SYNTHESIS

OF

CHIRAL PHOSPHORUS MUSTARDS

FROM

CHIRAL AMINO ALCOHOLS

by

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Submitted in Partial Fulfillment of the Requirements

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in the

Chemistry

Program

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SYNTHESIS OF CHIRAL PHOSPHORUS MUSTARDS

FROM CHIRAL AMINO ALCOHOLS

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ABSTRACT

The content of the research presented in this thesis is based on the synthesis, purification and characterization of chiral phosphorus mustards. The stereochemistry of the starting materials were varied to do a comparison study between pairs of enantiomers and diastereomers. Phosphorus mustards contain the bis(2-chloroethyl) group which is found in chemotherapeutic agents known as nitrogen mustards. Therefore, as such, they may be useful as a potential form of cancer treatment.

ACKNOWLEDGMENTS

I would like to thank my research advisor Dr. John Jackson for all the patience, help, encouragement and direction he has given to me over the past two years while completing my research and my thesis. I would also like to thank the members of my thesis committee Dr. Elmer Foldvary and Dr. Michael Serra, as well as, the chemistry department faculty, the secretaries and Bob and Sue for all their helpfulness and words of encouragement.

I would like to also thank my parents for without whom none of my achievements or education would have been possible; especially to my father who always believed I could do anything when I wasn't sure I could. We've come a long way since calculus! Finally, I would like to thank my fiance David for his patience and understanding.

At this time, I would like to dedicate my thesis to my mother, Darla Madison and to my uncle, William Forbes in the hopes that something I did will someday lead to more effective forms of cancer treatment.

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LIST OF ABBREVIATIONS

Abbreviation	Description
(#a)	fast diastereomer
$[\alpha]^{25}_{D}$	specific rotation
(#b)	slow diastereomer
C-	carbon atom
$CDCl_3$	deuterochloroform
^{13}C	carbon 13 nuclear magnetic resonance spectroscopy
CH_2Cl_2	methylene chloride
cm	centimeter
$(CH_3)_4$	tetramethylsilane
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
δ	parts per million
D_2O	deuterated water
DNA	deoxyribonucleic acid
FTIR	fourier transform infrared spectroscopy
g	gram
Hz	Hertz
^{1}H	hydrogen 1 nuclear magnetic resonance spectroscopy
J	coupling constant in Hertz
mL	milliliter
mmol	millimoles
mm Hg	millimeters of mercury

mp	**melting point**
m	**multiplicity**
MHz	**mega Hertz**
NA	**not applicable**
NMR	**nuclear magnetic resonance spectroscopy**
$-NH_2$	**amino group**
N-7	**nitrogen atom at seventh position in the molecule guanine**
N-3	**nitrogen atom at third position in the molecule adenine**
-OH	**hydroxyl group**
^{31}P	**phosphorus 31 nuclear magnetic resonance spectroscopy**
PMA	**phosphomolybdic acid**
$POCl_3$	**phosphoryl trichloride**
R_f	**ratio calculated in TLC analysis**
ratio a:b	**ratio of fast diastereomer to slow diastereomer**
s	**singlet**
-SH	**sulfhydryl group**
THF	**tetrahydrofuran**
TLC	**thin layer chromatography**

Chapter One

Introduction:

The History

of

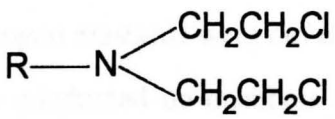
Chemotherapeutic Agents

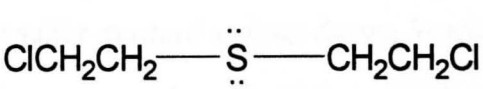
Historical Perspective of Cancer and Nitrogen Mustards

Statistical data shows that approximately 600,000 new cases of cancer are diagnosed each year in the U.S. and on average one out of every five of these new cases will result in death.[1] The indiscriminatory nature of cancer has led to a heightened fear because all ages, genders and races have approximately equal probability to develop and subsequently die from cancerous tendencies;[1] however, cancer is by no means a recent development in human health. The term cancer was derived from the word canker in the early 16th century and means to spread indefinitely and to eat away or corrode the part in which it is situated.[2] Historically, the chemical treatment of cancer began more than three thousand years ago with simple plant preparations. Approximately 200 years ago, the prescribed method of treatment contained heavy metals such as mercury and silver.[3] The first favorable response with a chemotherapeutic agent was detailed in 1865 with Lissauerer's report on potassium arsenite;[3] however, it has not been until recently that truly effective treatments have been derived. For example, one of the most successful has been the development of cyclophosphamide.[4]

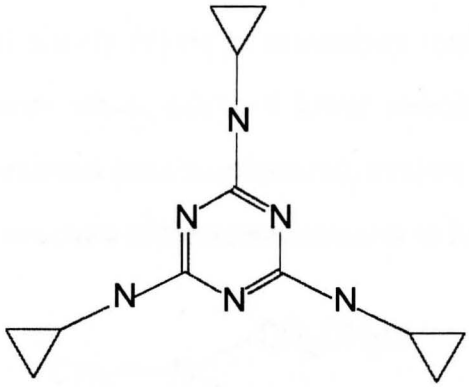
Cyclophosphamide belongs to one of the most widely used families of chemotherapeutics -- the alkylating agents.[5] Alkylating agents are categorized into distinct classes based on structural differences.[4] These classes are as follows:

1. Nitrogen and Sulfur mustards

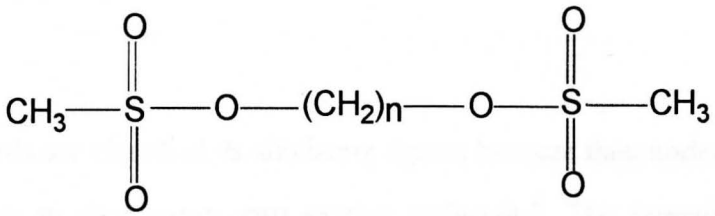




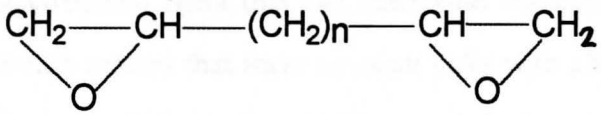
2. Ethylene imines



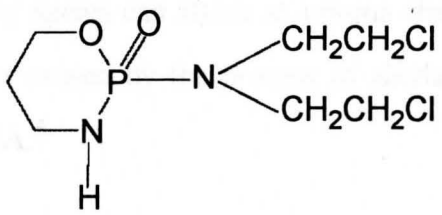
3. Esters of sulfonic acids



4. Epoxides[6]

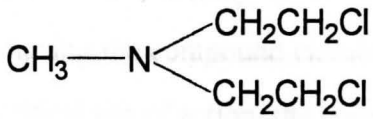


Cyclophosphamide is classified as a nitrogen mustard. It has the following structure:



Nitrogen mustards were developed from sulfur mustard analogs during World War II.[7] Studies conducted on members of the military exposed to chemical warfare agents (i.e. sulfur mustards), showed toxic effects such as depression of the lymphoid system and leucopenia.[8] U.S. medical officer S.F. Alexander, who made these observations, recognized the clinical significance of using these compounds to treat neoplasms of the

leukopoietic tissue. However, the toxic effects of chemical warfare agents on the central nervous system and high clinical activity prompted researchers toward the synthesis and development of nitrogen mustards which exhibited lower toxicity.[8] Thus, with the successful synthesis of nitrogen mustard (mechlorethamine), modern cancer chemotherapy began in the early 1940's.[3] The structure of mechlorethamine is as follows:



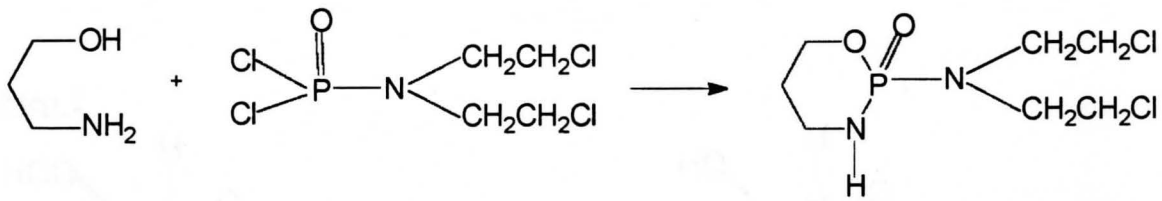
Alkylating Agents

Nitrogen mustards are classified as alkylating agents because they undergo reactions in which they introduce an alkyl group onto another molecule.[5] The formation of the alkyl group occurs when a H· atom is removed from an alkane. The once unreactive alkane is now transformed into an electrophilic agent that can react with nucleophilic or electron-rich centers.[6] The electron-rich centers that form covalent links with alkylating agents in biomolecules are the sulfhydryl (-SH), amino ($-NH_2$), and hydroxy groups (-OH).[3] Because the cells of the human body are primarily composed of molecules with these types of functional groups, the alkylating agents can attack at various chemical positions in the cell.[5] The major biological effect caused by the process of alkylation occurs when the alkylating agent interacts with DNA.[5]

Cyclophosphamide

Clinical tests have shown that alkylating agents are some of the most effective treatments against various forms of cancer.[9] Cyclophosphamide, which is a derivative of nitrogen mustard, is one of the most exhaustively studied alkylating agents.[6] Cyclophosphamide was first synthesized in Germany in 1958[10] under the premise of latentiation.[4] Latentiation involves having the compound circulate through the body in an inactive form and then, once at the critical site of action, the compound is transformed into the active alkylating agent.[7] Cyclophosphamide was designed specifically to be the transport for the nitrogen mustard.[10] Its design was based on the idea that malignant cells exhibit higher levels of phosphoramidase enzyme activity and since the inactive transport consisted of an ester amide of phosphoric acid, preferential activation should occur within the malignant cell.[11] Cyclophosphamide quickly showed a high degree of clinical success and became one of the most widely used alkylating agents. It can be administered either orally or intravenously,[6] and it is safer than other alkylating agents when given in large doses.[7]

Synthesis of Cyclophosphamide

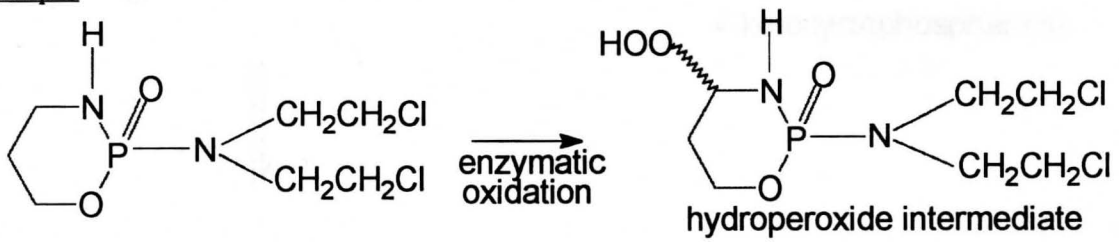


In the synthesis of cyclophosphamide, one mole of 3-propanolamine combines with one mole of dichloro-N-(dichloroethyl)phosphoramide in the presence of two equivalents of triethylamine to yield one mole of cyclophosphamide and two moles of triethylamine hydrochloride.[11]

Mechanism of Action of Cyclophosphamide

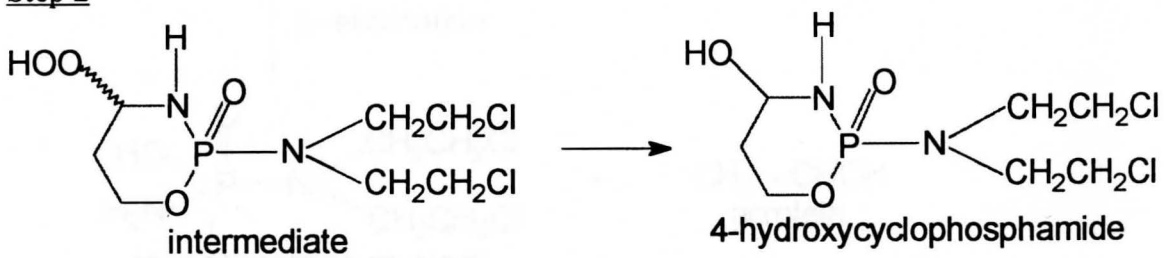
The bioactivation of cyclophosphamide is a complex process.[11] Unlike most alkylating agents that react immediately, cyclophosphamide must be activated.[5] This process of activation has been studied by a large number of researchers and volumes of information have been compiled.[10] The current mechanism put forth has been derived from experimentally isolated metabolites;[10] however, the mechanism is still not completely understood. It will require future investigations into the processes of hydrolysis, oxidation and rearrangement of the ring system as well as other activation steps before it can be considered completely accurate.[4] The following outlines the currently defined mechanism of activation for cyclophosphamide.

Step 1



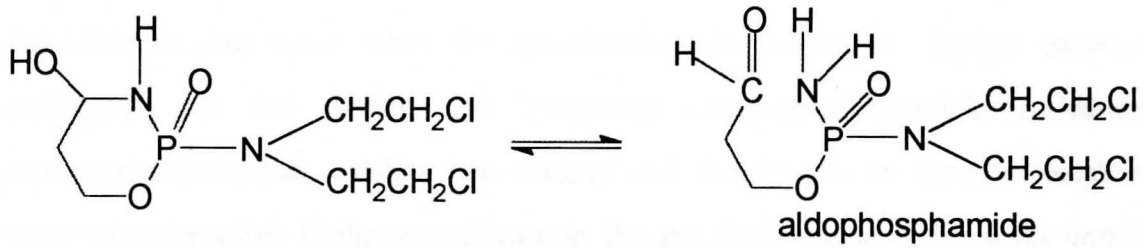
In step 1, cyclophosphamide undergoes microsomal oxidation[5] by the hepatic cytochrome P-450 oxidase system of the liver to form an intermediate hydroperoxide.[11]

Step 2



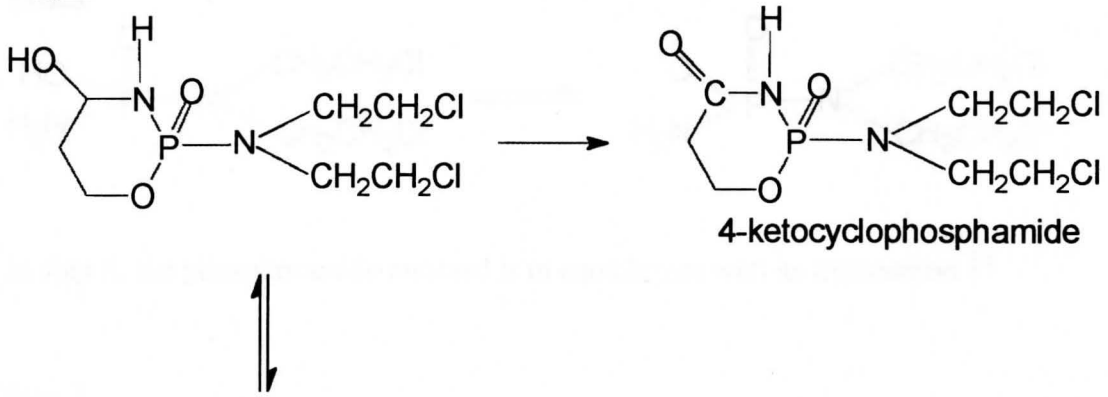
In step 2, the hydroperoxide intermediate undergoes more microsomal activity and is converted to the 4-hydroxycyclophosphamide.

Step 3

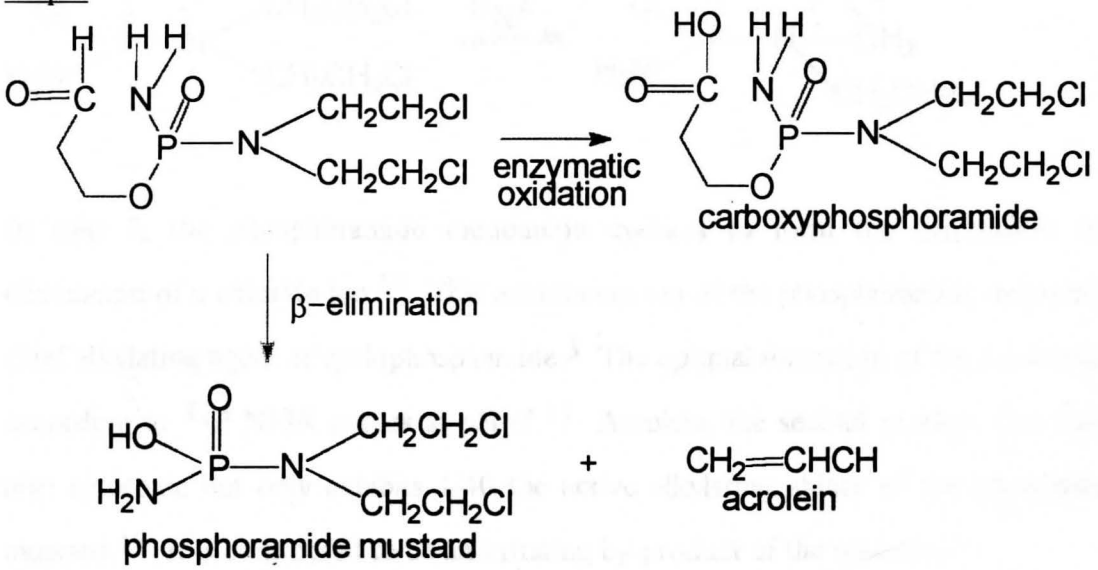


In step 3, the 4-hydroxycyclophosphamide (an aldehydeamine) is in equilibrium with its tautomer, the open chain amino aldehyde form, aldophosphamide.[10]

Step 4



Step 5



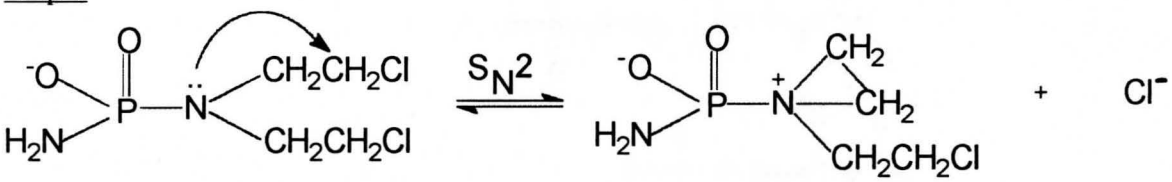
In step 4, 4-ketocyclophosphamide is formed. This compound exhibits low toxicity and is considered to be an inactive metabolite that is predominantly excreted in the urine.[10] In

step 5, the intermediate aldophosphamide can undergo one of two types of reactions. The first reaction can occur when the aldophosphamide undergoes further enzymatic oxidation. If this occurs, the compound carboxyphosphoramide is formed. Carboxyphosphoramide exhibits low toxicity and therefore is an inactive metabolite excreted in the urine. In the second reaction, the aldophosphamide is chemically unstable and can undergo β-elimination to form phosphoramide mustard and acrolein.[10] Note this β-elimination is not an enzymatically induced step.[11]

Step 6

In step 6, the phosphoramide mustard is in equilibrium with its monoanion.[11]

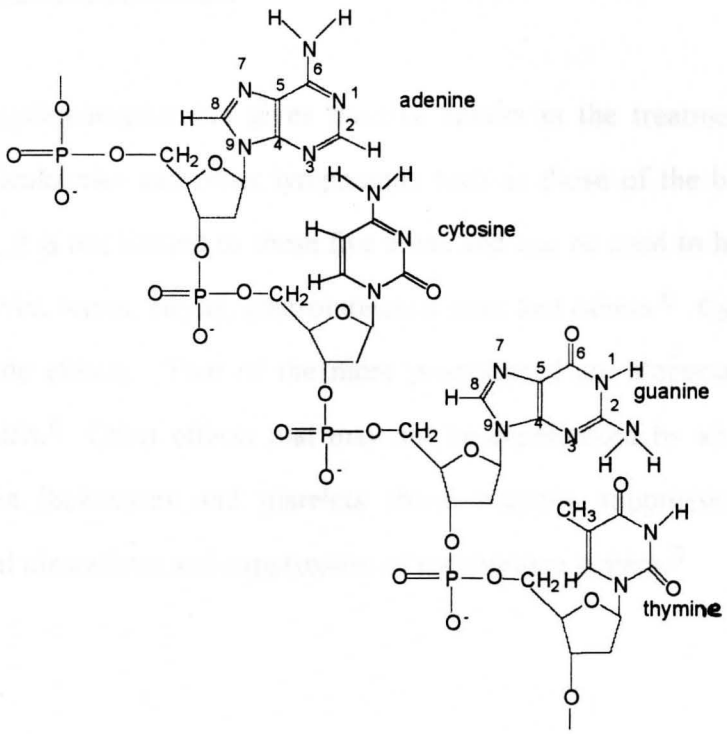
Step 7



In step 7, the phosphoramide monoanion cyclizes to form the aziridinium ion by elimination of a chloride ion.[11] This aziridinium ion of the phosphoramide mustard is the chief alkylating agent of cyclophosphamide.[3] The optimal formation of the aziridinium ion according to ^{31}P NMR occurs at pH=7.[11] Acrolein, the second product that forms, is also cytotoxic but only exhibits 1/40 the active alkylating ability of the phosphoramide mustard.[11] It is considered to be an irritating by-product of the reaction.[3]

Activity of Cyclophosphamide

The major biological effect that results from alkylating agents such as cyclophosphamide occurs as a result of their interaction with DNA. The specific site of interaction is the N-7 atom of guanine and to a smaller extent the N-3 position of adenine.[5] This alkylating ability occurs in the ratio of about 90% to 10% respectively.[3] A small section of DNA shown below allows for easy reference to the positions in the adenine and guanine molecules that undergo alkylation.[12]



Alkylating agents can have two effects on cells. They can either inhibit cell growth or they can kill the cell completely.[3] Therefore, alkylating agents are not cell cycle specific and they can kill both resting and proliferating cells.[3] Two types of damage may occur when alkylating agents interact with DNA. First a single alkylation of a guanine moiety may occur. In the second example, if a bifunctional agent is present, two guanine moieties may be alkylated concurrently. These two moieties may either be in the same strand of DNA or come from two completely different strands of DNA.[4] Concurrent alkylation

may lead to three results. First, cross-linking of two DNA strands may occur. In the second result, one strand of DNA may cross-link with a protein molecule. In the third result, single or double strand breaks of DNA may occur which leads to depurination.[3] Regardless of where the cross-linkages occur, interference of DNA replication leads to inhibition of cell growth which is the desired result.[5] Therefore, the cytotoxicity of the drug directly correlates with its ability to cause cross-linking and subsequent death of the cell.[3]

Clinical use of Cyclophosphamide

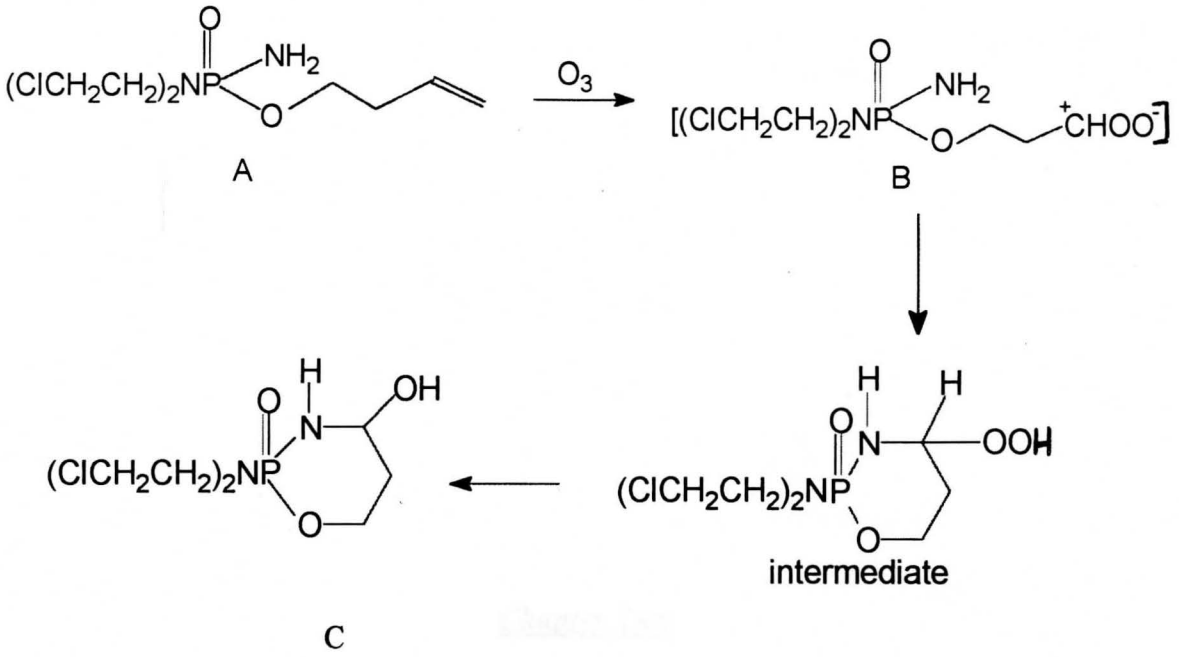
Clinically, cyclophosphamide gives positive results in the treatment of Hodgkin's disease, chronic leukemias and other lymphomas such as those of the breast, ovary and lung.[6] However, it is not limited to these five areas and can be used to help treat cancers of the uterus, cervix, testes, larynx, gastrointestinal tract and others.[6] Cyclophosphamide is not without side effects. Two of the more pronounced are alopecia (hair loss) and hemorrhagic cystitis.[6] Other effects that may not be experienced by all patients include depression of the leukocytes and platelets (bone marrow suppression), nausea and vomiting, mucosal ulcerations and suppression of the immune system.[3]

Summary

When cyclophosphamide was initially synthesized and clinically tested, it was found that the active metabolite displayed 200 times the cytotoxicity than was originally expected.[5] These positive results led researchers to develop several types of alkylating agents with different carrier groups that react at different intracellular sites and require different modes of activation.[9] To give a broad overview of the extent of the chemical spectrum that phosphorus mustards encompass a few examples follow. In the first series,

reactions of acetal-glycosides and their glycoconjugates are reacted with the usually labile intermediate, aldophosphamide. These compounds were designed under the premise that aldophosphamide would be released from these types of carrier structures by acid-catalyzed or pH selective enzymatic hydrolysis due to stimulation of tumor cell glycolysis. In this specific instance aldophosphamide was combined with α and β-D-glucose as well as with α-D-mannose. To further ensure that a high degree of acid-labile acetal-glycosides became present in tumor cells the compounds were synthesized with a carboxylate moiety that acted as a spacer to allow for easier and direct binding to peptides which in turn allowed the compounds to combine with tumor associated antibodies.[13] In another series, analogs of cyclophosphamide which contain ethoxy carbonyl groups were synthesized. This example demonstrates the fact that although compounds can be very similar, such as five and six membered rings which contain the same substituent groups in the same relative positions, one will be more active against inhibiting tumor cell growth.[14] In a physiologically important example, consideration is given to the extensive effects that hormones cause in the human body. By coupling cyclophosphamide to estrone a more selectively cytotoxic agent was hoped for which would be chemically attracted to neoplasms that display hormonal tendencies.[15] In another physiologically important example, the effect of chirality is investigated.[16] By definition, chirality means an object is nonsuperimposable on its mirror image.[17] It follows; therefore, by definition that the human body is chiral. When chiral compounds enter the body such as in the example of (+) and (-) thalidomide, both compounds undergo metabolic reactions; however, one compound can be beneficial with minimal side effects while the other with the opposite chirality can be detrimental with severe side effects. Therefore it is imperative that when new chiral drugs are designed, tests are conducted to determine which form is the least detrimental and exhibits the fewest side effects. These studies allow doctors to prescribe the safest form of the drug to treat patients.[18] In the previous examples the compounds were all cyclic in nature; however, the final example demonstrates that not all compounds

have to be cyclic.[19] For example, aliphatic compounds such as 3-buten-1-ol can also react to form phosphorus mustards.



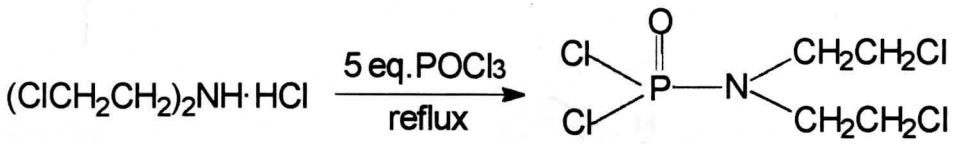
Upon ozonization of compound A, the zwitter ion of compound B is formed. This reaction gives a highly cytostatic result because compound B is easily converted to compound C by biological reduction. The aliphatic compounds have their own unique characteristics as far as reactivity and selectivity are concerned, and they also have their own degree of success as clinical treatments. These few examples demonstrate only a small spectrum of the chemical principles that need to be considered when synthesizing a new phosphorus mustard. However, the development of these new drugs has provided researchers with new understanding that high antitumor activity is directly proportional to toxicity.[5] Therefore, the degree of toxicity has to be controlled by developing drug administration techniques and continued modification of the carrier.[5] To date, the successful use of alkylating agents has led to the synthesis of thousands of variations of these compounds.[6]

Chapter Two

Results and Discussion

Results and Discussion

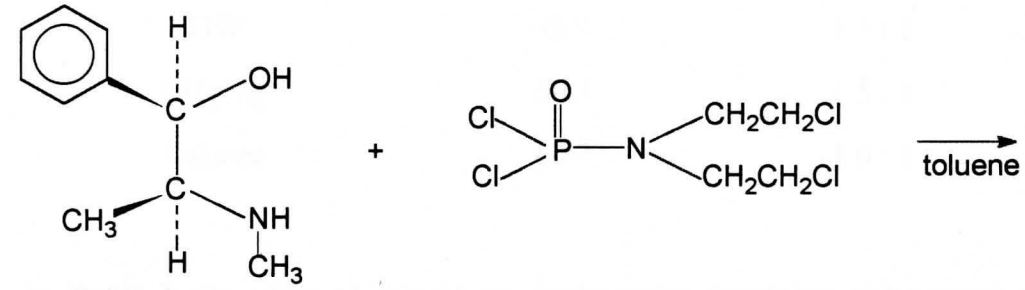
Synthesis of the starting material, bis-(2-chloroethyl)phosphoramidic dichloride (**3**) was performed according to literature methods.[20] The reaction shown below outlines the synthesis of compound (**3**):

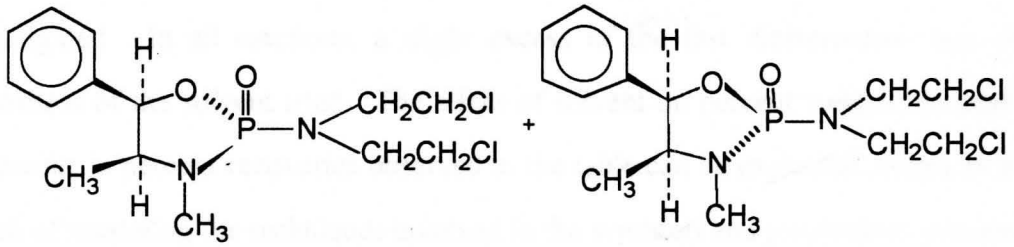


(**1**) (**2**) (**3**)

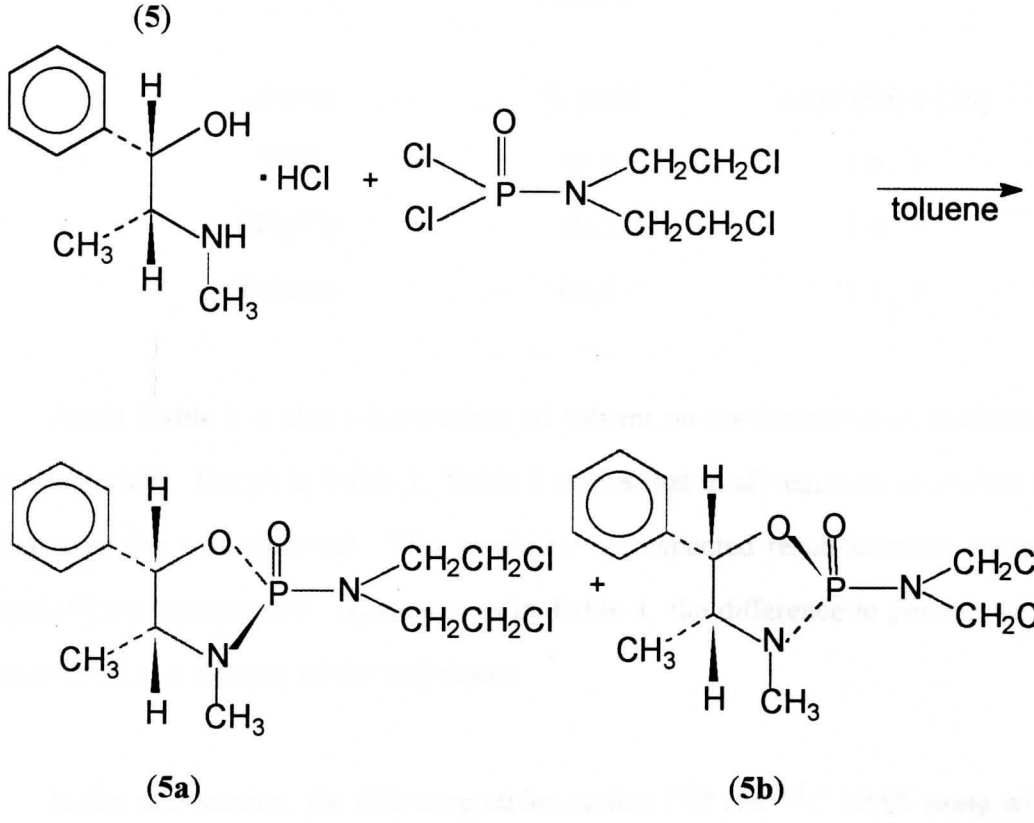
The first series of 1,3,2-oxazaphospholidin-2-ones to be synthesized contained (+) and (-)-ephedrine. As can be shown in the following syntheses, the compounds are very similar in structure except for the stereochemistry which differs at carbons 4 and 5 as well as at phosphorus. The first reaction involves the synthesis by use of (-)-ephedrine (**4**) while the second reaction uses (+)-ephedrine hydrochloride (**5**).

(**4**)





(**4a**) (**4b**)



(5a) **(5b)**

Table 1

solvent	% yield	ratio 4a : 4b
THF	45.2	1.1 : 1
CH_2Cl_2	50.7	1.5 : 1
Toluene	60.7	1.6 : 1

In **Table 1** the effect of solvent on diastereomer formation and percent yield is investigated. In all reactions, a slight excess of the fast diastereomer was observed, regardless of the solvent used. The effect of solvent on percent yield is minimal and the difference in percent recoveries observed in the table can be explained simply as being the result of mastering the techniques involved in the synthesis and purification processes.

Table 2

solvent	% yield	ratio (5a) : (5b)
THF	41.4	1.6 : 1
CH_2Cl_2	50.2	2.4 : 1
Toluene	60.9	1.1 : 1

Again **Table 2** is also a comparison of solvent on the formation of diastereomers and percent yield. Just as in **Table 1**, **Table 2** shows that in all reactions an excess of the fast diastereomer was observed. This would be the expected result because compounds (**4**) and (**5**) are enantiomers. Again just as in **Table 1**, the difference in percent yields can be attributed to a mastery of the techniques.

In the next section, the following tables outline ^{31}P and ^{13}C NMR along with mp and $[\alpha]^{25}_D$ data for compounds (**3**), (**4a**), (**4b**), (**5a**), and (**5b**) as well as for each starting material (**4**) and (**5**) where applicable. A tabular format was chosen to make the data easily presentable for future comparisons. Also for convenience and easy reference, the assignment of carbon atoms is given for each product that was recovered.

Table 3

^{31}P NMR

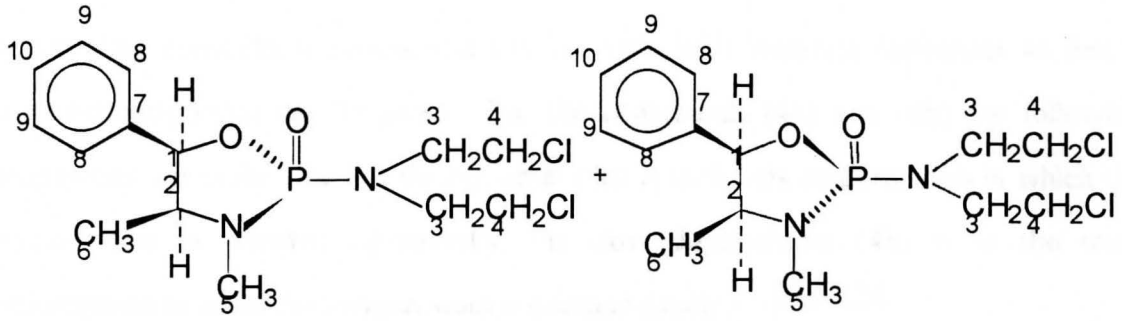
Compound Number	Chemical Shift	$[\alpha]^{25}_D$	mp
3	18.6	NA	53-54
4a	25.4	-28.5	105-109
4b	26.0	-47.0	129-134
5a	25.5	+42.4	106-110
5b	26.0	+78.1	133-135

In **Table 3** good agreement is observed when comparing the ^{31}P chemical shifts and melting points for each of the diastereomeric pairs. The diastereomeric pairs as determined by ^{31}P NMR are: (**4a**)-(**5a**) and (**4b**)-(**5b**). The slow diastereomer, designated as (**b**) shows the greatest chemical shift along with the highest melting point. A direct correlation between $[\alpha]^{25}_{D}$ values cannot be made due to the inconsistencies and lack of sensitivity associated with the antiquated equipment which was used to perform the analyses.

Table 4

^{13}C NMR

Carbon Number	(4)	(4a) (m, J)	(4b) (m, J)
7	142.23	135.97 (d, 6.9 Hz)	135.96 (d, 9.9 Hz)
9	127.85	128.53 (s)	128.65 (s)
8	126.77	128.31 (s)	128.28 (s)
10	126.04	125.96 (s)	125.57 (s)
1	73.56	81.65 (s)	78.28 (d, 3.8 Hz)
2	60.29	59.46 (d, 13.0 Hz)	59.61 (d, 11.5 Hz)
3	NA	49.79 (d, 4.6 Hz)	49.66 (d, 5.3 Hz)
4	NA	42.50 (s)	42.48 (s)
5	33.49	28.55 (d, 4.5 Hz)	29.49 (d, 6.1 Hz)
6	13.67	13.96 (s)	14.91 (d, 1.5 Hz)



(**4a**) (**4b**)

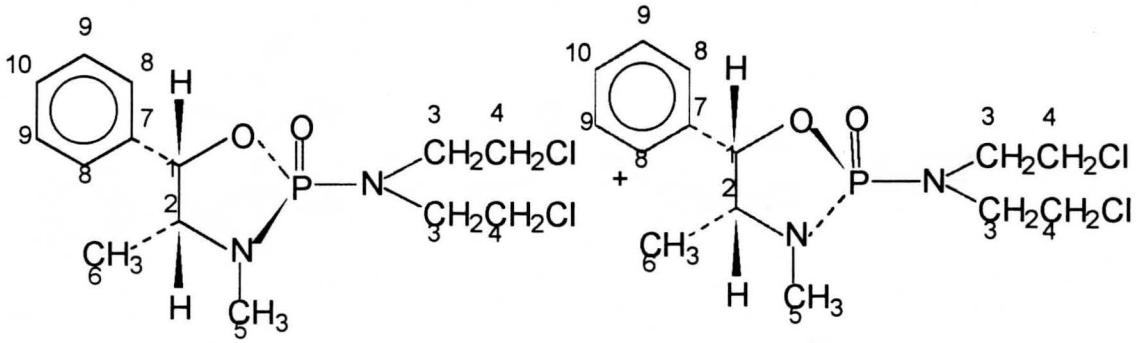
In **Table 4** the chemical shift, multiplicity and coupling constant is given for each carbon atom in compounds (**4a**) and (**4b**). From the table it can be noted that four carbon atoms are being acted upon by phosphorus in (**4a**) and six in (**4b**) because their multiplicities change from singlets to doublets. In compound (**4a**) the following carbon atoms are coupled to the phosphorus atom: C-7, C-5, C-3, C-2. In compound (**4b**) all of the previously mentioned carbon atoms in (**4a**) are coupled to phosphorus as well as C-1 and C-6. The coupling constants for their respective carbons are approximately of the same magnitude. Fom the coupling constants, the carbons can be ranked according to their distance away from the phosphorus atom either through space orientation or the number of actual bonds connecting the two atoms. In (**4a**) C-3 and C-5 are approximately the same distance away (4.6 Hz and 4.5 Hz respectively). The other two coupling constants are 6.9 Hz and 13.0 Hz at C-7 and C-2 respectively. In (**4b**) C-6 is the closest at 1.5 Hz, then C-1 at 3.8 Hz, then C-5 and C-3 at 6.1 and 5.3 respectively. Finally C-7 and C-2 are found to be the furthest distance away at 9.9 Hz and 11.5 Hz respectively. Compound (**4b**) would seem to be exhibiting a more crowded structure than compound (**4a**) because of the greater degree of interaction with the carbon atoms. If the structure of (**4b**) is crowded it would support the fact that (**4a**) occurs 1.5 times more because it is a less sterically hindered product. Due to the fact that complete data analysis (x-ray crystal structure) is incomplete at the current time, exact conformations have not been assigned to each diastereomer. However, by studying the ^{31}P chemical shifts and

the coupling constants a comparison can be made with literature references so that a structural assignment can be given. For the compounds (**4a**) and (**4b**) the following assignments are made: the fast diastereomer (**4a**) is in the cis conformation in which the oxygen atom is oriented equatorially, the slow diastereomer (**4b**) is in the trans conformation in which the oxygen atom is oriented axially.[21,22,23,24]

Table 5

^{13}C NMR

Carbon Number	(5)	(5a) (m, J)	(5b) (m, J)
7	141.39	136.29 (d, 6.1 Hz)	135.99 (d, 10.7 Hz)
9	128.19	128.46 (s)	128.62 (s)
8	127.16	128.23 (s)	128.24 (s)
10	126.14	125.93 (s)	125.54 (s)
1	72.94	81.54 (s)	78.23 (d, 3.8 Hz)
2	60.62	59.30 (d, 13.0 Hz)	59.56 (d, 11.5 Hz)
3	NA	49.63 (d, 4.6 Hz)	49.60 (d, 5.3 Hz)
4	NA	42.45 (s)	42.50 (s)
5	33.89	28.44 (d, 5.4 Hz)	29.45 (d, 5.3 Hz)
6	13.89	13.91 (s)	14.88 (d, 1.5 Hz)

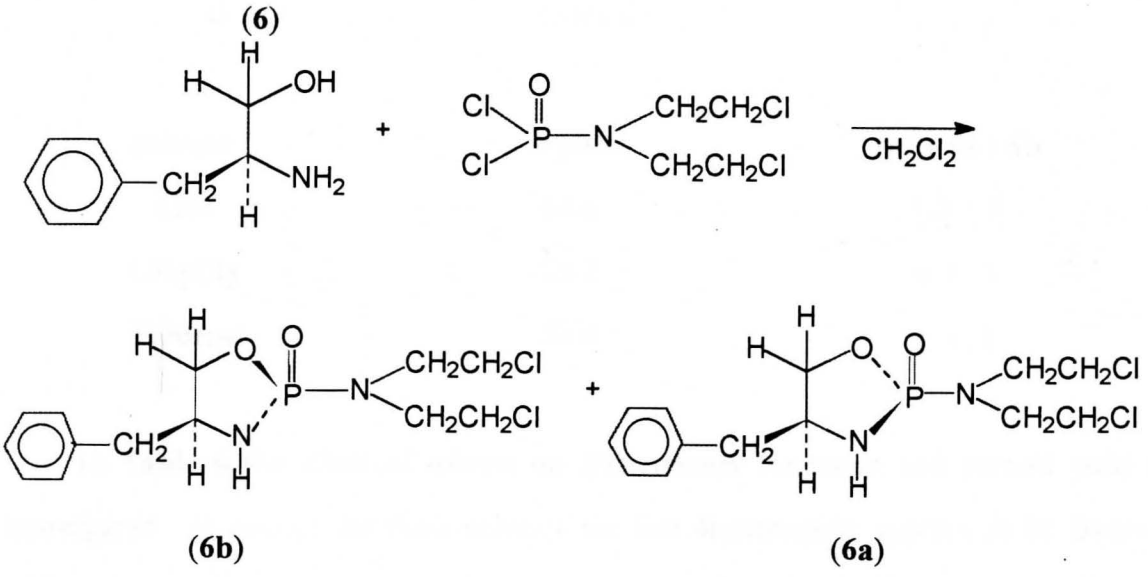


(5a)

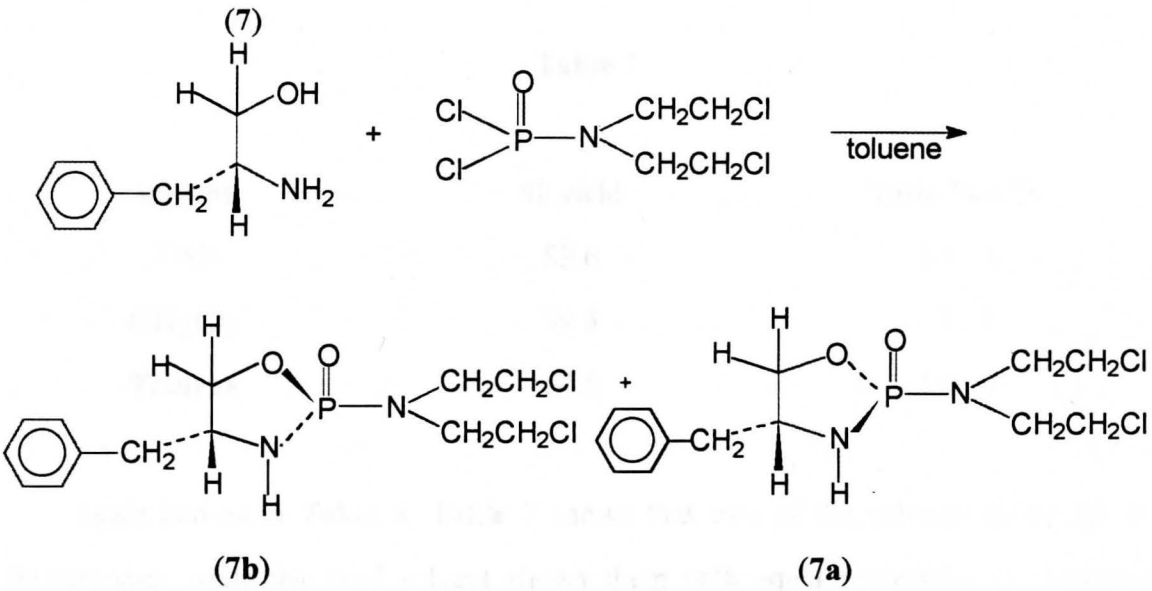
(5b)

Just as in **Table 4**, **Table 5** also illustrates chemical shift for each carbon atom along with the multiplicity and coupling constant. Also just as in **Table 4**, **Table 5** gives the same results as those discussed for compounds (**4a**) and (**4b**) because the compounds are enantiomeric pairs. The only difference is for a few **J** values which are still close enough to agree within experimental error. As discussed with (**4a**) and (**4b**) complete analysis has not been completed for these structures; therefore, the structural assignments are based primarily on comparison of ^{31}P NMR observed experimentally with those in literature. Due to the fact that compounds (**4a**), (**4b**), (**5a**) and (**5b**) are diastereomeric pairs it follows that (**5a**) must have the same relative stereochemistry as (**4a**) and (**5b**) must have the same relative stereochemistry as (**4b**). Therefore, (**5a**) is cis and (**5b**) is trans in their respective conformations.

The second series of 1,3,2-oxazaphospholidin-2-ones to be synthesized contained either (R)-(+)-2-amino-3-phenyl-1-propanol (**6**) or (S)-(-)-2-amino-3-phenyl-1-propanol (7) as the starting material. As can be shown in the following syntheses, the compounds are very similar in structure except for the stereochemistry at carbons 2 and 5 as well as at phosphorus. The first reaction involves the synthesis by use of compound (**6**) while the second involves compound (7).


(6)
OH
NH2
CH2
Cl
Cl
O
P
N
CH2CH2Cl
CH2CH2Cl
CH2Cl2
CH2
N
H
O
P
N
CH2CH2Cl
CH2CH2Cl

(6b) **(6a)**


(7)
OH
NH2
CH2
Cl
Cl
O
P
N
CH2CH2Cl
CH2CH2Cl
toluene
CH2
N
H
O
P
N
CH2CH2Cl
CH2CH2Cl

(7b) **(7a)**

Table 6

solvent	% yield	ratio 6a : 6b
THF	64.6	1.3 : 1
CH_2Cl_2	73.2	0.9 : 1
Toluene	54.0	1.9 : 1

In **Table 6** the effect of solvent on diastereomer formation and percent yield is investigated. In two of the three solvents the fast diastereomer appears to be favored while in the third solvent the slow diastereomer is slightly favored. However, the effect of solvent on percent yield and the formation of the diastereomers is minimal. Therefore, it will be assumed in all the reactions that an excess of the fast diastereomer is preferred and any discrepancies are due to unrefined techniques.

Table 7

solvent	% yield	ratio 7a : 7b
THF	53.6	1.1 : 1
CH_2Cl_2	59.5	1 : 1
Toluene	64.0	1.9 : 1

Again just as in **Table 6**, **Table 7** shows that two of the solvents favor the fast diastereomer while the third solvent shows them with equal probability of occurring. Again this follows from **Table 6** as should be expected because the compounds are diastereomeric pairs. Any discrepancies are simply the result of technique during purification.

In the next section, the following tables outline ^{31}P and ^{13}C NMR along with $[\alpha]^{25}_D$ and mp data for compounds (**3**), (**6a**), (**6b**), (**7a**) and (**7b**) as well as for each starting material (**6**) and (**7**) where applicable. A tabular format was chosen to make the data easily presentable for future comparisons. Also for convenience, the assignment of carbon atoms is given for each product that was recovered.

Table 8

^{31}P NMR

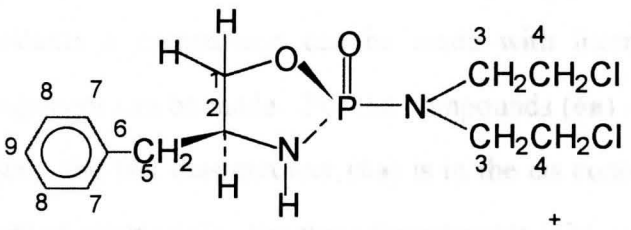
Compund Number	Chemical Shift	$[\alpha]^{25}_D$	mp
(3)	18.6	NA	53-54
(6a)	28.8	+19.9	NA
(6b)	30.2	+76.5	84-88
(7a)	28.5	+59.9	NA
(7b)	30.5	-34.1	86-88

In **Table 8** good agreement is observed when comparing the ^{31}P chemical shifts and melting points for each of the diasteromeric pairs. As can be seen from the table the fast diastereomer (**6a**) was a yellow oil at room temperature; therefore, it did not have a melting point. The slow diastereomer was a white, crystalline solid at room temperature; therefore, it did have a melting point. Again just as in the ephedrine series [compounds (**4**) and (**5**)], the slow diastereomer showed the greatest chemical shift and the highest melting point. Again just as with the previously mentioned compounds a direct correlation between $[\alpha]^{25}_D$ values cannot be made due to the antiquated equipment which was used to perform the analyses.

Table 9

^{13}C NMR

Carbon Number	(6)	(6a) (m, J)	(6b) (m, J)
6	138.8	136.7 s	136.6 s
7	129.3	129.1 s	129.1 s
8	128.6	129.0 s	129.0 s
9	126.5	127.1 s	127.2 s
1	66.2	70.7 (d, 1.5 Hz)	70.9 s
2	54.3	56.1 (d, 9.2 Hz)	54.3 (d, 9.9 Hz)
3	NA	49.3 (d, 4.5 Hz)	49.4 (d, 4.5 Hz)
4	NA	42.3 s	42.4 s
5	40.8	42.1 (d, 3.8 Hz)	41.8 (d, 9.2 Hz)



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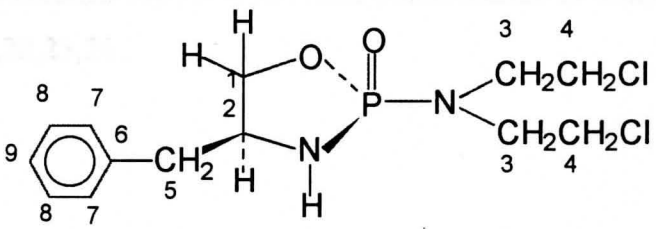


(6b) **(6a)**

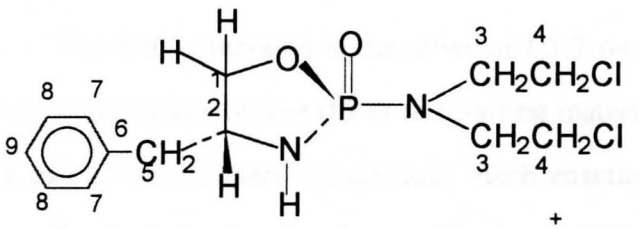
In **Table 9** the chemical shift, multiplicity and coupling constant is given for each carbon atom in compounds (**6a**) and (**6b**). From the table it can be noted that three carbon atoms are coupled to phosphorus in (**6a**) and four in (**6b**) because their multiplicities change from singlets to doublets. In compound (**6a**) the following carbon atoms are coupled to the phosphorus atom: C-2, C-3, C-5. In compound (**6b**) all of the

previously mentioned carbon atoms in (**6a**) are coupled to phosphorus as well as C-1. The coupling constants are of the same magnitude for C-2 and C-3; however, C-5 is almost 2.4 times greater in (**6a**) than in (**6b**). From the coupling constants, the carbons can be ranked according to their distance away from the phosphorus atom either through space orientation or the number of actual bonds connecting the two atoms. In (**6a**) C-2 and C-5 are approximately the same distance away, 9.9 Hz and 9.2 Hz respectively. C-3 is the closest at approximately half of the distance with a J value of 4.5 Hz. In (**6b**) the closest is C-1 at 1.5 Hz, then C-5 at 3.8 Hz followed by C-3 and 4.5 Hz and then C-2 at 9.2 Hz. Compound (**6b**) appears to be exhibiting a more crowded structure than compound (**6a**) because of the greater degree of interaction with the carbon atoms. If the structure of (**6b**) is crowded it would support the fact that (**6a**) occurs 1.5 times more because it is a less sterically hindered product. Due to the fact that complete data analysis (x-ray crystal structure) is incomplete at the current time, exact conformations have not been assigned to each diastereomer. However, by studying the ^{31}P chemical shifts and the coupling constants a comparison can be made with literature references so that a structural assignment can be made. For the compounds (**6a**) and (**6b**) the following assignments are made: the fast diastereomer (**6a**) is in the cis conformation in which the oxygen atom is oriented equatorially, the slow diastereomer (**6b**) is in the trans conformation in which the oxygen atom is oriented axially.[21,22,23,24]

Table 10

^{13}C NMR

Carbon Number	(7)	(7a) (m, J)	(7b) (m, J)
6	138.7	136.8 s	136.6 s
7	129.3	129.1 s	129.1 s
8	128.7	129.0 s	129.0 s
9	126.6	127.2 s	127.3 s
1	66.3	70.8 s	71.0 s
2	54.3	56.1 (d, 9.9 Hz)	54.4 (d, 9.9 Hz)
3	NA	49.2 s	49.3 s
4	NA	42.4 s	42.4 s
5	40.9	42.1 (d, 3.8 Hz)	41.8 (d, 8.4 Hz)



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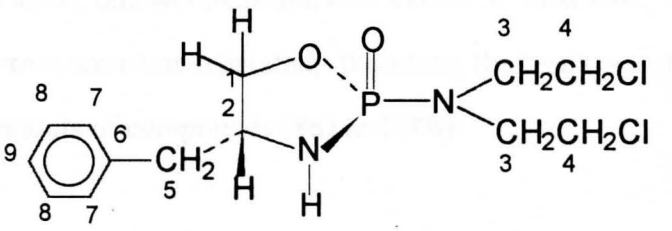
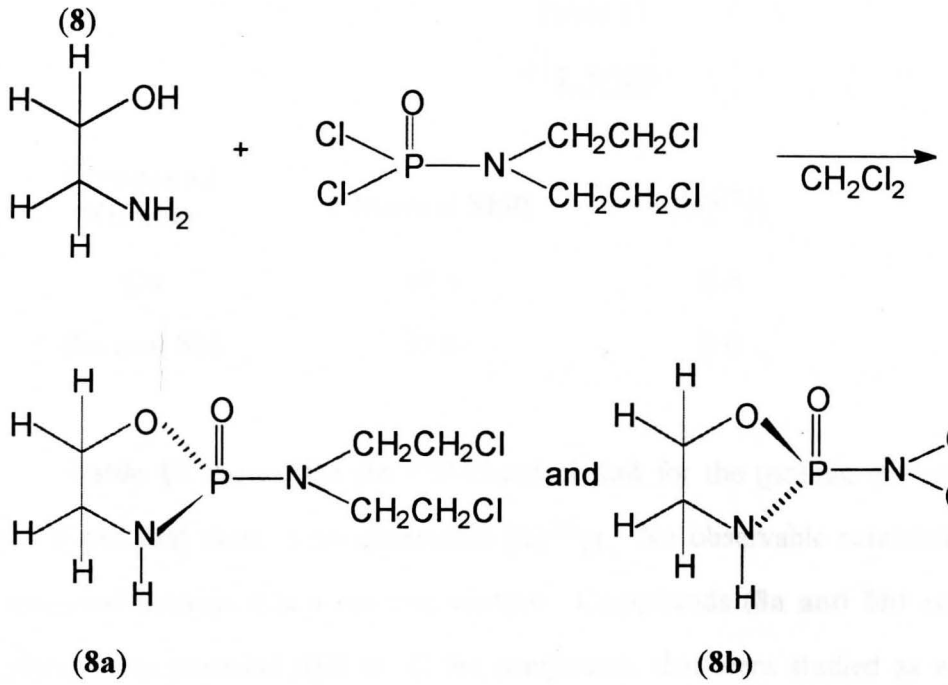


(7b) **(7a)**

Just as in **Table 9**, **Table 10** also illustrates chemical shift for each carbon atom along with the multiplicity and coupling constant. **Table 10** shows agreement with **Table 9** when chemical shifts are compared; however, they vary in the coupling of phosphorus to carbon atoms numbered C-3 and C-1. Compound (**7a**) should show the same coupling

pattern as that of compound (**6a**) because they are diastereomeric pairs. However, (**7a**) does not show coupling to C-3. Compound (**7b**) should display the same coupling pattern as (**6b**) because they are also diastereomeric pairs. However, (**7b**) does not show coupling to C-3 and C-1. Although there are a few inconsistencies they can be attributed to the NMR procedure. The NMR data obtained for compounds (**7a**) and (**7b**) was not as good as that obtained for compounds (**6a**) and (**6b**). There was less noise and better sensitivity in the latter and less sensitivity and increased noise in the former. Therefore, when comparing the structural configurations of (**6a**), (**6b**), (**7a**) and (**7b**) the same results and conclusions should have been reached just as they were for the ephedrine series which dealt with compounds (**4a**), (**4b**), (**5a**), and (**5b**). It also follows that the same assignments can be made in which the fast diastereomer is in the cis configuration and the slow diastereomer is in the trans configuration.

The final compound in the series of 1,3,2-oxazaphospholidin-2-ones to be synthesized contained ethanolamine (**8**) as the starting material. According to the following synthesis a pair of enantiomers is expected. Each enantiomer will have a different stereochemical configuration at the phosphorus atom; one will be R and one will be S. However, during this research project the enantiomers were not separated; therefore, the result is a racemic mixture which contains equal amounts of compounds (**8a**) and (**8b**).



(8a) **(8b)**

Table 10

solvent	**% yield**
THF	62.0
CH_2Cl_2	84.8
Toluene	30.7

In **Table 10** the percent yield versus the type of solvent used is shown.The methylene chloride solvent appears to give the highest percent yield of product while the toluene gives the lowest; however, these apparent solvent effects can be ascribed to inferior techniques while purifying by flash column chromatography.

The following tables outline ^{31}P NMR (where applicable) and ^{13}C NMR along with $[\alpha]^{25}_{D}$ and mp data for compounds (**8a and 8b**). Assignment of carbon atoms is also shown for easy reference.

Table 11

^{31}P NMR

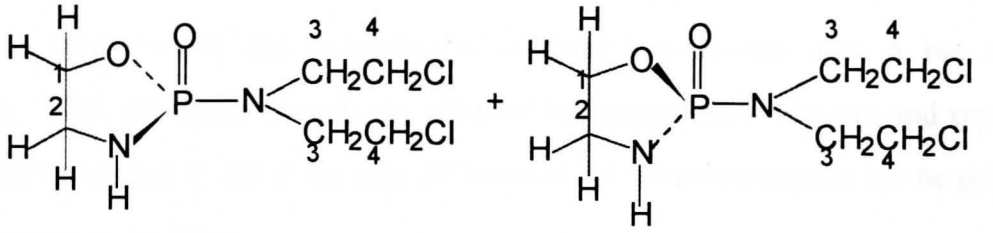
Compound Number	Chemical Shift	$[\alpha]^{25}_D$	mp
(3)	18.6	NA	53-54
(8a and 8b)	30.8	0.0	67-73

Table 11 shows that the ^{31}P chemical shift for the racemic mixture (**8a and 8b**) is 30.8 and that there is no observable $[\alpha]^{25}_D$. No observable rotational data should be expected because it is a racemic mixture. Compounds (**8a and 8b**) exhibit the greatest phosphorus chemical shift of all the compounds that were studied as well as the lowest melting point except when compared to (**6a**) which was an oil at room temperature

Table 12

^{13}C NMR

Carbon Number	(8)	(8a and 8b) (m, J)
1	57.8	66.3 (d, 2.2 Hz)
3	NA	49.3 (d, 4.6 Hz)
2	41.6	42.4 s
4	NA	42.3 (d, 9.2 Hz)



(8a) **(8b)**

In **Table 12** the chemical shift, multiplicity and coupling constant is given for each carbon atom in the racemic mixture (**8a / 8b**). From the table it can be noted that three of the four carbon atoms are coupled to phosphorus because their multiplicities change from singlets to doublets. From the coupling constants, the carbons can be ranked according to their distance away from the phosphorus atom either through space orientation or the number of bonds connecting the two atoms. As would be expected C-4 shows the greatest coupling constant because it is the farthest carbon away from the phosphorus atom. It also follows that C-3 has the second greatest coupling constant because it is two full bonds away from phosphorus. C-1 displays the smallest coupling constant and C-2 does not exhibit any coupling to the phosphorus atom at all; therefore, it cannot be ranked by its coupling constant. Therefore in this racemic mixture, the phosphorus atom can interact with almost all of the atoms which can influence the chemical properties of the compound.

Conclusion

In summary, the synthesis of chiral phosphorus mustards from chiral amino alcohols are reactions that are easily prepared and which give moderate percent yields after purification. They can also be shown to produce the expected diastereomers as witnessed by the fast and slow bands during flash column chromatography, different R_f values during TLC analysis, different melting points and other physical properties such as color and whether they were crystalline solids or yellow oils. According to ^{13}C NMR and ^{31}P NMR a preliminary assignment was made in regards to structural conformations of the

phosphorus atom which can be compared to x-ray crystal data once it has been completed. Also, for future projects the effect of low temperature reactions and type of solvent may be studied to see if the ratio of formation of the diastereomers can be shifted to prefer one over the other.

Chapter Three

Experimental

Experimental Section

General Methods. Melting points are uncorrected. All reactions were conducted under a positive pressure of argon. All solvents were dried by standard techniques. Flash chromatography was conducted with Merck grade 9385, 230-400 mesh silica. Analytical thin layer chromatography (TLC) was conducted on aluminum backed silica plates. Visualization was accomplished with an ultraviolet lamp and/or staining with 5% phosphomolybdic acid (PMA) in ether or 5% ninhydrin in absolute ethanol, with heating.

NMR spectra (^{1}H, ^{13}C, and ^{31}P) were recorded with a Varian Gemini 2000, 400 MHz spectrometer, with $CDCl_3$ or D_2O as the solvent. The ^{1}H and ^{13}C chemical shifts are reported in parts per million down field from $(CH_3)_4Si$, while ^{31}P chemical shifts are reported in parts per million down field from H_3PO_4 (external standard). Coupling constants are reported in Hertz. Fourier transform infrared spectra (FTIR) were recorded with a Perkin-Elmer 1600 or a Bio-Rad FTS 40.

Bis-(2-chloroethyl)phosphoramidic dichloride[20] (3)

To (9.99 g, 55.7 mmol) bis-(2-chloroethyl)amine hydrochloride (**1**) was added (30 mL, 49.4 g, 321.9 mmol) $POCl_3$ (**2**). The solution was heated to 100 °C with constant stirring and refluxed overnight. Upon completion of refluxing, the reaction mixture had turned a to coffee colored liquid. Phosphoryl chloride (8.0 mL) was removed by distillation under an aspirator at 32-33 °C. The remaining reaction mixture was purified by vacuum fractional distillation at 138 °C and 2.1 mm Hg. The product was a clear, colorless liquid that immediately crystallized into opaque, white, crystalline solid at room temperature (9.27 g 64% yield). Melting point 53-54 °C. IR 3020.1, 1459.6, 1353.4, 1281.4, 1119.9, 1084.4, and 979.2 cm^{-1}. ^{31}P NMR: δ 18.6. ^{13}C NMR: δ 49.25 (d, J=3.8), 40.86 (d, J=3.2). ^{1}H NMR: δ 3.67-3.54 (m, 8 H).

(5R, 4S, 2R and 5R, 4S, 2S)-2[bis-2(chloroethyl)amino]-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (4a and 4b)

To a 35 mL round bottomed flask was added bis-(2-chloroethyl)phosphoramidic dichloride (**3**) (1.20 g, 4.65 mmol), (-)-ephedrine (**4**) (0.82 g, 4.96 mmol), toluene (20 mL), and triethylamine (1.5 mL, 10.8 mmol) at 0° C. The solution was warmed to room temperature and allowed to stir overnight. The solution was filtered through a 2.0 cm layer of florisil and washed several times (60-80 mL CH_2Cl_2). Excess solvent was removed to yield a white, shiny, crystalline solid which was purified by flash column chromatography [55.0 g silica (200 mL 100% ether), (250 mL 1:1 ether-ethyl acetate), (400ml 100% ethyl acetate)]. Fast diastereomer (**4a**): 0.61 g, (37.4% yield), mp 105-109 °C, off white, crystalline solid. R_f = 0.5 (1:1 ether-ethyl acetate). $[\alpha]^{25}_D$ = -28.5°. ^{31}P NMR: δ 25.4. ^{13}C NMR: δ 135.97 (d, J=6.9 Hz), 128.53, 128.31, 125.96, 81.65, 59.46 (d, J=13.0 Hz), 49.79 (d, J=4.6 Hz), 42.50, 28.55, (d, J=4.5 Hz), 13.96. ^{1}H NMR: δ 7.41-7.26 (m, 5 H), 5.47 (dd, 1 H, J=6.2 , 2.3 Hz), 3.76-3.50 (m, 5 H), 3.47-3.32 (m, 4 H), 2.68 (d, 3 H, J=10.4 Hz), 0.84 (d, 3 H, J=6.6 Hz).

Slow diastereomer (**4b**): 0.38 g, (23.4% yield), mp 129-134 °C, off white, crystalline solid. R_f = 0.3 (1:1 ether-ethyl acetate). $[\alpha]^{25}_D$ = -47.0°. ^{31}P NMR: δ 26.0. ^{13}C NMR: δ 135.96 (d, J=9.9 Hz), 128.65, 128.28, 125.57, 78.28 (d, J=3.8 Hz), 59.61 (d, J=11.5 Hz), 49.66 (d, J=5.3 Hz), 42.48, 29.49 (d, J=6.1 Hz), 14.91 (d, J=1.5 Hz). ^{1}H NMR: δ 7.41-7.21 (m, 5 H), 5.75 (d, 1 H, J=6.8 Hz), 3.71-3.62 (m, 4 H), 3.60-3.38 (m, 5 H), 2.71 (d, 3 H, J= 9.6 Hz), 0.74 (d, 3 H, J=6.4 Hz).

(5S, 4R, 2R and 5S, 4R, 2S)-2-[bis-(2-chloroethyl)amino]-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (5a and 5b)

To a 25 mL round bottomed flask was added bis-(2-chloroethyl)phosphoramidic dichloride (**3**) (0.25 g, 0.98 mmol), (+)-ephedrine hydrochloride (**5**) (0.20 g, 1.01 mmol), toluene (10 mL), and triethylamine (0.6 mL, 4.31 mmol) at 0 °C. The solution was warmed to room temperature and allowed to stir overnight. A thick, white, opaque precipitate formed when the reaction was complete. The solution was filtered through a 1.0 cm layer of florisil and washed several times (60-80 mL CH_2Cl_2). Excess solvent was removed to yield a white, shiny crystalline solid which was purified by flash column chromatography [20.0 g silica (100 mL 100% ether), (150 mL 1:1 ether-ethyl acetate), (150 mL 100% ethyl acetate)]. Fast diastereomer (**5a**): 0.11 g, (31.9% yield), mp 106-110 °C, white, crystalline solid. R_f = 0.4 (1:1 ether-ethyl acetate). $[\alpha]^{25}_D$ = -47.1°. ^{31}P NMR: δ 25.5. ^{13}C NMR: δ 136.29 (d, J=6.1 Hz), 128.46, 128.23, 125.93, 81.54, 59.30 (d, J=13.0 Hz), 49.63 (d, J=4.6 Hz), 42.45, 28.44 (d, J=5.4 Hz), 13.93. ^{1}H NMR: δ 7.38-7.32 (m, 5 H), 5.48 (dd, 1 H, J=6.2 , 2.2 Hz), 3.78-3.61 (m, 4 H), 3.61-3.51 (m, 1 H), 3.49-3.36 (m, 4 H), 2.68 (d, 3 H, J=10.4 Hz), 0.85 (d, 3 H, J=6.6 Hz).

Slow diastereomer (**5b**): 0.10 g, (29.0% yield), mp 133-135 °C, white, crystalline solid. R_f = 0.2 (1:1 ether-ethyl acetate). $[\alpha]^{25}_D$ = +78.1°. ^{31}P NMR: δ 26.1. ^{13}C NMR: δ 135.99 (d, J=10.7 Hz), 128.62, 128.24, 125.54, 78.23 (d, J=3.8 Hz), 59.56 (d, J=11.5 Hz), 49.60 (d, J=5.3 Hz), 42.50, 29.45 (d, J=5.3 Hz), 14.88 (d, J=1.5 Hz). ^{1}H NMR: δ 7.40-7.19 (m, 5 H), 5.74 (d, 1 H, J=6.8 Hz), 3.70-3.63 (m, 4 H), 3.58-3.37 (m, 5 H), 2.70 (d, 3 H, J=9.7 Hz), 0.73 (d, 3 H, J=6.6 Hz).

(5R, 2S and 5R, 2R)-2[bis-(2-chloroethyl)amino]-4-benzyl-1,3,2-oxazaphospholidin-2-one (6a and 6b)

To a 35 mL round bottomed flask was added bis-(2-chloroethyl)phosphoramidic dichloride (**3**) (0.33 g, 1.29 mmol), (R)-(+)-2-amino-3-phenyl-1-propanol (**6**) (0.21 g, 1.36 mmol), CH_2Cl_2 (15 mL), and triethylamine (1.5 mL, 10.8 mmol) at 0° C. The solution was warmed to room temperature and allowed to stir overnight. The solution was filtered through a 1.0 cm layer of florisil and washed several times (60-80 mL CH_2Cl_2). Excess solvent was removed to yield a white, oily residue which was purified by flash column chromatography [12.3 g silica (250 mL 9:1 ether-ethyl acetate), (250 mL 1:1 ether-ethyl acetate), (120 mL 100% ethyl acetate)]. Fast diastereomer (**6a**): 0.15 g, (34.4% yield), clear, yellow oil. R_f = 0.2 (7:3 ether-ethyl acetate). $[\alpha]^{25}_D$ = +19.9°. ^{31}P NMR: δ 28.5. ^{13}C NMR: δ 136.74, 129.11, 129.06, 127.12, 70.74 (d, J=1.5 Hz), 56.06 (d, J=9.2 Hz), 49.26 (d, J=4.5 Hz), 42.33, 42.10 (d, J=3.8 Hz). ^{1}H NMR: δ 7.36-7.18 (m, 5 H), 4.24 (ddd, 1 H, J=13.6 , 9.1 , 6.4 Hz), 4.10 (ddd, 1 H, J=9.1 , 7.6 , 6.0 Hz), 3.91-3.83 (m, 1 H), 3.61-3.55 (m, 4 H), 3.50-3.28 (m, 4 H), 3.11 (d, 1 H, J=15.4 Hz), 2.94 (dd, 1 H, J=13.4 , 8.2 Hz), 2.87 (dd, 1 H, J=13.4 , 6.2 Hz).

Slow diastereomer (**6b**): 0.17 g, (39.0% yield), mp 84-88 °C, white, iridescent, crystalline solid. R_f = 0.09 (7:3 ether-ethyl acetate). $[\alpha]^{25}_D$ = +76.5°. ^{31}P NMR: δ 30.3. ^{13}C NMR: δ 136.57, 129.12, 128.98, 127.20, 70.90, 54.33 (d, J=9.9 Hz), 49.39 (d, J=4.5 Hz), 42.37, 41.76 (d, J=9.2 Hz). ^{1}H NMR: δ 7.37-7.14 (m, 5 H), 4.40 (ddd, 1 H, J=12.1 , 8.9 , 6.9 Hz), 4.14-4.02 (m, 1 H), 3.87 (dd, 1 H, J=16.6 , 8.2 Hz), 3.67-3.54 (m, 4 H), 3.47-3.33 (m, 4 H), 2.99 (d, 1 H, J=12.8), 2.84 (dd, 1 H, J=13.4 , 5.4 Hz), 2.72 (dd, 1 H, J=13.4 , 8.2 Hz).

(5S, 2S and 5S, 2R)-2[bis-(2-chloroethyl)amino]-4-benzyl-1,3,2-oxazaphospholidin-2-one (7a and 7b)

To a 35 mL round bottomed flask was added bis-(2-chloroethyl)phosphoramidic dichloride (**3**) (0.26 g, 1.01 mmol), (S)-(-)-2-amino-3-phenyl-1-propanol (**7**) (0.15 g, 1.01 mmol), toluene (15 mL), and triethylamine (1.5 mL, 10.8 mmol) at 0 °C. The solution was warmed to room temperature and allowed to stir over night. The solution was filtered through a 2.0 cm layer of florisil and washed several times (60-80 mL CH_2Cl_2). Excess solvent was removed to yield a white, shiny, iridescent residue which was purified by flash column chromatography [12.0g silica (375 mL 9:1 ether-ethyl acetate), (250 mL 1:1 ether-ethyl acetate), (125 mL 100% ethyl acetate)]. Fast diastereomer (**7a**): 0.14 g, (41.2% yield), clear, yellow oil. R_f = 0.20 (7:3 ether-ethyl acetate). $[\alpha]^{25}_D$ = +59.9°. ^{31}P NMR: δ 28.5. ^{13}C NMR: δ 136.76, 129.07, 128.99, 127.12, 70.79, 56.11 (d, J=9.9 Hz), 49.23, 42.35, 42.13 (d, J=3.8 Hz). ^{1}H NMR: δ 7.35-7.20 (m, 5 H), 4.24 (ddd, 1 H, J=13.7 , 9.1 , 6.4 Hz), 4.10 (ddd, 1 H, J=9.0 , 7.5 , 6.0 Hz), 3.95-3.84 (m, 4 H), 3.61-3.56 (m, 1 H), 3.43-3.30 (m, 4 H), 3.15 (d, 1 H, J=15.7 Hz), 2.95 (dd, 1 H, J=13.4 , 8.1 Hz), 2.87 (dd, 1 H, J=13.4 , 6.4 Hz).

Slow diastereomer (**7b**): 0.08 g, (22.8% yield), off white, iridescent, crystalline solid. mp 86-88 °C. R_f = 0.09 (7:3 ether-ethyl acetate). $[\alpha]^{25}_D$ = -34.1°. ^{31}P NMR: δ 30.50. ^{13}C NMR: δ 136.56, 129.09, 129.07, 127.30, 70.99, 54.40 (d, J=9.9 Hz), 49.34, 42.38, 41.80 (d, J=8.4 Hz). ^{1}H NMR: δ 7.40-7.12 (m, 5 H), 4.39 (ddd, 1 H, J=11.8 , 8.8 , 7.0 Hz), 4.13-4.02 (m, 1 H), 3.86 (dd, 1 H, J=16.6 , 8.2 Hz), 3.65-3.56 (m, 4 H), 3.49-3.34 (m, 4 H), 3.03 (d, 1 H, J=11.2 Hz), 2.83 (dd, 1 H, J=13.6 , 5.2 Hz), 2.72 (dd, 1 H, J=13.6 , 8.0 Hz).

(±)-2-[bis-(2-chloroethyl)amino]-1,3,2-oxazaphospholidin-2-one (8a/8b)

To a 35 mL round bottomed flask was added bis-(2-chloroethyl)phosphoramidic dichloride (**3**) (0.26 g, 1.00 mmol), ethanolamine hydrochloride (**8**) (0.14 g, 1.43 mmol), CH_2Cl_2 (18 mL), and triethylamine (2.5 mL, 18.0 mmol) at 0 °C. The solution was warmed to room temperature and allowed to stir overnight. The solution was filtered through a 1.0 cm layer of florisil and washed several times (60-80 mL CH_2Cl_2). Excess solvent was removed to yield a white solid which was purified by flash column chromatography [10.2 g silica (250 mL 100% ethyl acetae), (50 mL 1:1 ethyl acetate-acetonitrile), (100 mL 100% acetonitrile)]. 0.21 g, (84.8% yield), mp 63-70 °C, yellow, oily solid. R_f = 0.30 (1:1 ethyl acetate-acetonitrile). $[\alpha]^{25}_D$ = 0.0 ° ^{31}P NMR: δ 30.8. ^{13}C NMR: δ 66.33 (d, J=2.2 Hz), 49.27 (d, J=4.6 Hz), 42.4, 42.38 (d, J=9.2 Hz). ^{1}H NMR: δ 4.37 (dddd, 2 H, 13.9 Hz , 8.9 Hz , 7.6 Hz , 6.4 Hz), 4.22 (dddd, 2 H, 12.8 Hz , 8.8 Hz , 6.8 Hz , 6.0 Hz), 3.86 (m, 1 H), 3.66-3.50 (m, 4 H), 3.47-3.26 (m, 4 H).

References

(1) Friedberg, Errol C. In *Cancer Biololgy,* W.H. Freeman Co.: New York, **1986**; pp vii-2.
(2) Simpson, J.A., and Weimer, E.S.C, Eds. *The Oxford English Dictionary,* Oxford University Press: Claredon, **1989;** Vol. 2., pp 417.
(3) Creasy, William A. In *Cancer: An Introduction,* Oxford University Press: New York, **1981**; pp 186-196.
(4) Busch, Harris, Ed. In *Methods in Cancer Research,* Academic Press: New York, **1967**; Vol. 3., pp 180-605.
(5) Ambrose, E.J., and Roe, F.J., Eds. In *The Biology of Cancer,* Wiley and Sons: New York, **1975**; pp 180-194.
(6) Knock, Frances E. In *Anticancer Agents,* Charles C. Thomas: Springfield, Ill., **1967**; pp 105-552.
(7) Larinov, L.F. In *Cancer Chemotherapy,* Crozy, A., Trans.; Pergamon Press: Oxford, **1965**; pp 271-306.
(8) Amdur, Mary O.; Doull, John; Klaassen, and Curtis D., Eds. In *The Basic Science of Poisons,* M^{c}Graw-Hill: New York, **1993**; pp 284.
(9) Sartorelli, Alan C. In *Cancer Chemotherapy,* American Chemical Society: Washington, D.C., **1976**; pp 70-72.
(10) Niedle, Stephen and Waring, Michael J., Eds. In *Molecular Aspects of Anticancer Drug Action,* Weinheim: Deerfield Beach, Fla., **1983**; pp 270-272.
(11) Remers, William A., Ed. In *Antineoplastic Agents*, Wiley and Sons: New York, **1984**; pp 91-124.
(12) Toy, D.F. In *Phosphorus Chemistry In Everyday Living,* American Chemical Society: Washington, D.C., **1976**; pp 172-174.
(13) Tietze, Lutz F.; Fischer, Roland; Beller, Matthias; and Seele, Rainer. *Liebigs Ann. Chem.* **1990;** pp 151-157.
(14) Foster, Emerson L. *Journal of Pharmaceutical Sciences.* **1978**; *67*, pp 709-710.
(15) Foster, Emerson L. and Blinckenstaff, Robert T. *Steroids.* **1974**; *24*, pp 737-738.
(16) Thompson, Charles M.; Frick, Jeffrey A.; and Green, Diana L.C. *J. Org. Chem.* **1990**; *55*, pp 111-116.
(17) Morrison, Robert T. and Boyd, Robert N. In *Organic Chemistry,* Allyn and Bacon: Boston, **1987;** pp 131-134.
(18) Farmer, P.B. *Biochemical Pharmacology.* **1988**; *37*, pp 145-147.
(19) Takamizawa, Akira; Matsumoto, Saichi; *et al. J. Am. Chem. Soc.* **1973**; *95*, pp 985-986.
(20) Friedman, O.M. and Seligman, A.M. *J. Am. Chem. Soc.* **1954**; *76*, pp 655.
(21) Gorenstein, David G. In *Phosphorus-31 Chemical Shifts: Principles and Empirical Observations,* Academic Press: New York, **1984**; pp 18-22.
(22) Cox, P.J.; Farmer, P.B.; Jarman, M.; and Jones, M. *Biochemical Parmacology.* **1976**, *25*, pp 993-996.
(23) Ludeman, Susan M.; Bartlett, Desiree L.; and Zon, Gerald. *J. Org. Chem.* **1979**, *44*, pp 1163-1166.

(24) Jackson, John A.; Frick, Jeffrey A.; and Thompson, Charles M. *Bioorganic & Medicinal Chemistry Letters.* **1992**, *2*, pp 1547-1549.

Figure 1 ^{1}H NMR of Compound 3

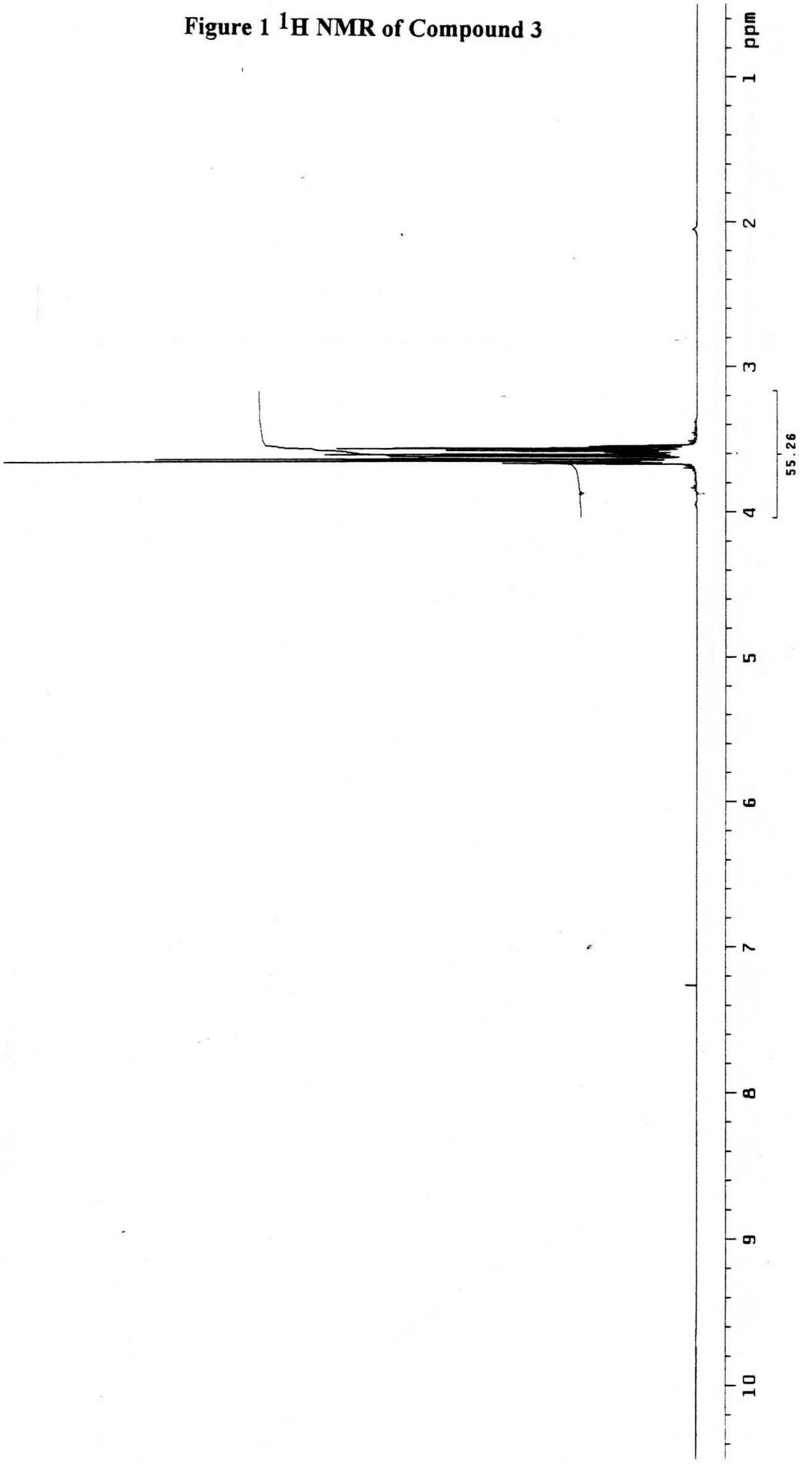


Figure 2 ^{13}C NMR of Compound 3

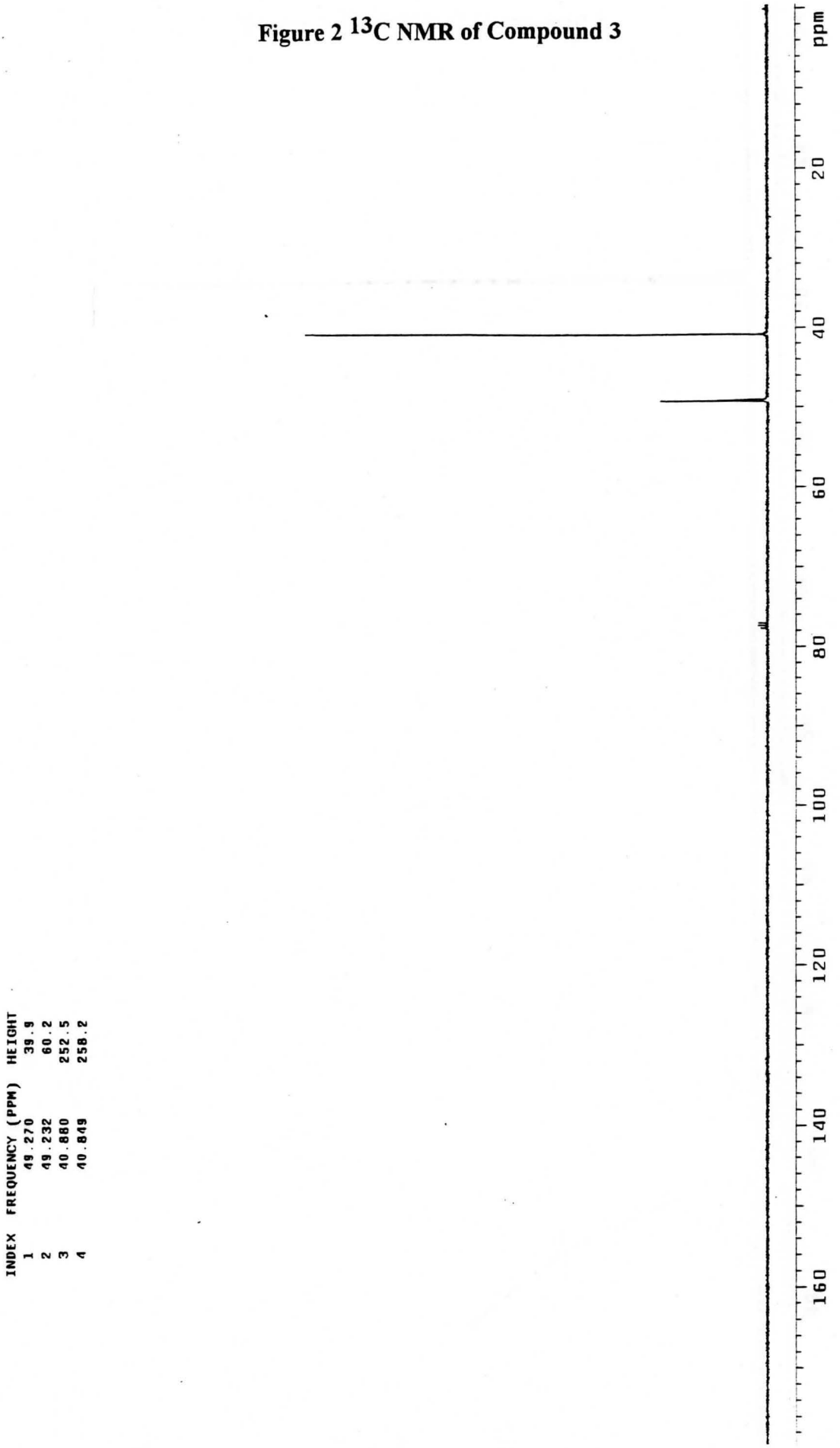


Figure 3 ^{31}P NMR of Compound 3

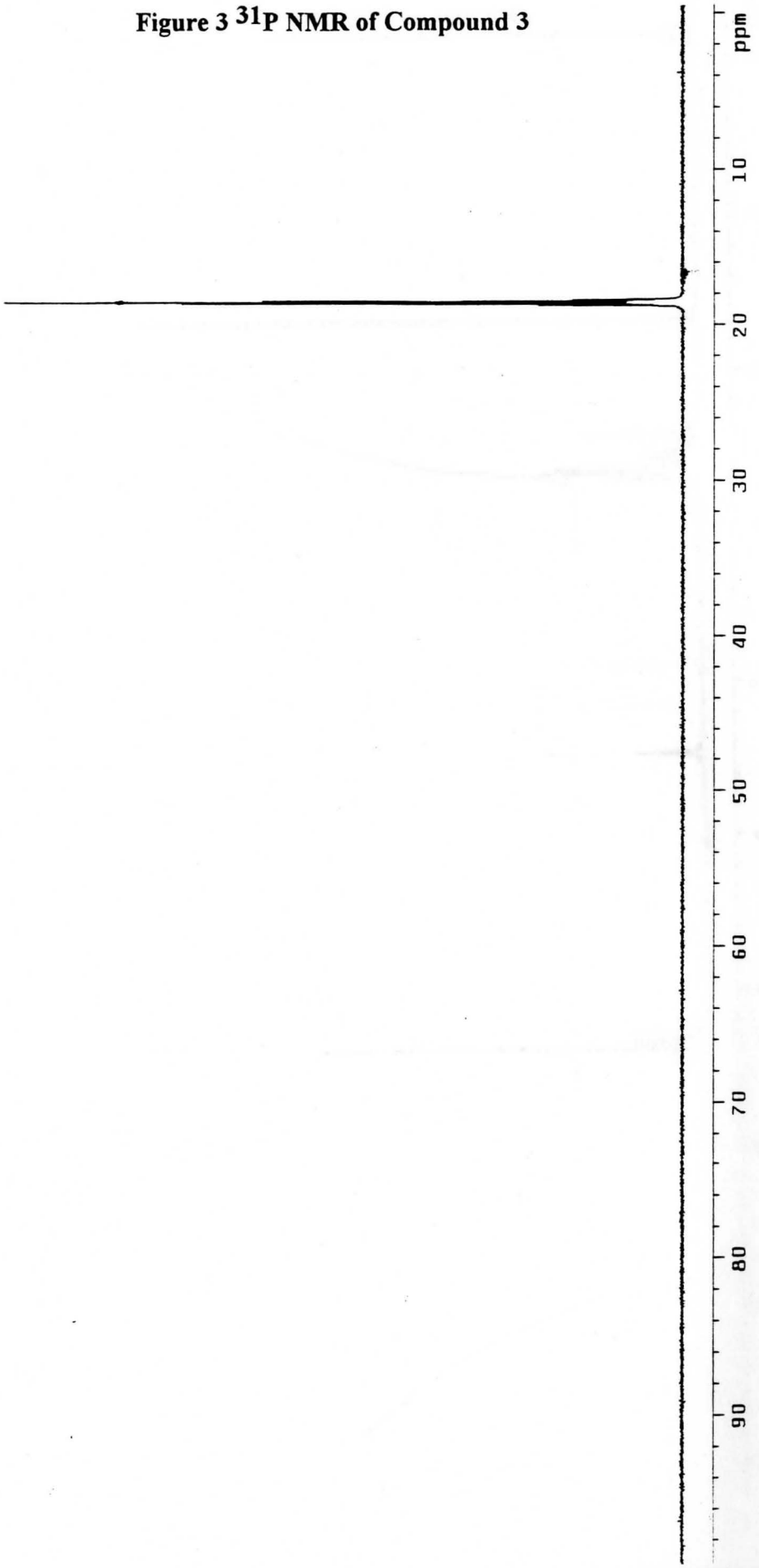


Figure 4 ^{1}H NMR of Compound 4a

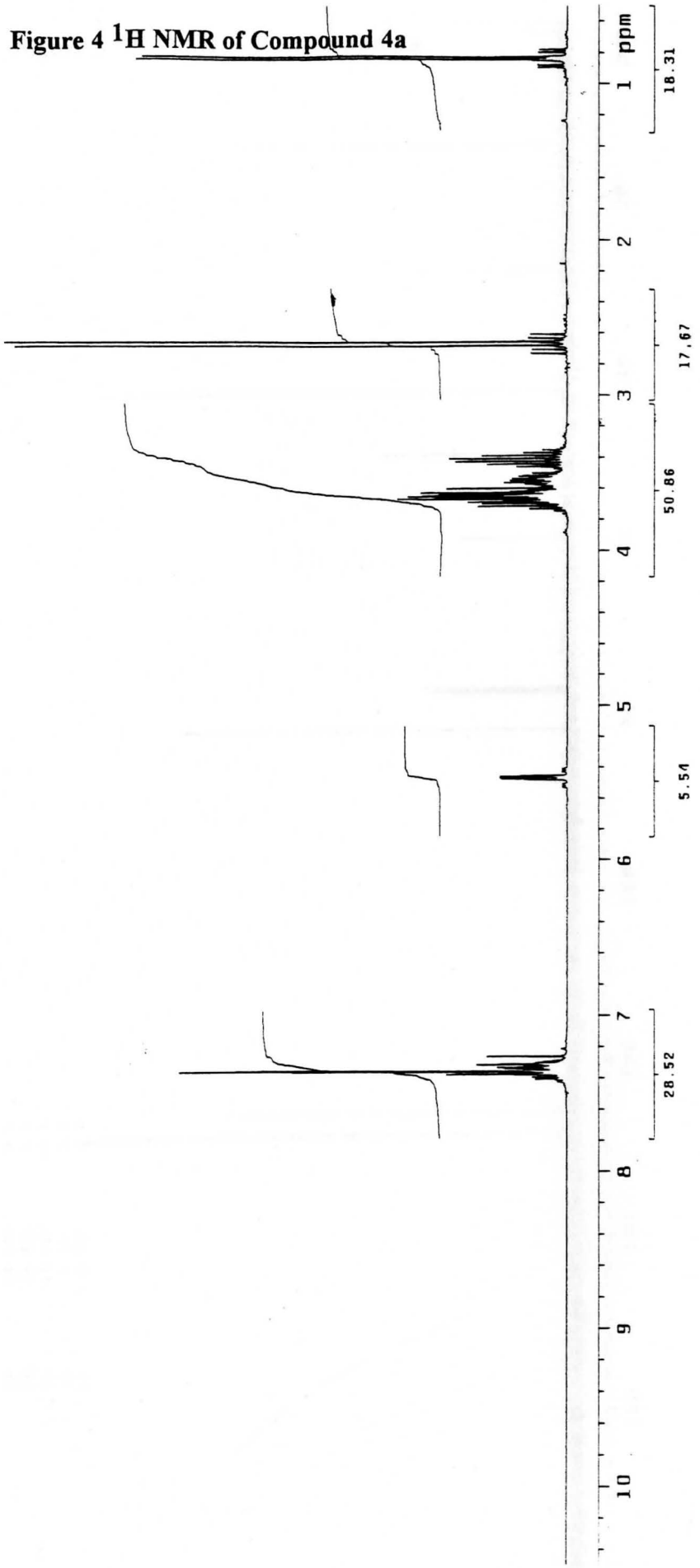
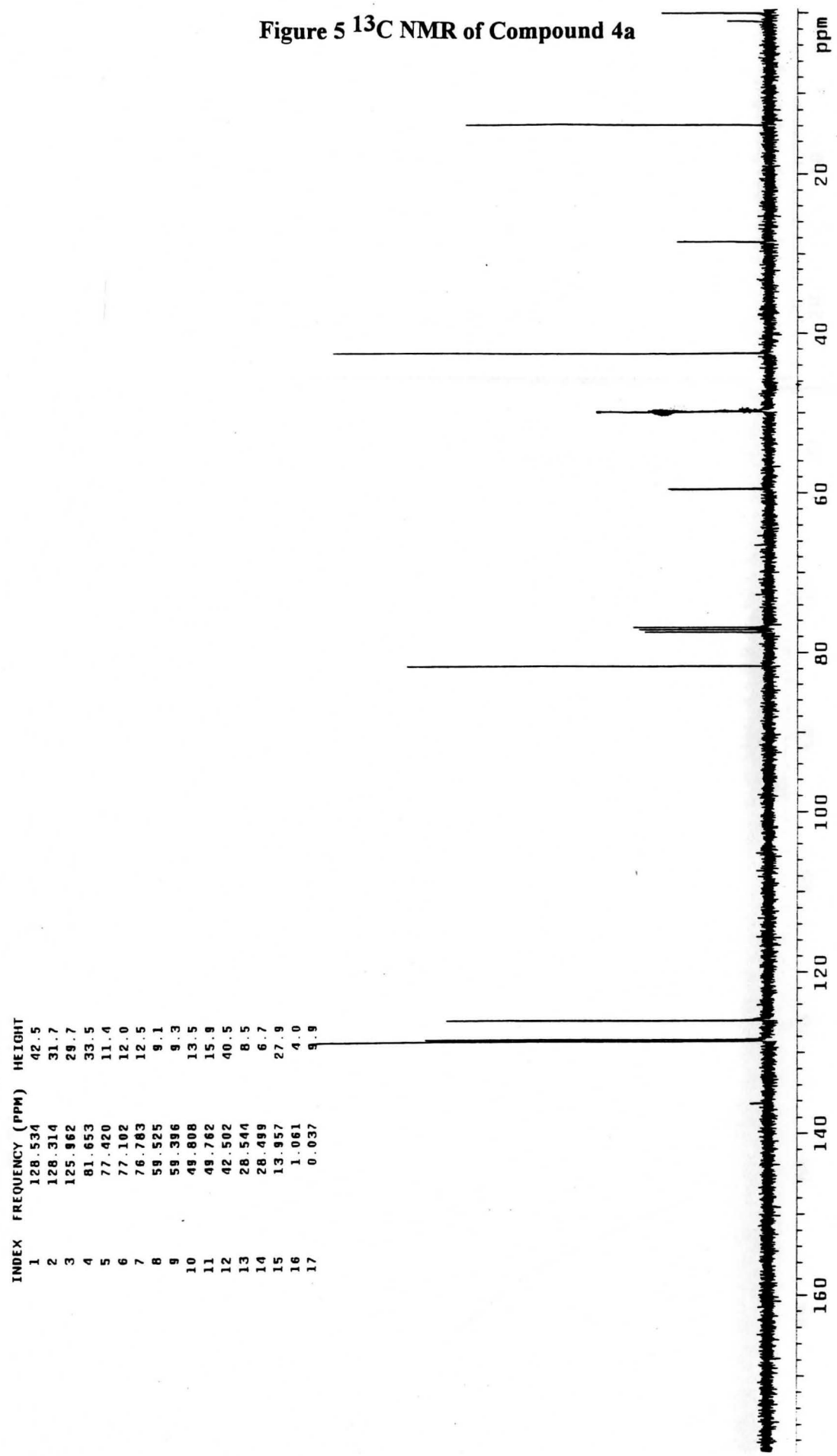


Figure 5 ^{13}C NMR of Compound 4a



INDEX	FREQUENCY (PPM)	HEIGHT
1	128.534	42.5
2	128.314	31.7
3	125.962	29.7
4	81.653	33.5
5	77.420	11.4
6	77.102	12.0
7	76.783	12.5
8	59.525	9.1
9	59.396	9.3
10	49.808	13.5
11	49.762	15.9
12	42.502	40.5
13	28.544	8.5
14	28.499	6.7
15	13.957	27.9
16	1.061	4.0
17	0.037	9.9

Figure 6 ^{31}P NMR of Compound 4a

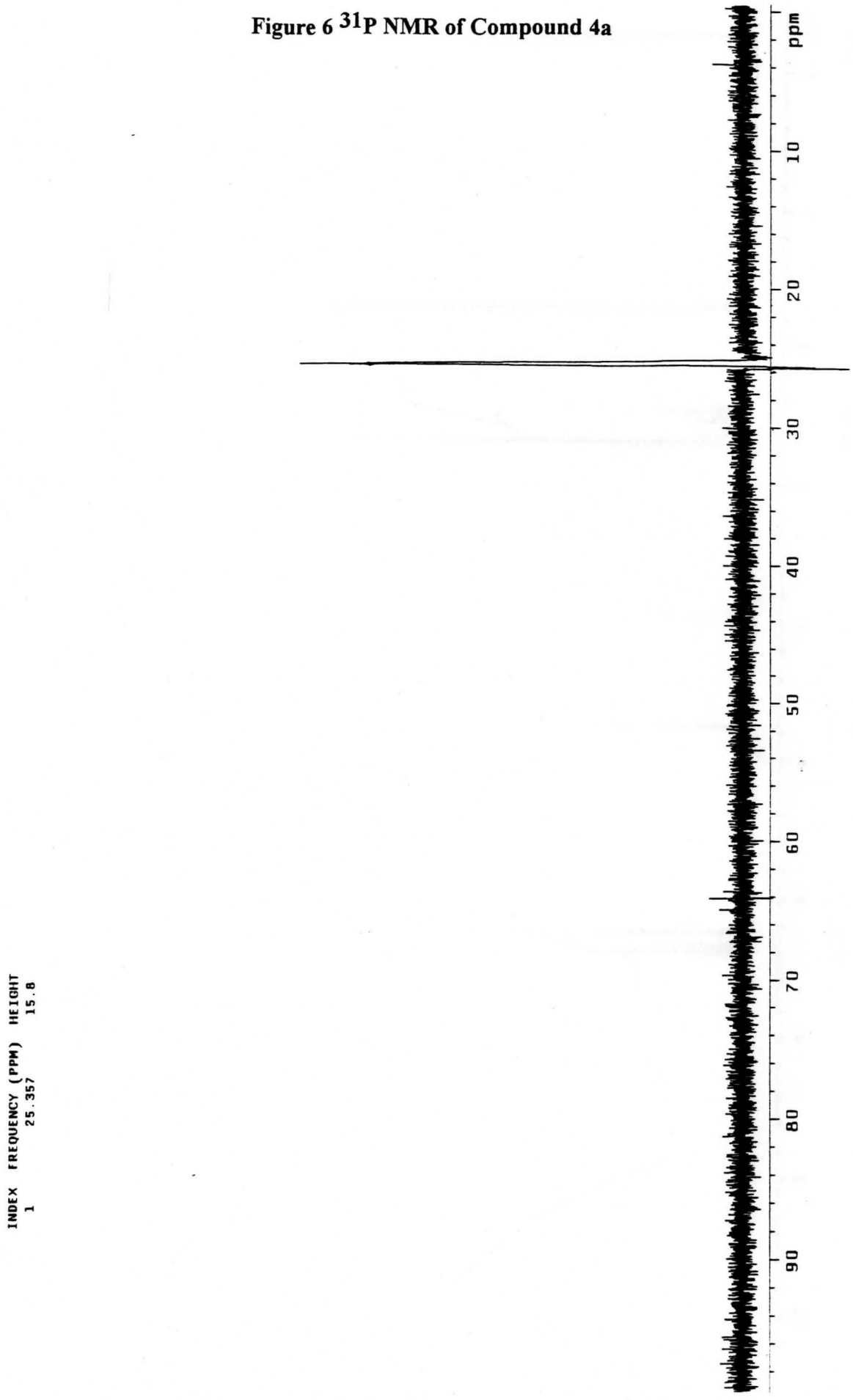


Figure 7 ^{1}H NMR of Compound 4b

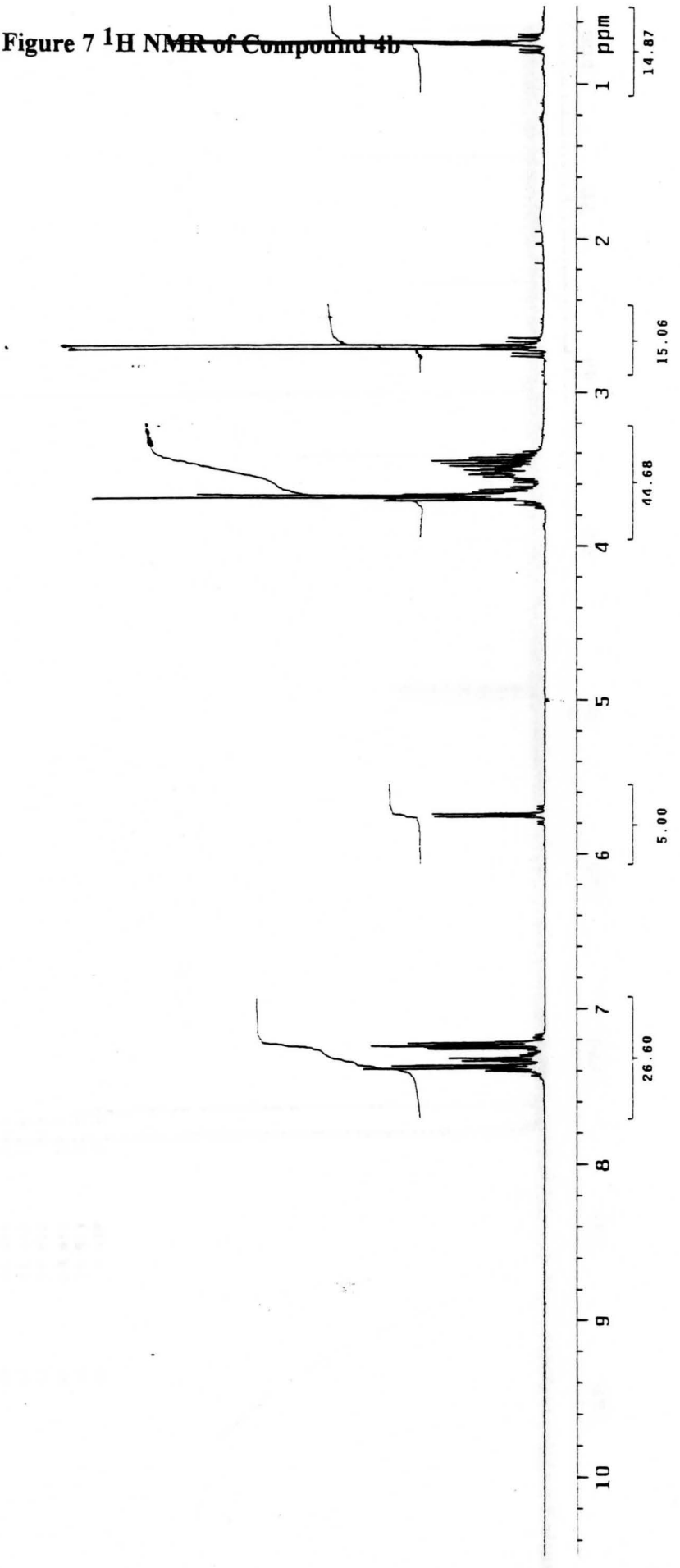
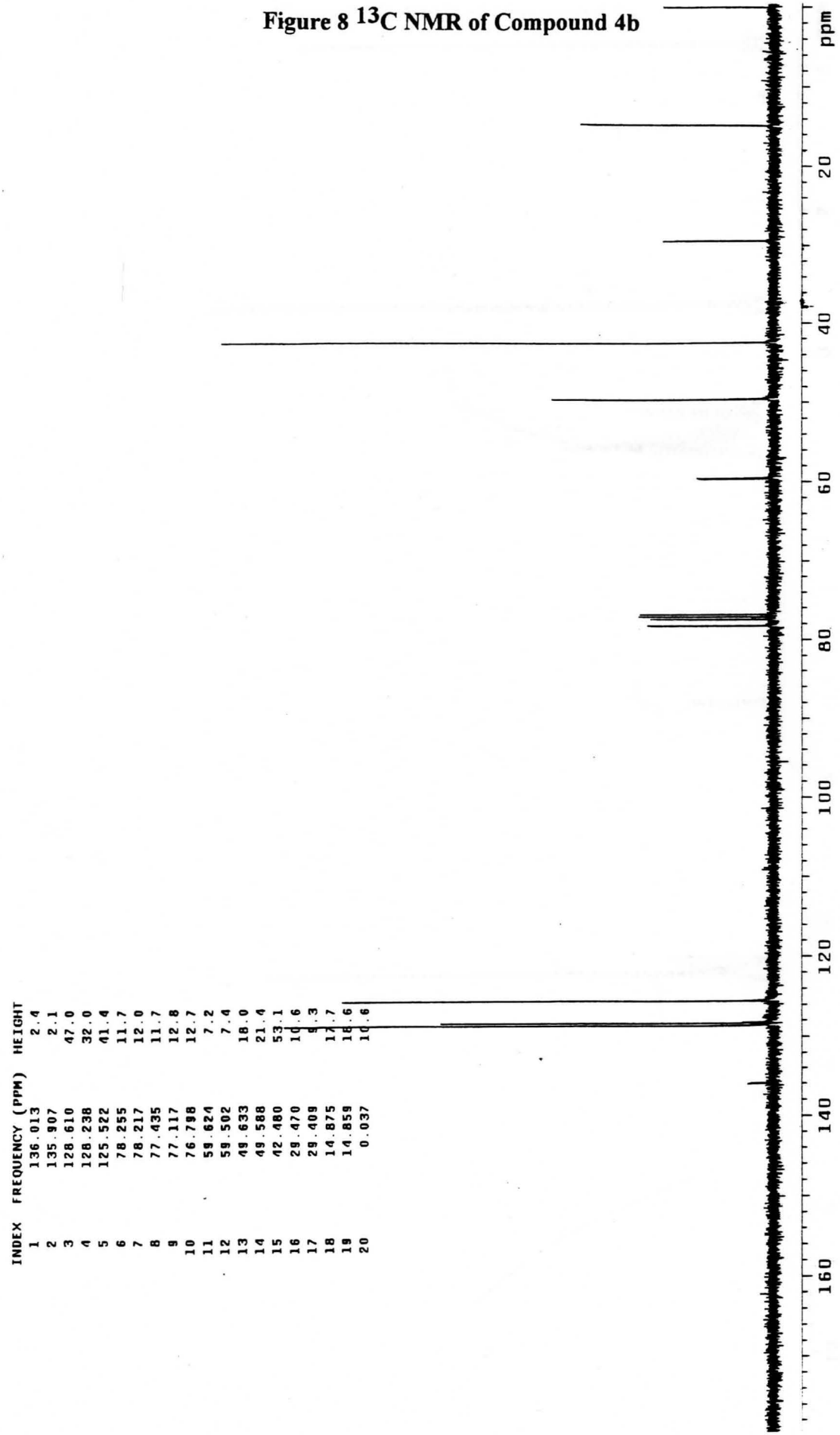


Figure 8 ^{13}C NMR of Compound 4b



INDEX	FREQUENCY (PPM)	HEIGHT
1	136.013	2.4
2	135.907	2.1
3	128.610	47.0
4	128.238	32.0
5	125.522	41.4
6	78.255	11.7
7	78.217	12.0
8	77.435	11.7
9	77.117	12.8
10	76.798	12.7
11	59.624	7.2
12	59.502	7.4
13	49.633	18.0
14	49.588	21.4
15	42.480	53.1
16	29.470	10.6
17	29.409	9.3
18	14.875	17.7
19	14.859	18.6
20	0.037	10.6

Figure 9 ^{1}H NMR of Compound 5a

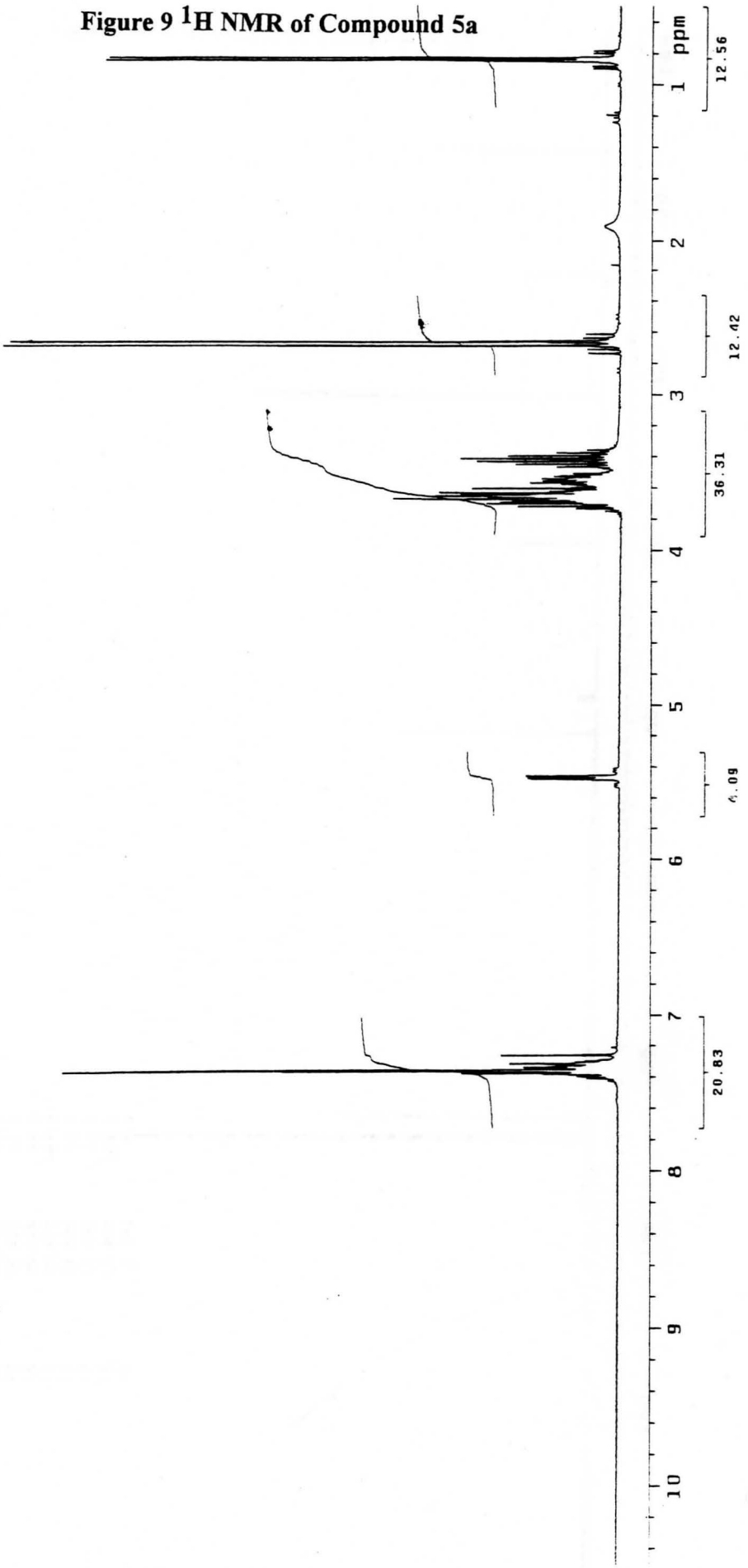


Figure 10 ^{13}C NMR of Compound 5a

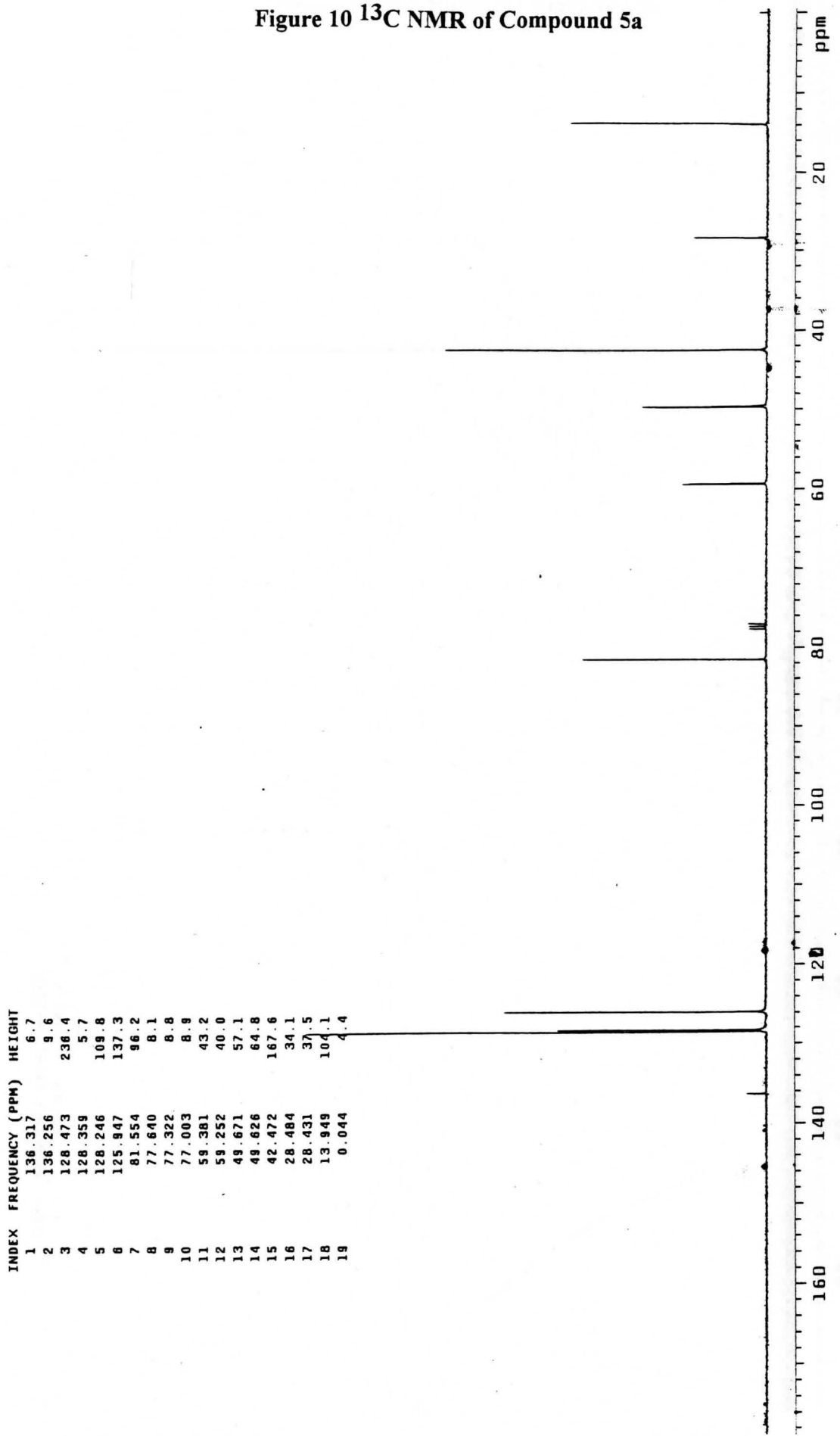


Figure 11 ^{31}P NMR of Compound 5a

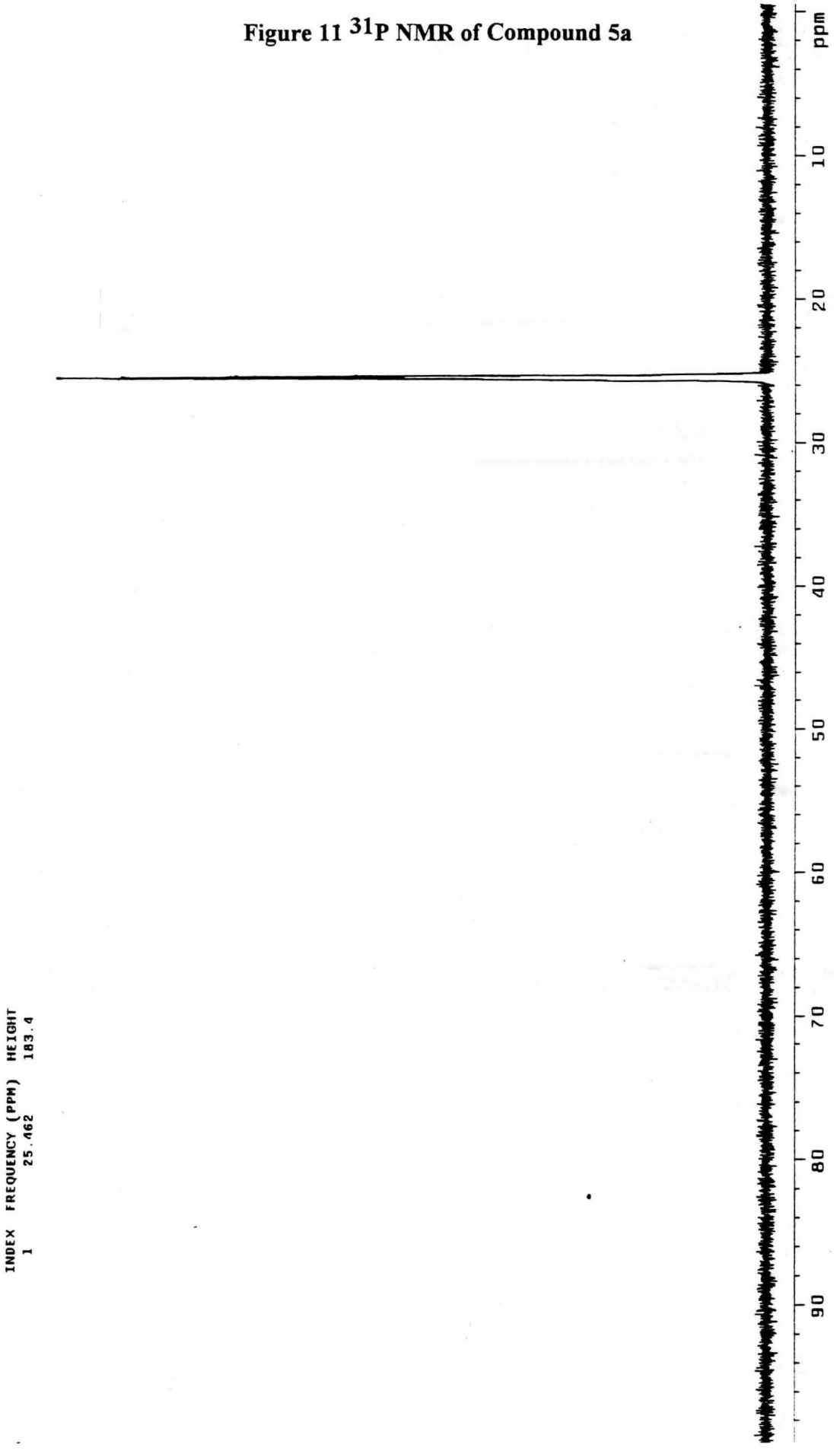
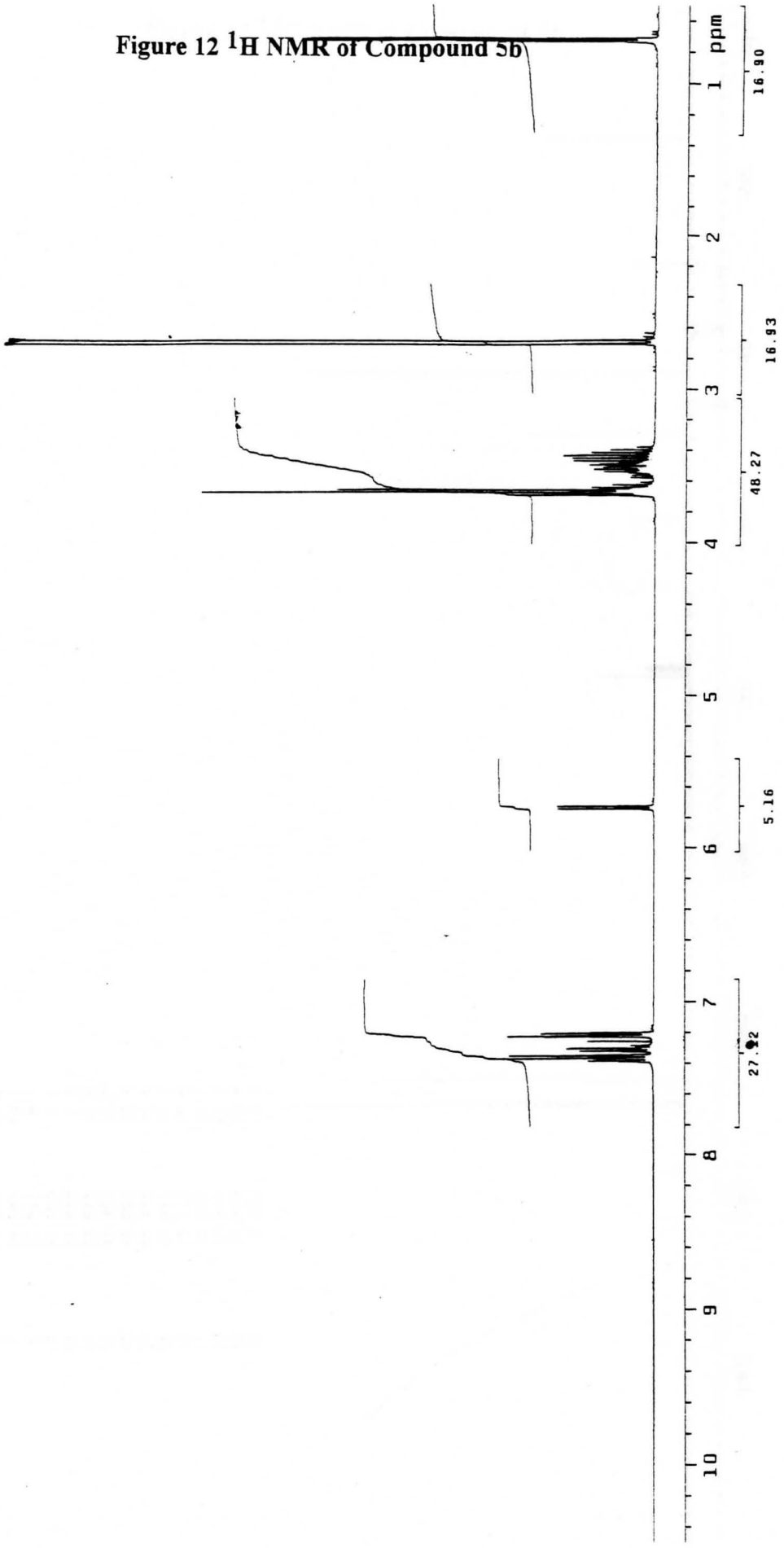


Figure 12 ^{1}H NMR of Compound 5b



INDEX	FREQUENCY (PPM)	HEIGHT
1	136.013	3.8
2	135.907	3.2
3	128.594	74.0
4	128.215	48.3
5	125.507	58.4
6	78.224	16.9
7	78.186	18.1
8	77.511	8.7
9	77.193	8.9
10	76.874	8.8
11	59.593	11.9
12	59.480	12.0
13	49.603	29.4
14	49.550	30.9
15	42.472	77.2
16	29.455	10.4
17	29.402	11.7
18	14.867	25.2
19	14.844	28.8
20	0.044	4.4

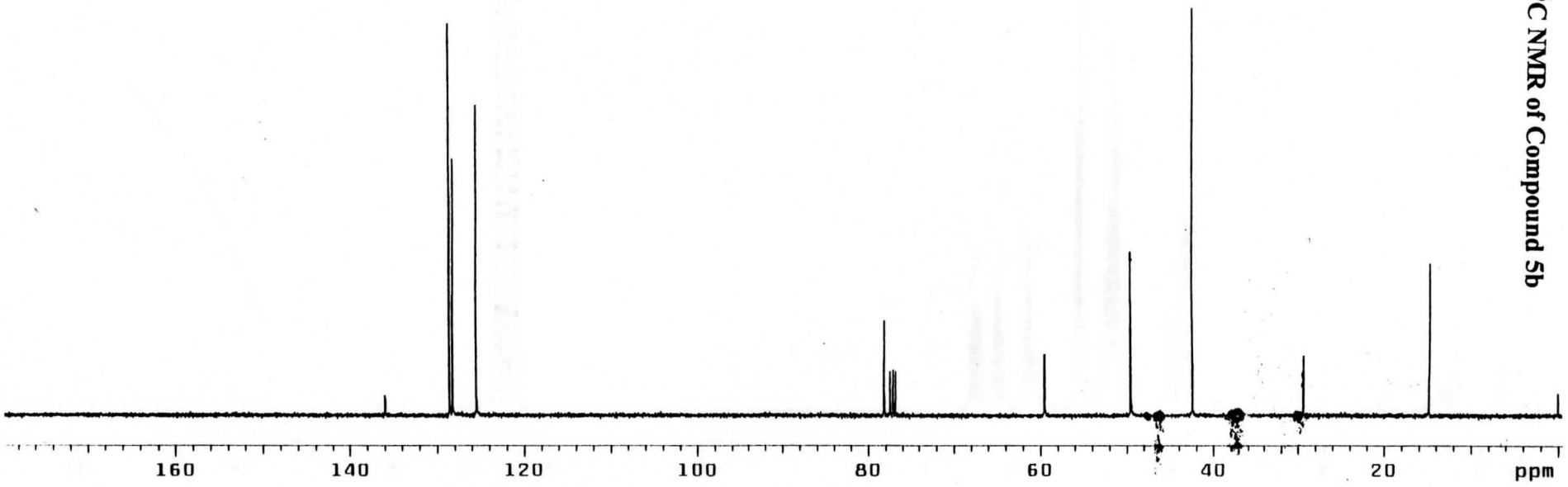


Figure 13 ^{13}C NMR of Compound 5b

Figure 14 ^{1}H NMR of Compound 6a

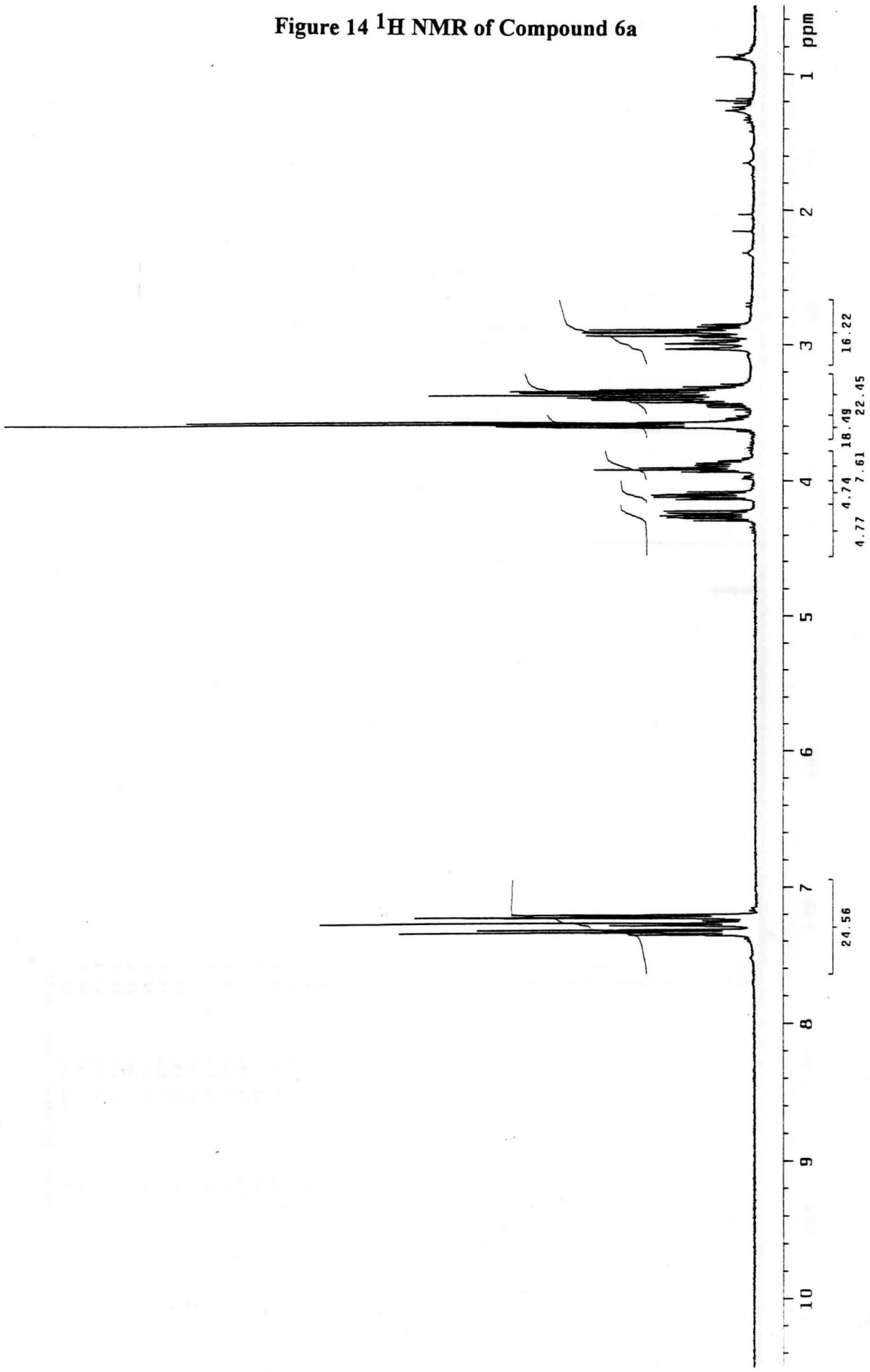
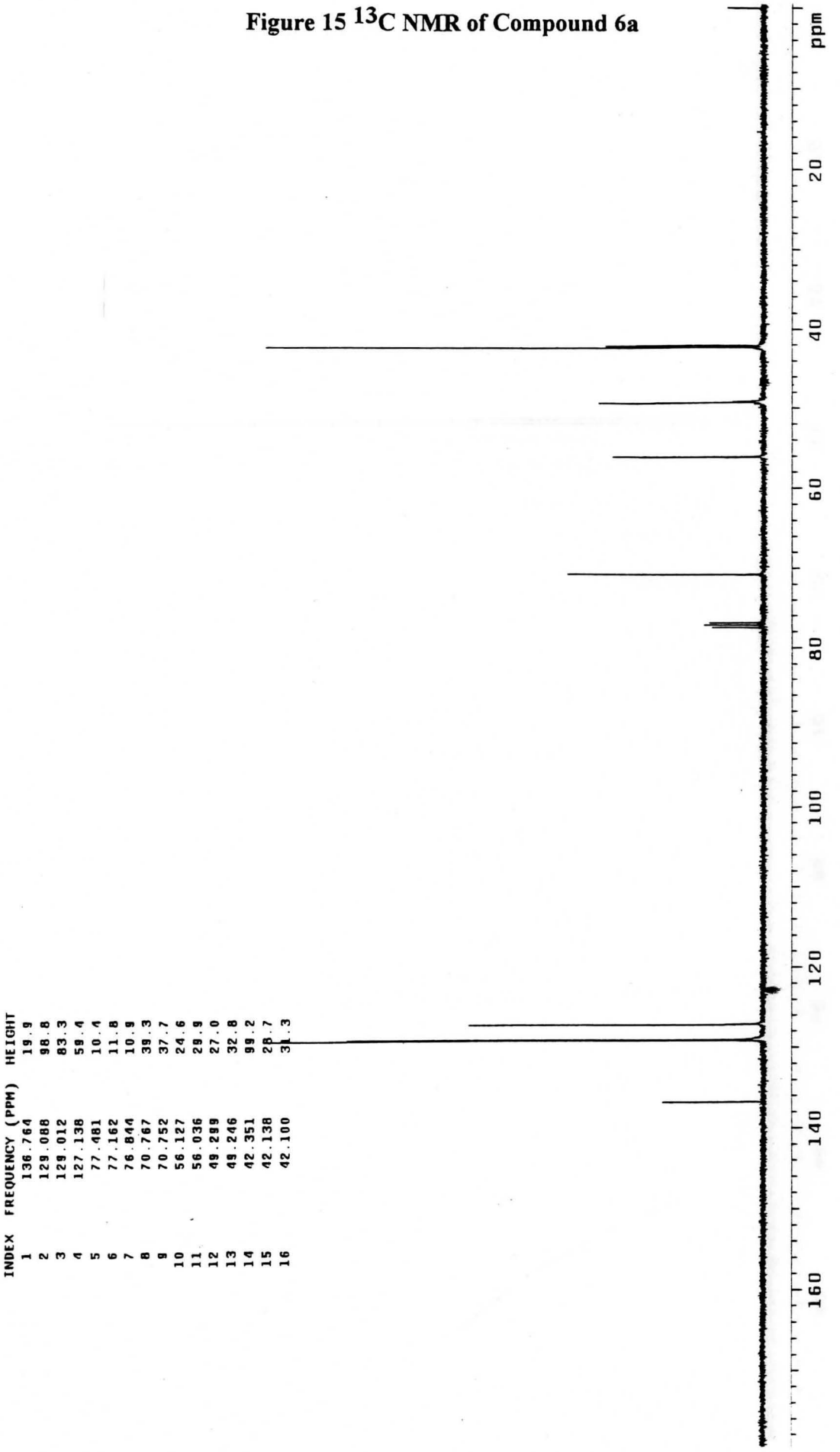


Figure 15 ^{13}C NMR of Compound 6a



INDEX	FREQUENCY (PPM)	HEIGHT
1	136.764	19.9
2	129.088	98.8
3	129.012	83.3
4	127.138	59.4
5	77.481	10.4
6	77.162	11.8
7	76.844	10.9
8	70.767	39.3
9	70.752	37.7
10	56.127	24.6
11	56.036	29.9
12	49.299	27.0
13	49.246	32.8
14	42.351	99.2
15	42.138	28.7
16	42.100	31.3

Figure 16 ^{31}P NMR of Compound 6a

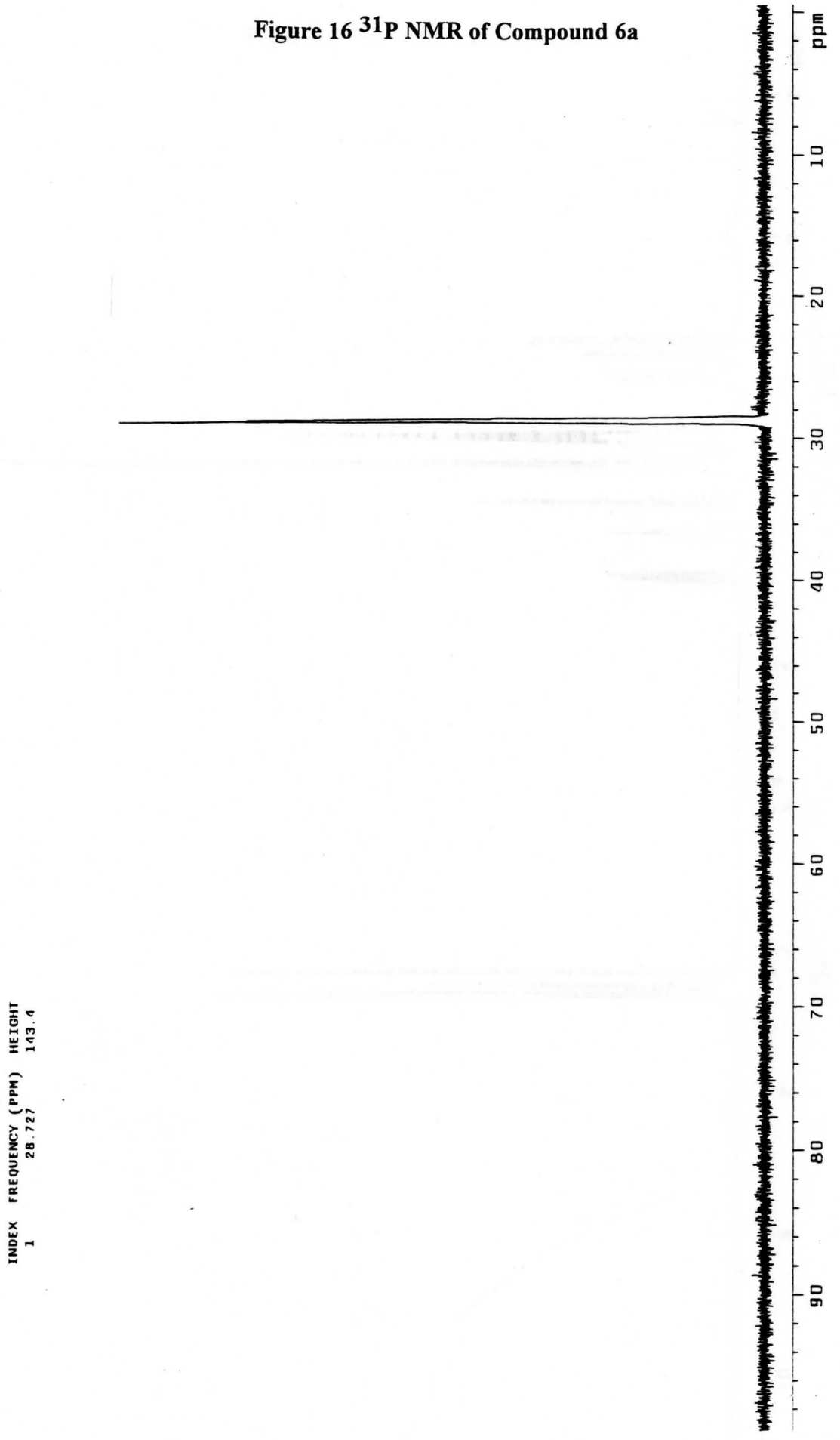


Figure 17 ^{1}H NMR of Compound 6b

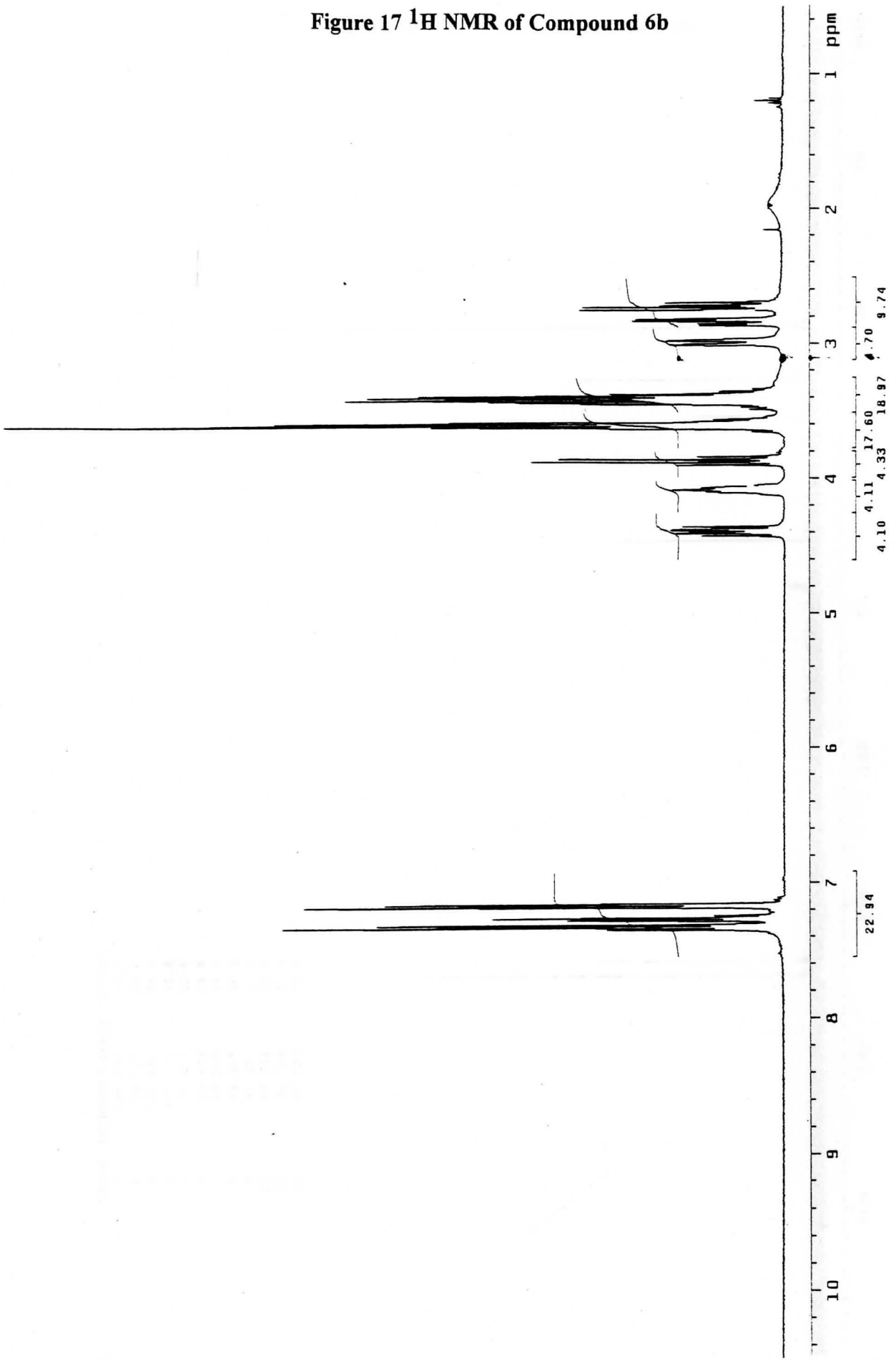
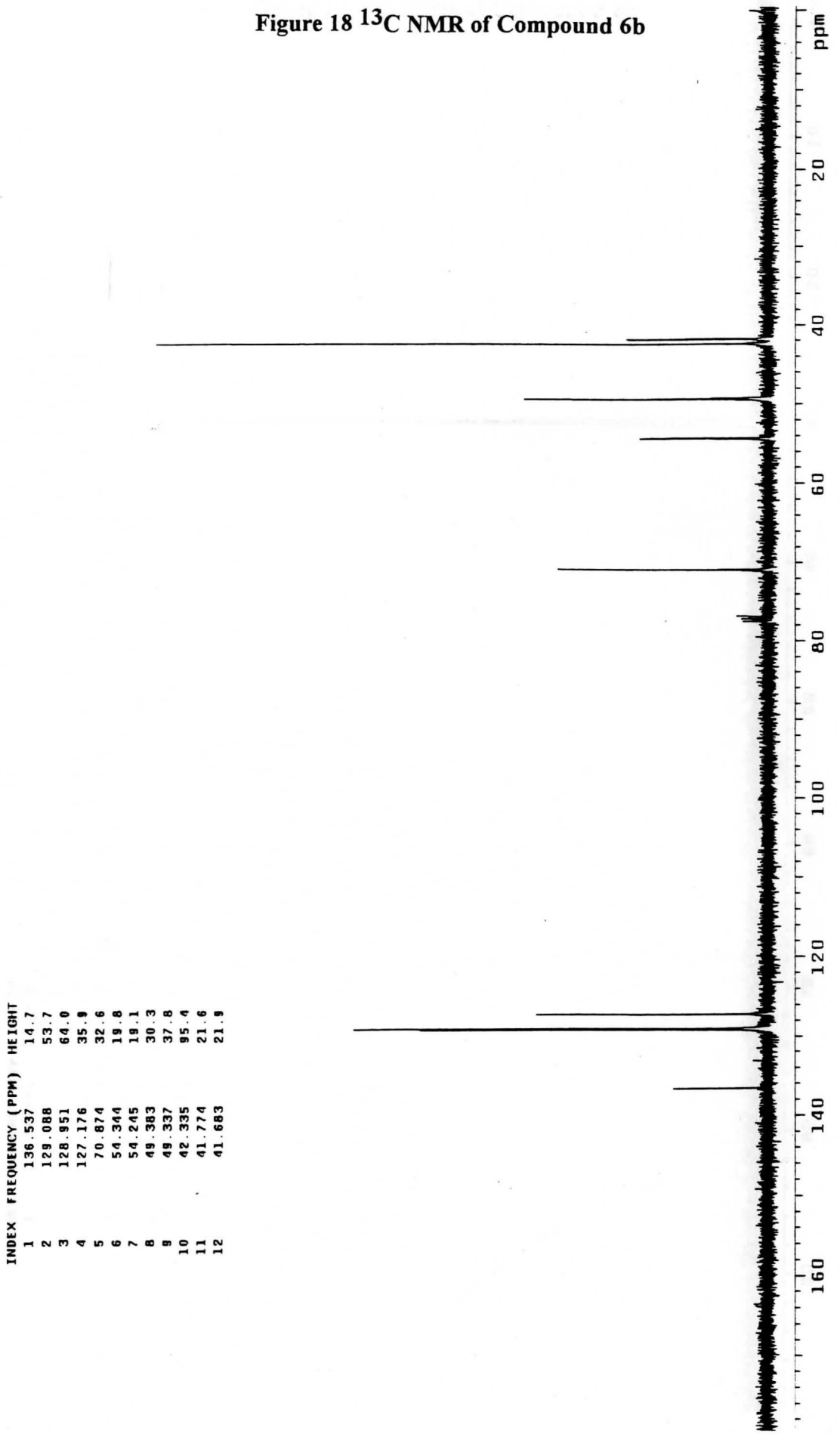


Figure 18 ^{13}C NMR of Compound 6b



INDEX	FREQUENCY (PPM)	HEIGHT
1	136.537	14.7
2	129.088	53.7
3	128.951	64.0
4	127.176	35.9
5	70.874	32.6
6	54.344	19.8
7	54.245	19.1
8	49.383	30.3
9	49.337	37.8
10	42.335	95.4
11	41.774	21.6
12	41.683	21.9

Figure 19 ^{31}P NMR of Compound 6b

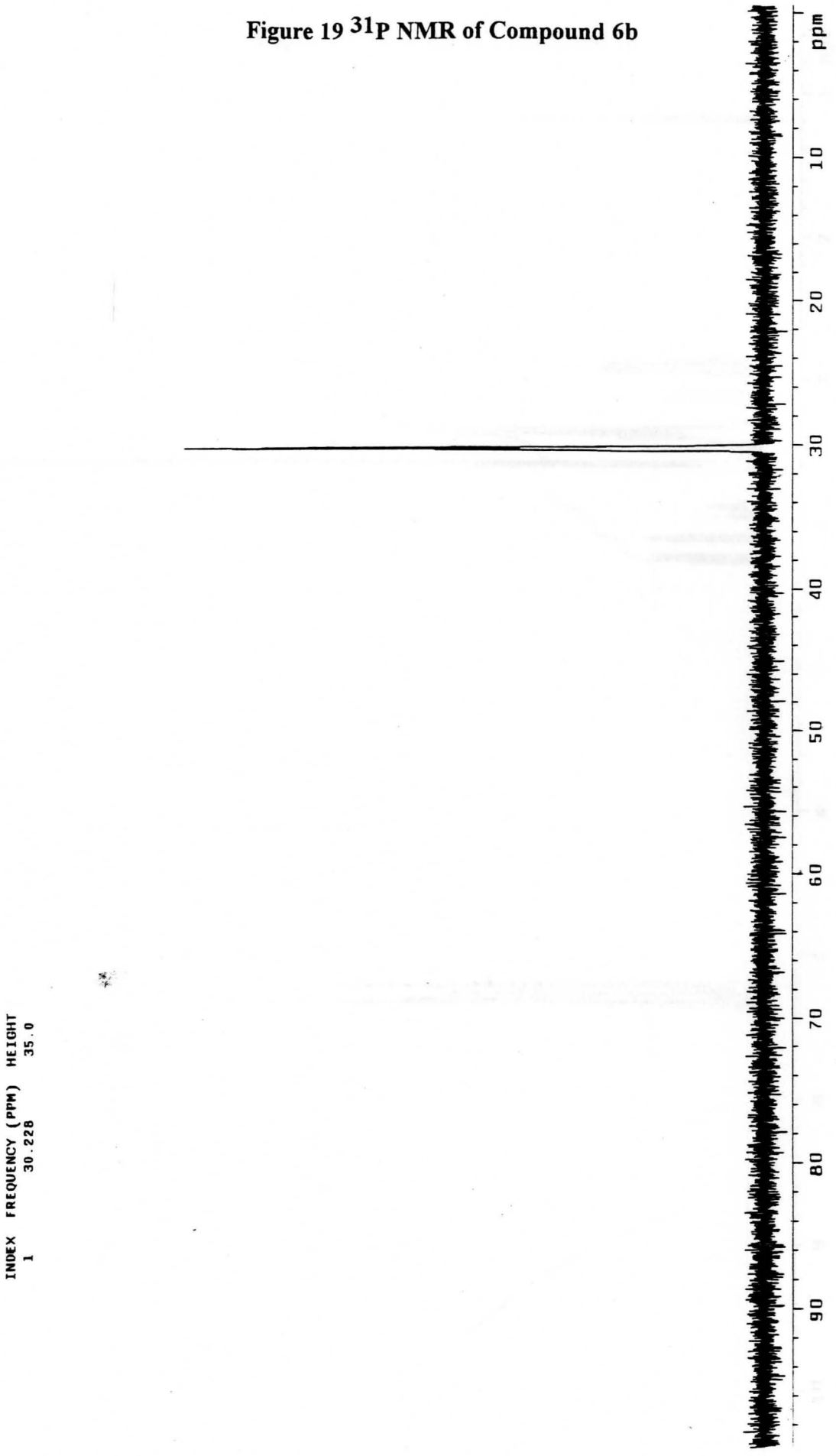


Figure 20 ^{1}H NMR of Compound 7a

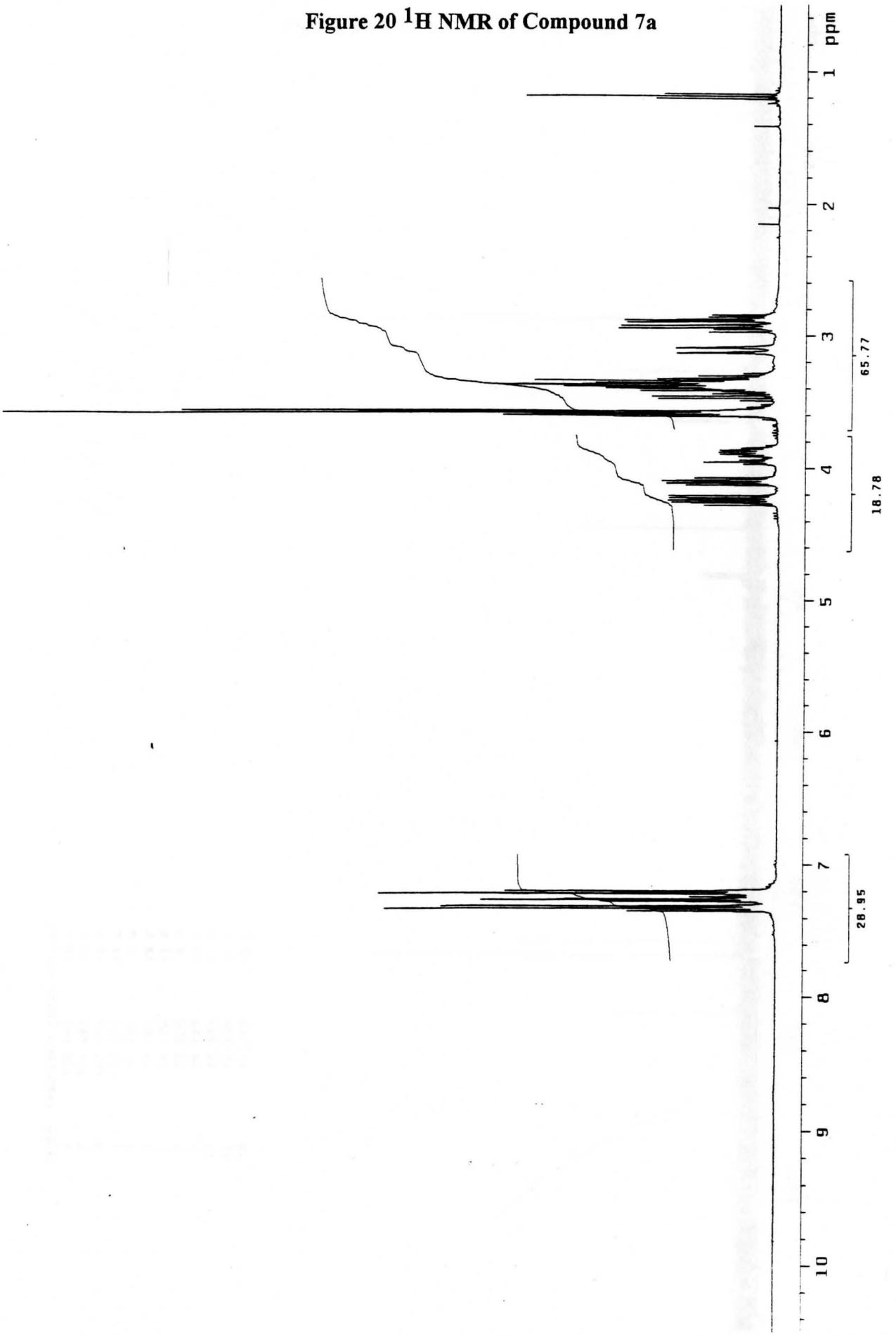
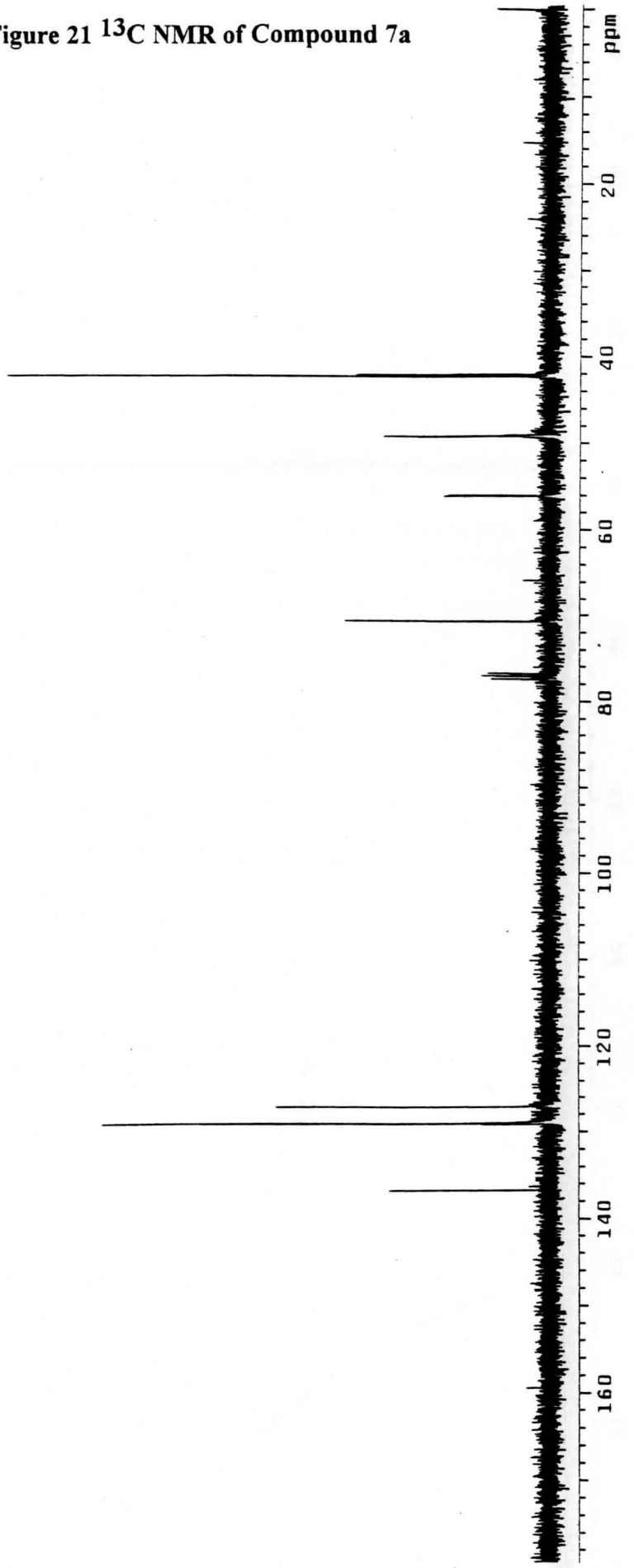


Figure 21 ^{13}C NMR of Compound 7a



INDEX	FREQUENCY (PPM)	HEIGHT
1	136.749	15.1
2	129.095	32.8
3	129.034	42.3
4	127.161	26.0
5	77.132	6.4
6	70.783	19.6
7	58.149	10.0
8	58.058	9.7
9	49.223	15.9
10	42.343	51.6
11	42.138	17.6
12	42.100	18.6

Figure 22 ^{31}P NMR of Compound 7a

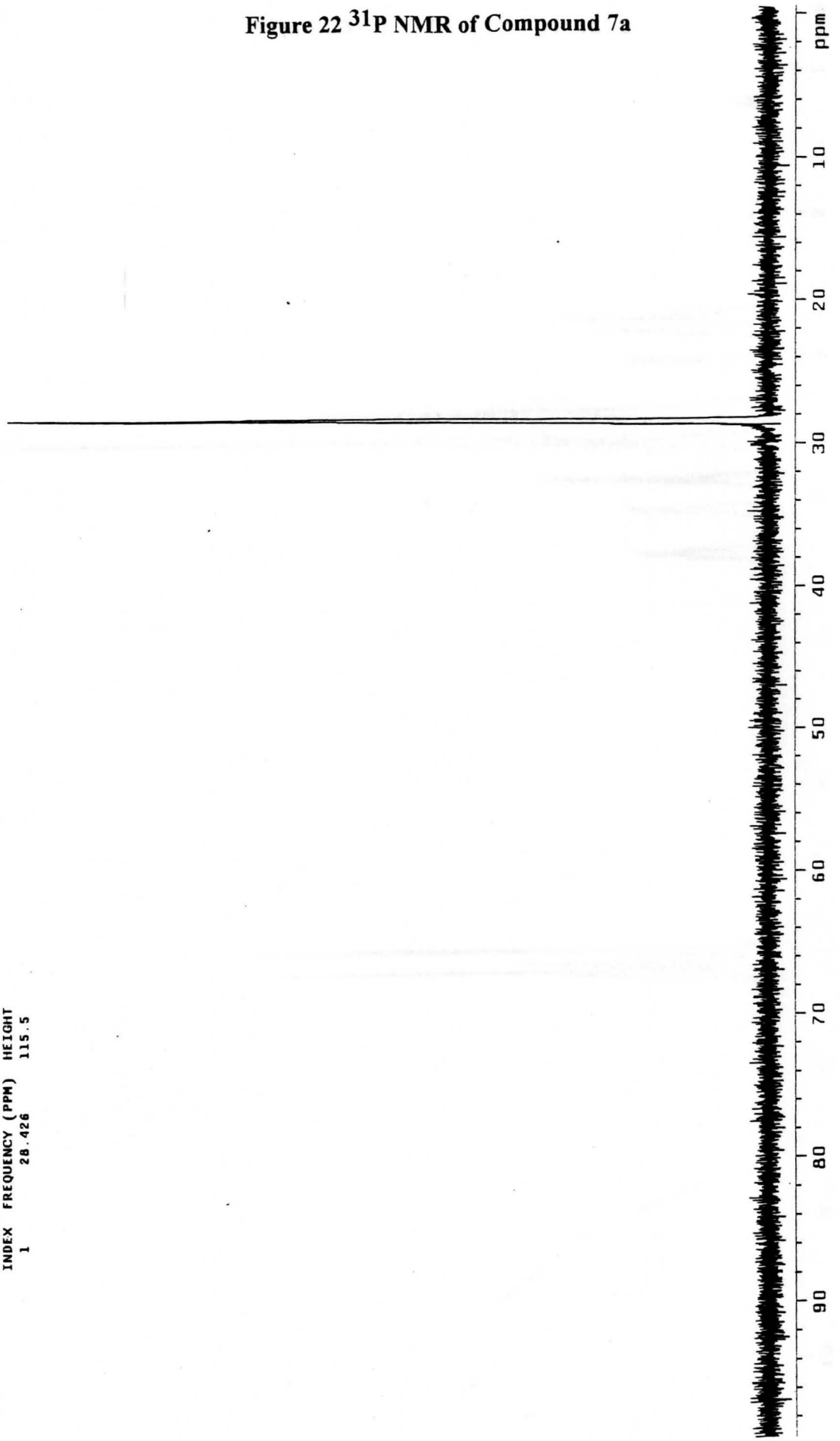


Figure 23 ^{1}H NMR of Compound 7b

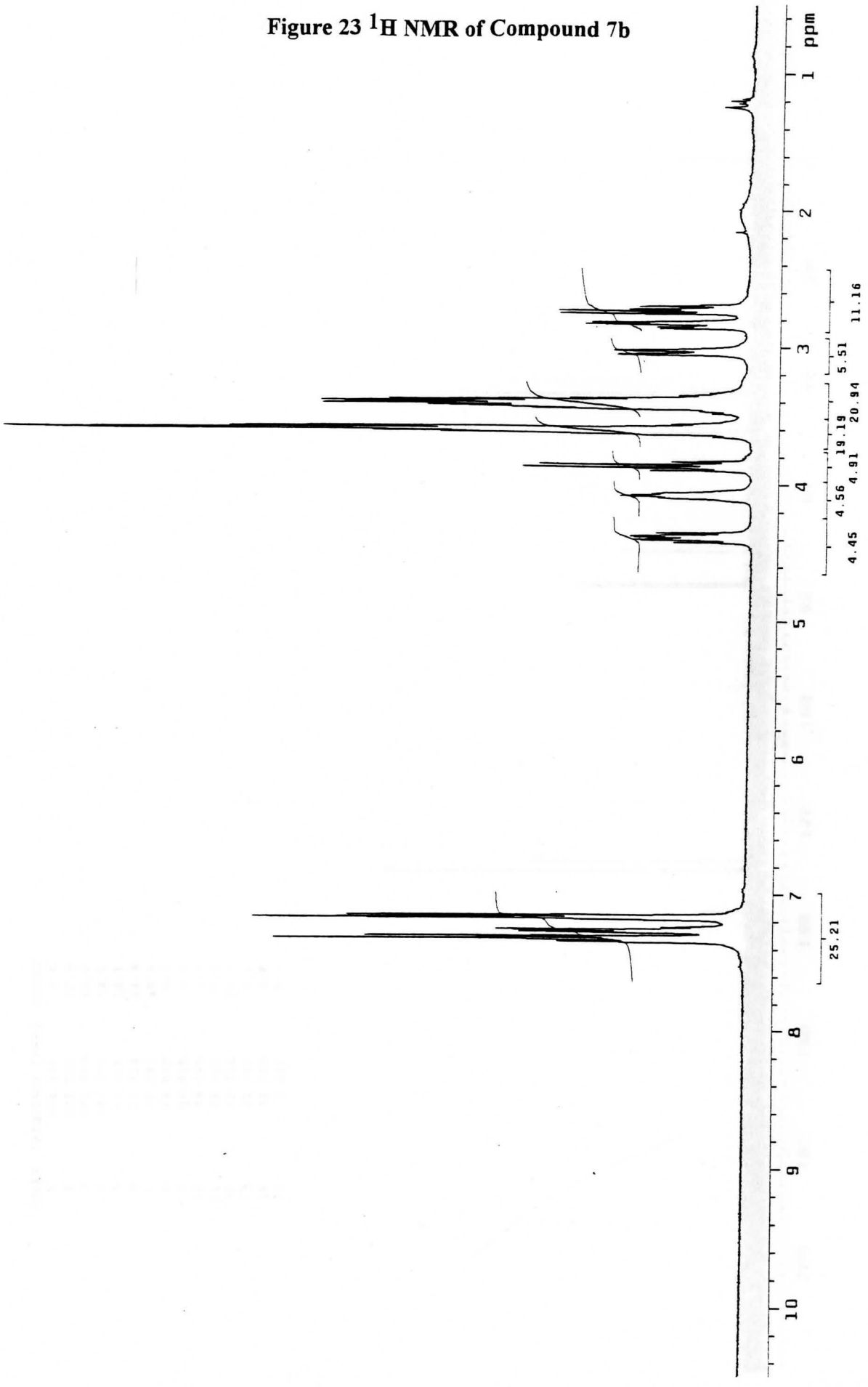
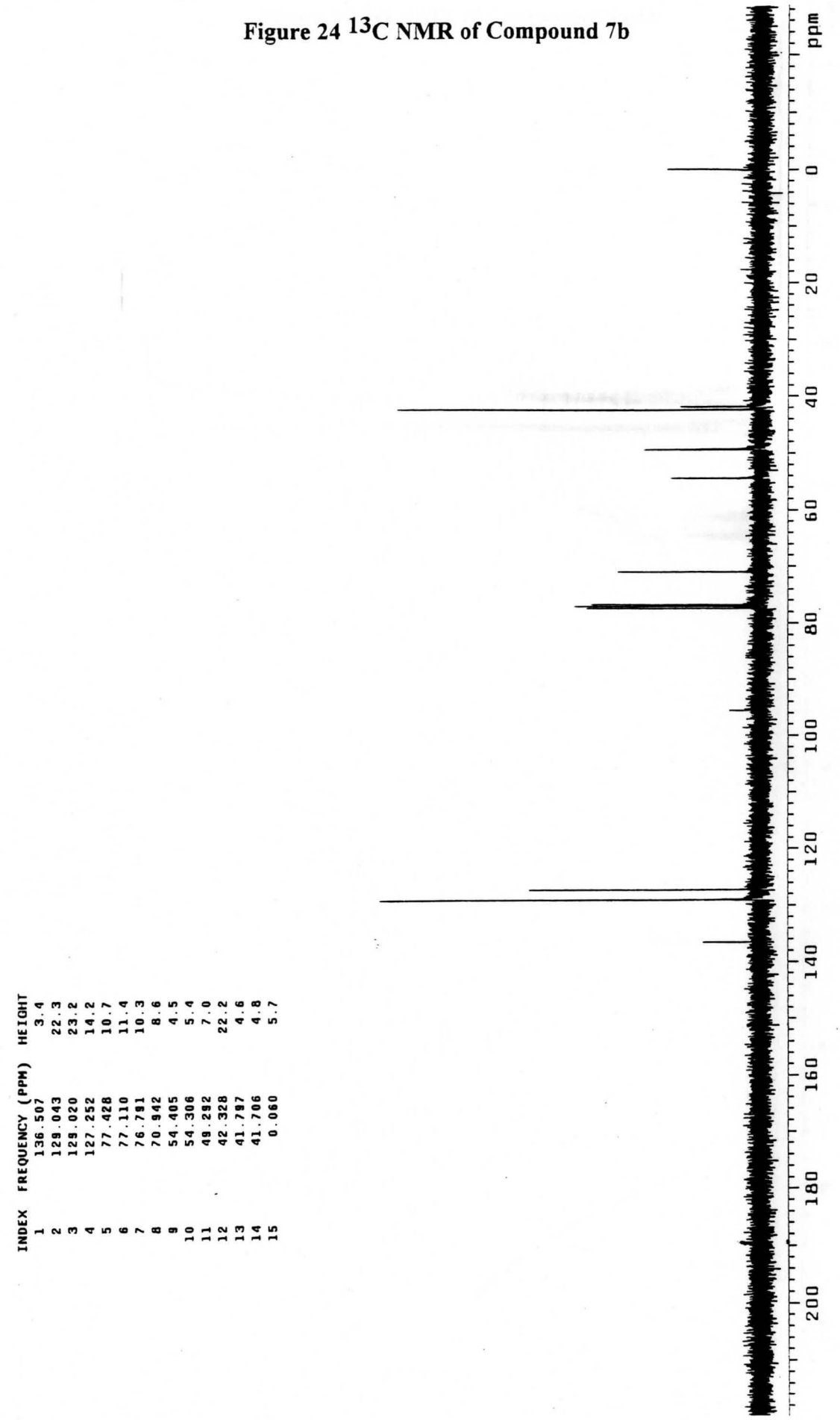


Figure 24 ^{13}C NMR of Compound 7b



INDEX	FREQUENCY (PPM)	HEIGHT
1	136.507	3.4
2	129.043	22.3
3	129.020	23.2
4	127.252	14.2
5	77.428	10.7
6	77.110	11.4
7	76.791	10.3
8	70.942	8.6
9	54.405	4.5
10	54.306	5.4
11	49.292	7.0
12	42.328	22.2
13	41.797	4.6
14	41.706	4.8
15	0.060	5.7

Figure 25 ^{1}H NMR of Compound 8a/8b

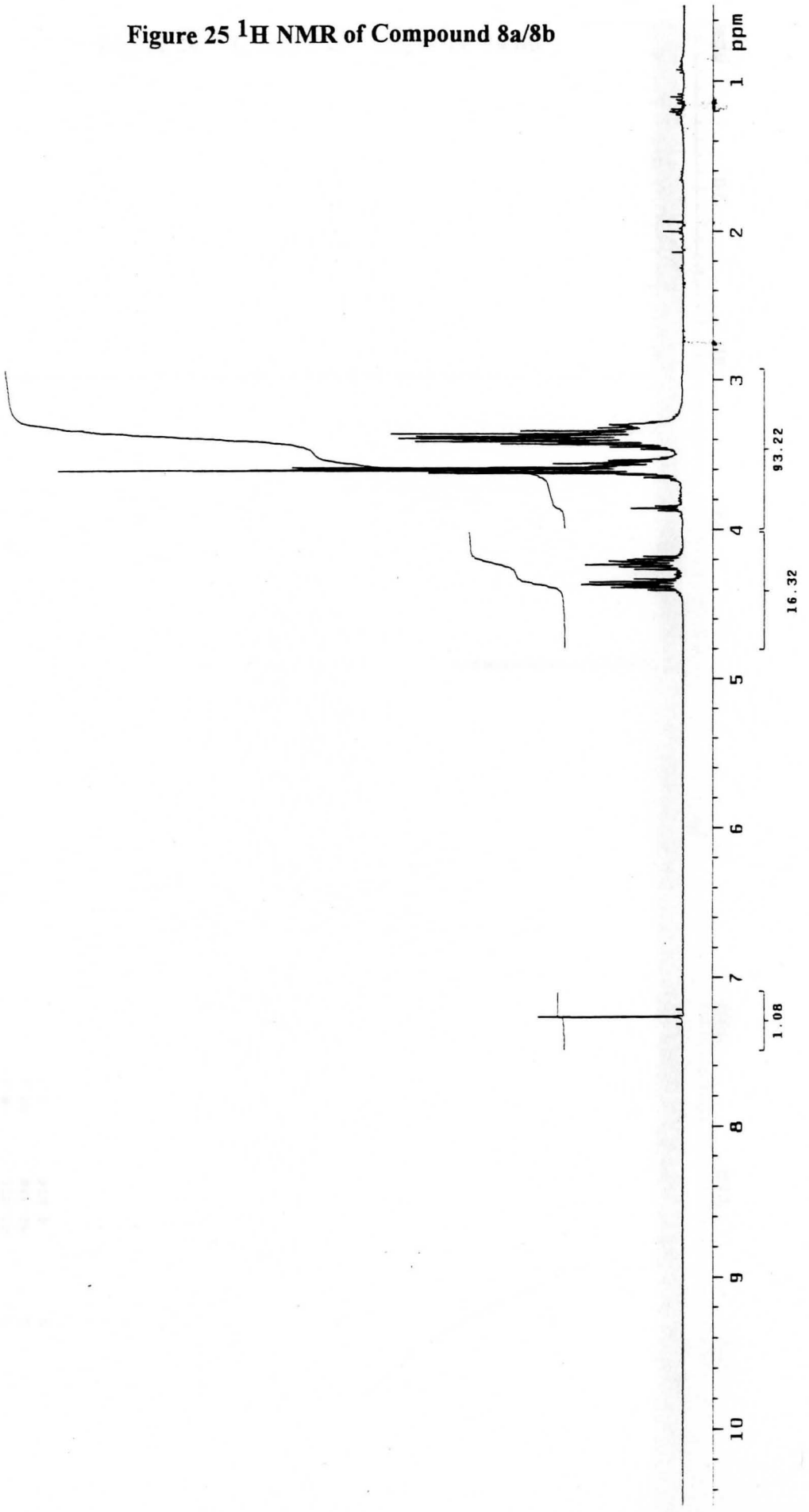
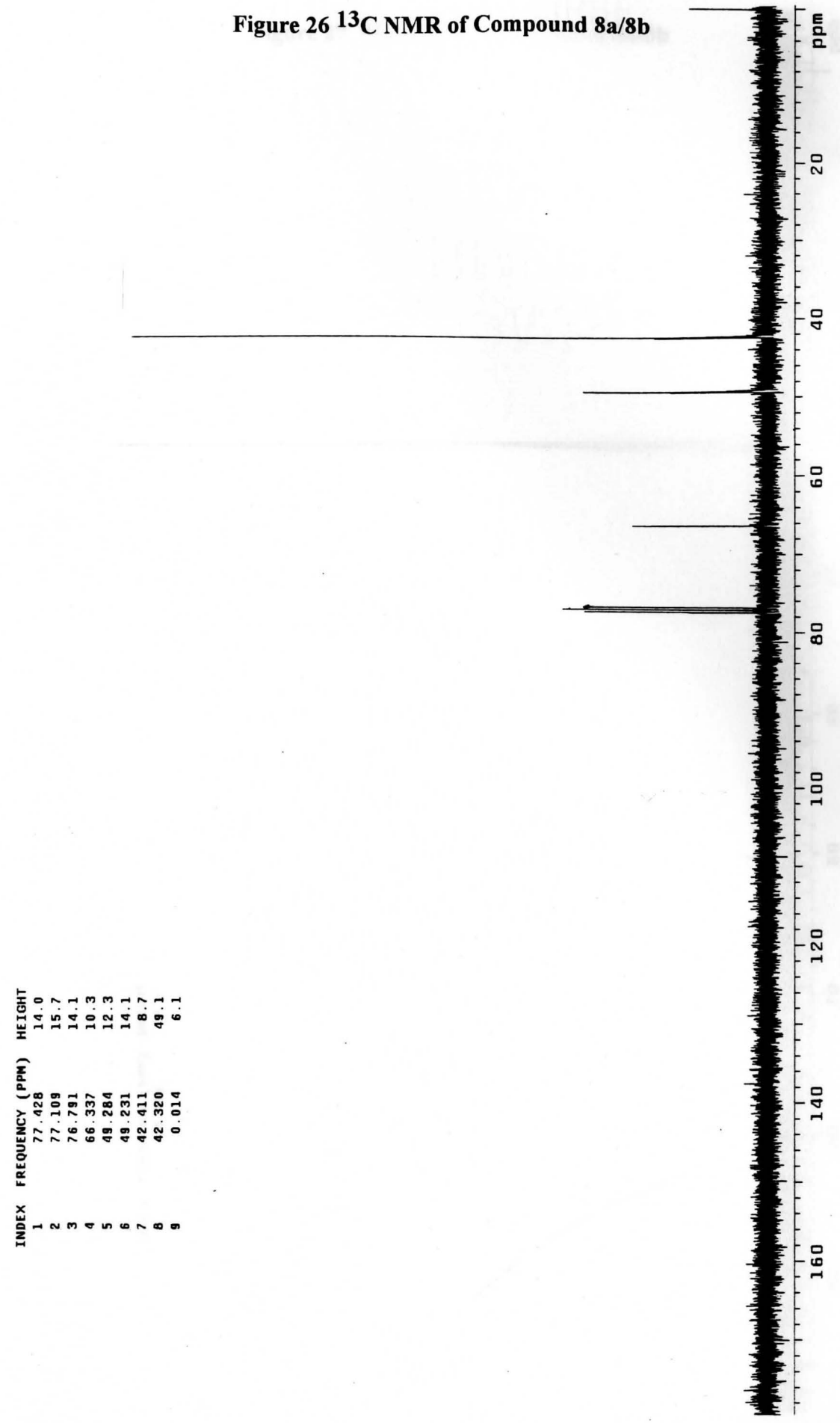


Figure 26 ^{13}C NMR of Compound 8a/8b



INDEX	FREQUENCY (PPM)	HEIGHT
1	77.428	14.0
2	77.109	15.7
3	76.791	14.1
4	66.337	10.3
5	49.284	12.3
6	49.231	14.1
7	42.411	8.7
8	42.320	49.1
9	0.014	6.1

Figure 27 ^{31}P NMR of Compound 8a/8b