2016-07-01

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

Jintao Jiang
Brigham Young University

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

Steven L. Castle, Chair
Merritt B. Andrus
Roger G. Harrison
Matt A. Peterson
Joshua L. Price

Department of Chemistry and Biochemistry

Brigham Young University

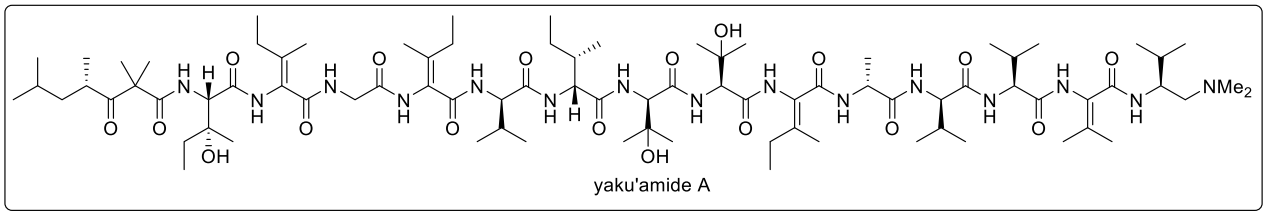
July 2016

ABSTRACT

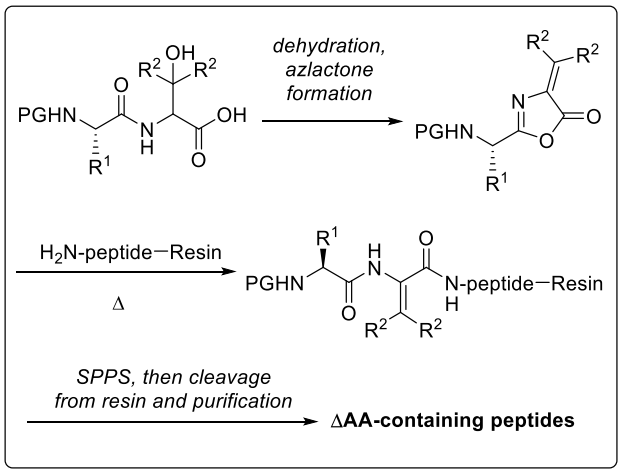
Studies Toward Yaku'amide A and Synthesis and Applications of
Bulky α,β-Dehydroamino Acids

Jintao Jiang
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→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 ΔVal and ΔEnv into small synthetic peptides via Solid Phase Peptide Synthesis (SPPS). We have also prepared two analogues of a monomeric 3_{10}-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, ΔVal and ΔEnv, SPPS, analogues

ACKNOWLEDGEMENTS

I gratefully acknowledge everyone that has supported me throughout my Ph.D. studies at Brigham Young University. Without the generous support of my mentors, colleagues, friends and family this dissertation would not have been possible.

First and foremost, I would like to thank my research advisor, Dr. Steven L. Castle, for his support and encouragement throughout the two projects I have worked on. When there were headwinds toward my research, he provided many constructive suggestions and solutions for the problems. And he inspired me to work efficiently and guided me through difficult times of my research.

I would also like to thank my graduate committee members, Dr. Merritt B. Andrus, Dr. Roger G. Harrison, Dr. Matt A. Peterson and Dr. Joshua L. Price for their suggestions and mentorship with my projects.

My thanks to Dr. Scott R. Burt for helping me with the NMR experiments and Dr. Brad Berges for running the assay experiments.

I also take this opportunity to thank all the staff of the Department of Chemistry and Biochemistry for providing a nice workplace. Janet Fonoimoana helped graduate students a lot from the very beginning.

It was an honor to work with a lot of great people in the Castle group and I would like to thank Joseph Cardon, Shi Luo, Zhiwei Ma for their contributions to the research.

I would like to thank the Department of Chemistry and Biochemistry at Brigham Young University for providing me a great opportunity to pursue a Ph.D. I gratefully acknowledge the financial support from BYU Graduate Studies and Simmons Center for Cancer Research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

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 ^{1}H, ^{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]

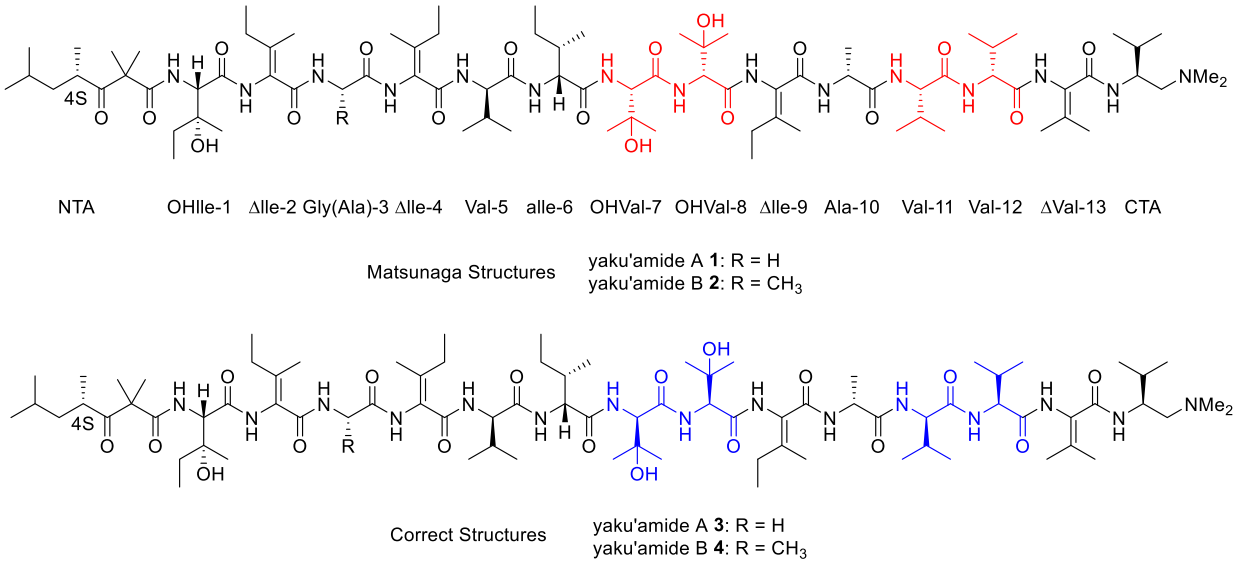
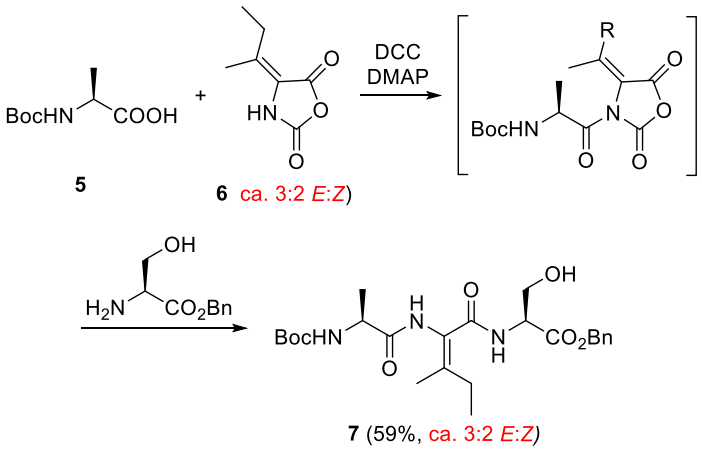


Figure 1.1 Structures of yaku'amide A (R = H) and yaku'amide B (R = CH_3)

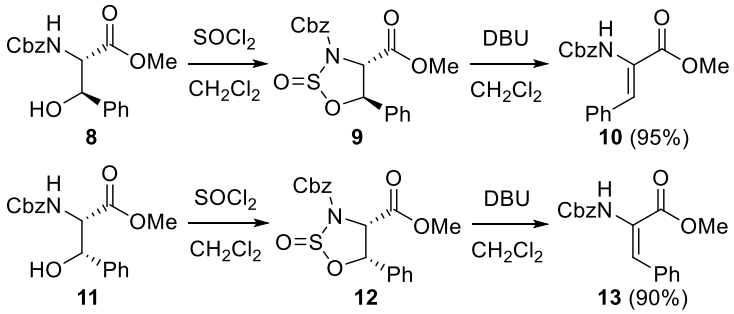
1.2 **Dehydroisoleucine Synthesis**

Yaku'amide A contains two Z-α,β-dehydroisoleucine (ΔIle) and one E-α,β-dehydroisoleucine (ΔIle) residues. It is challenging to selectively install Z- and E-α,β-dehydroisoleucine (ΔIle).[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 ΔIle-derived anhydride **6** as a linchpin to generate a mixture of alkene isomers **7** which limits its application.

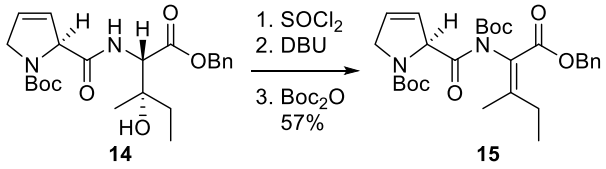


Scheme 1.1 Shin's Dehydroisoleucine Synthesis

Wandless and co-workers developed a two-step *anti* dehydration to stereoselectively construct bulky trisubstituted and tetrasubstituted ΔAAs.[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*-ΔPhe derivative **10** in very good yield (Scheme 1.2).[103] The same methodology yielded *E*-ΔPhe derivative **13** from **11**. Next, they applied this method to dipeptide **14** to provide *E*-ΔIle dipeptide **15** (Scheme 1.3), which was used as a building block for the total synthesis of phomopsin B[7]. 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.

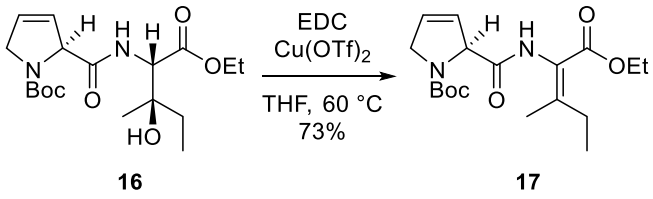


Scheme 1.2 Wandless Pre-Dehydroisoleucine Synthesis



Scheme 1.3 Wandless Dehydroisoleucine Synthesis

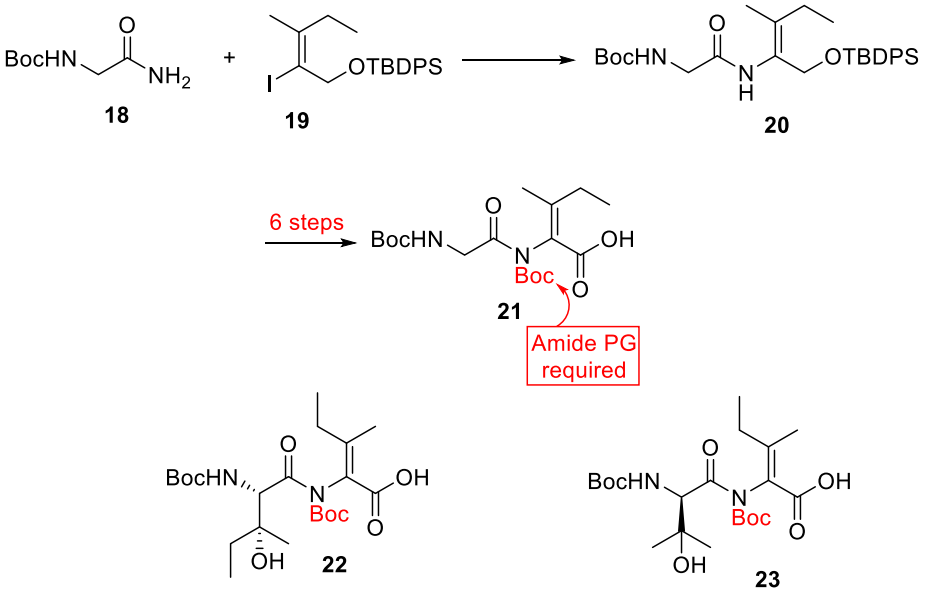
In order to synthesize phomopsin, Shangguan and Joullié developed a copper–carbodiimide *syn* dehydration to access the tripeptide side chain.[8] They synthesized compound **16** from commercially available material and screened the reaction conditions for the dehydration reaction. In the presence of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

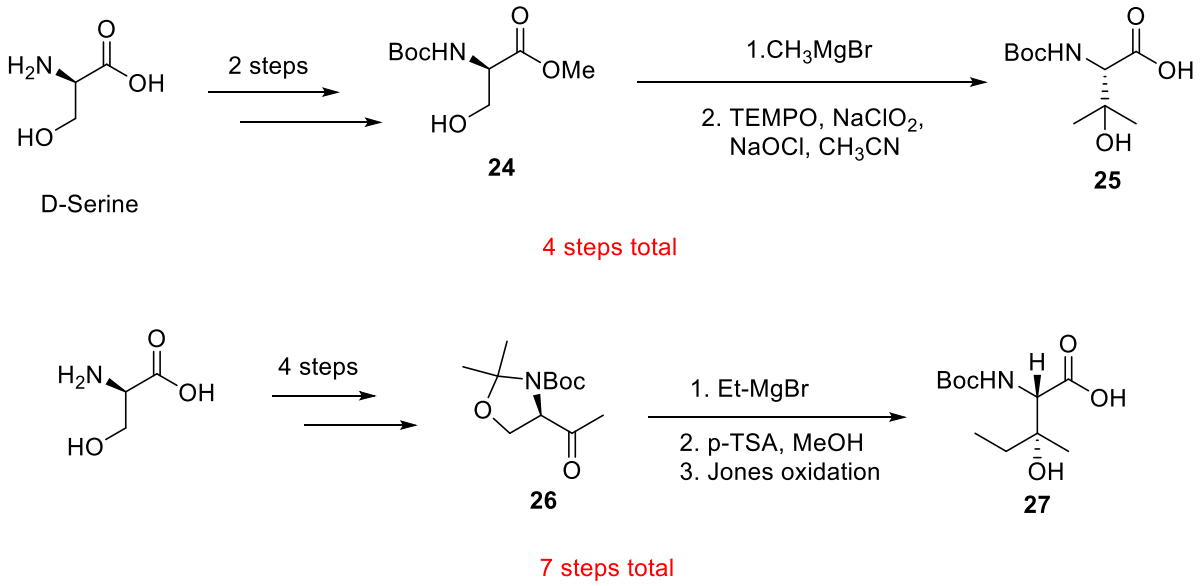
The Inoue group finished the first total synthesis of yaku'amide A. For the challenging dehydroisoleucine synthsis, they adopted a Cu-catalyzed amidation method with vinyl iodides[9] to generate the stereospecific subunits of yaku'amide A such as **20**. To continue the synthesis, they used a cumbersome 6-step processs 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

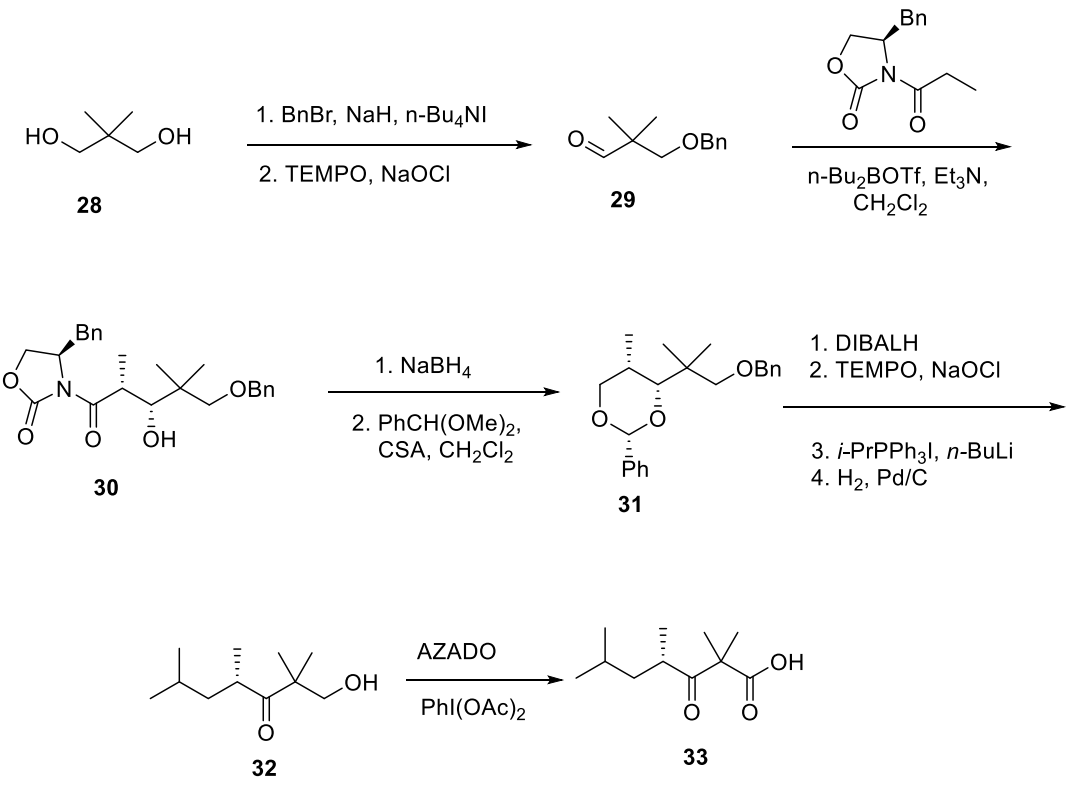
Inoue and co-workers used methods developed by Lubell[10] and Guanti[11] for the synthesis of β-OHVal and β-OHIle derivatives. N-(*tert*-Butoxycarbonyl)-D-serine methyl ester was generated in two steps from D-Serine. With a Grignard reaction and oxidation in the presence of TEMPO, they generated β-OHVal 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

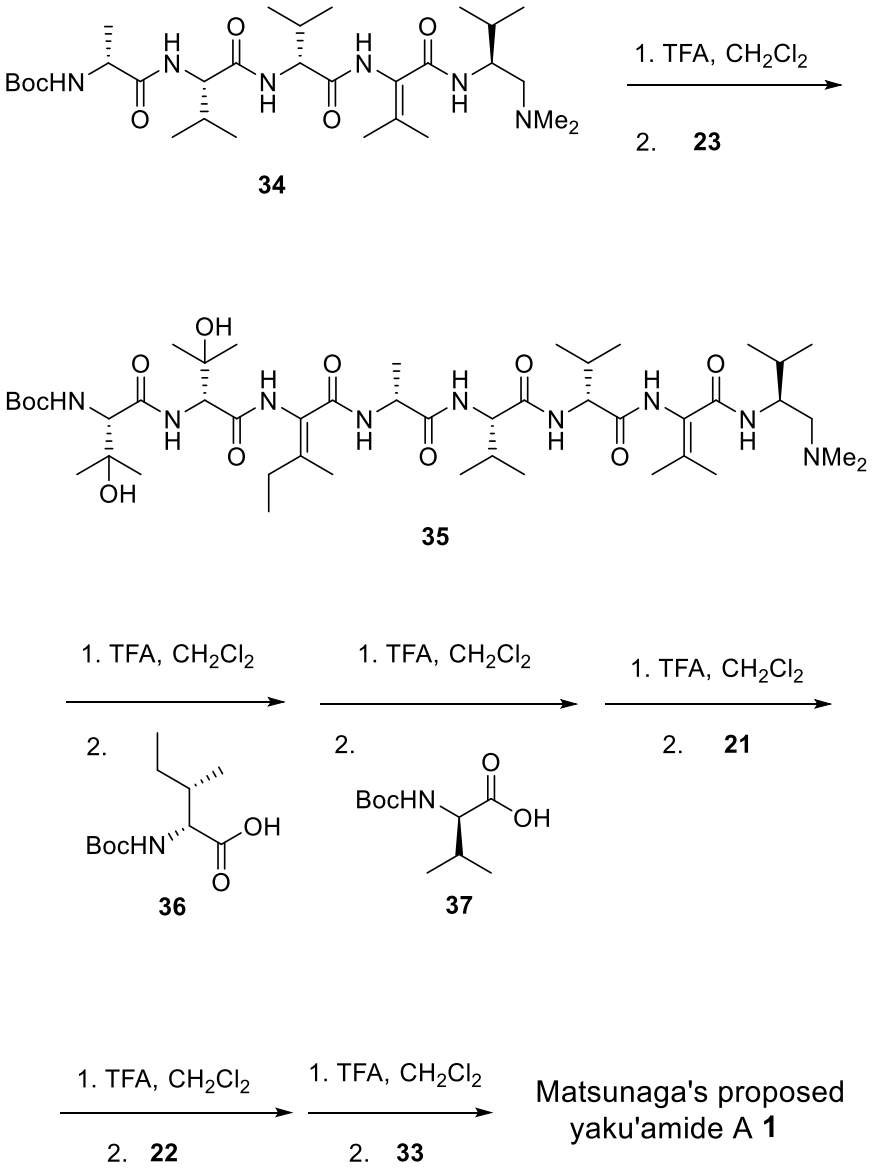
For the synthesis of NTA subunit **33**, Inoue and co-workers generated aldehyde **29** in two steps from diol **18** through monobenzylation and oxidation (Scheme 1.7). Then they adopted the Evans asymmetric aldol reaction[12] to install the C4 (S)-stereocenter. Reduction of syn-aldol intermediate **30** with $NaBH_4$ 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 AZADO[13] and $PhI(OAc)_2$[14] 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 occuring 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**

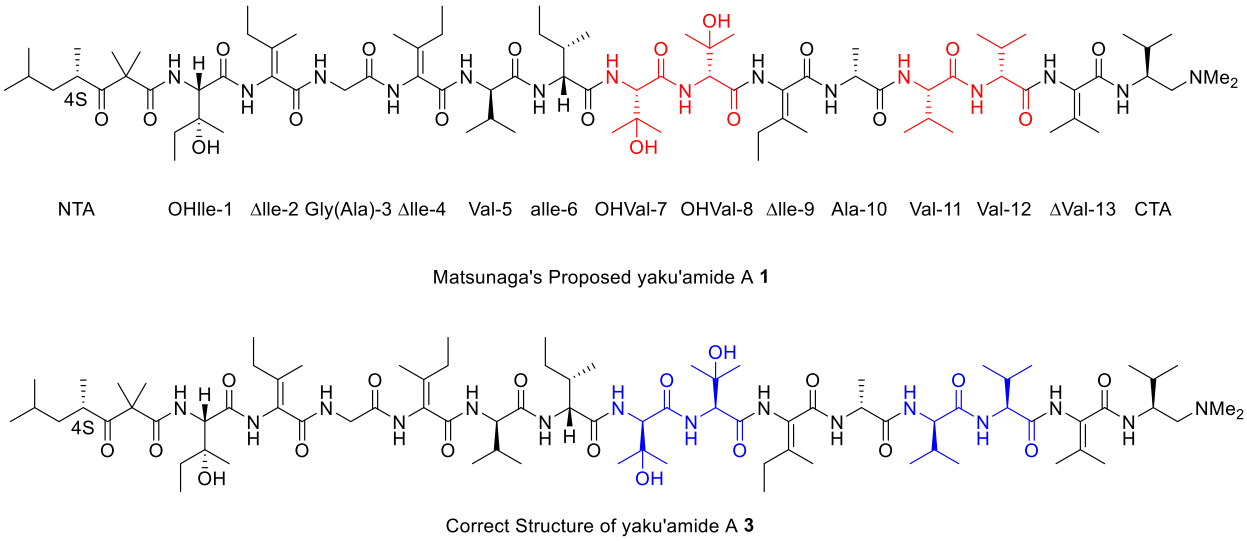


Figure 1.2 Structures of Matsunaga's Proposed and Naturally Occuring yaku'amide A

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-OHIle 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**

1. Ueoka, R.; Ise, Y.; Ohtsuka, S.; Okada, S.; Yamori, T.; Matsunaga, S. *J. Am. Chem. Soc.* **2010**, *132*, 17692.
2. Kuranaga, T.; Sesoko, Y.; Sakata, K.; Maeda, N.; Hayata, A.; Inoue, M. *J. Am. Chem. Soc.* **2013**, *135*, 5467.
3. Kuranaga, T.; Mutoh, H.; Sesoko, Y.; Goto, T.; Matsunaga, S.; Inoue, M. *J. Am. Chem. Soc.* **2015**, *137*, 9443.
4. Jiang, J.; Ma, Z.; Castle, S. L. *Tetrahedron* **2015**, 71, 5431.
5. Nakamura, Y.; Ito, A.; Shin, C. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2151.
6. Stohlmeyer, M. M.; Tanaka, H.; Wandless, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 6100.
7. Grimley, J. S.; Sawayama, A. M.; Tanaka, H.; Stohlmeyer, M. M.; Woiwode, T. F.; Wandless, T. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 8157.
8. Shangguan, N.; Joullié, M. *Tetrahedron Lett.* **2009**, *50*, 6748.
9. (a) Klapars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421. (b) Jiang, L.; Job, G. E.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 3667.
10. Dettwiler, J. E.; Lubell, W. D. *J. Org. Chem.* **2003**, *68*, 177.
11. Ageno, G.; Banfi, L.; Giuseppe Guanti, G. C.; Manghisi, E.; Riva, R.; Rocca, V. *Tetrahedron* **1995**, *51*, 8121.
12. (a) Evans, D. A.; Vogel, E.; Nelson, J. V. *J. Am. Chem. Soc.* **1979**, *101*, 6120-6123. (b) Evans, D. A.; Starr, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 13531.
13. Shibuya, M.; Tomizawa, M.; Suzuki, I.; Iwabuchi, Y. *J. Am. Chem. Soc.* **2006**, *128*, 8412.
14. Watanabe, T.; Imaizumi, T.; Chinen, T.; Nagumo, Y.; Shibuya, M.; Usui, T.; Kanoh, N.; Iwabuchi, Y. *Org. Lett.* **2010**, *12*, 1040.

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 (ΔIle); and (3) the installation of Z- and E-α,β-dehydroisoleucine (ΔIle) 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.

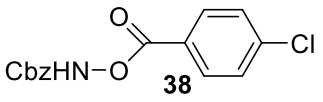
2.2 New Nitrogen Sources for Racemic Aminohydroxylation

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 aminohydroxylation[14-17] to prepare these β-hydroxyamino acids. Due to the regioselectivity,

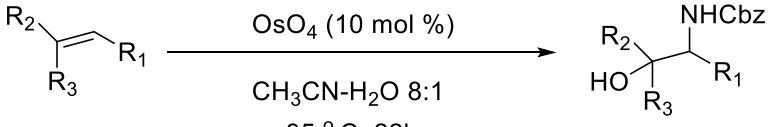
reactivity and substrate scope issues of the abovementioned methodologies, we plan to develop innovative methods to efficiently synthesize β-hydroxyamino acids.

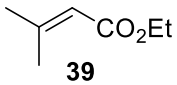
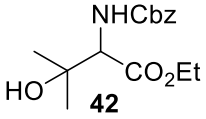
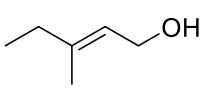
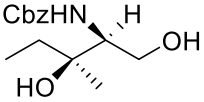
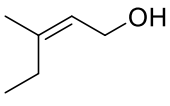
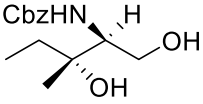
In preliminary studies, we have used a base-free intermolecular aminohydroxylation procedure to construct the β-hydroxyamino acids existing in yaku'amide A.[18] Aminohydroxylations of alkenes **39**-**41** employing acyloxycarbamate **38** as an effective nitrogen source generated racemic amino alcohols **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 ΔVal and Z-, E-ΔIle in yaku'amide A if we can also develop an efficient dehydration method.

Table 2.1 Regioselective Base-Free Aminohydroxylation



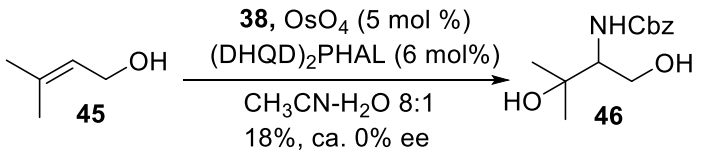
38



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

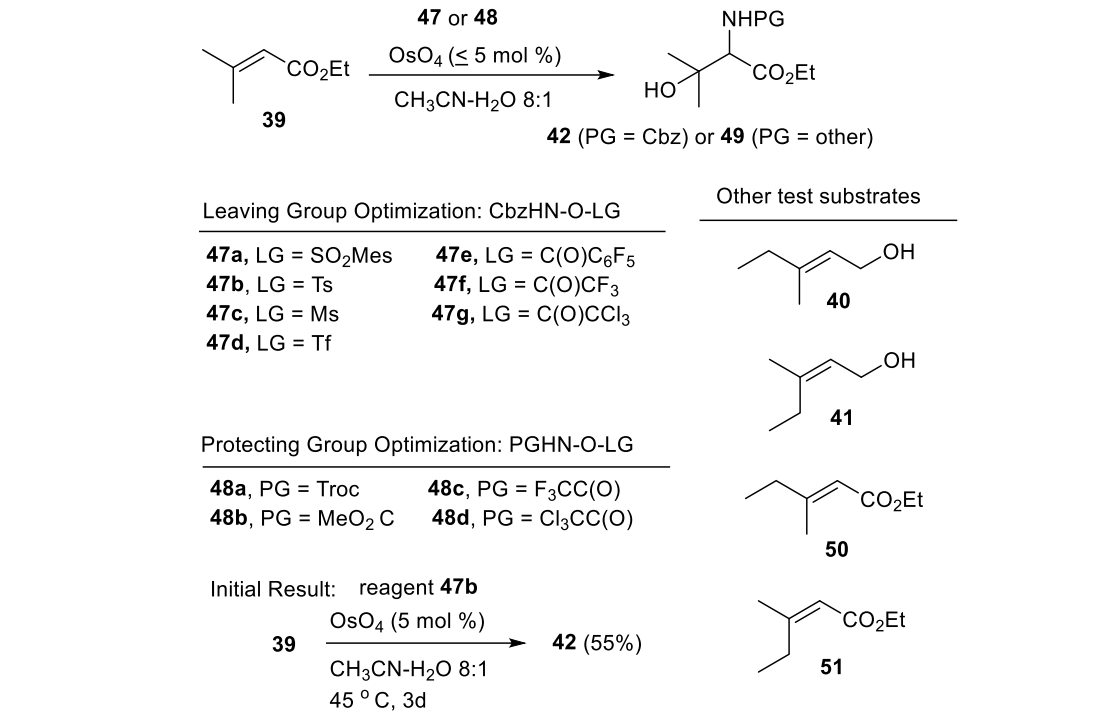
Compared with enoate **39**, trisubstituted allylic alcohol **45** is a better substrate for the racemic aminohydroxylation. So we started with allylic alcohol **45** as the testing substrate for attempted asymmetric aminohydroxylation to construct enantiopure β-hydroxyamino acids. However, we

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.

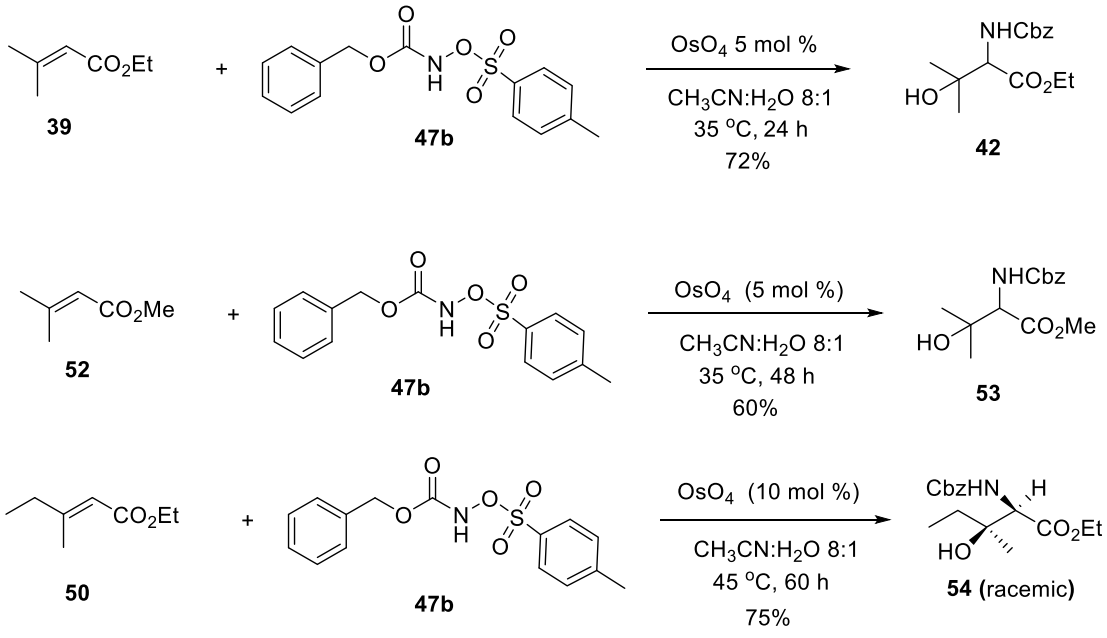


Scheme 2.1 Attempted Enantioselective Aminohydroxylation Reaction

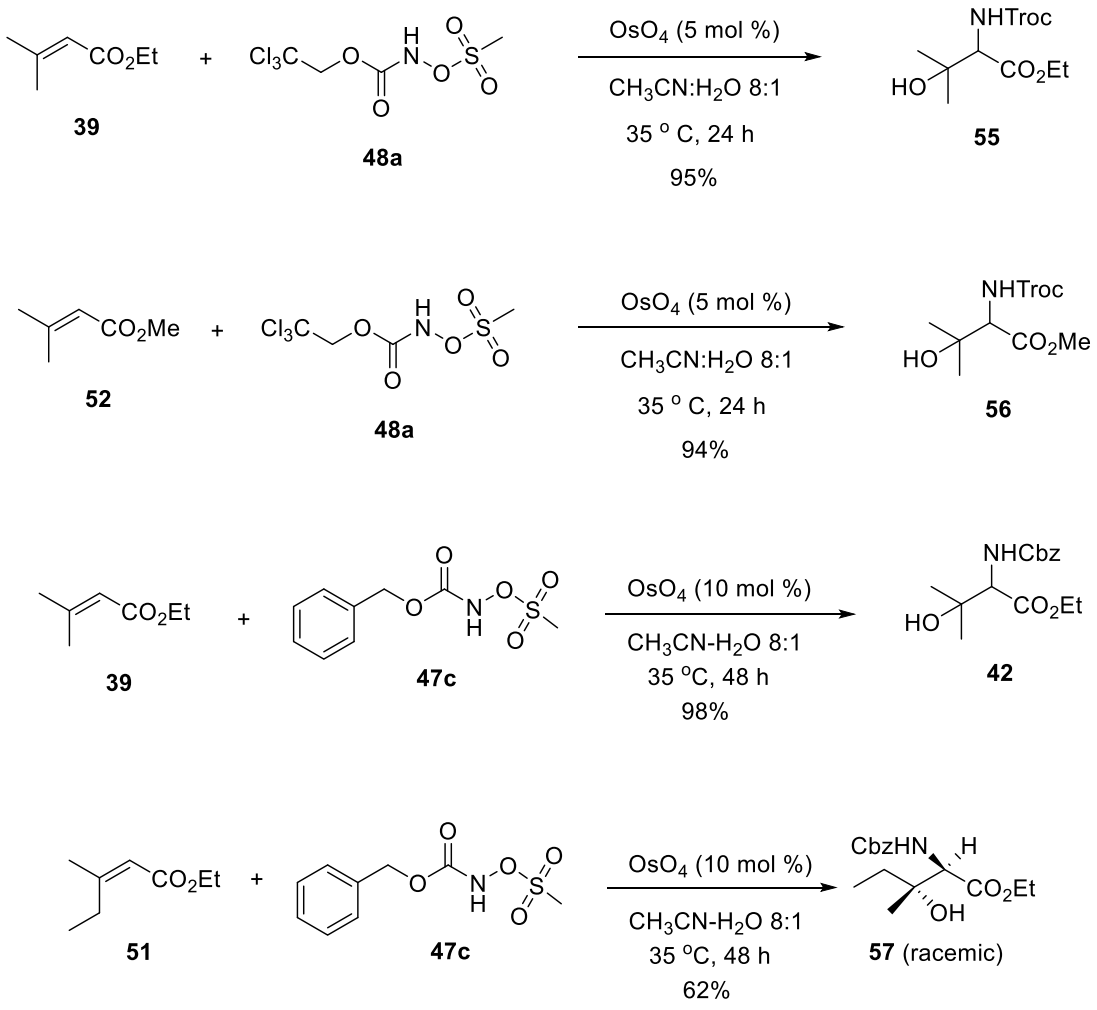
Although the racemic base-free aminohydroxylation is a useful process, there are still some limitations such as slow rate (significantly lower yields 20-40% were obtained when carried out at lower temperatures and catalyst loadings) and the formation of Cbz-NH_2 as a byproduct. We also used 10 mol % OsO_4 loading for all the reactions in our first-generation protocol. Our initial experiment with 5 mol % OsO_4 did not proceed very well. Motivated by Donohoe's work on tethered substrates[19] and Luxenburger's research on protecting groups[20] 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).



Scheme 2.2 New Nitrogen Sources for Aminohydroxylations



Scheme 2.3 Improvements with Tosyloxycarbamates

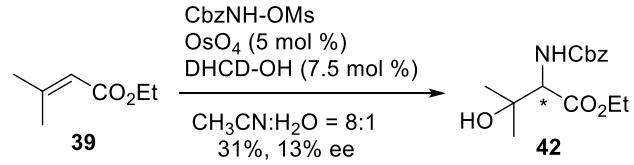


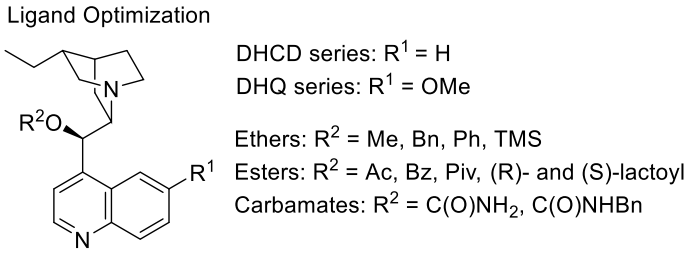
Scheme 2.4 Improvements with Mesyloxycarbamates

With **47b** (CbzHN-OTs), **47c** (CbzHN-OMs), and **48a** (TrocHN-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** (TrocHN-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.





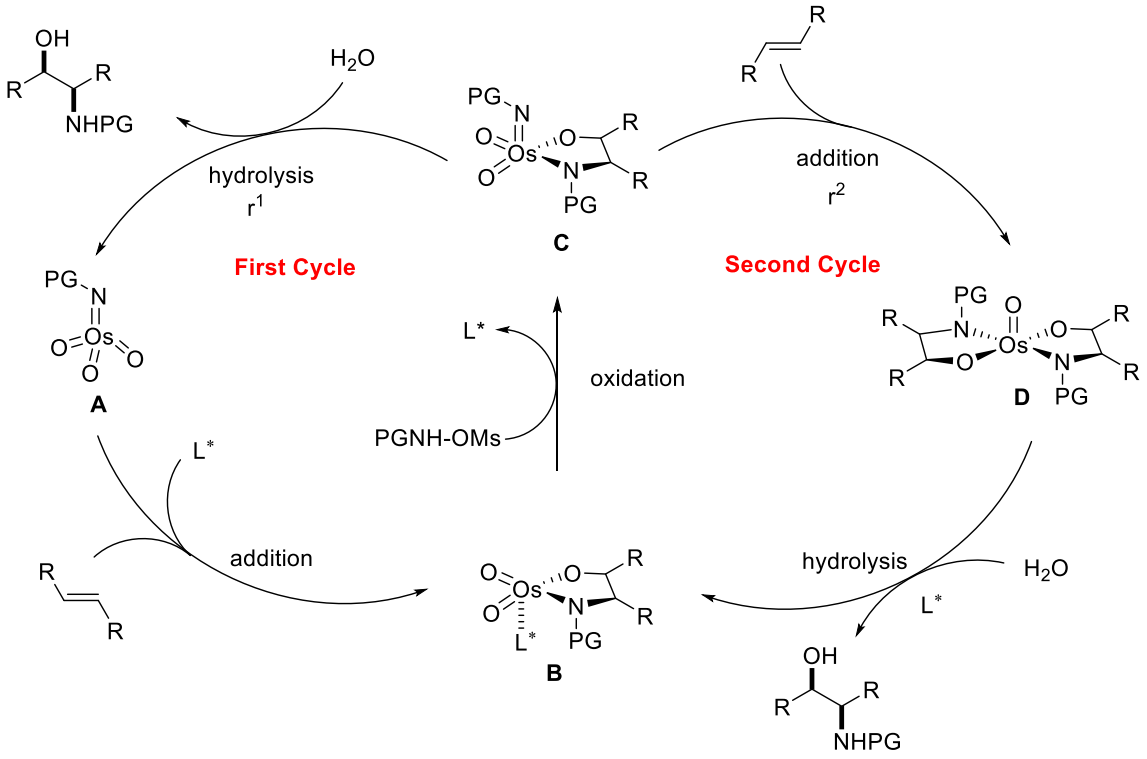
Scheme 2.5 Optimization of Ligand-Controlled Asymmetric Aminohydroxylation

Table 2.2 Initial Studies Toward Asymmetric Aminohydroxylation

Entry	Ligand	Yield	ee
1	DHQD-MEQ	64%	Racemic
2	DHQD-CLB	66%	Racemic
3	$DHQD_2PHAL$	54%	Racemic
4	$DHQD_2PYR$	70%	Racemic
5	DHQD-OH	52%	Racemic
6	DHCD-OH	31%	13%

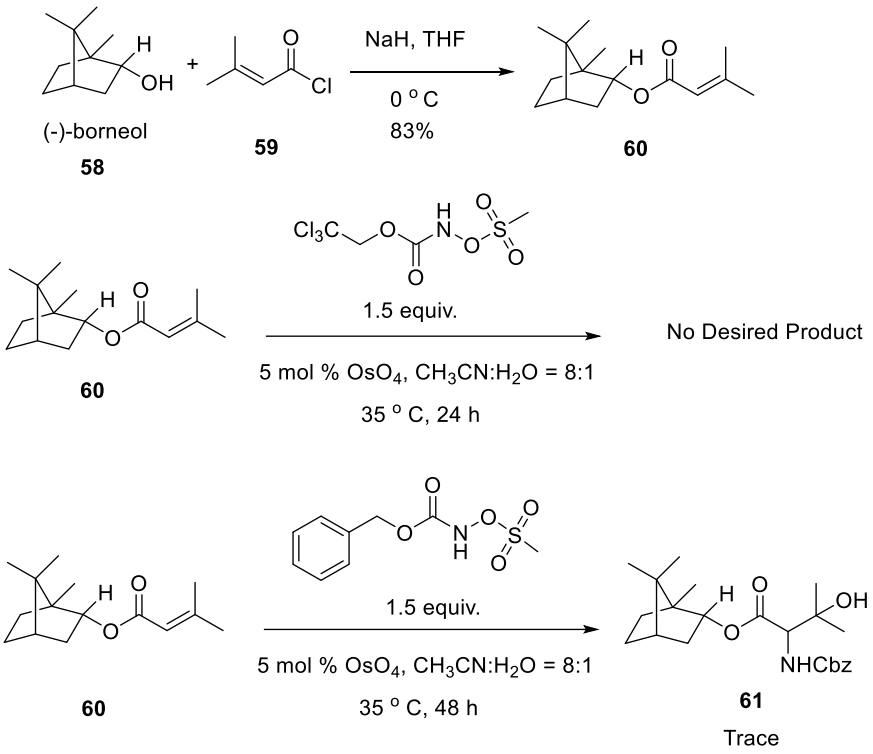
When we used dihydrocinchonidine (DHCD-OH) for this reaction, we only got low yield and 13% ee. But DHCD-OH may not be bulky enough, so we attached small and medium sized groups to DHCD-OH and DHQ-OH in order to get improved enantioselectivity. In addition, smaller nitrogen sources can provide higher 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]

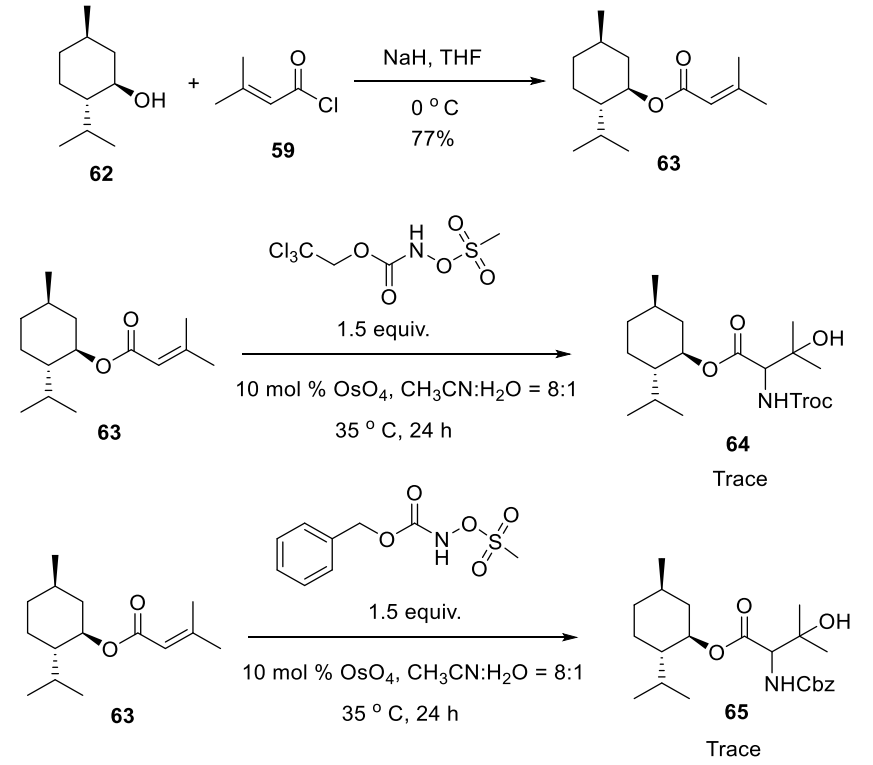


Scheme 2.6 Two Catalytic Cycles in Os-Catalyzed Aminohydroxylation

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** (TrocHN-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

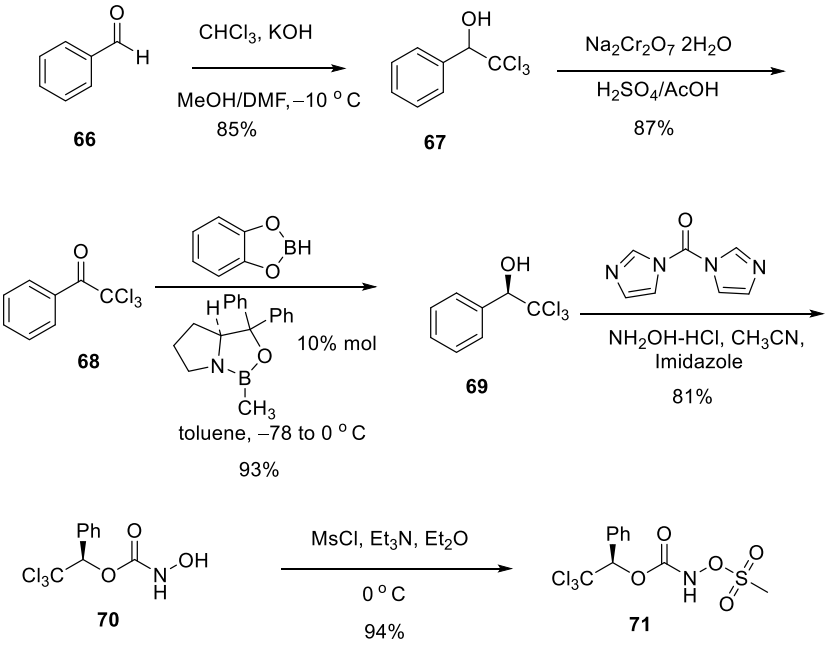


Scheme 2.8 Chiral Substrate from L-Menthol for Asymmetric Aminohydroxylation

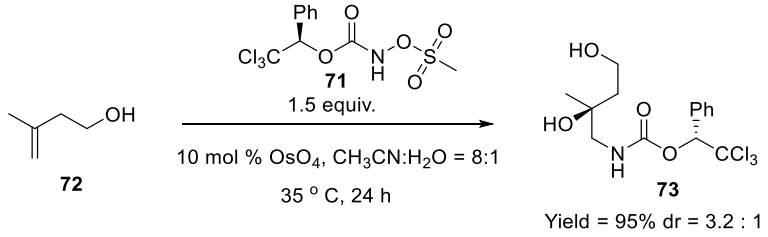
2.4 **Chiral Nitrogen Sources for Aminohydroxylation**

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.



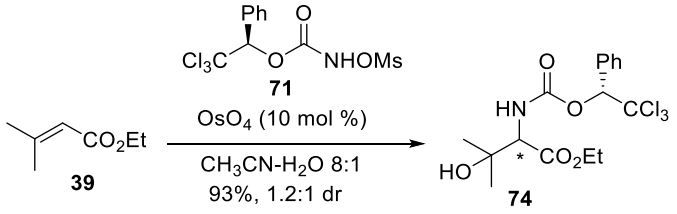
Scheme 2.9 Synthesis of Chiral Nitrogen Reagent



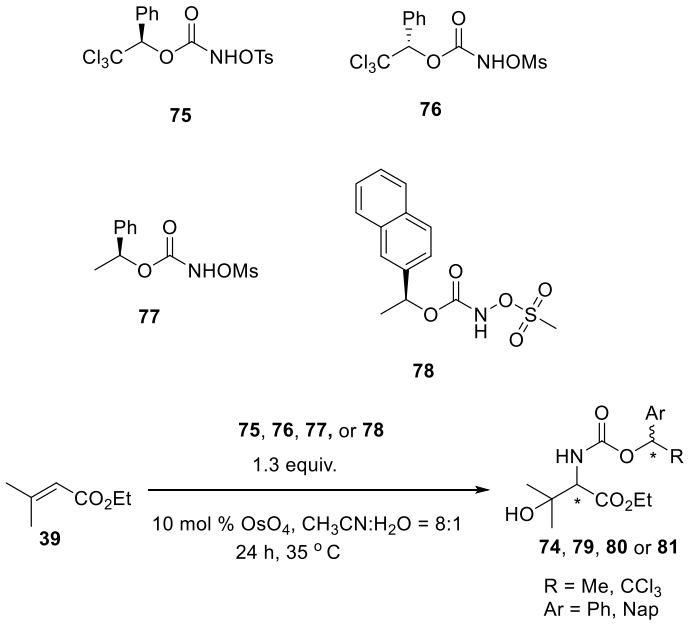
Scheme 2.10 Asymmetric Aminohydroxylation with Chiral Nitrogen Reagent

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 goups 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.



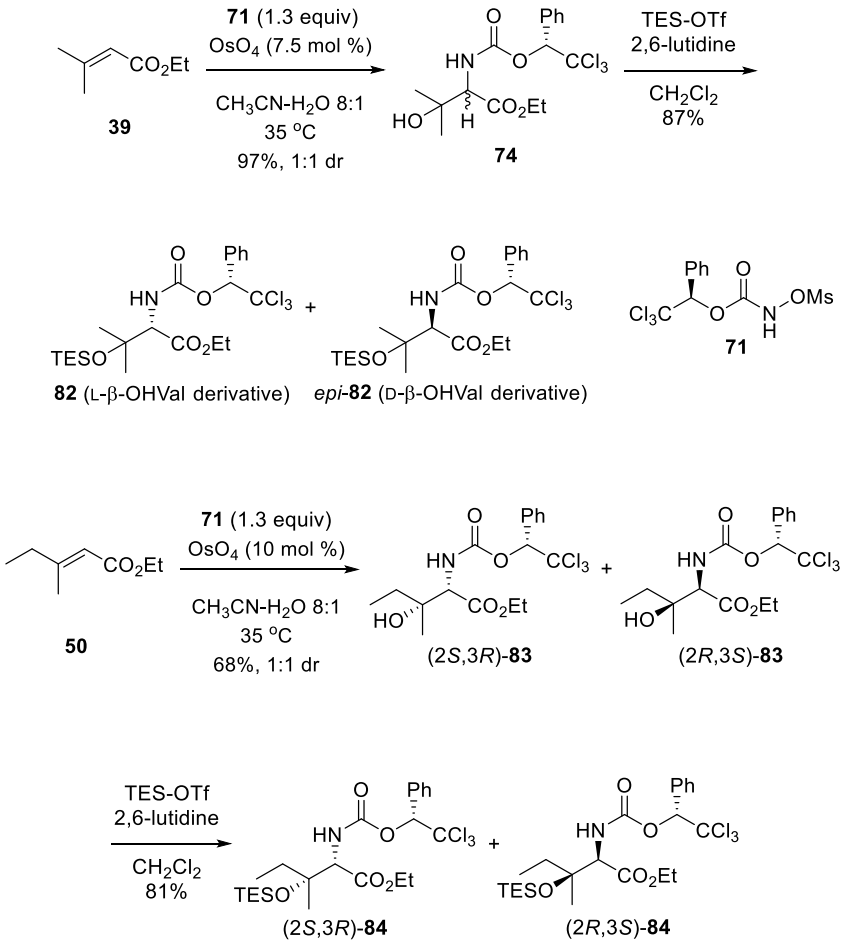
Scheme 2.11 Reagent-Controlled Asymmetric Aminohydroxylation



Scheme 2.12 Optimization of Reagent-Controlled Asymmetric Aminohydroxylations

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 SiO_2 after TES protection. The overall yield for the two steps was about 84%.

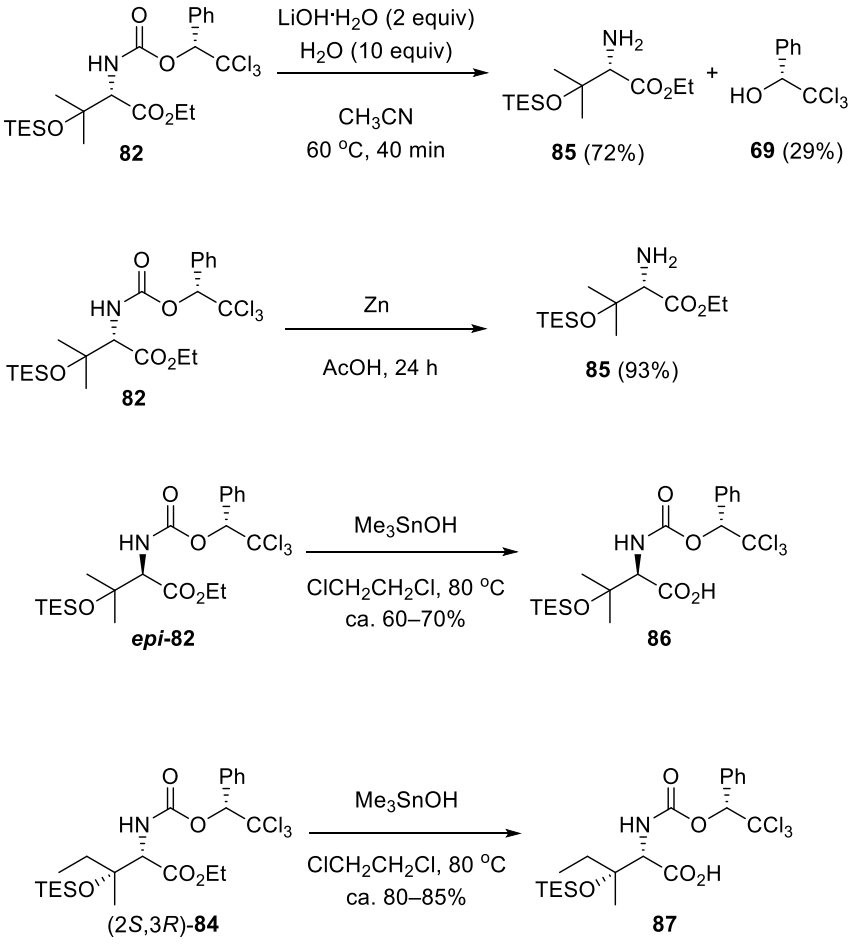


Scheme 2.13 Preliminary Studies on β-Hydroxy Amino Acid Synthesis

Using the same chemistry with enoate **50** as substrate, we prepared the β-OHIle derivatives (2S,3R)-**83** and (2R,3S)-**83**. Since **50** is bulkier than **39**, we obtained a lower yield for the first step. Product (2S,3R)-**83** was used directly for the synthesis of yaku'amide A while (2R,3S)-**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**

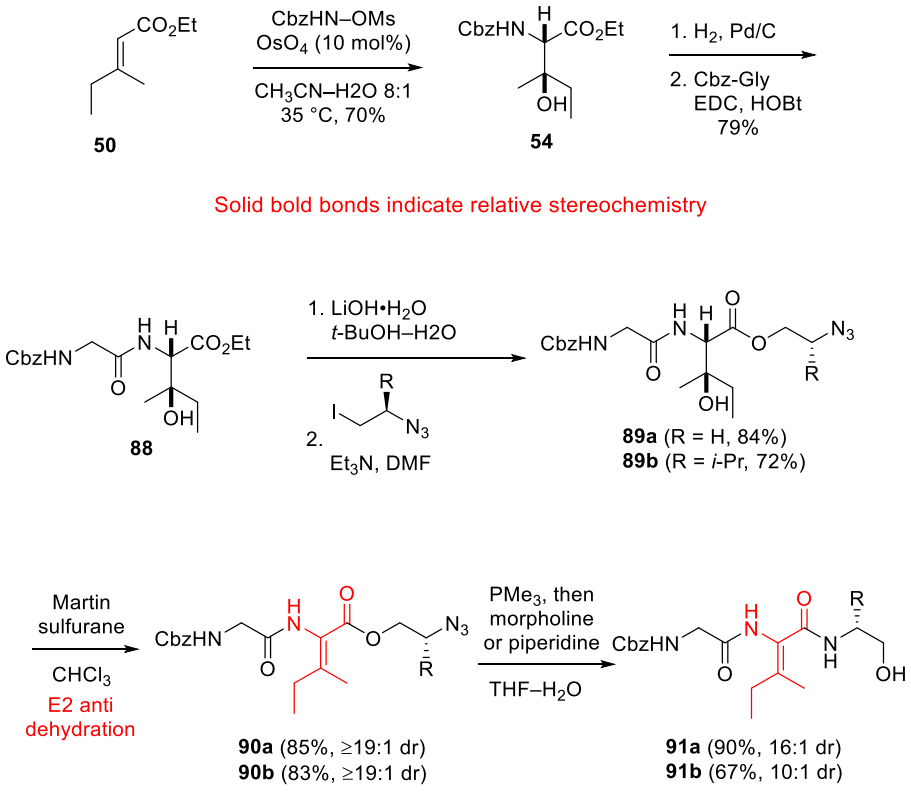
In order to perform peptide couplings, it is necessary to selectively remove the protecting groups of the chiral reagent-mediated aminohydroxylation product. To our surprise, the ethyl ester was slightly more stable than the chiral carbamate group of **82** to basic hydrolysis. So we could hydrolyze 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 Me_3SnOH to selectively hydrolyze epi-**82**, and (2S,3R)-**84**, we obtained pure acids with good yields (Scheme 2.14).



Scheme 2.14 Chemoselective Deprotections

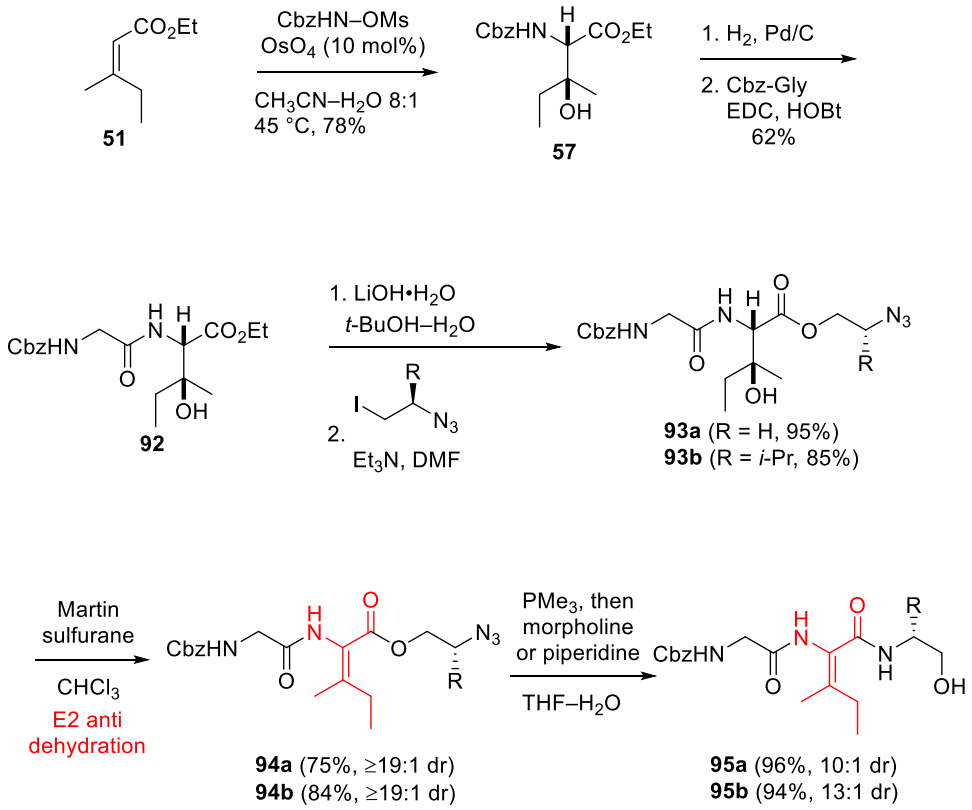
2.6 **Dehydroisoleucine Synthesis**

To synthesize Z- and E-ΔIle-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-ΔIle-containing peptides **91a** and **91b** with more than 10:1 diastereoselectivity (Z:E ratio).[27]



Scheme 2.15 Synthesis of Z-ΔIle-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-ΔIle-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→N acyl transfer sequence from **93a** and **93b**.[27]



Scheme 2.16 Synthesis of E-ΔIle-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-OHIle derivatives **25** and **27**, we only needed two steps to produce these residues. Also, our approach to install Z- and E-ΔIle requires no backbone amide protection and it would dramatically shorten the total synthesis of yaku'amide A.

2.8 **References**

1. Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490.
2. Harris, C. M.; Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915.
3. Koiso, Y.; Natori, M.; Iwasaki, S.; Sato, S.; Sonoda, R.; Fujita, Y.; Yaegashi, H.; Sato, Z. *Tetrahedron Lett.* **1992**, *33*, 4157.
4. Sharman, G. J.; Williams, D. H.; Ewing, D. F.; Ratledge, C. *Biochem. J.* **1995**, *305*, 187.
5. Renner, M. K.; Shen, Y.-C.; Cheng, X.-C.; Jensen, P. R.; Frankmoelle, W.; Kauffman, C. A.; Fenical, W.; Lobkovsky, E.; Clardy, J. *J. Am. Chem. Soc.* **1999**, *121*, 11273.
6. Li, L.; Klauber, E. G.; Seidel, D. *J. Am. Chem. Soc.* **2008**, *130*, 12248.
7. Patel, J.; Clavé, G.; Renard, P.-Y.; Franck, X. *Angew. Chem. Int. Ed.* **2008**, *47*, 4224.
8. Willis, M. C.; Cutting, G. A.; Piccio, V. J. D.; Durbin, M. J.; John, M. P. *Angew. Chem. Int. Ed.* **2005**, *44*, 1543.
9. Ooi, T.; Kameda, M.; Taniguchi, M.; Maruoka, K. *J. Am. Chem. Soc.* **2004**, *126*, 9685.
10. Makino, K.; Hiroki, Y.; Hamada, Y. *J. Am. Chem. Soc.* **2005**, *127*, 5784.
11. Makino, K.; Goto, T.; Hiroki, Y.; Hamada, Y. *Angew. Chem. Int. Ed.* **2004**, *43*, 882.
12. Lei, A.; Wu, S.; He, M.; Zhang, X. *J. Am. Chem. Soc.* **2004**, *126*, 1626.
13. Tellam, J. P.; Kociok-Köhn, G.; Carbery, D. R. *Org. Lett.* **2008**, *10*, 5199.
14. Bodkin, J. A.; Bacskay, G. B.; McLeod, M. D. *Org. Biomol. Chem.* **2008**, *6*, 2544.

15. Han, H.; Cho, C.-W.; Janda, K. D. *Chem. – Eur. J.* **1999**, *5*, 1565.

16. Morgan, A. J.; Masse, C. E.; Panek, J. S. *Org. Lett.* **1999**, *1*, 1949.

17. Tao, B.; Schlingloff, G.; Sharpless, K. B. *Tetrahedron Lett.* **1998**, *39*, 2507.

18. Ma, Z.; Naylor, B. C.; Loertscher, B. M.; Hafen, D. D.; Li, J. M.; Castle, S. L. *J. Org. Chem.* **2011**, *77*, 1208.

19. Donohoe, T. J.; Bataille, C. J. R.; Gattrell, W.; Kloesges, J.; Rossignol, E. *Org. Lett.* **2007**, *9*, 1725.

20. Harris, L.; Mee, S. P. H.; Furneaux, R. H.; Gainsford, G. J.; Luxenburger, A. *J. Org. Chem.* **2011,** *76*, 358.

21. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.

22. Bodkin, J. A.; McLeod, M. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2733.

23. Andersson, M. A.; Epple, R.; Fokin, V. V. *Angew. Chem. Int. Ed.* **2002**, *41*, 472.

24. Wu, P.; Hilgraf, R.; Fokin, V. V. *Adv. Synth. Catal.* **2006**, *348*, 1079.

25. Duan, P.-W.; Chiu, C.-C.; Lee, W.-D.; Pan, L. S.; Venkatesham, U.; Tzeng, Z.-H.; Chen, K. *Tetrahedron: Asymmetry* **2008**, *19*, 682.

26. Lebel, H.; Trudel, C.; Spitz, C. *Chem. Commun.* **2012**, *48*, 7799.

27. Ma, Z.; Jiang, J.; Luo, S.; Cai, Y.; Cardon, J. M.; Kay, B. M.; Ess, D. H.; Castle, S. L. *Org. Lett.* **2014**, *16*, 4044.

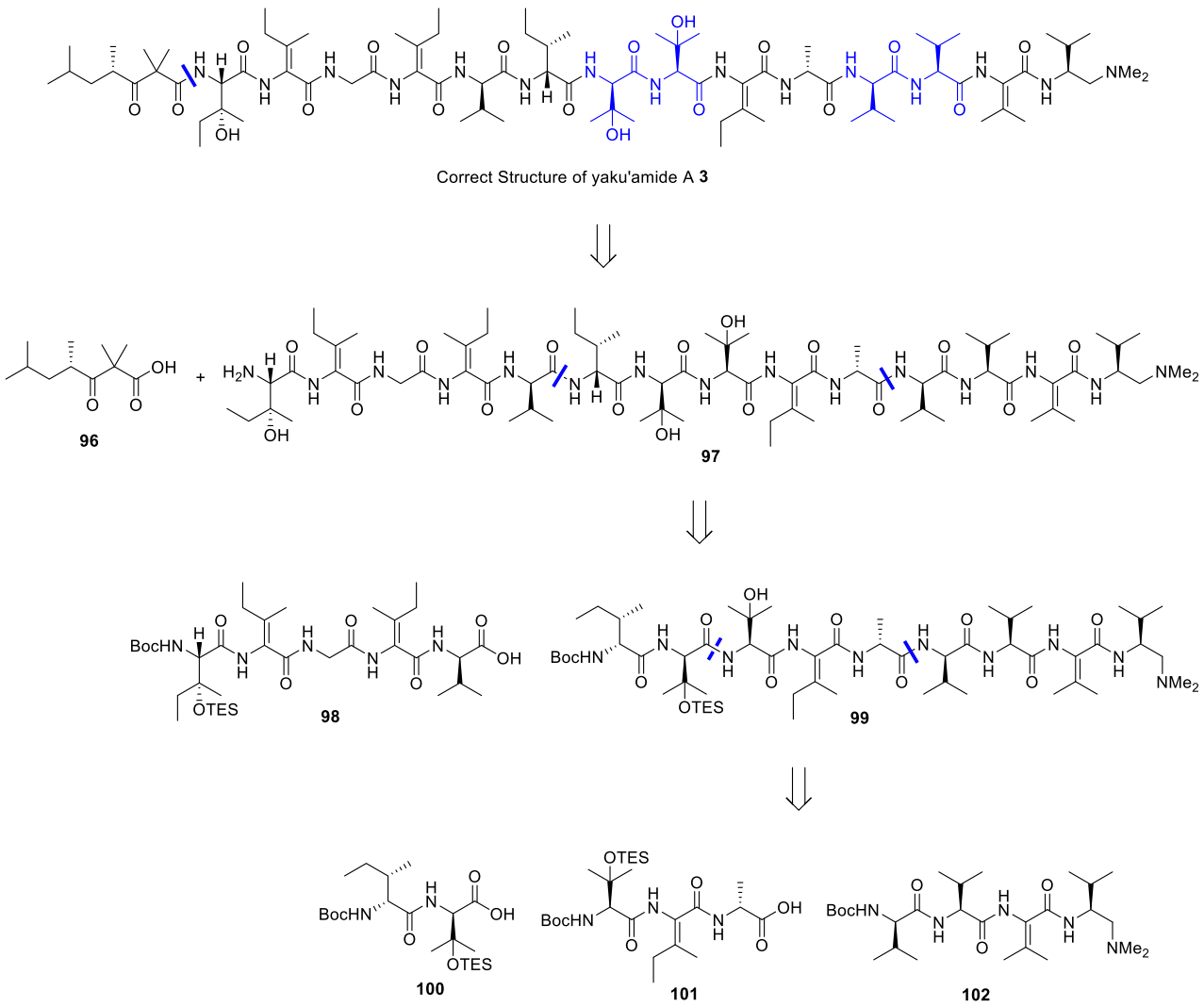
CHAPTER 3. PROGRESS TOWARD YAKU'AMIDE A

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-ΔIle 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-ΔIle and one E-ΔIle, while nonapeptide **99** includes one Z-ΔIle and one ΔVal. 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.



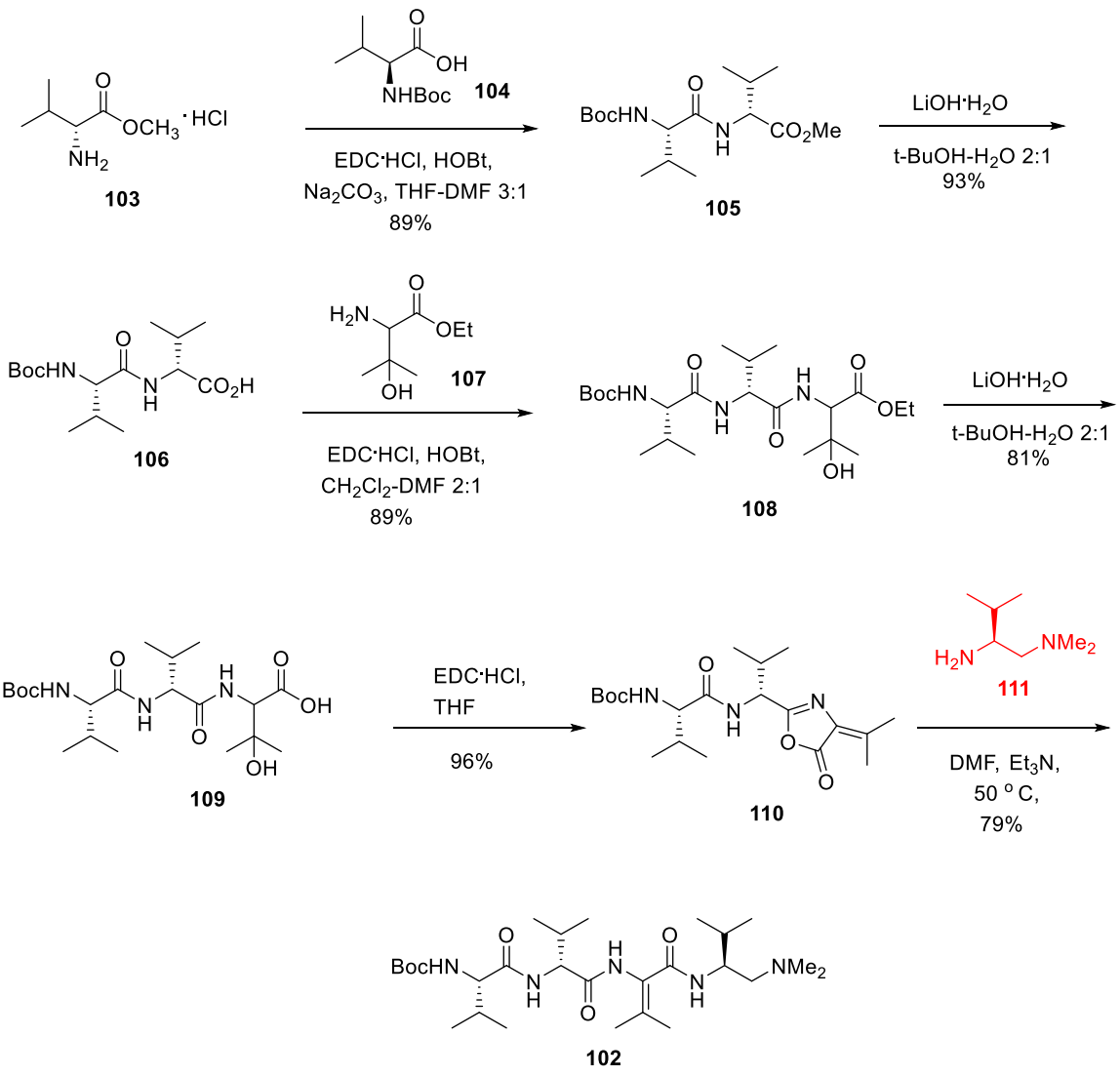
Scheme 3.1 Retrosynthetic Analysis of yaku'amide A **3**

3.3 **Synthesis of Right-hand Tetrapeptide**

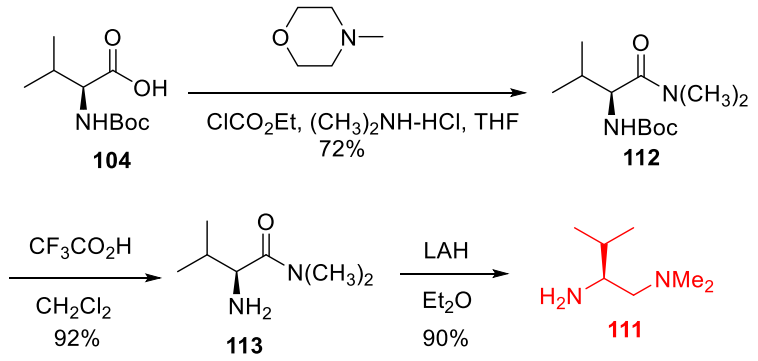
When we worked on the synthesis of tetrapeptide of yaku'amide A in 2012, only the structure proposed by Matsunaga[3] was known and the absolute C4-stereochemistry still needed to be determined. So our synthesis was toward the structure of **1** proposed by Matsunaga.

Since the right-hand tetrapeptide **102** contains one ΔVal, 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]

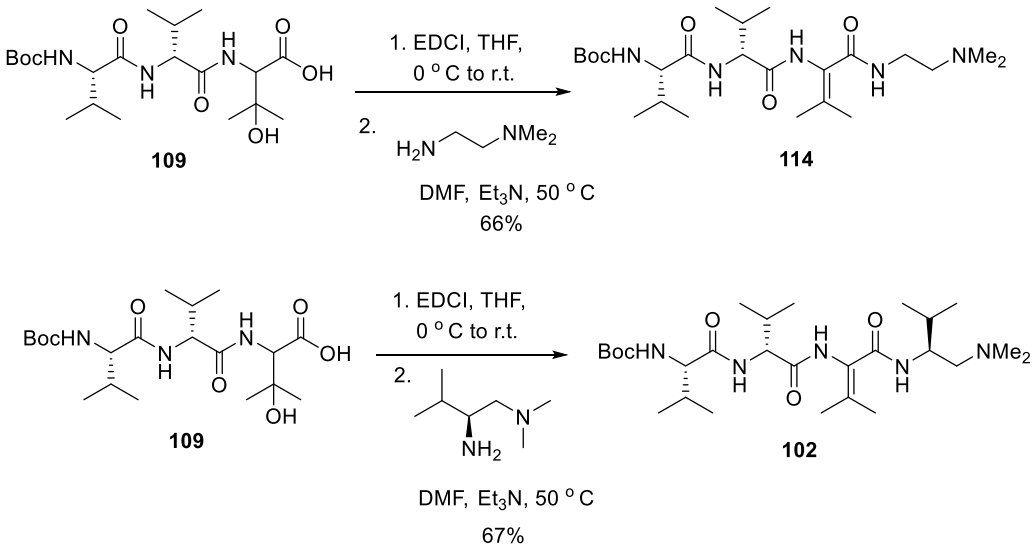


Scheme 3.2 Synthesis of Right-Hand Tetrapeptide **102**



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**.

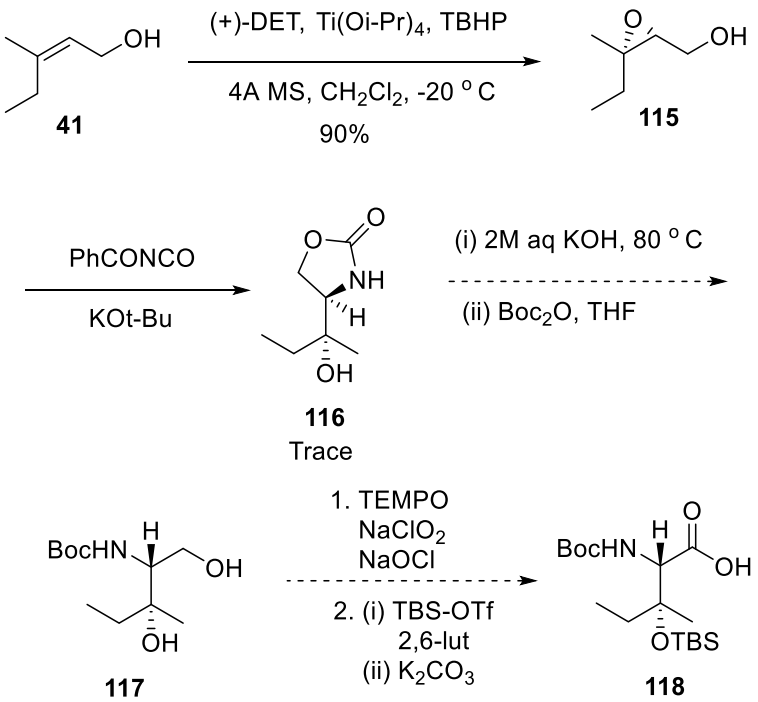


Scheme 3.4 Optimization of the Synthesis of **102**

3.4 **Studies Toward the Synthesis of (2S,3R)-β-OHIle 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-OHIle derivative, we designed a synthetic route to access **118** (Scheme 3.5).

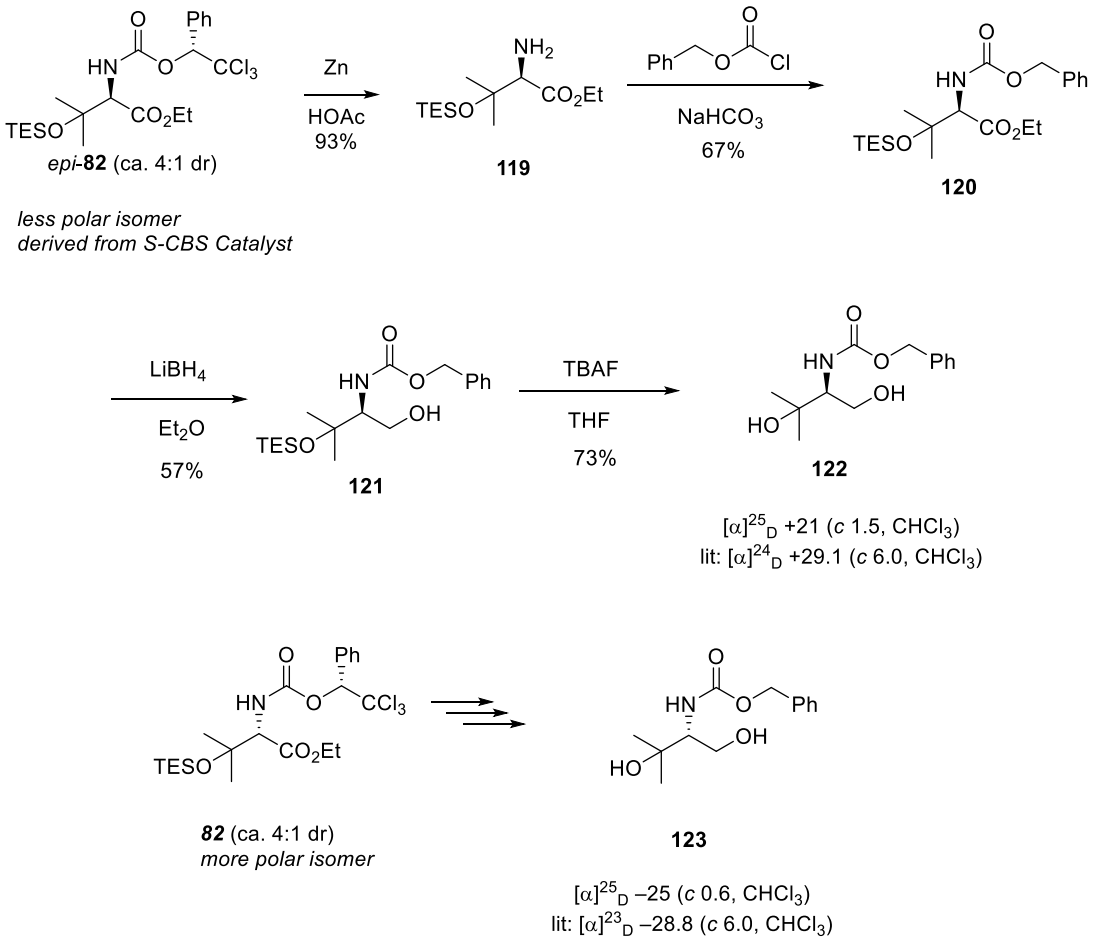
First, allylic alcohol **41** was obtained in two steps from commercially available ethyl 2-pentynoate **51**.[5,6] Next, we used Sharpless asymmetric epoxidation[7] to generate epoxide **115**. The enantioselective epoxidation of **41** was not reported before. While we were working on the next step 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-OHIle Derivative **118**

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

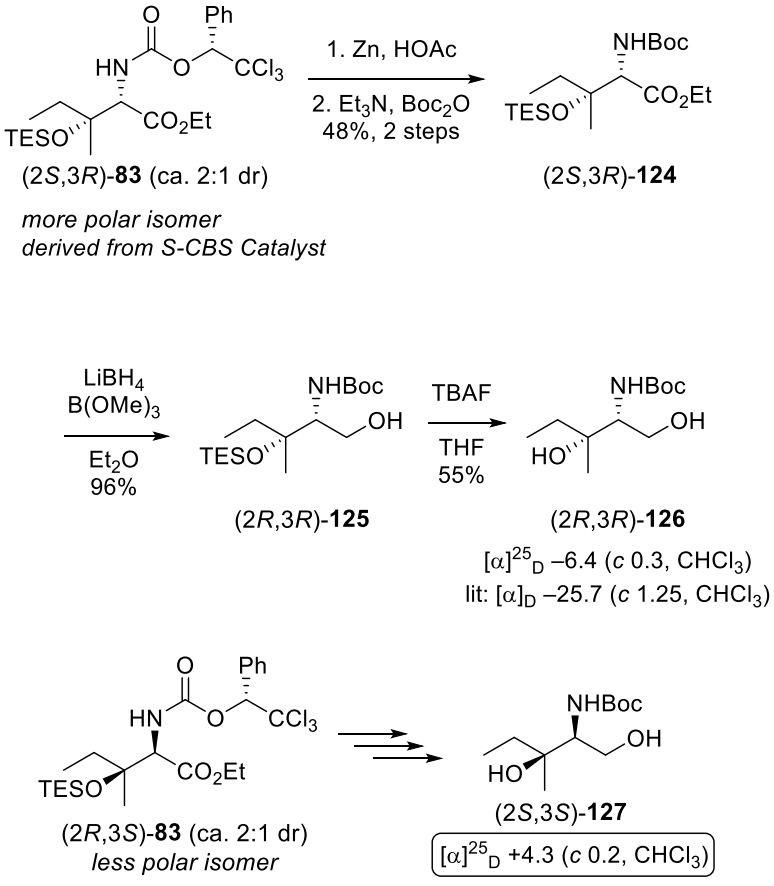
With the chiral reagent-based method in hand, we needed to assign the absolute configurations of the β-OHVal derivatives **82** and *epi*-**82** for the synthesis of dipeptide **100** and tripeptide **101**. We converted *epi*-**82** and **82** into diols **122** and **123** reported by Saalfrank and 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 $LiBH_4$, 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**

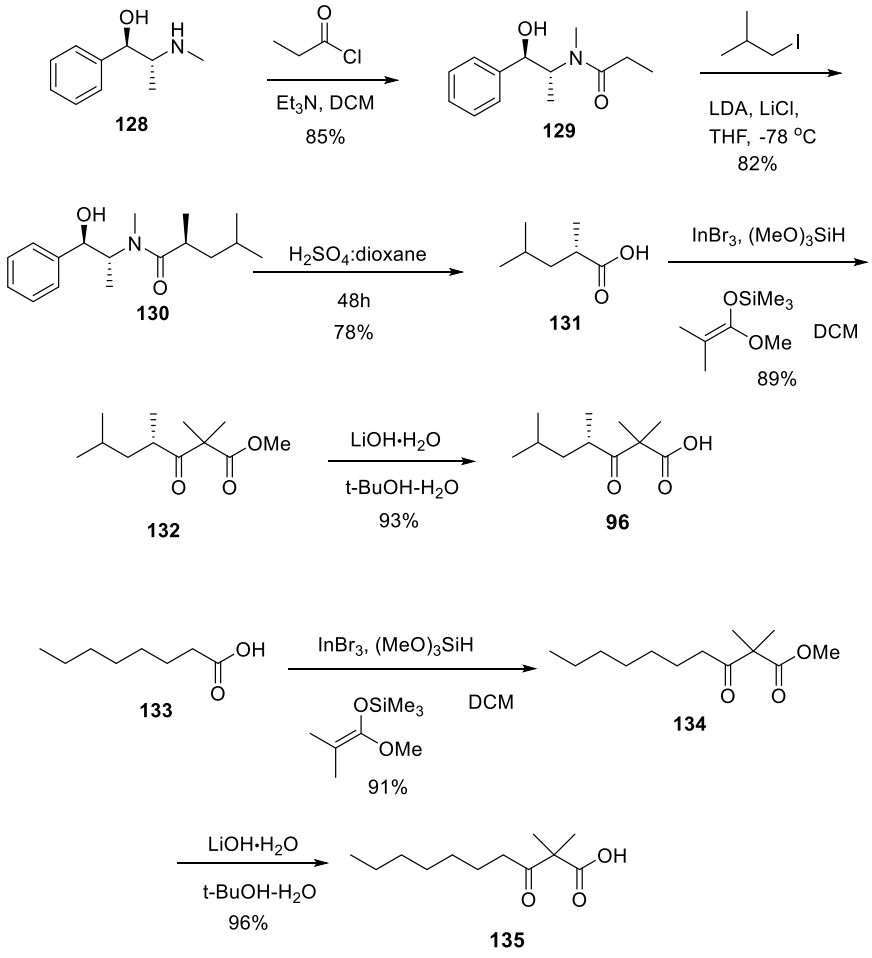
In order to synthesize pentapeptide **98**, co-worker Joseph M. Cardon and I assigned absolute configurations of (2*S*,3*R*)-**83** and (2*R*,3*S*)-**83** based on data reported by Guanti and co-workers.[9] Removal of the chiral carbamate and a subsequent Boc protection of the newly generated amine produced (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 (2S,3R)-**83** and (2R,3S)-**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-OHIle 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**, (2*S*,3*R*)-**83** and (2*R*,3*S*)-**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**

1. Kuranaga, T.; Sesoko, Y.; Sakata, K.; Maeda, N.; Hayata, A.; Inoue, M. *J. Am. Chem. Soc.* **2013**, *135*, 5467.
2. Kuranaga, T.; Mutoh, H.; Sesoko, Y.; Goto, T.; Matsunaga, S.; Inoue, M. *J. Am. Chem. Soc.* **2015**, *137*, 9443.
3. Ueoka, R.; Ise, Y.; Ohtsuka, S.; Okada, S.; Yamori, T.; Matsunaga, S. *J. Am. Chem. Soc.* **2010**, *132*, 17692.
4. Andrés, J. M.; Manzano, R.; Pedrosa, R. *Chem. Eur. J.* ***2008,*** *14*, 5116.
5. Shangguan, N.; Joullié, M. *Tetrahedron Lett.* **2009**, *50*, 6748.
6. Gilbert, J. C.; Kelly, T. A. *Tetrahedron* **1988**, *44*, 7587.
7. Rossiter, B. E.; Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 464.
8. Scheurer, A.; Mosset, P.; Bauer, W.; Saalfrank, Rolf W. *Eur. J. Org. Chem.* **2001**, *2001*, 3067.
9. Ageno, G.; Banfi, L.; Giuseppe Guanti, G. C.; Manghisi, E.; Riva, R.; Rocca, V. *Tetrahedron* **1995**, *51*, 8121.
10. Ghosh, A. K.; Anderson, D. D. *Org. Lett.* **2012,** *14*, 4730.
11. Turk, J. A.; Visbal, G. S.; Lipton, M. A. *J. Org. Chem.* **2003**, *68*, 7841.
12. Myers, A. G.; Yang, B. H.; Chen, H.; McKinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997,** *119*, 6496.
13. Nishimoto, Y.; Okita, A.; Yasuda, M.; Baba, A. *Angew. Chem. Int. Ed.* **2011,** *50*, 8623.

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

4.1 Introduction

There are no α,β-dehydroamino acids (ΔAAs) 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 ΔAA-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 ΔAAs-containing peptides have potential to target PPIs.

Previously, ΔAAs have been the topic of a series of reviews.[6] Some reviews focused on specific categories of ΔAAs,[7] while others centered on certain characteristics of their chemistry.[8,9] We wrote a review about bulky ΔAAs 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., ΔVal, ΔIle, ΔLeu, and ΔPhe). In

other words, the interactions between the main chain and the side chain of ΔAAs in a peptide would cause significant rigidity. This rigidity can induce more stable folded states of bulky ΔAA-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 ΔAAs and tetrasubstituted ΔAAs will be discussed in this section. Since there are too many trisubsituted ΔAA-containing natural products, I will only summarize selected compounds with interesting structures and/or potent bioactivity in this category.

4.2 **Bulky Trisubstituted ΔAA-Containing Natural Products**

Many bulky ΔAA-containing natural products are diketopiperazines. In this family, several dysamides isolated from *Dysidea herbacea* and *Dysidea chlorea* sponges contain δ-polychlorinated ΔLeu subunits (Figure 4.1, bulky ΔAAs 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]

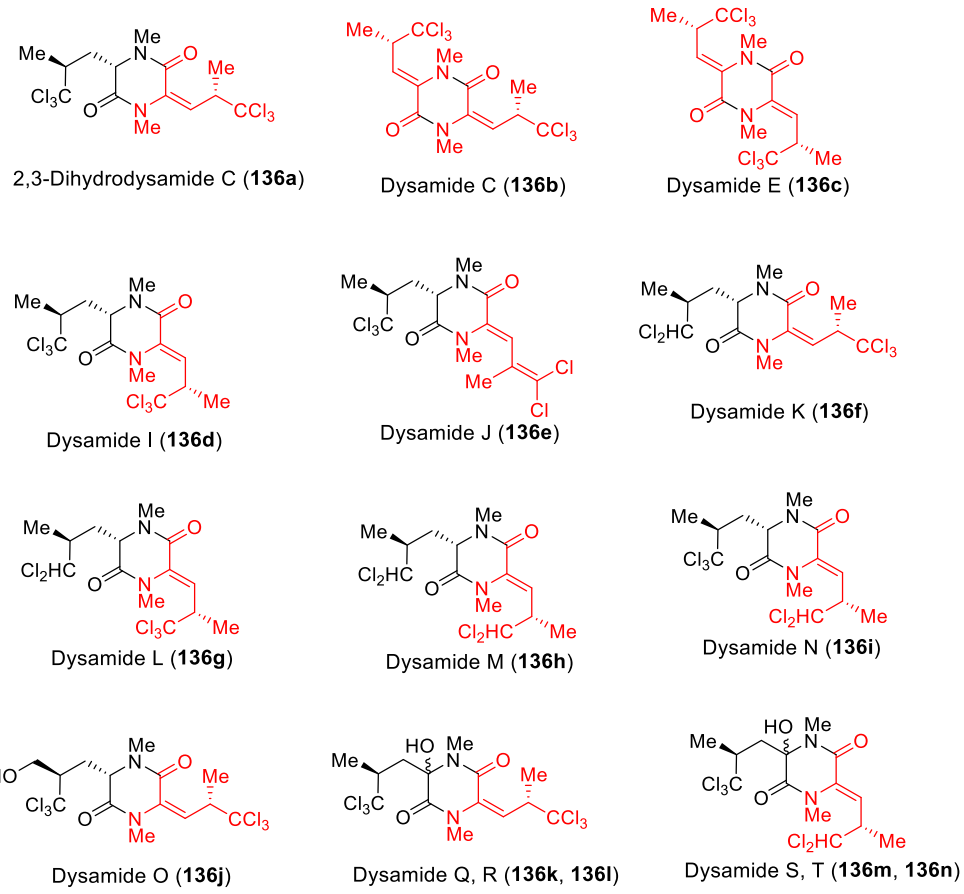


Figure 4.1 Structures of Dysamides

Albonoursin (**137**, Figure 4.2), obtained from the actinomycete species in the 1960's, includes one *Z*-ΔLeu and one *Z*-ΔPhe.[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]

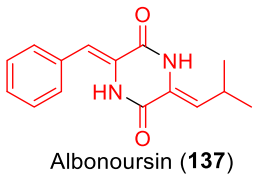
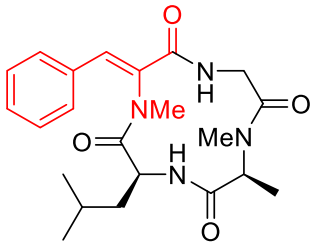


Figure 4.2 Stucture of Albonoursin

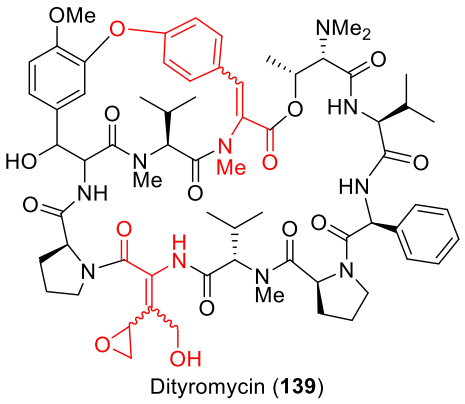
Templeton and co-workers obtained Tentoxin (**138**, Figure 4.3) from the fungus *Alternaria tenuis*. It is a cyclic tetrapeptide with a Z-ΔPhe residue and it is toxic to plants.[19] Both the Meyer[20] and Rich[21] groups helped to determine its structure. Tentoxin can induce chlorosis in certain plant species, which indicates that it is a potential herbicide.[22] Due to the high affinity of **138** to the chloroplast F_1 H^+-ATPase, it can inhibit energy transfer.[23]



Tentoxin (**138**)

Figure 4.3 Stucture of Tentoxin

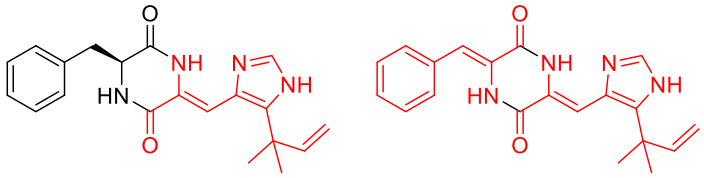
In 1977, Ōmura and co-workers isolated the antibiotic dityromycin.[24] Later its structure (**139**, Figure 4.4) was determined[25], but there were still uncertainties about the configurations of the two ΔAA residues and the β-OH-Tyr. Compound **139** possesses an *O*-arylated ΔPhe and an unusual tetrasubstituted ΔAA containing a hydroxymethyl group and an epoxide. With an extra degree of unsaturation, GE82832[26], 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]



Dityromycin (**139**)

Figure 4.4 Stucture 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*-ΔHis 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]



(–)-Phenylahistin (**140**) Dehydrophenylahistin (**141**)

Figure 4.5 Structures of (–)-Phenylahistin and Dehydrophenylahistin

From the fungus *Aspergillus effuses* H1-1, Li and co-workers isolated effusin A (**142**, Figure 4.6) and dihydrocryptoechinuline D (**143**) in 2012.[33] Compound **142** includes a spirocyclic *N*,*O*-acetal and both peptides contain a reverse prenylated *Z*-ΔTrp 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.

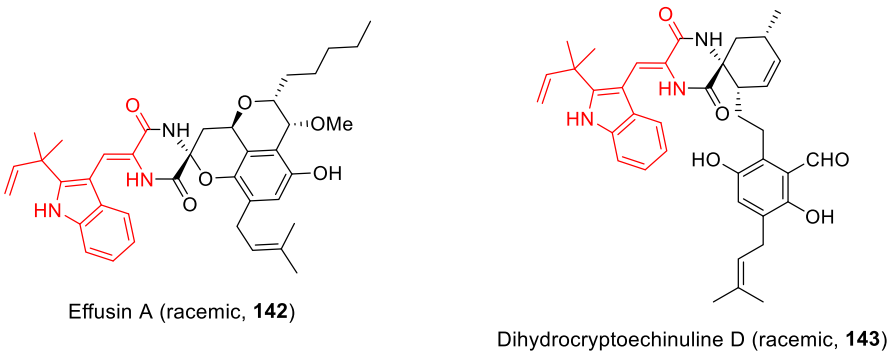


Figure 4.6 Structures of Effusin A and Dihydrocryptoechinuline D

4.3 Bulky Tetrasubstituted ΔAA-Containing Natural Products

In 1969, Marchand and co-workers isolated the ΔVal-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 ^{1}H 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]

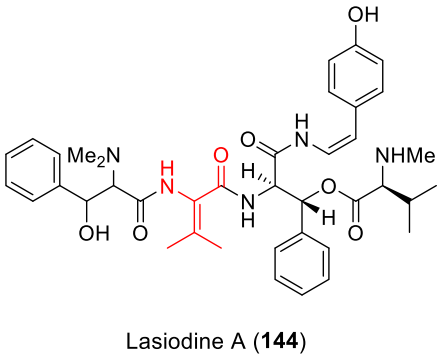
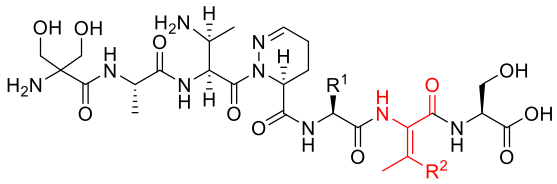


Figure 4.7 Structure of Lasiodine A

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.



Antrimycin	R^1	R^2
A (**145a**)	Me	Et
B (**145b**)	Et	Et
C (**145c**)	*n*-Pr	Et
D (**145d**)	*i*-Bu	Et
Av (**145e**)	Me	Me
Bv (**145f**)	Et	Me
Cv (**145g**)	*n*-Pr	Me
Dv (**145h**)	*i*-Bu	Me

Figure 4.8 Structures of Antrimycins

In 1983, Culvenor and co-workers isolated the antimitotic 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]

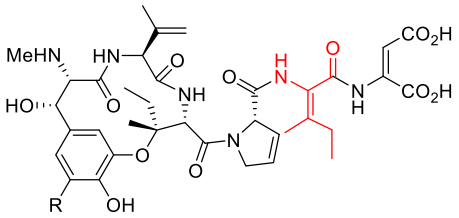


Figure 4.9 Structures of Phomopsins A and B

In the same year, Kemmer and co-workers isolated Myxovalargin A (**147**, Figure 4.10) from the myxobacterium *Myxococcus fulvus* and explored its activity against Gram-positive bacteria.[45] Although they discovered that ΔVal and ΔIle were present in the structure, the complete structure of **147** was proposed in 1989. They found that Myxovalargin 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]

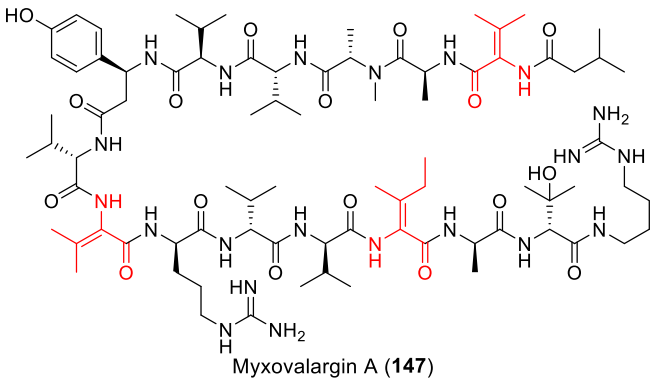
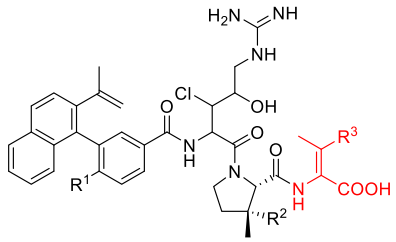


Figure 4.10 Structure of Myxovalargin A

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 ΔIle residue in **148b** adopts an *E*-geometry from NOE studies.

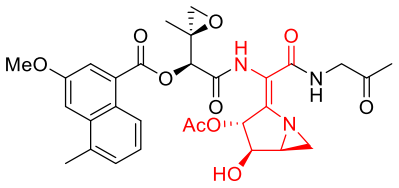
However, the researchers were not certain about the configuration of the revised arginine residue. Peptides **148a**–**e** showed inhibitory effects against glucagon-stimulated gluconeogenesis, and the serine/threonine protein phosphatases PP1 and PP2A[49a] were identified as targets after a modified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



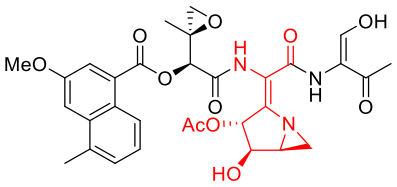
	R^1	R^2	R^3
148a	OH	OH	Me
148b	OH	OH	Et
148c	OH	H	Me
148d	OMe	OH	Et
148e	OMe	OH	Me

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 Moran[51] 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]



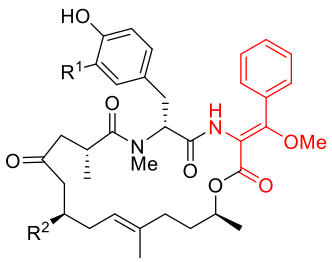
Azinomycin A (**149a**)



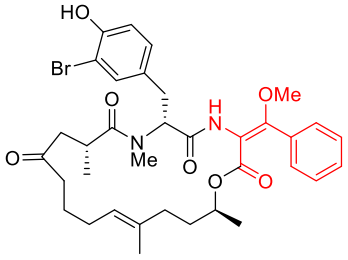
Azinomycin B (**149b**)

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 ΔAA 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]



Miuraenamide A (**150a**): R^1 = Br, R^2 = H
Miuraenamide B (**150b**): R^1 = I, R^2 = H
Miuraenamide C (**150c**): R^1 = Cl, R^2 = H
Miuraenamide F (**150d**): R^1 = Br, R^2 = OH



Miuraenamide D (**150e**)

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 ΔAAs. First, we included metabolites that possess trisubstituted ΔAAs. Next, we covered those that include tetrasubstituted ΔAAs.

4.5 **References**

1. Hajduk, P. J.; Greer, *J. Nat. Rev. Drug Discovery* **2007**, *6*, 211.

2. Lo Conte, L.; Chothia, C.; Janin, J. *J. Mol. Biol.* **1999**, *285*, 2177.

3. Bullock, B. N.; Jochim, A. L.; Arora, P. S., Assessing Helical Protein Interfaces for Inhibitor Design. *J. Am. Chem. Soc.* **2011,** *133*, 14220.

4. Bonauer, C.; Walenzyk, T.; König, B., α,β-Dehydroamino Acids. *Synthesis* **2006,** *2006*, 1.

5. Arkin, M. R.; Wells, J. A. *Nat. Rev. Drug Discovery* **2004**, *3*, 301.

6. (a) Schmidt, U.; Hausler, J.; Oehler, E.; Poisel, H. *Fortschr. Chem. Org. Naturst.* **1979**, *37*, 251. (b) Stammer, C. H. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1982; Vol. 6, pp 33–74. (c) Noda, K.; Shimohigashi, Y.; Izumiya, N. In *The Peptides: Analysis, Synthesis, Biology*; Gross, E. and Meienhofer, J., Eds.; Academic Press: New York, 1983; Vol. 5, pp 285–339. (d) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159. (e) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243. (f) Kazmeier, U. In *Amino Acids, Peptides, and Proteins in Organic Chemistry*; Hughes, A. B., Ed.; Wiley VCH: Weinheim, 2009; Vol. 2, pp 3–34.

7. (a) Dehydroalanine: Suzen, S. *Ankara Ecz. Fac. Derg.* **1999**, *28*, 129. (b) Dehydrotyrosine and derivatives: Sugumaran, M.; Robinson, W. E. *Mar. Drugs* **2010**, *8*, 2906. (c) Dehydrophenylalanine: Gupta, M.; Chauhan, V. S. *Biopolymers* **2011**, *95*, 161. (d) Dehydrotryptophan: Kaur, H.; Heapy, A. M.; Brimble, M, A. *Org. Biomol. Chem.,* **2011**, *9*, 5897.

8. For reviews of the conformations of peptides that contain α,β-dehydroamino acids, see: (a) Jain, R.; Chauhan, V. S. *Biopolymers (Pept. Sci.)* **1996**, *40*, 105. (b) Singh, T. P.; Kaur, P.

Prog. Biophys. Mol. Biol. **1996**, *66*, 141. (c) Mathur, P.; Ramakumar, S.; Chauhan, V. S. *Biopolymers (Pept. Sci.)* **2004**, *76*, 150.

9. For a review on the synthesis of α,β-dehydroamino acids via Cu-mediated C(sp^2)–N bond formation, see: Kuranaga, T.; Sesoko, Y.; Inoue, M. *Nat. Prod. Rep.* **2014**, *31*, 514.
10. Jiang, J.; Ma, Z.; Castle, S. L. *Tetrahedron* **2015**, *71*, 5431.
11. (a) English, M. L.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 1464. (b) English, M. L.; Stammer, C. H. *Biochem. Biophys. Res. Commun.* **1978**, 85, 780. (c) Shimohigashi, Y.; Chen, H.-C.; Stammer, C. H. *Peptides* **1982**, *3*, 985. (d) Shimohigashi, Y.; Stammer, C. H. *J. Chem. Soc., Perkin Trans. 1* **1983**, 803.
12. For recent reviews of peptide natural products, see: (a) Schwarzer, D.; Finking, R.; Marahiel, M. A. *Nat. Prod. Rep.* **2003**, *20*, 275. (b) Matsunaga, S.; Fusetani, N. *Curr. Org. Chem.* **2003**, *7*, 945. (c) McIntosh, J. A.; Donia, M. S.; Schmidt, E. W. *Nat. Prod. Rep.* **2009**, *26*, 537. (d) Velásquez, J. E.; van der Donk, W. A. *Curr. Opin. Chem. Biol.* **2011**, *15*, 11. (e) Bockus, A. T.; McEwen, C. M.; Lokey, R. S. *Curr. Top. Med. Chem.* **2013**, *13*, 821. (f) Arnison, P. G.; Bibb, M. J.; Bierbaum, G.; Bowers, A. A.; Bugni, T. S.; Bulaj, G.; Camarero, J. A.; Campopiano, D. J.; Challis, G. L.; Clardy, J.; Cotter, P. D.; Craik, D. J.; Dawson, M.; Dittmann, E.; Donadio, S.; Dorrestein, P. C.; Entian, K.-D.; Fischbach, M. A.; Garavelli, J. S.; Göransson, U.; Gruber, C. W.; Haft, D. H.; Hemscheidt, T. K.; Hertweck, C.; Hill, C.; Horswill, A. R.; Jaspars, M.; Kelly, W. L.; Klinman, J. P.; Kuipers, O. P.; Link, A. J.; Liu, W.; Marahiel, M. A.; Mitchell, D. A.; Moll, G. N.; Moore, B. S.; Müller, R.; Nair, S. K.; Nes, I. F.; Norris, G. E.; Olivera, B. M.; Onaka, H.; Patchett, M. L.; Piel, J.; Reaney, M. J. T.; Rebuffat, S.; Ross, R. P.; Sahl, H.-G.; Schmidt, E. W.; Selsted, M. E.; Severinov, K.; Shen, B.; Sivonen, K.; Smith, L.; Stein, T.; Süssmuth, R. D.; Tagg, J. R.; Tang, G.-L.; Truman, A. W.; Vederas, J. C.; Walsh,

C. T.; Walton, J. D.; Wenzel, S. C.; Willey, J. M.; van der Donk, W. A. *Nat. Prod. Rep.* **2013**, *30*, 108.

13. (a) Kazlauskas, R.; Murphy, P. T.; Walls, R. J. *Tetrahedron Lett.* **1978**, 4945. (b) Su, J.-Y.; Zhong, Y.-L.; Zeng, L.-M.; Wei, X.; Wang, Q.-W.; Mak, T. C. W.; Zhou. Z.-Y. *J. Nat. Prod.* **1993**, *56*, 637. (c) Dumdei, E. J.; Simpson, J. S.; Garson, M. J.; Byriel, K. A.; Kennard, C. H. L. *Aust. J. Chem.* **1997**, *50*, 139. (d) Fu, X., Ferreira, M. L. G.; Schmitz, F. J.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 1226.
14. (a) Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302. (b) Biskupiak, J. E.; Ireland, C. M. *Tetrahedron Lett.* **1984**, 2935. (c) de Laszlo, S. E.; Williard, P. G. *J. Am. Chem. Soc.* **1985**, *107*, 199. (d) Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336.
15. Khokhlov, A. S., Lokshin G. B. *Tetrahedron Lett.* **1963**, 1881.
16. Wang, P.; Xi, L.; Liu, P.; Wang, Y.; Wang, W.; Huang, Y.; Zhu, W. *Mar. Drugs* **2013**, *11*, 1035.
17. Fukushima, K.; Yazawa, K.; Arai, T. *J. Antibiot.* **1973**, *26*, 175.
18. (a) Kanzaki, H.; Imura, D.; Nitoda, T.; Kawazu, K. *J. Mol. Catal. B: Enzym* **1999**, *6*, 265. (b) Kanzaki, H.; Yanagisawa, S.; Nitoda, T. *J. Antibiot.* **2000**, *53*, 1257.
19. (a) Fulton, N. D.; Bollenbacher, K.; Templeton, G. E. *Phytopathology* **1965**, *55*, 49. (b) Templeton, G. E.; Grable, C. I.; Fulton, N. D.; Bollenbacher, K. *Phytopathology* **1965**, *55*, 1079. (c) Templeton, G. E.; Grable, C. I.; Fulton, N. D.; Bollenbacher, K. *Phytopathology* **1967**, *57*, 516.

20. (a) Meyer, W. L.; Templeton, G. E.; Grable, C. I.; Sigel, C. W.; Jones, R.; Woodhead, S. H.; Sauer, C. *Tetrahedron. Lett.* **1971**, 2357. (b) Meyer, W. L.; Kuyper, L. F.; Lewis, R. B.; Templeton, G. E.; Woodhead, S. H. *Biochem. Biophys. Res. Commun.* **1974**, *56*, 234.
21. (a) Koncewicz, M.; Mathiaparanam, P.; Uchytil, T. F.; Sparapano, L.; Tam, J.; Rich, D. H.; Durbin, R. D. *Biochem. Biophys. Res. Commun.* **1973**, *53*, 653. (b) Rich, D. H.; Mathiaparanam, P. *Tetrahedon Lett.* **1974**, 4037.
22. Cavelier, F.; Verducci, J.; André, F.; Haraux, F.; Sigalat, C.; Traris, M.; Vey, A. *Pestic. Sci.* **1998**, *52*, 81.
23. Prachyawarakorn, V.; Mahidol, C.; Sureram, S.; Sangpetsiripan, S.; Wiyakrutta, S.; Ruchirawat, S.; Kittakoop, K. *Planta Med.* **2008**, *74*, 69.
24. Ōmura, S.; Iwai, Y.; Hirano, A.; Awaya, J.; Suzuki, Y.; Matsumoto, K. *Agric. Biol. Chem*. **1977**, *41*, 1827.
25. Teshima, T.; Nishikawa, M.; Kubota, I.; Shiba, T.; Iwai, Y.; Ōmura, S. *Tetrahedron Lett*. **1988**, *29*, 1963.
26. Brandi, L., Fabbretti, A., Di Stefano, M., Lazzarini, A., Abbondi, M.; Gualerzi, C. O. *RNA* **2006**, *12*, 1262.
27. Brandi, L.; Maffioli, S.; Donadio, S.; Quaglia, F.; Sette, M.; Milón, P.; Gualerzi, C. O.; Fabbretti, A. *FEBS Lett.* **2012**, *586*, 3373.
28. (a) Bulkley, D.; Brandi, L.; Polikanov, Y. S.; Fabbretti A.; O'Connor, M.; Gualerzi, C.O.; Steitz, T. A. *Cell Rep.* **2014**, *6*, 357. (b) Lin, J.; Gagnon, M. G.; Bulkley, D.; Steitz, T. A. *Cell* **2015**, *160*, 219.
29. (a) Kanoh, K.; Kohno, S.; Asari, T.; Harada, T.; Katada, J.; Muramatsu, M.; Kawashima, H.; Sekiya, H.; Uno, I. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2847. (b) Kanoh, K.; Kohno, S.; Katada,

J.; Hayashi, Y.; Muramatsu, M.; Uno, I. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 1130. (c) Kanoh, K.; Kohno, S.; Katada, J.; Takahashi, J.; Uno, I. *J. Antibiot.* **1999**, *52*, 134.

30. Kanzaki, H.; Yanagisawa, S.; Kanoh, K.; Nitoda, T. *J. Antibiot.* **2002**, *55*, 1042.
31. Mita, M. M.; Spear, M. A.; Yee, L. K.; Mita, A. C.; Heath, E. I.; Papadopoulos, K. P.; Federico, K. C.; Reich, S. D.; Romero, O.; Malburg, L.; Pilat, M.; Lloyd, G. K.; Neuteboom, S. T. C.; Cropp, G.; Ashton, E.; LoRusso, P. M. *Clin. Cancer Res.* **2010**, *16*, 5892.
32. Yamazaki, Y.; Tanaka, K.; Nicholson, B.; Deyanat-Yazdi, G.; Potts, B.; Yoshida, T.; Oda, A.; Kitagawa, T.; Orikasa, S.; Kiso, Y.; Yasui, H.; Akamatsu, M.; Chinen, T.; Usui, T.; Shinozaki, Y.; Yakushiji, F.; Miller, B. R.; Neuteboom, S.; Palladino, M.; Kanoh, K.; Lloyd, G. K.; Hayashi, Y. *J. Med. Chem.* **2012**, *55*, 1056.
33. Gao, H.; Liu, W.; Zhu, T.; Mo, X.; Mándi, A.; Kurtán, T.; Li, J.; Ai, J.; Gu, Q.; Li, D. *Org. Biomol. Chem.* **2012**, *10*, 9501.
34. Marchand, J.; Pais, M.; Monseur, X.; Jarreau, F.-X. *Tetrahedron* **1969**, *25*, 937.
35. Pais, M.; Jarreau, F.-X.; González Sierra, M.; Mascaretti, O. A.; Ruveda, E. A.; Chang, C.-J.; Hagaman, E. W.; Wenkert, E. *Phytochemistry* **1979**, *18*, 1869.
36. Ravizzini, R. A.; Andreo, C. S.; Vallejos, R. H. *Plant Cell Physiol.* **1977**, *18*, 701.
37. (a) Shimada, N.; Morimoto, K.; Naganawa, H.; Takita, T.; Hamada, M ; Maeda, K.; Takeuchi T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 1613. (b) Morimoto, K.; Shimada, N.; Naganawa, H.; Takita T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 1615.
38. Morimoto, K.; Shimada, N.; Naganawa, H.; Takita T.; Umezawa, H. *J. Antibiot.* **1982**, *35*, 378.
39. (a) Shiroza, T.; Ebisawa, N.; Furihata, K.; Endo, T.; Seto, H.; Otake, N. *Agric. Biol. Chem.* **1982**, *46*, 865. (b) Shiroza, T.; Ebisawa, N.; Kojima, A.; Furihata, K.; Shimazu, A.; Endo, T.;

Seto, H.; Otake, N. *Agric. Biol. Chem*. **1982**, *46*, 1885. (c) Shiroza, T.; Ebisawa, N.; Furihata, K.; Endo, T.; Seto, H.; Otake, N. *Agric. Biol. Chem*. **1982**, *46*, 1891.

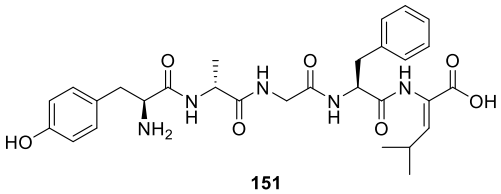
40. Culvenor, C. C. J.; Cockrum, P. A.; Edgar, J. A.; Frahn, J. L.; Gorst-Allman, C. P.; Jones, A. J.; Marasas, W. F. O.; Murray, K. E.; Smith, L. W.; Steyn, P. S.; Vleggaar, R.; Wessels, P. L. *J. Chem. Soc., Chem. Commun.* **1983**, 1259.
41. (a) Mackay, M. F.; Van Donkelaar, A.; Culvenor, C. C. J. *J. Chem. Soc., Chem. Commun.* **1986**, 1219. (b) Culvenor, C. C. J.; Edgar, J. A.; Mackay, M. F.; Gorst-Allman, C. P.; Marasas, W. F. O.; Steyn, P. S.; Vleggaar, R.; Wessels, P. L. *Tetrahedron* **1989**, *45*, 2351.
42. Edgar, L. E.; Culvenor, C. C. J. *Biochem. Pharmacol.* **1987**, *36*, 2133.
43. Ludueña, R. F.; Roach, M. C.; Prasad, V.; Lacey, E. *Biochem. Pharmacol.* **1990**, *39*, 1603.
44. Mitra, A.; Sept, D. *Biochemistry* **2004**, *43*, 13955.
45. Irschik, H., Gerth, K., Kemmer, T., Steinmetz, H., Reichenbach, H. *J. Antibiot.* **1983,** *36*, 6.
46. Steinmetz, H.; Irschik, H.; Reichenbach, H.; Höfle, G. *Chem. Pept. Proteins* **1989**, *4,* 13.
47. Irschik, H., Reichenbach, H. *J. Antibiot.* **1985,** *38*, 1237.
48. (a) Ohtsu, Y.; Sasamura, H.; Tsurumi, Y.; Yoshimura, S.; Takase, S.; Hashimoto, M.; Shibata, T.; Hino, M.; Fujii, T. *J. Antibiot*. **2003**, *56*, 682. (b) Ohtsu, Y.; Sasamura, H.; Shibata, T.; Nakajima, H.; Hino, M.; Fujii, T. *J. Antibiot*. **2003**, *56*, 689. (c) Zenkoh, T.; Ohtsu, Y.; Yoshimura, S.; Shigematsu, N.; Takase, S.; Hino, M. *J. Antibiot*. **2003**, *56*, 694.
49. (a) Hatori, H.; Zenkoh, T.; Kobayashi, M.; Ohtsu, Y.; Shigematsu, N.; Setoi, H.; Hino, M.; Handa, H. *J. Antibiot.* **2004**, *57*, 456 (correction: *J. Antibiot.* **2005**, *58*, C4). (b) Zenkoh, T.; Hatori, H.; Tanaka, H.; Hasegawa, M.; Hatakeyama, M.; Kabe, Y.; Setoi, H.; Kawaguchi, H.; Handa, H.; Takahashi, T. *Org. Lett.* **2004**, *6*, 2477.

50. (a) Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *39*, 1527. (b) Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554.
51. Moran, E. J.; Armstrong, R. W. *Tetrahedron Lett.* **1991**, *32*, 3807.
52. Hata, T.; Koga, F.; Sano, Y.; Kanamori, K.; Matsumae, A.; Sugawara, R.; Hoshi, T.; Shima, T.; Ito, S. Tomizawa, S. *J. Antibiot.*, *Ser. A* **1954**, *7*, 107.
53. (a) Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144. (b) Coleman, R. S.; Perez, R. J.; Burk, C. H.; Navarro, A. *J. Am. Chem. Soc.* **2002**, *124*, 13008.
54. Kelly, G. T.; Liu, C.; Smith, R., III; Coleman, R. S.; Watanabe, C. M. H. *Chem. Biol.* **2006**, 13, 485.
55. Iizuka, T.; Fudou, R., Jojima, Y.; Ogawa, S.; Yamanaka, S.; Inukai, Y.; Ojika, M. *J. Antibiot.* **2006,** *59*, 385.
56. Ojika, M.; Inukai, Y.; Kito, Y.; Hirata, M.; Iizuka, T.; Fudou, R. *Chem. Asian J.* **2008**, *3*, 126.
57. Sumiya, E.; Shimogawa, H.; Sasaki, H.; Tsutsumi, M.; Yoshita, K.; Ojika, M.; Suenaga, K.; Uesugi, M. *ACS Chem. Biol*. **2011**, *6*, 425.

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

5.1 Introduction

Researchers have discovered that α,β-dehydroamino acids (ΔAAs) 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.



151

Tyr-D-Ala-Gly-Phe-NH-ΔLeu

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*-ΔLeu-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 ΔLeu and ΔPhe in enkephalin, bulky tetrasubstituted ΔAAs such as ΔVal and ΔEnv 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 ΔVal and ΔEnv 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 ΔVal and ΔEnv in small synthetic peptides (Figure 5.2).

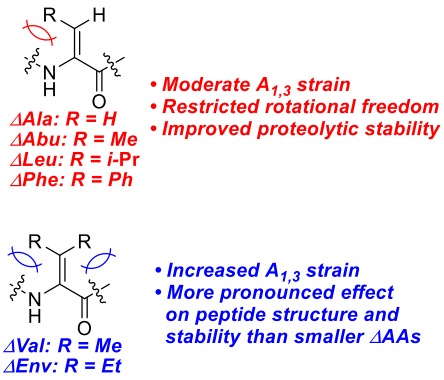


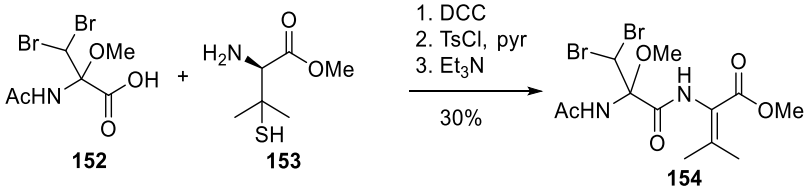
Figure 5.2 Bulky Dehydroamino Acids versus Normal Dehydroamino Acids

5.2 Methods of Synthesizing Bulky α,β-Dehydroamino Acids

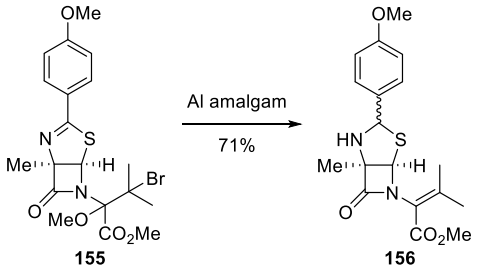
The classical method for the synthesis of ΔAAs involves the condensation of an azlactone (i.e., oxazolone) with an aldehyde.[4] In previous chapters of this dissertation, several newly developed methods of constructing ΔIle (Chapter 1 and 3) and ΔVal (Chapter 3) have been discussed. The synthesis and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

this chapter. In this section I will only discuss selected methods for the synthesis of bulky ΔAAs. 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 ΔVal-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 ΔVal subunit of β-lactam thiazolidine **156** from **155** in the presence of aluminum amalgam (Scheme 5.2).[7]



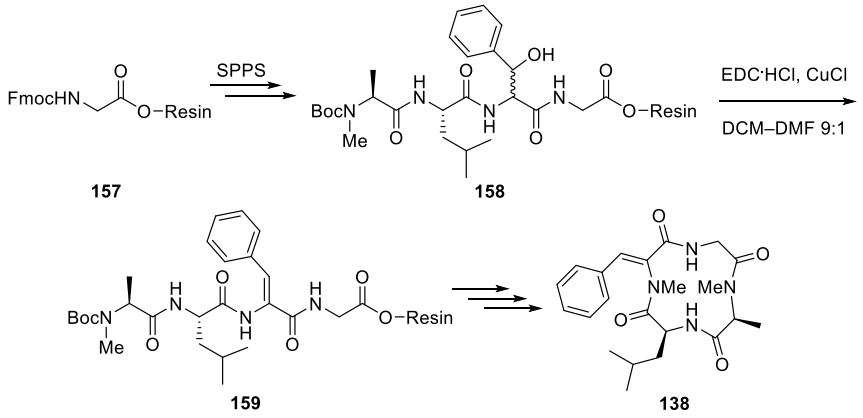
Scheme 5.1 Synthesis of ΔVal-containing Peptide **154**



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

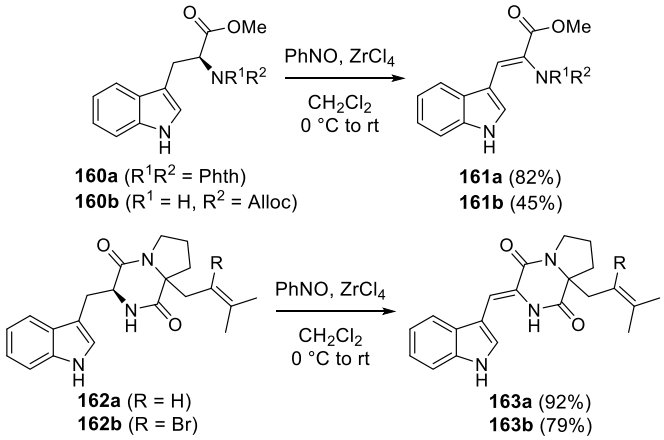
In 2003, Albericio and co-workers performed a copper–carbodiimide elimination procedure on a resin-bound peptide. Dehydration of **158** produced *Z*-ΔPhe containing peptide **159**, which was converted to tentoxin (**138**, Scheme 5.3).[8] Only the thermodynamically more stable **159** was

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

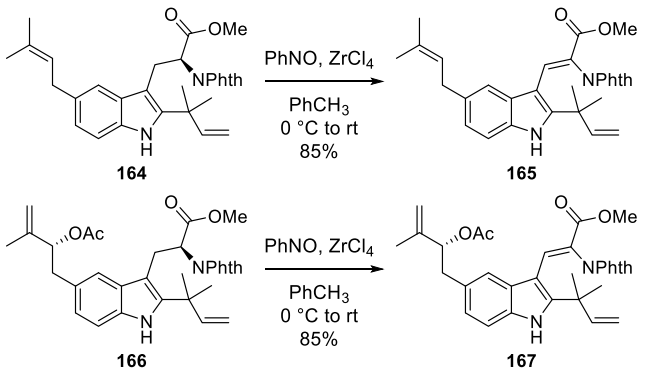


Scheme 5.3 Synthesis of Tentoxin

In 2006, Baran and co-workers synthesized *Z*-ΔTrp 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]

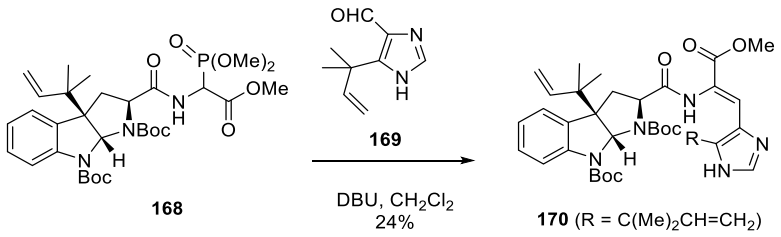


Scheme 5.4 Synthesis of *Z*-ΔTrp Derivatives **161** and **163**



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

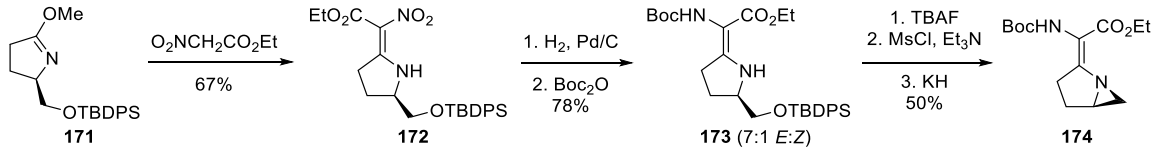
Olefinations are another important method of synthesizing bulky ΔAAs and their derivatives. In 2009, Shangguan and Joullié prepared the *Z*-ΔHis substructure of isoroquefortine E **170** via the Horner–Wadsworth–Emmons reaction of peptide phosphonate **168** and aldehyde **169** (Scheme 5.6).[11] Although compound **170** was generated as a single isomer, the yield was modest.



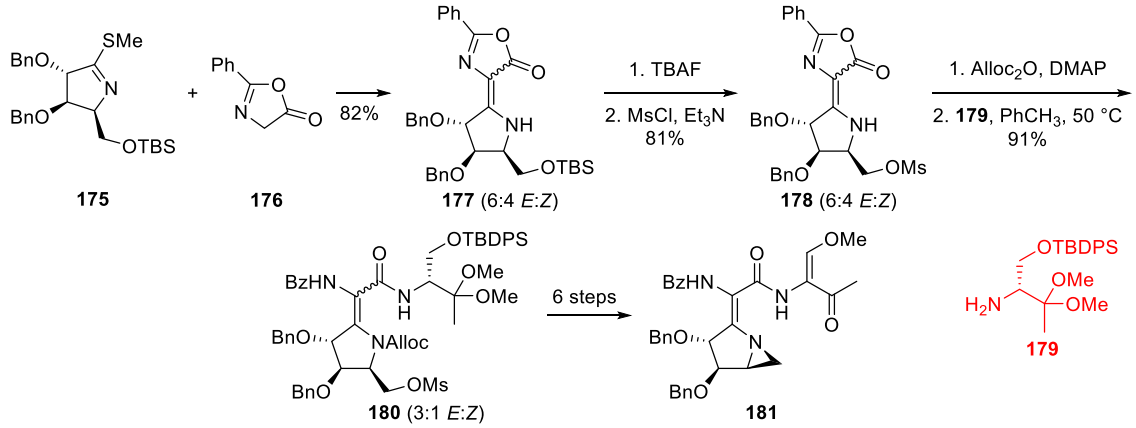
Scheme 5.6 Synthesis of the *Z*-ΔHis Substructure of Isoroquefortine E

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 ΔAA 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 ΔAA derivative **173** isomers in which the *E* isomer was the major product. A subsequent deprotection, mesylation, and cyclization generated the model of the azabicyclic ΔAA **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]

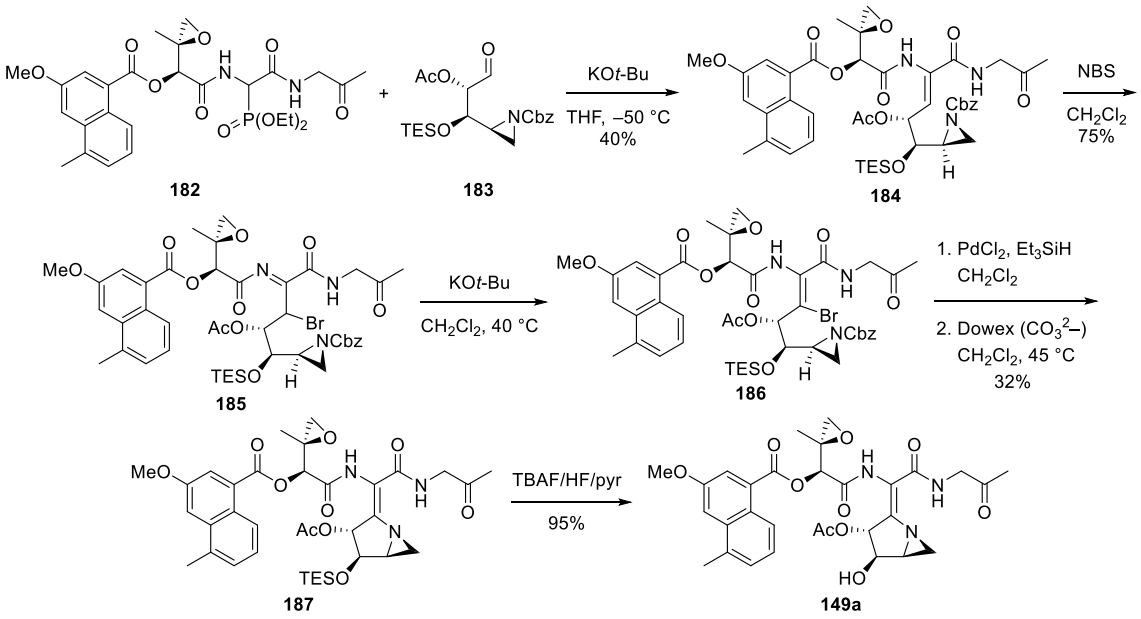


Scheme 5.7 Synthesis of the Model of Azabicyclic ΔAA **174**



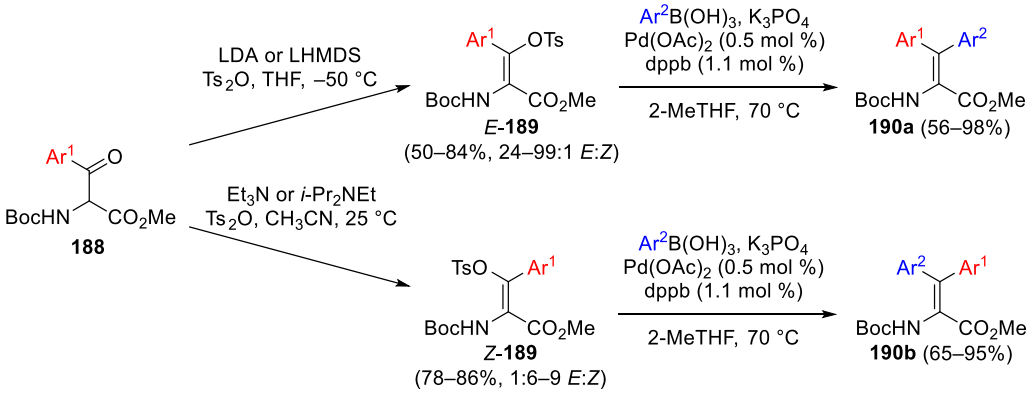
Scheme 5.8 Synthesis of the Right-hand Fragment of Azinomycin B

The Armstrong[14] and Coleman[15] groups simultaneously developed methods to install this unusual bulky ΔAA. 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*-ΔAA **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 clevage 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 ΔAAs. 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 ΔAAs **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**.



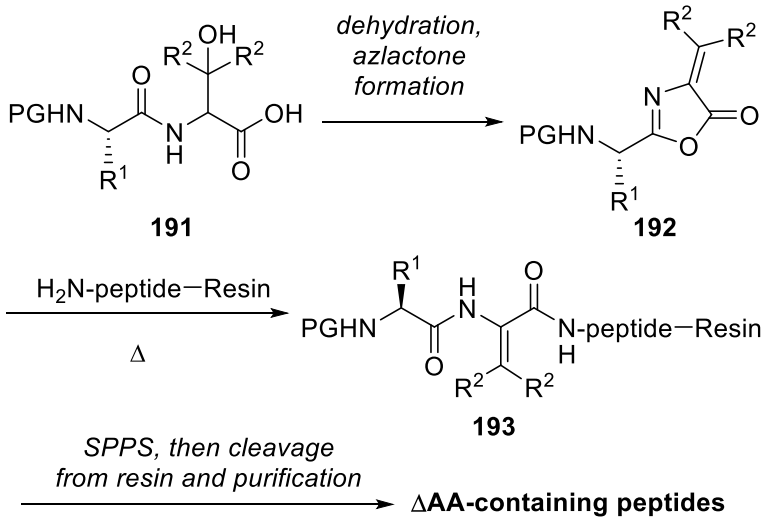
Scheme 5.10 Synthesis of Unsymmetrical β,β-diaryl Substituted ΔAAs

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

There are two major challenges involved in incorporating bulky ΔAAs 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*-ΔIle) to afford *Z*/*E* mixtures of a *C*-terminal ΔAA 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 ΔAA-containing peptides via solid-phase peptide synthesis (SPPS).

In the process of our effort to synthesize yaku'amide A, we became interested in incorporating ΔVal and ΔEnv into peptides for the purpose of studying the impact of these residues on peptide structure and stability. We have developed methods to include these ΔAAs in simple peptides using SPPS, setting the stage for further study of complex ΔAA-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*-ΔIle, it does not have such impact on ΔVal (R^2 = Me) and ΔEnv (R^2 = 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 ΔAAs placed at the chosen positions.

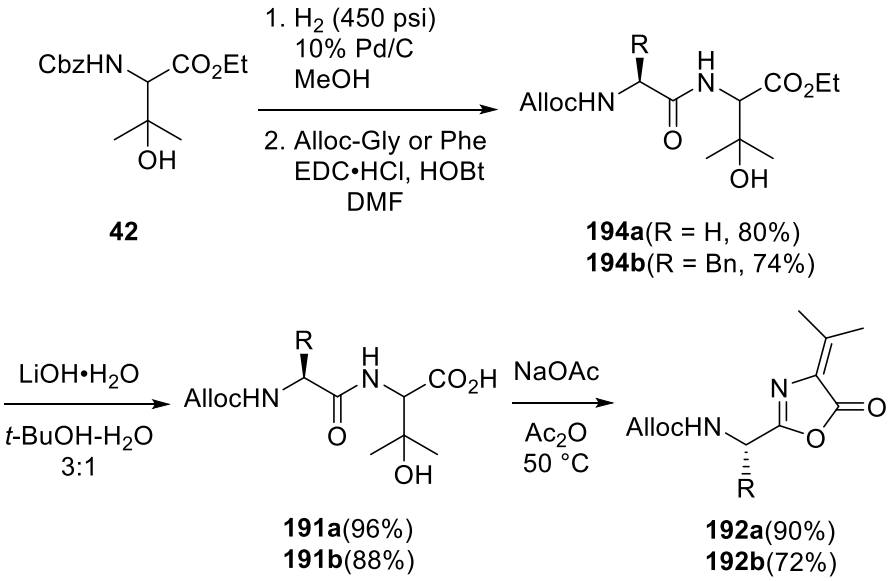


Scheme 5.11 Strategy for Synthesis of ΔAA-containing Peptides via SPPS

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.

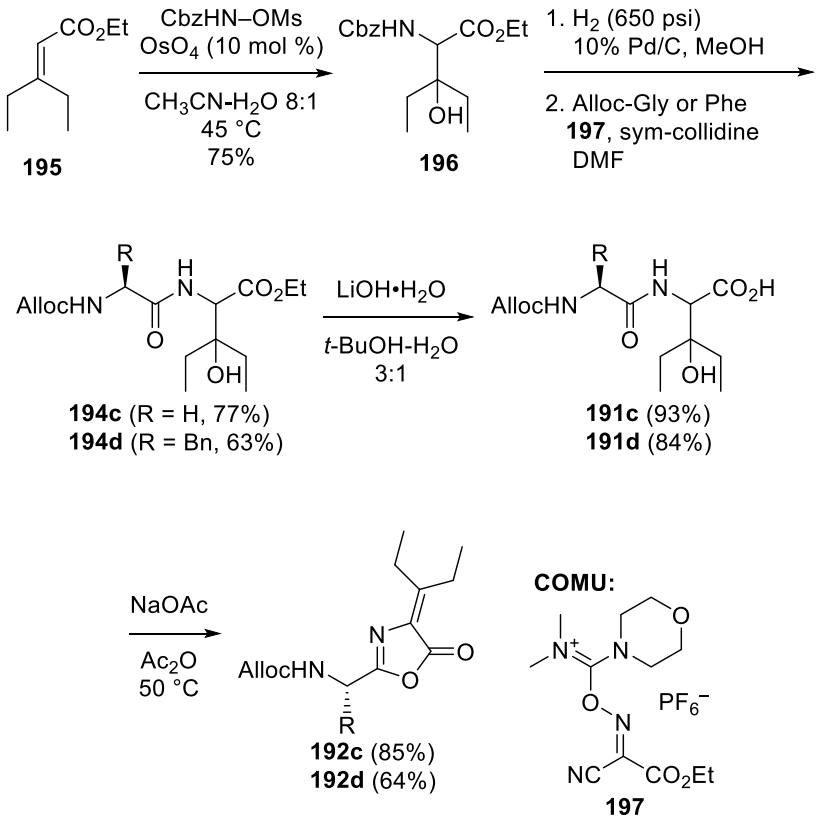
Based on this route, we prepared ΔVal-containing azlactones **192** from Cbz-protected β-OHVal derivative **42**[20] (Scheme 5.12). Hydrogenolysis of **42** provided an amine, which coupled with Alloc-Gly or Alloc-Phe separately to generate dipeptides **194a** and **194b** in good yields. Saponification 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 SiO_2 chromatography, they were typically used directly in peptide couplings without purification.



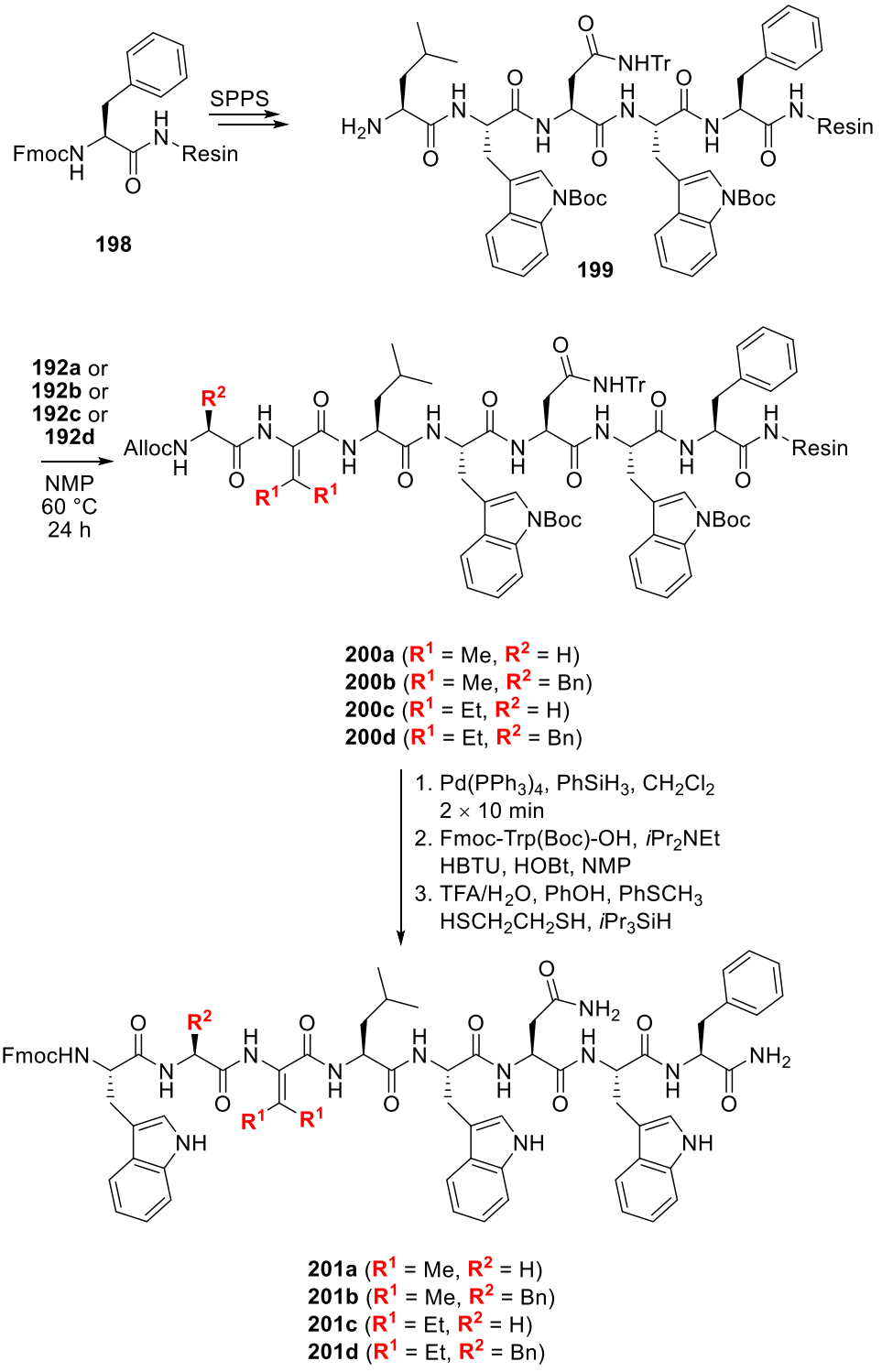
Scheme 5.12 Synthesis of ΔVal-based Azlactones

Similarly, we synthesized racemic β-hydroxy ethylnorvaline derivative **196** from known enoate **195**[22] via base-free aminohydroxylation[23] (Scheme 5.13). Subjection of **196** to the same sequence adopted with **42** produced the corresponding azlactones **192c** and **192d**. Notably, we discovered that COMU[24] 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 ΔEnv compared with ΔVal, the yields of some reactions in the ΔEnv sequence such as couplings and dehydration-cyclizations were lower.



Scheme 5.13 Synthesis of ΔEnv-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.



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 $Pd(PPh_3)_4$ and $PhSiH_3$.[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.

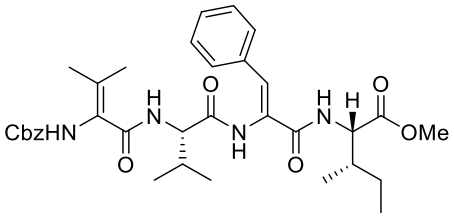
5.4 Applications of Bulky α,β-Dehydroamino Acids

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 ΔAAs 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 ΔVal and ΔEnv and incorporate them into peptides. Next, our plan was to replace certain proteinogenic residues in secondary structures with ΔVal and ΔEnv to check if they can provide stability to proteolytic degradation without negatively impacting bioactivity. We used SPPS to rapidly prepare these peptides.

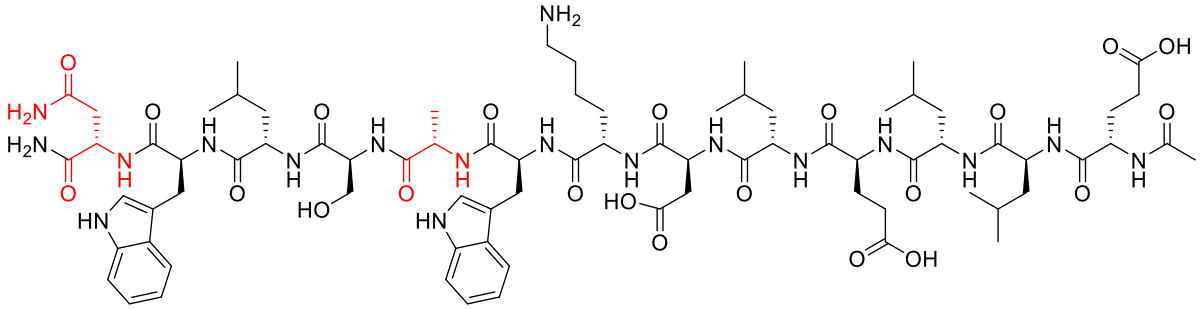
Since helical domains play an important role in mediating protein-protein interactions at protein interfaces, stable mimics are widely used as reagents for molecular biology and drug discovery. In 2003, Singh and co-workers synthesized the tetrapeptide *N*-Cbz-ΔVal-Val-*Z*-ΔPhe-Ile-OMe **202** (Figure 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 ΔVal or ΔEnv can be incorporated into helices. To continue our studies of bulky

ΔAAs, we chose a monomeric 3_{10}-helical peptide gp41$_{659–671}$ (**203**, Figure 5.4) stabilized by N- and C-capping as a system to evaluate whether ΔVal and ΔEnv would impart proteolytic stability to 3_{10} helices.[28]



202

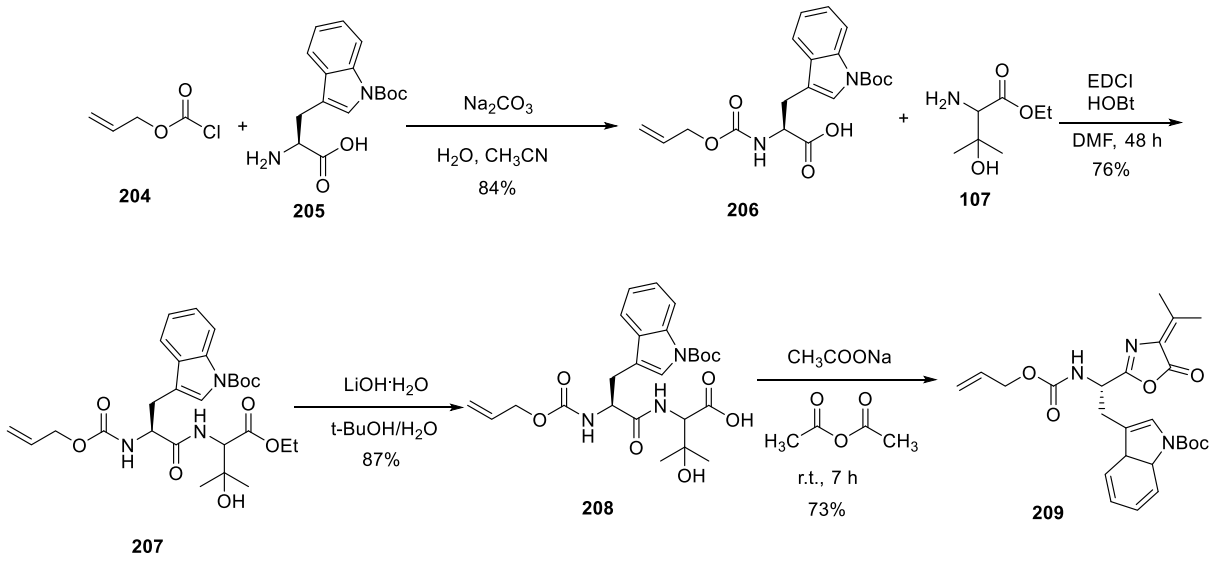
Figure 5.3 *N*-Cbz-ΔVal-Val-*Z*-ΔPhe-Ile-OMe



203

Figure 5.4 3_{10}-Helical Peptide Gp41$_{659–671}$

Our initial study involved replacing the alanine and asparagine residues of gp41$_{659–671}$ since alanine and asparagine are both adjacent to a tryptophan in this peptide, we could synthesize two analogues with a single ΔVal-based azlactone. Thus, we prepared ΔVal-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 ΔAA-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 ΔVal-based Azlactones that Contain Protected Tryptophan

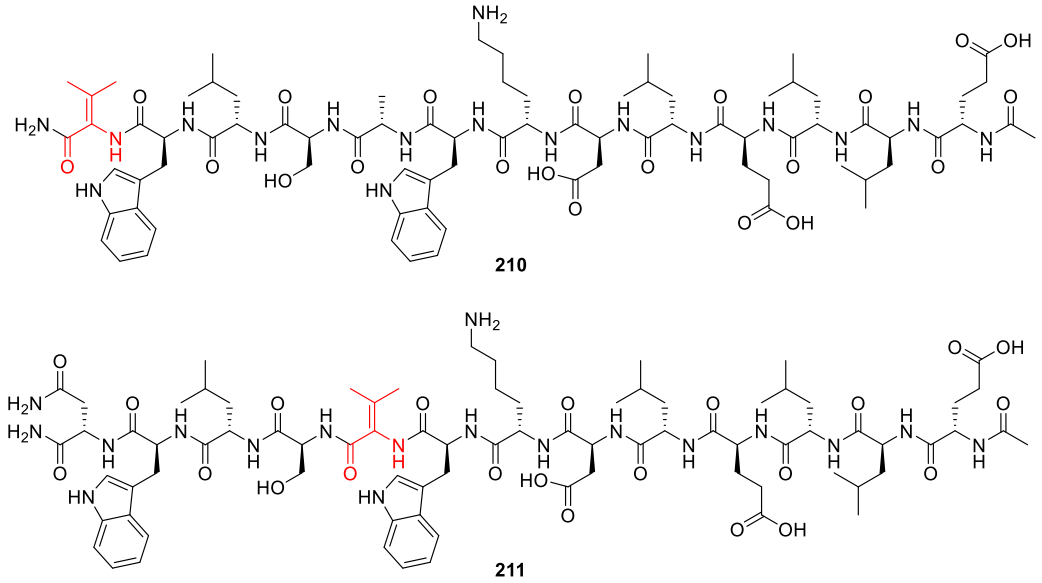


Figure 5.5 13-Residue Analogues

In 2003, the FDA approved the commercial use of enfuvirtide (FUZEON^{TM}) 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.

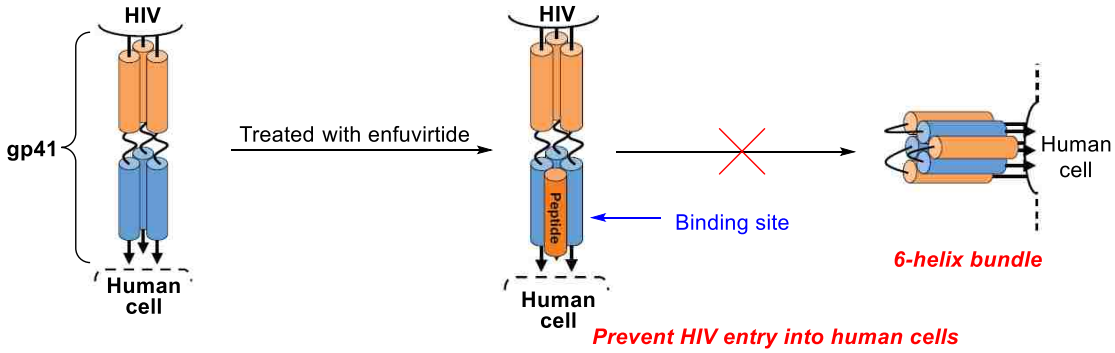


Figure 5.6 Enfuvirtide Prevents HIV Entry into Human Cells

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 ΔVal and ΔEnv 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]

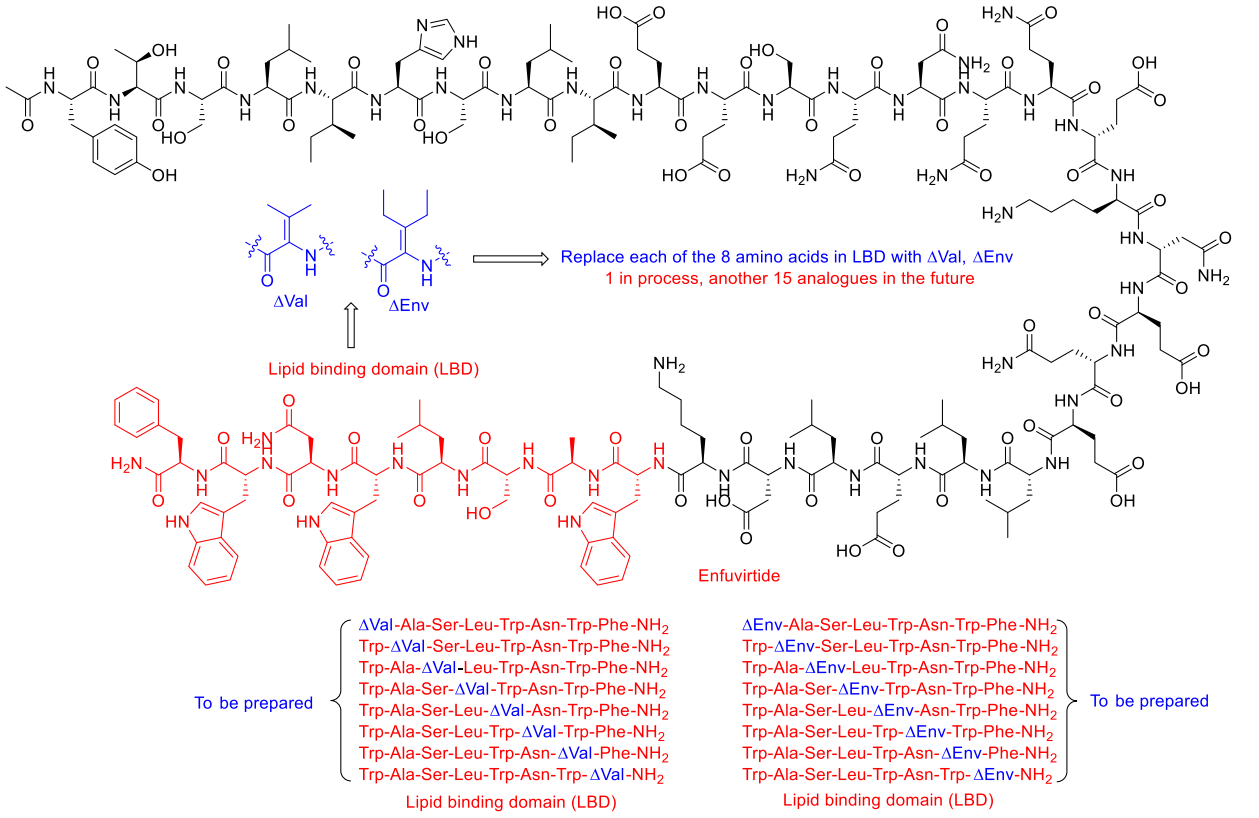


Figure 5.7 Enfuvirtide and 16 Future Analogues

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 ΔVal and ΔEnv. 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.

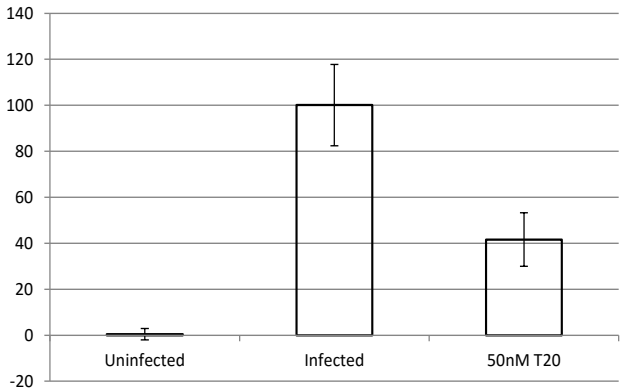


Figure 5.8 HIV-1 Infectivity Assay Control Experiment (T20 is another name for enfuvirtide)

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 ΔAAs 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 ΔVal and ΔEnv into small synthetic peptides. So far,

we have prepared two analogues of a monomeric 3_{10}-helical peptide. Analogues of the anti-HIV drug enfuvirtide will be made in the future.

5.6 References

1. Shimohiagashi, Y.; Stammer, C. H. *J. Chem. Soc, Perkin Trans 1* **1983**, 803.
2. M. L. English; C. H. Stammer, *Biochem. Biophys. Res. Commun.*, **1978**, *83*, 1464.
3. M. L. English; C. H. Stammer, *Biochem. Biophys. Res. Commun.*, **1978**, *85*, 780.
4. Schmidt, U.; Hausler, J.; Oehler, E.; Poisel, H. *Fortschr. Chem. Org. Naturst.* **1979**, *37*, 251.
5. Jiang, J.; Ma, Z.; Castle, S. L. *Tetrahedron* **2015**, *71*, 5431.
6. Nakatsuka, S.; Tanino, H.; Kishi, Y. *J. Am. Chem. Soc.* **1975**, *97*, 5008.
7. Nakatsuka, S.; Tanino, H.; Kishi, Y. *J. Am. Chem. Soc.* **1975**, *97*, 5010.
8. Jiménez, J. C.; Chavarría, B.; López-Macià, À.; Royo, M.; Giralt, E.; Albericio, F. *Org. Lett.* **2003**, *5*, 2115.
9. Baran, P. S.; Hafensteiner, B. D.; Ambhaikar, N. B.; Guerrero, C. A.; Gallagher, J. D. *J. Am. Chem. Soc.* **2006**, *128*, 8678.
10. Dai, Q.; Xie, X., Xu, S.; Ma, D.; Tang, S.; She, X. *Org. Lett.* **2011**, *13*, 2302.
11. Shangguan, N.; Joullié, M. M. *Tetrahedron. Lett.* **2009**, *50*, 6755.
12. Hashimoto, M.; Matsumoto, M.; Yamada, K.; Terashima, S. *Tetrahedron Lett.* **1994**, *35*, 2207.
13. Hashimoto, M.; Terashima, S. *Tetrahedron Lett.* **1994**, *35*, 9409.
14. (a) Armstrong, R. W.; Moran, E. J. *J. Am. Chem. Soc.* **1992**, *114*, 371. (b) Armstrong, R. W.; Tellew, J. E.; Moran, E. J. *J. Org. Chem.* **1992**, *57*, 2208. (c) Combs, A. P.; Armstrong, R. W. *Tetrahedron Lett.* **1992**, *33*, 6419. (d) Moran, E. J.; Tellew, J. E.; Zhao, Z.; Armstrong, R. W.

J. Org. Chem. **1993**, *58*, 7848. (e) Armstrong, R. W.; Tellew, J. E.; Moran, E. J. *Tetrahedron Lett.* **1996**, *37*, 447.

15. (a) Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1992**, *57*, 5813. (b) Coleman, R. S.; Carpenter, A. J. *J. Org. Chem.* **1993**, *58*, 4452. (c) Coleman, R. S.; Kong, J.-S. *J. Am. Chem. Soc.* **1998**, *120*, 3538. (d) Coleman, R. S.; Kong, J.-S.; Richardson, T. E. *J. Am. Chem. Soc.* **1999**, *121*, 9088. (e) Coleman, R. S.; Li, J.; Navarro, A. *Angew. Chem., Int. Ed.* **2001**, *40*, 1736.
16. Molinaro, C.; Scott, J. P.; Shevlin, M.; Wise, C.; Ménard, A.; Gibb, A.; Junker, E. M.; Lieberman, D. *J. Am. Chem. Soc*. **2015**, *137*, 999.
17. Stohlmeyer, M. M.; Tanaka, H.; Wandless, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 6100.
18. Shangguan, N.; Joullié, M. *Tetrahedron Lett.* **2009**, *50*, 6748.
19. Kuranaga, T.; Sesoko, Y.; Sakata, K.; Maeda, N.; Hayata, A.; Inoue, M. *J. Am. Chem. Soc.* **2013**, *135*, 5467.
20. (a) Ma, Z.; Naylor, B. C.; Loertscher, B. M.; Hafen, D. D.; Li, J. M.; Castle, S. L. *J. Org. Chem.* **2012**, *77*, 1208. (b) Ma, Z.; Jiang, J.; Luo, S.; Cai, Y.; Cardon, J. M.; Kay, B. M.; Ess, D. H.; Castle, S. L. *Org. Lett.* **2014**, *16*, 4044. (c) Jiang, J.; Luo, S.; Castle, S. L. *Tetrahedron Lett.* **2015**, *56*, 3311.
21. Abdel-Motaleb, R. M.; Bakeer, H. M.; Tamam, G. H.; Arafa, W. A. A. *J. Heterocycl. Chem.* **2012**, *49*, 1071.
22. Rawat, V.; Chouthaiwale, P. V.; Chavan, V. B.; Suryavanshi, G.; Sudalai, A. *Tetrahedron Lett.* **2010**, *51*, 6565.
23. (a) Harris, L.; Mee, S. P. H.; Furneaux, R. H.; Gainsford, G. J.; Luxenburger, A. *J. Org. Chem.* **2011**, *76*, 358. (b) Masuri; Willis, A. C.; McLeod, M. D. *J. Org. Chem.* **2012**, *77*, 8480.

24. El-Faham, A.; Subirós Funosas, R.; Prohens, R.; Albericio, F. *Chem. Eur. J.* **2009**, *15*, 9404.

25. (a) Cai, L.; Jiang, S. *ChemMedChem* **2010**, *5*, 1813. (b) He, Y. *Curr. Pharm. Des.* **2013**, *19*, 1800. (c) Tan, J. J.; Ma, X. T.; Liu, C.; Zhang, X. Y.; Wan, C. X. *Curr. Pharm. Des.* **2013**, *19*, 1810.

26. Thieriet, N.; Alsina, J.; Giralt, E.; Guibé, F.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 7275.

27. Makker, J.; Dey, S.; Mukherjee, S.; Vijayaraghavan, R.; Kumar, P.; Singh, T. P. *J. Mol. Struct.* **2003**, *654*, 119.

28. Biron, Z.; Khare, S.; Samson, A. O.; Hayek, Y.; Naider, F.; Anglister, J. *Biochemistry* **2002**, *41*, 12687.

29. Lalezari, J. P.; Eron, J. J.; Carlson, M.; Cohen, C.; DeJesus, E.; Arduino, R. C.; Gallant, J. E.; Volberding, P.; Murphy, R. L.; Valentine, F.; Nelson, E. L.; Sista, P. R.; Dusek, A.; Kilby, J. M. *AIDS* **2003,** *17* (5), 691.

30. Greenberg, M. L.; Cammack, N. *J Antimicrob Chemother* **2004,** *54* (2), 333.

31. Peisajovich, S. G.; Gallo, S. A.; Blumenthal, R.; Shai, Y. *J. Biol. Chem.* **2003,** *278* (23), 21012.

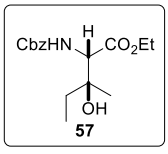
32. Huet, T.; Kerbarh, O.; Schols, D.; Clayette, P.; Gauchet, C.; Dubreucq, G.; Vincent, L.; Bompais, H.; Mazinghien, R.; Querolle, O.; Salvador, A.; Lemoine, J.; Lucidi, B.; Balzarini, J.; Petitou, M. *Antimicrob. Agents Chemother.* **2010**, *54*, 134.

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. ^{1}H 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). ^{13}C 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.

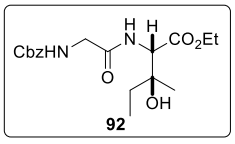
6.2 Experimental Procedures and Spectral Data



57

Ethyl (2*S,3*S**)-2-(((Benzyloxy)carbonyl)amino)-3-hydroxy-3 methylpentanoate (57).** A solution of benzyl ((methylsulfonyl)oxy)carbamate[2] (820 mg, 3.34

mmol, 1.4 equiv) in CH_3CN (18 mL) at rt was treated with OsO_4 (4 wt % solution in H_2O, 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 CH_3CN (6.0 mL) and H_2O (1.5 mL). The resulting mixture was stirred at 45 °C for 2.5 d, treated with sat aq $K_2S_2O_5$ (7 mL), and stirred for an additional 10 min. It was then diluted with H_2O (35 mL) and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with sat aq $NaHCO_3$ (2 × 50 mL) and brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (100 mL of SiO_2, 1–5% MeOH in CH_2Cl_2 gradient elution) afforded **57** (580 mg, 1.87 mmol, 78%) as a light yellow oil: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{16}H_{23}NO_5H^+$ requires 310.1654).

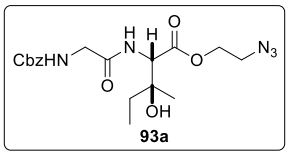


92

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 H_2 (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% H_2O 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 $NaHCO_3$ (12 mL) and diluted with H_2O (12 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL), and the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (70 mL of SiO_2, 1–5% MeOH in CH_2Cl_2 gradient elution) afforded **92** (220 mg, 0.600 mmol, 62%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{18}H_{26}N_2O_6H^+$ requires 367.1869).

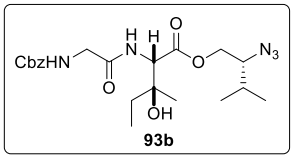


93a

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–H_2O (3:1, 1.6 mL) at 0 °C was treated with LiOH•H_2O (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 (Na_2SO_4) 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 Et_3N (49 μL, 36 mg,

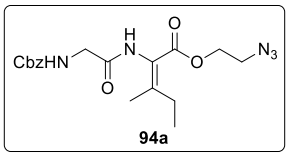
0.35 mmol, 3.1 equiv). The resulting mixture was stirred at 80 °C under Ar for 48 h, then concentrated *in vacuo*. The residue was dissolved in EtOAc (20 mL) and washed with brine (3 × 3 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (3 mL of SiO_2, 0–2% MeOH in CH_2Cl_2 gradient elution) afforded **93a** (43.6 mg, 0.107 mmol, 95% from **92**) as a colorless oil: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{18}H_{25}N_5O_6H^+$ requires 408.1883).



93b

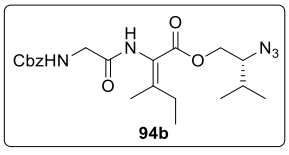
(*R*)-2-Azido-3-methylbutyl (2*S,3*S**)-2-(2-(((benzyloxy)carbonyl) amino)acetamido)-3-hydroxy-3-methylpentanoate (93b).** A solution of the acid derived from hydrolysis of ester **92** (prepared as described for azidoethyl ester **93a**, 30.7 mg, 0.0907 mmol) and iodide (44 mg, 0.184 mmol, 2.0 equiv) in anhydrous DMF (870 μL) at rt under Ar was treated with Et_3N (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 (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (5 mL of SiO_2, 0–1.5% MeOH in CH_2Cl_2 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: ^{1}H NMR ($CDCl_3$, 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^+, $C_{21}H_{31}N_5O_6H^+$ requires 450.2353).



94a

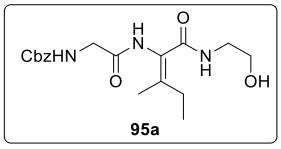
2-Azidoethyl (*E*)-2-(2-(((benzyloxy)carbonyl)amino)acetamido)-3-methylpent-2-enoate (94a). A solution of alcohol **93a** (19.3 mg, 0.0474 mmol) in anhydrous $CHCl_3$ (210 μL) was treated with Martin sulfurane (0.24 M in anhydrous $CHCl_3$, 400 μL, 0.096 mmol, 2.0 equiv) dropwise at 0 ºC under Ar. The resulting mixture was stirred at 0 ºC 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, 0.0354mmol, 75%, >19:1 dr) as a light yellow oil: ^{1}H 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^+, $C_{18}H_{23}N_5O_5H^+$ requires 390.1777).



94b

(*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

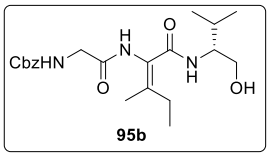
anhydrous $CHCl_3$ (240 μL) was treated with Martin sulfurane (0.24 M in anhydrous $CHCl_3$, 460 μL, 0.11 mmol, 2.0 equiv) dropwise at –20 °C. The resulting mixture was stirred at –20 °C under Ar for 1 h, warmed to rt, and concentrated *in vacuo*. Flash chromatography (10 mL of SiO_2, 0–1.5% MeOH in CH_2Cl_2 gradient elution) afforded **94b** (19.7 mg, 0.0457 mmol, 84%, >19:1 dr) as a colorless oil: $[\alpha]^{25}_D$ +3.7 (*c* 0.30, CH_2Cl_2); ^{1}H 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^+, $C_{21}H_{29}N_5O_5H^+$ requires 432.2247).



95a

Benzyl (*E*)-(2-((1-((2-hydroxyethyl)amino)-3-methyl-1-oxopent-2-en-2-yl)amino)-2-oxoethyl)carbamate (95a). A solution of azide **94a** (3.0 mg, 0.0077 mmol) in THF (210 μL) and H_2O (16 μL) at 0 °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 °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:^{1}H 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,

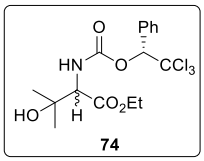
1H), 5.14 (s, 2H), 3.89 (d, J = 5.7 Hz, 2H), 3.73 (q, J = 5.1 Hz, 2H), 3.43 (q, J = 5.0 Hz, 2H), 3.24 (br s, 1H), 2.41 (q, J = 7.3 Hz, 2H), 1.70 (s, 3H), 1.09 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.4, 166.6, 157.2, 142.4, 135.6, 128.6 (2C), 128.5, 128.2 (2C), 123.7, 67.7, 61.4, 45.1, 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^+, $C_{18}H_{25}N_3O_5H^+$ requires 364.1872).



95b

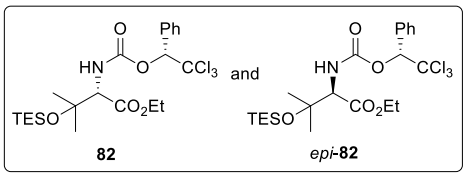
Benzyl (*R*,*E*)-(2-((1-((1-hydroxy-3-methylbutan-2-yl)amino)-3-methyl-1-oxopent-2-en-2-yl)amino)-2-oxoethyl)carbamate (95b). A solution of azide **94b** (6.1 mg, 0.014 mmol) in DMF (400 μL) and H_2O (31 μL) at 0 °C under Ar was treated dropwise with PMe_3 (1 M in THF, 43 μL, 0.043 mmol, 3.0 equiv). The resulting mixture was stirred at 0 °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 × 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: $[\alpha]^{25}_D$ +38 (*c* 0.12, $CHCl_3$); ^{1}H 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^+, $C_{21}H_{31}N_3O_5H^+$ requires 406.2342).



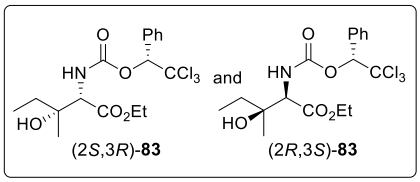
74

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) carbamate[4] (**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 °C for 24 h, then treated with sat aq $K_2S_2O_5$ (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^+, $C_{16}H_{20}Cl_3NO_5H^+$ requires 412.0485).



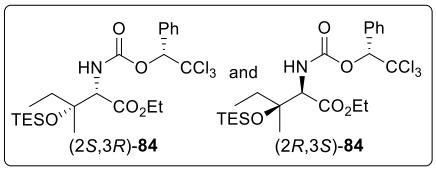
Ethyl (*S*)-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)butanoate (82) and **ethyl (*R*)-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)butanoate (*epi*-82)**. A solution of **74** (1.01 g, 2.45 mmol) in anhydrous CH_2Cl_2 (24 mL) at 0 ºC 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 $NaHCO_3$ (8 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (30 mL SiO_2, 1–2% MeOH in CH_2Cl_2 gradient elution) afforded **82** and *epi*-**82** (1.12 g, 2.13 mmol, 87%) as a 1:1 mixture of diastereomers. Additional flash chromatography (SiO_2, 10–50% CH_2Cl_2 in hexanes gradient elution) could be performed to afford **82** and *epi*-**82** (>10:1 dr) as colorless oils. For **82**: $[\alpha]^{25}_D$ –5.79 (*c* 1.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{22}H_{34}Cl_3NO_5SiH^+$ requires 526.1350). For *epi*-**82**: $[\alpha]^{25}_D$ –10.2 (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 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^+, $C_{22}H_{34}Cl_3NO_5SiH^+$ requires 526.1350).



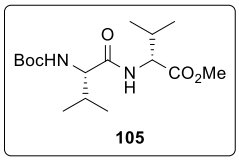
Ethyl (2*S*,3*R*)-3-hydroxy-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)pentanoate ((*2S,3R*)-83) and **ethyl (2*R*,3*S*)-3-hydroxy-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)pentanoate ((*2R,3S*)-83).** A solution of (*R*)-2,2,2-trichloro-1-phenylethyl ((methylsulfonyl)oxy)carbamate[4] (**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 $K_2S_2O_5$ (10 mL) and stirred for an additional 15 min. It was then diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with sat aq $NaHCO_3$ (2 × 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: ^{1}H 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).



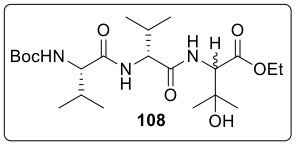
Ethyl (2*S*,3*R*)-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl)oxy)pentanoate (**(2*S*,3*R*)-84)** and **ethyl (2*R*,3*S*)-3-methyl-2-((((*R*)-2,2,2-trichloro-1-phenylethoxy)carbonyl)amino)-3-((triethylsilyl) oxy)pentanoate ((2*R*,3*S*)-84).** A solution of **83** (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 μL, 352 mg, 3.27 mmol, 2.4 equiv) followed by TES-OTf (500 μL, 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, $[\alpha]^{25}_D$ –22.1 (*c* 0.19, $CHCl_3$); ^{1}H 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

($M(NH_4)^+$, $C_{23}H_{36}Cl_3NO_5Si(NH_4)^+$ requires 557.1767). For (2*R*,3*S*)-**84**: less polar isomer, $[\alpha]^{25}_D$ –4.5 (*c* 0.22, $CHCl_3$); ^1H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 ($M(NH_4)^+$, $C_{23}H_{36}Cl_3NO_5Si(NH_4)^+$ requires 557.1767).



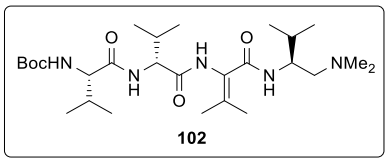
105

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% H_2O content, 760 mg, 4.5 mmol, 1.5 equiv), EDC•HCl (863 mg, 4.5 mmol, 1.5 equiv), and $NaHCO_3$ (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 $NaHCO_3$ (10 mL) and H_2O (20 mL), extracted with EtOAc (4 × 35 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (80 mL of SiO_2, 0–25% EtOAc in hexanes gradient elution) afforded **105** (889 mg, 2.7 mmol, 89%) as a white film: ^1H NMR ($CDCl_3$, 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^+, $C_{16}H_{30}N_2O_5H^+$ 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–H_2O (2:1, 21 mL) at 0 °C was treated with LiOH•H_2O (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 H_2O (5 mL), and extracted with EtOAc (5 × 20 mL). The combined organic layers were dried (Na_2SO_4) 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.

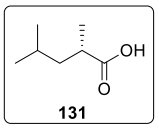
A solution of the acid in anhydrous DMF–CH_2Cl_2 (1:1, 30 mL) at 0 °C under Ar was treated with amine **107** (328 mg, 2.04 mmol, 1.2 equiv), HOBt (ca. 20% H_2O content, 456 mg, 2.7 mmol, 1.6 equiv), and EDC•HCl (489 mg, 2.6 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C under Ar for 24 h. The reaction was quenched by the addition of sat aq $NaHCO_3$ (10 mL) and H_2O (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 (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (50 mL of SiO_2, 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: ^{1}H NMR ($CDCl_3$, 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^+, $C_{22}H_{41}N_3O_7H^+$ requires 460.3023).



102

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–H_2O (1:1, 10 mL) at rt was treated with LiOH•H_2O (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 H_2O (3 mL), and extracted with EtOAc (6 × 10 mL). The combined organic layers were dried (Na_2SO_4) 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 NEt_3 (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 $NaHCO_3$ (4 mL), diluted with H_2O (4 mL), and extracted with EtOAc (8 × 10 mL). The combined organic layers were dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (15 mL of SiO_2, 0–4% MeOH in EtOAc with 1% Et_3N gradient elution) afforded **102** (94.7 mg, 0.18 mmol, 67% from **109**) as a white film: ^{1}H NMR ($CDCl_3$, 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^+, $C_{27}H_{51}N_5O_5H^+$ requires 526. 3968).



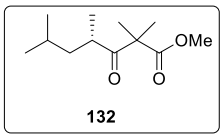
131

(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 CH_2Cl_2 (50 ml) at room temperature was added Et_3N (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 $NaHCO_3$ (40 ml), 1.0 M aqueous HCl (2 × 30 mL) and brine (40 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash chromatography (200 mL of SiO_2, 2–8% MeOH in CH_2Cl_2 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*Pr$_2$NH (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 NH_4Cl (20 ml) and saturated, aqueous $Na_2S_2O_3$ (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 Na_2SO_4, and filtered.

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

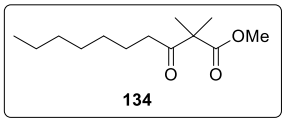
A 250-mL round-bottomed flask was charged with amide **130** (390 mg, 1.41 mmol, 1 equiv), dioxane (20 mL), and 9 N aqueous sulfuric acid solution (20 mL). The biphasic mixture was heated at reflux for 24 h and then cooled to 0 °C. The pH of the mixture was adjusted to pH > 10 by the slow addition of 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%): ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.8, 42.7, 37.3, 25.8, 22.5, 22.4, 17.3; HRMS (ESI) *m/z* 131.1076 (MH^+, $C_7H_{14}O_2H^+$ requires 131.1072).



132

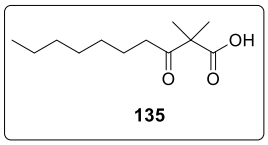
Methyl (S)-2,2,4,6-tetramethyl-3-oxoheptanoate (132). To a suspended solution of $InBr_3$ (35.5 mg, 0.1 mmol) and **131** (131 mg, 1 mmol) in dichloromethane (10 mL) was added $(MeO)_3SiH$ (134 uL, 1.05 mmol), and methyl trimethylsilyl dimethylketene acetal (406 uL, 2 mmol). The reaction mixture was stirred for 6 h at room temperature and then was quenched by 1 M HCl aq (10 mL). The resulting mixture was extracted with Et_2O (3 × 20 ml). The organic

layer was dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (15 mL of SiO_2, 1–5% MeOH in CH_2Cl_2 gradient elution) afforded **132** (190 mg, 0.89 mmol, 89%) as a light yellow liquid: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{12}H_{22}O_3H^+$ requires 215.1647).



134

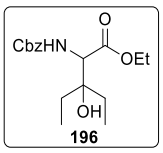
Methyl 2,2-dimethyl-3-oxodecanoate (134). To a suspended solution of $InBr_3$ (35.5 mg, 0.1 mmol) and **133** (145 mg, 1 mmol) in dichloromethane (10 mL) was added $(MeO)_3SiH$ (134 uL, 1.05 mmol), and methyl trimethylsilyl dimethylketene acetal (406 uL, 2 mmol). The reaction mixture was stirred for 6 h at room temperature and then was quenched by 1 M HCl aq (10 mL). The resulting mixture was extracted with Et_2O (3 × 20 ml). The organic layer was dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (15 mL of SiO_2, 1–5% MeOH in CH_2Cl_2 gradient elution) afforded **134** (208 mg, 0.91 mmol, 91%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{13}H_{24}O_3H^+$ requires 229.1804).



135

2,2-dimethyl-3-oxodecanoic acid (135). A solution of ester **134** (117 mg, 0.512 mmol) in *t*-BuOH–H_2O (2:1, 6 mL) at 0 °C was treated with LiOH•H_2O (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

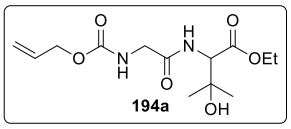
addition of 2 N HCl (4 mL) and extracted with EtOAc (6 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The crude carboxylic acid **135** (105 mg, 0.49 mmol, 96%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ COOH (1H), 2.54 (t, J = 7.5 Hz, 2H), 1.64–1.56 (m, 2H), 1.42 (s, 6H), 1.36–1.20 (m, 8H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 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^+, $C_{12}H_{22}O_3H^+$ requires 215.1647).



196

Ethyl 2-(((benzyloxy)carbonyl)amino)-3-ethyl-3-hydroxypentanoate (196). A solution of benzyl ((methylsulfonyl)oxy)carbamate[2] (1.472 g, 6.00 mmol, 1.3 equiv) in CH_3CN (50 mL) at rt was treated with OsO_4 (4 wt % solution in H_2O, 2.9 mL, 0.46 mmol, 0.10 equiv), stirred for 10 min, and then treated with a solution of ethyl 3-ethylpent-2-enoate[6] (**195**, 710 mg, 4.54 mmol) in CH_3CN (10.0 mL) and H_2O (4.7 mL). The resulting mixture was stirred at 45 °C for 4 d, treated with sat aq $K_2S_2O_5$ (20 mL), and stirred for an additional 20 min. It was then diluted with H_2O (40 mL) and extracted with EtOAc (4 × 80 mL). The combined organic layers were washed with sat aq $NaHCO_3$ (2 × 40 mL) and brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (300 mL of SiO_2, 0.25–5% MeOH in CH_2Cl_2 gradient elution) afforded **196** (1.10 g, 3.40 mmol, 75%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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,

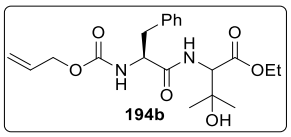
3033, 2972, 1719, 1509, 1334, 1051 cm^{-1}; HRMS (ESI) *m/z* 324.1808 (MH^+, $C_{17}H_{25}NO_5H^+$ requires 324.1811).



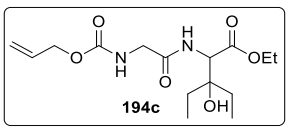
194a

Ethyl 2-(2-(((allyloxy)carbonyl)amino)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^+, $C_{13}H_{22}N_2O_6H^+$ requires 303.1556).



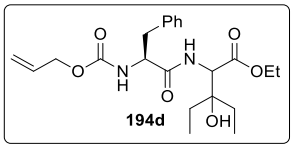
Ethyl 2-((*S*)-2-(((allyloxy)carbonyl)amino)-3-phenylpropanamido)-3-hydroxy-3-methylbutanoate (194b). A solution of the crude amine prepared via hydrogenolysis of **42** (73 mg, 0.45 mmol, 1.0 equiv) in DMF (2 mL) was added to a solution of ((allyloxy)carbonyl)-L-phenylalanine (127.7 mg, 0.512 mmol, 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 H_2O (15 mL) and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with brine (10 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 **194b** (132 mg, 0.336 mmol, 74%) as a light yellow oil that was a 1:1 mixture of diastereomers: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{20}H_{28}N_2O_6H^+$ 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 H_2 (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.

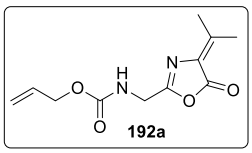
The crude amine (41 mg, 0.22mmol, 1.0 equiv) was dissolved in DMF (1 mL) and added to a solution of ((allyloxy)carbonyl)glycine (103.4 mg, 0.650 mmol, 3.0 equiv) in DMF (1 mL) at 0 °C under Ar. The mixture was stirred for 10 min, then treated with COMU (279.7 mg, 0.653 mmol, 3.0 equiv) and 2,4,6-collidine (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 $NaHCO_3$ (3 mL), and extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine (5 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 **194c** (59 mg, 0.18 mmol, 82%, 77% from **7**) as a light yellow oil: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{15}H_{26}N_2O_6H^+$ requires 331.1869).



194d

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 μL, 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 $NaHCO_3$ (3 mL), and extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine (5 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 **194d** (75 mg, 0.18 mmol, 68%, 63% from **7**) as a light yellow oil that was a 1:1 mixture of diastereomers: ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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^+, $C_{22}H_{32}N_2O_6H^+$ requires 421.2339).

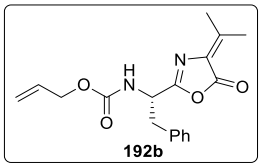


192a

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–H_2O (3:1, 4 mL) at 0 °C under Ar was treated with LiOH•H_2O (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 (Na_2SO_4) 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 H_2O (0.8 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na_2CO_3 (3× 3 mL) and brine (1.5 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford **192a** (43 mg, 0.18 mmol, 90%) as a light yellow solid: ^{1}H NMR ($CDCl_3$, 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^+, $C_{11}H_{14}N_2O_4H^+$ requires 239.1032).

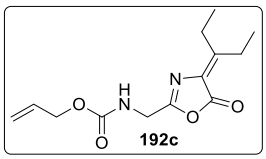


192b

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–H_2O (3:1, 2 mL) at 0 °C under Ar was treated with LiOH•H_2O (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 (Na_2SO_4) 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

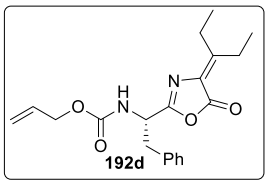
at 50 °C for 7 h, at which time the starting material had disappeared as evidenced by MS. The excess acetic anhydride was decomposed by the addition of H_2O (0.5 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na_2CO_3(3× 3 mL) and brine (1.5 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford **192b** (32 mg, 0.097 mmol, 72%) as a light yellow solid: ^{1}H NMR ($CDCl_3$, 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^+, $C_{18}H_{20}N_2O_4H^+$ 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–H_2O (3:1, 2 mL) at 0 °C under Ar was treated with LiOH•H_2O (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 (Na_2SO_4) 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 H_2O (0.5 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na_2CO_3 (3× 3 mL) and brine (1.5 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford **192c** (35 mg, 0.13 mmol, 85%) as a light yellow

solid: ^{1}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^+, $C_{13}H_{18}N_2O_4H^+$ requires 267.1345).



192d

Allyl (S)-(1-(5-oxo-4-(pentan-3-ylidene)-4,5-dihydrooxazol-2-yl)-2-phenylethyl)carbamate (192d). A solution of **194d** (52 mg, 0.12 mmol) in *t*-BuOH–H_2O (3:1, 2 mL) at 0 °C under Ar was treated with LiOH•H_2O (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 (Na_2SO_4) 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 H_2O (0.5 mL). The mixture was then extracted with EtOAc (4 × 5 mL), washed with sat aq Na_2CO_3 (3 × 3 mL) and brine (1.5 mL), dried (Na_2SO_4), and concentrated *in vacuo* to afford **192d** (24 mg, 0.067 mmol, 64%) as a light yellow solid: ^{1}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^+, $C_{20}H_{24}N_2O_4H^+$ 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 °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 × 3 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*Pr_2NEt (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 °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 × 3 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, Et_3N was later found to provide better results. The resulting mixture was stirred at 60 °C for 24 h. The solution was drained from the resin using a vacuum manifold, and the resin was rinsed with DMF (5 × 2–3 mL).

Alloc deprotection. The resin-bound peptide **200** (10 μmol) was washed with CH_2Cl_2 (10 × 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 $Pd(PPh_3)_4$ (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 × 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 × 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 × 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

1. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.

2. Qin, L.; Zhou, Z.; Wei, J.; Yan, T.; Wen, H. *Synth. Commun.***2010**, *40*, 642.

3. Shangguan, N.; Joullié, M.*Tetrahedron Lett.***2009**, *50*, 6748.

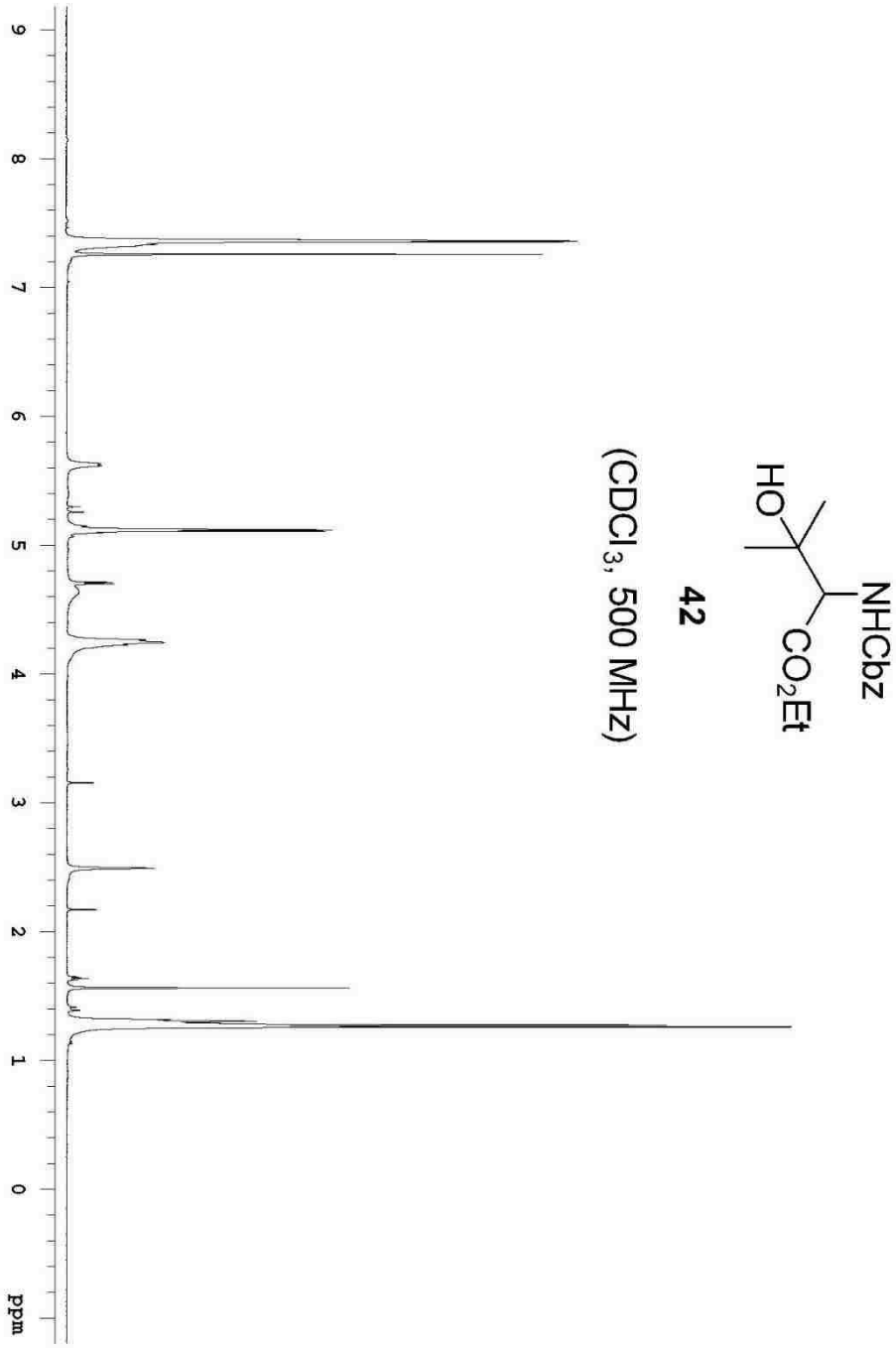
4. H. Lebel, C. Trudel, C. Spitz, *Chem. Commun.* **2012**, *48*, 7799.

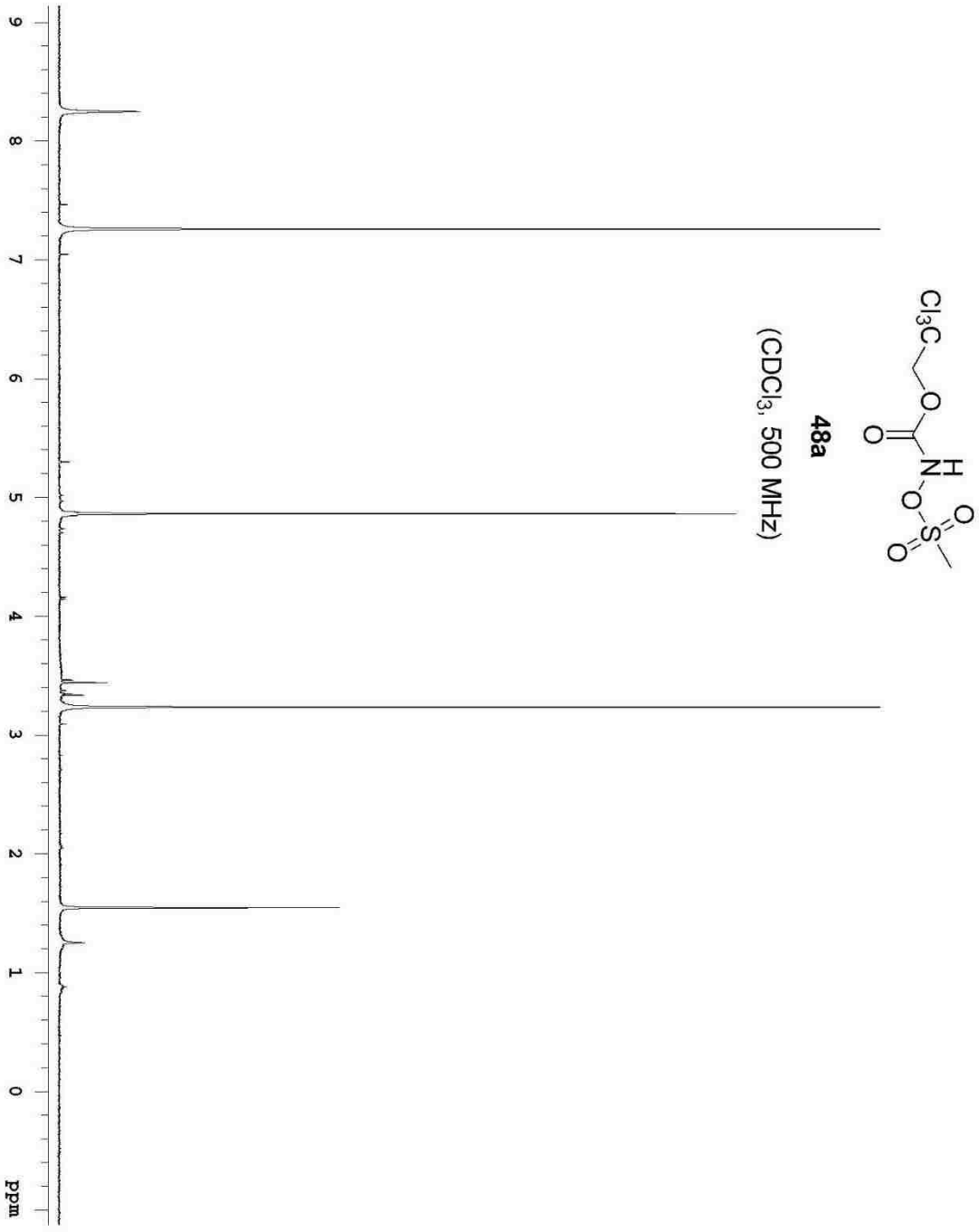
5. L. Wu, H. Ye, D. Xiao, *Zhongguo Yiyao Gongye Zazhi* **2004**, *35*, 67. Alternatively, **50** could be prepared via the procedure employed by Inoue and co-workers (*J. Am. Chem. Soc.* **2013**, *135*, 5467) for synthesis of the analogous vinyl iodide (listed as compound **33** in their paper), using MeOH or H_2O trapping instead of I_2 trapping.

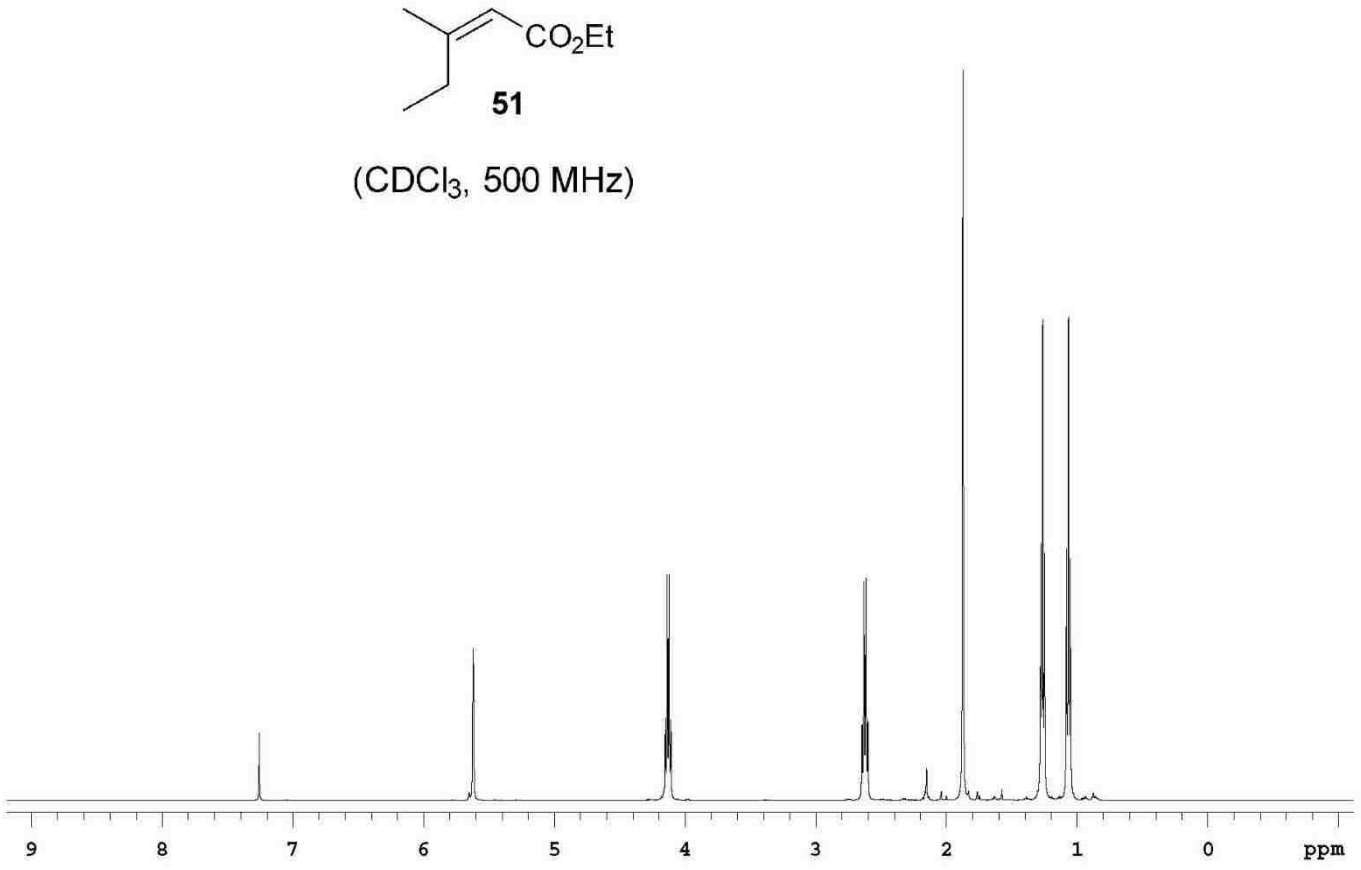
6. Rawat, V.; Chouthaiwale, P. V.; Chavan, V. B.; Suryavanshi, G.; Sudalai, A. *Tetrahedron Lett.* **2010**, *51*, 6565.

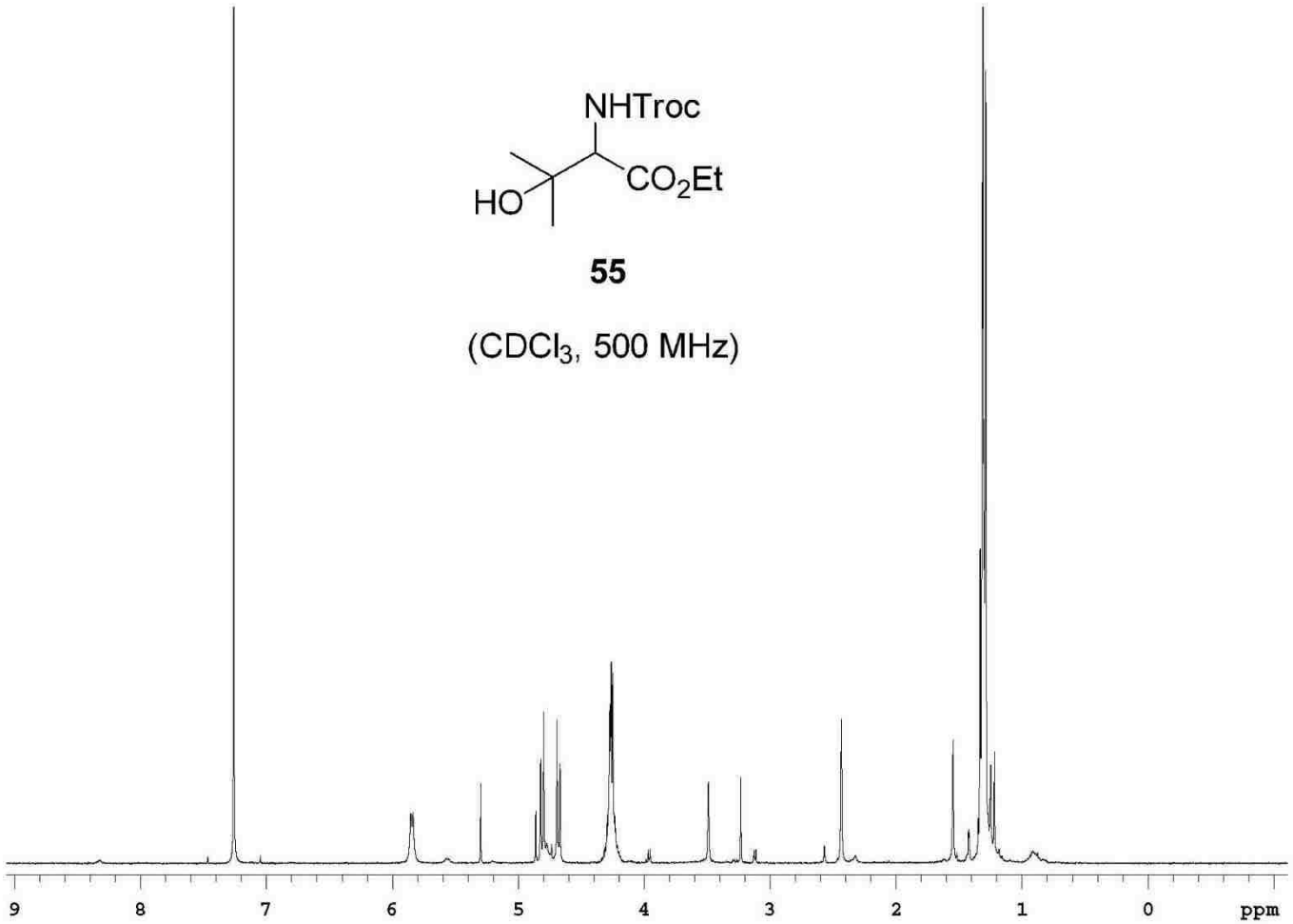
7. Ma, Z.; Naylor, B. C.; Loertscher, B. M.; Hafen, D.D.; Li, J. M.; Castle, S. L. *J. Org. Chem.* **2012**, *77*, 1208.

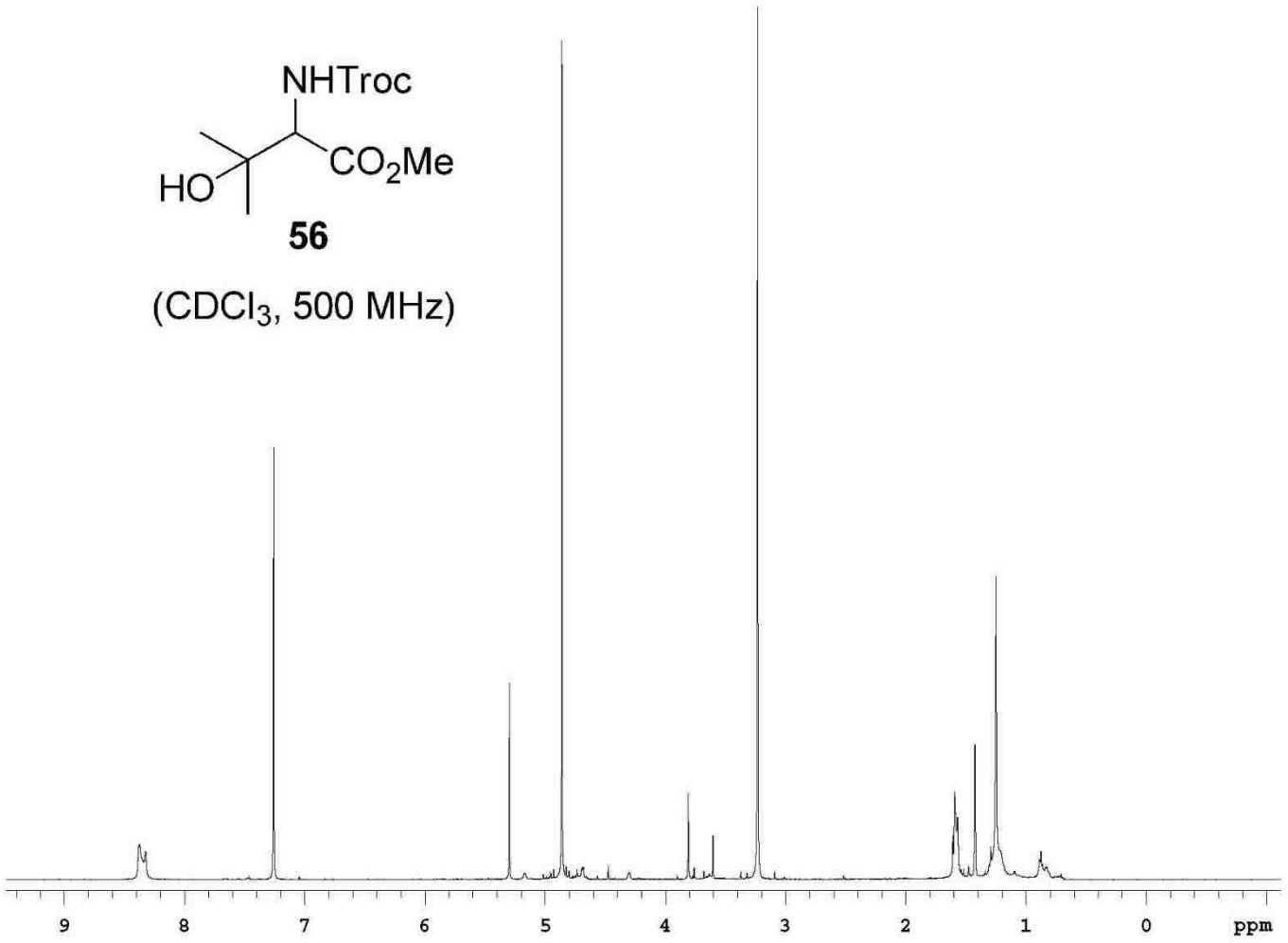
6.4 **Spectra**

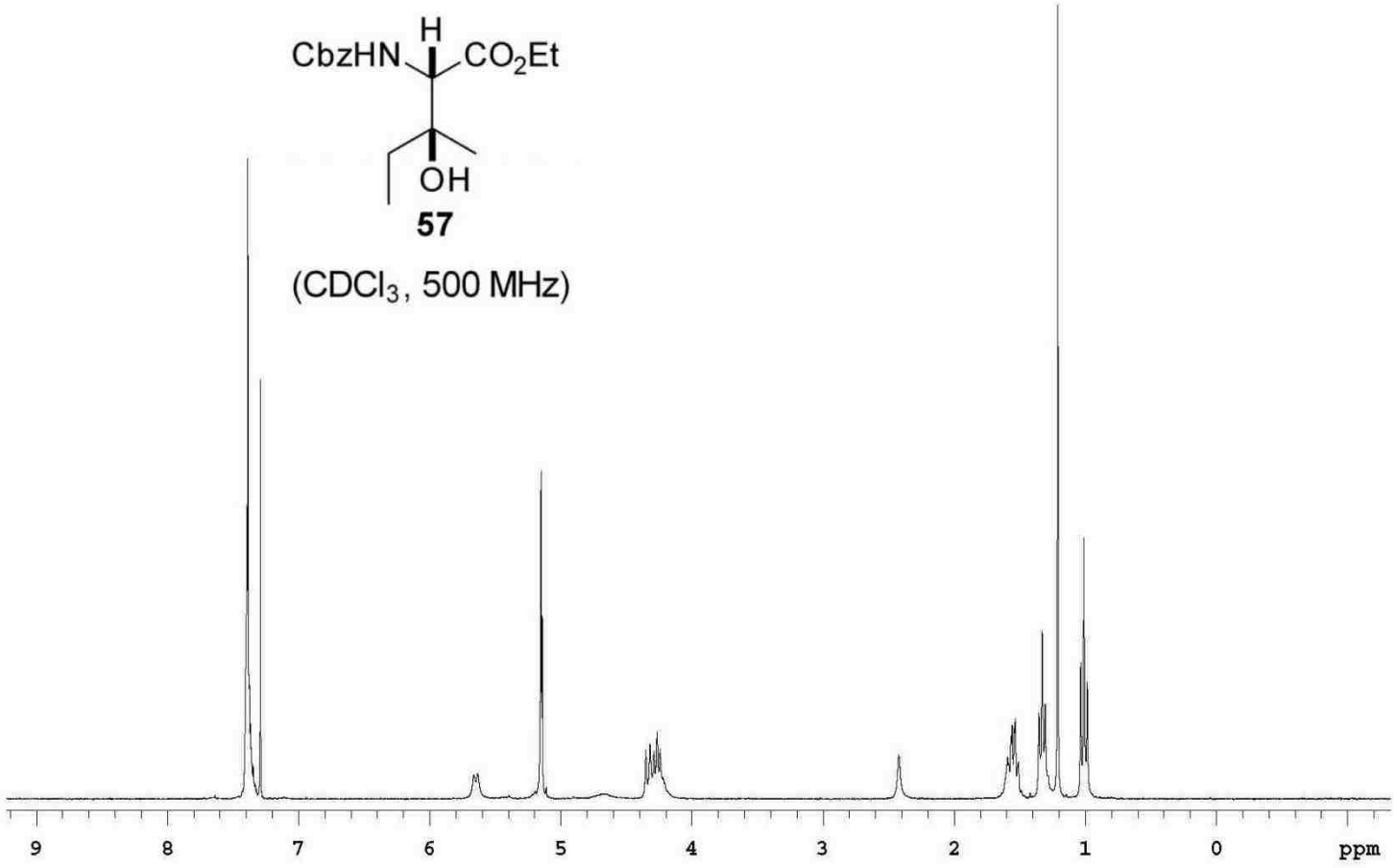


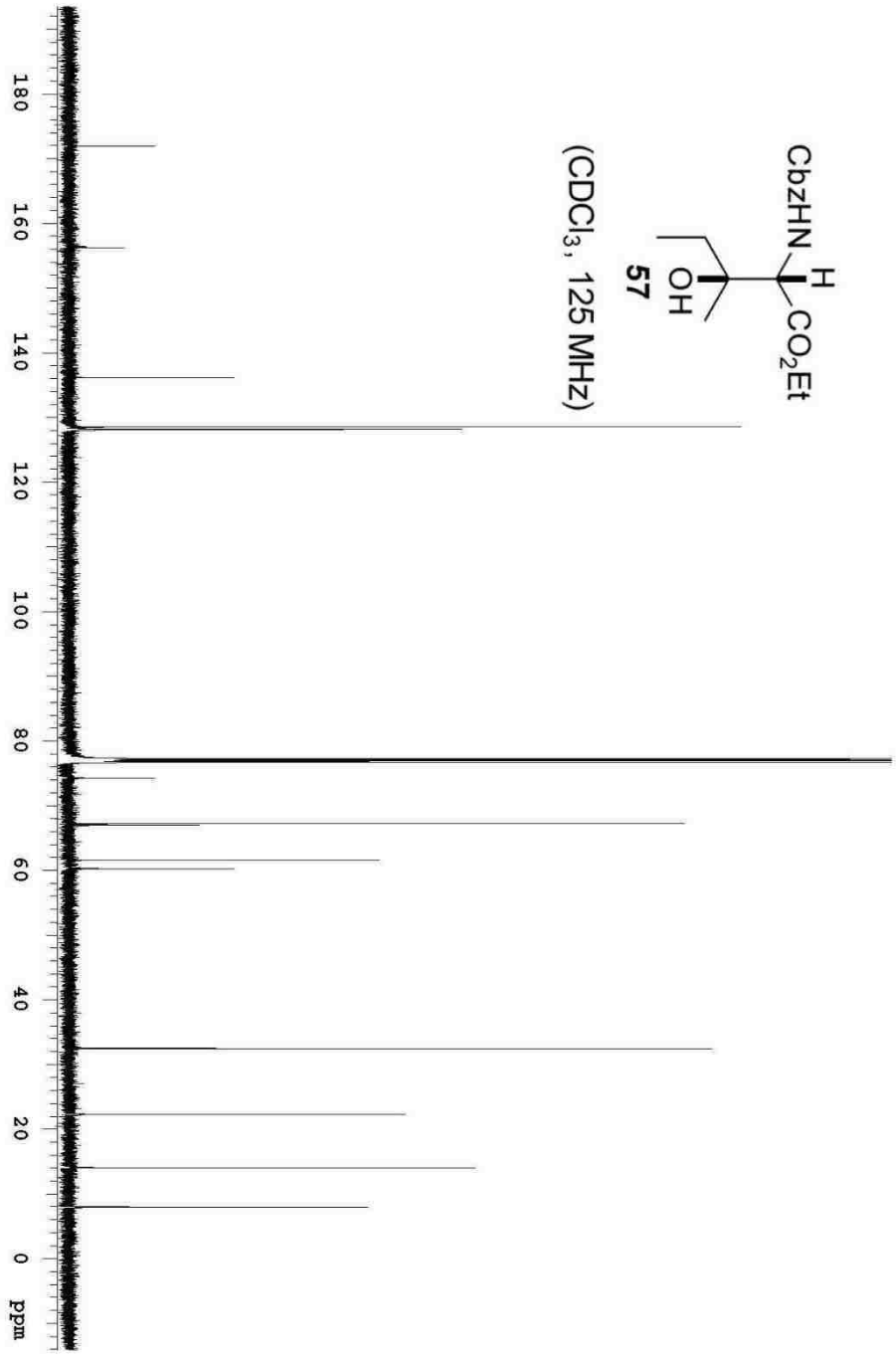

Cl3C
O
O
H
N
O
O
S
O
O
48a
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

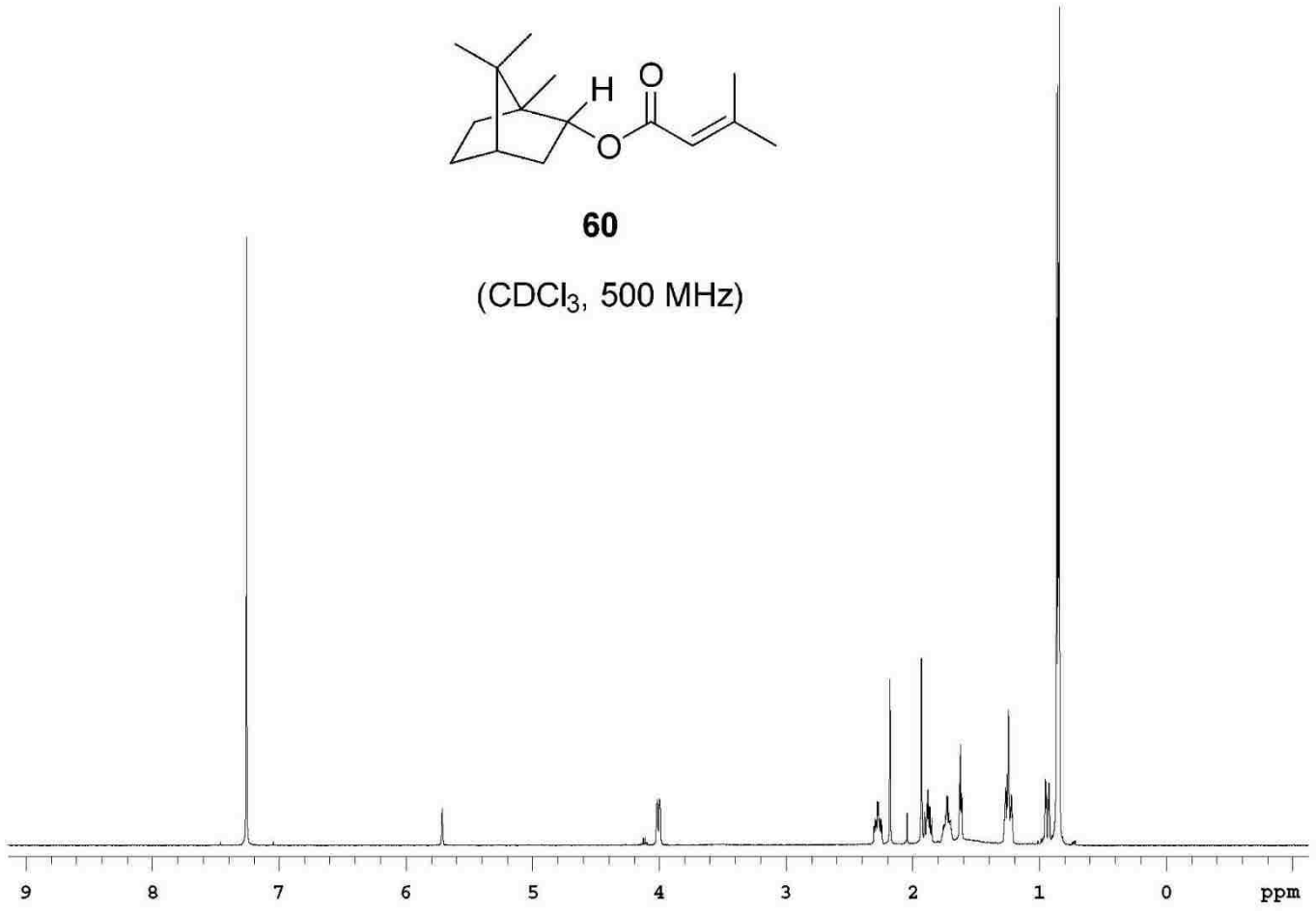

CO2Et
51
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm


NHTroc
HO
CO2Et
55
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm


NHTroc
HO
CO_2Me
56
($CDCl_3$, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

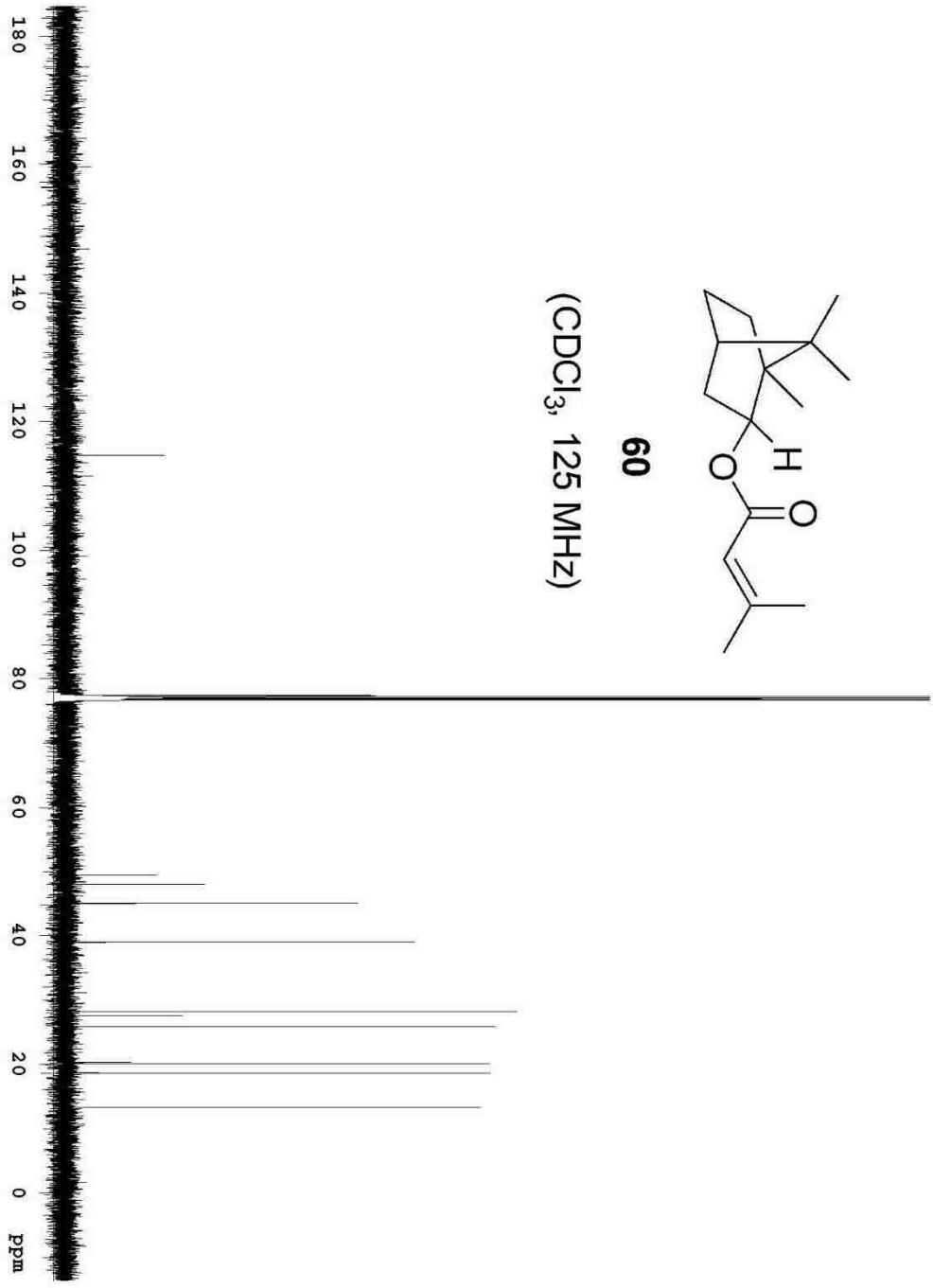

CbzHN
H
CO2Et
OH
57
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm


CbzHN
H
CO2Et
OH
57
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
0
ppm



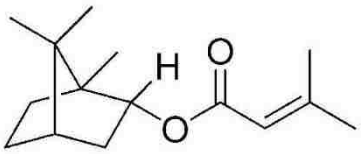
60

($CDCl_3$, 500 MHz)



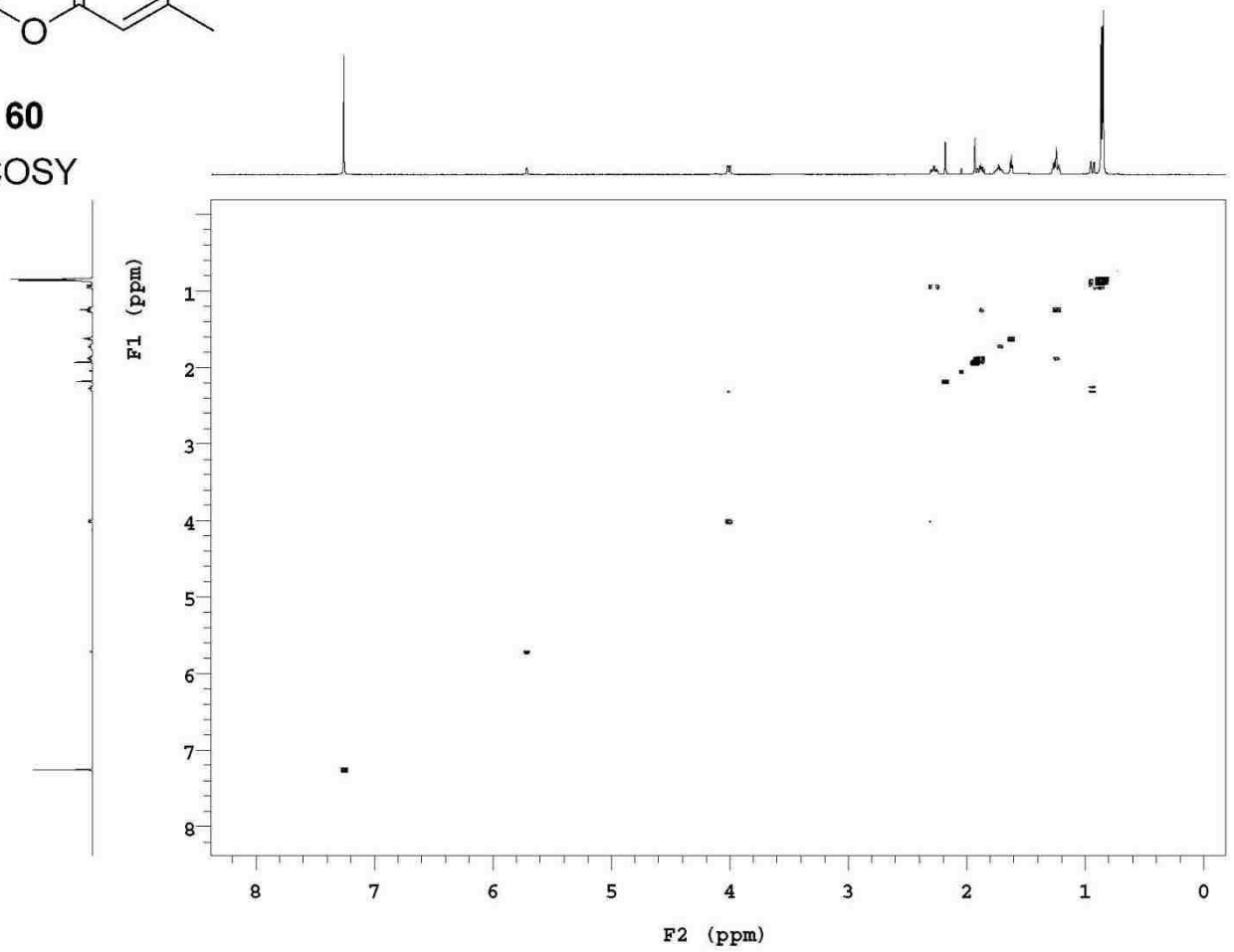
60

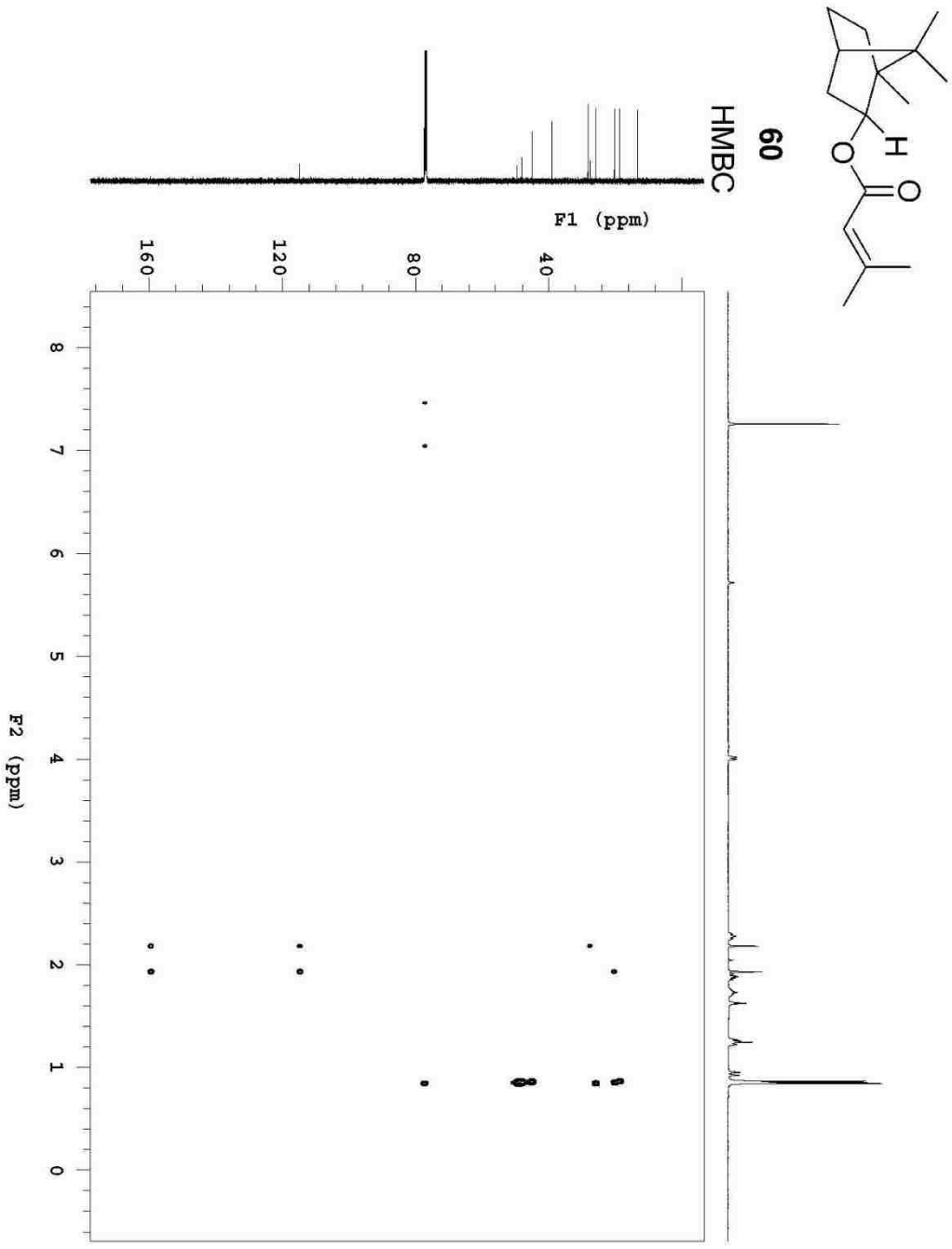
($CDCl_3$, 125 MHz)

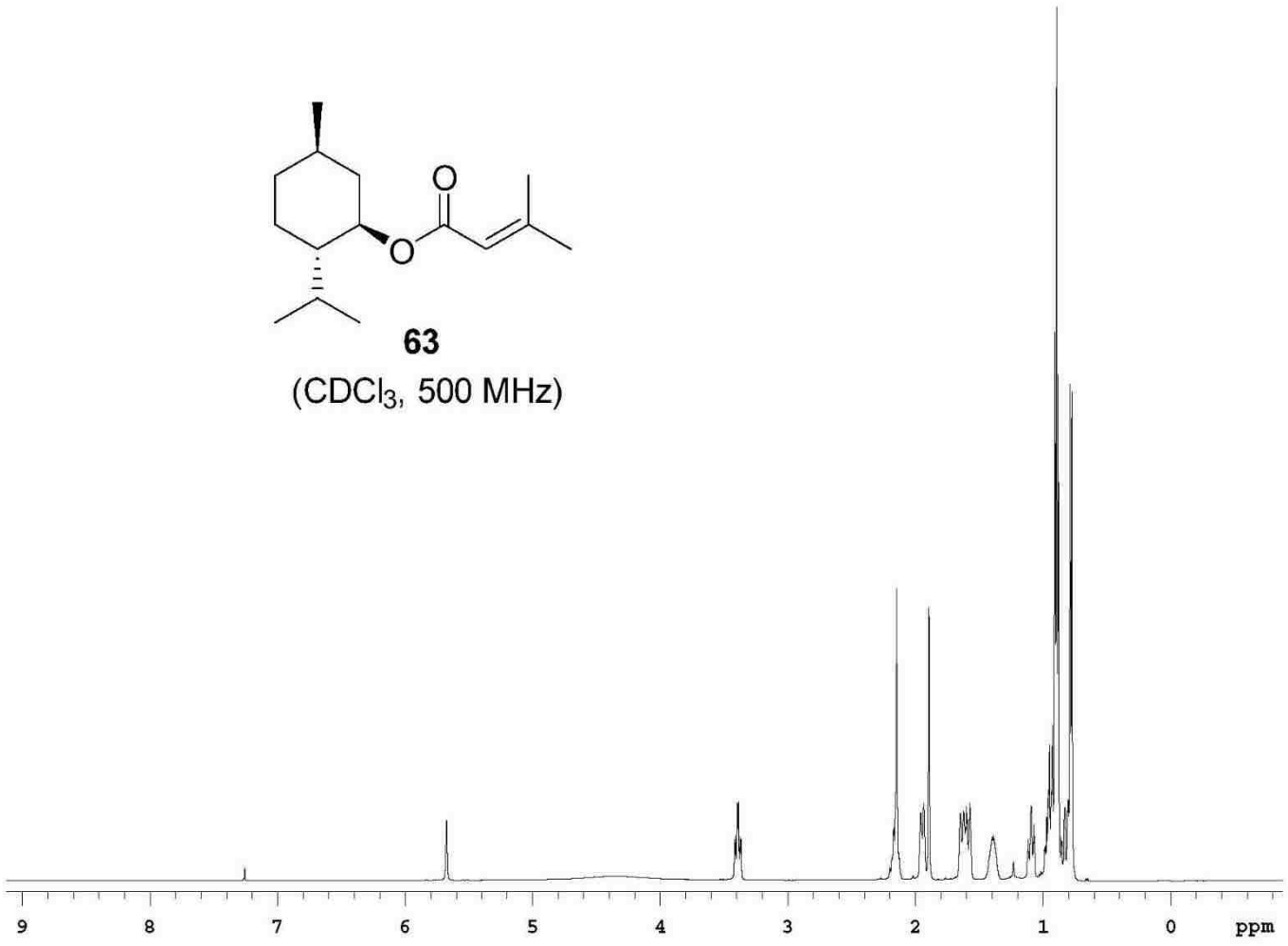


60

COSY

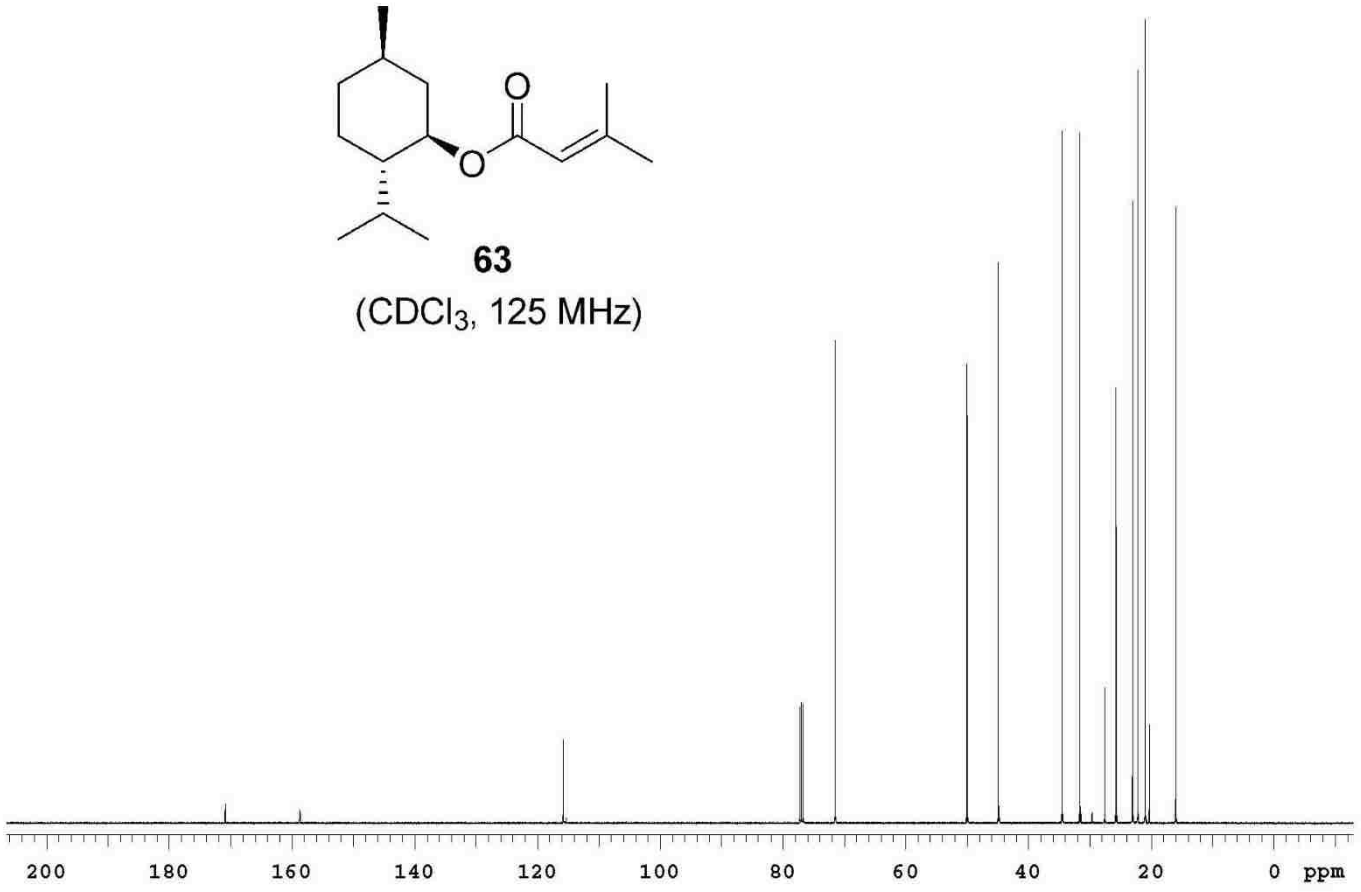


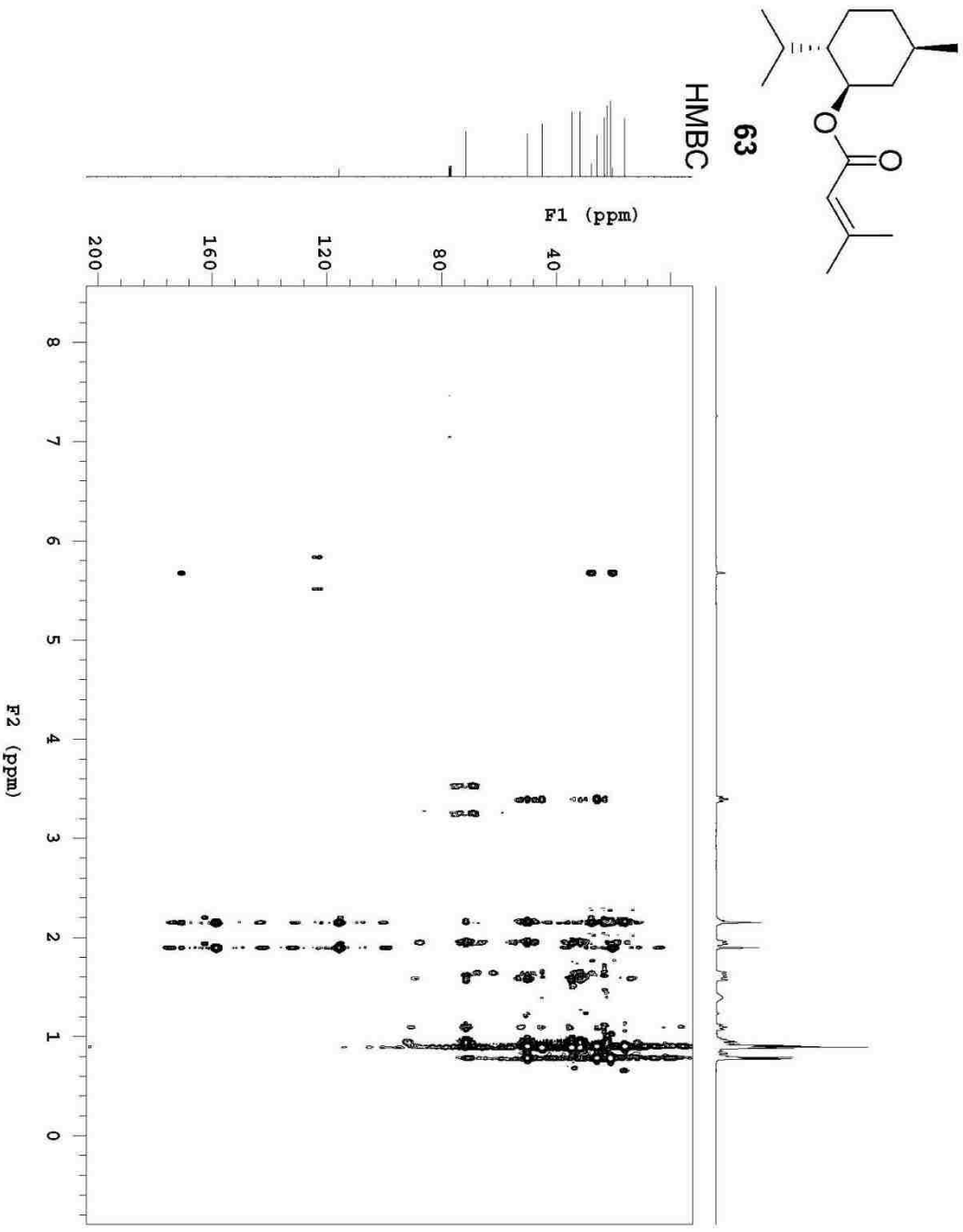

60
HMBC
F1 (ppm)
F2 (ppm)

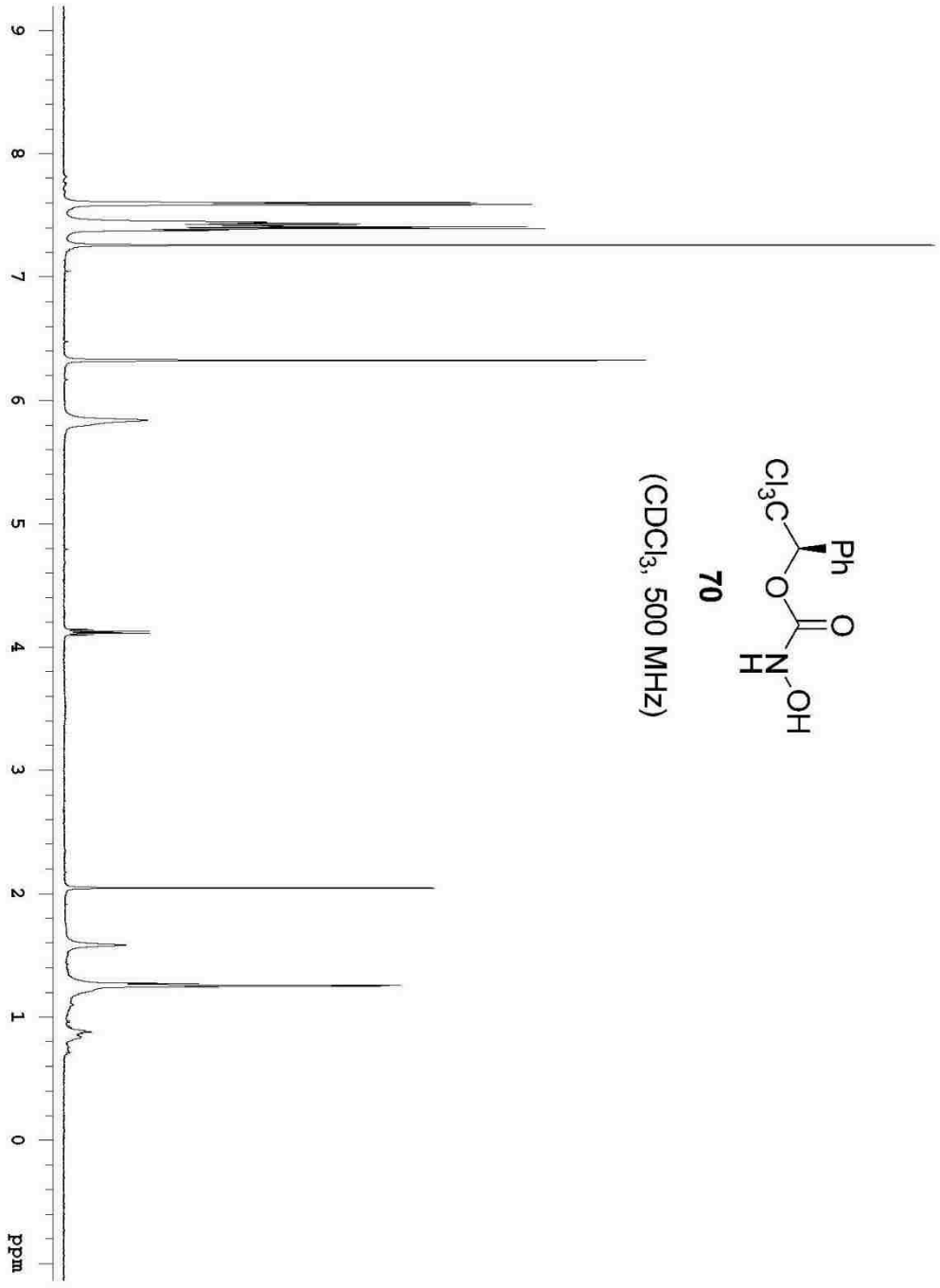


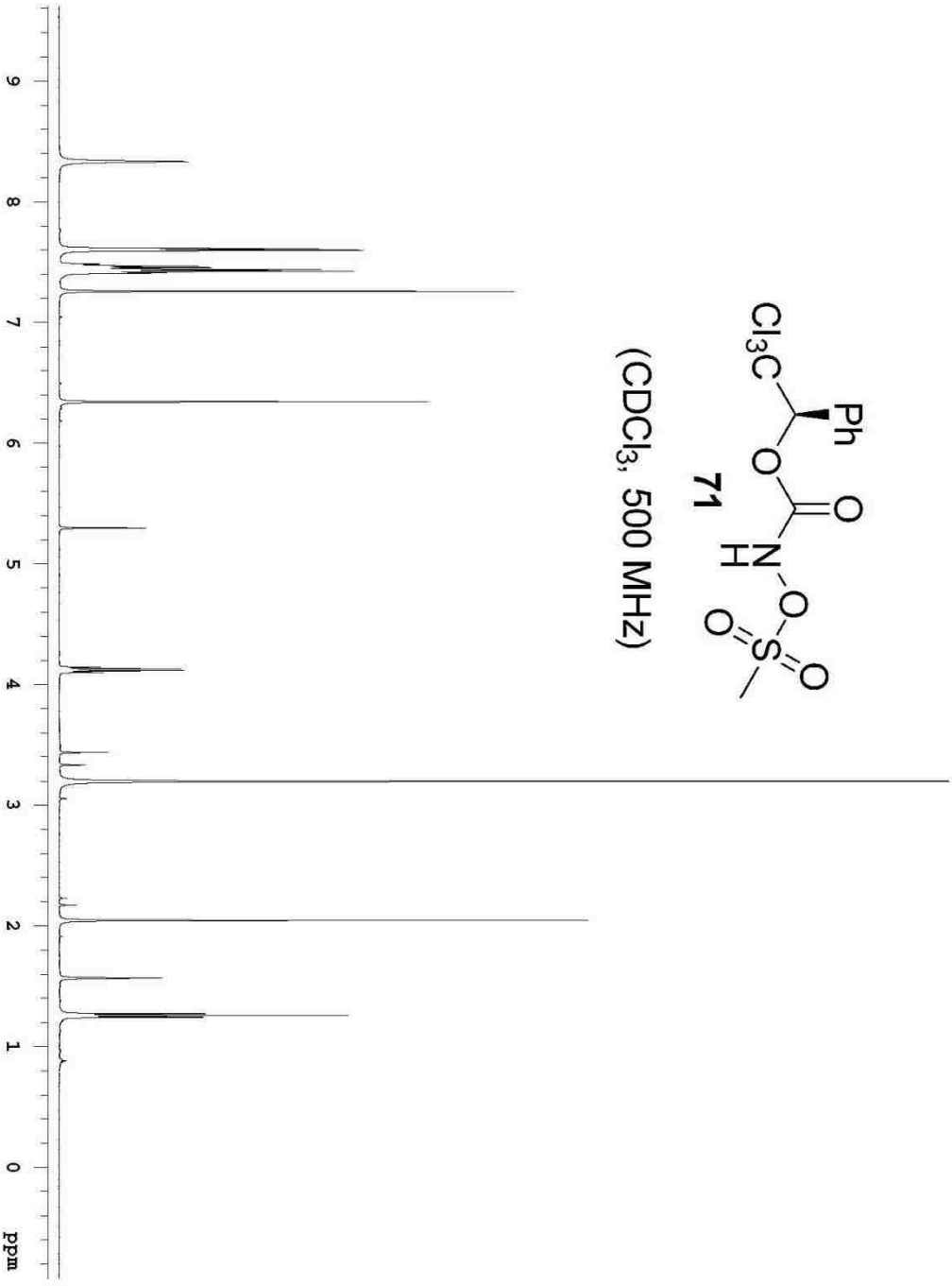
63

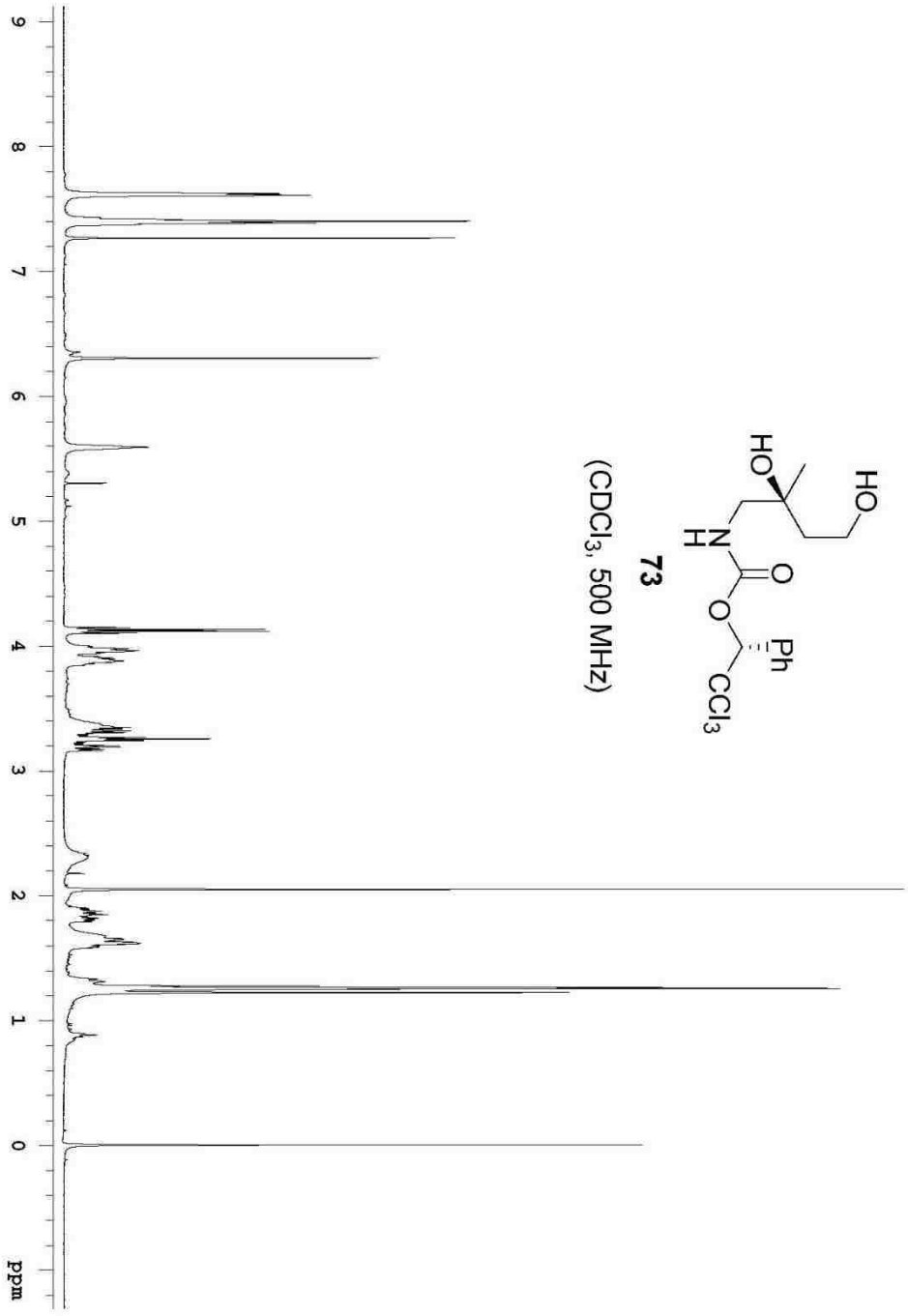
($CDCl_3$, 500 MHz)

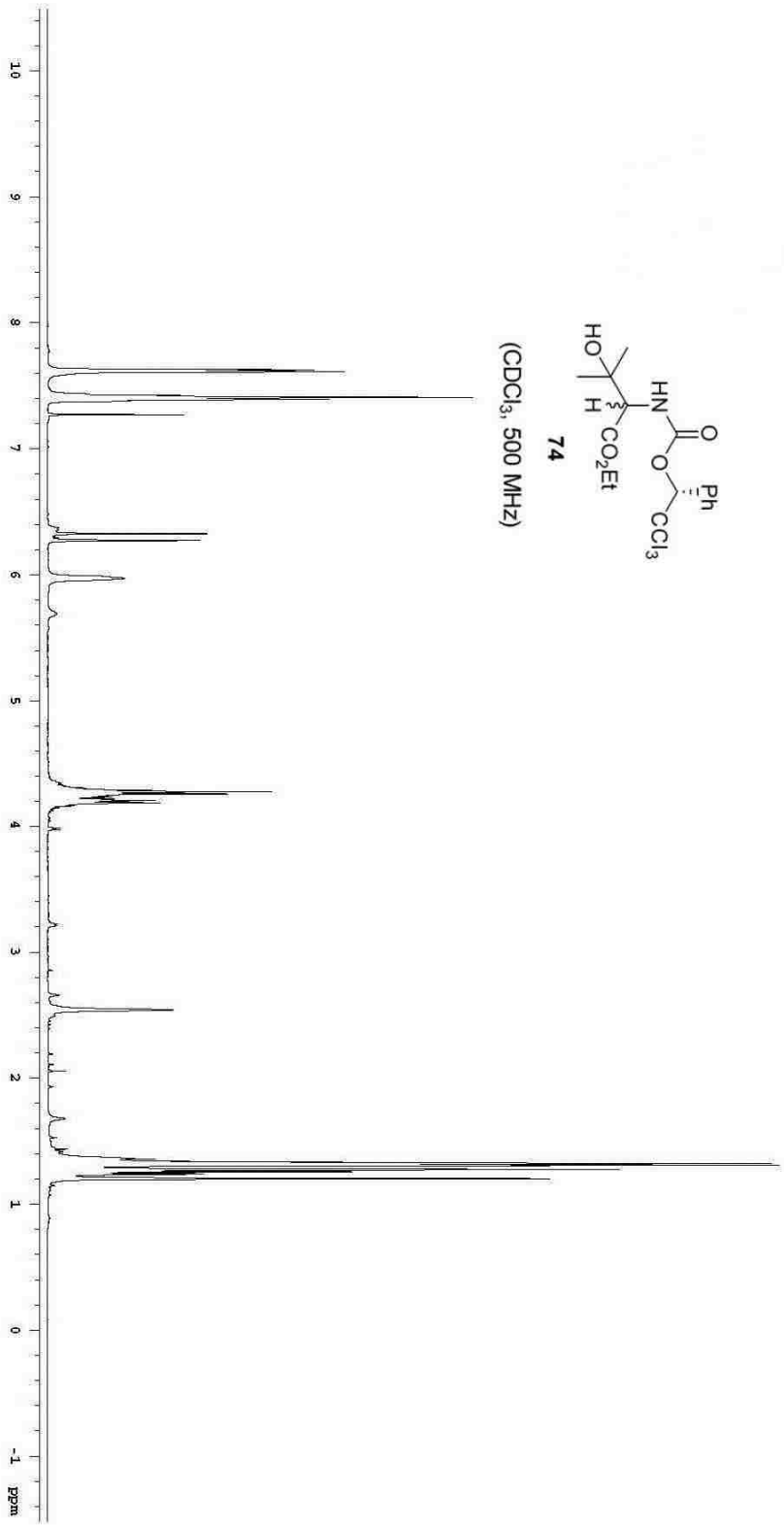

O
O
63
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
0
ppm

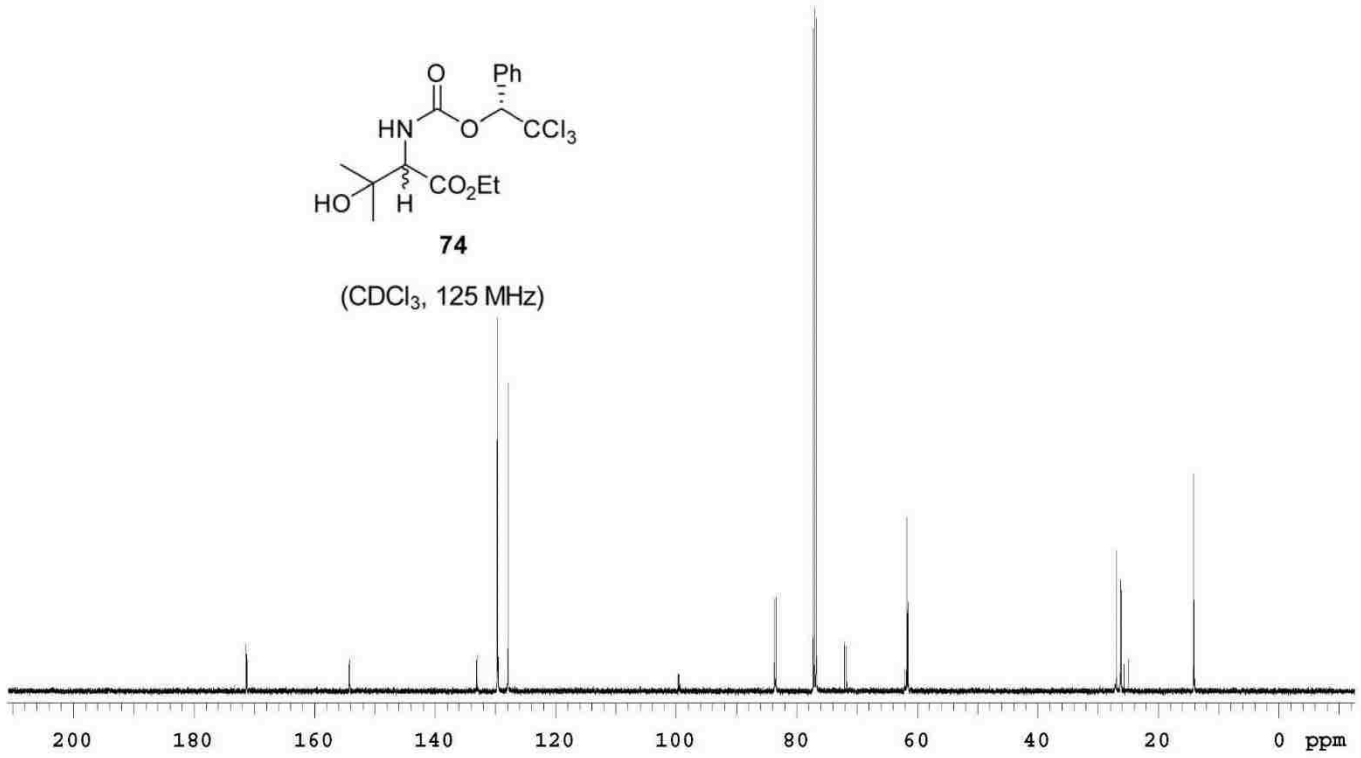

63
HMBC
F1 (ppm)
F2 (ppm)

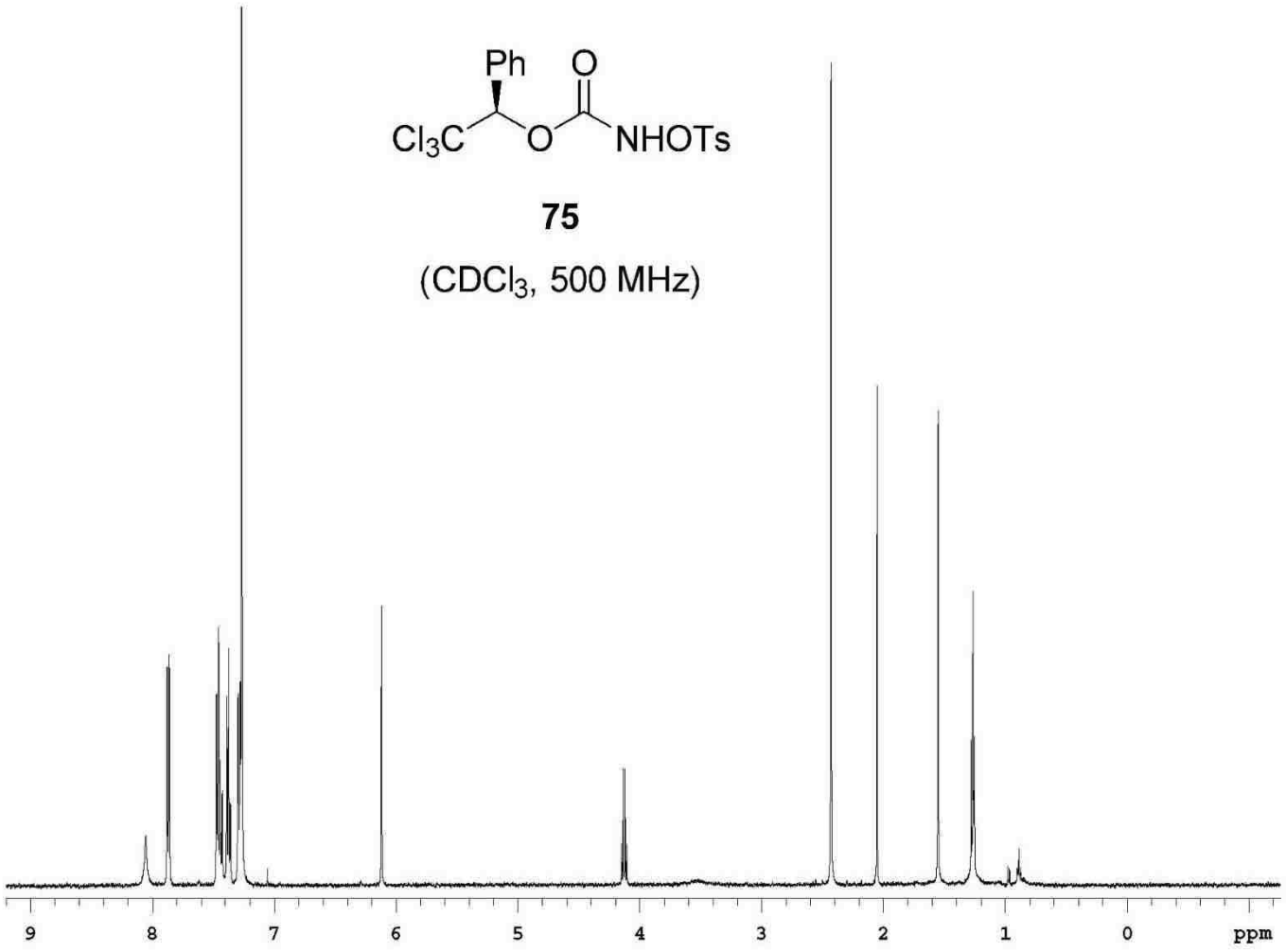

Cl3C
Ph
O
O
N
H
OH
70
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

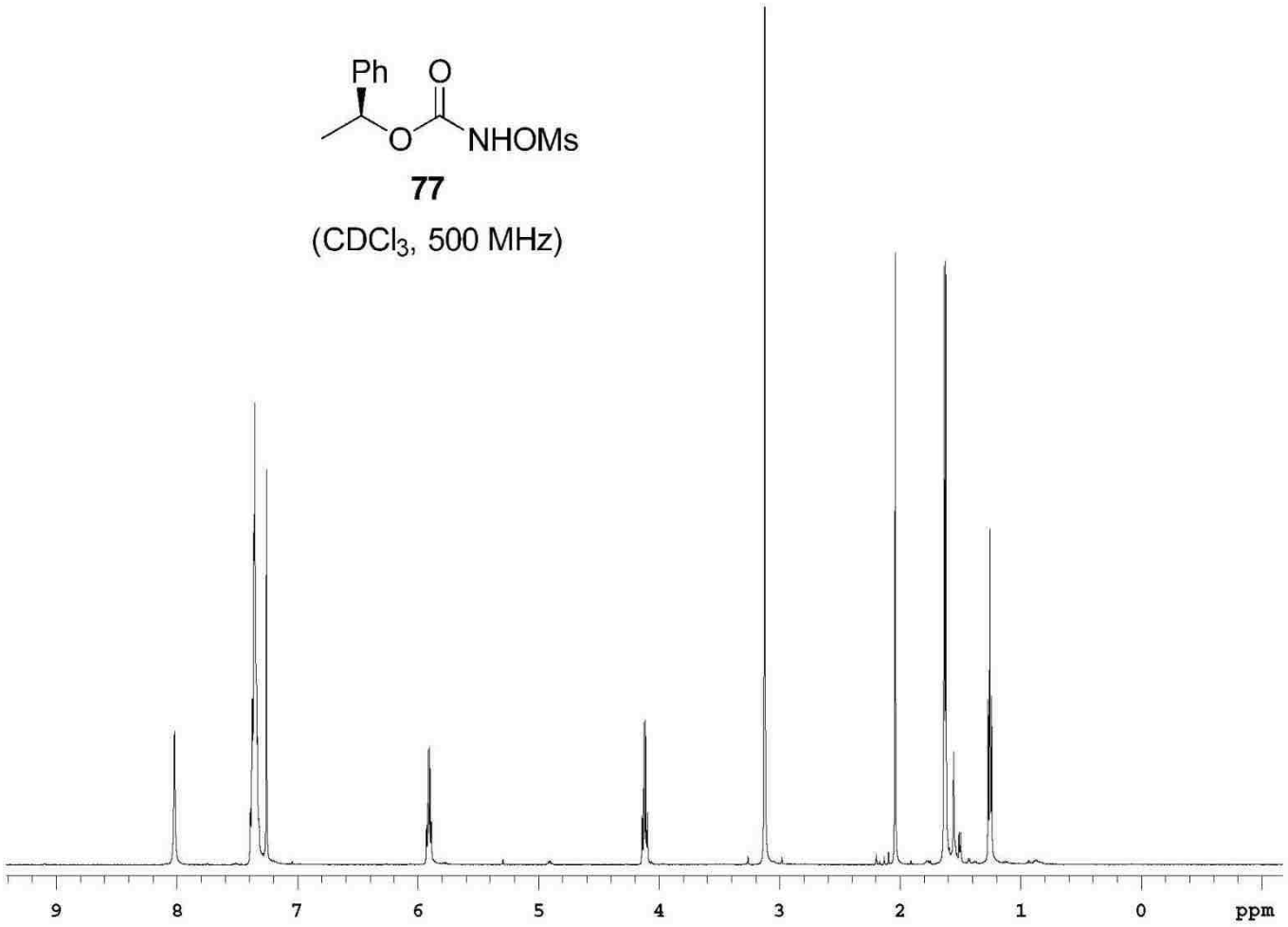

Ph
O
Cl3C
O
N
H
O
O
S
O
O
71
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

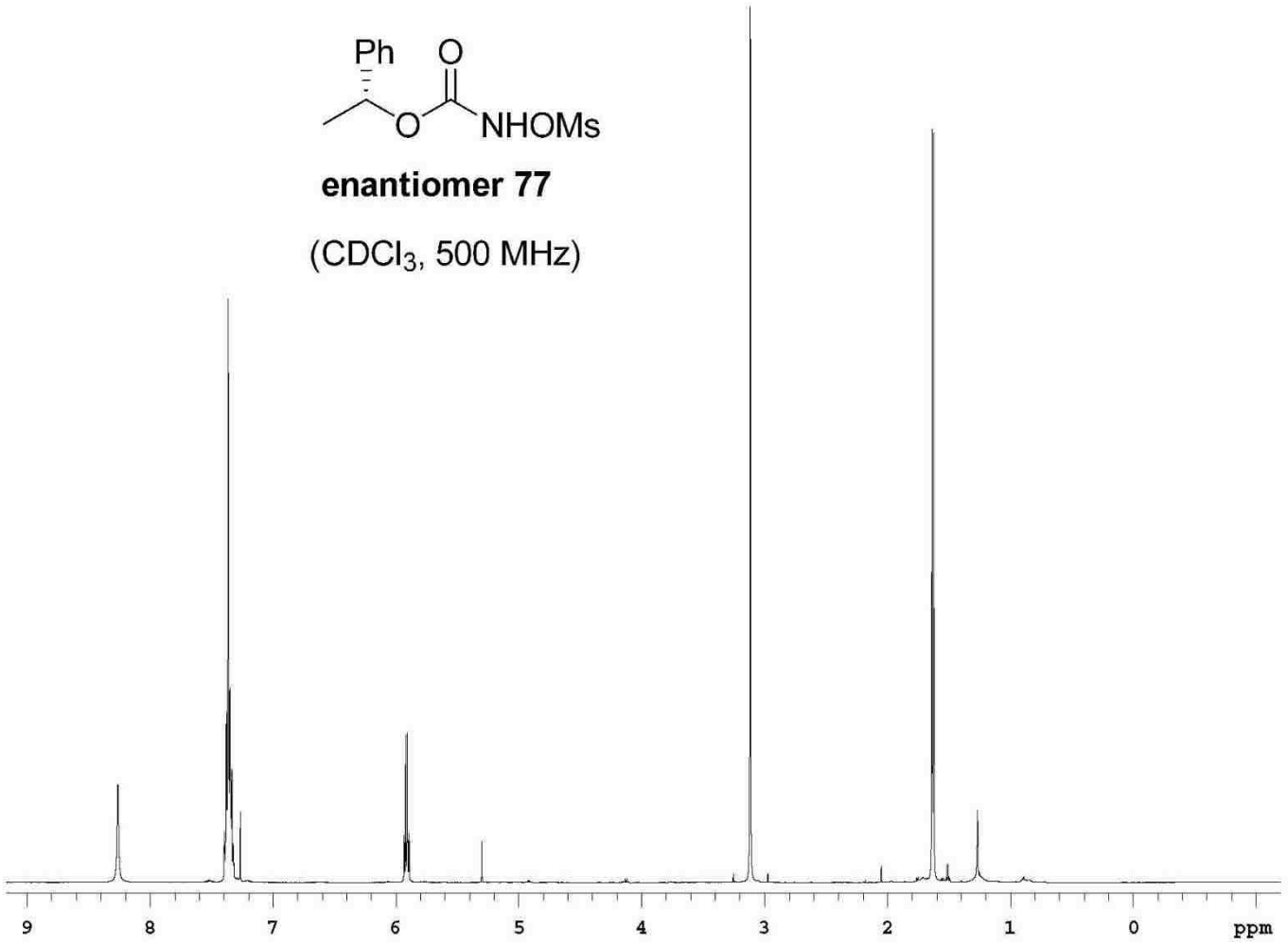

HO
HO
N
H
O
O
Ph
CCl3
73
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

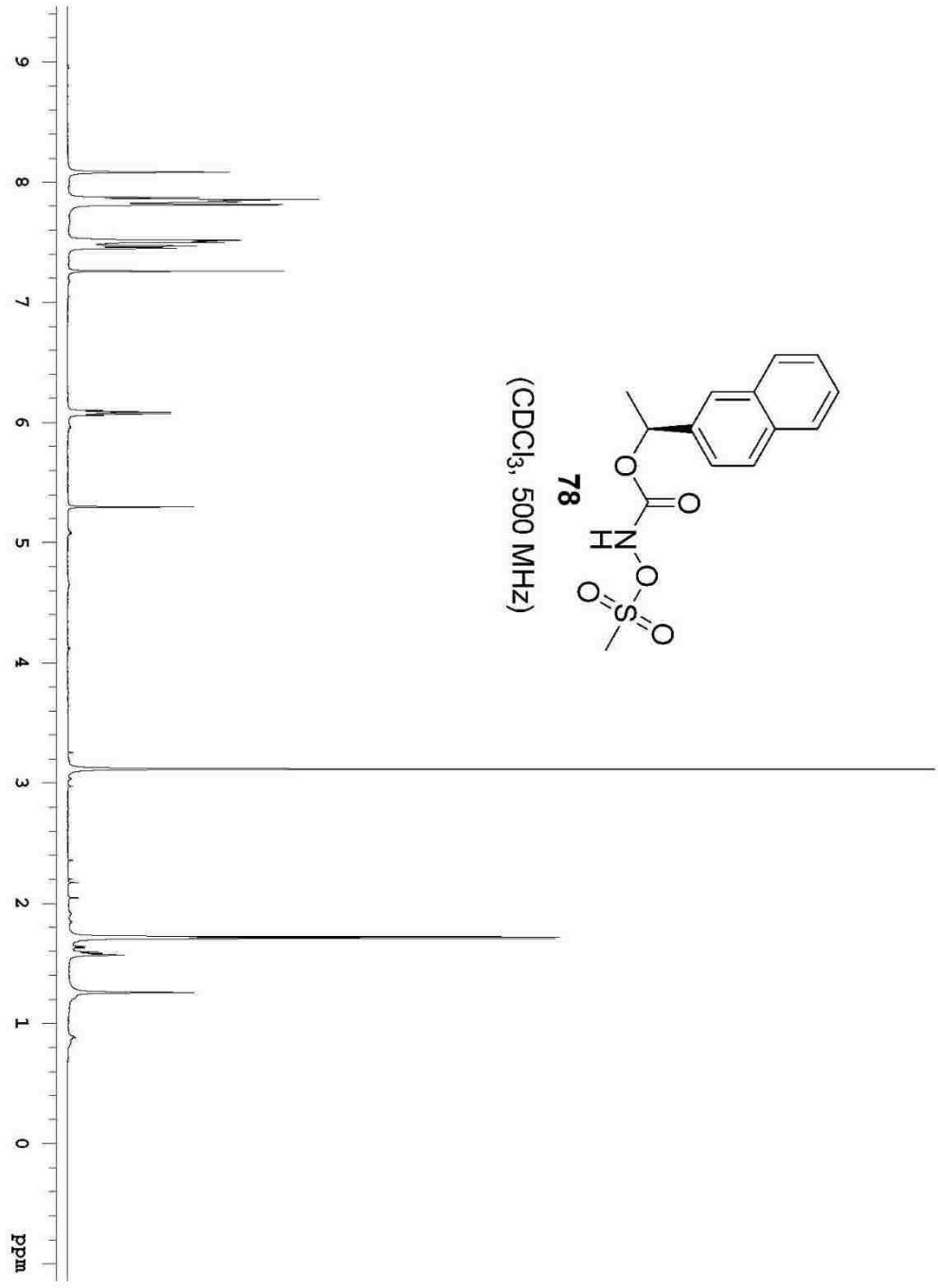

HO
H
HN
O
O
Ph
CCl3
CO2Et
74
(CDCl3, 500 MHz)
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

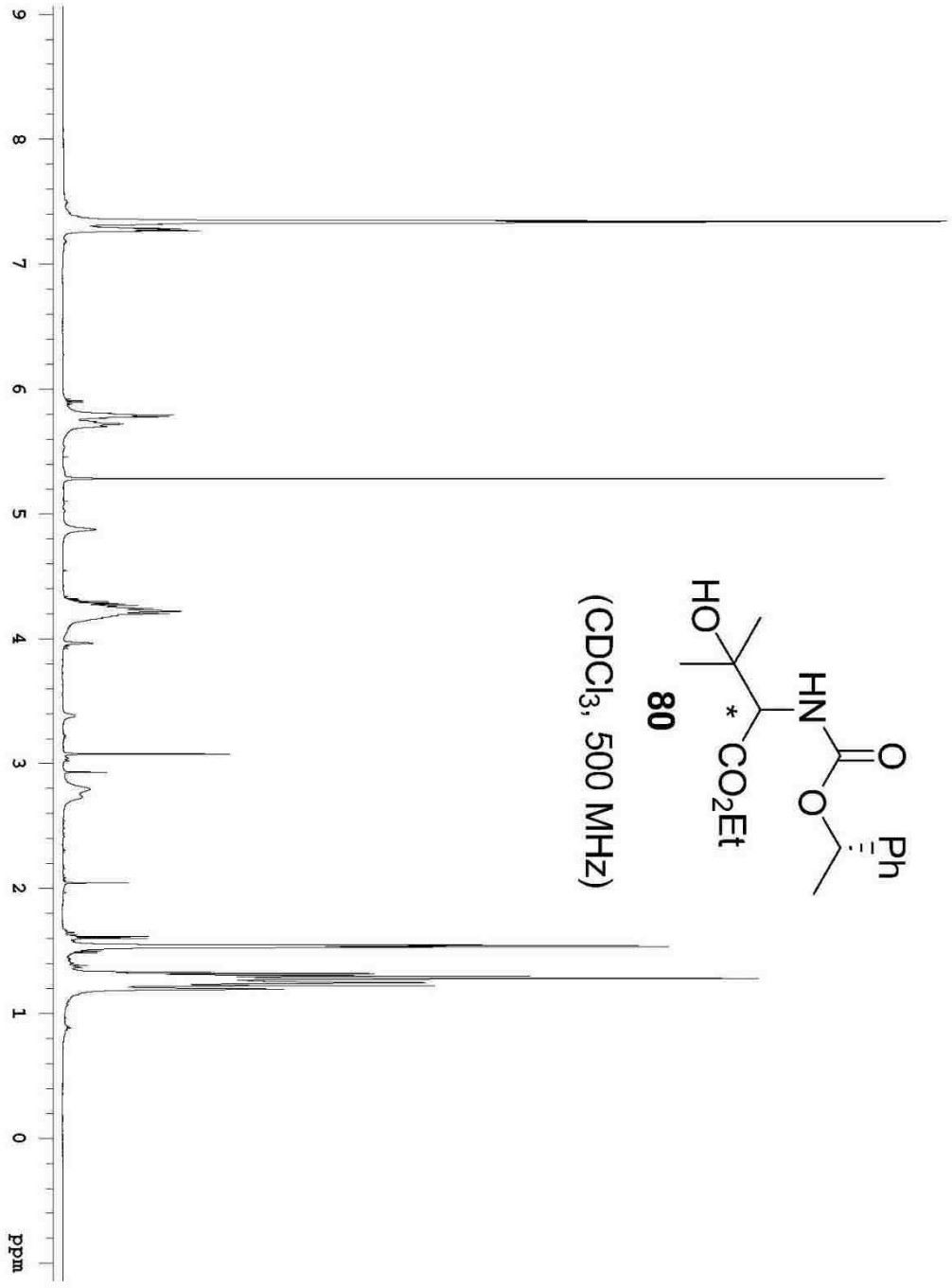

O
Ph
HN
O
CCl3
HO
H
CO2Et
74
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
0 ppm

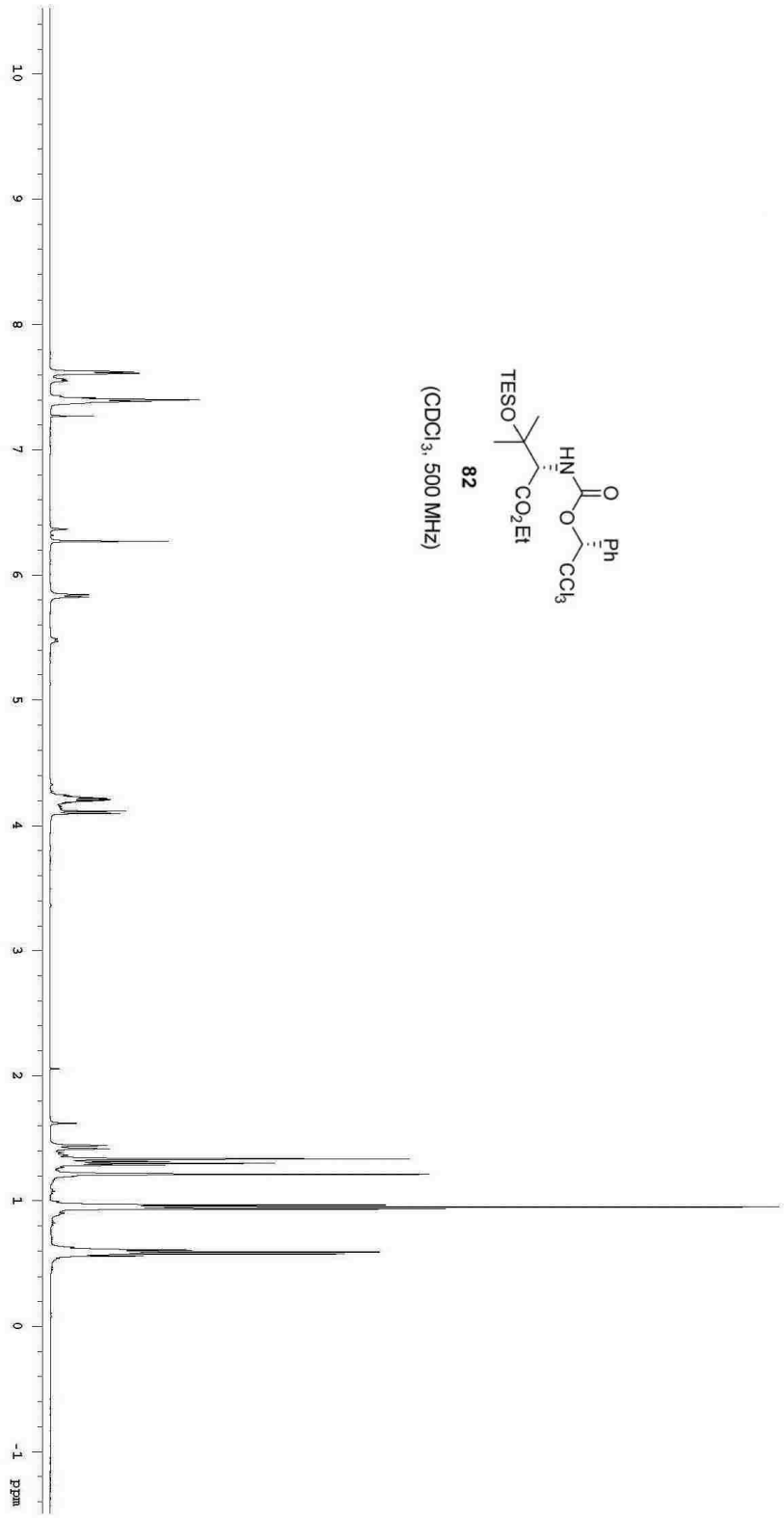

Ph
O
Cl3C
O
NHOTs
75
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

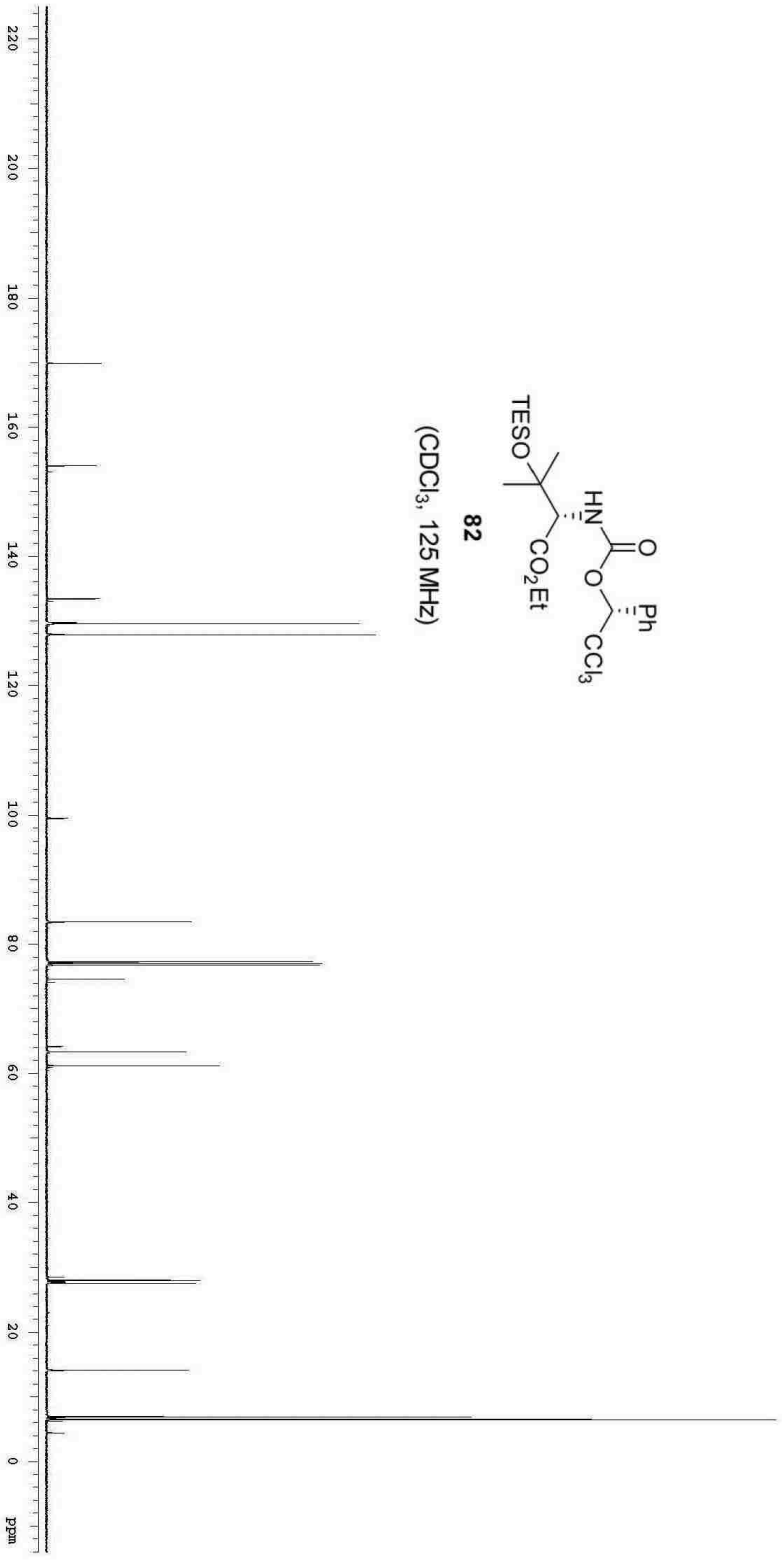

Ph
O
O
NHOMs
77
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

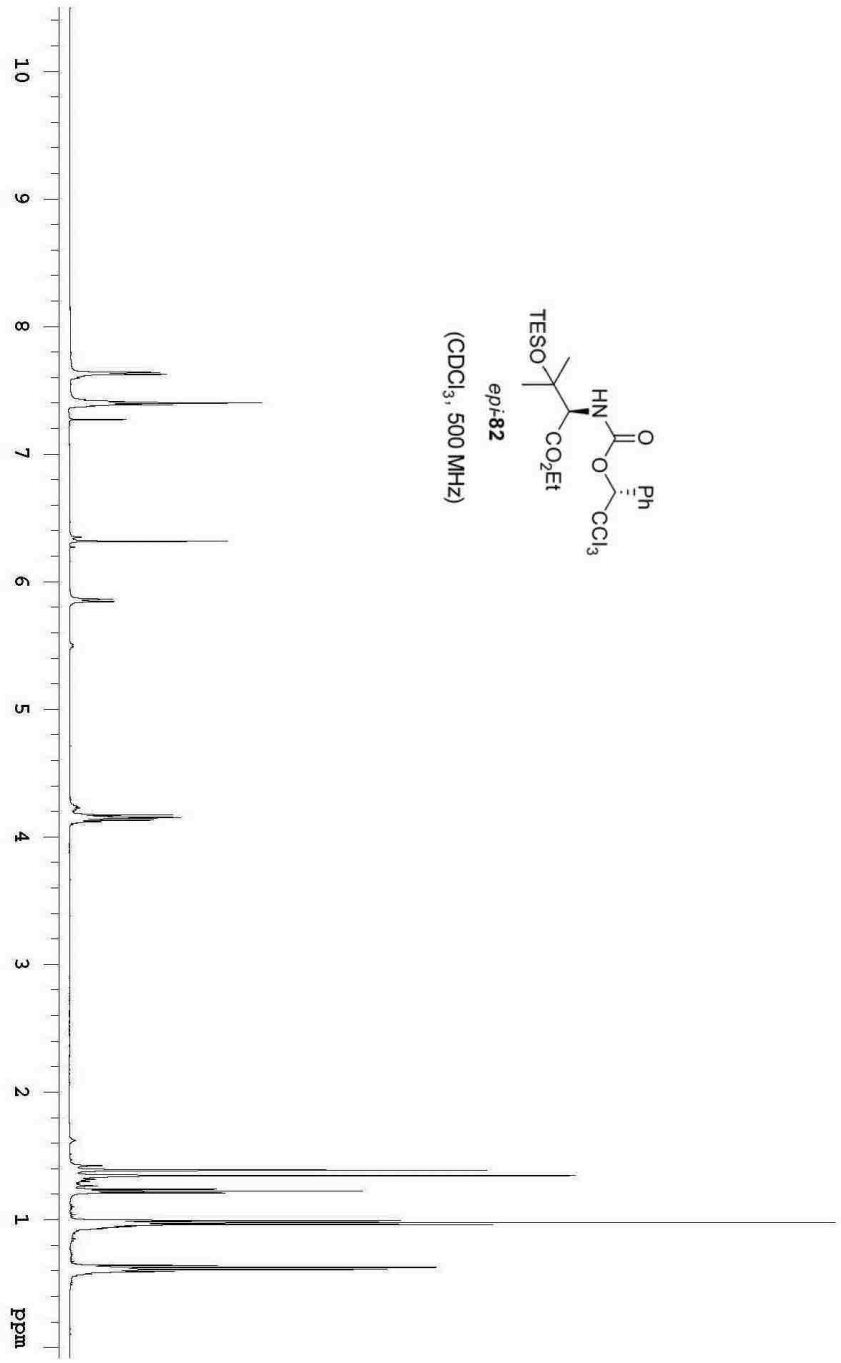

Ph
O
O
NHOMs
enantiomer 77
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

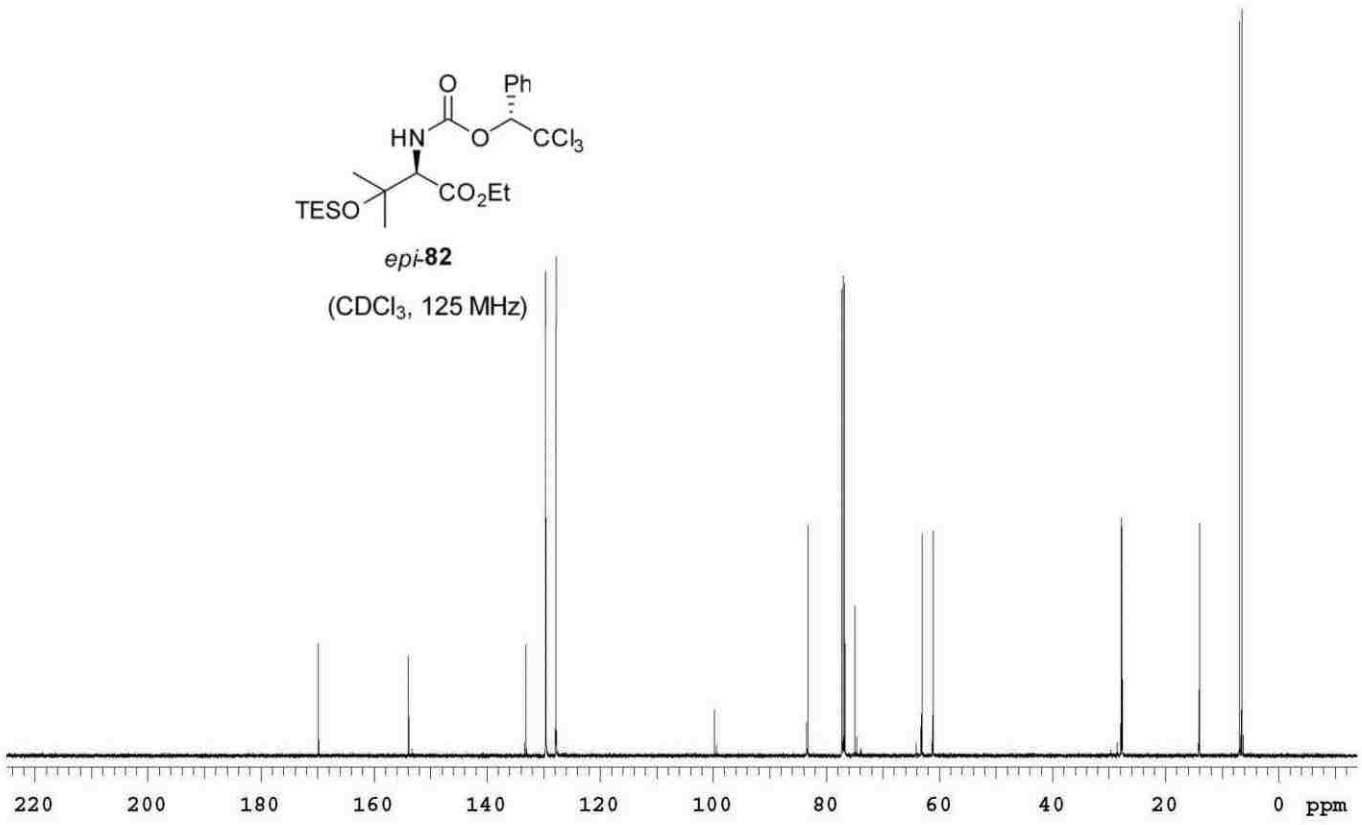

78
(CDCl3, 500 MHz)
ppm

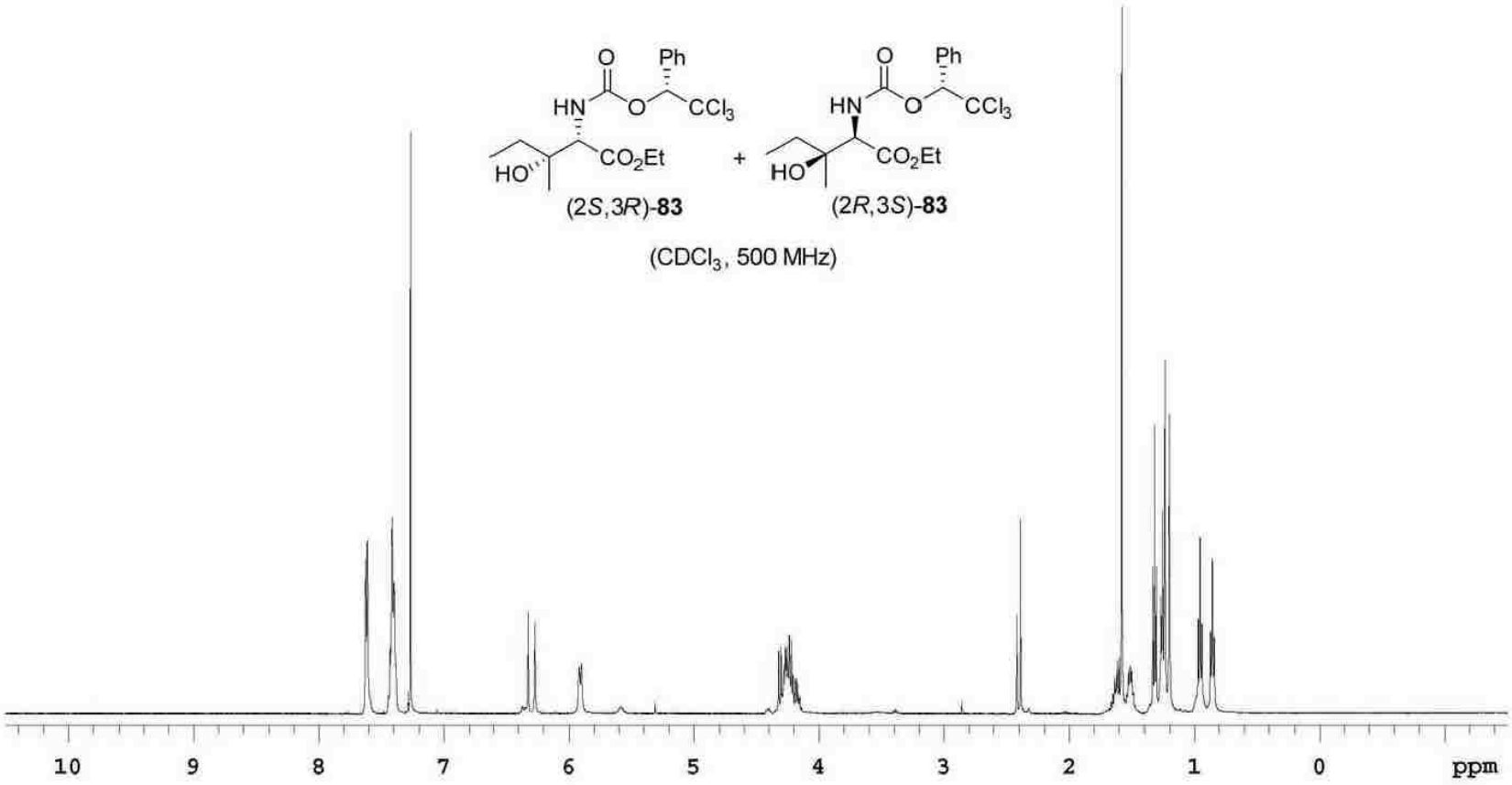

9
8
7
6
5
4
3
2
1
0
ppm
HO
HN
O
O
Ph
CO2Et
*
80
(CDCl3, 500 MHz)

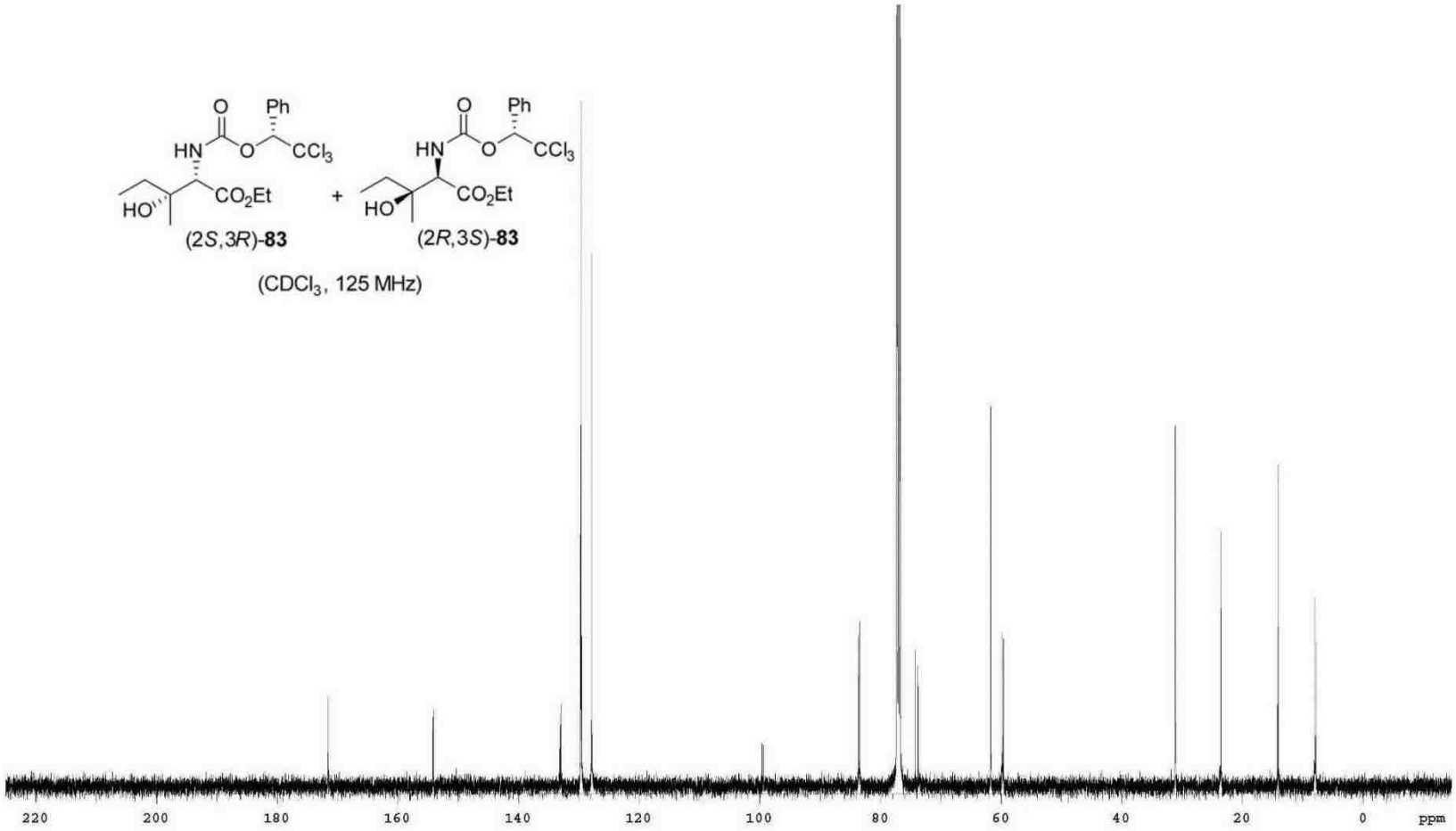

TESO
HN
O
O
Ph
CCl3
CO2Et
82
(CDCl3, 500 MHz)
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

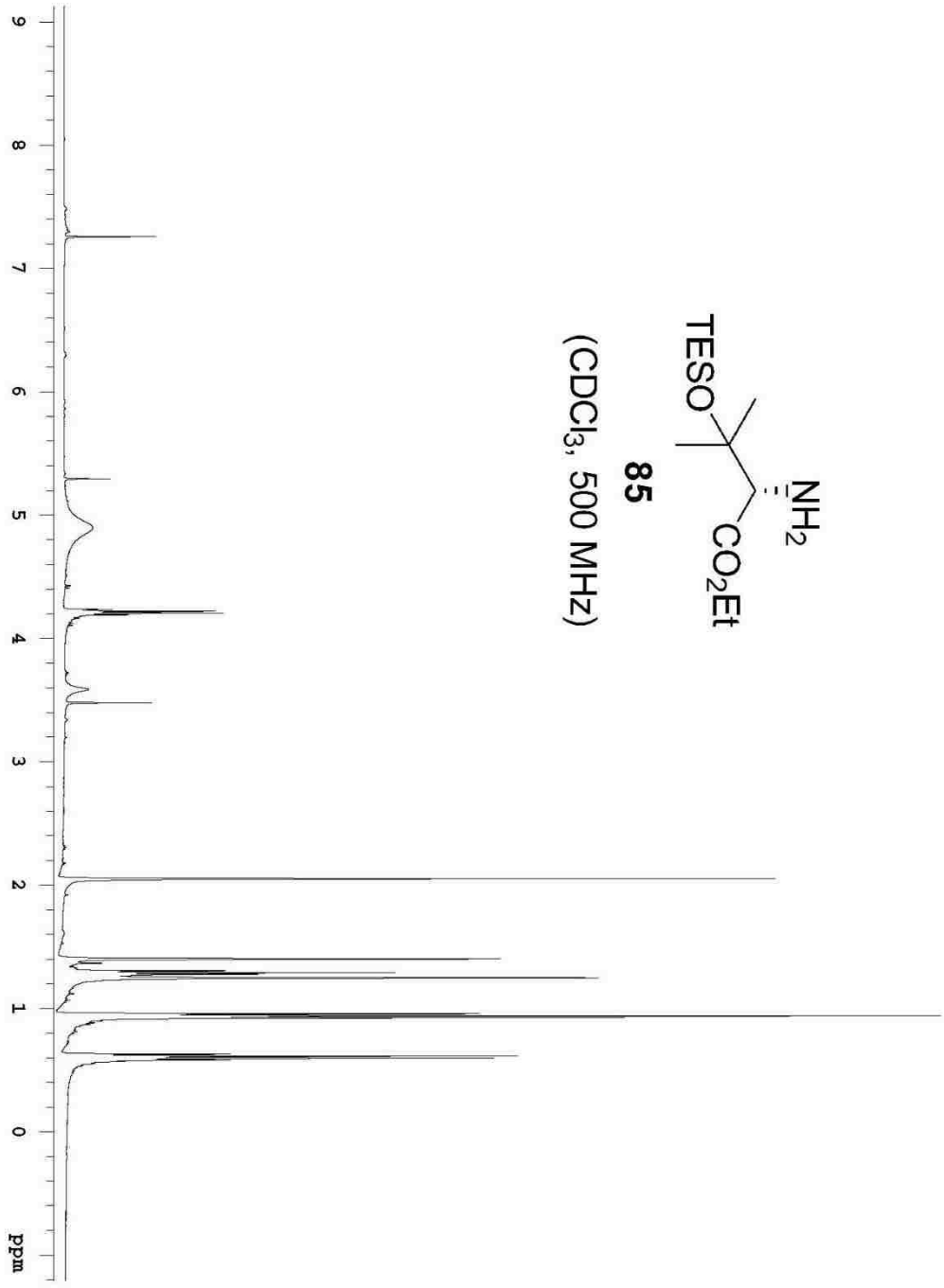

TESO
HN
O
O
Ph
CCl3
CO2Et
82
(CDCl3, 125 MHz)
220
200
180
160
140
120
100
80
60
40
20
0
ppm

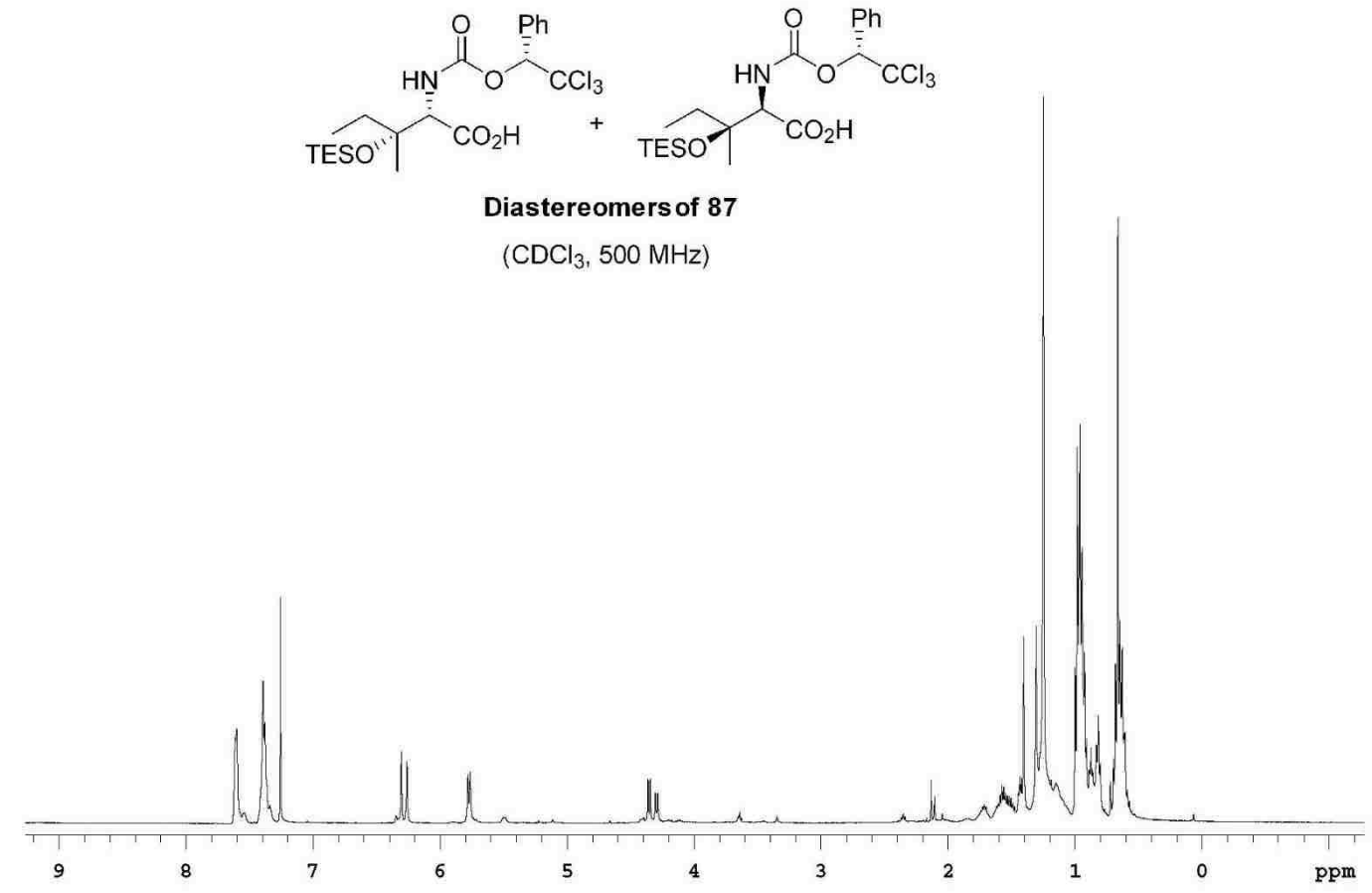

TESO
HN
O
O
Ph
CCl3
CO2Et
epi-82
(CDCl3, 500 MHz)
10
9
8
7
6
5
4
3
2
1
ppm

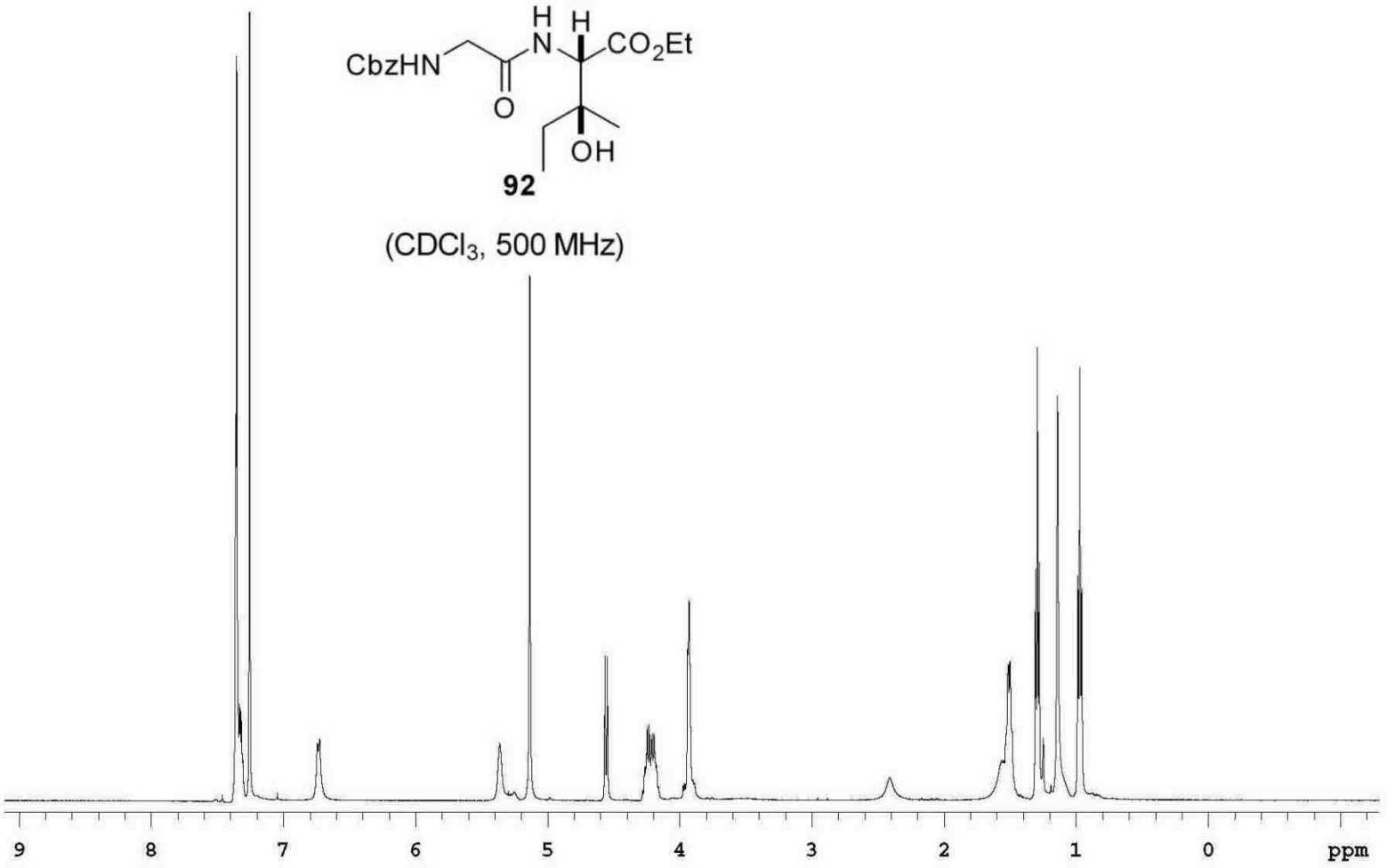

O
Ph
HN
O
CCl3
TESO
CO2Et
epi-82
(CDCl3, 125 MHz)
220
200
180
160
140
120
100
80
60
40
20
0
ppm

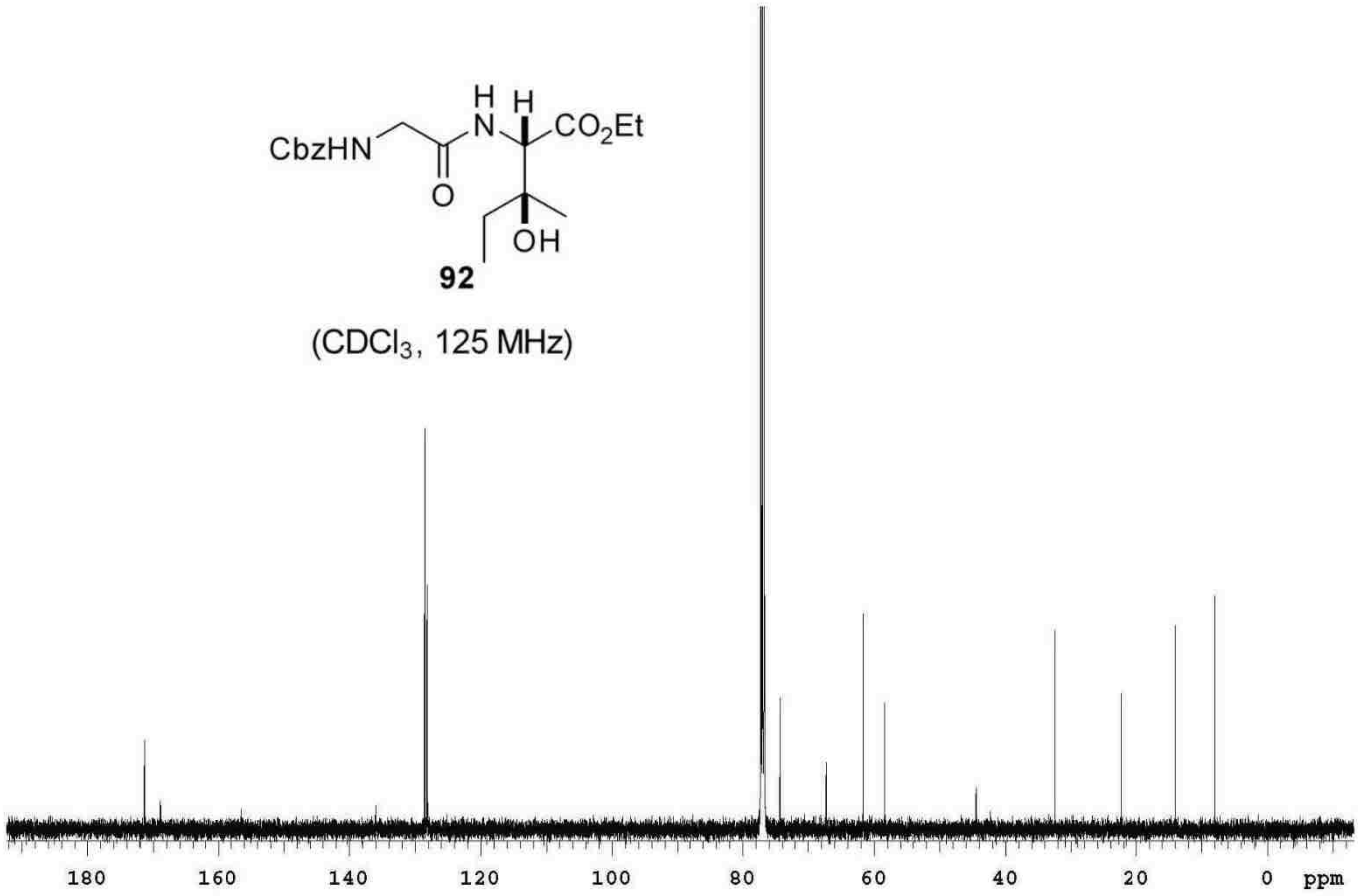

O
Ph
HN
O
CCl3
CO2Et
HO
(2S,3R)-83
+
(2R,3S)-83
(CDCl3, 500 MHz)
10
9
8
7
6
5
4
3
2
1
0
ppm

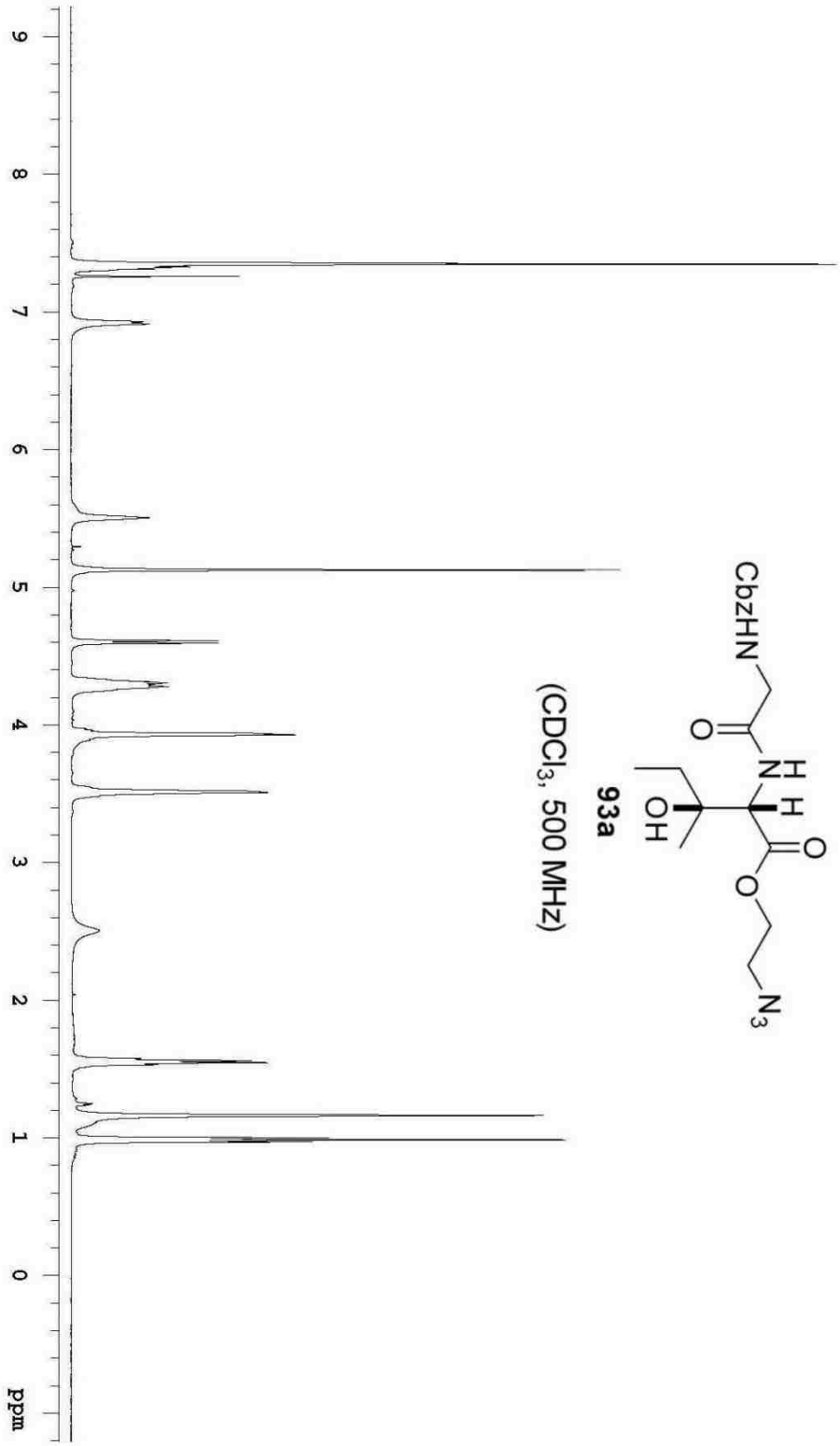

O
Ph
HN
O
CCl3
HO
CO2Et
(2S,3R)-83
+
(2R,3S)-83
(CDCl3, 125 MHz)
220
200
180
160
140
120
100
80
60
40
20
0
ppm

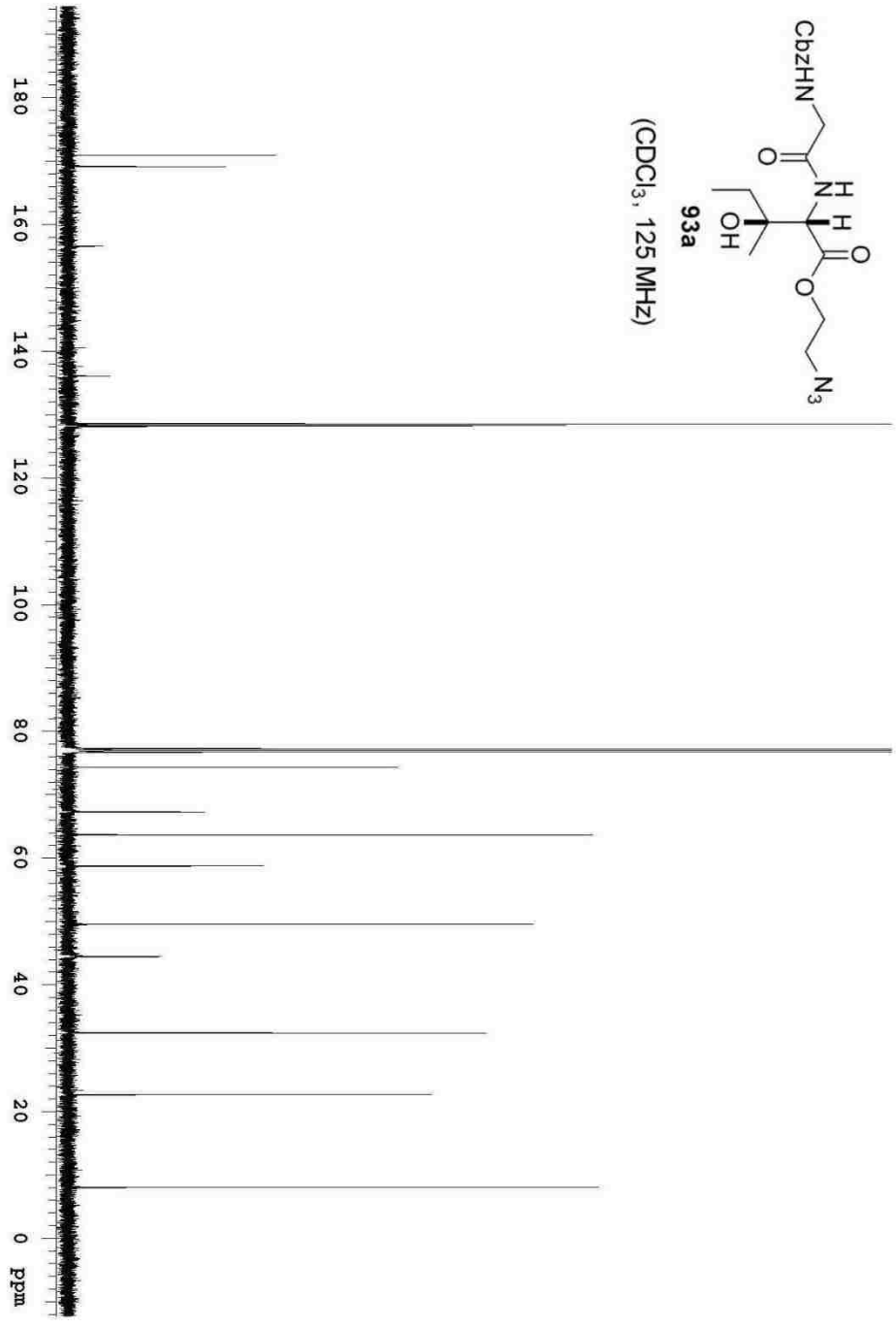

TESO
NH2
CO2Et
85
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

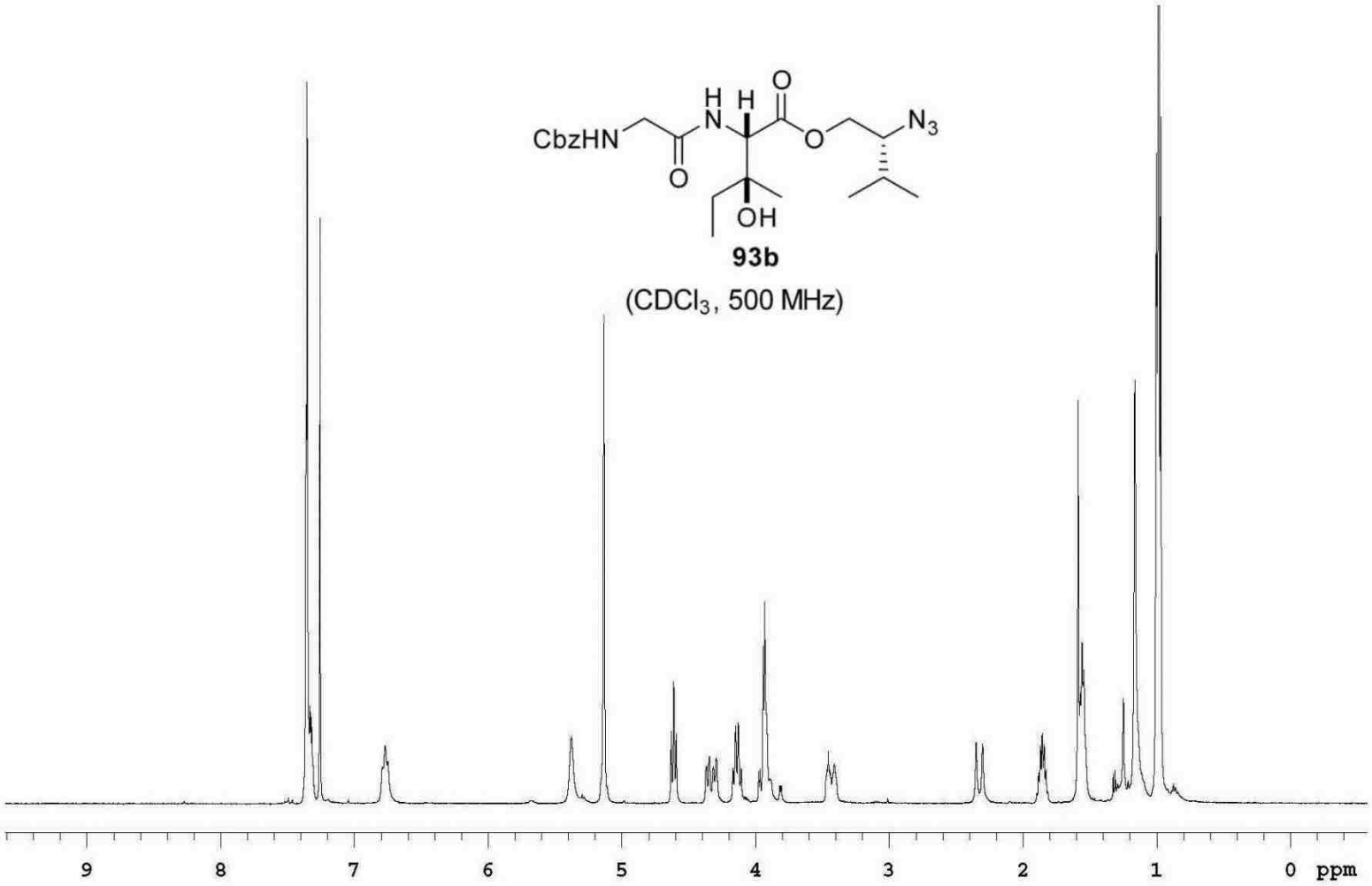

O
Ph
HN
O
CCl3
CO2H
TESO
+
O
Ph
HN
O
CCl3
CO2H
TESO
Diastereomers of 87
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

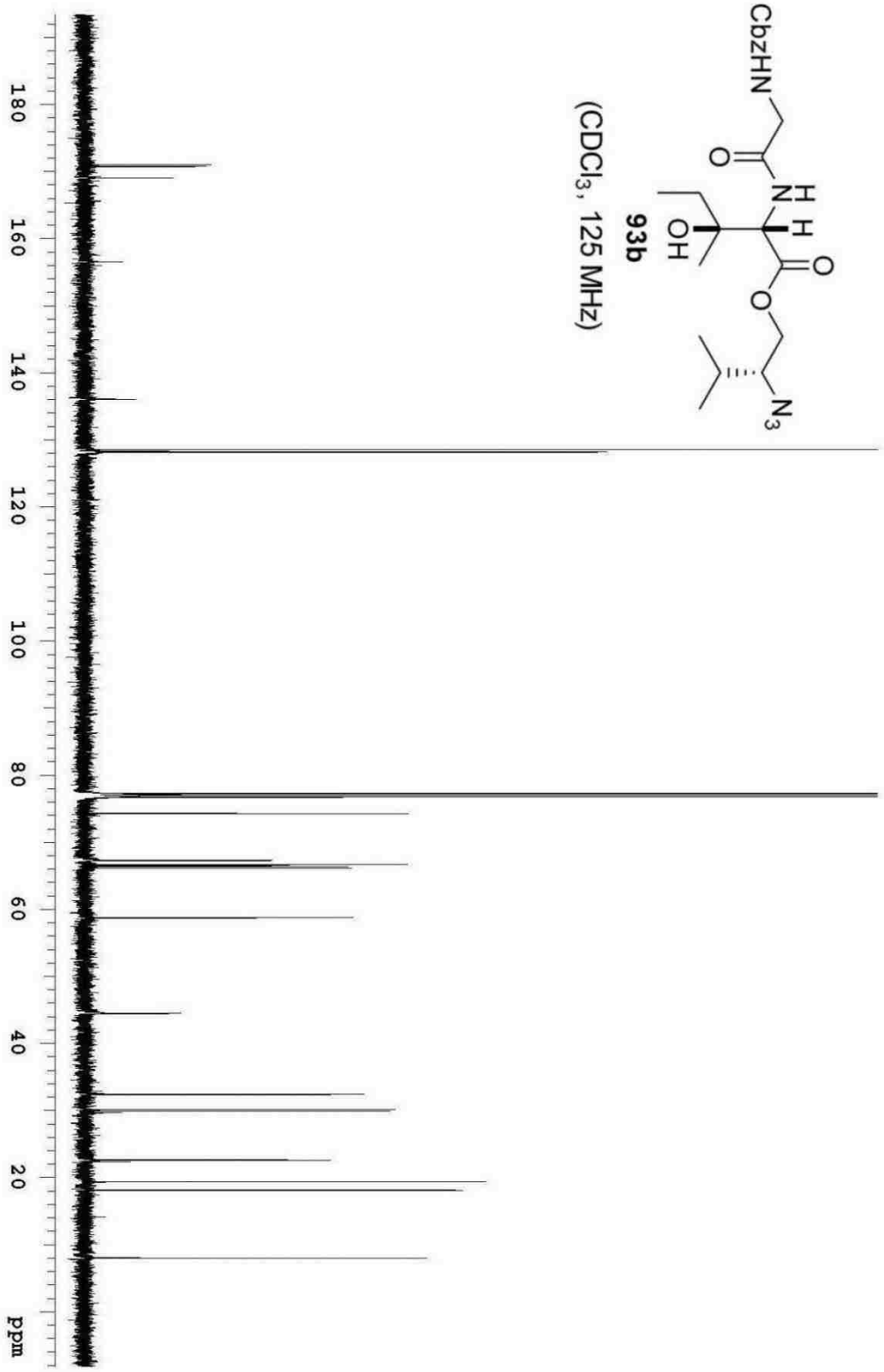

CbzHN
H
N
H
CO2Et
O
OH
92
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

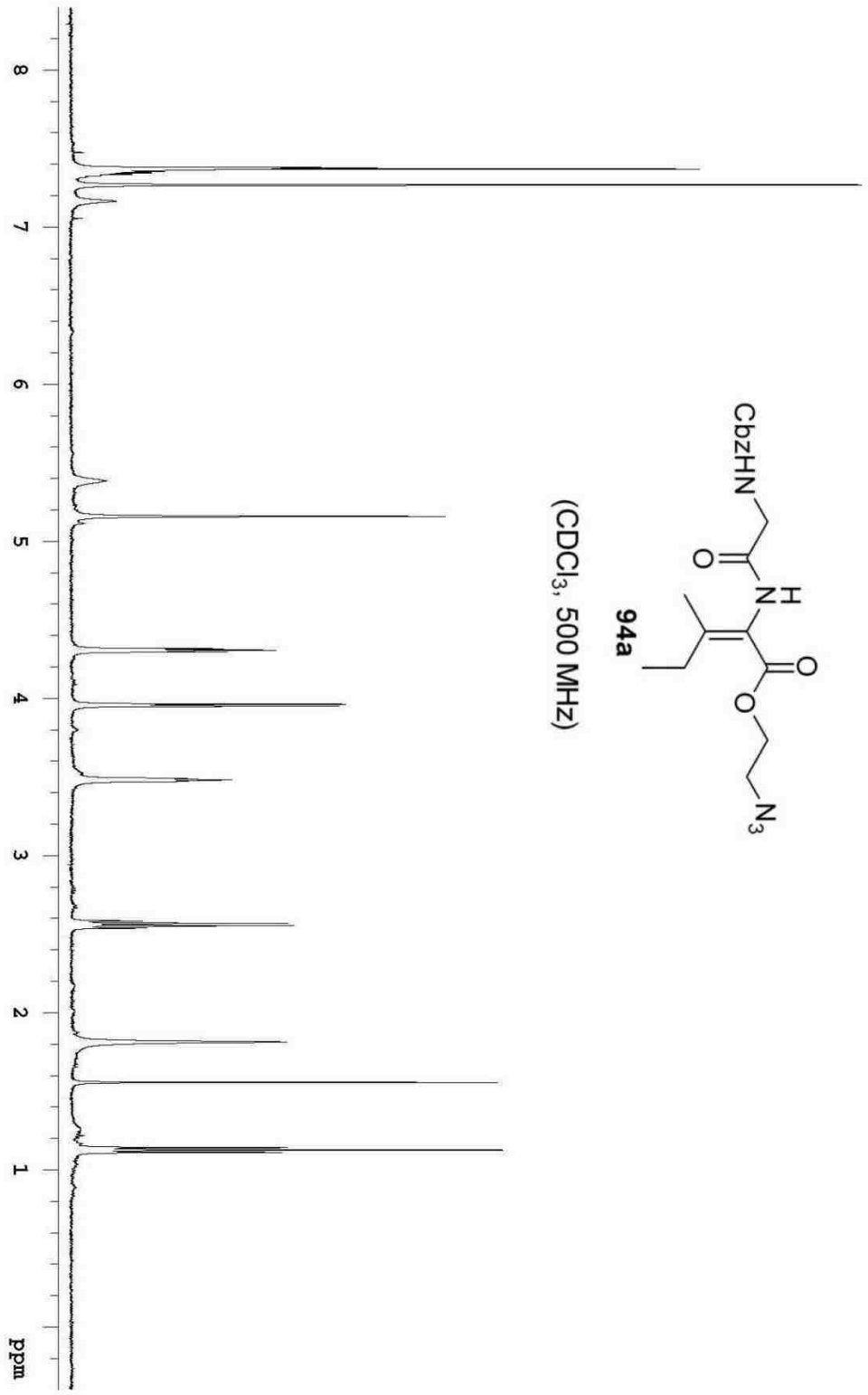

CbzHN
H
N
H
CO2Et
O
OH
92
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
0
ppm

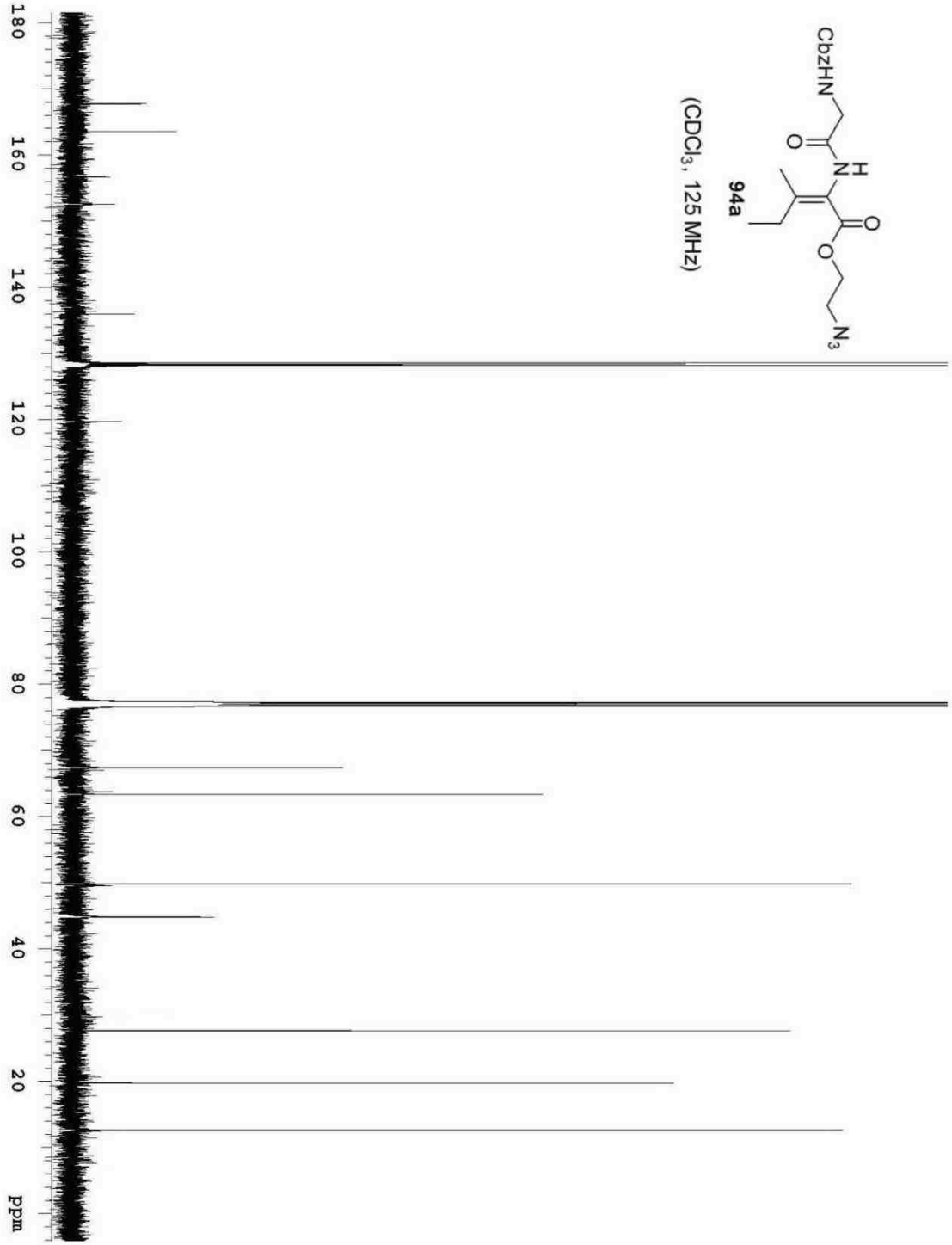

CbzHN
O
N
H
H
OH
O
O
O
N3
93a
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

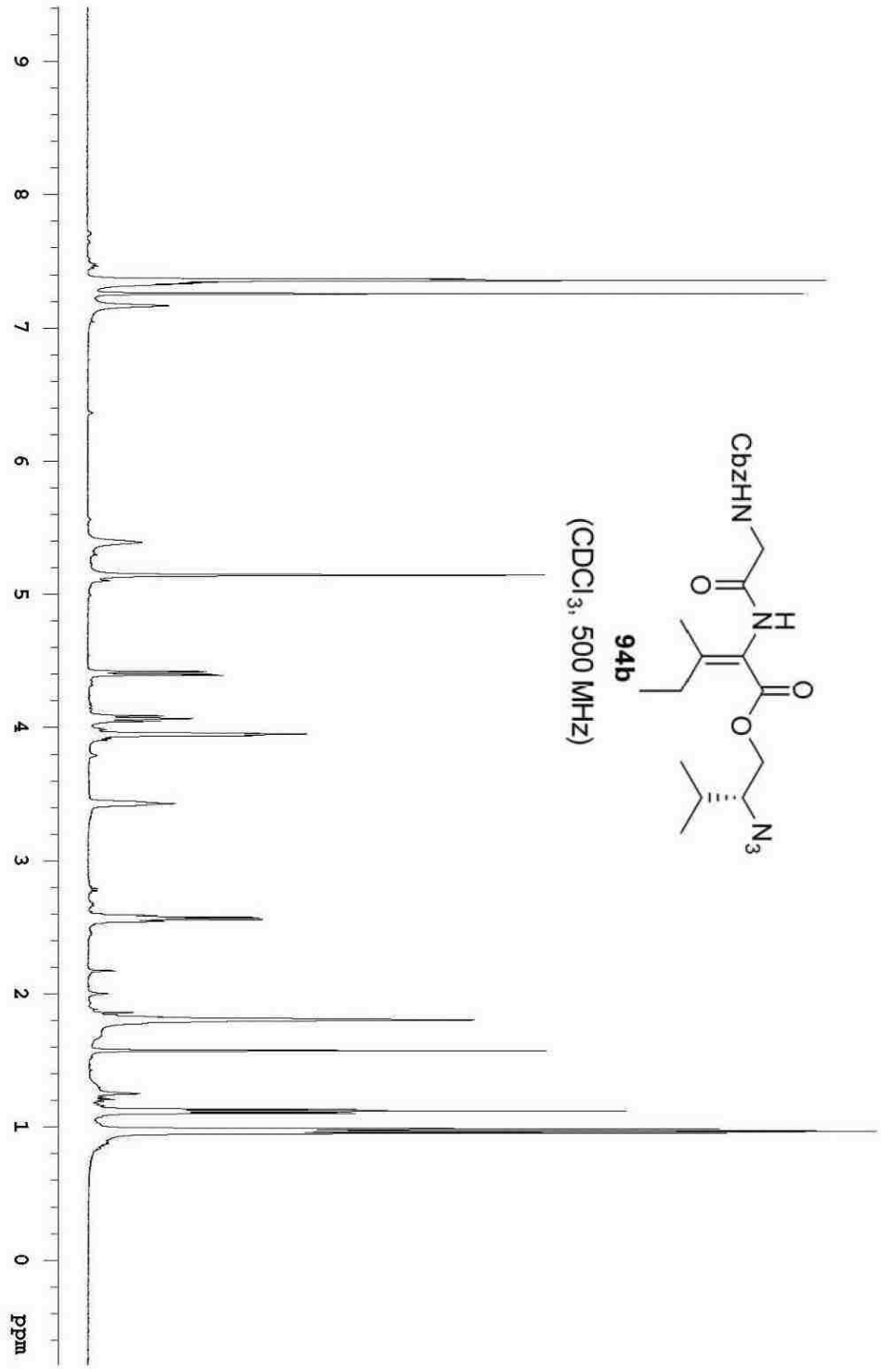

CbzHN
O
N
H
H
OH
O
O
N3
93a
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
0
ppm

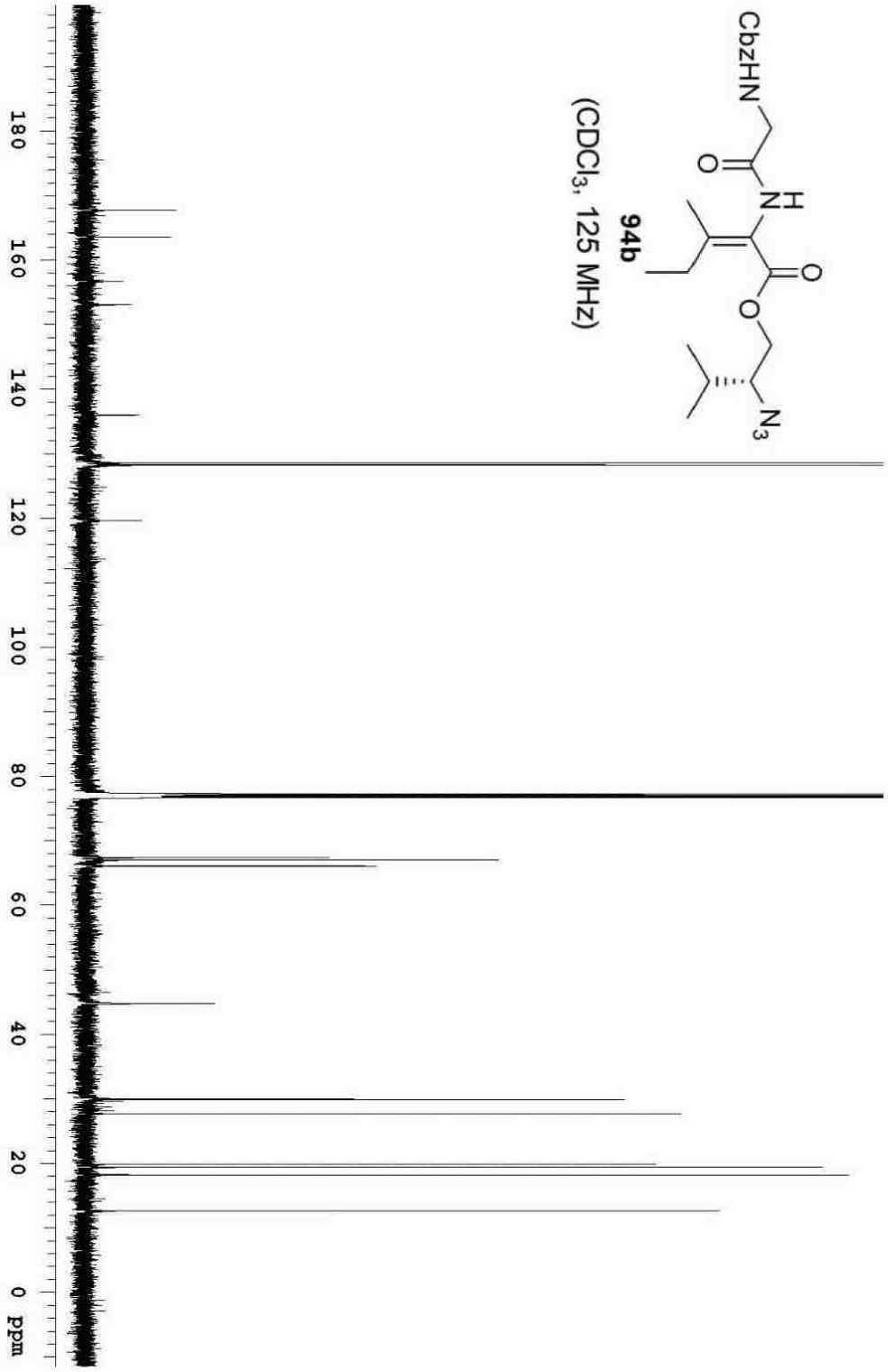

CbzHN
H
N
H
O
O
O
N3
OH
93b
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0 ppm

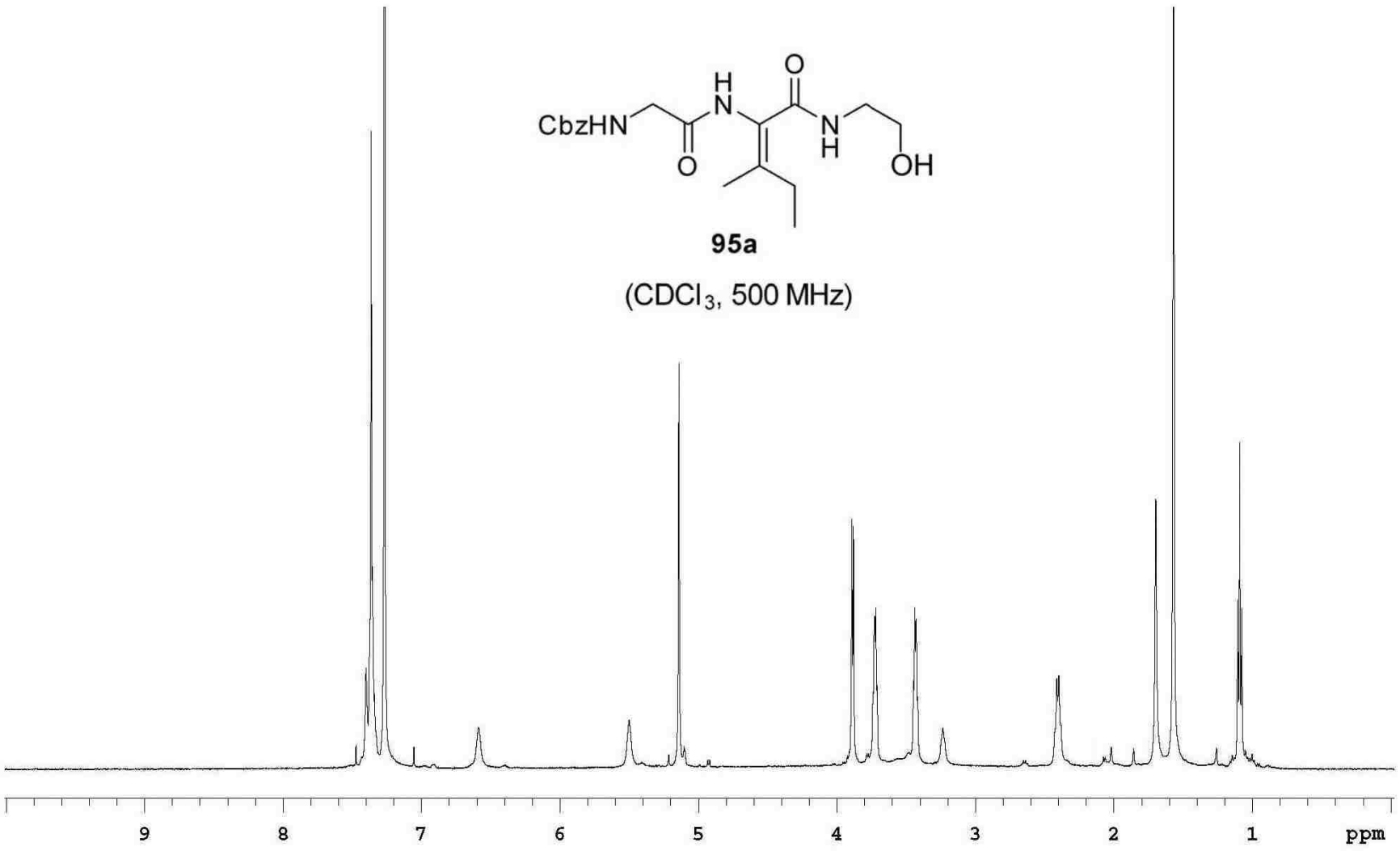

CbzHN
O
H
N
H
N
O
OH
O
N3
93b
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
ppm

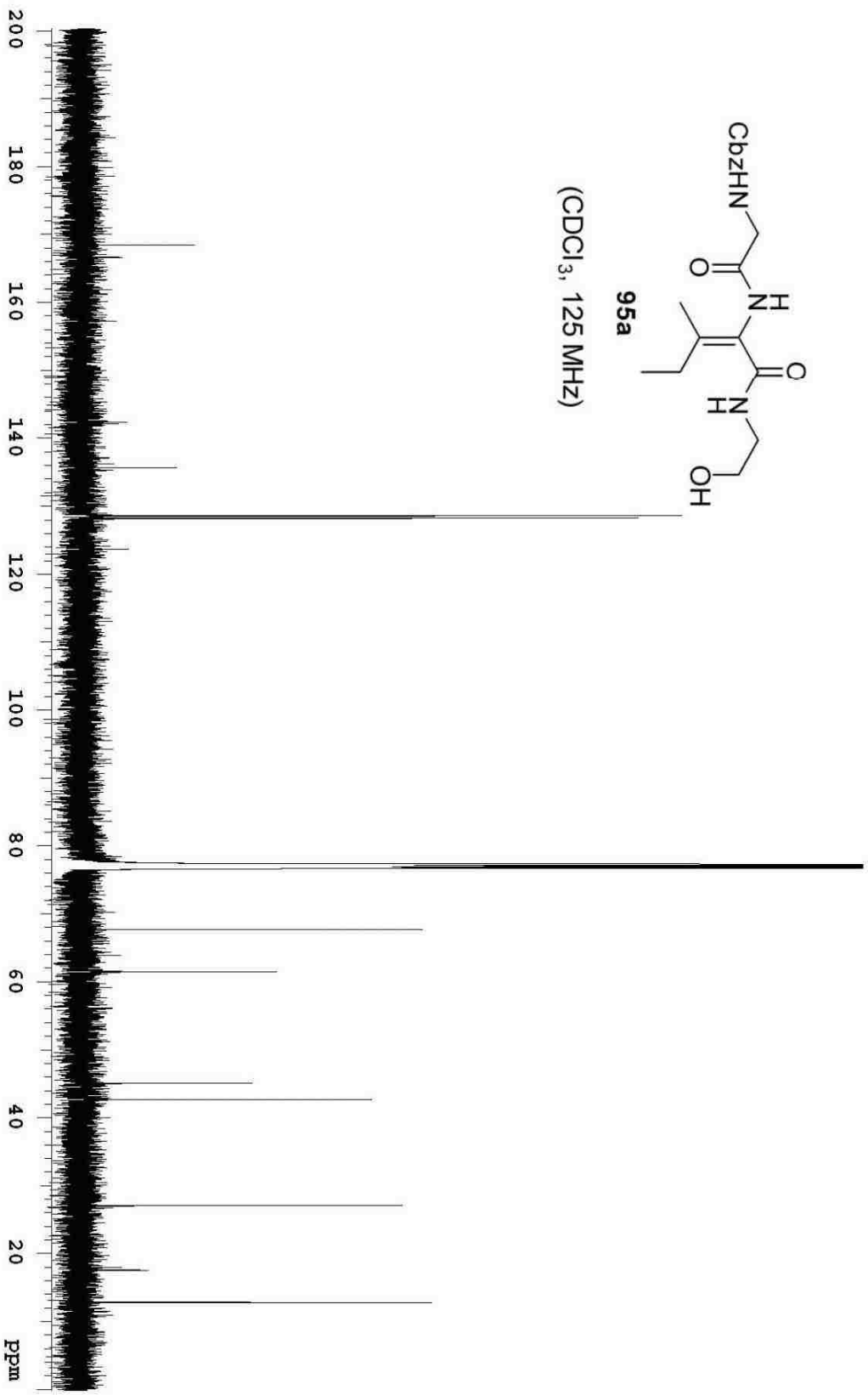

CbzHN
O
H
N
O
O
O
N3
94a
(CDCl3, 500 MHz)
8
7
6
5
4
3
2
1
ppm

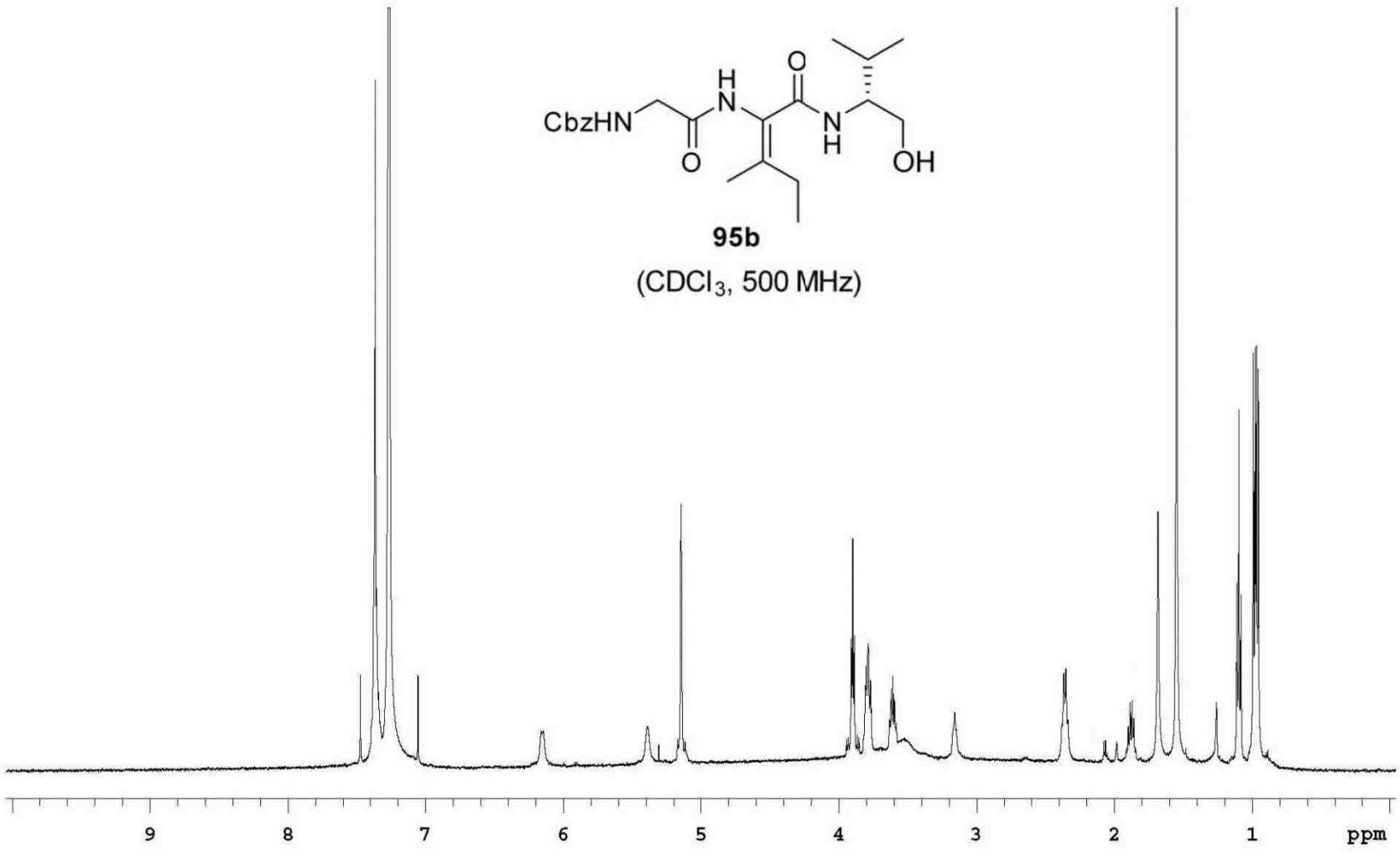

CbzHN
H
N
O
O
O
O
N3
94a
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
ppm

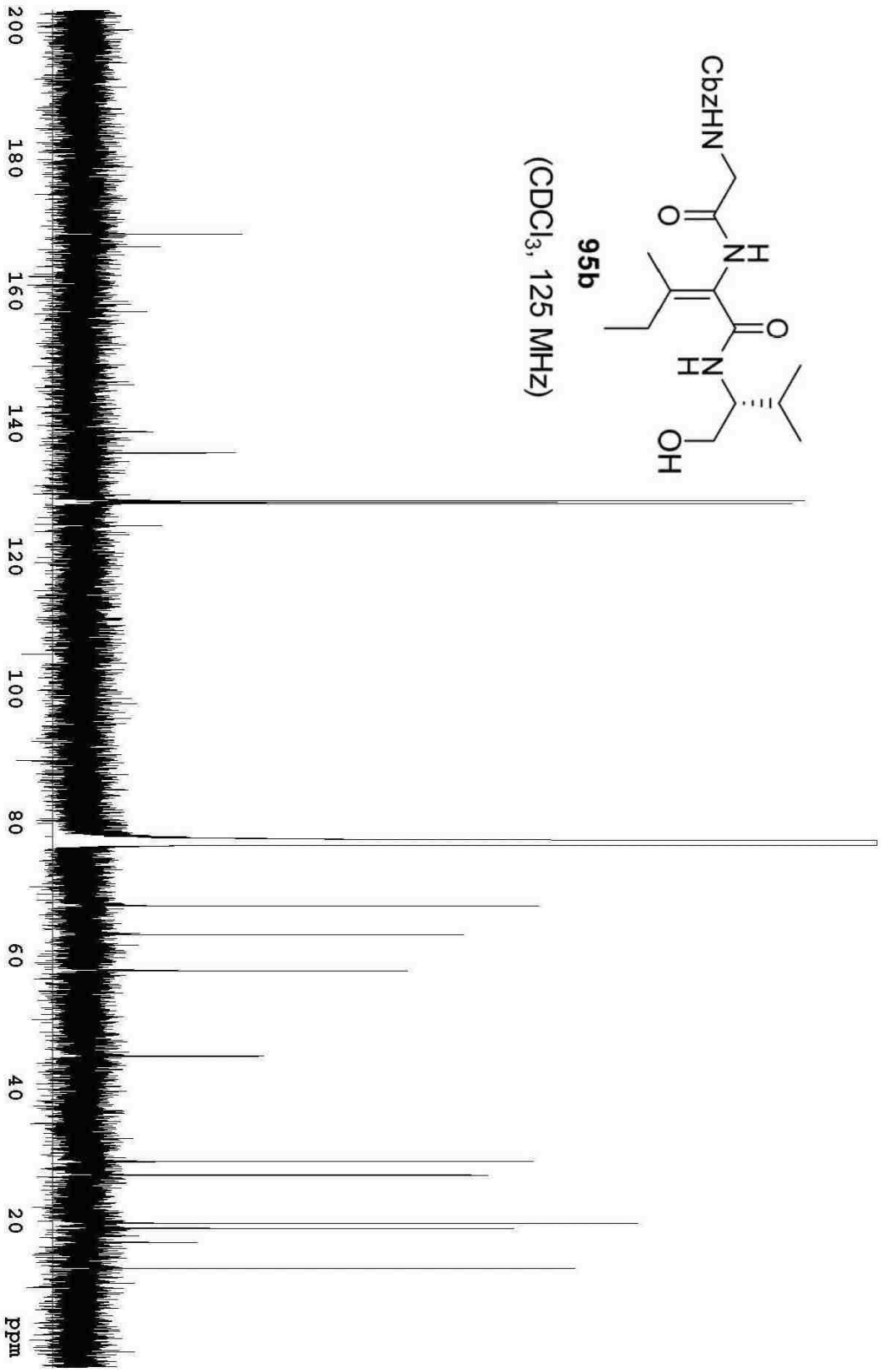

CbzHN
O
H
N
O
O
O
N3
94b
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

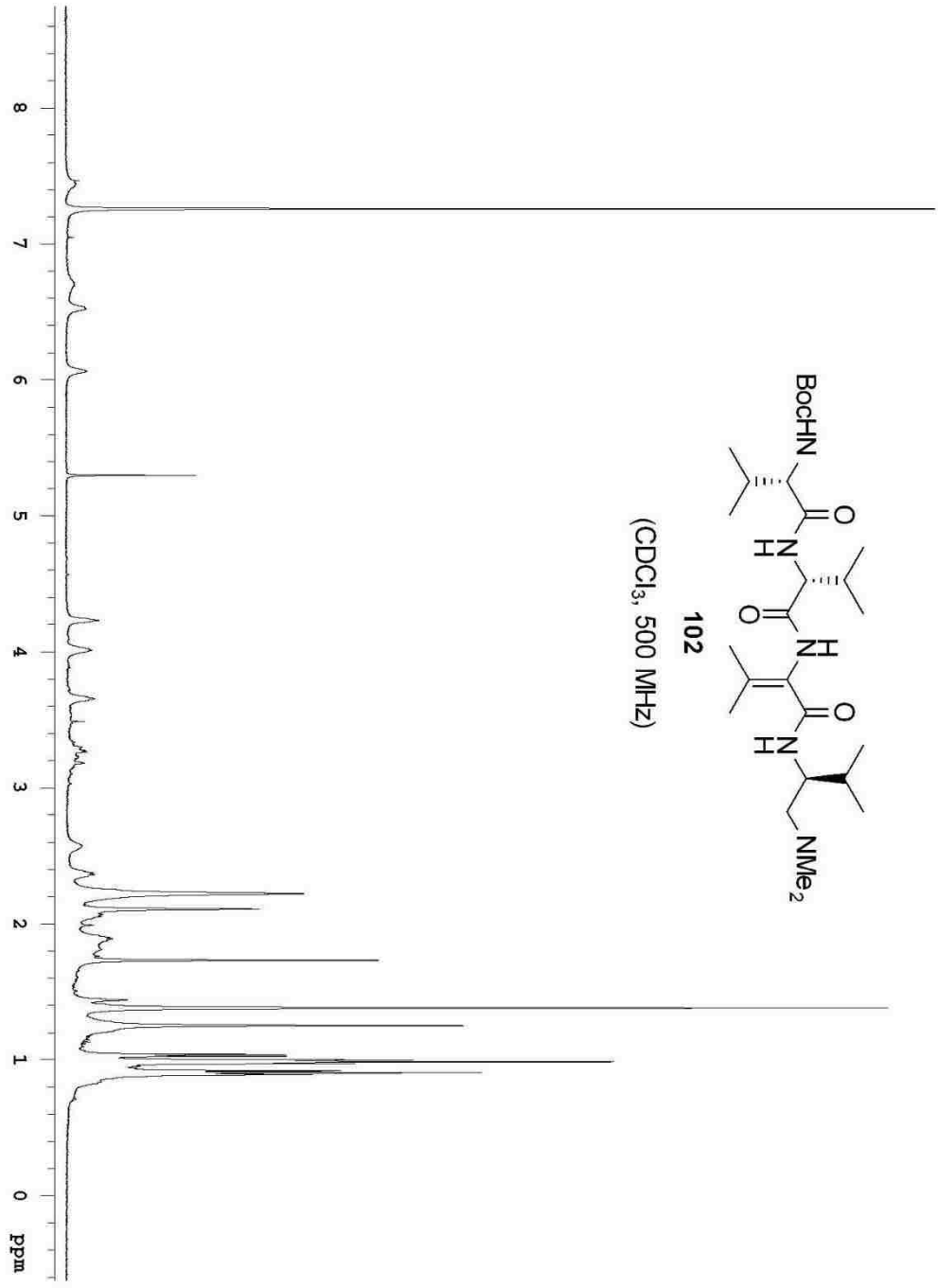

CbzHN
O
H
N
O
O
O
N3
94b
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
0 ppm

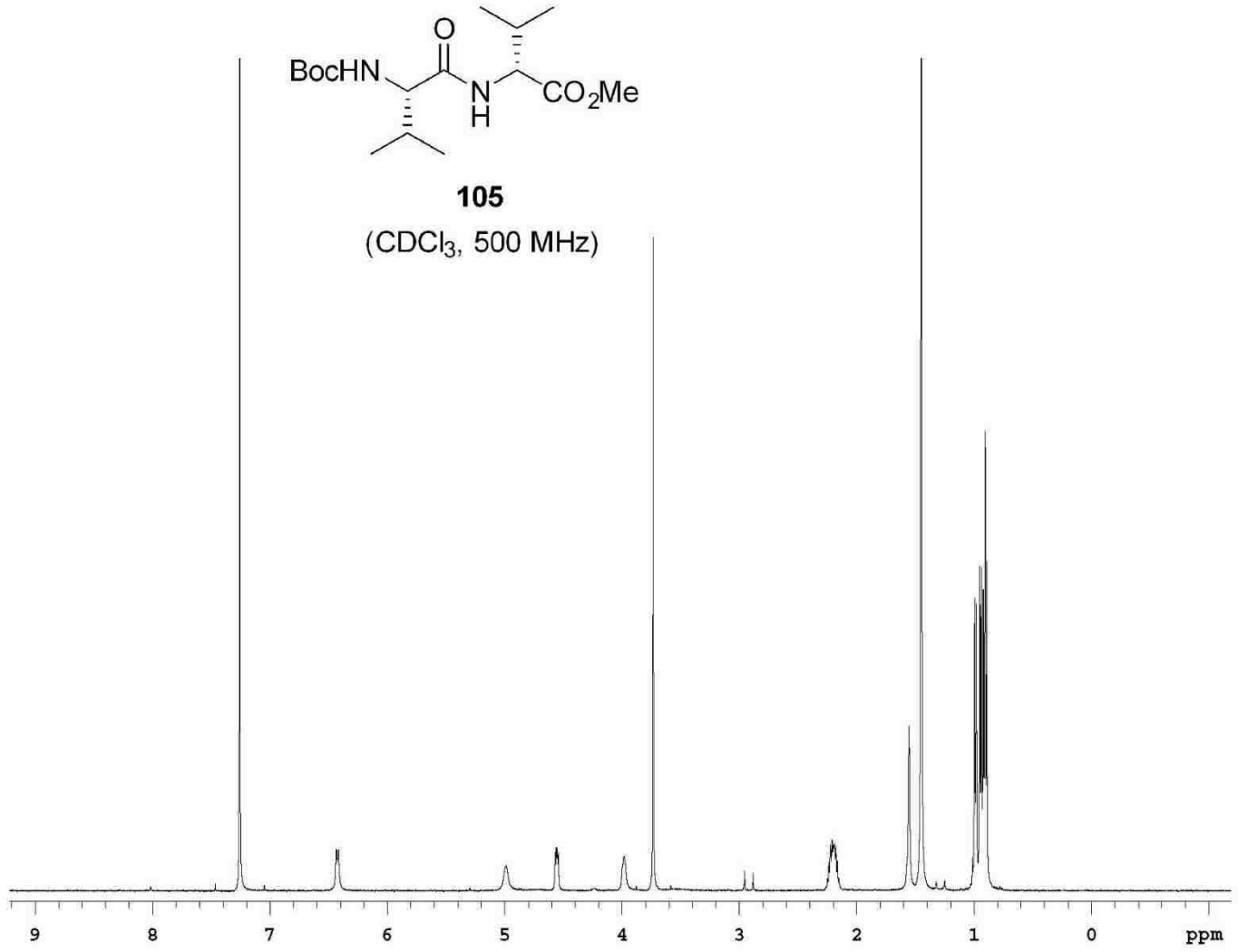

CbzHN
H
N
O
O
N
H
OH
95a
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
ppm

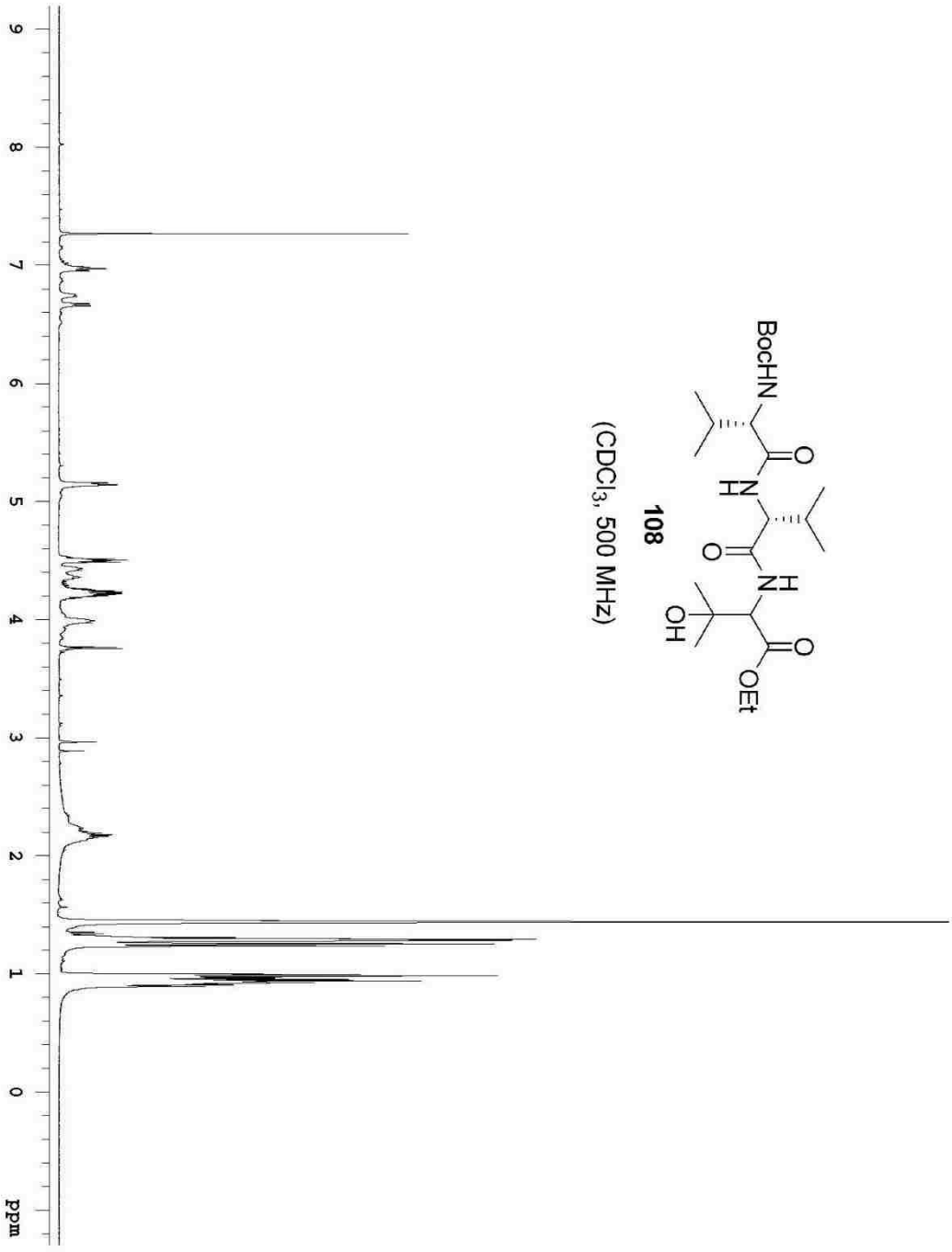

CbzHN
O
H
N
O
H
N
OH
95a
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
ppm

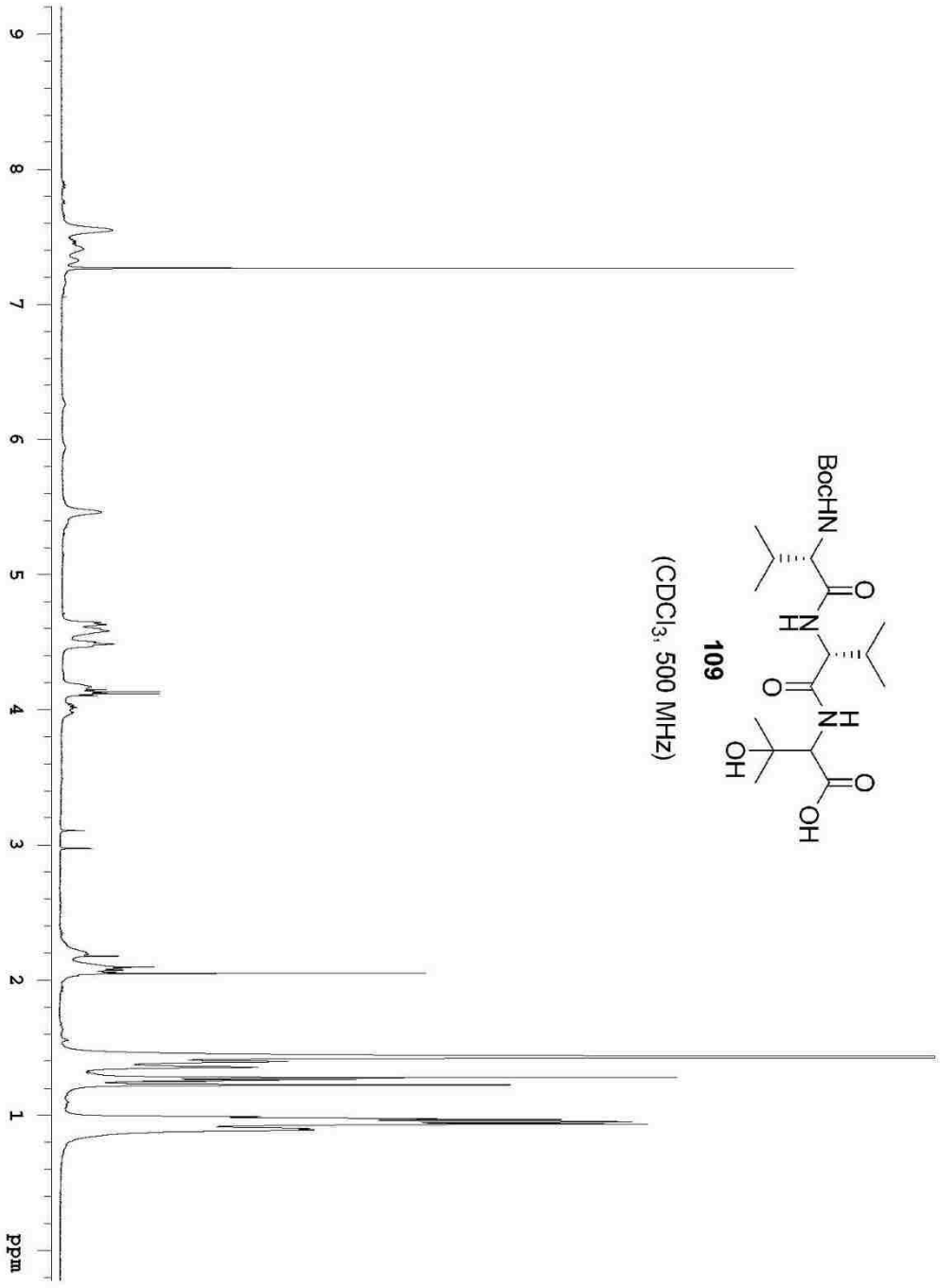

CbzHN
H
N
O
O
N
H
OH
95b
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
ppm

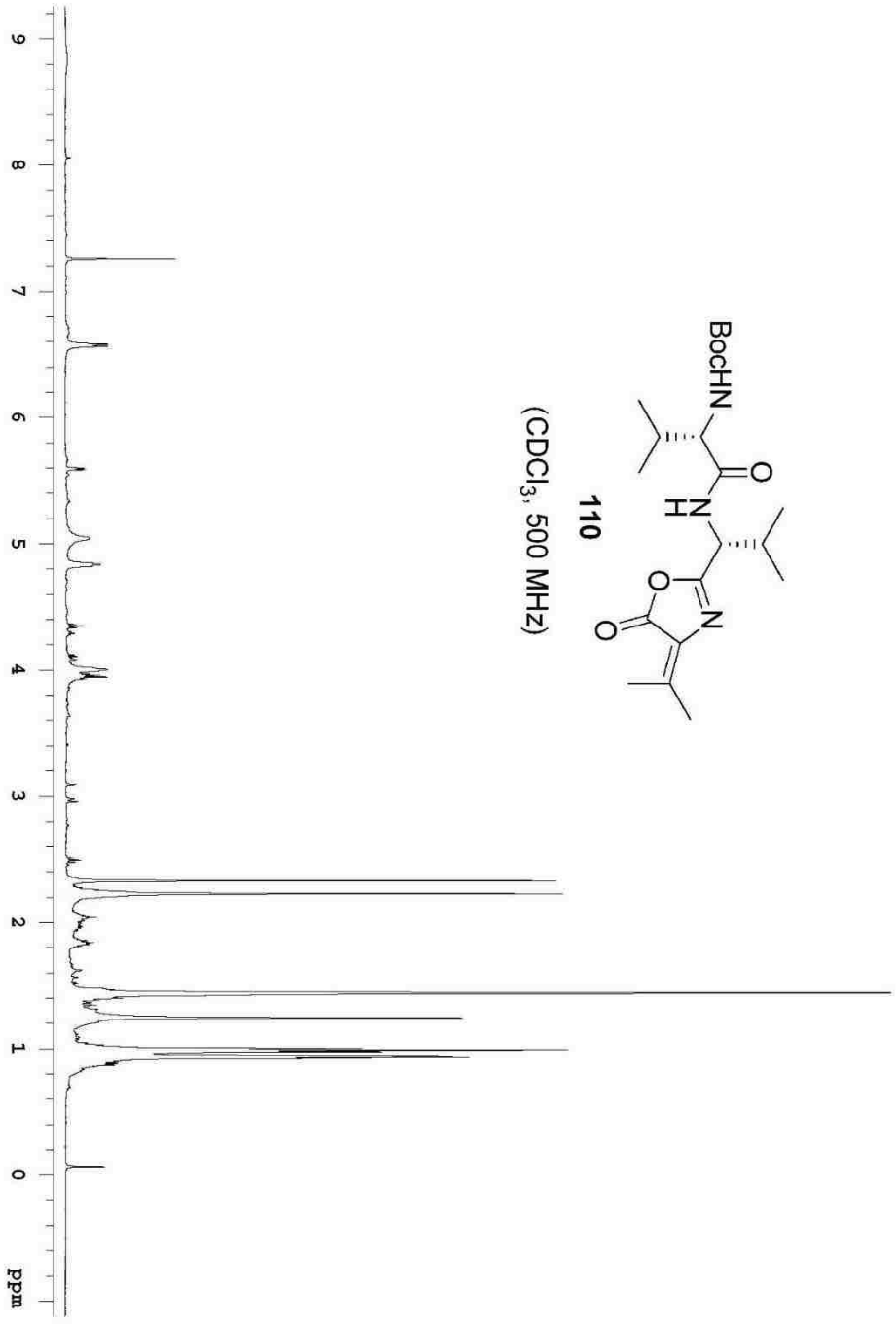

CbzHN
O
H
N
O
H
N
OH
95b
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
ppm

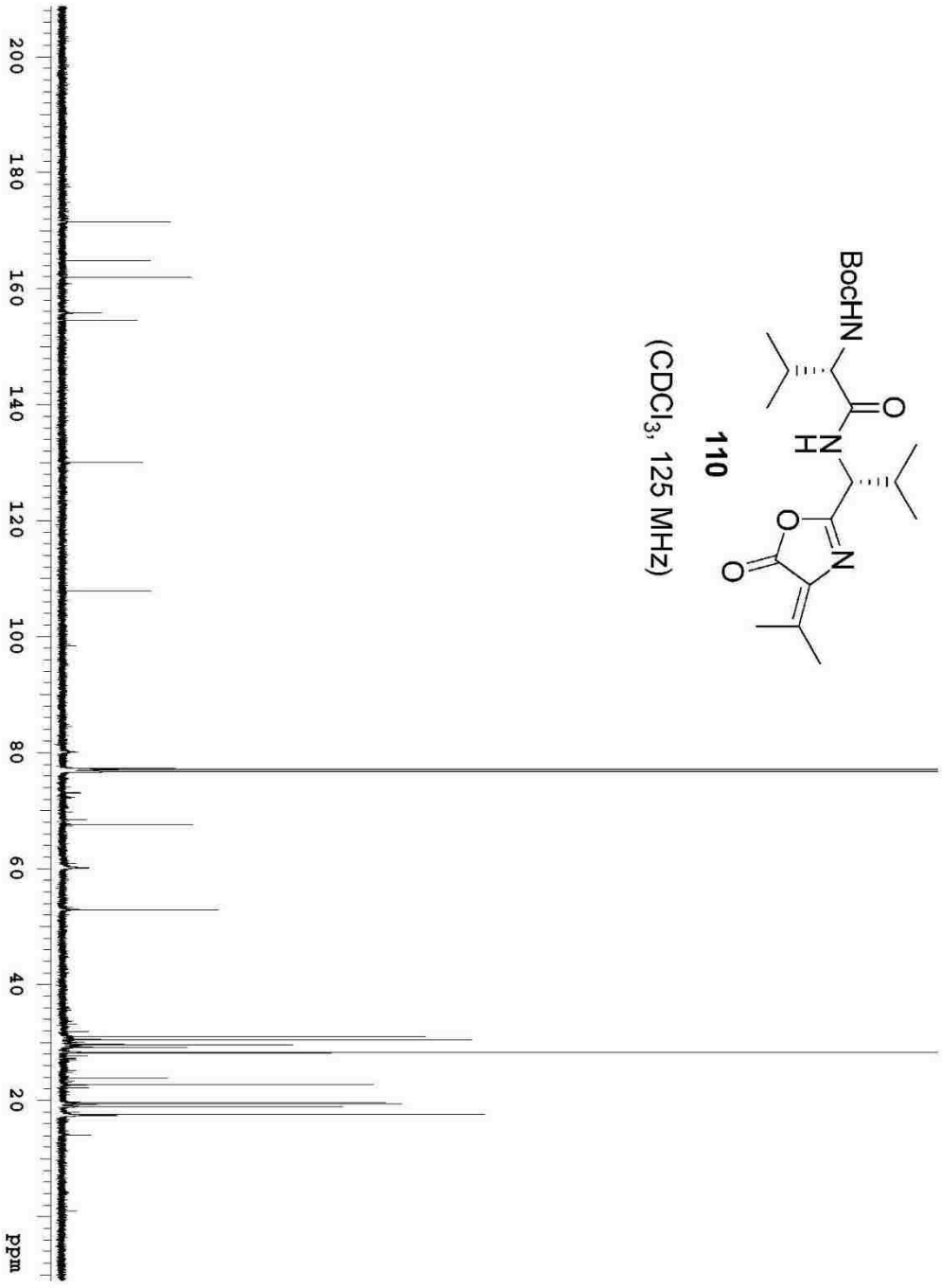

BocHN
O
N
H
O
N
H
O
N
H
NMe2
102
(CDCl3, 500 MHz)
8
7
6
5
4
3
2
1
0
ppm

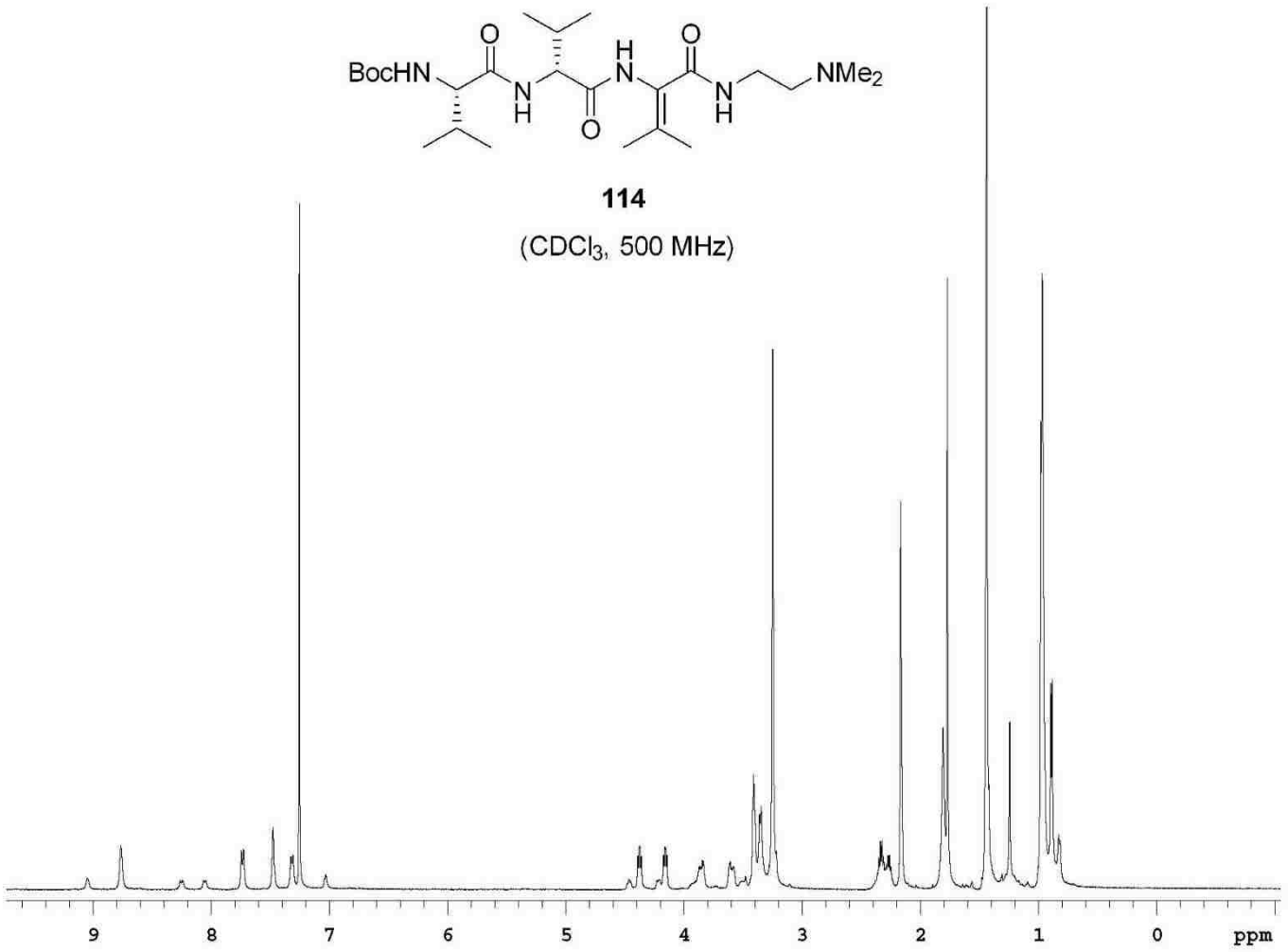

BocHN
O
N
H
CO2Me
105
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

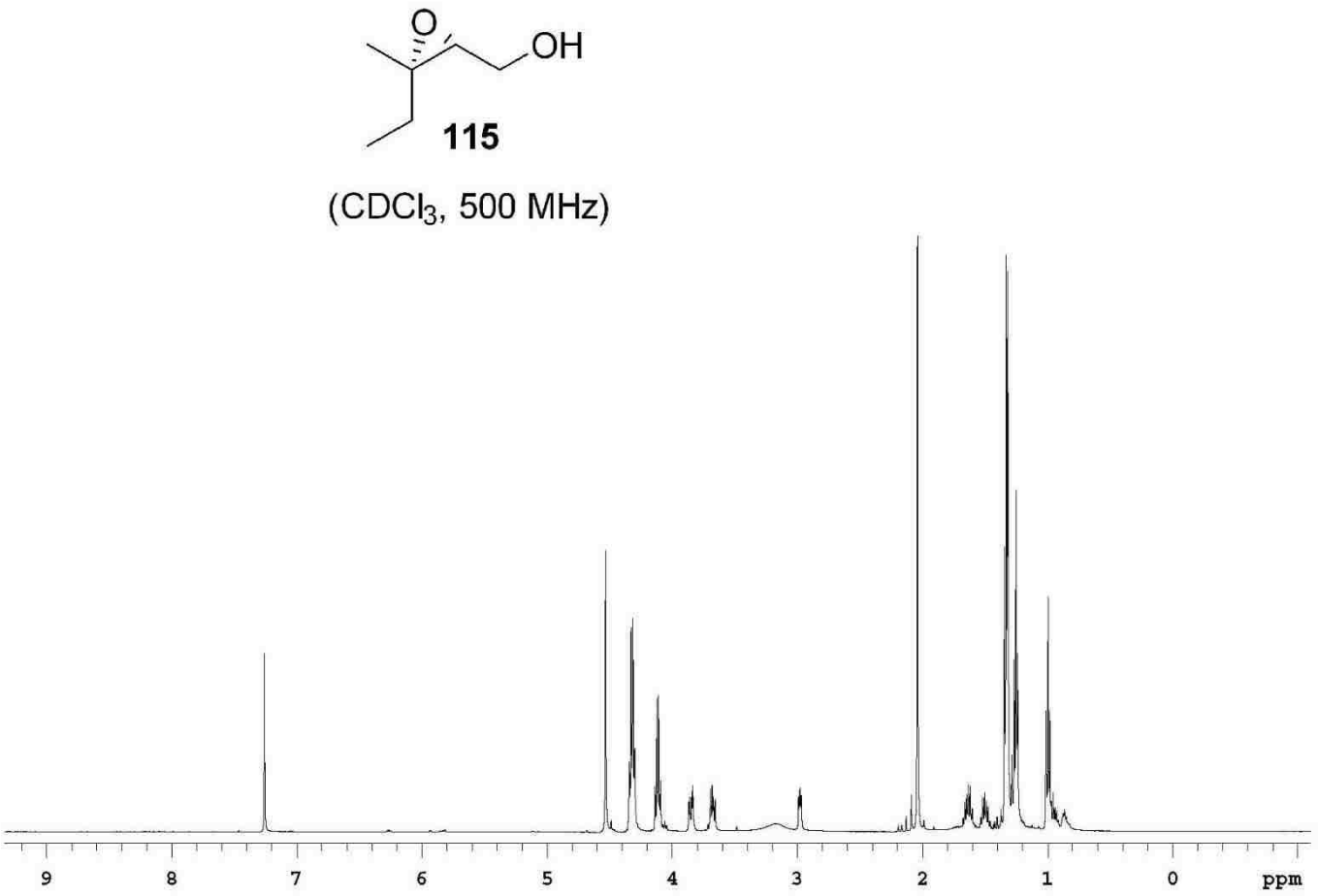

BocHN
O
H
N
O
H
N
OH
O
OEt
108
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

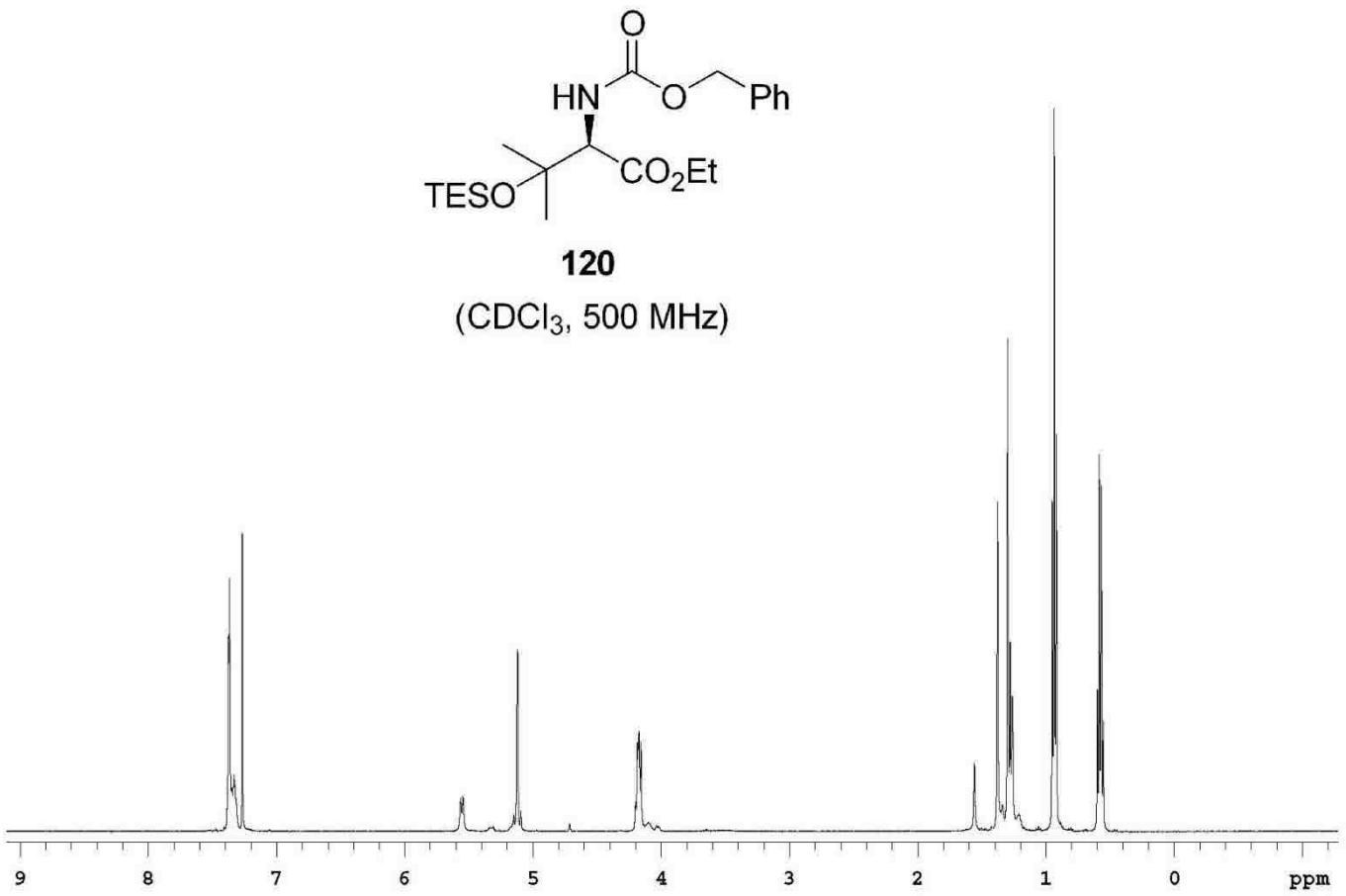

BocHN
O
H
N
O
H
N
OH
O
OH
109
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
ppm

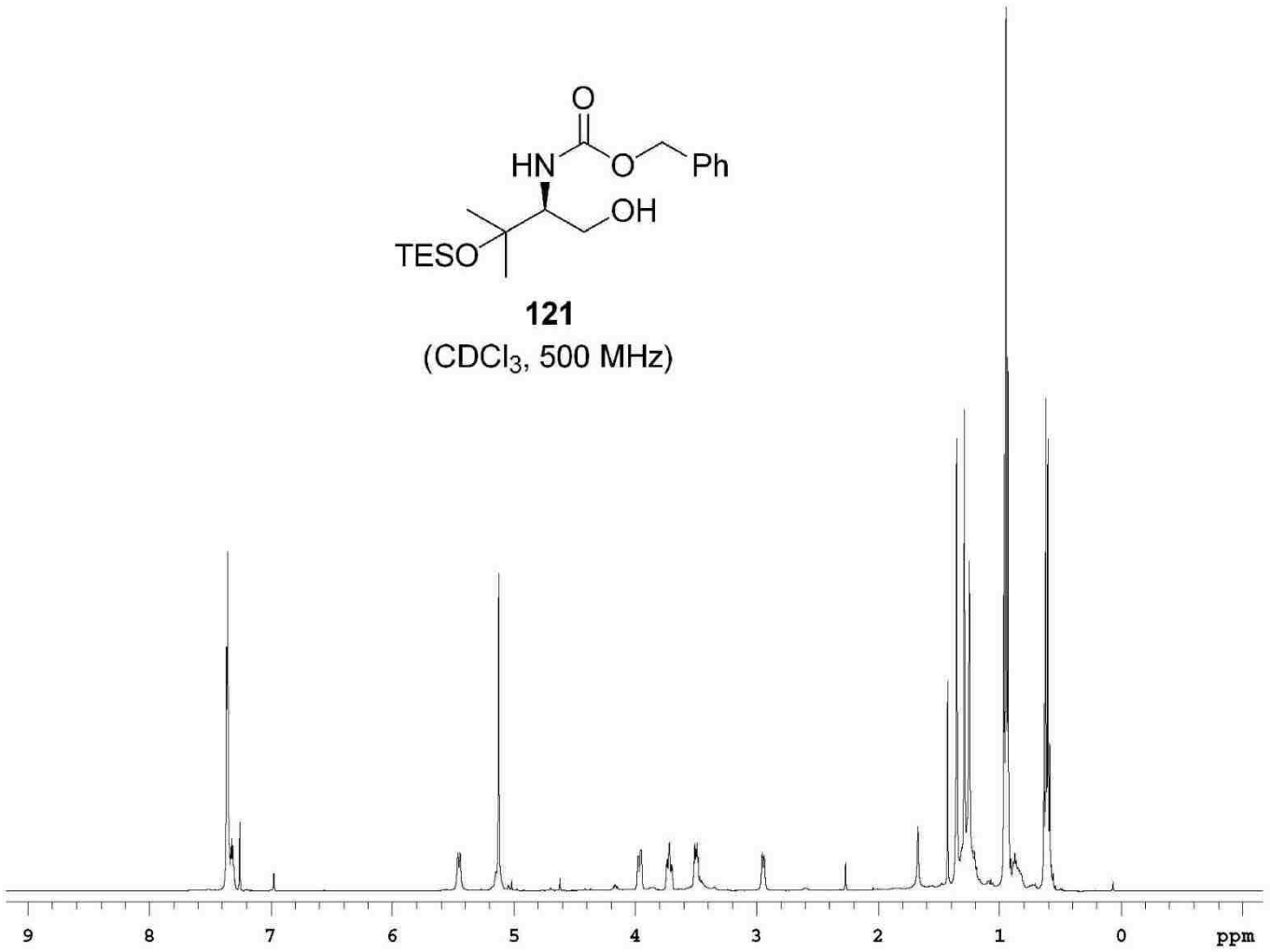

BocHN
O
N
H
O
O
N
O
110
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm


BocHN
O
N
H
O
O
N
O
110
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
ppm


BocHN
O
N
H
O
H
N
O
N
H
NMe2
114
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

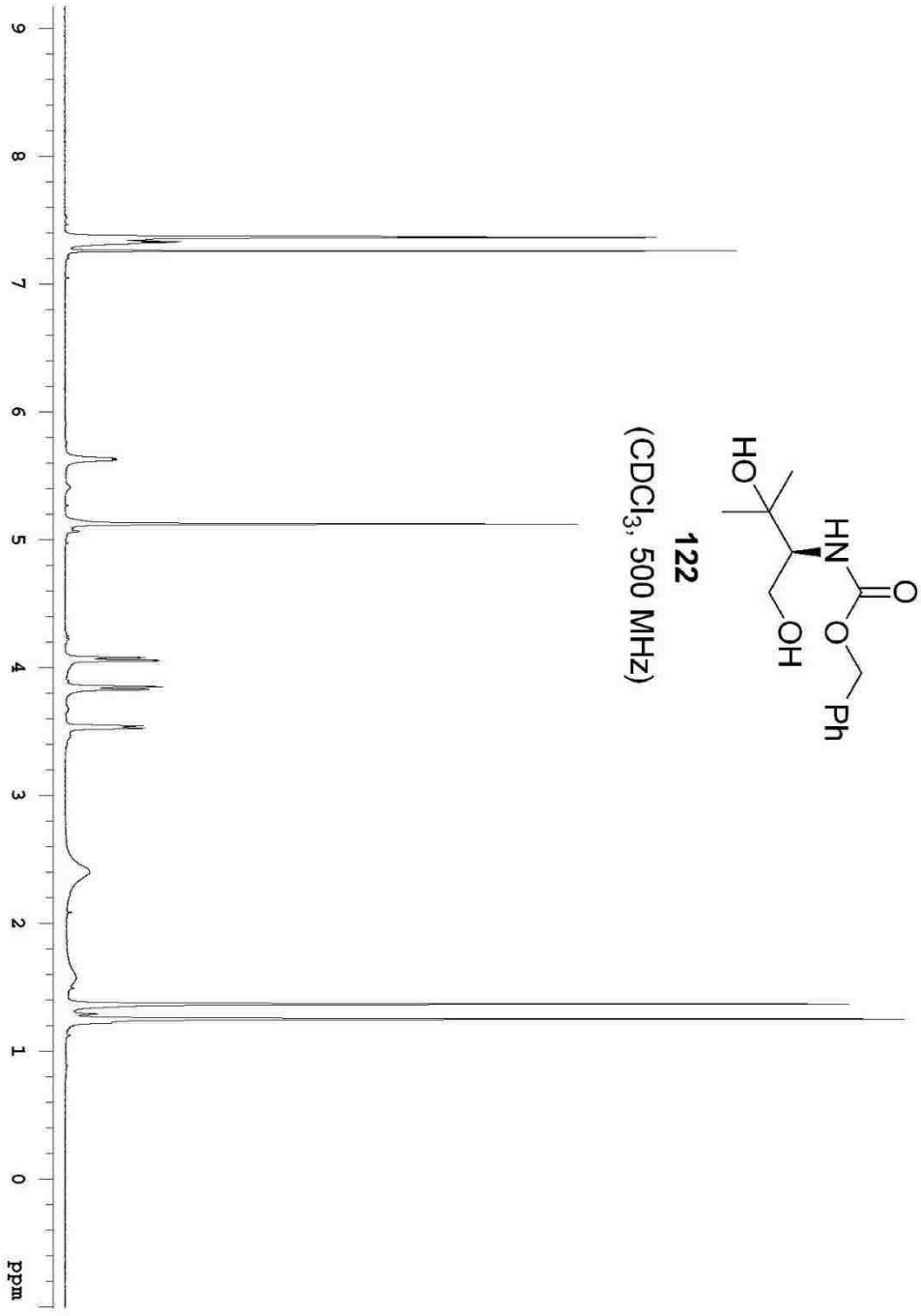

O
OH
115
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm

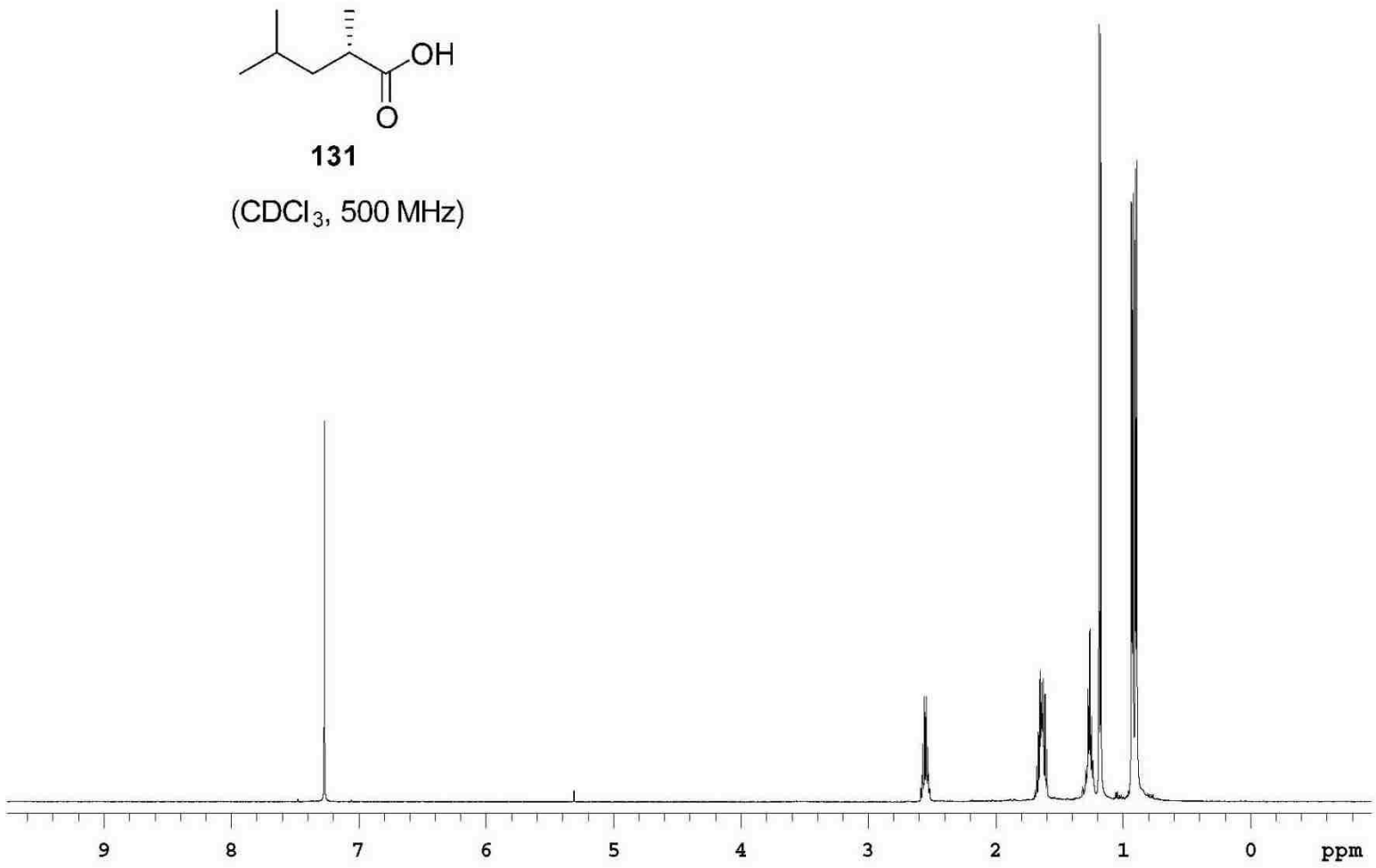

O
HN
O
Ph
TESO
CO2Et
120
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm



121

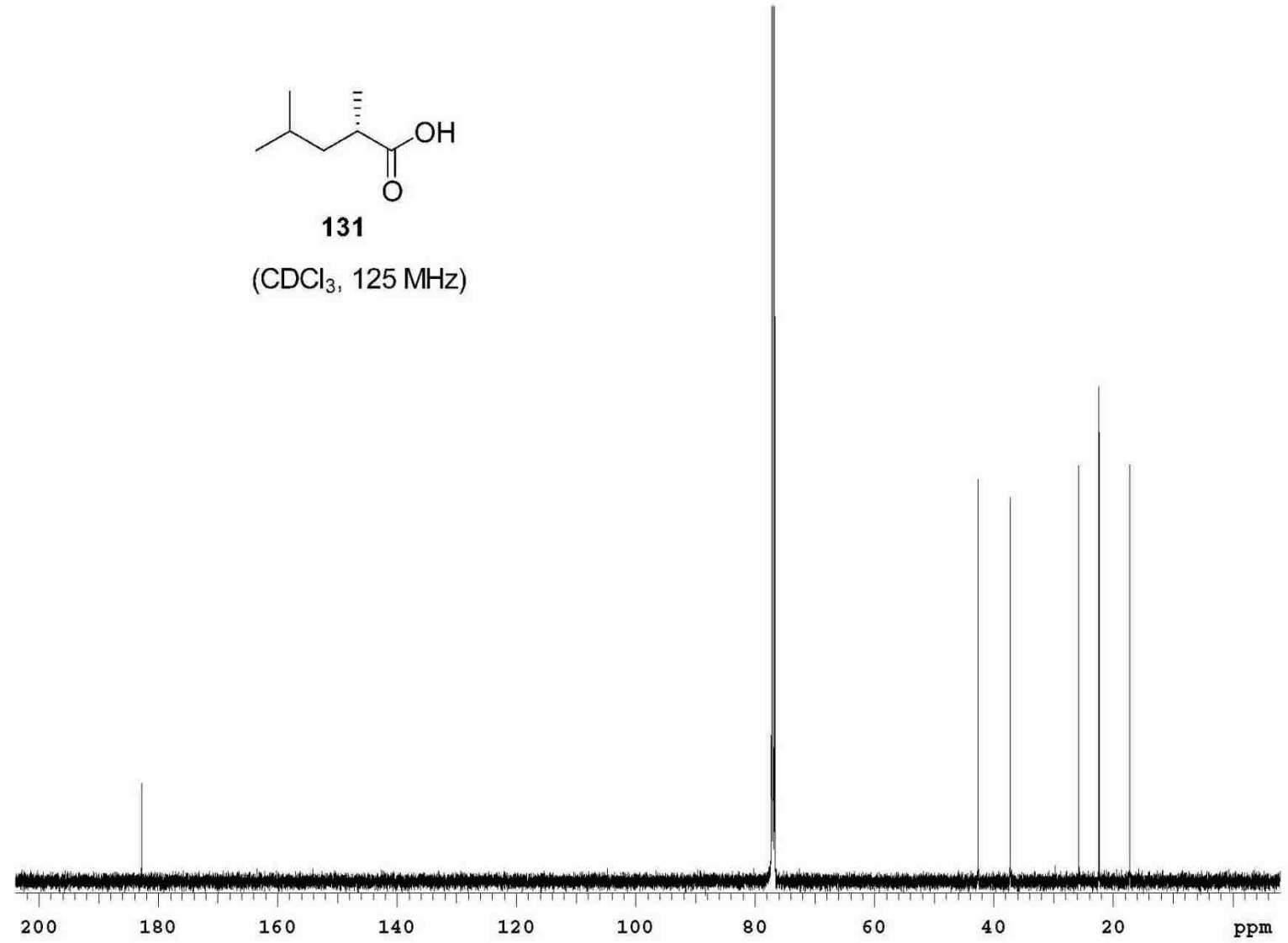
($CDCl_3$, 500 MHz)


HO
HN
O
O
OH
Ph
122
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm



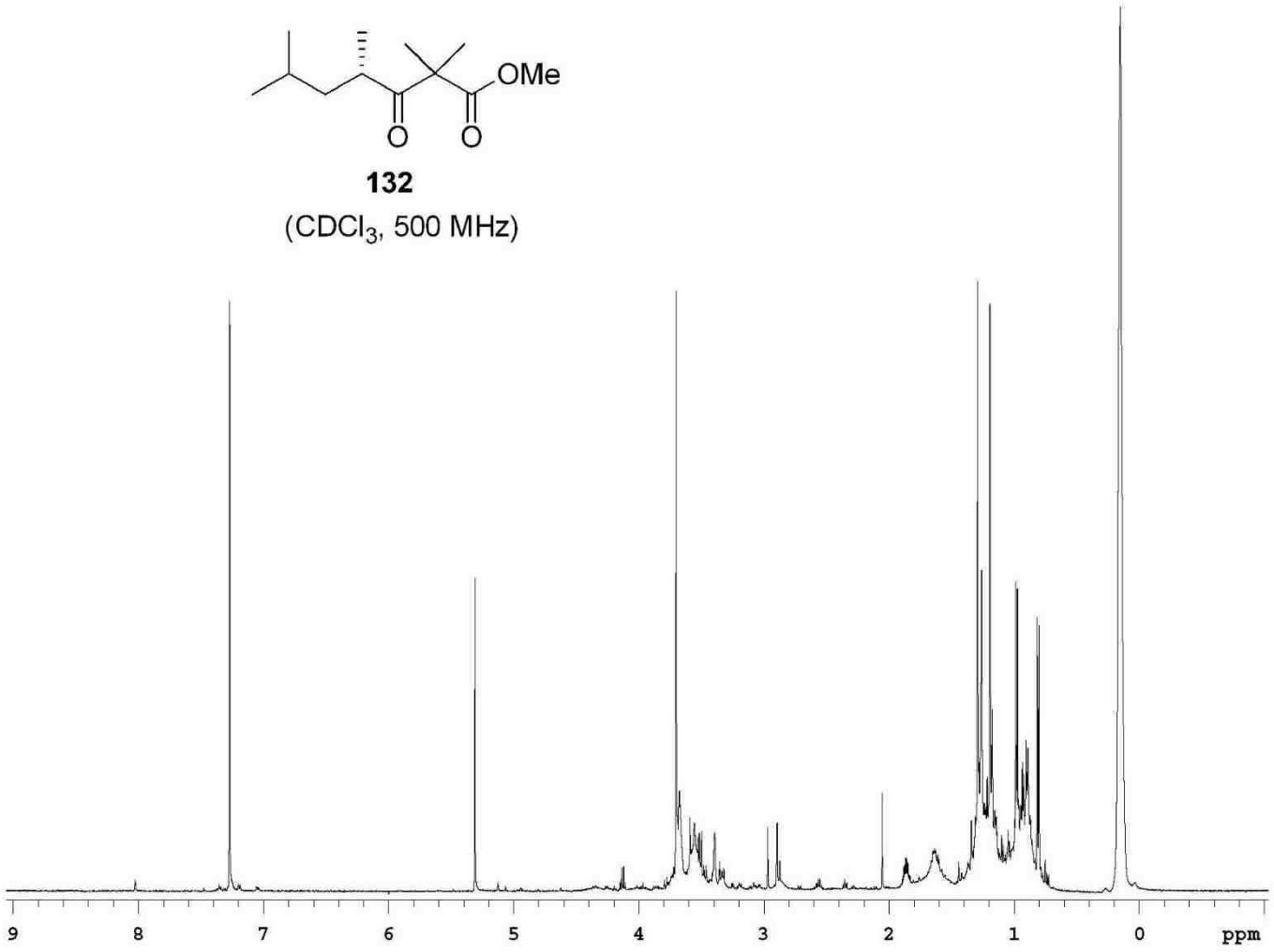
131

($CDCl_3$, 500 MHz)



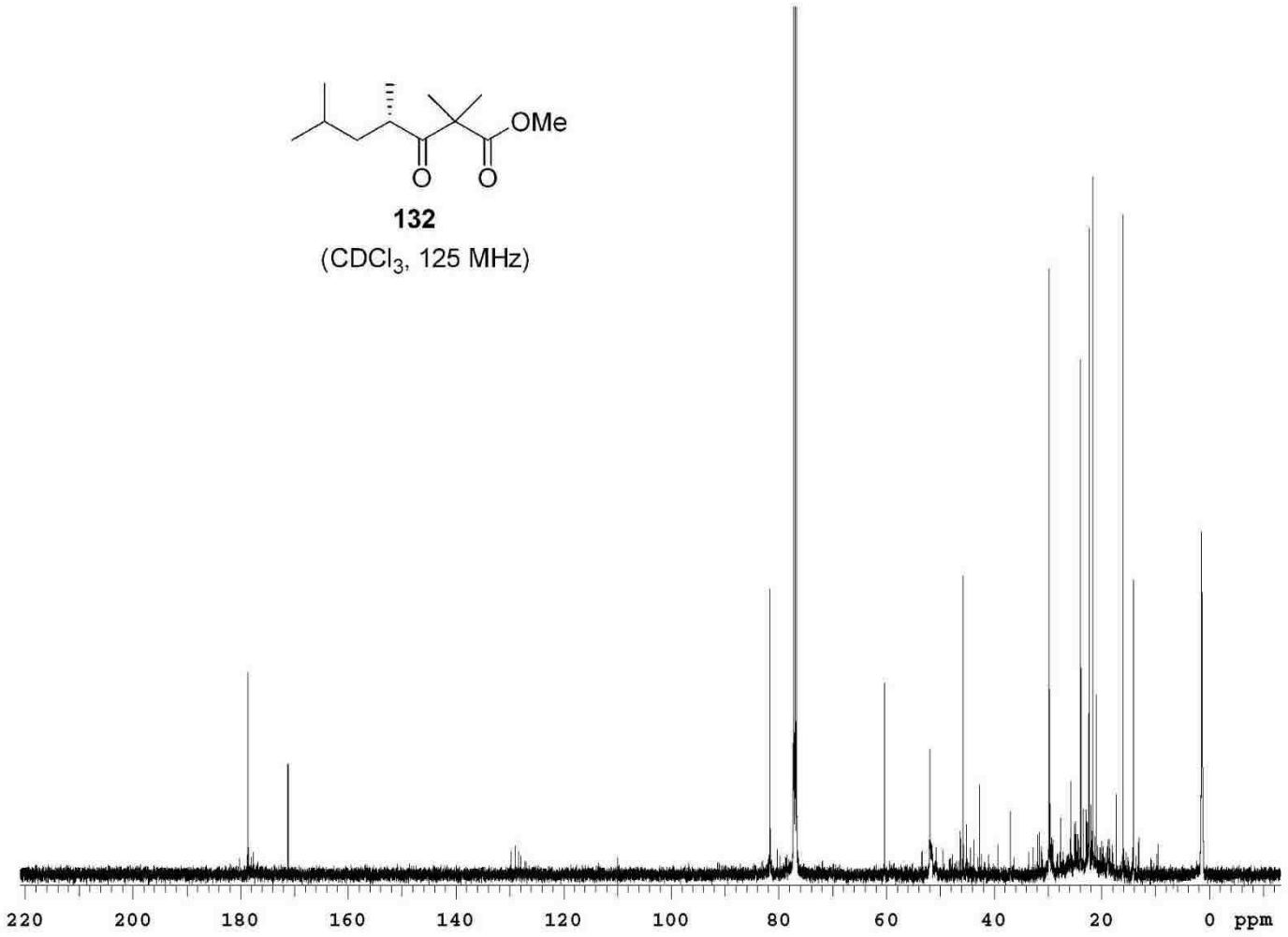
131

($CDCl_3$, 125 MHz)



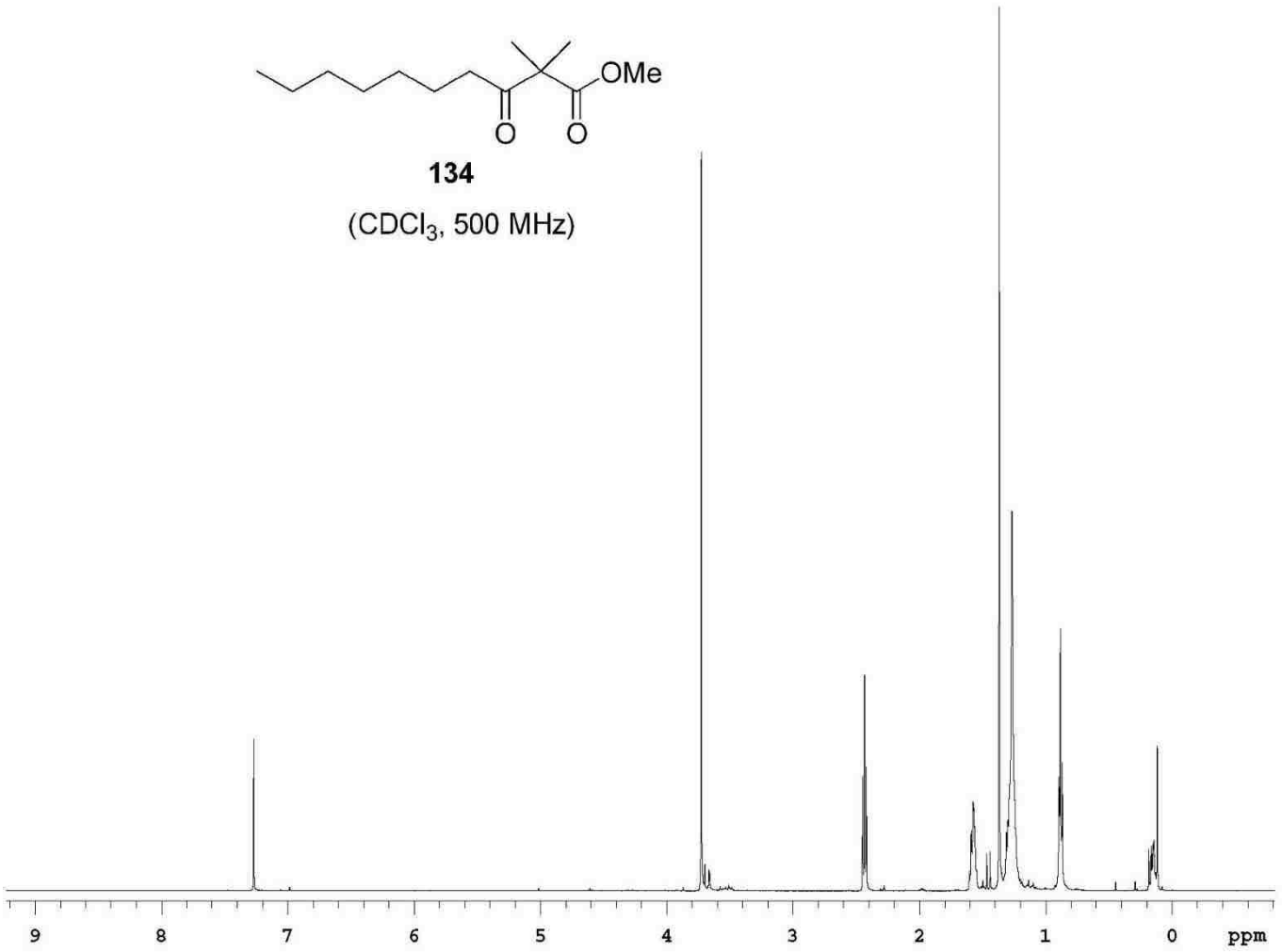
132

($CDCl_3$, 500 MHz)



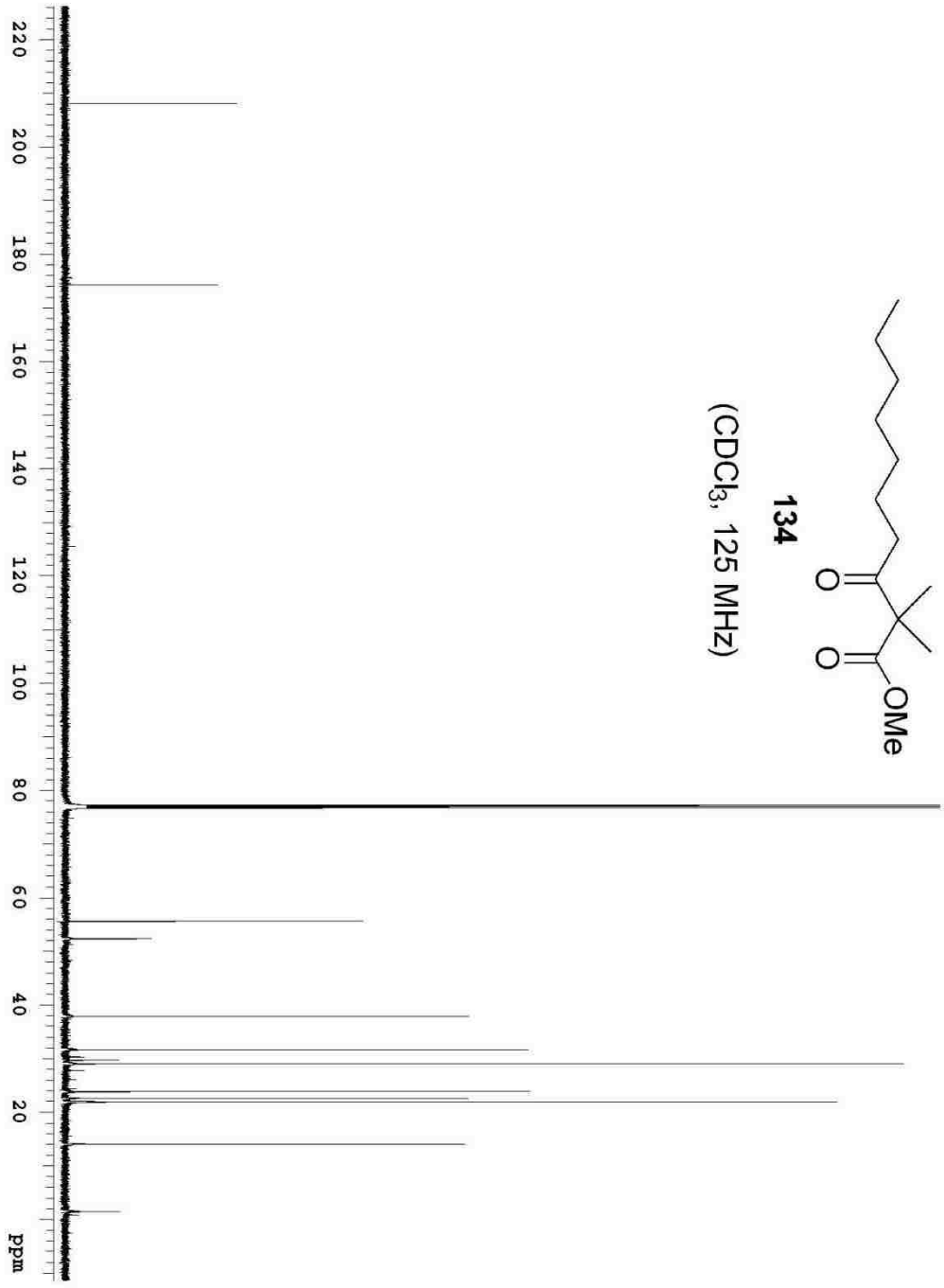
132

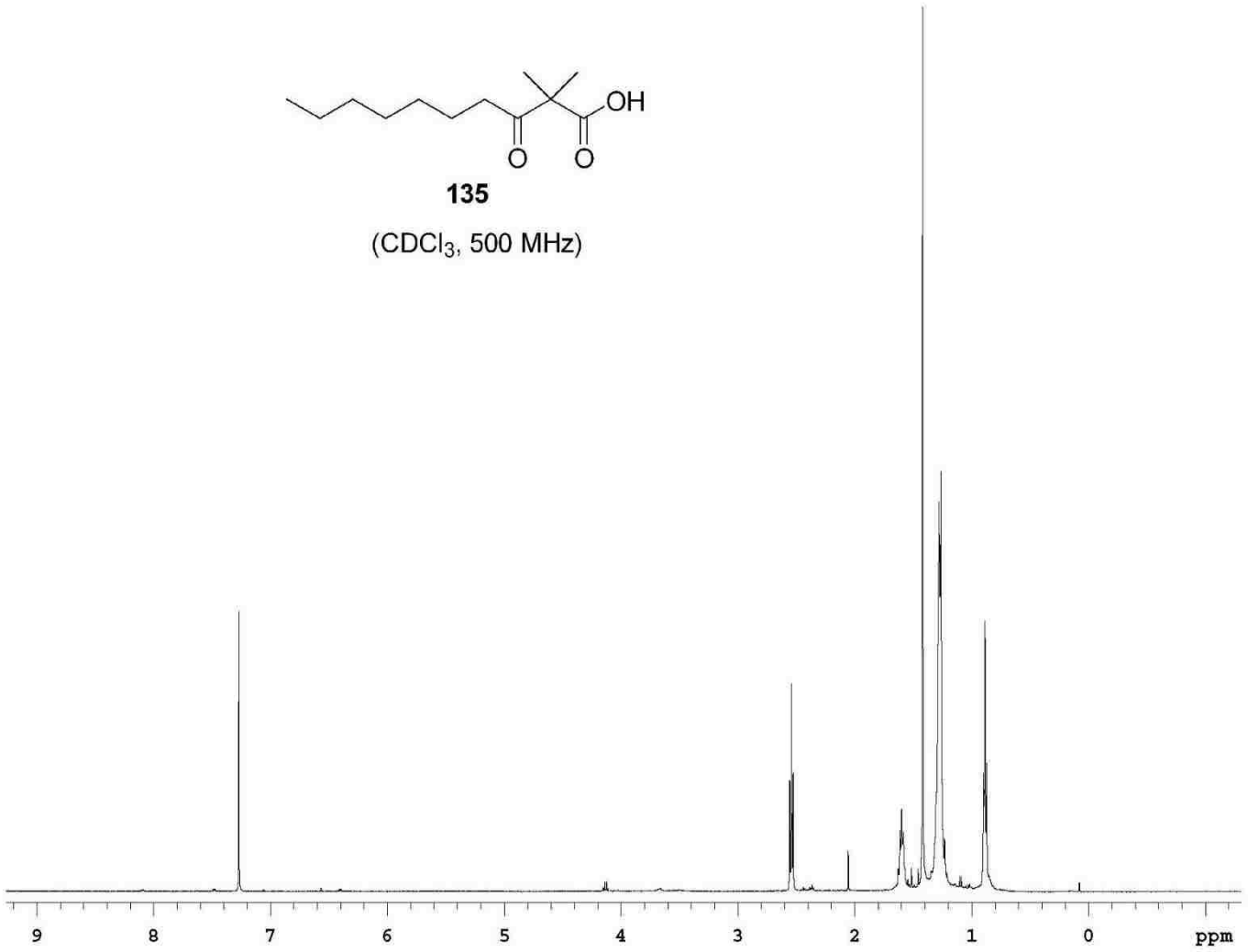
($CDCl_3$, 125 MHz)



134

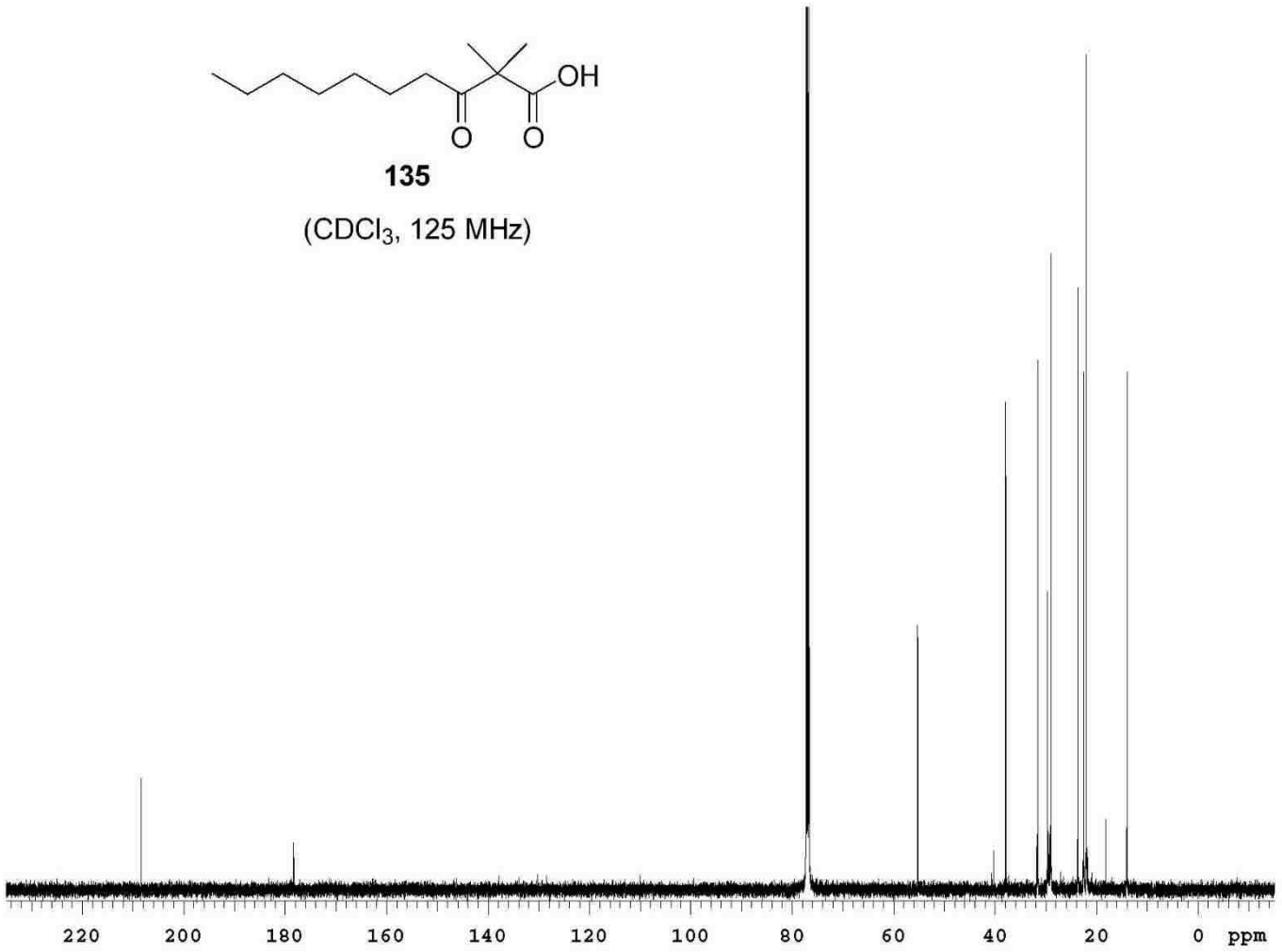
($CDCl_3$, 500 MHz)


OMe
O
O
134
(CDCl3, 125 MHz)
220
200
180
160
140
120
100
80
60
40
20
ppm



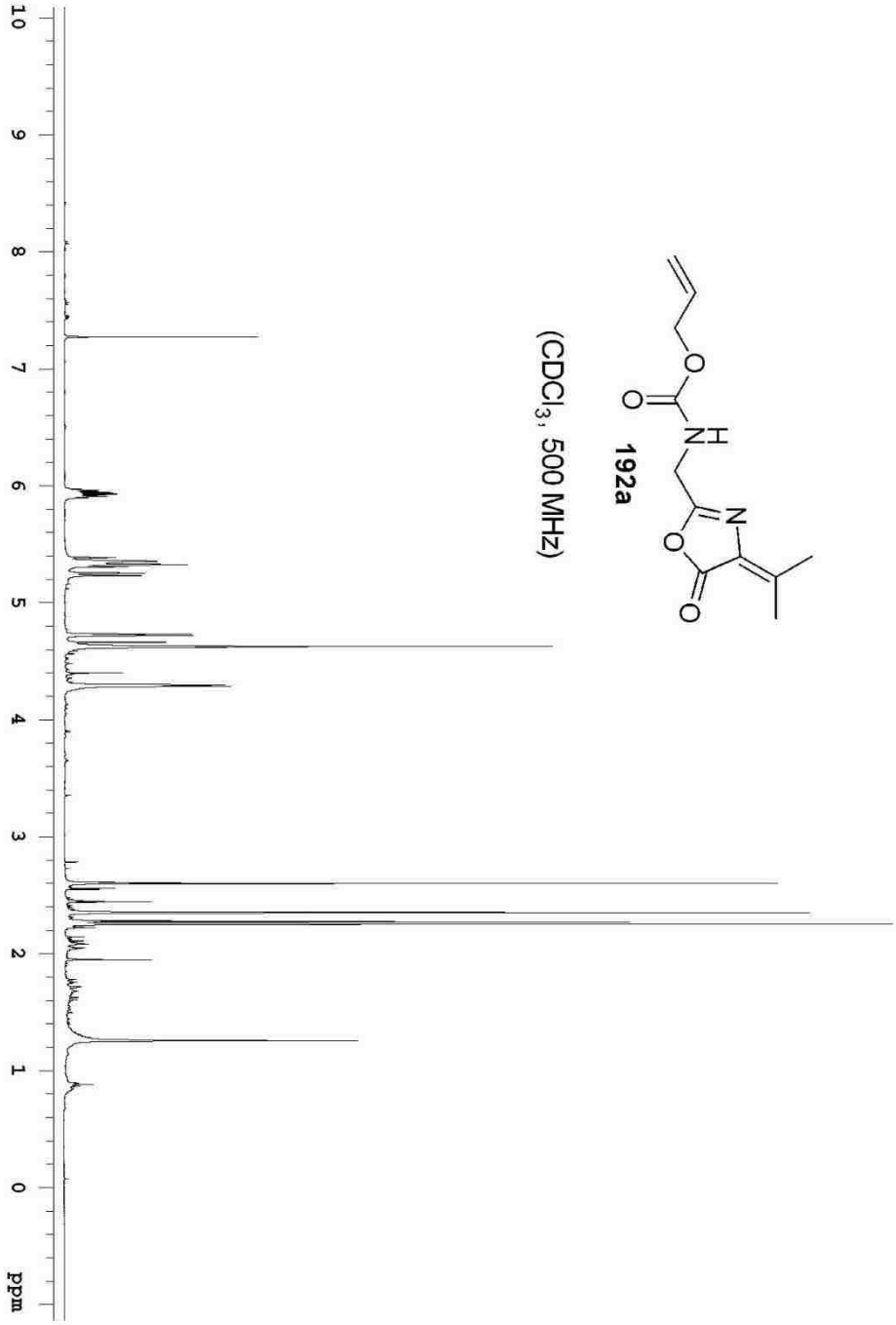
135

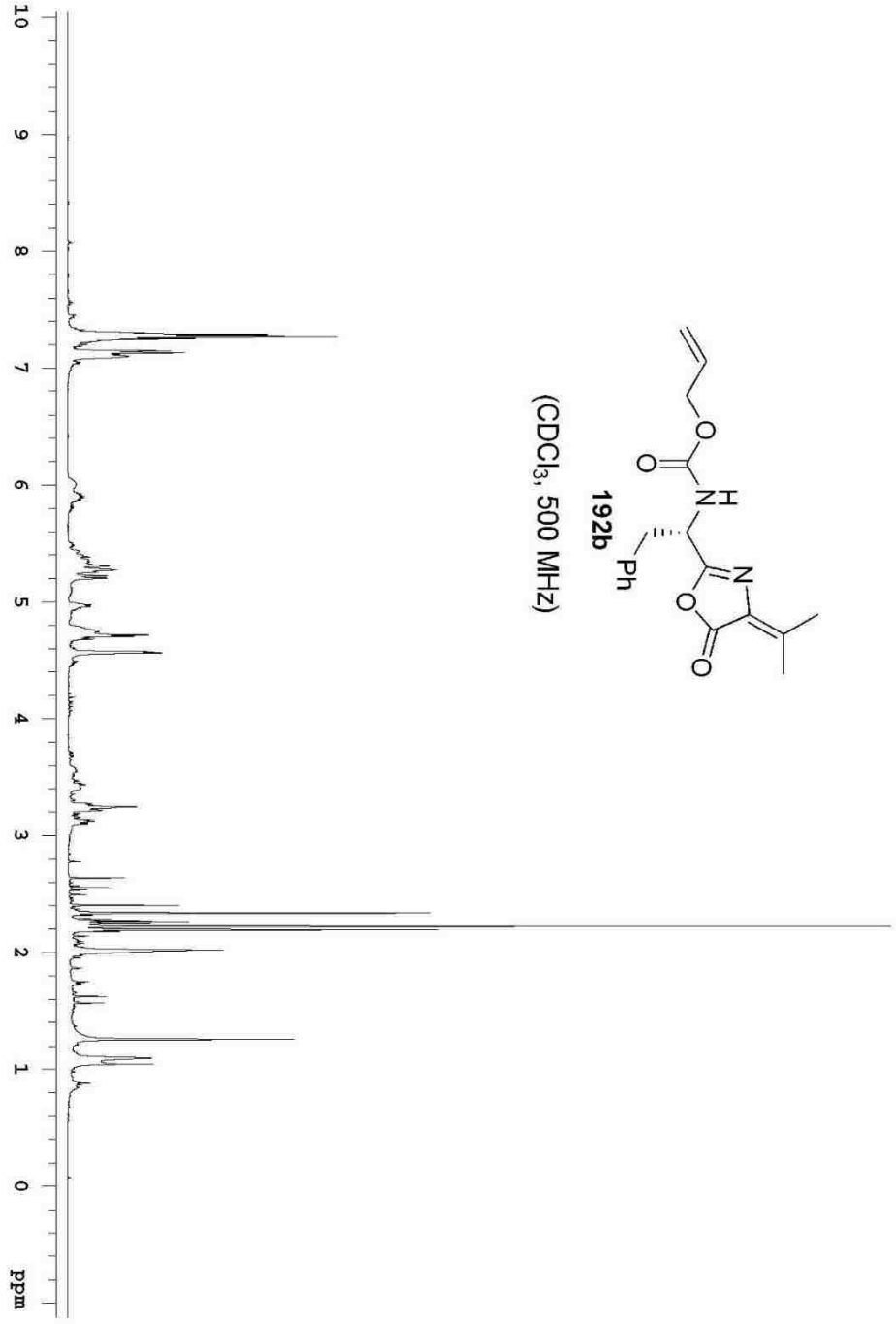
($CDCl_3$, 500 MHz)



135

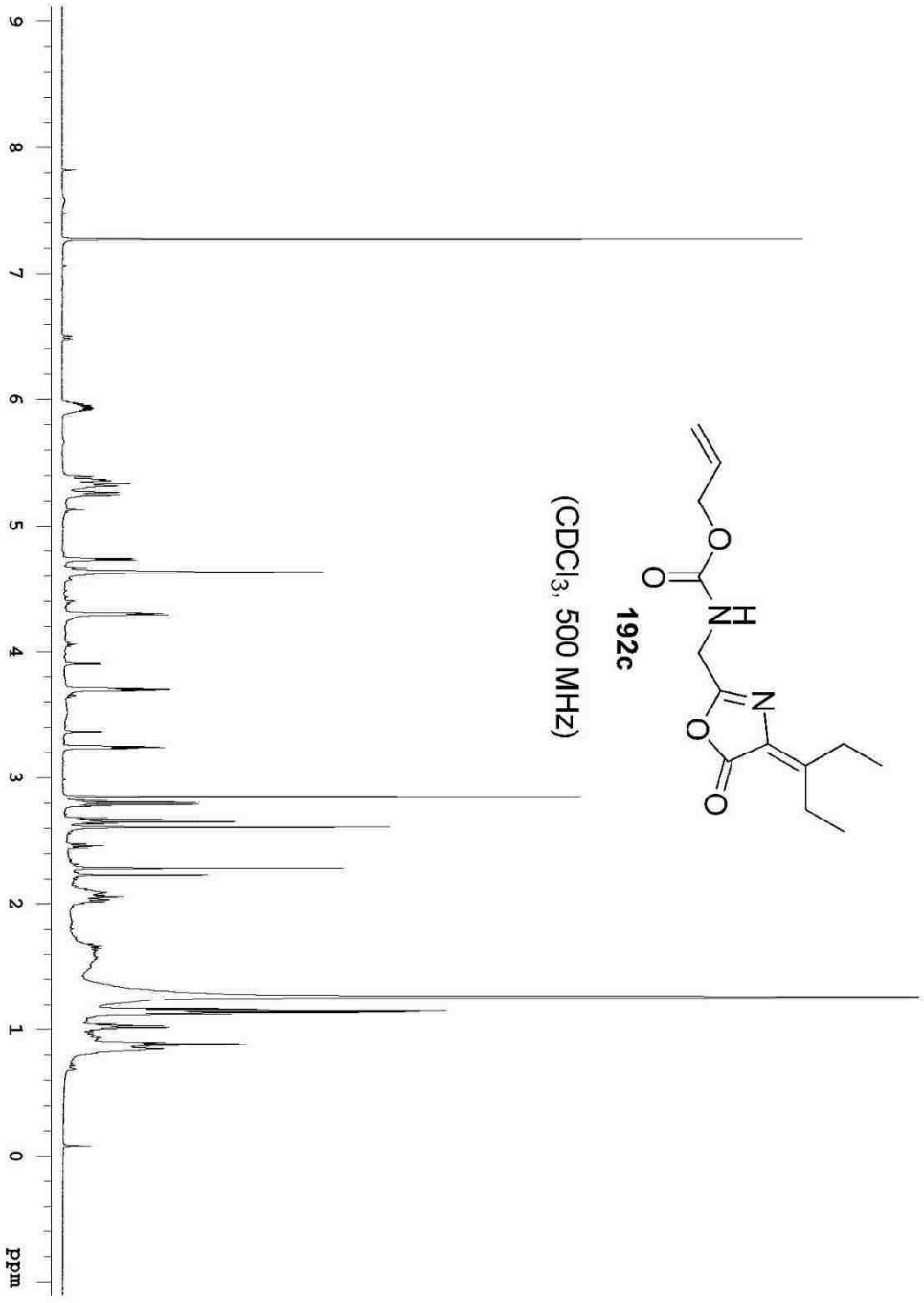
($CDCl_3$, 125 MHz)


192a
(CDCl3, 500 MHz)
ppm



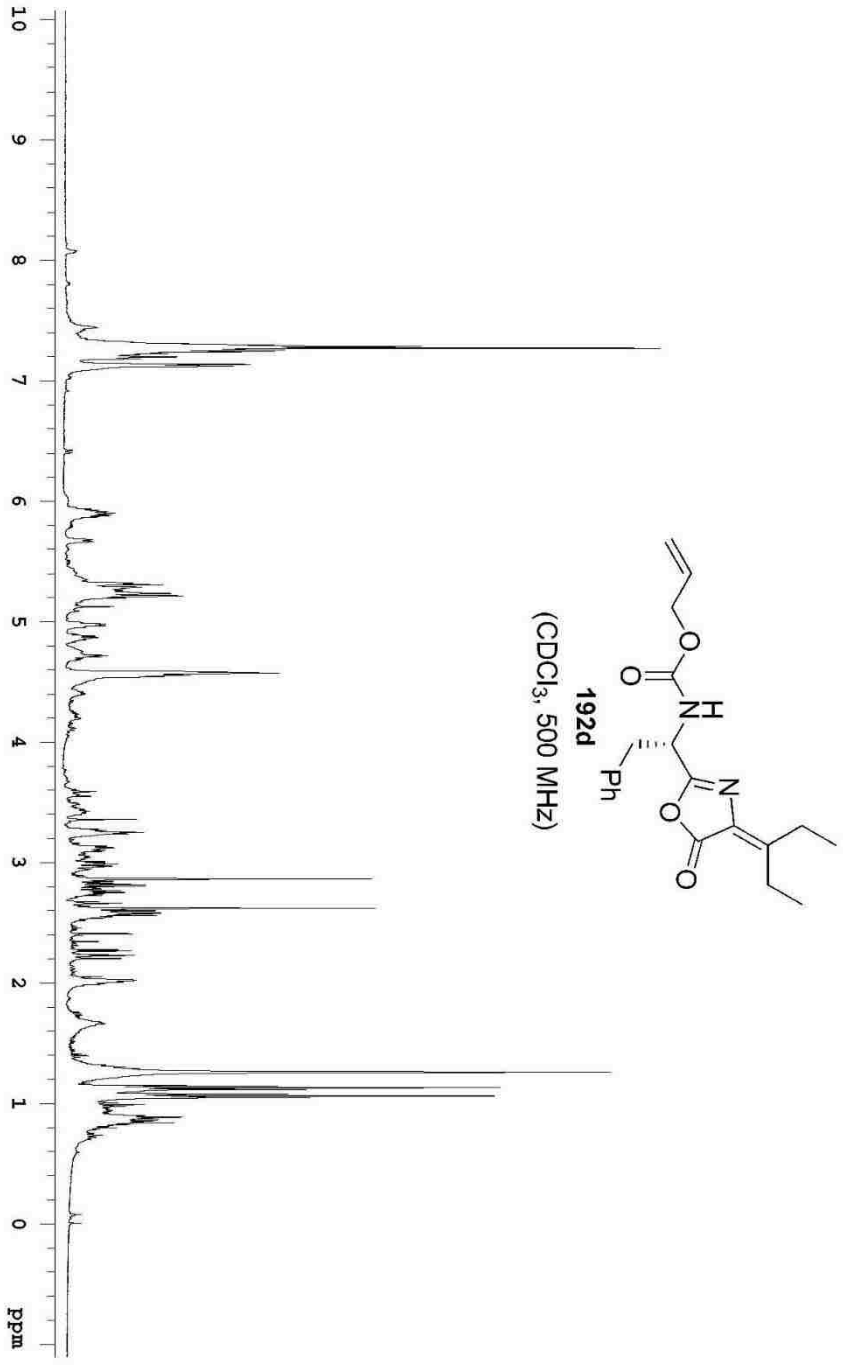
192b

($CDCl_3$, 500 MHz)



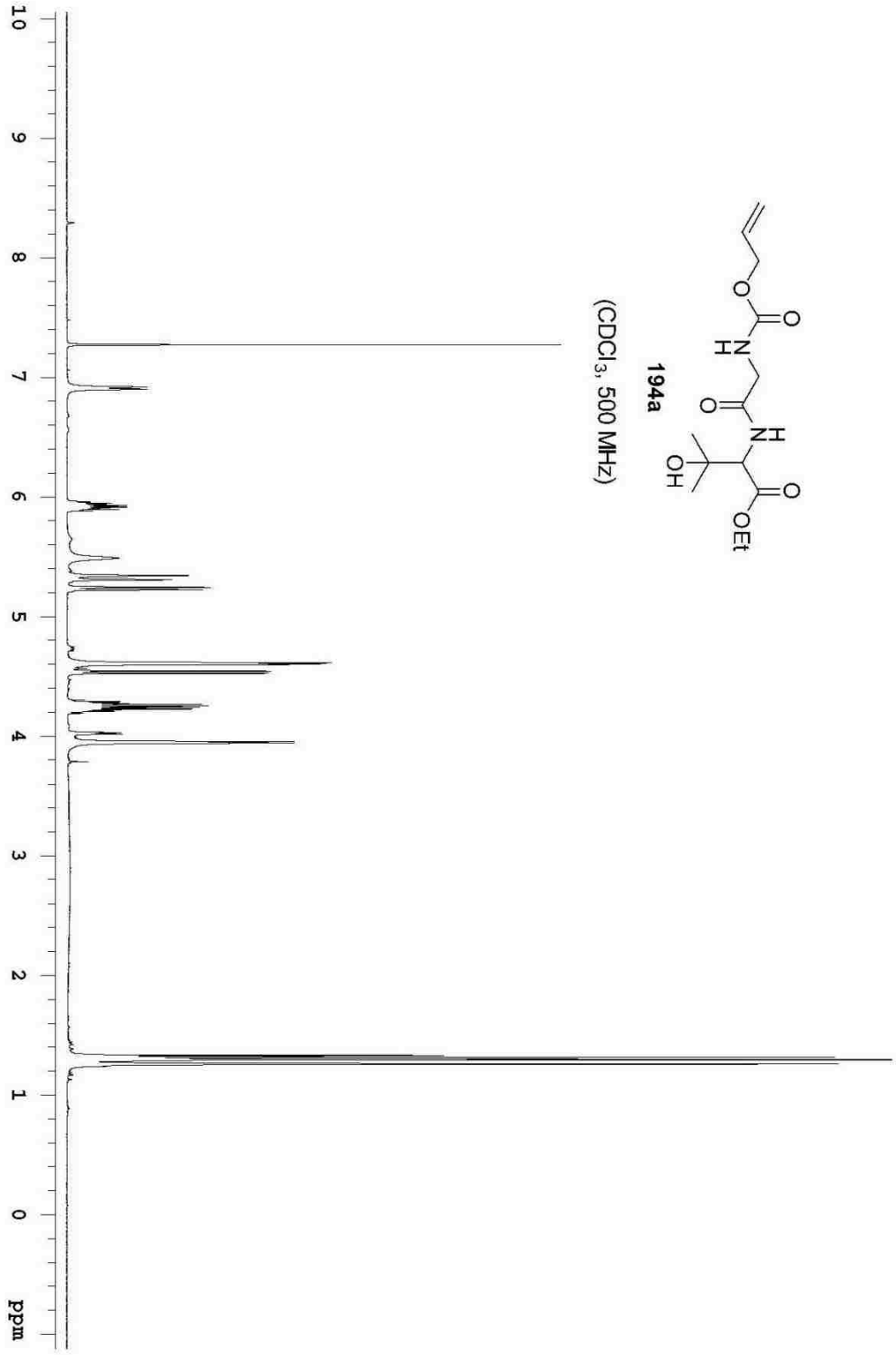
192c

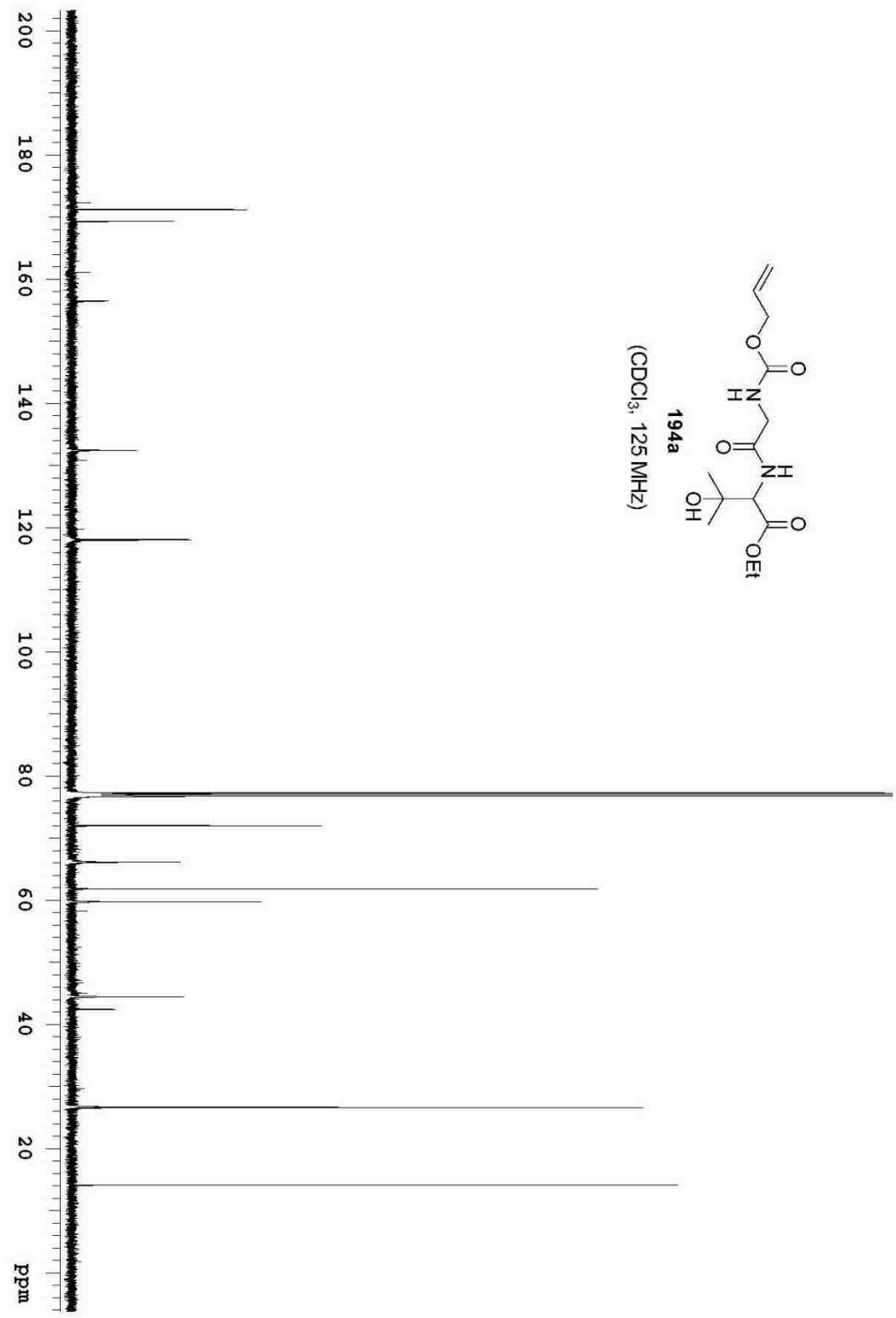
($CDCl_3$, 500 MHz)

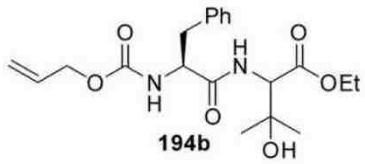


192d

($CDCl_3$, 500 MHz)

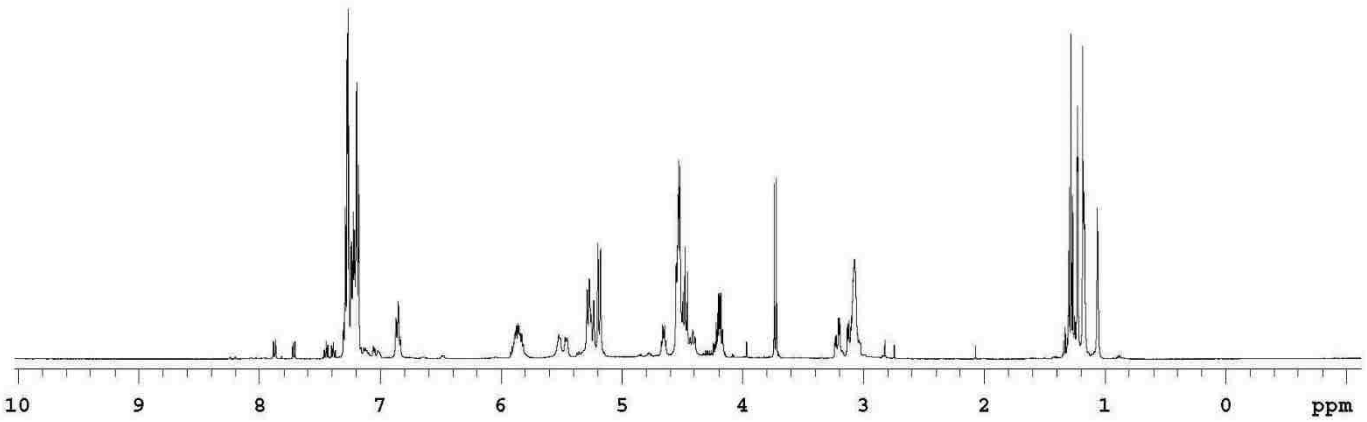

194a
(CDCl3, 500 MHz)
OEt
OH
ppm

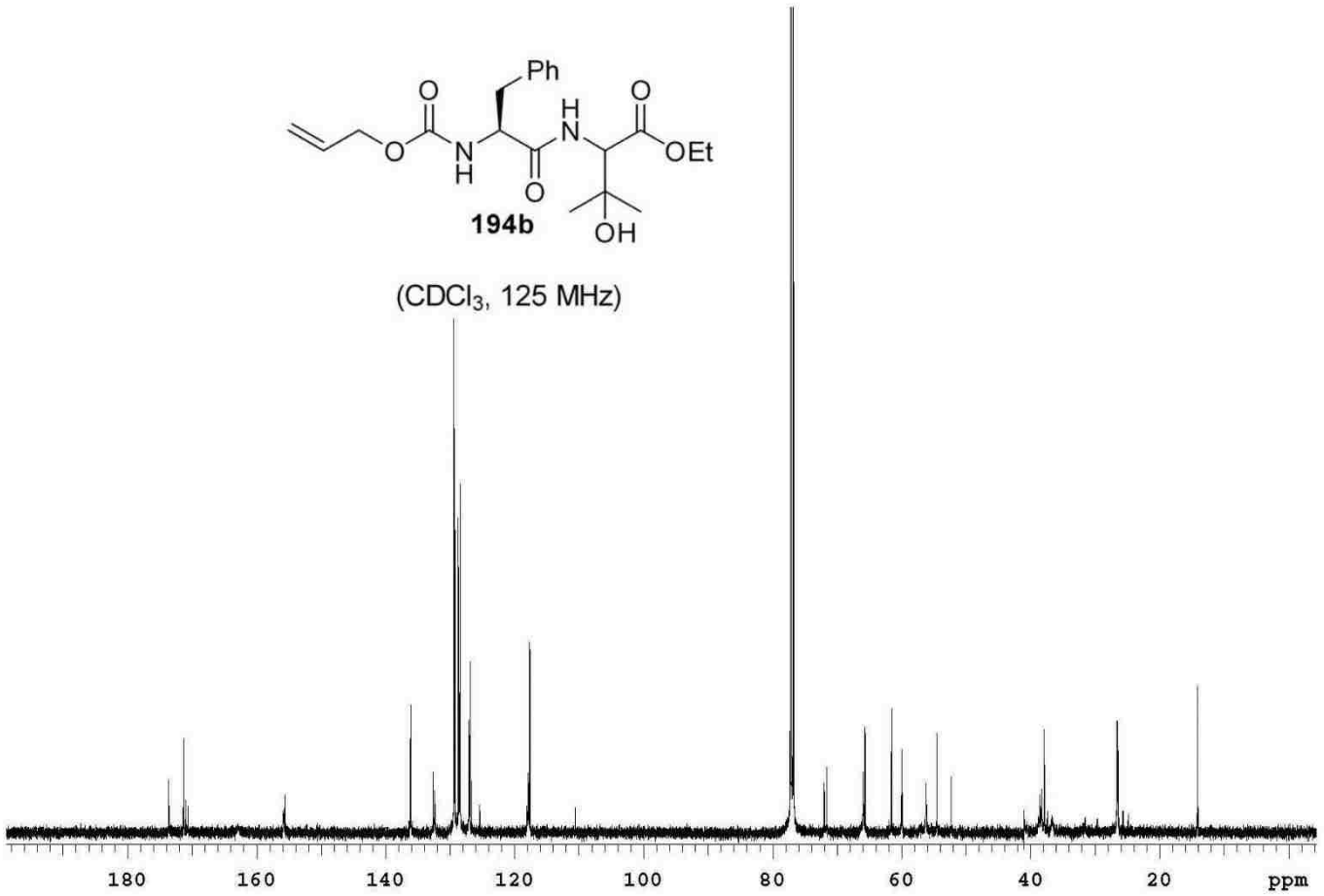

194a
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
ppm

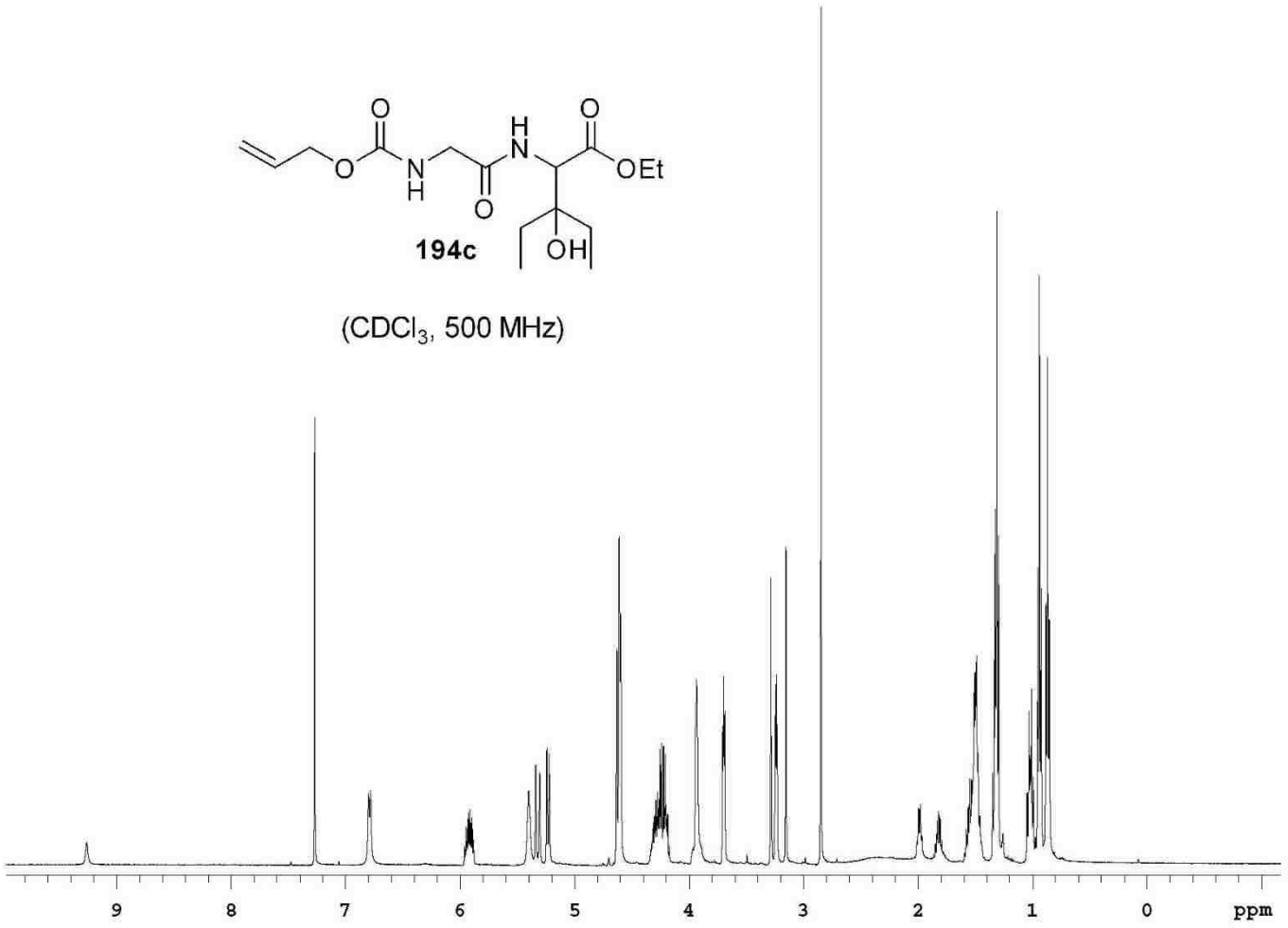


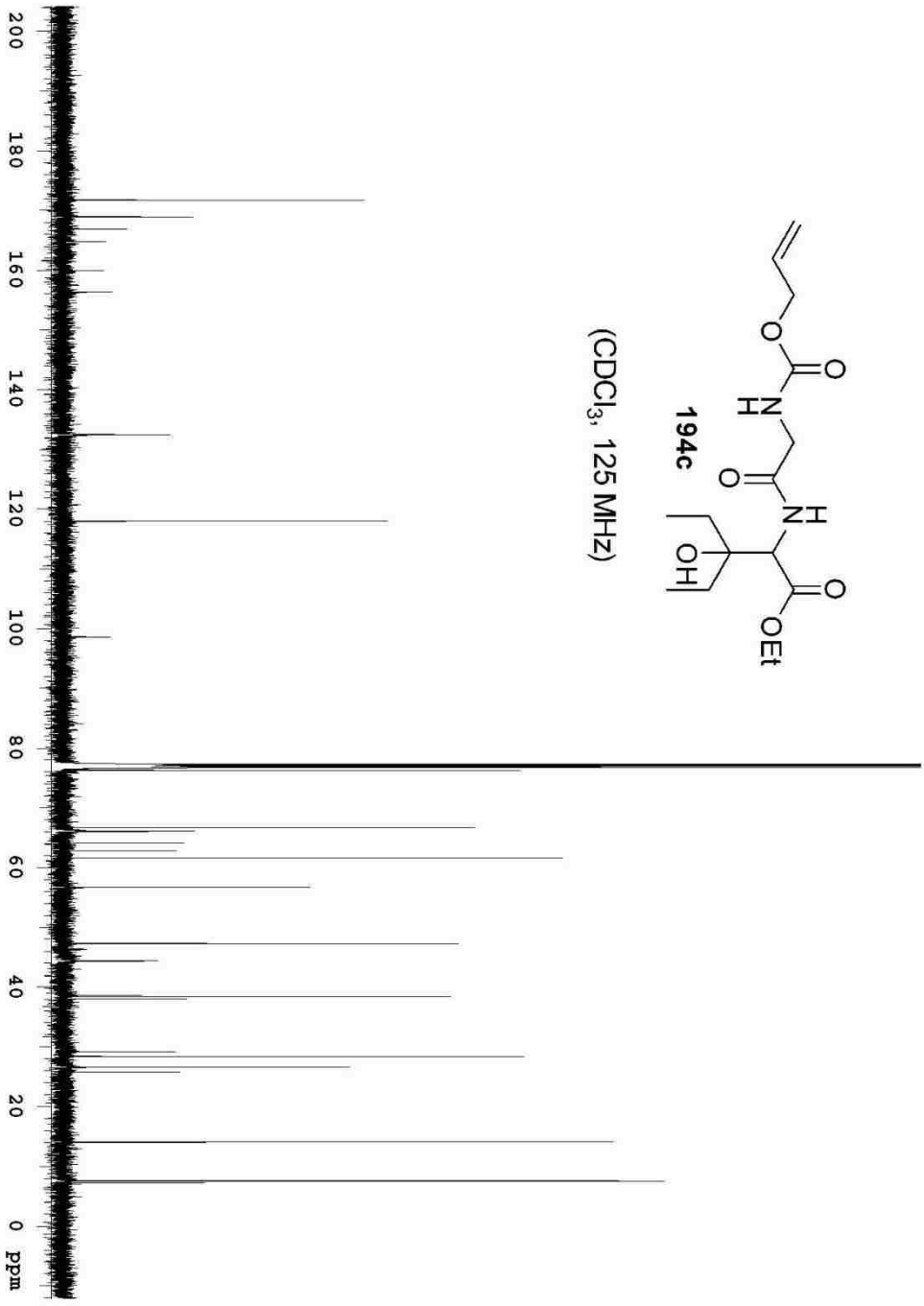
194b

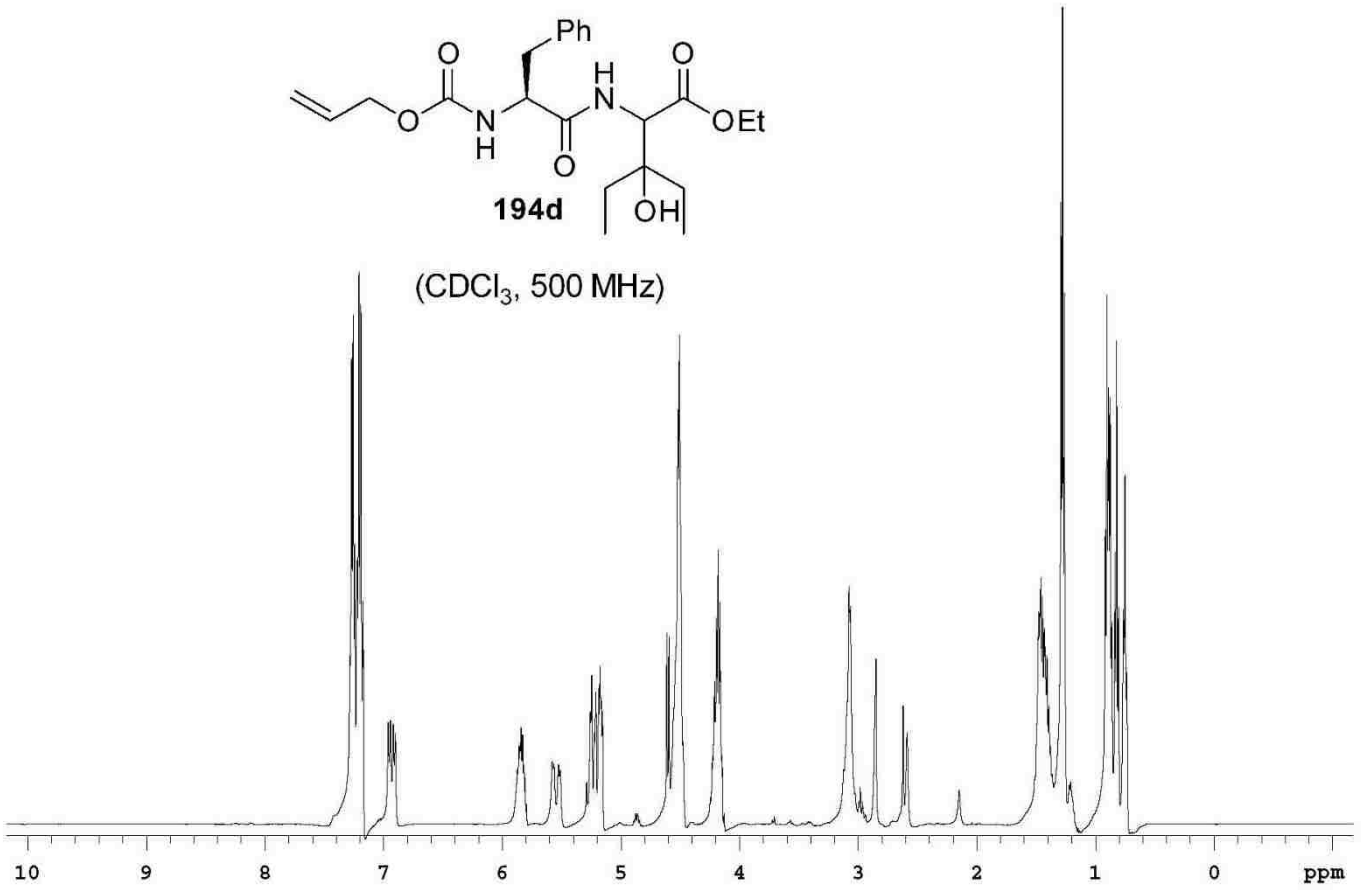
($CDCl_3$, 500 MHz)

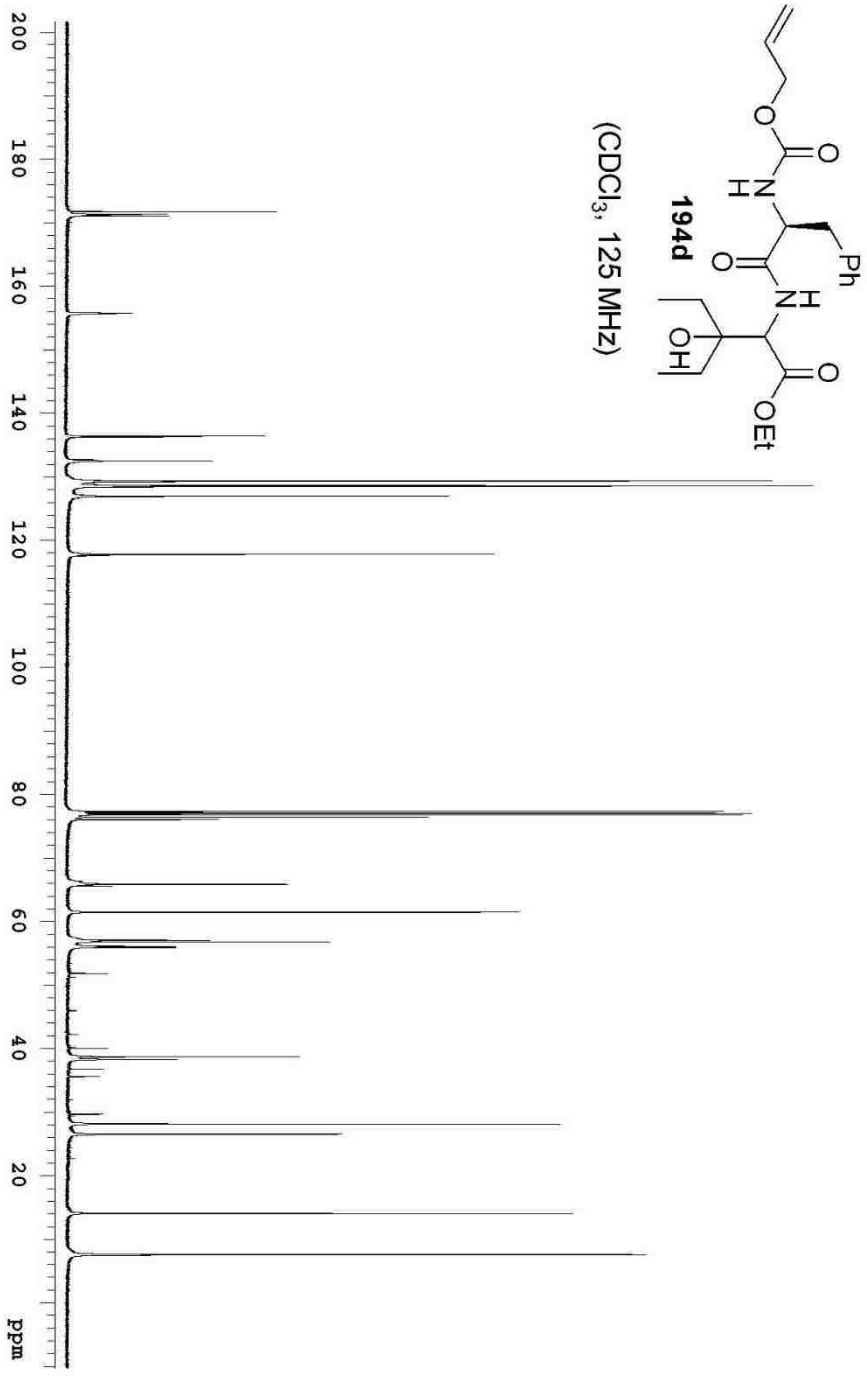


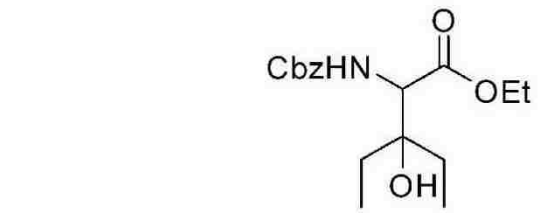

Ph
O
H
N
O
OEt
O
N
H
O
OH
194b
(CDCl3, 125 MHz)
180
160
140
120
100
80
60
40
20
ppm


194c
(CDCl3, 500 MHz)
9
8
7
6
5
4
3
2
1
0
ppm


194c
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
0 ppm

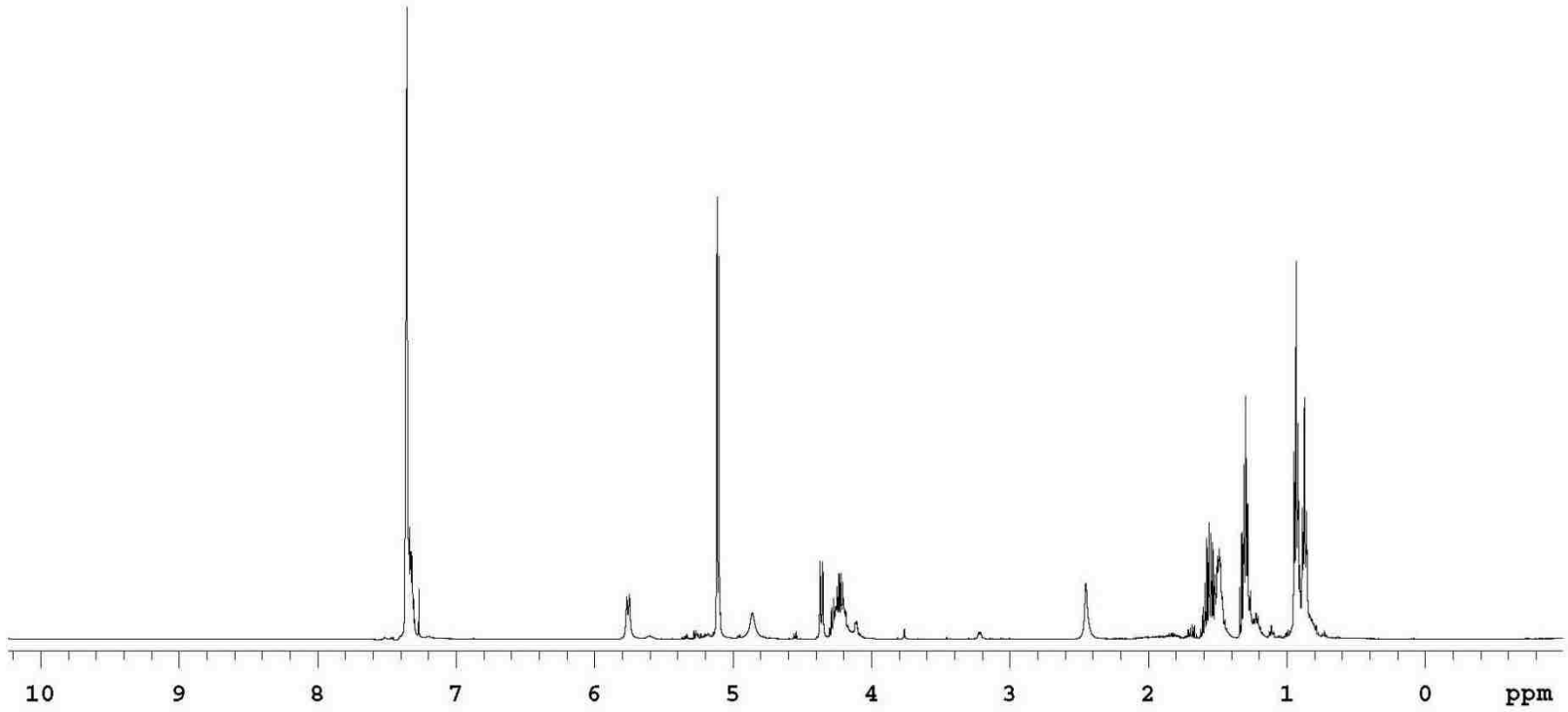

Ph
O
O
H
N
O
OEt
O
N
H
O
OH
194d
(CDCl3, 500 MHz)
10
9
8
7
6
5
4
3
2
1
0
ppm

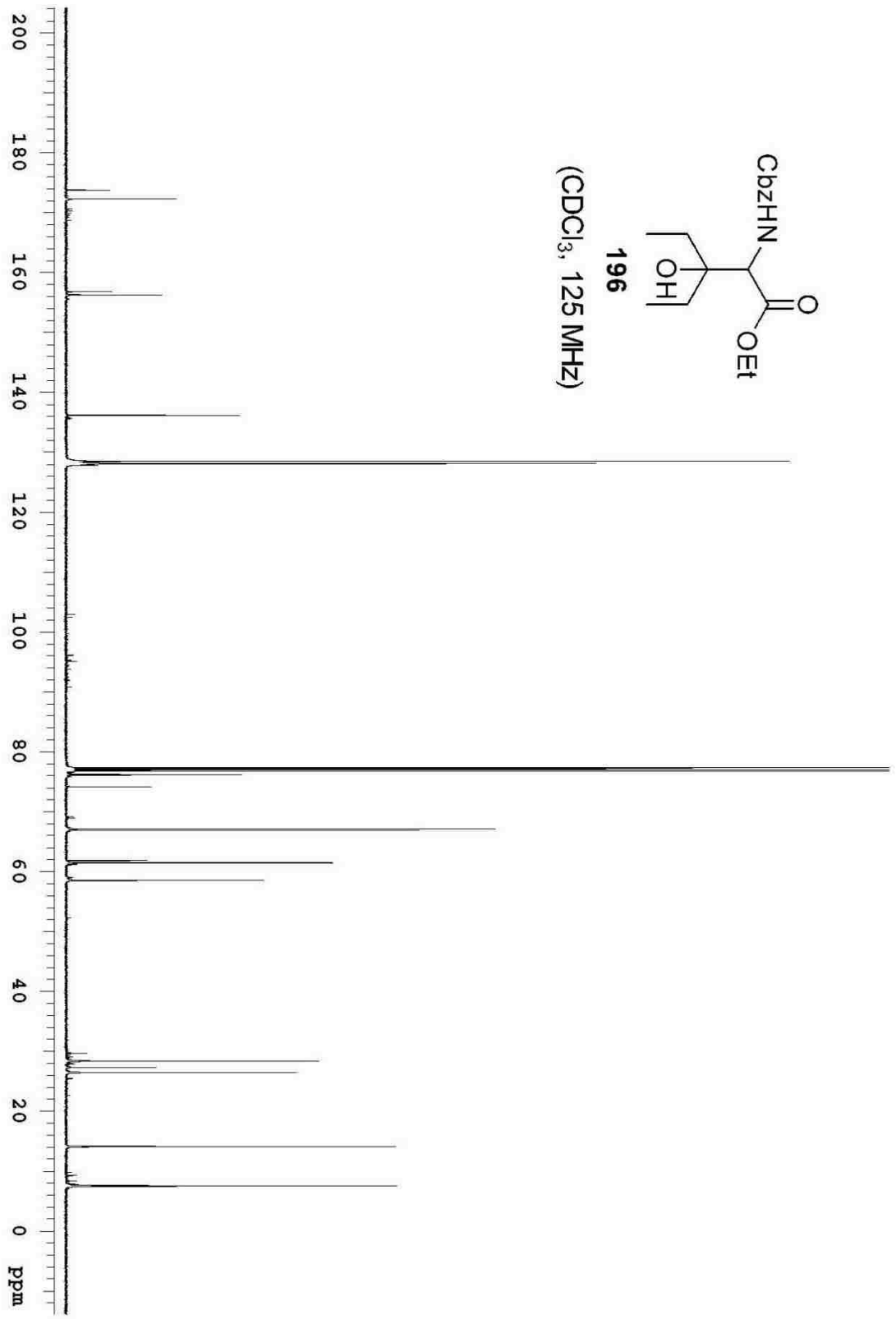

194d
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
ppm

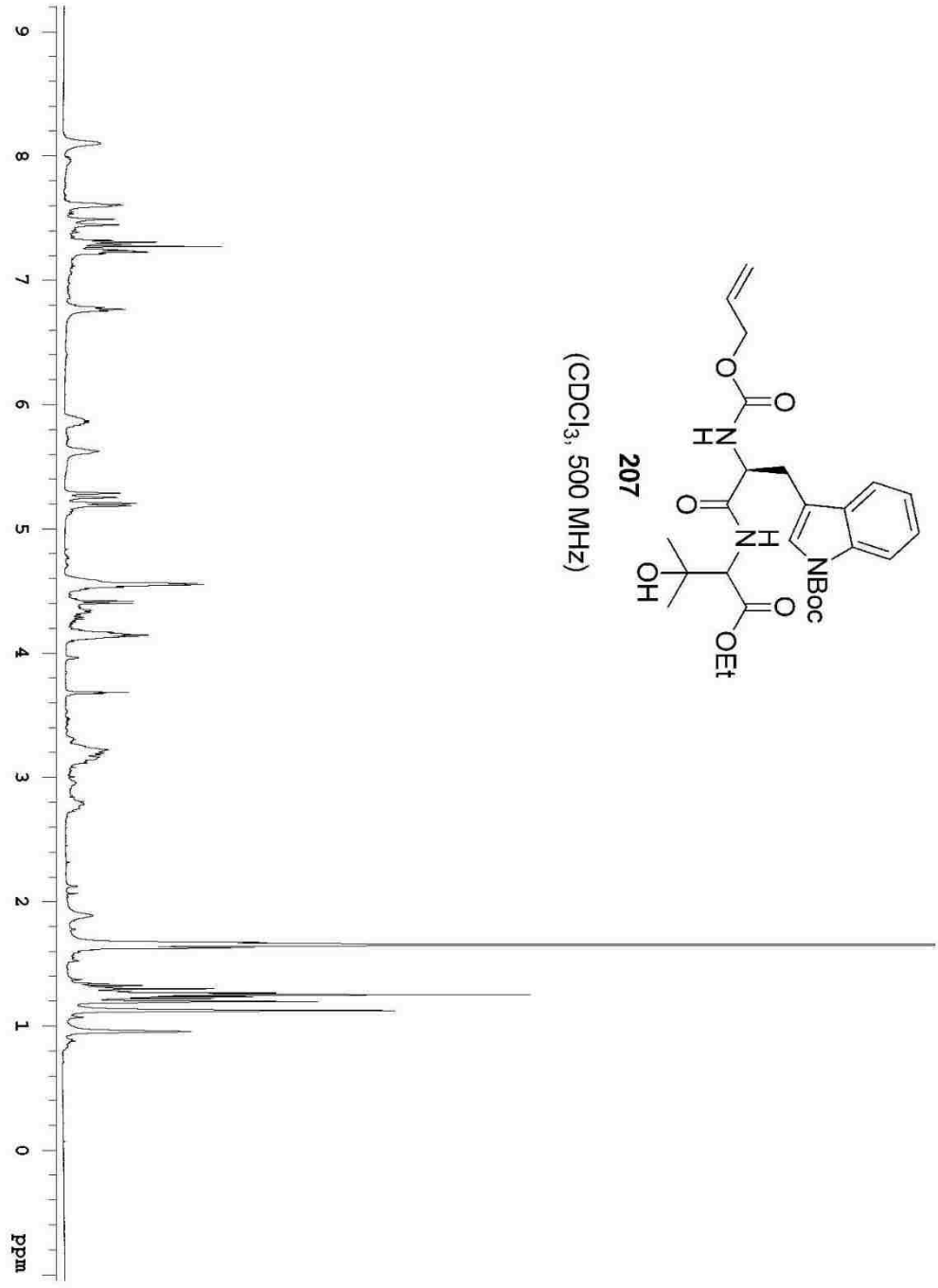


196

($CDCl_3$, 500 MHz)

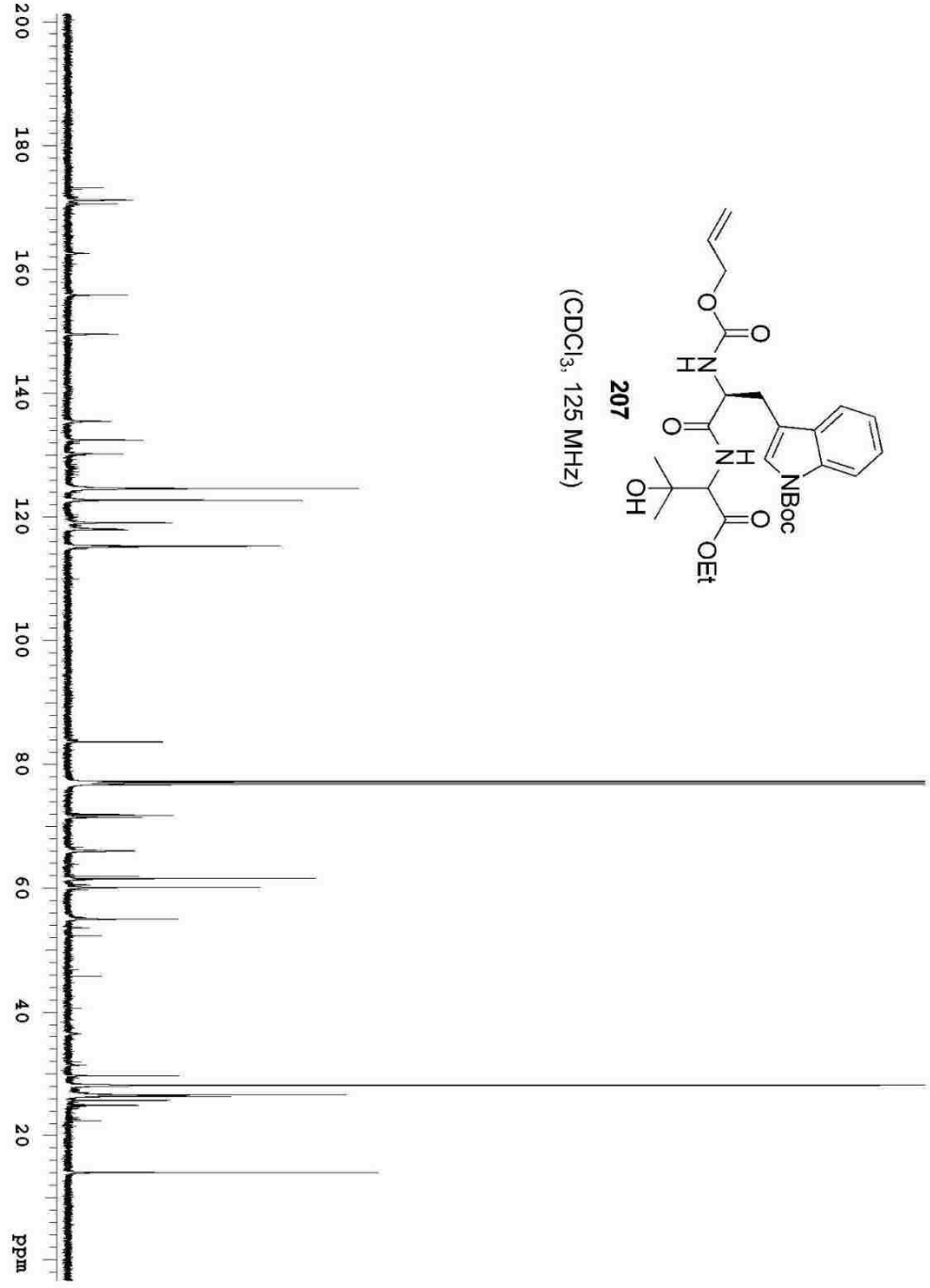



CbzHN
O
OEt
OH
196
(CDCl3, 125 MHz)
200
180
160
140
120
100
80
60
40
20
0
ppm



207

($CDCl_3$, 500 MHz)



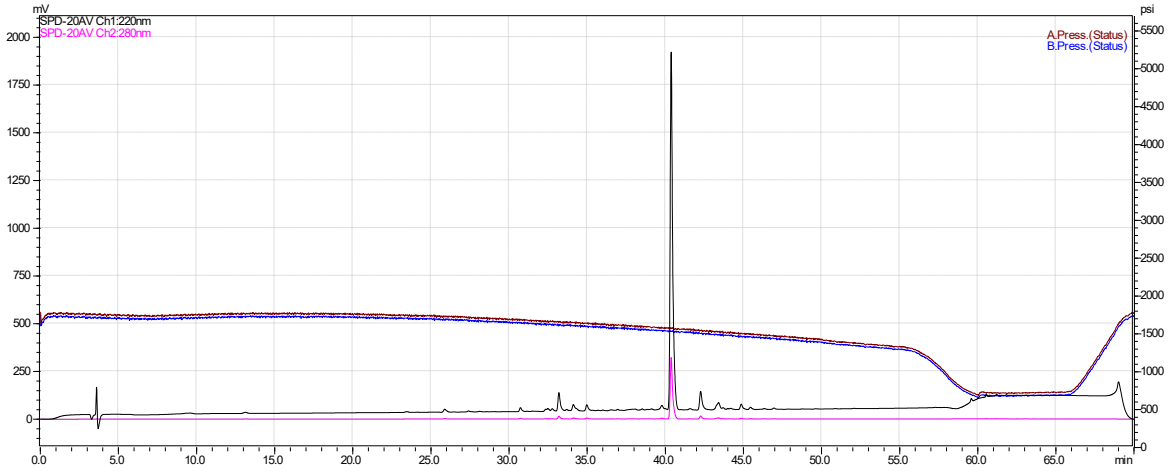
207

($CDCl_3$, 125 MHz)

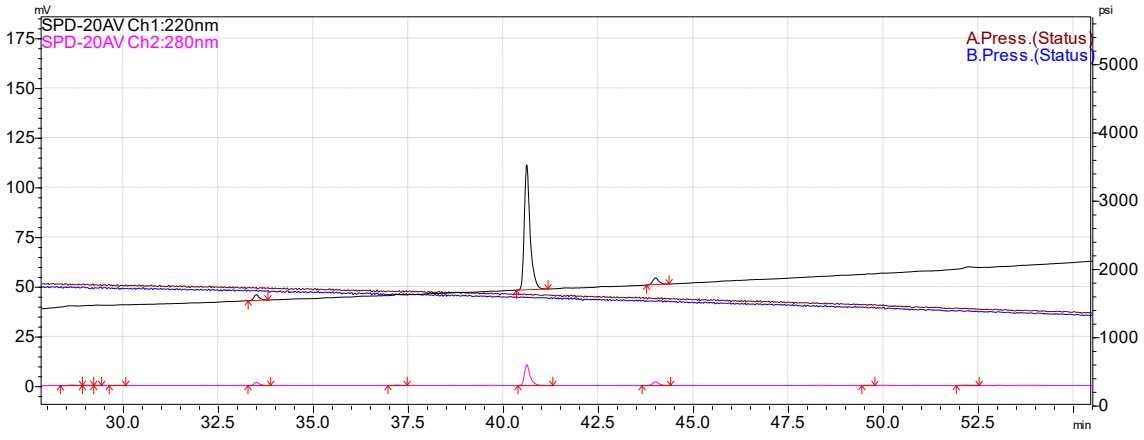
6.5 HRMS Data and HPLC Traces of Peptides 201a–d

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 × 4.6 mm, 5µm), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

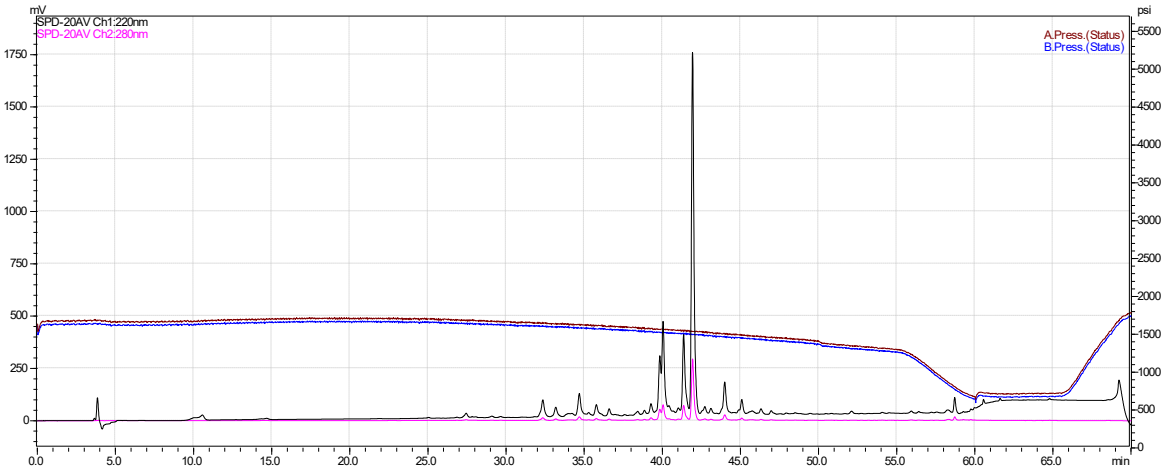


Purified **201a** (Discovery C18 Column (25 cm × 4.6 mm, 5µm), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

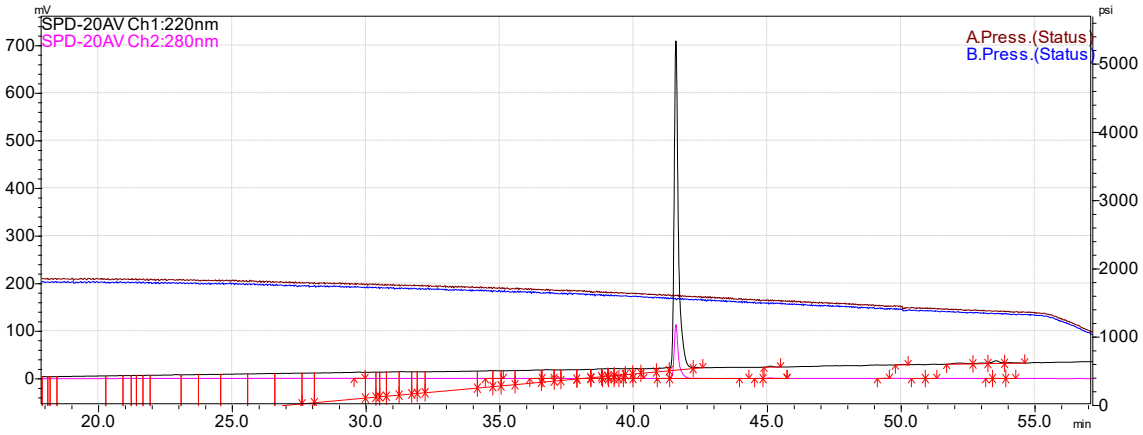


201b: HRMS (ESI) *m/z* 1416.6556 (MH^+, $C_{81}H_{85}N_{13}O_{11}H^+$ requires 1416.6570)

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

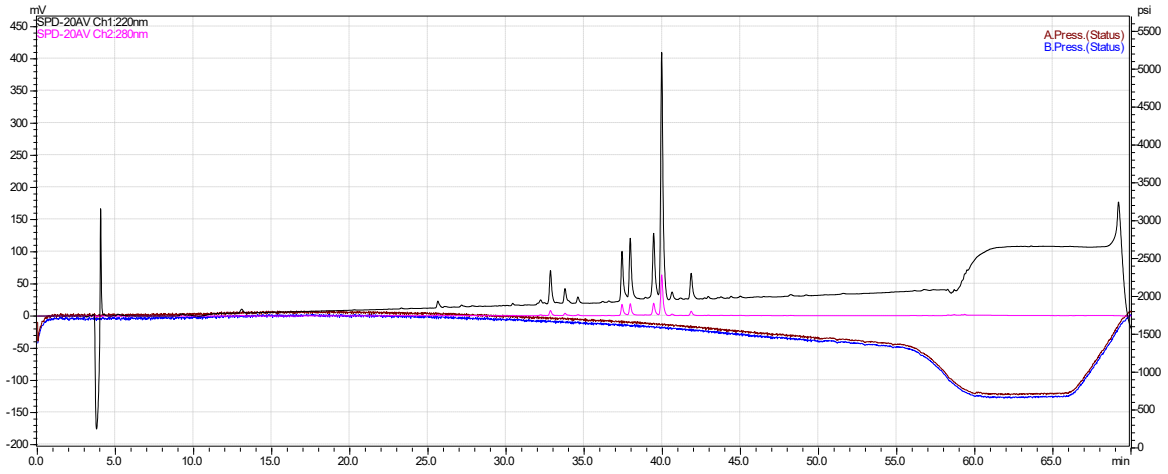


Purified **201b** (Discovery C18 Column (25 cm × 4.6 mm, 5µm), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)

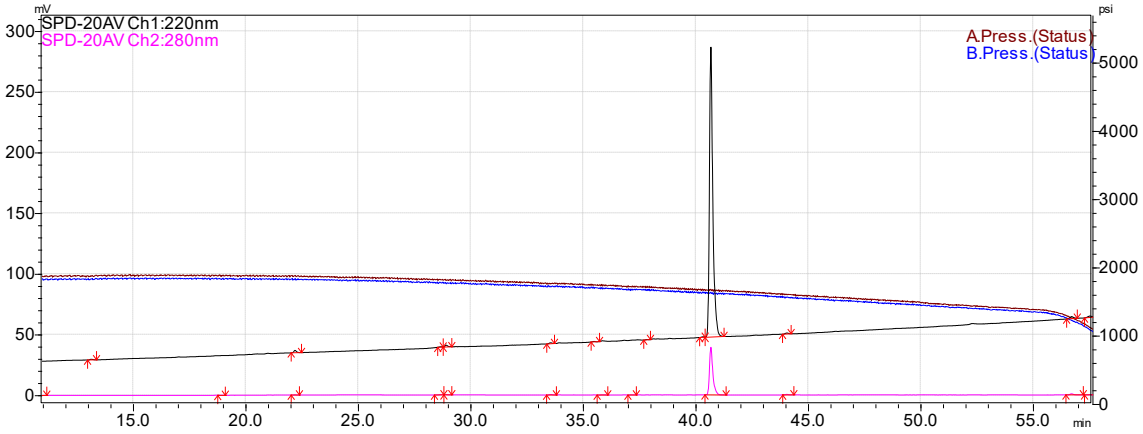


201c: HRMS (ESI) *m/z* 1354.6430 (MH^+, $C_{76}H_{83}N_{13}O_{11}H^+$ requires 1354.6413)

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

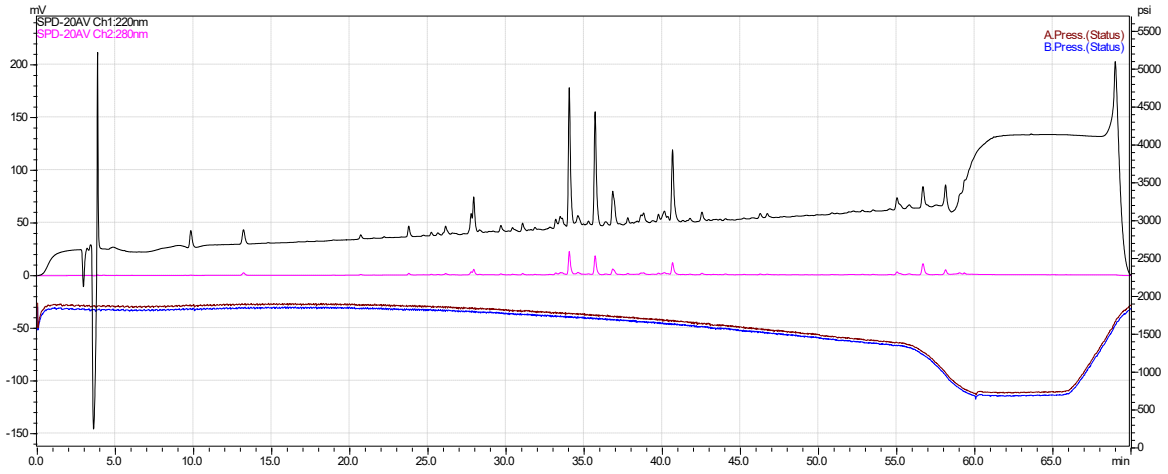


Purified **201c** (Discovery C18 Column (25 cm × 4.6 mm, 5µm), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)



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 × 4.6 mm, 5µm), 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 × 4.6 mm, 5µm), 10–60% CH_3CN (0.1% TFA) in H_2O (0.1% TFA) gradient over 50 min, 1 mL/min)