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By Lindsey Gayle Fischer

Entitled Solid-Phase Synthesis of N-Carboxyalkyl Unnatural Amino Acids

For the degree of Master of Science

Is approved by the final examining committee:

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SOLID-PHASE SYNTHESIS OF N-CARBOXYALKYL UNNATURAL AMINO ACIDS

A Thesis

Submitted to the Faculty

of

Purdue University

by

Lindsey Gayle Fischer

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

August 2010

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Indianapolis, Indiana

To my beloved grandmother, Phyllis Leidolf, who passed away on August 10, 2009 from congestive heart failure. I will see you again one day.

ACKNOWLEDGMENTS

I would like to acknowledge with thanks the assistance and encouragement from my two mentors, Dr. Martin J. O'Donnell and Dr. William L. Scott. Thank you to my group members Geno Samaritoni, Ziniu Zhou and Jim McCarthy for their advice and input into my project. The assistance from Dr. Karl Dria with the instrumentation and Dr. Robert Minto with NMR interpretations is greatly appreciated. Thank you to Dr. Michael VanNieuwehnze at Indiana University for assistance with optical rotations and to all of my previous co-workers at Dow AgroSciences and Eli Lilly for their instruction in making me the research chemist I am today. Thank you to my family and friends for their love and support. Lastly, a grateful thank you to the Lord who has brought me to places in life I never dreamed imaginable.

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LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AA	Amino acid
Phe	Phenylalanine
Ala	Alanine
Pro	Proline
Lys	Lysine
NEP	Neutral endopeptidase
ECPPA	*N*-[1-(*S*)-Ethoxycarbonyl-3-phenylpropyl)-(*S*)-Alanine
Cbz	Carboxybenzyl
HPLC	High-performance liquid chromatography
rt	Room temperature
HMPA	Hexamethylphosphoramide
DMSO	Dimethylsulfoxide
DCC	Dicyclohexylcarbodiimide
THF	Tetrahydrofuran
DMF	Dimethylformamide
TFA	Trifluoroacetic acid
DPPA	Diphenyl phosphoryl azide
NMM	*N*-Methylmorpholine
TxS	Thromboxane synthase

DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
MMP	Matrix metalloproteinase
BCA	Bifunctional chelating agent
Equiv	Equivalents
NMDA	*N*-Methyl-D-aspartate
DHPP	Dihydroxyphenylpyruvate
HATU	1-Hydroxy-7-aza-benzotriazole
DIEA	*N*,*N*-Diisopropylethylamine
NMR	Nuclear magnetic resonance
DIC	Diisoproylcarbodiimide
HOBt	1-Hydroxybenzotriazole
DMAP	4-Dimethylamino pyridine
BAL	Backbone amide linker
NMP	1-Methyl-2-pyrrolidone
BTPP	*tert*-Butylimino-tri(pyrrolidino)phosphorane
Fmoc	Fluorenylmethoxycarbonyl
LC/MS	Liquid chromatography-mass spectrometry
BIG	Benzophenone imine of glycine on wang resin
HRMS	High resolution mass spectrometry
Pip	Piperidine
TBAI	Tetrabutylammonium iodide
Pyr	Pyridine
dr	Diastereomeric ratio

ABSTRACT

Fischer, Lindsey Gayle M.S., Purdue University, August 2010, Solid-Phase Synthesis of N-Carboxyalkyl Unnatural Amino Acids. Major Professor: Martin J. O'Donnell, Ph.D.

A novel route has been developed for the solid-phase synthesis of N-carboxyalkyl unnatural amino acids as potential metalloprotease inhibitors. The key step involves a nitrogen alkylation of resin-bound amino acids with α-bromoesters. Alkylation of the benzophenone imine of glycine on Wang resin was used to introduce unnatural amino acid side chains onto the resin-bound glycine. The benzyl α-bromoesters [$BrCH(R^2)CO_2Bn$], starting materials for the C-N bond construction, were prepared in solution by diazotization of naturally-occurring amino acids to form the α-bromoacids, followed by benzylation of the carboxylic acid to form the benzyl α-bromoesters. N-Alkylation of the resin-bound, unnatural amino acids with the benzyl α-bromoesters and subsequent cleavage from resin gave the benzyl ester monoacid intermediates. Exploration of reverse-phase cyano-silica gel chromatography and preparative liquid chromatography provided effective purification of the benzyl ester intermediates. Hydrolysis of the analytically pure benzyl ester monoacids afforded clean products as the diacids. The two points of variation introduced through the two on-resin alkylation steps, C-alkylation of the benzophenone imine of glycine and N-alkylation with the benzyl α-bromoesters, allow for the combinatorial synthesis of a library of target compounds.

CHAPTER 1. BACKGROUND

1.1. Introduction

N-Carboxyalkyl dipeptides have been shown to be active metalloprotease inhibitors of the angiotensin-converting enzyme (ACE).[1] Examples include commercially-available drugs for hypertension and heart disease, such as enalapril **1** and lisinopril **2** (Figure 1). Both of these drugs are tripeptidomimetics that consist of a dipeptide where the N-terminus has been alkylated by a carboxyalkyl substituent and contain a proline residue at the C-terminus. Enalapril is a prodrug of the active dicarboxylic acid enalaprilat in which the ethyl ester is enzymatically cleaved to a carboxylic acid in the body. Enalapril contains an alanine residue in the middle position, whereas lisinopril contains a lysine residue. Since these drugs have been effective in treating hypertension and heart disease, peptidomimetic chemistry has been used to synthesize potential analogs for biological screening. However, the isolation of these analogs has been challenging due to the polarity of the amine and carboxylic acid functionalities.

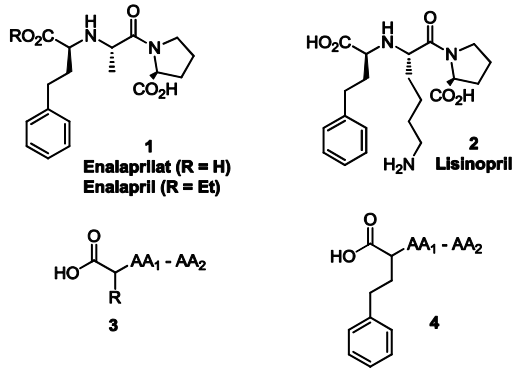
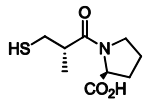


Figure 1. Commercially Available ACE inhibitors and Generic Structure of Drug Analogs.

The generic structure **3** as N-carboxyalkyl dipeptides is consistent in many ACE inhibitor analogs reported in the literature, especially incorporating the phenethyl side chain as R (**4**, Figure 1).[1-4] This hydrophobic side chain is believed to have a strong interaction with the S_1 hydrophobic pocket (Figure 2) at the active site of ACE.[1]

Structure-activity relationships have been explored by several research groups by varying the three residue portions of enalaprilat.[1-2,5-7] Since enalaprilat contains the phenethyl side chain followed by alanine (AA_1) and proline (AA_2), many analogs have been prepared by keeping one of the portions of **3** (the side chain R, the AA_1, or the AA_2) constant and varying other portions of this generic structure. A complete review by Wyvratt and Patchett of the drug discovery of ACE inhibitors is available.[1]

The first potent ACE inhibitor captopril (**5**) was made by Cushman and Ondetti in the early 1970s.[5,7] Its development showed the application of intelligent drug design by devising a molecular structure to specifically bind in the enzymatic pocket of ACE (Figure 2). It was known that ACE was a carboxydipeptidase enzyme containing a Zn^{2+} ion. These researchers hypothesized that the active site of ACE may be similar to other carboxypeptidase enzymes such as bovine pancreatic carboxypeptidase A.



Captopril (5)

By comparison to the bovine pancreatic carboxypeptidase A, it appeared that a positively charged residue at the active site binds with the peptide at the negatively charged C-terminal carboxyl group of the substrate seen below (Figure 2). The Zn^{2+} ion is believed to assist in the peptide bond cleavage and is separated from the positively charged residue in the active site by two amino acids, whereas the Zn^{2+} ion in bovine carboxypeptidase A is separated by only a single amino acid residue. Due to an additional amino acid separating the Zn^{2+} ion and the positively charged residue of the active site, Cushman and Ondetti assumed the carbonyl group of the central amino acid residue interacted with the enzyme through hydrogen bonding. The rigid structure of proline seems to impact the strong binding in the deeper portion of the active site pocket.

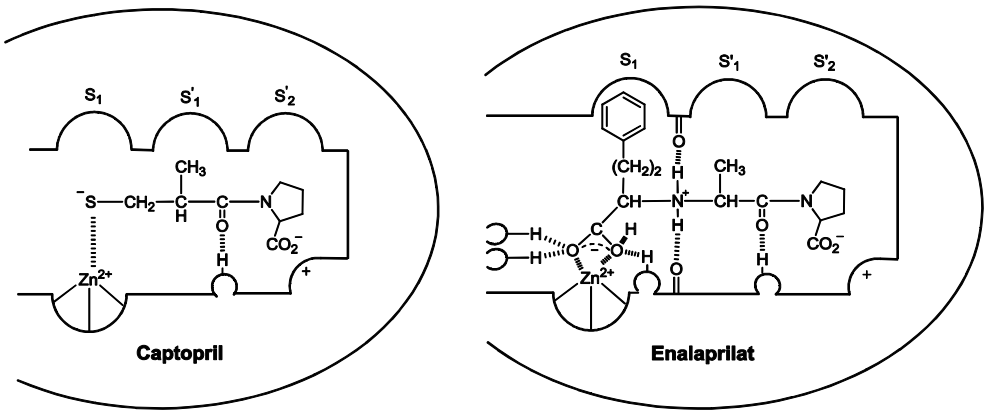


Figure 2. The Binding Interactions of Captopril and Enalaprilat within the ACE Active Site.[1]

Based on this structural model for the active site of ACE, potential drug targets have been designed and tested by various groups. Cushman and Ondetti tested carboxyalkanoyl (**6**) and mercaptoalkanoyl amino acids (**7**).[5] Succinyl-(*S*)-proline and glutaryl-(*S*)-proline derivatives were tested, and the methyl-substituted mercaptopropanoyl derivative **8**, later marketed as captopril, exhibited very high levels of activity against ACE. Since sulfur binds more strongly to zinc than oxygen, these results implied that the mercapto functionality binds to the Zn^{2+} ion since the mercaptoalkanoyl analogs were more potent than the carboxyalkanoyl analogs. Cushman and Ondetti also showed that the (*S*)-proline analogs were more active than the (*R*)-proline analogs. This suggested that the interaction between the carboxyl group of the C-terminus of the (*S*)-amino acid with the positively charged residue of the active site required a very specific three-dimensional interaction.

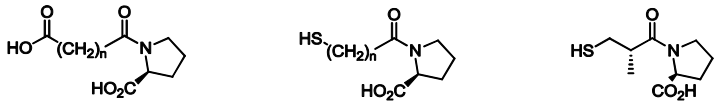


Figure 3. Carboxyalkanoyl and Mercaptoalkanoyl Proline Analogs.

Following Cushman and Ondetti's work, researchers at Merck Sharp and Dohme Research Laboratories and the Merck Institute for Therapeutic Research focused their efforts on synthesizing N-carboxymethyl dipeptide derivatives.[2] The original extract from the South American pit viper *Bothrops jararaca* that is responsible for ACE inhibitory

activity was known to contain a peptide with the C-terminus sequence of Phe-Ala-Pro. From these results the Merck group first analyzed potential targets by keeping the Ala-Pro residues of the dipeptide portion constant. They varied the substituent on the N-carboxyalkyl group directly attached to the N-terminus of the alanine and established that the phenethyl substituent was the most active side chain. Later, numerous efforts by other groups exploited this finding by incorporating the phenethyl side chain into their analogs.[8-12] Two commercially available drugs, enalapril (**1**) and lisinopril (**2**), were developed based on this finding. Next, the Merck group kept the phenethyl substituted N-carboxyalkyl group constant and varied the two amino acid residues of the dipeptide portion. It was confirmed that the C-terminal residue as proline, thiaproline, and hydroxylprolines were the most potent, again suggesting that the cyclic residue locks the substrate into the active site for highest affinity. The most active amino acids in the middle position proved to be (*S*)-alanine, (*S*)-fluoroalanine, (*S*)-lysine, and (*S*)-arginine. This study showed the first synthesis of the eventual commercial drug lisinopril (**2**) with the phenethyl substituted N-carboxyalkyl group directly attached to the N-terminus of the Lys-Pro dipeptide.

Pasha and associates studied tripeptidomimetics where proline was kept constant at the C-terminus and showed ornithine bonded stronger than lysine in the S_1' pocket of the enzyme site.[6,13] Ornithine is the unnatural amino acid similar to lysine but with one less carbon on the side chain (**9**). The ornithine was acylated on the α-amino group with thiophene or indole heterocylic moieties with varying chain lengths to give analogs **9**. These heterocycles were employed to interact with the Zn^{2+} ion located in the enzyme active site. Biological results showed that the analogs containing three carbons between the carboxylic acid and heterocyclic ring had poor activity because the alkyl chain was too long to fit into the active site pocket. Likewise, analogs containing zero or one carbons between the carboxylic acid and heterocyclic ring were able to fit into the active site of ACE, but they exhibited poor activity because the side chains were too short to bind strongly. On the other hand, analogs containing two carbons between the carboxylic acid and heterocyclic ring showed the highest inhibition, suggesting these substrates contain the appropriate number of carbons to form a tight fit in the active site, with analog **10** being the most active. The two carbon chain length of the substituent is consistent with the results from the Merck laboratories, where the phenethyl substituent of the N-carboxyalkyl group was most active.

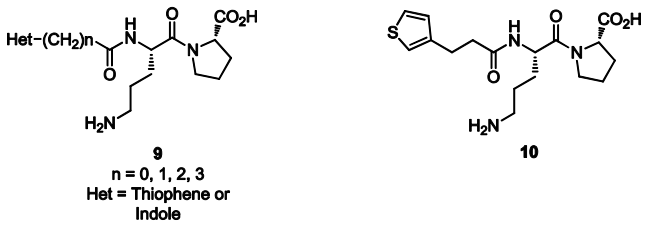


Figure 4. Ornithine-based Tripeptidomimetic Analogs.

Many examples incorporating substituted prolines have appeared in literature (Figure 5). Researchers from Schering-Plough Co. explored 4-substituted proline derivatives of the commercial drug captopril (**11**).[14] Monosubstituted and disubstituted prolines were synthesized and tested for ACE inhibition. Their findings explained the spatial requirements for the binding affinity of substituted prolines in the active site. Cyclic acetals and thioacetals were also tested and gave the best activity. Researchers from the Squibb Institute for Medical Research also explored 4-substituted proline analogs, including analogs of N-carboxyalkyl dipeptides incorporating the phenethyl substituent (**12**).[15] Bhagwat *et al.* substituted the proline of captopril with a thiorphan derivative in **13** to increase dual ACE and NEP activity, which acts as a diuretic and has shown to significantly improve the efficacy of ACE inhibitors.[16] Mencel *et al.* also investigated 4-substituted prolines by incorporating an aryl sulfonamide diuretic moiety into enalaprilat (**14**).[17]

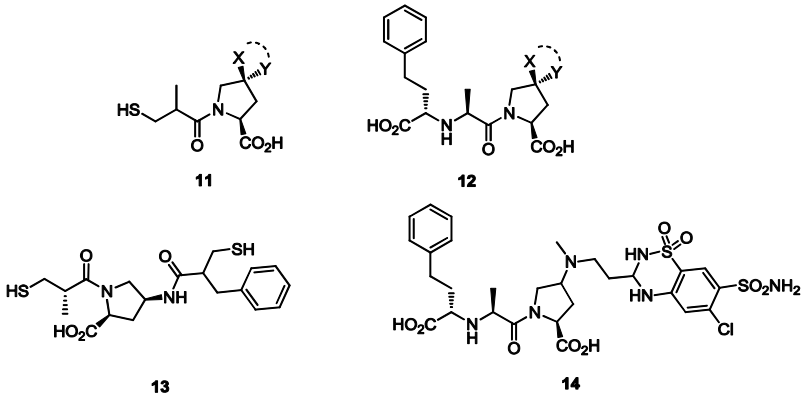


Figure 5. Structures of 4-Substituted Proline Analogs.

Work has been done by substituting fused ring structures for the proline portion of the ACE analogs to fit into the S_2' pocket of the active site.[18-19] Wyvratt and Patchett reported conformationally restricted analogs of captopril and enalaprilat in their review

on ACE inhibitors.[1] The benzo-fused analogs of captopril such as **15** greatly increased the potency, while benzolactams **16** were also shown to be potent inhibitors. Bicyclic lactam and benzolactam analogs of enalaprilat, such as **17** and **18**, also showed high inhibition of ACE.

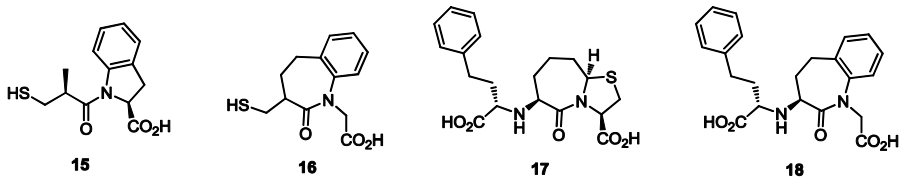


Figure 6. Fused Ring Structures of ACE Inhibitor Analogs.

In summary, various analogs and structure-activity relationship studies of captopril (**5**), enalapril (**1**) and lisinopril (**2**) have given insights into the structure of the enzyme active site. The presence of the phenethyl substituent on the tripeptidomimetics was of particular interest for the research reported in this thesis to make N-carboxyalkyl amino acids. The introduction of this alkyl substituent has been synthetically derived in various manners, as discussed below.

1.2. Examples of Solution-Phase Synthesis of N-Carboxyalkyl Dipeptides and Amino Acids in the Chemical Literature: Introducing the N-Carboxyalkyl Group onto Nitrogen

When surveying the synthetic strategies for preparing the N-carboxyalkyl dipeptides, the formation of the carbon-nitrogen bond on the amino acid has been the focal point for synthesis. Typically, the two types of reactions commonly used are the N-alkylation with α-haloesters and reductive amination with α-ketoesters (**19** to **20**, Figure 7). The esters **20** could then be hydrolyzed or hydrogenolyzed to give the diacid products **21**.

A comparison of the diastereoselectivity between these two synthetic routes shows no diastereoselectivity when employing reductive amination methods. When using an optically active α-haloester, however, various diastereoselectivity results have been reported based on the reaction conditions. These results are discussed below.

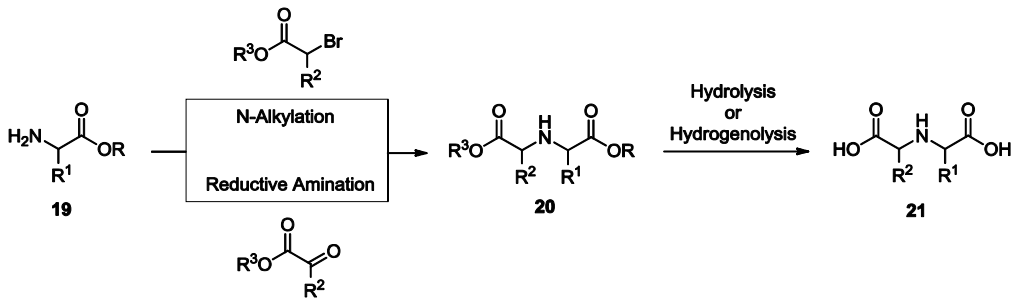
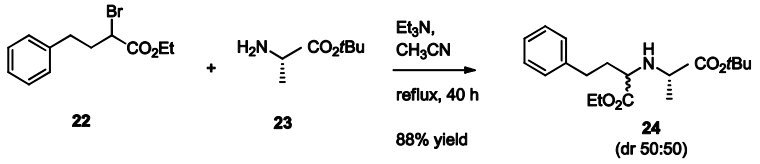


Figure 7. Synthetic Methods for Preparing N-Carboxyalkyl Amino Acids through the Use of α-Haloesters or α-Ketoesters.

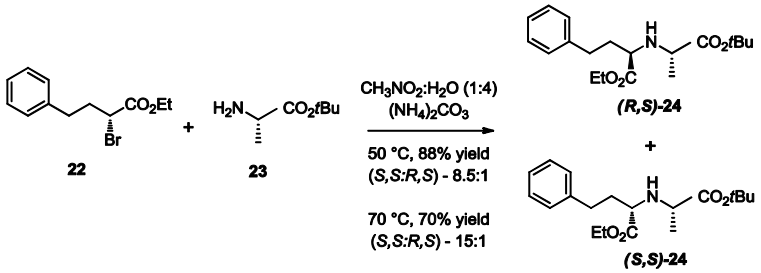
1.2.1. Solution-Phase Synthesis of ACE Inhibitor Analogs by N-Alkylation with α-Halocarbonyl Compounds

Kaltenbronn *et al.* reported the synthesis of the major intermediate **24** by N-alkylation of the *t*-butyl ester of (*S*)-alanine (**23**) with ethyl 2-bromo-4-phenylbutanoate (**22**).[20] The reaction was refluxed in acetonitrile in the presence of triethylamine for 40 hours to give an 88% yield of equal amounts of diastereomers of **24** (Scheme 1). This intermediate has been important to make ACE inhibitor analogs by deprotecting and coupling the carboxylic acid with various amino acids such as proline to afford enalapril.



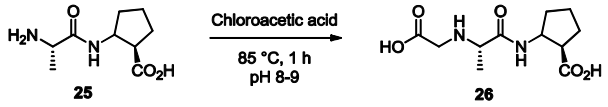
Scheme 1. Synthesis of **24** as an Important Intermediate for the Synthesis of ACE Inhibitors.

Iwasaki *et al.*[21] also reported the synthesis of diastereomers **24** through a diastereoselective synthesis involving the optically active enantiomer of ethyl 2-bromo-4-phenylbutanoate (**22**). Various solvents and bases were evaluated to optimize the yield and diastereoselectivity. The mixed solvent CH_3NO_2:H_2O (1:4) and the base ammonium carbonate [$(NH_4)_2CO_3$] gave the highest yield and diastereoselectivity of **24** (Scheme 2).



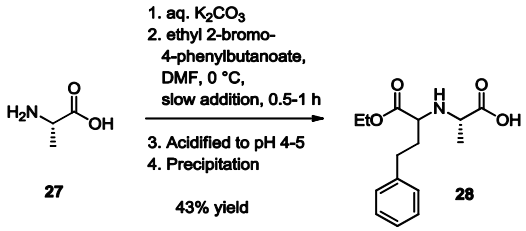
Scheme 2. Diastereoselective Synthesis of ACE Inhibitors.

Patchett *et al.*[2] were the first to synthesize the ACE inhibitor enalapril (**1**). Reductive amination with the α-ketoester was used to prepare enalapril, but they were also interested in confirming the activity of the unsubstituted N-carboxyalkyl dipeptide. To prepare this analog, they alkylated the dipeptide (*S*)-alanyl-(*S*)-proline **25** with chloroacetic acid to give the unsubstituted analog **26** (Scheme 3).



Scheme 3. Synthesis of Unsubstituted N-Carboxyalkyl Dipeptides via N-Alkylation.

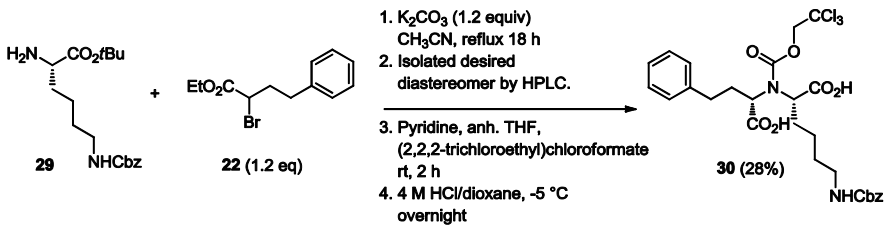
Jian-hong *et al.*[8] used the N-alkylation method to generate *N*-[1-(*S*)-ethoxycarbonyl-3-phenylpropyl]-(*S*)-alanine (ECPPA, **28**), the intermediate generated by the angiotensin-converting enzyme. (*S*)-Alanine (**27**) was alkylated with ethyl 2-bromo-4-phenylbutanoate in the presence of potassium carbonate to form the carbon-nitrogen bond in **28** (Scheme 4).



Scheme 4. Synthesis of ECPPA by N-Alkylation.

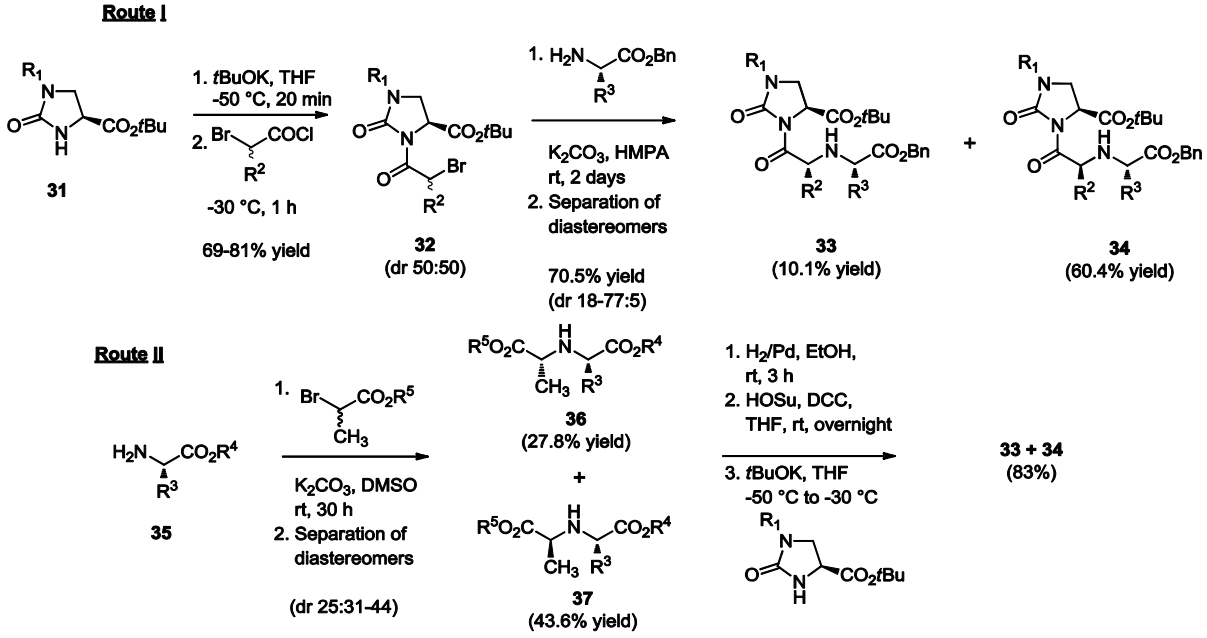
Barton *et al.*[9] also used ethyl 2-bromo-4-phenylbutanoate to make analogs of enalapril. One example involved alkylation of the amino acid *N*-Cbz-(*S*)-lysine (**29**) with the α-bromoester **22** under anhydrous basic conditions in acetonitrile (Scheme 5). The desired diastereomer was isolated by HPLC, and the amine was acylated before the

ester was hydrolyzed with hydrochloric acid. The diacid product **30** was isolated in 28% yield.



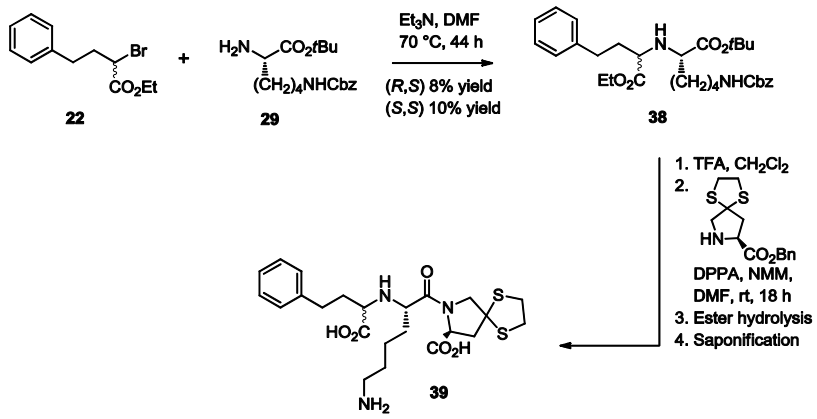
Scheme 5. Synthesis of ACE Inhibitor Analogs by N-Alkylation of Lysine.

Hayashi *et al.*[10] used two synthetic methods involving the N-alkylation with α-bromoesters to make 2-oxoimidazolidine analogs to test for ACE inhibition. The first route (Scheme 6) involved N-acylation of the 2-oxoimidazolidine **31** with an α-bromoacyl chloride to give intermediate **32**, which was then used to alkylate the amine of various benzyl protected amino acids to give diastereomers **33** and **34**. The second route involved initial alkylation of the nitrogen of various protected amino acids **35** with 2-bromopropanoate esters to give diastereomers **36** and **37**, followed by coupling with the 2-oxoimidazolidines to give diastereomers **33** and **34**. The protected esters of **33** and **34** were deprotected to the carboxylic acids by hydrogenolysis or acid hydrolysis.



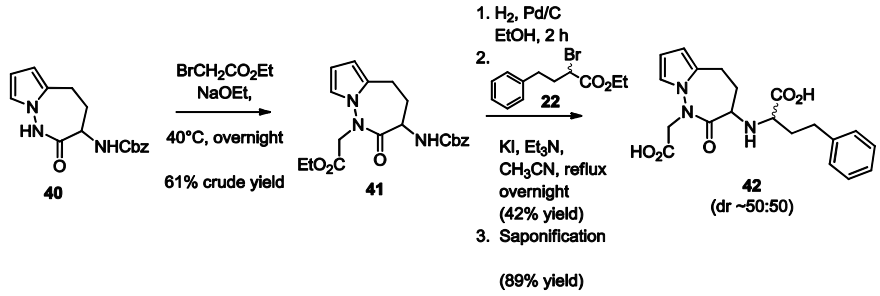
Scheme 6. The Synthesis of 2-Oxoimidazolidine Analogs by Two Synthetic Routes.

Smith *et al.*[11] focused their efforts on synthesizing spirapril related analogs of enalapril and lisinopril. Their synthesis of the lisinopril derivative **39** involved N-alkylation of *t*-butyl *N*-Cbz-(*S*)-lysine (**29**) with ethyl 2-bromo-4-phenylbutanoate **22** to give the mixture of diastereomers **38**. This mixture was then coupled with the spirapril proline amino acid to give the target compounds **39** (Scheme 7).



Scheme 7. The Use of N-Alkylation to Make Spirapril Analogs of Lisinopril.

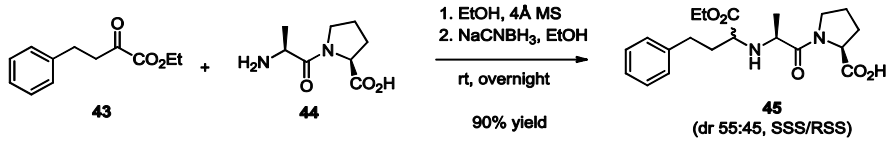
Ortiz *et al.*[12] utilized ethyl 2-bromo-4-phenylbutanoate as an alkylating reagent to synthesize pyrrole[1,2-*b*][1,1]diazepine derivatives **42** as bicyclic lactam analogs of commercially available benazepril and cilazapril. The diazepine **40** was first N-alkylated with ethyl bromoacetate to give intermediate **41**. The Cbz-protecting group was removed by hydrogenolysis and then alkylated with ethyl 2-bromo-4-phenylbutanoate, followed by saponification of the ethyl ester to give the desired target **42** (Scheme 8).



Scheme 8. Synthesis of Pyrrole[1,2-*b*][1,1]diazepine Derivatives.

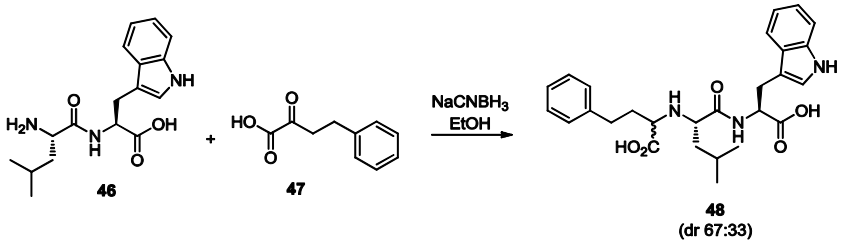
1.2.2. Solution-Phase Synthesis of ACE Inhibitor Analogs by Reductive Amination with α-Ketocarbonyl Compounds

Wyvratt *et al.*[22] reported the first synthesis of the commercial drug enalapril by reductive amination with ethyl 2-oxo-4-phenylbutanoate (**43**), using an excess of the α-ketoester to avoid reduction to the α-hydroxyester. The dipeptide (*S*)-Alanyl-(*S*)-proline (**44**) was coupled with the α-ketoester in the presence of sodium cyanoborohydride to give a 90% yield of diastereomers **45** (Scheme 9).



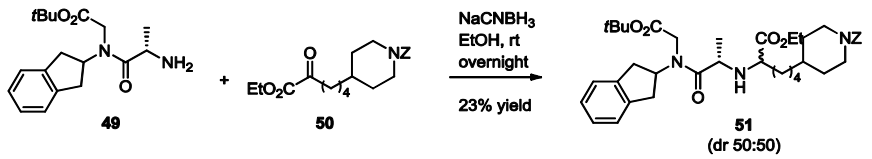
Scheme 9. Synthesis of Enalapril by Reductive Amination.

Maycock *et al.*[23] utilized the reductive amination method with an α-ketoacid to produce thermolysins analogs, which had previously shown activity for the angiotensin-converting enzyme. (*S*)-Leucyl-(*S*)-tryptophan **46** was condensed with 2-oxo-4-phenylbutanoic acid (**47**) in the presence of sodium cyanoborohydride to give analog **48** (Scheme 10), which was shown to be the most active analog. The separate diastereomers were tested with the more abundant diastereomers being the most active. The exact configuration of this diastereomer was not determined but hypothesized to be the *S*,*S*,*S* diastereomer from previous studies.



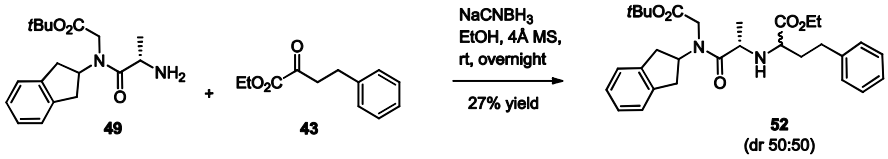
Scheme 10. Synthesis of Thermolysin Inhibitors as Compared to ACE Inhibitors.

Kori *et al.*[24] designed ACE inhibitors by replacing the phenethyl side chain with a 4-piperidylpentyl group. In one example, the amino amide **49** was condensed with the corresponding α-ketoester **50** in the presence of sodium cyanoborohydride to prepare such analogs **51** in a 50:50 diastereomeric mixture (Scheme 11).



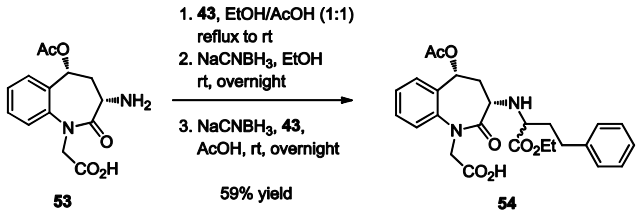
Scheme 11. Synthesis of ACE Inhibitors Containing a 4-Piperidylpentyl Group.

Suh *et al.*[25] made N-substituted glycine derivatives to explore ACE inhibition. One route employed the amino amide **49**, which was condensed with ethyl 2-oxo-4-phenylbutanoate (**43**) with sodium cyanoborohydride to afford **52** (Scheme 12). The esters could then be hydrolyzed to the carboxylic acids.



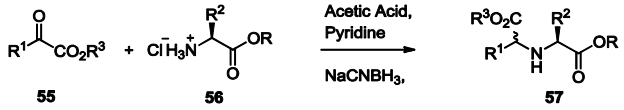
Scheme 12. Synthesis of N-Substituted Glycine Derivatives.

Ksander *et al.*[26] were interested in incorporating dual ACE and TxS (thromboxane synthase) inhibition into the same molecule. One set of analogs were the 5-oxy-substituted benazepines **54**, which were prepared by reductive amination of **53** with ethyl 2-oxo-4-phenylbutanoate **43** (Scheme 13).



Scheme 13. Synthesis of Dual ACE/TxS Inhibitors by Reductive Amination.

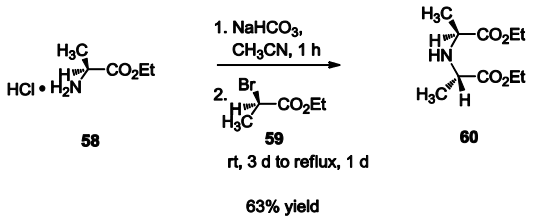
Hagmann *et al.*[27] prepared N-carboxyalkyl peptides as matrix metalloproteinase (MMP) inhibitors. Synthetic routes commonly used to prepare ACE inhibitors were employed by reductive amination of an amino acid ester **56** with α-ketoester **55** in the early part of the synthetic sequence to afford **57** (Scheme 14).



Scheme 14. Synthesis of N-Carboxyalkyl Peptides as MMP Inhibitors.

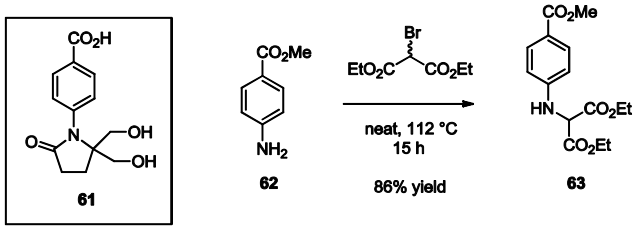
1.2.3 Solution-Phase Synthesis of Substituted Amines by N-Alkylation with α-Halocarbonyl Compounds

Harfenist *et al.*[28] were interested in making piperazinyl carbazoles for testing as antipsychotic drugs. Structure-activity relationships were explored to minimize the side effects commonly seen due to extended use of neuroleptic drugs. The enantioselective synthesis of **60** was key in this synthesis. The same synthetic route was used for both enantiomers of the alanine ethyl ester **58** (Scheme 15). The authors reported the formation of the by-product meso compound of **60** by attack of bromide ion formed during the reaction on the unreacted α-bromoester to give the other enantiomer of **59**. The meso by-product was separated by HPLC. The α-bromoester **59** was prepared from the α-bromoacid of alanine through the diazotization and bromide displacement.



Scheme 15. N-Alkylation of Alanine Ethyl Ester with an α-Bromoester.

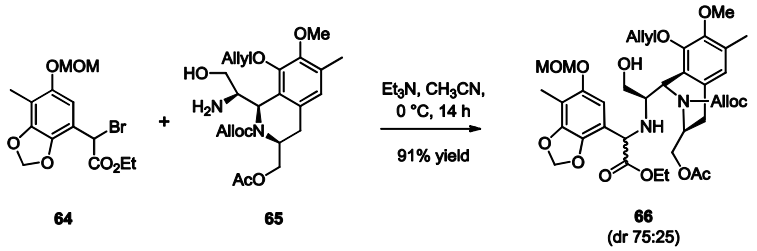
Brouoillette *et al.*[29] made analogs of anti-influenza drugs for influenza A and B of neuraminidase. Structure-activity relationship studies of benzoic acids of neuraminidase inhibitors containing pyrrolidinone rings were investigated based on the previous lead **61**. The first step in synthesizing a targeted analog involved the N-alkylation of methyl *p*-aminobenzoate **62** with diethyl bromomalonate to afford intermediate **63** (Scheme 16).



Scheme 16. N-Alkylation with Diethyl Bromomalonate.

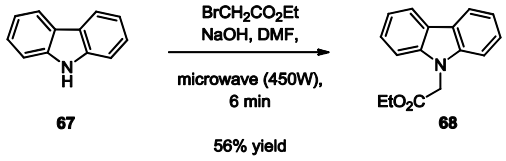
Zhu *et al.*[30] reported the total synthesis of Ecteinascidin 743 that had been isolated from the Caribbean tunicate *Ecteinascidia turbinate*. This was found to have potent cytotoxicity against tumor cells and was in clinical trials for various cancer

treatments. A variety of reaction conditions for the N-alkylation were tested including solvents (CH_3CN, trifluoroethanol, THF), bases (Et_3N, pyridine, DBU, Ag_2O), and temperature (from -45 °C to rt). The optimal conditions were applied to the alkylation of amine **65** with the α-bromoester **64** in acetonitrile with triethylamine at 0 °C (Scheme 17). The isolated diastereomers **66** showed a 75:25 diastereomeric ratio suggesting an S_N1 mechanism for the formation of the major diastereomer with absolute configuration of *R*.



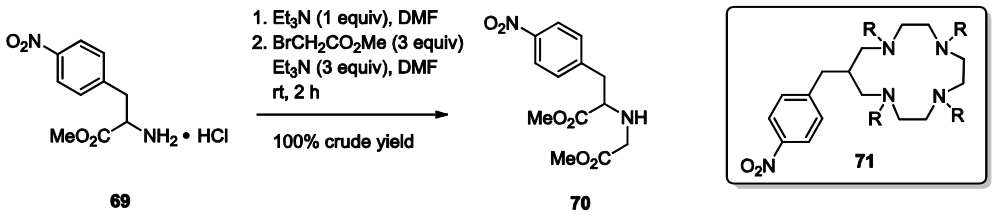
Scheme 17. Use of N-Alkylation in the Total Synthesis of Ecteinascidin 743.

Ou *et al.*[31] reported the synthesis of 9H-carbazole-9-carboxylic acids using microwave irradiation in solution-phase reactions. Previous work had reported the N-alkylation of 9H-carbazoles under "dry" conditions in the absence of solvent. Some previous analogs had been tested as potential immunodulating agents. Reactions were conducted using α-bromoesters where the 9H-carbazole **67** was alkylated with ethyl bromoacetate for 6 minutes to give compounds **68** (Scheme 18).



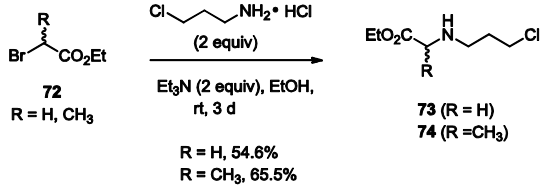
Scheme 18. Microwave Synthesis of N-Alkylated Carbazoles.

Kato and Kimura *et al.*[32] prepared novel chelating agents for radiolabeling of monoclonal antibodies for tumor imaging and cancer treatment. Kinetic and stability studies were used to evaluate the complex formation of the bifunctional chelating agents (BCAs), such as **71** with yttrium (III). The key intermediate **70** was made by N-alkylation of *p*-nitrophenylalanine methyl ester **69** with methyl bromoacetate (Scheme 19).



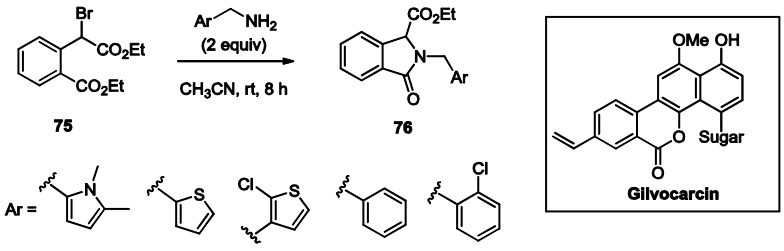
Scheme 19. The Synthesis of Bifunctional Chelating Agents by N-Alkylation.

Aladzheva *et al.*[33] prepared *N*-3-chloropropyl amino acid esters as starting materials for the synthesis of 1,2-azaphospholane-containing amino acid targets. 3-Chloropropylamine was alkylated by α-bromoesters **72** to yield the *N*-3-chloropropylglycine ethyl ester **73** or *N*-3-chloropropylalanine ethyl ester **74** for use as a starting reagent in their synthesis (Scheme 20). These products were explored as novel compounds for biological activity.



Scheme 20. Synthesis of *N*-3-Chloropropylglycine Ethyl Ester and *N*-3-Chloro-propylalanine Ethyl Ester as Precursors to 1,2-Azaphospholanes.

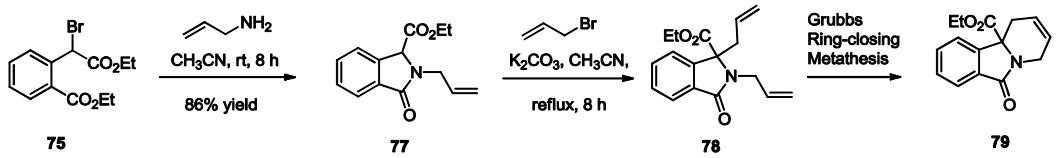
Decroix *et al.*[34] used diethyl α-bromohomophthalate **75** with various benzyl amines to make compounds **76** as precursors to analogs of gilvocarcin (Scheme 21). Gilvocarcin and related tetracyclic aromatic compounds are metabolites of some *Streptomyces* species and were evaluated as a potential new class of antibiotics.



Scheme 21. Synthesis of Isoindolones as Precursors to Tetracyclic Gilvocarcin Analogs.

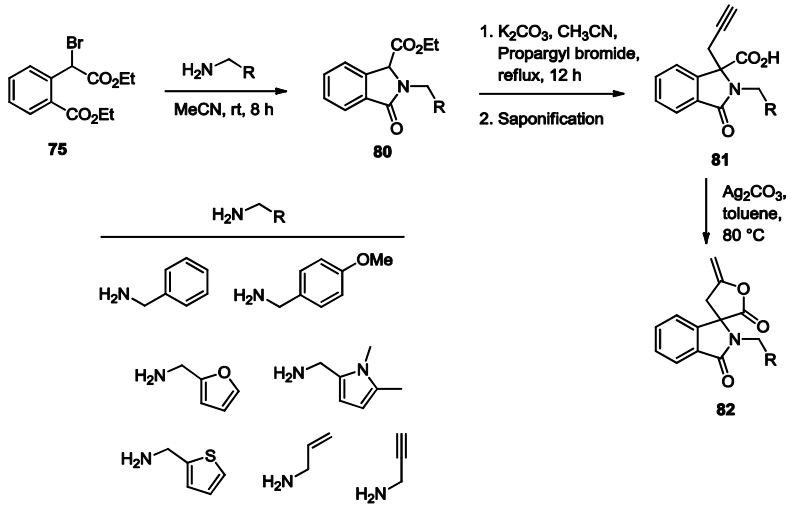
Othman *et al.*[35] used the N-alkylation reaction to synthesize various olefin-containing phthalimidines **79**. Diethyl bromohomophthalate **75** was coupled with

allylamine to give isoindolones **77** (Scheme 22). The α-carbon of the ethyl ester was then alkylated with allyl or propargyl bromide to introduce a second olefin in **78**, which could then react in one-pot Grubbs ring-closing metatheses to give phthalimidines **79**.



Scheme 22. Synthesis of Olefin-Containing Phthalimidines as Precursors for Grubbs Ring-Closing Metathesis Reactions.

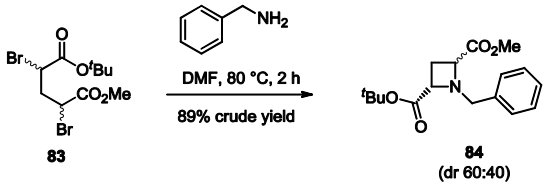
Othman *et al.*[36] synthesized the phthalimidines **80** from diethyl bromohomophthalate **75** and various amines (Scheme 23) as intermediates to spirolactones. These intermediates were converted to γ-acetylenic carboxylic acids **81** that could be cyclized to the spirolactone compounds **82**. Such spirolactones have been shown to have cytotoxic, insecticidal, and antibiotic activity.



Scheme 23. Synthesis of Phthlamidine Compounds for Synthesis of Spirolactones.

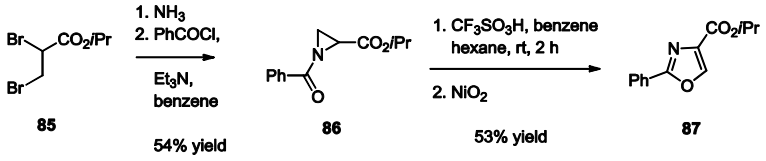
Baldwin *et al.*[37] synthesized azetidines as intermediates for the formation of γ-lactam analogs of carbapenicillanic acids to develop a new class of antibiotics. The α,α'-dibromo diester **83** was reacted with benzylamine to give the four-membered ring systems of **84** seen in penicillin analogs (Scheme 24). A diastereomeric ratio of 60:40 was observed with the trans isomer being more abundant. Further chemistry on the esters gave carbapenicillanic acid analogs. Although the x-ray crystallographic studies

showed the analogs closely resembled the carbapenicillanic acids, the targeted compounds did not show any antibacterial or β-lactamase inhibitory activity.



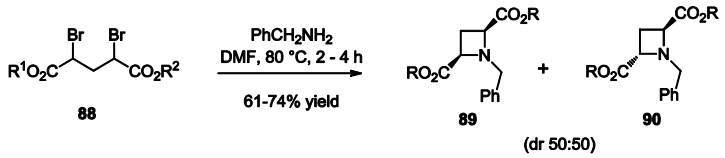
Scheme 24. Synthesis of Azetidines as Precursors to Carbapenicillanic Acid Analogs.

Eastwood *et al.*[38] prepared N-acylaziridines **86**. The dibromoester **85** and ammonia were coupled to form the three-membered ring system of **86** (Scheme 25). These were then used to explore various reaction conditions for ring enlargements of the three-membered rings to the 2,4-disubstituted oxazoles. Optimal conditions utilized a nickel peroxide oxidation to give compounds **87**. This chemistry was used to make halichondramide scaffolds containing multiple 2,4-disubstituted oxazoles as potential anti-fungal agents.



Scheme 25. Synthesis of 2,4-Disubstituted Oxazoles from N-Acylaziridines.

Kozikowski *et al.*[39] explored the synthesis of azetidine-2,4-dicarboxylic acids for stimulus of $^{45}Ca^{2+}$ uptake in cerebellar granule cells. The *N*-methyl-D-aspartate (NMDA) receptors have been linked to memory loss and degeneration after strokes and heart attacks. The azetidine-2,4-dicarboxylic acids were made from 2,4-dibromoglutaric acid diesters **88** with benzylamine to give diastereomers **89** and **90** (Scheme 26). These targets were of interest to explore possible modulation of the NMDA receptors and their impact on combating neurological degeneration.



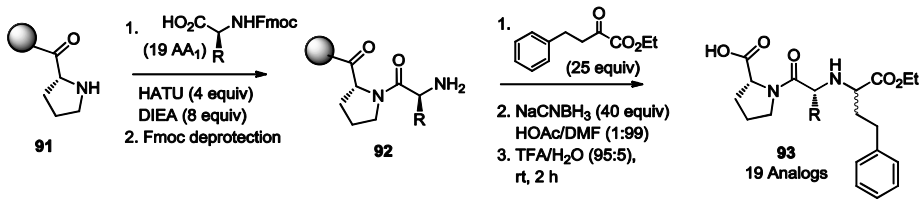
Scheme 26. Synthesis of Azetidine-2,4-Diesters.

1.3. Examples of Solid-Phase Synthesis of Substituted Amines

The above examples demonstrate the wide synthetic application of N-alkylation with α-halocarbonyl compounds by solution-phase chemistry. The utilization of solid-phase methods and combinatorial chemistry for the synthesis of amino acid derivatives has become an important methodology since the beginnings of solid-phase peptide synthesis by Merrifield in the 1960s.[40] Considerable work in the field has involved the synthesis of oligopeptides by coupling amines and carboxylic acids to form peptide bonds. Various resins and linkers have been developed to produce the desired functionalities after cleavage of the modified peptides or their derivatives. Only a few examples have been reported for the alkylation of amines with α-halocarbonyl compounds.[41] In general, most involve reaction of a resin-bound α-bromocarbonyl compound with excess amine in solution. This process of using excess amine in solution capitalizes on solid-phase methodology to minimize dialkylation of the resin-bound substrate. On the other hand, it cannot take advantage of the variety of resin-bound amines accessible by solid-phase combinatorial chemistry. The route reported in this thesis utilizes resin-bound amino acids as the nucleophile and excess α-bromoester as the electrophile in solution. Few examples were found that used solid-phase synthesis to produce ACE inhibitors,[13,42] with only one targeting the N-carboxyalkyl dipeptides by reductive amination.[43] Thus, the utilization of solid-phase synthesis and combinatorial chemistry is an area of potential utility for the synthesis of such analogs.

1.3.1. Solid-Phase Synthesis of ACE Inhibitors with α-Ketocarbonyl Compounds

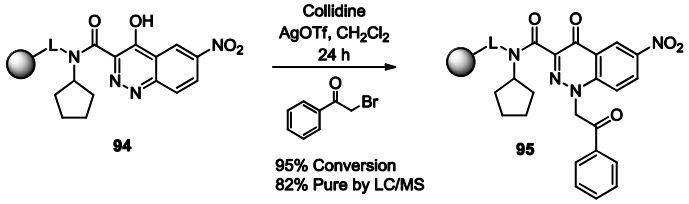
Blackburn *et al.*[43] synthesized a library of N-carboxyalkyl dipeptides as ACE inhibitors using solid-phase synthesis. In order to avoid the premature cyclitative cleavage of the dipeptide as the diketopiperazine from the resin when the starting resin-bound amino acid was proline, a DHPP linker was employed. The resin-bound proline (**91**) was coupled with 19 different amino acids to give the resin-bound dipeptides **92**. These were then condensed with ethyl 2-oxo-4-phenylbutanoate in the presence of sodium cyanoborohydride, followed by TFA cleavage from the resin to afford a library of 19 compounds (Scheme 27, **93**). Enalapril was made as a model compound for the synthetic route. The mixture of cleaved products were analyzed by mass spectrometry and screened for biological activity.



Scheme 27. Solid-Phase Synthesis of ACE Inhibitors.

1.3.2. N-Alkylation of a Resin-Bound Nucleophile with α-Bromocarbonyl Compounds

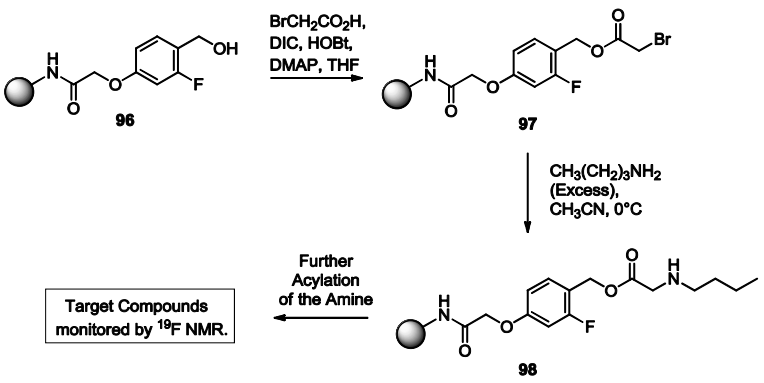
Brill *et al.*[44] reported one of the few examples for N-alkylation of a resin-bound nucleophilic nitrogen with α-bromocarbonyl compounds to synthesize cinnoline derivatives **95**. These compounds have shown a broad spectrum of activity in many pharmaceutical, antibacterial and agricultural applications. A resin-bound cinnoline **94** was alkylated on the heterocylic nitrogen with alkyl halides, including bromomethyl phenyl ketone, to give compound **95** (Scheme 28).



Scheme 28. N-Alkylation of Cinnoline Derivatives.

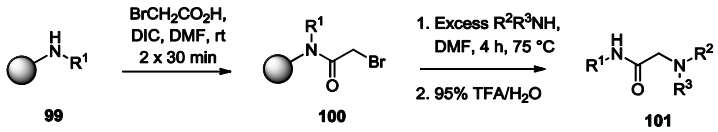
1.3.3. Resin-Bound Electrophilic α-Bromocarbonyl Compounds in Reaction with Excess Amines in Solution

Many examples have been reported for coupling of a resin-bound alcohol with bromoacetic acid to incorporate a resin-bound α-bromocarbonyl compound, which are then reacted with excess amine (to avoid polyalkylation). Kihlberg *et al.*[45] used this chemistry to make fluorinated linkers for monitoring solid-phase synthesis by ^{19}F NMR spectroscopy. A resin-bound benzylic alcohol **96** was coupled to bromoacetic acid to give the resin-bound α-bromoester **97**, which was then used to alkylate *n*-butylamine to give compound **98** (Scheme 29). The amine was then further acylated to give the final targets that were monitored by ^{19}F NMR.



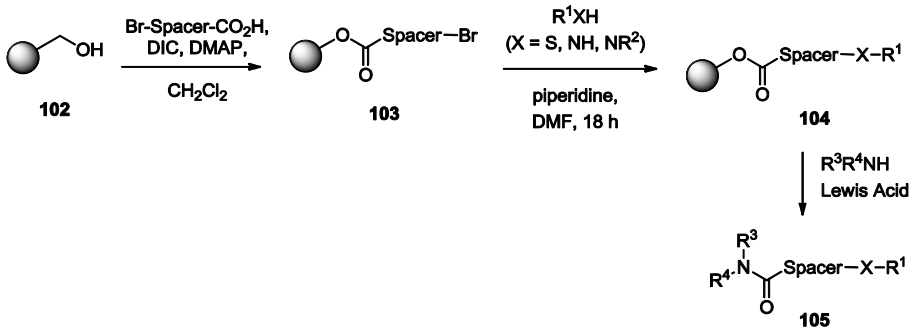
Scheme 29. Amine Alkylation by a Resin-Bound α-Bromoester.

Barany *et al.*[46] used the BAL (Backbone Amide Linker) resin to develop a new synthetic strategy for the synthesis of lidocaine and procainamide analogs. The resin-bound amine **99** was acylated with bromoacetic acid to form the resin-bound α-bromoamides **100**. These intermediates were then reacted with secondary amines and cleaved from the resin to afford target compounds **101** (Scheme 30).



Scheme 30. Synthesis of Lidocaine and Procainamide Analogs on BAL Resin.

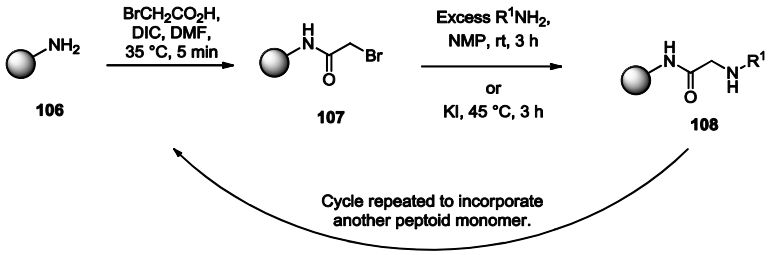
Barn *et al.*[47] explored methods to cleave resin-bound esters from Wang resin with Lewis acids to produce amide functionalities. They used resin-bound α-bromoesters to test different Lewis acids for cleavage from the Wang resin to afford the amide products **105**. The resin-bound alcohol **102** was coupled to a bromoacid to produce the resin-bound bromide **103**. These were then used to alkylate amines and thiols to give intermediates **104**. Secondary amines with various Lewis acids were then tested as a route to the cleaved amide products **105** (Scheme 31).



Scheme 31. Lewis Acid-Catalyzed Cleavage from Wang Resin to Afford Amides.

1.3.4. Synthesis of Peptoids from Resin-Bound α-Bromoesters and Amines

Zuckermann *et al.*[48-49] pioneered the solid-phase synthesis of peptoids, N-substituted glycine oligomers. A resin-bound amine **106** was acylated with bromoacetic acid to give **107**, which was reacted with excess amine in solution to give **108**. The alkylated amine **108** was then acylated again with bromoacetic acid and this two-step cycle was repeated to give N-substituted glycine oligomers (Scheme 32).



Scheme 32. Synthesis of Peptoid Oligomers using Bromoacetic Acid and Primary Amines.

The synthesis of peptoid oligomers has also been performed with the use of microwave irradiation.[50-51] The same synthetic route as described above (Scheme 32) was carried out with each monomer addition step requiring less than one minute to complete.

1.4. The Mechanism of the N-Alkylation with α-Halocarbonyl Compounds

Markovnik *et al.*[52] discuss the mechanism for reaction of nitrogen nucleophiles with α-halocarbonyl compounds. Figure 8 shows the possible transition states (**109**-**111**) for nucleophilic attack by the amine. The second order reaction of the N-acylalkylation is frequently viewed as an S_N2 nucleophilic substitution. However, a nucleophilic substitution on the carbonyl followed by an elimination (S_NAdE) mechanism was also discussed. The electron-withdrawing effect of the carbonyl helps explain the stability of the transition states. The S_NAdE mechanism was indirectly confirmed by evidence that the carbonyl is a competitive center for nucleophilic attack. As cited by Markovnik *et al.*, a wide variety of nucleophiles have demonstrated this principle for the haloalkylaminocarbinol intermediate **116**. It was concluded that N-alkylation can occur by either an S_N2 mechanism with weaker nucleophiles or an S_NAdE mechanism with stronger nucleophiles.

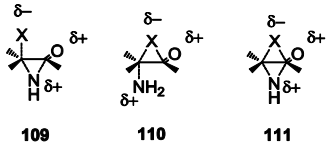
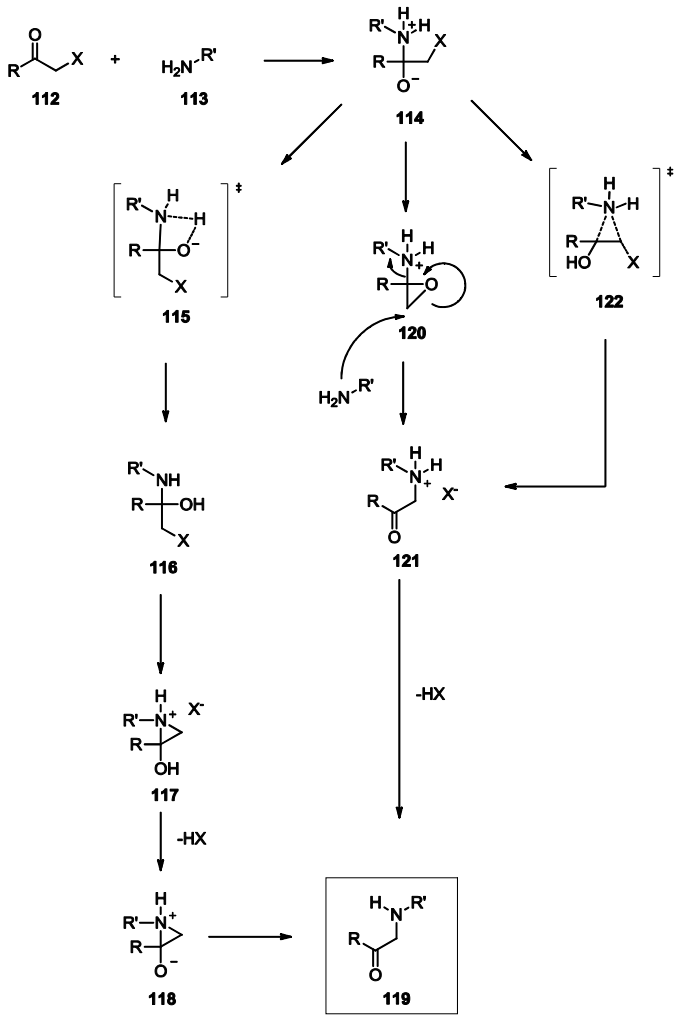


Figure 8. Possible Transition States for N-Acylalkylation with Ammonia.[52]

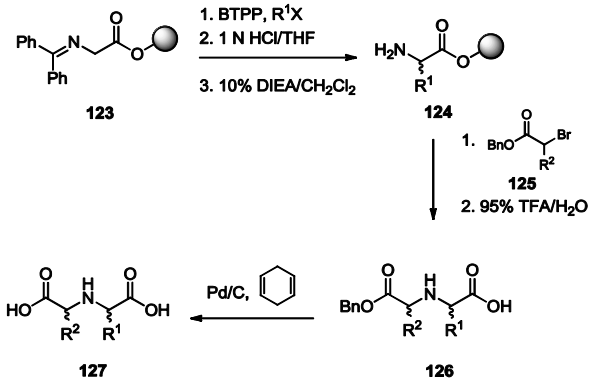
The S_NAdE mechanism, as outlined below (Scheme 33), has many possible routes. The primary amine can first attack the carbonyl forming the tetrahedral intermediate **114**. Three routes can then be envisioned. The anionic oxygen of **115** could deprotonate the cationic nitrogen to form **116**. The neutral amine could then attack the carbon alpha to the carbonyl with elimination of the halide and formation of the three-membered ring intermediate **117**. Loss of HX and subsequent ring-opening and reformation of the carbonyl would afford product **119**. In a second route from **114**, the anionic oxygen could attack the carbon alpha to the carbonyl with elimination of the halide to form the epoxide intermediate **120**. A second amine could attack the epoxide to reform the carbonyl with loss of the protonated amine on the α-carbon to give product **119**. In a third route, the nitrogen of the tetrahedral intermediate **114** could form a three-membered ring transition state **12** with loss of the halide to form intermediate **121**, followed by subsequent loss of HX to give product **119**.



Scheme 33. S_NAdE Mechanism of N-Acylalkylation.[52]

CHAPTER 2. PLAN OF STUDY

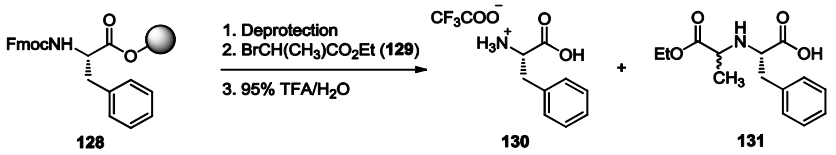
This study will focus on the synthesis of N-carboxyalkyl unnatural amino acids **127** from the benzophenone imine of glycine on Wang resin **123**. O'Donnell and Scott[53-55] reported the formation of unnatural amino acids **124** on resin by alkylation of the α-carbon of glycine with alkyl halides. These resin-bound unnatural amino acids have been used as key intermediates to many different combinatorial scaffolds. The overall purpose of this study is to expand the use of resin-bound unnatural amino acids by establishing a novel route for the N-alkylation of the amino acids with α-bromoesters **125** to prepare a generic scaffold for potential ACE inhibitors **127** through intermediate **126**. The usefulness of the methodology will be demonstrated by the synthesis and isolation of a combinatorial library of ACE analogs by varying the inputs R^1 and R^2 in **127** (Scheme 34).



Scheme 34. Proposed Synthesis of N-Carboxyalkyl Unnatural Amino Acids.

Initially, the reaction conditions (solvent, temperature and reagent stoichiometry) for N-alkylation of the resin-bound amino acid with an α-bromoester will be studied. The resin-bound natural amino acid Fmoc-Phe-Wang **128** will be deprotected, N-alkylated and then cleaved from the resin to compare the crude yield of N-alkylated product **131** to

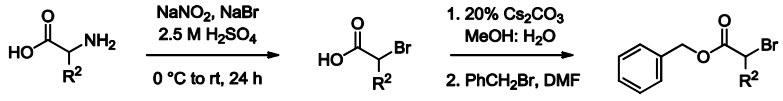
the unalkylated phenylalanine **130** by LC/MS (Scheme 35). Any presence of dialkylated product will be evaluated as well. Racemic ethyl 2-bromopropanoate [**(±)-129**], which is commercially available, will be used as the generic α-bromoester. The crude products will be used to establish a chromatographic purification procedure. Since a resin-bound, optically active amino acid will be used, the isolation and characterization of the two diastereomers formed during the alkylation will also be explored.



Scheme 35. Proposed Reaction Sequence to Find Optimal Conditions for N-Alkylation.

Following optimization of the N-alkylation of the resin-bound phenylalanine with racemic ethyl 2-bromopropanoate, a synthetic route to prepare various α-bromoesters will be investigated. Benzyl esters **125** will be prepared since these can be cleaved by hydrogenolysis in the absence of aqueous base or acid to give the final products **127**.

A convenient route for converting an optically active amino acid **132** to its α-bromoacid **133** (Scheme 36) while retaining the chiral center was first described by Izumiya and Nagamatsu[56] and later refined by Karoyan *et al*.[57] The double inversion of this transformation was studied by Koch *et al.*[58] and has been cited by many other groups that have successfully used this method. This route will be used to prepare α-bromoacids from the amino acids (*S*)-phenylalanine, (*S*)-homophenylalanine, and (*S*)-leucine. These α-bromoacids (**133b**-**d**) and racemic ethyl 2-bromopropanoic acid (**133a**) as the commercially available product will then be converted to their benzyl esters (**125a**-**d**) in the presence of cesium carbonate and benzyl bromide (Scheme 36) adapted from a procedure for the benzylation of α-hydroxyacids by Shin *et al.*[59]



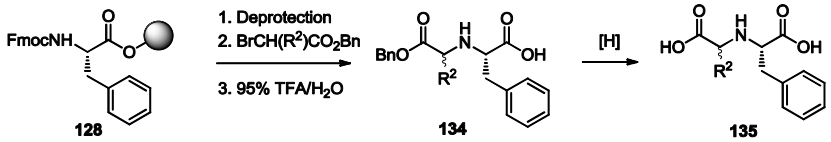
(±)-132a, R = CH_3
(*S*)-132b, R = CH_2Ph
(*S*)-132c, R = CH_2CH_2Ph
(*S*)-132d, R = $CH(CH_3)_2$

(±)-133a, R = CH_3
(*S*)-133b, R = CH_2Ph
(*S*)-133c, R = CH_2CH_2Ph
(*S*)-133d, R = $CH(CH_3)_2$

(±)-125a, R = CH_3
(*S*)-125b, R = CH_2Ph
(*S*)-125c, R = CH_2CH_2Ph
(*S*)-125d, R = $CH(CH_3)_2$

Scheme 36. Synthesis of Benzyl α-Bromoesters by Diazotization of Naturally-Occurring Amino Acids.

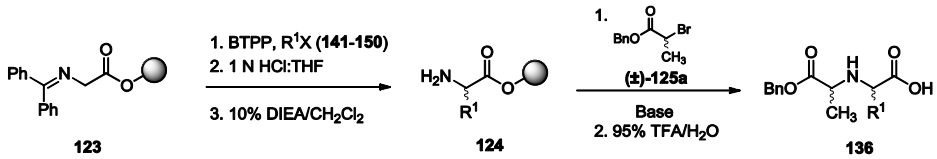
The benzyl α-bromoesters **125a-d** will be reacted with Phe-Wang, prepared from Fmoc-Phe-Wang, to study the effects of steric hindrance on the benzyl α-bromoesters in the N-alkylation reaction (Scheme 37). These compounds will also be used to establish a standard procedure for the hydrogenolysis of the benzyl ester **134** to the diacid **135** in order to isolate and characterize the amino diacids products.



128 134 135

Scheme 37. N-Alkylation of Phe-Wang and Hydrogenolysis to the Diacid.

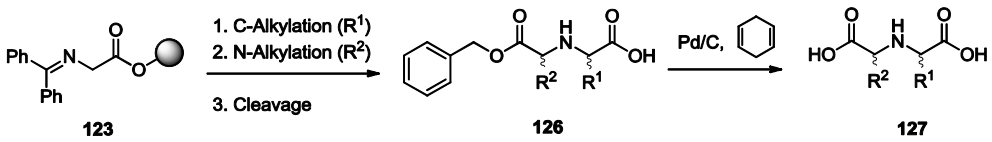
Once the optimal reaction conditions for the N-alkylation with benzyl α-bromoesters have been established, ten alkyl halides (**141**-**150**) will be used in the alkylation of the resin-bound benzophenone imine of glycine (**123**, Scheme 38) to prepare racemic, unnatural amino acids **124**. These intermediates will then be reacted with racemic benzyl 2-bromopropanoate **(±)-125a** to give **136**.



123 124 136

Scheme 38. Proposed Scheme and Alkyl Halides to Rehearse on the BIG-Wang Resin.

Finally, the rehearsed alkyl halides will be used on an appropriate scale to make 10-20 mg of the N-carboxyalkyl amino acids analogs. A combinatorial approach will be developed with the alkyl halides (**141**-**150**) as the input R^1 and the benzyl α-bromoesters as the input R^2. The analogs will be made in a combinatorial fashion represented in Scheme 39. Products **126** will be purified and fully characterized by LC/MS, ^1H and ^{13}C NMR, and HRMS. Each compound **126** will be further hydrogenolyzed to give the corresponding N-carboxyalkyl amino acids **127**, which will also be fully characterized.



R^1X =	141	142	143	144	145	146	147	148	149	150
(±)-125a										
(*S*)-125d										
(*S*)-125c										

Scheme 39. Final Combinatorial Synthesis of N-Carboxyalkyl Amino Acid Analogs.

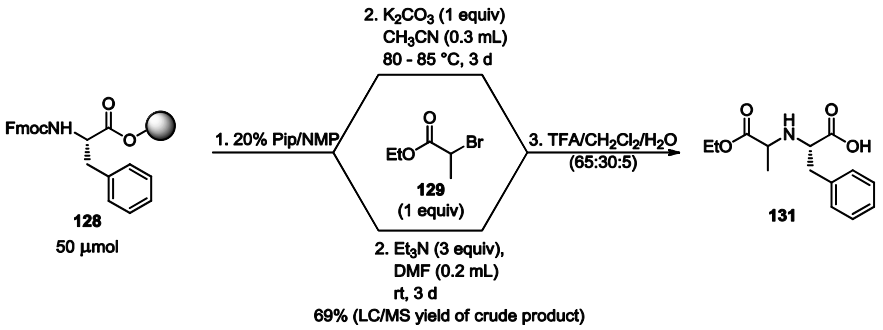
CHAPTER 3. RESULTS AND DISCUSSION

3.1. Experiments to Optimize the N-Alkylation Reaction

3.1.1. Optimization of the N-Alkylation of Phe-Wang with Ethyl 2-Bromo-propanoate

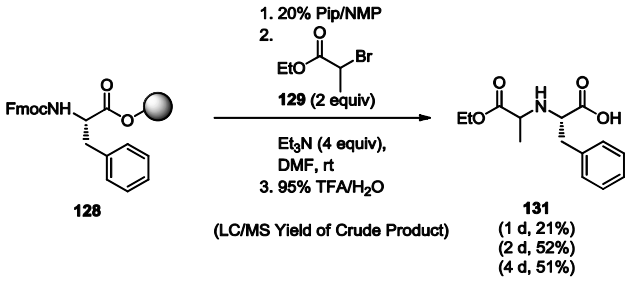
In order to find optimal conditions for the N-alkylation of a resin-bound amino acid with an α-bromoester, the literature background discussed in Chapter 1 was analyzed and select reaction conditions were tested. Ethyl 2-bromopropanoate was found to be an inexpensive, commercially available α-bromoester that could be used to first test these literature conditions. Two common conditions found for the alkylation of an amine with an α-bromoester were the use of potassium carbonate in acetonitrile and the use of triethylamine in DMF (Scheme 40). These were tested on two side-by-side reactions for each of the separate reaction conditions.

Resin-bound phenylalanine was treated with one equivalent of ethyl 2-bromopropanoate and allowed to react for 3 days under both reaction conditions. Following cleavage of the product from the resin, the potassium carbonate and acetonitrile reactions showed a crude yield of 50 mg that was much higher than the theoretical yield, likely due to the presence of the inorganic salt. It appeared that the potassium carbonate did not dissolve well in the acetonitrile, and this poor solubility likely led to poor conversion as evidenced by negligible product in the LC/MS. In contrast, the triethylamine and DMF conditions led to crude yields comparable to the theoretical yield, and the LC/MS showed 69% of the desired product **131**. The diastereomeric ratio of the products in this reaction and the subsequent optimization reactions covered in this section was 52:48 to 55:45.



Scheme 40. N-Alkylation using K_2CO_3/CH_3CN or Et_3N/DMF.

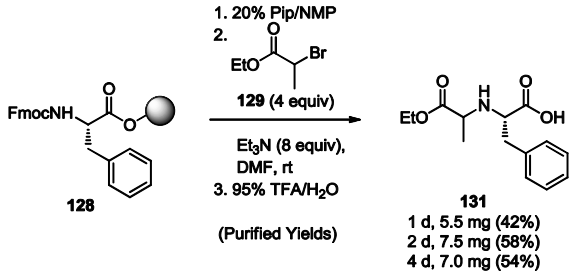
After observing a good conversion using triethylamine and DMF with only one equivalent of the α-bromoester, a second reaction was performed using 2 equivalents of the α-bromoester. A reaction vessel containing Fmoc-Phe-Wang was deprotected and treated with the α-bromoester (2 equiv) and triethylamine (4 equiv) in 0.5 mL of DMF (Scheme 41). In order to monitor the reactions, small aliquots were removed from the reaction vessel and cleaved after one, two and four days to determine product yields. The cleaved products at the three time points were analyzed by LC/MS. The LC/MS results after one day showed that 21% of the product had formed, after two days 52% of the product had formed, and after four days 51% of the product had formed. These results suggested that the maximum amount of N-alkylated product was formed after two days. The crude products were combined and purified to give 8 mg (30% yield) of product **131**.



Scheme 41. Monitoring the Reaction Time: One, Two and Four Days.

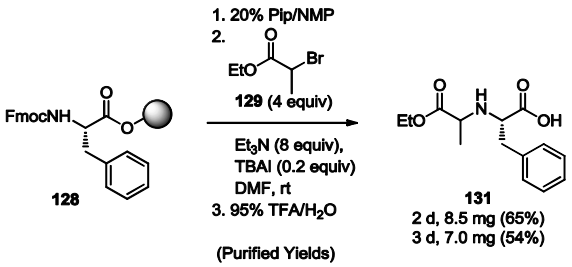
The above results were promising for these reaction conditions, but the removal of sample aliquots may have been misleading since the exact amount of resin and removed reagent solution may have been inconsistent. Another set of three separate reactions was set up to analyze the time points of two, three and four days (Scheme 42).

The three reactions were run simultaneously, and the equivalents of the α-bromoester and triethylamine were increased to four and eight, respectively, while keeping the total volume constant in order to double the concentration of reagents. The resin in each reaction vessel was undisturbed until it was cleaved from the resin. The first reaction vessel was washed and cleaved after two days, and the LC/MS results of the crude product showed 59% product. The second reaction vessel was washed and cleaved after three days, and the LC/MS results of the crude product showed 67% product. Likewise, the third reaction vessel was washed and cleaved after four days, and the LC/MS of the crude product showed 68% product. These results suggested that the optimal reaction time at room temperature is two days, and an increase in the concentration of reagents led to a higher yield of crude product by LC/MS from 52% (Scheme 41) to 67%. Each of the three crude products was purified to give 5.5 mg (42% yield) for the two-day reaction, 7.5 mg (58% yield) for the three-day reaction, and 7.0 mg (54% yield) for the four-day reaction.



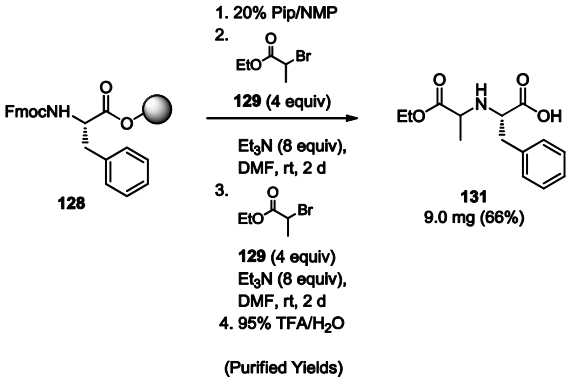
Scheme 42. Increased Concentration of α-Haloester and Base at Varying Reaction Times.

Next, the same reaction was conducted using tetrabutylammonium iodide (TBAI) through an in situ Finkelstein reaction in an attempt to increase the reactivity of the α-bromoester. Two reactions, one for two days and one for three days, were run simultaneously (Scheme 43). The LC/MS results showed poor UV detection, which were commonly observed for these reactions due to the lack of chromophores present in the target compound. The purified yields, however, were comparable to yields reported above. The two-day reaction gave 8.5 mg (65%) and the three-day reaction gave 7.0 mg (54%). It appeared the presence of TBAI did not have a measurable effect on the reaction rate.



Scheme 43. Addition of Tetrabutylammonium Iodide to Increase the Reaction Rate.

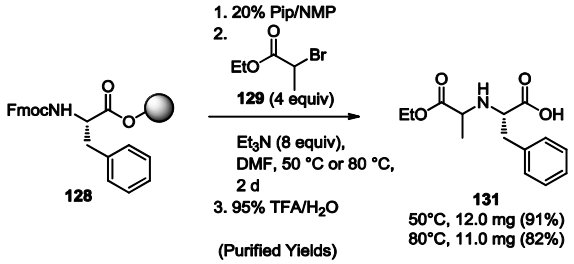
The next method tested to increase the overall conversion was to treat the resin-bound phenylalanine with the α-bromoester and triethylamine at room temperature for two days, wash the resin to remove all of the reagents, and then treat the resin again with more α-bromoester and triethylamine for an additional two days (Scheme 44). This technique is commonly used in solid-phase synthesis to decrease the reaction time by continually supplying an excess amount of reagent to the resin. The LC/MS results of the crude product showed 69% of the product formed. The crude material was purified to afford 9.0 mg (66% yield). This method did slightly increase the isolated yield, but the potential use of excess reagents that will require lengthy preparation may be problematic.



Scheme 44. Introducing a Second Charge of Reagents to the Resin.

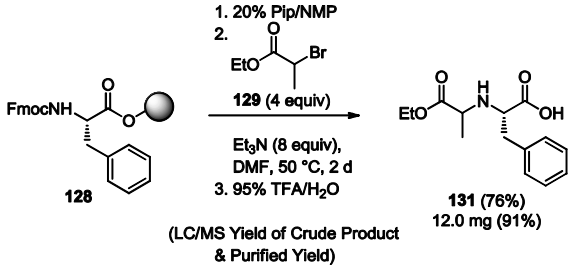
Next, as a final variable, the reaction temperature was explored. Two reactions were set up simultaneously, one at 50 °C and one at 80 °C (Scheme 45). The LC/MS results of the crude materials showed a 76% conversion for the reaction at 50 °C and a 70% conversion for the reaction at 80 °C. The crude products were purified to give 12.0 mg (91%) for the 50 °C reaction and 11.0 mg (82%) for the 80 °C reaction. Comparing

the LC/MS yield of crude products to the isolated yield, the higher isolated yield is likely due to the low UV absorbance of product **131**.



Scheme 45. Results from Heating the N-Alkylation Reaction.

The optimal reaction conditions determined from these sets of experiments (Schemes 40-45): reaction of **128** (50 μmol) with ethyl 2-bromopropanoate (4 equiv) and triethylamine (8 equiv) in 0.5 mL of DMF at 50 °C for 2 days to give a conversion of 76% (LC/MS) and a 91% purified yield of product **131** (Scheme 46).

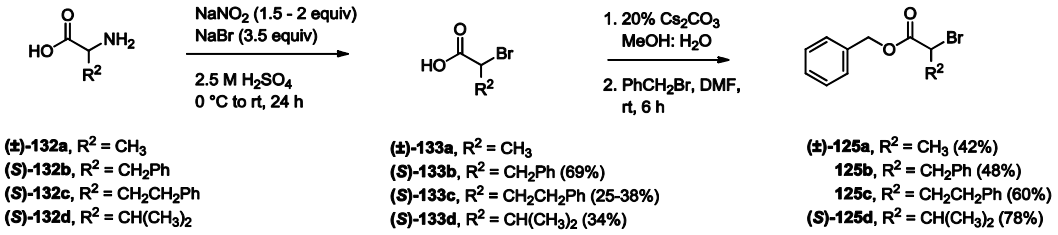


Scheme 46. Optimized N-Alkylation of Phe-Wang with Ethyl 2-Bromopropanoate.

3.1.2. Synthesis of Benzyl α-Bromoesters as Reagents for the N-Alkylation Reaction

Benzyl α-bromoesters were prepared by diazotization of naturally-occurring amino acids[57] (*S*)-phenylalanine, (*S*)-homophenylalanine and (*S*)-leucine to give the α-bromoacids **133a-d** (Scheme 47). This transformation has been reported to occur with retention of configuration by a double inversion previously discussed (Chapter 2).[56,58,60-61] The prepared α-bromoacids (**133b-d**) and commercially available racemic 2-bromopropanoic acid (**(±)-133a**) were converted to their benzyl esters (**125a-d**) in the presence of cesium carbonate and benzyl bromide.[59]

In order to confirm the stereochemistry of the α-bromoesters **125b-d**, the optical rotations of these starting reagents was measured. The optical rotations of **125b** and **125c** were +0.2° and -0.3°, respectively. Literature values were not available and, unless the optical rotations of both pure compounds were near zero, this result suggests possible racemization during preparation. The measured optical rotation of **(*R*)-125d**, however, was previously reported in literature (+31.8°, *c* = 1.0, methanol).[62] The optical rotation of **(*S*)-125d** was -22.5° (*c* = 1.0, methanol), suggesting partial racemization of the starting chiral center. Since the optical rotations of the α-bromoacids **133a**-**d** were not measured, it cannot be determined whether the racemization occurred during the diazotization step or the benzyl ester formation step. Given the literature precedence for the retention of configuration for the diazotization step (Chapter 2), it is speculated the racemization occurs during the benzylation step due to the presence of cesium carbonate. Since the exact stereochemistry for **125b** and **125c** is unknown based on these results, the side chain R is drawn with a straight bond throughout the rest of this thesis.[63] Since the optical rotation for **125d** appears to have been partially preserved, it is given as the *S* enantiomer.

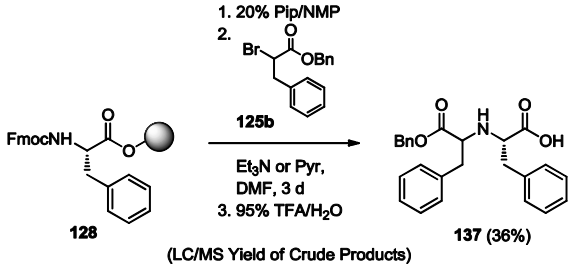


Scheme 47. Preparation of Benzyl α-Bromoesters.

3.1.3. N-Alkylation of Phe-Wang with Benzyl 2-Bromo-3-phenylpropanoate

Following the optimized synthesis of benzyl α-bromoesters **125a-d** (Section 3.1.2), these prepared reagents were used to alkylate the resin-bound phenylalanine to further optimize the reaction conditions. (*S*)-Benzyl 2-bromo-3-phenylpropanoate (**125b**) was first subjected to the optimal reaction conditions described above preparing **131** (Section 3.1.1). Fmoc-Phe-Wang was deprotected and treated with the α-bromoester (4 equiv) and triethylamine (9 equiv) in DMF at 50 °C (Scheme 48). A sample was removed and cleaved after 2 days (19%, LC/MS), and the remaining resin was cleaved after 3

days (36%, LC/MS). The amount of product observed by LC/MS was much lower than the amount observed with ethyl 2-bromopropanoate (Scheme 46).



Scheme 48. N-Alkylation of Phe-Wang with Benzyl 2-Bromo-3-phenylpropanoate.

The reduced yield of product **137** was likely due to β-elimination of the α-bromoester to the conjugated α,β-unsaturated carbonyl compound due to the large excess of base present and acidity of the benzylic proton. As a control, a sample of the α-bromoester was allowed to react under identical reaction conditions in the absence of the resin-bound phenylalanine overnight. The reaction mixture was extracted with ether and concentrated. ^{1}H NMR analysis of the product showed 67% of the α-bromoester had undergone elimination. It is noted that the other α-bromoesters **125a** and **125c** showed negligible elimination. This competing reaction consumed the starting reagent, resulting in the lower yield of N-alkylated product. The results from the additional studies described below are reported in Table 1.

Table 1. Results from the N-Alkylation of Phe-Wang with Benzyl 2-Bromo-3-phenyl-propanoate.

Entry	Equiv of 125b	Base (equiv)	TBAI (equiv)	Solvent	Temp. (°C)	LC/MS (%)[a]
1	4	Et_3N (9)	----	DMF	50	36
2	4	Pyr (8)	----	DMF	70	22
3	4	Pyr (8)	0.2	DMF	50	26
4	6	Pyr (10.5)	----	DMF	55	39
5	6	Pyr (10.5)	2.0	DMF	55	28
6	4	Pyr (8.5)	2.0	DMF	rt	16
7	4	Pyr (8.8)	----	NMP	50	30

[a] % Product in the reaction mixture. Diastereomeric ratios ranged from 56:44 to 46:54.

Due to the possible elimination of the α-bromoester, the subsequent reactions were repeated in the presence of the weaker base pyridine. Phe-Wang was treated with benzyl 2-bromo-3-phenylpropanoate (4 equiv) and pyridine (8 equiv) in DMF and allowed to react at 70 °C (Table 1, entry 2). This higher temperature was used in an attempt to increase the amount of alkylation; however, the rate of elimination may have also increased. After cleaving the product from the resin, the LC/MS results showed 22% of the desired product. It appeared the pyridine also led to elimination of the starting α-bromoester because of the presence of such a large amount of unalkylated phenylalanine observed by LC/MS.

Next, the addition of tetrabutylammonium iodide (TBAI) was tested. The N-alkylation of Phe-Wang was conducted with benzyl 2-bromo-3-phenylpropanoate (4 equiv) and pyridine (8 equiv) at 50 °C in the presence of TBAI (0.2 equiv). LC/MS results showed 26% product (Table 1, entry 3). Thus, as observed earlier with ethyl 2-bromopropanoate, TBAI did not lead to an increased yield of product.

Because of the competing elimination reaction and consumption of the α-bromoester, Phe-Wang resin was next treated with six equivalents of benzyl 2-bromo-3-phenylpropanoate (Table 1, entry 4). The amount of pyridine was also increased slightly, but not in the 2:1 ratio of base to α-bromoester used before. LC/MS results of the reaction mixture showed 39% of the product had formed. The use of more equivalents of the α-bromoester only slightly increased the amount of product observed by LC/MS.

Next, Phe-Wang was treated again benzyl 2-bromo-3-phenylpropanoate (6 equiv), but also in the presence of 2 equivalents of TBAI (Table 1, entry 5). LC/MS results indicated 28% of the product had formed, and this result again confirmed that the use of TBAI did not increase the N-alkylation.

Since the use of six equivalents of benzyl 2-bromo-3-phenylpropanoate seemed to only slightly increase the amount of N-alkylation observed by LC/MS and considering several steps were needed to prepare this starting material (Section 3.1.2), the benzyl 2-bromo-3-phenylpropanoate was used in 4 equivalents for subsequent reactions. The reaction had been conducted with 0.2 equivalents of TBAI with 4 equivalents of the α-bromoester, thus the reaction was repeated in the presence of 2.0 equivalents of TBAI at room temperature to possibly avoid elimination of the starting reagent. The 16% of the

product seen by the LC/MS analysis ultimately confirmed that the use of TBAI, even in greater than stoichiometric amounts, did not increase N-alkylation (Table 1, entry 6).

Finally, N-methylpyrrolidone (NMP) was used as a solvent in place of dimethylformamide (DMF). Although the competing elimination reaction with the starting α-bromoester would still occur, NMP was tested with the other α-bromoesters and this reaction was conducted for comparison. Fmoc-Phe-Wang was deprotected and treated with benzyl 2-bromo-3-phenylpropanoate (4 equiv) and pyridine (8.8 equiv) in NMP at 50 °C. The LC/MS results showed 30% of the product had formed (Table 1, entry 7).

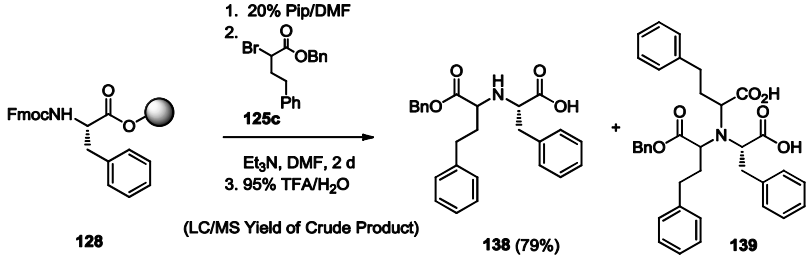
After analyzing the results from these reactions with benzyl 2-bromo-3-phenylpropanoate, it appeared that the competing elimination reaction could not readily be overcome to produce a reasonable yield of alkylated material. Considering the 50-100 μmol scale of these reactions, isolating enough material when only 30-35% of the resin-bound phenylalanine was being alkylated was not practical and this electrophile was not used further.

3.1.4 N-Alkylation of Phe-Wang with Benzyl 2-Bromo-4-phenylbutanoate

Incorporation of the phenethyl substituent into the N-carboxyalkyl amino acid scaffold was of considerable interest due to the known inhibitory activity of similar compounds against the angiotensin-converting enzyme (for example, see **1**, **12, 14**, **17**, **18**, Figures 1, 5 and 6). The reagent benzyl (*S*)-2-bromo-4-phenylbutanoate (**125c**) was synthesized as described previously (Scheme 48). Elimination of the bromide from this α-bromoester was not expected to be a competing reaction because an acidic β-proton was no longer present.

Initially, the optimal reaction conditions determined earlier for ethyl 2-bromopropanoate (Scheme 46) were tested. Fmoc-Phe-Wang was deprotected and treated with benzyl 2-bromo-4-phenylbutanoate (4.5 equiv) and triethylamine (7.5 equiv) in DMF at 50 °C for 2-3 days (Scheme 49, Table 2, entry 1). A sample aliquot was removed after 2 days, washed and cleaved to give 63% of the desired product by LC/MS. There was also a small amount of dialkylated material **139** (<5%) in which one of the benzyl esters had been converted to the carboxylic acid that was only visible by the mass spectrum and not by UV absorption. The resin was heated an additional day and cleaved after 3 days to give 79% of product by LC/MS. These initial results suggested

that the α-bromoester was not being consumed in the elimination side reaction with triethylamine. The results for the following optimization experiments are shown in Table 2.



Scheme 49. N-Alkylation of Phe-Wang with Benzyl 2-Bromo-4-phenylbutanoate.

Table 2. Results from the N-Alkylation of Phe-Wang with Benzyl 2-Bromo-4-phenyl-butanoate.

Entry	Equiv of 125c	Base (equiv)	TBAI	Solvent	Temp (°C)	LC/MS %[a]
1	4.5	Et_3N (7.5)	----	DMF	50	79
2	3	Et_3N (7)	----	DMF	70	44
3	6	Et_3N (11)	----	DMF	55	54
4	6	Et_3N (10.5)	2.0	DMF	55	49
5	4	Et_3N (7.5)	2.0	DMF	rt	33
6	4	Et_3N (7.5)	----	NMP	50	34
7	4	Et_3N (8)	2.0	CH_2Cl_2	rt	0
8	4	Et_3N (8)	2.0	CH_3CN	rt	0
9	4	Et_3N (8)	2.0	THF/MeOH (2:1)	rt	0
10	4	Et_3N (8)	----	DMSO	50	59
11	4	Et_3N (4)	----	DMF	50	62
12	4	Et_3N (4)	----	DMSO	50	63
13	4	Et_3N (2.5)	----	DMF	50	67
14	4	Et_3N (1.5)	----	DMF	50	69
15	4	Et_3N (1.5)	----	DMF	70	73

[a] % Product in the reaction mixture. Diastereomeric ratio 56:44.

Next, the above reaction was repeated with benzyl 2-bromo-4-phenylbutanoate (3 equiv) and triethylamine (7 equiv) in DMF at 70 °C. After 3 days, LC/MS analysis

showed 44% of the product had formed (Table 2, entry 2). There also appeared to be a negligible amount of the dialkylated material **139** apparent in the mass spectrum. From these results, the increase in temperature appeared to give a lower percentage of alkylated product.

The number of equivalents of the α-bromoester and triethylamine were increased to 6 and 11 equivalents, respectively, in an effort to increase the amount of alkylated product in the absence of and presence of TBAI (Table 2, entries 3-4). The LC/MS results showed 54% of the product had formed for the reaction in the absence of TBAI, while the reaction containing TBAI gave 49% of the desired product. It appeared an increase in the α-bromoester and triethylamine did not significantly increase the amount alkylated product, and similar conversions were seen with or without the presence of TBAI. Due to the several steps required to make this starting α-bromoester, four equivalents of the α-bromoester were used in all further studies.

Next, the same reaction was repeated with TBAI at room temperature with 4 equivalents of the α-bromoester (Table 2, entry 5). The results from this reaction would indicate whether TBAI could achieve the same conversion as the reaction at 50-55 °C. LC/MS results showed only 33% of the product formed, confirming that elevated temperatures were necessary to achieve the optimal amount of alkylated product.

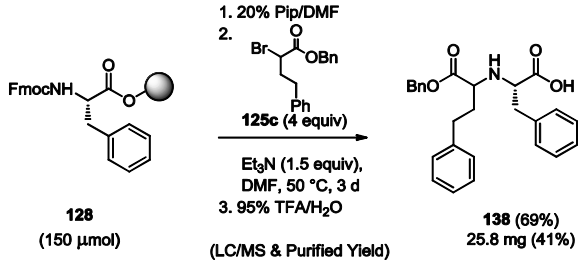
A solvent study was conducted next by running the reaction in NMP, methylene chloride, acetonitrile, THF/methanol, and DMSO (Table 2, entries 6-10). The reactions in methylene chloride, acetonitrile, and THF/methanol were conducted at room temperature for three days due to the relatively low boiling points of these solvents. TBAI was added to these reactions (entries 7-9) in an attempt to increase the N-alkylation. The reactions in NMP and DMSO were conducted at 50 °C for three days. All of these reactions involved 4 equivalents of the α-bromoester and 8 equivalents of triethylamine. LC/MS results showed only 34% of the resin-bound phenylalanine was alkylated for the reaction in NMP (entry 6) and 59% of the product for the reaction in DMF (entry 10). The reactions conducted in the other three solvents (entries 7-9) showed no conversion of the starting material to product. The results for the reaction in DMSO were comparable to the yields seen using DMF; however, NMR analysis of the crude material showed the presence of DMSO. Numerous washes prior to cleavage of the product from the resin

did not eliminate all of the DMSO from the alkylation reaction. Because of this contamination problem, DMF still appeared to be the best solvent for the N-alkylation.

Next, the number of equivalents of triethylamine was examined in the two best solvents, DMF and DMSO (Table 2, entries 11-14). Although complete removal of the DMSO had been problematic, reactions were still tested in DMSO to compare the overall yield observable by LC/MS. Phe-Wang was treated with the α-bromoester (4 equiv) and triethylamine (4, 2.5 or 1.5 equiv) in DMF and DMSO at 50 °C for 3 days (entries 11-13). LC/MS results (62-69%) again showed comparable results for the corresponding reactions in DMF and DMSO with a decrease in the amount of triethylamine having no effect on the amount of product observed by LC/MS. The fewer equivalents of triethylamine seemed to be sufficient for the neutralization of the HBr produced in the alkylation step.

The reaction temperature was explored as a final variable by repeating the reaction with α-bromoester (4 equiv) and triethylamine (1.5 equiv) at 70 °C for three days (Table 2, entry 15). Previous reactions conducted at 70 °C had given slightly lower yields, and LC/MS results showed 73% of the product had been formed (entry 15). These results were comparable to the reaction conducted at 50 °C (entry 14), and the increase in temperature did not seem to substantially increase the conversion.

The optimal reaction conditions with benzyl 2-bromo-4-phenylbutanoate used the α-bromoester (4 equiv) and triethylamine (1.5 equiv) in DMF at 50° for 3 days. In order to obtain at least the desirable amount of 10 mg of purified product, the reaction was scaled up to 150 μmol of the starting Fmoc-Phe-Wang (Scheme 50). LC/MS results indicated 69% of the product was formed and 25.8 mg (41% yield) was isolated.

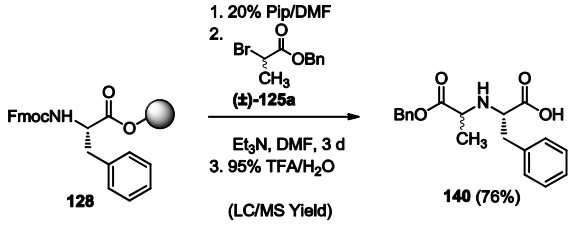


Scheme 50. Optimized N-Alkylation of Phe-Wang with Benzyl 2-Bromo-4-phenyl-butanoate.

3.1.5. N-Alkylation of Fmoc-Phe-Wang with Benzyl 2-Bromopropanoate

The use of benzyl 2-bromopropanoate as an alkylating reagent was of interest in order to evaluate the steric demands between the side chain on the amino acid and the side chain introduced through the N-alkylation with the α-bromoesters. The methyl side chain used earlier (Section 3.1.1) is the least hindered alkyl side chain, which mimics the alanine residues seen in many of the ACE analogs previously discussed (Section 1.1-1.2). Racemic benzyl 2-bromopropanoate (**(±)-125a**) was synthesized from commercially available 2-bromopropanoic acid (Scheme 48, **(±)-133a**). It is noted that, as a control, these experiments were conducted simultaneously alongside those with benzyl 2-bromo-4-phenylbutanoate (**125c**).

First, the optimized reaction conditions from the studies involving ethyl 2-bromopropanoate were used. Fmoc-Phe Wang was deprotected and treated with the α-bromoester (4 equiv) and triethylamine (8 equiv) in DMF at 50 °C for 3 days (Scheme 51). The crude LC/MS results showed 76% of crude product (Table 3, entry 1). These results were similar to those from the N-alkylation with ethyl 2-bromopropanoate (Scheme 46). The complete list of results from this set of optimization experiments is given in Table 3.



Scheme 51. N-Alkylation of Phe-Wang with Benzyl 2-Bromopropanoate.

Table 3. Results from the N-Alkylation of Phe-Wang with Benzyl 2-Bromopropanoate.

Entry	Equiv of 125a	Base (equiv)	TBAI	Solvent	Temp. (°C)	LC/MS (%)[a]
1	4	Et_3N (8)	----	DMF	50	76
2	4	Et_3N (7.5)	2.0	DMF	rt	66
3	4	Et_3N (7.5)	----	NMP	50	82
4	4	Et_3N (8)	2.0	CH_2Cl_2	rt	15
5	4	Et_3N (8)	2.0	CH_3CN	rt	0
6	4	Et_3N (8)	2.0	THF/MeOH (2:1)	rt	0
7	4	Et_3N (8)	----	DMSO	50	62
8	4	Et_3N (4)	----	DMF	50	81
9	4	Et_3N (4)	----	DMSO	50	64
10	4	Et_3N (2.5)	----	DMF	50	82
11	4	Et_3N (1.5)	----	DMF	50	83
12	4	Et_3N (1.5)	----	DMF	70	84

[a] % Product in the reaction mixture. Diastereomeric ratio ranged from 52:48 to 45:54.

The reaction was repeated in the presence of TBAI at room temperature to see if the reaction could be run at ambient temperature (Table 3, entry 2). The resin was treated with benzyl 2-bromopropanoate (4 equiv), triethylamine (7.5 equiv) and TBAI (2 equiv) in DMF. LC/MS results showed 66% product. It is unclear whether the presence of TBAI increased the reaction rate since a reaction at room temperature without TBAI was not conducted; however, the reaction at 50 °C in the absence of TBAI gave a higher conversion of alkylated product.

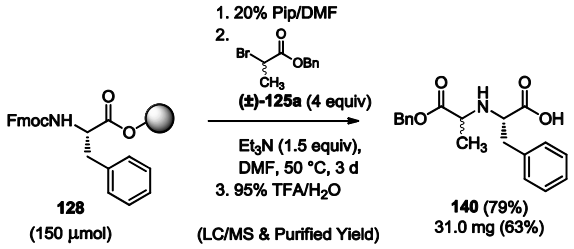
Next, a solvent study was conducted by running the reaction in NMP, methylene chloride, acetonitrile, THF/methanol, and DMSO (Table 3, entries 3-7). As before, the reactions in methylene chloride, acetonitrile, and THF/methanol were conducted at room temperature for three days due to their relatively low boiling points of these solvents. In addition, TBAI was added to these reactions in an attempt to increase the N-alkylation. The reactions in NMP and DMSO were conducted at 50 °C for three days. All of these reactions used the α-bromoester (4 equiv) and triethylamine (8 equiv). LC/MS results showed a high conversion (82%) for the reaction in NMP (entry 3). The reaction in methylene chloride only gave 15% of the desired product, while those run in acetonitrile and THF/methanol did not give any product (entry 4-6). The reaction in DMSO also gave

a reasonable yield of 62% (entry 7). Although NMP gave the highest conversion for benzyl 2-bromopropanoate (entry 3), reactions with the other α-bromoesters worked best in DMF. Thus, DMF was chosen as the best overall solvent. DMSO was also considered a possible solvent for conducting these reactions; however, the contamination discussed earlier was still problematic.

Next, the stoichiometry of triethylamine was explored with DMF and DMSO. The number of equivalents of triethylamine was reduced to 4, 2.5 and 1.5 equivalents, and the reactions were conducted in DMF and in DMSO for 3 days at 50 °C (Table 3, entries 8-11). LC/MS results indicated that reactions run in DMF gave higher conversion than DMSO, and the decrease in the number of equivalents of triethylamine did not affect the yield, confirming only 1.5 equivalents of triethylamine are required for the N-alkylation.

The reaction temperature was explored as a final variable by conducting the reaction at 70 °C. Phe-Wang was deprotected and treated with benzyl 2-bromopropanoate (4 equiv) and triethylamine (1.5 equiv) in DMF (Table 3, entry 12) at 70 °C for 3 days. LC/MS results showed 84% of crude products. Since this conversion was almost identical to the same reaction conducted at 50 °C, it appeared the increase in temperature did not increase the rate of N-alkylation.

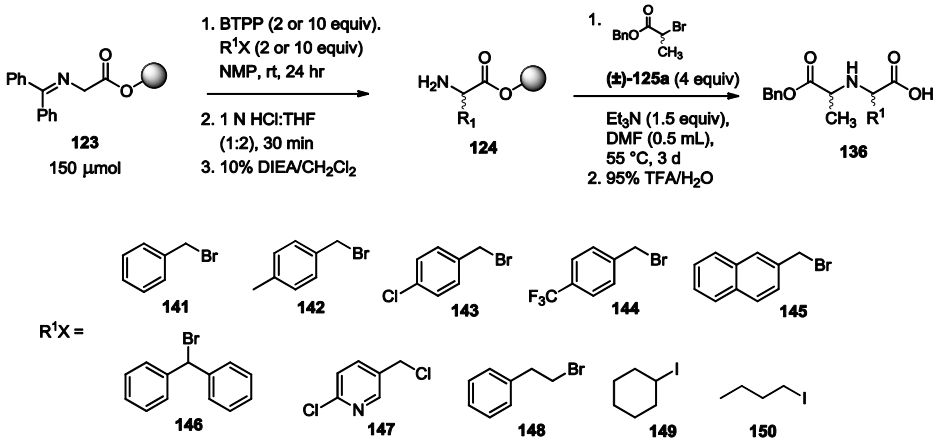
The optimal reaction conditions for the N-alkylation were benzyl 2-bromopropanoate (4 equiv) and triethylamine (1.5 equiv) in DMF at 50 °C for 3 days (Scheme 52). The reaction was scaled-up to 150 μmol of Fmoc-Phe-Wang to determine the amount of isolable product possible after purification. The LC/MS results showed 79% of crude product, which was purified to give 31.0 mg (63% yield) of **140**.



Scheme 52. Optimized N-Alkylation of Phe-Wang with Benzyl 2-Bromopropanoate.

3.2 Evaluation of Alkyl Halides in the Synthesis of Unnatural Amino Acids

Once the N-alkylation of a resin-bound amino acid had been optimized, the application of this step to unnatural amino acids was explored. O'Donnell and Scott *et al.*[54-55,64] have reported the C-alkylation of the resin-bound benzophenone imine of glycine to generate unnatural amino acid side chains. Since this methodology utilizes alkyl halides that are commercially available, a combinatorial approach is easily accessible. A range of alkylating reagents (**141**-**150**) of varying types was tested in conjunction with the N-alkylation of the resulting resin-bound, unnatural amino acid with racemic benzyl 2-bromopropanoate (**(±)-125a**, Scheme 53). Good overall conversions for this four-step reaction sequence were seen by LC/MS analysis of the crude products (49-71%). LC/MS analysis also showed a diastereomeric mixture of each benzyl ester **136**. Isolation of these products was accomplished by cyano-silica chromatography to give 7.5-22.4 mg of product (14-47% purified yield, Table 4).



Scheme 53. Synthesis of Unnatural Amino Acids by C-Alkylation of Glycine.

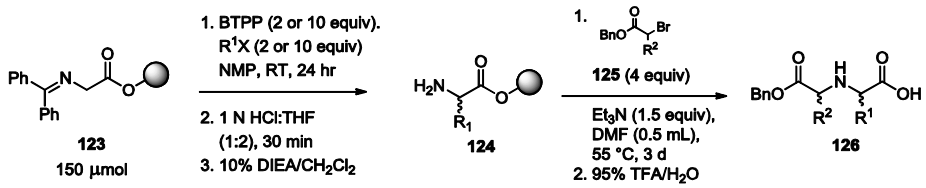
Table 4. Results of C-Alkylation with Alkylating Reagents Followed by N-Alkylation.

R^1X	Conversion by LC/MS	Purified Yield of 126 (%)
141	60%	16.3 mg (33%)
142	71%	18.7 mg (37%)
143	63%	7.5 mg (14%)
144	48%	10.9 mg (18%)
145	67%	13.5 mg (24%)
146	55%	15.1 mg (25%)
147	49%	8.2 mg (15%)
148	70%	17.5 mg (34%)
149	67%	22.4 mg (47%)
150	56%	13.8 mg (31%)

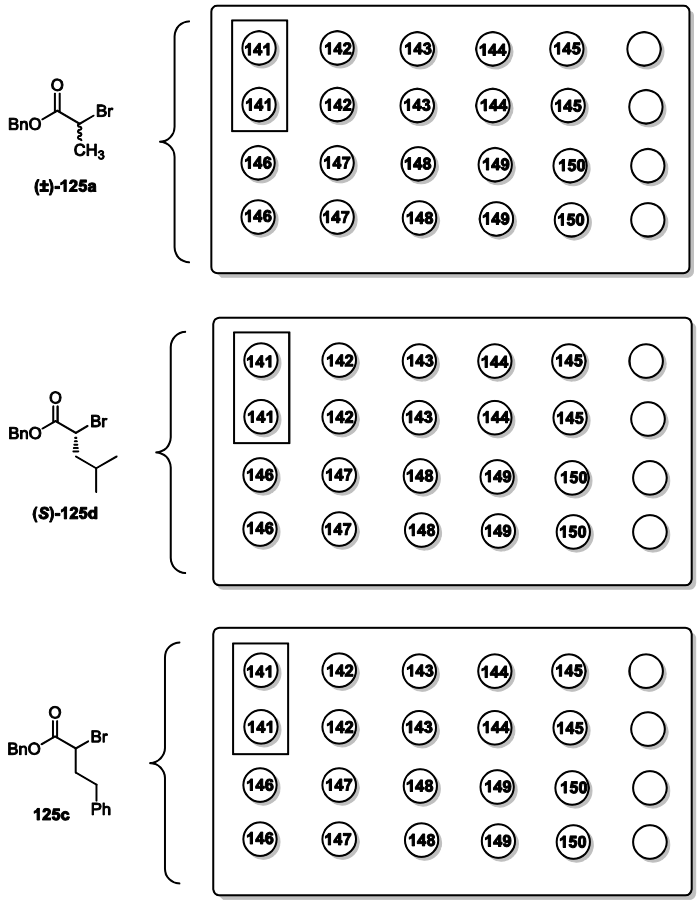
Compared to the percent conversion observed by LC/MS, the isolated yields are much lower. It was difficult to analyze the crude samples for the presence of the trifluoroacetic acid salt of products **136** due to the known presence of the trifluoracetic acid salt of the unalkylated amino acid. ^{19}F NMR of the crude samples showed the presence of fluorine due to this trifluoroacetic acid salt, while a ^{19}F NMR of the purified samples confirmed the isolated material was not the trifluoroacetic acid salt form of product **136**.

3.3. Combinatorial Synthesis of N-Carboxyalkyl Amino Acid Analogs

Next, the combinatorial synthesis of N-carboxyalkyl amino acid analogs **126** was performed using the alkylating reagents (**141**-**150**, Scheme 53) as the input for R^1 and the benzyl α-bromoesters **125a**, **125c** and **125d** (Scheme 47) as the input for R^2 (Scheme 54). Two samples of each analog were prepared to ensure the reproducibility of the synthetic sequence and to provide an additional sample for biological testing. Three 4 x 6 combinatorial reaction boards, one for each of the benzyl α-bromoesters (Scheme 55), with 20 reactions per board (the 10 alkylating reagents in duplicate) afforded two samples of each of the 30 analogs. The benzophenone imine of glycine on Wang resin was alkylated with 2 equivalents of the alkyating reagents **141**-**147** and **150** and 2 equivalents of BTPP. For the less-activated alkylating reagents **148**-**149**, 10 equivalents each of R^1X and BTPP were used following the procedure previously reported by O'Donnell *et al.*[64] The benzophenone imine activating group was then hydrolyzed with hydrochloric acid, and the hydrochloride salt of the unnatural amino acid was neutralized with *N*,*N*-diisopropylethylamine (DIEA). The free amine was alkylated with the corresponding α-bromoester (4 equiv) and triethylamine (1.5 equiv) in DMF at 55 °C. After 3 days the reagents and solvents were removed by filtration and the resin was washed. The crude products were cleaved from the resin with trifluoroacetic acid, and the results are shown in Table 5.

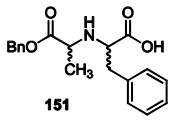
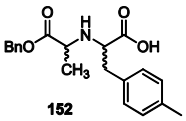
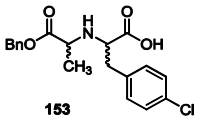
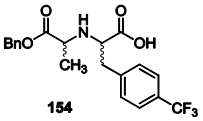
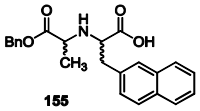
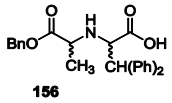
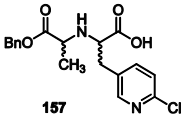
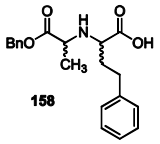
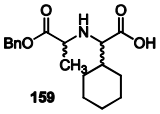
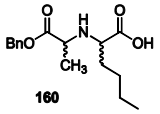


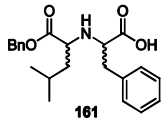
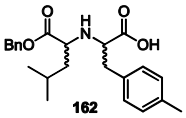
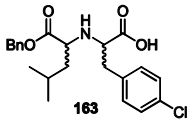
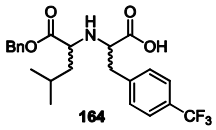
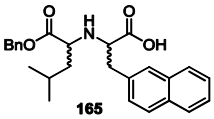
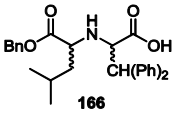
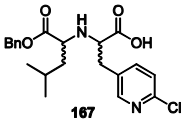
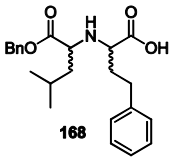
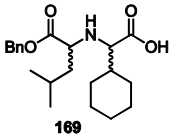
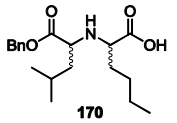
Scheme 54. Combinatorial Synthesis of N-Carboxyalkyl Amino Acid Analogs **126**.

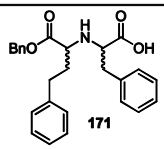
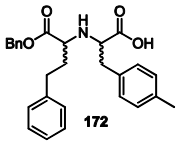
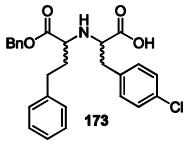
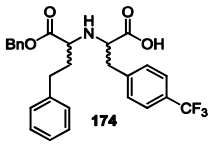
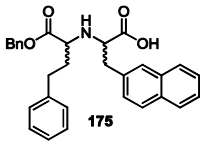
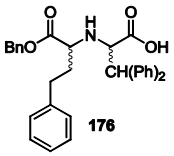
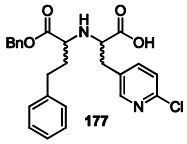
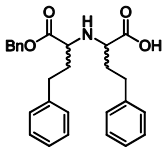
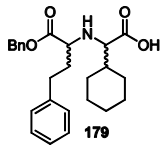
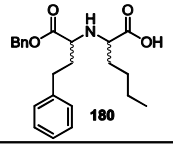


Scheme 55. Combinatorial Set-Up for Synthesizing N-Carboxyalkyl Amino Acid Analogs **126**.

Table 5. Results of the Combinatorial Synthesis of N-Carboxyalkyl Amino Acid Analogs.

Entry	Structure	LC/MS % (Crude)[a]	Crude Yield (mg)	Purified Yield (mg) (% Yield)	LC/MS % Purity
1	 151	72% 66%	53.6 52.0	25.8 (53%) 25.1 (51%)	98% 98%
2	 152	68% 76%	55.0 58.8	22.5 (44%) 20.3 (40%)	98% 100%
3	 153	67% 72%	58.6 58.8	14.6 (27%) 7.0 (13%)	95% 92%
4	 154	64% 59%	64.4 64.4	18.5 (31%) 21.6 (36%)	99% 99%
5	 155	67% 67%	64.5 63.0	20.0 (35%) 13.5 (24%)	89% 98%
6	 156	34% 32%	46.8 52.1	6.0 (10%) 10.0 (17%)	90% 90%
7	 157	55% 60%	62.2 62.0	11.9 (22%) 15.2 (28%)	100% 100%
8	 158	69% 75%	49.8 48.5	11.1 (22%) 19.4 (38%)	100% 100%
9	 159	65% 65%	45.0 42.3	10.0 (21%) 7.6 (16%)	100% 100%
10	 160	59% 63%	43.4 44.1	6.2 (14%) 18.9 (43%)	100% 95%

Entry	Structure	LC/MS % (Crude)[a]	Crude Yield (mg)	Purified Yield (mg) (% Yield)	LC/MS % Purity
11	 161	39% 39%	43.5 44.1	16.9 (30%) 11.0 (20%)	90% 96%
12	 162	41% 41%	46.0 45.1	12.2 (21%) 15.2 (26%)	98% 96%
13	 163	33% 34%	46.5 46.6	13.4 (22%) 15.6 (26%)	87% 93%
14	 164	29% 28%	49.3 49.4	13.8 (21%) 7.5 (11%)	93% 93%
15	 165	40% 37%	45.5 51.2	15.0 (24%) 15.0 (24%)	96% 92%
16	 166	20% 23%	47.6 48.3	4.7 (8%) 5.7 (9%)	96% 95%
17	 167	21% 19%	49.8 50.0	9.4 (15%) 8.4 (14%)	87% 87%
18	 168	35% 35%	32.7 34.0	4.2 (7%) 12.6 (22%)	94% 90%
19	 169	34% 37%	29.6 29.3	18.0 (33%) 2.6 (5%)	93% 93%
20	 170	40% 38%	34.7 33.0	14.4 (29%) 14.2 (29%)	91% 95%

Entry	Structure	LC/MS % (Crude)[a]	Crude Yield (mg)	Purified Yield (mg) (% Yield)	LC/MS % Purity
21	 171	51% 45%	61.5 60.8	4.6 (7%)	100%
22	 172	55% 52%	65.5 69.0	8.4 (13%) 12.6 (19%)	100% 100%
23	 173	49% 54%	67.1 73.7	12.9 (19%) 8.7 (13%)	100% 100%
24	 174	43% 48%	76.1 72.3	3.0 (4%) 5.7 (8%)	100% 100%
25	 175	47% 44%	81.8 73.3	9.8 (14%) 8.0 (11%)	100% 100%
26	 176	28% 27%	50.0 47.3	3.8 (5%) 2.6 (4%)	100% 100%
27	 177	30% 28%	80.1 72.5	4.0 (6%) 5.0 (7%)	100% 100%
28	 178	56% 56%	46.5 50.7	8.2 (13%) 8.8 (14%)	100% 100%
29	 179	72% 72%	41.6 46.1	8.6 (14%) 7.2 (12%)	100% 100%
30	 180	68% 67%	47.3 43.8	7.3 (13%) 9.4 (16%)	100% 100%

[a] Diastereomeric ratios ranged from 68:33 to 44:56. See Experimental Section for individual values.

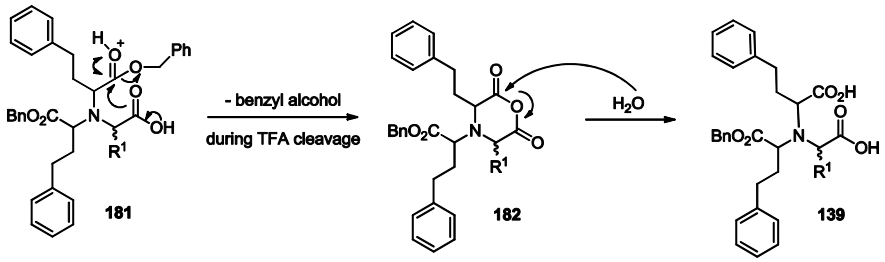
In general, the unnatural amino acids that were N-alkylated with benzyl 2-bromopropanoate (**(±)-125a**) gave the highest conversions to products **151**-**160** (Table 5, entries 1-10) by LC/MS analysis. The analogs **161**-**170** (Table 5, entries 11-20) prepared using benzyl 2-bromo-4-methylpentanoate (**(*S*)-125d**) gave the lowest conversions of the three α-bromoesters, rationalized by the disubstituted branching on the γ-carbon. Benzyl 2-bromo-4-phenylbutanoate (**(±)-125c**) gave reasonable conversions to the N-alkylated products **171-180** (Table 5, entries 21-30), but not as high as **(±)-125a**, likely due to the increased steric hindrance by the phenethyl side chain on the α-bromoester. Overall, the LC/MS yields indicated in Table 5 were much higher than the isolated yields. Quantitative NMR of the crude mixture of **157** gave a 34% yield, much closer to the purified yield. This result suggests the LC/MS results are higher than the actual yield due to higher UV absorption of the products versus the unalkylated amino acids. This indicates all of the N-alkylated product was eluted from the column and no trifluoroacetic acid salt of the product formed, contrary to the discussion in Section 3.2.

The more sterically demanding alkylating reagents 2-(bromomethyl)naphthalene **145** (product entries 5, 15 and 25) and bromodiphenylmethane **146** (product entries 6, 16 and 26) gave poorer yields of products, likely due to the increased steric hindrance employed by these unnatural side chains on the resin-bound amino acid. 4-Chlorobenzyl bromide **143** (product entries 3, 13 and 23) and 2-chloro-5-(chloromethyl)pyridine **147** (product entries 7, 17 and 27) alkylating reagents gave comparable conversion (LC/MS) to the analogs prepared with the same α-bromoester; however, the isolated yields after column chromatography were consistently lower for these alkylating reagents. These analogs were more insoluble in organic solvents seen by their salt-like character after purification, likely making it more difficult for all of the product to elute from the column resulting in the lower yields.

For most cases the duplicate samples gave consistent results for crude LC/MS results, crude yields and purified yields. These results confirm the reproducibility and robust synthetic sequence for isolating compounds **126**. A few cases, however, showed similar results for the crude LC/MS and crude yield but gave very different purified yields (Table 1, entries 8, 10, 18, and 19). These inconsistencies are likely a result of conducting the purifications in a combinatorial fashion by purifying multiple samples at once, usually 5 or 10 samples at a time. The lower isolated yields for one sample may

have been due to contaminated fractions during the column chromatography, ultimately determined by how evenly the sample was loaded on the prepacked cyano-silica gel cartridges. Some crude samples were insoluble and required more solvent to transfer the crude material to the column. This would create a larger bandwidth for the loaded material, causing a poorer separation.

The analogs made with the α-bromoesters **(±)-125a** and **(*S*)-125d** were easily isolated using cyano-silica gel chromatography. However, the LC/MS results for the purified analogs alkylated with the α-bromoester **(*S*)-125c** showed a large amount of dialkylated material **139** (~10-25%) that had also been debenzylated by an intramolecular loss of one equivalent of benzyl alcohol, likely through the anhydride **182** (Scheme 56). Cyano-silica gel and silica gel chromatography did not give separation of the desired monoalkylated product **138** from the dialkylated by-product **139**. These analogs (entries 21-30) were first purified on cyano-silica gel to remove impurities, and then the mixture of the monoalkylated and dialkylated products were separated by preparative liquid chromatography using reverse-phase conditions. This purification method also allowed separation of the diastereomers for most analogs, and each of the diastereomers were isolated and characterized. The purified yields of these analogs (entries 21-30) reflect the combined yields of the two diastereomers and are slightly lower due to the requirement of two purifications to isolate the desired products from the by-product of the dialkylated material.



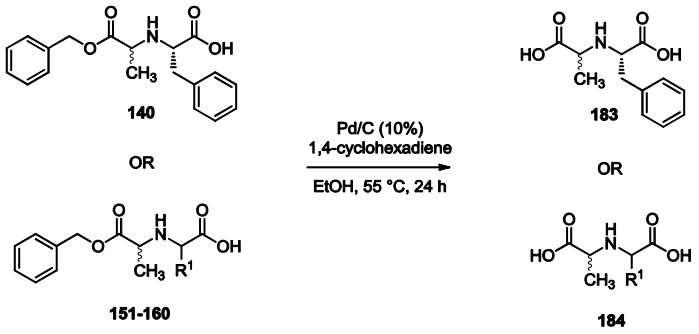
Scheme 56. Mechanism of the Formation of the Dialkylated By-Product **136**.

The analogs alkylated with the α-bromoester containing the isobutyl group [**(*S*)-125d**, entries 11-20] showed <5% of the corresponding dialkylated products by LC/MS. The identification of this contaminant was not determined until after complete purification and analysis of each analog and the large amount of this by-product had been observed in the synthesis of analogs prepared with **(*S*)-125c** (entries 21-30). The isobutyl analogs

(entries 11-20) were not purified a second time by preparative liquid chromatography to remove the dialkylated material, and the LC/MS results of the purified compounds reflect this in Table 5. The analogs made with the α-bromoester containing the methyl group [**(±)-125a**, entries 1-10] did not show the presence of dialkylated material. Taken together, these results indicate that there is more dialkylation with the larger side chain on the α-bromoester. All of the purified samples were analyzed by LC/MS, ^{1}H NMR, ^{13}C NMR, and HRMS. The NMR spectra for the products alkylated with the α-bromoester **(*S*)-125d** (entries 11-20) show contaminant peaks for the dialkylated by-product.

3.4 Deprotection of Benzyl-Protected N-Carboxyalkyl Amino Acids to the Diacid

The conversion of the benzyl ester intermediates **140** and **151**-**160** (where R^2 = CH_3) to the N-carboxyalkyl amino acids was explored using hydrogenolysis conditions (Scheme 57). The benzyl ester was dissolved in ethanol and Pd/C (10%, 20-35 mg) was added depending on the amount of starting material (5–25 mg). 1,4-Cyclohexadiene (20 equiv) was then added by a syringe. The reaction temperature (room temperature to 55 °C) and length of the reaction (6-24 h) were evaluated. Reactions conducted at room temperature did not always show complete conversion to the carboxylic acid, and many of the benzyl esters were not soluble in ethanol unless heated at elevated temperatures. Thus, a reaction temperature of 55 °C was used for optimal conversion. Reactions conducted for 6-12 hours did not always show complete conversion by LC/MS either, and reactions were allowed to proceed for 24 hours to ensure complete deprotection.



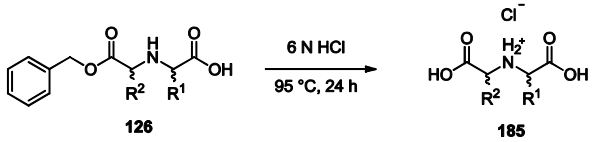
Scheme 57. Hydrogenolysis of Benzyl Ester Intermediates to the N-Carboxyalkyl Amino Acids.

The crude reaction mixtures were filtered through celite with ethanol and concentrated under a stream of nitrogen or under vacuum. LC/MS analyses of these reactions often showed the desired products **183** and **184** streaking on the column with poor ionization through the mass spectrometer. However, the disappearance of the starting benzyl ester was observed. Initial attempts to obtain NMR data showed these compounds were very hygroscopic and lyophilization was required to remove all water from the samples. After lyophilization, it was still difficult to obtain clean spectra because the hygroscopic character of these compounds due to the presence of a small amount of water interfering with the proton shifts. Attempts to obtain ^{13}C NMR of **183** and **184** also gave inconsistent results. Some samples gave clear carbon signals while others gave no carbon signals at all. Indications that the presence of palladium in the samples was a potential problem became apparent when palladium was observed settling out of the DMSO-d_6 solution of one compound in its NMR tube. It was hypothesized that the presence of palladium in the samples was interfering with the ability to acquire NMR spectra.

Micron syringe filters (0.2-0.5 micron) were used in an attempt to remove the palladium, but the quality of the ^{13}C NMR spectra was not improved. SciFinder searches of the N-carboxyalkylamino acids show a large number of these scaffolds as ligands to metals, suggesting the amine and two carboxylic acids were complexing with palladium. An elemental analysis was performed on a sample that had been hydrogenolyzed under the conditions described above, and the results showed 12.6% palladium present in the sample.

The metal scavengers Smopex-234 and DARKO were used in an attempt to remove the palladium from the reaction mixture,[65] but without success. One sample was heated with Smopex-234, but NMR analysis showed no evidence of the desired product present. Other samples were stirred in DARKO at room temperature, and NMR analyses did not show any improvement in the acquisition of carbon signals. An elemental analysis was obtained for one of the samples stirred with DARKO for four days, and the presence of palladium was confirmed at 17.2%. It appeared the interaction between the carboxylic acids and amine of the N-carboxyalkylamino acid compounds and palladium was too strong to completely remove the palladium from the samples that had been hydrogenolyzed.

Therefore, hydrolysis conditions were used for deprotection of the benzyl ester intermediates. Initial tests were performed on analogs **161**-**170** and then the hydrolysis was carried out on the remaining analogs (Scheme 58). Heating the benzyl esters in 6 N HCl (2 mL) at 95 °C in an oil bath or oven for 24 hours gave complete debenzylation by LC/MS. Initially hydrolysis was carried out in a 1:1 mixture of 6 N HCl/dioxane; however, the dioxane also hydrolyzed to side products as observed by the high yield and ^{1}H NMR. The dioxane was removed and hydrolysis was carried out in 6 N HCl at 95 °C.



126 185

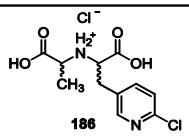
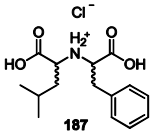
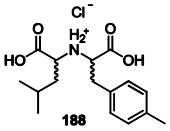
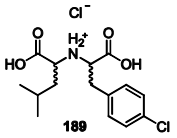
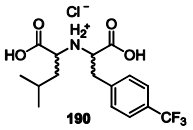
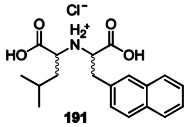
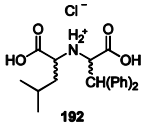
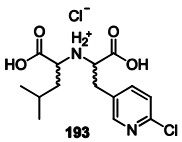
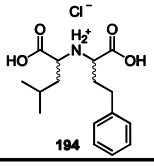
Scheme 58. Hydrolysis of Benzyl Ester Intermediates to the N-Carboxyalkyl Amino Acids.

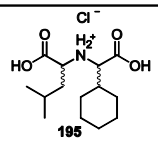
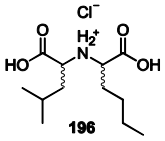
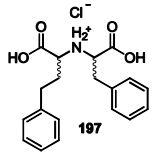
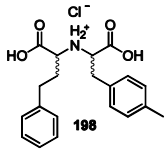
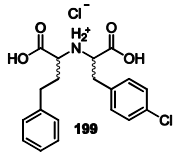
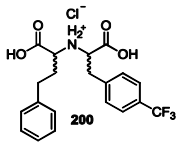
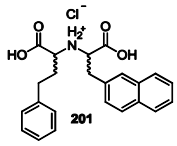
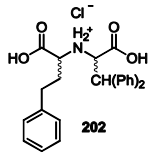
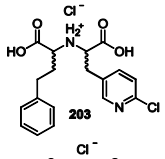
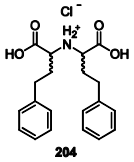
Hydrolysis of intermediates **157** and **161-170** showed complete conversion of the starting benzyl esters by LC/MS, and the corresponding ions for the diacids were observed in the mass spectra. After evidence of complete hydrolysis, the reaction mixtures were concentrated under vacuum and lyophilized to afford white or yellow solids as the hydrochloride salt products. The isolated yields were consistently higher than those that were previously observed under the hydrogenolysis conditions (**186-196**, Table 6).

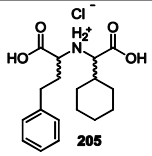
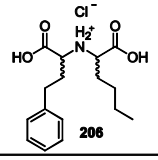
Since the diastereomers of the benzyl esters **171-180** were separated by preparative liquid chromatography, the duplicate samples of each separate diastereomer (**171**-**180**) were combined and hydrolyzed to the diacids **197**-**206**. In some cases complete separation of the benzyl ester diastereomers was not possible with the reverse-phase conditions used, and these samples were hydrolyzed as mixtures (indicated in Table 6). For the diacids **186**-**196** (Table 6, entries 1-11), hydrolysis was performed on the mixture of diastereomers. For **189** (entry 4), the initial purity of the benzyl ester starting material was only 87%, giving a lower purity for the isolated product. A few examples (**198**-**200**, entry 13-15) were insoluble in the 6 N HCl solution and showed incomplete hydrolysis after 24 hours. A small amount of DMSO was added to these samples to assist in solubilizing the benzyl ester. Even after the addition of DMSO, these samples still did not completely dissolve in solution, resulting in incomplete hydrolysis. These samples were not further characterized. All other samples were

analyzed by ^{1}H and ^{13}C NMR, when quantities permitted (>6 mg), and high-resolution mass spectrometry.

. **Table 6.** Results from the Hydrolysis of the Benzyl Ester Intermediates to the Diacids.

Entry	Structure	Overall Isolated Yield mg (% Yield)	LCMS %
1	 186	12.4 (27%)	100%
2	 187	12.4 (26%)	100%
3	 188	6.0 (12%)	99%
4	 189	10.0 (19%)	88%[a]
5	 190	5.0 (9%)	97%
6	 191	11.8 (22%)	100%
7	 192	4.0 (7%)	100%
8	 193	7.8 (15%)	100%
9	 194	11.0 (22%)	95%

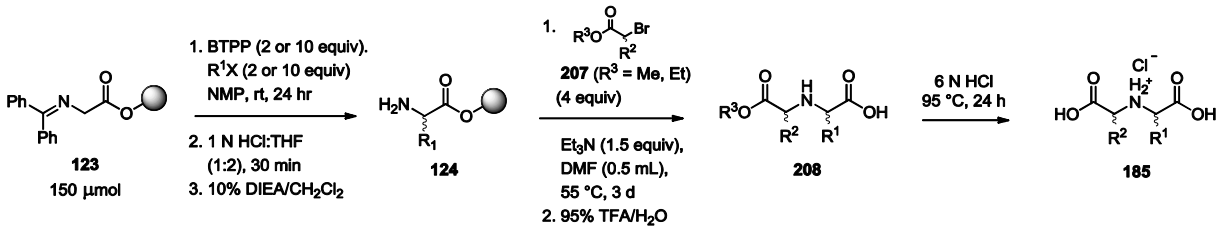
Entry	Structure	Overall Isolated Yield mg (% Yield)	LCMS %
10	 195	------[b]	100%
11	 196	8.9 (21%)	100%
12	 197	4.5 (8%) 3.7 (7%)	Diast. 1: 100% Diast. 2: 100%
13	 198	Insoluble 12.0 (21%)	Diast. 1: Incomplete Diast. 2: 100%
14	 199	Insoluble 8.4 (14%)	Diast. 1: Incomplete Diast. 2: 100%
15	 200	Insoluble	Mixture: Incomplete
16	 201	5.0 (8%) 6.0 (10%)	Diast. 1: Incomplete Diast. 1: 100%
17	 202	3.6 (5%) 4.3 (7%)	Diast. 1: 100% Diast. 2: 100%
18	 203	3.5 (6%) 4.1 (7%)	Diast. 1: 100% Diast. 2: 100%
19	 204	5.5 (10%) 8.5 (15%)	Diast. 1: 100% Mixture: 100%

Entry	Structure	Isolated Yield (mg) (Overall % Yield)	LCMS %
20	 205	6.8 (13%) 4.6 (9%)	Diast. 1: 100% Diast. 2: 100%
21	 206	10.8 (22%) 4.4 (9%)	Mixture:100% Diast. 2: 100%

[a] Initial sample purity – 87%.
[b] Tare lost during hydrolysis.

3.5. Synthesis of an Ethyl Ester Analog and Subsequent Hydrolysis to the Amino Diacid

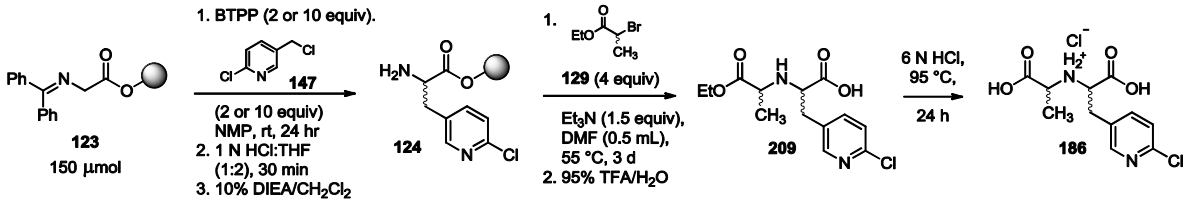
Since hydrogenolysis conditions were not successful for conversion of the benzyl ester intermediates **126** to the amino diacids **185** (Section 3.4) and hydrolysis conditions were successful, methyl or ethyl α-bromoesters **207** could be used to prepare alkyl ester intermediates **208** (Scheme 59). The benzophenone imine of glycine **123** could be alkylated with various alkylating reagents to give the resin-bound unnatural amino acid **124**. N-Alkylation could then be performed using a methyl or ethyl α-bromoester **207**, and cleavage from the resin would give the alkyl ester intermediate **208**. Hydrolysis of this intermediate would then give the amino diacids **185**.



Scheme 59. Use of Alkyl α-Bromoesters to Prepare Amino Diacids **185**.

Analog **186** was made through the ethyl ester intermediate **209** using racemic ethyl 2-bromopropanoate **129**. The benzophenone imine of glycine **123** was alkylated with 2-chloro-5-(chloromethyl)pyridine **147** to give the unnatural amino acid **124**, which was then N-alkylated with **129** and cleaved from the resin. The crude ethyl ester intermediate **209** was purified over cyano-silica gel to give 14 mg (31% yield). The ester

was then hydrolyzed under acidic conditions to give 15.7 mg of amino diacid **186** (34% overall yield, Scheme 60).



Scheme 60. Synthesis of Amino Diacid **186** through Ethyl Ester **209**.

CHAPTER 4. CONCLUSIONS

The solid-phase synthesis of N-carboxyalkyl unnatural amino acids was accomplished through the N-alkylation of resin-bound unnatural amino acids with α-bromoesters. The unnatural amino acids were obtained by C-alkylation of resin-bound glycine. The N-alkylation step with α-bromoesters to give N-carboxyalkyl unnatural amino acids that mimic commercially available drugs for ACE inhibitors, especially those incorporating the phenethyl side chain, was optimized. The preparation of the benzyl α-bromoester starting reagents for the N-alkylation was carried out in order to permit hydrogenolysis conditions for the debenzylation step. However, the complexation of palladium catalyst was problematic and was overcome by acidic hydrolysis of the benzyl esters to the carboxylic acids. Since it was demonstrated that the benzyl ester hydrogenolysis was unsuccessful, other alkyl α-bromoesters would likely be preferable to the benzyl α-bromoesters for future experiments. The synthesis of analog **186** with the ethyl α-bromoester was completed; however, if the synthetic route to prepare the α-bromoesters would be used, control experiments would need to be conducted to determine the stereochemical fate of the α-carbon on the starting amino acid. The combinatorial synthesis of the thirty analogs was shown to be a powerful tool for synthesizing many analogs at one time. Considering the stereospecificity of the ACE active site, separation of the diastereomers was possible in order to test each racemic diastereomer separately in biological assays, but the relative configuration of these diastereomers would need to be determined in the future.

CHAPTER 5. EXPERIMENTAL SECTION

5.1 General Methods

All chemicals and organic solvents were reagent grade and used directly without further purification. Acetone, 2-(bromoethyl)benzene, chloroform-d_1, 5-(chloromethyl)-2-chloropyridine, cyclohexyl iodide, triethylamine, 4-trifluoromethylbenzyl bromide, hexanes, *N*,*N*-dimethylformamide (DMF), and dimethylsulfoxide-d_6 were purchased from Acros Organics. Benzyl bromide, 2-(bromomethyl)naphthalene, *n*-butyl iodide, 4-chlorobenzyl bromide, trifluoroacetic acid (TFA), *N*,*N*-diisopropylethylamine (DIEA), 4-methylbenzyl bromide, diphenylbromomethane, and piperidine were purchased from Aldrich Chemical Co. Acetonitrile, hydrochloric acid, methanol, and 1-methyl-2-pyrrolidone (NMP) were purchased from Fisher Scientific. Dichloromethane, absolute ethanol and tetrahydrofuran (THF) were purchased from Pharmco-Aaper. *tert*-Butylimino-tri(pyrrolidino)phosphorane (BTPP) was purchased from Fluka. Fmoc-Phenylalanine on Wang resin (Fmoc-Phe-W, 0.70 mmol/g, 75–150 μm) and benzophenone imine of glycine Wang resin (PL-BIG-W, 0.83 mmol/g, 75–150 μm) were purchased from Polymer Laboratories. DARKO (20-40 mesh) was purchased from EM Science. SMOPEX-234 was purchased from Johnson Matthey. Solution- and solid-phase organic transformations and resin washes were carried out at ambient temperatures unless otherwise indicated. Ratios in all solvents and reagent mixtures prepared are volume to volume unless otherwise noted.

Manual solid-phase organic syntheses were carried out in two types of reaction vessels. Small scale reactions (50 μmol) were performed in 3.5 mL fritted glass reaction vessels (Chemglass, IUP-0305-270H) equipped with polypropylene screw caps (Chemglass, IUP-0305-280H) with Teflon-faced silicon septa (Chemglass, IUP-0305-281H) on the Bill-Board set. Small scale reactions (150 μmol) were performed in 5.0 mL fritted glass reaction vessels (Chemglass, IUP-0905-210PS) equipped with polypropylene screw caps (Chemglass, IUP-0305-280H) with Teflon-faced silicon septa

(Chemglass, IUP-0305-281H) on the Bill-Board set. The Bill-Board set was designed by Professor Scott as inexpensive equipment to simplify and expedite multiple, manual solid-phase syntheses.[66] Motor rotators were used as rotation apparatus for small scale reactions.

Depending on the number of reactions performed, the starting resin was distributed either by weight (≤ 6 reactions) or as aliquots from an isopycnic suspension (>6 reactions). The resin for the optimization reactions, performing one to six reactions at a time, was distributed by weight, typically 50 μmol (with a known loading) unless otherwise noted, and the resin was swelled for 30 min to 1 h in 2:1 CH_2Cl_2/DMF (or CH_2Cl_2/NMP). The resin for the combinatorial syntheses of N-carboxyalkyl amino acid analogs in the 6 x 4 Bill-Board set was distributed as aliquots from an isopycnic suspension from a neutral buoyancy suspension in CH_2Cl_2/NMP, distributing 150 μmol of the starting resin (with a known loading) by repeated aliquots (1–2 mL) to each of the reaction vessels in the Bill-Board. During the distribution of the resin, the isopycnic solvent was allowed to drain through the frit in the reaction vessels. When distribution was complete, residual solvent was removed with an “air-push” from a disposable plastic pipet (Fisher, 13-711-23) fitted with a pierced septum (Aldrich, Z12743-4). The bottom of each reaction vessel was then capped, and a new calibrated pipet (Fisher, 13-711-24) was used for adding each reagent in the following step. The tops of all reaction vessels were capped and the Bill-Board was placed on an appropriate rotation apparatus. Following the reaction, the reagents and solvents were drained and the resin product was washed with the indicated solvents. For washing of small scale reactions, at least 30 sec was normally used after addition of solvents to the reaction vessels (with bottom end open for draining) followed by an “air-push.” Solid-phase reactions at elevated temperatures were carried out in an Isotemp® Oven Model 280A (Fisher Scientific) with the reaction vessels capped to finger tightness.

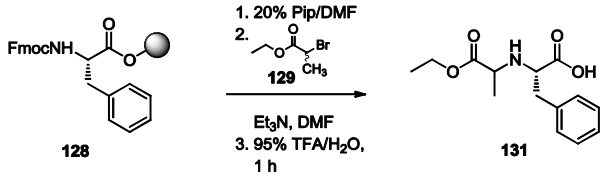
Analytical thin layer chromatography (TLC) was performed on EM Science silica gel 60 F_{254}, 0.25 mm pre-coated glass plates (EMD Chemical Inc., 5715-7) or EM Science cyano-silica gel F_{254}, 0.15-0.20 pre-coated glass plates (EMD Chemical Inc., 16464-5). TLC plates were visualized using UV_{254}. Column chromatography was performed on HyperSep Cyano-Silica 3 mL cartridges (60108-748) pre-loaded with 500 mg of silica gel 60 (irregular particles 40 – 63 μm) from ThermoScientific for 50 μmol small scales reactions. For 150 μmol small scale reactions, the cyano-silica gel from two

of the HyperSep Cyano-Silica® 3 mL cartridges were combined to form 1 g columns in one of the 3 mL cartridges. Reverse-phase chromatography was performed on a Waters PrepLC 400 System with ISCO Absorbance Detector and an Agilent Eclipse XDB-C_{18} columns (5 μm, 9.4 x 250 mm, PN 990967-202). The isocratic mobile phase used for these purifications was 1:1 0.1% TFA/CH_3CN:H_2O. Yields of the final compounds, after chromatographic purification, were calculated on the basis of the initial loading of the starting resins and are the overall yields for all reaction steps starting from these resins.

LC/MS analyses were conducted using an Agilent system, consisting of a 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion electrospray ionization. The LC/MS samples were analyzed as solutions in CH_3CN or a mixture of CH_3CN:CH_3OH. The LC/MS samples analyzed for the hydrolysis reactions were run as aliquots from the aqueous reaction mixture. The LC/MS-derived composition of mixtures was determined based on UV integration at 210 nm. The LC/MS chromatography was carried out on an Agilent Eclipse XDB-C_{18} column (5 μm, 4.6 x 250 mm, PN 990967-902) eluting with 1:1 acetonitrile/methanol containing 5 mM NH_4OAc as the organic mobile phase and water containing 5 mM aqueous NH_4OAc as the aqueous mobile phase. This system was used for all compounds except those purified by PrepLC, which were eluted with an Agilent Eclipse C_{18} column (5 μm, 4.6 x 150 mm, PN 993967-902) eluting with 0.1% TFA/CH_3CN solvent system.

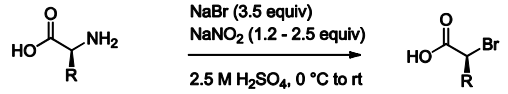
^1H NMR and ^{13}C NMR spectra were recorded at 500 MHz (Bruker Advance III 500 spectrometer) using TMS (0.00 ppm) and chloroform-d_1 or dimethylsulfoxide-d_6. ^{13}C NMR were run with broadband decoupling, and the carbon signals for the carbonyl carbons were not always visible in the spectra. Copies of the NMR spectra reported in this experimental are located in Appendix A. High-resolution mass spectrometry results were obtained on a MAT 95XP (Thermo Electron Corp.) with electron impact (EI) or chemical ionization (CI) methods at Indiana University, Bloomington, IN. Optical rotations were performed using a PerkinElmer Model 343 Polarimeter with a polarimeter cell (1-mL capacity and 100-mm pathlength) at Indiana University, Bloomington, IN. Concentrations are reported in g per 100 mL. Elemental analyses were performed by Midwest Microlabs, Indianapolis, IN.

5.2 General Procedure for the Optimization of N-Alkylation of Resin-Bound Amino Acids with Ethyl 2-Bromopropanoate: *N*-[1-Methyl-2-oxo-2-(ethoxy)ethyl]phenylalanine



Fmoc-Phe-Wang resin **128** (72 mg, 0.70 mmol/g, 50 μmol) was added to a solid-phase reaction vessel (3 mL) and swelled in 2:1 CH_2Cl_2/NMP for 1 h. The resin was filtered, a solution of 20% piperidine/DMF (2 mL) was added and the vessel was rotated at room temperature for 30 min. The resin was filtered and washed with DMF (3 x 2 mL). A mixture of the ethyl 2-bromopropanoate (200 or 400 μmol, 2 or 4 equiv) and triethylamine (400 or 800 μmol, 4 or 8 equiv) in DMF (0.5 mL) was added. If tetrabutylammonium iodide (TBAI) was used, it was then added to the vessel as a solid. The resin was heated at 50 °C for the indicated time (1-4 d) referenced in Chapter 3.1.1. The vessels were allowed to cool to room temperature and the resin was washed with DMF (3 x 2 mL), MeOH (3 x 2 mL), THF (3 x 2 mL), and MeOH (3 x 2 mL). A solution of 95% TFA/H_2O (2 mL) was added to each vessel followed by rotation for 1 h. The filtrate-containing product was collected in tared vials and the cleavage solution was evaporated with a stream of nitrogen. Each sample was analyzed by LC/MS and cyano-silica TLC plates (30% acetone/hexanes). The products were purified by loading the crude product (13-19 mg) onto a dry 500 mg cyano-silica cartridge with 200-300 μL of acetone and allowing the column to dry with a stream of nitrogen. The column was then washed with hexanes (2 x 2 mL) and eluted with 30% acetone/hexanes (8 x 1 mL). Fractions were analyzed by cyano-silica TLC plates (30% acetone/hexanes) and pooled to afford the product as a white solid. For the reaction under optimal conditions (Scheme 47), **131** was obtained as a white solid (12.0 mg, 91% isolated yield). Crude LC/MS purity 76%, t_R = 3.8, 4.0 min (dr 52:48 – 55:45); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24 (t, *J* = 14.3 Hz, 3H), 1.33 (d, *J* = 7.2 Hz, 1.5H), 1.42 (d, *J* = 7.2 Hz, 1.5H), 3.17 (m, 1H), 3.34 – 3.48 (m, 2H), 3.68 (m, 1H), 4.15 (m, 2H), 7.30 – 7.35 (m, 5H).

5.3 General Procedure for the Conversion of Amino Acids to α-Bromoacids (**133b-d**)

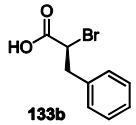


(*S*)-132b, R = CH_2Ph
(*S*)-132c, R = CH_2CH_2Ph
(*S*)-132d, R = $CH(CH_3)_2$

(*S*)-133b, R = CH_2Ph (69%)
(*S*)-133c, R = CH_2CH_2Ph (25-38%)
(*S*)-133d, R = $CH(CH_3)_2$ (34%)

To the amino acid **(*S*)-132b-d** (2.4-13.8 mmol) in a 100 – 250 mL round-bottom flask equipped with a magnetic stirrer was added 2.5 M H_2SO_4 (1.3–1.5 mL/mmol) at room temperature. Sodium bromide (3.5 equiv) was added with stirring and the mixture was placed in an ice bath (0 °C), where it rapidly became a thick solution. Sodium nitrite (1.2 equiv) in water (2–5 mL) was added in small portions (about 5 drops every 5 min), with each releasing a yellow gas. If the solution had not changed from a heterogeneous, white mixture to a clear solution after 1 h, additional sodium nitrite was added as a solid until no color change was observed. Stirring was continued for 6-24 h. Experimentation with the reaction conditions showed that over time a polymer-like solid formed, especially when the reaction was allowed to continue overnight. (This solid may be a polymer-like compound due to alkylation on the nitrogen of the starting amino acid with the α-bromoacid product.) Also, when colder reaction conditions were maintained (-10 °C to 0 °C), higher yields were observed, particularly for converting **(*S*)-132c** to **(*S*)-133c**. The reaction was quenched with brine and extracted three times with ethyl acetate. The organic layers were washed with brine and dried over Na_2SO_4. The crude material was loaded on a silica column and eluted with 0–5% CH_3OH/CH_2Cl_2 (0.2% TFA). The purified products were obtained as oils.

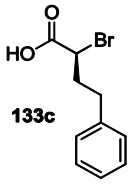
(*S*)-2-bromo-3-phenylpropanoic acid (133b)



133b

Compound **133b** was obtained from **132b** (2.00 g, 12.1 mmol) as a yellow oil (1.90 g, 69% isolated yield) following chromatographic purification over silica gel with 0.2% TFA/CH_2Cl_2. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.23 – 3.27 (m, 1H), 3.44 – 3.49 (m, 1H), 4.42 (t, *J* = 8.0 Hz, 1H), 7.22 – 7.32 (m, 5H).[56]

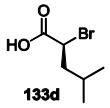
(*S*)-2-bromo-4-phenylbutanoic acid (133c)



133c

Compound **133c** was obtained from **132c** (2.00 g, 11.2 mmol) as a pale yellow oil (1.464 g, 54% isolated yield) following chromatographic purification over silica gel with 0.2-0.3% TFA/CH_2Cl_2. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.31 – 2.39 (m, 2H), 2.77 – 2.85 (m, 2H), 4.19 (t, *J* = 6.2 Hz, 1H), 7.21 – 7.31 (m, 5H).[65]

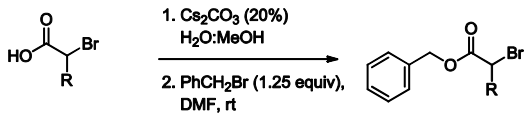
(*S*)-2-bromo-4-methylpentanoic acid (133d)



133d

Compound **133d** was obtained from **132d** (10.15 g, 77.4 mmol) as a clear oil (5.075 g, 34% isolated yield) following chromatographic purification over silica gel with 0.2% TFA/CH_2Cl_2. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H), 1.82 (m, 1H), 1.92 (t, *J* = 7.3 Hz, 2H), 4.30 (t, *J* = 7.7 Hz, 1H).[56]

5.4 General Procedure for Conversion of α-Bromoacids to Benzyl α-Bromoesters (**125a-d**)



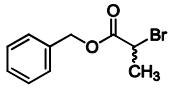
(±)-133a, R = CH_3
(*S*)-133b, R = CH_2Ph
(*S*)-133c, R = CH_2CH_2Ph
(*S*)-133d, R = $CH(CH_3)_2$

(±)-125a, R = CH_3
125b, R = CH_2Ph
125c, R = CH_2CH_2Ph
(*S*)-125d, R = $CH(CH_3)_2$

The α-bromoacid **133a-d** (0.96–65.6 mmol) in a round-bottom flask was dissolved in H_2O:MeOH (1.5:0.15 mL/mmol) and stirred at room temperature. A solution of 20% aq Cs_2CO_3 was added until the pH was ~8–9 as measured by indicator paper. The solution was concentrated and benzyl bromide (1.25 equiv) in DMF (1 mL/mmol) was added. The solution was stirred at room temperature for 5-24 h. The reaction was quenched with brine and extracted three times with ether. The organic layers were washed three times with excess water to remove any remaining DMF and dried over

Na_2SO_4 and concentrated. The crude product was purified on silica gel chromatography with 1–3% ethyl acetate/hexanes to give pure product **125a-d** as an oil (R = CH_3, 42%; R = CH_2Ph, 48%; R = CH_2CH_2Ph, 60%; R = $CH_2CH(CH_3)_2$, 78%).

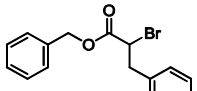
Benzyl (±)-2-bromopropanoate (125a)



(±)-125a

Compound **(±)-125a** was obtained from **(±)-133a** (10.03 g, 65.6 mmol) as an oil (6.77 g, 42% isolated yield) following chromatographic purification over silica gel with 1% ethyl acetate/hexanes. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.70 (d, *J* = 7.0 Hz, 3H), 4.27 – 4.31 (m, 1H), 5.05 – 5.11 (m, 2H), 7.21 – 7.27 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.7, 40.2, 67.6, 128.2, 128.4, 128.5, 128.7, 135.4, 170.0.

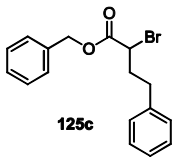
Benzyl 2-bromo-3-phenylpropanoate (125b)



125b

Compound **125b** was obtained from **133b** (1.685 g, 8.09 mmol) as an oil (1.235 g, 48% isolated yield) following chromatographic purification over silica gel with 3% ethyl acetate/hexanes. $[\alpha]^{27}_D$ +0.2° (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.24 – 3.28 (m, 1H), 3.45 – 3.49 (m, 1H), 4.44 (t, *J* = 7.0 Hz, 1H), 5.14 (s, 2H), 7.16 – 7.18 (m, 2H), 7.24 – 7.28 (m, 5H), 7.32 – 7.34 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 41.2, 45.3, 67.6, 127.4, 128.3, 128.5, 128.7, 128.8, 129.3, 135.1, 136.7, 169.3.

Benzyl 2-bromo-4-phenylbutanoate (125c)

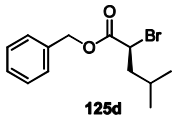


125c

Compound **125c** was obtained from **(*S*)-133c** (3.140 g, 12.9 mmol) as an oil (2.585 g, 60% isolated yield) following chromatographic purification over silica gel with 1% ethyl acetate/hexanes. $[\alpha]^{27}_D$ -0.3° (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.28 – 2.42 (m, 2H), 2.67 – 2.81 (m, 2H), 4.20 (t, *J* = 6.4 Hz, 1H), 5.19 (s, 2H), 7.14 – 7.16 (m, 2H),

7.19 – 7.22 (m, 1H), 7.26 – 7.29 (m, 3H), 7.30 – 7.38 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 32.1, 35.2, 44.1, 66.5, 125.4, 127.2, 127.5, 127.5, 127.6, 134.1, 138.7, 168.4.

(*S*)-Benzyl 2-bromo-4-methylpentanoate (125d)

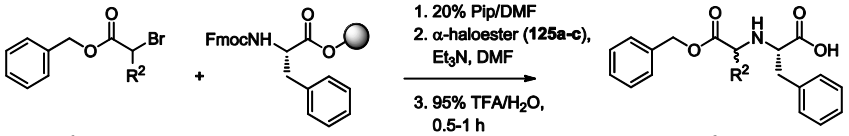


125d

Compound **125d** was obtained from **(*S*)-133d** (5.075 g, 26.0 mmol) as an oil (5.760 g, 78% isolated yield) following chromatographic purification with 1-2% ethyl acetate/hexanes. $[\alpha]^{27}_D$ -22.5° (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.92 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 6.6 Hz, 3H), 1.78 (m, 1H), 1.90 – 1.94 (m, 2H), 4.34 (t, *J* = 7.3 Hz, 1H), 5.23 (s, 2H), 7.40 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.5, 22.3, 26.3, 43.4, 44.5, 67.5, 128.2, 128.4, 128.6, 135.2, 169.8.

Anal. Calcd. for $C_{13}H_{17}Br$: C, 54.75%; H, 6.01%; Br, 28.02%. Found: C, 54.81%; H, 5.92%; Br, 28.06%.

5.5 General Procedure for the Optimization of the N-Alkylation of Resin-Bound Amino Acids with Benzyl α-Bromoesters



125b ($R^2 = CH_2Ph$)
125c ($R^2 = CH_2CH_2Ph$)
125a ($R^2 = CH_3$)

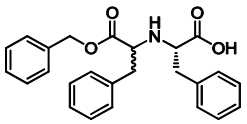
128

137 ($R^2 = CH_2Ph$)
138 ($R^2 = CH_2CH_2Ph$)
140 ($R^2 = CH_3$)

Fmoc-Phe-Wang resin **128** (72 mg, 0.70 mmol/g, 50 μmol) was added to a solid-phase reaction vessel (3 mL) and swelled in 2:1 CH_2Cl_2/DMF for 1 h. The resin was filtered, a solution of 20% piperidine/DMF (2 mL) was added, and the vessel was rotated at room temperature for 30 min. The resin was filtered and washed with DMF (3 x 2 mL). A mixture of the α-bromoester (**125a-c**) and triethylamine in DMF (0.5 mL) was added at the equivalents indicated (Section 3.1.3-4). If the presence of tetrabutylammonium iodide (TBAI) was being tested, it was then added to the vessel as a solid. The resin was heated at 50 °C for the indicated time (2-4 d). The vessels were allowed to cool to room

temperature and the resin was washed with DMF (3 x 2 mL), MeOH (2 x 2 mL), THF (2 x 2 mL), and MeOH (3 x 2 mL). A solution of 95% TFA/H_2O (2 mL) was added to each vessel followed by rotation for 30 min to 1 h. The filtrate-containing product was collected in tared vials and the cleavage solution was evaporated with a stream of nitrogen. Each sample was analyzed by LC/MS and cyano-silica TLC plates (30% acetone/hexanes). The products were purified by loading the crude product (12–18 mg) onto a dry 500 mg cyano-silica cartridge with 200–300 μL of acetone and allowing the column to dry with a stream of nitrogen. The column was washed with hexanes (2 x 2 mL) and eluted with 30% acetone/hexanes (8 x 1 mL). Fractions were analyzed by cyano-silica TLC (30% acetone/hexanes) and pooled to afford the product as a white solid upon evaporation.

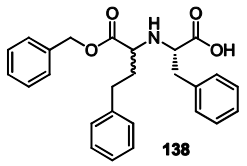
***N*-[1-Phenylmethyl-2-oxo-2-(phenylmethoxy)ethyl]phenylalanine (137)**



137

Compound **137** was obtained by N-alkylation with **125b** (for conditions, see Table 1, entry 1). Crude LC/MS purity 36%, t_R = 7.8, 8.2 (dr 56:44 to 46:54); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.64 – 2.78 (m, 1H), 2.87 – 3.06 (m, 3H), 3.18 – 3.51 (m, 2H), 5.00 – 5.15 (m, 2H), 6.75 (d, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 7.5 Hz, 1.5H), 7.04 (d, *J* = 7.2 Hz, 1.5H), 7.11 – 7.19 (m, 4H), 7.28 – 7.31 (m, 4H), 7.36 – 7.38 (m, 3H).

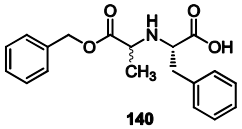
Mono(phenylmethyl) α-[(1-carboxy-2-phenylethyl)amino]benzenebutanoate (138)



138

Compound **138** was obtained by N-alkylation with **125c** (for optimal conditions, see Scheme 50 and Table 2, entry 15). Crude LC/MS purity 63%, t_R = 9.6, 10.1 (dr 56:44); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.97 – 2.04 (m, 2H), 2.24 – 2.50 (m, 2H), 3.08 – 38 (m, 2.5H), 3.71 – 3.72 (m, 1.5H), 4.94 – 5.06 (m, 2H), 6.85 (d, *J* = 6.9 Hz, 1H), 6.90 (d, *J* = 7.0 Hz, 1H), 7.06 – 7.23 (m, 13H).

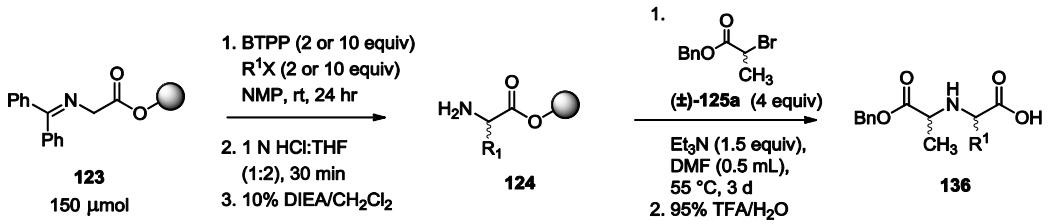
***N*-[1-Methyl-2-oxo-2-(phenylmethoxy)ethyl]phenylalanine (140)**



140

Compound **140** was obtained by N-alkylation with **(±)-125a** (for optimal conditions, see Scheme 52 and Table 3, entry 12). Crude LC/MS purity 81%, t_R = 6.5, 6.8 (dr 52:48 to 45:54); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37 (d, J = 7.0 Hz, 1.2H), 1.41 (d, J = 7.1 Hz, 1.7H), 3.13 – 3.20 (m, 1H), 3.32 – 3.48 (m, 1.5H), 3.80 – 3.89 (m, 1.5H), 5.01 – 5.06 (m, 2H), 7.21 – 7.33 (m, 10H).

5.6 General Procedure for the Evaluation of Alkylating Agents in the Synthesis of Unnatural Amino Acids



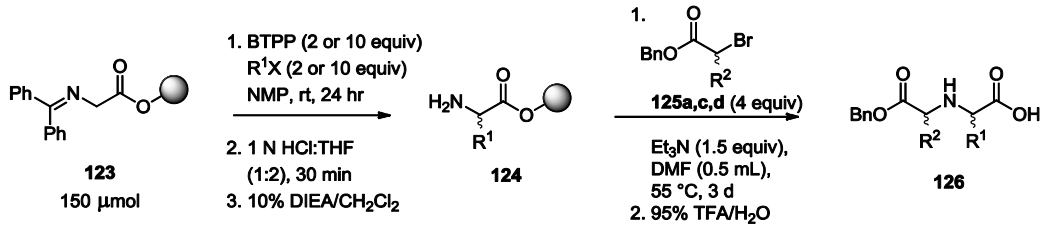
An isopycnic solution of the benzophenone imine of glycine resin (BIG-Wang, 0.84 mmol/g) was prepared by mixing the resin in a solution of NMP/CH_2Cl_2 (13:7). The isopycnic solution was distributed evenly (150 μmol each) to six solid-phase reaction vessels (5 mL) by equally repeated dispenses using an autopipetter. The solvent was drained from the resin. For the reactions involving 2 equivalents of the alkylating reagent and BTPP, a solution of 0.2 M BTPP (1.5 mL, 300 μmol) was added to the resin and allowed to mix with the resin for 5 min. A solution of 0.2 M of each alkylating reagent (R^1X, 1.5 mL, 300 μmol) was then added to its corresponding reaction vessel. For the reactions involving 10 equivalents of the alkylating reagent and BTPP, 0.375 mL of NMP was added to the resin followed by 0.75 mL of 2.0 M BTPP (1.5 mmol, 10 equiv) and mixed for 5 min. A solution of 1.33 M of each alkylating reagent (R^1X, 1.2 mL, 1.5 mmol, 10 equiv) was added to its corresponding reaction vessel. The reactions were rotated at room temperature for 24 hrs.

The reagents were filtered off, and the resin was washed with NMP (2 x 2 mL) and THF (2 x 2 mL). The benzophenone imine was hydrolyzed by adding 1 N HCl/THF (2 mL, 1:2) to each reaction vessel and rotating the resin for 20 min. The reagents were filtered off, and the resin was washed with THF (2 x 2 mL), 10% DIEA/CH_2Cl_2 (3 x 2 mL), and DMF (3 x 2 mL). To each reaction vessel was added a solution of benzyl 2-bromopropanoate (0.4 M, 1.5 mL, 600 μmol, 4 equiv) followed by triethylamine (31 μL, 225 μmol. 1.5 equiv). The reactions were heated in an oven at 55 °C for 3 d.

The reagents were filtered off, and the resin was washed with DMF (2 x 2 mL), THF (2 x 2 mL), and CH_2Cl_2 (3 x 2 mL). The products were cleaved from the resin by 95% TFA/H_2O (2 mL) to each reaction vessel and rotating at room temperature for 1 h. The cleavage solutions were collected in tared vials, washed with CH_2Cl_2 (1 x 2 mL), and evaporated down under a stream of nitrogen. The crude yield was recorded and the purity was analyzed by LC/MS.

The crude products were analyzed by cyano-silica gel TLC (30% acetone/hexanes). Each sample was purified by loading the crude product (29–74 mg) onto a dry 500 mg cyano-silica cartridge with 200–300 μL of acetone and allowing the column to dry with a stream of nitrogen or by standing overnight. The column was equilibrated with hexanes (2 x 2 mL) and eluted with a gradient of 10–40% acetone/hexanes based on the TLC profile. Fractions were analyzed by cyano-silica TLC (30% acetone/hexanes) and pooled to afford products **138**. Analytical data for these products is given following their combinatorial preparation (see Section 5.7).

5.7 General Procedure for the Synthesis of N-Carboxyalkyl Amino Acid Analogs



For each of the 3 Bill-Boards depicted in Scheme 56, an isopycnic solution of the benzophenone imine of glycine resin (BIG-Wang, 0.84 mmol/g) was prepared by mixing

the resin (3.61 g, 3.03 mmol) in a solution of NMP/CH_2Cl_2 (13:7). The isopycnic solution was distributed evenly (150 μmol) to the twenty solid-phase reaction vessels (5 mL) by equally repeated dispenses using an autopipetter. The solvent was drained and the resin for each reaction vessel was washed with CH_2Cl_2 (1 mL). For the reactions involving 2 equivalents of the alkylating reagent and BTPP, a solution of 0.2 M BTPP in NMP (1.5 mL, 300 μmol) was added to the resin and allowed to mix with the resin for 5 min. A solution of 0.2 M of each alkylating reagent in NMP (R^1X, 1.5 mL, 300 μmol) was then added to its corresponding reaction vessel. For the reactions involving 10 equivalents of the alkylating reagent and BTPP, 0.375 mL of NMP was added to the resin followed by 2.0 M BTPP in NMP (0.75 mL, 1.5 mmol, 10 equiv) and mixed for 5 min. A solution of 1.33 M of each alkylating reagent in NMP (R^1X, 1.2 mL, 1.5 mmol, 10 equiv) was added to its corresponding reaction vessel. The reactions were rotated at room temperature for 24 hrs.

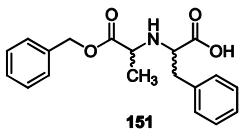
The reagents were filtered off, and the resin was washed with NMP (2 x 2 mL) and THF (2 x 2 mL). The benzophenone imine was hydrolyzed by the addition of 1 N HCl/THF (2 mL, 1:2) to each reaction vessel and rotating the resin for 20 min. The hydrolysis solution was filtered off, and the resin was washed with THF (2 x 2 mL), 10% DIEA/CH_2Cl_2 (3 x 2 mL), and DMF (3 x 2 mL). To each reaction vessel was added a 0.4 M solution of the α-bromoester in DMF (1.5 mL, 600 μmol, 4 equiv – Bill-Board #1: benzyl 2-bromopropanoate; Bill-Board #2: benzyl 2-bromo-4-methylpentanoate; Bill-Board #3: benzyl 2-bromo-4-phenylbutanoate), followed by triethylamine (31 μL, 225 μmol. 1.5 equiv). The reactions were heated in an oven at 55 °C for 3 d.

The reagents were filtered off, and the resin was washed with DMF (2 x 2 mL), THF (2 x 2 mL), and CH_2Cl_2 (3 x 2 mL). The products were cleaved from the resin by adding 95% TFA/H_2O (2 mL) to each reaction vessel and rotating at room temperature for 1 h. The cleavage solutions were collected in tared vials, washed with CH_2Cl_2 (1 x 2 mL), and evaporated under a stream of nitrogen. The crude yield was recorded and the purity of each sample was analyzed by LC/MS.

The crude products were analyzed by cyano-silica gel TLC with acetone-hexanes (1:2). Each sample was purified by loading the crude product (29 – 82 mg) onto a dry 1 g cyano-silica cartridge with acetone (300-400 μL) and allowing the column to dry with a stream of nitrogen or by standing overnight. The column was equilibrated with hexanes

(2 x 2 mL) and eluted with a gradient of 10-40% acetone/hexanes based on the TLC profile. Fractions were analyzed by cyano-silica TLC (30% acetone/hexanes) and pooled to afford products **126** as a mixture of diastereomers. For Bill-Board #3 where the α-bromoester was benzyl 2-bromo-4-phenylbutanoate and the dialkylated by-product was observed, the samples, following purification on the cyano-silica cartridges, were further purified on reverse-phase PrepLC using an isocratic system of 30:70 organic/aqueous for more polar analogs, 50:50 organic/aqueous for most of the analogs, and 70:30 organic/aqueous for the more lipophilic analogs. The dialkylated material was eluted with 80:20 organic/aqueous and the column washed with 100:0 organic/aqueous. The diastereomers were separated by this method for most of the analogs from Bill-Board #3. Results for each of the 30 analogs, produced in duplicate, are shown in Table 1 (page 69). The diastereomeric ratios in the LC/MS results below are reported in the order of elution from the column. The identification of the diastereomers in the ^{1}H NMR data below is designated as diastereomer A being the more abundant diastereomer and diastereomer B being the least abundant diastereomer based on integration areas. The more abundant diastereomer in the ^{1}H NMR spectra does not necessarily correspond to the more prevalent diastereomer indicated by LC/MS, since the purification of the crude material may have altered this ratio due to possible salt formation of the diastereomers that did not elute from the column (see discussion on page 46). Analog **157** was also analyzed by 2D NMR experiments (COSY, DEPT-90, DEPT-135, HSQC, and HMBC) to try and separate the diastereomer signals in the ^{1}H and ^{13}C NMR. A table correlating the proton and carbon signals can be seen in Appendix B. For analogs **169**-**178** where diastereomers were separated, the HRMS data is reported for both diastereomers in which diastereomer 1 corresponds to the diastereomer that eluted first from the column and first on the LC/MS and diastereomer 2 corresponds to the diastereomer that eluted second from the column and second on the LC/MS.

***N*-[1-Methyl-2-oxo-2-(phenylmethoxy)ethyl]phenylalanine (151)**

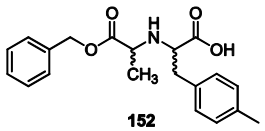


151

Compound **151** was obtained with benzyl bromide and benzyl (±)-2-bromopropanoate as a white solid (sample 1: 25.8 mg, 53% isolated yield; sample 2:

25.1 mg, 51% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 72% (sample 1) and 66% (sample 2), t_R = 6.3, 6.6 min (dr 50:50); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37 (diastereomer B, d, *J* = 7.2 Hz, 1.5 H), 1.41 (diastereomer A, d, *J* = 7.2 Hz, 1.5 H), 3.12 – 3.21 (m, 1H), 3.31 (diastereomer A, dd, *J* = 14.4 Hz, *J* = 5.4 Hz, 0.59 H), 3.42 (diastereomer B, dd, *J* = 14.5 Hz, 5.2 Hz, 0.48 H), 3.52 (diastereomer B, q, *J* = 7.2 Hz, 0.47H), 3.80 (diastereomer A, q, *J* = 7.1 Hz, 0.56H), 3.82 – 3.87 (m, 1H), 5.04 – 5.11 (m, 2H), 7.23 – 7.35 (m, 10H), 7.95 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 15.4, 16.7, 36.4, 37.0, 55.7, 55.9, 61.9, 68.0, 127.5, 127.6, 128.4, 128.4, 128.7, 128.7, 128.8, 129.0, 129.0, 129.2, 129.4, 134.5, 134.5, 134.9, 135.8. HRMS calcd for $(M + H)^+$: $C_{19}H_{21}NO_4$ 328.1549, found 328.1545.

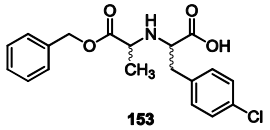
***N*-[1-Methyl-2-oxo-2-(phenylmethoxy)ethyl]-4-methylphenylalanine (152)**



152

Compound **152** was obtained with 4-methylbenzyl bromide and benzyl (±)-2-bromopropanoate as a white solid (sample 1: 22.5 mg, 44% isolated yield; sample 2: 20.3 mg, 40% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 68% (sample 1) and 76% (sample 2), t_R = 7.3, 7.6 min (dr 54:46); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (diastereomer B, d, *J* = 7.1 Hz, 2H), 1.38 (diastereomer A, d, *J* = 7.1 Hz, 1H), 2.29 (diastereomer B, s, 2H), 2.30 (diastereomer A, s, 1H), 3.06 – 3.10 (m, 1H), 3.22 (diastereomer A, dd, *J* = 14.3 Hz, *J* = 10.4 Hz, 0.3H), 3.35 (diastereomer B, dd, *J* = 14.5 Hz, *J* = 4.9 Hz, 0.7H), 3.45 (diastereomer B, q, *J* = 7.1 Hz, 0.7H), 3.63 – 3.70 (m, 0.7H), 3.74 – 3.76 (m, 0.6H), 5.13 – 5.05 (m, 2H), 7.09 – 7.15 (m, 4H), 7.25 – 7.38 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.0, 21.1, 36.3, 55.9, 56.0, 61.8, 61.9, 67.8, 67.9, 128.4, 128.7, 128.7, 128.8, 129.0, 129.2, 129.7, 129.8, 131.0, 131.7, 132.5, 134.6, 134.7, 137.2, 137.3, 172.3. HRMS calcd for $(M + H)^+$: $C_{20}H_{23}NO_4$ 342.1705, found 342.1721.

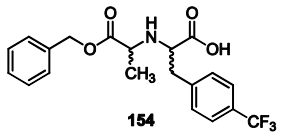
***N*-[1-Methyl-2-oxo-2-(phenylmethoxy)ethyl]-4-chlorophenylalanine (153)**



153

Compound **153** was obtained with 4-chlorobenzyl bromide and benzyl (±)-2-bromopropanoate as a white solid (sample 1: 14.6 mg, 27% isolated yield; sample 2: 7.0 mg, 13% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 67% (sample 1) and 72% (sample 2), t_R = 7.6, 7.9 min (dr 51:49); ^{1}H NMR (500 MHz, DMSO): δ 1.14 (diastereomer B, d, *J* = 6.9 Hz, 1.4H), 1.17 (diastereomer A, d, *J* = 6.9 Hz, 1.5H), 2.77 – 2.91 (m, 2H), 3.31 – 3.35 (m, 0.5 H), 3.38 – 3.46 (m, 1.5H), 5.05 – 5.12 (m, 2H), 7.19 – 7.23 (m, 2H), 7.28 – 7.39 (m, 7H). ^{13}C NMR (500 MHz, DMSO): δ 18.3, 18.4, 37.7, 37.8, 54.2, 59.7, 60.0, 65.5, 65.6, 127.7, 127.7, 127.8, 127.9, 128.3, 130.8, 130.8, 130.9, 131.1, 135.9, 136.0, 136.8, 137.0. HRMS calcd for (M + H)$^+$: $C_{19}H_{20}ClNO_4$ 362.1159, found 362.1176.

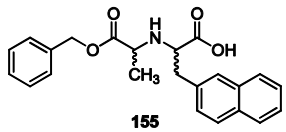
***N*-[1-Methyl-2-oxo-2-(phenylmethoxy)ethyl]-4-trifluoromethylphenyl-alanine (154)**



154

Compound **154** was obtained with 4-trifluoromethylbenzyl bromide and benzyl (±)-2-bromopropanoate as a white solid (sample 1: 18.5 mg, 31% isolated yield; sample 2: 21.6 mg, 36% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 64% (sample 1) and 59% (sample 2), t_R = 8.0, 8.3 min (dr 49:51); ^{1}H NMR (500 MHz, DMSO): δ 1.15 (diastereomer B, d, *J* = 6.9 Hz, 1.4H), 1.19 (diastereomer A, d, *J* = 6.9 Hz, 1.6H), 2.89 – 3.02 (m, 2H), 3.38 (diastereomer A, q, *J* = 6.8 Hz, 0.6H), 3.45 (diastereomer B, q, *J* = 6.9 Hz, 0.6H), 3.51 – 3.56 (m, 1H), 5.05 – 5.13 (m, 2H), 7.32 – 7.38 (m, 5H), 7.41 – 7.45 (m, 2H), 7.58 – 7.62 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 18.3, 18.3, 38.0, 38.1, 54.1, 54.2, 59.5, 59.7, 65.5, 65.7, 123.3, 124.6, 124.6, 124.7, 124.7, 124.7, 125.4, 126.5, 126.7, 127.0, 127.8, 127.8, 127.9, 128.3, 129.9, 130.1, 135.9, 135.9, 142.7, 143.0. HRMS calcd for (M + H)$^+$: $C_{20}H_{20}F_3NO_4$ 396.1423, found 396.1441.

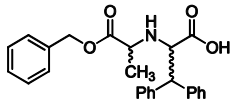
α-[[2-Oxo-2-(phenylmethoxy)-1-(methyl)ethyl]amino]-3,2'-naphthylpropanoic acid (155)



155

Compound **155** was obtained with 2-(bromomethyl)naphthalene and benzyl (±)-2-bromopropanoate as a yellow oil (sample 1: 20.0 mg,35% isolated yield; sample 2: 13.5 mg, 24% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 67% (sample 1) and 67% (sample 2), t_R= 8.1, 8.3 (dr unavailable due to overlapping peaks); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.15 (diastereomer B, d, *J* = 7.0 Hz, 1.9H), 1.30 (diastereomer A, d, *J* = 7.0 Hz, 0.9H), 3.14 – 3.19 (m, 0.7H), 3.20 – 3.25 (m, 0.5H), 3.30 (diastereomer B, q, *J* = 7.2 Hz, 0.8H), 3.33 – 3.37 (m, 0.5H), 3.44 – 3.45 (m, 0.6H), 3.47 – 3.51 (m, 0.5H), 3,61 – 3.65 (m, 1H), 3.68 – 3.71 (m, 1H), 5.01 – 5.11 (m, 1H), 7.21 – 7.24 (m, 2H), 7.32 – 7.36 (m, 4H), 7.46 – 7.52 (m, 2H), 7.68 (s, 1H), 7.77 – 7.82 (m, 3H). ^{13}C NMR (500MHz, $CDCl_3$) : δ 15.7, 29.3, 31.7, 36.6, 53.8, 55.8, 56.0, 51.8, 68.0, 68.0, 126.1, 126.4, 126.5, 126.6, 126.8, 127.7, 127.8, 127.8, 128.3, 128.4, 128.7, 128.7, 128.8, 129.0, 132.2, 132.5, 133.1, 133.4, 133.5, 134.3, 172.0. HRMS calcd for $(M + H)^+$: $C_{23}H_{23}NO_4$ 378.1705, found 378.1712.

α-[[2-Oxo-2-(phenylmethoxy)-1-(methyl)ethyl]amino]-3-diphenylpropanoic acid (156)

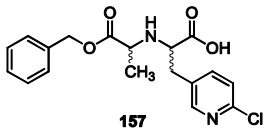


156

Compound **156** was obtained with bromodiphenylmethane and benzyl (±)-2-bromopropanoate as a yellow oil (sample 1: 6.0 mg, 10% isolated yield; sample 2: 10.0 mg, 17% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 82% (sample 1) and 89% (sample 2), t_R = 5.5, 6.0 min (dr 55:45); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (diastereomer B, d, *J* = 7.1 Hz, 1.4H), 1.32 (diastereomer A, d, *J* = 7.1 Hz, 1.6H), 3.48 – 3.52 (diastereomer B, m, 0.4H), 3.85 – 3.90 (diastereomer A, m, 0.6H), 4.34 (d, *J* = 9.0 Hz, 0.5H), 4.42 –

4.47 (m, 1H), 4.58 (d, *J* = 9.1 Hz, 0.5H), 5.02 (diastereomer B, d, *J* = 12.2 Hz, 0.45H), 5.08 (diastereomer B, d, *J* = 12.2 Hz, 0.45H), 5.16 (diastereomer A, d, *J* = 12.1 Hz, 0.8H), 5.23 (diastereomer A, d, *J* = 12.0 Hz, 0.6H), 7.21 – 7.38 (m,15H), 8.56 (s). HRMS calcd for $(M + H)^+$: $C_{25}H_{25}NO_4$ 404.1862, found 404.1871.

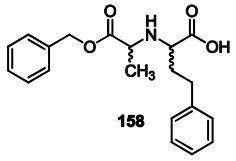
α-[[2-Oxo-2-(phenylmethoxy)-1-(methyl)ethyl]amino]-3,5'-(2-chloro-pyridinyl)-propanoic acid (157)



157

Compound **157** was obtained with 5-(chloromethyl)-2-chloropyridine and benzyl (±)-2-bromopropanoate as a yellow, oily solid (sample 1: 11.9 mg, 22% isolated yield; sample 2: 15.2 mg, 28% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 55% (sample 1) and 60% (sample 2), t_R = 5.6, 5.9 (dr 53:47); ^{1}H NMR (500 MHz, DMSO): δ 1.14 (diastereomer B, d, *J* = 7.0 Hz, 1.1H), 1.18 (diastereomer A, d, *J* = 6.9 Hz, 1.8H), 2.79 – 2.95 (m, 2H), 3.38 – 3.49 (m, 2H), 5.05 – 5.13 (m, 2H), 7.30 – 7.42 (m, 6H), 7.69 – 7.72 (m, 1H), 8.24 (s, 1H). ^{13}C NMR (500 MHz, DMSO): δ 18.3, 18.4, 34.2, 34.5, 54.1, 54.1, 59.2, 59.4, 65.5, 65.7, 123.3, 123.4, 127.7, 127.8, 127.9, 127.9, 128.3, 133.0, 133.1, 135.9, 135.9, 140.4, 140.6, 148.1, 148.1, 150.3, 150.4, 173.6, 174.1, 174.3. HRMS calcd for $(M + H)^+$: $C_{18}H_{19}ClN_2O_4$ 363.1111, found 363.1130.

α-[[2-Oxo-2-(phenylmethoxy)-1-(methyl)ethyl]amino]-4-phenylbutanoic acid (158)

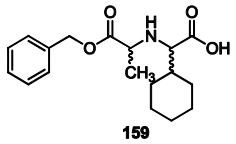


158

Compound **158** was obtained with (2-bromoethyl)benzene and benzyl (±)-2-bromopropanoate as a yellow oil (sample 1: 11.1 mg, 22% isolated yield; sample 2: 19.4 mg, 38% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 69% (sample 1) and 75% (sample 2), t_R = 7.2, 7.4 (dr 45:55); ^{1}H NMR (500 MHz, DMSO): δ 1.25 (diastereomer B, d, *J* = 6.9 Hz, 0.7H), 1.29 (diastereomer A, d, *J* = 6.9 Hz, 2.2H), 1.81 – 1.91 (m, 2H), 2.57 –

2.71 (m, 2H), 3.27 (diastereomer B, t, *J* = 5.9 Hz, 0.3H), 3.35 (diastereomer A, s, 0.8H), 3.50 – 3.52 (diastereomer B, m, 0.3H), 3.59 – 3.60 (diastereomer A, m, 0.8H), 5.07 – 5.16 (m, 2H), 7.16 – 7.19 (m, 3H), 7.26 – 7.29 (m, 2H), 7.32 – 7.35 (m, 1H), 7.36 – 7.38 (m, 4H). ^{13}C NMR (500 MHz, DMSO): δ 17.8, 31.0, 31.1, 33.7, 33.9, 54.3, 54.2, 57.9, 58.3, 65.8, 125.8, 127.8, 128.0, 128.2, 128.2, 128.2 128.3, 135.8, 141.2, 172.9, 174.3. HRMS calcd for $(M + H)^+$: $C_{20}H_{23}NO_4$ 342.1705, found 342.1718.

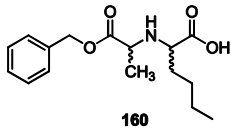
Mono(phenylmethyl) α-[[(1-carboxy-1-methyl)ethyl]amino]-2-cyclohexylethanoate (159)



159

Compound **159** was obtained with cyclohexyl iodide and benzyl (±)-2-bromopropanoate as a yellow oil (sample 1: 10.0 mg, 21% isolated yield; sample 2: 7.6 mg, 16% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 65% (sample 1) and 65% (sample 2), t_R = 6.9, 7.3 (dr 56:44); ^{1}H NMR (500 MHz, DMSO): δ 1.05 – 1.21 (m, 5H), 1.34 – 1.36 (m, 3H), 1.61 (s, 3H), 1.67 – 1.69 (m, 3H), 3.37 (s, 1H), 3.71 (s, 1H), 5.11 – 5.20 (m, 2H), 7.34 – 7.39 (m, 5H). ^{13}C NMR (500 MHz, DMSO): δ 25.4, 25.4, 25.6, 28.3, 54.9, 64.0, 128.0, 128.1, 128.3, 135.6. HRMS calcd for $(M + H)^+$: $C_{18}H_{25}NO_4$ 320.1862, found 320.1864.

α-[[2-Oxo-2(phenylmethoxy)-1-(methyl)ethyl]amino]hexanoic acid (160)

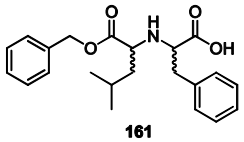


160

Compound **160** was obtained with *n*-butyl iodide and benzyl (±)-2-bromopropanoate as a yellow oil (sample 1: 6.2 mg, 14% isolated yield; sample 2: 18.9 mg, 43% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 59% (sample 1) and 63% (sample 2), t_R = 6.1, 6.2 (dr unavailable due to overlapping peaks); ^{1}H NMR (500 MHz, DMSO): δ 0.84 (t, *J* = 6.9 Hz, 3H), 1.25 – 1.28 (m, 4H), 1.32 (d, *J* = 6.9 Hz, 3H), 1.57 – 1.65 (m,

2H), 3.45 (s, 1H), 3.69 (s, 1H), 5.12 – 5.19 (m, 2H), 7.33 – 7.36 (m, 1H), 7.38 – 7.39 (m, 4H). ^{13}C NMR (500 MHz, DMSO): δ 13.7, 17.2, 21.7, 27.0, 54.2, 58.6, 66.1, 127.9, 128.1, 128.4, 135.6. HRMS calcd for $(M + H)^+$: $C_{16}H_{23}NO_4$ 294.1705, found 294.1731.

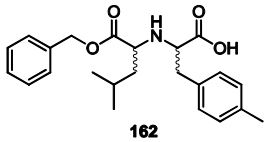
***N*-[1-Isobutyl-2-oxo-2-(phenylmethoxy)ethyl]phenylalanine (161)**



161

Compound **161** was obtained with benzyl bromide and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 16.9 mg, 30% isolated yield; sample 2: 11.0 mg, 20% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 39% (sample 1) and 39% (sample 2), t_R = 8.4, 9.3 min (dr 62:38); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.66 (d, *J* = 6.5 Hz, 1.1H), 0.71 (d, *J* = 6.5 Hz, 1.2H), 0.81 – 0.84 (diastereomer A, m, 3.4H), 1.40 (diastereomer B, m, 0.4H), 1.62 – 1.68 (m, 2H), 1.70 – 1.77 (diastereomer A, m, 0.6H), 3.19 – 3.27 (m, 1H), 3.34 (diastereomer B, t, *J* = 7.1 Hz, 0.4H), 3.43 (diastereomer A, dd, *J* = 14.6 Hz, *J* = 4.9 Hz, 0.6H), 3.52 (diastereomer B, dd, *J* = 14.8 Hz, *J* = 4.2 Hz, 0.4H), 3.79 – 3.82 (diastereomer B, m, 0.4H), 3.85 – 3.88 (diastereomer A, 0.6H), 3.93 (diastereomer A, t, *J* = 6.7 Hz, 0.6H), 5.08 – 5.15 (m, 2H), 7.26 – 7.37 (m, 10H), 8.94 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.1, 21.7, 22.2, 22.6, 24.6, 24.6, 35.5, 36.4, 39.0, 39.6, 59.3, 59.5, 62.2, 62.8, 68.4, 68.5, 127.9, 128.0, 128.6, 128.6, 128.8, 129.0, 129.0, 129.1, 129.2, 129.3, 129.4, 134.1, 134.1, 135.2, 169.3, 169.4, 170.3, 170.9. HRMS calcd for $(M + H)^+$: $C_{22}H_{27}NO_4$ 370.2018, found 370.2010.

***N*-[1-Isobutyl-2-oxo-2-(phenylmethoxy)ethyl]-4-methylphenylalanine (162)**

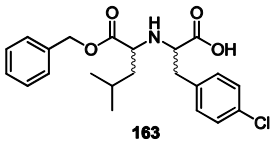


162

Compound **162** was obtained with 4-methylbenzyl bromide and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 12.2 mg, 21% isolated yield; sample 2: 15.2 mg, 26% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 41% (sample 1) and 41%

(sample 2), t_R = 9.2, 10.0 min (dr 62:38); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.66 (d, *J* = 6.5 Hz, 1.2H), 0.71 (d, *J* = 6.5 Hz, 1.3H), 0.81 – 0.83 (diastereomer A, m, 3.5H), 1.40 (diastereomer B, septet, *J* = 6.7 Hz, 0.4H), 1.61 – 1.68 (m, 2H), 1.70 – 1.76 (diastereomer A, m, 0.6H), 2.28 (diastereomer B, s, 1.3H), 2.32 (diastereomer A, s, 1.7H), 3.16 – 3.24 (m, 1H), 3.35 (diastereomer B, t, *J* = 7.1 Hz, 0.4H), 3.39 (diastereomer A, dd, *J* = 14.8 Hz, *J* = 4.9 Hz, 0.6H), 3.49 (diastereomer B, dd, *J* = 14.8 Hz, *J* = 4.1 Hz, 0.4H), 3.81 (diastereomer B, dd, *J* = 9.8 Hz, *J* = 4.2 Hz, 0.4H), 3.89 (diastereomer A, dd, *J* = 9.2 Hz, *J* = 4.9 Hz, 0.6H), 3.98 (diastereomer A, t, *J* = 6.7 Hz, 0.6H), 5.10 – 5.16 (m, 2H), 7.09 – 7.12 (m, 1H), 7.14 (s, 2H), 7.19 – 7.21 (m, 1H), 7.25 – 7.29 (2H), 7.34 – 7.38 (m, 3H), 9.29 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.0, 21.0, 21.1, 21.7, 22.2, 22.5, 24.6, 24.7, 35.1, 35.9, 38.9, 39.4, 59.3, 59.5, 62.2, 62.9, 68.5, 68.6, 114.8, 117.1, 128.6, 128.6, 128.8, 129.0, 129.0, 129.3, 129.9, 130.0, 130.7, 131.8, 134.1, 137.7, 137.8, 169.1, 169.1, 170.2, 170.8. HRMS calcd for $(M + H)^+$: $C_{23}H_{29}NO_4$ 384.2175, found 384.2171.

_N_-[1-Isobutyl-2-oxo-2-(phenylmethoxy)ethyl]-4-chlorophenylalanine (163)

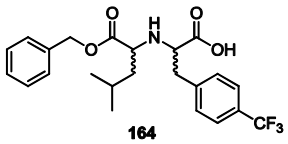


163

Compound **163** was obtained with 4-chlorobenzyl bromide and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 13.4 mg, 22% isolated yield; sample 2: 15.6 mg, 26% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 33% (sample 1) and 34% (sample 2); t_R = 9.3, 10.0 min (dr 61:39); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.70 (d, *J* = 6.6 Hz, 1.2H), 0.74 (d, *J* = 6.5 Hz, 1.2H), 0.82 – 0.84 (diastereomer A, m, 3.5H), 1.44 (diastereomer B, septet, *J* = 6.8 Hz, 0.4H), 1.62 – 1.73 (m, 2.6H), 3.18 – 3.26 (m, 1H), 3.36 – 3.40 (m, 1H), 3.46 (diastereomer B, dd, *J* = 14.9 Hz, 4.6 Hz, 0.4H), 3.77 – 3.80 (diastereomer B, m, 0.4H), 3.85 – 3.88 (diastereomer A, m, 0.6H), 3.97 (Diastereomer A, t, *J* = 6.5 Hz, 0.6H), 5.12 – 5.17 (m, 2H), 7.18 – 7.19 (m, 1H), 7.23 – 7.25 (m, 1H), 7.26 – 7.31 (m, 4H), 7.35 – 7.39 (m, 3H), 9.40 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.0, 21.7, 22.1, 22.5, 24.6, 24.7, 34.7, 35.6, 38.9, 39.4, 59.4, 59.4, 61.9, 62.5, 68.7, 68.8, 128.6, 128.7, 128.8, 128.9, 129.1, 129.2, 129.4, 129.5, 129.8, 130.1, 130.8, 132.4, 132.5,

133.6, 133.9, 133.9, 134.1, 169.0, 169.2, 170.0, 170.6. HRMS calcd for $(M + H)^+$: $C_{22}H_{26}ClNO_4$ 404.1629, found 404.1614.

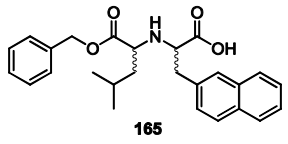
_N_-[1-Isobutyl-2-oxo-2-(phenylmethoxy)ethyl]-4-trifluoromethylphenylalanine (164)



164

Compound **164** was obtained with 4-trifluoromethylbenzyl bromide and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 13.8 mg, 21% isolated yield; sample 2: 7.5 mg, 11% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 29% (sample 1) and 28% (sample 2), t_R = 9.5, 10.2 (dr 62:38); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.66 (d, *J* = 6.5 Hz, 1.1H), 0.71 (d, *J* = 6.6 Hz, 1.2H), 0.82 – 0.84 (diastereomer A, m, 3.6H), 1.40 (diastereomer B, m, 0.4H), 1.60 – 1.72 (m, 2.6H), 3.27 – 3.33 (m, 1.4H), 3.43 (diastereomer A, dd, *J* = 14.7 Hz, *J* = 5.6 Hz, 0.6H), 3.54 (diastereomer B, dd, *J* = 14.7 Hz, *J* = 4.4 Hz, 0.4H), 3.77 – 3.80 (diastereomer B, m, 0.4H), 3.86 – 3.88 (diastereomer A, m, 0.6H), 3.92 (diastereomer A, t, *J* = 6.4 Hz, 0.6H), 5.15 (m, 2H), 7.25 – 7.31 (m, 2H), 7.35 – 7.38 (m, 4H), 7.42 – 7.44 (m, 1H), 7.55 – 7.60 (m, 2H), 9.00 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.0, 21.8, 22.1, 22.5, 24.6, 24.7, 35.5, 36.3, 39.2, 39.9, 59.3, 61.7, 62.4, 68.5, 68.5, 114.8, 117.1, 122.9, 125.0, 125.1, 126.0, 126.0, 126.1, 128.6, 128.6, 128.8, 128.8, 129.1, 129.1, 129.6, 129.8, 130.0, 130.3, 131.1, 134.0, 134.1, 138.6, 139.7, 161.6, 161.9, 169.6, 170.0, 170.7, 171.0. HRMS cald for $(M + H)^+$ $C_{23}H_{26}F_3NO_4$ 438.1892, found 438.1881.

Mono(phenylmethyl) α-[[(1-Carboxy-2,2'-naphthyl)ethyl]amino]-4-methyl-pentanoate (165)

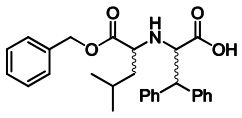


165

Compound **165** was obtained with 2-(bromomethyl)naphthalene and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 15.0 mg, 24% isolated yield; sample 2: 15.0 mg, 24% isolated yield) following chromatographic purification over

cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 40% (sample 1) and 37% (sample 2), t_R = 9.6, 10.2 (dr 59:41); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.41 (d, *J* = 6.5 Hz, 1.2H), 0.51 (d, *J* = 6.5 Hz, 1.2H), 0.77 – 0.80 (diastereomer A, m, 3.5H), 1.24 – 1.30 (diastereomer B, m, 0.4H), 1.55 – 1.68 (m, 2.6H), 3.25 (diastereomer B, t, *J* = 7.2 Hz, 0.4H), 3.35 – 3.42 (m, 1.H), 3.54 (diastereomer B, dd, *J* = 14.6 Hz, *J* = 4.9 Hz, 0.6H), 3.67 (diastereomer B, dd, *J* = 14.7 Hz, *J* = 3.8 Hz, 0.4H), 3.87 – 3.94 (m, 1.6H), 4.95 – 5.10 (m, 2H), 7.16 – 7.20 (m, 2H), 7.30 – 7.37 (m, 4H), 7.45 – 7.49 (m, 2H), 7.77 – 7.83 (m, 3.6H), 7.89 (Diastereomer B, s, 0.4H), 9.13 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 20.9, 21.8, 22.1, 22.2, 24.5, 24.6, 36.0, 36.9, 39.2, 40.0, 53.4, 59.4, 59.5, 61.9, 62.7, 68.2, 68.2, 126.2, 126.3, 126.5 126.6, 126.7, 127.6, 127.7, 127.8, 127.9, 128.5, 128.7, 128.7, 128.8, 128.9, 128.9, 129.1, 129.2, 131.8, 132.7, 132.7, 132.8, 133.5, 133.6, 134.1, 134.2, 169.7, 170.0, 171.0, 171.3. HRMS calcd for $(M + H)^+$ $C_{26}H_{29}NO_4$ 420.2175, found 420.2172.

Mono(phenylmethyl) α-[[(1-Carboxy-2-diphenyl)ethyl]amino]-4-methylpentanoate (166)

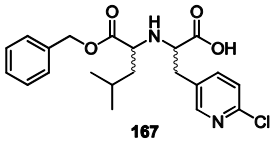


166

Compound **166** was obtained with bromodiphenylmethane and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 4.7 mg, 7% isolated yield; sample 2: 5.7 mg, 9% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 20% (sample 1) and 23% (sample 2), t_R = 9.9, 10.9 min (dr 55:45); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.63 (d, *J* = 6.5Hz, 1.4H), 0.66 (d, *J* = 6.5 Hz, 1.3H), 0.74 – 0.76 (diastereomer A, m, 3.3H), 1.26 (diastereomer B, m, 0.45H), 1.45 – 1.57 (m, 2H), 1.62 – 1.68 (diastereomer A, m, 0.55H), 3.51 (diastereomer B, t, *J* = 7.1 Hz, 0.45H), 4.02 – 4.04 (diastereomer A, 0.55H), 4.37 – 4.41 (m, 1H), 4.52 – 4.55 (m, 1H), 5.11 (diastereomer B, d, *J* = 12.0 Hz, 0.4H), 5.13 (diastereomer B, d, *J* = 12.0 Hz, 0.5H), 5.23 (diastereomer A, d, *J* = 12.0 Hz, 0.6H), 5.29 (diastereomer A, d, *J* = 12.0 Hz, 0.5H), 7.17 – 7.42 (m, 15H), 9.32 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.6, 22.2, 22.3, 24.5, 24.7, 39.8, 29.9, 52.3, 53.0, 59.1, 59.8, 64.5, 64.9, 68.2, 68.7, 127.7, 127.8, 128.0, 128.1, 128.1, 128.4, 128.5, 128.6, 128.7, 128.7,

128.8, 128.9, 128.9, 129.0, 129.4, 129.7, 134.2, 134.4, 136.9, 138.2, 138.4, 138.7, 169.0, 169.9, 171.7. HRMS calcd for $(M + H)^+$: $C_{28}H_{31}NO_4$ 446.2331, found 446.2309.

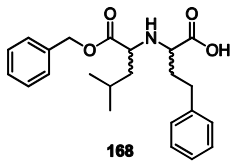
Mono(phenylmethyl) α-[[(1-Carboxy-2,5'-(2-chloropyridine))ethyl]-amino]-4-methyl-pentanoate (167)



167

Compound **167** was obtained with 5-(chloromethyl)-2-chloropyridine and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 9.4 mg, 15% isolated yield; sample 2: 8.4 mg, 14% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 21% (sample 1) and 19% (sample 2), t_R = 7.6, 8.5 min (dr 67:33); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (d, *J* = 3.1 Hz, 1.1H), 0.83 (d, *J* = 3.1 Hz, 1.2H), 0.85 – 0.86 (diastereomer A, m, 3.4H), 1.58 (diastereomer B, m, 0.4H), 1.66 – 1.80 (m, 2.6H), 3.32 – 3.34 (m, 2H), 3.67 – 3.71 (diastereomer B, m, 0.4H), 3.91 – 3.93 (m, 1.6H), 5.13 – 5.25 (m, 2H), 7.28 – 7.38 (m, 6H), 7.68 – 7.72 (m, 1H), 8.33 (s, 1H), 10.3 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.4, 21.9, 22.0, 22.4, 24.7, 24.8, 32.5, 33.0, 39.2, 39.7, 59.5, 61.1, 68.5, 68.6, 124.9, 125.0, 128.6, 128.7, 128.8, 128.8, 129.0, 129.0, 130.0, 130.6, 134.1, 141.2, 141.4, 150.0, 150.0, 169.8, 170.0, 170.4. HRMS calcd for $(M + H)^+$: $C_{21}H_{25}ClN_2O_4$ 405.1581, found 405.1574.

Mono(phenylmethyl) α-[[(1-Carboxy-3-phenyl)propyl]amino]-4-methylpentanoate (168)

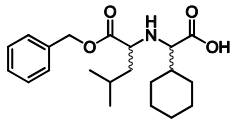


168

Compound **168** was obtained with (2-bromoethyl)benzene and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 4.2 mg, 7% isolated yield; sample 2: 12.6 mg, 22% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 35% (sample 1) and 35% (sample 2), t_R = 8.9, 9.9 min (dr 65:35); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82 – 0.84 (m,

6H), 1.68 – 1.69 (m, 3H), 2.11 – 2.17 (m, 1H), 2.22 – 2.28 (m, 1H), 2.71 – 2.84 (m, 2H), 3.52 (t, *J* = 6.0 Hz, 1H), 3.79 (t, *J* = 6.0 Hz, 1H), 5.13 – 5.22 (m, 2H), 7.12 – 7.13 (m, 2H), 7.16 – 7.19 (m, 1H), 7.22 – 7.25 (m, 2H), 7.30 – 7.33 (m, 2H), 7.34 – 7.36 (m, 3H), 9.10 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.9, 22.2, 24.6, 31.5, 32.6, 39.7, 59.4, 60.6, 68.5, 126.5, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 134.1, 139.5, 169.5, 171.9. HRMS calcd for $(M + H)^+$: $C_{23}H_{29}NO_4$ found 384.2165.

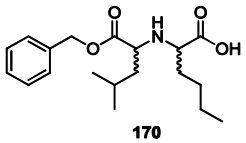
Mono(phenylmethyl) α-[[(1-Carboxy-1-cyclohexyl)methyl]amino]-4-methyl-pentanoate (169)



169

Compound **169** was obtained with cyclohexyl iodide and benzyl (*S*)-2-bromo-4-methylpentaoate as a yellow oil (sample 1: 18.0 mg, 33% isolated yield; sample 2: 2.6 mg, 5% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 34% (sample 1) and 37% (sample 2), t_R = 9.2, 10.8 min (dr 66:34); ^{1}H NMR (500 MHz, DMSO): δ 0.84 – 0.88 (m, 6H), 1.03 – 1.23 (m, 5H), 1.55 – 1.69 (m, 8H), 1.75 – 1.80 (m, 1H), 3.53 (s, 1H), 3.77 (s, 1H), 5.13 – 5.24 (m, 2H), 7.35 – 7.41 (m, 5H). ^{13}C NMR (500 MHz, DMSO): δ 21.7, 22.1, 22.3, 24.2, 24.2, 25.4, 25.6, 28.4, 28.5, 58.2, 64.4, 128.1, 128.2, 128.2, 128.3, 135.2, 157.7, 157.9. HRMS calcd for $(M + H)^+$: $C_{21}H_{31}NO_4$ 362.2331, found 362.2327.

α-[[2-Oxo-2-(phenylmethoxy)-1-(isobutyl)ethyl]amino]hexanoic acid (170)

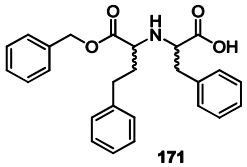


170

Compound **170** was obtained with *n*-butyl iodide and benzyl (±)-2-bromopropanoate as a yellow oil (sample 1: 14.4 mg, 29% isolated yield; sample 2: 14.2 mg, 28% isolated yield) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5). Crude LC/MS purities 40% (sample 1) and 38% (sample 2), t_R = 8.3, 9.4 min (dr 60:40); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 – 0.95 (m, 2H), 1.25 – 1.33 (m, 2H), 1.35 – 1.45 (m, 2H), 1.60 – 1.66 (m, 0.5H), 1.71 – 1.81 (m, 2H), 1.82 –

1.96 (m, 2.5H), 3.58 (diastereomer A, t, *J* = 6.1 Hz, 0.6H), 3.65 (diastereomer B, t, *J* = 6.2 Hz, 0.4H), 3.88 – 3.92 (m, 1H), 5.16 – 5.30 (m, 2H), 7.31 – 7.33 (m, 1H), 7.34 – 7.39 (m, 4H), 10.2 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.5, 13.5, 19.0, 21.3, 21.8, 22.1, 22.2, 22.2, 22.8, 24.6, 24.9, 27.3, 27.5, 29.5, 30.4, 38.6, 39.5, 58.9, 59.4, 60.8, 61.4, 68.6, 68.6, 128.7, 128.8, 128.8, 129.1, 134.1, 168.9, 169.0, 171.7, 171.9. HMRS calcd for $(M + H)^+$: $C_{19}H_{29}NO_4$ 336.2175, found 336.2181.

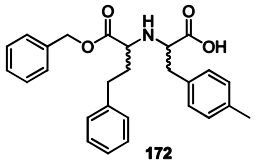
Mono(phenylmethyl) 1-carboxy-2-(phenyl)ethyl]amino]benzenebutanoate (171)



171

Compound **171** was obtained with benzyl bromide and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white solid (5.6 mg, 9% isolated yield – 2.3 mg and 2.2 mg of separate diastereomers and 1.1 mg of a mixture of diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50) by preparative liquid chromatography. Crude LC/MS purities 51% (sample 1) and 45% (sample 2), t_R = 9.3, 9.9 min (dr 56:44); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.92 – 1.94 (m, 1H), 1.99 – 2.03 (m, 1H), 1.57 (t, *J* = 7.2 Hz, 2H), 3.04 – 3.08 (m, 1H), 3.14 – 3.16 (m, 1H), 3.37 (s, 1H), 3.50 (s, 1H), 5.08 (d, *J* 12.0 Hz, 1H), 5.11 (d, *J* = 12.0 Hz, 1H), 7.05 (d, *J* = 14.6 Hz, 2H), 7.20 – 7.22 (m, 3H), 7.25 – 7.27 (m, 2H), 7.30 – 7.34 (m, 5H), 7.38 – 7.41 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 31.7, 34.8, 38.0, 60.6, 61.7, 67.2, 126.3, 127.5, 128.3, 128.6, 128.6, 128.7, 129.0, 129.4, 135.1, 135.3, 140.1, 173.0, 173.5. Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82 – 1.88 (m, 2H), 2.31 – 2.34 (m, 2H), 2.91 – 2.96 (m, 1H), 3.19 – 3.22 (m, 1H), 3.29 – 3.32 (m, 1H), 3.5 (s, 1H), 5.09 (d, *J* = 11.8 Hz, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 6.93 (d, *J* = 14.6 Hz, 2H), 7.17 – 7.20 (m, 1H), 7.23 – 7.26 (m, 4H), 7.32 – 7.35 (m, 4H), 7.36 – 7.40 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 29.7, 31.2, 33.9, 38.4, 60.4, 67.4, 126.2, 127.4, 128.2, 128.5, 128.6, 128.7, 129.0, 129.3, 135.0, 136.4, 173.2. HRMS calcd for $(M + H)^+$: $C_{26}H_{27}NO_4$ 418.2018, found 418.2017.

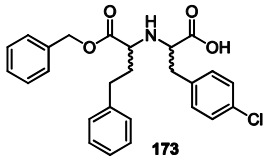
Mono(phenylmethyl) α-[[1-carboxy-2-(4-methylphenyl)ethyl]amino]benzene-butanoate (172)



172

Compound **172** was obtained with 4-methylbenzyl bromide and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white solid (sample 1: 8.4 mg, 13% isolated yield – 5.4 mg and 3.0 mg of separate diastereomers; sample 2: 12.6 mg, 19% isolated yield – 7.2 mg and 5.4 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50 organic/aqueous) by preparative liquid chromatography. Crude LC/MS purities 55% (sample 1) and 52% (sample 2), t_R = 9.9, 10.4 min (dr 56:44); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.90 – 1.97 (m, 1H), 1.99 – 2.07 (m, 1H), 2.34 (s, 3H), 2.47 – 2.59 (m, 2H), 2.98 – 3.04 (m, 1H), 3.09- 3.15 (m, 1H), 3.49 – 3.50 (m, 1H), 3.56 – 3.58 (m, 1H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.11 (d, *J* = 12.6 Hz, 1H), 7.02 (d, *J* = 14.7 Hz, 2H), 7.08 – 7.14 (m, 4H), 7.17 – 7.20 (m, 1H), 7.23 – 7.26 (m, 2H), 7.32 – 7.34 (m, 2H), 7.36 – 7.42 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.1, 31.3, 33.6, 37.0, 60.0, 61.6, 67.6, 126.4, 128.3, 128.6, 128.6, 128.8, 128.8, 129.2, 129.2, 129.7, 129.9, 132.0, 134.8, 137.2, 139.8, 171.8, 173.2. Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.89 (s, 2H), 2.29 (s, 3H), 2.33 – 2.37 (m, 2H), 2.92 – 2.97 (m, 1H), 3.27 – 3.29 (m, 2H), 3.56 (s, 1H), 5.06 (d, *J* = 12.0 Hz, 1H), 5.14 (d, *J* = 12.0 Hz, 1H), 6.91 (d, *J* = 7.3 Hz, 2H), 7.10 (s, 4H), 7.15 – 7.18 (m, 1H), 7.20 – 7.23 (m, 2H), 7.30 – 7.32 (m, 2H), 7.34 – 7.37 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.1, 29.7, 31.1, 33.5, 37.4, 60.2, 62.1, 67.5, 126.2, 128.2, 128.5, 128.6, 128.7, 129.1, 129.7, 133.2, 134.9, 137.1, 140.1, 172.4, 174.0. HRMS calcd for $(M + H)^+$: $C_{27}H_{29}NO_4$ 432.2175, found 432.2189 (diastereomer 1) and 432.2168 (diastereomer 2).

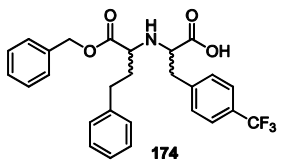
Mono(phenylmethyl) α-[[1-carboxy-2-(4-chlorophenyl)ethyl]amino]benzene-butanoate (173)



173

Compound **173** was obtained with 4-chlorobenzyl bromide and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white solid (sample 1: 11.8 mg, 17% isolated yield – 5.8 mg and 6.0 mg of separate diastereomers; sample 2: 7.0 mg, 10% isolated yield – 3.0 mg and 4.0 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50) by preparative liquid chromatography. Crude LC/MS purities 55% (sample 1) and 52% (sample 2), t_R = 9.9, 10.4 min (dr 59:41); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99 – 2.07 (m, 2H), 2.58 (s, 2H), 3.08 – 3.14 (m, 2H), 3.53 – 3.56 (m, 2H), 5.12 (d, *J* = 13.0 Hz, 1H), 5.13 (d, *J* = 13.0 Hz, 1H), 6.28 (s), 7.03 (d, *J* = 3.4 Hz, 2H), 7.13 (d, *J* = 4.0 Hz, 2H), 7.17 – 7.20 (m, 1H), 7.23 – 7.26 (m, 2H), 7.28 – 7.29 (m, 2H), 7.33 – 7.35 (m, 2H), 7.39 – 7.43 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 31.5, 34.7, 38.6, 59.3, 60.4, 66.2, 125.7, 128.1, 128.2, 128.2, 128.2, 128.4, 130.6, 132.1, 135.5, 135.6, 141.0, 173.6, 174.8. Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.13 (s, 2H), 2.39 – 2.41 (m, 1H), 2.55 – 2.58 (m, 1H), 3.03 – 3.04 (m, 1H), 3.36 – 3.38 (m, 1H), 3.45 (s, 1H), 3.80 (s, 1H), 5.06 (d, *J* = 11.8 Hz, 1H), 5.17 (d, *J* = 11.8 Hz, 1H), 6.95 (d, *J*= 7.1 Hz, 2H), 7.12 – 7.14 (m, 2H), 7.19 – 7.26 (m, 5H), 7.30 – 7.32 (m, 2H), 7.39 – 7.40 (m, 3H), 7.53 (s). ^{13}C NMR (500 MHz, $CDCl_3$): δ 29.7, 30.9, 32.4, 35.8, 59.6, 68.2, 126.5, 127.0, 128.3, 128.6, 128.8, 128.8, 129.0, 129.2, 130.5, 133.5, 134.4, 139.3, 170.4, 172.5. HRMS calcd for $(M + H)^+$: $C_{26}H_{26}ClNO_4$ 452.1629, found 452.1635 (diastereomer 1) and 452.1650 (diastereomer 2).

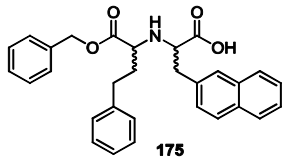
Mono(phenylmethyl) α-[[1-carboxy-2-(4-trifluoromethylphenyl)ethyl]amino]-benzenebutanoate (174)



174

Compound **174** was obtained with 4-trifluoromethylbenzyl bromide and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white solid (sample 1: 3.0 mg, 4% isolated yield as a mixture of diastereomers; sample 2: 5.7 mg, 8% isolated yield – 3.0 mg and 5.7 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50) by preparative liquid chromatography. Crude LC/MS purities 43% (sample 1) and 48% (sample 2), t_R = 10.2, 10.6 min (dr 58:42); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.97 – 2.05 (m, 2H), 2.59 (s, 2H), 3.16 (s, 2H), 3.42 – 3.44 (m, 1H), 3.55 (s, 1H), 5.10 – 5.16 (m, 2H), 7.04 – 7.07 (m, 2H), 7.18 – 7.21 (m, 1H), 7.25 – 7.27 (m, 2H), 7.31 – 7.36 (m, 4H), 7.38 – 7.41 (m, 3H), 7.58 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 29.7, 31.7, 34.7, 37.8, 60.6, 61.3, 67.4, 125.8, 126.4, 128.2, 128.6, 128.8, 128.8, 129.8, 135.0, 139.6, 140.0, 173.2, 173.3. HRMS calcd for $(M + H)^+$: $C_{27}H_{26}F_3NO_4$ 486.1892, found 486.1912.

Mono(phenylmethyl) α-[[(1-Carboxy-2,2'-naphthyl)ethyl]amino]benzenebutanoate (175)

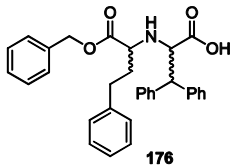


175

Compound **175** was obtained with 2-(bromomethyl)naphthalene and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a yellow-white solid (sample 1: 9.0 mg,13% isolated yield – 2.8 mg and 6.2 mg of separate diastereomers; sample 2: 7.7 mg, 11% isolated yield – 2.5 mg and 5.2 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50) by preparative liquid chromatography. Crude LC/MS purities 47% (sample 1) and 44% (sample 2), t_R = 10.2, 10.6 min (dr 55:45); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.89 – 2.03 (m, 2H), 2.53 (s, 2H), 3.19 – 3.46 (m, 2H), 3.61 (s, 2H), 4.96 – 5.03 (m, 2H), 6.99 (s, 2H), 7.15 – 7.25 (m, 5H), 7.32 – 7.36 (m, 4H), 7.49 – 7.52 (m, 2H), 7.69 (s, 1H), 7.82 – 7.86 (m, 3H). Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.97 (s, 2H), 2.25 – 2.29 (m, 2H), 2.34 – 2.37 (m, 1H), 3.26 – 3.29 (m, 1H), 3.37 (s, 1H), 3.56 – 3.59 (m, 1H), 3.86 (s, 1H), 4.94 (d, *J* = 11.9 Hz, 1H), 5.11 (d, *J* = 11.99 Hz, 1H), 6.77 – 6.78 (m, 2H), 7.11 – 7.12 (m, 3H), 7.21 – 7.22 (m, 2H),

7.32 – 7.37 (m, 4H), 7.48 – 7.52 (m, 2H), 7.76 – 7.83 (m, 4H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 31.0, 32.8, 37.3, 60.0, 62.2, 67.8, 126.1, 126.2, 126.5, 126.7, 127.0, 127.7, 127.7, 128.1, 128.2, 128.4, 128.6, 128.6, 128.7, 128.8, 129.0, 132.6, 133.5, 133.6, 134.6, 139.6, 171.2, 172.2. HRMS calcd for $(M + H)^+$: $C_{30}H_{29}NO_4$ found 468.2175 (diastereomer 1) and 468.2195 (diastereomer 2).

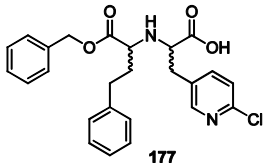
Mono(phenylmethyl) α-[[(1-Carboxy-2-diphenyl)ethyl]amino]benzenebutanoate (176)



176

Compound **176** was obtained with bromodiphenylmethane and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a clear oil (sample 1: 3.8 mg, 5% isolated yield – 2.0 mg and 1.8 mg of separate diastereomers; sample 2: 2.6 mg, 4% isolated yield – 1.2 mg and 1.4 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (70:30 to 100:0) by preparative liquid chromatography. Crude LC/MS purities 28% (sample 1) and 27% (sample 2), t_R = 10.5, 10.9 min (dr 54:46); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.93 – 2.03 (m, 2H), 2.49 (t, *J* = 15.8 Hz, 2H), 3.65 (s, 1H), 4.24 (d, *J* = 9.9 Hz, 1H), 4.41 (d, *J* = 10.0 Hz, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 5.22 (d, *J* = 11.9 Hz, 1H), 6.96 (d, *J* = 7.3 Hz, 2H), 7.13 – 7.40 (m, 18 H). Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.75 – 1.88 (m, 2H), 2.27 (t, *J* = 7.9 Hz, 2H), 3.24 (t, *J* = 5.7 Hz, 1H), 3.99 (d, *J* = 8.7 Hz, 1H), 4.43 (d, *J* = 8.7 Hz, 1H), 5.07 (d, *J* = 12.0 Hz, 1H), 5.14 (d, *J* = 12.0 Hz, 1H), 6.90 (d, *J* = 14.3 Hz, 2H), 7.15 – 7.38 (m, 18H). HRMS calcd for $(M + H)^+$: $C_{32}H_{31}NO_4$ 494.2331, found 494.2314 (diastereomer 1) and 494.2321 (diastereomer 2).

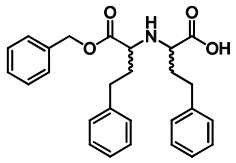
Mono(phenylmethyl) α-[[(1-Carboxy-2,5'-(2-chloropyridine))ethyl]-amino]benzene-butanoate (177)



177

Compound **177** was obtained with 5-(chloromethyl)-2-chloropyridine and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white solid (sample 1: 4.0 mg, 6% isolated yield as a mixture of diastereomers; sample 2: 5.0 mg, 7% isolated yield – 2.2 mg and 2.8 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (30:70 to 50:50) by preparative liquid chromatography. Crude LC/MS purities 30% (sample 1) and 28% (sample 2), t_R = 8.6, 9.2 min (dr 61:39); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.95 - 1.96 (m, 1H), 2.05 (s, 1H), 2.59 – 2.62 (m, 2H), 3.05 (s, 2H), 3.45 (s, 1H), 3.52 (s, 1H), 5.11 (d, *J* = 12.0 Hz, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 2H), 7.16 – 7.19 (m, 1H), 7.23 – 7.24 (m, 2H), 7.33 – 7.40 (m, 5H), 7.50 (d, *J* = 7.3 Hz, 1H), 8.24 (s, 1H). Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.09 – 2.17 (m, 2H), 2.50 – 2.52 (m, 1H), 2.70 (s, 1H), 3.10 – 3.21 (m, 2H), 3.55 (s, 1H), 3.76 (s, 1H), 5.08 (d, *J* = 11.9 Hz, 1H), 5.20 (d, *J* = 11.9 Hz, 1H), 6.99 (d, *J* = 7.2 Hz, 2H), 7.17 – 7.20 (m, 1H), 7.22 – 7.25 (m, 3H), 7.32 – 7.33 (m, 2H), 7.36 – 7.37 (m, 3H), 7.54 (d, *J* = 7.3 Hz, 1H), 8.25 (s, 1H). HRMS calcd for $(M + H)^+$: $C_{25}H_{25}ClN_2O_4$ 453.1581, found 453.1571 (diastereomer 1) and 453.1566 (diastereomer 2).

Mono(phenylmethyl) α-[[(1-Carboxy-3-phenyl)propyl]amino]benzenebutanoate (178)

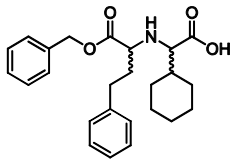


178

Compound **178** was obtained with (2-bromoethyl)benzene and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white solid (sample 1: 8.2 mg, 13% isolated yield – 5.3 mg and 2.9 mg of separate diastereomers; sample 2: 8.8 mg, 14% isolated yield –5.6 mg and 3.2 mg of separate diastereomers) following chromatographic purification over

cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50) by preparative liquid chromatography. Crude LC/MS purities 56% (sample 1) and 56% (sample 2), t_R = 9.7, 10.4 min (dr 58:42); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): 2.11 – 2.19 (m, 4H), 2.53 – 2.57 (m, 2H), 2.73 (s, 2H), 3.57 (s, 1H), 3.77 (s, 1H), 5.08 (d, *J* = 11.9 Hz, 1H), 5.19 (d, *J* = 11.9 Hz, 1H), 6.96 (d, *J* = 7.3 Hz, 2H), 7.09 – 7.23 (m, 8H), 7.27 – 7.34 (m, 2H), 7.35 – 7.36 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 30.8, 31.4, 32.2, 32.3, 60.0, 60.6, 68.5, 126.5, 126.6, 128.2, 128.5, 128.6, 128.8, 128.9, 129.1, 134.2, 139.1, 139.5, 169.4, 172.2. Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.05 – 2.13 (m, 1H), 2.18 – 2.19 (m, 2H), 2.24 – 2.29 (m, 1H), 2.47 – 2.53 (m, 1H), 2.56 – 2.69 (m, 1H), 2.70 – 2.79 (m, 2H), 3.45 (t, *J* = 5.9 Hz, 1H), 3.64 (t, *J* = 5.8 Hz, 1H), 4.99 (d, *J* = 12.0 Hz, 1H), 5.13 (d, *J* = 11.9 Hz, 1H), 6.99 (d, *J* = 7.3 Hz, 2H), 7.08 (d, *J* = 7.3 Hz, 7.14 – 7.24 (m, 6H), 7.27 – 7.28 (m, 2H), 7.32 – 7.37 (m, 3H). HRMS calcd for $(M + H)^+$: $C_{27}H_{29}NO_4$ 432.2175, found 432.2166.

Mono(phenylmethyl) α-[[(1-Carboxy-1-cyclohexyl)methyl]amino]benzenebutanoate (179)

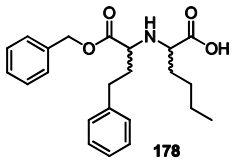


179

Compound **179** was obtained with cyclohexyl iodide and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a clear oil (sample 1: 7.5 mg, 12% isolated yield – 4.4 mg and 3.1 mg of separate diastereomers; sample 2: 7.2 mg, 12% isolated yield –1.2 mg and 6.0 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (50:50) by preparative liquid chromatography. Crude LC/MS purities 72% (sample 1) and 72% (sample 2), t_R = 10.1, 11.1 min (dr 60:40); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 – 1.27 (m, 5H), 1.58 – 1.68 (m, 5H), 1.94 – 1.99 (m, 1H), 2.09 – 2.12 (m, 2H), 2.49 (t, *J* = 7.9 Hz, 2H), 3.45 – 3.46 (m, 1H), 3.61 – 3.63 (m, 1H), 5.12 (d, *J* = 11.8 Hz, 1H), 5.27 (d, *J* = 11.8, 1H), 6.94 (d, *J* = 7.4 Hz, 2H), 7.07 – 7.10 (m, 1H), 7.16 – 7.19 (m, 2H), 7.35 – 7.40 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 25.4, 25.6, 25.9, 28.5, 28.7, 30.7, 31.9, 39.6, 61.0, 67.2, 68.8, 126.7, 128.1, 128.7, 128.9, 129.1, 129.2, 134.0, 139.1, 168.6, 171.3. Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.03 – 1.10

(m, 2H), 1.14 – 1.23 (m, 3H), 1.61 – 1.71 (m, 5H), 1.87 – 1.91 (m, 1H), 2.15 – 2.22 (m, 2H), 2.54 – 2.60 (m, 1H), 2.65 – 2.71 (m, 1H), 3.31 – 3.32 (m, 1H), 3.68 (t, *J* = 5.8 Hz, 1H), 5.05 (d, *J* = 11.9 Hz, 1H), 5.18 (d, *J* = 12.0 Hz, 1H), 7.04 (d, *J* = 7.2 Hz, 2H), 7.16 – 7.19 (m, 1H), 7.22 – 7.26 (m, 2H), 7.32 – 7.37 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 25.6, 25.8, 26.0, 28.8, 31.4, 32.2, 39.8, 60.5, 65.8, 68.1, 126.5, 128.3, 128.7, 128.8, 128.8, 128.9, 134.6, 139.6, 170.5, 172.6. HRMS calcd for $(M + H)^+$: $C_{25}H_{31}NO_4$ 410.2331, found 410.2344 (diastereomer 1) and 410.2318 (diastereomer 2).

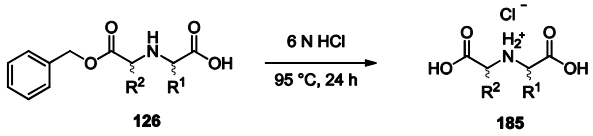
α-[[2-Oxo-2(phenylmethoxy)-1-(2-phenylethyl)ethyl]amino]hexanoic acid (180)



178

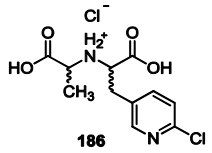
Compound **180** was obtained with *n*-butyl iodide and (*S*)-benzyl 2-bromo-4-phenylbutanoate as a white, oily solid (sample 1: 7.3 mg, 13% isolated yield – 1.3 mg and 6.0 mg of separate diastereomers; sample 2: 9.4 mg, 16% isolated yield – 5.0 mg and 4.4 mg of separate diastereomers) following chromatographic purification over cyano-silica gel with acetone-hexanes (1:4 to 2:5) and over C_{18}-silica gel with 0.1% TFA/CH_3CN and H_2O (30:70 to 50:50) by preparative liquid chromatography. Crude LC/MS purities 68% (sample 1) and 67% (sample 2), t_R = 9.2, 10.0 min (dr 58:42); Diastereomer 1 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.5 Hz, 3H), 1.25 – 1.35 (m, 4H), 1.82 – 1.84 (m, 2H), 2.17 – 2.18 (m, 2H), 2.63 (s, 2H), 3.47 (s, 1H), 3.72 (s, 1H), 5.14 (d, *J* = 12.3 Hz, 1H), 5.25 (d, *J* = 11.8 Hz, 7.03 – 7.05 (m, 2H), 7.15 – 7.18 (m, 1H), 7.21 – 7.24 (m, 2H), 7.34 – 7.39 (m, 5H). Diastereomer 2 – ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (t, *J* = 7.3 Hz, 3H), 1.23 (sextet, *J* = 7.4 Hz, 2H), 1.31 – 1.39 (m, 2H), 1.73 – 1.87 (m, 2H), 2.09 – 2.17 (m, 2H), 2.54 – 2.65 (m, 2H), 3.45 (t, *J* = 6.1 Hz, 1H), 3.69 (t, *J* = 5.9 Hz, 1H), 5.12 (d, *J* = 11.9 Hz,12H), 5.23 (d, *J* = 11.9 Hz, 1H), 6.99 (d, *J* = 7.3 Hz, 2H), 7.13 – 7.15 (m, 1H), 7.19 – 7.21 (m, 2H), 7.34 – 7.39 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.6, 22.2, 27.3, 30.9, 31.0, 32.8, 60.2, 61.5, 68.3, 126.5, 128.3, 128.6, 128.8, 128.9, 129.0, 134.3, 139.4, 170.0, 172.6. HRMS calcd for $(M + H)^+$: $C_{23}H_{29}NO_4$ 384.2175, found 384.2162 (diastereomer 1) and 384.2185 (diastereomer 2).

5.8 General Procedure for the Hydrolysis of the Benzyl Ester Intermediates



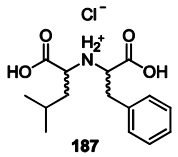
To the benzyl ester intermediate **126** was added to 6 N HCl (2 mL) and the mixture was heated at 95 °C in an oil bath (≤ 4 samples) or in an oven (>4 samples). After 24 hrs the samples were cooled to room temperature and an aliquot was removed for analysis by LC/MS. If the hydrolysis was complete, the reaction mixture was concentrated under vacuum, frozen in water (1–2 mL) and then lyophilized to give products **127** as a white or yellow solid. If the hydrolysis was not complete, heating of the reaction mixture was continued for an additional 48–72 hrs until the reaction was complete by LC/MS.

α-[[(1-Carboxy-1-methyl)ethyl]amino]-3,5'-(2-chloropyridinyl)propanoic acid hydrochloride (186)



Compound **186** was obtained from **155** as a solid (12.4 mg, 27% overall yield). LC/MS purity 100%, t_R = 2.8, 3.1 (dr unavailable due to overlapping peaks); ^{1}H NMR (500 MHz, DMSO): δ 1.51 – 1.54 (m, 3H), 3.23 – 3.27 (m, 1H), 3.41 (diastereomer A, dd, *J* = 14.5 Hz, *J* = 5.8 Hz, 0.7H), 3.51 (diastereomer B, dd, *J* = 14.3 Hz, *J* = 5.1 Hz, 0.4H), 4.07 – 4.13 (m, 1H), 4.29 – 4.32 (m, 0.5H), 4.35 – 4.38 (m, 0.7H), 7.50 – 7.53 (m, 1H), 7.86 – 7.90 (m, 1H), 8.36 – 8.37 (m, 1H). ^{13}C NMR (500 MHz, DMSO): δ 14.2, 14.9, 31.1, 31.2, 53.7, 54.1, 58.1, 58.2, 128.9, 130.4, 131.0, 140.6, 140.8, 148.9, 149.0, 150.5, 150.6, 168.9, 169.0, 170.4. HRMS calcd for $(M + H)^+$: $C_{11}H_{13}ClN_2O_4$ 273.0642, found 273.0652.

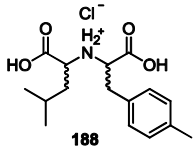
***N*-[(1-Isobutyl-2-carboxy)ethyl]phenylalanine hydrochloride (187)**



187

Compound **187** was obtained from **159** as a solid (12.4 mg, 26% overall yield). LC/MS purity 100%, t_R = 4.3, 5.5 (dr 60:40); ^{1}H NMR (500 MHz, DMSO): δ 0.47 (d, *J* = 6.5 Hz, 0.3H), 0.54 (d, *J* = 6.6 Hz, 0.3H), -.65 – 0.71 (m, 0.3H), 0.79 – 0.81 (m, 1.3H), 0.86 – 0.89 (m, 2.6H), 1.28 – 1.34 (m, 0.2H), 1.41 – 1.46 (m, 0.2H), 1.51 – 1.56 (m, 0.5H), 1.59 – 1.66 (m, 1H), 1.80 (m, 0.5H), 3.04 – 3.18 (m, 2H), 3.62 (s, 1H), 3.79 (s, 0.4H), 3.93 (s, 0.6H), 7.22 – 7.45 (m, 5H). ^{13}C NMR (500 MHz, DMSO): δ 21.1, 21.8, 22.1, 22.4, 24.1, 24.2, 57.3, 57.5, 59.9, 126.8, 128.2, 128.3, 128.5, 129.1, 129.2, 135.9, 136.0. HRMS calcd for (M + Na)$^+$: $C_{15}H_{21}NO_4$ 302.1368, found 302.1369.

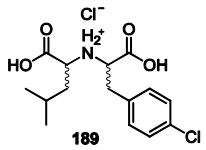
***N*-[(1-Isobutyl-2-carboxy)ethyl]-4-methylphenylalanine hydrochloride (188)**



188

Compound **188** was obtained from **160** as a solid (6.0 mg, 12% overall yield). LC/MS purity 99%, t_R = 5.3, 6.4 (dr 60:40); ^{1}H NMR (500 MHz, DMSO): δ 0.47 (d, *J* = 6.5 Hz, 0.2H), 0.51 (d, *J* = 6.6 Hz, 0.2H), 0.63 – 0.72 (m, 0.2H), 0.78 – 0.81 (m, 2H), 0.86 – 0.88 (m, 3.5H), 1.48 – 1.50 (m, 1H), 1.55 – 1.57 (m, 1H), 1.62 (m, 0.4H), 1.78 (m, 0.6H), 2.26 (s, 3H), 2.97 – 3.08 (m, 2H), 3.30 (s, 0.5H), 3.54 (s, 0.8H), 3.68 (s, 0.5H), 3.82 (s, 0.6H), 7.09 – 7.14 (m, 4H). ^{13}C NMR (500 MHz, DMSO): δ 20.5, 21.9, 21.9, 22.1, 22.4, 24.1, 24.1, 36.4, 57.3, 57.5, 60.1, 60.3, 128.7, 128.8, 129.0, 129.1, 132.9, 135.5, 135.7, 171.5, 172.7. HRMS calcd for (M + Na)$^+$: $C_{16}H_{23}NO_4$ 316.1525, found 316.1522.

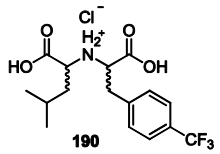
***N*-[(1-Isobutyl-2-carboxy)ethyl]-4-chlorophenylalanine hydrochloride (189)**



189

Compound **189** was obtained from **161** as a solid (10.0 mg, 19% overall yield). LC/MS purity 88%, t_R = 5.6, 6.6 (dr 60:40; ^{1}H NMR (500 MHz, DMSO): δ 0.78 – 0.82 (diastereomer B, m, 2.3H), 0.86 – 0.89 (diastereomer A, m, 3.7H), 1.48 – 1.51 (m, 1H), 1.57 – 1.60 (m, 1H), 1.52 – 1.66 (diastereomer B, m, 0.4H), 1.77 – 1.82 (diastereomer A, m, 0.6H), 3.02 – 3.15 (m, 2H), 3.32 (s), 3.61 (s), 3.90 (s), 7.29 – 7.30 (m, 2H), 7.35 – 7.39 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 21.9, 22.1, 22.3, 24.1, 24.1, 57.4, 57.5, 59.8, 60.0, 128.0, 128.2, 131.1, 131.2, 131.3, 131.5. HRMS calcd for (M + Na)$^+$: $C_{15}H_{20}ClNO_4$ 336.0979, found 336.0992.

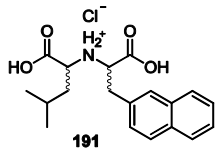
***N*-[(1-Isobutyl-2-carboxy)ethyl]-4-trifluoromethylphenylalanine hydrochloride (190)**



190

Compound **190** was obtained from **162** as a solid (5.0 mg, 9% overall yield). LC/MS purity 97%, t_R = 6.3, 7.3 (dr 70:30); ^{1}H NMR (500 MHz, DMSO): δ 0.44 (d, *J* = 6.4 Hz, 0.4H), 0.52 (d, *J* = 6.5 Hz, 0.4H), 0.66 – 0.70 (m, 0.5H), 0.74 (d, *J* = 6.5 Hz, 0.6H), 0.78 (d, *J* = 6.5 Hz, 0.6H), 0.86 – 0.89 (m, 3.6H), 1.26 – 1.45 (m, 0.8H), 1.50 – 1.57 (m, 1.4H), 1.79 (m, 0.6H), 7.50 – 7.52 (m, 2H), 7.65 – 7.69 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 20.9, 21.7, 22.0, 22.1, 23.5, 24.1, 57.4, 57.6, 59.6, 125.0, 125.3, 130.1, 130.2, 130.2, 130.3. HRMS calcd for (M + Na)$^+$: $C_{16}H_{20}F_3NO_4$ 370.1242, found 370.1260.

α-[[(1-Carboxy-2,2'-naphthyl)ethyl]amino]-4-methylpentanoic acid hydrochloride (191)

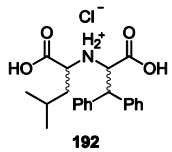


191

Compound **191** was obtained from **163** as a solid (11.8 mg, 22% overall yield). LC/MS purity 100%, t_R = 6.4, 7.2, dr 46:54); ^{1}H NMR (500 MHz, DMSO): δ 0.76 – 0.77 (m, 2.1H), 0.81 – 0.91 (m, 3.9H), 1.60 – 1.71 (m, 2.7H), 1.81 – 1.87 (diastereomer B, m, 0.3H), 3.22 – 3.36 (m, 2H), 3.42 – 3.46 (m, 1H), 3.56 (s), 3.81 (s), 4.11 (s), 4.23 (s), 7.34 – 7.39 (m, 2H), 7.48 – 7.53 (m, 2H), 7.80 – 7.86 (m, 2H), 7.88 – 7.91 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 21.6, 22.1, 22.2, 22.5, 24.1, 24.2, 57.4, 59.8, 125.6, 125.8, 126.0,

126.1, 127.3, 127.4, 127.4, 127.7, 127.9, 127.9, 128.0, 128.2, 128.4, 131.9, 132.0, 132.0, 132.8, 132.8. HRMS calcd for $(M + Na)^+$: $C_{19}H_{23}NO_4$ 352.1525, found 352.1525.

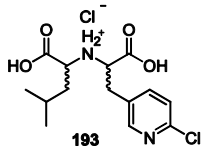
α-[[(1-Carboxy-2-diphenyl)ethyl]amino]-4-methylpentanoic acid hydrochloride (192)



192

Compound **192** was obtained from **164** as a solid (4.0 mg, 7% overall yield). LC/MS purity 100%, t_R = 4.9, 6.3 (dr 66:34); ^{1}H NMR (500 MHz, DMSO): δ 0.60 (d, *J* = 5.9 Hz, 1.3H), 0.64 (d, *J* = 6.4 Hz, 1.3H), 0.80 – 0.82 (m, 3.4H), 1.30 – 1.44 (m, 2.5H), 1.66 – 1.71 (m, 0.6H), 3.02 (s), 3.26 (s), 4.25 – 4.34 (m, 1H), 7.16 – 7.18 (m, 1H), 7.23 – 7.29 (m, 4H), 7.30 – 7.34 (m, 2H), 7.38 – 7.40 (m, 1H), 7.46 – 7.49 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 21.8, 22.0, 22.2, 23.8, 24.0, 57.3, 58.7, 62.9, 126.5, 126.8, 128.1, 128.2, 128.2, 128.3, 128.4, 128.5. HRMS calcd for $(M + Na)^+$: $C_{21}H_{25}NO_4$ 378.1681, found 378.1699.

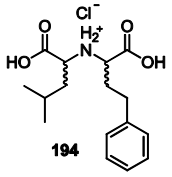
α-[[(1-Carboxy-2,5'-(2-chloropyridine))ethyl]amino]-4-methylpentanoic acid hydrochloride (193)



193

Compound **193** was obtained from **165** as a solid (7.8 mg, 15% overall yield). LC/MS purity 100%, t_R = 3.8, 4.8 (dr 65:35); ^{1}H NMR (500 MHz, DMSO): δ 0.86 – 0.92 (m, 6H), 1.68 – 1.77 (m, 2.3H), 1.85 – 1.90 (m, 0.6H), 3.16 – 3.40 (m, 2H), 3.93 (t, *J* = 6.6 Hz, 1.4H), 4.32 (t, *J* = 6.4 Hz, 0.6H), 7.51 – 7.54 (m, 1H), 7.81 – 7.84 (m, 1H), 8.34 – 8.35 (m, 1H). ^{13}C NMR (500 MHz, DMSO): δ 21.2, 21.6, 22.2, 22.7, 24.1, 24.4, 57.6, 58.9, 123.9, 130.4, 140.8, 149.0, 150.6. HRMS calcd for $(M – H + 2Na)^+$: $C_{14}H_{18}ClN_2O_4$ 359.0750, found 359.0761.

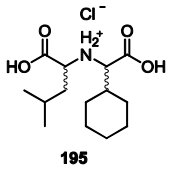
Mono(phenylmethyl) α-[[(1-Carboxy-3-phenyl)propyl]amino]-4-methylpentanoic acid hydrochloride (194)



194

Compound **194** was obtained from **166** as a solid (11.0 mg, 22% overall yield). LC/MS purity 95%, t_R = 5.2, 6.2 (dr 53:47); ^{1}H NMR (500 MHz, DMSO): δ 0.83 - 0.93 (m, 6H), 1.65 – 1.79 (m, 2.2H), 1.88 – 1.94 (m, 0.6H), 2.07 – 2.18 (m, 2H), 2.64 – 2.87 (m, 2H), 3.89 – 3.92 (m, 1H), 7.21 – 7.26 (m, 3H), 7.30 – 7.34 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 21.7, 22.1, 24.0, 30.5, 57.5, 58.3, 126.1, 128.1, 128.2, 128.2, 128.4, 132.9, 140.2. HRMS calcd for $(M - H + 2Na)^+$: $C_{16}H_{22}NO_4$ 338.1344, found 338.1346.

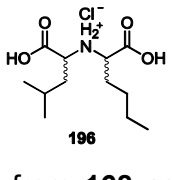
α-[[(1-Carboxy-1-cyclohexyl)methyl]amino]-4-methylpentanoic acid hydrochloride (195)



195

Compound **195** was obtained from **167** as a solid. LC/MS purity 100%, t_R = 4.1, 5.7 (dr unavailable due to lack of chromaphore); ^{1}H NMR (500 MHz, DMSO): δ 0.86 – 0.93 (m, 6H), 1.05 – 1.30 (m, 4H), 1.61 – 1.88 (m, 9H), 3.59 (s), 3.67 (s). ^{13}C NMR (500 MHz, DMSO): δ 21.4, 21.8, 22.2, 22.2, 24.2, 25.1, 25.2, 25.3, 25.3, 25.6, 27.6, 27.8, 28.7, 58.2, 64.5, 172.0. HRMS calcd for $(M - H + 2Na)^+$: $C_{14}H_{24}NO_4$ 316.1501, found 316.1499.

α-[[1-carboxy-1-(isobutyl)methyl]amino]hexanoic acid hydrochloride (196)

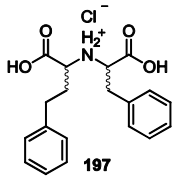


196

Compound **196** was obtained from **168** as a solid (8.9 mg, 21% overall yield). LC/MS purity 100%, t_R = 3.4 (dr unavailable due to lack of chromaphore); ^{1}H NMR (500

MHz, DMSO): δ 0.84 – 0.92 (m, 9H), 1.23 – 1.43 (m, 4H), 1.50 – 1.54 (m ,1H), 1.57 – 1.68 (m, 2H), 1.72 – 1.87 (m, 2H), 3.37 – 3.41 (m, 1H), 3.58 – 3.60 (m, 1H). ^{13}C NMR (500 MHz, DMSO): δ 13.5, 13.6, 13.7, 21.6, 21.7, 21.8, 22.0, 22.2, 24.1, 24.2, 26.7, 26.8, 30.3, 30.5, 57.2, 57.7, 38.4, 59.0. HRMS calcd for $(M + H)^+$: $C_{12}H_{23}NO_4$ 246.1705, found 246.1695.

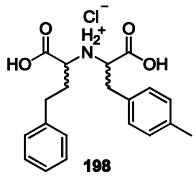
α-[[1-carboxy-2-(phenyl)ethyl]amino]benzenebutanoic acid hydrochloride (197)



197

Compound **197** was obtained from **169** as a solid (Diastereomer 1: 4.5 mg (8% overall yield); Diastereomer 2: 3.7 mg (7% overall yield)). LC/MS purities: Diastereomer 1 – 100%; Diastereomer 2 – 100%, t_R = 5.9 (Diastereomer 1) and 6.8 (Diastereomer 2); Diastereomer 1 – ^{1}H NMR (500 MHz, DMSO): δ 1.56 - 1.72 (m, 2H), 2.09 - 2.10 (m, 2H), 2.36 – 2.64 (m, 2H), 2.74 – 2.87 (m, 2H), 3.07 – 3.11 (m, 1H), 3.47 (t, *J* = 6.1 Hz, 1H), 7.13 – 7.39 (m, 10H). Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 1.72 – 1.92 (m, 2H), 2.18 – 2.32 (m, 1H), 3.07 – 3.11 (m, 1.5H), 3.46 – 3.50 (m, 0.5H), 7.00 (d, *J* = 7.2 Hz, 1H), 7.13 – 7.19 (m, 1H), 7.22 – 7.35 (m, 7H), 7.45 (d, *J* = 7.3 Hz, 1H). HRMS calcd for $(M + H)^+$: $C_{19}H_{21}NO_4$ 328.1549, found 328.1564 (diastereomer 1). HRMS calcd for (M – H + 2Na): $C_{19}H_{21}NO_4$ 372.1188, found 372.1196 (diastereomer 2).

α-[[1-carboxy-2-(4-methylphenyl)ethyl]amino]benzenebutanoic acid hydrochloride (198)

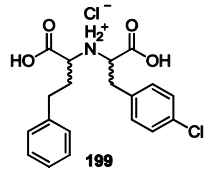


198

Compound **198** was obtained from **170** as a solid (Diastereomer 2: 12.0 mg, 21% overall yield). LC/MS purity: Diastereomer 2 – 100%; t_R = 7.3; Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 1.94 (s, 2H), 2.26 (s, 3H), 2.46 – 2.54 (m, 1H), 2.58 – 2.64 (m, 1H), 3.00 – 3.01 (m, 2H), 3.49 (s, 1H), 3.76 (s, 1H). ^{13}C NMR (500 MHz, DMSO): δ 20.6, 30.7,

58.3, 60.3, 125.8, 127.9, 128.2, 128.2, 128.7, 129.2, 135.6, 140.9, 172.5. HRMS calcd for $(M + H)^+$: $C_{20}H_{23}NO_4$ 342.1705, found 342.1715 (diastereomer 2).

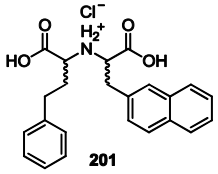
α-[[1-carboxy-2-(4-chlorophenyl)ethyl]amino]benzenebutanoic acid hydrochloride (199)



199

Compound **199** was obtained from **171** as a solid (Diastereomer 2: 8.4 mg, 14% overall yield). LC/MS purity: Diastereomer 2 – 100%; t_R = 7.7; ^{1}H NMR (500 MHz, DMSO): δ 1.72 – 1.74 (m, 2H), 2.37 – 2.48 (m, 2H), 2.79 – 2.83 (m, 1H), 2.92 – 2.95 (m, 1H), 3.13 – 3.19 (m, 1H), 3.35 (s), 700 – 7.02 (m, 2H), 7.14 – 7.17 (m, 1H), 7.18 – 7.35 (m, 6H). ^{13}C NMR (500 MHz, DMSO): δ 30.7, 34.0, 58.4, 60.5, 125.6, 127.8, 127.9, 138.1, 128.2, 128.3, 130.9, 131.2, 141.5. HRMS calcd for $(M + H)^+$: $C_{19}H_{20}ClNO_4$ 384.0979, found 384.0998.

Mono(phenylmethyl) α-[[(1-Carboxy-2,2'-naphthyl)ethyl]amino]benzenebutanoic acid hydrochloride (201)

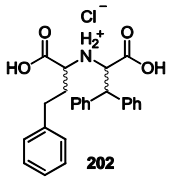


201

Compound **201** was obtained from **173** as a solid (Diastereomer 1: 5.0 mg (8% overall yield); Diastereomer 2: 6.0 mg (10% overall yield)). LC/MS purities: Diastereomer 1 – 100%; Diastereomer 2 – 100%, t_R = 7.6 (Diastereomer 1) and 8.1 (Diastereomer 2); Diastereomer 1 – ^{1}H NMR (500 MHz, DMSO): δ 1.04 – 1.23 (m, 1H), 1.43 – 1.49 (m, 1H), 1.94 – 2.36 (m, 4H), 2.56 – 2.66 (m, 2H), 7.02 – 7.33 (m, 6H), 7.41 – 7.55 (m, 3H), 7.78 – 7.95 (m, 3H). Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 2.00 – 2.15 (m, 2H), 2.47 – 2.54 (m, 1H), 2.60 – 2.64 (m, 1H), 3.08 – 3.15 (m, 1H), 3.25 – 3.30 (m, 1H), 6.63 (s, 0.3H), 7.06 – 7.10 (m, 1.5H), 7.17 – 7.31 (m, 4H), 7.45 – 7.61 (m, 2.5H), 7.71 – 7.82 (m, 1H), 7.85 – 7.93 (m, 2H), 8.00 – 8.01 (m, 0.5H), 8.42 (s, 0.3H). ^{13}C NMR (500 MHz, DMSO): δ 30.8, 58.3, 127.4, 127.6, 128.1, 128.1, 128.2, 128.2, 128.2, 128.3,

128.4, 132.8. HRMS calcd for $(M + H)^+$: $C_{23}H_{23}NO_4$ 378.1705, found 378.1704 (diastereomer 1) and 378.1720 (diastereomer 2).

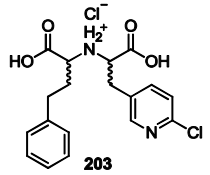
α-[[(1-Carboxy-2-diphenyl)ethyl]amino]benzenebutanoic acid hydrochloride (202)



202

Compound **202** was obtained from **174** as a solid (Diastereomer 1: 3.6 mg (5% overall yield); Diastereomer 2: 4.3 mg (7% overall yield)). LC/MS purities: Diastereomer 1 – 100%; Diastereomer 2 – 100%, t_R = 7.4 (Diastereomer 1) and 8.5 (Diastereomer 2); ^{1}H NMR (500 MHz, DMSO): δ 1.88 (s, 2H), 2.52 – 2.64 (m, 2H), 3.34 (s, 1H), 4.41 (s, 1H), 7.10 – 7.37 (m, 13H), 7.50 (d, *J* = 7.5 Hz, 2H). Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 1.63 – 1.81 (m, 2H), 2.14 – 2.28 (m, 2H), 3.08 – 3.11 (m, 1H), 4.18 – 4.20 (m, 1H), 6.98 (d, *J* = 7.2 Hz, 2H), 7.14 – 7.16 (m, 3H), 7.23 – 7.28 (m, 6H), 7.41 (d, *J* = 7.4 Hz, 2H), 7.48 (d, *J* = 7.5 Hz, 2H). HRMS calcd for $(M + H)^+$: $C_{25}H_{25}NO_4$ 404.1862, found 404.1869 (diastereomer 1) and 404.1879 (diastereomer 2).

α-[[(1-Carboxy-2, 5'-(2-chloropyridine))ethyl]-amino]benzenebutanoic acid hydrochloride (203)

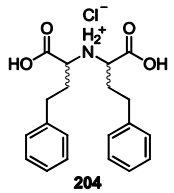


203

Compound **203** was obtained from **175** as a solid (Diastereomer 1: 3.5 mg (6% overall yield); Diastereomer 2: 41. mg (7% overall yield)). LC/MS purities: Diastereomer 1 – 100%; Diastereomer 2 – 100%, t_R = 7.4 (Diastereomer 1) and 8.5 (Diastereomer 2); ^{1}H NMR (500 MHz, DMSO): δ 2.10 – 2.17 (m, 2H), 2.60 – 2.66 (m, 1H), 2.79 – 2.85 (m, 1H), 3.15 – 3.27 (m, 1H), 3.32 – 3.40 (m, 1H), 3.98 (s, 1H), 4.34 (s, 1H), 7.04 – 7.11 (m, 1H), 7.16 – 7.35 (m, 2H), 7.44 – 7.54 (m, 1H), 7.81 – 7.90 (m, 1H), 8.35 – 7.36 (m, 1H). Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 1.84 – 2.01 (m, 2H), 2.35 – 2.54 (m, 2H), 3.17 – 3.31 (m, 2H), 3.37 (t, *J* = 6.1 Hz, 0.4H), 4.48 – 4.53 (m, 0.6H), 7.07 (d, *J* = 6.4 Hz, 1H), 7.17 – 7.23 (m, 2H), 7.27 – 7.49 (m, 2H), 7.46 – 7.54 (m, 1H), 7.77 – 7.82

(m, 0.4H), 7.90 – 7.92 (m, 0.6H), 8.17 – 8.18 (m, 0.6H), 8.43 – 8.44 (m, 0.6H). HRMS calcd for $(M - H + 2Na)^+$: $C_{18}H_{18}ClN_2O_4$ 407.0750, found 407.0765 (diastereomer 1) and 407.0758 (diastereomer 2).

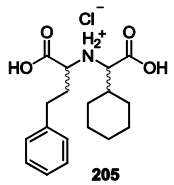
Mono(phenylmethyl) α-[[(1-Carboxy-3-phenyl)propyl]amino]benzenebutanoic acid hydrochloride (204)



204

Compound **204** was obtained from **176** as a solid (Diastereomer 1: 5.5 mg (10% overall yield); Mixture: 8.5 mg (15% overall yield). LC/MS purities: Diastereomer 1 – 100%, Diastereomer 2 – 100%, t_R = 6.6, 7.4 (dr 14:86). Diastereomer 1 – ^{1}H NMR (500 MHz, DMSO): δ 1.83 – 1.91 (m, 4H), 2.64 – 2.67 (m, 4H), 3.26 (s, 2H), 7.16 – 7.23 (m, 6H), 7.26 – 7.31 (4H). Mixture 2 – ^{1}H NMR (500 MHz, DMSO): δ 1.88 – 2.19 (m, 4H), 2.60 – 2.79 (m, 4H), 3.89 – 3.90 (m, 0.5H), 7.17 – 7.32 (m, 10H). ^{13}C NMR (500 MHz, DMSO): δ 30.9, 31.2, 31.8, 58.2, 64.8, 125.8, 126.1, 128.2, 128.2, 128.3, 128.4, 140.4, 141.2, 171.5. HRMS calcd for $(M + H)^+$: $C_{20}H_{24}NO_4$ 342.1705, found 342.1709 (diastereomer 1) and 342.1714 (diastereomer 2).

α-[[(1-Carboxy-1-cyclohexyl)methyl]amino]benzenebutanoic acid hydrochloride (205)

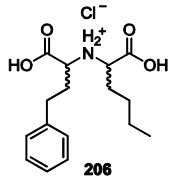


205

Compound **205** was obtained from **177** as a solid (Diastereomer 1: 6.8 mg, (13% overall yield); Diastereomer 2: 4.6 mg (9% overall yield)). LC/MS purities: Diastereomer 1 – 100%; Diastereomer 2 – 100%, t_R = 6.0 (Diastereomer 1) and 7.0 (Diastereomer 2); Diastereomer 1 – ^{1}H NMR (500 MHz, DMSO): δ 1.08 – 1.17 (m, 5H), 1.58 – 1.61 (m, 3H), 1.68 – 1.74 (m, 3H), 1.80 -1.91 (m, 2H), 2.58 – 2.92 (m, 2H), 3.89 – 3.97 (m, 1H), 7.16 – 7.33 (m, 5H). Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 0.98 – 1.24 (m,

5H), 1.39 – 1.45 (m, 0.5H), 1.60 – 1.73 (m, 5H), 1.89 – 1.97 (m, 1.5H), 2.08 – 2.17 (m, 2H), 2.62 – 2.87 (m, 2H), 3.76 – 3.77 (m, 1H), 3.90 – 3.92 (m, 1H), 7.20 – 7.25 (m, 3H), 7.29 – 7.33 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 24.7, 25.2, 25.6, 27.7, 28.5, 30.9, 31.4, 31.7, 126.1, 128.2, 128.4, 140.6, 170.6, 171.5. HRMS calcd for $(M + H)^+$: $C_{18}H_{25}NO_4$ 320.1862, found 320.1873 (diastereomer 1) and 320.1852 (diastereomer 2).

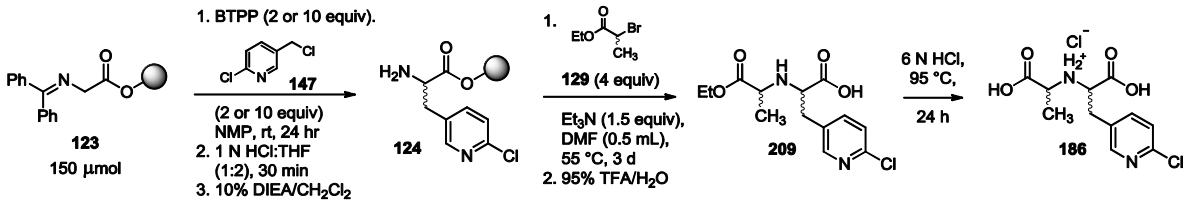
α-[[1-Carboxy-3-phenyl)propyl]amino]hexanoic acid hydrochloride (206)



206

Compound **206** was obtained from **178** as a solid (Mixture 1: 10.7 mg, (22% overall yield); Diastereomer 2: 4.4 mg (9% overall yield)). LC/MS purities: Diastereomer 2 – 100%, t_R = 5.5 (Diastereomer 1) and 6.3 (Diastereomer 2); Mixture 1 – ^{1}H NMR (500 MHz, DMSO): δ 0.85 - 0.89 (m, 3H), 1.29 – 1.33 (m, 3H), 1.37 – 1.42 (m, 1H), 1.63 – 1.67 (m, 0.6H), 1.73 – 1.78 (m, 1H), 1.82 – 1.86 (m, 0.4H), 1.92 – 2.14 (m, 2H), 2.54 – 2.87 (m, 2H), 3.40 (s, 1H), 3.65 (s, 1H), 7.19 – 7.24 (m, 3H), 7.27 – 7.32 (m, 2H). ^{13}C NMR (500 MHz, DMSO): δ 13.6, 13.7, 21.7, 21.8, 26.7, 26.9, 27.3, 30.7, 30.8, 58.6, 59.1, 126.0, 128.1, 128.2, 128.2, 128.3, 128.3, 128.4, 140.7, 171.7. Diastereomer 2 – ^{1}H NMR (500 MHz, DMSO): δ 0.85 – 0.89 (m, 3H), 1.26 – 1.33 (m, 3H), 1.49 – 1.44 (m, 1H), 1.76 (s, 2H), 2. 00 – 2.06 (m, 2H), 2.63 – 2.67 (m, 1H), 2.76 – 2.79 (m, 1H), 3.68 (s, 1H), 3.93 – 3.99 (m, 0.5H), 4.11 – 4.14 (m, 0.2H), 7.19 – 7.24 (m, 3H), 7.29 – 7.32 (m, 2H). HRMS calcd for $(M – H + 2Na)^+$: $C_{16}H_{22}NO_4$ 338.1344, found 338.1352 (mixture 1). HRMS calcd for (M + H): $C_{16}H_{22}NO_4$ 338.1344 294.1705, found 294.1703 (diastereomer 2).

5.9 General Procedure for the Preparation of Ethyl Ester Intermediate **209** and Hydrolysis to the Amino Diacid



The benzophenone imine of glycine resin (BIG-Wang, 180 mg, 150 μmol, 0.84 mmol/g) was weighed into a 5-mL reaction vessel and swelled in CH_2Cl_2 for 30 min. The solvent was drained and a solution of 0.2 M BTPP in NMP (1.5 mL, 300 μmol, 2 equiv) was added to the resin and allowed to mix with the resin for 5 min. A solution of 0.2 M of 2-chloro-5-(chloromethyl)pyridine (**147**) in NMP (1.5 mL, 300 μmol, 2 equiv) was then added, and the reaction was rotated at room temperature for 24 hrs.

The reagents were filtered off, and the resin was washed with NMP (2 x 2 mL) and THF (2 x 2 mL). The benzophenone imine was hydrolyzed by the addition of 1 N HCl/THF (2 mL, 1:2) to the reaction vessel and rotating the resin for 20 min. The hydrolysis solution was filtered off, and the resin was washed with THF (2 x 2 mL), 10% DIEA/CH_2Cl_2 (3 x 2 mL), and DMF (3 x 2 mL). To the reaction vessel was added a 0.4 M solution of ethyl 2-bromopropanoate in DMF (1.5 mL, 600 μmol, 4 equiv), followed by triethylamine (31 μL, 225 μmol. 1.5 equiv). The reaction was heated in an oven at 55 °C for 3 d.

The reagents were filtered off, and the resin was washed with DMF (2 x 2 mL), THF (2 x 2 mL), and CH_2Cl_2 (3 x 2 mL). The product was cleaved from the resin by adding 95% TFA/H_2O (2 mL) to the reaction vessel and rotating at room temperature for 1 h. The cleavage solution was collected in a tared vial, the resin was washed with CH_2Cl_2 (1 x 2 mL), the washes were collected in the tared vial, and the solution was evaporated under a stream of nitrogen to give a crude yield of 66 mg. The LC/MS of the crude mixture showed 58% of the product.

The crude product was analyzed by cyano-silica gel TLC with acetone-hexanes (1:3) and purified by loading the crude product (66 mg) onto a dry 1 g cyano-silica cartridge with acetone (300 μL) and allowing the column to dry with a stream of nitrogen for 1.5 h. The column was equilibrated with hexanes (2 x 2 mL) and eluted with a

gradient of 10-40% acetone/hexanes. Fractions were analyzed by cyano-silica TLC (30% acetone/hexanes) and pooled to afford product **209** as a mixture of diastereomers (14.0 mg, 31%).

α-[[2-oxo-2-(ethoxy)-1-(methyl)ethyl]amino]-3,5'-(2-chloro-pyridinyl)-propanoic acid (209)

LC/MS purity of purified product 100%, t_R = 4.4, 4.6 (dr unavailable due to overlapping peaks). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.17 – 1.25 (m, 3H), 1.49 – 1.52 (m, 3H), 3.25 – 3.36 (m, 2H), 3.75 – 3.76 (m, 0.4H), 3.90 – 3.95 (m, 1.5H), 4.03 – 4.20 (m, 2H), 7.20 – 7.21 (m, 1H), 7.72 – 7.76 (m, 1H), 8.59 – 8.66 (m, 1H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 14.0, 15.3, 16.6, 33.2, 55.5, 55.8, 61.0, 62.6, 62.7, 124.5, 130.4, 131.0, 140.7, 150.2, 150.3, 150.4, 150.5, 170.4, 171.2, 171.3. HRMS calcd for $(M + H)^+$: $C_{13}H_{17}ClN_2O_4$ 301.0955, found 301.0959.

To the ethyl ester intermediate **209** was added to 6 N HCl (2 mL) and the mixture was heated at 95 °C in an oil bath for 24 h. The sample was cooled to room temperature and an aliquot was analyzed by LC/MS, showing 100% of the amino diacid product **186**. The reaction mixture was concentrated under vacuum, frozen in water (1 mL) and then lyophilized to give product **186** as a yellow solid (15.7 mg, 34% overall yield).

α-[[(1-Carboxy-1-methyl)ethyl]amino]-3,5'-(2-chloropyridinyl)propanoic acid hydrochloride (186)

LC/MS purity 100%, t_R = 2.86, 3.08 (dr unavailable due to overlapping peaks). ^{1}H NMR (500 MHz, DMSO): δ 1.540 – 1.53 (m, 3H), 3.21 – 3.26 (m, 1H), 3.38 (dd, *J* = 14.6 Hz, *J* = 5.7 Hz, 0.6H), 3.48 (dd, *J* = 14.3 Hz, *J* = 5.2 Hz, 0.4H), 4.09 – 4.14 (m, 1H), 4.31 – 4.34 (m, 0.4H), 4.36 – 4.39 (m, 0.6H), 7.51 – 7.53 (m, 1H), 7.84 – 7.88 (m, 1H), 8.36 – 8.37 (m, 1H). ^{13}C NMR (500 MHz, DMSO): δ 14.7, 15.4, 31.6, 31.8, 54.4, 547, 58.7, 58.7, 124.4, 124.5, 130.9, 131.4, 141.2, 141.4, 149.5, 149.6, 151.1, 151.2, 169.6, 169.6, 171.0, 171.1. HRMS calcd for $(M + H)^+$:$C_{11}H1_{13}ClN_2O_4$ 273.0642, found 273.0650.

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BIBLIOGRAPHY

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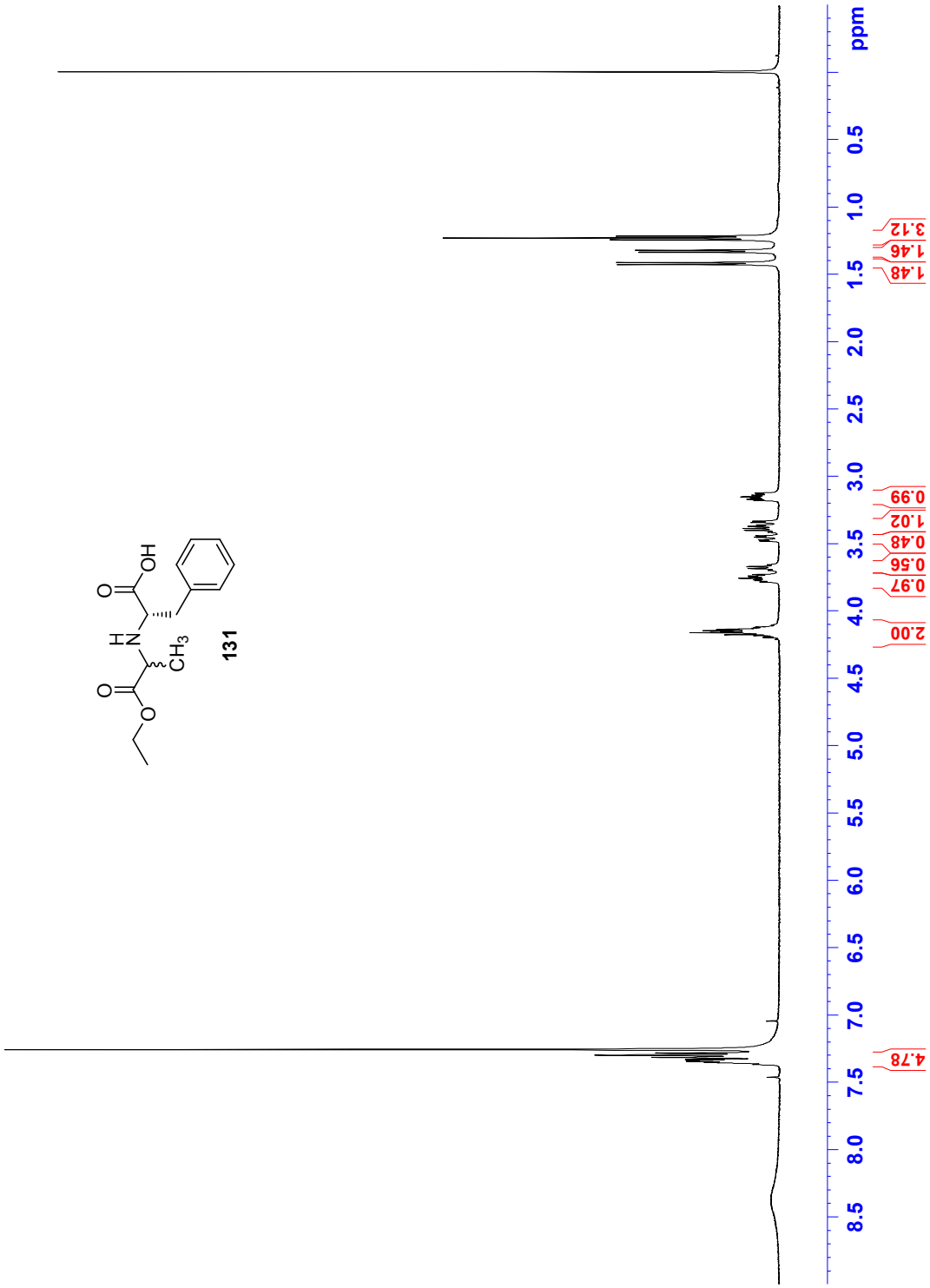
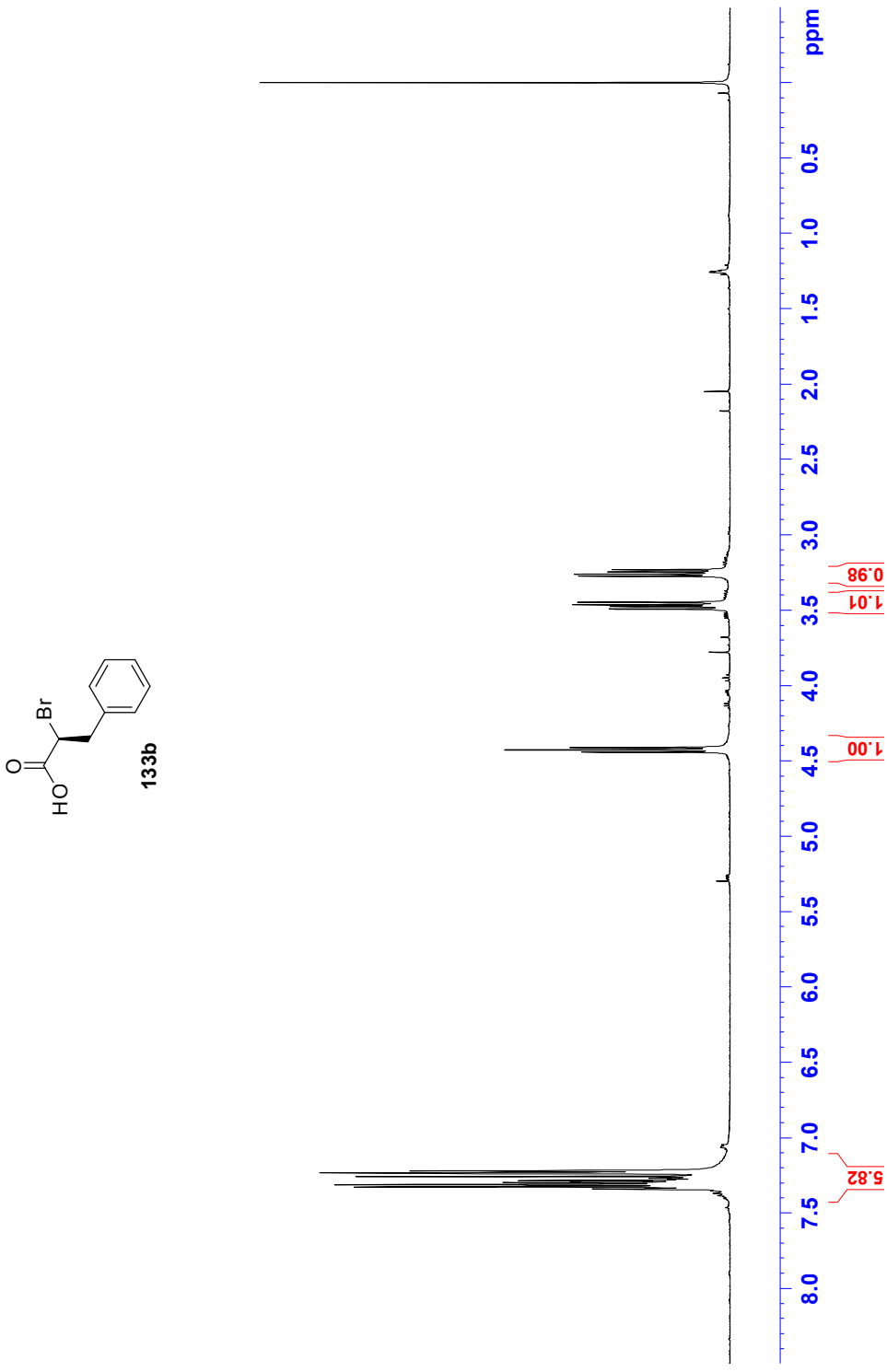
APPENDICES

Appendix A. NMR Spectra

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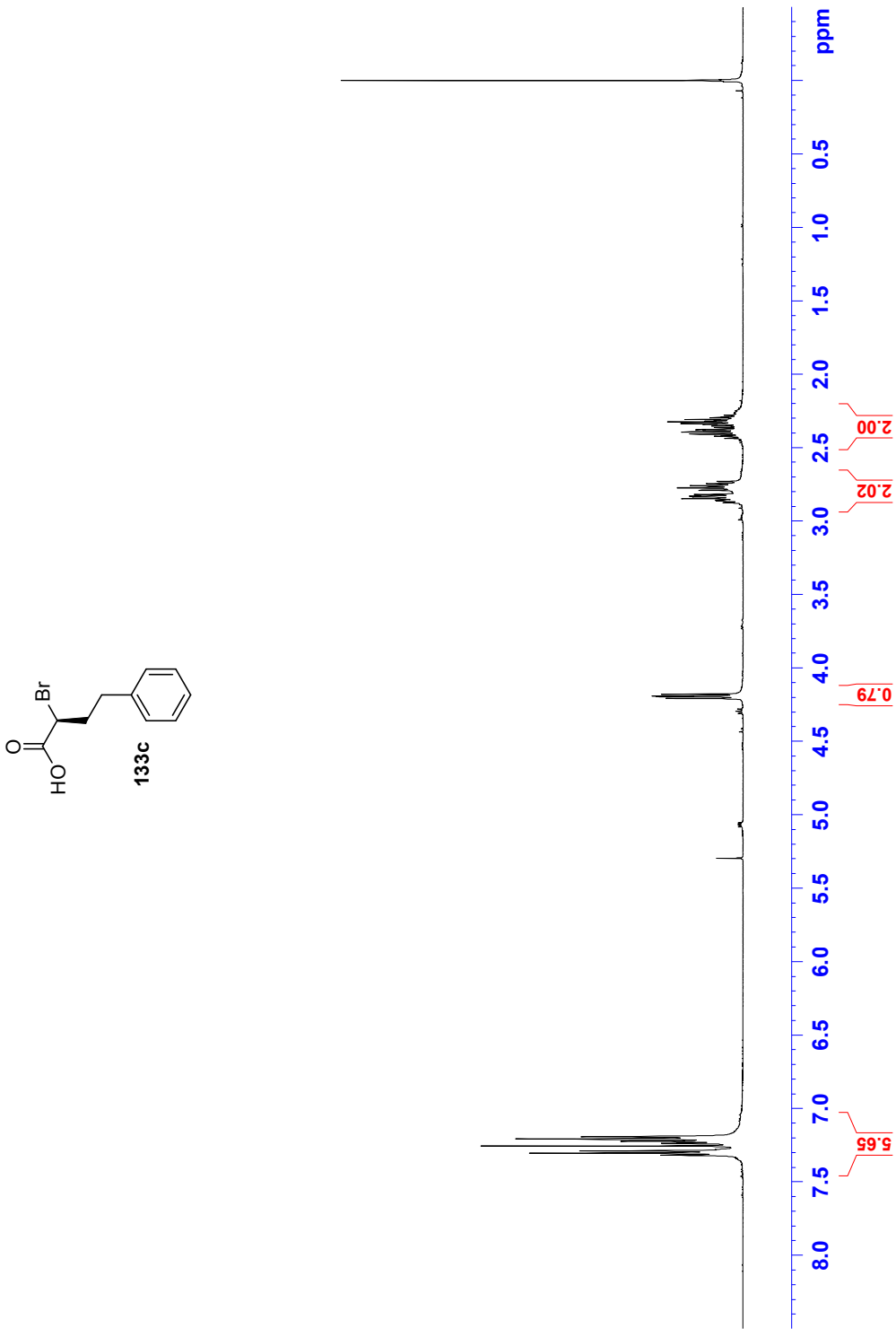
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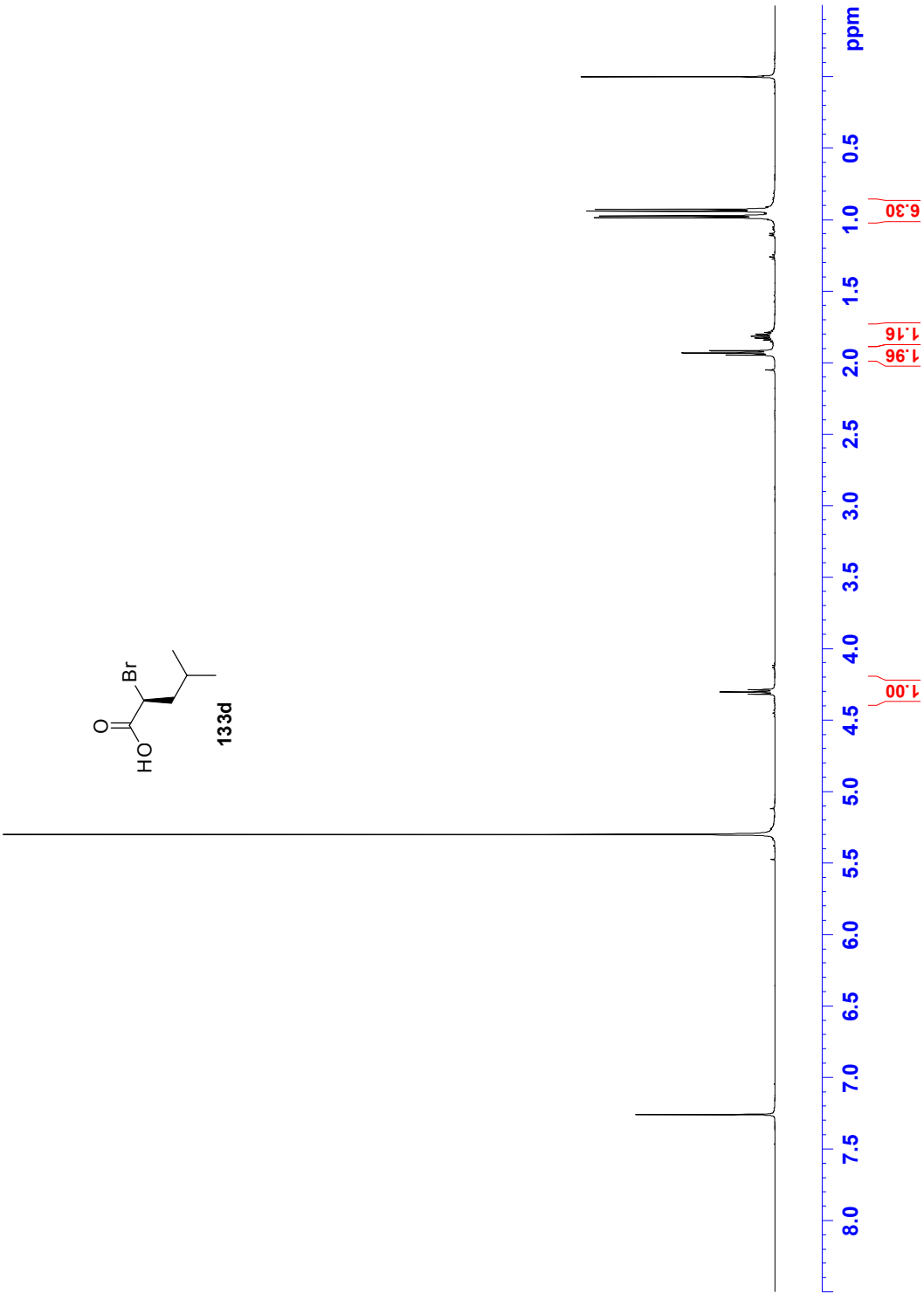
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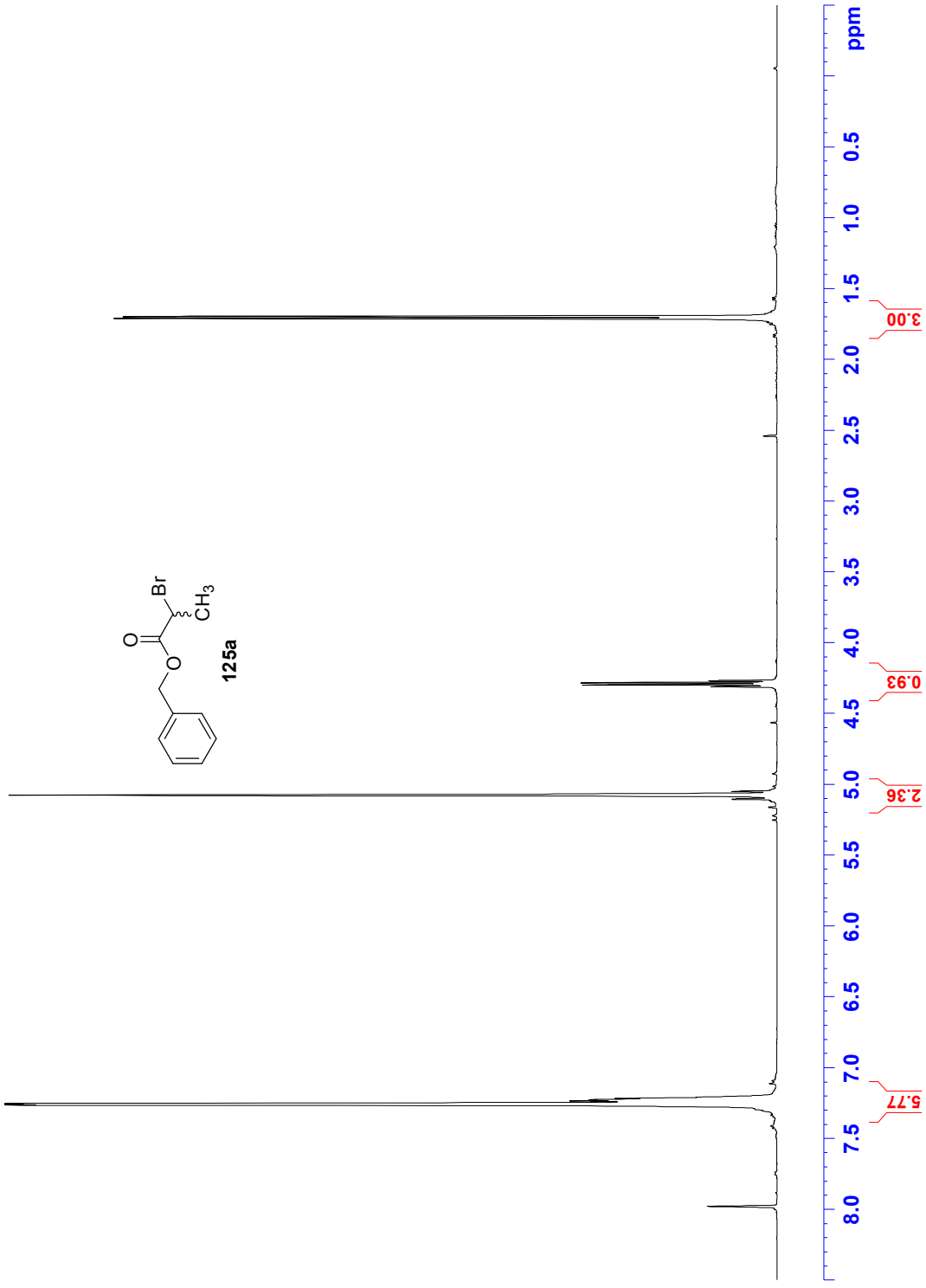
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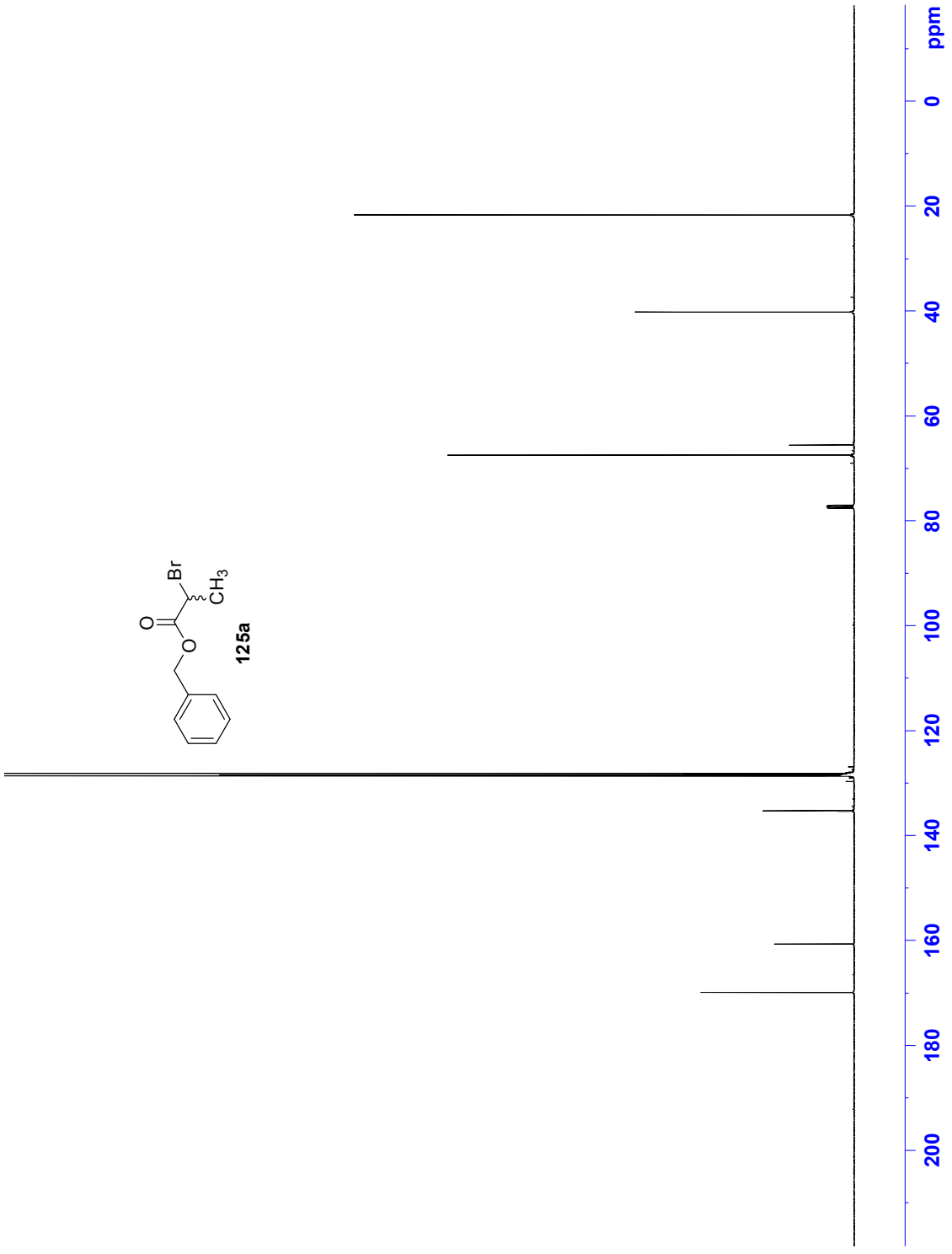
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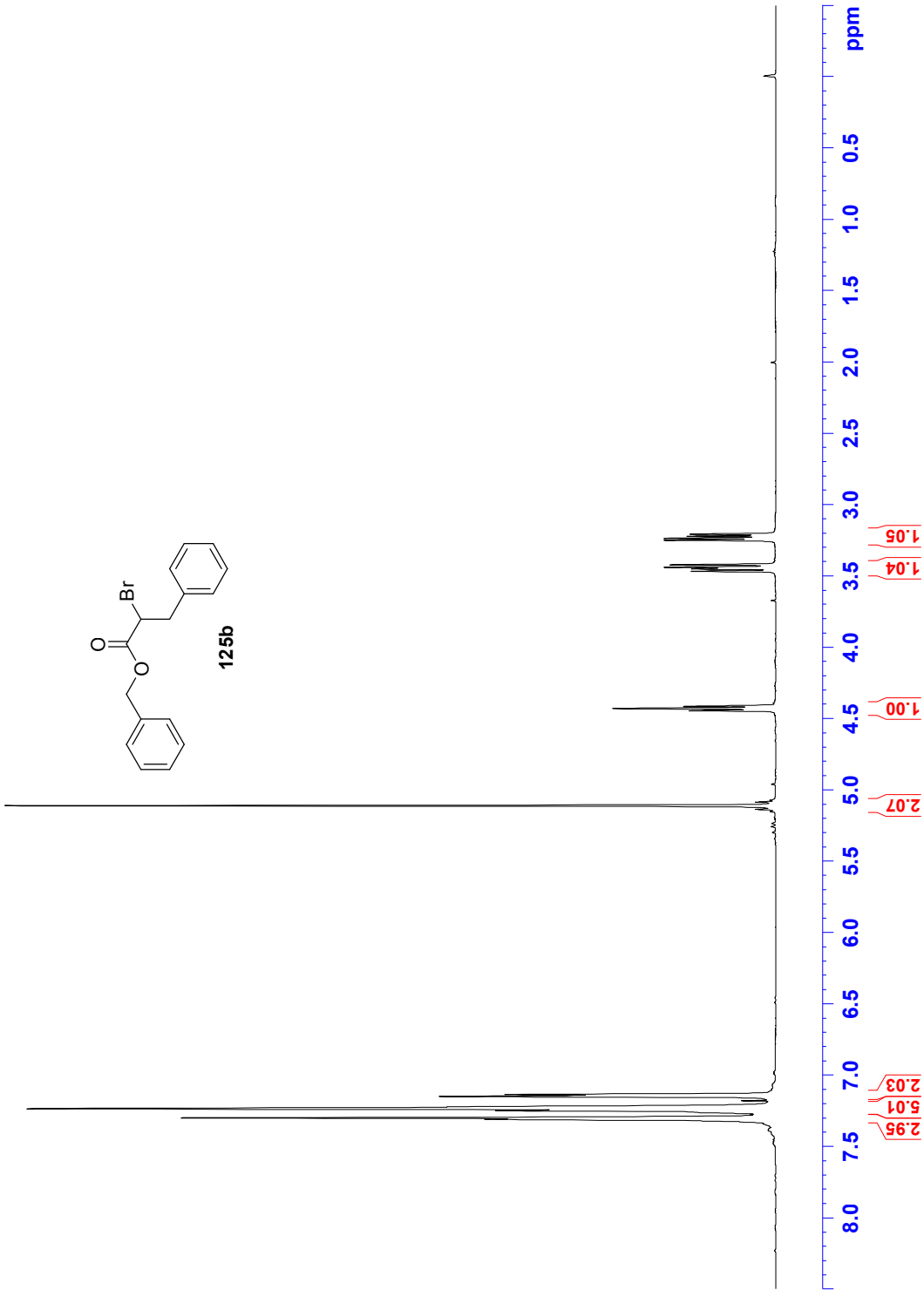
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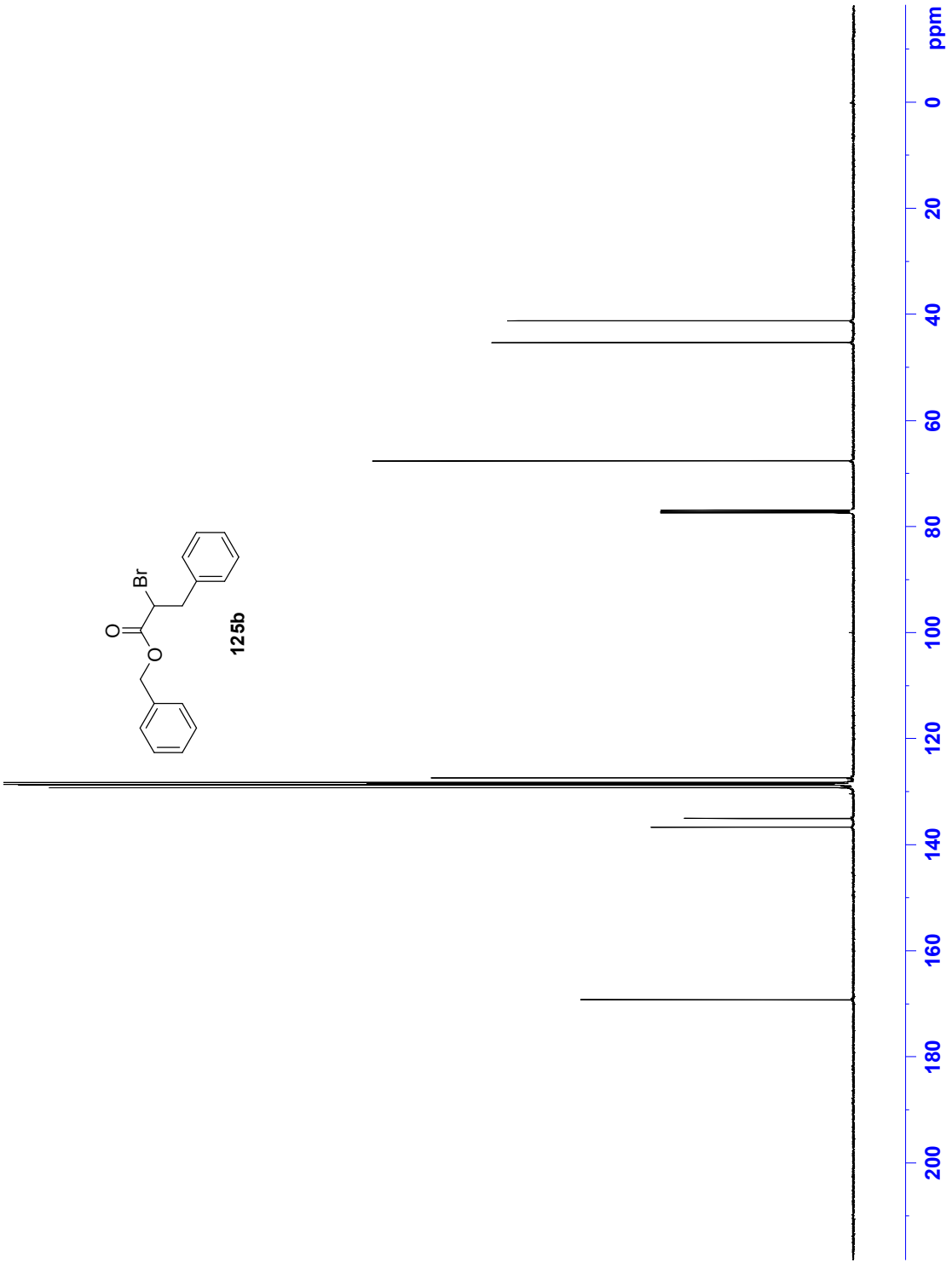
2.95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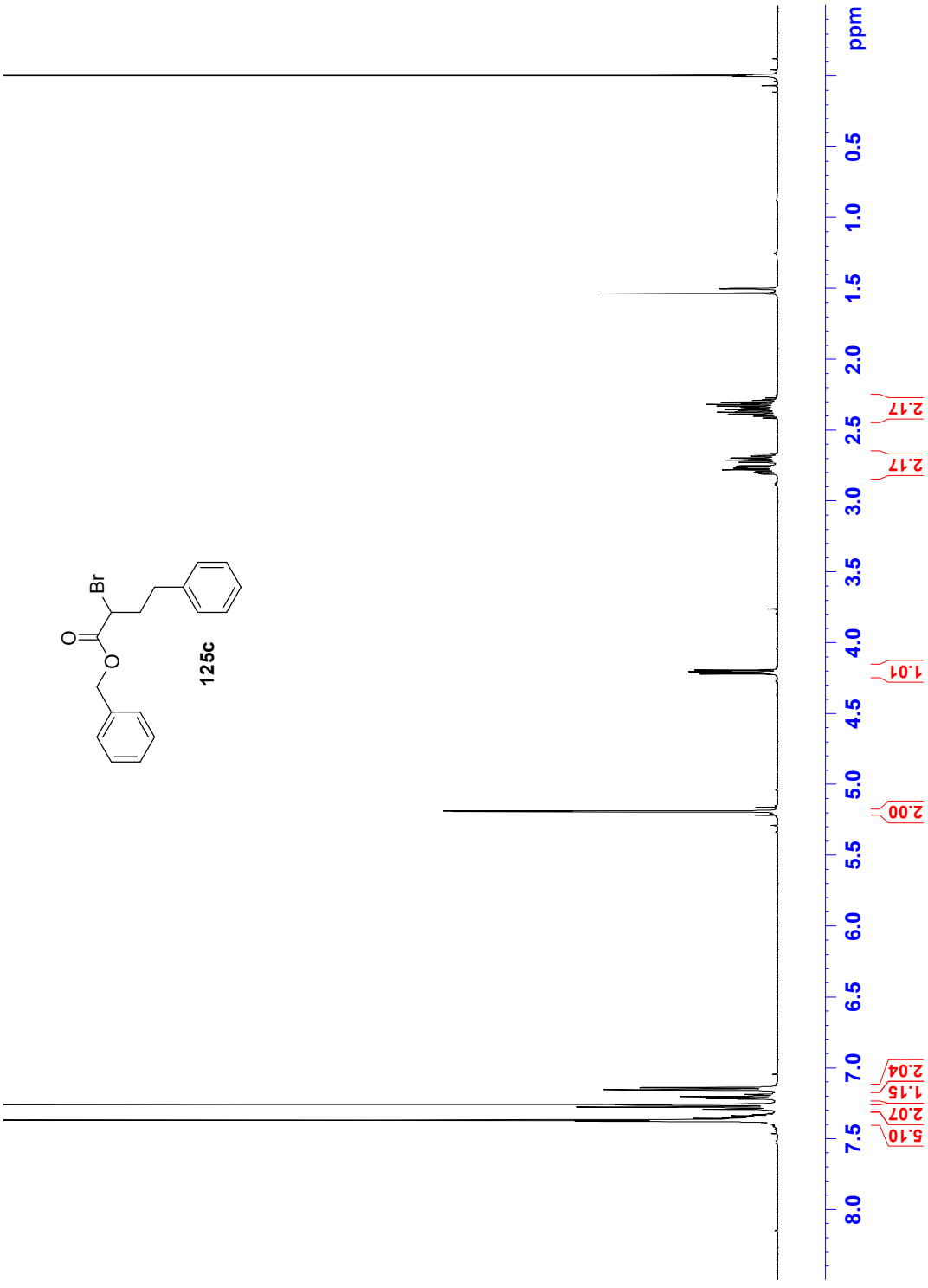
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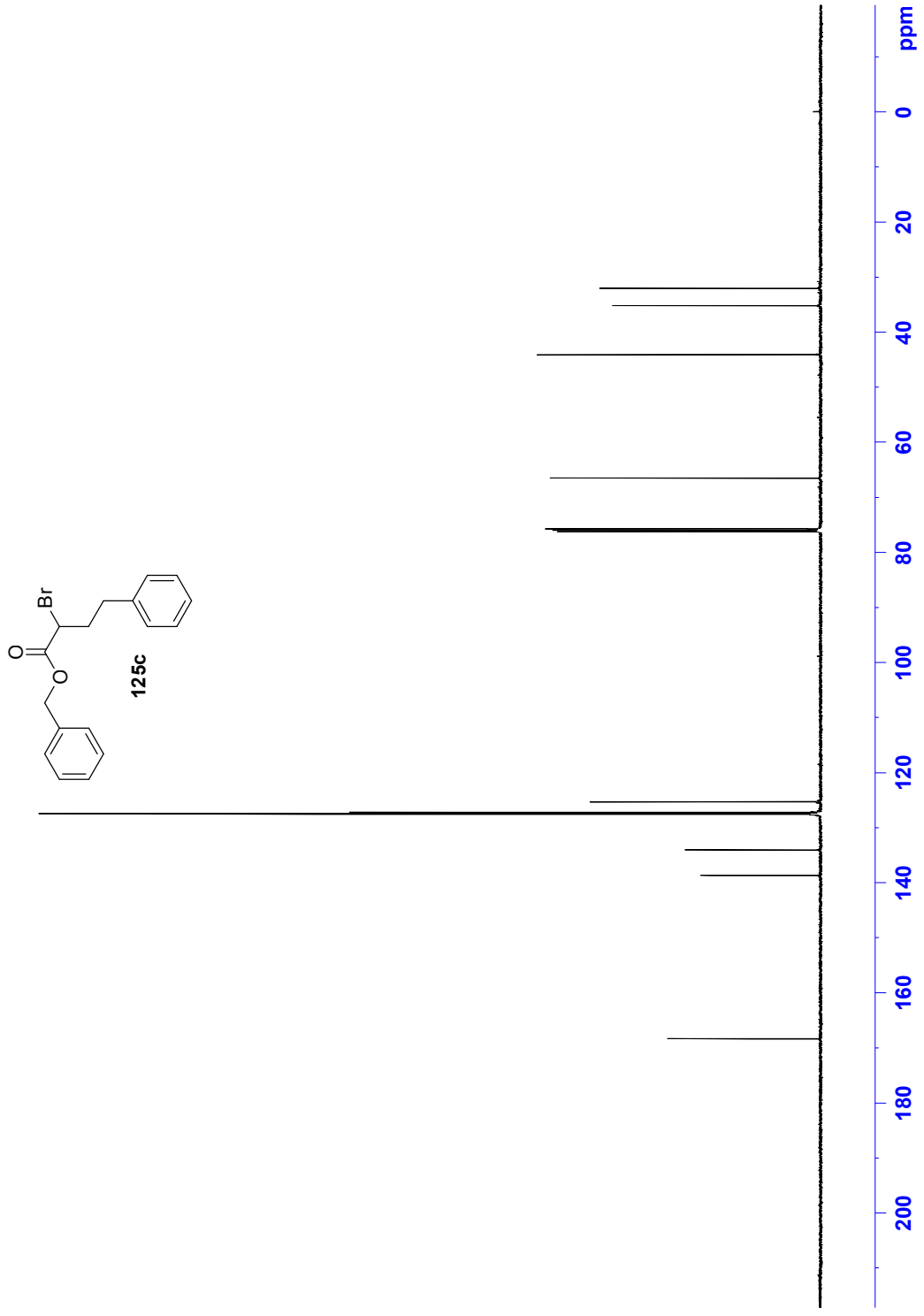
2.04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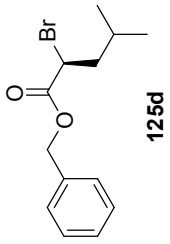
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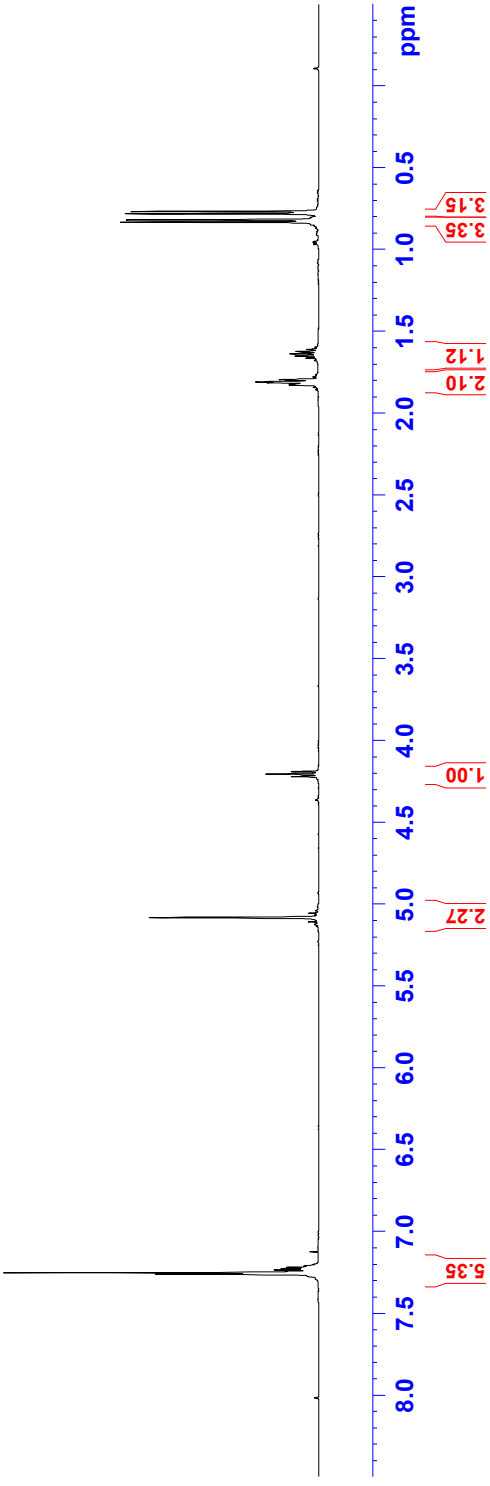
125d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6.0
6.5
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7.5
8.0
3.15
3.35
1.12
2.10
1.00
2.27
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Br
O
O
125d
ppm
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60
80
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140
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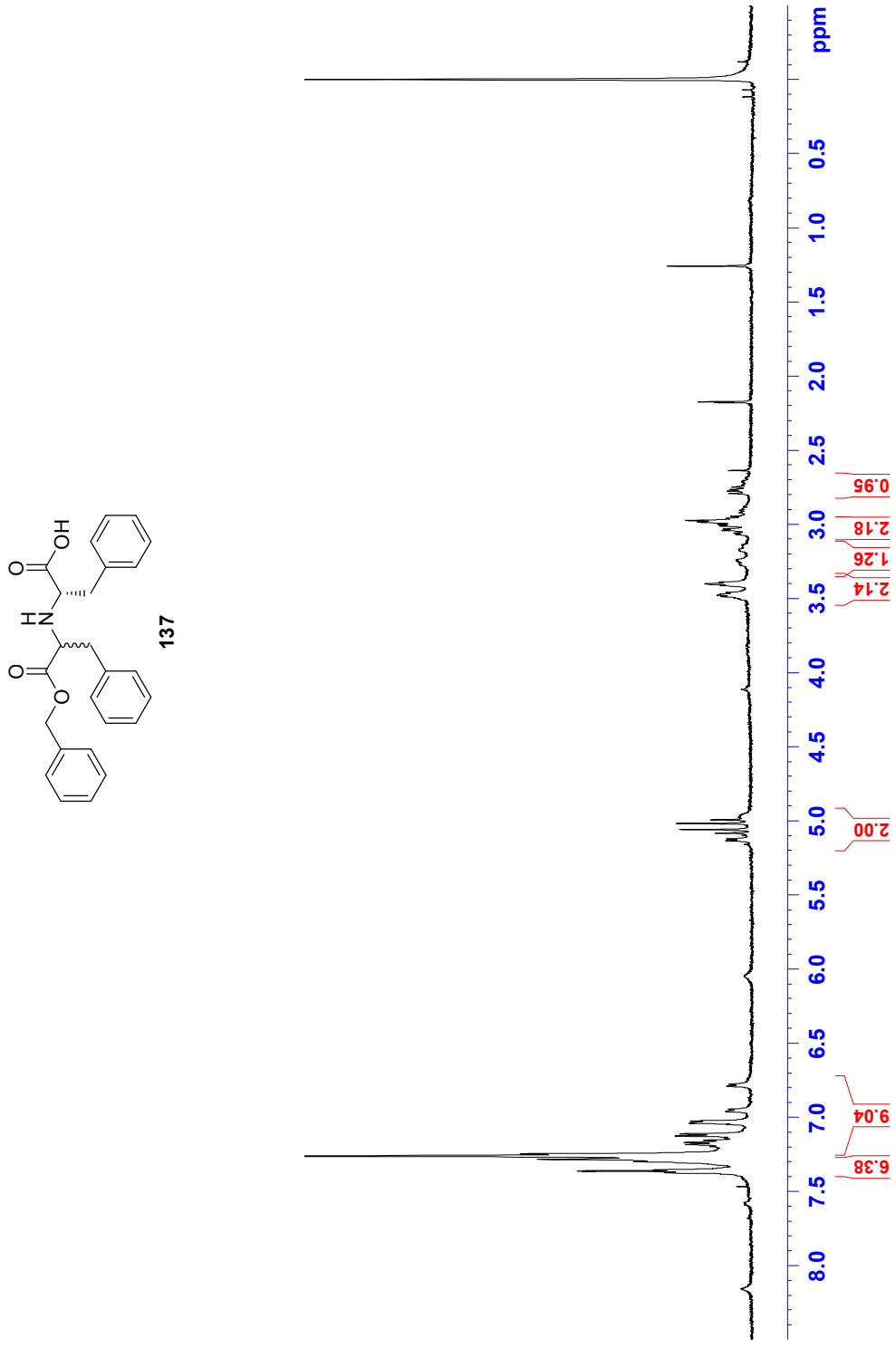
180
200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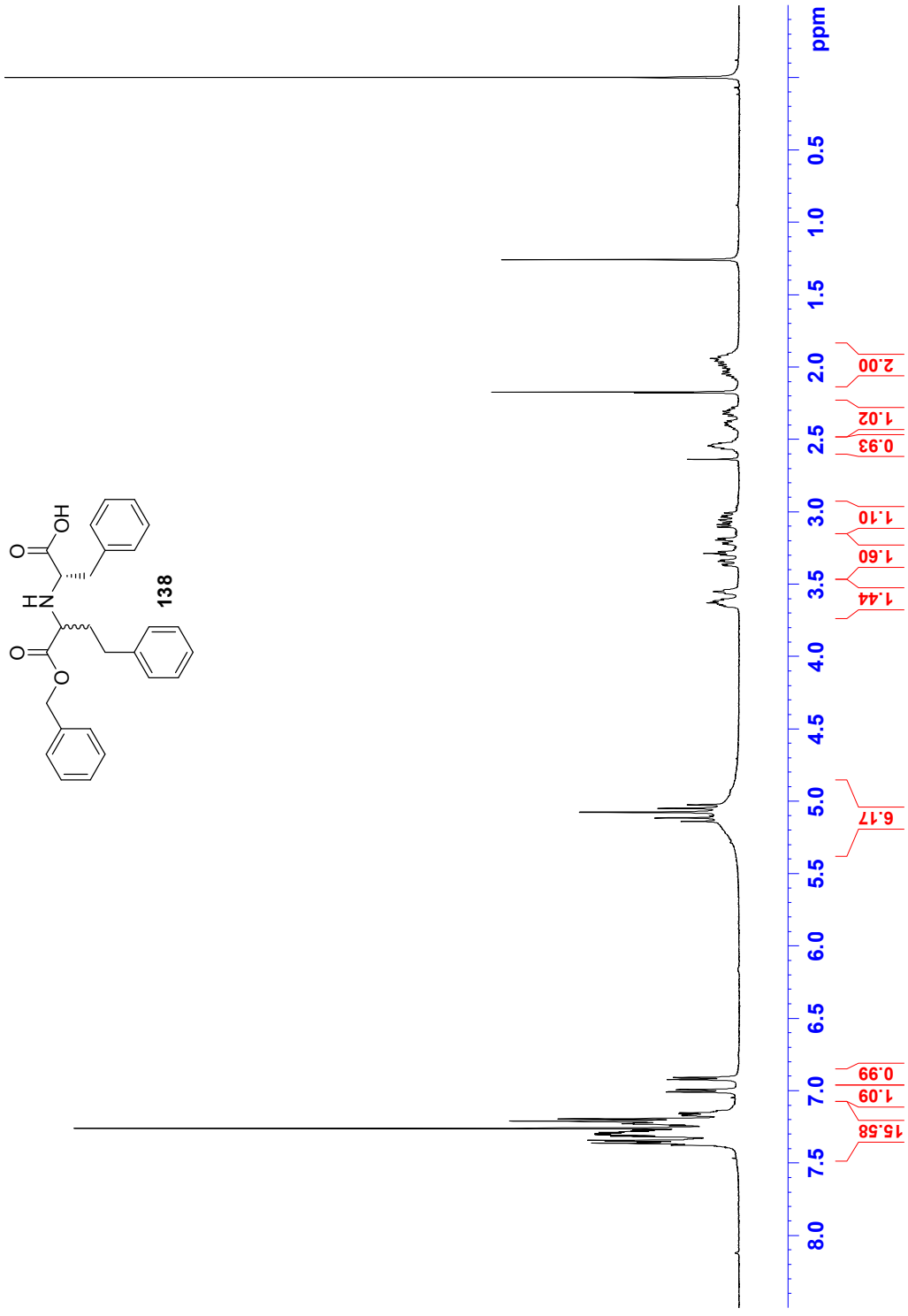
6.38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6.5
7.0
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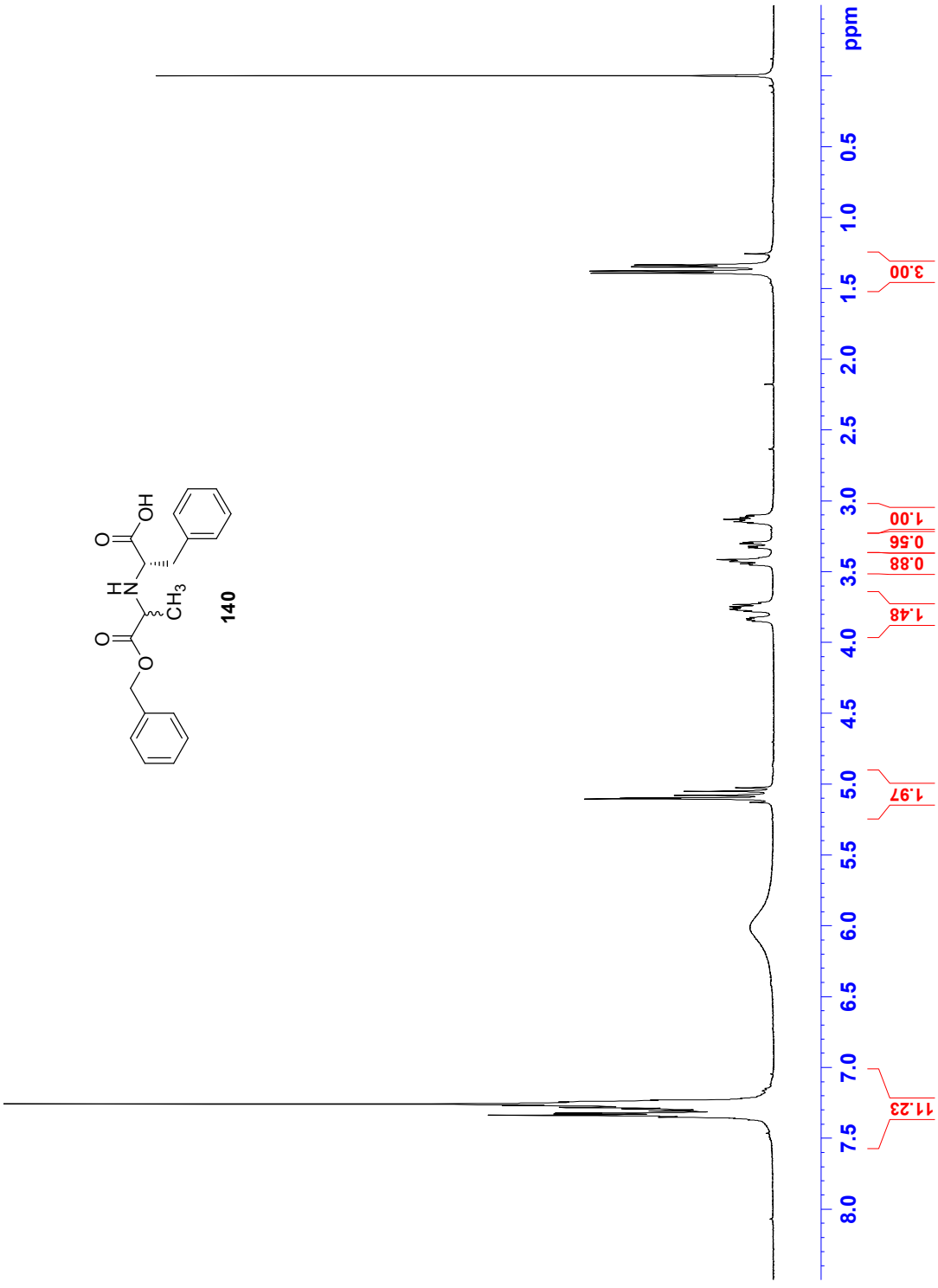
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6.5
7.0
7.5
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1.48
1.97
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HN
CH3
O
O
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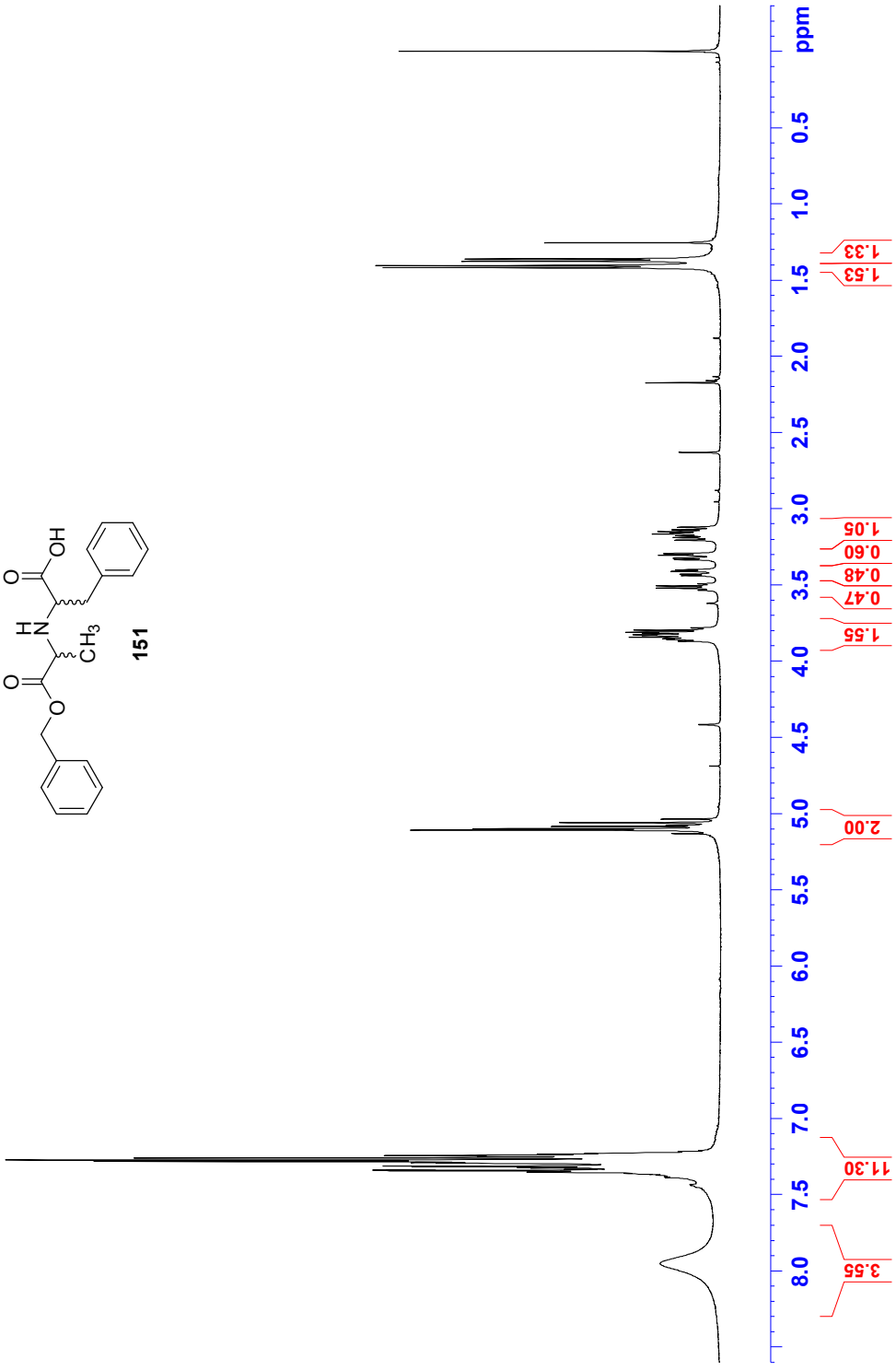
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4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
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1.53
1.05
0.60
0.48
0.47
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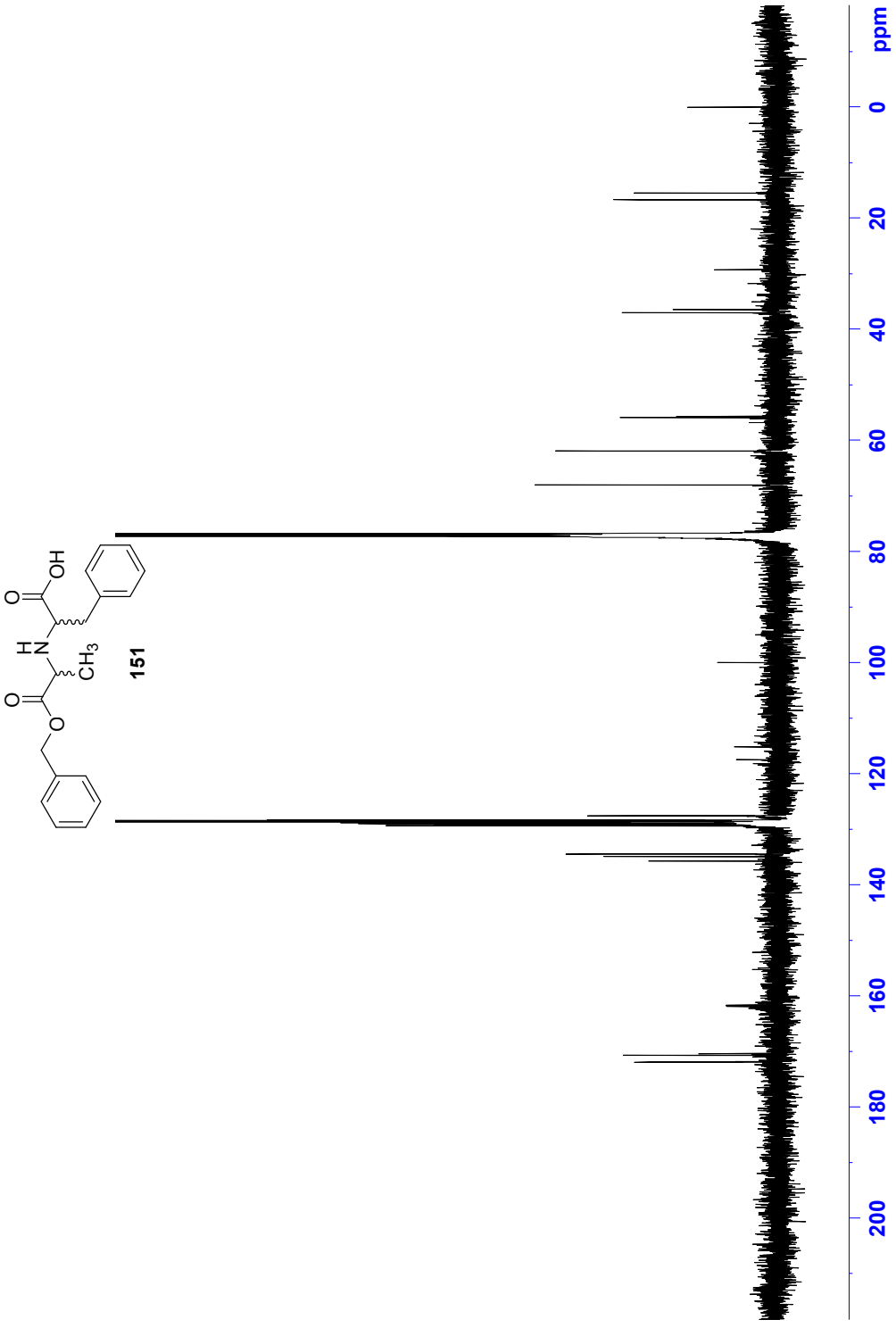
2.00
11.30
3.55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CH3
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ppm
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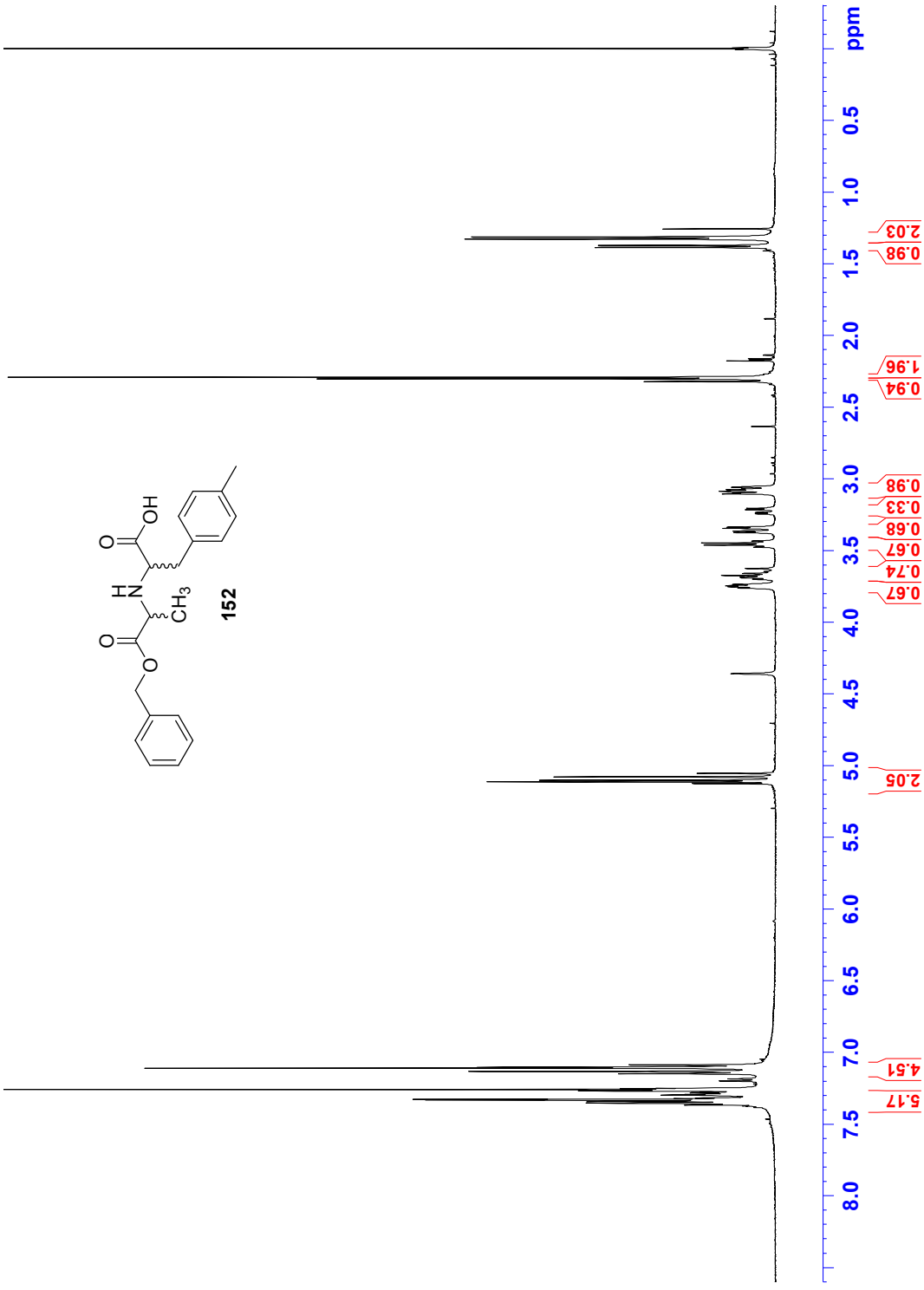
200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4.0
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2.0
1.5
1.0
0.5
2.03
0.98
1.96
0.94
0.98
0.33
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0.67
0.74
0.67
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CH3
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O
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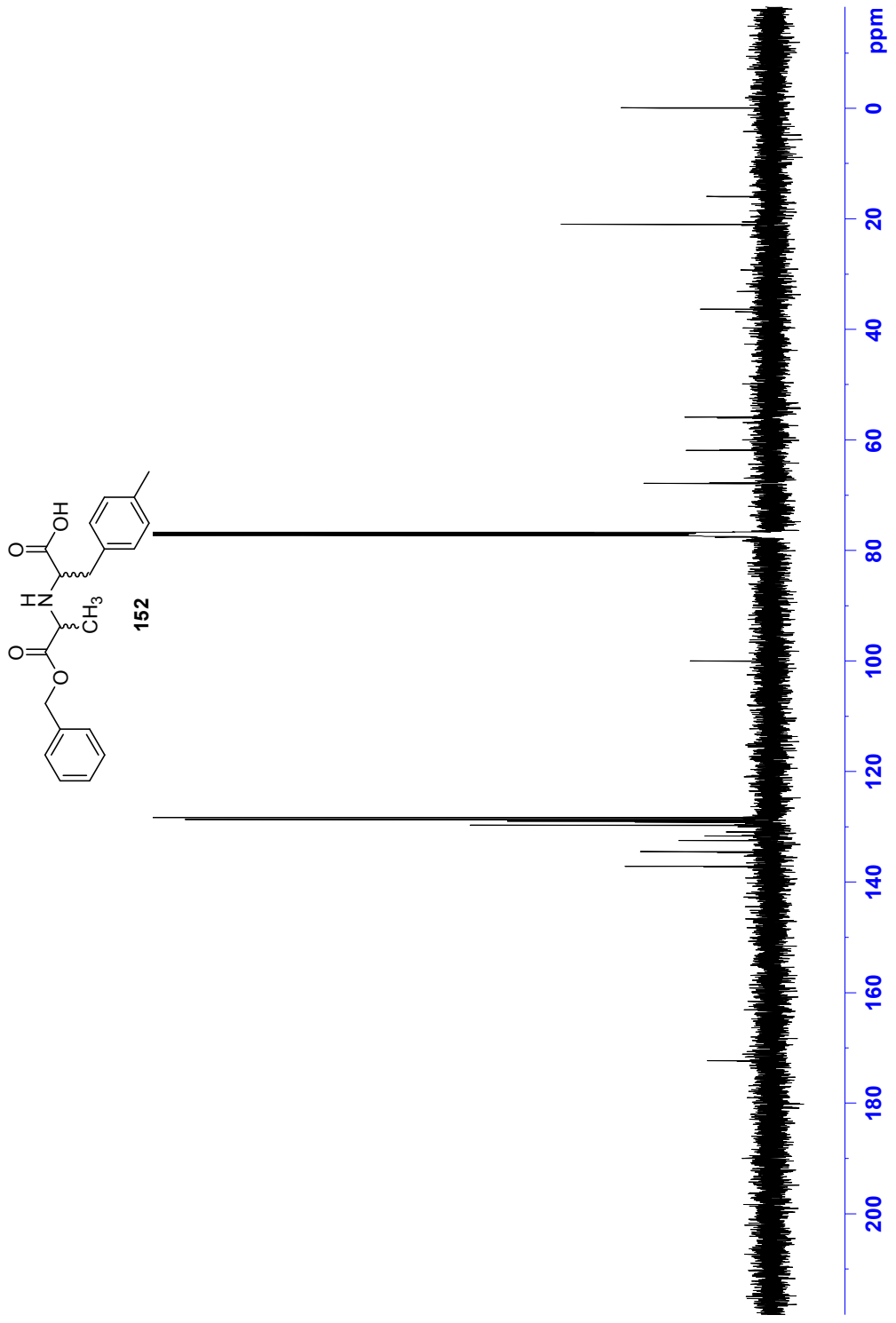
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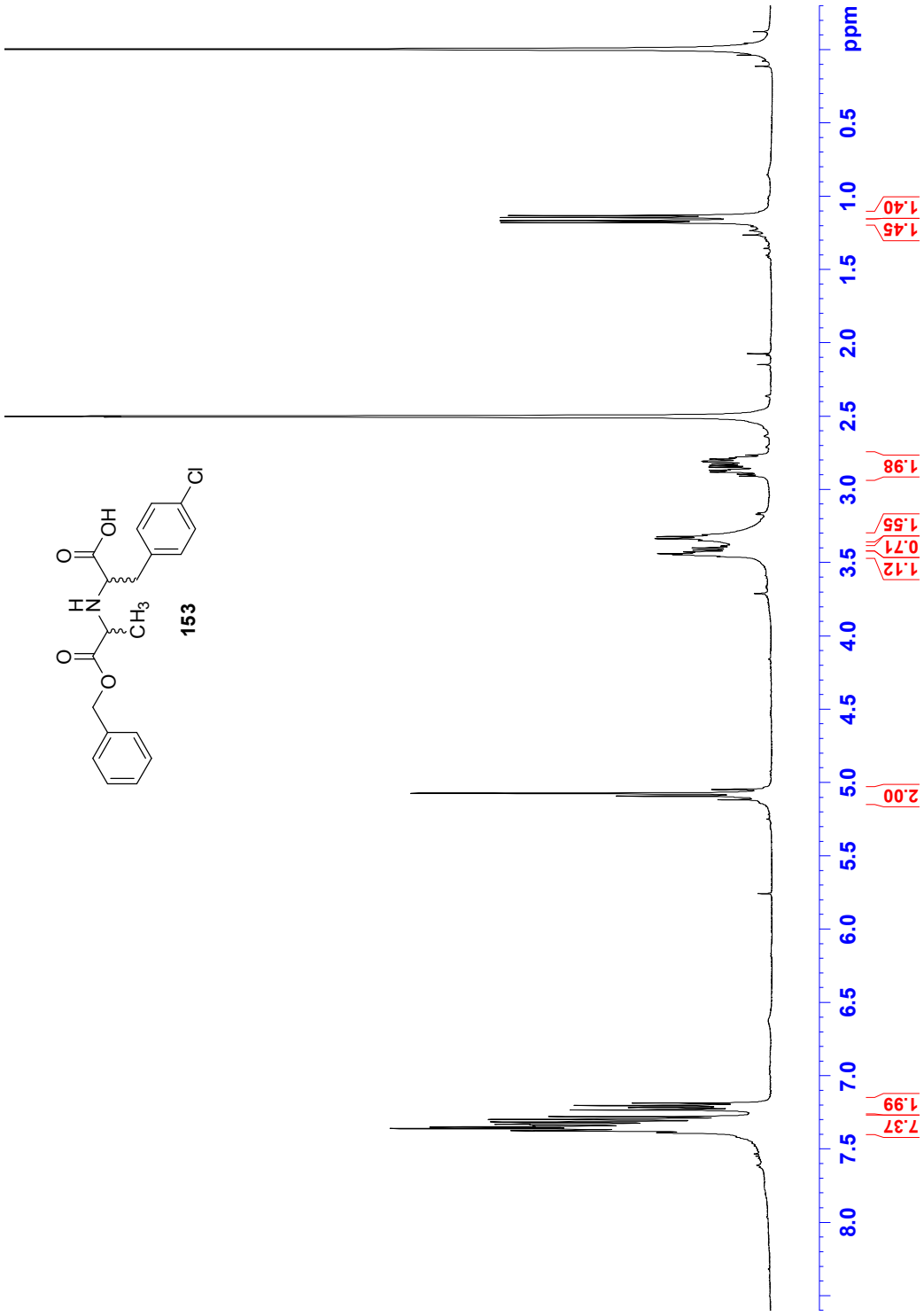
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1.45
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7.37
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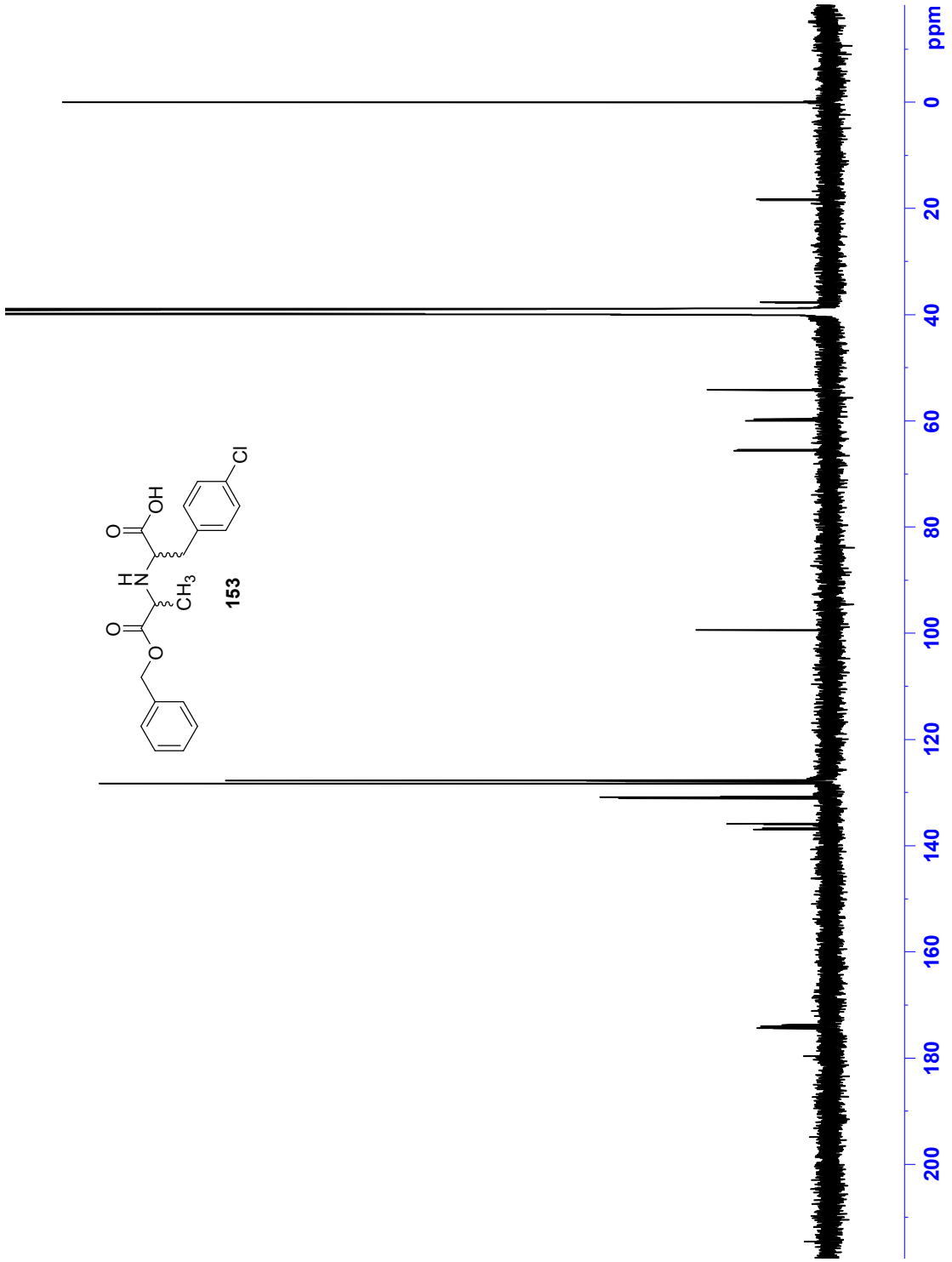
N
CH3
O
O
153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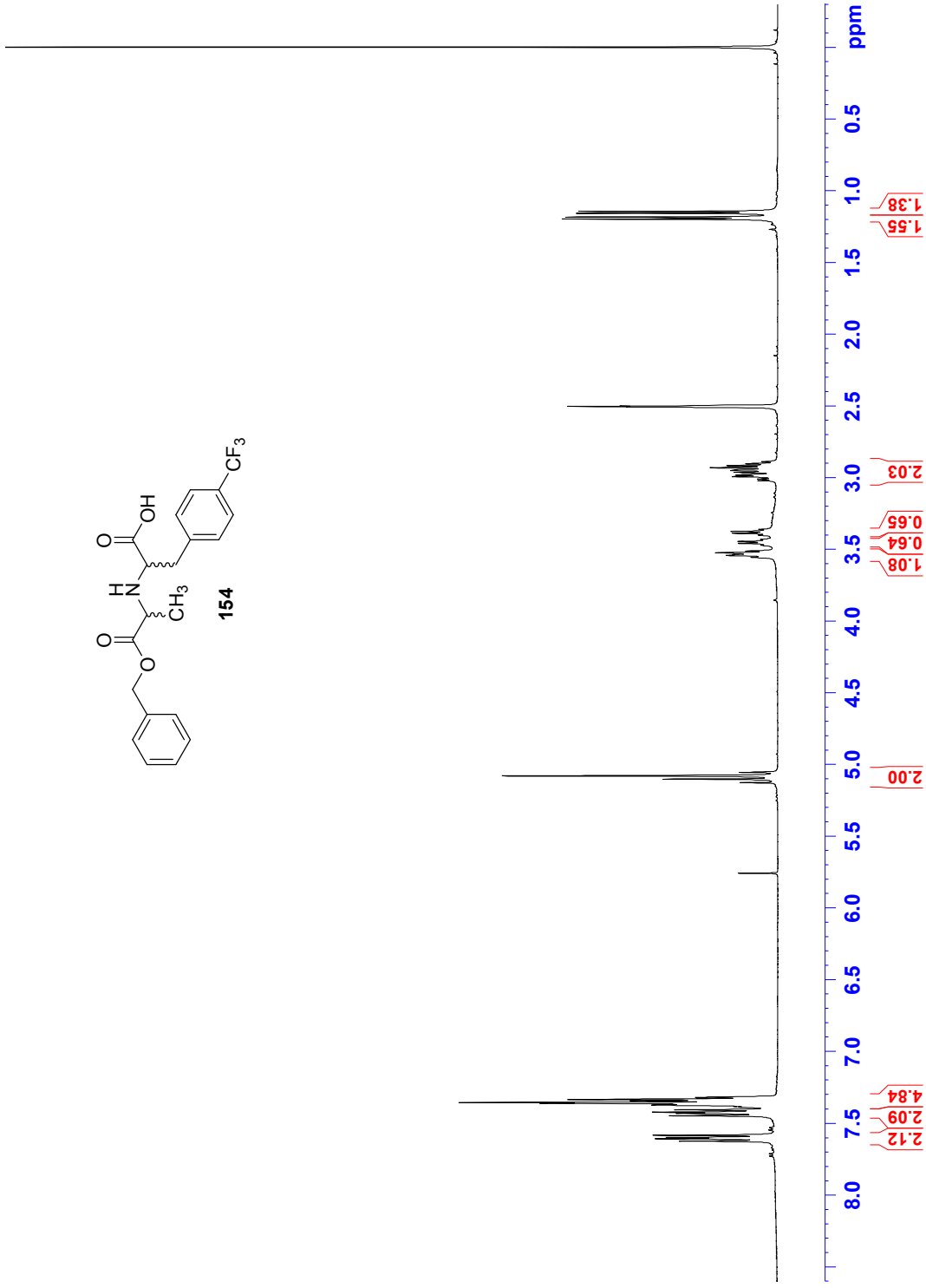
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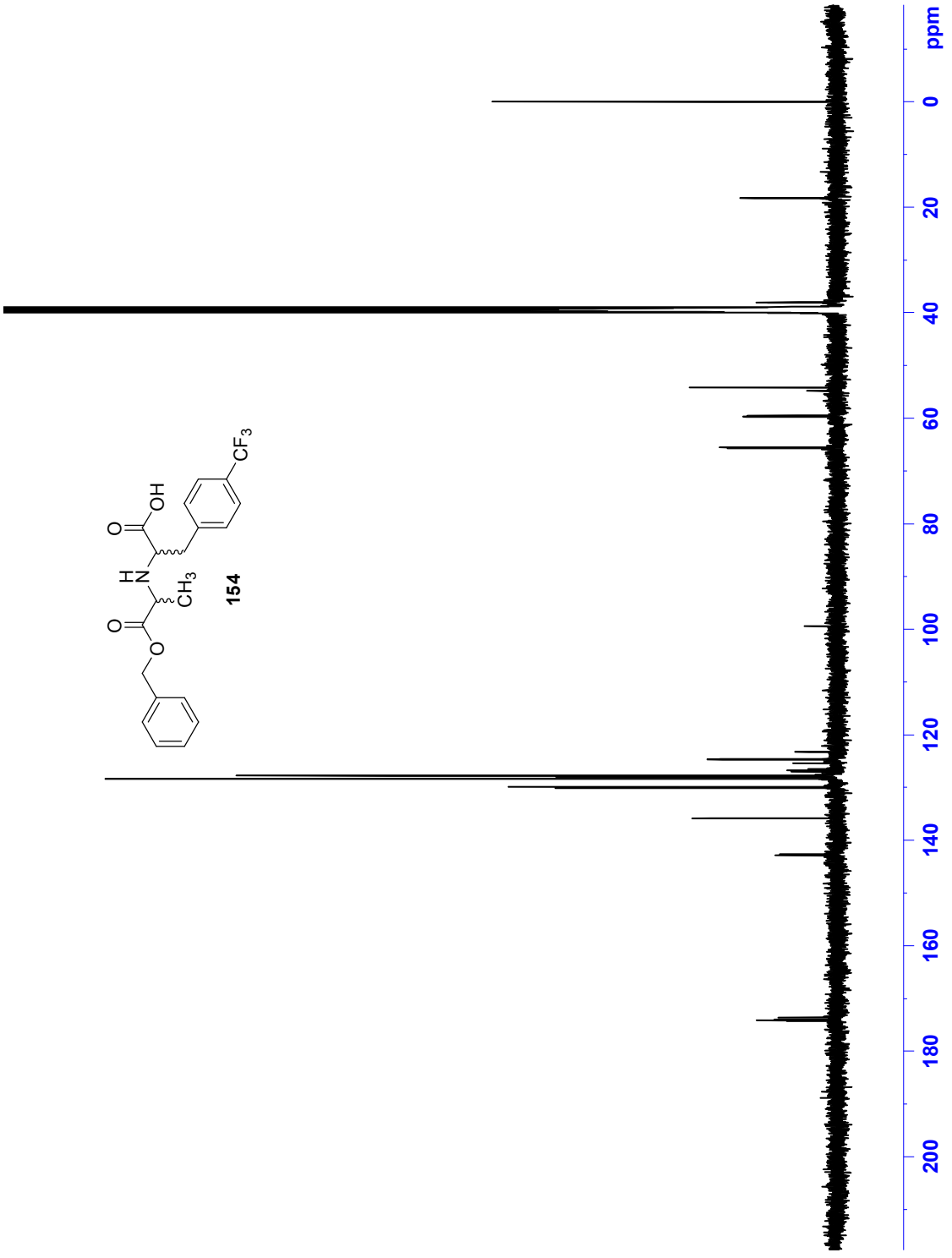
CF3

154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N
CH3
O
O
CF3
154

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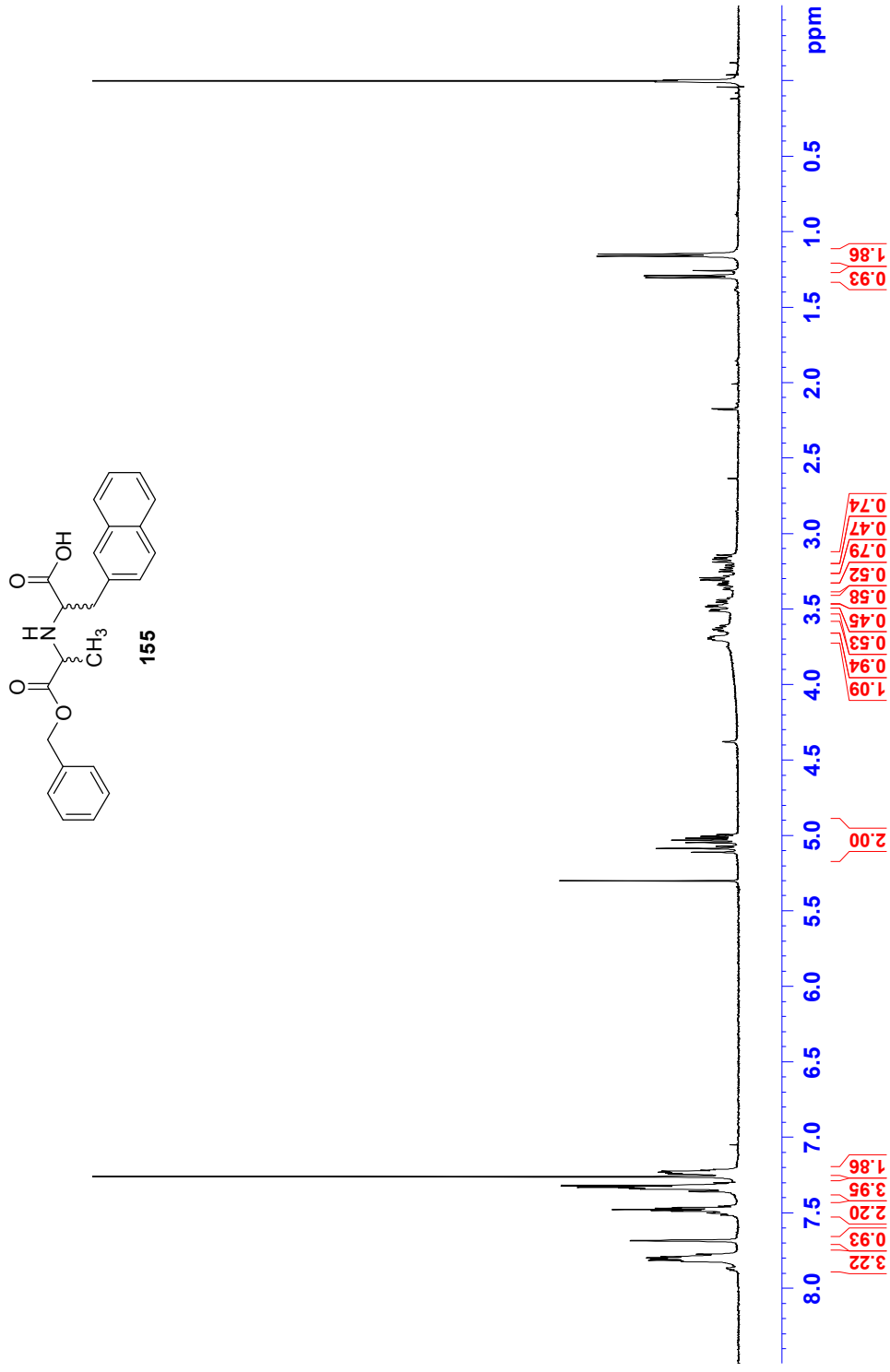
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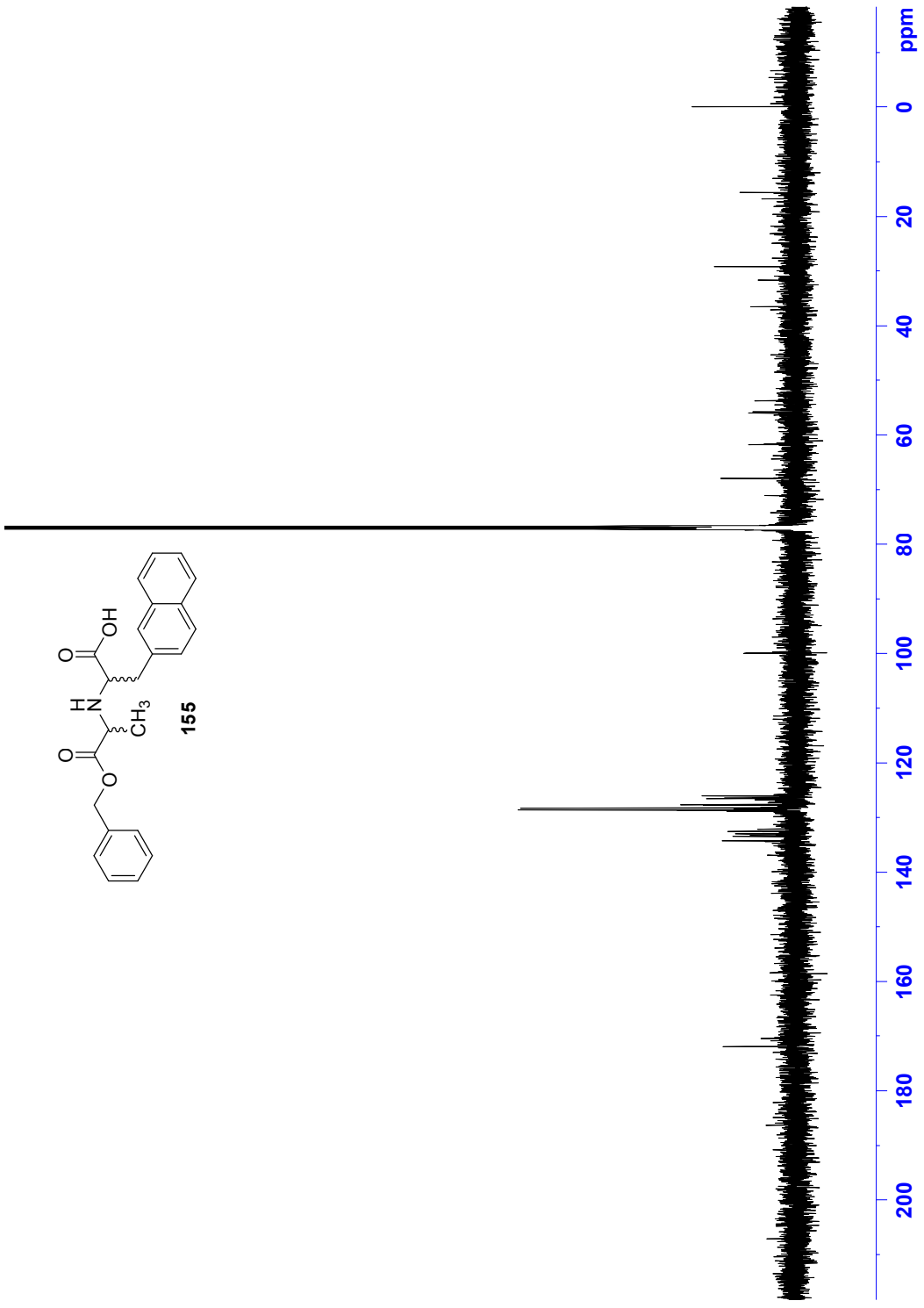
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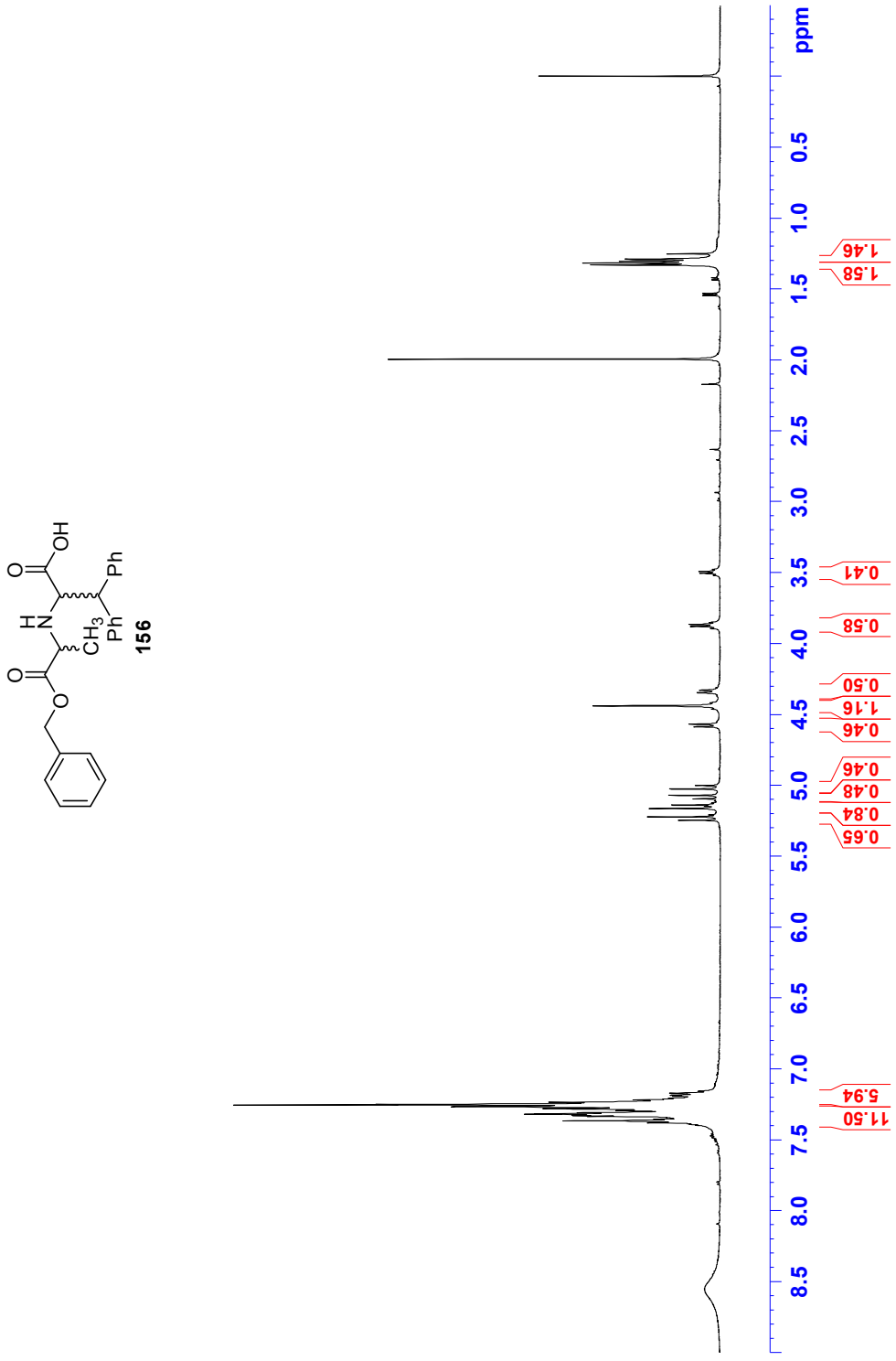
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O
O
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Ph
Ph
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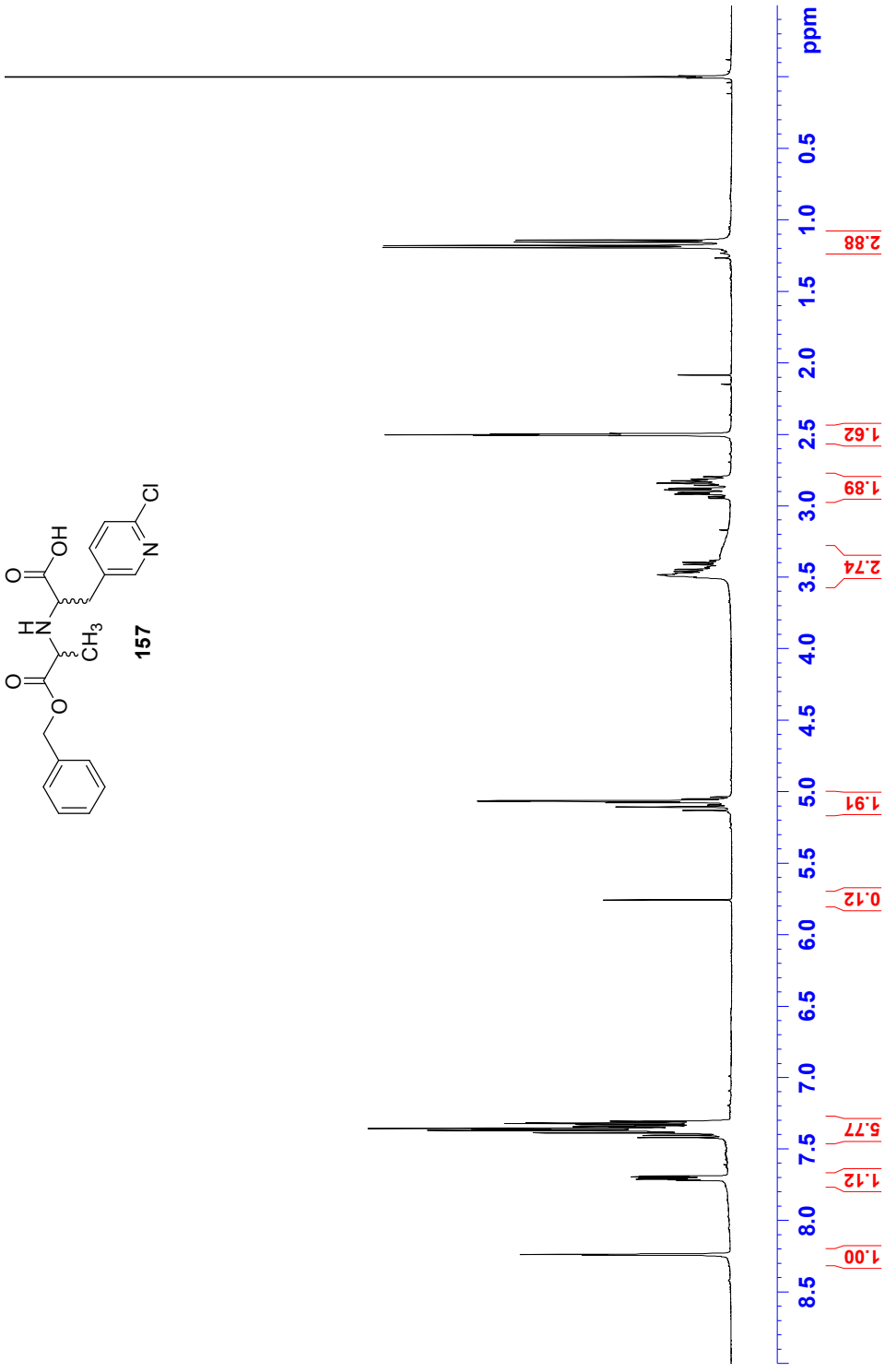
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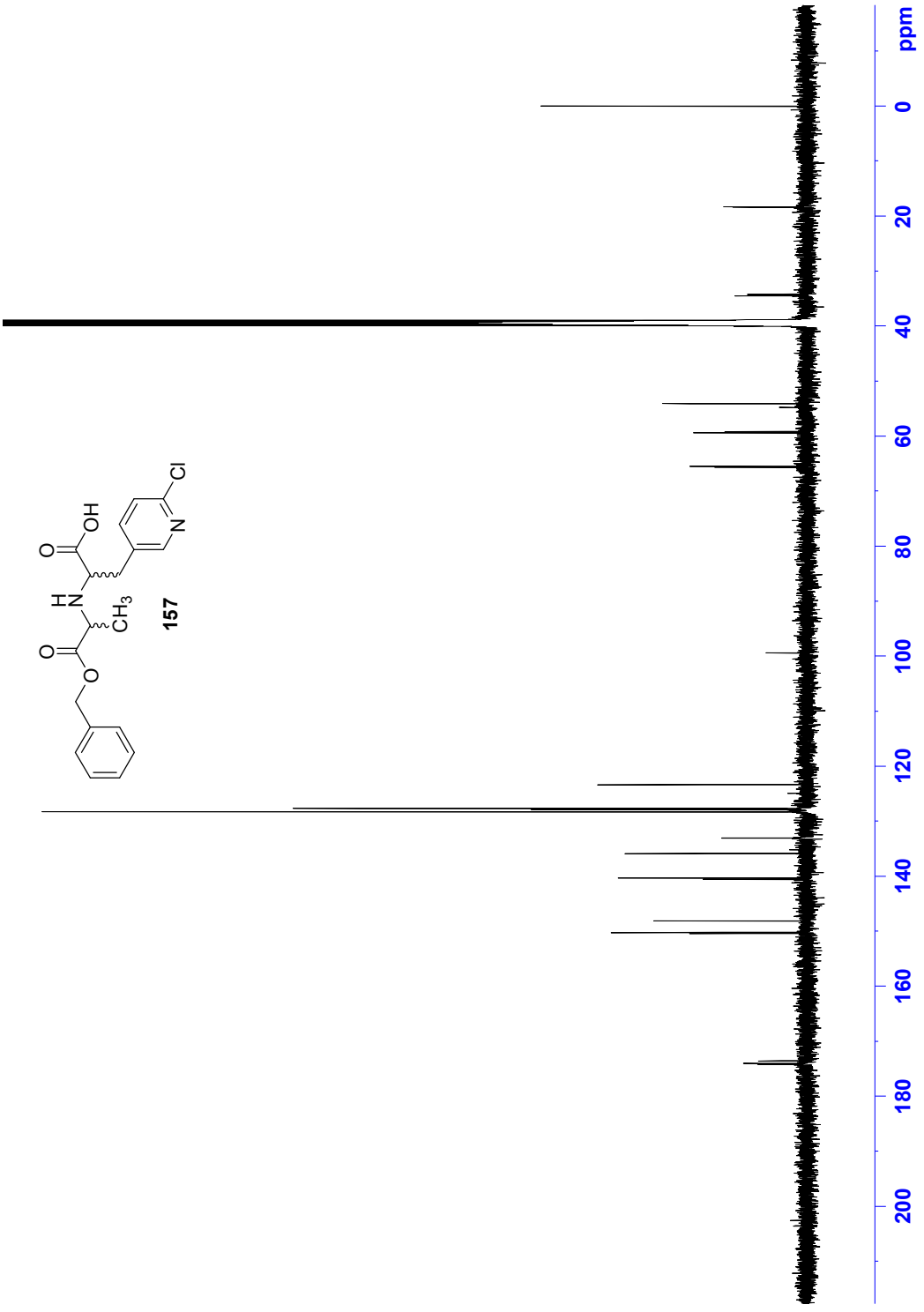
O
Cl
N

157


ppm
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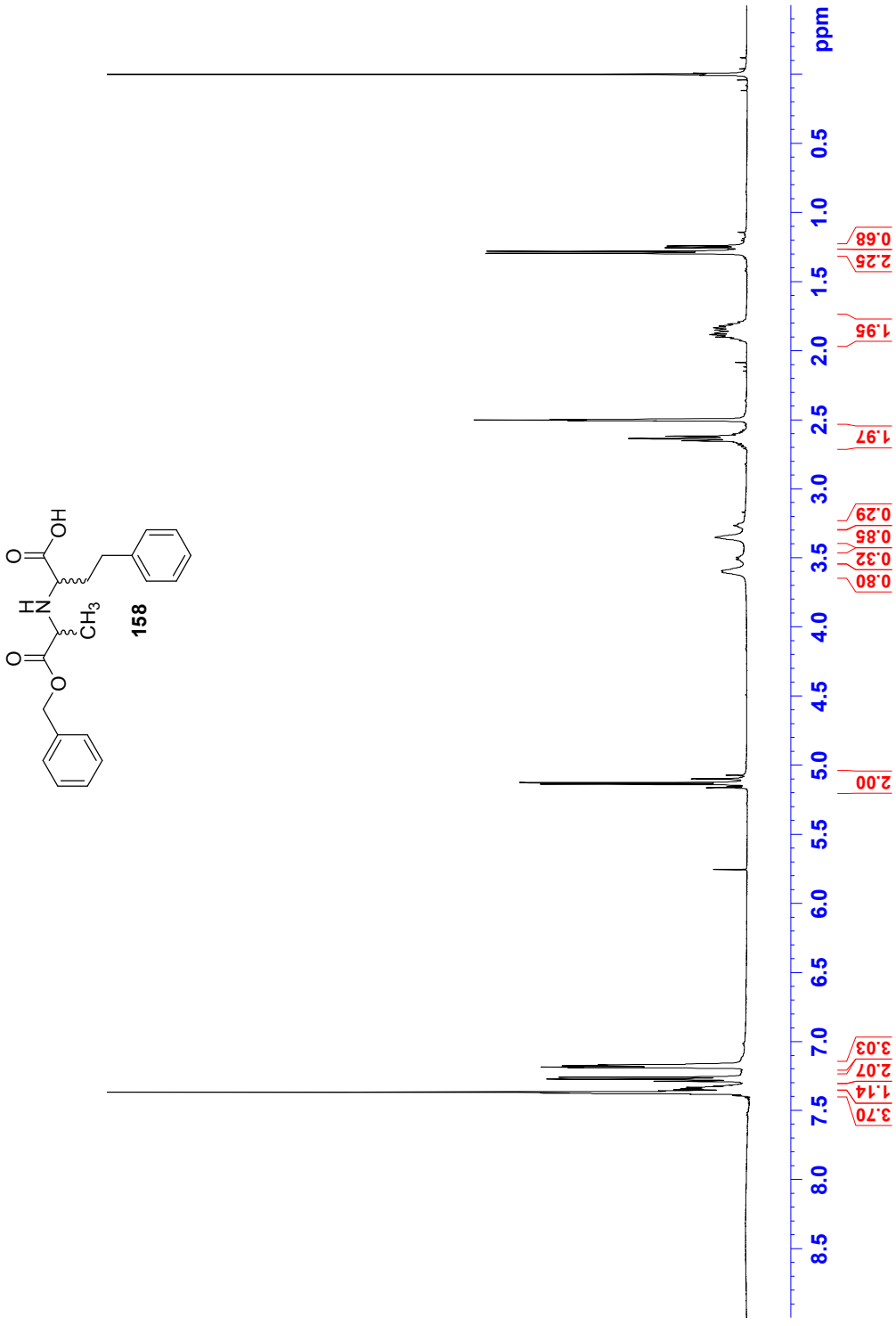
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8.5
0.68
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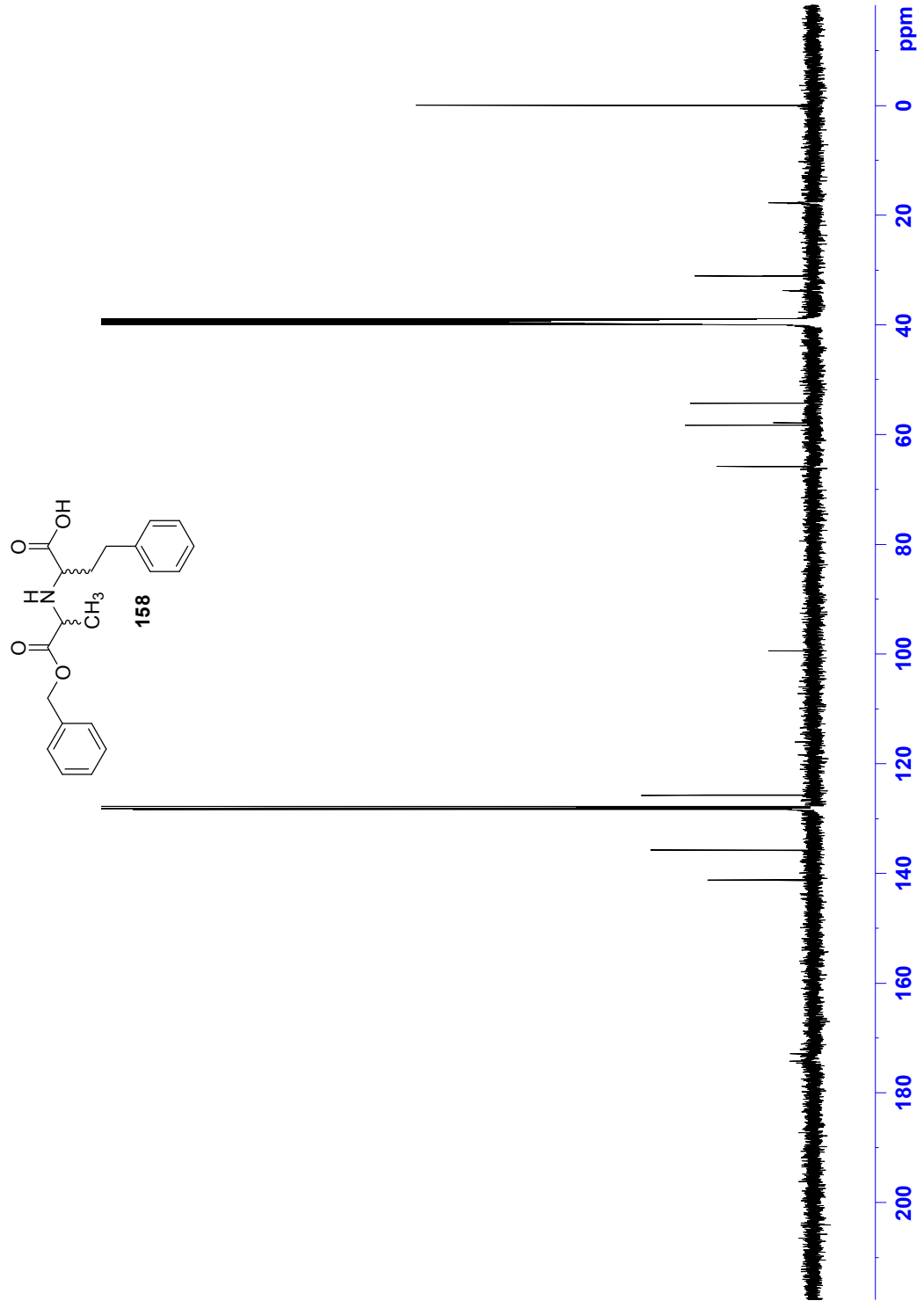
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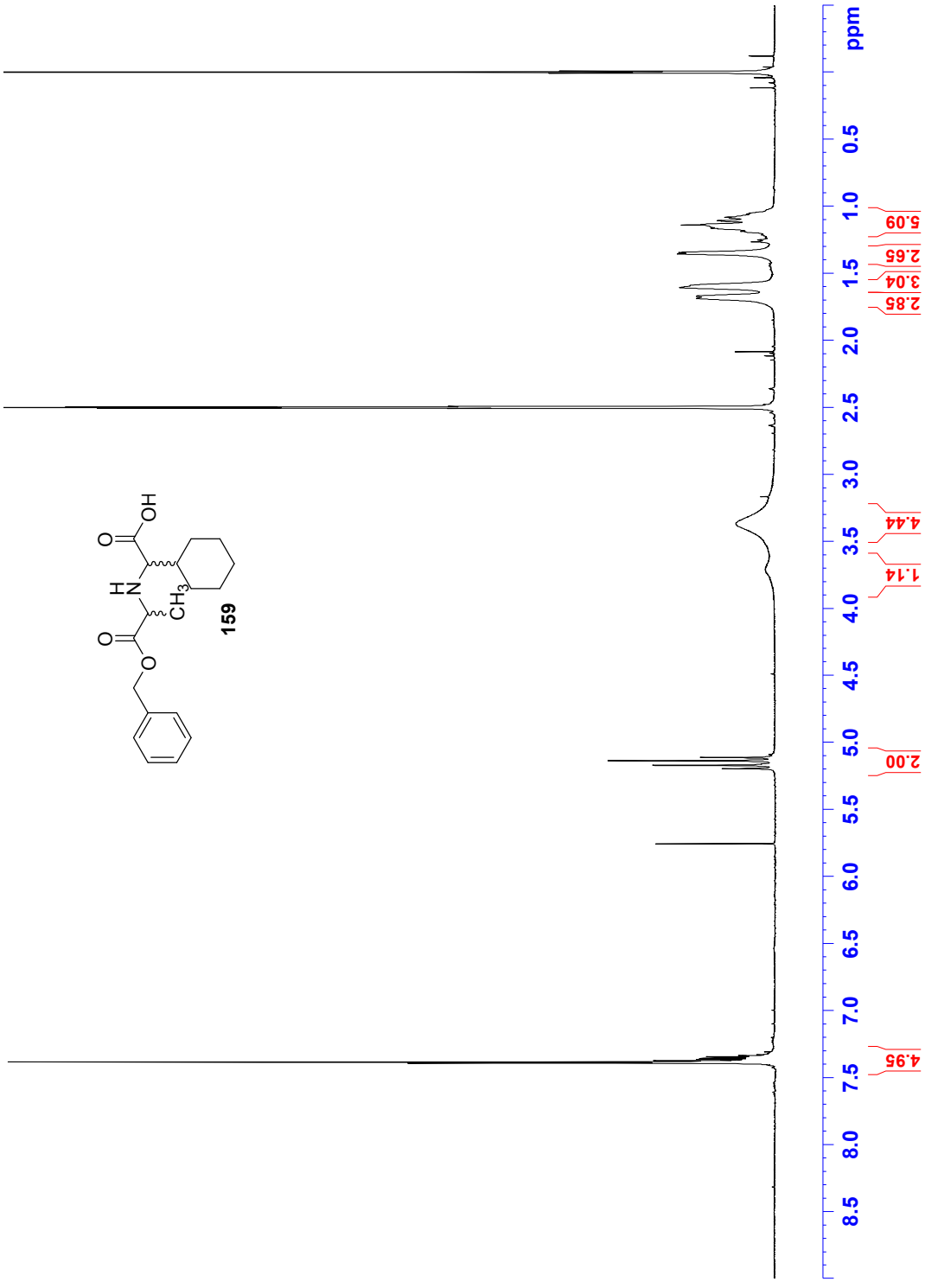
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3.04
2.85
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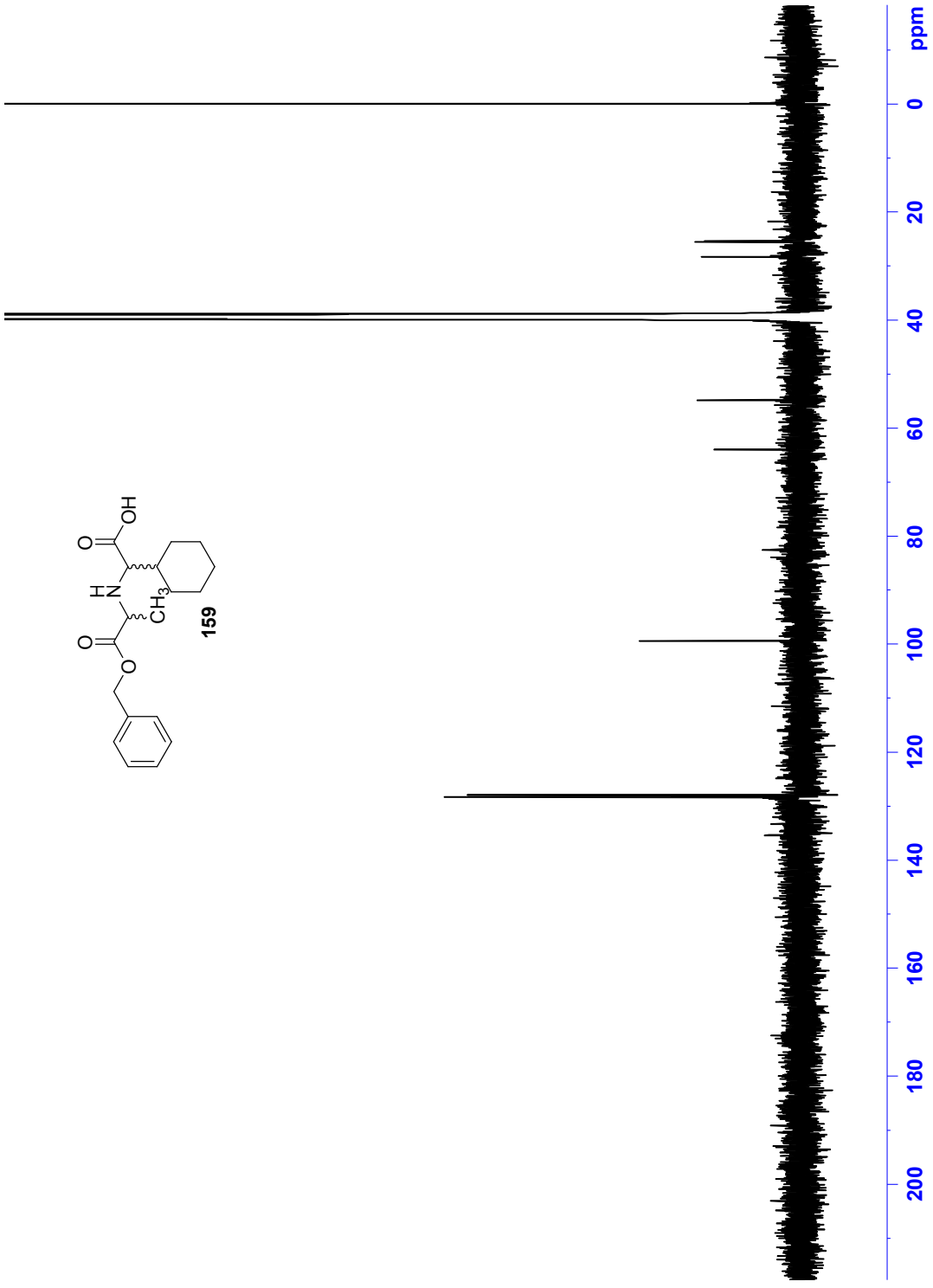
2.00
4.95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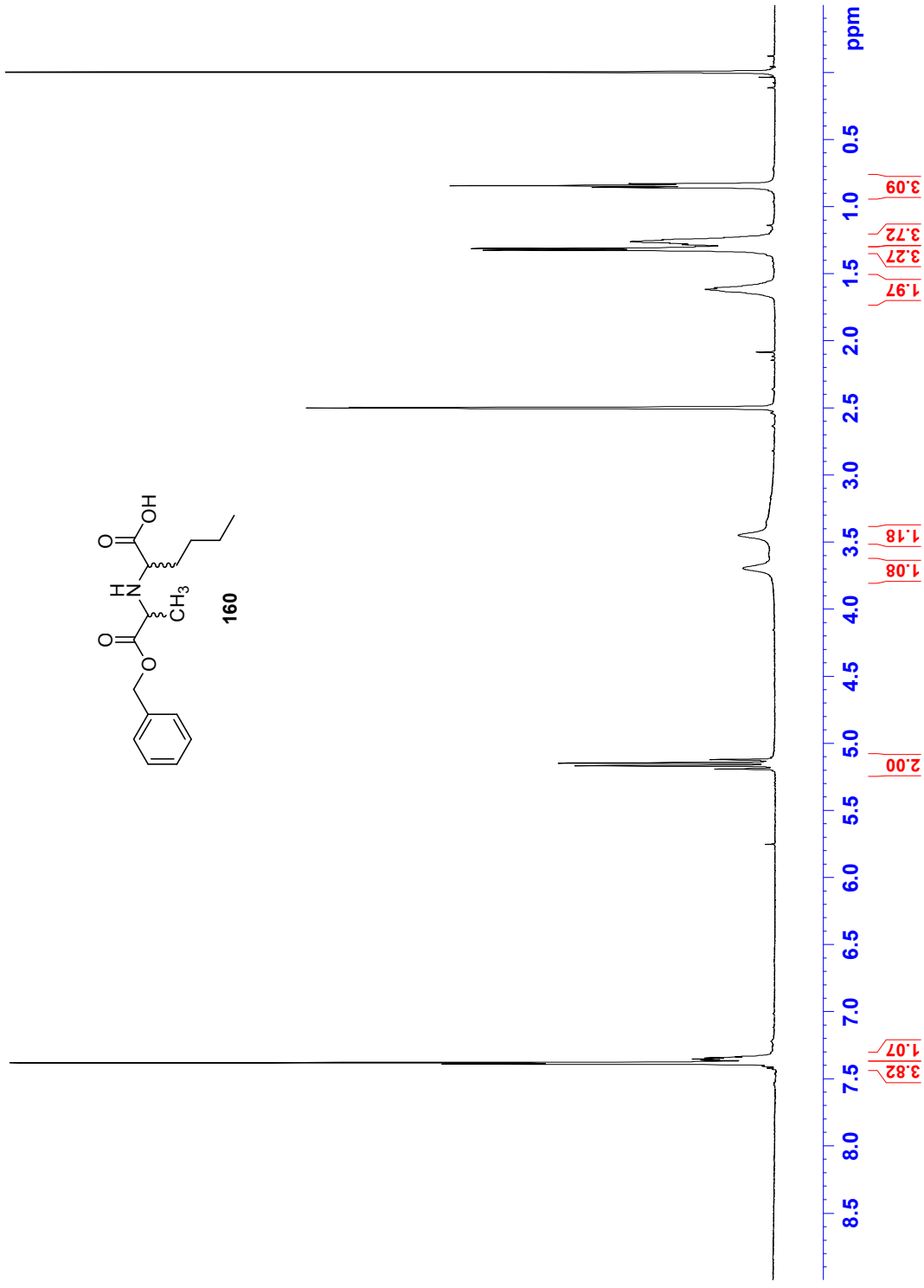
O
O
CH3
159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7.5
8.0
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CH3
O
O
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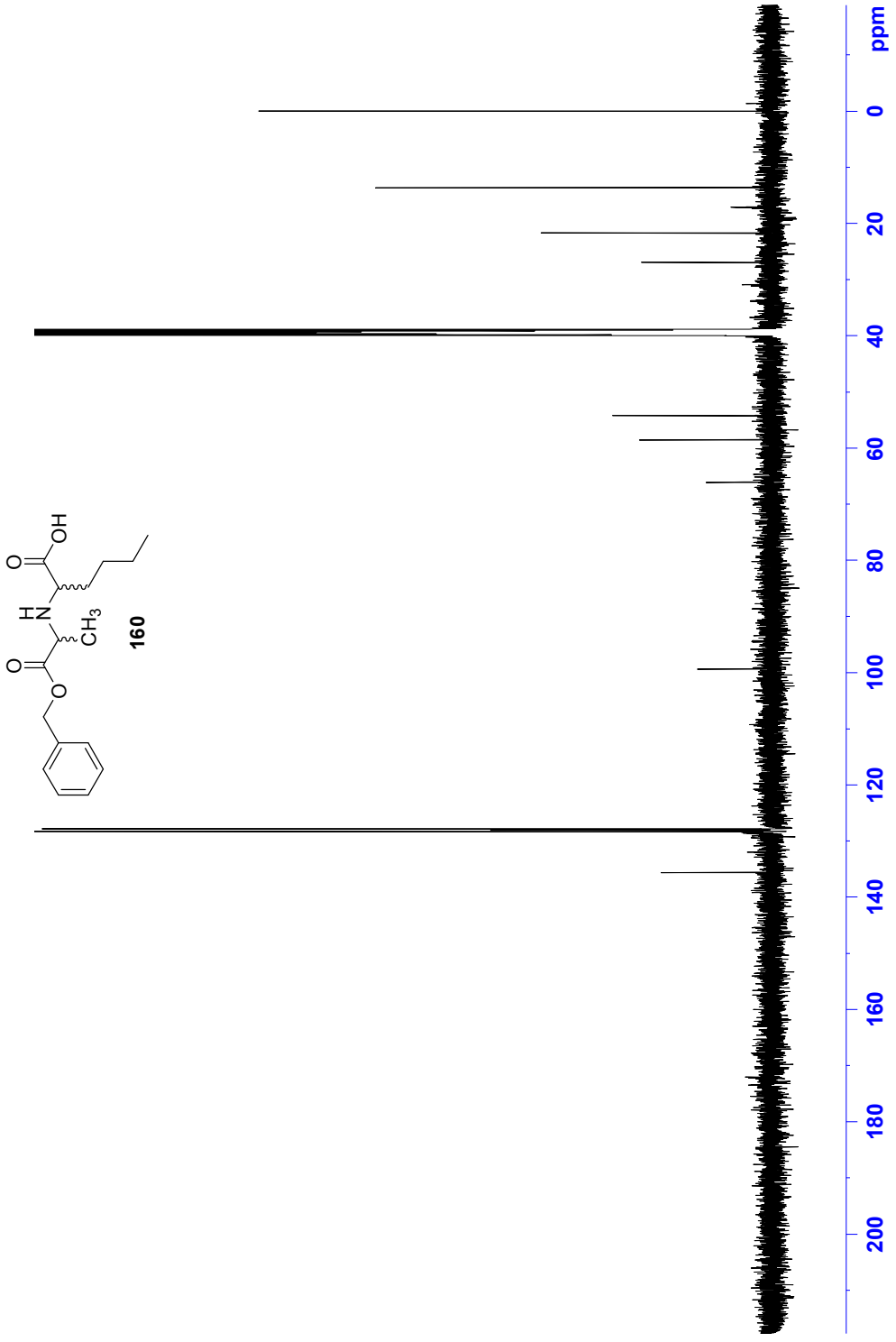
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CH3
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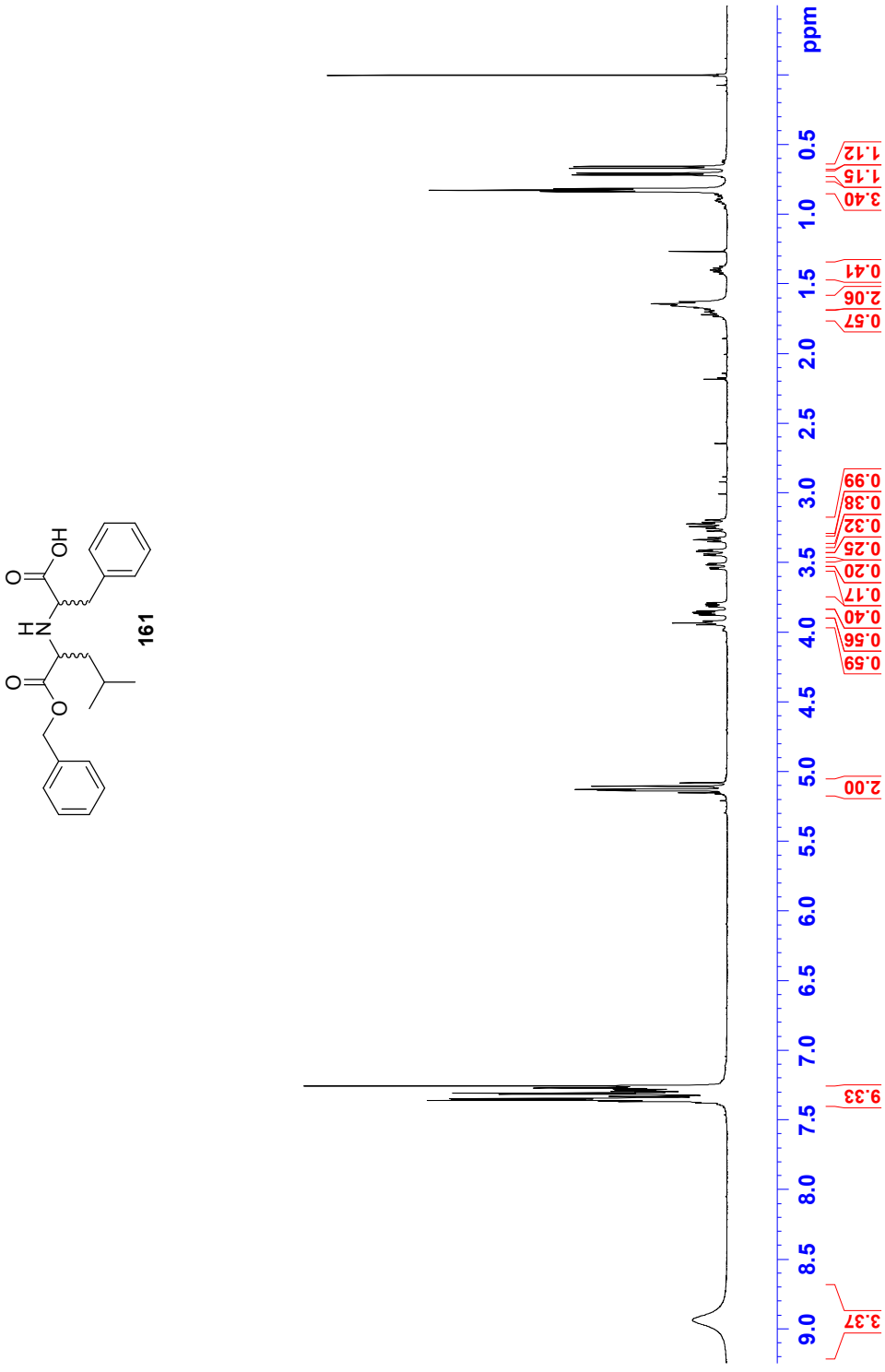
180
200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7.0
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8.5
9.0
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1.15
3.40
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0.57
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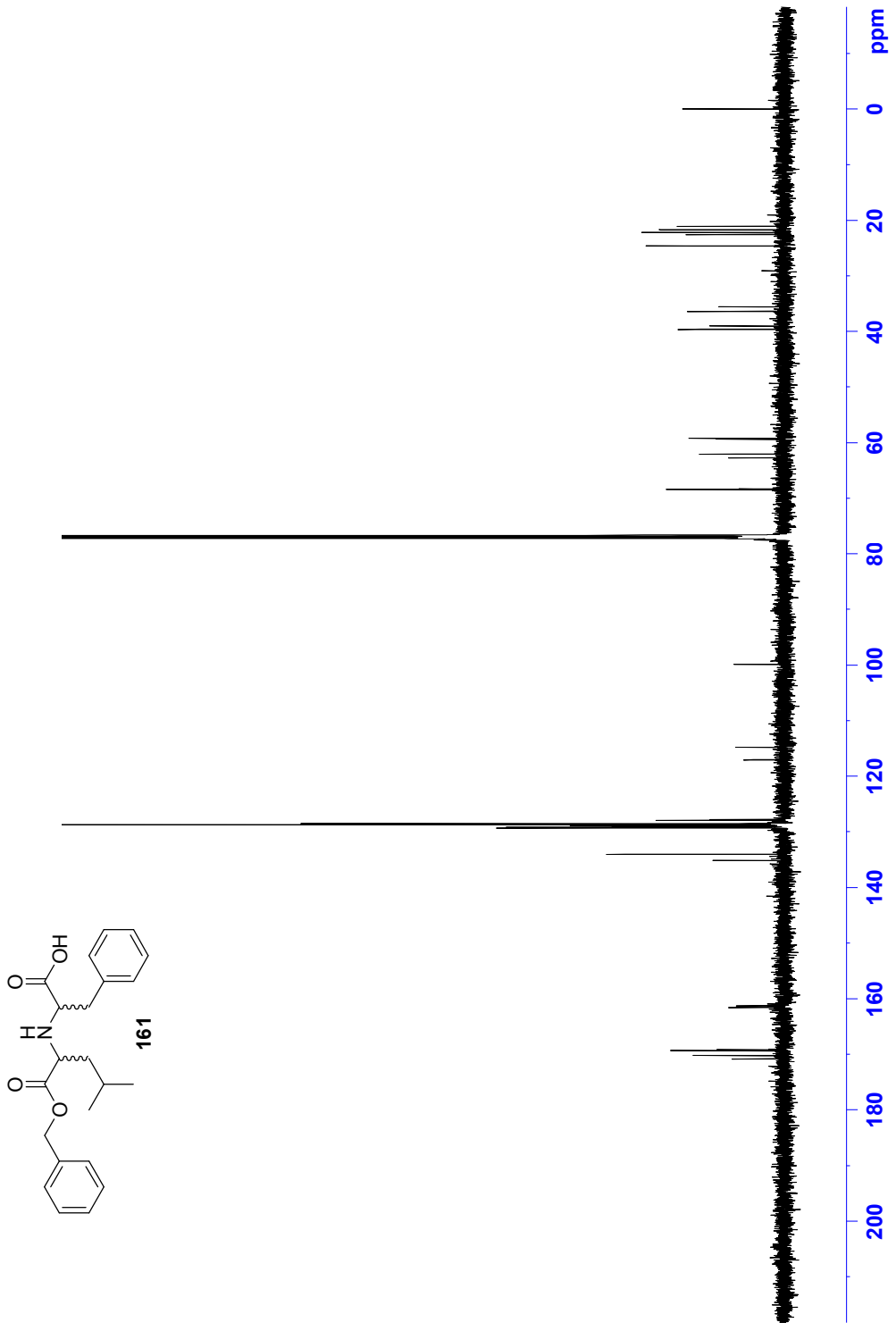
3.37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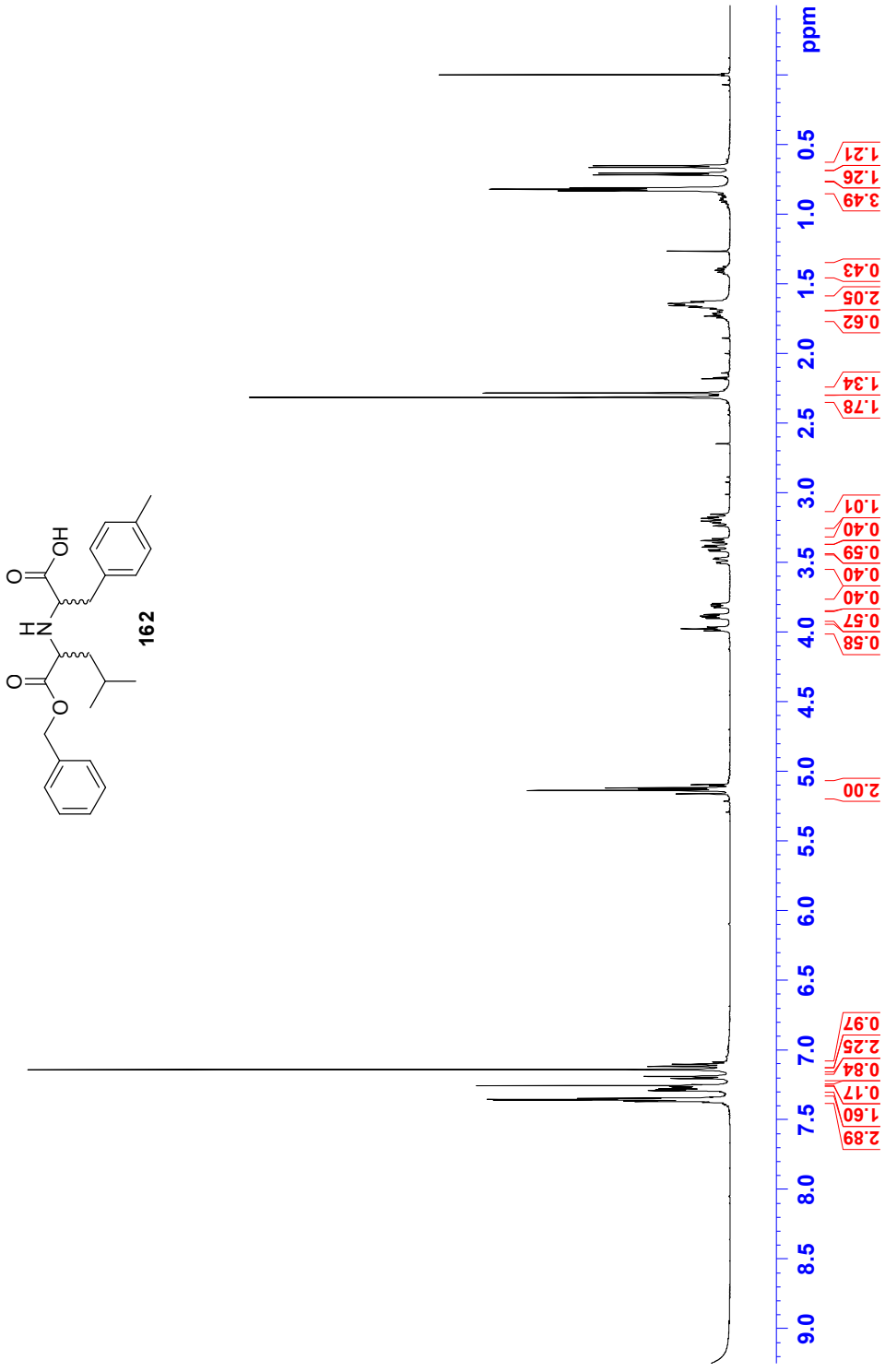
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0.57
0.40
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0.40
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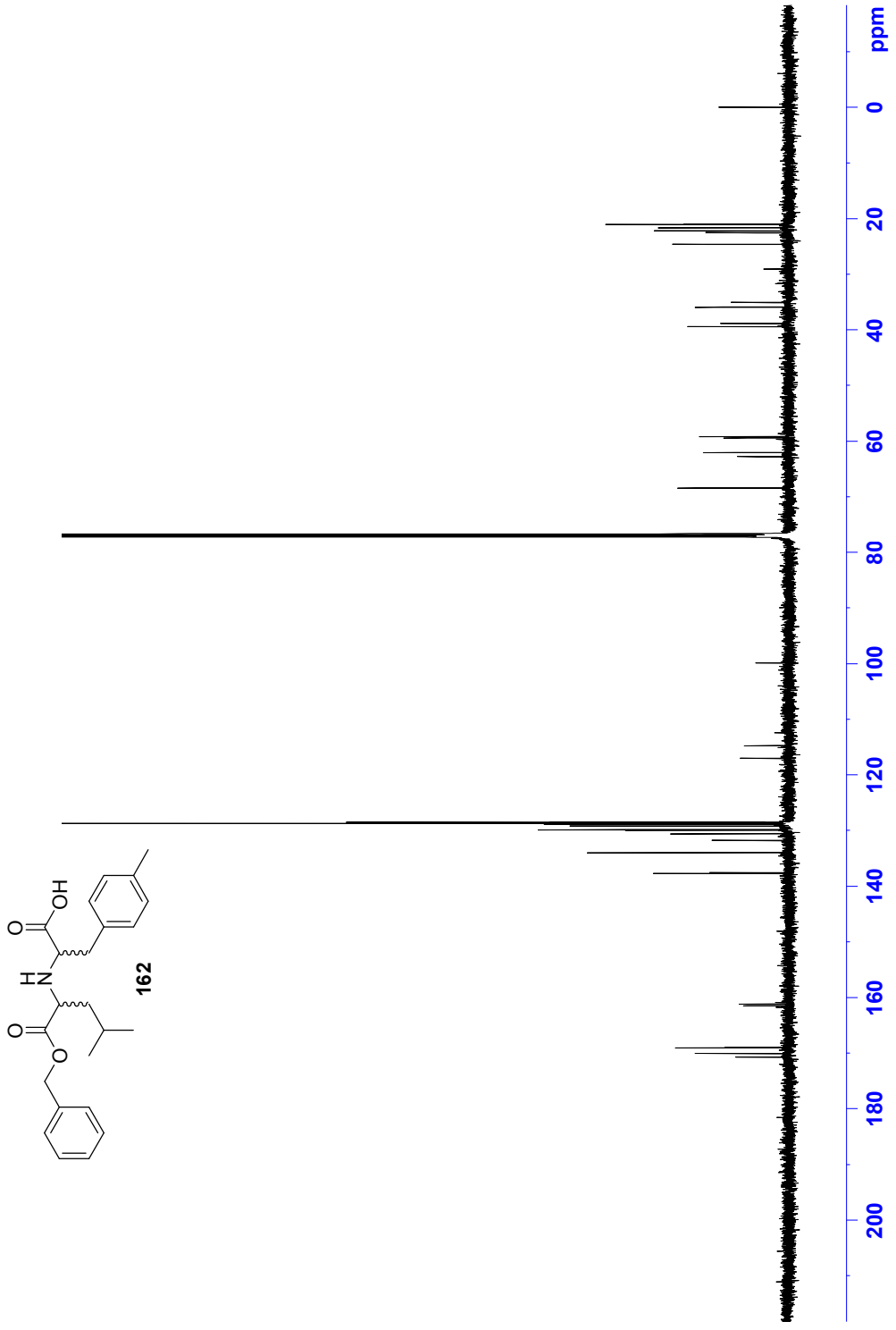
1.78
1.34
0.62
2.05
0.43
3.49
1.26
1.21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60
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100
120
140
160
180
200
OH
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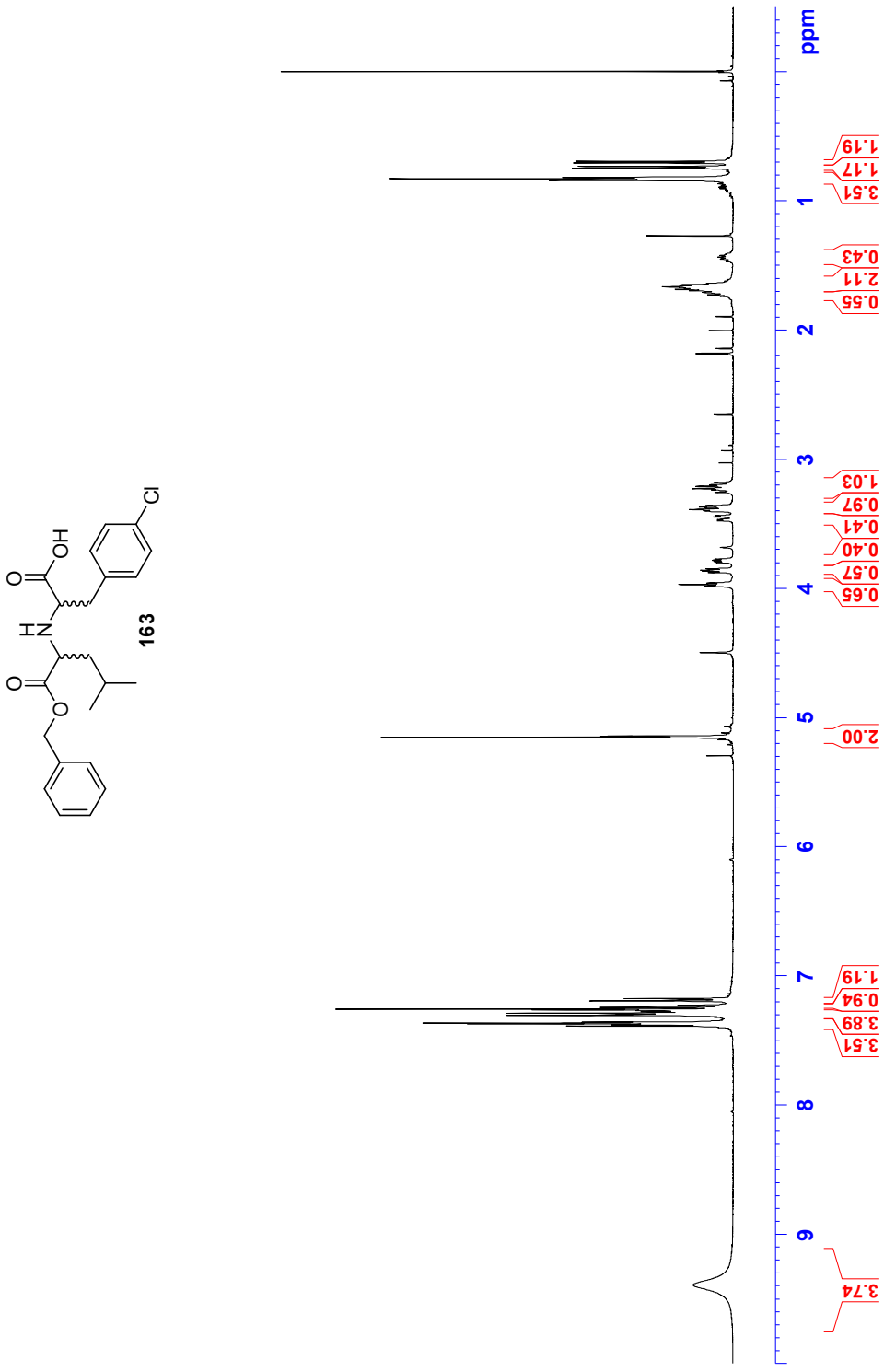
N
O
O
162


Cl
OH
O
HN
O
O
163
ppm
1
2
3
4
5
6
7
8
9
3.51
1.17
1.19
0.55
2.11
0.43
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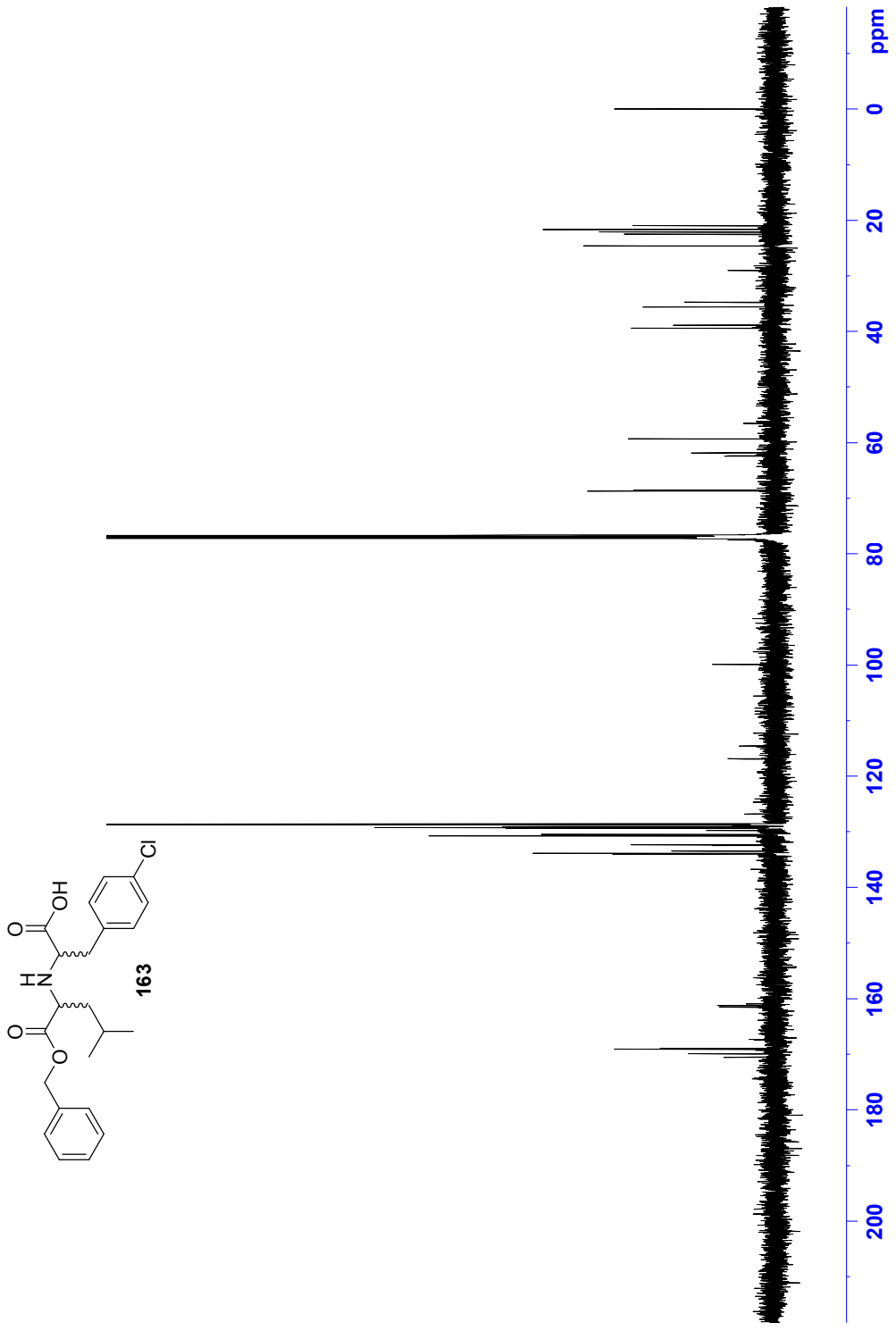
0.57
0.40
0.41
0.97
1.03
2.00
3.51
3.89
0.94
1.19
3.74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OH
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H
N
O
O
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ppm
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60
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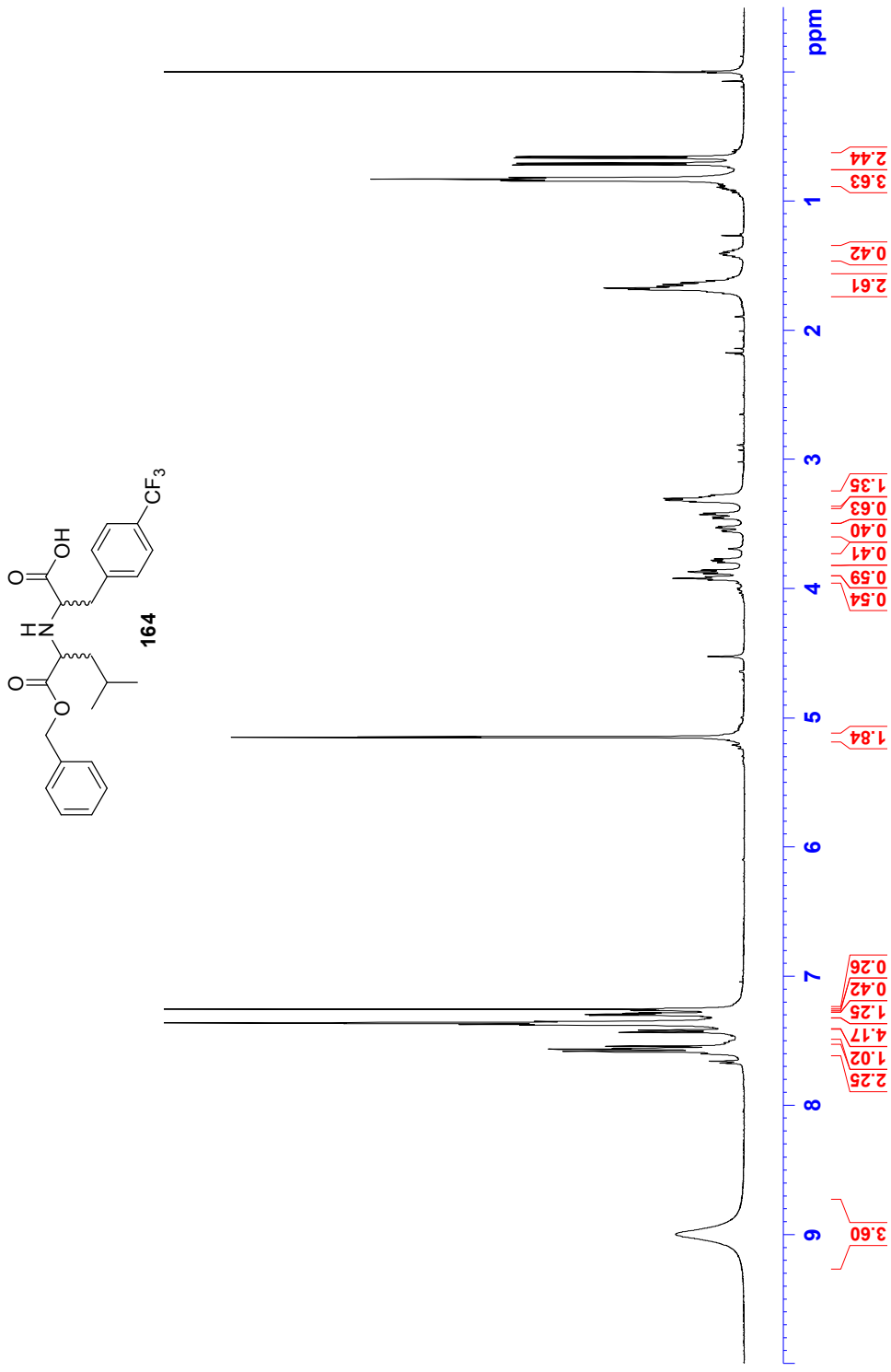
180
200


O
OH
H
N
O
O
CF3
164
ppm
1
2
3
4
5
6
7
8
9
2.44
3.63
0.42
2.61
1.35
0.63
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0.54
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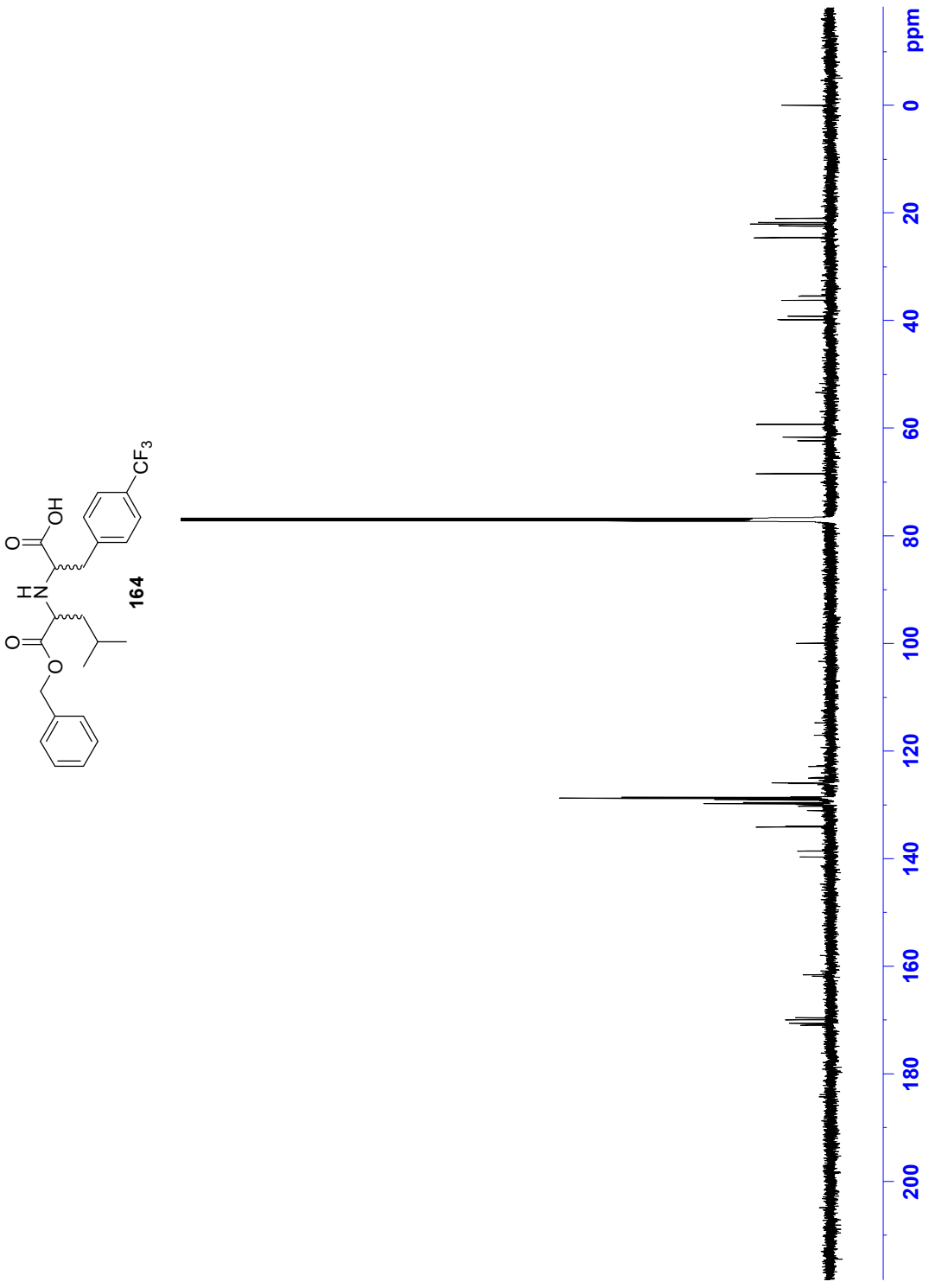
2.25
3.60


O
OH
H
N
O
O
O
CF3
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ppm
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60
80
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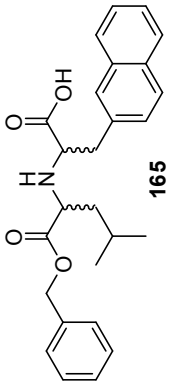
160
180
200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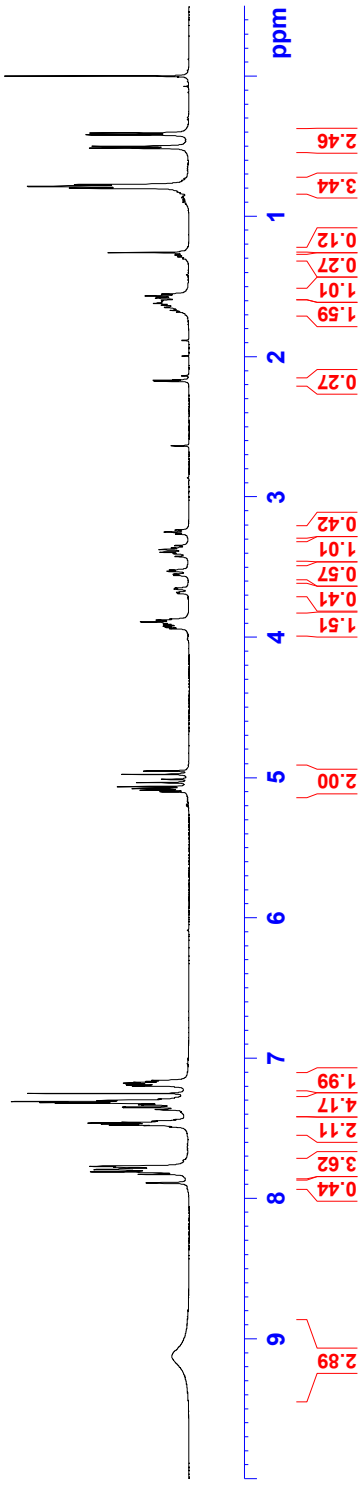
OH
H
N
O
O
165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3
4
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6
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8
9
2.46
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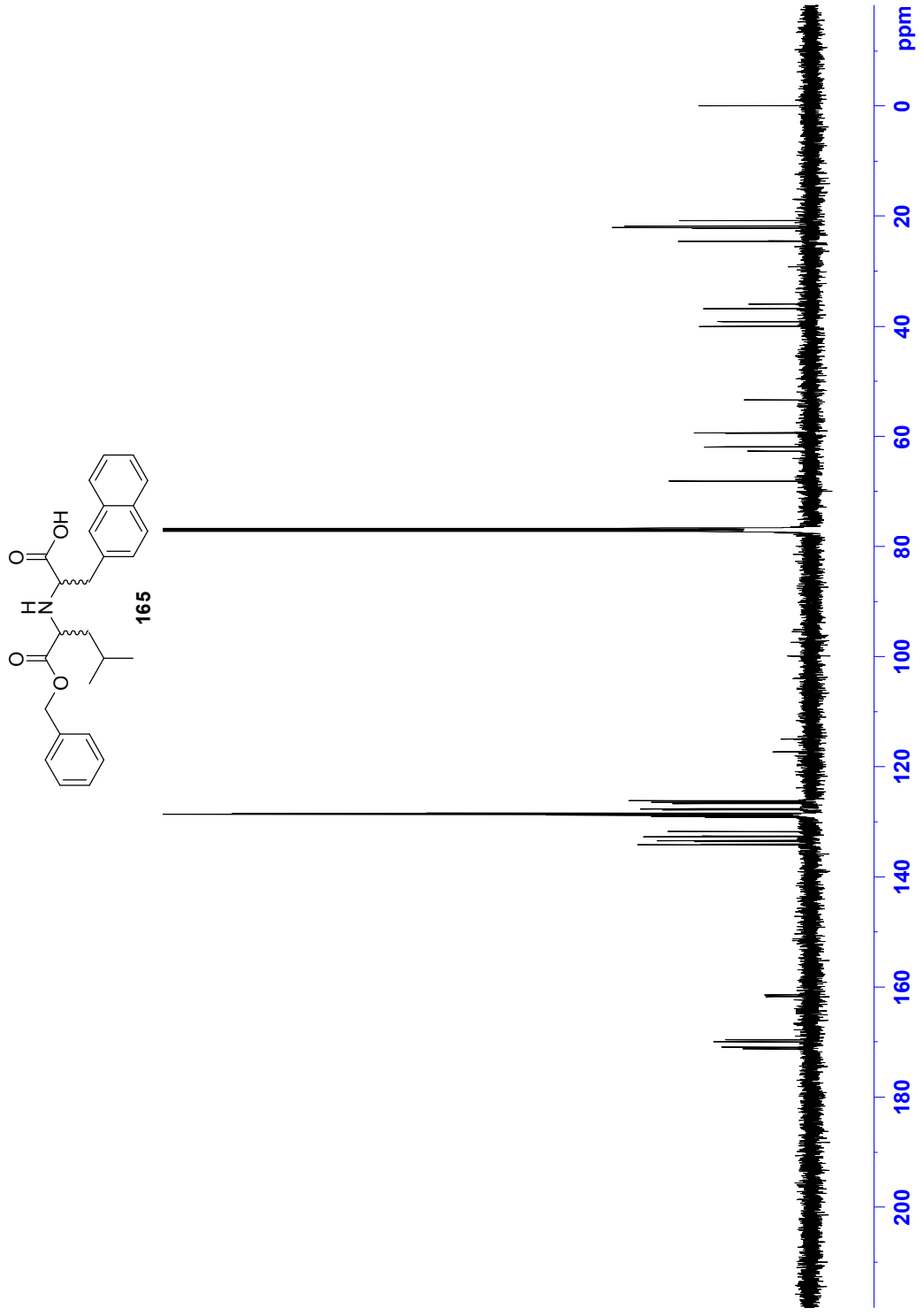
0.27
1.01
1.59
0.27
0.42
1.01
0.57
0.41
1.51
2.00
1.99
4.17
2.11
3.62
0.44
2.89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180
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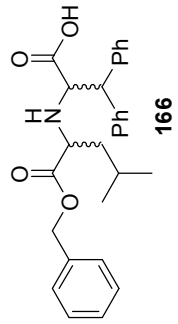
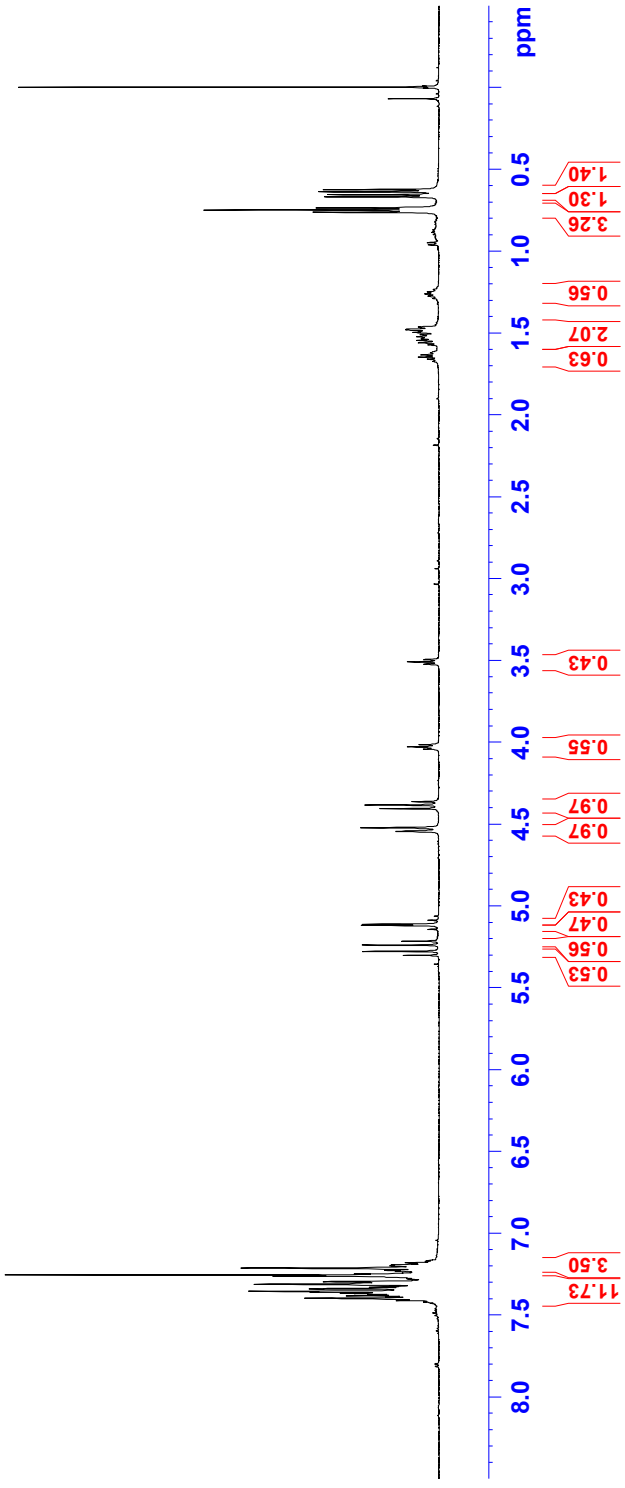

O
OH
H
N
Ph
Ph
O
O

166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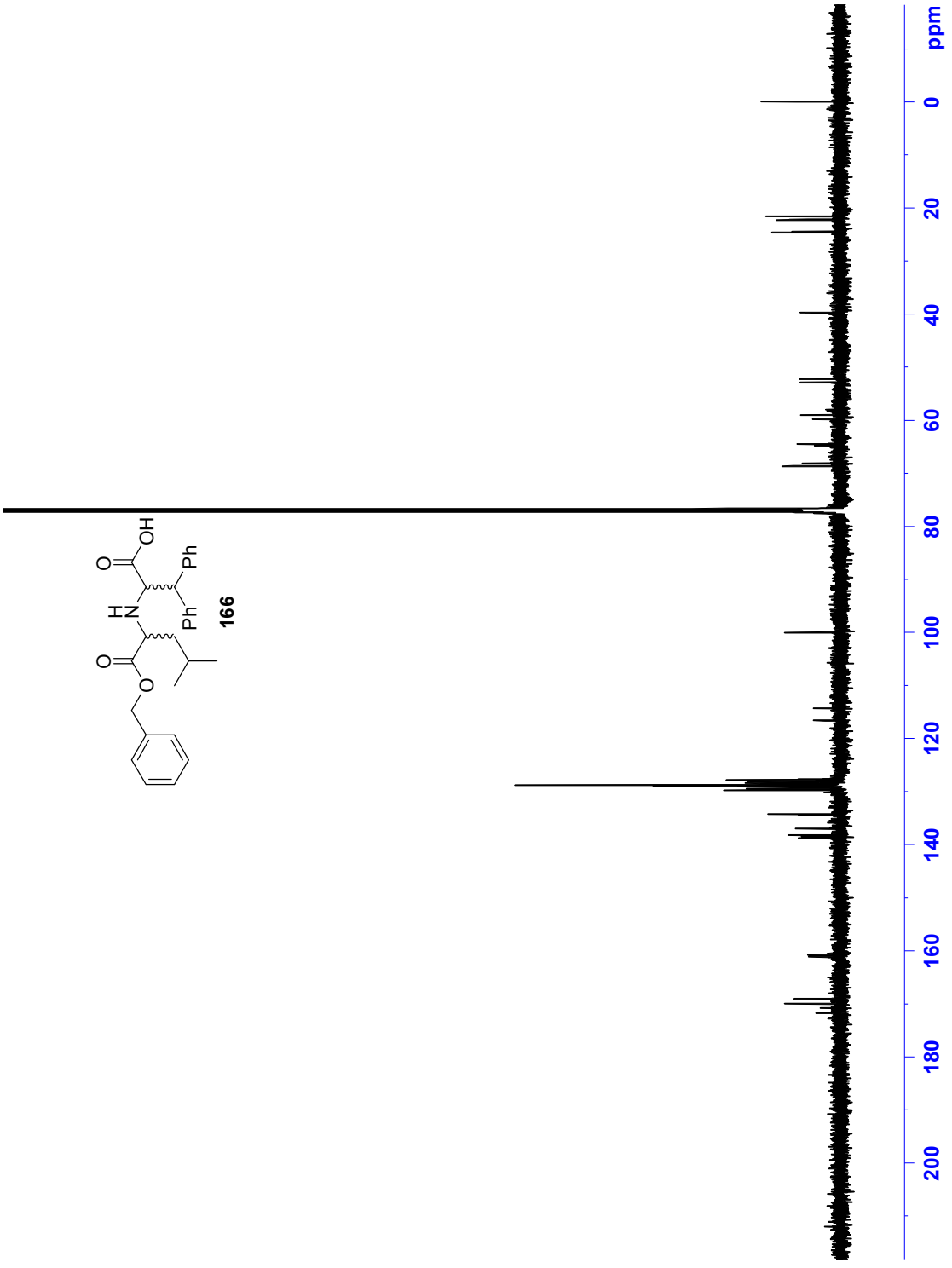
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.40
1.30
3.26
0.56
2.07
0.63
0.43
0.55
0.97
0.97
0.43
0.47
0.56
0.53
3.50
11.73


ppm
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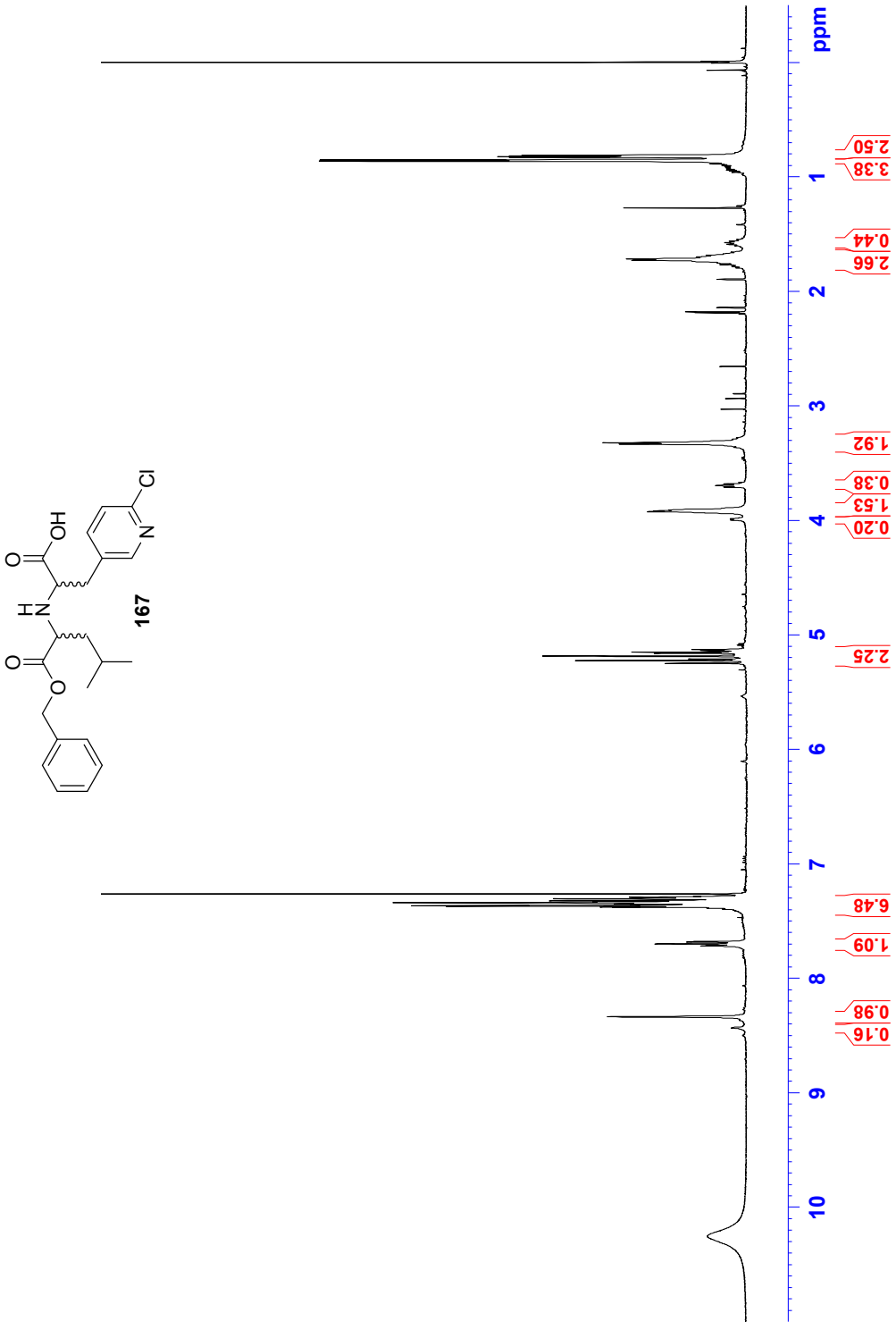
80
100
120
140
160
180
200
O
OH
H
N
Ph
Ph
O
O
166


ppm
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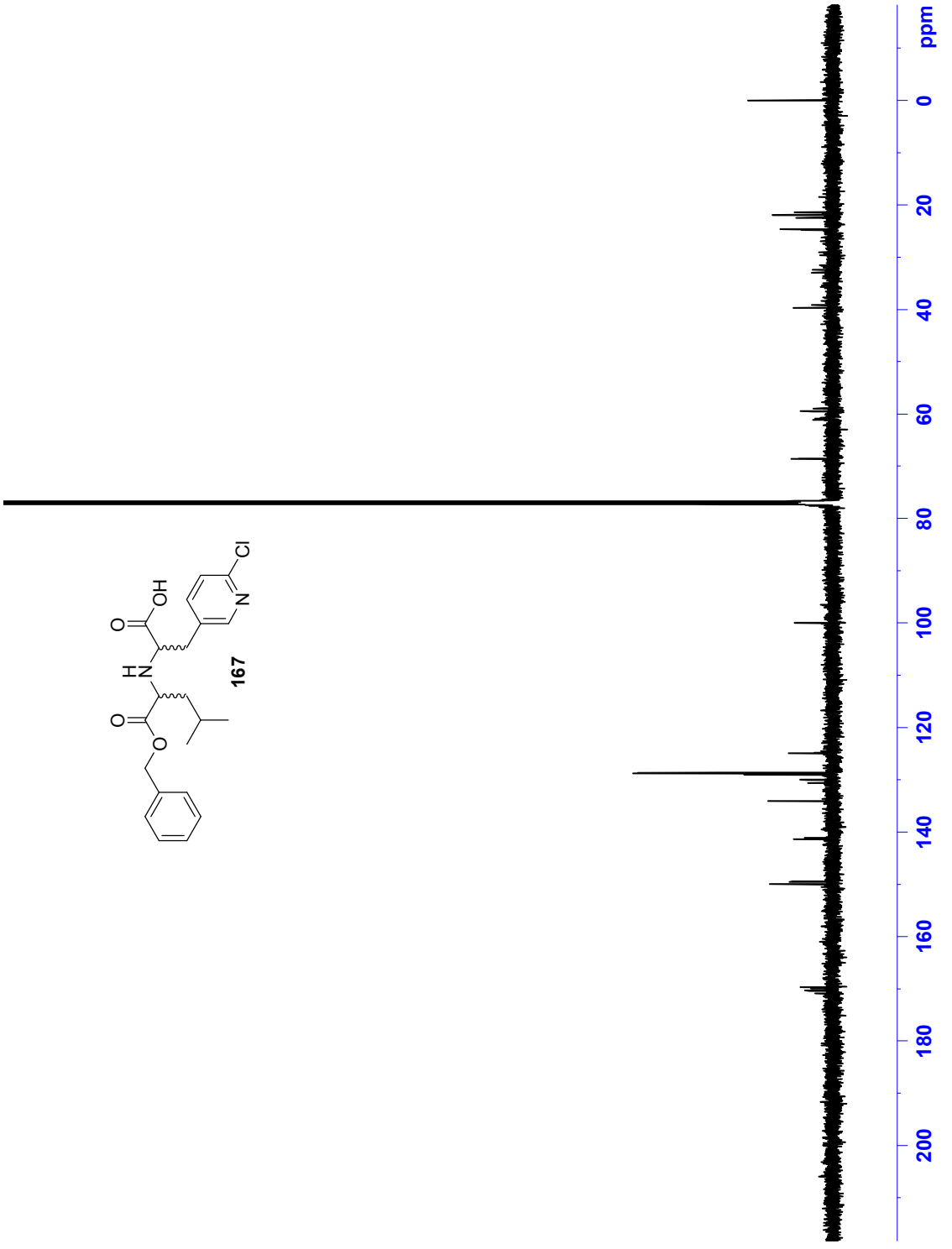
3
4
5
6
7
8
9
10
2.50
3.38
0.44
2.66
1.92
0.38
1.53
0.20
2.25
6.48
1.09
0.98
0.16
O
OH
N
H
O
O
Cl
N
167


ppm
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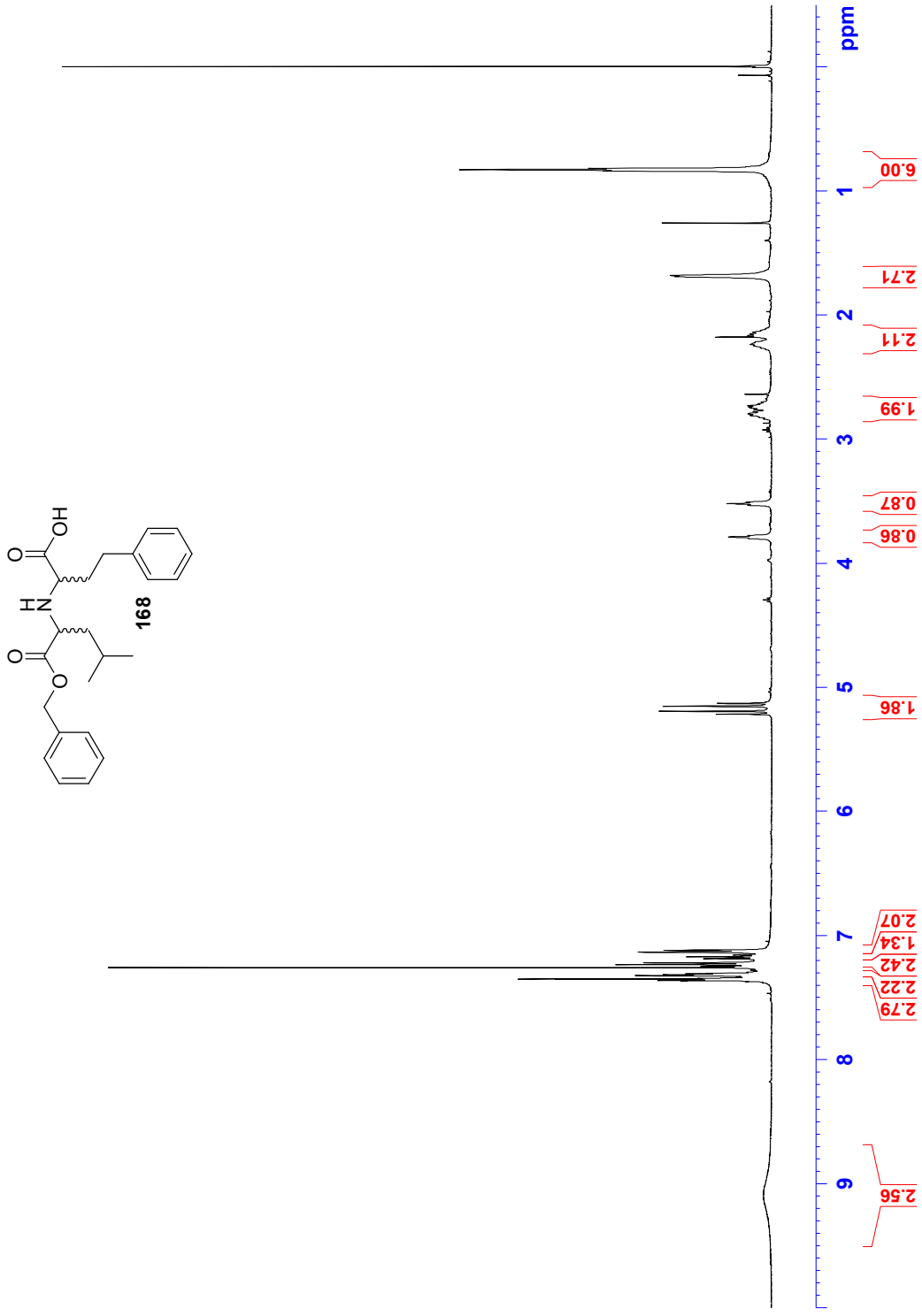
180
200
Cl
N
OH
O
H
N
O
O
167


168
ppm
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8
7
6
5
4
3
2
1
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2.42
1.34
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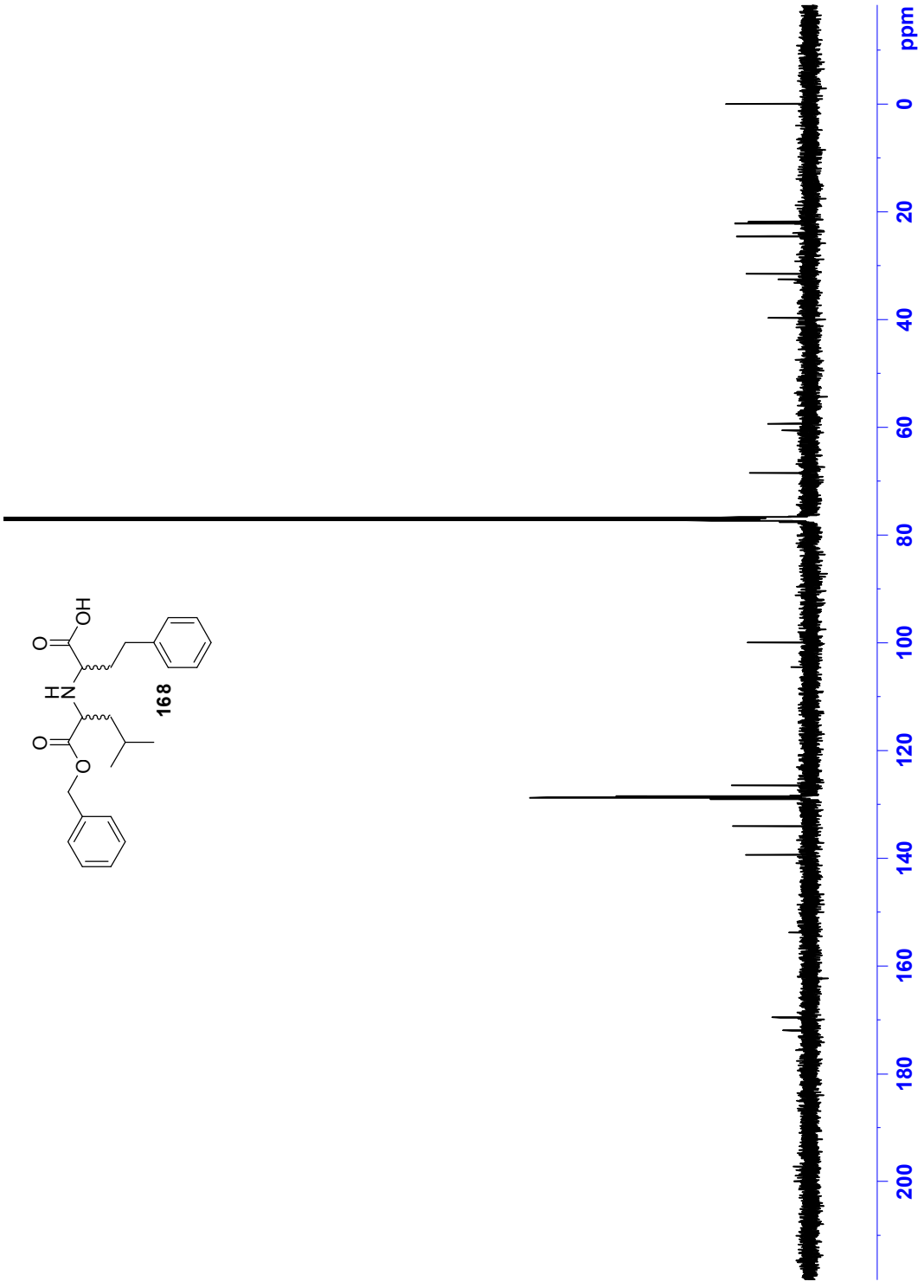
0.86
0.87
1.99
2.11
2.71
6.00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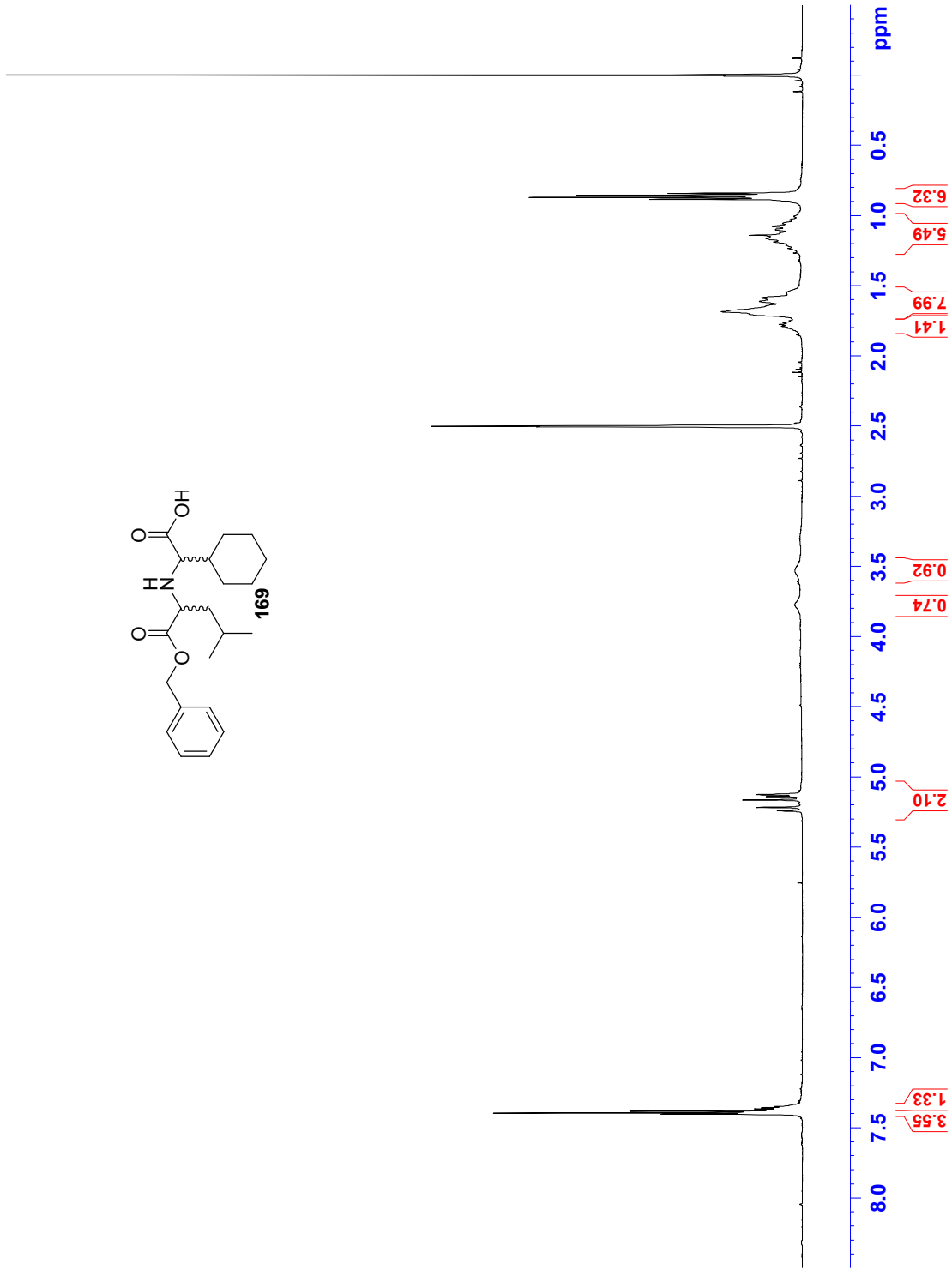
180
200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ppm
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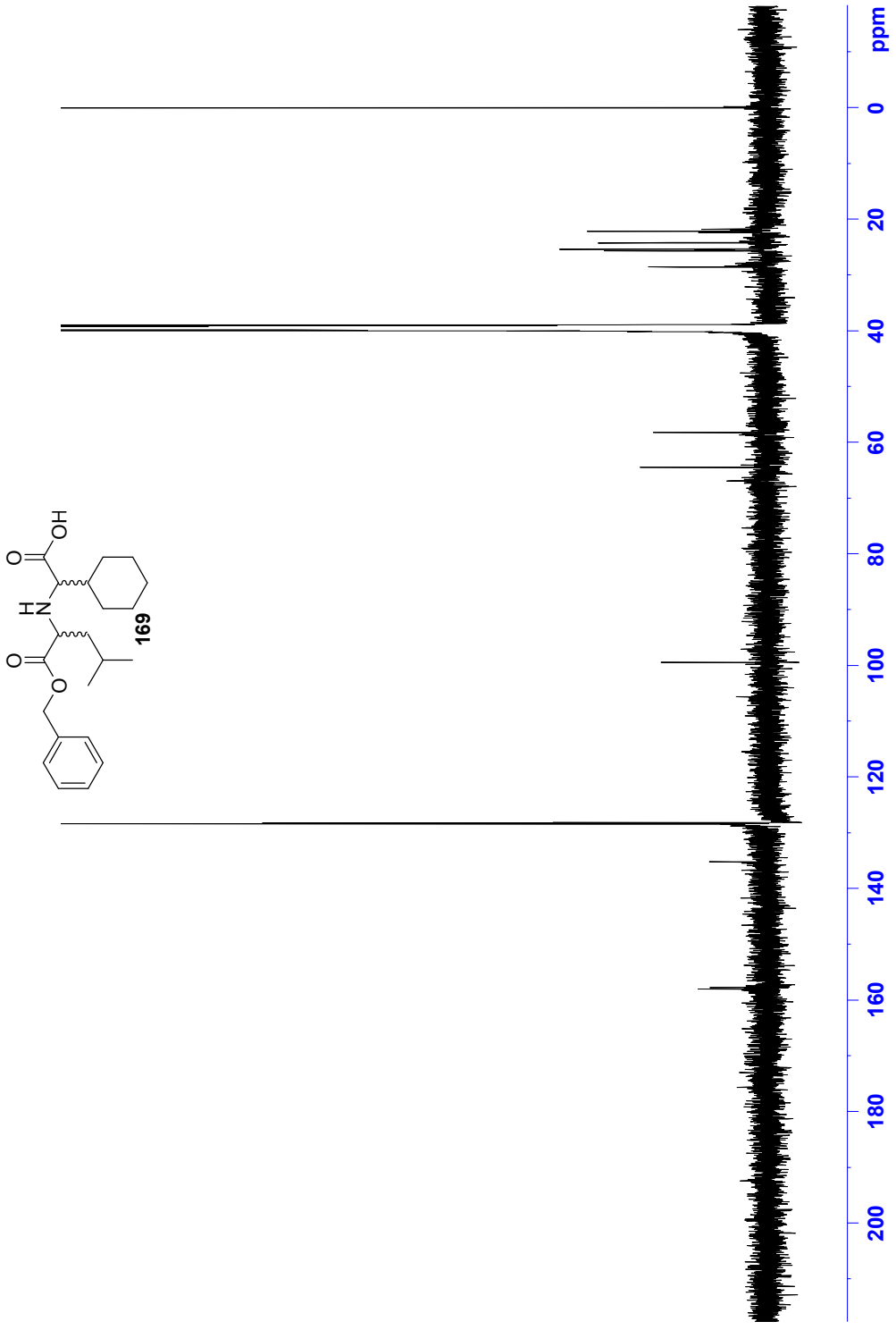
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
6.32
5.49
7.99
1.41
0.92
0.74
2.10
1.33
3.55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ppm
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100
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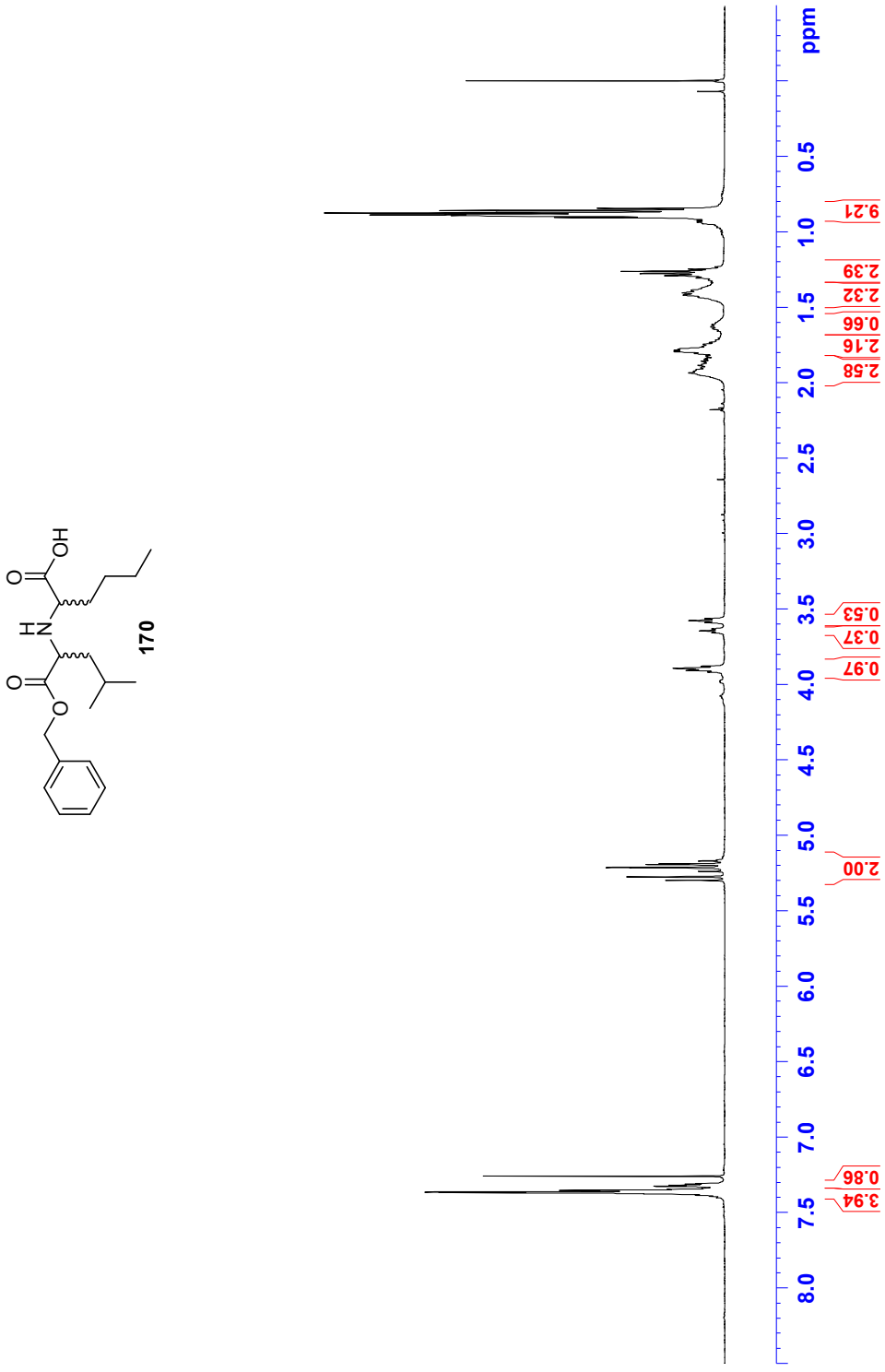
170
ppm
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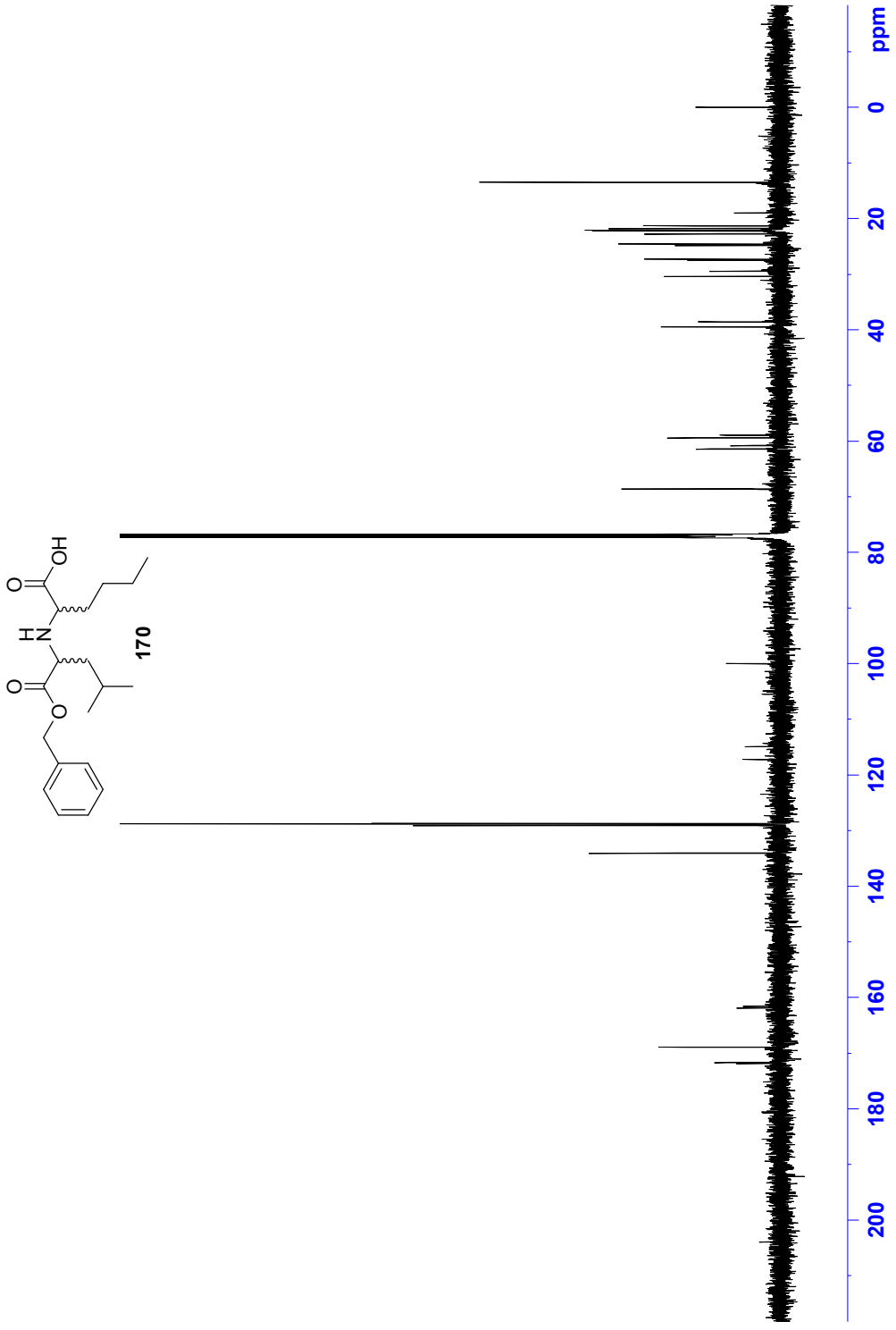
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
9.21
2.39
2.32
0.66
2.16
2.58
0.53
0.37
0.97
2.00
0.86
3.94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ppm
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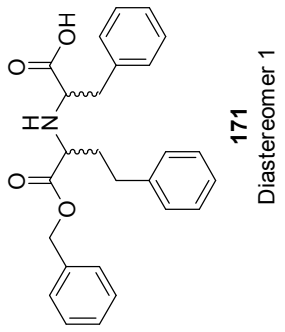
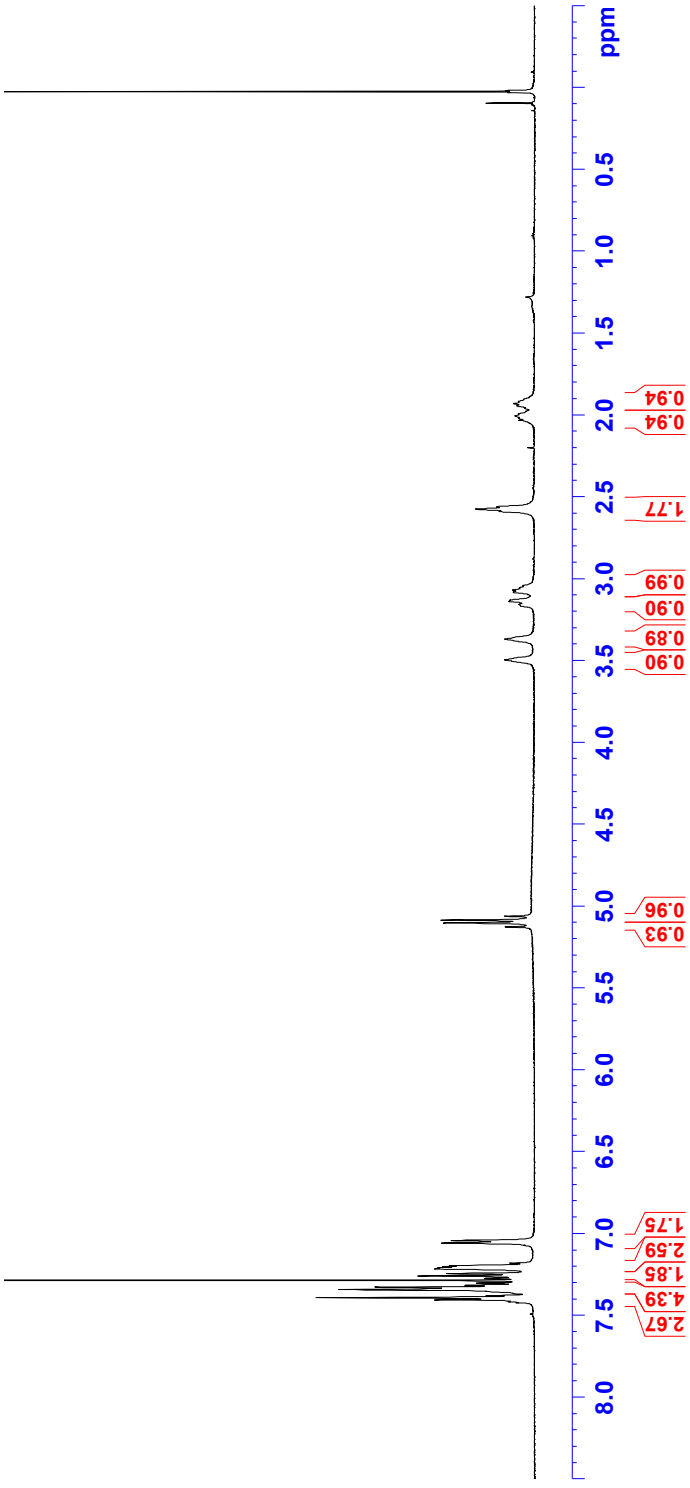
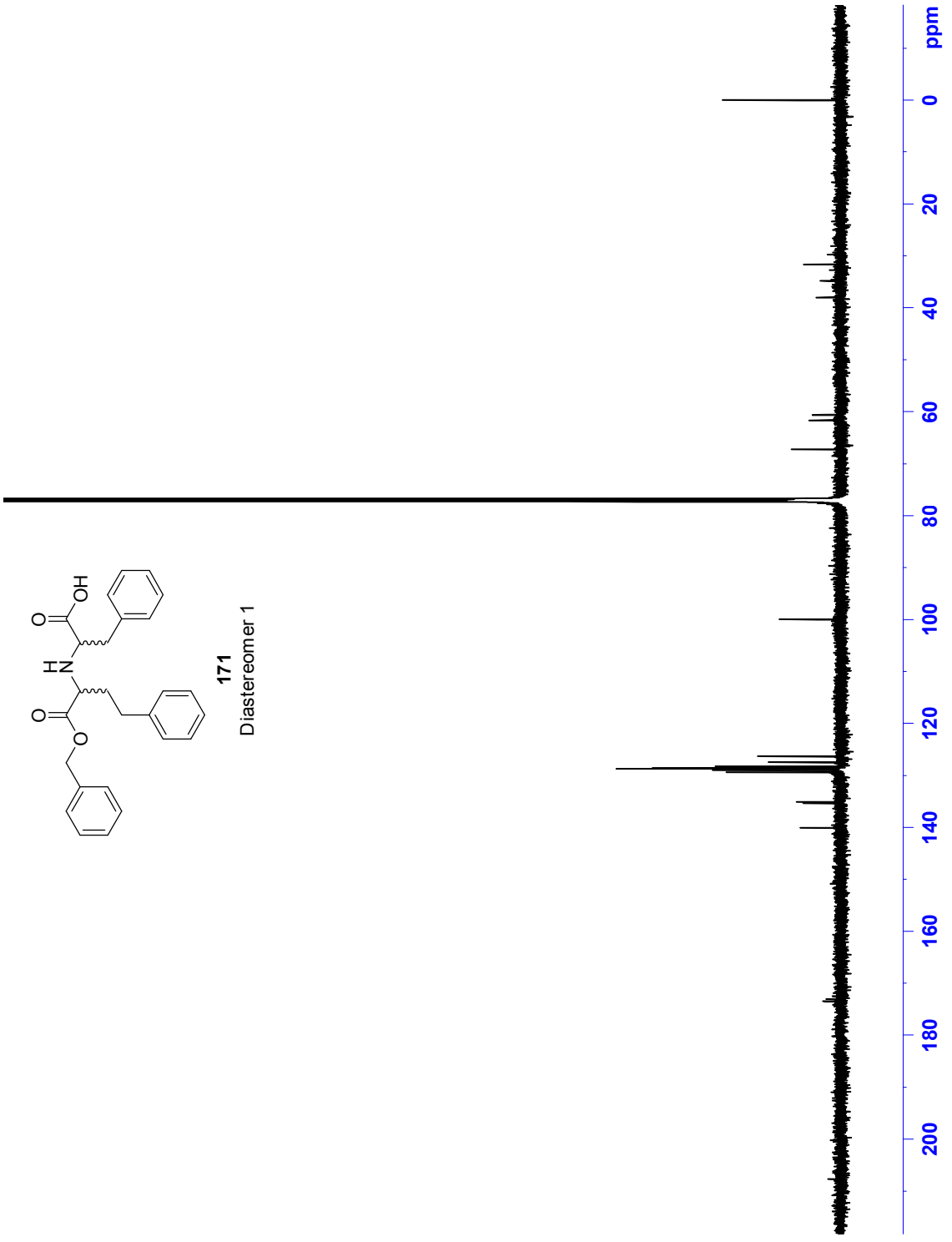


171

Diastereomer 1




171
Diastereomer 1
ppm
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60
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140
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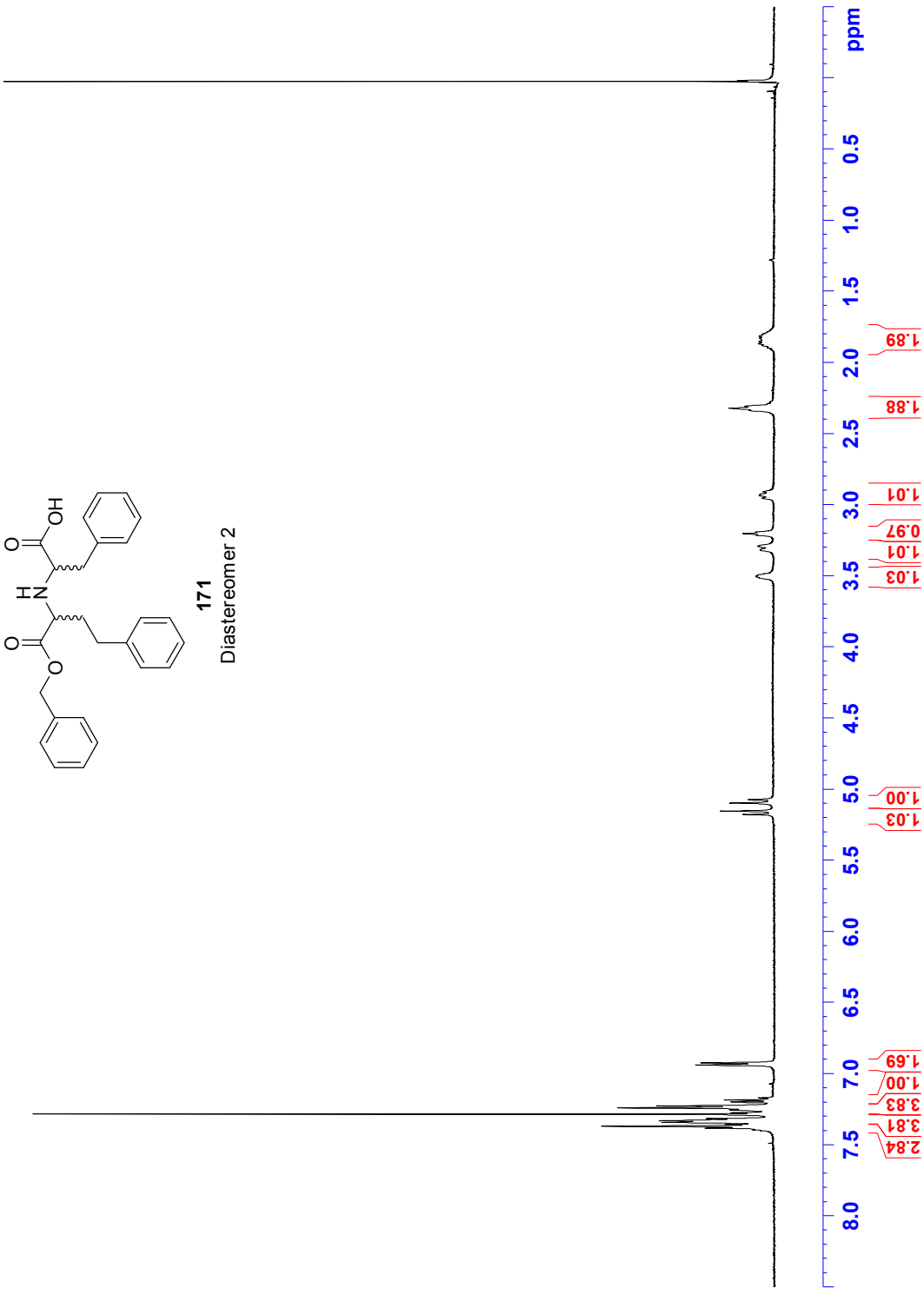
180
200


171
Diastereomer 2
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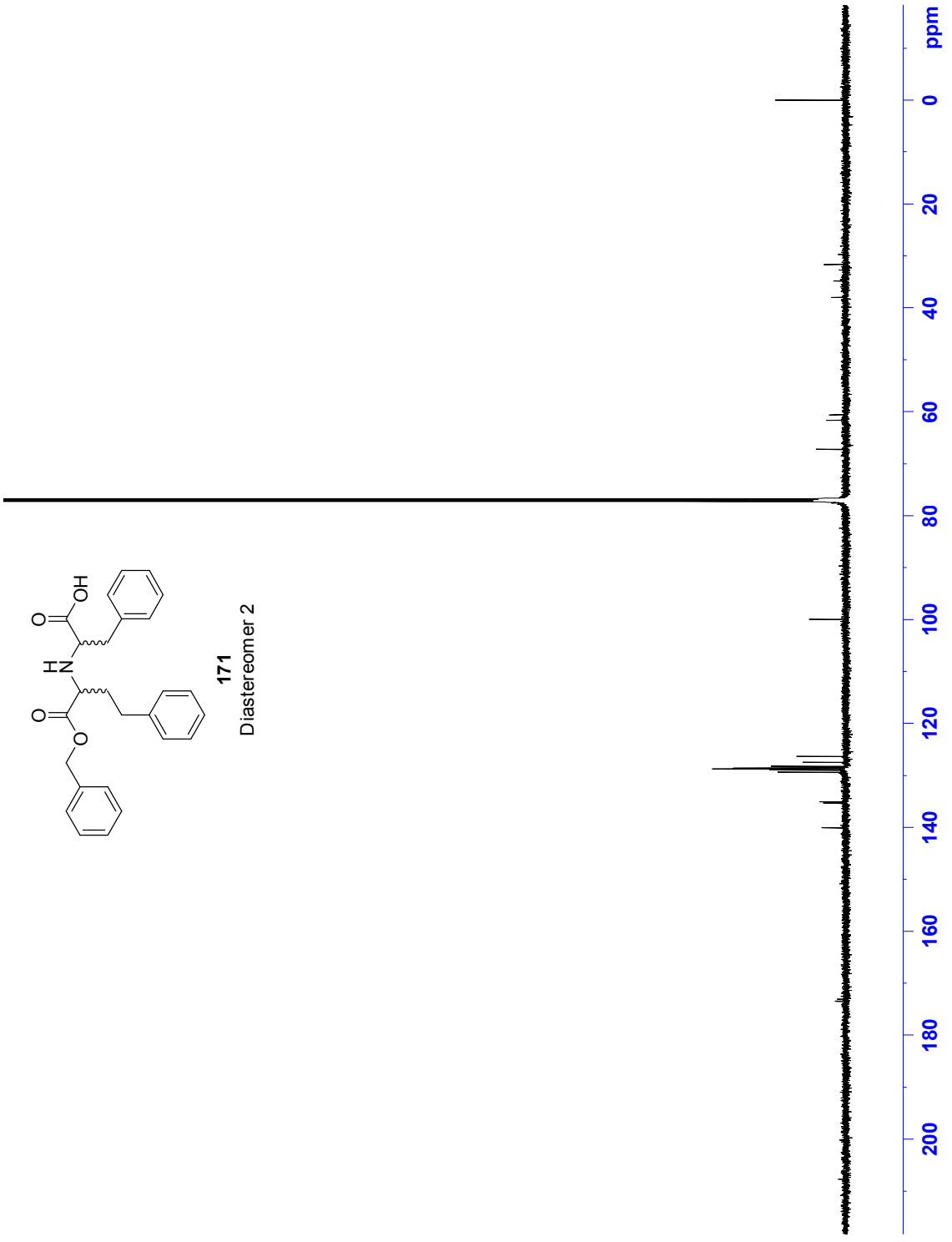
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.89
1.88
1.01
0.97
1.01
1.03
1.00
1.03
1.69
1.00
3.83
3.81
2.84


171
Diastereomer 2
ppm
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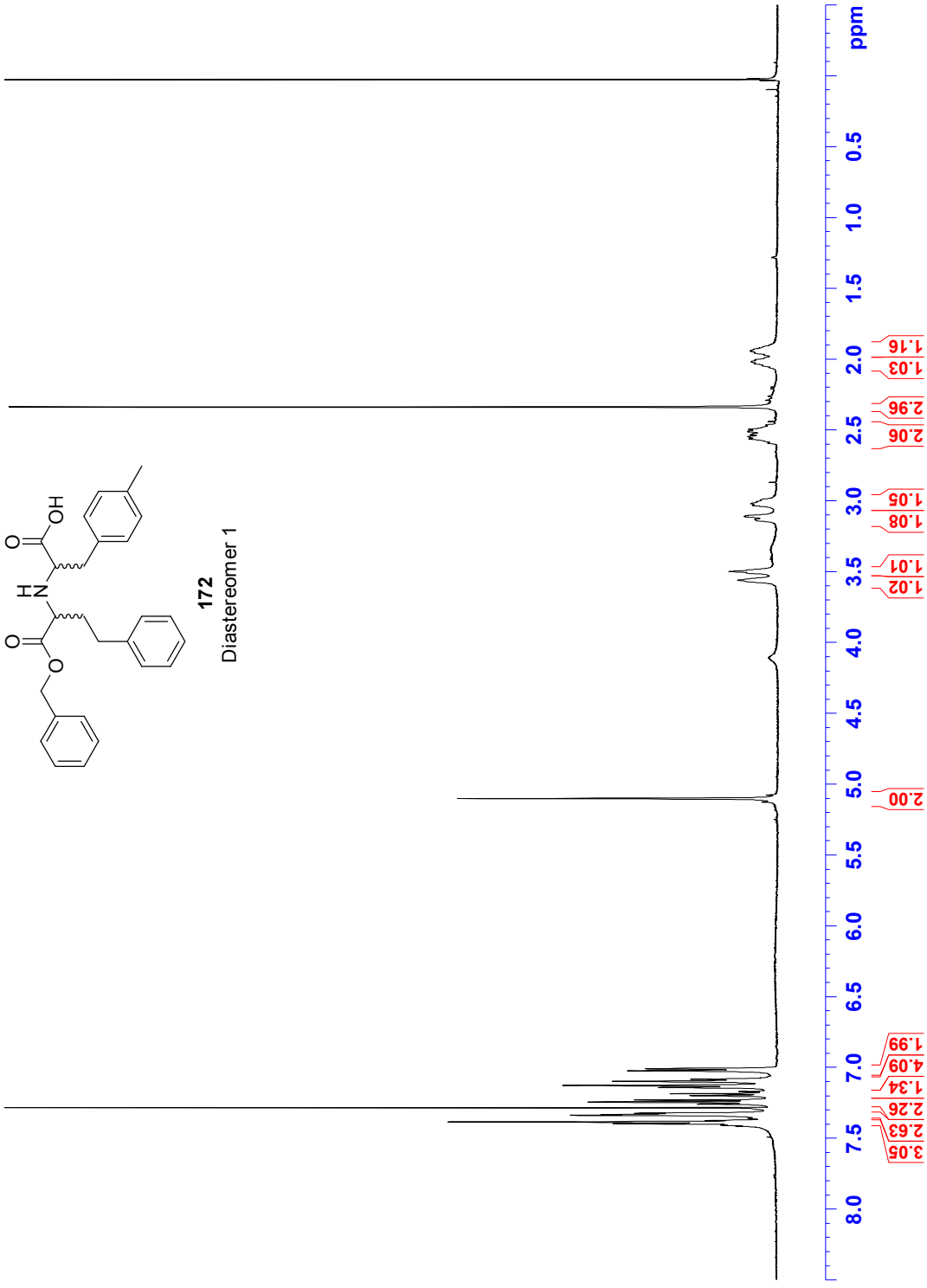
172
Diastereomer 1
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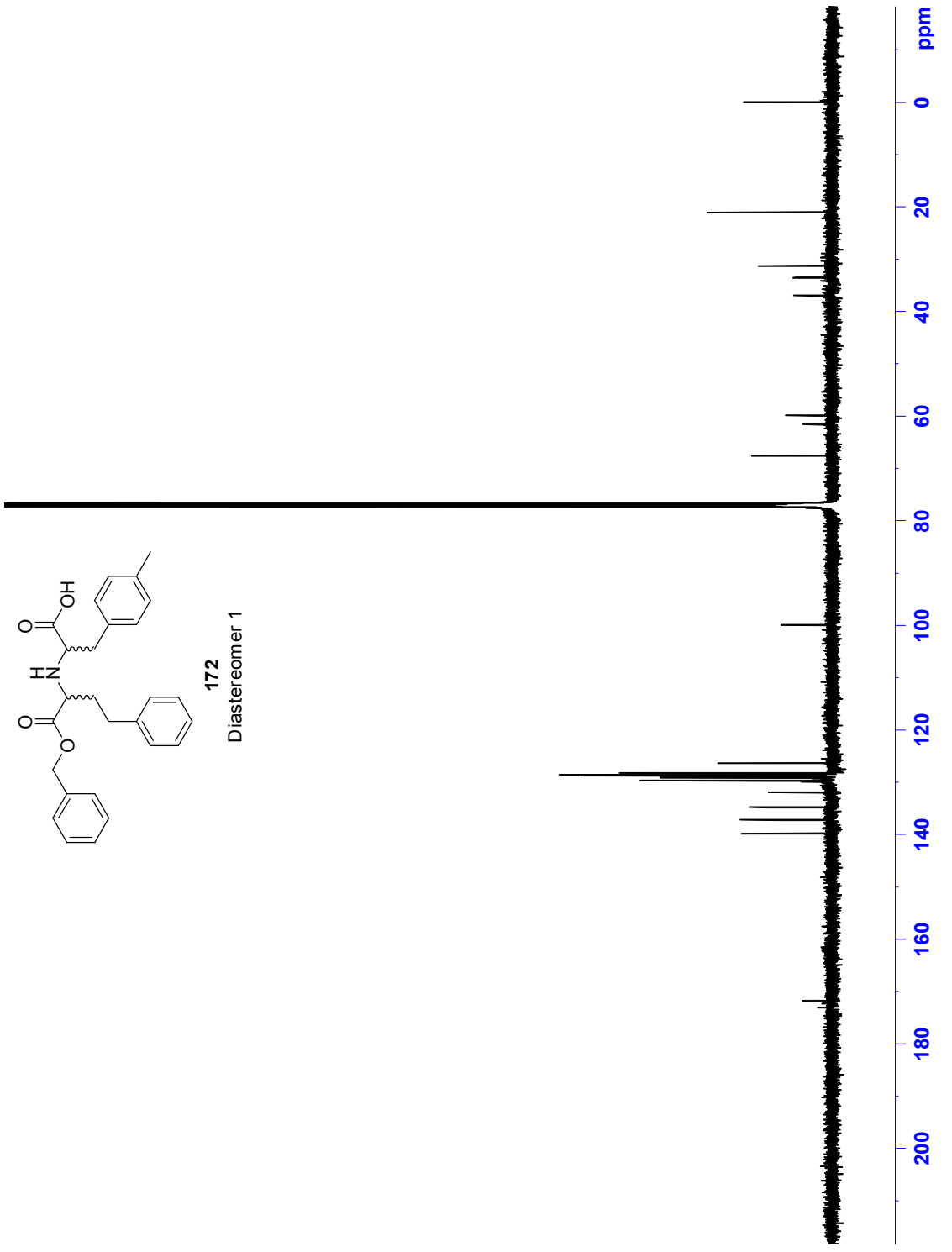
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.16
1.03
2.96
2.06
1.05
1.08
1.01
1.02
2.00
1.99
4.09
1.34
2.26
2.63
3.05


ppm
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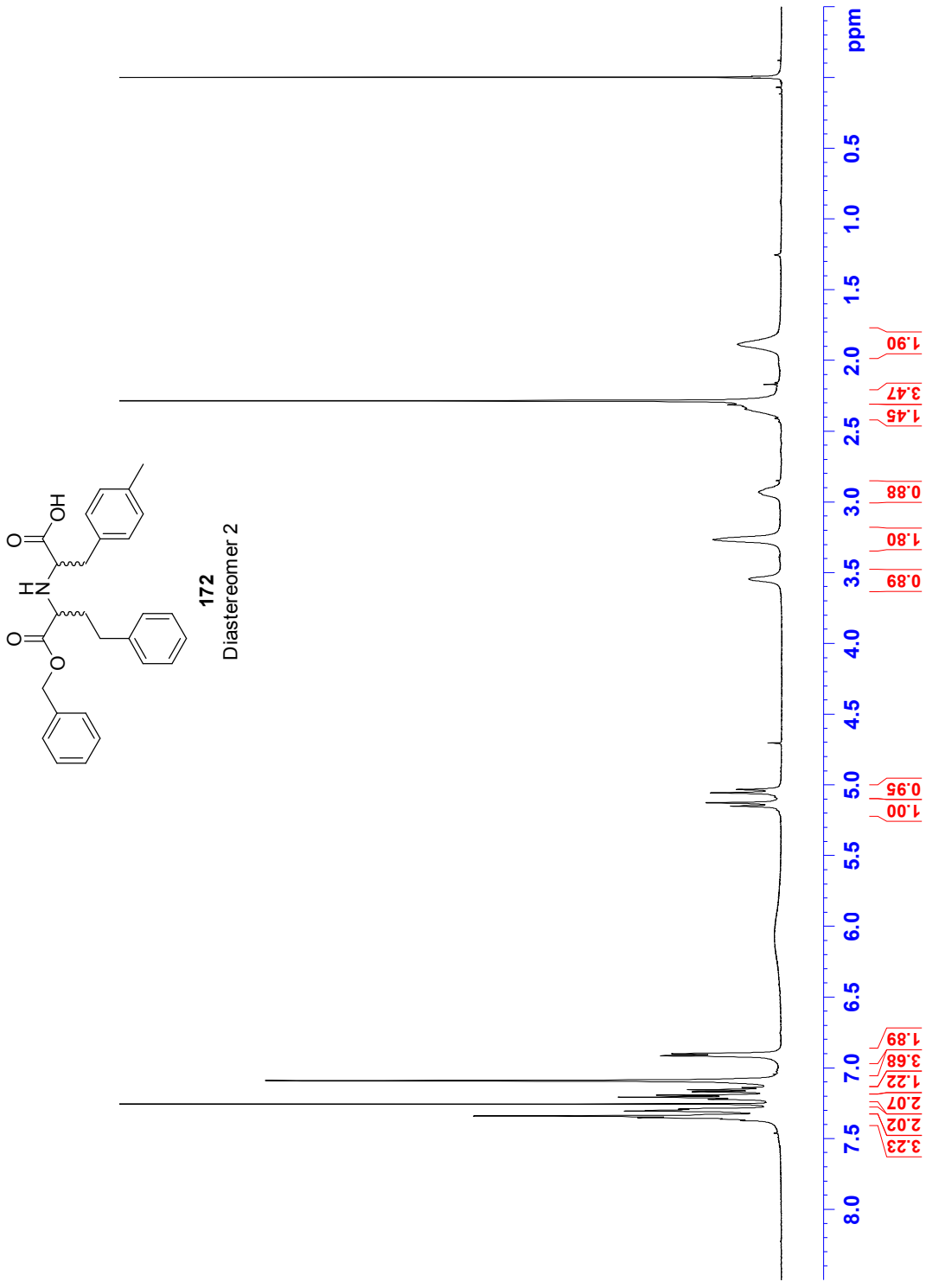
100
120
140
160
180
200
OH
O
HN
O
O
172
Diastereomer 1


172
Diastereomer 2
ppm
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2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.90
3.47
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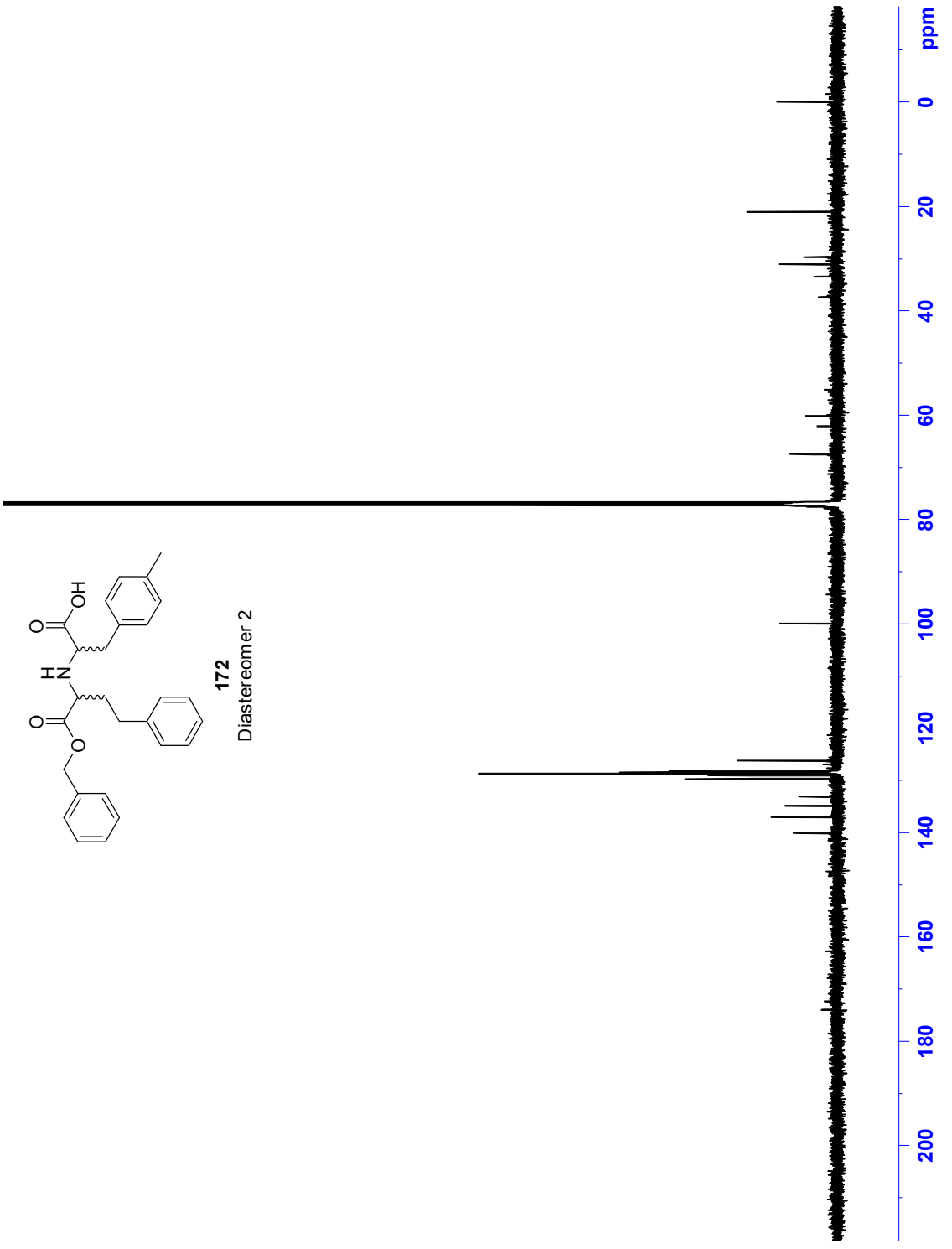
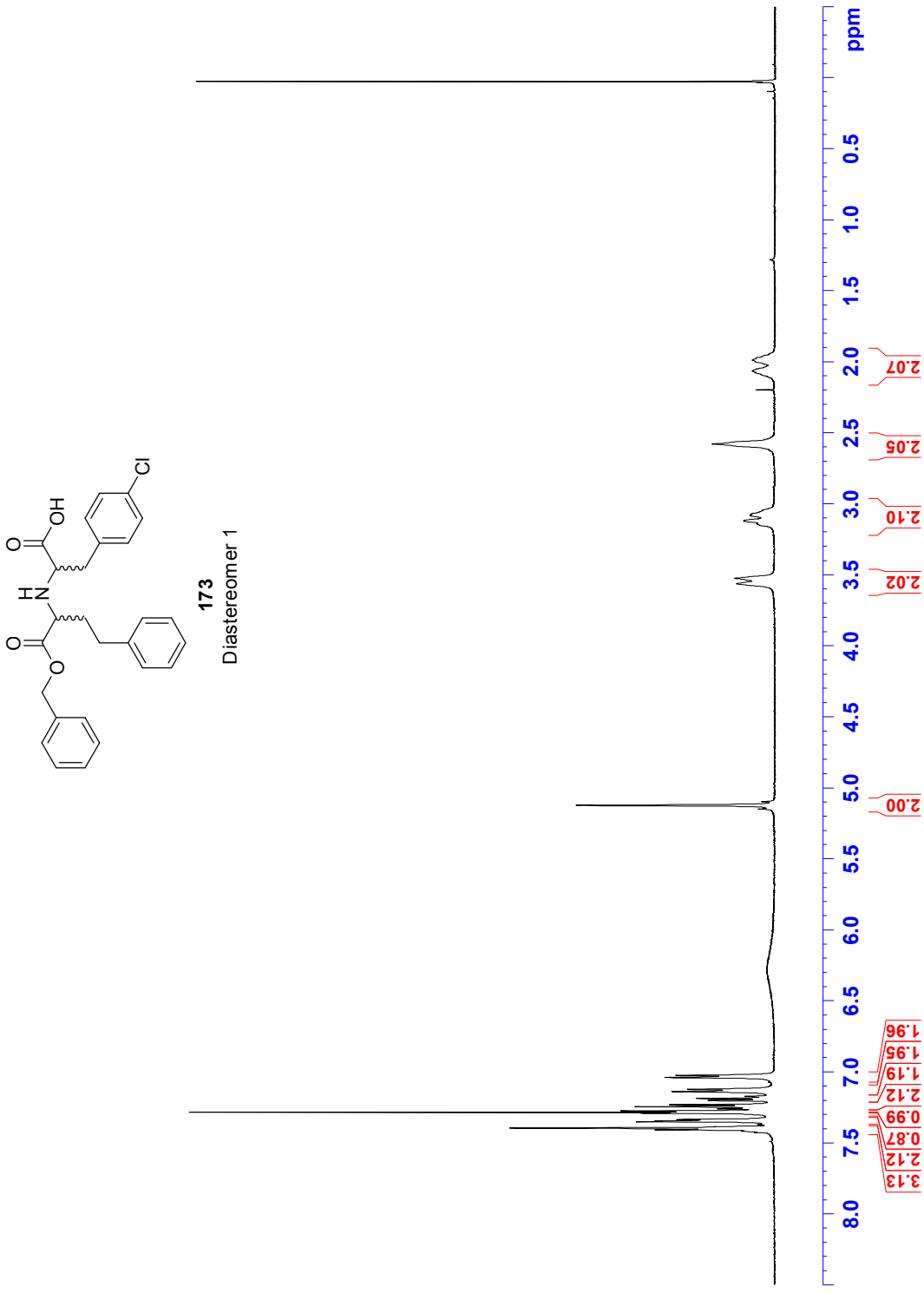
1.80
0.89
1.00
0.95
1.89
3.68
1.22
2.07
2.02
3.23



172
Diastereomer 2


Cl
OH
O
HN
O
O
O
173
Diastereomer 1
ppm
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
3.13
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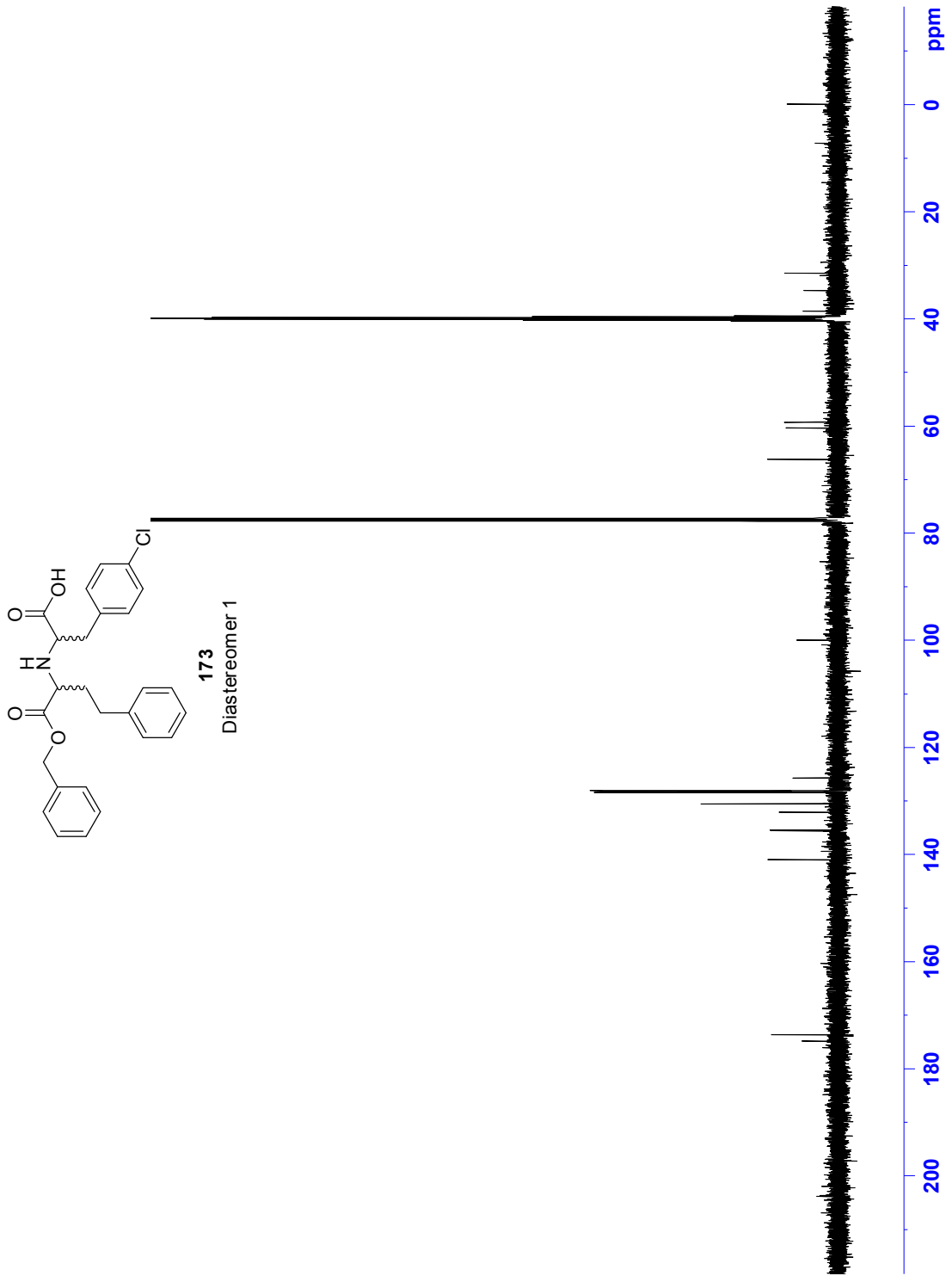
2.12
1.19
1.95
1.96
2.00
2.02
2.10
2.05
2.07


Cl
OH
O
H
N
O
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O
173
Diastereomer 1
ppm
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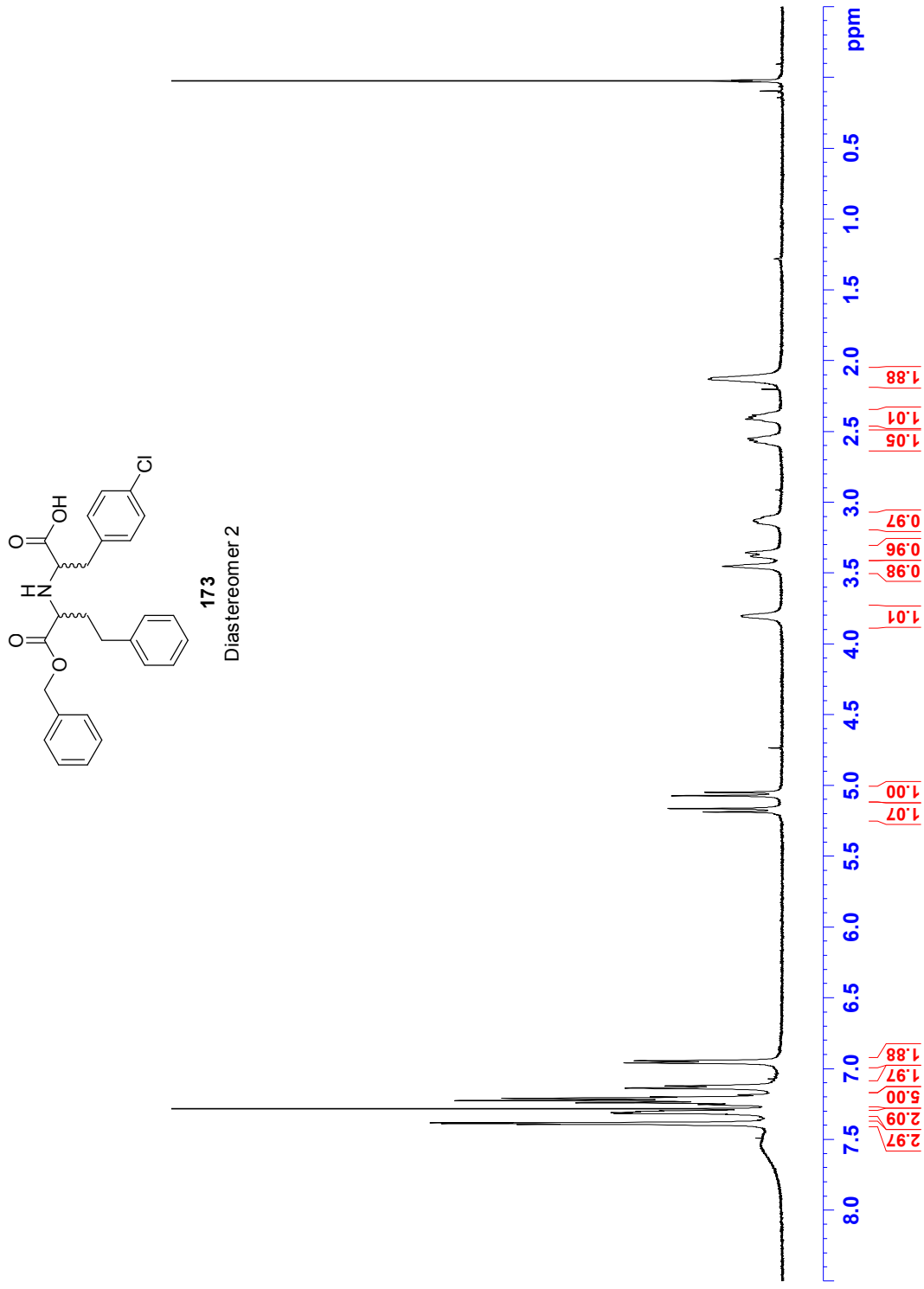
180
200


O
OH
HN
O
O
Cl
173
Diastereomer 2
ppm
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1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.88
1.01
1.05
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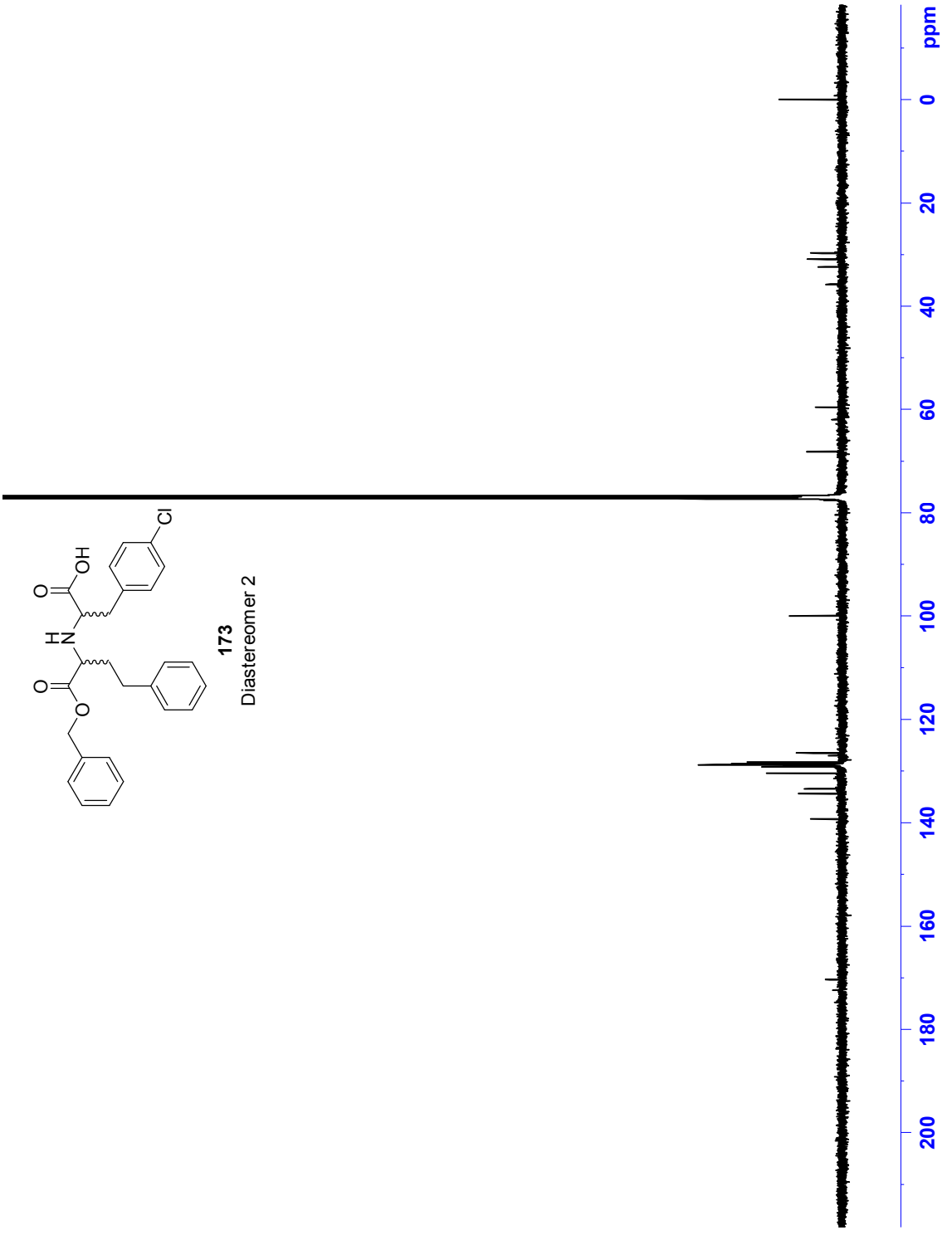
0.98
1.01
1.00
1.07
1.88
1.97
5.00
2.09
2.97


ppm
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40
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100
120
140
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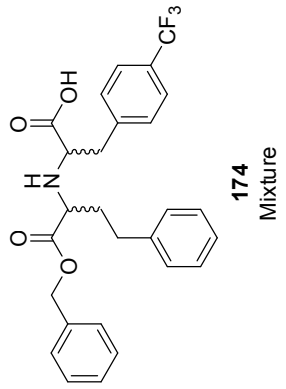
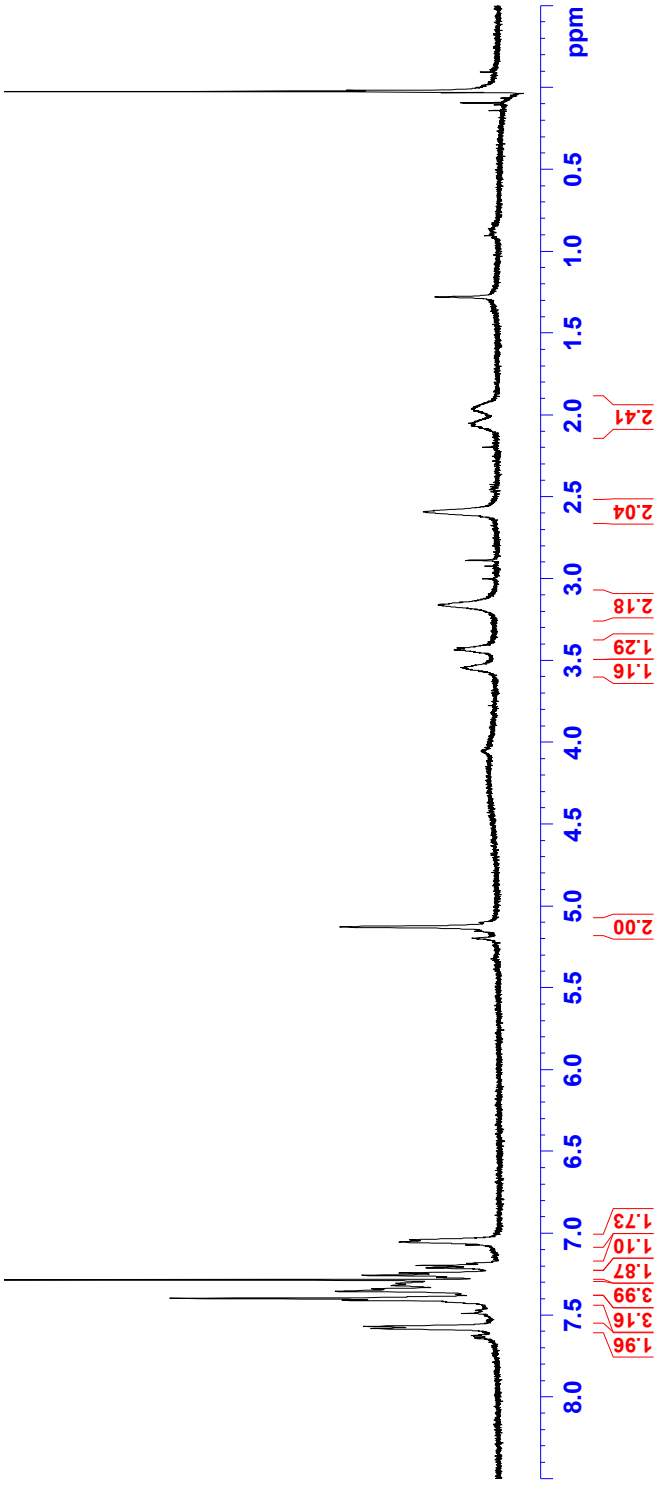
180
200
O
OH
H
N
O
O
Cl
173
Diastereomer 2


O
OH
HN
O
O
CF3

174
Mixture


ppm
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2.5
3.0
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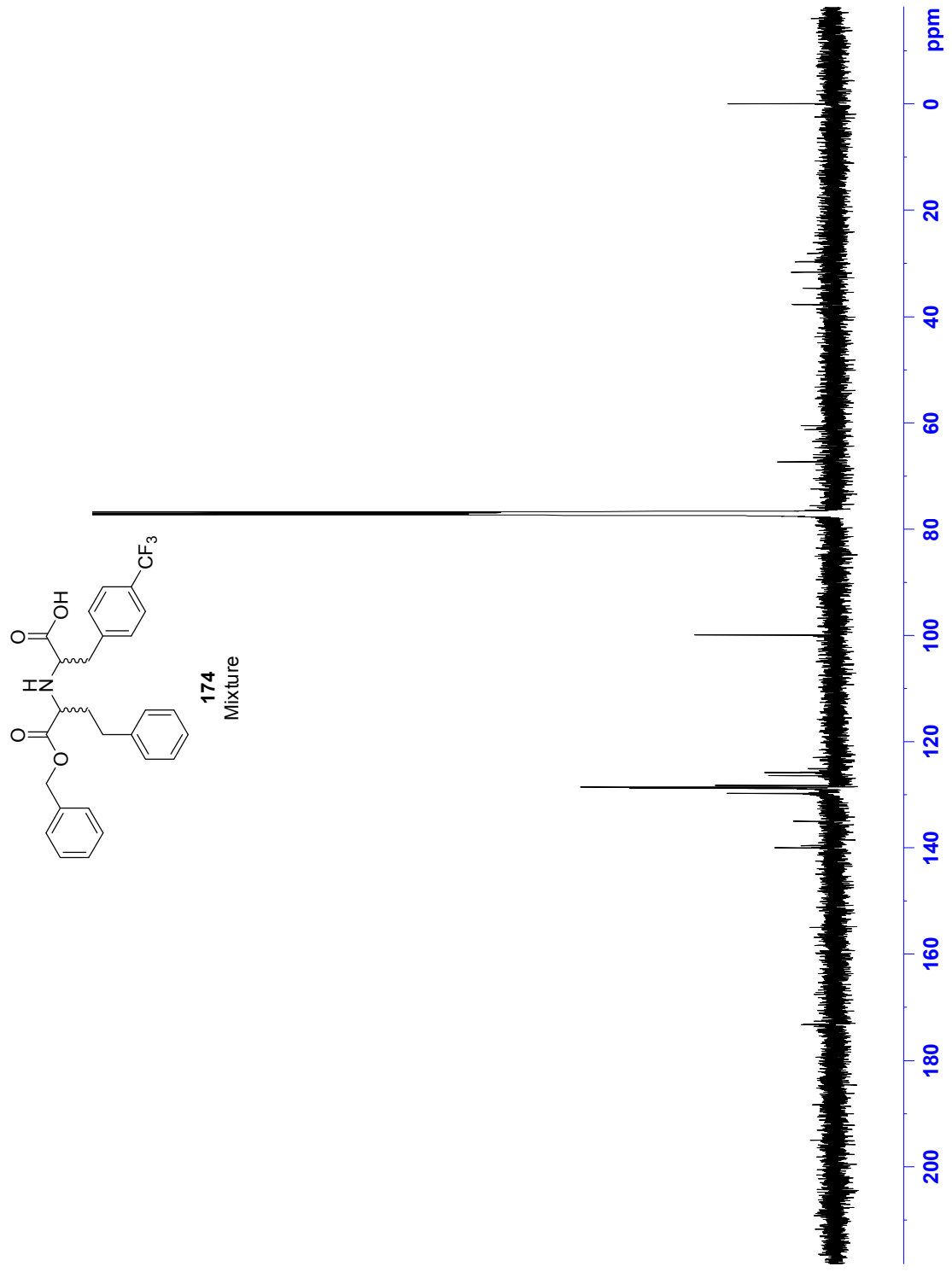
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
2.41
2.04
2.18
1.29
1.16
2.00
1.73
1.10
1.87
3.99
3.16
1.96


CF3
OH
O
H
N
O
O
174
Mixture
ppm
0
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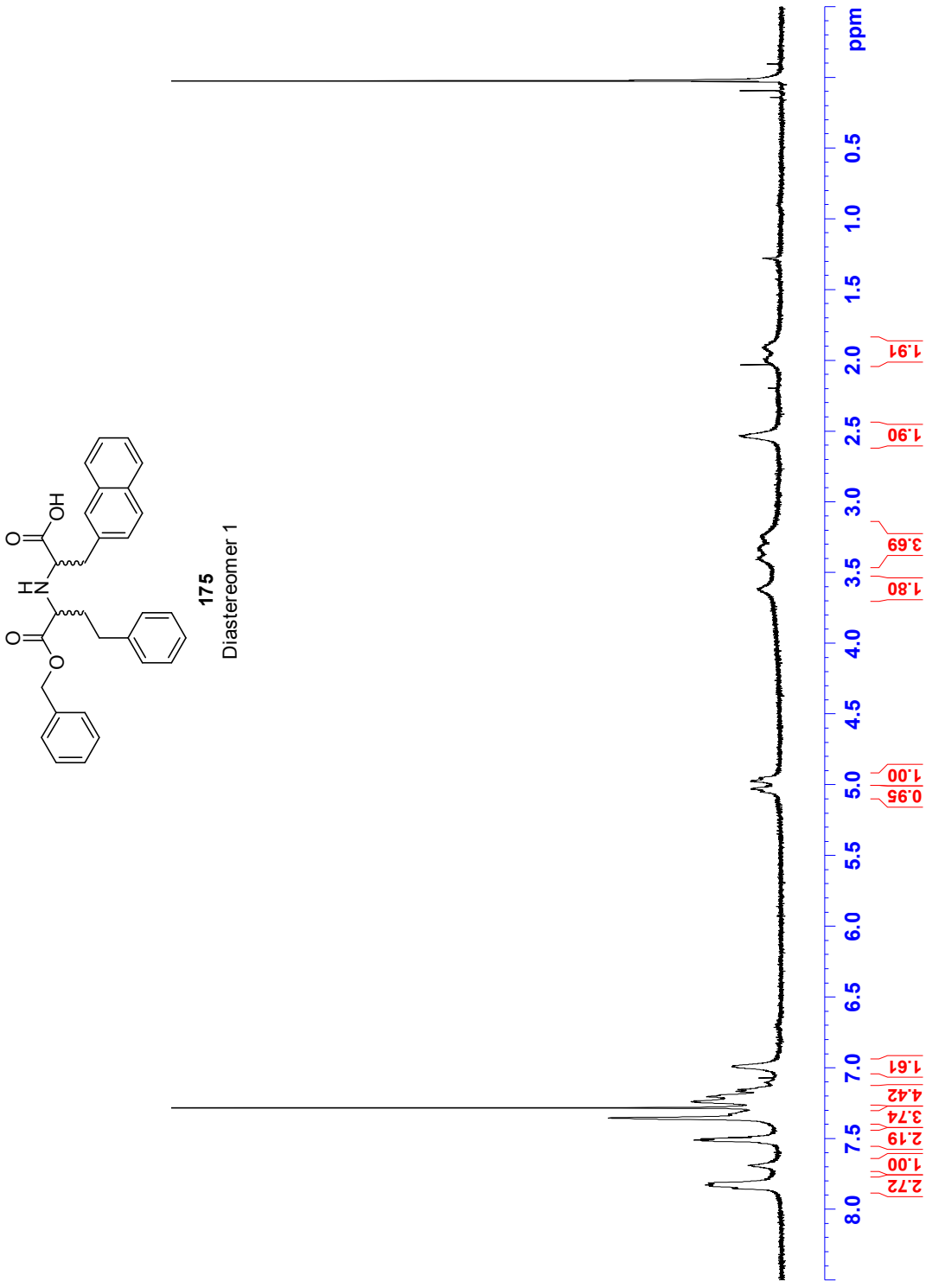
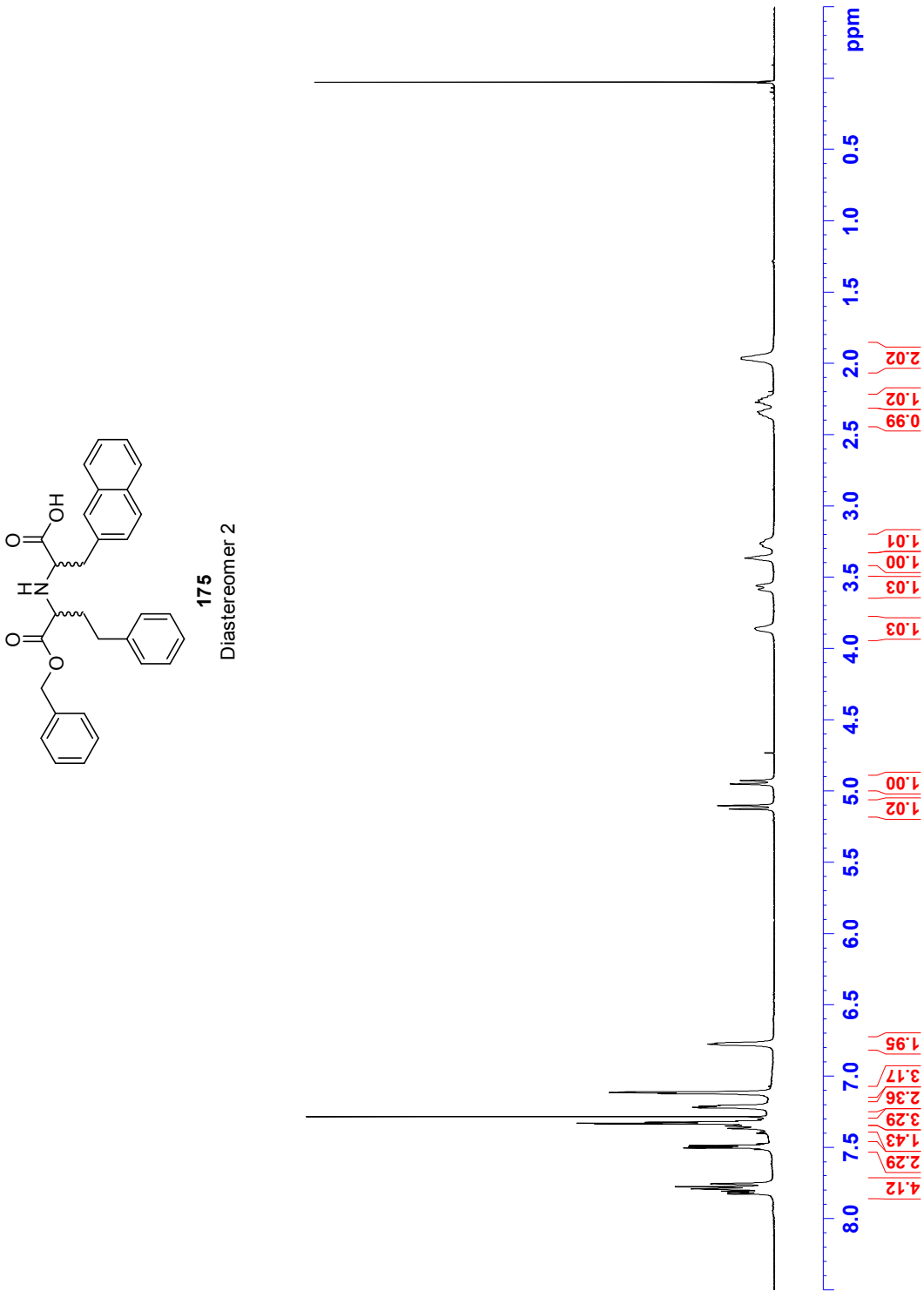
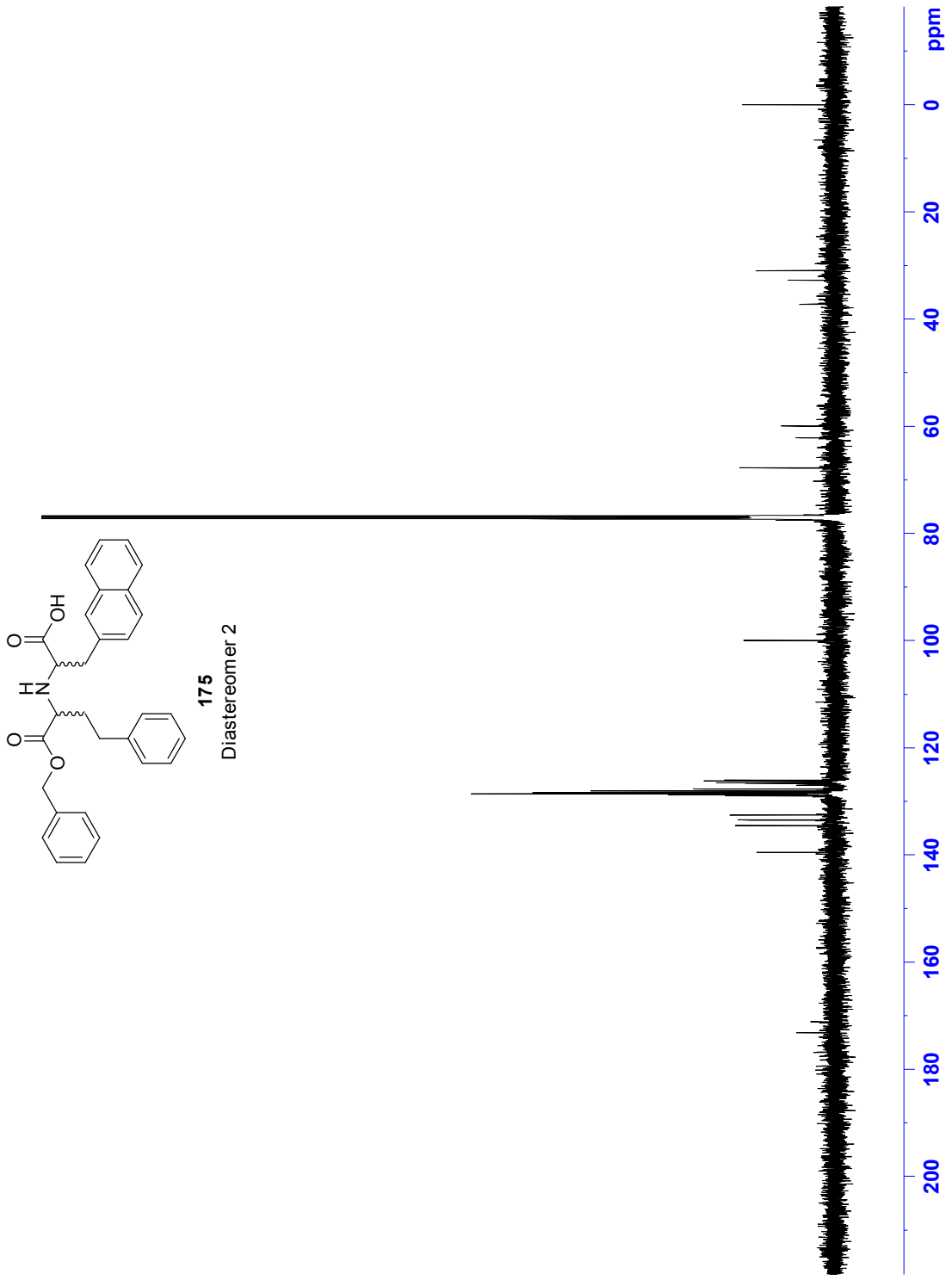
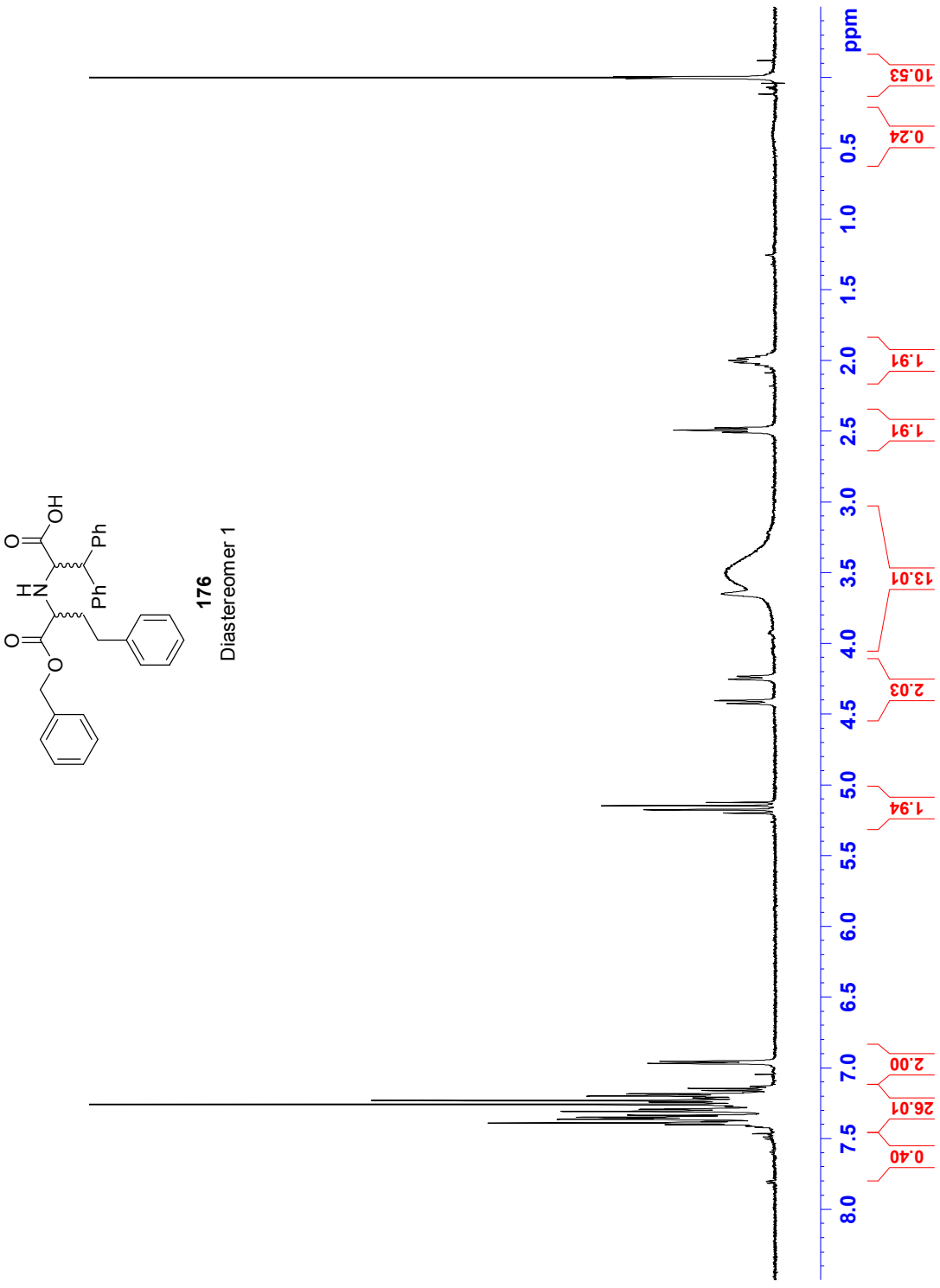
60
80
100
120
140
160
180
200

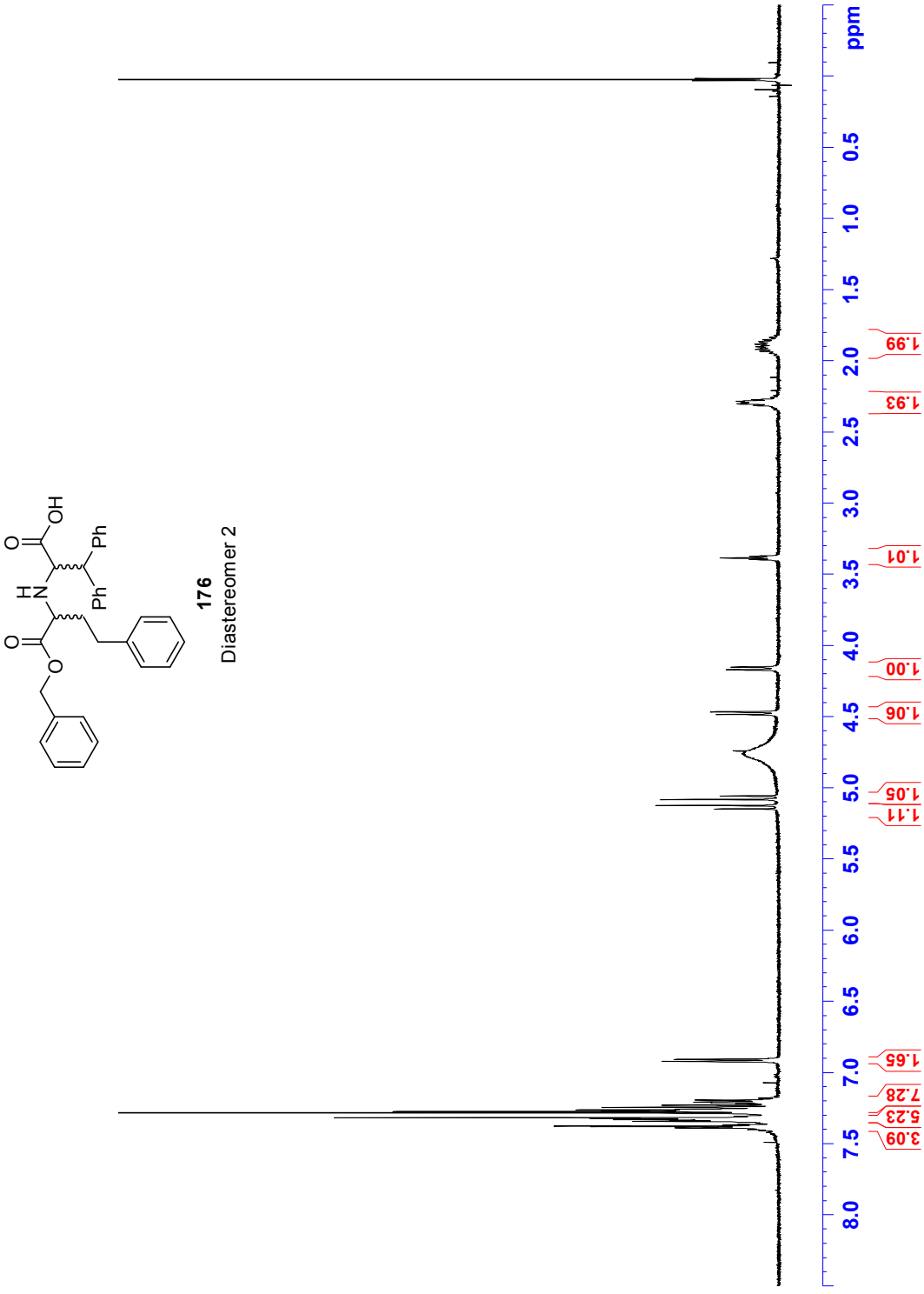

175
Diastereomer 1
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.91
1.90
3.69
1.80
0.95
1.00
1.61
4.42
3.74
2.19
1.00
2.72

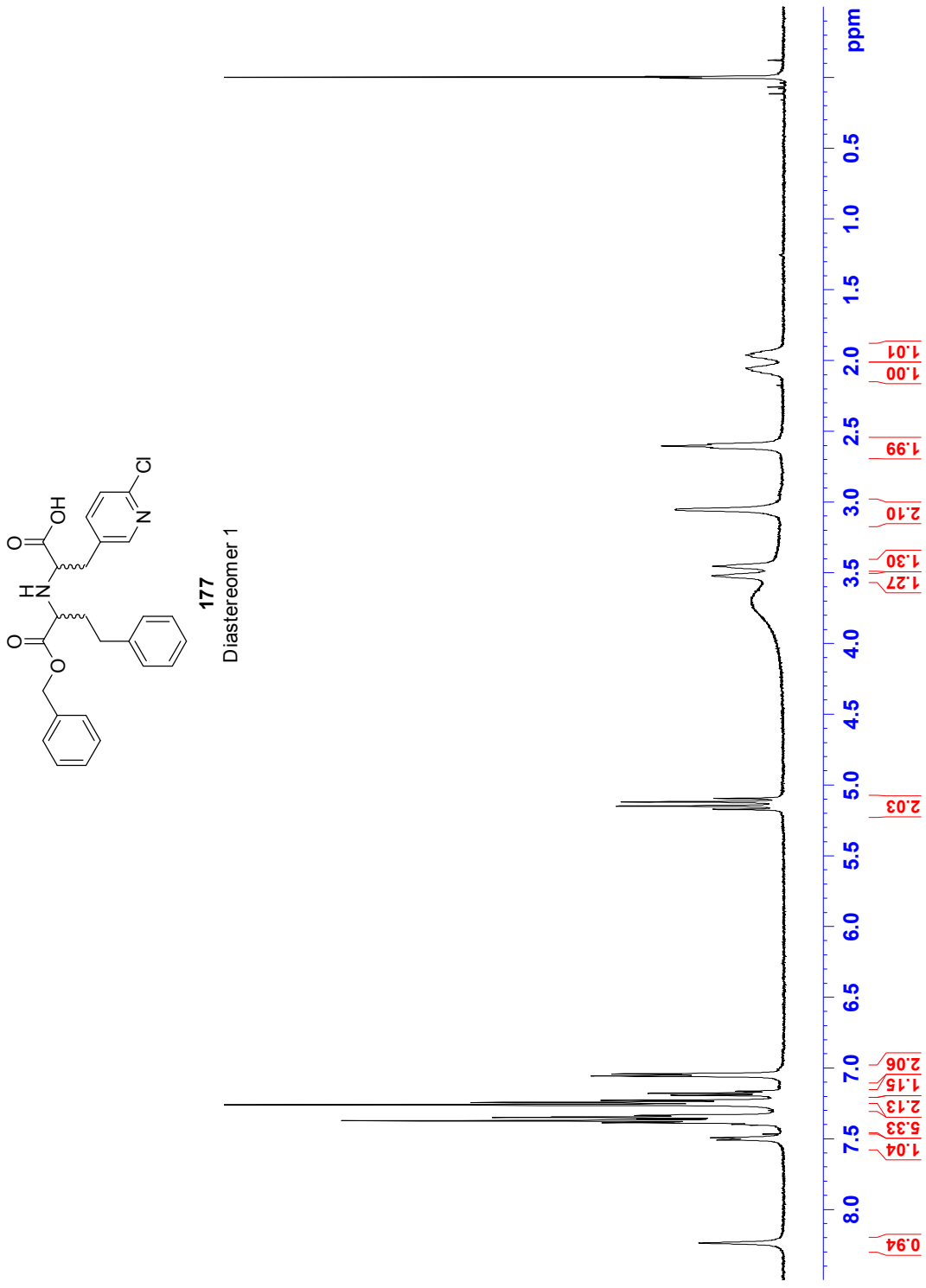


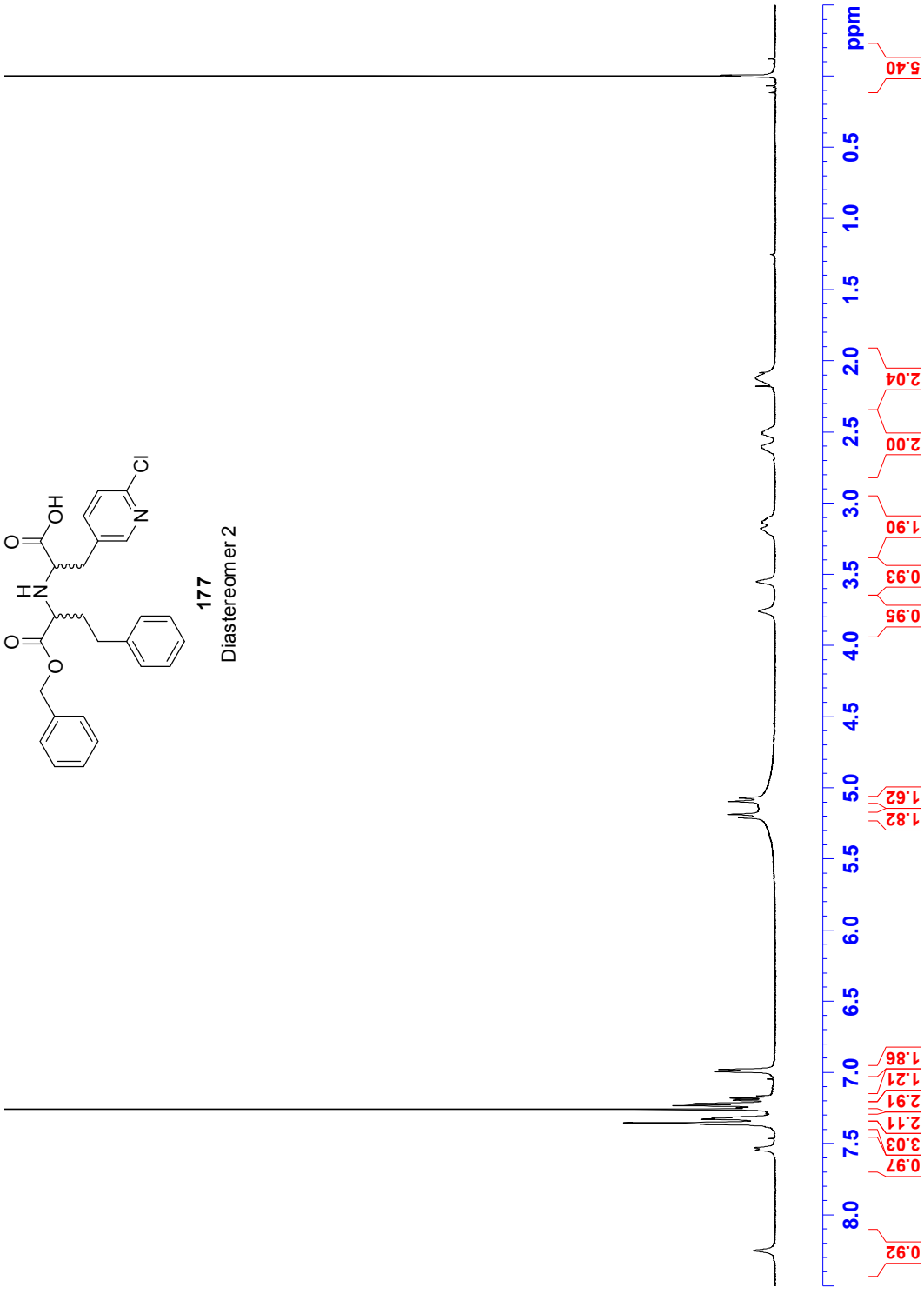
175
Diastereomer 2

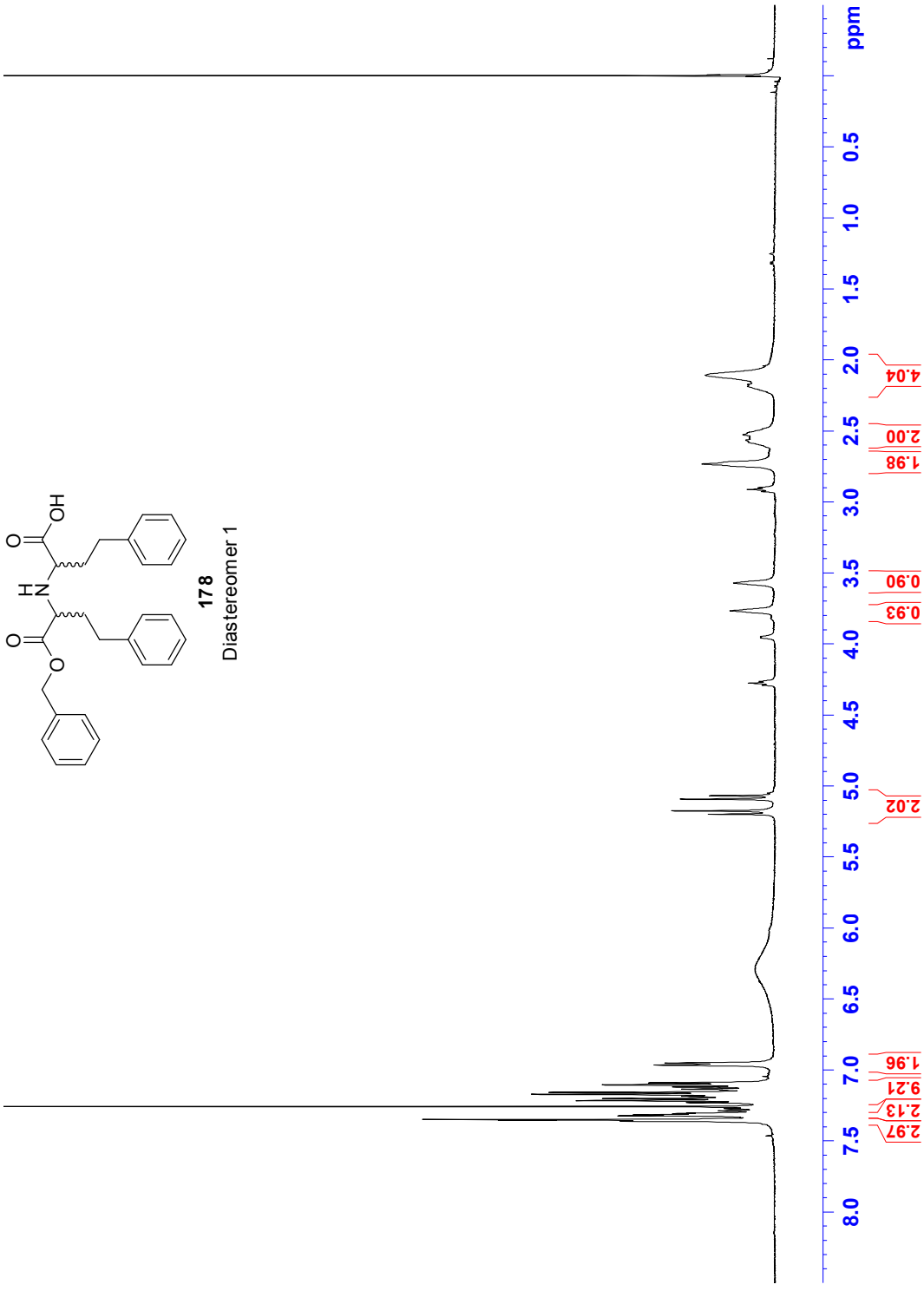

OH
O
H
N
O
O
175
Diastereomer 2
ppm
0
20
40
60
80
100
120
140
160
180
200

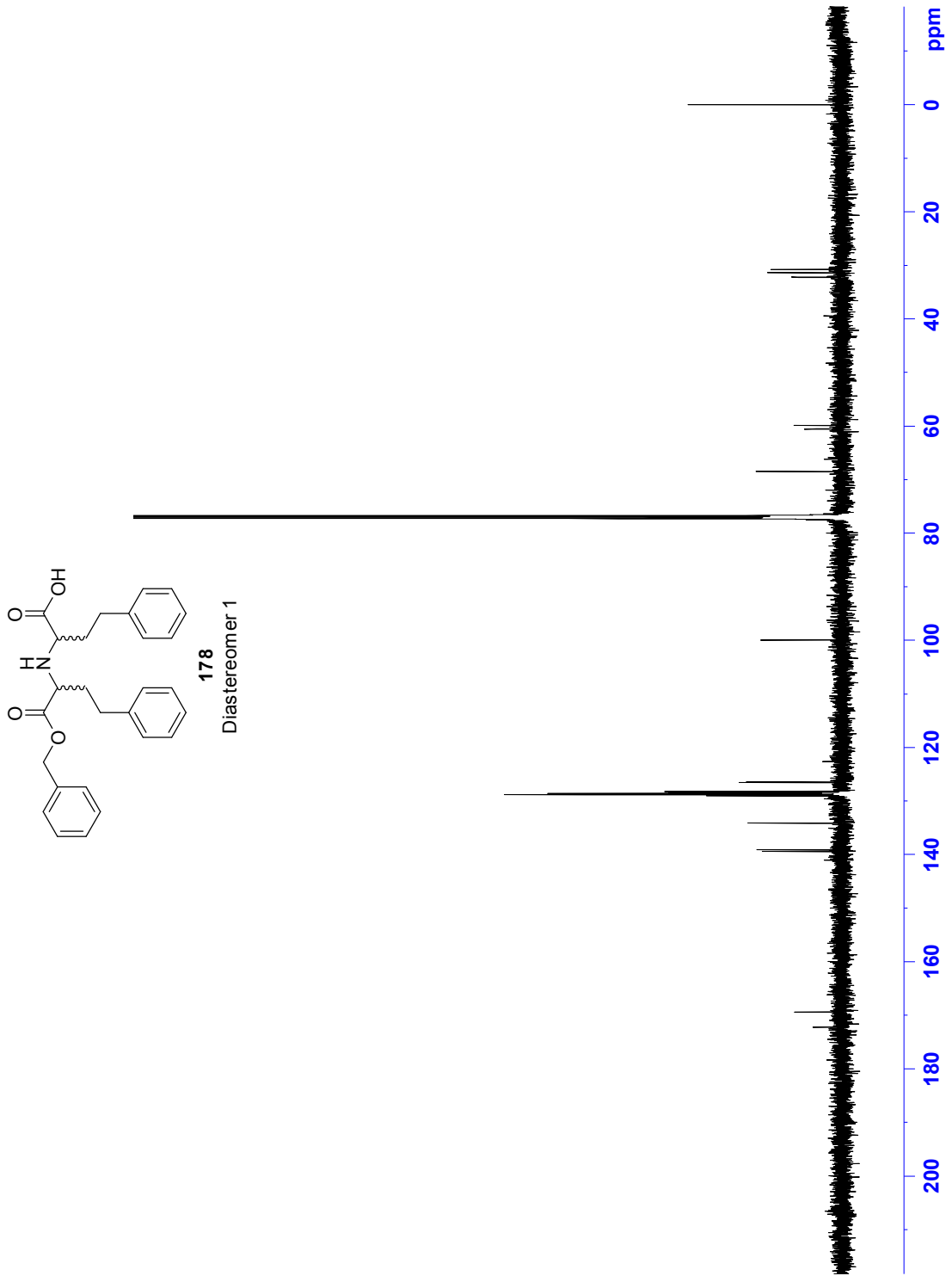

176
Diastereomer 1
ppm
10.53
0.24
1.91
1.91
13.01
2.03
1.94
2.00
26.01
0.40

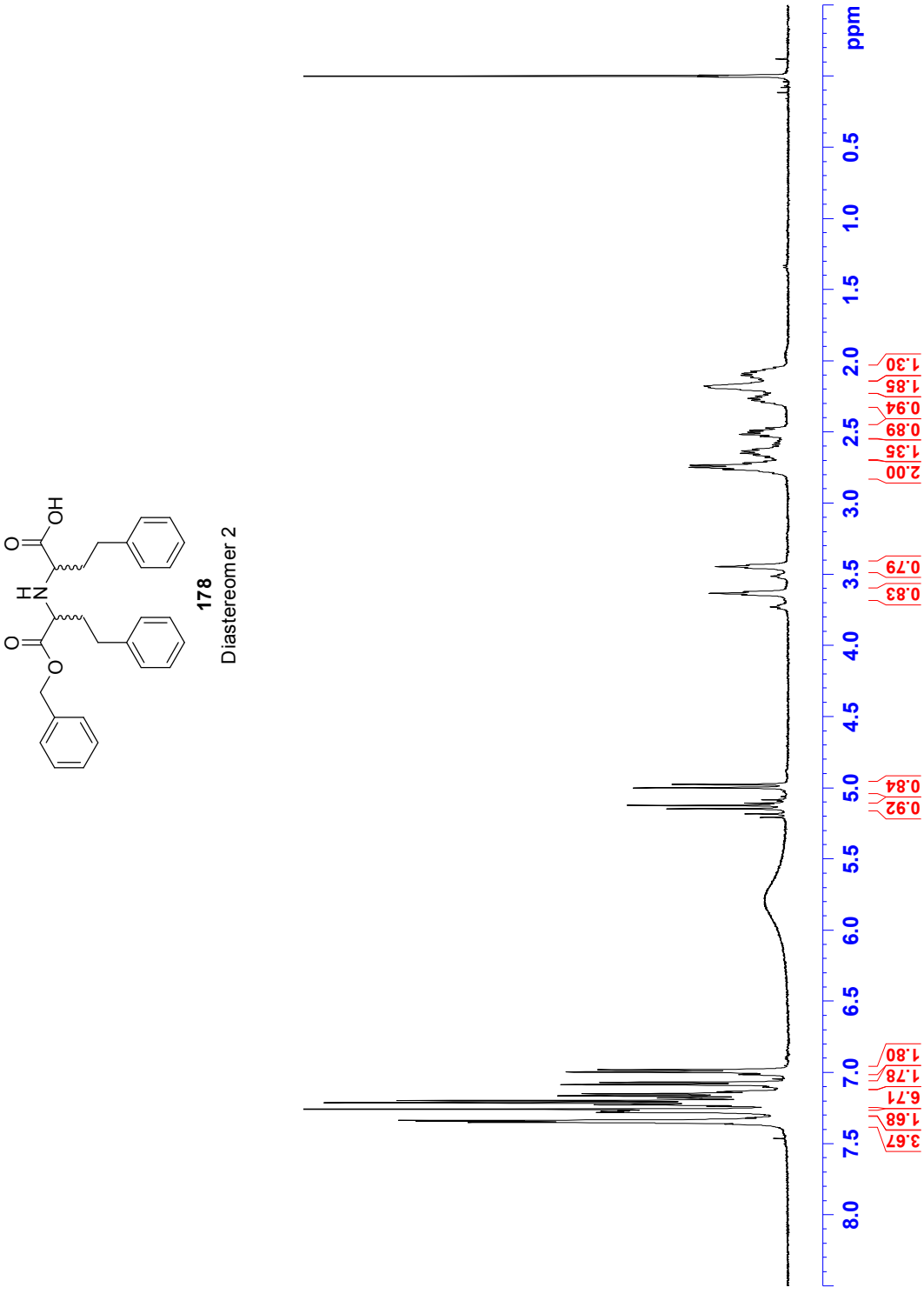

176
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.99
1.93
1.01
1.00
1.06
1.05
1.11
1.65
7.28
5.23
3.09

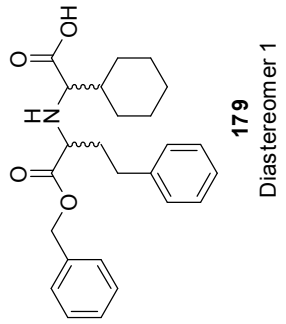

177
Diastereomer 1
ppm
0.94
1.04
5.33
2.13
1.15
2.06
2.03
1.27
1.30
2.10
1.99
1.00
1.01


177
Diastereomer 2
ppm
5.40
2.04
2.00
1.90
0.93
0.95
1.62
1.82
0.97
3.03
2.11
2.91
1.21
1.86
0.92


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
4.04
2.00
1.98
0.90
0.93
2.02
1.96
9.21
2.13
2.97
OH
O
HN
O
O
178
Diastereomer 1

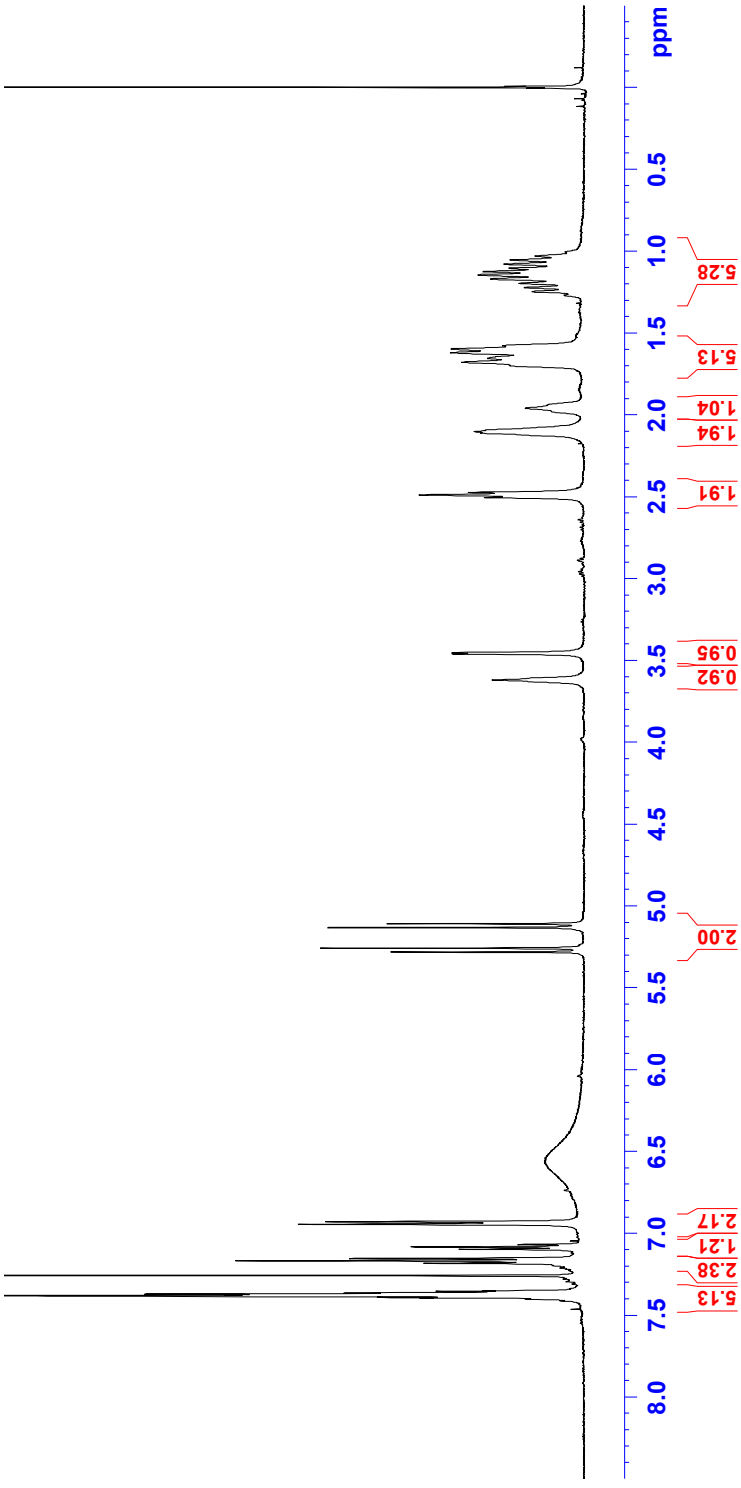

178
Diastereomer 1
ppm
0
20
40
60
80
100
120
140
160
180
200

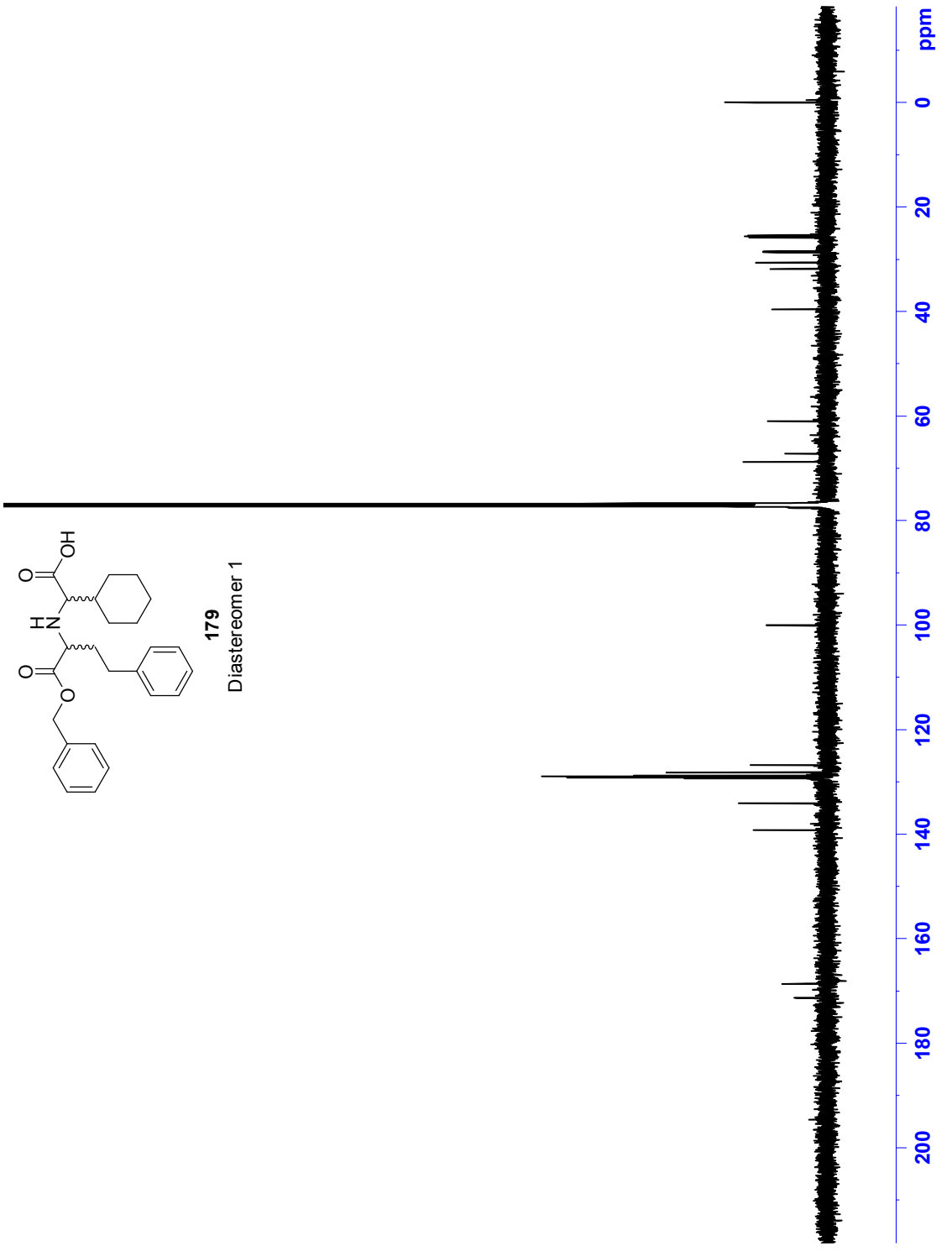

178
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.30
1.85
0.94
0.89
1.35
2.00
0.79
0.83
0.84
0.92
1.80
1.78
6.71
1.68
3.67

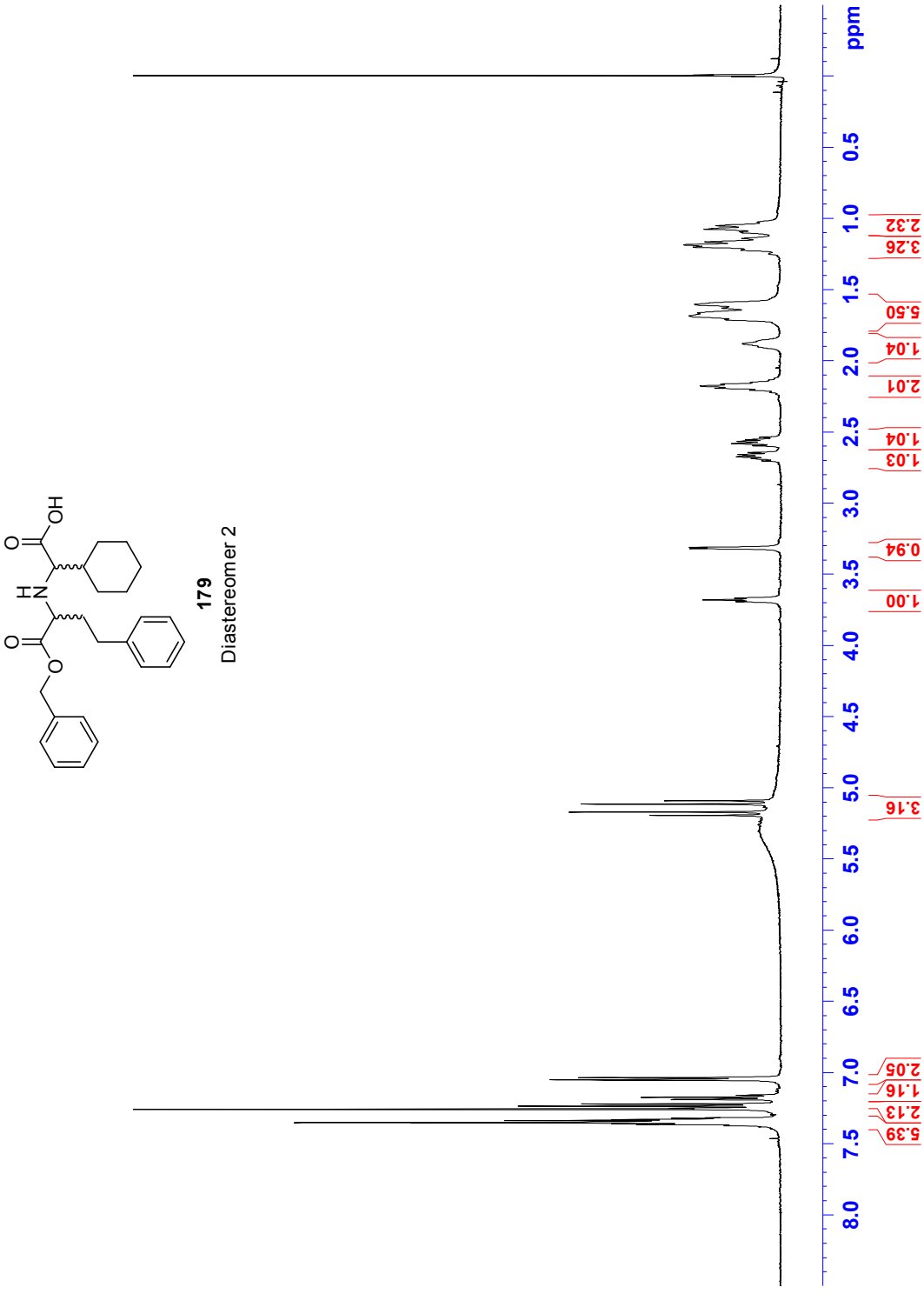


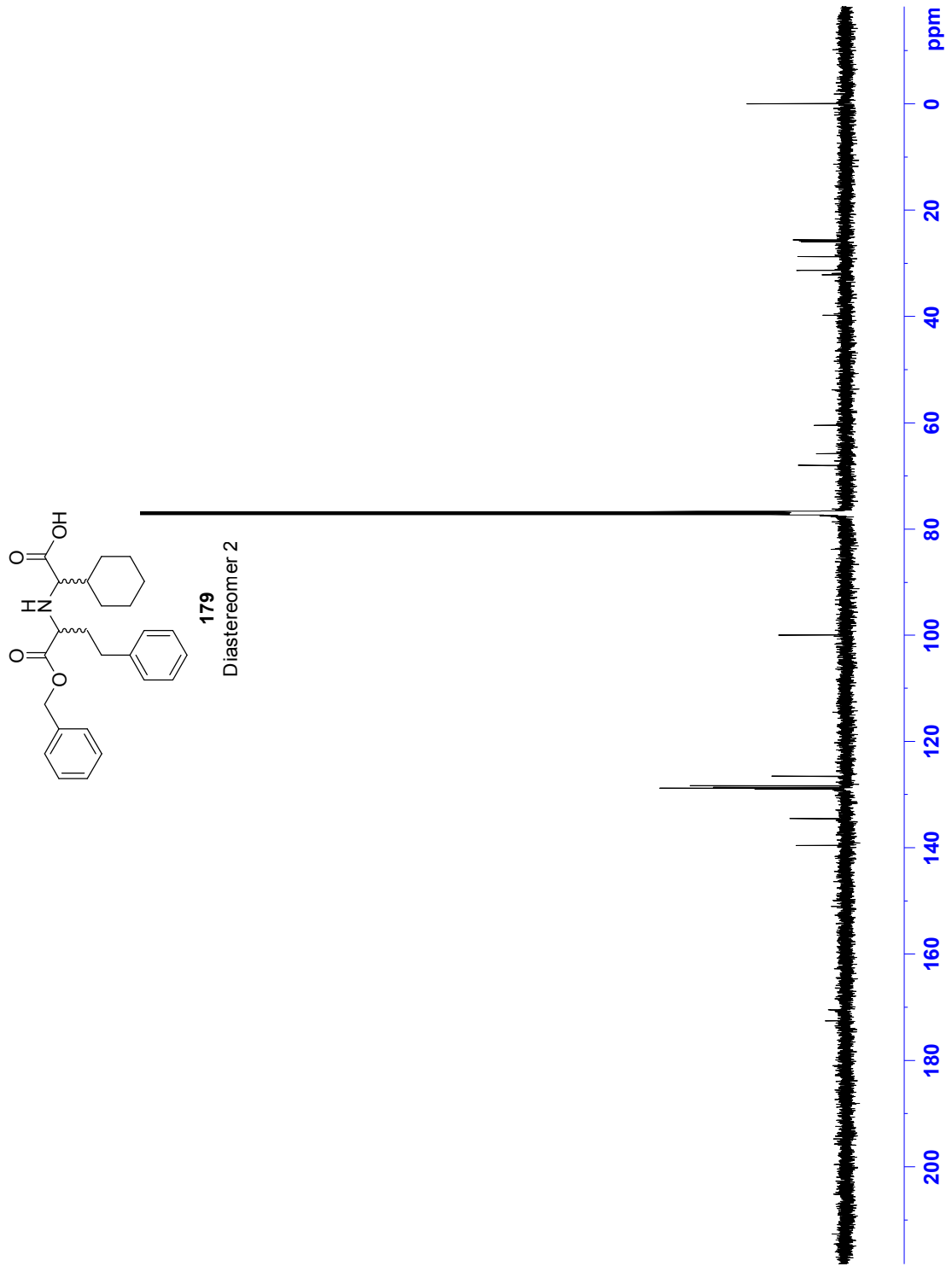
179

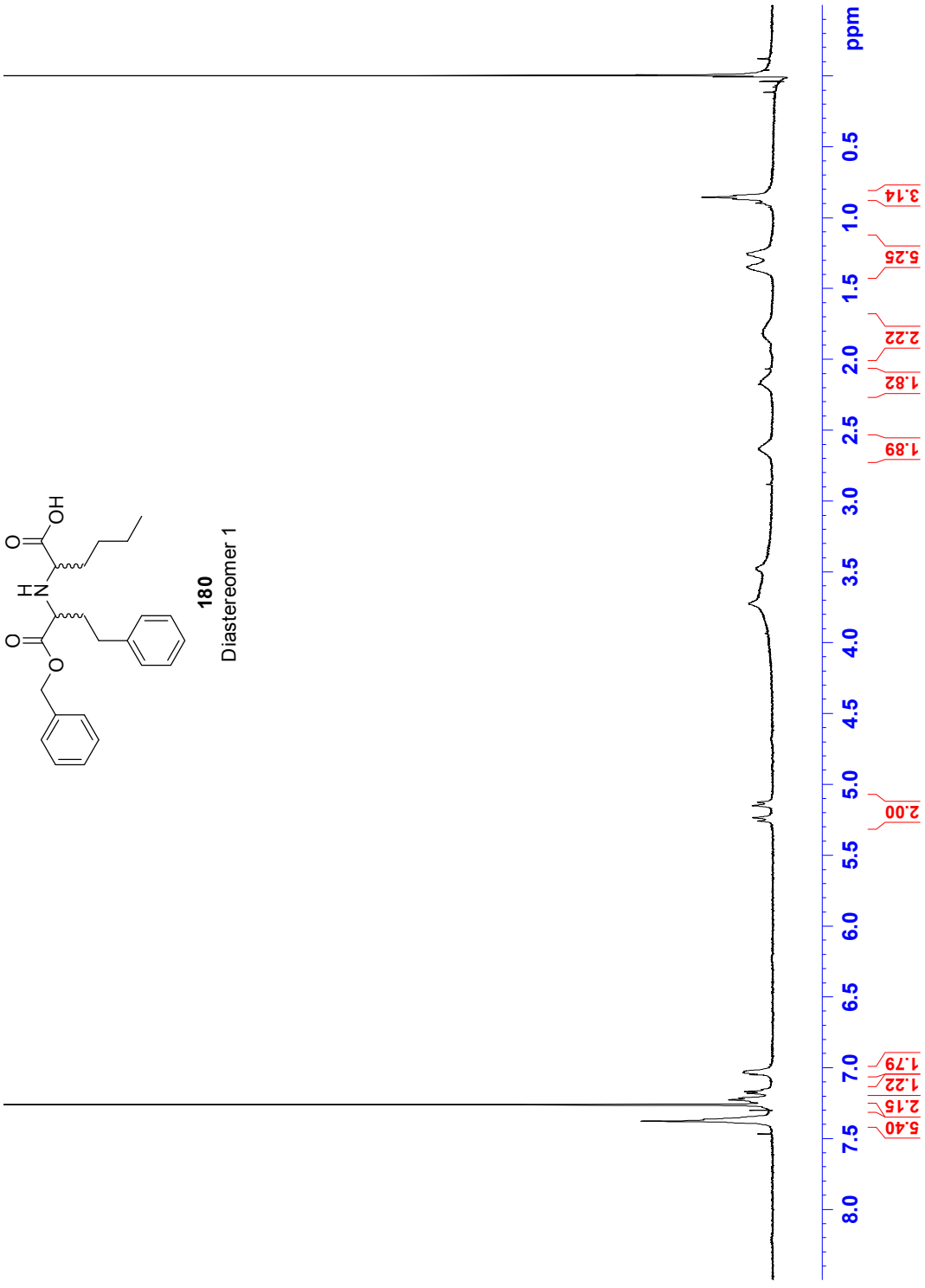
Diastereomer 1

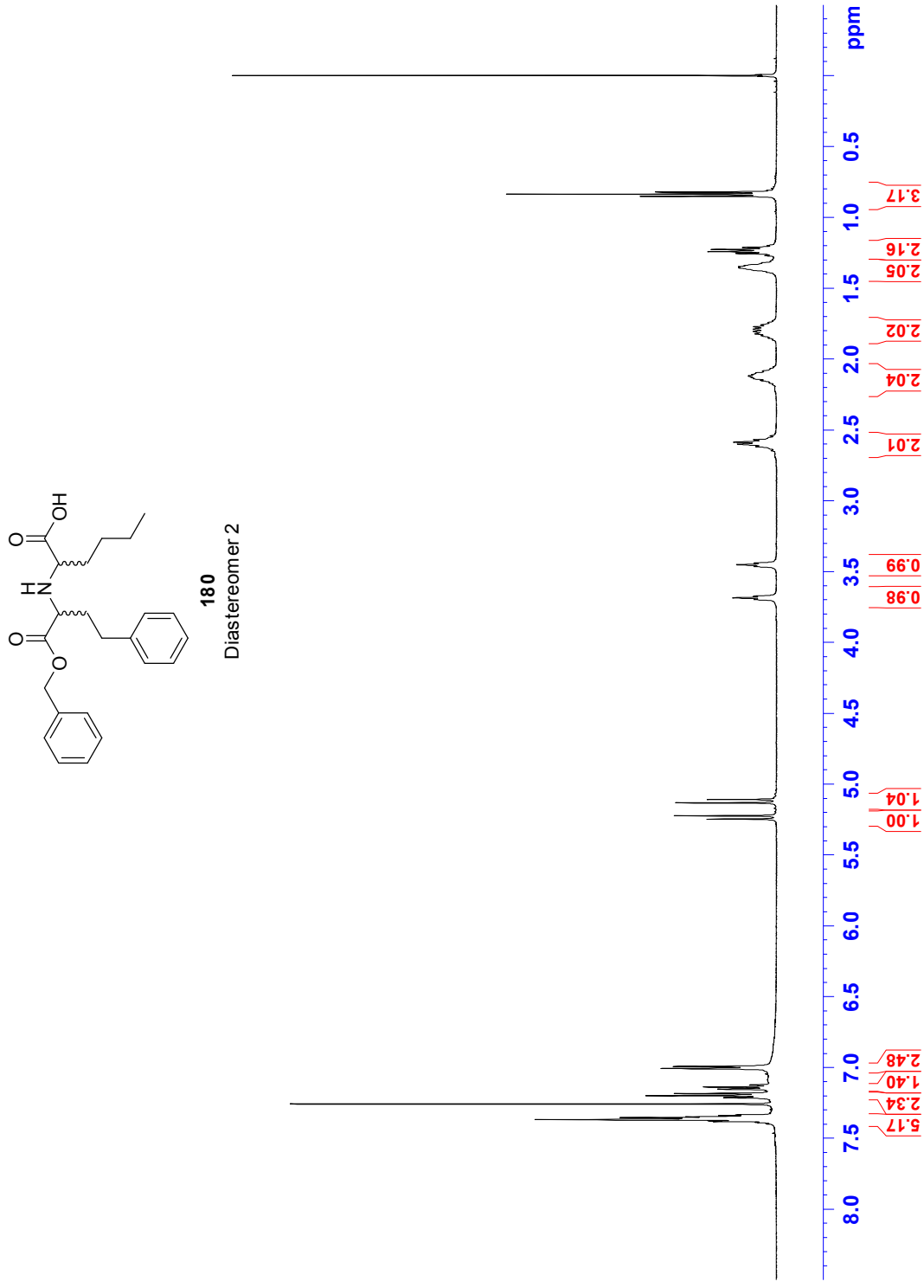


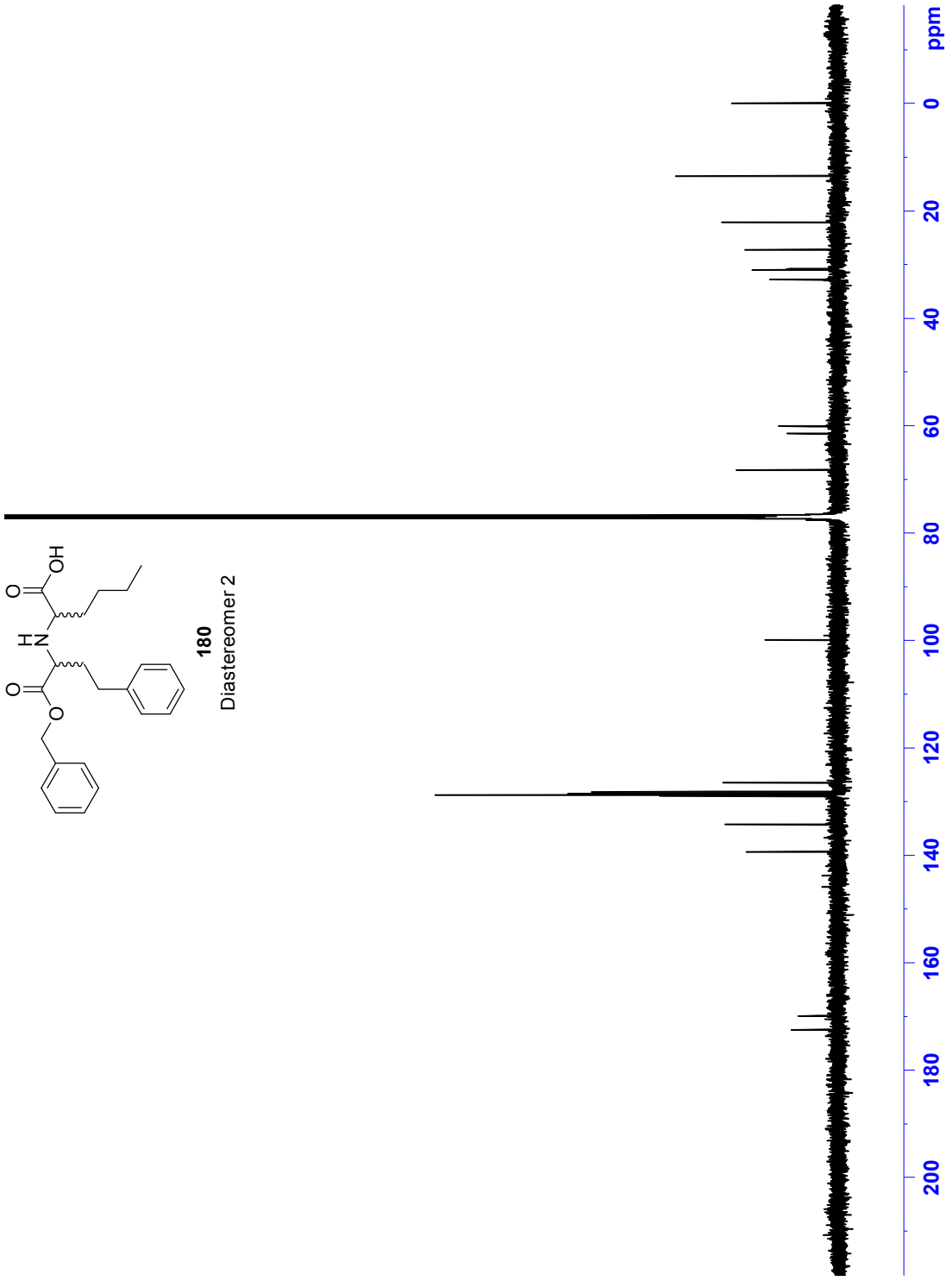

ppm
0
20
40
60
80
100
120
140
160
180
200
OH
O
HN
O
O
179
Diastereomer 1

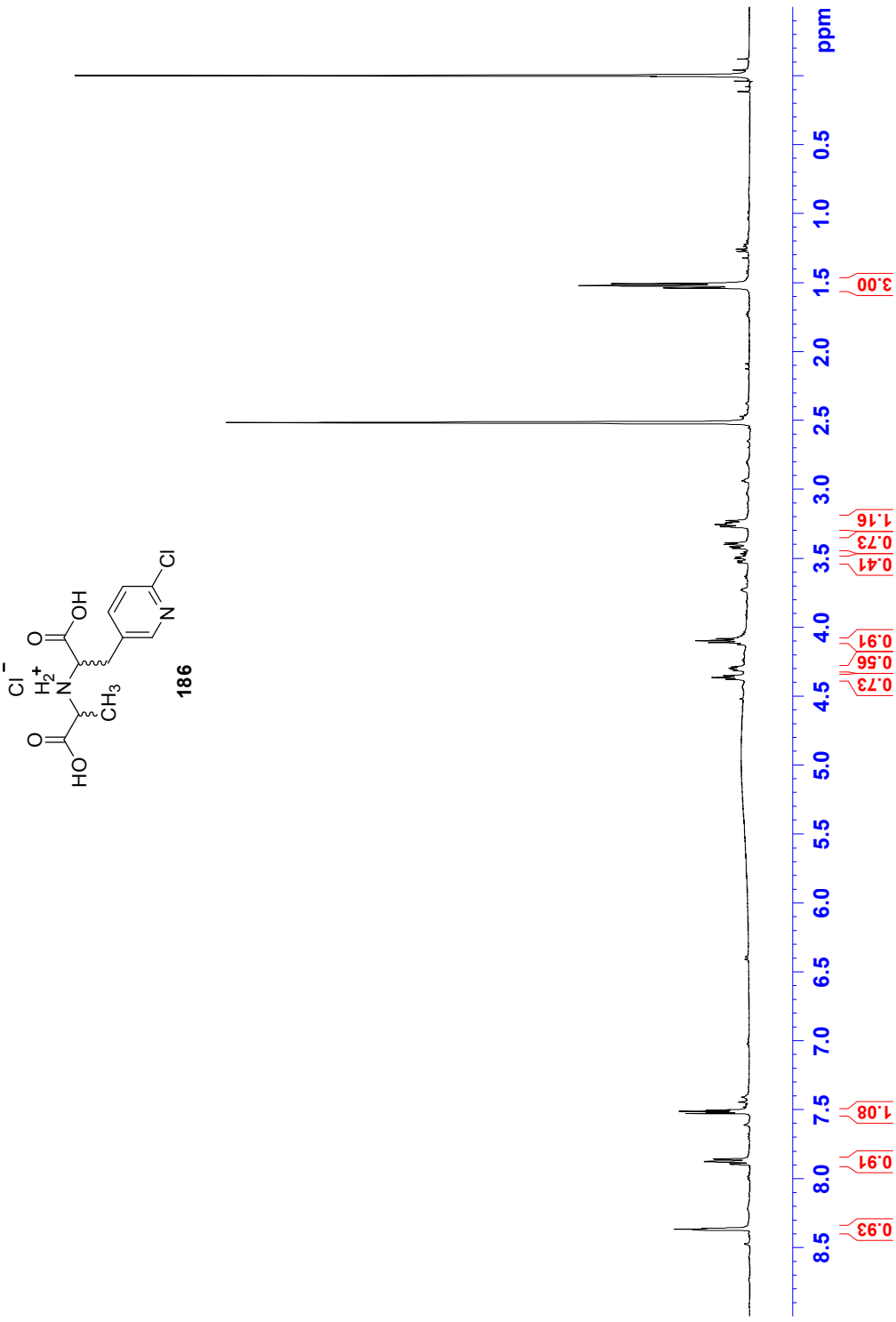

179
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
2.32
3.26
5.50
1.04
2.01
1.04
1.03
0.94
1.00
3.16
2.05
1.16
2.13
5.39

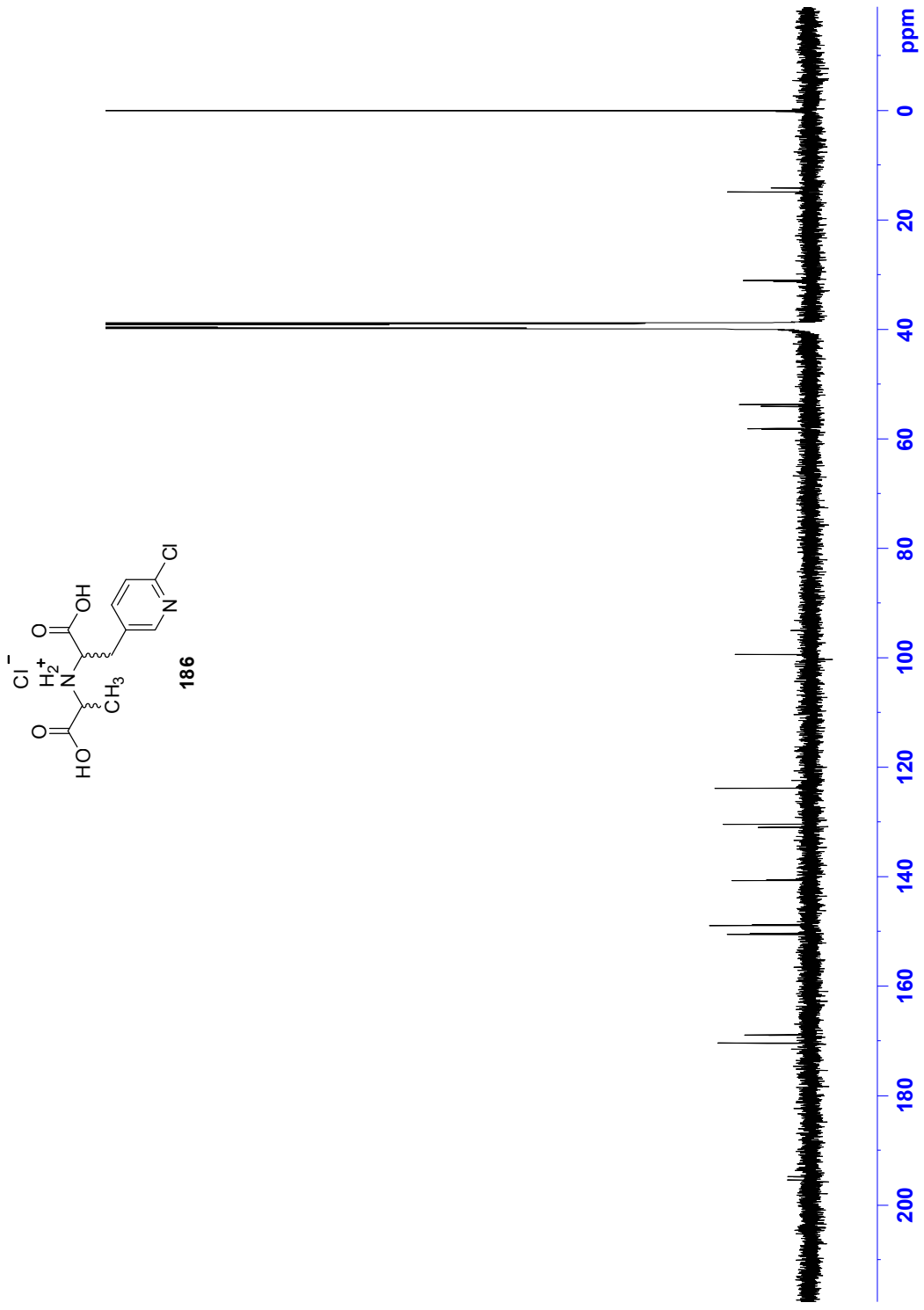

179
Diastereomer 2
ppm
0
20
40
60
80
100
120
140
160
180
200


180
Diastereomer 1
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.14
5.25
2.22
1.82
1.89
2.00
1.79
1.22
2.15
5.40

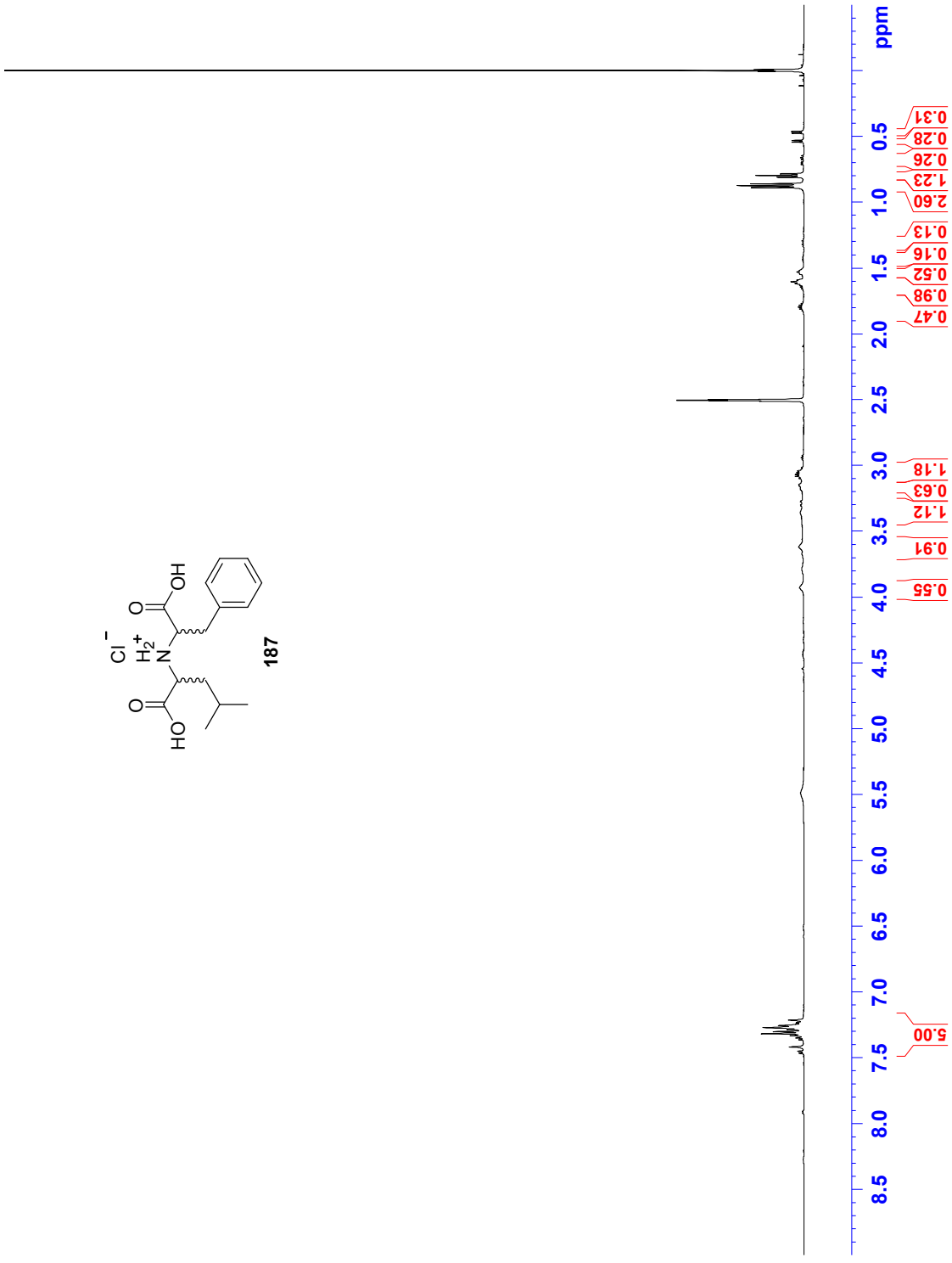

180
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.17
2.16
2.05
2.02
2.04
2.01
0.99
0.98
1.04
1.00
2.48
1.40
2.34
5.17

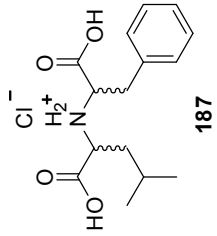

ppm
0
20
40
60
80
100
120
140
160
180
200
180
Diastereomer 2
OH
O
N
H
O
O


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
3.00
1.16
0.73
0.41
0.91
0.56
0.73
1.08
0.91
0.93
Cl
OH
O
N
H_2^+
Cl^-
CH_3
O
HO
186

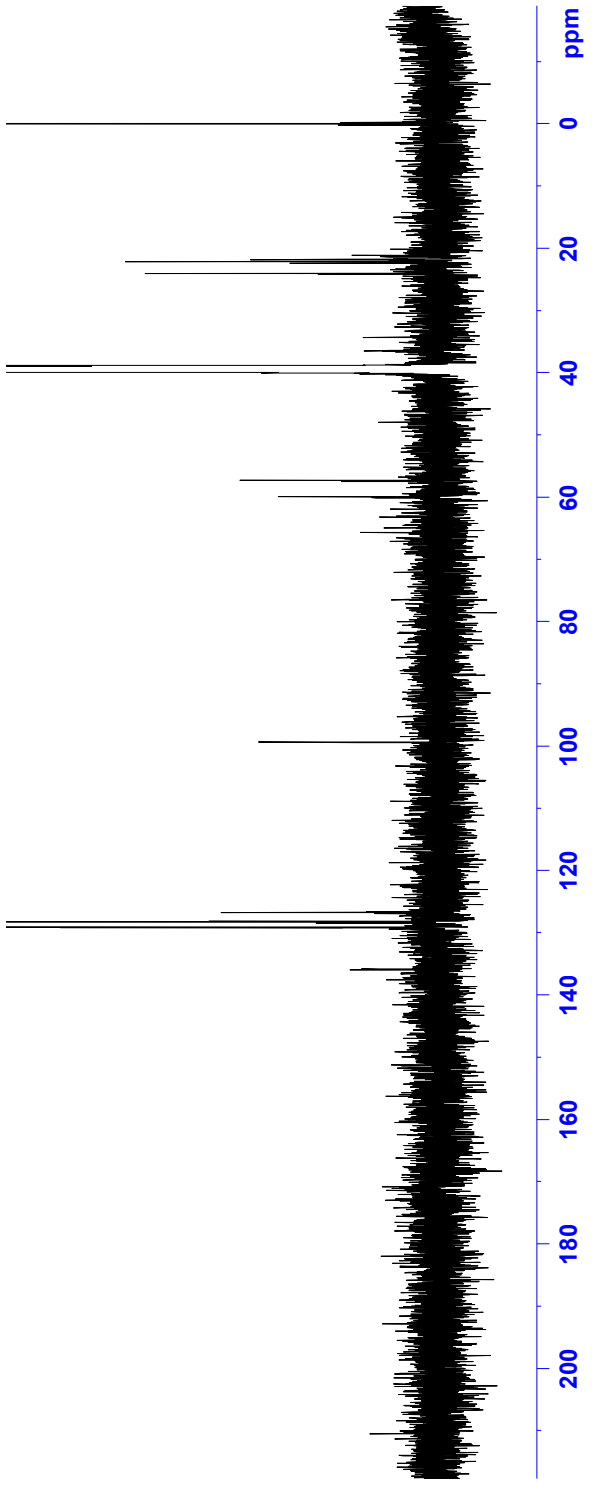


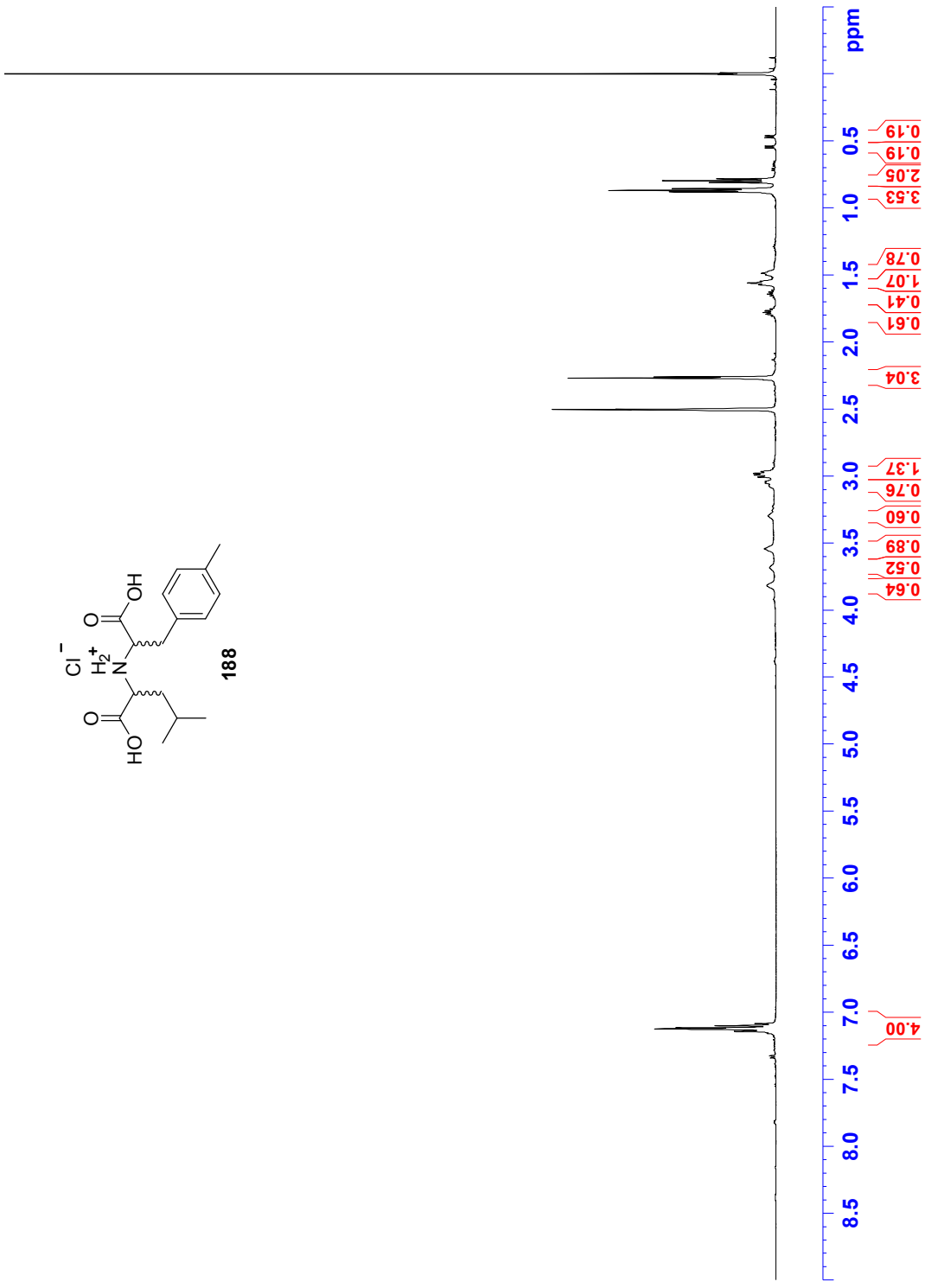
186

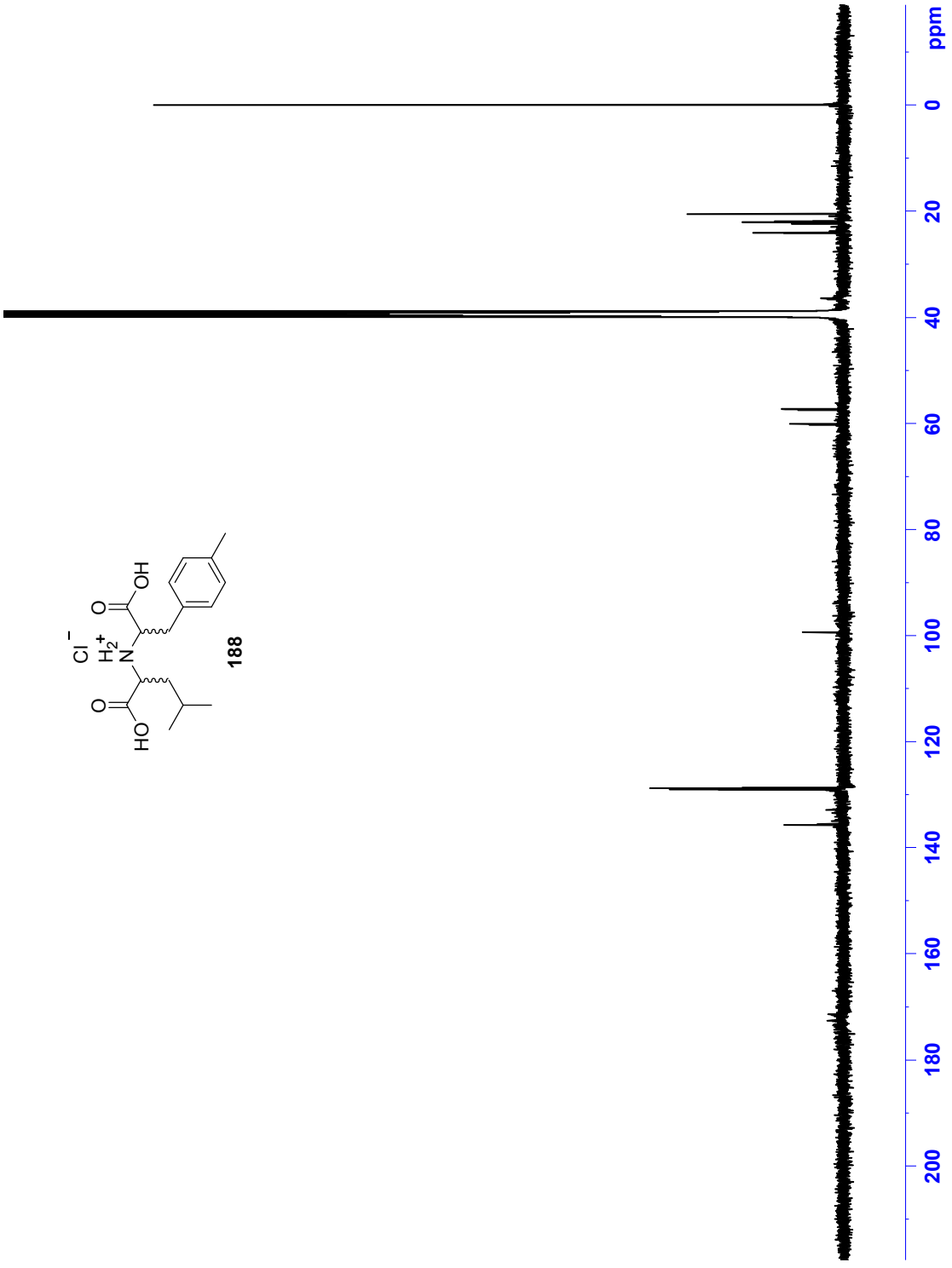

187
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
0.31
0.28
0.26
1.23
2.60
0.13
0.16
0.52
0.98
0.47
1.18
0.63
1.12
0.91
0.55
5.00

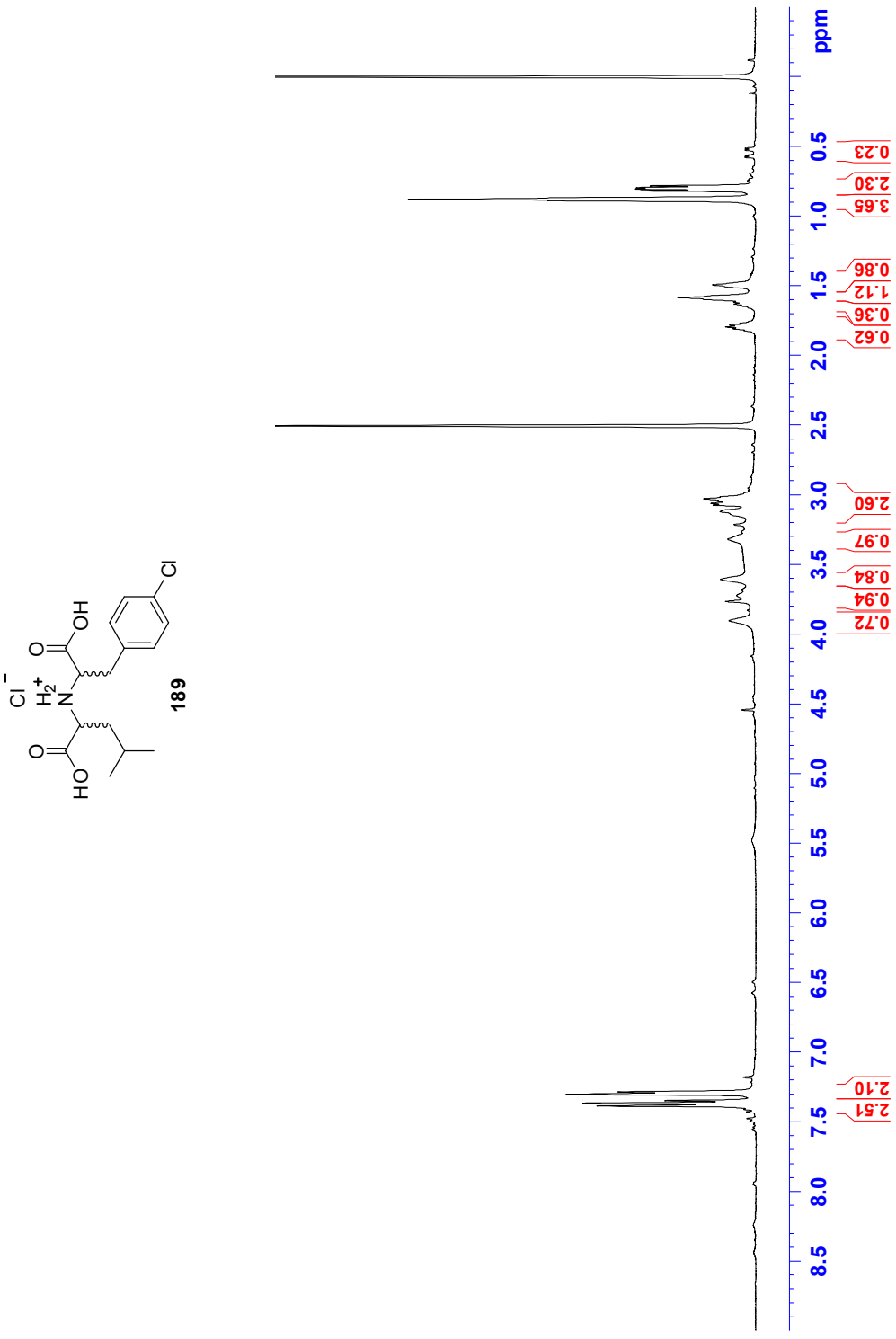


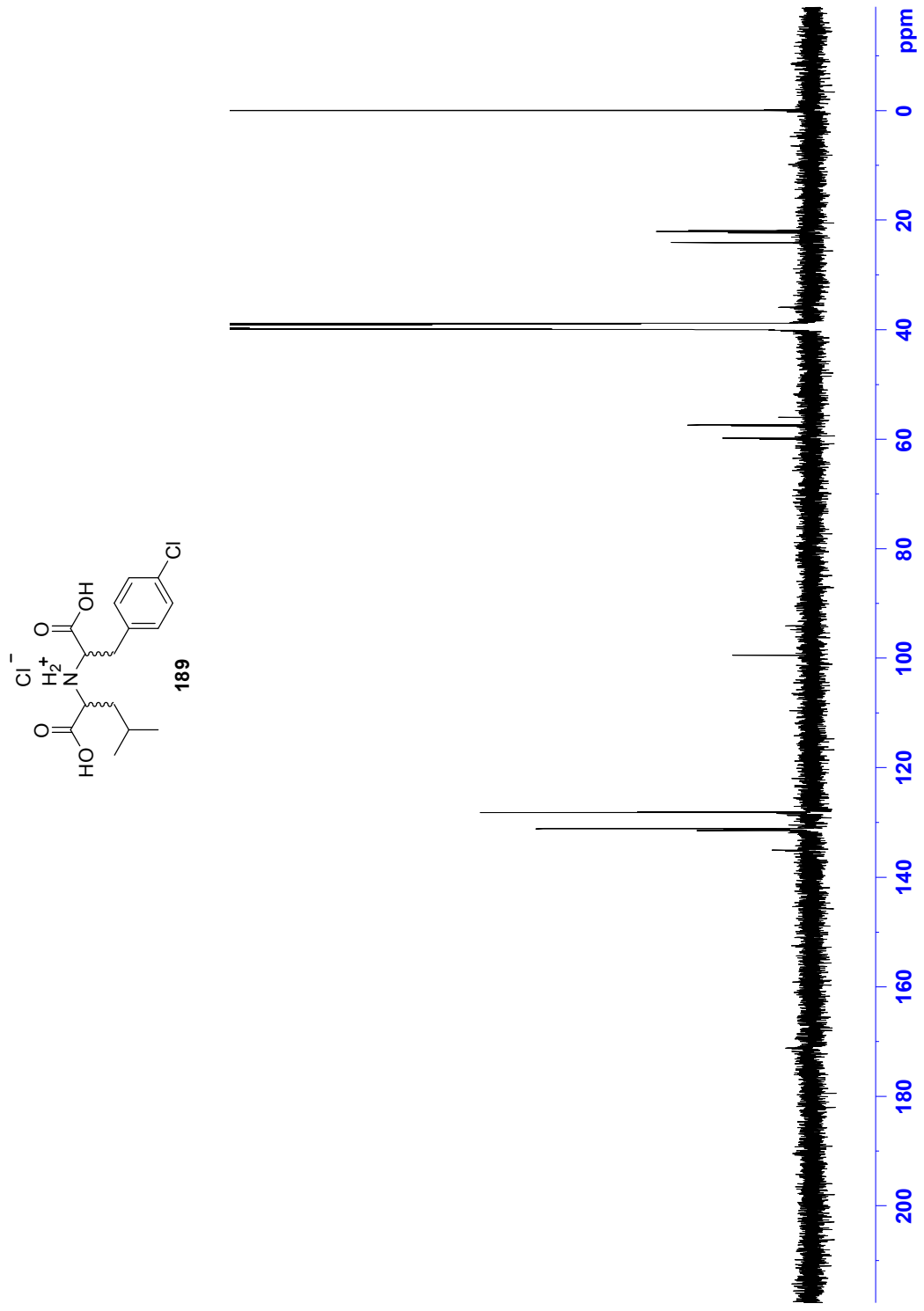
187

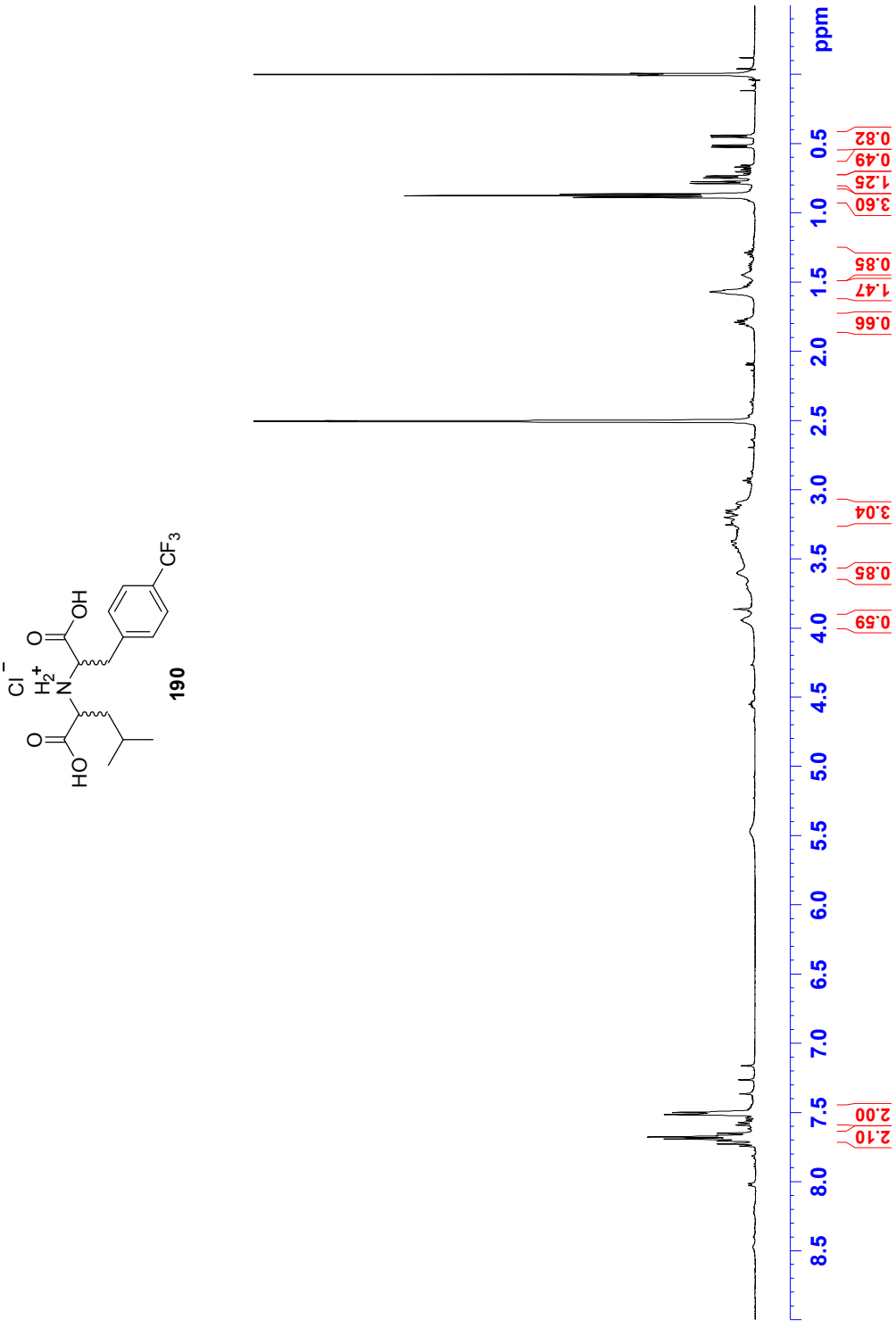


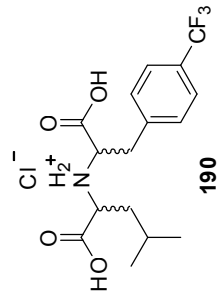

188
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
0.19
0.19
2.05
3.53
0.78
1.07
0.41
0.61
3.04
1.37
0.76
0.60
0.89
0.52
0.64
4.00


ppm
0
20
40
60
80
100
120
140
160
180
200
188

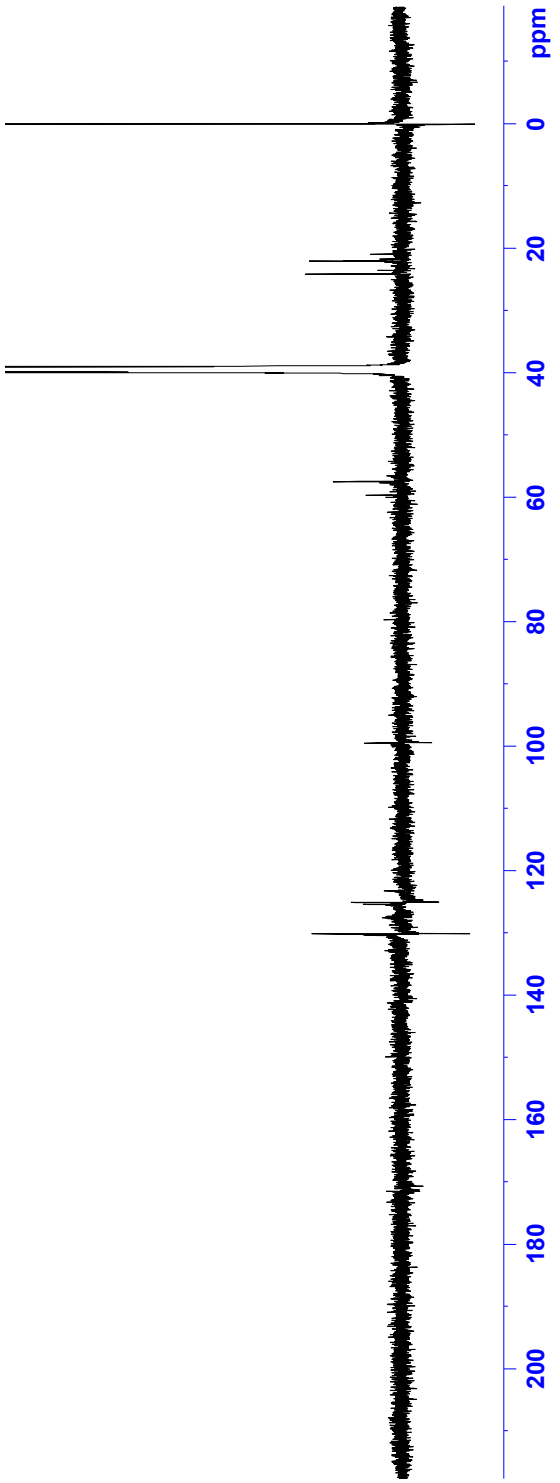

Cl
OH
O
H_2^+
N
Cl^-
O
HO
189
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.23
2.30
3.65
0.86
1.12
0.36
0.62
2.60
0.97
0.84
0.94
0.72
2.10
2.51

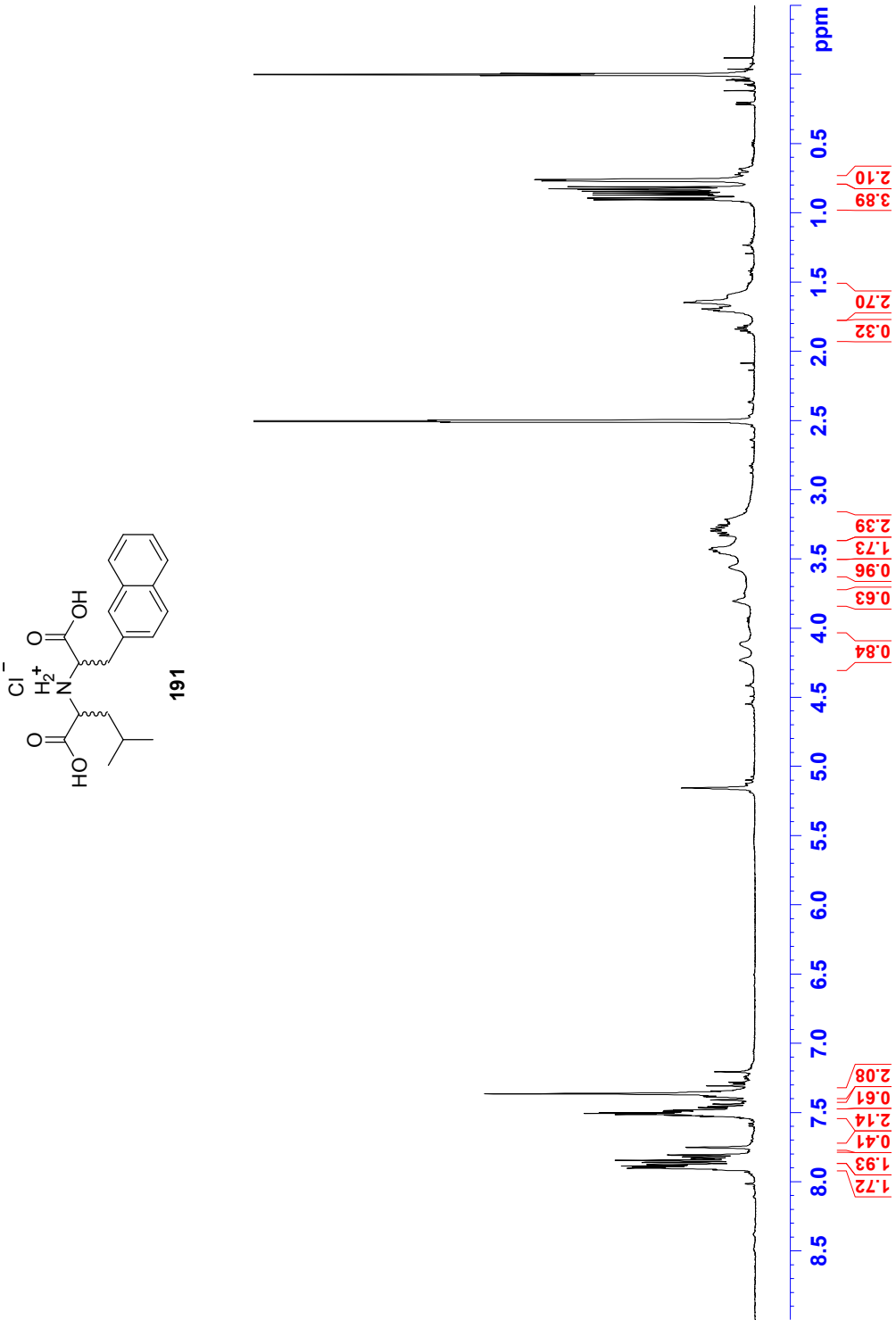

Cl
OH
O
Cl^- H_2^+N
O
HO
189
ppm
0
20
40
60
80
100
120
140
160
180
200

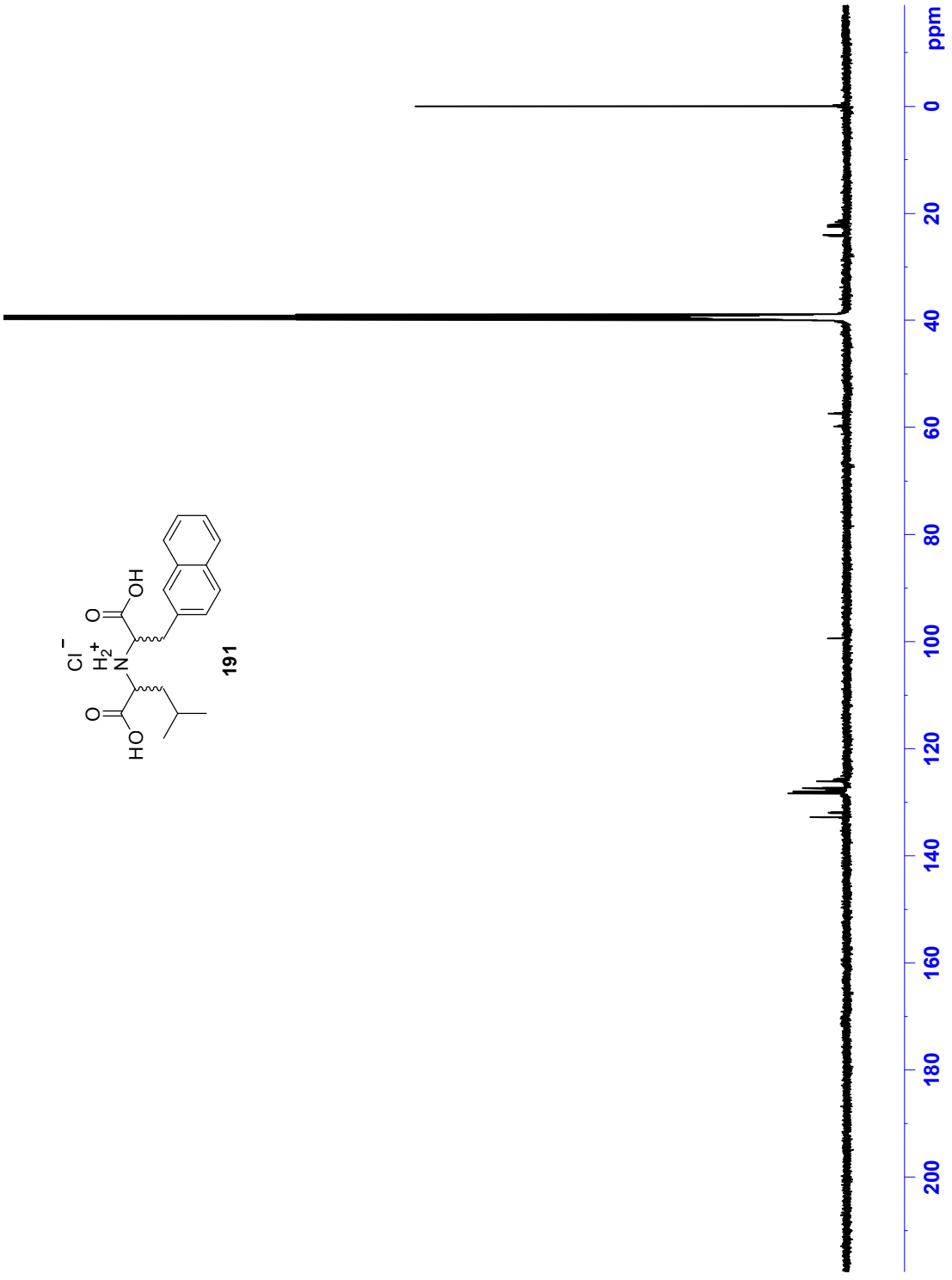

Cl⁻
H2+N
O
OH
O
HO
CF3
190
ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.10
2.00
0.59
0.85
3.04
0.66
1.47
0.85
3.60
1.25
0.49
0.82

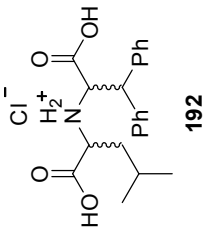


190

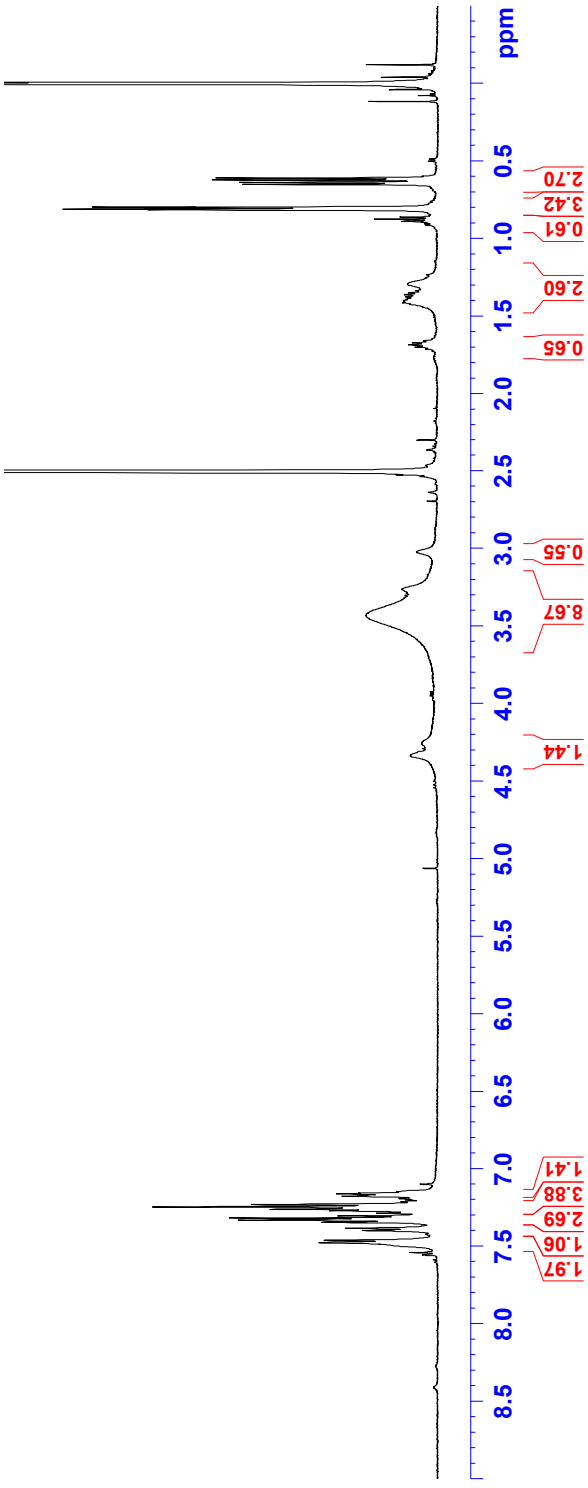


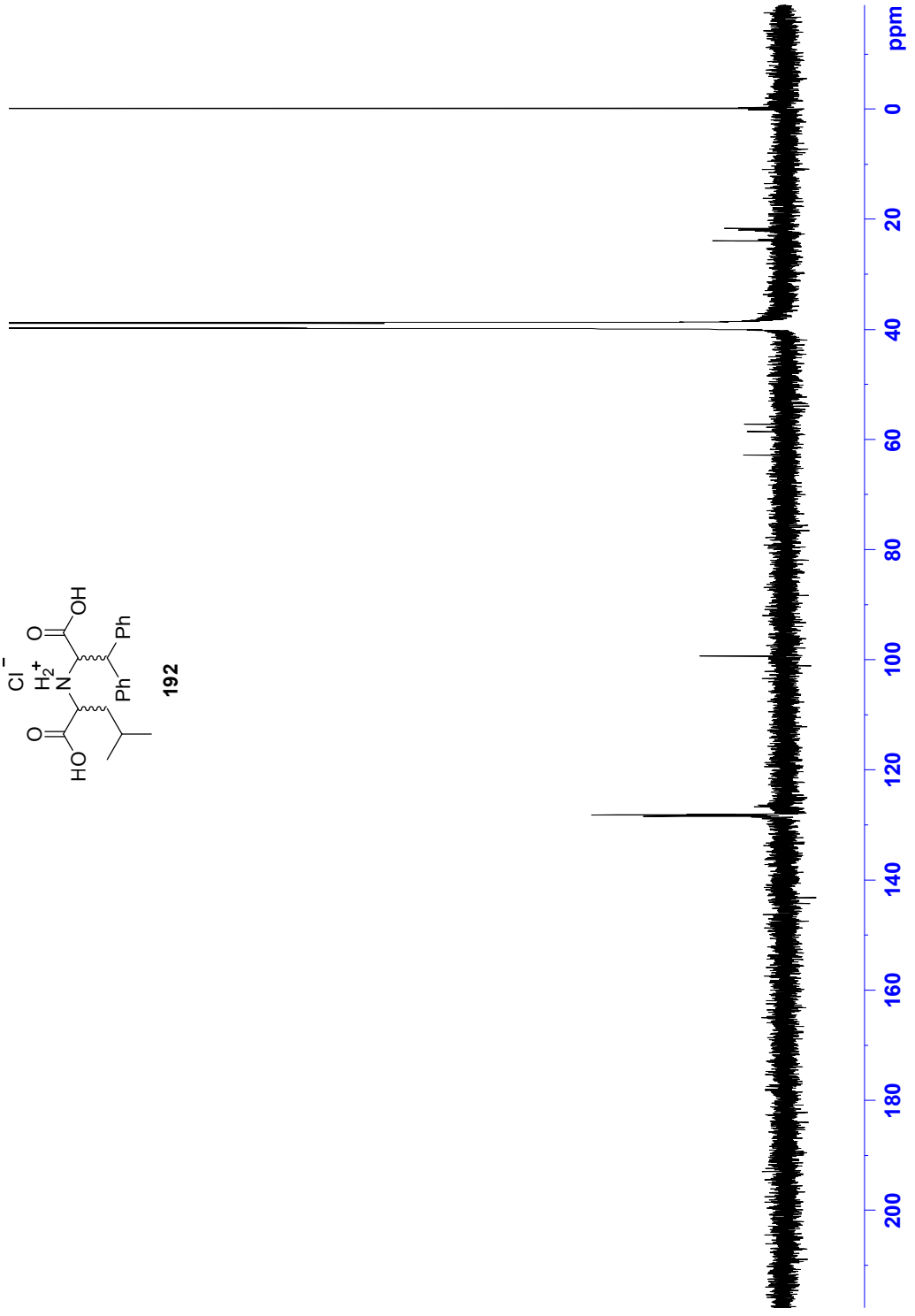

191
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
2.10
3.89
2.70
0.32
2.39
1.73
0.96
0.63
0.84
1.72
1.93
0.41
2.14
0.61
2.08

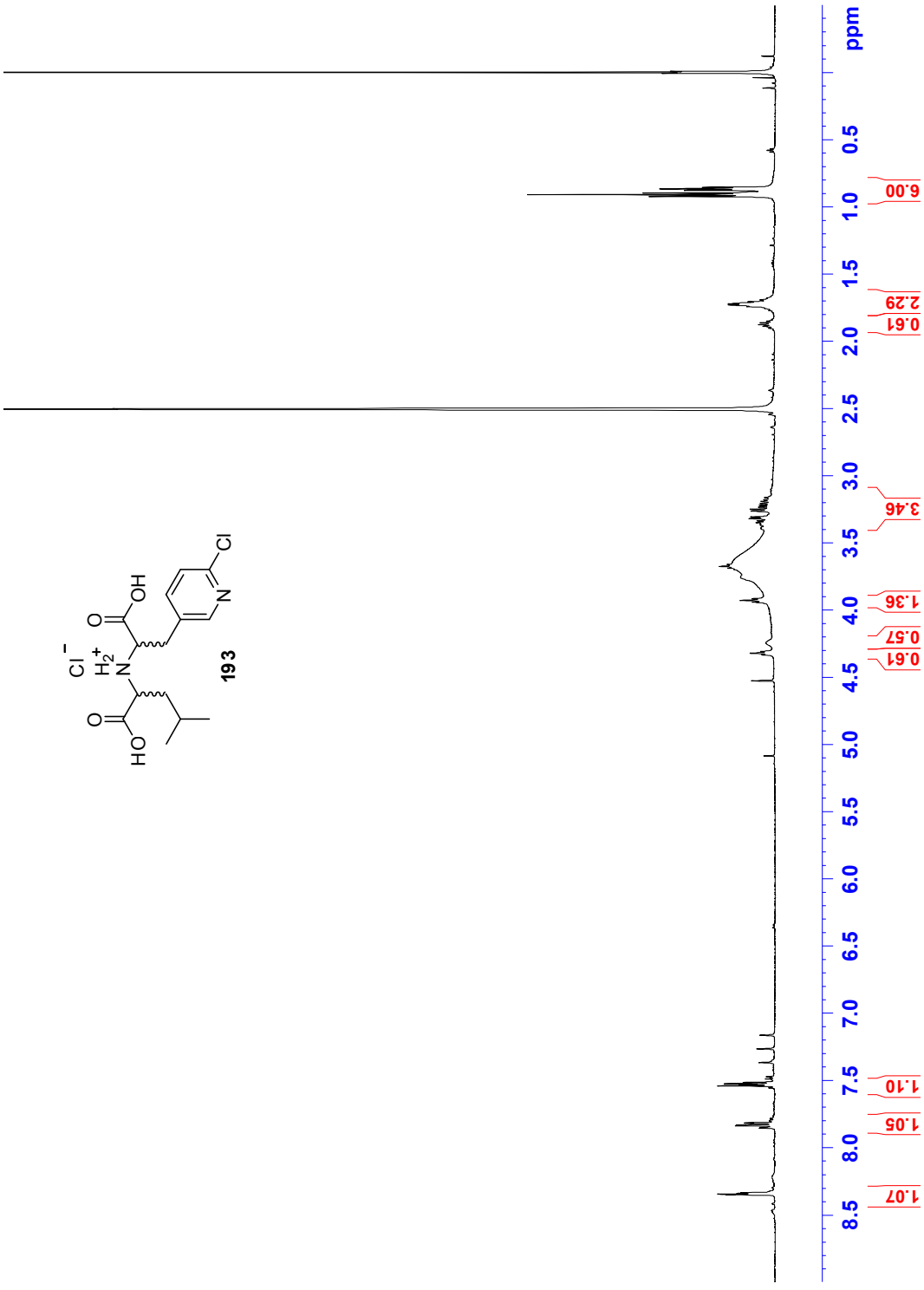

ppm
0
20
40
60
80
100
120
140
160
180
200
191

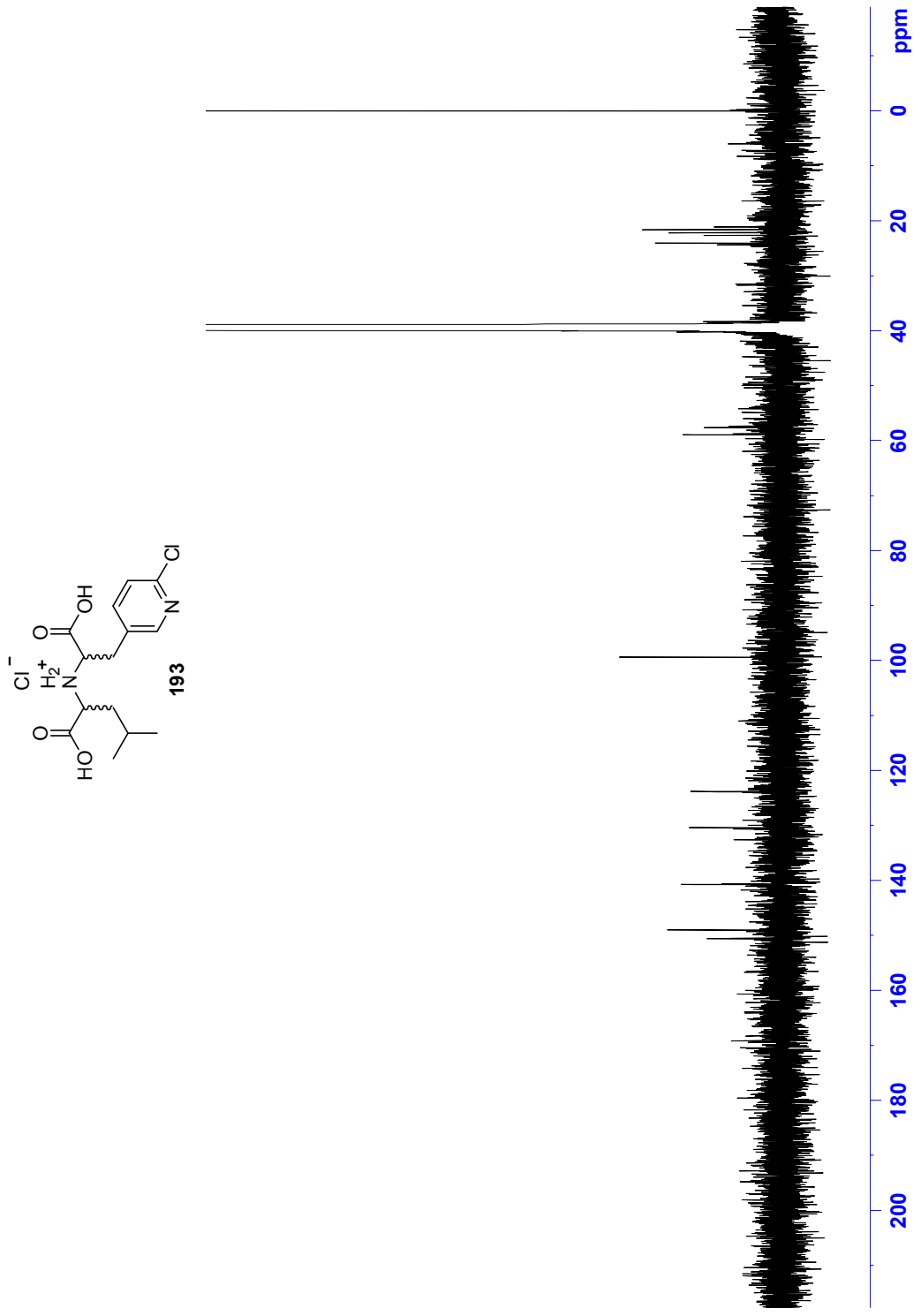

OH
O
Ph
Cl⁻
H2+
N
Ph
O
HO

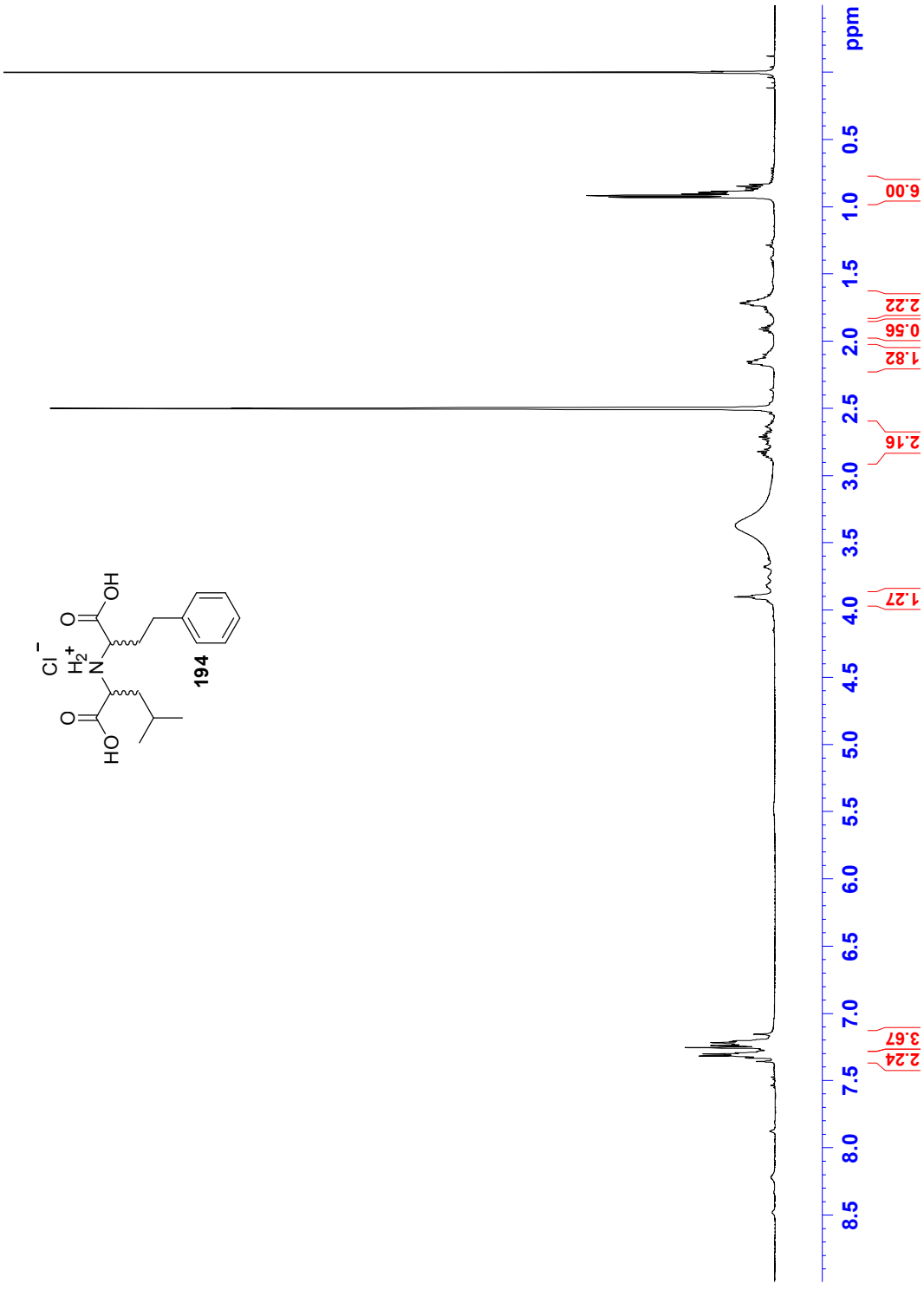
192

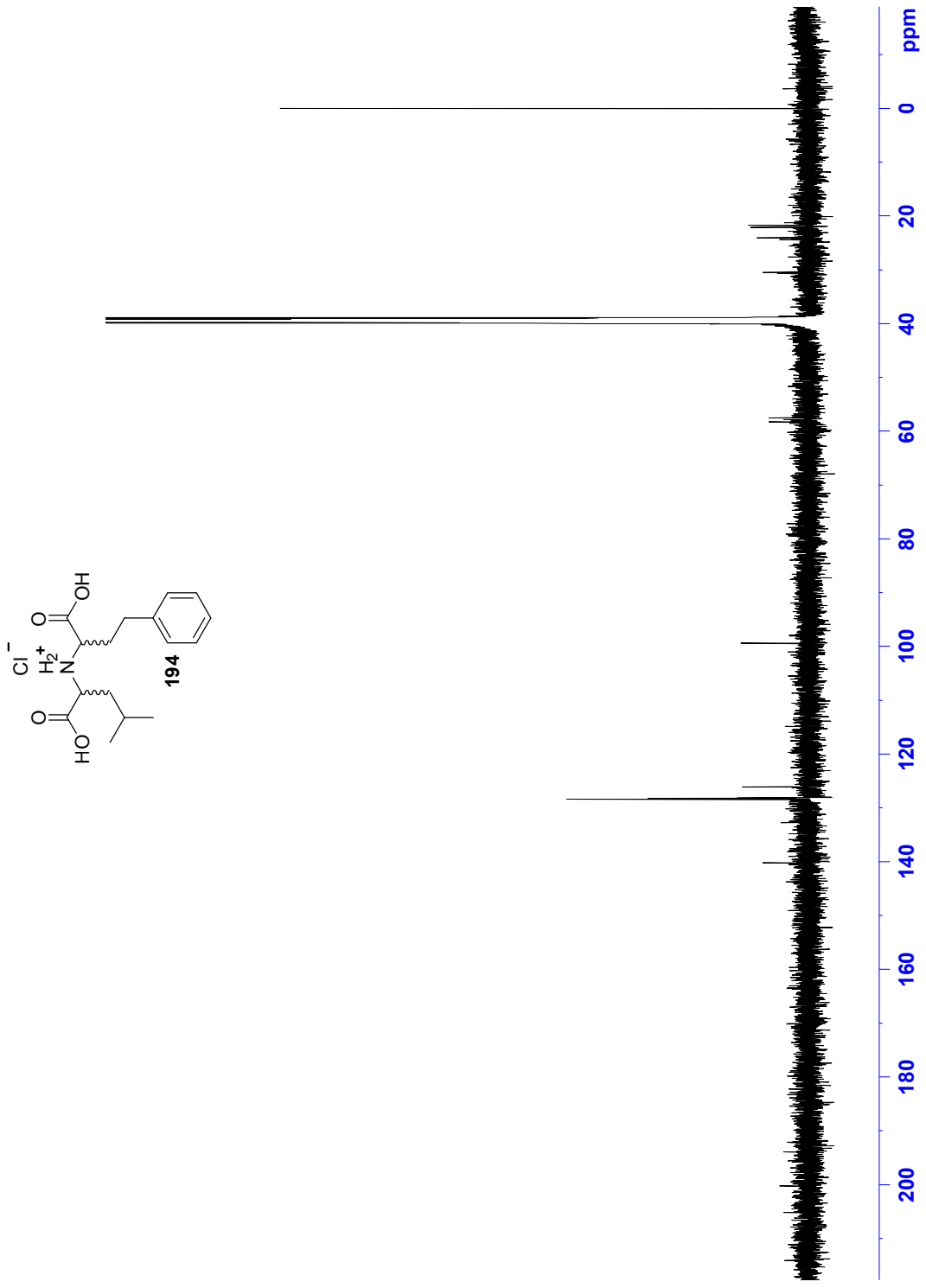

ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
2.70
3.42
0.61
2.60
0.65
0.55
8.67
1.44
1.41
3.88
2.69
1.06
1.97

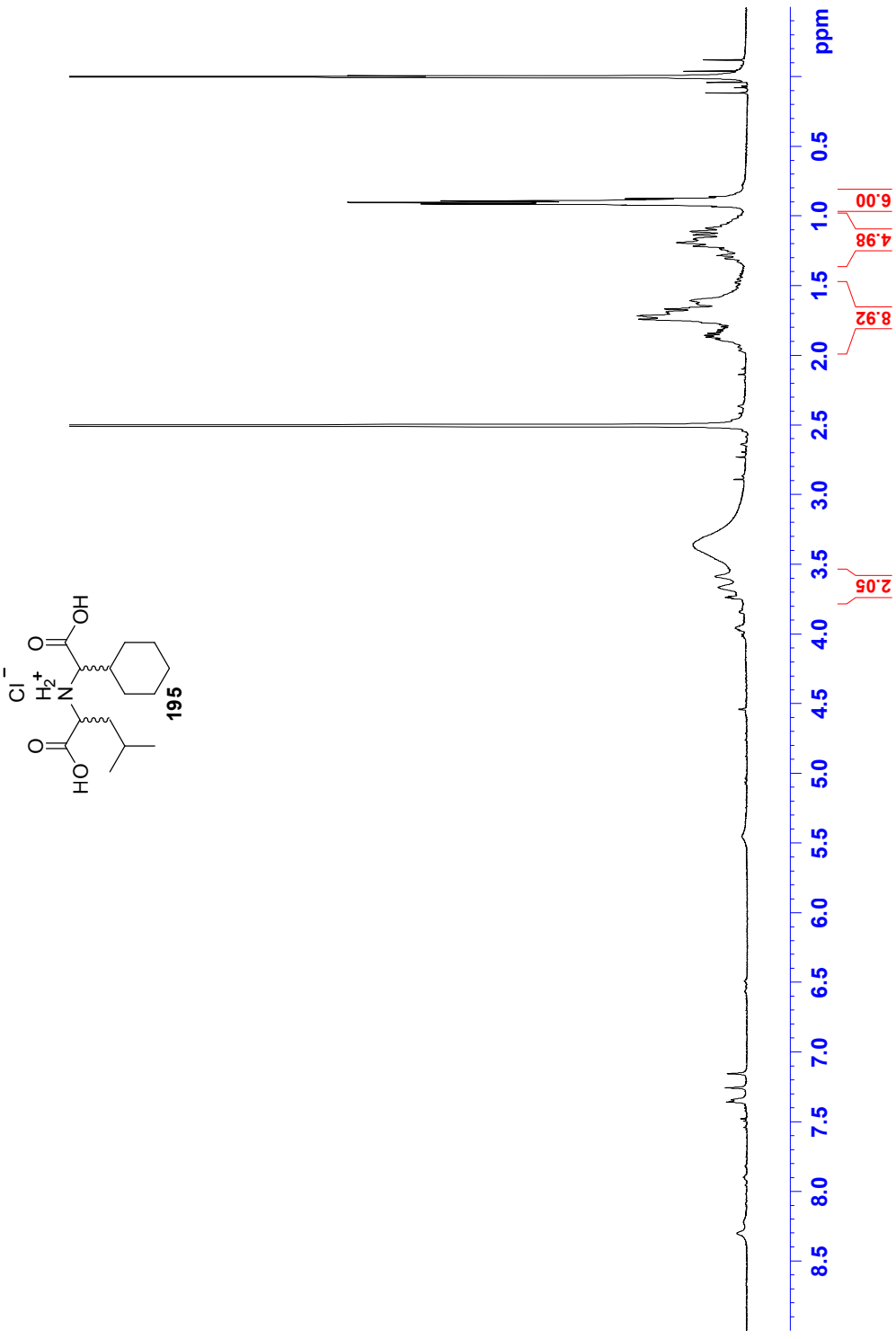

Cl⁻
H2+
N
O
OH
Ph
Ph
O
HO
192
ppm
0
20
40
60
80
100
120
140
160
180
200

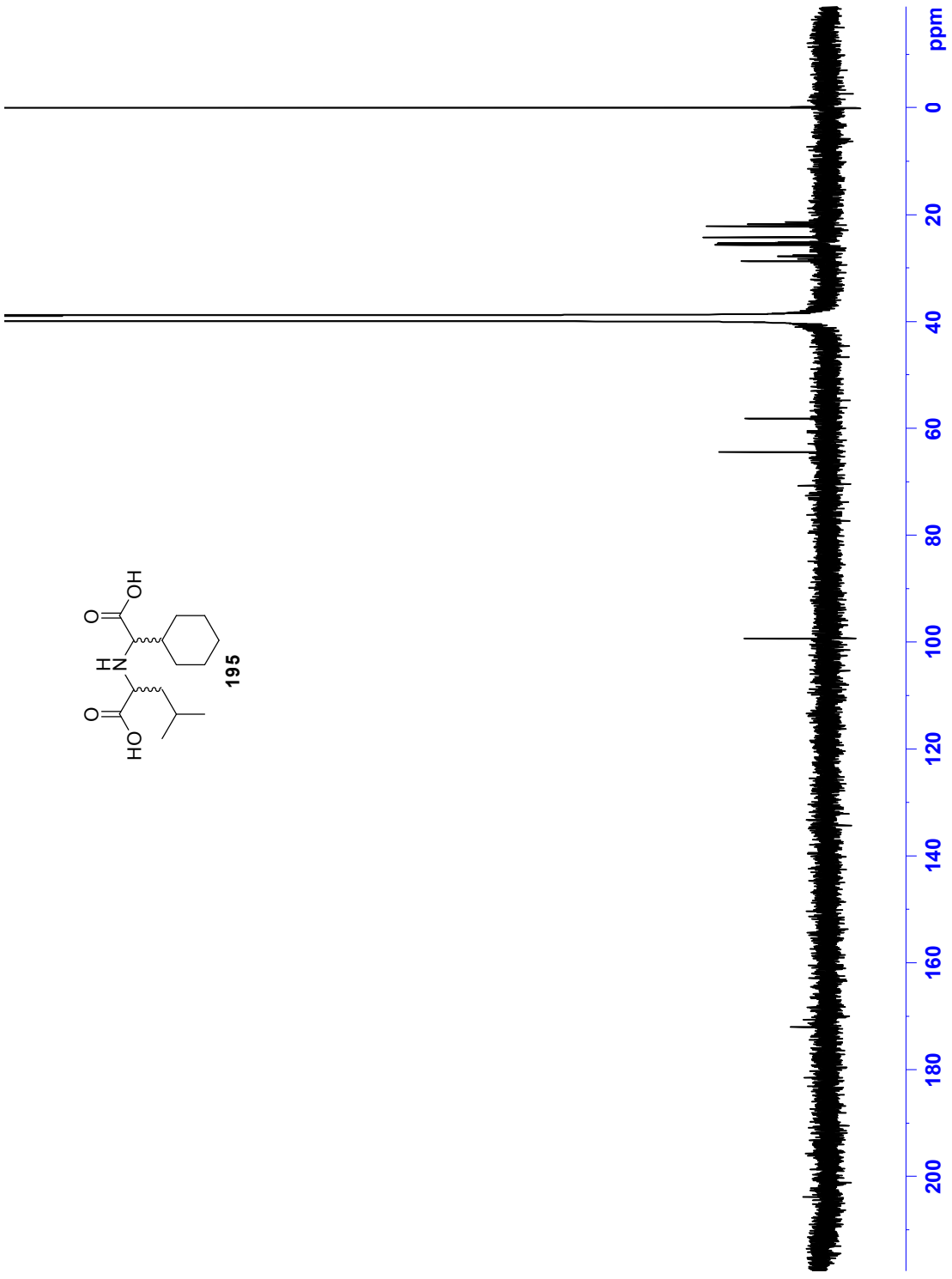

ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
6.00
2.29
0.61
3.46
1.36
0.57
0.61
1.10
1.05
1.07
193

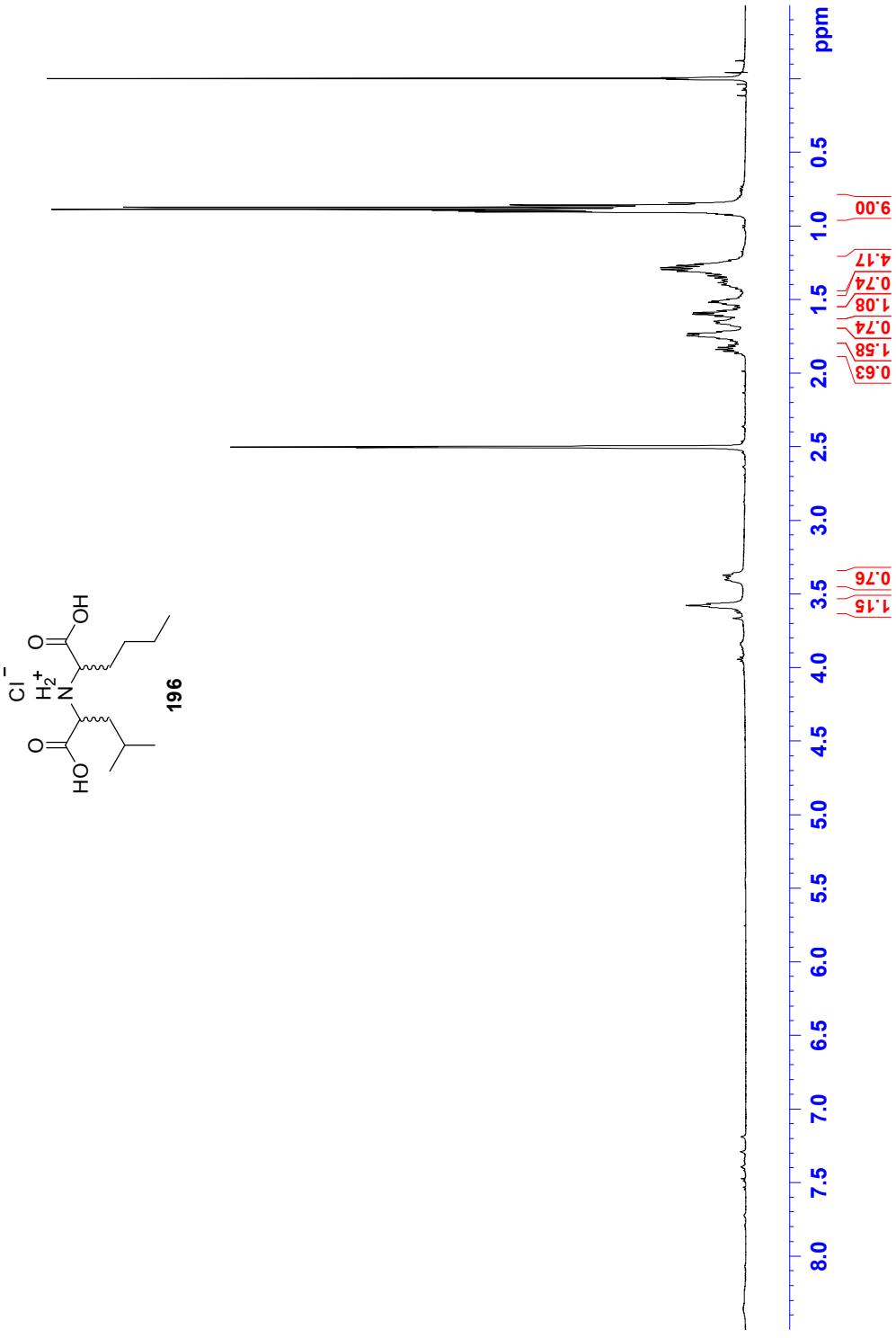

Cl
H2
N
O
OH
N
Cl
O
HO
193
ppm
0
20
40
60
80
100
120
140
160
180
200

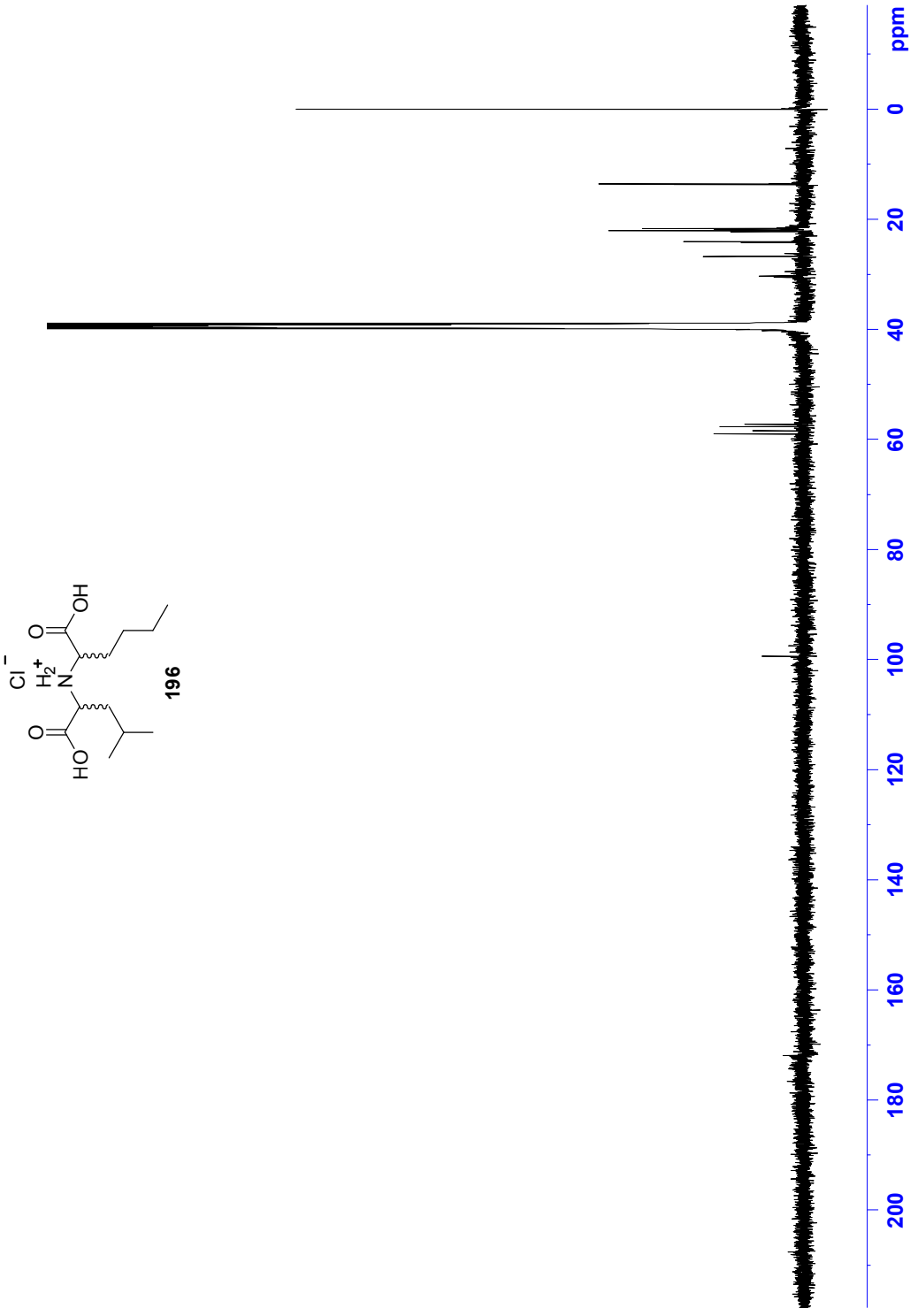

194
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
6.00
2.22
0.56
1.82
2.16
1.27
3.67
2.24

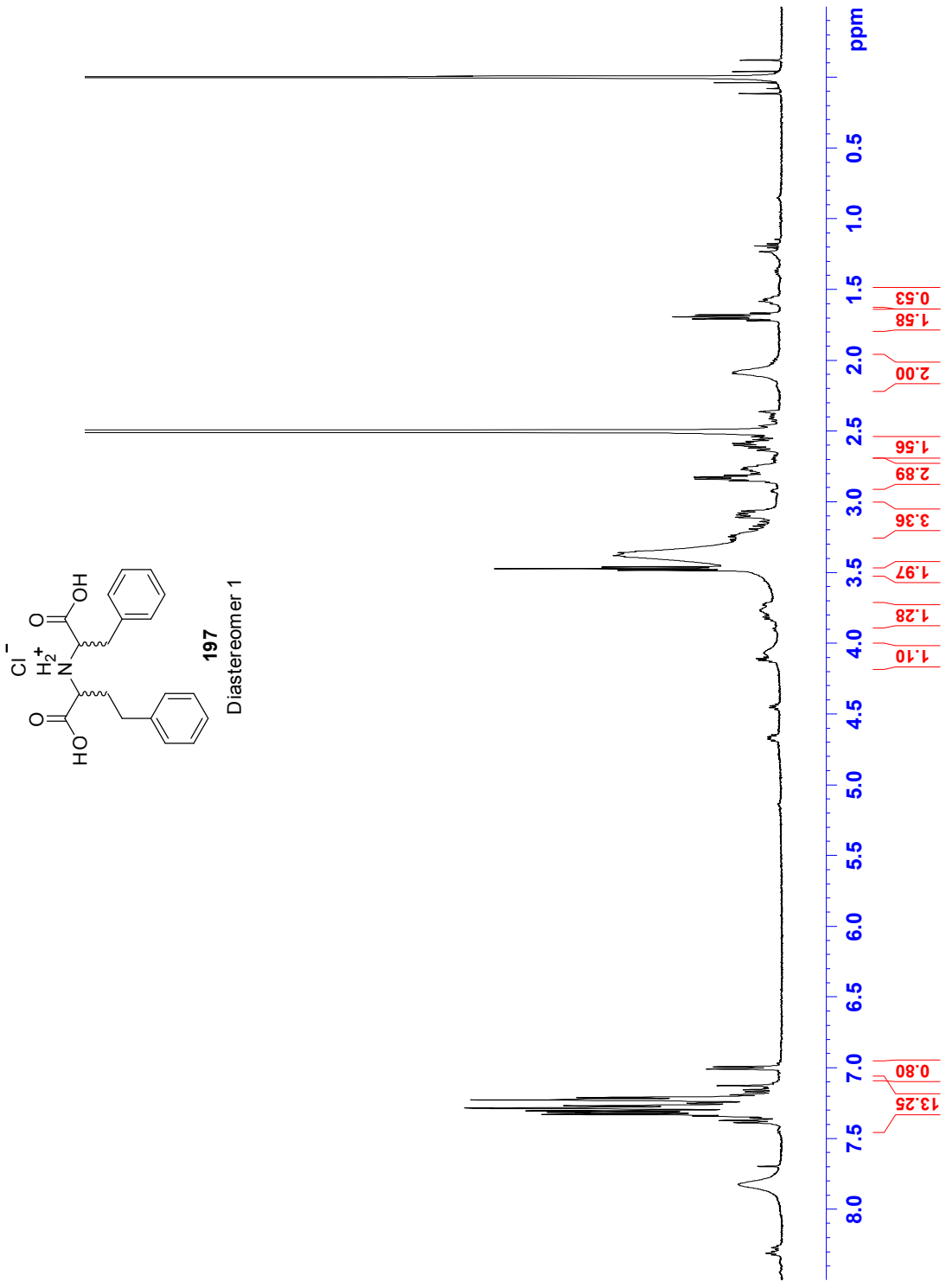

194
ppm
0
20
40
60
80
100
120
140
160
180
200

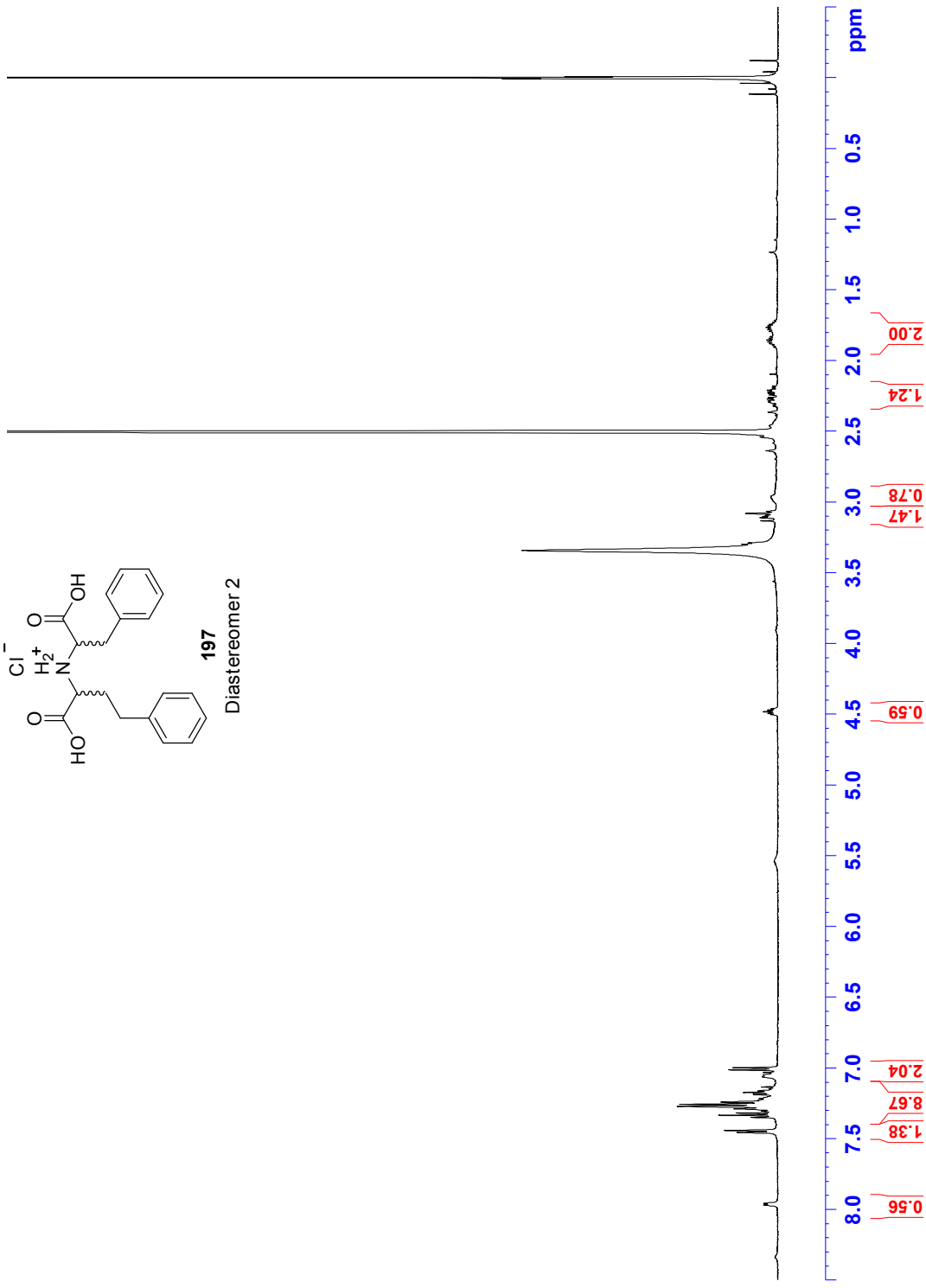

195
ppm
6.00
4.98
8.92
2.05

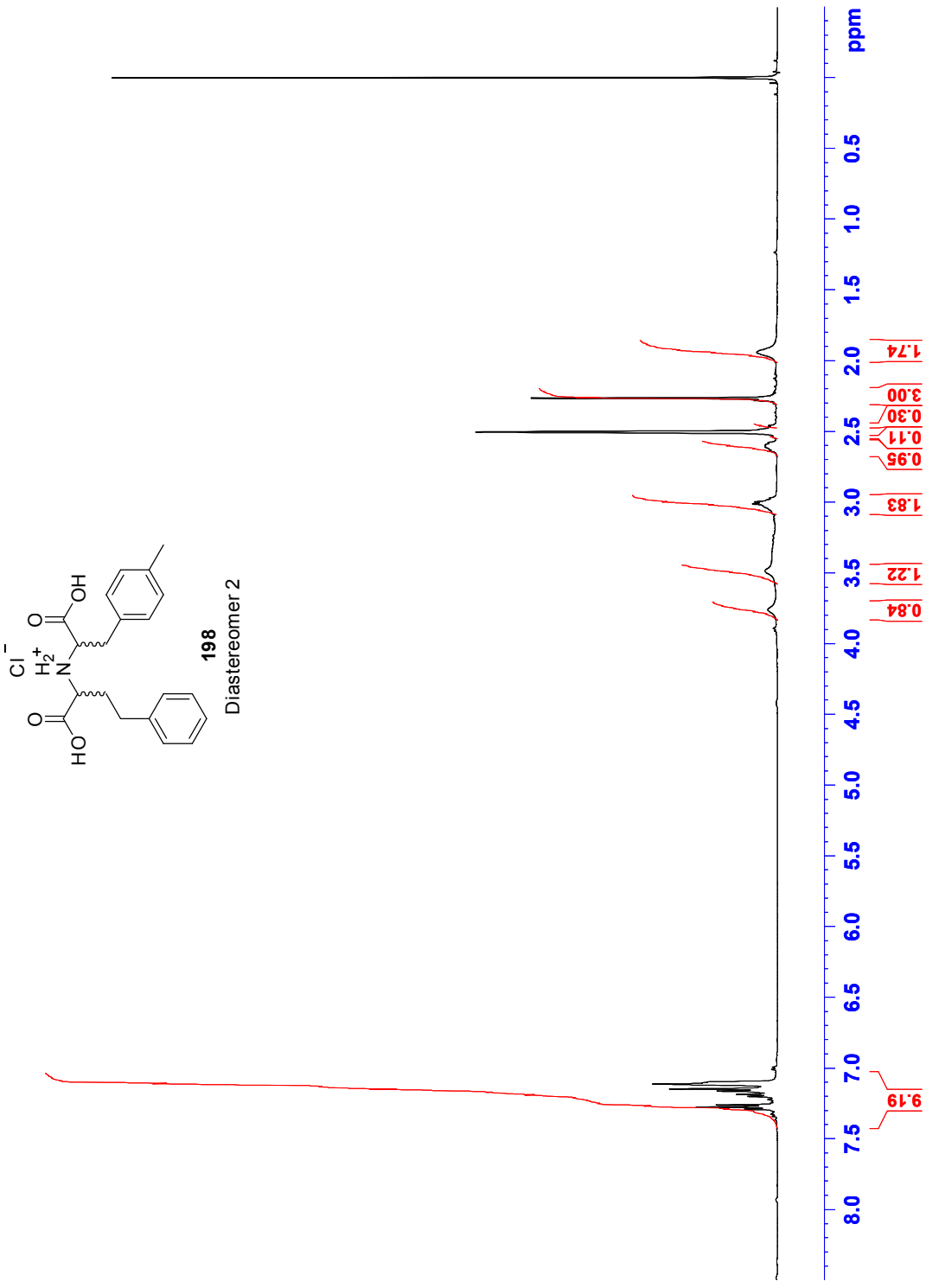

ppm
0
20
40
60
80
100
120
140
160
180
200
O
OH
H
N
O
HO
195

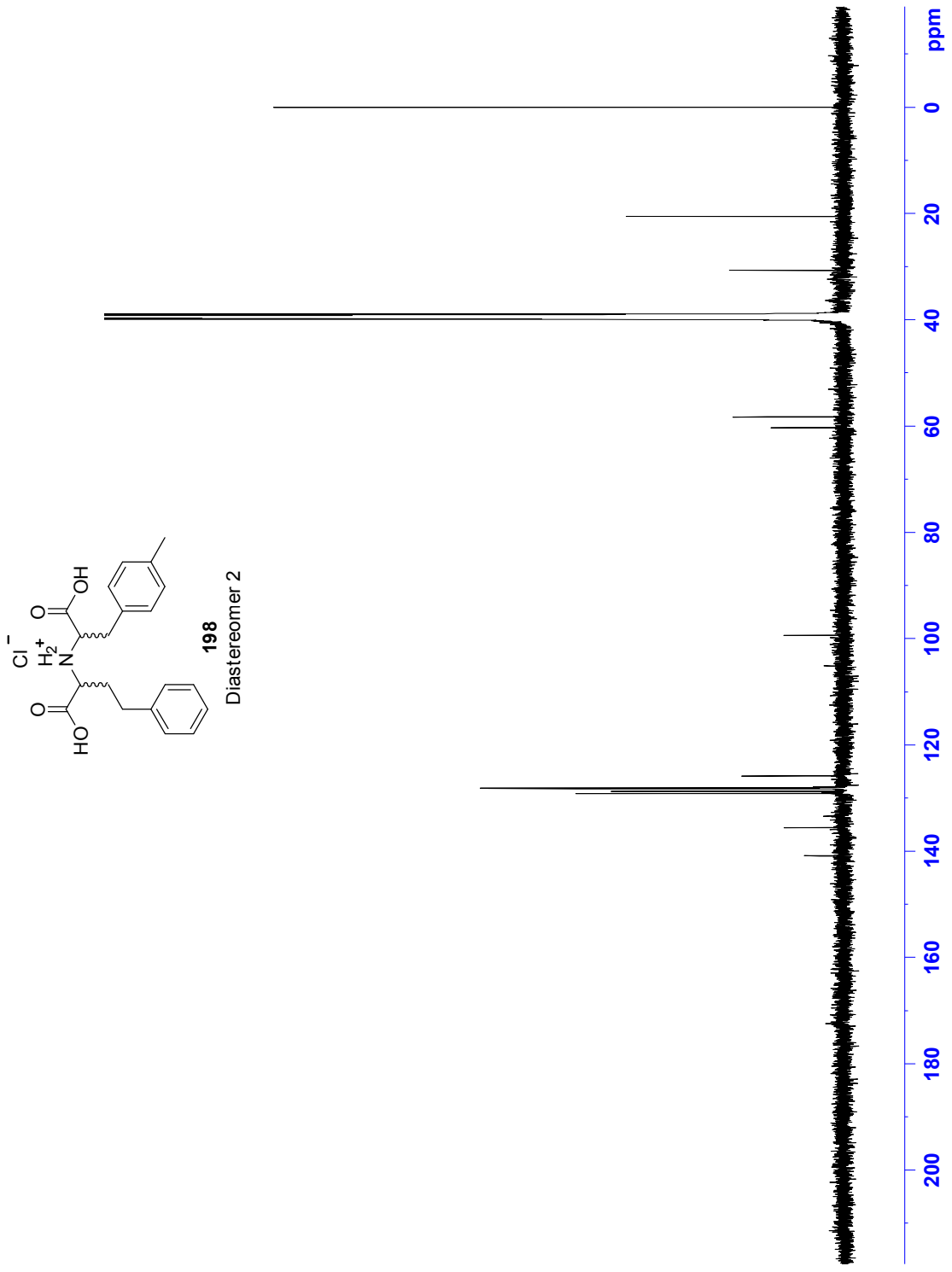

196
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
9.00
4.17
0.74
1.08
0.74
1.58
0.63
0.76
1.15

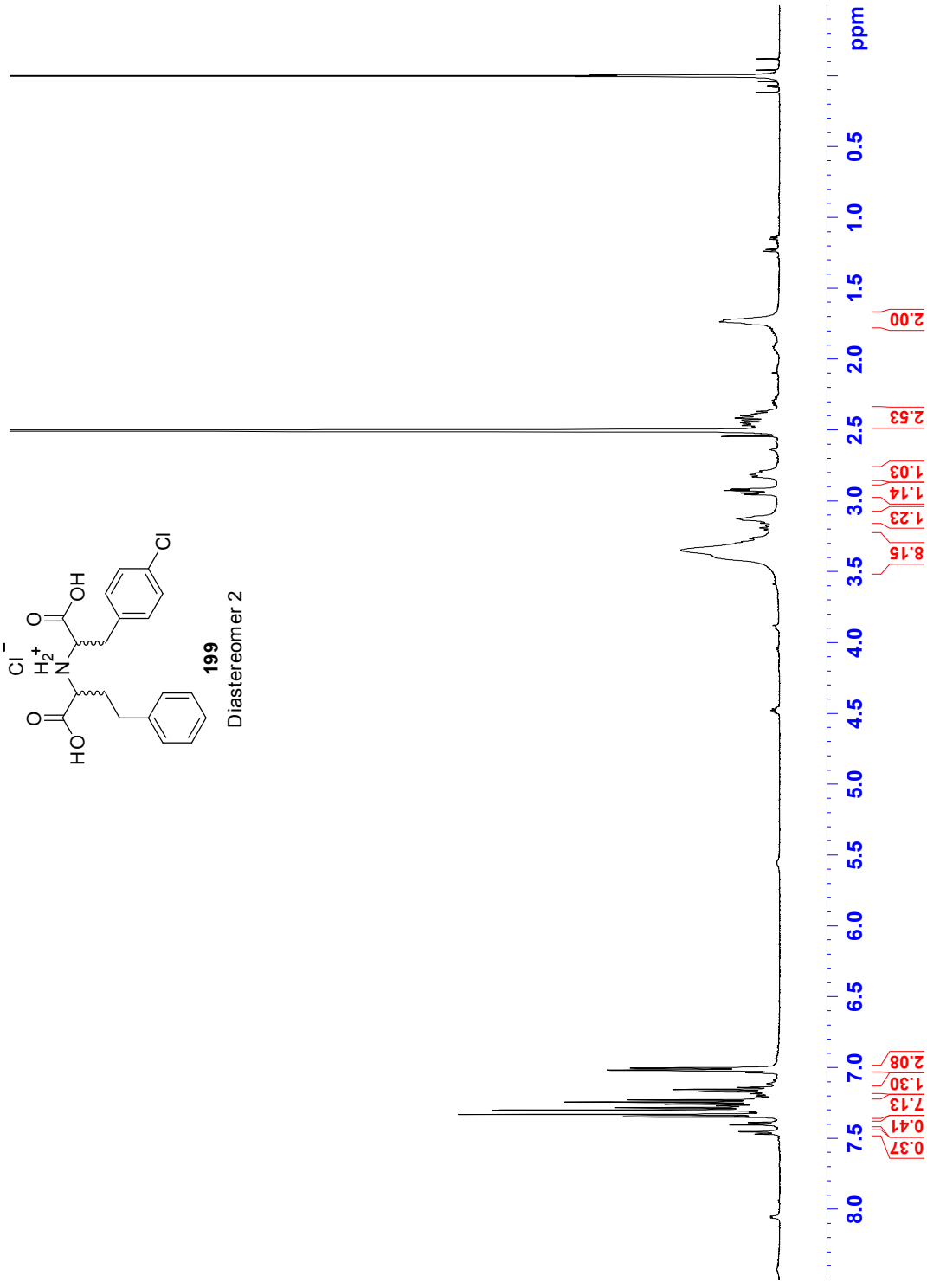

ppm
0
20
40
60
80
100
120
140
160
180
200
196

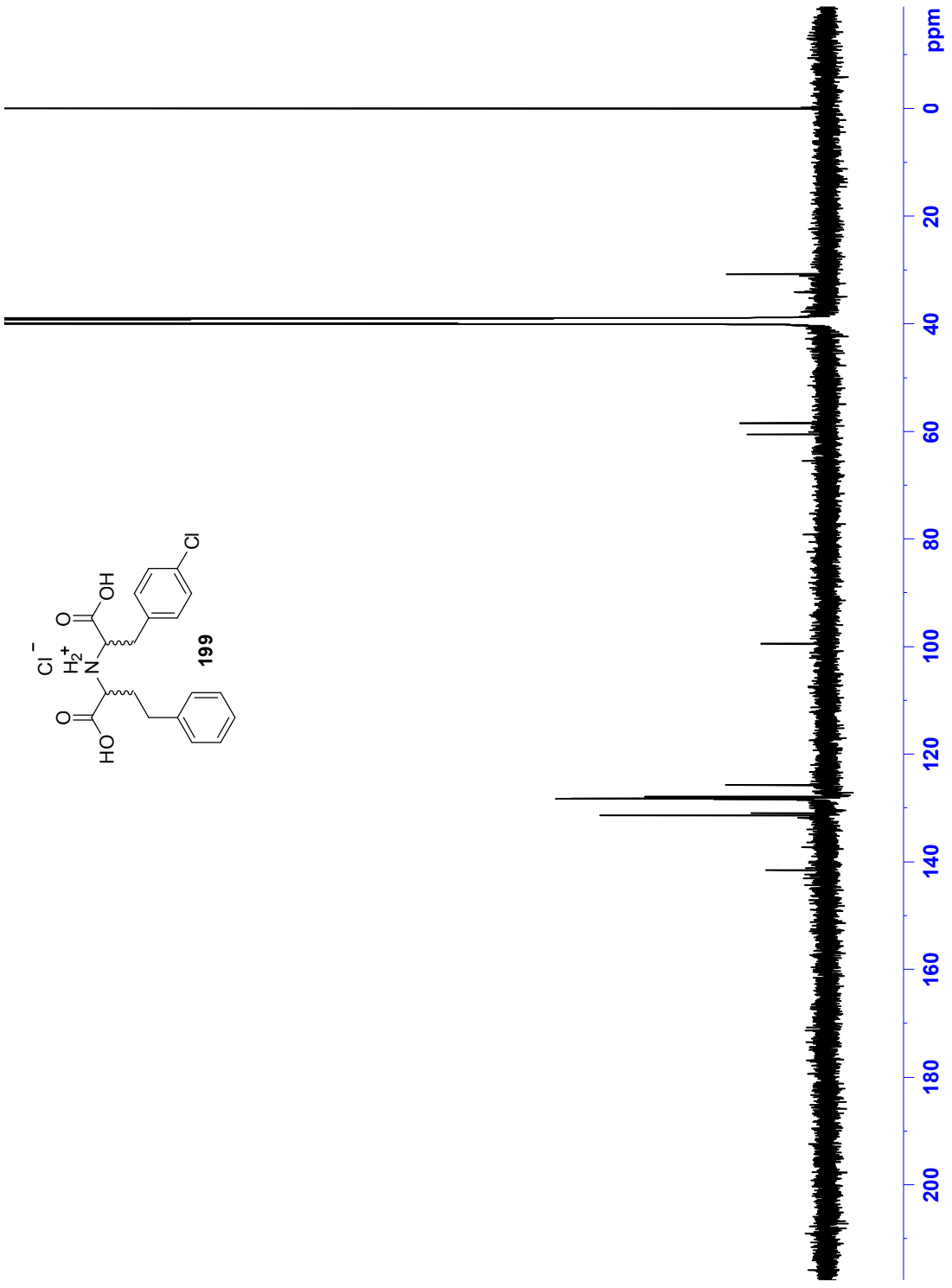

197
Diastereomer 1
ppm
0.53
1.58
2.00
1.56
2.89
3.36
1.97
1.28
1.10
0.80
13.25

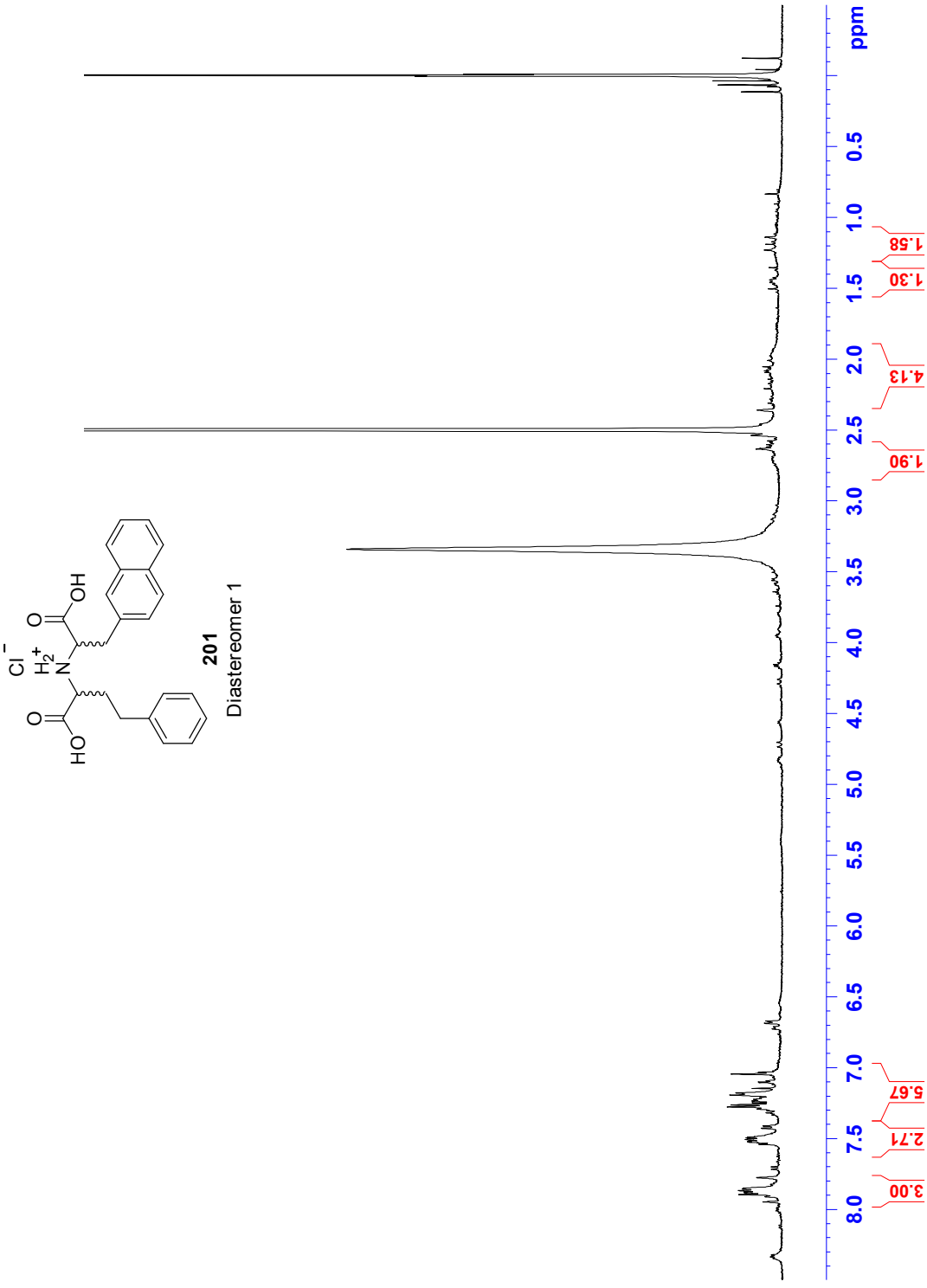

197
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
2.00
1.24
0.78
1.47
0.59
2.04
8.67
1.38
0.56

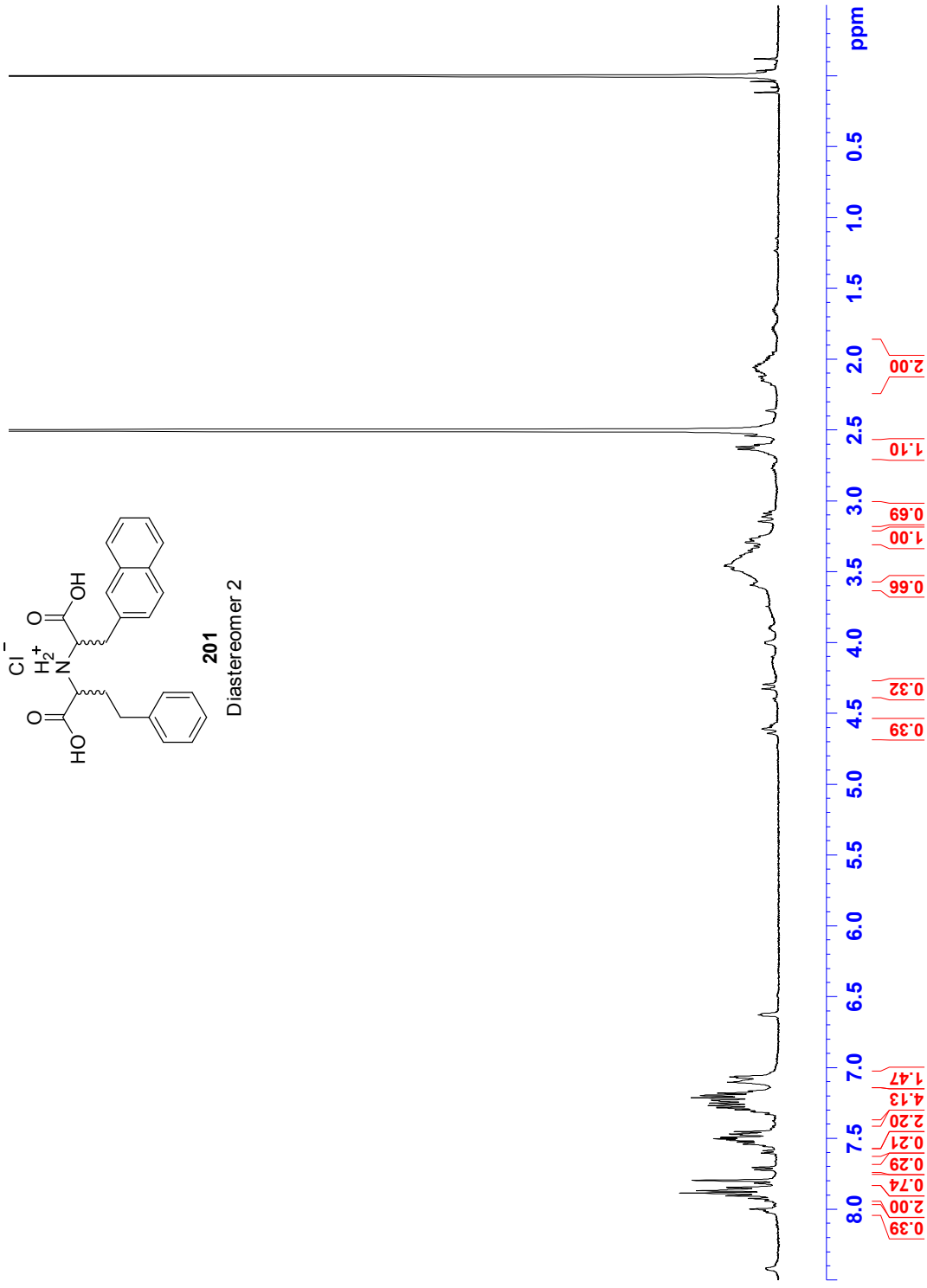

ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.74
3.00
0.30
0.11
0.95
1.83
1.22
0.84
9.19
198
Diastereomer 2


198
Diastereomer 2
ppm
0
20
40
60
80
100
120
140
160
180
200


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
2.00
2.53
1.03
1.14
1.23
8.15
0.37
0.41
7.13
1.30
2.08
Cl
OH
O
H_2^+
N
Cl^-
O
HO
199
Diastereomer 2


Cl
OH
O
Cl⁻
H2+
N
O
HO
199
ppm
0
20
40
60
80
100
120
140
160
180
200


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
1.58
1.30
4.13
1.90
5.67
2.71
3.00
201
Diastereomer 1


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
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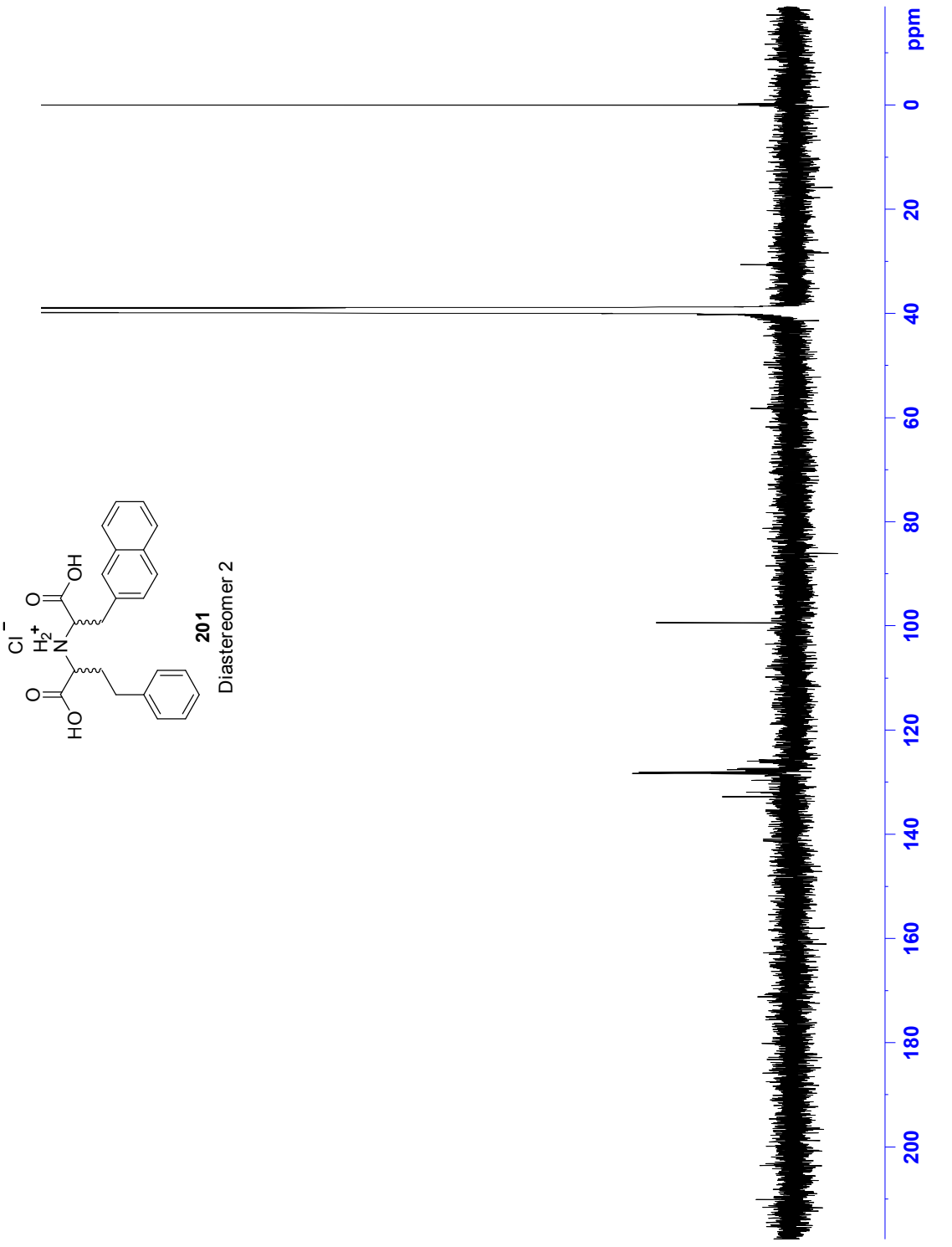
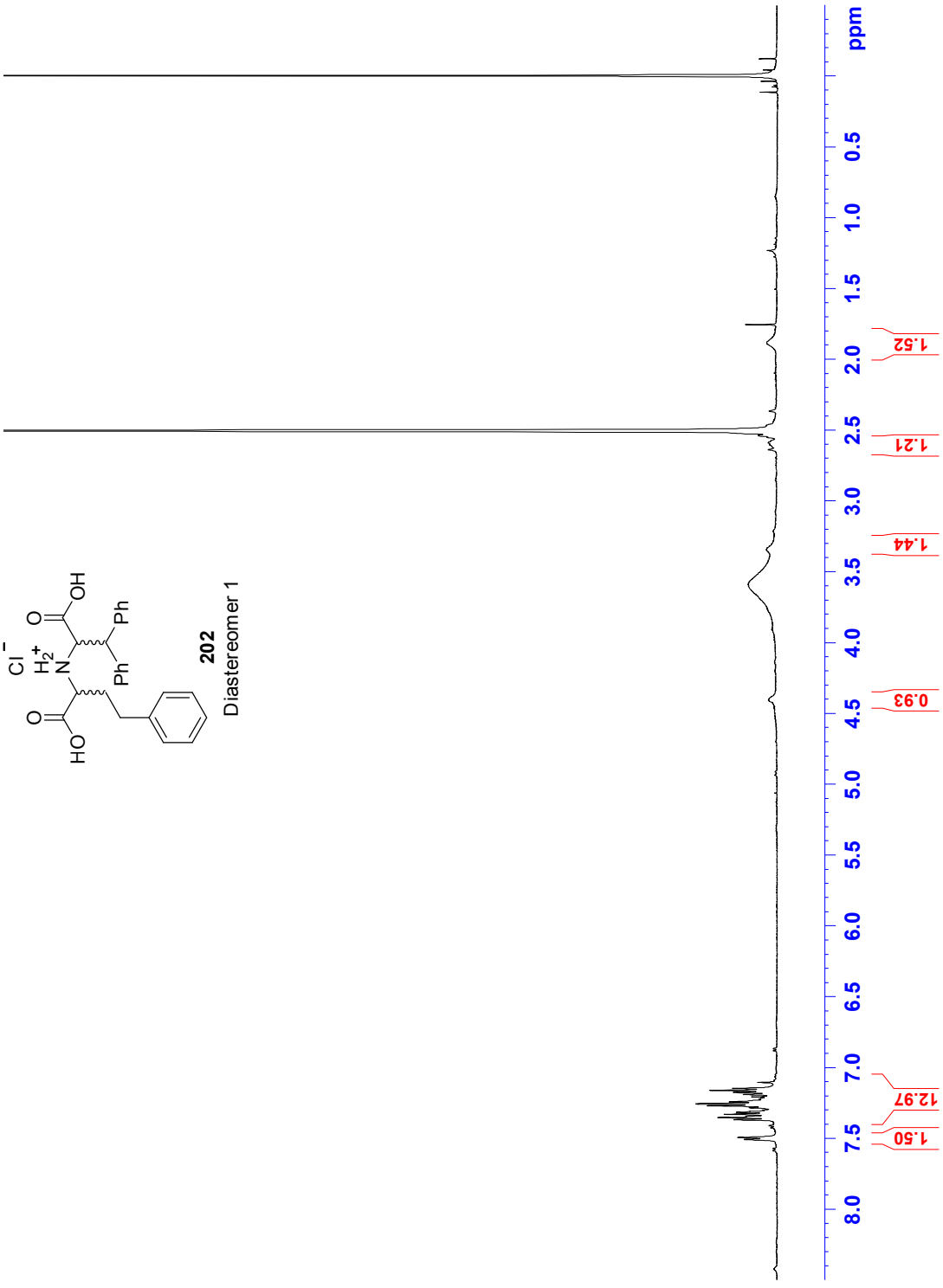
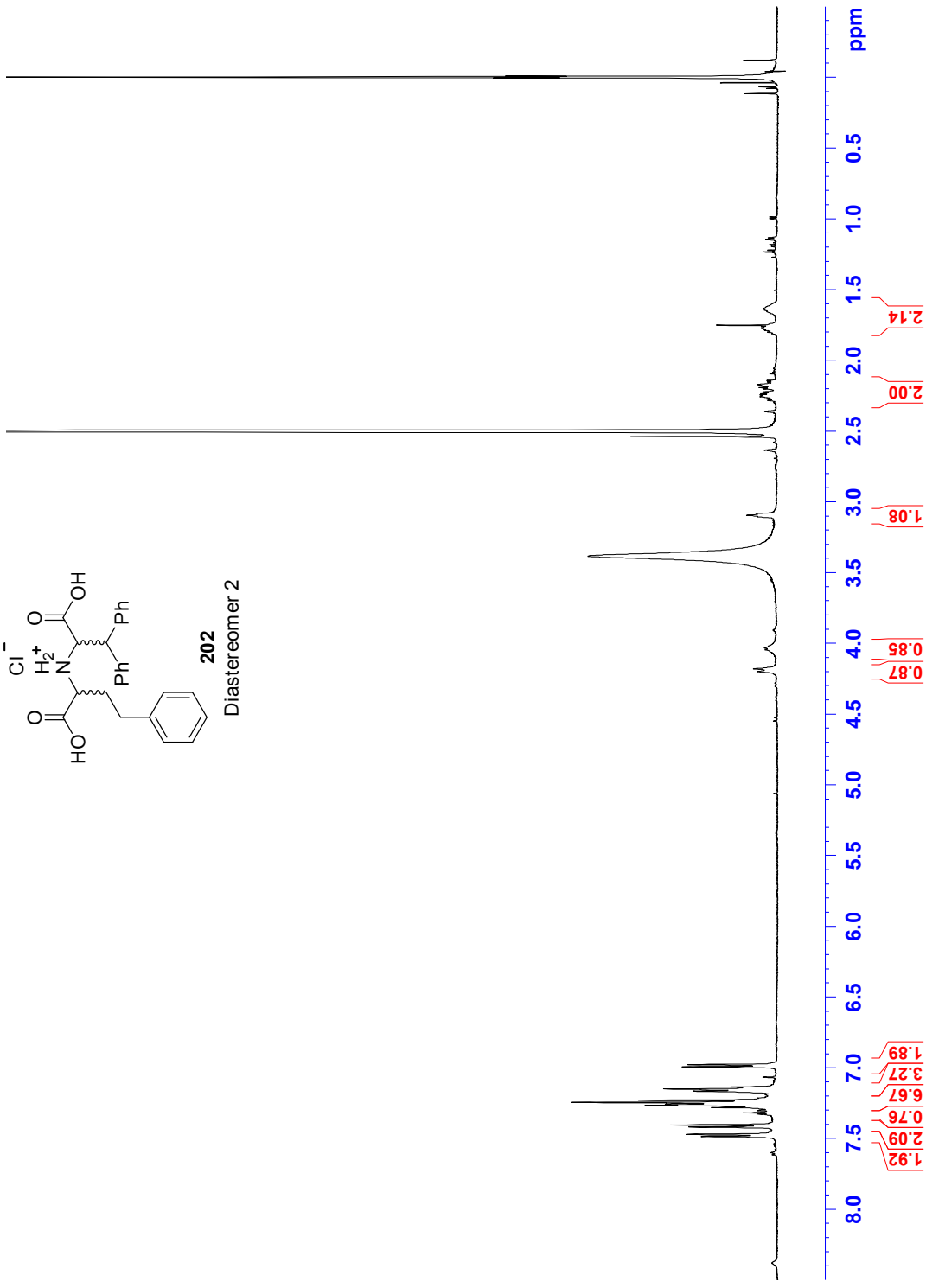
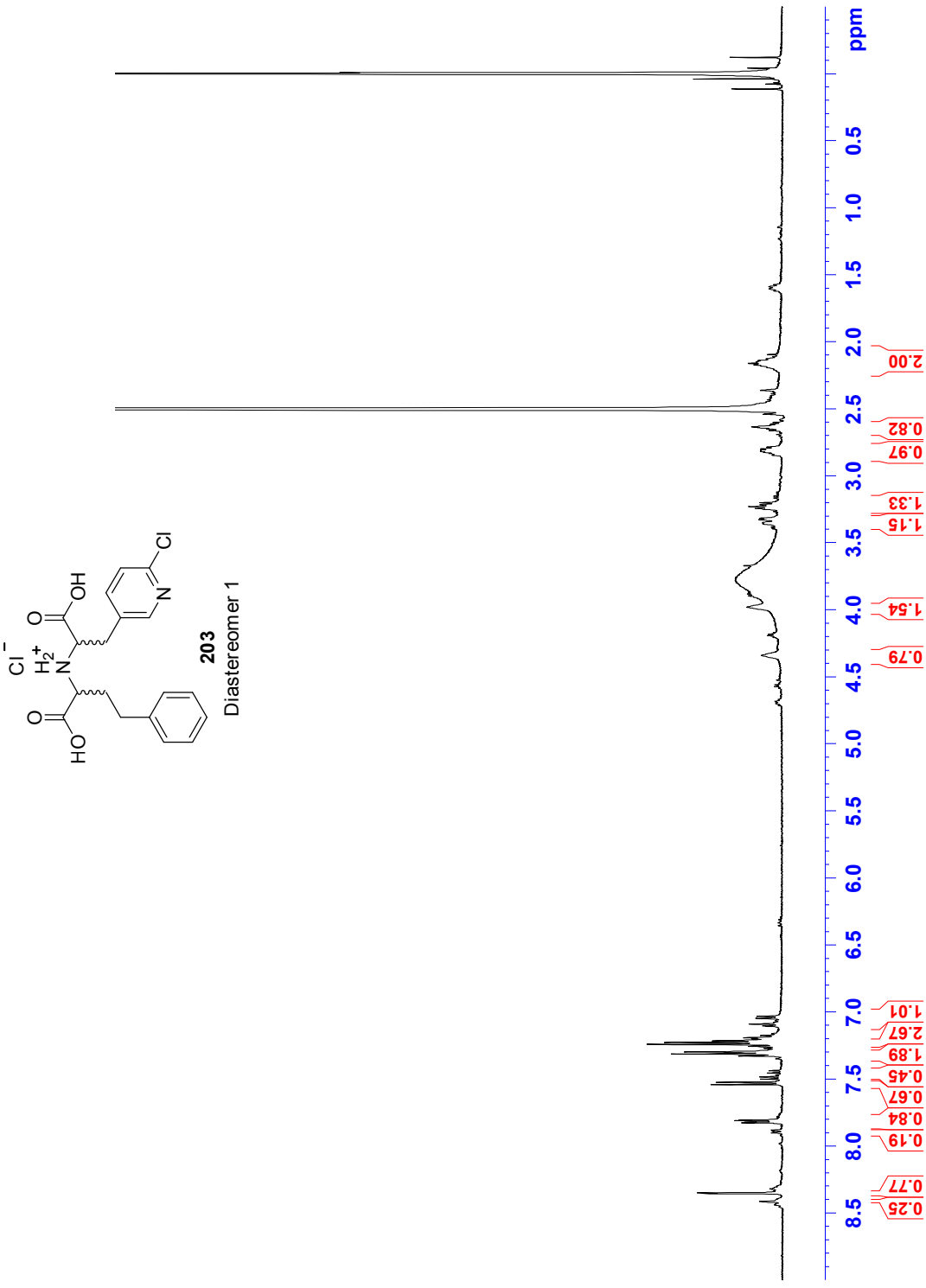
6.5
7.0
7.5
8.0
2.00
1.10
0.69
1.00
0.66
0.32
0.39
1.47
4.13
2.20
0.21
0.29
0.74
2.00
0.39
201
Diastereomer 2


ppm
0
20
40
60
80
100
120
140
160
180
200
201
Diastereomer 2

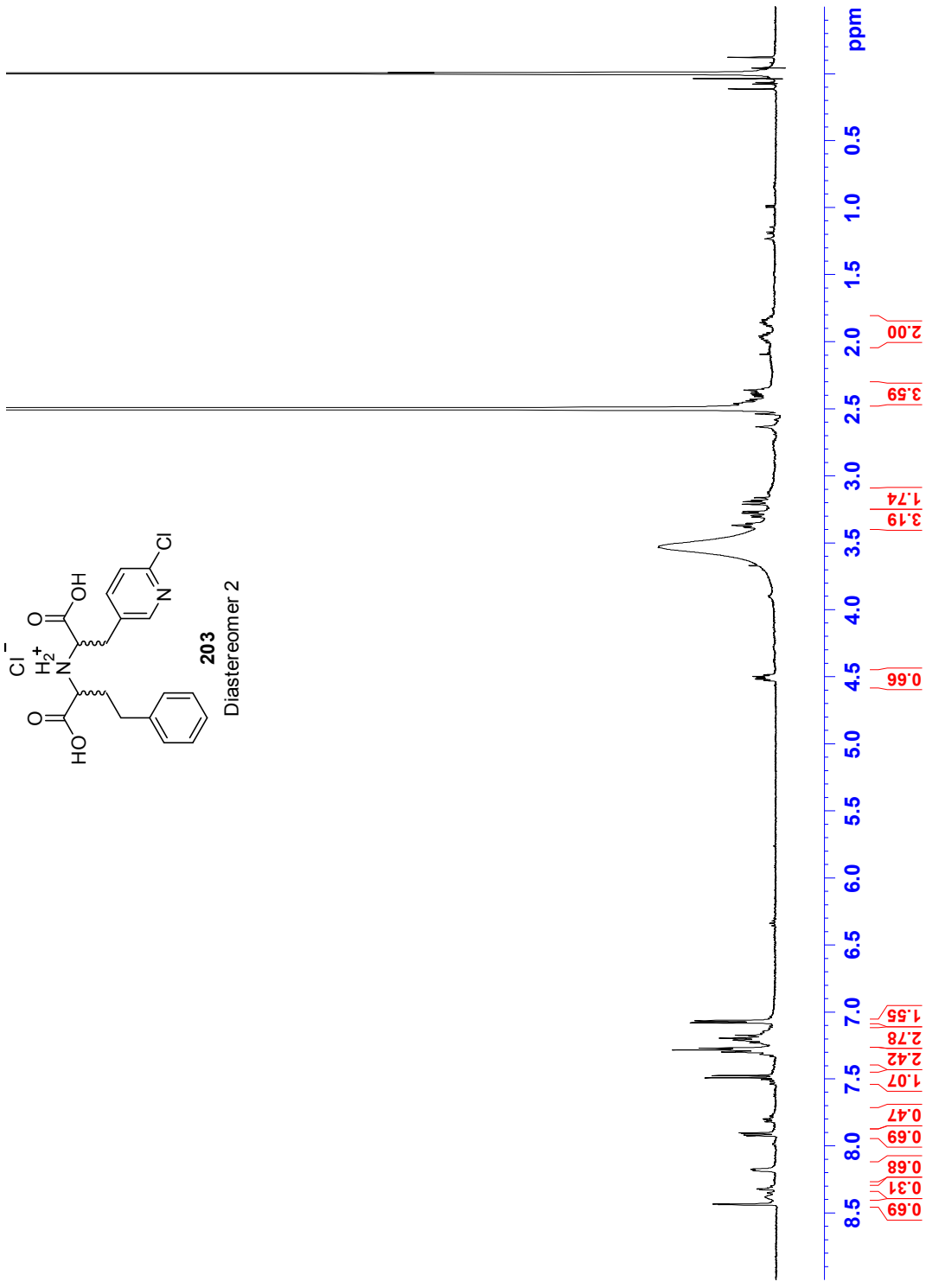

202
Diastereomer 1
ppm
1.52
1.21
1.44
0.93
12.97
1.50

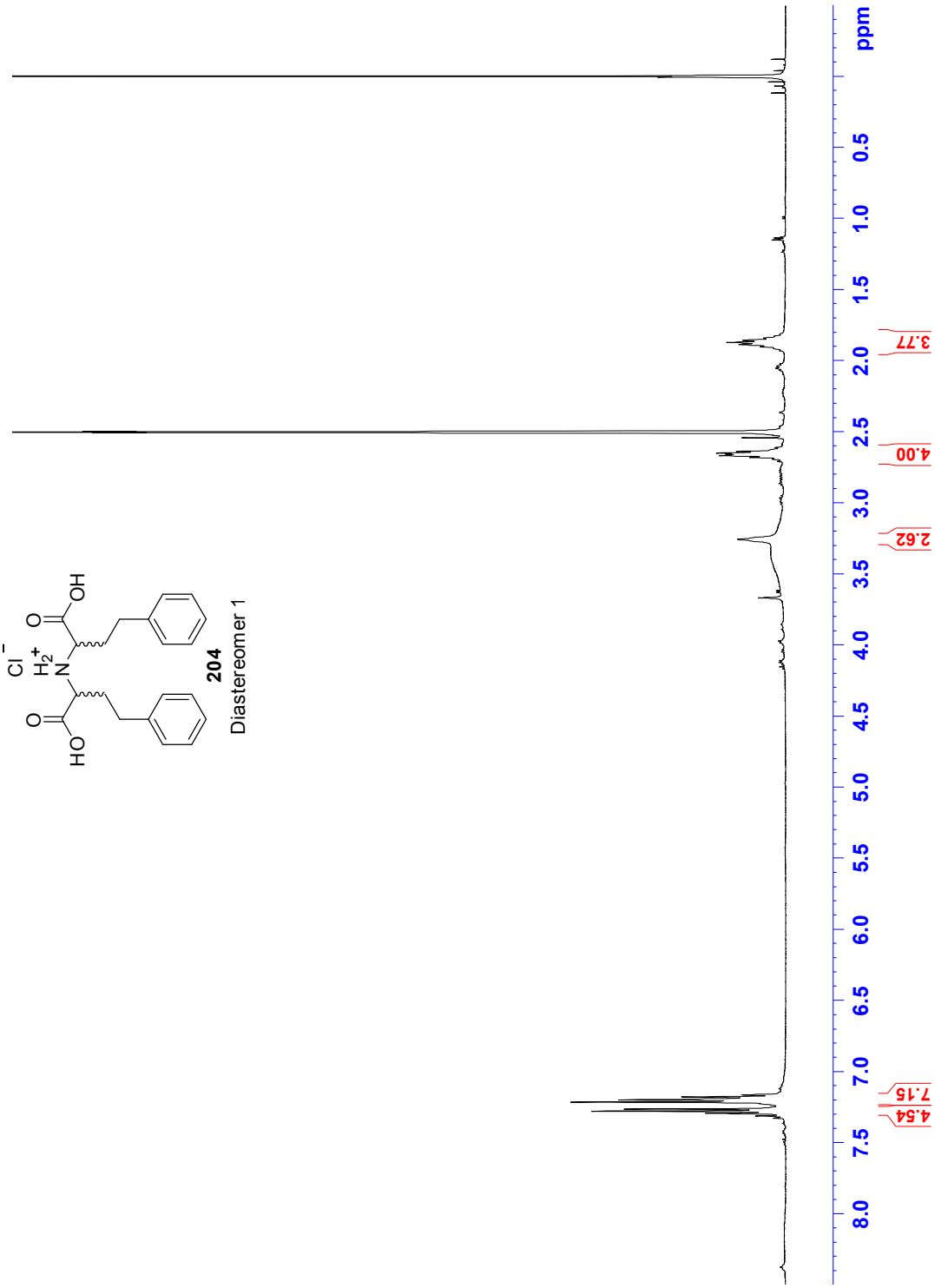

ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
2.14
2.00
1.08
0.87
0.85
1.92
2.09
0.76
6.67
3.27
1.89
202
Diastereomer 2



203

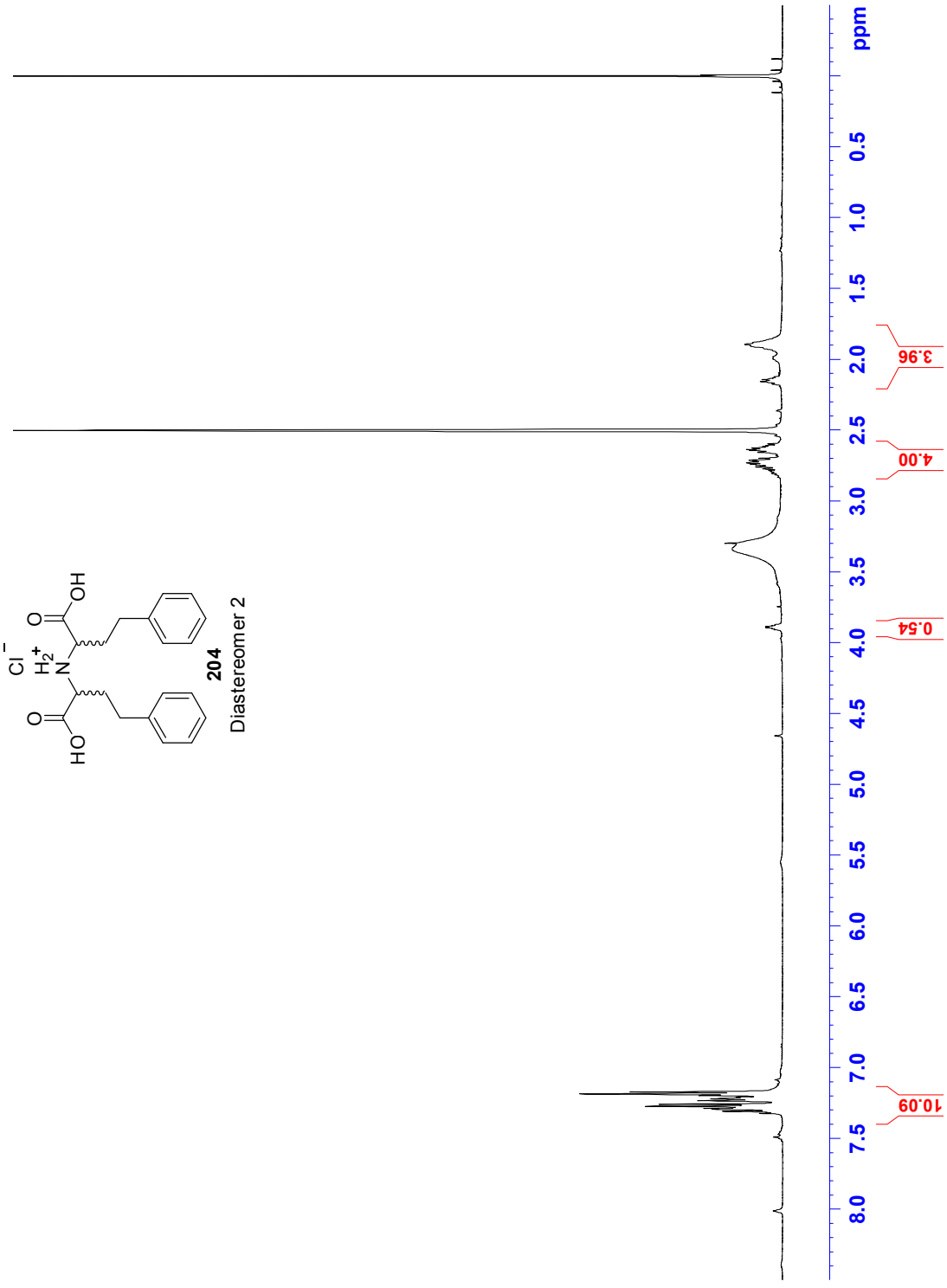
Diastereomer 1

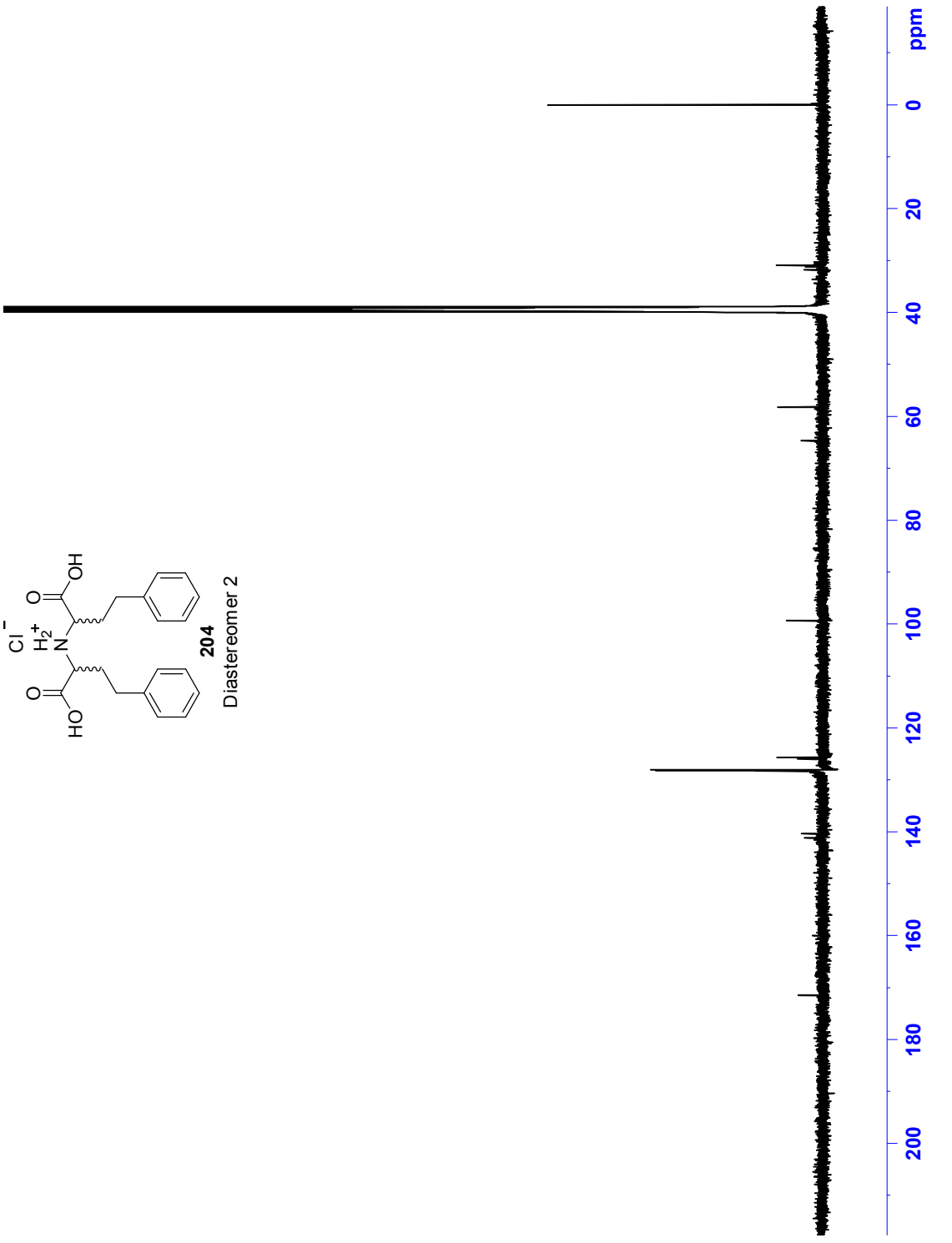

ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.69
0.31
0.68
0.69
0.47
1.07
2.42
2.78
1.55
0.66
3.19
1.74
3.59
2.00
Cl
N
OH
O
Cl
H2
N
O
HO
203
Diastereomer 2



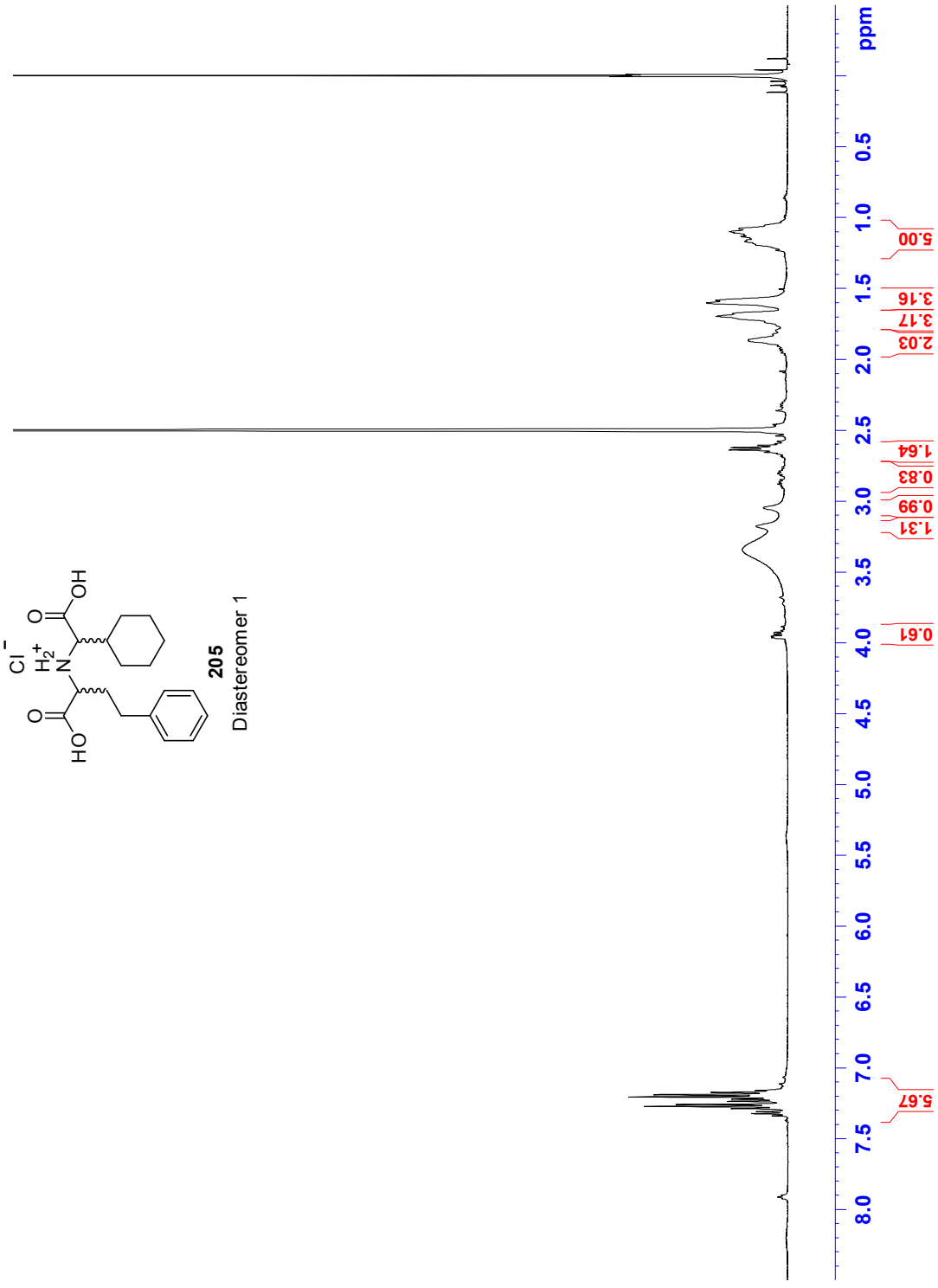
204

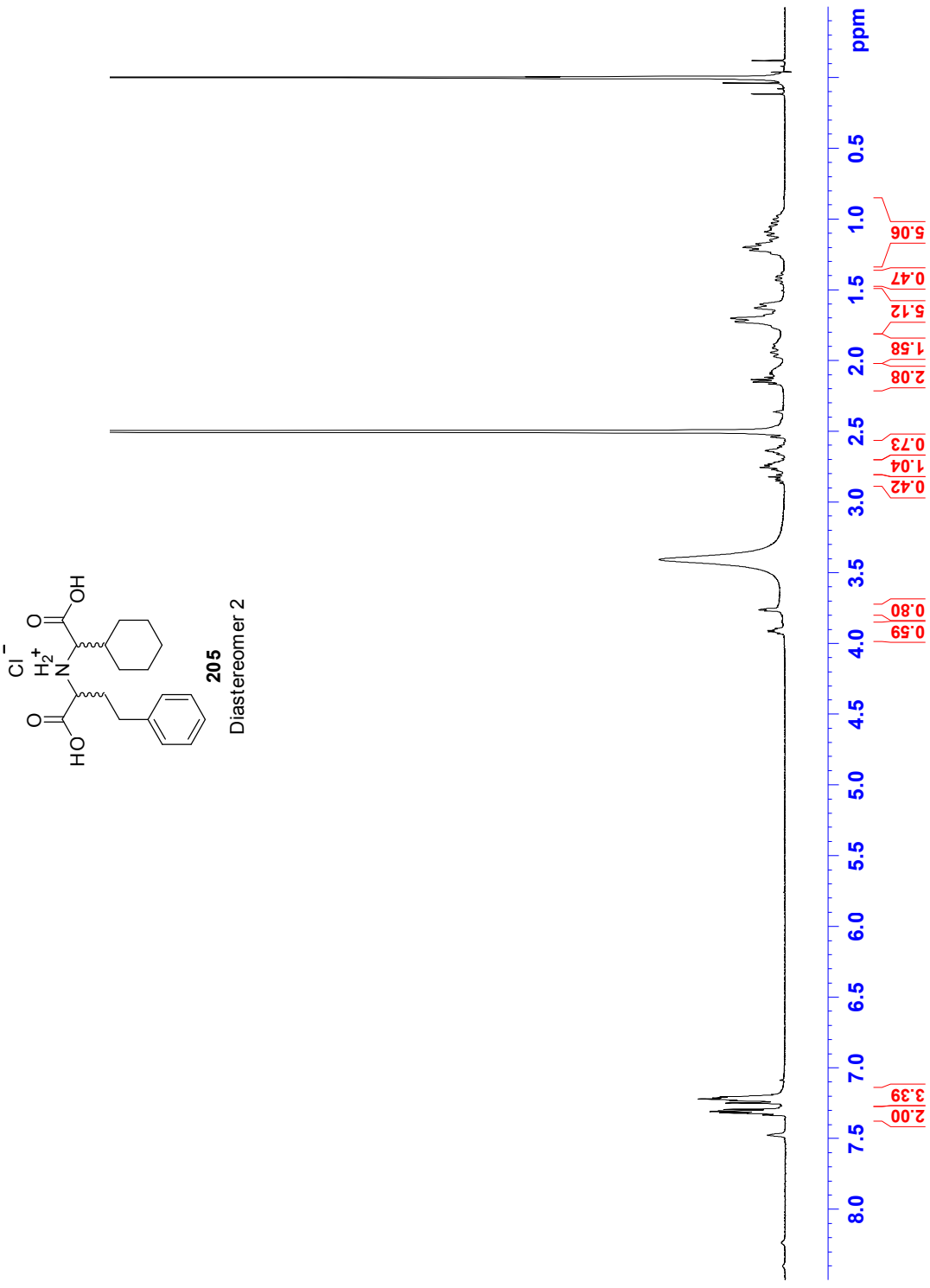
Diastereomer 1

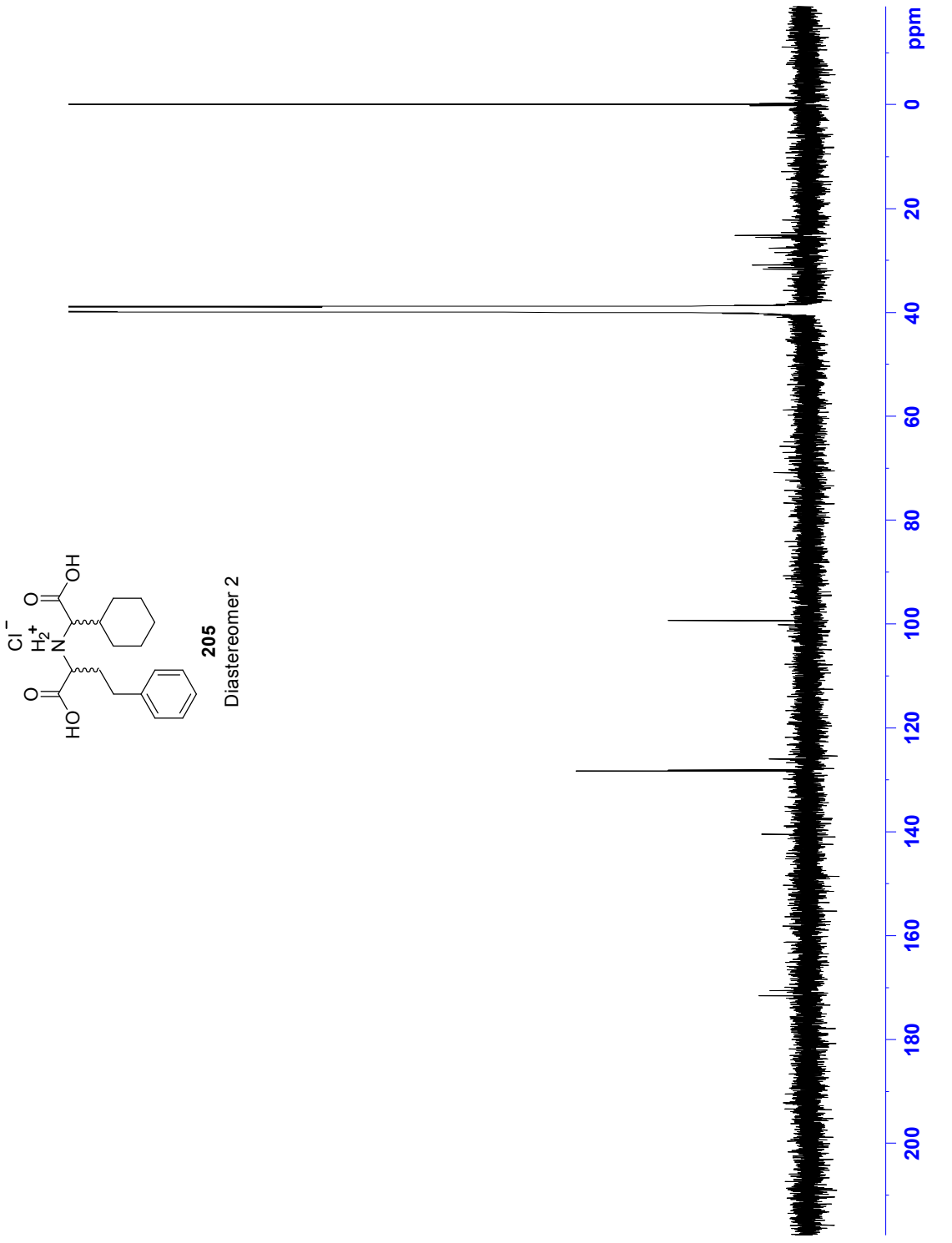

ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.96
4.00
0.54
10.09
Cl⁻
H2+N
O
OH
HO
204
Diastereomer 2

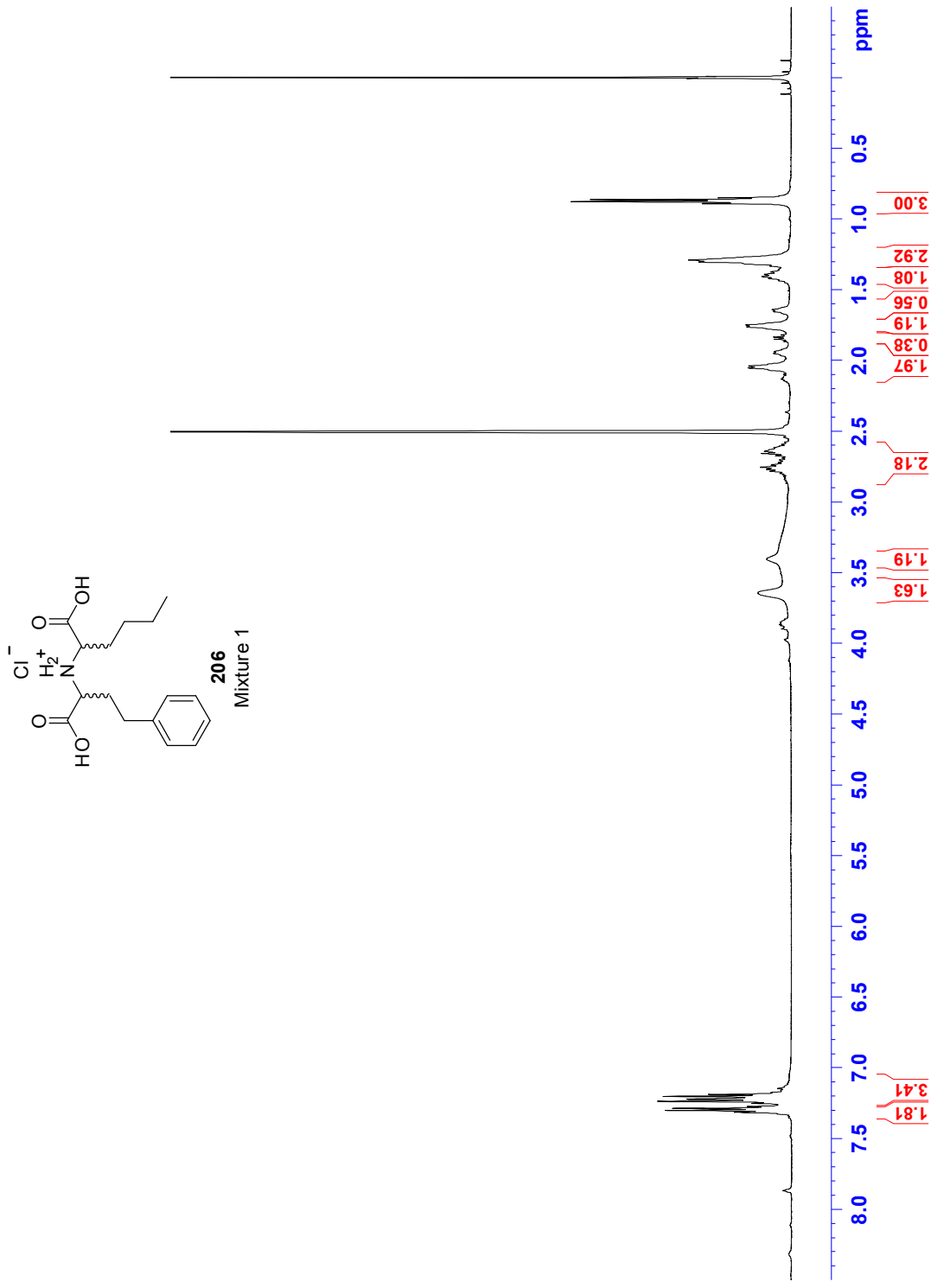


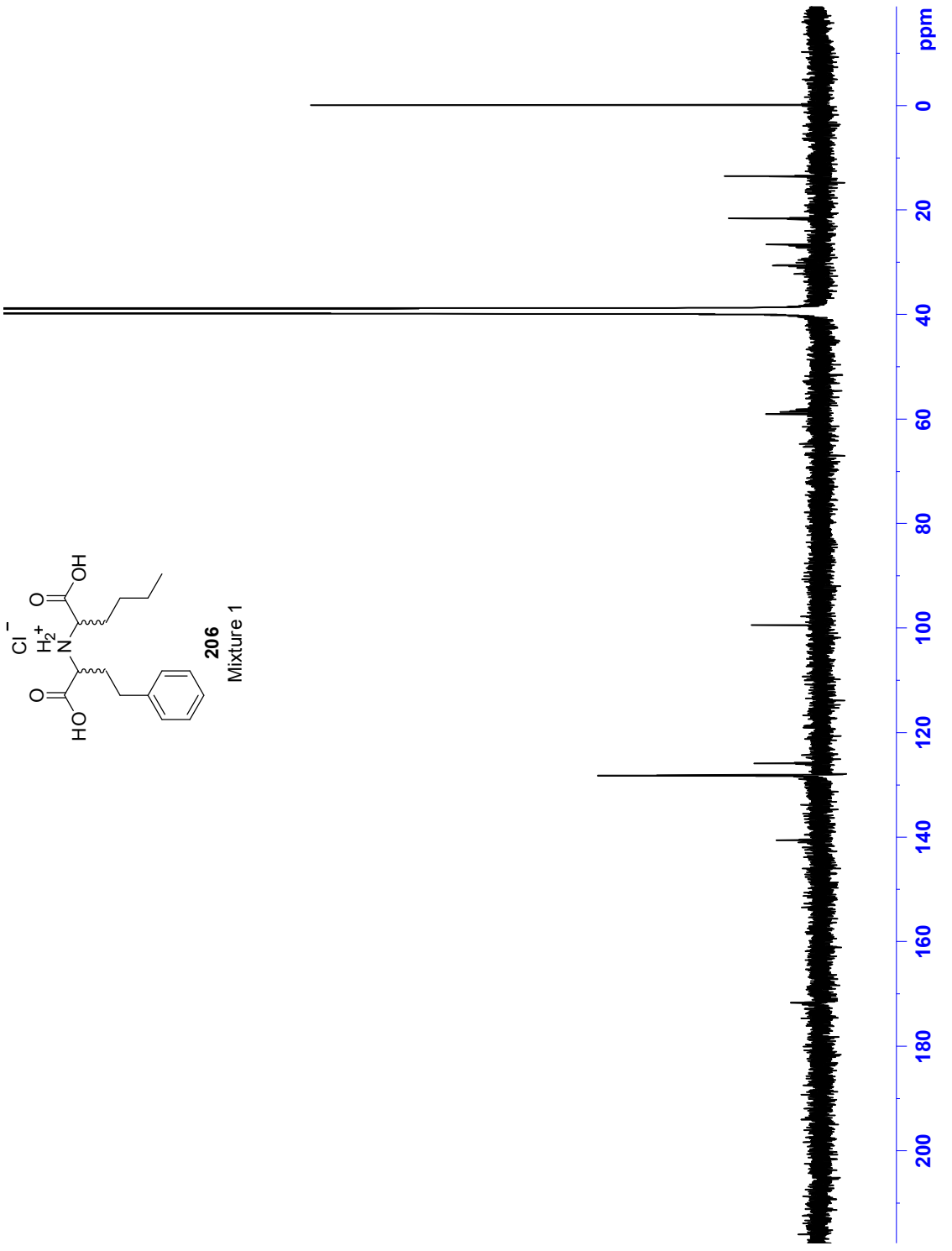
204
Diastereomer 2

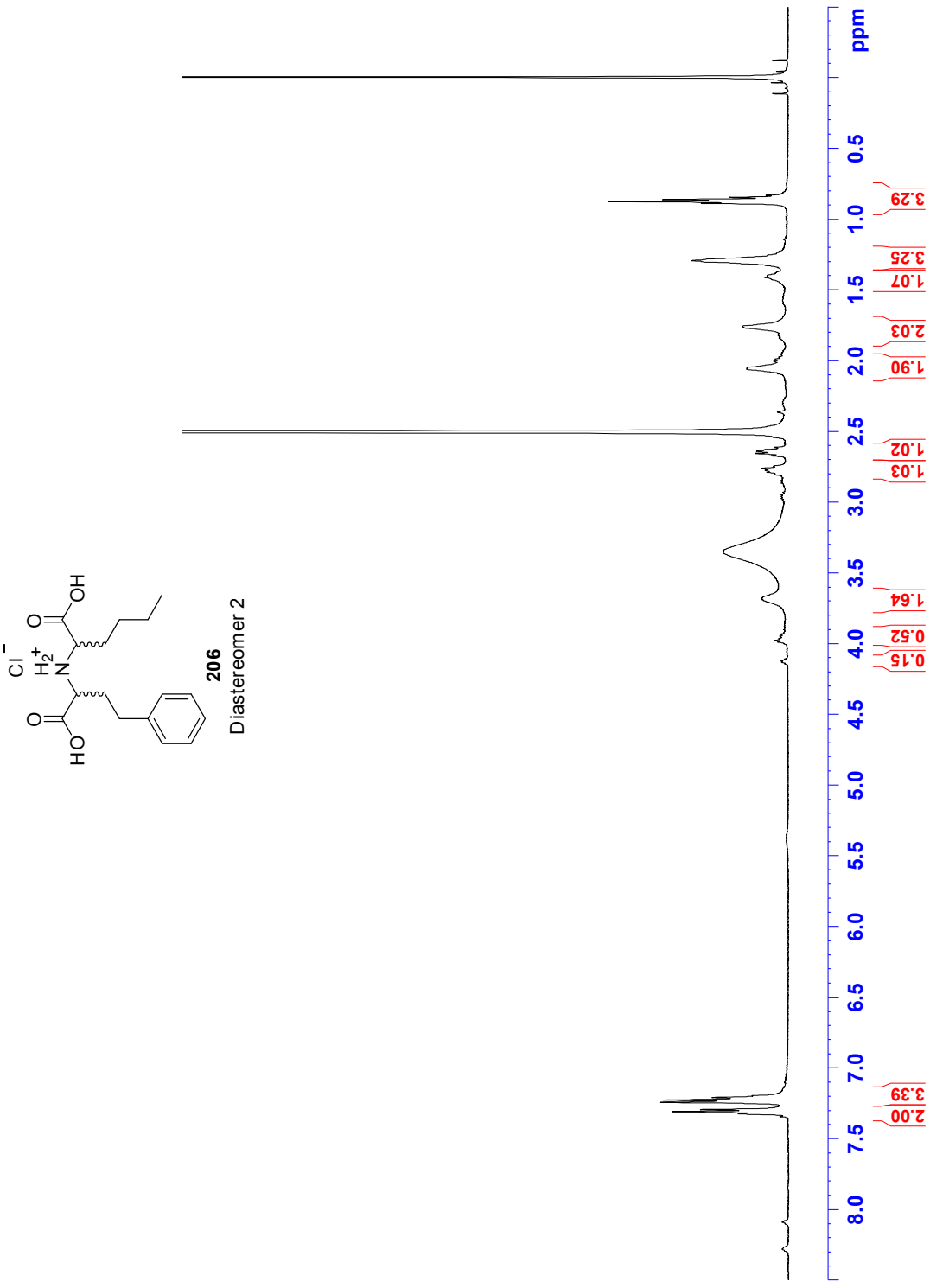

205
Diastereomer 1
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
5.00
3.16
3.17
2.03
1.64
0.83
0.99
1.31
0.61
5.67

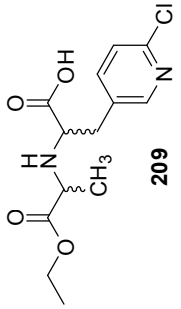

205
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
5.06
0.47
5.12
1.58
2.08
0.73
1.04
0.42
0.80
0.59
3.39
2.00


205
Diastereomer 2
ppm
0
20
40
60
80
100
120
140
160
180
200

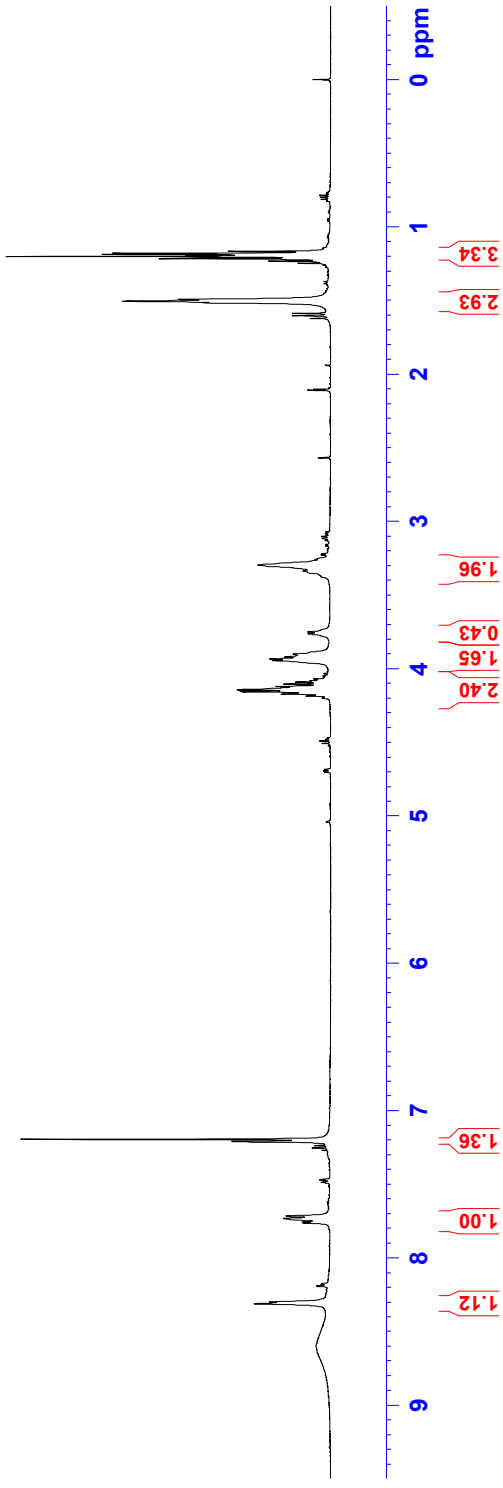

206
Mixture 1
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.00
2.92
1.08
0.56
1.19
0.38
1.97
2.18
1.19
1.63
3.41
1.81

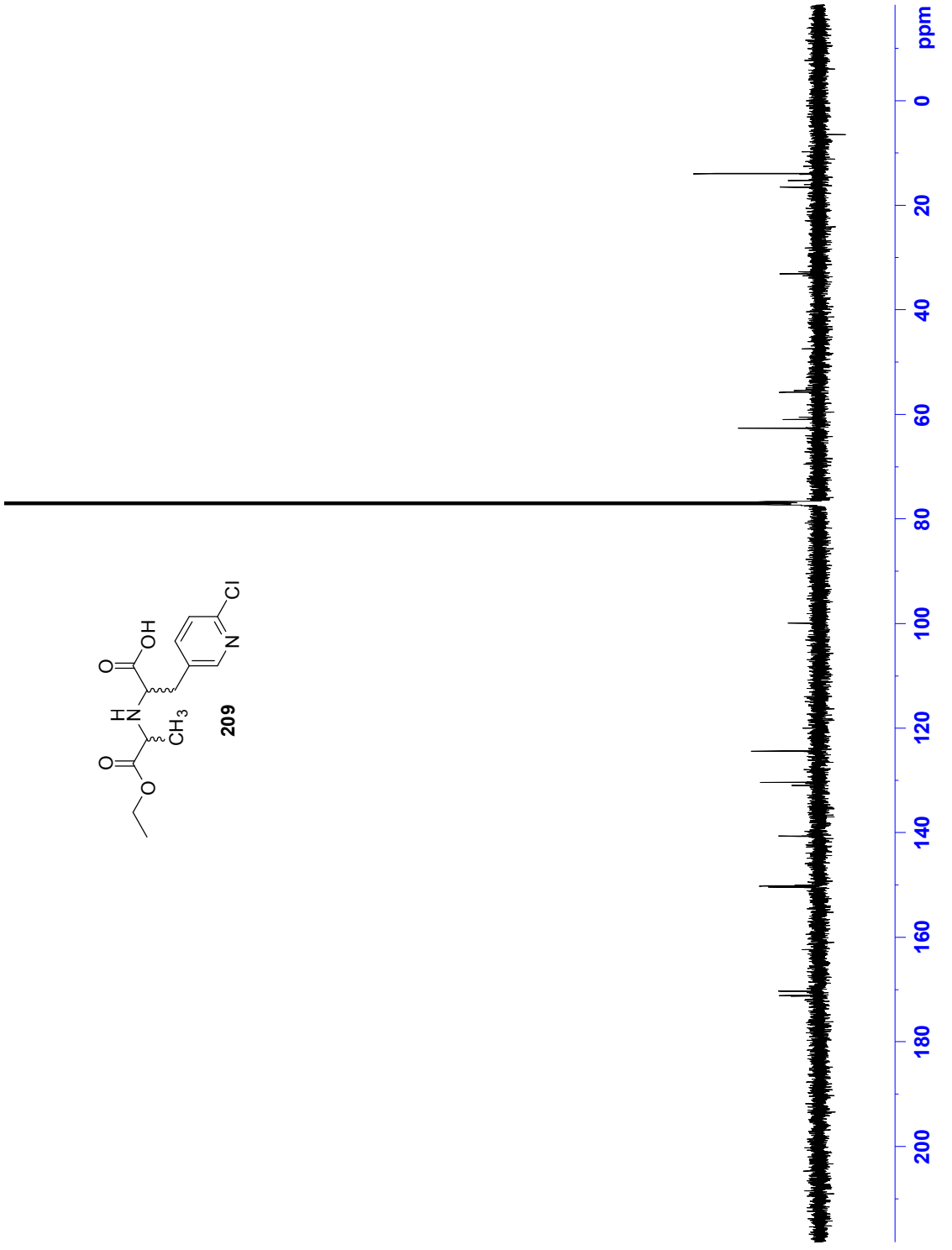

206
Mixture 1
ppm
0
20
40
60
80
100
120
140
160
180
200

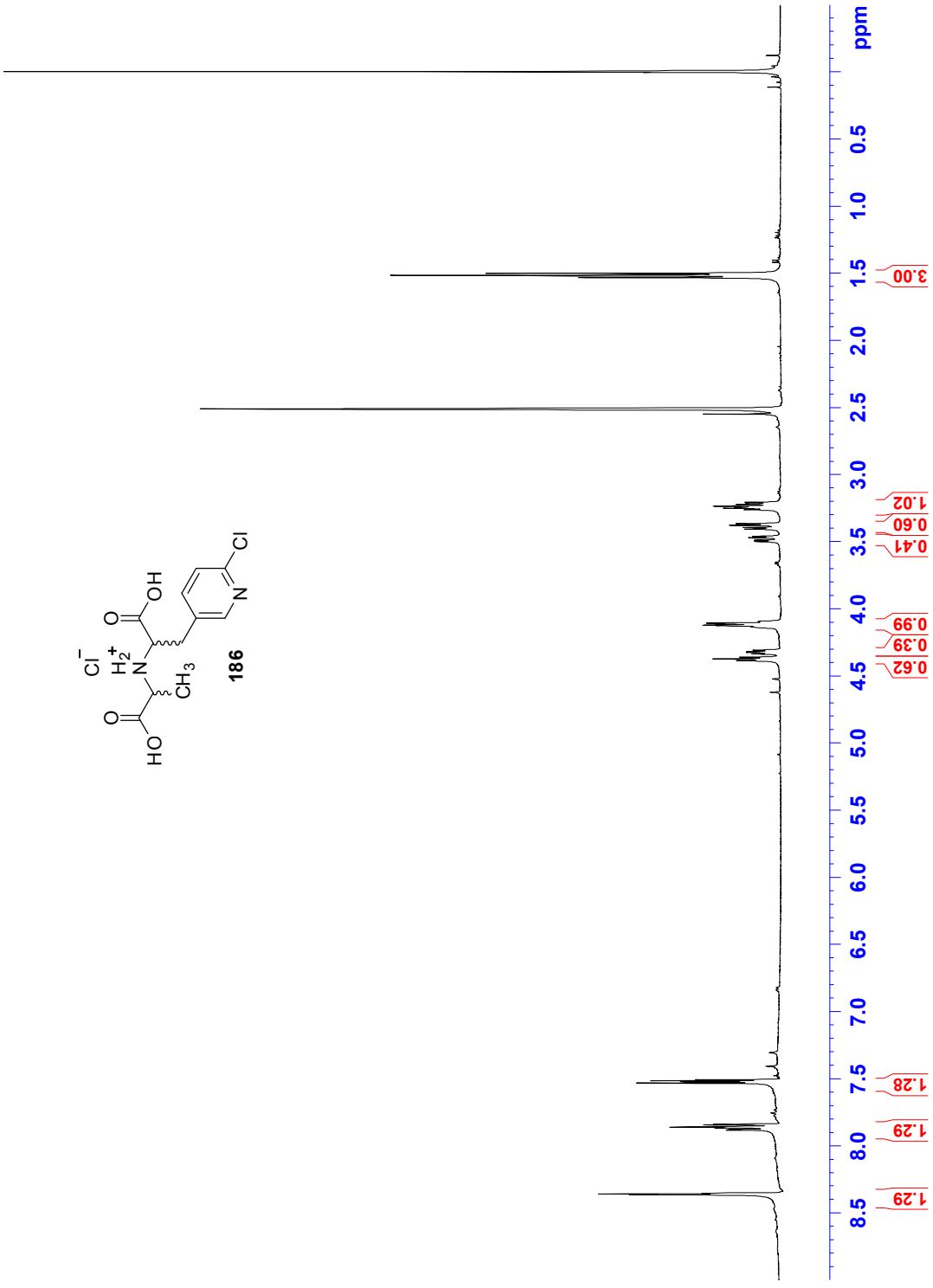

206
Diastereomer 2
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.29
3.25
1.07
2.03
1.90
1.02
1.03
1.64
0.52
0.15
3.39
2.00

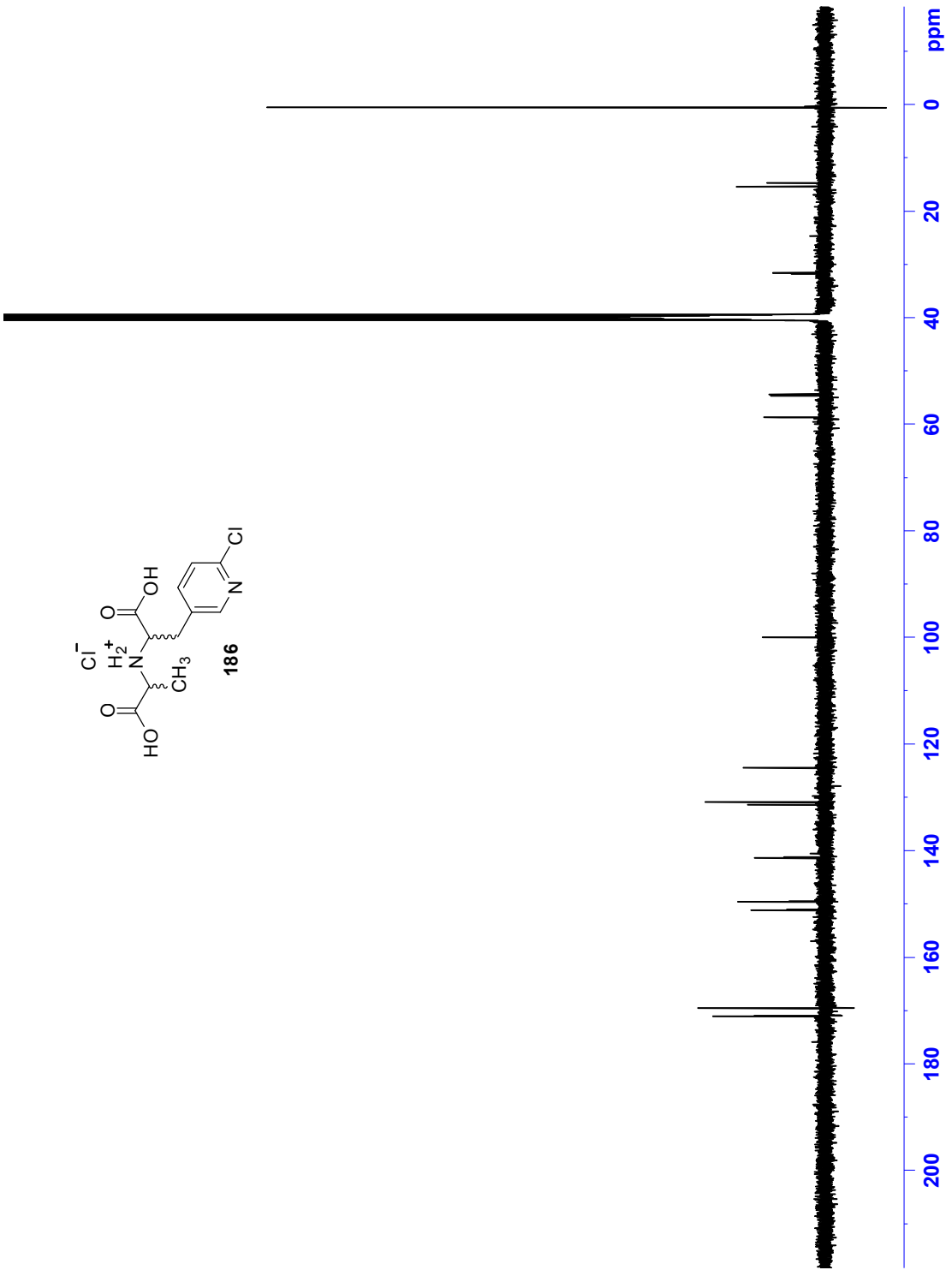

O
OH
H
N
CH3
O
O
Cl
N

209


0 ppm
1
2
3
4
5
6
7
8
9
3.34
2.93
1.96
0.43
1.65
2.40
1.36
1.00
1.12


ppm
0
20
40
60
80
100
120
140
160
180
200
O
OH
N
H
CH3
O
O
Cl
N
209


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
3.00
1.02
0.60
0.41
0.99
0.39
0.62
1.28
1.29
1.29
Cl
OH
O
N
H2+
N
CH3
O
HO
Cl−
186


ppm
0
20
40
60
80
100
120
140
160
180
200
Cl
N
OH
O
H_2^+
N
CH_3
O
HO
Cl^-
186

Appendix B. 2D NMR Experiments

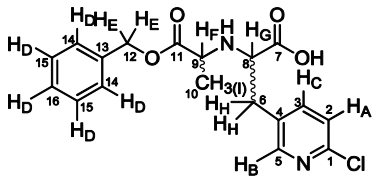


Table 7. Correlation of Proton and Carbon Shifts from the 2D NMR Experiments.

Carbon Shift	Carbon Number	Proton Shift	Proton Letter	Carbon Shift	Carbon Number	Proton Shift	Proton Letter
174.3	7	----	----	127.9	14, 15,16	7.31-7.39	H_D
174.1	7	----	----	127.8	14, 15,16	7.31-7.39	H_D
174.1	11	----	----	127.7	14, 15,16	7.31-7.39	H_D
173.6	11	----	----	123.4	3	7.41	H_C
150.4	2	8.24	H_B	123.3	3	7.41	H_C
150.3	2	8.24	H_B	65.7	12	5.06	H_E
148.2	1	----	----	65.5	12	5.12	H_E
148.1	1	----	----	59.4	8	3.47	H_G
140.6	5	7.70	H_C	59.2	8	3.47	H_G
140.4	5	7.70	H_C	54.1	9	3.43	H_F
135.9	13	----	----	54.1	9	3.39	H_F
135.9	13	----	----	34.5	6	2.88, 2.80	H_H
133.1	4	----	----	34.2	6	2.90, 2.82	H_H
133.1	4	----	----	18.4	10	1.18	H_I
128.3	14, 15, 16	7.31-7.39	H_D	18.3	10	1.14	H_I
127.9	14, 15,16	7.31-7.39	H_D				

Red = More abundant diastereomer
Green = Less abundant diastereomer
Black = Indistinguishable