Bis (2,2,2-trifluoroethoxy) Phosphorochloridate: A New Method for the Synthesis of Peptides

by

Russell A. Bowman

Submitted in Fulfillment of the Requirements

for the Degree of

Masters of Science

in the

Chemistry

Program

Youngstown State University

August 1996

Bis (2,2,2-trifluoroethoxy) Phosphorochloridate:

A New Method

For The

Synthesis

of

Peptides

Russell A. Bowman

I hereby release this thesis to the public. I understand this thesis will be housed at the Circulation Desk of the University library and will be available for public access. I also authorize the University or other individuals to make copies of this thesis as needed for scholarly research.

Signature:

Russell A. Bowman — 8/5/96 Date

Approvals:

Dr. John A. Jackson — 8/5/96 Date
Thesis Advisor

Dr. Daryl Mincey — 8/5/96 Date
Committee Member

Dr. Jeff A. Smiley — 8/5/96 Date
Committee Member

Dr. Peter J. Kasvinsky — 8/6/96 Date
Dean of Graduate Studies

Abstract

The focus of the research presented here is the use of bis (2,2,2-trifluoroethoxy) phosphorochloridate as a new coupling reagent for the synthesis of peptides. Bis (2,2,2-trifluoroethoxy) phosphorochloridate is modeled after diethyl phosphorocyanide (DEPC). DEPC converts the carboxyl portion of an amino acid to an O-acyl phosphate, which acts as a leaving group when attacked by a good nucleophile such as an amino group from a second amino acid, resulting in the formation of a peptide. The trifluoroethyl groups would enhance the electrophilicity of the phosphorus, thus increasing the yield of the intermediate, O-acyl phosphate, and augment the leaving group ability of the phosphate moiety in the peptide coupling.

Acknowledgments

I would like to thank Dr. John A. Jackson for his guidance in my research and thesis. In addition, I would like to acknowledge Dr. Daryl Mincey and Dr. Jeff Smiley for their contributions as members of my thesis committee. I would like to extend gratitude to my family for their moral support and encouragement especially my brother, Bob, who has always been there for me when I needed him the most.

Table of Contents

List of Structures

List of Figures

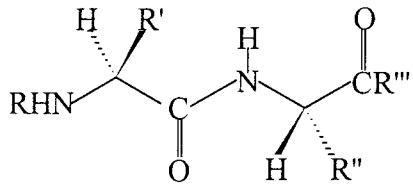
List of Abbreviations

Abbreviation	Description
^{13}C	carbon-13
Cbz	benzyloxycarbonyl
EDC	1-ethyl-3-[3-(diethylamino)propyl]carbodiimide
Et_3N	triethylamine
^{1}H	hydrogen-1
HCl(aq)	hydrochloric acid
Hz	Hertz
Ile	isoleucine
J	coupling constant (in Hertz)
Leu	leucine
Me	methyl
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NMR	nuclear magnetic resonance
^{31}P	phosphorus-31
Pro	proline
Ser	serine
THF	tetrahydrofuran
TLC	thin layer chromatography
Val	valine

Chapter 1: Introduction

Part A: Peptides and their uses

A peptide **1** consists of amino acid residues linked by amide bond. In today's usage, a protein is any macromolecule incorporating more than fifty α-amino acid residues while a peptide is classified as any molecule with less than fifty α-amino acid residues[1].

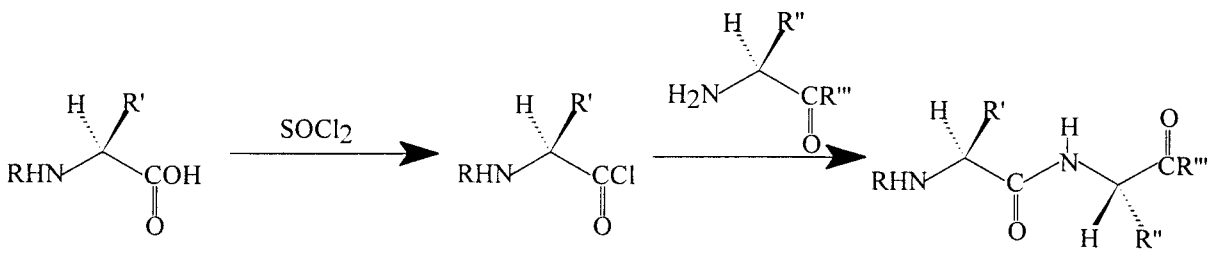


1

Peptides or proteins exist in all living organisms and have the ability to influence the endocrine, neurological, immune, and enzymatic processes with high specificity and prodigious potency. This gives them a wide variety of applications in the medicinal field as drugs for many physiological processing such as the regulation of pain and stimulating the immune system. Their applications are seemingly endless and the demand for more peptides increases all the time since there are still more diseases than there are cures.

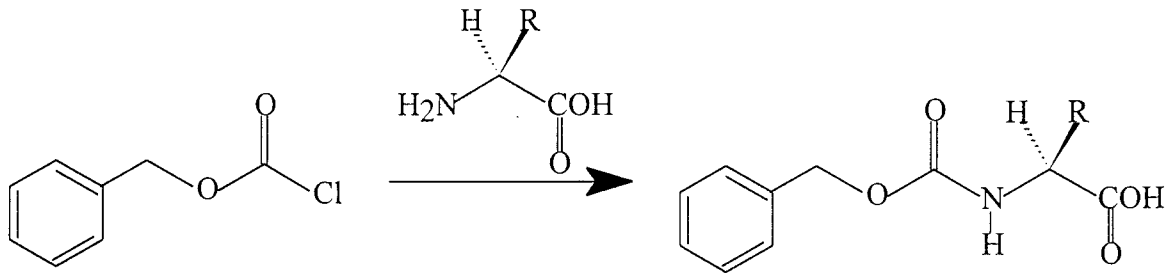
Part B: Historical perspective

Early in this century, the synthesis of peptides by the acid chloride method[2] or by the azide procedure led to molecules containing as many as 19 amino acids; but after this initial success, from 1911 to 1920, there were no significant developments reported. In the 1920s, E. Abderhalden enriched the literature mainly with new compounds rather than



Acid Chloride Method

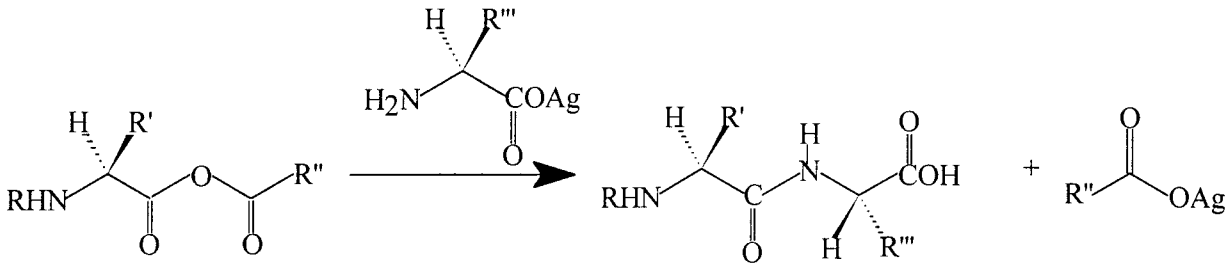
new methods of synthesis. It was not until 1932 that the discovery of the easily removable protecting group, the carbobenzoxy group[3], led to revolutionary developments.



Carbobenzoxy Protection

As a consequence of this unsurpassed contribution, the syntheses of naturally occurring small peptides were soon achieved. The carbobenzoxy protection, with coupling of amino acids through their azides or chlorides, was successful when simple amino acids were involved. Unfortunately, amino acids that have a third functional group, in addition to having the amino and the carboxyl group, required additional methods of protection

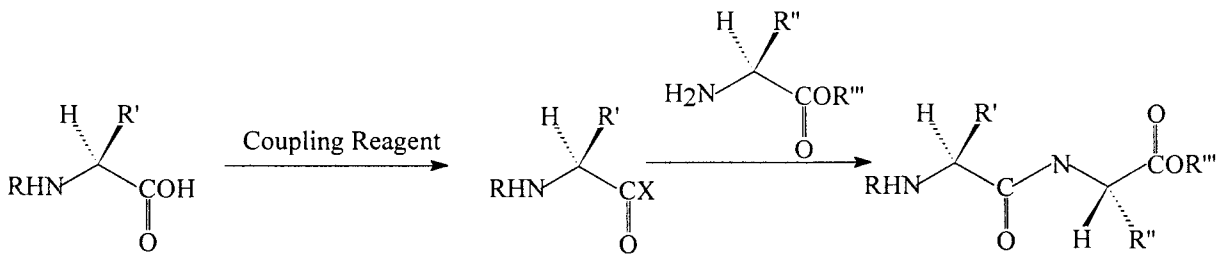
and coupling. A major breakthrough in synthetic methods finally came around 1950 with the advent of the mixed anhydride procedures[4]. In 1953, V. du Vigneaud and his



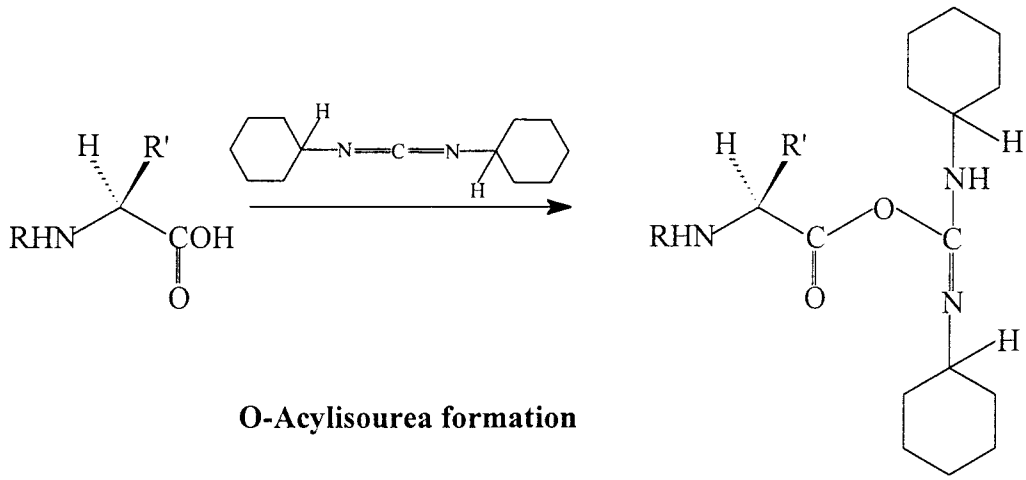
Mixed Anhydride Method

associates introduced mixed anhydrides of protected amino acids in peptide chemistry, the utility this method can best be illustrated by noting that the elucidation of the structure of the hormone oxytocin was announced simultaneously with its synthesis. In this memorable synthesis of a cyclic nonapeptide with hormonal activity[5], advantage was taken of the newly discovered mixed anhydride procedures.

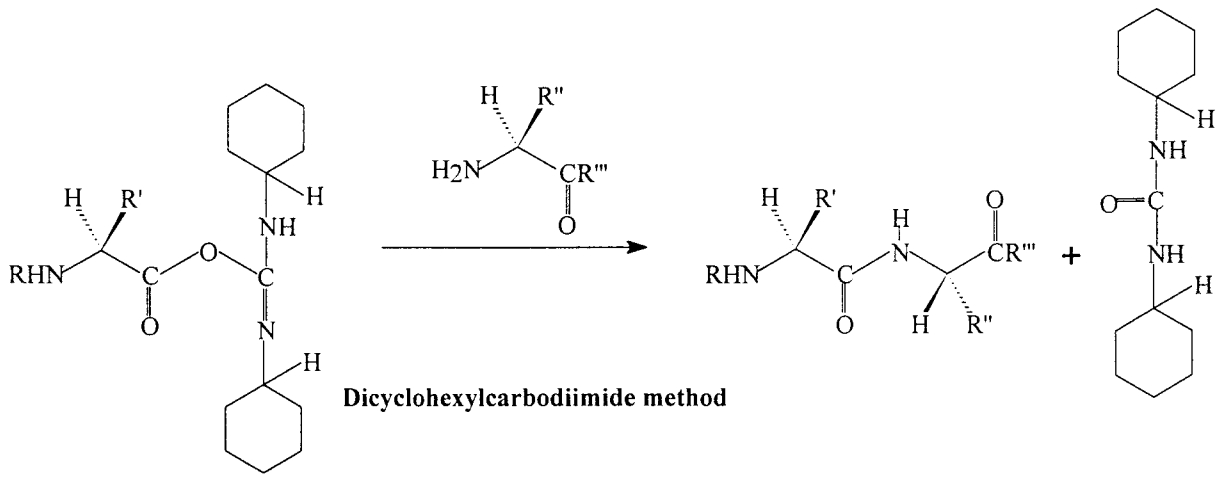
Soon after the development of the mixed anhydride procedures, the appearance of active esters and coupling reagents brought an unprecedented richness to the peptide



scene. Thiophenyl esters, activated methyl esters, nitrophenyl esters, nitrothiophenyl esters, the coupling reagent dicyclohexylcarbodiimide[6] (DCC), ethoxyacetylene, and somewhat later, carbonyldiimidazole were all introduced. The 1950s also brought new ideas in the area of protecting groups, the most notable being the tert-butoxycarbonyl

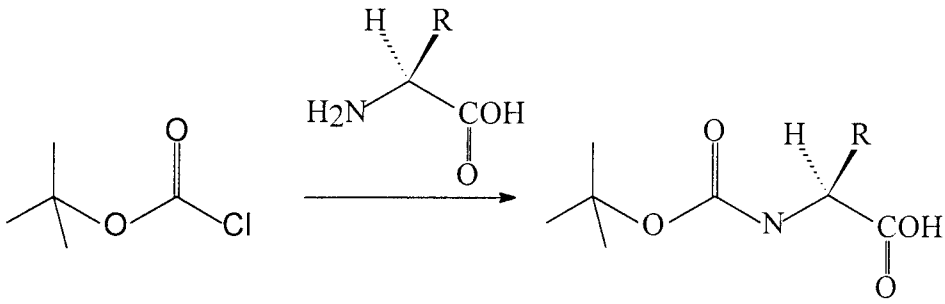


O-Acylisourea formation



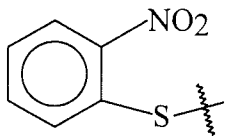
Dicyclohexylcarbodiimide method

group[7], for the protection of amino groups and the masking of the carboxyl function by tert-butyl esters.

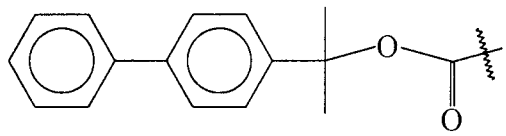


tert-Butoxy Carbonyl Protection

The 1960s witnessed a remarkable further evolution of peptide synthesis. It is impossible to do justice to the numerous new protecting groups, while two of the most important were the o-nitrophenylsulfenyl[8] and the biphenylisopropyloxycarbonyl groups.



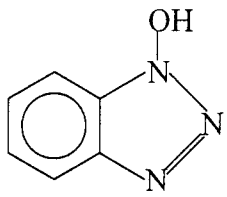
o-Nitrophenylsulfenyl group



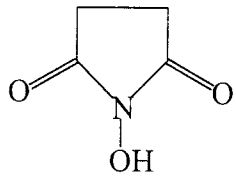
Biphenylisopropyloxycarbonyl group

From the methods of activation, new active esters, O-acyl derivatives of hydroxylamines[9,10], and aryl esters with negative substituents must be pointed out in this enumeration. New coupling reagents were introduced, among them the convenient and therefore promising 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline or EEDQ[11]. Additives capable of efficiently reducing racemization that occurs during coupling of

peptides gained major significance. Hydroxysuccinimide[12] and 1-hydroxybenzotriazole are the most efficient in this respect. The original four-center condensation method of



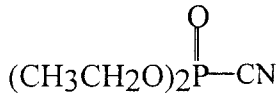
1-Hydroxybenzotriazole



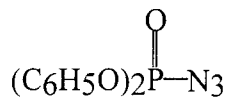
N-Hydroxysuccinimide

Ugi appeared as real promise because it provided optically pure peptides. None of these innovations had the revolutionary effect of that exerted by a technique of the Merrifield synthesis.

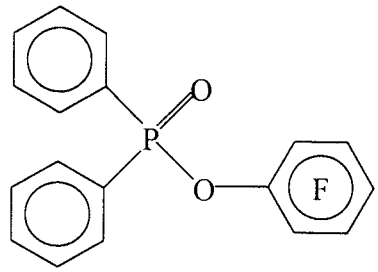
From 1970 to 1990, a new breed of coupling reagents emerged; these reagents allowed increased product yields within shorter time frames with no racemization when using conventional methods, or the Merrifield approach. This new breed of reagent consisted of organophosphorus reagents. Three of the most noted or more commonly used are diphenyl phosphorazidate (DPPA), diethyl phoshorocyanidate[13] (DEPC), and pentafluorophenyl diphenylphosphinate[14] (FDPP).



DEPC



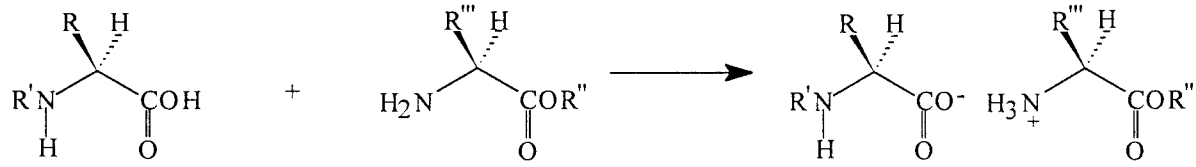
DPPA



FDPP

Part C: Problems of Peptide Synthesis

There are three main problems which have to be confronted when peptides are synthesized: (1) coupling, (2) protecting, and (3) racemization. First, the formation of peptide bonds needs to employ coupling reagents; in order, to activate the carboxyl component of an amino acid that enables the attack by the amino group of the second amino acid. This is necessary since the formation of the peptide bond is highly thermodynamically unfavorable and thus forms an amine salt.

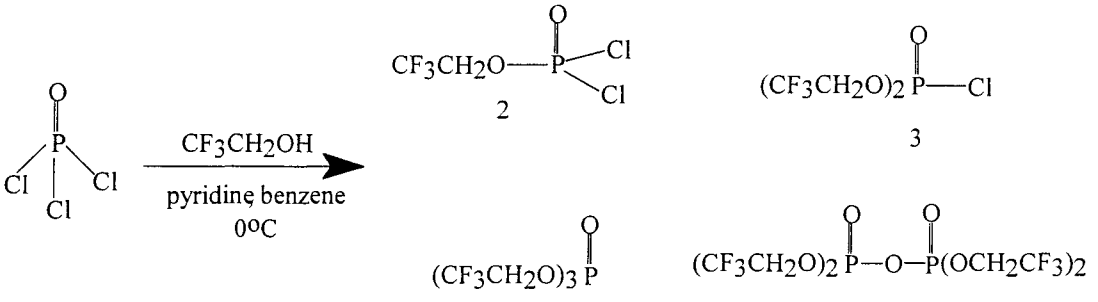


Secondly, linking amino acids together to produce the desired sequence requires the specific condensation between the carboxylic acid of amino acid and the amine of another. Because each amino acid contains both functional groups, the protection of the other is required. Otherwise, a complex mixture would result consisting of two homogeneous and two heterogeneous dipeptides.

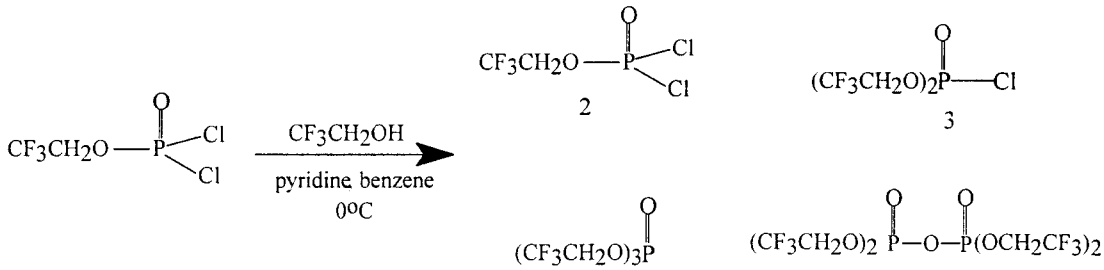
Thirdly, amino acid derivatives are prone to epimerization of the α carbon by a deprotonation-protonation sequence. Loss of stereochemical purity as the multistep synthesis proceeds leads to a large number of diastereomeric peptide impurities with structures nonetheless very similar to the desired product. Note: Many peptides are quite insoluble in common solvents that would make separation of these impurities nearly impossible.

Chapter 2: Results and Discussion

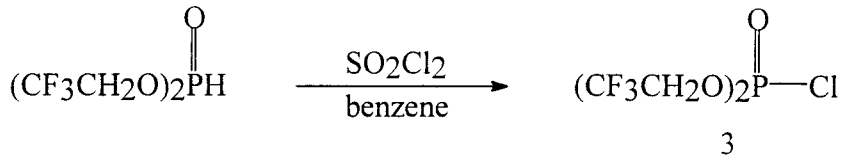
The purpose of this project was to use bis (2,2,2-trifluoroethoxy) phosphorochloridate as a peptide coupling reagent. The first part of the project was to actually synthesize the organophosphorus reagent: bis-(2,2,2-trifluoroethoxy) phosphorochloridate by using a literature method developed by K. Sellars[15]. This method consisted of a two-step reaction sequence with fractional vacuum distillation of each product. The first step began with the addition of 2,2,2-trifluoroethanol, pyridine, and benzene to phosphorus oxychloride at 0 °C for one hour formed four



different products, one of them being the desired product being 2,2,2-trifluoroethoxy phosphorodichloridate (**2**) while the impurities were bis-(2,2,2-trifluoroethoxy) phosphorochloridate (**3**), tris-(2,2,2-trifluoroethoxy) phosphate, and tetrakis-(2,2,2-trifluoroethoxy) pyrophosphate along with unreacted starting materials.



In the second step of the reaction sequence, the pure 2,2,2-trifluoroethoxy phosphorodichloridate (**2**) was treated with a mixture of 2,2,2-trifluoroethanol, pyridine, and benzene thus forming a mixture which includes the desired bis-(2,2,2-trifluoroethoxy) phosphorochloridate (**3**), along with tris-(2,2,2-trifluoroethoxy) phosphate, tetrakis-(2,2,2-trifluoroethoxy) pyrophosphate, and unreacted starting materials. Unfortunately, purification by vacuum distillation was incredibly difficult, obtaining pure product was nearly impossible. The reported yields by Sellars were not reproducible. For the first step, the synthesis of 2,2,2-trifluoroethoxy phosphorodichloridate only yielded product in a 23.9% yield while for the second step, bis (2,2,2-trifluoroethoxy) phosphorochloridate could only be isolated in the crude product mixture as a small quantity and was never purified by fractional vacuum distillation since the boiling points of the impurities overlapped the boiling point of the desired product. Therefore, a new method had to be devised; in order to give a better yield with less impurities in it.



3

The new synthesis of bis (2,2,2-trifluoroethoxy) phosphorochloridate (**3**) utilized the treatment of bis (2,2,2-trifluoroethoxy) phosphite with sulfuryl chloride in benzene at O ^{o}C which resulted in the quantitative formation of the desired product. Upon monitoring the crude reaction mixture by ^{31}P NMR, the bis (2,2,2-trifluoroethoxy) phosphite peak at 9.05 ppm diminished over a two period while the bis (2,2,2-trifluoroethoxy) phosphorochloridate appeared at 6.73 ppm, thus giving a 95 to 100% conversion, before purification. Distillation of the product produced analytically pure material in an 88.6% yield.

Figure 1: ^{31}P NMR: bis (2,2,2-trifluoroethoxy) phosphorochloridate

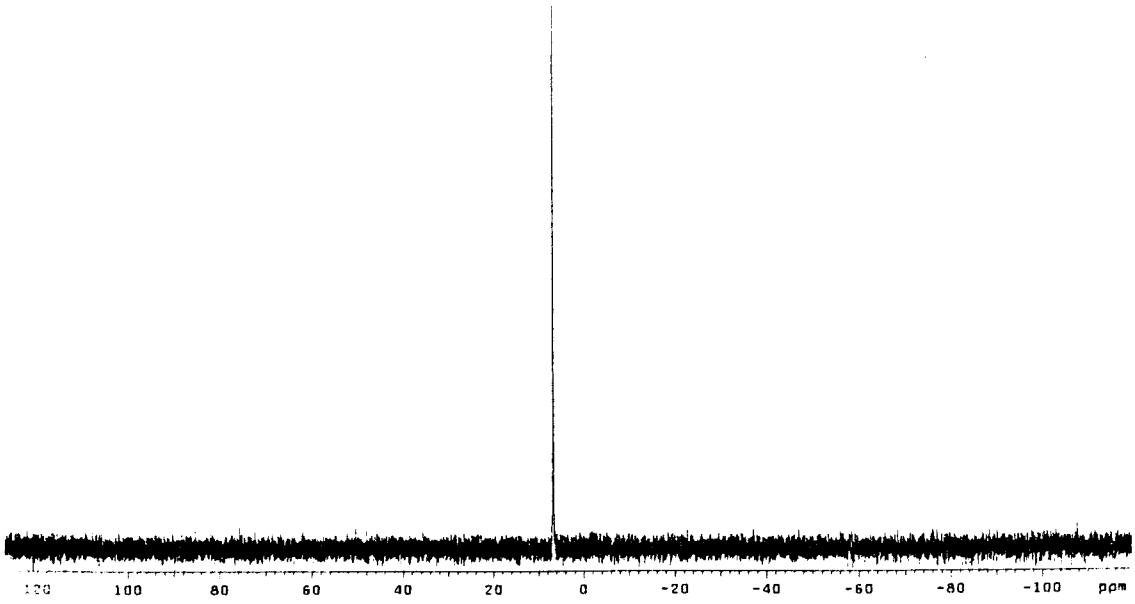


Figure 2: ^{13}C NMR: bis (2,2,2-trifluoroethoxy) phosphorochloridate

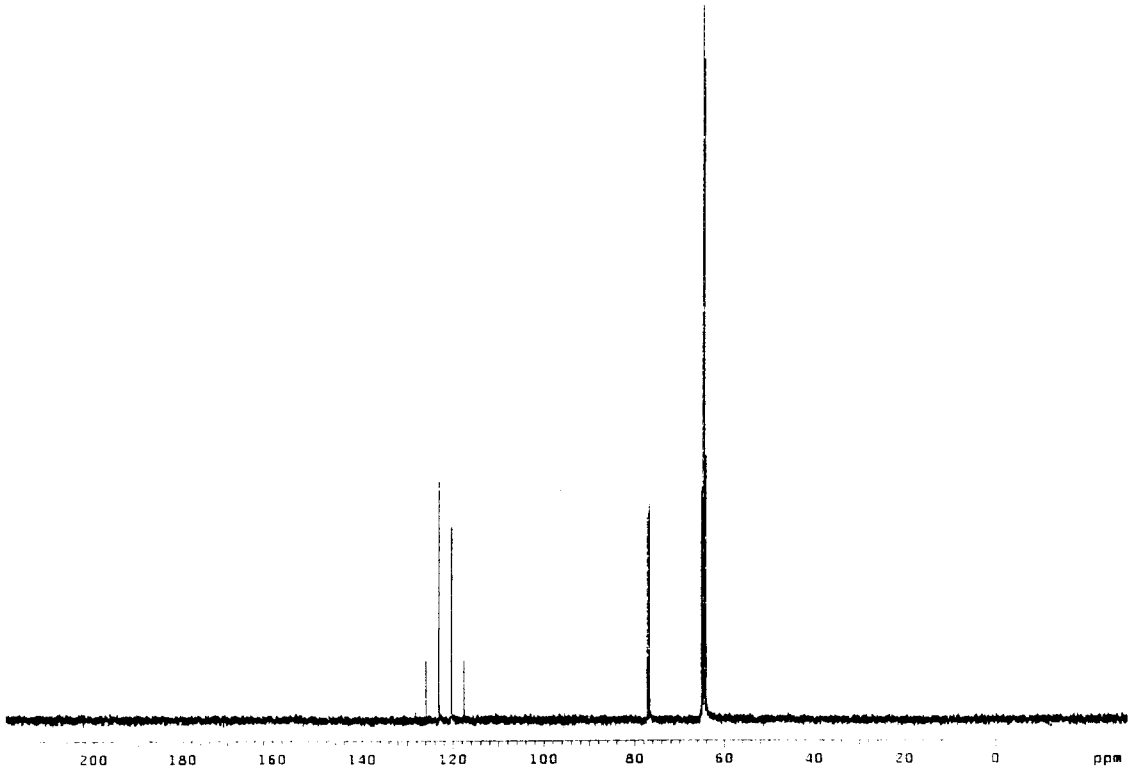
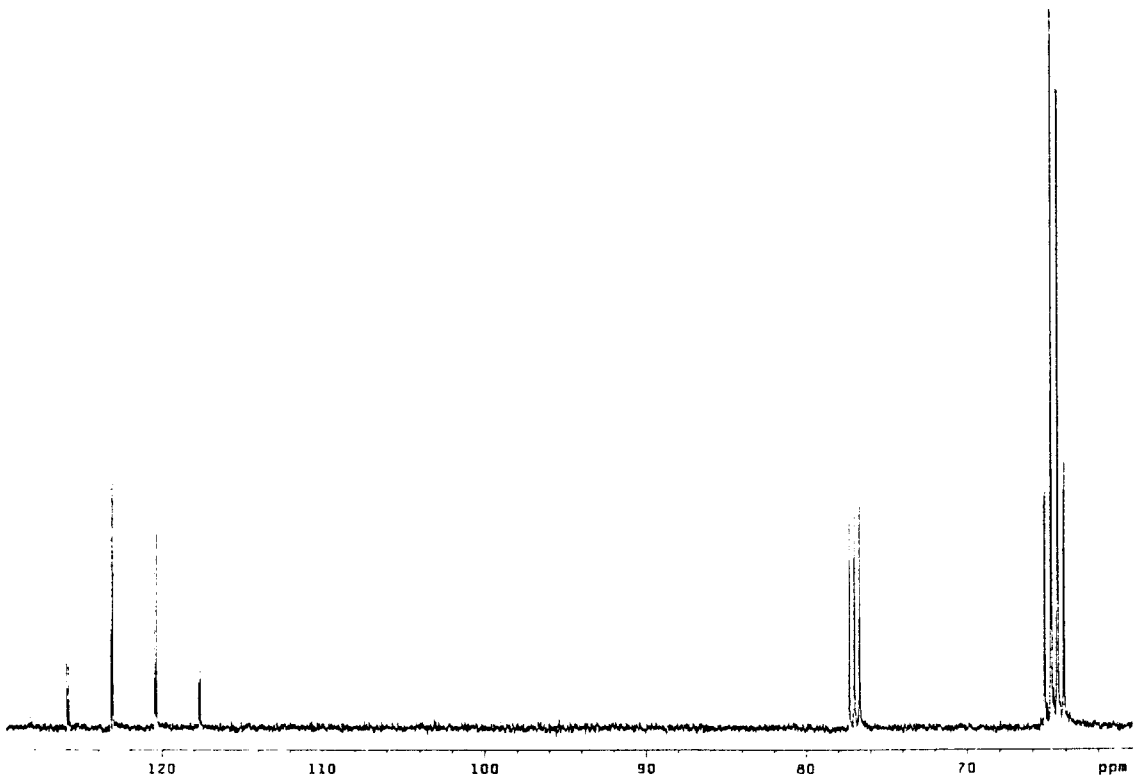
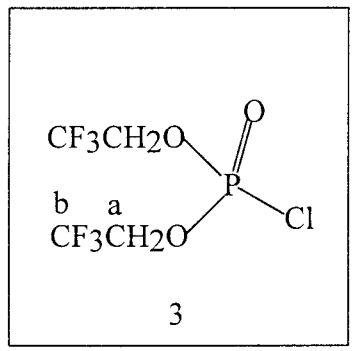


Figure 3: ^{13}C NMR: Expanded bis (2,2,2-trifluoroethoxy) phosphorochloridate



The extensive couplings observed in the ^{13}C NMR spectra were a result of the spin 1/2 phosphorus and fluorine nuclei present in the molecule. Rather than two singlets, two doublets of quartets, set **a** and **b** (64.7 and 128.1 ppm), were observed. The set **a**, doublet of quartets (64.7 ppm), exhibited two bond coupling to the fluorine (J= 39.2 Hz) and phosphorus (J=5.5 Hz); whereas, set **b** had one bond coupling to the fluorine (J= 277.0 Hz) and three bond coupling to the phosphorus (J=11.3 Hz). Set **b**'s J coupling constants were substantially larger than set **a**'s due to the direct coupling to fluorine.

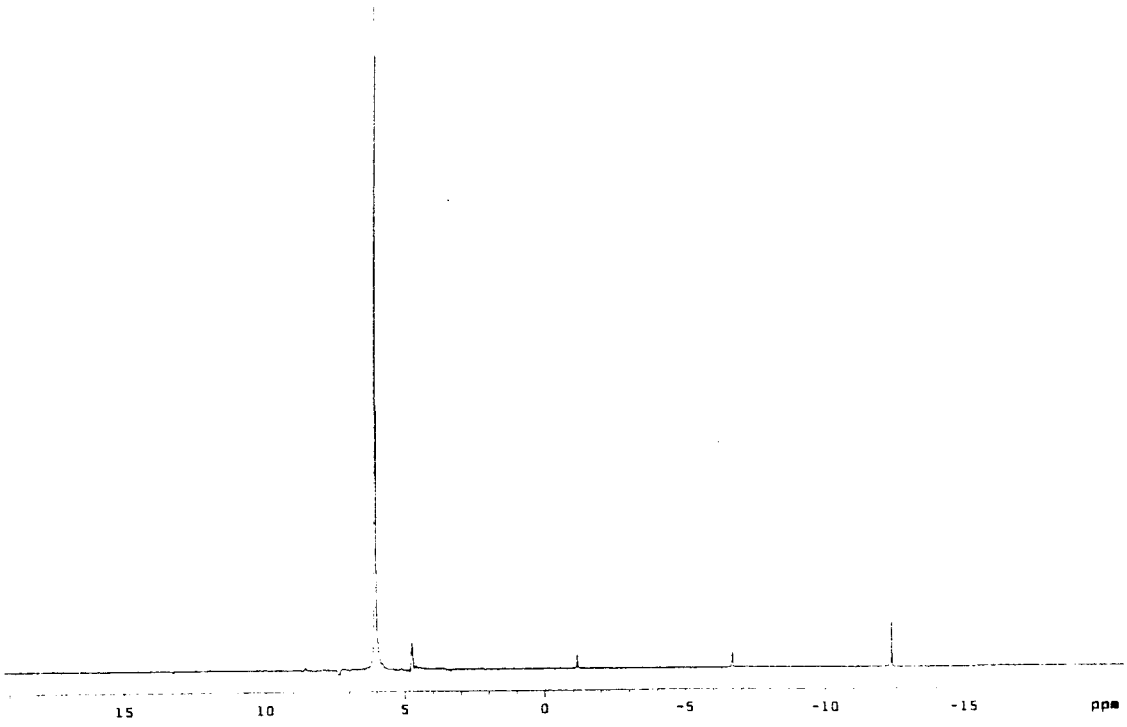


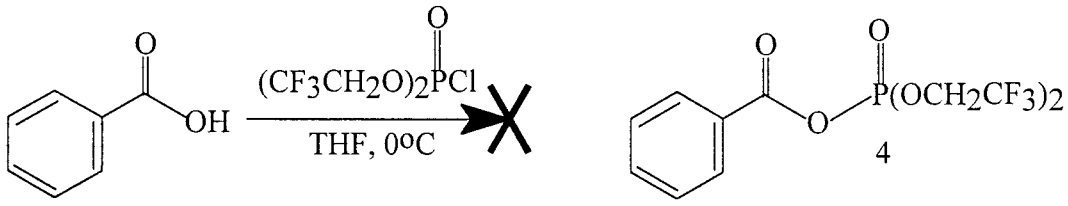
3

The next step was to study the usefulness of bis (2,2,2-trifluoroethoxy) phosphorochloridate. This was done in two parts: (1) the formation and characterization of the O-acyl phosphate, and (2) the formation and characterization of a simple amide. For the formation of the O-acyl phosphate, benzoic acid was chosen to be the simple carboxylic acid. To the benzoic acid in THF at 0 ^{o}C, the bis

(2,2,2-trifluoroethoxy) phosphorochloridate was added (dropwise for ten minutes) to it. Examination of the crude mixture by ^{31}P NMR indicated that over a nine-day period ~90% of the crude mixture was still the starting materials. The reaction conditions were modified by the addition of one equivalent of triethylamine in the

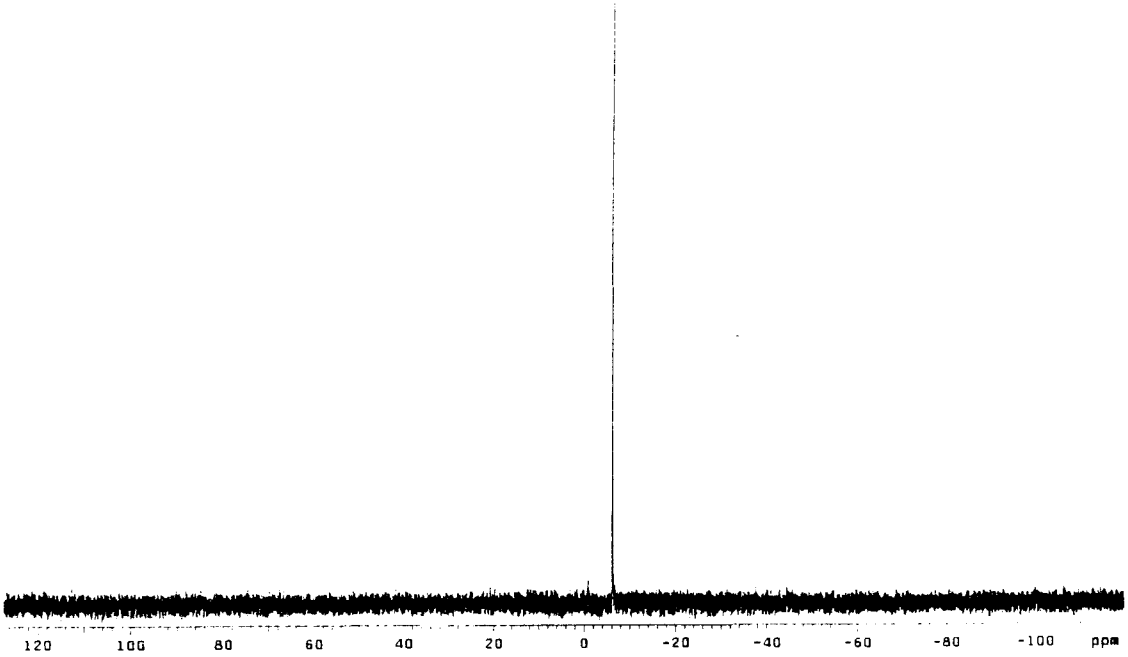
Figure 4: ^{31}P NMR: O-acyl phosphate over a nine day period



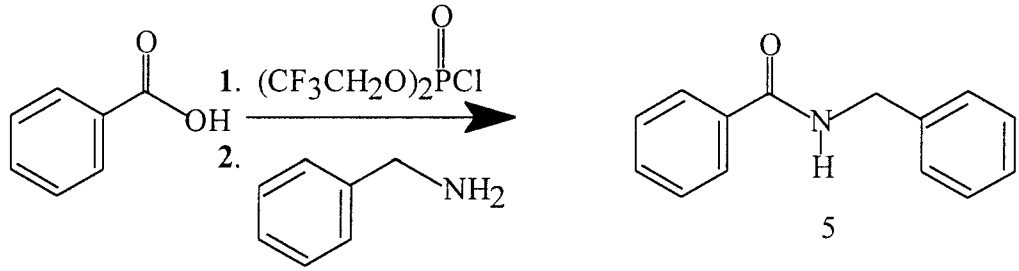


reaction. The triethylamine was used to generate a carboxylate anion from a carboxylic acid. The reaction was repeated again except this time triethylamine was added dropwise for ten minutes to the benzoic acid and bis (2,2,2-trifluoroethoxy) phosphorochloridate in THF at 0 °C. The formation of the O-acyl phosphate **4** was monitored by ^{31}P NMR with an ~90% conversion after only one hour. Unfortunately,

Figure 5: ^{31}P NMR: O-acyl phosphate 4 over a one hour period



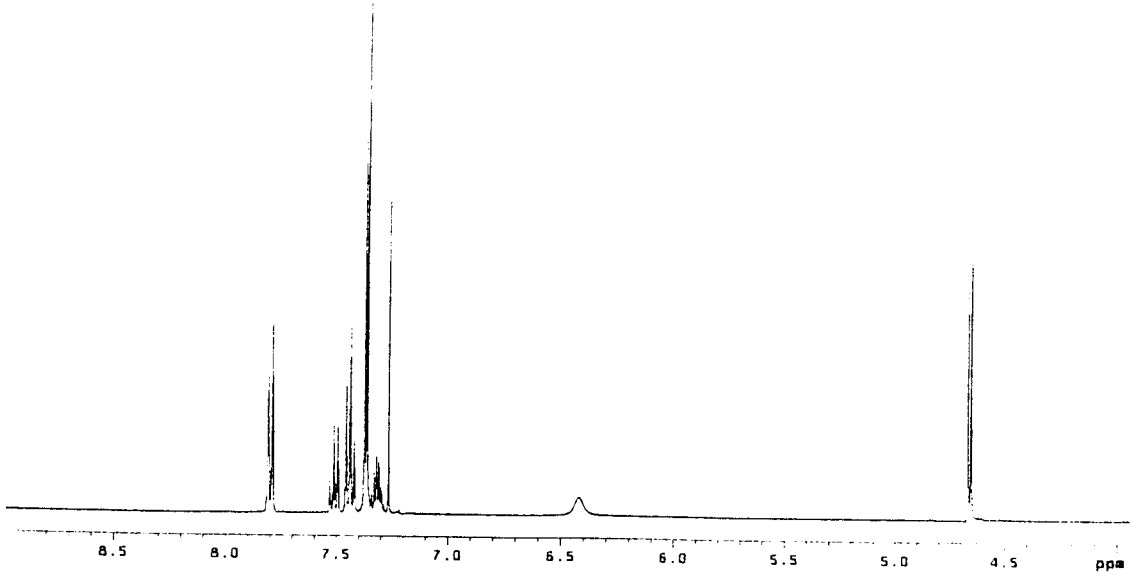
purification of the O-acyl phosphate **4** proved to be difficult for it would decompose on the column during flash chromatography.



5

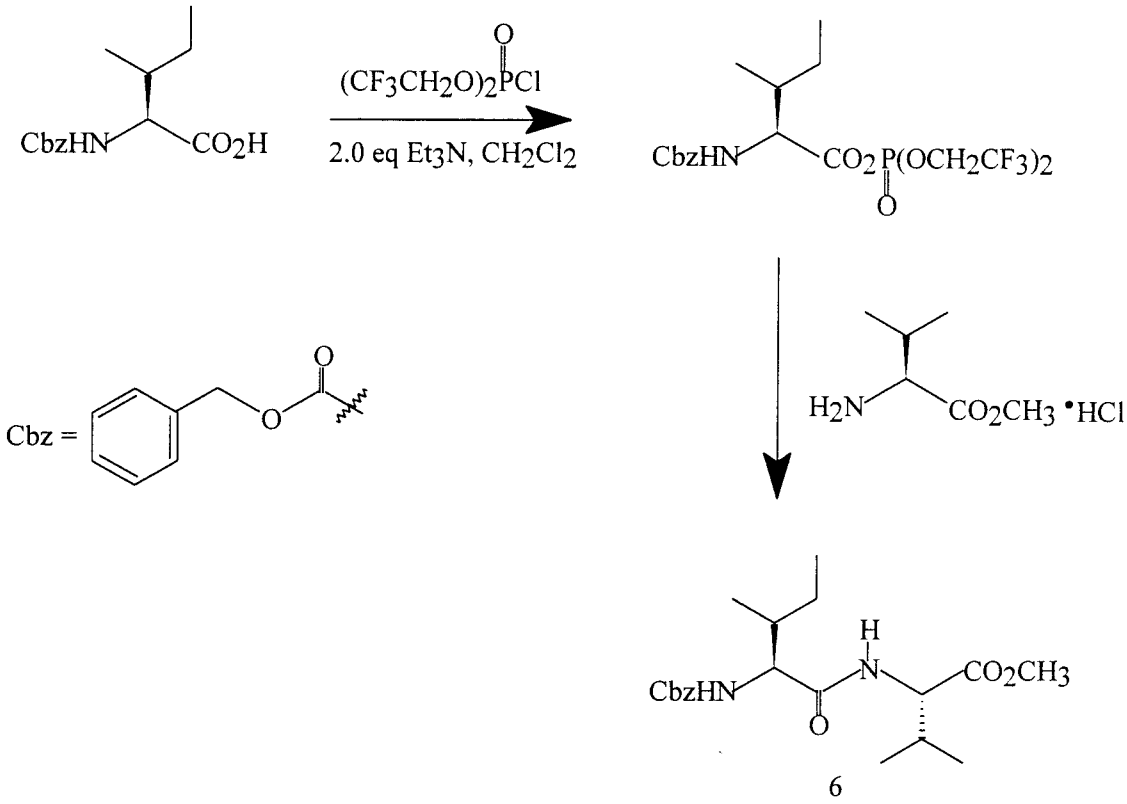
For the formation of an amide, a 'one flask' approach was used. The O-acyl phosphate was formed *in situ*, and benzylamine was added to the mixture and allowed to stir for four hours. After a series of aqueous washes and purification by flash column chromatography, white crystals of N-benzyl benzamide (**5**) were collected with a 48.3 % yield. Figure 6: ^{1}H NMR, the benzylic protons appeared as a doublet at 4.64 ppm while the amide proton appeared as a broad singlet at 6.42 ppm, with the aromatic protons appearing as a wide multipet from 7.30-7.82 ppm.

Figure 6: ^{1}H NMR: N-Benzyl benzamide (**5**) expanded



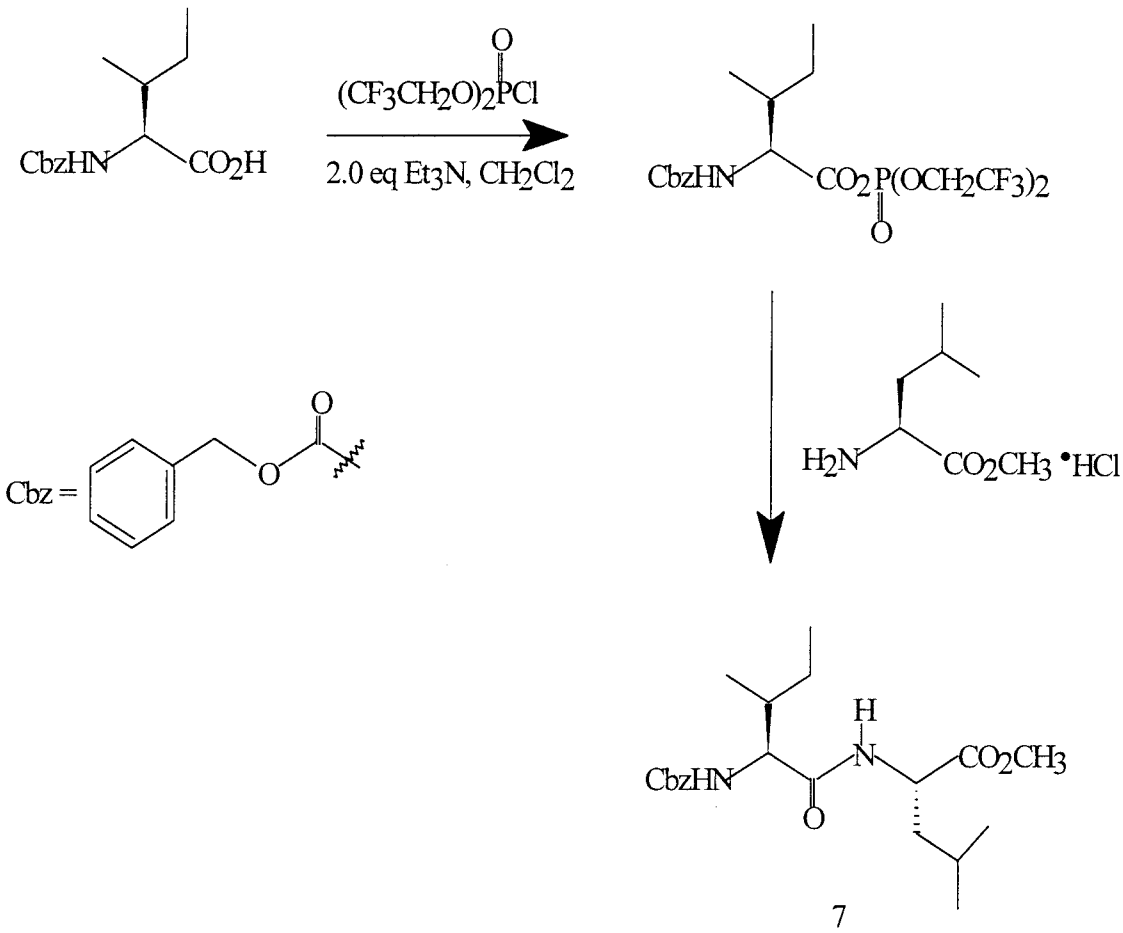
These preliminary studies paved the way for the synthesis of dipeptides. The scope of this project was to take six protected amino acids (three amino-protected and three carboxyl-protected amino acids) and form nine different dipeptides in all. The three amino-protected amino acids (L-isoleucine, L-serine, and L-valine) were protected with the benzyloxycarbonyl (Cbz) group, according to standard literature methods while the three carboxyl-protected amino acids (L-leucine, L-proline, and L-valine) were protected with the methyl ester group as purchased.

The first dipeptide to be synthesized with the bis (2,2,2-trifluoroethoxy) phosphorochloridate was Cbz-isoleucine-valine-methyl ester (Cbz-Ile-ValOMe) **6**. The Cbz-isoleucine and bis (2,2,2-trifluoroethoxy) phosphorochloridate were dissolved in methylene chloride while two equivalents of triethylamine was added dropwise to the mixture for ten minutes at 0 °C. After the addition, the mixture was stirred for one hour at room temperature. The mixture was then cooled to 0 °C and the L-valine methyl ester hydrochloride was added to the mixture. After the second addition, the mixture was allowed to stir for twelve hours. After aqueous washes and purification by flash column chromatography, a 63% yield of Cbz-Ile-Val-OMe was obtained. This dipeptide was previously synthesized using EDC as the coupling reagent in 57% yield. Other solvents tested were THF and DMF, but methylene chloride proved to be the best solvent because the O-acyl phosphate formed rapidly with a 90% conversion after only one hour.



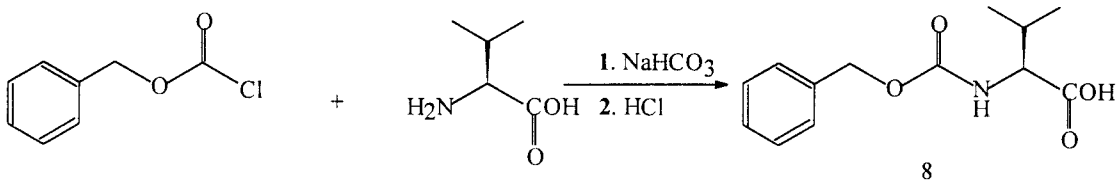
6

The next dipeptide to be synthesized was Cbz-isoleucine-leucine methyl ester (Cbz-Ile-LeuOMe) **7**. It was done in the same manner as the previous synthesis except L-leucine methyl ester hydrochloride was used. After purification, white crystals of Cbz-Ile-LeuOMe were obtained in a 42 % yield.



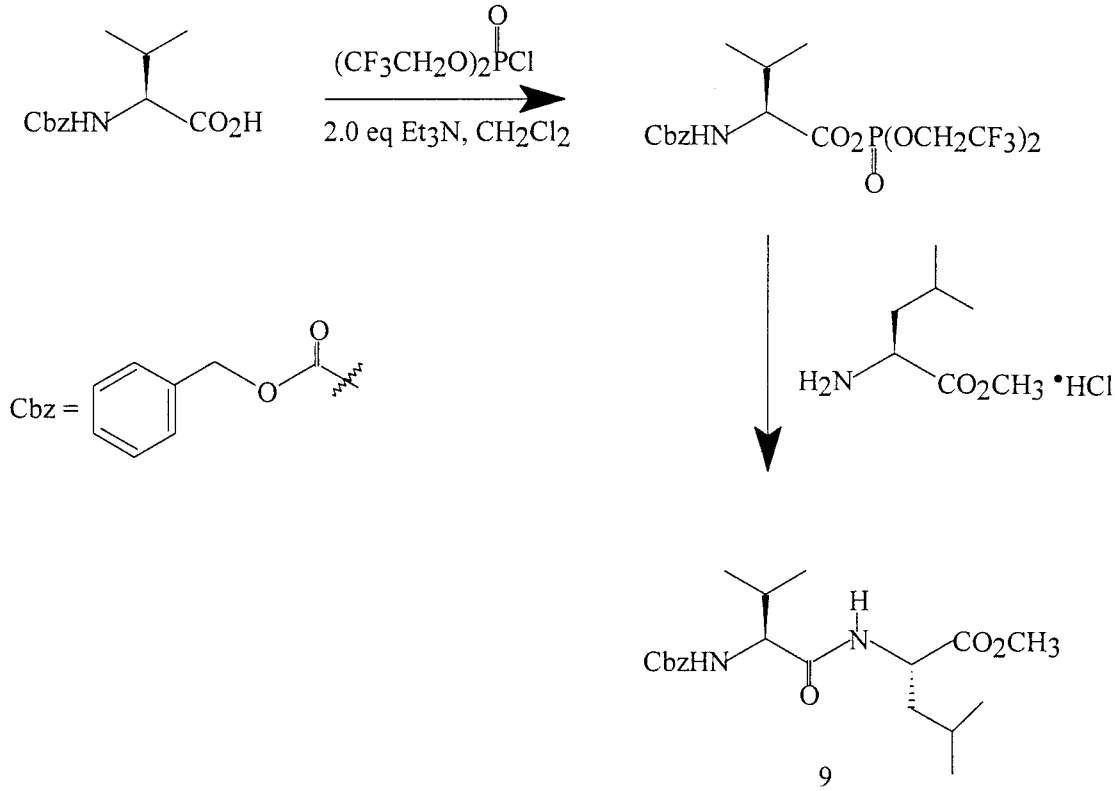
7

Before the next series of dipeptides could be synthesized, the protected amino acid, Cbz-valine was synthesized using the Fruton method[16] which stated that benzyl chloroformate (Cbz-Cl) was added to L-valine dissolved in 1/4 saturated sodium bicarbonate. After the addition, the pH was maintained at 10 followed by a diethyl ether wash, the aqueous phase was then acidified with 5M HCl, and the product was extracted with ethyl acetate. White crystals of Cbz-valine were collected with a 37.4% yield.

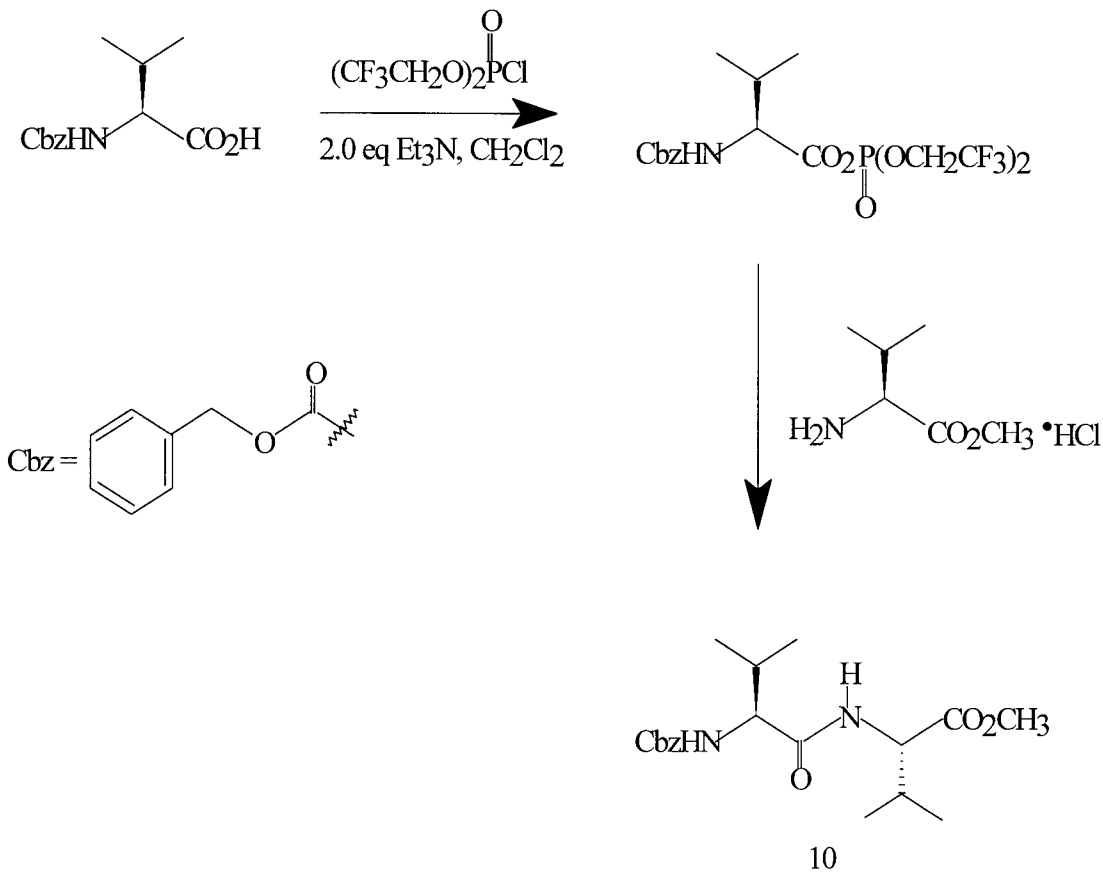


8

Since the protected amino acid, Cbz-valine, was now produced, the next series of dipeptides could be synthesized. The Cbz-Val-LeuOMe **9** was synthesized in a 49% yield in the same manner as the previous dipeptides, and Cbz-Val-ValOMe **10** was produced in a 59% yield.

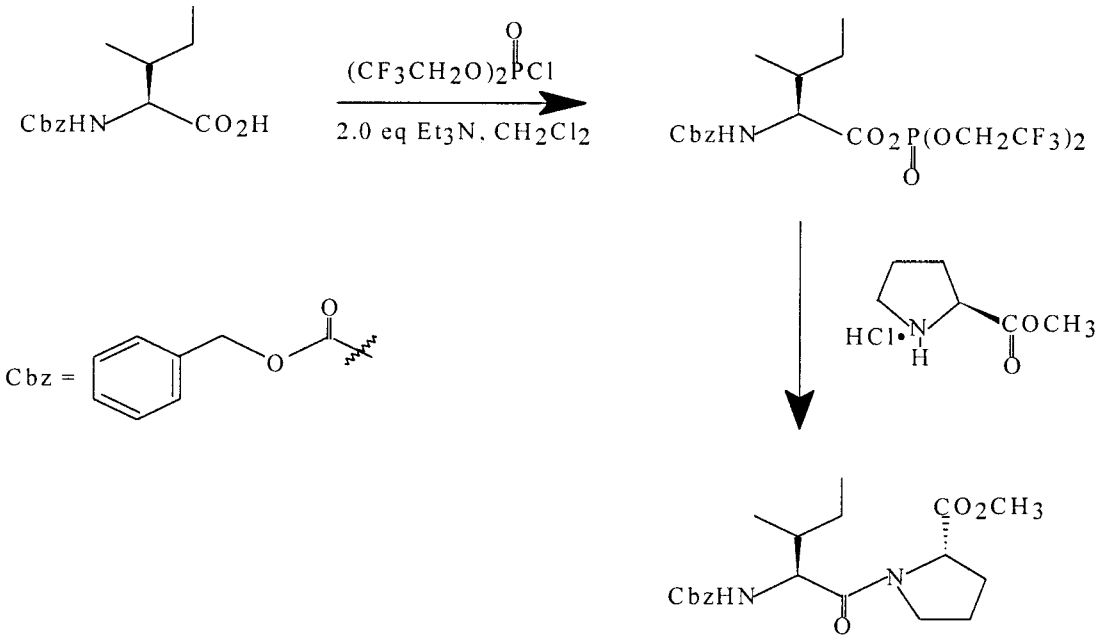


9

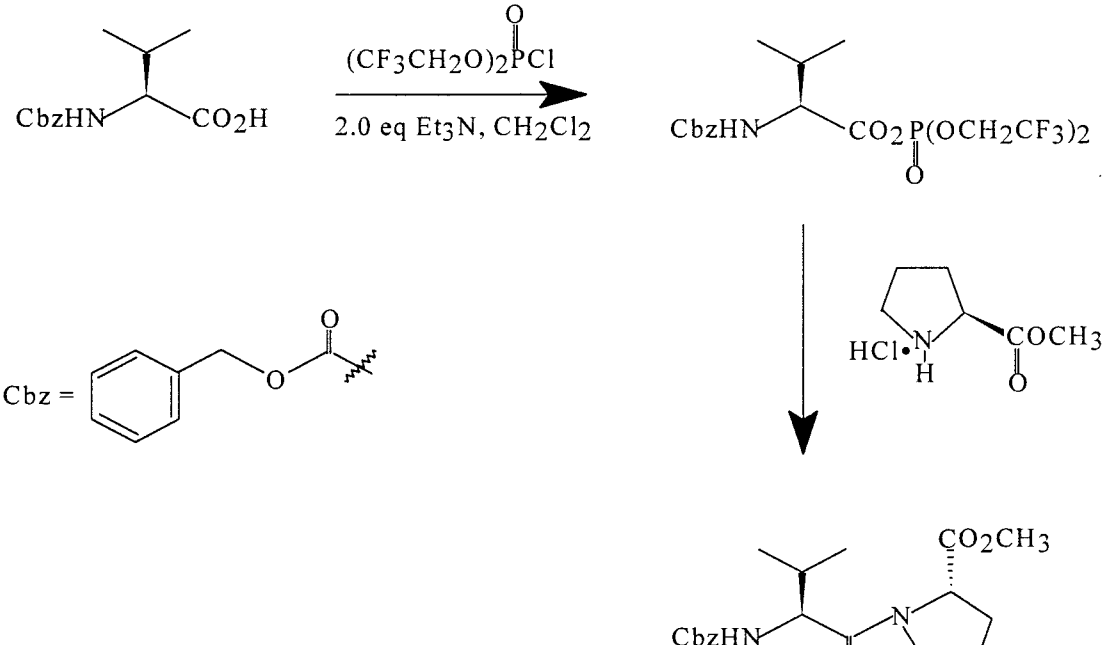


10

The last set of dipeptides to be synthesized were those combining to L-proline methyl ester hydrochloride. Cbz-Ile-ProOMe **11** was produced in a 22% yield while Cbz-Val-ProOMe **11** was synthesized in a 40% yield.


CbzHN
CO2H
(CF3CH2O)2PCl
2.0 eq Et3N, CH2Cl2
CbzHN
CO2P(OCH2CF3)2
Cbz =
HCl•N
H
COCH3
CO2CH3
CbzHN
N

11

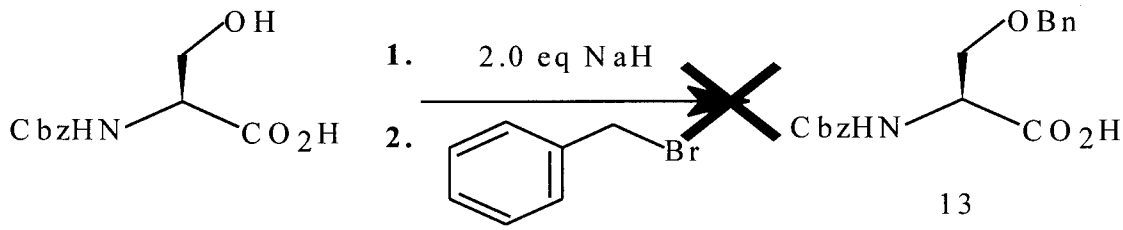

CbzHN
CO2H
(CF3CH2O)2PCl
2.0 eq Et3N, CH2Cl2
CbzHN
CO2P(OCH2CF3)2
Cbz =
HCl•N
H
COCH3
CO2CH3
CbzHN
N

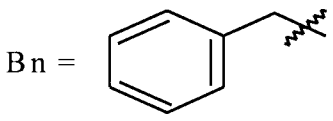
12

Table 1: Product Yields

Product	Percent yield
Cbz-Ile-ValOMe	63
Cbz-Ile-LeuOMe	42
Cbz-Ile-ProOMe	22
Cbz-Val-ValOMe	59
Cbz-Val-LeuOMe	49
Cbz-Val-ProOMe	40

With six of the nine dipeptides produced, the Cbz-serine series was to be the most challenging since preliminary studies indicated that the hydroxyl group of the amino acid had to be protected. By using the Sugano[17] method, the hydroxyl group would be protected with a benzyl group. The Cbz-serine was dissolved in DMF and cooled to 0 °C, two equivalents of sodium hydride was then added to the mixture. After the solution stopped bubbling, benzyl bromide was added to the mixture and was allowed to stir overnight. After a series of washes and purification by flash column chromatography, tan crystals were collected, but the product proved not to be the desired product and has not yet been identified. Since this amino acid wasn't produced, the last three dipeptides weren't attempted at all.





Chapter 3: Experimental

General Methods. Melting points are not corrected. All products purchased by Aldrich were used without purification. The triethylamine was distilled from CaH_2 prior to use. All reactions were conducted under a positive pressure of argon. All solvents were dried or distilled 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 staining with 5% phosphomolybdic acid (PMA) in ethanol or 5% ninhydrin in absolute ethanol, with heating.

NMR spectra (^1H, ^{13}C, and ^{31}P) were recorded with a Varian Gemini 2000, 400 MHz spectrometer, with $CDCl_3$ or D_2O as the solvent. The ^1H and ^{13}C chemical shifts are reported in parts per million downfield from $(CH_3)_4Si$. The ^{31}P chemical shifts are reported in parts per million downfield from H_3PO_4 (external standard). Coupling constants are reported in Hertz.

Bis (2,2,2-trifluoroethoxy) phosphorochloridate. (3)

Method A. To a solution of bis (2,2,2-trifluoroethoxy) phosphite (24.6 g, 100 mmol) in benzene (20 mL) was added dropwise a solution of sulfuryl chloride (8.03 mL, 100 mmol) in benzene (20 mL) at 0 °C for one hour. After the addition, the mixture was allowed to warm to room temperature for two hours. The benzene was removed by evaporation, and purification by fractional distillation yielded bis (2,2,2-trifluoroethoxy) phosphorochloridate (24.7 g, 88.6%).

^{1}H NMR: δ 4.47-4.55 (m, 4H)

^{13}C NMR: δ 64.7 (dq, J= 39.2, 5.5 Hz), 121.8 (dq, J= 277.0, 11.3 Hz)

^{31}P NMR: δ 6.73

Method B. To a solution of phosphorus oxychloride (74.4 mL, 800 mmol) in benzene (40 mL) was added dropwise a mixture of 2,2,2-trifluoroethanol (29.4 mL, 400 mmol), pyridine (32.4 mL, 400 mmol), and benzene (40 mL) at 0 °C over a period of one hour. The mixture was then allowed to warm to room temperature, heated at 50 °C for 15 minutes, and stirred for two hours. The pyridine hydrochloride was removed by filtration and washed with benzene. The solvent was removed by evaporation yielding crude product of 2,2,2-trfluroethoxy phosphorodichloridate. This crude product was purified by fractional vacuum distillation affording a clear liquid of 2,2,2-trifluoroethoxy phosphorodichloridate (20.8 g, 23.9%).

A mixture of 2,2,2-trifluoroethanol (3.5 mL, 48.0 mmol), pyridine (3.9 mL, 48.0 mmol) in benzene (14.5 mL) was added dropwise to 2,2,2-trifluoroethoxy phosphorodichloridate (20.8 g, 96.0 mmol) in benzene (14.5 mL) at 0 °C for 30 minutes. After the addition, the mixture was allowed to warm to room temperature for one hour. The pyridine hydrochloride was removed by filtration and washed with 50 mL benzene. The mixture was removed by evaporation yielding crude product of bis (2,2,2-trifluoroethoxy) phosphorochloridate. Fractional vacuum distillation was performed on the crude product, but no pure product was ever obtained.

N-Benzyl benzamide (5). Triethylamine (0.7 mL, 5.0 mmol) was added dropwise for ten minutes to a stirred mixture of benzoic acid (611 mg, 5.0 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.78 mL, 5.0 mmol) in THF (15 mL) at 0 °C. After the addition, the mixture was allowed to warm to room temperature for one hour. The mixture was cooled once again to 0 °C, and benzylamine (0.55 mL, 5.0 mmol) was added dropwise over ten minutes to the mixture. After the addition, the mixture was allowed to warm to room temperature for four hours. The triethylamine hydrochloride was removed by filtration and washed with 50 mL THF. The THF was removed by evaporation yielding 2.02 g of crude product. The product was then dissolved in 50 mL ether and washed successively with 25 mL aliquots of 5% HCl (4X), water (4X), sat. $NaHCO_3$ (4X), and sat. NaCl (4X). The product was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation yielding 0.610 g of crude product. Purified by flash column chromatography (25.0 g silica, 3:2 diethyl ether:petroleum ether) affording white crystals of N-benzyl benzamide (520 mg, 48.3%). Melting point: 103-105 °C

^{1}H NMR: δ 4.64 (d, 2H, J=5.68 Hz), 6.42 (br s, 1H), 7.30-7.53 (m, 8H), 7.79-7.82 (m, 2H)

^{13}C NMR: δ 44.2, 126.9, 127.7, 127.9, 128.6, 128.8, 131.5, 134.5, 138.2, 167.3

Carbobenzoxy L-valine (8). To a solution of L-valine (4.69 g, 40.0 mmol) in 1/4 saturated sodium bicarbonate (200 mL) was added benzyl chloroformate (7.51 g, 44.0 mmol). The solution was maintained at pH 10 with the addition of 10% sodium hydroxide. The mixture was then washed with ether (2X40 mL) and the aqueous fraction acidified to pH 3 with 5M HCl. The aqueous solution was extracted with ethyl

acetate (2X30 mL) and the solvent was removed by rotary evaporation yielding 3.76 g (15.0 mmol, 37.4%) of the protected amino acid.

^{1}H NMR: δ 0.92 (d, 3H, J=7.20 Hz), 1.00 (d, 3H, J=6.80 Hz), 2.20-2.28 (m, 1H), 4.35 (dd, 1H, J=9.07, 4.49 Hz), 5.14 (d, 1H, J=11.20 Hz), 5.34 (d, 1H, J=9.20 Hz), 6.37 (d, 1H, J=8.40 Hz), 7.31-7.40 (m, 5H), 10.40 (s, 1H)

^{13}C NMR: δ 17.35, 18.95, 31.03, 58.87, 67.21, 128.1, 128.2, 128.5, 136.1, 156.4, 176.8

Cbz-Isoleucine-Valine methyl ester dipeptide (6). At 0 °C, to a solution of Cbz-isoleucine (0.66 g, 2.5 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.4 mL, 2.5 mmol) in methylene chloride (20 mL) was added triethylamine (0.7 mL, 5.0 mmol) for ten minutes. After the addition, the mixture was allowed to warm to room temperature for two hours. The solution was then cooled to 0 °C, and L-valine methyl ester HCl (0.42 g, 2.5 mmol) was added to the solution. After the addition, the mixture was allowed to warm to room temperature for twelve hours. The methylene chloride was removed by evaporation yielding a viscous yellow oil. The product was then dissolved in 50 mL ethyl acetate and washed successively with 25 mL aliquots of 5% HCl, water, sat. $NaHCO_3$, and sat. NaCl. The product was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation yielding 1.2 g of the crude dipeptide. Purified by flash column chromatography (48 g silica, 7:3 n-hexane:ethyl acetate) affording white crystals of Cbz-Ile-ValOMe (600 mg, 1.6 mmol, 63%). Melting point: 129-131 °C

^{1}H NMR: δ 0.89-0.95 (m, 9H), 0.94 (t, 3H, J=7.20 Hz), 1.13-1.20 (m, 1H), 1.50-1.56 (m, 1H), 1.85-1.90 (m, 1H), 2.15-2.20 (m, 1H), 3.74 (s, 3H), 4.06 (dd, 1H, J=8.00, 7.20 Hz), 4.54 (dd, 1H, J=8.80, 4.80 Hz), 5.12

(s, 2H), 5.33 (d, 1H, J=8.06 Hz), 6.25 (d, 1H, J=8.42 Hz), 7.30-7.37 (m, 5H)

^{13}C NMR: δ 11.31, 15.42, 17.75, 18.87, 24.83, 31.17, 37.39, 52.10, 57.13, 59.79, 67.05, 128.0, 128.1, 128.5, 136.3, 156.3, 171.1, 172.0

Cbz-Isoleucine-Leucine methyl ester dipeptide (7). For ten minutes at 0 °C, to a stirred mixture of Cbz-isoleucine (1.3 g, 5.0 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.8 mL, 5.0 mmol) in methylene chloride (20 mL) was added dropwise triethylamine (1.4 mL, 10 mmol). After the addition, the mixture was allowed to stir and warm to room temperature for one hour. The mixture was then cooled to 0 °C, and the addition of L-leucine methyl ester HCl (0.91 g, 5.0 mmol) followed. After the addition, the mixture was allowed to stir and warm to room temperature for twelve hours. The methylene chloride was removed by evaporation yielding a viscous yellow oil. The product was then dissolved in 50 mL ethyl acetate and washed successively with 25 mL aliquots of 5% HCl, water, sat. $NaHCO_3$, and sat. NaCl. The product was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation yielding 1.5 g of the crude dipeptide. Purified by flash column chromatography (59 g silica, 13:7 n-hexane:ethyl acetate) affording white crystals of Cbz-Ile-LeuOMe (820 mg, 2.1 mmol, 42%). Melting point: 109-111 °C

^{1}H NMR: δ 0.89-0.95 (m, 12H), 1.12-1.26 (m, 1H), 1.50-1.60 (m, 2H), 1.62-1.67 (m, 2H), 1.85-1.87 (m, 1H), 3.73 (s, 3H), 4.04 (dd, 1H, J=8.00, 7.20 Hz), 4.59-4.64 (m, 1H), 5.11 (s, 2H), 5.35 (d, 1H, J=8.06 Hz), 6.17 (d, 1H, J=6.77 Hz), 7.31-7.37 (m, 5H)

^{13}C NMR: δ 11.34, 15.37, 21.91, 22.73, 24.79, 24.84, 37.54, 41.52, 50.77, 52.25, 59.65, 67.05, 128.0, 128.2, 128.5, 136.3, 156.2, 170.9, 173.0

Cbz-Valine-Leucine methyl ester dipeptide (9). Triethylamine (0.7 mL, 5.0 mmol) was added dropwise over ten minutes to a stirred mixture of Cbz-valine (0.63 g, 2.5 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.4 mL, 2.5 mmol) in methylene chloride (20 mL) at 0 °C. After the addition, the mixture was allowed to stir and warm to room temperature for one hour. The mixture was cooled to 0 °C, and L-leucine methyl ester HCl (0.45 g, 2.5 mmol) was added to it. After the addition, the mixture was allowed to warm to room temperature for twelve hours. The methylene chloride was removed by evaporation yielding a viscous yellow oil. The product was then dissolved in 50 mL ethyl acetate and washed successively with 25 mL aliquots of 5% HCl, water, sat. $NaHCO_3$, and sat. NaCl. The product was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation yielding 0.80 g of the crude dipeptide. Purified by flash column chromatography (32 g silica, 13:7 n-hexane:ethyl acetate) affording white crystals of Cbz-Val-LeuOMe (460 mg, 1.2 mmol, 49%). Melting point: 97 °C (sublimes)

^{1}H NMR: δ 0.91-0.99 (m, 9H), 0.98 (d, 3H, J=6.80 Hz), 1.52-1.59 (m, 1H), 1.60-1.67 (m, 2H), 2.10-2.14 (m, 1H), 3.73 (s, 3H), 4.05 (dd, 1H, J=8.00, 7.20 Hz), 4.59-4.64 (m, 1H), 5.11 (s, 2H), 5.42 (d, 1H, J=8.60 Hz), 6.34 (d, 1H, J=7.87 Hz), 7.30-7.36 (m, 5H)

^{13}C NMR: δ 17.79, 19.08, 21.88, 22.72, 24.83, 31.20, 41.46, 50.76, 52.21, 60.32, 67.03, 128.0, 128.1, 128.5, 136.3, 156.3, 171.0, 173.1

Cbz-Valine-Valine methyl ester dipeptide (10). To a stirred mixture of Cbz-valine (0.63 g, 2.5 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.4 mL, 2.5

mmol) in methylene chloride (20 mL) was added dropwise triethylamine (0.7 mL, 5.0 mmol) at 0 °C for fifteen minutes. After the addition, the mixture was allowed to warm to room temperature for one hour. The mixture was cooled to 0 °C and L-valine methyl ester HCl (0.42 g, 2.5 mmol) was added to it. After the addition, the mixture was allowed to warm to room temperature for twelve hours. The methylene chloride was removed by rotary evaporation yielding a viscous yellow oil. The product was then dissolved in 50 mL ethyl acetate and washed successively with 25 mL aliquots of 5% HCl, water, sat. $NaHCO_3$, and sat. NaCl. The product was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation yielding 0.85 g of the crude dipeptide. Purified by flash column chromatography (35 g silica, 13:7 n-hexane:ethyl acetate) affording white crystals of Cbz-Val-ValOMe (540 mg, 1.5 mmol, 59%). Melting point: 102-106 °C

^{1}H NMR: δ 0.88-1.00 (m, 9H), 0.98 (d, 3H, J=6.80 Hz), 2.10-2.19 (m, 2H), 3.74 (s, 3H), 4.07 (dd, 1H, J=8.00, 7.20 Hz), 4.54 (dd, 1H, J= 8.79, 4.94 Hz), 5.12 (s, 2H), 5.41 (d, 1H, J=8.06 Hz), 6.41 (d, 1H, J=8.97 Hz), 7.30-7.36 (m, 5H)

^{13}C NMR: δ 17.75, 17.83, 18.87, 19.13, 31.05, 31.14, 52.08, 57.14, 60.49, 67.04, 128.0, 128.1, 128.5, 136.3, 156.4, 171.2, 172.1

Cbz-Isoleucine-Proline methyl ester (11). For fifteen minutes at 0 °C, triethylamine (0.7 mL, 5.0 mmol) was added dropwise to a stirred mixture of Cbz-isoleucine (0.66 g, 2.5 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.4 mL, 2.5 mmol) in methylene chloride (20 mL). After the addition, the mixture was allowed to warm to room temperature for one hour. The mixture was then cooled to 0 °C, and the addition

mixture was allowed to warm to room temperature for twelve hours. The methylene chloride was removed by evaporation yielding a viscous yellow oil. The product was then dissolved in 50 mL 2:1 ethyl acetate:benzene solution and washed successively with 25 mL aliquots of 5% HCl, water, sat. $NaHCO_3$, and sat. NaCl. The product was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation yielding 0.73 g of the crude dipeptide. The crude product was then purified by flash column chromatography (30 g silica, 11:9 n-hexane:ethyl acetate) affording a clear oil of Cbz-Ile-ProOMe (210 mg, 0.56 mmol, 22%).

^{1}H NMR: δ 0.91 (t, 3H, J= 7.42 Hz), 1.04 (d, 3H, J= 6.96 Hz), 1.12-1.18 (m, 1H), 1.57-1.63 (m, 1H), 1.77-1.81 (m, 1H), 1.96-2.08 (m, 3H), 2.21-2.26 (m, 1H), 3.65-3.69 (m, 1H), 3.72 (s, 3H), 3.81-3.85 (m, 1H), 4.35 (dd, 1H, J=9.25, 7.60 Hz), 4.53 (dd, 1H, J=8.62, 4.67 Hz), 5.08 (dd, 2H, J= 12.27 Hz), 5.43 (d, 1H, J= 9.34 Hz), 7.30-7.38 (m, 5H)

^{13}C NMR: δ 11.12, 15.20, 24.26, 24.93, 29.06, 37.89, 47.25, 52.11, 56.81, 58.85, 66.87, 128.0, 128.1, 128.5, 136.4, 156.4, 171.1, 172.3

Cbz-Valine-Proline methyl ester dipeptide (12). To a stirred mixture of Cbz-valine (0.63 g, 2.5 mmol) and bis (2,2,2-trifluoroethoxy) phosphorochloridate (0.4 mL, 2.5 mmol) in methylene chloride (20 mL) was added dropwise triethylamine (0.7 mL, 5.0 mmol) at 0 °C for fifteen minutes. After the addition, the mixture was allowed to warm to room temperature for one hour. The mixture was cooled to 0 °C and L-proline methyl ester HCl (0.41 g, 2.5 mmol) was added to it. After the addition, the mixture was allowed to warm to room temperature for twelve hours. The methylene chloride was removed by rotary evaporation yielding a viscous yellow oil. The product was dissolved

HCl, water, sat. $NaHCO_3$, and sat. NaCl. The product was dried over anhydrous Na_2SO_4 and removed by rotary evaporation yielding 0.82 g of the crude dipeptide. Flash column chromatography (17 g silica, 11:9 n-hexane:ethyl acetate) purified the crude product affording a clear oil of Cbz-Ile-ProOMe (360 mg, 0.99 mmol, 40%).

^{1}H NMR: δ 0.95 (d, 3H, J= 6.78 Hz), 1.05 (d, 3H, J=6.77 Hz), 1.96-2.09 (m, 4H), 2.22-2.25 (m, 1H), 3.66-3.70 (m, 1H), 3.72 (s, 3H), 4.34 (dd, 1H, J=9.25, 6.50 Hz), 4.53 (dd, 1H, J=8.51, 4.85 Hz), 5.09 (dd, 2H, J= 12.36 Hz), 5.51 (d, 1H, J= 9.34 Hz), 7.30-7.36 (m, 5H)

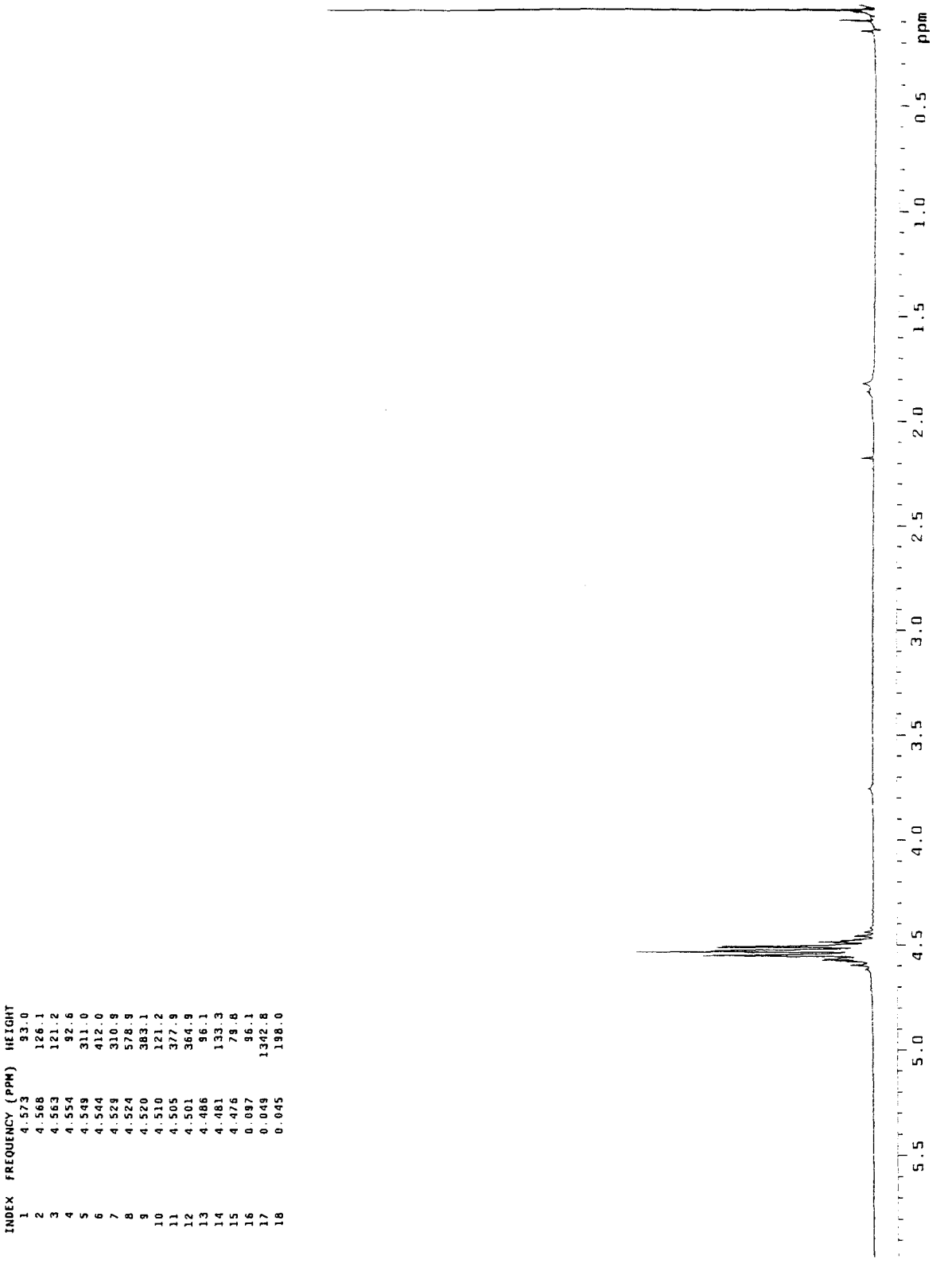
^{13}C NMR: δ 17.46, 19.20, 24.95, 29.01, 31.35, 47.17, 52.11, 57.49, 58.80, 66.84, 127.9, 128.0, 128.5, 136.4, 156.4, 170.9, 172.3

Attempted Synthesis of Cbz-O-benzyl-serine (13). To a solution of Cbz-serine (6.70 g, 30.0 mmol) in DMF (150 mL) was added sodium hydride (60%) (2.64 g, 66.0 mmol) at 0 °C. After the evolution of hydrogen gas ceased, benzyl bromide (3.90 mL, 33.0 mmol) was added to the solution. The reaction was allowed to stir overnight. The solvent was removed by evaporation below 40 °C. The residue was dissolved in water (150 mL) and the solution was extracted with diethyl ether (2X60 mL). The aqueous phase was acidified to pH 3.5 with 3N HCl and extracted with ethyl acetate (5X60 mL). The combined organic layers were dried with anhydrous sodium sulfate and the solvent was removed by evaporation. The reaction mixture was purified by flash column chromatography, but the diprotected amino acid **13** was not isolated.

References

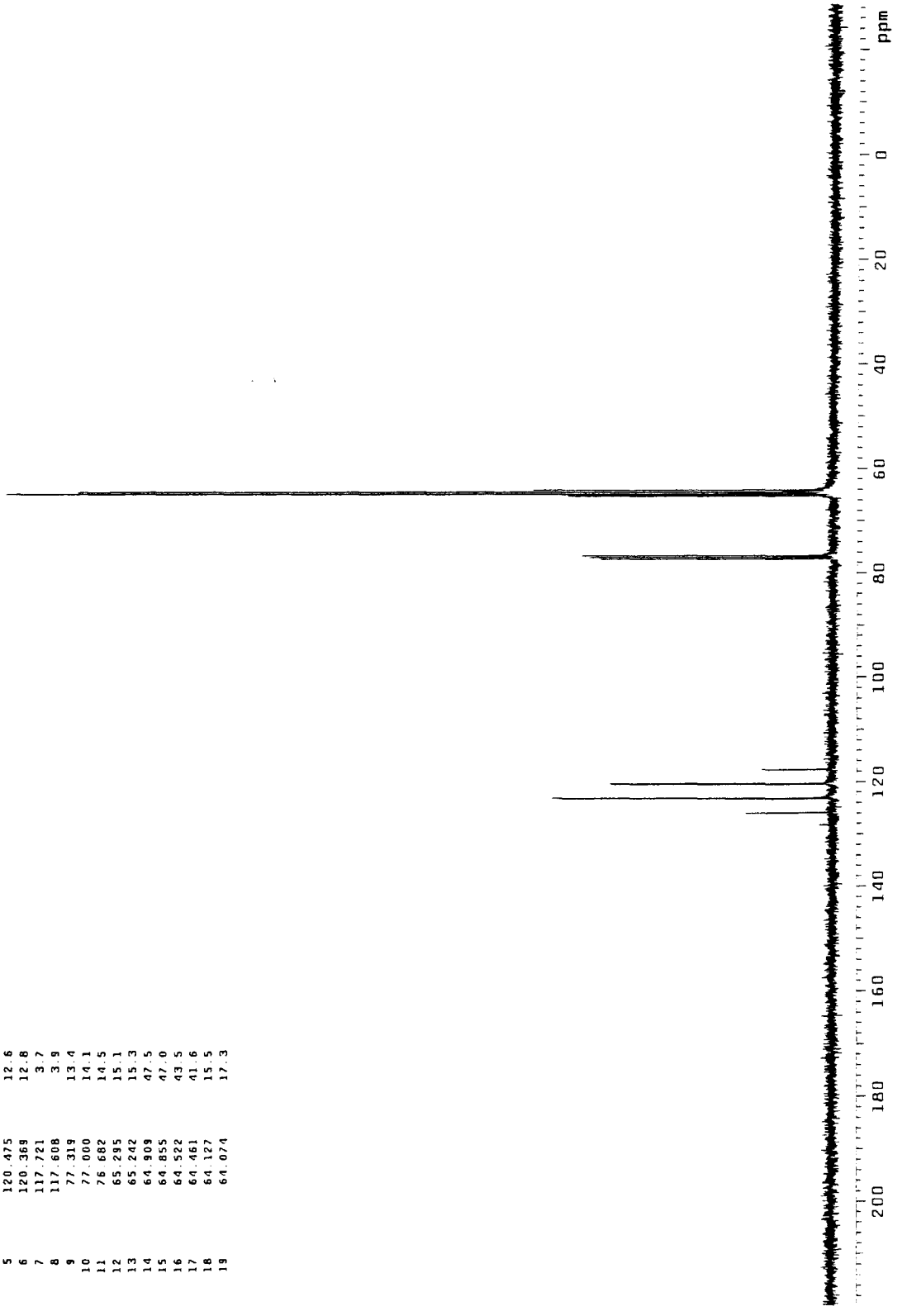
(1) Jones, J., In *Amino Acid and Peptide Synthesis*; Oxford University Press, **1992**.
(2) Fischer, E. *Ber.* **1906**, *39*, 530
(3) Bergmann, M.; Zervas, L. *Ber.* **1932**, *65*, 1192
(4) Vaughan, J.R. *J. Amer. Chem. Soc.* **1951**, *73*, 3547
(5) Vigneaud, V.; Ressler, C.; Swan, J.M.; Roberts, C.W.; Katsoyannis, P.G.; Gordon, S. *J. Amer. Chem. Soc.* **1953**, *75*, 4879
(6) Sheehan, J.C.; Hess, G.P. *J. Amer. Chem. Soc.* **1955**, *77*, 1067
(7) Carpino, L.A. *J. Amer. Chem. Soc.* **1957**, *79*, 98
(8) Zervas, L.; Boravas, D.; Gazis, E. *J. Amer. Chem. Soc.* **1963**, *85*, 3660
(9) Nefkens, G.H.; Tesser, G.I. *J. Amer. Chem. Soc.* **1961**, *83*, 1263
(10) Anderson, G.W.; Zimmerman, J.E.; Callahan, F.M. *J. Amer. Chem. Soc.* **1963, *85***, 3039
(11) Belleau, B; Malek, G. *J. Amer. Chem. Soc.* **1968**, *90*, 1651
(12) Bodanszky, M.; Klausner, Y.; Ondetti, M. In *Peptide Synthesis*; John Wiley & Sons, Inc., **1976**; 2nd ed.
(13) Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Letters* **1973**, *18*, 1595-1598
(14) Chen, S.; Xu, J. *Tetrahedron Letters* **1991**, *32, No. 46*, 6711-6714
(15) Sellars, K. *J. Appl. Chem.* **1956**, *6*, January, 45-48
(16) Fruton, J.S. *J. Bio. Chem.* **1942**, *146*, 463
(17) Sugano, H.; Miyoshi, M. *J. Org. Chem.* **1976**, *41*, 2352

Figure 7 ^{1}H NMR: bis (2,2,2-trifluoroethoxy) phosphorochloridate



INDEX	FREQUENCY (PPM)	HEIGHT
1	4.573	93.0
2	4.568	126.1
3	4.563	121.2
4	4.554	92.6
5	4.549	311.0
6	4.544	412.0
7	4.529	310.9
8	4.524	578.9
9	4.520	383.1
10	4.510	121.2
11	4.505	377.9
12	4.501	364.9
13	4.486	96.1
14	4.481	133.3
15	4.476	79.8
16	0.097	96.1
17	0.049	1342.8
18	0.045	198.0

Figure **8** ^{13}C NMR: bis (2,2,2-trifluoroethoxy) phosphorochloridate



INDEX	FREQUENCY (PPM)	HEIGHT
1	125.990	4.9
2	125.876	3.9
3	123.236	15.7
4	123.123	16.2
5	120.475	12.6
6	120.369	12.8
7	117.721	3.7
8	117.608	3.9
9	77.319	13.4
10	77.000	14.1
11	76.682	14.5
12	65.295	15.1
13	65.242	15.3
14	64.909	47.5
15	64.855	47.0
16	64.522	43.5
17	64.461	41.6
18	64.127	15.5
19	64.074	17.3

Figure **9** ^{31}P NMR: bis (2,2,2-trifluoroethoxy) phosphorochloridate

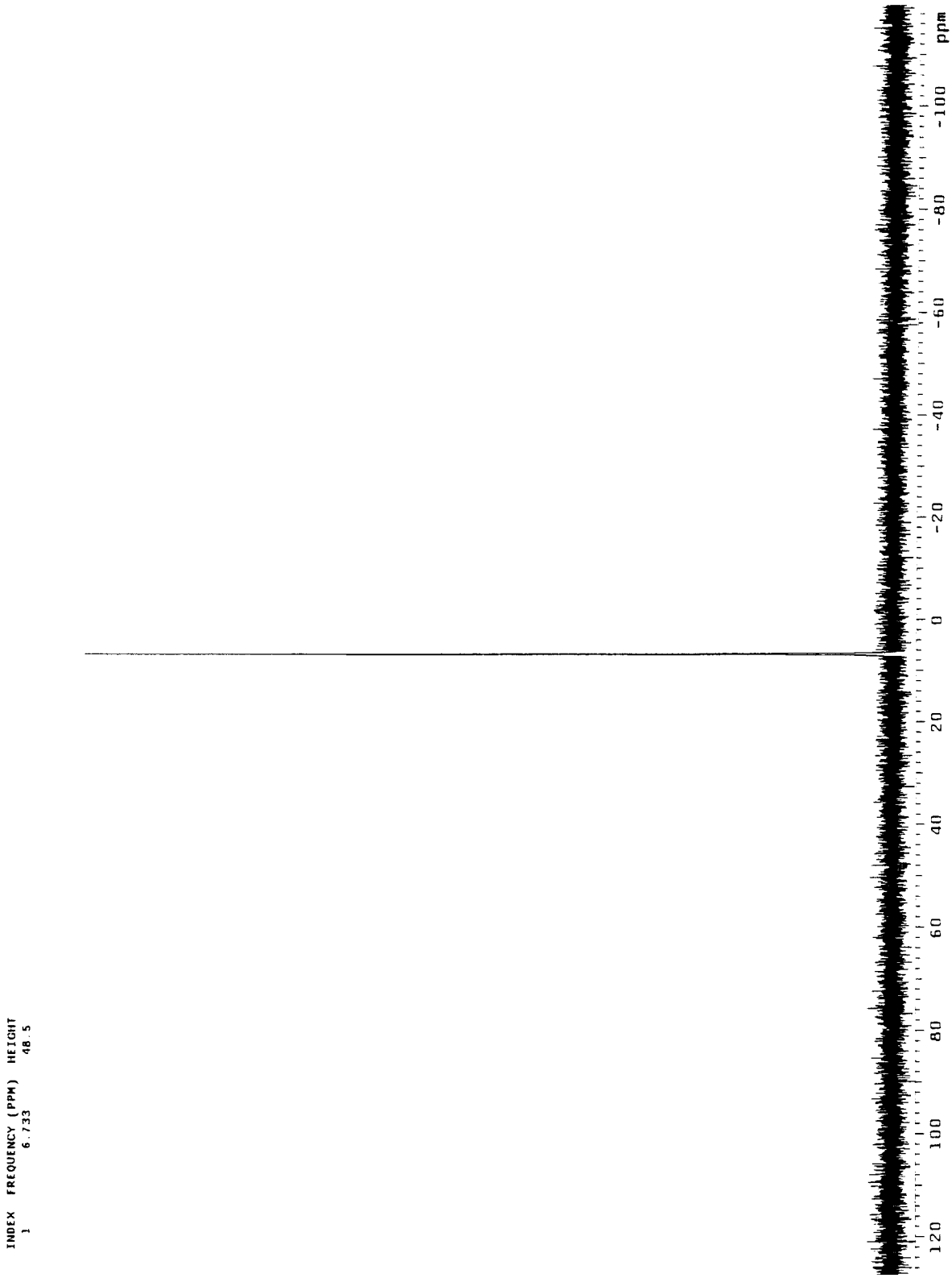


Figure **10** ^{1}H NMR: N-benzyl benzamide

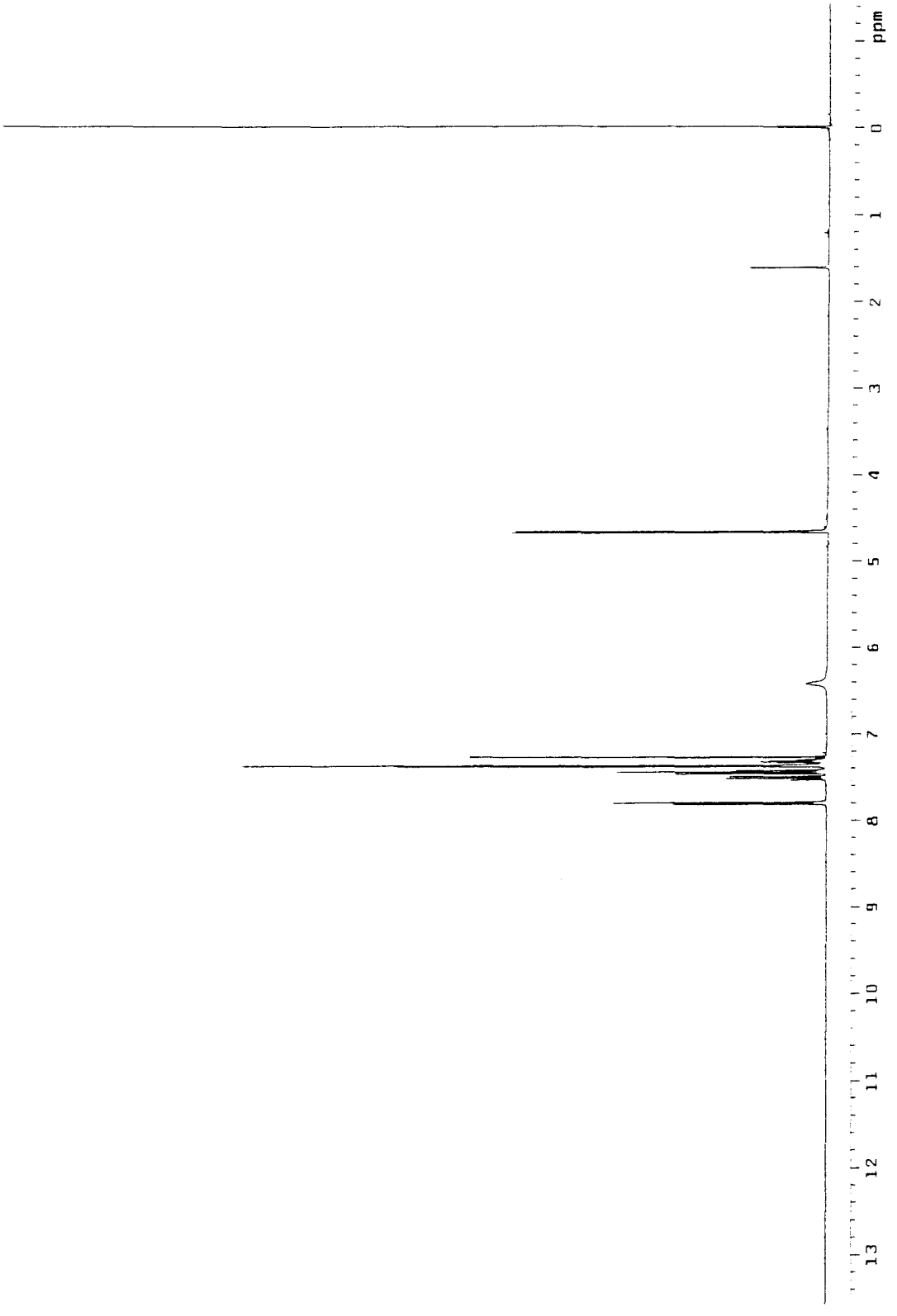
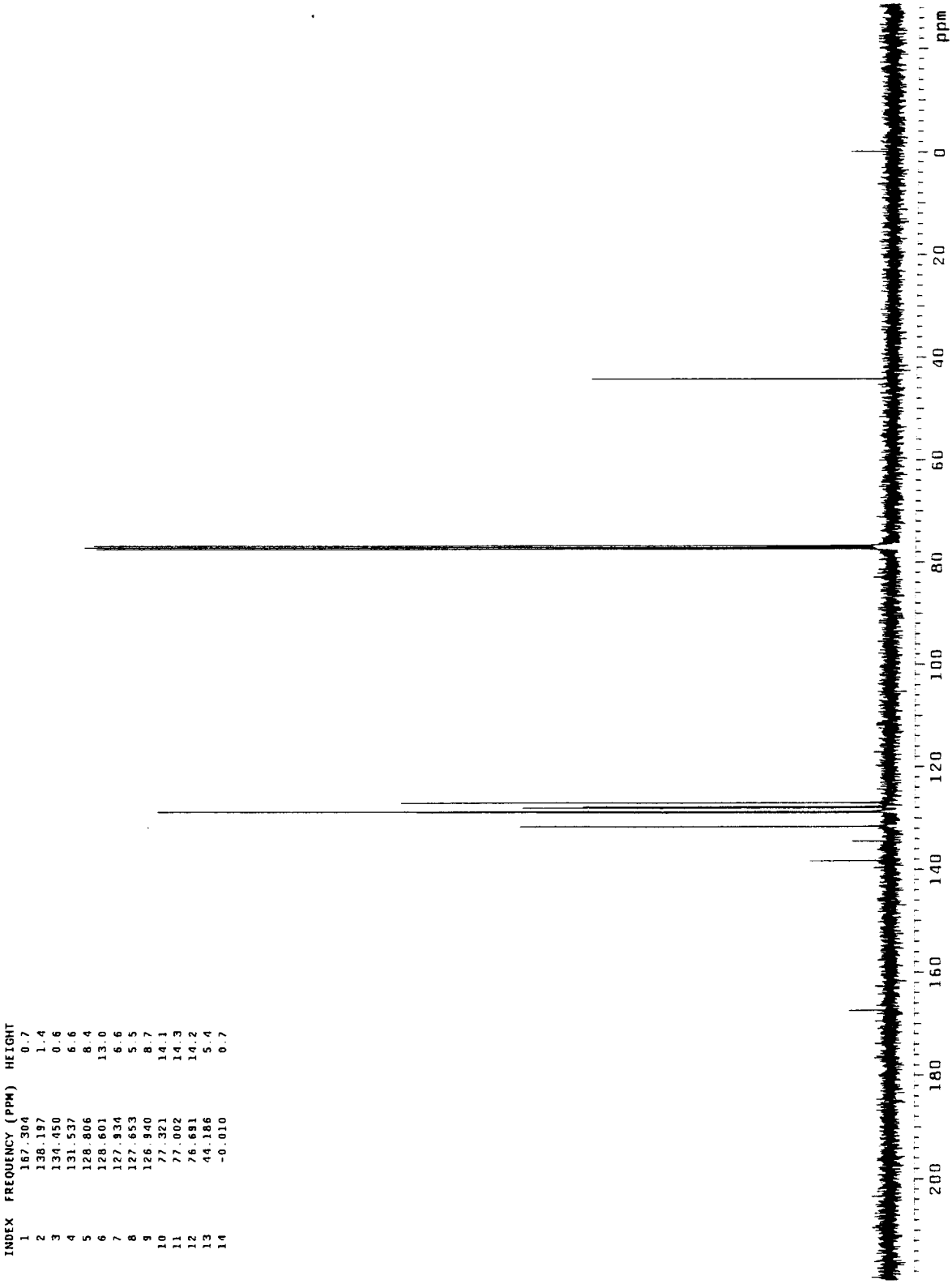


Figure 11 ^{13}C NMR: N-benzyl benzamide



INDEX	FREQUENCY (PPM)	HEIGHT
1	167.304	0.7
2	138.197	1.4
3	134.450	0.6
4	131.537	6.6
5	128.806	8.4
6	128.601	13.0
7	127.934	6.6
8	127.653	5.5
9	126.940	8.7
10	77.321	14.1
11	77.002	14.3
12	76.691	14.2
13	44.186	5.4
14	-0.010	0.7

Figure 12 ^{1}H NMR: Cbz-Ile-ValOMe

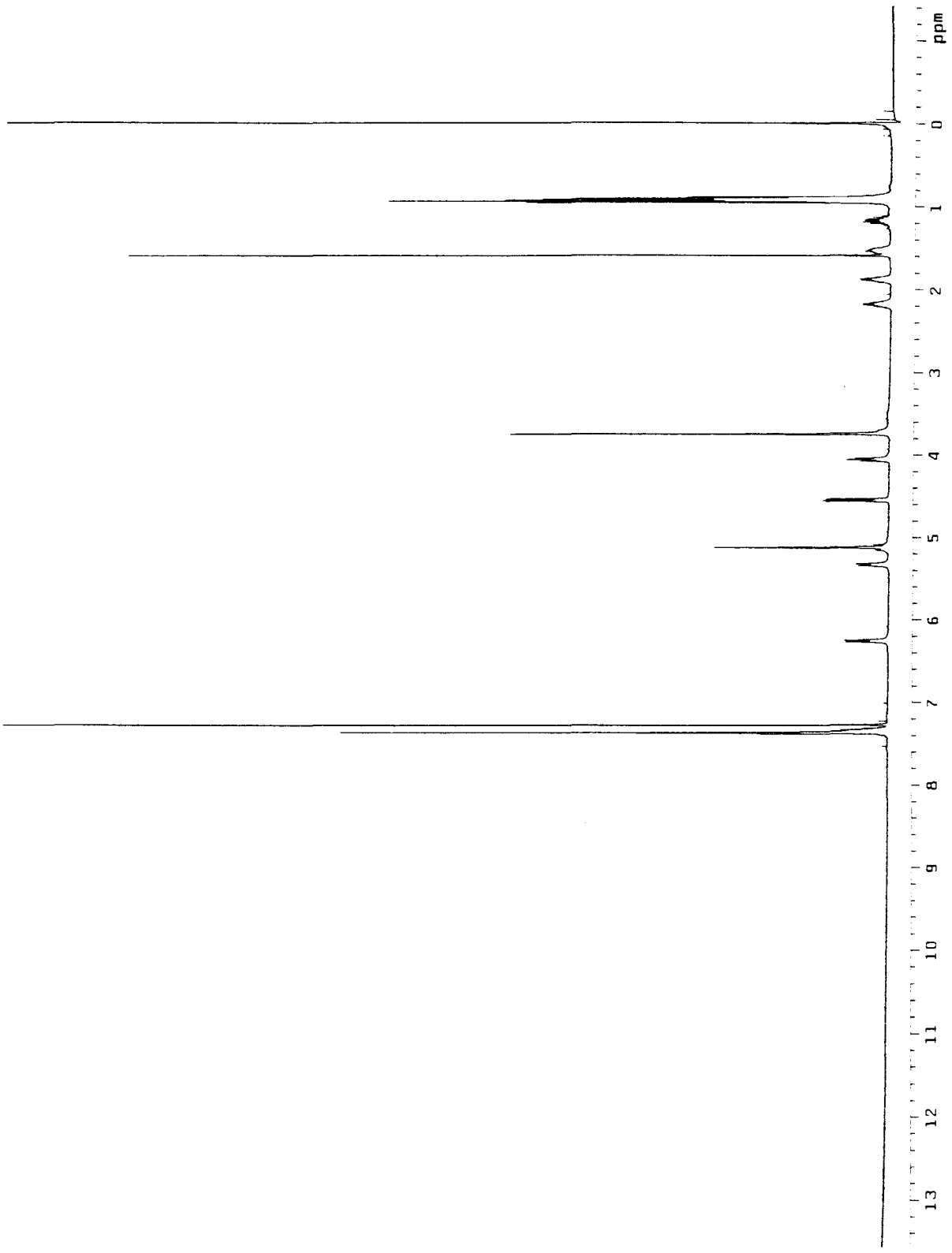
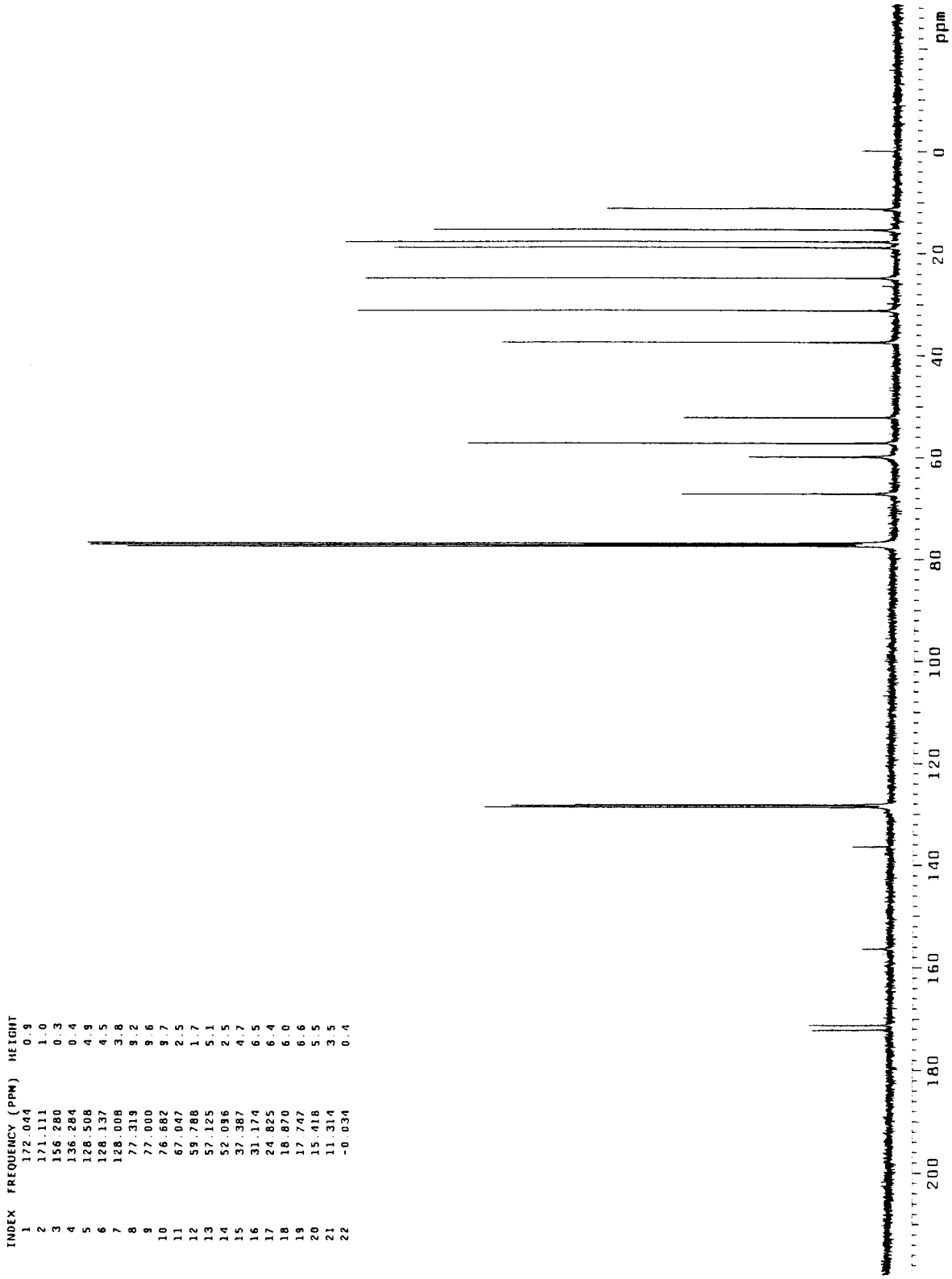


Figure **13** ^{13}C NMR: Cbz-Ile-ValOMe



INDEX	FREQUENCY (PPM)	HEIGHT
1	172.044	0.9
2	171.111	1.0
3	156.280	0.3
4	136.284	0.4
5	128.508	4.9
6	128.137	4.5
7	128.008	3.8
8	77.319	9.2
9	77.000	9.6
10	76.682	9.7
11	67.047	2.5
12	59.788	1.7
13	57.125	5.1
14	52.096	2.5
15	37.387	4.7
16	31.174	6.5
17	24.825	6.4
18	18.870	6.0
19	17.747	6.6
20	15.418	5.5
21	11.314	3.5
22	-0.034	0.4

Figure **14** ^{1}H NMR: Cbz-Ile-LeuOMe

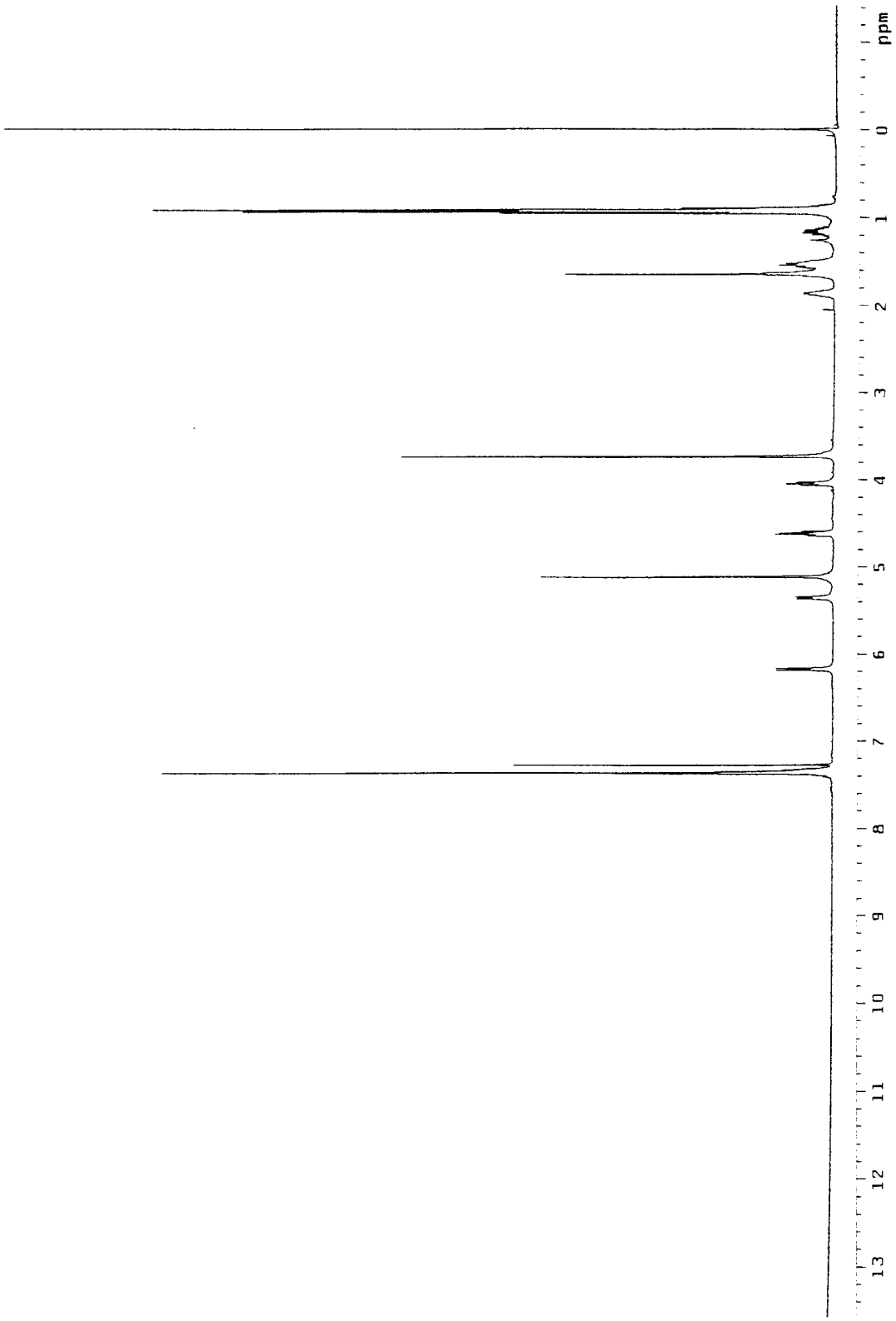
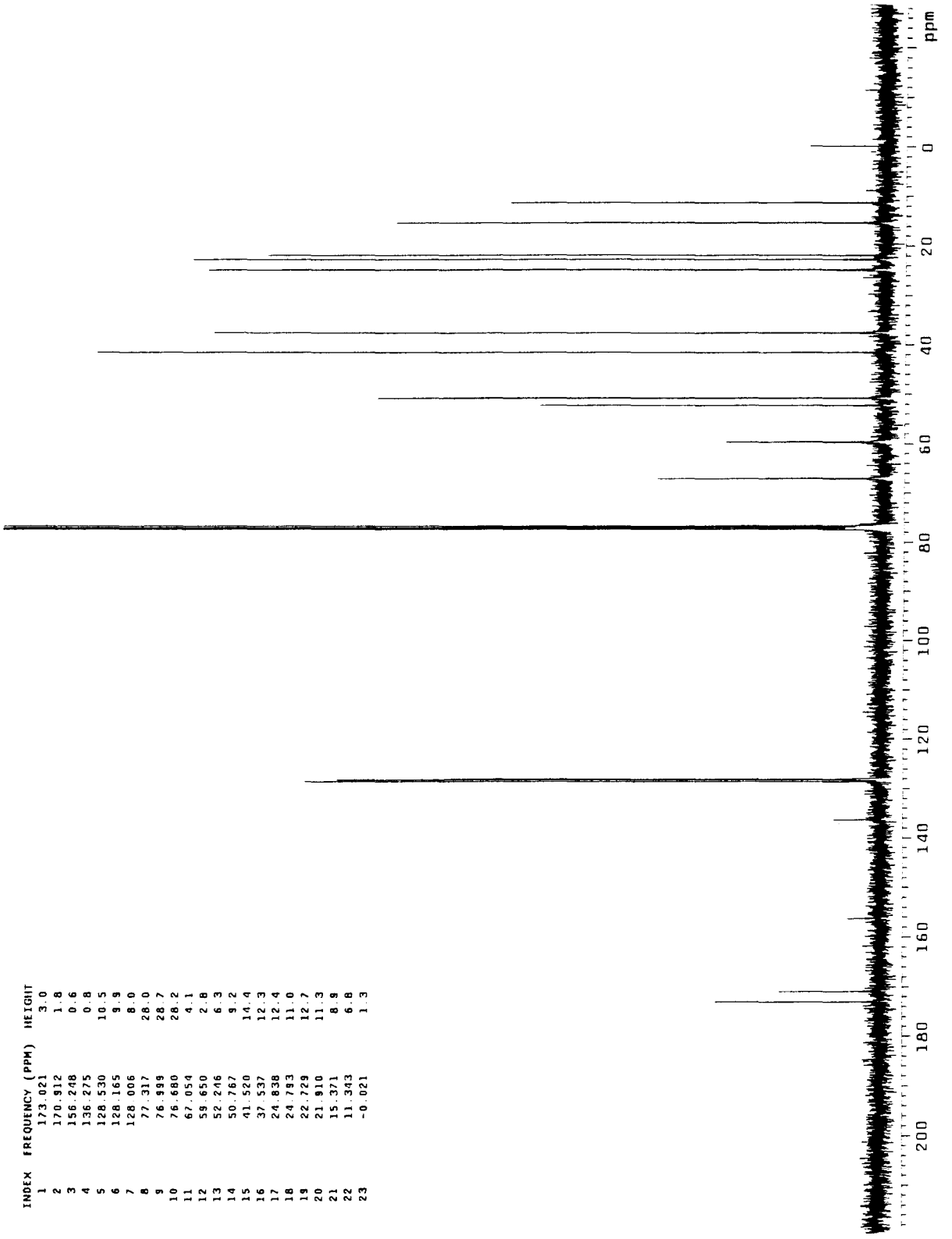


Figure **15** ^{13}C NMR: Cbz-Ile-LeuOMe



INDEX	FREQUENCY (PPM)	HEIGHT
1	173.021	3.0
2	170.912	1.8
3	156.248	0.6
4	136.275	0.8
5	128.530	10.5
6	128.165	9.9
7	128.006	8.0
8	77.317	28.0
9	76.999	28.7
10	76.680	28.2
11	67.054	4.1
12	59.650	2.8
13	52.246	6.3
14	50.767	9.2
15	41.520	14.4
16	37.537	12.3
17	24.838	12.4
18	24.793	11.0
19	22.729	12.7
20	21.910	11.3
21	15.371	8.9
22	11.343	6.8
23	-0.021	1.3

Figure **16** ^{1}H NMR: Cbz-Valine

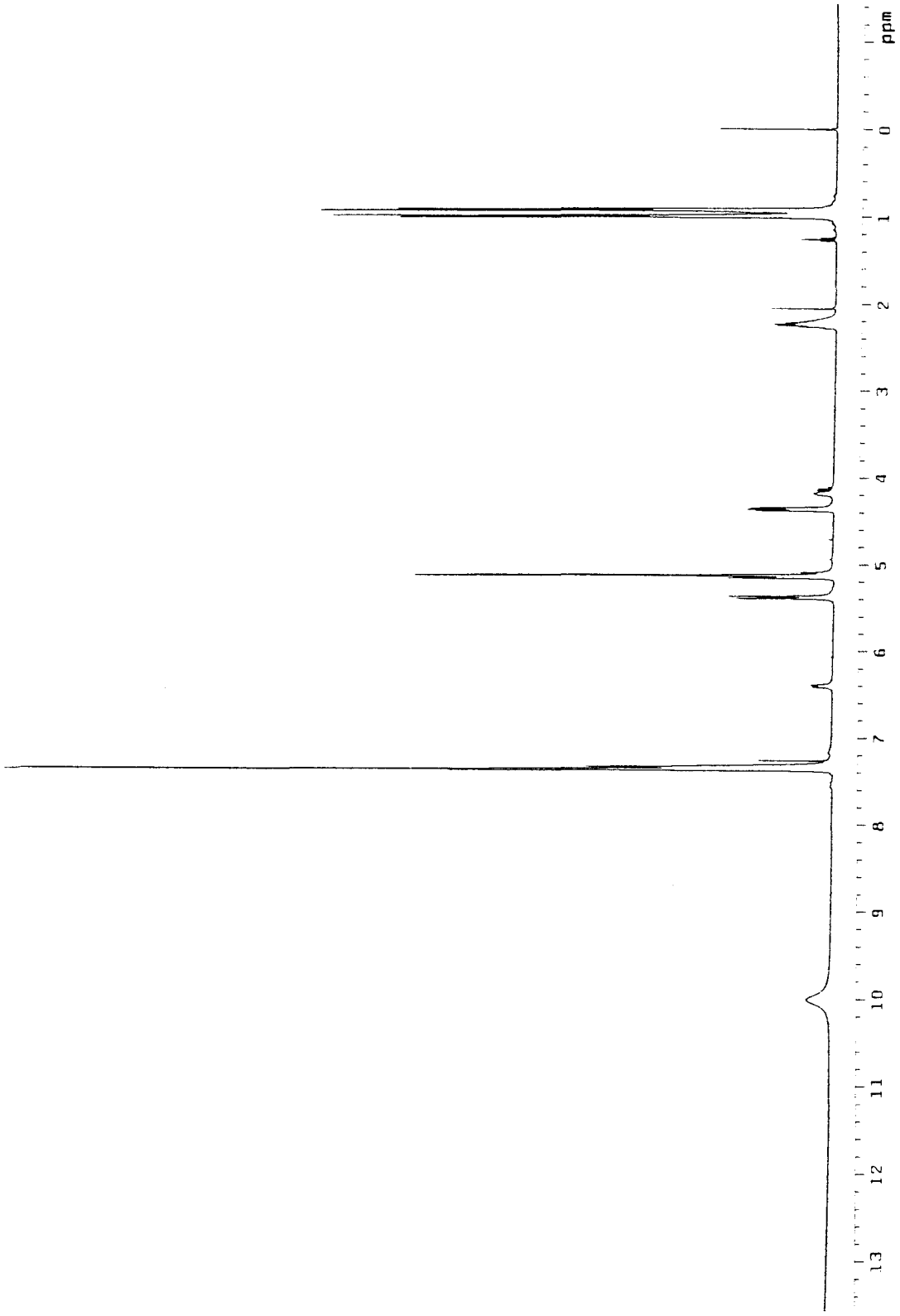
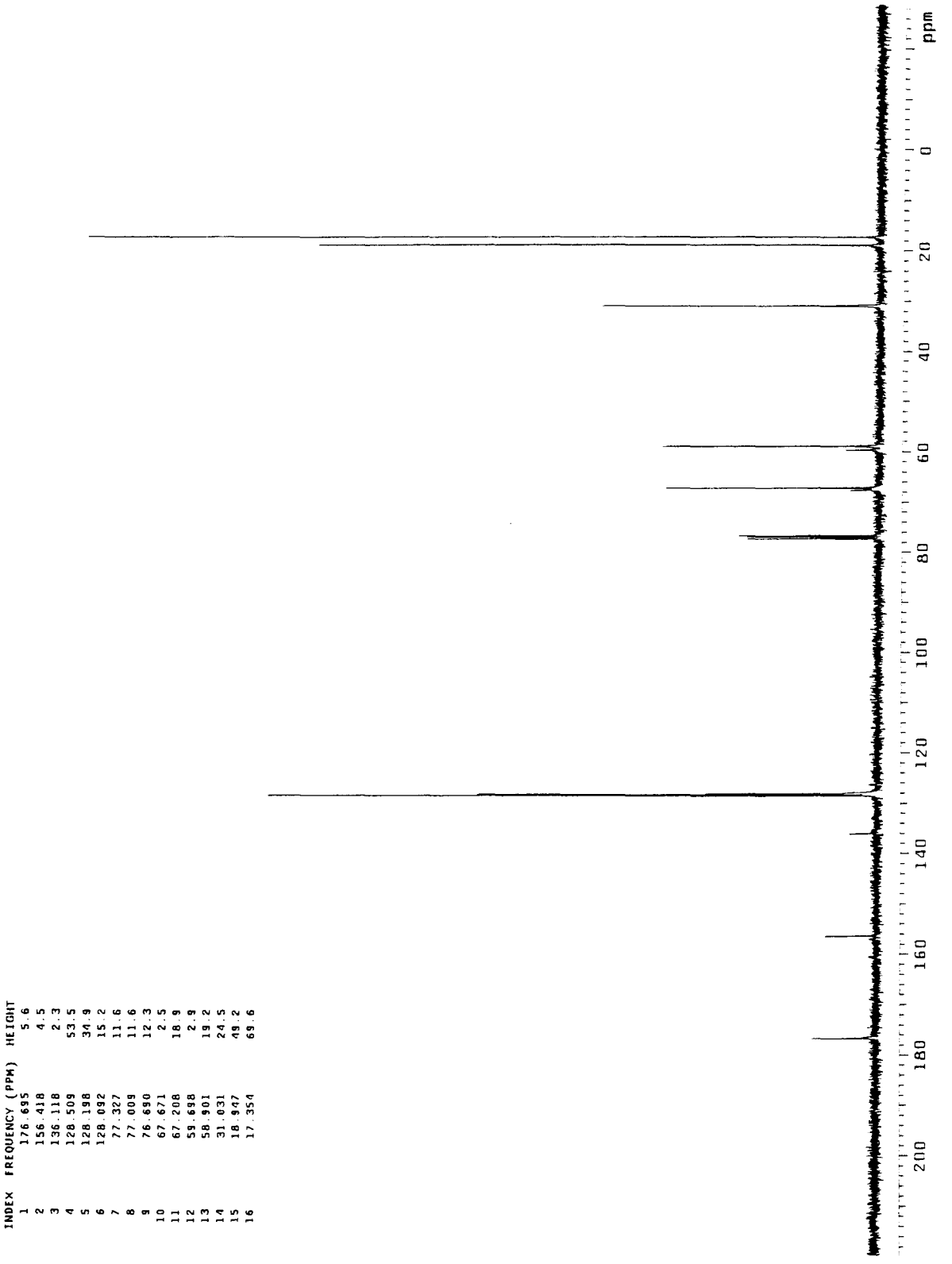


Figure 17 ^{13}C NMR: Cbz-Valine



INDEX	FREQUENCY (PPM)	HEIGHT
1	176.695	5.6
2	156.418	4.5
3	136.118	2.3
4	128.509	53.5
5	128.198	34.9
6	128.092	15.2
7	77.327	11.6
8	77.009	11.6
9	76.690	12.3
10	67.671	2.5
11	67.208	18.9
12	59.698	2.9
13	58.901	19.2
14	31.031	24.5
15	18.947	49.2
16	17.354	69.6

Figure **18** ^{1}H NMR: Cbz-Val-LeuOMe

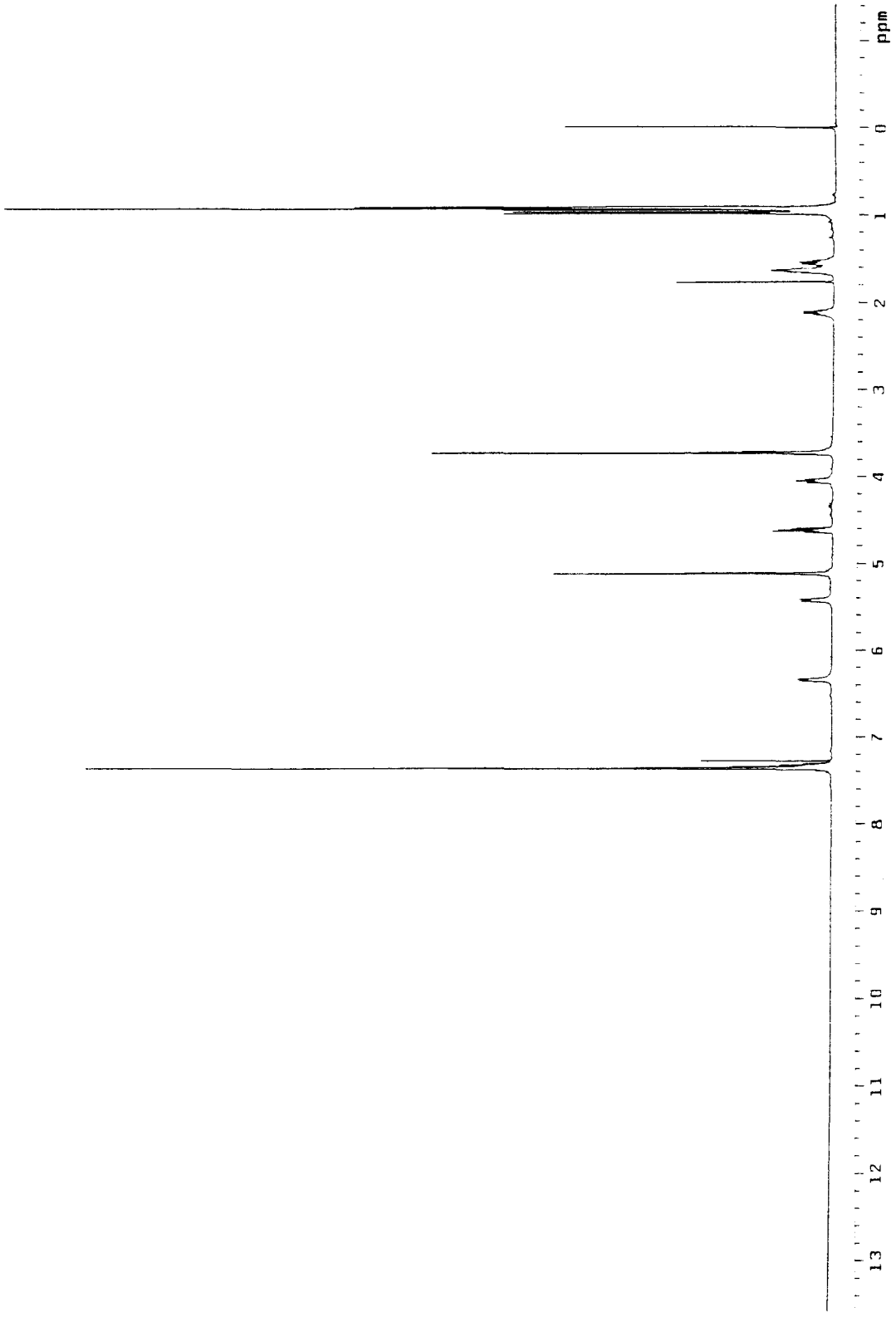
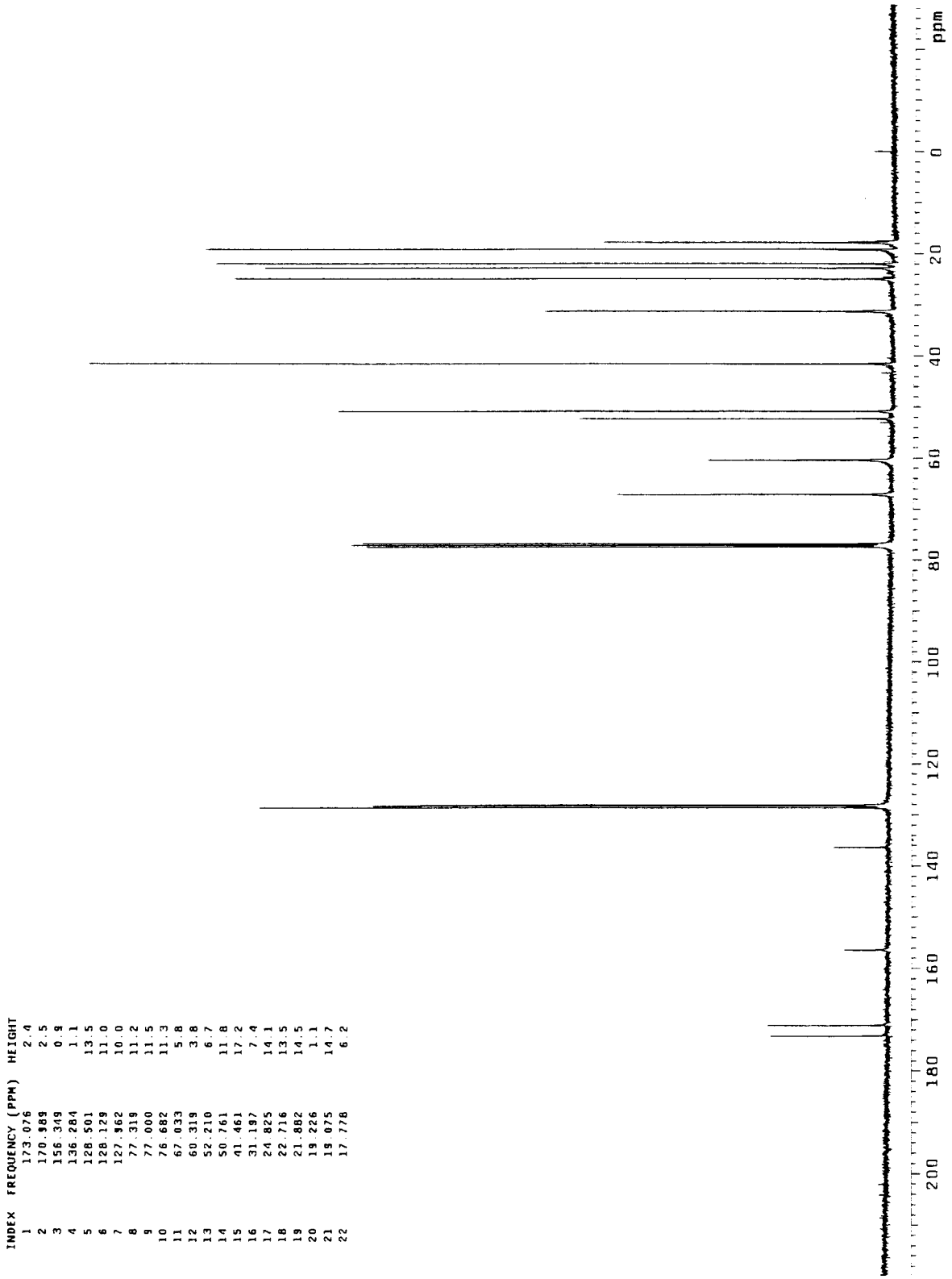


Figure 19 ^{13}C NMR: Cbz-Val-LeuOMe



INDEX	FREQUENCY (PPM)	HEIGHT
1	173.076	2.4
2	170.989	2.5
3	156.349	0.9
4	136.284	1.1
5	128.501	13.5
6	128.129	11.0
7	127.962	10.0
8	77.319	11.2
9	77.000	11.5
10	76.682	11.3
11	67.033	5.8
12	60.319	3.8
13	52.210	6.7
14	50.761	11.8
15	41.461	17.2
16	31.197	7.4
17	24.825	14.1
18	22.716	13.5
19	21.882	14.5
20	19.226	1.1
21	19.075	14.7
22	17.778	6.2

Figure **20** ^{1}H NMR: Cbz-Val-ValOMe

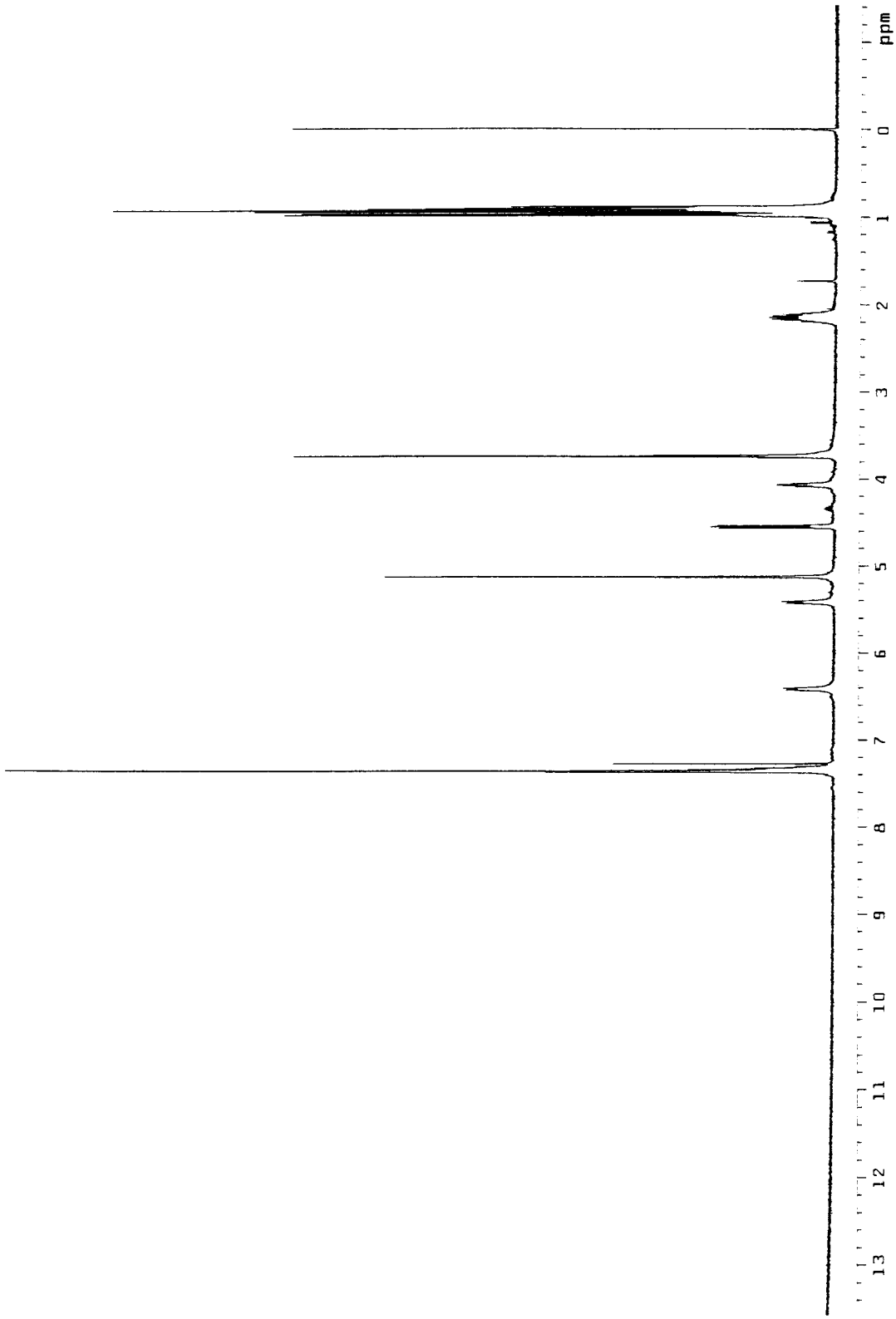
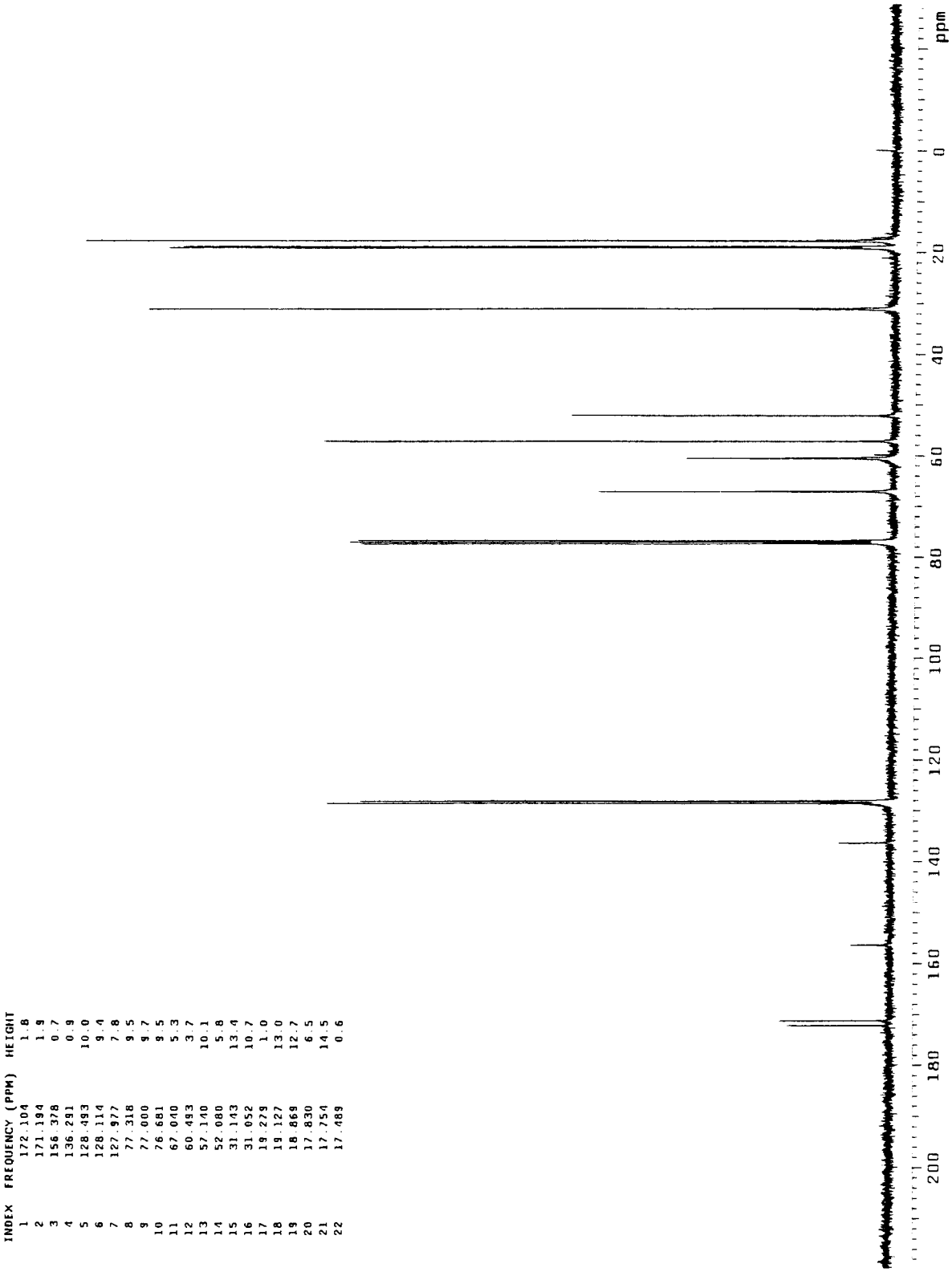


Figure **21** ^{13}C NMR: Cbz-Val-ValOMe



INDEX	FREQUENCY (PPM)	HEIGHT
1	172.104	1.8
2	171.194	1.9
3	156.378	0.7
4	136.291	0.9
5	128.493	10.0
6	128.114	9.4
7	127.977	7.8
8	77.318	9.5
9	77.000	9.7
10	76.681	9.5
11	67.040	5.3
12	60.493	3.7
13	57.140	10.1
14	52.080	5.8
15	31.143	13.4
16	31.052	10.7
17	19.279	1.0
18	19.127	13.0
19	18.869	12.7
20	17.830	6.5
21	17.754	14.5
22	17.489	0.6

Figure **22** ^{1}H NMR: Cbz-Ile-ProOMe

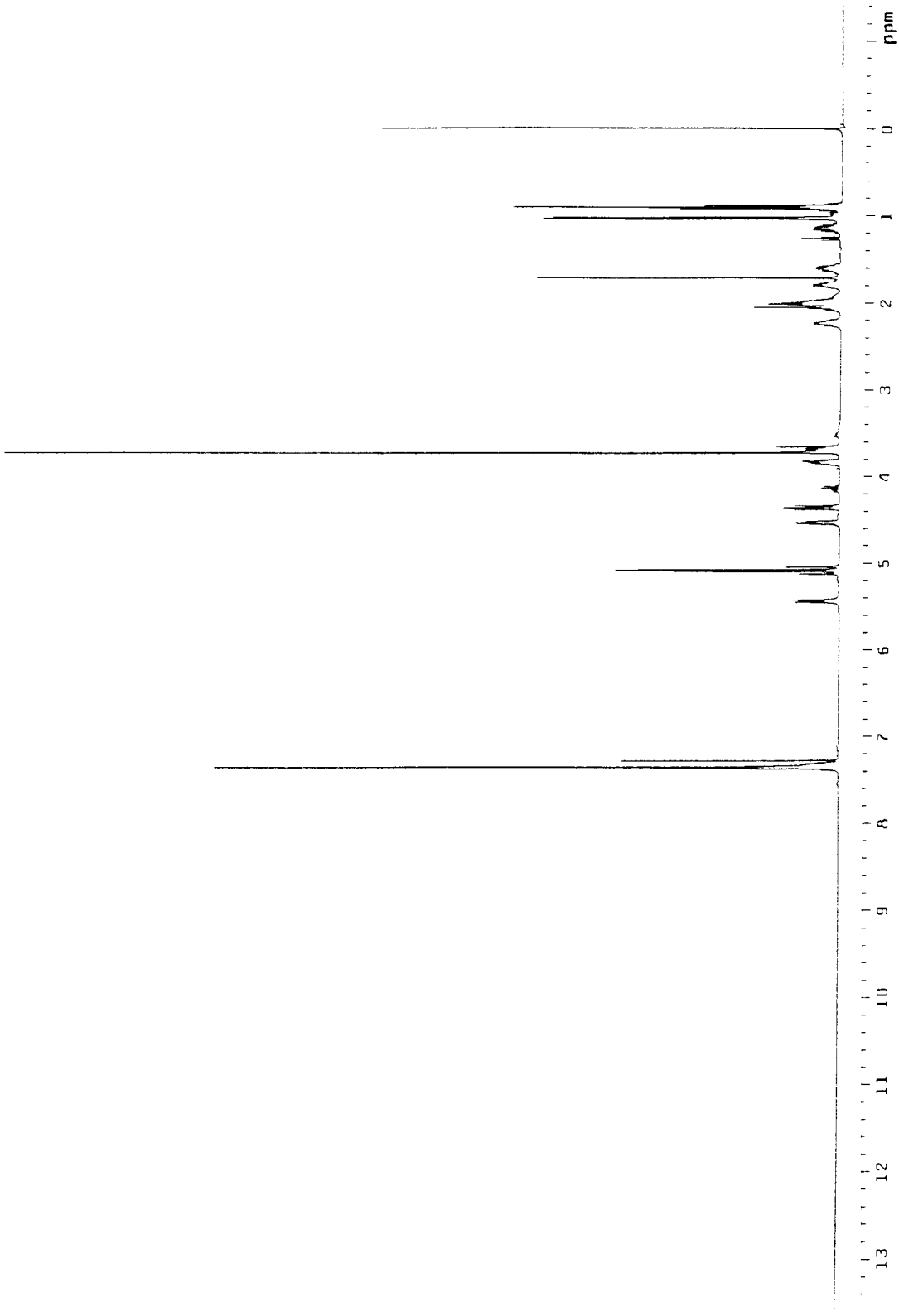
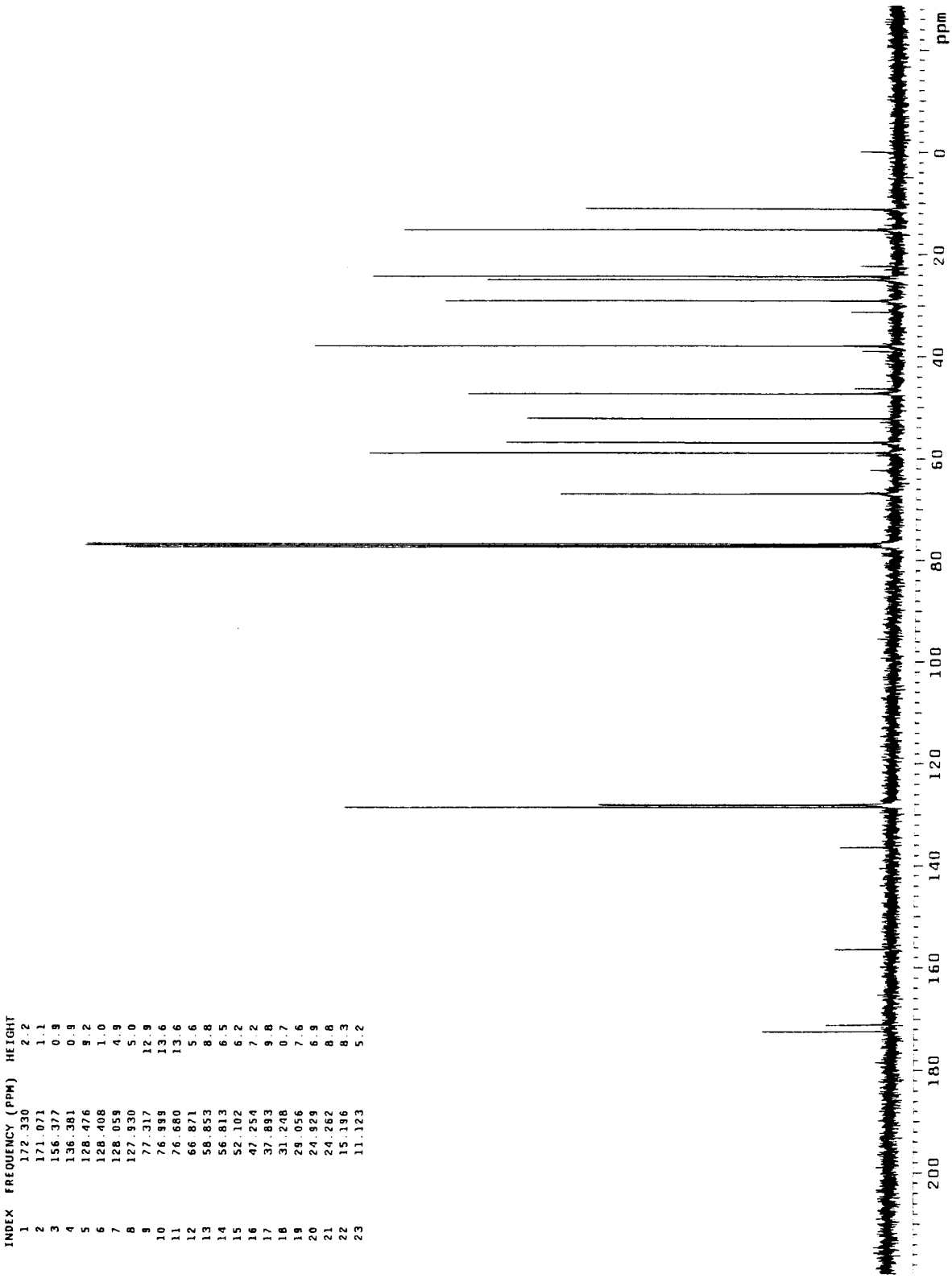


Figure **23** ^{13}C NMR: Cbz-Ile-ProOMe



INDEX	FREQUENCY (PPM)	HEIGHT
1	172.330	2.2
2	171.071	1.1
3	156.377	0.9
4	136.381	0.9
5	128.476	9.2
6	128.408	1.0
7	128.059	4.9
8	127.930	5.0
9	77.317	12.9
10	76.999	13.6
11	76.680	13.6
12	66.871	5.6
13	58.853	8.8
14	56.813	6.5
15	52.102	6.2
16	47.254	7.2
17	37.893	9.8
18	31.248	0.7
19	29.056	7.6
20	24.929	6.9
21	24.262	8.8
22	15.196	8.3
23	11.123	5.2

Figure **24** ^{1}H NMR: Cbz-Val-ProOMe

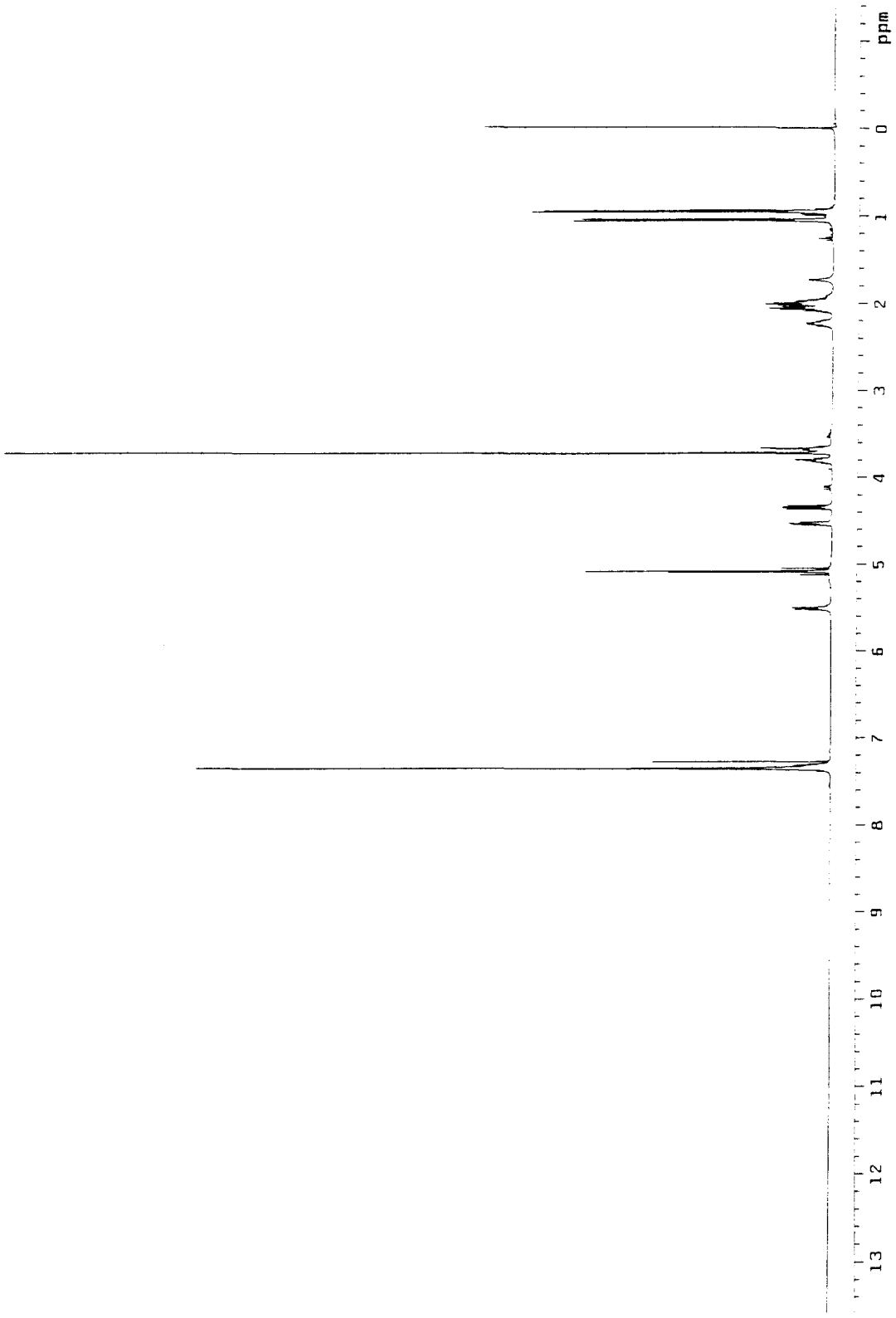


Figure **25** ^{13}C NMR: Cbz-Val-ProOMe