SYNTHESIS OF PHOSPHONOPEPTIDE TRANSITION STATE ANALOGS OF HIV-1 PROTEASE

by

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TRANSITION STATE ANALOGS

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ABSTRACT

The focus of the research presented here is on the multi-step synthesis, isolation and characterization of phosphorous containing transition state analogs. Several procedures for the the synthesis of the aminophosphonate core structure and side chain dipeptides were explored. These structures were designed to mimic the tetrahedral intermediate formed during the protease catalyzed hydrolysis of polypeptide substrates. As such, they are potential inhibitors of the HIV-1 protease enzyme.

ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS

Abbreviation	Description
Ala	alanine
Asn	asparagine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Cbz	benzyloxycarbonyl
DCC	1,3-dicyclohexylcarbodiimide
DMF	dimethylformamide
DNA	deoxyribonucleic acid
EDC	1-ethyl-3-[3-(diethylamino)propyl]-carbodiimide
Et	ethyl
Et_3N	triethylamine
EtOAc	ethylacetate
FTIR	fourier transform infrared spectroscopy
GC	gas chromatography
Gln	glutamine
HIV-1	human immunodeficiency virus type 1
Hz	Hertz
Iaa	isoamylamide
Ile	isoleucine
Iva	isovaleryl
J	coupling contant (in Hz)
Leu	leucine

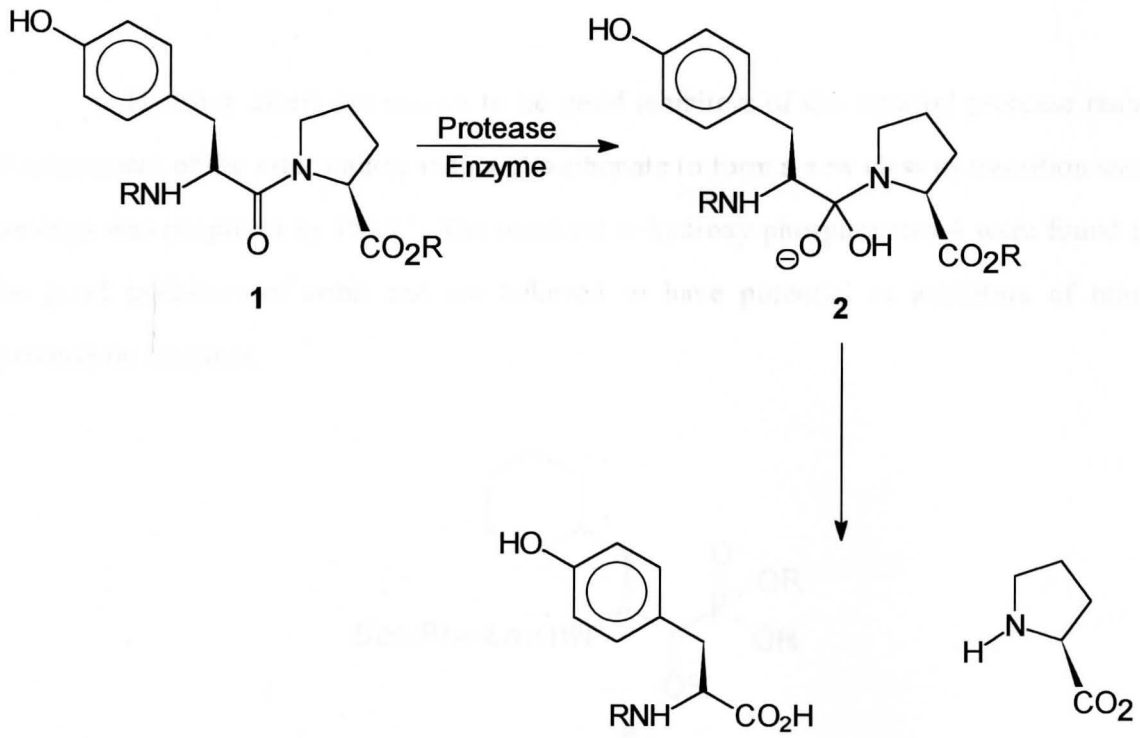
Me	methyl
MeOH	methanol
MS	mass spectrometery
mmol	millimoles
m/z	mass to charge ratio
NMR	nuclear magnetic resonance
Phe	phenylalanine
ppm	parts per million
Pro	proline
RNA	ribonucleic acid
Ser	serine
TBAB	tetrabutylammonium bromide
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TMSBr	trimethylsilyl bromide
Tyr	tyrosine
Val	valine

Chapter 1 Introduction

A transition state analog is a mimic of an intermediate structure in a chemical transformation. The true intermediate is a high energy state structure that is produced during a reaction, prior to the formation of products. The transition state intermediate tends to react readily to form low energy products. Pauling's[1] work on enzyme catalysis lead to the transition state theory. It states that a stable structure resembling the transition state (the high energy intermediate) would have a much greater affinity for the enzyme than would the natural substrate (the low energy structure). As a result of this relationship, transition state analogs have been found to be responsible for inhibition of various enzymes.

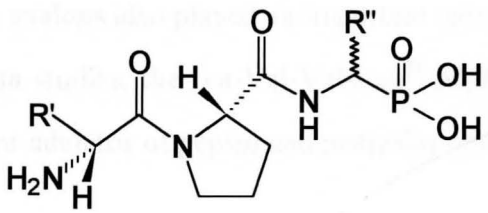
An example of a transition state intermediate is illustrated by protease catalyzed hydrolysis of polypeptide chains. The low energy natural substrate **1** binds with the active site of the protease enzyme, proceeds through a high energy intermediate structure **2** and is subsequently hydrolyzed at the carbonyl-nitrogen bond. The function of a well designed transition state analog would be first, to mimic the true intermediate **2** and secondly, to block the active site of the protease enzyme by having a much lower rate of hydrolysis.

Transition state analogs were originally developed to elucidate mechanisms of enzymatic reactions. The transition state analog locks the enzyme's active site in mid-catalysis, allowing for the use of X-ray crystallography to determine the structure. This evidence was then used to develop mechanisms for catalysis.



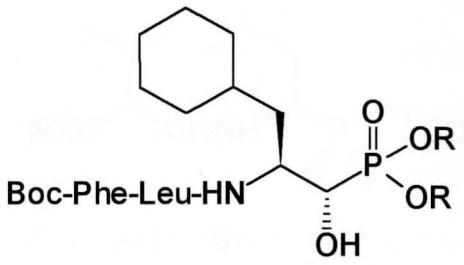
1 2

Phosphonopeptide transition state analogs have demonstrated inhibitory action in similar biological systems.[2] Phosphorous containing inhibitors are classical inhibitors of serine protease. In research conducted by Cheng,[3] phosphonopeptide analogs such as **3**, were designed and synthesized as inhibitors of thrombin, a serine protease. Substrate related peptidyl α-amino phosphonic acids formed stable transition state analogs with the active site of these enzymes. Because the structure of the transition state analog closely resembles that of the natural intermediate, there is great potential for the selective inhibition of this serine protease.



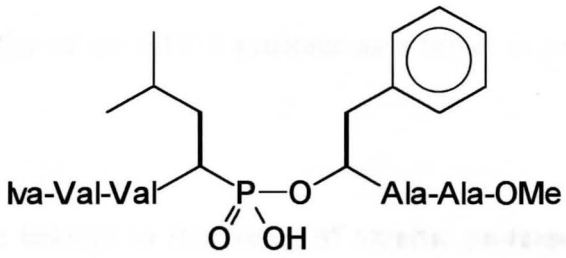
3

α-Hydroxy esters are known to be good inhibitors of the aspartyl protease renin. Replacement of the ester moiety with a phosphonate to form a new class of transition state analogs was proposed by Patel.[4] The resultant α-hydroxy phosphonates **4** were found to be good inhibitors of renin and are believed to have potential as inhibitors of other proteolytic enzymes.



4

Bartlett[5] recognized the critical step in aspartic protease catalyzed hydrolysis to be the formation of the tetrahedral intermediate. This occurred after the catalyzed addition of water to the amide linkage. Phosphorus-containing peptide analogs **5** and **6** (R = Iva, R' = Ala-Iaa, Y = OH or NH_2) were designed and synthesized as slow binding inhibitors to mimic such tetrahedral intermediates. It was felt that the incorporation of the electrophilic phosphorous moiety into various peptide fragments could improve the selectivity of the inhibitors. Furthermore, it was thought that the tetrahedral phosphorous moiety closely mimiced the tetrahedral intermediate formed during the hydrolysis of the peptides, increasing its relevance as a transition state analog. The composition of the peptide fragments utilized in these analogs also played an important role in their selectivity. Upon completion of the inhibition studies, the Iva-Val-Val-Leu^{P}-(O)Phe-Ala-Ala-OMe (**5**) was found to be the most potent inhibitor of pepsin and penicillopepsin.



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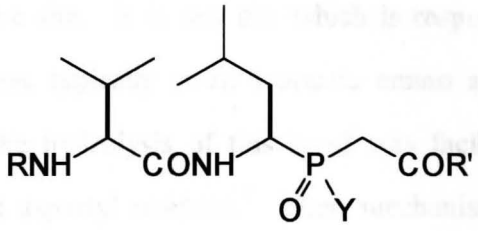


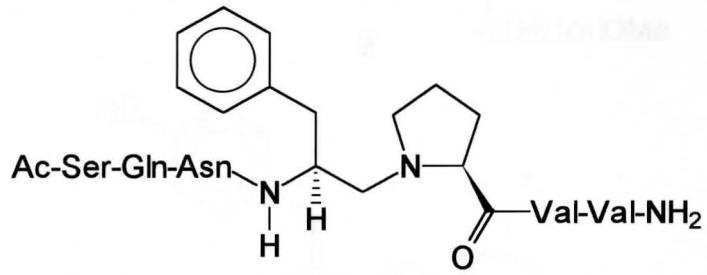
6

The Human Immunodeficiency Virus Type 1 (HIV-1) protease enzyme functions in the fragmenting of polyproteins produced during viral replication. This results in mature structural proteins and enzymes to form new infectious virions. More specifically, after translation, there are two precursor polyprotein products, designated Pr55 and Pr160. These originate from DNA encoded RNA and are subsequently fragmented into either new structural proteins of the virion core or enzymes of retroviral replication, respectively. It is this site specific cleaving of the RNA polyproteins for which the protease is responsible.

Implication of the HIV-1 protease enzyme as a potential target for inhibition, stems from work conducted by Seelmeier.[6] The basis for this research involves the site-directed mutation of the catalytically active sites in the enzymes precursor proteins. The consequence of this alteration was the formation of non-infectious virions. This evidence

confirms the vulnerability of the HIV-1 protease as a target in preventing replication of infectious virions.

HIV-1 protease belongs to the family of aspartic proteases. Two individual 99 amino acid polypeptides are assembled into a homodimer containing two C terminal aspartyl residues at the interface.[7] These residues, designated Asp-25 and Asp-25' constitute the enzyme's active site. It is this site which is responsible for the catalyzed cleavage of peptide sequences, typically at an aromatic amino acid and proline peptide bond. Hyland found that the hydrolysis of this bond was facilitated by the acid/base chemistry at the two distinct aspartyl residues.[8] Their mechanism was elucidated using synthetic analogs of the oligopeptide substrates. These analogs contain isosteric replacements for the scissile dipeptide amide bonds. The isosteres, **7** and **8** act as competitive inhibitors of the HIV-1 protease.



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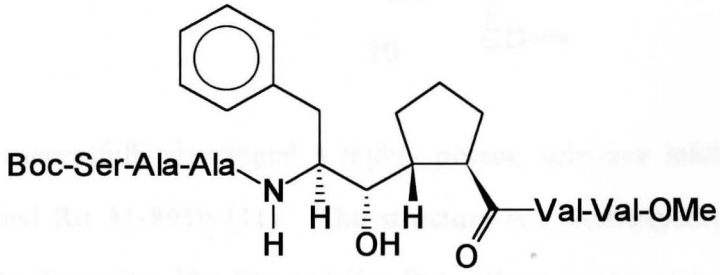
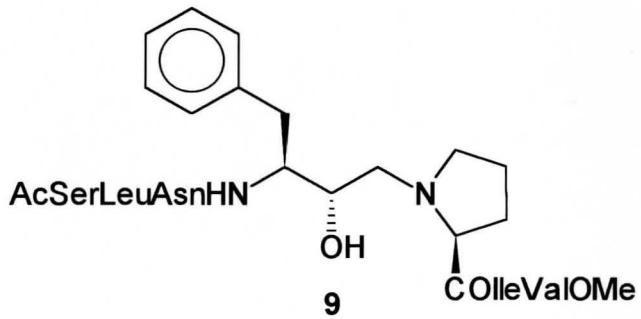


8

The transition state analog hypothesis[9] proposed by Wolfenden has been used to design inhibitors of HIV-1 protease. Rich[10] used this approach to design several hydroxyethylamine dipeptidyl isosteres **9** as mimics of the tetrahedral intermediate **10**. The subsequent evaluation of these inhibitors revealed that the essential constituent in the most potent analogs was the hydroxyl group adjacent to the tetrahedral carbon. This hydroxyl moiety binds to the C terminal catalytic aspartic acid residue of the enzyme consequently blocking the active site. Due to the hydroxyl groups relevance in mimicing the transition state intermediate, analogs designed without this function were found to be much weaker protease inhibitors.



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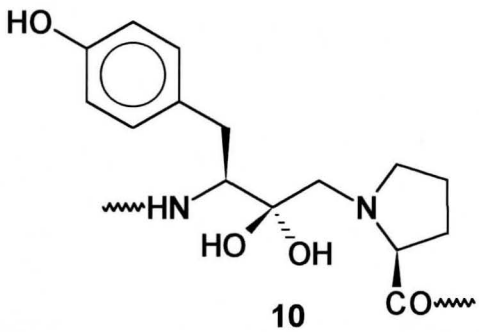
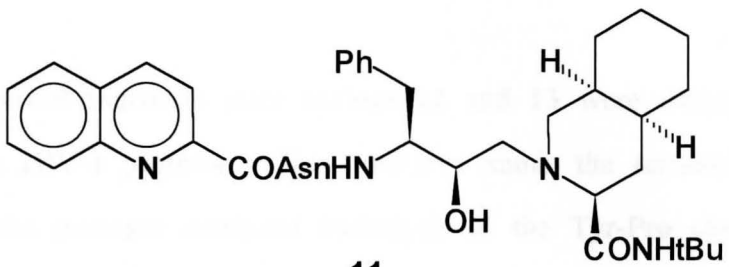


10

Parkes[11] successfully developed a highly potent, selective inhibitor of the HIV protease designated Ro 31-8959 (**11**). The structure is a hydroxyethylamine transition state analog of the dipeptides Phe-Pro and Tyr-Pro. These peptides are natural cleavage sequences in the viral polyprotein. It was reasoned that these analogs would exhibit high

selectivity for the viral enzymes since amide bonds N-terminal to proline are generally not cleaved by mammalian endopeptidases. Ro 31-8959 is now in phase III clinical trials.



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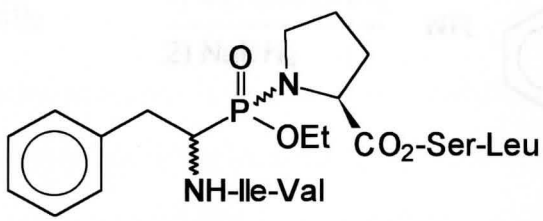
Chapter 2 Results and Discussion

The proposed transition state analogs **12** and **13** were designed as potential inhibitors of the HIV-1 protease. The structures mimic the tetrahedral intermediate formed during the protease catalyzed hydrolysis of the Tyr-Pro cleavage sequence. Several synthetic procedures leading to the core aminophosphonate structure and the side chain dipeptides were studied.



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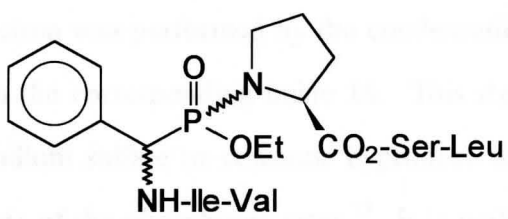
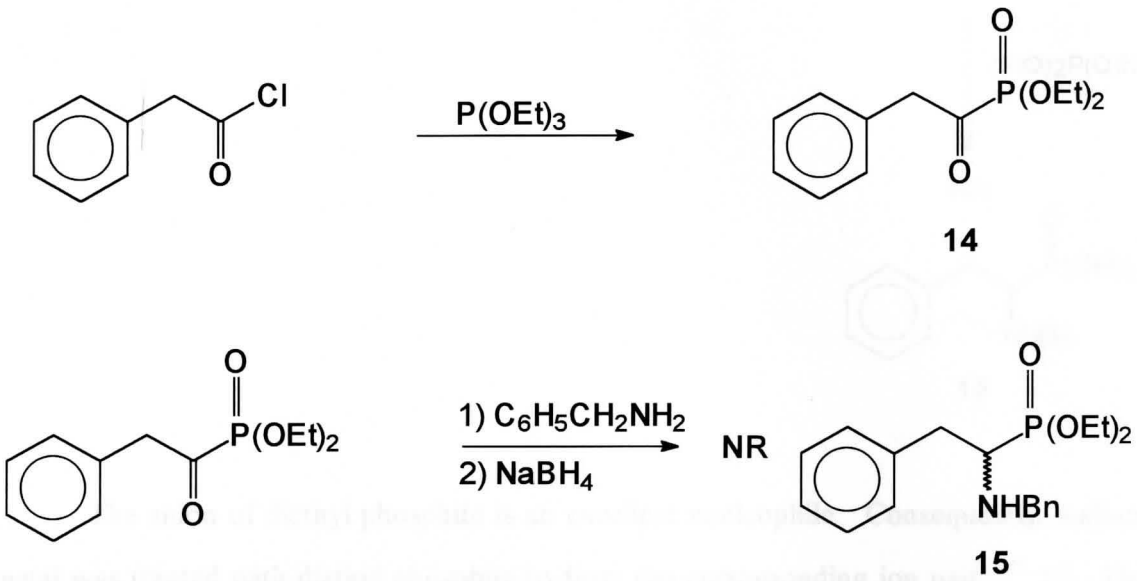


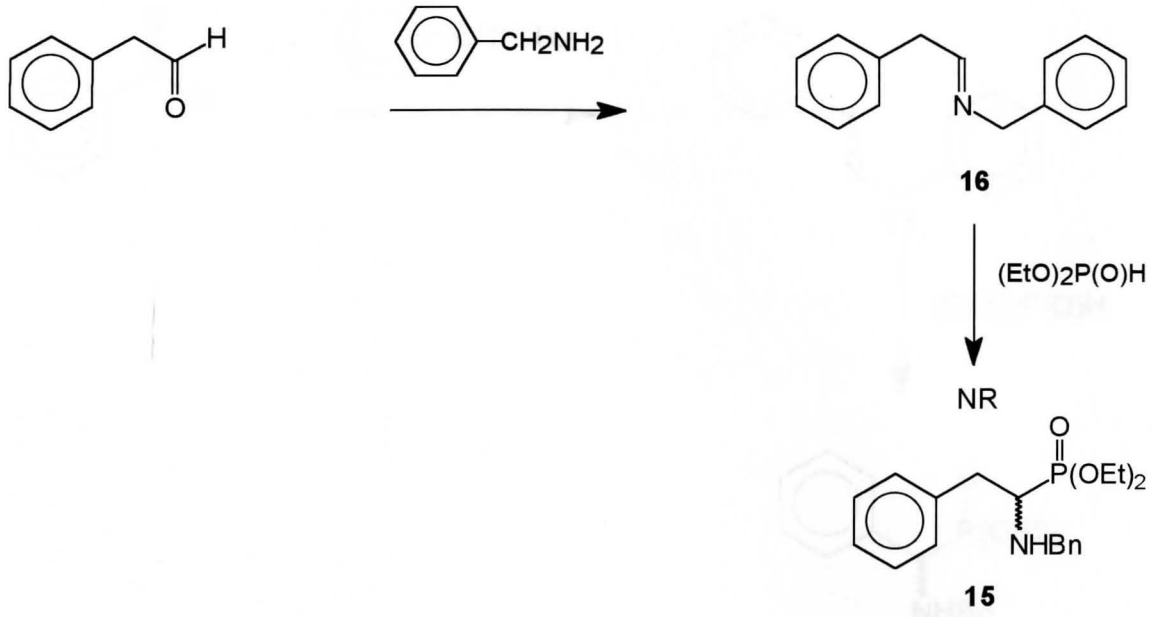
13

The α-ketophosphonate approach to the formation of the aminophosphonate **15** was the first synthesis attempted. This procedure began with the reaction of phenyl acetyl chloride with triethyl phosphite to form the corresponding α-ketophosphonate **14**. The product was purified and isolated by fractional distillation in low yields (25%). Next, was the addition of benzylamine to form the desired imine *in situ* followed by reductive amination with sodium borohydride.[12] Subsequent quenching and aqueous work-up

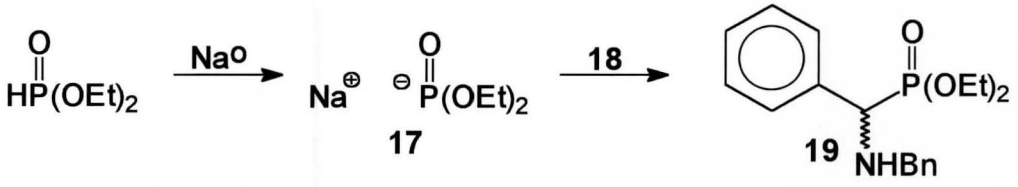
followed by flash column chromatography failed to produce the α-aminophosphonic acid diester **15**. After several attempts at this procedure, new approaches were investigated.



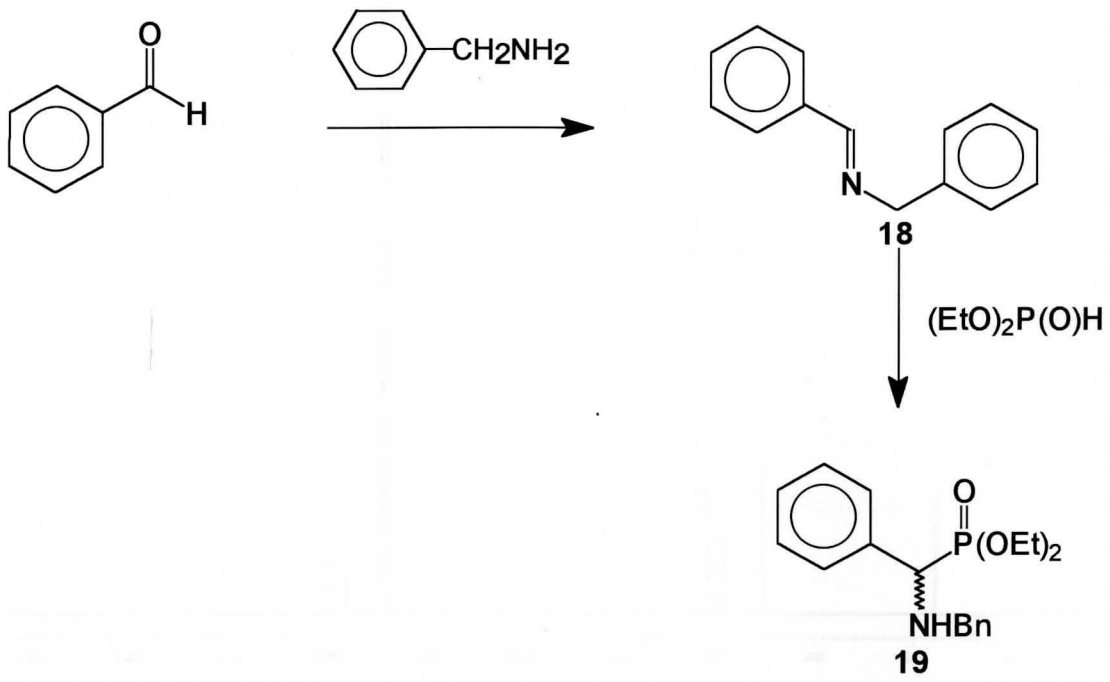
Another possible method for the formation of **15** was by the phenylacetaldehyde imine approach. The reaction was performed by the condensation of phenylacetaldehyde with benzylamine to form the corresponding imine **16**. This step was carried out in the presence of anhydrous sodium sulfate to consume byproduct water. This measure was taken to prevent hydrolysis of the phosphonic ester.[13] It is well known that the carbon-nitrogen double bond is analogous in many respects to a carbonyl, thus susceptible to nucleophilic attack.[14] Next, the imine was treated with diethyl phosphite in an attempt to form product **15** under a variety of conditions.[13,15,16] Reactions were performed in a NMR tube to monitor product formation. Imine formation was observed almost instantly but the diethyl phosphite was not consumed even over extended periods (5 days). The enolizability of the phenylacetaldehyde may have resulted in a mixture of imine products which apparently polymerized readily in an Aldol-like condensation.[2]



The anion of diethyl phosphite is an excellent nucleophile. Consequently, sodium metal was treated with diethyl phosphite to form the corresponding ion pair[17,18] **17**. The diethyl phosphite anion was then added to a solution of the pre-formed imine and for the first time, desired product **19** was isolated although yields were extremely low.



To eliminate the problem of polymer formation, the nonenolizable benzaldehyde was substituted for phenylacetaldehyde in the reaction with benzylamine. As with the phenylacetaldehyde, quantitative yields were obtained in short periods of time although polymerization was not a problem. The pre-formed imine **18** underwent reaction with diethyl phosphite at reflux[15] to give the desired product in excellent yields (93%). When a two fold excess of diethyl phosphite was used, all of the imine was consumed and after aqueous work-up, the aminophosphonate **19** required no further purification.



Complete consumption of the imine was monitored by ^{13}C NMR. As the azomethine signal at 161.41 ppm diminished, the chiral carbon doublet (**e**, bonded to phosphorous) at 58.19 ppm appeared. This transformation was used as an indicator of aminophosphonate formation (Figure 1 and 2).

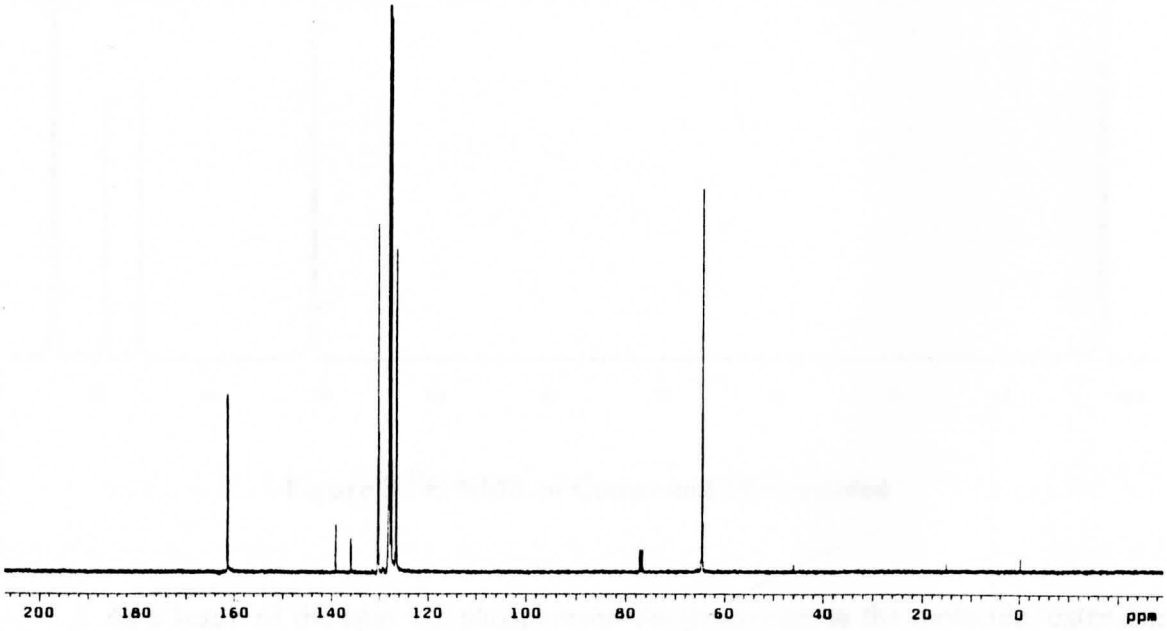


Figure 1 ^{13}C NMR of Compound **18**

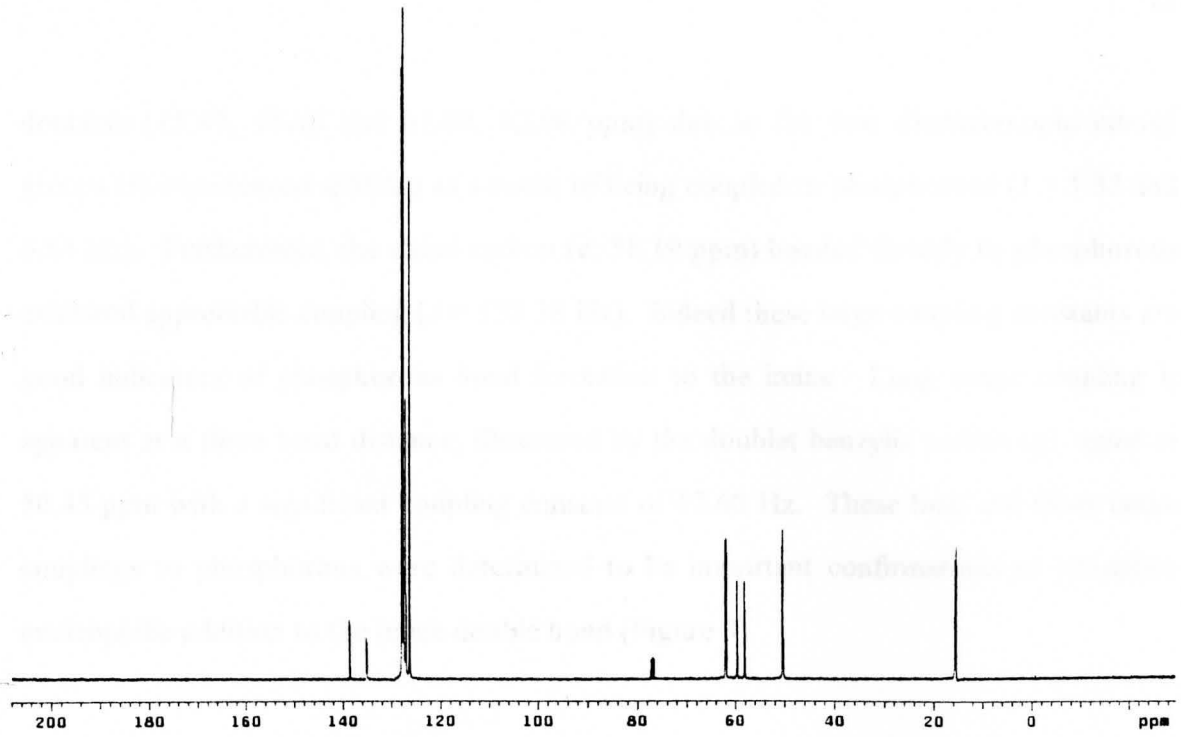


Figure 2 ^{13}**C NMR of Compound 19**

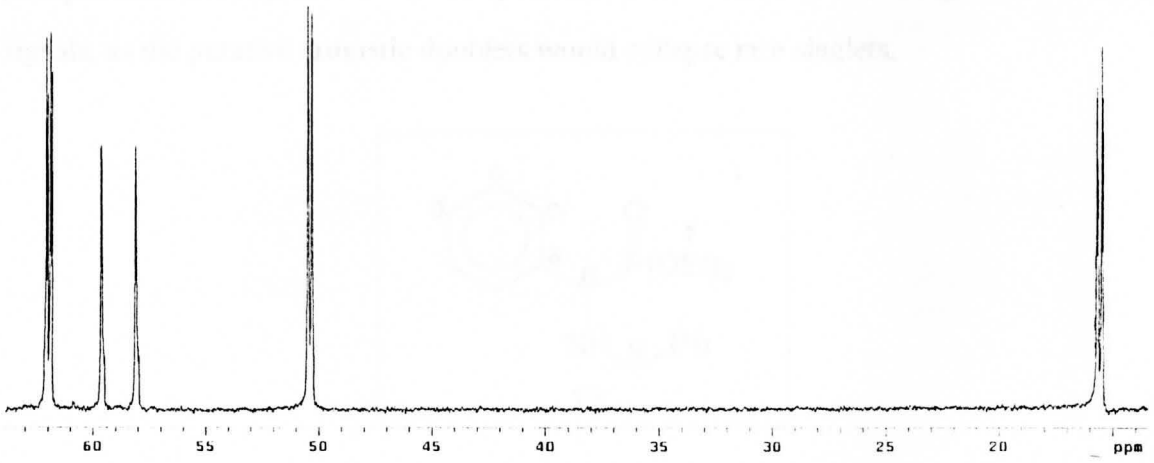
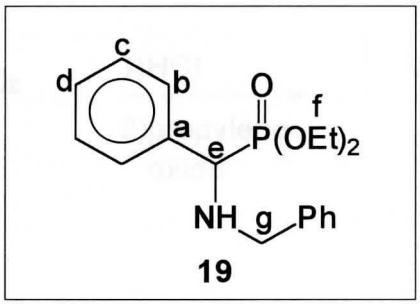


Figure 3 ^{13}**C NMR of Compound 19 Expanded**

As a result of the spin 1/2 phosphorous nuclei present in the molecule, extensive couplings were observed in the NMR spectra. For example, in the ^{13}C spectrum, the four

doublets (15.47, 15.68 and 61.89, 62.08 ppm) due to the two diastereotopic ethoxy groups (**f**) experienced splitting as a result of being coupled to phosphorous (J = 5.33 and 6.84 Hz). Furthermore, the chiral carbon (**e**, 58.19 ppm) bonded directly to phosphorous exhibited appreciable coupling (J = 153.38 Hz). Indeed these large coupling constants are good indicators of phosphorous bond formation to the imine. Long range coupling is apparent at a three bond distance, illustrated by the doublet benzylic carbon (**g**) signal at 50.45 ppm with a significant coupling constant of 17.60 Hz. These long and short range couplings to phosphorous were determined to be important confirmations of phosphite nucleophilic addition to the imine double bond (Figure 3).

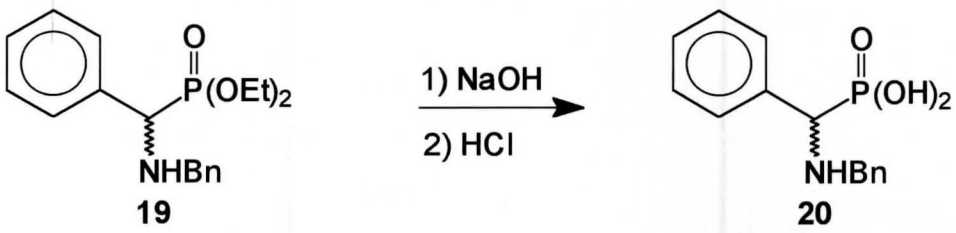
The assignment of the signals in the aromatic region of the ^{13}C NMR are tentative. Upon initial inspection of the of the ^{13}C NMR spectrum of **19**, it appears that there are some extraneous signals. One possible explanation for this is that there may be two, three and four bond couplings of phosphorous with the aromatic carbons (**a**, **b** and **c**). A phosphorous decoupled ^{13}C NMR spectrum would simplify the assignment of these signals, as the putative aromatic doublets would collapse into singlets.



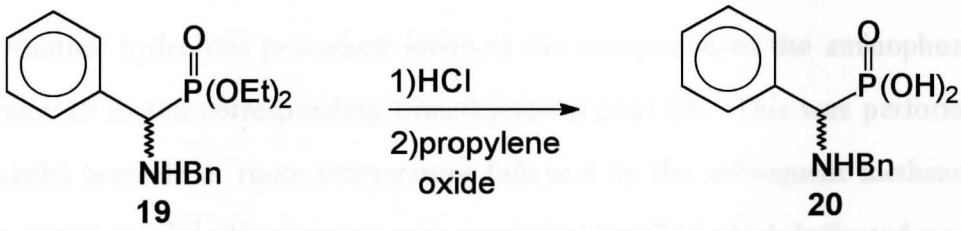
19

Several methods of hydrolysis of **19** were attempted beginning with general text book procedures and eventually attempting more radical approaches. Base catalyzed hydrolysis of the aminophosphonate **19** was tried at room temperature with NaOH. Evaluation of this reaction after several days indicated minimal reaction and mostly

starting material. The phase transfer catalyst tetrabutylammonium bromide (TBAB) was also utilized in the NaOH catalyzed reaction[19,20] without appreciable product formation. Acid catalyzed hydrolysis was also attempted at room temperature with HCl. This also resulted in minimal hydrolysis of **19**.



Acid catalyzed hydrolysis of **19** with concentrated HCl was also performed at reflux. Upon evaporation of the HCl, the residue was dissolved in ethanol and the product was subsequently precipitated using propylene oxide.[15,21,22] This procedure gave excellent yields (87%) of pure phosphonic acid **20**. The use of **20** in the formation of the phosphonopeptide was limited by low solubility in standard coupling solvents.



NMR confirmation of **20** was made by observing the disappearance of the signals due to the ethoxy groups (**f**) present in **19**. In the proton spectrum, this was indicated by the loss of the doublet of triplets (1.06, 1.23 ppm) upfield and the corresponding multiplets (4.02-4.13 ppm) downfield. ^{13}C NMR (Figure 4) verified the absence of the

four ethoxy doublets (15.47, 15.68 and 61.89, 62.08 ppm) that were present in the spectrum of the diester. Furthermore, the ^{31}P NMR indicated an upfield chemical shift (8.78 ppm) compared to the diester (24.55 ppm).

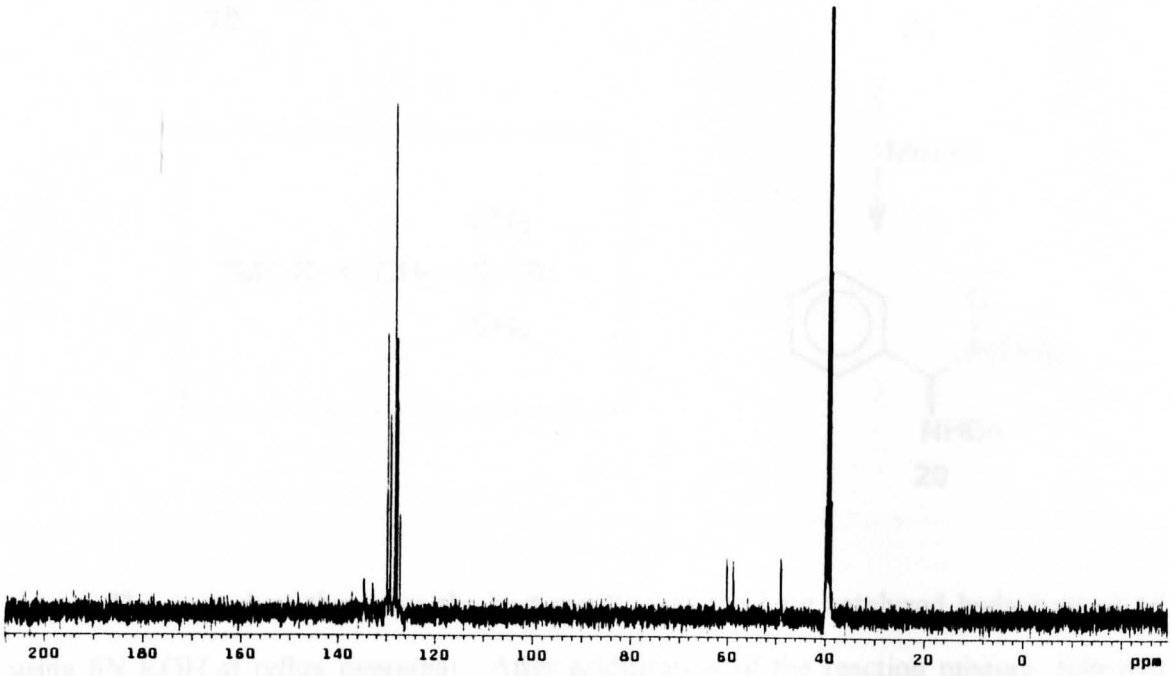
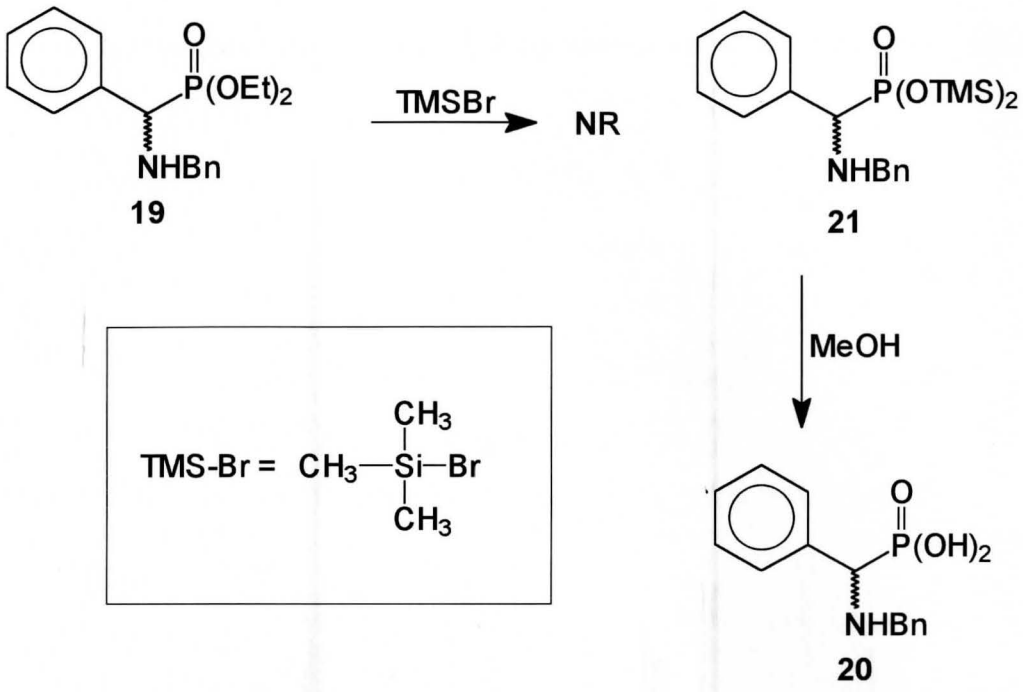


Figure 4 ^{13}C **NMR of Compound 20**

Another hydrolysis procedure involved the conversion of the aminophosphonate diethylester **19** to the corresponding trimethylsilyl diester **21**. This was performed with trimethylsilyl bromide at room temperature followed by the subsequent methanolysis to yield the diacid **20**.[23,24] The reaction was monitored by TLC which indicated no product was formed.



The procedure that gave the best results was the base catalyzed hydrolysis of **19** using 8N KOH at reflux overnight. After acidification of the reaction mixture, followed by aqueous work-up and removal of methanol *en vacuo*, the monoester **22** was isolated as a crystalline solid. The solubility of the monoester in standard coupling solvents increased its utility in subsequent peptide coupling reactions. Characterization by NMR indicated formation of the monoester **22** in excellent yields (98%). An analytical sample was obtained by recrystallization from methanol.

The formation of monoester **22** (the hydrolysis of one ethoxy group, **f**) was illustrated in the proton NMR by the absence of one triplet at 1.23 ppm and the formation of a doublet of doublets of quartets at 3.68 and 3.77 ppm. In the ^{13}C spectrum (Figure 5), the two pair of doublets due to **f** (15.47, 15.48 and 61.89, 62.08 ppm), were each reduced to a single doublet. ^{31}P NMR revealed an upfield chemical shift (10.76 ppm) from that of the diester (24.55 ppm). These transformations are consistent with the hydrolysis of one of the diastereotopic ethoxy groups.

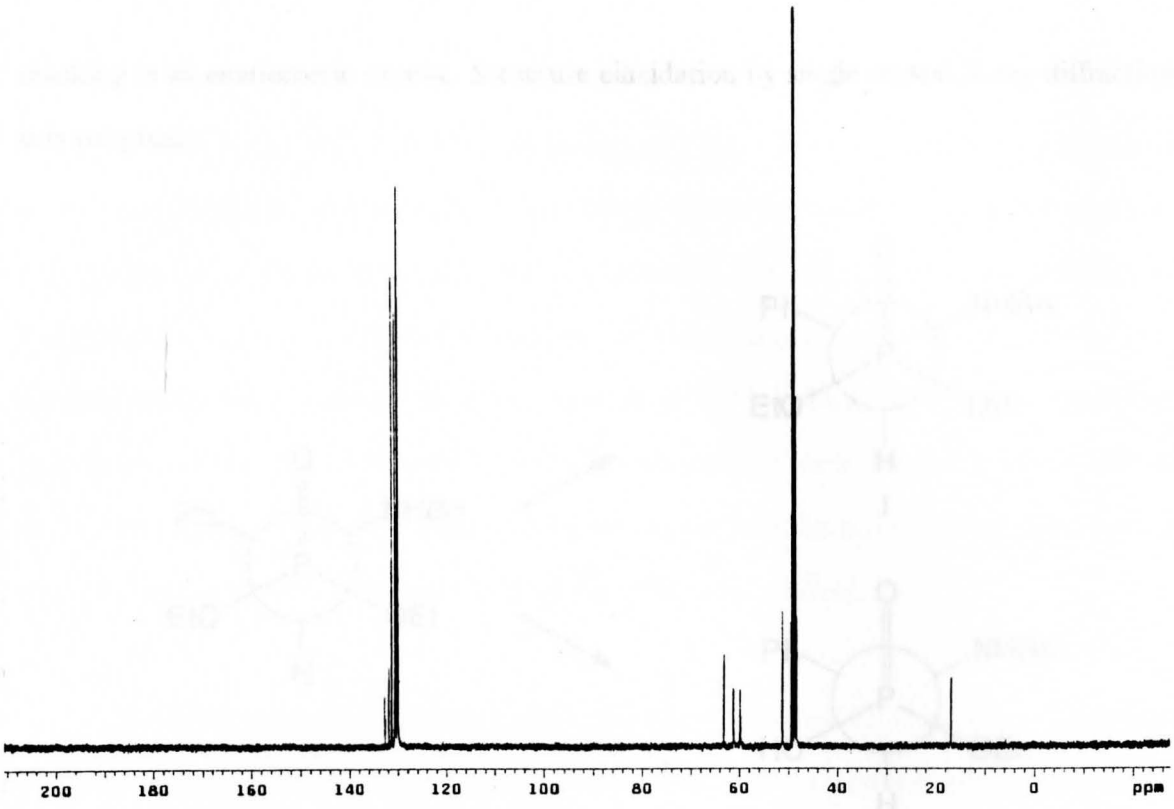
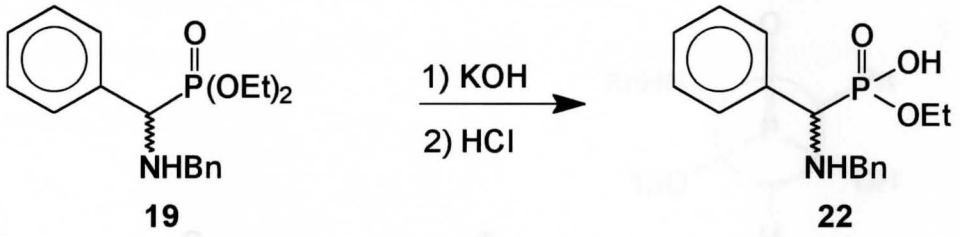
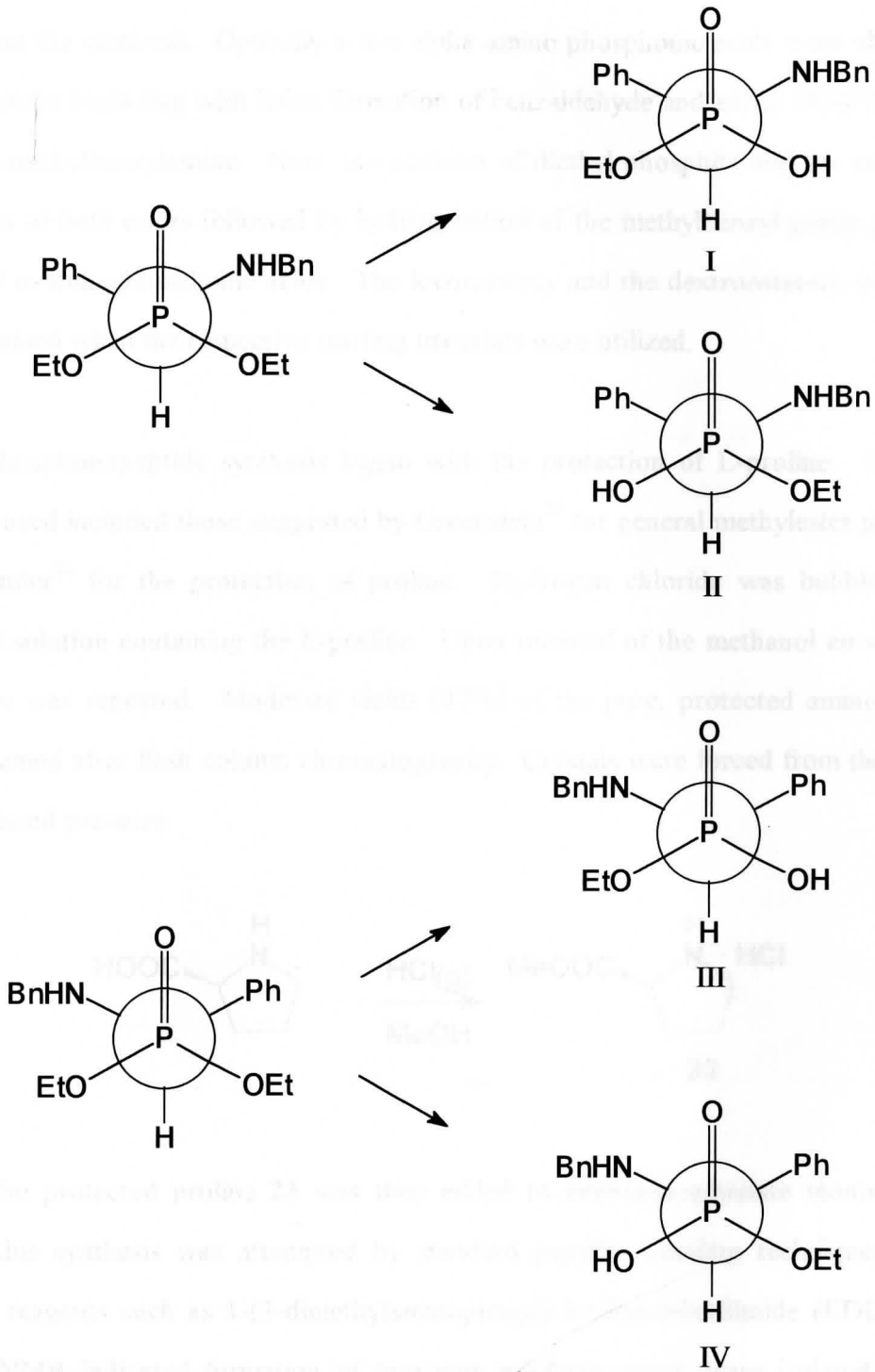


Figure 5 ^{13}C NMR of Compound **22**



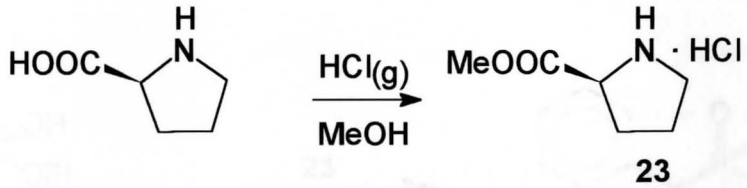
Monohydrolysis of the racemic diethyl ester **19** poses a question of the reaction's diastereoselectivity. Racemic starting materials were used during the imine formation and the addition of diethyl phosphite. As a result, the single chiral carbon gave two possible enantiomers, R and S, in a racemic mixture prior to hydrolysis. NMR analysis indicated that the two ethyl esters are non equivalent, i.e. diastereotopic. Hydrolysis of the racemic mixture creates a second chiral center at phosphorous affording two pairs of enatiomers, II & III and I & IV. One pair of enatiomers should be favored due to steric hindrance,

resulting in an enatiomeric excess. Structure elucidation by single crystal X-ray diffraction is in progress.



Although non chiral starting materials were used in the synthesis of **22**, studies conducted by Gilmore and McBride[15] using chiral substrates illustrated diastereoselectivity throughout the synthesis. Optically active alpha-amino phosphonic acids were obtained in multiple steps beginning with imine formation of benzaldehyde and either (R)-(+)- or (S)-(-)-alpha-methylbenzylamine. Next, the addition of diethyl phosphite and the subsequent hydrolysis of both esters followed by hydrogenation of the methylbenzyl group produced the chiral α-aminophosphonic acids. The levorotatory and the dextrorotatory enatiomers were obtained when the respective starting materials were utilized.

Phosphonopeptide synthesis began with the protection of L-proline. Literature methods used included those suggested by Greenstein[25] for general methylester protection and Erlander[26] for the protection of proline. Hydrogen chloride was bubbled into a methanol solution containing the L-proline. Upon removal of the methanol *en vacuo* the procedure was repeated. Moderate yields (47%) of the pure, protected amino acid **23** were obtained after flash column chromatography. Crystals were forced from the viscous oil at reduced pressure.

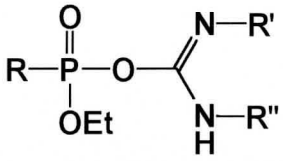


23

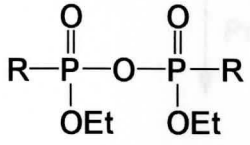
The protected proline **23** was then added to aminophosphonate monoester **22.** Initially this synthesis was attempted by standard peptide coupling techniques[27] using coupling reagents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). TLC and ^{31}P NMR indicated formation of two new products which were isolated by flash

column chromatography. The exact identity of the new products is still uncertain, however, ^{13}C, ^{31}P and ^{1}H NMR suggests one of these products may be phosphorous anhydride **25** and the other product may be the phosphonopeptide **26**.

Examination of similar couplings by Bartlett[28] suggested an alternative activated intermediate to the standard *O*-phosphorylisourea **24**. He proposed that the actual intermediate is a phosphorous anhydride **25**. The final product in the coupling would be identical except that the maximum yield of the phosphonopeptide would be 50%.

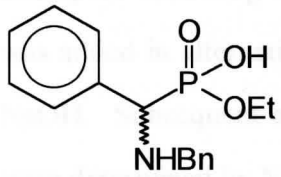


24

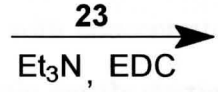


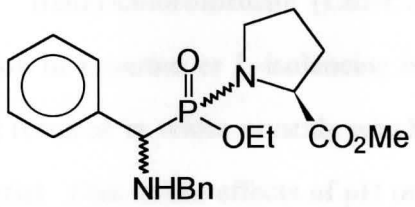
25

R=1-benzylamino-1-phenyl
R'=dimethylaminopropyl
R''=ethyl



22

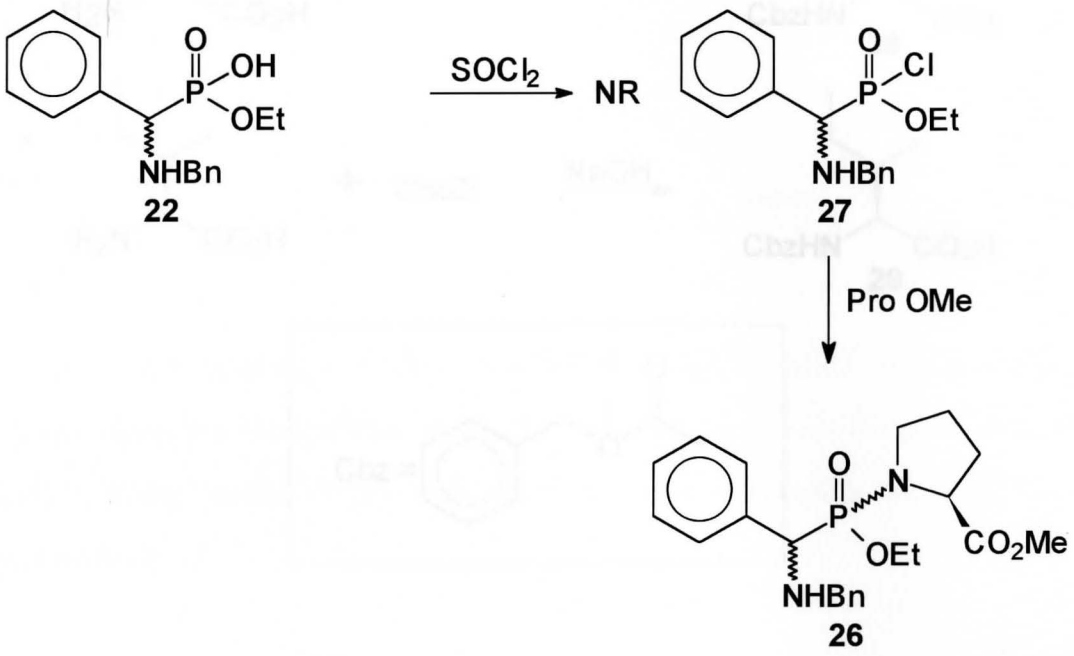




26

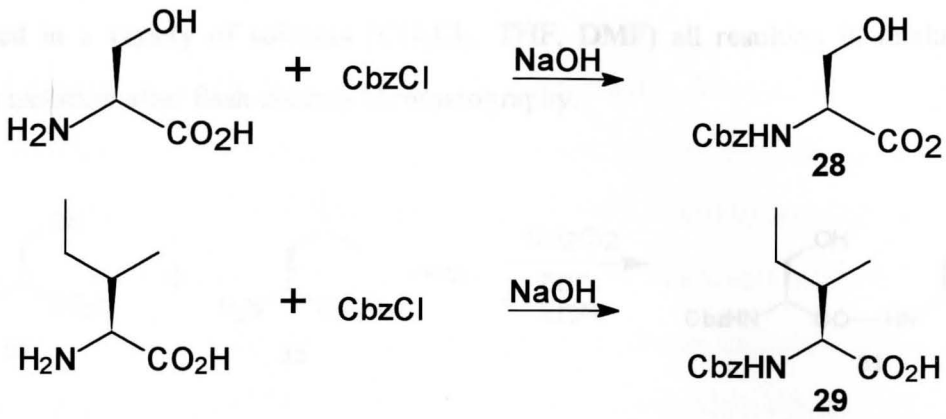
Another approach in the formation of the phosphonopeptide **26** involved the conversion of the aminophosphonic acid to the corresponding phosphonic acid chloride **27**

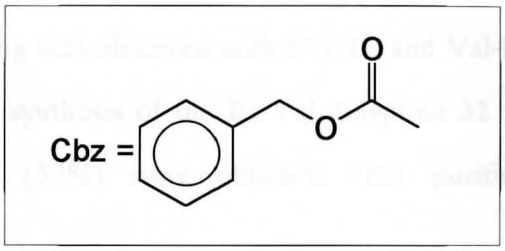
using thionyl chloride[28] or phosphorous pentachloride. This reaction was monitored by TLC and upon consumption of aminophosphonate **22**, the volatiles were removed by evaporation. The aminophosphonic acid chloride was stirred with proline methyl ester **(23)** in an attempt to form phosphonopeptide **26**.



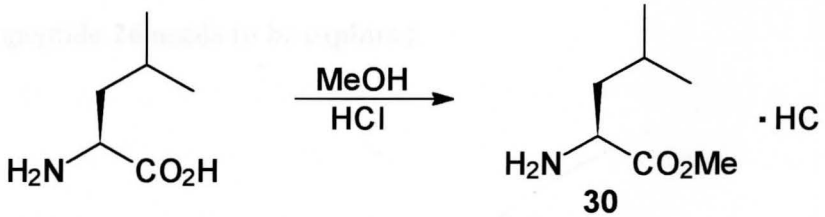
Side chain dipeptide formation began with the protection of the amino acids at their respective functional groups. The N terminal protection of serine and isoleucine was attempted following a procedure published by Fruton.[29] Benzylchloroformate (Cbz-Cl) was added in alternating aliquots with NaOH to a solution of L-serine or L-isoleucine in NaOH. Subsequent acidification with concentrated HCl resulted in white crystals which were determined by NMR to contain mostly starting material. Due to the effects of pH on the structure of amino acids and the pH fluctuations observed during the reaction, a buffer of saturated $NaHCO_3$ was used to dissolve the amino acid prior to the additions of Cbz-Cl. The products obtained after acidification were, in fact, the protected amino acids

(Cbz-Ser **28** and Cbz-Ile **29**) and recrystallization afforded the pure products in good yields (69% and 86% respectively).

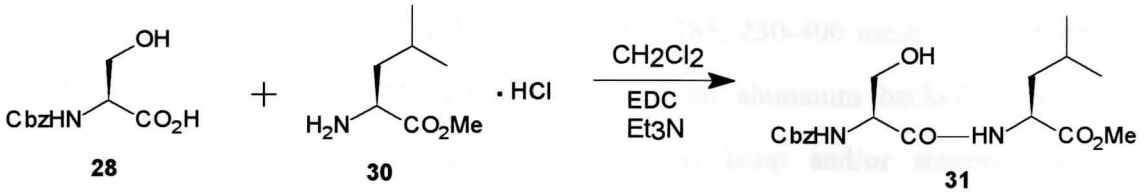




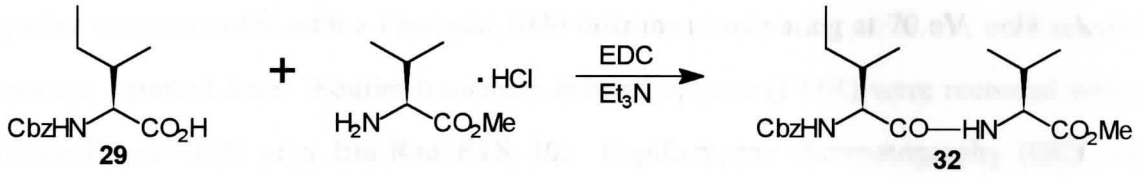
Carboxyl function protection of L-leucine as the methyl ester was performed by general amino acid protection techniques.[25,27] The saturation of a solution of the amino acid in methanol with hydrogen chloride, followed by removal of the solvent, afforded the protected product as an oil. The oil was dissolved in a minimal amount of the parent alcohol and forced to crystallize by the addition of diethyl ether. Pure, white crystals of leucine methyl ester hydrochloride (**30**) were obtained in excellent yields (90%).



A number of procedures were carried out in an attempt to couple the protected amino acids. For the Ser-Leu dipeptide **31**, coupling reagents such as 1,3-dicyclohexylcarbodiimide (DCC), 1,3-diisopropylcarbodiimide (DIPC), and EDC were evaluated in a variety of solvents (CH_2Cl_2, THF, DMF) all resulting in minimal to no peptide isolation after flash column chromatography.



Successful coupling was observed with Cbz-Ile and Val-MeO. The most effective coupling reagent for the synthesis of the Ile-Val dipeptide **32** was found to be EDC in CH_2Cl_2. Good yields (57%) were obtained after purification by flash column chromatography.



Successful synthesis of aminophosphonate **22** as a precursor to phosphonopeptide **26** has been completed. Further research is required to optimize the reaction conditions in the synthesis of **26**. In addition, coupling of the side chain dipeptide sequences **31** and **32** to phosphonopeptide **26** needs to be explored.

Chapter 3 Experimental

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 $CDCl_3$, CD_3OD, DMSO or D_2O as the solvent. The ^{1}H and ^{13}C chemical shifts are reported in parts per million downfield from $(CH_3)_4Si$, while ^{31}P chemical shifts are reported in parts per million downfield from H_3PO_4 (external standard). Coupling constants are reported in Hertz. Low resolution electron impact (EI) mass spectra were recorded with a Finnegan 1020 instrument operating at 70 eV; only selected ions are reported here. Fourier transform infrared spectra (FTIR) were recorded with a Perkin-Elmer 1600 or a Bio-Rad FTS 40. Capillary gas chromatography (GC) was performed on a 25m, HP-1 capillary column (column ID: 0.2 mm) at gas flow rates of 300 mL/min (air), 30 mL/min (hydrogen), and 15 mL/min (helium). Injector and detector temperatures were 275 °C, ramped oven temperatures of 50-250 °C at 20 °C/min were used.

Diethyl(1-oxo-2-phenylethyl)phosphonate (14). To phenylacetyl chloride (6.61 mL, 50 mmol) was added triethyl phosphite (8.57 mL, 50 mmol) dropwise via syringe. The initially exothermic reaction was heated at 70 °C overnight. Fractional distillation through a 10 cm vigreux column produced a yellow oil, 155-180 °C (0.800 mmHg), which

was further purified by flash column chromatography (100 g silica gel) eluted with ether:petroleum ether (50:50) yielding 3.2 g (12.5 mmol, 25%) of clear oil. Characterization by NMR and GC-MS indicated pure phosphonate **14**. ^{1}H NMR δ 1.29 (t, 6, J = 7.00), 3.79 (s, 2), 4.04-4.19 (m, 4), 7.27-7.36 (m, 5); ^{13}C NMR δ 15.47 (d, J = 6.14), 40.98, 62.62 (d, J = 4.63), 127.16, 128.36 (d, J = 7.64), 129.21 (d, J = 6.03), 133.61 (d, J = 26.65), 167.61; EIMS, *m/z* (relative intensity) 256 (M^+, 35), 228 (20), 118 (100), 91 (80), 65, (30).

Attempted Synthesis of Diethyl(1-benzylamino-2-phenylethyl)phosphonate (15). Method A. To a solution of diethyl (1-oxo-2-phenylethyl) phosphonate (**14**) (780 mg, 3.0 mmol) in 20 mL methanol was added benzylamine (0.60 mL, 5.5 mmol) at room temperature. After 4 h, sodium borohydride (163 mg, 3.6 mmol) was added in small portions. Vigorous effervescence was observed for 1 hour after which time the reaction was quenched with 25 mL 10% HCl and washed with diethyl ether (2 x 25 mL). The organic layers were then backwashed with 10% HCl (2 x 15 mL). The combined aqueous layers were neutralized with solid sodium carbonate and extracted with diethyl ether (3 x 15 mL). The organic layer was washed with brine and dried over anhydrous sodium sulfate. Purification of the crude mixture (610 mg) by flash column chromatography failed to produce **15**.

Method B. To phenylacetaldehyde (1.30 g, 10.0 mmol) over anhydrous potassium carbonate (1.0 g) was added benzylamine (1.1 mL, 10.0 mmol) at room temperature. The mixture became yellow with the evolution of heat and the reaction was complete after 30 minutes. The viscous mixture was dissolved in methylene chloride and gravity filtered to remove the potassium carbonate. The filtrate was concentrated *en vacuo* giving a crude yield of 1.89 g (9.0 mmol, 94%) for the imine **16**.

To the above imine **16** (1.05 g, 5.0 mmol) was added diethyl phosphite (1.3 mL, 10 mmol) in THF. The mixture was refluxed for 2.5 hours and then stirred overnight at room temperature. The THF was removed *en vacuo* and the residual oil dissolved in 25 mL diethyl ether and washed with water (3 x 50 mL). The organic fraction was concentrated *en vacuo* yielding 1.95 g crude. ^{1}H, ^{13}C and ^{31}P NMR indicated no coupling of the imine with phosphorous.

Diethyl(1-benzylamino-1-phenyl)phosphonate (19). To benzaldehyde (10.16 mL, 100 mmol) in diethyl ether (30 mL) over anhydrous sodium sulfate (10 g) was added benzylamine (10.92 mL, 100 mmol) at room temperature. The mixture became light yellow with the evolution of heat and the reaction was complete after 30 minutes. The mixture was gravity filtered to remove the sodium sulfate. The filtrate was concentrated *en vacuo* yielding 19.49 g, (100 mmol, 100%) of the imine **18**. ^{1}H NMR δ 4.71 (s, 2), 7.15-7.30 (m, 8), 7.71-7.73 (m, 2), 8.23 (s, 1); ^{13}C NMR δ 64.65, 126.64, 127.67, 127.99, 128.17, 128.25, 130.40, 136.02, 139.18, 161.41.

To the above imine **18** (19.49 g, 100 mmol) was added diethyl phosphite (25.76 mL, 200 mmol) in THF (25 mL). The mixture was refluxed overnight. The THF was removed *en vacuo* and the residual oil dissolved in 35 mL diethyl ether and washed with water (5 x 50 mL). The organic fraction was concentrated *en vacuo* yielding 30.99 g (93.0 mmol 93%) of the pure aminophosphonate **19**. ^{1}H NMR δ 1.06 (t, 3, J = 1.81), 1.23 (t, 3, J = 1.81), 2.61 (br s, 1), 3.65 (AB q, 2, J_1 =13.40, J_2 = 109.99), 3.73-3.83 (m, 1), 3.88-3.96 (m, 1), 4.01 (d, 1, J = 20.40), 4.02-4.13 (m, 2), 7.18-7.44 (m, 10); ^{13}C NMR δ 15.47 (d, J = 5.33), 15.68 (d, J = 5.33), 50.45 (d, J = 17.60), 58.19 (d, J = 153.38), 61.89 (d, J = 6.84), 62.08 (d, J = 6.84), 126.32, 127.11 (d, J = 3.02), 127.53, 127.58, 127.66 (d, J = 2.01), 127.96 (d, J = 6.03), 135.18, 138.63; ^{31}P NMR δ 24.55.

1-Benzylamino-1-phenylphosphonic acid (20). Method A. To aminophosphonate **19** (1.67 g, 5.0 mmol) was added concentrated HCl (50 mL). The mixture was refluxed for 48 hours at 130 °C with stirring. The solution was diluted with water (40 mL) and the mixture was evaporated to near dryness *en vacuo* at 60 °C. The white residue was dissolved in a minimal amount of ethanol and propylene oxide was added dropwise with good stirring until precipitation was complete. The mixture was filtered, washed with 100 mL ethanol and dried *en vacuo* yielding 1.21 g (4.36 mmol, 87%) phosphonic acid **20**, mp 236-238 °C. ^{1}H NMR δ 3.60-4.42 (br s, 1), 3.84 (d, 1, J = 16.80), 3.95 (AB q, 2, J_1 = 13.80, J_2 = 137.78), 7.33-7.40 (m, 10), 7.44-7.46 (m, 2); ^{13}C NMR δ 49.03 (d, J = 6.24), 59.53 (d, J = 133.57), 127.22, 127.81, 128.34, 129.13, 129.18, 129.78, 132.77, 134.58; ^{31}P NMR, δ 8.78.

Method B. To a solution of diethyl(1-benzylamino-1-phenyl)phosphonate (**19**) (670 mg, 2.0 mmol) in methylene chloride was added trimethylsilylbromide (0.90 mL, 6.8 mmol). The mixture was stirred for two hours at room temperature then methanol (5 mL) was added. This solution was stirred for 10 minutes, concentrated *en vacuo* and the addition of methanol repeated. TLC indicated no product formation.

Method C. To a solution of diethyl(1-benzylamino-1-phenyl)phosphonate (**19**) (670 mg, 2.0 mmol) in THF was added 1N NaOH (4.5 mL, 4.4 mmol) dropwise with stirring. The mixture was stirred overnight at room temperature then diluted with water (20 mL) and acidified with HCl to pH 6. The solution was then extracted with diethyl ether (2 x 25 mL), dried over sodium sulfate and evaporated *en vacuo* yielding no product.

Ethyl(1-benzylamino-1-phenyl)phosphonic acid (22). To the amino phosphonate **19** (3.33 g, 10.0 mmol) in methanol (40 mL) was added 8N KOH (25 mL).

The solution was refluxed overnight, diluted with water (50 mL) and washed with CH_2Cl_2 (25 mL). The aqueous layer was acidified to pH 1 with concentrated HCl and extracted with CH_2Cl_2 (3 x 45 mL). The organic layer was dried over sodium sulfate, filtered and evaporated at reduced pressure yielding 3.00 g (9.82 mmol, 98%). The white crystals (mp 70-74 °C) were evaluated by NMR revealing pure aminophosphonic acid monoester **22**, mp 70-74 °C. ^{1}H NMR δ 1.10 (t, 3, J = 1.71), 3.68 (ddq, 1, J_1 = 7.20, J_2 = 7.60, J_3 = 10.07), 3.77 (ddq, 1, J_1 = 7.20, J_2 = 7.60, J_3 = 10.07), 4.19 (AB q, 2, J = 50.59), 4.27 (d, 1, J = 16.40), 7.42-7.46 (m, 11), 7.53-7.55 (m, 1); ^{13}C NMR δ 16.91 (d, J = 6.04), 51.33 (d, J = 6.04), 60.54 (d, J = 140.41), 63.20 (d, J = 6.84), 130.10, 130.15, 130.21 (d, J = 1.01), 130.61, 130.65 (d, J = 2.01), 131.35, 131.94, 132.73 (d, J = 4.53); ^{31}P NMR δ 10.76.

Proline methyl ester hydrochloride (23). To a solution of L-proline (11.5 g, 100 mmol) in methanol (125 mL) was added hydrogen chloride gas for 20 minutes. The solution was concentrated *en vacuo* and the prior procedure repeated twice. The residual oil crystallized under high vacuum and purified by flash column chromatography (100 g silica, eluted with a gradient). Final yield 7.75 g (46.8 mmol, 47%) of the protected amino acid **19**. ^{1}H NMR δ 2.07-2.26 (m, 3), 2.38-2.51 (m, 1), 3.41-3.68 (m,2), 3.85 (s, 3), 4.49-4.57 (m, 1), 9.30 (br s, 1); ^{13}C NMR δ 23.59, 28.63, 45.80, 53.42, 59.15, 169.01.

Phosphonopeptide (26). Method A. To a solution of the aminophosphonic acid mono ester **22** (310 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) was added L-proline methyl ester hydrochloride (**23**) (170 mg, 1.0 mmol) and EDC (210 mg, 1.1 mmol). The mixture was stirred until completely dissolved then Et_3N (0.31 mL, 2.2 mmol) was added to the solution with almost immediate formation of a white precipitate. The reaction was stirred overnight and TLC indicated formation of at least three new products. The mixture was diluted with 150 mL EtOAc 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 over anhydrous sodium sulfate, filtered and evaporated *en vacuo*. The residue was dissolved in a minimal amount of CH_2Cl_2, applied to 15 g silica and eluted with a solvent gradient from diethyl ether to methanol. NMR analysis indicated possible formation of the corresponding phosphorous anhydride **25**.

Method B. To a solution of the aminophosphonic acid **22** (310 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) was added thionyl chloride (0.1 mL, 1.3 mmol) with stirring. The mixture was allowed to stir for four hours at rt, then all volatile materials were removed first by a stream of argon gas and then *en vacuo*. To this residue was added L-proline methyl ester hydrochloride (**23**) (170 mg, 1.0 mmol) with Et_3N (0.15 mL, 1.1 mmol) in CH_2Cl_2 (5 mL). This mixture was allowed to stir for two days at room temperature. Flash column chromatography was performed on 25 g silica and eluted with a gradient from petroleum ether:diethylether to methanol. ^1H, ^{13}C and ^{31}P NMR indicated the formation of new products, but assignment of the spectra was inconclusive.

Carboxybenzyl L-serine (28). Method A. To a solution of L-serine (10.65 g, 100 mmol) in 4 M NaOH (25 mL, 100 mmol) at 0 °C was added via an addition funnel, 4 M NaOH (30 mL, 120 mmol) and benzylchloroformate (16 mL, 110 mmol) alternately in 6 mL and 4 mL aliquots respectively with stirring for 30 minutes. The white solution was extracted with diethyl ether (2 x 30 mL) and the aqueous fraction was acidified with 5M HCl to pH 3. The white precipitate filtered, dried and recrystalized from chloroform yielding 3.58 g (12.9 mmol, 13%).

Method B. The use of saturated sodium bicarbonate to initially dissolve the L-serine allowed the pH to be maintained at 10 without the addition of sodium hydroxide to the reaction mixture. Yields were increased to 16.55 g (69.2 mmol, 69 %), mp 116-118 °

C. ^{1}H NMR δ 3.20-4.22(br s, 2), 3.64-3.66 (m, 2), 4.04-4.60 (m, 1), 5.02 (s, 2), 6.85-6.89 (m, 1), 7.30-7.35 (m, 5); ^{13}C NMR δ 57.22, 61.93, 66.05, 128.25, 128.33, 128.88, 137.54, 156.59, 172.61.

Carbobenzoxy isoleucine (29). To a solution of L-isoleucine (1.32 g, 10.0 mmol) in 1/4 saturated sodium bicarbonate (50 mL) was added benzylchloroformate (1.6 mL, 11.0 mmol). The solution was maintained at pH 10 with the addition of sodium hydroxide. The mixture was then washed with ether (2 x 40 mL) and the aqueous fraction acidified to pH 3 with 5M HCl. The aqueous solution was then extracted with ethyl acetate (2 x 30 mL) and evaporated *en vacuo* yielding 2.27 g (8.6 mmol, 86%) of the protected amino acid **29**. ^{1}H NMR δ 0.88-0.95 (m, 6), 1.13-1.26 (m, 2), 1.41-1.52 (m, 1), 1.88-1.97 (m, 1), 4.36-4.39 (m, 1), 5.10-5.11 (m, 2), 7.20-7.38 (m, 5), 9.21 (br s, 1); ^{13}C NMR δ 11.41, 15.27, 24.74, 37.69, 58.18, 66.96, 127.91, 127.99, 128.34, 136.09, 156.29, 175.45.

L-leucine methylester hydrochloride (30). To a solution of L-leucine (10.62 g , 81.0 mmol) in methanol (250 mL) was added HCl gas until all leucine had dissolved. At this point the reaction vessel was placed in a ice bath with the continued addition of hydrogen chloride for 20 min. The solution was then stirred overnight at room temperature followed by degassing by vacuum aspirator and removal *en vacuo* of residual methanol maintaining a temperature below 50 °C. The residual yellow/brown oil was dissolved in 30 mL ethanol and tirturated with 300 mL diethyl ether. The white crystals were vacuum filtered and dried *en vacuo*. The remaining white solid weighed 13.3 g (90%) with a melting point range determined to be 145-149 °C. ^{1}H NMR δ 0.93 (d, 6), 1.78 (m, 3), 3.79 (s, 3), 3.88 (m, 1), 8.81 (br s, 2); ^{13}C NMR δ 22.11, 22.20, 23.86, 39.18, 50.72, 52.47, 170.10.

Attempted Synthesis of Cbz Serine-Leucine methyl ester dipeptide (31). To a solution of Cbz serine (**28**) (1.42 g, 5.1 mmol) and leucine methyl ester hydrochloride (**30**) (920 mg, 5.1 mmol) was added DCC (1.14 g ,5.5 mmol) and triethylamine (0.80 mL, 5.7 mmol). The mixture was stirred at room temperature overnight. Flash column chromatography resulted in recovery of starting materials.

Valine methyl ester-Cbz Isoleucine dipeptide (32). To a solution of L-valine methyl ester hydrochloride (251 mg, 1.5 mmol) and Cbz-isoleucine (**29**) (400 mg, 1.5 mmol) in methylene chloride (35 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (327 mg, 1.7 mmol) and triethylamine (0.45 mL, 3.0 mmol). After stirring overnight at room temperature, the solution was extracted into diethyl ether (30 mL) and washed with water (2 x 50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated *en vacuo* yielding a white solid (500 mg, 1.4 mmol, 92%). This crude product was purified by flash column chromatography (20 g silica, ethyl acetate) affording white crystals of Val MeO-Cbz Ile (**32**) (311 mg, 0.86 mmol, 57%). ^{1}H NMR δ 0.87-1.03 (m, 12), 1.14-1.22 (m, 2), 1.50-1.59 (m, 1), 1.74-1.83 (m, 1), 2.09-2.20 (m, 1), 3.66-3.71 (s, 3), 4.06-4.08 (m, 1), 4.34-4.35 (m, 1), 5.08-5.12 (m, 2), 7.27-7.35 (m, 5), 8.23-8.27 (m, 1); ^{13}C NMR δ 11.25, 15.82, 18.51, 19.37, 25.82, 31.70, 38.08, 52.32, 59.08, 60.84, 67.62, 128.70, 128.90, 129.37, 138.25, 158.43, 173.24, 174.40.

References

(1) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375.

(2) a) Bartlett, P. A.; Kezer, W. B. *J. Am. Chem. Soc.* **1984**, *106*, 4282. b) Sampson, N. S.; Bartlett, P. A. *Biochemistry* **1991**, *30*, 2255. c) Bartlett, P. A.; Archer, F. *Bulletin De La Societe Chimique De France* **1986**, *5*, 771. d) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618. e) Jacobson, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654.

(3) Cheng, L; Goodwin, C. A.; Scully, M. F.; Kakkar, V. V.; Claeson, G. *Tetrahedron Letters* **1991**, *32*, 7333.

(4) Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E. *Tetrahedron Letters* **1990**, *39*, 5587.

(5) Bartlett, P. A.; Hanson, J. E.; Giannousis, P. P. *J. Org. Chem.* **1990**, *55*, 6268.

(6) Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 6612.

(7) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schmeider, J.; Keny, S. B. H. *Science* **1989**, *245*, 616.

(8) Hyland, L. J.; Tomaszek, T. A.; Roberts, G. D.; Carr, S. A.; Magaard, V. W.; Bryan, H. L.; Fakhoury, S. A.; Moore, M. L.; Minnich, M. D.; Culp, J. S.; DesJarlais, R. L.; Meek, T. D. *Biochemistry* **1991**, *30*, 8441.

(9) a) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10. b) Wolfenden, R. *Ann. Rev. Biophys. Bioeng.* **1976**, *5*, 271.

(10) Rich, D. H.; Green, J.; Toth, M. V.; Marshall, G. R.; Kent, S. B. H. *J. Med. Chem.* **1990**, *33*, 1288.

(11) Parkes, K. E. B.; Bushnell, D. J.; Crackett, P. H.; Dunsdon, S. J.; Freeman, A. C.; Gunn, M. P.; Hopkins, R. A.; Lambert, R. W.; Martin, J. A.; Merrett, J. H.; Redshaw, S.; Spurden, W. C.; Thomas, G. J. *J. Org. Chem.* **1994**, *59*, 3656.

(12) a) Schellenberg, K. A. *J. Org. Chem.* **1963**, *28*, 3259. b) Billman, J. H.; Diesing, A. C. *J. Org. Chem.* **1957**, *22*, 1068.

(13) a) Fields, E. K. *J. Am. Chem. Soc.* **1952**, *74*, 1528. b) Wagner, E. C. *J. Org. Chem.* **1954**, *19*, 1862.

(14) Patai, S. In *The Chemistry of the Carbonyl Group*, John Wiley & Sons Inc., **1966**.

(15) Gilmore, W. F.; McBride, H. A. *J. Am. Chem. Soc.* **1972**, *94*, 4361.

(16) Glowiak, T.; Sawka-Dobrowolska, W. *Tetrahedron Letters* **1977**, *45*, 3968.

(17) Ladd, E. C.; Harvey, M. P. *Canadian Patent* **1955**, 509,034.

(18) Kotynski, A.; Stec, W. J. *J. Chem. Research* **1978**, 41.

(19) Yuan, C.; Wang, G.; Chen, S. *Synthesis* **1990**, 522.

(20) Sjoberg, K. *Aldrichimica Acta* **1980**, *13*, 55.

(21) Chambers, J. R.; Isbell, A. F. *J. Org. Chem.* **1964**, *29*, 832.

(22) Hoffman, M. *Polish J. Chem.* **1978**, *52*, 851.

(23) Rudinskas, A. J.; Hullar, T. L. *J. Med. Chem.* **1976**, *19*, 1367.

(24) McKenna, C. E.; Higa, M. T.;Chueng, N. H.; McKenna, M. C. *Tetrahedron Letters* **1977**, *2*, 155.

(25) Greenstein, J. P.; Winitz, M., In *Chemistry of the Amino Acids*; John Wiley & Sons, Inc.,**1961**; vol. 2.

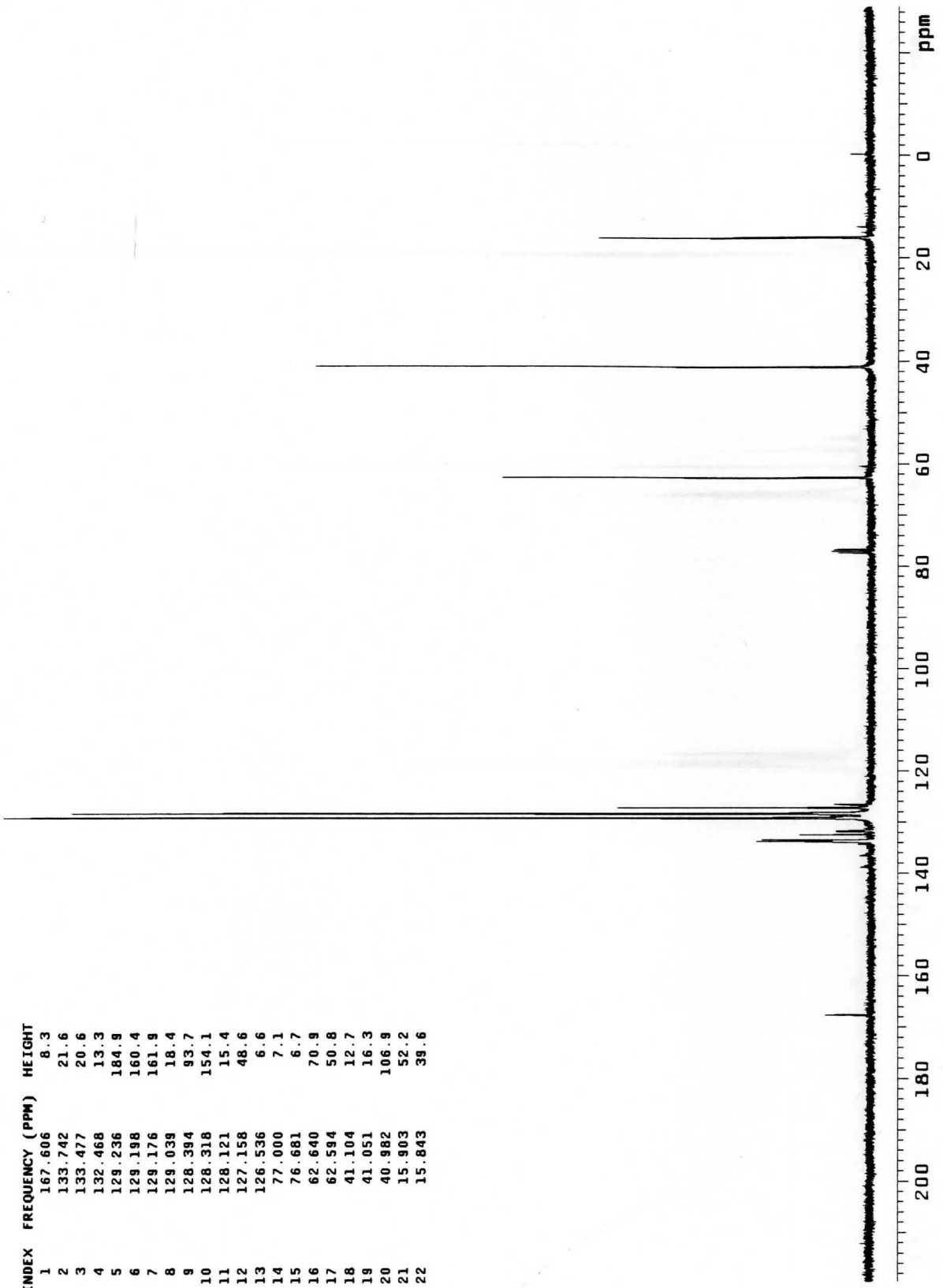
(26) Erlanger, B. F.; Sach, H.; Brand, E. *J. Am. Chem. Soc.* **1954**, *76*, 1806.

(27) Bodanszky, M.; Bodanszky, A., In *The Practice of Peptide Synthesis*; Springer-Verlag, **1984**.

(28) Bartlett, P. A.; Hanson, J. E.; Giannousis, P. P. *J. Org. Chem.* **1990**, *55*, 6268.

(29) Fruton, J. S. *J. Bio. Chem.* **1942**, *146*, 463.

Figure 6 ^{13}C NMR of Compound 14



INDEX	FREQUENCY (PPM)	HEIGHT
1	167.606	8.3
2	133.742	21.6
3	133.477	20.6
4	132.468	13.3
5	129.236	184.9
6	129.198	160.4
7	129.176	161.9
8	129.039	18.4
9	128.394	93.7
10	128.318	154.1
11	128.121	15.4
12	127.158	48.6
13	126.536	6.6
14	77.000	7.1
15	76.681	6.7
16	62.640	70.9
17	62.594	50.8
18	41.104	12.7
19	41.051	16.3
20	40.982	106.9
21	15.903	52.2
22	15.843	39.6

Figure 7 ^{1}**H NMR of Compound 14**

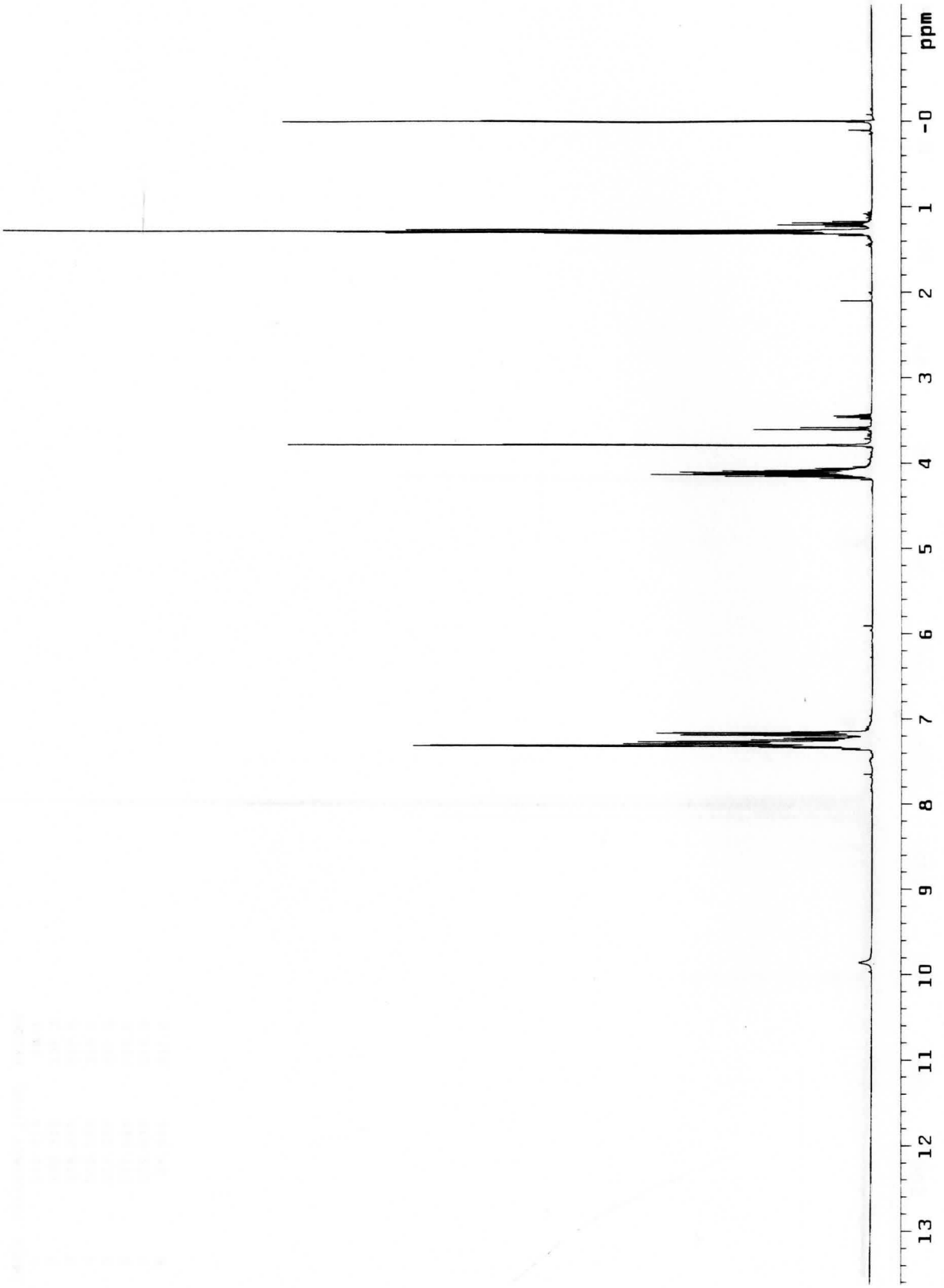
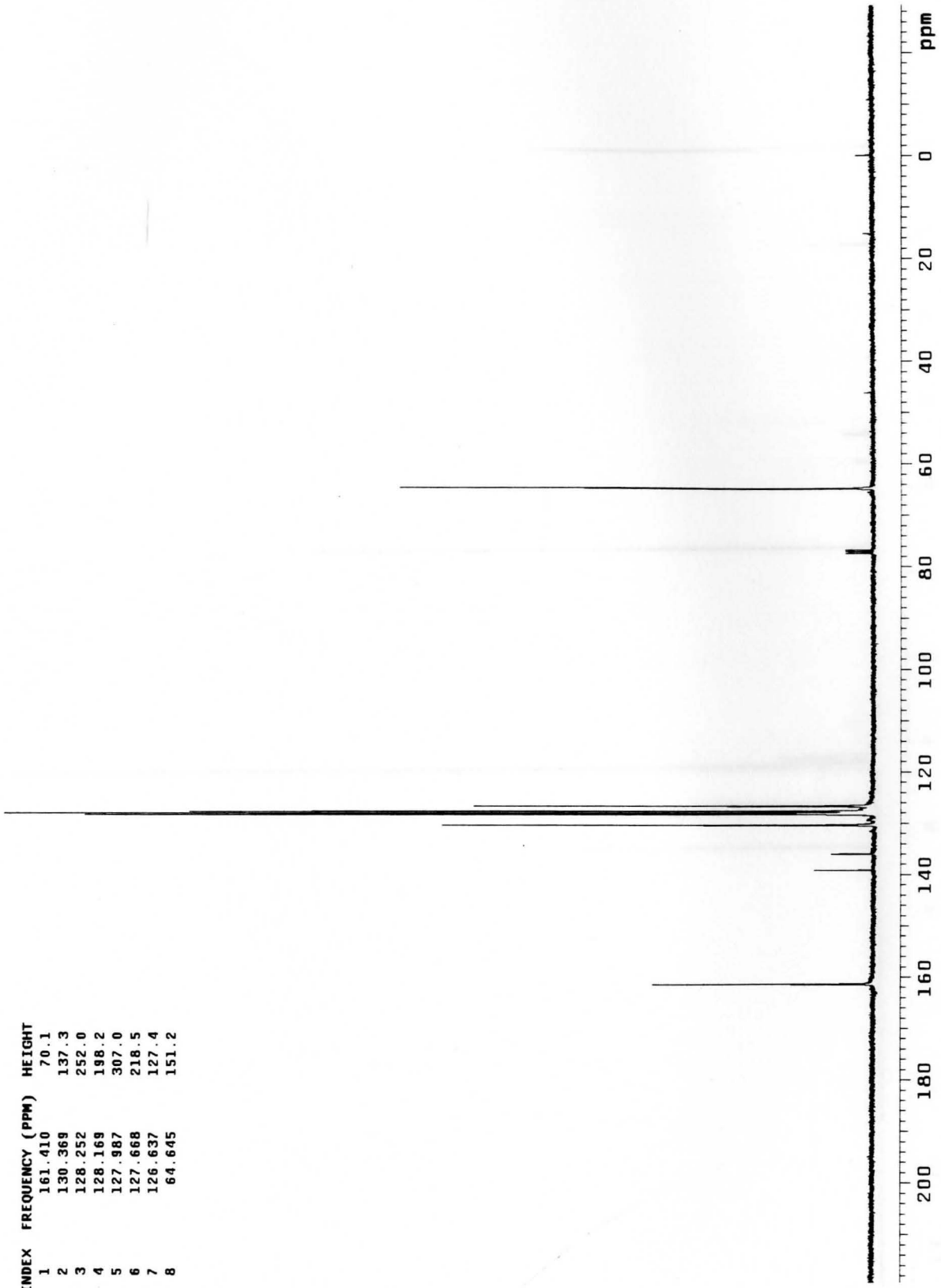
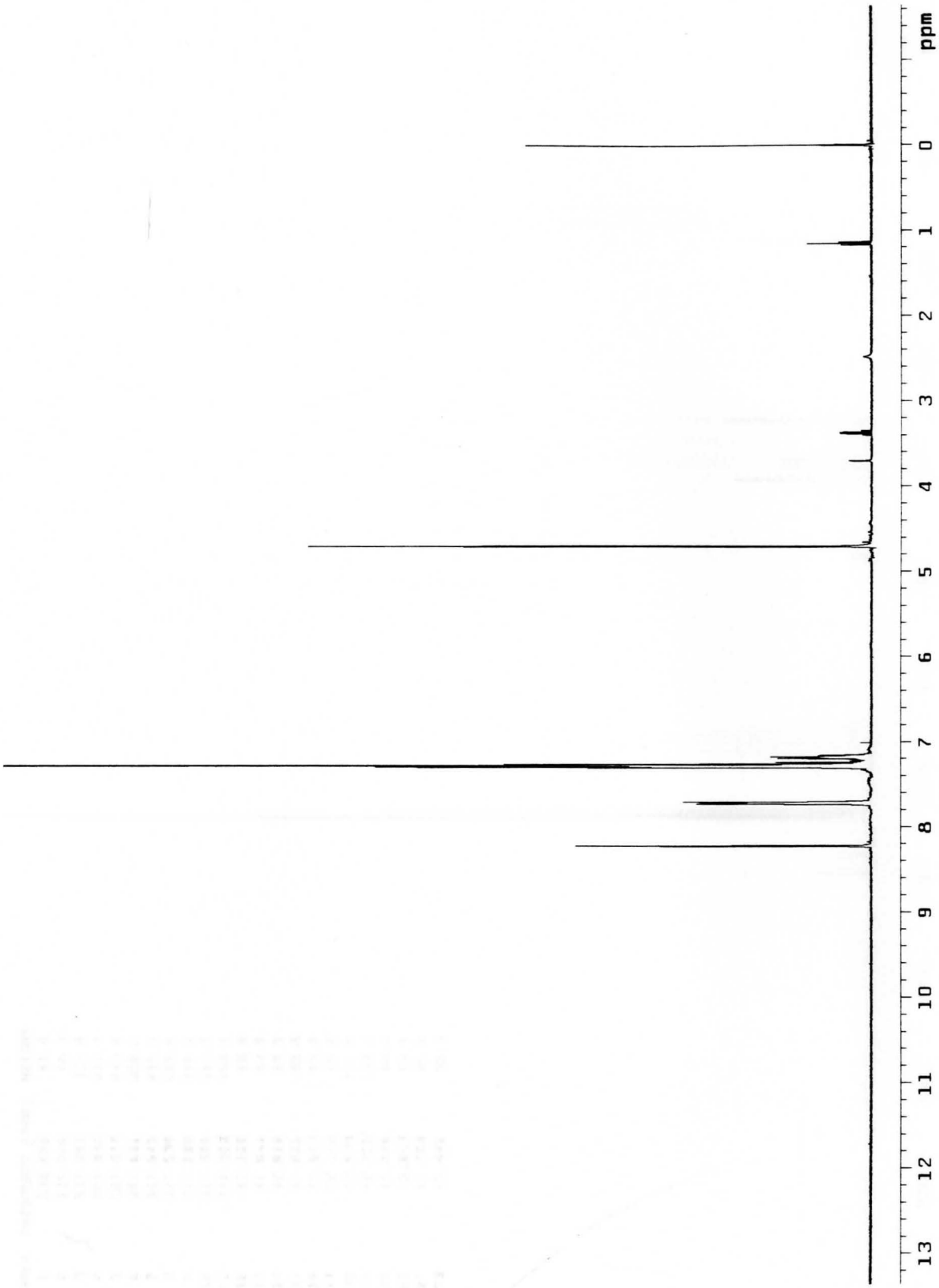


Figure 8 ^{13}C **NMR of Compound 18**



INDEX	FREQUENCY (PPM)	HEIGHT
1	161.410	70.1
2	130.369	137.3
3	128.252	252.0
4	128.169	198.2
5	127.987	307.0
6	127.668	218.5
7	126.637	127.4
8	64.645	151.2

Figure 9 ^{1}H NMR of Compound **18**



INDEX	FREQUENCY (PPM)	HEIGHT
1	138.628	41.0
2	135.176	28.3
3	127.992	434.0
4	127.932	437.4
5	127.674	445.9
6	127.651	380.2
7	127.575	695.5
8	127.530	595.6
9	127.120	200.1
10	127.090	162.7
11	126.323	356.2
12	62.109	98.0
13	62.041	83.0
14	61.919	99.5
15	61.851	88.8
16	59.674	69.0
17	58.149	68.8
18	50.533	106.0
19	50.358	104.3
20	15.706	84.4
21	15.653	85.1
22	15.494	95.2
23	15.441	81.3

Figure 10 ^{13}C NMR of Compound 19

Figure 11 ^{1}H NMR of Compound 19

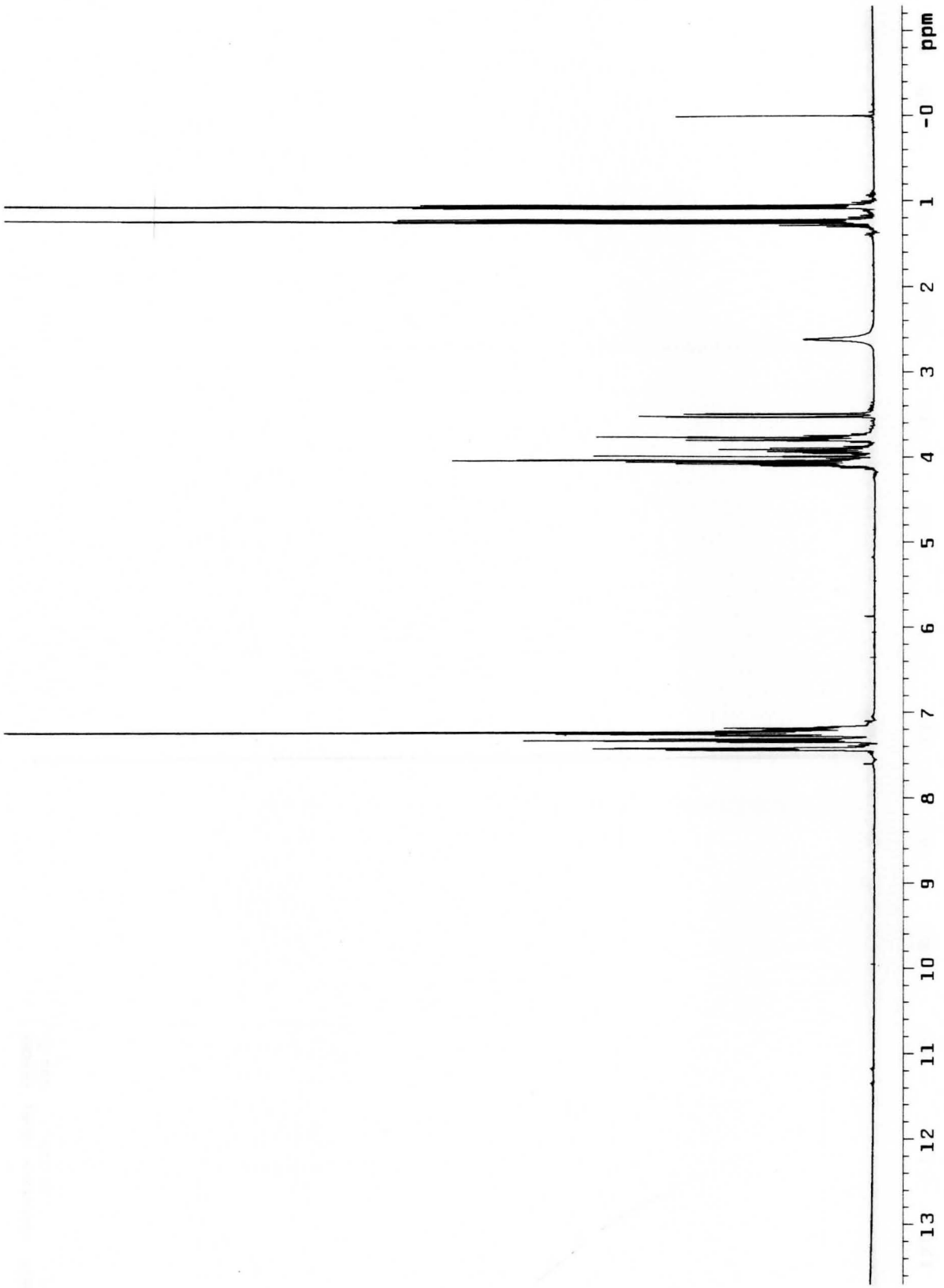
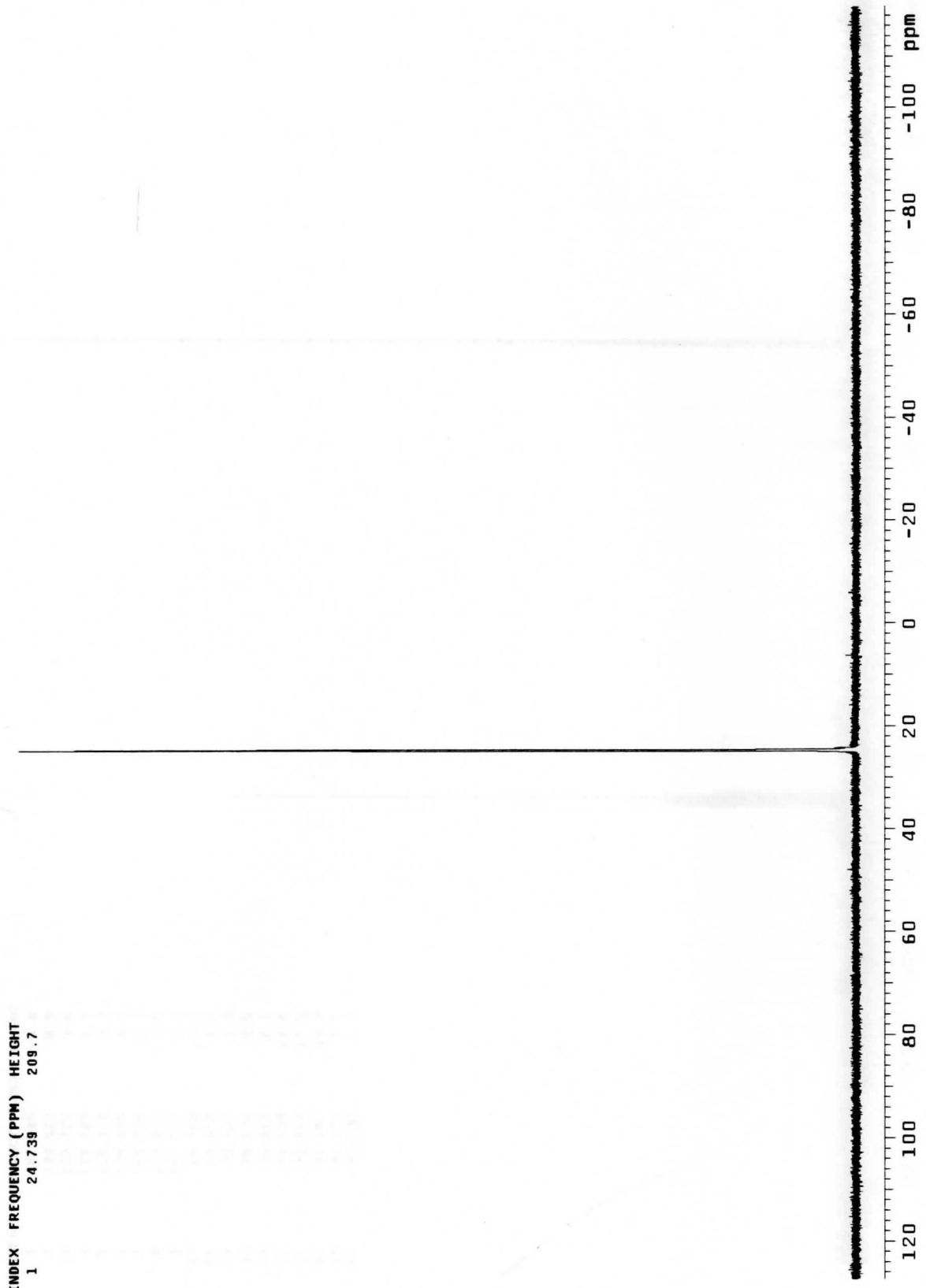


Figure 12 ^{31}P NMR of Compound 19



INDEX	FREQUENCY (PPM)	HEIGHT
1	202.865	-0.6
2	134.577	0.8
3	132.772	0.6
4	129.783	6.2
5	129.176	4.4
6	129.130	4.0
7	128.235	11.3
8	127.811	6.1
9	127.219	2.5
10	60.198	1.3
11	58.870	1.2
12	49.001	1.3
13	40.126	2.5
14	39.913	7.1
15	39.709	14.8
16	39.504	17.3
17	39.291	15.3
18	39.087	7.7
19	38.867	2.6

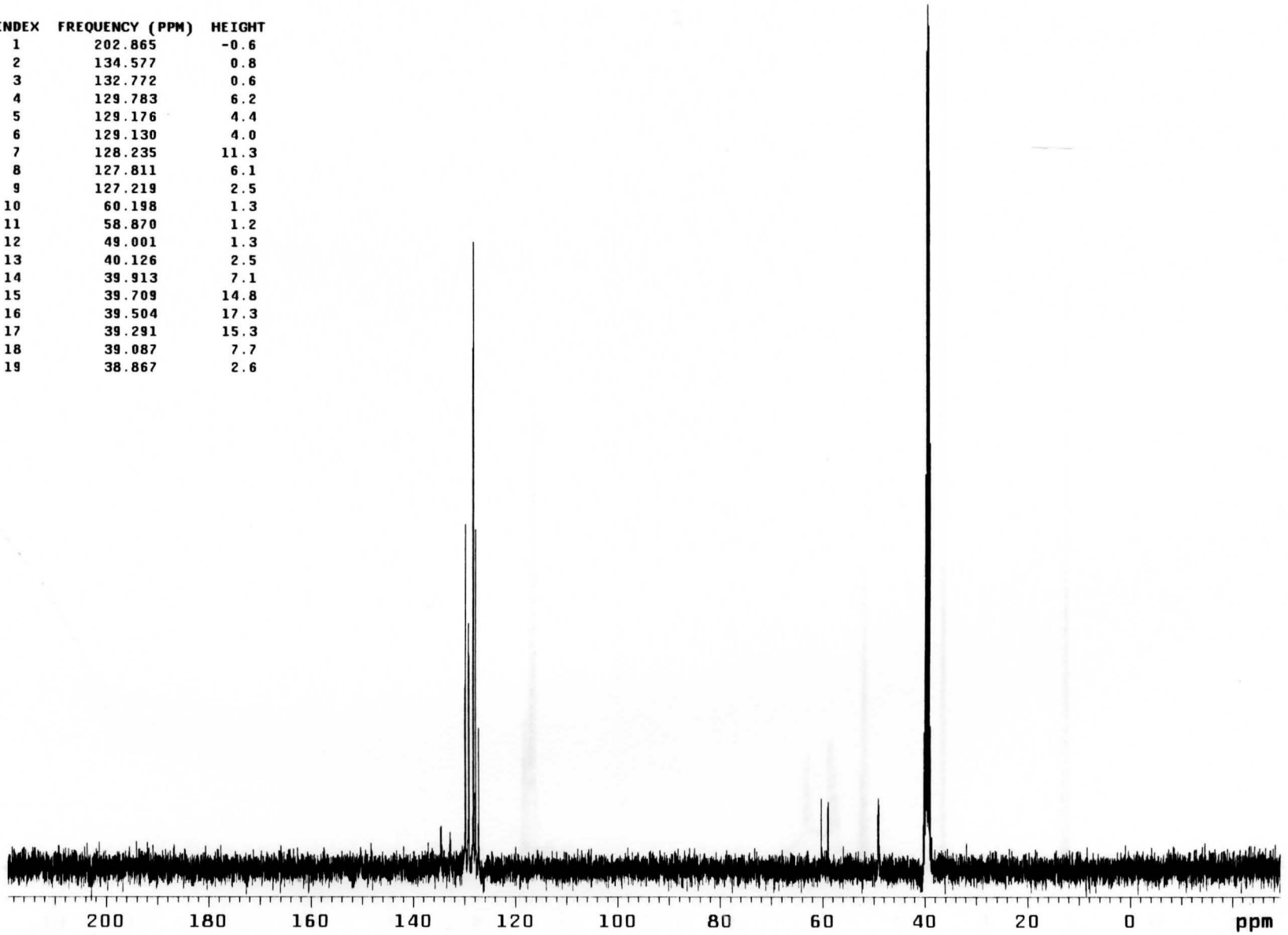


Figure 13 ^{13}C NMR of Compound **20**

Figure 14 ^{1}H NMR of Compound 20

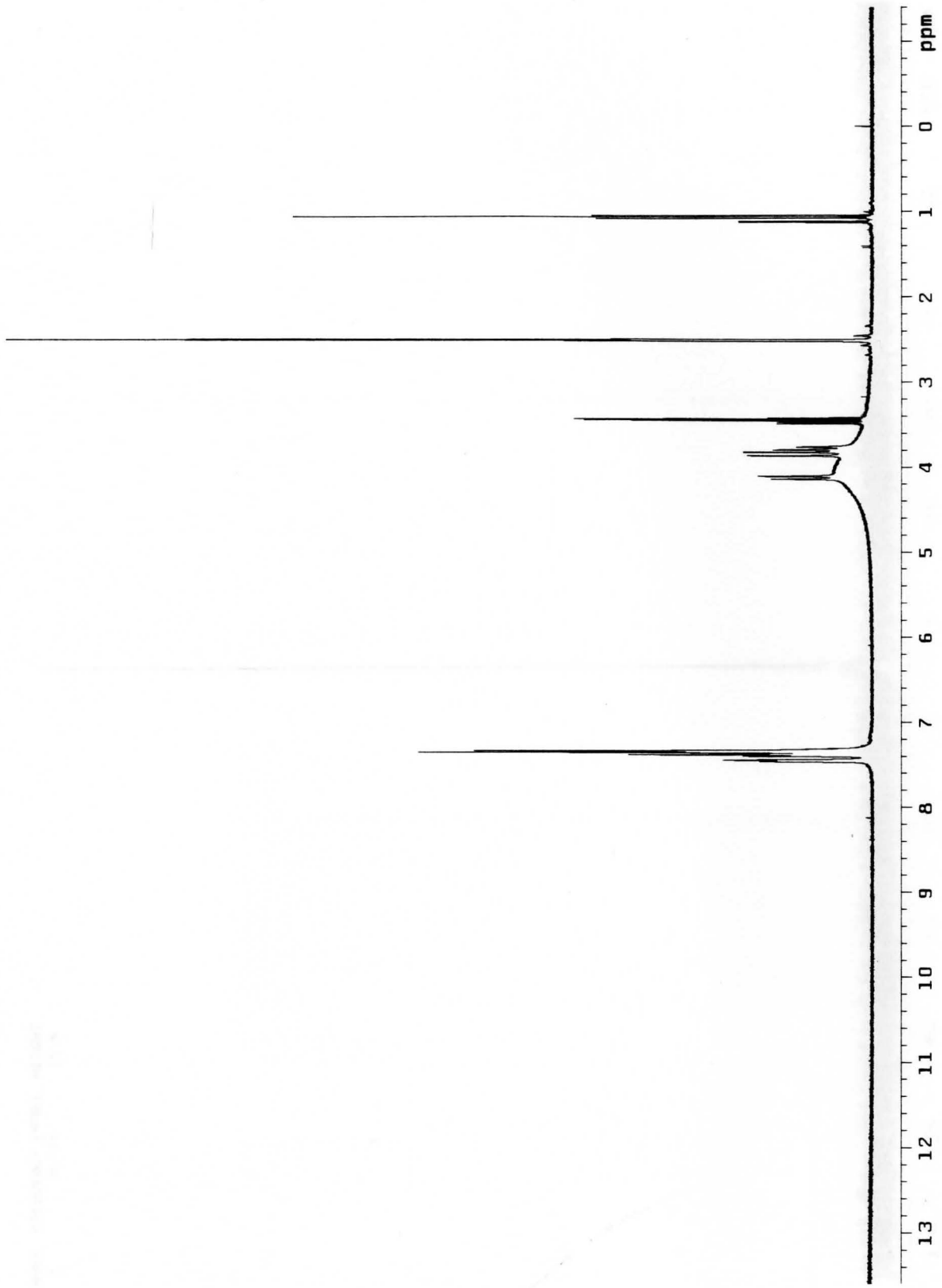
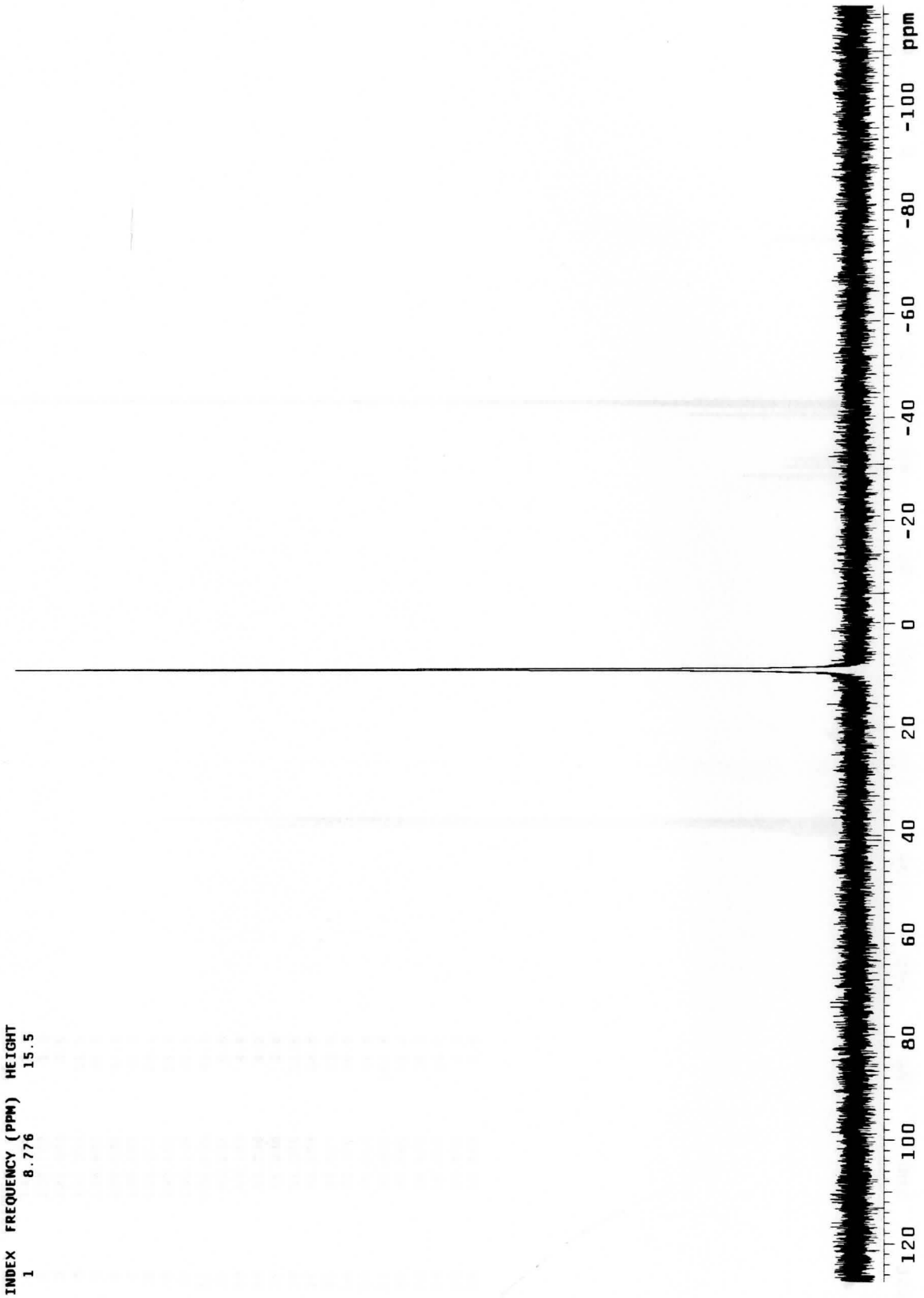


Figure 15 ^{31}P NMR of Compound **20**



INDEX	FREQUENCY (PPM)	HEIGHT
1	132.755	5.0
2	132.710	5.3
3	131.944	8.5
4	131.352	51.5
5	130.662	46.5
6	130.639	47.6
7	130.609	40.0
8	130.214	26.1
9	130.199	26.4
10	130.146	61.4
11	130.055	49.2
12	63.231	8.8
13	63.163	9.9
14	61.236	6.2
15	59.840	6.0
16	51.359	12.5
17	51.299	14.6
18	49.637	13.5
19	49.425	46.0
20	49.212	97.8
21	49.000	106.4
22	48.788	81.8
23	48.575	38.3
24	48.355	14.0
25	16.942	7.3
26	16.882	7.4

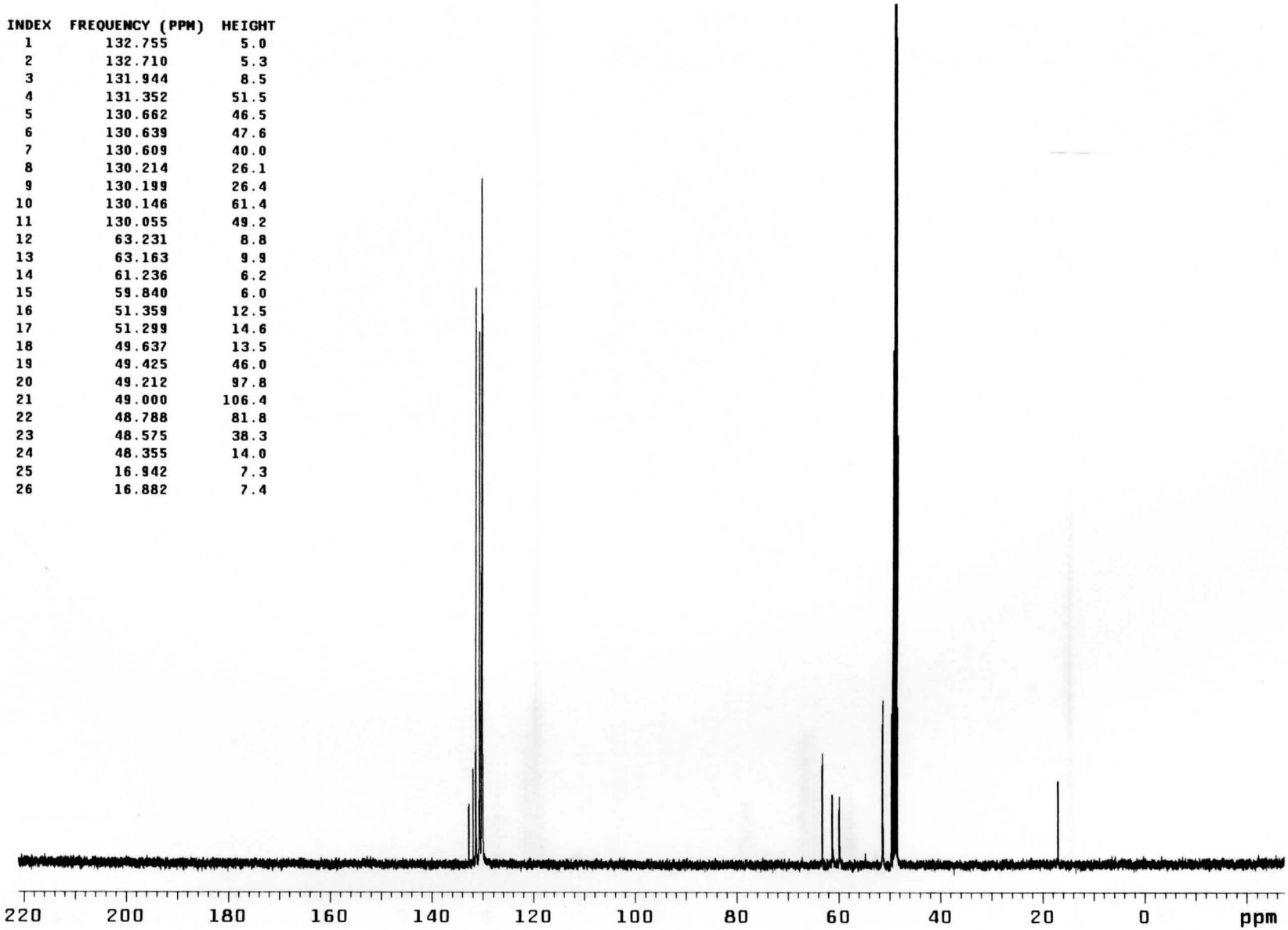


Figure 16 ^{13}C NMR of Compound **22**

Figure 17 ^{1}H NMR of Compound 22

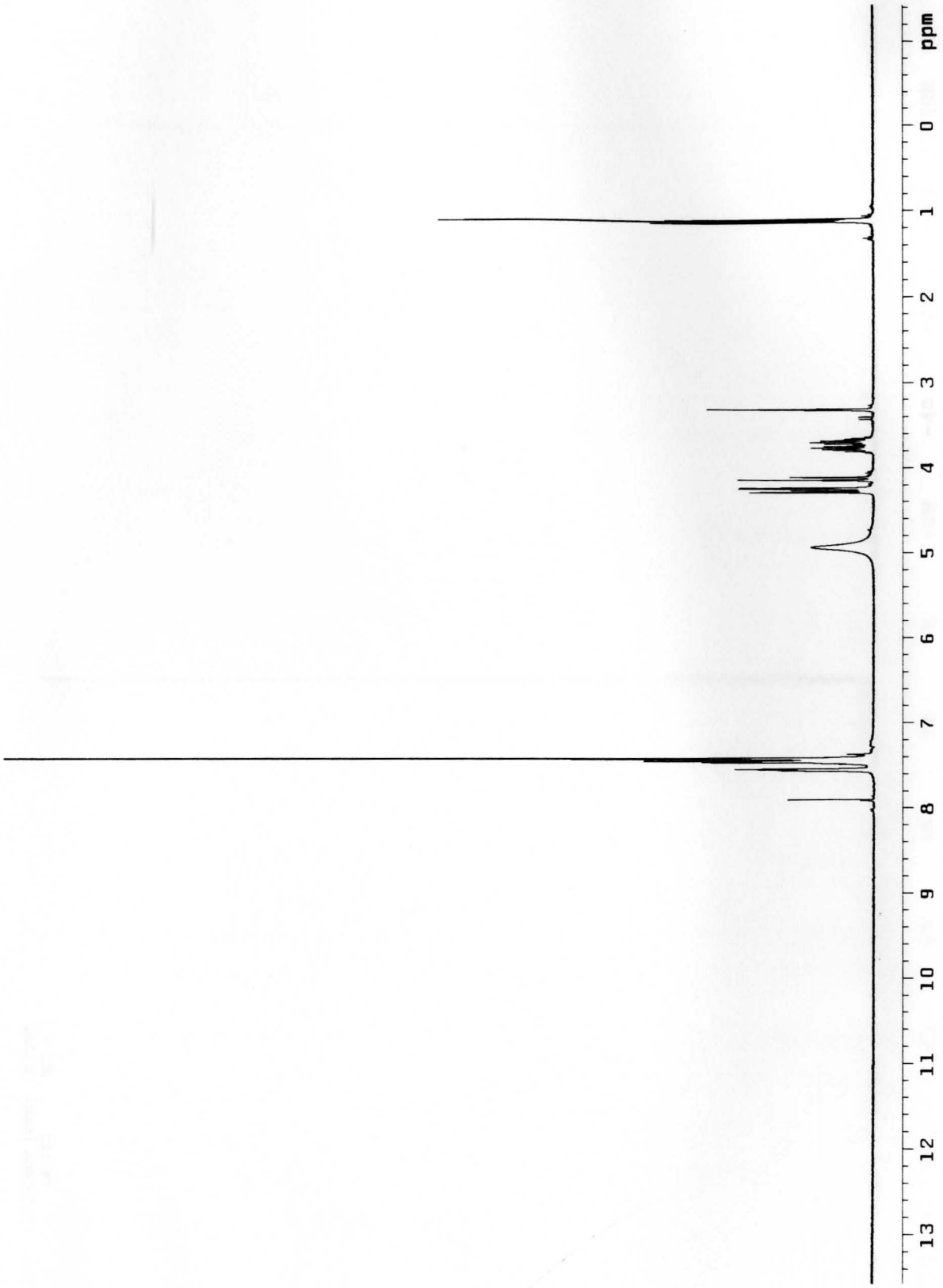


Figure 18 ^{31}P **NMR of Compound 22**

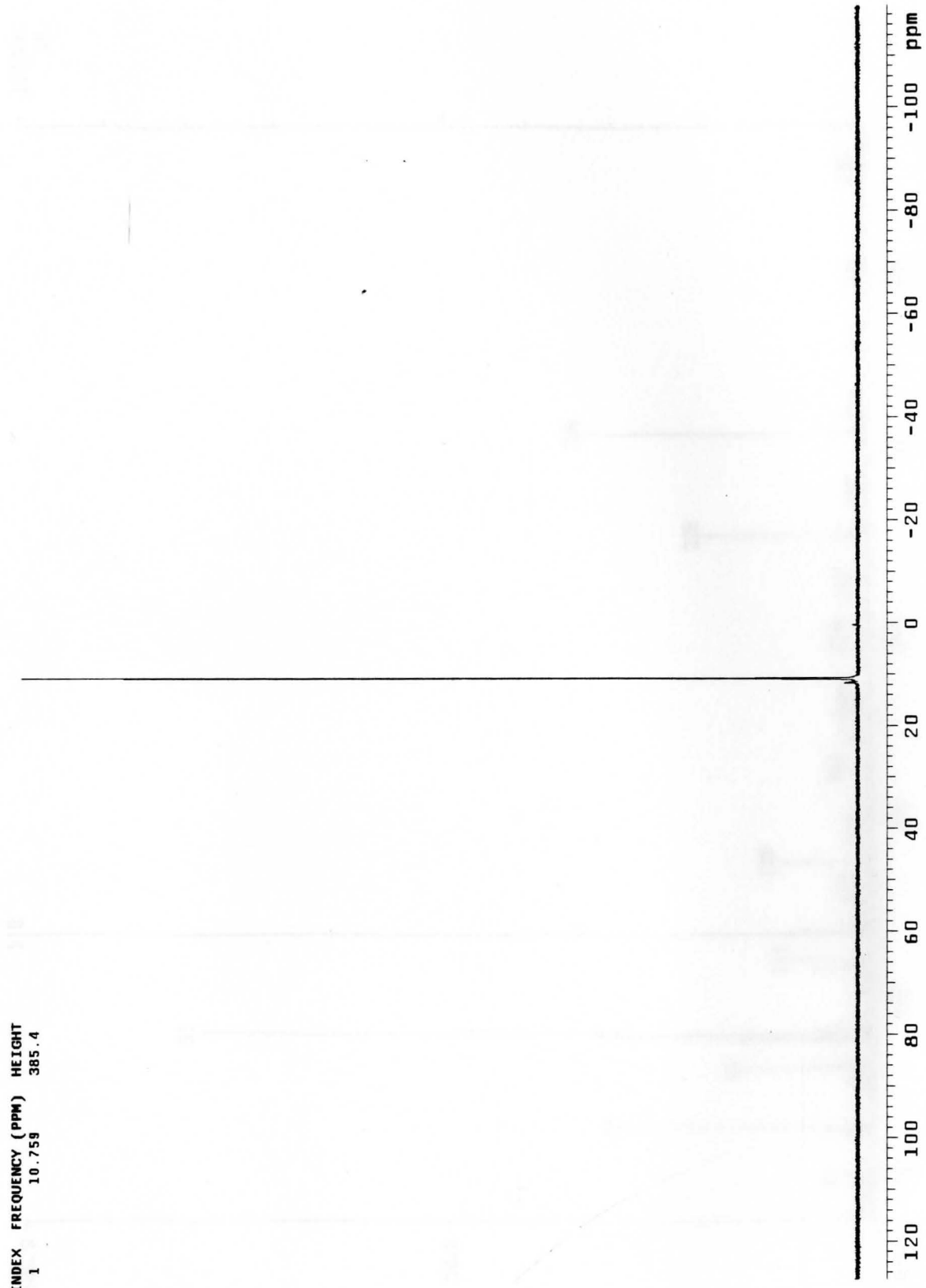


Figure 19 EIMS of Compound 14