SYNTHESIS AND CHARACTERIZATION OF NOVEL PHOSPHONOPEPTIDES

by

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ABSTRACT

The focus of the research that is presented in this thesis is the formation and characterization of a novel phosphonopeptide that was designed as a potential inhibitor of HIV-1 protease. Standard coupling reagents and techniques were employed in trying to link the side chain peptides to the core phosphonate structures. The core phosphonate structures and the side chain peptides were synthesized using cited reference methods. Finally, determination and confirmation of structural identity of these peptides was made using ^{1}H, ^{13}C, ^{31}P NMR, and IR spectroscopy.

ACKNOWLEDGMENTS

I would like to thank Dr. Jackson for the generous help and guidance that he has provided during this research project. I would also like to acknowledge and thank Dr. Mincey and Dr. Mike who helped me on this project by being members of my thesis committee. And lastly, I would like to say how much I appreciate all my friends and family for their support and understanding.

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LIST OF ABBREVIATIONS

Abbreviation	Description
Bn	benzyl
Cbz	benzyloxycarbonyl
DCC	1,3-dicyclohexylcarbodiimide
DCU	dicyclohexylurea
DMF	dimethylformamide
EDC	1-ethyl-3-[3-(diethylamino)propyl]-carbodiimide
Et	ethyl
Et_3N	triethylamine
EtOAc	ethylacetate
g	gram
FTIR	fourier transform infrared spectroscopy
GC	gas chromatography
HIV-1	human immunodeficiency virus type1
HCl	hydrochloric acid
HOBt	1-hydroxybenzotriazole
Hz	Hertz
Ile	isoleucine
J	coupling constant (in Hz)
KH_2PO_4	potassium phosphate
KOH	potassium hydroxide
Leu	leucine
Me	methyl
MeOH	methanol
mg	milligram

mL	milliliter
MS	mass spectrometry
mmol	millimoles
$NaHCO_3$	sodium bicarbonate
NaCl	sodium chloride
NMR	nuclear magnetic resonance
ppm	parts per million
Phe	phenylalanine
Pro	proline
ProOMe	proline methyl ester hydrochloride
Ser	serine
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Tyr	tyrosine
Val	valine

Chapter 1 Introduction

Viruses are obligate intracellular parasites. This means they can only multiply by using other cell's metabolic machinery. Viruses fall into 2 general classes based on what type of genetic material they use, DNA or RNA. They never contain both types of nucleic acids.

For a virus (also called a virion) to multiply it must enter the host cell. It can do this by attaching itself to the plasma membrane of the host cell by interacting with specific receptors on the cell surface. The virion can then penetrate into the interior of the cell by a process sometimes called viropexis. This is an invagination by the cell membrane with pinocytosis of the virion particle. After this, the virus penetrates the cell membrane, uncoating occurs, and the viral nucleic acid is released into the cytoplasm of the cell. Now the viral genome is replicated to provide genetic material for its progeny by using DNA or RNA polymerases. This results in the formation of what is called messenger RNA. Messenger RNA translates the message it is carrying to protein by incorporating itself into the host cell DNA. The results of this message are enzymes needed to produce nucleic acids and proteins for the viral core. The last two stages of viral replication are the assembly of the virion particle and the budding of the mature viruses. In the process of assembly, new virial RNA and structural proteins are brought together on a newly formed membrane. Then the mature viral particles are released from the cell through a process called budding.[1,2]

The AIDS virus in particular belongs to a class of viruses called retroviruses which use RNA as their genetic material. The distinguishing feature of the retroviral infection of a host cell is the initial conversion of their RNA genome to DNA by virus mitigated reversed transcription followed by the stable integration of the retroviral DNA genome into the host cell's chromosome. All retroviruses have 3 genes. These genes code for coat proteins, which make up the inner virus particle (called the *gag* gene), the

enzyme reverse-transcriptase (called the *pol* gene), and the proteins of the viral envelope (called the *env* gene). The AIDS virus also has extra genes that specify for four or five additional proteins, which are regulator proteins that give finer levels of control and versatility. Two of the best known of these genes are the *tat* gene which is an up-regulator or amplifier of viral gene expression in the infected cell, and the *rev* gene, which shifts the balance from production of viral regulatory proteins to proteins that make up virus particles.

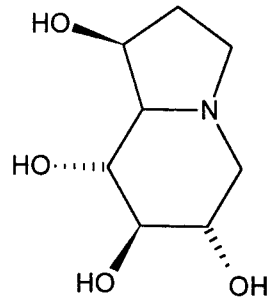
The AIDS virus displays tropism for the human T4-positive lymphocytes and some macrophages, which perform functions vital to the human immune system. These cells have the CD4 receptor protein while most other cells in the body do not. HIV binds to the CD4 receptor protein and since no other cells in the body contain this protein, HIV only infects T4-positive lymphocytes and these macrophages. The primary effect of the AIDS virus on the T4-positive lymphocytes is cytopathic. This gradual depletion of cell population leads to the eventual collapse of the host's immune system, allowing opportunistic infections to take hold, eventually leading to death. The effect of HIV infection on macrophages is different however. It does not kill them but uses their cellular machinery to produce more virions.[3,4]

The life cycle of the AIDS virus is much like that of other retroviruses:

- Bind to the surface of the cell.
- Virus brought into the cytoplasm of the cell.
- Viral envelope removed.
- Reverse-transcriptase is activated.
- Conversion of viral RNA to DNA.
- Viral DNA moves into the nucleus of the cell.
- Viral DNA is incorporated into the host cell DNA.
- Host cell reads integrated viral DNA and makes more copies of viral RNA.
- RNA functions either as a viral messenger sent to program the formation of viral proteins or becomes genetic material for new virion particles.
- Formation of new virion particles.

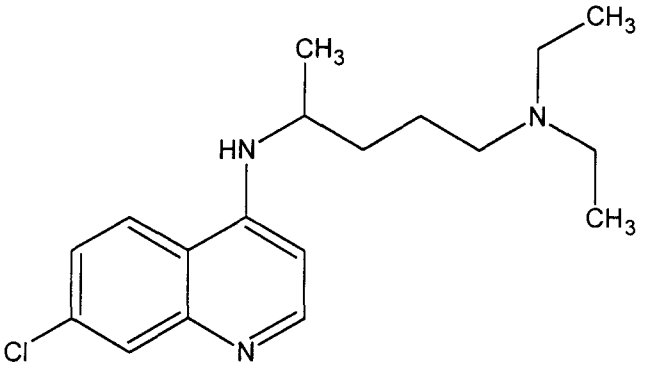
- Release of mature viruses from the host cell.[4]

The clinical use of antiviral drugs is a fairly recent development in comparison to antibacterial agents. In the 1950's a number of antiviral drugs had been discovered[1], but their clinical application was delayed until the early 1960's. The reason for the slowness in development was due to the difficulties in finding specific drugs that would target viral infections without adversely effecting the host. The difficulties arose because the virus actually uses the host cells metabolic system. Continuing research by scientists[5] led to the discovery that viruses contain enzymes that are distinct from the host cell while others carry the information for synthesizing virus specific enzymes in the genetic code. This allowed points for attack by antiviral agents. Based on this information, the steps in the replicative cycle of the AIDS virus or HIV, as it will now be called, could be considered as targets for drug chemotherapy. These include the binding stage where HIV attaches to the CD4 receptor protein. CD4 receptor decoys such as castanospermine (shown as **Structure 1)** would prevent viral attachment by acting as decoys, thus blocking HIV/CD4 protein interaction.[3]

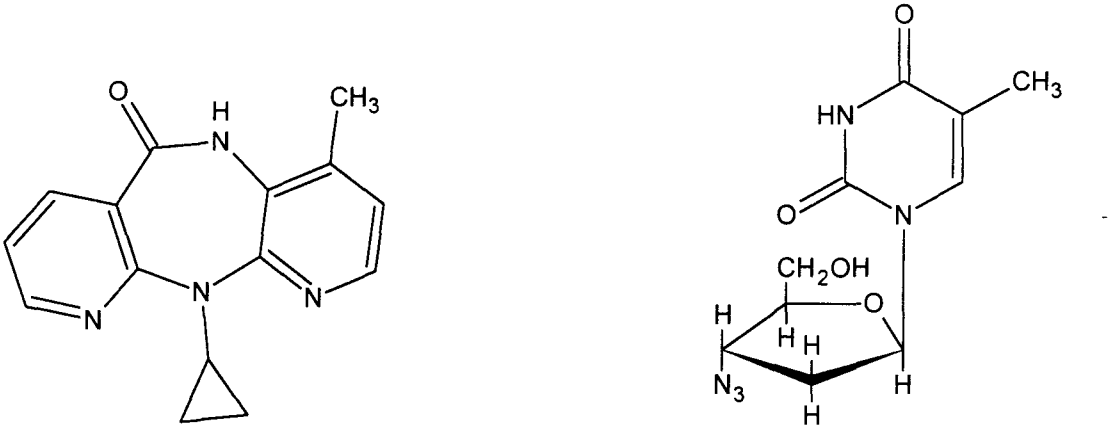


Structure 1. (Castanospermine).

After a virus binds to a CD4 receptor protein, it must penetrate into the cell and uncoat so as to expose it's RNA to be transcribed to DNA. Drugs such as chloroquinone (shown as **Structure 2**) prevent this from happening by inhibiting enzymes responsible for this function.[3] The transcription of RNA to DNA is regulated by reverse transcriptase. Inhibitors such as nevirapine and azidothymidine (shown as **Structure 3**) prevent this enzyme regulated transcription.[3]



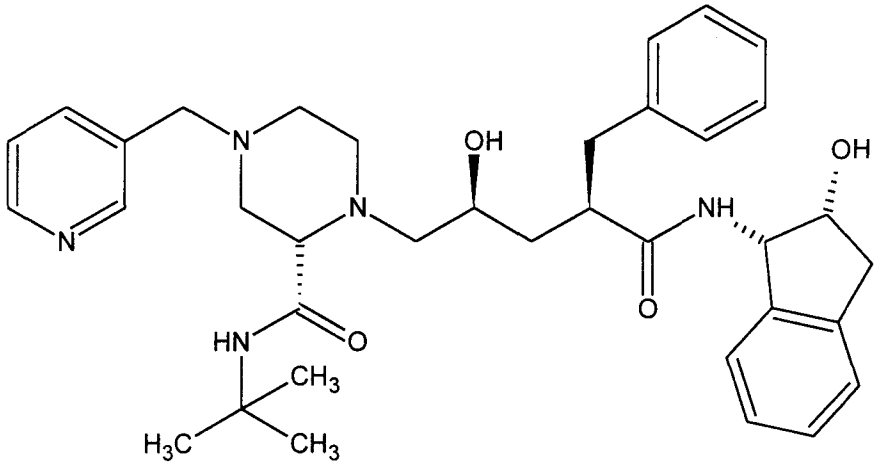
Structure 2. (Chloroquinone).



Structure 3. (Nevirapine and Azidothymidine).

The integration of the proviral DNA into the host cell DNA and expression of viral genes is essential for the synthesis of proteins that will make up new viruses. Drugs such as phosphonoacetic acid and phosphonoformic acid (shown as **Structure 4**) inhibit this integration and expression.[6] Viral protein production and assembly could be halted by protease inhibitors such as Indinavir (shown as **Structure 5**) which prevent maturation of the virions by binding up HIV-1 protease.[7] Lastly, interferons have shown promise in preventing the release of the viruses.[6,8,9]

Structure 4. (Phosphonoacetic acid and Phosphonoformic acid).



Structure 5. (Indinavir).

HIV-1 protease, in particular, is a good target for ezyme inhibition due to it's important functions in viral reproduction. The functional protease is required for the maturation of viral proteins, for the appearance of characteristic structural feactures in the virion, for the final assembly of the mature virus, and most importantly for the development of infectivity.

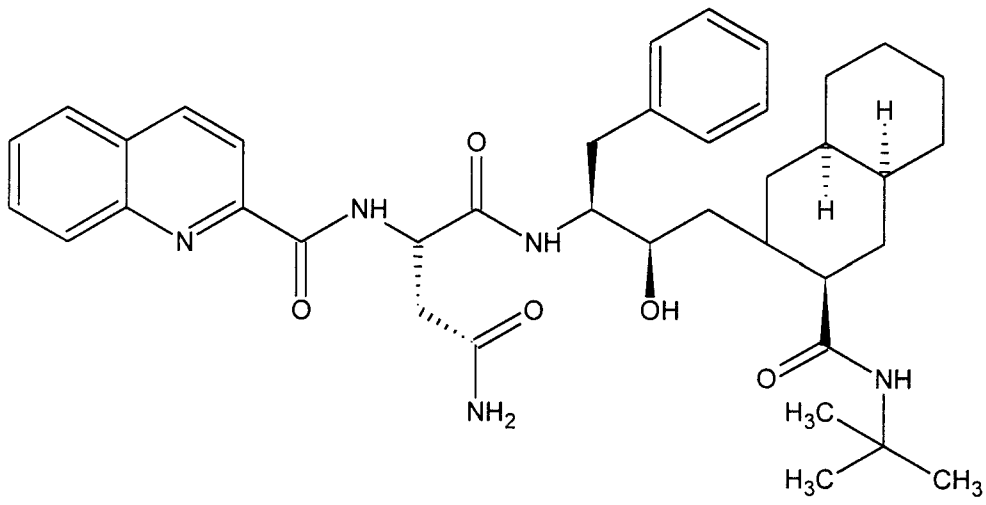
HIV-1 protease is classified as an aspartyl protease. The indicative feacture of a aspartyl protease is the Asp-Thr-Gly sequence that is present in the HIV-1 enzyme peptide structure. It is a 99 aminoacid polypeptide monomer, but the active protease exists as a dimer with each monomer contributing a aspartyl residue to the active site. These two residues assume opposite roles in general acid-base catalysis. The protonated aspartyl group effects protonation of the carbonyl oxygen of the scissile amide bond,

while the unprotonated aspartyl residue deprotonates the lytic water molecule which subsequently attacks the scissile carbonyl as a hydroxide ion.

The proteins that comprise the virion core and and enzymes essential to viral replication are the products of the *gag* and *pol* genes respectively. HIV-1 protease cleaves the translation products of these gene at specific sequences and activates the structural proteins and genes, thus rendering the virions mature and infectious. HIV-1 protease is also suspected of having a important role in the early events of the viral life cycle such as destabilization of the virion capsid or activation of various enzymes that will help in this destabilization. Based on this premise, inhibition of HIV-1 protease would also prevent proviral integration.[7,10]

Inhibition of the HIV-1 protease was recognized early as a potential chemotherapeutic strategy for treating AIDS.[11] The design of inhibitors has followed two distinct pathways. One relies upon the traditional principles of incorporating transition state mimics into substrate analogs and the second strategy is a result of the early availability of structural data on the enzyme and enzyme-inhibitor complexes (structure based design inhibitors).

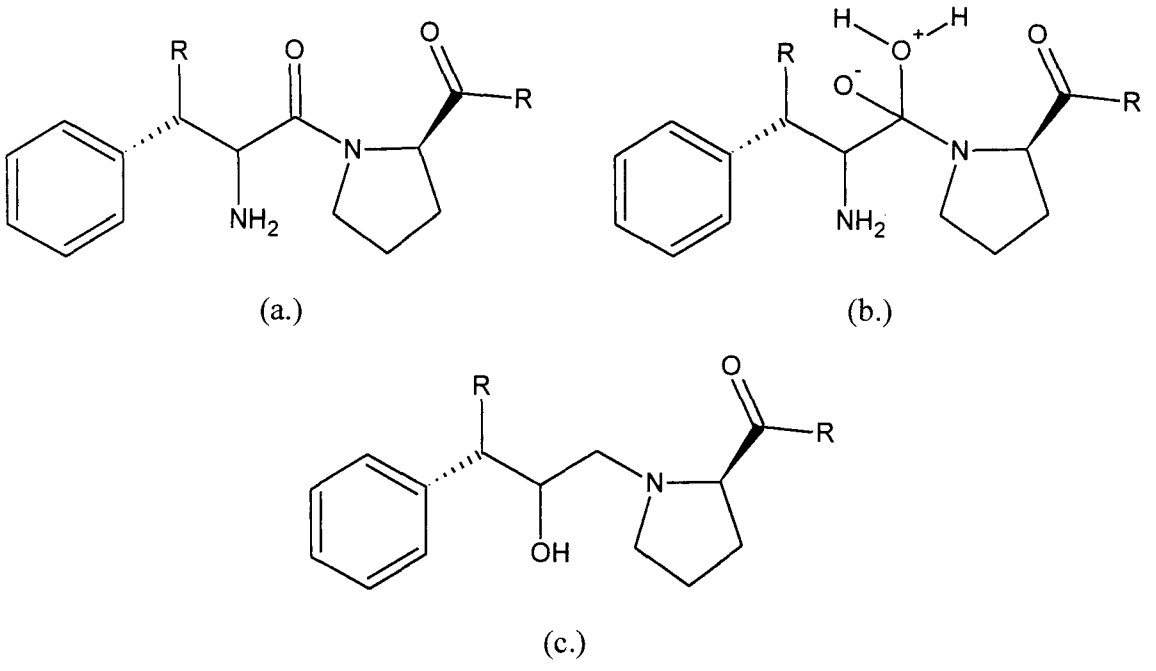
Given the mechanistic classification of HIV-1 protease as a aspartic protease, one might choose to investigate oligopeptide inhibitors that replace the hydrolyzable dipeptide linkage with a chemically stable mimic of an aspartic proteolysis transition state or reaction intermediate. One would then expect the resulting peptide analogs to inhibit HIV-1 protease, and given the distinct cleavage sequences of the enzyme, confer some selectivity. Design of a structural based inhibitor (shown as **Structure 6**) relied on the structural information regarding HIV-1 protease and inhibitor complexes.[12] Non peptide inhibitors were developed which incorporated a surrogate for the water molecule observed in almost all inhibitor-enzyme complexes that bridge the protein and inhibitor through a network of hydrogen bonds to the flaps of the protease.



Structure 6. (Ro31-8959; a HIV-1 protease inhibitor developed by scientists at Hoffman-LaRoche).

Inhibition of HIV-1 protease results in the formation of replication incompetent, non-infectious virions. The virions are characterized by the presence of unprocessed *gag* polyproteins and by the absence of reverse transcriptase activity. The unavailability of protease renders the virus permanently immature. Based on this evidence, the validity of this enzyme as a suitable target for inhibition is confirmed. Protease inhibitors may offer therapeutic advantages over inhibitors of reverse transcriptase such as azidothymidine (AZT) and may also prove less toxic, most likely due to their specificity for the retroviral proteases.[3,7,11]

The design of transition state analog enzyme inhibitors is based on the hypothesis that molecules which resemble a substrate in its transition state geometry have a much higher affinity for an enzyme than the substrate itself.[13] Therefore when going about designing transition state analog inhibitors it is necessary to find stable molecules that resemble the metastable reaction intermediates (see **Structure 7**).[7] These transition state analogs may be structured in such a way that they resemble substrates to the extent that they are bound very tightly to an enzyme without undergoing catalytic conversion, thereby inhibiting it from performing further catalysis.[14,15]

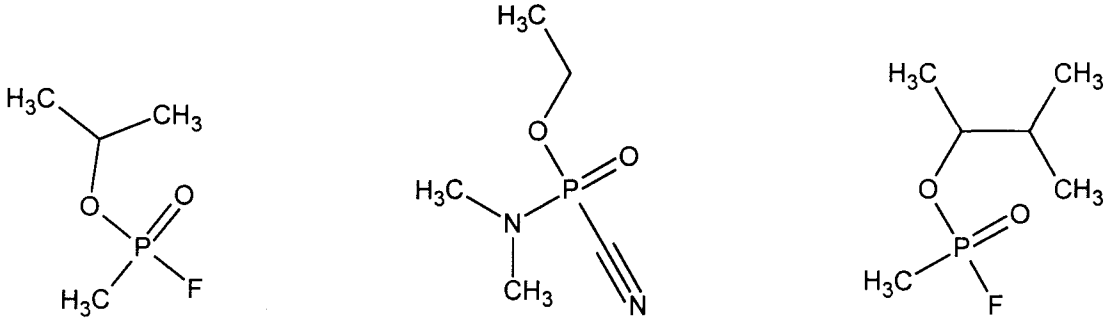


Structure 7. ((a.) Phe-Pro peptide substrate; (b.) the Phe-Pro peptide reaction intermediate; (c.) and a hydroxyethylamine-containing peptide transistion state analog of the Phe-Pro peptide reaction intermediate).

The transition state geometry of a peptide undergoing hydrolysis is tetrahedral. Hydrolysis occurs when a peptide bond is catalytically cleaved. Therefore when designing a transition state analog to inhibit this cleavage, the tetrahedral geometry would have to be accounted for in some way. Organophosphorus compounds, where the phosphorus is pentavalent, satisfy this requirement. In recent years numerous investigations have been performed using analogs in which a normal carboxylic acid functional group has been replaced by another type of acidic linkage.[16] This was done with the anticipation that changing the carboxylic acid linkage to another type of acid, of similar or different strength, might cause the analog to serve as a false substrate for normal metabolic processes. Phosphorus-centered analog acids can provide regulation of biological processes by several routes, including the introduction of a site of modified acidity, the formation of modified amide and ester linkages, and by resisting ordinary

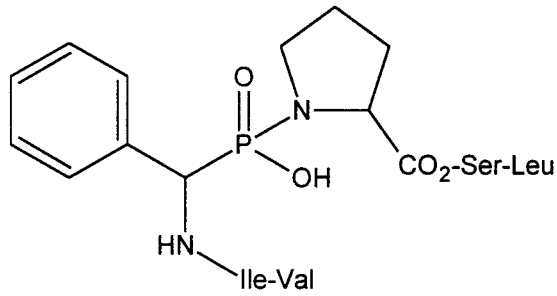
decarboxylation processes. In general this involves the replacement of a C-COOH linkage with a C-P(OH) linkage. These phosphorus-centered acid sites could include both dibasic linkages [-$P(OH)_2$], which will exhibit very different acidic characteristics than the normal carboxylic acids, as well as monobasic linkages [-P(OH)], expected to exhibit characteristics more closely corresponding to those of the natural substrates.

The history of organophosphorus chemistry probably began during the middle ages. Paracelsus (1493-1541) and the Arabic alchemists may have had phosphorus available to them, but the discovery of elemental phosphorus is attributed to Hennig Brand between 1667 and 1677.[17] The scientifically planned study of organophosphorus compounds did not systematically begin until the early 19th century. The esterification of dihydrated phosphoric acid represents the first significant research in this field (In 1820, Lassaigne synthesized triethyl phosphite from ethanol and phosphoric acid). Thenard, in 1847, prepared several phosphines[18] and von Hofman synthesized the first alkylphosphonic acids in 1872.[19] The first systematic investigation of organophosphorus compounds did not begin until 1874 with the work of A. Michaelis. His work gave the foundation for the formation of P-C, P-S, P-Se, and P-N bonds and the Michaelis-Becker[20] reaction is widely used for the synthesis of alkylphosphonates. Michaelis and Kaehne[21] discovered the reaction between the ester of a trivalent phosphorus compound with an alkyl halide, which is one of the most versatile methods of P-C bond formation. It is now known as the Michaelis-Arbuzov reaction due to the detail in which Arbuzov explored this reaction.[22] During the 1930's organophosphorus compounds were intensively studied as potential chemical warfare agents by British and German scientists. Scientists in Germany, headed by Schrader[24] and Saunders[25], succeeded in synthesizing



Structure 8. (Nerve gases sarin, tabun, and soman).

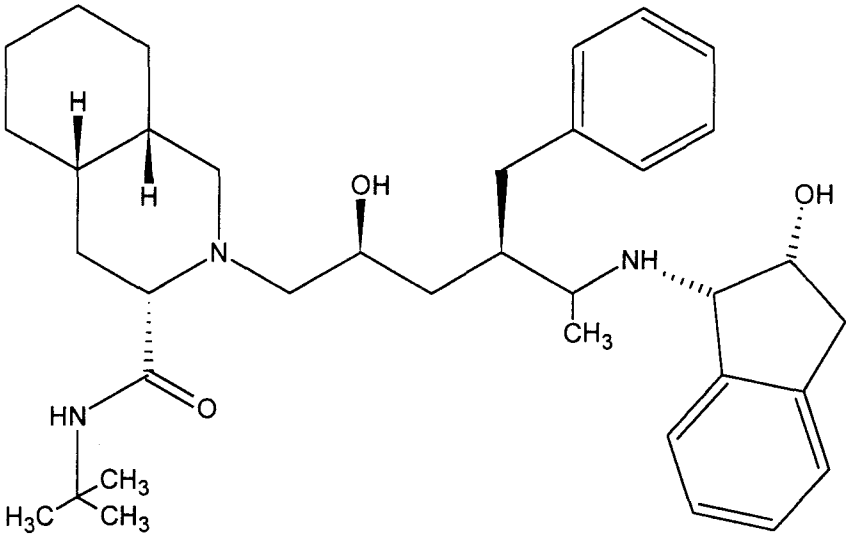
three important nerve gases; tabun, sarin, and soman (see **Structure 8**). These nerve gases are classical inhibitors of the enzyme acetylcholinesterase. In the 1950's the conversion of an aldehyde or ketone into a olefin (alkene) by reaction with a phosphine-ylid opened up a whole new field of chemistry. This reaction is called the Wittig reation.[25] This led to the preparation of optically active phosphines by Horner and his group.[26,27-30] Recently in the 1990's organophosphorus based transition state analogs have been shown to be potent inhibitors of aspartic proteases.[31] Based on this information, the determined structural makeup of HIV-1 protease, and the known peptide substrates of the HIV-1 protease, the following phosphorus based transition state analog was determined (See **Structure 9**). This peptide analog was suggested as a possible inhibitor of the catalyzed hydrolysis of the bond between phenylalanine and proline by HIV-1 protease.



Structure 9. (Possible transition state analog inhibitor).

Much of the structural information known about HIV-1 protease and how it interacts with the substrate is derived from X-ray crystal studies. This was possible since the crystal structure of HIV-1 protease was determined in 1989.[32] Insights derived from previous studies of the HIV-1 protease as well as the comparison of the present structure to another retroviral protease may help in understanding the details of the functioning of HIV-1 protease. Crystal structure studies of how HIV-1 protease functions in cleaving peptide bonds and on the conformational changes that take place when substrates and inhibitors bind to the enzyme may give clues on how to design HIV-1 protease inhibitors

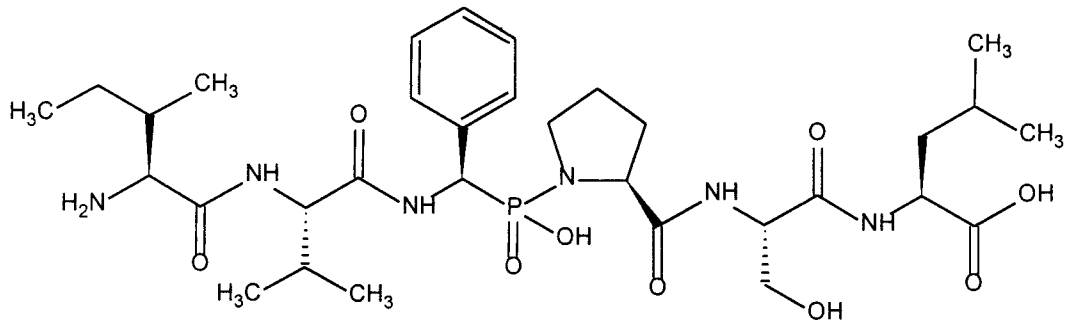
which could be used as effective agents in the control of AIDS (see **Structure 10** for a example of a structurally designed enzyme inhibitor).



Structure 10. (L-704,486, Hydroxyaminopentaneamide; a structurally designed protease inhibitor).

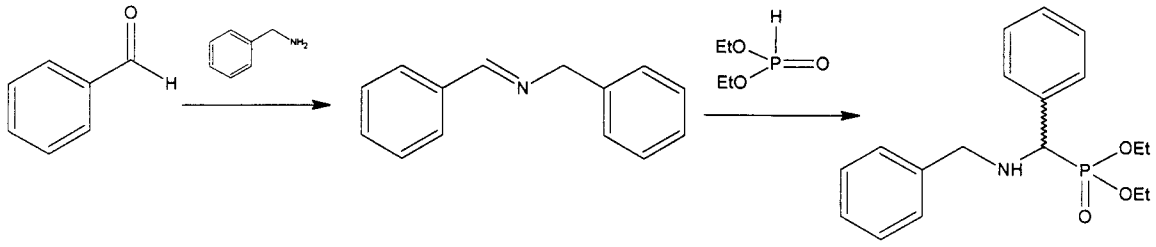
Chapter 2 Results and Discussion

The aim of this project was to synthesize the phosphonopeptide **11**, a potential transition state analog inhibitor of HIV-1 protease. The core of this particular phosphonopeptide is a modified form of phenylalanine with the carbonyl group replaced with a phosphoryl group. In doing this, HIV-1 protease may preferentially bind to this phosphonopeptide, and thus be inhibited. The side chain peptide sequences were chosen in regard to known active site peptide residues that HIV-1 protease hydrolyzes. The core phosphonates were synthesized using known reaction pathways. Modification and linking of the phosphonates to the peptides and peptide synthesis was also investigated.



11

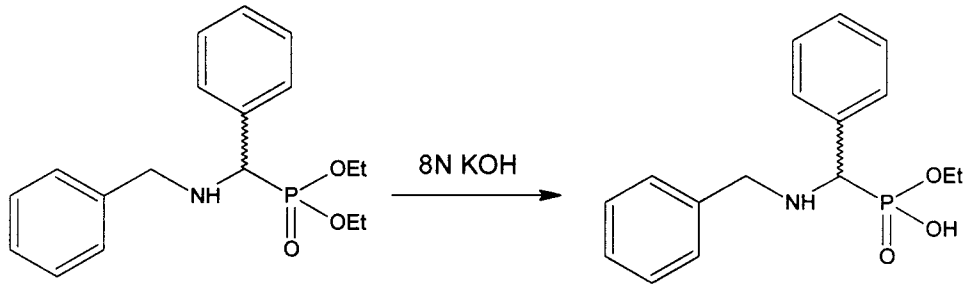
The first syntheses attempted were those involving the phosphonopeptide's core phosphonate structure.[33] This was done by reacting benzaldehyde with benzylamine to produce the isolible imine. This imine was then coupled with triethyl phosphite to form the aminophosphonate diester **12**.[34] This diester was isolated in very good yields (97%) and with good purity following a simple aqueous workup. This aminophosphonate



12a **12**

Scheme 1

diester **12** was then modified to form two related compounds by hydrolyzing the phosphonate ester. The first one formed was the aminophosphonic acid monoester **13**. This was done by refluxing diester **12** in 8N KOH for 24 hours, followed by a simple aqueous workup. High yields were obtained (75%) with very good purity after recrystallization from methanol. The disappearance of the triplet (CH_3) at 1.29 ppm and the multiplet (CH_2) at 3.93-4.03 ppm, due to the loss of one of the two ethoxy groups, in

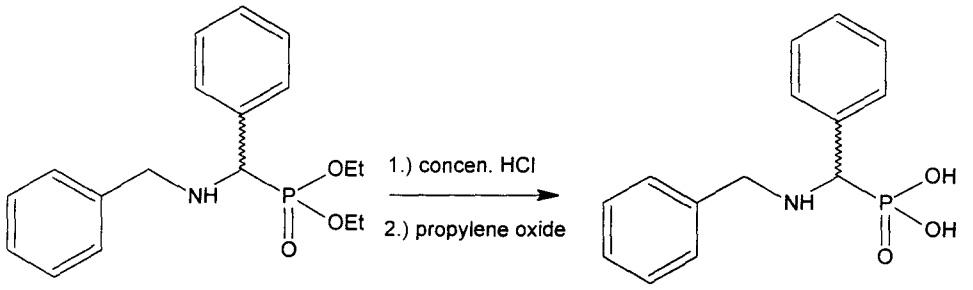


12 **13**

Scheme 2

the ^1H spectra confirmed product formation. The second related compound formed was the aminophosphonic diacid **14**. The conversion from diester **12** to diacid 14 was relatively facile. It was achieved by refluxing diester **12** in concentrated HCl for 48 hours. Upon removal of the solvent, the residue was dissolved in a minimum of ethanol and precipitated out of solution by dropwise addition of propylene oxide.[34,35] This procedure afforded phosphonate **14** in yields averaging around 94%. The loss of the two

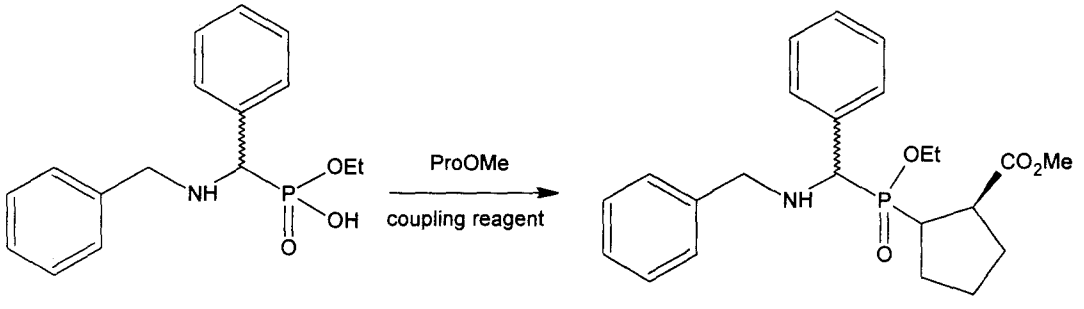
triplets (CH_3) at 1.18 ppm and 1.29 ppm as well as the 2 multiplets (CH_2) at 3.84-3.92 ppm and 3.93-4.03 ppm belonging to the two ethoxy groups confirmed the conversion of diester **12** into diacid **14**.



12 **14**

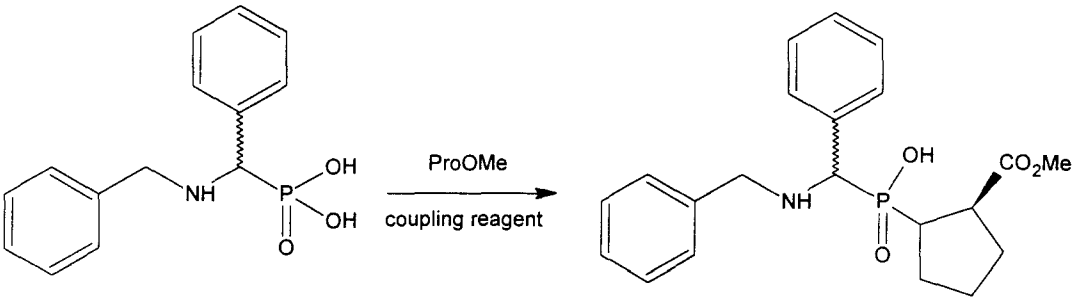
Scheme 3

Having made the two phosphonic acids needed for further syntheses, the coupling of compounds **13** and **14** to proline methyl ester to form the phosphonopeptides **15** and **16**



13 **15**

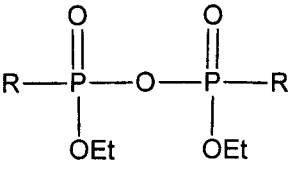
Scheme 4



14 **16**

Scheme 5

was attempted. A wide variety of coupling reagents (EDC, DCC, HOBt, DIPC, EEDC, Carbonyldiimidazole, and Woodward's reagent) were tried in order to couple the aminophosphonic acids to proline as well as aqueous and nonaqueous workups without success. This may be attributed to the low solubility of diacid **14** in methylene chloride, THF, and even DMF. However monoester **13** has appreciable solublity in these various solvents. Sterics may have also played a part in this reaction. Due to proline's bulky size and the two phenyl groups present on the two aminophosphonic acids, coupling of the two may have been more difficult. This is not to say that the phosphonopeptides **15** and **16** did not form. According to ^{31}P NMR 2 or 3 products did form having shifts of 6.64 ppm, 21.39 ppm, and 24.50 ppm. One of them could have been **15** or **16**, but even if they had formed they were in very low yields. Two of the other products formed may have been the phosphorus anhydride **17** or the *O*-phosphorylurea **18**.[33]



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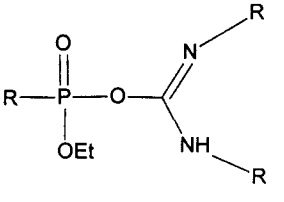
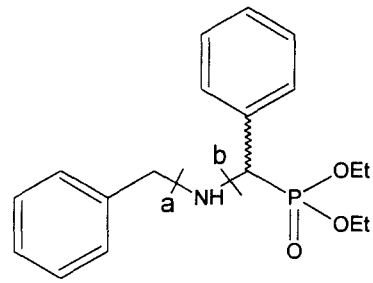


18

The benzyl deprotection of the amino portion of these molecules and the subsequent coupling to a dipeptide was investigated next.

Deprotection of phosphonates **12-14** was a facile reaction involving Pearlman's catalyst which preferentially cleaves benzyl groups.[36,37] This catalyst could have possibly cleaved bonds in two different locations (see **19**). Reaction results show that the catalyst



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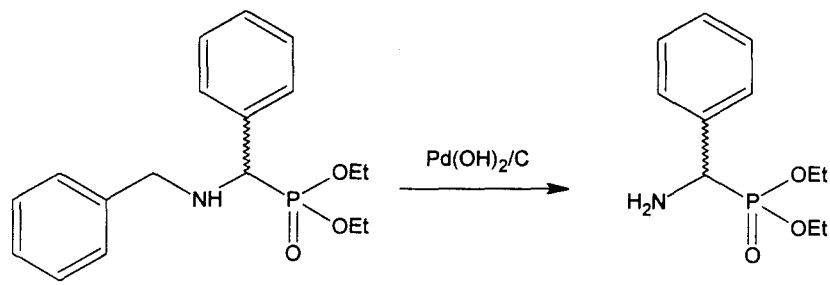
cleaves bond *a* preferentially over bond *b*. This could be the result of electronic and steric factors. Bond *a* may be cleaved preferentially due to the enhanced electronic character of the benzyl pi system and sterically due to having less hinderance as compared to cleavage at bond *b*. Based on this information the benzyl group would be adsorbed to the catalyst preferentially and thus cleaved at bond *a*. Debenzylation of phosphonates **12-14** was performed in methanol with a reactant to catalyst ratio of approximately 1:1. This mixture was placed in a Parr hydrogenation apparatus and under a positive pressure of 40 psi of hydrogen the deprotection occured. Debenzylation of diester **12** to yield the deprotected diester **20** was performed under these conditions.



12 **20**

Scheme 6

After purification by flash column chromatography debenzylated diester **20** was obtained in 70% yield. Product formation was confirmed by ^{1}H NMR where there was the absence of the NH singlet at 2.52 ppm and the appearance of a broad singlet at 4.85 ppm due to the free amine as well as the disappearance of the benzyl CH_2 doublet at 4.03 ppm. ^{13}C NMR also confirmed this due to the absence of the benzyl CH_2 peak at 51.15 ppm in **20** as well as the appearance of four peaks in the aromatic region instead of eight.

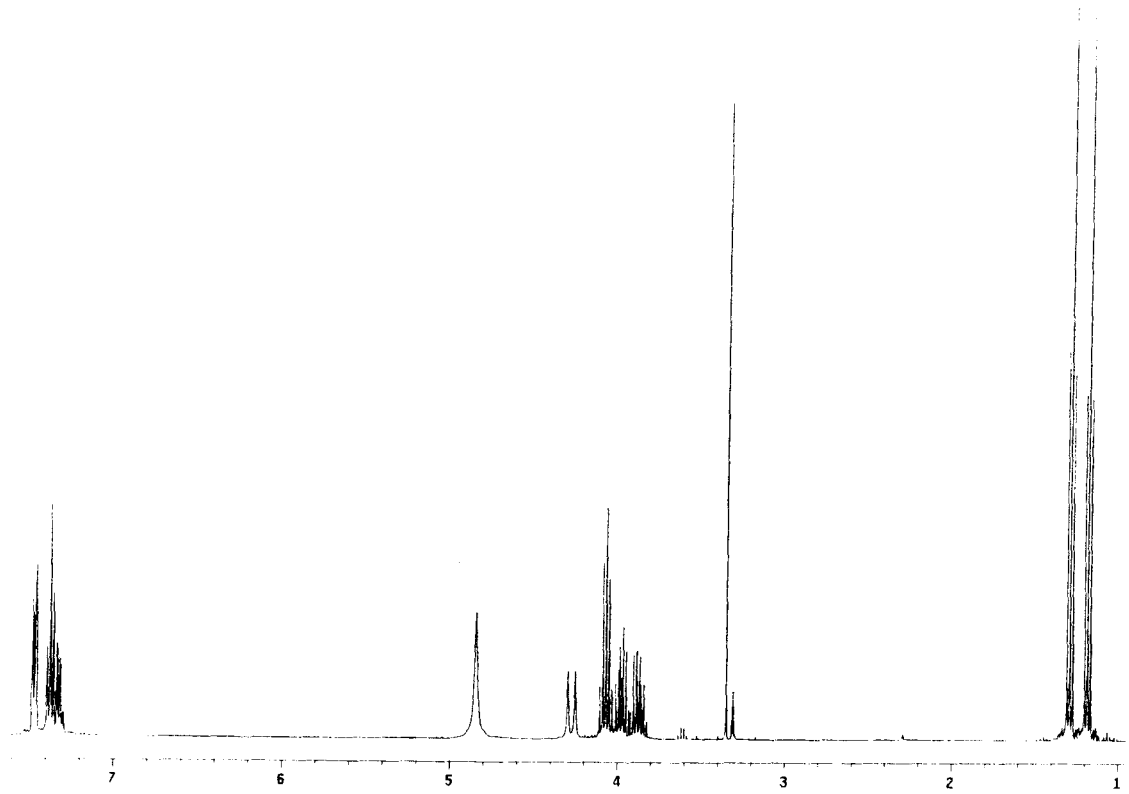
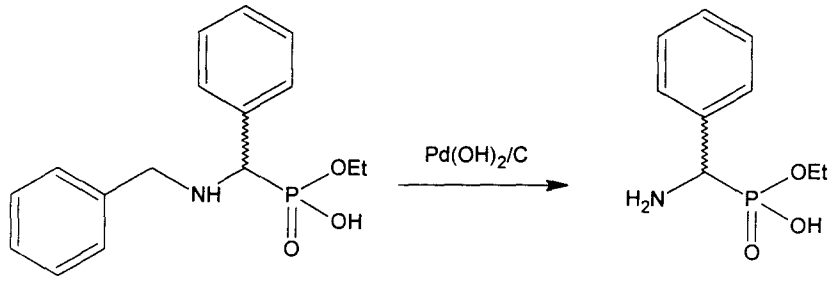


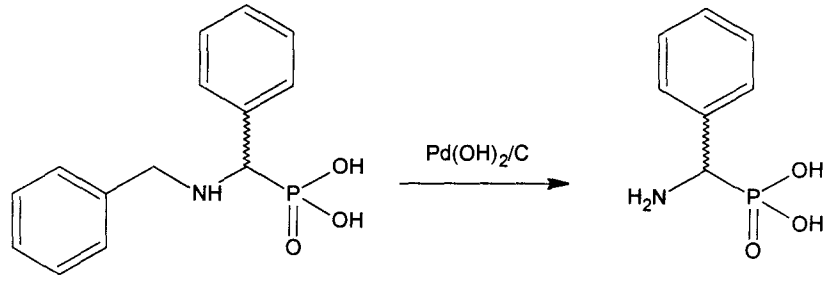
Figure 1 ^{1}H NMR of Compound **20**

Deprotection of phosphonates **13** and **14** was performed under the same conditions as **12** but since the resulting products were more polar, the workup involved was much simpler. Debenzylation of **13** and **14** to yield the deprotected aminophosphonic acid monoester **21** and diacid **22** occured readily. The resulting products were washed with hot methanol and 1-propanol respectively, then vacuum filtered. Good yield of debenzylated monoester **21** was obtained (69%), but lesser yields



13 **21**

Scheme 7



14 **22**

Scheme 8

(50%) of debenzylated diacid **22** were observed. This was most likely due to the low solubility of diacid **14** in methanol (Some of the starting product **14** was filtered off when the catalyst was removed). Formation of debenzylated phosphonates **21** and **22** was confirmed by ^{1}H NMR and ^{13}C NMR. For Compound **21** most notably in the ^{1}H NMR spectra was the absence of the doublet at 4.19 ppm and the absence of the peak at 51.26 ppm in the ^{13}C NMR spectra. The absence of these two peaks is due to the loss of the benzyl CH_2. There was also a reduced number of peaks in the aromatic regions of both spectra. For compound **22** the absence of the doublet at 3.84 ppm in the 1H NMR spectra and the doublet at 49.43 ppm in the ^{13}C NMR spectra a number of peaks in the aromatic regions of both spectra. The peaks at 3.84 ppm and 49.43 ppm were due to the benzyl CH_2 and their absence signifies that the cleavage was successful.

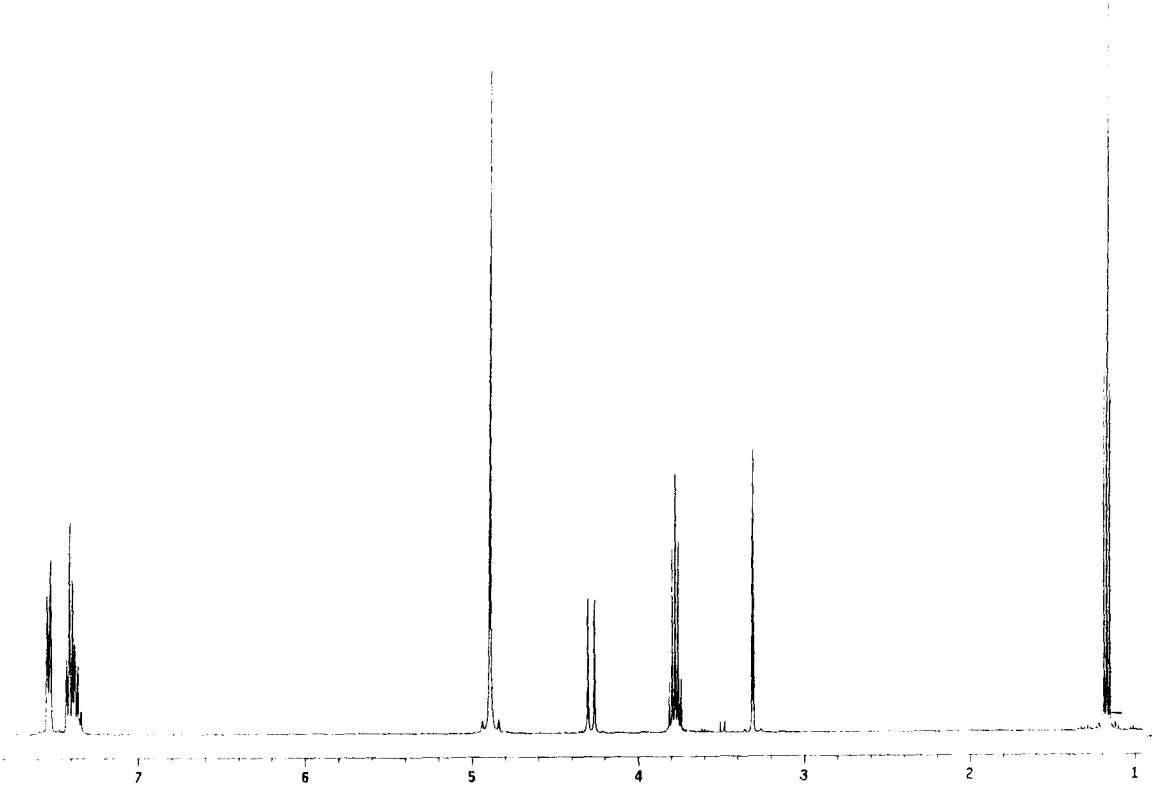
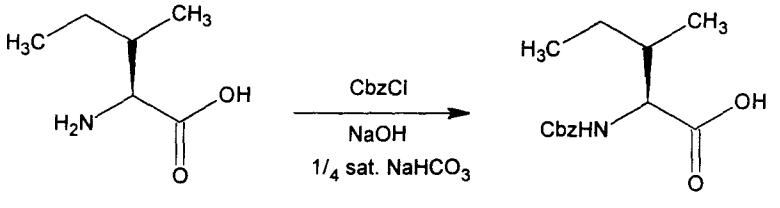


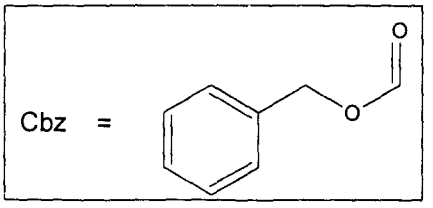
Figure 2 ^{1}H NMR of Compound **21**

After the deprotection of the core phosphonates was completed, the dipeptide that would be coupled to it was made. This dipeptide was carbobenzoxy Isoleucine-Valine. The first step was to protect Isoleucine with benzyl chloroformate.[38] This was done by dissolving by dissolving the Isoleucine in ¼ saturated $NaHCO_3$ solution (which acted as a buffer to the control the pH changes that would occur during coupling) then adding benzyl chloroformate. Upon acidification of the solution after a few hours, Cbz-Isoleucine(**23**) was obtained in good yields (93%). Cbz-Isoleucine (**23**) was next coupled



23

Scheme 9



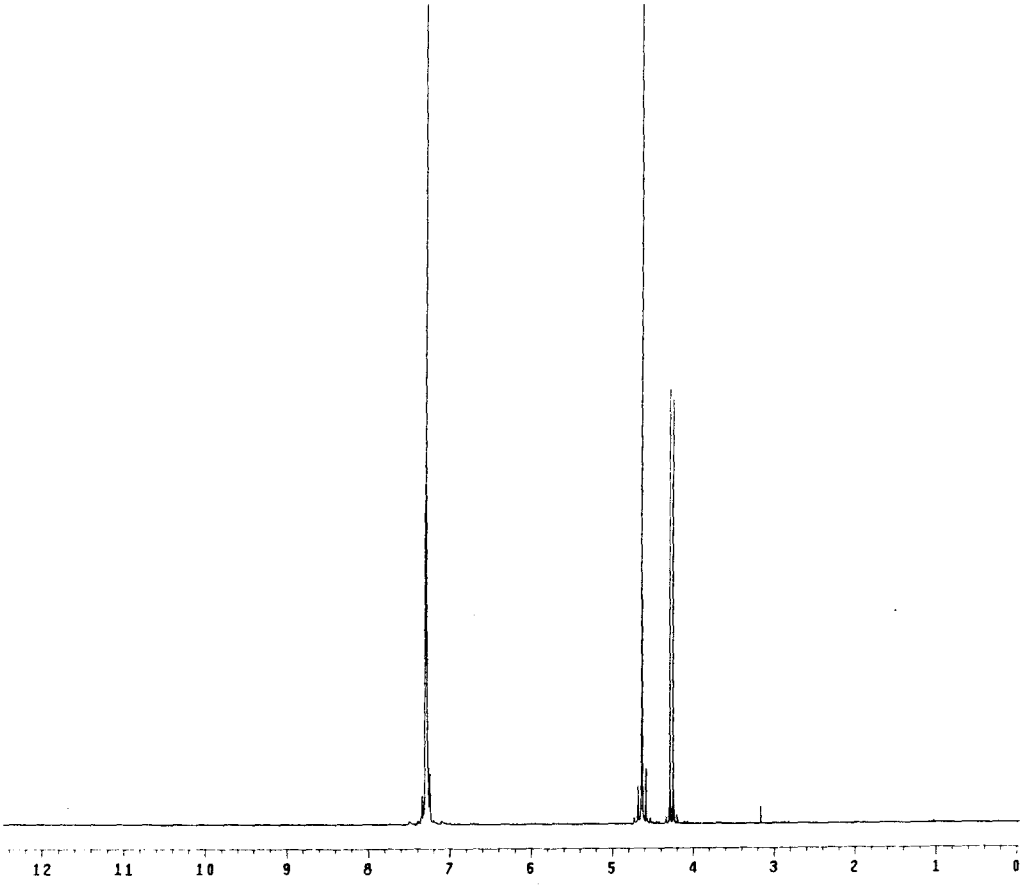
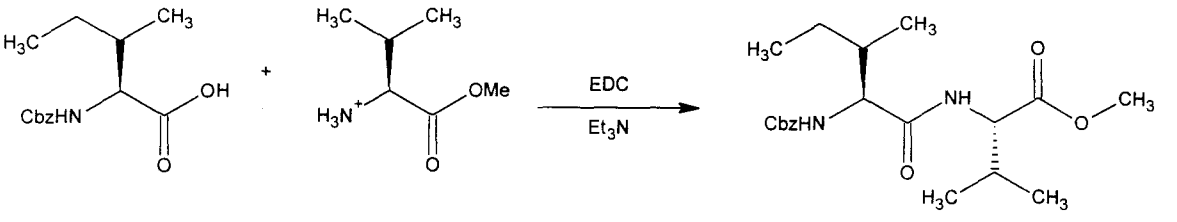


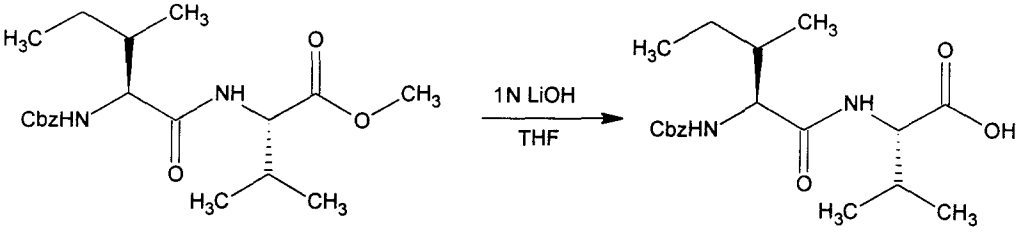
Figure 3 ^{1}H NMR of Compound **22**

with Valine methyl ester using standard peptide coupling techniques.[38] EDC and DCC were both tried but the EDC was the coupling reagent of choice due to the good yields it produced. The dipeptide Cbz-Isoleucine-Valine methyl ester (**24**) was produced with a yield of 76% after purification by flash column chromatography.



23 **24**

Scheme 10



24 **25**

Scheme 11

To couple the dipeptide to the debenzylated phosphonate core structures, the methyl ester had to be hydrolyzed. This occurred readily in the presence of 1N LiOH. Upon acidification the deprotected dipeptide **25** was obtained (73% yield). This hydrolysis can readily be seen in the ^{1}H spectra by the absence of the methyl ester peak at 3.71 ppm as well as in the IR spectrum were there is an absence of the peak at 1734cm^{-1} due to the methyl ester and the presence of a broad peak at 3431cm^{-1} due to the OH now present. Also still present is the peak at 1674cm^{-1} due to the carbonyl portion of the carboxylic acid.

The problem next encountered was coupling the dipeptide **25** to the debenzylated phosphonates **21** and **22** to form the intermediate phosphonopeptides **26** and **27**. Various coupling reagents were experimented with to get this to occur (EDC, DCC, DIPC as well as with or without the presence of HOBt), but with little success. The ^{1}H and ^{13}C NMR of the products of **scheme 12** indicated contamination with HOBt and the urea side

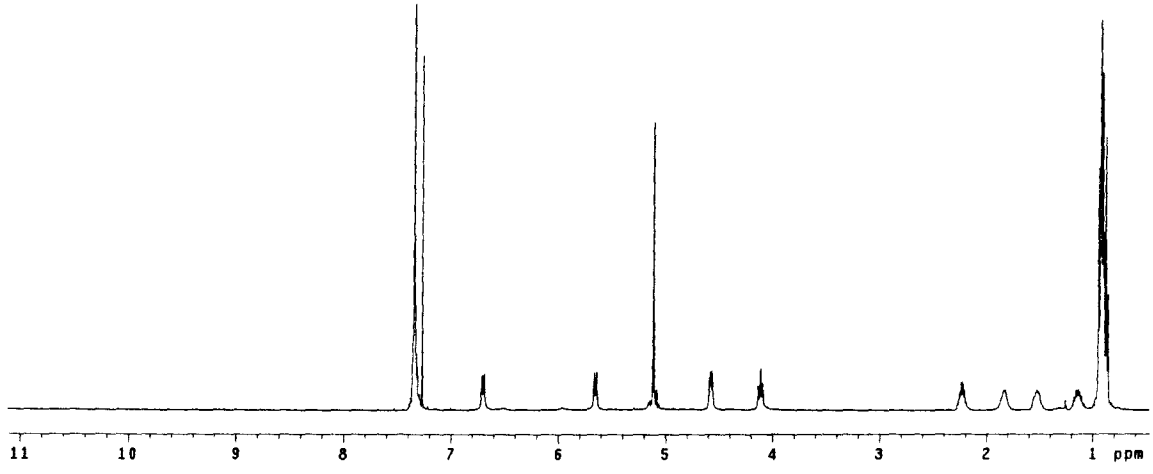


Figure 4 ^{1}H NMR of Compound **25**

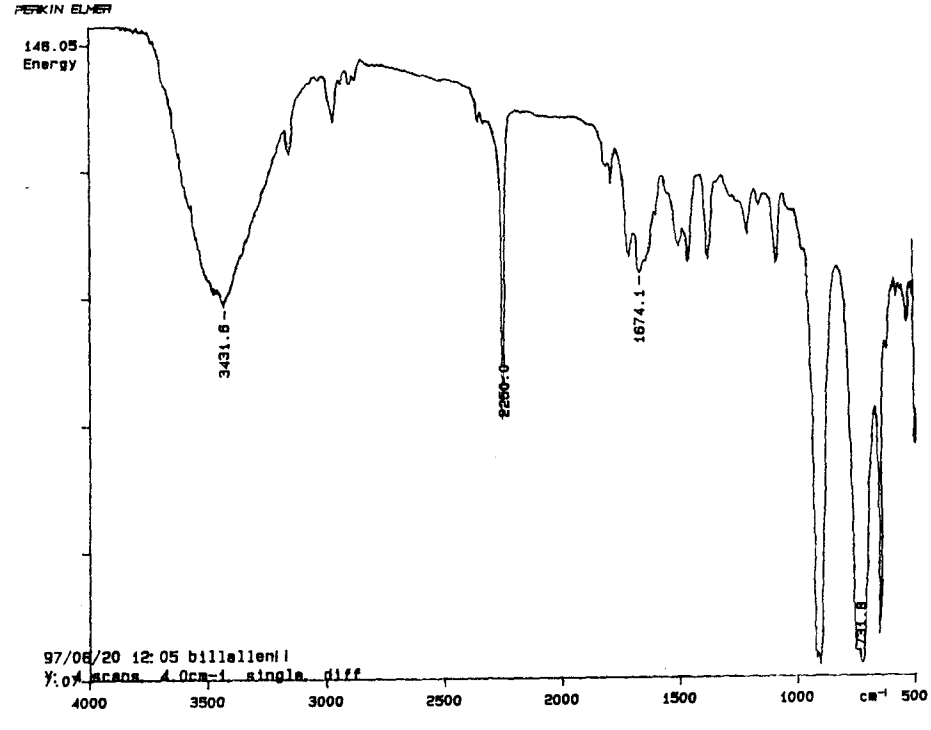
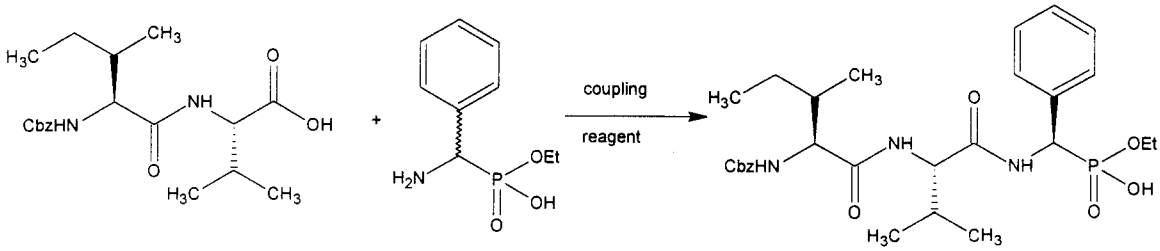
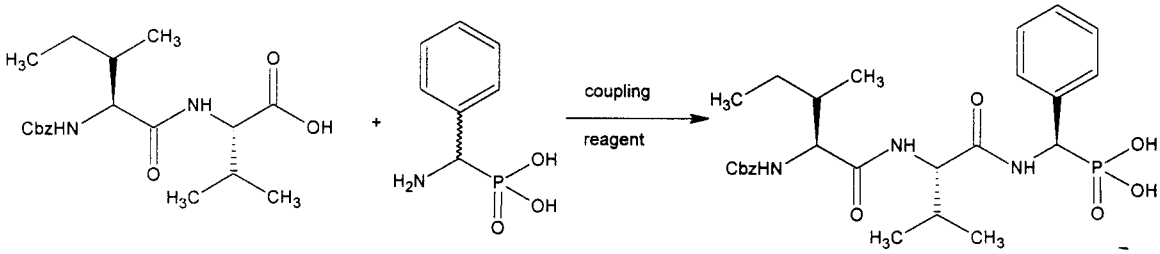


Figure 5 IR spectrum of Compound **25**



25 **21** **26**

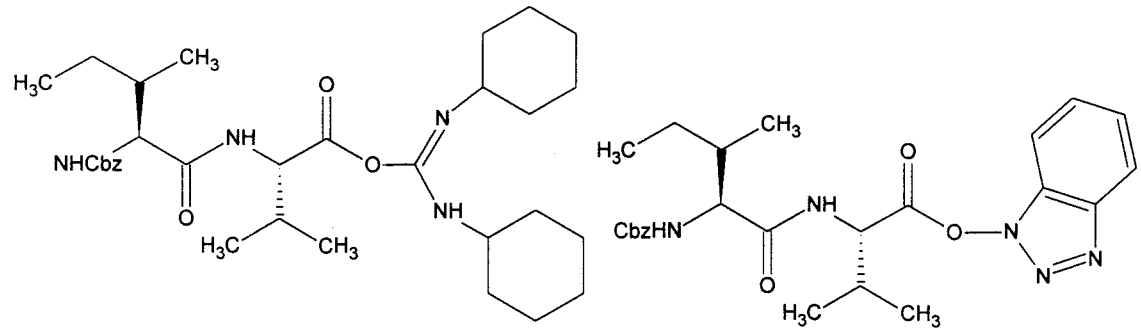
Scheme 12



25 **22** **27**

Scheme 13

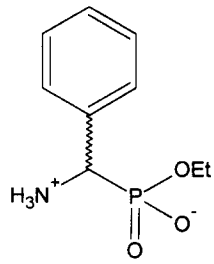
products of the coupling reagents. These contaminants on the other hand may be the active intermediates such as the *O*-acyl urea **28** or the *O*-acyl benzotriazole **29** that did not



28 **29**

react with the debenzylated phosphonates **21** or **22**. This may be the case due to compound **21** and **22** having zwitterionic character as shown in structures **30** and **31**. If

this is so, the presence of triethyl amine or some other weak base would be needed to consume the interchangable proton and free the amine so the coupling could occur. Based on this knowledge better results should occur with product **20** due to the fact that there is not a interchangable proton present.



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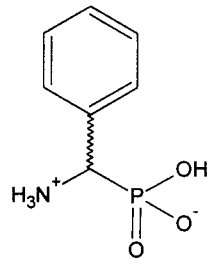

31

Conclusion

In summary, synthesis of the core phosphonates, as well as the side chain peptides, were easily accomplished and in good yields. Difficulty arose when the side chain peptides were reacted with aminophosphonic acid **14** and aminophosphonic acid monoester **13**. Actual coupling between the two has not been confirmed, but there is evidence based on ^{31}P NMR that a small amount of CbzIleVal dipeptide may have been coupled with the aminophosphonic acid monoester **13**. More investigation into different coupling reagents and more attention placed on monitering of reaction conditions by TLC may help produce higher yields of these phosphonopeptides so that confirmation of existence can be made. Another useful tool that would help in planning reactions and make final structural confirmations is X-ray diffration studies of the intermediates and final products. For example, preliminary X-ray diffraction data for the aminophosphonic acid monoester **13** shows that the carboxyl proton transfers to the amino portion of the molecule. X-ray diffraction studies on the debenzylated monoester **19** and diacid **20** could confirm that the carboxyl proton transfers to the amine in these molecules also. One planning a synthetic strategy involving these molecules could then account for this exchange.

Chapter 3 Experimental

General Methods. All nonaqueous reactions were conducted under a inert atmosphere of Argon. All solvents used were dried by standard techniques. All melting points reported are uncorrected. Merck grade 9385, 230-400A mesh silica was used for all flash chromatography. Analytical thin-layer chromatography (TLC) was conducted on Whatman alumina backed silica plates. Visualization was accomplished with an ultraviolet lamp and/or staining with 5% phosphomolybdic acid (PMA) in ethanol 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. Deuterated solvents used include $CDCl_3$, CD_3OD, DMSO, and D_2O. The ^{1}H and ^{13}C chemical shifts are reported in parts per million downfield from the standard $(CH_3)_4Si$. ^{31}P chemical shifts are reported in parts per million downfield from the standard H_3PO_4. Coupling constants are reported in Hertz. Fourier transform infrared spectra (FTIR) were recorded with a Perkin-Elmer 1600 series II instrument.

Diethyl(1-benzylamino-1-phenyl)phosphonate (12). To a mixture of anhydrous sodium sulfate (20 g) and diethyl ether (60 mL) was added benzaldehyde (20.32 mL, 200 mmol) and benzylamine (21.84 mL, 200 mmol). This mixture was stirred at room temperature for 1 hour. This mixture became a light yellow and heat was evolved. The mixture was gravity filtered to remove the anhydrous sodium sulfate. The filtrate was concentrated *en vacuo* yielding 38.95 g (200 mmol, 100%) of imine **12a**. To the imine **12a** (38.95 g, 200 mmol) was added diethyl phosphite (51.52 mL, 400 mmol) in THF (50 mL). This mixture was stirred and refluxed overnight. The THF was removed under reduced pressure. To the residual yellow oil that remained was added diethyl ether (70 mL) and the ether solution was washed with water (5 x 50 mL). The organic layer was dried with anhydrous sodium sulfate, gravity filtered, and the solvent was removed by rotary evaporation yielding 64.88 g (194 mmol, 97%) of the diethyl aminophosphonate

12. ^{1}H NMR δ 1.13 (t, 3, *J*=7.20), 1.28 (t, 3, *J*=7.20), 2.52 (br s, 1), 3.70 (AB q, 2, J_1=13.20, J_2=107.59), 3.79-3.87 (m, 1), 3.92-4.03 (m, 1), 4.03 (d, 1, *J*=20.00), 4.04-4.13 (m, 2), 7.23-7.45 (m, 10); ^{13}C NMR δ 16.19 (d, *J*=6.13), 16.38 (d, *J*=6.13), 51.15 (d, *J*=17.60), 59.55 (d, *J*=153.38), 62.72 (d, *J*=6.94), 62.894 (d, *J*=6.84), 127.05, 127.84 (d, *J*=3.12), 128.28, 128.30, 128.41 (d, *J*=2.01), 128.63 (d, *J*=6.13), 135.66, 139.26; ^{31}P NMR δ 24.66.

1-Benzylamino-1-phenylphosphonic acid (14). To diethyl(1-benzylamino-1-phenyl)phosphonate (3.33 g, 10 mmol) was added concentrated 12N HCl (100 mL). This mixture was refluxed at 130°C for 48 hours. The solution was diluted with water (40 mL) and the solvent was evaporated to near dryness *en vacuo* with heat. The resulting residue was dissolved in a small amount of 95% ethanol and propylene oxide was added dropwise with stirring until precipitation was complete. This mixture was vacuum filtered, washed with 200 mL of 95% ethanol, and dried yielding 2.60 g (9.40 mmol, 94%) of aminophosphonic acid **14**, mp 236-239°C. ^{1}H NMR δ 3.84 (d, 2, *J*=16.40), 3.93 (AB q, 1, J_1=13.60, J_2=141.58), 7.304-7.388 (m, 8), 7.439-7.452 (m, 2); ^{13}C NMR δ 49.43 (d, *J*=6.31), 59.64 (d, *J*=128.73), 127.29, 127.92, 128.34, 129.17, 129.89, 135.01; ^{31}P NMR δ 9.13.

Ethyl(1-benzylamino-1-phenyl)phosphonic acid (13). To a solution of diethyl(1-benzylamino-1-phenyl)phosphonate (6.66 g, 20 mmol) in methanol (50 mL) was added 8N KOH (35 mL). This solution stirred and refluxed overnight, diluted with water (100 mL), and washed with methylene chloride (50 mL). The aqueous layer was acidified to pH 1 with concentrated HCl and extracted with methylene chloride (4 x 90 mL). The organic layer was dried over anhydrous sodium sulfate, gravity filtered, and the organic solvent was removed under reduced pressure yielding 5.99 g (19.32 mmol, 96.6 %) of the aminophosphonic acid monoester **13**. This product was further recrystallized from methanol yielding 4.70 g (15.16 mmol, 75.8%) of pure white product, mp 71-73°C. ^{1}H NMR δ 1.09 (t, 3, *J*=7.20), 3.66 (ddq, 1, J_1=7.19, J_2=7.60, J_3=10.00), 3.72 (ddq, 1, J_1=7.19, J_2=7.60, J_3=10.00), 4.19 (d, 2, *J*=15.60), 4.20 (AB q, 1, J_1=13.60,

J_2=35.20), 4.88 (s, 2), 7.42-7.47 (m, 8), 7.51-7.53 (m, 2); ^{13}C NMR δ 16.98, 51.26 (d, J=5.33), 60.78 (d, J=138.80), 62.70 (d, J=6.03), 129.97, 130.16, 130.55 (d, J=4.62), 130.62, 131.34, 132.13, 133.37; ^{31}P NMR δ 9.99.

Phosphonopeptide 16. Method A. To a solution of aminophosphonic acid **14** (0.091 g, 0.337 mmol)in methylene chloride (5 mL) was added L-proline methyl ester hydrochloride(0.060 g, 0.353 mmol), 1,3-dicyclohexylcarbodiimide (0.110 g, 0.524 mmol), and triethyl amine (0.20 mL, 1.29 mmol) in that sequence. This solution was stirred at room temperature for 24 hours, diluted with ethyl acetate (150 mL), and washed with saturated KH_2PO_4 (3 x 30 mL), saturated $NaHCO_3$ (2 x 20 mL), and saturated NaCl (20 mL). The organic fraction was dried with anhydrous sodium sulfate, gravity filtered, and the solvent was removed by rotary evaporation. TLC and NMR analysis on the resulting residue indicated minimal product formation along with DCU byproduct.

Method B. To a solution of aminophosphonic acid (0.270 g, 1.00 mmol) **14** in methylene chloride (10 mL) was added L-proline methyl ester hydrochloride (0.170 g, 1.00 mmole), 1,3-dicyclohexylcarbodiimide (0.230 g, 1.10 mmol), and 1-hydroxybenzotriazole hydrate (0.135 g, 1.00 mmol). This mixture was stirred, triethyl amine (0.15 mL, 1.10 mmol) was added, and then it was left to stir for 48 hours at room temperature. The solution was diluted with ethyl acetate (150 mL), washed with saturated KH_2PO_4 (3 x 30 mL), saturated $NaHCO_3$ (2 x 20 mL), and saturated NaCl (20 mL) solutions. The organic fraction was dried over anhydrous sodium sulfate, gravity filtered, and reduced *en vacuo*. The residual oil was purified by flash column chromatography (35g of silica eluted with a gradient from petroleum ether to methylene chloride). Recovered 10 mg of product. TLC and NMR analysis of the isolated product after chromatography showed that it was just a mixture of starting products.

Method C. To a solution of aminophosphonic acid **14** (0.270 g, 1.0 mmol) in THF (5 mL) was added carbonyldiimidazole (0.162 g, 1.0 mmol). This mixture stirred for 30 minutes at room temperature, L-proline methyl ester hydrochloride (0.165 g, 1.0

mmol) was added, and then the solution was left to stir at room temperature for 48 more hours. The solvent was removed under reduced pressure yielding a white solid. This white solid was recrystallized with 95% ethanol yielding 0.104 g of a fine white powder. TLC and NMR analysis indicated no product formation, just the presence of the starting material aminophosphonic acid **14**. The melting point of the solid, 236-239°C, confirmed this as well.

Method D. To a solution of aminophosphonic acid **14** (0.275 g, 1.0 mmol) in acetonitrile (40 mL) was added N-ethyl-5-phenylisoxazolium-3'-sulfonate (0.258 g, 1.0 mmol). This solution was cooled to 0°C, treated with triethyl amine (0.15 mL, 1.0 mmol), and stirred for one hour. L-proline methyl ester hydrochloride (0.170 g, 1.0 mmol) as well as triethyl amine (0.150 mL, 1.0 mmol) were added, then the solution was left to stir for 24 hours. The solvent was removed by rotary evaporation, chloroform was added to dissolve the residue, and the solution was filtered through a sintered glass funnel with the help of Celite filter aid. Flash column chromatography was performed on 25 g of silica and eluted with a solvent gradient from ethyl acetate to methanol. Obtained 180 mg of product. TLC and NMR indicated little product formation, only the presence of starting materials proline and Woodward's reagent K.

Phosphonopeptide 15. Method A. To a solution of aminophosphonic monoester **13** (0.050 g, 0.162 mmol) in methylene chloride (5 mL) was added sequentially L-proline methyl ester hydrochloride (0.028 g, 0.167 mmol), 1,3-dicyclohexylcarbodiimide (0.038 g, 0.183 mmol), and triethyl amine (0.052 mL, 0.367 mmol). This solution was stirred at room temperature for 24 hours. The solution was then diluted with ethyl acetate (150 mL), washed with saturated KH_2PO_4 (3 x 10 mL), saturated $NaHCO_3$ (2 x 10 mL), and saturated NaCl (10 mL). The organic fraction was dried over anhydrous sodium sulfate, gravity filtered, and the solvent was removed *en vacuo* leaving 27 g of a white residue. NMR analysis did not indicate product formation but it did show the presence of DCU byproduct.

Method B. To a solution of aminophosphonic monoester **13** (0.310 g, 1.0 mmol) in methylene chloride (10 mL) was added L-proline methyl ester hydrochloride (0.170 g, 1.0 mmol), 1,3-dicyclohexylcarbodiimide (0.230 g, 1.1 mmol), and 1-hydroxybenzotriazole hydrate (0.135 g, 1.0 mmol). Triethyl amine (0.15 mL, 1.1 mmol) was added, and the solution was stirred for 48 hours. The solution was then diluted with ethyl acetate(150 mL), washed with saturated KH_2PO_4 (3 x 10 mL), saturated $NaHCO_3$ (2 x 10 mL), and saturated NaCl (10 mL). The organic fraction was dried over anhydrous sodium sulfate, gravity filtered, and the solvent was removed under reduced pressure leaving a white residue. This residue was applied to 34 g of silica in a column and eluted with a solvent gradient ranging from petroleum ether to methylene chloride. Recovered 7 mg of byproduct. NMR analysis indicated that it was residual starting material HOBt and a small amount of DCU byproduct.

Method C. To a solution of aminophosphonic acid monoester **13** (0.310 g, 1.0 mmol) in THF (10 mL) was added carbonyldiimidazole (0.165 g, 1.0 mmol). This solution was stirred for 30 minutes, L-proline methyl ester hydrochloride (0.164 g, 1.0 mmol) was added, and then it was stirred for another 24 hours at room temperature. The solvent was removed by rotary evaporation, 2N HCl was added to the residual white material, the solution was cooled in a ice bath, and the resulting solid was suction filtered. The white solid was washed with water and dried *en vacuo*. The resulting product was recrystallized from 95% ethanol. 0.105 g of white crytalline powder with the melting point of 199-201°C was recovered. NMR analysis of this white powder indicated no product formation, just a mix of starting products mainly the monoester **13**.

Method D. Aminophosphonic acid monoester **13** (0.310 g, 1.0 mmol) and N-ethyl-5-phenylisoxazolium-3'-sulfonate (0.255 g, 1.0 mmol) were dissolved in acetonitrile (40 mL) and cooled to 0°C in a ice bath. Triethyl amine (0.14 mL, 1.0 mmol) was added to this solution which was stirred for one hour. L-proline methyl ester hydrochloride (0.170 g, 1.0 mmol) and triethyl amine (0.14 mL, 1.0 mmol) were then added. This resulting solution was left to stir for 24 hours at room temperature. The

solvent was then removed under reduced pressure, chloroform was added to the residue, and the solution was filtered through a sintered glass funnel. Flash column chromatography was performed on 30 g of silica and eluted with a solvent gradient form chloroform to methanol. TLC indicated the presence of unreacted starting products, proline and monoester **13** with no product formation.

Method E. To Aminophosphonic acid monoester **13** (0.310 mg, 1.0 mmol) in methylene chloride (10 mL) was added L-proline methyl ester hydrochloride (0.170 g, 1.0 mmol), DIPC (0.139 g, 0.180 mL, 1.1 mmol), and triethyl amine (0.15 mL, 1.1 mmol). This mixture was then stirred for 24 hours. The resulting solution was diluted with 150 mL of ethyl acetate, washed with saturated KH_2PO_4 (3 x 30 mL), saturated $NaHCO_3$ (2 x 20 mL), and saturated NaCl solution (20 mL). The organic fraction was dried over anhydrous sodium sulfate, gravity filtered, and the organic solvent was removed by rotary evaporation under reduced pressure. The resulting residue was applied to 20 g of silica in a column and eluted with ethyl acetate as the solvent. 160 mg of product was isolated. Structural analysis by NMR showed it to be diisopropylurea byproduct.

Diethyl(1-amino-1-phenyl)phosphonate (20). $Pd(OH)_2/C$ (1.50 g) was added to a solution of diethyl aminophosphonate **12** (4.00 g, 12.00 mmol) in methanol (50 mL). This mixture was placed in a Parr bottle and hooked up to a Parr hydrogenation apparatus. The air above the mixture was then evacuated and 40 psi of hydrogen was added to the parr bottle. The bottle was shook for 24 hours and then the mixture was gravity filtered to remove the catalyst. TLC on the resulting mixture indicated good product formation with little or no starting material present. Flash column chromatography was performed on 150 g of silica and eluted with 12.5% methanol/ ethyl acetate to purify the crude product. 2.03 g (8.35 mmol, 70%) of diethyl(1-amino-1-phenyl)phosphonate (**20**) was isolated. ^{1}H NMR δ 1.18 (t, 3, *J*=7.20), 1.29 (t, 3, *J*=7.00), 3.84-3.92 (m, 1), 3.98 (q, *J*=7.10), 4.03-4.11 (m, 2), 4.27 (d, 1, *J*=17.60), 4.85 (s, 2), 7.31-7.39 (m, 3), 7.45-7.48 (m, 2); ^{13}C NMR δ 16.66 (q, *J*=5.87), 54.50 (d,

J=148.75), 64.23 (d, *J*=3.11), 64.31 (d, *J*=3.02), 129.01 (d, *J*=3.02), 129.14 (d, *J*=6.03), 129.43 (d, *J*=3.02), 138.56; ^{31}P NMR δ 26.44.

Ethyl(1-amino-1-phenyl)phosphonic acid (21). $Pd(OH)_2/C$ (1.00 g) was added to a solution of aminophosphonic acid monoester **13** (1.00 g, 3.19 mmol) in 50 mL of a 1:1methanol/ethanol solution. This mixture was transferred to a Parr bottle and hooked up to a Parr hydrogenation apparatus. The air in the bottle was evacuated and 40 psi of hydrogen was added to the atmosphere above the mixture. The bottle was shaken for 24 hours and then the mixture was gravity filtered to remove the catalyst. The solvent was removed by rotary evaporation. The crude product was washed with hot 1-propanol and vacuum filtered yielding 0.48g (2.19 mmol, 69%) of Ethyl(1-amino-1-phenyl)phosphonic acid (**21**), mp 232-235 °C. ^{1}H NMR δ 1.17 (t, 3, *J*=6.80), 3.78 (q, 2, *J*=7.20), 4.28 (d, 1, *J*=16.00), 4.89 (s, 3), 7.36-7.43 (m, 3), 7.52-7.54 (m, 2); ^{13}C NMR δ 17.12 (d, *J*=6.13), 54.57 (d, *J*=140.40), 62.55 (d, *J*=6.13), 129.34 (d, *J*=4.63), 129.49 (d, *J*=2.31), 129.70, 135.31; 31P NMR δ 11.27.

1-amino-1-phenylphosphonic acid (22). $Pd(OH)_2/C$ (1.00 g) was added to a solution of aminophosphonic acid **14** (1.00 g, 3.61 mmol) in methanol (50 mL). This mixture was transferred to a Parr bottle and hooked up to Parr hydrogenation apparatus. The air in the bottle was evacuated and 40 psi of hydrogen was added to the atmosphere above the mixture. The bottle was shook for 24 hours and then the mixture was gravity filtered to remove the catalyst. The catalyst was then washed with hot water to ensure that any residual product was removed. The solvent was removed *en vacuo* and the residual grey flakes were washed with hot methanol. The product was subsequently vacuum filtered yielding 0.338 g (1.81 mmol, 50%) of grey-white 1-amino-1-phenylphosphonic acid (**22**), mp 268-272 °C. ^{1}H NMR δ 4.26 (d, 1, *J*=16.00), 7.24-7.34 (m, 5); ^{13}C NMR δ 54.56 (d, *J*=138.79), 128.71 (d, *J*=5.33), 129.93 (d, *J*=16.80), 133.79; ^{31}P NMR δ 11.33.

Carbobenzoxy Isoleucine (23). Benzyl chloroformate (3.2 mL, 22 mmol) was added to a solution of L-isoleucine (2.64 g, 20 mmol) in 1/4 saturated sodium bicarbonate solution (100 mL) and sodium hydroxide (10 mL). The solution was maintained at pH 10 with additional 4M sodium hydroxide as needed. This solution was stirred for 3 hours. The resulting solution was washed with diethyl ether (2 x 50 mL) and the aqueous fraction was acidified to pH 3 with 5M hydrochloric acid. The aqueous fraction was extracted with ethyl acetate (3 x 40 mL), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure with a rotary evaporator yielding 4.95g (18.67 mmol, 93%) of carbobenzoxy Isoleucine (**23**). ^{1}H NMR δ 0.88-1.01 (m, 6), 1.13-1.28 (m, 1), 1.42-1.54 (m, 1), 1.90-2.02 (m, 1), 4.40 (dd, 1, J_1=4.80, J_2=9.20), 5.13 (s, 2), 5.37 (d, J=8.80), 7.30-7.41 (m, 5), 9.48 (br s 1); ^{13}C NMR δ 11.80, 16.05, 26.11, 38.42, 60.01, 67.65, 128.77, 128.95, 129.42, 138.20, 158.72, 175.23.

Carbobenzoxy Isoleucine-Valine methyl ester dipeptide (24). To a solution of Valine methyl ester hydrochloride (1.67 g, 10 mmol) in methylene chloride (50 mL) was added Cbz-Isoleucine (**23**) (2.65 g, 10 mmol), EDC (2.30 g, 12 mmol), HOBt (1.35 g, 10 mmol), and Et_3N (2.79 mL, 20 mmol). This solution was then stirred overnight at room temperature. The resulting solution was extracted into diethyl ether (75 mL) and washed with water (2 x 75 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure yielding a yellow-white residue. This residue was purified by flash column chromatography (60 g of silica eluted with ethyl acetate). Yield of bright white crystals of Cbz-Isoleucine-Valine methyl ester (**24**) was 1.39 g (7.68 mmol, 76.8%), mp 120-124°C. ^{1}H NMR δ 0.85-0.92 (m, 12), 1.09-1.17 (m, 1), 1.47-1.51 (m, 1), 1.82-1.86 (m, 1), 2.12-2.16 (m, 1), 3.71 (s, 3), 4.41 (t, 1, J=8.00), 4.51 (dd, 1, J_1=4.80, J_2=8.80), 5.09 (s, 2), 5.35 (d, 1, J=8.80), 6.33 (d, 1, J=8.80), 7.24-7.33 (m, 5); ^{13}C NMR δ 11.31, 15.40, 17.73, 18.87, 24.78, 31.14, 37.36, 52.13, 57.09, 59.71, 67.02, 127.99, 128.13, 128.50, 171.16, 172.07.

Carbobenzoxy Isoleucine-Valine dipeptide (25). To a solution of peptide **24** (0.525 g, 1.39 mmol) in THF (7 mL) was added 1N lithium hydroxide (3 mL, 3.0 mmol). This mixture was stirred at room temperature for 48 hours. The solvent was removed with a rotary evaporator then the residual yellow-white solid was dissolved in methylene chloride (75 mL) and washed with water (5 x 50 mL). The pH of the aqueous phase was then adjusted to 3 with 5M HCl. A white solid precipitated out of the solution. This white solid was then vacuum filtered, washed with water, and left to dry. The yield of Cbz-Isoleucine-Valine (**25**) was 0.370 g (1.02 mmol, 73.7%), mp 193-196°C. ^{1}H NMR δ 0.82-0.94 (m, 12), 1.10-1.17 (m, 1), 1.50-1.54 (m, 1), 1.80-1.82 (m, 1), 2.20-2.25 (m, 1), 4.13 (t, 1, J=8.40), 4.58 (dd, 1, J_1=4.40, J_2=8.40), 5.11 (s, 2), 5.78 (d, 1, J=9.20), 6.84 (s, 1, J= 8.40), 7.29-7.37 (m, 5); ^{13}C NMR δ 11.03, 15.32, 17.59, 18.89, 24.76, 31.01, 37.10, 57.03, 59.67, 67.16, 127.95, 128.17, 128.50, 156.63, 171.97, 174.61.

Attempted Synthesis of CbzIleVal-Aminophosphonic acid Monoester (26). To a solution of Cbz-IleVal (**25**) (100 mg, 0.275 mmol) in methylene chloride (7 mL) was added DCC (62 mg, 0.300 mmol), HOBt (37 mg, 0.275 mmol), and ethyl(1-amino-1-phenyl)phosphonic acid (**21**) (60 mg, 0.275 mmol). This mixture was stirred at room temperature for 48 hours. The urea byproduct was then filtered off and the solvent was removed under reduced pressure. The resulting residue was applied to 10 g of silica in a flash column and eluted with ethyl acetate. Recovered 11 mg of a mixture of HOBt and DCU as indicated by ^{1}H and ^{13}C NMR.

Attempted Synthesis of CbzIleVal-Aminophosphonic Diacid (27). To a solution of Cbz-Isoleucine-Valine (**25**) (100 mg, 0.275 mmol) in DMF (7 mL) was added DCC (62 mg, 0.300 mmol), 1-amino-1-phenylphosphonic acid (**22**) (52 mg, 0.275 mmol), and Et_3N (0.07 mL, 0.500 mmol). This mixture was stirred for 48 hours at room temperature. The urea byproduct was then filtered off and the solvent was removed *en vacuo*. The residue that remained was purified by flash column chromatography (25 g of

silica eluted with methylene chloride). Recovered 65 mg of product. ^{1}H and ^{13}C NMR revealed that the product was a mixture of DCU and starting materials.

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Figure 6 ^{1}H NMR of Compound **12**

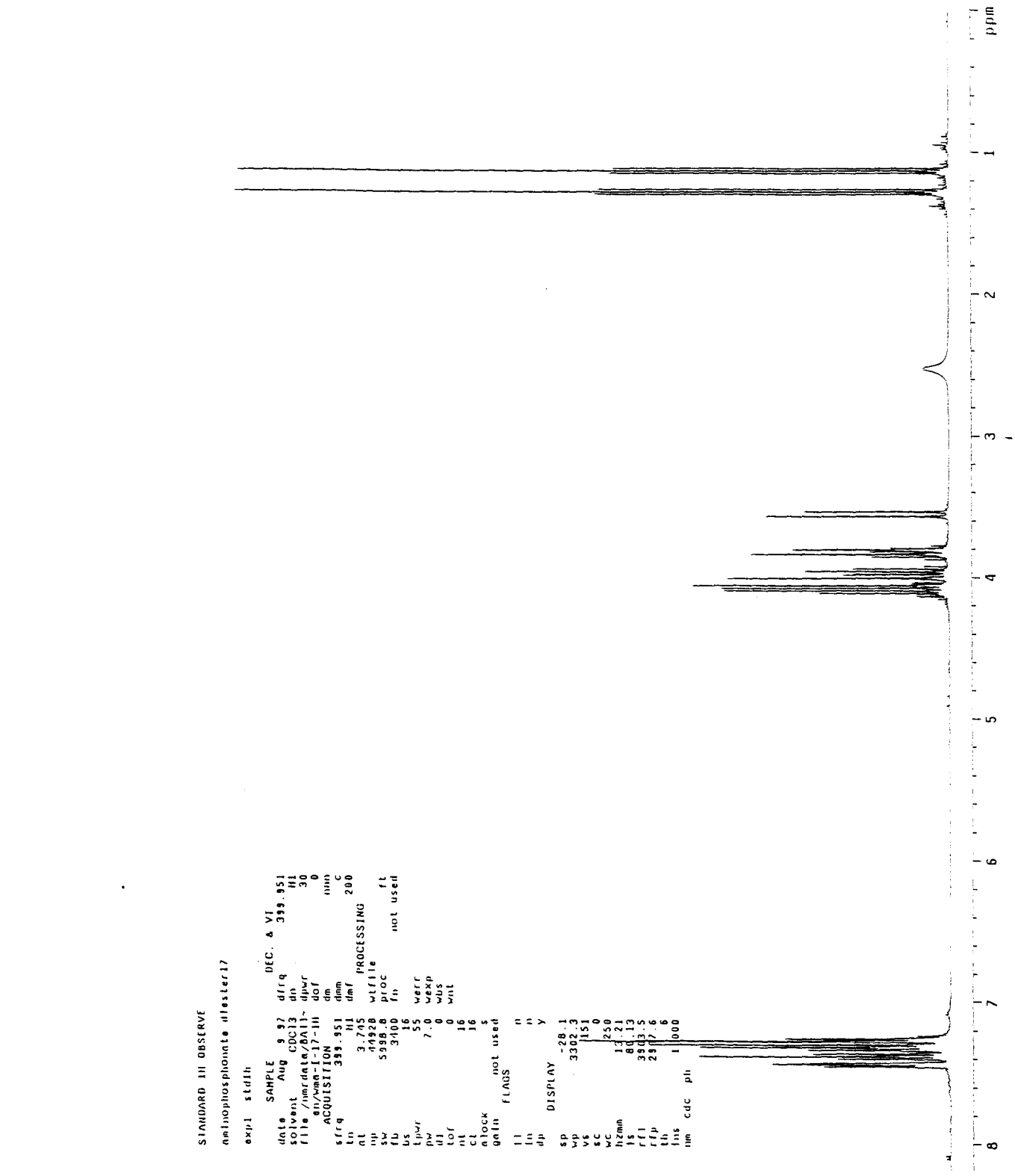


Figure 7 ^{13}C NMR of Compound **12**

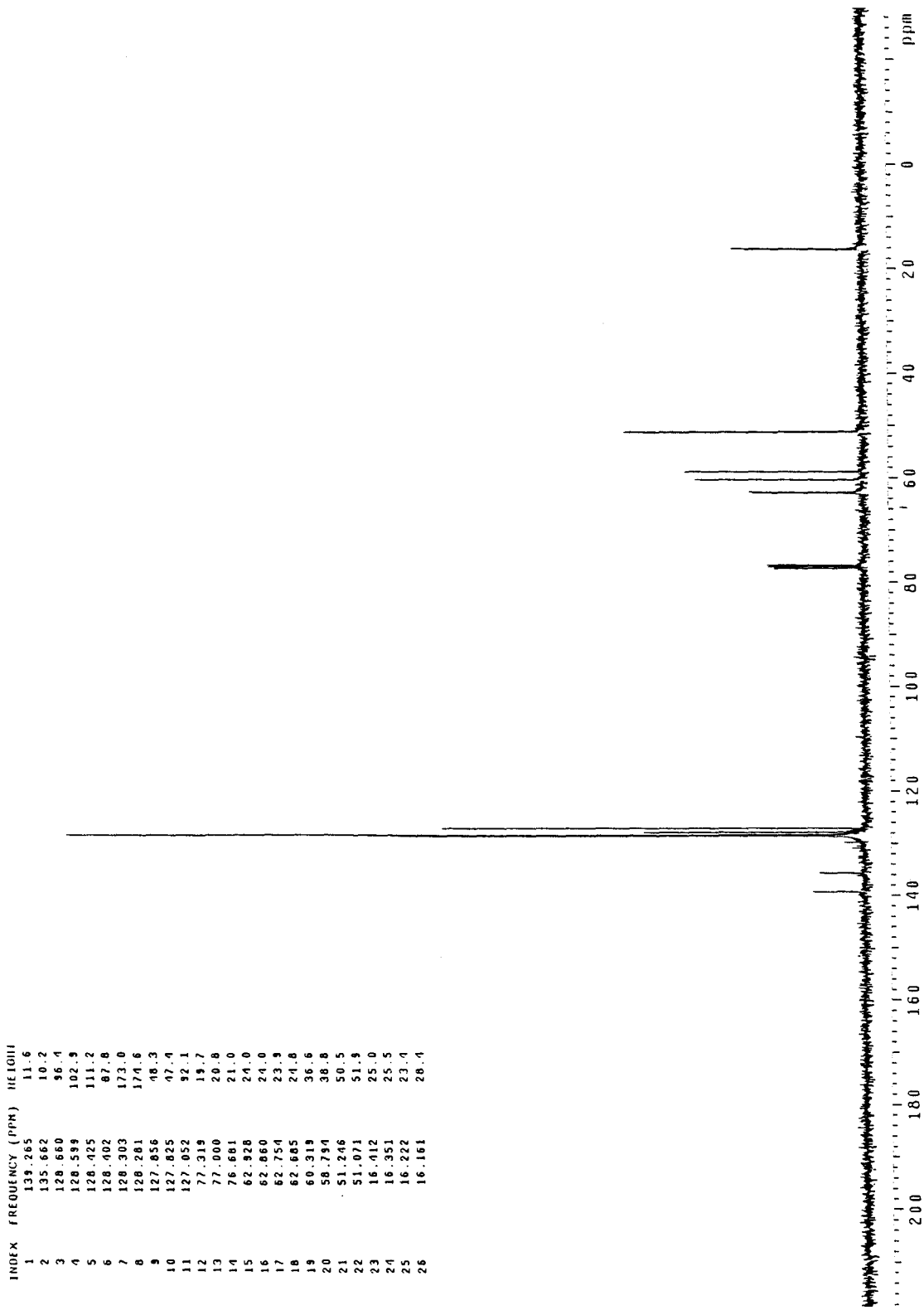


Figure 8 ^{1}H NMR of Compound **13**

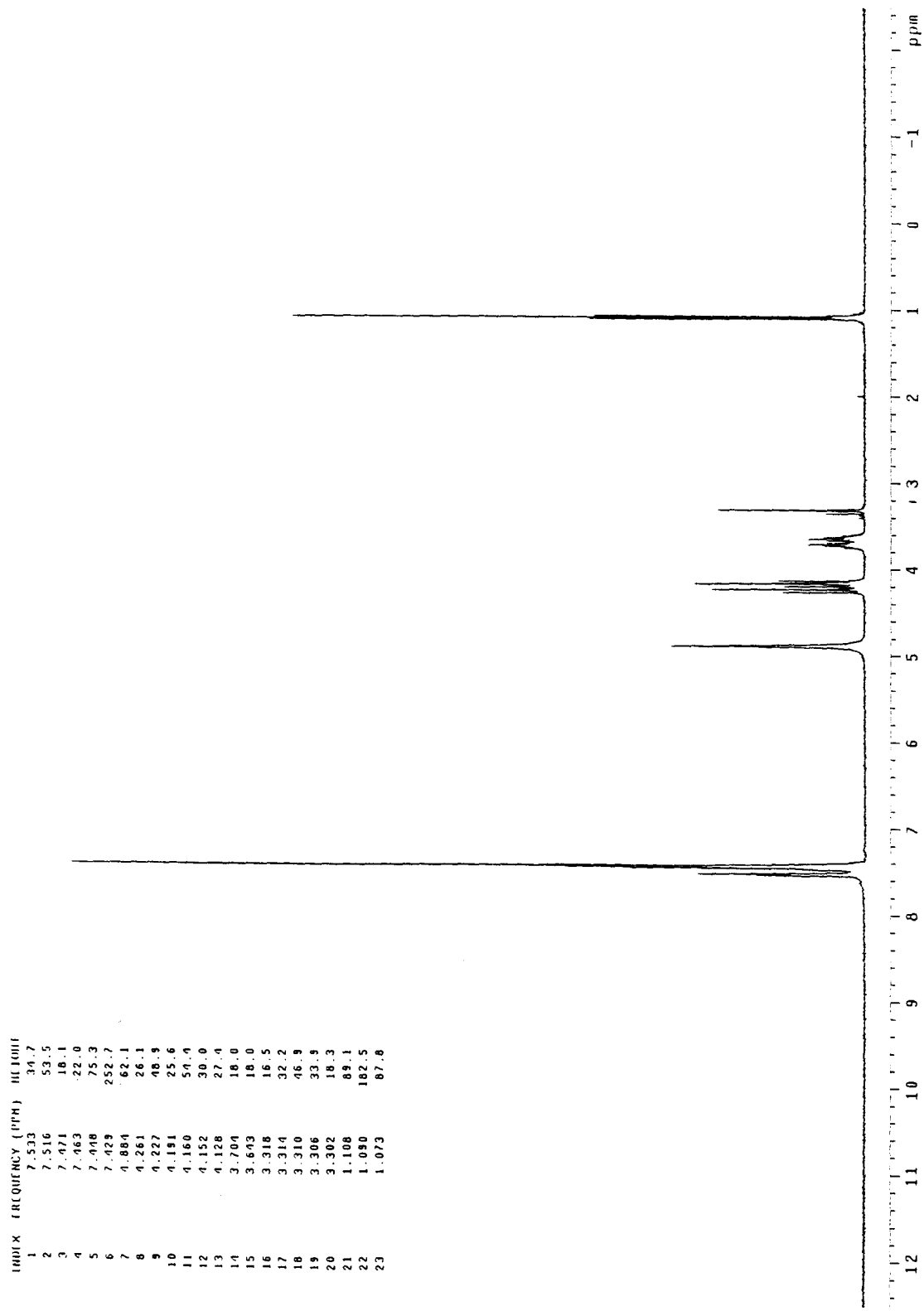


Figure 9 ^{13}C NMR of Compound **13**

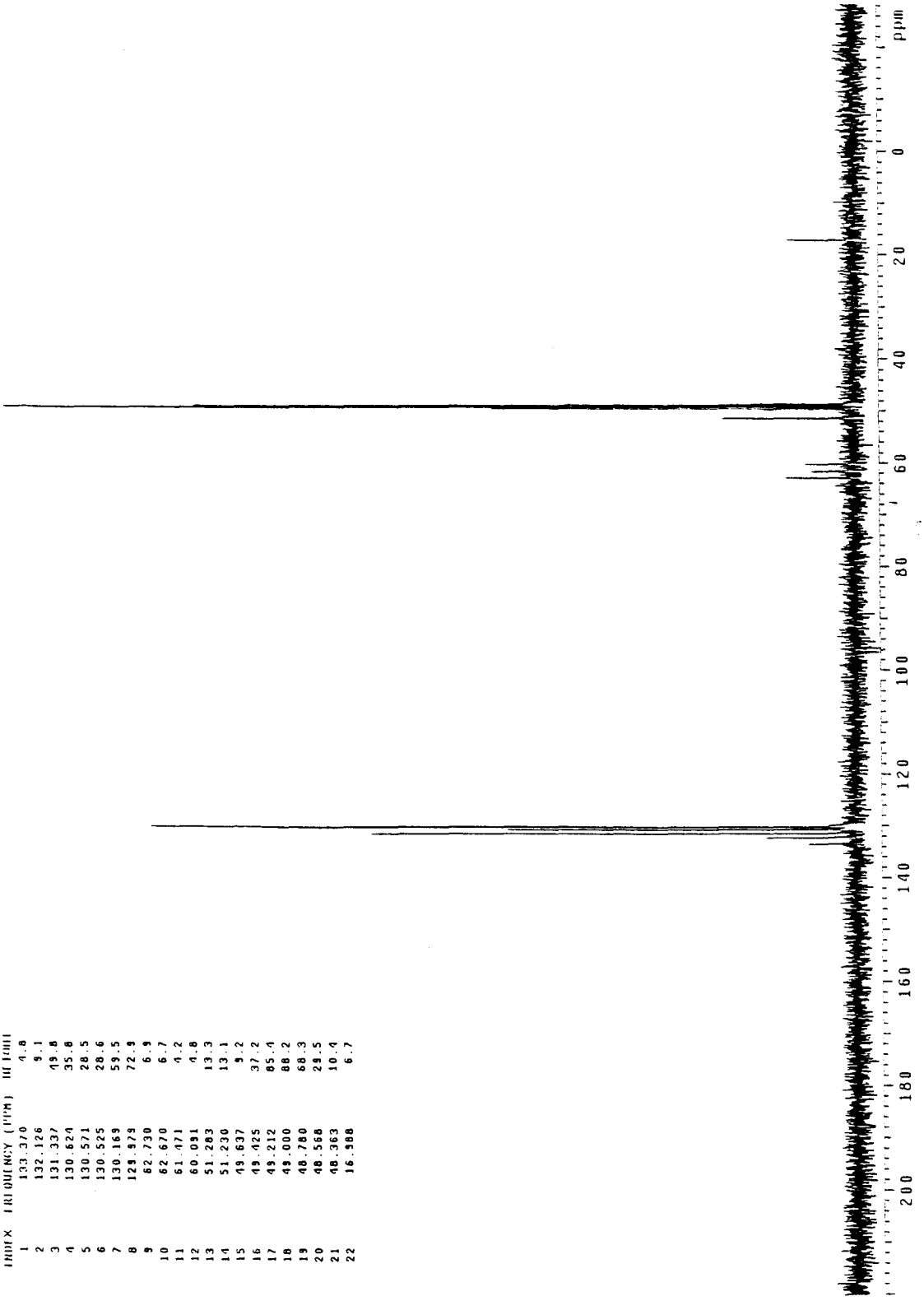


Figure 10 ^{1}H NMR of Compound **14**

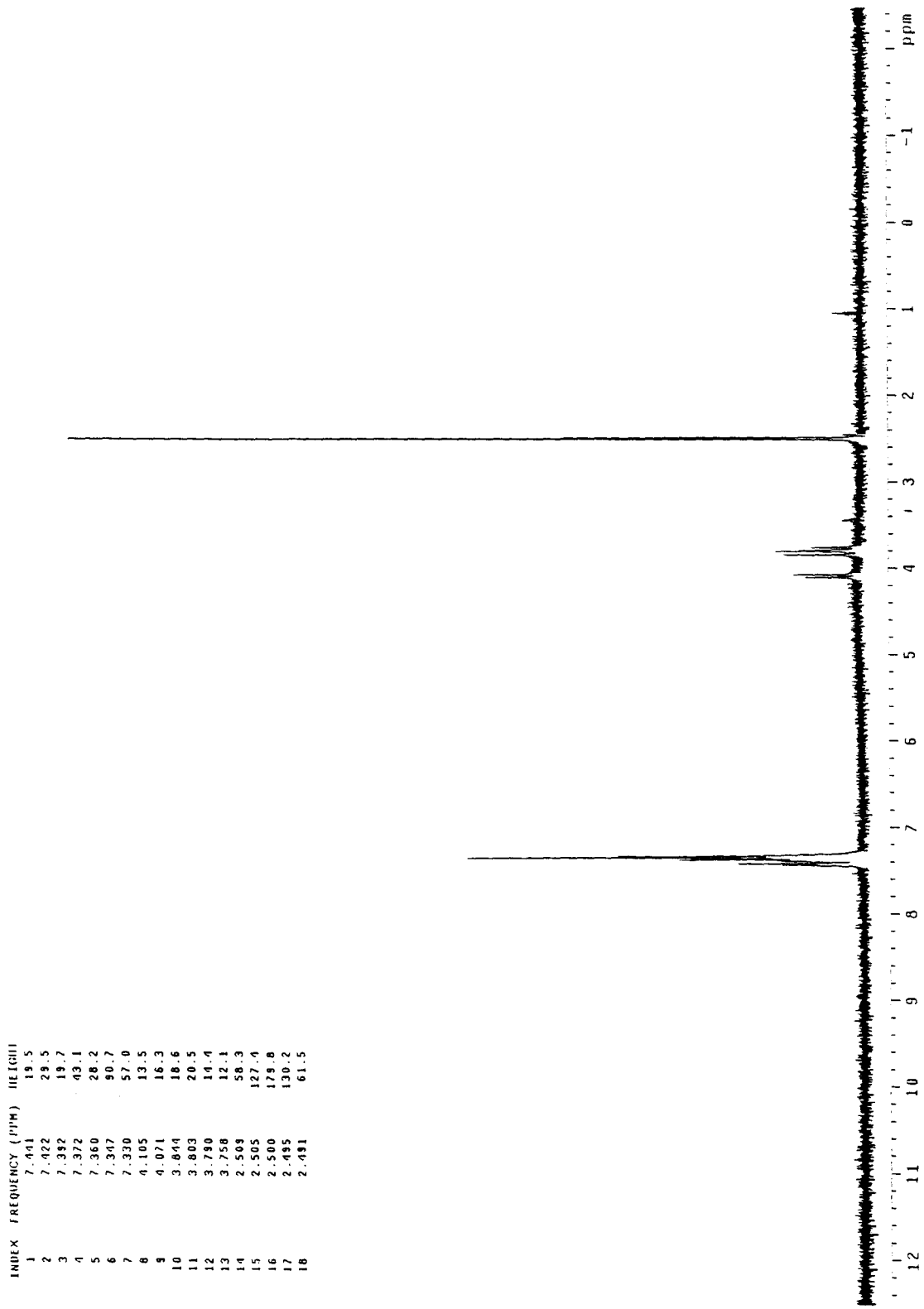


Figure 11 ^{1}H NMR of Compound **20**

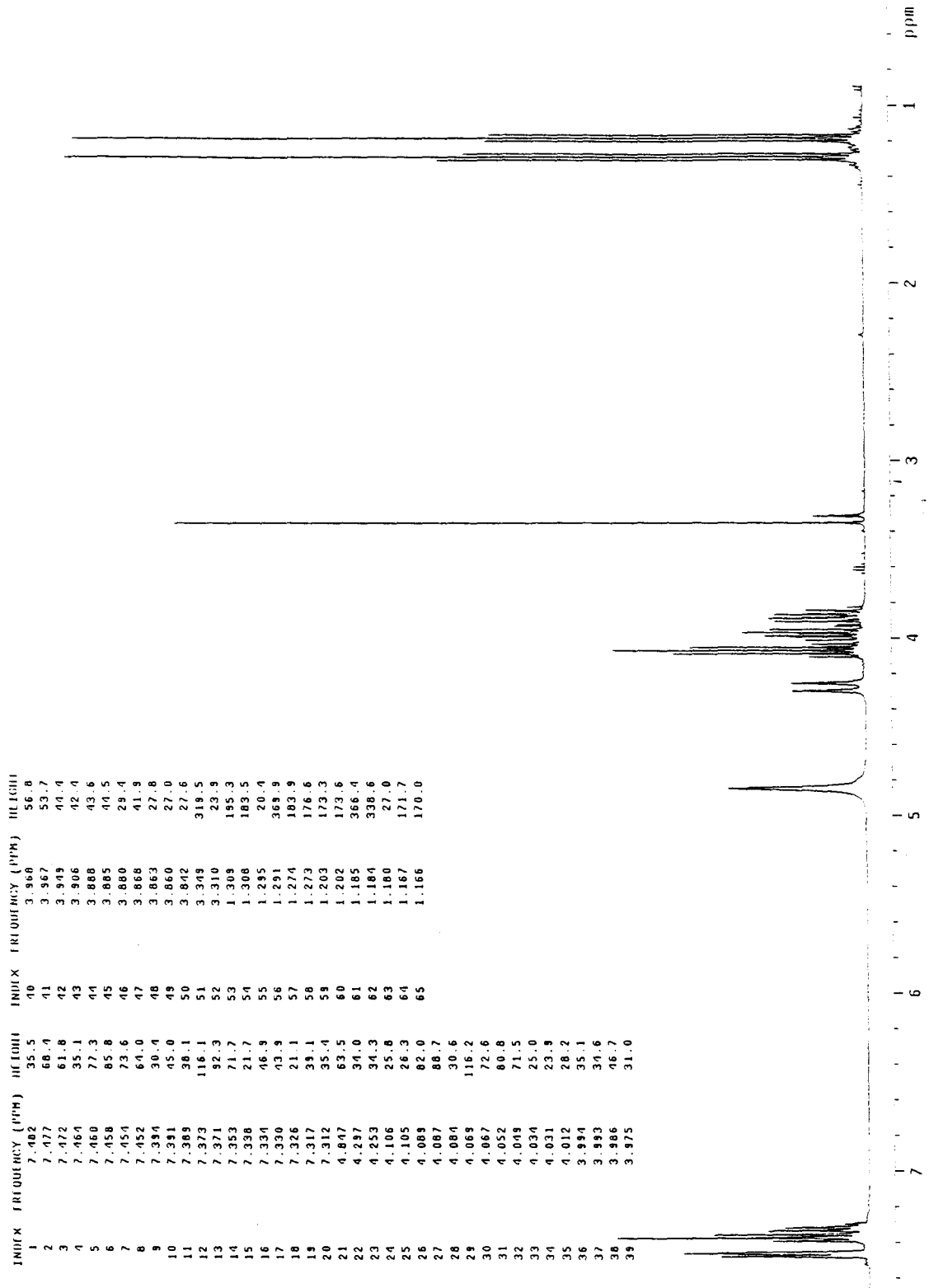


Figure 12 ^{13}C NMR of Compound **20**

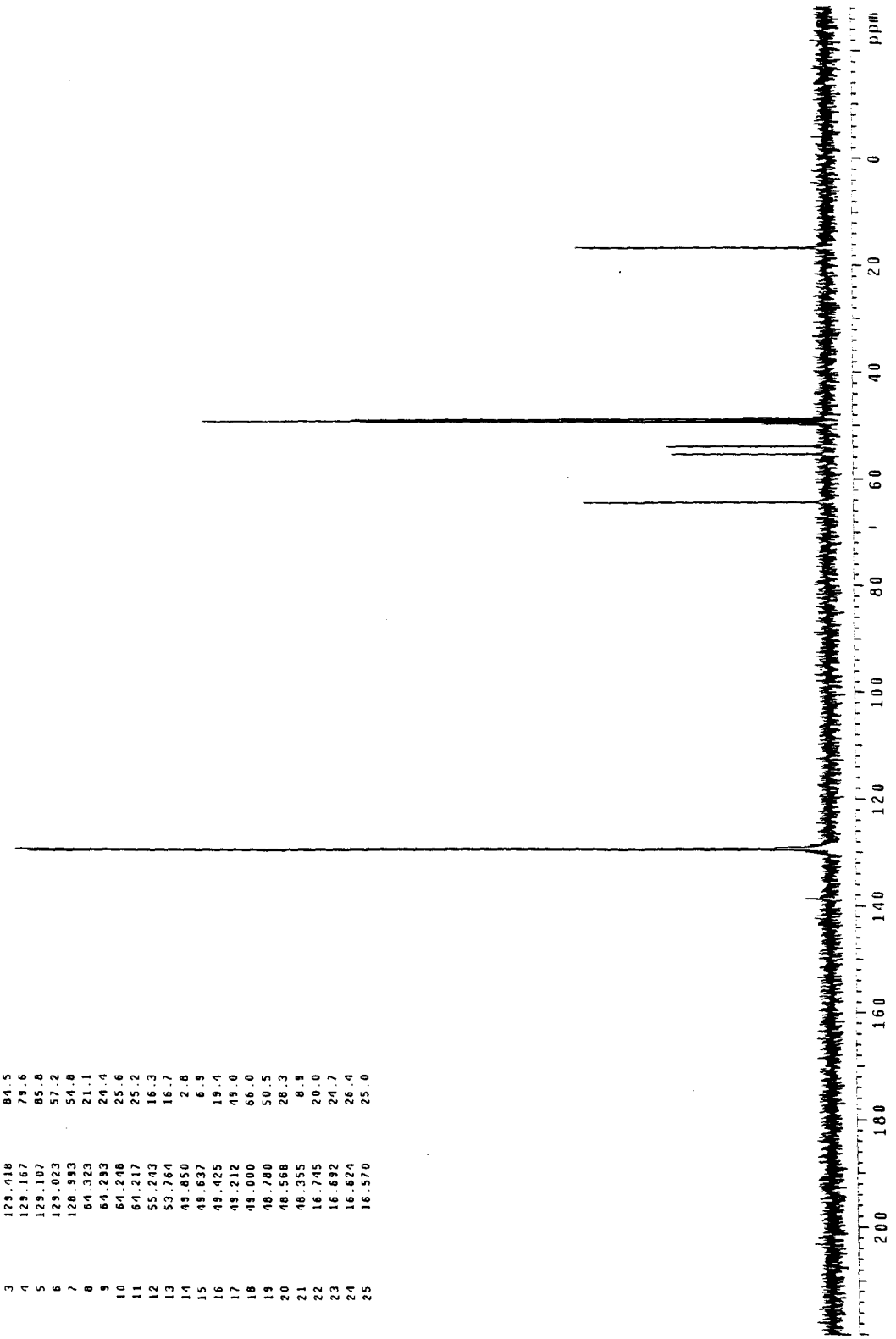


Figure 13 ^{31}P NMR of Compound **20**

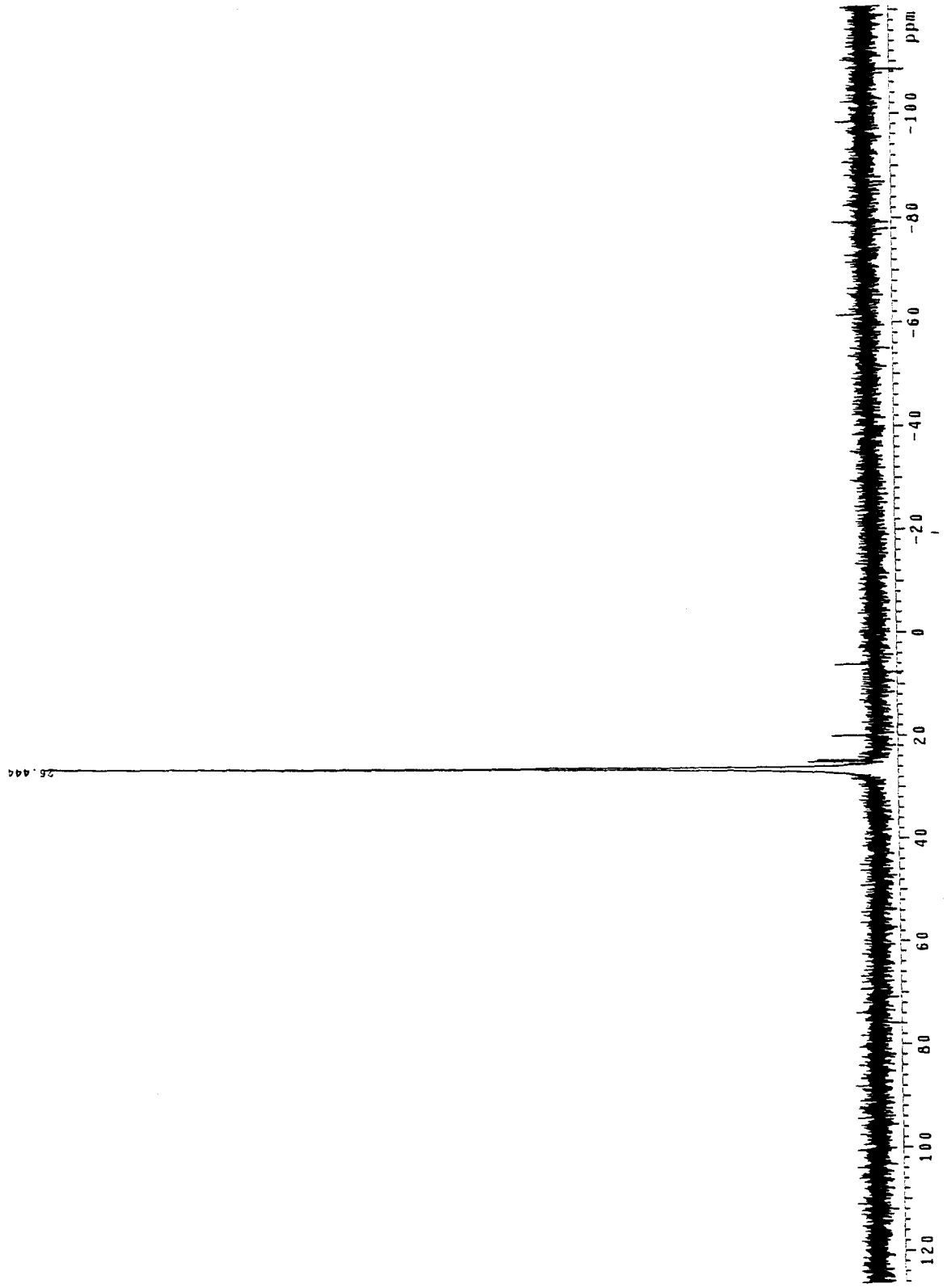
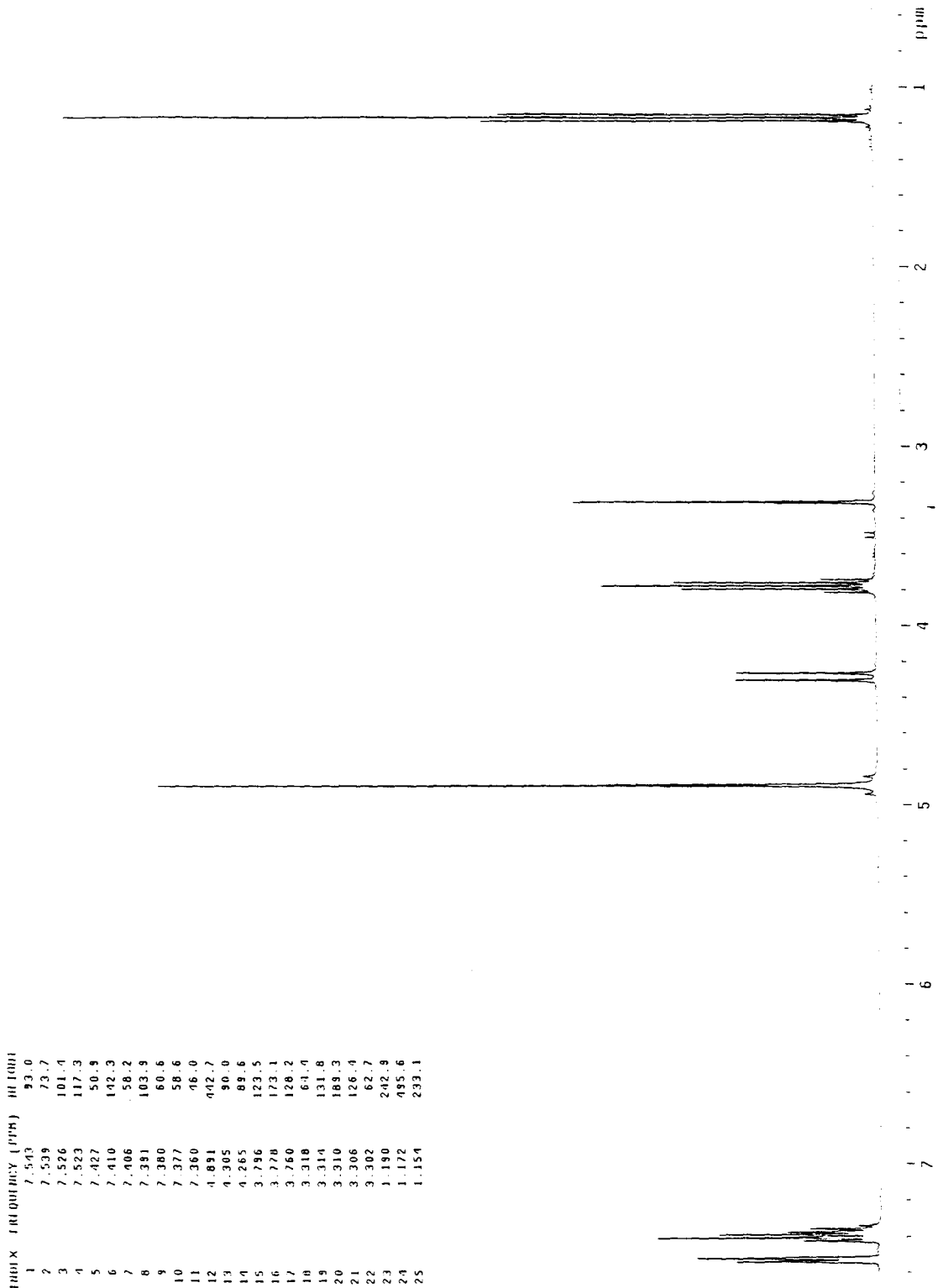


Figure 14 ^{1}H NMR of Compound **21**



INDEX	FREQUENCY (PPM)	HEIGHT
1	7.543	93.0
2	7.539	73.7
3	7.526	101.4
4	7.523	117.3
5	7.427	50.9
6	7.410	142.3
7	7.406	58.2
8	7.391	103.9
9	7.380	60.6
10	7.377	58.6
11	7.360	46.0
12	4.891	942.7
13	4.305	90.0
14	4.265	89.6
15	3.796	123.5
16	3.778	173.1
17	3.760	128.2
18	3.318	61.4
19	3.314	131.8
20	3.310	189.3
21	3.306	126.4
22	3.302	62.7
23	1.190	242.8
24	1.172	495.6
25	1.154	233.1

Figure 15 ^{13}C NMR of Compound **21**

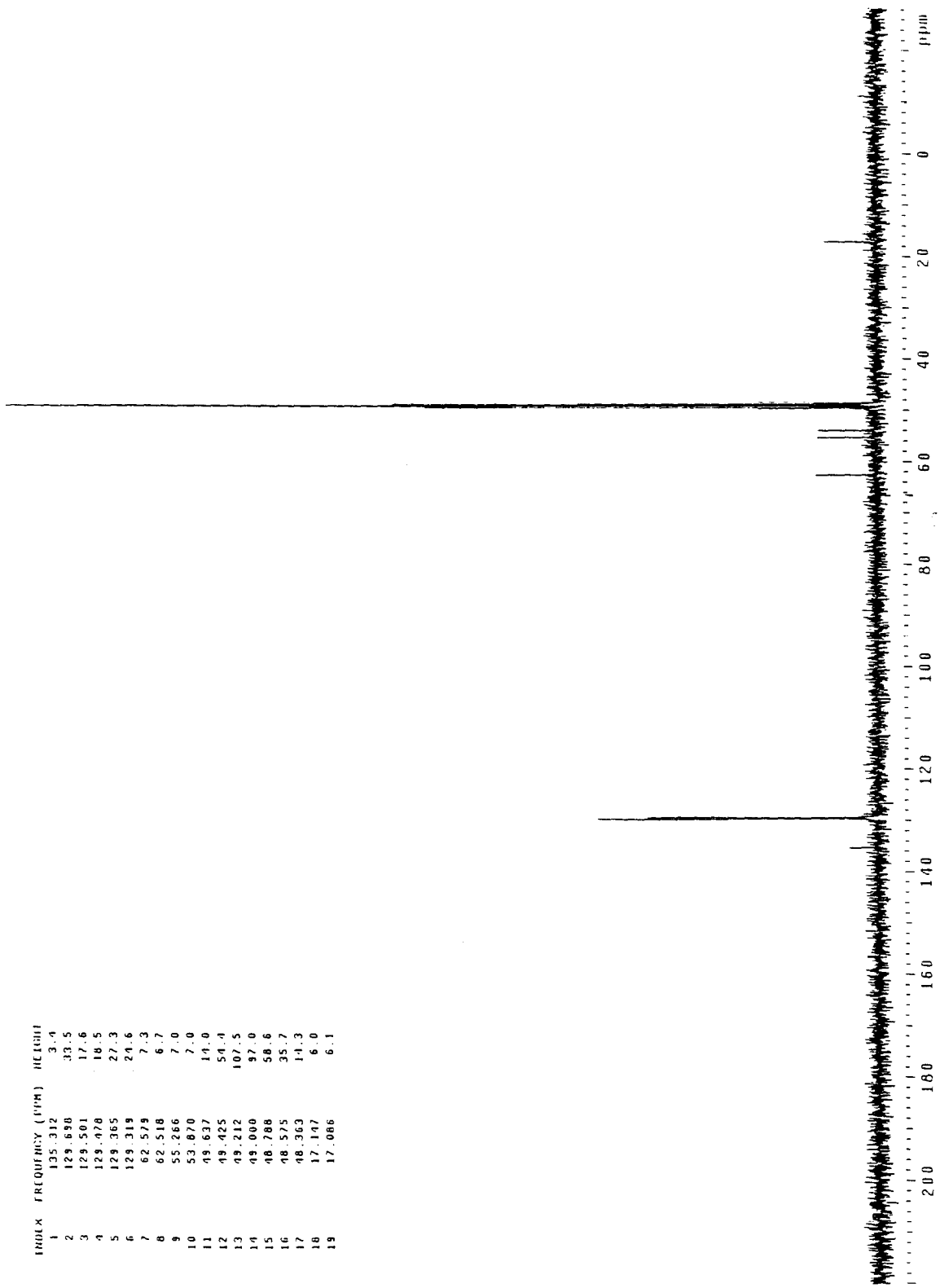


Figure 16 ^{31}P NMR of Compound **21**

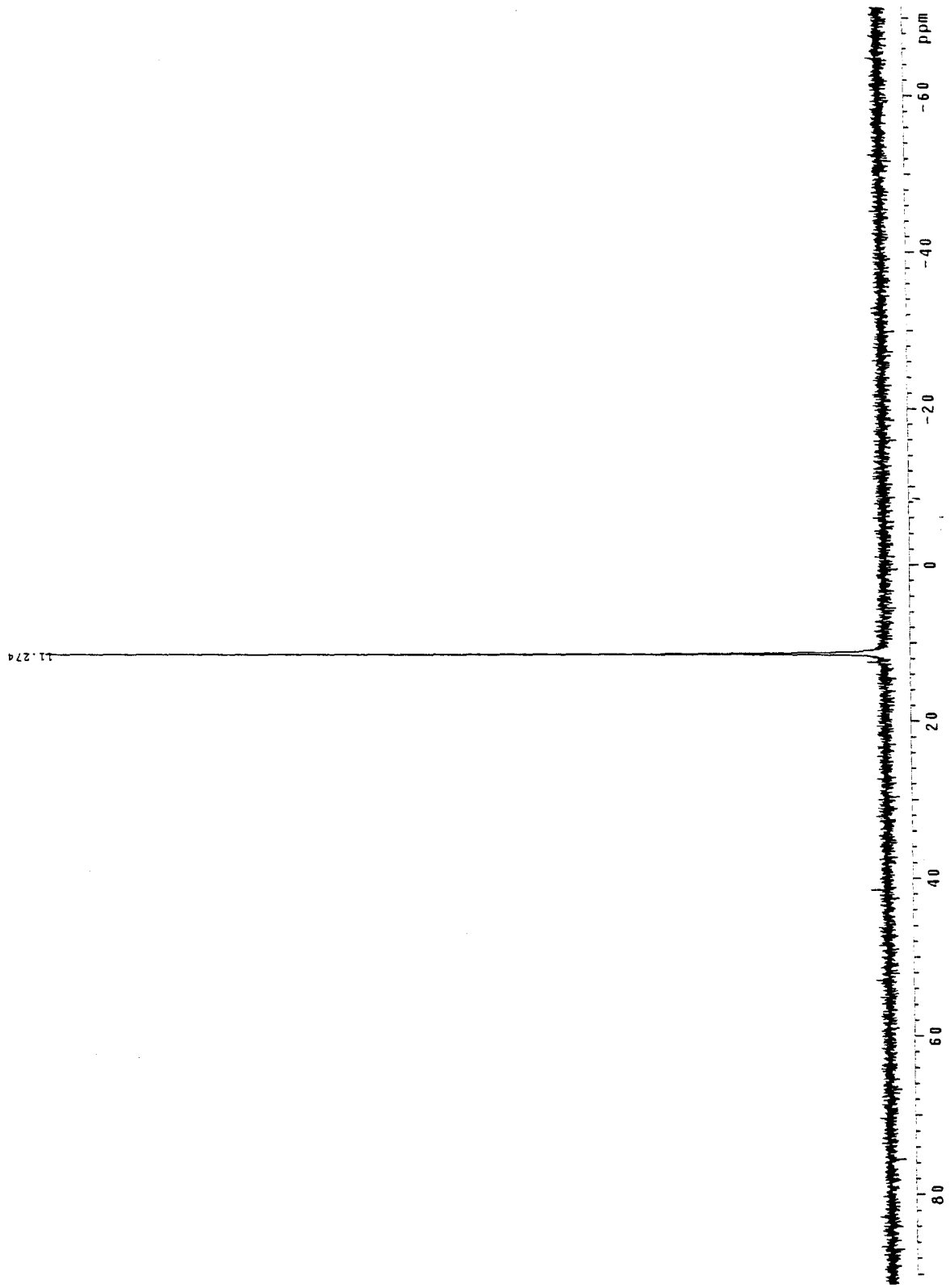


Figure 17 ^{1}H NMR of Compound **22**

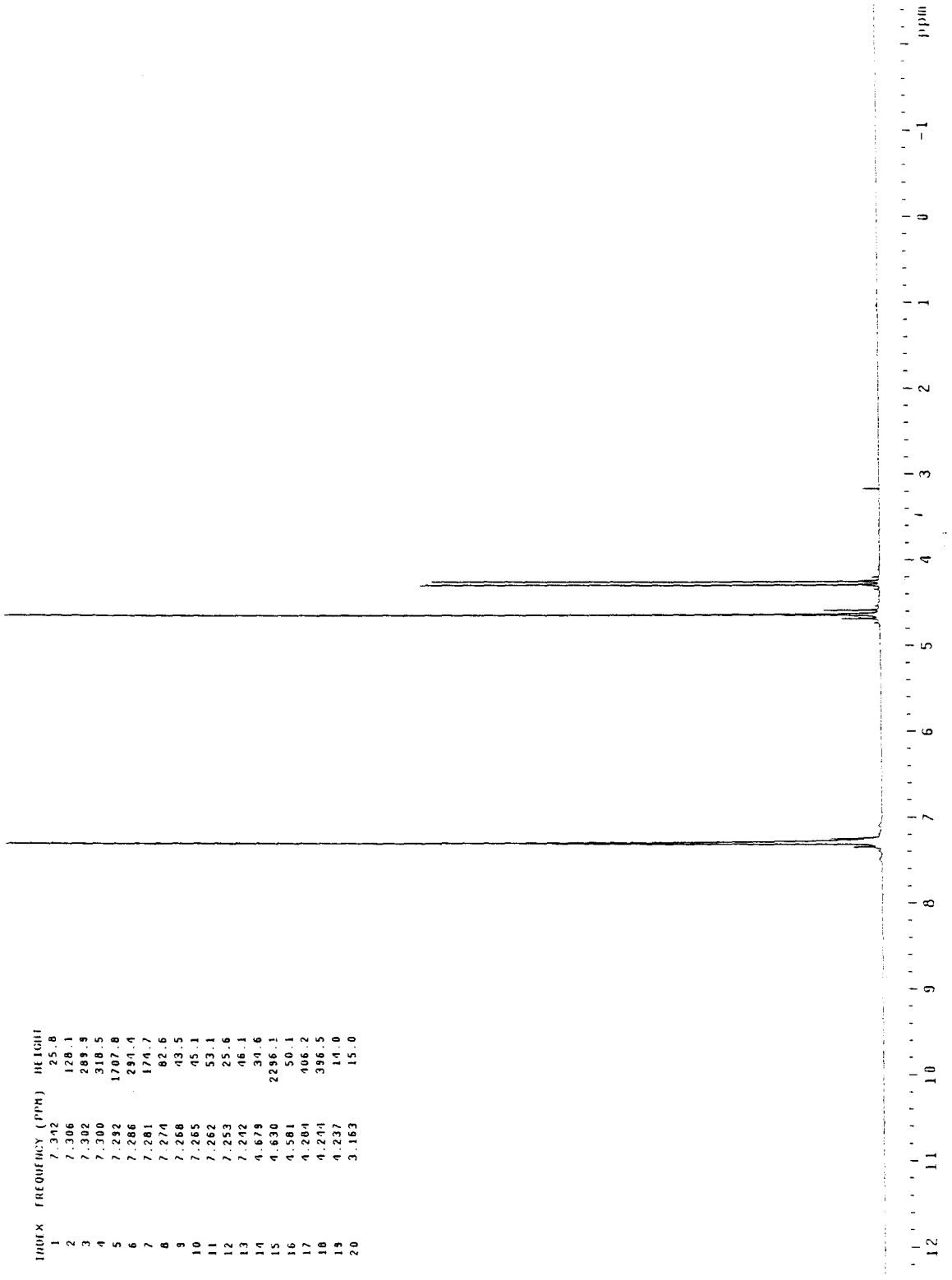


Figure 18 ^{13}C NMR of Compound **22**

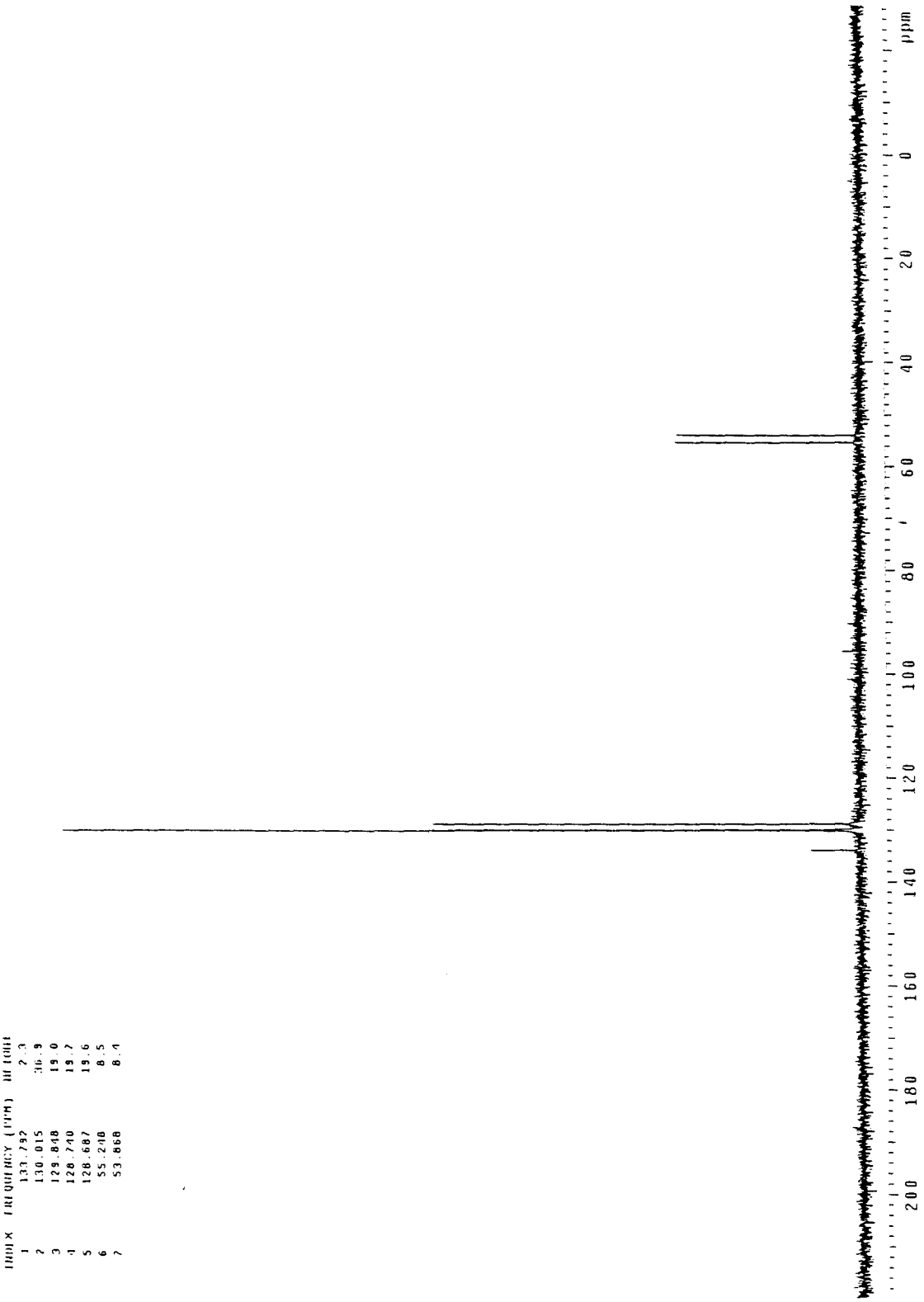


Figure 19 ^{31}P NMR of Compound **22**

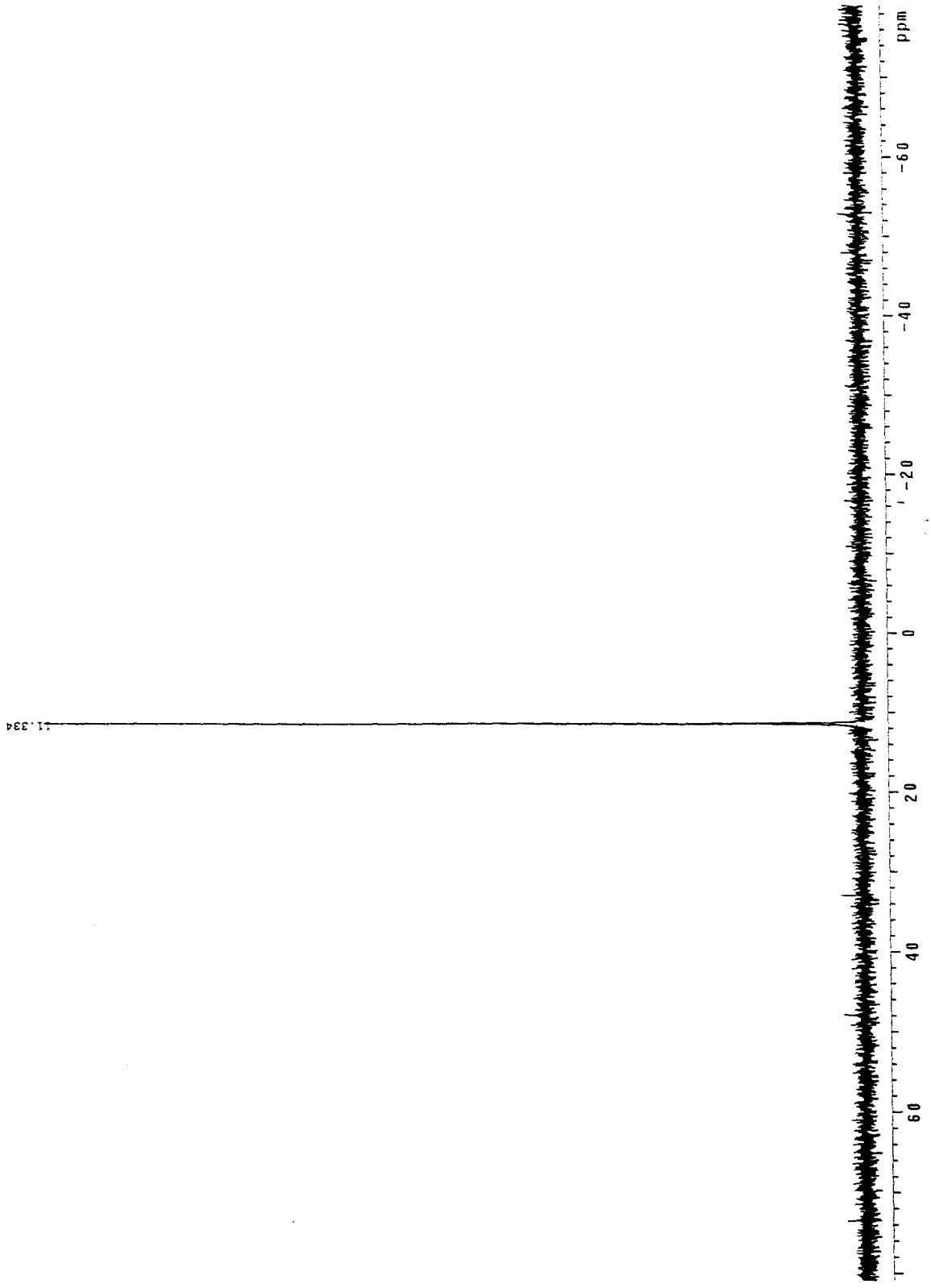


Figure 20 ^{1}H NMR of Compound **23**

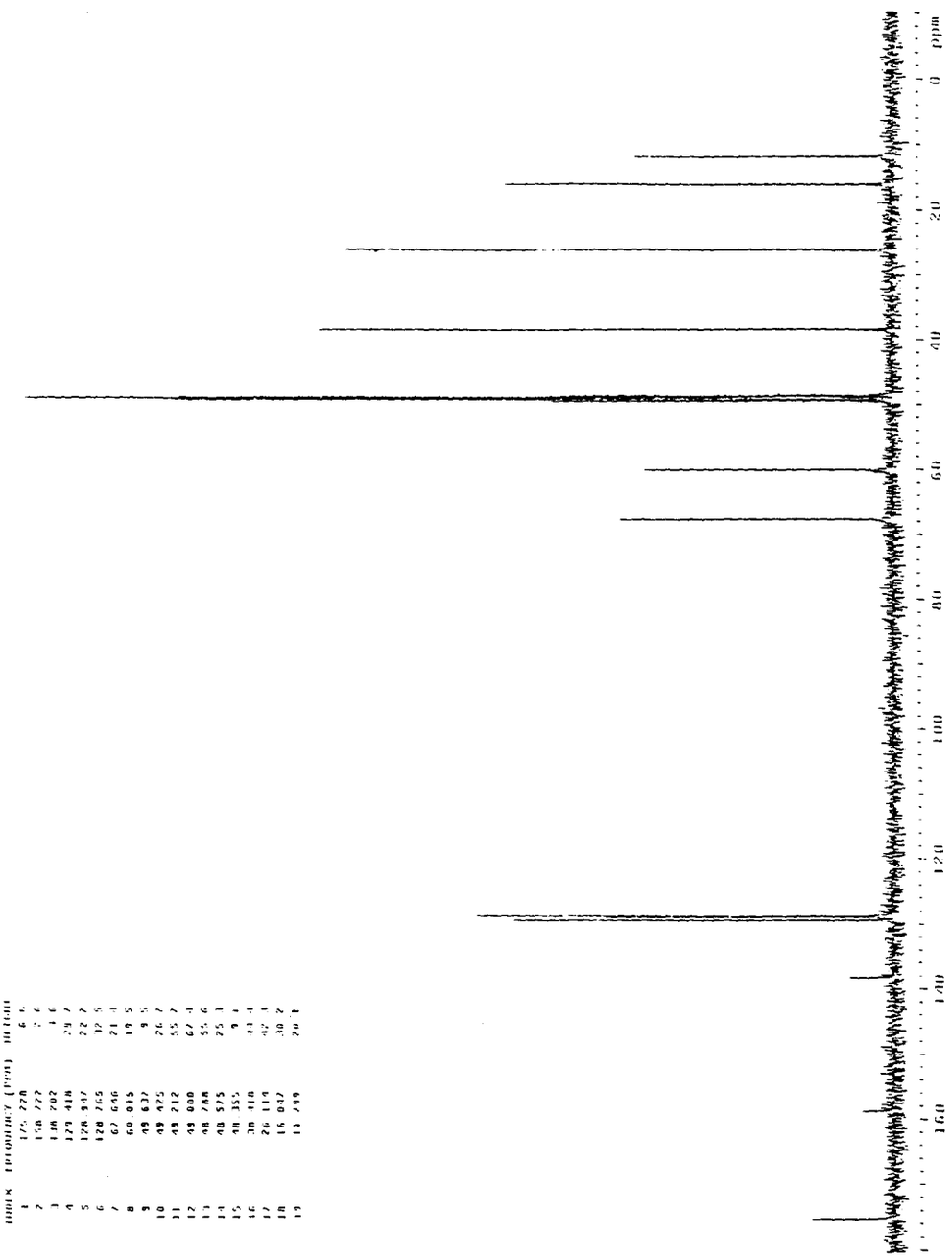


Figure 21 ^{13}C NMR of Compound **24**

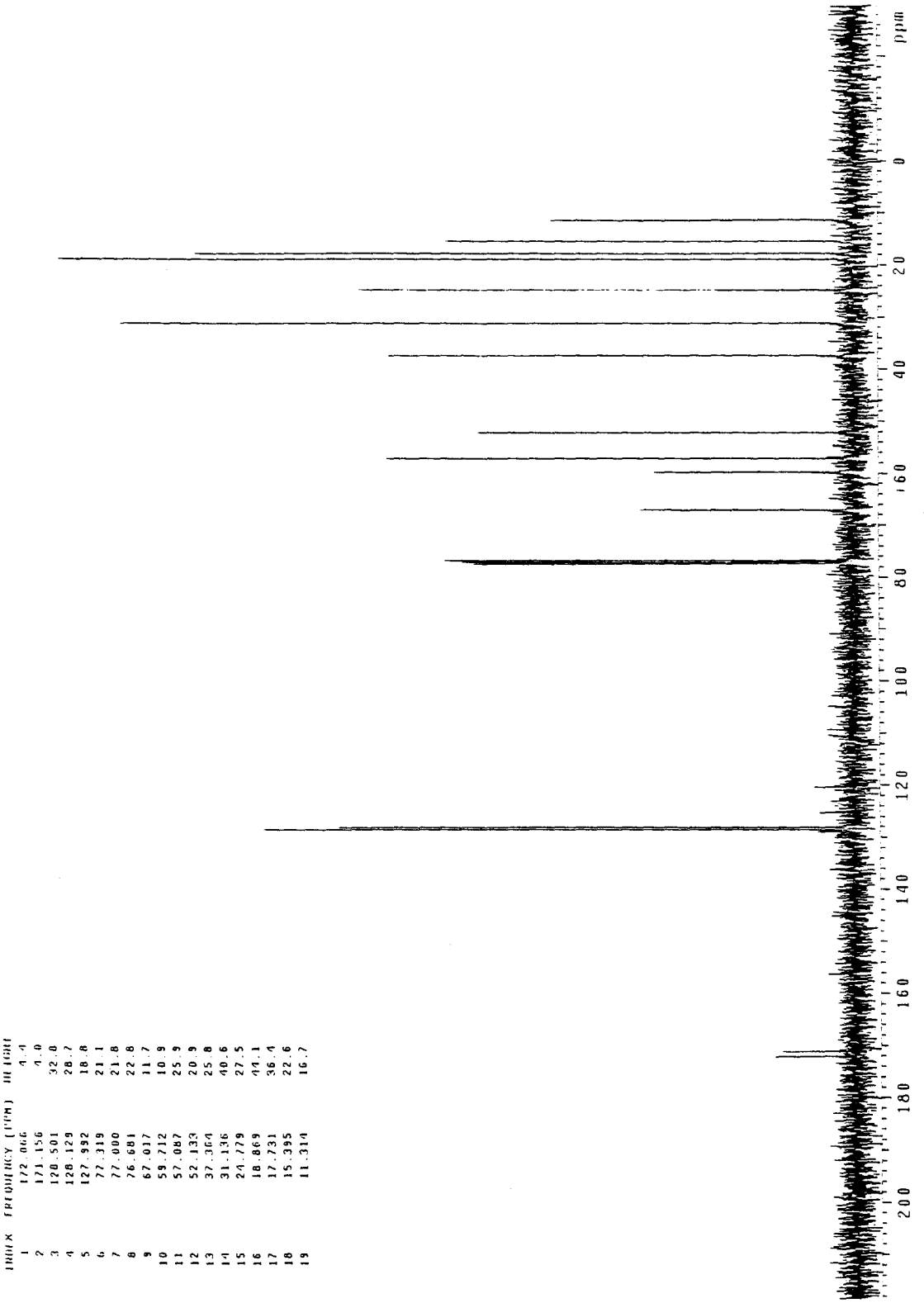


Figure 22 ^{1}H NMR of Compound **25**

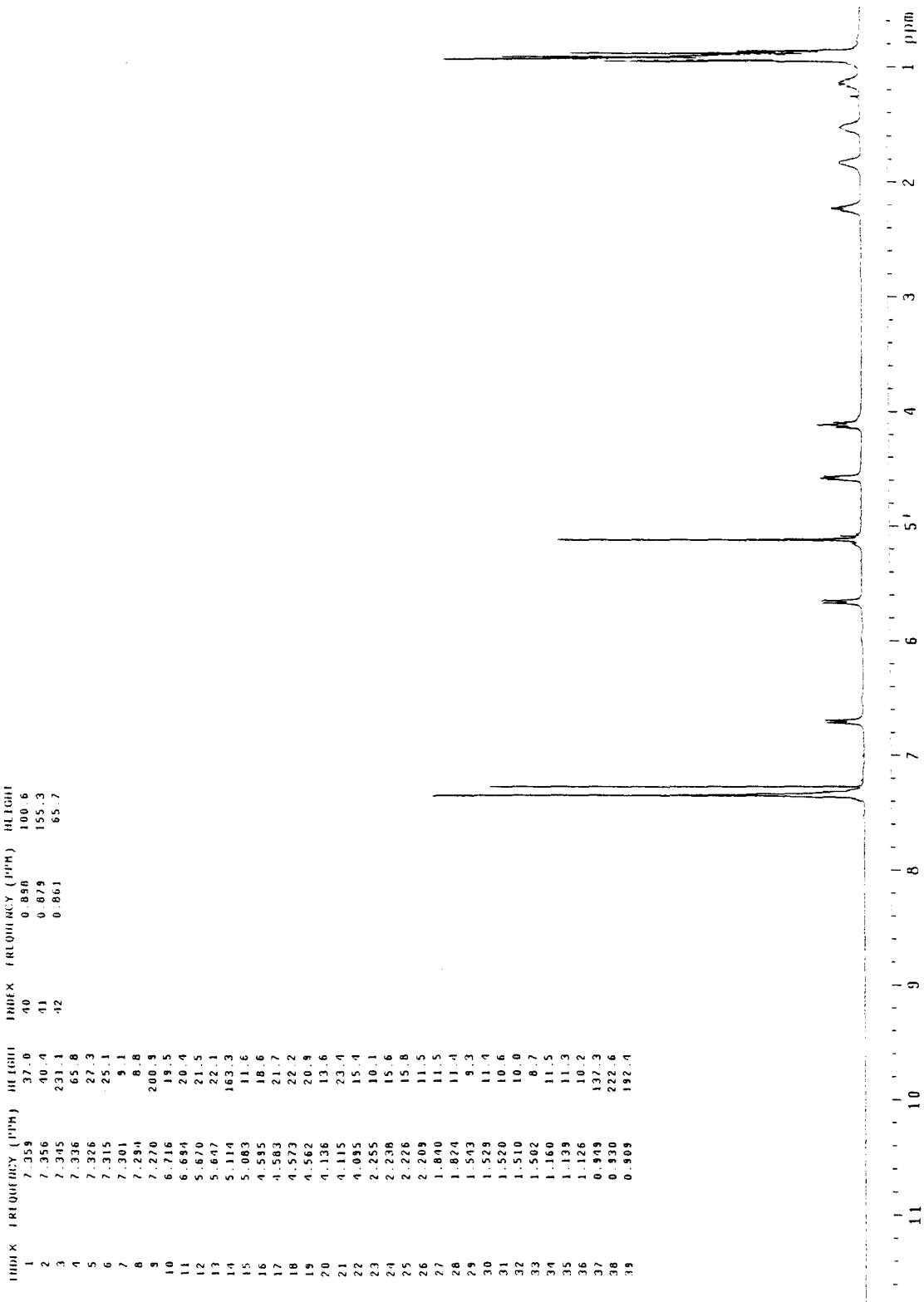


Figure 23 ^{13}C NMR of Compound **25**

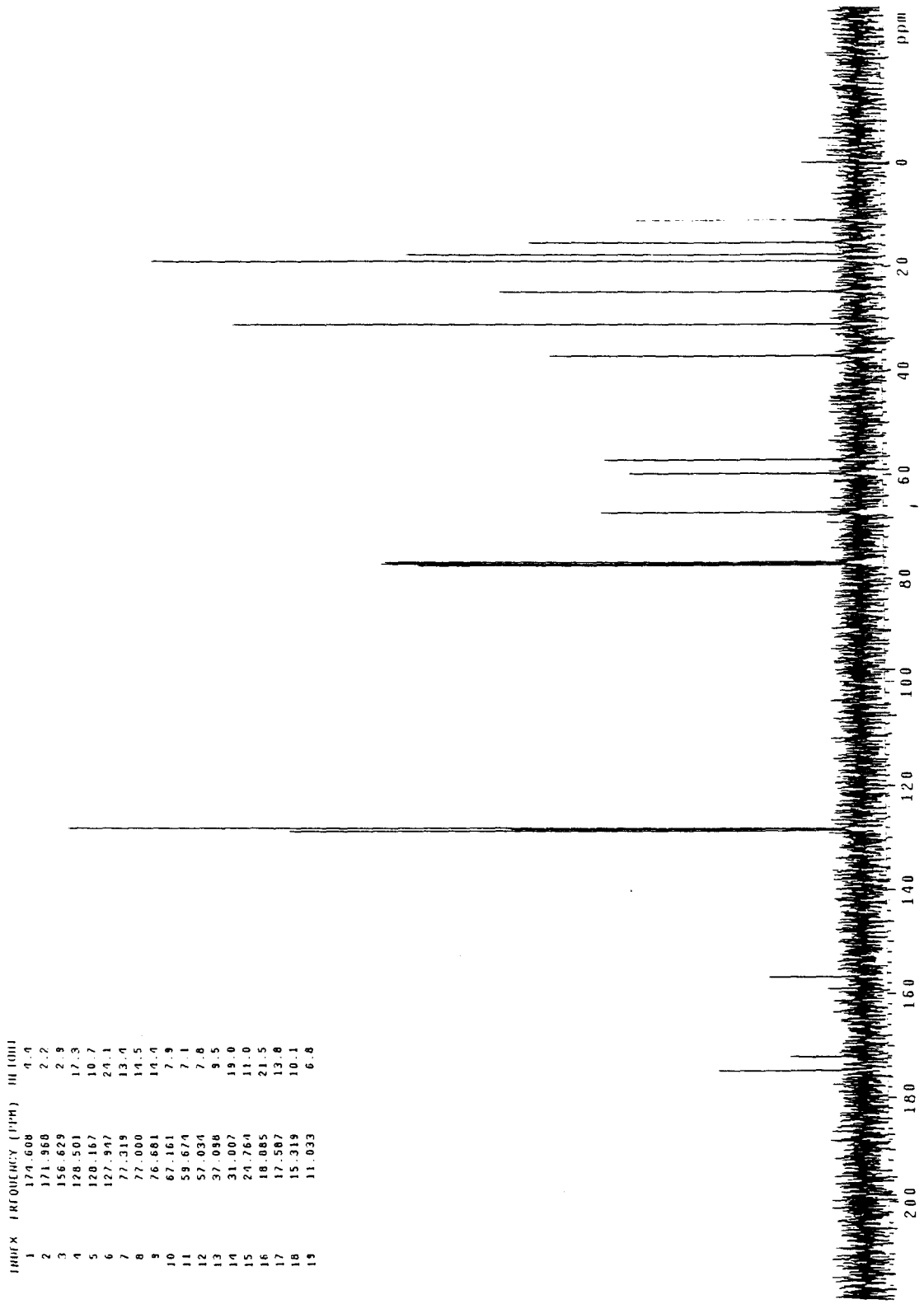


Figure 24 IR of Compound **24**

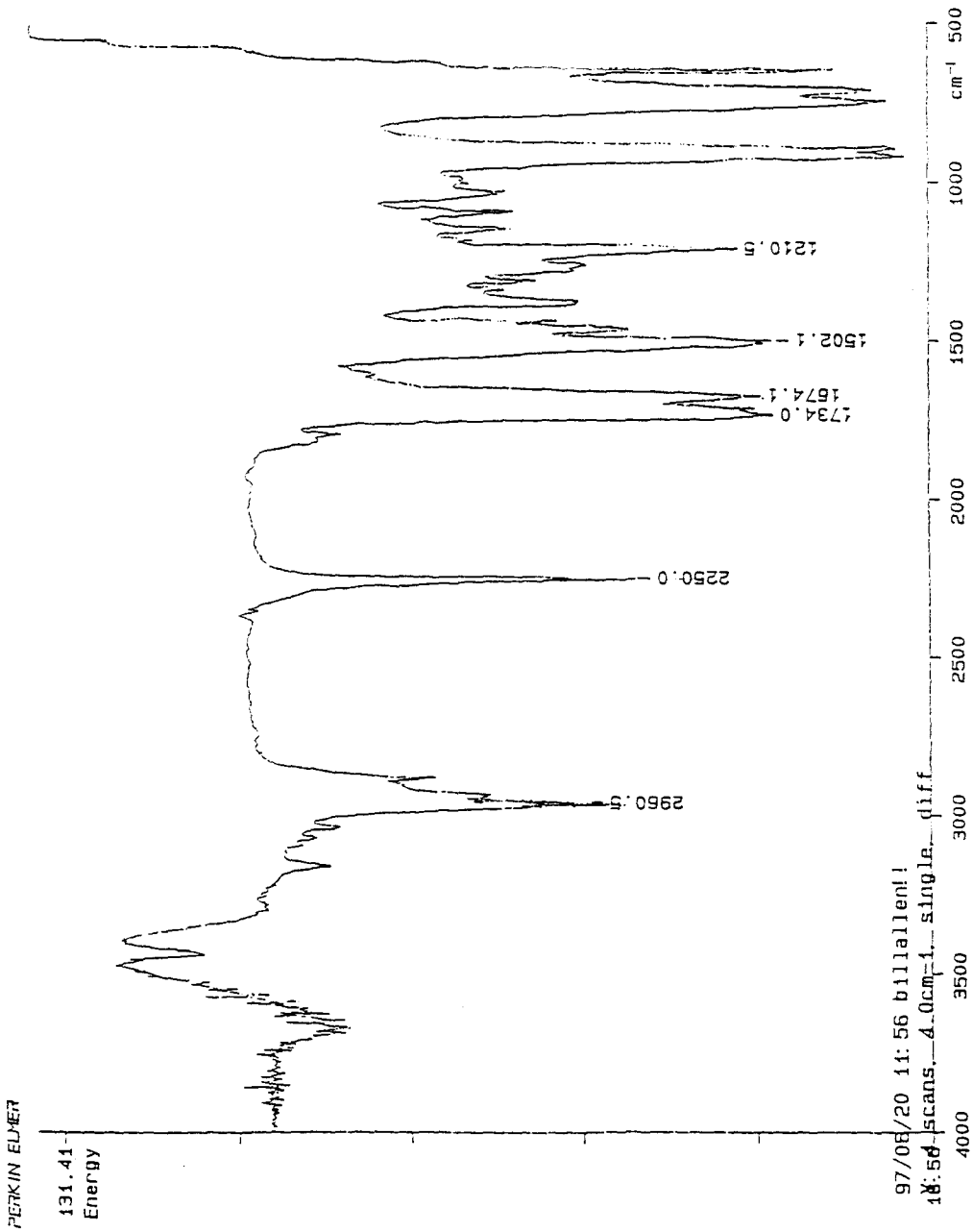


Figure 25 IR of Compound **25**