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Studies Toward Yaku'amide A and Synthesis and Applications of Bulky α,β -Dehydroamino Acids

Jintao Jiang
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Studies Toward Yaku'amide A and Synthesis and Applications of
Bulky α,β -Dehydroamino Acids

Jintao Jiang

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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July 2016

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ABSTRACT

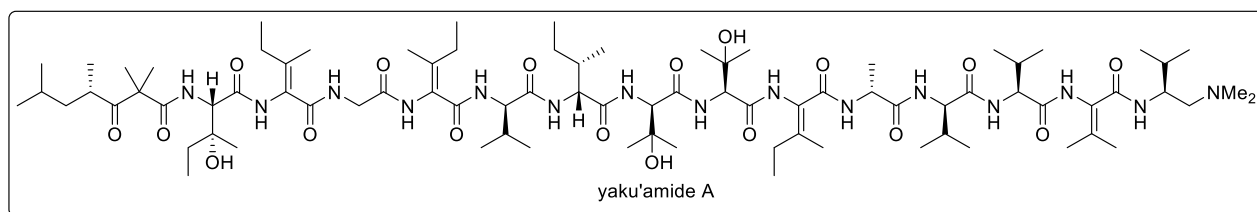
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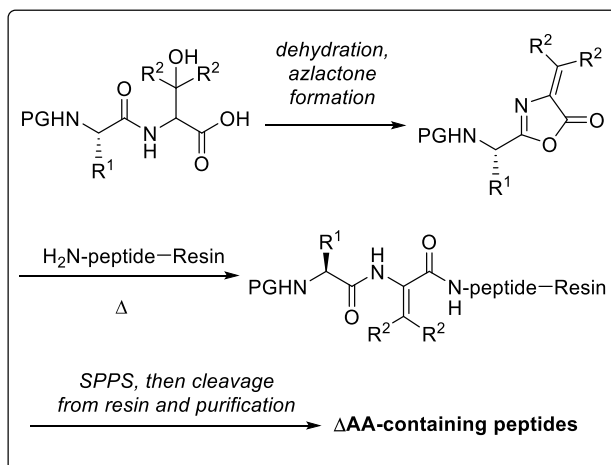
Department of Chemistry and Biochemistry, BYU

Doctor of Philosophy

Yaku'amide A shows a unique inhibitory profile against a series of 39 human cancer cell lines (JFCR39). In our efforts to synthesize yaku'amide A, we have optimized our regioselective base-free aminohydroxylation method with a series of nitrogen sources, developed a chiral reagent-mediated aminohydroxylation strategy and chemoselective deprotections of the resulting aminohydroxylation product, and explored a stereospecific E2 dehydration and O \rightarrow N acyl transfer sequence. In addition, we have prepared the right-hand tetrapeptide and the NTA subunit.



For our bulky α,β -dehydroamino acids project, we have developed strategies to incorporate α,β -dehydroamino acids such as Δ Val and Δ Env into small synthetic peptides via Solid Phase Peptide Synthesis (SPPS). We have also prepared two analogues of a monomeric 3₁₀-helical peptide with 13 residues.



Keywords: yaku'amide A, inhibitory profile, chiral reagent-mediated aminohydroxylation, chemoselective deprotections, stereospecific E2 dehydration, right-hand tetrapeptide, NTA, bulky α,β -dehydroamino acids, Δ Val and Δ Env, SPPS, analogues

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TABLE OF CONTENTS

TITLE PAGE	i
ABSTRACT.....	ii
TABLE OF CONTENTS.....	iv
LIST OF TABLES.....	vi
LIST OF FIGURES	vii
LIST OF SCHEMES.....	ix
Chapter 1. Yaku'amide A Introduction	1
1.1 Structure and Bioactivity.....	1
1.2 Dehydroisoleucine Synthesis	3
1.3 Inoue Group Synthesis of Dehydroisoleucine and Yaku'amide A	5
1.4 Summary	9
1.5 References	10
Chapter 2. Synthetic Strategies Toward Yaku'amide A.....	11
2.1 Introduction	11
2.2 New Nitrogen Sources for Racemic Aminohydroxylation	11
2.3 Initial Studies Toward Asymmetric Aminohydroxylation.....	16
2.4 Chiral Nitrogen Sources for Aminohydroxylation.....	20
2.5 Selective Deprotection of Chiral Reagent-Mediated Aminohydroxylation Product	24
2.6 Dehydroisoleucine Synthesis	25
2.7 Summary	26
2.8 References	27
Chapter 3. Progress Toward Yaku'amide A.....	29
3.1 Introduction	29
3.2 Retrosynthetic Analysis of Yaku'amide A.....	29
3.3 Synthesis of Right-hand Tetrapeptide.....	30
3.4 Studies Toward the Synthesis of (2S,3R)- β -OHlle Derivative.....	33
3.5 Assignment of Absolute Configurations of β -OHVal Derivatives 82 and <i>epi</i> -82	34
3.6 Assignment of Absolute Configurations of (2S,3R)-83 and (2R,3S)-83	35
3.7 Synthesis of The NTA.....	36
3.8 Summary	37
3.9 References	38

Chapter 4. Introduction to Bulky α,β -Dehydroamino Acids.....	39
4.1 Introduction	39
4.2 Bulky Trisubstituted Δ AA-Containing Natural Products	40
4.3 Bulky Tetrasubstituted Δ AA-Containing Natural Products.....	44
4.4 Summary	48
4.5 References	49
Chapter 5. Synthesis and Applications of Bulky α,β -Dehydroamino Acids.....	56
5.1 Introduction	56
5.2 Methods of Synthesizing Bulky α,β -Dehydroamino Acids.....	57
5.3 Methods of Incorporating Bulky α,β -Dehydroamino Acids into Peptides.....	63
5.4 Applications of Bulky α,β -Dehydroamino Acids.....	68
5.5 Summary	73
5.6 References	74
Chapter 6. Experimental Section	77
6.1 General Experimental Details	77
6.2 Experimental Procedures and Spectral Data	77
6.3 References	103
6.4 Spectra.....	105
6.5 HRMS Data and HPLC Traces of Peptides 201a–d.....	186

LIST OF TABLES

Table 2.1 Regioselective Base-Free Aminohydroxylation	12
Table 2.2 Initial Studies Toward Asymmetric Aminohydroxylation	16

LIST OF FIGURES

Figure 1.1 Structures of yaku'amide A (R = H) and yaku'amide B (R = CH ₃).....	2
Figure 1.2 Structures of Matsunaga's Proposed and Naturally Occuring yaku'amide A	9
Figure 4.1 Structures of Dysamides.....	41
Figure 4.2 Structure of Albonoursin	41
Figure 4.3 Structure of Tentoxin.....	42
Figure 4.4 Structure of Dityromycin.....	43
Figure 4.5 Structures of (-)-Phenylahistin and Dehydrophenylahistin	43
Figure 4.6 Structures of Effusin A and Dihydrocryptoechinuline D.....	44
Figure 4.7 Structure of Lasiodine A	44
Figure 4.8 Structures of Antrimycins.....	45
Figure 4.9 Structures of Phomopsins A and B.....	46
Figure 4.10 Structure of Myxovalargin A.....	46
Figure 4.11 Structures of Peptides 148a-e	47
Figure 4.12 Structures of Azinomycins A and B.....	47
Figure 4.13 Structures of Miuraenamides.....	48
Figure 5.1 Structure of Enkephalin Analogue in the Z-configuration	56
Figure 5.2 Bulky Dehydroamino Acids versus Normal Dehydroamino Acids	57
Figure 5.3 <i>N</i> -Cbz- Δ Val-Val-Z- Δ Phe-Ile-OMe	69
Figure 5.4 ³ ₁₀ -Helical Peptide Gp41 ₆₅₉₋₆₇₁	69
Figure 5.5 13-Residue Analogues.....	70
Figure 5.6 Enfuvirtide Prevents HIV Entry into Human Cells.....	71
Figure 5.7 Enfuvirtide and 16 Future Analogues.....	72

Figure 5.8 HIV-1 Infectivity Assay Control Experiment (T20 is another name for enfuvirtide). 73

LIST OF SCHEMES

Scheme 1.1 Shin's Dehydroisoleucine Synthesis.....	3
Scheme 1.2 Wandless Pre-Dehydroisoleucine Synthesis	4
Scheme 1.3 Wandless Dehydroisoleucine Synthesis.....	4
Scheme 1.4 Joullie's Dehydroisoleucine Synthesis	4
Scheme 1.5 Inoue's Dehydroisoleucine Synthesis	5
Scheme 1.6 Inoue's Synthesis of β -Hydroxy Amino Acids.....	6
Scheme 1.7 Inoue's Synthesis of NTA Subunit 33	7
Scheme 1.8 Inoue's Total Synthesis of Matsunaga's Proposed yaku'amide A 1.....	8
Scheme 2.1 Attempted Enantioselective Aminohydroxylation Reaction.....	13
Scheme 2.2 New Nitrogen Sources for Aminohydroxylations.....	14
Scheme 2.3 Improvements with Tosyloxycarbamates.....	14
Scheme 2.4 Improvements with Mesyloxycarbamates.....	15
Scheme 2.5 Optimization of Ligand-Controlled Asymmetric Aminohydroxylation	16
Scheme 2.6 Two Catalytic Cycles in Os-Catalyzed Aminohydroxylation.....	18
Scheme 2.7 Chiral Substrate from (-)-Borneol for Asymmetric Aminohydroxylation	19
Scheme 2.8 Chiral Substrate from L-Menthol for Asymmetric Aminohydroxylation.....	19
Scheme 2.9 Synthesis of Chiral Nitrogen Reagent.....	21
Scheme 2.10 Asymmetric Aminohydroxylation with Chiral Nitrogen Reagent	21
Scheme 2.11 Reagent-Controlled Asymmetric Aminohydroxylation	22
Scheme 2.12 Optimization of Reagent-Controlled Asymmetric Aminohydroxylations	22
Scheme 2.13 Preliminary Studies on β -Hydroxy Amino Acid Synthesis	23
Scheme 2.14 Chemoselective Deprotections.....	24

Scheme 2.15 Synthesis of Z- Δ Ile-Containing Peptides	25
Scheme 2.16 Synthesis of E- Δ Ile-Containing Peptides	26
Scheme 3.1 Retrosynthetic Analysis of yaku'amide A 3	30
Scheme 3.2 Synthesis of Right-Hand Tetrapeptide 102	31
Scheme 3.3 Synthesis of Diamine 111	32
Scheme 3.4 Optimization of the Synthesis of 102	32
Scheme 3.5 Proposed Synthesis of (2S, 3R)- β -OHIle Derivative 118	33
Scheme 3.6 Assignment of Absolute Configurations of epi-82 and 82.....	34
Scheme 3.7 Assignment of Absolute Configurations of (2S,3R)-83 and (2R,3S)-83	35
Scheme 3.8 Synthesis of NTA 96 and Model Study of Similar Acid.....	36
Scheme 5.1 Synthesis of Δ Val-containing Peptide 154.....	58
Scheme 5.2 Synthesis of β -lactam Thiazolidine 156.....	58
Scheme 5.3 Synthesis of Tentoxin.....	59
Scheme 5.4 Synthesis of Z- Δ Trp Derivatives 161 and 163	59
Scheme 5.5 Synthesis of the Precursors of Isoechinulin A and Variecolorin C.....	60
Scheme 5.6 Synthesis of the Z- Δ His Substructure of Isoroquefortine E	60
Scheme 5.7 Synthesis of the Model of Azabicyclic Δ AA 174	61
Scheme 5.8 Synthesis of the Right-hand Fragment of Azinomycin B	61
Scheme 5.9 Synthesis of Azinomycin A.....	62
Scheme 5.10 Synthesis of Unsymmetrical β,β -diaryl Substituted Δ AAs.....	62
Scheme 5.11 Strategy for Synthesis of Δ AA-containing Peptides via SPPS	64
Scheme 5.12 Synthesis of Δ Val-based Azlactones.....	65
Scheme 5.13 Synthesis of Δ Env-based Azlactones	66

Scheme 5.14 Solid-phase Synthesis of Δ AA-containing Peptides	67
Scheme 5.15 Synthesis of Δ Val-based Azlactones that Contain Protected Tryptophan	70

CHAPTER 1. YAKU'AMIDE A INTRODUCTION

1.1 Structure and Bioactivity

Marine sponges produce a variety of peptides and polyketides which have potent biological activities. In 2010 Matsunaga and co-workers isolated two cytotoxic compounds, yaku'amide A and B, from a deep-sea sponge *Ceratopsion* sp. at Yakushinsone located in the East China Sea.¹ These two peptides include unique N- and C-termini and are rich in dehydroamino acids and β -hydroxyamino acids. HR-ESIMS experiments established the molecular formulas of these two peptides. The initial structures of these two peptides (**1** and **2**, Figure 1.1) were proposed with the aid of ^1H , ^{13}C , COSY, TOCSY, HSQC, HMBC, NOESY, 2D NMR spectra and chemical degradations. Of the 13 amino acid residues, one is α,β -dehydrovaline (ΔVal), one is E- α,β -dehydroisoleucine (ΔIle), two are Z- α,β -dehydroisoleucine (ΔIle), three are β -hydroxyamino acids and six are common amino acids. These two peptides are capped with C-terminal amine (CTA) and N-terminal acyl group (NTA). With these data in hand, Matsunaga and co-workers proposed the structures of yaku'amide A and B without defining the C4-configuration of NTA.

Importantly, these two linear peptides exhibit inhibitory effects against P388 murine leukemia cells (IC_{50} = 14 ng/mL for yaku'amide A and 4 ng/mL for yaku'amide B). Also noteworthy is that yaku'amide A shows a unique inhibitory profile against a series of 39 human cancer cell lines (JFCR39). With a strong cancer cell growth inhibitory activity (IC_{50} < 1 μM) against several cancer cell lines including breast, colon, gastrointestinal and lung cancer cells, it possesses a unique

profile of activity in this cell line relative to other known anticancer agents. All of these facts indicate that yaku'amide can be utilized as a tool for discovering a new anticancer target.

In order to research the anticancer mode of action of yaku'amide A, large quantities need to be used. Unfortunately, there is a limited natural supply of yaku'amide A just like many marine natural products. The unusual structure, potent bioactivity, and limited supply combine to justify a total synthesis of yaku'amide A. Motivated by these factors, the Inoue group synthesized the two isomers of the yaku'amide A structure proposed by Matsunaga and determined the absolute C4 (S)-stereochemistry in 2013.² Two years later, they established the correct structures via the reassignment of four residues (OHVal-7, OHVal-8, Val-11, Val-12, highlighted in red and blue) of yaku'amide A and B.³

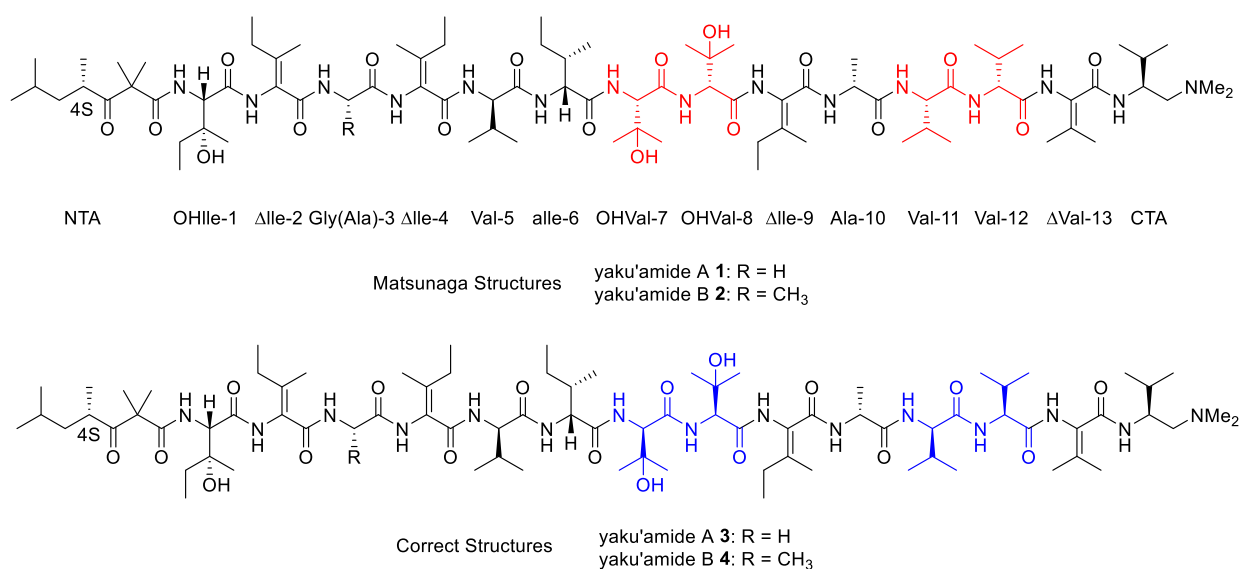
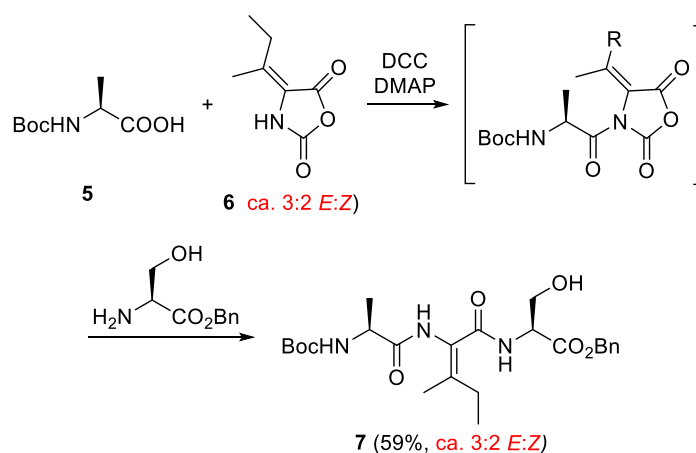


Figure 1.1 Structures of yaku'amide A (R = H) and yaku'amide B (R = CH₃)

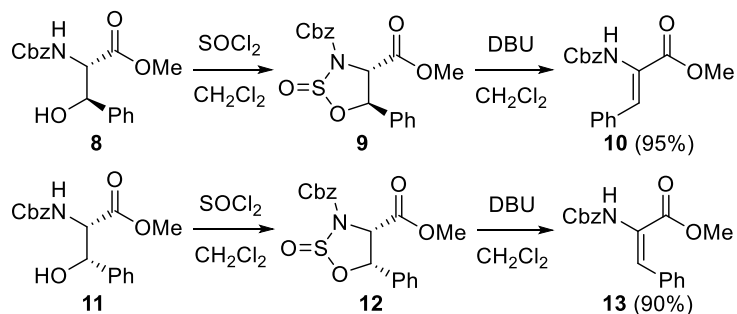
1.2 Dehydroisoleucine Synthesis

Yaku'amide A contains two *Z*- α,β -dehydroisoleucine (Δ Ile) and one *E*- α,β -dehydroisoleucine (Δ Ile) residues. It is challenging to selectively install *Z*- and *E*- α,β -dehydroisoleucine (Δ Ile).⁴ Shin and co-workers have adopted dehydroamino acids-containing-*N*-carboxyanhydride derivatives for the one-pot synthesis of tripeptides **7** (Scheme 1.1).⁵ They established Δ Ile-derived anhydride **6** as a linchpin to generate a mixture of alkene isomers **7** which limits its application.

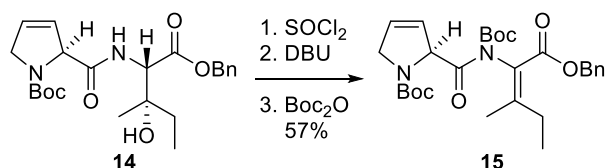


Scheme 1.1 Shin's Dehydroisoleucine Synthesis

Wandless and co-workers developed a two-step *anti* dehydration to stereoselectively construct bulky trisubstituted and tetrasubstituted Δ AAs.⁶ They used SOCl_2 to generate cyclic sulfamidite **9** from β -phenylserine derivative **8**. When treated with DBU, **9** underwent *anti* elimination to produce *Z*- Δ Phe derivative **10** in very good yield (Scheme 1.2).¹⁰³ The same methodology yielded *E*- Δ Phe derivative **13** from **11**. Next, they applied this method to dipeptide **14** to provide *E*- Δ Ile dipeptide **15** (Scheme 1.3), which was used as a building block for the total synthesis of phomopsin B⁷. In order to use **15** in the peptide coupling step without isomerization of the double bond, a Boc group was introduced to protect the backbone amide.

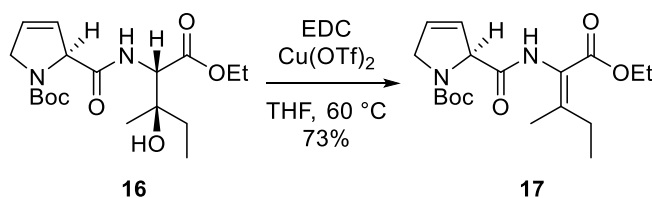


Scheme 1.2 Wandless Pre-Dehydroisoleucine Synthesis



Scheme 1.3 Wandless Dehydroisoleucine Synthesis

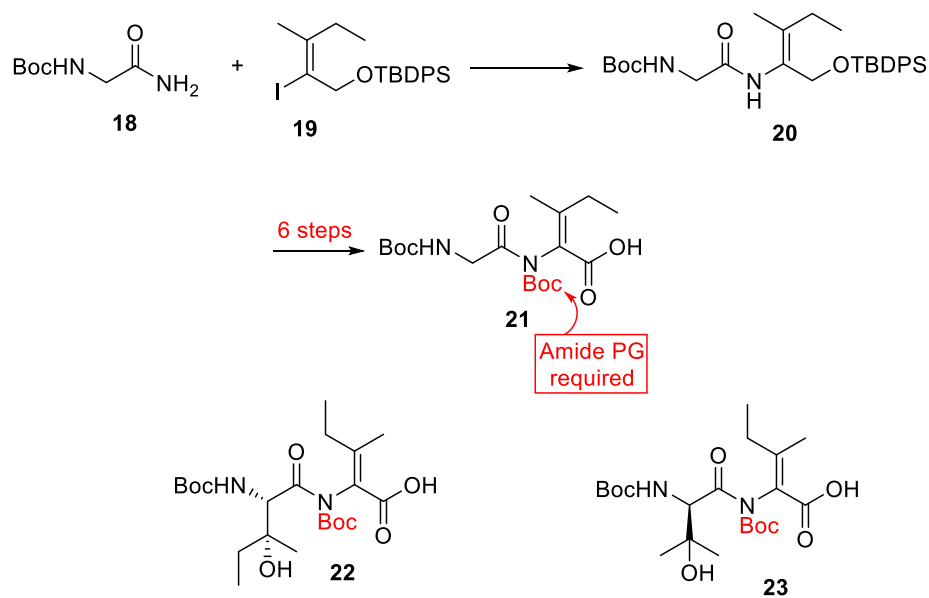
In order to synthesize phomopsin, Shangguan and Joullié developed a copper–carbodiimide *syn* dehydration to access the tripeptide side chain.⁸ They synthesized compound **16** from commercially available material and screened the reaction conditions for the dehydration reaction. In the presence of $\text{Cu}(\text{OTf})_2$ with THF as solvent, they generated the *E*-Alle residue. Intermediate **17** was later protected to prevent isomerization of the *E*-Alle to provide the phomopsin tripeptide building block (Scheme 1.4).



Scheme 1.4 Joullie's Dehydroisoleucine Synthesis

1.3 Inoue Group Synthesis of Dehydroisoleucine and Yaku'amide A

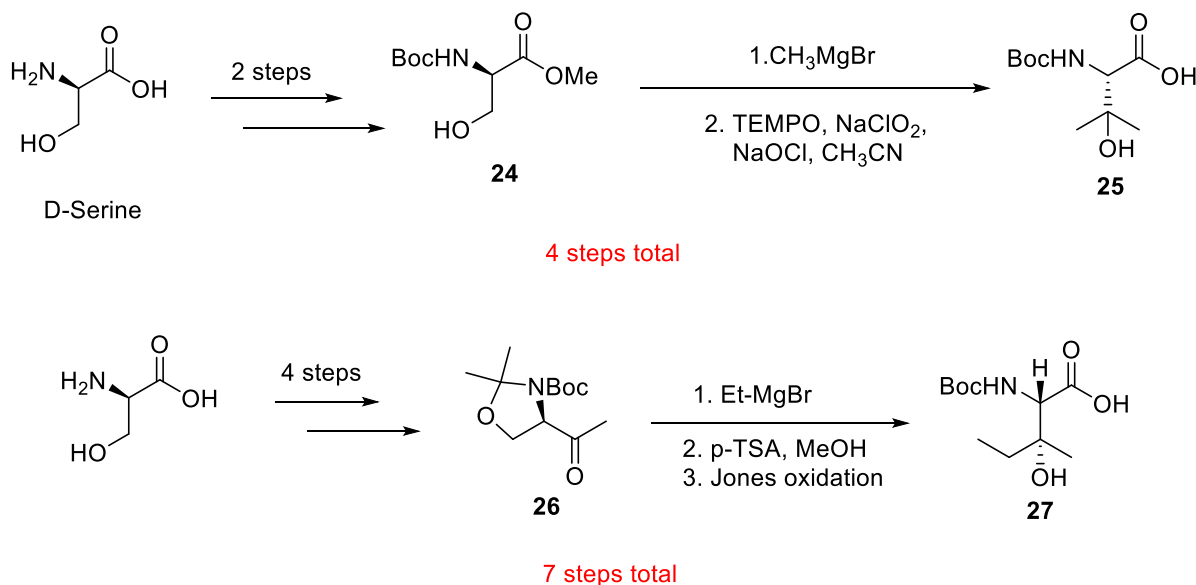
The Inoue group finished the first total synthesis of yaku'amide A. For the challenging dehydroisoleucine synthesis, they adopted a Cu-catalyzed amidation method with vinyl iodides⁹ to generate the stereospecific subunits of yaku'amide A such as **20**. To continue the synthesis, they used a cumbersome 6-step process to convert **20** to **21** for the reason that amide protection was required to prevent azlactone formation (Scheme 1.5). Using similar approaches, they prepared building blocks **22** and **23**.



Scheme 1.5 Inoue's Dehydroisoleucine Synthesis

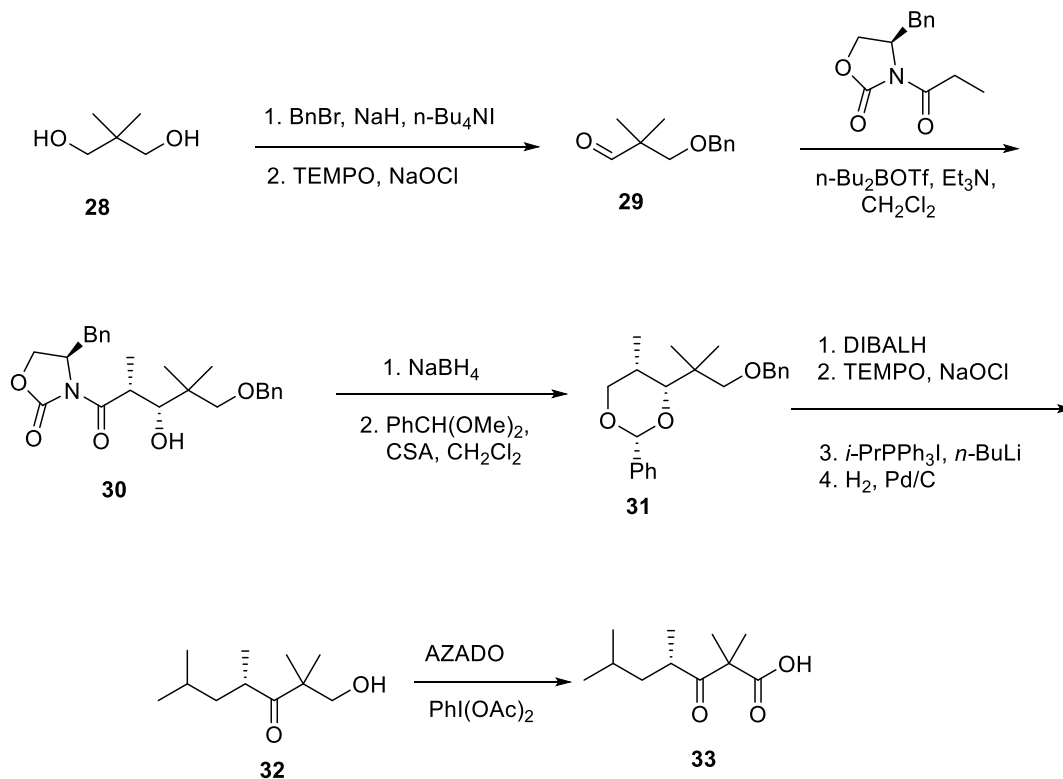
Inoue and co-workers used methods developed by Lubell¹⁰ and Guanti¹¹ for the synthesis of β -OHVal and β -OHlle derivatives. N-(*tert*-Butoxycarbonyl)-D-serine methyl ester was generated in two steps from D-Serine. With a Grignard reaction and oxidation in the presence of TEMPO, they generated β -OHVal derivative **25** (Scheme 1.6). The enantiomer of **25** was synthesized in 4 steps from L-Serine. Another 4 steps from D-Serine delivered methyl ketone **26**. A subsequent

chelation-controlled addition with Et-MgBr, acetonide cleavage and a Jones oxidation of the newly formed diol furnished β -OHile derivative **27**.



Scheme 1.6 Inoue's Synthesis of β -Hydroxy Amino Acids

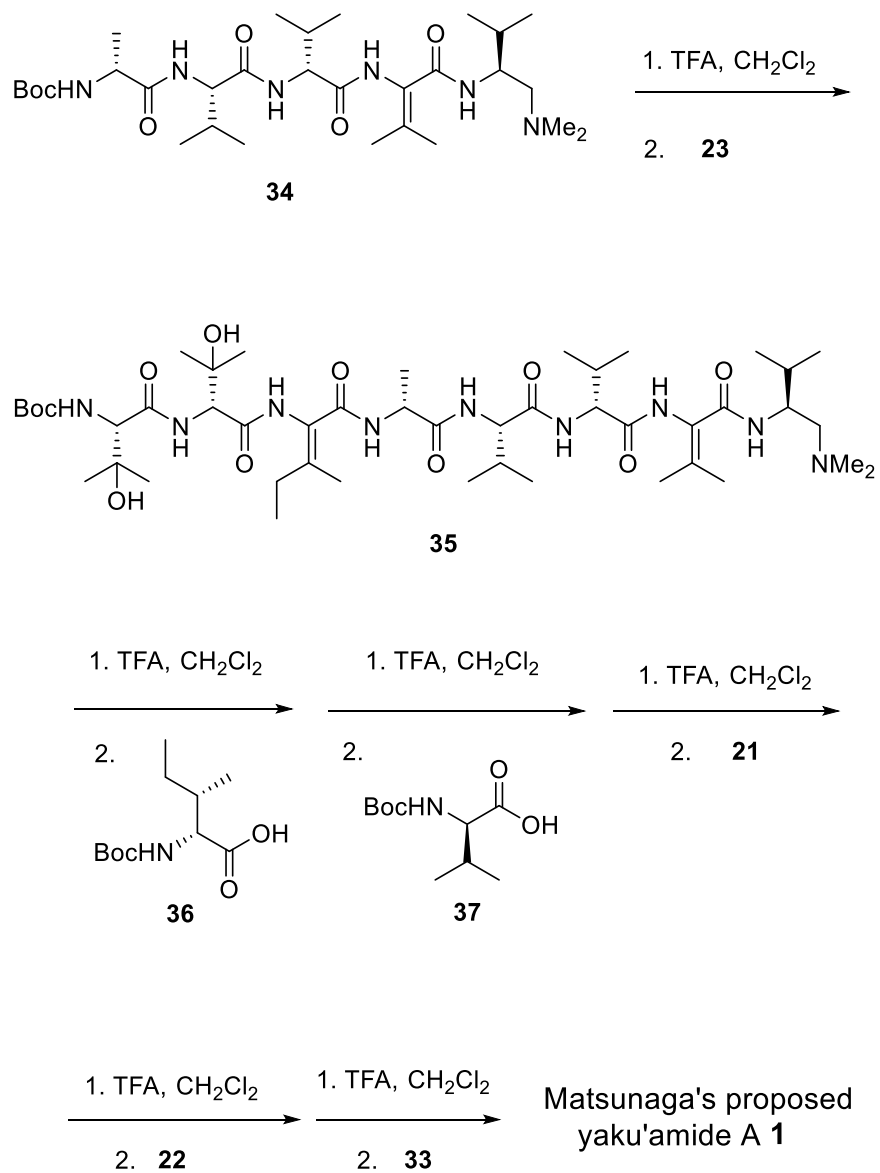
For the synthesis of NTA subunit **33**, Inoue and co-workers generated aldehyde **29** in two steps from diol **18** through monobenylation and oxidation (Scheme 1.7). Then they adopted the Evans asymmetric aldol reaction¹² to install the C4 (S)-stereocenter. Reduction of syn-aldol intermediate **30** with NaBH₄ and protection of the resulting diol yielded benzylidene acetal **31**. Acetal removal and oxidation set the stage for a Wittig reaction. Next, the newly generated olefin was treated with Pd/C to provide the primary alcohol **32**. A final oxidation in the presence of AZADO¹³ and PhI(OAc)₂¹⁴ furnished the desired NTA **33**.



Scheme 1.7 Inoue's Synthesis of NTA Subunit 33

With all the building blocks in hand, the Inoue group finished the total synthesis of Matsunaga's proposed yaku'amide A structure with multiple amidation reactions (Scheme 1.8). Six Boc-removal and coupling procedures led to the formation of the final peptide. For the last step, they prepared both NTA isomers and obtained two possible yaku'amide A structures. After comparing the spectra, they determined the C4 (S)-stereochemistry of naturally occurring yaku'amide A. They moved on to synthesize yaku'amide B. However, they found differences in the retention times of the synthesized structure and the natural product using UHPLC. Then, Inoue and co-workers elucidated the correct structure of yaku'amide B with Marfey's analysis and chemical synthesis. By applying the same synthetic method, they generated the revised yaku'amide A (**3**, Figure 1.2). The chromatographical data are identical with the re-isolated yaku'amide A natural product. The difference between the correct structure and Matsunaga's

proposed structure of yaku'amide A is that the orders of OHVal-7 and OHVal-8 and Val-11 and Val-12 (highlighted in red in Figure 1.2) are switched.



Scheme 1.8 Inoue's Total Synthesis of Matsunaga's Proposed yaku'amide A 1

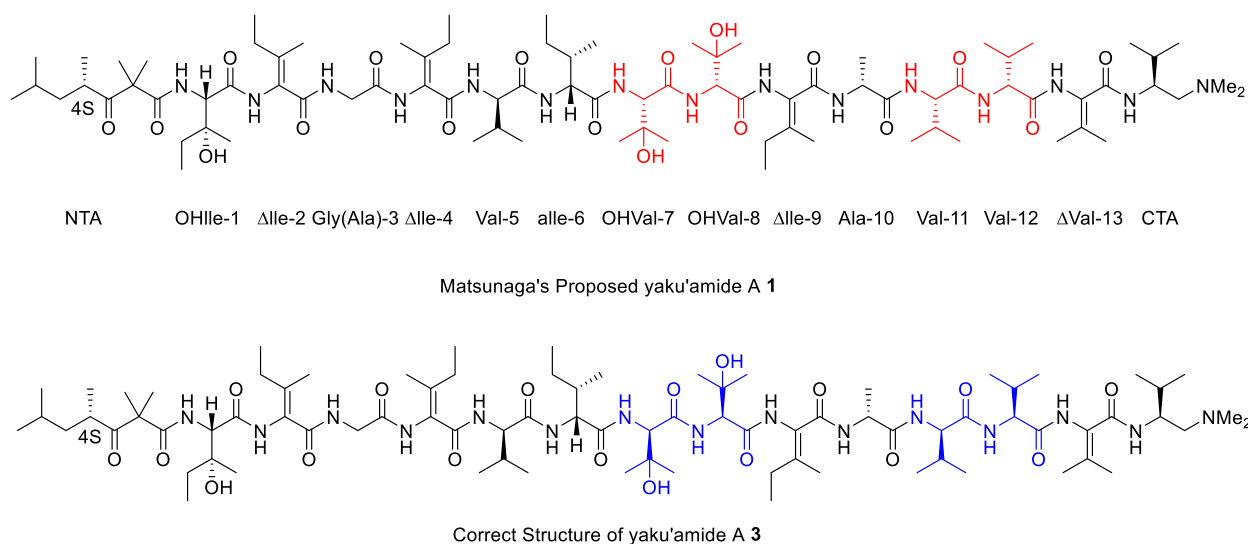


Figure 1.2 Structures of Matsunaga's Proposed and Naturally Occuring yaku'amide A

1.4 Summary

Inoue's synthesis of yaku'amide A includes several key transformations such as the application of the Cu-catalyzed cross-coupling of primary amides and vinyl iodides to install several important building blocks of yaku'amide A including three Z- or E-dehydroisoleucine derivatives and the dehydrovaline-containing right-hand tetrapeptide and the preparation of two NTA enantiomers to determine the C4 (S)-stereochemistry.

However, it takes four and seven steps to construct β -OHVal and β -OHlle derivatives **25** and **27** from D-serine, respectively. Also, it adds another four steps to access *Ent*-**25** from L-Serine. In order to research the biological mode of action of a series of yaku'amide analogues, it is vital to develop new and more efficient methods to access its challenging dehydroamino and β -hydroxyamino acids.

1.5 References

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CHAPTER 2. SYNTHETIC STRATEGIES TOWARD YAKU'AMIDE A

2.1 Introduction

There are several challenges for the synthesis of yaku'amide A: (1) the preparation of β -hydroxyamino acids and dehydroamino acids; (2) the selective synthesis of Z- and E- α,β -dehydroisoleucine (Δ Ile); and (3) the installation of Z- and E- α,β -dehydroisoleucine (Δ Ile) into peptides without alkene isomerization. Inoue's synthesis of yaku'amide A was completed in 25 longest linear steps with 86 steps overall. The total number of steps makes Inoue's synthesis not efficient to provide enough material for performing cellular assays on yaku'amide A. In order to identify the pharmacophore and target of yaku'amide A, more efficient synthesis of this type of peptide needs to be developed.

2.2 New Nitrogen Sources for Racemic Aminohydroxylation

β -hydroxyamino acids are key building blocks of many biologically active compounds such as polyoxins,¹ vancomycin,² ustiloxins,³ exochelins,⁴ and cyclomarins.⁵ Many studies have been carried out to explore the stereoselective synthesis of this unit. Chemists have used aldol reactions,⁶⁻⁹ asymmetric hydrogenations,¹⁰⁻¹² Claisen rearrangement,¹³ and enantioselective aminohydroxylation¹⁴⁻¹⁷ to prepare these β -hydroxyamino acids. Due to the regioselectivity,

reactivity and substrate scope issues of the abovementioned methodologies, we plan to develop innovative methods to efficiently synthesize β -hydroxyamino acids.

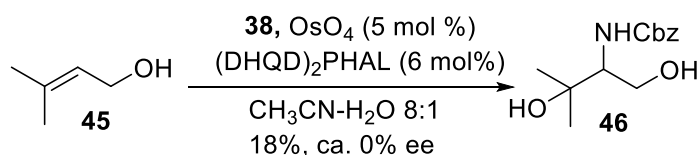
In preliminary studies, we have used a base-free intermolecular aminohydroxylation procedure to construct the β -hydroxyamino acids existing in yaku'amide A.¹⁸ Aminohydroxylations of alkenes **39-41** employing acyloxycarbamate **38** as an effective nitrogen source generated racemic amino alcohols **42-44** with excellent regioselectivity and moderate to good yields (Table 2.1). Compounds **42-44** can function as precursors to create the dehydroamino acids Δ Val and Z-, E- Δ Alle in yaku'amide A if we can also develop an efficient dehydration method.

Table 2.1 Regioselective Base-Free Aminohydroxylation

Entry	Alkene	Product	Yield (%)
1	 39	 42	79
2	 40	 43 (racemic)	55
3	 41	 44 (racemic)	59

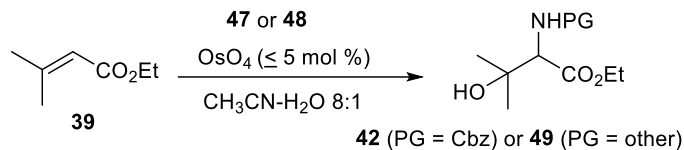
Compared with enoate **39**, trisubstituted allylic alcohol **45** is a better substrate for the racemic aminohydroxylation. So we started with allylic alcohol **45** as the testing substrate for attempted asymmetric aminohydroxylation to construct enantiopure β -hydroxyamino acids. However, we

could not effectively produce enantiopure compound **46** from allylic alcohol **45** using known asymmetric aminohydroxylation conditions (Scheme 2.1). This may have something to do with the steric hindrance at the Os center. If the nitrogen source and chiral ligand are too bulky, they might hinder this reaction.

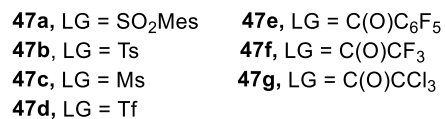


Scheme 2.1 Attempted Enantioselective Aminohydroxylation Reaction

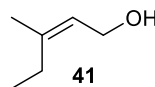
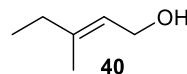
Although the racemic base-free aminohydroxylation is a useful process, there are still some limitations such as slow rate (significantly lower yields 20-40% were obtained when carried out at lower temperatures and catalyst loadings) and the formation of Cbz-NH₂ as a byproduct. We also used 10 mol % OsO₄ loading for all the reactions in our first-generation protocol. Our initial experiment with 5 mol % OsO₄ did not proceed very well. Motivated by Donohoe's work on tethered substrates¹⁹ and Luxemburger's research on protecting groups²⁰ we optimized this type of reaction by screening a variety of nitrogen source reagents (Scheme 2.2). We chose commercially available enoate **39** as the testing substrate, screening reagents **47a-g** to solve the previous problems of this reaction. We also employed different protecting group as shown in **48a-d** to pair with the best leaving groups. After screening all the leaving groups and protecting groups, we discovered that tosyloxy- and mesyloxycarbamates (CbzHN-OTs and CbzHN-OMs) as our second generation nitrogen sources afforded amino alcohols in good to excellent yields (**47b**, Scheme 2.3 and **47c**, Scheme 2.4).



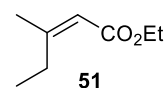
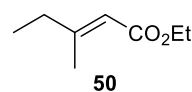
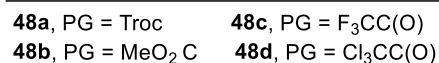
Leaving Group Optimization: CbzHN-O-LG



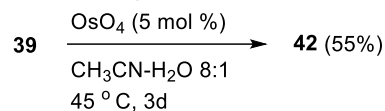
Other test substrates



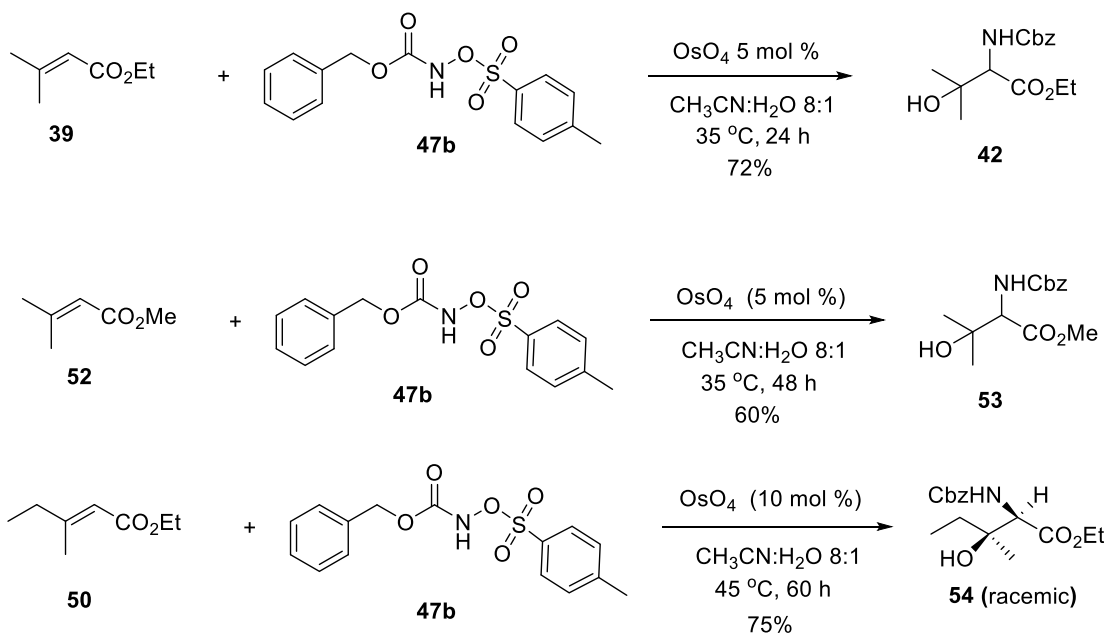
Protecting Group Optimization: PGHN-O-LG



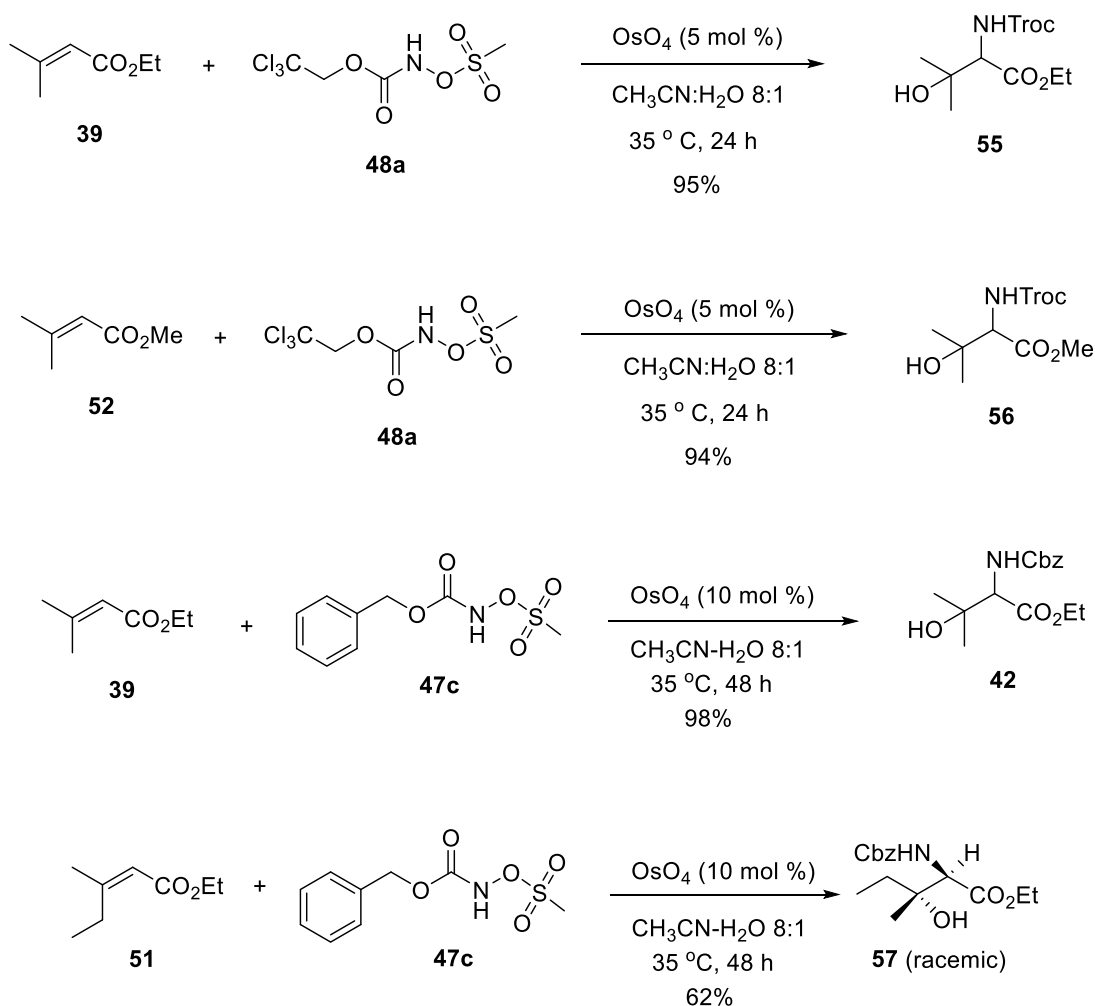
Initial Result: reagent **47b**



Scheme 2.2 New Nitrogen Sources for Aminohydroxylations



Scheme 2.3 Improvements with Tosyloxycarbamates

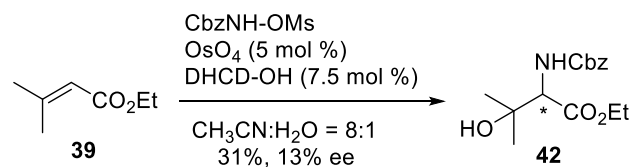


Scheme 2.4 Improvements with Mesyloxycarbamates

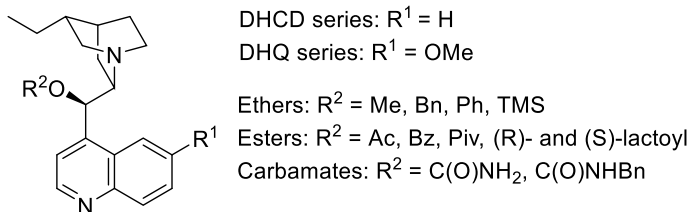
With **47b** (CbzHN-OTs), **47c** (CbzHN-OMs), and **48a** (TrochHN-OMs), we also optimized the temperature, carbamate ratio and catalyst loading for the aminohydroxylation reactions. The general trend is that bulkier substrates required longer reaction times and higher catalyst loadings. For example, with alkene **50** as substrate, it took 60 h and 10 mol % OsO_4 to get 75% yield. When we switched to mesyloxycarbamate **47c** for aminohydroxylation with alkene **51** (the isomer of **50**), we obtained 62% yield (Scheme 2.4). And we got the best results with **48a** (TrochHN-OMs) as the nitrogen source. We obtained 95% and 94% yields with ethyl ester **39** and methyl ester **52** as the substrates, respectively.

2.3 Initial Studies Toward Asymmetric Aminohydroxylation

Next, we started to develop a method for the asymmetric aminohydroxylation (AA) to yield enantiopure β -OHVal and β -OHlle directly. This process has never been developed to access these valuable amino acids. Co-worker Joseph M. Cardon and I explored a series of chiral ligands for this reaction such as Sharpless ligands (DHQD-PHAL, DHQD-MEQ, DHQD-PYR, DHQD-CLB)²¹ and chiral amines (Scheme 2.5 and Table 2.2). From this previous study, the traditional Sharpless ligands are apparently too bulky to give high ee values with trisubstituted alkenes.



Ligand Optimization



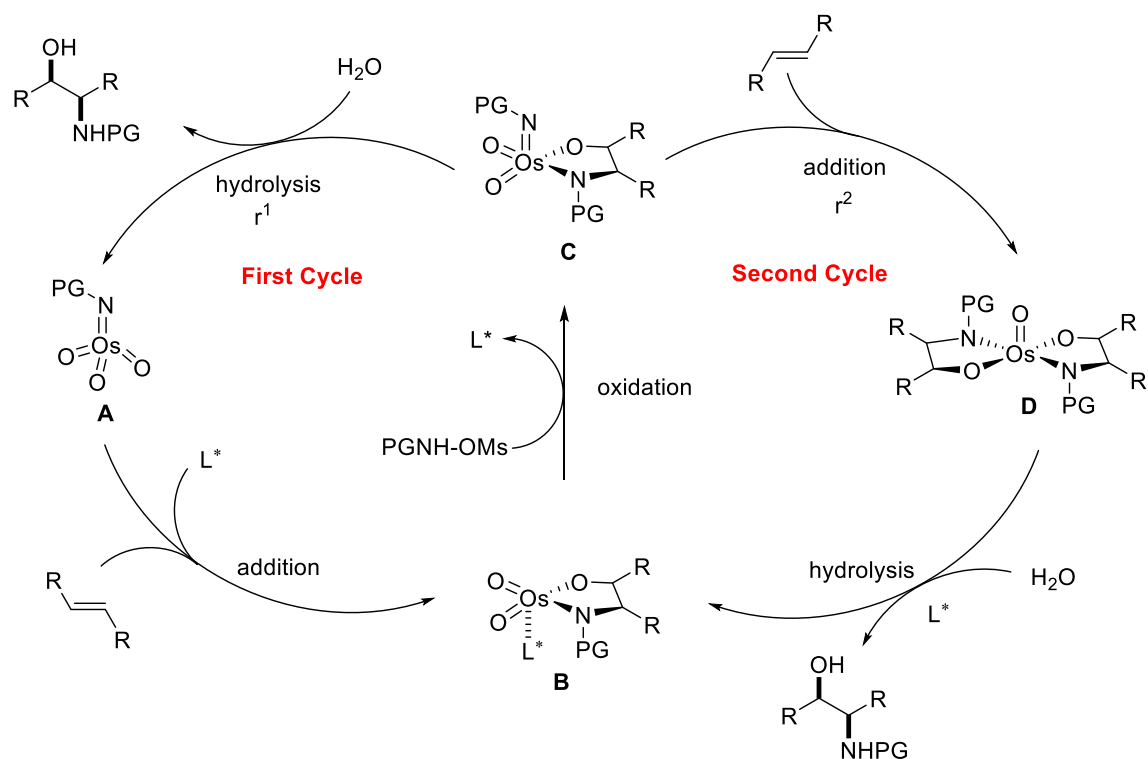
Scheme 2.5 Optimization of Ligand-Controlled Asymmetric Aminohydroxylation

Table 2.2 Initial Studies Toward Asymmetric Aminohydroxylation

Entry	Ligand	Yield	ee
1	DHQD-MEQ	64%	Racemic
2	DHQD-CLB	66%	Racemic
3	DHQD ₂ PHAL	54%	Racemic
4	DHQD ₂ PYR	70%	Racemic
5	DHQD-OH	52%	Racemic
6	DHCD-OH	31%	13%

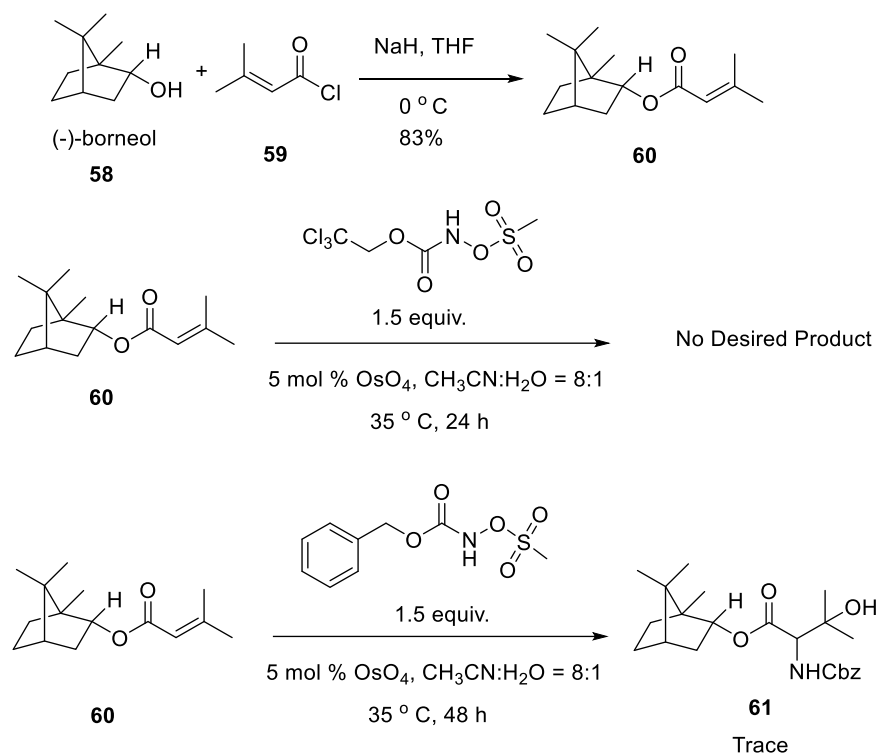
When we used dihydrocinchonidine (DHCD-OH) for this reaction, we only got low yield and 13% ee. But DHCD-OH may not be bulky enough, so we attached small and medium sized groups to DHCD-OH and DHQ-OH in order to get improved enantioselectivity. In addition, smaller nitrogen sources can provide higher ee in other aminohydroxylation reactions,²² so we switched to N-mesyloxycarbamate **47c** to improve the ee as well. With our best nitrogen reagent, we started to optimize the chiral ligands. After screening all the ligands available, we also optimized solvent and temperature. Unfortunately, we did not get higher ee values for attempted asymmetric aminohydroxylations.

Although disappointing, these results can be explained by the accepted mechanism of the Os-catalyzed AA reaction (Scheme 2.6). Highly selective aminohydroxylations are assumed to progress via sequential additions of the olefin and the chiral ligand (L^*) to imidoosmium species A. Accompanied by the dissociation of L^* , the resulting Os(VI) azaglycolate B is reoxidized by the nitrogen source reagent to afford Os(VIII) azaglycolate C. The “first cycle”^{23,24} is completed by hydrolysis to produce the enantioenriched product and regenerate A. This pathway is active when the rate of alkene addition to C (r^2) is slower than the rate of hydrolysis (r^1). When r^2 is greater than r^1 , a “second cycle” takes place to add the olefin to an unligated complex (C). As a result, aminohydroxylations through the second cycle usually provide racemic products. The characteristics of such reactions are either abnormally slow hydrolysis (r^1) or abnormally fast addition (r^2) rates. Moreover, these reactions are not accelerated in the presence of the chiral ligands. Our CbzNH-OMs mediated aminohydroxylations of trisubstituted alkenes display all of the features of “second cycle” routes as described by Sharpless.^{23,24}

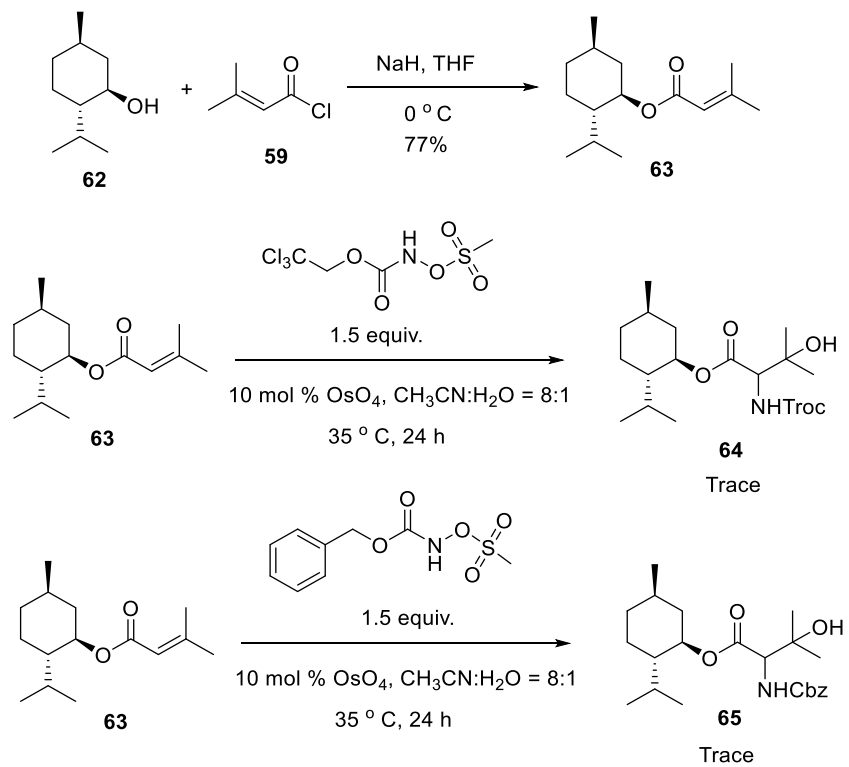


Scheme 2.6 Two Catalytic Cycles in Os-Catalyzed Aminohydroxylation

After failing to develop an asymmetric method with a series of chiral ligands to provide the important subunits of yaku'amide A, we tried to use chiral substrates to synthesize the enantiopure β -OHVal and β -OHlle (Scheme 2.7). We prepared the trisubstituted alkenes from (-)-borneol, L-menthol and 3,3-dimethylacryloyl chloride.²⁵ Then, we used **47c** (CbzHN-OMs) and **48a** (TrochHN-OMs) as the nitrogen sources to test the chiral substrate-controlled aminohydroxylations. Unfortunately, we obtained either no desired product or trace amount of the desired product with chiral substrate **60**. When we increased the catalyst loading to 10 mol % with alkene **63**, we also obtained poor results (Scheme 2.8). Although we could try to design new chiral ligands for better ee results, we decided to change to chiral nitrogen sources first before investing effort into this area.



Scheme 2.7 Chiral Substrate from (-)-Borneol for Asymmetric Aminohydroxylation

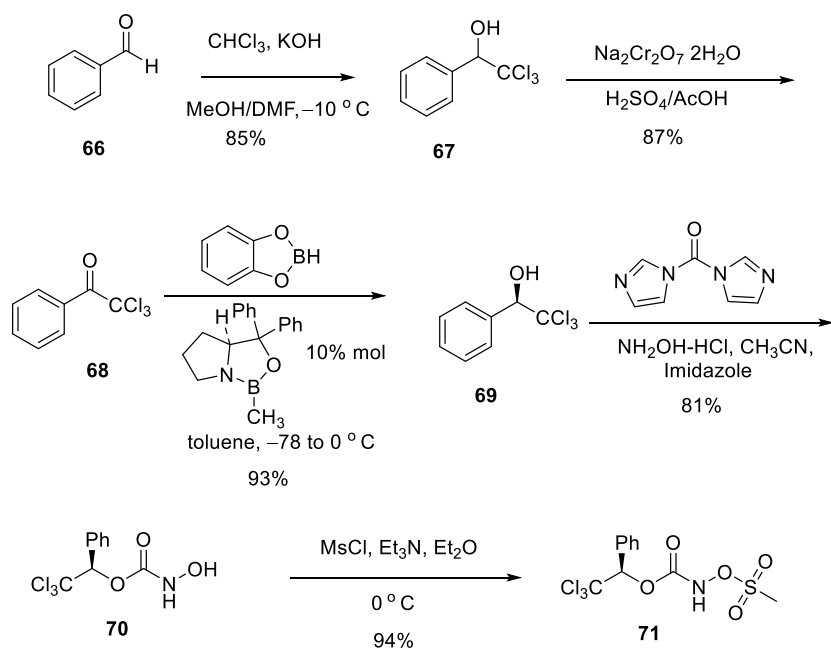


Scheme 2.8 Chiral Substrate from L-Menthol for Asymmetric Aminohydroxylation

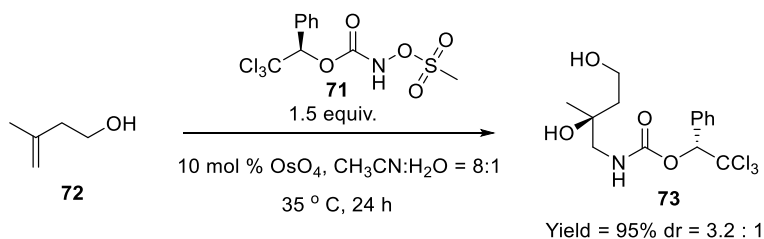
2.4 Chiral Nitrogen Sources for Aminohydroxylation

After failing to develop a method directly for the asymmetric aminohydroxylation to yield enantiopure β -OHVal and β -OHlle, we worked toward the synthesis of chiral nitrogen reagents for asymmetric aminohydroxylation. Described below are the detailed procedures for the synthesis of the chiral nitrogen source **71** (Scheme 2.9).²⁶ Coupling of the benzaldehyde (**66**) with chloroform generated a racemic secondary alcohol **67**. A subsequent oxidation with Jones reagent yielded phenyltrichloromethyl ketone **68**. Upon treatment with chiral CBS catalyst, ketone **68** was converted to a chiral secondary alcohol **69**. Next, in the presence of 1,1-carbonyldiimidazole and hydroxylamine hydrochloride, we obtained primary alcohol **70** as the precursor of the first chiral nitrogen source for this project. After attaching the mesylate group, we generated the chiral reagent (R)-1-Phenyl-2,2,2-trichloroethyl-N-mesyloxycarbamate (**71**). When we used HPLC to check the enantiopurity of **71**, we got more than 95% ee for all batches.

Then, we tested the asymmetric aminohydroxylation with disubstituted alkene **72** as substrate first and got 95% yield and 3.2:1 diastereoselectivity (Scheme 2.10). We also optimized the temperature, solvent and reaction time to get better results. Surprisingly, when we ran the reaction for 48 h, we got slightly lower yield. Our hypothesis was that the longer reaction time might cause some side reactions.



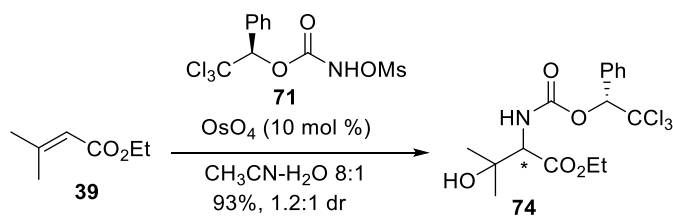
Scheme 2.9 Synthesis of Chiral Nitrogen Reagent



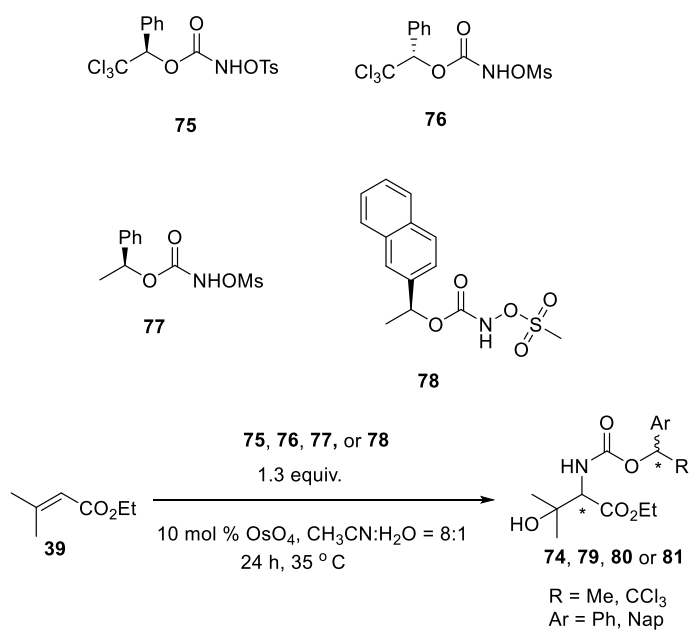
Scheme 2.10 Asymmetric Aminohydroxylation with Chiral Nitrogen Reagent

When we switched to enoate **39** as our testing substrate for asymmetric aminohydroxylation with the optimized reaction conditions, we only obtained a low diastereomeric ratio 1.2:1 (dr) with chiral N-mesyloxycarbamate **71** as the nitrogen source (Scheme 2.11). Based on the mechanism of the aminohydroxylation reaction,^{23,24} we reasoned that it would be possible to get a higher dr by changing the size of the substituents of the chiral reagent. So we prepared a series of chiral reagents with smaller and bulkier alkyl groups and bulkier aryl groups (Scheme 2.12). Later, we tested these chiral reagents with enoate **39** as the substrate under the same conditions. We obtained slightly higher 2:1 dr with **76**, but we only obtained about 1:1 dr with reagent **77**. Nevertheless,

the key benefit from this approach using chiral nitrogen source is that we could separate the diastereomeric products for a productive use of this amino alcohol product even at a moderate dr.



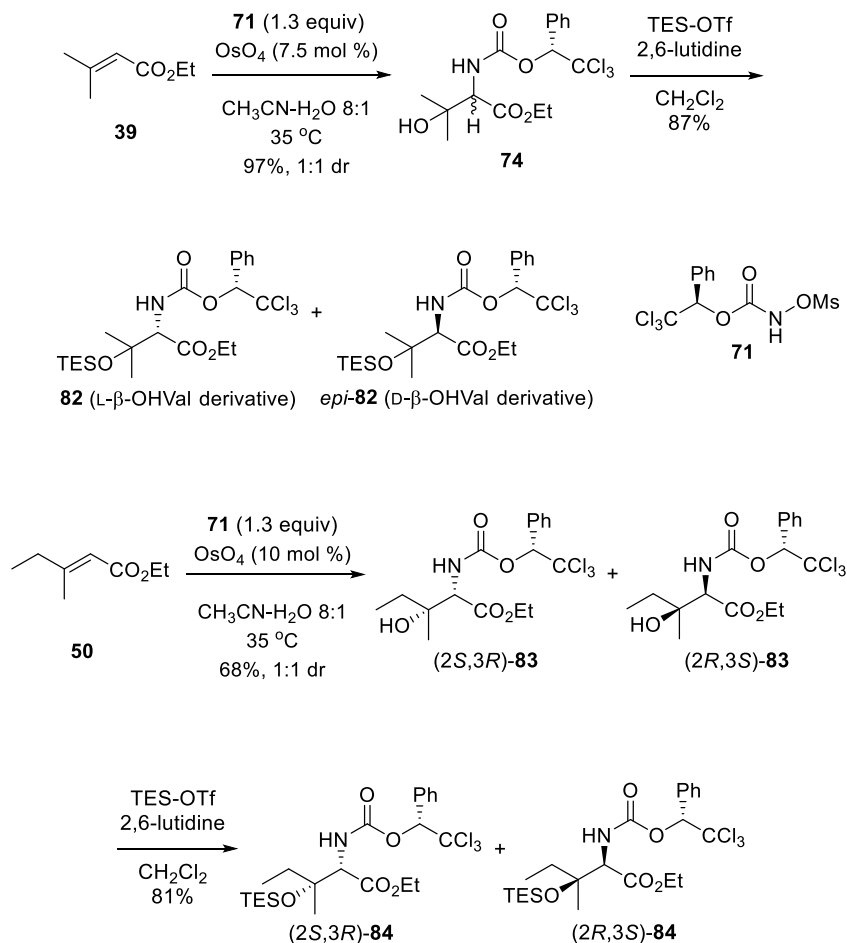
Scheme 2.11 Reagent-Controlled Asymmetric Aminohydroxylation



Scheme 2.12 Optimization of Reagent-Controlled Asymmetric Aminohydroxylation

To continue our study, we adopted this newly-developed chiral reagent-based method to synthesize the β -OHVal and β -OHlle residues of yaku'amide A. Starting from enoate **39**, aminohydroxylation with Lebel's mesyloxycarbamate **71**²⁶ provided **74** in 97% yield with about 1:1 diastereoselectivity (Scheme 2.13). Since both enantiomers of β -OHVal exist in yaku'amide A, we could theoretically use both products for the total synthesis without discarding any material. Unfortunately, the separation of the resulting diastereomers of **74** was not possible.

Notwithstanding, the diastereomeric mixture of D-β-OHVal derivative **82** and L-β-OHVal derivative *epi*-**82** was separable on SiO₂ after TES protection. The overall yield for the two steps was about 84%.

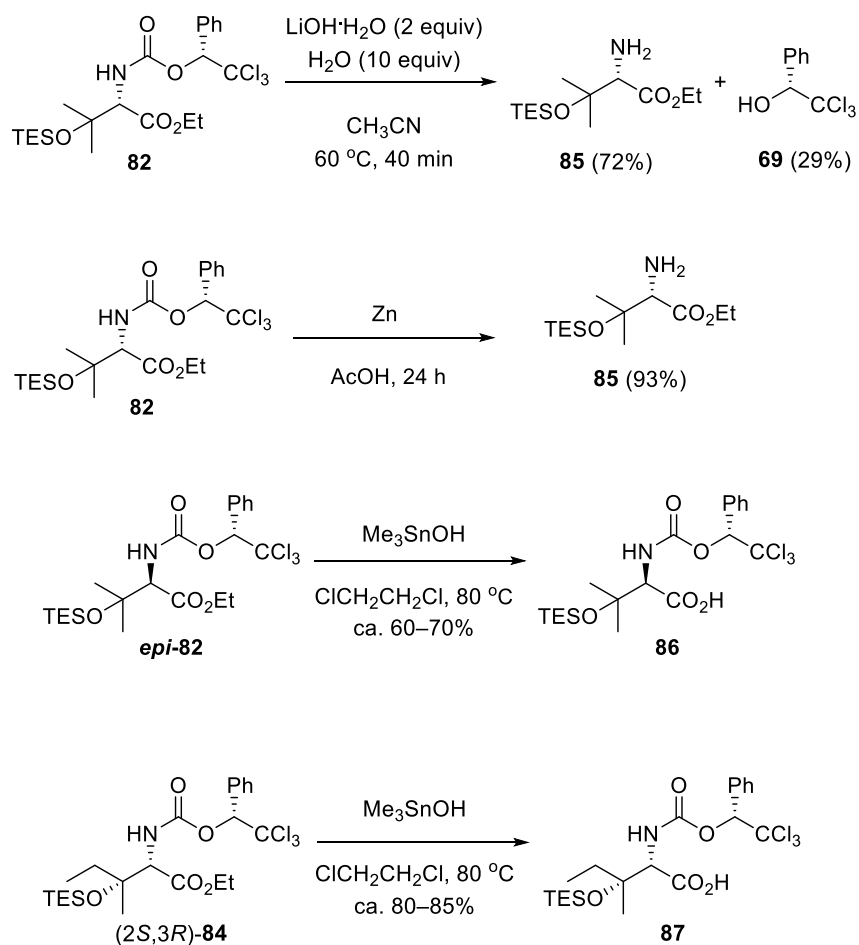


Scheme 2.13 Preliminary Studies on β-Hydroxy Amino Acid Synthesis

Using the same chemistry with enoate **50** as substrate, we prepared the β-OH Ile derivatives (2*S*,3*R*)-**83** and (2*R*,3*S*)-**83**. Since **50** is bulkier than **39**, we obtained a lower yield for the first step. Product (2*S*,3*R*)-**83** was used directly for the synthesis of yaku'amide A while (2*R*,3*S*)-**83** was converted to a synthetic intermediate in the synthesis of ΔIle. The synthesis of yaku'amide will be discussed in chapter 3.

2.5 Selective Deprotection of Chiral Reagent-Mediated Aminohydroxylation Product

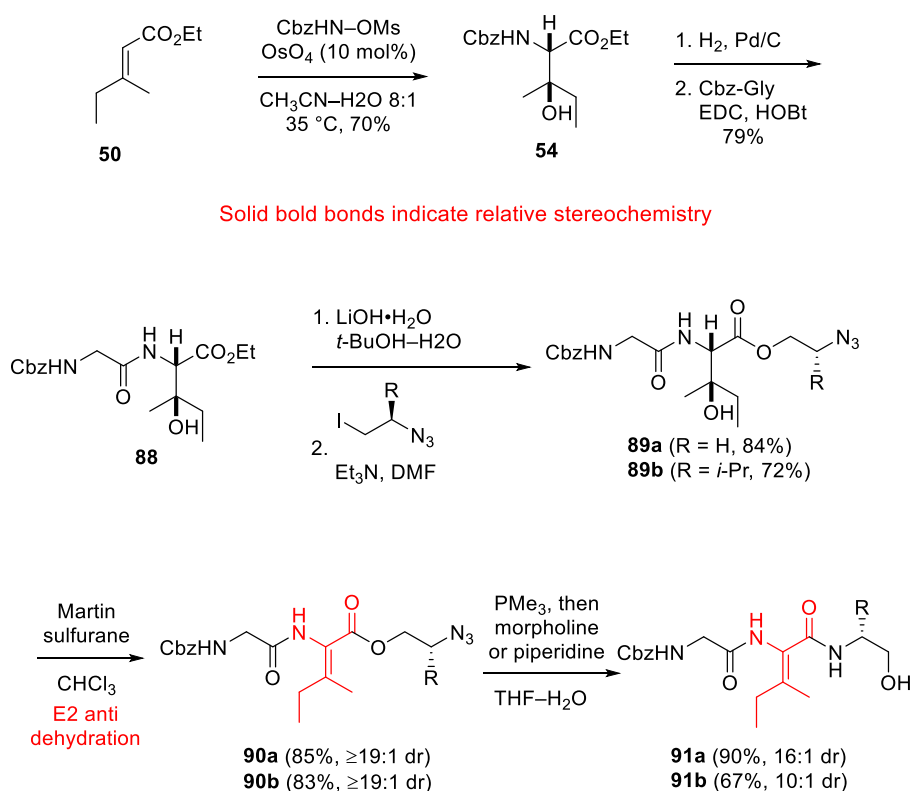
In order to perform peptide couplings, it is necessary to selectively remove the protecting groups of the chiral reagent-mediated aminohydroxylation product. To our surprise, the ethyl ester was slightly more stable than the chiral carbamate group of **82** to basic hydrolysis. So we could hydrolyze the carbamate of **82** without the cleavage of the ester. Also, we could recover chiral alcohol **69** and recycle it in the synthesis of chiral reagent **71**, thus making the process more efficient. Upon treatment with Zn/AcOH, **82** could also be converted to amine **85** cleanly, but without recovery of alcohol **69**. When we used Me₃SnOH to selectively hydrolyze *epi*-**82**, and (2*S*,3*R*)-**84**, we obtained pure acids with good yields (Scheme 2.14).



Scheme 2.14 Chemoselective Deprotections

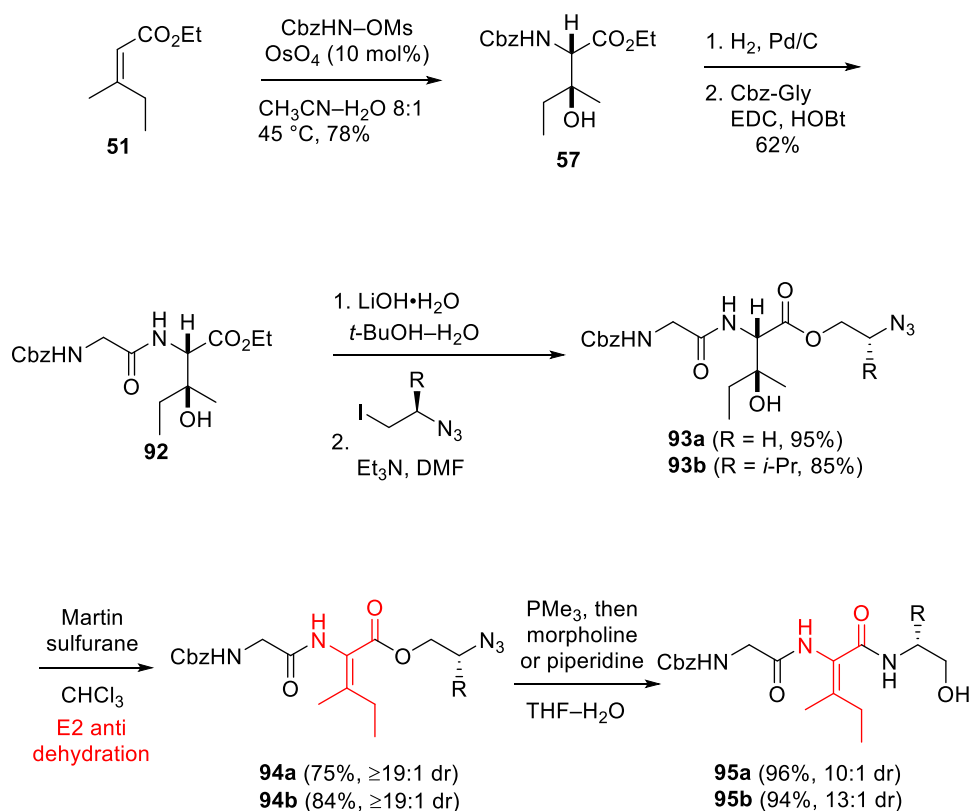
2.6 Dehydroisoleucine Synthesis

To synthesize *Z*- and *E*-Alle-containing model peptides, we combined our improved aminohydroxylation with a unique *anti* dehydration. Co-worker Zhiwei Ma investigated a series of *anti* dehydration conditions and found Martin sulfurane would give the best results, providing a high yield of a single isomer. Aminohydroxylation of *E*-enoate **50** with CbzHN-OMs generated racemic tertiary alcohol **54** in good yield (Scheme 2.15). Removal of the Cbz protecting group and coupling with Cbz-Gly afforded a dipeptide **88**. Next, basic hydrolysis and alkylation with azido iodides produced the precursors **89a** and **89b** for later *anti* dehydrations in the presence of Martin sulfurane. With subsequent Staudinger reductions of **90a** and **90b**, the resulting primary amines underwent O→N acyl transfers to deliver *Z*-Alle-containing peptides **91a** and **91b** with more than 10:1 diastereoselectivity (*Z*:*E* ratio).²⁷



Scheme 2.15 Synthesis of *Z*-Alle-Containing Peptides

Similarly, we obtained racemic alcohol **57**, the diastereomer of **83**, with our standard aminohydroxylation (Scheme 2.16). Using the same methods for the synthesis of *Z*- Δ Ile-containing peptides, we synthesized β -azido esters **93a** and **93b** with very good yields. Next, **95a** and **95b** were provided through our stereospecific dehydration and O \rightarrow N acyl transfer sequence from **93a** and **93b**.²⁷



Scheme 2.16 Synthesis of *E*- Δ Ile-Containing Peptides

2.7 Summary

To access the β -hydroxyamino acids and dehydroamino acids in yaku'amide A we have optimized our regioselective base-free aminohydroxylation method with a series of nitrogen sources, developed a chiral reagent-mediated aminohydroxylation strategy and chemoselective deprotections of the resulting aminohydroxylation product, and explored a stereospecific E2

dehydration and O→N acyl transfer sequence. Compared with Inoue's lengthy method of constructing β -OHVal and β -OHlle derivatives **25** and **27**, we only needed two steps to produce these residues. Also, our approach to install Z- and E- Δ Ile requires no backbone amide protection and it would dramatically shorten the total synthesis of yaku'amide A.

2.8 References

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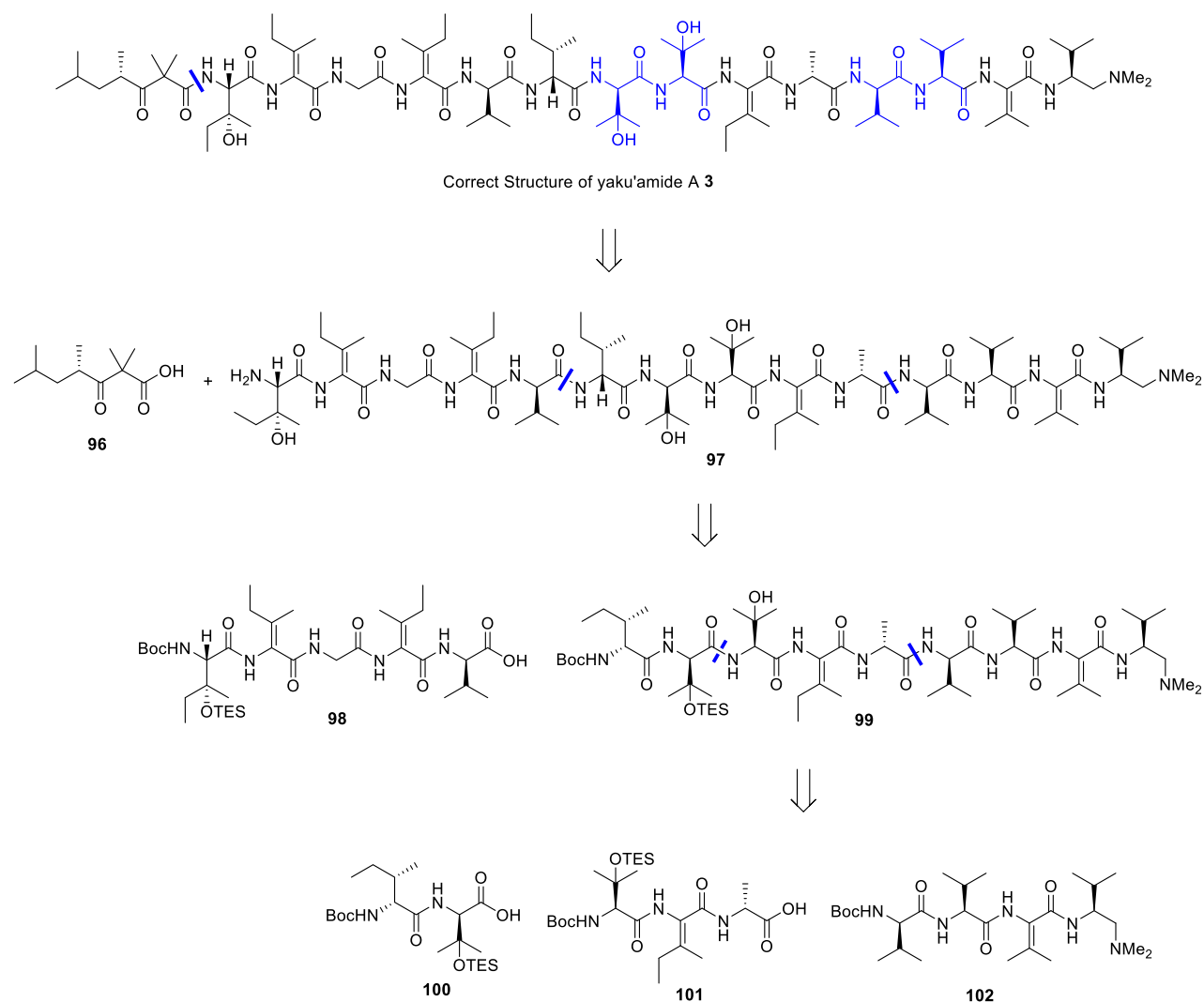
CHAPTER 3. PROGRESS TOWARD YAKU'AMIDE A

3.1 Introduction

With lengthy routes to construct β -hydroxyamino acids and dehydroamino acids, Inoue's group synthesized yaku'amide A in 86 steps. After developing methodologies to access these important subunits in our group, we designed a detailed plan to achieve the total synthesis of yaku'amide A in a more efficient manner.

3.2 Retrosynthetic Analysis of Yaku'amide A

Based on the previously mentioned results, we have designed a retrosynthesis of yaku'amide A (Scheme 3.1). Following Inoue's work,^{1,2} the NTA (**96**) and tetradecapeptide **97** could be assembled to give target molecule **3**. So we planned to couple the NTA with **97** in the final step of our total synthesis as well. Since we could prepare Z- and E- Δ Ile residues without backbone amide protection, we disconnected compound **97** to give left-hand pentapeptide **98** and nonapeptide **99** to increase the synthetic efficiency. Pentapeptide **98** contains one Z- Δ Ile and one E- Δ Ile, while nonapeptide **99** includes one Z- Δ Ile and one Δ Val. Based on the structural complexity, it would take a similar number of steps to access both **98** and **99**, thus maximizing convergency of our total synthesis. After a careful examination, we divided building block **99** into three intermediates—dipeptide **100**, tripeptide **101**, and right-hand tetrapeptide **102**—to minimize the number of steps.



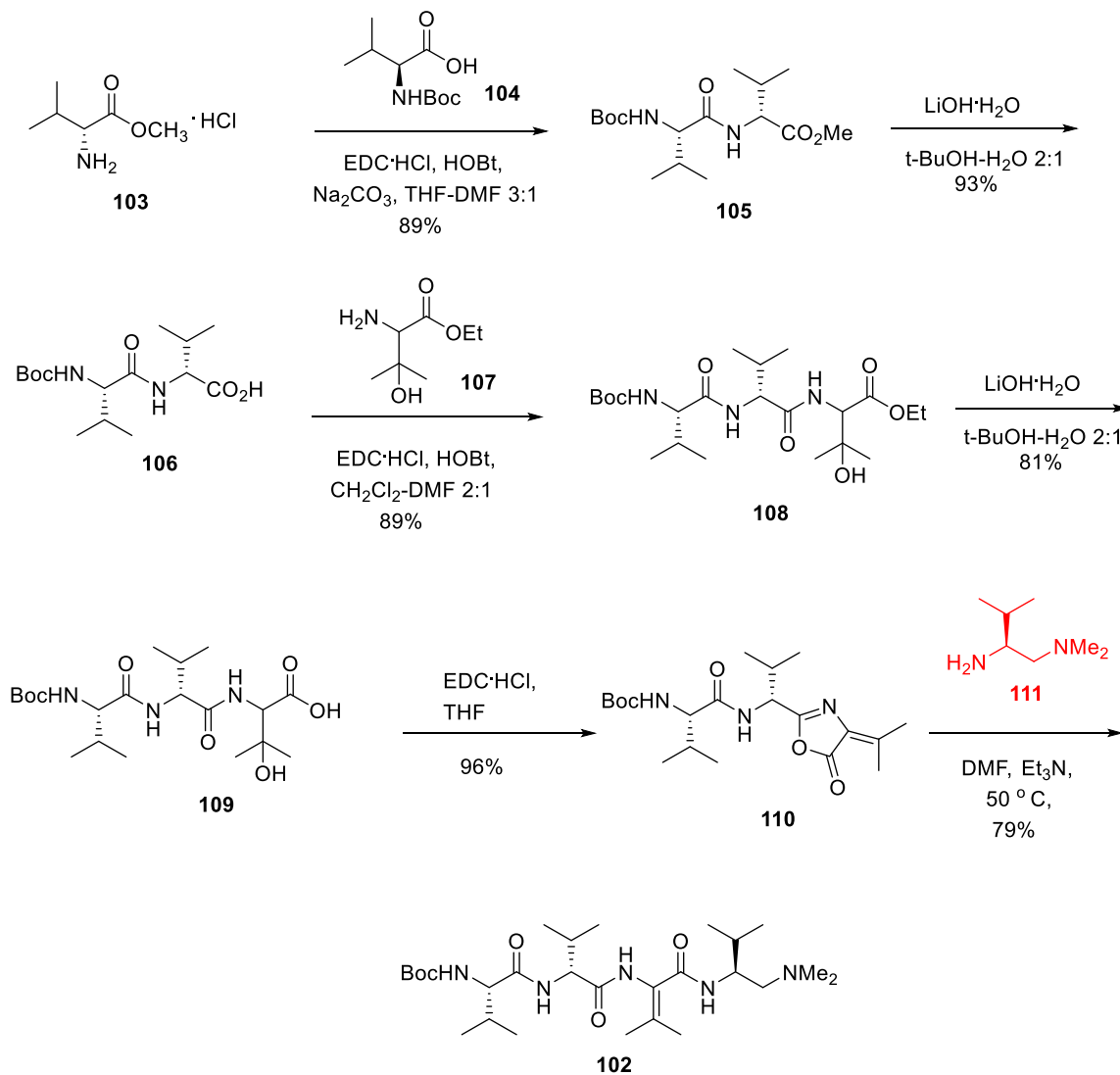
Scheme 3.1 Retrosynthetic Analysis of yaku'amide A 3

3.3 Synthesis of Right-hand Tetrapeptide

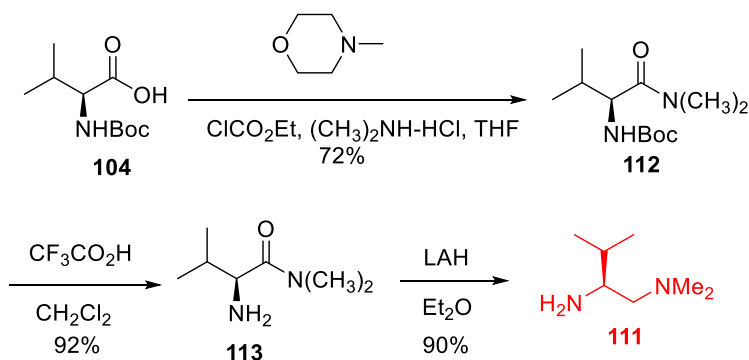
When we worked on the synthesis of tetrapeptide of yaku'amide A in 2012, only the structure proposed by Matsunaga³ was known and the absolute C4-stereochemistry still needed to be determined. So our synthesis was toward the structure of **1** proposed by Matsunaga.

Since the right-hand tetrapeptide **102** contains one Δ Val, we do not need to worry about alkene isomerization for the synthesis. Coupling of Boc-L-Val **104** with D-Val-OMe-HCl **103** produced dipeptide **105** (Scheme 3.2). Saponification of dipeptide **105** delivered an acid **106**. Next, coupling

of this newly generated acid **106** with racemic β -OHVal derivative **107** furnished tripeptide **108**. After another saponification, the produced acid **109** formed a five-membered azlactone ring intermediate **110** in the presence of peptide coupling agent EDCI. Next, coupling of **110** with diamine **111** afforded the right-hand tetrapeptide **102** of the originally proposed yaku'amide A. Known diamine **111** was synthesized in three steps from commercially available Boc-L-Val **104** (Scheme 3.3).⁴

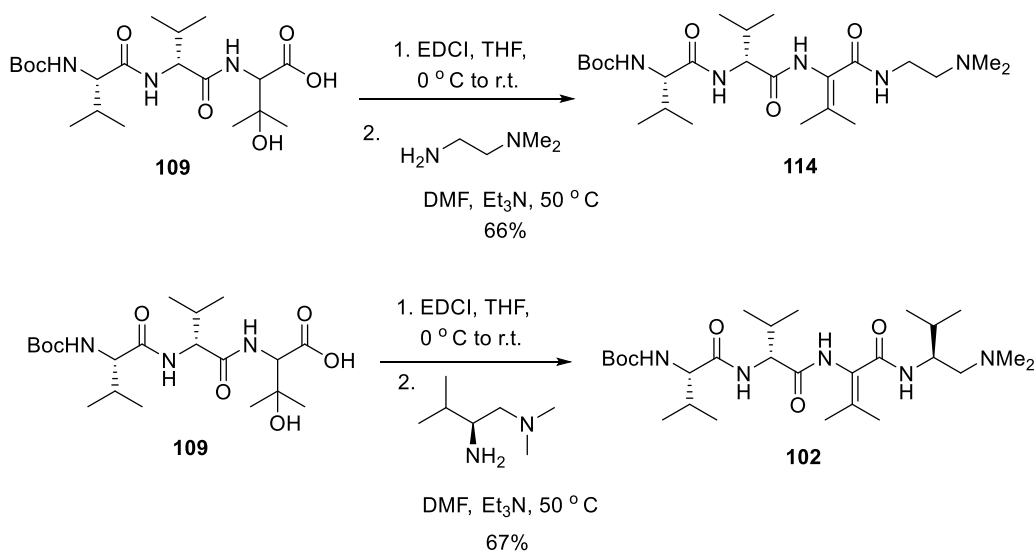


Scheme 3.2 Synthesis of Right-Hand Tetrapeptide **102**



Scheme 3.3 Synthesis of Diamine **111**

After finishing the synthesis of **102**, we intended to combine the last two steps together. Since it took three steps to prepare diamine **111**, we used a commercially available N,N-dimethylethylenediamine to optimize the last two steps. After screening different solvents, temperatures, reaction times, and reagent ratios, the last two steps could be achieved in a one-pot procedure with a 66% yield in our model study. When we applied the reaction conditions to the synthesis of **102**, we obtained 67% yield over two steps (Scheme 3.4). Co-worker Zhiwei Ma used this route to prepare the actual tetrapeptide of **3** by making the enantiomer of **106**.

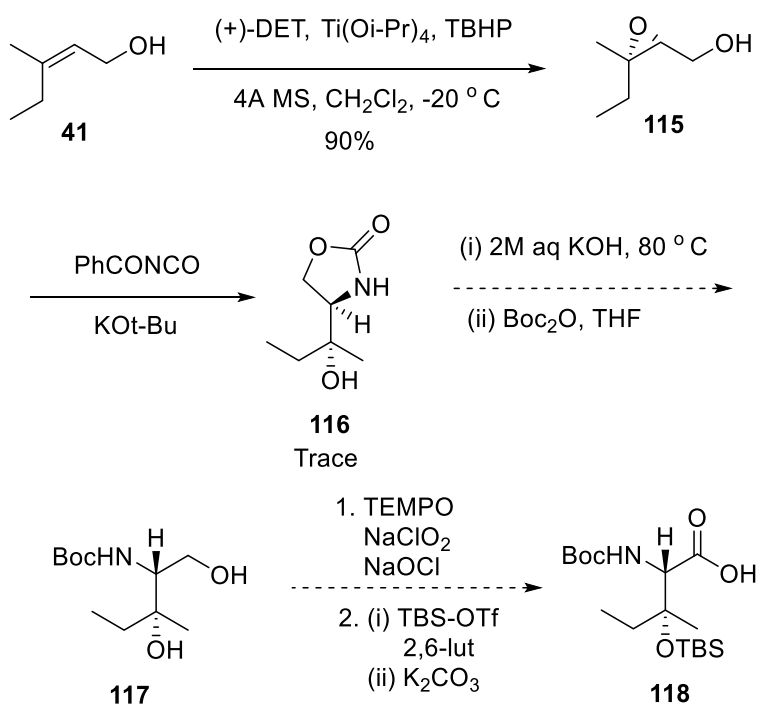


Scheme 3.4 Optimization of the Synthesis of **102**

3.4 Studies Toward the Synthesis of (2S,3R)- β -OHlle Derivative

When we started the total synthesis of **1**, there was no published total synthesis of yaku'amide **A**. In order to synthesize a (2S,3R)- β -OHlle derivative, we designed a synthetic route to access **118** (Scheme 3.5).

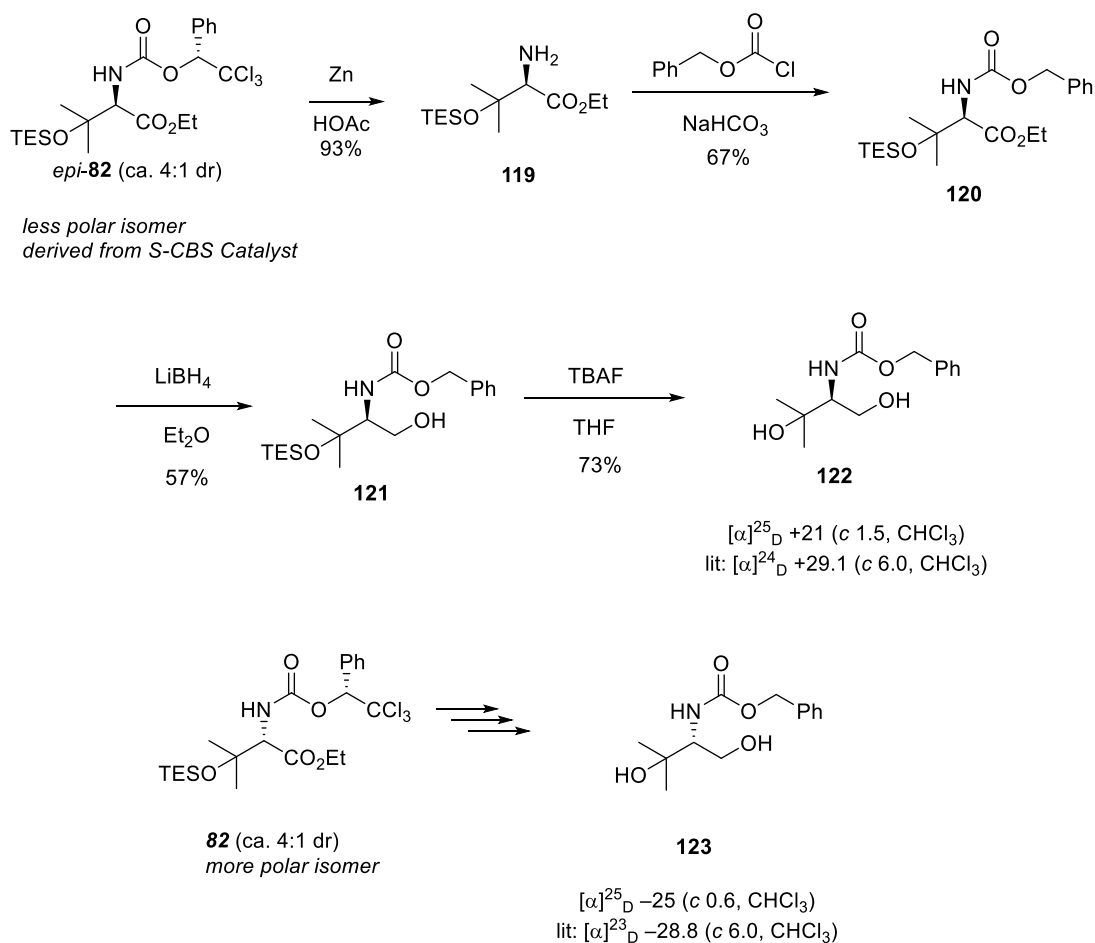
First, allylic alcohol **41** was obtained in two steps from commercially available ethyl 2-pentynoate **51**.^{5,6} Next, we used Sharpless asymmetric epoxidation⁷ to generate epoxide **115**. The enantioselective epoxidation of **41** was not reported before. While we were working on the next step to generate oxazolidinone **116**, we discovered a chiral reagent-controlled aminohydroxylation to access **118** in shorter steps (Chapter 2.4). So this synthetic route was discontinued in our lab without calculating the ee of **115**.



Scheme 3.5 Proposed Synthesis of (2S, 3R)- β -OHlle Derivative **118**

3.5 Assignment of Absolute Configurations of β -OHVal Derivatives **82** and *epi-82*

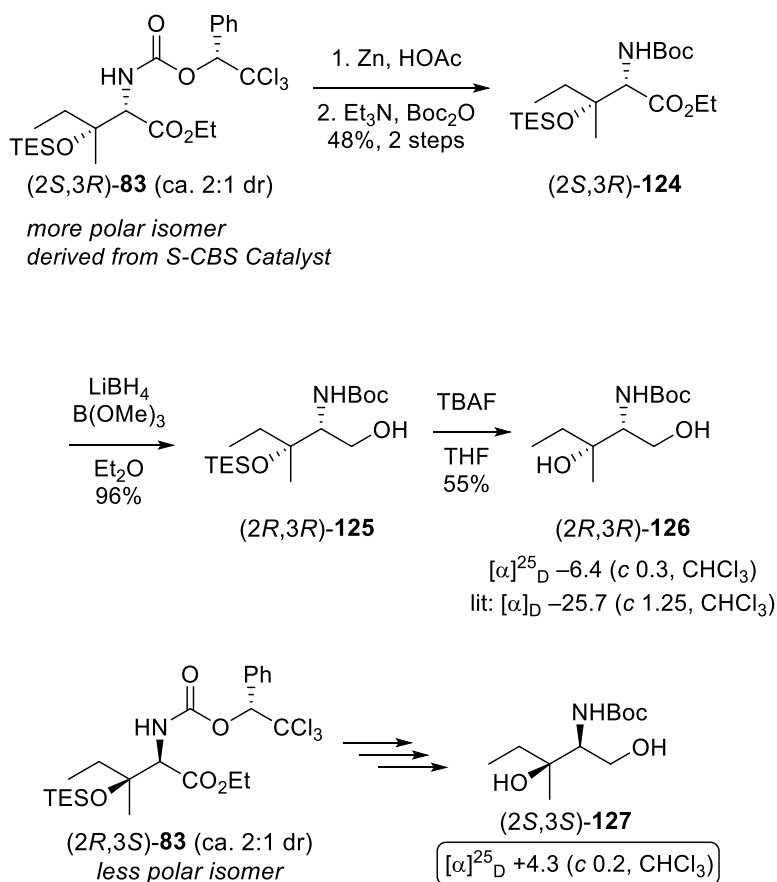
With the chiral reagent-based method in hand, we needed to assign the absolute configurations of the β -OHVal derivatives **82** and *epi-82* for the synthesis of dipeptide **100** and tripeptide **101**. We converted *epi-82* and **82** into diols **122** and **123** reported by Saalfrank and co-workers (Scheme 3.6).⁸ In the presence of Zn/AcOH, *epi-82* was cleanly converted to amine **119**. A subsequent Cbz protection of the primary amine **119** generated intermediate **120**. Upon treatment of LiBH₄, the ester was reduced to a primary alcohol **121**. A final TES deprotection afforded diol **122**. Similarly, we generated diol **123** and matched the optical rotations with the data found in the literature.



Scheme 3.6 Assignment of Absolute Configurations of *epi-82* and **82**

3.6 Assignment of Absolute Configurations of (2*S*,3*R*)-**83** and (2*R*,3*S*)-**83**

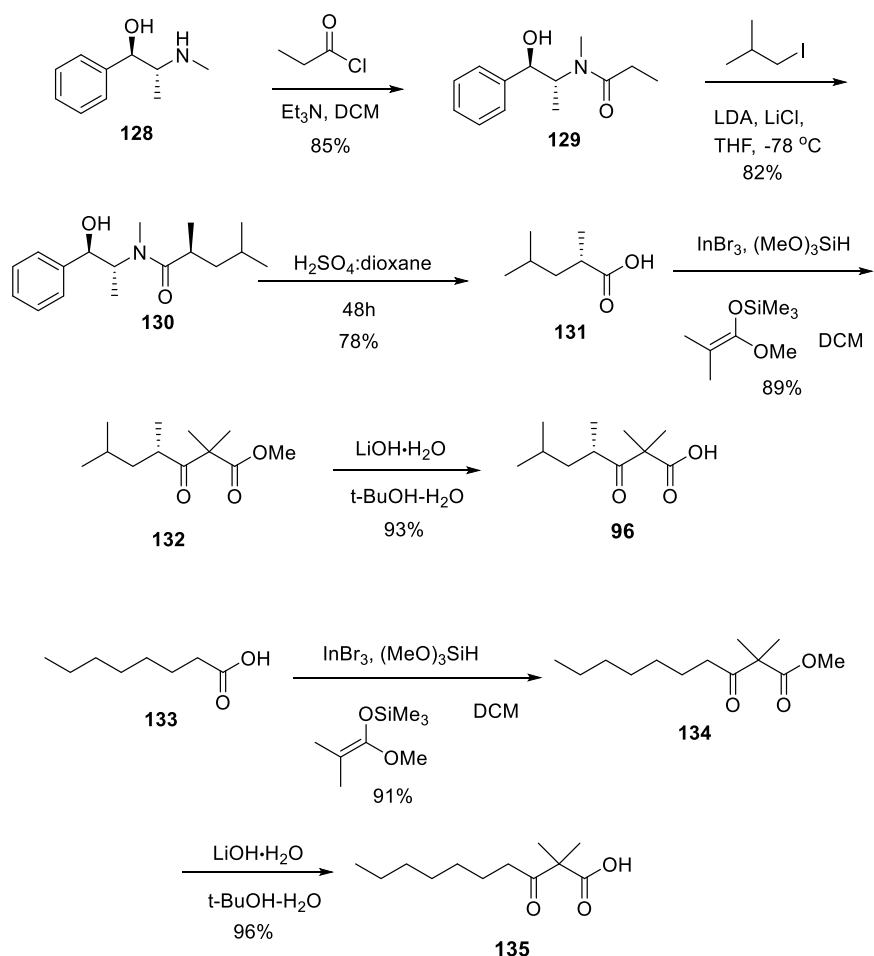
In order to synthesize pentapeptide **98**, co-worker Joseph M. Cardon and I assigned absolute configurations of (2*S*,3*R*)-**83** and (2*R*,3*S*)-**83** based on data reported by Guanti and co-workers.⁹ Removal of the chiral carbamate and a subsequent Boc protection of the newly generated amine produced (2*S*,3*R*)-**124** (Scheme 3.7). A reduction of **124** and a final TES deprotection afforded (2*S*,3*R*)-**126**. With similar chemistry, we prepared diol **127** and measured the optical rotations.



Scheme 3.7 Assignment of Absolute Configurations of (2*S*,3*R*)-**83** and (2*R*,3*S*)-**83**

3.7 Synthesis of The NTA

While co-workers were working on the synthesis of building blocks **98**, **100**, and **101** of yaku'amide A, we also designed a route to synthesize NTA **96** in shorter steps. The proposed synthesis of **96** is shown in Scheme 3.8.



Scheme 3.8 Synthesis of NTA **96** and Model Study of Similar Acid

Starting from pseudoephedrine **128**, an acylation with propionyl chloride delivered amide **129**.¹⁰ Next, an asymmetric alkylation with 1-iodo-2-methylpropane yielded known compound **130**.¹¹ The reported de value of **130** was more than 99%. Validation of the de will be performed in the future. In the presence of sulfuric acid, the chiral auxiliary pseudoephedrine was removed to generate a carboxylic acid **131**.¹² This set the stage for an indium-catalyzed cross-Claisen

condensation with a ketene silyl acetal to produce β -ketoester **132**.¹³ Finally, a basic hydrolysis generated NTA **96**. We used commercially available octanoic acid **133** as a model compound to optimize the last two steps of the synthesis of NTA **96**. β -ketoester **134** and acid **135** were characterized to help us with the purification and reaction details. NTA **96** will be coupled with tetradecapeptide **97** to finish the total synthesis of yaku'amide A in near future.

3.8 Summary

In our efforts to synthesize yaku'amide A, we have successfully achieved the synthesis of the right-hand tetrapeptide. For the synthesis of (2S,3R)- β -OHlle derivative in our early days of this project, we prepared an interesting epoxide which could undergo epoxide ring-opening chemistry to provide useful compounds. In addition, we have assigned the absolute configurations of β -OHVal derivatives **82**, *epi*-**82**, (2S,3R)-**83** and (2R,3S)-**83**, which would be used to prepare building blocks of yaku'amide A. For the synthesis of the NTA subunit, we used 5 linear and total steps compared with 10 linear and 11 total steps adopted by Inoue's group.

3.9 References

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CHAPTER 4. INTRODUCTION TO BULKY α,β -DEHYDROAMINO ACIDS

4.1 Introduction

There are no α,β -dehydroamino acids (Δ AAs) among the 20 proteinogenic amino acids, but they exist in some toxins and antibiotics in nature. Their planar geometry at the α and β carbons greatly impacts the structures of Δ AA-containing peptides. For example, they can strongly induce certain folded conformations in peptides.¹ Replacement of a saturated residue with a dehydroamino acid would introduce significant constraints into the peptide backbone due to the interactions between the main chain and the side chain. Protein-protein interactions (PPIs) are very important targets for drug design since they control fundamental cellular process.²⁻⁴ Researchers have discovered that protein secondary structures contribute greatly to the free energy of binding in organizing the "hot spots" in proteins.⁵ And synthetic peptides containing key amino acids of energetically important protein secondary structures show inhibitory profile against protein interfaces specifically. So bulky Δ AAs-containing peptides have potential to target PPIs.

Previously, Δ AAs have been the topic of a series of reviews.⁶ Some reviews focused on specific categories of Δ AAs,⁷ while others centered on certain characteristics of their chemistry.^{8,9} We wrote a review about bulky Δ AAs in which we define them as residues that contain either a trisubstituted alkene with a large β substituent or a tetrasubstituted alkene.¹⁰ High levels of $A_{1,3}$ strain are triggered by the group or groups at the β carbon (e.g., Δ Val, Δ Ile, Δ Leu, and Δ Phe). In

other words, the interactions between the main chain and the side chain of Δ AAs in a peptide would cause significant rigidity. This rigidity can induce more stable folded states of bulky Δ AA-containing peptides. Also, the backbone geometry is altered from the non-planar tetrahedral configurations to the planar trigonal configurations so active sites of proteases do not accept bulky dehydroamino acids efficiently, thus stabilizing peptides to proteases.¹¹

Bulky α,β -dehydroamino acids are key components of many peptide natural products.¹² The structures and properties of naturally occurring peptides that include trisubstituted Δ AAs and tetrasubstituted Δ AAs will be discussed in this section. Since there are too many trisubstituted Δ AA-containing natural products, I will only summarize selected compounds with interesting structures and/or potent bioactivity in this category.

4.2 Bulky Trisubstituted Δ AA-Containing Natural Products

Many bulky Δ AA-containing natural products are diketopiperazines. In this family, several dysamides isolated from *Dysidea herbacea* and *Dysidea chlorea* sponges contain δ -polychlorinated Δ Leu subunits (Figure 4.1, bulky Δ AAs shown in red).¹³ The conjugated diene existing in dysamide J (**136e**) could likely be generated via elimination of HCl from dysamide M (**136h**). With the help of X-ray crystallography and total synthesis, the absolute stereochemistry of these compounds were assigned.¹⁴

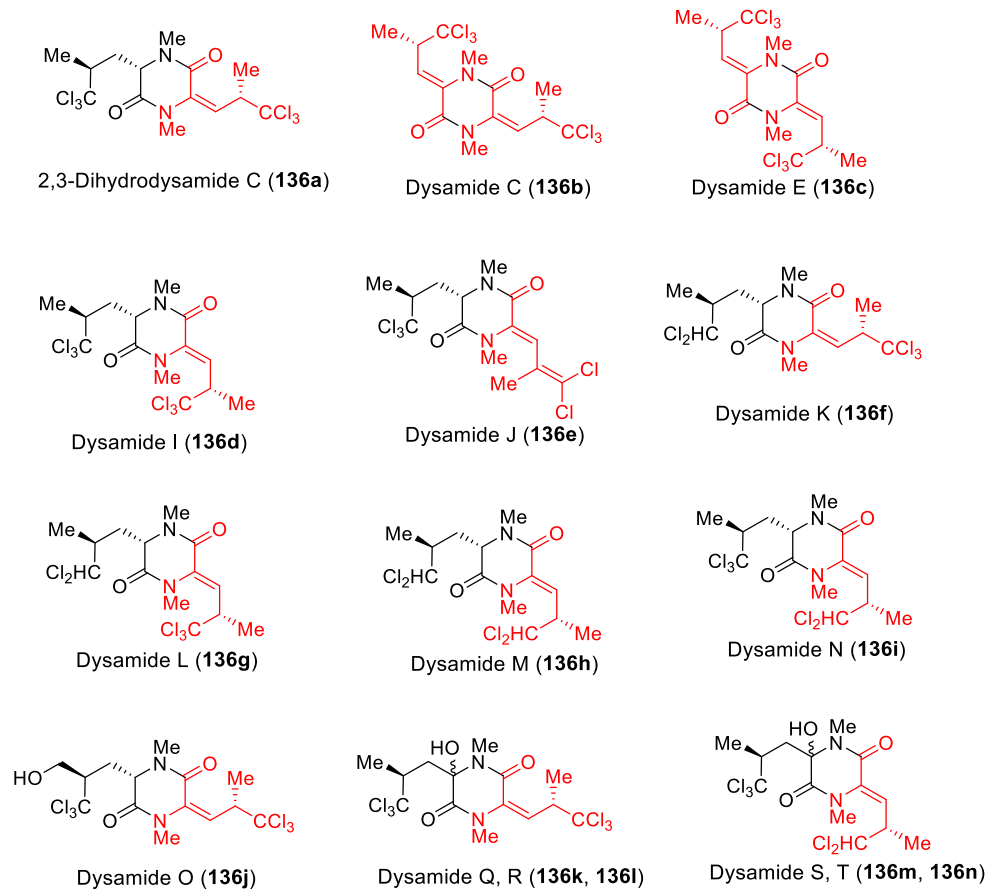


Figure 4.1 Structures of Dysamides

Albonoursin (**137**, Figure 4.2), obtained from the actinomycete species in the 1960's, includes one *Z*- Δ Leu and one *Z*- Δ Phe.¹⁵ It shows inhibitory profile against bacteria, the H1N1 strain of influenza,¹⁶ and tumor cells.¹⁷ Kanzaki and co-workers used cell-free bacterial extracts to prove that **137** is generated via enzymatic dehydrogenation of cyclo(L-Phe-L-Leu).¹⁸

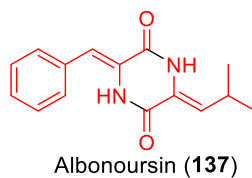


Figure 4.2 Structure of Albonoursin

Templeton and co-workers obtained Tentoxin (**138**, Figure 4.3) from the fungus *Alternaria tenuis*. It is a cyclic tetrapeptide with a Z- Δ Phe residue and it is toxic to plants.¹⁹ Both the Meyer²⁰ and Rich²¹ groups helped to determine its structure. Tentoxin can induce chlorosis in certain plant species, which indicates that it is a potential herbicide.²² Due to the high affinity of **138** to the chloroplast F₁ H⁺-ATPase, it can inhibit energy transfer.²³

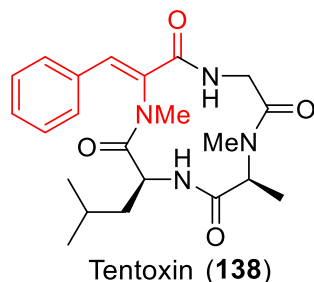


Figure 4.3 Structure of Tentoxin

In 1977, Ōmura and co-workers isolated the antibiotic dityromycin.²⁴ Later its structure (**139**, Figure 4.4) was determined²⁵, but there were still uncertainties about the configurations of the two Δ AA residues and the β -OH-Tyr. Compound **139** possesses an *O*-arylated Δ Phe and an unusual tetrasubstituted Δ AA containing a hydroxymethyl group and an epoxide. With an extra degree of unsaturation, GE82832²⁶, an inhibitor of translocation in bacteria, has a very similar structure to **139**.²⁷ Both **139** and GE82832 can bind to the ribosomal protein S12, resulting in disruption of an interaction between S12 and elongation factor G (EF-G), thereby inhibiting the process of EF-G-catalyzed translocation.²⁸

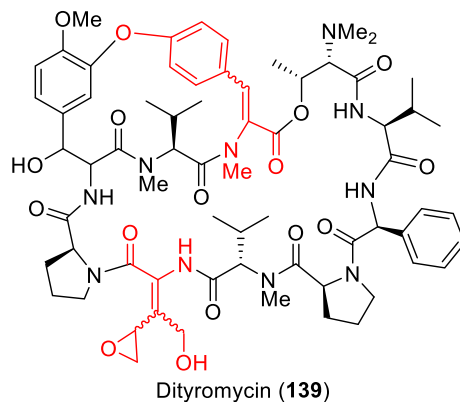


Figure 4.4 Structure of Dityromycin

In 1997, Kanoh and co-workers derived phenylahistin (**140**, Figure 4.5) from culture broths of *Aspergillus ustus* NSC-F038 and elucidated its structure.^{29a} They used chiral HPLC to separate the mixture of enantiomers ($R:S = 3:1$) of this Z - Δ His containing compound. (–)-Phenylahistin ((S)-enantiomer) showed cell cycle inhibitory and antitumor activities by disrupting tubulin polymerization.²⁹ In 2002, dehydrophenylahistin (**141**) was synthesized from **140** through an enzymatic conversion.³⁰ This compound exhibited outstanding anticancer activity (ca. 1000 times more potent than **140**), and clinical trials were conducted under the name plinabulin (NPI-2358).³¹ Further structure–activity studies indicated the potency of **141** could be strengthened by introducing a benzoyl or a fluoro substituent to the phenyl subunits.³²

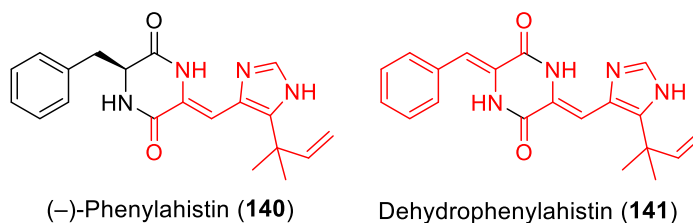


Figure 4.5 Structures of (–)-Phenylahistin and Dehydrophenylahistin

From the fungus *Aspergillus effusus* H1-1, Li and co-workers isolated effusin A (**142**, Figure 4.6) and dihydrocryptochinuline D (**143**) in 2012.³³ Compound **142** includes a spirocyclic N,O -acetal and both peptides contain a reverse prenylated Z - Δ Trp structure. In addition, both **142** and

143 occur in nature as racemates that could be separated by chiral HPLC. Racemic **143** displayed antitumor activity, and (+)-**143** was discovered to inhibit topoisomerase I.

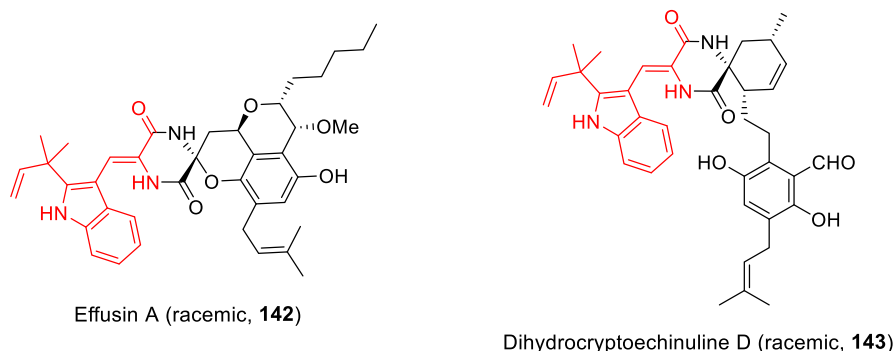


Figure 4.6 Structures of Effusin A and Dihydrocryptochinuline D

4.3 Bulky Tetrasubstituted Δ AA-Containing Natural Products

In 1969, Marchand and co-workers isolated the Δ Val-containing acyclic depsipeptide lasiodine A (**144**, Figure 4.7) from the African buckthorn plant *Lasiodiscus marmoratus*.³⁴ The structures of the *D*-*threo*- β -phenylserine and *N*-Me-*D*-Val residues were elucidated by degradation studies, and the *Z*-enamide configuration was determined by ¹H NMR spectroscopy.³⁵ Nevertheless, the stereochemistry of the *N,N*-dimethyl- β -phenylserine residue remains unclear. Lasiodine A showed inhibitory activity against photophosphorylation in spinach chloroplasts.³⁶

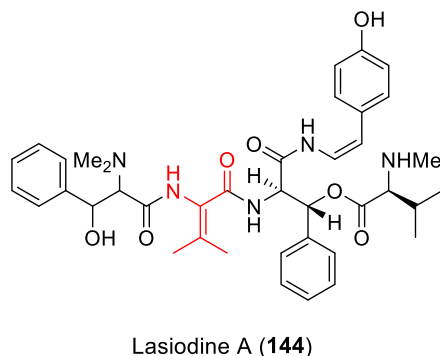


Figure 4.7 Structure of Lasiodine A

In 1981, Shimada and co-workers isolated the antibiotic heptapeptide antrimycin A (**145a**, Figure 4.8) from *Streptomyces xanthocidicus*.³⁷ One year later, they isolated relatively small amounts of peptides antrimycins B–C and Av–Dv (**145b–h**) from the same antrimycin A-producing microorganism.³⁸ In 1982, Shiroza and co-workers isolated cirratiomycin A and B from a different microorganism, *Streptomyces cirratus*. Cirratiomycin B is identical to **145a**, while cirratiomycin A possesses a Leu instead of the Ala residue found in **145a**.³⁹ Antrimycins A–D contain a *E*-ΔIle residue, while Antrimycins Av–Dv contain a ΔVal residue.

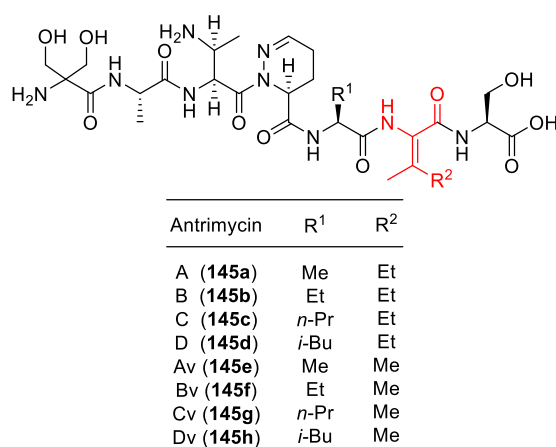


Figure 4.8 Structures of Antrimycins

In 1983, Culvenor and co-workers isolated the antimetabolic peptides phomopsins A and B (**146a** and **146b**, Figure 4.9) from the cultivated fungus *Phomopsis leptostromiformis*.⁴⁰ The originally proposed structures were revised with new data obtained from chemical degradation, mass spectrometry and X-ray crystallography studies.⁴¹ Their uncommon structure contains a 13-membered lactam as well as an acyclic side chain. This family possesses six nonproteinogenic amino acids and one *E*-ΔIle. Phomopsin A activates lupinosis in livestock, and it binds to β-tubulin to inhibit the generation of microtubules.^{42–44}

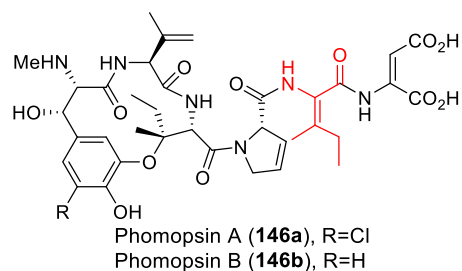


Figure 4.9 Structures of Phomopsins A and B

In the same year, Kemmer and co-workers isolated Myxoalargin A (**147**, Figure 4.10) from the myxobacterium *Myxococcus fulvus* and explored its activity against Gram-positive bacteria.⁴⁵ Although they discovered that Δ Val and Δ Ile were present in the structure, the complete structure of **147** was proposed in 1989. They found that Myxoalargin A includes an *E*-Ile residue.⁴⁶ Ensuing studies established that **147** inhibits the synthesis of bacterial protein and causes damage to cell membranes. Interestingly, it is likely to contribute to its toxicity in mice due to the latter bioactivity.⁴⁷

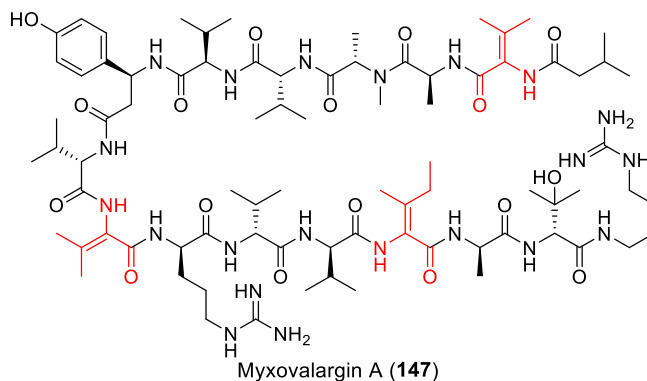


Figure 4.10 Structure of Myxoalargin A

In 2003, FR225659 (**148a**, Figure 4.11) and four similar peptides (**148b–e**) were isolated from a strain of fungus grown on a decayed leaf by chemists at Fujisawa Pharmaceutical Co.⁴⁸ With chemical degradation and X-ray crystallographic analysis, they were able to determine the configuration of the stereocenter of the methylated hydroxyproline residue in **148a**. In addition, they established that the *C*-terminal Δ Ile residue in **148b** adopts an *E*-geometry from NOE studies.

However, the researchers were not certain about the configuration of the revised arginine residue. Peptides **148a–e** showed inhibitory effects against glucagon-stimulated gluconeogenesis, and the serine/threonine protein phosphatases PP1 and PP2A^{49a} were identified as targets after a modified **148a** was attached to affinity latex beads^{49b}.

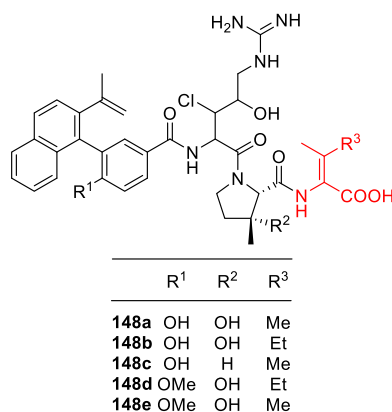


Figure 4.11 Structures of Peptides **148a-e**

In 1986, Nagaoka and co-workers isolated Azinomycins A and B (**149a** and **149b**, Figure 4.12) from *Streptomyces griseofuscus*.⁵⁰ Later, Armstrong and Moran⁵¹ found that **149b** is spectroscopically the same as carzinophilin,⁵² a peptide discovered earlier. These peptides feature a tetrasubstituted dehydroamino acid containing a substituted aziridino[1,2-*a*]pyrrolidine ring structure. Azinomycin B can generate cross-links between strands of DNA, leading to potent activity against several tumor types.⁵³ Watanabe and co-workers conducted in vivo experiments to demonstrate that **149b** can cause broad DNA damage within cells.⁵⁴

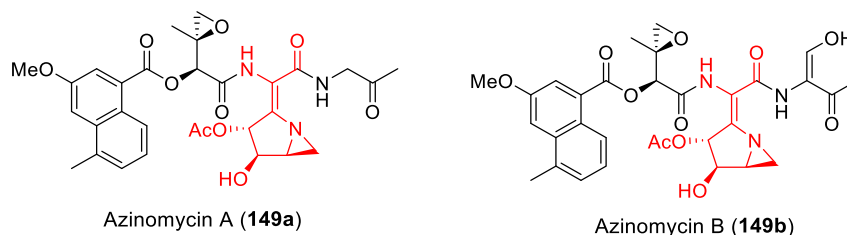


Figure 4.12 Structures of Azinomycins A and B

Miuraenamides A and B (**150a** and **150b**, Figure 4.13) were isolated by Fudou and co-workers from a myxobacterial strain SMH-27-4 in 2006.⁵⁵ These two macrocyclic depsipeptides include *E*- β -methoxydehydrophenylalanine and show antifungal activity. Two years later, they isolated miuraenamides C, F, and D (**150c–e**, Figure 4.13) and elucidated the structures of these peptides.⁵⁶ Compound **150e** was considerably less potent than **150a–d** due to the change of the Δ AA geometry to a *Z*- β -methoxydehydrophenylalanine. A cell morphology profiling of **150a** established that it could function as an actin filaments stabilizer *in vitro*.⁵⁷

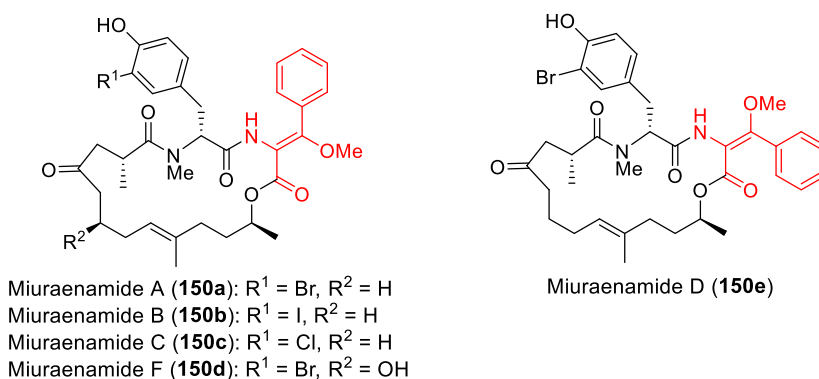


Figure 4.13 Structures of Miuraenamides

4.4 Summary

Peptide natural products have attracted substantial attention from biologists and chemists alike as a result of their remarkable molecular structure and potent biological activity. Bulky α,β -dehydroamino acids are components of many naturally occurring peptides. In this section, we have summarized the structures and bioactivities of important compounds that contain bulky Δ AAs. First, we included metabolites that possess trisubstituted Δ AAs. Next, we covered those that include tetrasubstituted Δ AAs.

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CHAPTER 5. SYNTHESIS AND APPLICATIONS OF BULKY α,β - DEHYDROAMINO ACIDS

5.1 Introduction

Researchers have discovered that α,β -dehydroamino acids (Δ AAs) can stabilize peptides to degradation by proteases without diminishing their bioactivity.¹ In 1983, Stammer reported that a *Z*-dehydroleucine-containing analogue of enkephalin resisted enzymatic degradation while maintaining the enkephalin-like activity (Figure 5.1). The incorporation of dehydroleucine in the enkephalin molecule produced a peptide with unchanged selectivity that could interact with both μ and δ receptors with full activity.

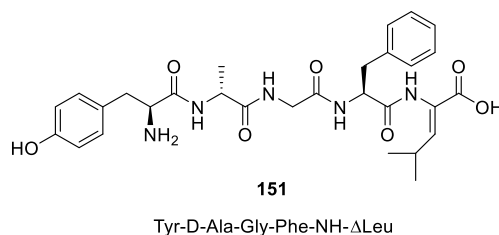


Figure 5.1 Structure of Enkephalin Analogue in the *Z*-configuration

Previously, methods involving conversion of the carboxylic acid of enkephalin to the corresponding amide, ester or alcohol would stabilize the peptide to degradation.^{2,3} However, the receptor selectivity and *in vivo* biological activity would be changed invariably. In comparison, the *Z*- Δ Leu-enkephalin was about four times more active compared with its saturated form (Leu)

in the assay with etorphine as tracer. Also, the authors had reported that some synthetic dehydrophenylalanine-containing peptides could be stabilized to enzymatic hydrolysis by thermolysin and chymotrypsin.^{2,3}

Compared with the trisubstituted Δ Leu and Δ Phe in enkephalin, bulky tetrasubstituted Δ AAs such as Δ Val and Δ Env should have a larger effect on peptide structure and stability due to their increased levels of $A_{1,3}$ strain causing significant rigidity and more stable folded states (Figure 5.2). Our hypothesis is that the bulky dehydroamino acids Δ Val and Δ Env should stabilize certain peptides without changing the biological activities. Thus, we will incorporate these dehydroamino acids into a variety of small peptides to determine which types of peptide are favored and stabilized. In this way, we will develop rules of how to incorporate Δ Val and Δ Env in small synthetic peptides (Figure 5.2).

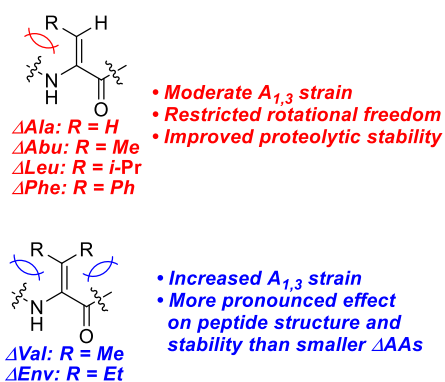


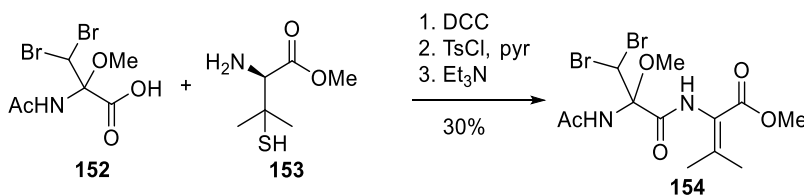
Figure 5.2 Bulky Dehydroamino Acids versus Normal Dehydroamino Acids

5.2 Methods of Synthesizing Bulky α,β -Dehydroamino Acids

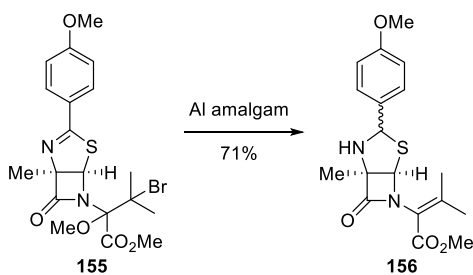
The classical method for the synthesis of Δ AAs involves the condensation of an azlactone (i.e., oxazolone) with an aldehyde.⁴ In previous chapters of this dissertation, several newly developed methods of constructing Δ Ile (Chapter 1 and 3) and Δ Val (Chapter 3) have been discussed. The synthesis and applications of Δ Env are very similar to those of Δ Val and will be included later in

this chapter. In this section I will only discuss selected methods for the synthesis of bulky Δ AAs. A more comprehensive treatment of this subject may be found in our review article that was published in 2015.⁵

A series of elimination approaches have been adopted to construct bulky α,β -dehydroamino acid derivatives. In 1975, Kishi and co-workers prepared Δ Val-containing peptide **154** in 3 steps from methoxydibromo acid **152** and penicillamine methyl ester **153** through an elimination of the generated dipeptide in the final step (Scheme 5.1).⁶ This route was mostly based on the biosynthetic pathways to the cephalosporins and penicillins. In related work targeting these antibiotics, the same researchers generated the Δ Val subunit of β -lactam thiazolidine **156** from **155** in the presence of aluminum amalgam (Scheme 5.2).⁷



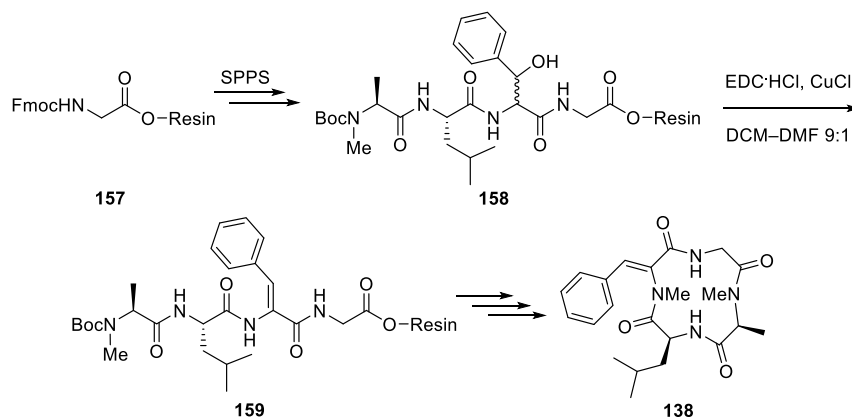
Scheme 5.1 Synthesis of Δ Val-containing Peptide **154**



Scheme 5.2 Synthesis of β -lactam Thiazolidine **156**

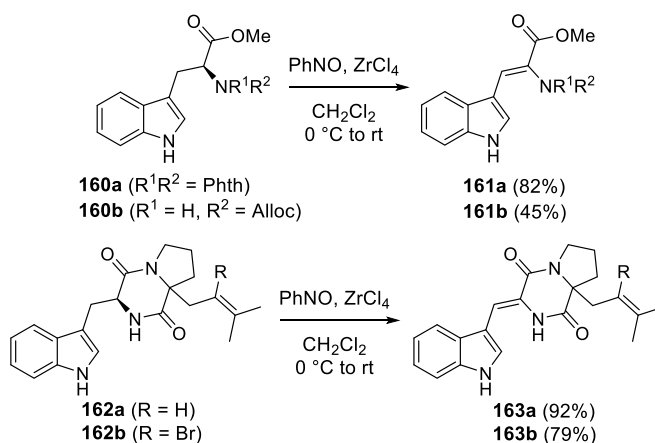
In 2003, Albericio and co-workers performed a copper–carbodiimide elimination procedure on a resin-bound peptide. Dehydration of **158** produced *Z*- Δ Phe containing peptide **159**, which was converted to tentoxin (**138**, Scheme 5.3).⁸ Only the thermodynamically more stable **159** was

generated from the diastereomeric mixture of **158**. A library of tentoxin analogues was prepared via this route.

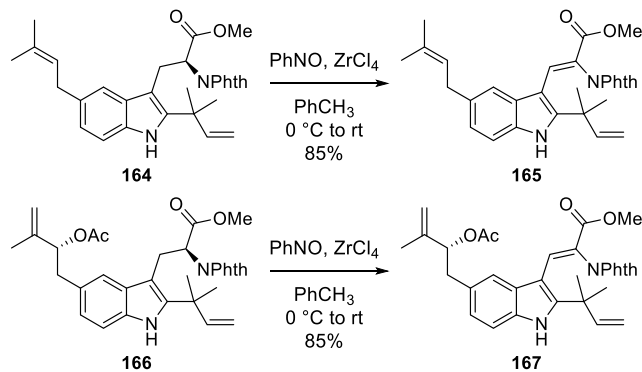


Scheme 5.3 Synthesis of Tentoxin

In 2006, Baran and co-workers synthesized *Z*- Δ Trp derivatives **161** and **163** from protected tryptophans (**160**, Scheme 5.4) or diketopiperazines (**162**) with nitrosobenzene and ZrCl_4 .⁹ A possible Lewis acid induced attack of the indole ring of nitrosobenzene, elimination, and tautomerization were involved in the reaction process. She and co-workers applied this approach to construct the intermediates **165** and **167** separately for the total syntheses of alkaloids isoechinulin A and variecolorin C (Scheme 5.5).¹⁰



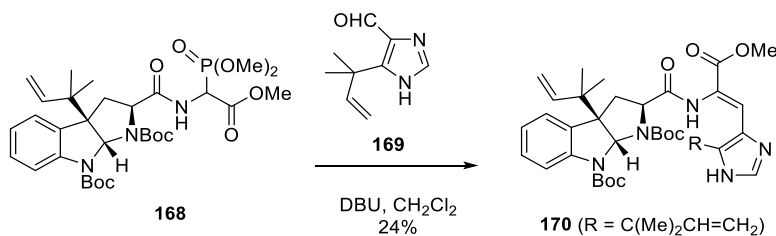
Scheme 5.4 Synthesis of *Z*- Δ Trp Derivatives **161** and **163**



Scheme 5.5 Synthesis of the Precursors of Isoechinulin A and Variecolorin C

Olefinations are another important method of synthesizing bulky Δ AAs and their derivatives.

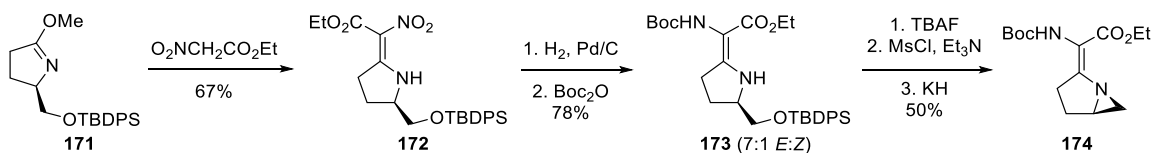
In 2009, Shangguan and Joullié prepared the *Z*- Δ His substructure of isoroquefortine E **170** via the Horner–Wadsworth–Emmons reaction of peptide phosphonate **168** and aldehyde **169** (Scheme 5.6).¹¹ Although compound **170** was generated as a single isomer, the yield was modest.



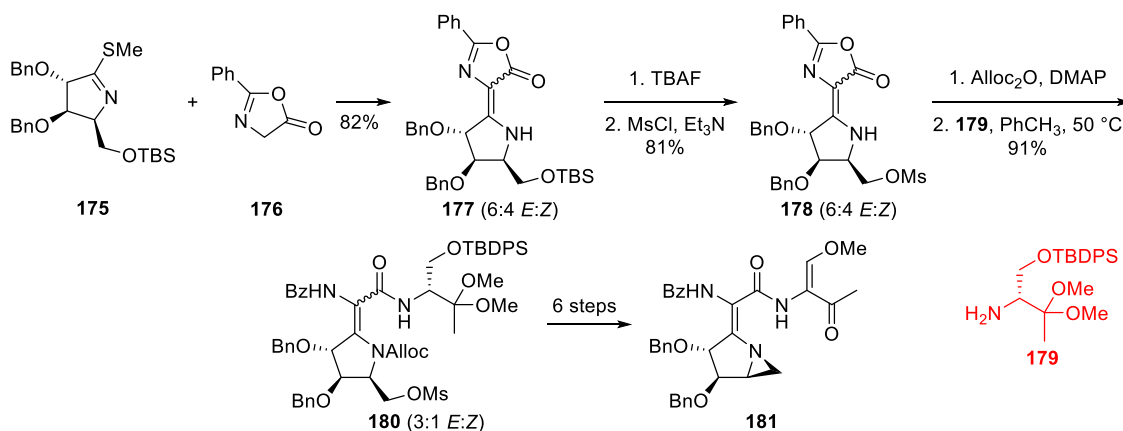
Scheme 5.6 Synthesis of the *Z*- Δ His Substructure of Isoroquefortine E

The unique structures of the azinomycins (**149a** and **149b**, Figure 4.12) have drawn the attention of synthetic chemists. In 1994, Terashima and co-workers reported the first synthesis of the unusual azabicyclic bulky Δ AA existing in these compounds. Condensation of imidate **171** and ethyl nitroacetate provided the *Z* isomer **172** in good yield (Scheme 5.7).¹² Reduction of **172** and a following protection afforded a mixture of Δ AA derivative **173** isomers in which the *E* isomer was the major product. A subsequent deprotection, mesylation, and cyclization generated the model of the azabicyclic Δ AA **174** included in the azinomycins. An adaptation of this method was

used to furnish the right-hand fragment of azinomycin B from thioimidate **175** and azlactone **176** (Scheme 5.8).¹³

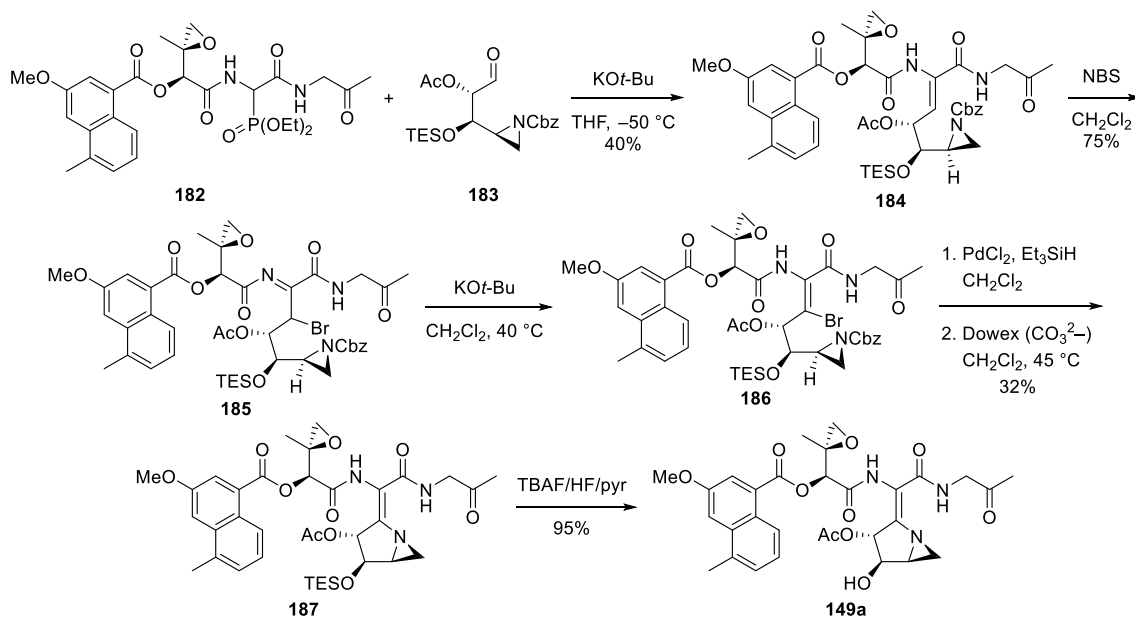


Scheme 5.7 Synthesis of the Model of Azabicyclic Δ AA **174**



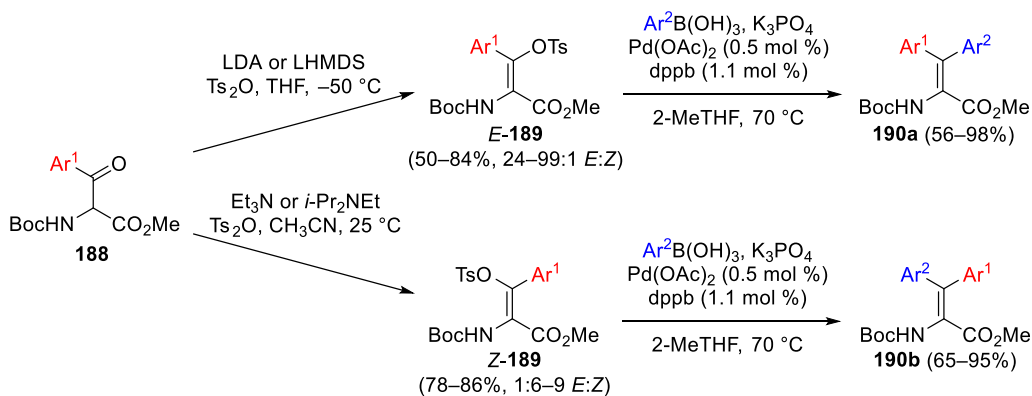
Scheme 5.8 Synthesis of the Right-hand Fragment of Azinomycin B

The Armstrong¹⁴ and Coleman¹⁵ groups simultaneously developed methods to install this unusual bulky Δ AA. Coleman's method led to the total synthesis of azinomycin A (Scheme 5.9).^{15e} From phosphonate **182** and aldehyde **183**, Coleman and co-workers synthesized *Z*- Δ AA **184** with minor amounts of the *E* isomer via Horner–Wadsworth–Emmons olefination. A subsequent bromination of **184** and base-promoted tautomerization later generated vinyl bromide *E*-**186**, which could be separated from the minor *Z* isomer. Removal of the Cbz group, treatment with Dowex anion exchange resin, and cleavage of the silyl ether delivered the relatively unstable natural product (**149a**).



Scheme 5.9 Synthesis of Azinomycin A

In 2015, researchers at Merck discovered a stereoselective method to prepare unsymmetrical β,β -diaryl substituted Δ AAs. Under kinetic conditions, α -amino β -keto esters **188** were converted to enol tosylates *E*-**189** with excellent stereoselectivity (Scheme 5.10).¹⁶ Suzuki couplings with *E*-**189** afforded Δ AAs **190a**. Similarly, a thermodynamic enol tosylation method transformed **188** to *Z*-**189**, although with decreased levels of stereoselectivity. Adducts **190b** were obtained through Suzuki couplings from these enol tosylates *Z*-**189** with similar efficiency to *E*-**189**.



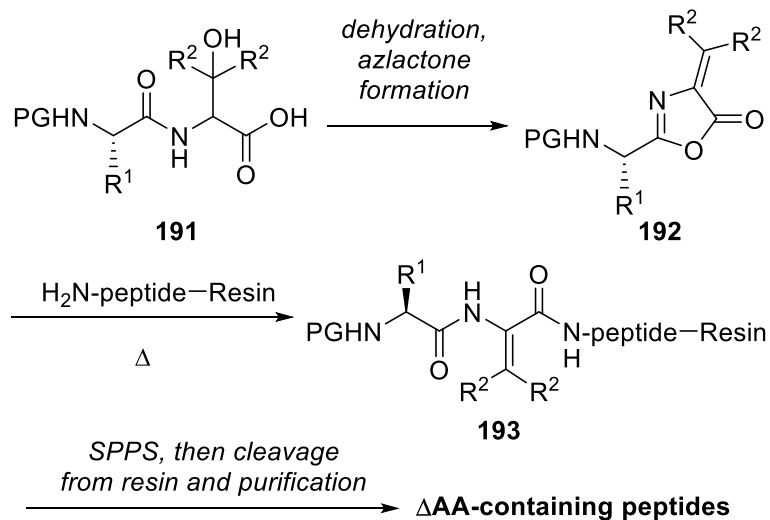
Scheme 5.10 Synthesis of Unsymmetrical β,β -diaryl Substituted Δ AAs

5.3 Methods of Incorporating Bulky α,β -Dehydroamino Acids into Peptides

There are two major challenges involved in incorporating bulky Δ AAAs into peptides. First, isomerization occurs when there is a minor energy difference between the *Z* and *E* isomers of a residue (i.e., *Z*- and *E*- Δ Ile) to afford *Z/E* mixtures of a *C*-terminal Δ AA through an azlactone intermediate. Previously, this problem has been solved by backbone amide protection, which lengthens the synthetic route.¹⁷⁻¹⁹ Another challenge is the lack of methods to prepare bulky Δ AA-containing peptides via solid-phase peptide synthesis (SPPS).

In the process of our effort to synthesize yaku'amide A, we became interested in incorporating Δ Val and Δ Env into peptides for the purpose of studying the impact of these residues on peptide structure and stability. We have developed methods to include these Δ AAAs in simple peptides using SPPS, setting the stage for further study of complex Δ AA-containing therapeutic peptides.

Our synthetic strategy is summarized in Scheme 5.11. Upon activation of its carboxylate group, carboxylic acid **191** would be transformed into an azlactone **192**. Although this process can scramble the stereochemistry of unsymmetrical *Z*- and *E*- Δ Ile, it does not have such impact on Δ Val ($R^2 = \text{Me}$) and Δ Env ($R^2 = \text{Et}$). Next, coupling of resin-bound peptide with **192** would produce elongated resin-bound peptide **193**. Subjection of **193** to SPPS methods, subsequent cleavage from the resin, and purification would generate the targeted peptides with bulky Δ AAAs placed at the chosen positions.

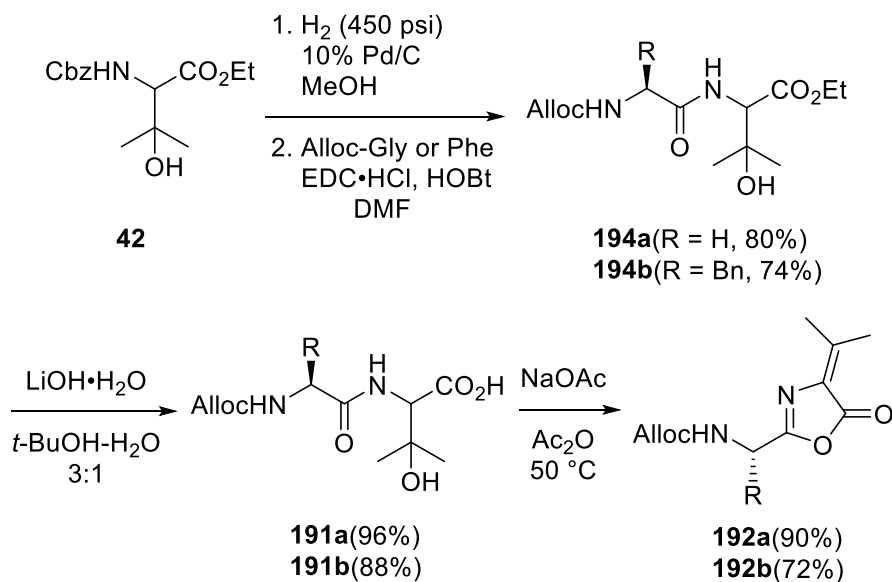


Scheme 5.11 Strategy for Synthesis of Δ AA-containing Peptides via SPPS

Initially, we employed Fmoc-protected dipeptides of type **191**. Although generation of the azlactones was successful, the coupling of resin-bound peptides with the azlactones under heating afforded only trace amounts of the desired peptides based on MS analysis. Further studies indicated that the Fmoc protecting group was not very stable under the reaction conditions. When we switched to Alloc-protected substrates, we found that these compounds were more stable under the basic coupling conditions. Moreover, cleavage of the Alloc group from solid-supported peptides has been achieved under conditions that do not remove the acid-labile side-chain protecting groups adopted in Fmoc-based SPPS.

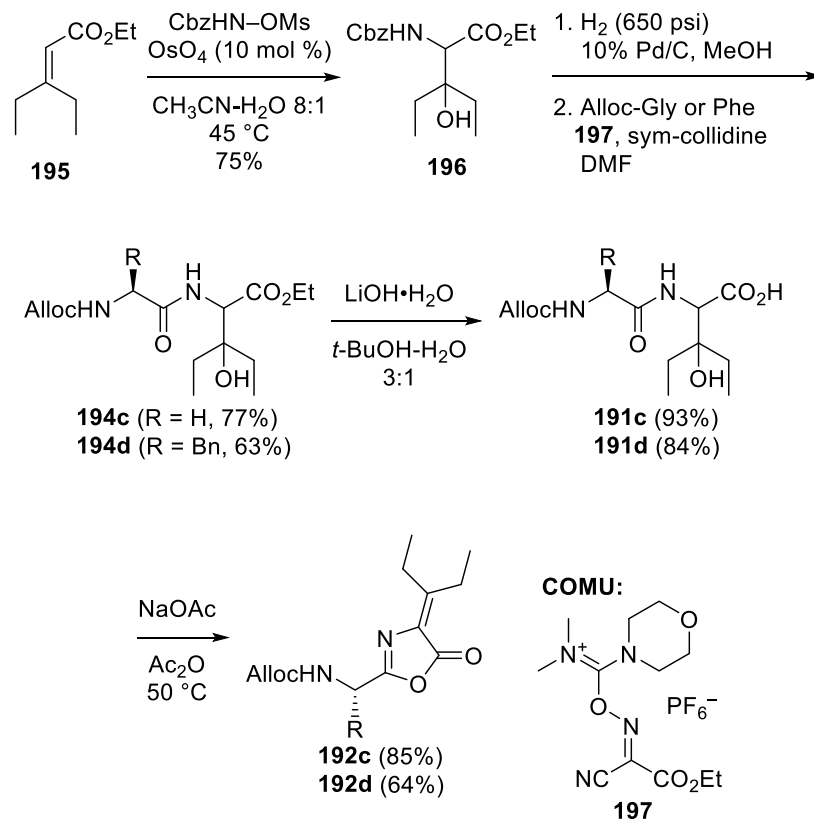
Based on this route, we prepared Δ Val-containing azlactones **192** from Cbz-protected β -OHVal derivative **42**²⁰ (Scheme 5.12). Hydrogenolysis of **42** provided an amine, which coupled with Alloc-Gly or Alloc-Phe separately to generate dipeptides **194a** and **194b** in good yields. Saponification afforded the free acids **191a** and **191b**. Subsequent dehydration and cyclization occurred to yield **192a** and **192b** in the presence of Ac_2O and NaOAc .²¹ Since the azlactones **192**

were sensitive to SiO₂ chromatography, they were typically used directly in peptide couplings without purification.



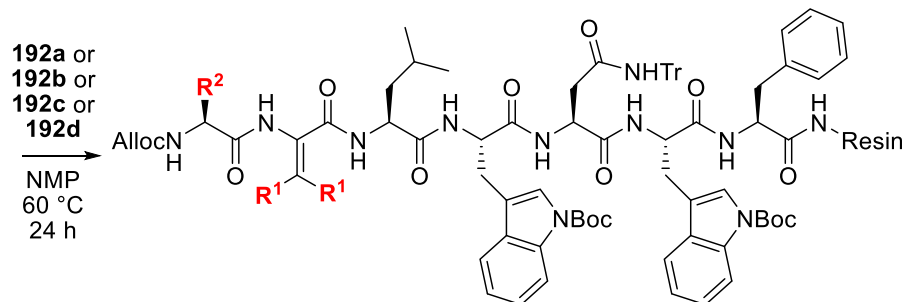
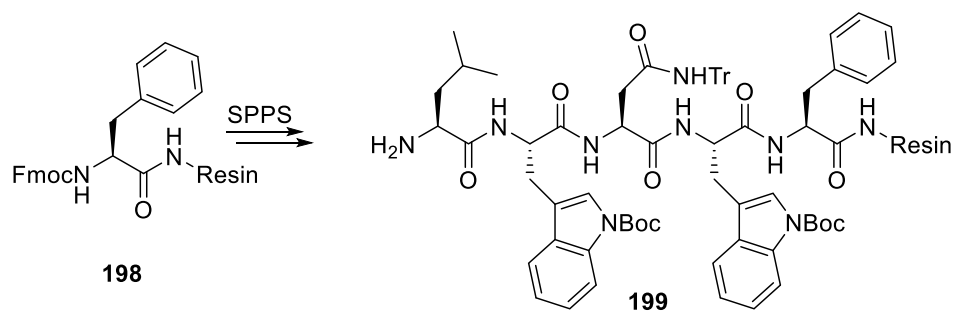
Scheme 5.12 Synthesis of Δ Val-based Azlactones

Similarly, we synthesized racemic β -hydroxy ethylnorvaline derivative **196** from known enoate **195**²² via base-free aminohydroxylation²³ (Scheme 5.13). Subjection of **196** to the same sequence adopted with **42** produced the corresponding azlactones **192c** and **192d**. Notably, we discovered that COMU²⁴ was superior to EDC·HCl and HOBT in preparation of the dipeptides **194c** and **194d** from the hindered amine derived from **196**. Due to the bulkier nature of Δ Env compared with Δ Val, the yields of some reactions in the Δ Env sequence such as couplings and dehydration-cyclizations were lower.



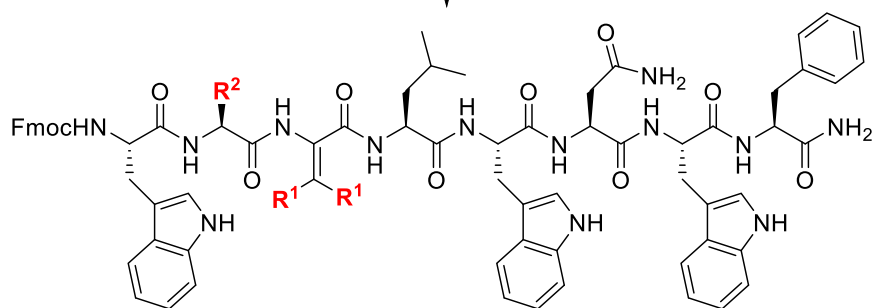
Scheme 5.13 Synthesis of Δ Env-based Azlactones

We prepared analogues of the C-terminal structure of enfuvirtide, the first inhibitor of HIV membrane fusion,²⁵ with four azlactones **192a–d**. In order to explore the incorporation of azlactones into resin-bound peptides, we attached Fmoc-Phe-OH to Rink amide resin and extended the chain with standard Fmoc-based SPPS techniques to provide resin-bound pentapeptide **199** (Scheme 5.14). Next, we screened the coupling reaction of **199** and **192a** with different additives (DMAP versus no additive), solvents (NMP versus DMF), and reaction temperatures (50 °C versus 60 °C). We found that heating the reaction mixture at 60 °C in NMP without DMAP for 24 h produced the best results through analysis of the reactions by LC/MS. Hence, we applied these conditions to mediate the couplings of azlactones **192b–192d** with **199**. Each reaction delivered the anticipated product as evidenced by MS. However, the yields decreased somewhat based on HPLC traces with larger size of the azlactones.



- 200a** ($R^1 = \text{Me}$, $R^2 = \text{H}$)
200b ($R^1 = \text{Me}$, $R^2 = \text{Bn}$)
200c ($R^1 = \text{Et}$, $R^2 = \text{H}$)
200d ($R^1 = \text{Et}$, $R^2 = \text{Bn}$)

1. $\text{Pd}(\text{PPh}_3)_4$, PhSiH_3 , CH_2Cl_2
 $2 \times 10 \text{ min}$
 2. Fmoc-Trp(Boc)-OH, $i\text{Pr}_2\text{NEt}$
 HBTU, HOBT, NMP
 3. TFA/ H_2O , PhOH, PhSCH_3
 $\text{HSCH}_2\text{CH}_2\text{SH}$, $i\text{Pr}_3\text{SiH}$



- 201a** ($R^1 = \text{Me}$, $R^2 = \text{H}$)
201b ($R^1 = \text{Me}$, $R^2 = \text{Bn}$)
201c ($R^1 = \text{Et}$, $R^2 = \text{H}$)
201d ($R^1 = \text{Et}$, $R^2 = \text{Bn}$)

Scheme 5.14 Solid-phase Synthesis of ΔAA -containing Peptides

To continue our study, we removed the Alloc groups of resin-bound peptides **200a–d** with Pd(PPh₃)₄ and PhSiH₃.²⁶ Coupling of the newly generated resin-bound amines with Fmoc-Trp(Boc)-OH was completed under standard conditions. After a TFA-mediated deprotection, we removed the crude octapeptides **201a–d** from the solid support and purified them by reverse-phase HPLC.

5.4 Applications of Bulky α,β -Dehydroamino Acids

Due to the significant challenges in synthesizing these bulky dehydroamino acids, no studies have been published about incorporating them into bioactive peptides. To date, not much is known about the properties of bulky Δ AAs incorporated in various secondary structures such as β -turns and helices. Also, their ability to stabilize medium- or large-sized bioactive peptides to proteases has not been demonstrated.

Recently, we have developed methods to access α , β -dehydroamino acids such as Δ Val and Δ Env and incorporate them into peptides. Next, our plan was to replace certain proteinogenic residues in secondary structures with Δ Val and Δ Env to check if they can provide stability to proteolytic degradation without negatively impacting bioactivity. We used SPPS to rapidly prepare these peptides.

Since helical domains play an important role in mediating protein-protein interactions at protein interfaces, stable mimics are widely used as reagents for molecular biology and drug discovery. In 2003, Singh and co-workers synthesized the tetrapeptide *N*-Cbz- Δ Val-Val-*Z*- Δ Phe-Ile-OMe **202** (Figure 5.3).²⁷ The conformation of this tetrapeptide resembles that of a 3_{10} -helix, with two intramolecular hydrogen bonds. However, this tetrapeptide is too small to conclusively demonstrate that Δ Val or Δ Env can be incorporated into helices. To continue our studies of bulky

Δ AAs, we chose a monomeric 3_{10} -helical peptide gp41₆₅₉₋₆₇₁ (**203**, Figure 5.4) stabilized by N- and C-capping as a system to evaluate whether Δ Val and Δ Env would impart proteolytic stability to 3_{10} helices.²⁸

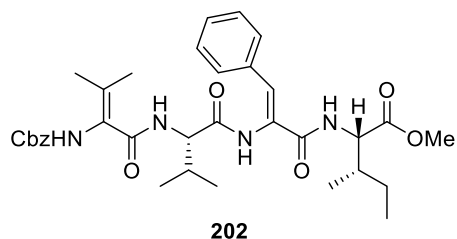


Figure 5.3 *N*-Cbz- Δ Val-Val-Z- Δ Phe-Ile-OMe

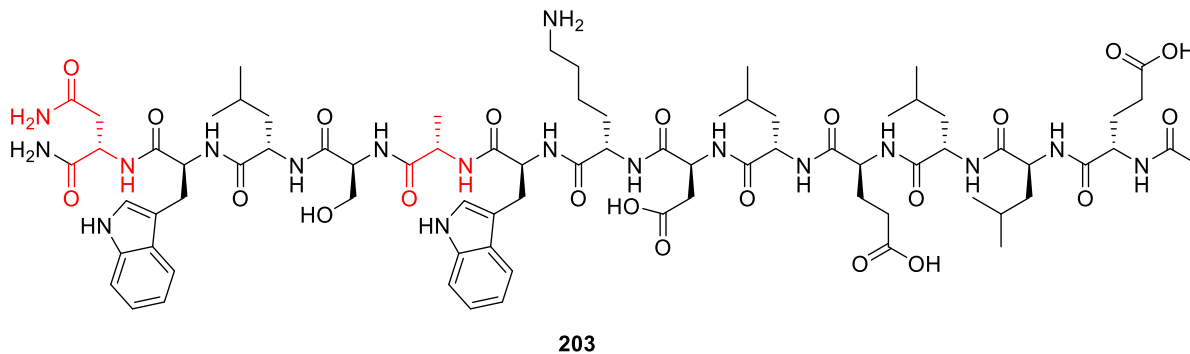
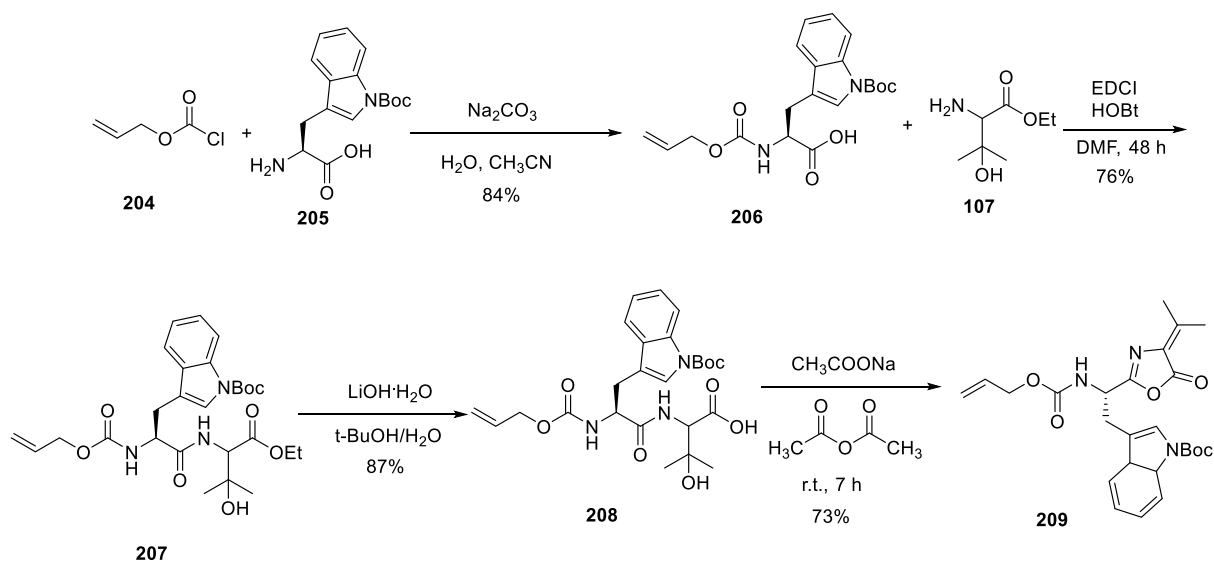


Figure 5.4 3_{10} -Helical Peptide Gp41₆₅₉₋₆₇₁

Our initial study involved replacing the alanine and asparagine residues of gp41₆₅₉₋₆₇₁ since alanine and asparagine are both adjacent to a tryptophan in this peptide, we could synthesize two analogues with a single Δ Val-based azlactone. Thus, we prepared Δ Val-based azlactone **209** that contains a protected tryptophan using the methods we developed earlier (Scheme 5.15). Next, we constructed two analogues **210** and **211** (Figure 5.5) of the 3_{10} -helical peptide. To the best of our knowledge, these two 13-residue peptides represent the longest bulky Δ AA-containing peptides that have been prepared to date. Assays using pronase to determine the proteolytic stability of these peptides are underway in our lab.



Scheme 5.15 Synthesis of Δ Val-based Azlactones that Contain Protected Tryptophan

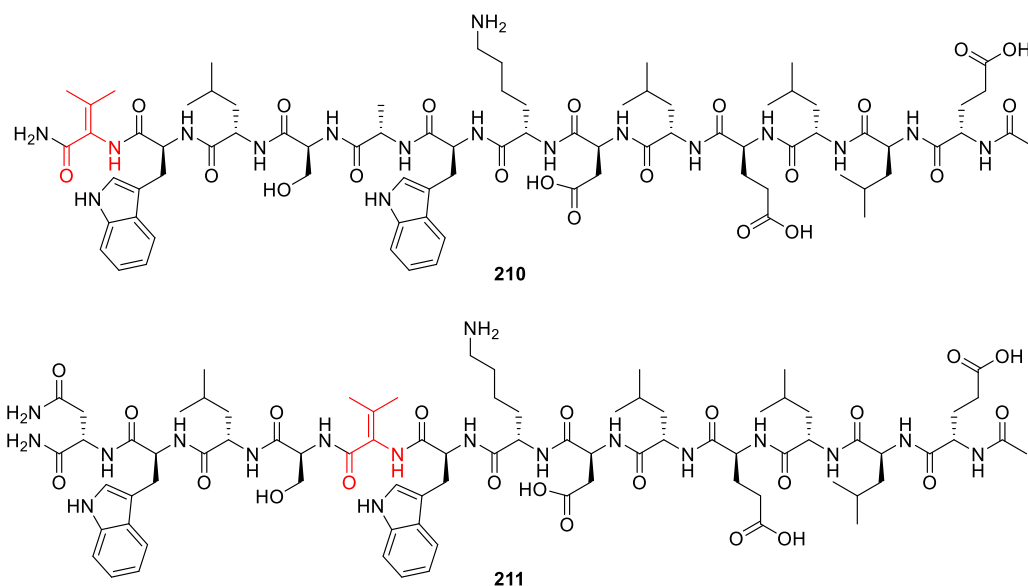


Figure 5.5 13-Residue Analogues

In 2003, the FDA approved the commercial use of enfuvirtide (FUZEONTM) as a new type of antiretroviral drug targeting HIV. Enfuvirtide is the first inhibitor of HIV membrane fusion, a peptide mimetic of an important fragment within gp41 which could block this glycoprotein's structural transformation prior to the fusion process (Figure 5.6).²⁹ A fragment containing 10

amino acids from residues 36 to 45 in gp41 is very important for viral fusion and could form a binding site for enfuvirtide.

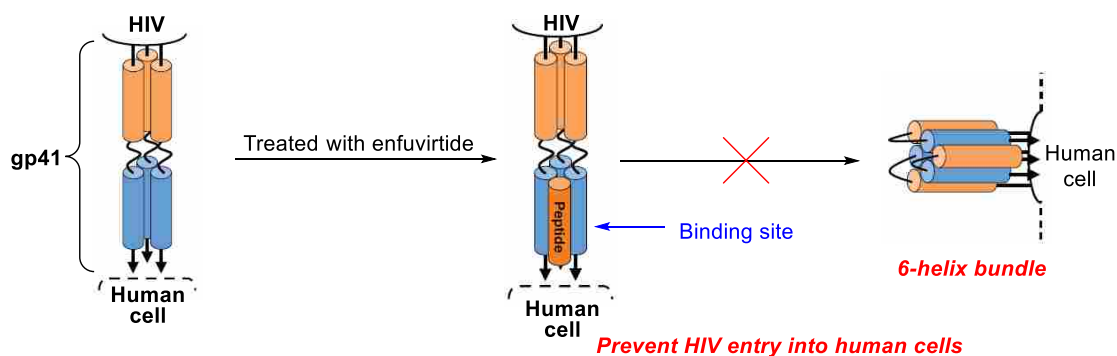


Figure 5.6 Enfuvirtide Prevents HIV Entry into Human Cells

The mechanism of action is as follows: HIV could bind to the host CD4 cell receptor via the surface glycoprotein. The viral transmembrane protein gp41 then undergoes conformational changes to facilitate the virus–cell membrane fusion. However, binding of enfuvirtide to gp41 prevents the required protein folding, inhibiting the HIV-1 6-helix bundle fusion process with the target cell and preventing the formation of an entry pore for the virus.³⁰

However, due to its rapid enzymatic degradation by proteolysis *in vivo*, the half-life of enfuvirtide is very short once in circulation. Therefore, the therapeutic applications of this drug are limited. It is administrated in injectable form and the subcutaneous injection should be performed twice daily. Because this kind of therapy is typically required for a long time, this dosage form is a hurdle for adherence to the drug regimen. In addition, enfuvirtide can cause local skin reactions at the injection site.

Our hypothesis is that replacing common amino acids in enfuvirtide with bulky dehydroamino acids Δ Val and Δ Env should stabilize the corresponding analogues of this drug to proteases without changing the potency against HIV. Next, our plan is to replace each of the eight common

amino acids of the lipid binding domain in enfuvirtide with ΔVal and ΔEnv to increase the stability of this drug and at the same time maintain the anti-HIV bioactivity. The eight amino acids from the nonpolar lipid binding domain (LBD) are highlighted in red (Figure 5.7). Its function is to interact with lipids in the membrane of the human cell, and it mainly contains common nonpolar amino acids. Studies have been carried out to modify this region with other nonpolar groups. The potency of the analogues is very similar to the wild type enfuvirtide although the shape is changed due to the modifications of this region.³¹

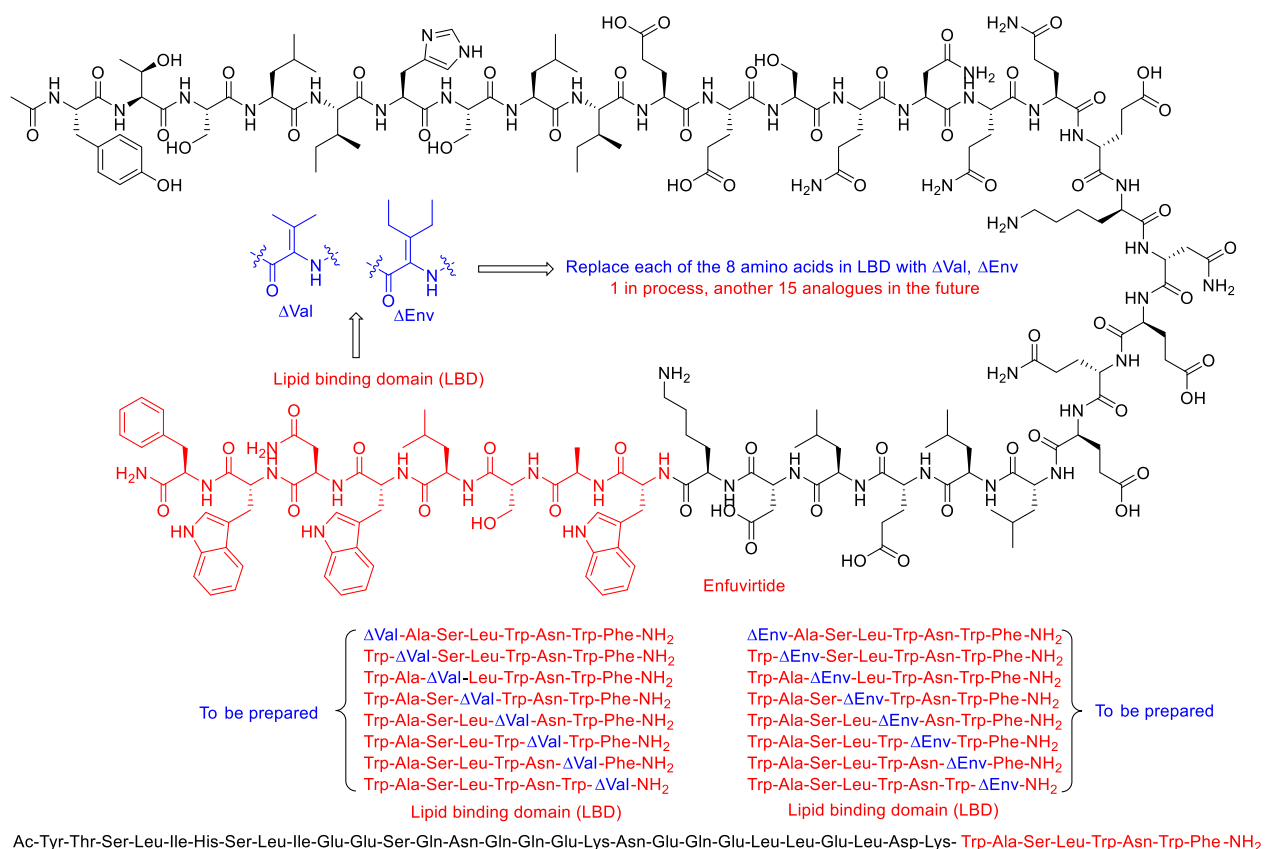


Figure 5.7 Enfuvirtide and 16 Future Analogues

Based on this previous work, enfuvirtide analogues produced by modifications of the amino acid sequence from the LBD should maintain the drug's anti-HIV activity when the nonpolar nature of this LBD area is unchanged. Since bulky dehydroamino acids ΔVal and ΔEnv are nonpolar, the

polarity of this region would not be changed in enfuvirtide after substituting each of the eight amino acids with Δ Val and Δ Env. Hence, incorporation of them in enfuvirtide should not reduce potency of this drug. As mentioned above, the rigidifying effects of these residues should improve the stability of enfuvirtide. Evaluation of a sample of enfuvirtide prepared by us showed anti-HIV properties, with results that are consistent with the EC₅₀ values for the drug considering the variations in HIV-1 isolates and cell lines (Figure 5.8).³² Currently we are working on purification of the crude material of two of the 16 analogues. Afterwards, our collaborator Dr. Brad Berges will use in vitro HIV-1 infectivity assay to test the anti-HIV activities of the purified analogues as well.

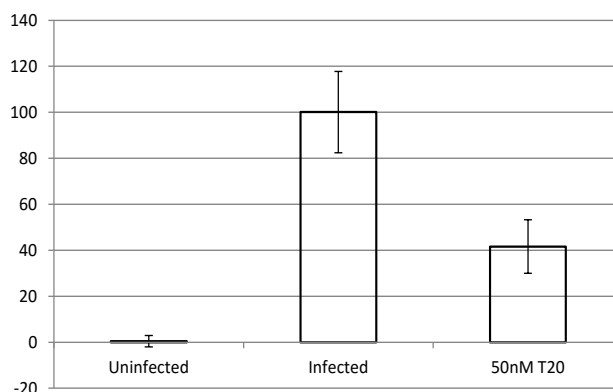


Figure 5.8 HIV-1 Infectivity Assay Control Experiment (T20 is another name for enfuvirtide)

5.5 Summary

Bulky α,β -dehydroamino acids are present in many important biologically active natural products. A variety of synthetic methods have been developed to synthesize bulky Δ AAs and incorporate them into peptides. In this regard, we have covered different synthetic methods to construct bulky α,β -dehydroamino acids in this chapter. In our lab, we have developed methods to incorporate α,β -dehydroamino acids such as Δ Val and Δ Env into small synthetic peptides. So far,

we have prepared two analogues of a monomeric 3₁₀-helical peptide. Analogues of the anti-HIV drug enfuvirtide will be made in the future.

5.6 References

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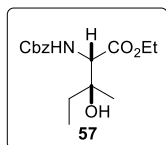
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CHAPTER 6. EXPERIMENTAL SECTION

6.1 General Experimental Details

Dichloromethane, dimethylformamide, methanol, tetrahydrofuran, toluene and triethylamine were dried by passage through a solvent drying system containing cylinders of activated alumina.¹ Other solvents and reagents were purchased from commercial vendors and used without purification. Flash chromatography was carried out using 60–230 mesh silica gel. ¹H NMR spectra were acquired on a 500 MHz spectrometer with chloroform (7.27 ppm), methanol (3.34 ppm), or benzene (7.15 ppm) as internal reference. Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), br s (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). ¹³C NMR spectra were acquired on a spectrometer operating at 125 MHz with chloroform (77.23 ppm), methanol (49.86 ppm), or benzene (128.62 ppm) as internal reference. Infrared spectra were obtained on an FT-IR spectrometer. Mass spectral data were obtained using ESI techniques.

6.2 Experimental Procedures and Spectral Data

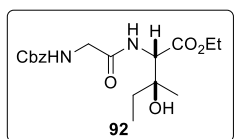


Ethyl

(*2S**,*3S**)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxy-3-

methylpentanoate (57). A solution of benzyl ((methylsulfonyl)oxy)carbamate² (820 mg, 3.34

mmol, 1.4 equiv) in CH₃CN (18 mL) at rt was treated with OsO₄ (4 wt % solution in H₂O, 1.5 mL, 0.24 mmol, 0.099 equiv), stirred for 10 min, and then treated with a solution of enoate **51**³ (340 mg, 2.39 mmol) in CH₃CN (6.0 mL) and H₂O (1.5 mL). The resulting mixture was stirred at 45 °C for 2.5 d, treated with sat aq K₂S₂O₅ (7 mL), and stirred for an additional 10 min. It was then diluted with H₂O (35 mL) and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with sat aq NaHCO₃ (2 × 50 mL) and brine (40 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (100 mL of SiO₂, 1–5% MeOH in CH₂Cl₂ gradient elution) afforded **57** (580 mg, 1.87 mmol, 78%) as a light yellow oil: ¹H NMR (CDCl₃, 500 MHz) δ 7.37–7.30 (m, 5H), 5.62 (d, *J* = 9.3 Hz, 1H), 5.12 (s, 2H), 4.31 (d, *J* = 9.4 Hz, 1H), 4.28–4.18 (m, 2H), 2.39 (br s, 1H), 1.56–1.46 (m, 2H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.18 (s, 3H), 0.98 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.9, 156.3, 136.1, 128.5 (2C), 128.2 (2C), 128.1, 74.3, 67.2, 61.6, 60.3, 32.5, 22.3, 14.1, 8.0; IR (film) ν_{max} 3363, 2978, 1716, 1519, 1337, 1208, 1058 cm⁻¹; HRMS (ESI) *m/z* 310.1711 (MH⁺, C₁₆H₂₃NO₅H⁺ requires 310.1654).

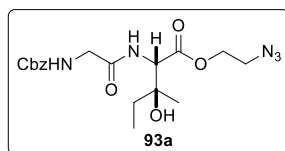


Ethyl (2*S,3*S**)-2-(2-(((Benzyloxy)carbonyl)amino)acetamido)-3-**

hydroxy-3-methylpentanoate (92). A solution of **57** (353 mg, 1.14 mmol) in MeOH (10 mL) was treated with 10% Pd/C (114.6 mg, 0.32 wt equiv) and stirred at rt under H₂ (550 psi) for 2.5 d. The mixture was filtered through a pad of Celite (washed with 125 mL of MeOH), and the filtrate was concentrated *in vacuo* to afford the crude amine (198 mg, 1.13 mmol), a portion of which was used without further purification.

A solution of the amine (170 mg, 0.970 mmol) in anhydrous DMF (12 mL) at 0 °C under Ar was treated with *N*-Cbz-glycine (401.6 mg, 1.92 mmol, 2.0 equiv), HOBt (ca. 14% H₂O content,

327.5 mg, 2.08 mmol, 2.1 equiv), and EDC•HCl (375.7 mg, 1.96 mmol, 2.0 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 48 h. The reaction was quenched by the addition of sat aq NaHCO₃ (12 mL) and diluted with H₂O (12 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL), and the combined organic layers were washed with brine (20 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (70 mL of SiO₂, 1–5% MeOH in CH₂Cl₂ gradient elution) afforded **92** (220 mg, 0.600 mmol, 62%) as a colorless oil: ¹H NMR (CDCl₃, 500 MHz) δ 7.38–7.30 (m, 5H), 6.74 (d, *J* = 8.4 Hz, 1H), 5.37 (s, 1H), 5.14 (s, 2H), 4.56 (d, *J* = 8.9 Hz, 1H), 4.28–4.16 (m, 2H), 3.98–3.89 (m, 2H), 2.42 (br s, 1H), 1.51 (q, *J* = 7.1 Hz, 2H), 1.30 (t, *J* = 7.2 Hz, 3H), 1.14 (s, 3H), 0.98 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.4, 169.0, 156.5, 136.0, 128.5 (2C), 128.3, 128.2 (2C), 74.3, 67.3, 61.7, 58.4, 44.5, 32.5, 22.4, 14.1, 8.0; IR (film) ν_{\max} 3344, 2979, 1728, 1525, 1212, 1051 cm⁻¹; HRMS (ESI) *m/z* 367.1862 (MH⁺, C₁₈H₂₆N₂O₆H⁺ requires 367.1869).

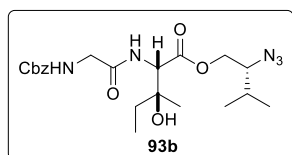


2-Azidoethyl(2*S,3*S**)-2-(2-(((benzyloxy)carbonyl)amino)acetamido)-**

3-hydroxy-3-methylpentanoate (93a). A solution of ester **92** (163.4 mg, 0.446mmol) in *t*-BuOH–H₂O (3:1, 1.6 mL) at 0 °C was treated with LiOH•H₂O (93.1 mg, 2.22 mmol, 5.0 equiv), then stirred at 0 °C for 2 h. The resulting mixture was acidified to pH 1~2 by the addition of 2 N HCl (2 mL) and extracted with EtOAc (6 × 3 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid (152.2 mg, 150.9 mg theoretical yield, quant.) was used directly without further purification.

A solution of the crude carboxylic acid (38.3 mg, 0.113 mmol) and iodide (47.7 mg, 0.242 mmol, 2.1 equiv) in anhydrous DMF (1.1 mL) at rt under Ar was treated with Et₃N (49 μL, 36 mg,

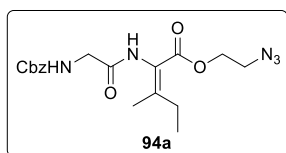
0.35 mmol, 3.1 equiv). The resulting mixture was stirred at 80 °C under Ar for 48 h, then concentrated *in vacuo*. The residue was dissolved in EtOAc (20 mL) and washed with brine (3 × 3 mL). The organic layer was dried (Na₂SO₄) and concentrated *in vacuo*. Flash chromatography (3 mL of SiO₂, 0–2% MeOH in CH₂Cl₂ gradient elution) afforded **93a** (43.6 mg, 0.107 mmol, 95% from **92**) as a colorless oil: ¹H NMR (CDCl₃, 500 MHz) δ 7.38–7.30 (m, 5H), 6.92 (d, *J* = 8.2 Hz, 1H), 5.51 (s, 1H), 5.13 (s, 2H), 4.60 (d, *J* = 8.9 Hz, 1H), 4.34–4.24 (m, 2H), 3.97–3.89 (m, 2H), 3.56–3.46 (m, 2H), 2.50 (br s, 1H), 1.55 (q, *J* = 7.2 Hz, 2H), 1.16 (s, 3H), 0.99 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 170.9, 169.2, 156.6, 136.1, 128.6 (2C), 128.3 (2C), 128.1, 74.3, 67.3, 63.7, 58.7, 49.5, 44.5, 32.4, 22.7, 8.0; IR (film) ν_{max} 3353, 2925, 2106, 1728, 1522, 1259, 1050 cm⁻¹; HRMS (ESI) *m/z* 408.1836 (MH⁺, C₁₈H₂₅N₅O₆H⁺ requires 408.1883).



(R)-2-Azido-3-methylbutyl (2S*,3S*)-2-(2-(((benzyloxy)carbonyl)

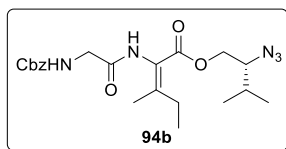
amino)acetamido)-3-hydroxy-3-methylpentanoate (93b). A solution of the acid derived from hydrolysis of ester **92** (prepared as described for azidoethyl ester **93a**, 30.7 mg, 0.0907 mmol) and iodide (44 mg, 0.184 mmol, 2.0 equiv) in anhydrous DMF (870 μL) at rt under Ar was treated with Et₃N (38 μL, 28 mg, 0.27 mmol, 3.0 equiv). The resulting mixture was stirred at 80 °C under Ar for 24 h, then the reaction mixture was dissolved in EtOAc (20 mL) and washed with brine (3 × 3 mL). The organic layer was dried (Na₂SO₄) and concentrated *in vacuo*. Flash chromatography (5 mL of SiO₂, 0–1.5% MeOH in CH₂Cl₂ gradient elution) afforded **93b** (34.8 mg, 0.0774 mmol, 85% from **92**) as a yellow oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.40–7.29 (m, 5H), 6.85 and 6.83 (2d, *J* = 10.6 Hz, 1H), 5.44 (s, 1H), 5.13 (s, 2H), 4.62 and 4.61 (2d, *J* = 9.6 Hz, 1H), 4.39–4.27 (m, 1H), 4.19–4.08 (m, 1H), 3.98–3.88 (m, 2H), 3.48–3.43 and

3.43–3.37 (2m, 1H), 2.44 and 2.40 (2s, 1H), 1.92–1.79 (m, 1H), 1.62–1.50 (m, 2H), 1.17 (s, 3H), 1.03–0.95 (m, 9H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.0 and 170.7, 169.1 and 169.0, 156.6, 136.1, 128.6 (2C), 128.3 (2C), 128.2, 74.3, 67.3, 66.7 and 66.6, 66.4 and 66.1, 58.7, 44.5, 32.4, 30.2 and 30.0, 22.7 and 22.6, 19.4, 18.1, 8.0; IR (film) ν_{max} 3344, 2968, 2101, 1735, 1522, 1262 cm^{-1} ; HRMS (ESI) m/z 450.2345 (MH^+ , $\text{C}_{21}\text{H}_{31}\text{N}_5\text{O}_6\text{H}^+$ requires 450.2353).



2-Azidoethyl (E)-2-(2-(((benzyloxy)carbonyl)amino)acetamido)-3-

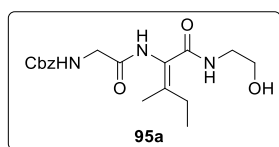
methylpent-2-enoate (94a). A solution of alcohol **93a** (19.3 mg, 0.0474 mmol) in anhydrous CHCl_3 (210 μL) was treated with Martin sulfurane (0.24 M in anhydrous CHCl_3 , 400 μL , 0.096 mmol, 2.0 equiv) dropwise at 0 $^\circ\text{C}$ under Ar. The resulting mixture was stirred at 0 $^\circ\text{C}$ for 1 h and concentrated *in vacuo*. Flash chromatography (3 mL of SiO_2 , 0–2% MeOH in CH_2Cl_2 gradient elution) afforded **93a** (13.8 mg, 0.0354 mmol, 75%, >19:1 dr) as a light yellow oil: ^1H NMR (CDCl_3 , 500 MHz) δ 7.39–7.30 (m, 5H), 7.16 (s, 1H), 5.38 (s, 1H), 5.15 (s, 2H), 4.30 (t, $J = 5.0$ Hz, 2H), 3.95 (d, $J = 5.9$ Hz, 2H), 3.47 (t, $J = 4.6$ Hz, 2H), 2.55 (q, $J = 7.5$ Hz, 2H), 1.81 (s, 3H), 1.12 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 167.7, 163.5, 156.7, 152.6, 136.0, 128.6 (2C), 128.4, 128.2 (2C), 119.7, 67.4, 63.4, 49.8, 44.8, 27.7, 19.8, 12.6; IR (film) ν_{max} 3313, 2936, 2107, 1722, 1515, 1264 cm^{-1} ; HRMS (ESI) m/z 390.1776 (MH^+ , $\text{C}_{18}\text{H}_{23}\text{N}_5\text{O}_5\text{H}^+$ requires 390.1777).



(R)-2-azido-3-methylbutyl (E)-2-(2-(((benzyloxy)carbonyl)amino)

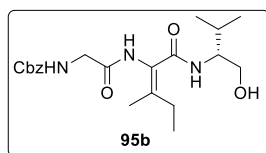
acetamido)-3-methylpent-2-enoate (94b). A solution of alcohol **93b** (24.5 mg, 0.0545 mmol) in

anhydrous CHCl_3 (240 μL) was treated with Martin sulfurane (0.24 M in anhydrous CHCl_3 , 460 μL , 0.11 mmol, 2.0 equiv) dropwise at $-20\text{ }^\circ\text{C}$. The resulting mixture was stirred at $-20\text{ }^\circ\text{C}$ under Ar for 1 h, warmed to rt, and concentrated *in vacuo*. Flash chromatography (10 mL of SiO_2 , 0–1.5% MeOH in CH_2Cl_2 gradient elution) afforded **94b** (19.7 mg, 0.0457 mmol, 84%, >19:1 dr) as a colorless oil: $[\alpha]_D^{25} +3.7$ (*c* 0.30, CH_2Cl_2); ^1H NMR (CDCl_3 , 500 MHz) δ 7.40–7.30 (m, 5H), 7.17 (br s, 1H), 5.40 (br s, 1H), 5.15 (s, 2H), 4.44–4.37 (m, 1H), 4.12–4.01 (m, 1H), 4.00–3.89 (m, 2H), 3.47–3.40 (m, 1H), 2.57 (qd, $J = 7.5, 1.9$ Hz, 2H), 1.88–1.72 (m, 4H), 1.12 (t, $J = 7.4$ Hz, 3H), 0.98 (d, $J = 6.9$ Hz, 3H), 0.96 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 167.7, 163.6, 156.7, 153.1, 136.0, 128.6 (2C), 128.3, 128.2 (2C), 119.6, 67.4, 67.0, 66.1, 44.8, 29.9, 27.7, 19.9, 19.4, 18.2, 12.6; IR (film) ν_{max} 3321, 2966, 2101, 1725, 1514, 1264 cm^{-1} ; HRMS (ESI) m/z 432.2261 (MH^+ , $\text{C}_{21}\text{H}_{29}\text{N}_5\text{O}_5\text{H}^+$ requires 432.2247).



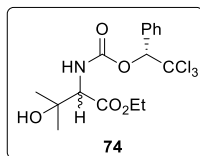
Benzyl (E)-2-((1-((2-hydroxyethyl)amino)-3-methyl-1-oxopent-2-en-2-yl)amino)-2-oxoethylcarbamate (95a). A solution of azide **94a** (3.0 mg, 0.0077 mmol) in THF (210 μL) and H_2O (16 μL) at $0\text{ }^\circ\text{C}$ under Ar was treated dropwise with PMe_3 (1 M in THF, 23 μL , 0.023 mmol, 3.0 equiv). The resulting mixture was stirred at $0\text{ }^\circ\text{C}$ to rt for 20 h, at which time the starting material had disappeared as evidenced by MS. The mixture was then treated dropwise with morpholine (24 μL , 24 mg, 0.28 mmol), and stirred at rt for 60 h followed by concentration *in vacuo*. The residue was dissolved in EtOAc (5 mL), washed with H_2O (2×1 mL) and brine (1 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (2 mL of SiO_2 , 0–3% MeOH in CH_2Cl_2 gradient elution) afforded **95a** (2.7 mg, 0.0074 mmol, 96%, 10:1 dr) as a white film: ^1H NMR (CDCl_3 , 500 MHz) δ 7.40 (br s, 1H), 7.39–7.31 (m, 5H), 6.59 (br s, 1H), 5.46 (br s,

1H), 5.14 (s, 2H), 3.89 (d, $J = 5.7$ Hz, 2H), 3.73 (q, $J = 5.1$ Hz, 2H), 3.43 (q, $J = 5.0$ Hz, 2H), 3.24 (br s, 1H), 2.41 (q, $J = 7.3$ Hz, 2H), 1.70 (s, 3H), 1.09 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 168.4, 166.6, 157.2, 142.4, 135.6, 128.6 (2C), 128.5, 128.2 (2C), 123.7, 67.7, 61.4, 45.1, 42.7, 27.0, 17.5, 12.8; IR (film) ν_{max} 3316, 2919, 1685, 1522, 1248, 1050 cm^{-1} ; HRMS (ESI) m/z 364.1871 (MH^+ , $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_5\text{H}^+$ requires 364.1872).



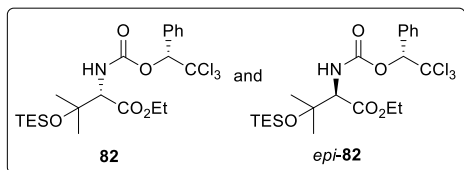
Benzyl (*R,E*)-(2-((1-((1-hydroxy-3-methylbutan-2-yl)amino)-3-methyl-1-oxopent-2-en-2-yl)amino)-2-oxoethyl)carbamate (95b). A solution of azide **94b** (6.1 mg, 0.014 mmol) in DMF (400 μL) and H_2O (31 μL) at 0 $^\circ\text{C}$ under Ar was treated dropwise with PMe_3 (1 M in THF, 43 μL , 0.043 mmol, 3.0 equiv). The resulting mixture was stirred at 0 $^\circ\text{C}$ to rt for 24 h, at which time the starting material had disappeared as evidenced by MS. The mixture was then treated dropwise with morpholine (173 μL , 174 mg, 2.0 mmol) and stirred at rt for 80 h followed by concentration *in vacuo*. The residue was dissolved in EtOAc (10 mL), washed with H_2O (2 \times 2 mL) and brine (2 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (3 mL of SiO_2 , 0–3% MeOH in CH_2Cl_2 gradient elution) afforded **95b** (5.4 mg, 0.013 mmol, 94%, 13:1 dr) as a white film: $[\alpha]_{\text{D}}^{25} +38$ (c 0.12, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz) δ 7.44–7.31 (m, 6H), 6.15 (d, $J = 5.0$ Hz, 1H), 5.39 (br s, 1H), 5.19–5.09 (m, 2H), 3.97–3.85 (m, 2H), 3.84–3.76 (m, 2H), 3.65–3.57 (m, 1H), 3.16 (br s, 1H), 2.36 (q, $J = 7.5$ Hz, 2H), 1.94–1.84 (m, 1H), 1.69 (s, 3H), 1.09 (t, $J = 7.5$ Hz, 3H), 0.98 (d, $J = 6.7$ Hz, 3H), 0.97 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 168.7, 166.8, 157.1, 139.0, 135.8, 128.6 (2C), 128.4, 128.2 (2C), 124.8, 67.6, 63.2, 57.8, 44.9, 29.0, 27.0, 19.7, 19.0, 16.8, 12.9; IR (film) ν_{max} 3284, 2923, 2360,

1653, 1522, 1232, 1048 cm^{-1} ; HRMS (ESI) m/z 406.2338 (MH^+ , $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5\text{H}^+$ requires 406.2342).



Ethyl 3-hydroxy-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)

amino)butanoate (74). A solution of (*R*)-2,2,2-trichloro-1-phenylethyl ((methylsulfonyl)oxy) carbamate⁴ (**71**, 1.29 g, 3.56 mmol, 1.4 equiv) in CH_3CN (24 mL) at rt was treated with OsO_4 (4 wt % solution in H_2O , 1.2 mL, 48 mg OsO_4 , 0.189 mmol, 0.075 equiv), stirred at rt for 10 min, then treated with ethyl 3,3-dimethylacrylate (**39**, 350 μL , 323 mg, 2.52 mmol) and H_2O (1.46 mL). The resulting mixture was stirred at 35 $^\circ\text{C}$ for 24 h, then treated with sat aq $\text{K}_2\text{S}_2\text{O}_5$ (6.0 mL) and stirred for an additional 5 min. It was then diluted with H_2O (60 mL) and extracted with EtOAc (3×60 mL). The combined organic layers were washed with sat aq NaHCO_3 (2×60 mL) and brine (60 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (45 mL of SiO_2 , 1–3% MeOH in CH_2Cl_2 gradient elution) afforded **74** (1.01 g, 2.45 mmol, 97%) as a colorless oil that was a 1:1 mixture of diastereomers: ^1H NMR (CDCl_3 , 500 MHz) δ 7.62 (d, $J = 6.5$ Hz, 2H), 7.46–7.35 (m, 3H), 6.33 and 6.27 (2s, 1H), 5.97 (br s, 1H), 4.43–4.11 (m, 3H), 2.54 (m, 1H), 1.38–1.19 (m, 9H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.3 and 171.2, 154.3 and 154.2, 133.1 and 133.0, 129.7 (2C), 129.6, 127.9 (2C), 99.7 and 99.4, 83.7 and 83.5, 72.1 and 71.7, 61.8, 61.6 and 61.5, 27.0, 26.3 and 26.2, 14.1 and 14.0; IR (film) ν_{max} 3432, 2981, 1727, 1511, 1375, 1202, 1063, 1026 cm^{-1} ; HRMS (ESI) m/z 412.0503 (MH^+ , $\text{C}_{16}\text{H}_{20}\text{Cl}_3\text{NO}_5\text{H}^+$ requires 412.0485).



Ethyl (S)-3-methyl-2-((((R)-2,2,2-trichloro-1-

phenylethoxy)carbonyl)amino)-3-((triethylsilyloxy)butanoate (82) and ethyl (R)-3-methyl-2-

((((R)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyloxy)butanoate (epi-

82). A solution of **74** (1.01 g, 2.45 mmol) in anhydrous CH₂Cl₂ (24 mL) at 0 °C under Ar was

treated dropwise with 2,6-lutidine (850 μL, 786 mg, 7.34 mmol, 3.0 equiv) followed by TES-OTf

(1.1 mL, 1.3 g, 4.9 mmol, 2.0 equiv). The resulting mixture was stirred at 0 °C for 2 h, then

quenched by the addition of sat aq NaHCO₃ (8 mL). The layers were separated, and the aqueous

layer was extracted with CH₂Cl₂ (3 × 15 mL). The combined organic layers were washed with

brine (15 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (30 mL SiO₂,

1–2% MeOH in CH₂Cl₂ gradient elution) afforded **82** and *epi-82* (1.12 g, 2.13 mmol, 87%) as a

1:1 mixture of diastereomers. Additional flash chromatography (SiO₂, 10–50% CH₂Cl₂ in hexanes

gradient elution) could be performed to afford **82** and *epi-82* (>10:1 dr) as colorless oils. For **82**:

[α]²⁵_D –5.79 (*c* 1.26, CHCl₃); ¹H NMR (CDCl₃, 500 MHz, ca. 4.7:1 mixture of rotamers, data for

major rotamer) δ 7.62 (d, *J* = 6.5 Hz, 2H), 7.44–7.35 (m, 3H), 6.27 (s, 1H), 5.83 (d, *J* = 9.0 Hz,

1H), 4.27–4.17 (m, 2H), 4.11 (d, *J* = 9.5 Hz, 1H), 1.35 (s, 3H), 1.30 (t, *J* = 7.0 Hz, 3H), 1.21 (s,

3H), 0.95 (t, *J* = 8.0 Hz, 9H), 0.58 (q, *J* = 8.0 Hz, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 169.8, 154.0,

133.4, 129.7, 129.6 (2C), 127.8 (2C), 99.5, 83.5, 74.6, 63.3, 61.2, 28.0, 27.7, 14.1, 6.9 (3C), 6.3

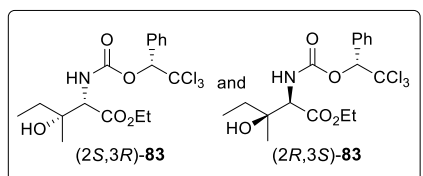
(3C); IR (film) ν_{max} 3440, 3357, 2956, 2876, 1740, 1505, 1371, 1203, 1066 cm^{–1}; HRMS (ESI)

m/z 526.1350 (MH⁺, C₂₂H₃₄Cl₃NO₅SiH⁺ requires 526.1350). For *epi-82*: [α]²⁵_D –10.2 (*c* 2.0,

CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ 7.63 (d, *J* = 7.5 Hz, 2H), 7.44–7.36 (m, 3H), 6.32 (s, 1H),

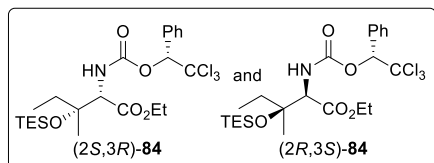
5.85 (d, *J* = 9.7 Hz, 1H), 4.19–4.09 (m, 3H), 1.39 (s, 3H), 1.35 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 3H),

0.98 (t, $J = 7.5$ Hz, 9H), 0.62 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 169.9, 153.9, 133.2, 129.7 (2C), 129.6, 127.9 (2C), 99.8, 83.3, 75.0, 63.1, 61.2, 27.7, 27.6, 14.0, 7.0 (3C), 6.5 (3C); IR (film) ν_{max} 3444, 3348, 2956, 2876, 1741, 1506, 1371, 1335, 1202, 1063 cm^{-1} ; HRMS (ESI) m/z 526.1351 (MH^+ , $\text{C}_{22}\text{H}_{34}\text{Cl}_3\text{NO}_5\text{SiH}^+$ requires 526.1350).



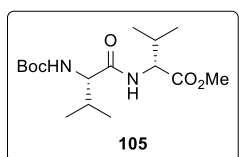
Ethyl (2S,3R)-3-hydroxy-3-methyl-2-((((R)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)pentanoate ((2S,3R)-83) and **ethyl (2R,3S)-3-hydroxy-3-methyl-2-((((R)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)pentanoate ((2R,3S)-83)**. A solution of (*R*)-2,2,2-trichloro-1-phenylethyl ((methylsulfonyl)oxy)carbamate⁴ (**71**, 740 mg, 2.04 mmol, 1.2 equiv) in CH_3CN (12 mL) at rt was treated with OsO_4 (4 wt % solution in H_2O , 1.1 mL, 44 mg OsO_4 , 0.17 mmol, 0.10 equiv), stirred at rt for 30 min, then treated with enoate **50**⁵ (240 mg, 1.69 mmol) and H_2O (0.43 mL). The resulting mixture was stirred at 45 °C for 60 h, then treated with sat aq $\text{K}_2\text{S}_2\text{O}_5$ (10 mL) and stirred for an additional 15 min. It was then diluted with H_2O (50 mL) and extracted with EtOAc (3 \times 50 mL). The combined organic layers were washed with sat aq NaHCO_3 (2 \times 40 mL) and brine (40 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (40 mL of SiO_2 , 1–3% MeOH in CH_2Cl_2 gradient elution) afforded **83** (490 mg, 1.15 mmol, 68%) as a colorless oil that was a 1:1 mixture of the (2*S*,3*R*)- and (2*R*,3*S*)-diastereomers: ^1H NMR (CDCl_3 , 500 MHz) δ 7.62 (d, $J = 6.5$ Hz, 2H), 7.46–7.35 (m, 3H), 6.32 and 6.27 (2s, 1H), 5.91 (d, $J = 8.5$ Hz, 1H), 4.38–4.17 (m, 3H), 2.42 and 2.39 (2s, 1H), 1.75–1.55 (m, 1H), 1.55–1.43 (m, 1H), 1.39–1.14 (m, 6H), 0.96 and 0.86 (2t, $J = 7.5$ and 7.5 Hz, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.6 and 171.5, 154.2 and 154.1, 133.1 and 133.0, 129.7 (2C), 129.6,

127.9 (2C), 99.7 and 99.4, 83.7 and 83.4, 74.2 and 73.8, 61.7, 59.9 and 59.6, 31.1, 23.5, 14.1 and 14.0, 8.0 and 7.8; HRMS (ESI) m/z 426.0651 (MH^+ , $C_{17}H_{22}Cl_3NO_5H^+$ requires 426.0642).



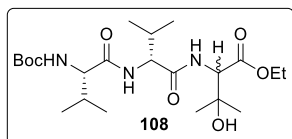
Ethyl (2*S*,3*R*)-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)pentanoate ((2*S*,3*R*)-84) and ethyl (2*R*,3*S*)-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)pentanoate ((2*R*,3*S*)-84). A solution of **83** (587 mg, 1.38 mmol) in anhydrous CH_2Cl_2 (11 mL) at 0 °C under Ar was treated dropwise with 2,6-lutidine (380 μ L, 352 mg, 3.27 mmol, 2.4 equiv) followed by TES-OTf (500 μ L, 585 mg, 2.21 mmol, 1.6 equiv). The resulting mixture was stirred at 0 °C for 5 h, then treated with sat aq $NaHCO_3$ (3 mL) and CH_2Cl_2 (6 mL). The aqueous layer was extracted with CH_2Cl_2 (3×6 mL), and the combined organic layers were washed with brine (6 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (100 mL of SiO_2 , 50% CH_2Cl_2 in hexanes elution) afforded **84** (600 mg, 1.11 mmol, 81%) as a 1:1 mixture of diastereomers. Additional flash chromatography (50 mL SiO_2 , 10–50% CH_2Cl_2 in hexanes gradient elution) could be performed to afford (2*S*,3*R*)-**84** and (2*R*,3*S*)-**84** (>10:1 dr) as colorless oils. For (2*S*,3*R*)-**84**: more polar isomer, $[\alpha]_D^{25} -22.1$ (c 0.19, $CHCl_3$); 1H NMR ($CDCl_3$, 500 MHz, ca. 3:1 mixture of rotamers, data for major rotamer) δ 7.62 (d, $J = 6.5$ Hz, 2H), 7.45–7.34 (m, 3H), 6.27 (s, 1H), 5.79 (d, $J = 7.5$ Hz, 1H), 4.25–4.16 (m, 2H), 4.15–4.08 (m, 1H), 1.68–1.57 (m, 1H), 1.48–1.40 (m, 1H), 1.35–1.25 (m, 6H), 1.01–0.91 (m, 9H), 0.78 (t, $J = 7.5$ Hz, 3H), 0.60 (q, $J = 7.9$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz, data for major rotamer) δ 170.2, 154.0, 133.4, 129.6 (3C), 127.8 (2C), 99.5, 83.4, 77.2, 61.2, 60.0, 34.0, 24.5, 14.2, 9.0, 7.0 (3C), 6.7 (3C); IR (film) ν_{max} 3442, 2956, 2877, 1743, 1501, 1371, 1335, 1200, 1067 cm^{-1} ; HRMS (ESI) m/z 557.1791

(M(NH₄)⁺, C₂₃H₃₆Cl₃NO₅Si(NH₄)⁺ requires 557.1767). For (2*R*,3*S*)-**84**: less polar isomer, [α]²⁵_D –4.5 (*c* 0.22, CHCl₃); ¹H NMR (CDCl₃, 500 MHz, ca. 7:1 mixture of rotamers, data for major rotamer) δ 7.63 (d, *J* = 6.2 Hz, 2H), 7.45–7.35 (m, 3H), 6.32 (s, 1H), 5.81 (d, *J* = 9.8 Hz, 1H), 4.28 (d, *J* = 9.8 Hz, 1H), 4.18–4.06 (m, 2H), 1.85–1.75 (m, 1H), 1.55–1.46 (m, 1H), 1.33 (s, 3H), 1.20 (t, *J* = 7.2 Hz, 3H), 0.98 (t, *J* = 8.0 Hz, 9H), 0.92 (t, *J* = 7.4 Hz, 3H), 0.63 (q, *J* = 7.9 Hz, 6H); ¹³C NMR (CDCl₃, 125 MHz, data for major rotamer) δ 170.2, 153.9, 133.2, 129.7 (2C), 129.6, 127.8 (2C), 99.8, 83.3, 77.6, 61.1, 59.8, 33.9, 24.6, 14.0, 9.2, 7.0 (3C), 6.7 (3C); IR (film) ν_{max} 3446, 2956, 1742, 1503, 1371, 1201, 1066 cm⁻¹; HRMS (ESI) *m/z* 557.1757 (M(NH₄)⁺, C₂₃H₃₆Cl₃NO₅Si(NH₄)⁺ requires 557.1767).



Methyl (tert-butoxycarbonyl)-L-valyl-D-valinate (105). A solution of Boc-

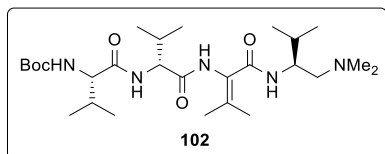
D-Val (507 mg, 3.02 mmol) in anhydrous THF–DMF (5:1, 30 mL) at 0 °C under Ar was treated sequentially with L-Val-OMe•HCl (982 mg, 4.5 mmol, 1.5 equiv), HOBT (ca. 20% H₂O content, 760 mg, 4.5 mmol, 1.5 equiv), EDC•HCl (863 mg, 4.5 mmol, 1.5 equiv), and NaHCO₃ (636 mg, 6.0 mmol, 2.0 equiv). The resulting mixture was warmed to rt slowly and stirred under Ar for 24 h. The reaction was quenched by the addition of sat aq NaHCO₃ (10 mL) and H₂O (20 mL), extracted with EtOAc (4 × 35 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (80 mL of SiO₂, 0–25% EtOAc in hexanes gradient elution) afforded **105** (889 mg, 2.7 mmol, 89%) as a white film: ¹H NMR (CDCl₃, 500 MHz) δ 6.43 (d, *J* = 9.0 Hz, 1H), 4.99 (s, 1H), 4.56 (dd, *J* = 4.5, 5.0 Hz, 1H), 3.99 (br s, 1H), 3.74 (s, 3H), 2.25–2.15 (m, 2H), 1.45 (s, 9H), 1.02–0.88 (m, 12H); HRMS (ESI) *m/z* 331.2236 (MH⁺, C₁₆H₃₀N₂O₅H⁺ requires 331.2233).



Ethyl (6S,9R)-12-(2-hydroxypropan-2-yl)-6,9-diisopropyl-2,2-

dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (108). A solution of ester **105** (600.0 mg, 1.82 mmol) in *t*-BuOH–H₂O (2:1, 21 mL) at 0 °C was treated with LiOH•H₂O (343 mg, 8.2 mmol, 4.5 equiv) and stirred at 0 °C for 6 h. The resulting mixture was acidified to pH 4~5 by the addition 2N HCl, diluted with H₂O (5 mL), and extracted with EtOAc (5 × 20 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **106** (536 mg, 1.7 mmol, 93%) was used directly in the next step without further purification.

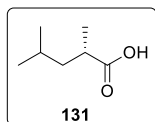
A solution of the acid in anhydrous DMF–CH₂Cl₂ (1:1, 30 mL) at 0 °C under Ar was treated with amine **107** (328 mg, 2.04 mmol, 1.2 equiv), HOBt (ca. 20% H₂O content, 456 mg, 2.7 mmol, 1.6 equiv), and EDC•HCl (489 mg, 2.6 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C under Ar for 24 h. The reaction was quenched by the addition of sat aq NaHCO₃ (10 mL) and H₂O (10 mL), and the organic solvent was removed *in vacuo*. The remaining aqueous layer was extracted with EtOAc–*t*-BuOH (10:1, 10 × 20 mL), and the combined organic layers were washed with brine (2 × 10 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO₂, 50–100% EtOAc in hexanes gradient elution, then 1% MeOH in EtOAc elution) afforded **108** (695.4 mg, 1.5 mmol, 89%) as a colorless oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.02–6.93 (m, 1H), 6.78–6.70 and 6.70–6.64 (m, 1H), 5.19–5.10 (m, 1H), 4.55–4.32 (m, 2H), 4.31–4.15 (m, 2H), 3.99 (s, 1H), 2.21 (s, 1H), 2.21–2.03 (m, 2H), 1.41 (s, 9H), 1.28–1.17 (m, 9H), 0.99–0.82 (m, 12H); HRMS (ESI) *m/z* 460.3029 (MH⁺, C₂₂H₄₁N₃O₇H⁺ requires 460.3023).



tert-Butyl ((4S,10R,13S)-4,10-diisopropyl-2,14-dimethyl-

6,9,12-trioxo-7-(propan-2-ylidene)-2,5,8,11-tetraazapentadecan-13-yl)carbamate (102). A solution of ester **108** (152 mg, 0.331 mmol) in *t*-BuOH–H₂O (1:1, 10 mL) at rt was treated with LiOH•H₂O (62.5 mg, 1.49 mmol, 4.5 equiv), then stirred at rt for 5 h. The resulting mixture was acidified to pH 4~5 by the addition of 2 N HCl, diluted with H₂O (3 mL), and extracted with EtOAc (6 × 10 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **109** (116 mg, 0.269 mmol, 81%) was used directly without further purification.

A solution of the crude acid in anhydrous DMF (10 mL) at rt under Ar was treated with EDC•HCl (103 mg, 0.537 mmol, 2.0 equiv) and stirred at rt for 15 h, at which point the starting material had been consumed as evidenced by MS. The mixture was then treated with amine **111** (77 mg, 0.591 mmol, 2.2 equiv) followed by DMF (1.0 mL) and NEt₃ (376 μL, 273 mg, 2.7 mmol, 10.0 equiv). The resulting mixture was stirred at rt for 4 h. The reaction was quenched by the addition of sat aq NaHCO₃ (4 mL), diluted with H₂O (4 mL), and extracted with EtOAc (8 × 10 mL). The combined organic layers were dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (15 mL of SiO₂, 0–4% MeOH in EtOAc with 1% Et₃N gradient elution) afforded **102** (94.7 mg, 0.18 mmol, 67% from **109**) as a white film: ¹H NMR (CDCl₃, 500 MHz) δ 7.44 (s, 1H), 6.71 (s, 1H), 6.57 (s, 1H), 6.08 (s, 1H), 4.22 (s, 1H), 4.03 (s, 1H), 3.63 (s, 1H), 3.38–3.10 (m, 1H), 2.61–2.53 (m, 1H), 2.40–2.33 (m, 1H), 2.21 (s, 6H), 2.08 (s, 3H), 2.07–2.01 (m, 1H), 1.97–1.85 (m, 1H), 1.72 (s, 3H), 1.39 (s, 9H), 1.07–0.82 (m, 18H); HRMS (ESI) *m/z* 526.3967 (MH⁺, C₂₇H₅₁N₅O₅H⁺ requires 526.3968).

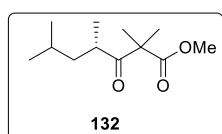


(S)-2,4-dimethylpentanoic acid (131). To a solution of (1*R*,2*R*)-(-)-pseudoephedrine (5.0 g, 30.3 mmol, 1.00 equiv) in CH₂Cl₂ (50 ml) at room temperature was added Et₃N (4.7 ml, 34 mmol, 1.12 equiv) and propionic chloride (3.0 ml, 34 mmol, 1.12 equiv) in 1 ml portions over 30 min. After stirring for 1 h, the mixture was washed with saturated, aqueous NaHCO₃ (40 ml), 1.0 M aqueous HCl (2 × 30 mL) and brine (40 ml). The organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. Flash chromatography (200 mL of SiO₂, 2–8% MeOH in CH₂Cl₂ gradient elution) afforded a white solid which was dried under vacuum for 12 h. This solid was dissolved in refluxing toluene (20 ml), placed in a water bath at 80 °C and allowed to slowly cool to ambient temperature. Further cooling to –18 °C overnight followed by filtration and drying afforded known propionamide **129** (5.7 g, 25.8 mmol, 85%) as white crystals.

To an ice-cooled suspension of flame-dried lithium chloride (1.79 g, 42.3 mmol, 6.00 equiv) in THF (20 ml) was added *i*Pr₂NH (2.3 ml, 16.2 mmol, 2.3 equiv) followed by *n*BuLi (1.6 N in hexane, 9.3 ml, 14.8 mmol, 2.1 equiv). The yellow slurry was stirred for 15 min at 0 °C, 20 min at room temperature and then cooled to –78 °C. A solution of amide **129** (1.56 g, 7.05 mmol, 1.00 equiv) in THF (20 ml) was added via cannula over 30 min and the solution was vigorously stirred for 45 min. After 15 min at 0 °C, 15 min at room temperature, and recooling to –78 °C, iodide (1.22 ml, 10.6 mmol, 1.50 equiv) was added neat and the reaction mixture was stirred for 1 h at –78 °C and an additional 60 min at 0 °C. The reaction mixture was quenched by the addition of saturated, aqueous NH₄Cl (20 ml) and saturated, aqueous Na₂S₂O₃ (2 ml). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 40 ml). The combined organic solutions were washed with brine (30 ml), dried over anhydrous Na₂SO₄, and filtered.

Concentration under reduced pressure provided known amide **130** (1.6 g, 82%) as a solid, which was used without further purification.

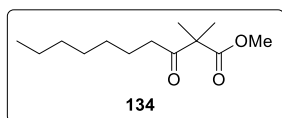
A 250-mL round-bottomed flask was charged with amide **130** (390 mg, 1.41 mmol, 1 equiv), dioxane (20 mL), and 9 N aqueous sulfuric acid solution (20 mL). The biphasic mixture was heated at reflux for 24 h and then cooled to 0 °C. The pH of the mixture was adjusted to pH > 10 by the slow addition of 50% (w/w) aqueous sodium hydroxide solution, and the resulting mixture was partitioned between water (30 mL) and dichloromethane (30 mL). The aqueous layer was separated and extracted with dichloromethane (30 mL). The aqueous layer was acidified to pH < 2 by the slow addition of 6 N aqueous sulfuric acid solution and then extracted with dichloromethane (3 × 40 ml). The latter organic extracts were combined and concentrated to a volume of ca. 50 mL, and the concentrate was then washed with 1 N aqueous hydrochloric acid solution to remove residual dioxane. The resulting organic layer was dried over sodium sulfate and concentrated to afford acid **131** as a clear liquid (143 mg, 78%): ¹H NMR (CDCl₃, 500 MHz) δ COOH (1H), 2.58–2.52 (m, 1H), 1.67–1.60 (m, 1H), 1.28–1.23 (m, 2H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.90 (d, *J* = 6.0 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 182.8, 42.7, 37.3, 25.8, 22.5, 22.4, 17.3; HRMS (ESI) *m/z* 131.1076 (MH⁺, C₇H₁₄O₂H⁺ requires 131.1072).



Methyl (S)-2,2,4,6-tetramethyl-3-oxoheptanoate (132). To a suspended

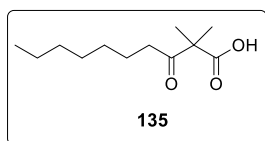
solution of InBr₃ (35.5 mg, 0.1 mmol) and **131** (131 mg, 1 mmol) in dichloromethane (10 mL) was added (MeO)₃SiH (134 uL, 1.05 mmol), and methyl trimethylsilyl dimethylketene acetal (406 uL, 2 mmol). The reaction mixture was stirred for 6 h at room temperature and then was quenched by 1 M HCl aq (10 mL). The resulting mixture was extracted with Et₂O (3 × 20 ml). The organic

layer was dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (15 mL of SiO₂, 1–5% MeOH in CH₂Cl₂ gradient elution) afforded **132** (190 mg, 0.89 mmol, 89%) as a light yellow liquid: ¹H NMR (CDCl₃, 500 MHz) δ 3.70 (s, 3H), 1.90–1.83 (m, 1H), 1.70–1.56 (m, 1H), 1.33–1.13 (m, 8H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.95–0.85 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 178.6, 171.2, 52.0, 45.8, 37.0, 29.9, 23.9, 22.4 (2C), 21.7 (2C), 16.0; HRMS (ESI) *m/z* 215.1653 (MH⁺, C₁₂H₂₂O₃H⁺ requires 215.1647).



Methyl 2,2-dimethyl-3-oxodecanoate (134). To a suspended solution of

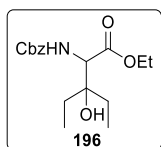
InBr₃ (35.5 mg, 0.1 mmol) and **133** (145 mg, 1 mmol) in dichloromethane (10 mL) was added (MeO)₃SiH (134 uL, 1.05 mmol), and methyl trimethylsilyl dimethylketene acetal (406 uL, 2 mmol). The reaction mixture was stirred for 6 h at room temperature and then was quenched by 1 M HCl aq (10 mL). The resulting mixture was extracted with Et₂O (3 × 20 ml). The organic layer was dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (15 mL of SiO₂, 1–5% MeOH in CH₂Cl₂ gradient elution) afforded **134** (208 mg, 0.91 mmol, 91%) as a colorless oil: ¹H NMR (CDCl₃, 500 MHz) δ 3.73 (s, 3H), 2.44 (t, *J* = 7.5 Hz, 2H), 1.60–1.54 (m, 2H), 1.37 (s, 6H), 1.32–1.20 (m, 8H), 0.88 (t, *J* = 7.0 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 208.1, 174.3, 55.6, 52.4, 37.9, 31.7, 29.1, 29.0, 23.9, 22.6, 21.9 (2C), 14.1; HRMS (ESI) *m/z* 229.1805 (MH⁺, C₁₃H₂₄O₃H⁺ requires 229.1804).



2,2-dimethyl-3-oxodecanoic acid (135). A solution of ester **134** (117 mg,

0.512 mmol) in *t*-BuOH–H₂O (2:1, 6 mL) at 0 °C was treated with LiOH•H₂O (96.8 mg, 2.3 mmol, 4.5 equiv), then stirred at 0 °C for 5 h. The resulting mixture was acidified to pH 1~2 by the

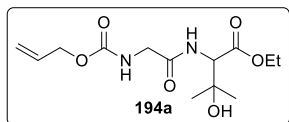
addition of 2 N HCl (4 mL) and extracted with EtOAc (6 × 5 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **135** (105 mg, 0.49 mmol, 96%) was obtained as a colorless oil: ¹H NMR (CDCl₃, 500 MHz) δ COOH (1H), 2.54 (t, *J* = 7.5 Hz, 2H), 1.64–1.56 (m, 2H), 1.42 (s, 6H), 1.36–1.20 (m, 8H), 0.88 (t, *J* = 7.0 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 208.3, 178.3, 55.3, 38.0, 31.9, 29.1, 29.0, 23.8, 22.7, 21.1 (2C), 14.1; HRMS (ESI) *m/z* 215.1652 (MH⁺, C₁₂H₂₂O₃H⁺ requires 215.1647).



Ethyl 2-(((benzyloxy)carbonyl)amino)-3-ethyl-3-hydroxypentanoate (196). A

solution of benzyl ((methylsulfonyl)oxy)carbamate² (1.472 g, 6.00 mmol, 1.3 equiv) in CH₃CN (50 mL) at rt was treated with OsO₄ (4 wt % solution in H₂O, 2.9 mL, 0.46 mmol, 0.10 equiv), stirred for 10 min, and then treated with a solution of ethyl 3-ethylpent-2-enoate⁶ (**195**, 710 mg, 4.54 mmol) in CH₃CN (10.0 mL) and H₂O (4.7 mL). The resulting mixture was stirred at 45 °C for 4 d, treated with sat aq K₂S₂O₅ (20 mL), and stirred for an additional 20 min. It was then diluted with H₂O (40 mL) and extracted with EtOAc (4 × 80 mL). The combined organic layers were washed with sat aq NaHCO₃ (2 × 40 mL) and brine (40 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (300 mL of SiO₂, 0.25–5% MeOH in CH₂Cl₂ gradient elution) afforded **196** (1.10 g, 3.40 mmol, 75%) as a yellow oil: ¹H NMR (CDCl₃, 500 MHz) δ 7.37–7.30 (m, 5H), 5.76 (d, *J* = 9.0 Hz, 1H), 5.11 (d, *J* = 7.5 Hz, 2H), 4.36 (d, *J* = 9.5 Hz, 1H), 4.27–4.20 (m, 2H), 2.45 (br s, 1H), 1.61–1.47 (m, 4H), 1.31 (t, *J* = 7.0 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H), 0.87 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 172.3, 156.3, 136.2, 128.5 (2C), 128.2 (2C), 128.1, 76.1, 67.1, 61.5, 58.5, 27.31, 27.29, 14.1, 7.59, 7.55; IR (film) ν_{max} 3366,

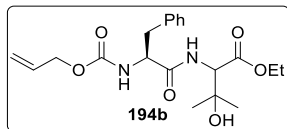
3033, 2972, 1719, 1509, 1334, 1051 cm^{-1} ; HRMS (ESI) m/z 324.1808 (MH^+ , $\text{C}_{17}\text{H}_{25}\text{NO}_5\text{H}^+$ requires 324.1811).



Ethyl 2-((allyloxy)carbonylamino)acetamido-3-hydroxy-3-

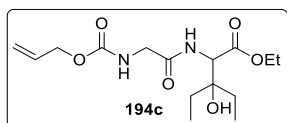
methylbutanoate (194a). A solution of **42**⁷ (353 mg, 1.20 mmol) in MeOH (10 mL) was treated with 10% Pd/C (114.6 mg, 0.32 wt equiv) and stirred at rt under H_2 (650 psi) for 1 d. The mixture was filtered through a pad of Celite (washed with 125 mL of MeOH), and the filtrate was concentrated *in vacuo* to afford the crude amine (198 mg, 193 mg theoretical yield, quant.), a portion of which was used without further purification in the next reaction.

The crude amine (81 mg, 0.50 mmol, 1.0 equiv) was dissolved in DMF (2 mL) and added to a solution of ((allyloxy)carbonyl)glycine (88 mg, 0.55 mmol, 1.1 equiv) in DMF (3 mL) at 0 °C under Ar. The mixture was stirred for 10 min, then treated with EDC•HCl (115.7 mg, 0.604 mmol, 1.2 equiv) and HOBT (81.5 mg, 0.532 mmol, 1.1 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 24 h, then diluted with H_2O (15 mL) and extracted with EtOAc (4 × 30 mL). The combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO_2 , 0.5–5 % MeOH in CH_2Cl_2 gradient elution) afforded **194a** (122 mg, 0.404 mmol, 80%) as a light yellow oil: ^1H NMR (CDCl_3 , 500 MHz) δ 6.91 (d, $J = 9.0$ Hz, 1H), 5.96–5.88 (m, 1H), 5.49 (br s, 1H), 5.33 (d, $J = 17.0$ Hz, 1H), 5.24 (d, $J = 13.5$ Hz, 1H), 4.64–4.56 (m, 3H), 4.54 (d, $J = 9.0$ Hz, 1H), 4.30–4.20 (m, 2H), 3.95 (d, $J = 6.0$ Hz, 2H), 1.32 (t, $J = 7.0$ Hz, 3H), 1.30 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.2, 169.3, 156.5, 132.5, 118.0, 72.0, 66.1, 61.8, 59.8, 44.4, 26.7, 26.6, 14.1; IR (film) ν_{max} 3338, 2982, 1728, 1531, 1213 cm^{-1} ; HRMS (ESI) m/z 303.1534 (MH^+ , $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_6\text{H}^+$ requires 303.1556).



Ethyl 2-((S)-2-(((allyloxy)carbonyl)amino)-3-phenylpropanamido)-3-

hydroxy-3-methylbutanoate (194b). A solution of the crude amine prepared via hydrogenolysis of **42** (73 mg, 0.45 mmol, 1.0 equiv) in DMF (2 mL) was added to a solution of ((allyloxy)carbonyl)-L-phenylalanine (127.7 mg, 0.512 mmol, 1.1 equiv) in DMF (3 mL) at 0 °C under Ar. The mixture was stirred for 10 min, then treated with EDC•HCl (96.1 mg, 0.501 mmol, 1.1 equiv) and HOBT (68.4 mg, 0.447 mmol, 0.99 equiv). The resulting mixture was stirred at 0 °C to rt under Ar for 24 h, then diluted with H₂O (15 mL) and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with brine (10 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO₂, 0.5–5 % MeOH in CH₂Cl₂ gradient elution) afforded **194b** (132 mg, 0.336 mmol, 74%) as a light yellow oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.31–7.19 (m, 5H), 6.81–6.75 (m, 1H), 5.94–5.82 (m, 1H), 5.47 (d, *J* = 7.0 Hz, 1H), 5.29 (d, *J* = 10.0 Hz, 1H), 5.26 (d, *J* = 8.5 Hz, 1H), 4.71–4.64 and 4.59–4.40 (2m, 5H), 4.23–4.17 (m, 2H), 3.25–3.19 (m, 1H), 3.16–3.11 (m, 1H), 1.29 (t, *J* = 7.5 Hz, 3H), 1.23 (s, 3H), 1.19 (s, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.4, 171.0 and 170.7, 155.7, 136.2 and 136.1, 132.7 and 132.4, 129.5 and 129.3 (2C), 128.8 and 128.5 (2C), 127.1 and 127.0, 117.9 and 117.7, 72.0 and 71.9, 66.0, 61.62 and 61.58, 56.2, 54.6, 38.6 and 38.3, 26.63 and 26.58, 26.5 and 26.4, 14.1; IR (film) ν_{\max} 3338, 2982, 1728, 1531, 1213, 1050 cm⁻¹; HRMS (ESI) *m/z* 393.1989 (MH⁺, C₂₀H₂₈N₂O₆H⁺ requires 393.2026).

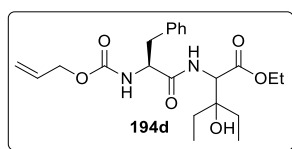


Ethyl 2-(2-(((allyloxy)carbonyl)amino)acetamido)-3-ethyl-3-

hydroxypentanoate (194c). A solution of **196** (470 mg, 1.45 mmol) in MeOH (14 mL) was treated

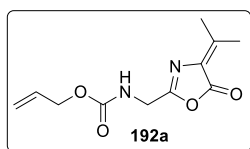
with 10% Pd/C (190 mg, 0.4 wt equiv) and stirred at rt under H₂ (650 psi) for 2 d. The mixture was filtered through a pad of Celite (washed with 200 mL of MeOH), and the filtrate was concentrated *in vacuo* to afford the crude amine (258 mg, 1.36 mmol, 94%), a portion of which was used without further purification in the next reaction.

The crude amine (41 mg, 0.22 mmol, 1.0 equiv) was dissolved in DMF (1 mL) and added to a solution of ((allyloxy)carbonyl)glycine (103.4 mg, 0.650 mmol, 3.0 equiv) in DMF (1 mL) at 0 °C under Ar. The mixture was stirred for 10 min, then treated with COMU (279.7 mg, 0.653 mmol, 3.0 equiv) and 2,4,6-collidine (172 μL, 158 mg, 1.30 mmol, 6.0 equiv). The resulting mixture was warmed to rt and stirred for 12 h. It was then cooled to 0 °C, quenched with sat aq NaHCO₃ (3 mL), and extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine (5 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO₂, 0.5–5 % MeOH in CH₂Cl₂ gradient elution) afforded **194c** (59 mg, 0.18 mmol, 82%, 77% from **7**) as a light yellow oil: ¹H NMR (CDCl₃, 500 MHz) δ 6.79 (d, *J* = 8.5 Hz, 1H), 5.97–5.88 (m, 1H), 5.40 (br s, 1H), 5.32 (d, *J* = 17.0 Hz, 1H), 5.23 (d, *J* = 11.0 Hz, 1H), 4.64–4.58 (m, 3H), 4.32–4.28 (m, 1H), 4.27–4.19 (m, 2H), 3.94 (s, 2H), 1.58–1.46 (m, 4H), 1.31 (t, *J* = 7.5 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H), 0.87 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.8, 169.0, 156.4, 132.5, 118.0, 76.3, 66.7, 61.6, 56.7, 44.4, 28.4, 26.6, 14.1, 7.6, 7.5; IR (film) ν_{max} 3347, 2975, 1729, 1521, 1255 cm⁻¹; HRMS (ESI) *m/z* 331.1879 (MH⁺, C₁₅H₂₆N₂O₆H⁺ requires 331.1869).



Ethyl 2-((S)-2-(((allyloxy)carbonyl)amino)-3-phenylpropanamido)-3-ethyl-3-hydroxypentanoate (194d). A solution of the crude amine prepared via hydrogenolysis

of **196** (50 mg, 0.26 mmol, 1.0 equiv) in DMF (1 mL) was added to a solution of ((allyloxy)carbonyl)-L-phenylalanine (198.5 mg, 0.796 mmol, 3.0 equiv) in DMF (1 mL) at 0 °C under Ar. The mixture was stirred for 10 min, then treated with COMU (340.2 mg, 0.794 mmol, 3.0 equiv) and 2,4,6-collidine (210 μ L, 193 mg, 1.59 mmol, 6.0 equiv). The resulting mixture was warmed to rt and stirred for 12 h. It was then cooled to 0 °C, quenched with sat aq NaHCO₃ (3 mL), and extracted with EtOAc (4 \times 10 mL). The combined organic layers were washed with brine (5 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO₂, 0.5–5 % MeOH in CH₂Cl₂ gradient elution) afforded **194d** (75 mg, 0.18 mmol, 68%, 63% from **7**) as a light yellow oil that was a 1:1 mixture of diastereomers: ¹H NMR (CDCl₃, 500 MHz) δ 7.30–7.17 (m, 5H), 6.95 and 6.91 (2d, *J* = 9.0 and 8.5 Hz, 1H), 5.89–5.79 (m, 1H), 5.57 and 5.52 (2d, *J* = 7.0 and 7.5 Hz, 1H), 5.26–5.16 (m, 2H), 4.61 (d, *J* = 9.0 Hz, 1H), 4.57–4.46 (m, 3H), 4.23–4.14 (m, 2H), 3.14–3.03 (m, 2H), 2.85 (s, 1H), 1.51–1.37 (m, 4H), 1.28 (t, *J* = 7.5 Hz, 3H), 0.93–0.73 (m, 6H); ¹³C NMR (CDCl₃, 125 MHz) δ 171.8, 171.2 and 171.1, 155.8 and 155.7, 136.4 and 136.3, 132.5, 129.4 and 129.3 (2C), 128.6 and 128.5 (2C), 126.9, 117.8, 76.4, 65.9, 61.5 and 61.4, 57.0 and 56.8, 56.1 and 55.9, 38.7 and 38.3, 26.6, 26.5, 14.1 and 14.0, 7.63 and 7.60, 7.57 and 7.50; IR (film) ν_{max} 3320, 2972, 2942, 1728, 1532, 1258, 1032 cm⁻¹; HRMS (ESI) *m/z* 421.2338 (MH⁺, C₂₂H₃₂N₂O₆H⁺ requires 421.2339).

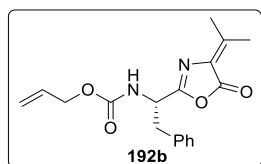


Allyl ((5-oxo-4-(propan-2-ylidene)-4,5-dihydrooxazol-2-yl)methyl)carbamate (192a).

A solution of **194a** (112 mg, 0.370 mmol) in *t*-BuOH–H₂O (3:1, 4 mL) at 0 °C under Ar was treated with LiOH•H₂O (70 mg, 1.7 mmol, 4.5 equiv), then stirred at 0 °C for 5 h. The resulting mixture was acidified to pH 1–2 by the addition of 2 N HCl (1.5 mL)

and extracted with EtOAc (6 × 6 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **191a** (98 mg, 0.36 mmol, 96%) was used directly in the next reaction without further purification.

A solution of **191a** (55 mg, 0.20 mmol) in acetic anhydride (1.2 mL) at rt under Ar was treated with sodium acetate (25 mg, 0.30 mmol, 1.5 equiv). The resulting mixture was stirred at 50 °C for 7 h, at which time the starting material had disappeared as evidenced by MS. The excess acetic anhydride was decomposed by the addition of H₂O (0.8 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na₂CO₃ (3 × 3 mL) and brine (1.5 mL), dried (Na₂SO₄), and concentrated *in vacuo* to afford **192a** (43 mg, 0.18 mmol, 90%) as a light yellow solid: ¹H NMR (CDCl₃, 500 MHz) δ 5.96–5.91 (m, 1H), 5.39–5.32 (m, 2H), 5.25 (d, *J* = 10.5 Hz, 1H), 4.63 (br s, 2H), 4.30 (d, *J* = 5.5 Hz, 2H), 2.36 (s, 3H), 2.26 (s, 3H); HRMS (ESI) *m/z* 239.1076 (MH⁺, C₁₁H₁₄N₂O₄H⁺ requires 239.1032).

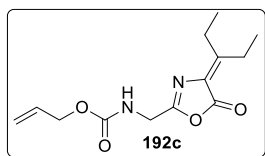


Allyl (S)-1-(5-oxo-4-(propan-2-ylidene)-4,5-dihydrooxazol-2-yl)-2-

phenylethyl)carbamate (192b). A solution of **194b** (60 mg, 0.15 mmol) in *t*-BuOH–H₂O (3:1, 2 mL) at 0 °C under Ar was treated with LiOH·H₂O (30.7 mg, 0.732 mmol, 4.8 equiv), then stirred at 0 °C for 5 h. The resulting mixture was acidified to pH 1–2 by the addition of 2 N HCl (0.8 mL) and extracted with EtOAc (6 × 6 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **191b** (49 mg, 0.13 mmol, 88%) was used directly in the next reaction without further purification.

A solution of **191b** (49 mg, 0.13 mmol) in acetic anhydride (1 mL) at rt under Ar was treated with sodium acetate (17.4mg, 0.212 mmol, 1.6 equiv). The resulting mixture was stirred

at 50 °C for 7 h, at which time the starting material had disappeared as evidenced by MS. The excess acetic anhydride was decomposed by the addition of H₂O (0.5 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na₂CO₃ (3 × 3 mL) and brine (1.5 mL), dried (Na₂SO₄), and concentrated *in vacuo* to afford **192b** (32 mg, 0.097 mmol, 72%) as a light yellow solid: ¹H NMR (CDCl₃, 500 MHz) δ 7.30–7.20 (m, 5H), 5.94–5.85 (m, 1H), 5.40–5.24 (m, 2H), 5.21 (d, *J* = 10.5 Hz, 1H), 4.99–4.93 (m, 1H), 4.60–4.54 (m, 2H), 3.27–3.21 (m, 1H), 3.14–3.10 (m, 1H), 2.34 (s, 3H), 2.20 (s, 3H); HRMS (ESI) *m/z* 329.1517 (MH⁺, C₁₈H₂₀N₂O₄H⁺ requires 329.1501).

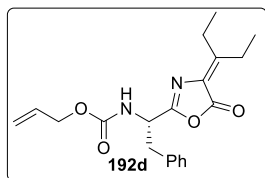


Allyl ((5-oxo-4-(pentan-3-ylidene)-4,5-dihydrooxazol-2-

yl)methyl)carbamate(192c). A solution of **194c** (55 mg, 0.17 mmol) in *t*-BuOH–H₂O (3:1, 2 mL) at 0 °C under Ar was treated with LiOH•H₂O (32.4 mg, 0.772 mmol, 4.6 equiv), then stirred at 0 °C for 5 h. The resulting mixture was acidified to pH 1–2 by the addition of 2 N HCl (0.8 mL) and extracted with EtOAc (6 × 6 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **191c** (47 mg, 0.16 mmol, 93%) was used directly in the next reaction without further purification.

A solution of **191c** (47 mg, 0.16 mmol) in acetic anhydride (1 mL) at rt under Ar was treated with sodium acetate (19.7 mg, 0.240 mmol, 1.5 equiv). The resulting mixture was stirred at 50 °C for 7 h, at which time the starting material had disappeared as evidenced by MS. The excess acetic anhydride was decomposed by the addition of H₂O (0.5 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na₂CO₃ (3 × 3 mL) and brine (1.5 mL), dried (Na₂SO₄), and concentrated *in vacuo* to afford **192c** (35 mg, 0.13 mmol, 85%) as a light yellow

solid: $^1\text{H NMR}$ (CDCl_3 , 500 MHz) δ 5.99–5.90 (m, 1H), 5.40–5.35 (m, 1H), 5.32 (d, $J = 10.5$ Hz, 1H), 5.25 (d, $J = 10.5$ Hz, 1H), 4.63 (br s, 2H), 4.30 (d, $J = 5.0$ Hz, 2H), 2.80 (q, $J = 7.5$ Hz, 2H), 2.66 (q, $J = 7.5$ Hz, 2H), 0.89 (t, $J = 7.0$ Hz, 3H), 0.87–0.82 (m, 3H); HRMS (ESI) m/z 267.1363 (MH^+ , $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_4\text{H}^+$ requires 267.1345).



Allyl (S)-1-(5-oxo-4-(pentan-3-ylidene)-4,5-dihydrooxazol-2-yl)-2-phenylethylcarbamate (192d). A solution of **194d** (52 mg, 0.12 mmol) in *t*-BuOH–H₂O (3:1, 2 mL) at 0 °C under Ar was treated with LiOH·H₂O (26.6 mg, 0.634 mmol, 5.1 equiv), then stirred at 0 °C for 5 h. The resulting mixture was acidified to pH 1–2 by the addition of 2 N HCl (0.8 mL) and extracted with EtOAc (6 × 6 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude carboxylic acid **191d** (41 mg, 0.10 mmol, 84%) was used directly in the next reaction without further purification.

A solution of **191d** (41 mg, 0.104 mmol) in acetic anhydride (1 mL) at rt under Ar was treated with sodium acetate (14.1 mg, 0.172 mmol, 1.7 equiv). The resulting mixture was stirred at 50 °C for 7 h, at which time the starting material had disappeared as evidenced by MS. The excess acetic anhydride was decomposed by the addition of H₂O (0.5 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na₂CO₃ (3 × 3 mL) and brine (1.5 mL), dried (Na₂SO₄), and concentrated *in vacuo* to afford **192d** (24 mg, 0.067 mmol, 64%) as a light yellow solid: $^1\text{H NMR}$ (CDCl_3 , 500 MHz) δ 7.33–7.22 (m, 5H), 5.96–5.85 (m, 1H), 5.34–5.19 (m, 3H), 5.00–4.95 (m, 1H), 4.60–4.54 (m, 2H), 3.29–3.22 (m, 1H), 3.14–3.08 (m, 1H), 2.81 (q, $J = 7.5$ Hz, 2H), 2.59 (q, $J = 8.0$ Hz, 2H), 0.91–0.82 (m, 6H); HRMS (ESI) m/z 357.1832 (MH^+ , $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_4\text{H}^+$ requires 357.1814).

General Procedures for Solid-Phase Peptide Synthesis

Attachment of C-terminal amino acid to resin. Rink amide MBHA resin (100–200 mesh, 10 μmol) was added to a fritted polypropylene syringe. The resin was swelled in CH_2Cl_2 (10 min), and then in DMF (3 min). The swelling solvents were drained from the resin using a vacuum manifold. After Fmoc deprotection (see below for procedure, repeated twice), the amino acid was coupled to the resin (see below for procedure, repeated twice).

Fmoc Deprotection. The resin (10 μmol) was treated with piperidine (20% solution in DMF, 250 μL) and allowed to stand for 1 min. The solution was drained from the resin using a vacuum manifold, and additional piperidine (20% solution in DMF, 250 μL) was added. The resulting mixture was stirred at 80 $^\circ\text{C}$ in a microwave oven for 4 min. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF ($5 \times 3 \text{ mL}$).

Peptide coupling. The Fmoc-protected amino acid (50 μmol , 5 equiv) and HBTU (9.5 mg, 50 μmol , 5 equiv) were dissolved in a 0.1 M HOBt solution in NMP (250 μL , 0.25 μmol , 5 equiv). *i*Pr₂NEt (8.5 μL , 50 μmol , 10 equiv) was added to this solution, and it was allowed to stand for ca. 1 min. The solution was added to the resin (10 μmol), and the resulting mixture was stirred at 70 $^\circ\text{C}$ in a microwave oven for 10 min. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF ($5 \times 3 \text{ mL}$).

Coupling of azlactone 2 with the resin-bound peptide. A solution of **192** (50 μmol , 5 equiv) and DMAP (5 μmol , 0.1 equiv) in NMP (300 μL) was added to the resin-bound peptide **199** (10 μmol). Compared with DMAP, Et₃N was later found to provide better results. The resulting mixture was stirred at 60 $^\circ\text{C}$ for 24 h. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF ($5 \times 2\text{--}3 \text{ mL}$).

Alloc deprotection. The resin-bound peptide **200** (10 μmol) was washed with CH_2Cl_2 (10 \times 4 mL) and placed under an Ar atmosphere. A solution of PhSiH_3 (29.7 μL , 26.1 mg, 241 μmol , 24 equiv) in CH_2Cl_2 (1 mL) was added with stirring, followed by a solution of $\text{Pd}(\text{PPh}_3)_4$ (2.4 mg, 2 μmol , 0.2 equiv) in CH_2Cl_2 (2 mL). The resulting mixture was stirred at rt under Ar for 10 min. The resin was washed with CH_2Cl_2 (8 \times 4 mL), and the deprotection reaction was repeated once.

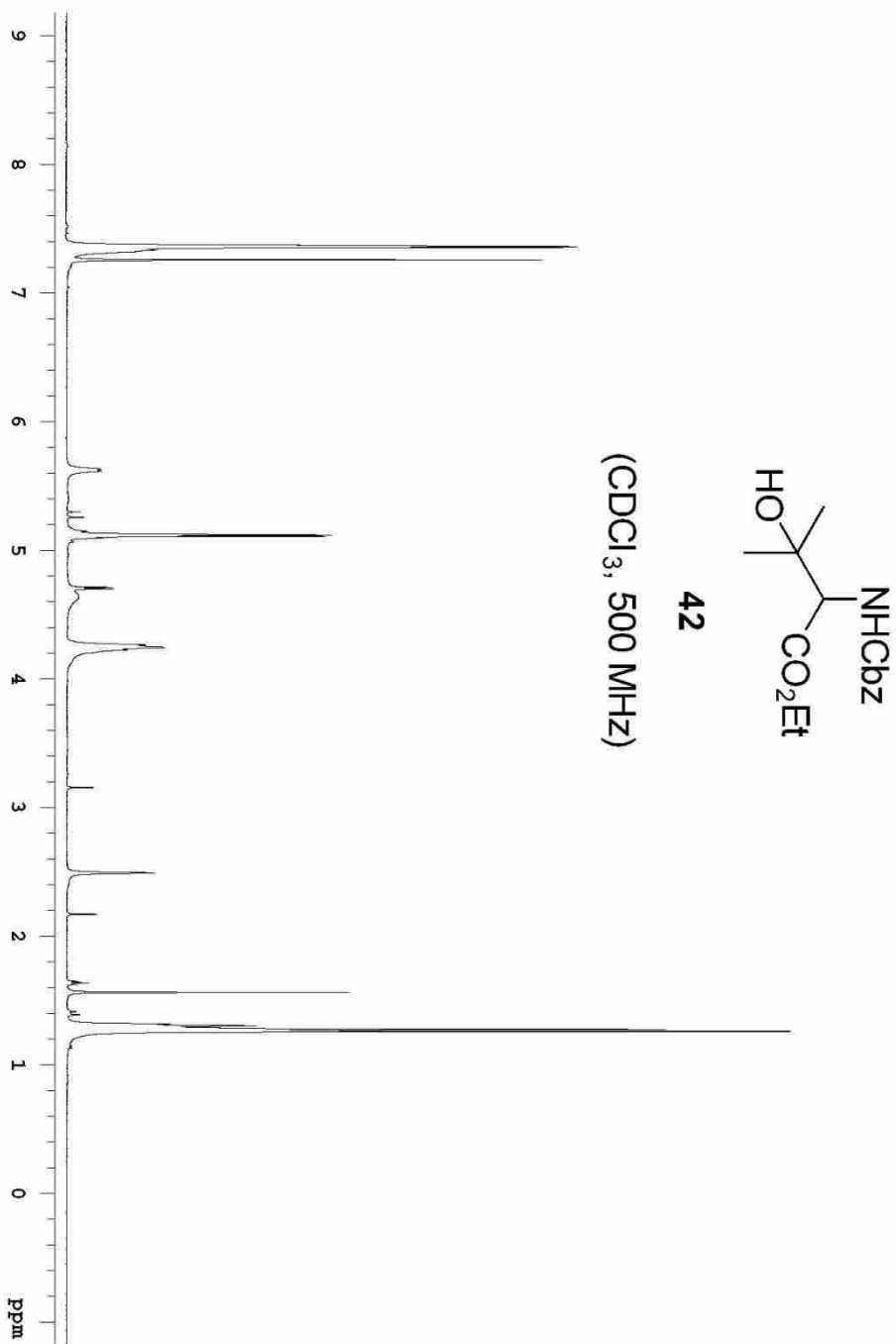
Cleavage of peptide 201 from resin and purification. The resin was rinsed with CH_2Cl_2 (10 \times 2 mL), then treated carefully with a solution of phenol (50 mg, 530 μmol), H_2O (50 μL), thioanisole (50 μL , 53 mg, 430 μmol), ethanedithiol (25 μL , 28 mg, 300 μmol), and triisopropylsilane (10 μL , 7.7 mg, 50 μmol) in trifluoroacetic acid (800 μL) to avoid the buildup of excess CO_2 pressure in the reaction vessel. The resulting mixture was stirred at rt for 4 h, and the peptide was precipitated by filtering the mixture and pouring the filtrate into cold Et_2O (40 mL). The resin was rinsed with TFA (1 mL), and the precipitate was collected by centrifugation. The crude peptide was lyophilized and purified by HPLC (Discovery BIO Wide Pore C18-10 Column (25 cm \times 10 mm, 10 μm), 30–50% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 16 min, 6 mL/min flow rate, then 100% CH_3CN (0.1% TFA) for 15 min, 15 mL/min flow rate).

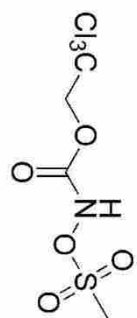
6.3 References

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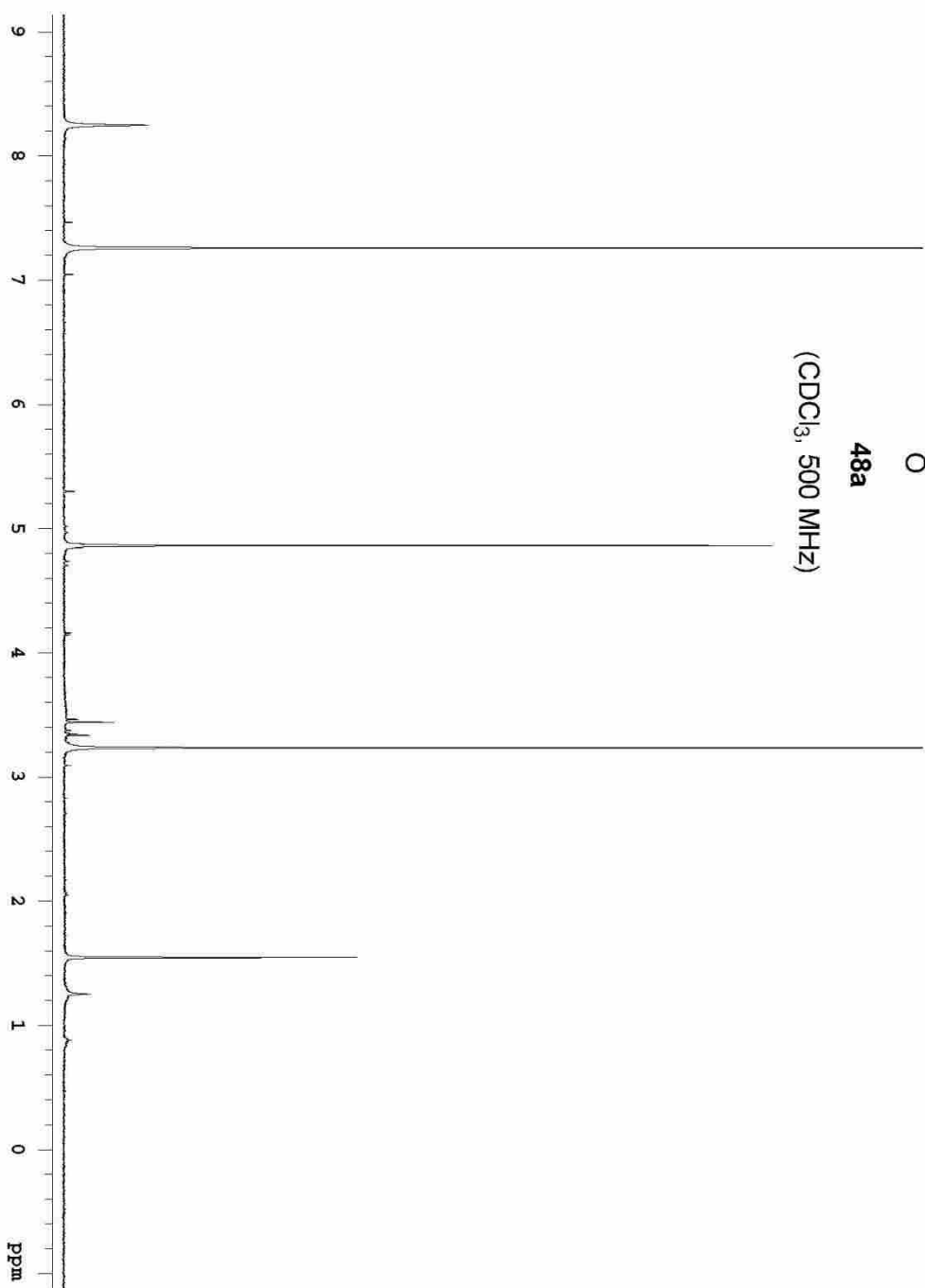
6.4 Spectra

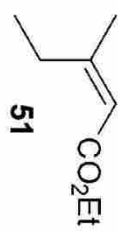




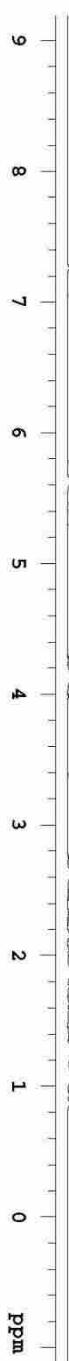
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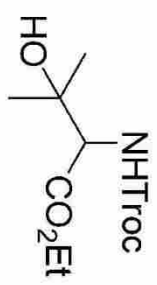
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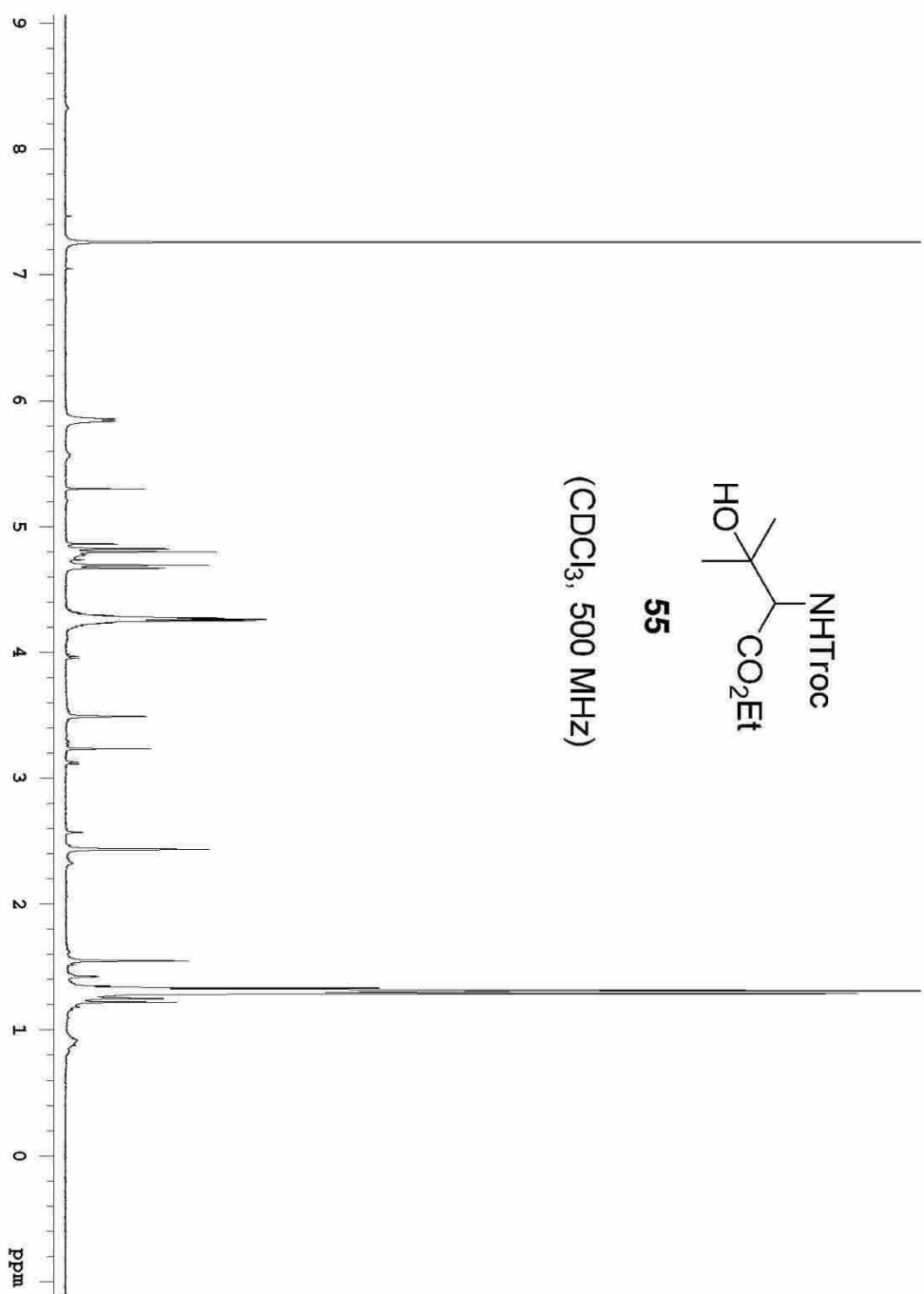
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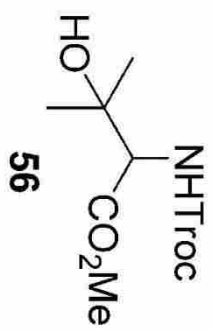




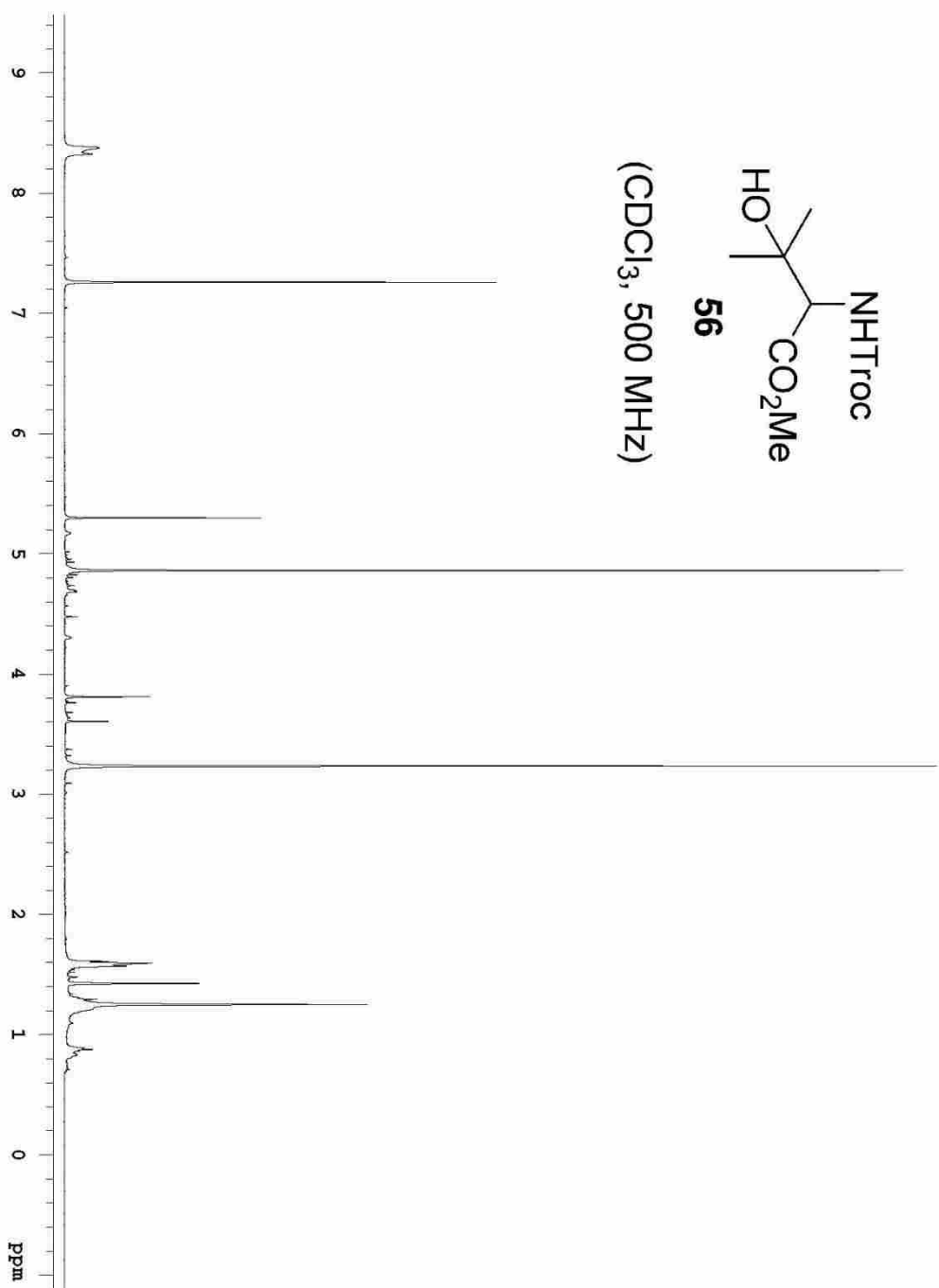
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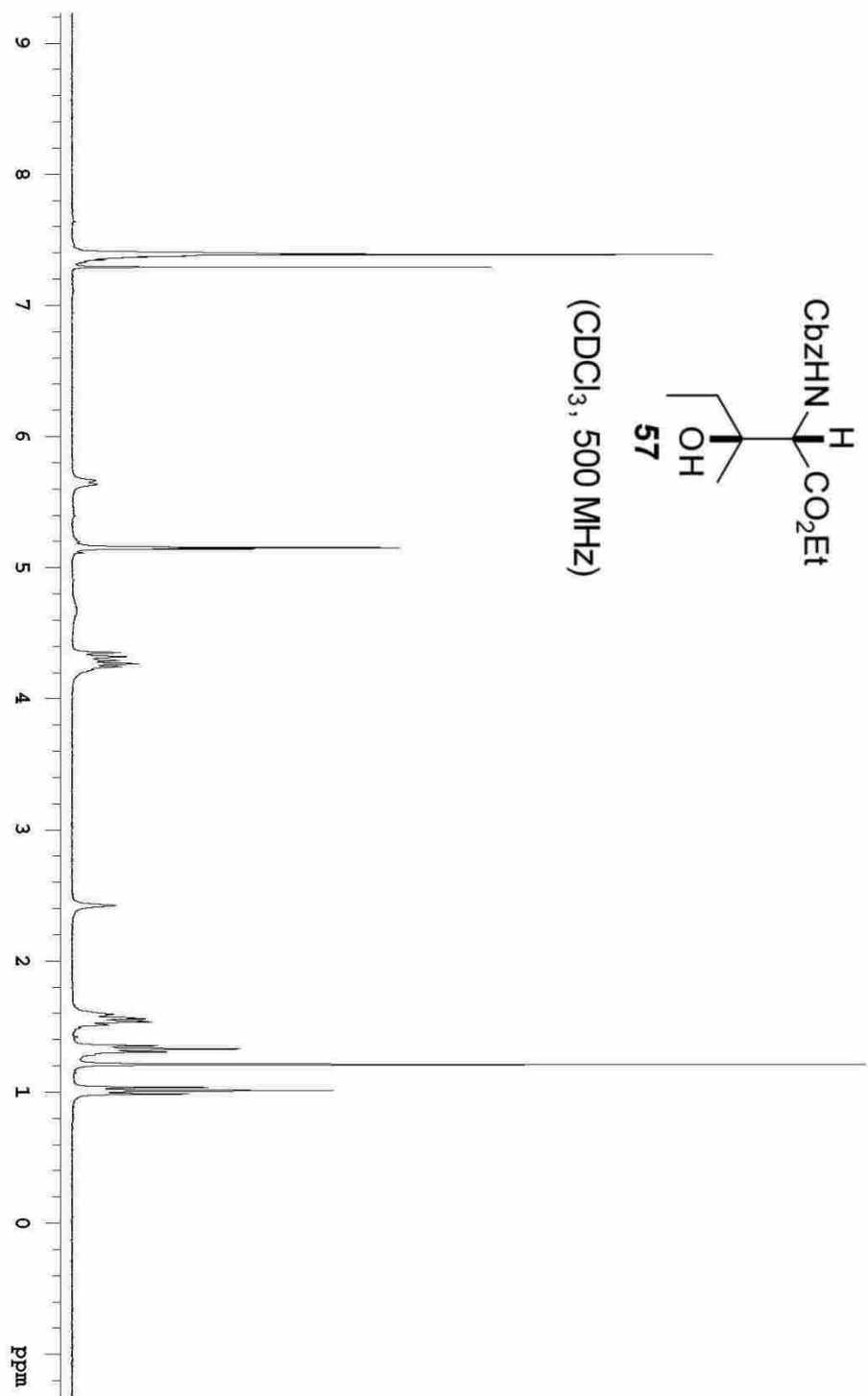
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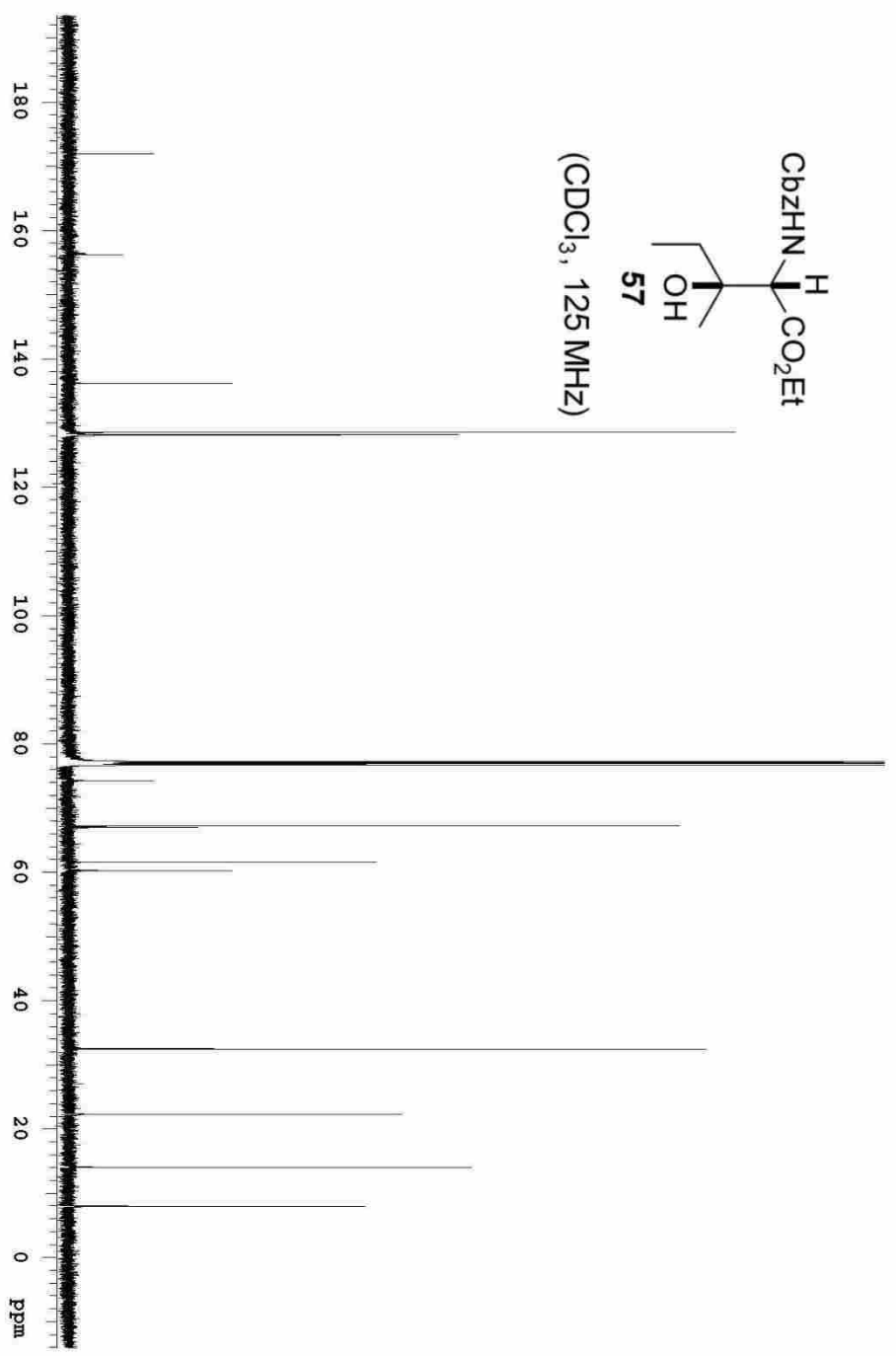
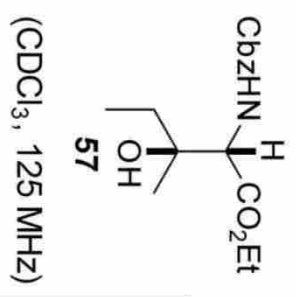


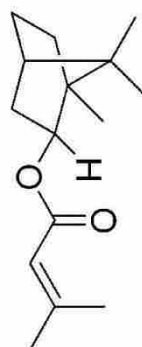


(CDCl₃, 500 MHz)



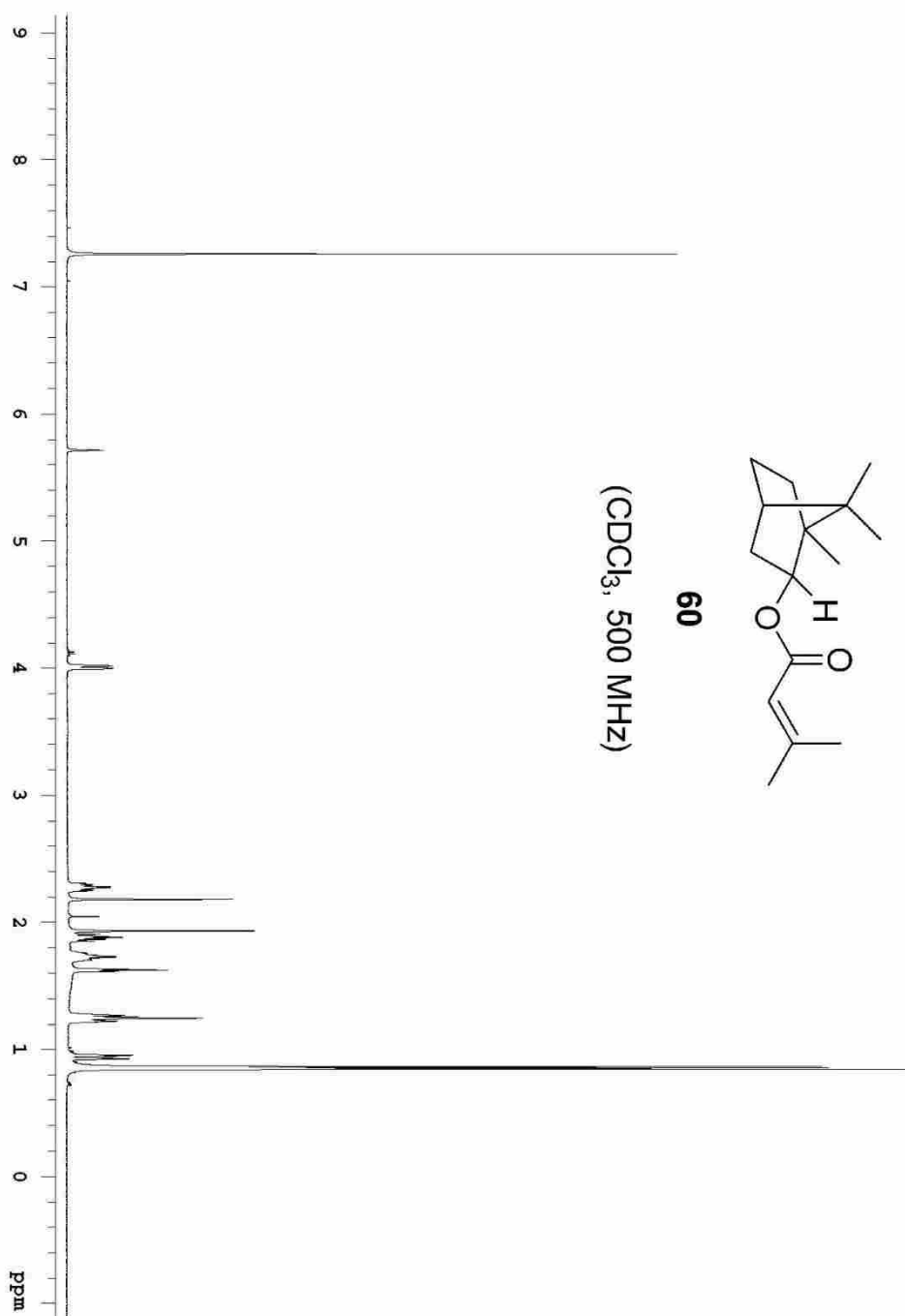


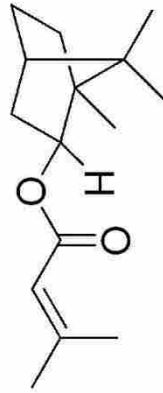




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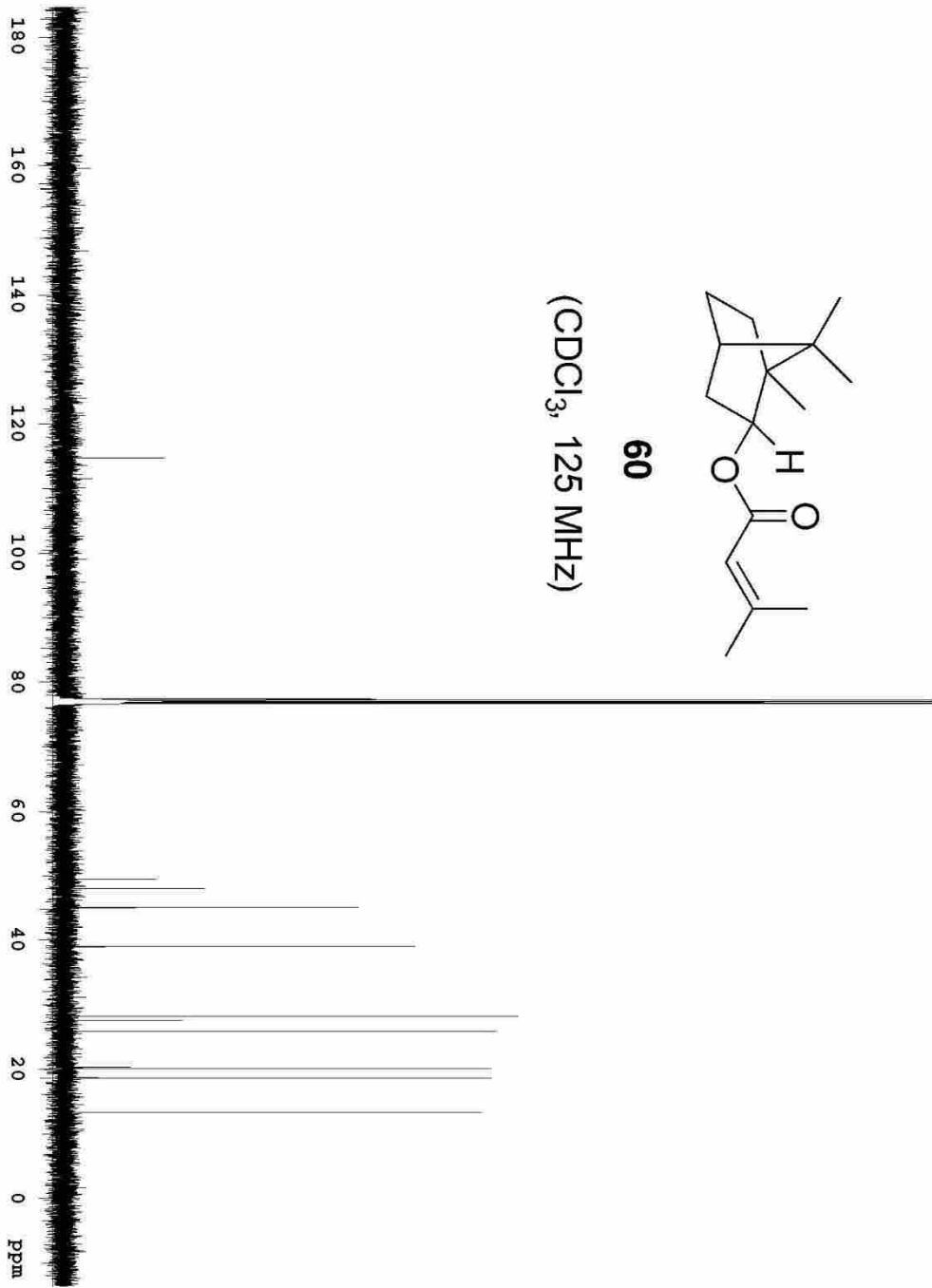
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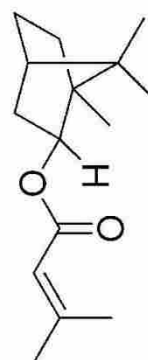




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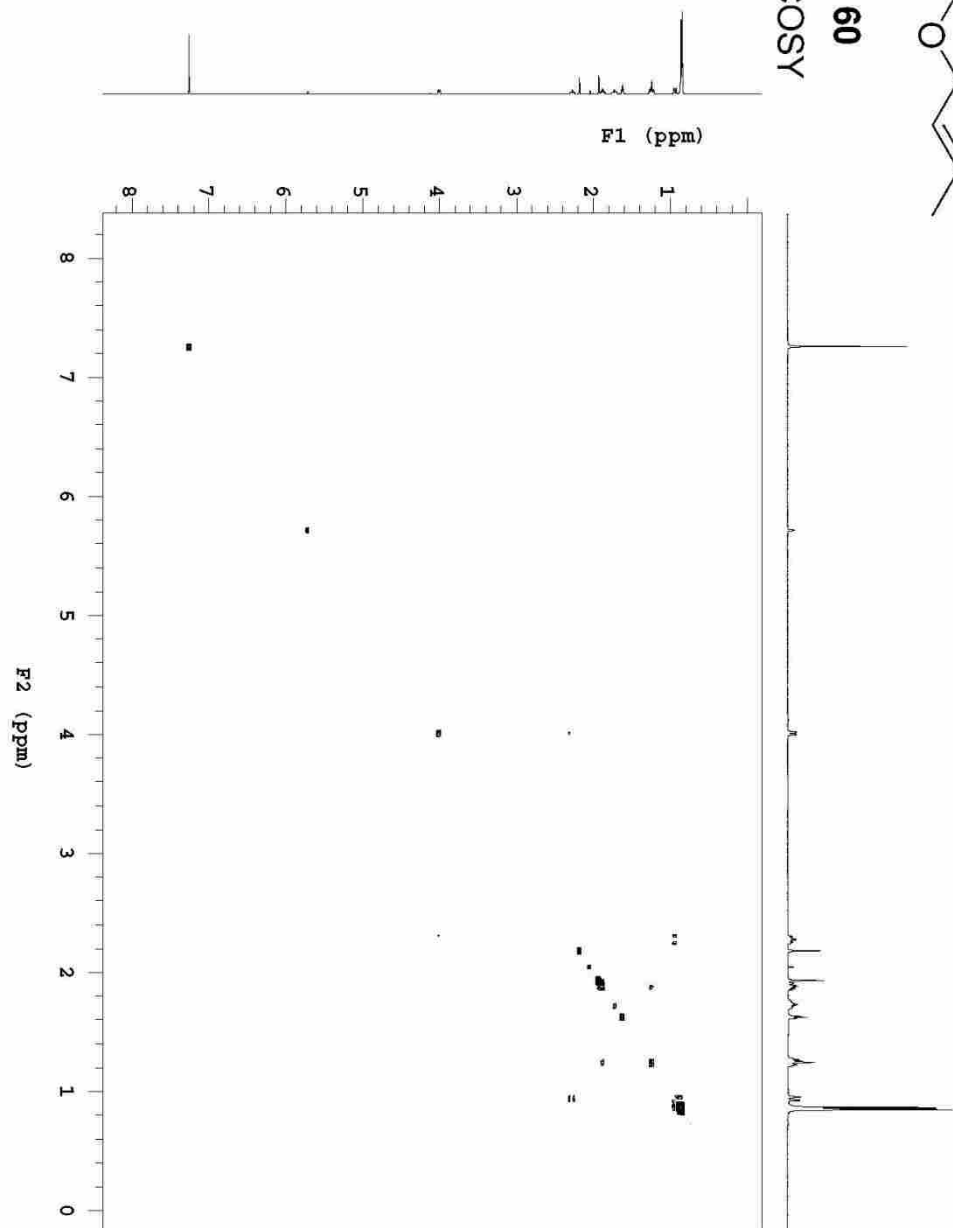
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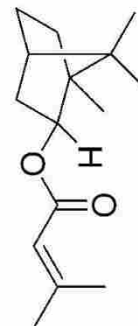




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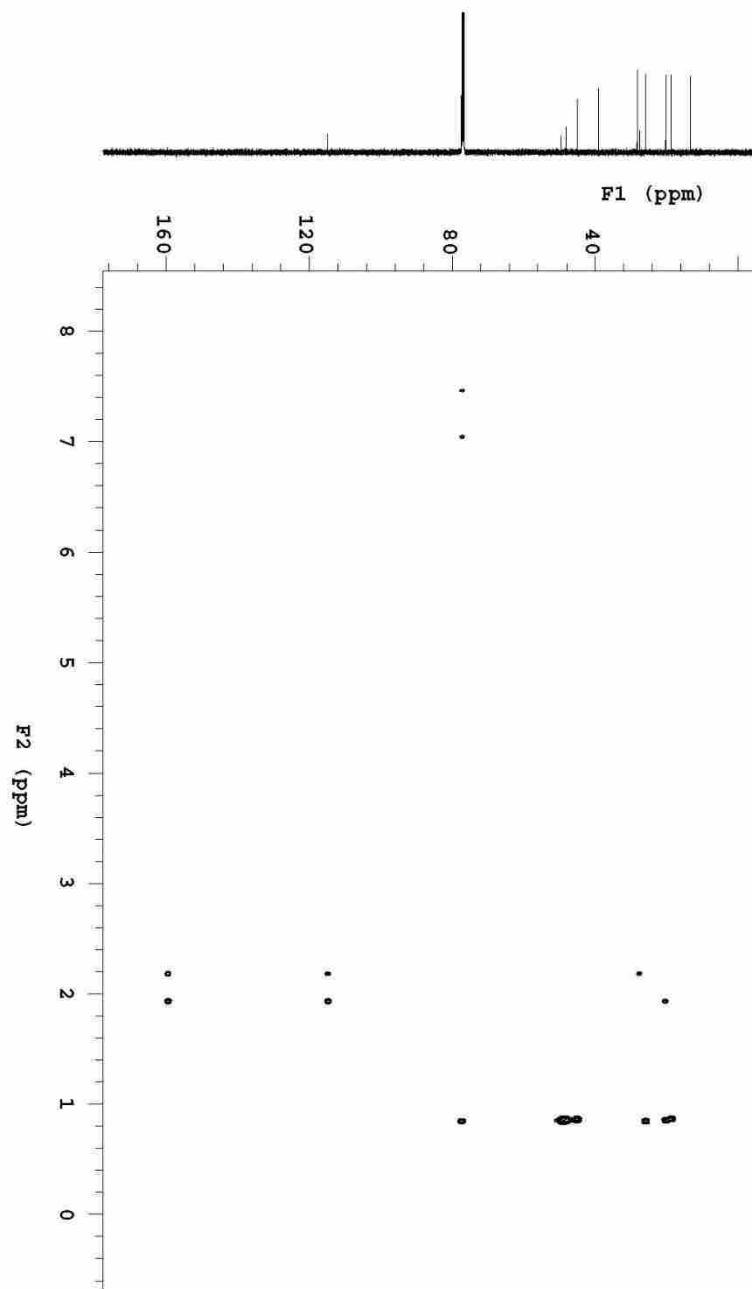
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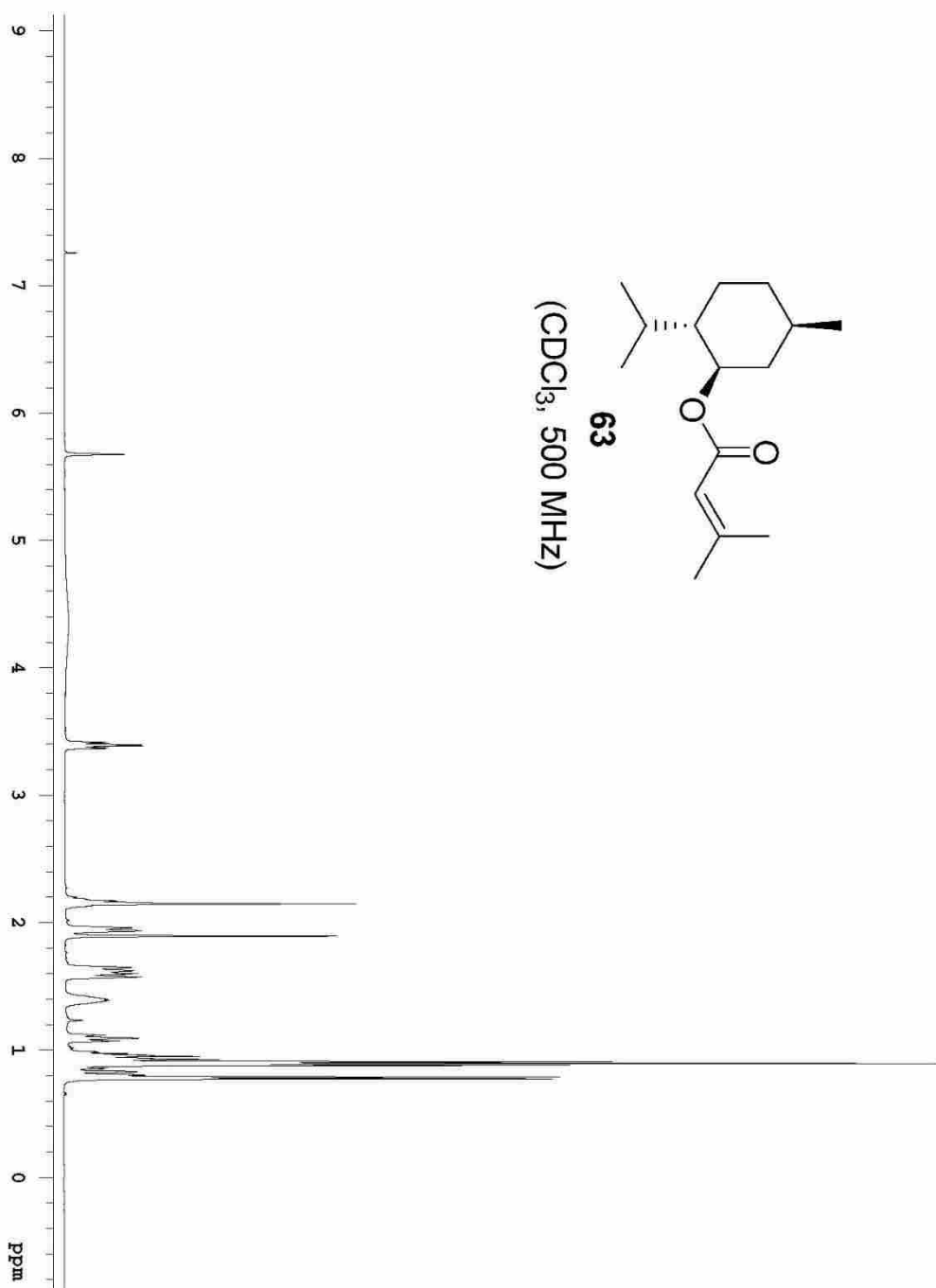
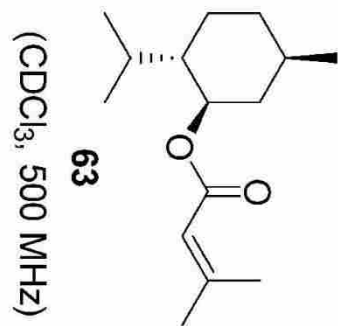


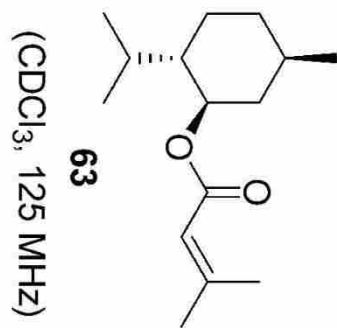


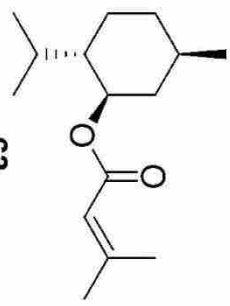
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HMBC



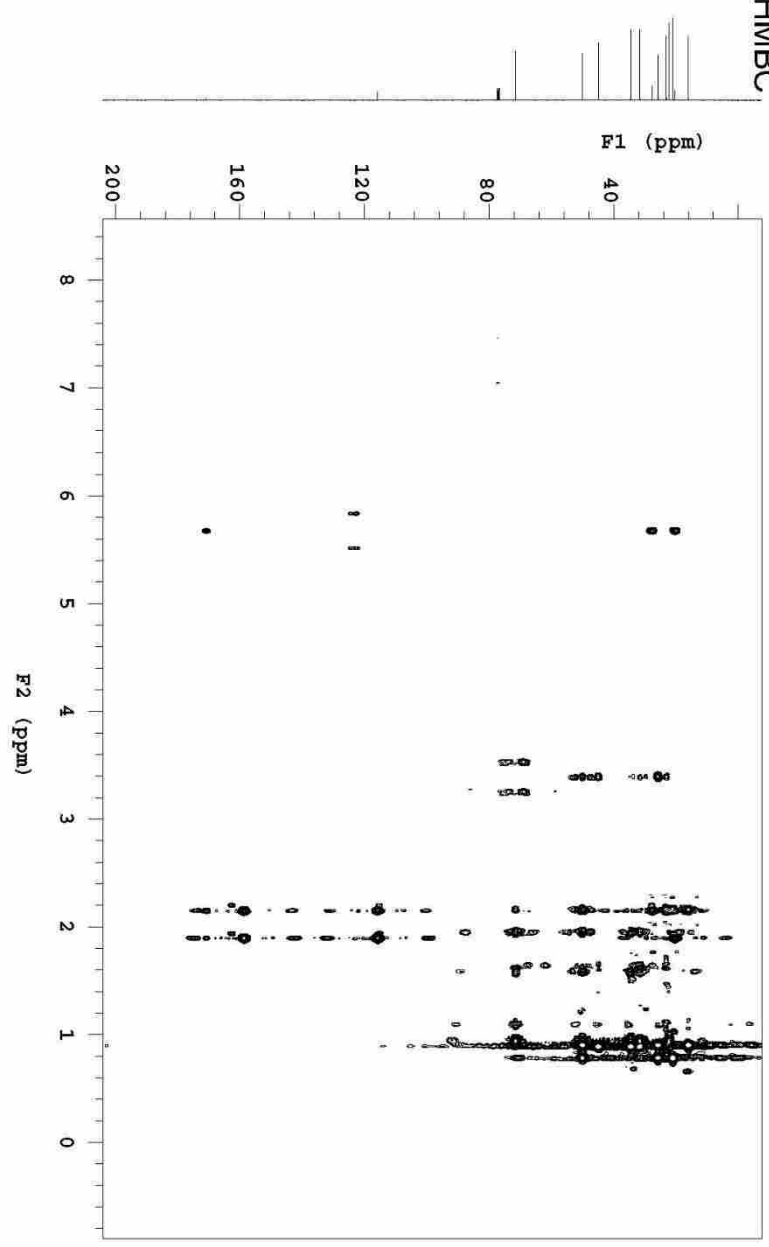


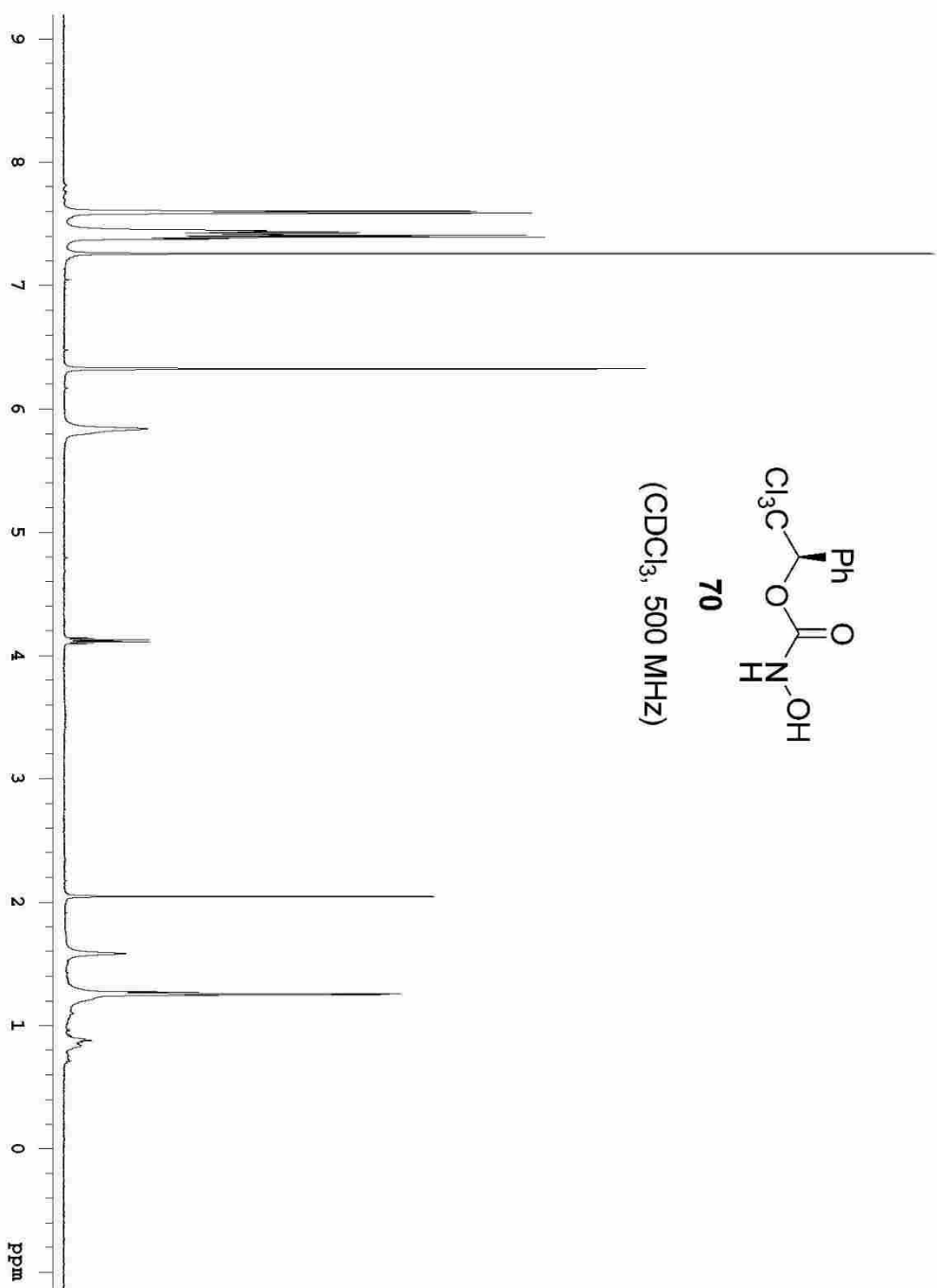
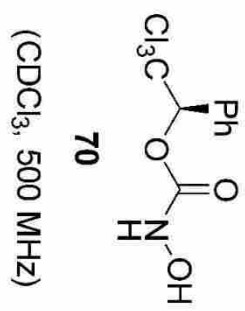


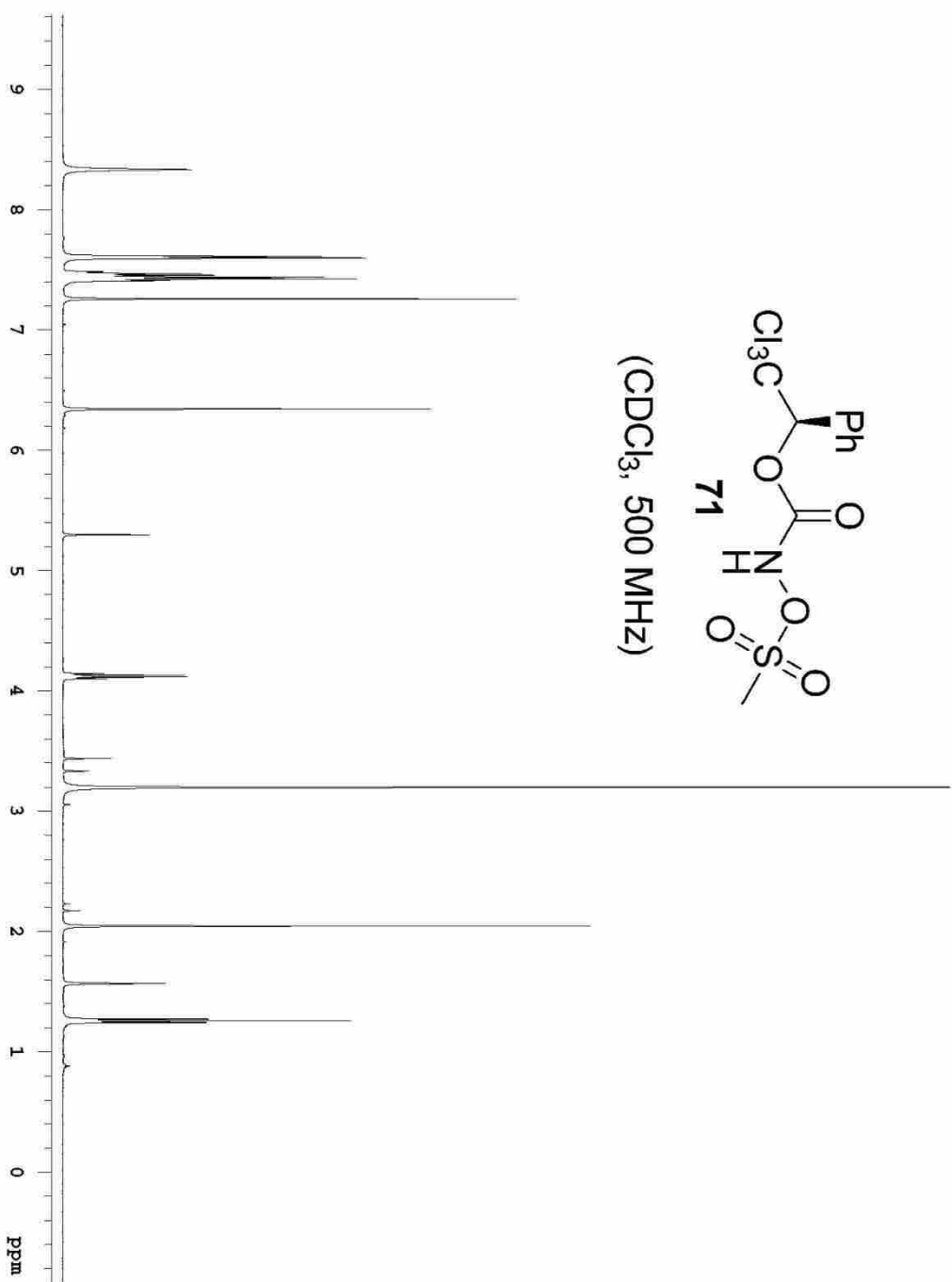
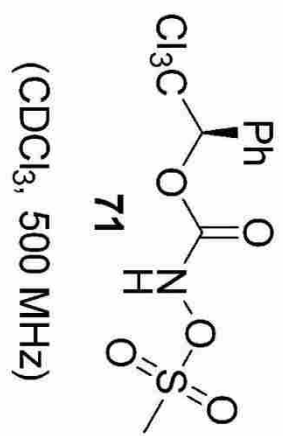


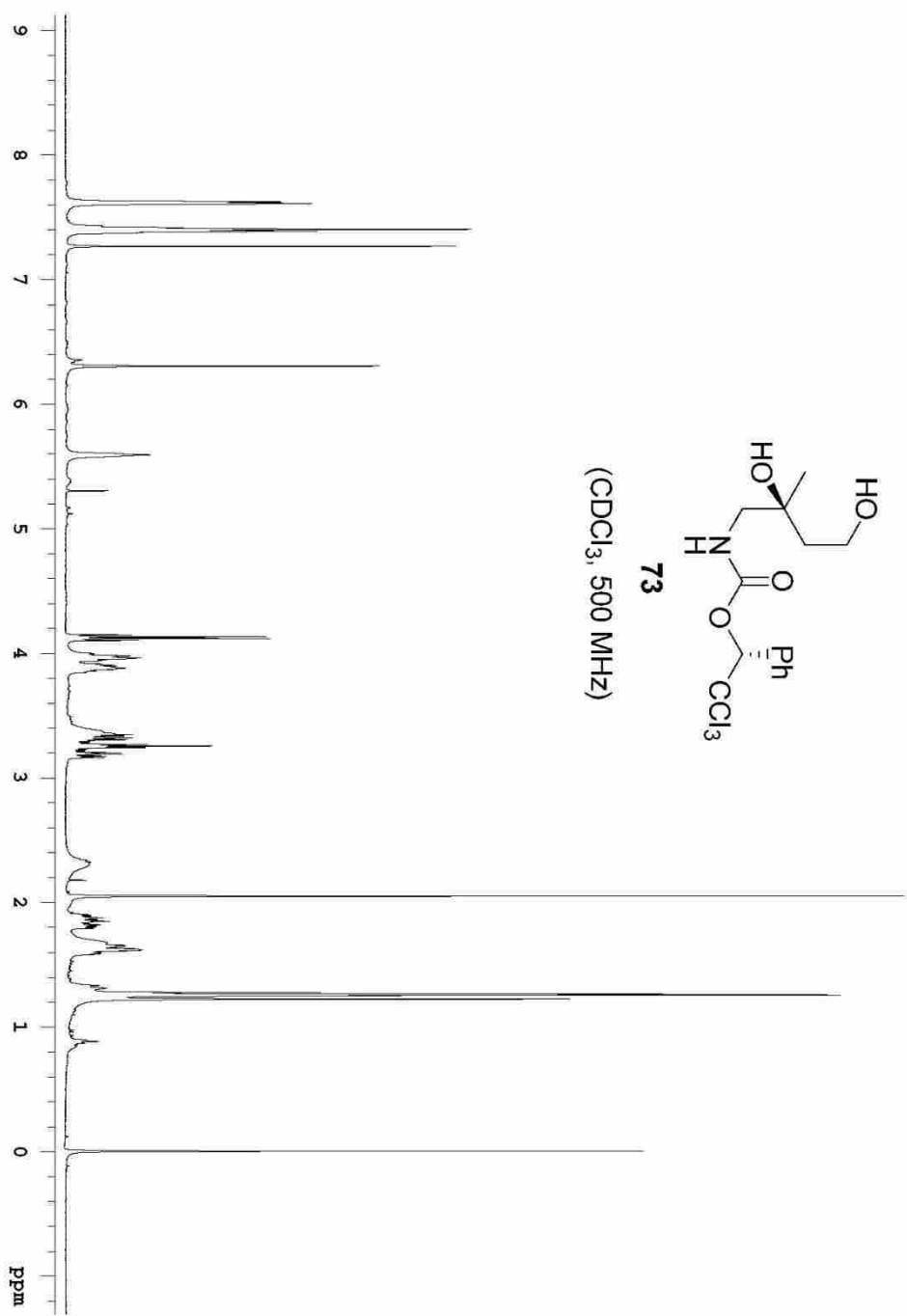
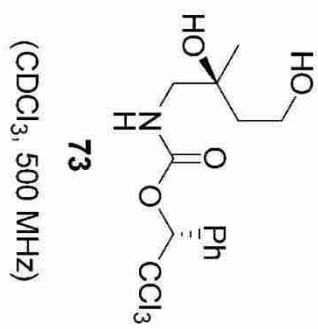
63

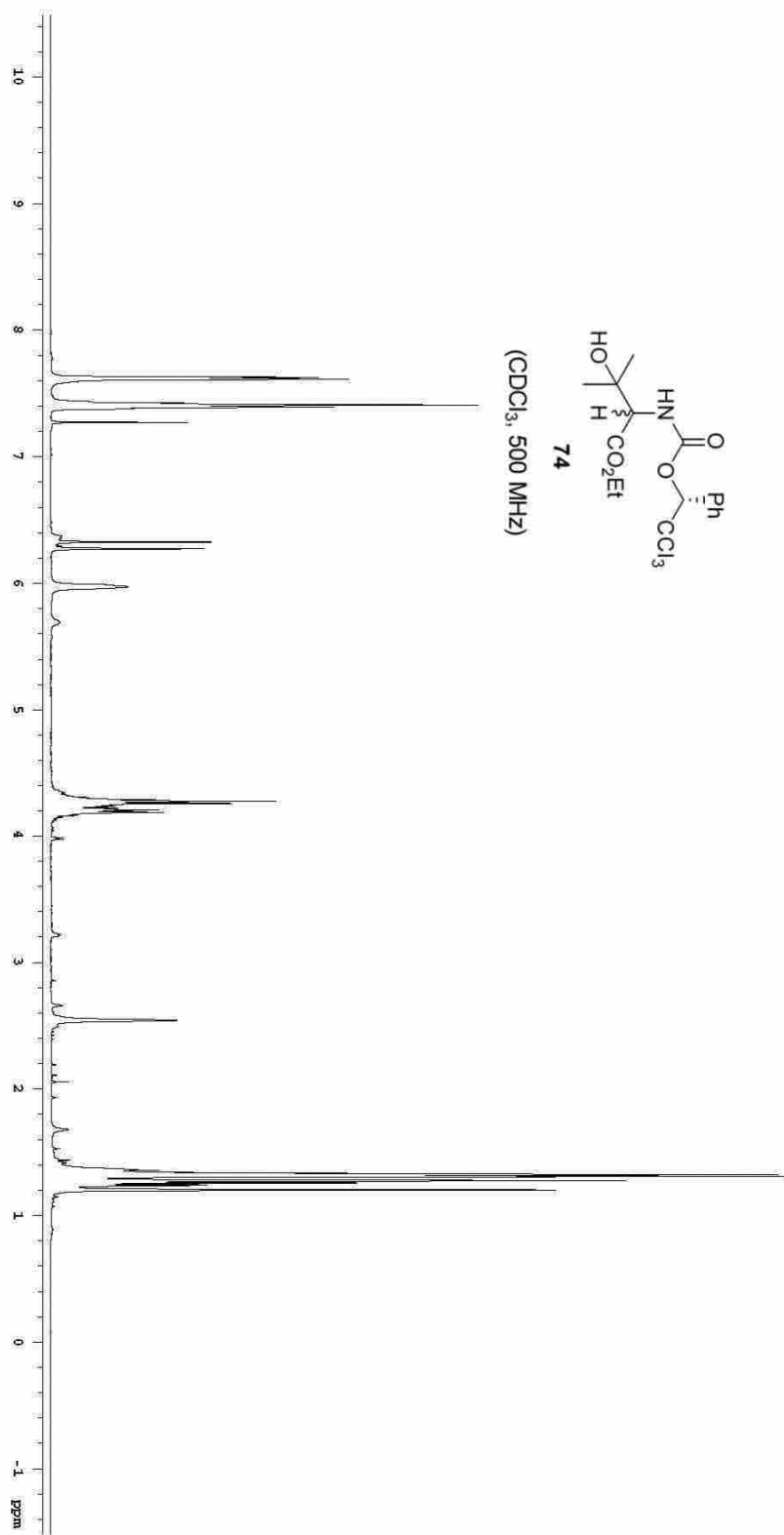
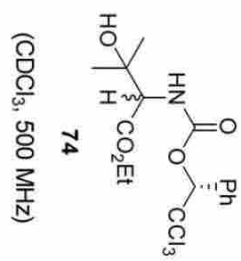
HMBC

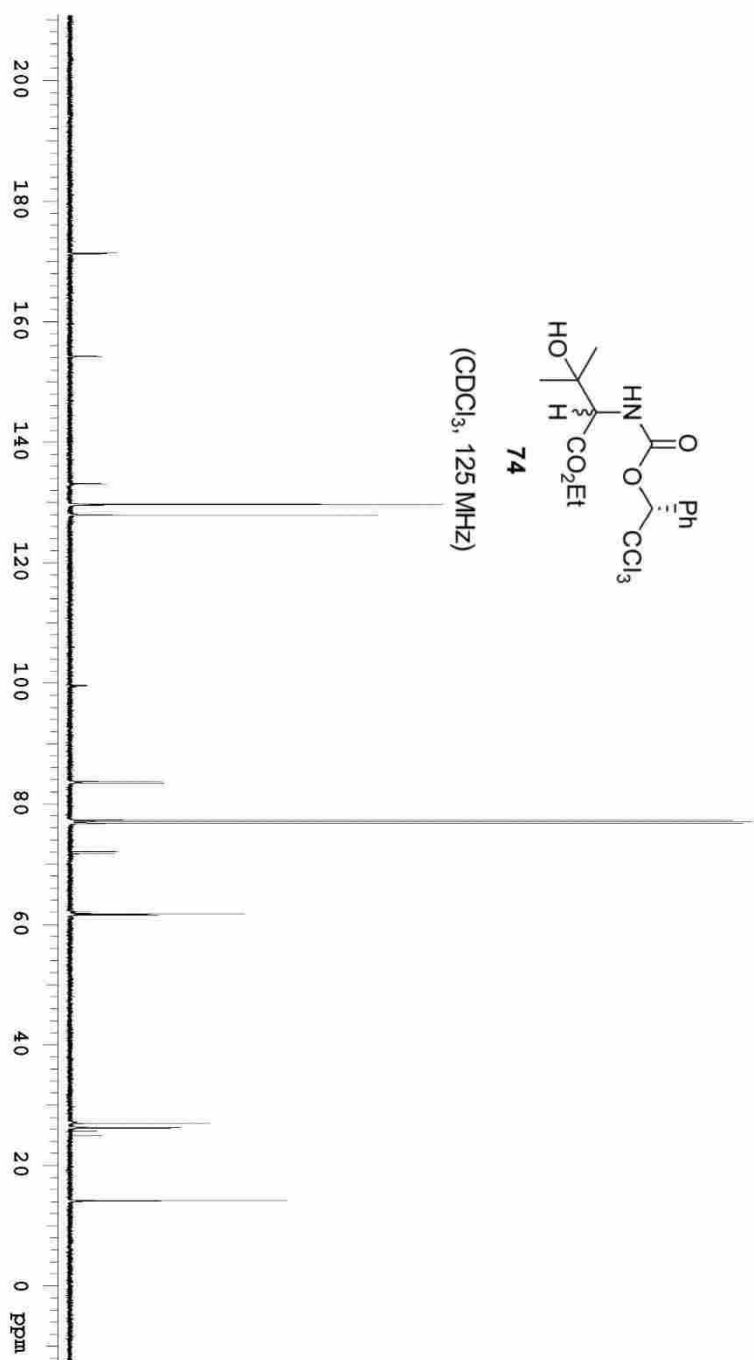


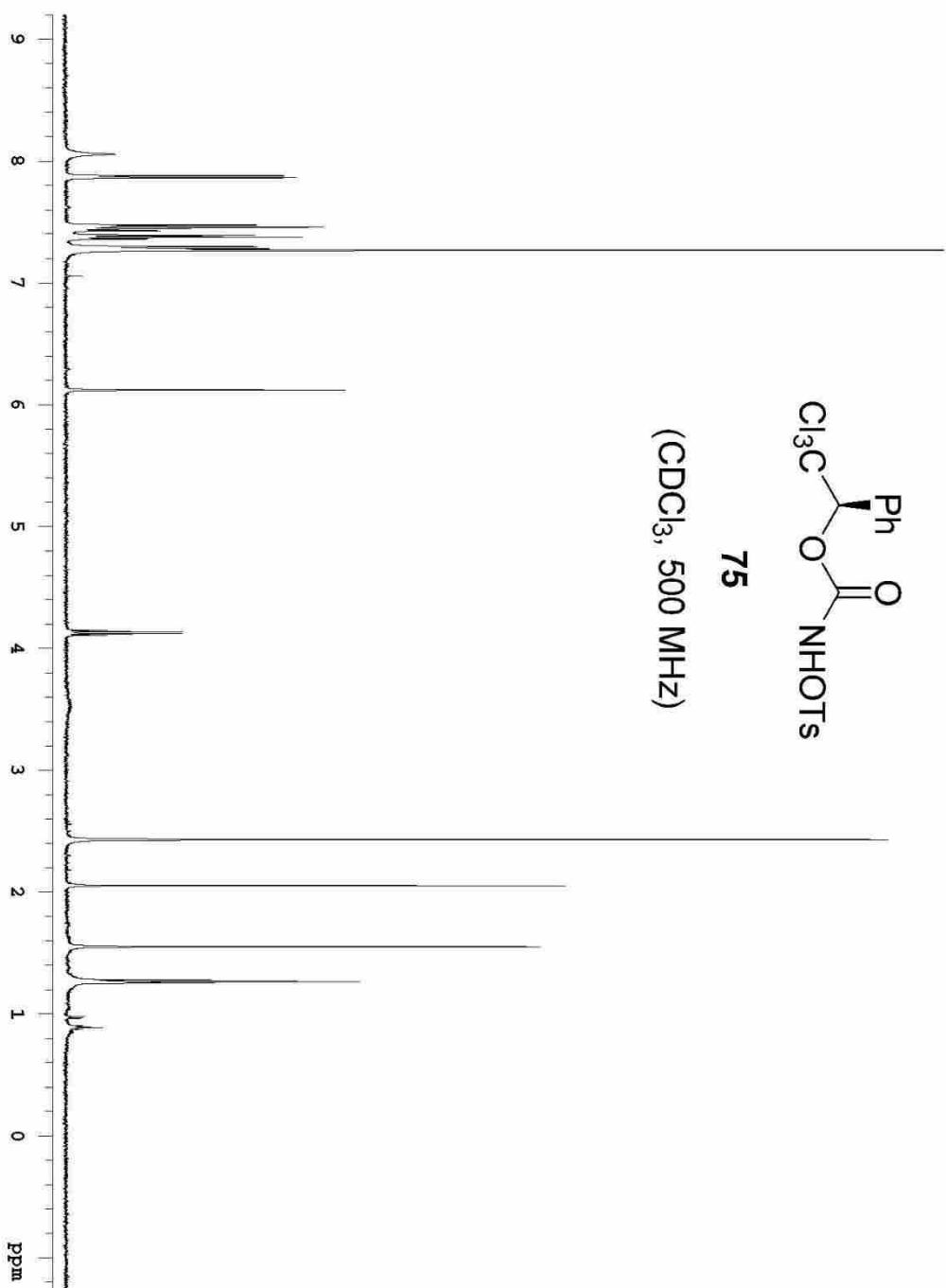
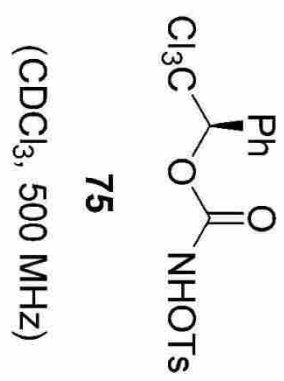


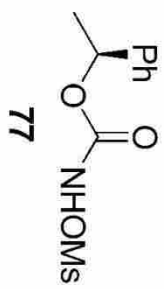




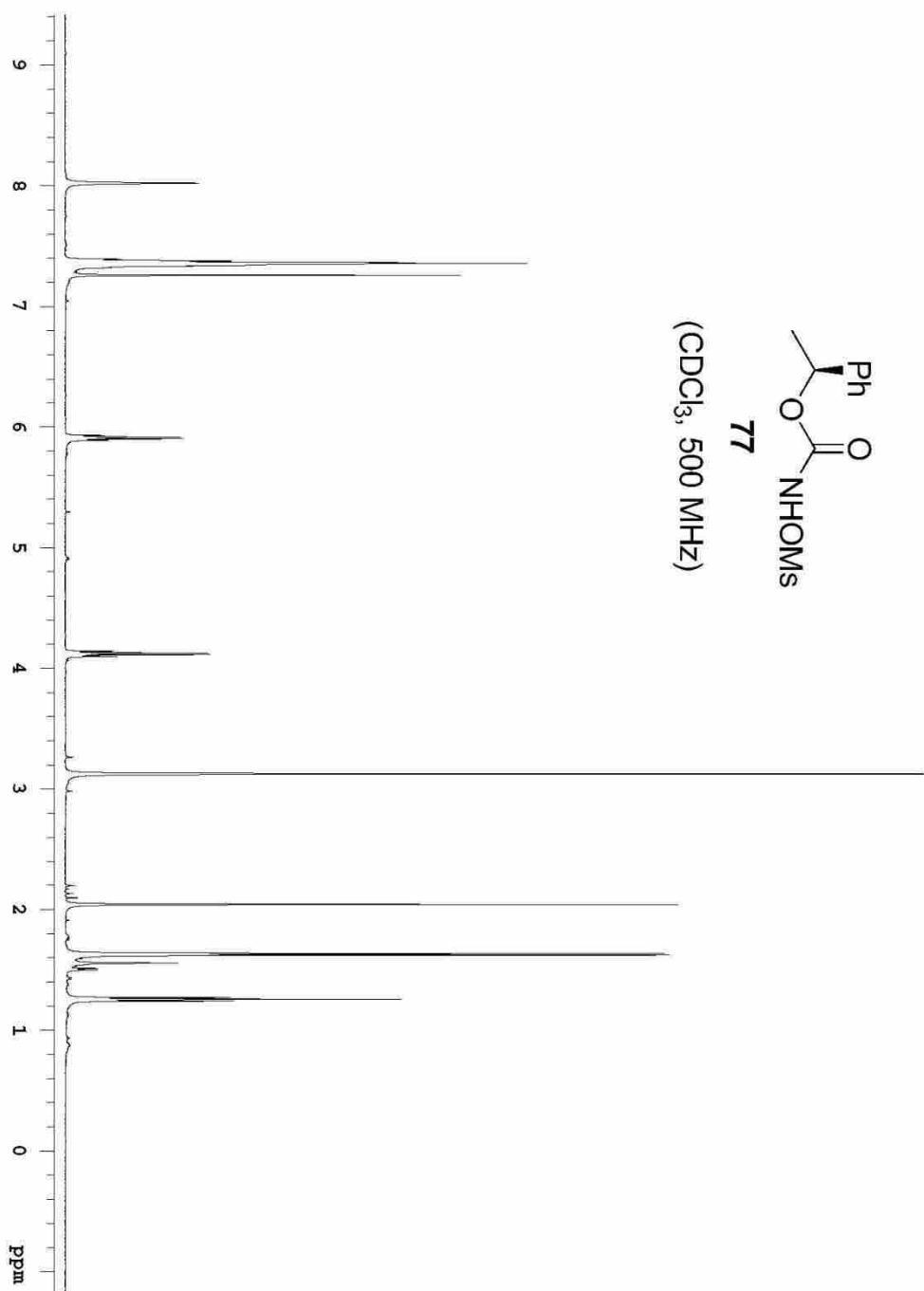


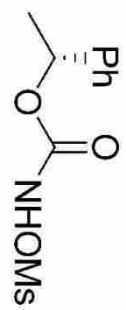






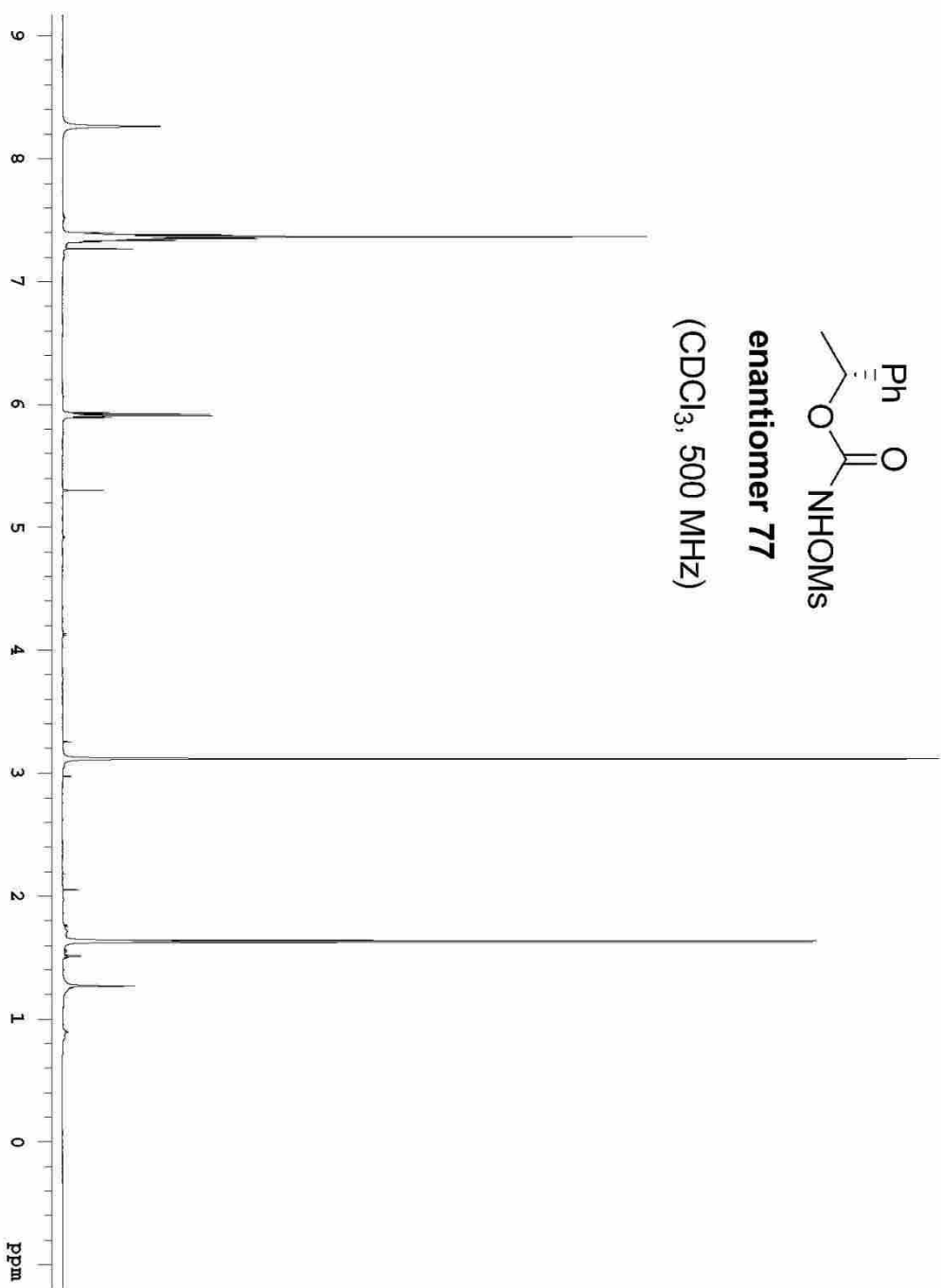
(CDCl₃, 500 MHz)

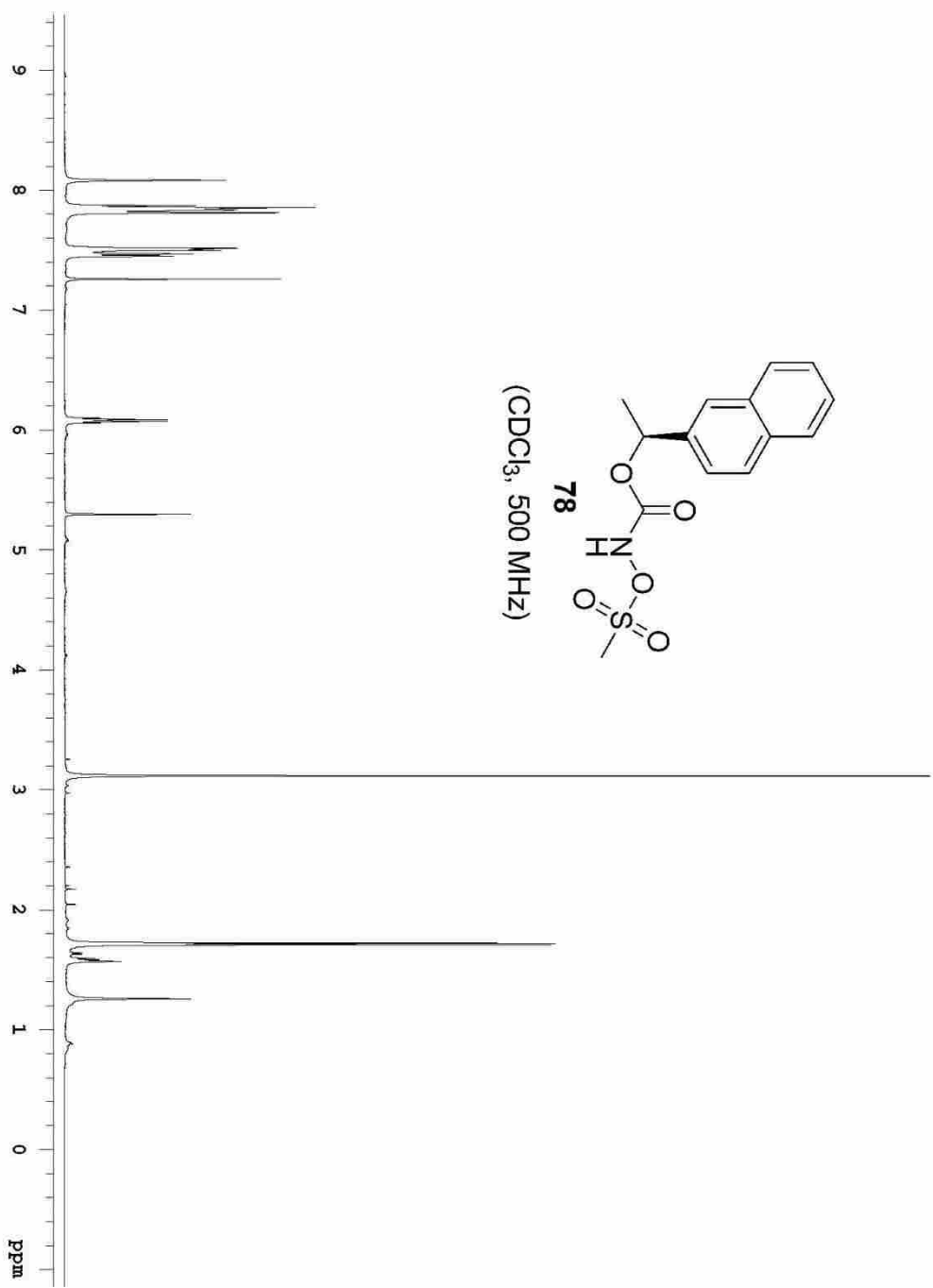
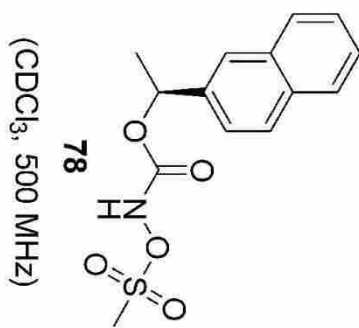


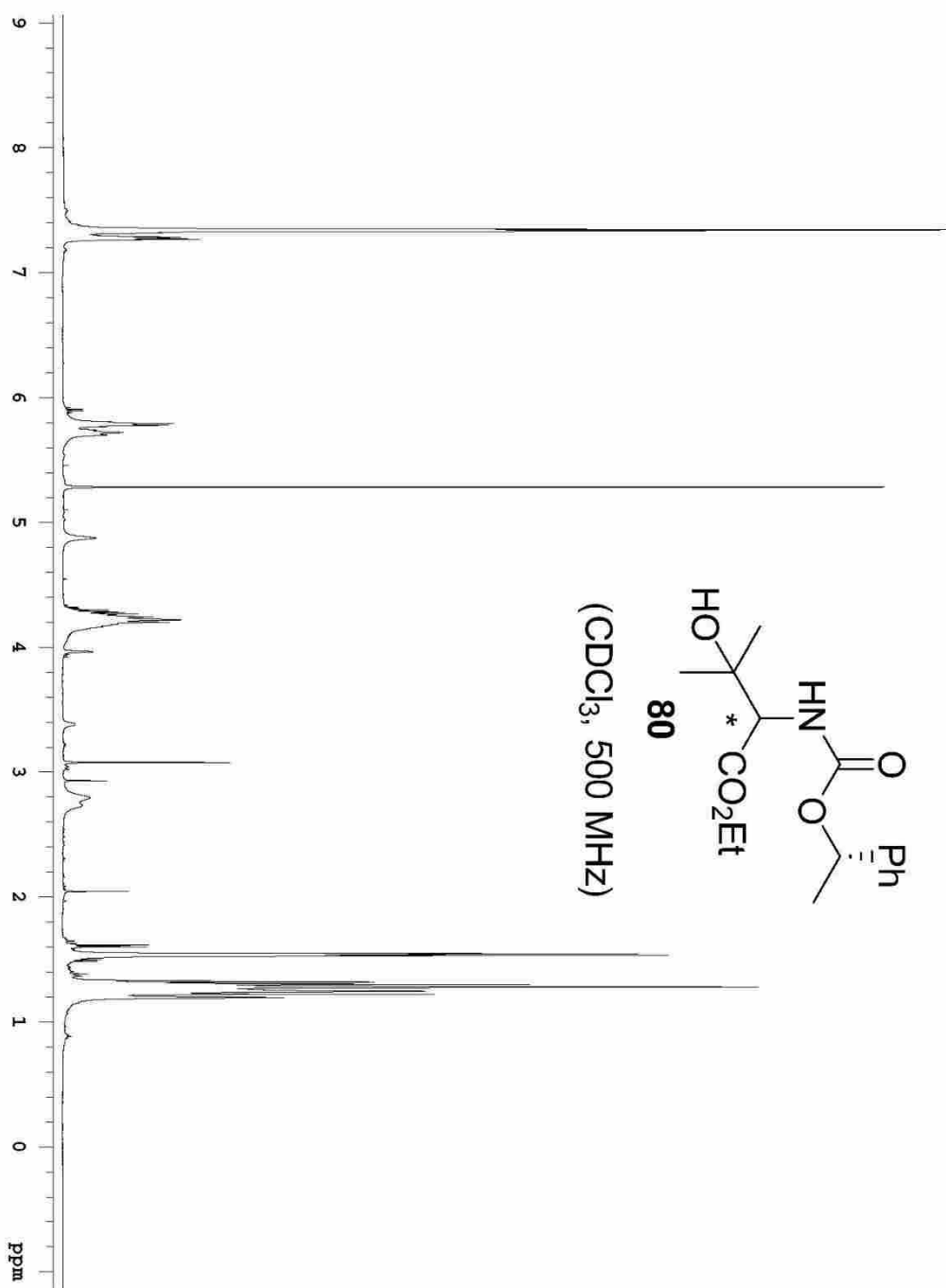
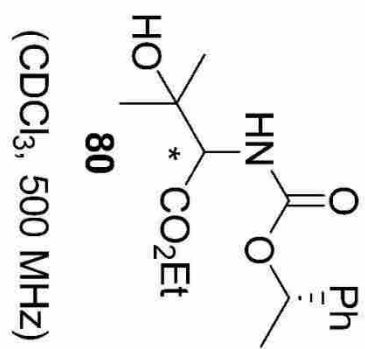


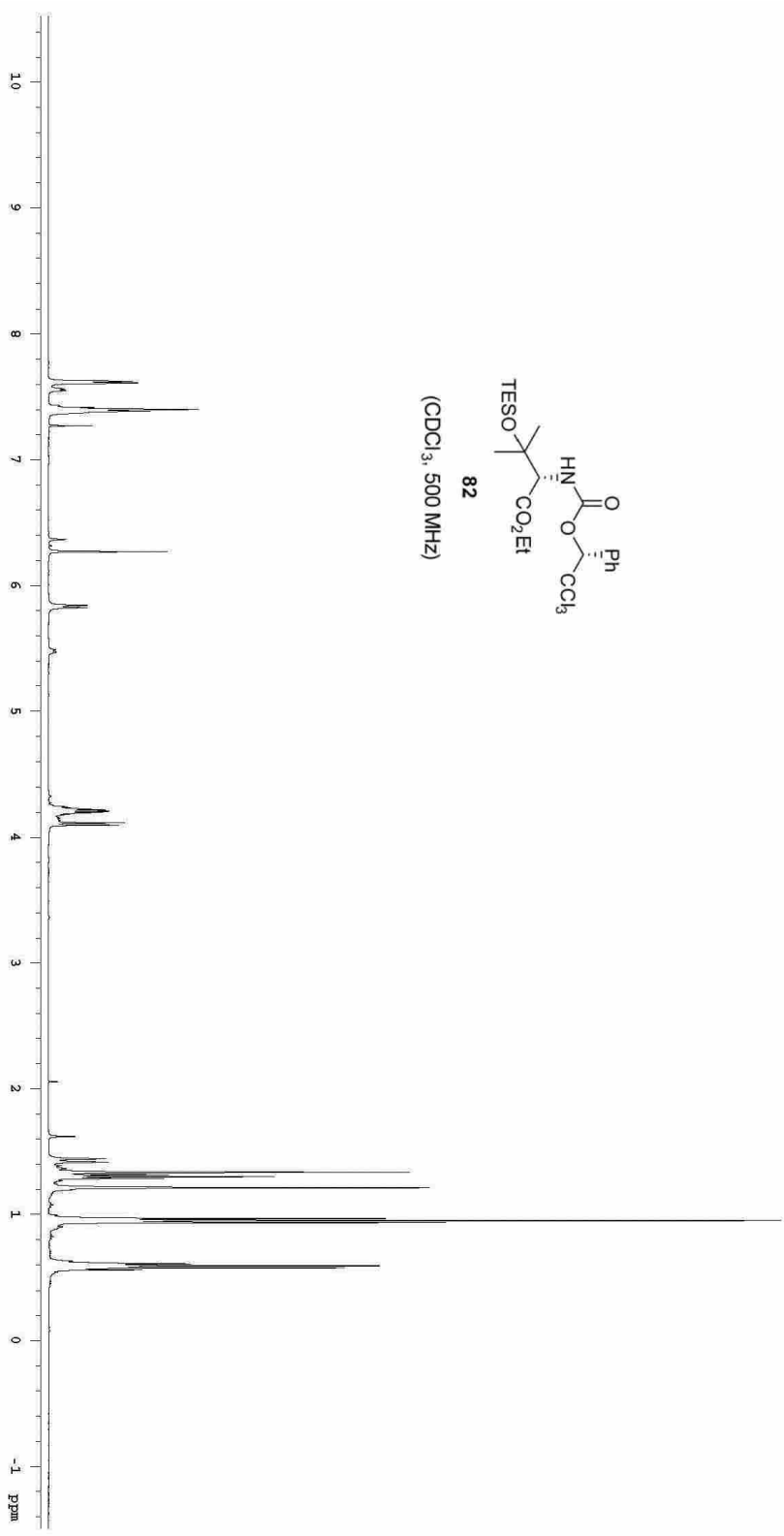
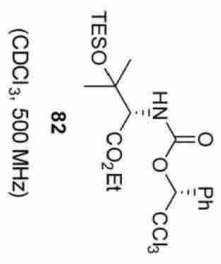
enantiomer 77

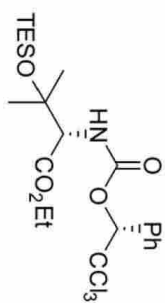
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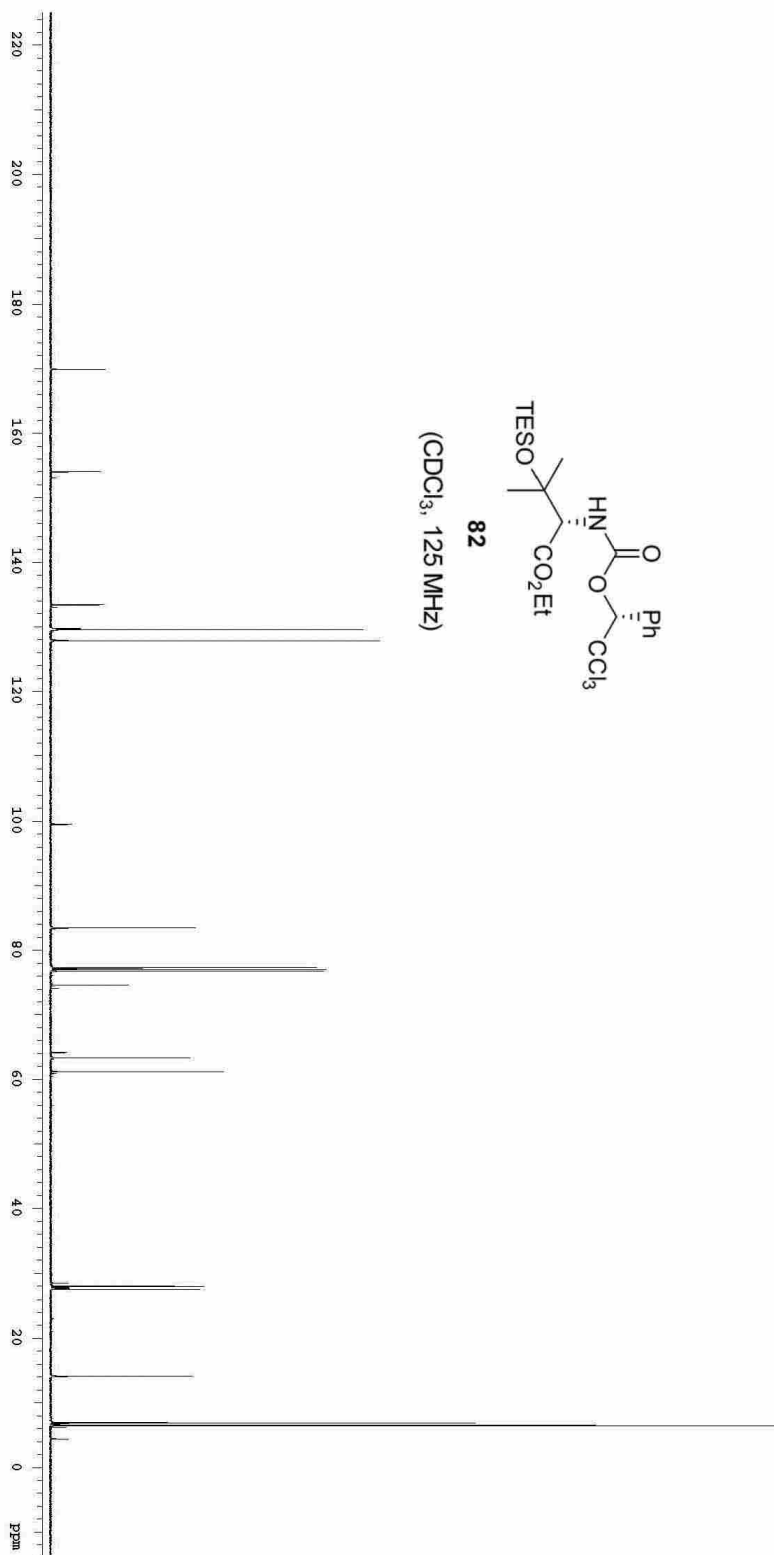


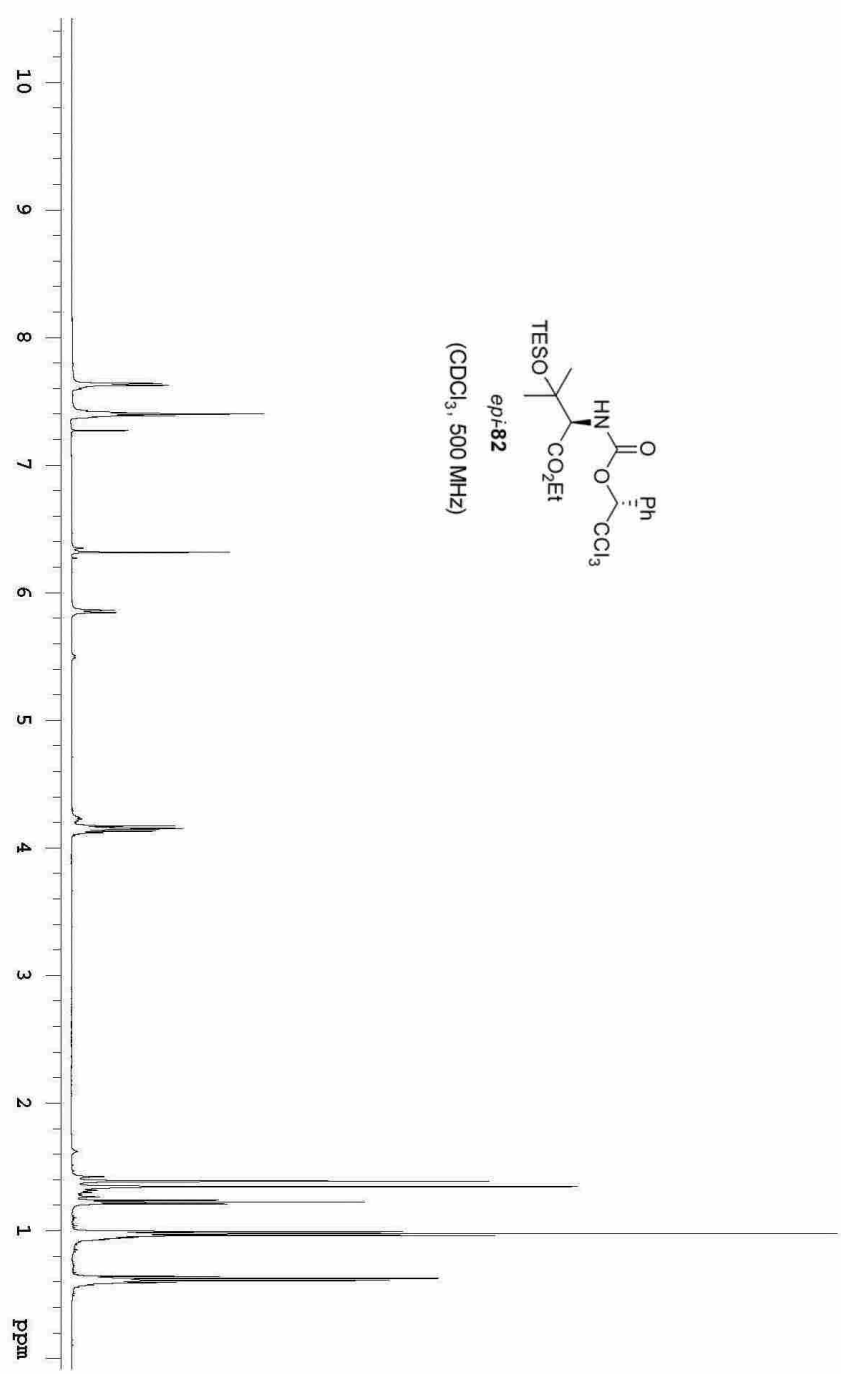
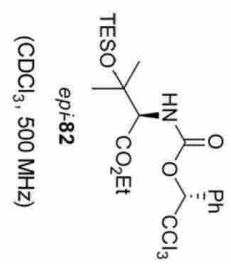


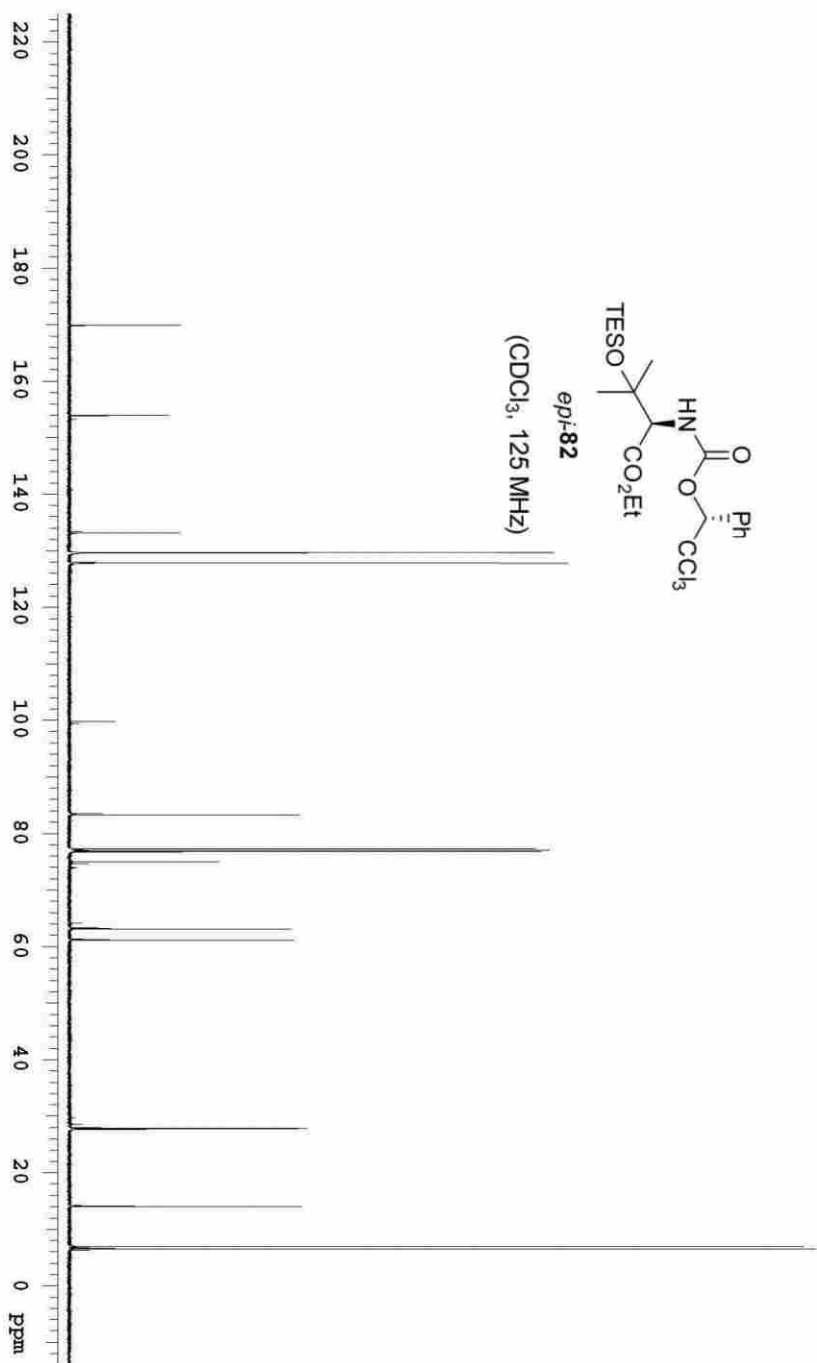


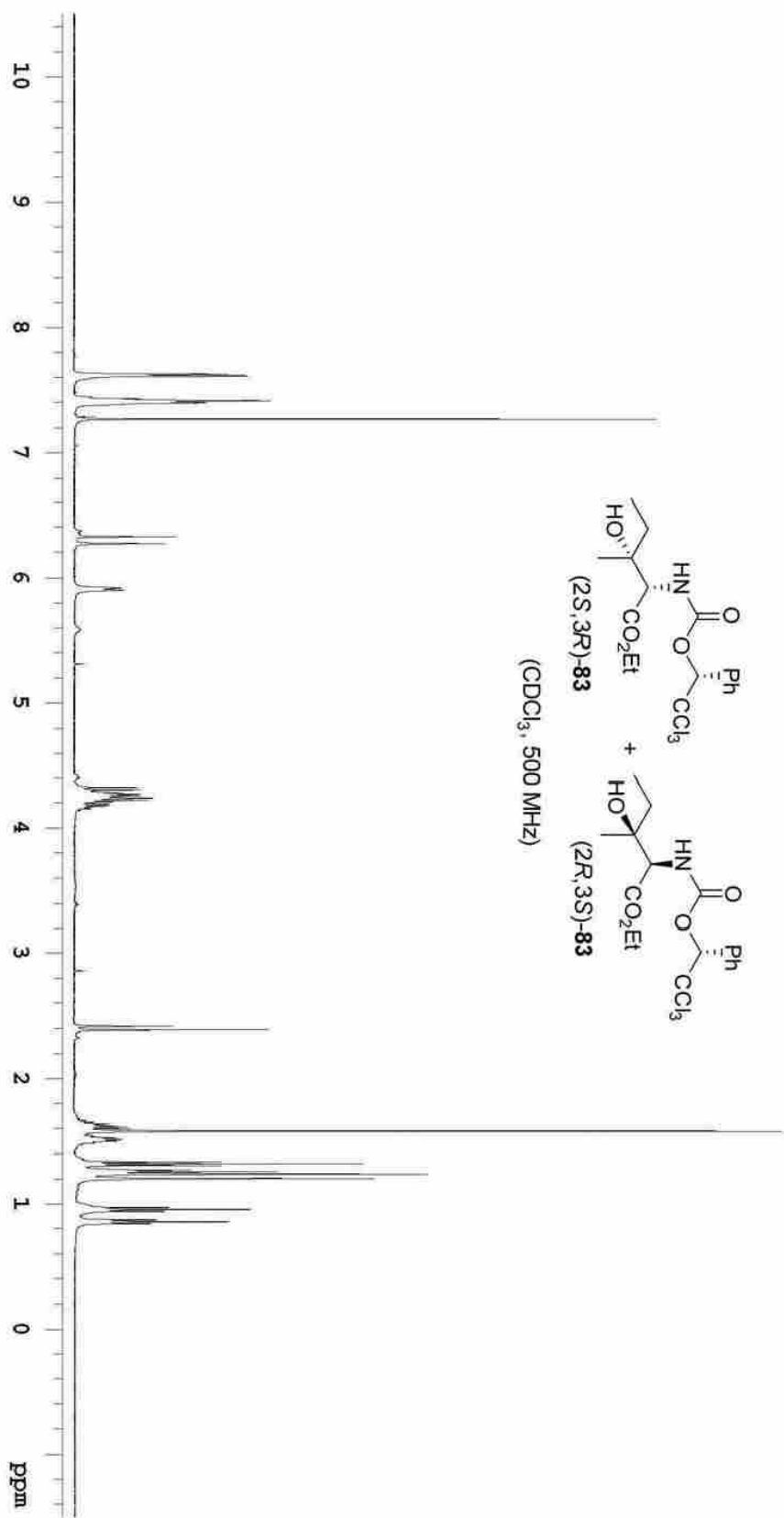


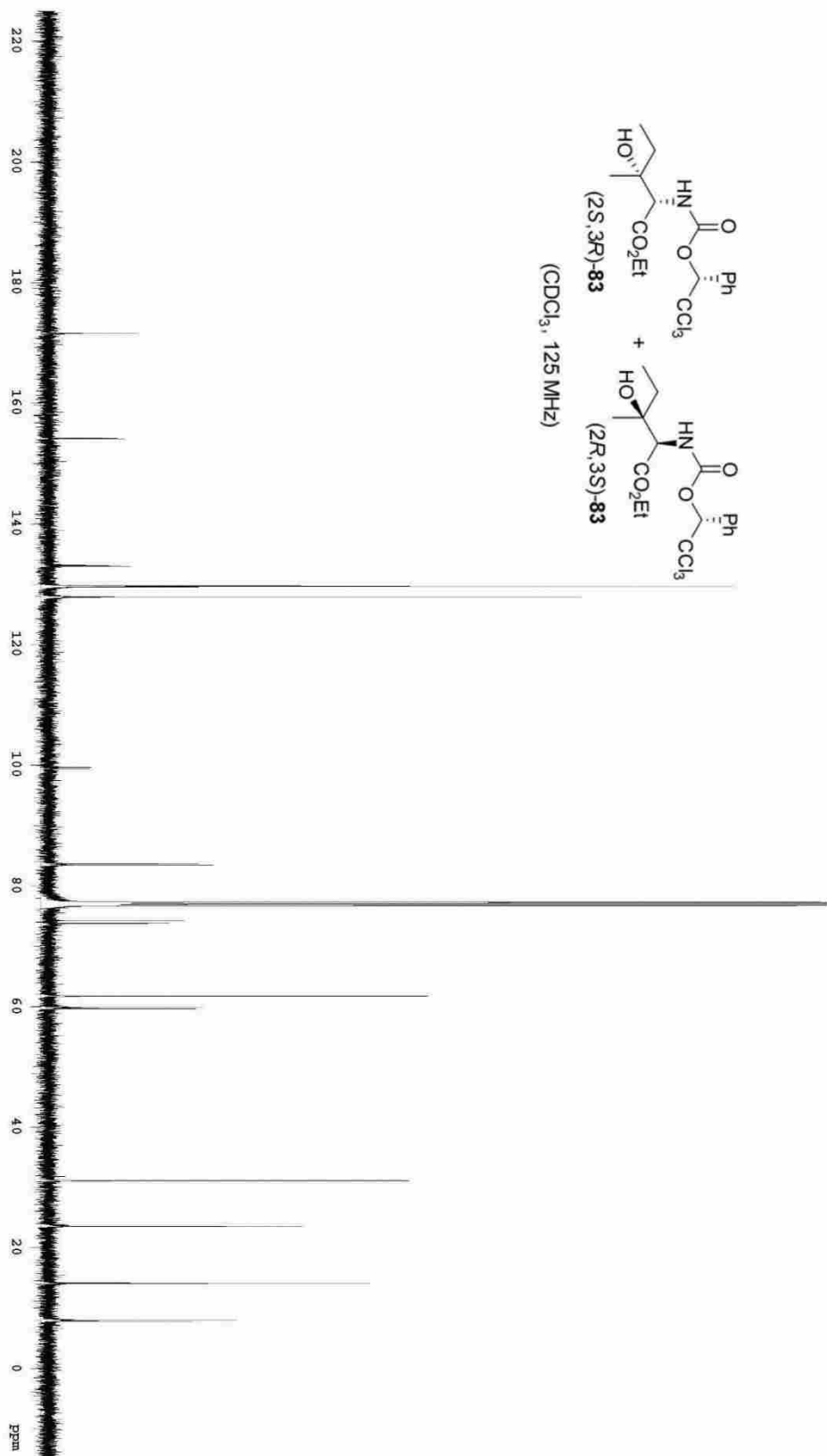
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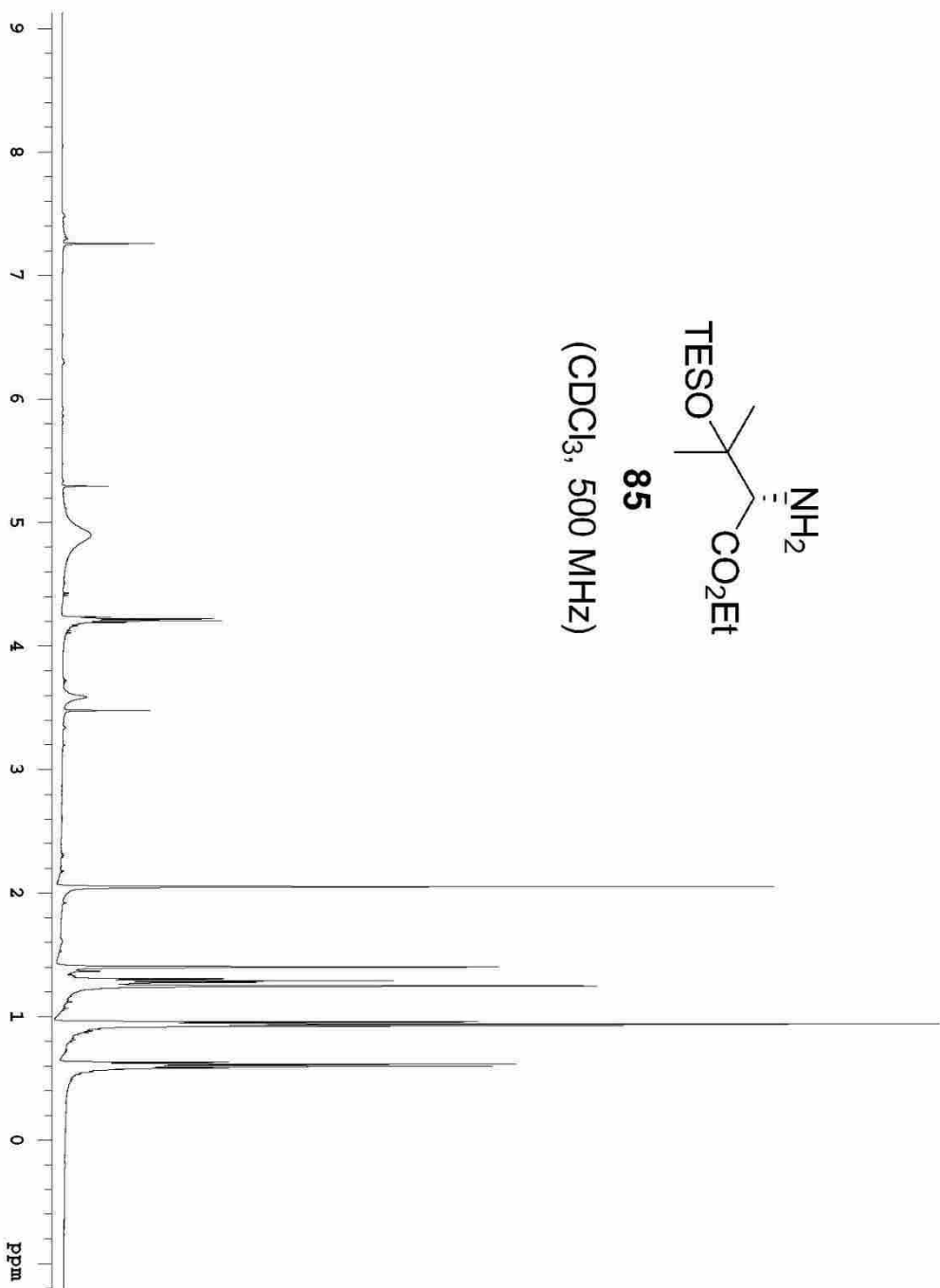
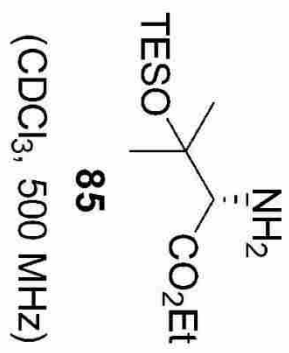


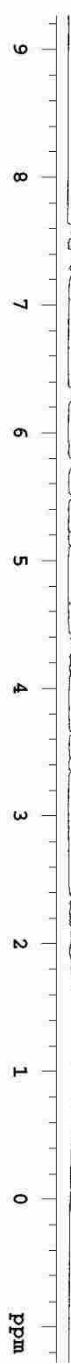
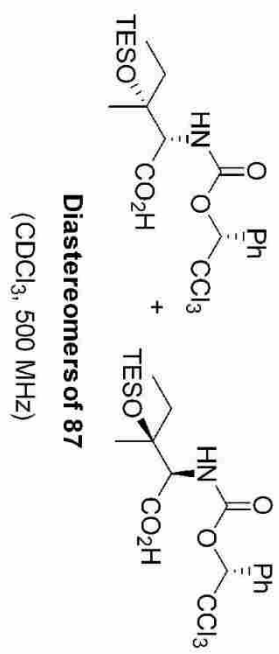


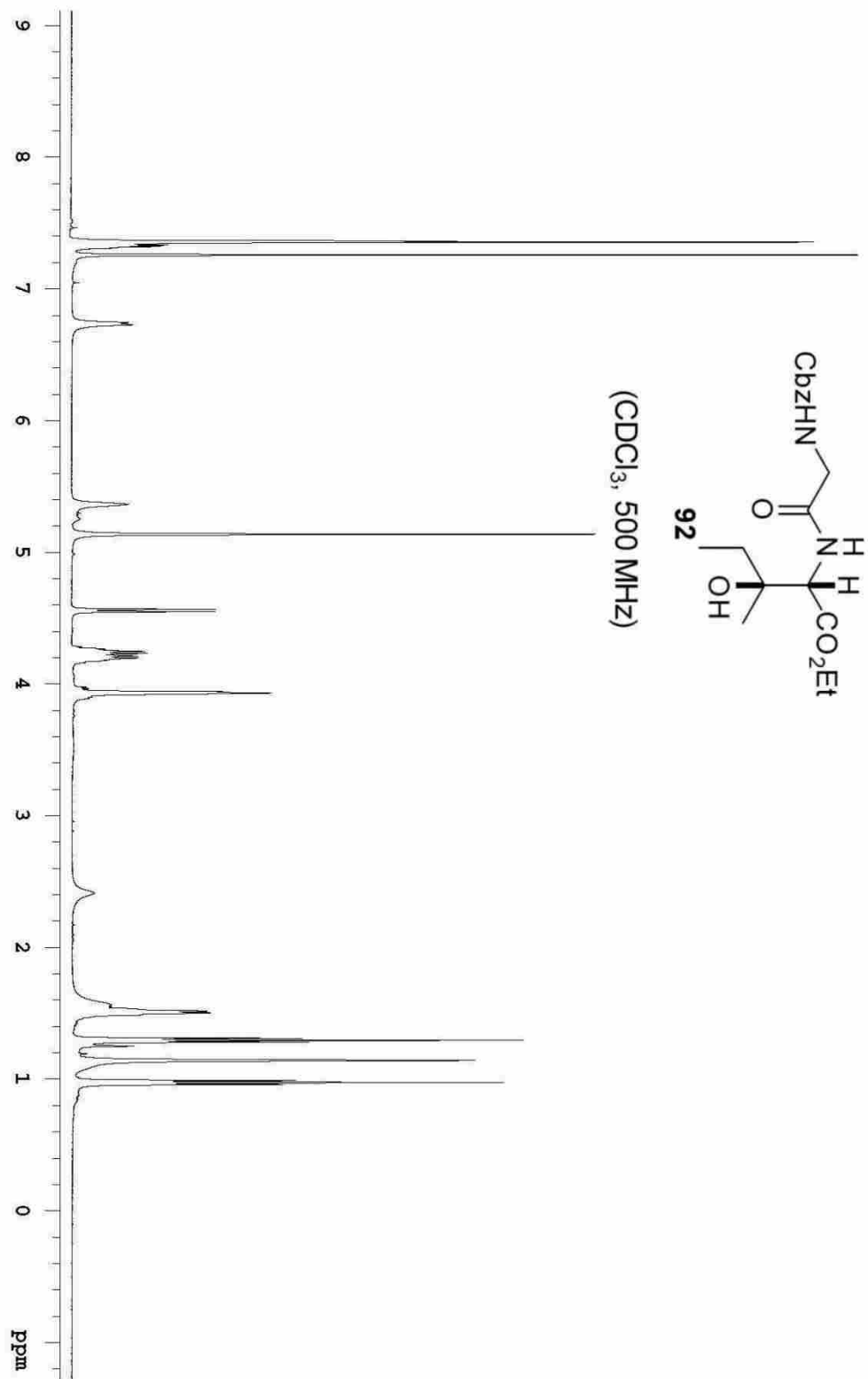


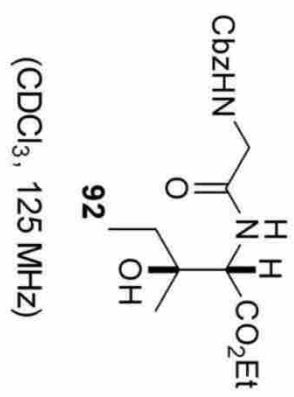




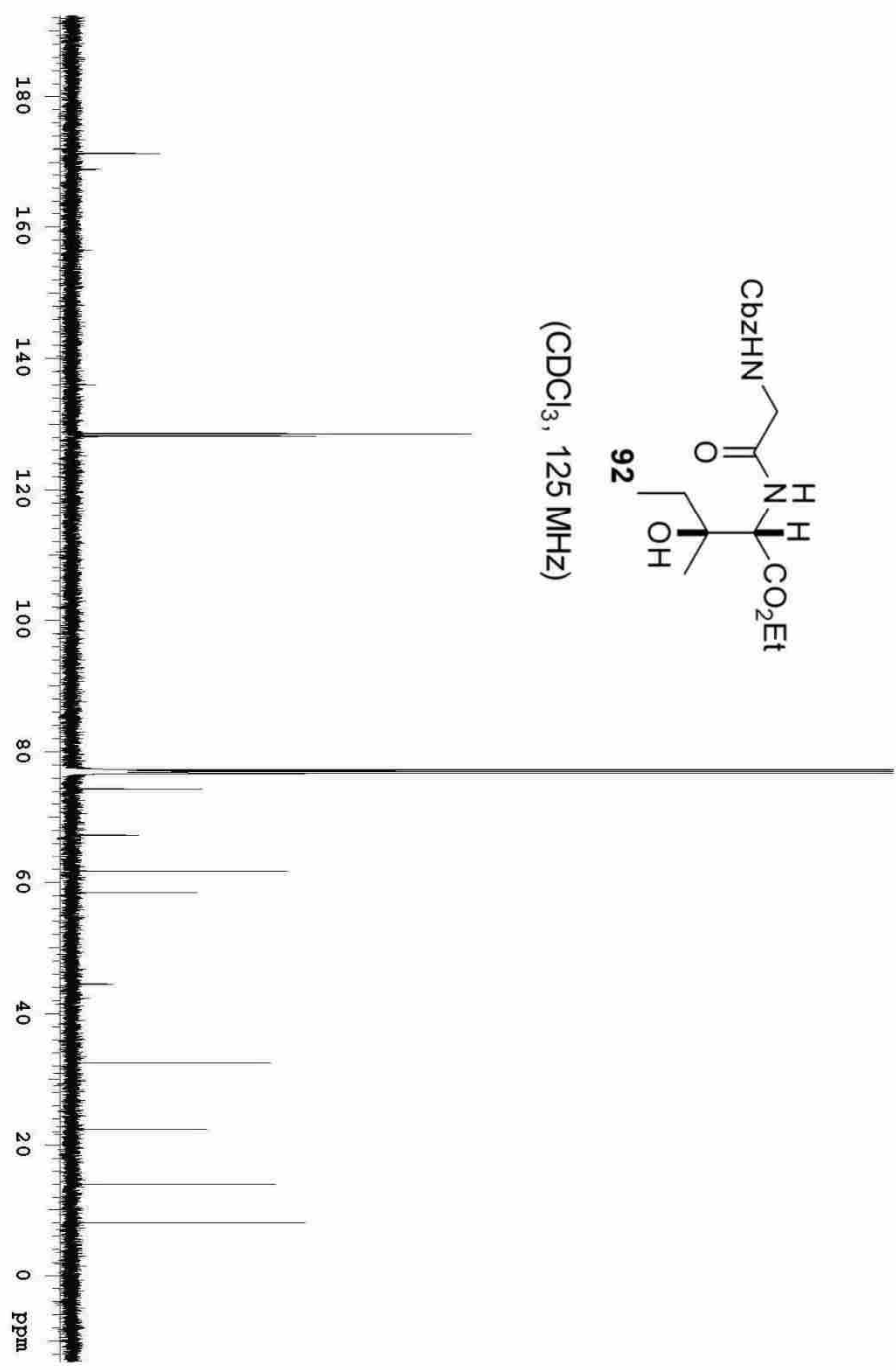


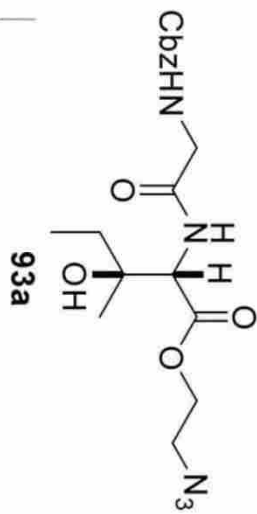






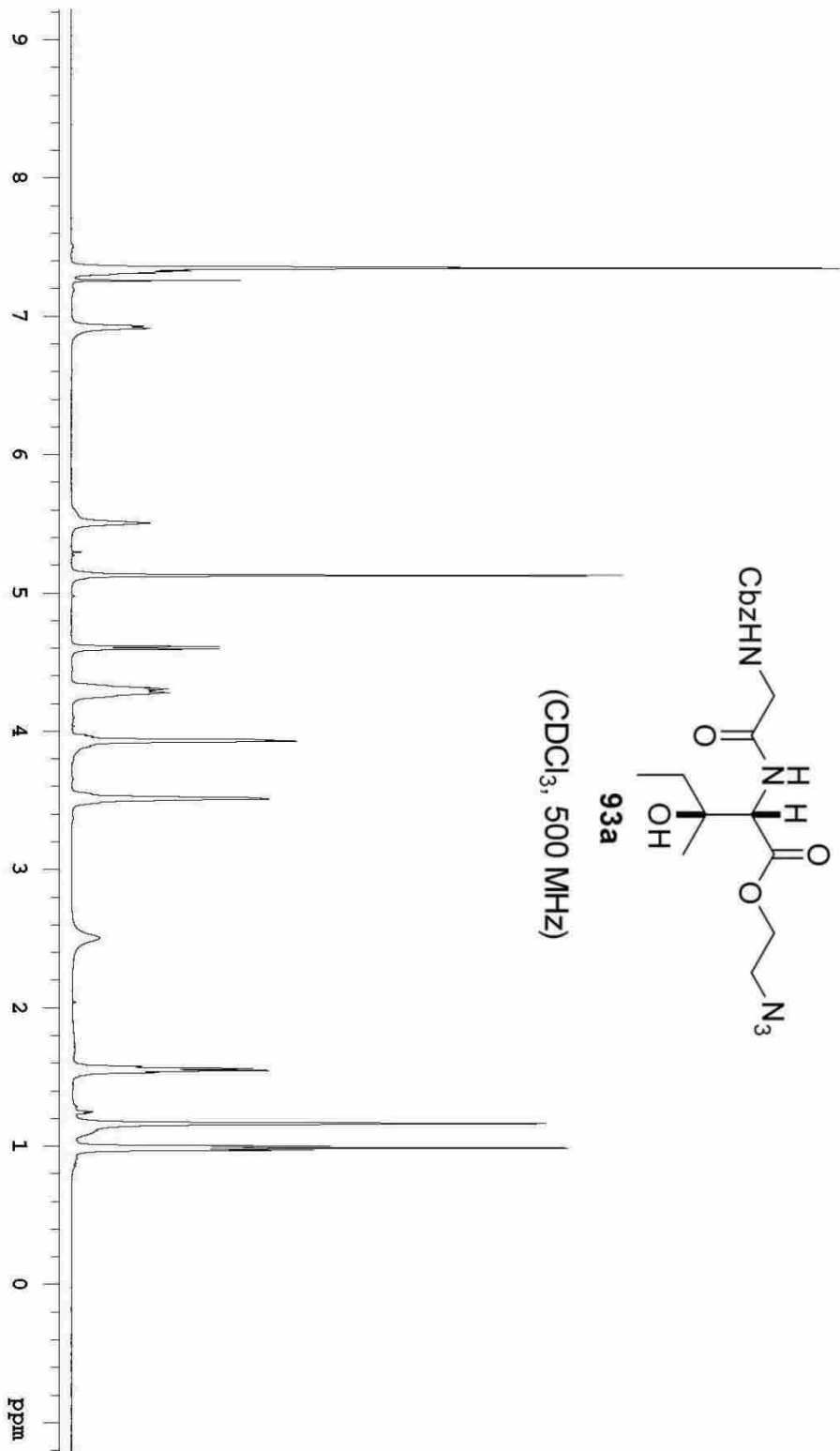
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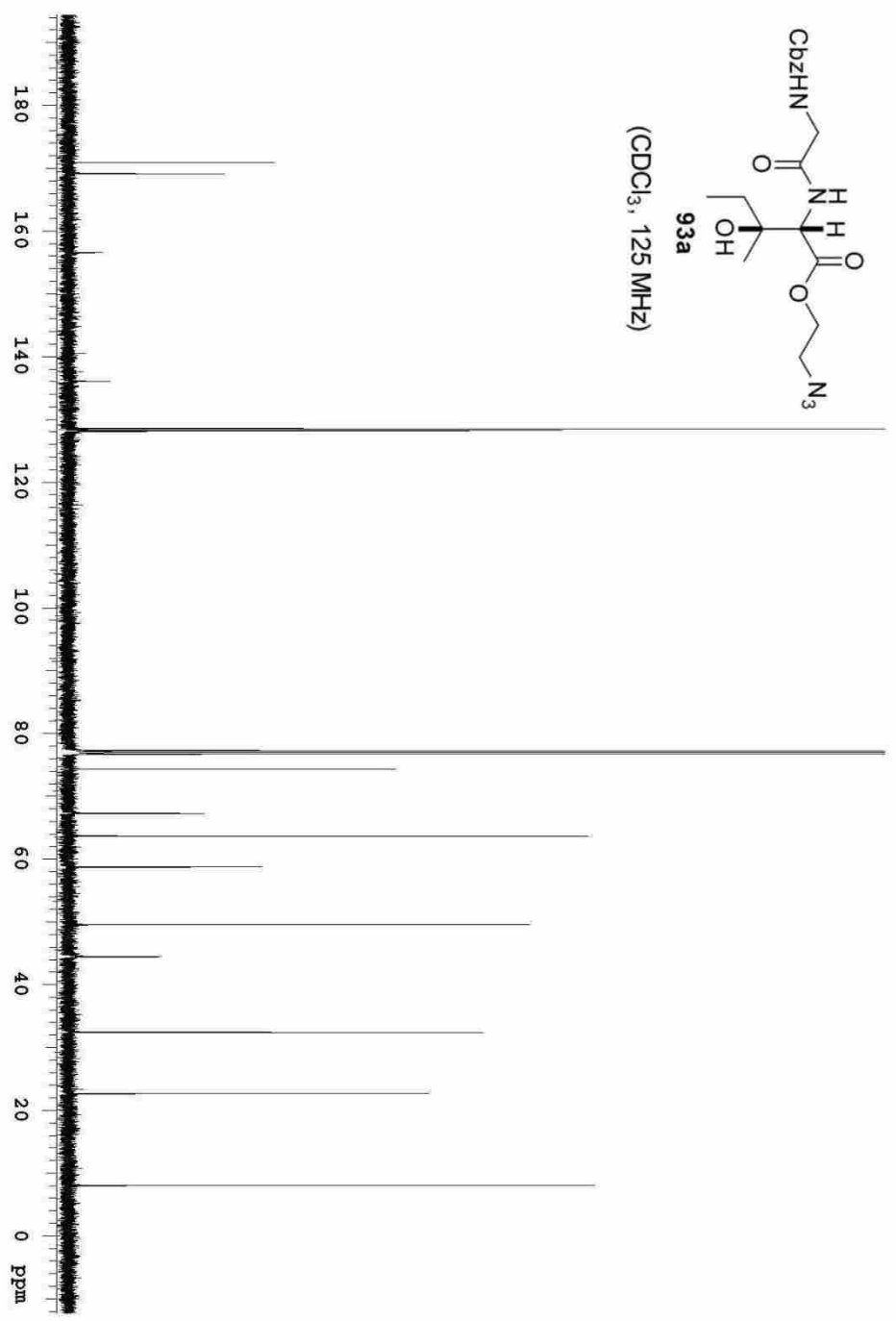
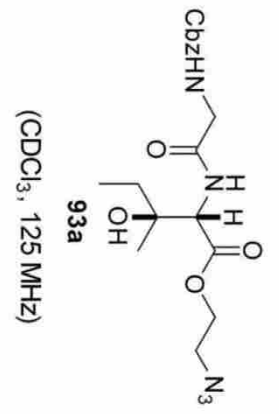


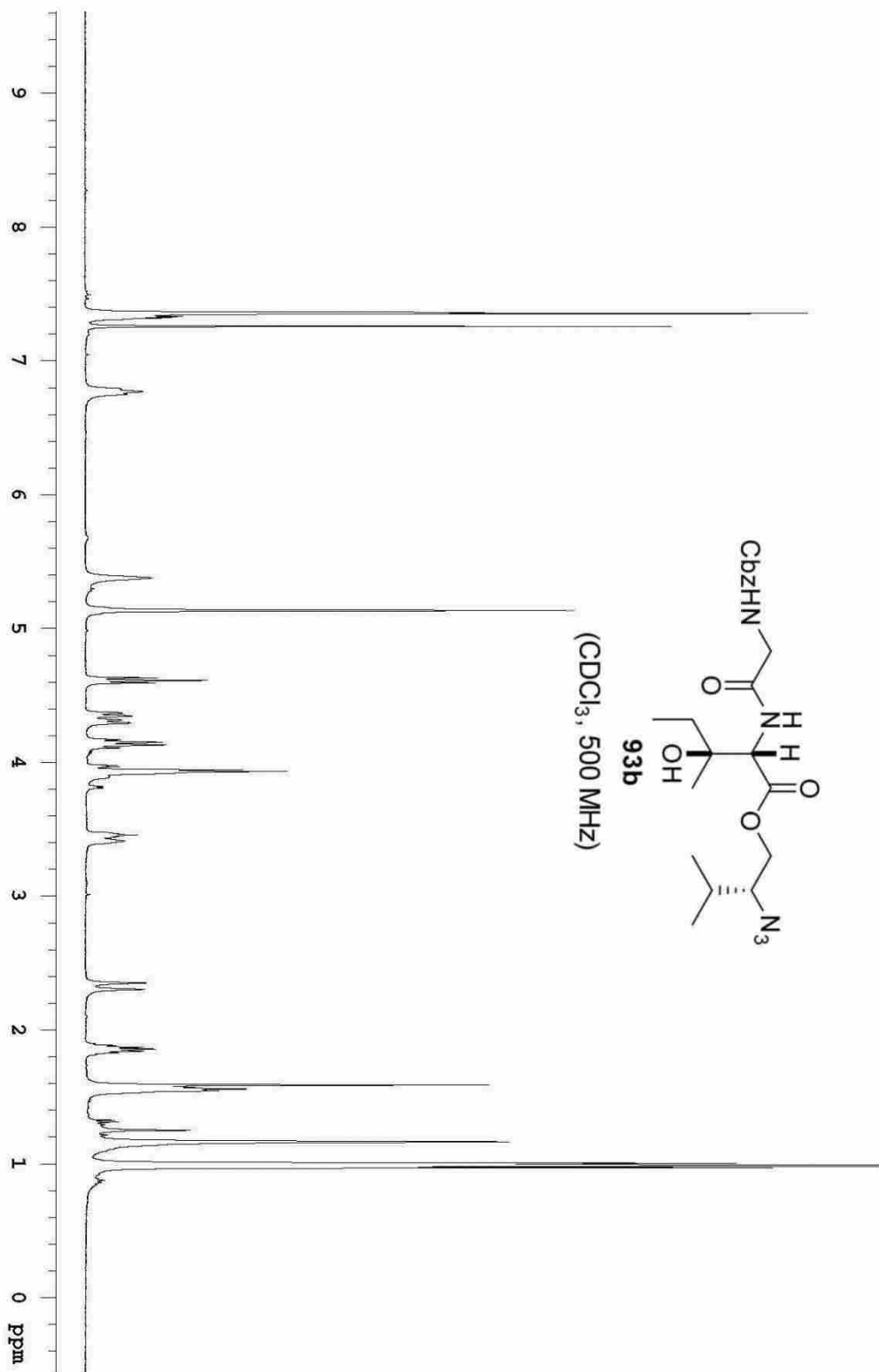


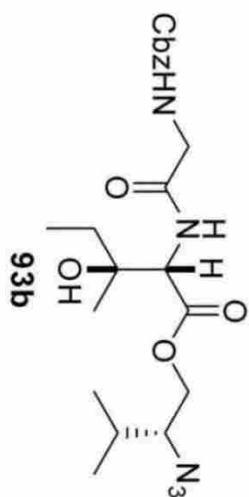
93a

(CDCl₃, 500 MHz)

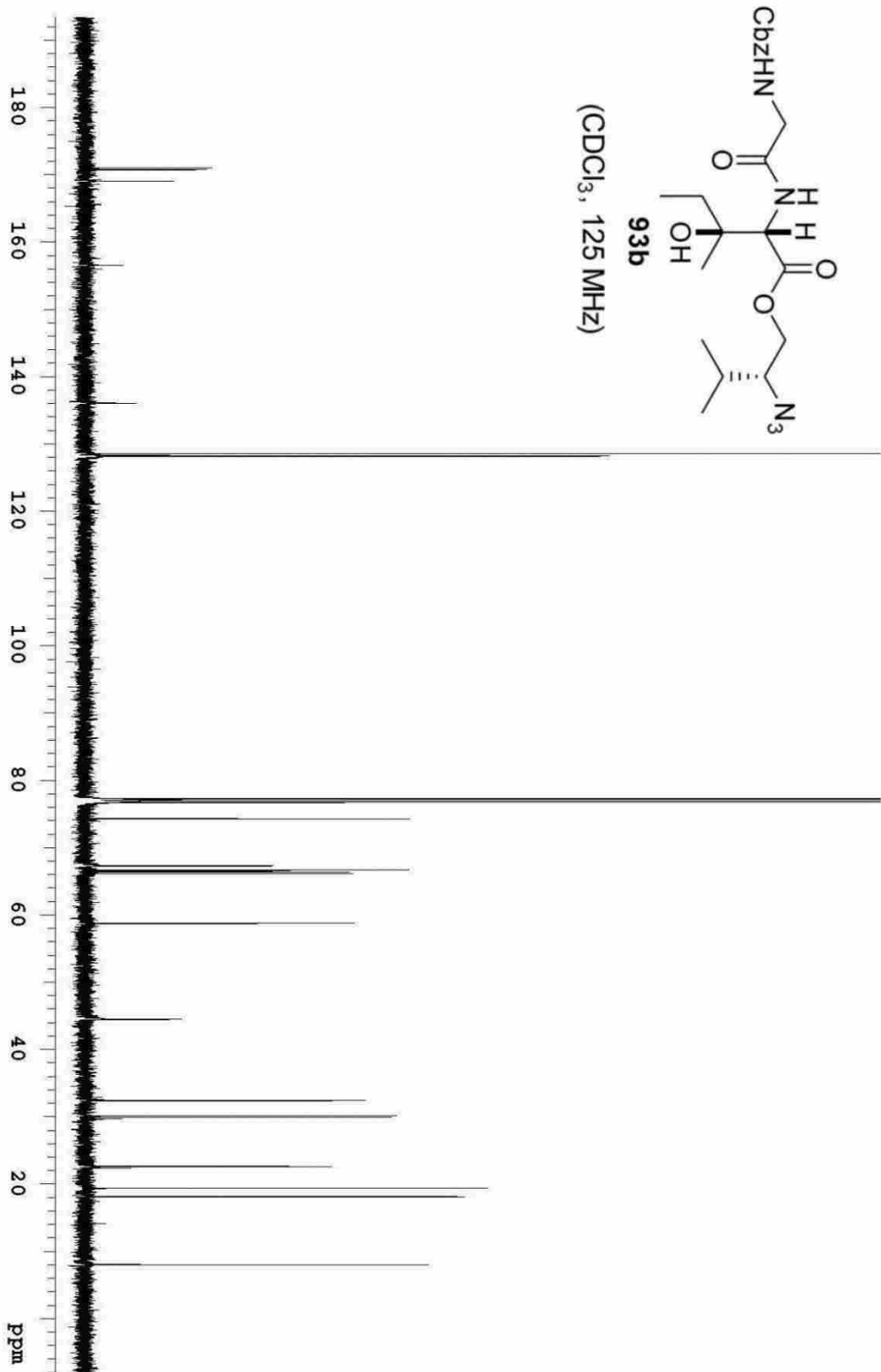


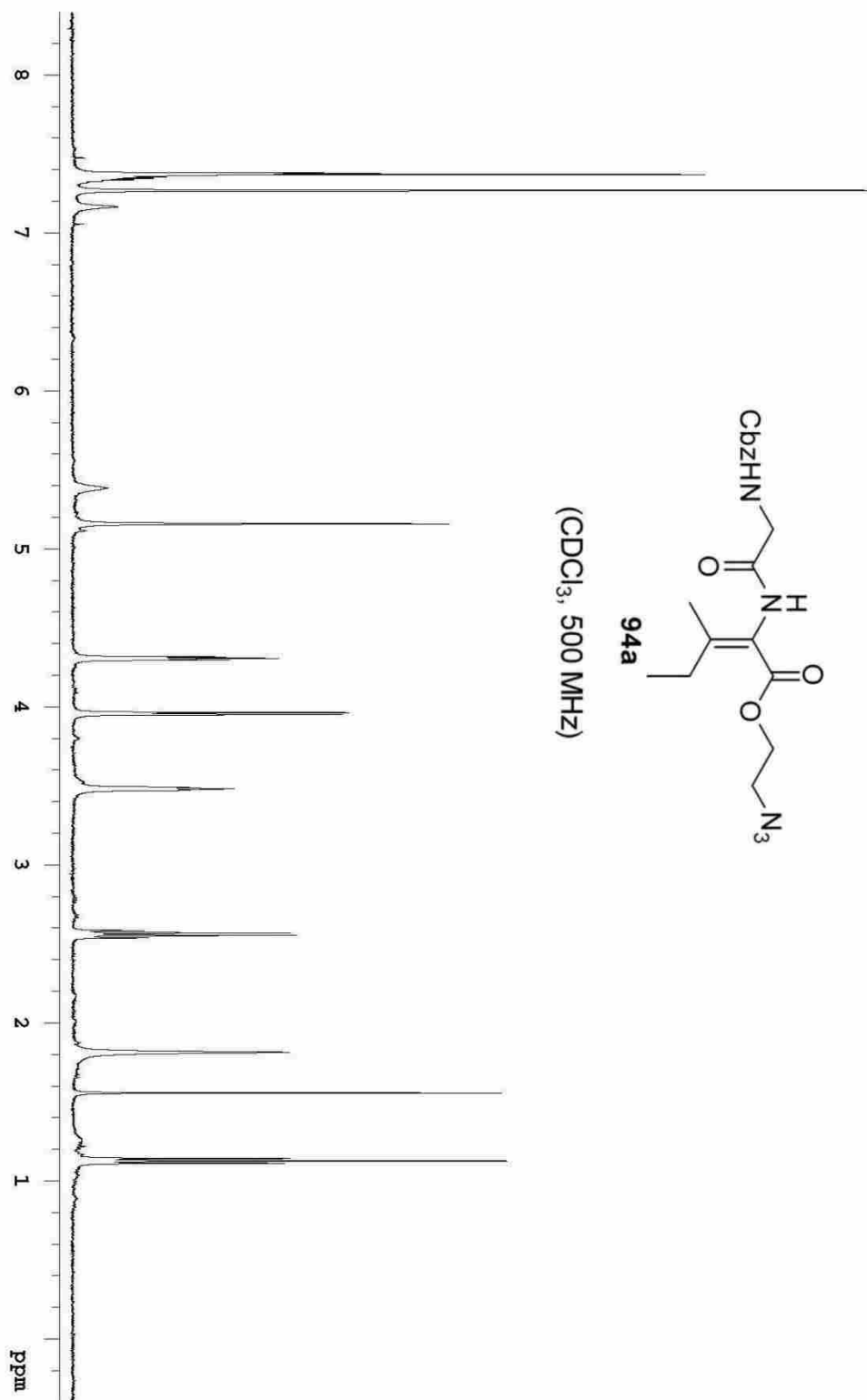


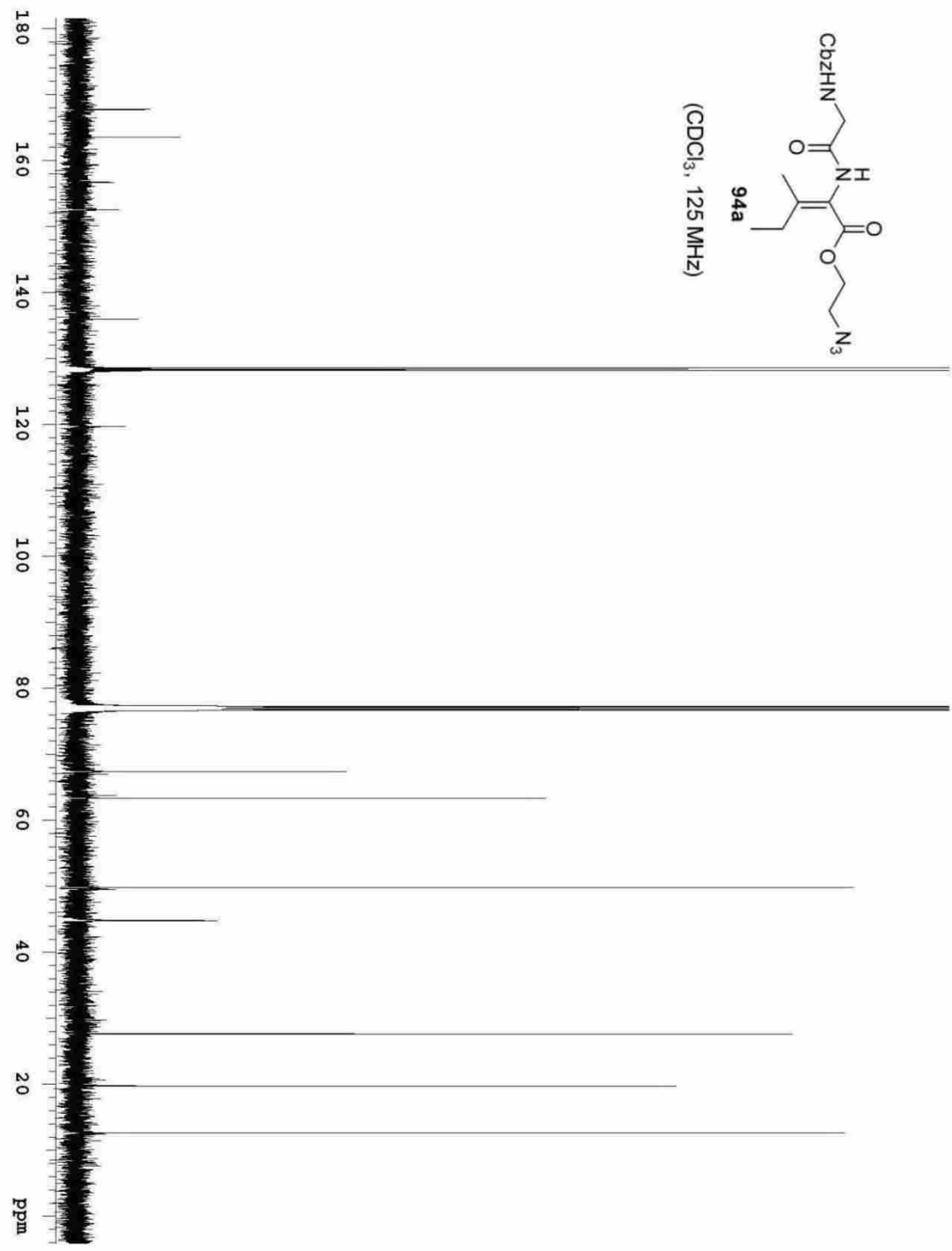
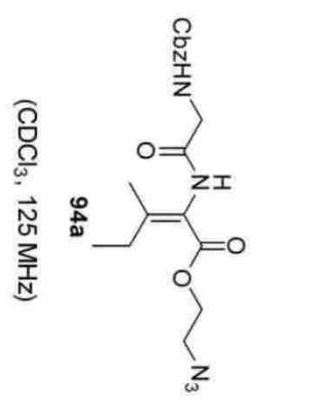


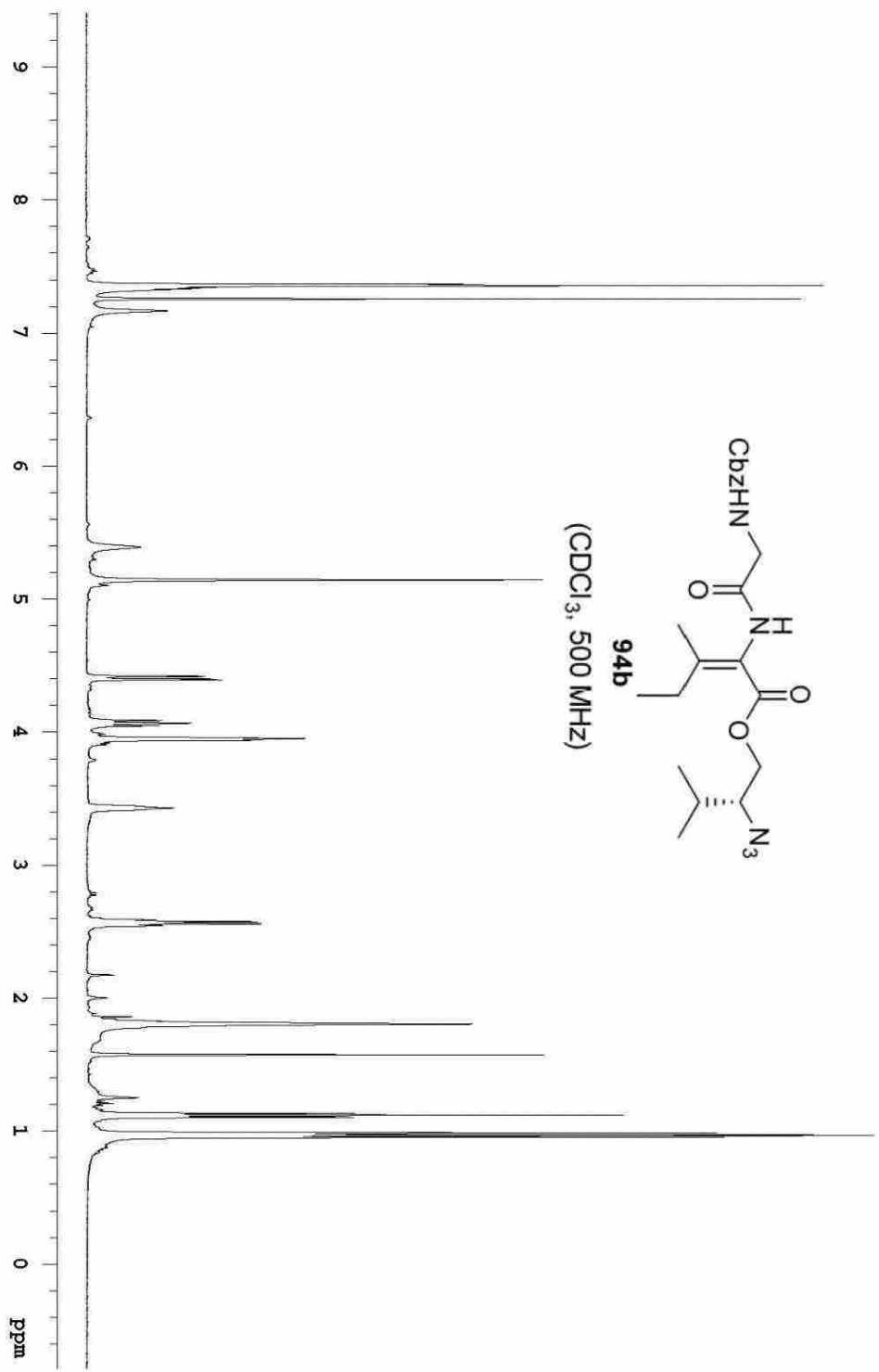


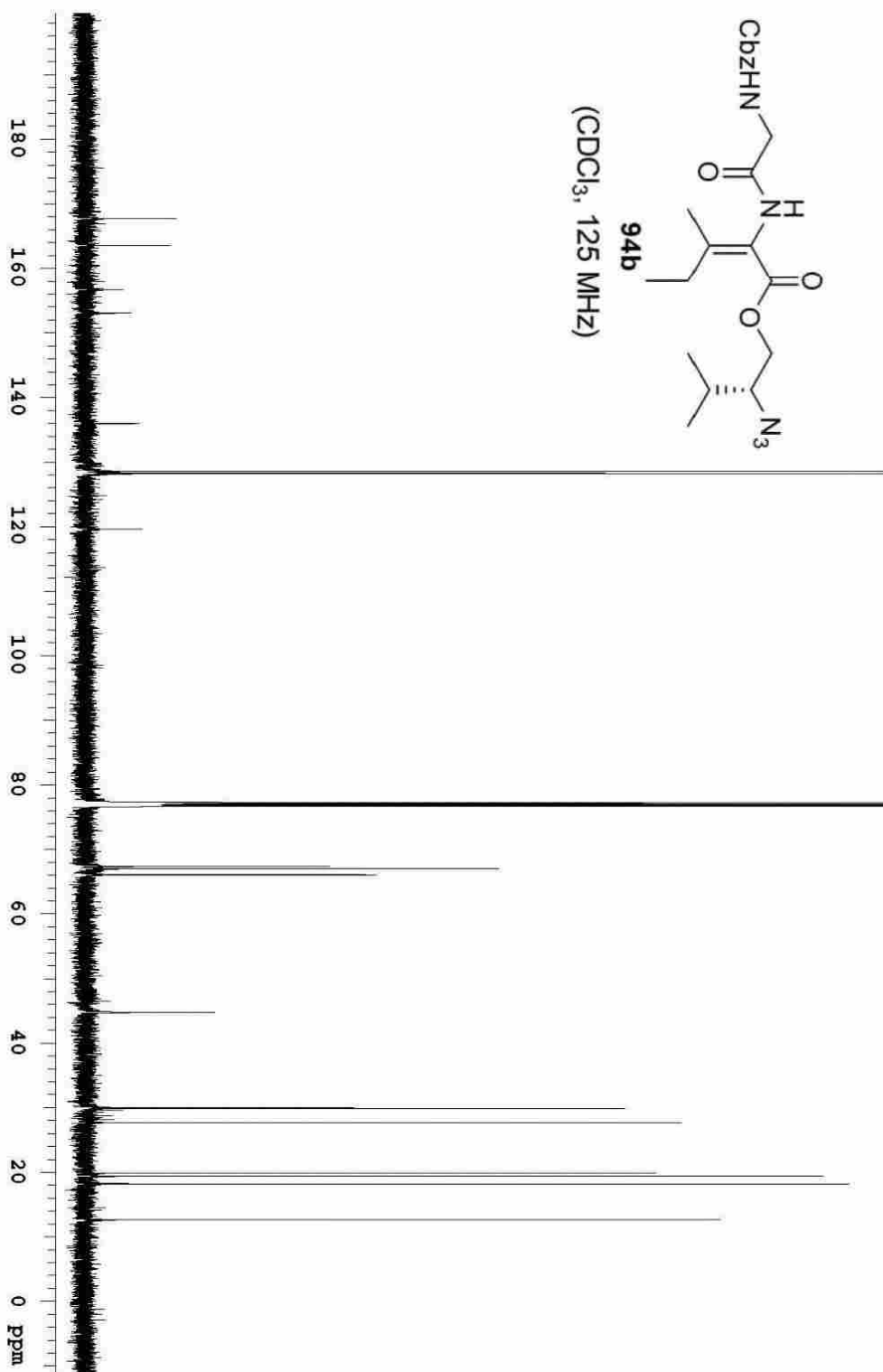
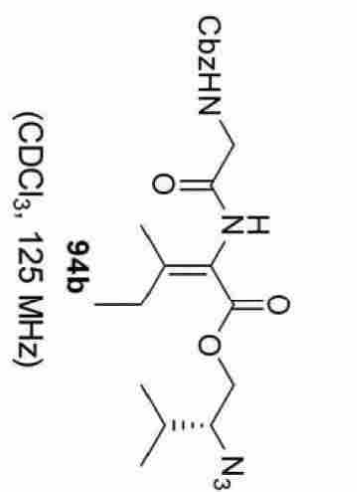
(CDCl₃, 125 MHz)

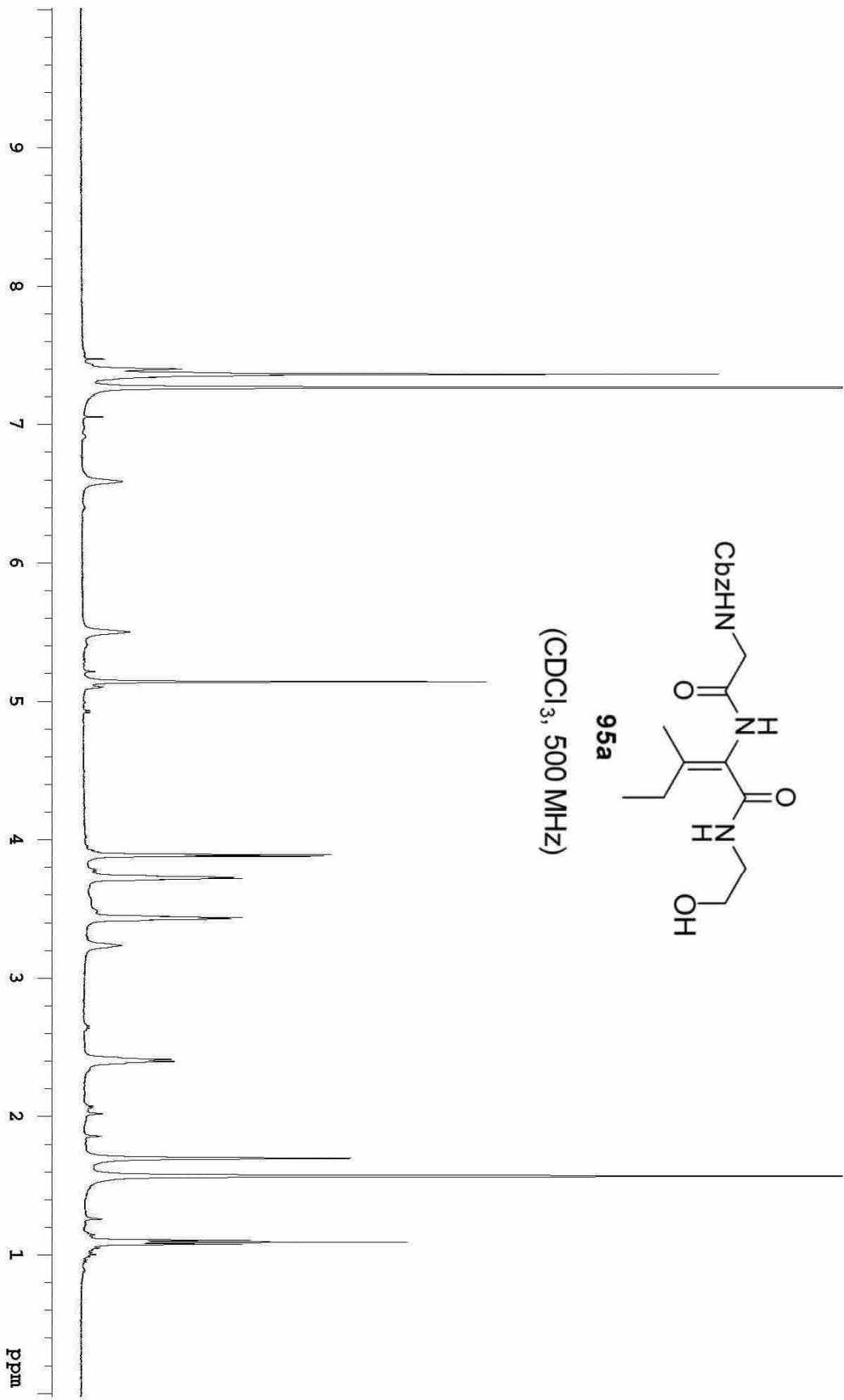


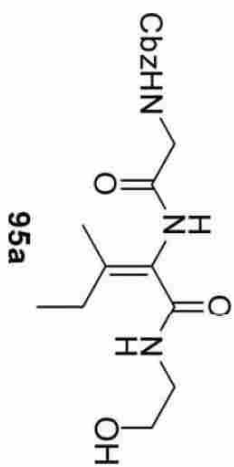




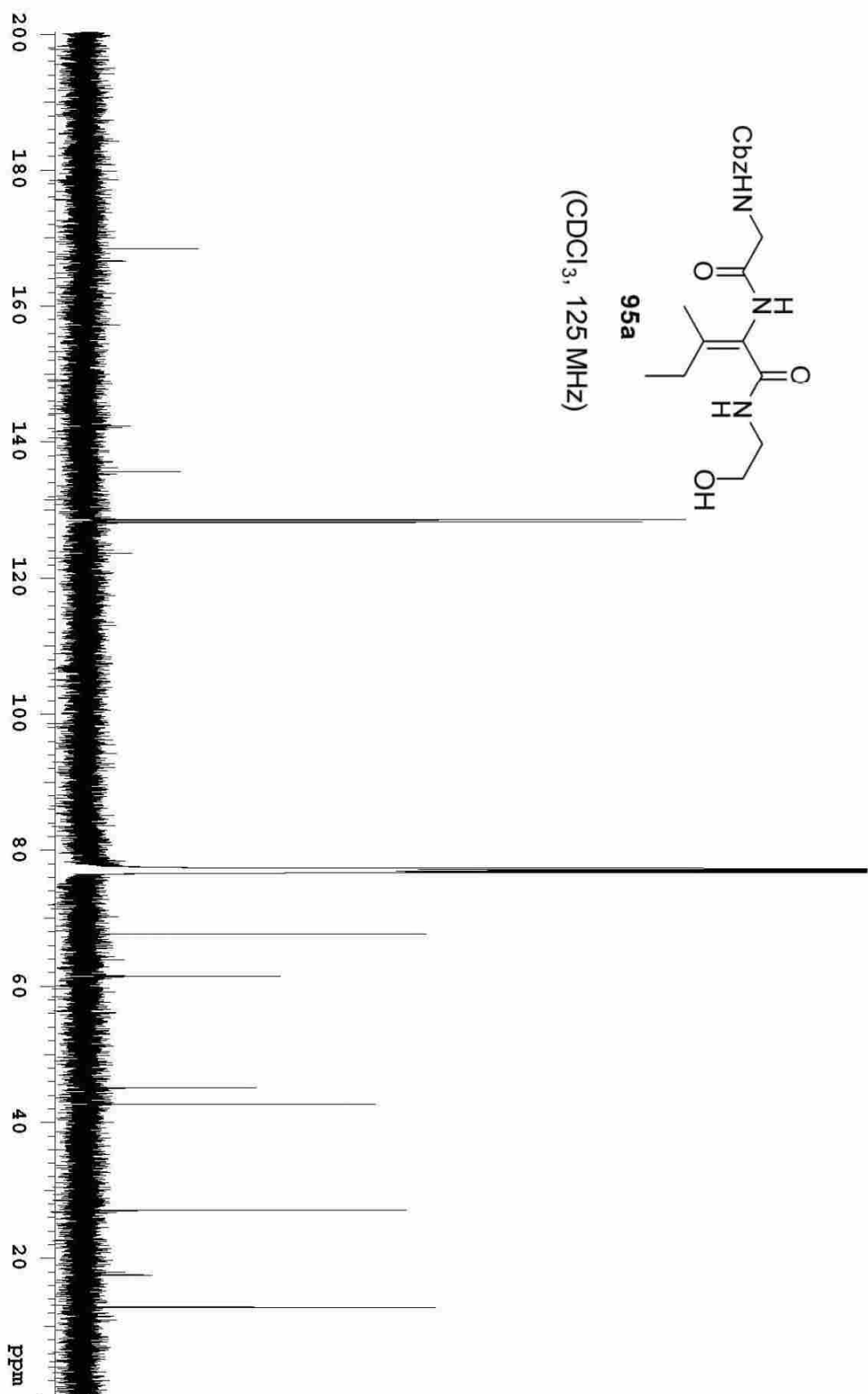


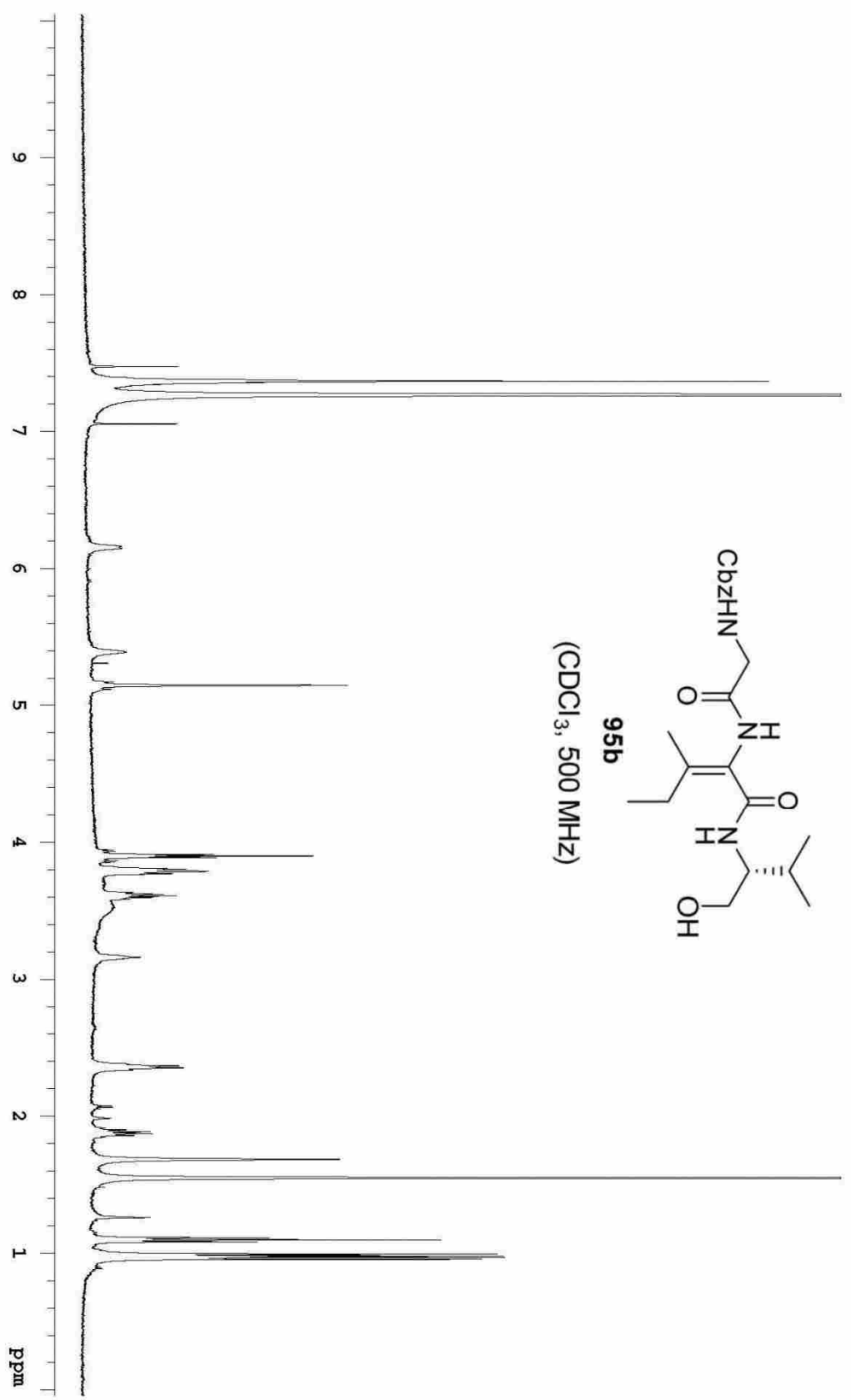
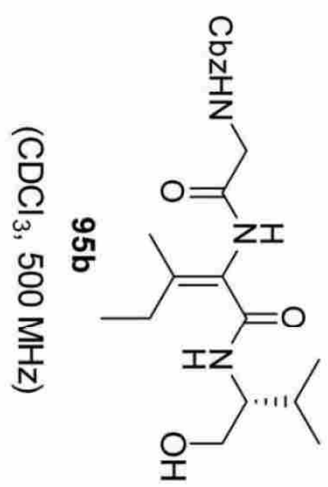


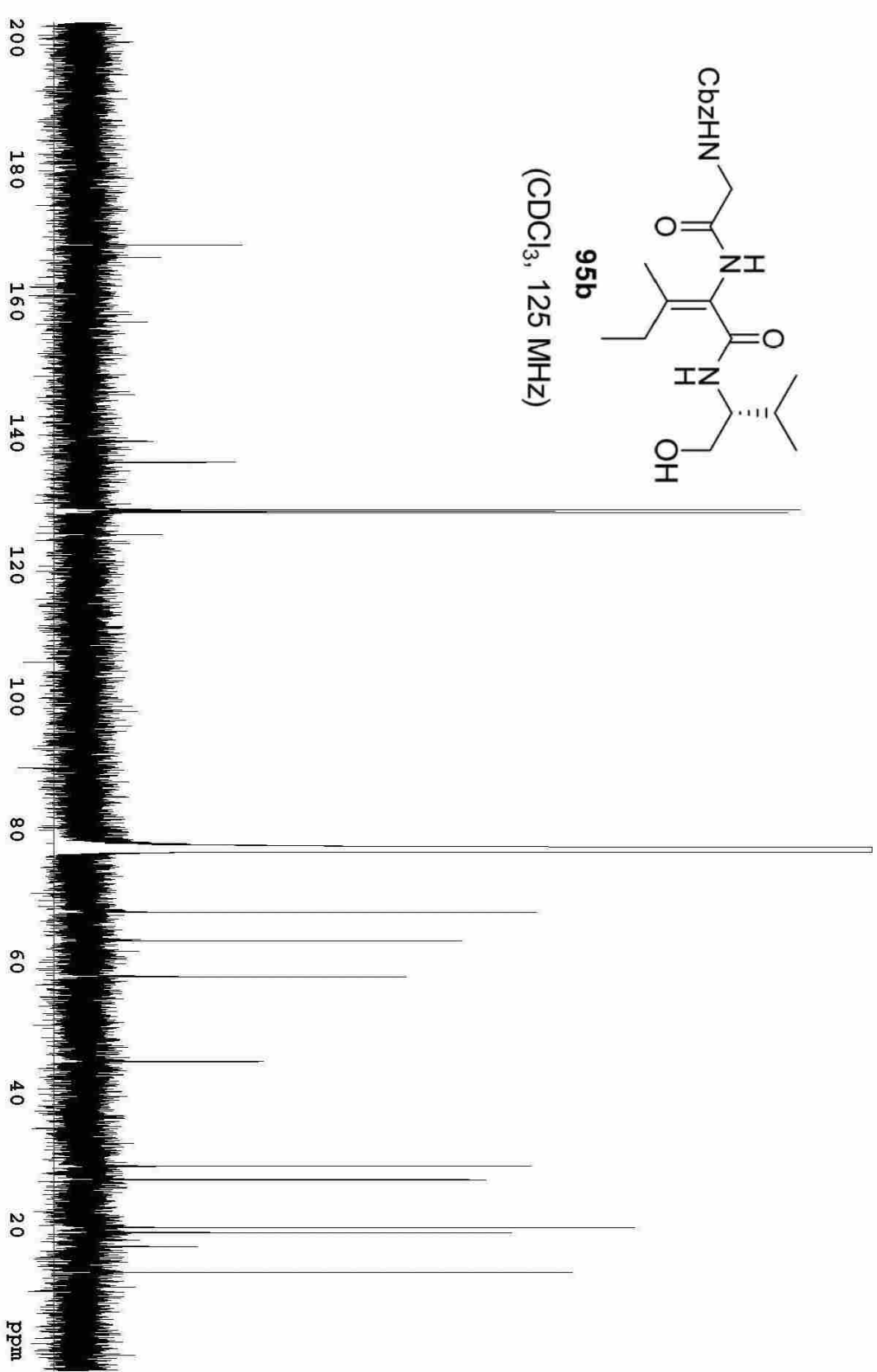
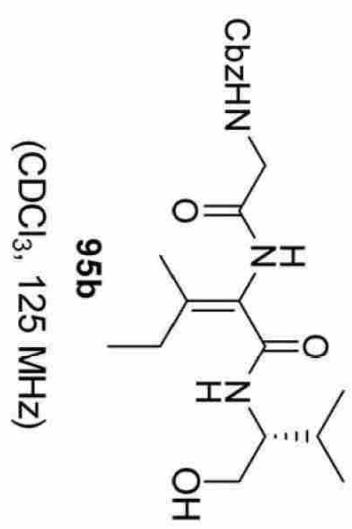


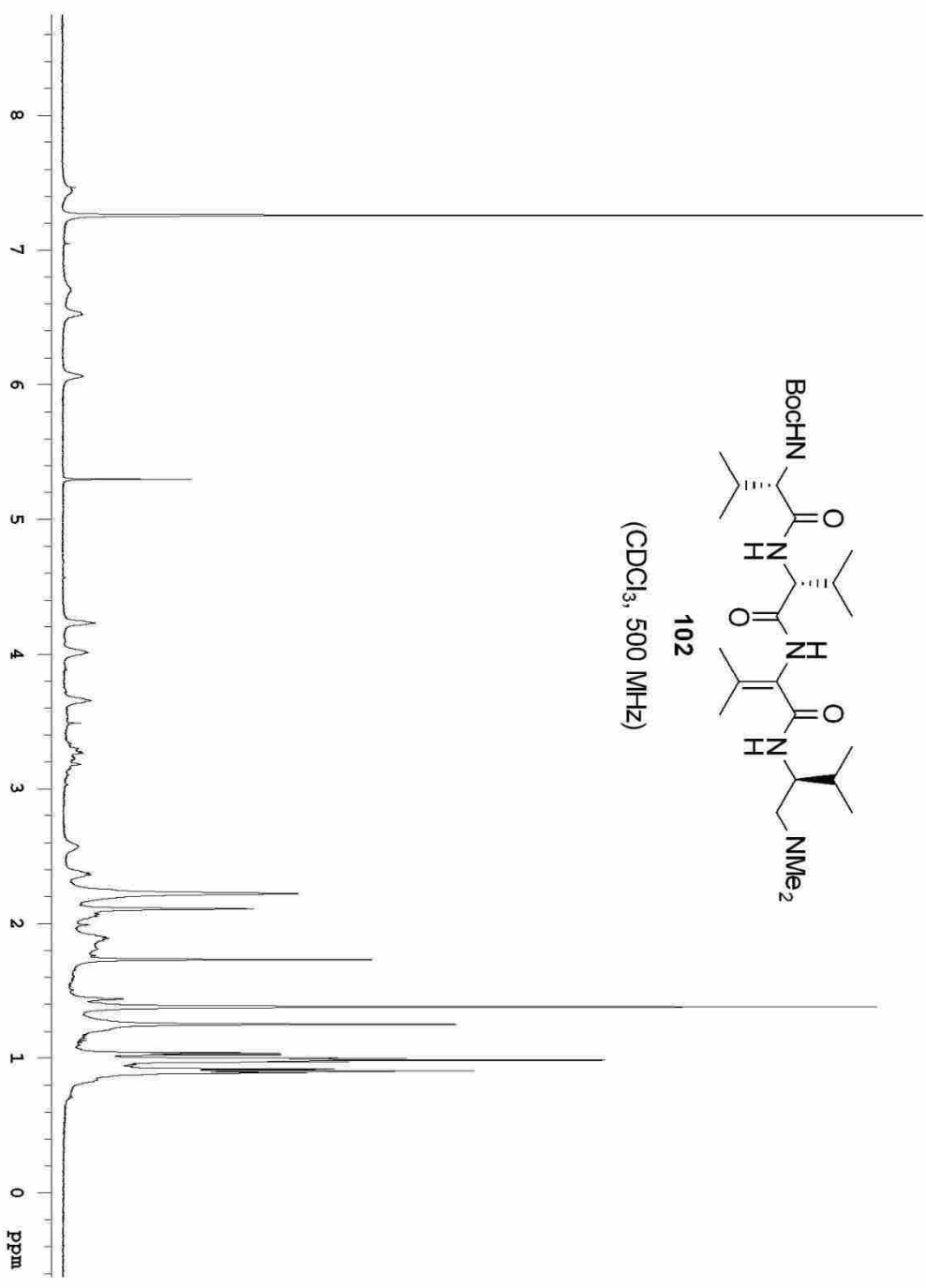
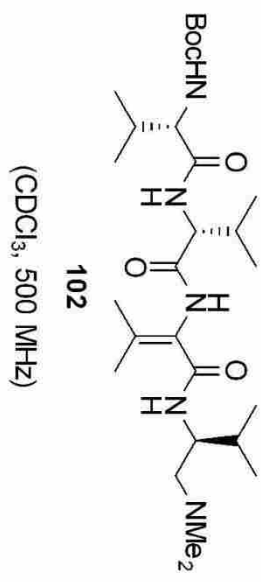


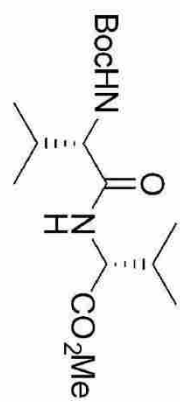
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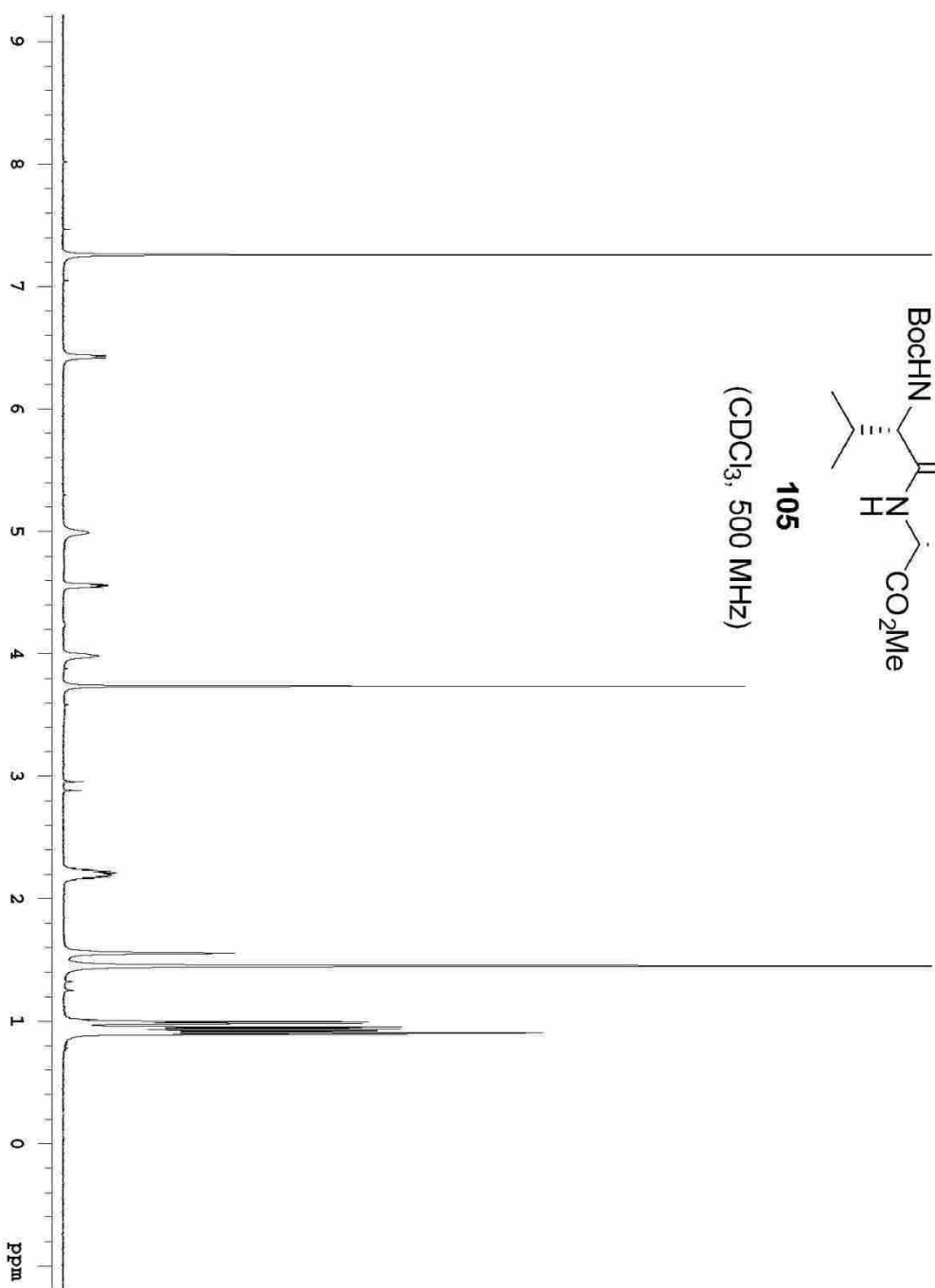


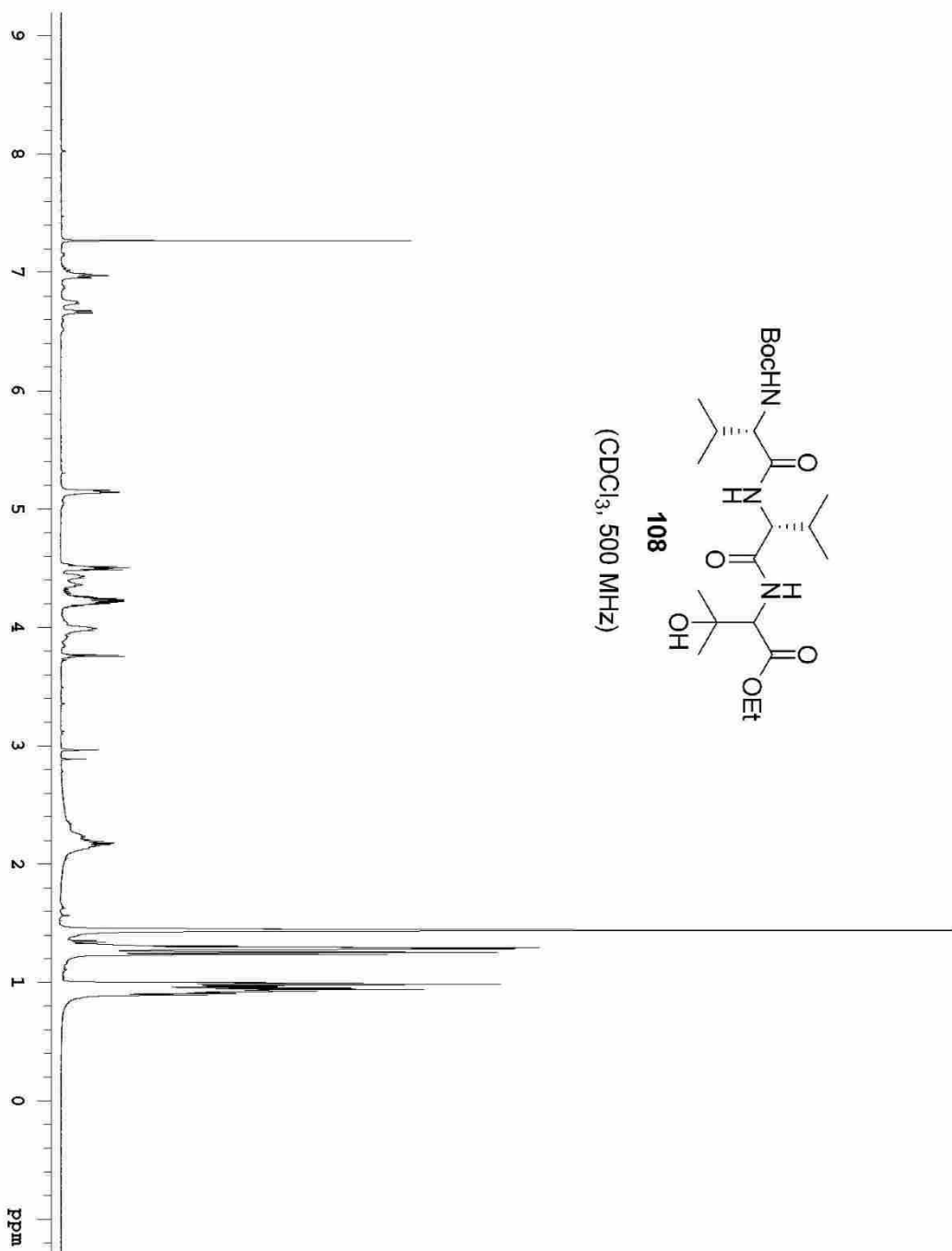
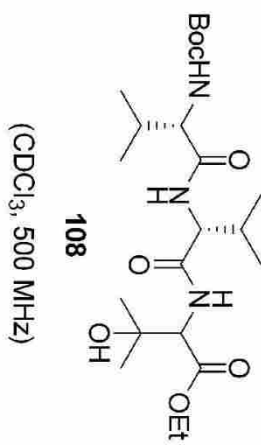


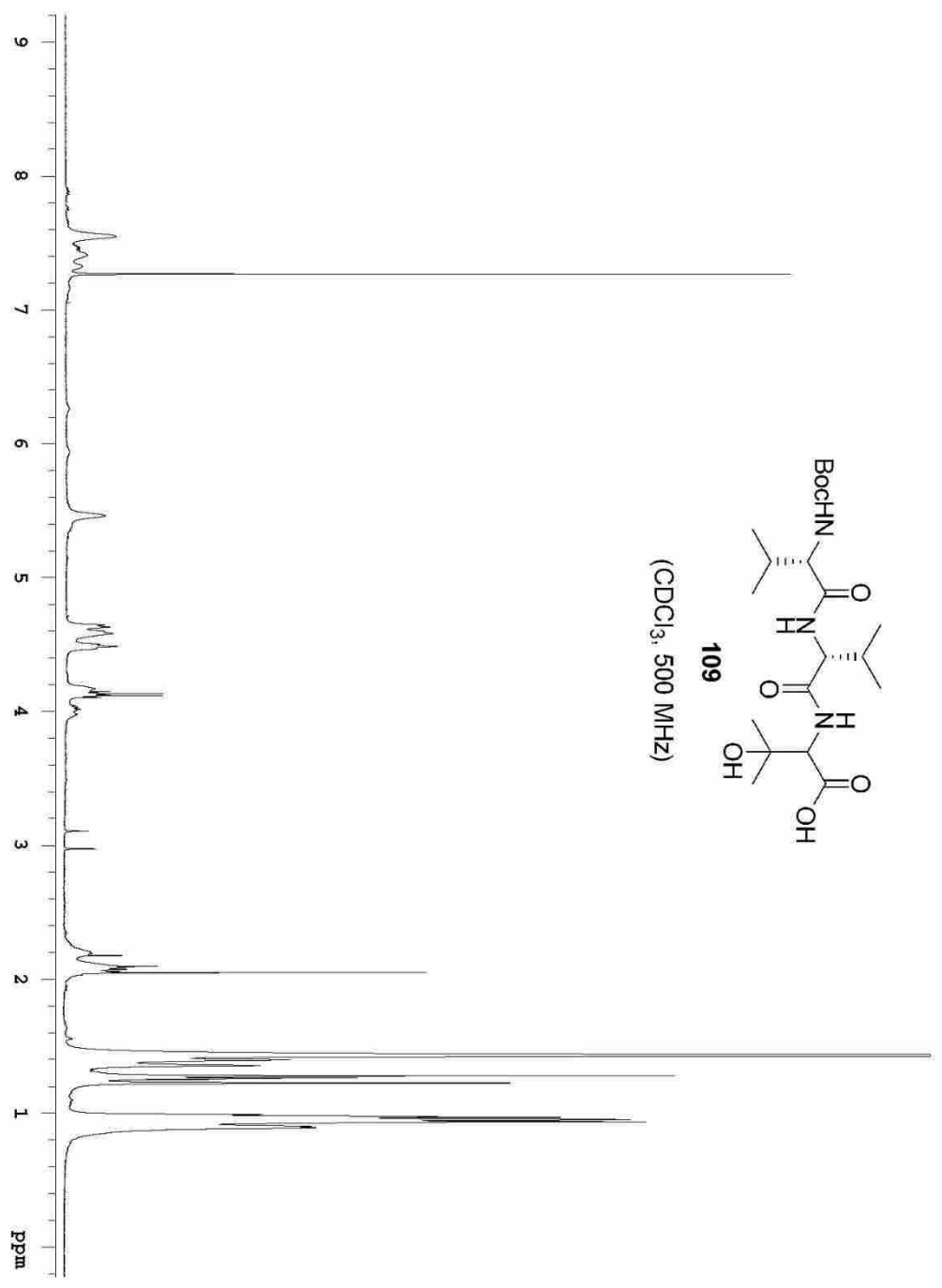
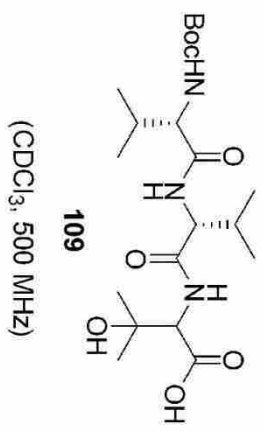


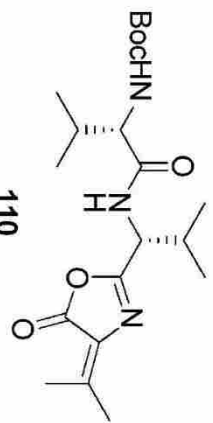
105

(CDCl₃, 500 MHz)

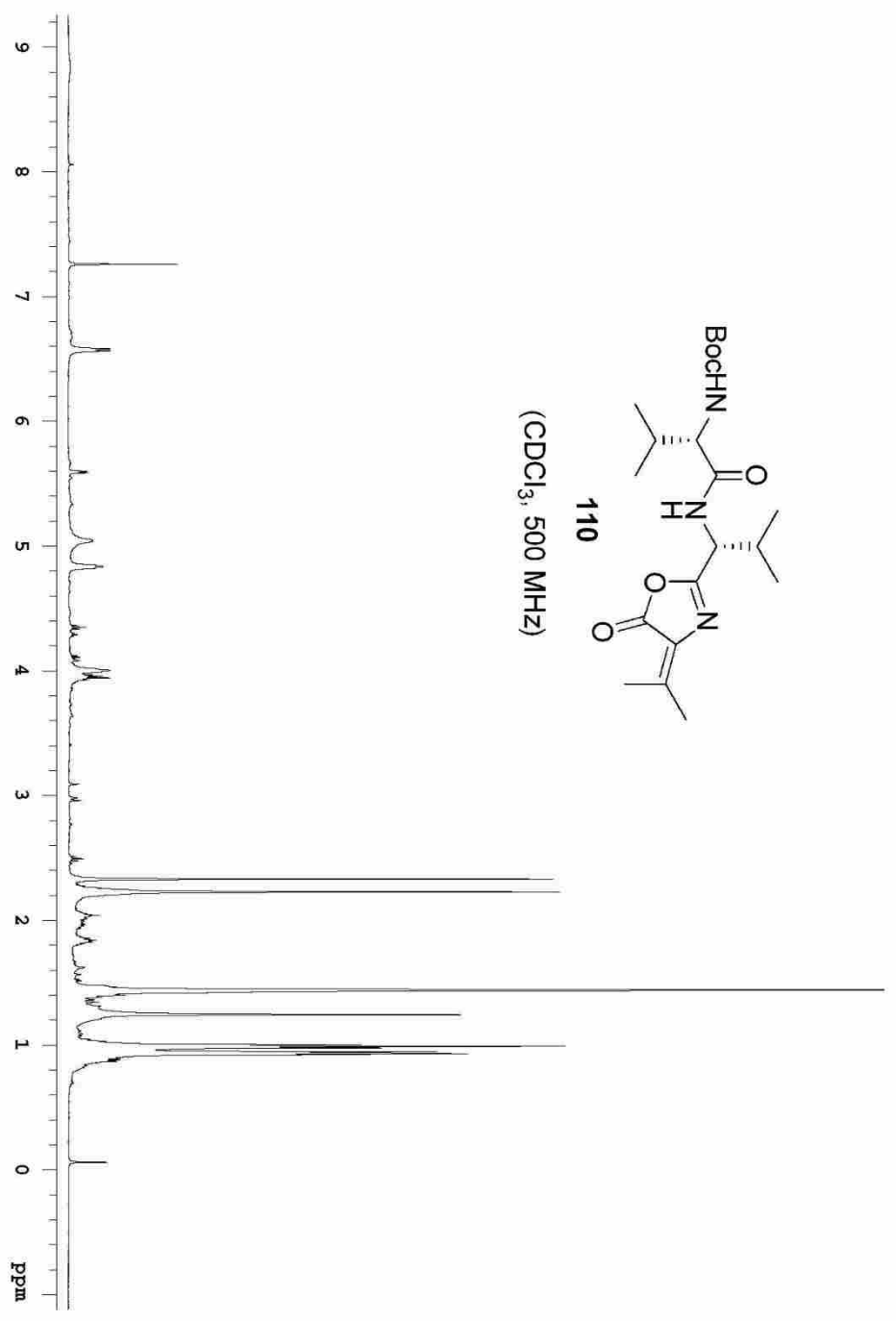


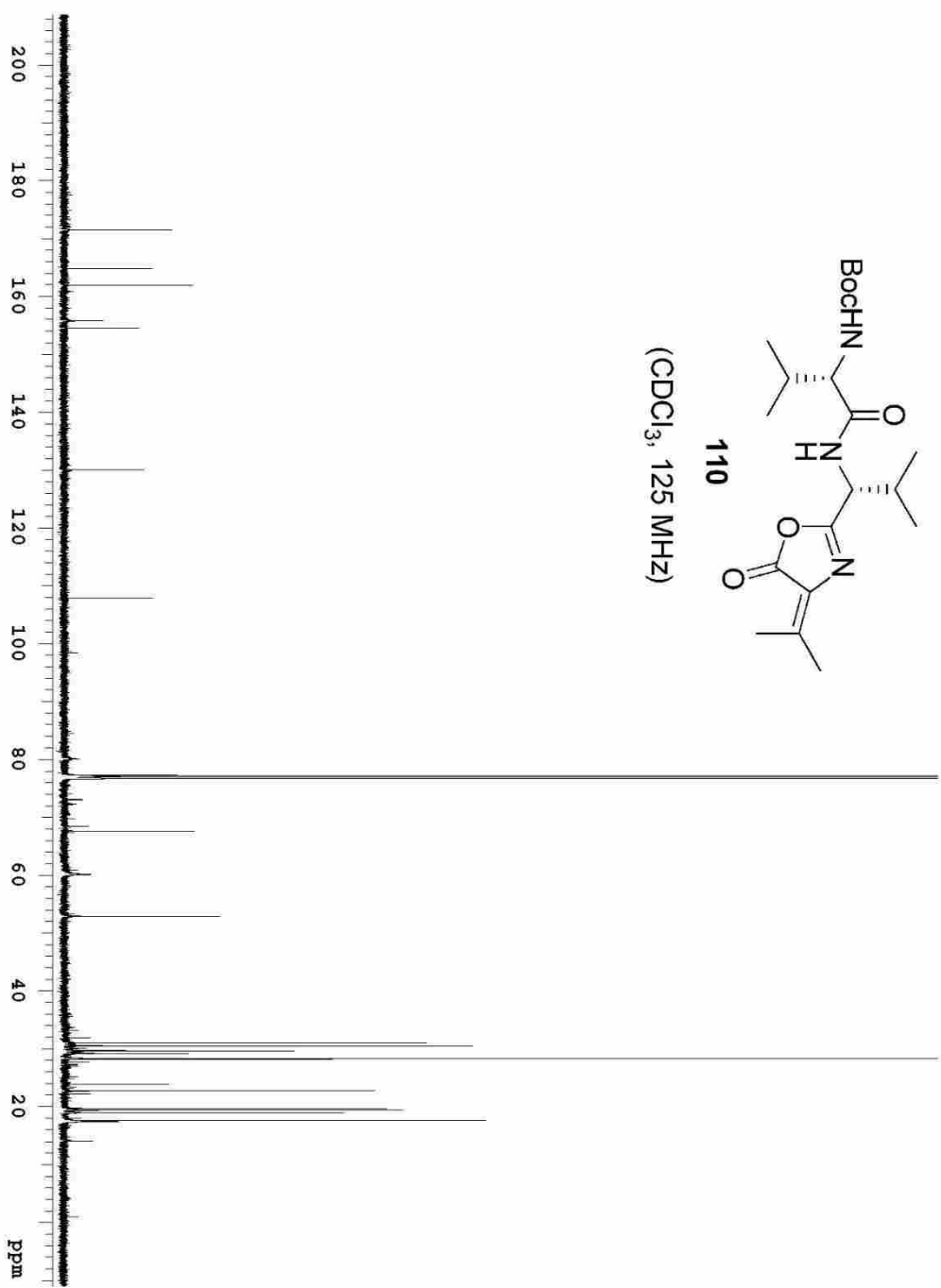
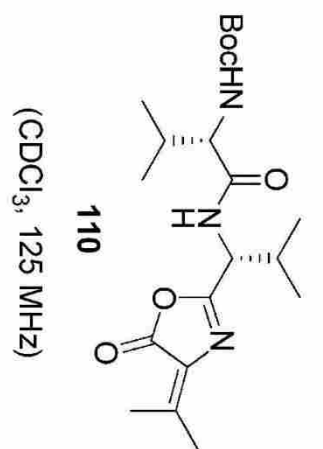


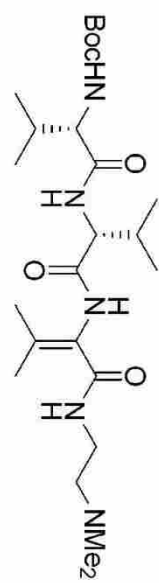




110
(CDCl₃, 500 MHz)

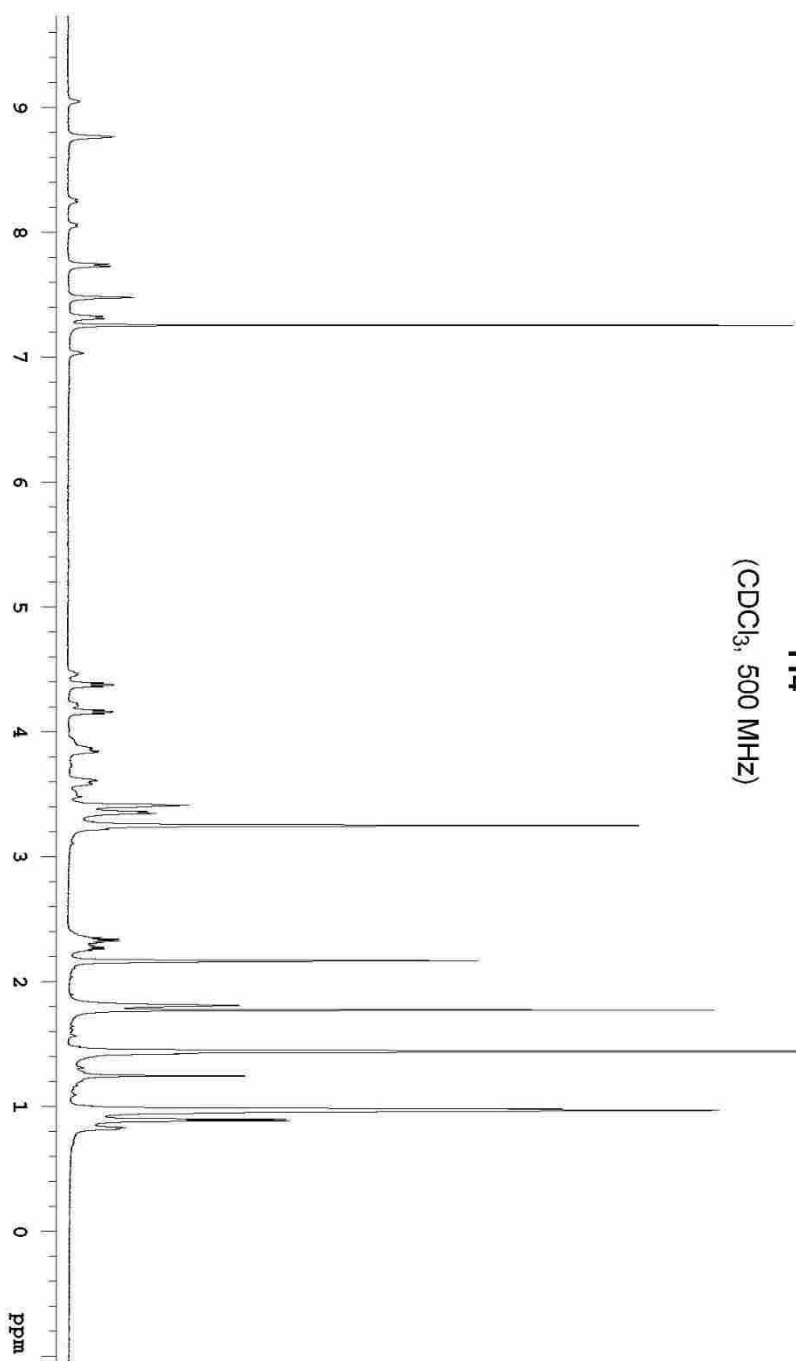


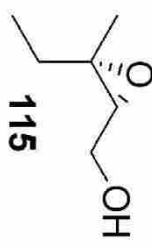




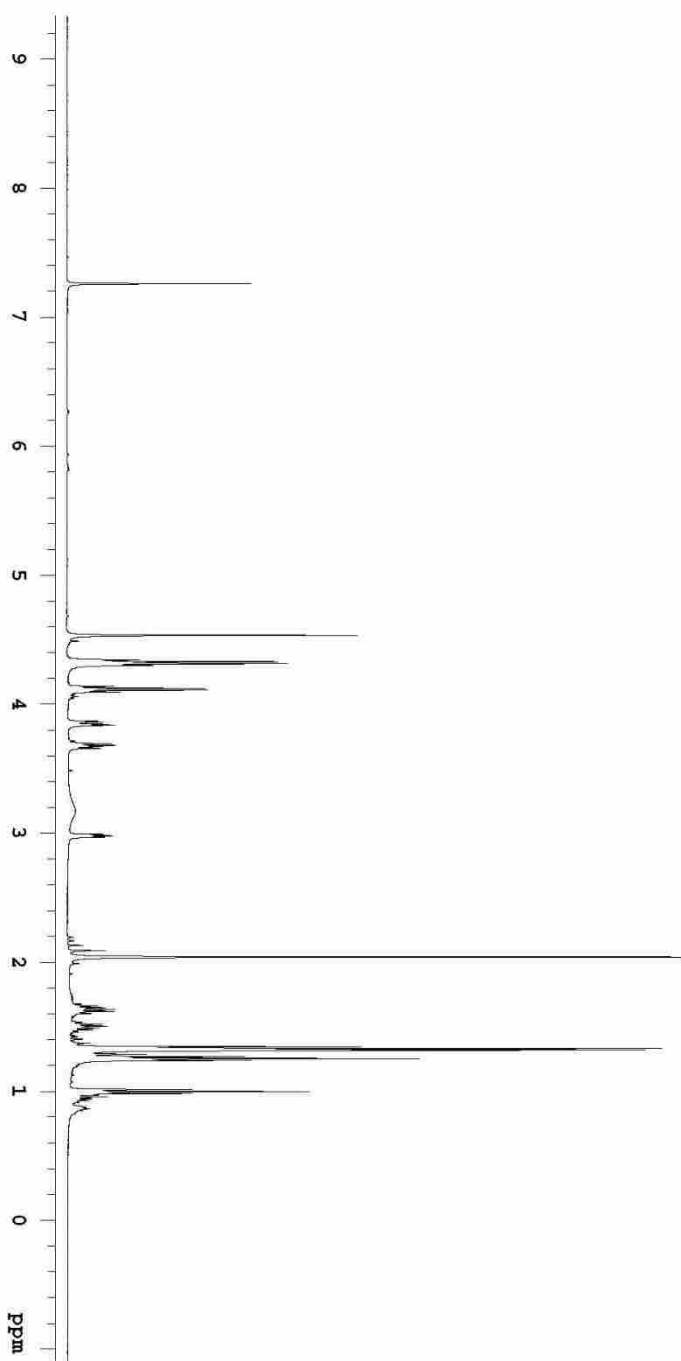
114

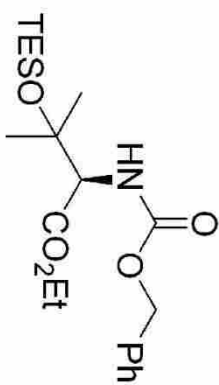
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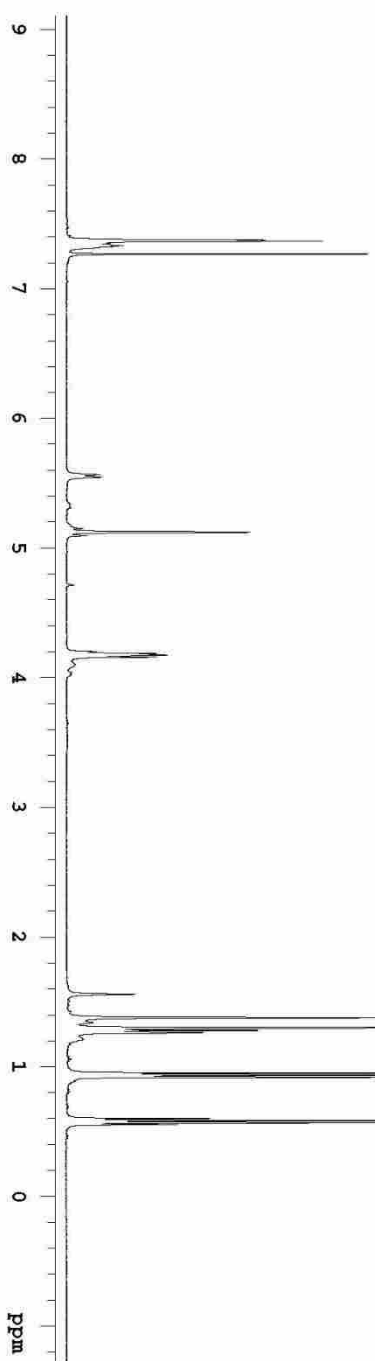
(CDCl₃, 500 MHz)

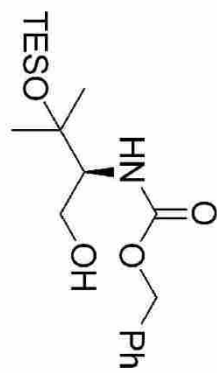




120

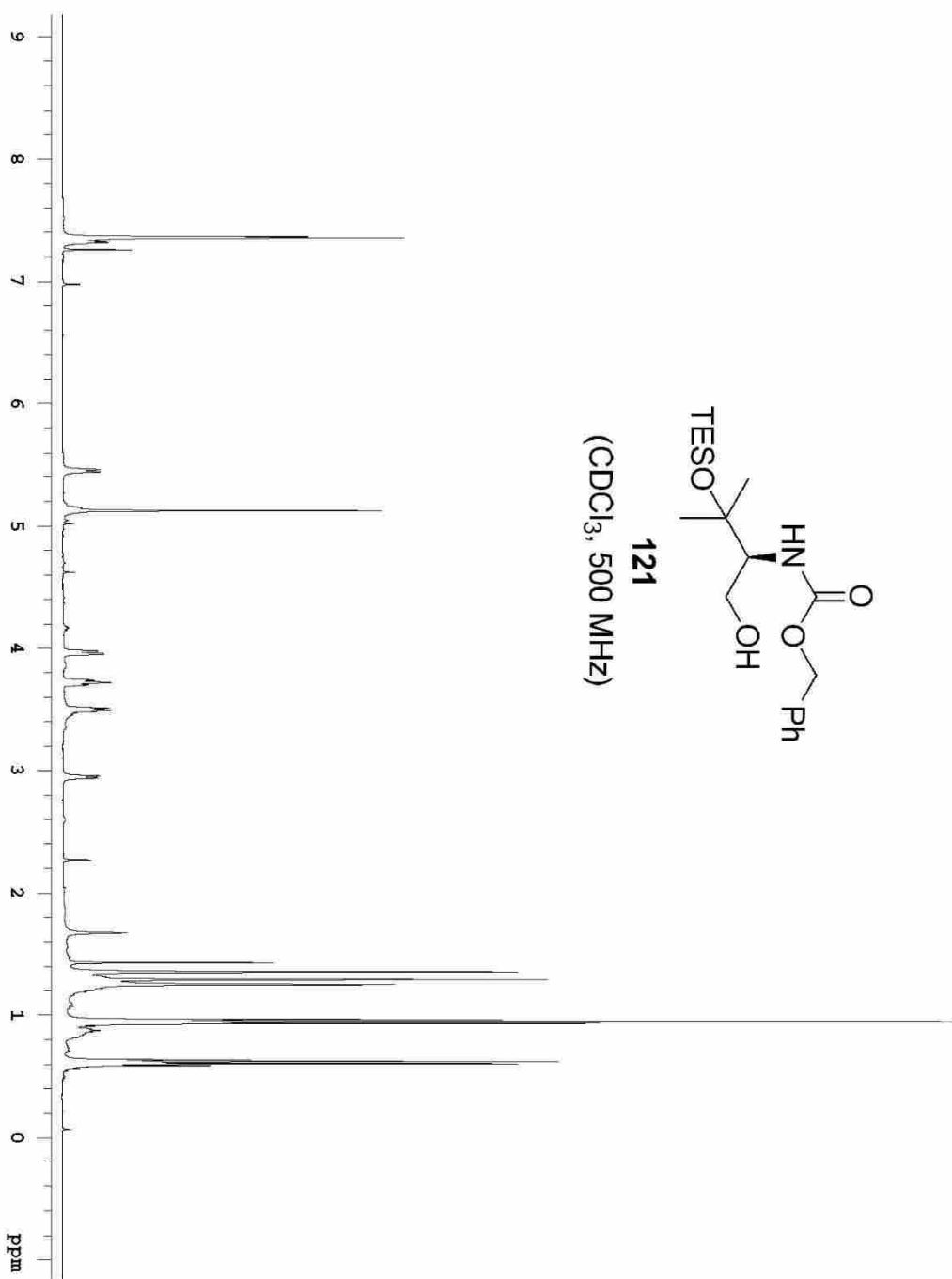
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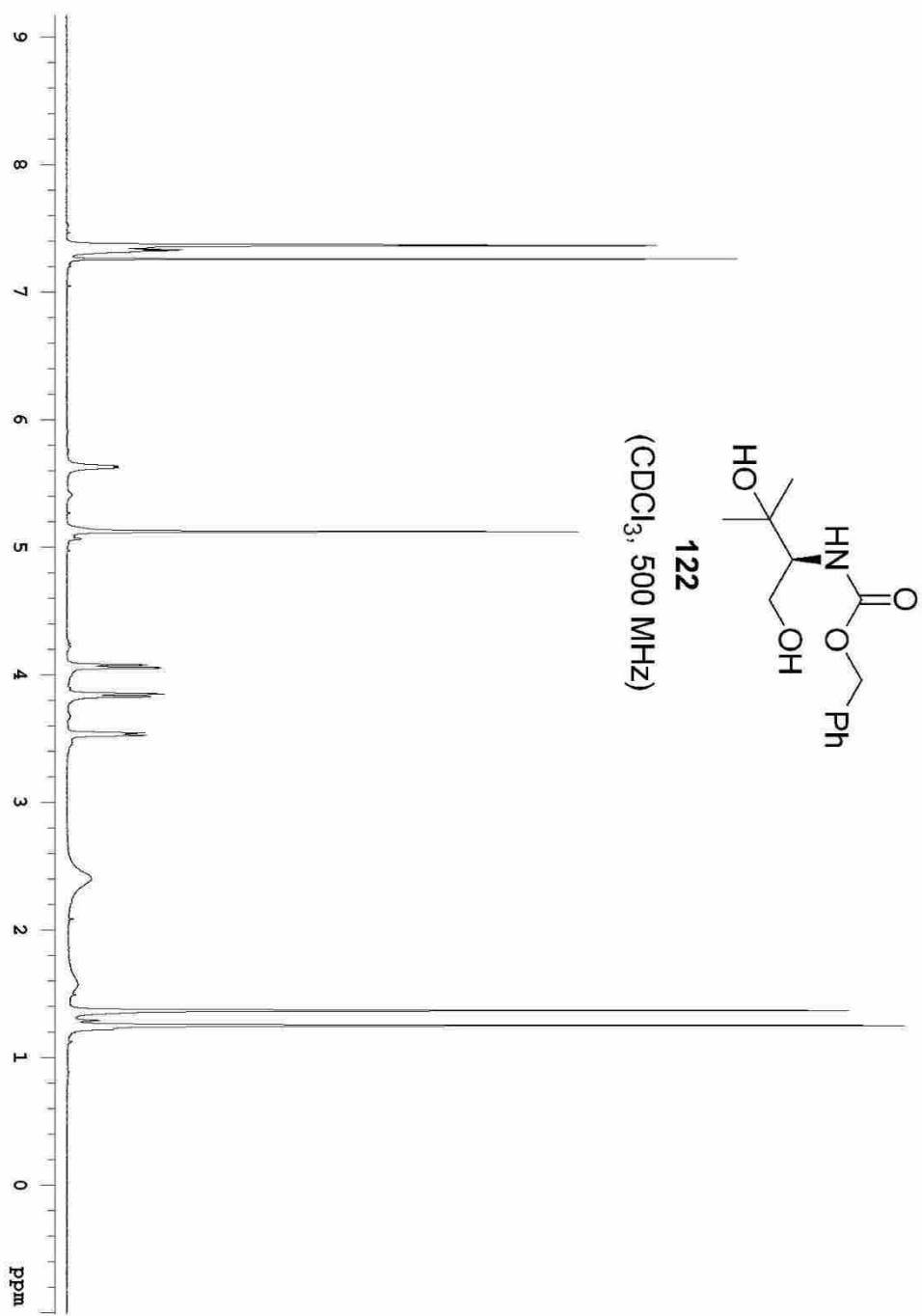


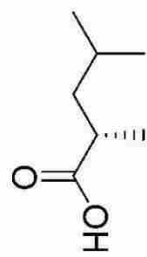


121

(CDCl₃, 500 MHz)

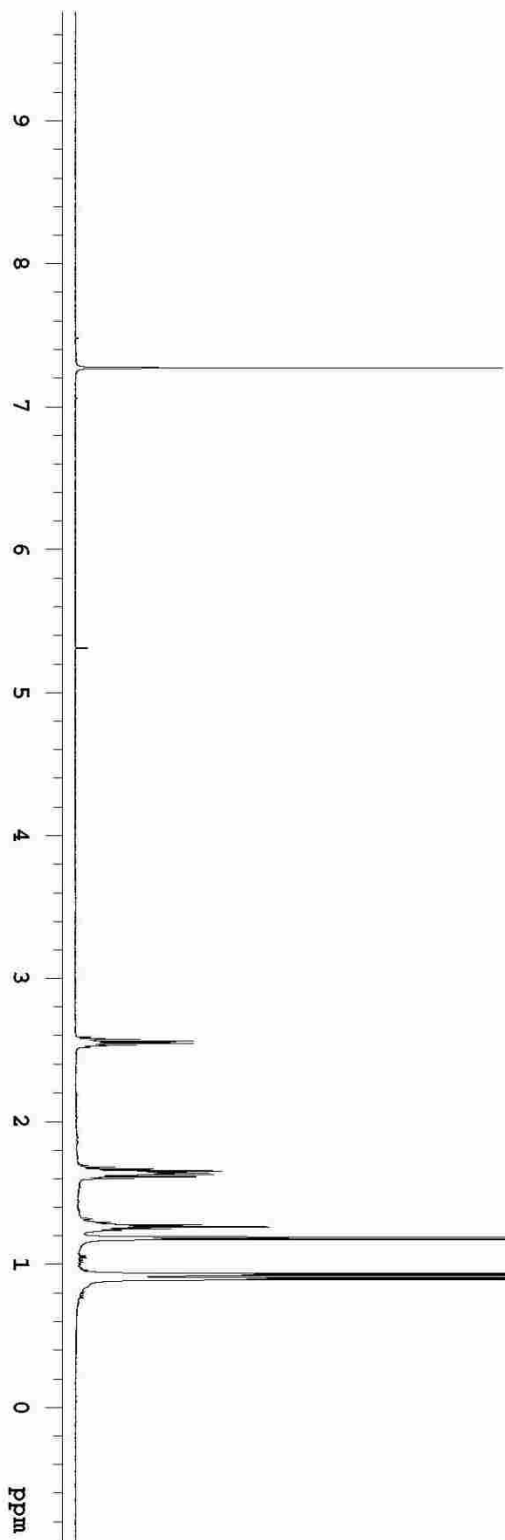


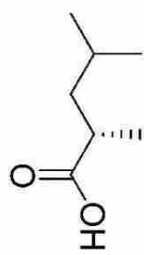




131

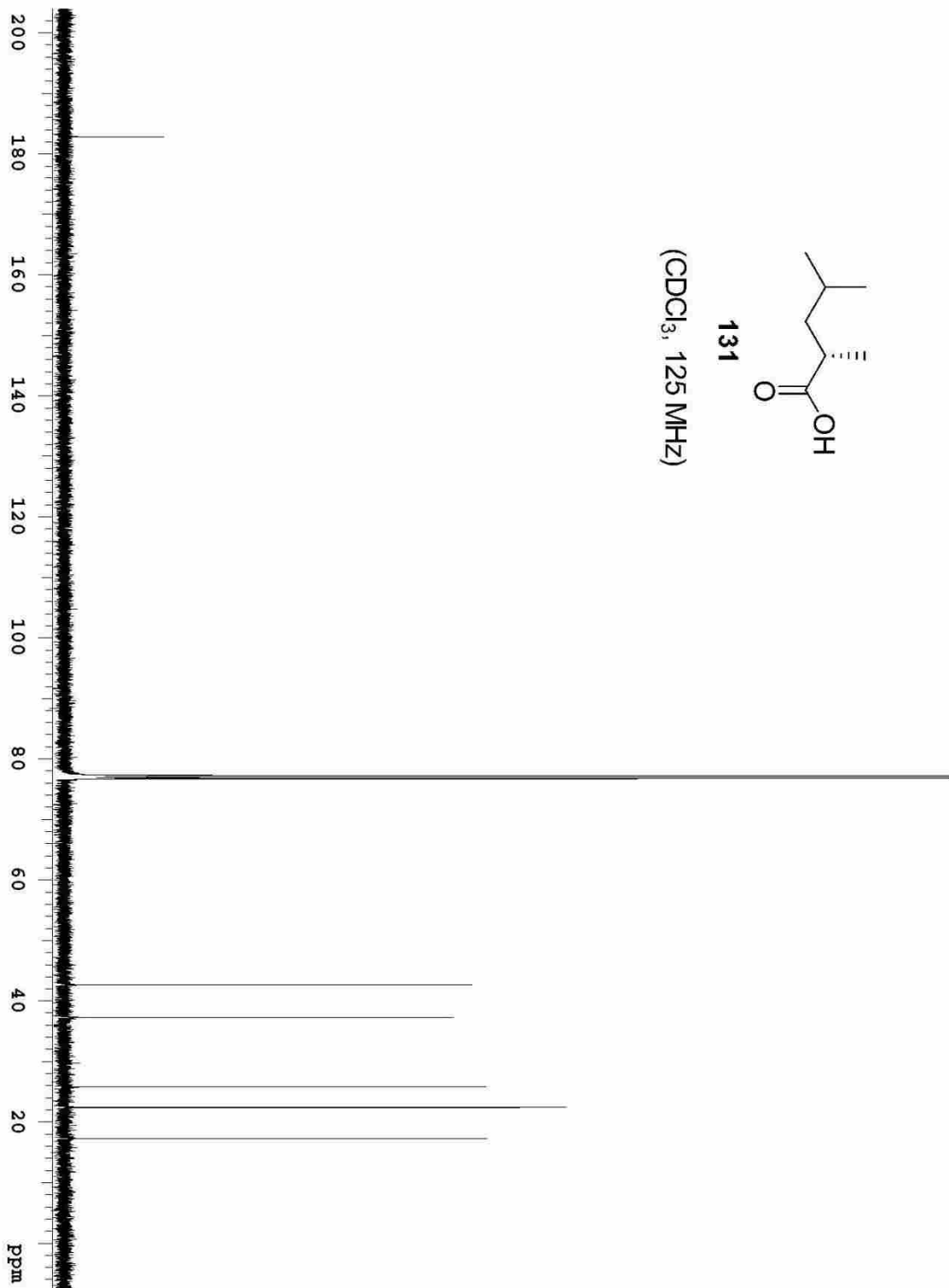
(CDCl₃, 500 MHz)

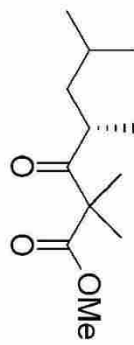




131

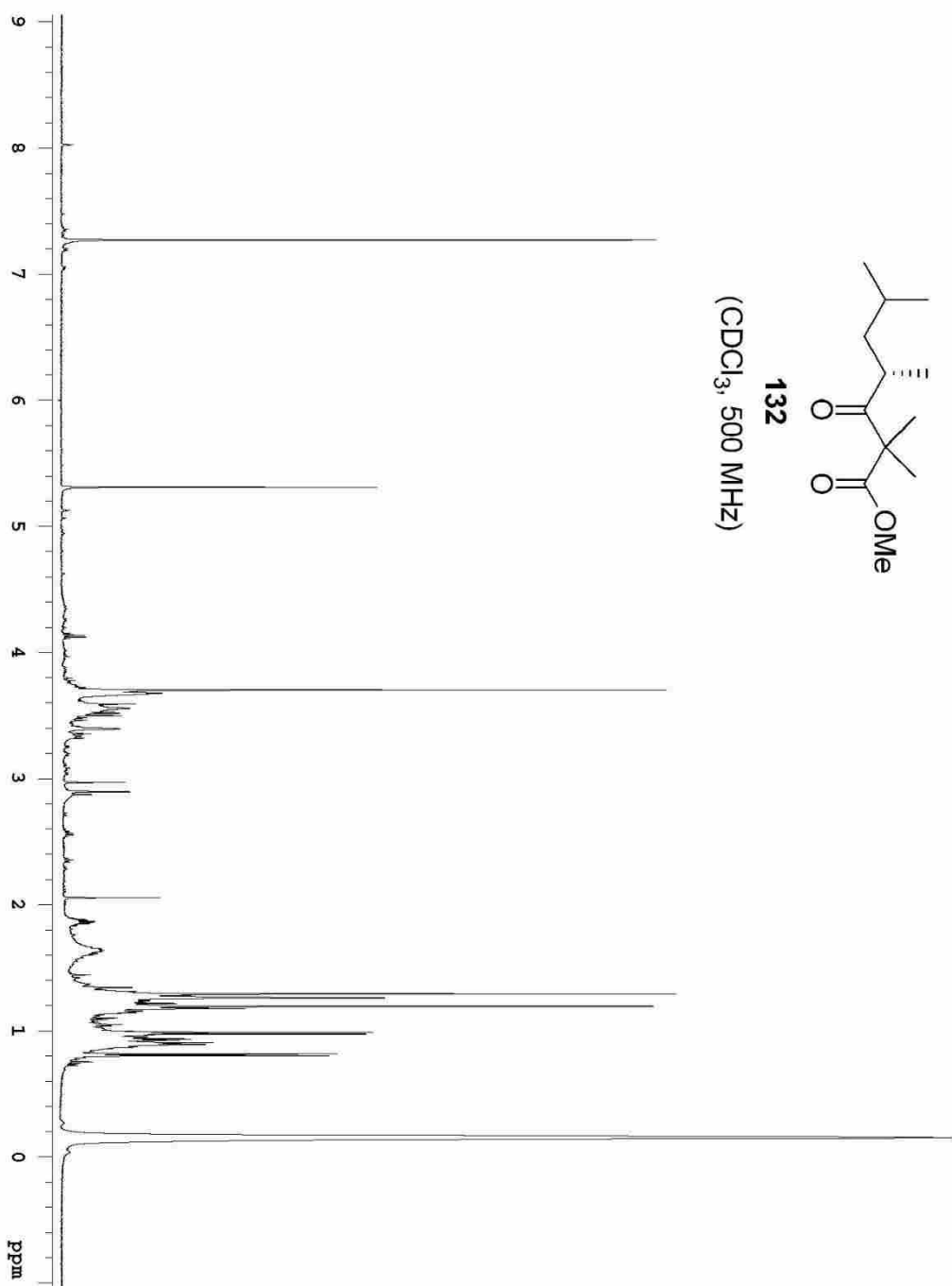
(CDCl₃, 125 MHz)

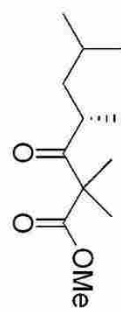




132

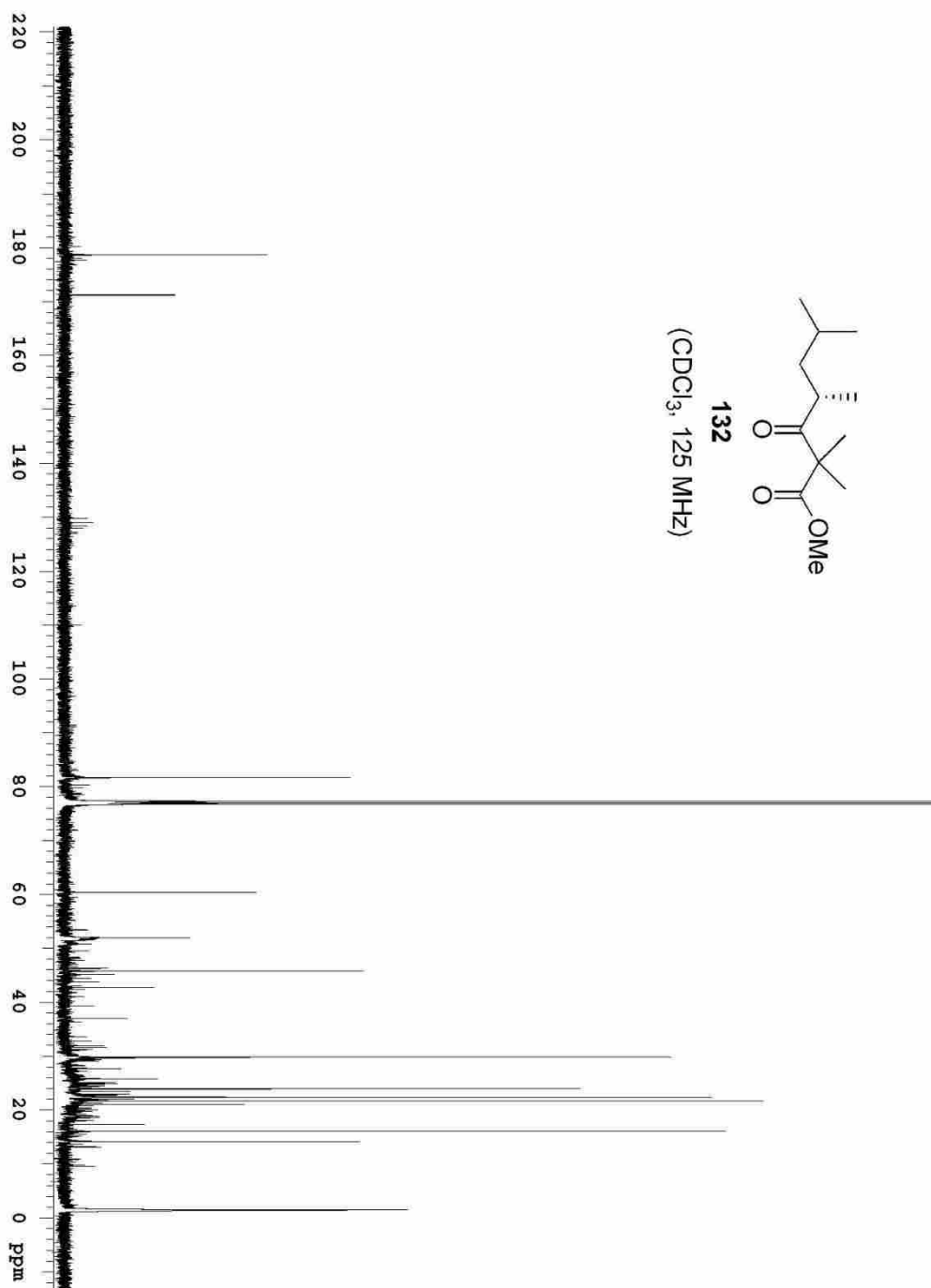
(CDCl₃, 500 MHz)

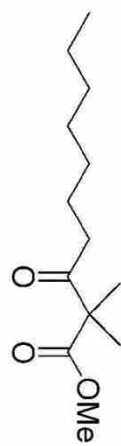




132

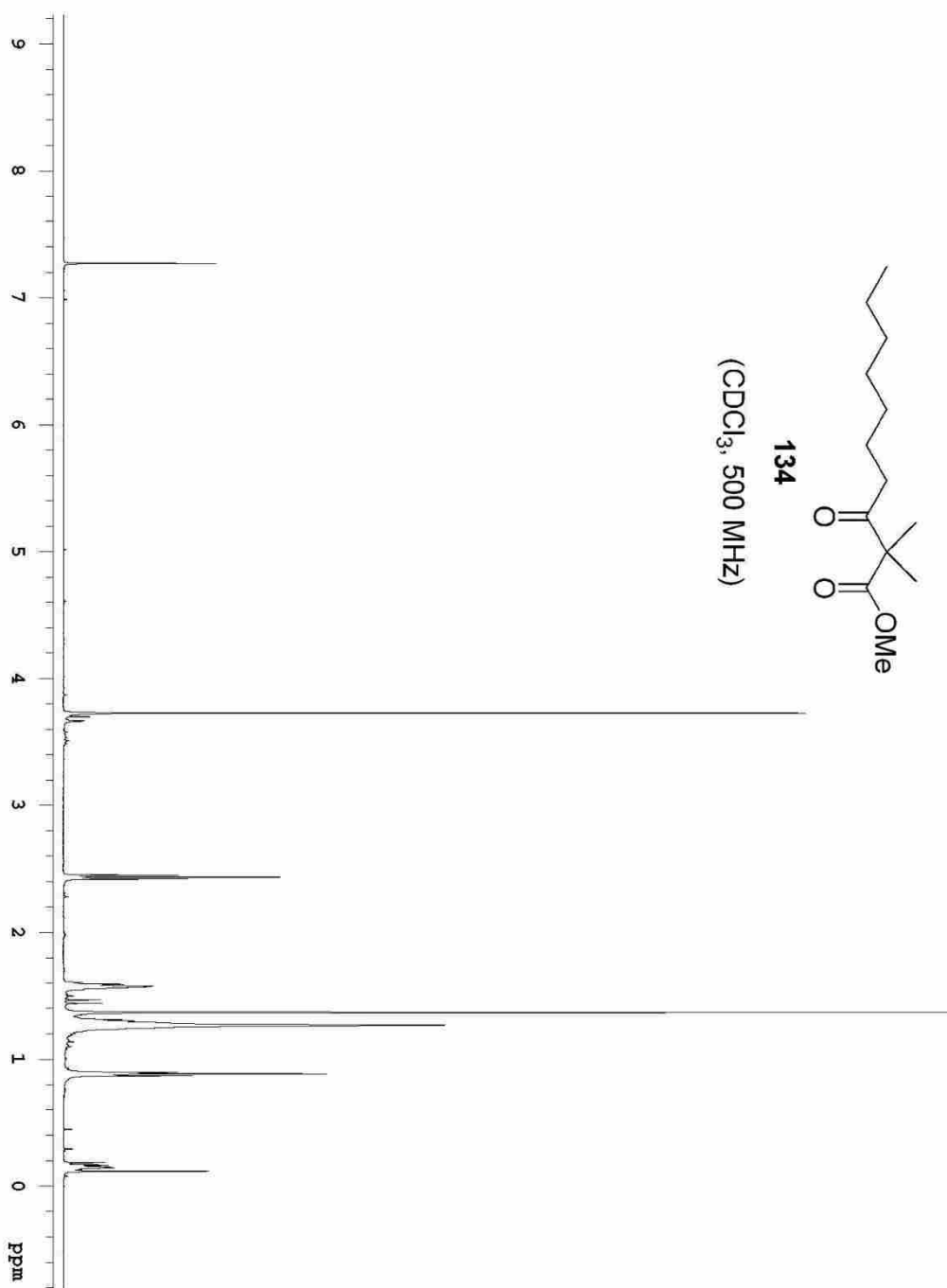
(CDCl₃, 125 MHz)

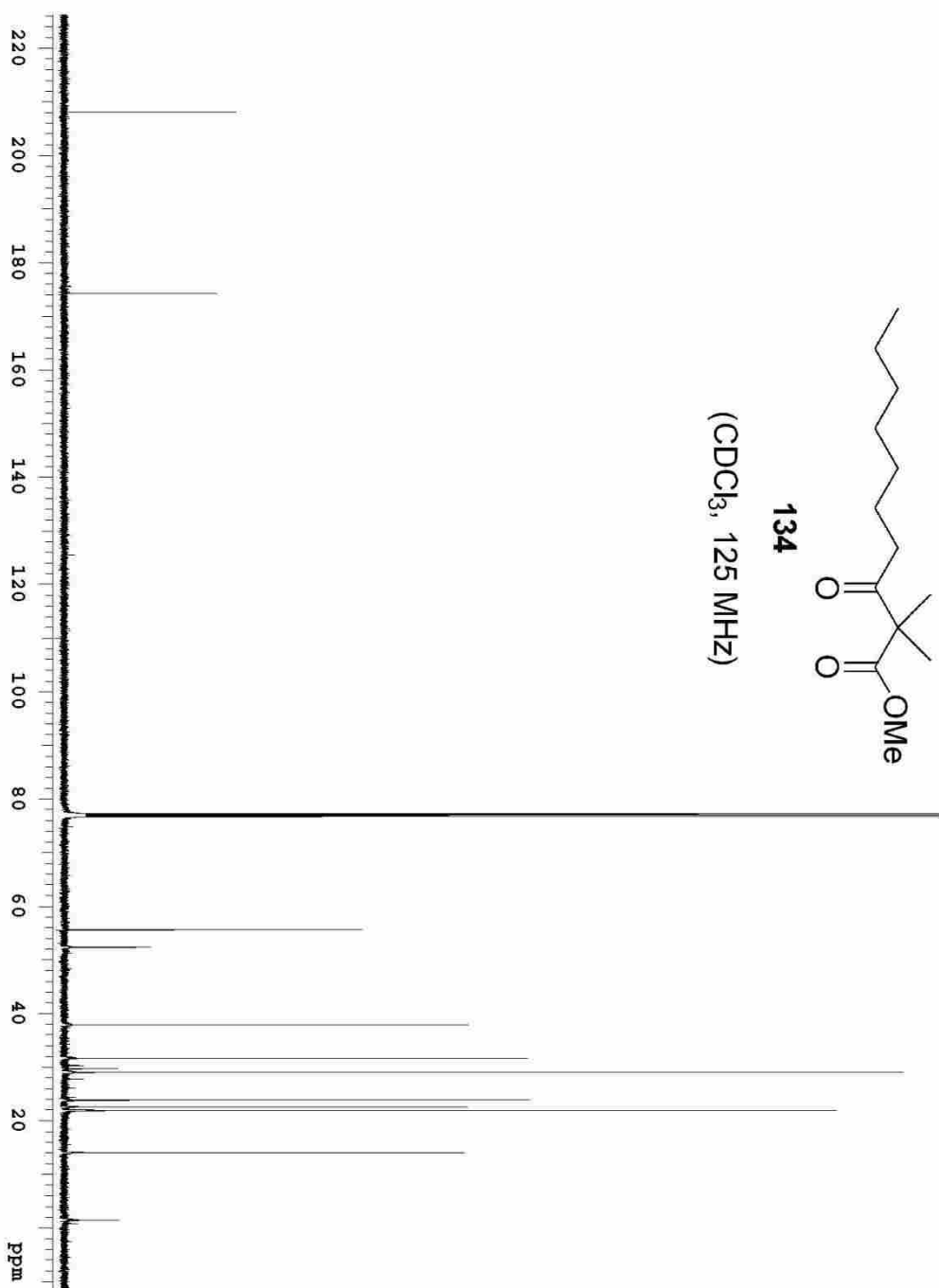
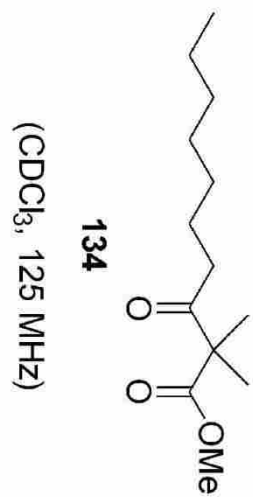


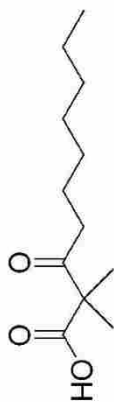


134

(CDCl₃, 500 MHz)

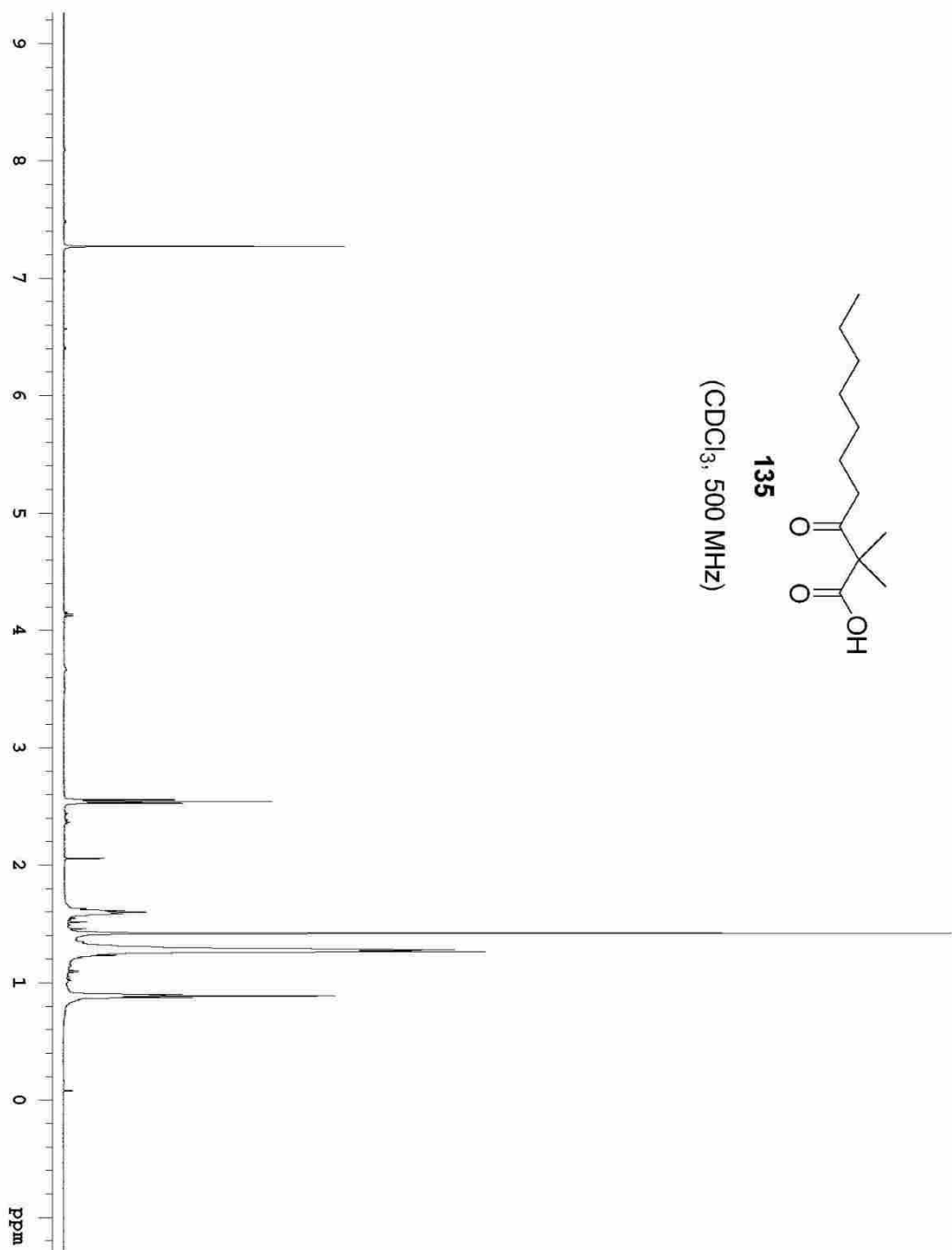


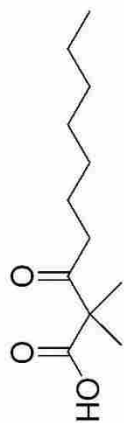




135

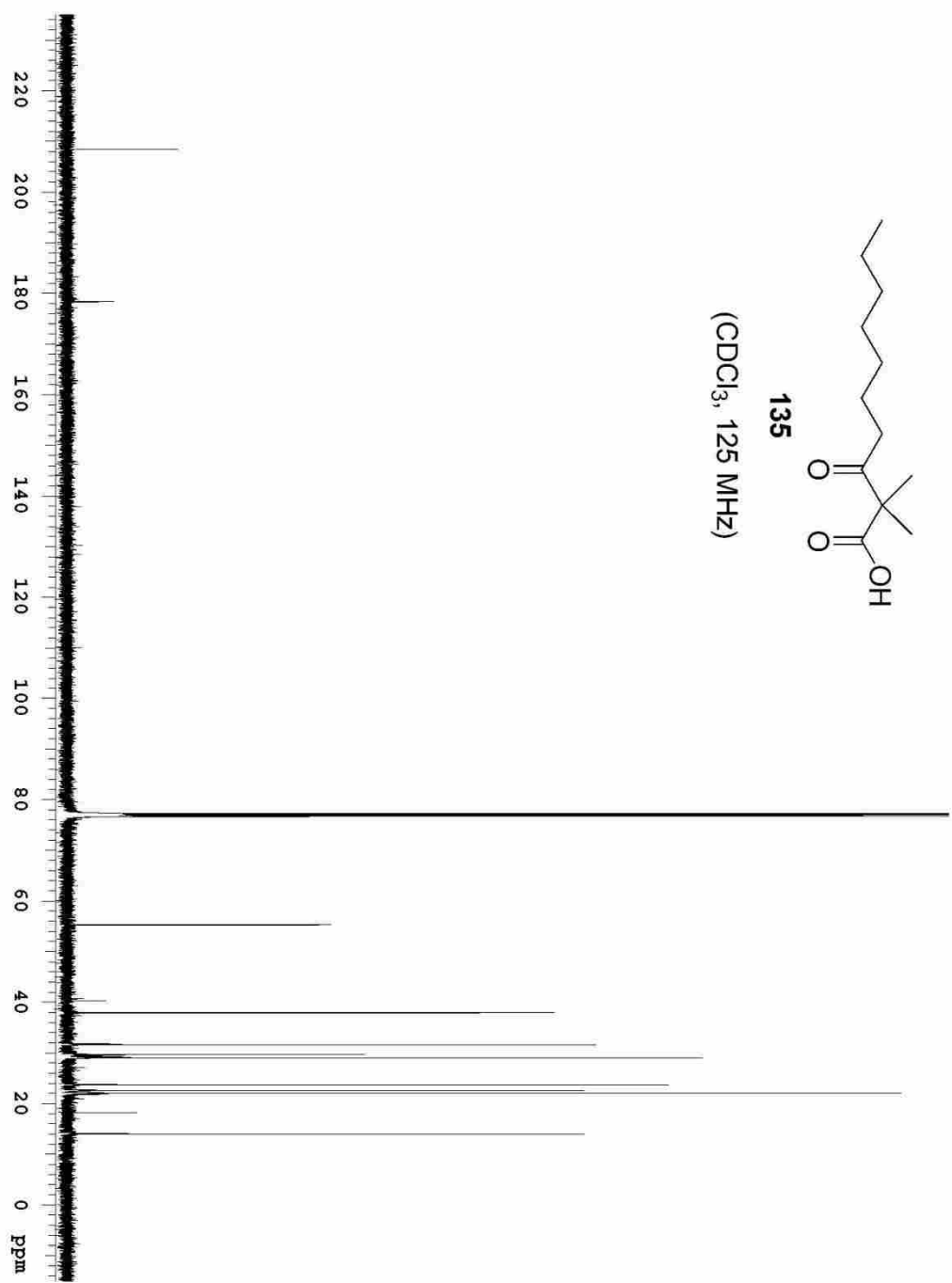
(CDCl₃, 500 MHz)

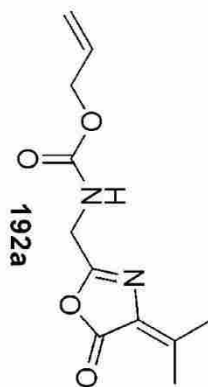




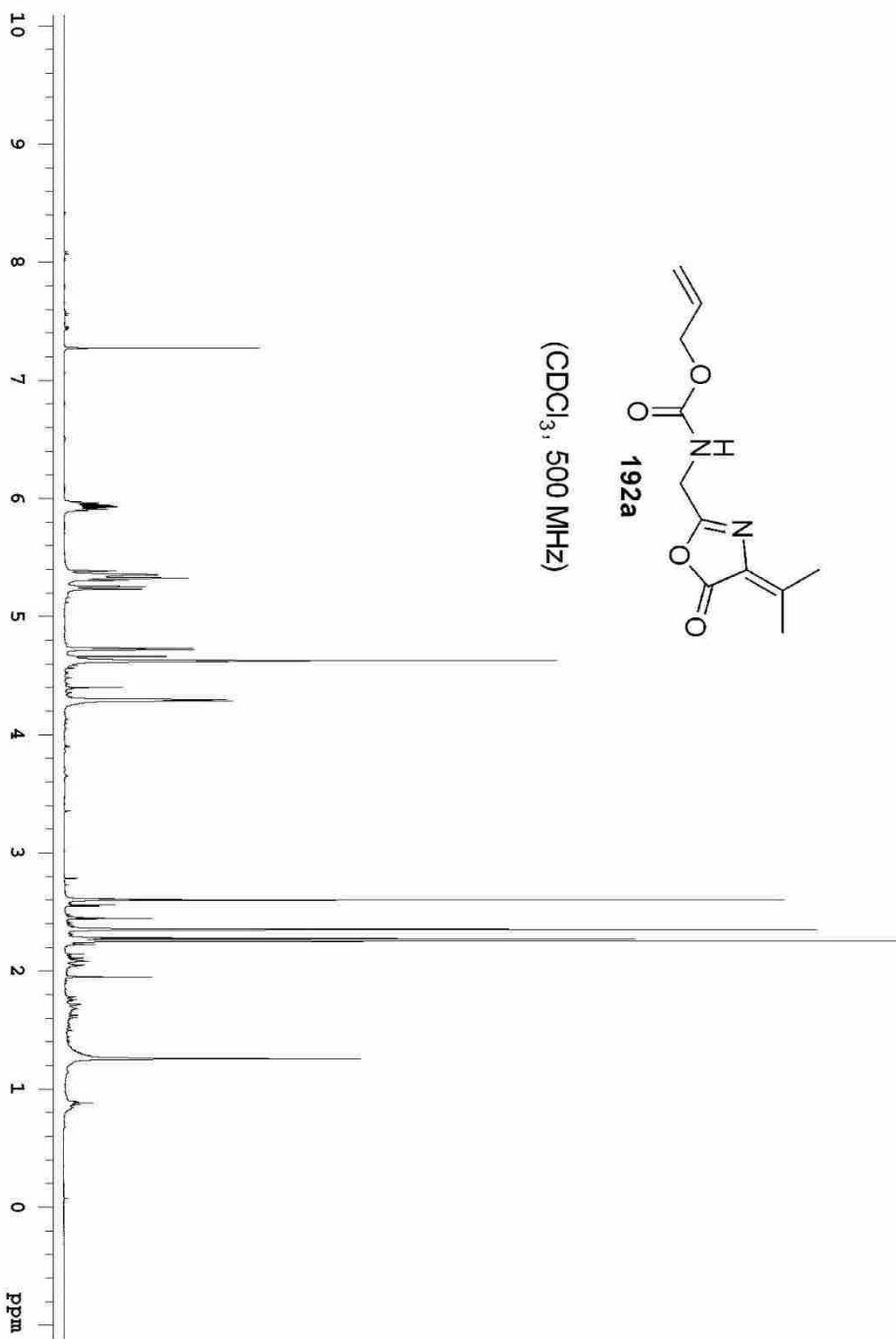
135

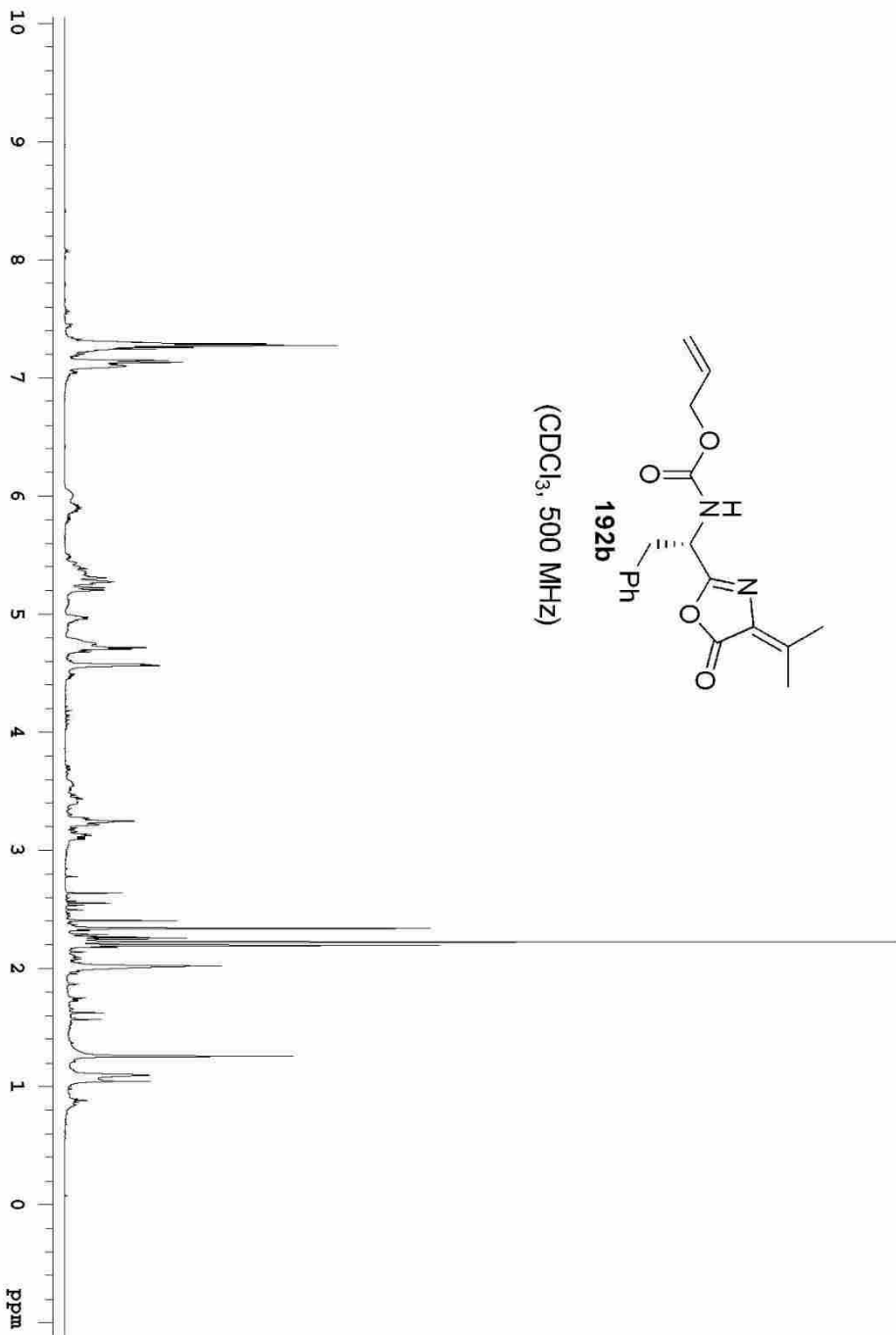
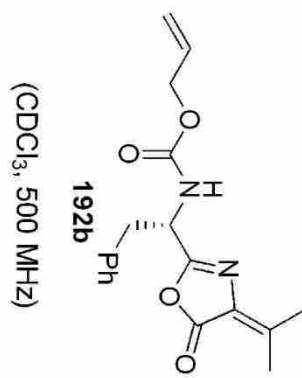
(CDCl₃, 125 MHz)

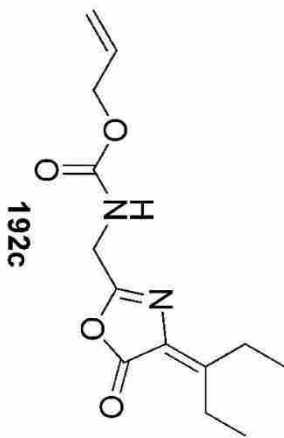




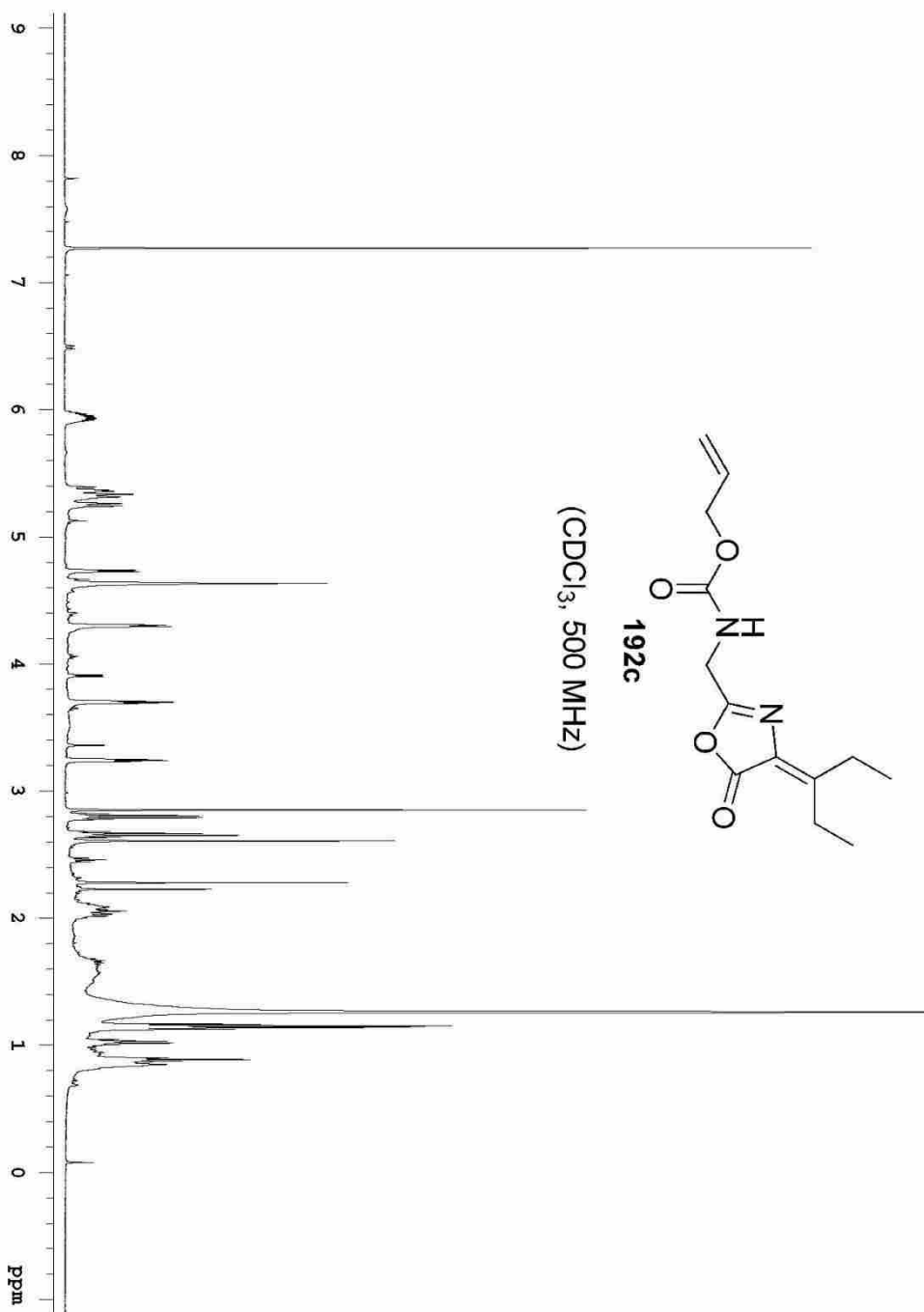
(CDCl₃, 500 MHz)

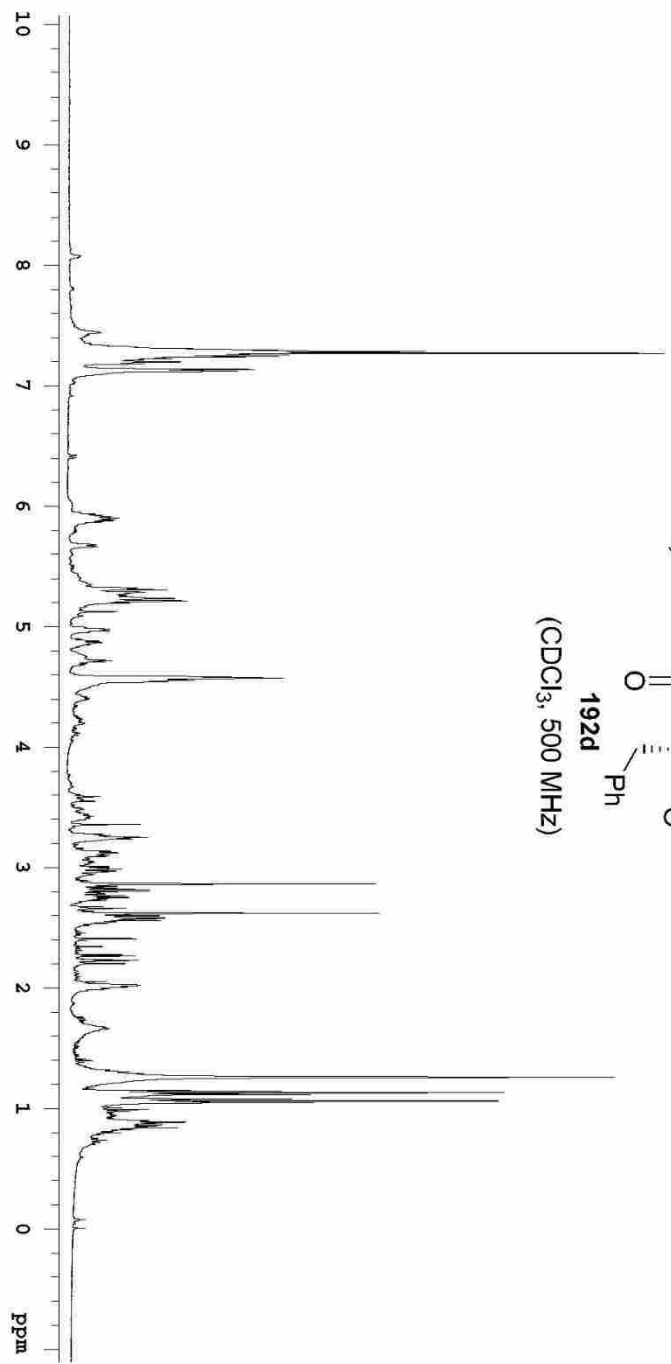
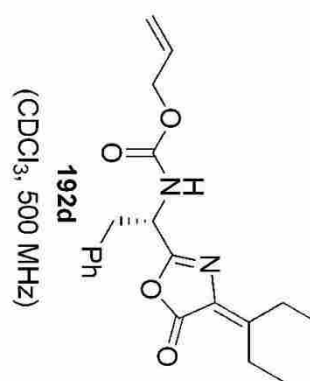


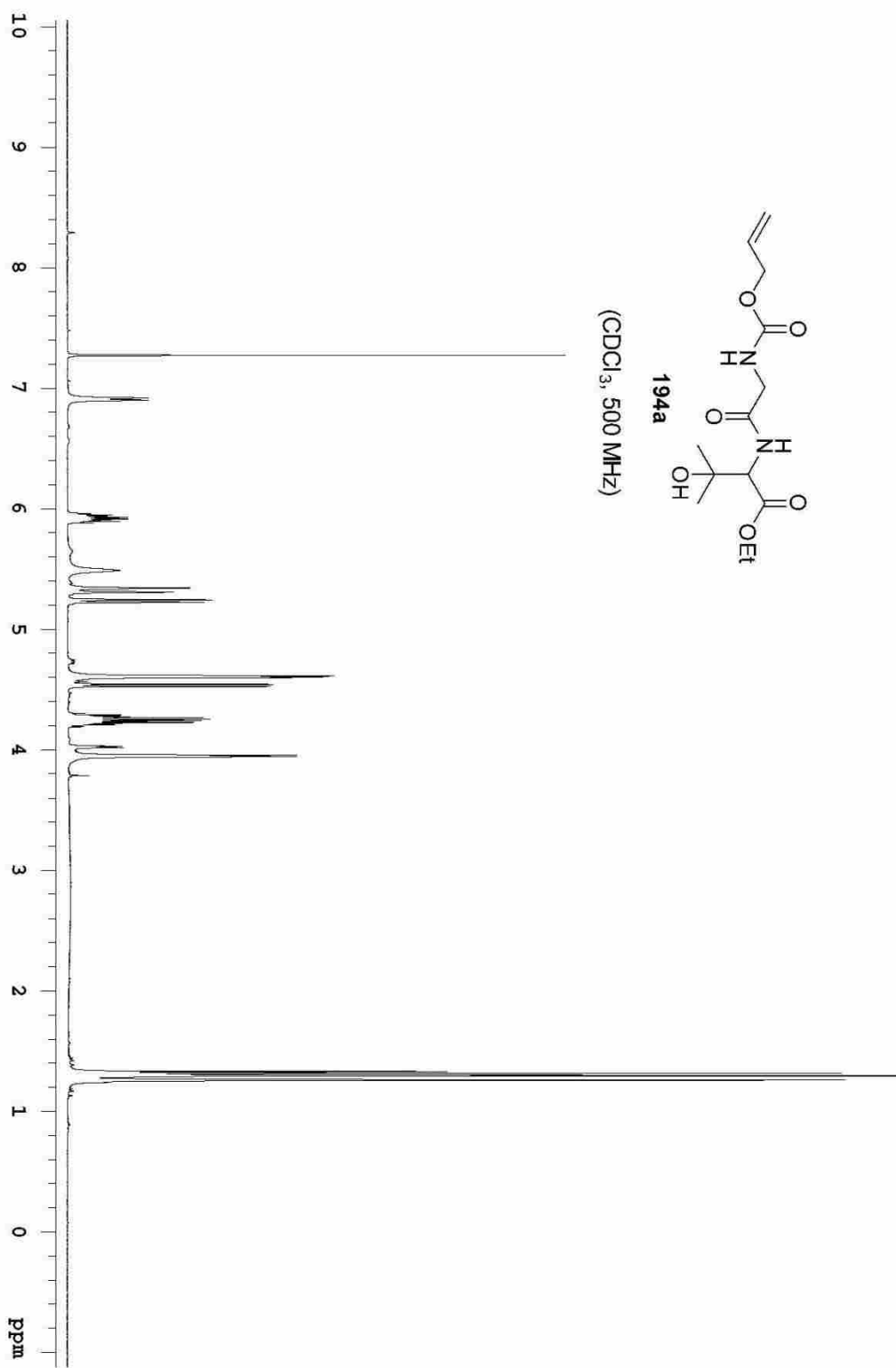
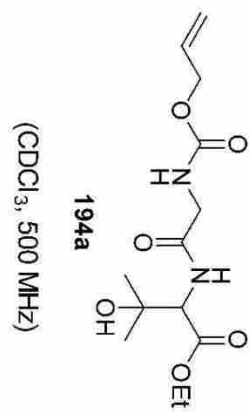


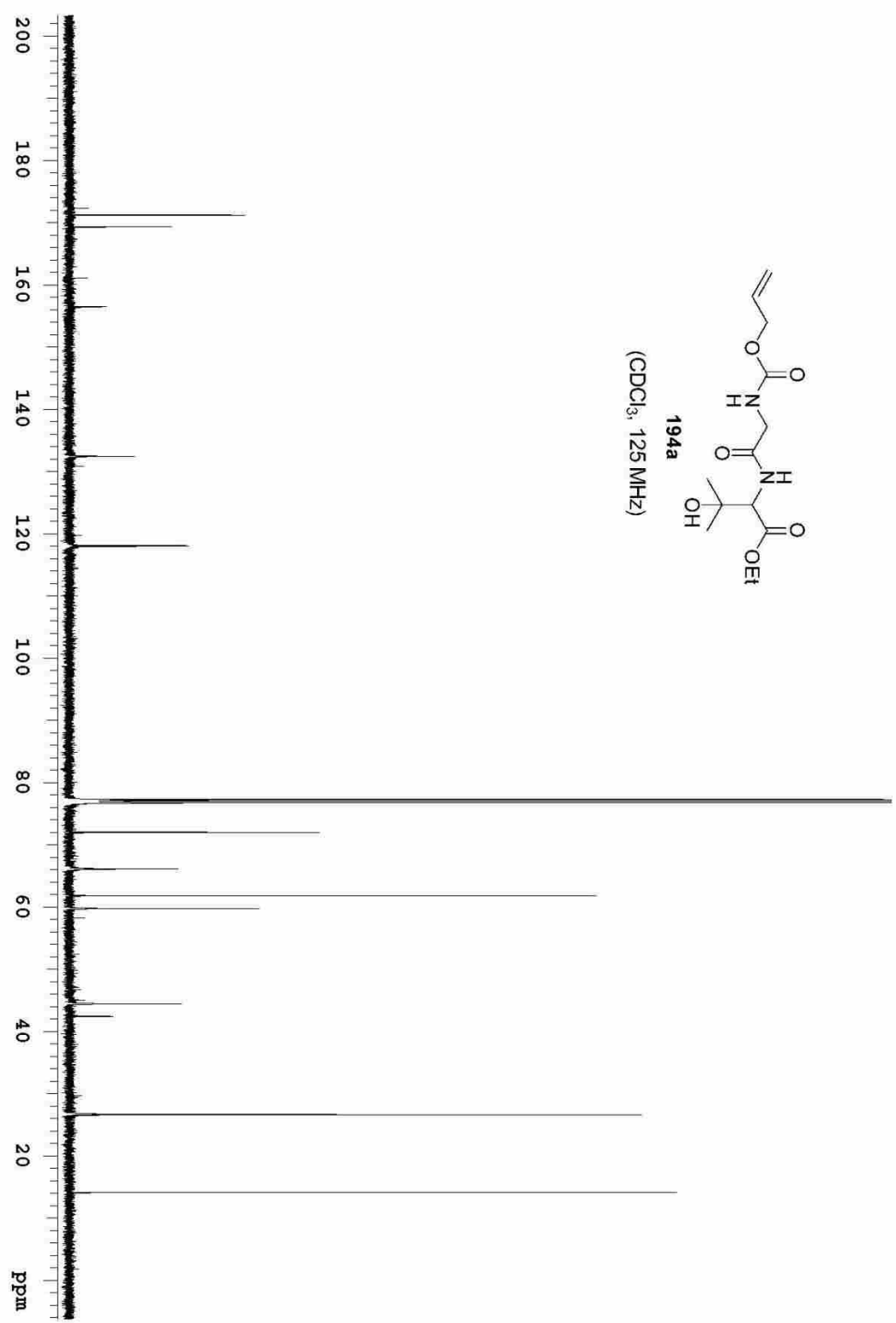
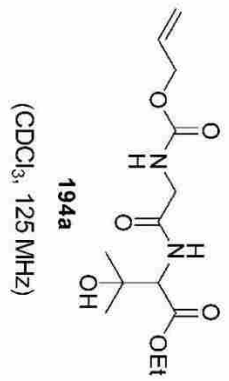


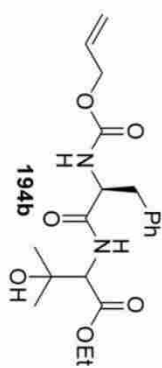
(CDCl₃, 500 MHz)



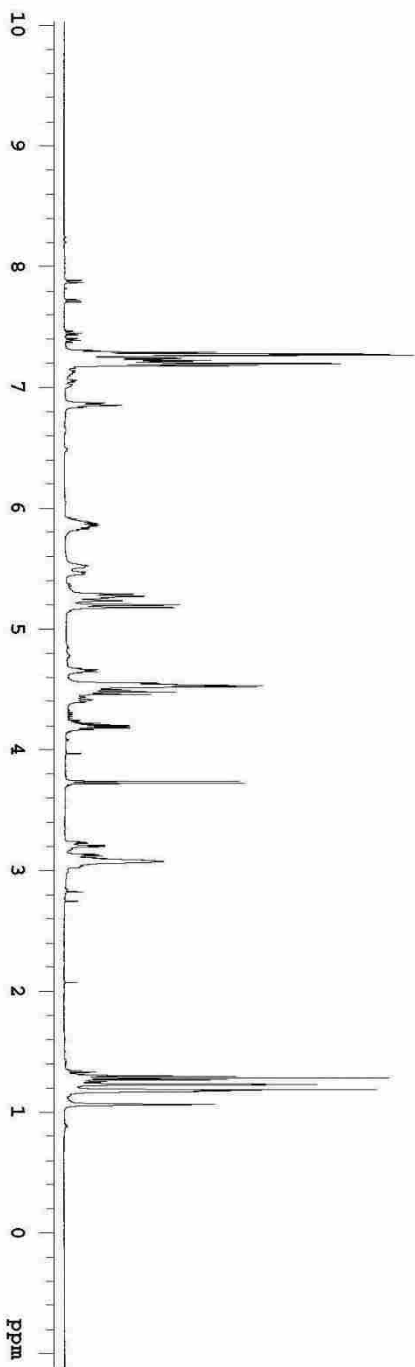


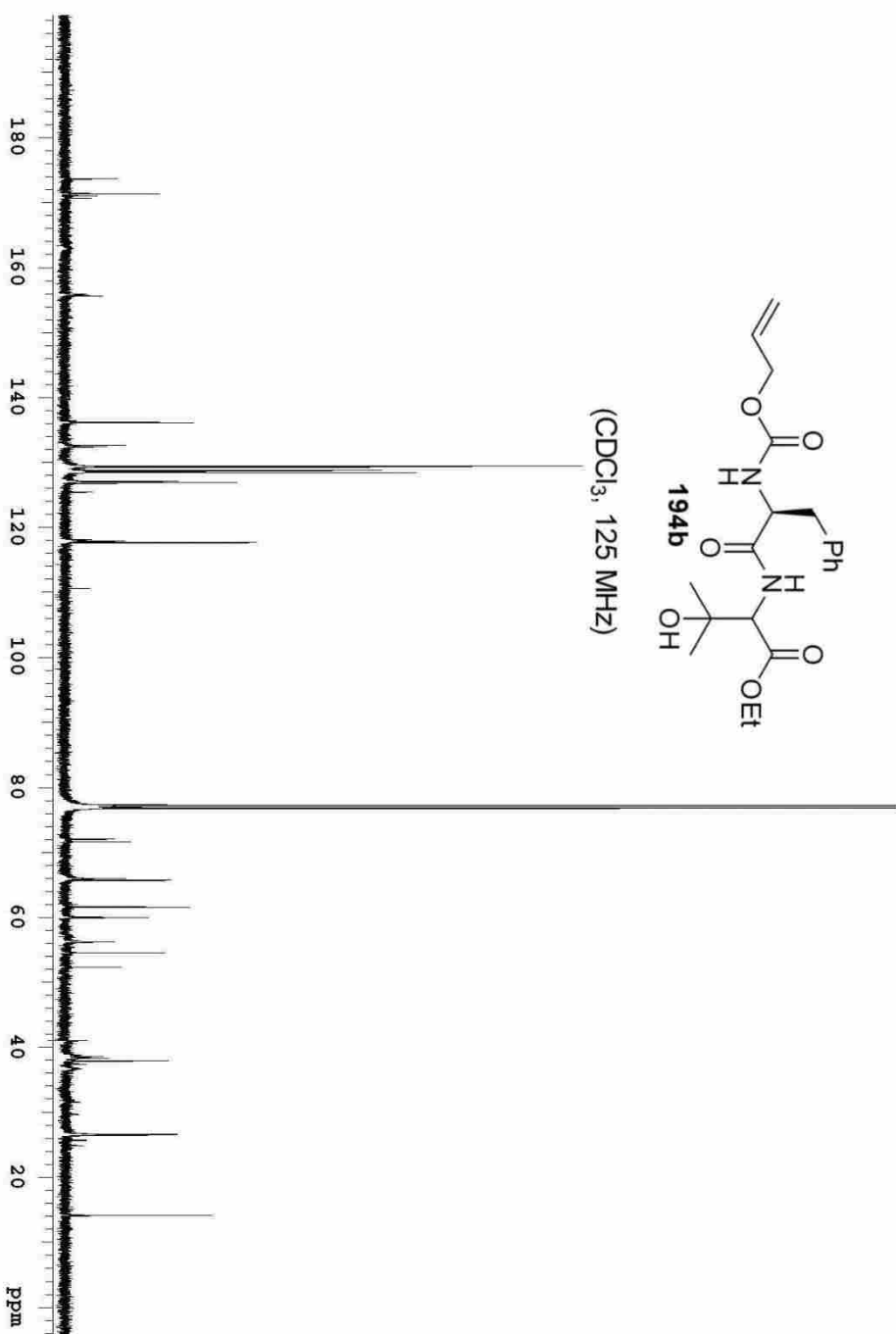


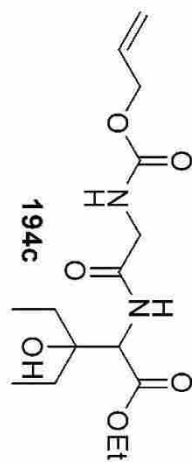




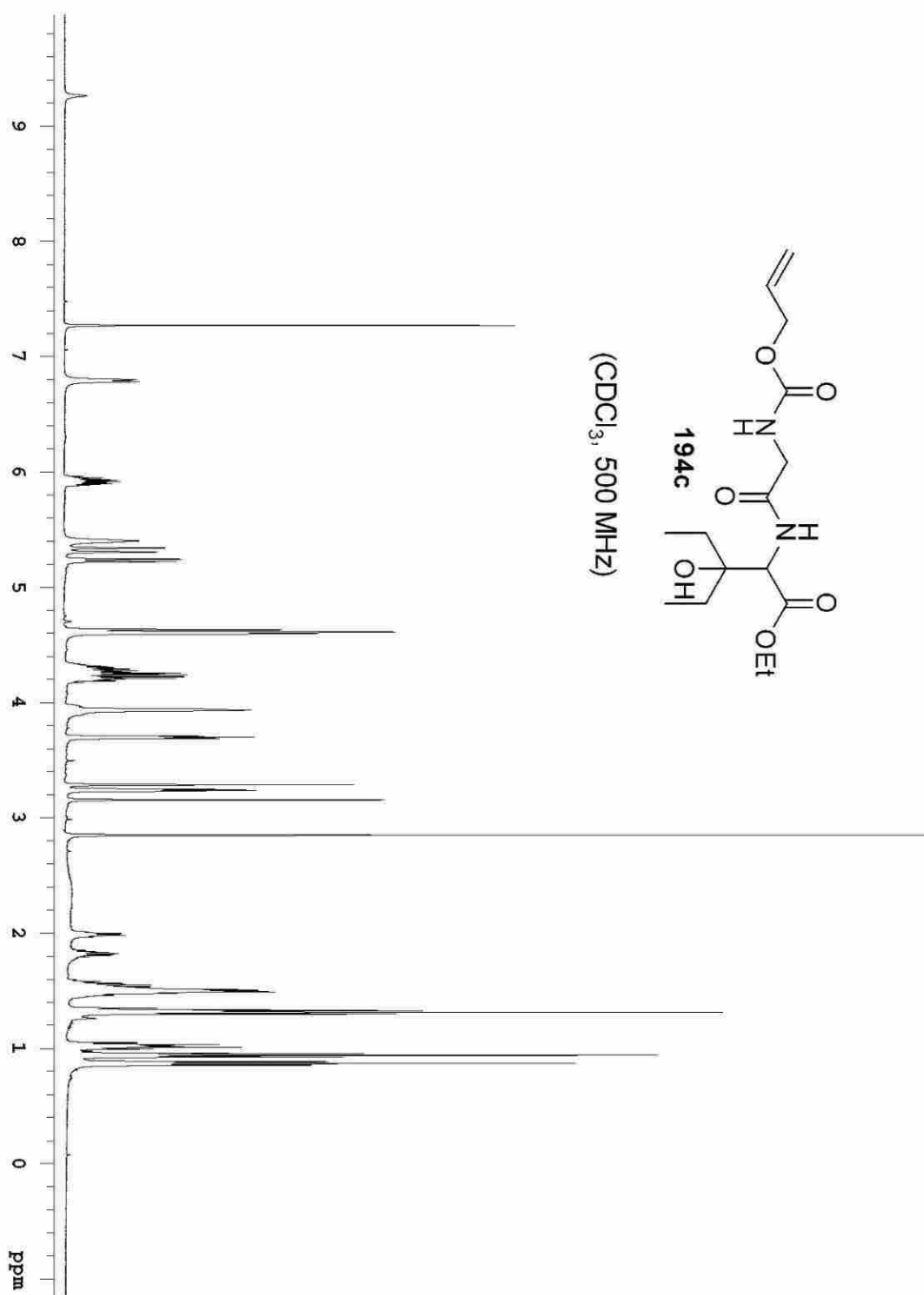
(CDCl₃, 500 MHz)

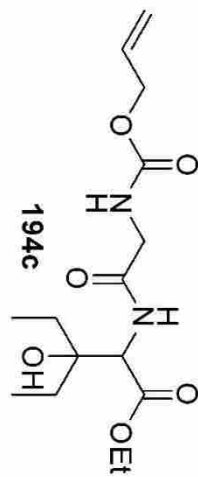




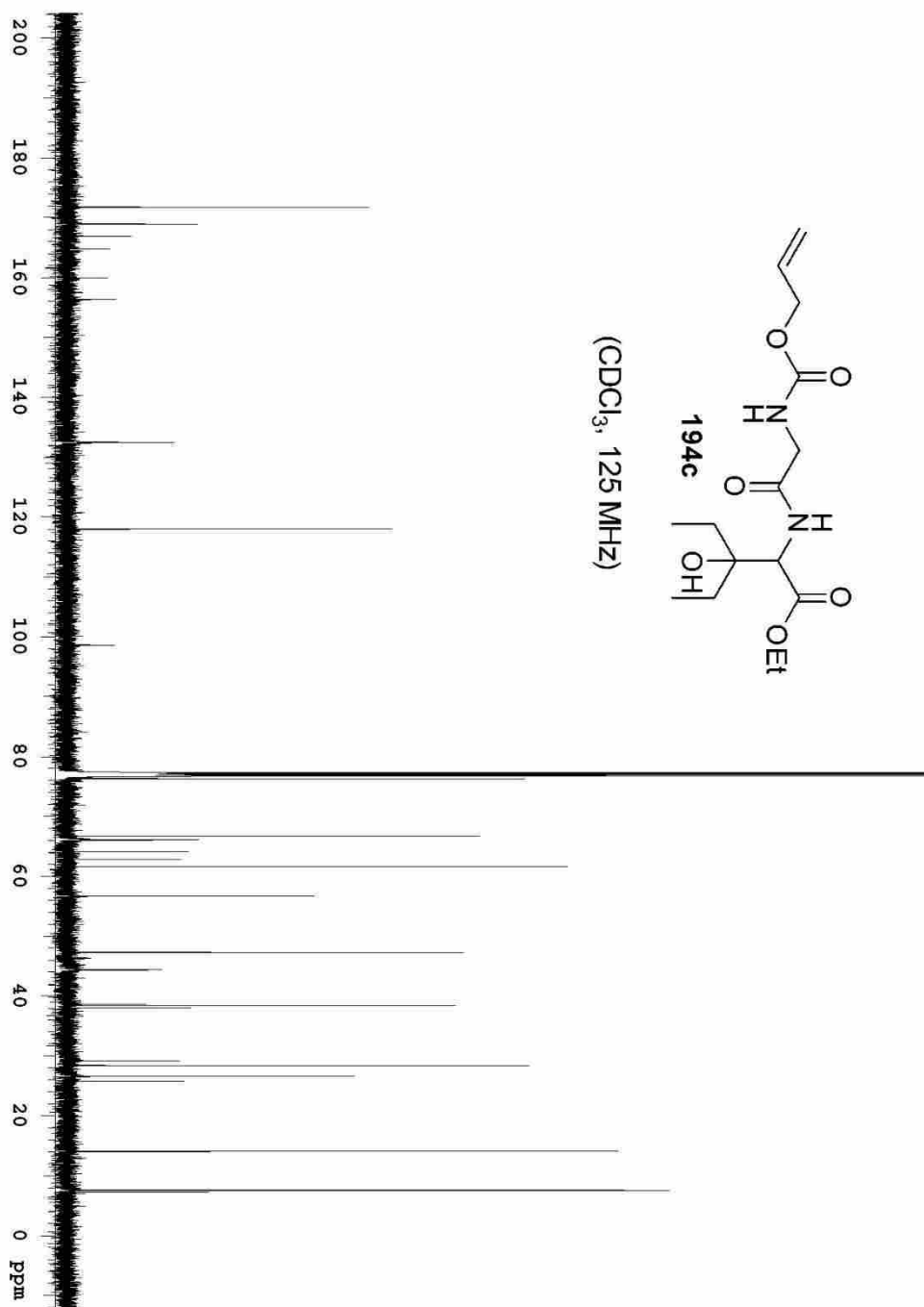


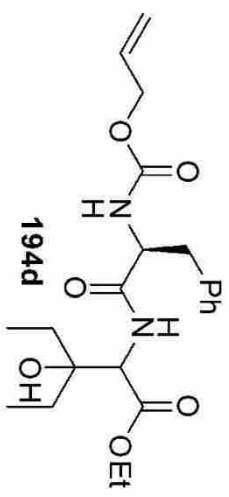
(CDCl₃, 500 MHz)



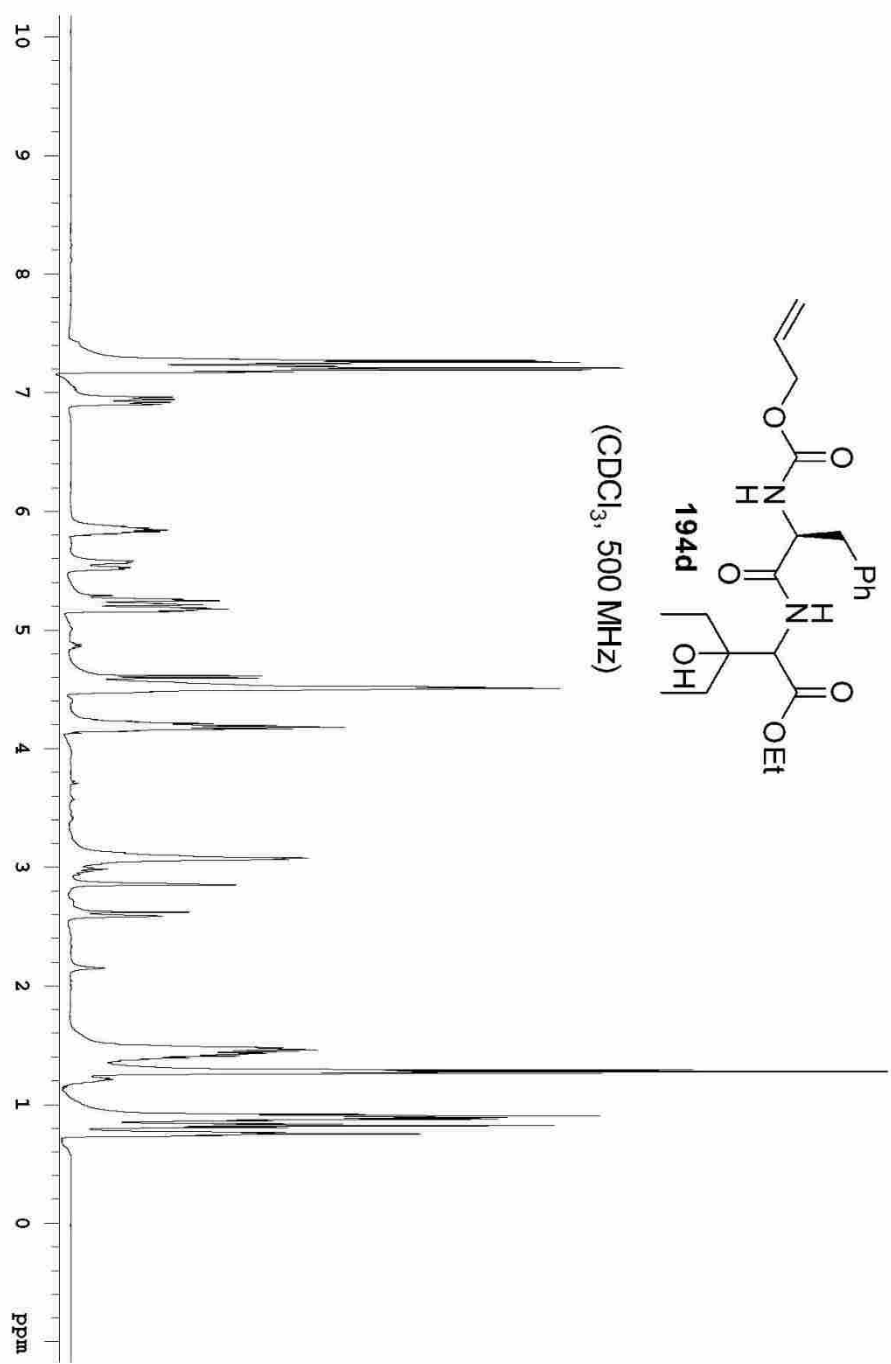


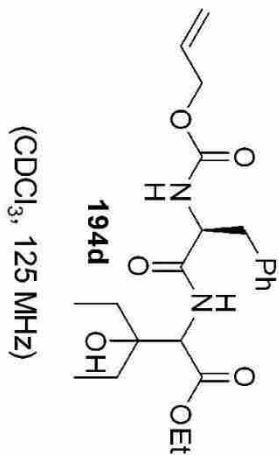
(CDCl₃, 125 MHz)

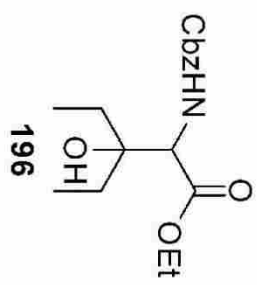




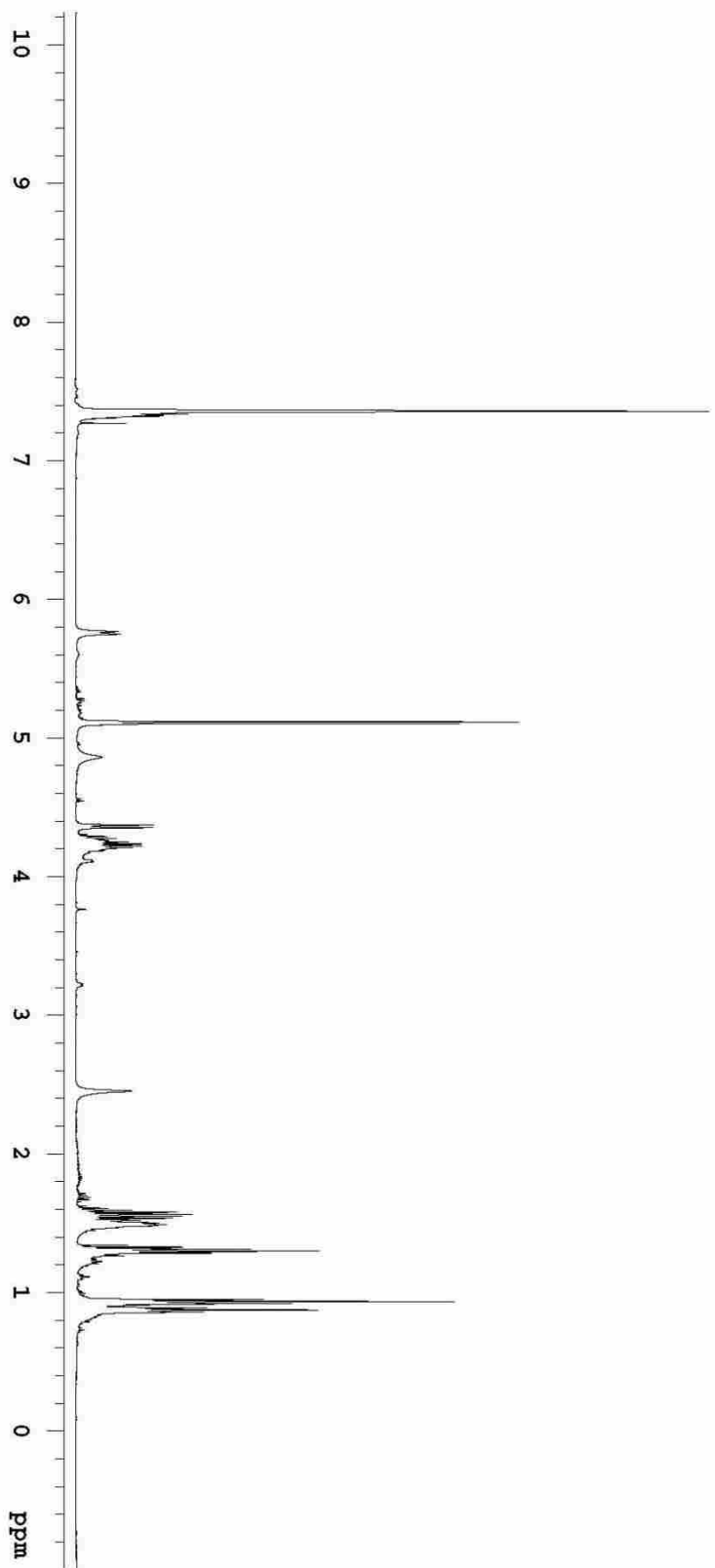
(CDCl₃, 500 MHz)

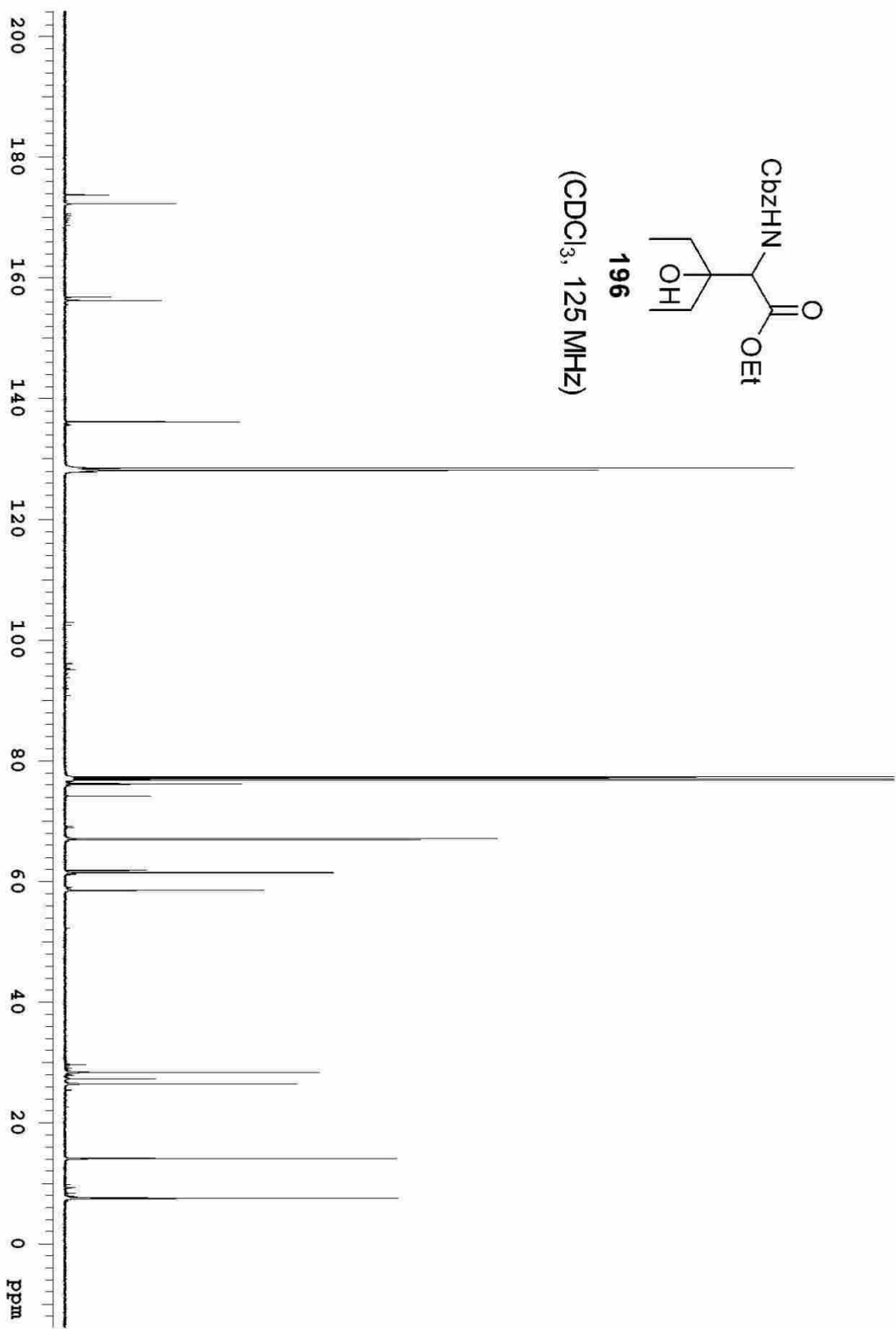
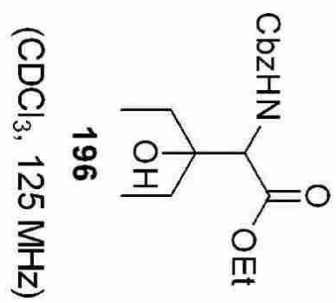


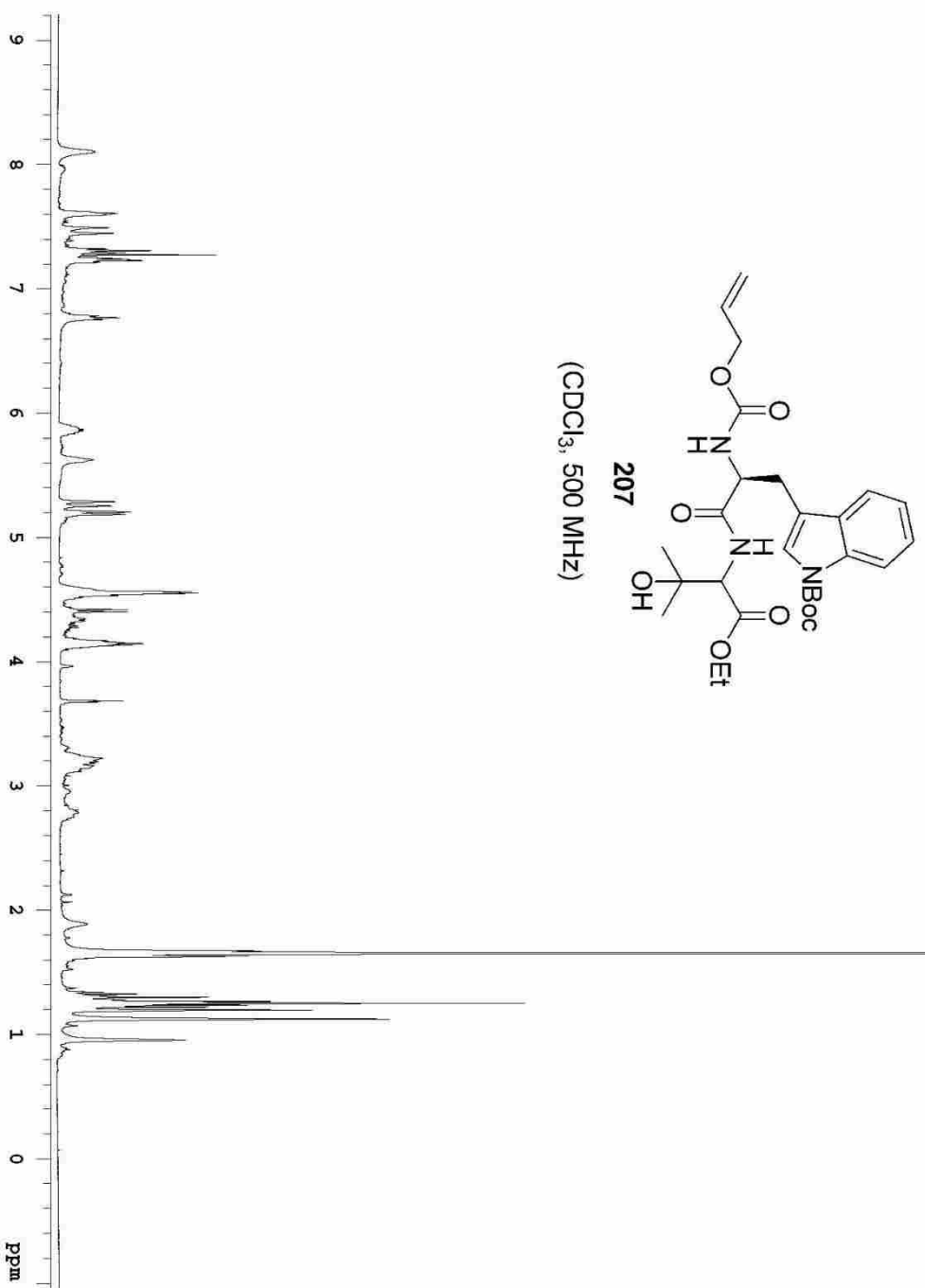
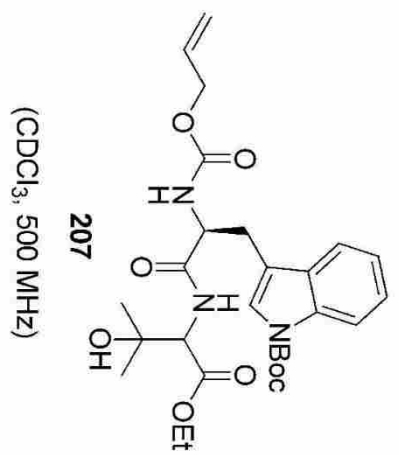


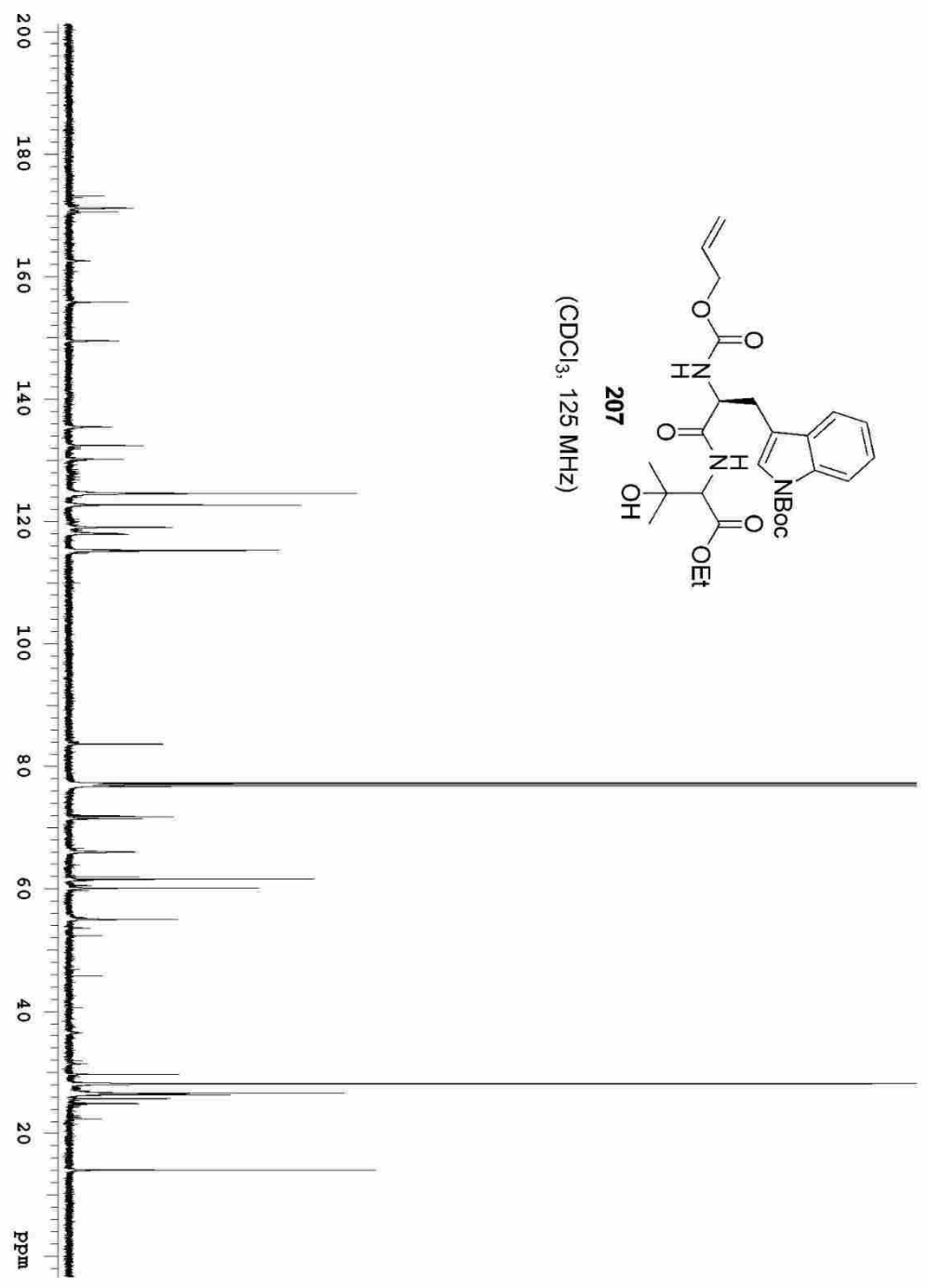
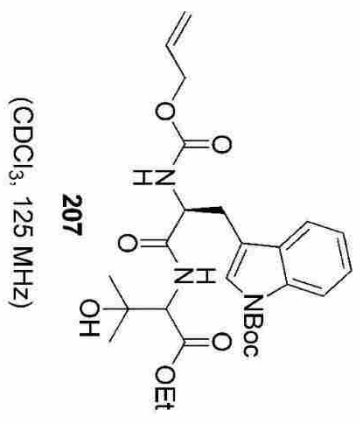


(CDCl₃, 500 MHz)





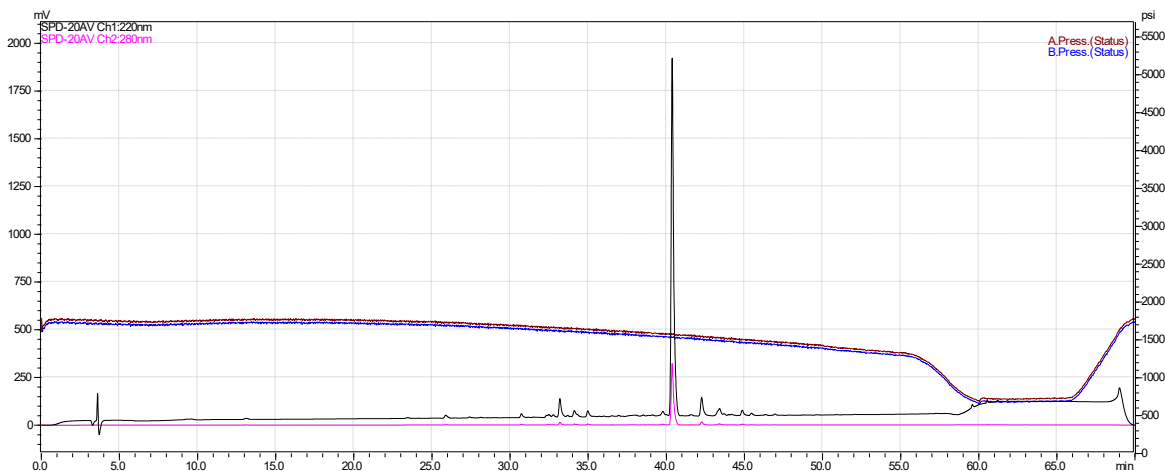




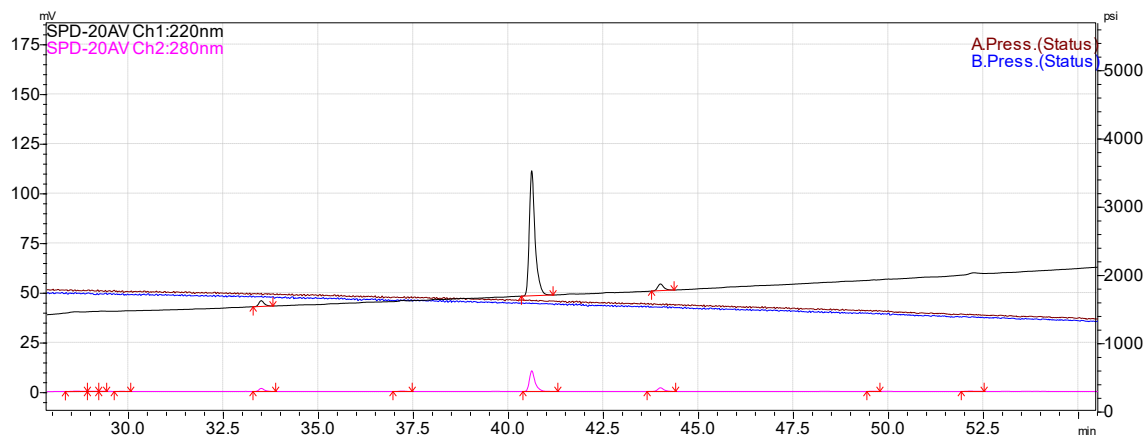
6.5 HRMS Data and HPLC Traces of Peptides 201a–d

201a: HRMS (ESI) m/z 1326.6124 (MH^+ , $C_{74}H_{79}N_{13}O_{11}H^+$ requires 1326.6100)

Crude **201a** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

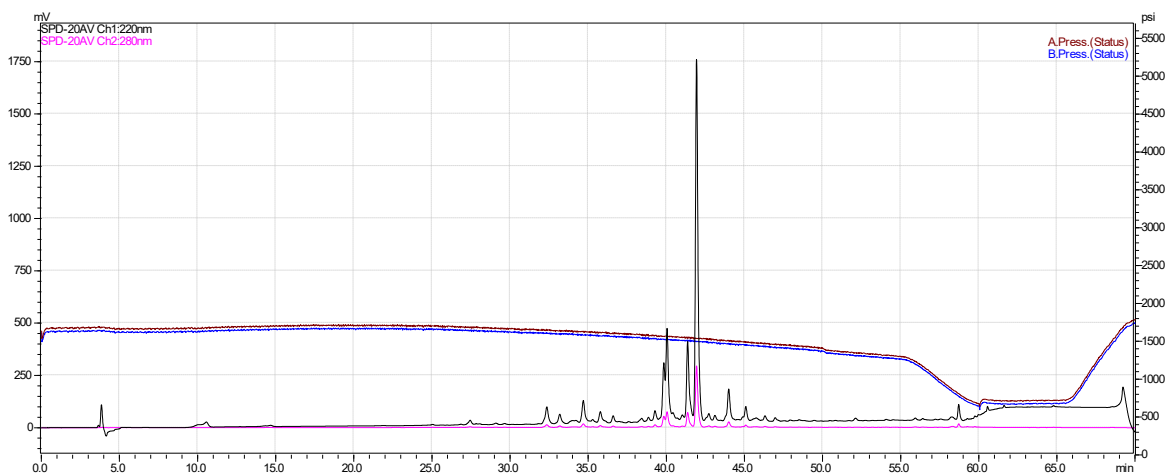


Purified **201a** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

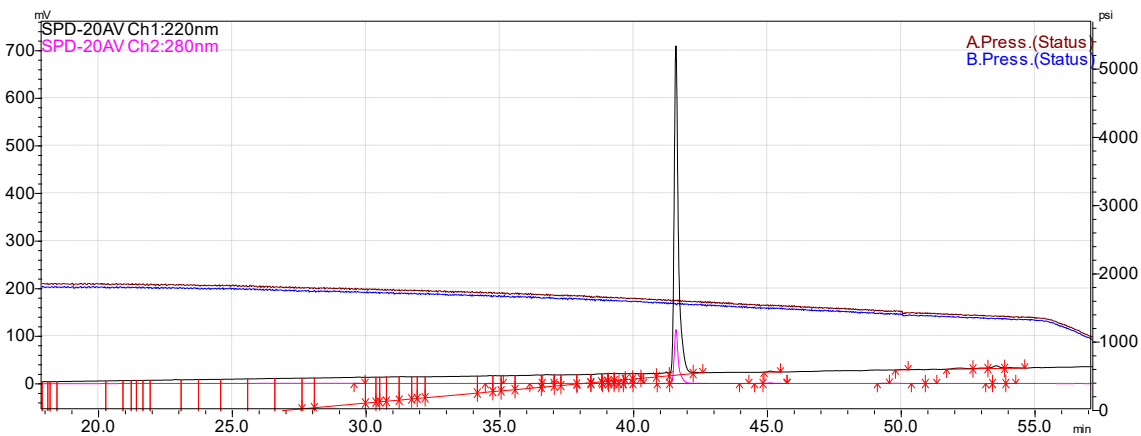


201b: HRMS (ESI) m/z 1416.6556 (MH^+ , $C_{81}H_{85}N_{13}O_{11}H^+$ requires 1416.6570)

Crude **201b** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

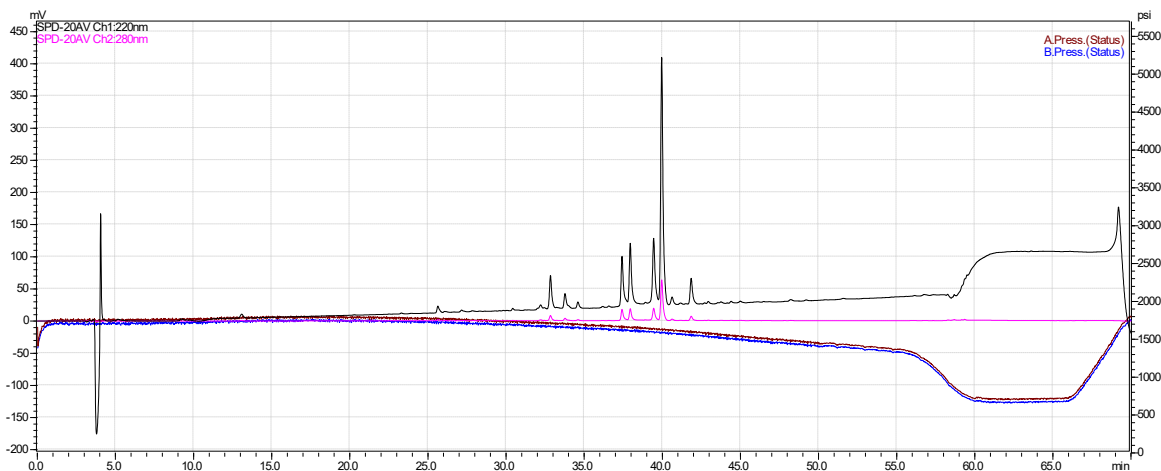


Purified **201b** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

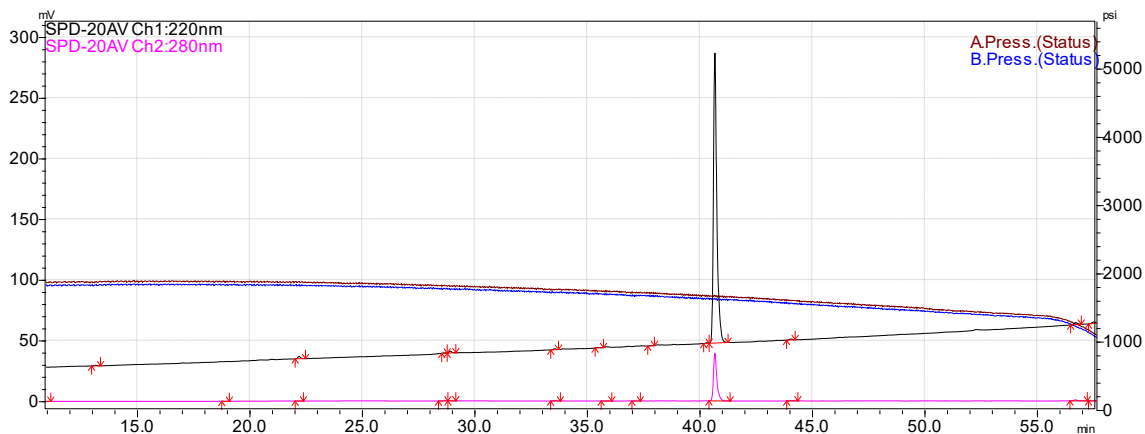


201c: HRMS (ESI) m/z 1354.6430 (MH^+ , $C_{76}H_{83}N_{13}O_{11}H^+$ requires 1354.6413)

Crude **201c** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

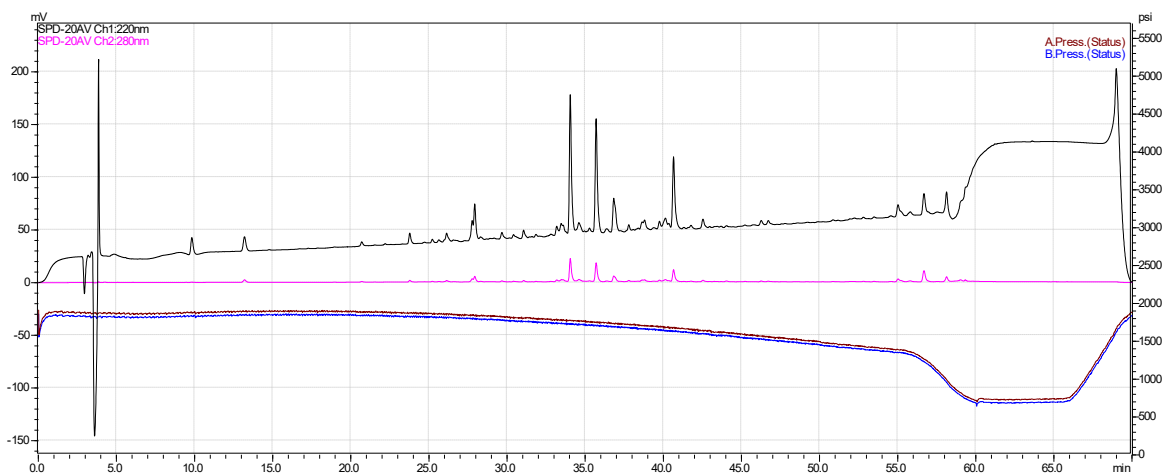


Purified **201c** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)



201d: HRMS (ESI) m/z 1444.6921 (MH^+ , $C_{83}H_{89}N_{13}O_{11}H^+$ requires 1444.6883)

Crude **201d** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)



Purified **201d** (Discovery C18 Column (25 cm \times 4.6 mm, 5 μ m), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

