at 254 nm. This procedure resulted in three compounds i.e., compound 6, compound 7, and compound 8, being isolated.

3.7.A Physical data of compound 5

Name: Tyramine [2-(p-hydroxyphenyl)ethylamine]

Yield: 8 mg

Physical description: colourless crystals

No melting point was obtained owing to the low yield.

Literature melting point: 164.0 – 164.5 °C.

^{1}H NMR (CD_3OD) (300 MHz) δ (ppm) 2.86 (t, 2H, *J* 4.34 Hz) 3.30 (t, 2H, *J* 4.46 Hz) 6.80 (d, 2H, *J* 8.49 Hz) 7.12 (d, 2H, *J* 8.42 Hz) [spectrum 5.1]

Proton signals (δ ppm)	**Proton No.**
2.86	$C\underline{H_2}$-Ar
3.30	$C\underline{H_2}$-NH_2
6.80	H-3
7.12	H-2

^{13}C NMR (CD_3OD) (300 MHz) δ (ppm) 34.00 (CH_2) 42.5 (CH_2) 117.00 (CH) 128.50 (C) 131.00 (CH) 157.80 (C) [spectrum 5.5]

Carbon signals (δ ppm)	**Carbon No.**
34.00	$\underline{C}H_2$-Ar
42.50	$\underline{C}H_2$-NH_2
117.00	C-3
128.50	C-2
131.00	C-4
157.00	C-1

Preparation of O-acetyl mandelic acid chloride[45]

O-Acetyl mandelic acid chloride was first prepared by heating 1.0 g of acetyl mandelic acid with 0.60 ml (2.10 mol) of thionyl chloride under reflux for four hours. The crude acid chloride was distilled under reduced pressure to give a product as a colourless oil, bp 150 °C at 25 mmHg.

Preparation of mandelic ester derivative of compound 7[45]

The acid chloride (0.14 mmol) was mixed with compound 9 in 300 μl pyridine and 300 μl carbon tetrachloride. The reaction was allowed to stand until completion (1 hour), after which it was diluted with 20 ml ether, washed twice with cold 3 M HCl , cold saturated Na_2CO_3 and saturated NaCl. The ether fraction was dried using $MgSO_4$ and then concentrated *in vacuo* repeatedly using carbon tetrachloride to remove traces of ether. A NMR spectrum of the mixture was recorded but no evidence for the formation of the mandelic ester was found.

The physical data of the original compounds i.e., compound 6, compound 7, and compound 8 are given below.

3.7.B Physical data of compound 6

Name: cytosine ribonucleoside

Yield: 8 mg

Physical description: colourless needles

No melting points were obtained

^{1}H NMR (DMSO) (400 MHz) δ (ppm) 3.55 (m, 2H) 3.81 (q, 1H, *J* 3.69) 3.95 (q, 1H, *J* 4.25) 4.05 (q, 1H, *J* 5.22) 5.10 (d, 1H. *J* 5.11) 5.40 (d, 1H, *J* 5.49) 5.65 (d, 1H, *J* 8.06) 5.79 (d, 1H, *J* 8.06 [spectrum 6.1]

Proton signals (δ ppm)	**Proton No.**
3.55	H-5
3.81	H-4
3.95	H-3
4.05	H-2
5.10	OH(C-3)
5.40	OH(C-2)
5.65	H-5'
5.79	H-1
7.89	H-6'

^{13}C NMR (DMSO) (400 MHz) δ (ppm) 61.00 (CH_2) 70.00 (CH) 73.80 (CH) 85.00 (CH) 87.80 (CH) 102.00 (CH) 140.00 (C) 150.00 (CH) 163.00 (C) [spectrum 6.4]

Carbon signals (δ ppm)	Carbon No.
61.00	C-5
70.00	C-2
73.80	C-3
85.00	C-4
87.80	C-1
102.00	C-5'
140.00	C-6'
150.00	C-4'
163.00	C-2'

3.7.C Physical data of compound 7

Name: inosine ribonucleoside

Yield: 7 mg

Physical description: colourless needles

Melting points were not obtained.

^{1}H NMR (CD_3OD) (500 MHz) δ (ppm) 3.75 – 3.95 (dd, 2H, J 44.44Hz) 4.25 (q, 1H, J 3.31 Hz) 4.35 (q, 1H, J 3.69 Hz) 4.65 (t, 1H, J 5.39 Hz) 6.06 (d, 1H, J 5.84 Hz) 8.10 (s, 1H) 8.40 (s, 1H) [spectrum 7.1]

Proton signals (δ ppm)	Proton No.
3.75 – 3.95	H-5
4.25	H-4
4.35	H-3
4.65	H-2
6.06	H-1
8.10	H-7'
8.40	H-2'

^{13}C NMR (CD3OD) (500 MHz) δ (ppm) 62.96 (CH) 72.13 (CH) 76.11 (CH) 90.58 (CH_2) 119.81 (C) 126.28 (C) 143.18 (CH) 146.85 (CH) 158.90 (C) [spectrum 7.6]

Carbon signals (δ ppm)	Carbon No.
62.96	C-5
72.13	C-3
76.11	C-2
87.54	C-4
90.58	C-1
119.81	C-5'
126.28	C-4'
142.18	C-2'
146.85	C-7'
158.90	C-9'

3.7.D Physical data of compound 8

Name: thymine ribonucleoside

Yield: 8 mg

Physical description: white needles

^{1}H NMR (CD_3OD) (500 MHz) δ (ppm) 1.90 (s, 3H, *J* 0 Hz) 2.25 (m, 2H, *J* 5.86 Hz) 2.85 - 3.71 (dd, 2H, *J* 27.75 Hz) 3.95 (q, 1H, *J* 6.76 Hz) 4.45 (p, 1H, *J* 3.29 Hz) 6.30 (t, 1H, *J* 6.78 Hz) 7.85 (s, 1H) [spectrum 8.1]

Proton signals (δ ppm)	Proton No.
1.90	$C_{5'}$-$C\underline{H}_3$
2.25	H-2
2.85 – 3.71	H-5
3.95	H-4
4.45	H-3
6.30	H-1
7.85	H-6'

^{13}C NMR (CD_3OD) (500 MHz) δ (ppm) 3.00 (CH_3) 32.00 (CH_2) 54.00 ($CH_{2)}$) 63.00 (CH) 79.00 (CH) 129.00 (C) 144.00 (CH) 157.00 (C) [spectrum 8.6]

Carbon signals (δ ppm)	Carbon No.
3.00	$C_{5'}$-$\underline{C}H_3$
32.00	C-2
54.00	C-5
63.00	C-4
79.00	C-1
129.00	C-2'
144.00	C-6'
157.00	C-4'

3.8 Acetylation of compounds

Some mixtures could not be resolved into individual components and, thus, they were acetylated. This procedure was performed by adding 2 ml of pyridine and 4 ml of acetic anhydride to a sample. This was agitated for couple of minutes and was allowed to stand for approximately 48 hours at room temperature. The reaction was then stopped by adding 10 ml of methanol to decompose acetic anhydride and the reaction mixture concentrated to dryness *in vacuo*.

Toluene was added to form an azeotrope with pyridine. Methanol was added in turn to remove toluene. After the sample was dry, ^{1}H NMR spectroscopy was used to confirm acetylation had taken place and the mixture introduced onto a column for further purification.

Compound 8 was acetylated using this method and compounds 9 and 10 were the products after running the procedure on fraction P3-10 (spectra 9.1 and 10.1).

3.8.A Physical data of compound 9

Name: O-acetyltyramine

Yield: 7 mg

Physical description: Amorphous solid (yellow)

Melting points were not obtained

^{1}H NMR (CD_3OD) (400 MHz) δ (ppm) 1.97 (s, 3H) 2.71 (t, 2H, *J* 7.40 Hz) 3.35 (t, 2H, *J* 7.14 Hz) 6.68 (d, 1H, *J* 8.42 Hz) 7.01 (d, 1H, *J* 8.61) [spectrum 9.1]

Proton signals (δ ppm)	Proton No.
1.97	-C<u>H</u>$_3$
2.71	C<u>H</u>$_2$-Ar
3.35	C<u>H</u>$_2$-NH
6.68	H-3
7.01	H-2

^{13}C NMR (CD_3OD) (400 MHz) δ (ppm) 22 (CH_3) 34.50 (CH_2) 40.50 (CH_2) 115.00 (CH)121.50 (C) 129.80 (CH) 157.00 (C) 173.00 (C=O) [spectrum 9.3]

Carbon signals (δ ppm)	Carbon No.
22.00	-$\underline{C}H_3$
34.50	$\underline{C}H_2$-Ar
40.50	$\underline{C}H_2$-NH
115.00	C-3
121.50	C-4
129.80	C-2
157.00	C-1
173.00	-$\underline{C}$=O

Mass spectrum: $C_{10}H_{13}NO_2$ requires 179, EIMS *m/z* (relative intensities): 179 (3), 162 (2), 120 (100), 107 (34), 77 (8), 60 (4) [spectrum 9.7]

I.R data: ν_{max} [NaCl] (cm^{-1}): 3350 (O-H stretching) 2950 (C-H aromatic stretching) 1650 (C=O stretching) 1475, 1525 (CH_2 bending) [spectrum 9.9]

3.8.B Physical data of compound 10

Name: N-acetyltyramine

Yield: 7mg

Physical description: Amorphous solid (yellow)

Melting points were not obtained.

^{1}H NMR (CD_3OD) (400 MHz) δ (ppm) 2.21 (s, 3H) 2.78 (t, 2H, *J* 7.33 Hz) 3.38 (t, 2H, *J* 7.33 Hz) 7.01 (d, 2H, *J* 8.61 Hz) 7.21 (d, 2H *J* 8.61 Hz) [spectrum 10.1]

Proton signals (δ ppm)	Proton No.
2.21	-C<u>H</u>$_3$
2.78	C<u>H</u>$_2$-Ar
3.38	C<u>H</u>$_2$-NH
7.01	H-2
7.21	H-3

^{13}C NMR (CD_3OD) (400 MHz) δ (ppm) 20.00 (CH_3) 34.50 (CH_2) 41.00 (CH_2) 121.50 (CH) 129.00 (CH) 130.00 (C) 149.50 (C) 170.00 (C) [spectrum 10.4]

Mass spectrum: $C_{10}H_{13}NO_2$ requires 179, EIMS *m/z* (relative intensities): 179 (2), 162 (28), 120 (100), 107 (32), 91 (10) 72 (7), [spectrum 10.6]

Carbon signals (δ ppm)	Carbon No.
20.00	-<u>C</u>H$_3$
34.50	<u>C</u>H$_2$-Ar
41.00	<u>C</u>H$_2$-NH
121.50	C-3
129.00	C-2
130.00	C-4
149.50	C-1
170.00	-<u>C</u>=O

I.R data: ν_{max} [NaCl] (cm^{-1}): 2950 (C-H aromatic stretching) 1650, 1750 (C=O stretching) 1500 (CH_2 bending) 1200 (C-O stretching) [spectrum 10.7]

3.8.C Physical data of compound 11

Name: 3,5-diacetylthymidine

Yield: 8 mg

Physical description: amorphous white

^{1}H NMR (CD_3OD) (400 MHz) δ (ppm) 1.94 (s, 3H, *J* 0 Hz) 2.25 (m, 2H) 4.30 (q, 1H) 4.40 (dd, 2H) 5.30 (p, 1H) 6.30 (t, 1H) 7.55 (s, 1H) [spectrum 8A]

Since spectrum 8A was not clear enough with poor resolution, *J* values of some peaks could not be assigned.

Proton signals (δ ppm)	Proton No.
1.94	C-5'-C<u>H</u>$_3$
2.25	H-2
4.30	H-4
4.40	H-5
5.30	H-3
6.30	H-1
7.55	H-6'

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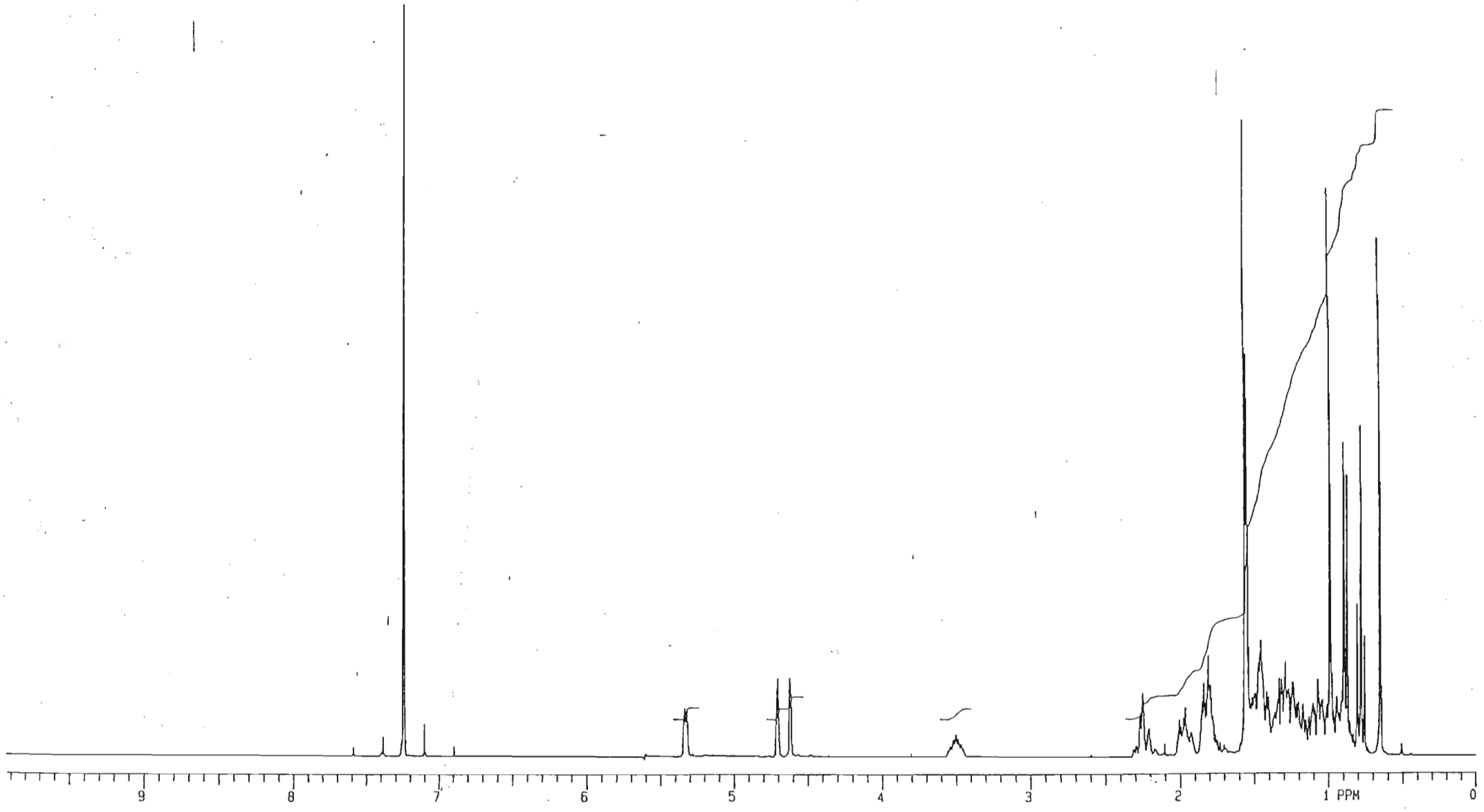
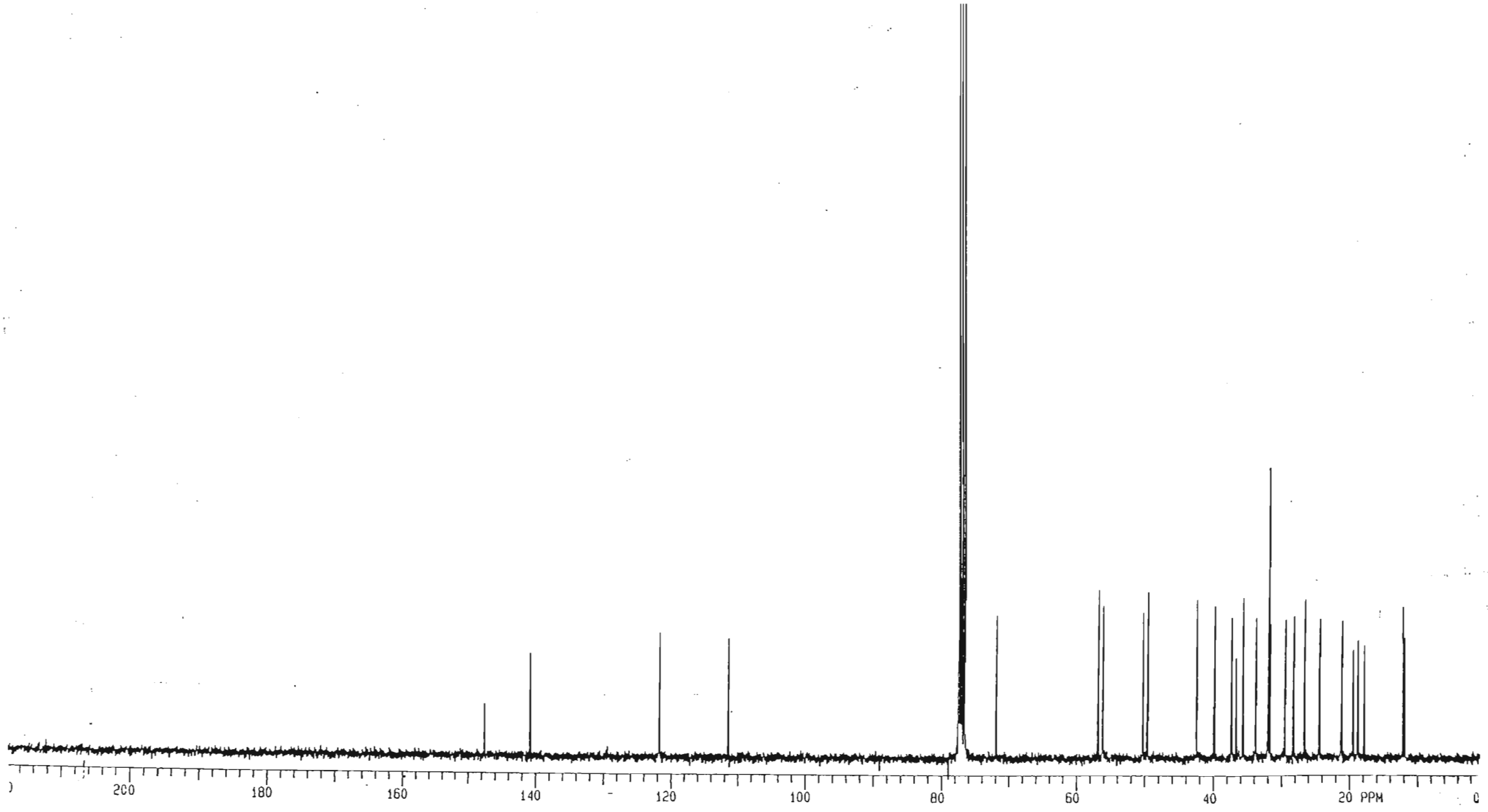
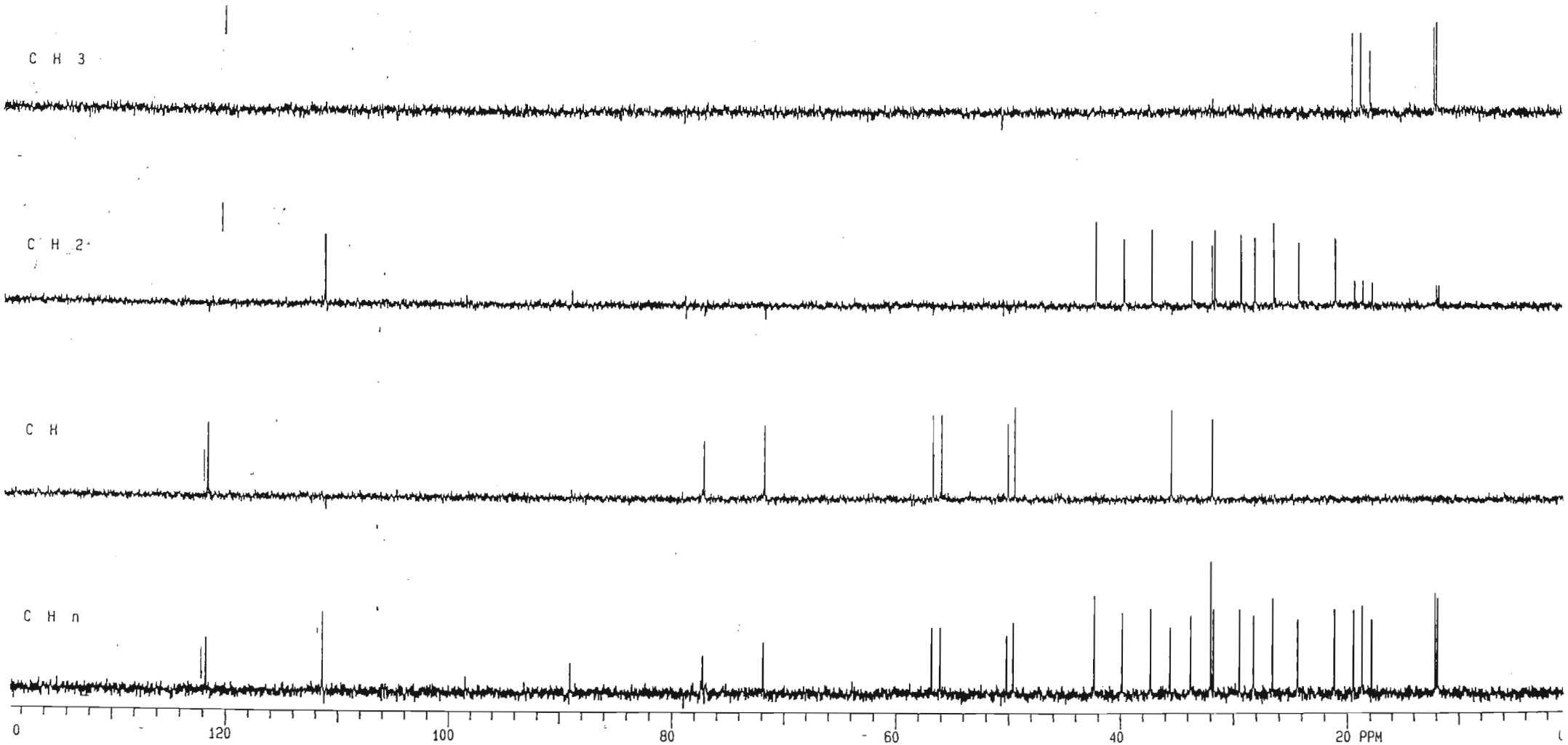
SPECTRA



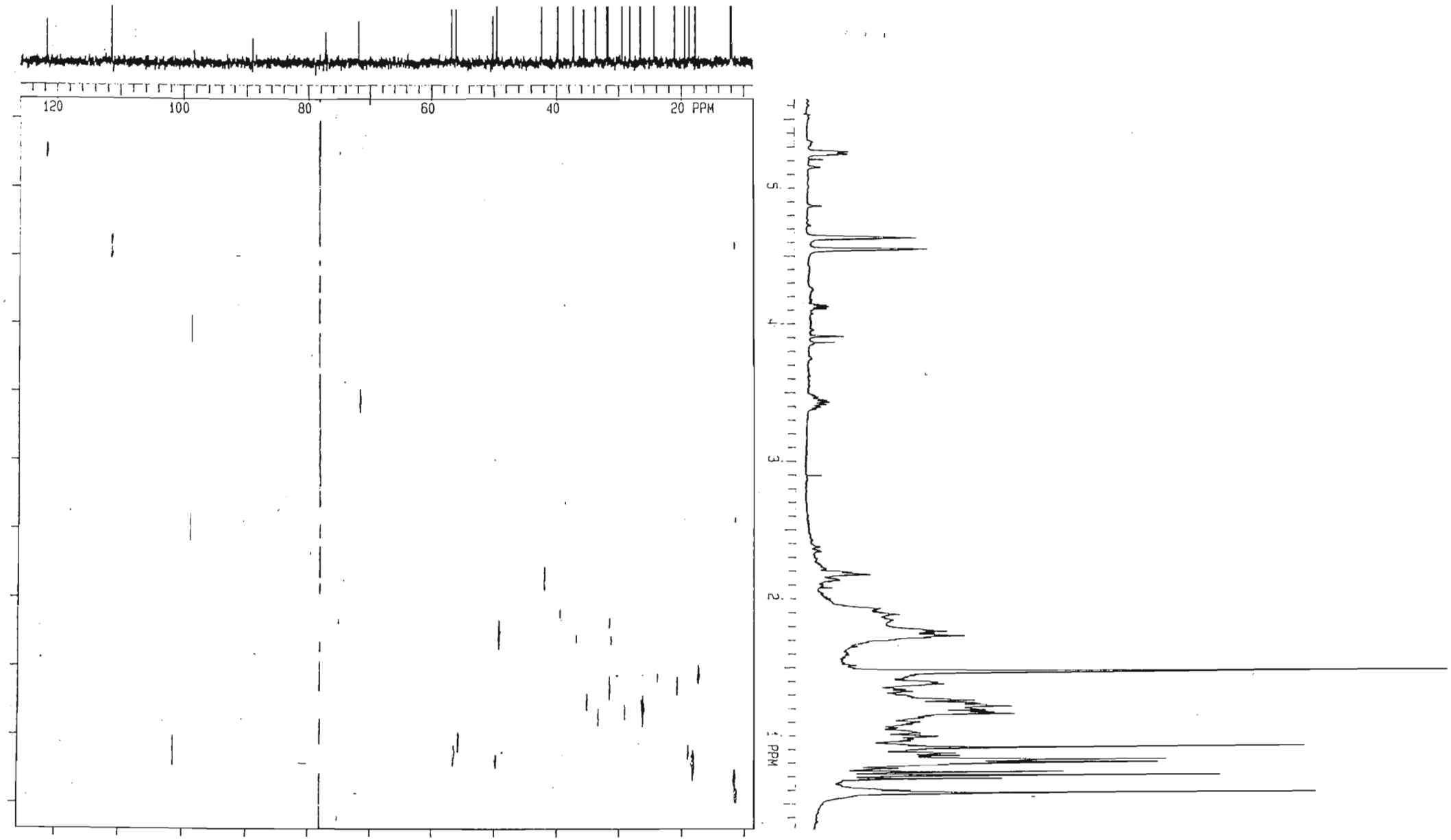
Spectrum 1.1: ^{1}H NMR spectrum of compound 1 ($CDCl_3$)



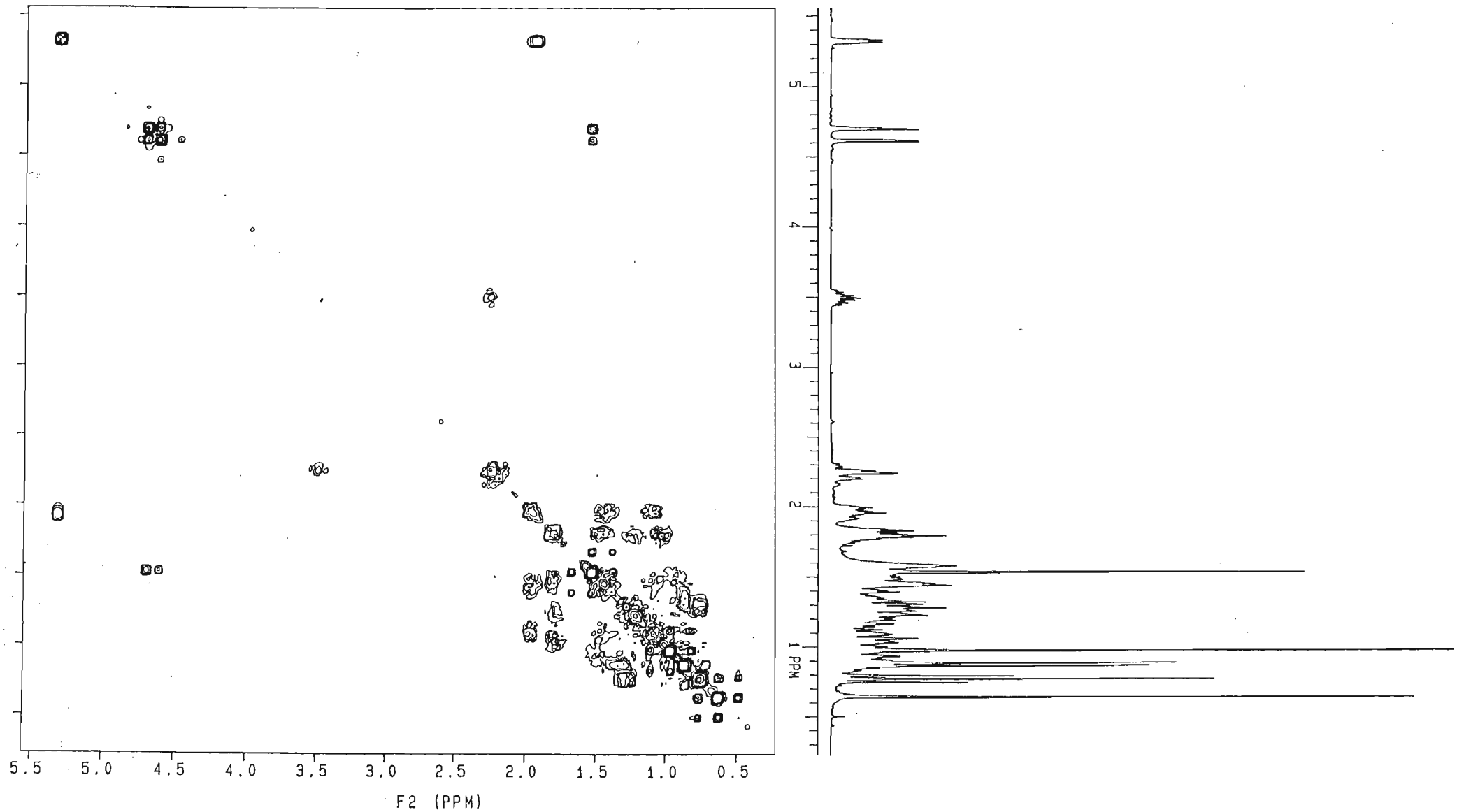
Spectrum 1.2: ^{13}C NMR spectrum of compound 1 ($CDCl_3$)



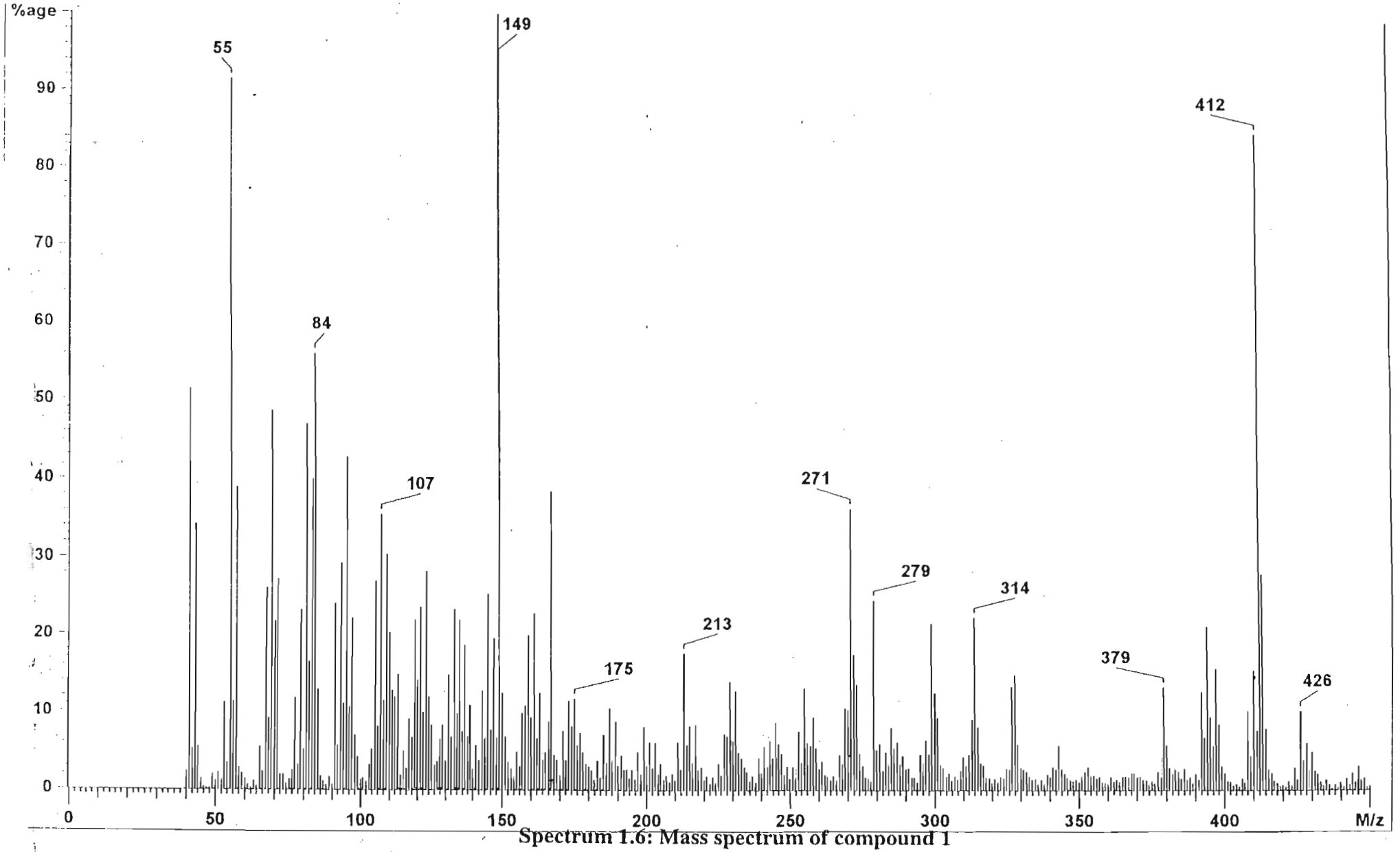
Spectrum 1.3: DEPT spectrum of compound 1 ($CDCl_3$)



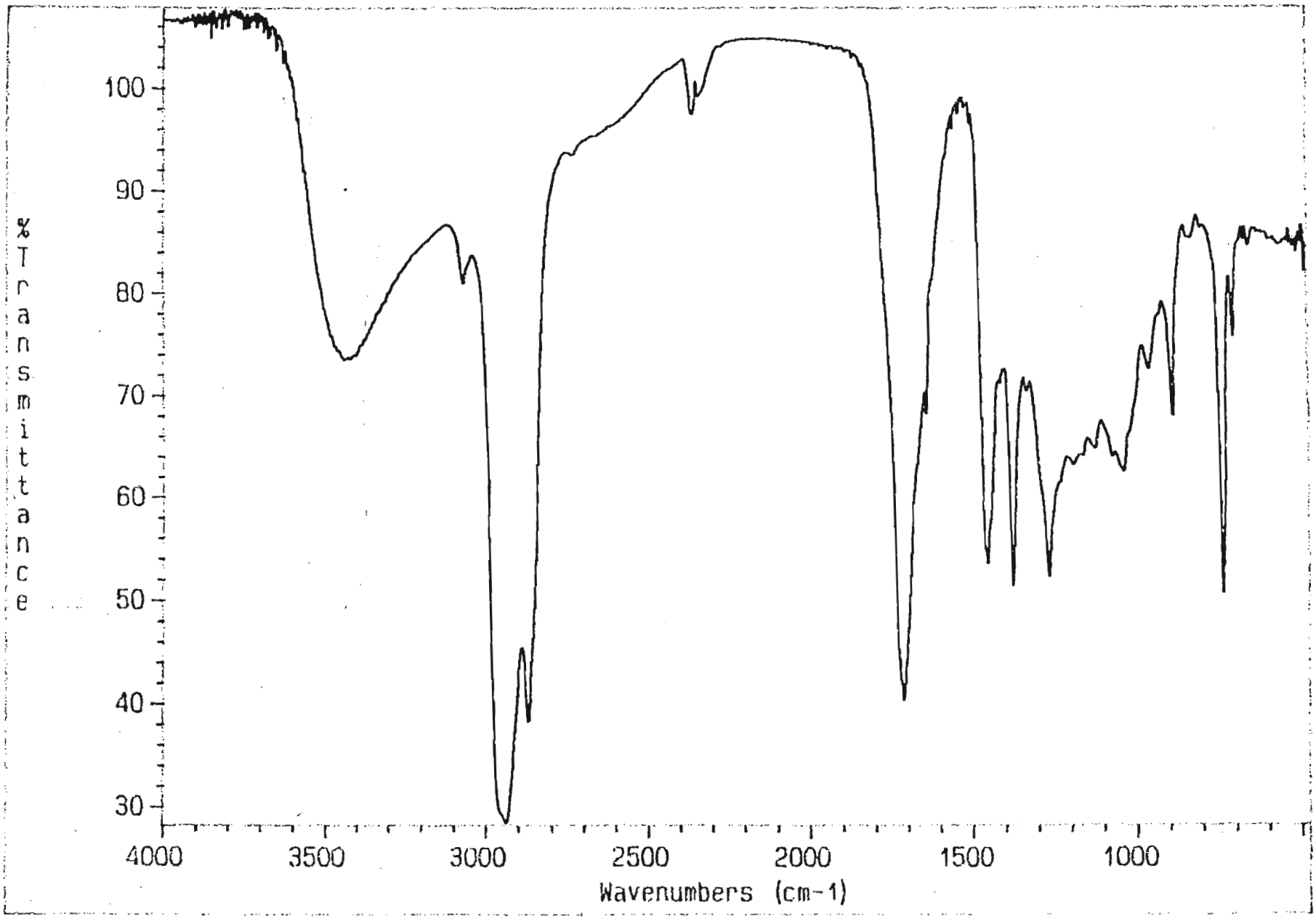
Spectrum 1.4: HETCOR spectrum of compound 1 ($CDCl_3$)



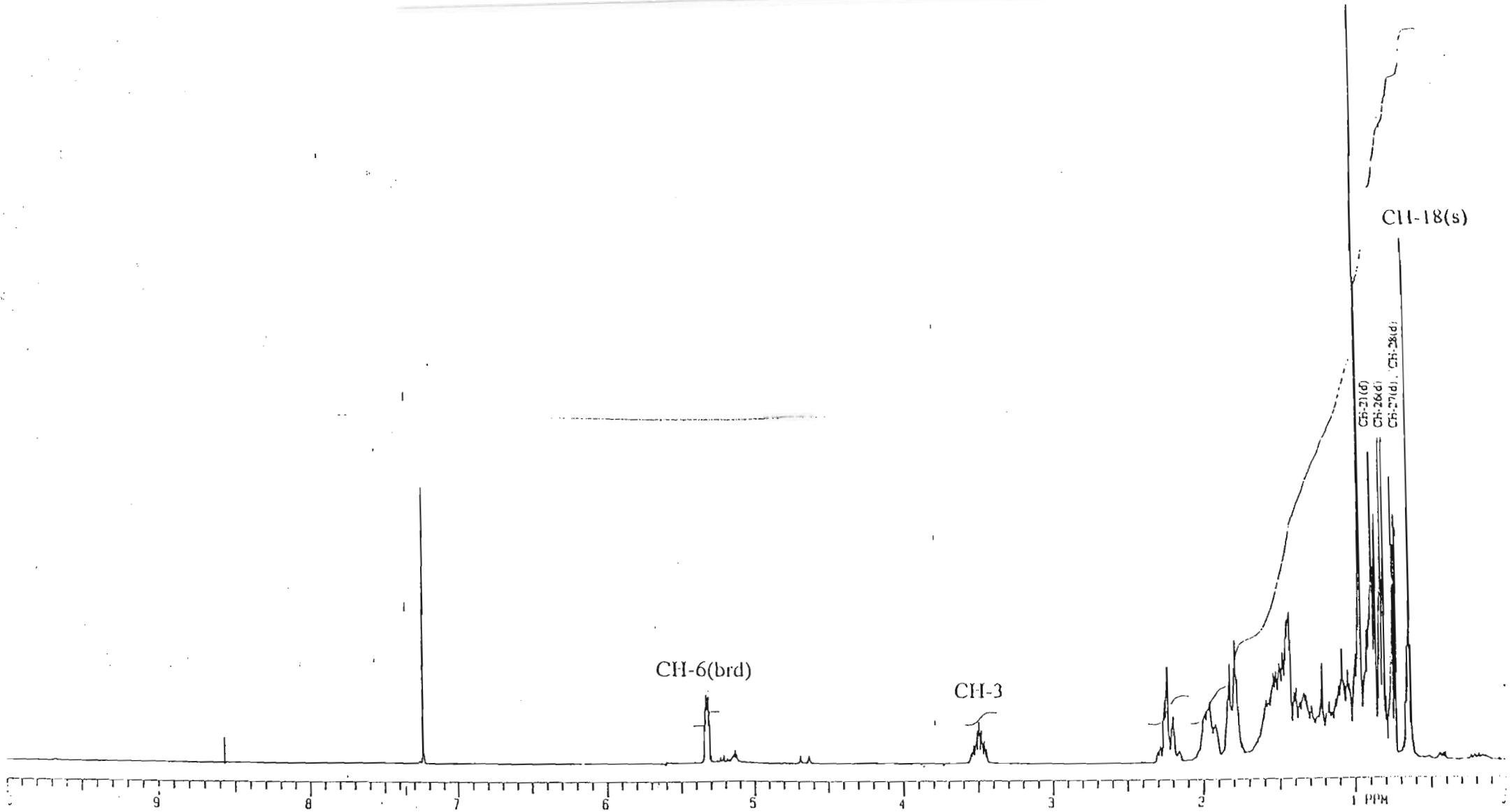
Spectrum 1.5: COSY spectrum of compound 1 ($CDCl_3$)



Spectrum 1.6: Mass spectrum of compound 1

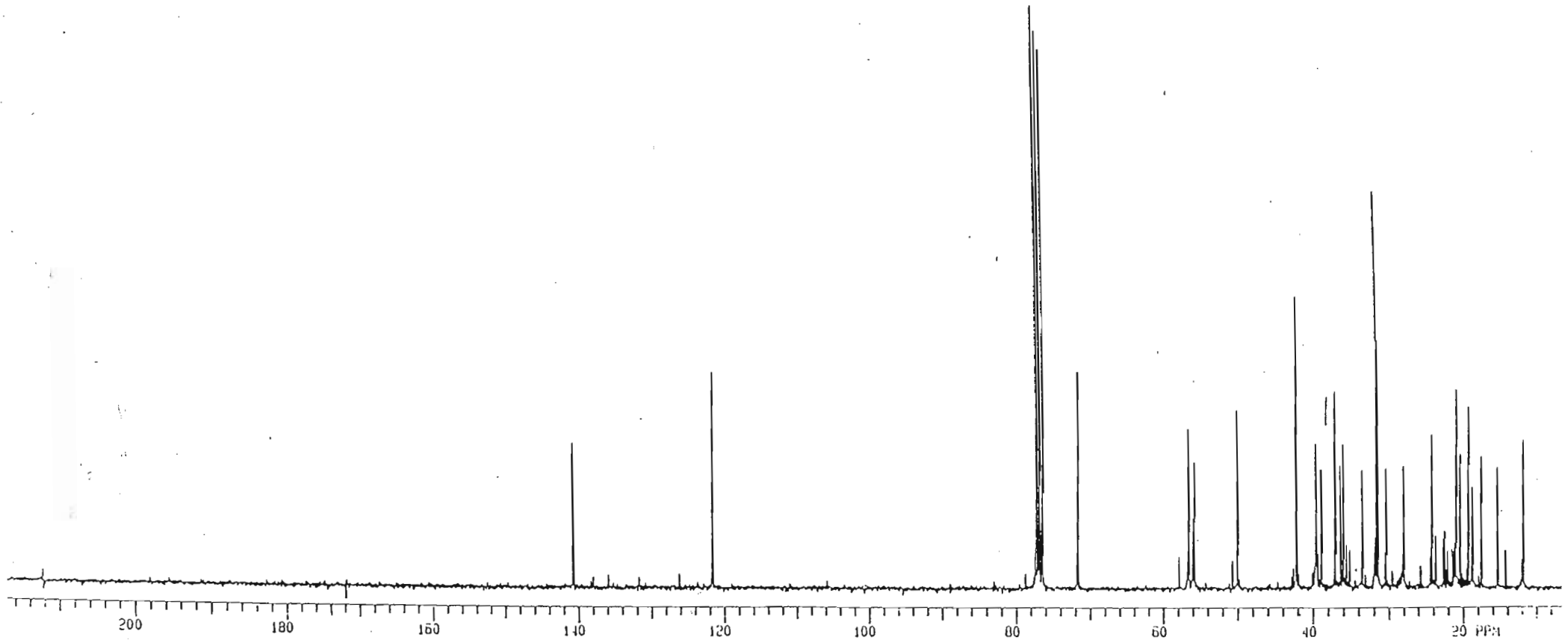


Spectrum 1.7: Infrared spectrum of compound 1

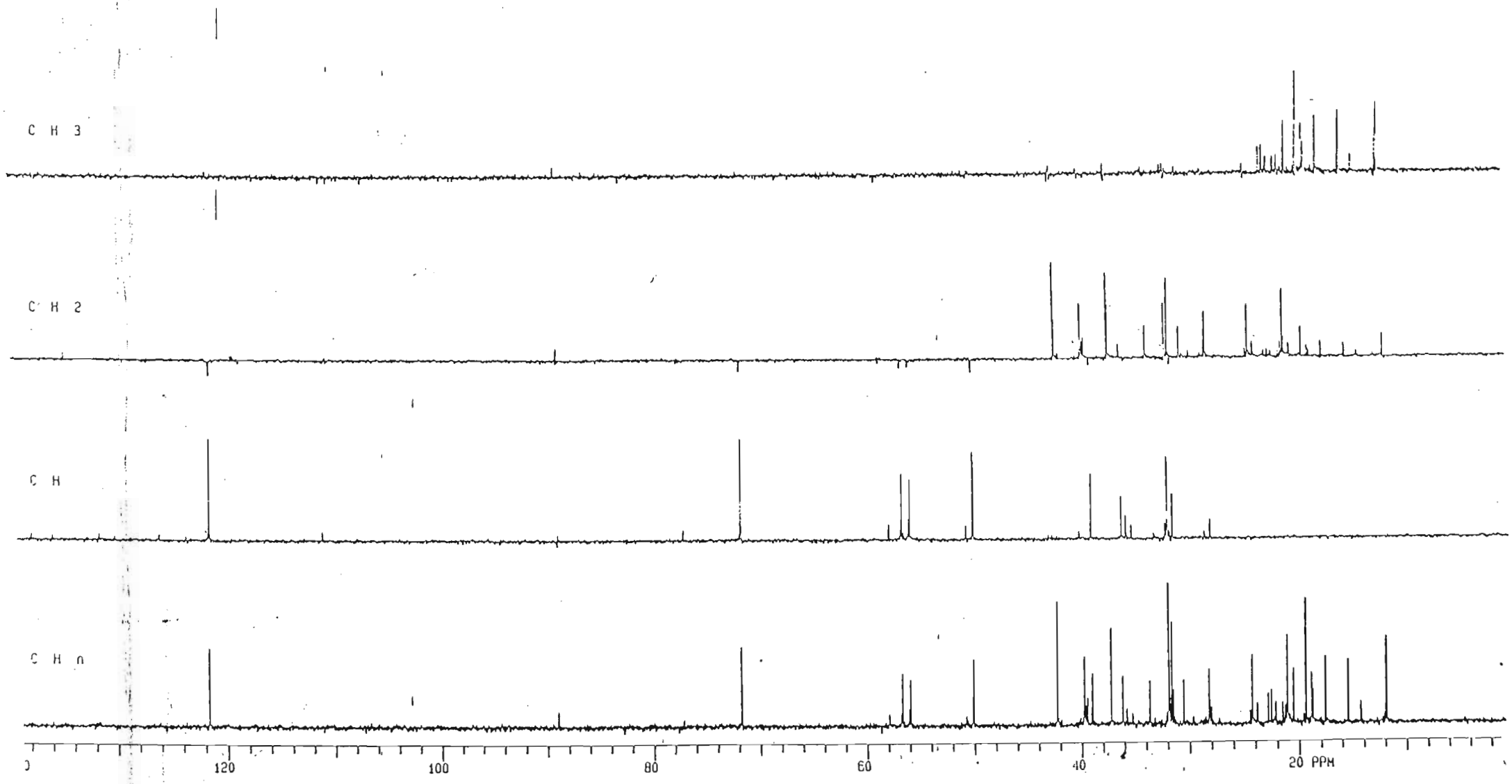


S2.1

Spectrum 2.1: ^{1}H NMR spectrum of compound 2 ($CDCl_3$)

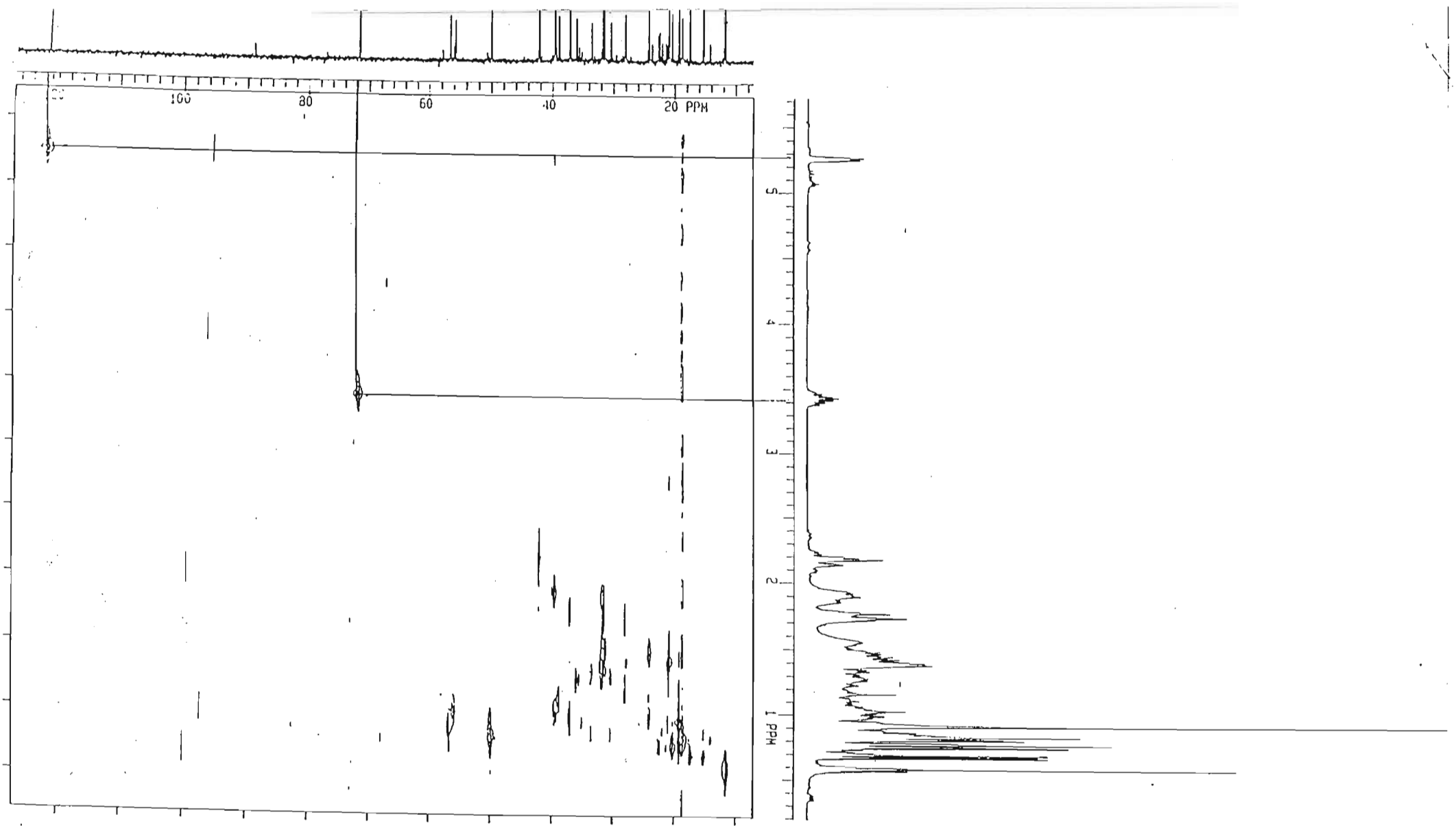


Spectrum 2.2: ^{13}C NMR spectrum of compound 2 ($CDCl_3$)

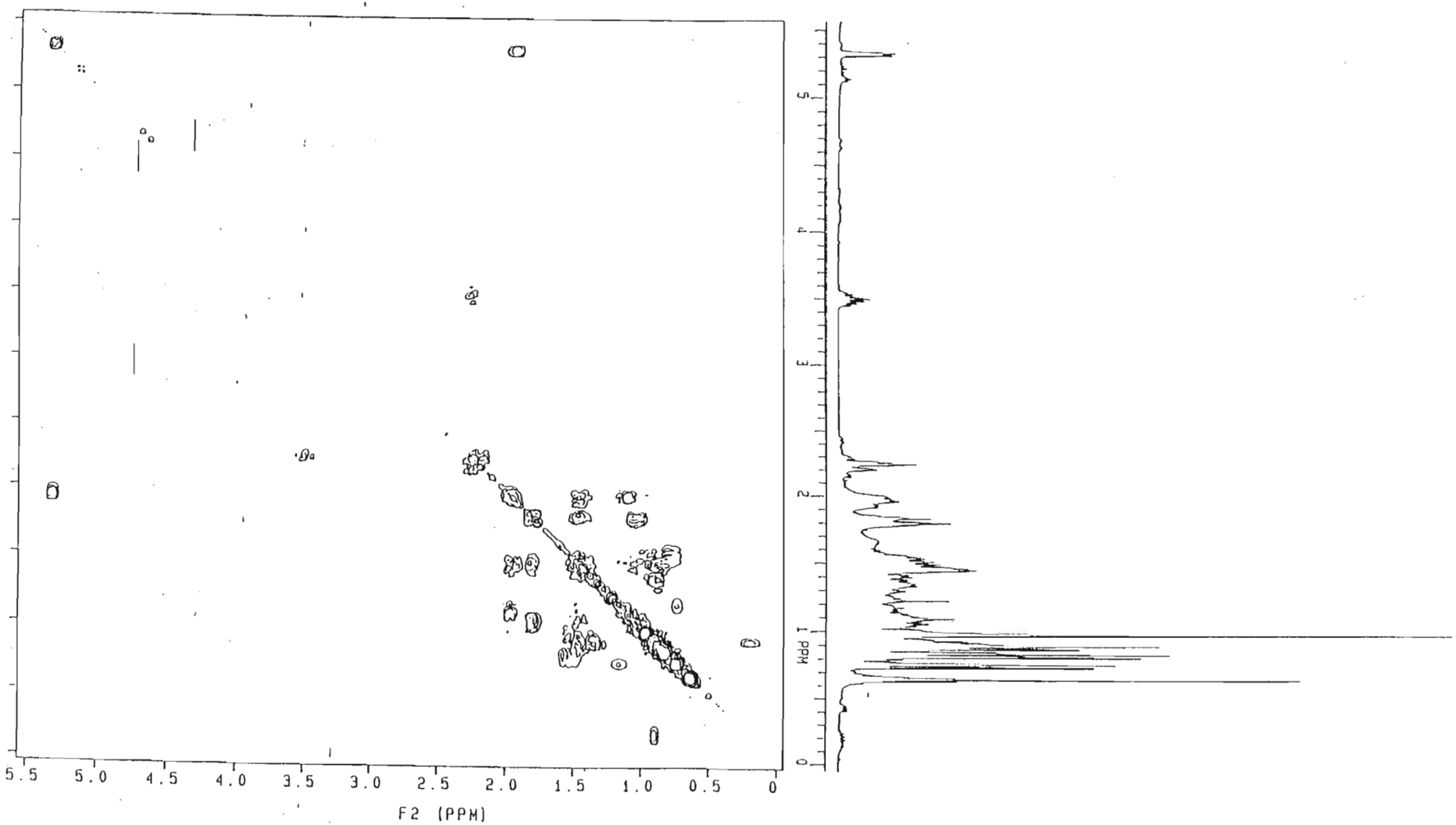


Spectrum 2.3: DEPT spectrum of compound 2 ($CDCl_3$)

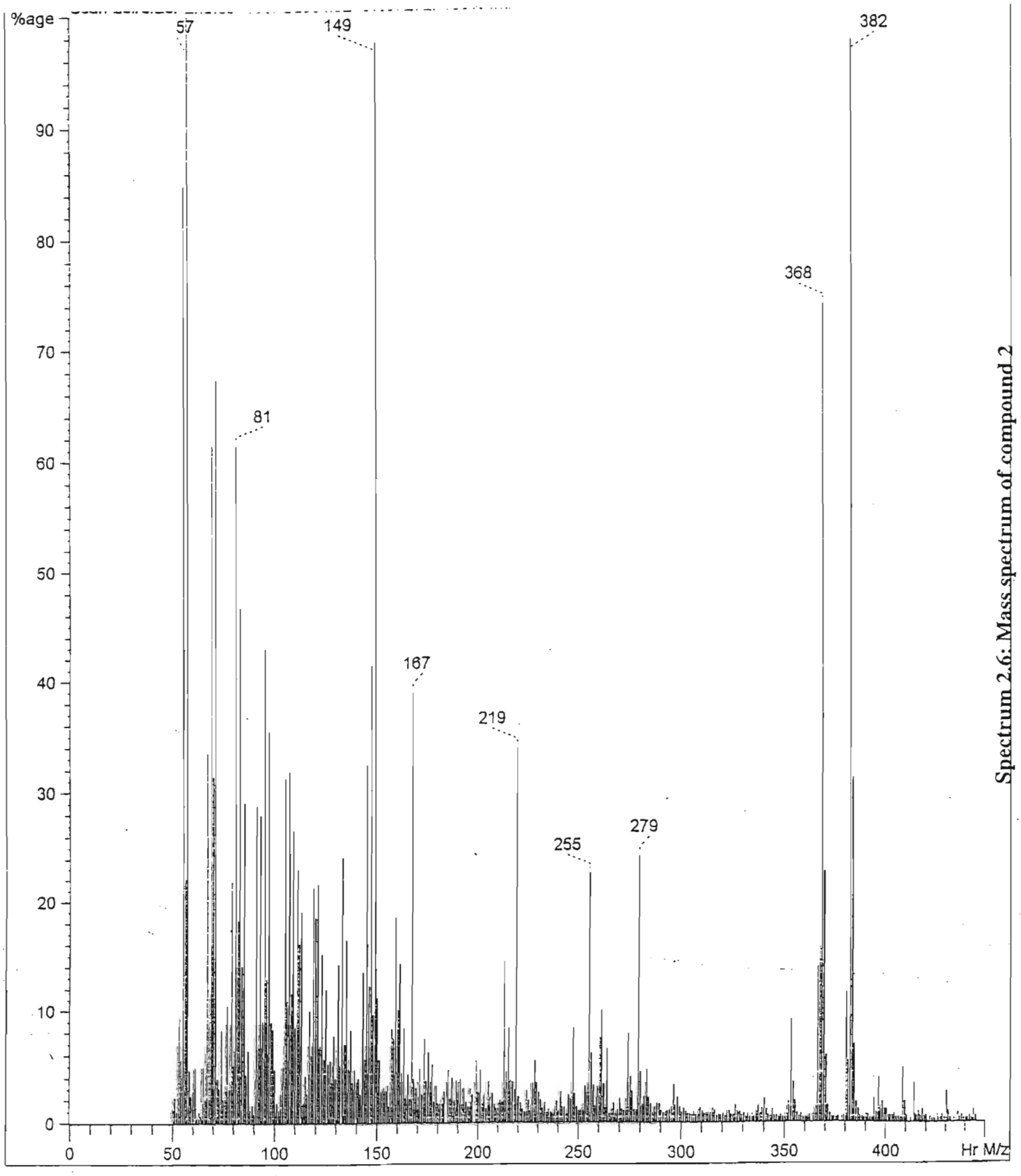
S2.3



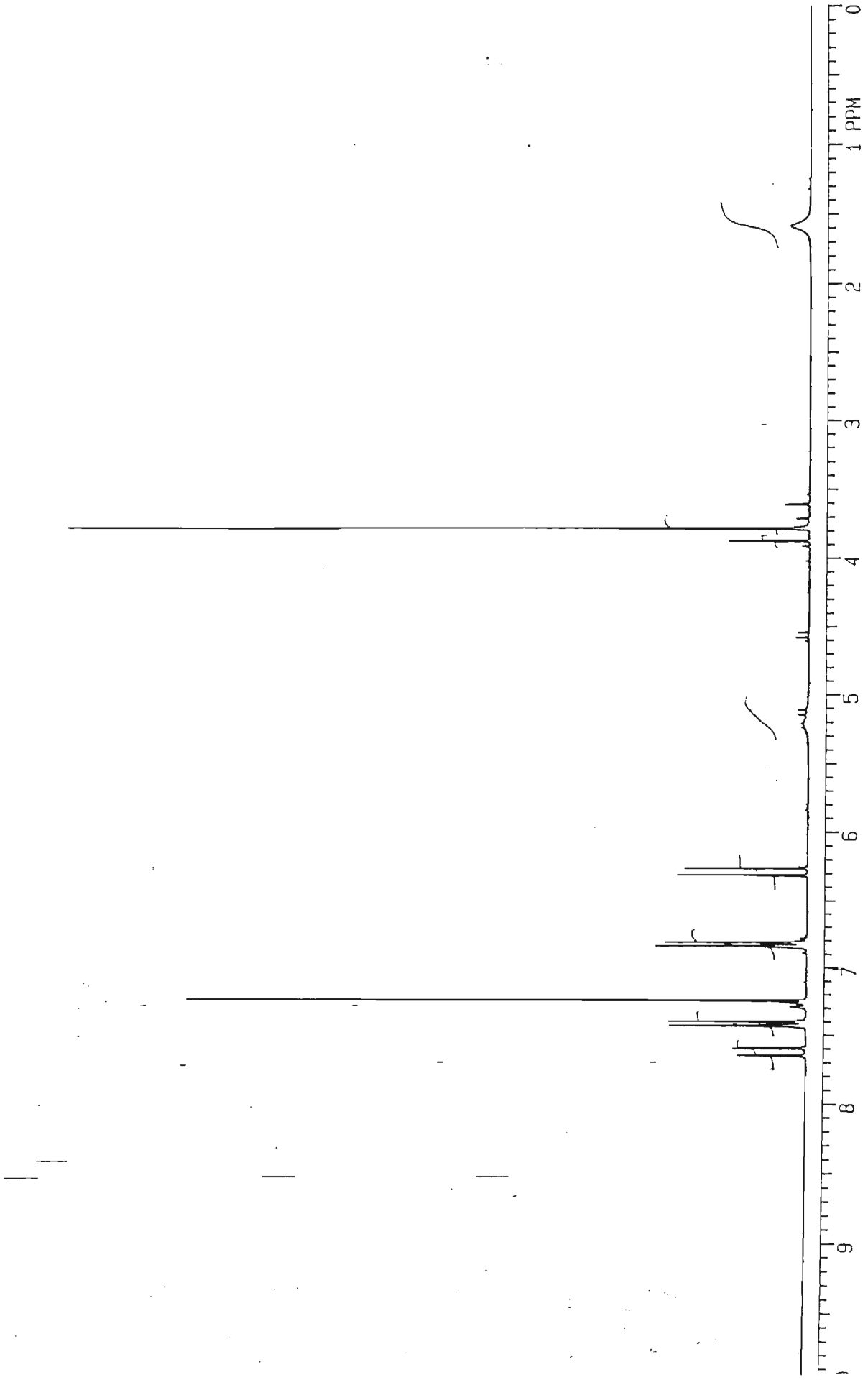
Spectrum 2.4: HETCOR spectrum of compound 2 ($CDCl_3$)



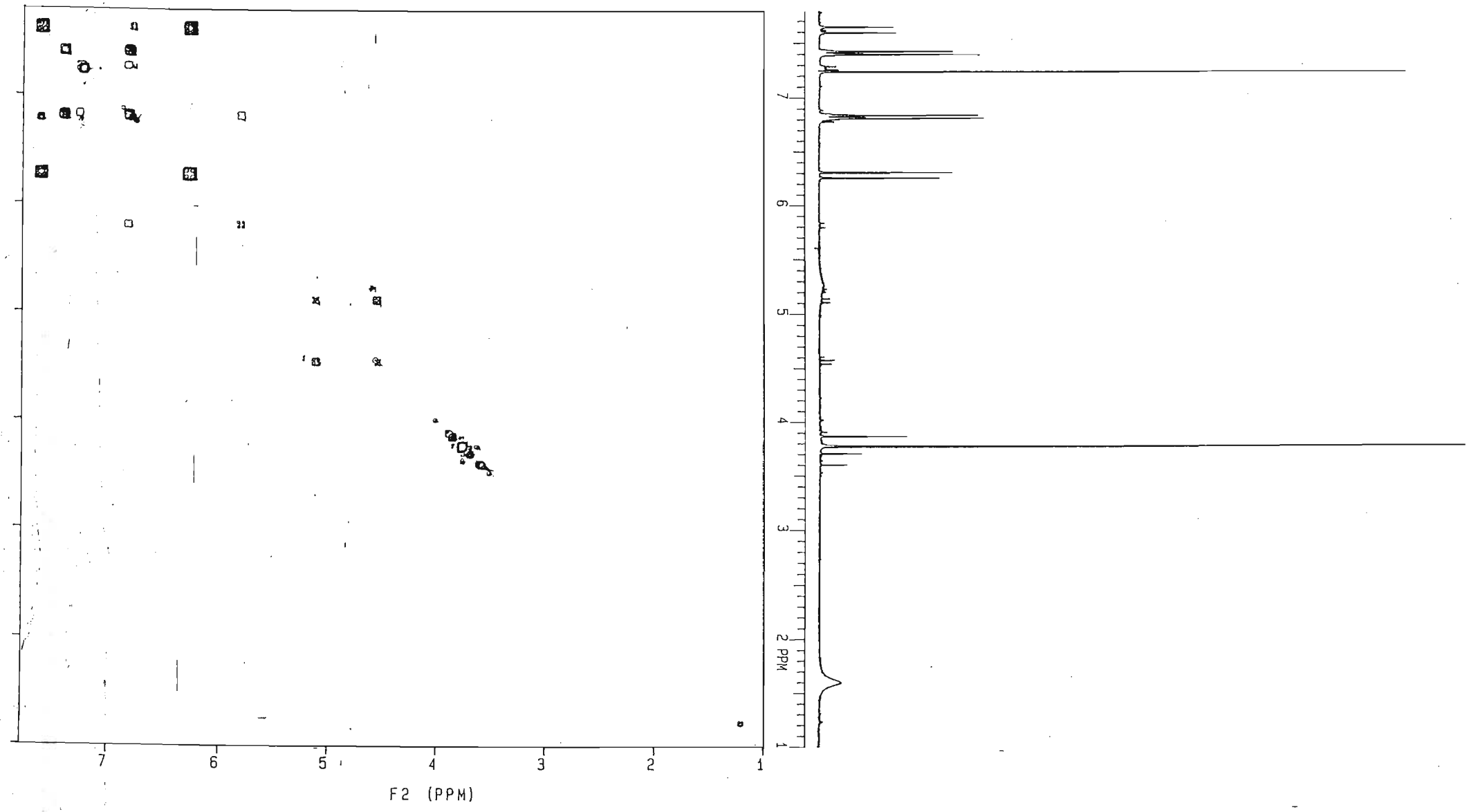
Spectrum 2.5: COSY spectrum of compound 2 ($CDCl_3$)



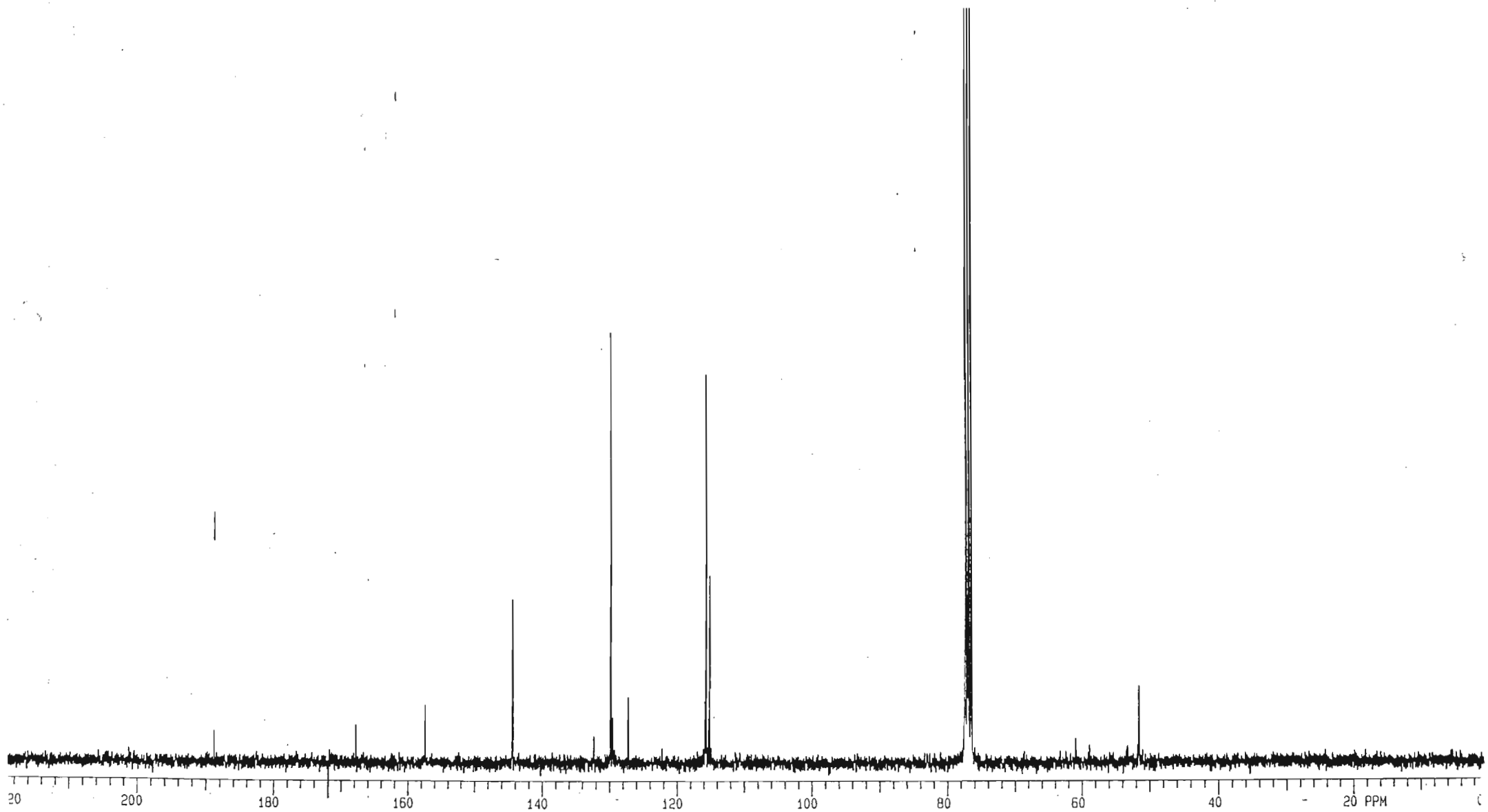
Spectrum 2.6: Mass spectrum of compound 2



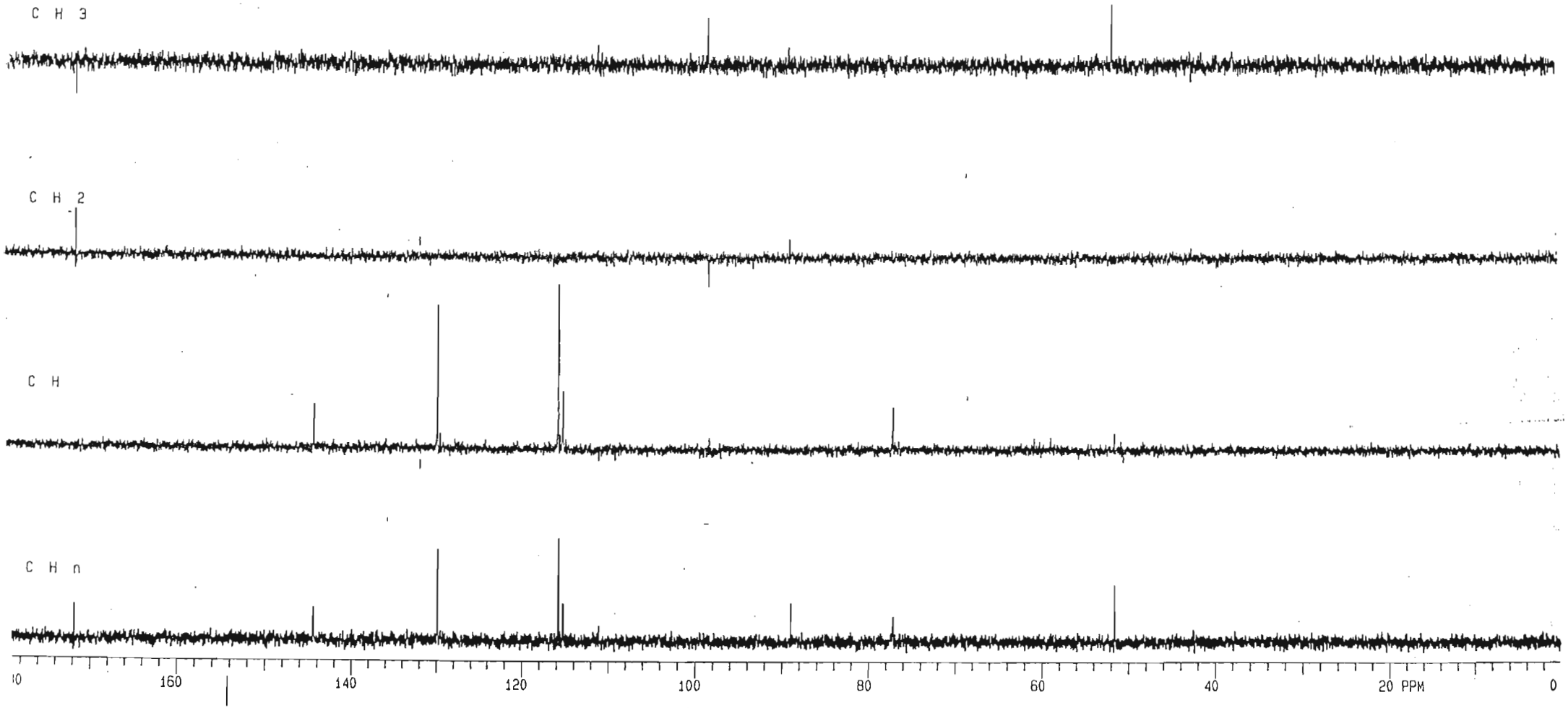
Spectrum 3.1: ^{1}H NMR spectrum of compound 3 ($CDCl_3$)



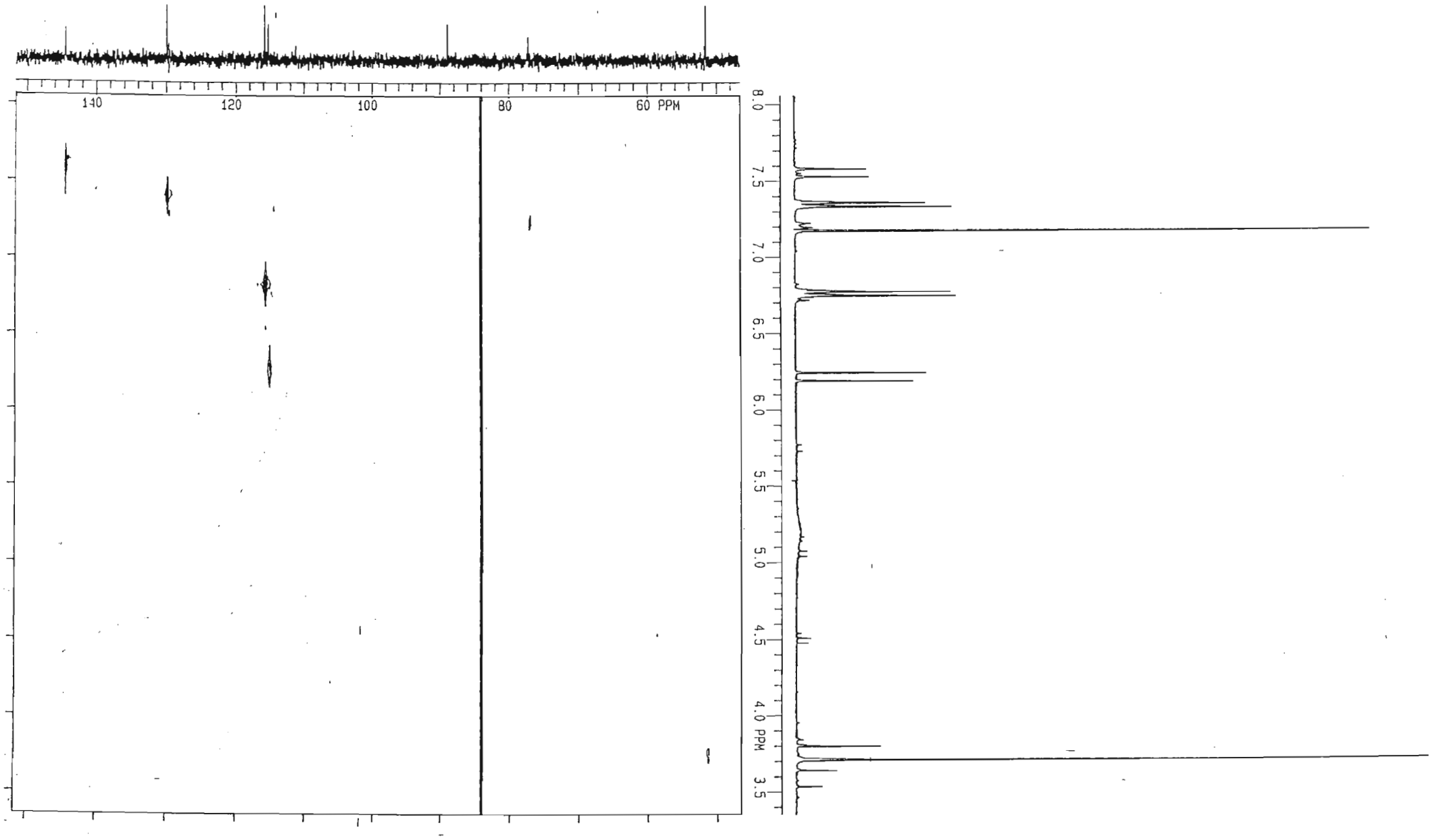
Spectrum 3.2: COSY spectrum of compound 3 ($CDCl_3$)



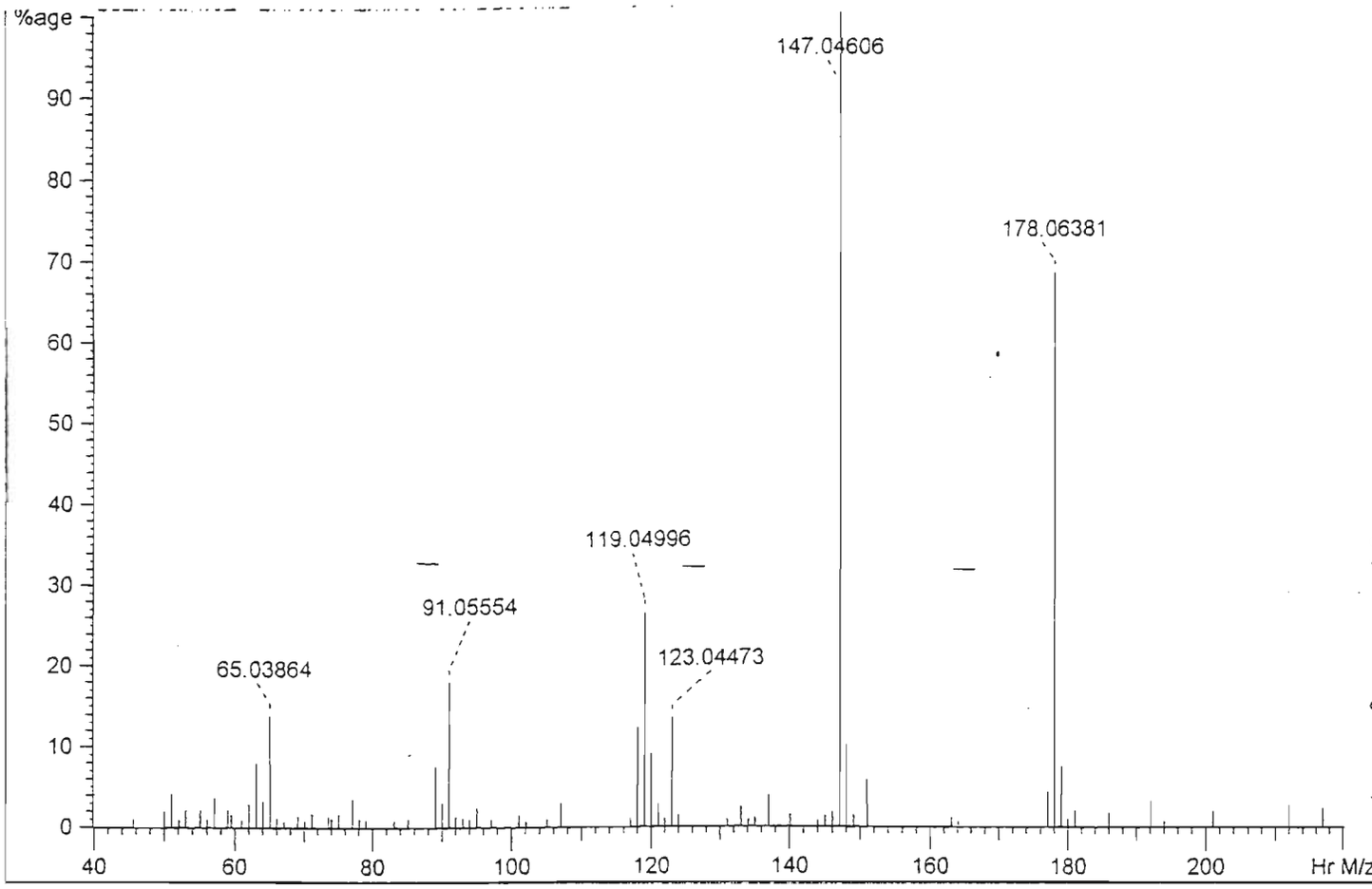
Spectrum 3.3: ^{13}C NMR spectrum of compound 3 ($CDCl_3$)



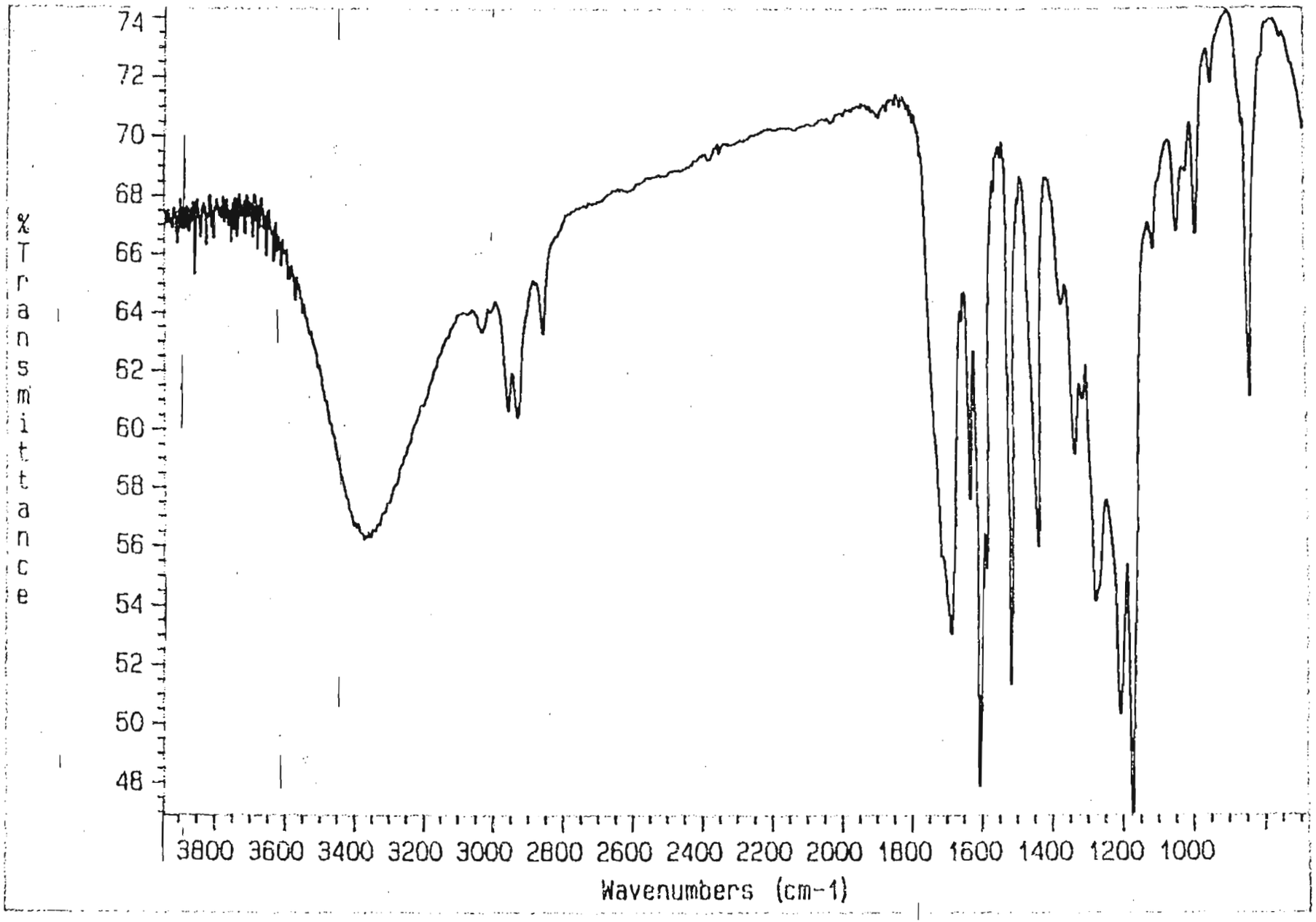
Spectrum 3.4: DEPT spectrum of compound 3 ($CDCl_3$)



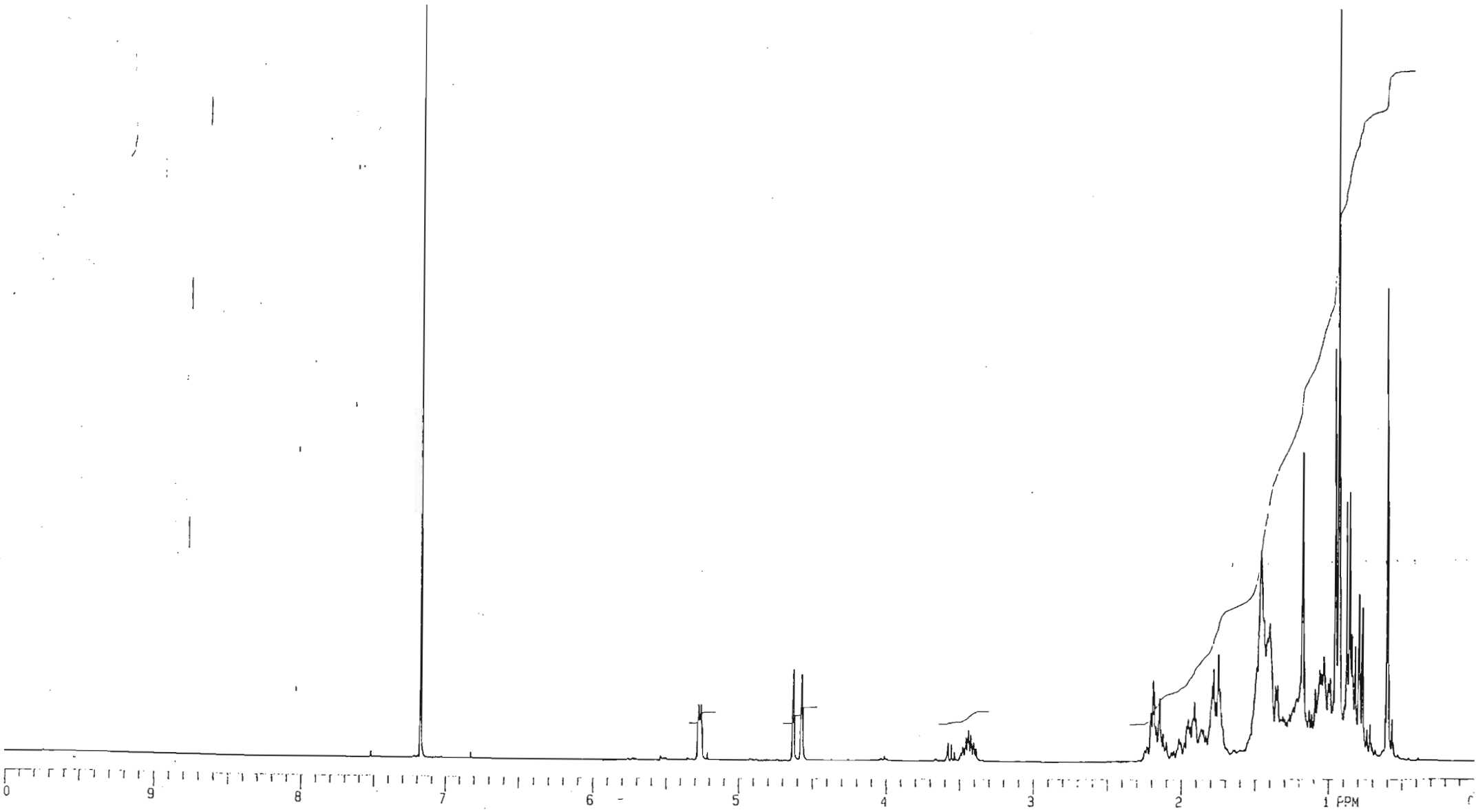
Spectrum 3.5: HETCOR spectrum of compound 3 ($CDCl_3$)



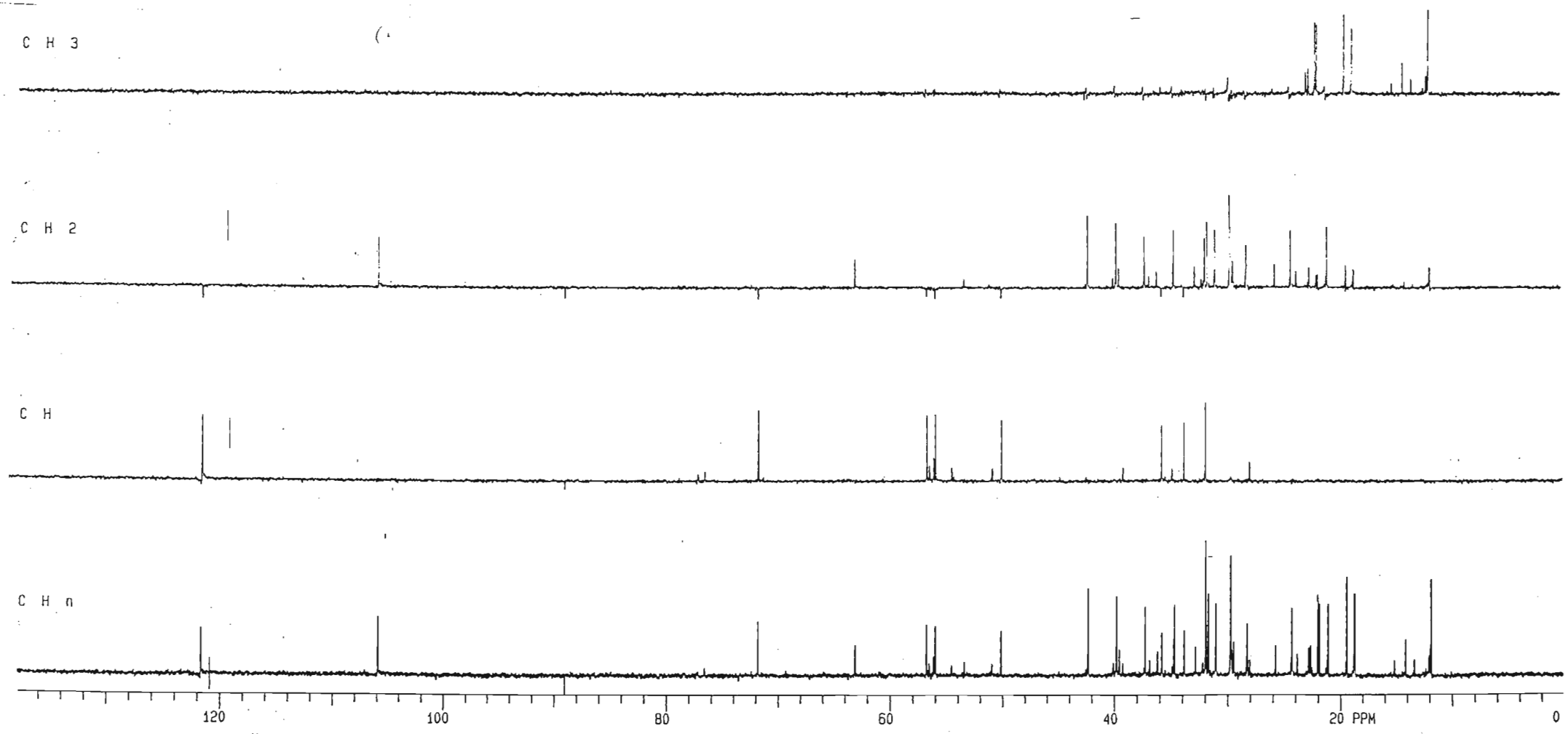
Spectrum 3.6: Mass spectrum of compound 3



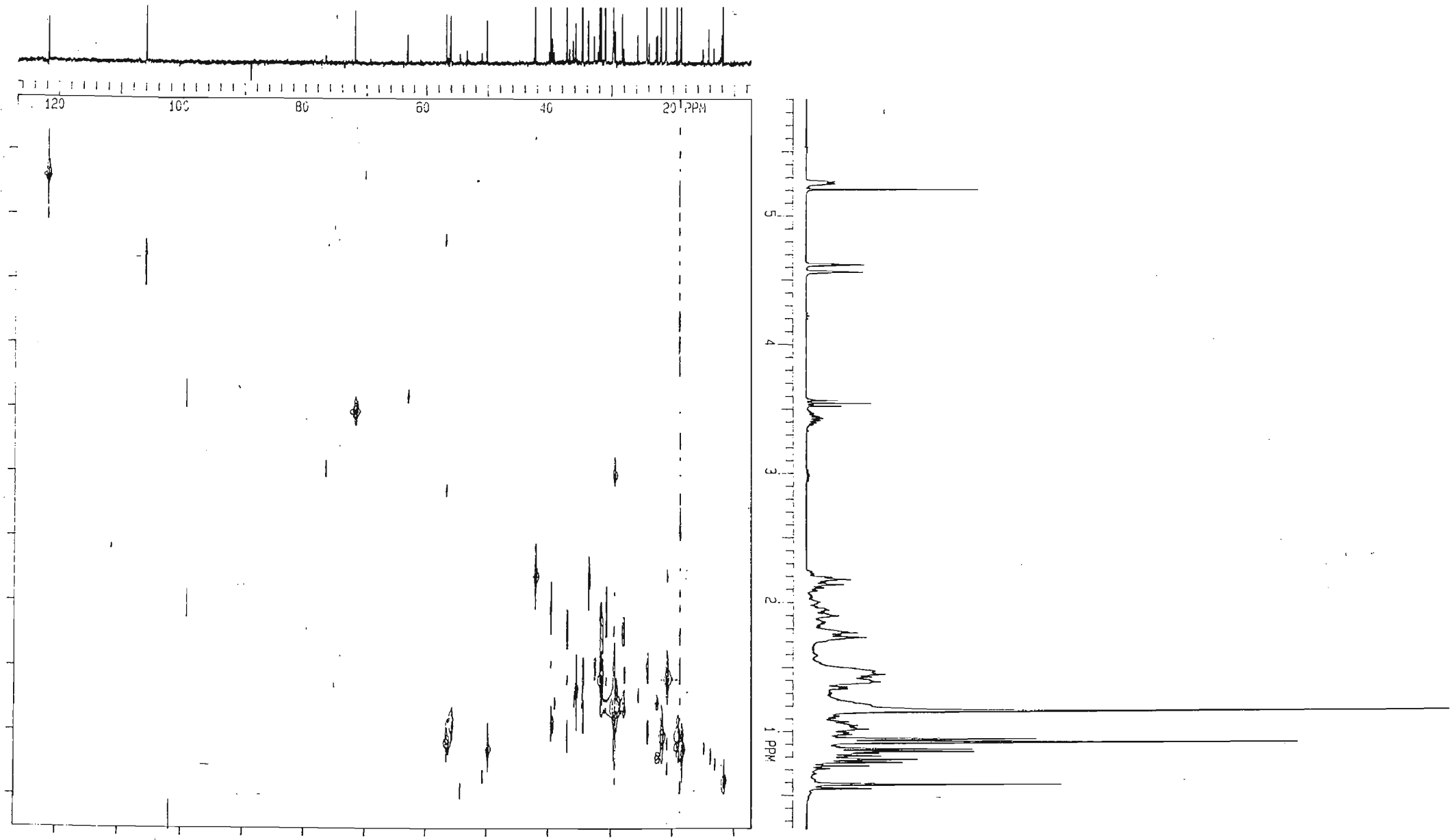
Spectrum 3.7: Infrared spectrum of compound 3



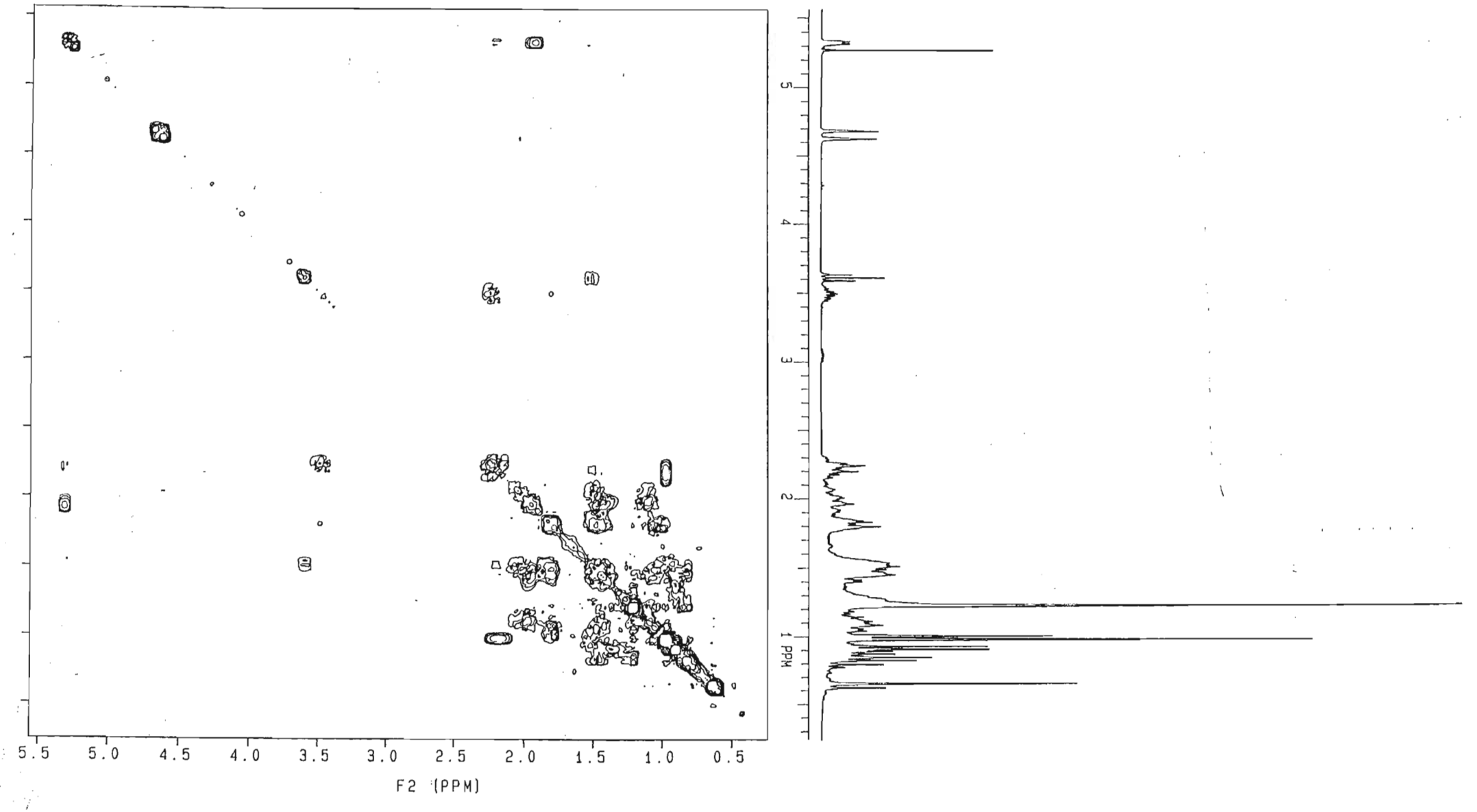
Spectrum 4.1: ^{1}H NMR spectrum of compound 4 ($CDCl_3$)



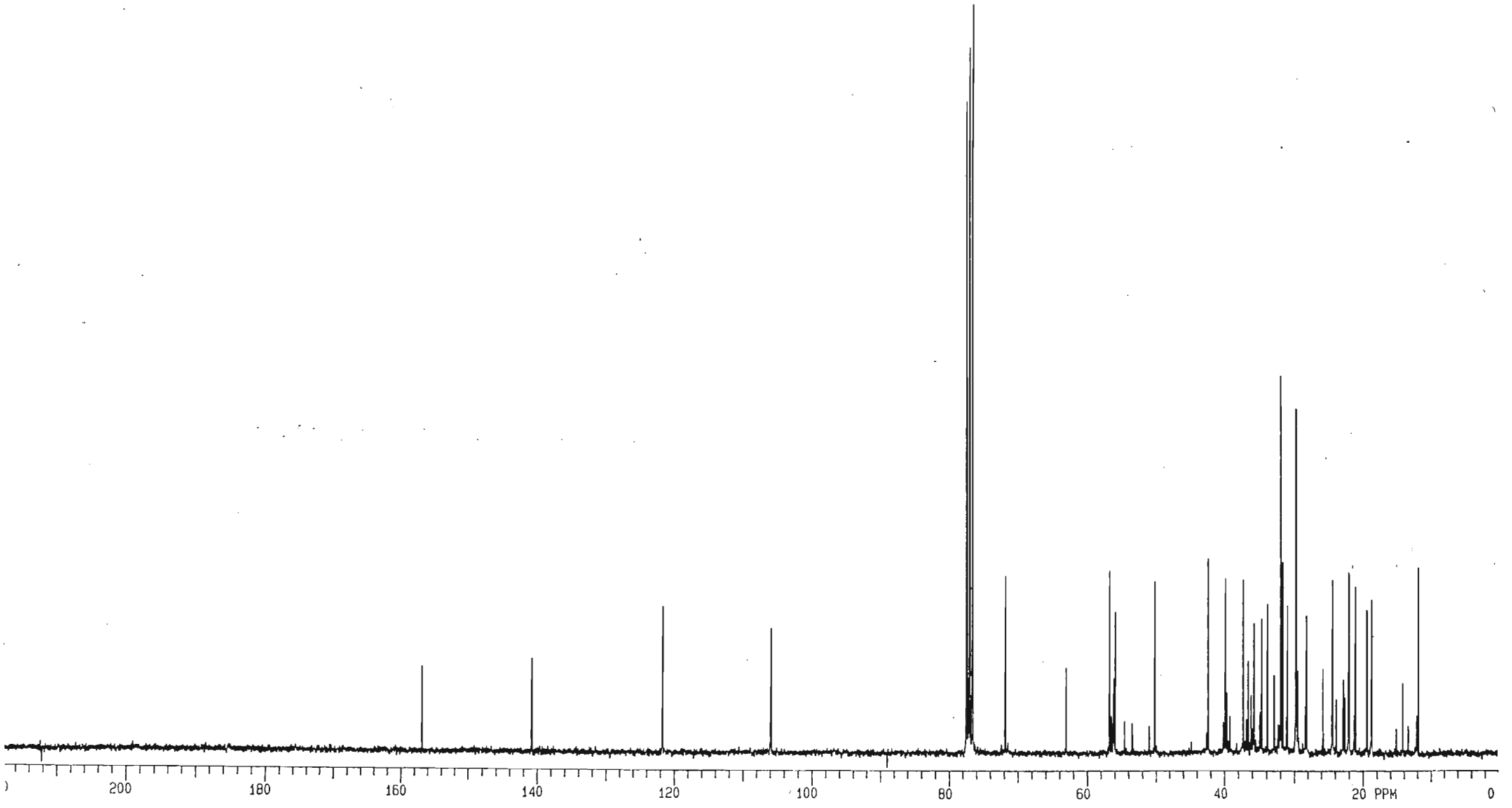
Spectrum 4.2: DEPT spectrum of compound 4 ($CDCl_3$)



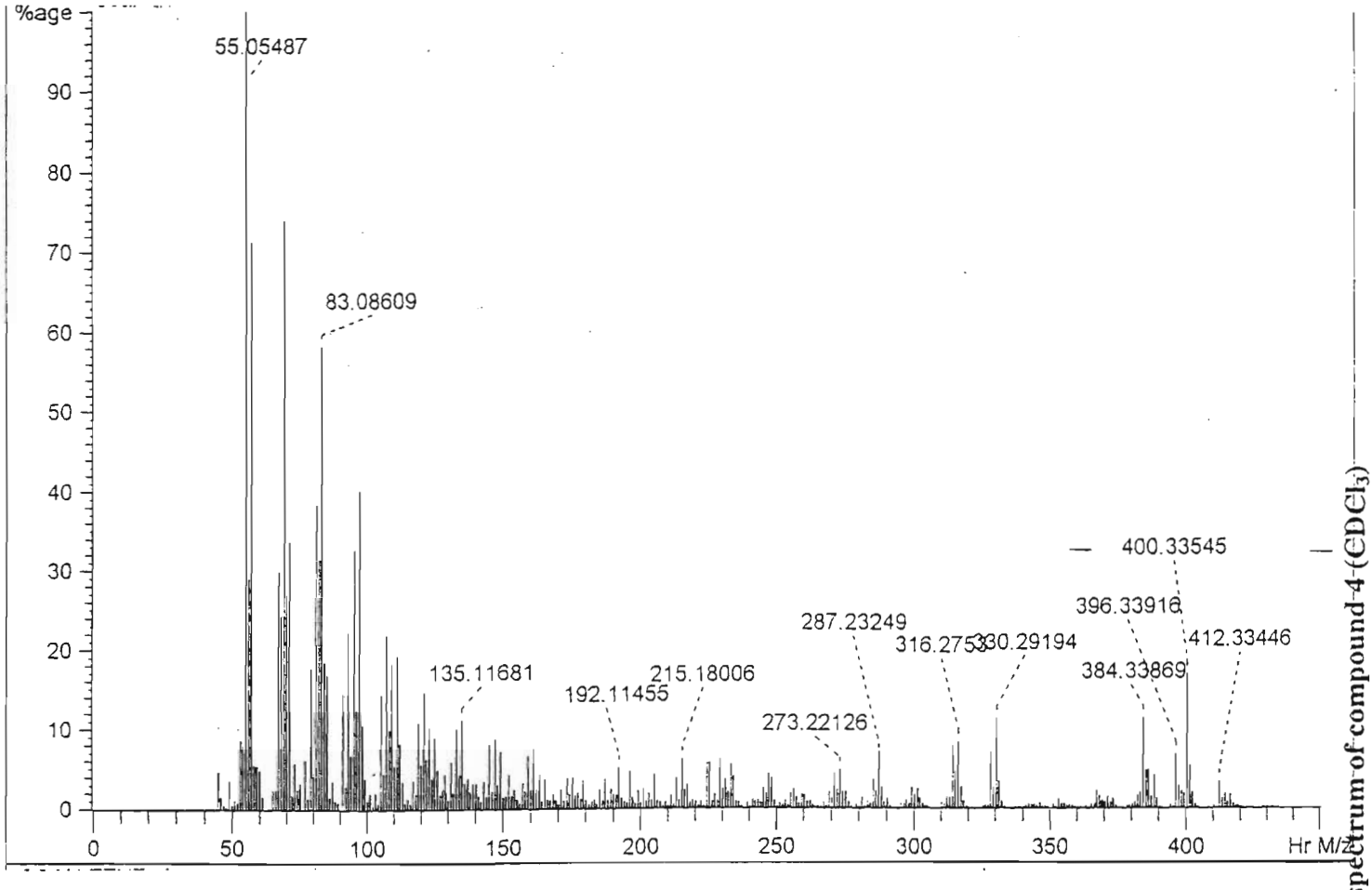
Spectrum 4.3: HETCOR spectrum of compound 4 ($CDCl_3$)



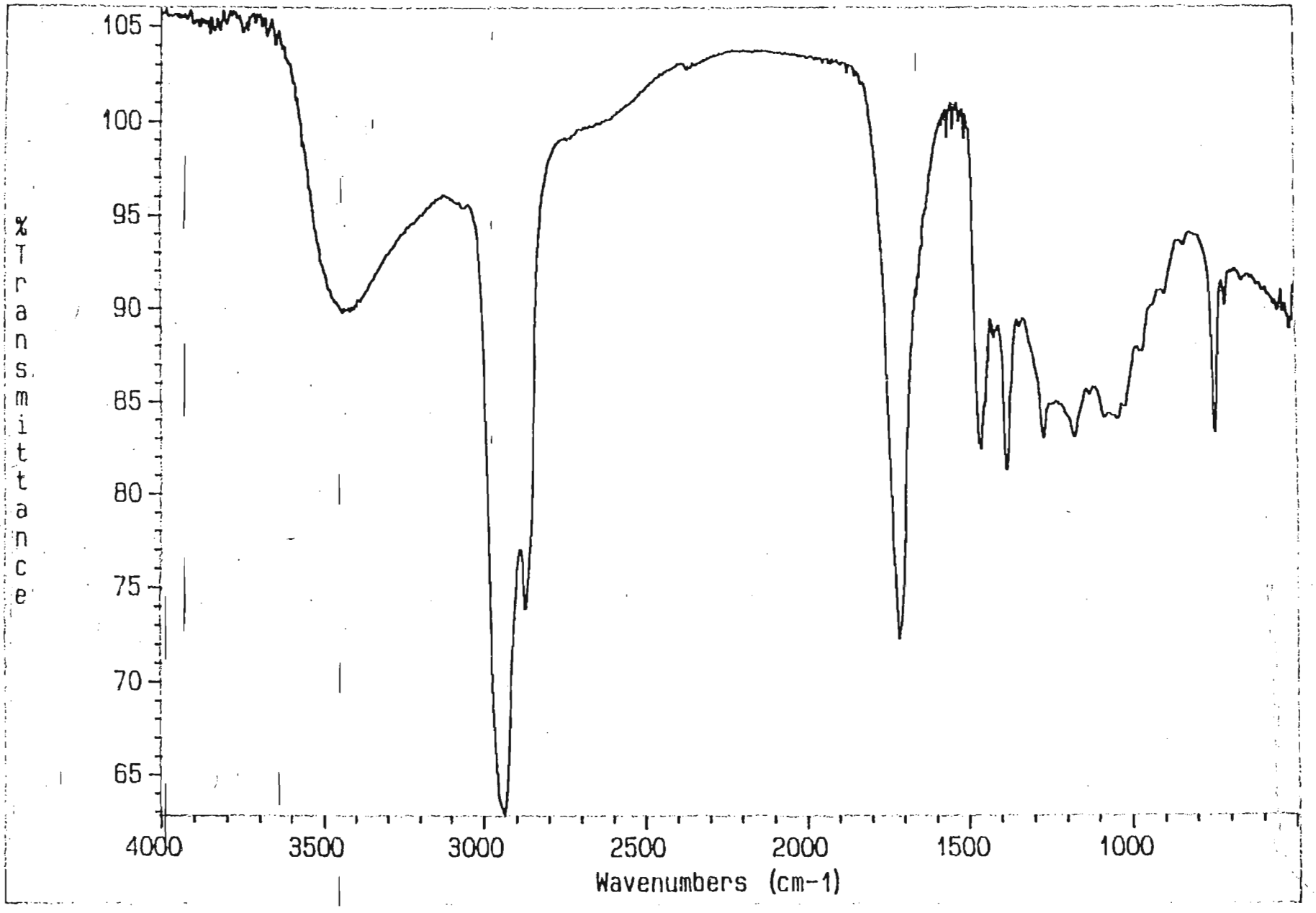
Spectrum 4.4: COSY spectrum of compound 4 ($CDCl_3$)



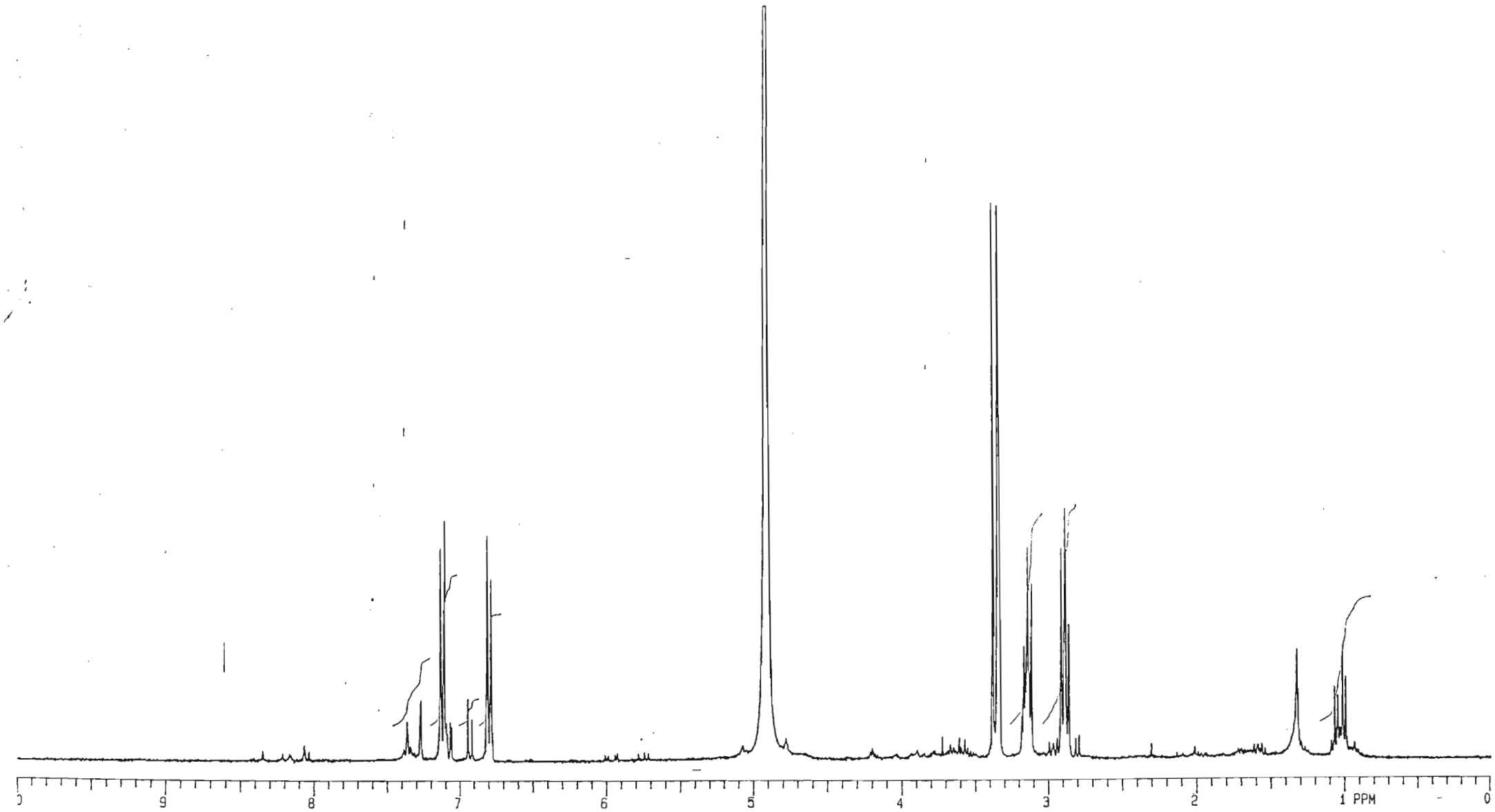
Spectrum 4.5: ^{13}C NMR spectrum of compound 4 ($CDCl_3$)



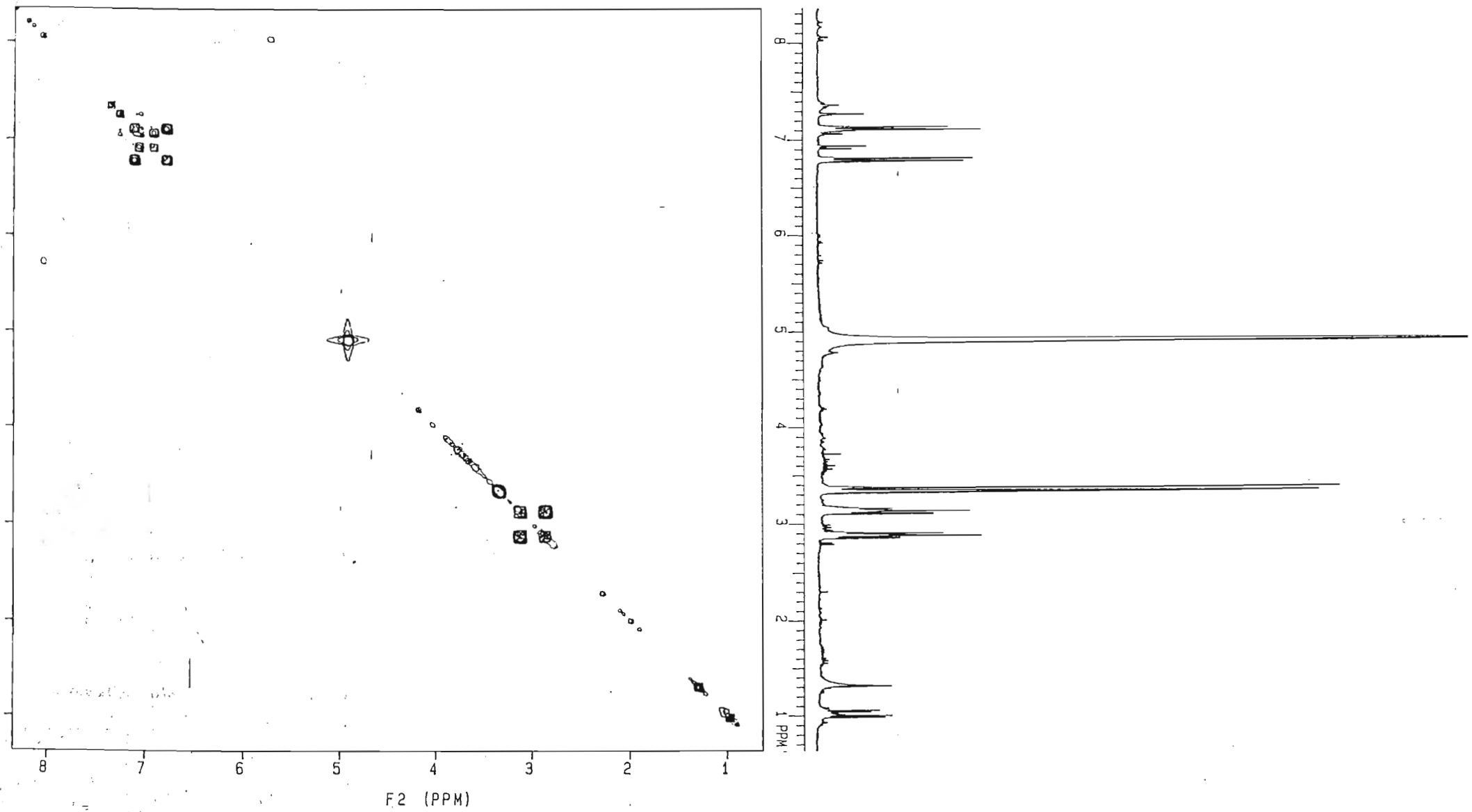
Spectrum 4.6: Mass spectrum of compound-4-($CDCl_3$)



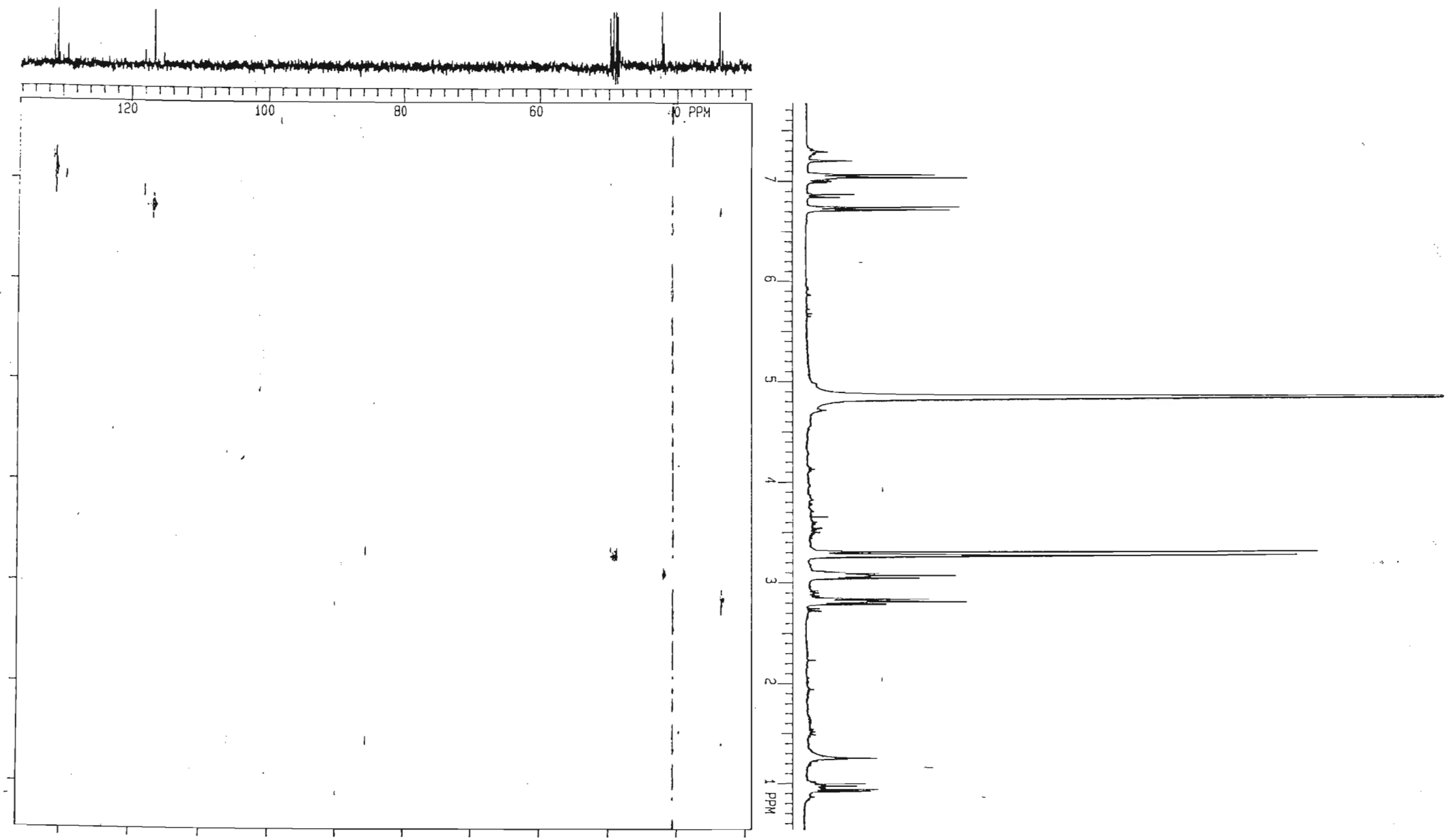
Spectrum 4.7: Infrared spectrum of compound 4 ($CDCl_3$)



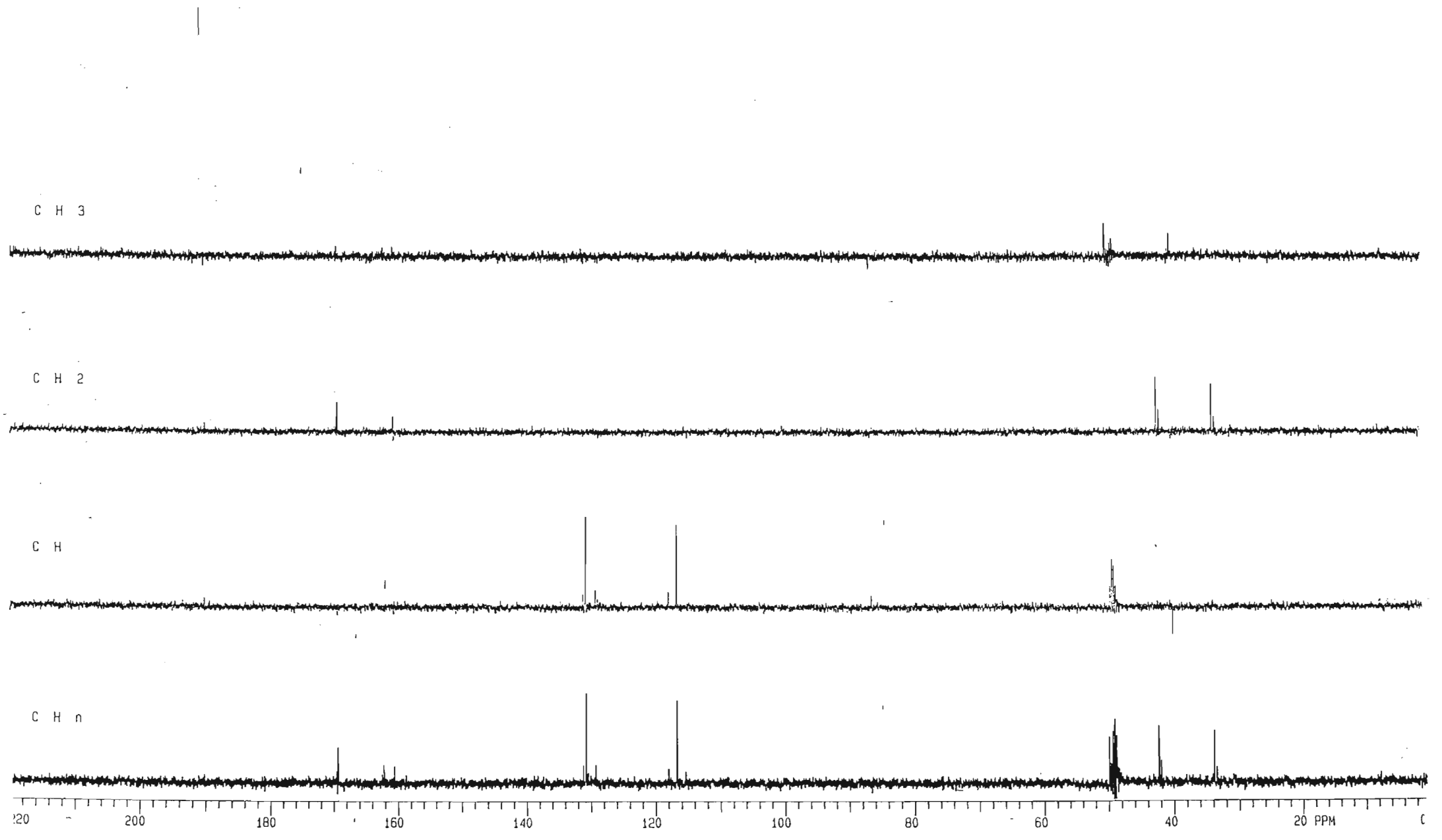
Spectrum 5.1: ^{1}H NMR spectrum of compound 5 (CD_3OD)



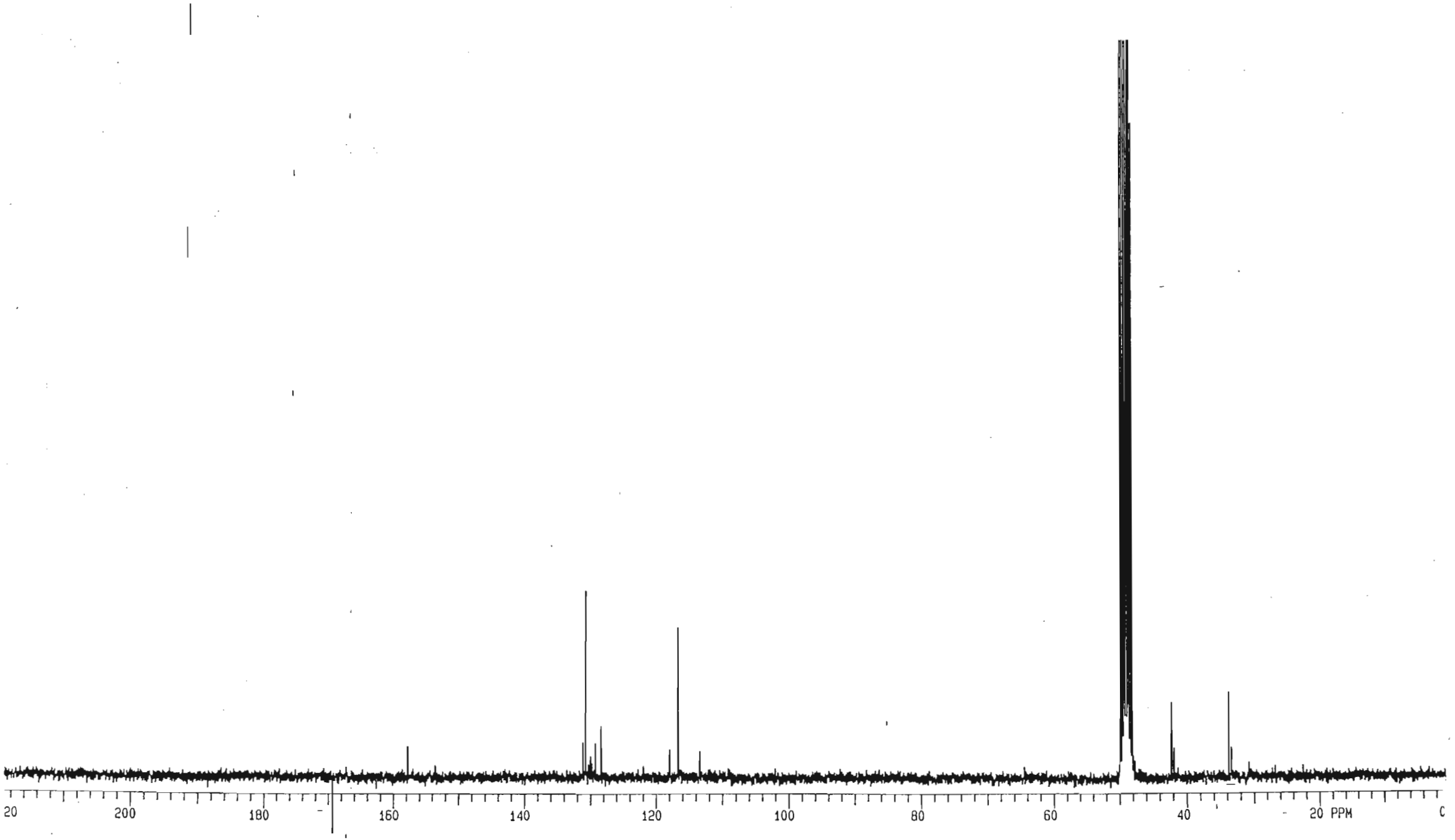
Spectrum 5.2: COSY spectrum of compound 5 (CD_3OD)



Spectrum 5.3: HETCOR spectrum of compound 5 (CD_3OD)

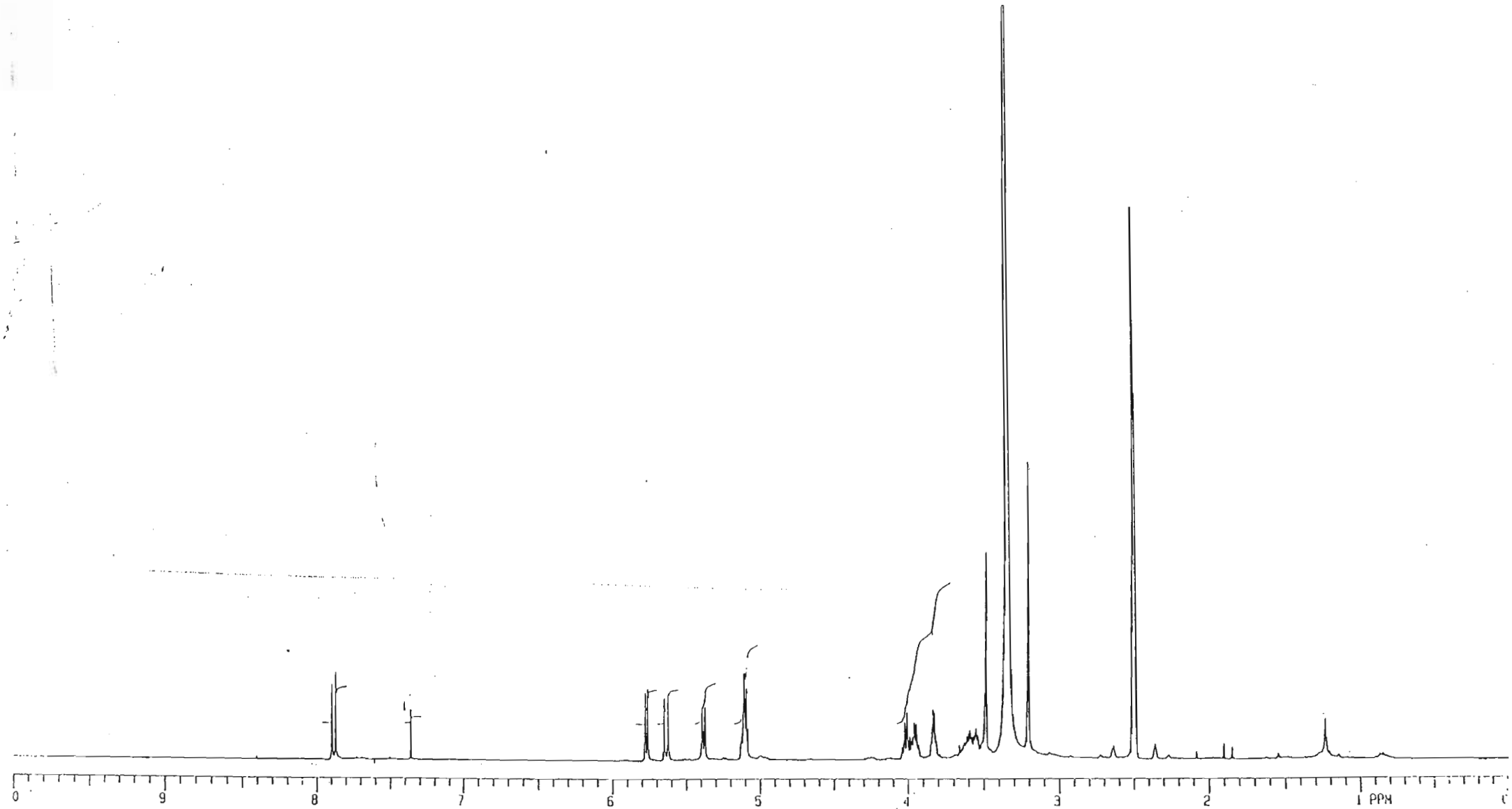


Spectrum 5.4: DEPT spectrum of compound 5 (CD_3OD)

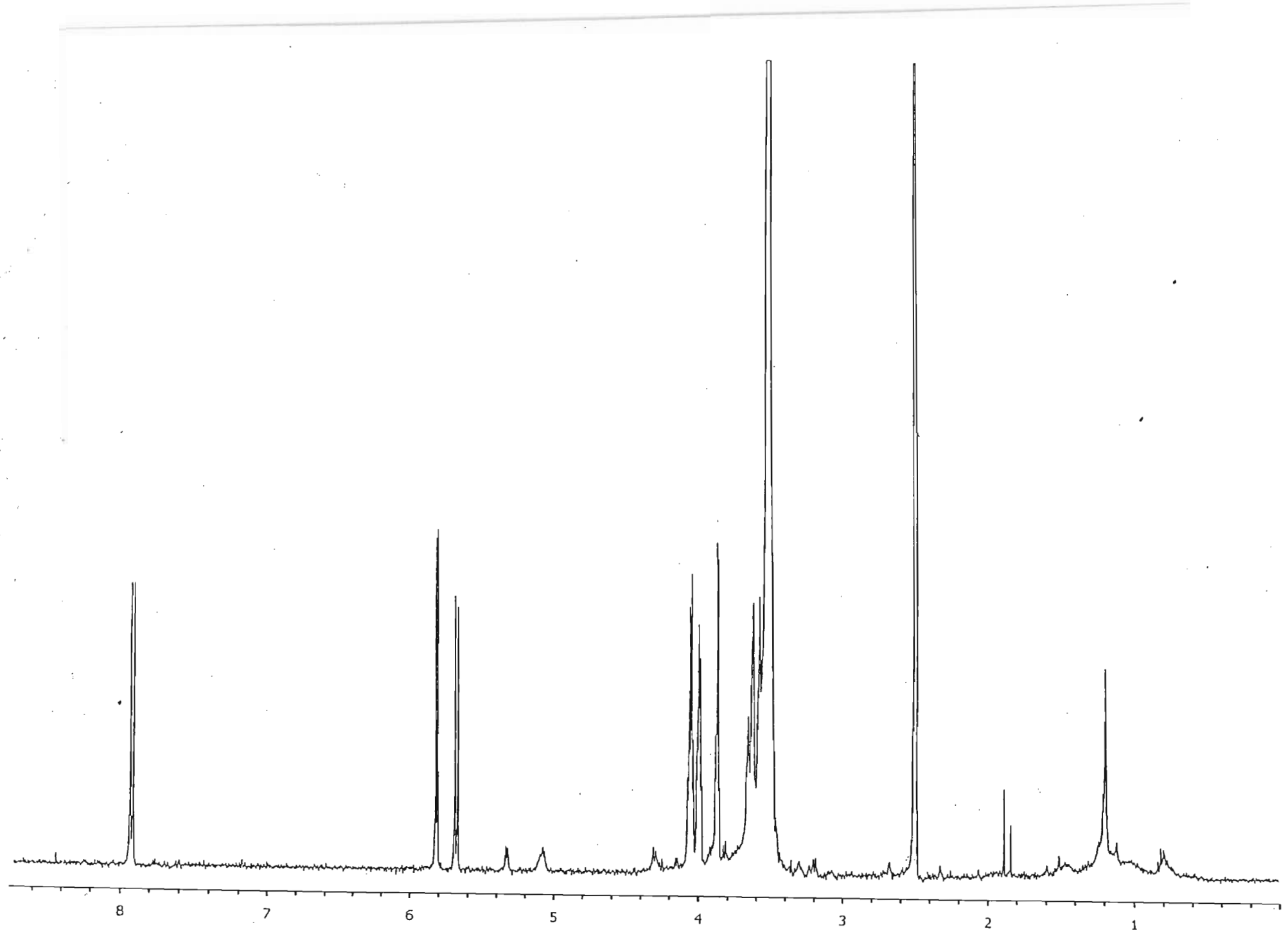


Spectrum 5.5: ^{13}C NMR spectrum of compound 5 (CD_3OD)

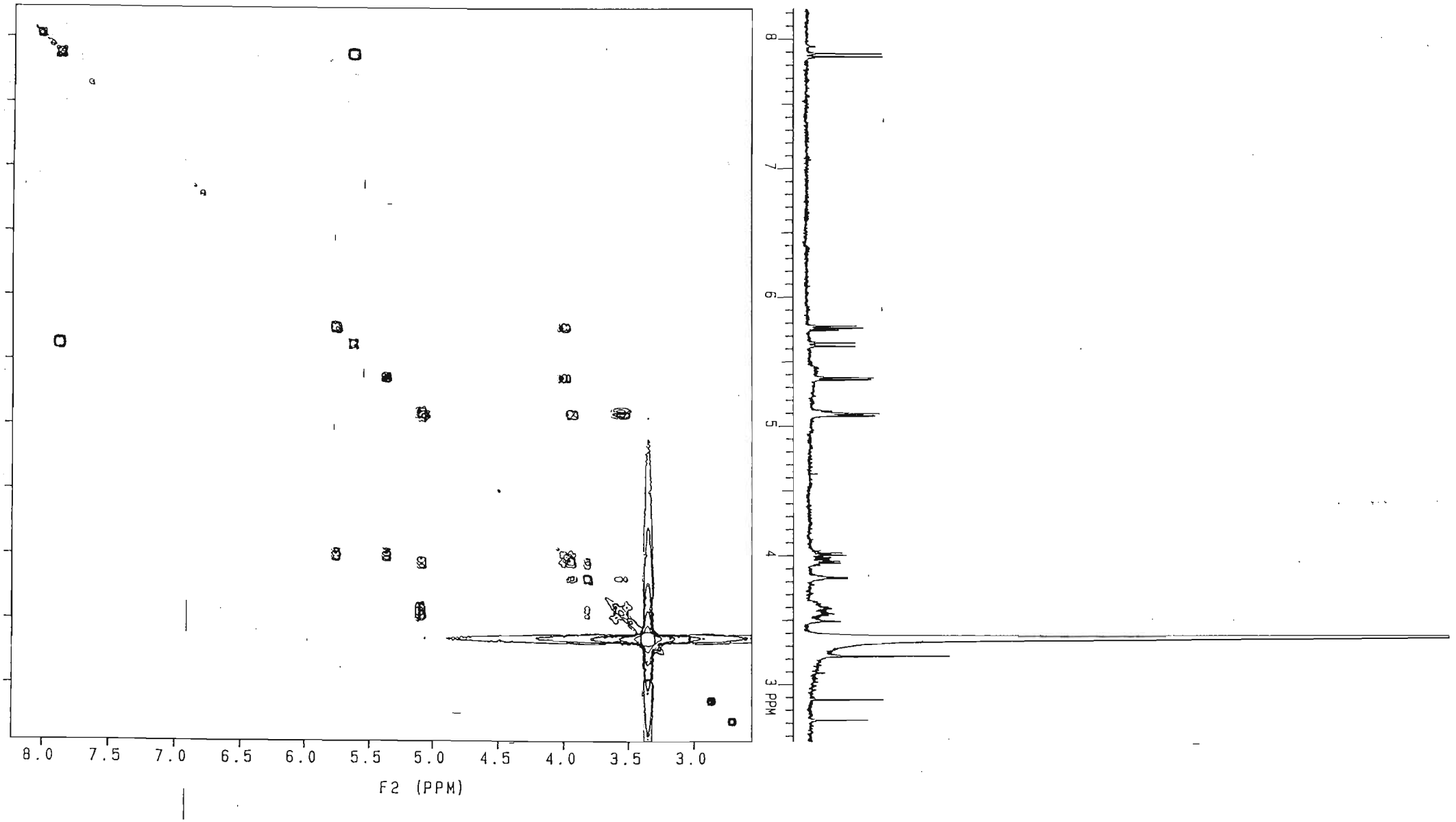
S5.5



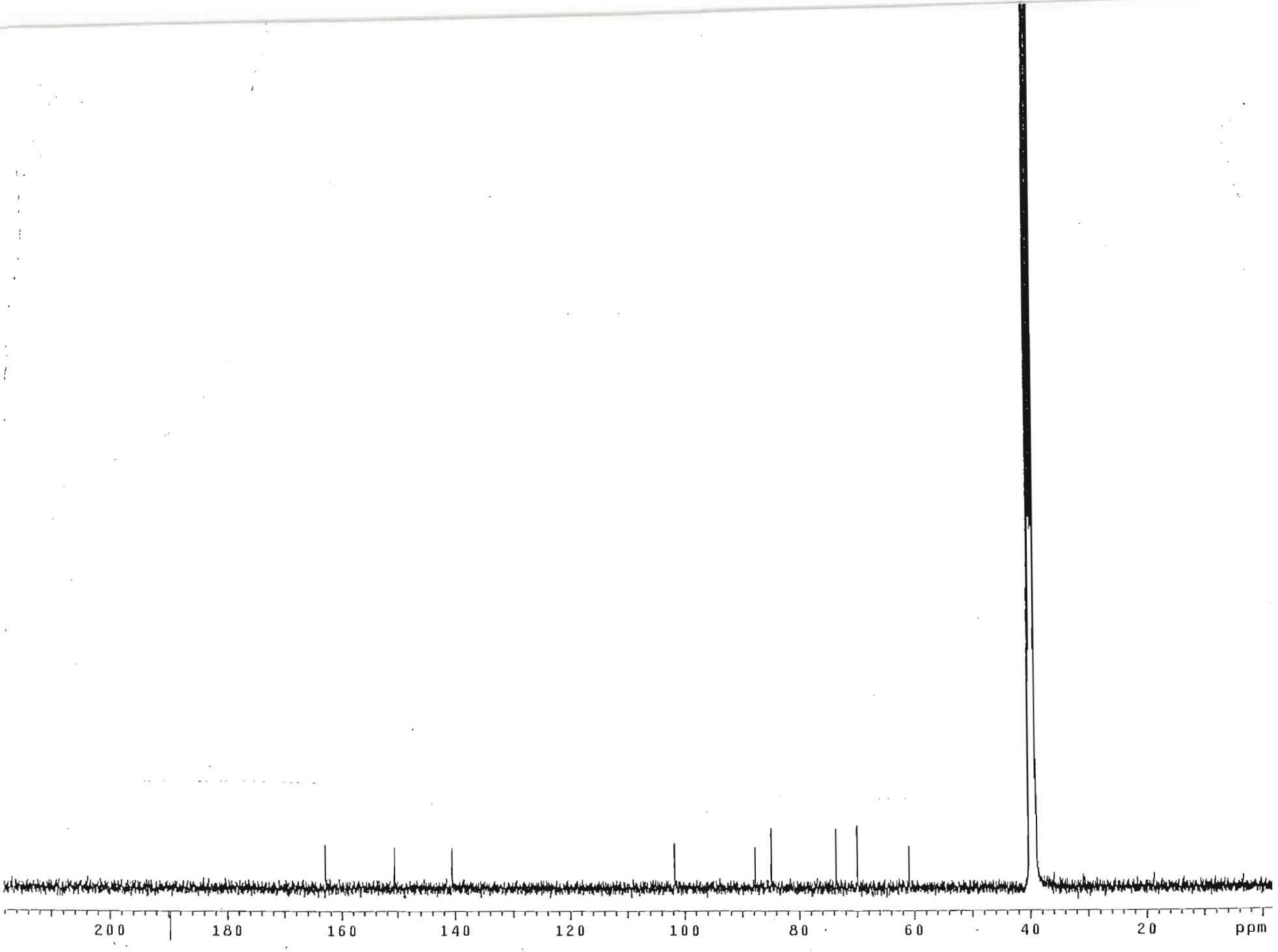
Spectrum 6.1: ^{1}H NMR spectrum of compound 6 (DMSO)



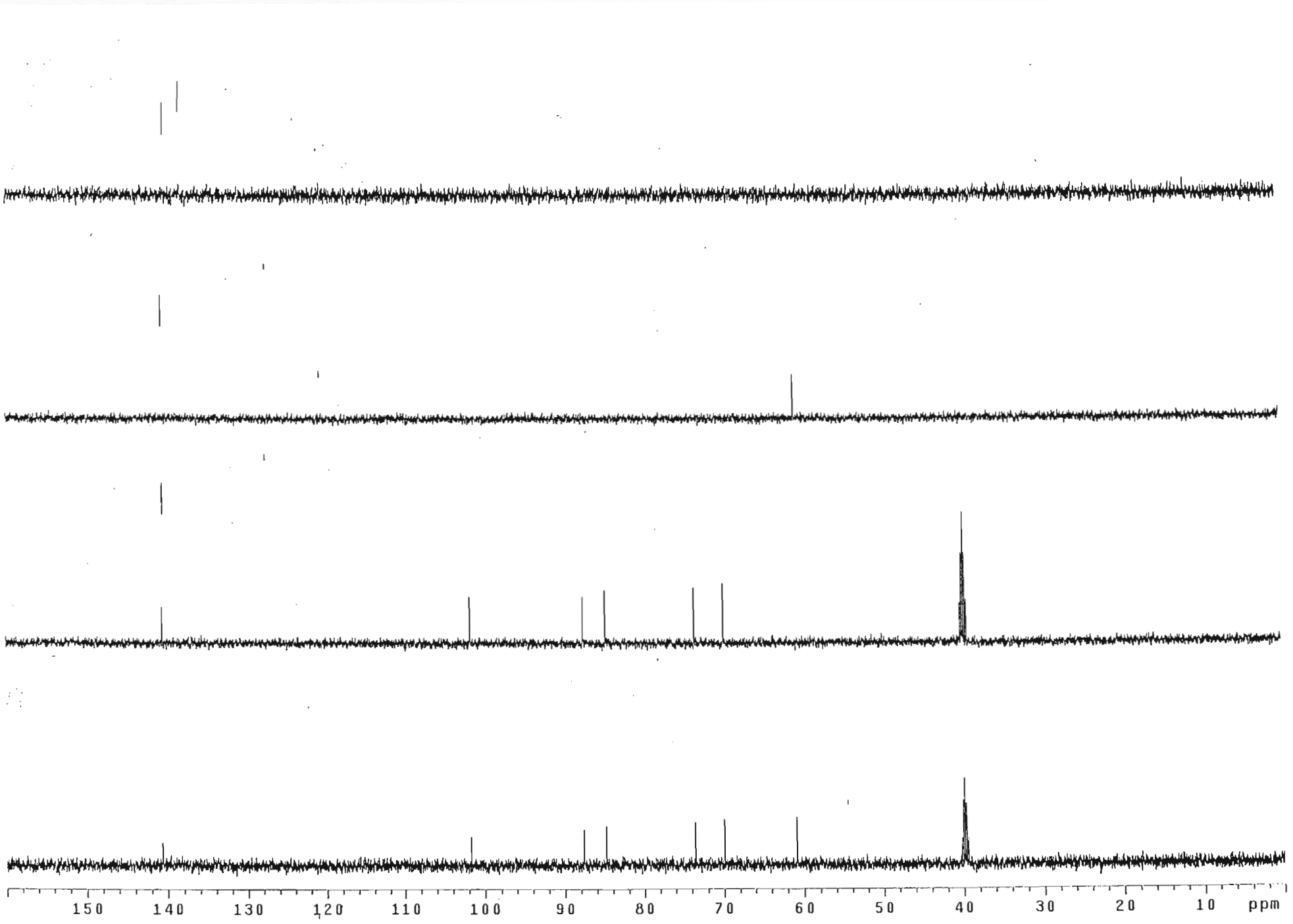
Spectrum 6.2: ^{1}H NMR spectrum of compound 6 (D_2O)



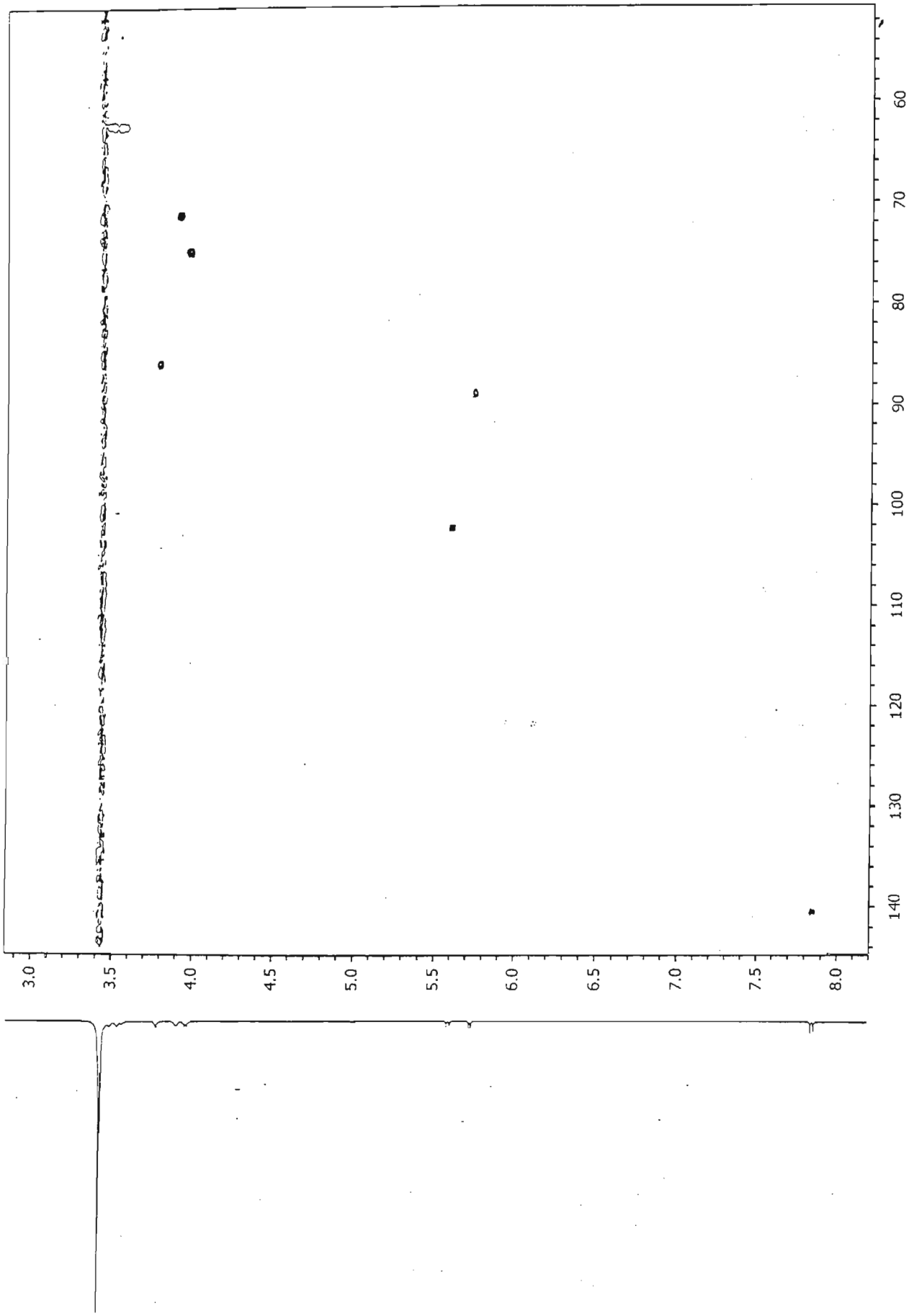
Spectrum 6.3: COSY spectrum of compound 6 (DMSO)



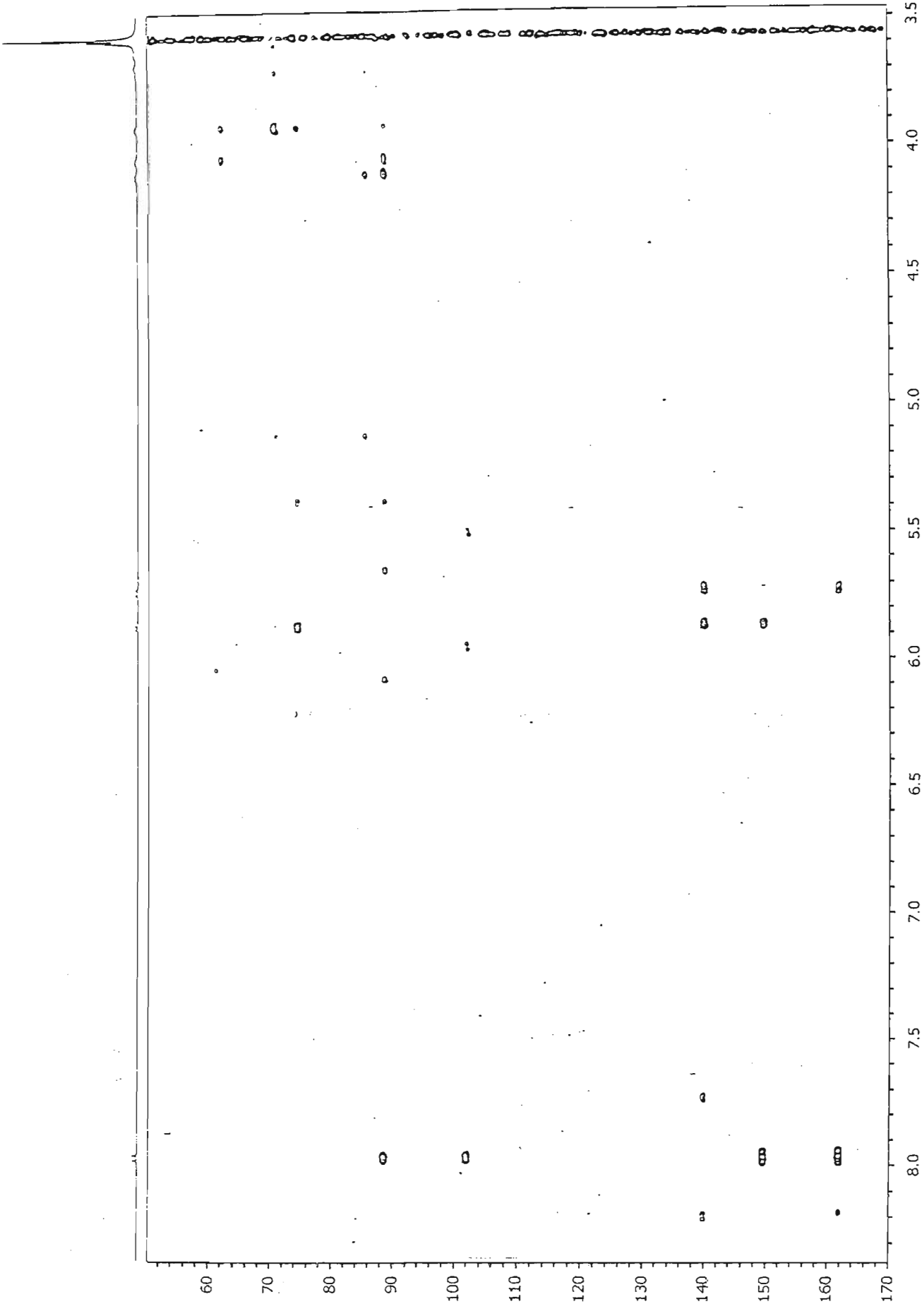
Spectrum 6.4: ^{13}C NMR spectrum of compound 6 (DMSO)



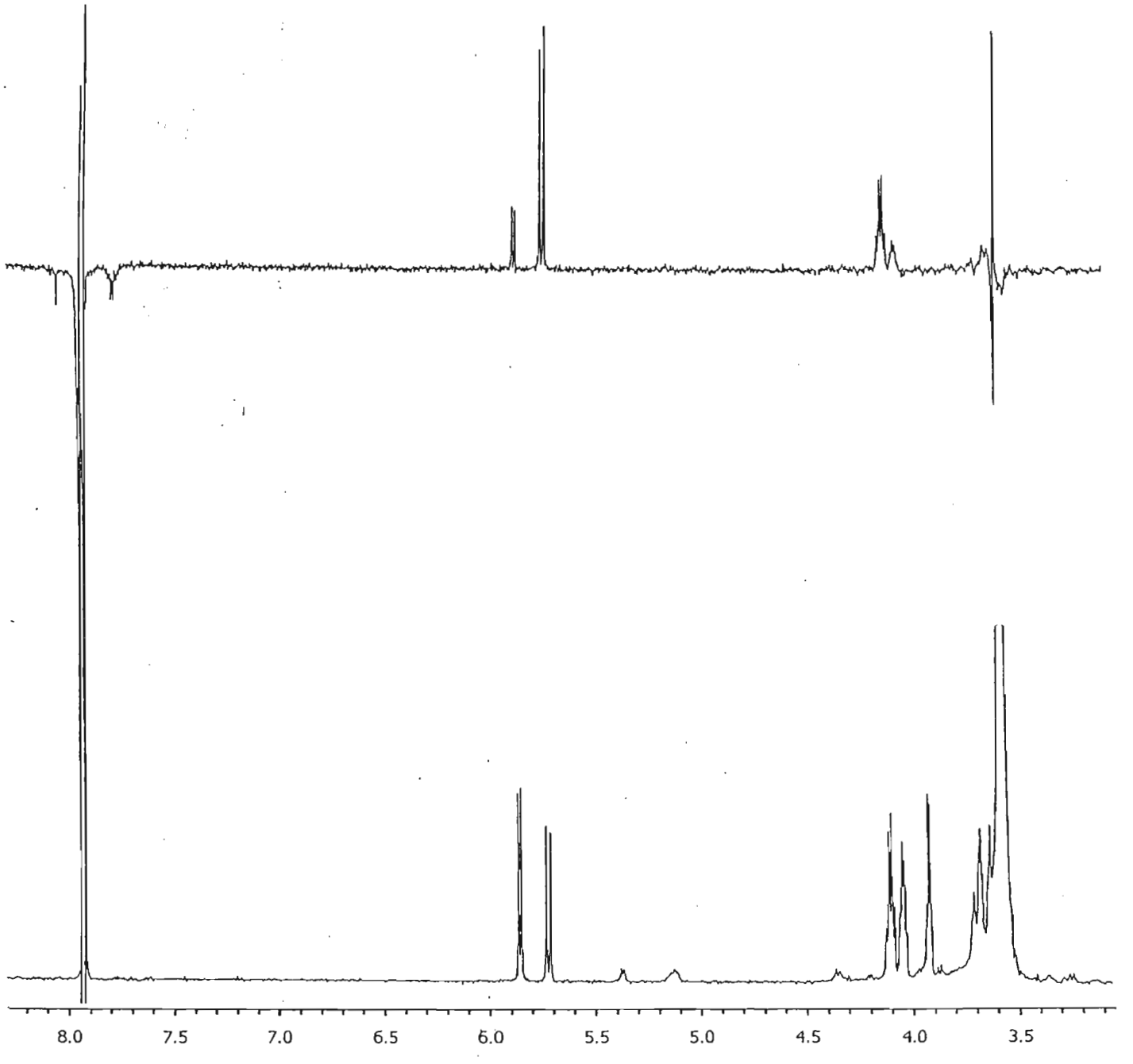
Spectrum 6.5: DEPT spectrum of compound 6 (DMSO)



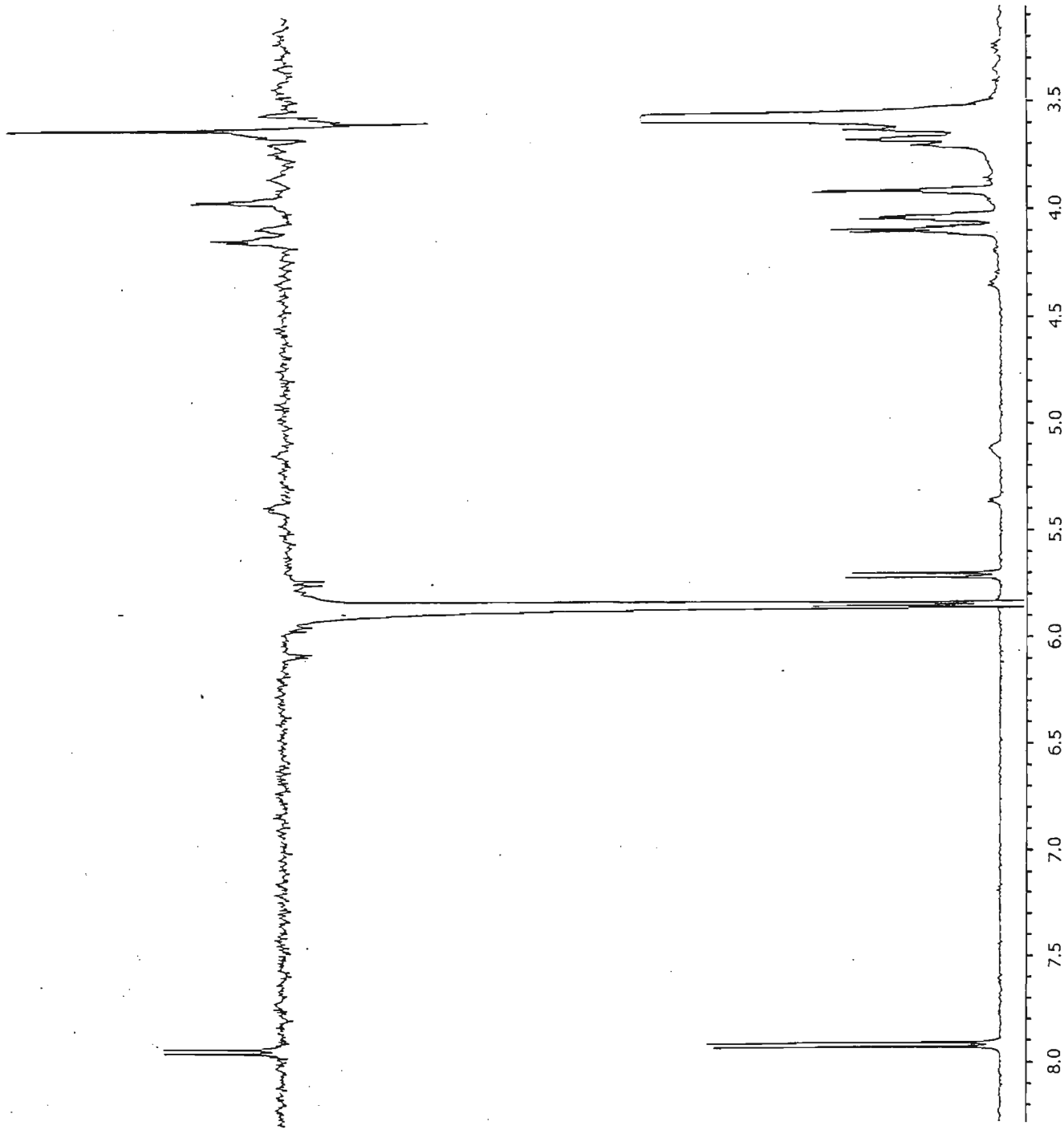
Spectrum 6.6: HSQC spectrum of compound 6



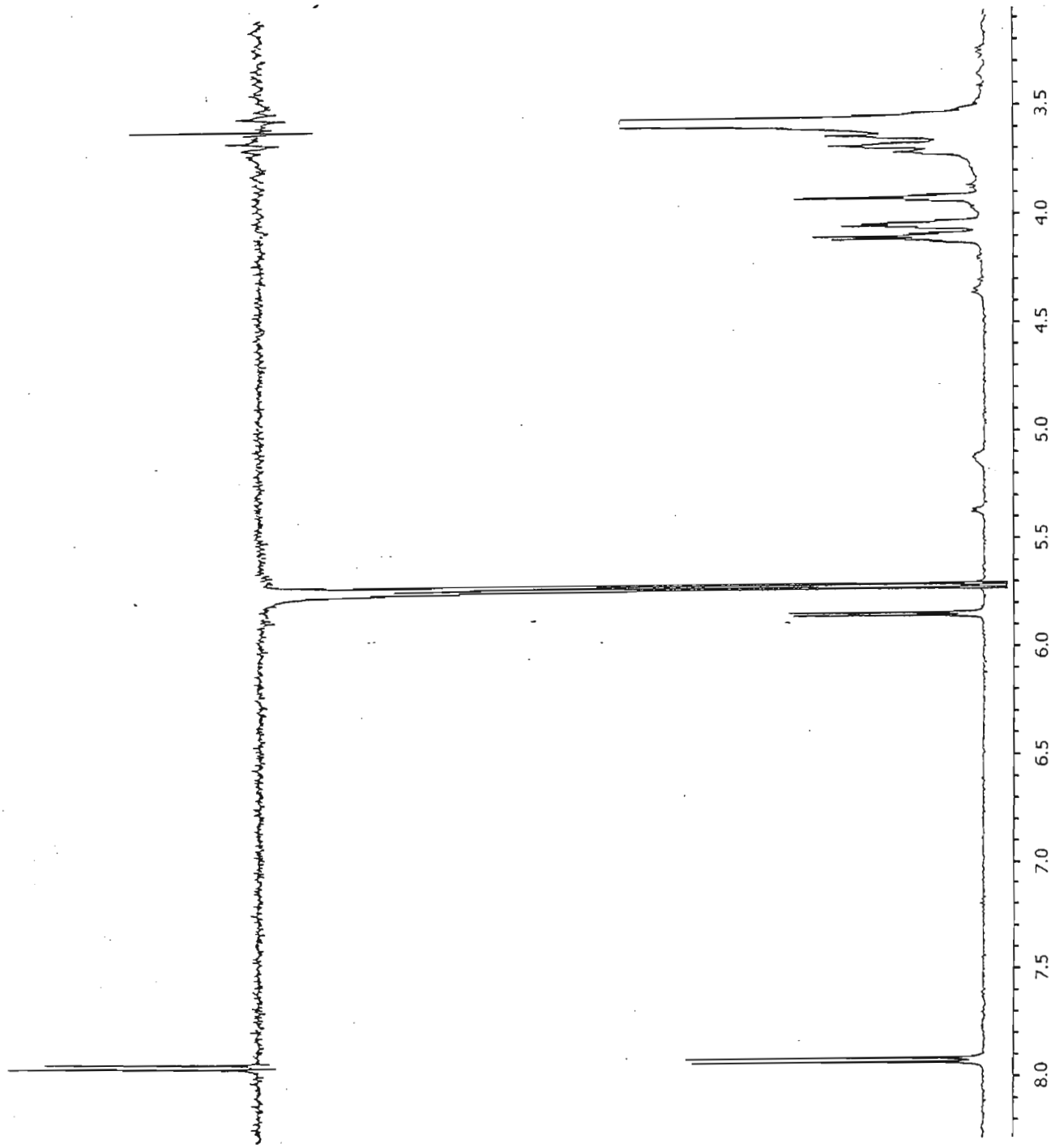
Spectrum 6.7: HMBC spectrum of compound 6



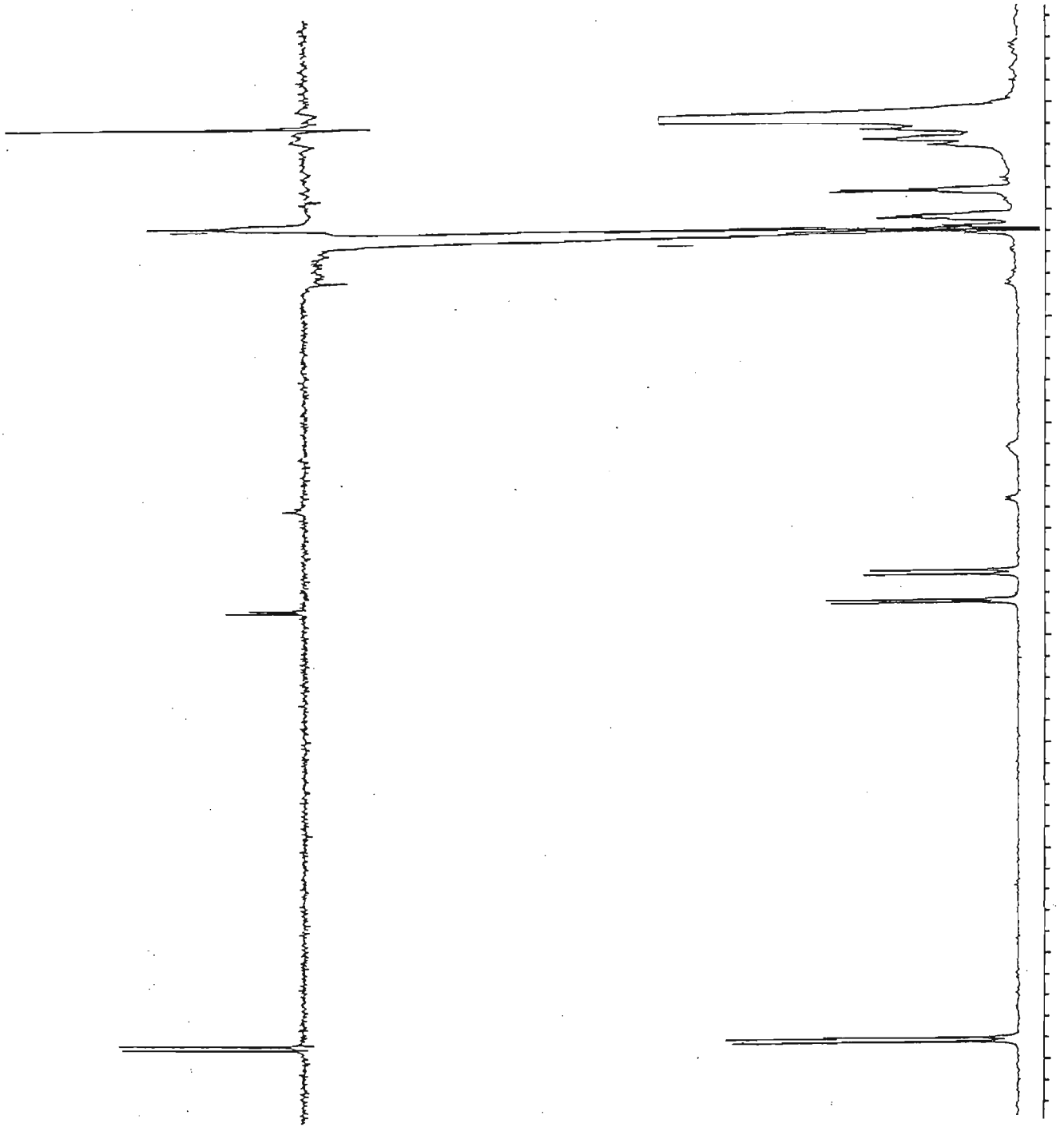
Spectrum 6.8: NOE spectrum of compound 6 (DMSO)



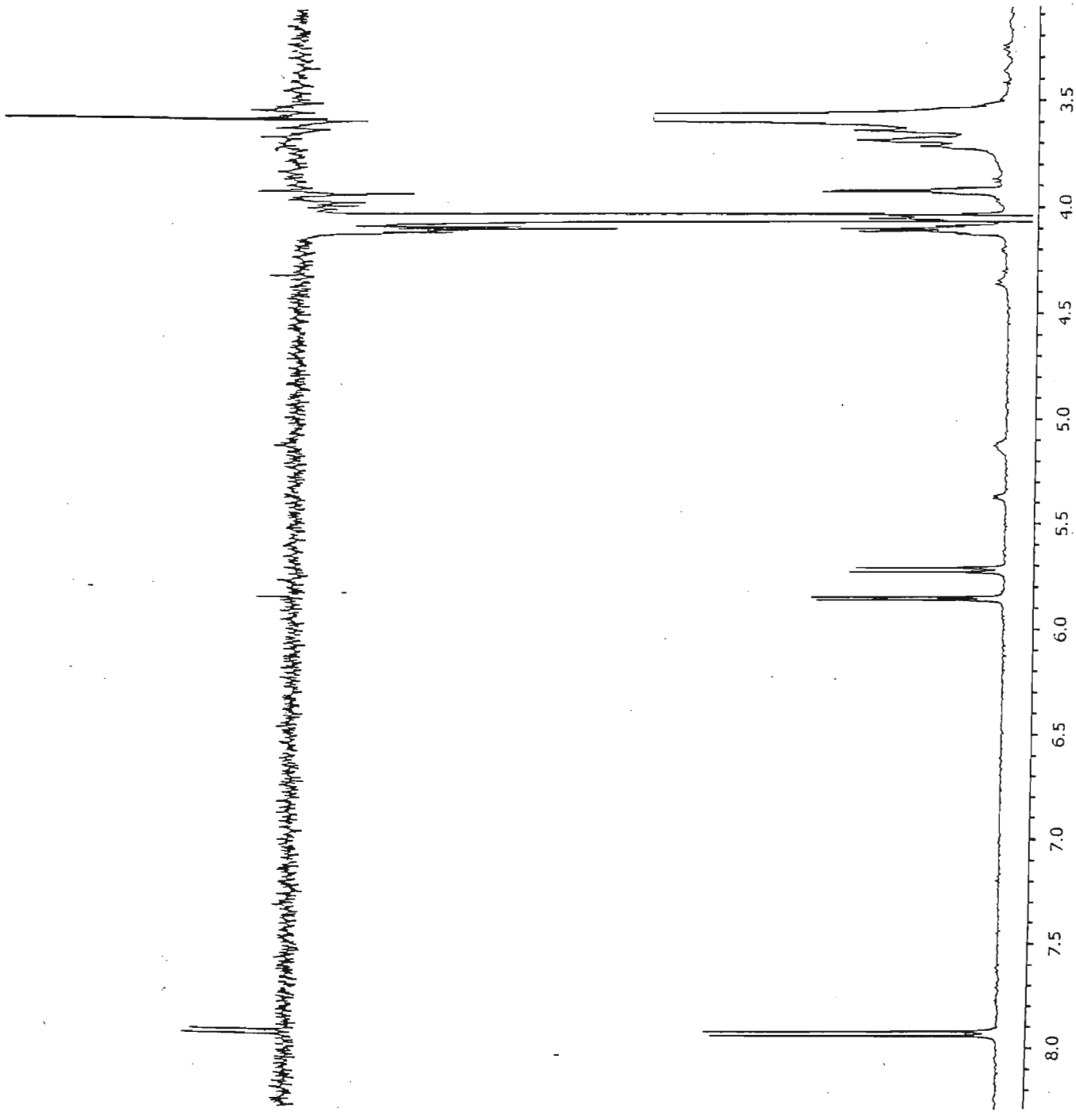
Spectrum 6.9: NOE spectrum of compound 6 (DMSO)



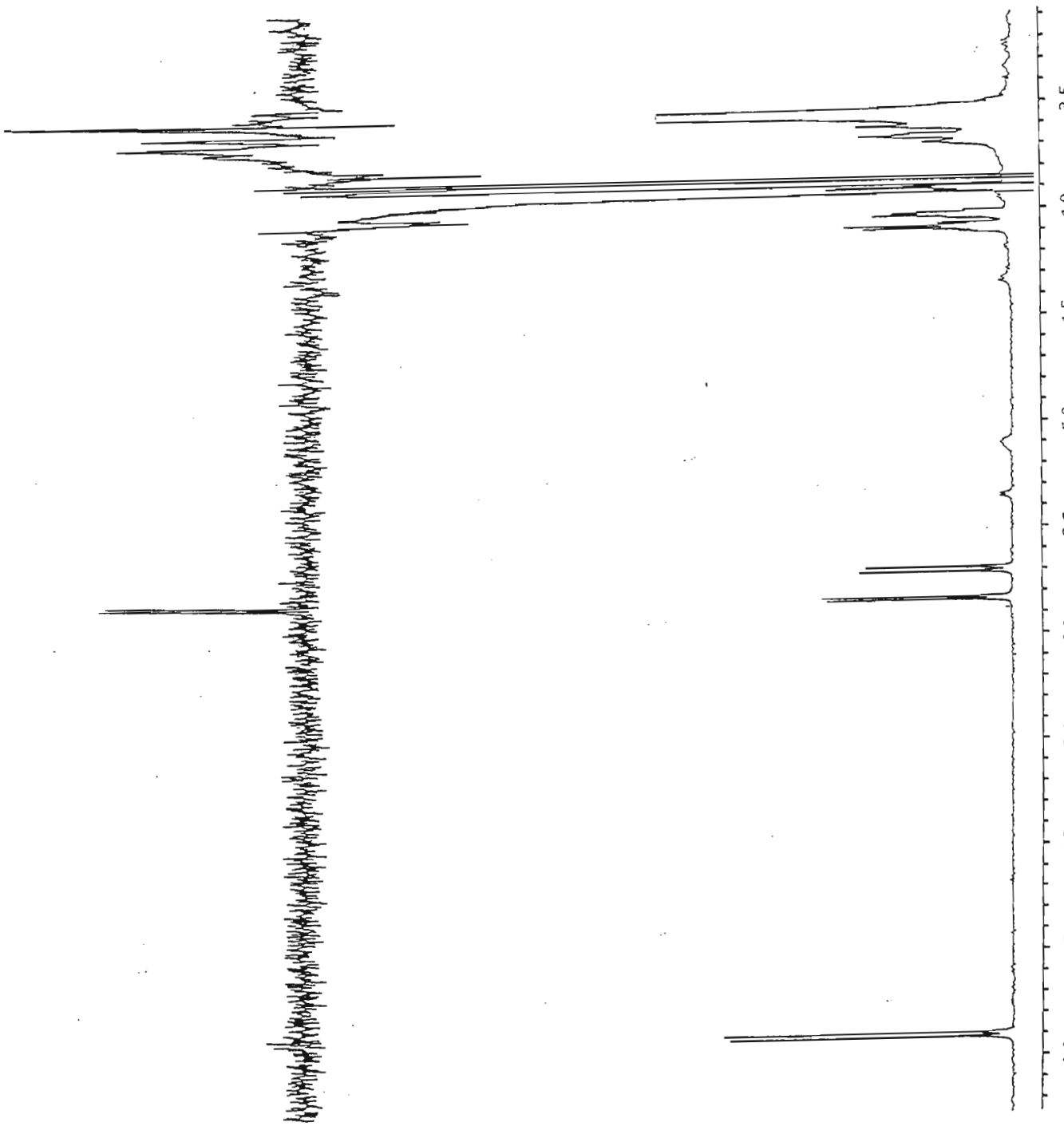
Spectrum 6.10: NOE spectrum of compound 6 (DMSO)



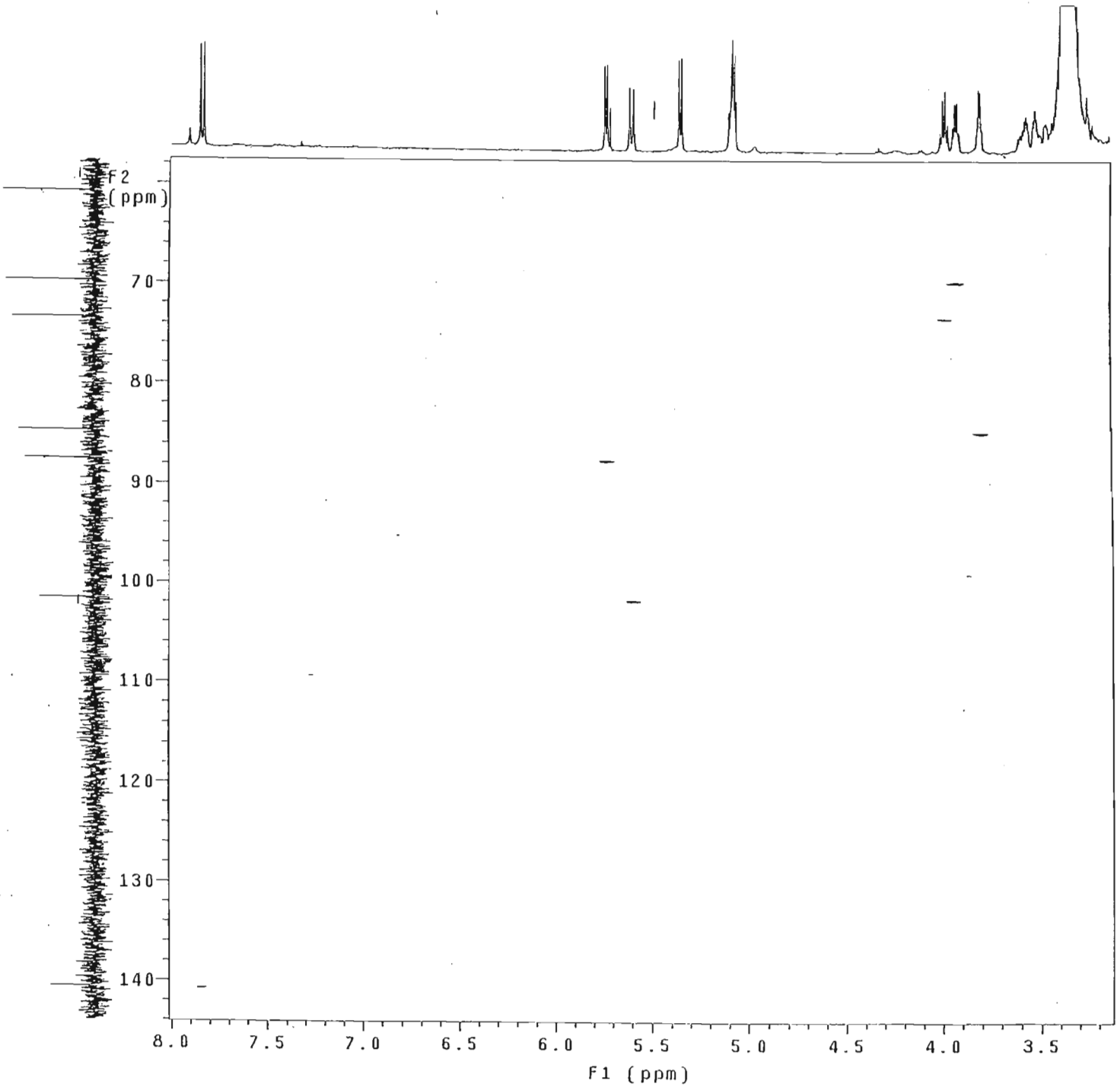
Spectrum 6.11: NOE spectrum of compound 6 (DMSO)



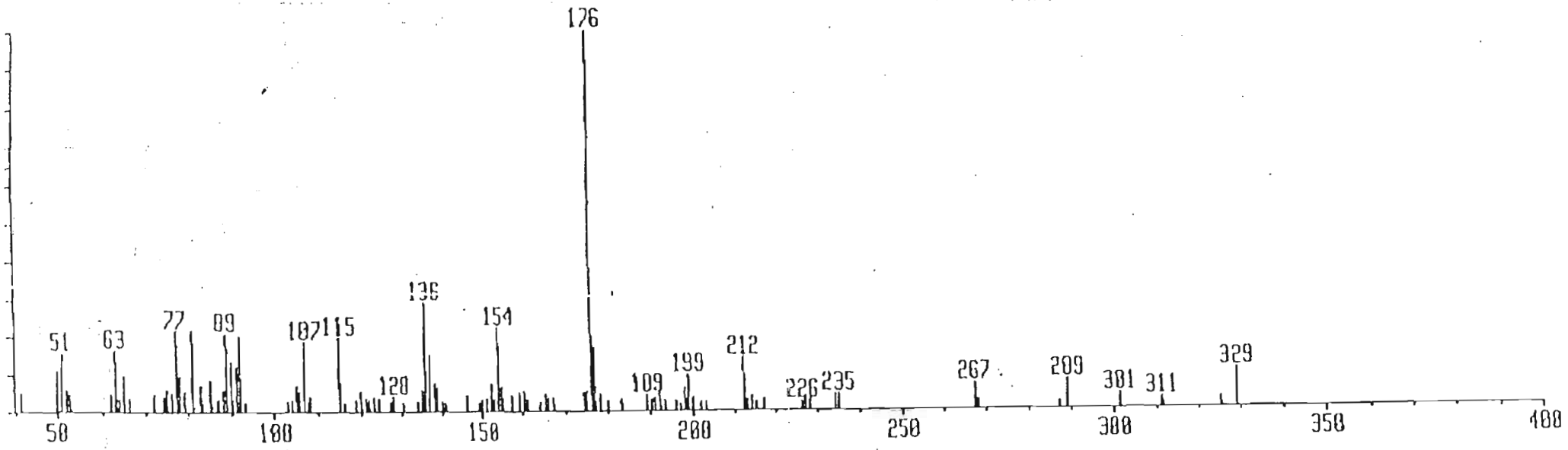
Spectrum 6.12: NOE spectrum of compound 6 (DMSO)



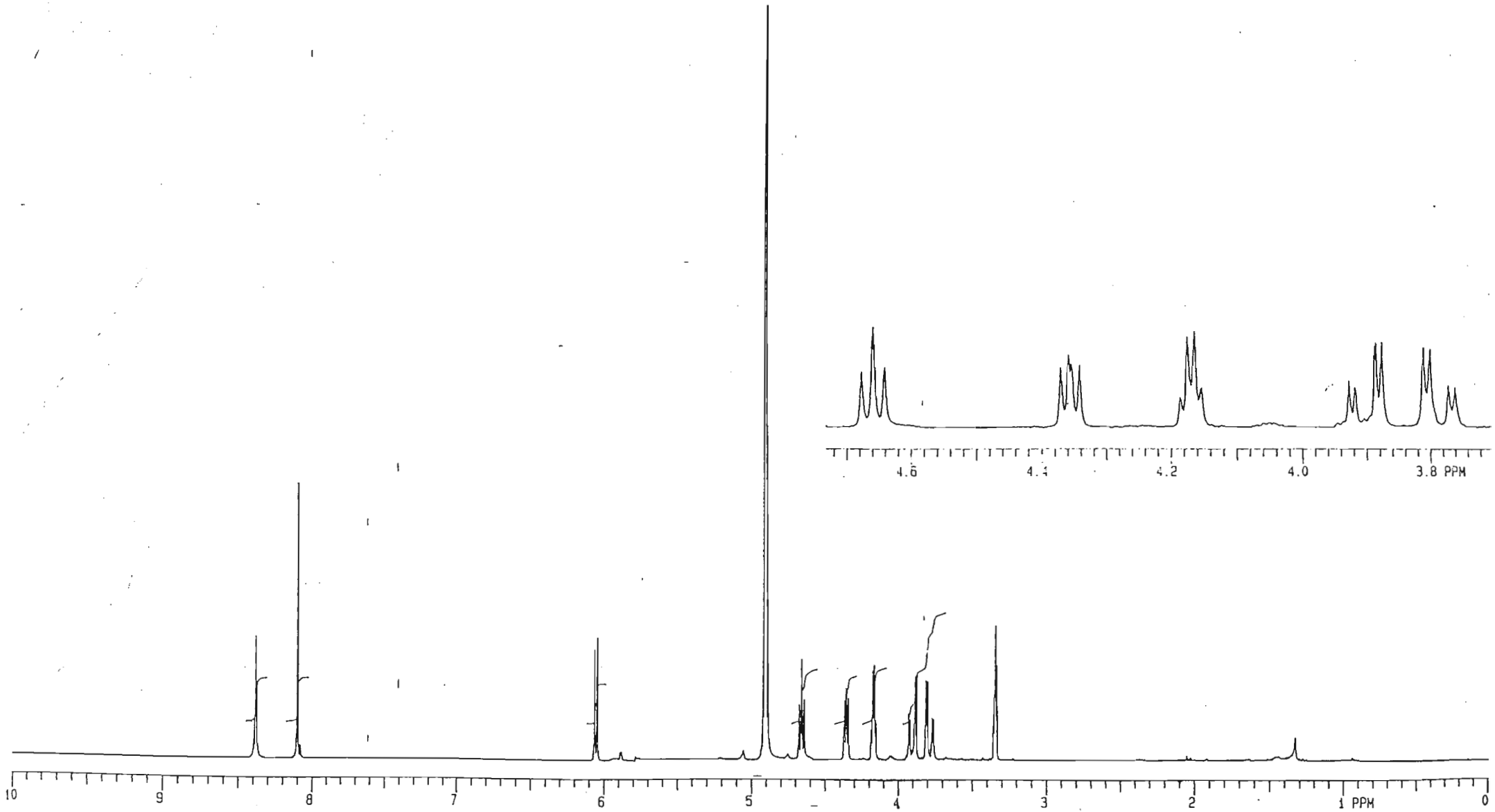
Spectrum 6.13: NOE spectrum of compound 6 (DMSO)



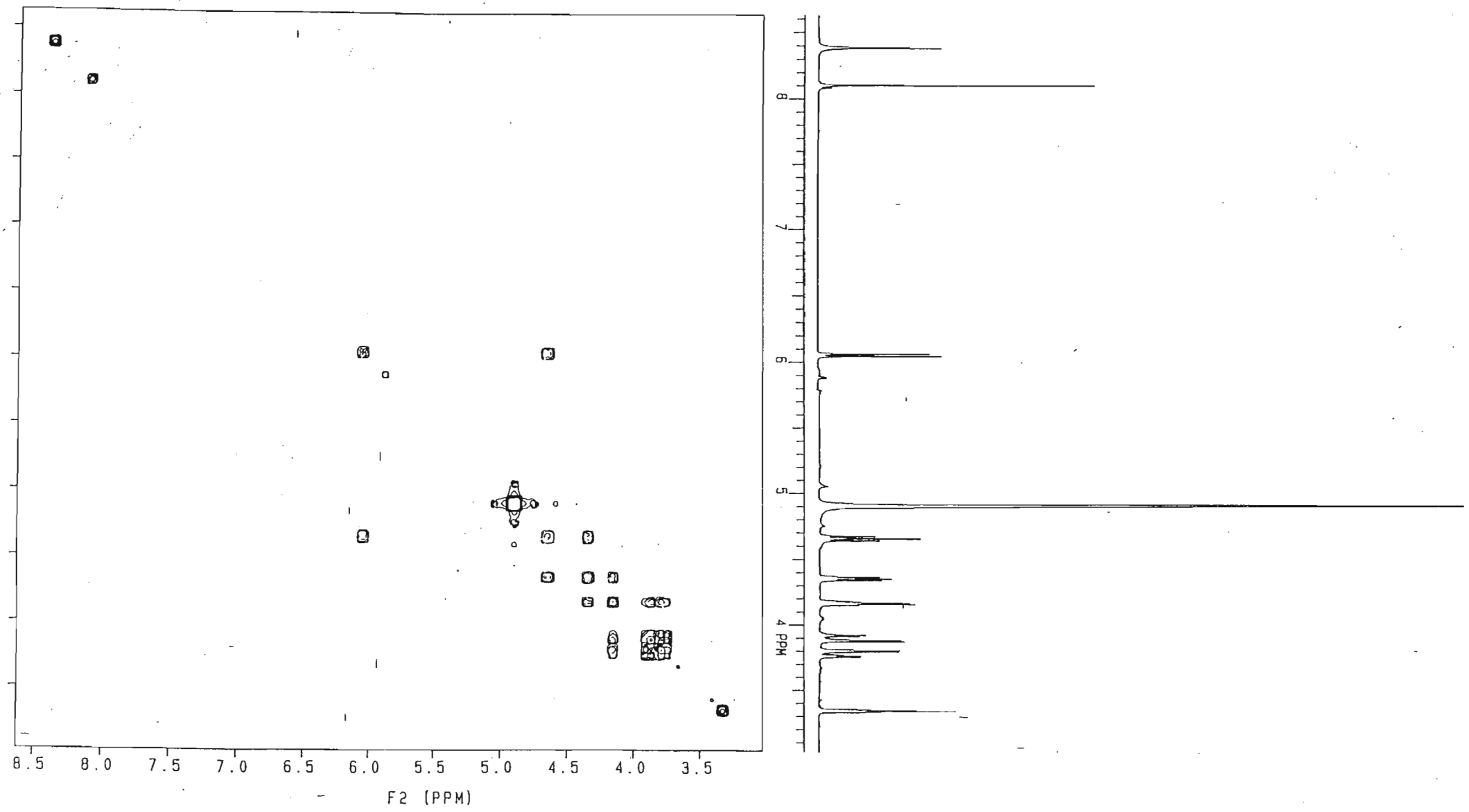
Spectrum 6.14: HETCOR spectrum of [illegible]



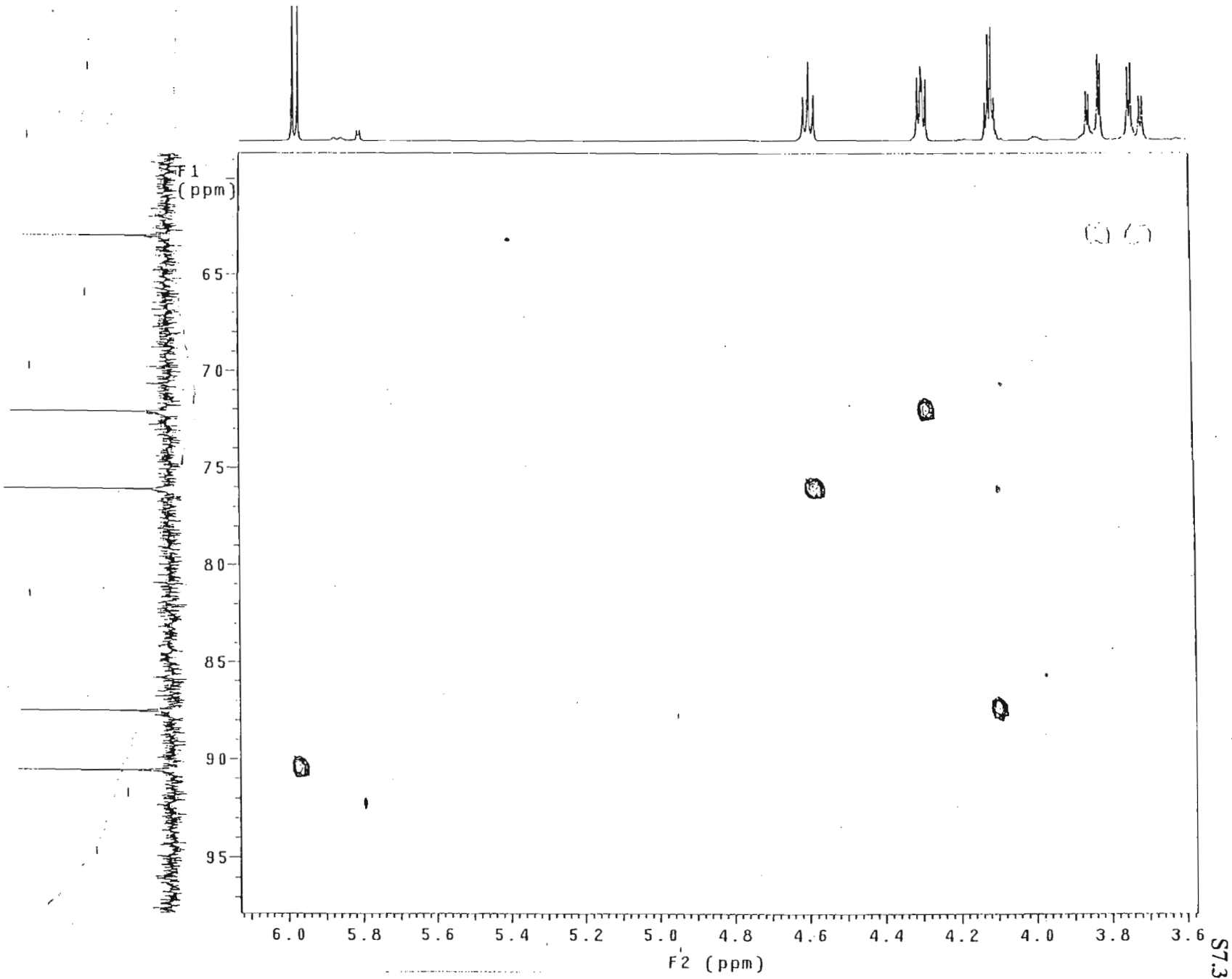
Spectrum 6.15: Mass spectrum of compound 6



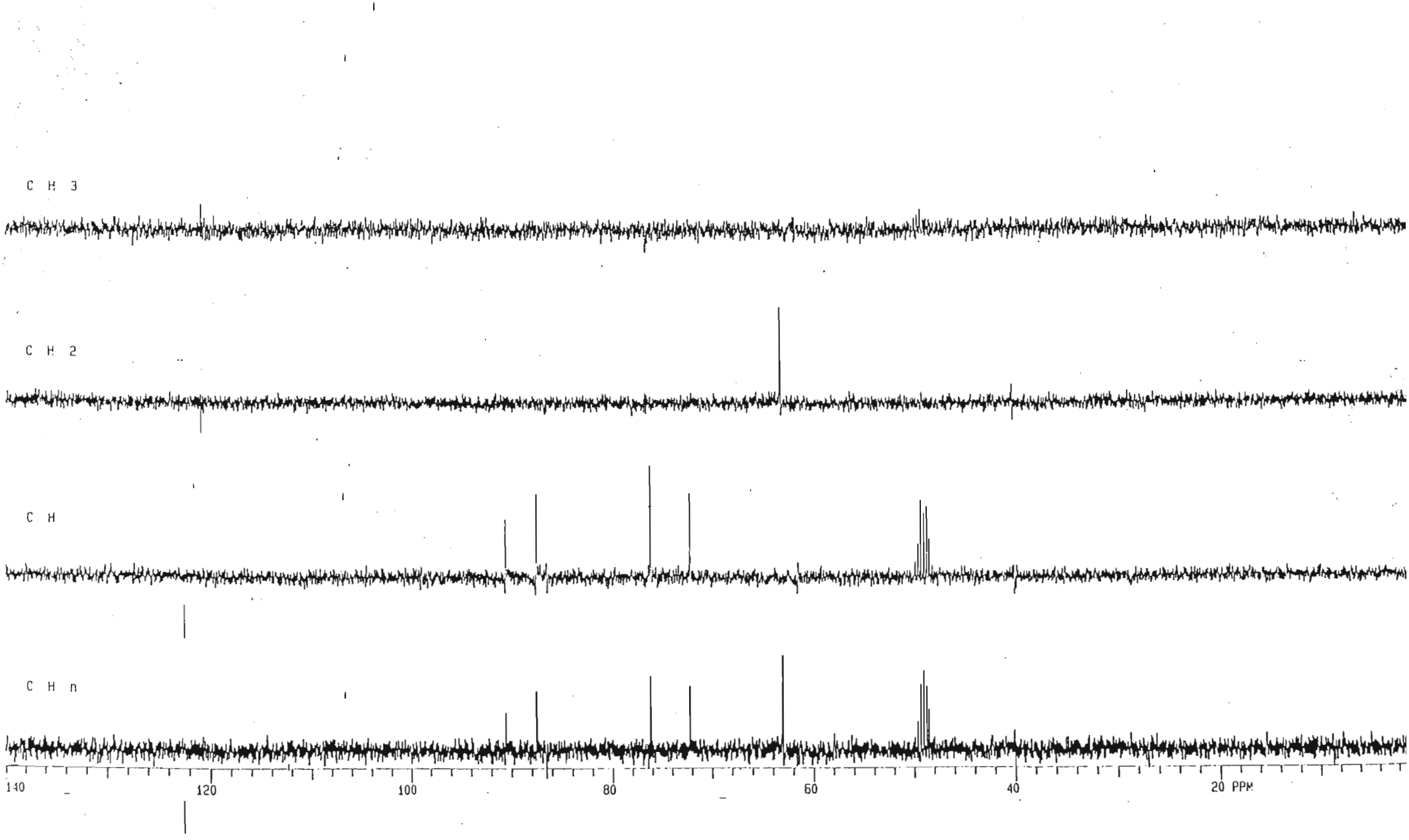
Spectrum 7.1: ^{1}H NMR spectrum of compound 7 (CD_3OD)



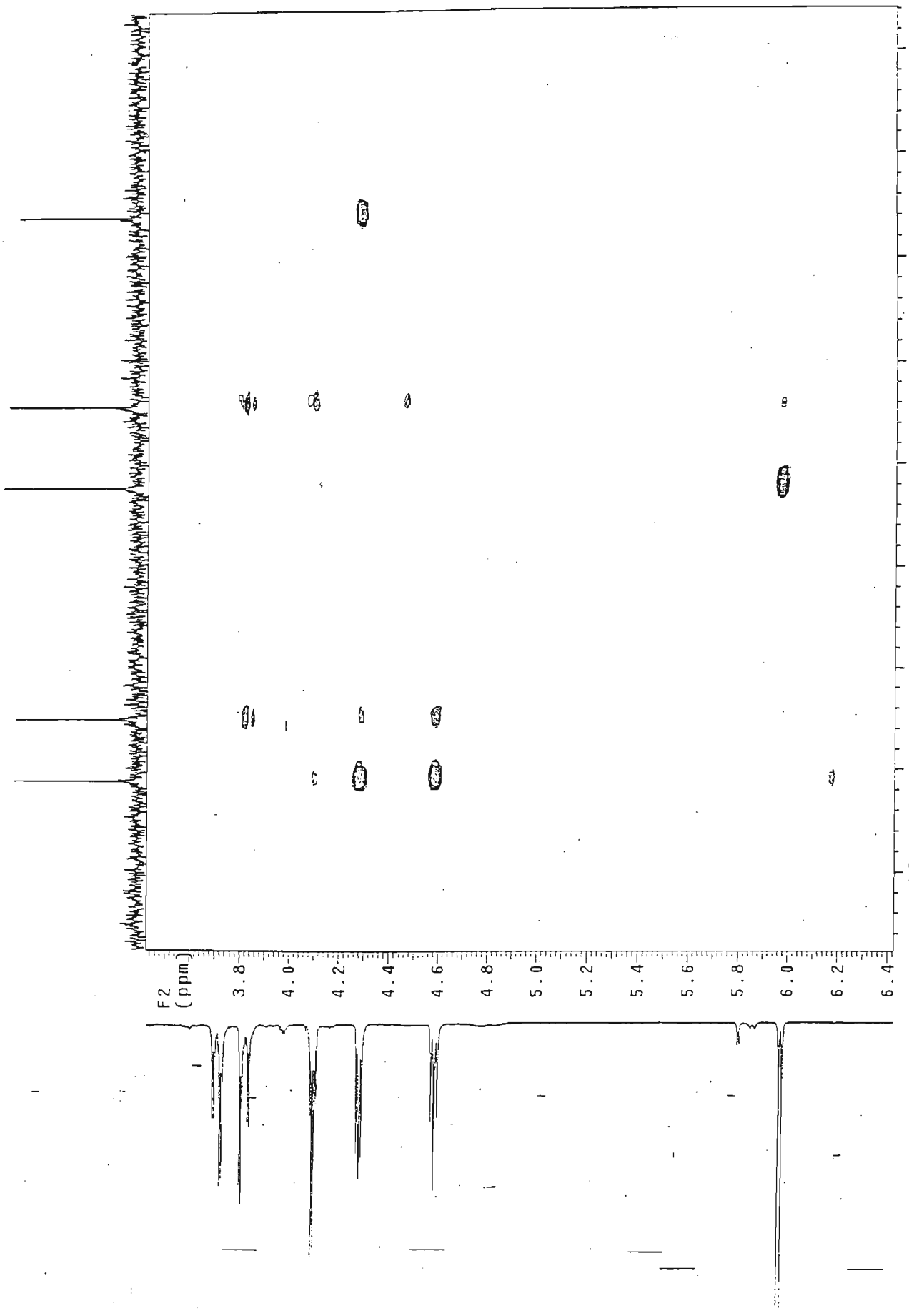
Spectrum 7.2: COSY spectrum of compound 7 (CD_3OD)



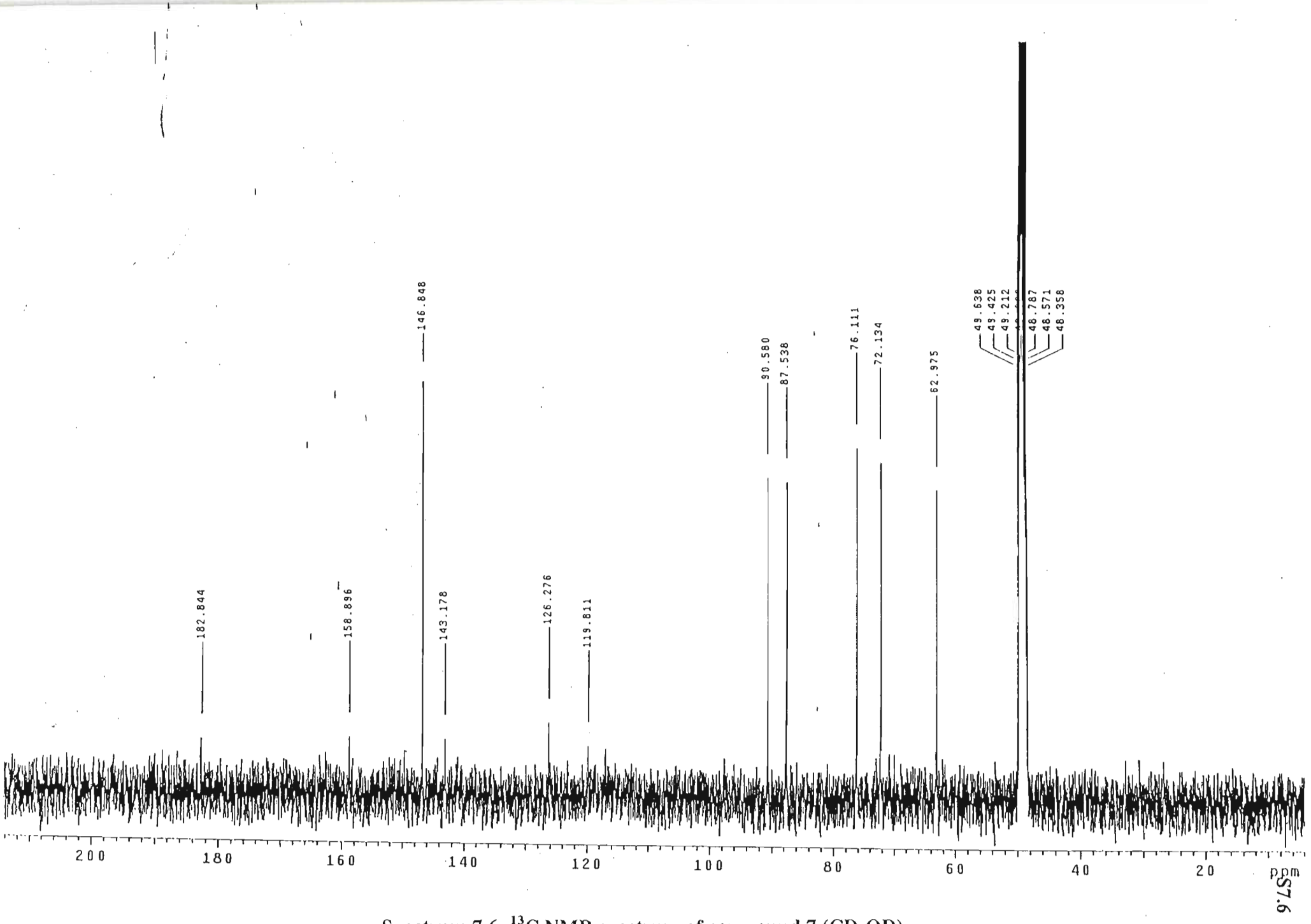
Spectrum 7.3: HSQC spectrum of compound 7 (CD_3OD)



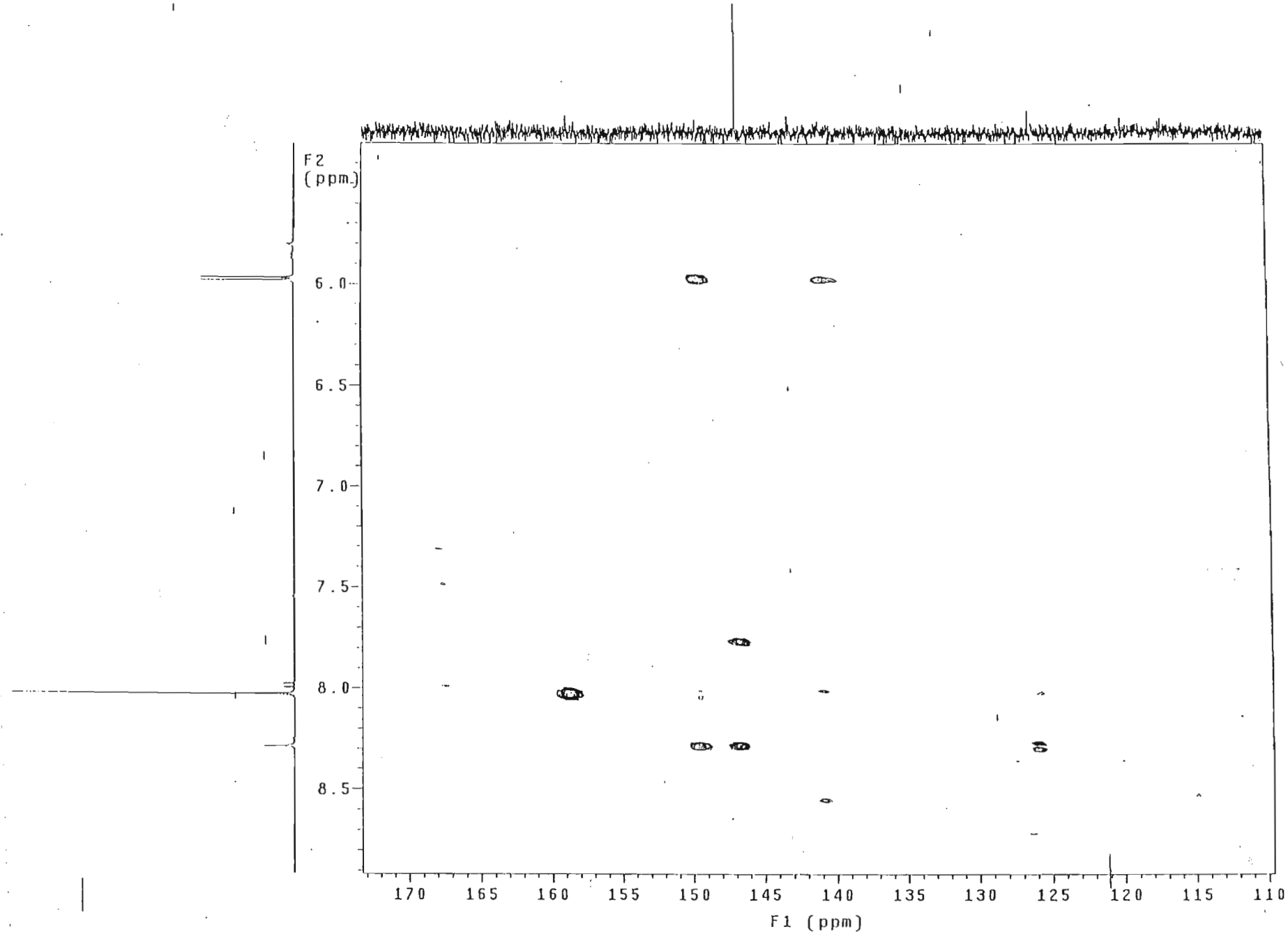
Spectrum 7.4: DEPT spectrum of compound 7 (CD_3OD)



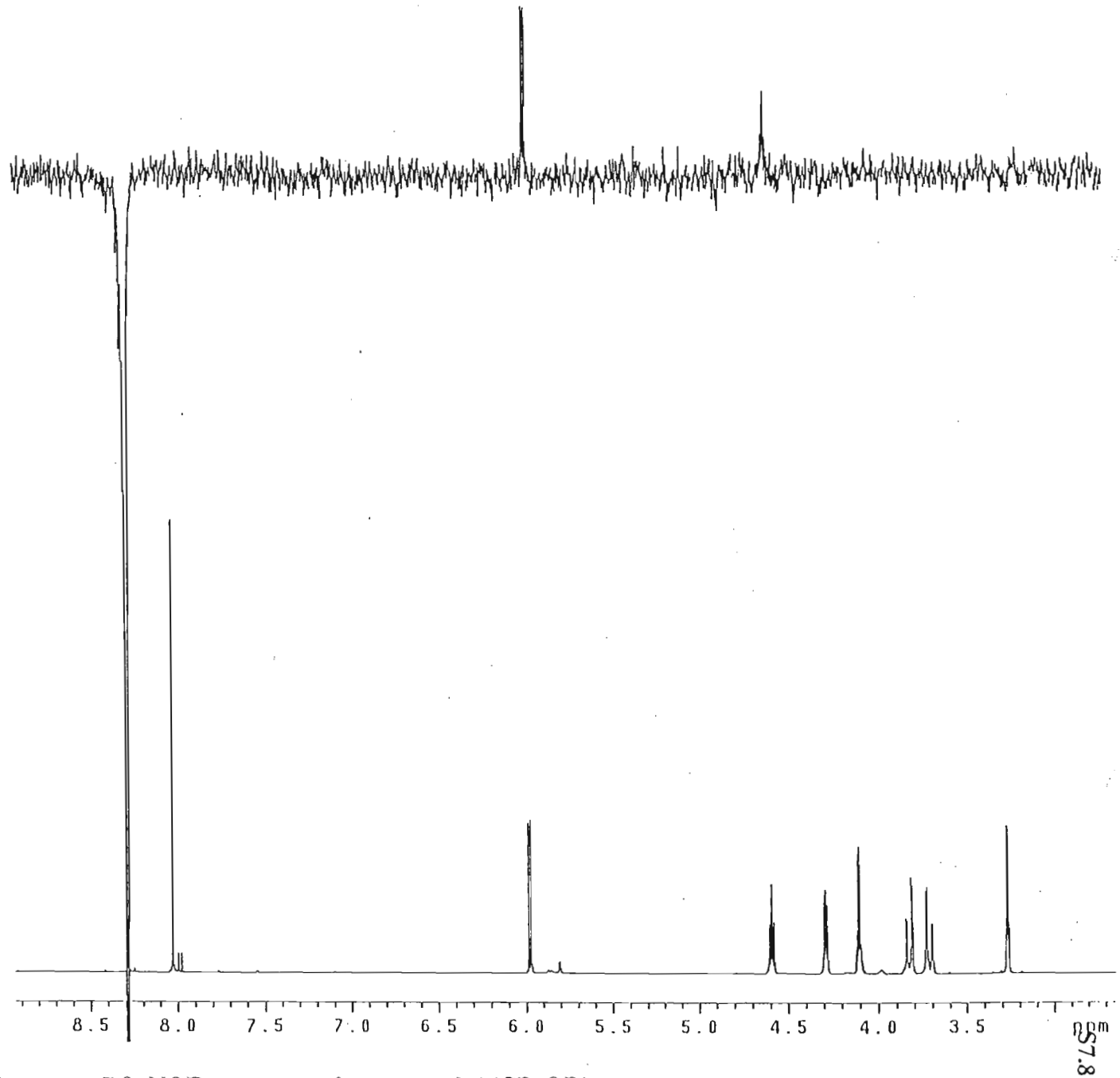
Spectrum 7.5: HMBC spectrum of compound 7 (CD_3OD)



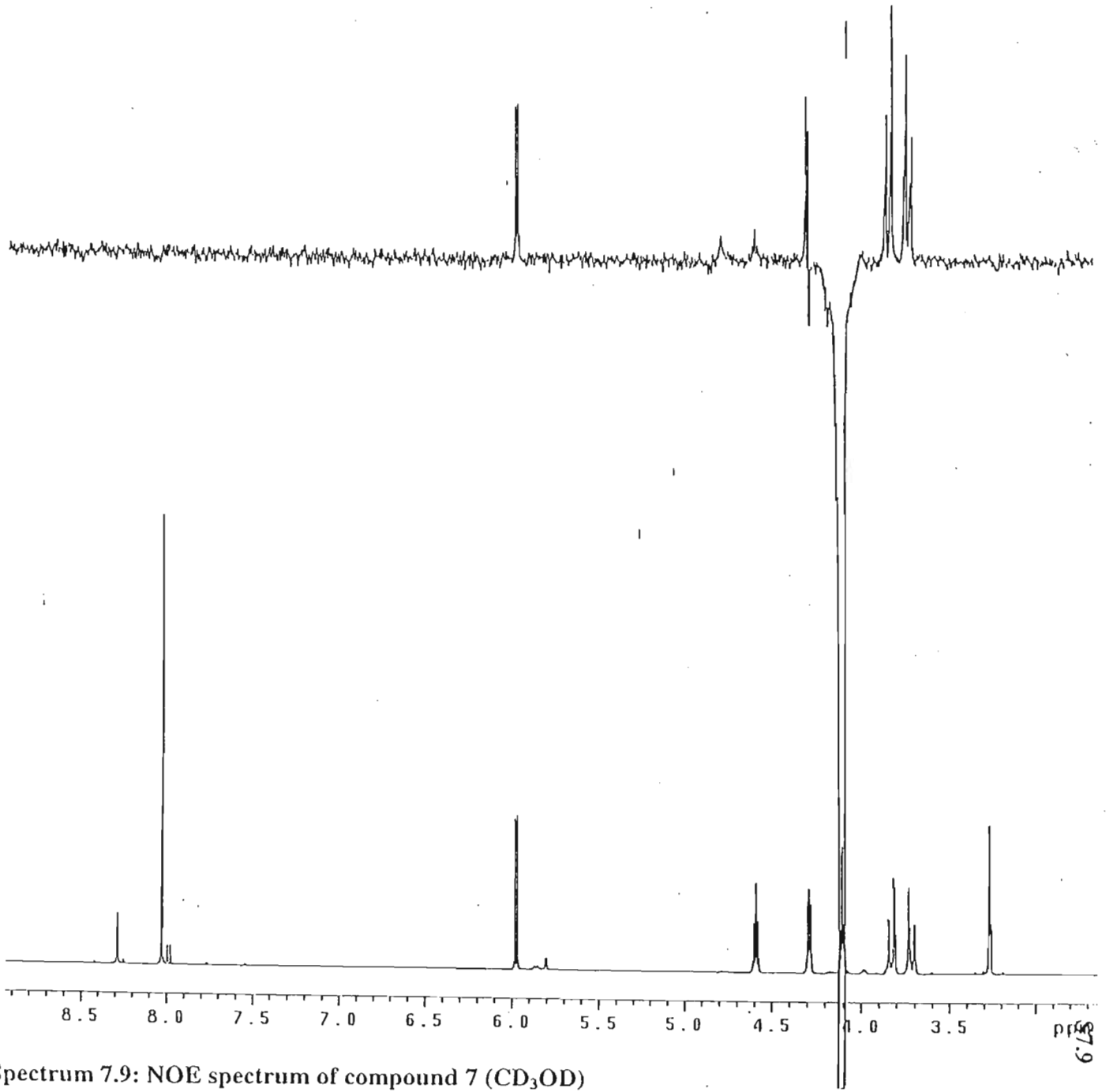
Spectrum 7.6: ^{13}C NMR spectrum of compound 7 (CD_3OD)



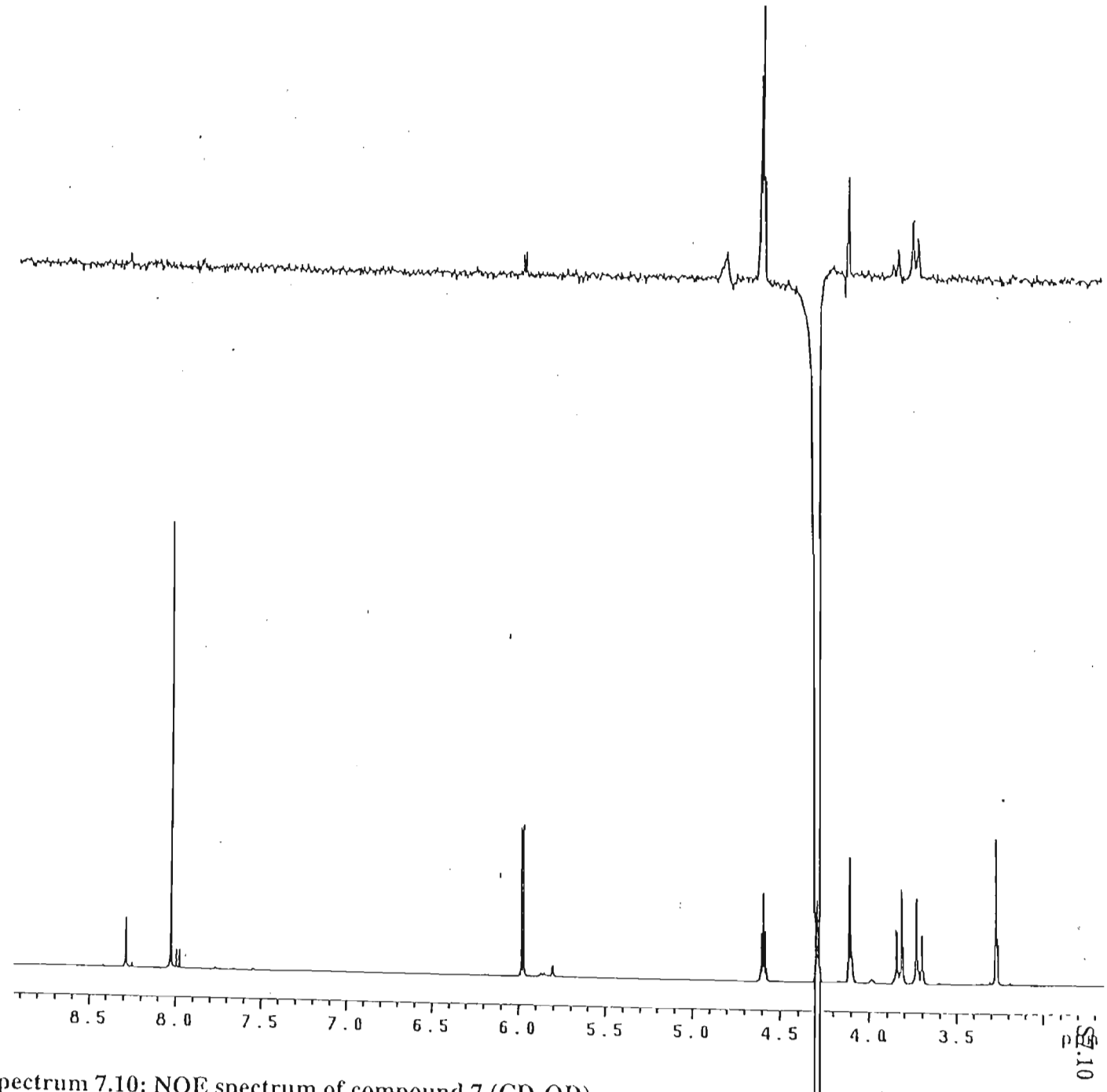
Spectrum 7.7: HMBC spectrum of compound 7 (CD_3OD)



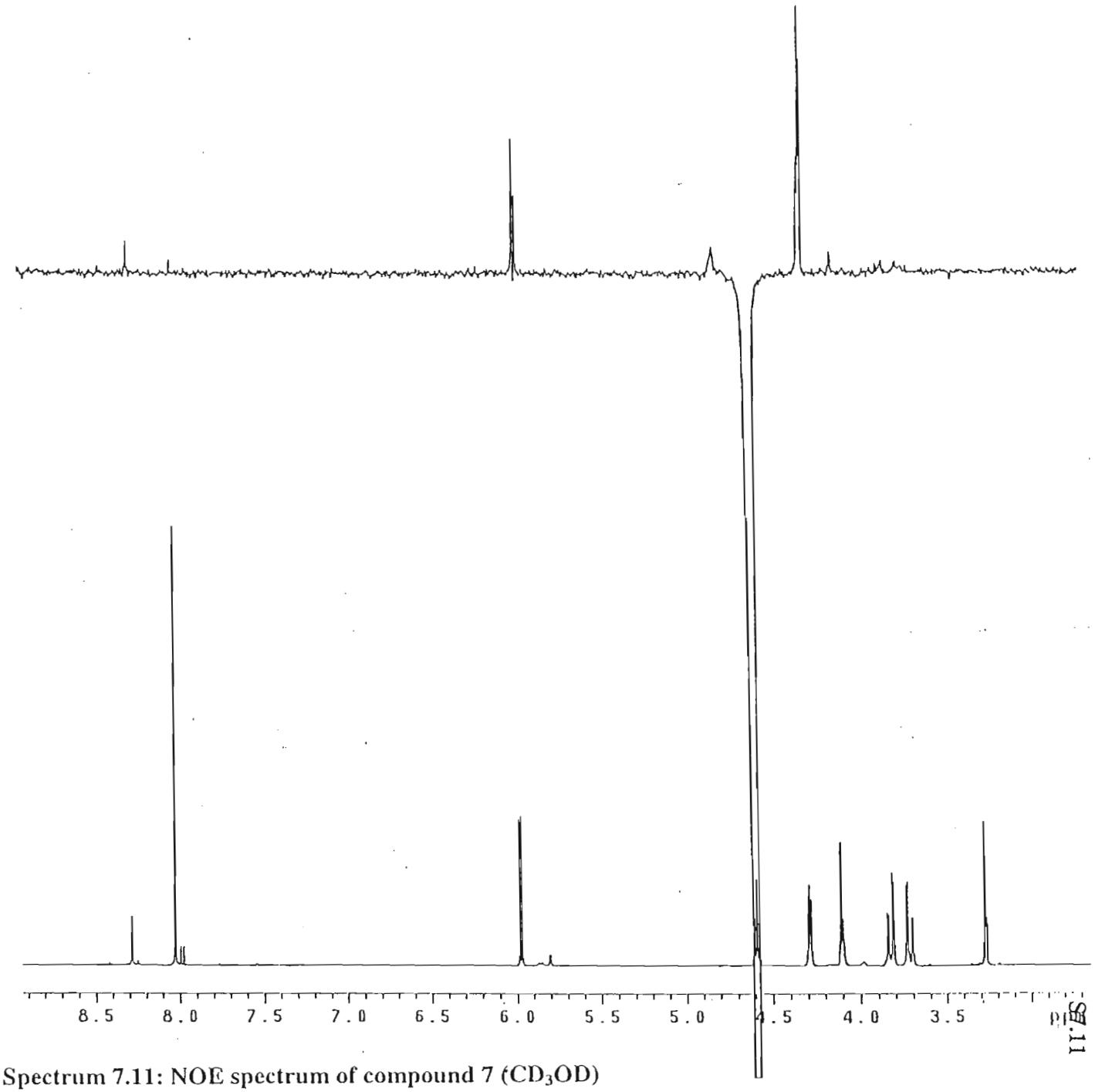
Spectrum 7.8: NOE spectrum of compound 7 (CD_3OD)



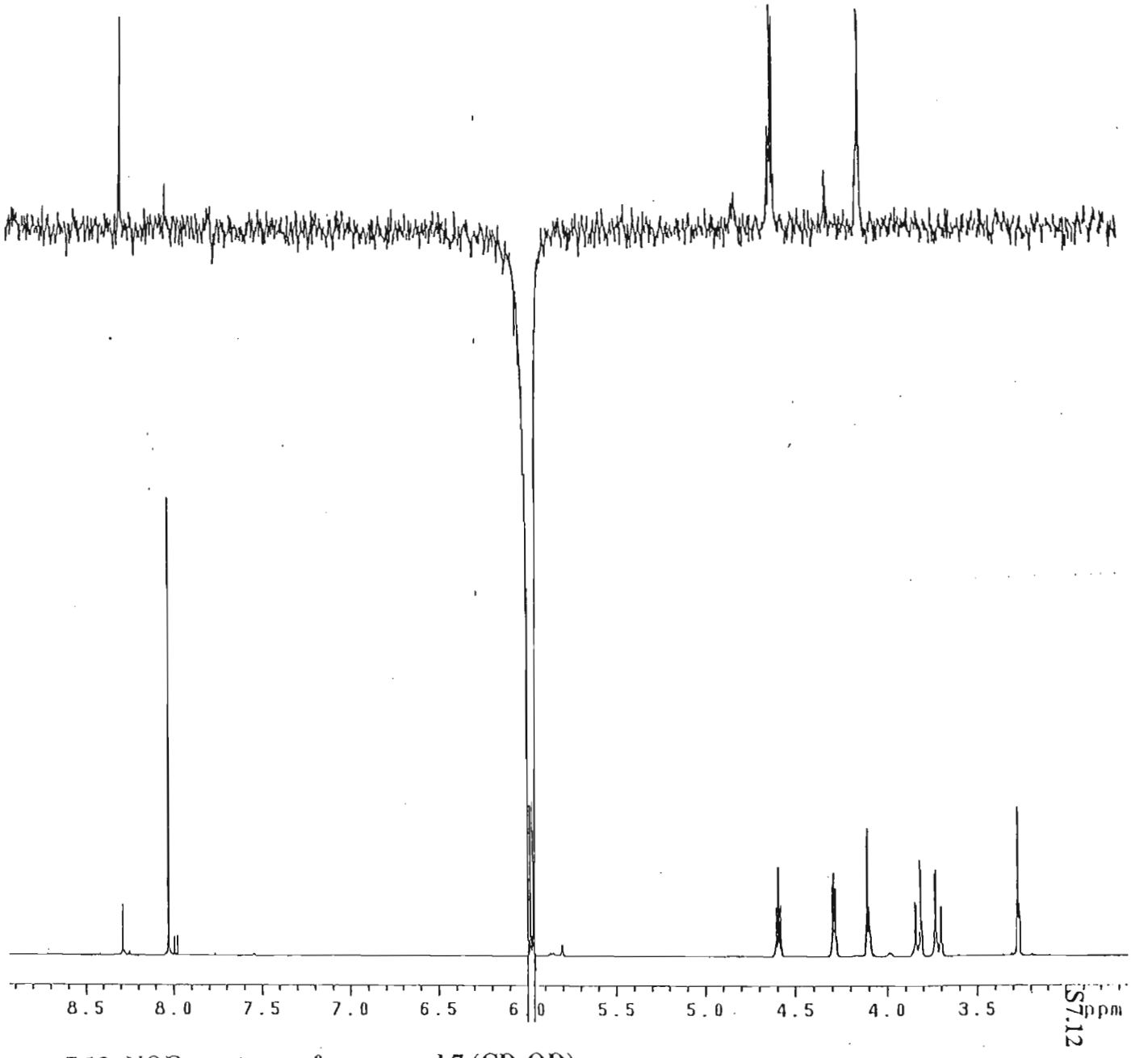
Spectrum 7.9: NOE spectrum of compound 7 (CD_3OD)



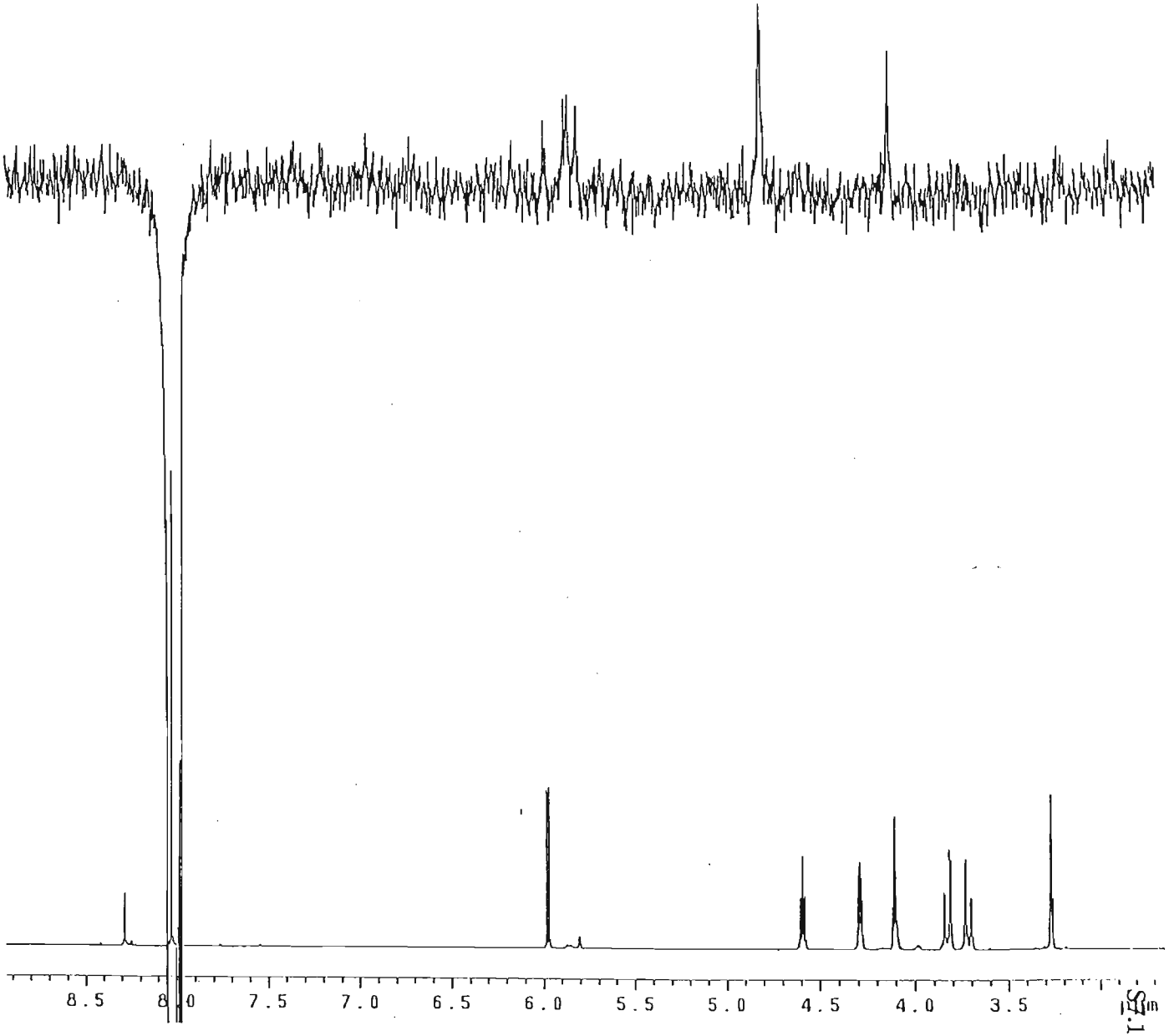
Spectrum 7.10: NOE spectrum of compound 7 (CD_3OD)



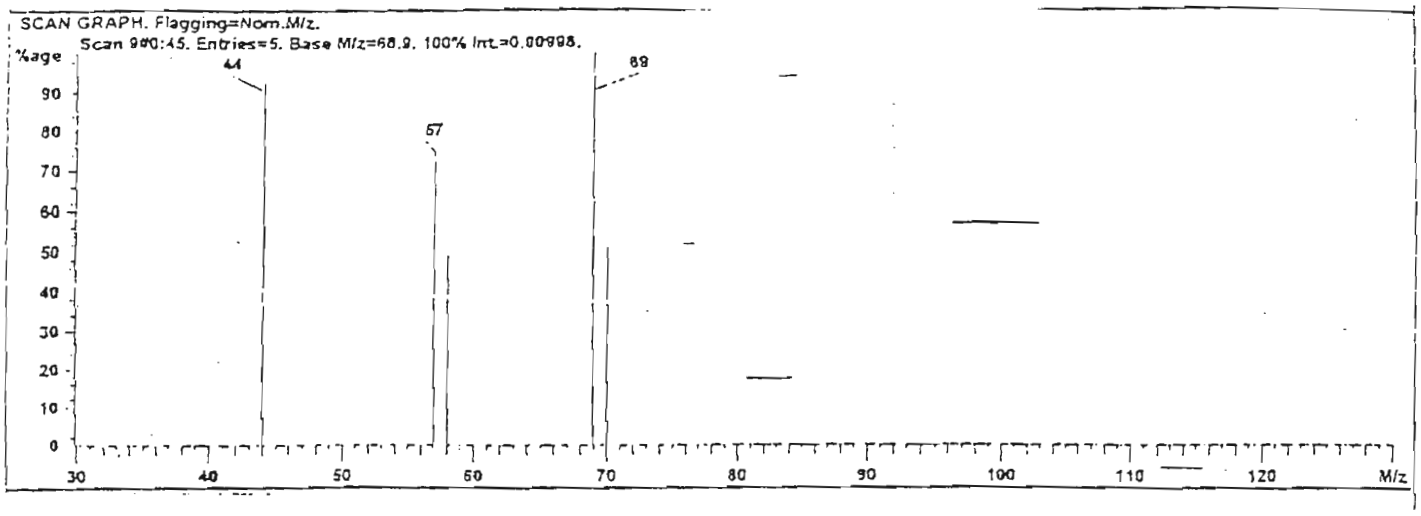
Spectrum 7.11: NOE spectrum of compound 7 (CD_3OD)

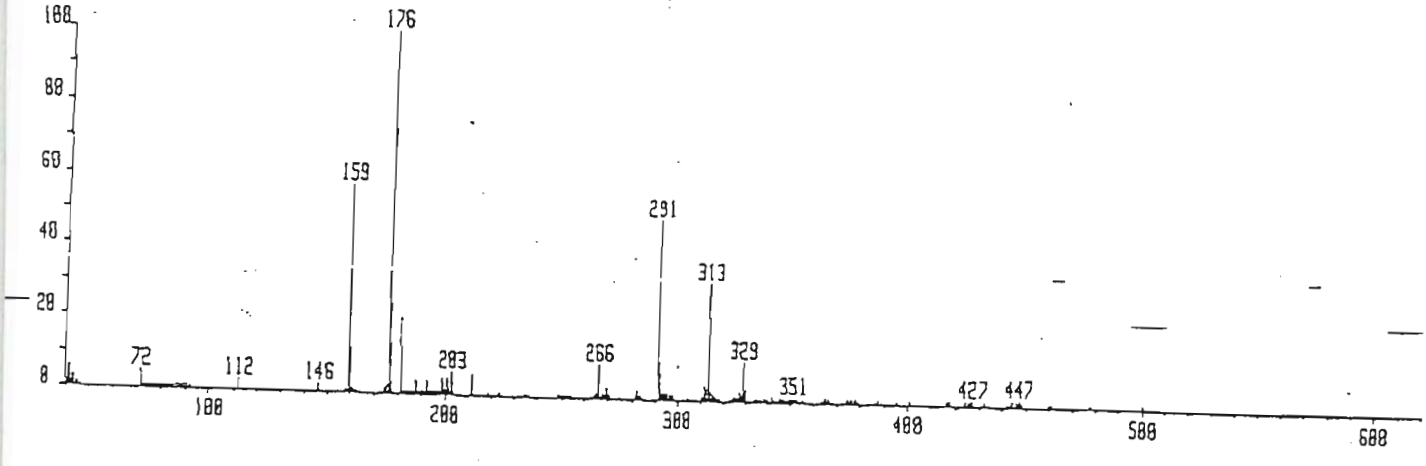


Spectrum 7.12: NOE spectrum of compound 7 (CD_3OD)

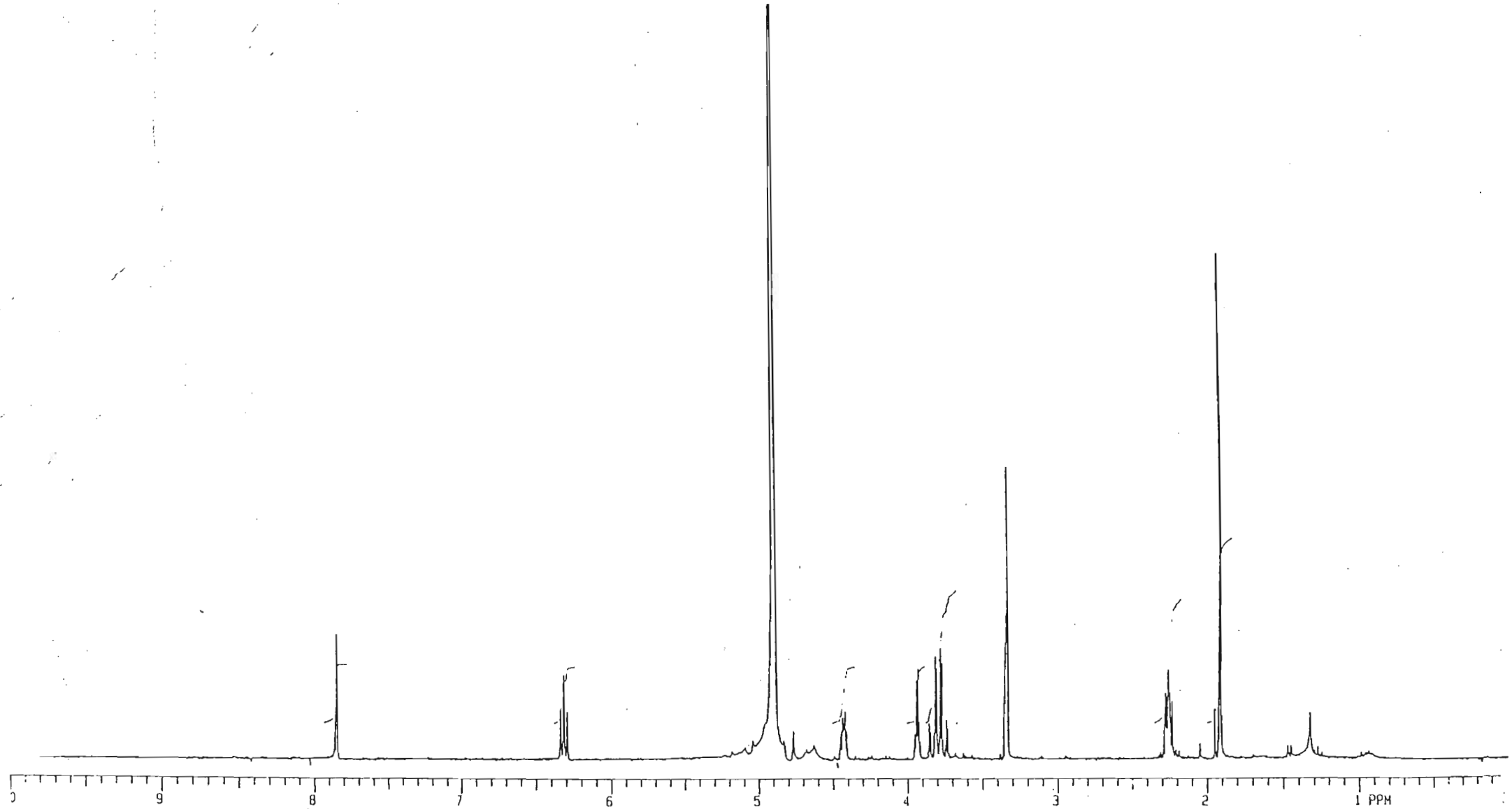


Spectrum 7.13: NOE spectrum of compound 7 (CD_3OD)

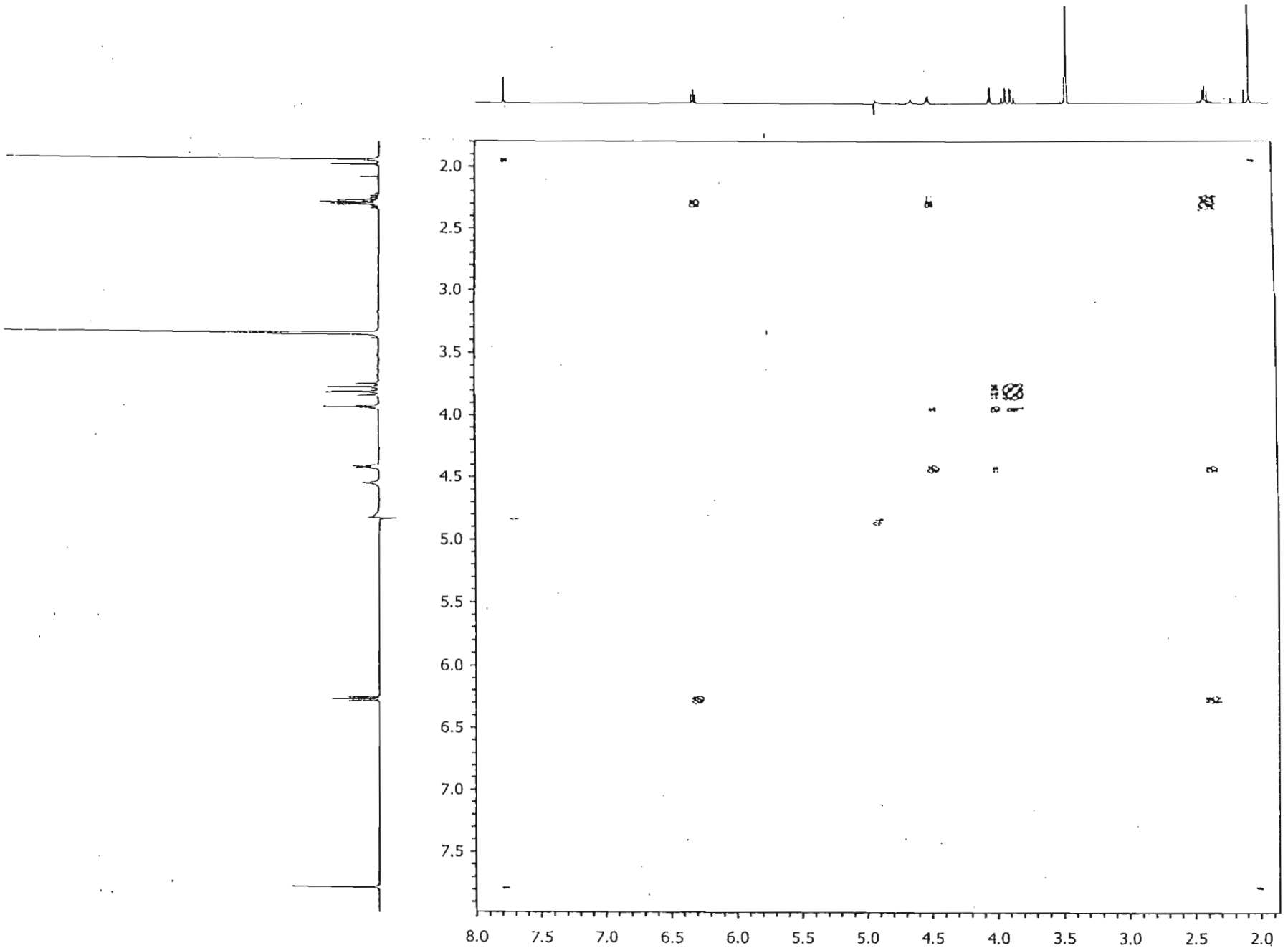




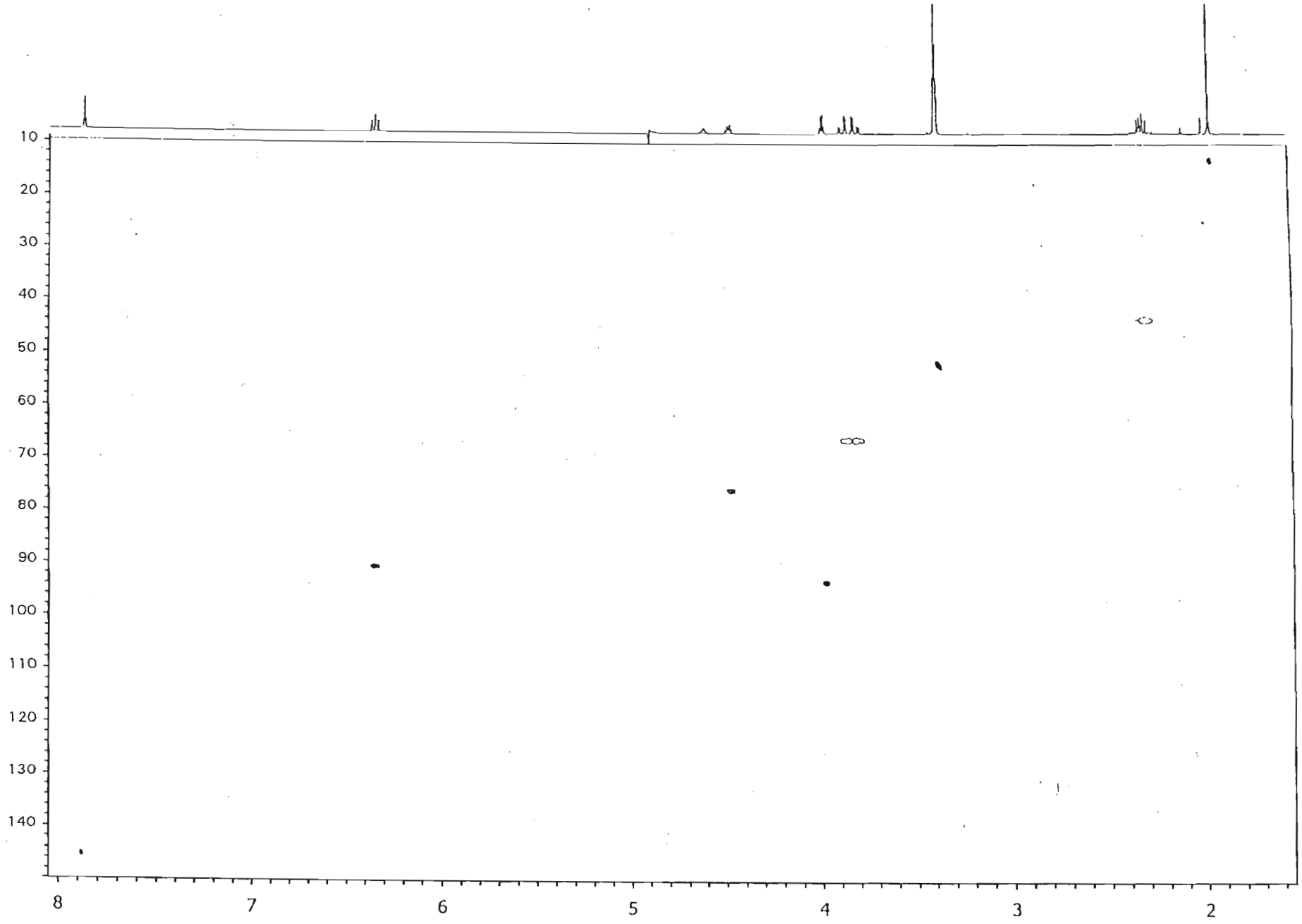
Spectrum 7.14: Mass spectrum of compound 7 (CD_3OD)



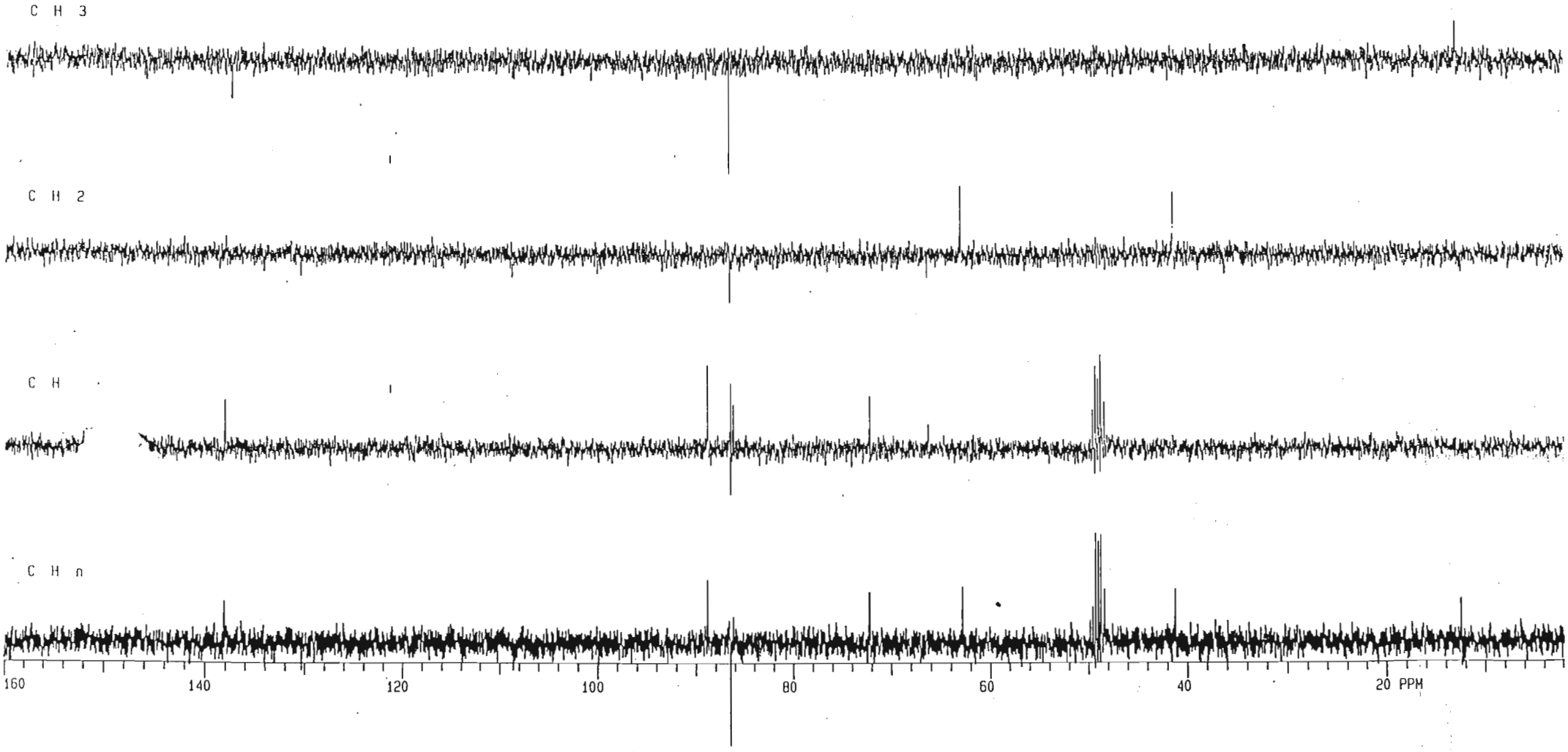
Spectrum 8.1: ^{1}H NMR spectrum of compound 8 (CD_3OD)



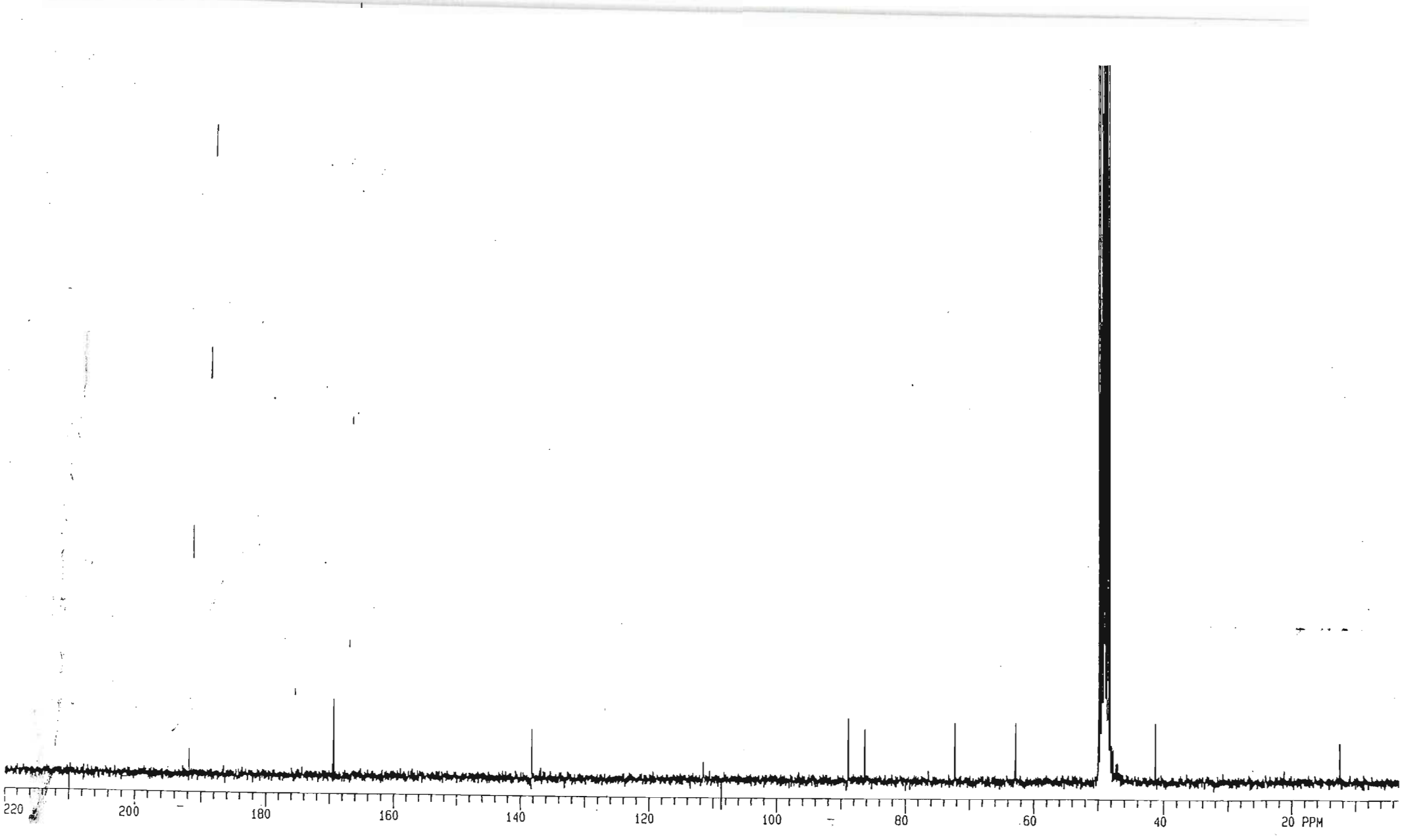
Spectrum 8.2: COSY spectrum of compound 8 (CD_3OD)



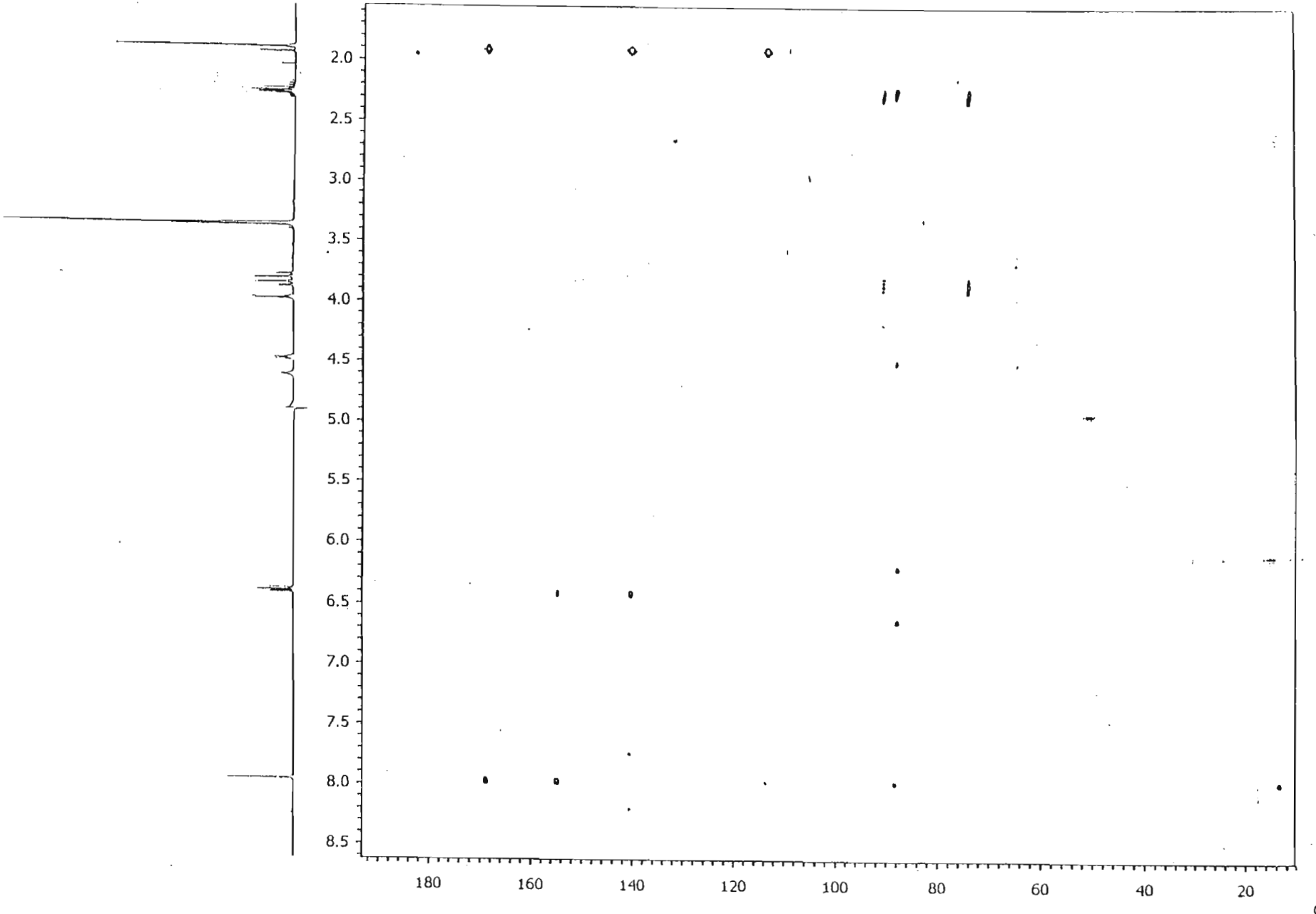
Spectrum 8.3: HSQC spectrum of compound 8 (CD_3OD)



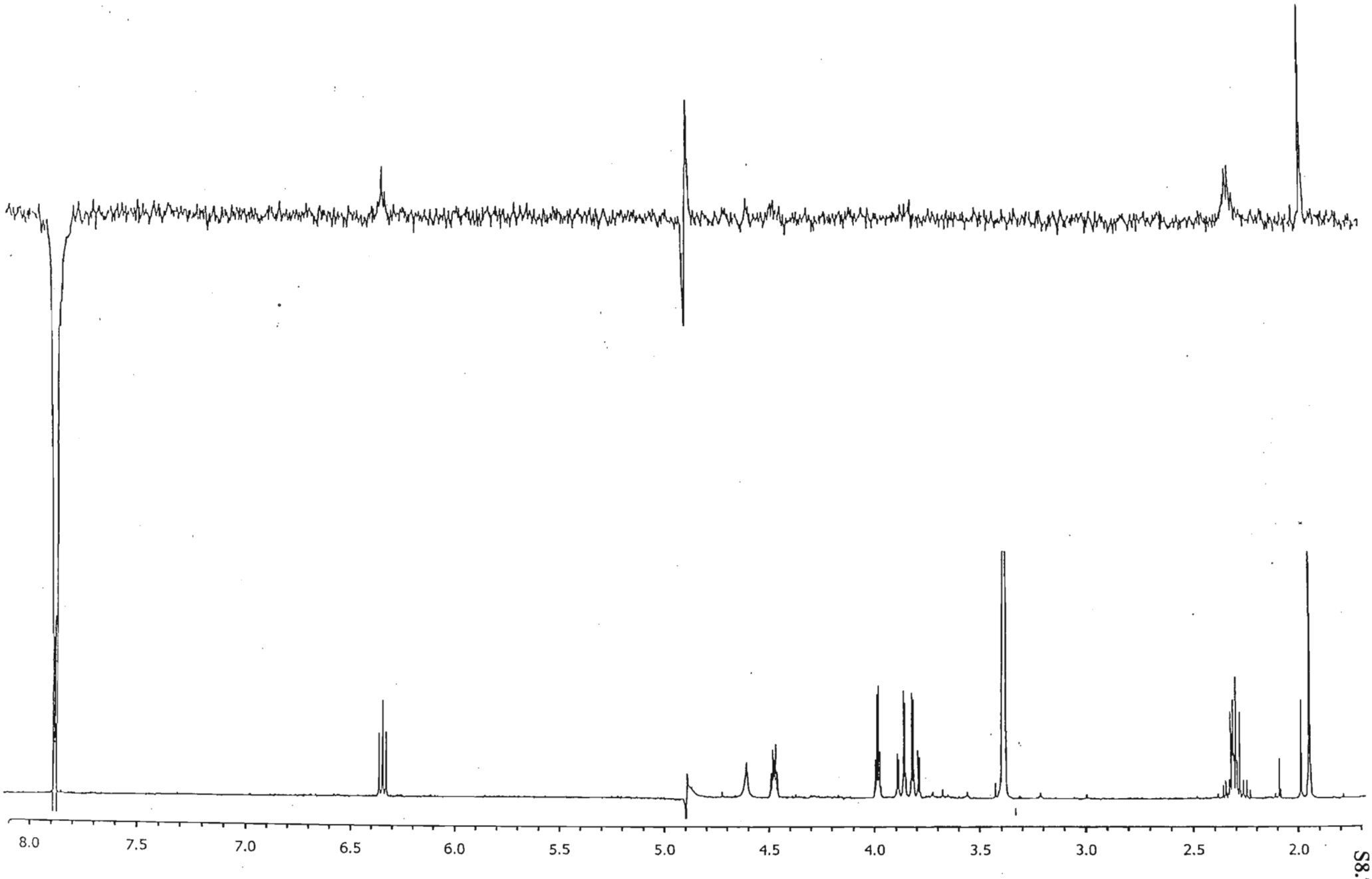
Spectrum 8.4: DEPT spectrum of compound 8 (CD_3OD)



Spectrum 8.5: ^{13}C NMR spectrum of compound 8 (CD_3OD)

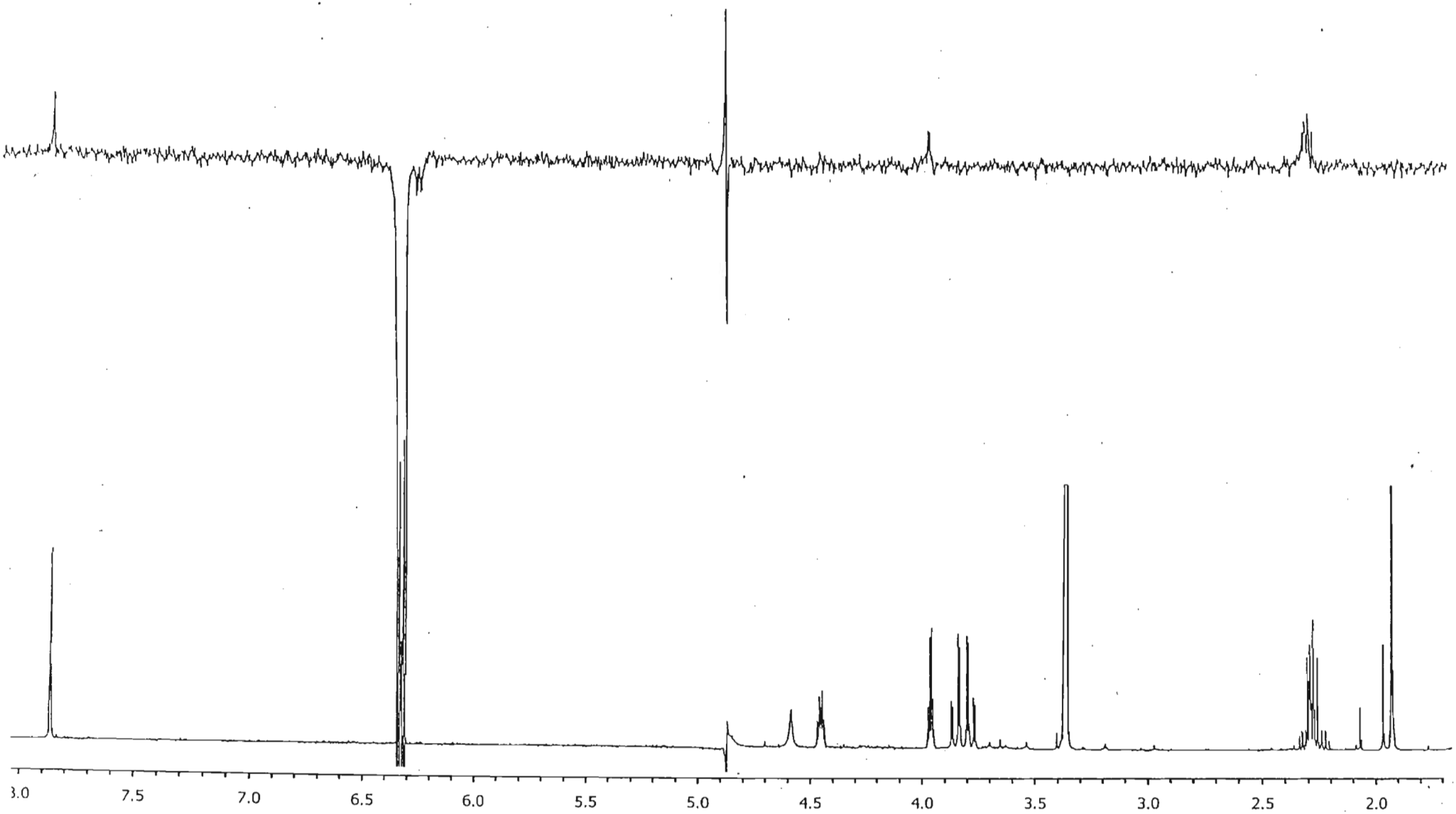


Spectrum 8.6: HMBC spectrum of compound 8 (CD_3OD)

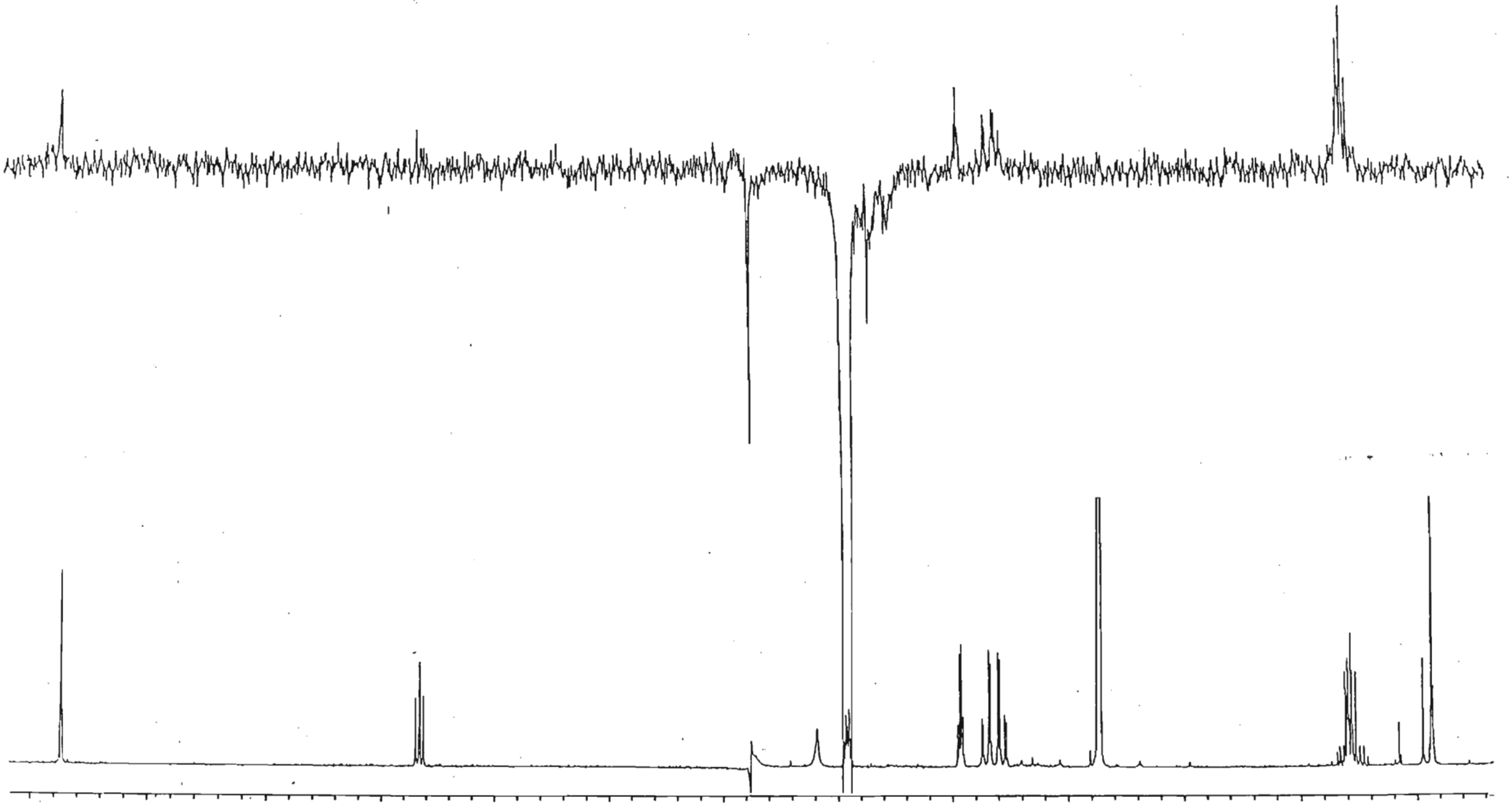


S8.7

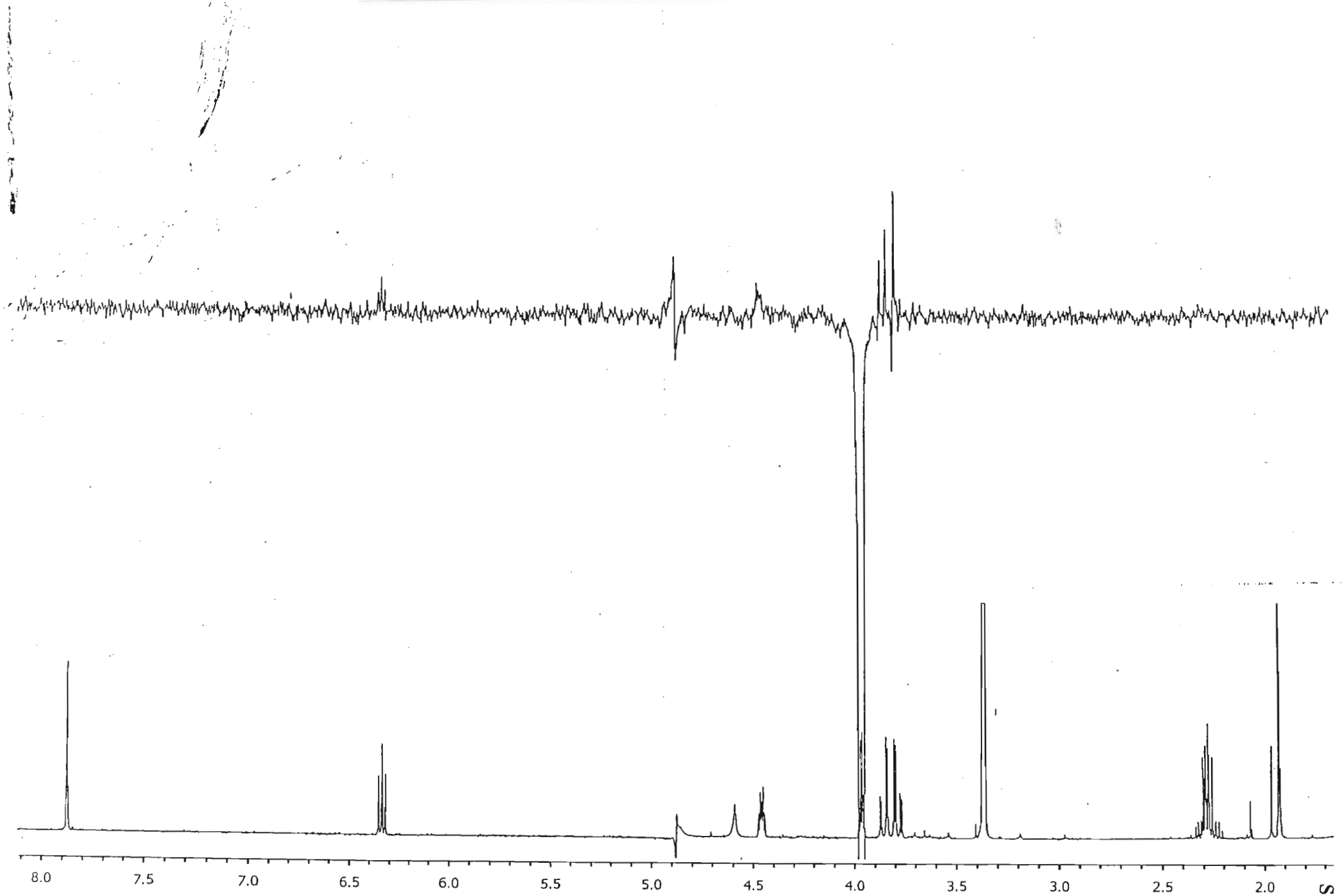
Spectrum 8.7: NOE spectrum of compound 8 (CD_3OD)



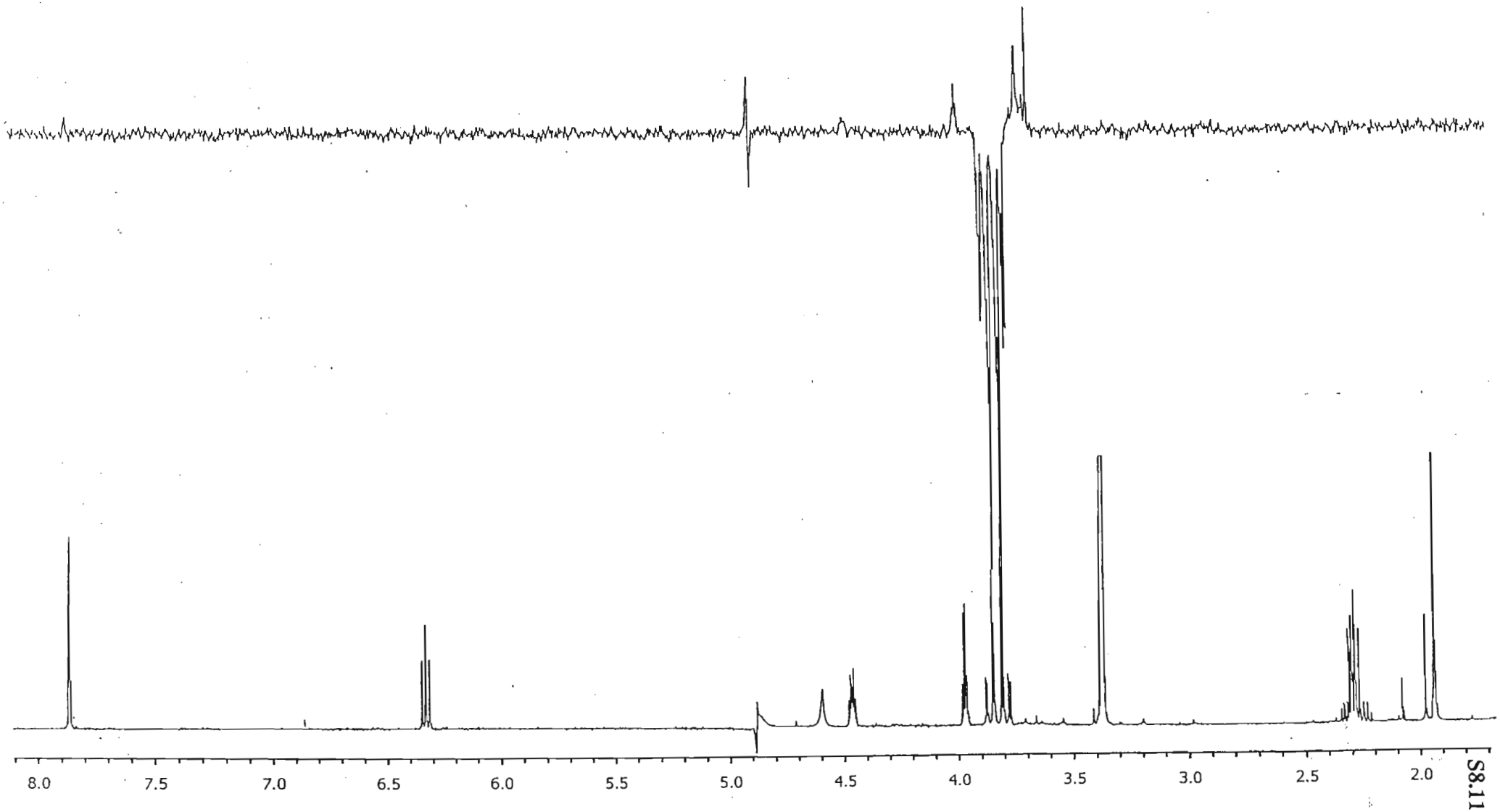
Spectrum 8.8: NOE spectrum of compound 8 (CD_3OD)



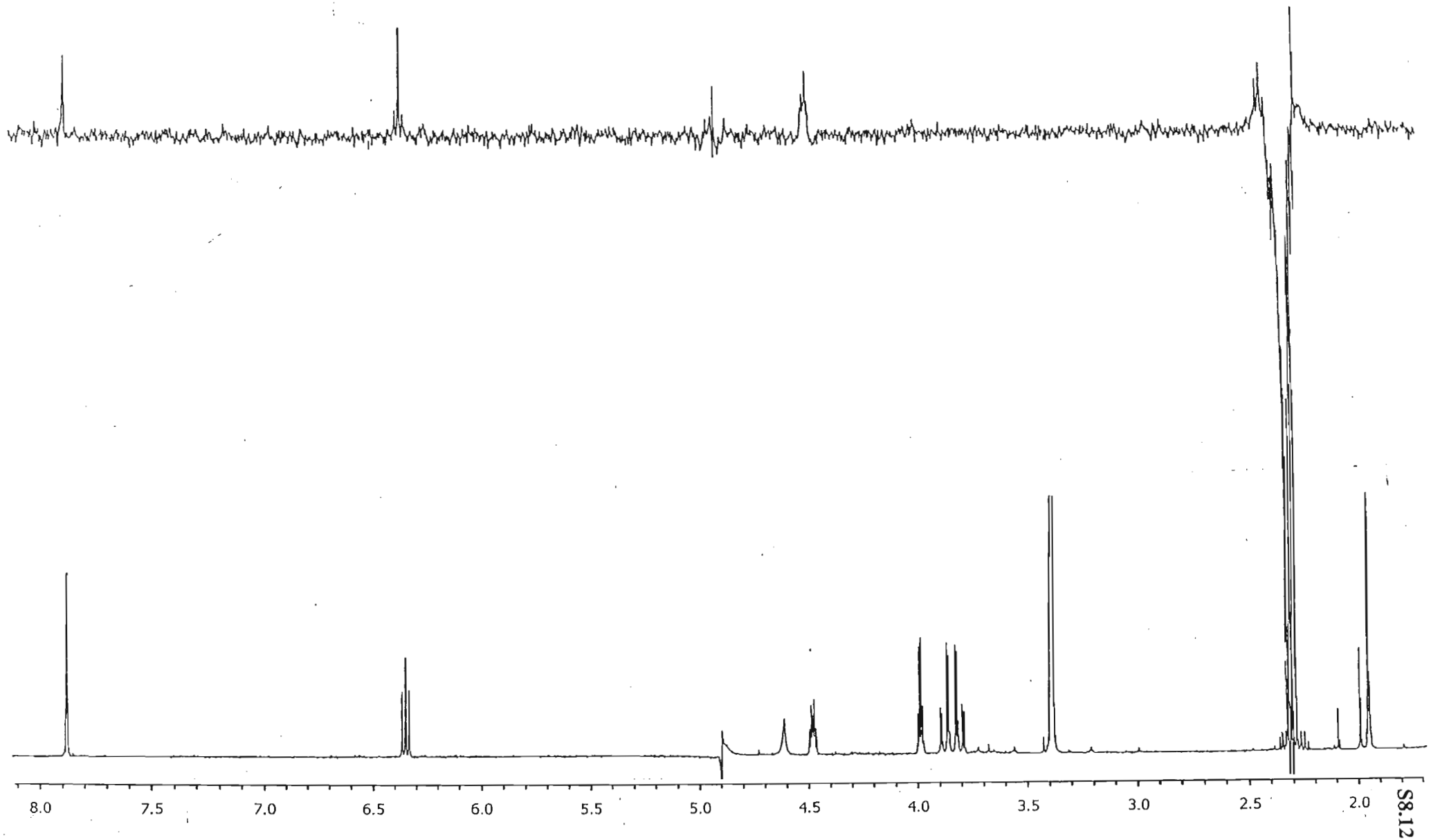
Spectrum 8.9: NOE spectrum of compound 8 (CD_3OD)



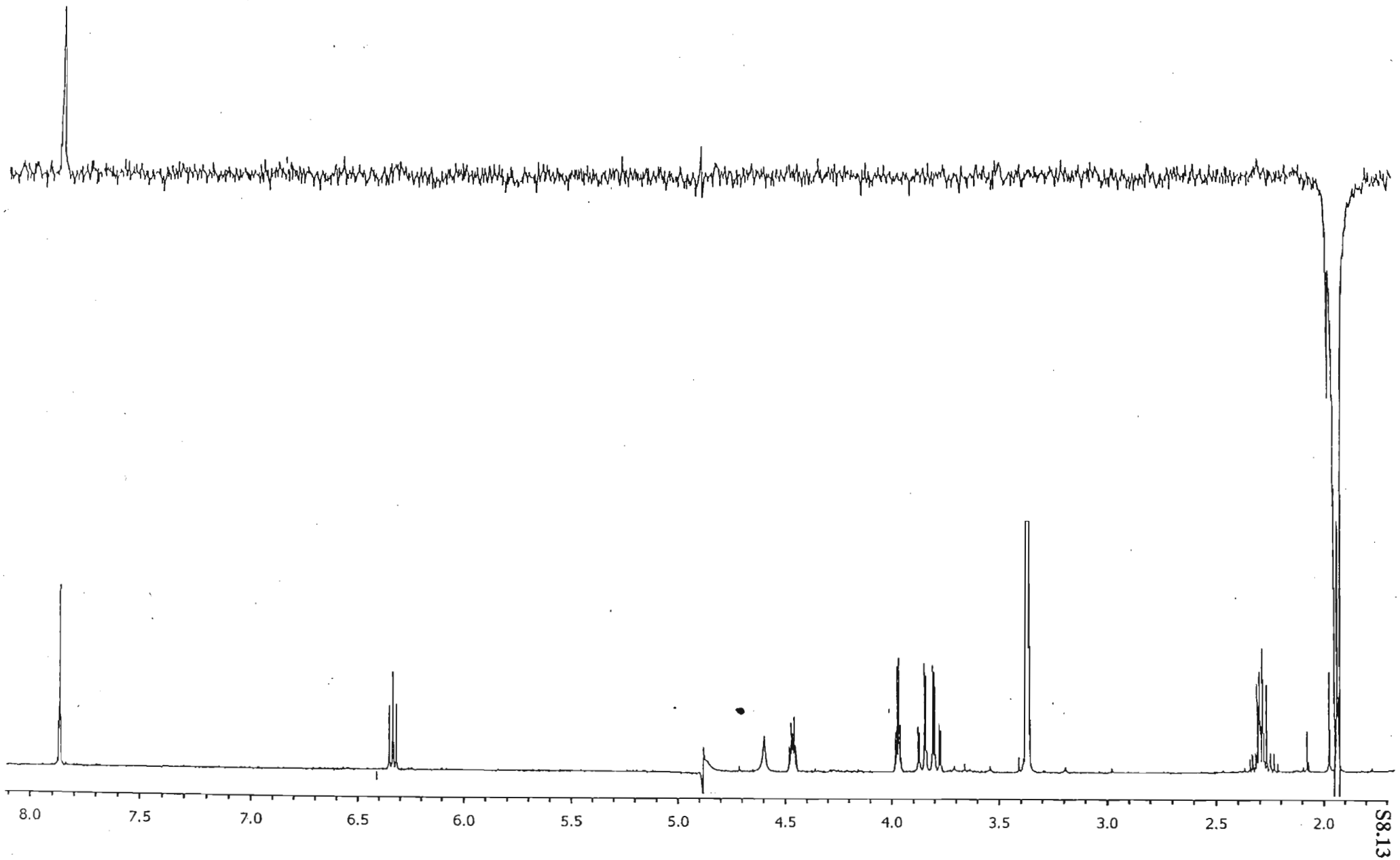
Spectrum 8.10: NOE spectrum of compound 8 (CD_3OD)



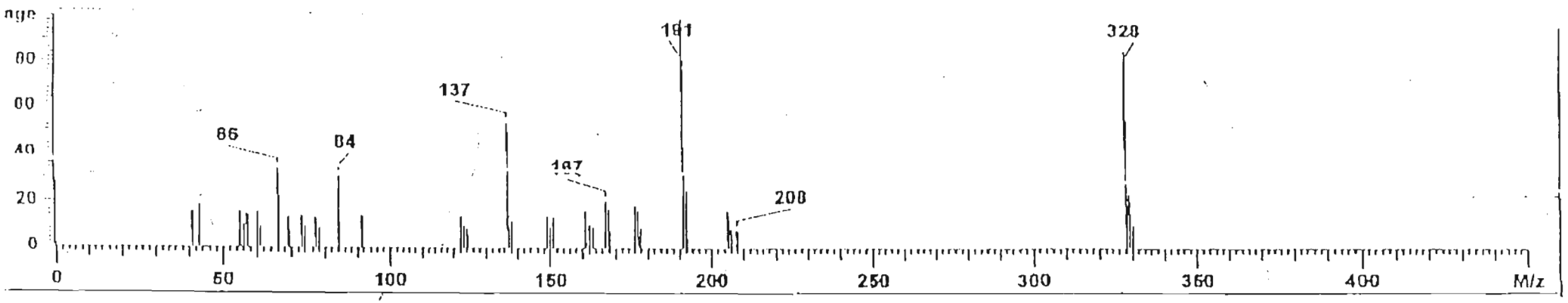
Spectrum 8.11: NOE spectrum of compound 8 (CD_3OD)



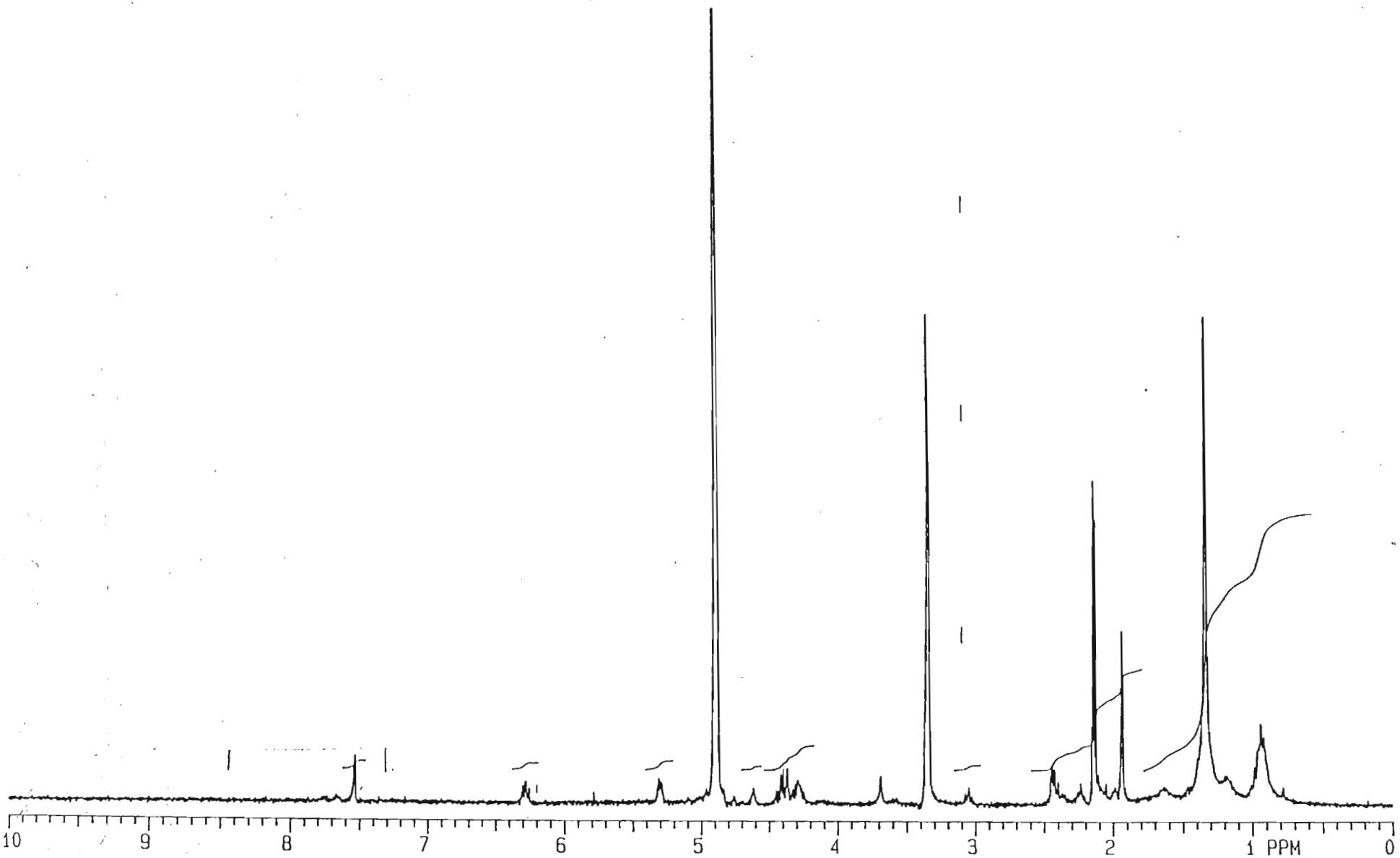
Spectrum 8.12: NOE spectrum of compound 8 (CD_3OD)



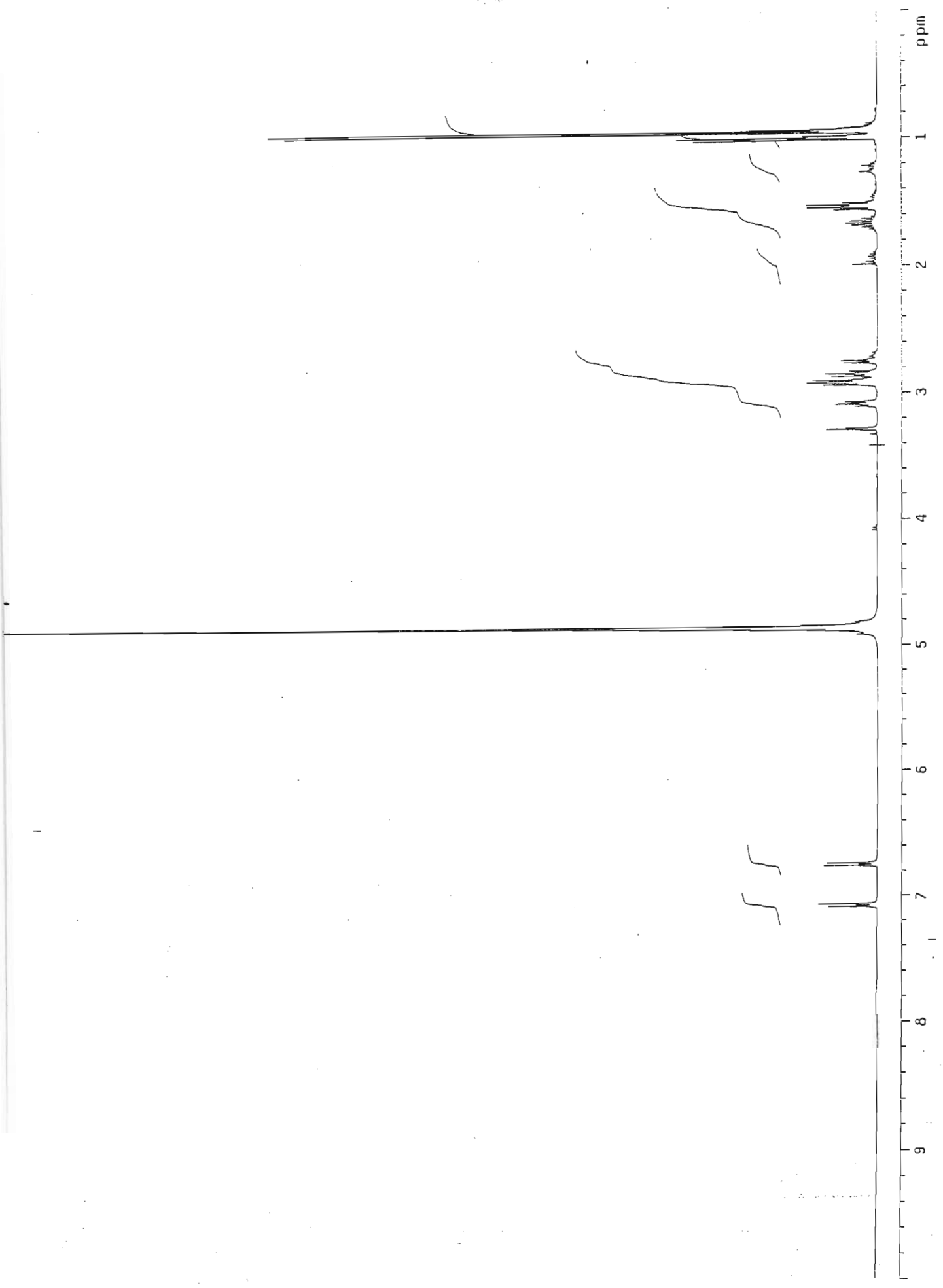
Spectrum 8.13: NOE spectrum of compound 8 (CD_3OD)



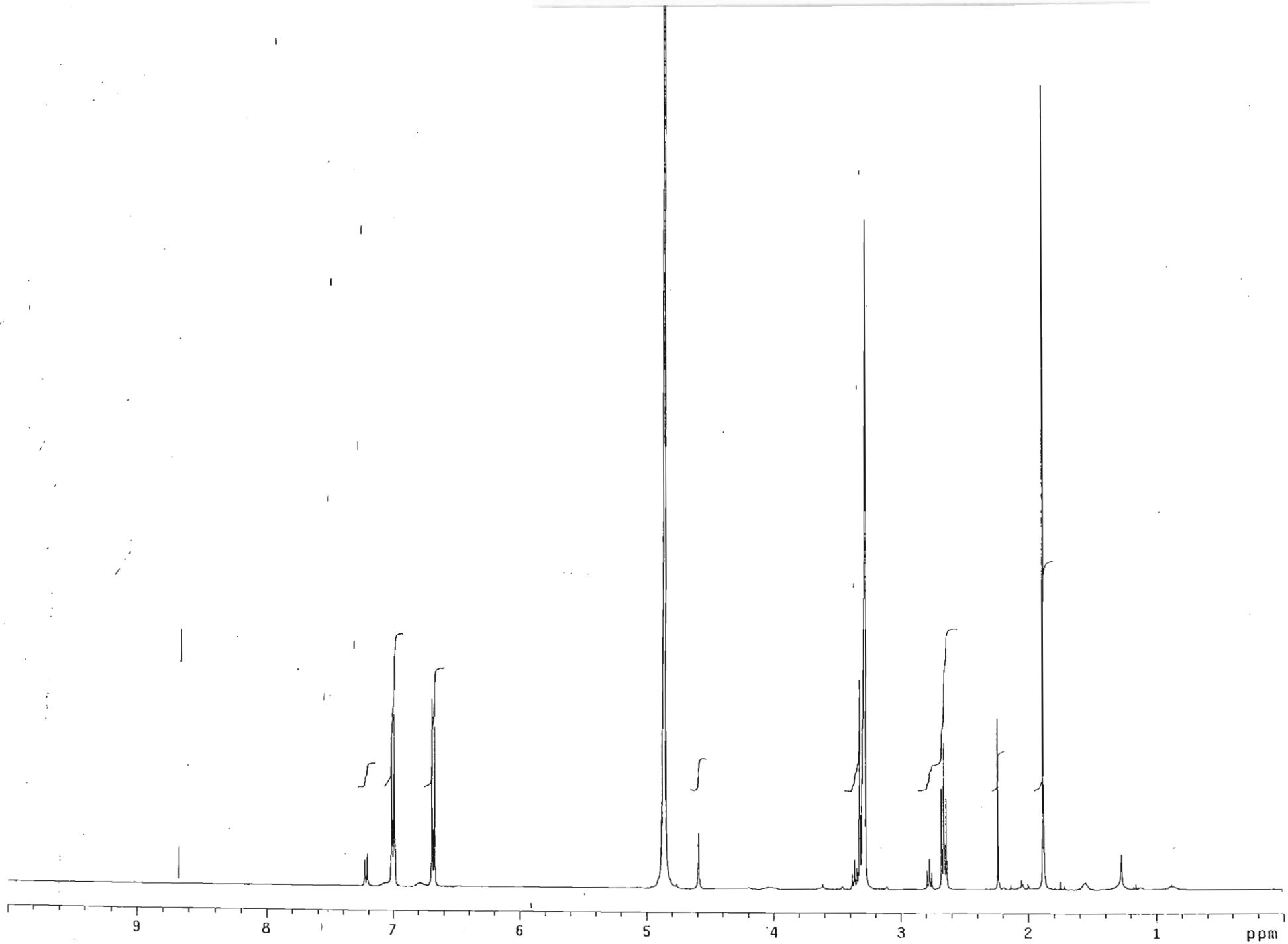
Spectrum 8.14: Mass spectrum of compound 8



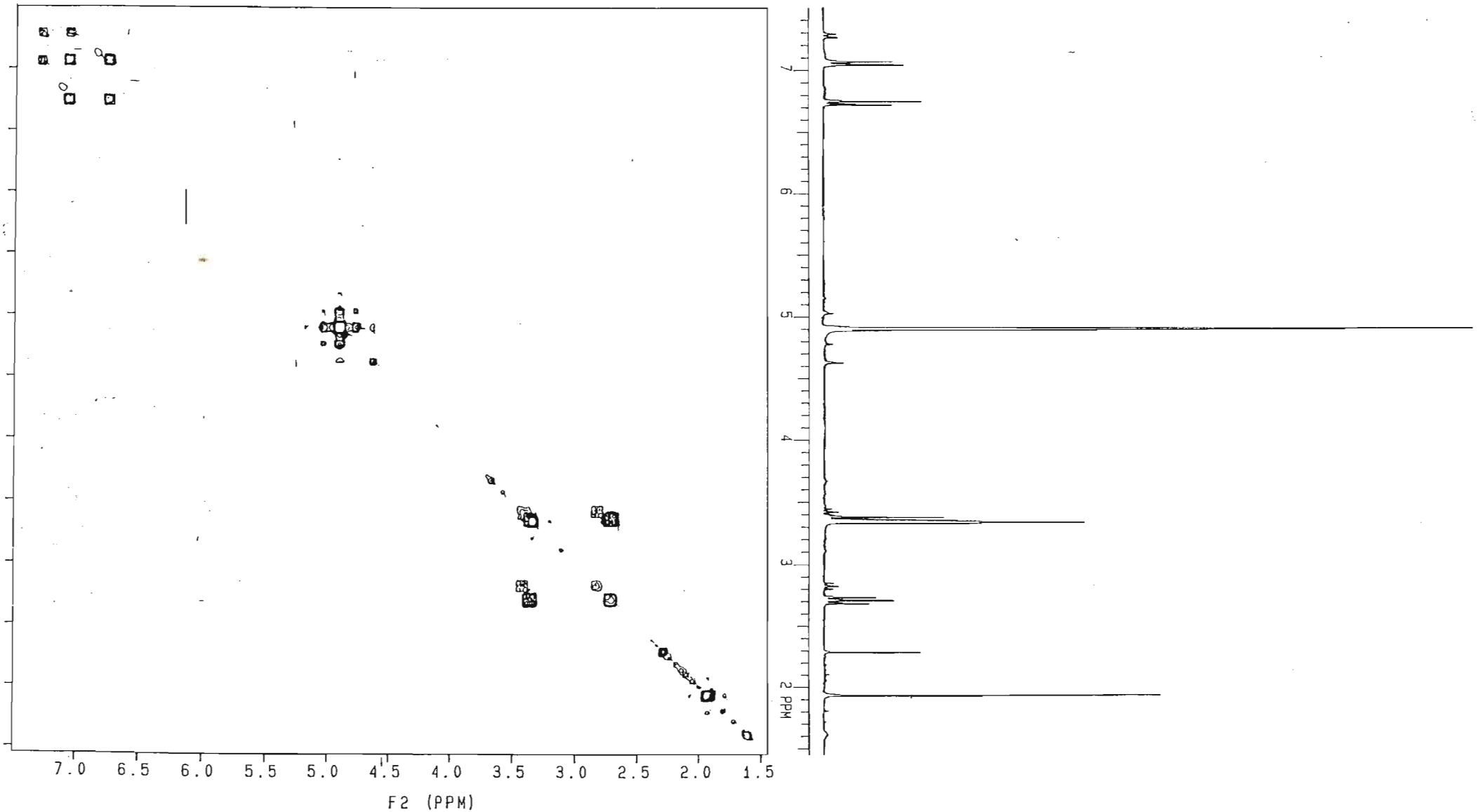
Spectrum 8A: ^{1}H NMR spectrum of acetylated compound 8 (CD_3OD)



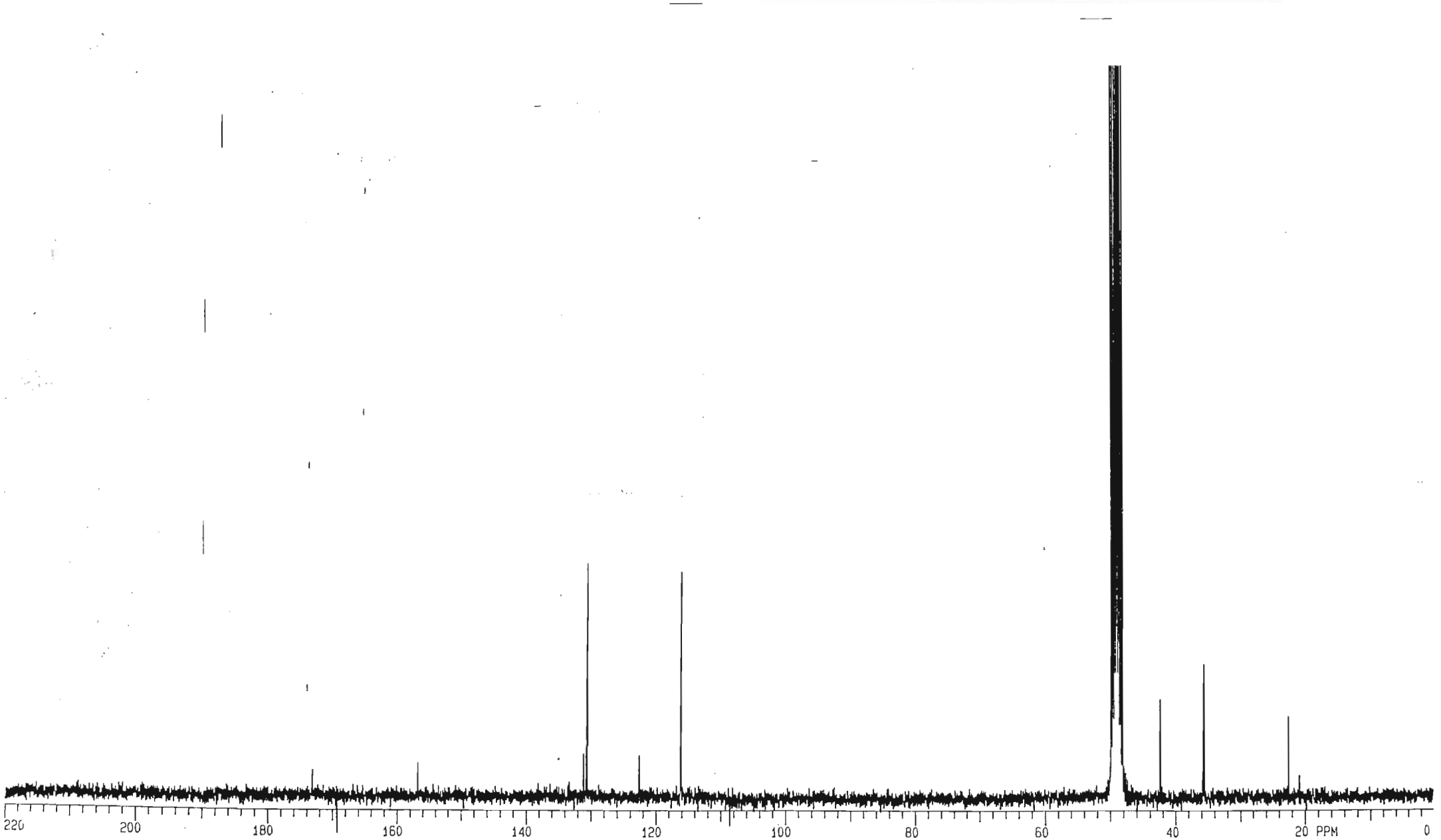
Spectrum A: ^{1}H NMR spectrum of fraction P3-10 (CD_3OD)



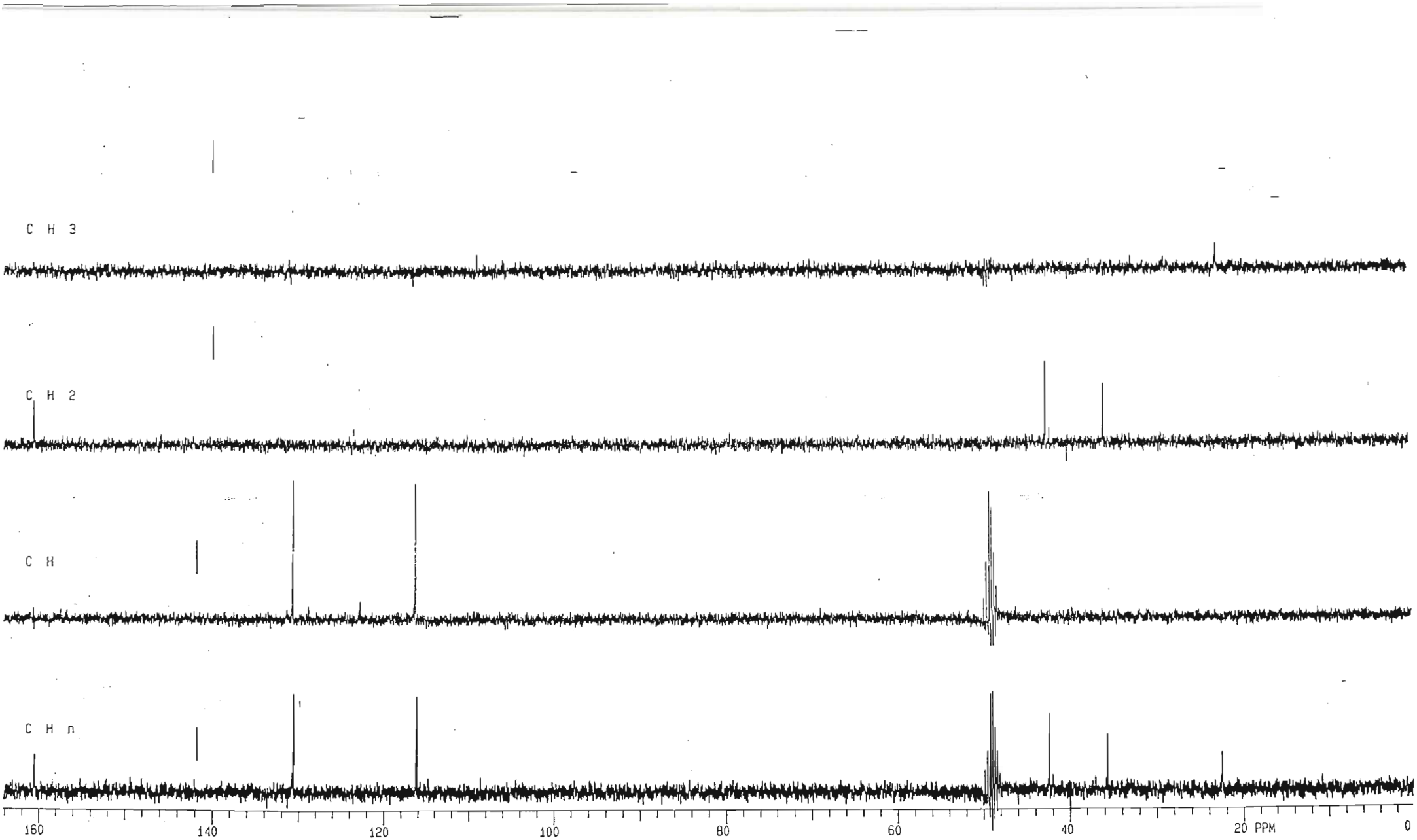
Spectrum 9.1: ^{1}H NMR spectrum of compound 9 (CD_3OD)



Spectrum 9.2: COSY spectrum of compound 9 (CD_3OD)

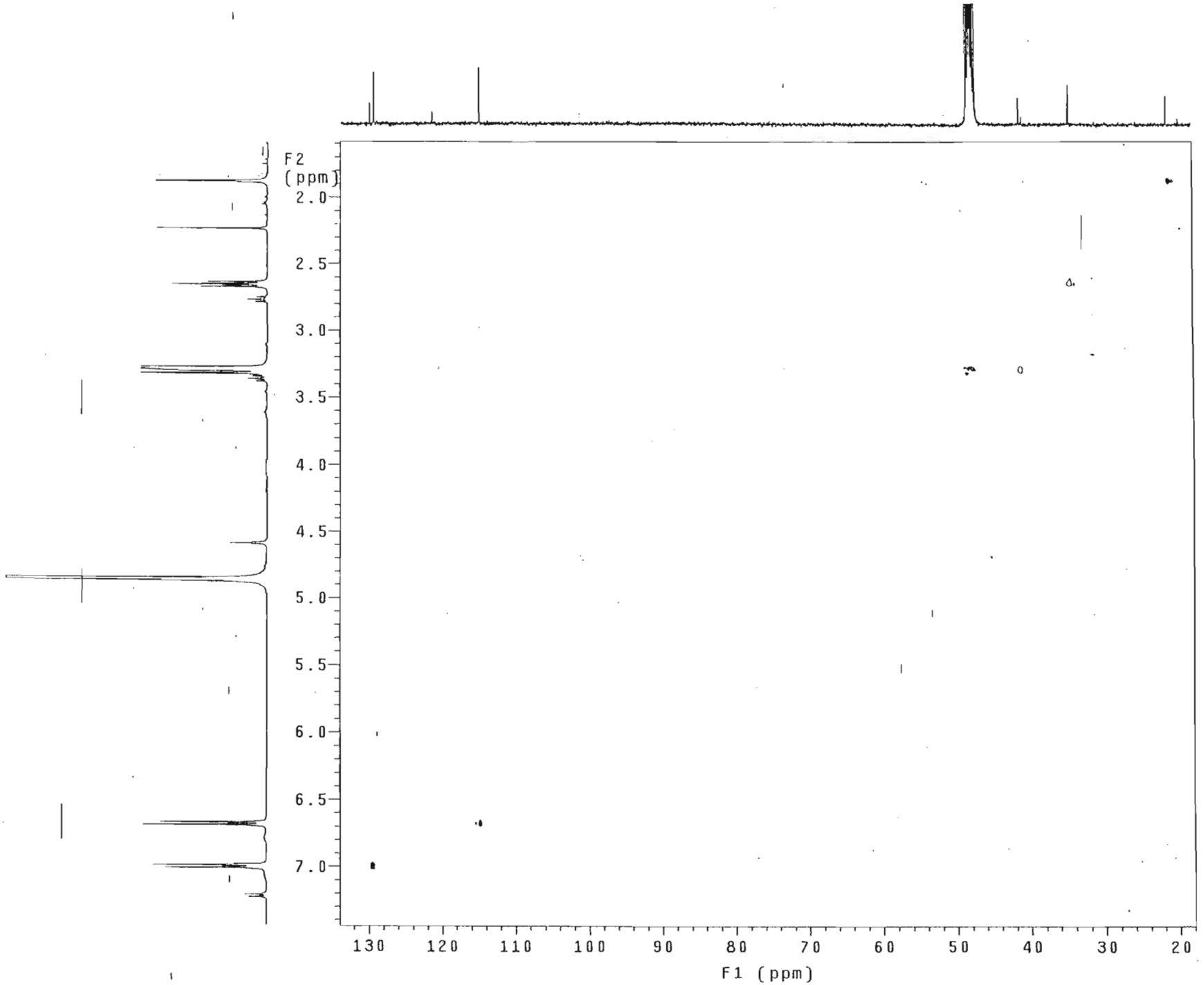


Spectrum 9.3: ^{13}C spectrum of compound 9 (CD_3OD)

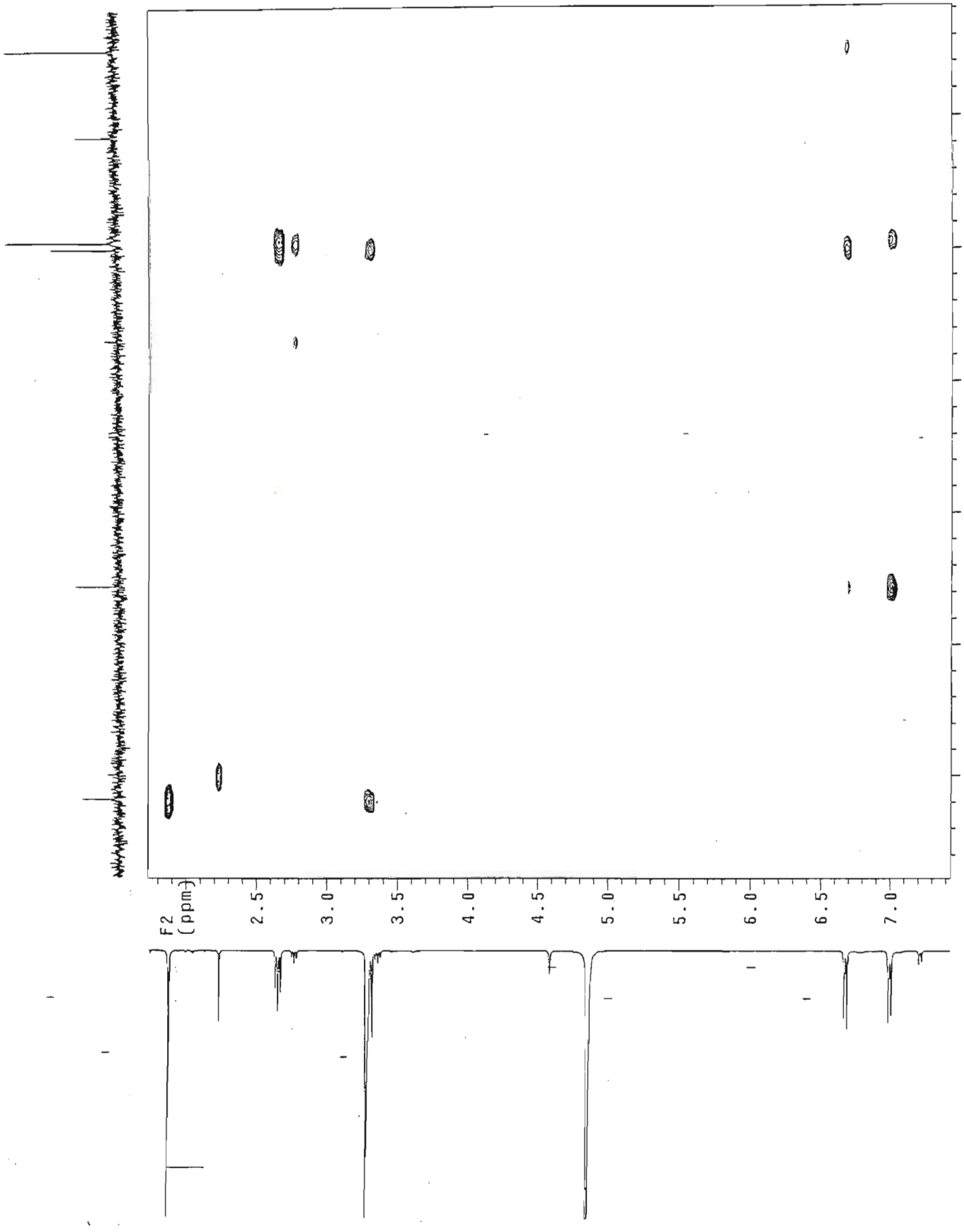


S9.4

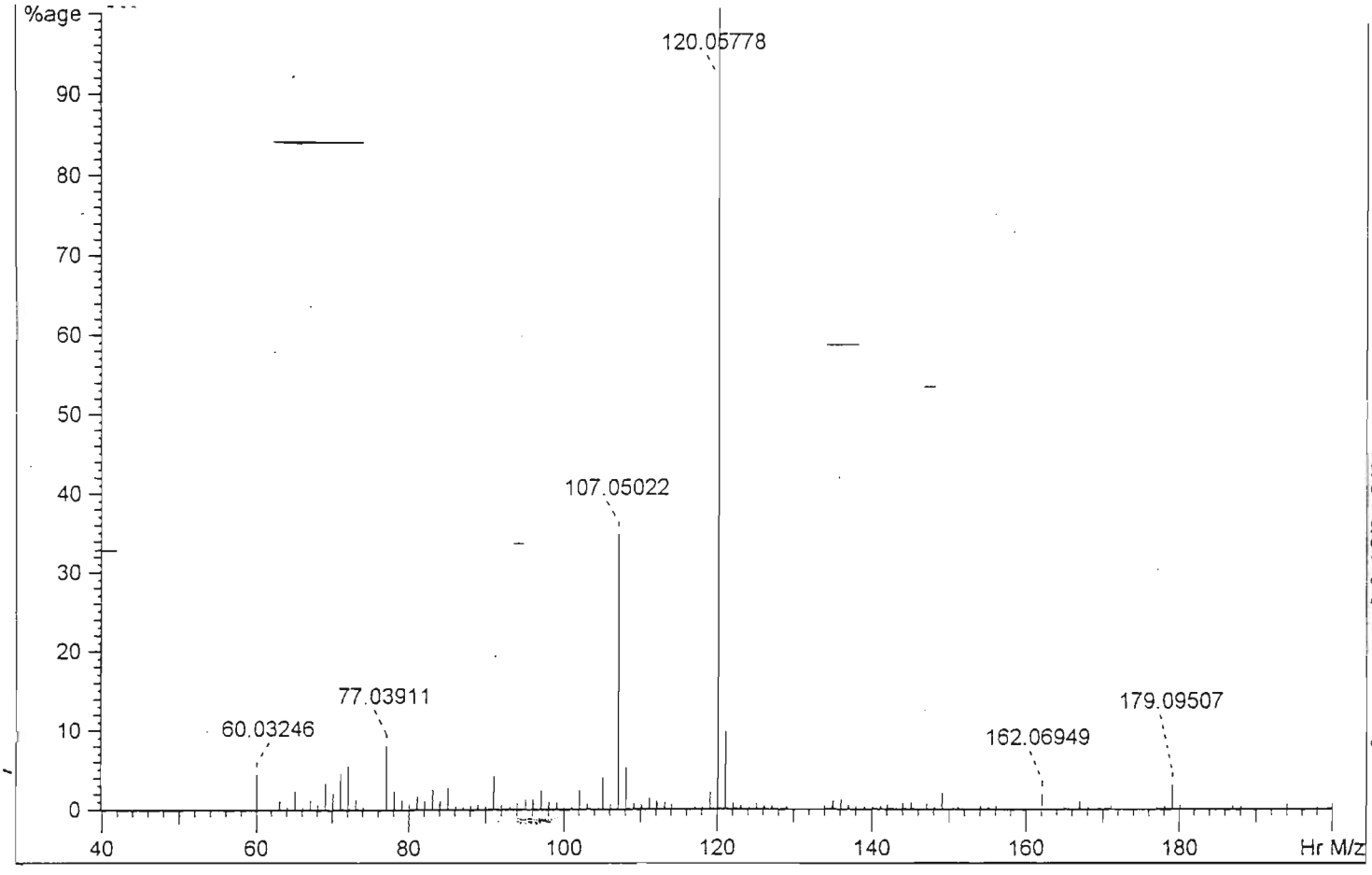
Spectrum 9.4: DEPT spectrum of compound 9 (CD_3OD)



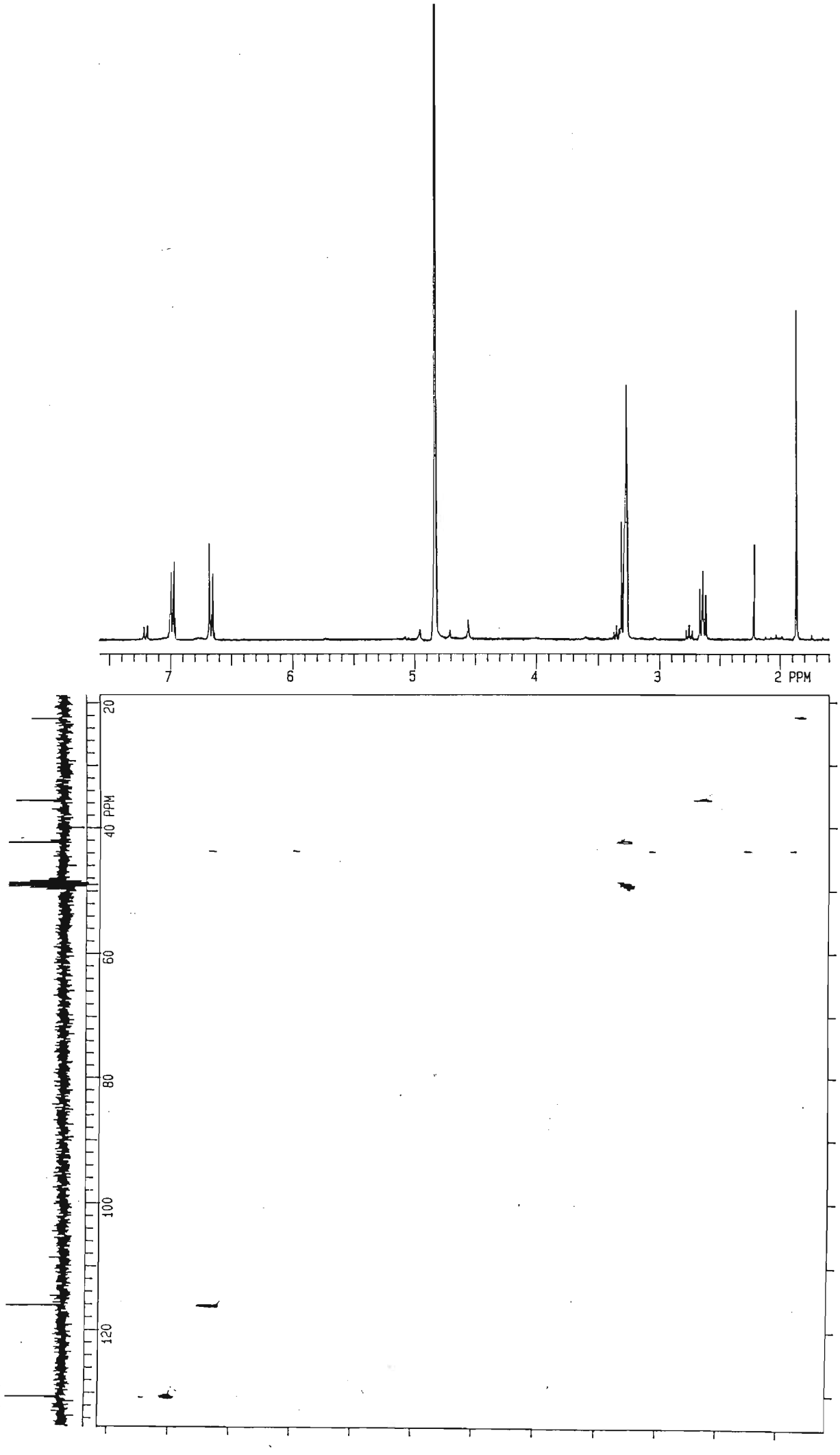
Spectrum 9.5: HSQC spectrum of compound 9 (CD_3OD)



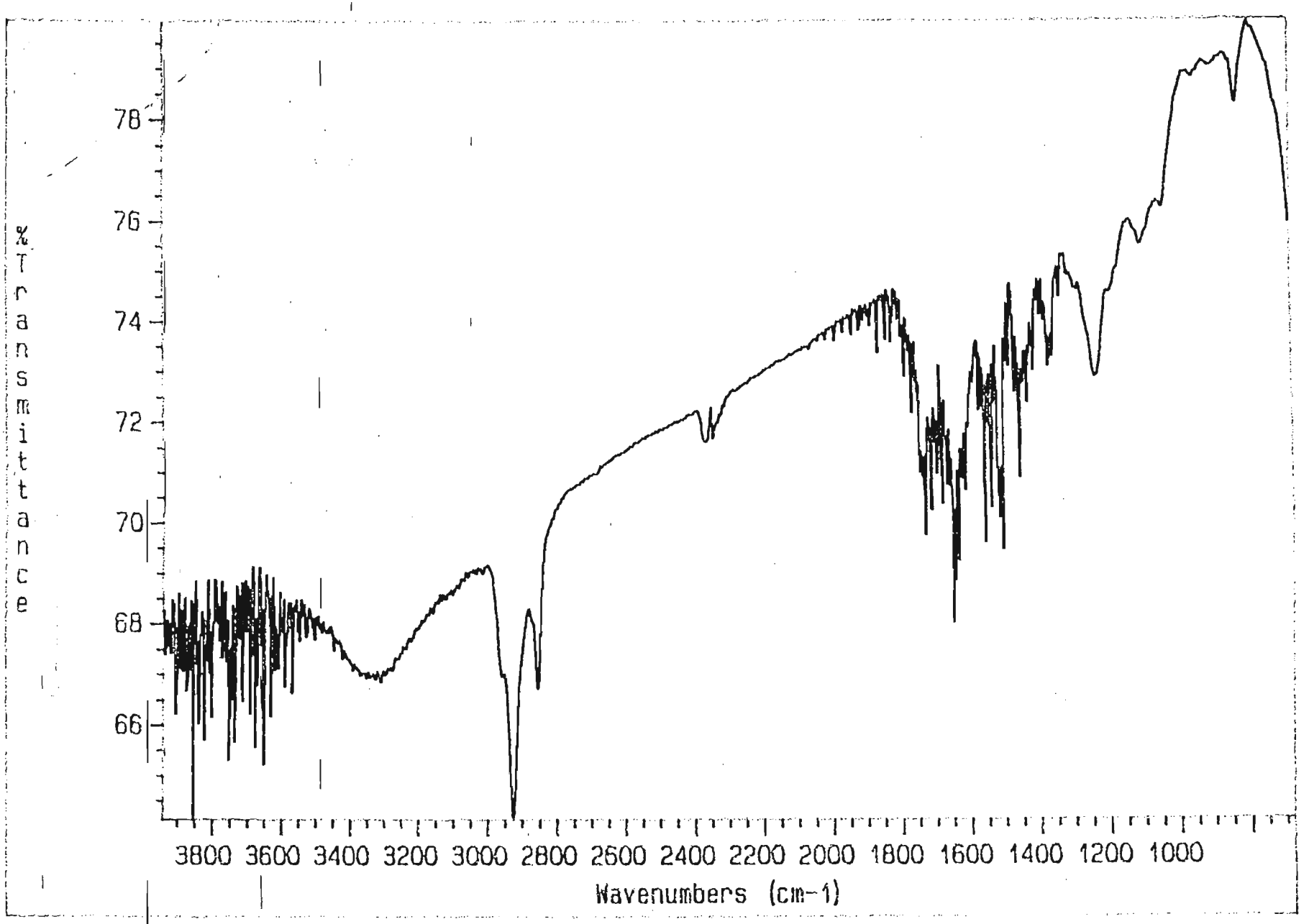
Spectrum 9.6: HMBC spectrum of compound 9 (CD_3OD)



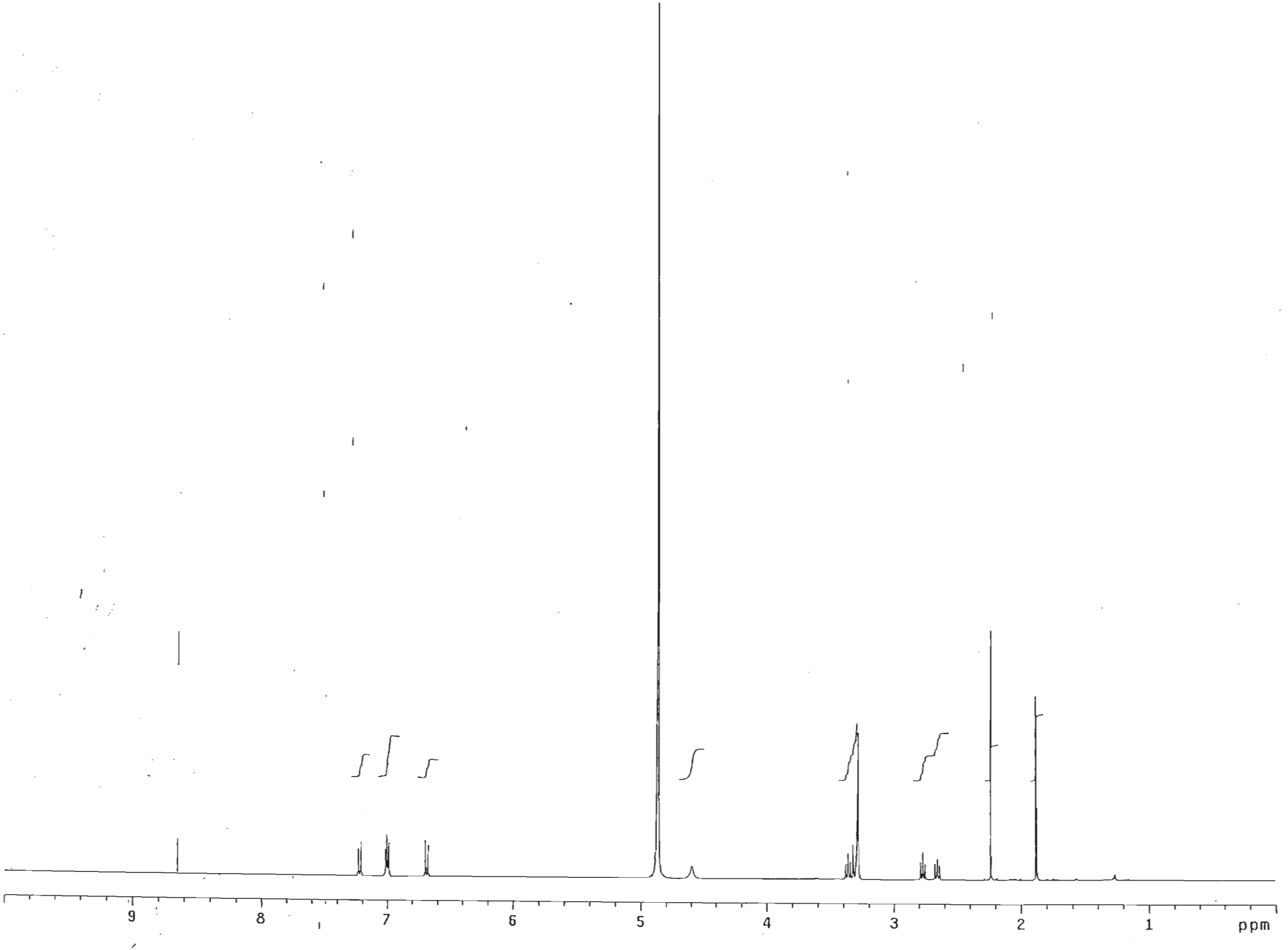
Spectrum 9.7: Mass spectrum of compound 9 (CD_3OD)



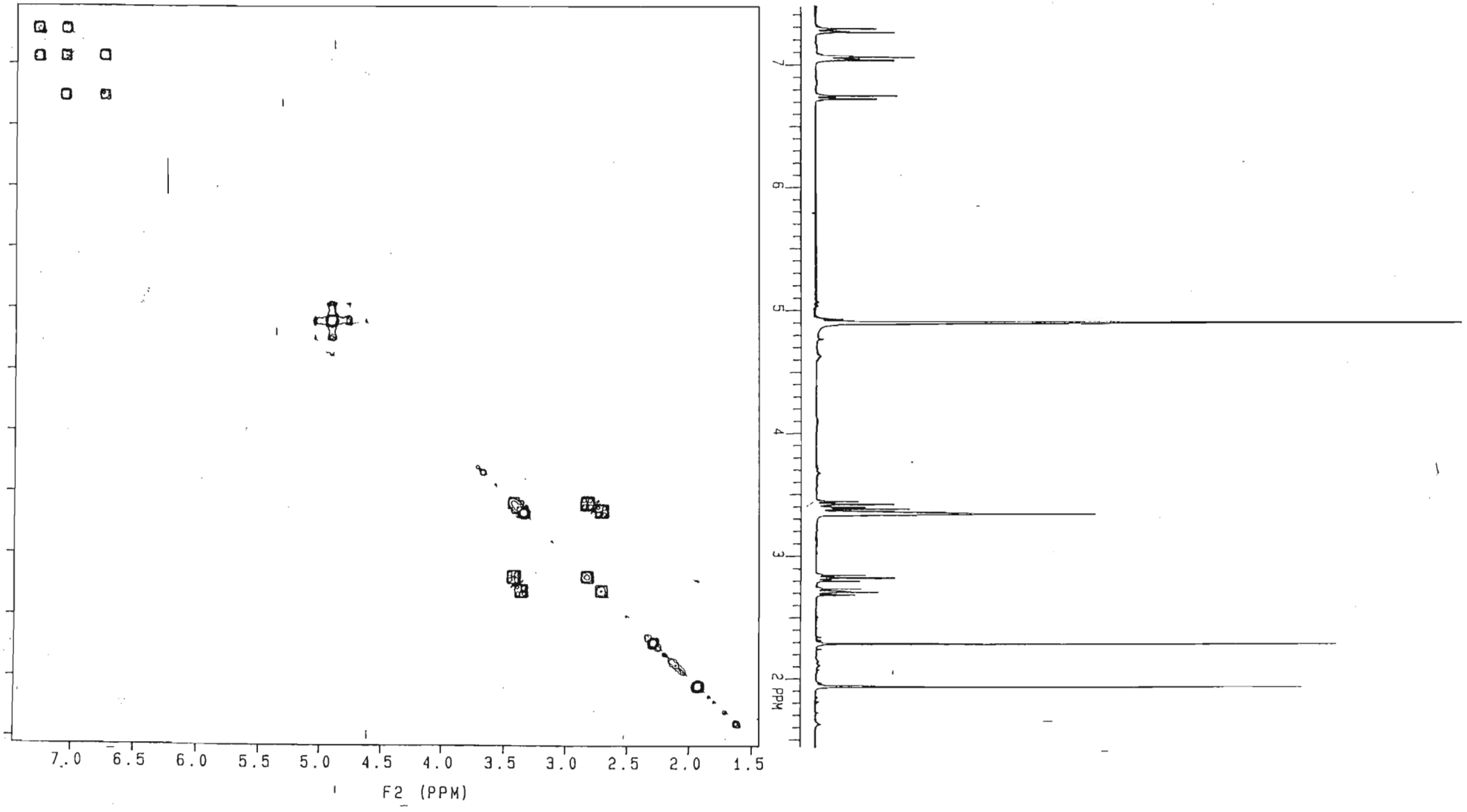
Spectrum 9.8: HETCOR spectrum of compound 9 (CD_3OD)



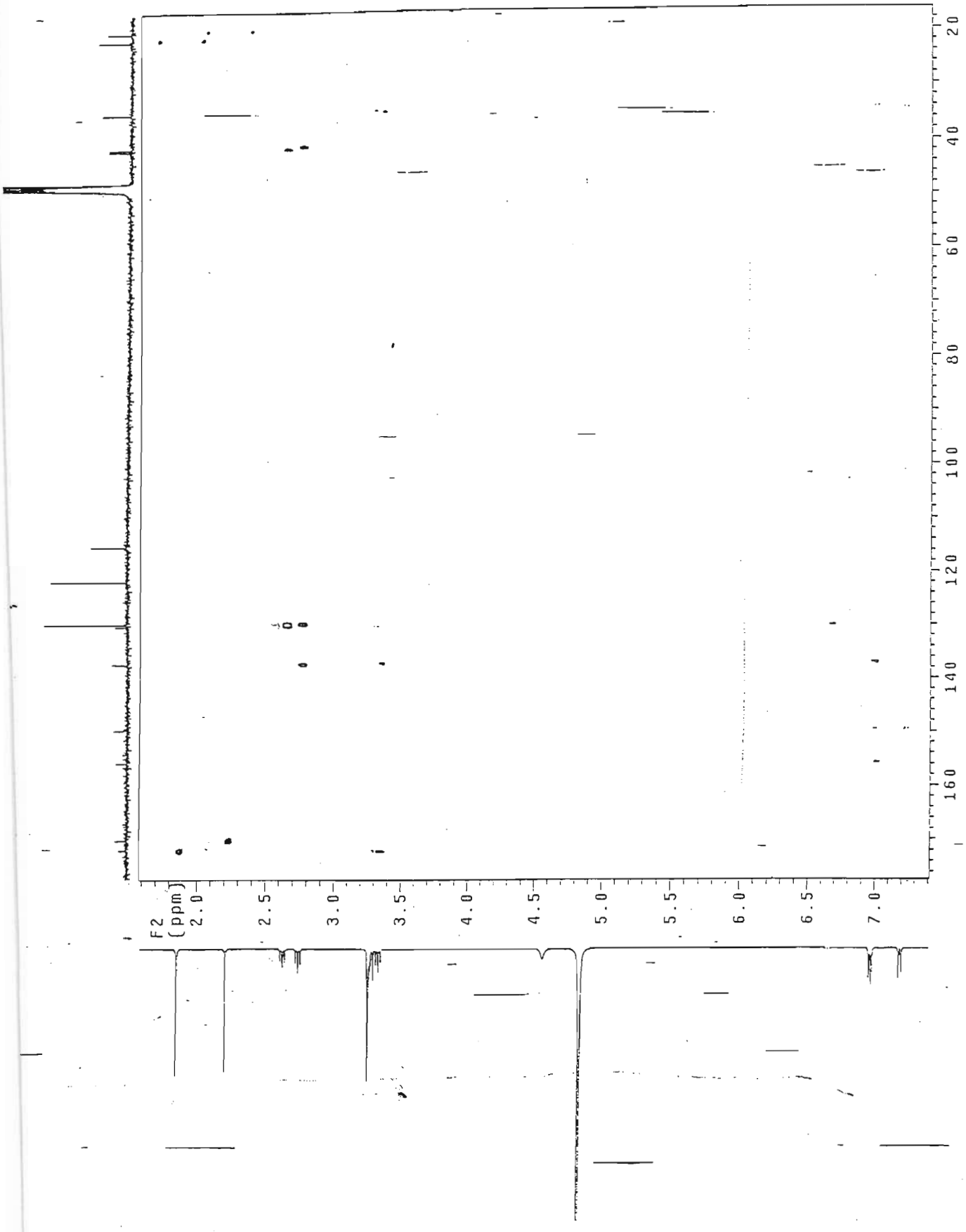
Spectrum 9.9: Infrared spectrum of compound 9



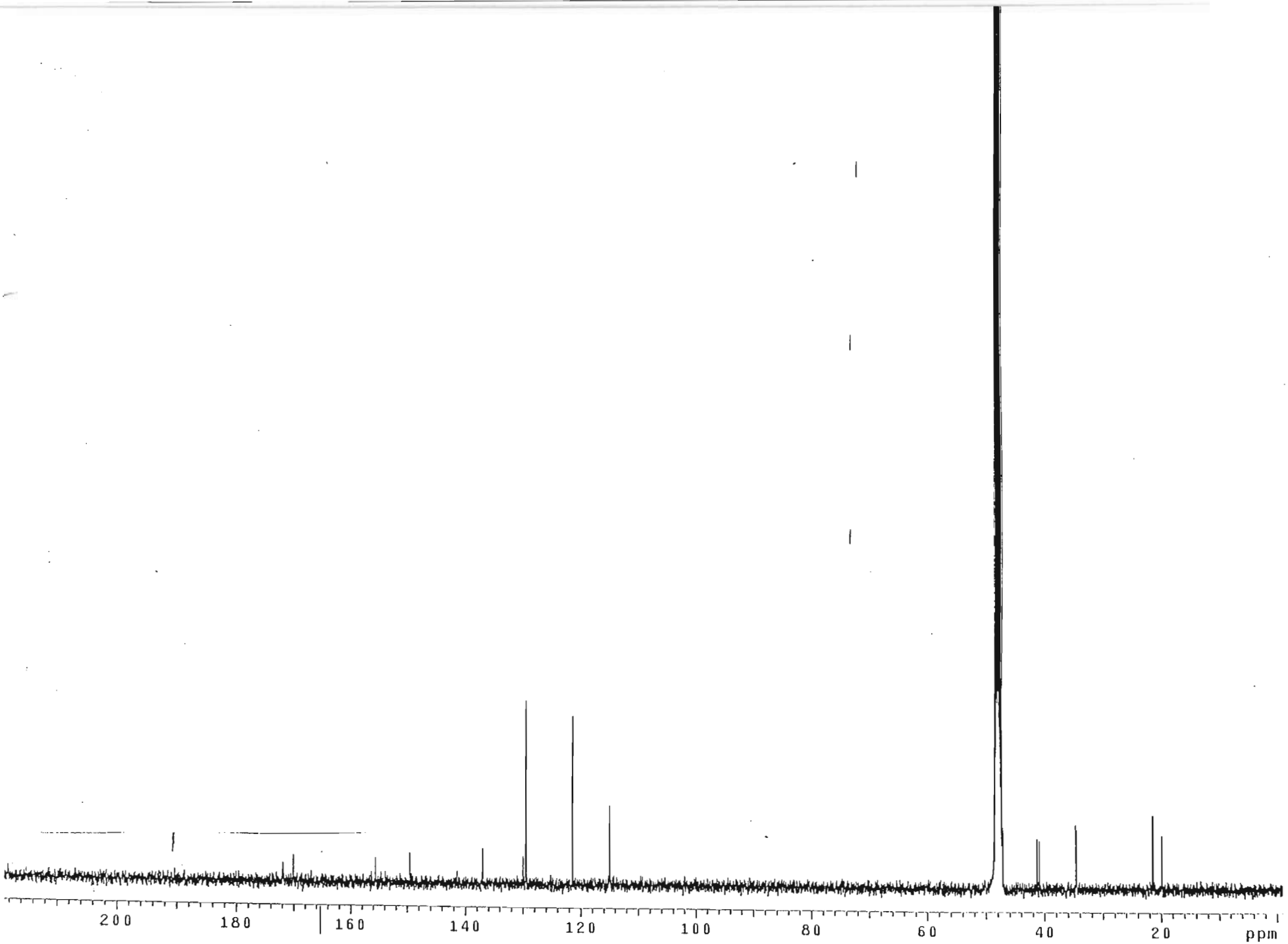
Spectrum 10.1: ^{1}H NMR spectrum of compound 10 (CD_3OD)



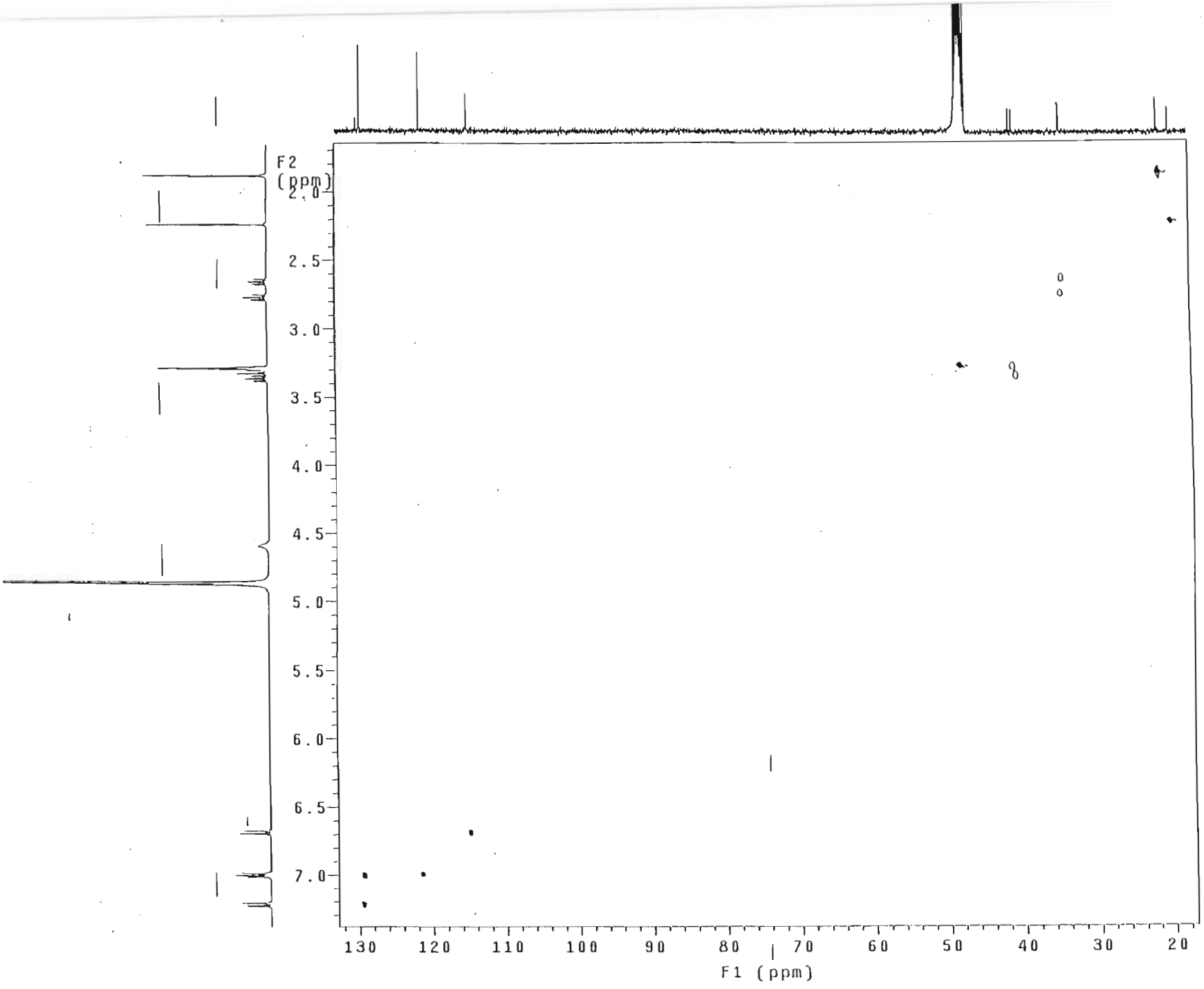
Spectrum 10.2: COSY spectrum of compound 10 (CD_3OD)



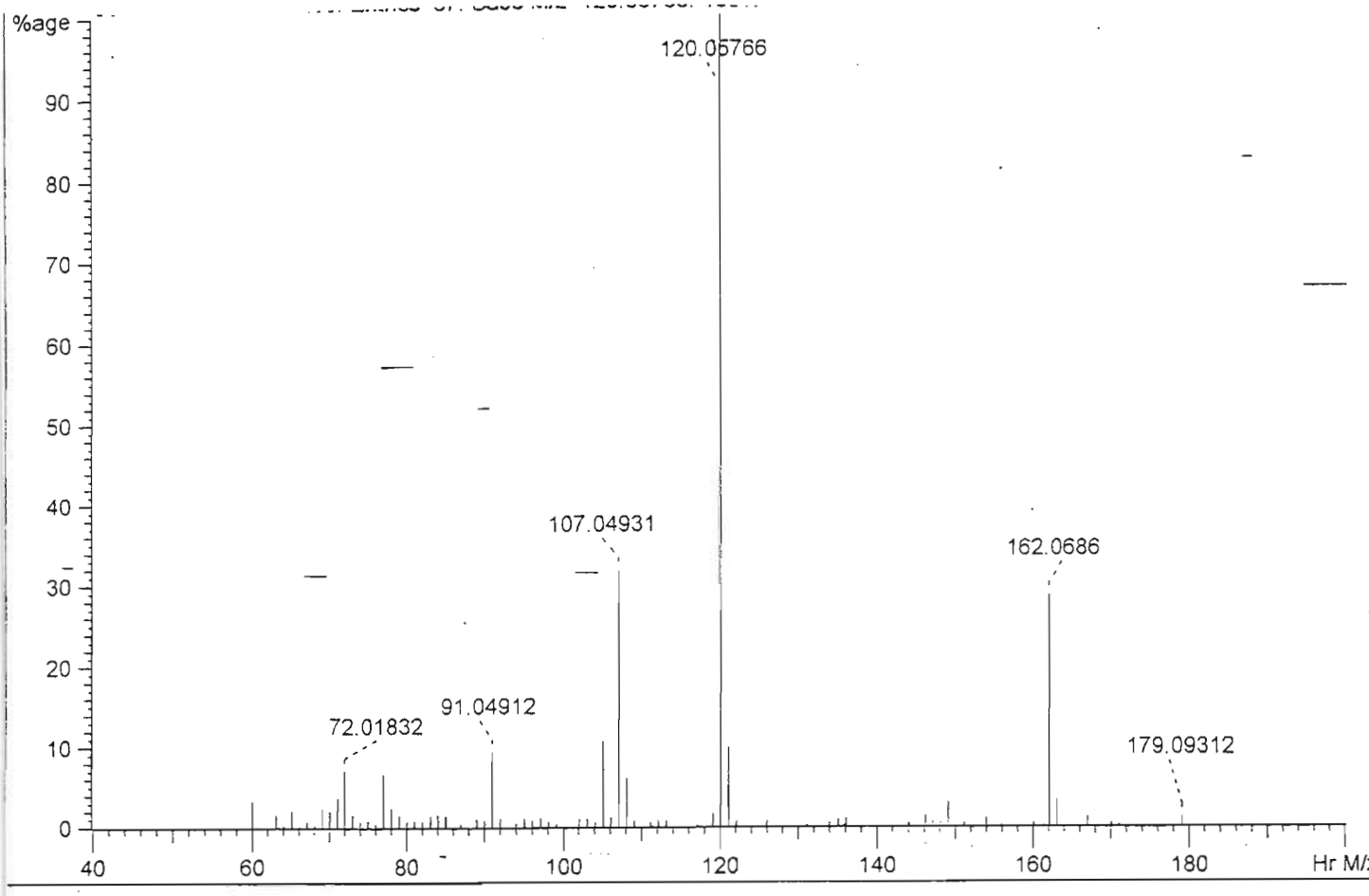
Spectrum 10.3: HMBC spectrum of compound 10 (CD_3OD)



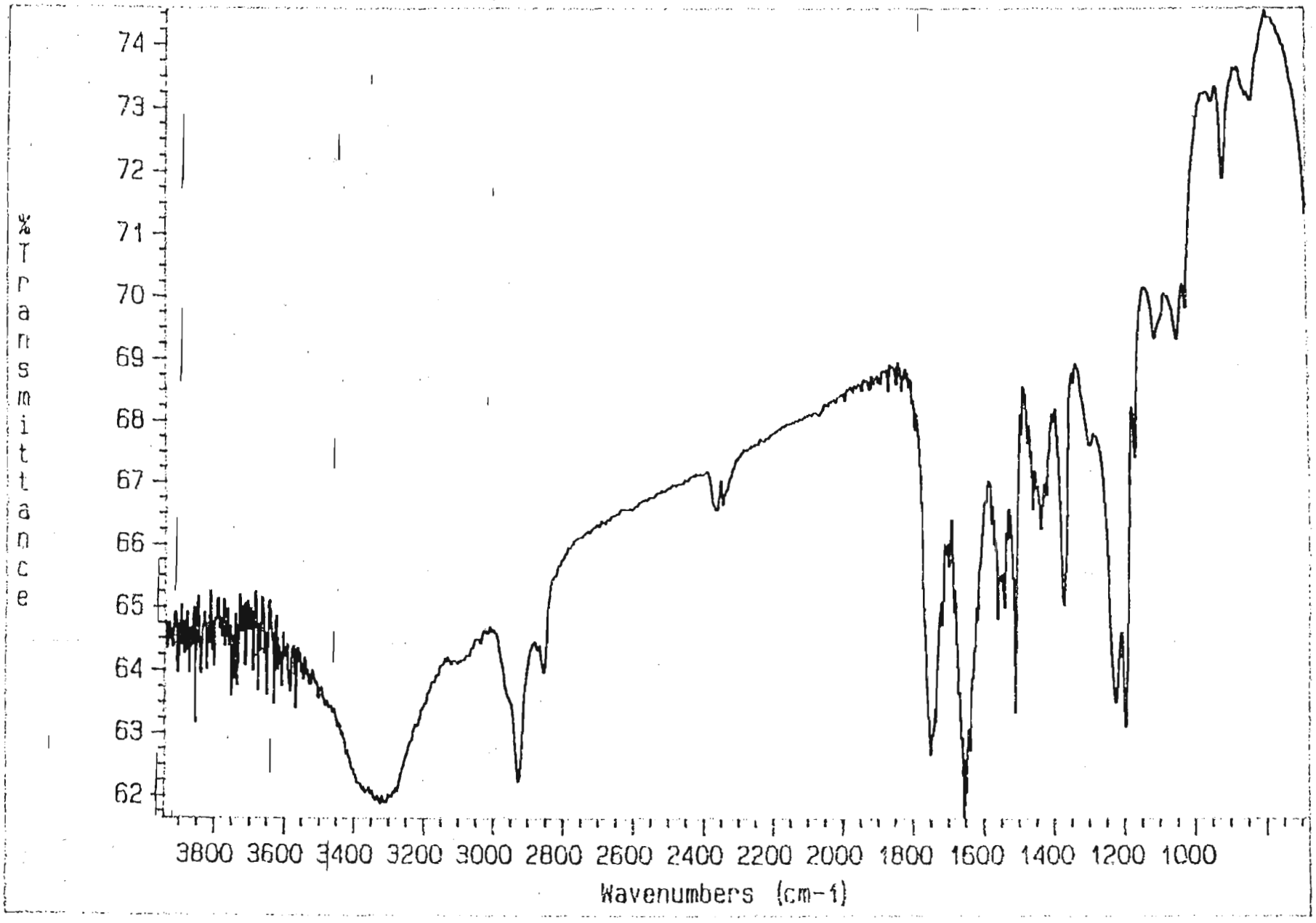
Spectrum 10.4: ^{13}C NMR spectrum of compound 10 (CD_3OD)



Spectrum 10.5: HSQC spectrum of compound 10 (CD_3OD)



Spectrum 10.6: Mass spectrum of compound 10



Spectrum 10.7: Infrared spectrum of compound 10