UNIVERSITY OF CALIFORNIA SAN DIEGO

SAN DIEGO STATE UNIVERSITY

Total Synthesis of Marine Natural Products: Micromide, Its Analogs, and Lagunamide A

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Chemistry

by

Lee Wang

Committee in charge:

University of California San Diego

Professor Stanley Opella
Professor Emmanuel Theodorakis

San Diego State University

Professor B. Mikael Bergdahl, Chair
Professor Thomas Cole
Professor Forest Rohwer

2019

The Dissertation of Lee Wang is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

_____

_____

_____

_____

Chair

University of California, San Diego

San Diego State University

2019

Dedication

To my grandparents, who were unable to witness this work;

To my parents, who have shown decades of patience and support;

To my daughter, whom I hope to inspire patience and persistence.

Table of Contents

List of Figures

List of Schemes

List of Abbreviations

APCI	Atmospheric Pressure Chemical Ionization
BOP(-Cl)	bis(2-oxo-3-oxazolidinyl)phosphinic (chloride)
COSY	Correlation Spectroscopy
CTC	2-chlorotritylchloride
DCC	*N,N'*-dicyclohexylcarbodiimide
DIAD	diisopropyl azodicarboxylate
DIC	*N,N'*-diisopropylcarbodiimide
(D)IPC	(di)isopinocampheyl
DIPEA	diisopropylethylamine
DKP	diketopiperazine
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMS	dimethylsulfide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	ethyl acetate
FID	Free Induction Decay
Fmoc	fluorenylmethyloxycarbonyl
HATU	hexafluorophosphate azabenzotriazole tetramethyl uronium
Hila	hydroxyisoleucic acid
HMBC	Heteronuclear Multiple Bond Coherence
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
HRFABMS	High Resolution Fast Atom Bombardment Mass Spectrometry
HRMALDI	High Resolution Matrix-Assisted Laser Desorption/Ionization
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
HWE	Horner-Wadsworth-Emmons
LAH	lithium aluminum hydride
LCMS	Liquid Chromatography Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy

PDC	pyridinium dichromate (Cornforth Reagent)
p-NBA	*para*-nitrobenzoic acid
p-NBCl	*para*-nitrobenzoyl chloride
PTSA	*para*-toluenesulfonic acid
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBroP	bromotripyrrolidinophosphonium hexafluorophosphate
ROESY	Rotating Frame Nuclear Overhauser Effect Spectroscopy
SMEAH	sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al®)
SPPS	Solid-Phase Peptide Synthesis
TBS	*tert*-butyldimethylsilyl
TBTU	*N,N,N′,N′*-Tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate
TDPS	*tert*-butyldiphenylsilyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMSD	trimethylsilyldiazomethane
VMAR	Vinylogous Mukaiyama Aldol Reaction

Acknowledgements

I would like to acknowledge Professor Mikael Bergdahl for his many years of support as mentor and as chair of my committee, and to the many undergrads that assisted me in the lab, especially Nick Hernandez and Rukman Thota, whose work was indispensable.

I would also like to acknowledge Dr. LeRoy Lafferty and Dr. Bennett Addison, former NMR Facility Directors, for their assistance; Professor Jeffery Gustafson for the use of his equipment, which saved me countless hours of extra work; members of the Holland lab for their assistance with the peptide synthesizer; Professor Andrew Cooksy for his support and collaboration. Mass spectra was acquired by Josh Swider.

Special acknowledgement to Michael Kelly, Scott Burley, David Schmit, Timothy Montgomery, Brent Banasik, Arielle Kanner, *vale* David Jones, and the many members of the Bergdahl lab for their camaraderie and companionship.

Chapter 2 includes material which appeared in Tetrahedron, 2016, Banasik, Brent; Wang, Lee; Kanner, Arielle; Bergdahl; B. Mikael, Elsevier, 2016.

Vita

2003-2007	U.S. Air Force
2007	Associate in Applied Science, Electronic Systems Technology, Community College of the Air Force, Maxwell-Gunter AFB, AL
2010	Bachelor of Science, Chemical Physics, San Diego State University, San Diego, CA
2011-2017	Teaching Associate, San Diego State University, San Diego, CA
2016	Master of Arts, Chemistry, San Diego State University, San Diego, CA
2018-2019	Scientist I, AxisPharm LLC, San Diego, CA
2019	Doctor of Philosophy, University of California San Diego, San Diego State University, San Diego, CA

Publications

"Further Insight into the Asymmetric Vinylogous Mukaiyama Aldol Reaction (VMAR); Application to the Synthesis of the C27-C45 Segment of Lagunamide A" Tetrahedron, Issue 19, Volume 72, May 2016, Pages 2481-2490, https://doi.org/10.1016/j.tet.2016.03.079.

ABSTRACT OF THE DISSERTATION

Total Synthesis of Marine Natural Products:
Micromide, Its Analogs and Lagunamide A

by

Lee Wang

Doctor of Philosophy in Chemistry

University of California, San Diego, 2019
San Diego State University, 2019

Professor B. Mikael Bergdahl, Chair

The total syntheses of two peptidic cytotoxins, micromide and lagunamide A was completed employing both solid-phase and solution-phase methods. Micromide was isolated from an unknown species of marine cyanobacterium of the genus *Symploca*. Its synthesis was accomplished using both solid-phase and solution-phase chemistry. Comparison of the synthetic and natural product's NMR spectra reveal that the reported structure of micromide was incorrect. Syntheses of multiple analogs of micromide was completed to resolve the discrepancies in the NMR spectra. The new data suggests that micromide requires a structural revision.

Lagunamide A is a cyclic depsipeptide also isolated from marine cyanobacteria, originally identified as *Lyngbya majuscula*. Recent genetic analysis found that a separate

cyanobacterium, *Moorea procudens*, exhibited very similar morphology to *L. majuscula* and many natural products were mistakenly attributed to *L. majuscula*. The origin of lagunamide A has not been confirmed after this new revelation. Synthesis of the peptide portion was achieved through solid-phase peptide synthesis (SPPS) and the polyketide moiety was achieved using Kobayashi's vinylogous Mukaiyama aldol reaction (VMAR).

Introduction

The toxicity of the mollusk *Dolabella auricularia*, commonly known as “sea hare”, was well known and in 1972, Pettit and co-workers discovered that extracts of *D. auricularia* showed strong anti-cancer effects.[1] Further investigation resulted in the discovery and isolation of dolastatins. Unfortunately, the amount of this bioactive material was so small, it took 1000 kilograms of *D. auriculari* and 15 years to isolate and characterize the most potent component of these extracts: dolatstatin 10. Dolastatins existed in such small amounts that only 28.7 mg of product was isolated from the massive bulk. Much the same as tetradotoxin from pufferfish, it was later discovered that these potent peptides existed in *D. auricularia* through bioaccumulation and were actually produced by microbes, specifically, the cyanobacteria *Symploca hydnoides* and *Lyngbya majuscula*[*] that the sea hare consumed. This is unsurprising since many marine cyanobacteria are well-known to be toxic. Since then, a plethora of natural products have been isolated from cyanobacteria such as pulau’amide, belamide A, grassystatins, lagunamides, apramides, among many others.[2–6]

Since the discovery of the cytotoxin source, obtaining useful amounts of these natural products has become much easier. In some cases, the cyanobacteria could even be cultured. However, microorganisms mutate frequently and while many natural products were discovered, their expression of particular natural products are not consistent.[7] These compounds are the result of post-translational modifications i.e. secondary metabolites which means that their production is not necessarily conserved across all populations. Much the same as other microorganisms like yeast, various populations or strains will express different metabolites. Even the same strain will change due to environmental stressors. In the case of apramide,

[*] *Lyngbya majuscula* was previously categorized based on traditional morphology and has recently been divided into several new genera based on genetic analysis. Natural products originally derived from *L. majuscula* are most likely from *Moorea procudens*.

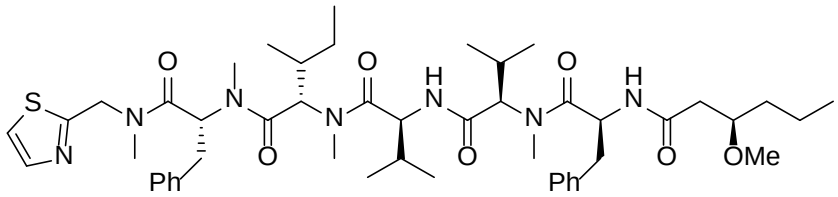
repeated collection of *Symploca* samples over time ceased to provide apramide.[8] For these reasons, it is necessary to develop synthetic methods to produce these molecules.

In addition to addressing the difficulty of obtaining sufficient amounts of these natural products, elucidation of these secondary metabolites remains a difficult task. It is common practice to determine the chirality of the amino acid residues by hydrolysis and then compare its retention time to a reference on chiral HPLC. However, due to the harsh hydrolysis conditions, it is common for amino acids to epimerize during hydrolysis.[9] When hydrolysis products are compared to standards by resolution on chiral HPLC, it is possible that false positives lead to the incorrect assignment of stereochemistry.[10] These corrections to the stereochemistry are only verified by comparison to their synthetic counterparts. Indeed, a simple search on Web of Science of journal articles with the terms “stereochemical revision” or “structural revision” in the title, published in the past five years returned nearly a hundred matches. In fact, both natural products discussed in the following chapters were initially mischaracterized.

Due to its initial mischaracterization, the discussion of each molecule will begin with their original proposed structures, as the focus of this dissertation pertains to the chemical strategies used in the total synthesis, followed by the modifications used in the revised structures.

Chapter 1. Micromide

In early 2003, the marine natural product micromide (**1**) was described by Williams et al. as a linear lipopeptide, containing a thiazole-modified glycine unit, five amino acid residues, and a β-methoxyhexanoyl tail (Figure 1).[11] Micromide was isolated from marine cyanobacteria of the genus Symploca and showed excellent potency against solid-tumor KB cells (IC_{50} = 260 nM).



1

Figure 1. Micromide as Initially Published

The same cyanobacteria have been a prolific source of bioactive natural products, most of which are post-translationally modified peptides. Among these bioactive molecules discovered, include apramides,[6] ulongamides,[12] grassystatins,[4] symplostatins,[13,14] and dolastatins,[15] the last of which reached phase II clinical trials. Currently, no molecular pharmacology studies on micromide have been performed, however, based on the trend followed by this class of natural products, we anticipate that micromide is an antimitotic agent, preventing tumor growth by inhibition of microtubule polymerization.[16]

The total synthesis of micromide was completed in 2009 by our group, but remained published only in students' theses because a comparison of the synthetic and natural products indicated that a structural revision was necessary. While our efforts to determine the necessary structural changes, a separate total synthesis of micromide was completed by Han and co-workers in 2014.[17] Their synthesis features many similar methods used in our synthesis including the use of the nosyl protecting group for site-selective *N*-methylations, and acid

chloride couplings, but despite dramatic differences in the reported spectral data, no effort was made to reconcile the differences between the natural and synthetic products.

The journey to revise the structure of micromide has been an arduous one. Based on the data provided in their paper, there was no reason to doubt their assessment of micromide until the synthetic product was completed, and it was clear that it did not match the natural product. Micromide was synthesized utilizing multiple routes in our lab and combined with the Han synthesis, the total of four syntheses via various methods firmly establishes that **1** is not the structure of the natural product. Since the work produced by Williams et al. did not show any obvious flaws, there was not a solid starting point for our investigation. We contacted Williams in hopes of obtaining the original FID files for the NMR spectra. Unfortunately, the backups at the

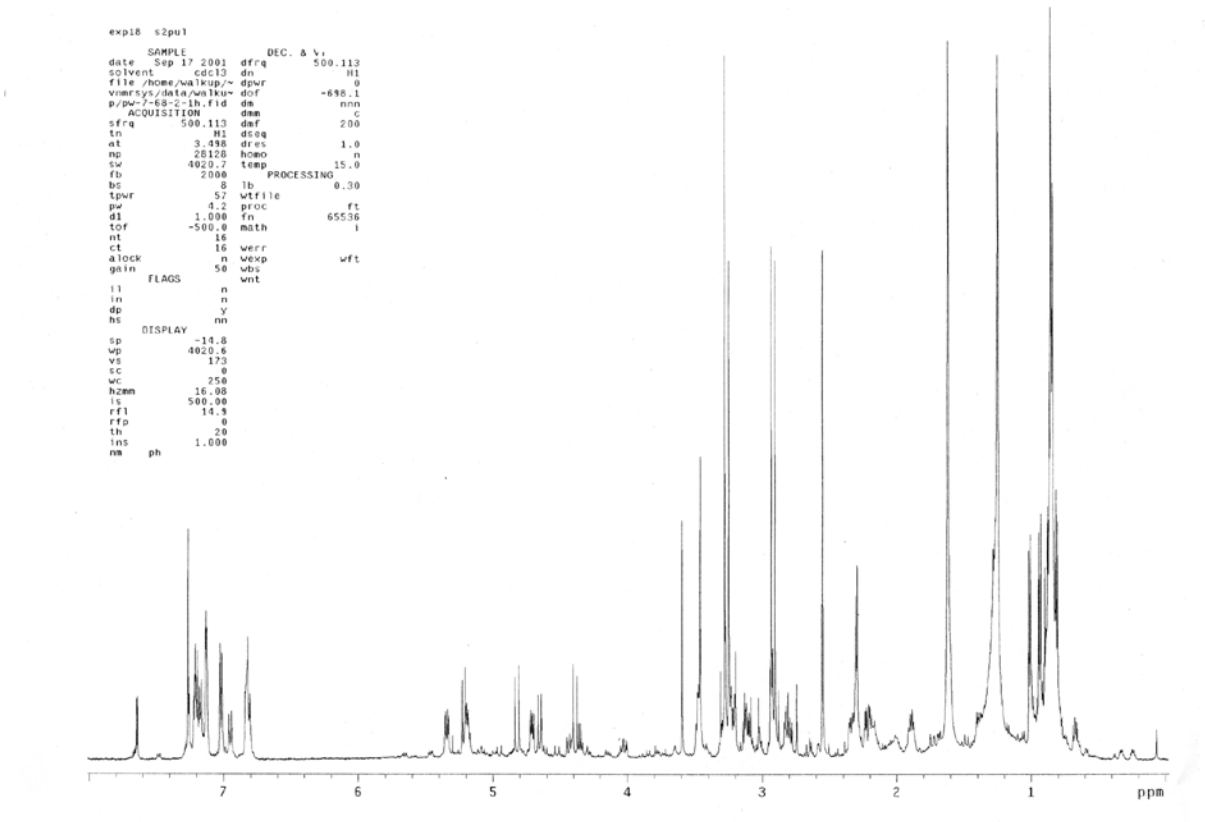
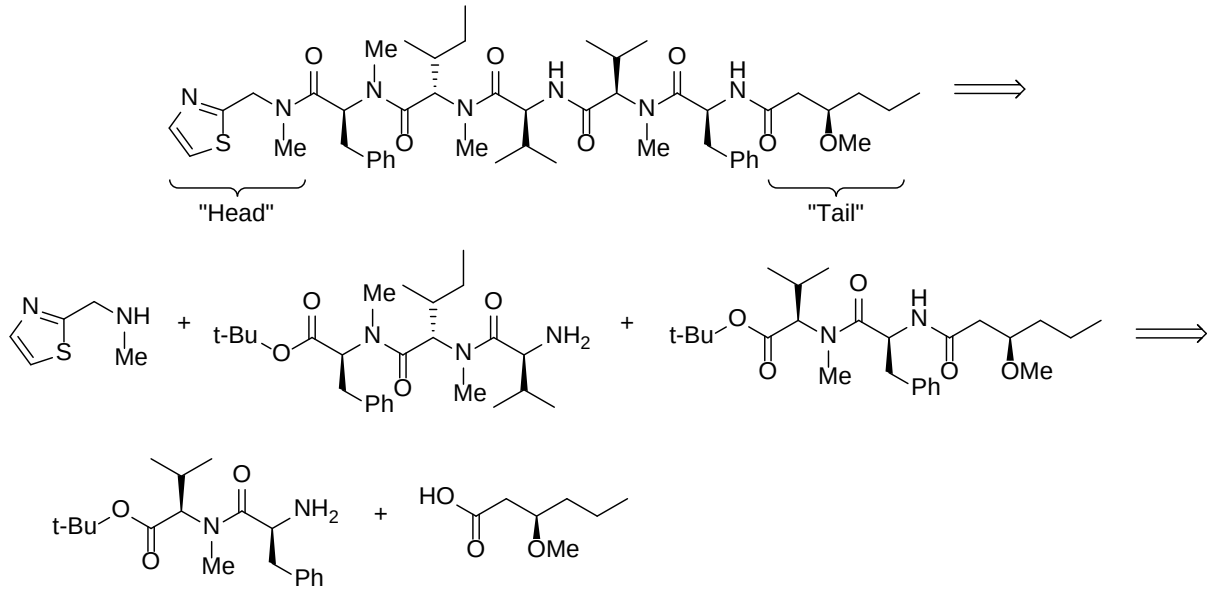


Figure 2. Sole Surviving Spectrum of Micromide

University of Hawai'i had become corrupted and we were left with a single ^{1}H spectrum of the natural product (Figure 2) and the tabulated data from the publication. Thus, our only option was to systematically probe potential faults in their various methods of analysis, based on differences between the natural and synthetic products. This chapter describes our various syntheses of multiple analogs, made in our attempts to produce a structural revision of micromide.

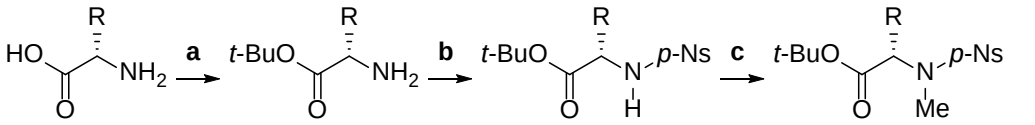
1.1 Retrosynthesis (Original Structure)



Scheme 1. Retrosynthesis of Micromide

Our initial synthesis of **1** was fairly straightforward. Due to the difficult nature of coupling *N*-methylated amino acids, the sterically hindered residues would be coupled via the acid chloride. We would take advantage of the secondary amide in the center by producing the left tripeptide and right lipopeptide and coupling them in a convergent manner (Scheme 1). The modified glycine-thiazole unit would be produced by reductive amination of 2-thiazolecarboxaldehyde and the β-methoxyhexanoyl moiety by asymmetric conjugate addition.

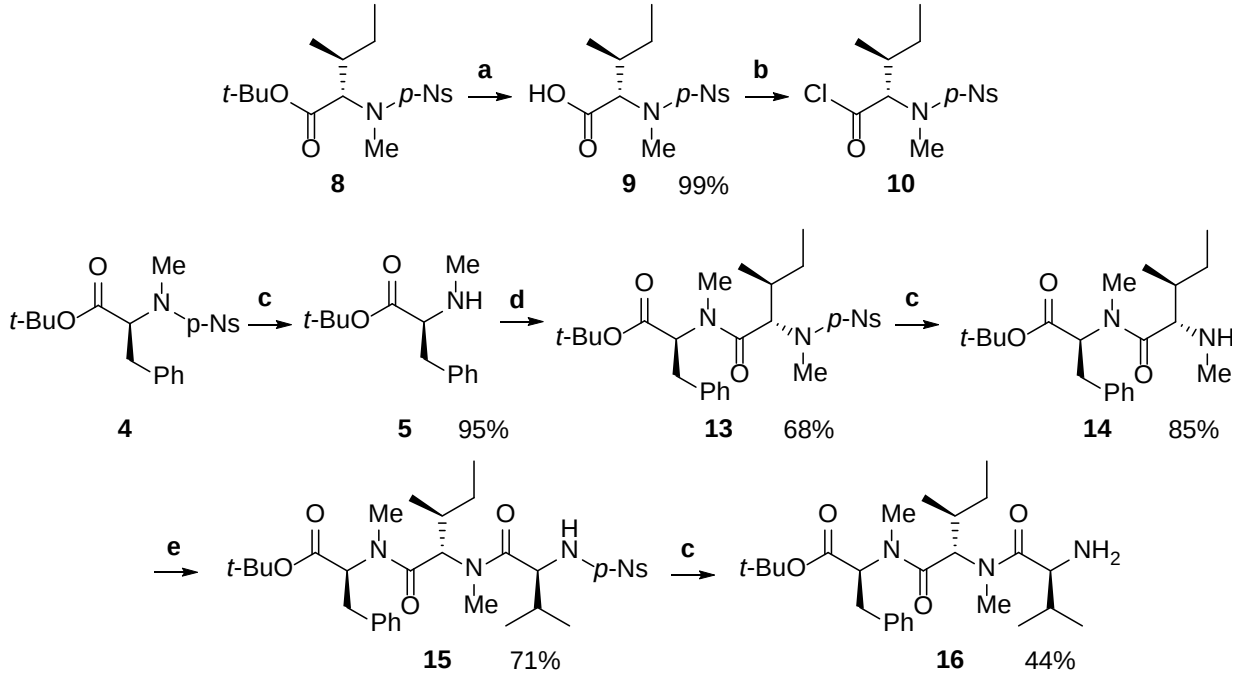
1.2 Synthesis of the Peptide



Conditions: a) *t*-BuOAc, $HClO_4$; b) *p*-NsCl, Et_3N, CH_2Cl_2; c) CH_3I, K_2CO_3, DMF

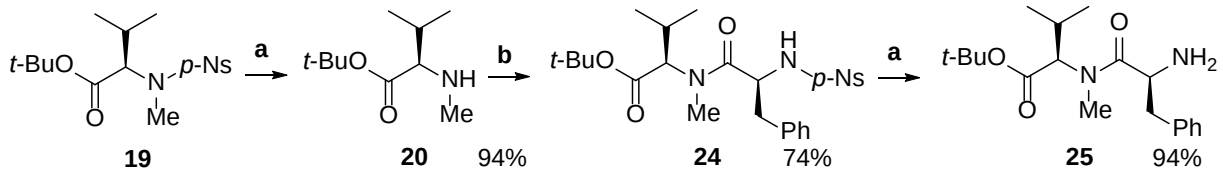
Scheme 2. *N*-Methylation and Peptide Coupling

The initial work on micromide was initiated by prior members of the group, who first produced the *N*-methyl amino acids modifying a procedure reported by Fukuyama (Scheme 2).[18] The use of the nosyl protecting group provides two purposes: protection of the amine and increasing the acidity of the *N*-H proton, reducing risk of epimerization of the α-proton under



Conditions: a) TFA, CH_2Cl_2; b) $SOCl_2$; c) mercaptoethanol, K_2CO_3, DMF; d) **10**, DIEA, CH_2Cl_2; e) **12**, DIEA, CH_2Cl_2

Scheme 3. Synthesis of the Tripeptide



19 **20** 94% **24** 74% **25** 94%

Conditions: a) mercaptoethanol, K_2CO_3, DMF; b) **23**, Et_3N, CH_2Cl_2

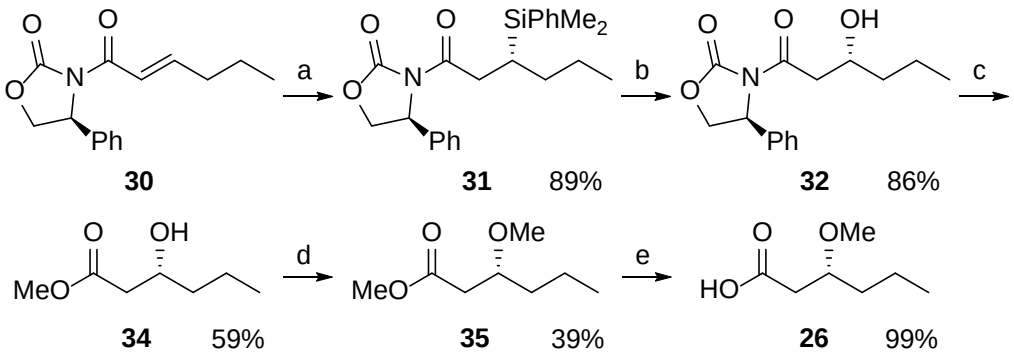
Scheme 4. Synthesis of the Dipeptide

basic conditions. However, care must be used for nosyl-protected amino acids that have not undergone methylation. The increased acidity of the *N*-H proton promotes self-condensation of the acid chloride in the presence of base. The protected *N*-Me-Phe **4** was de-nosylated with 2-mercaptoethanol to give free amine **5**, while isoleucinate **8** was converted to free acid **9** in 1:1 TFA/CH_2Cl_2 (Scheme 3). The nosylated isoleucine was converted to acyl chloride **10** and coupled to phenylalanine fragment **5**, initially under Schotten-Baumann conditions. These coupling reactions were later optimized using anhydrous conditions and triethylamine as the base, giving dipeptide **13**. The dipeptide was de-nosylated and the process was repeated with L-Val to give the tripeptide **16**.

In a fashion similar to the tripeptide, the dipeptide was produced by methylating D-Val under the same Fukuyama conditions (Scheme 4). Denosylation of **19** gave amine **20**, and coupling to the acid chloride **23** gave protected dipeptide **24**. Deprotection of the dipeptide gave **25**.

1.3 Synthesis of the β-methoxyhexanoyl Fragment

Initial work by previous members produced fragment **26** by asymmetric aldol coupling using 2-hydroxy-1,2,2-triphenylethyl acetate as a chiral auxiliary as described by Braun.[19,20] This method produced the desired product in excellent yields (>90%) however, the resulting product was an inseparable mixture of diastereomers with a dr of 84:16. Subsequently, the synthesis of this fragment was improved by using a conjugate addition of a silylzincate to an α,β-unsaturated imide in excellent yield and a dr of 98:2 (Scheme 5). In this case, the diastereomers were separable by column chromatography to give the pure product as a single diastereomer.



Conditions: a) $Me_2PhSiZnEt_2Li$, THF, -78°C; b) $Hg(OAc)_2$, HO_2Ac, HOAc; c) NaOMe, MeOH; d) $CF_3SO_3CH_3$, di-*t*-Bu-4-MePyr, CH_2Cl_2; e) LiOH, THF, 0°C

Scheme 5. Synthesis of (*R*)-3-methoxyhexanoic acid **26**

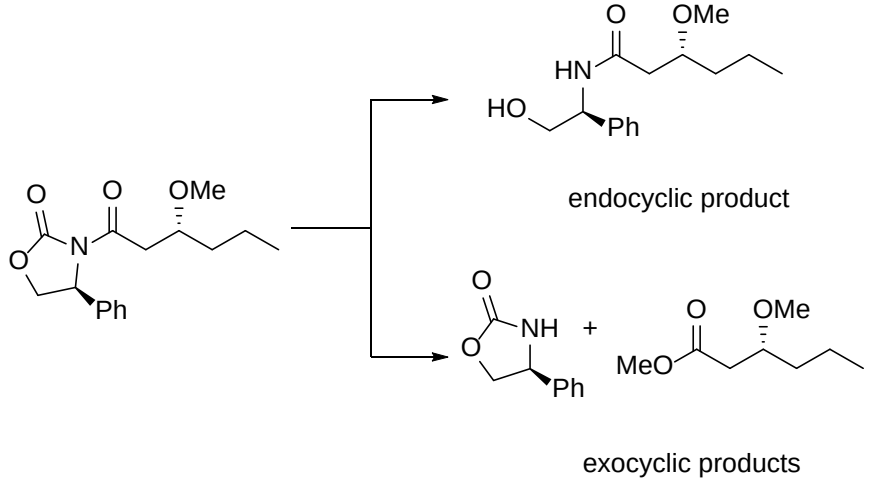
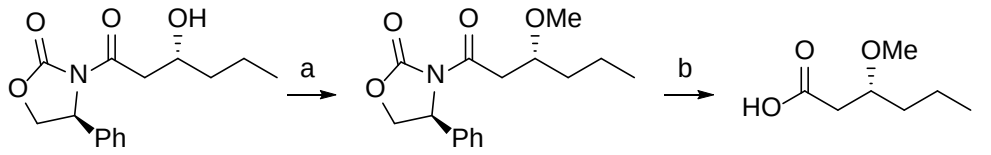


Figure 3. Regioslectivity of theMethoxide Nucleophile



32 **33** 73% **26** 90%

Conditions: a) TMSD, HBF_4, CH_2Cl_2, 0°C; b) LiOH, H_2O_2, THF, H_2O, 0°C

Scheme 6. Improved Synthesis of **26**

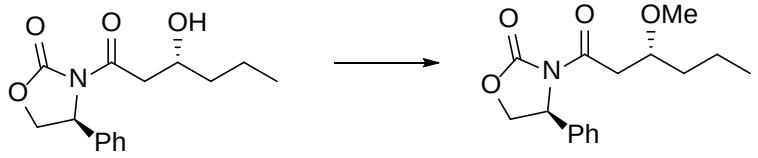
The phenylglycine-derived 2-oxazolidinone **28** was condensed with (*E*)-hex-2-enoic acid to give α,β-unsaturated imide **30**, which underwent conjugate addition by a silylzincate to give silane **31**. The resulting β-silane underwent Tamao-Fleming oxidation with mercury (II) acetate to give corresponding alcohol **32** with retention of stereochemistry.[21,22] In previous work, removal of the chiral auxiliary was achieved by methanolysis of the imide to methyl ester **34**, followed by methylation of the hydroxyl group, and finally saponification of the ester to give carboxylic acid **26**. This procedure produced inconsistent results, due to the poor regioselectivity of the methoxide. In most instances, the methoxide preferred endocyclic attack, resulting in the ring opened product (Figure 3).

The existing scheme was a work-around for a single complication: difficulty in methylating the β-oxygen. The auxiliary inhibits the methylation of the β-oxygen, but removal of the auxiliary prior to methylation would result in methylation of the carboxylic acid back to the ester and require a second saponification. To improve regioselectivity and reduce the number of synthetic steps, a modified route was proposed (Scheme 6). The hydroxyl group would be methylated prior to the removal of the auxiliary, and the product could be taken directly to the carboxylic acid via Evans' lithium hydroperoxide method.[23]

The original protocol using methyl triflate gave lackluster yields, suggesting that this particular hydroxy group would be difficult to methylate. Various conditions were surveyed (Table 1) and the mild conditions of trimethylsilyldiazomethane (TMSD) catalyzed with fluoroboric acid gave the best result, producing a 73% yield (77% converted yield) in one step—

a marked improvement on the previous 23% over two steps. With **33** in hand, the chiral auxiliary was oxidized with $LiOH/H_2O_2$ to give the necessary acid.

Table 1. Survey of Methylating Reagents

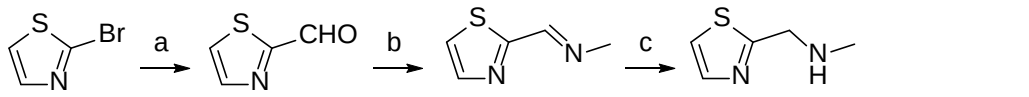


Entry	Methylating Reagent	Base/Catalyst	Yield
1	MeOTf	2,6-lutidine	No reaction
2	MeOTf	2,6-di-*tert*-butyl-4-methylpyridine	24%
3	MeOTf	imidazole	No reaction
4	Me_2SO_4	NaH	β-elimination
5	$TMSCHN_2$	HBF_4	77%

1.4 Final Couplings

The thiazole-modified glycine unit was produced through reductive amination of 2-thiazolecarboxaldehyde, inspired by Kempf et al. (Scheme 7).[24] Kempf's one-pot method provided a convenient route but it only provided a meager 18% yield. Mr. Changchun Jiang, a previous group member, elected to use a step-wise method to improve the yield. 2-Thiazolecarboxaldehyde was reacted with methylamine to give Schiff base **37**, followed by reduction using Cho's solvent-free conditions, to give thiazolamine **38**.[25] Jiang was unable to couple his thiazole to the peptide and it was later discovered that the thiazole was still complexed to boron. To completely remove the boron, it was necessary to reflux the product in methanol, defeating the purpose of the solvent-free method. In continuing the work on micromide, the solvent-free method was abandoned and the Schiff base was reduced with $NaBH_4$ in ethanol. While the imine was successfully isolated by flash chromatography, the majority of product reverted to the aldehyde, presumably catalyzed by the acidity of the silica gel. Therefore, subsequent reactions were immediately reduced without purification, giving amine **38** in 63% over two steps.

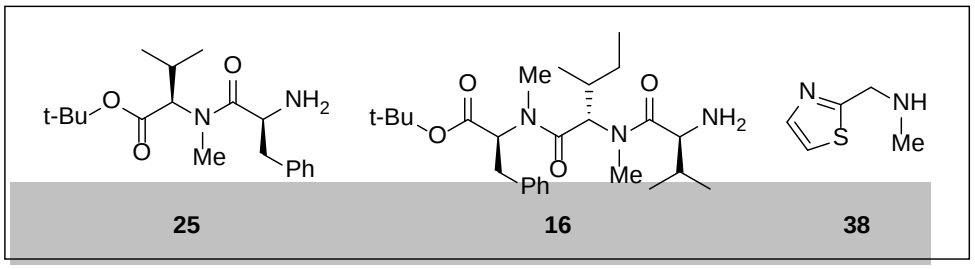
With all the necessary fragments in hand, β-ether **26** was coupled to dipeptide **25** with EDC/HOBt to give lipopeptide **39** (Scheme 8). Lipopeptide **39** was deprotected in TFA/CH_2Cl_2 to give free acid **40**. Tripeptide **16** was coupled to **40** using a mixture of HATU/TBTU, giving the lipopeptide precursor **41**. Deprotection of **41** in TFA/CH_2Cl_2 gave the free acid **42**, which was coupled to thiazolamine **38** to give micromide **1**, the structure as proposed by Williams.

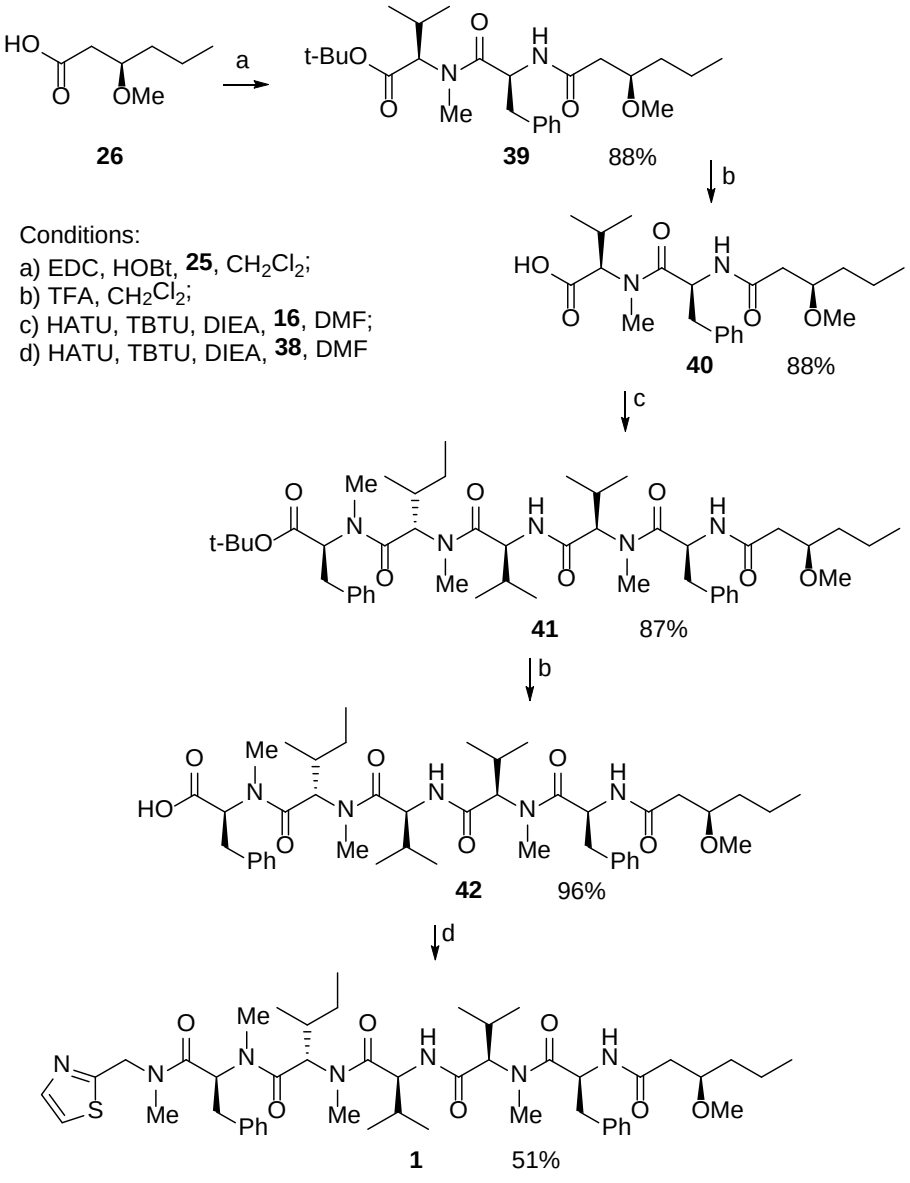


36 54% **37** 83% **38** 13% (solvent-free) 49%

Conditions: a) n-BuLi, DMF, Et_2O; b) $MeNH_2$·HCl, NaOH, MeOH; c) $B(OH)_3$, $NaBH_4$ *or* $NaBH_4$, EtOH

Scheme 7. Synthesis of the Thiazole-Glycine Unit





Scheme 8. Assembly of Micromide, **1**

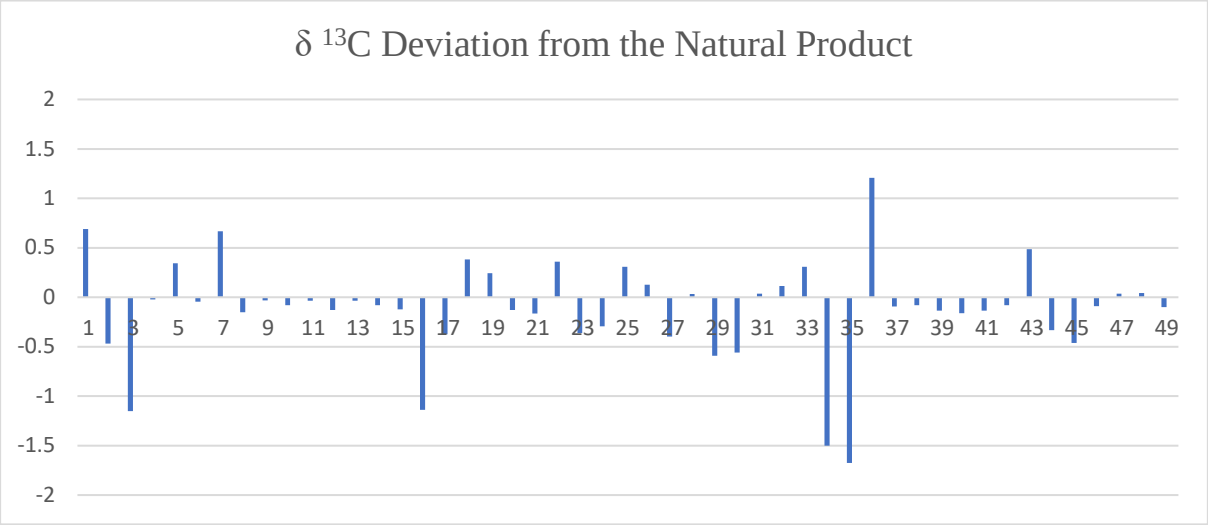


Figure 4. ^{13}C Chemical Shift Difference Between 1 and Natural Micromide

A stereoselective synthesis of the published structure for micromide was completed, and at this point it was discovered that the chemical properties of **1** did not match the reported natural product (Figure 5). Spectroscopically, the ^{1}H NMR spectrum exhibited significant differences in chemical shift, and exhibited a different optical rotation, though the sign was still the same. Biologically, the synthetic product showed no bioactivity against KB cells as originally described. It was evident that a structural revision was necessary.

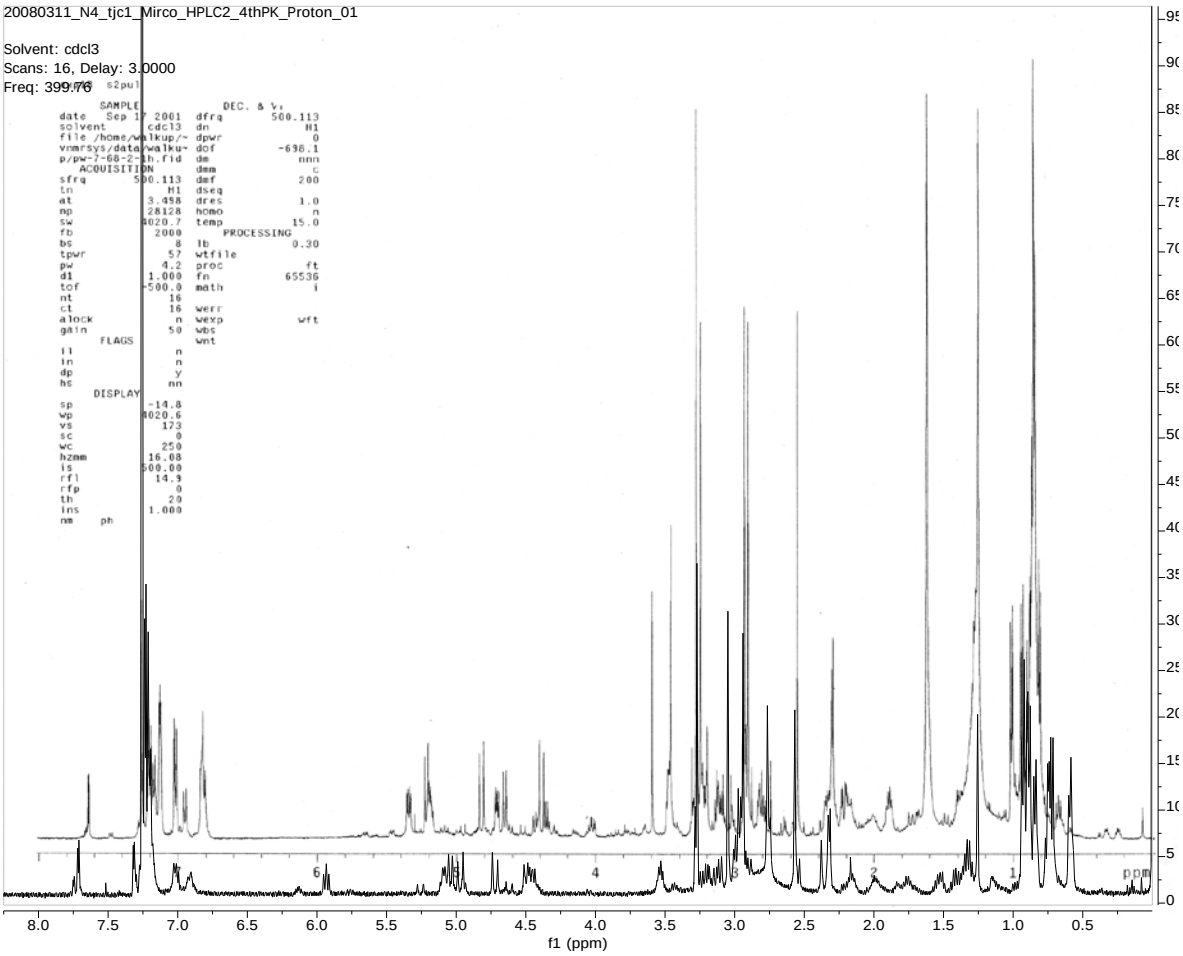


Figure 5. Comparison of **1** (Foreground) to Natural Micromide (Background)

1.5 Stereochemical Revision

Professor Williams at University of Hawai'i was contacted in hopes of obtaining the original NMR FID files. Williams notified us that the digitally archived files had unfortunately become corrupted over the years and he was unable to locate any other copies. Resources on hand were the published NMR spectral data table and a copy of the ^{1}H spectrum. Armed with what little was available, we began by examining the major differences in the spectral data. Within the ^{1}H spectra, the most evident difference was the chemical shift of *α*-proton of the *N*-Me-L-Phe residue (C7). In the natural product, this signal was found at 5.34 ppm but was shifted downfield to 5.93 ppm in the synthetic product. Comparison of the Δ-^{13}C signals between the synthetic product and the natural product found the largest differences in the chemical shifts of C34-C36, one of the phenylalanine residues. We reasoned that this could be the result of two possibilities: either the phenylalanine residue could be the D-enantiomer, or the shift was induced by a conformational change by the neighboring D-Val unit and should be replaced with L-Val.

For the first case, substituting the *N*-Me-L-Phe with *N*-Me-D-Phe gives the proposed epimer **1a** (Figure 6). But complicating things further, the ^{1}H data and ^{13}C data were not in agreement. Where the ^{1}H NMR data suggested a change at C7, the ^{13}C NMR data suggested a change at C35. The initial report of micromide described difficulty in resolving the Phe carbon signals, finding only 44 of the 49 necessary signals. It was inferred that two phenyl rings existed from the elemental composition, determined by HRMS data. It would make sense if the phenyl peaks overlapped and integration of the peak areas should provide insight into the number of protons. However, connectivity was determined by $^{3}J_{C\text{-}H}$ couplings between the H-15 to C-7 and

C-16, and H-22 to C-17 and C-23. Given that two Phe and two Val residues exist, and the close proximity of the peaks, their correlations may have been mis-assigned. Without the original HSQC and HMBC spectra, we were unable to verify if the original spectroscopic assignments were reported correctly. Given the possibility that the phenylalanine assignments were transposed, isomers **1b** and **1c** were included in the list of potential candidates.

For the second case, it can be argued that the existence of a D-amino acid seems out of place. While it is not uncommon to find D-amino acids in secondary metabolites due to post-translational modifications or epimerization, similar lipopeptides from *symploca spp*. do not contain D-amino acids, suggesting that no biosynthetic pathway exists for enzyme-catalyzed

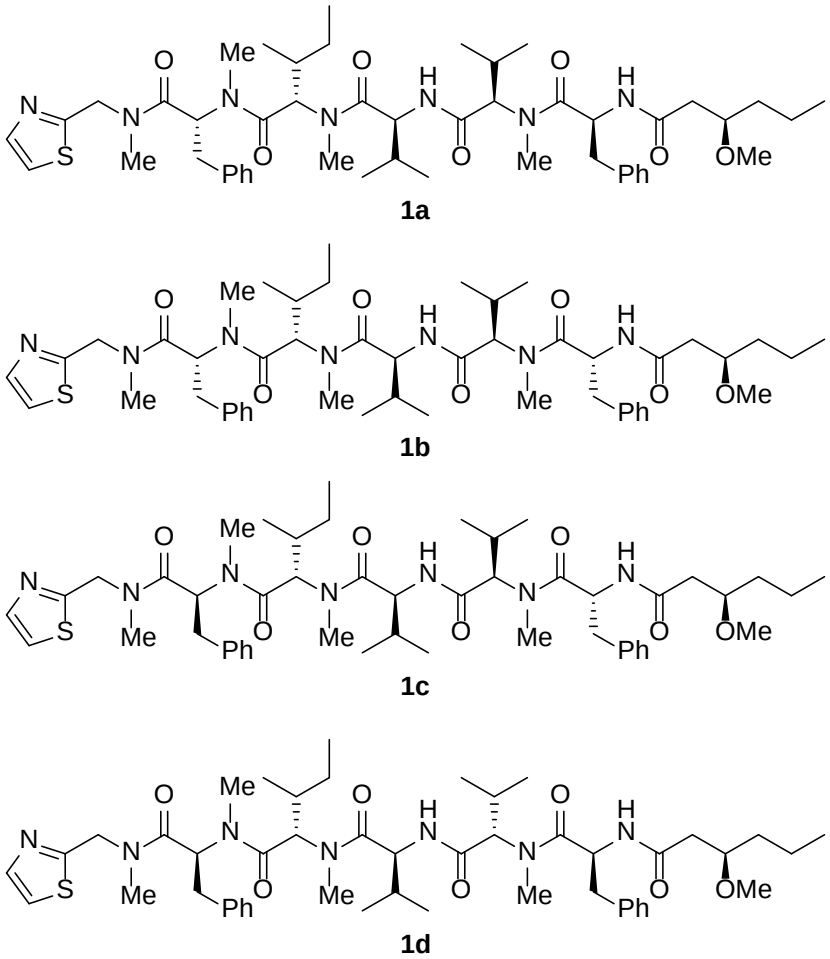
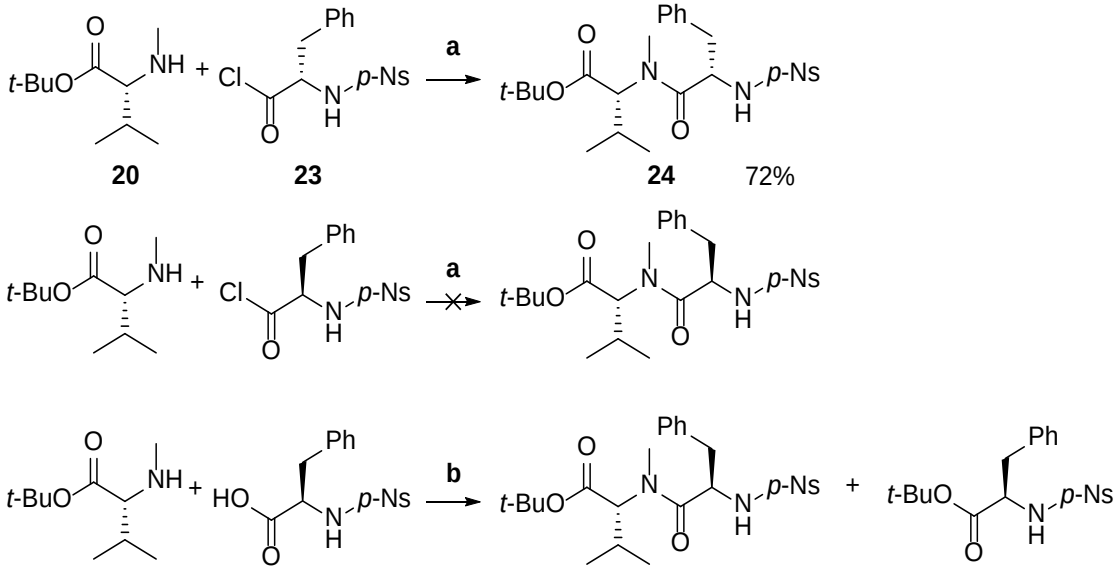


Figure 6. Stereoisomers of Micromide

interconversion of L to D amino acids in this particular genus of cyanobacteria. Similar lipopeptides, including dolastatins, apratoxins, lyngbyapeptins and apramides, none of which contain a D-residue. The absolute stereochemistry of micromide was determined by ozonolysis and acid hydrolysis, which can cause epimerization.[9] These facts suggest that micromide could consist of solely L-amino acids and therefore stereoisomer **1d** was included as well.

As a preliminary investigation into the effects of stereochemistry on secondary structure and subsequently, chemical shifts, we attempted to produce the diastereomer of dipeptide **24** (*N*-Me-D-Val, D-Phe). Surprisingly, the reaction produced no desired product; instead, giving a precipitate that was insoluble in $CDCl_3$, DMSO-d6, CD_3OD, and D_2O (Scheme 9). It is suspected that the precipitate is the result of diketopiperazine formation. It is well-known that *N*-methylated amino acids are more stable in the *cis* conformation, which increases the favorability of forming DKPs.[26] When the coupling was performed via the activated ester using HATU instead of the acid chloride, a precipitate formed with the same insolubility, however, a side product was discovered where the tert-butyl ester from the valine portion had migrated to the phenylalanine.



Conditions: a) DIEA, CH_2Cl_2; b) HATU, Et_3N, DMF

Scheme 9. Idiosyncrasy of Dipeptide Diastereomer

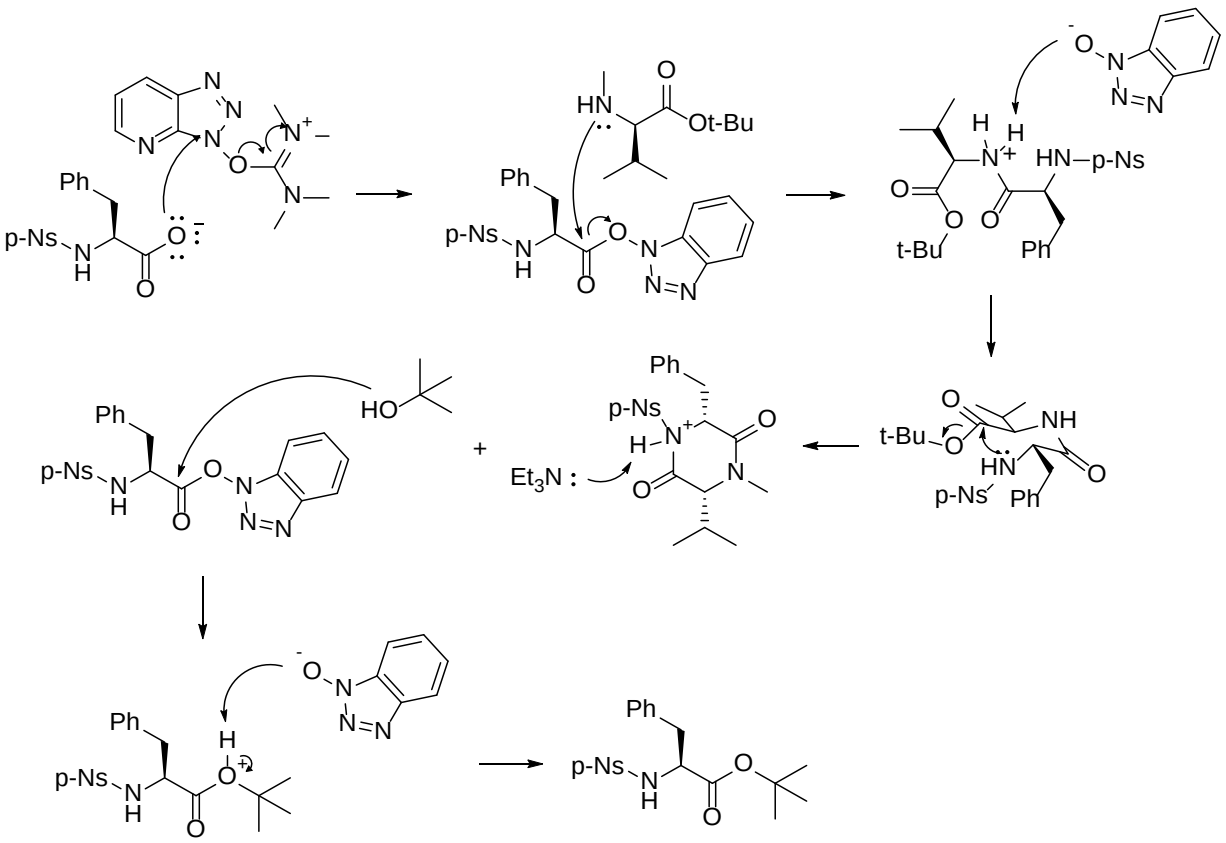


Figure 7. Proposed Mechanism of *tert*-Butyl Migration.

This could be explained by the formation of DKP and elimination of tert-butanol, followed by transesterification of the activated ester (Figure 7).

While the previous synthesis provided a route to an enantiopure product, producing the *N*-methylated amino acids was slow and laborious, and the propensity to form DKPs was unpredictable as the stereochemistry was altered. A more expedient and reliable process was required.

1.6 Synthesis on Solid Support

The need to quickly produce multiple isomers of micromide led us to investigate the possibility of producing the peptide fragment on solid support. Initially, the Wang linker on polystyrene solid support was chosen as the anchor and the fluorenylmethyloxycarbonyl (Fmoc) protecting group was chosen for its ease of use. *N*-Me-L-Phe was loaded on the resin. The bound amino ester was deprotected under standard conditions with 20% piperidine in DMF, followed by coupling to *N*-Me-Ile using PyBroP. A small amount was cleaved from the resin in 50% TFA/CH_2Cl_2 and the coupling appeared to be successful when the crude product was analyzed by LCMS. Unfortunately, attempts to couple the next valine residue were unsuccessful and no material was isolated after cleavage.

Considering the previous issues with DKPs, we hypothesized that the *cis* conformation directs the *N*-terminus towards the Wang linker, and because the amino acid is bound to the Wang linker as a benzyl ester, the ester provides a labile leaving group. Upon deprotection of the dipeptide, it cyclizes to the diketopiperazine and detaches from the resin (Figure 8). To

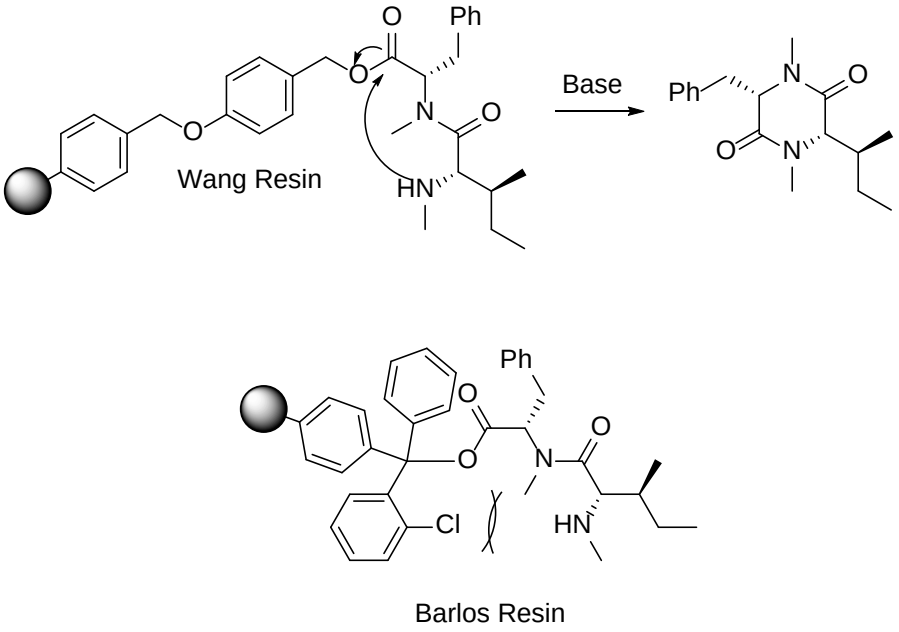
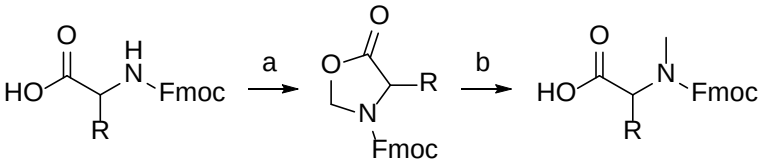


Figure 8. Comparison of Wang and Barlos Resins

investigate this idea, the anchored peptide was deprotected and the washes of the deprotection step were collected. Analysis by LCMS observed the mass of the DKP and verified that the dipeptide was cleaving itself from the resin. The solubility of this DKP suggests that the insolubility of the previous one is due to the nosyl moiety. The Wang resin was replaced with Barlos's 2-chlorotritylchloride (CTC) resin, which is known to inhibit DKP formation.[27]

The CTC resin was successfully loaded with Fmoc-*N*-Me-L-Phe in quantitative yield. In the coupling with isoleucine, the reaction never reached completion, despite two repeated couplings, and it was necessary to cap the peptide by acetylation with 10% acetic anhydride in pyridine. Coupling of the hexanoyl tail marked the end of the solid phase synthesis and thus, the peptide was cleaved from the resin with 1% TFA in CH_2Cl_2 and purified, giving **42** in 11.5% overall yield. Finally, the peptide was coupled to the thiazole head unit to give micromide **1**.

Micromide (initial proposed structure) was successfully synthesized on solid support, which was verified against the product produced by solution phase chemistry. While the solid-phase method was more facile, it consumed a large excess of reagents, forcing us to synthesize the Fmoc-amino acids. A literature search discovered Friedringer's method of accessing *N*-methyl amino acids by the reduction of oxazolidinones (Scheme 10).[28] Initially, the microwave-assisted method described by Govender and Arvidsson was used, which took only 4 minutes of total reaction time, giving yields of 70-95%.[29] The *N*-methyl amino acids could be

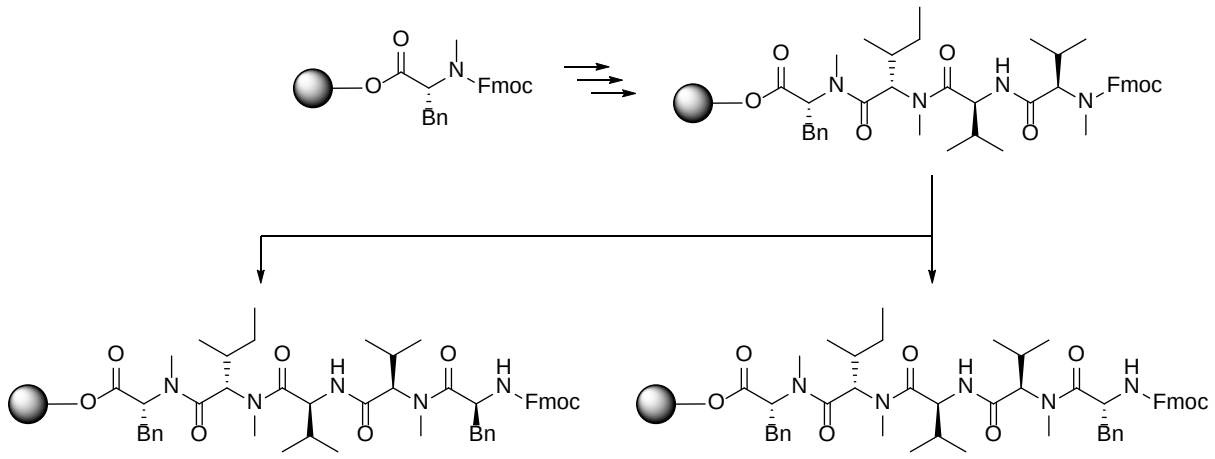


Conventional	88-97%	51-73%
Microwave	77-84%	59-80%

Friedinger Conditions: a) $HO(CH_2O)_nH$, TsOH, MePh; b) TFA, Et_3SiH, CH_2Cl_2

Govender Conditions: a) $HO(CH_2O)_nH$, TsOH, MeCN, *mw*; b) $AlCl_3$, Et_3SiH, CH_2Cl_2, *mw*

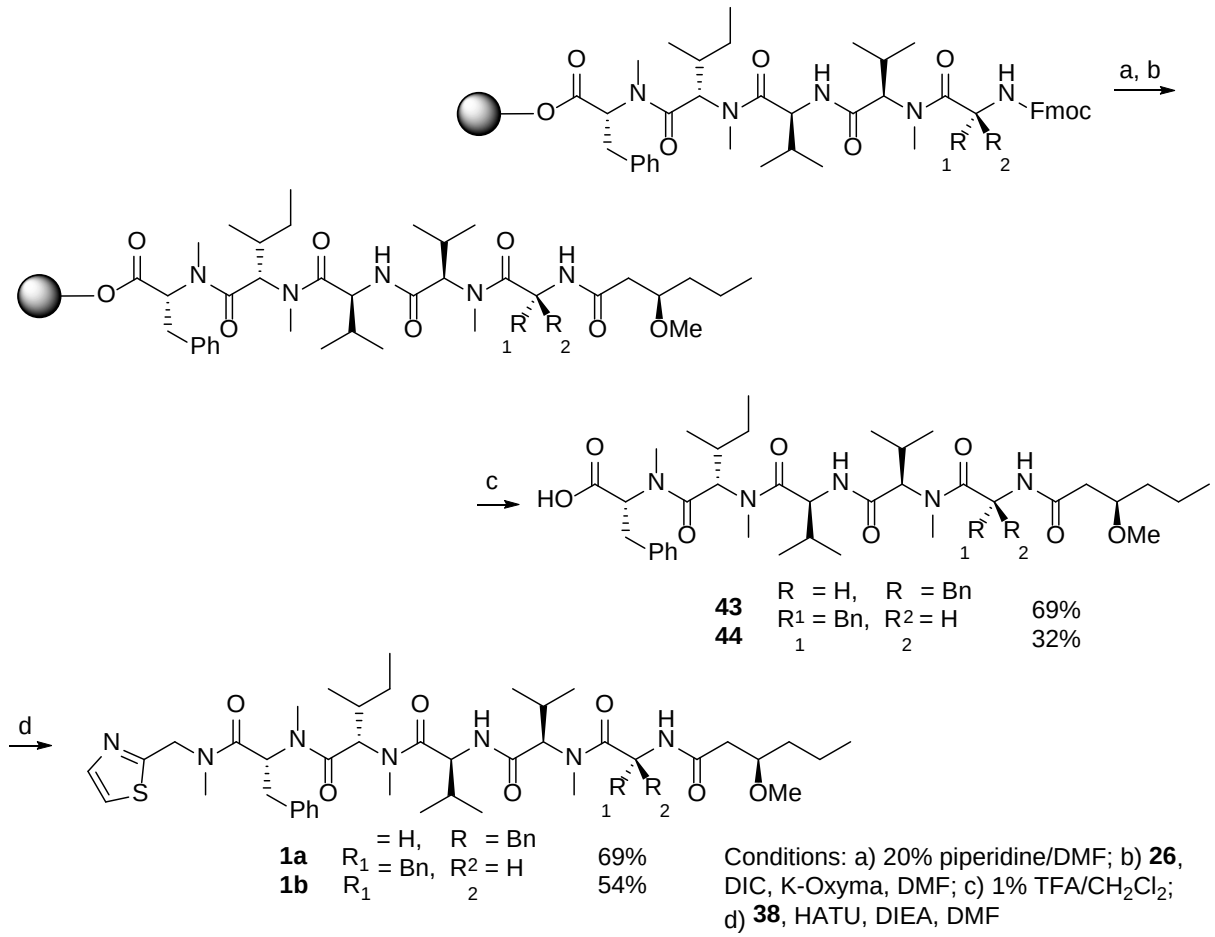
Scheme 10. Synthesis of N-Methyl Amino Acids via Oxazolidin-5-ones



Scheme 11. Diversification of Resin-Bound Peptides

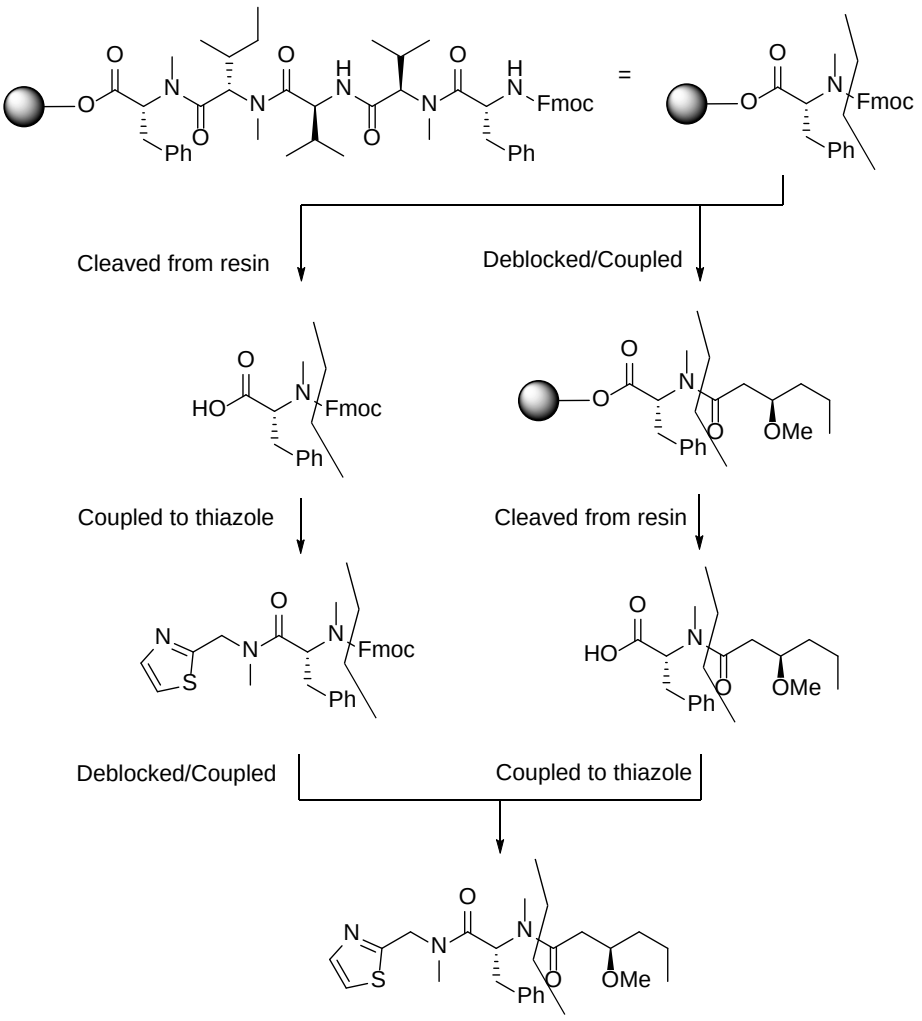
produced *and purified* in only one hour using the microwave-assisted method. However, the size of our microwave limited our scale to one gram at a time. Yields dropped precipitously when the reaction vessels were loaded beyond one gram. In contrast, the Friedinger method was scalable to multigram scales (10-20 g), generating the oxazolidinones in 76-80% yield and *N*-Me amino acids in 80-99% yield.

With a robust supply of the necessary amino acids in hand, work proceeded on isomers **1a** and **1b**. In the same manner previously described, the first four amino acids were loaded and coupled on Barlos resin. Since the two isomers differ only on the last residue, after the fourth residue was attached, the resin was divided into two equal portions (Scheme 11). One half was subjected to Fmoc-*N*-Me-L-Phe and the other half to its antipode. The hexanoyl tail was coupled to each and cleaved from the resin to give **43** and **44** (Scheme 12). Despite being conducted under identical conditions and in parallel, the NMR spectrum of **43** was more convoluted than **44**, exhibiting significantly more signals. It is suspected that these additional signals were the result of rotamers, since the two materials were not divided until the last three steps, epimerization would not lead to such complex spectra. Mass spectrometry confirmed that these were not the result of additions or deletions in the sequence.



Scheme 12. Synthesis of **1a** and **1b**

Although the linear approach on solid phase was successful, many reactions required multiple repetitions of the coupling. In addition, standard reaction conditions required the use of three equivalents of carboxylic acid, meaning that the precious chiral hexanoyl tail **26** had to be used in excess. We anticipated that the reaction kinetics would be improved in solution phase and would require less reagents. The synthesis of **1a** was repeated but with the head and tail coupled in solution-phase. The peptide was cleaved from solid support with the Fmoc protecting group still attached (Scheme 13). The peptide was coupled to the thiazole, deblocked (Fmoc deprotected), and coupled to the hexanoyl tail.



Scheme 13. Revised Solid-Phase Pathway

1.7 Revised Solution Phase Chemistry

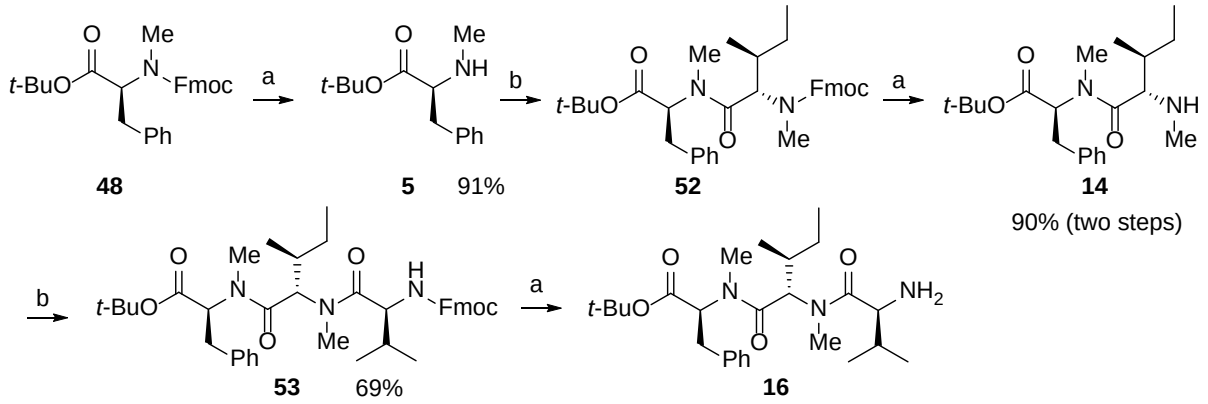
With two isomers produced, little change was observed in the chemical shift of the C7 proton. Between the isomers, changes of less than 0.1 ppm was observed and it seemed unlikely that a simple stereochemical change could cause such a drastic change in chemical shift. Solid-phase synthesis provided a quicker route but was difficult to analyze throughout the synthesis. In addition, the benefits of solid-phase synthesis are a double-edged sword. In reducing the number of purification steps, higher quality reagents are required, otherwise the formation of significant side-products arises and side-products such as deletions become very difficult to separate from the final product. The initial decision to avoid solution phase chemistry was primarily due to the long reaction times and laborious nature of applying the nosyl chemistry.

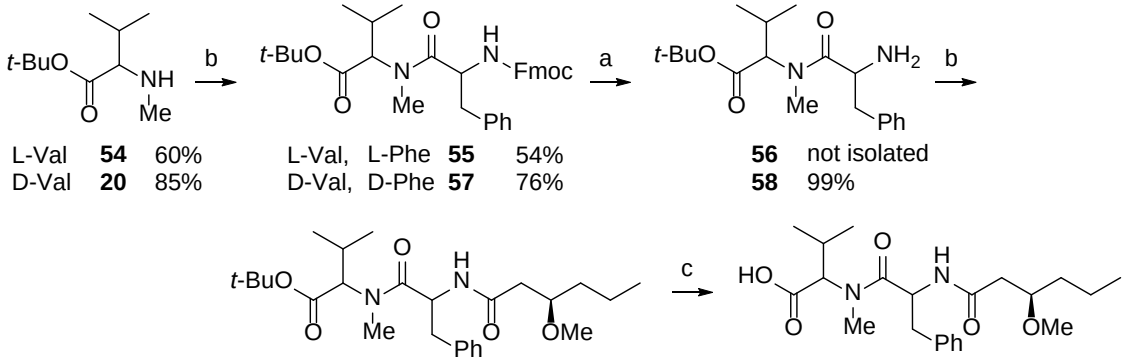
From the experience gained from using the Fmoc protecting group, and the large amount of Fmoc-*N*-Me amino acids now on hand, the convergent solution-phase strategy became much more facile (Scheme 14). The next two isomers were produced using solution-phase chemistry to better understand the change in chemical shifts of the α-protons with each coupling, as well as identifying in which step do the extra proton signals in **43** appear. The Fmoc amino acids were protected as the *tert*-butyl ester using isobutylene, catalyzed by sulfuric acid in CH_2Cl_2. The Fmoc group could be deprotected using diethylamine and coupled to the next carboxylic acid without purification, however, the Fmoc group frequently complicated the NMR spectrum by creating multiple stable rotamers and severely broadening signals in the spectrum for single amino acids. As the peptide was extended, the peaks decreased to two or three rotamers and broadening of the peaks was reduced as well. This was evident due to the presence of multiple *tert*-butyl signals, whose integrations corresponded to the multiple signals of the other moieties, specifically, the α-proton and side-chain groups. In order to exclude the possibility that these were diastereomers, the free amines were isolated after Fmoc

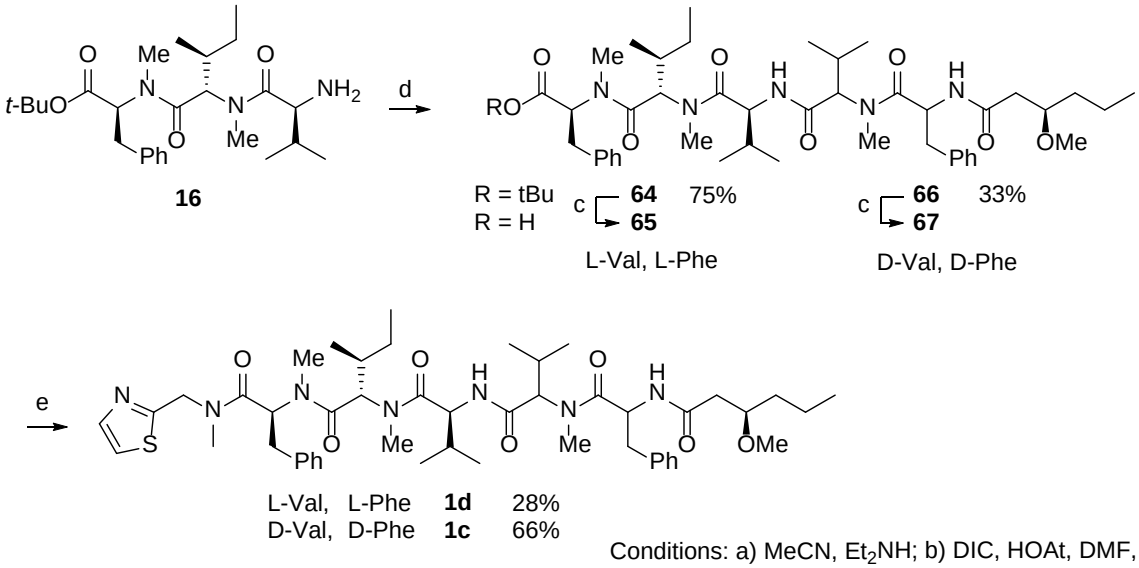
deprotection. Once Fmoc was absent, the signals converged. As further evidence, NOE NMR experiments were performed, with selective pulses focused on the α-proton signals. A selective pulse at the targeted signal resulted in appearance of the rotameric peak in the same phase due to chemical exchange.[30]

Using the solution-phase method, the Phe-Ile coupling provided consistently low yields. When using carbodiimides as coupling reagents, reaction times were too slow, resulting in a large amount of urea side product. Other coupling reagents did not show much improvement. Interestingly, the mass spectrometry of the isolated product under APCI mode showed almost undetectable amounts of the parent ion. The compound readily fragmented between the amide connected to C16. It can be assumed that the large steric hindrance between the benzyl and sec-butyl side chains was detrimental to the stability of the amide bond. This result is consistent with results from the solid-phase syntheses, where the coupling reaction never reached completion and it was necessary to cap the unreacted *N*-termini as the acetamide. In addition, in each subsequent coupling product always fragmented at the same position in unless the phenylalanine residue existed as the free acid.

Using the modified Fmoc solution-phase strategy, the final isomers of micromide, **1c** and **1d** were completed. To our disappointment, comparison of the NMR spectral data showed that none of the isomers correctly matched the natural product. Comparison of the ^{13}C shifts (Figure 9) did not give further insight into the corrections that would be necessary to establish the true structure of micromide. When L-phenylalanine was substituted with its enantiomer at C6-7, there was no deviation from the natural product. However, C34-36 signals in **1a** was brought more in line with the natural product. Due to lack of conformational constraint at the terminal points, it is not surprising that stereotopic changes would have miniscule effects given the larger degrees of freedom in bond rotation.







Conditions: a) MeCN, Et_2NH; b) DIC, HOAt, DMF, appropriate carboxylic acid; c) TFA, CH_2Cl_2; d) **61** or **63**, HATU, DIPEA, DMF; e) **38**, EDC, HOAt, Et_3N, CH_2Cl_2

Scheme 14. Fmoc Solution-Phase Synthesis

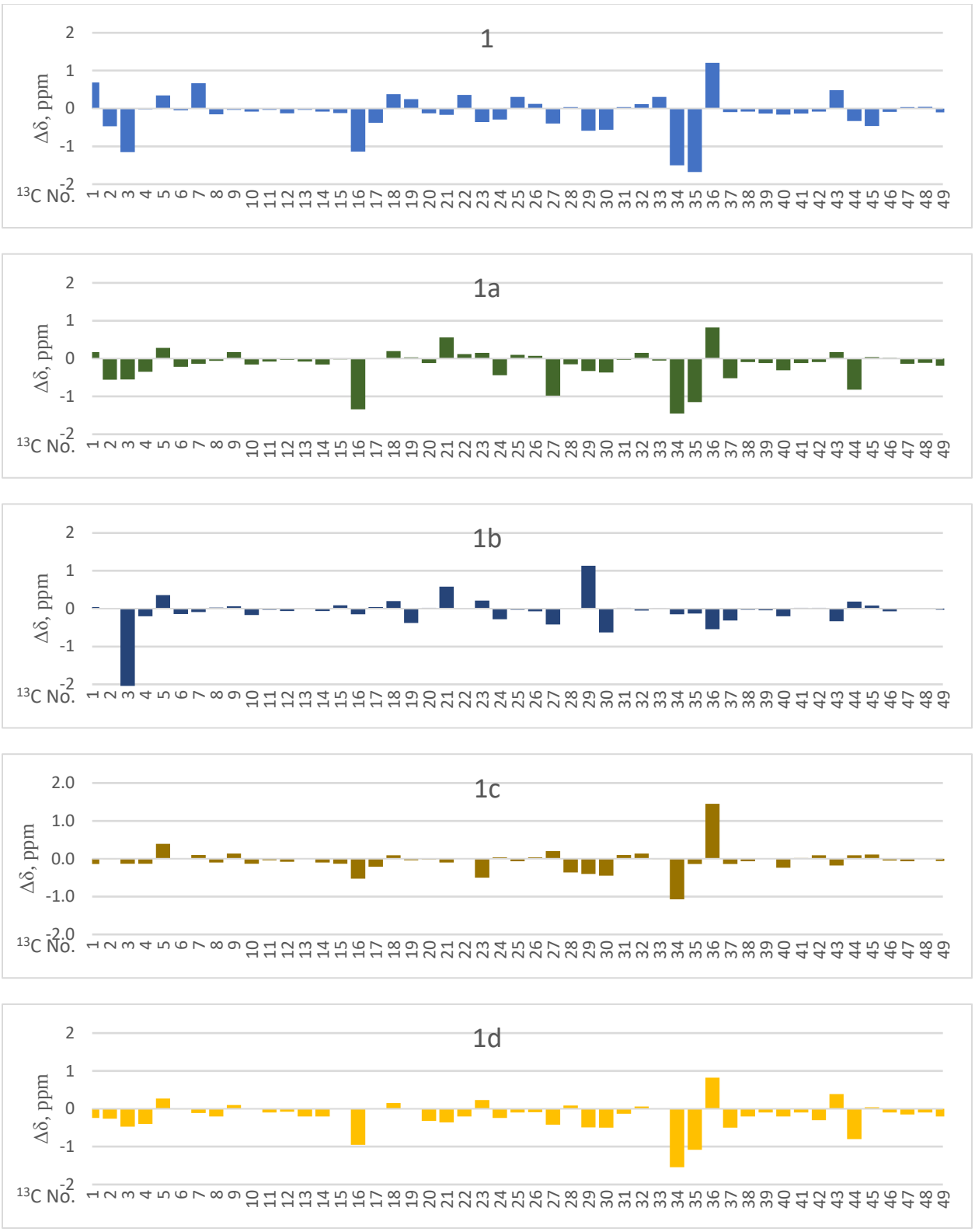


Figure 9. $\Delta^{13}C$ Comparison Across All Stereoisomers

1.8 Beyond Stereochemical Revisions: Structural Revision

In all isomers produced, there was little change in the chemical shift of the α proton at 5.9 ppm and the dramatic difference could not be explained by a change in stereochemistry. We re-examined the ^{1}H spectrum of the natural product and discovered an anomaly. Williams identified ^{1}H peaks at 6.8 ppm as the ortho-aromatic protons of the phenyl ring (C38, C42), upfield of the amide proton signals, and the peak at 7.02 as aromatic protons (C10, C11).[11] In the synthetic product, the phenyl signals are overlapped in the region above 7.1 ppm, downfield of the amide signals, with little distinction between the two phenylalanine residues. By comparison (Figure 4) it appears that the supposed aromatic protons are actually amide protons, which was confirmed by HMBC correlations. Typically, phenyl moieties present as a cluster of overlapping triplets and doublets. Additional substitutions on the ring give distinct peak shapes. Only when the substitution is an electronegative heteroatom, do the peaks become deshielded below 7 ppm. The spectrum of compound **68** contains an unsubstituted benzyl moiety, as well as both *ortho* and *para* substituted phenyl moieties, providing a clear comparison of the different types. The widely spaced doublets in the natural product spectrum suggest that, at least one phenyl ring is substituted.

The most obvious replacement for phenylalanine would be tyrosine. DNA codons of phenylalanine and tyrosine residues differ by a single nucleoside (TTT/TTC and TAT/TAC, respectively) and a single point mutation would result in the expression of a tyrosine version of micromide. We investigated literature of other lipopeptides from cyanobacteria. Indeed, the substitution of phenylalanine residues with tyrosine is a common motif. Dolastatin 11 and 12 exhibit this exact phenomenon, differing by a single methoxy substitution on the phenyl ring.[31] Ulongamide A and B also differ solely in a Phe/Tyr substitution. Apramide A-C contains the same *N*-Me-Gly-thz moiety adjacent to a tyrosine, with striking similarities to micromide (Figure 10). Given this information, a tyrosine residue could account for the difference between the ^{1}H

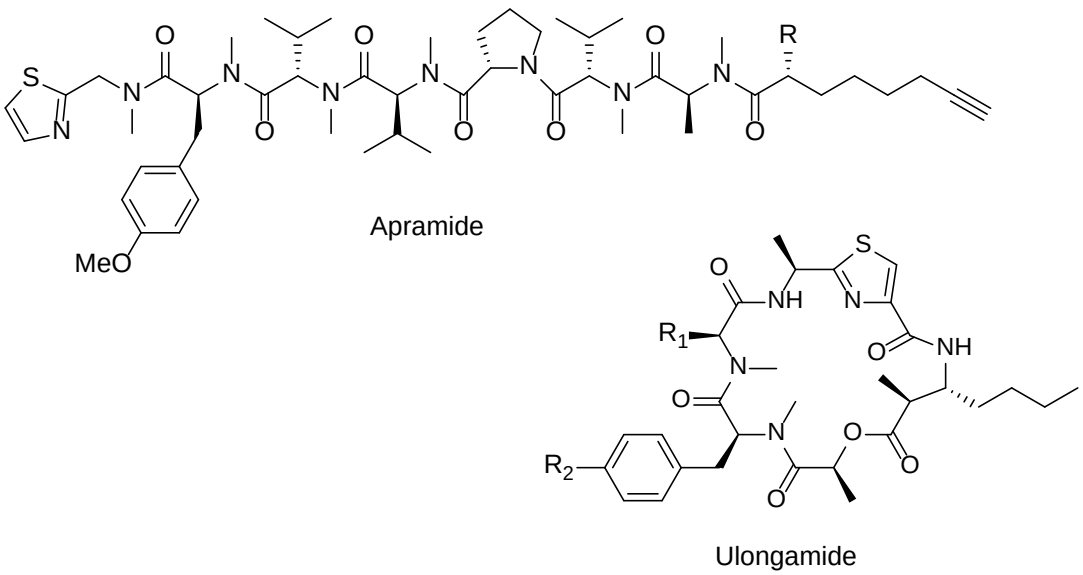
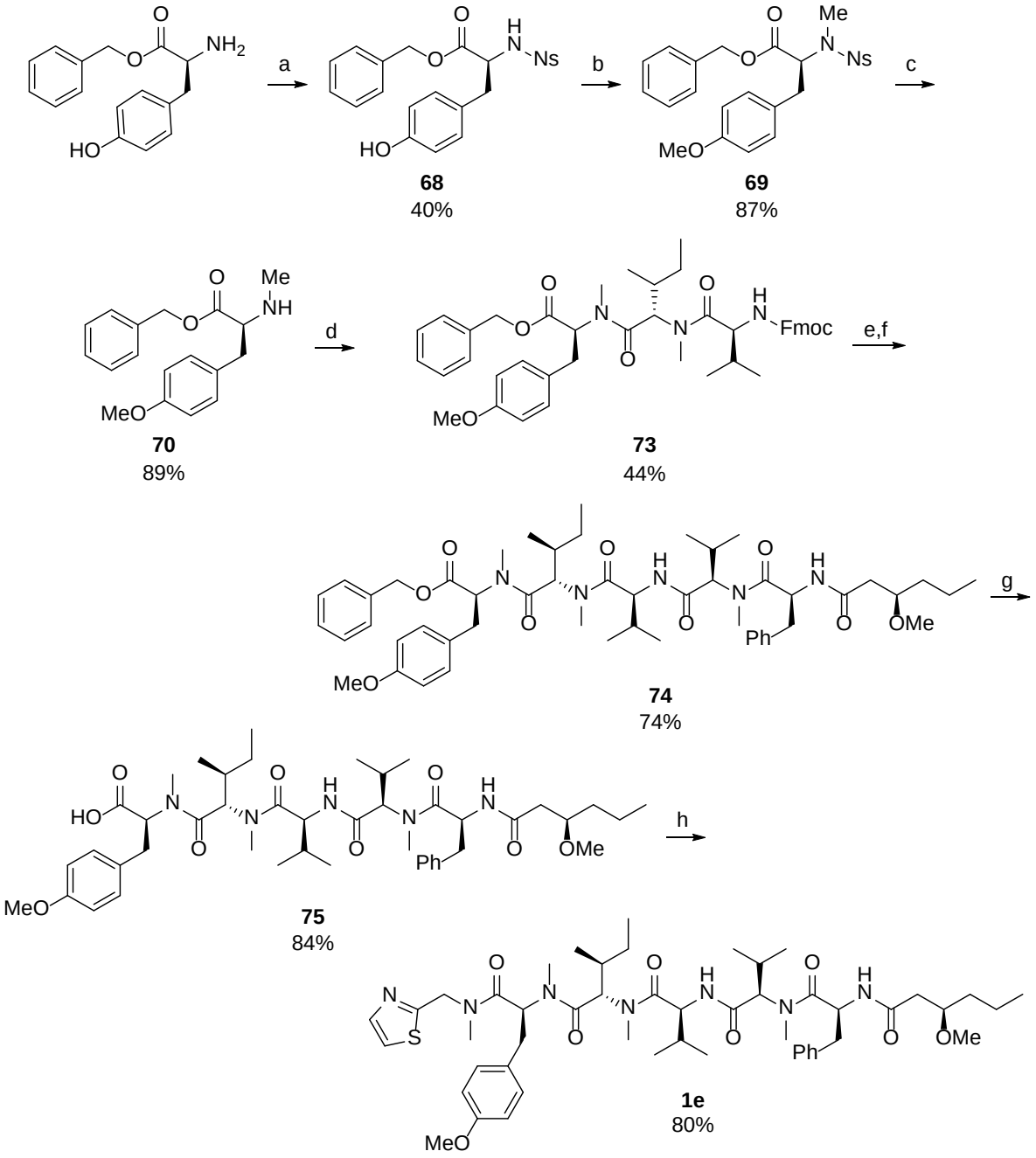


Figure 10. Marine Natural Products Containing Phe/Tyr Substitutions

spectra. In other marine natural products, tyrosine residues also existed as the *O*-methylated form. Between the hydroxy and methoxy versions of tyrosine, we chose the methoxy since it appears to be more common but also for its simplicity in synthesis. The phenol and amine could be methylated simultaneously, and the need for protections and deprotections later on would be avoided. We decided to incorporate an *N,O*-dimethyl tyrosine into the lipopeptide by replacing the *N*-Me-Phe. Such a change would undoubtedly have dramatic changes to the ^{13}C spectrum, most notably, a tyrosine residue would give a very distinct signal around 158 ppm which would not be obscured by any other signals. On the other hand, the hypothetical carbon would have no bonded hydrogens and may require longer relaxation times to acquire a decent signal. Given the small amount of natural product isolated, Williams most likely left the relaxation delay at one second to allow for more scans. But because the original ^{13}C spectrum was no longer available, we were unable to corroborate this conjecture.

The synthesis began and tyrosine was methylated using the same Fukuyama method as previously described. Attempts to protect the amine as the nosyl amide under the same conditions as the other amino acids failed. The difference in nucleophilicity between nitrogen

and oxygen was not sufficient to provide the necessary chemoselectivity, as the nosyl chloride was too electrophilic and resulted in di-nosylation of both the amino and phenolic moieties.



Conditions: a) *o*-NsCl, Et_3N, THF, DMF; b) MeI, K_2CO_3, DMF; c) 2-mercaptoethanol, DBU, DMF; d) **72**, BOP-Cl, DIEA, CH_2Cl_2; e) Et_2NH, MeCN; f) **61**, HATU, HOAt, DIEA, DMF; g) H_2, $Pd(OH)_2$/C, EtOAc; h) **26**, HATU, HOAt, DMF

Scheme 15. Synthesis of the Tyrosine-based Analog

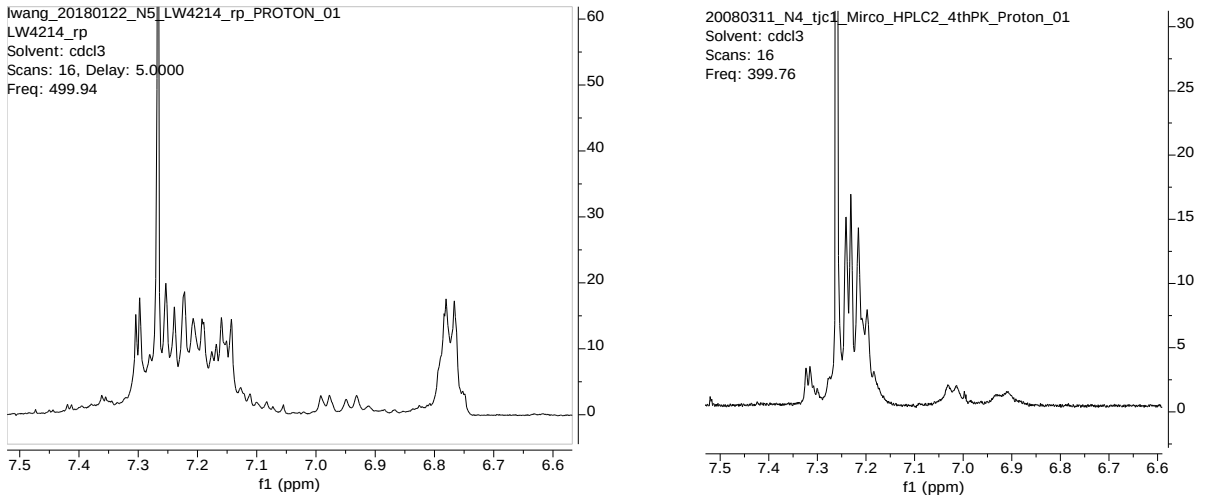
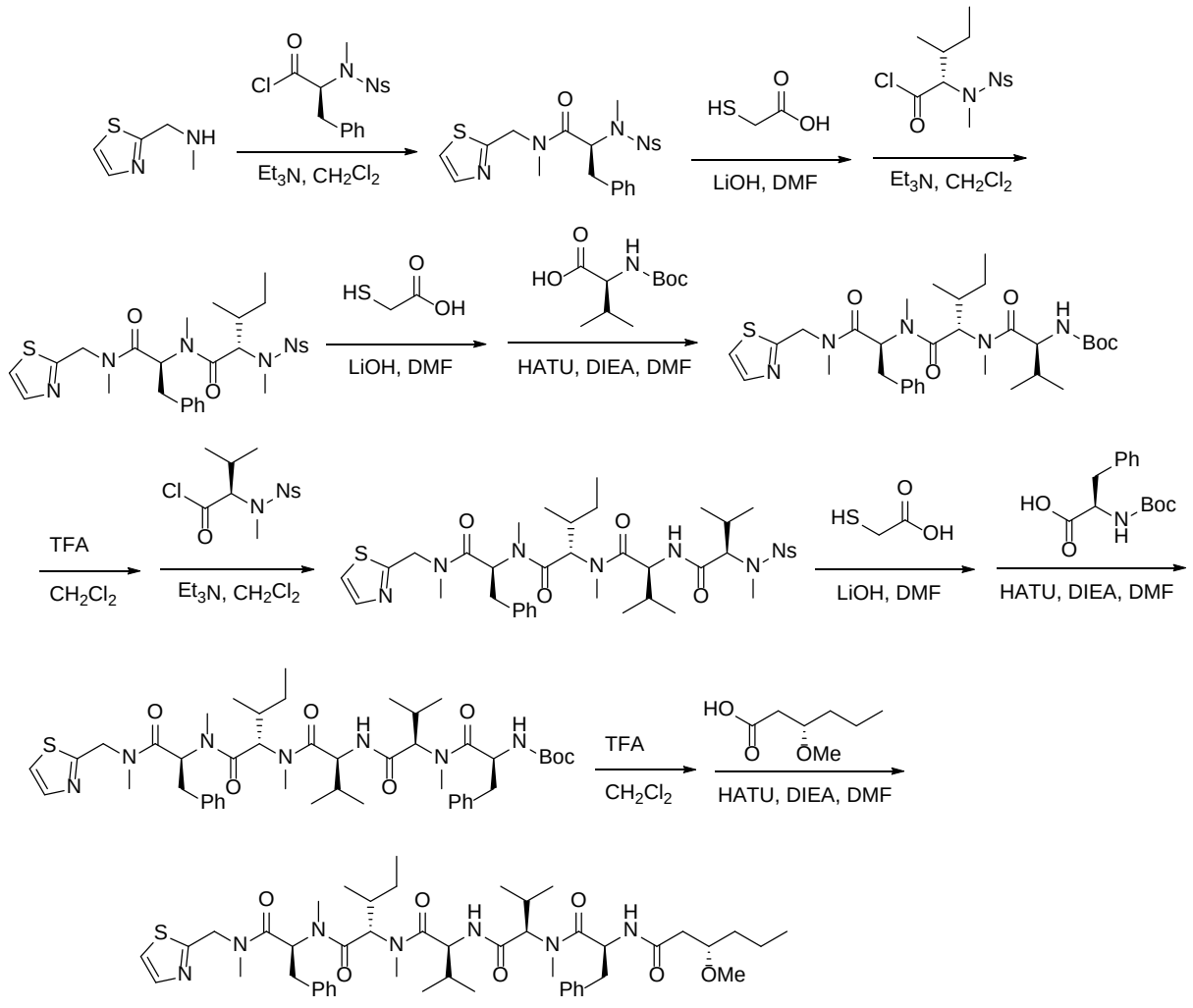


Figure 11. Comparison of Aromatic Regions. (Left: **1e**, Right: **1**)

Following the work of Penso et al., the addition of DMF modulated the solvent effects sufficiently enough to produce **68** in acceptable yield (Scheme 15).[32] The resulting product was di-methylated with iodomethane giving **69** in 86.5% yield. Since the ester protecting group was less sterically hindered than a *tert*-butyl group, similar to the Wang resin, we anticipated that deprotection of a dipeptide would result in formation of the diketopiperazine. Thus, the isoleucine residue was coupled to valine first to make the dipeptide, and then coupled to tyrosine benzyl ester. The remaining steps were conducted in the same manner as the other analogs. The resulting product gave a spectrum that coincides much better with the natural product in the aromatic region (Figure 11), but the anomalous 5.8 ppm peak remained.

In our synthesis, the signal remains in the 5.8 ppm range while the phenylalanine is protected as the *tert*-butyl ester. It is not until the final thiazole coupling that the α-proton moves downfield. In comparison to the Han synthesis (Scheme 16), the molecule was built linearly and the α-proton signal does not move downfield until the condensation with isoleucine, however, this was an artifact of the electronic effects of the nosyl protecting group. Stereochemical alterations did not provide the necessary adjustments to the chemical shifts, nor did the change to the side chain. Our last investigation was the effect of *N*-methylation of the phenylalanine residue. While a search of literature for the effect of *N*-alkylation on α-proton shifts showed that

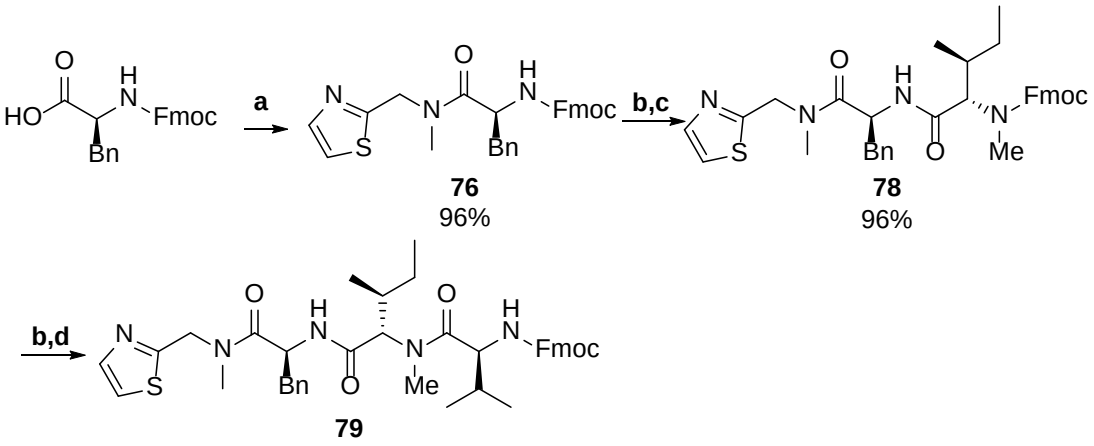


Scheme 16. Han Synthesis of Micromide

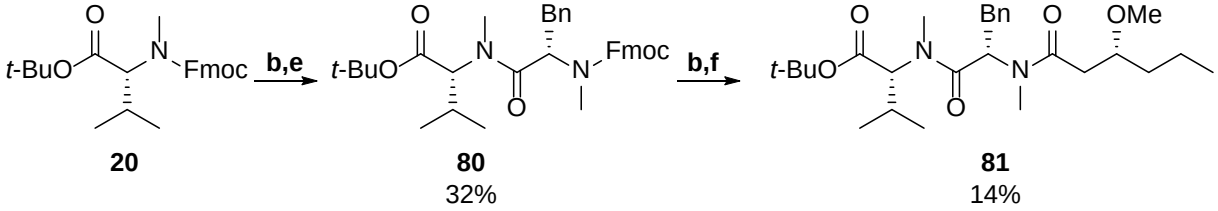
is it possible for signals to exist down in the 6 ppm range, there were no known compounds containing the GlyThz-Phe-Ile sequence, *N*-alkylated or otherwise, other than intermediates of micromide itself.

To investigate the effect of *N*-methylation on micromide's chemical shifts, the *N*-desmethyl fragment **79** was synthesized. Each amino acid was coupled in a linear fashion. When repeating the route using Fmoc as a protecting group, multiple rotamers are seen and the α-proton is found both at 4.9 and 5.5 ppm, suggesting that such drastic changes in chemical shifts can occur solely from secondary structure. Once the Fmoc protecting group was removed, the signals converged at 3.98 ppm in the free amine. Condensation with isoleucine moved the

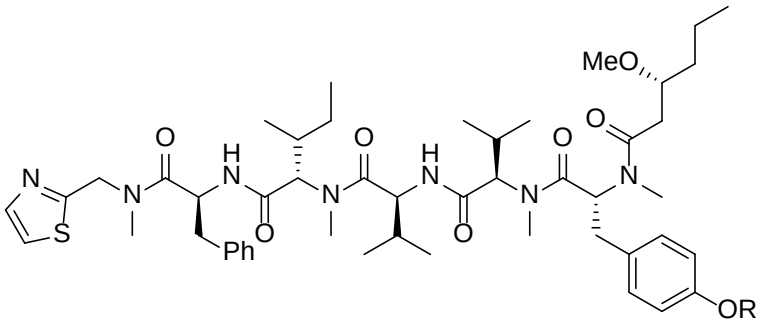
signal to 5.13 ppm. This result suggested that problem with the downshifted signal at 5.8 ppm was the result of the methyl positioning. Excited by this new data, the theory was explored further. A tyrosine substitution was not introduced here to reduce the number of variables, and to avoid the assumption that the HRMS data was incorrect (Scheme 17). In producing fragment **81**, the isolated product appeared to show two isomers. Duplicate α proton peaks were present in fragment **80**, which was typical of Fmoc-protected species but NMR of the crude material after deprotection showed convergence to a single peak, showing that the substrate's stereochemistry was intact (no sign of the diastereomer). After the coupling of the hexanoyl tail, two peaks re-emerged in a 5:6 ratio. Two 1D-NOE experiments were performed with a selective pulse in the region of the α-protons and the *N*-methyl groups. No signals were observed from the α protons as the signal-to-noise ratio was too low, but signals in the same phase were observed for the methyl groups, indicating that the duplication of signals was the result of



Conditions: a) **38**, HATU, HOAt, DIEA, DMF; b) MeCN, Et_2NH; c) Fmoc-N-Me-Ile, HATU, HOAt, DIEA, DMF; d) Fmoc-L-Val-OH, HATU, HOAt, DIEA, DMF e) Fmoc-N-Me-L-Phe, HATU, HOAt, DIEA, DMF; f) **26**, HATU, HOAt, DIEA, DMF



Scheme 17. *N*-Methyl Shifted Fragments



R = Me or H

Figure 12. Proposed Aryl Substitution

cis-trans amide conformations. Since the phenylalanine's α proton was once again shifted downfield, it suggests that the downfield shifts are primarily caused by electronic effects due to *N*-alkylated amides.

The change in chemical shifts suggest that both phenylalanines should exist as secondary amides i.e. non-methylated, to prevent the downfield shift. Williams et al. identified the phenylalanine protons as a dd at 5.34 ppm for the methylated residue, and as a ddd at 5.19 ppm for the unmethylated residue. This is a reasonable conclusion since one would expect to see coupling between the two benzylic protons and the amide proton. An *N*-alkylated residue would only have the benzylic protons and present as dd. However, the dd splitting pattern is the result of the benzylic protons being diastereotopic. If the bond angle between the protons was small enough, following the Karplus relation, the dd pattern would merge to a triplet, a phenomenon frequently seen in the isomers produced. In our intermediates, the C7 protons were often seen as two rotamers, one as a doublet of doublets and the other as a triplet. Therefore, it is possible for the peptide to contain two un-alkylated phenylalanines and show both a dd and a ddd. But the ddd assignment itself is questionable. The signal at 5.19 ppm is overlapped with the doublet from C17 and the available spectrum lacks the resolution to distinguish the peaks.

The loss of a methyl group would mean an additional *N*-H signal is required. We have already established that Williams incorrectly assigned the doublet at 7.02 ppm an aromatic proton. In our studies of the synthetic product, the isolated doublets around 7 ppm are always the amide protons. Since the peak height of the signal at 7.02 is twice that of the doublet at 6.95 ppm, it is conceivable that there are two amide protons overlapped in that region.

1.9 Conclusion and Future Work

Results of the tyrosine analog were encouraging, since replacing one residue gave the necessary changes to the aromatic region. Along with the data from changes to positions of *N*-methylation, the next candidate for investigation would be incorporating both changes by removing the methyl group at C15 and replacing the C34 phenylalanine with tyrosine (Figure 12). Recall that connectivity was established solely by HMBC correlations between the *N*-Me/*N*-H and carbonyl carbons. The close proximity of the carbon signals makes it easy to transpose two like residues. However, the ^{1}H-NMR distinctly shows one α-proton signal splitting as dd due to $^{3}J_{H\text{-}H}$ coupling to the benzylic protons. The other shows a ddd due to coupling from *N*-H and benzyl protons, and the other as dd benzyl protons, suggesting that one Phe/Tyr residue is *N*-methylated, while the other is not. Thus, the proposed changes results in a standard Phe and a *N*-methylated tyrosine.

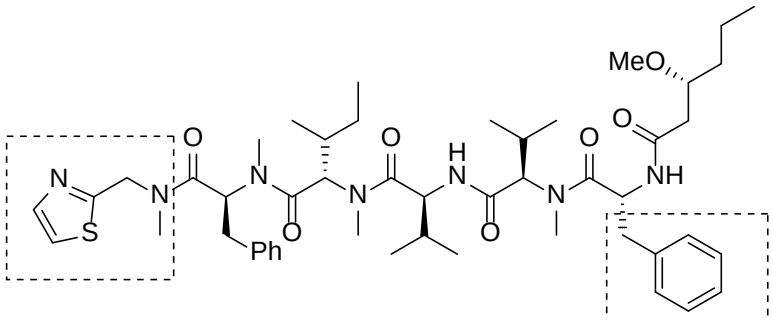
Despite the implications of a dd vs ddd splitting pattern, without confirmation by a synthetic molecule, the phenolic moiety should be methylated or not remains uncertain. The minimum coupling constant given by the Karplus relationship is 2 Hz. If the dihedral angle between the alpha proton and any of the adjacent protons is close to 90 degrees, then the splitting may not be observable, reducing the ddd to a dd. The spectrum from the natural product contains singlets at 3.6 and 3.45 ppm, whose intensity is only about half that of other methyl singlets in the spectrum but without integral values, it is thus not clear if the singlets are the result of impurities. The 3.45 ppm signal could easily be residual methanol and it is also possible that the two singlets are conformers of an *O*-Me. The *O*-methylated tyrosine signal in the tyrosine analog **1e** appeared at 3.77 ppm and at 3.57 ppm in the H NMR spectrum of apramide A. Consequently the 3.6 ppm singlet is very well in the range of methoxybenzene moieties.

In these proposals, we have completely disregarded gravimetric data. The original isolation of micromide was not completely pure. Apramides A, B, and G were found with micromide and even after multiple purifications, apramide G can be seen on the baseline of micromide's ^{1}H-NMR spectrum. With mass spectral data, it is standard procedure to state the mass-to-charge ratio. In this case, MALDI and HRMALDI was used to determine the mass of the isolated material. While MALDI is a soft ionization technique, it is still known to produce fragments in certain samples. The resulting mass found could be a false positive due to fragmentation or contamination. However, if we wished to maintain fidelity with the gravimetric data, substituting the thiazole with oxazole while incorporating tyrosine would give a mass close enough to the original structure to be within error by HRMALDI (Figure 13). Since oxazoles produce NMR spectra nearly identical to thiazoles, the substitution could potentially give the desired NMR spectra, but the trend with other marine natural products from these particular cyanobacteria appear to exhibit thiazoles exclusively.

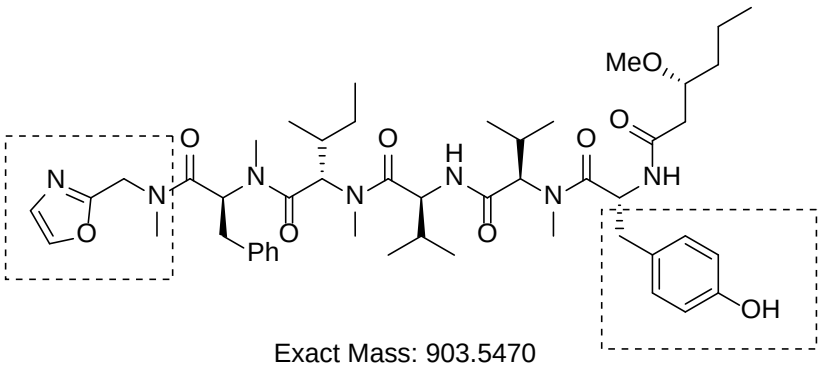
It is unfortunate that the true structure of micromide remains unsolved. To identify the error in micromide's structure, it must be presumed that Williams's interpretation of the data was in error. Our dilemma lies in that a complete set of Williams's data is no longer available to review, thus our only option was to explore each area of analysis by systematically assuming a flaw existed in that set: 1) Stereochemistry, 2) Methylations, 3) Structural/Functional groups. Micromide was analyzed using chiral HPLC for stereotopic identification, NMR spectral data for functional groups and connectivities, and high-resolution mass spectrometry for elemental composition. We began with the most common source of error: stereotopic changes, producing 4 stereoisomers of micromide. Though many other stereoisomers exist, none of the isomers produced resulted in a change significant enough to support this idea. It should be noted

however, that rotamers of Fmoc-protected intermediates did show the potential to cause such a change in chemical shift.

Based on the spectral data and similarities to apramide, a tyrosine-based analog was also produced. The resulting NMR spectrum exhibited signals in the aromatic region that is more in line with the natural product. The only method used by Williams to determine the connectivity of the amino acid residues was HMBC. Considering that two valine and two phenylalanine residues exist, it is a curiosity why no additional experiments such as NOESY, ROESY or TOCSY data was acquired as complimentary data to support their proposed structure. Just three years prior to the publication of micromide, Williams's co-author Yoshida, also co-authored a publication on the characterization of the apramides. Three apramides were found in the isolation of micromide. In this paper, HRFABMS and NOESY experiments were used as part of the characterization, but the same was not done for micromide.



Exact Mass: 903.5292



Exact Mass: 903.5470

Figure 13. Comparison of **1** to Hypothetical Oxazole Analog

Any sort of additional fragmentation data would have been immensely useful in narrowing the possibilities. If FABMS was not available, simple MS/MS or even a simple APCI-MS analysis would have been particularly useful to reinforce the results given by the hydrolysis of micromide. As we observed in the synthetic product, Phe -Ile bond was easily broken by chemical ionization and showed distinct mass-charge signals. If these fragments were also observed in the natural product, it would support the proposed connectivities based on HMBC data, as well as verify the positions of *N*-methylation.

In our work, we have ruled out stereotopic errors in the Phe and Val residues i.e. diastereomers of **1** as the source of discrepancies between the synthetic and natural versions of micromide. Changes in stereochemistry failed to bring the chemical shift of the Phe α-proton upfield enough to match the natural product. Changes in stereochemistry also failed to resolve the discrepancy in carbon chemical shifts as well. A carbonyl carbon resides at 173 ppm in all stereoisomers, far outside the range of natural micromide. Additionally, new data from synthetic modifications suggest that a para-substitution one aromatic ring may be appropriate, as well as a change in the location of *N*-methylations.

Chapter 2. Lagunamide A

Despite the many advances in the chemical and life sciences today, malaria continues to be a major contributor to mortality rates in the world and is the most lethal parasitic threat to humans. According to the World Health Organization, nearly half of the world's population is at risk of malaria. Rising drug-resistance and the low number of effective treatments threatens the progress made in controlling malaria over the last fifty years. The first synthetic drug, chloroqine, was discovered after a herculean effort by Johann Andersag and co-workers after screening of 12,000 compounds.[33] Chloroquine was initially considered too toxic to use and was shelved until it was independently discovered in the United States in 1943, after the government commissioned the Board for the Coordination of Malarial Studies. Chloroquine provided effective treatment against malaria, resulting in the elimination of malaria in North America and Europe. Unfortunately, beginning in 1957, the first chloroquine-resistant *P. falciparum* was discovered and chloroquine resistance spread quickly across the tropics of South America, Southeast Asia, and Africa. Though chloroquine resistance remains limited to the falciparum species, rates of infection and mortality began to rise in the 1980's.

Two decades after the discovery of chloroquine, Tu Youyou discovered artemisinin in 1979, which would quickly become the first-line defense against malaria.[34] Artemisinin would become a first-line defense against *P. falciparum* due to its ability to fight *P. falciparum* in all its stages. However, in the recent years, *P. falciparum* has been developing resistance to artemisinin as well. Today, artemisinin-resistant strains threaten the progress made in the last century and there is a need for new drugs to combat the growing threat.

As microbes develop drug-resistance, modern treatment often involves multi-drug approaches that utilize different mechanisms. Chloroquine was a derivative of the alkaloid quinine. Chloroquine and its related products work by inhibiting the formation of hemozoin from heme, a waste product of metabolizing hemoglobin. Unable to remove its waste, heme builds up

in the parasite and results in lysing of its membranes. Artemisinin works through highly reactive radicals formed upon exposure to heme, which react with many of the parasite's proteins.[35,36] To add to existing arsenal against malaria, peptidic natural products found as secondary metabolites of marine cyanobacteria have shown the ability to inhibit *Plasmodium spp*.[37]

One of these metabolites is lagunamide A (**147**), which shows anti-malarial activity (IC_{50} = 0.19 μM) against *P. falciparum*, as well as high potency against P388 murine leukemia cell lines (IC_{50} = 6.4 nM).[5] This made lagunamide A an excellent target for studies into its mechanism of action and the needed total synthesis. First isolated by Tripathi and co-workers from *Moorea procudens* (formerly *Lyngbya majuscula*),[38,39] lagunamide A is a cyclic depsipeptide featuring six amino acid residues and an unsaturated lipid chain containing four contiguous stereocenters. During the course of this work, three other total syntheses and one informal synthesis of lagunamide A have been published, including its stereochemical revision. This section will discuss synthetic strategies used prior to the stereochemical revision, followed by modifications after the revisions were made known.

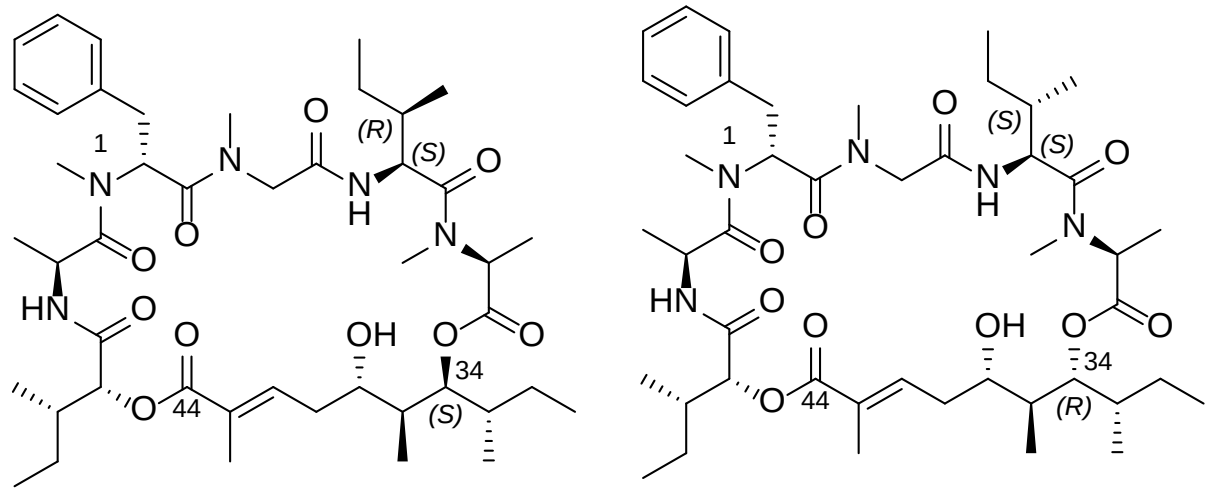
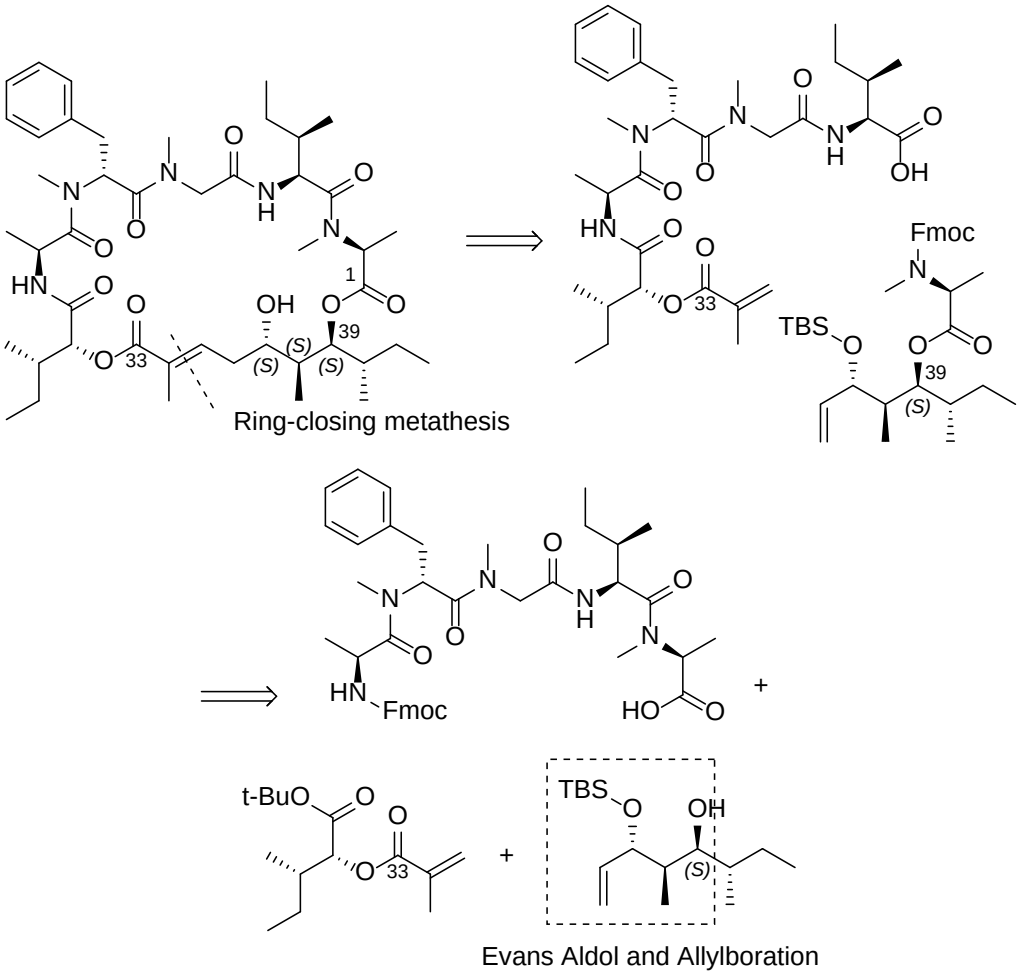


Figure 14. Original and Revised Structures of Lagunamide A

2.1 Retrosynthesis

It was natural to envisage the synthesis of lagunamide as two major fragments: the peptide portion and the polyketide portion (Scheme 18). Ideally, the cyclization would be performed through ring-closing metathesis under neutral conditions to reduce any chances of hydrolysis or epimerization. Two alternative options would also be possible: cross-metathesis with macrolactamization as the final step, or ozonolysis of the olefin and coupling with a phosphonium ylide. The peptide portion would be produced using SPPS methods previously used in the process of producing micromide. The polyketide fragment and its key stereocenters at C38-40 would be established through Evans' aldol with (*S*)-2-methylbutanal to give the

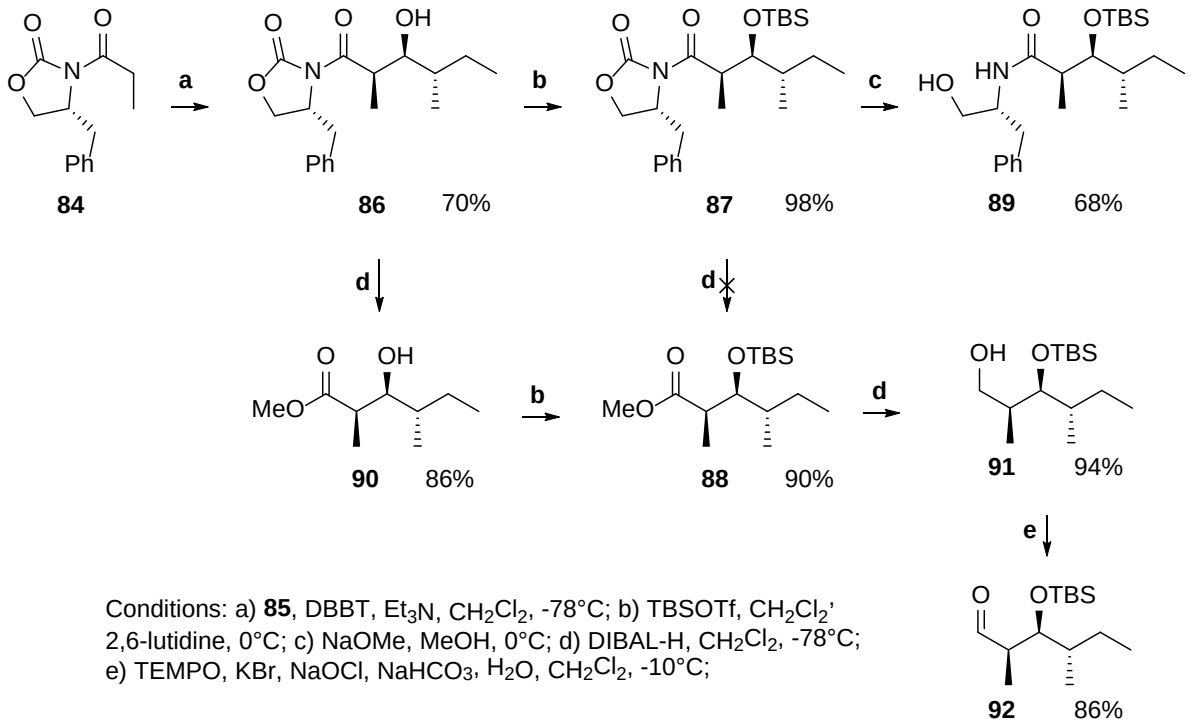


Scheme 18. Key Fragments of Lagunamide A

needed *S,S* relationship on C38,C39 described by Tripathi. Reagent-controlled asymmetric homologation via Brown's allylborane would establish the stereocenter on the second hydroxyl group at C37.

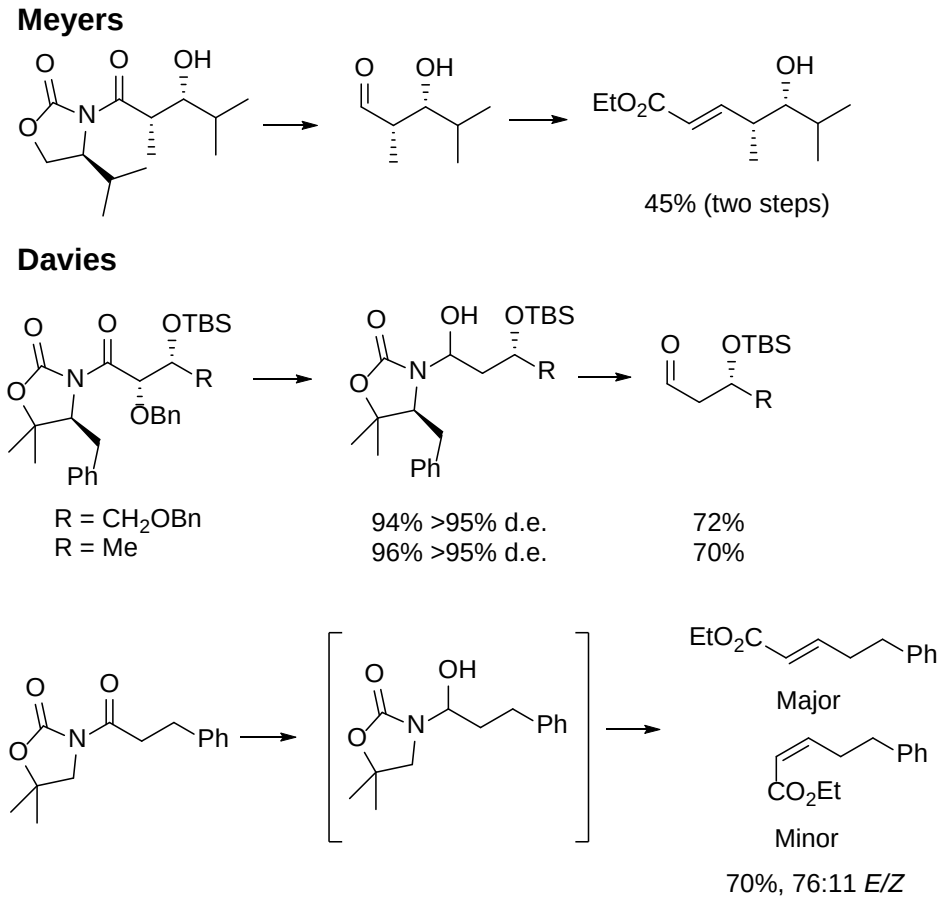
2.2 Synthesis of the C27-C45 Polyketide Fragment

The initial synthesis began smoothly with the synthesis of Evans chiral imide **84** (Scheme 19).[40] TEMPO oxidation of commercially available (*S*)-methylbutanol gave the corresponding aldehyde **85** in 70% yield.[41] By performing the oxidation with care, the resulting aldehyde was sufficiently pure to use without further treatment. The transformation by Evans' aldol reaction gave **86** in good yields. Protection of the β-hydroxy group as the TBS-silyl ether **87** using TBS-Cl was unsuccessful and the more powerful triflate reagent was required, giving a nearly quantitative yield. Attempts to cleave the auxiliary using sodium methoxide gave desired ester **88**, but showed poor regioselectivity. The competitive attack on the endo-cyclic carbonyl resulted in ring-opening of the oxazolidinone, giving **89** as the major product. Like the chiral imide used in micromide (compound **32**; Figure 3), steric congestion at the β-oxygen was detrimental to the regioselectivity of the methoxide nucleophile, and it was necessary to cleave the auxiliary prior to protection of the β-hydroxy moiety. Thus, the chiral auxiliary was cleaved



Conditions: a) **85**, DBBT, Et_3N, CH_2Cl_2, -78°C; b) TBSOTf, CH_2Cl_2', 2,6-lutidine, 0°C; c) NaOMe, MeOH, 0°C; d) DIBAL-H, CH_2Cl_2, -78°C; e) TEMPO, KBr, NaOCl, $NaHCO_3$, H_2O, CH_2Cl_2, -10°C;

Scheme 19. Synthesis of Intermediate **92** via Evans' Aldol Chemistry



Scheme 20. Accessing Aldehydes Directly from Acyl Oxazolidinones

from **86** with sodium methoxide to give ester **90**. Protection of the hydroxyl group with TBS-OTf afforded silyl ether **88** in excellent yield. The ester was reduced to alcohol **91**, and subsequently oxidized to aldehyde **92** under both Swern and TEMPO conditions, the latter giving a cleaner product.

Typically, converting esters or amides to aldehydes gives the best yields in a two-step process by reduction to the alcohol, followed by oxidation. However, examples of acyl oxazolidinones being cleaved directly to the aldehyde are known. For lagunamide A, the two-step process gave aldehyde **92** in 80.2% overall yield. Despite the good yields afforded by the existing strategy, the ability to shorten the synthesis by three steps was enticing. Direct reductive cleavage to aldehydes had been performed as early as 1985 by Albert Meyers in the

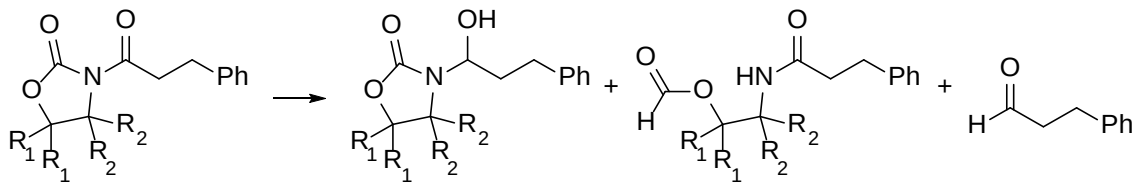
synthesis of a madumycin II fragment (Scheme 20).[42] Meyers and co-workers reduced their substrate with SMEAH to the *N*-1'-hydroxy species, which spontaneously decomposed to the aldehyde after reduction with Red-Al. Meyers was unable to purify the aldehyde without it decomposing, so the crude was directly subjected to HWE conditions to give the alpha-beta unsaturated ester.

The work of Stephen G. Davies expanded on the Evans auxiliary by introducing *gem*-dimethyl groups on the oxazolidinone, enhancing stereoselectivity and regioselectivity during cleavage (Table 2). Naming this chiral auxiliary as SuperQuat,[43] the Davies group investigated its and other chiral imides use as latent aldehydes, choosing DIBAL-H as the reducing agent.[44,45] The substrate used by Davies produced a *N*-1'-hydroxy species stable enough to be isolated by chromatography and required treatment with K_2CO_3/MeOH to induce fragmentation to the desired aldehyde. Davies was able to take this method a step further by using a lithium phosphonate to promote fragmentation and perform an HWE reaction "*in situ*" without workup.

Interestingly, the same issue with regioselectivity arises with the reductive cleavage as the transesterification. The imide presents two reactive carbonyls. In the ester cleavage, discrimination of the nucleophilic attack hinges on sterics. Cleavage of oxazolidinones has been thoroughly examined by Evans.[23] However, reductive cleavage by Evans was done with strong reducing agents such as LAH, to give the alcohol product. The Davies SuperQuat oxazolidinone (5,5-dimethyloxazolidi-2-none) was used to direct selectivity to the *N*-acyl carbonyl. The substitutions at the 4 position of the ring, critical for chiral induction, are slightly detrimental to the isolation of the *N*-1'-hydroxy intermediate.

Following these examples, the silyl-protected aldol product **87** was subjected to the same reduction conditions, utilizing DIBAL and SMEAH independently. Substrate **87** was

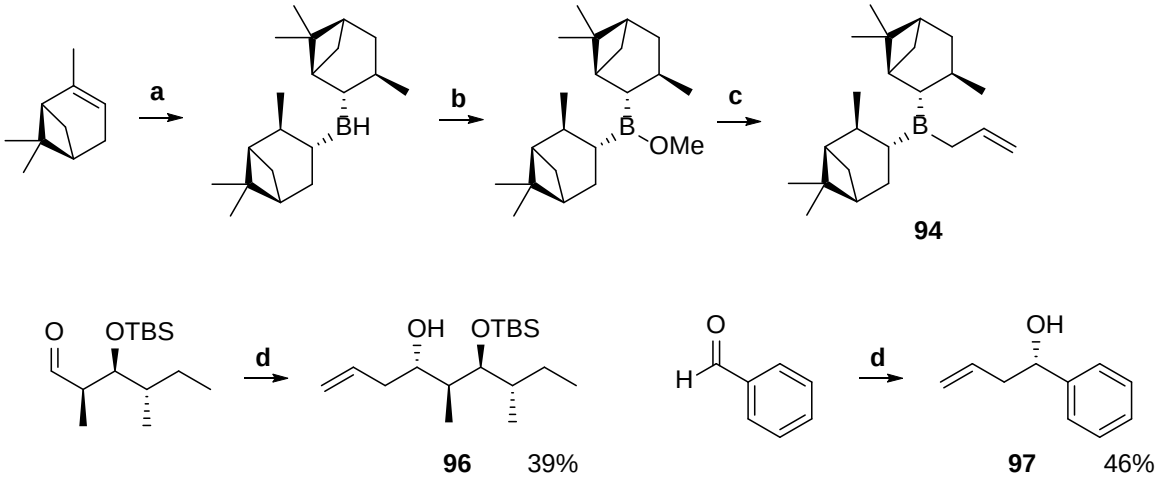
Table 2. Effects of Oxazolidinon Substituent Groups Demonstrated by Davies



R_1,R_1; R_2,R_2			
H,H; H,H	65%	5%	17%
H,H; Me,Me	45%	5%	27%
Me,Me; H,H	100%	--	--

unaffected by DIBAL, returning only the starting material—an unsurprising result, considering the steric congestion produced by being bounded by the oxazolidinone and the TBS ether. The congestion presumably prevents the bridged DIBAL dimer from complexing with the carbonyl. A distinct aldehyde peak was observed by NMR when SMEAH was used as the reducing agent. While the sizes of DIBAL and SMEAH are not drastically different, SMEAH does not require complexation to donate a hydride. Like Meyers, attempts to isolate the aldehyde resulted in decomposition, while attempts to use the crude directly did not yield the desired olefin. Given that the aldehyde was generated, there is potential for direct reductive cleavage to work, but with aldehyde **92** already in hand, the concept was set aside.

The next task was producing the Brown's allylborane for the allylboration.[46,47] To begin, borane-DMS complex was treated with (+)-α-pinene to give (-)-diisopinocampheylborane as a white solid (Scheme 21), however, the formation of solid was not consistent, giving doubts as to quality of the starting material. When solid did form, the DMS was removed in vacuo and excess pinene with THF was added to allow for equilibration to higher enantiopurity as described by Brown. The residual (-)-Ipc_2BH was treated with methanol to give the methylborinate ester.



Conditions: a) BH_3·DMS, THF *then* Et_2O, 0°C; b) MeOH, 0°C; c) allylMgBr, Et_2O, -78°C; d) **94**, Et_2O, -78°C

Scheme 21. Allylboration via Brown's DIPC Reagent

Grignard addition of allyl magnesium bromide to the borinate gave the desired allyl diisopinocampheylborane reagent.

The results of allylboration did not fare well; yields were inconsistent, ranging from 12-38%. It is suspected that congestion due to the large protecting group on the β-oxygen inhibits the reaction, much in the same way it inhibited the regioselectivity of the oxazolidinone cleavage. Much slower reaction rates were observed in reactions when using aldehydes with bulky β-substitutions. When the reagent was tested with unhindered aldehydes such as benzaldehyde and pentanaldehyde, the reaction proceeded with much greater ease, giving isolated yields of 65% and 81% yields respectively. However, allylborations have been performed using more substituted allyl groups such as 1,3-dimethylallyl, producing yields greater than 70%.[47] Allylborations are also known to proceed nearly instantaneously, even at temperatures as low as -80°C. If there was a mismatch between the stereocontrol of the reagent and the substrate, we would expect higher conversions with lower dr. Three-dimensional modelling shows that enough room is available for the borane to complex to the aldehyde but

steric bulk from the TBS moiety prevents the cyclic transition state from forming (Figure 15).

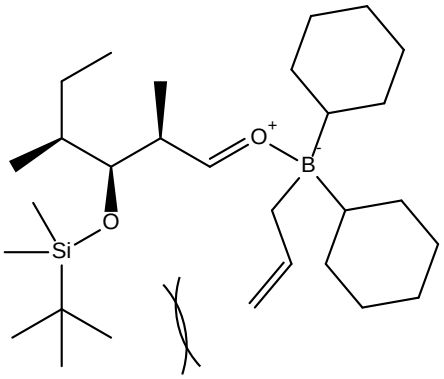


Figure 15. Steric Congestion Inhibiting Sigmatropic Rearrangement

2.3 Other Syntheses and Accessing Anti-Aldol Products

As efforts to optimize the addition at C37 progressed, Dai et al. presented the first total synthesis and stereochemical revision of lagunamide A.[10] The work by Dai et al. produced the synthetic lagunamide A, which showed discrepancies in the ^{13}C spectra against the natural lagunamide A. In their stereochemical revision, six total stereoisomers were produced before the correct structure was confirmed (Figure 16).

Shortly after the release of the work, other groups soon presented their efforts on lagunamide A as well including the Wei group at Fudan University in Shanghai, China; Chang et. al, a multi-institution collaboration in Taiwan; Jorges and Kazmaier from Saarland University in Germany. In each synthesis, the authors relied on the olefinic moiety as a key coupling step. Three of the syntheses featured the homoallylic alcohol **Error! Reference source not found.** as the intermediate (Figure 17) for the flexibility to proceed by metathesis or by oxidative cleavage, followed by HWE. With the exception of Jorges and Kazmaier, all other syntheses began with (*S*)-2-methylbutanal and built the fragment towards the olefinic end. It is not surprising, since the Evans aldol reaction is such a staple of polyketide chemistry, that it was the first choice by every group. The major limitation of the Evans chemistry is that it can only access aldol products with *syn* selectivity.[48] When Dai identified that the C38 and C39 stereocenters were configured in an *anti* relationship, each group diverged in their approaches to completing the fragment. Dai's route required agility, as the stereochemistry had not been verified yet, so while the methods they used produced less than ideal yields and selectivities, the intermediates were easily modified to produce different stereoisomers or the minor diastereomer was used.

The second report by the Wei group [49] presented two key features. First, a substrate-directed method was used to access the homoallylic alcohol. By adding $ZnCl_2$ as a Lewis acid with an allyl Grignard reagent, the "desired" i.e. pre-revision stereochemistry was obtained at C37 with a 90:10 dr. (Figure 18). Under the Felkin-Ahn model, 1,2-asymmetric

induction leads to the 1,2-*syn* product and the effect of 1,3-induction is generally too weak on its own. However, chelation of the beta substituent results in 1,3-*anti* control. Through this rationale, introduction of a Lewis acid would generate selectivity through the Cram-chelate intermediate to overpower 1,2-induction to give the *anti*-Felkin product. Interestingly, ten other

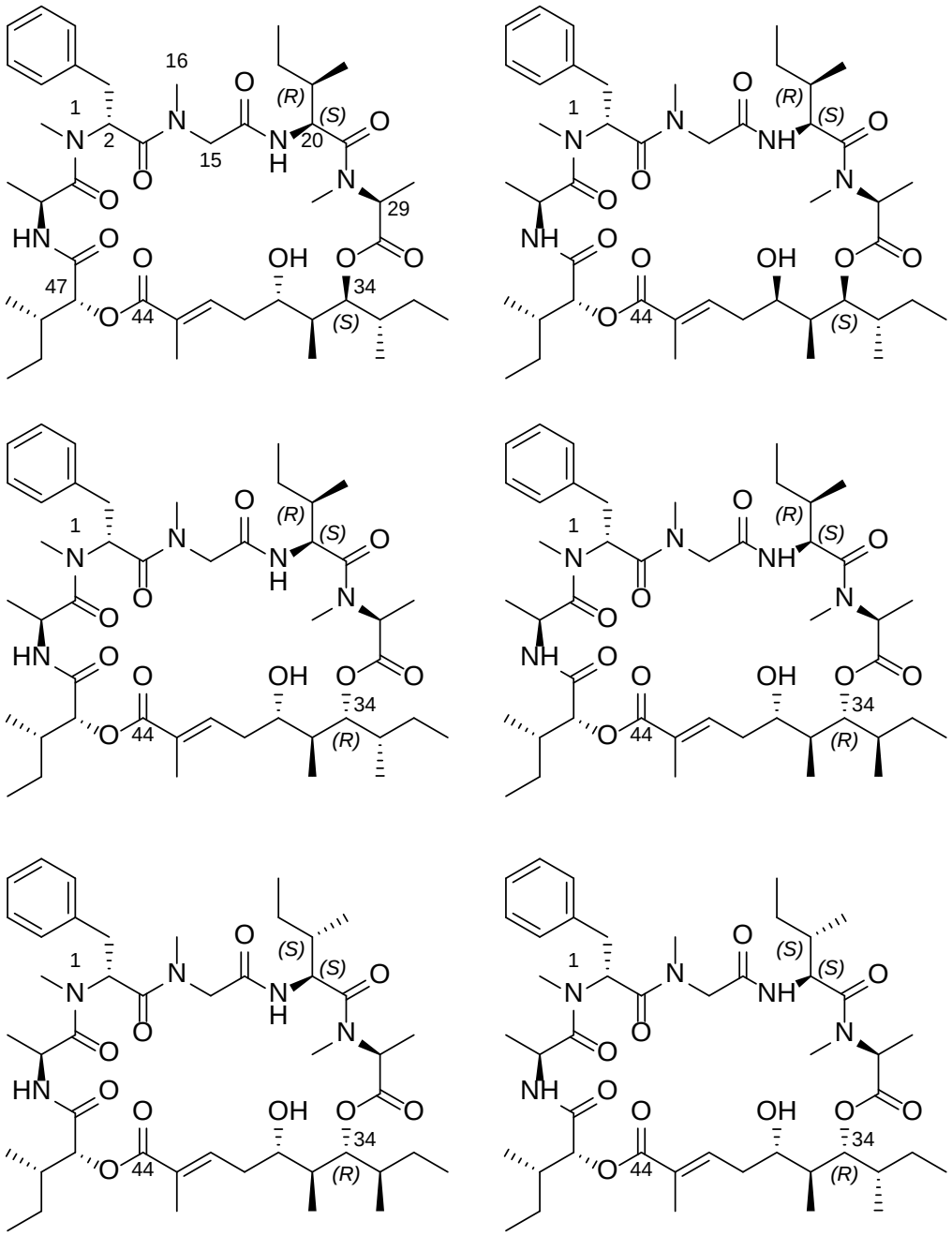
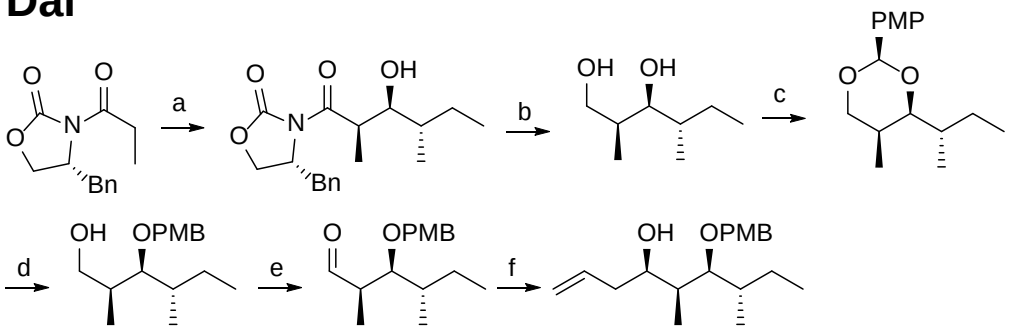


Figure 16. Six Isomers of Lagunamide A Produced by Dai

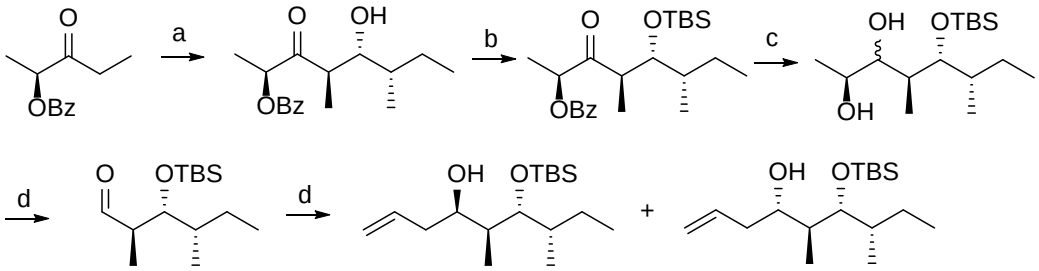
Lewis acids surveyed did not provide any stereoselective enhancement. One possible explanation is that the Lewis acid must provide stronger ligation than the magnesium generated

Dai



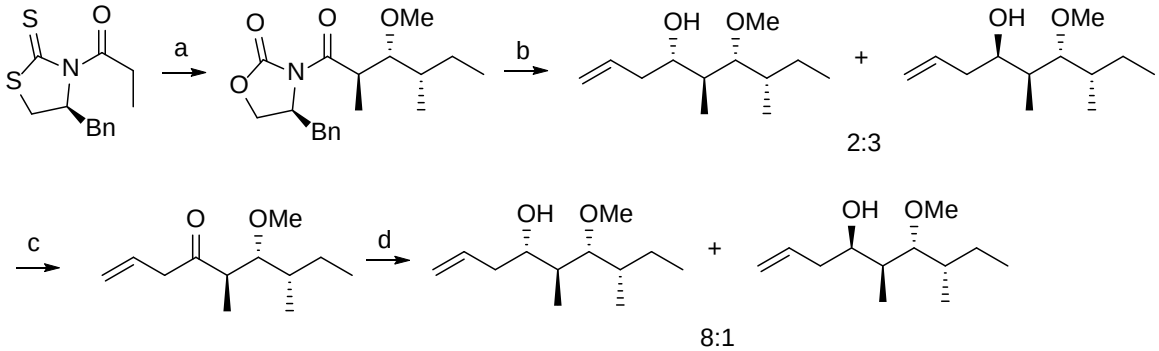
a) Bu_2BOTf, DIPEA, (S)-methylbutanal, CH_2Cl_2; b) $NaBH_4$, Et_2O, MeOH;
c) anisaldehyde dimethyl acetal, PPTS; d) DIBAL-H, CH_2Cl_2; e) DMP, $NaHCO_3$, CH_2Cl_2; f) allyltributylstannane, $BF_3 \cdot OEt_2$, CH_2Cl_2

Huang



a) (S)-methylbutanal, Cy_2BCl, Me_2NEt; b) TBSOTf, 2,6-lutidine;
c) $NaBH_4$, MeOH, THF; d) $NaIO_4$, MeOH, H_2O; e) AllylMgCl, THF

Liu



a) $TiCl_4$, DIPEA, then $SnCl_4$, (S)-methylbutanal dimethyl acetal; b) DIBAL-H; CH_2Cl_2;
c) AllylMgCl, THF; d) $NaBH_4$, MeOH

Figure 17. Various Methods Towards the Homoallylic Alcohol Intermediate

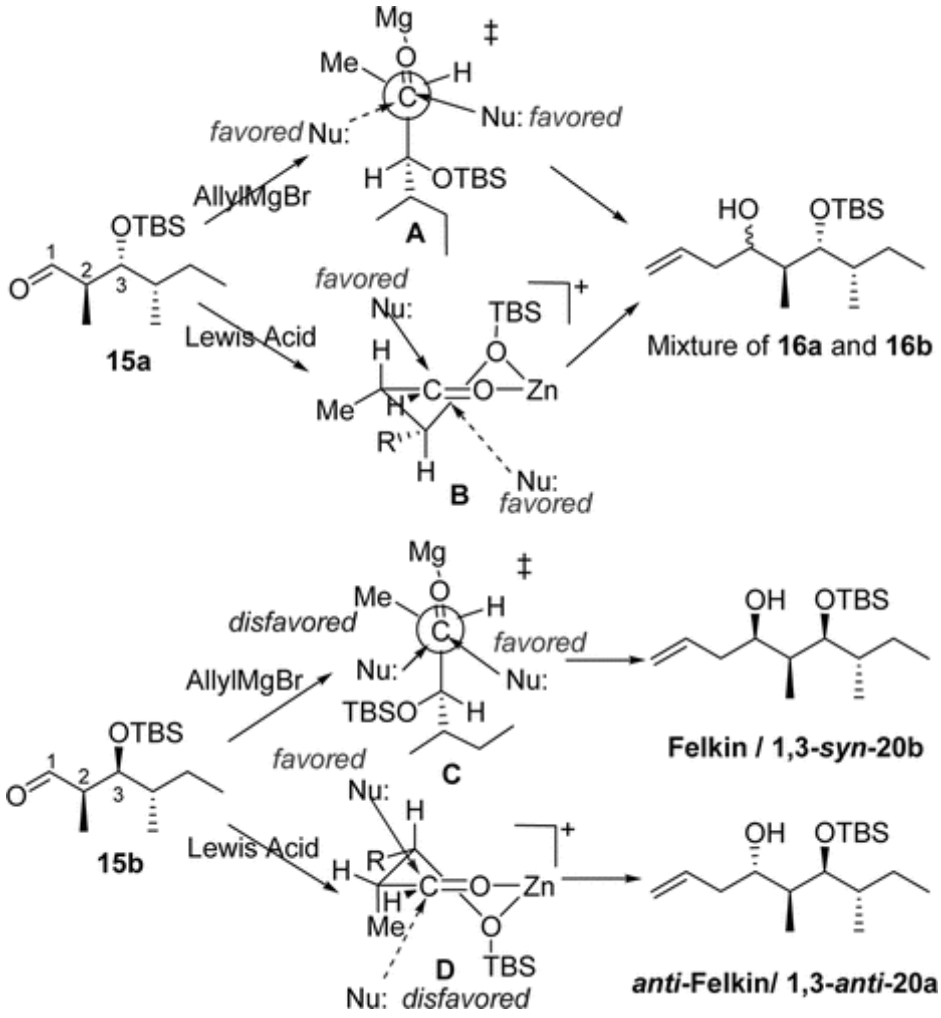


Figure 18. Predictions of Felkin-Ahn and Cram Models.

by the Grignard reagent. Based on the Irving-Williams series, zinc provides one of the more stable divalent complexes.[50] Since the proposed transition state requires a configuration leading to strong 1,3-diaxial interactions that block the approach of the nucleophile, it is reasonable that a strong chelator would be required to overcome these 1,3-diaxial interactions. Unfortunately, as a substrate-directed method, this only worked with the pre-revision product. Therefore, it was necessary for the Wei group to oxidize and reduce the alcohol at C39 to obtain the desired stereochemistry. They found that the use of lithium triethylborohydride provided the desired stereochemistry in 86% yield with greater than 99:1 dr. The second feature, the Wei group attempted to cyclize the product by ring-closing metathesis, but no cyclized material was found

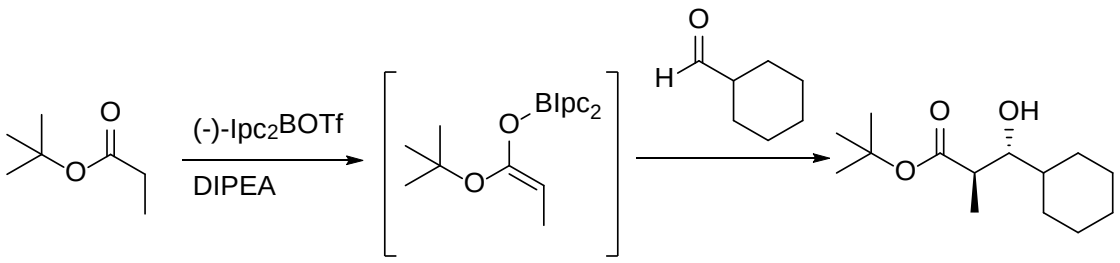


Figure 19. Anti-selective Aldol via a Boron Enolate

after repeated attempts. However, cross-metathesis with methacrylaldehyde provided the desired product in 80% yield.

The third report was the synthesis of the C27-C45 fragment by Chang and co-workers, who successfully produced the desired fragment, but the final deprotection necessary for a formal synthesis was not reported. Their work utilized the Crimmins auxiliary for chiral induction to obtain the *anti* relationship between the alpha-methyl and the beta-hydroxy groups. The homoallylic alcohol was accessed via the Grignard reagent with little enantioselectivity. Like the previous example, the Chang group also resorted to the oxidation-reduction motif. However, their manipulation was to the homoallylic alcohol. They showed that oxidizing the alcohol at C37, followed by reduction with $NaBH_4$ without external chiral induction gave the desired stereochemistry in 8:1 dr.

The final report by Jorges and Kazmaier produced lagunamide A through Matteson homologation. Their method was too laborious to be considered for a scalable synthesis but it demonstrated the versatility of Matteson homologation.

We had previously considered the Crimmins auxiliary since it was the quickest way to access the anti product. Treating our existing Evans auxiliary with Lawesson's reagent to give the oxazolidine-2-thione, but the literature suggested that such a modification would not give diastereomeric ratios of acceptable levels. Indeed, the Crimmins method was undertaken by Chang and coworkers, who were only able to obtain their anti-aldol product in 62% yield with 82:18 dr.

The original intent in using allylboration was to take advantage of its versatility, as reactions involving DIPC is known to be strictly reagent-controlled.[51] Therefore, it was expected that the antipode would allow access to the opposite diastereomer. The failure of the original allylboration to provide desirable yields necessitated a different strategy. Since the necessary reagents were already on-hand, other methods utilizing α-pinene were examined. The enol-borinate described by P.V. Ramachandran[52] was investigated as potential route to the desired aldol product (Figure 19). Simultaneously, a sulfonamide auxiliary developed by Arun Ghosh and Jae-Hun Kim, utilizing a chiral aminoindanol was investigated by co-workers in the lab (Figure 20).[53] Though the enol-borinate provided the expected product on a test substrate, yields were once again low and inconsistent, perhaps due to the sensitivity of the reagent to water and oxygen. Ghosh's sulfonamide was more tolerant of external factors and became the lead candidate for accessing fragment **117**. Ghosh and Kim later described acenaphthene-derived aminoalcohols with greater performance superior to their original sulfamide but the original sulfamide worked exceptionally well for our purposes.[54]

(1R,2S)-1-Amino-2,3-dihydro-1H-inden-2-ol was tosylated to give sulfonamide **101**, followed by acylation with propionyl chloride to give ester **102** (Scheme 22). The titanium-mediated aldol reaction provided **103** in 98% yield and 95:5 dr. Cleavage of the auxiliary by methoxide gave **104**, followed by protection of the β-alcohol with TBS-Cl to give **105**. The ester was reduced to alcohol **106** with DIBAL-H. Oxidation of **106** with PDC gave the

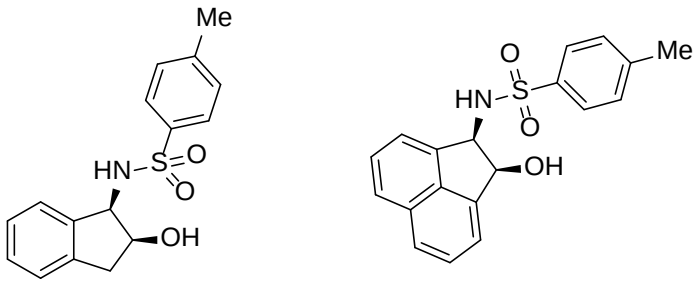
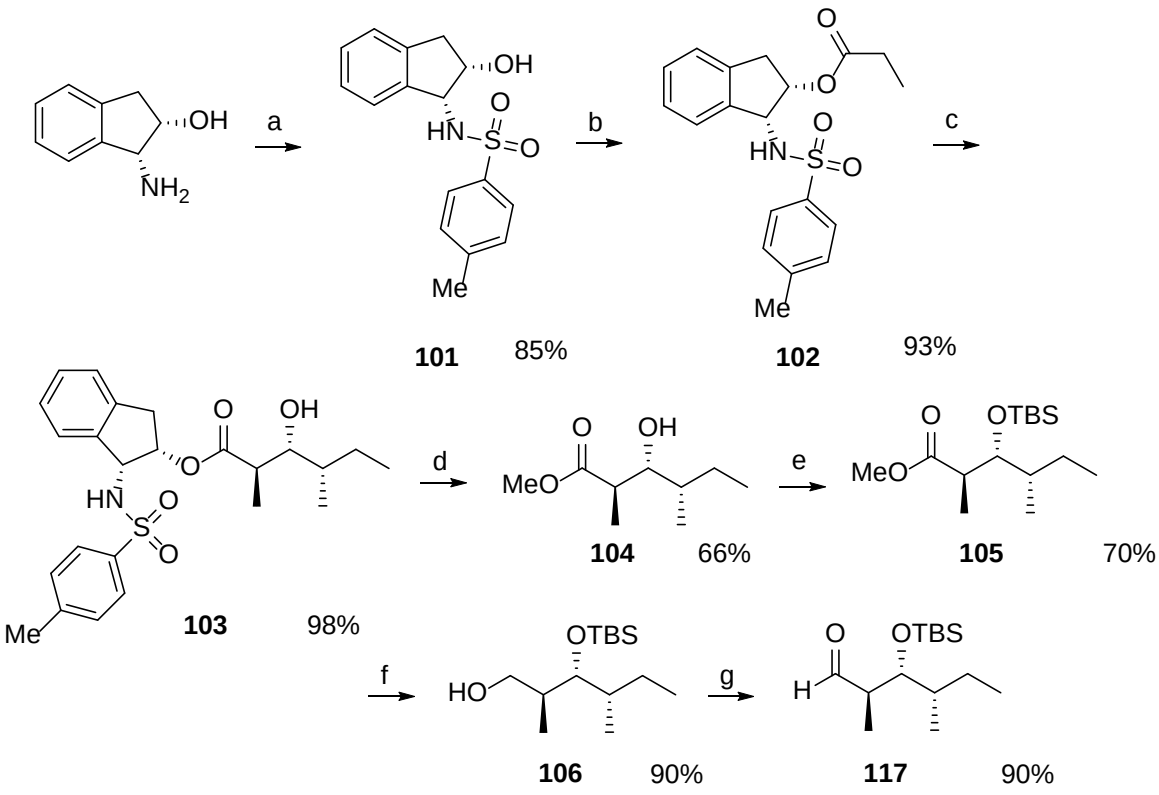


Figure 20. Chiral Aminoindanol and Acenapthene Auxiliaries

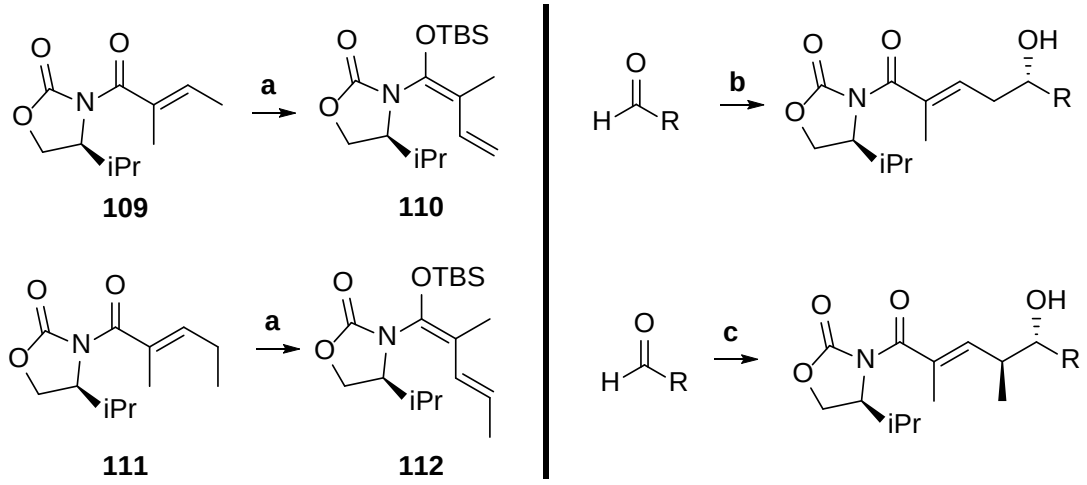


Conditions: a) TsCl, Na_2CO_3, H_2O, EtOAc, THF; b) EtCOCl, C_5H_5N, CH_2Cl_2; c) $TiCl_4$, (S)-methylbutanal, CH_2Cl_2; d) NaOMe, MeOH; e) TBSCl, imidazole, CH_2Cl_2; f) DIBAL-H, CH_2Cl_2; g) PDC, CH_2Cl_2

Scheme 22. Synthesis of Fragment via the Ghosh Aldol Reaction

desired aldehyde **117**. With the corrected aldol product in hand, work proceeded towards accessing the olefinic fragment of lagunamide.

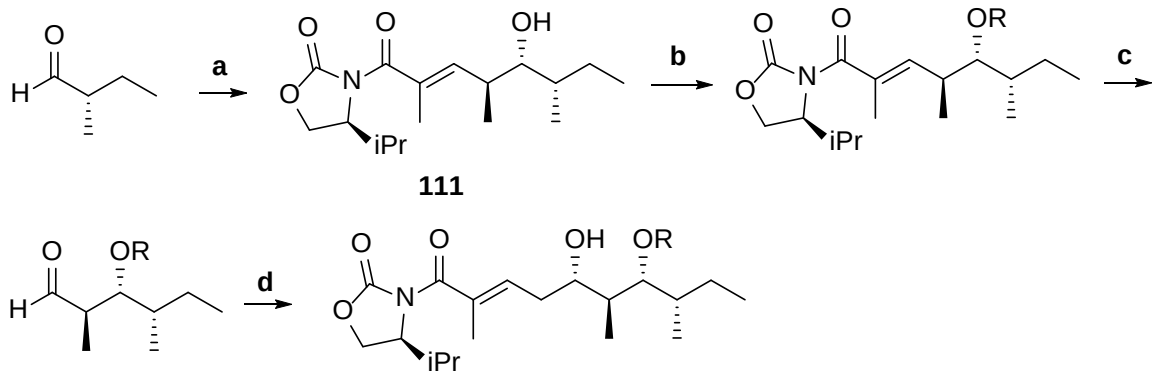
The lack of positive results from the allylborane reagent and the Wei group demonstrating that cyclization by ring-closing metathesis was inexpedient, the unsubstituted homoallylic alcohol was no longer a critical intermediate. We considered the possibility of accessing the olefinic portion directly using vinylogy and surveyed literature for a suitable method. The most interesting was a variant of the vinylogous Mukaiyama aldol reaction (VMAR). First described by Susumu Kobayashi, an Evans auxiliary was used to create vinyl ketene silyl *N,O*-acetal **110**, which provided 1,6,7 chiral induction (Scheme 23).[55] The Kobayashi method demonstrated high enantioselectivity and could possibly give the desired fragment **140**, without the need to couple by HWE or cross-metathesis.



Conditions: a) NaHMDS or KHMDS, TBS-Cl, THF;
b) **110**, $TiCl_4$, CH_2Cl_2; c) **112**,$TiCl_4$, CH_2Cl_2

Scheme 23. Remote Asymmetric 1,6,7-Induction by Kobayashi

Following Kobayashi's protocol, the VMAR chemistry was found to be easily reproducible. In addition, it was realized that Kobayashi's vinylogy could be extended to produce the anti-aldol product **117** by subjecting the product to ozonolysis (Scheme 24). While using the VMAR would reduce the atom economy due to ozonolysis, the ubiquitous use of the Evans oxazolidinone meant the chiral reagent was significantly cheaper—100-fold less expensive than the Ghosh aminoindanol. In addition, the method was more than twice as enantioselective. Thus, the Ghosh method was supplanted with the Kobayashi method.



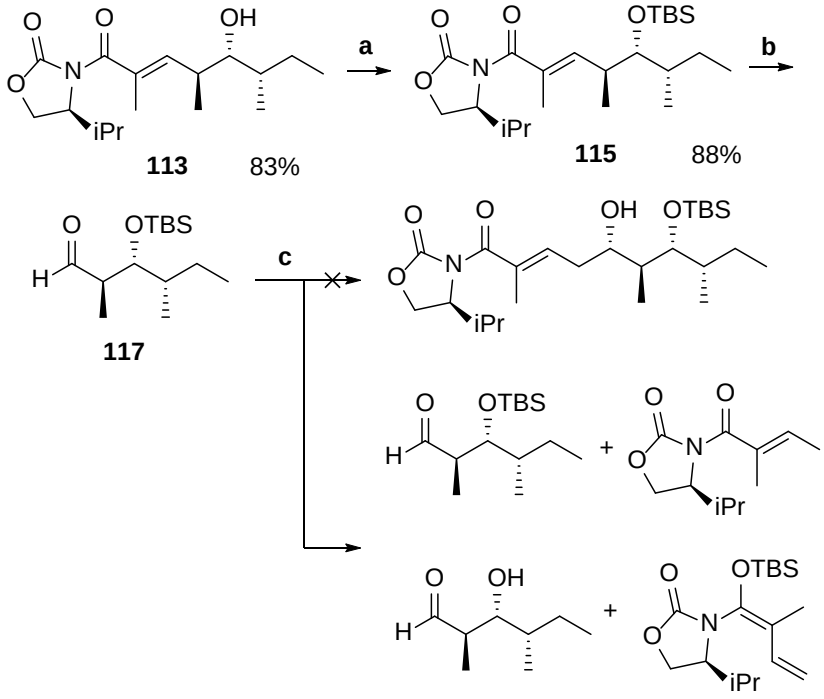
Conditions: a) **112**, $TiCl_4$, CH_2Cl_2, -78; b) protection;
c) O_3, CH_2Cl_2; d) **110**, $TiCl_4$, CH_2Cl_2

Scheme 24. Accessing the Anti-Homocrotyllic alcohol by Kobayashi's VMAR

2.4 Incorporation of the Kobayashi VMAR

Using the Kobayashi method, the goal would be to produce the polyketide fragment through two iterative VMARs (Scheme 25). After optimization, Kobayashi's VMAR produced **113** easily, isolated as a single diastereomer in 83% yield, but the choice of protecting group soon became the primary obstacle. Protection of the aldol product as the silyl ether **115**, followed by ozonolysis to aldehyde **117** proceeded in excellent yields. Unfortunately, the second iteration of the VMAR reaction produced no product, and aldehyde **117** was recovered, along with vinyl ketene acetal **112** and its hydrolysis product **110**, unless the reaction temperature was raised above -40°C. Higher temperatures were successful in producing aldol products but results in the complete loss of stereoselectivity.

Various other protecting groups were surveyed including bulkier silyl ethers, ethers, and acetals. While bulky silyl ethers are generally compatible with $TiCl_4$ and has been used by



Conditions: a) TBSOTf, 2,6-lutidine, CH_2Cl_2; b) O_3, pyridine, CH_2Cl_2; c) **110**, $TiCl_4$, CH_2Cl_2

Scheme 25. Polyketide Fragment through Iterative VMARs

Evans in titanium enolate chemistry, [56] both TBS- and TDPS-protected aldehydes were deprotected under typical Kobayashi conditions while providing no desired product. The THP ether also proved to be too sensitive to the Lewis acid. Only when the β-hydroxy group was protected as an ester, were we able to isolate any aldol product.

It is likely that the steric congestion due to the α methyl group and the large β-silyl ether inhibited the reaction. Activation of the aldehyde with $TiCl_4$ forms a complex similar to the allylborane previously investigated, preventing attack on the aldehyde by the nucleophile. The issue with steric congestion was also previously observed in the synthesis of micromide. Steric bulk at the β position can severely limit nucleophilic access to the carbonyl; conversely, the presence of steric bulk at the carbonyl can reduce reactivity of the β alcohol, requiring a more reactive silyl triflate over the more common silyl chloride. Unfortunately, only silyl ethers that are bulky are robust enough to withstand the $TiCl_4$ Lewis acid used in the VMAR. Smaller groups such as TES have been used to protect hydroxyl groups temporarily in VMARs but are hydrolyzed in the process.[57] The ester provides a more robust protecting group while being more compact at the same time.

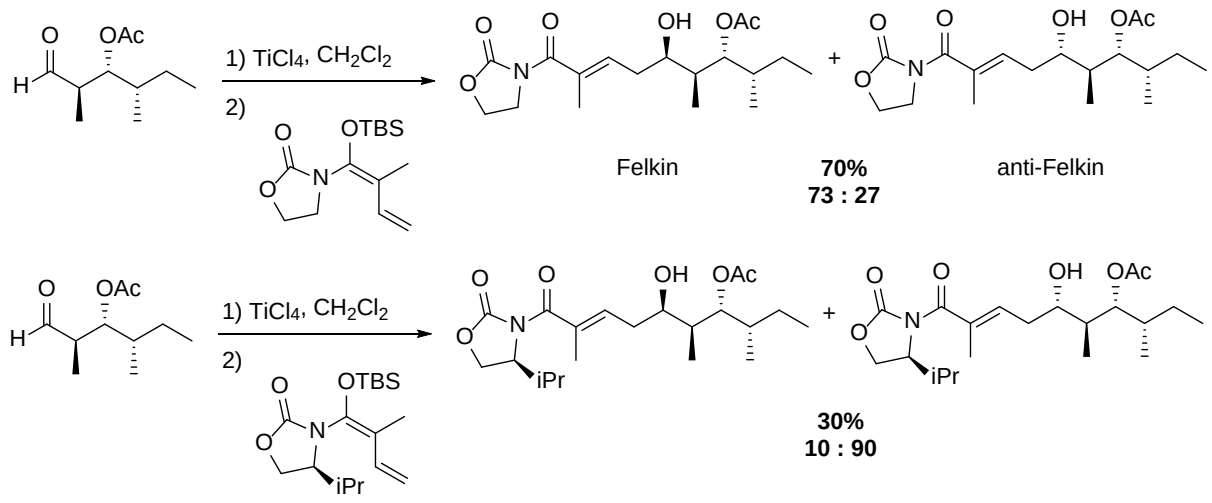
However, the ester provided its own challenges. The acetate-protected aldehyde was much more volatile than the silyl ether. The silyl ether protecting group more than doubles the mass of the aldehyde and provides roughly the same steric bulk. The large mass made the silyl ether-protected aldehyde a viscous oil, while the acetylated aldehyde was a light oil and surprisingly volatile. To reduce the volatility of the aldehyde, the propionate and decanoate were produced and subjected to the VMAR as well. The decanoate was no longer volatile, and its unbranched structure was expected to provide less steric congestion than the TBS group, however, it diminished the already poor yield of the second VMAR. Thus, the propionate was chosen as a compromise. The propionate was still volatile and even with carefully controlled concentrations, evaporative losses still ranged from 10-15% of the yield.

Attempts to optimize the second VMAR failed to reliably produce yields over 35%. The Kobayashi VMAR has been used extensively in total syntheses,[58–61] and many variations on it have been reported, including hetero-substitutions at the α position, *syn*-selective acetals,[62] and *E,E*-vinylketene acetals.[63] However, very few have used it on an aldol product, which contains a β-oxygenated moiety. In the one instance where we found its use, the same issue with low yields persisted.[64] To further understand the cause of these low yields, we can consider some parallels drawn between the VMAR and the related titanium enolate chemistry. Many of the variations on VMAR gave results consistent with titanium enolates.

Titanium enolates used by Ghosh were unreactive with aldehydes unless the aldehydes were pre-coordinated with $TiCl_4$. The Kobayashi reaction also provides better yields when the aldehyde is pre-coordinated with $TiCl_4$. It is known that the concentration of $TiCl_4$ affects the facial selectivity with α-substituted heteroatoms as a result of mono-dentate and bi-dentate titanium ligands.[65] Presumably, Kobayashi had anticipated this effect, since he reported the same effect in the VMAR reaction, years later.[66] In aldehydes that are unable to form bidentate species, a large excess of $TiCl_4$ reduced stereoselectivity and the same effect was observed with the VMAR by Hosokawa. In fact, at four equivalents of $TiCl_4$, selectivity in the VMAR was reversed from *anti* to *syn* with a diastereomeric ratio greater than 50:1.[67]

Another consideration is the directing effect of the substrate itself. As previously observed in the allylation by Huang and co-workers, chiral induction based on the substrate can be explained by application of the Felkin-Ahn model for 1,2-asymmetric induction. While typically a weaker effect, 1,3-asymmetric induction must also be considered. The effects of the two combined have been explored by Evans and co-workers.[68]

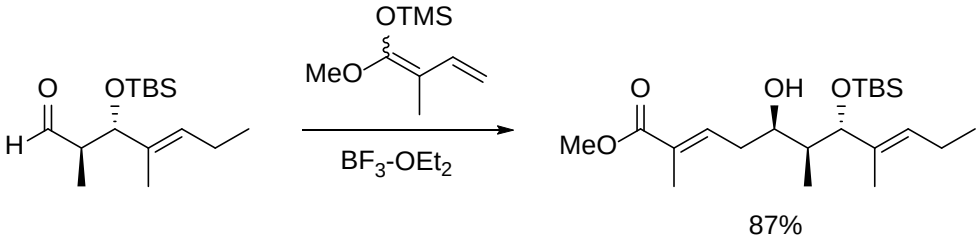
We found that the existing stereochemistry is “mismatched” with the directing ability of the chiral auxiliary. When the reaction was performed with an achiral reagent, the yield improved dramatically, giving 70% yield in 73:27 dr, with the major diastereomer being the antipode of the desired product (Scheme 26). The reaction also performed well using the chiral



Scheme 26. Chiral vs Achiral Imide VMAR

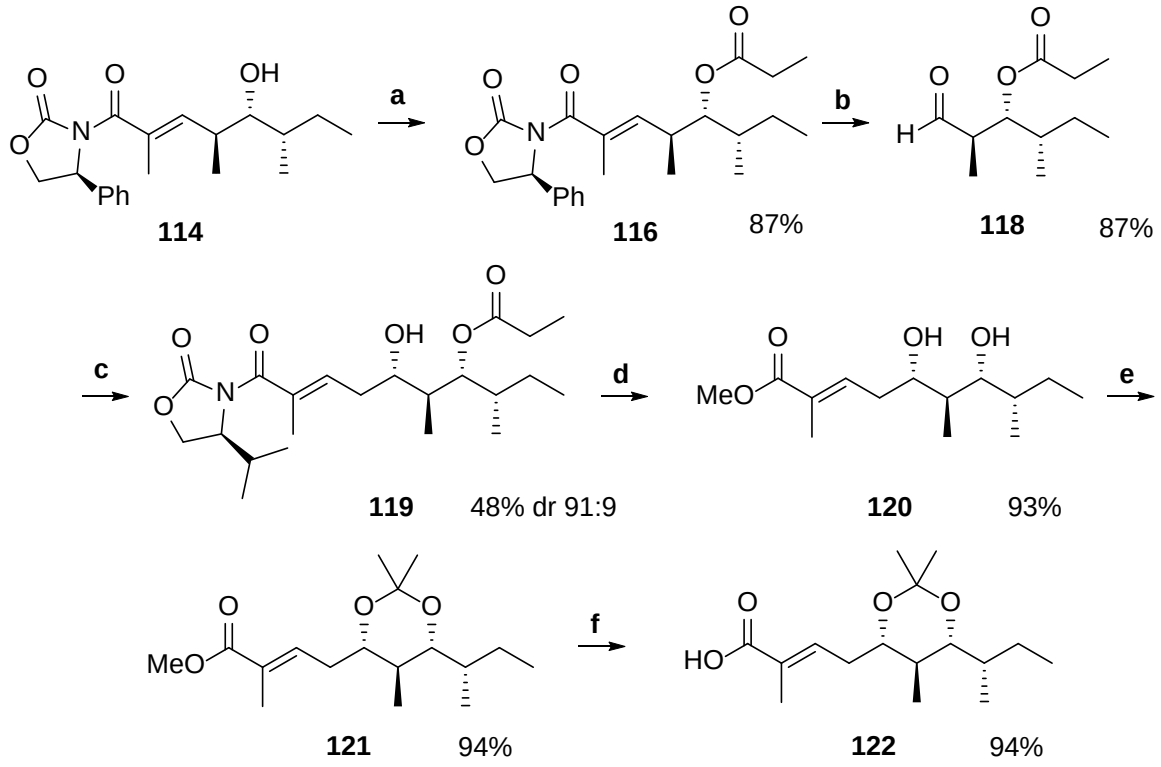
auxiliary with an aldehyde lacking an α methyl substitution, giving 68% yield of the matching diastereomer with 96:4 dr. This second reaction was performed on the decanoate, illustrating that hindrance to the reaction requires steric bulk at both the α and β positions. In the synthesis of aurilide, substrate-controlled VMAR gave the undesired (*syn*) stereochemistry as a single diastereomer (Scheme 27).[69]

When the 1,3-position is protected as a TBS ether, it appears that the large bulk of the silyl group and branched aldehyde leaves little space for the nucleophile to attack, once coordinated with $TiCl_4$. Using the propionate reduces steric crowding and provides some product, but it is still uncertain if the presence of more electron-rich moieties are detrimental to the efficacy of $TiCl_4$, especially when considering the oxophilic nature of titanium.



Scheme 27. Application of a VMAR in the Synthesis of Aurilide

Although attempts to improve the yield of the second VMAR continues, our current method is limited to 48% yield at best, using the propionate (Scheme 28). The chiral auxiliary was removed by methanolysis, which simultaneously cleaved the propionate, to give diol **120**. The diol was converted to acetonide **121**, allowing for verification of the stereochemistry by NMR. Saponification of ester gave carboxylic acid **122**.



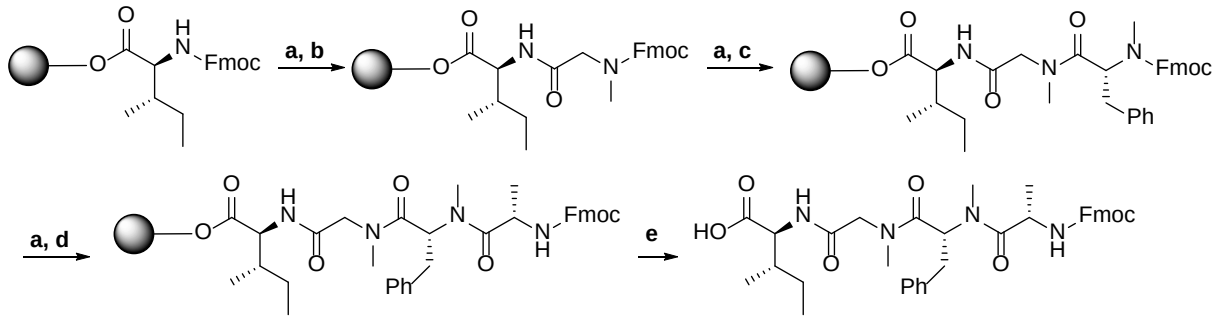
Conditions: a) EtCOCl, DMAP, C_5H_5N, CH_2Cl_2, 0°C; b) O_3, pyridine, CH_2Cl_2, -78°C;
c) **112**, $TiCl_4$, CH_2Cl_2, H_2O, -40°C; d) NaOMe, MeOH, 0°C; e) 2,2-dimethoxypropane, PTSA

Scheme 28. Synthesis of Acetonide Intermediate

2.5 Synthesis of the Peptide Fragment

In contrast to micromide, the peptidic portion of lagunamide was synthesized without incidence on solid support, presumably due to the lack of large adjacent side chains. Micromide consisted of large side chain residues (Phe, Ile) which were *N*-methylated, creating an extreme level of steric hindrance. While lagunamide also contains *N*-methylated amino acids and the same residues (Phe, Ile), the methylation occurs on unhindered amino acids (Ala, Gly) and each bulky residue is separated by an Ala or Gly.

Once again, we chose to use the Fmoc protecting group and the CTC linker. To produce the peptide, Fmoc-isoleucine was anchored to 2-CTC on polystyrene support. Removal of the Fmoc group was done by treating the polystyrene beads with 20% piperidine in DMF. After the necessary washes with DMF, Fmoc-sarcosine was coupled using DIC and K-Oxyma. Each successive coupling was performed in the same manner: deprotection with piperidine followed by coupling by DIC/K-Oxyma to give the peptide sequence Fmoc-L-Ala-*N*-Me-D-Phe-Sar-L-Ile.



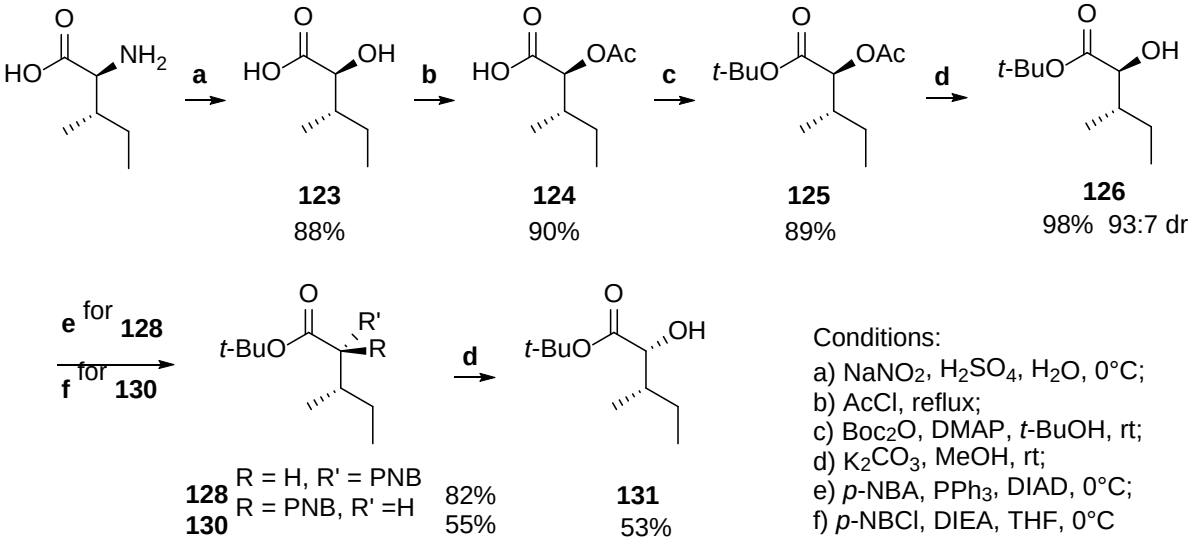
138 74% over 8 steps

Conditions: a) DMF, piperidine; b) Fmoc-Sar, DIC, K-Oxyma, DMF; c) Fmoc-N-Me-D-Phe, DIC, K-Oxyma, DMF; d) Fmoc-N-Me-L-Ala, DIC, K-Oxyma, DMF; e) 1% TFA/CH_2Cl_2

Scheme 29. Solid-Phase Synthesis of the Peptide Fragment

2.6 Synthesis of the D-Hydroxyisoleucic Acid Fragment

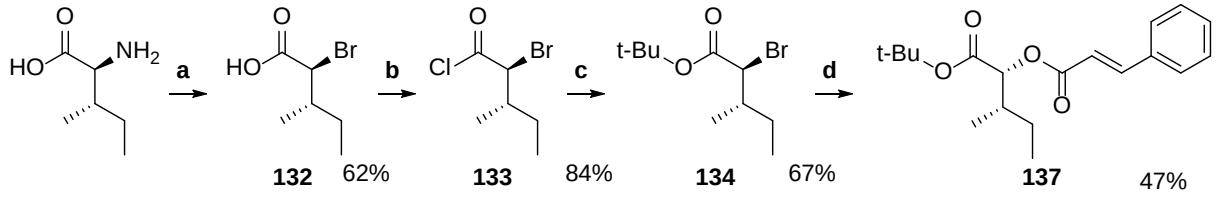
With peptide **138** in hand, only the D-hydroxyisoleucic acid fragment remained. Since the isoleucine derivative was the D-enantiomer, we accessed the necessary molecule by S_N2 inversion of the L-enantiomer. Diazotization of L-Ile to hydroxyisoleucic acid (Hila) **123** occurred in quantitative yield (Scheme 30). Attempts to directly esterify the carboxylic acid without first protecting the hydroxy group did not yield significant product. After acetylating the α-hydroxy moiety to produce compound **124**, Steglich esterification using tert-butanol achieved the tert-butyl ester **125** in 45% yield. A change of protocol to transesterification using Boc anhydride, catalyzed by DMAP improved yields to 89% (over two steps). Compound **125** was deacetylated with K_2CO_3 and MeOH to give free alcohol **126**. Tiglic acid was activated with DIAD and PPh_3, and alcohol **126** was added under these Mitsunobu conditions but did not give complete conversion to **128**. Presumably, the bulky alcohol is slow to activate and the higher pKa of the α,β-unsaturated acid results in faster decomposition of the DIAD-PPh_3 adduct, as described by Hughes and Reamer.[70] An increase to four equivalents of carboxylic acid did result in complete conversion, however, that would require using an excess of the C33-39 fragment, so instead, the stereochemistry was inverted using 4-nitrobenzoic acid, then cleaved. Stereochemical



Scheme 30. Mitsunobu Conversion of L-Isoleucic Acid to D-Isoleucic Acid

inversion was verified by coupling **126** with 4-nitrobenzoyl chloride to give the opposing diastereomer.

Other means to convert the amine to leaving group suitable for chiral inversion were also investigated. Parrish et al. successfully *O*-alkylated carboxylic acids with alkylbromides in the presence of $CsCO_3$, with one example of chiral inversion using neocholesteryl bromide.[71] Parrish reported that the conditions were mild enough such that no racemization was detected with alpha-substituted esters, so an attempt was made with the diazotization of L-Ile in the presence of excess bromide, giving the α-bromide. After protecting the carboxylic acid as the tert-butyl ester, treatment of the bromide with cinnamic acid, $CsCO_3$, in DMF gave the desired product in moderate yield. Though this method was not pursued further, it exhibited better yields with an electron-rich substrate compared to Mitsunobu conditions.

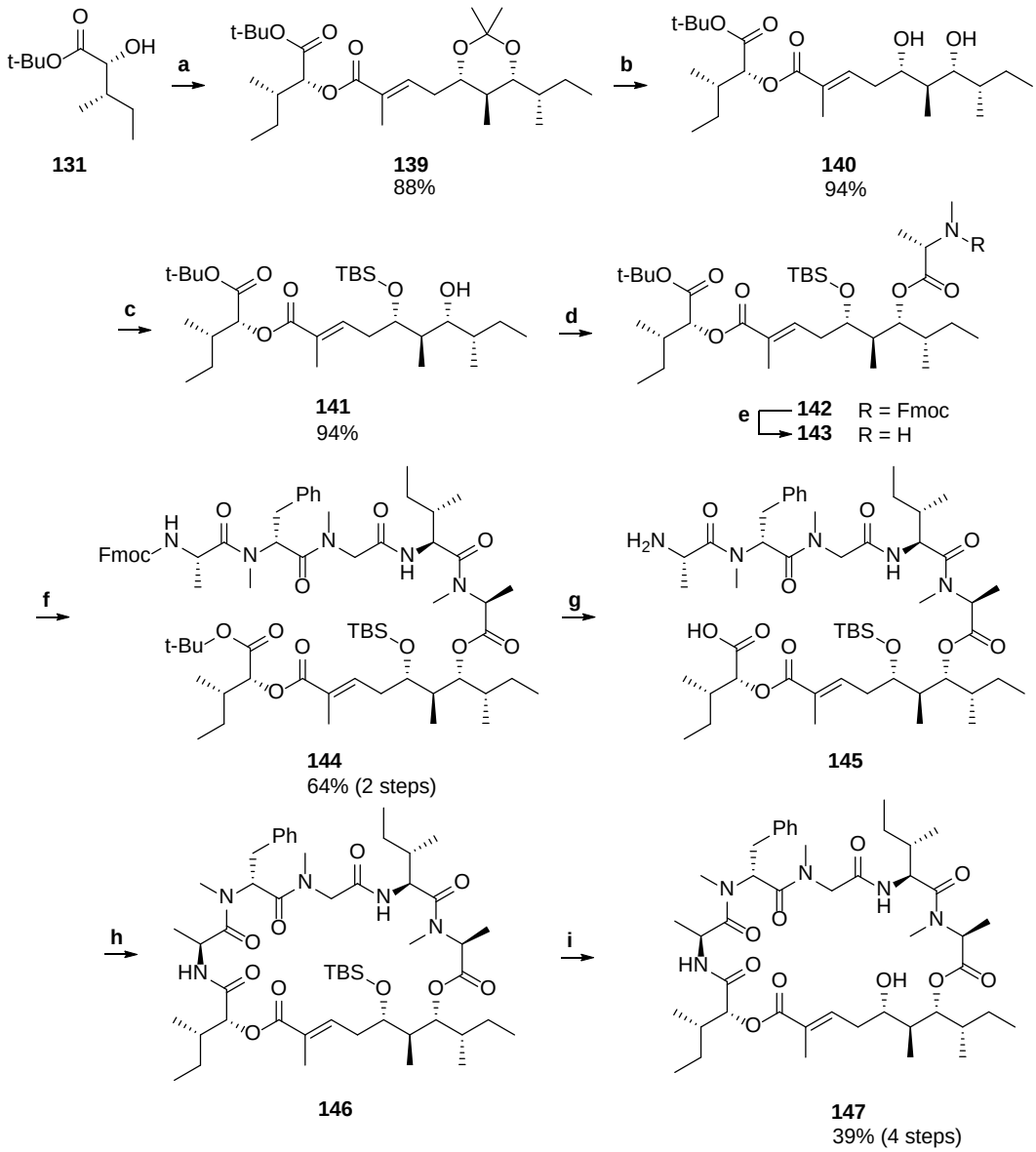


Conditions: a) HBr, KBr, H_2O; b) $SOCl_2$, CH_2Cl_2, reflux; c) t-BuOH, pyridine, CH_2Cl_2; d) (E)-cinnamic acid, $CsCO_3$, DMF

Scheme 31. Inversion of Isoleucine via the α-Bromide

2.7 Final Couplings

With all the necessary fragments in hand, compound **131** was coupled to acetonide **122** by Steglich esterification to give **139** (Scheme 32). Cleavage of the acetonide with PTSA afforded diol **140** followed by selective protection of the C27 alcohol with TBSOTf. Acylation of



Conditions: a) **122**, DCC, DMA, CH_2Cl_2, rt; b) PTSA, MeOH, rt; c) TBSOTf, 2,6-lutidine, CH_2Cl_2, -78°C; d) Fmoc-N-Me-L-Ala-Cl, DIEA, CH_2Cl_2, 0°C; e) Et_2NH, MeCN, rt; f) **139**; HATU, HOAt, collidine, DMF, rt; g) TFA, CH_2Cl_2, then Et_2NH, MeCN, rt; h) HATU, DIEA, CH_2Cl_2, rt; i) HF, MeCN, rt

Scheme 32. Assembly of lagunamide A 147

the remaining free alcohol **141** with Fmoc-*N*-Me-Ala-Cl gave **142**. The Fmoc group was removed with Et_2NH giving free amine **143**, which was used without purification. Coupling to the peptide **138** gave **144** in 64% yield. In preparation for the final cyclization, the Fmoc and tert-butyl ester moieties of **144** were deprotected with TFA, followed by Et_2NH to give **145**. Macrolactamization with HATU gave **146** and a final desilylation gave lagunamide A in 39% over four steps.

2.8 Conclusion and Future Work

Lagunamide A was produced in 5% overall yield, exceeding previously reported methods, using two iterative vinylogous Mukaiyama aldol reactions. Various other methods were explored in producing the southern fragment, including the Ghosh aminoindanol. Unfortunately, strictly-reagent controlled methods were not amenable as the molecule expanded and chiral induction was over-powered by the substrate. While it is fortuitous that the substrate naturally produces the desired stereochemistry through unsymmetric oxidations-reductions, it implies that modifications deviating from the natural product would be more challenging. The next obvious step would be to probe the mechanism of lagunamide A's toxicity. One such method would be to modify the depsipeptide with a traceable linker.

Tripathi et al., who originally reported the discovery of lagunamides A and B, also extended their study of lagunamides to human foreskin fibroblast cells (BJ) and p53 tumor suppressor oncogene knocked down fibroblast cells (BJ shp53). It should be noted that this study was reported the same year as the structural revision by Dai et al. and was reported without the corrected structures, but the sample of lagunamide used was the natural product. Lagunamide B shows less activity compared to lagunamide A, suggesting that the saturated polyketide group is important in the suppression of HeLa cells. However, potency in other aurilide-class compounds across other cell lines, show that unsaturation may simply attune the molecule to other cell lines. In a study of kulokekahilide, Takada et al. provided a systematic comparison of SARs between aurilides, pulau'amide, lagunamides, and kulokekohilides. Various modifications suggest that the stereochemistries at C2 is critically important. The epimer tends to lose almost all activity but differences in side-chain still retains activity, suggesting that the effect is the result of changes to ring-conformation. The same applies to changes at C50. Structural changes to either isoleucine moieties do not appear to have

significant impact to the SARs, while a stereochemical change in the respective position of aurilide caused it to lose all activity.

An assay of various natural products of the aurilide class showed that the hydroxy group was unchanged across the various forms. In kulokekahilide, transesterification from the 26-membered ring to the 24-membered ring did not exhibit significant change in activity, suggesting that the hydroxyl group would make a potential candidate for use as a future linker anchor point; however, the addition of a linker must not affect the ring conformation. If indeed, the Phe residue can be altered, substitution with tyrosine may provide an alternative anchor point for a linker. If the Phe provides critical hydrophobic interactions, then the Ala could be substituted with either lysine or glutamic acid, both of which are used commonly in linker chemistry.

Chapter 3. Experimental

This chapter contains the experimental procedures used. General procedures are given for protocols used across several compounds. Any deviations or notes are given explicitly. If no detail is given for a particular compound, it can be assumed that the general procedure was used without incidence.

3.1 Micromide (Nosyl Solution Phase)

3.1.1 General Procedure for the Esterification of Amino Acids

The amino acid (1 eq, 5.0 mmol) was combined with *t*-butyl acetate (17.3 eq, 86.5 mmol) and cooled to 0 °C. To the heterogenous mixture, $HClO_4$ was added, turning the reaction homogenous. The reaction vessel was equipped with a dry-ice condenser and the reaction was stirred for 8 hours at ambient temperature. The solution poured into an Erlenmeyer flask containing saturated aqueous Na_2CO_3, then extracted with CH_2Cl_2. The aqueous layer was separated and extracted two more times with CH_2Cl_2. The organic layers were combined and then extracted five times with 2% HCl (25 mL). The combined aqueous layers with solid Na_2CO_3 (until no bubbling and pH is basic). The free amine extracted two times with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and condensed.

3.1.2 General Procedure for the Nosylation of Amino Acid Esters

The amino ester was dissolved in CH_2Cl_2 and cooled to 0°C. 4-nitrobenzenesulfonyl chloride (1.1 eq) was added, followed by triethylamine (1.1 eq). The reaction was allowed to reach ambient temperature and stirred for 15 hours. The organic layer was washed with 1M HCl, water and dried over Na_2SO_4. The resulting solution was condensed and purified by flash chromatography.

3.1.3 General Procedure for the *N*-Methylation of Nosyl Amino Esters

Nosylated amine (1.0 eq) and K_2CO_3 (2.1 eq) was stirred in DMF at 0°C. After 10 minutes of stirring, iodomethane (3.2 eq) was added dropwise to the solution via syringe and the reaction was stirred at ambient temperature for 12 hours. The mixture was the poured into Et_2O and washed extensively with water to remove DMF.

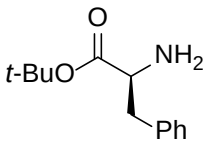
3.1.4 General Procedure for the Removal of Nosyl Protecting Groups

The nosylated amino ester (1.0 eq), K_2CO_3 (3.0 eq), mercaptoethanol (2.0 eq) and water (7.8 eq) was stirred in DMF for 12 hours at ambient temperature. The mixture was poured into Et_2O and the organic layer was washed extensively with water to remove DMF. Acid-base extraction followed by drying ($MgSO_4$) and condensation gave a sufficiently pure product for use without further treatment.

3.1.5 General Procedure for the Formation of Acyl Chloride

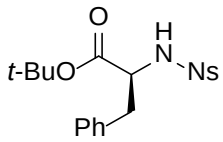
The carboxylic acid was stirred in neat thionyl chloride or oxalyl chloride for 14 hours at ambient temperature. The excess reagent was removed by rotary evaporation. The residue was dissolved in dry CH_2Cl_2 and evaporated repeatedly. The residue(*S*) were used without further treatment.

3.1.6 Tripeptide Fragment



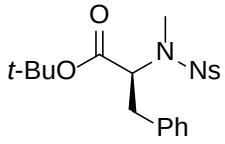
tert-butyl L-phenylalaninate (**2**). L-phenylalanine (10.0 g, 60.5 mmol) was treated as described in General Procedures to give 11.5 g of clear, colorless oil. 86% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.17 (m, 5H), 3.61 (dd, *J* = 7.7, 5.7 Hz, 1H), 3.03 (dd, *J* = 13.5, 5.7 Hz, 1H), 2.84 (dd, *J* = 13.5, 7.7 Hz, 1H), 1.42 (d, *J* = 0.7 Hz, 9H).



tert-butyl ((4-nitrophenyl)sulfonyl)-L-phenylalaninate (**3**). Phenylalanine ester **2** (11.5 g, 52.1 mmol) was treated as described in General Procedures. Purification by flash chromatography on silica (Et_2O/hexanes) gave 11.0 g of yellow oil which crystallized on standing. 52% yield.

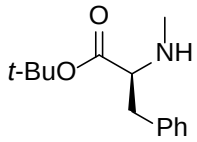
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 – 8.09 (m, 2H), 7.96 – 7.77 (m, 2H), 7.25 – 7.18 (m, 3H), 7.13 – 7.07 (m, 2H), 5.50 (d, *J* = 9.3 Hz, 1H), 4.13 (ddd, *J* = 9.4, 7.1, 5.7 Hz, 1H), 3.06 (dd, *J* = 13.8, 5.7 Hz, 1H), 2.96 (dd, *J* = 13.9, 7.1 Hz, 1H), 1.29 (s, 9H).



tert-butyl *N*-methyl-*N*-((4-nitrophenyl)sulfonyl)-L-phenylalaninate (*N*-Ns-*N*-Me-Phe-OtBu) (**4**). Protected phenylalanine **3** (10.0 g, 24.6 mmol) was treated as described in General Procedures. The crude was dissolved in a small amount of CH_2Cl_2 and recrystallized in Et_2O/hexanes to give 8.39 g of yellow needles. 81% yield.

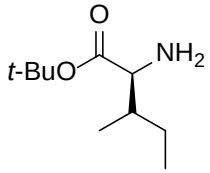
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, 2H), 7.61 (d, 2H), 7.30 – 7.22 (m, 2H), 7.20 – 7.12 (m, 3H), 4.89 (dd, *J* = 10.2, 5.8 Hz, 1H), 3.28 (dd, *J* = 14.4, 5.8 Hz, 1H), 2.88 (s, 3H), 2.88 (dd, *J* = 14.3, 10.3 Hz, 1H), 1.37 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.89, 152.46, 145.19, 136.51, 129.02, 128.76, 128.37, 127.08, 123.96, 82.77, 61.49, 35.56, 30.11, 27.86.



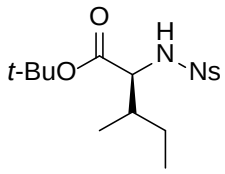
tert-butyl methyl-L-phenylalaninate (**5**). Methylated phenylalanine **4** (5.00 g, 11.9 mmol) was deprotected as described in General Procedures to give 1.09 g of light-yellow oil. 38.8% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.23 (m, 2H), 7.20 (td, *J* = 6.4, 1.6 Hz, 3H), 3.31 (t, *J* = 7.0 Hz, 1H), 2.94 (dd, *J* = 13.5, 6.5 Hz, 1H), 2.85 (dd, *J* = 13.6, 7.6 Hz, 1H), 2.37 (s, 3H), 1.35 (s, 9H).

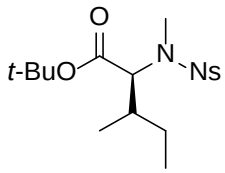


tert-butyl L-isoleucinate (**6**). L-isoleucine (2.00 g, 15.3 mmol) was treated as described in General Procedures to give 2.08 g of clear, colorless oil. 62% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.22 (d, *J* = 4.9 Hz, 1H), 1.71 (dqt, *J* = 9.2, 6.9, 4.9 Hz, 1H), 1.54 – 1.40 (m, 1H), 1.47 (s, 9H), 1.20 (ddq, *J* = 13.4, 9.2, 7.4 Hz, 1H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).

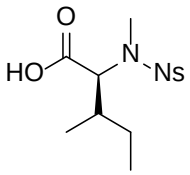


tert-butyl ((4-nitrophenyl)sulfonyl)-L-isoleucinate (**7**). See reference.[20]

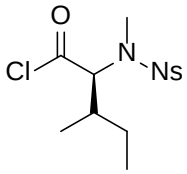


tert-butyl *N*-methyl-*N*-((4-nitrophenyl)sulfonyl)-L-isoleucinate (**8**). See reference.[20]

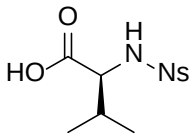
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, 2H), 8.02 (d, 2H), 4.17 (d, *J* = 10.4 Hz, 1H), 2.93 (s, 3H), 1.90 (ddtd, *J* = 16.7, 10.0, 6.8, 3.3 Hz, 1H), 1.66 – 1.51 (m, 1H), 1.26 (s, 9H), 1.25 – 1.08 (m, 1H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H).



N-methyl-*N*-((4-nitrophenyl)sulfonyl)-L-isoleucine (**9**). See reference.[20]

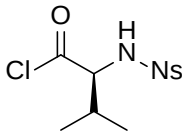


N-methyl-*N*-((4-nitrophenyl)sulfonyl)-L-isoleucinoyl chloride (**10**). Previous compound **9** was treated as described in General Procedures and used without purification.

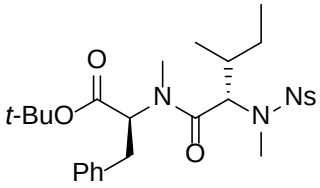


((4-nitrophenyl)sulfonyl)-L-valine (**11**). L-valine (1.00 g, 8.54 mmol) and Et_3N (16.6 mL, 20 eq) was dissolved in dioxane/water and cooled to 0°C while stirring. In 10 mL of dioxane, 4-nitrobenzenesulfonyl chloride was dissolved and added to the amino acid solution dropwise. After 1 hour, the dioxane was removed under reduced pressure. The residue was basified with 5% Na_2CO_3, and washed three times with Et_2O. The aqueous phase was acidified with 5% $KHSO_4$ and extracted three times with EtOAc. The organic layer was washed with brine, dried, and condensed to give 0.87 g of white solid. 33.7% yield.

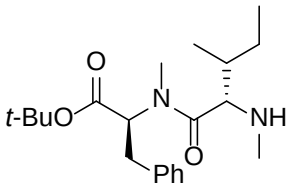
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 8.46 (d, *J* = 8.8 Hz, 1H), 8.38 (d, 2H), 8.03 (d, 2H), 3.61 (t, *J* = 6.6 Hz, 1H), 1.98 (dq, *J* = 13.6, 6.7 Hz, 1H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.88, 149.39, 146.74, 128.19, 124.27, 61.43, 30.29, 19.06, 17.74.



((4-nitrophenyl)sulfonyl)-L-valinoyl chloride (**12**). Previous compound was treated as described in General Procedures and used without purification.

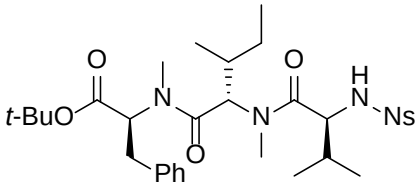


N-Ns-*N*-Me-L-Ile-*N*-Me-L-Phe-OtBu (**13**). Isoleucine derivative **10** was dissolved in CH_2Cl_2 and phenylalanine derivative **5** was added, followed by Et_3N. The reaction was stirred at ambient temperature for 12 hours.



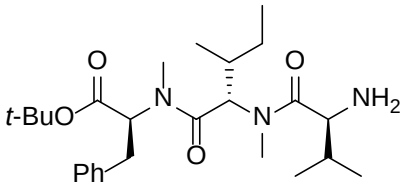
N-Me-L-Ile-*N*-Me-L-Phe-OtBu (**14**). The dipeptide **13** was denosylated as described under General Procedures and purified by flash chromatography.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 – 7.08 (m, 5H), 5.58 (dd, *J* = 11.4, 5.1 Hz, 1H), 3.38 (dd, *J* = 14.7, 5.2 Hz, 1H), 3.03 – 2.98 (m, 1H), 2.94 (dd, *J* = 14.7, 11.5 Hz, 1H), 2.89 (s, 3H), 1.72 (s, 3H), 1.64 (dtd, *J* = 15.1, 7.5, 3.2 Hz, 2H), 1.50 (ddd, *J* = 9.8, 6.6, 3.1 Hz, 1H), 1.46 (s, 9H), 1.17 – 1.05 (m, 1H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H).



N-p-Ns-L-Val-*N*-Me-L-Ile-*N*-Me-L-Phe-OtBu (**15**). *N*-Nosyl-L-valine **11** was stirred in neat thionyl chloride at ambient temperature for 12 hours. The excess thionyl chloride was removed

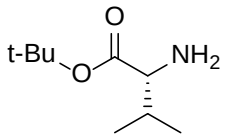
by rotary evaporation. The residue was dissolved in dry CH_2Cl_2 and evaporated repeatedly. The residue was dissolved in THF and a solution of free amine **14** in THF, was added, followed by Et_3N. Note: Under anhydrous conditions, the sulfonamide is sufficiently reactive to polymerize or self-condense. Order of addition is of critical importance to prevent side product. This is not an issue under Schotten-Baumann conditions. The reaction was stirred at ambient temperature for 12 hours. The reaction was quenched with water and THF was removed by rotary evaporation. The aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$ and dried over $MgSO_4$. After condensing, the residue was purified by flash chromatography (Et_2O/CH_2Cl_2) to give a light yellow solid.



L-Val-*N*-Me-L-Ile-*N*-Me-L-Phe-OtBu (**16**). Tripeptide **15** (160 mg, 0.247 mmol) was denosylated as described in the General Procedures. Purification by flash chromatography on silica gave 50 mg of the desired material. 44% yield.

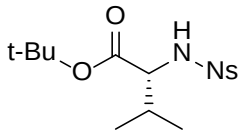
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.14 (m, 5H), 5.56 (dd, *J* = 11.7, 4.9 Hz, 1H), 5.12 (d, *J* = 10.7 Hz, 1H), 3.37 (dd, *J* = 15.1, 4.9 Hz, 1H), 3.31 (d, *J* = 3.9 Hz, 1H), 2.91 (dd, *J* = 15.2, 12.0 Hz, 1H), 2.83 (s, 3H), 2.44 (s, 3H), 2.06 (tdd, *J* = 12.7, 6.4, 3.0 Hz, 1H), 1.46 (s, 9H), 1.25 – 1.14 (m, 1H), 1.02 – 0.91 (m, 1H), 0.88 (dd, *J* = 6.6, 3.6 Hz, 6H), 0.85 (t, *J* = 7.5 Hz, 3H), 0.74 (d, *J* = 6.7 Hz, 3H).

3.1.7 Dipeptide Fragment



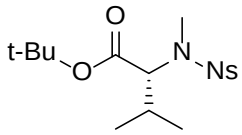
tert-butyl D-valinate (**17**). D-Valine (3.94 g, 33.66 mmol) was treated as described in General Procedures to give 5.83 g of off-white solid and used without further purification. 81% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.16 (d, *J* = 4.8 Hz, 1H), 2.00 (pd, *J* = 6.9, 4.8 Hz, 1H), 1.47 (s, 9H), 1.35 (s, 2H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H).



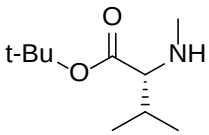
tert-butyl ((4-nitrophenyl)sulfonyl)-D-valinate (**18**). Valine ester **17** (4.71 g, 27.2 mmol) was treated as described in General Procedures to give 8.48 g of brownish-yellow crystals. 87% yield.

^{1}H NMR (400 MHz, CDCl3) δ 8.33 (d, *J* = 9.1 Hz, 2H), 8.03 (d, *J* = 9.1 Hz, 2H), 3.70 (dd, *J* = 9.8, 4.4 Hz, 1H), 2.10 (pd, *J* = 6.9, 4.4 Hz, 1H), 1.24 (s, 9H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H).



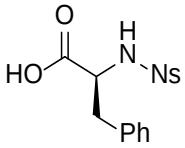
tert-butyl *N*-methyl-*N*-((4-nitrophenyl)sulfonyl)-D-valinate (**19**). Protected D-valine **18** (3.12 g, 8.7 mmol) was methylated as described in General Procedures to give a dark reddish-yellow oil. 96.2% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 9.1 Hz, 2H), 8.02 (d, *J* = 9.1 Hz, 2H), 4.07 (d, *J* = 10.4 Hz, 1H), 2.93 (s, 3H), 2.21 – 2.03 (m, 1H), 1.27 (s, 9H), 1.00 (d, *J* = 3.6 Hz, 3H), 0.98 (d, *J* = 3.5 Hz, 3H).



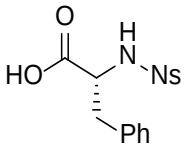
tert-butyl methyl-D-valinate (**20**). Compound **19** (2.41 g, 6.61 mmol) was deprotected as described in General Procedures to give 1.16 g of clear colorless oil. 93.6% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (d, *J* = 6.1 Hz, 1H), 2.36 (s, 3H), 1.93 – 1.79 (m, 1H), 1.48 (s, 9H), 0.95 (d, *J* = 2.1 Hz, 3H), 0.94 (d, *J* = 2.1 Hz, 3H).

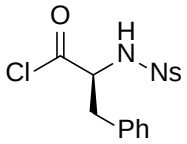


((4-nitrophenyl)sulfonyl)-L-phenylalanine (**21**). L-phenylalanine (1.00 g, 5.96 mmol) was suspended in 10 mL dioxane and water was added until amino acid dissolves. The solution was cooled to 0°C and Et_3N (16.6 mL) was added to the solution. 4-nitrobenzenesulfonyl chloride (1.98 g, 8.94 mmol), dissolved in 10 mL dioxane, was added slowly. The solution was stirred for 1 hour at 0°C. The dioxane was then removed under vacuum and the residue was treated with 100 mL 5% aqueous Na_2CO_3. The aqueous layer was washed with Et_2O and acidified with $NaHSO_4$. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were washed with water, brine and dried over Na_2SO_4. Condensed to give 1.56 g of a light-yellow solid. 75% yield.

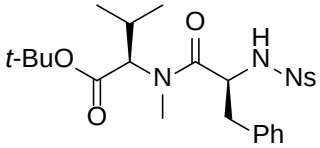
1H NMR (400 MHz, DMSO-d6) δ 8.72 (s, 1H), 8.20 (d, *J* = 8.9 Hz, 2H), 7.74 (d, *J* = 8.9 Hz, 2H), 7.16 – 7.05 (m, 5H), 3.96 (s, 1H), 2.98 (dd, *J* = 13.7, 4.8 Hz, 1H), 2.71 (dd, *J* = 13.8, 10.0 Hz, 1H).



((4-nitrophenyl)sulfonyl)-D-phenylalanine (**22**). D-phenylalanine (1.00 g, 5.96 mmol) was treated in the same manner as the previous compound to give 1.53 g of a light-yellow solid. 75% yield. Spectrum identical to enantiomer **21**.

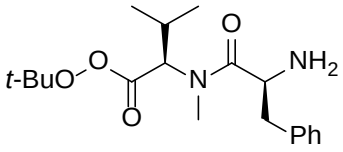


((4-nitrophenyl)sulfonyl)-L-phenylalaninoyl chloride (**23**). *N*-protected L-phenylalanine **21** (200 mg, 0.571 mmol) was treated as described in General Procedures and used without further treatment.



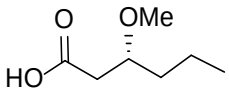
p-Ns-L-Phe-*N*-Me-D-Val-OtBu (**24**). Acid chloride **23** (0.571 mmol) was dissolved in CH_2Cl_2 and derivative **20** (89 mg, 0.476 mmol) was added, followed by Et_3N (0.27 mL). The reaction was stirred at ambient temperature for 12 hours. Water was added and the THF was removed under vacuum. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with saturated Na_2CO_3, water, and brine. The organic layer was dried over $MgSO_4$ and condensed. The residue was purified by flash chromatography on silica (Et_2O/CH_2Cl_2) to give 183 mg of light-yellow solid. 74% yield.

1H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.13 (m, 2H), 7.82 – 7.76 (m, 2H), 7.24 – 7.16 (m, 5H), 4.64 (d, *J* = 10.3 Hz, 1H), 3.80 – 3.67 (m, 1H), 2.98 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.94 (s, 3H), 2.79 (dd, *J* = 14.0, 8.0 Hz, 1H), 2.06 (dq, *J* = 10.4, 6.7 Hz, 1H), 1.45 (s, 9H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.56 (d, *J* = 6.8 Hz, 3H).



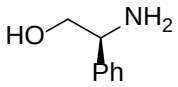
L-Phe-*N*-Me-D-Val-OtBu (**25**). The previous compound was denosylated as described in the General Procedures.

3.1.8 Terminal Fragments



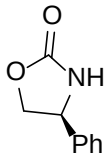
(*R*)-3-methoxyhexanoic acid (**26**). From compound **33**: the starting compound (500 mg) was stirred in 35 mL of 3:1 THF in water and cooled to 0°C. Hydrogen peroxide (30%, 778 mg) was added, followed by lithium hydroxide (2M, 1.7 mL). No starting material was detectable by TLC after 5 minutes, but the reaction was allowed to continue for 30 minutes. The excess peroxide was quenched with 1.5M sodium sulfite and the THF removed by evaporation. The aqueous layer was extracted with 10 mL CH_2Cl_2 twice to remove the oxazolidinone and acidified to pH 1 with dilute HCl. The acidic aqueous layer was extracted twice with 70 mL EtOAc. The combined organic layers were washed with brine, dried over sodium sulfate, and condensed. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$) to give 226 mg of a clear oil. 90% yield. From ester **35**: The β-methoxy methyl ether (450 mg, 2.05 mmol) was dissolved in 20 mL THF and cooled to 0°C. Lithium hydroxide (2M, 2.8 mL) was added and the reaction was stirred for two hours. The base was neutralized with NH_4Cl and THF was removed *in vacuo*. The aqueous layer was extracted with EtOAc, washed with acidic brine, and dried of Na_2SO_4. Purification by flash chromatography gave 284 mg of desired product. 69% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (br s, 1H), 3.64 (p, *J* = 5.9 Hz, 1H), 3.35 (s, 3H), 2.54 (dd, *J* = 15.4, 7.3 Hz, 1H), 2.45 (dd, *J* = 15.4, 5.3 Hz, 1H), 1.61 – 1.51 (m, 1H), 1.51 – 1.42 (m, 1H), 1.42 – 1.29 (m, 2H), 0.91 (t, *J* = 7.3 Hz, 3H).



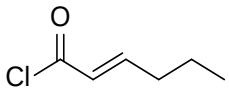
(*S*)-2-amino-2-phenylethan-1-ol (**27**). In a 2 L round-bottomed flask, LAH tablets (15.1 g, 397 mmol) were suspended in 500 mL of THF under argon and cooled to 0°C on an ice bath. L-phenylglycine (30.0 g, 19.8 mmol) was added in portions, allowing for the solid to disperse before the next portion. The flask was then equipped with a reflux condenser. The heterogenous mixture was stirred at 0°C until the evolution of gas was no longer vigorous (ca. 3 hours). The ice bath was replaced with a heating mantle and refluxed for 18 hours. Note: refluxing was halted while reaction was unattended. The reaction was then cooled to 0°C and carefully quenched with 60 mL saturated K_2CO_3. The aluminum salts were filtered off and stirred in 300 mL of THF for 10 minutes. The process was repeated 2 more times. The organic layers were combined and dried over sodium sulfate (Warning: $MgSO_4$ will chelate the product result in reduced yields). The solution was condensed under reduced pressure and further dried by azeotroping with toluene (crystallization is inhibited by the presence of water). Application of high vacuum induced crystallization, which is highly exothermic, causing the flask to reach ca. 100°C. Allowed the product to cool before continuing to remove residual solvent. The solid was dissolved in 1 L of 3:1 hexanes and ethyl acetate. Insoluble impurities were removed by hot filtration. Addition of a seed crystal was necessary to induce crystallization to produce 16.8 g of pale yellow crystals. The filtrate was condensed under reduced pressure and purified by flash chromatography to give an additional 5.16 g of the desired product. 81.5% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.26 (m, 5H), 4.05 (dd, *J* = 8.3, 4.5 Hz, 1H), 3.75 (dd, *J* = 10.7, 4.5 Hz, 1H), 3.55 (dd, *J* = 10.7, 8.3 Hz, 1H), 1.87 (br s, 3H).

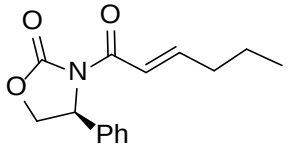


(*S*)-4-phenyloxazolidin-2-one (**28**). An oven dried 100 mL, one-neck round bottom flask was loaded with (*S*)-phenylglycinol (48.5 mmol, 5.00 g), K_2CO_3 (4.85 mmol, 0.67 g), and diethyl carbonate (193.9 mmol, 23.5 mL). The reaction was stirred at 80°C and monitored by TLC until starting material is consumed. Excess diethyl carbonate and ethanol was removed by rotary evaporation with a water bath at 80°C. The residue was dissolved in 100 mL EtOAc, washed with 2×20 mL H_2O and 3×30 mL brine, dried and condensed. Recrystallization in 3:2 EtOAc/hexanes afforded 5.01 g of white crystals. 80% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.26 (m, 5H), 4.95 (dd, *J* = 9.5, 6.7 Hz, 1H), 4.75 (t, *J* = 8.7 Hz, 1H), 4.20 (dd, *J* = 8.6, 6.9 Hz, 1H).



(E)-hex-2-enoyl chloride (**29**). Commercially available (E)-hex-2-enoic acid (7.6 g, 66.6 mmol) was dissolved in 50 mL of CH_2Cl_2 and 0.5 mL of DMF. The solution was cooled to 0°C and thionyl chloride (9.6 mL, 133.1 mmol) was added. The clear solution was allowed to reach ambient temperature and then refluxed until SO_2 ceases to evolve. The resulting yellow-brown solution was condensed by rotary evaporation and resulting oil was distilled *in vacuo* to give 7.16 g of light yellow oil in 81.1% yield. NB: Using the product without distillation resulted in significantly reduced yields in the following reaction.

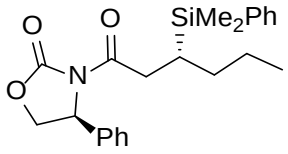


(S,E)-3-(hex-2-enoyl)-4-phenyloxazolidin-2-one (**30**). Method A:[72] Oxazolidinone **28** (21.5 mmol, 3.50 g) was stirred in THF and cooled to -78°C. To the solution, n-butyl lithium

(22.5 mmol, 9.0 mL, 2M in hexanes) was added dropwise. The lithium amide precipitates out as the addition of n-butyl lithium proceeds. The acid chloride **29** was added dropwise. The reaction was stirred for 1.5 hours, then quenched with 10 mL saturated Na_2CO_3 and allowed to warm to ambient temperature. The mixture was diluted with H_2O until all salts were dissolved. The aqueous layer was extracted 3 times with 100 mL MTBE. The organic layers were combined and dried over Na_2SO_4 and condensed to give a light-yellow oil which solidified upon standing. The product was purified by column chromatography, 10-100% Et_2O/hexanes to give 5.03 g of while crystalline solid. 90.4% yield.

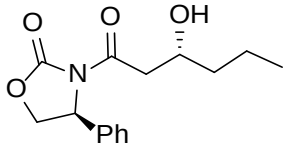
Method B:[73] Oxazolidinone **28** (64.2 mmol, 10.0 g), DIC (67.4 mmol, 10.4 mL), and DMAP (7.4 mmol, 0.90 g) was stirred in CH_2Cl_2. (E)-hex-2-enoic acid was dissolved in 20 mL CH_2Cl_2 and added dropwise over 1 hour. After stirring for 48 hours at ambient temperature, 20 mL saturated $NaHCO_3$ was added and stirred for 15 minutes. The mixture was poured into a separation funnel with 20 mL H_2O and 50 mL CH_2Cl_2 and separated. The aqueous layer was extracted two more times with 50 mL CH_2Cl_2. The organic layers were combined and washed two times with 30 mL 1M HCl, then dried over Na_2SO_4 and condensed. The residue was triturated in 150 mL Et_2O to remove residual urea. 100 mL of hexanes was added and the product was allowed to crystallize spontaneously, giving a first crop of 12.6 g. A second crystallization of the mother liquor yielded a crop of 1.05 g. Column chromatography of the residual supernatant yielded an additional 0.591 g of product. Total yield 89.6%.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 5H), 7.26 (dt, *J* = 15.2, 1.4 Hz, 1H), 7.09 (dt, *J* = 15.3, 6.9 Hz, 1H), 5.49 (dd, *J* = 8.7, 3.9 Hz, 1H), 4.70 (t, *J* = 8.8 Hz, 1H), 4.28 (dd, *J* = 8.9, 3.9 Hz, 1H), 2.24 (qd, *J* = 7.1, 1.5 Hz, 2H), 1.50 (h, *J* = 7.4 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).



(*S*)-3-((*R*)-3-(dimethyl(phenyl)silyl)hexanoyl)-4-phenyloxazolidin-2-one (**31**). In a pear-shaped flask, lithium metal (0.8 g) was stirred in 20 mL THF and cooled to 0°C under argon. To the mixture, chlorophenyldimethylsilane was added. The mixture was stirred at 0°C for 5 hours and was left standing for 18 hours at -10°C. Excess lithium was removed by transferring the dark burgundy silyl lithium solution via cannula to a round-bottomed flask, both at -78°C. Diethyl zinc (1M in hexanes, 18.0 mL) was added dropwise. The solution was warmed to and stirred at 0°C for 30 minutes, then returned to -78°C, at which time, enone **30** dissolved in THF, was added dropwise to the silyl zincate solution. The resulting solution was stirred at -78°C for 12 hours. The reaction was quenched with 8 mL of saturated aqueous ammonium chloride and allow to warm to ambient temperature. THF was removed rotary evaporation and the resulting zinc slurry was extracted with three portions of 100 mL diethyl ether. The combined organic layer was washed with water, then brine, dried with sodium sulfate, and condensed. The residue was purified by flash chromatography (CH_2Cl_2/hexanes) to give 2.21 g of pale yellow oil. 89% yield.

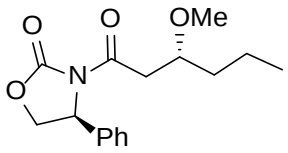
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 – 7.45 (m, 2H), 7.38 – 7.27 (m, 6H), 7.26 – 7.21 (m, 2H), 5.27 (dd, *J* = 8.6, 3.8 Hz, 1H), 4.58 (t, *J* = 8.8 Hz, 1H), 4.22 (dd, *J* = 8.9, 3.7 Hz, 1H), 2.94 (dd, *J* = 16.8, 5.6 Hz, 1H), 2.88 (dd, *J* = 16.8, 8.2 Hz, 1H), 1.59 – 1.49 (m, 1H), 1.40 – 1.28 (m, 1H), 1.22 – 1.09 (m, 3H), 0.73 (t, *J* = 7.1 Hz, 3H), 0.23 (s, 3H), 0.22 (s, 3H).



(*S*)-3-((*R*)-3-hydroxyhexanoyl)-4-phenyloxazolidin-2-one (**32**). Silyl oxazolidinone **31** (4.02 g, 10.2 mmol) was dissolved in 60 mL glacial acetic acid and cooled on an ice bath. Water

(20 mL) was added and then mercury (II) acetate (4.21 g, 13.2 mmol) was added in one portion. Peracetic acid (32% in dilute acetic acid, 12.4 mL, 59.0 mmol) was added over 5 minutes. The solution was stirred at ambient temperature for 5 hours. The solution was diluted with water, cooled to 0°C and quenched with saturated sodium sulfite. (Note: Substituting sodium sulfite with sodium thiosulfate results in the formation of colloidal sulfur that cannot be removed by filtration or flash chromatography.) Complete quenching of the peracid is indicated by the formation of dark grey precipitate, presumably mercury (I) oxide. The mixture was extracted 3 times with 100 mL diethyl ether. The combined organic extracts were washed with 3 portions of 1M NaOH (50 mL), brine, and filtered over a pad of celite. The solution was then dried over sodium sulfate and condensed. The residue was purified by flash chromatography on silica (EtOAc/hexanes) to give 1.06 g of clear oil. 75% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.27 (m, 5H), 5.45 (dd, *J* = 8.7, 3.7 Hz, 1H), 4.70 (t, *J* = 8.8 Hz, 1H), 4.28 (dd, *J* = 8.9, 3.7 Hz, 1H), 4.10 – 3.99 (m, 1H), 3.15 (dd, *J* = 17.3, 2.8 Hz, 1H), 3.04 (dd, *J* = 17.3, 9.2 Hz, 1H), 2.73 (d, *J* = 4.5 Hz, 1H), 1.61 – 1.29 (m, 4H), 0.91 (t, *J* = 7.0 Hz, 3H).

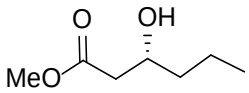


(*S*)-3-((*R*)-3-methoxyhexanoyl)-4-phenyloxazolidin-2-one (**33**). Using TMSD: β-hydroxyl **32** (997 mg, 3.6 mmol) was dissolved in 10 mL CH_2Cl_2 and cooled to 0°C in a Teflon flask. Aqueous fluoroboric acid (50% w/w, 0.45 mL, 3.6 mmol) was added. With vigorous stirring, TMSD (7.2 mL, 14.4 mmol) was added by syringe pump over one hour. Following complete addition, the reaction was allowed to stir another hour and then quenched with 10% acetic acid in methanol. The solvent was removed by rotary evaporation and residue dissolved in EtOAc. The organic layer was washed with water three times, then saturated bicarbonate, brine, dried over sodium sulfate, and concentrated. The residue was purified by flash chromatography on

silica gel (EtOAc/CH_2Cl_2) to give the β-ether as a colorless oil (766 mg, 73%) and starting material (72 mg). NB: Starting material remains despite use of excess reagent and prolonged reaction times.

Using MeOTf: β-hydroxyl **32** (865 mg, 3.12 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (1.80 g, 8.73 mmol) was stirred in 50 mL CH_2Cl_2 under argon at 0°C. Methyl triflate (0.96 mL, 8.73 mmol) was added dropwise and the reaction was allowed to reach ambient temperature. After 18 hours, the reaction was quenched with saturated sodium bicarbonate. The mixture was transferred to a separation funnel and washed three times with 1M HCl, brine, and dried over Na_2SO_4. Isolated 256 mg desired product and 383 mg of starting material. 55.7% converted yield.

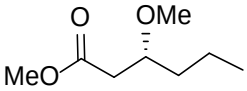
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 5H), 5.45 (dd, *J* = 8.8, 3.9 Hz, 1H), 4.69 (t, *J* = 8.8 Hz, 1H), 4.27 (dd, *J* = 8.9, 4.0 Hz, 1H), 3.67 (ddd, *J* = 10.3, 6.7, 4.9 Hz, 1H), 3.42 (dd, *J* = 15.7, 7.2 Hz, 1H), 3.23 (s, 3H), 2.86 (dd, *J* = 15.7, 5.5 Hz, 1H), 1.53 – 1.24 (m, 4H), 0.88 (t, *J* = 7.1 Hz, 3H).



Methyl (*R*)-3-hydroxyhexanoate (**34**). Compound **32** (300 mg, 1.08 mmol) was dissolved in methanol (9 mL) and cooled to 0°C. Sodium methoxide (70 mg, 1.30 mmol) dissolved in methanol was added and stirred for 1 hour at 0°C. The reaction was acidified with saturated ammonium chloride and extracted with diethyl ether. The combined organic layers were washed with water and brine, then dried over sodium sulfate. The solution was carefully condensed at 10°C by rotary evaporation and purified by flash chromatography (Et_2O/petroleum ethers) to give 122 mg of colorless volatile oil containing residual Et_2O. 39% yield calculated by NMR.

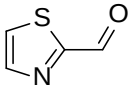
^{1}H NMR (599 MHz, $CDCl_3$) δ 4.06 – 3.95 (m, 1H), 3.74 – 3.64 (br m, 3H), 2.55 – 2.45 (m, 1H), 2.39 (ddd, *J* = 16.4, 9.1, 1.8 Hz, 1H), 1.54 – 1.42 (m, 2H), 1.42 – 1.31 (m, 2H), 0.94 – 0.89 (m, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 173.47, 67.73, 51.70, 41.15, 38.66, 18.67, 13.92.

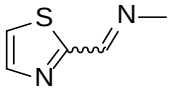


Methyl (*R*)-3-methoxyhexanoate (**35**). Ester **34** (1.00 g, 6.85 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (3.93 g, 19.1 mmol) was stirred in 50 mL CH_2Cl_2 under argon at 0°C. Methyl triflate (2.10 mL, 19.1 mmol) was added dropwise and the reaction was allowed to reach ambient temperature. After 18 hours, the reaction was quenched with saturated sodium bicarbonate. The mixture was transferred to a separation funnel and washed three times with 1M HCl, brine, and dried over Na_2SO_4. The organic layers were gently condensed and purified by flash chromatography (Et_2O/petroleum ether) on silica. Isolated 651 mg desired product as a clear volatile oil. 59.4% yield

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (d, *J* = 0.8 Hz, 3H), 3.64 (ddtd, *J* = 7.4, 6.2, 5.3, 0.8 Hz, 1H), 3.35 (d, *J* = 0.8 Hz, 3H), 2.61 – 2.48 (m, 1H), 2.42 (ddd, *J* = 15.1, 5.4, 0.8 Hz, 1H), 1.63 – 1.25 (m, 4H), 0.93 (t, *J* = 7.2 Hz, 3H).



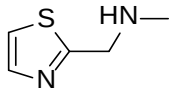
thiazole-2-carbaldehyde (**36**). Prepared from 2-bromothiazole as described by Dondoni and Perrone.[74] 54.2% yield.



N-methyl-1-(thiazol-2-yl)methanimine (**37**). Methylamine hydrochloride (85.9 mmol, 5.50 g), aldehyde **36** (14.3 mmol, 1.62 g), and NaOH (81.6 mmol, 3.26 g) were stirred in 50 mL of anhydrous methanol with 4Å powdered molecular sieves. After 16 hours, the MeOH was removed and replaced with Et_2O. The organic layer was washed with H_2O and brine, dried over

Na_2SO_4 and filtered. Concentration gave 1.52 g of yellow oil, which was used without further purification. 83% yield.

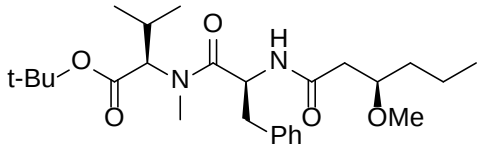
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.91 (d, *J* = 3.2 Hz, 1H), 7.40 (dd, *J* = 3.2, 1.0 Hz, 1H), 3.57 (d, *J* = 1.7 Hz, 3H).



N-methyl-1-(thiazol-2-yl)methanamine (**38**). Schiff base **37** (12.1 mmol, 1.52 g) was dissolved in 100 mL of anhydrous ethanol. $NaBH_4$ (18.1 mmol, 0.68 g) was added in one portion and stirred under argon for 24 hours. The excess borohydride was hydrolyzed with 50 mL of 1M NaOH. The ethanol was evaporated and the aqueous layer was extracted with 3×70 mL Et_2O. The organic layers were combined and washed with brine. Dried over $NaSO_4$ and condensed. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$) on silica to give 759 mg of light-brown oil. 49% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 3.3 Hz, 1H), 7.28 (d, *J* = 2.8 Hz, 1H), 4.10 (s, 2H), 2.53 (s, 3H).

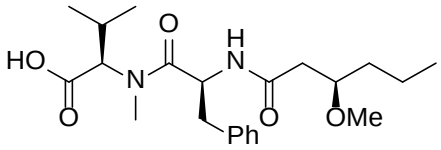
3.1.9 Final Couplings



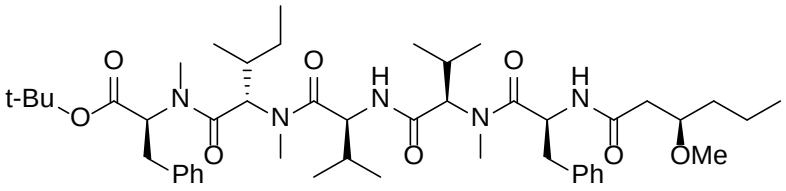
tert-butyl *N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-D-valinate (**39**). From **25**: see reference.[20] From Fmoc-dipeptide **59**: 100 mg of the dipeptide was deprotected by dissolving in 4 mL of MeCN and stirring in 2 mL of Et_2NH for 2 hours. The volatiles were removed by rotary evaporation and the residue was dissolved in 8 mL DMF. Acid **26** (28 mg, 0.19 mmol) and PyBOP (100 mg, 0.19 mmol) was dissolved in 2 mL DMF. The two solutions were combined, followed by the addition of DIEA. The reaction was stirred for 16 hours and

quenched with 10% citric acid and water. The mixture was extracted 3 times with 40 mL Et_2O. The combined organic layers were washed 3 times with water, once with brine, dried over Na_2SO_4 and condensed. The residue was purified by flash chromatography on silica (Et_2O/hexanes) to give 73 mg of product in 88% yield.

1H NMR (400 MHz, $CDCl_3$) existed as rotational conformers: δ 7.32 – 7.10 (m, 11H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 5.36 – 5.21 (m, 2H), 4.63 (d, *J* = 10.4 Hz, 1H), 4.14 (d, *J* = 10.5 Hz, 1H), 3.57 – 3.38 (m, 2H), 3.27 (s, 3H), 3.17 (s, 2H), 3.07 (dd, *J* = 13.4, 8.0 Hz, 1H), 2.94 (s, 3H), 2.89 (s, 3H), 2.31 (d, *J* = 1.3 Hz, 1H), 2.30 – 2.25 (m, 3H), 2.04 (dp, *J* = 10.4, 6.6 Hz, 1H), 1.58 – 1.47 (m, 1H), 1.46 (s, 7H), 1.42 (s, 3H), 1.41 (s, 10H), 1.39 – 1.26 (m, 4H), 1.25 (s, 1H), 1.04 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.91 (s, 1H), 0.88 (t, *J* = 7.1 Hz, 7H), 0.62 (d, *J* = 6.7 Hz, 3H).

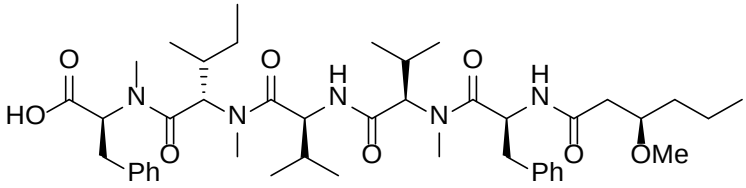


N-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-D-valine (**40**). See reference.[20]

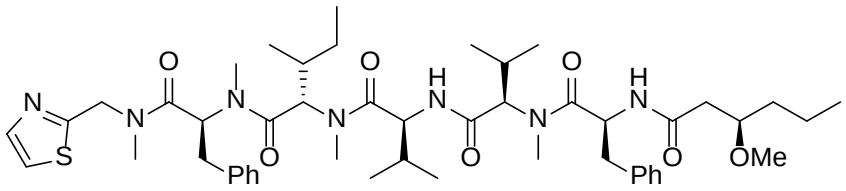


tert-butyl *N*-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-L-phenylalaninate (**41**). Compound **39** (153 mg, 0.38 mmol) was stirred in 5 mL CH_2Cl_2 and 3 mL TFA for 2 hours at 0°C. The volatiles were removed *in vacuo* and the residue was dissolved in CH_2Cl_2. To the solution, HATU (116 mg, 0.38 mmol), HOAt (51 mg, 0.38 mmol), and DIPEA (26 µL, 1.51 mmol) was added. After 5 minutes of stirring, tripeptide **16** (174 mg, 0.38 mmol), dissolved in CH_2Cl_2, was added. After stirring for 15 hours at ambient temperature, the reaction was complete by TLC. The volatiles were removed, and the crude residue was purified by flash chromatography on silica (CH_2Cl_2/MeOH), followed by

reverse-phase flash chromatography on C18-modified silica (MeCN/H_2O, 0.1% TFA) to give 280 mg of amorphous white solid. 87% yield.



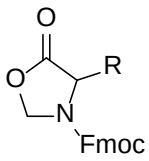
N-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-L-phenylalanine (**42**). Compound **41** (172 mg, 0.202 mmol) was stirred in 5 mL CH_2Cl_2 and 2 mL TFA for 3 hours. The volatiles were removed by rotary evaporation. The residue was redissolved in CH_2Cl_2 and condensed. This process was repeated once more with CH_2Cl_2 and once with toluene. The residue was used without further treatment.



micromide isomer (**1**). Crude compound **42** from above was dissolved in 2 mL DMF. HATU (85 mg, 0.22 mmol) and DIEA (0.07 mL, 0.40 mmol) were added, turning the colorless solution yellow. A solution of **38** in Et_2O (2.38 mL, 0.22 mmol) was added and stirred for 20 hours. The reaction was quenched with water and extracted with 100 mL EtOAc. The organic layer was washed 3 times with 30 mL of pH 10 carbonate buffer, then brine, dried over Na_2SO_4, and condensed. The residue was purified by column chromatography on silica (CH_2Cl_2/MeOH), then by reverse-phase chromatography on C18-modified silica (H_2O/MeCN/0.1% TFA) to give 94 mg of amorphous white solid in 51% yield. $[\alpha]_D^{20} = -32.23°$ (c=5.0, $CHCl_3$)

3.2 Micromide (Solid Phase)

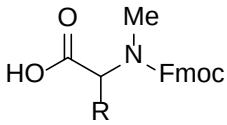
3.2.1 General Procedure for the Synthesis of Fmoc-Oxooxazolidines



Conventional method: The Fmoc-protected amino acid was refluxed in toluene with paraformaldehyde (200 mg/mol amino acid) and catalytic *p*-toluenesulfonic acid (20 mol %) in a Dean-Stark apparatus for one hour. The solution was diluted with ethyl acetate and washed with 20% sodium bicarbonate and then brine. The organic layer was dried over sodium sulfate and condensed under reduced pressure to give clear oils. 88-97% yield. Products have previously been characterized.[75]

Microwave method[29]: Combined Fmoc-amino acid (1.3 mmol), paraformaldehyde (400 mg), and p-toluenesulfonic acid (30 mg) with 10 mL MeCN in a microwave vial. The mixture was heated to 120°C for 2 minutes using a Biotage Initiator microwave. The cooled mixture was diluted with EtOAc and washed with dilute $NaHCO_3$, water, and brine. Dried over Na_2SO_4 and condensed. Product was dried slowly under reduced pressure. Product foams if placed under high vacuum. 77-84% yield.

3.2.2 General Procedure for the Synthesis of Fmoc-*N*-Methyl Amino Acids



Conventional method: To a solution of Fmoc-oxooxazolidine dissolved in CH_2Cl_2 and TFA, triethylsilane was added. The solution was stirred for 18 hours at ambient temperature. The volatiles were removed by rotary evaporation and the residue was dissolved in ethyl acetate and washed extensively with water to remove residual TFA. The resulting material was

dried over Na_2SO_4, condensed and purified by flash chromatography (EtOAc/MeOH/MeCN/H_2O). 51-73% yield. Products have been previously characterized.[75]

Microwave method: The respective amino acid derived Fmoc-oxooxazolidine was suspended in 5 mL CH_2Cl_2 in a 20 mL microwave vial and cooled to -40°C. $AlCl_3$ (2 eq.) was added in portions. Et_3SiH was added dropwise and the mixture was removed from cooling. The suspension was stirred until bubbling ceased. The vial was then capped and the suspension irradiated for 1 minute at 100°C in a Biotage Initiator microwave reactor to give a homogenous solution. The reaction was uncapped and poured into 50 mL CH_2Cl_2 and the organic layer was washed with 1 M HCl and acidic brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/20% AcOH in MeOH). 59-80% yield.

3.2.3 General Procedure for the Solid-Phase Loading

In a tared 20 mL polyethylene fritted syringe, 1.00 g of 2-chlorotrityl chloride (CTC) resin was swelled in anhydrous CH_2Cl_2 for 30 minutes, then drained. Fmoc-*N*-methyl-phenylalanine (0.77 g) dissolved in 10 mL CH_2Cl_2 was taken up into the syringe, followed by 0.71 mL of DIPEA. The mixture was agitated for one hour. The syringe was drained and the resin was washed with CH_2Cl_2. The resin was capped by treating with 0.2 mL MeOH and 0.2 mL DIPEA in 10 mL CH_2Cl_2 for 20 min. The syringe was then drained and fresh CH_2Cl_2 was taken up and agitated for 2 min. This process was repeated three times. The resin was dried in vacuo and the difference in mass was used to estimate loading. Spectrophotometry of the cleaved piperidine-Fmoc adduct may be performed to quantitatively determine loading, however, loading was generally quantitative relative to the manufacturer's specifications.

3.2.4 Quantification of Solid-Phase Reactions

Resin loading with amino acids was quantified indirectly by spectrophotometry of the Fmoc-piperidine adduct. The resin was dried in vacuo and 4 mg of resin was stirred in 1.00 mL of 20% piperidine in DMF for 1 hour. The supernatant was diluted ten-fold with DMF and transferred to a 1 cm quartz cuvette. The Fmoc substitution was calculated by the following equation:[76]

$$\mathrm{S_{Fmoc}\ [mmol\ g^{-1}]} = \frac{\mathrm{E_{289.8\ nm}} \times 10^{6}\mathrm{mmol} \times \mathrm{mol^{-1}} \times \mathrm{g^{-1}} \times \mathrm{V} \times \mathrm{D}}{\varepsilon_{289.8\ \mathrm{nm}} \times \mathrm{m}_{resin} \times \mathrm{L}}$$

$\mathrm{S_{Fmoc}} = \mathrm{Fmoc\ substitution\ [mmol\ g^{-1}]}$

$\varepsilon_{289.8\ \mathrm{nm}} = \mathrm{Molar\ absorption\ coefficient\ at\ 289.8\ nm}$

$\mathrm{E_{289.8\ nm}} = \mathrm{Absorption\ of\ the\ sample\ solution\ at\ 289.8\ nm}$

$\mathrm{m_{resin}} = \mathrm{Sample\ weight\ of\ the\ resin\ [mg]}$

$\mathrm{V} = \mathrm{Sample\ volume\ [L]}$

$\mathrm{L} = \mathrm{Optical\ path\ length\ of\ the\ cell\ [cm]}$

$\mathrm{D} = \mathrm{Dilution\ factor}$

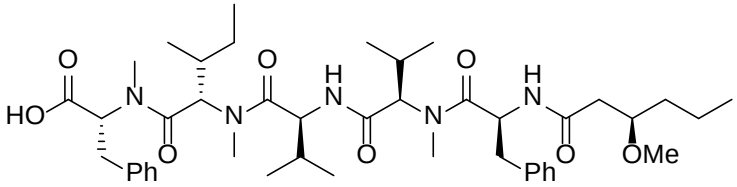
3.2.5 General Procedure for Solid-Phase Peptide Couplings

The resin-anchored peptide was deprotected by drawing up a solution of 20% piperidine/DMF into the syringe and agitated for 20 min. The solution was drained and fresh piperidine/DMF solution was added and agitated for 10 minutes. The appropriate Fmoc amino acid, DIC, and K-Oxyma (3 eq. each, relative to resin) was dissolved in 15 mL DMF and drawn into the syringe. The mixture was agitated for 20 hours at ambient temperature. The syringe was then drained and washed 3 times with DMF, then 3 times with CH_2Cl_2. The resin beads were tested for free amines using the appropriate test (Kaiser/Chloranil). The coupling

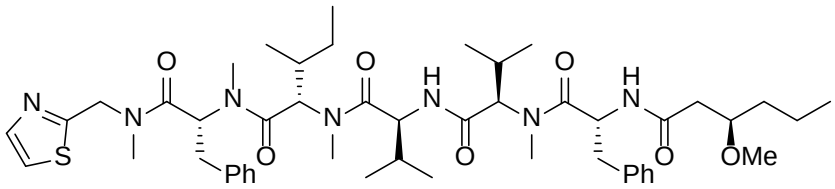
procedure was repeated if positive. Otherwise, the resin was capped with 10% acetic anhydride/pyridine solution over 20 minutes and washed with CH_2Cl_2.

3.2.6 General Procedure for Removal of Peptide from Solid Support

A 20% solution of HFIP in CH_2Cl_2 was drawn into the syringe and the mixture was agitated for 1 hour. The liquid was expelled into a round bottom flask. The resin was washed repeatedly with CH_2Cl_2 and the washings were combined in the round-bottom flask. The combined washings were condensed, and the residue was purified by reverse phase flash chromatography (MeCN/H_2O, 0.1% TFA) on Biotage SNAP C18 columns.

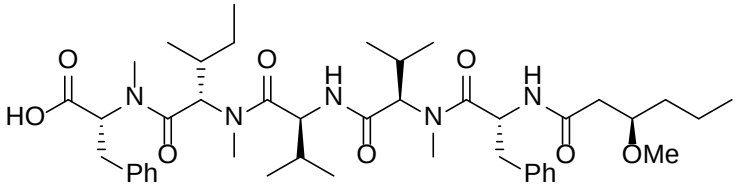


N-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-D-phenylalanine (**43**). Produced following general procedures. Theoretical resin loading was 0.76 mmol. Isolated 416 mg of amorphous white solid. 68.9% yield over 12 steps. APCIMS m/z [M+H] 794.5062 (calcd for $C_{44}H_{68}N_5O_8$), found 794.5

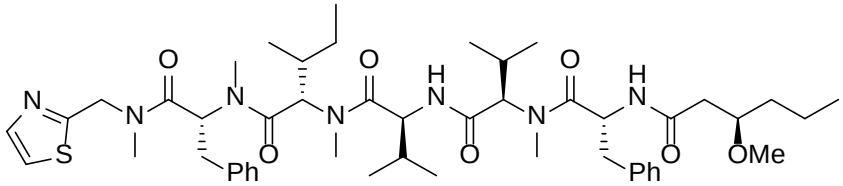


micromide isomer (**1a**). Lipopeptide **43** (109 mg, 0.14 mmol) and HOAt (37 mg, 0.27 mmol) was dissolved in 5 mL anhydrous DMF. DIC (0.043 mL, 0.27 mmol) was added, followed by thiazole **38** (26 mg, 0.21 mmol), and the solution was stirred under argon at ambient temperature for 20 hours. The reaction was quenched with water and diluted with 20 mL Et_2O. The organic layer was washed with 0.25 M HCl, water, brine, and dried over Na_2SO_4. The organic layer was concentrated and the residue was purified by flash chromatography on C18-

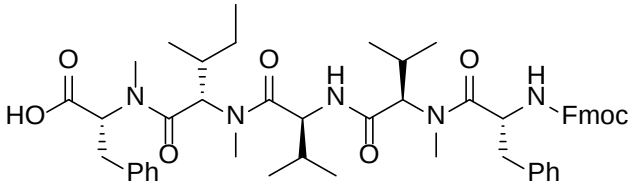
modified silica (MeCN/H_2O, 0.1% TFA) to give 86 mg of an amorphous white solid. 69% yield. APCIMS m/z [M+H] 904.23 (calcd for $C_{49}H_{73}N_7O_7S$), found 904.7.



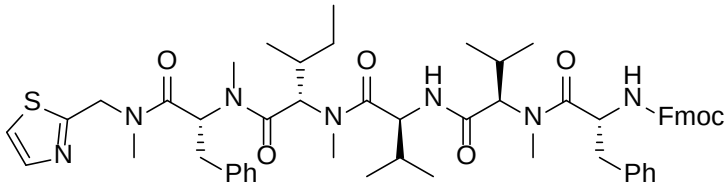
N-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-D-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-D-phenylalanine (**44**). Produced following general procedures. Theoretical resin loading was 0.76 mmol. Isolated 192 mg of amorphous white solid. 31.8% yield over 12 steps. APCIMS m/z [M+H] 794.51 (calcd for $C_{44}H_{68}N_5O_8$), found 794.5



micromide isomer (**1b**). Reaction conditions and purification identical to **1a**. Isolated 67 mg, 54% yield. APCIMS m/z [M+H] 904.23 (calcd for $C_{49}H_{73}N_7O_7S$), found 904.7.



N-(*N*-*N*-((((9H-fluoren-9-yl)methoxy)carbonyl)-D-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-D-phenylalanine (**45**).

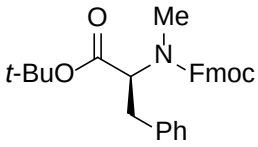


(9H-fluoren-9-yl)methyl ((4R,7S,10S,13R,16R)-4-benzyl-7-((*S*)-sec-butyl)-10,13-diisopropyl-2,5,8,14-tetramethyl-3,6,9,12,15-pentaoxo-17-phenyl-1-(thiazol-2-yl)-2,5,8,11,14-pentaazaheptadecan-16-yl)carbamate (**46**).

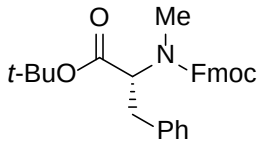
3.3 Micromide (Fmoc Solution Phase)

3.3.1 General Procedure for the *tert*-Butyl Esterification of Amino Acids

In a pressure flask, the amino acid was stirred in CH_2Cl_2 and cooled to -78°C. Isobutylene was added by flowing isobutylene gas into a dry-ice condenser and dropping the condensate into the pressure flask. A catalytic amount (1-2 mL) of concentrated sulfuric acid was added. The pressure flask was sealed and allowed to warm to ambient temperature. Caution: liquid isobutylene should not exceed 60 psi (pressure flask rating) under normal ambient temperatures, however, a protective shield should be used. The heterogenous mixture was stirred for 2-3 hours. The reaction is generally complete when the reaction becomes homogenous. The flask was then carefully opened and poured into a saturated solution of sodium bicarbonate. See specific compounds for additional information.

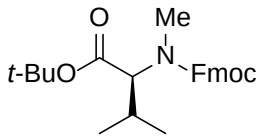


Fmoc-*N*-Me-L-Phe-OtBu (**47**). Fmoc-*N*-Me-L-Phe was treated as described in General Procedures.



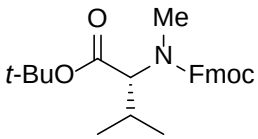
Fmoc-*N*-Me-D-Phe-OtBu (**48**). Fmoc-*N*-Me-D-Phe (1.00 g, 2.49 mmol) was dissolved in 50 mL of CH_2Cl_2 and 1 drop of DMF. Oxalyl chloride (0.52 mL, 4.98 mmol) was added and the reaction was refluxed for 1 hour. The volatiles were removed by rotary evaporation and the residue was re-dissolved in CH_2Cl_2. To the solution, tert-butanol (0.71 ml, 7.47 mmol) and pyridine (0.40 mL, 4.98 mmol) was added and stirred for 20 hours. The reaction was quenched with water and the volatiles were removed by rotary evaporation. The aqueous mixture was extracted 2 times with 50 mL Et_2O. The combined organic layers was washed with 1M $NaHCO_3$, H_2O, 1M HCl, brine, and dried over Na_2SO_4. The residue was purified by flash chromatography on silica (Et_2O/hexanes) to give 0.956 g of clear oil. 83.9%

^{1}H NMR (500 MHz, cdcl$_3$) δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, ^{1H}, *rotamer*), 7.44 (d, *J* = 7.5 Hz, ^{1H}, *rotamer*), 7.39 (t, *J* = 7.4, 7.4 Hz, 2H), 7.34 – 7.16 (m, 7H), 7.04 (d, *J* = 7.4 Hz, 1H), 4.94 (dd, *J* = 10.7, 5.5 Hz, 1H), 4.72 (dd, *J* = 10.4, 5.4 Hz, 1H, *rotamer*), 4.50 – 4.44 (m, 1H, *rotamer*), 4.34 (dd, *J* = 10.5, 7.2 Hz, 1H), 4.27 (dd, *J* = 10.5, 7.4 Hz, 1H), 4.21 (q, *J* = 5.9, 5.9, 5.6 Hz, 1H, *rotamer*), 4.14 (t, *J* = 6.2, 6.2 Hz, 1H, *rotamer*), 3.33 (dd, *J* = 14.5, 5.5 Hz, 1H), 3.17 (dd, *J* = 14.4, 5.4 Hz, 1H, *rotamer*), 3.02 (dd, *J* = 14.4, 10.8 Hz, 1H), 2.85 (s, 3H), 1.46 (s, 9H), 1.43 (s, 9H, *rotamer*).



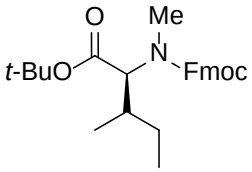
Fmoc-*N*-Me-L-Val-OtBu (**49**). Fmoc-*N*-Me-L-Val (2.00 g, 5.66 mmol) and tert-butyl N,N'-diisopropylcarbamimidate (2.26 g, 11.2 mmol) were refluxed in 50 mL toluene for 20 hours. Quenched with 10% citric acid and washed repeatedly with water. The organic layer was then washed with sat. Na_2CO_3 and brine, dried over $MgSO_4$, filtered, and condensed. The residue was stirred in Et_2O and the insoluble material was filtered off. The solution was injected onto a

100 g silica column and purified (Et_2O/hexanes) to give 1.06 g of clear oil, 45.2% yield. Spectrum identical to following compound.



Fmoc-*N*-Me-D-Val-OtBu (**50**). Fmoc-*N*-Me-D-Val-OH was treated as described in General Procedures. After neutralizing with bicarbonate, the CH_2Cl_2 was replaced with EtOAc and washed with water and brine. Purified by flash chromatography on silica (Et_2O/hexanes). 77.2% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62 (dd, *J* = 20.7, 7.2 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 4.56 (dd, *J* = 10.6, 6.4 Hz, 1H), 4.50 – 4.39 (m, 1H), 4.36 (dd, *J* = 23.8, 10.4 Hz, 1H), 4.27 (dt, *J* = 12.8, 6.8 Hz, 1H), 4.08 (d, *J* = 10.7 Hz, 1H, *rotamer*), 2.89 (d, *J* = 6.2 Hz, 3H), 2.13 (ddt, *J* = 24.3, 11.6, 6.5 Hz, 1H), 1.46 (s, 9H), 1.45 (s, 9H, *rotamer*), 1.00 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.5 Hz, 3H, *rotamer*), 0.87 (d, *J* = 6.7 Hz, 3H), 0.76 (d, *J* = 6.7 Hz, 3H, *rotamer*).



tert-butyl *N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-L-isoleucinate (**51**). Fmoc-*N*-Me-L-Ile-OH was treated as described in General Procedures. The resulting mixture was poured into 100 mL CH_2Cl_2 and the organic layer was washed with dilute bicarbonate, water, brine, and dried over $MgSO_4$. Purified by flash chromatography on silica (20% EtOAc/hexanes, isocratic) to give a clear, colorless, viscous oil. Formed crystals on standing for 2 weeks at 4°C. 3.25 g, 94% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.64 (dd, *J* = 15.5, 7.8 Hz, 2H), 7.42 (t, *J* = 7.5, 7.5 Hz, 2H), 7.33 (t, *J* = 7.5, 7.5 Hz, 2H), 4.62 – 4.42 (m, 2H), 4.40 – 4.17 (m, 2H), 2.91 (s, 3H), 2.03 – 1.87 (m, 1H), 1.48 (s, 9H), 1.43 – 1.30 (m, 2H), 1.02 – 0.88 (m, 6H).

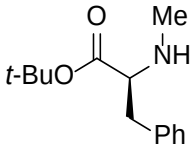
3.3.2 General Procedure for Removal of Fmoc Groups

The Fmoc-protected amino acid ester/peptide was stirred in 40% diethylamine in acetonitrile for 2 hours. The volatiles were removed by rotary evaporation. The residue was re-dissolved in acetonitrile and condensed. This process was repeated and then the residue was placed under high vacuum to ensure removal of any residual diethylamine. In some instances, the product was purified by flash chromatography for spectroscopic data but no significant change in yield was observed if purification was not performed.

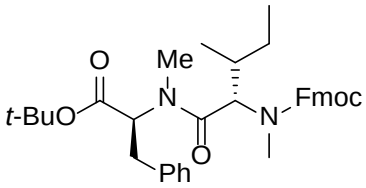
3.3.3 General Procedure for Peptide Coupling Using HATU/HOAt

The *N*-protected amino acid was pre-activated with HATU (1.5 eq), HOAt (1.5 eq), and DIEA (3.2 eq) in DMF for 10 minutes. The free amine (1 eq) in DMF was added and the solution was stirred for 4 hours (primary amine) or 20 hours (secondary amine). The solution was poured into dilute HCl and extracted with 3 portions of Et_2O. The combined organic layers were washed 4 times with dH_2O, then brine, dried over $MgSO_4$, filtered, and condensed.

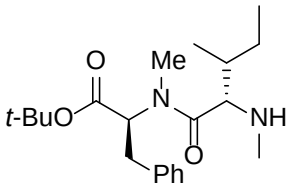
3.3.4 Tripeptide Fragment



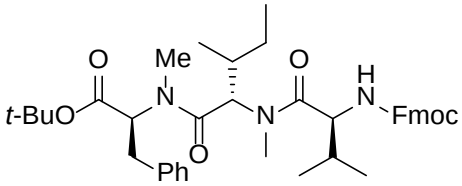
tert-butyl methyl-L-phenylalaninate (**5**). From Fmoc-*N*-Me-Phe-OtBu: **47** (3.49 g, 7.63 mmol) was deprotected as described above. The residue was purified by flash chromatography on silica (CH_2Cl_2/Et_2O) to give 1.63 g of clear, colorless oil. 90.8% yield. NMR spectrum identical to nosyl derivative (see Chapter 3.1.6).



Fmoc-*N*-Me-L-Ile-*N*-Me-L-Phe-OtBu (**52**). *N*-Me-Fmoc-L-Isoleucine (1.02 g, 2.77 mmol) was pre-activated by dissolving in 5 mL of anhydrous DMF, adding DIC (0.434 mL, 2.77 mmol), HOAt (0.377 g, 2.77 mmol), and stirred for 10 minutes. To the solution, **5** was added. The reaction was stirred at ambient temperature for 18 hours. TLC show presence of the deprotected product **14**. Without workup, the entire reaction was taken to the next step.



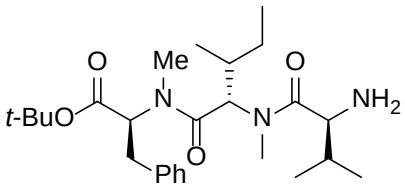
N-Me-L-Ile-*N*-Me-L-Phe-OtBu (**14**). From **52**: to the previous reaction, 6 mL of Et_2NH was added and stirred for 1 hour. Excess Et_2NH was removed by rotary evaporation and the flask contents were poured into 150 mL Et_2O. The organic layer was washed with water and saturated aqueous LiCl and dried over $NaSO_4$. Condensing the solution gave white precipitate (urea) in clear oil. The mixture was diluted in CH_2Cl_2 and the urea was filtered off. The filtrate was condensed and purified by flash chromatography on silica (CH_2Cl_2/Et_2O) to give 693 mg of clear oil. 89.6% yield over two steps. NMR spectrum identical to nosyl derivative (see Chapter 3.1.6).



Fmoc-L-Val-*N*-Me-L-Ile-*N*-Me-L-Phe-OtBu (**53**). Fmoc-L-Val (796 mg, 2.35 mmol), DIC (0.37 mL, 2.35 mmol), HOAt (319 mg, 2.35 mmol), and **14** (810 mg, 2.23 mmol) were treated in

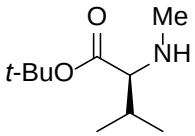
the same manner as compound **52**. Purification by flash chromatography on silica (EtOAc/hexanes) gave 863 mg of colorless resin in 69.4% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.41 (td, *J* = 7.4, 2.2 Hz, 2H), 7.35 – 7.29 (m, 2H), 7.25 (d, *J* = 7.3 Hz, 2H), 7.23 – 7.17 (m, 3H), 5.60 (dd, *J* = 11.7, 4.9 Hz, 1H), 5.44 (d, *J* = 9.2 Hz, 1H), 5.10 (d, *J* = 10.8 Hz, 1H), 4.43 – 4.32 (m, 3H), 4.23 (t, *J* = 7.3 Hz, 1H), 3.41 (dd, *J* = 15.2, 4.9 Hz, 1H), 3.00 – 2.90 (m, 1H), 2.85 (s, 3H), 2.54 (s, 3H), 1.60 (d, *J* = 6.0 Hz, 3H), 1.48 (s, 9H), 1.23 – 1.12 (m, 1H), 0.88 (t, *J* = 7.0 Hz, 6H), 0.84 – 0.75 (m, 6H).

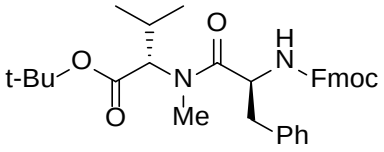


L-Val-*N*-Me-L-Ile-*N*-Me-L-Phe-OtBu (**16**). From **53**: Fmoc-protected peptide was deprotected as described in General Procedures. 76.2% yield. See Chapter 3.1.6 for spectral data.

3.3.5 Dipeptide Fragment



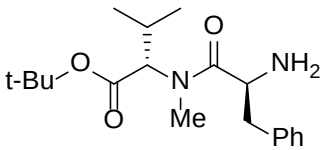
tert-butyl methyl-L-valinate (**54**). Compound **49** was stirred in 40% diethylamine in acetonitrile for 2 hours. The volatiles were removed by rotary evaporation and the residue was purified by flash chromatography. 60% yield. See Chapter 3.1.7 for spectral data (identical to enantiomer).



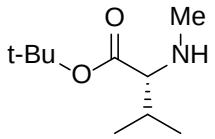
Fmoc-L-Phe-*N*-Me-L-Val-OtBu (**55**). Fmoc-L-phenylalanine (612 mg, 1.58 mmol) was preactivated with DIC (0.25 mL, 1.58 mmol) and HOAt (215 mg, 1.58 mmol) in DMF for 10 minutes. Amino ester **54** was added and the solution was stirred for 16 hours at ambient temperature. Water was added until a white emulsion appeared and the heterogenous mixture was stirred until the insoluble product adhered to the flask and the solution was transparent. The liquid was decanted off and the flask with residue was rinsed with water. The residue was dissolved in Et_2O and the organic layer was washed with water and brine, dried over $NaSO_4$, and condensed. The residue was purified by flash chromatography on silica (CH_2Cl_2/Et_2O) to give 450 mg of a clear, colorless resin in 53.7% yield.

^{1}H NMR (500 MHz, $CDCl_3$) (major rotamer) δ 7.73 (d, *J* = 7.6 Hz, 2H), 7.54 (t, *J* = 8.2 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.32 – 7.15 (m, 7H), 5.78 (d, *J* = 8.8 Hz, 1H), 4.96 (dt, *J* = 8.8, 6.6 Hz, 1H), 4.80 (d, *J* = 10.4 Hz, 1H), 4.34 (dd, *J* = 10.6, 7.4 Hz, 1H), 4.25 (dd, *J* = 10.6, 7.2 Hz, 1H), 4.16 (t, *J* = 7.5 Hz, 1H), 3.11 (dd, *J* = 13.5, 6.9 Hz, 1H), 2.98 – 2.92 (m, 1H), 2.92 (s, 3H), 2.14 (dp, *J* = 10.4, 6.7 Hz, 1H), 1.45 (s, 9H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.81 (d, *J* = 6.7 Hz, 3H).

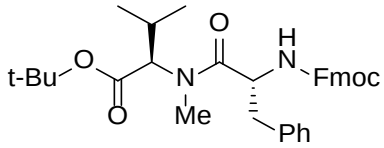
^{13}C NMR (126 MHz, $CDCl_3$) (major rotamer) δ 172.23, 169.79, 155.71, 143.87, 141.27, 136.02, 129.60, 129.53, 128.50, 127.68, 126.98, 125.20, 119.95, 82.39, 81.57, 66.99, 62.63, 47.12, 38.87, 31.05, 28.06, 27.80, 19.68, 18.94.



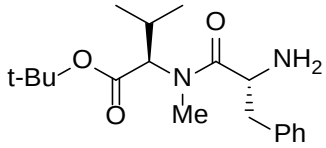
L-Phe-*N*-Me-L-Val-OtBu (**56**). Fmoc-protected peptide (100 mg) was deprotected as described in General Procedures and used without purification.



N-Me-D-Val-OtBu (**20**). From **50**: was stirred in 40% diethylamine in acetonitrile for 2 hours. The volatiles were removed by rotary evaporation and the residue was purified by flash chromatography on silica (Et_2O/CH_2Cl_2) to give 1.17 g in 85% yield. See Chapter 3.1.7 for spectral data.



Fmoc-D-Phe-*N*-Me-D-Val-OtBu (**57**). Method A: Prepared in the same manner as **55** with HOBt in lieu of HOAt. 67.9% yield. Method B: Fmoc-D-Phe (1.03 g, 2.67 mmol) was suspended in CH_2Cl_2 at 0°C. DIPEA (0.97 mL, 5.59 mmol) was added to the suspension. BOP-Cl (0.318 g, 2.80 mmol) was added, followed immediately by **20**. The reaction was allowed to reach ambient temperature and stirring was maintained for 18 hours. The reaction was then quenched with H_2O and the organic layer was removed by rotary evaporation. The residue was dissolved in EtOAc and the organic layer was washed with H_2O, saturated Na_2CO_3, brine, and dried over Na_2SO_4. The organic layer was condensed and the residue was purified by flash chromatography on silica gel (Et_2O/CH_2Cl_2) to give 1.08 g of resinous oil. 76.0% yield.

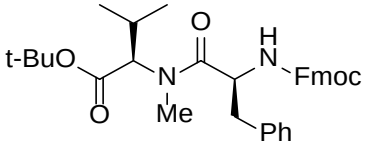


D-Phe-*N*-Me-D-Val-OtBu (**58**). Compound **57** (1.04 g, 18.6 mmol) was deprotected as described in General Procedures. Purification by flash chromatography on silica ($MeOH/CH_2Cl_2$) gave 650 mg of colorless oil that crystallized upon standing. 99% yield.

1H NMR (400 MHz, $CDCl_3$) (major rotamer) δ 7.38 – 7.14 (m, 5H), 4.85 (d, *J* = 10.4 Hz, 1H), 3.94 (dd, *J* = 8.2, 5.1 Hz, 1H), 3.82 (d, *J* = 10.7 Hz, 2H), 3.02 (dd, *J* = 13.7, 5.3 Hz, 1H),

2.96 (s, 3H), 2.68 (dd, *J* = 13.6, 8.2 Hz, 1H), 2.17 (dtt, *J* = 16.8, 6.5, 3.3 Hz, 1H), 1.45 (s, 9H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.7 Hz, 3H).

13C NMR (101 MHz, $CDCl_3$) δ 175.44, 170.23, 137.83, 129.39, 128.65, 126.78, 81.46, 62.50, 53.25 (d, *J* = 25.9 Hz), 42.06, 30.84, 28.12, 27.42, 19.85, 19.10.

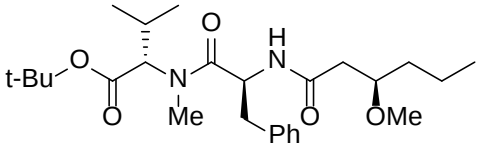


Fmoc-L-Phe-*N*-Me-D-Val-OtBu (**59**). Fmoc-L-Phe-OH (0.50 g, 1.29 mmol) was dissolved in 15 mL DMF. To the solution, HOBt (198 mg, 1.29 mmol) and DIC (0.20 mL, 1.29 mmol) was added and stirred for 10 min. A solution of **20** (0.23 g, 1.23 mmol) in DMF (5 mL) was added and stirred for 18 hours at ambient temperature. The solution was poured into 20 mL H_2O and extracted 3 times with 20 mL Et_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on silica (Et_2O/hexanes) 3.92 mg of amorphous solid in 57.3% yield. Application of a strong vacuum to the solvated product results in foaming of the product and small particulates floating into the vacuum line. This may be avoided by heating the product past its glass-transition temperature before application of the vacuum or letting the product stand under mild vacuum until the solvent is removed. Note: HOBt generally should not be used for the coupling of hindered amines, as .its reactivity is much lower than HOAt.[77]

1H NMR (500 MHz, $CDCl_3$) (*rotamer* 1) δ 7.78 (dd, *J* = 7.7, 2.3 Hz, 2H), 7.63 – 7.52 (m, 2H), 7.42 (td, *J* = 7.5, 2.8 Hz, 2H), 7.33 (td, *J* = 7.4, 5.9 Hz, 2H), 7.30 – 7.11 (m, 5H), 5.62 (d, *J* = 8.9 Hz, 1H), 5.05 – 4.96 (m, 1H), 4.68 (d, *J* = 10.4 Hz, 1H), 4.40 (dt, *J* = 10.4, 6.7 Hz, 1H), 4.32 – 4.23 (m, 1H), 4.23 – 4.14 (m, 1H), 3.10 (dd, *J* = 13.4, 7.9 Hz, 1H), 3.03 – 2.94 (m, 1H), 2.92 (s, 3H), 2.14 – 2.02 (m, 1H), 1.47 – 1.39 (m, 9H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.65 (d, *J* = 6.7 Hz, 3H).

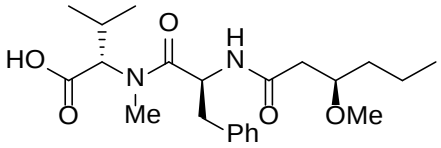
1H NMR (500 MHz, $CDCl_3$) (*rotamer* 2) δ 7.78 (dd, *J* = 7.7, 2.3 Hz, 2H), 7.63 – 7.52 (m, 2H), 7.42 (td, *J* = 7.5, 2.8 Hz, 2H), 7.33 (td, *J* = 7.4, 5.9 Hz, 2H), 7.30 – 7.11 (m, 5H), 5.62 (d, *J* = 8.9 Hz, 1H), 5.11 – 5.03 (m, 1H), 4.40 (dt, *J* = 10.4, 6.7 Hz, 1H), 4.32 – 4.23 (m, 1H), 4.23 – 4.14 (m, 1H), 4.09 (d, *J* = 10.5 Hz, 1H), 3.10 (dd, *J* = 13.4, 7.9 Hz, 1H), 3.03 – 2.94 (m, 1H), 2.98 (s, 3H), 2.37 – 2.25 (m, 1H), 1.54 – 1.48 (m, 9H), 1.06 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 3H).

3.3.6 Final Couplings

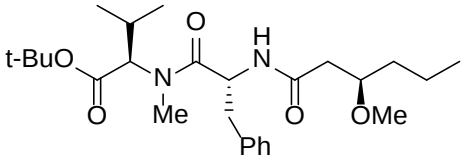


tert-butyl *N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-L-valinate (**60**). Hexanoic acid fragment **26** (28 mg, 0.19 mmol) and PyBOP (100 mg, 0.19 mmol) was dissolved in 2 mL DMF. The crude material OtBu (**56**, dissolved in 8 mL DMF, was added to the solution, followed by DIPEA (0.072 mL, 0.41 mmol). Stirred for 16 hours at ambient temperature. Quenched with 10% citric acid and extracted with Et_2O. The organic layer was washed with water, brine, and dried over Na_2SO_4 and condensed. The residue was purified by flash chromatography on silica (Et_2O/hexanes) to give 73 mg of clear, colorless oil in 88% yield.

1H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.16 (m, 5H), 6.87 (d, *J* = 8.2 Hz, 1H), 5.20 (ddd, *J* = 8.2, 7.2, 5.8 Hz, 1H), 4.80 (d, *J* = 10.5 Hz, 1H), 3.53 – 3.43 (m, 1H), 3.24 (d, *J* = 1.0 Hz, 3H), 3.12 (dd, *J* = 13.6, 7.2 Hz, 1H), 2.94 – 2.89 (m, 4H), 2.32 (d, *J* = 5.8 Hz, 2H), 2.13 (dp, *J* = 10.4, 6.6 Hz, 1H), 1.56 – 1.47 (m, 1H), 1.45 (s, 9H), 1.42 – 1.37 (m, 1H), 1.32 (qd, *J* = 7.3, 5.5 Hz, 2H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H).

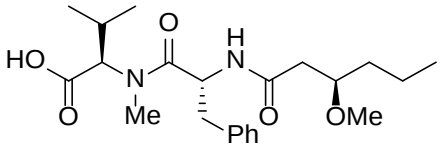


N-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-L-valine (**61**). Compound **60** was stirred in 1:1 CH_2Cl_2/TFA for 16 hours. The volatiles were removed by rotary evaporation. The residue was dissolved in CH_2Cl_2 and evaporated repeatedly to remove residual TFA. The crude material was used without further purification.



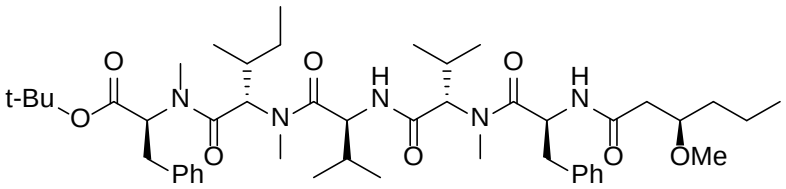
tert-butyl *N*-(((*R*)-3-methoxyhexanoyl)-D-phenylalanyl)-*N*-methyl-D-valinate (**62**). The title compound was produced in the same manner as compound **60**. 53.6% yield.

1H NMR (400 MHz, $CDCl_3$) (major rotamer) δ 7.31 – 7.15 (m, 5H), 6.88 (d, *J* = 8.3 Hz, 1H), 5.20 (ddd, *J* = 8.3, 7.0, 6.4 Hz, 1H), 4.79 (d, *J* = 10.5 Hz, 1H), 3.48 (p, *J* = 5.9 Hz, 1H), 3.30 (s, 3H), 3.09 (dd, *J* = 13.6, 7.0 Hz, 1H), 2.92 (s, 3H), 2.91 (dd, 1H, partially hidden) 2.35 – 2.28 (m, 2H), 2.13 (dp, *J* = 10.5, 6.7 Hz, 1H), 1.45 (s, 9H), 1.44 (m, 1H, partially hidden) 1.38 – 1.21 (m, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.89 (t, *J* = 7.1 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H).

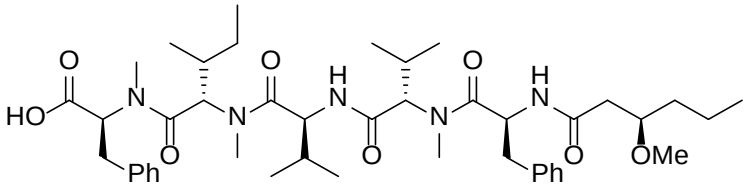


N-(((*R*)-3-methoxyhexanoyl)-D-phenylalanyl)-*N*-methyl-D-valine (**63**). Ester **62** (163 mg) was dissolved in 5 mL CH_2Cl_2 and 2 mL TFA was added with stirring at ambient temperature. After 4 hours, no starting material was visible by TLC. The solution was poured into 50 mL H_2O and separated. The aqueous layer was extracted 3 times with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solution was condensed, and the crude was used without further purification.

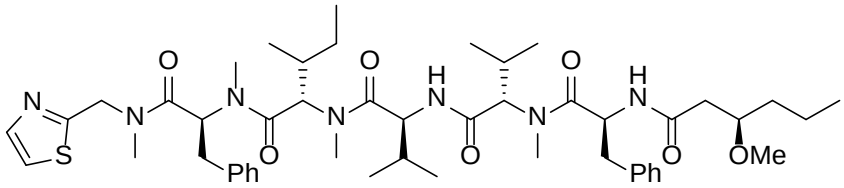
1H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.13 (m, 5H), 5.16 (td, *J* = 7.9, 6.8 Hz, 1H), 4.78 (d, *J* = 10.4 Hz, 1H), 3.49 (p, *J* = 5.6 Hz, 1H), 3.29 (s, 3H), 3.06 (dd, *J* = 13.5, 7.8 Hz, 1H), 2.95 (dd, *J* = 13.4, 6.9 Hz, 1H), 2.87 (s, 3H), 2.45 – 2.26 (m, 2H), 2.20 (ddt, *J* = 13.1, 10.8, 6.6 Hz, 1H), 1.52 – 1.40 (m, 1H), 1.38 – 1.16 (m, 3H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.88 (t, *J* = 7.2 Hz, 3H), 0.81 (d, *J* = 6.8 Hz, 3H).



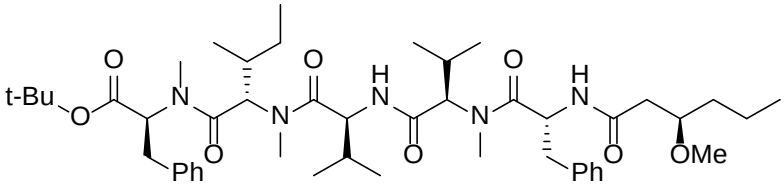
tert-butyl *N*-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-L-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-L-phenylalaninate (**64**). Lipopeptide **61** (23 mg) was dissolved in 1 mL DMF with 24 mg of HATU. DIPEA (20 µL) was added, followed by a solution of tripeptide free amine **16** in 1 mL DMF. The solution was stirred for 18 hours. The reaction was quenched with 5 mL H_2O and extracted with 2 × 35 mL EtOAc. The combined organic layers were washed repeatedly with H_2O, then brine. After drying over Na_2SO_4.and condensing, the residue was purified by flash chromatography oh silica gel (CH_2Cl_2/Et_2O) to give 36 mg of amorphous white solid. 75% yield. APCIMS *m/z* [M+H] 850.5, (calcd for $C_{48}H_{76}N_5O_8$ 850.57)



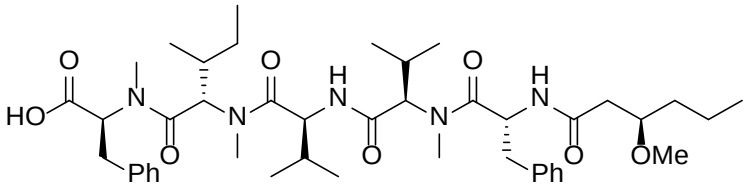
N-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-L-phenylalanyl)-*N*-methyl-L-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-L-phenylalanine (**65**). In a scintillation vial, the previous compound was stirred in 0.8 mL $CDCl_3$ and 0.5 mL TFA at ambient temperature. The reaction was monitored by TLC. Reaction was complete after 6 hours. The excess liquid was removed by rotary evaporation. The residue was repeatedly dissolved in CH_2Cl_2, dried and used immediately in the next step.



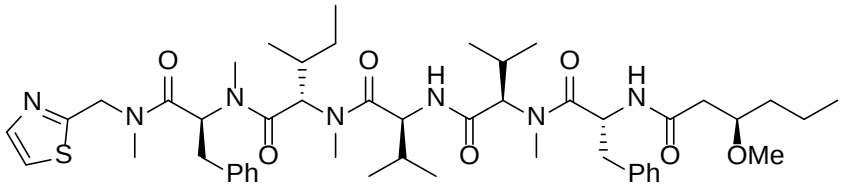
micromide isomer, all L-residues (**1d**). The previous compound (32 mg, 0.04 mmol) was combined with **38** (7 mg, 0.06 mmol), HOAt (8 mg, 0.06 mmol), EDCI (11 mg, 0.06 mmol) in 3 mL of CH_2Cl_2 with stirring. Et_3N (22 µL, 0.16 mmol) was added and the reaction was stirred at ambient temperature for 18 hours. The solution was poured into 70 mL Et_2O and the organic layer was washed with 1M HCl, H_2O, brine, dried over Na_2SO_4, then condensed. Purification on C18 silica (MeCN/H_2O) gave 24 mg of amorphous solid. 66% yield. APCIMS *m/z* [M+H] 904.2, (calcd for $C_{49}H_{73}N_7O_7S$ 904.54)



tert-butyl *N*-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-D-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-L-phenylalaninate (**66**). Lipopeptide **63** (117 mg, 0.288 mmol) was dissolved in 2 mL DMF with HATU (120 mg, 0.317 mmol). DIPEA (20 µL, 0.576 mmol) was added, followed by a solution of tripeptide free amine **16** in 1 mL DMF. The reaction was monitored by APCI-MS and complete after 6 hours. The reaction was diluted with EtOAc and washed repeatedly with H_2O. After drying over Na_2SO_4.and condensing, the residue was purified by flash chromatography on silica gel (CH_2Cl_2/Et_2O) followed by purification on C18 modified silica (MeCN/H_2O) to give 83 mg of amorphous white solid. 33.4% yield. APCIMS *m/z* [M+H] 850.5, (calcd for $C_{48}H_{75}N_5O_8$ 850.57)

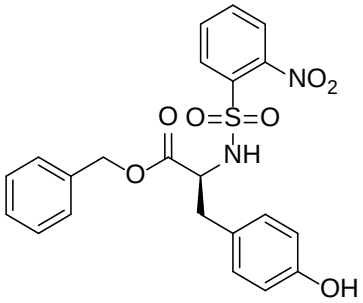


N-(*N*-*N*-(((*R*)-3-methoxyhexanoyl)-D-phenylalanyl)-*N*-methyl-D-valyl-L-valyl-*N*-methyl-L-isoleucyl)-*N*-methyl-L-phenylalanine (**67**). The



micromide isomer, D-Phe substitution (**1c**). Washed with water, 1M HCl. The residue was purified by reverse phase chromatography. Acetonitrile in water; 0.1% formic acid. 10% 1CV, 10-100% over 11 CV, 100% until product is off column. The isolated product is the thiazolium form. A second purification on a silica plug with MeOH/CH_2Cl_2. The final isolated material was then dissolved in ethyl acetate and washed with 1M sodium hydroxide. 28% yield.

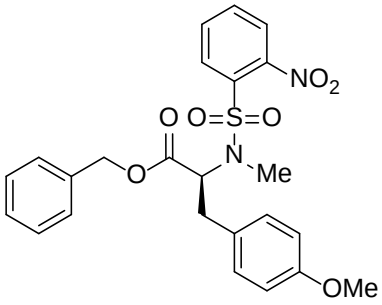
3.3.7 Tyrosine-based Analog



benzyl ((2-nitrophenyl)sulfonyl)-L-tyrosinate (**68**). (1.17 g, 4.31 mmol) was dissolved in 1 mL DMF and 5 mL anhydrous THF. 2-nitrobenzenesulfonyl chloride was dissolved in 3 mL anhydrous THF and added dropwise to the tyrosine solution with stirring. After 16 hours of stirring at ambient temperature, the reaction was quenched with 1 mL H_2O. THF was removed by rotary evaporation and replaced with EtOAc. The organic layer was washed with 2M HCl, brine, and dried over Na_2SO_4 to give a mixture of mono- and dinosylated products. Purification

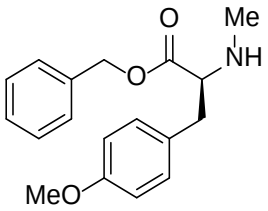
by flash chromatography on silica (EtOAc/hexanes) isolated 0.60 g of desired product. 40% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.76 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.62 (td, *J* = 7.7, 1.7 Hz, 1H), 7.57 (td, *J* = 7.6, 1.5 Hz, 1H), 7.36 – 7.30 (m, 3H), 7.17 – 7.10 (m, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 8.5 Hz, 2H), 6.01 (d, *J* = 9.0 Hz, 1H), 4.90 (d, *J* = 4.1 Hz, 2H), 4.46 (dt, *J* = 9.0, 6.1 Hz, 1H), 3.06 (t, *J* = 6.2 Hz, 2H).



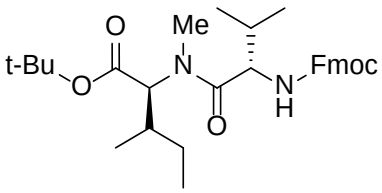
benzyl (*S*)-3-(4-methoxyphenyl)-2-((*N*-methyl-2-nitrophenyl)sulfonamido)propanoate (*N,O*-diMe-(*N*-nosyl)Tyr-OBn) (**69**). Previous compound **68** (131 mg, 0.29 mmol) was dissolved in 5 mL anhydrous DMF and stirred with K_2CO_3 (237 mg, 1.72 mmol). Freshly distilled MeI (0.16 mL, 2.58 mmol) was added and stirred at 40°C for 10 hours. The reaction was quenched with water and dissolved in Et_2O. The organic layer was washed with 5×30 mL H_2O, brine, and dried over Na_2SO_4. Condensing gave 87 mg of desired product. The resulting oil was sufficiently pure to use directly. 86.5% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.60 – 7.41 (m, 3H), 7.35 – 7.29 (m, 3H), 7.20 (ddt, *J* = 5.6, 2.9, 1.1 Hz, 2H), 7.14 – 7.07 (m, 2H), 6.77 – 6.70 (m, 2H), 5.05 (d, *J* = 4.1 Hz, 2H), 4.91 (dd, *J* = 9.1, 6.5 Hz, 1H), 3.76 (s, 3H), 3.30 (dd, *J* = 14.2, 6.4 Hz, 1H), 3.02 (s, 3H), 2.98 – 2.86 (m, 1H).



benzyl (*S*)-3-(4-methoxyphenyl)-2-(methylamino)propanoate (*N,O*-diMe-Tyr-OBn) (**70**). Previous compound **69** (87 mg, 0.18 mmol) was stirred in 4 mL anhydrous DMF and 2-mercaptoethanol (126 µL, 1.8 mmol). Addition of DBU (134 µL, 0.90 mmol) changed the color of the solution to a blue-green hue, indicative of the Meisenheimer complex. After 2 hours, the color changed to a bright yellow (thioether cleavage product). The solution was poured into 60 mL Et_2O. The organic layer was washed with 5×20 mL H_2O, brine, and dried over MgSO4. The crude was purified by flash chromatography on silica (MeOH/CH_2Cl_2) to give 59 mg of yellow oil, containing the desired product and a small amount of thioether contaminant (>90% purity by NMR) in 98% yield. Product was successfully isolated from thioether contaminant with the following method: equilibrated column with 2 CV of CH_2Cl_2. The product was dissolved in CH_2Cl_2 and wet-loaded onto the column. Isocratic 20% EtOAc/CH_2Cl_2 was pushed until the thioether was off the column. The eluent was changed to 80% EtOAc/CH_2Cl_2 and pushed until the desired product was isolated. The resulting light-yellow oil was free of all sulfurous products.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 3H), 7.25 (dd, *J* = 7.3, 2.3 Hz, 2H), 7.05 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 5.11 (d, *J* = 1.6 Hz, 2H), 3.79 (s, 3H), 3.47 (t, *J* = 6.8 Hz, 1H), 2.92 (t, *J* = 6.8 Hz, 2H), 2.38 (s, 3H).

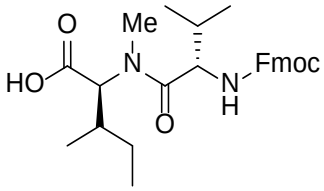


tert-butyl *N*-((((9H-fluoren-9-yl)methoxy)carbonyl)-L-valyl)-*N*-methyl-L-isoleucinate (Fmoc-L-Val-*N*-Me-L-Ile-OtBu) (**71**). *Method A:* Fmoc-protected isoleucine **51** (1.00 g, 2.36 mmol) was stirred in 10 mL MeCN and 5 mL Et_2NH for 2 hours. Volatiles were removed and the

residue was dissolved in CH_2Cl_2. Fmoc-L-Val (0.801 g, 2.36 mmol) was stirred in 20 mL CH_2Cl_2 with BOP-Cl (0.631 g, 2.48 mmol) and DIPEA (0.49 mL, 2.8 mmol) for 5 minutes. To the pre-activated valine solution, the isoleucine solution was added and stirred for 24 hours at ambient temperature. The mixture was condensed and the residue was dissolved in EtOAc. The organic layer was washed with 5 × 20 mL H_2O and dried over $MgSO_4$. Purification by flash chromatography on silica (Et_2O/hexanes) gave 613 mg of desired product. 49.7% yield.

Method B: Fmoc-protected isoleucine **51** (1.00 g, 2.36 mmol) was stirred in 10 mL MeCN and 5 mL Et_2NH for 2 hours. Volatiles were removed and the residue was dissolved in DMF. Fmoc-L-Val (.801 g, 2.36 mmol) was stirred in 20 mL DMF with HATU (0.943 g, 2.48 mmol) and DIPEA (0.49 mL, 2.8 mmol) for 5 minutes. To the pre-activated valine solution, the isoleucine was added and stirred for 24 hours at ambient temperature. The flask contents were poured into 100 mL Et_2O. The organic layer was washed with 5 × 20 mL H_2O, 20 mL 1M HCl, brine, and dried over $MgSO_4$. Purification as above, gave 822 mg of desired product. 66.6% yield.

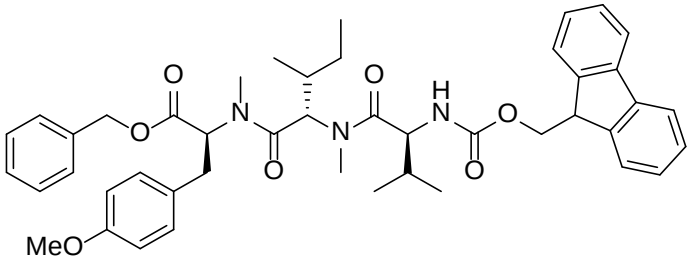
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.31 (tdd, *J* = 7.4, 2.6, 1.2 Hz, 2H), 5.56 (d, *J* = 9.3 Hz, 1H), 4.90 (d, *J* = 10.4 Hz, 1H), 4.55 (dd, *J* = 9.2, 6.2 Hz, 1H), 4.36 (dd, *J* = 7.3, 3.1 Hz, 2H), 4.22 (t, *J* = 7.1 Hz, 1H), 3.04 (s, 3H), 2.10 – 1.90 (m, 1H), 1.44 (s, 9H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.92 – 0.84 (m, 3H), 0.83 (t, *J* = 7.6 Hz, 3H).



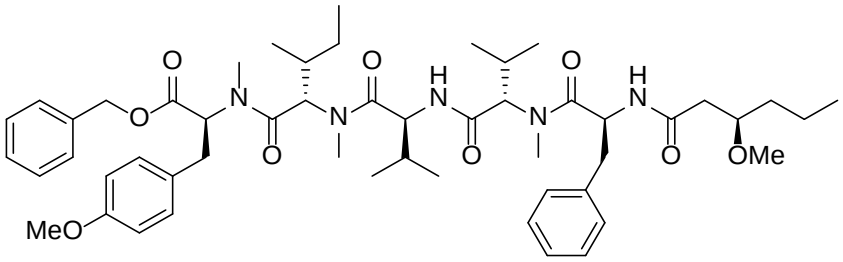
N-((((9H-fluoren-9-yl)methoxy)carbonyl)-L-valyl)-*N*-methyl-L-isoleucine (Fmoc-L-Val-*N*-Me-L-Ile-OH) (**72**). Dipeptide **71** (600 mg, 1.15 mmol) was stirred in 5 mL CH_2Cl_2 and 5 mL TFA for 6 hours. Volatiles were removed by rotary evaporation and the residue was dissolved in

EtOAc. The organic layer was washed with H_2O, brine, and dried over Na_2SO_4. Condensing gave 658 mg of clear oil. The residue was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.4 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.42 (td, *J* = 7.4, 2.3 Hz, 2H), 7.33 (tdd, *J* = 7.5, 2.7, 1.2 Hz, 2H), 5.87 (d, *J* = 9.3 Hz, 1H), 4.87 (d, *J* = 10.2 Hz, 1H), 4.56 (dd, *J* = 9.4, 6.9 Hz, 1H), 4.38 (dd, *J* = 7.4, 2.3 Hz, 2H), 4.24 (t, *J* = 7.2 Hz, 1H), 3.15 (s, 3H), 2.15 – 2.00 (m, 2H), 1.45 – 1.35 (m, 1H), 1.14 – 1.01 (m, 1H), 1.03 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H).

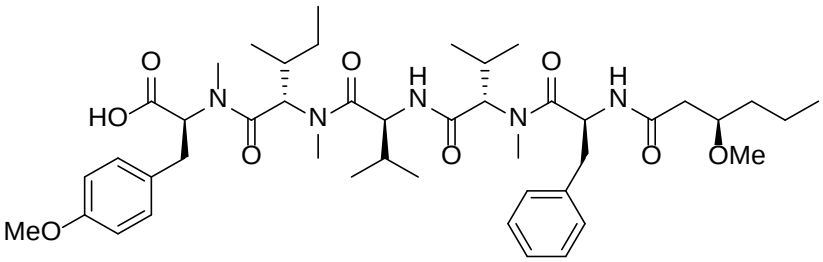


Fmoc-L-Val-*N*-Me-L-Ile-*N,O*-diMe-Tyr-OBn (**73**). Dipeptide **72** (27 mg, 0.06 mmol), BOP-Cl (16 mg, 0.06 mmol), and DIPEA (12 µL, 0.07 mmol) was stirred in DMF for 5 minutes. Tyrosine derivative **70** was dissolved in 1 mL DMF and added to the dipeptide. After stirring for 24 hours at ambient temperature, the reaction was diluted in 20 mL Et_2O. The organic layer was washed with 5×5 mL H_2O, 5 mL 1M HCl, brine, and dried over Na_2SO_4. Purification by flash chromatography on silica (Et_2O/hexanes) gave 19 mg of desired product. 44% yield.

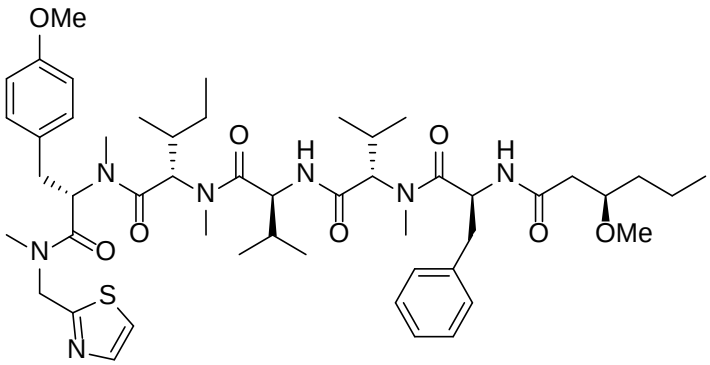


benzyl (3R,7S,10S,13S,16S,19S)-7-benzyl-16-((*S*)-sec-butyl)-10,13-diisopropyl-19-(4-methoxybenzyl)-9,15,18-trimethyl-5,8,11,14,17-pentaoxo-3-propyl-2-oxa-6,9,12,15,18-pentaazaicosan-20-oate (**74**). Dipeptide fragment **40** (48 mg, 0.12 mmol) was stirred in 1 mL DMF with HATU (52 mg, 0.17 mmol), HOAt (23 mg, 0.17 mmol), and DIPEA (60 µL, 0.34

mmol). Tripeptide **73** was deprotected as described under General Procedures and the resulting residue was dissolved in 1 mL DMF. The two solutions were combined and stirred for 4 hours at ambient temperature. The reaction was quenched with 3 mL H_2O and mixed with 5 mL EtOAc. The organic layer was washed with 3×20 mL 1M HCl, brine, and condensed. The residue was dried by azeotropic evaporation with MeCN. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography (MeOH/CH_2Cl_2) to give 80 mg of amorphous brown solid. 74% yield.

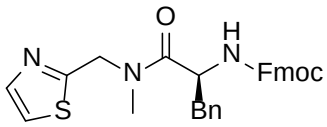


(3R,7S,10S,13S,16S,19S)-7-benzyl-16-((*S*)-sec-butyl)-10,13-diisopropyl-19-(4-methoxybenzyl)-9,15,18-trimethyl-5,8,11,14,17-pentaoxo-3-propyl-2-oxa-6,9,12,15,18-pentaazaicosan-20-oic acid (**75**). $Pd(OH)_2$ (2 mg) was suspended in 3 mL EtOAc under H_2 atmosphere for 30 minutes. Benzyl-protected peptide **74** (80.4 mg, 0.06 mmol) was dissolved in EtOAc and added to the mixture and stirred. The reaction was monitored by TLC until no more starting material was detected. The mixture was filtered through a 100 mg C8 SPE cartridge and condensed. The residue was purified by reverse-phase flash chromatography on C18-modfied silica (MeCN/H_2O, 0.1% formic acid). Despite TLC analysis, the reaction was incomplete. Isolated 18.5 mg of desired product and recovered 56 mg of starting material. 84% converted yield.



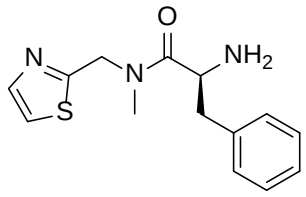
Micromide, tyrosine analog (**1e**). Peptide **75** (18.5 mg, 0.02 mmol) and thiazole **38** (8.4 mg, 0.07 mmol) were stirred in 1 mL anhydrous DMF. Pre-made 0.5 M solutions of HATU (0.044 mL, 0.02 mmol) and HOAt (0.044 mL, 0.02 mmol in DMF) were added and the mixture wazs stirred at ambient temperature for 4 hours. No starting material was observed by mass spectrometry (APCI mode). The reaction was quenched with 1 mL H_2O, turning the solution heterogenous. Sufficient MeCN was added to homogenize the solution. The resulting solution was purified by flash chromatography on C18 (MeCN/H_2O, 0.1% formic acid) without work-up. Isolated 16.5 g of amorphous white solid. 80% yield.

3.3.8 Other Structural Modifications



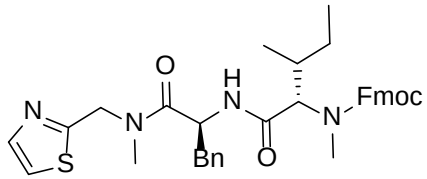
(9H-fluoren-9-yl)methyl (*S*)-(1-(methyl(thiazol-2-ylmethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**76**). Fmoc-L-Phe (453 mg, 1.17 mmol) was pre-activated with HATU (445 mg, 1.17 mmol), HOAt (159 mg, 1.17 mmol), and DIEA (0.40 mL, 2.34 mmol) in 7 mL of DMF for 10 minutes. Thiazole **38** (100 mg, 0.78 mmol) in 3 mL of DMF was added and the solution was stirred for 20 hours. The solution was poured into dilute HCl and extracted with 3 portions of Et_2O, totaling 150 mL. The combined organic layers were washed with 4×10 mL dH_2O, brine, dried over $MgSO_4$, filtered, and condensed. Attempts to dissolve the residue in EtOAc produced a precipitate, which was soluble in water. The organic layer was re-washed and dried.

Purification by column chromatography on silica (EtOAc/Et_2O) gave 372 mg of amorphous solid in 96% yield.

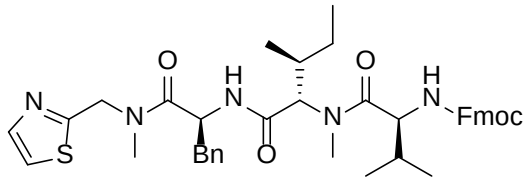


(*S*)-2-amino-*N*-methyl-3-phenyl-*N*-(thiazol-2-ylmethyl)propenamide (**77**). The previous compound was deprotected as described in General Procedures 3.3.2.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 3.3 Hz, ^{1}H, *rotamer*), 7.70 (d, *J* = 3.3 Hz, 1H), 7.31 (d, *J* = 3.3 Hz, 1H), 7.32 – 7.12 (m, 5H), 4.86 (d, *J* = 15.0 Hz, 1H), 4.77 (d, *J* = 15.0 Hz, 1H), 4.59 (d, *J* = 17.3 Hz, ^{1}H, *rotamer*), 4.44 (d, *J* = 17.2 Hz, ^{1}H, *rotamer*), 4.02 – 3.93 (m, 1H), 3.00 (s, 3H, *rotamer*), 3.06 – 2.94 (m, 1H), 2.87 (s, 3H), 2.86 – 2.75 (m, 1H), 1.93 (s, 2H).

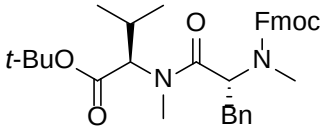


(9H-fluoren-9-yl)methyl methyl((2S,3S)-3-methyl-1-(((*S*)-1-(methyl(thiazol-2-ylmethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)carbamate (**78**). The previous compound was condensed with Fmoc-*N*-Me-L-Ile following the same protocol as compound **76**. 96% yield.

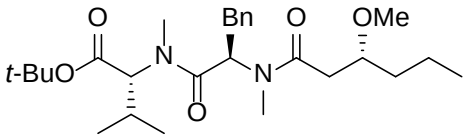


(9H-fluoren-9-yl)methyl ((*S*)-3-methyl-1-(methyl((2S,3S)-3-methyl-1-(((*S*)-1-(methyl(thiazol-2-ylmethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (**79**). The previous compound was deprotected as described in

General Procedures 3.3.2 and condensed with Fmoc-L-Val following the same protocol as compound **76**.



tert-butyl *N*-(*N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-L-phenylalanyl)-*N*-methyl-D-valinate (**80**). Fmoc-*N*-Me-L-Phe, prepared as described in 3.2.2, was pre-activated in 4 mL anhydrous DMF with HATU (609 mg, 1.6 mmol), HOAt (218 mg, 1.6 mmol), and DIPEA (0.55 mL, 3.2 mmol) for 5 minutes. *N*-Me-D-Val *tert*-butyl ester **20**, dissolved in 2 mL DMF, was added to the reaction and stirred at ambient temperature for 24 hours. The reaction was quenched with 20 mL of H_2O. The product precipitated out and adhered to the sides of the flask. The supernatant was decanted off and the residue dissolved in Et_2O. The organic layer was washed with H_2O, 10% citric acid, brine, and then dried over Na_2SO_4. The condensed residue was purified by flash chromatography (EtOAc/hexanes) to give 196 mg of amorphous white powder. 32% yield.



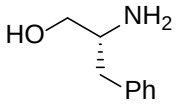
tert-butyl *N*-(*N*-((*R*)-3-methoxyhexanoyl)-*N*-methyl-L-phenylalanyl)-*N*-methyl-D-valinate (**81**). The previous compound was deprotected as described in General Procedures 3.3.2 and condensed with **26** following the same protocol as compound **76**. 13.8% yield. Major rotamer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.20 (m, 4H), 7.18 – 7.13 (m, 1H), 5.83 (dd, *J* = 8.6, 6.4 Hz, 1H), 4.69 (d, *J* = 10.5 Hz, 1H), 3.64 (p, *J* = 6.0, 6.0, 6.0, 6.0 Hz, 1H), 3.25 (s, 3H), 3.10 (s, 2H), 3.01 (s, 3H), 2.83 (s, 3H), 2.56 (dd, *J* = 15.3, 6.7 Hz, 1H), 2.28 (dd, *J* = 15.3, 5.6 Hz, 1H), 2.25 – 2.17 (m, 1H), 1.48 – 1.37 (m, 9H), 0.94 (dd, *J* = 6.5, 2.8 Hz, 4H), 0.91 (d, *J* = 7.3 Hz, 3H), 0.88 (t, *J* = 7.1, 7.1 Hz, 3H), 0.56 (d, *J* = 6.7 Hz, 3H).

3.4 Lagunamide

3.4.1 General Procedure for Oxidations Utilizing TEMPO

Commercial bleach (NaOCl, 5% free chlorine, 2.0 equivalents) was adjusted to pH 9.1 with solid sodium bicarbonate. The alcohol to be oxidized (1 equivalent) was stirred in CH_2Cl_2, KBr (10 mol %) with minimal water to dissolve, and TEMPO (1 mol %) and cooled to -10°C on a salt-ice bath. The bleach solution was added in aliquots, keeping the reaction temperature below 20°C. After complete addition of the bleach, the reaction was stirred for an additional 10 minutes. The organic layer was separated and washed with 10% HCl, containing potassium iodide (2 mol %), turning the solution violet. The organic layer was then washed with 10% sodium thiosulfate until the violet color dissipated, then with water and brine. The organic layer was dried over $MgSO_4$. Product is generally sufficiently pure to be used without purification.

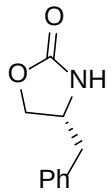
3.4.2 Polyketide Fragment



D-Phenylalinol (**82**). D-Phenylalanine (2.51 g, 15.2 mmol) was stirred in 100 mL anhydrous THF under argon and cooled to 0°C, to which, $NaBH_4$ (1.38 g, 36.5 mmol) was added. An addition funnel was charged with elemental iodine (4.05 g, 253.8 mmol), dissolved in 70 mL THF. Iodine was added dropwise, keeping the color as light as possible (ca. 30 minutes). Stir at ambient temperature until gas formation slows. Heat to 50°C and stir for 18 hours. Quench with 50 mL MeOH at 0°C and stir for 1 hour at ambient temperature. The borate esters were hydrolyzed with 100 mL of 20% KOH, stirred for 3 hours at ambient temperature. The THF was removed by rotary evaporation and the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 (Caution: Use of $MgSO_4$ will chelate and remove the

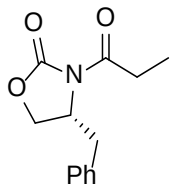
product) and concentrated. The residue was recrystallized in hot ether to give 1.49 g of white crystals. 64.7% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.14 (m, 5H), 3.63 (dd, *J* = 10.6, 3.9 Hz, 1H), 3.38 (dd, *J* = 10.5, 7.2 Hz, 1H), 3.18 – 3.06 (m, 1H), 2.79 (dd, *J* = 13.5, 5.3 Hz, 1H), 2.53 (dd, *J* = 13.5, 8.5 Hz, 1H).



(*R*)-4-benzyloxazolidin-2-one (**83**). D-Phenylalinol **82** (20.0 g, 132.2 mmol), diethyl carbonate (41.7 mL, 343.9 mmol), and K_2CO_3 (1.83 g, 13.2 mmol) were combined in a round-bottom flask and equipped with a distillation head. The mixture was stirred with heating (oil bath temp 120°C) until no more ethanol distills over. The pot residue was washed into a separatory funnel with EtOAc and water. The aqueous layer was extracted two more times with EtOAc. The organic layer was washed with water and brine, then dried over $MgSO_4$ and condensed. Residual diethyl carbonate was removed under high vacuum to give 22.9 g of crude solid. Recrystallization in EtOAc/hexanes gave 18.6 g of white crystals in a single crop. 79.2% isolated yield.

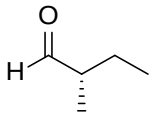
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.12 (m, 5H), 6.00 (s, 1H), 4.47 – 4.36 (m, 1H), 4.17 – 4.03 (m, 2H), 2.90 (dd, *J* = 13.6, 6.8 Hz, 1H), 2.84 (dd, *J* = 13.6, 6.3 Hz, 1H).



(*R*)-4-benzyl-3-propionyloxazolidin-2-one (**84**). Oxazolidinone **83** (10.0 g, 56.4 mmol) was dissolved in 100 mL anhydrous THF and cooled to -78°C. To the solution, n-Butyl lithium (2 M solution, 29.6 mL, 59.3 mmol) was added dropwise. The yellow solution was allowed to warm

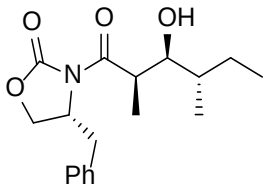
to ambient temperature for 30 minutes, then cooled back to -78°C. Propionyl chloride (5.17 mL, 59.3 mmol) was added dropwise. The reaction was stirred for 30 minutes at -78°C and then at ambient temperature for 2 hours. The reaction was quenched with saturated NH_4Cl and THF was removed by rotary evaporation. The aqueous mixture was extracted 3 times with CH_2Cl_2 and the combined organic layers were washed with water and brine, then dried over Na_2SO_4. Condensation gave a yellow solid that was purified by flash chromatography on silica to give 9.69 g of white crystals. 73.6% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.09 (m, 5H), 4.68 (ddt, *J* = 9.5, 7.2, 3.3 Hz, 1H), 4.26 – 4.11 (m, 2H), 3.31 (dd, *J* = 13.4, 3.3 Hz, 1H), 3.08 – 2.86 (m, 2H), 2.77 (dd, *J* = 13.4, 9.6 Hz, 1H), 1.21 (t, *J* = 7.4 Hz, 3H).



(*S*)-2-methylbutanal (**85**). Commercial (*S*)-2-methylbutanol (5.0 mL, 46.2 mmol) was produced as described in General Procedures. The resulting solution was partially condensed carefully, as the product is volatile and will evaporate with the solvent. Product was sufficiently pure and used without purification. Estimated 2.8 g of aldehyde in CH_2Cl_2 by NMR. 70% yield.

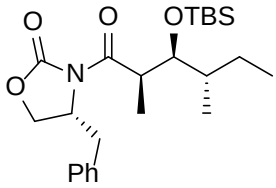
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (d, *J* = 1.9 Hz, 1H), 2.27 (hd, *J* = 6.9, 2.0 Hz, 1H), 1.75 (dqd, *J* = 13.9, 7.5, 6.5 Hz, 1H), 1.45 (dtd, *J* = 13.8, 7.5, 6.9 Hz, 1H), 1.09 (d, *J* = 7.0 Hz, 3H), 0.95 (t, *J* = 7.5 Hz, 3H).



(*R*)-4-benzyl-3-((2R,3S,4S)-3-hydroxy-2,4-dimethylhexanoyl)oxazolidin-2-one (**86**). In a round-bottomed flask, **84** (16.4 g, 70.37 mmol) was dissolved in 40 mL CH_2Cl_2, freshly distilled over calcium hydride, with stirring and cooled to 0°C. DBBT (18.2 mL, 84.4 mmol) was added

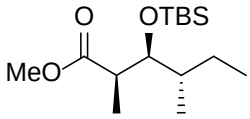
dropwise, followed by Et_3N (13.7 mL, 98.5 mmol). After 30 minutes, the reaction was cooled to -78°C and aldehyde **85** (7.97 g, 92.5 mmol) was added. The reaction was warmed to 0°C for 2 hours, then ambient temperature for 30 minutes. The reaction was quenched with 100 mL of pH 7 phosphate buffer and cooled to 0°C. The borinate ester was hydrolyzed with 100 mL of 2:1 30% H_2O_2/MeOH. The volatile solvents were removed by rotary evaporation and the aqueous slurry was extracted 3 times with Et_2O. The combined organic layers were washed with 5% bicarbonate, brine, dried over Na_2SO_4, and condensed. The residue was recrystallized in CH_2Cl_2/hexanes to give 15.8 g of white crystals. 70.4% yield. The resulting supernatant may be further purified by flash chromatography on silica (Et_2O/hexanes) if desired.

1H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.05 (m, 5H), 4.70 (ddd, *J* = 9.4, 7.2, 3.6 Hz, 1H), 4.27 – 4.12 (m, 2H), 3.96 (qd, *J* = 7.1, 2.2 Hz, 1H), 3.63 (dt, *J* = 9.0, 2.8 Hz, 1H), 3.26 (dd, *J* = 13.5, 3.5 Hz, 1H), 2.90 (d, *J* = 3.3 Hz, 1H), 2.79 (dd, *J* = 13.4, 9.4 Hz, 1H), 1.80 (dqd, *J* = 15.3, 7.7, 3.3 Hz, 1H), 1.61 – 1.45 (m, 2H), 1.23 (d, *J* = 7.0, 3H), 0.91 (t, *J* = 7.4 Hz, 3H), 0.87 (d, *J* = 6.8 Hz, 3H).



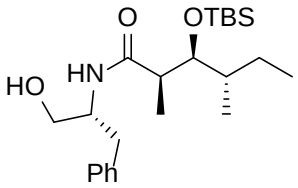
(*R*)-4-benzyl-3-((2R,3S,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexanoyl)oxazolidin-2-one (**87**). Dissolved **86** (1.00 g, 3.13 mmol) in 50 mL CH_2Cl_2 and 2,6-lutidine (1.46 mL, 12.5 mmol) at 0°C. TBS-OTf was added dropwise and the reaction was allowed to come to ambient temperature. The reaction was quenched with 6 mL H_2O and 2 mL MeOH. After 30 minutes of stirring, the volatiles were removed by rotary evaporation. The residue was extracted 3 times with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and condensed. The residue was purified by flash chromatography on silica (Et_2O/hexanes) to give 1.33 g of clear oil. 98.4% yield.

1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.18 (m, 5H), 4.63 (dtd, *J* = 9.8, 5.0, 3.3 Hz, 1H), 4.17 (d, *J* = 4.8 Hz, 2H), 4.01 – 3.91 (m, 2H), 3.27 (dd, *J* = 13.4, 3.3 Hz, 1H), 2.76 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.53 – 1.41 (m, 2H), 1.23 (d, *J* = 6.6 Hz, 3H), 1.09 – 0.96 (m, 1H), 0.93 (t, *J* = 6.7 Hz, 3H), 0.92 (s, 9H), 0.87 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H).



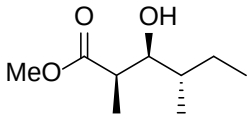
methyl (2R,3S,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexanoate (**88**). From **87** (as minor product): See compound **89**. From **90**: In the same method as compound **87**, the β-alcohol **90** (0.439 g, 2.52 mmol) was protected using TBS-OTf. Purification by flash chromatography on silica (Et_2O/hexanes) gave 0.657 g of clear, colorless oil. 90.4% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (t, *J* = 4.9 Hz, 1H), 3.65 (s, 3H), 2.61 (p, *J* = 6.9 Hz, 1H), 1.55 – 1.40 (m, 1H), 1.14 (d, *J* = 7.0 Hz, 3H), 1.11 – 0.95 (m, 1H), 0.88 (s, 9H), 0.87 (*overlapped*, d, 3H), 0.86 (*overlapped*, t, 3H), 0.05 (s, 3H), 0.01 (s, 3H).



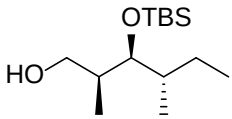
(2R,3S,4S)-3-((tert-butyldimethylsilyl)oxy)-*N*-((*R*)-1-hydroxy-3-phenylpropan-2-yl)-2,4-dimethylhexanamide (**89**). Sodium metal (10 mg, 0.435 mmol) was placed in a round-bottom flask under argon and dissolved in 2.5 mL anhydrous MeOH at 0°C. Dissolved in 5 mL, **87** (171 mg, 0.395 mmol) was added to the methoxide solution and stirred until the starting material was consumed (ca. 1 hour, monitored by TLC). The reaction was quenched with saturated NH_4Cl. The volatiles were removed by rotary evaporation and the residue was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by flash chromatography on silica (EtOAc/hexanes) gave 110 mg of colorless oil. 68.3% yield.

1H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.16 (m, 5H), 6.06 (d, *J* = 7.4 Hz, 1H), 4.24 – 4.02 (m, 2H), 3.76 (t, *J* = 4.7 Hz, 1H), 3.67 (d, *J* = 11.0 Hz, 1H), 3.60 (s, 1H), 2.97 – 2.76 (m, 2H), 2.38 (p, *J* = 6.8 Hz, 1H), 1.55 – 1.42 (m, 2H), 1.07 (d, *J* = 7.0 Hz, 3H), 0.91 – 0.82 (m, 15H), 0.07 (s, 3H), 0.04 (s, 3H).



methyl (2R,3S,4S)-3-hydroxy-2,4-dimethylhexanoate (**90**). In the same method as the previous compound, **86** (2.49 g, 7.80 mmol) was treated with sodium methoxide. Purification by flash chromatography on silica (1:1 Et_2O/hexanes) gave 0.949 g of the ester. 69.9% yield. The mobile phase was changed to 1:1 EtOAc/CH_2Cl_2 to recover the oxazolidinone (1.19 g, 85.8%).

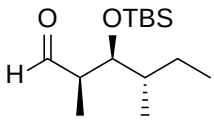
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.67 (dd, *J* = 8.5, 3.2 Hz, 1H), 2.68 (qd, *J* = 7.2, 3.2 Hz, 1H), 1.86 – 1.69 (m, 1H), 1.53 – 1.38 (m, 1H), 1.25 – 1.10 (m, 1H), 1.17 (d, *J* = 7.2 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H).



(2S,3S,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexan-1-ol (**91**). Ester **88** (1.56 g, 5.44 mmol) was dissolved in 50 mL CH_2Cl_2 and cooled to -78°C. DIBAL (1 M solution in hexanes, 11.4 mL) was added dropwise and stirred for 1 hour following complete addition. The reaction was quenched with saturated Rochelle salt and allowed to warm to ambient temperature. The mixture was separated and the aqueous layer extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and condensed. The residue was purified by flash chromatography on silica (Et_2O/hexanes) to give 1.32 g of clear, colorless oil. 93.5% yield.

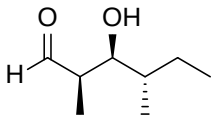
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (dd, *J* = 5.2, 2.5 Hz, 1H), 3.58 (ddd, *J* = 10.3, 7.8, 4.9 Hz, 1H), 3.46 (dt, *J* = 10.3, 5.7 Hz, 1H), 1.95 – 1.81 (m, 1H), 1.74 (t, *J* = 5.4 Hz, 1H), 1.60 – 1.47

(m, 2H), 1.17 – 1.00 (m, 1H), 0.91 (s, 9H), 0.90 (t, *J* = 7.0 Hz, 3H), 0.90 (d, *J* = 9.9 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.07 (s, 3H), 0.06 (s, 3H).

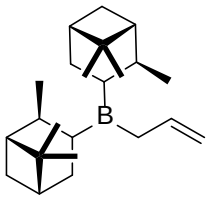


(2R,3S,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexanal (**92**). Alcohol **91** (0.497 g, 1.91 mmol) was oxidized utilizing TEMPO as described in General Procedures. Purification by flash chromatography on silica (Et_2O/hexanes) gave 0.423 g of clear, colorless oil. 85.8% yield.

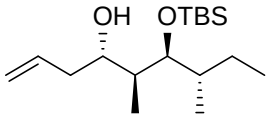
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (d, *J* = 1.1 Hz, 1H), 4.02 (dd, *J* = 5.6, 3.6 Hz, 1H), 2.47 (qdd, *J* = 6.9, 3.6, 1.1 Hz, 1H), 1.57 (dddd, *J* = 10.8, 5.5, 4.0, 2.5 Hz, 1H), 1.46 (dtt, *J* = 14.8, 7.4, 4.1 Hz, 1H), 1.11 (d, *J* = 7.0 Hz, 3H), 1.16 – 1.02 (m, 1H), 0.94 – 0.86 (m, 15H), 0.07 (s, 3H), 0.01 (s, 3H).



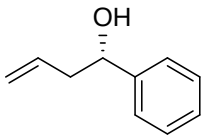
(2R,3S,4S)-3-hydroxy-2,4-dimethylhexanal (**93**). Imide **86** (500 mg, 1.57 mmol) was dissolved in 10 mL anhydrous THF and cooled to -78°C. Red-Al (65% w/w in toluene, 0.95 mL, 3.13 mmol) was added dropwise and stirred for 15 minutes. The reaction was raised to -40°C for 1 hour. The reaction was quenched with 5 mL of 4:1 EtOAc/MeOH at -40°C. The mixture was poured into 0.25M HCl and Et_2O and swirled. The aqueous layer was frozen using the remaining dry ice bath and the organic layer was decanted off. The solvent was evaporated and the residue was stirred in 20 mL of 4:1 MeOH/1 M K_2CO_3 for 15 minutes. The mixture was extracted 3 times with Et_2O. the combined organic layers were washed with brine, dried over Na_2SO_4, and condensed. The isolated material after flash chromatography was an unidentified complex mixture.



(-)-Allyldiisopinocampheylborane (**94**). All steps were performed under argon. Borane DMS complex (10.0-10.2 M, 1 equiv.) was cooled to 0°C with 4 times the volume of THF. To the solution, (+)-α-pinene (88% e.e., 2 equivalents) was added and stirred until the mixture becomes a white slurry. The solvent and DMS was removed *in vacuo* and replaced with the same volume of THF and 0.3 equiv. of (+)-α-pinene. The mixture was equilibrated in a freezer for 3 days. The solvent was removed *in vacuo* and replaced with Et_2O. Methanol was added slowly at 0°C to give the B-methoxydiisopinocampheyl borane. Excess methanol and solvent were removed *in vacuo* and the residue was re-dissolved in Et_2O to give a 0.5 M solution and cooled to -78°C. Allylmagnesium bromide (1.1 equiv.) was added, then stirred at ambient temperature for 2 hours. The resulting solution was used directly with the appropriate aldehyde.

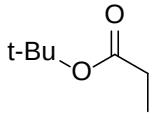


(4S,5S,6S,7S)-6-((tert-butyldimethylsilyl)oxy)-5,7-dimethylnon-1-en-4-ol (**95**). Allylborane **94** (0.387 mmol) was diluted in 5 mL Et_2O and cooled to -78°C. Aldehyde **92** in 1 mL Et_2O was added and the reaction was stirred for one hour, then allowed to warm to ambient temperature and stirred for an additional hour. The borane complex was hydrolyzed with $NaOH/H_2O_2$.



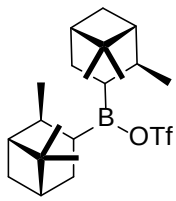
(*S*)-1-phenylbut-3-en-1-ol (**96**). Allyl-DIPC **94** (10 mmol) was dissolved in Et_2O (20 mL) and cooled to -78°C. Benzaldehyde (0.99 mmol) dissolved in Et_2O was added dropwise. The reaction was allowed to warm to ambient temperature and stirred for an additional hour. The borane complex was hydrolyzed with 3 M NaOH (5 mL) and 30% H_2O_2 (10 mL) for one hour.

The layers were separated and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with H_2O, brine, dried over Na_2SO_4, and condensed. The residue was purified by flash chromatography on silica gel (Et_2O/hexanes) to give 681 mg of colorless oil. 46.0% yield.



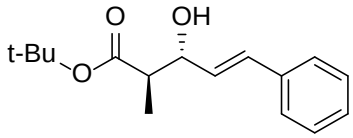
tert-Butyl propionate (**97**).[78] In an oven-dried flask equipped with a reflux condenser, 15.0 mL of tert-butanol and *N*,*N*-dimethylaniline (22.1 mL, 174 mmol) were combined with 20 mL Et_2O under argon. Heat was applied to the solution until reflux. Propionyl chloride (14.4 mL, 165 mmol) was added by syringe at a speed quick enough to maintain reflux. As the addition of acid chloride progresses, the HCl salt of dimethylaniline forms spontaneously. No rapid exotherm evolves with the propionate in contrast to the acetate, however, an ice bath was still applied immediately, before completing the addition of propionyl chloride. After complete addition, the mixture was stirred at 30°C for 1 hour. The organic layer was washed repeatedly with 1M H_2SO_4 in 5 mL portions until the washes no longer became opaque when made basic. The organic layer was then washed with saturated sodium bicarbonate, brine and dried over Na_2SO_4. The solution was distilled fractionally to give 10.8 mL of clear, colorless oil.

1H NMR (400 MHz, $CDCl_3$) δ 2.23 (q, *J* = 7.6 Hz, 2H), 1.45 (s, 9H), 1.09 (t, *J* = 7.6 Hz, 3H).



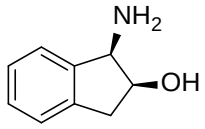
(-)-Diisopinocamphylboron triflate (**98**). All steps were performed under argon. Borane DMS complex (10.0-10.2 M, 1 equiv.) was cooled to 0°C with 4 times the volume of THF. To the solution, (+)-α-pinene (88% *ee*, 2 equivalents) was added and stirred until the mixture becomes

a white slurry. The solvent and DMS was removed *in vacuo* and replaced with the same volume of THF and 0.3 equiv. of (+)-α-pinene. The mixture was equilibrated in a freezer for 3 days. The solvent was removed *in vacuo* and replaced with CH_2Cl_2. The solution was cooled to -78°C and triflic acid was slowly added. The reaction was allowed to reach ambient temperature and stirred for an additional hour. The resulting triflate was used directly.

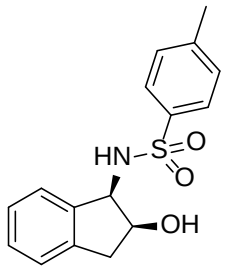


tert-butyl (2R,3R,E)-3-hydroxy-2-methyl-5-phenylpent-4-enoate (**99**). Boron triflate **98** (3.13 mmol) was cooled to -78°C. To the solution, ester **97** (2.4 mmol) and DIEA (5.3 mmol) were added and stirred for 3 hours. Cinnamaldehyde (2.4 mmol) was added and the reaction was maintained at -78°C for 8 hours. The reaction was quenched with water and the boron complex hydrolyzed with NaOH and 30% H_2O_2.

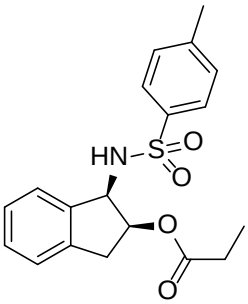
1H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.19 (m, 5H), 6.64 (dd, *J* = 15.9, 1.2 Hz, 1H), 6.20 (dd, *J* = 15.9, 6.6 Hz, 1H), 4.33 (tdd, *J* = 6.8, 5.7, 1.3 Hz, 1H), 2.90 (d, *J* = 5.8 Hz, 1H), 2.56 (p, *J* = 7.2 Hz, 1H), 1.47 (s, 9H), 1.21 (d, *J* = 7.2 Hz, 3H).



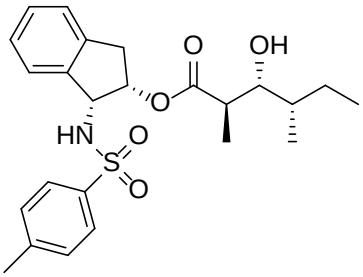
(1R,2S)-1-amino-2,3-dihydro-1H-inden-2-ol (**100**). Obtained from commercial sources.



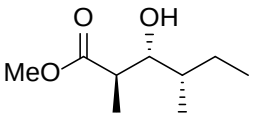
N-((1R,2S)-2-hydroxy-2,3-dihydro-1H-inden-1-yl)-4-methylbenzenesulfonamide (**101**). See reference.[79]



(1R,2S)-1-((4-methylphenyl)sulfonamido)-2,3-dihydro-1H-inden-2-yl propionate (**102**). See reference.[79]



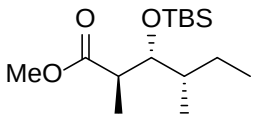
(1R,2S)-1-((4-methylphenyl)sulfonamido)-2,3-dihydro-1H-inden-2-yl (2R,3R,4S)-3-hydroxy-2,4-dimethylhexanoate (**103**). See reference.[79]



methyl (2R,3R,4S)-3-hydroxy-2,4-dimethylhexanoate (**104**). Dissolved **103** (2.00 g, 4.49 mmol) in 40 mL MeOH at 0 °C. A solution of sodium methoxide in methanol (0.500 g, 22.442 mmol in 20 mL MeOH) was added dropwise and the reaction was stirred for 16 hours. The reaction mixture was quenched with 50 mL sat. NH_4Cl and diluted with 50 mL H_2O. The mixture was extracted with 5 × 15 mL CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, concentrated, and purified by flash chromatography on silica gel (Et_2O/pentanes) giving 0.506 g of a colorless oil. 66% yield.

1H NMR (500 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.69 – 3.61 (m, 1H), 2.58 – 2.50 (m, 1H), 2.48 (d, J = 6.8 Hz, 1H), 1.54 – 1.41 (m, 2H), 1.37 – 1.29 (m, 1H), 1.21 (d, J = 7.2 Hz, 3H), 1.18 (d, J = 7.2 Hz, 1H), 0.91 (t, J = 7.1 Hz, 3H)

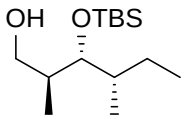
^{13}C NMR (101 MHz, $CDCl_3$) δ 176.55, 73.48, 51.77, 45.36, 34.55, 27.81, 22.74, 14.39, 14.10.



methyl (2R,3R,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexanoate (**105**). The title compound was prepared by the general procedure for silyl ether protection of hydroxyl **104** (0.450 g, 2.612 mmol) with TBSCl/imidazole. Purification by flash chromatography on silica gel (EtOAc/hexanes) gave 0.425 g of clear oil. 70% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 – 3.93 (m, 1H), 3.66 (t, *J* = 1.2 Hz, 3H), 2.69 – 2.60 (m, 1H), 1.52 – 1.38 (m, 1H), 1.32 – 1.27 (m, 2H), 1.08 (dd, *J* = 7.1, 0.9 Hz, 3H), 0.93 – 0.88 (m, 3H), 0.87 (m, 12H), 0.04 (m, 6H)

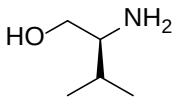
^{13}C NMR (101 MHz, $CDCl_3$) δ 175.55, 73.55, 51.53, 45.53, 32.97, 25.93, 23.00, 18.16, 14.17, 12.10, -4.28, -4.74.



(2S,3R,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexan-1-ol (**106**). The previous compound (0.370 g, 1.28 mmol) was dissolved in 30 mL dry CH_2Cl_2 and cooled to 0°C. DIBAL-H (2.56 mL, 2.56 mmol) was added dropwise. The reaction was monitored by TLC. After 14 hours the reaction was quenched with 30 mL saturated sodium potassium tartrate, stirred for 1 hr and then diluted with 20 mL H_2O. The mixture was extracted with 4 × 30 mL CH_2Cl_2, and the combined organic layers were dried over $MgSO_4$. The organic layer was filtered, concentrated and purified by flash chromatography on silica gel (EtOAc/hexanes) to afford 0.227 g of pale yellow oil. 90% yield

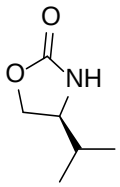
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 – 4.05 (m, 1H), 2.04 (d, J =2.6 Hz, 2H), 1.55 (s, 1H), 1.40 (m, 3H), 1.25 (m, 1H), 0.91 (m, 18H), 0.08 – 0.01 (m, 6H).

3.4.3 Vinylagous Mukaiyama



L-Valinol (**107**). In a 1 L round-bottom flask charged with 300 mL of THF and cooled to 0°C, LAH (25.9 g, 68.3 mmol) was added. L-Valine (40.0 g, 34.1 mmol) was added in portions with stirring. After complete addition, the mixture was refluxed for 20 hours. The mixture was then diluted with an additional 400 mL of THF and quenched at 0°C with 40 mL of water. The salts were filtered off and resuspended in 200 mL 4:1 THF/H_2O and stirred for 30 min. The salts were filtered off and the process was repeated. The filtrate was combined and condensed. The residue was dried azeotropically by refluxing with a Dean-Stark in benzene. The product was purified by vacuum distillation to give 27.9 g of clear, colorless oil that crystallized upon standing. 79.3% yield. Caution: A Fieser workup leaves a significant amount of product on the salts; the use of magnesium salts for drying results in significant loss of product due to chelation of the product to magnesium.

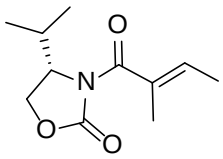
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (dd, *J* = 10.6, 3.9 Hz, 1H), 3.30 (dd, *J* = 10.5, 8.6 Hz, 1H), 2.56 (ddd, *J* = 8.6, 6.3, 3.9 Hz, 1H), 1.59 (dq, *J* = 13.4, 6.7 Hz, 1H), 0.92 (dd, *J* = 6.8, 4.1 Hz, 6H).



(*S*)-4-isopropyloxazolidin-2-one (**108**). L-Valinol **107** (12.1 g, 117.3 mmol), diethyl carbonate (27.7 mL, 234.6 mmol), and K_2CO_3 (3.24 g, 23.5 mmol) were combined in a round-bottom flask and equipped with a distillation head. The mixture was stirred with heating (oil bath temp 120°C) until no more ethanol distills over (ca. 3 hours). The pot residue was washed into a separatory funnel with CH_2Cl_2 and sufficient water to dissolve the inorganic salts. The organic

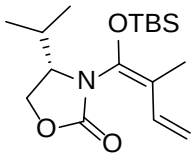
layer was washed with 25 mL of saturated $NaHCO_3$ two times, then dried over $MgSO_4$ and condensed. The residue formed pale yellow crystals upon standing in the freezer. The crystals were washed with cold hexanes. The product was purified by dissolving in minimal CH_2Cl_2 and diluting with 200 mL of boiling hexanes. The solution was boiled until CH_2Cl_2 was driven off and allowed to cool slowly to give 11.72 g of colorless crystals. 77.4% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.51 (s, 1H), 4.45 (t, *J* = 8.6 Hz, 1H), 4.11 (dd, *J* = 8.8, 6.2 Hz, 1H), 3.60 (dddd, *J* = 8.3, 7.1, 6.2, 1.1 Hz, 1H), 1.74 (h, *J* = 6.8 Hz, 1H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H).



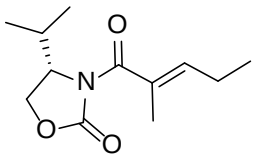
(S,E)-4-isopropyl-3-(2-methylbut-2-enoyl)oxazolidin-2-one (**109**). Oxazolidinone **108** (1.00 g, 7.74 mmol) was dissolved in THF and cooled to -78°C. n-Butyl lithium (3.87 mL, 8.51 mmol) was added slowly and stirred for 30 minutes. Freshly distilled (E)-but-2-enoyl chloride (0.918 g, 7.74 mmol) was added dropwise and after 20 minutes, the temperature was raised to 0°C and stirred for 30 additional minutes. The reaction was then quenched with 20 mL of 20% Na_2CO_3. 60 mL of water was added and the aqueous layer was extracted 4 times with 50 mL MTBE. The organic layers were combined, washed twice with brine, dried over $NaSO_4$, and condensed. The residue was purified by flash chromatography on silica (EtOAc/hexanes) to give 1.03 g of white solid. 62.7% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.20 (qq, *J* = 6.9, 1.4 Hz, 1H), 4.52 (ddd, *J* = 8.7, 5.5, 4.4 Hz, 1H), 4.31 (t, *J* = 8.9 Hz, 1H), 4.17 (dd, *J* = 9.0, 5.5 Hz, 1H), 2.36 (pd, *J* = 6.9, 4.4 Hz, 1H), 1.90 (t, *J* = 1.3 Hz, 3H), 1.80 (dd, *J* = 6.9, 1.2 Hz, 3H), 0.91 (dd, *J* = 7.0, 6.1 Hz, 6H).



(S,E)-3-(1-((*tert*-butyldimethylsilyl)oxy)-2-methylbuta-1,3-dien-1-yl)-4-isopropyloxazolidin-2-one (**110**). Imide **109** (0.500 g, 2.37 mmol) was dissolved in THF and cooled to -78°C. KHMDS (25 mL, 12.5 mmol) was added by syringe and the reaction was stirred for 90 minutes. TBS-Cl dissolved in 5 mL THF was added by syringe and stirred for 30 minutes. The reaction was quenched with saturated NH_4Cl and water and diluted with 20 mL EtOAc. The layers were separated and the aqueous layer was extracted two more times with 10 mL EtOAc. The combined organic layers were washed with twice with brine and dried over Na_2SO_4. The condensed residue was purified by flash chromatography (EtOAc/hexanes) to give 1.03 g of colorless oil containing silyl ether impurity. 98.5% yield (calcd by NMR internal standard).

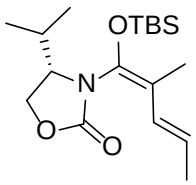
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.54 (t, *J* = 14.6 Hz, 1H), 5.14 (dd, *J* = 17.3, 1.3 Hz, 1H), 5.03 (dd, *J* = 10.9, 1.3 Hz, 1H), 4.32 (t, *J* = 8.8 Hz, 1H), 4.11 (t, *J* = 9.2 Hz, 1H), 4.01 (s, 1H), 1.95 (ddd, *J* = 14.1, 7.1, 4.8 Hz, 1H), 1.80 (s, 3H), 0.99 (s, 9H), 0.95 – 0.89 (m, 6H), 0.20 (s, 3H), 0.16 (s, 3H).



(S,E)-4-isopropyl-3-(2-methylpent-2-enoyl)oxazolidin-2-one (**111**). Oxazolidinone **108** (7.80 g, 60.4 mmol) was dissolved in 50 mL THF and cooled to -78°C. n-Butyl lithium (26.6 mL, 66.4 mmol) was added slowly and stirred for 30 minutes. Freshly distilled (E)-pent-2-enoyl chloride (8.8 g, 66.4 mmol) was added dropwise and after 20 minutes, the temperature was raised to 0°C and stirred for 30 additional minutes. The reaction was then quenched with 20 mL of 20% Na_2CO_3. 60 mL of water was added and the aqueous layer was extracted 4 times with 50 mL MTBE. The organic layers were combined, washed twice with brine, dried over $NaSO_4$,

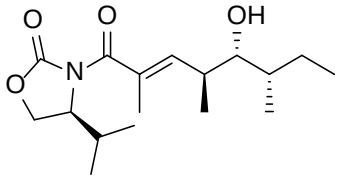
and condensed. The residue was purified by flash chromatography on silica (EtOAc/hexanes) to give 12.0 g of white solid. 87.9% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 – 5.90 (m, 1H), 4.51 (ddd, *J* = 8.8, 5.4, 4.4 Hz, 1H), 4.31 (t, *J* = 8.9 Hz, 1H), 4.17 (dd, *J* = 9.0, 5.4 Hz, 1H), 2.36 (pd, *J* = 7.0, 4.4 Hz, 1H), 2.20 (p, *J* = 7.6 Hz, 2H), 1.90 (q, *J* = 1.0 Hz, 3H), 1.05 (t, *J* = 7.6 Hz, 3H), 0.92 (dd, *J* = 7.0, 5.7 Hz, 6H).



(*S*)-3-((1E,3E)-1-((*tert*-butyldimethylsilyl)oxy)-2-methylpenta-1,3-dien-1-yl)-4-isopropyloxazolidin-2-one (**112**). Imide **111** (2.0 g, 8.88 mmol) was dissolved in 100 mL THF and cooled to -78°C. KHMDS (25 mL, 12.5 mmol) was added by cannula and the reaction was stirred for 90 minutes. TBS-Cl dissolved in 20 mL THF was added by cannula and stirred for 30 minutes. The reaction was quenched with saturated NH_4Cl and water and diluted with 100 mL EtOAc. The layers were separated and the aqueous layer was extracted two more times with 50 mL EtOAc. The combined organic layers were washed with twice with brine and dried over Na_2SO_4. The condesned residue was purified by flash chromatography (EtOAc/hexanes) to give 2.97 g of colorless oil. 98.5% yield.

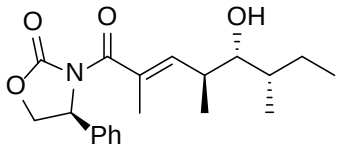
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (d, *J* = 15.6 Hz, 1H), 5.69 – 5.51 (m, 1H), 4.29 (t, *J* = 8.6 Hz, 1H), 4.09 (t, *J* = 8.5 Hz, 1H), 3.97 (s, 1H), 1.92 (q, *J* = 10.2, 6.6 Hz, 1H), 1.81 – 1.71 (m, 6H), 0.95 (t, *J* = 0.9 Hz, 9H), 0.89 (hidden, dd, 6H), 0.17 (s, 3H), 0.12 (s, 3H).



(*S*)-3-((4S,5R,6S,E)-5-Hydroxy-2,4,6-trimethyloct-2-enoyl)-4-isopropyloxazolidin-2-one (**113**). Aldehyde **85** (1.50 g, 17.5 mmol) was dissolved in 18 mL CH_2Cl_2 and cooled to -78°C.

$TiCl_4$ (0.96 mL, 8.75 mmol) was added to give a bright yellow solution. Ketene acetal **112** (2.97 g, 8.75 mmol) dissolved in 18 mL CH_2Cl_2 was added dropwise, turning the solution reddish-brown. The reaction was stirred for about 40 hours at -78°C at which point all the dry ice had sublimed and the reaction warmed to ambient temperature. The mixture was cooled to -40°C and quenched with 3 mL pyridine and 6 mL saturated Rochelle salt. The mixture was stirred until spontaneously warmed to ambient temperature. The aqueous layer was separated and extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and condensed. Purification by flash chromatography on silica (EtOAc/hexanes) gave 1.84 g of clear oil. 68% yield. 29:10 dr

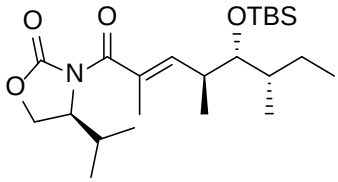
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (dq, *J* = 10.3, 1.5 Hz, 1H), 4.57 (ddd, *J* = 8.9, 5.7, 4.5 Hz, 1H), 4.33 (t, *J* = 9.0 Hz, 1H), 4.18 (dd, *J* = 9.0, 5.8 Hz, 1H), 3.30 (dt, *J* = 8.9, 2.7 Hz, 1H), 3.00 (dd, *J* = 2.9, 1.3 Hz, 1H), 2.73 (ddq, *J* = 10.3, 8.9, 6.6 Hz, 1H), 2.34 (pd, *J* = 7.0, 4.5 Hz, 1H), 1.95 (d, *J* = 1.5 Hz, 3H), 1.59 – 1.43 (m, 2H), 1.38 (dt, *J* = 13.2, 7.3 Hz, 1H), 0.97 – 0.87 (m, 15H).



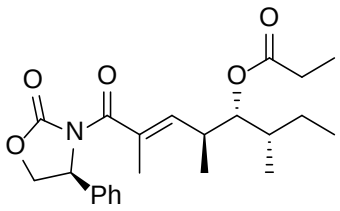
(*S*)-3-((4S,5R,6S,E)-5-hydroxy-2,4,6-trimethyloct-2-enoyl)-4-phenyloxazolidin-2-one (**114**). In each of four separate reaction vessels, aldehyde **85** (6.00 g, 3.87 mmol) was dissolved in CH_2Cl_2 and cooled to -78°C. The ***phenyl analog*** of ketene acetal **112** (1.50 g, 3.87 mmol) dissolved in 90 mL CH_2Cl_2 was added over 30 minutes. After complete addition of ketene acetal, 74 µL of H_2O was added and the reactions were stirred for 68 hours at -78°C. Each reaction was quenched with 5 mL of 1:1 saturated Rochelle salt/bicarbonate and 20 mL of water and allowed to warm to room temperature. All four reactions were combined and the organic layer was separated. The organic layer was condensed and re-dissolved in EtOAc. The aqueous layer was extracted with 100 mL EtOAc. The organic layers were combined and

washed with Rochelle salt until the organic layer was no longer turbid, then washed with brine, and dried over Na_2SO_4. Condensing gave 7.53 g of crude oil that solidified upon standing. The solid was dissolved in minimal Et_2O and an equal volume of hexanes was added. Crystals formed overnight, which were filtered and washed with Et_2O to give 0.945 g of white needles. The filtrate was condensed and purified by flash chromatography on silica (EtOAc/hexanes) to give 3.69 g of crystalline white solid. Total 4.64 g, 83.6% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 – 7.30 (m, 5H), 5.90 (dd, *J* = 10.4, 1.5 Hz, 1H), 5.53 (dd, *J* = 9.1, 7.8 Hz, 1H), 4.73 (t, *J* = 9.0 Hz, 1H), 4.26 (dd, *J* = 9.0, 7.9 Hz, 1H), 3.34 (dt, *J* = 8.9, 2.8 Hz, 1H), 2.96 (dd, *J* = 2.9, 1.3 Hz, 1H), 2.78 – 2.68 (m, 1H), 1.91 (d, *J* = 1.5 Hz, 3H), 1.62 – 1.46 (m, 2H), 1.39 (dt, *J* = 13.2, 7.3 Hz, 1H), 0.97 – 0.89 (m, 9H).



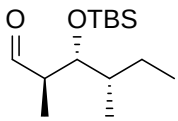
(*S*)-3-((4S,5R,6S,E)-5-((tert-butyldimethylsilyl)oxy)-2,4,6-trimethyloct-2-enoyl)-4-phenyloxazolidin-2-one (**115**). Protected using TBSOTf in the same manner as **87**, giving the desired product in 88% yield. $[\alpha]_D^{20}$ = +21.9^o (c 1.14, CH_2Cl_2); FTIR (neat, cm^{-1}) 2965.9, 2876.5, 1789.8, 1682.6, 1464.0, 1362.2, 1300.6, 1205.6, 1054.9, 772.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 (dd, *J* = 9.8, 1.6 Hz, 1H), 4.47 (dt, *J* = 9.0, 4.7 Hz, 1H), 4.29 (t, *J* = 8.8 Hz, 1H), 4.16 (dd, *J* = 8.9, 5.2 Hz, 1H), 3.49 (t, *J* = 3.9Hz, 1H), 2.70 (ddt, *J* = 10.1, 6.8, 3.5 Hz, 1H), 2.44 – 2.32 (m, 1H), 1.94 – 1.88 (m, 3H), 1.53 – 1.43 (m, 1H), 1.30 – 1.23 (m, R_3CH, 1H), 1.11 – 1.04 (m, RCH_2R, 1H), 1.01 (d, *J* = 7.0 Hz, RCH_3, 3H), 0.94 – 0.82 (m, 21H), 0.06 – 0.05, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.25, 53.60, 141.67, 129.66, 79.67, 63.51, 58.58, 40.02, 36.58, 28.37, 26.26, 26.09, 18.53,18.09, 18.04, 15.27, 15.06, 13.95, 12.30, -3.58, -3.85.



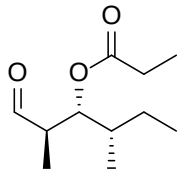
(3S,4R,5S,E)-3,5,7-trimethyl-8-oxo-8-((*S*)-2-oxo-4-phenyloxazolidin-3-yl)oct-6-en-4-yl propionate (**116**). Aldol product **114** (1.50 g, 4.34 mol) was dissolved in 20 mL CH_2Cl_2 and cooled to 0°C. Pyridine (1.4 mL, 17.4 mmol) and DMAP (0.27 g, 2.17 mmol) was added. Propionyl chloride (1.5 mL, 17.4 mmol) was added dropwise. The reaction was allowed to warm to ambient temperature and stirred for 20 hours. The reaction was quenched with water at 0°C. The volatiles were removed by rotary evaporation and the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed 3 times each with 1M HCl, saturated Na_2CO_3, water, and brine. Drying over Na_2SO_4 and condensing gave a crude yellow oil. Purification by flash chromatography on silica (EtOAc/hexanes) gave 1.51 g of white crystalline solid. 86.6% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.28 (m, 5H), 5.98 (dd, *J* = 9.7, 1.5 Hz, 1H), 5.42 (dd, *J* = 8.7, 6.4 Hz, 1H), 4.88 (dd, *J* = 6.8, 5.3 Hz, 1H), 4.69 (t, *J* = 8.8 Hz, 1H), 4.23 (dd, *J* = 8.9, 6.4 Hz, 1H), 2.87 (dp, *J* = 9.8, 6.9 Hz, 1H), 2.33 (qd, *J* = 7.6, 2.7 Hz, 2H), 1.88 (d, *J* = 1.4 Hz, 3H), 1.66 (ddt, *J* = 8.0, 6.7, 5.3 Hz, 1H), 1.35 (dqd, *J* = 12.6, 7.5, 5.2 Hz, 1H), 1.21 – 1.14 (m, 1H), 1.12 (t, *J* = 7.6 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.48, 171.07, 153.24, 140.30, 138.23, 131.19, 129.22, 129.08, 128.83, 126.23, 78.79, 77.41, 77.16, 76.91, 69.96, 58.30, 36.14, 34.90, 27.77, 26.32, 16.41, 13.65, 13.42, 11.48, 9.35.

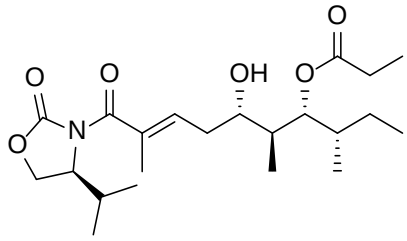


(2R,3R,4S)-3-((tert-butyldimethylsilyl)oxy)-2,4-dimethylhexanal. (**117**). See reference.[79]

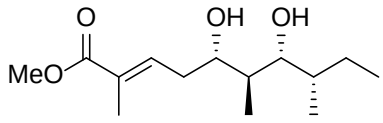


(2R,3R,4S)-2,4-dimethyl-1-oxohexan-3-yl propionate (**118**). α,β-Unsaturated imide **114** (0.500 g, 1.45 mmol) and pyridine (0.35 mL, 4.34 mmol) were dissolved in 25 mL CH_2Cl_2 and cooled to -78°C. An ozone generator was used to bubble ozone through the solution until it became powder blue. The solution was sparged with air until all color dissipated. The mixture was diluted with CH_2Cl_2 and the organic layer was washed with 10% citric acid, saturated $NaHCO_3$, brine, and then dried over Na_2SO_4. Condensing and purification by flash chromatography (CH_2Cl_2/Et_2O) gave 0.251 g of desired product. 86.6% yield.

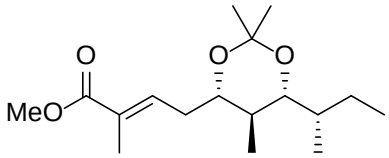
1H NMR (500 MHz, $CDCl_3$) δ 9.60 (d, *J* = 3.3 Hz, 1H), 5.13 (dd, *J* = 7.6, 4.5 Hz, 1H), 2.65 (pd, *J* = 7.2, 3.3 Hz, 1H), 2.33 (q, *J* = 7.5 Hz, 2H), 1.74 – 1.65 (m, 1H), 1.45 – 1.32 (m, 1H), 1.24 – 1.15 (m, 1H), 1.13 (t, *J* = 7.6 Hz, 3H), 1.08 (d, *J* = 7.1 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H).



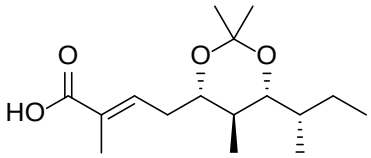
(3*S*,4*R*,5*S*,6*S*,*E*)-6-hydroxy-3,5,9-trimethyl-10-oxo-10-((*S*)-2-oxo-4-phenyloxazolidin-3-yl)dec-8-en-4-yl propionate (**119**). See reference.[80]



methyl (5S,6S,7R,8S,E)-5,7-dihydroxy-2,6,8-trimethyldec-2-enoate (**120**). See reference.[80]

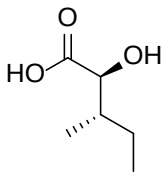


methyl (E)-4-((4S,5S,6R)-6-((*S*)-sec-butyl)-2,2,5-trimethyl-1,3-dioxan-4-yl)-2-methylbut-2-enoate (**121**). See reference.[80]



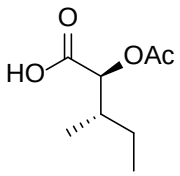
(E)-4-((4S,5S,6R)-6-((*S*)-sec-butyl)-2,2,5-trimethyl-1,3-dioxan-4-yl)-2-methylbut-2-enoic acid (**122**). See reference.[80]

3.4.4 Isoleucic Acid Fragment

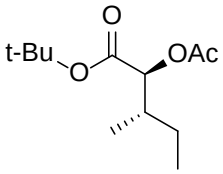


(2S,3S)-2-hydroxy-3-methylpentanoic acid (**123**). L-Isoleucine (20.0 g, 0.15 mol) was dissolved in 200 mL of 2M H_2SO_4 at 0°C. Sodium nitrite (42.1 g, 0.61 mol), dissolved in 300 mL water, was added dropwise by addition funnel over an hour. The solution was stirred for 18 hours at ambient temperature. The solution was then saturated with sodium chloride and extracted with three portions of 150 mL EtOAc. The combined organic layers were dried over sodium sulfate and condensed to give 17.6 g of yellow oil that crystallized on standing after two days. 88% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (d, *J* = 3.7 Hz, 1H), 1.97 – 1.82 (m, 1H), 1.44 (dqd, *J* = 13.4, 7.5, 4.6 Hz, 1H), 1.38 – 1.22 (m, 1H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).

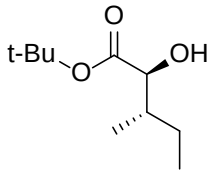


(2S,3S)-2-acetoxy-3-methylpentanoic acid (**124**). Acetyl chloride (23 mL) was slowly added to hydroxyisoleucic acid **123** (10.7 g, 81 mmol) under argon with a bubbler charged with saturated aqueous sodium carbonate. The solution was refluxed until HCl gas no longer evolved (ca. 1 hour), then stirred at ambient temperature for 18 hours. The reaction was diluted in CH_2Cl_2. With vigorous stirring, water was slowly added and the mixture was stirred for two hours. The organic layer was then separated and washed with water, brine, followed by drying over sodium sulfate. Condensing gave 12.7 g of oil, which gave a single spot by TLC. The product was used without further purification. 90.0% yield.



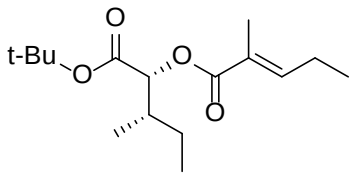
tert-butyl (2S,3S)-2-acetoxy-3-methylpentanoate (**125**). The previous crude **124** (5.00 g, 28.7 mmol) was dissolved in 75 mL of anhydrous *tert*-butyl alcohol under argon. BOC anhydride (9.4 g, 43 mmol) and DMAP (0.88 g, 7.2 mmol) was added and the reaction was stirred for 14 hours. The *tert*-butyl alcohol was removed by rotary evaporation and the residue dissolved in 100 mL of diethyl ether. The organic layer was washed with two 100 mL portions of water, and two 100 mL portions of brine, and dried of sodium sulfate. The organic layer was condensed and the residue was purified by flash chromatography to give 4.44 g of clear, colorless oil in 88.8% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.77 (d, *J* = 4.6 Hz, 1H), 2.12 (s, 3H), 1.93 (dqt, *J* = 9.2, 6.9, 4.6 Hz, 1H), 1.59 – 1.41 (m, 1H), 1.47 (s, 9H), 1.37 – 1.23 (m, 1H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.93 (t, *J* = 7.5 Hz, 3H).



tert-butyl (2S,3S)-2-hydroxy-3-methylpentanoate (**126**). In 50 mL MeOH, 72 mL water, and K_2CO_3 (18.0 g, 130 mmol), **125** (10.0 g, 43.4 mmol) were dissolved and stirred vigorously. The reaction was monitored by TLC and after 2 days, the mixture was extracted with three 100 mL portions of CH_2Cl_2. The combined organic layers were dried over sodium sulfate and condensed. The residue was distilled *in vacuo* to give 8.0 g of clear oil. A small degree of epimerization at the α carbon was observed, giving the desired product in 98% yield and 93:7 dr.

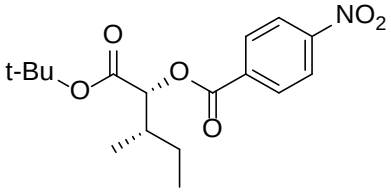
^{1}H NMR (500 MHz, $CDCl_3$) δ 3.96 (dd, *J* = 5.8, 3.5 Hz, 1H), 2.83 (d, *J* = 5.8 Hz, 1H), 1.83 – 1.72 (m, 1H), 1.49 (s, 9H), 1.42 – 1.32 (m, 1H), 1.31 – 1.19 (m, 1H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H).



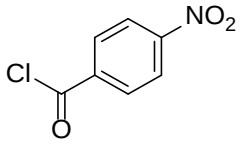
(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl (E)-2-methylpent-2-enoate (**127**). **By Mitsunobu:** (E)-2-methylpent-2-enoic acid (0.909 g, 7.97 mmol) was dried by azeoptropic evaporation of dioxane, then combined with ester **126** (0.500 g, 2.66 mmol), and PPh_3 (1.18 g, 4.51 mmol) at -20°C. DIAD was added and stirred for 1 hour. The reaction was allowed to warm to ambient temperature and stirred for 20 hours. The solution was condensed without workup and purified by flash chromatography on silica to give 237 mg of colorless oil. 31% yield. **From 134:** (E)-2-methylpent-2-enoic acid (0.500 g, 4.38 mmol), **134** (1.65 g, 6.57 mmol), and $CsCO_3$ (2.14 g, 6.57 mmol) were combined and stirred in 20 mL of anhydrous DMF. Molecular sieves (4Å, 20-50 mesh) was added and the reaction mixture was stirred for 24 hours at ambient temperature. The reaction was quenched with 1 M HCl and extracted with 3 × 100 mL Et_2O. The

combined organic layers were washed with saturated $NaHCO_3$, water, and brine. Dried over Na_2SO_4, condensed, and purified by flash chromatography on silica gel (Et_2O/hexanes) to give 162 mg of colorless oil.

^1H NMR (400 MHz, $CDCl_3$) δ 6.82 (tq, *J* = 7.4, 1.5 Hz, 1H), 4.95 (d, *J* = 3.4 Hz, 1H), 2.27 – 2.14 (m, 2H), 2.00 (dtd, *J* = 7.7, 6.7, 3.4 Hz, 1H), 1.86 (q, *J* = 1.0 Hz, 3H), 1.47 (s, 9H), 1.53 – 1.39 (m, 1H), 1.39 – 1.24 (m, 1H), 1.06 (t, *J* = 7.6 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.94 (t, *J* = 7.4 Hz, 3H).



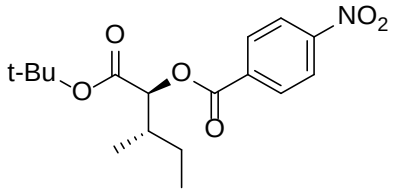
(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl 4-nitrobenzoate (**128**). 4-Nitrobenzoic acid (2.26 g, 13.5 mmol), **126** (1.50 g, 7.97 mmol), and PPh_3 (3.55 g, 13.5 mmol) were stirred in 25 mL of THF and cooled to 0°C on an ice bath. DIAD (2.67 mL, 13.5 mmol) was added dropwise, keeping the reaction temperature below 10°C. After the complete addition of DIAD, the ice bath was removed and the reaction was stirred for 20 hours. The reaction was then quenched with water. The THF was replaced with EtOAc and the organic layer was washed twice with saturated Na_2CO_3, and three times with water and brine. The organic layer was dried over Na_2SO_4, condensed, and the residue was purified by flash chromatography on silica to give 2.20 g of bright yellow oil. 81.8% yield.



4-Nitrobenzoyl chloride (**129**). 4-Nitrobenzoic acid (5.00 g, 29.9 mmol) was suspended in 20 mL THF and $SOCl_2$ (4.3 mL, 59.8 mmol) was added with 2 drops of catalytic DMF. The suspension was refluxed for one hours, after which the mixture became homogenous. Refluxing was continued for 4 hours. The solvent and $SOCl_2$ were removed by rotary evaporation to give a

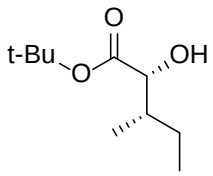
yellow oil. The oil was distilled *in vacuo* with a large condenser, as the oil immediately crystallizes when cooled. The crystals were washed off with CH_2Cl_2, condensed, and recrystallized in CCl_4.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 – 8.34 (m, 2H), 8.34 – 8.28 (m, 2H).



(2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl 4-nitrobenzoate (**130**). 4-Nitrobenzoyl chloride (542 mg, 2.92 mmol) was dissolved in THF and cooled to 0°C. DIPEA (0.93 mL, 5.31 mmol) was added, followed by **126** (542 mg, 2.92 mmol). The reaction was stirred at ambient temperature and monitored by TLC. After 16 hours, the reaction was still incomplete. A reflux condenser was attached and another 0.25 equivalents of acid chloride was added. The reaction was refluxed for 12 hours then quenched with 30 mL H_2O. The mixture was extracted three times with 75 mL CH_2Cl_2. The combined organic layers were dried over Na_2SO_4. Condensation by rotary evaporation gave 1.12 g of orange oil. Purified by flash chromatography on silica (EtOAc/hexanes) did not give complete separation of impurities. Isolated 275 mg of pure product and 317 mg of mixed material. 55% yield.

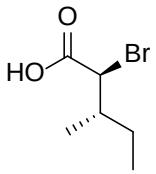
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 – 8.27 (m, 2H), 8.26 – 8.22 (m, 2H), 5.07 (d, *J* = 4.3 Hz, 1H), 2.13 (dddt, *J* = 11.4, 6.8, 4.5, 2.2 Hz, 1H), 1.63 (dqd, *J* = 13.5, 7.5, 4.7 Hz, 1H), 1.49 (d, *J* = 5.9 Hz, 9H), 1.46 – 1.33 (m, 1H), 1.08 (d, *J* = 6.9 Hz, 3H), 0.99 (q, *J* = 7.2 Hz, 3H).



tert-butyl (2R,3S)-2-hydroxy-3-methylpentanoate (**131**). Ester **128** (1.00 g, 2.96 mmol) was stirred in 15 mL of MeOH and cooled to 0°C. K_2CO_3 (0.819 g, 5.93 mmol) was added and

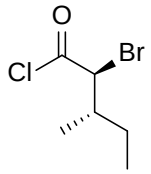
the reaction was monitored by TLC. After 1 hour, the MeOH was removed and replaced with EtOAc. The organic layer was washed with water and brine. Purification by flash chromatography on silica using EtOAc or Et_2O with hexanes failed to provide clean separation. Purification was repeated using 10-80% CH_2Cl_2/toluene affording 294 mg of pure product. 52.7% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 – 4.01 (m, 1H), 2.78 (d, *J* = 5.1 Hz, 1H), 1.77 (dpd, *J* = 7.9, 6.8, 2.8 Hz, 1H), 1.61 – 1.45 (m, 1H), 1.50 (s, 9H), 1.30 (dp, *J* = 13.5, 7.5 Hz, 1H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.81 (d, *J* = 6.8 Hz, 3H).



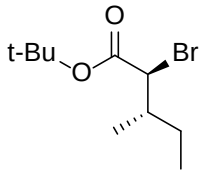
(2S,3S)-2-bromo-3-methylpentanoic acid (**132**).[81] L-Isoleucine (5.0 g, 38.1 mmol) and KBr (15.42 g, 130 mmol) were suspended in 31 mL of water. HBr (48%, 9.4 mL, 83.1 mmol) was added and the solution was cooled to -13°C. Argon was bubbled through the solution and $NaNO_2$ (3.26 g, 47.3 mmol) was added in portions every 5 minutes over 2.5 hours. The reaction was warmed to 0°C, the gas purge was stopped and the reaction was stirred for 6 hours. The solution was purged once more with argon gas, then extracted extensively with Et_2O. The combined organic layers were dried over $MgSO_4$ and condensed. The resulting solid was purified by flash chromatography (EtOAc/hexanes) to give 4.62 g of a burnished orange solid. 62.1% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.41 (s, 1H), 4.14 (d, *J* = 8.0 Hz, 1H), 2.05 (qd, *J* = 8.0, 6.7, 3.6 Hz, 1H), 1.76 (dqd, *J* = 14.9, 7.5, 3.7 Hz, 1H), 1.34 (ddt, *J* = 16.0, 14.5, 7.4 Hz, 1H), 1.07 (d, *J* = 6.7 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H).



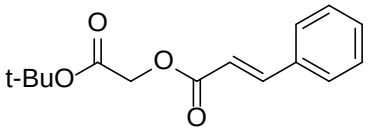
(2S,3S)-2-bromo-3-methylpentanoyl chloride (**133**). α-Bromocarboxylic acid **132** (4.62 g, 23.7 mmol) was dissolved in 30 mL CH_2Cl_2 at 0°C. An excess of $SOCl_2$ (20 mL, 280 mmol) was added. The solution was warmed to ambient temperature. The reaction vessel was affixed with a reflux condenser and a bubbler containing 10% NaOH. After refluxing for 20 hours, the excess $SOCl_2$ was removed by rotary evaporation, giving 4.26 g of residue. The product was of good purity and used without purification. 84.2% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.40 (d, *J* = 7.7 Hz, 1H), 2.15 (tqd, *J* = 8.8, 7.7, 7.2, 6.7, 3.5 Hz, 1H), 1.73 (dqd, *J* = 13.6, 7.5, 3.5 Hz, 1H), 1.35 (ddq, *J* = 13.6, 8.8, 7.4 Hz, 1H), 1.11 (d, *J* = 6.7 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H).



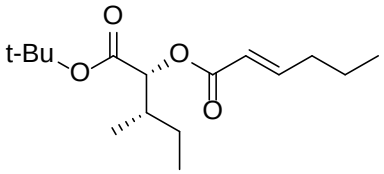
tert-butyl (2S,3S)-2-bromo-3-methylpentanoate (**134**). Acid chloride **133** (6.63 g, 31.0 mmol) was stirred in 90 mL CH_2Cl_2 and cooled to -40°C. tert-Butanol (6.90 g, 93.1 mmol) was added, followed by dropwise addition of pyridine (2.58 mL, 32.6 mmol). After 5 hours of stirring at ambient temperature, the reaction was quenched with water. The organic layer was washed with 1 M HCl, saturated Na_2SO_4, and brine. The organic layer was dried over Na_2SO_4, condensed, and then distilled *in vacuo* to give 5.19 g of oil. 66.6% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99 (d, *J* = 8.4 Hz, 1H), 2.06 – 1.94 (m, 1H), 1.79 – 1.68 (m, 1H), 1.49 (s, 9H), 1.38 – 1.23 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H).

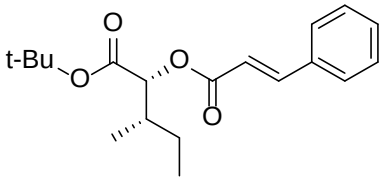


2-(tert-butoxy)-2-oxoethyl cinnamate (**135**). Cinnamic acid (1.00 g, 6.75 mmol) was dissolved in34 mL of anhydrous DMF. $CsCO_3$ (3.30 g, 10.1 mmol) was added, followed by *tert*-butyl bromoacetate (1.50 mL, 10.1 mmol). The mixture was stirred overnight at ambient temperature. The reaction was diluted with 100 mL Et_2O. The organic layer was washed with 3×100 mL water, then brine, and dried over Na_2SO_4. The organic layer was condensed to give an oil that crystallized upon standing. The solid was tritirated and recrystallized in hot hexanes to give 1.53 g of colorless, cubic crystals. 55.9% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 16.0 Hz, 1H), 7.57 – 7.51 (m, 2H), 7.39 (tdd, *J* = 4.2, 3.1, 2.2 Hz, 3H), 6.53 (d, *J* = 16.0 Hz, 1H), 4.64 (s, 2H), 1.50 (s, 9H).



(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl (E)-hex-2-enoate (**136**). (E)-hex-2-enoic acid (140 mg, 1.27 mmol), **126** (463 mg, 1.84 mmol), and Cs_2CO_3 (600 mg, 1.84 mmol) were combined in a reaction vessel with 10 mL DMF. The suspension was reacted in a Biotage Initiator microwave reactor at 160°C for 2 minutes. Purification by flash chromatography on silica gave 37 mg of desired product in 7.4% yield.

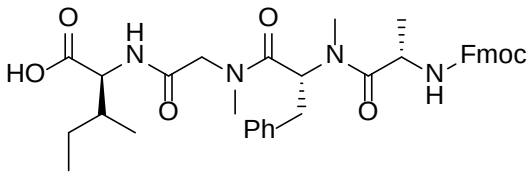


tert-butyl (2R,3S)-2-(cinnamoyloxy)-3-methylpentanoate (**137**). (E)-cinnamic acid (.500 g, 3.38 mmol) was dissolved in 7 mL of anhydrous DMF at ambient temperature. Anhydrous

Cs_2CO_3 (1.65 g, 5.05 mmol) was added in one portion, followed by **126** (1.27 g, 5.05 mmol). The mixture was stirred for 3 days at ambient temperature. The reaction was diluted with 70 mL Et_2O and washed with 4×30 mL water, brine, and dried over Na_2SO_4. The organic layer was condensed to give 1.19 g of colorless oil, consisting of the α-bromide and title compound. Estimated 0.64 g product by NMR; 47% yield.

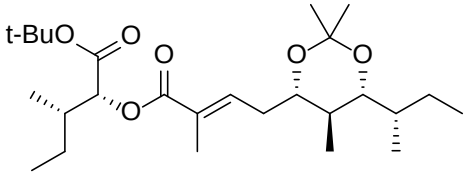
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 16.0 Hz, 1H), 7.58 – 7.48 (m, 2H), 7.44 – 7.33 (m, 3H), 6.52 (dd, *J* = 16.0, 0.5 Hz, 1H), 5.05 (d, *J* = 3.5 Hz, 1H), 2.01 (dddd, *J* = 17.0, 8.6, 6.7, 3.6 Hz, 1H), 1.51 (s, 1H), 1.48 (s, 9H), 1.39 – 1.29 (m, 1H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.96 (t, *J* = 7.5, 7.5 Hz, 3H).

3.4.5 Peptide Fragment

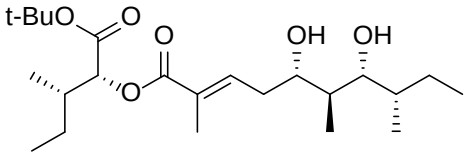


N-(*N*-((((9H-fluoren-9-yl)methoxy)carbonyl)-L-alanyl)-*N*-methyl-D-phenylalanyl)-*N*-methylglycyl-L-alloisoleucine (Fmoc-L-Ala-*N*-Me-D-Phe-Sar-L-Ile-OH) (**138**) was produced on solid phase as described in General Procedures 3.2.3-3.2.4 with the following exceptions: K-Oxyma was substituted with HOAt. The competed peptide was cleaved from the resin with 1:1 TFE/CH_2Cl_2 followed by five washes with 15 mL CH_2Cl_2. The combined organic layers were condensed to give a yellow solid. Purification by flash chromatography on silica with EtOAc and a mixture of 1:1:1 MeCN/MeOH/H_2O. The collected fractions were condensed and dissolved in CH_2Cl_2. Petroleum ether was added until product precipitates out. The suspension was centrifuged and the amorphous white solid was collected. 332 mg, 74% overall yield.

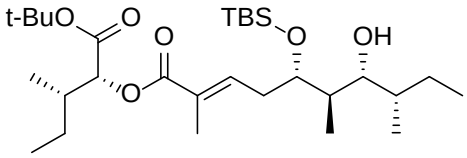
3.4.6 Final Couplings and Macrolactamization



tert-butyl (2R,3S)-2-(((E)-4-((4S,5S,6R)-6-((*S*)-sec-butyl)-2,2,5-trimethyl-1,3-dioxan-4-yl)-2-methylbut-2-enoyl)oxy)-3-methylpentanoate (**139**). See reference.[80]

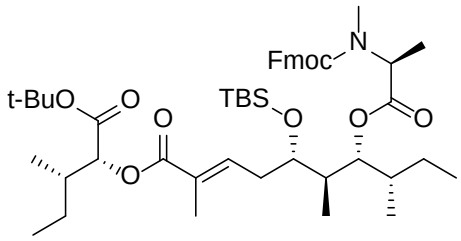


(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl (5S,6S,7R,8S,E)-5,7-dihydroxy-2,6,8-trimethyldec-2-enoate (**140**). Acetonide **139** (0.075 g, 0.1650 mmol) was dissolved in 3.0 mL anhydrous MeOH with 1 mol % PTSA and stirred 16 hours. The reaction was quenched with 8 mL saturated. $NaHCO_3$ and the methanol was evaporated. The aqueous layer was extracted with 4 x 10 mL EtOAc and the combined organic layers were washed with 30 mL brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel. (EtOAc/hexanes) to give 63 mg of colorless oil. 94% yield

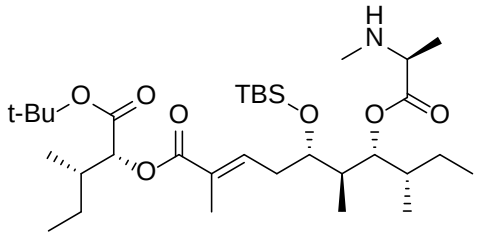


(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl (5S,6R,7R,8S,E)-5-((tert-butyldimethylsilyl)oxy)-7-hydroxy-2,6,8-trimethyldec-2-enoate (**141**). The previous compound (0.030 g, 0.072 mmol) was stirred in 1.0 mL anhydrous CH_2Cl_2 under argon and cooled to -78 °C. To the solution, 2,6-lutidine (0.017 mL, 0.144 mmol) and TBSOTf (0.016 mL, 0.080 mmol) was added and stirred for 1 hour. The reaction mixture was quenched with 5 mL cold DI water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were were washed with 1M HCl, brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash

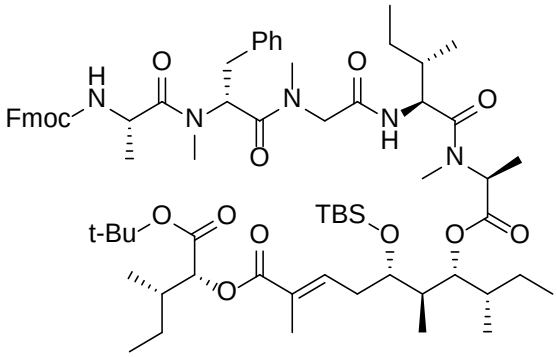
chromatography on silica gel. (EtOAc/hexanes) to give 36 mg of clear amorphous solid. 94% yield.



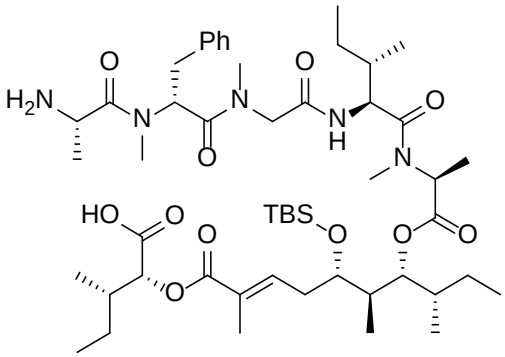
(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl (5S,6R,7R,8S,E)-7-((*N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-L-alanyl)oxy)-5-((tert-butyldimethylsilyl)oxy)-2,6,8-trimethyldec-2-enoate (**142**). The previous compound was stirred in CH_2Cl_2 at 0°C. To the solution, DIPEA was added followed by dropwise addition of Fmoc-*N*-Me-L-Ala-Cl dissolved in CH_2Cl_2.



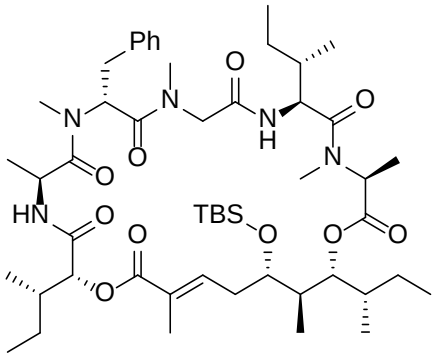
(2R,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl (5S,6R,7R,8S,E)-5-((tert-butyldimethylsilyl)oxy)-2,6,8-trimethyl-7-((methyl-L-alanyl)oxy)dec-2-enoate (**143**). The previous compound was stirred in 10% Et_2NH in MeCN. The reaction was monitored by TLC. Upon completion, the volatiles were removed by rotary evaporation. Solvent was repeatedly added and evaporated in vacuo until Et_2NH was completely removed. The crude residue was used without further treatment.



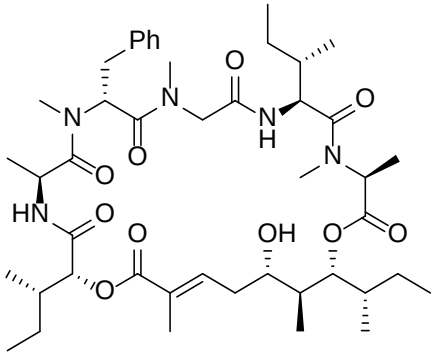
Compound (**144**). The previous compound and peptide **138** (54 mg, 0.084 mmol) were dissolved in 3.5 mL DMF and cooled to 0°C under argon. To the solution, HATU (46 mg, 0.122 mmol) and HOAt (11 mg, 0.081 mmol) were added, followed by collidine (27 µL, 0.203 mmol). The reaction was stirred at ambient temperature for 16 hours. The reaction was quenched with water and extracted 3 times with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$, saturated ammonium chloride, brine, and dried over Na_2SO_4. After concentration, the residue was purified by flash chromatography on silica gel (EtOAc/hexanes) to give 34 mg of amorphous white solid. 64% yield.



Compound (**145**). The previous compound was stirred in 4 mL 15% TFA in CH_2Cl_2 for 2 hours at 0°C. The volatiles were removed by rotary evaporation and then under high vacuum. The residue was then dissolved in MeCN with diethyl amine and stirred for 50 minutes. The volatiles were removed by rotary evaporation and the residue was dried in vacuo. LCMS (ESI) *m/z* calcd for $C_{51}H_{86}N_5O_{11}Si$ [M-H]- 972.60, found 972.47.



Compound (**146**). The previous compound **145** (0.0159 mmol) was stirred in 10 mL CH_2Cl_2. To the solution, HATU (60 mg, 0.1588 mmol) and DIPEA (55 µL, 3.175 mmol) was added. The reaction was stirred at ambient temperature until the starting material was no longer detectable by TLC. The mixture was concentrated by rotary evaporation and dried *in vacuo* for 2 h to give a brown residue. LCMS (ESI) m/z calcd for $C_{51}H_{85}N_5O_{10}SiNa$ [M+Na] 978.60, found 978.67.



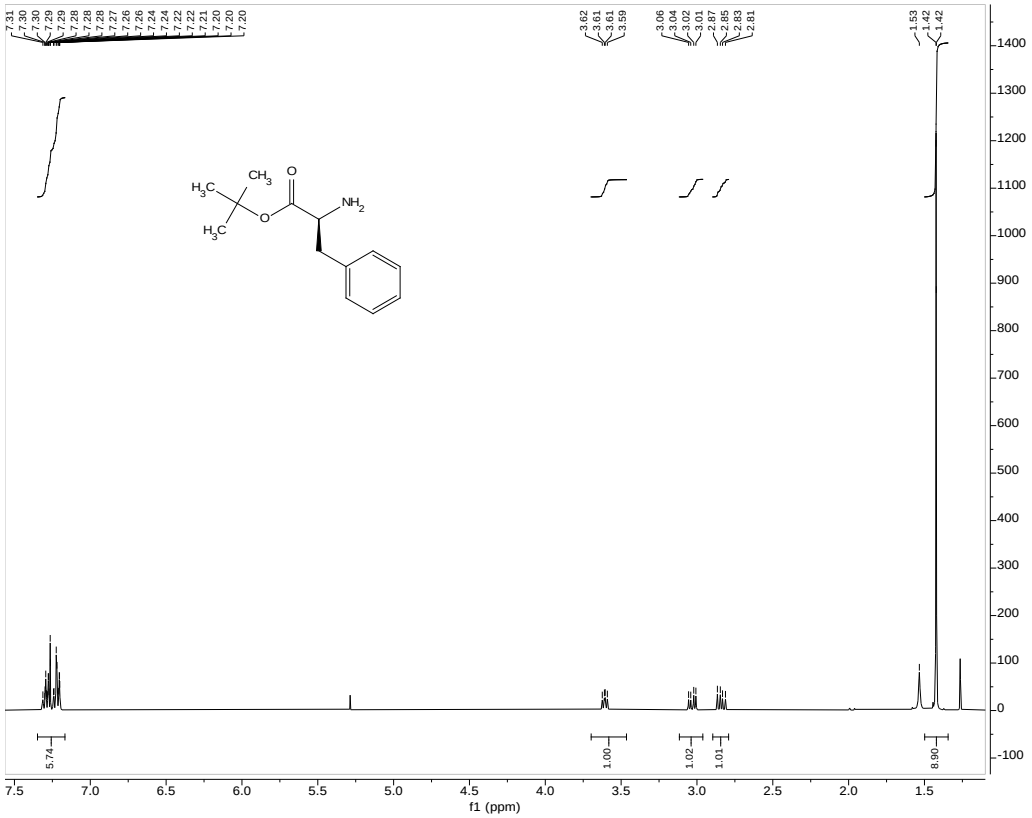
Compound (**147**). The previous compound was dissolved in MeCN (8.0 mL) and cooled to 0 °C, followed by addition of 49% aq. HF (2.0 mL). The reaction was allowed to come to ambient temperature and stirred for 1 hr. Reaction mixture was diluted with EtOAc (100 mL) and the washed with sat. aq. $NaHCO_3$ (2 x 20 mL), brine (2 x 20 mL), and then dried over Na_2SO_4. The organic layer was concentrated in vacuo to give 34 mg of brown oil. The residue was purified twice by flash chromatography on C18-modified silica (MeOH/H_2O, 0.1% formic acid), followed by silica gel (acetone/hexanes) to give the desired product (5 mg, 39% yield over 4 steps) as an amorphous solid.

$[\alpha]_D^{20} = -33.3°$ (c 0.1, MeOH); lit. $[\alpha]_D^{20} = -33.8°$ (c 0.1, MeOH).[10]; ^{1}H-NMR (500 MHz, MeOD): δ 7.32 (m, 1H), 7.26-7.14 (m, 5H), 5.46 (dd, J = 10.3, 5.2 Hz, 1H), 5.05 (d, J = 6.2 Hz, 1H), 4.92 (m, 1H), 4.84 (m, 1H), 4.52 (q, J = 6.9 Hz, 1H), 4.20 (d, J = 18.4 Hz, 1H), 3.95 (q, J = 6.8 Hz, 1H), 3.76 (m, 1H), 3.57 (d, J = 18.3 Hz, 1H), 3.30 (m, 3H), 3.09-3.02 (m, 1H), 3.04 (m, 3H), 2.96 (dd, J = 12.1, 5.8 Hz, 1H), 2.89 (m, 3H), 2.28-2.20 (m, 1H), 2.20-2.11 (m, 1H), 2.09-2.05 (m, 1H), 1.94 (m, 3H), 1.90-1.80 (m, 2H), 1.77-1.60 (m, 2H), 1.60-1.45 (m, 1H), 1.43 (d, J = 7.5 Hz, 3H), 1.38-1.26 (m, 3H), 1.19-1.10 (m, 2H), 1.05 (d, J = 6.8 Hz, 3H), 1.01-0.89 (m, 21H), 0.86 (d, J = 7.1 Hz, 3H) ppm; FTIR (neat, cm^{-1}) 3436, 2929, 2600, 2341, 2055, 1740, 1634, 1521, 1246, 1120, 750; LC-MS (ESI) *m/z* calcd for $C_{45}H_{70}N_5O_{10}$ $[M-H]^-$ 840.51, found 840.67.

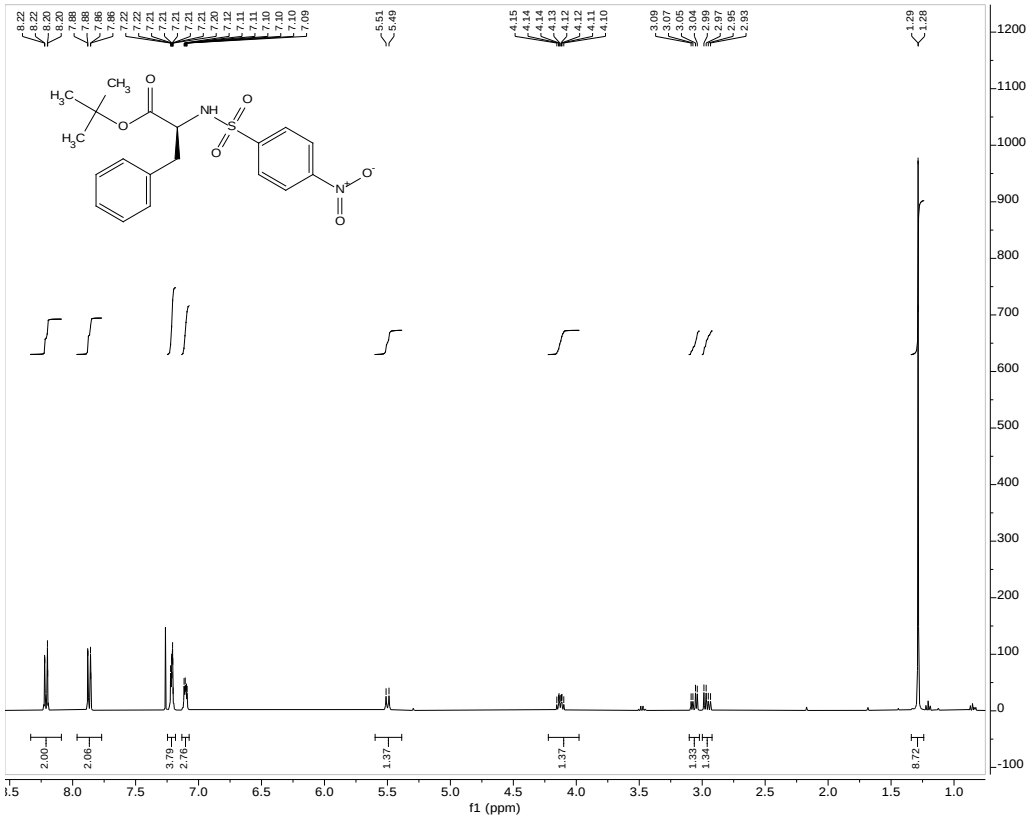
Spectra

Micromide (Nosyl Solution Phase)

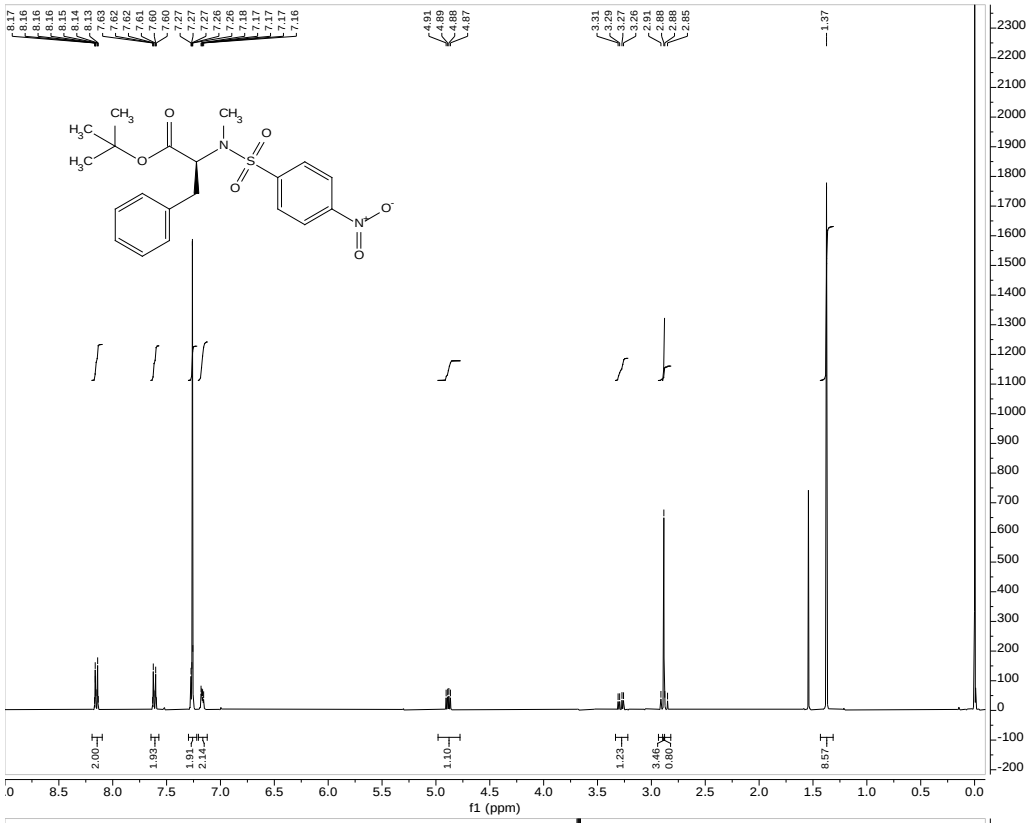
Compound **2**

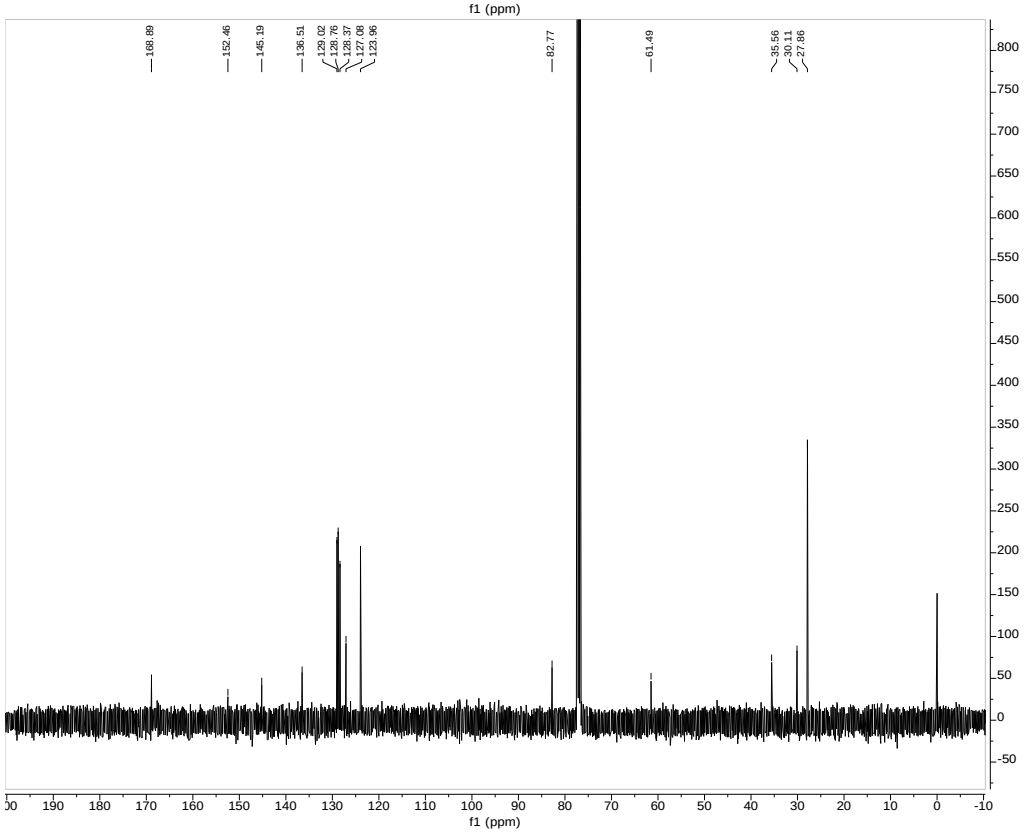


Compound **3**

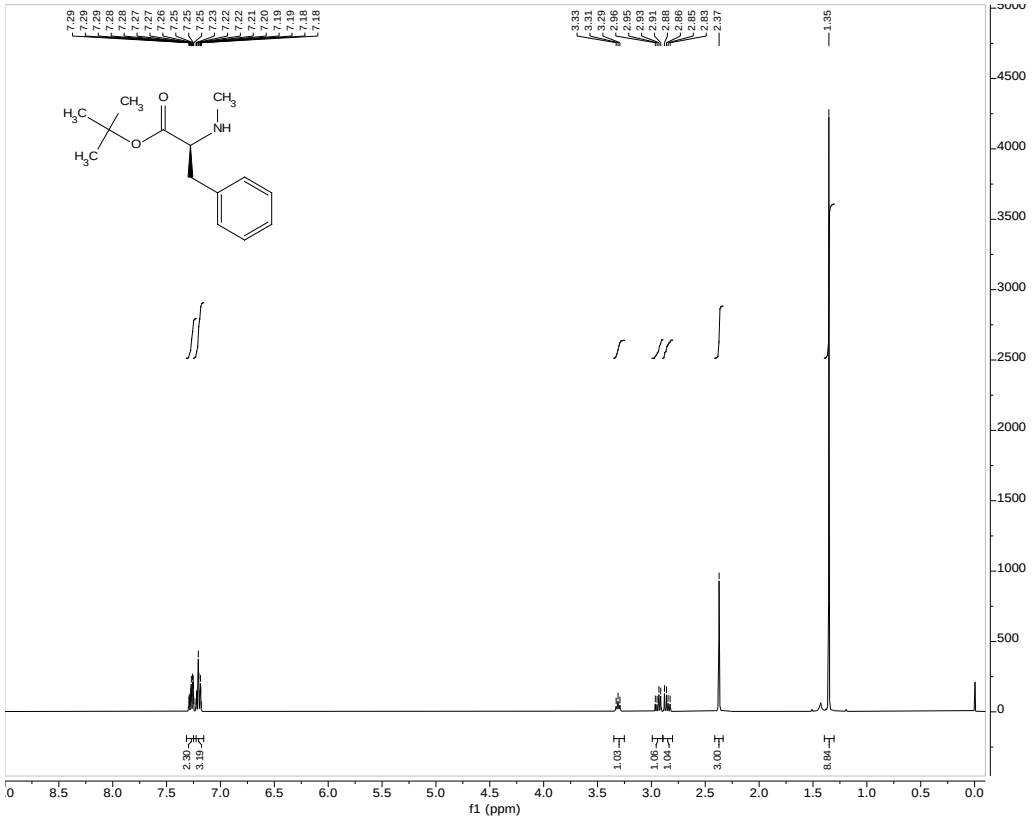

8.22
8.22
8.20
8.20
7.88
7.88
7.86
7.86
7.22
7.22
7.21
7.21
7.21
7.21
7.21
7.20
7.12
7.11
7.10
7.10
7.10
7.09
5.51
5.49
4.15
4.14
4.14
4.13
4.12
4.12
4.11
4.10
3.09
3.07
3.05
3.04
2.99
2.97
2.95
2.93
1.29
1.28
CH3
H3C
H3C
O
O
NH
S
O
O
N+
O-
O
2.00
2.06
3.79
2.76
1.37
1.37
1.33
1.34
8.72
f1 (ppm)

Compound **4**

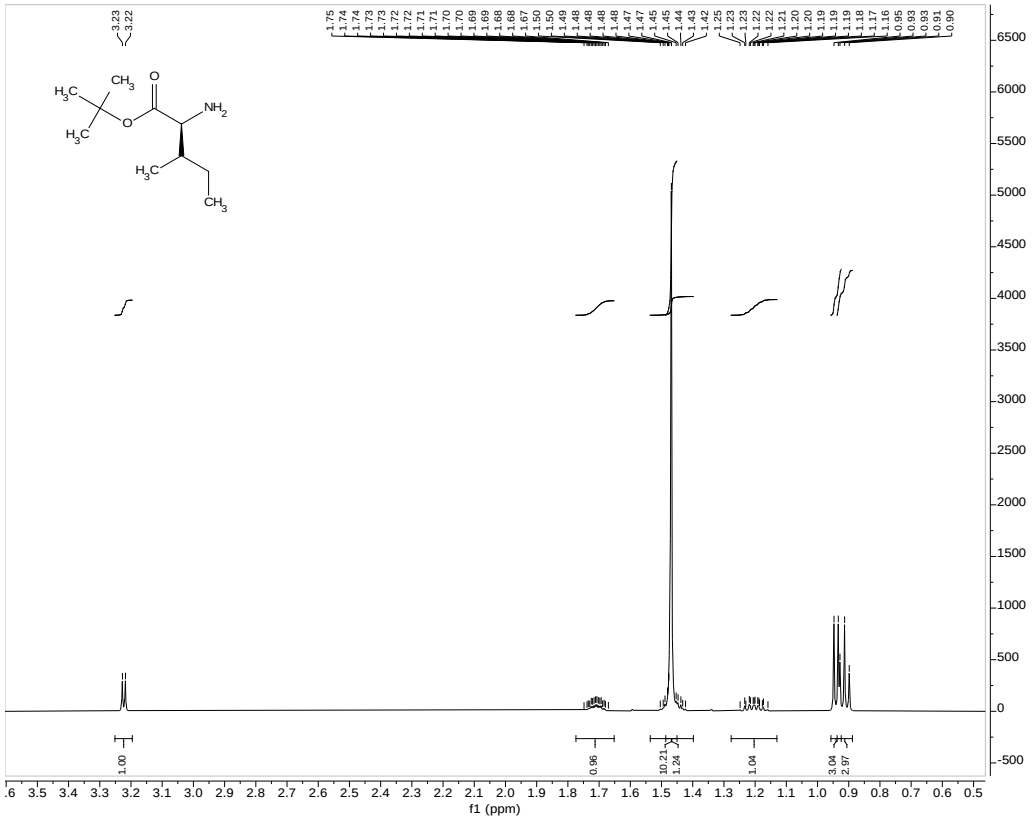

8.17
8.16
8.16
8.16
8.15
8.14
8.13
7.63
7.62
7.62
7.61
7.60
7.60
7.27
7.27
7.27
7.26
7.26
7.18
7.17
7.17
7.17
7.16
4.91
4.89
4.88
4.87
3.31
3.29
3.27
3.26
2.91
2.88
2.88
2.85
1.37
2.00
1.93
1.91
2.14
1.10
1.23
3.46
0.80
8.57
f1 (ppm)


168.89
152.46
145.19
136.51
129.02
128.76
128.37
127.98
123.96
82.77
61.49
35.56
30.11
27.86
f1 (ppm)

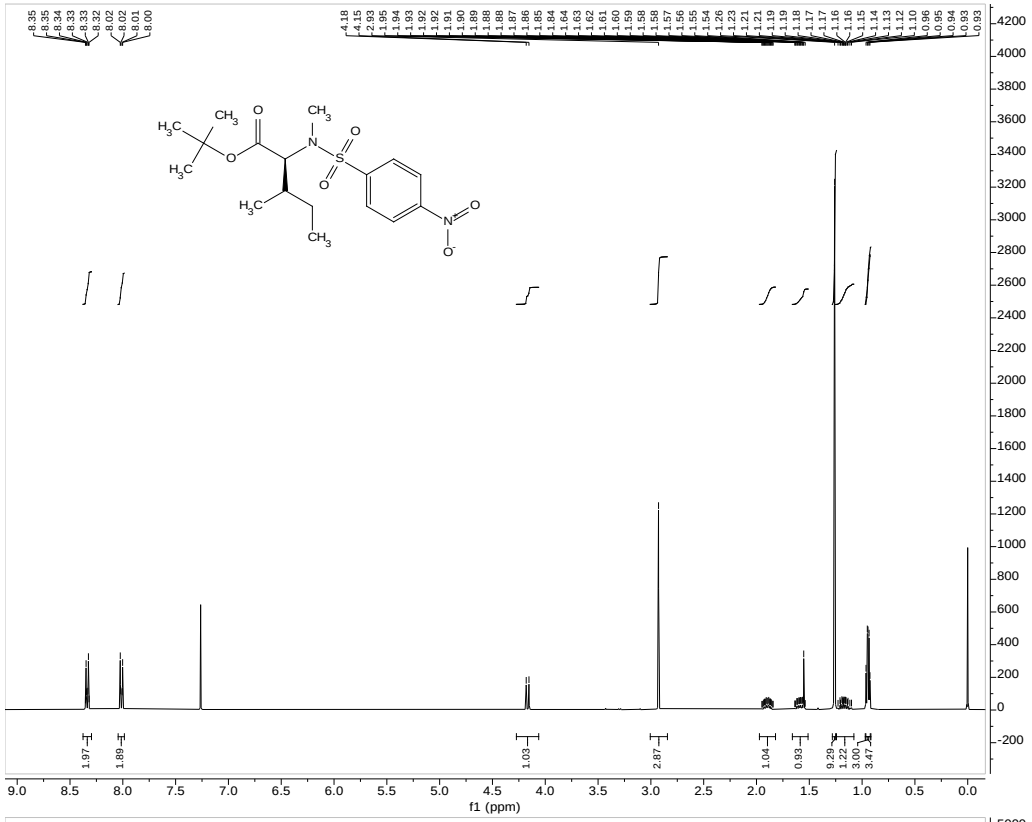
Compound **5**

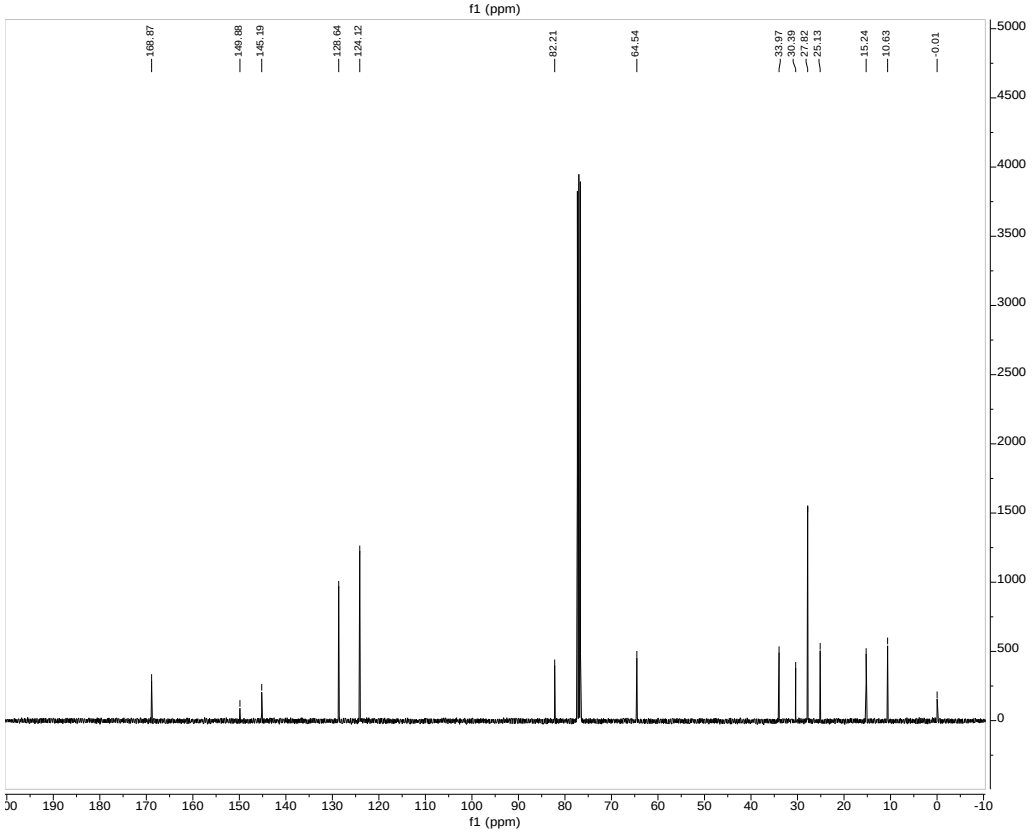

7.29
7.29
7.29
7.28
7.28
7.27
7.27
7.26
7.25
7.25
7.25
7.23
7.22
7.22
7.21
7.20
7.19
7.19
7.18
7.18
3.33
3.31
3.29
2.96
2.95
2.93
2.91
2.88
2.86
2.85
2.83
2.37
1.35
CH3
O
CH3
H3C
NH
O
H3C
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
2.30
3.19
1.03
1.06
1.04
3.00
8.84
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Compound **6**

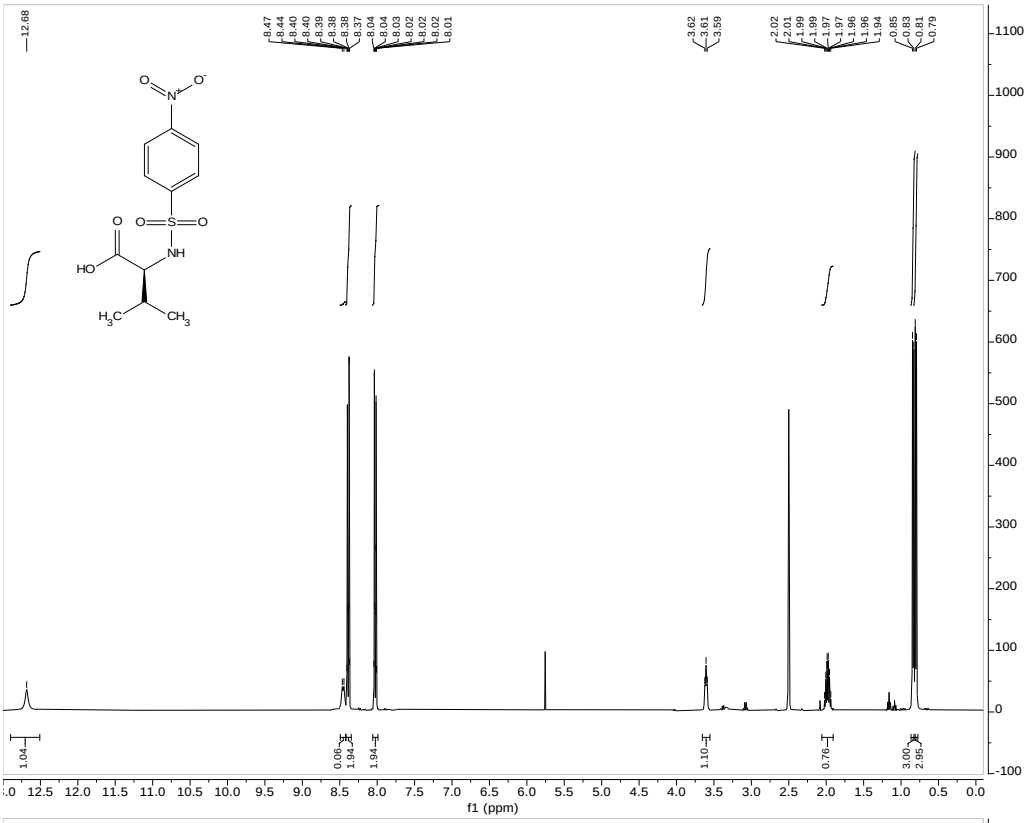


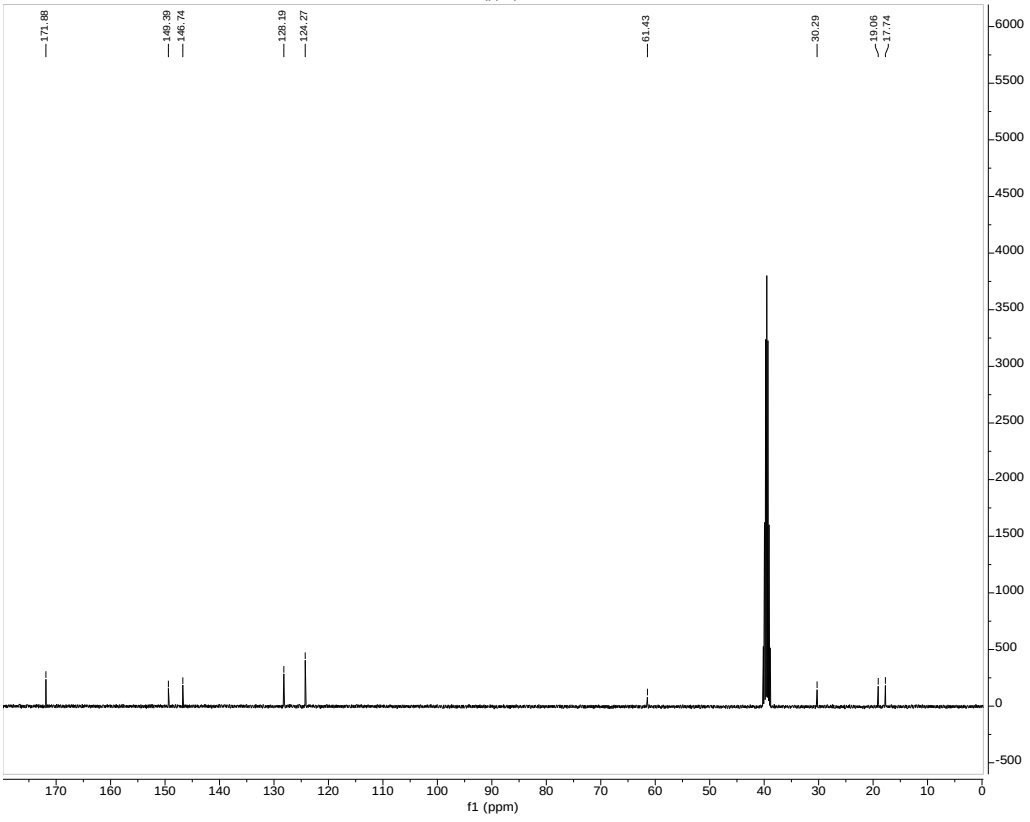
Compound **8**



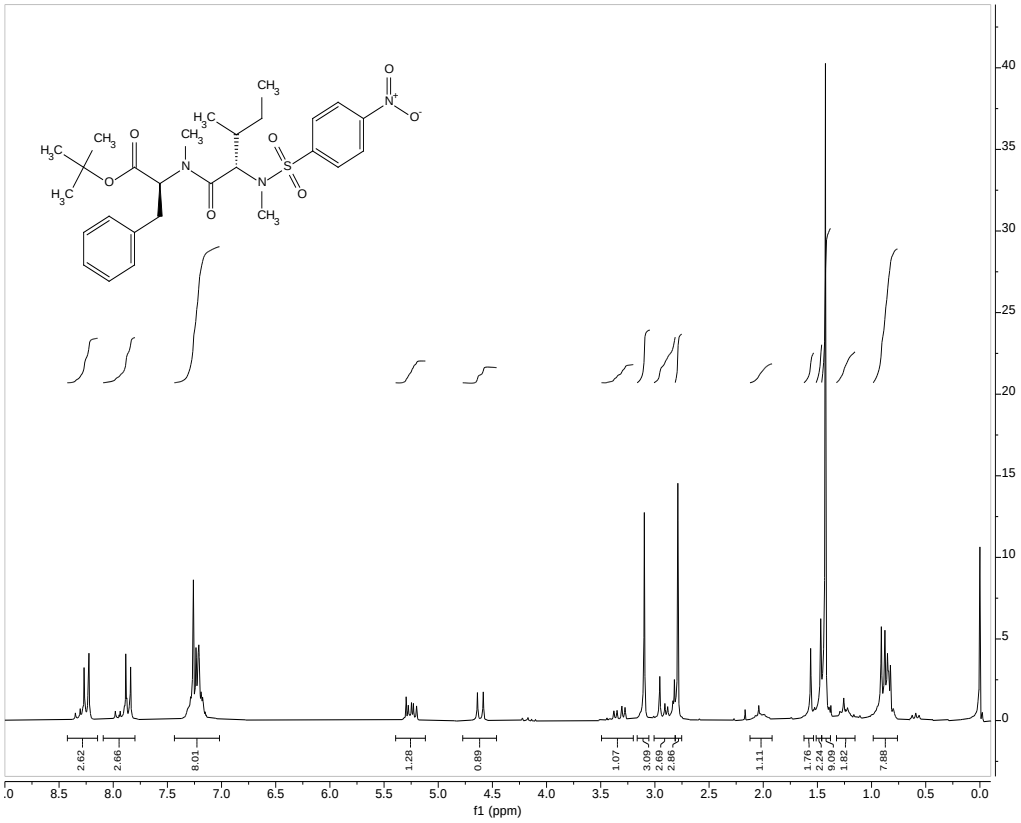


Compound **11**

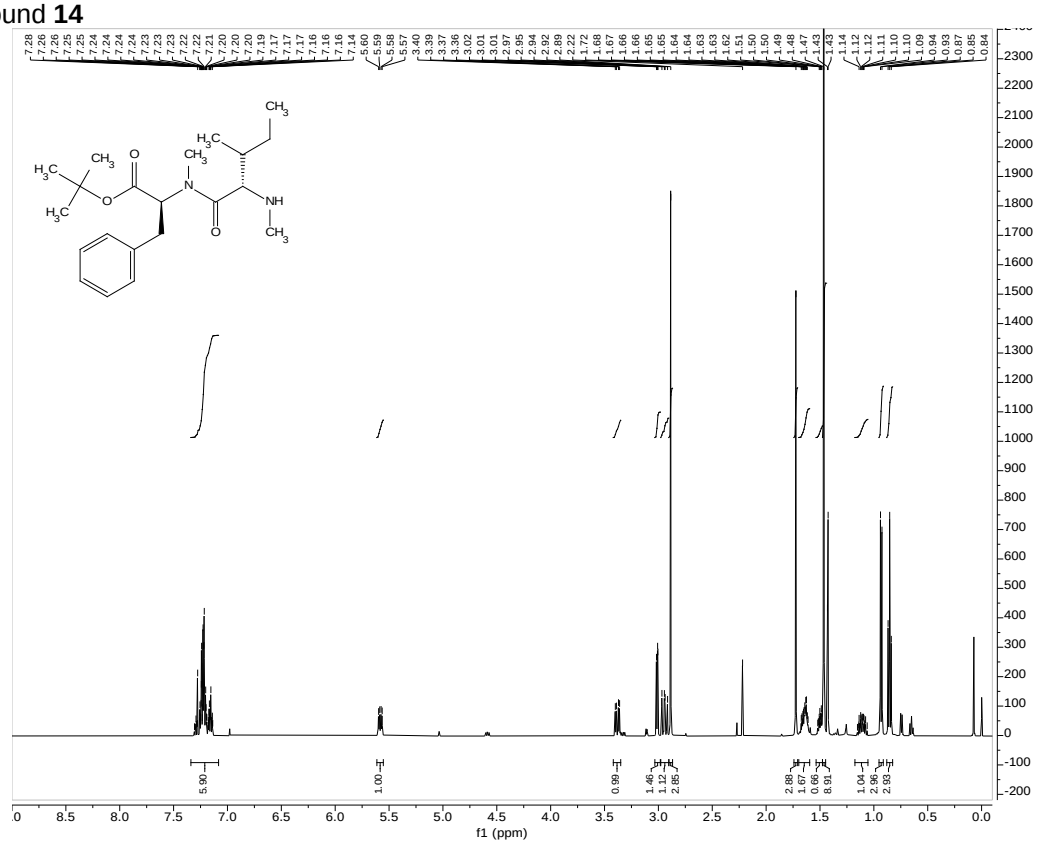

12.68
8.47
8.44
8.40
8.40
8.39
8.38
8.39
8.37
8.04
8.04
8.03
8.02
8.02
8.02
8.01
3.62
3.61
3.59
2.02
2.01
1.99
1.99
1.97
1.97
1.96
1.96
1.94
0.85
0.83
0.81
0.79
1.04
0.06
1.94
1.94
1.10
0.76
3.00
2.95
f1 (ppm)


171.88
149.39
146.74
128.19
124.27
61.43
30.29
19.06
17.74
f1 (ppm)

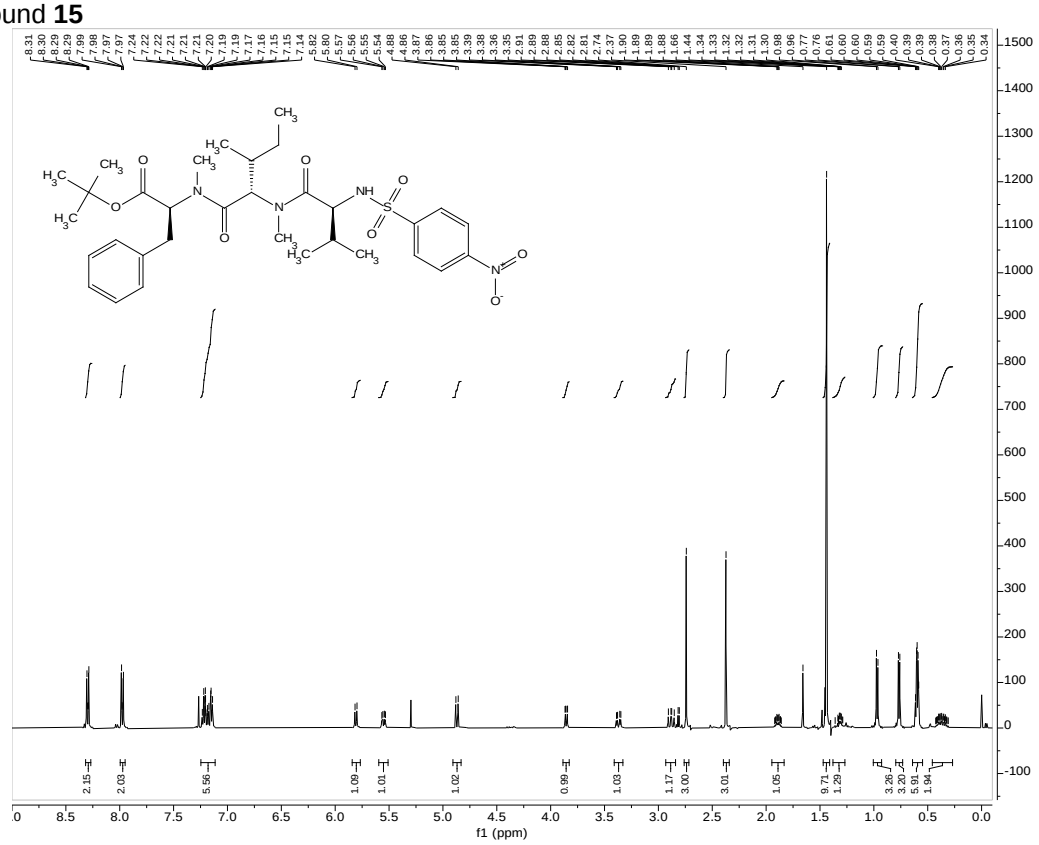
Compound **13**


f1 (ppm)
2.62
2.66
8.01
1.28
0.89
1.07
3.09
2.69
2.86
1.11
1.76
2.24
9.09
1.82
7.88

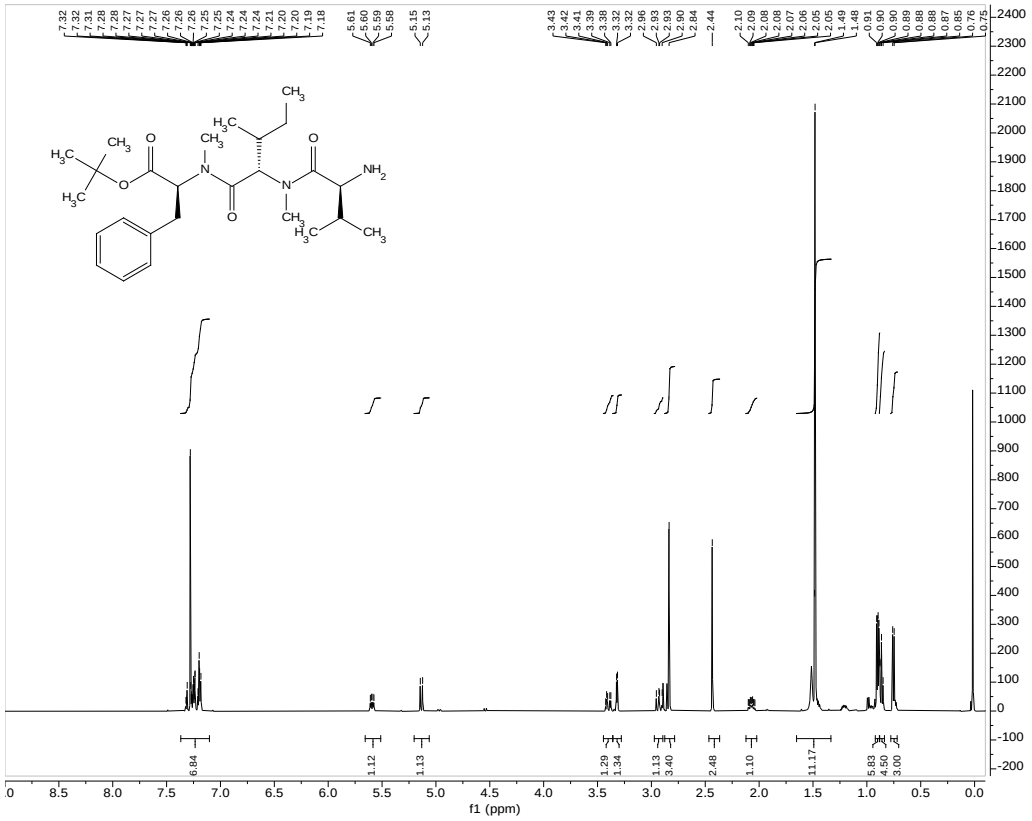
Compound **14**



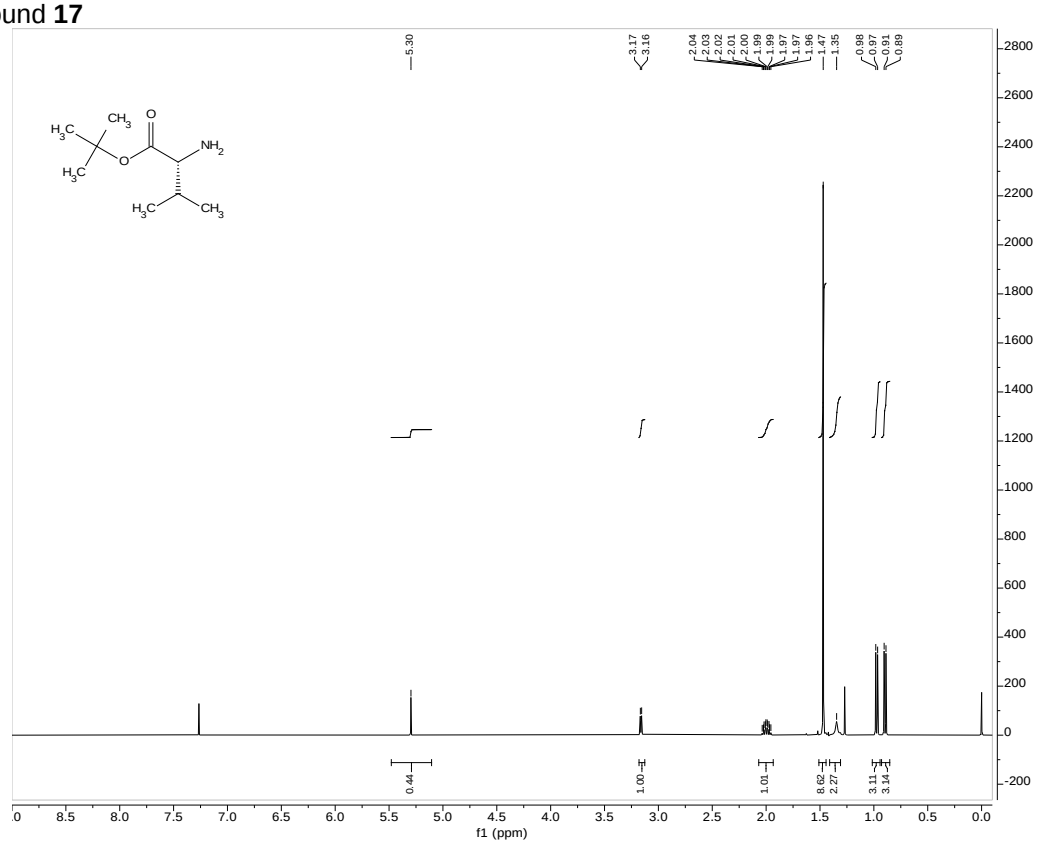
Compound **15**



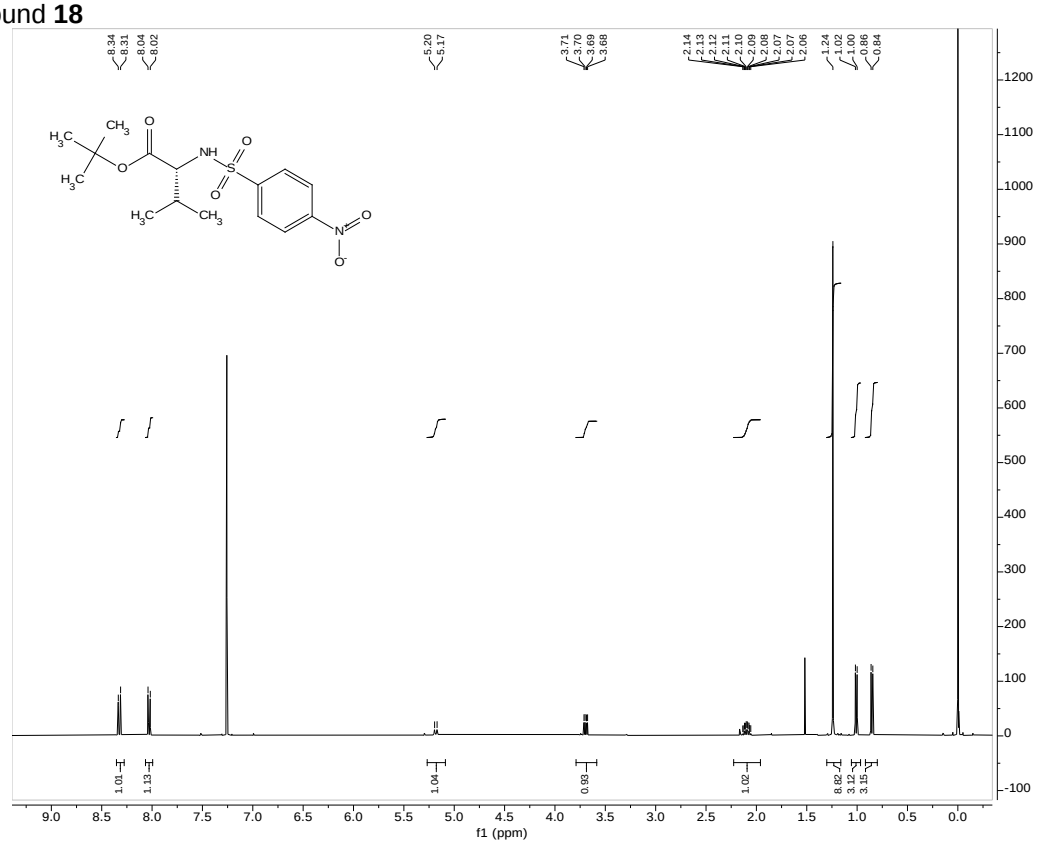
Compound **16**



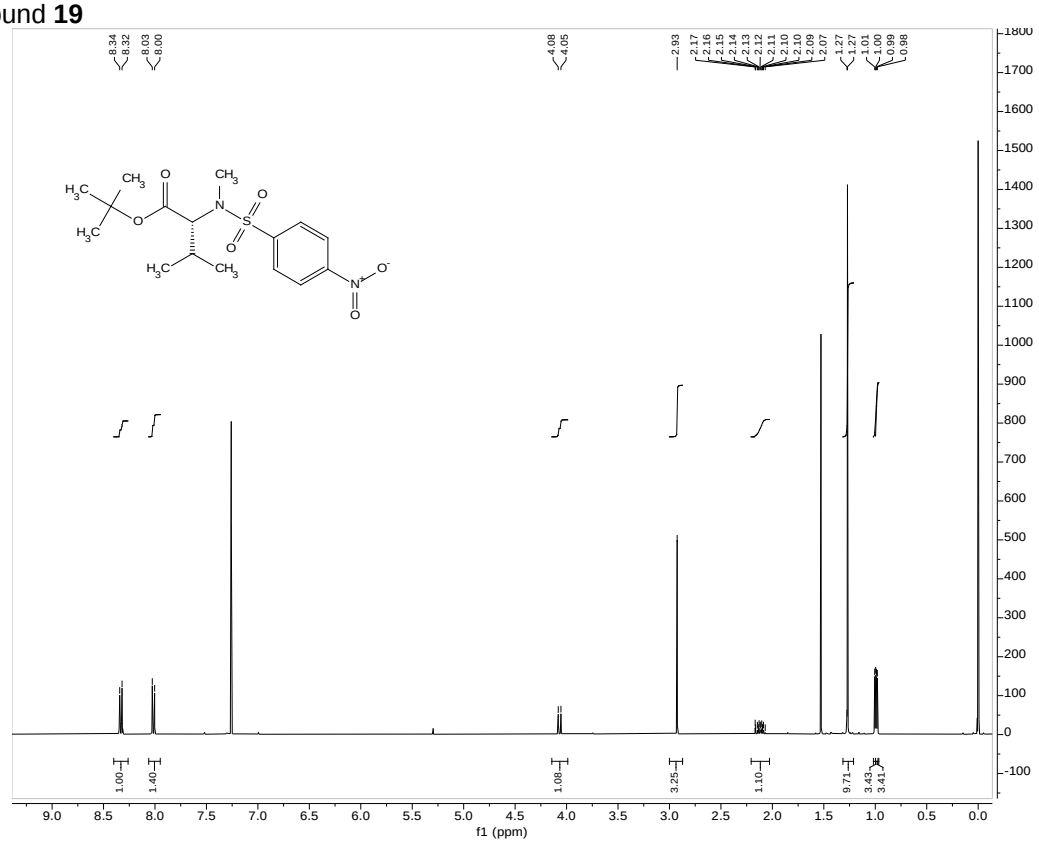
Compound **17**


5.30
3.17
3.16
2.04
2.03
2.02
2.01
2.00
1.99
1.99
1.97
1.97
1.96
1.47
1.35
0.98
0.97
0.91
0.89
0.44
1.00
1.01
8.62
2.27
3.11
3.14
f1 (ppm)

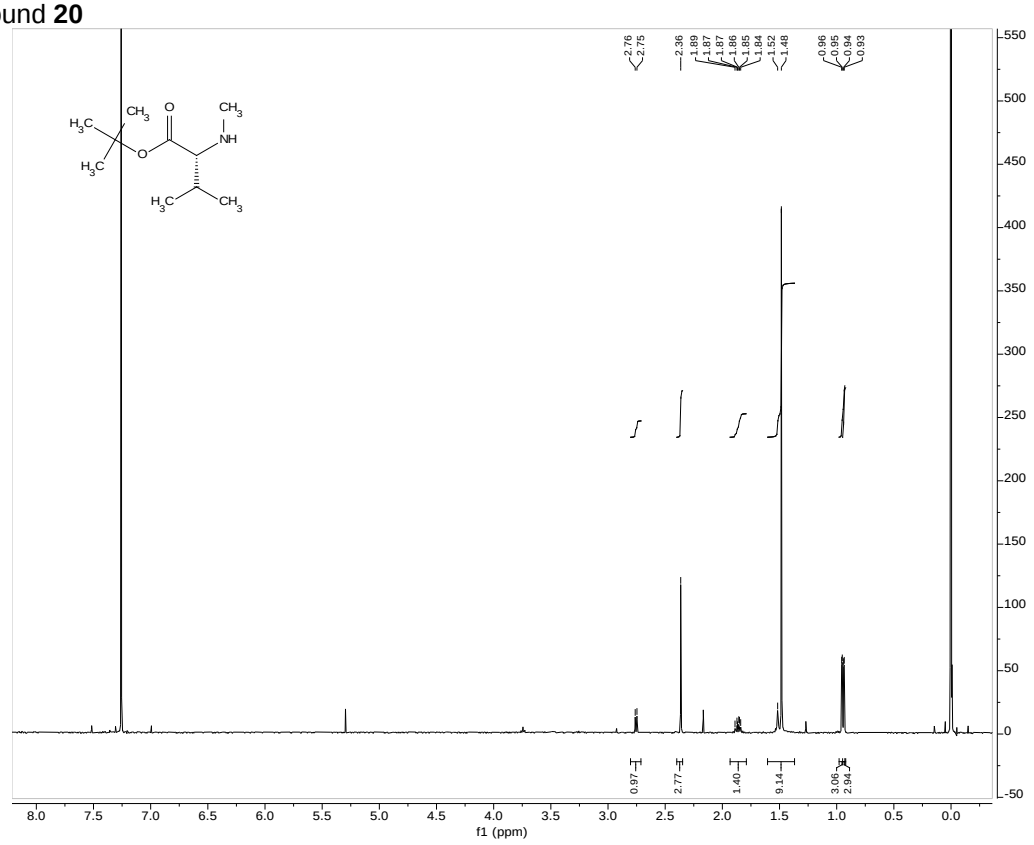
Compound **18**


8.34
8.31
8.04
8.02
5.20
5.17
3.71
3.70
3.69
3.68
2.14
2.13
2.12
2.11
2.10
2.09
2.08
2.07
2.07
2.06
1.24
1.02
1.00
0.86
0.84
1.01
1.13
1.04
0.93
1.02
8.82
3.12
3.15
f1 (ppm)

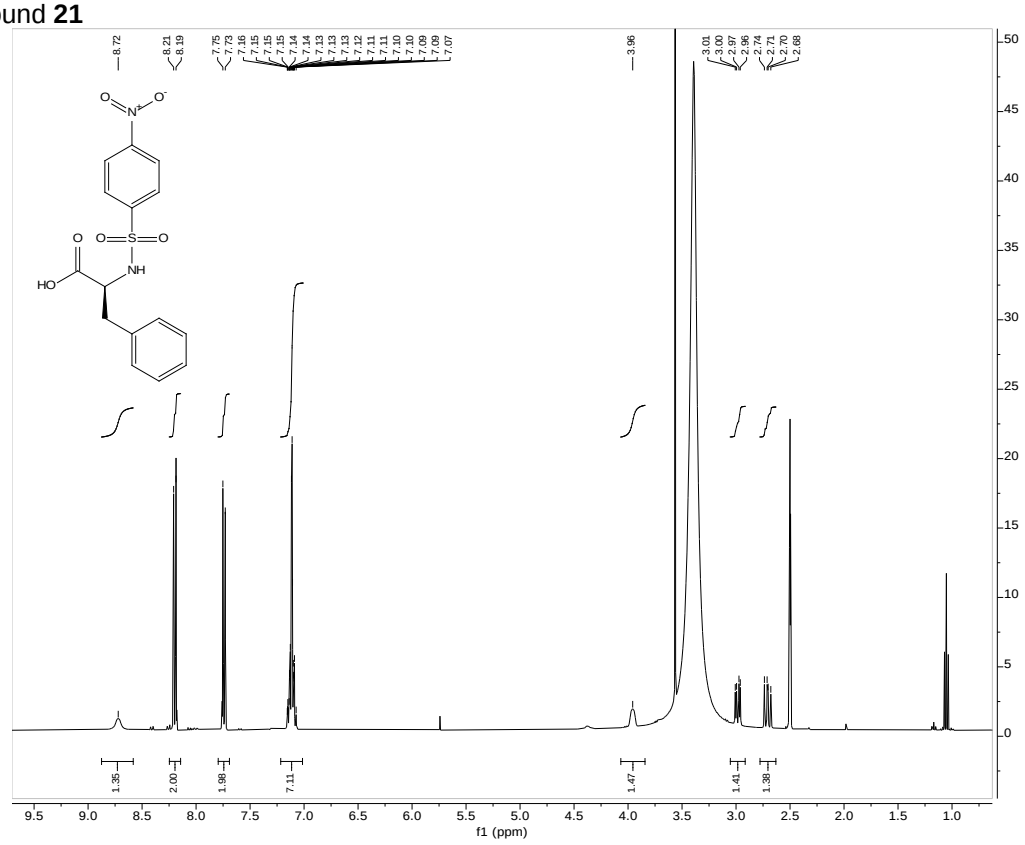
Compound **19**


8.34
8.32
8.03
8.00
4.08
4.05
2.93
2.17
2.16
2.15
2.14
2.13
2.12
2.11
2.10
2.10
2.09
2.07
1.27
1.27
1.01
1.00
0.99
0.98
1.00
1.40
1.08
3.25
1.10
9.71
3.43
3.41
f1 (ppm)

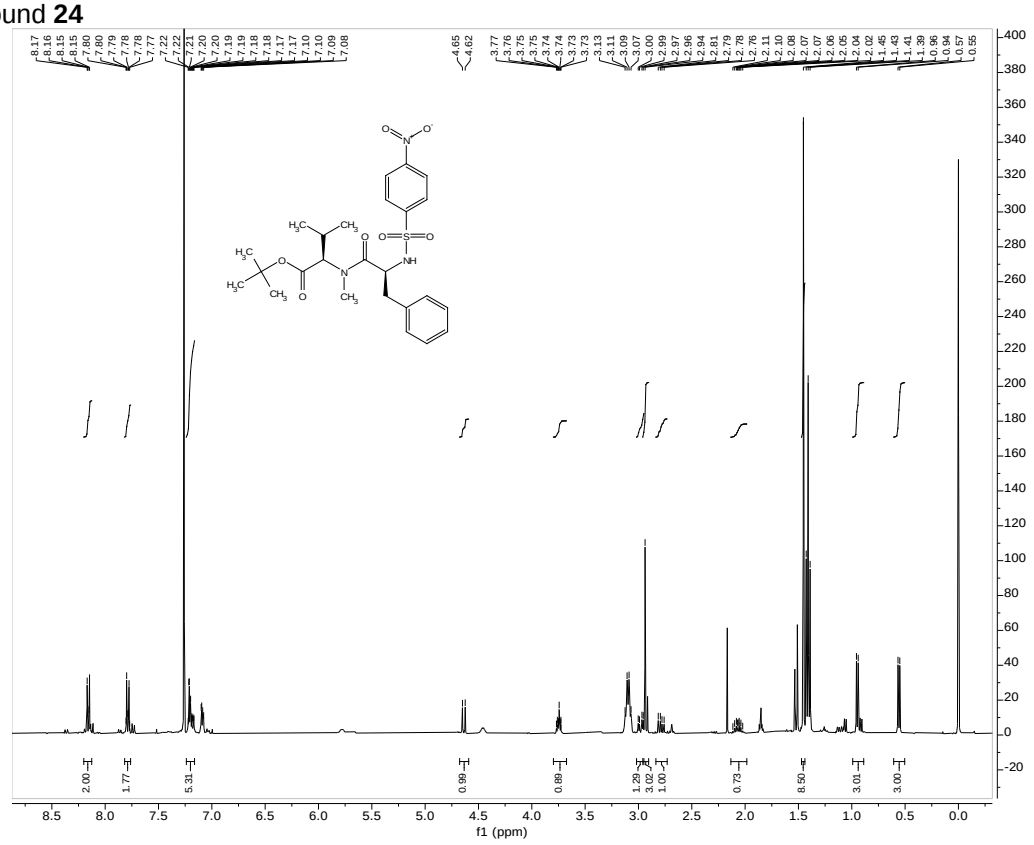
Compound **20**


2.76
2.75
2.36
1.89
1.87
1.87
1.86
1.85
1.84
1.52
1.48
0.96
0.95
0.94
0.93
0.97
2.77
1.40
9.14
3.06
2.94
f1 (ppm)

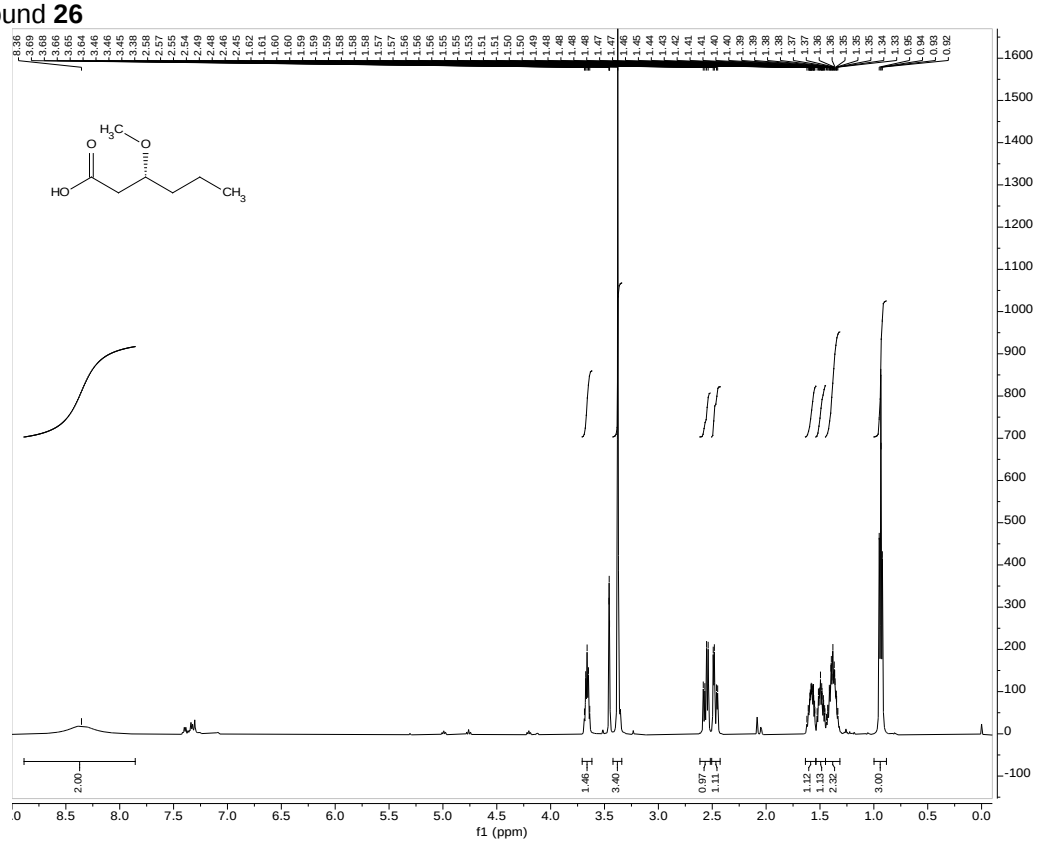
Compound **21**


8.72
8.21
8.19
7.75
7.73
7.16
7.15
7.15
7.15
7.14
7.14
7.13
7.13
7.12
7.11
7.11
7.10
7.10
7.09
7.09
7.07
3.96
3.01
3.00
2.97
2.96
2.74
2.71
2.70
2.68
1.35
2.00
1.98
7.11
1.47
1.41
1.38
f1 (ppm)

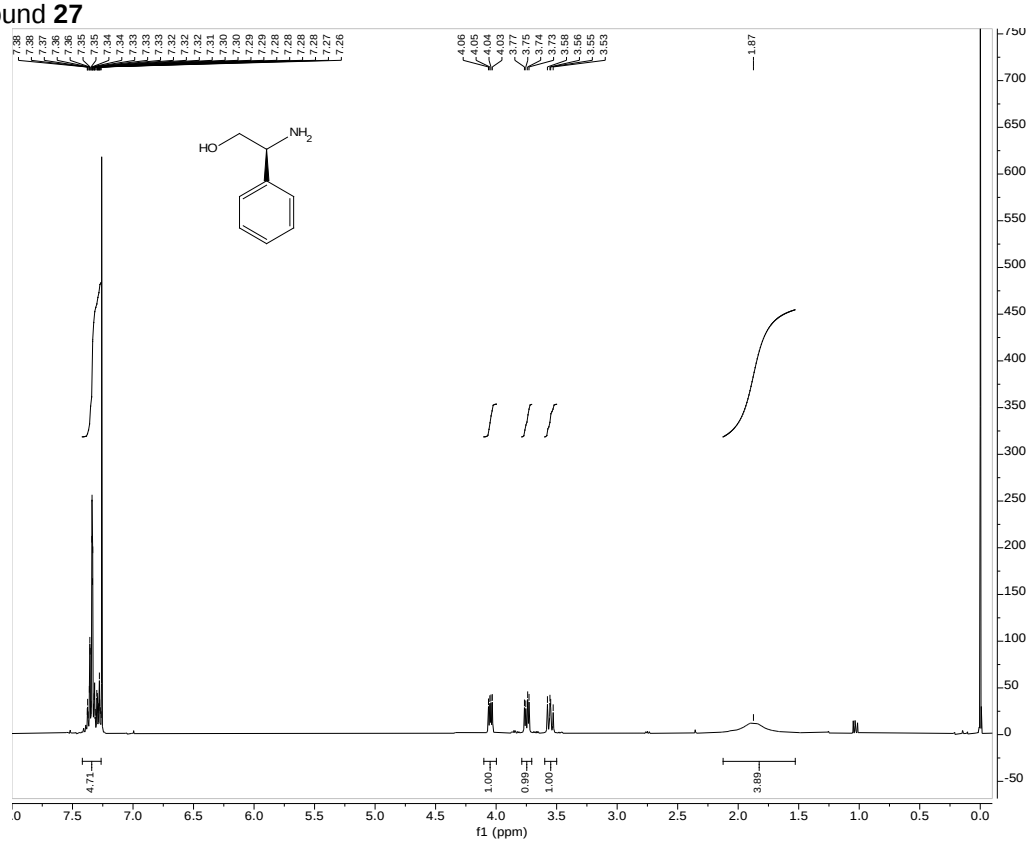
Compound **24**


8.17
8.16
8.15
8.15
7.80
7.79
7.78
7.78
7.77
7.22
7.21
7.20
7.20
7.19
7.19
7.18
7.18
7.17
7.17
7.10
7.09
7.08
4.65
4.62
3.77
3.76
3.75
3.75
3.74
3.74
3.73
3.73
3.13
3.11
3.09
3.07
3.00
2.99
2.97
2.96
2.94
2.81
2.79
2.78
2.76
2.11
2.10
2.08
2.07
2.07
2.06
2.05
2.04
2.02
1.45
1.43
1.41
1.39
0.96
0.94
0.57
0.55
2.00
1.77
5.31
0.99
0.89
1.29
3.02
1.00
0.73
8.50
3.01
3.00
f1 (ppm)

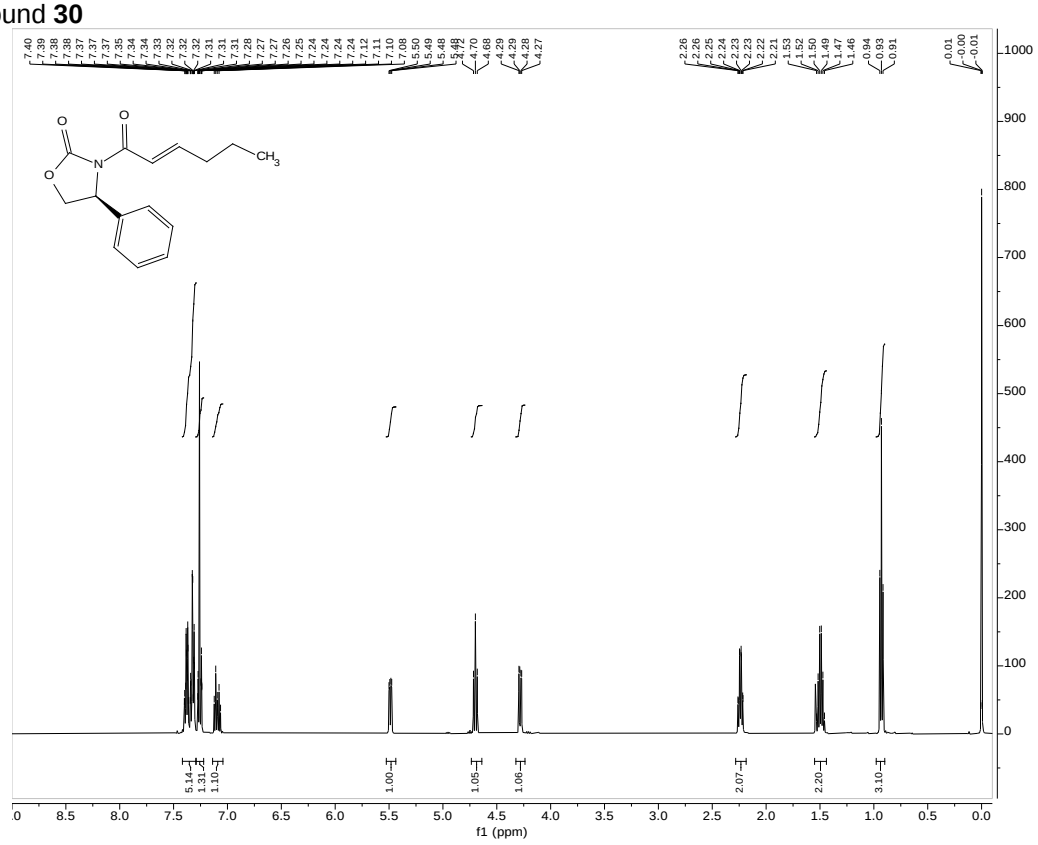
Compound **26**



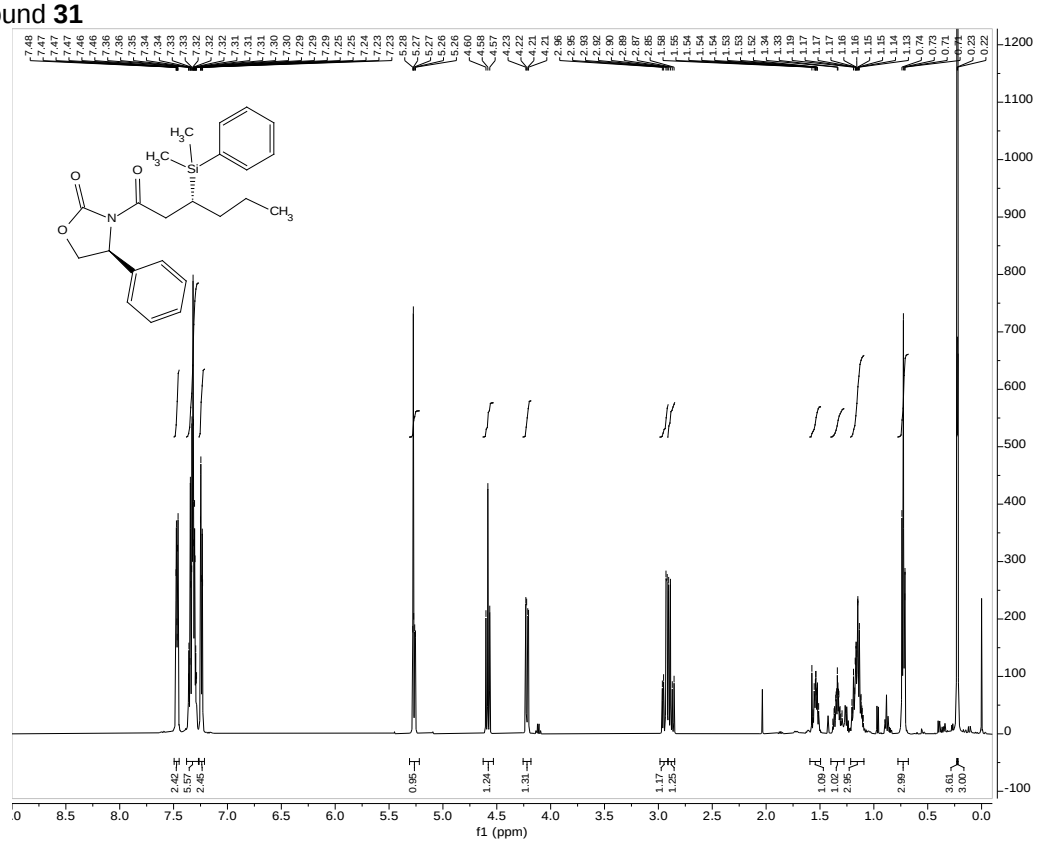
Compound **27**


7.38
7.38
7.37
7.36
7.36
7.35
7.35
7.34
7.34
7.33
7.33
7.33
7.32
7.32
7.32
7.31
7.30
7.30
7.29
7.29
7.28
7.28
7.28
7.28
7.27
7.26
4.06
4.05
4.04
4.03
3.77
3.75
3.74
3.73
3.58
3.56
3.55
3.53
1.87
HO
NH2
4.71
1.00
0.99
1.00
3.89
f1 (ppm)

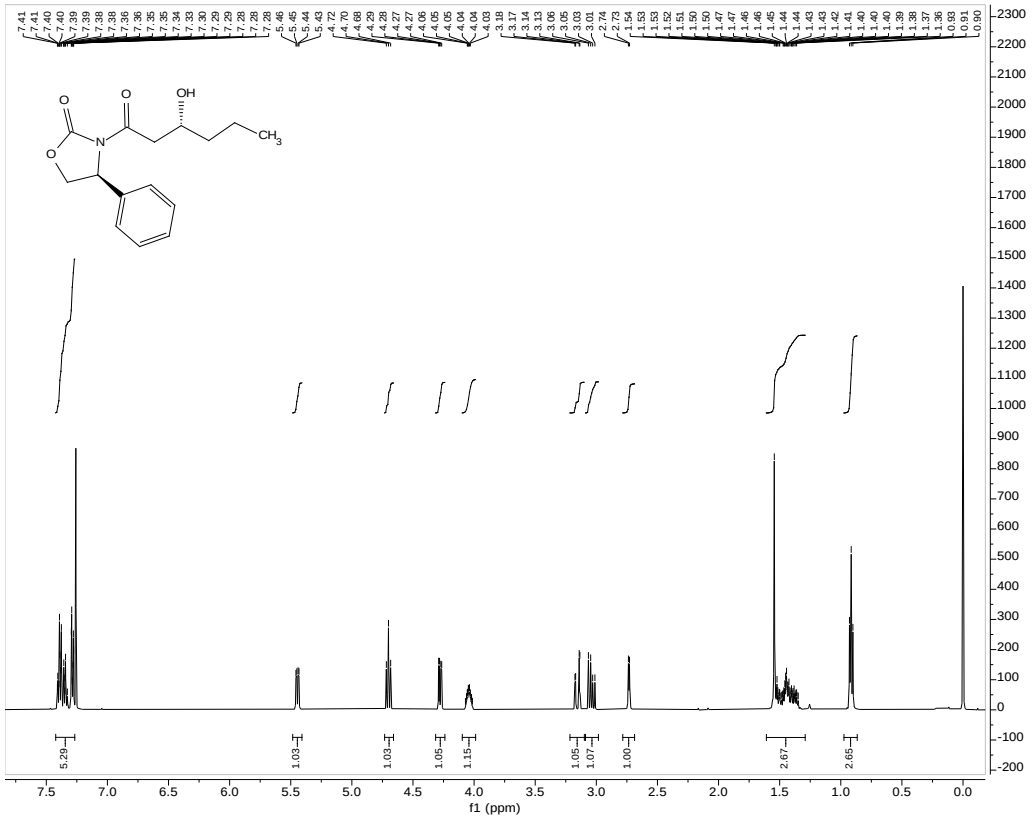
Compound **30**



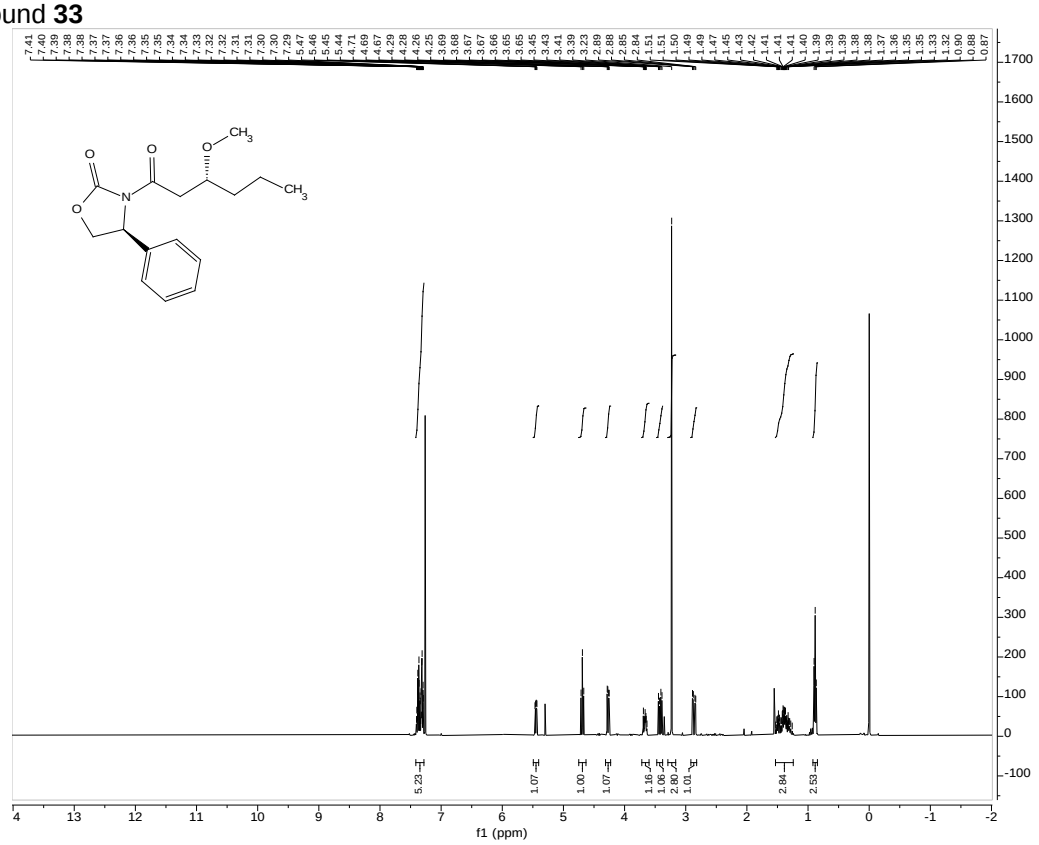
Compound **31**



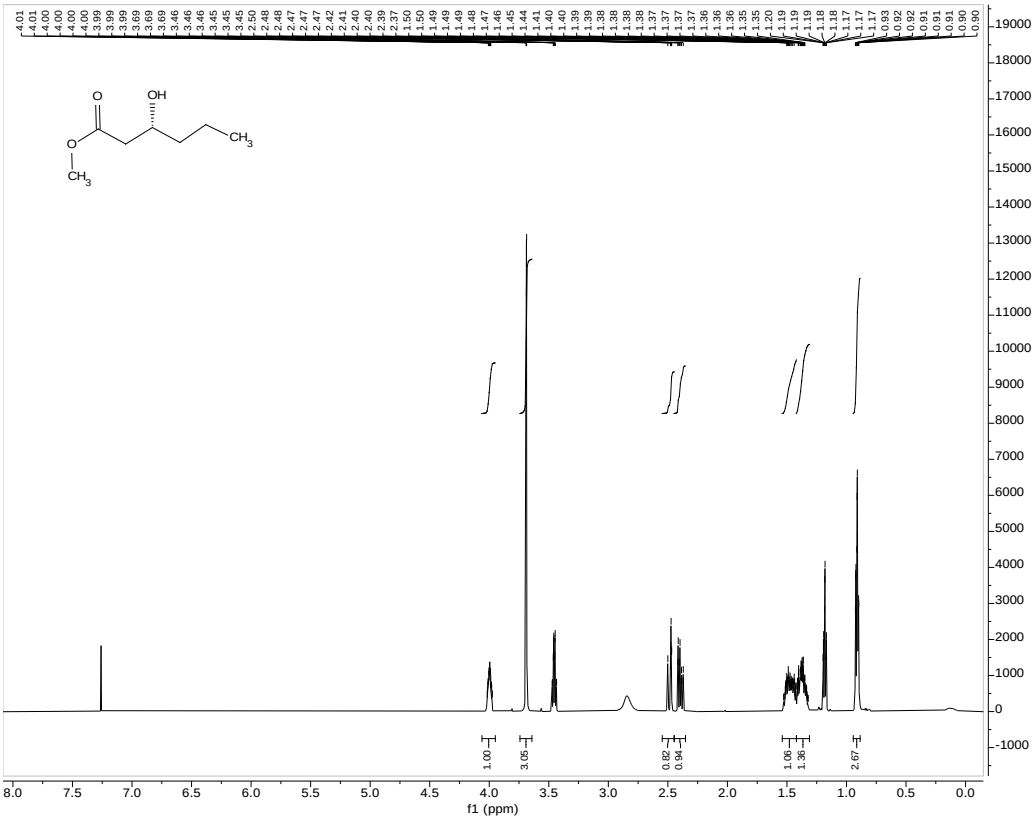
Compound **32**

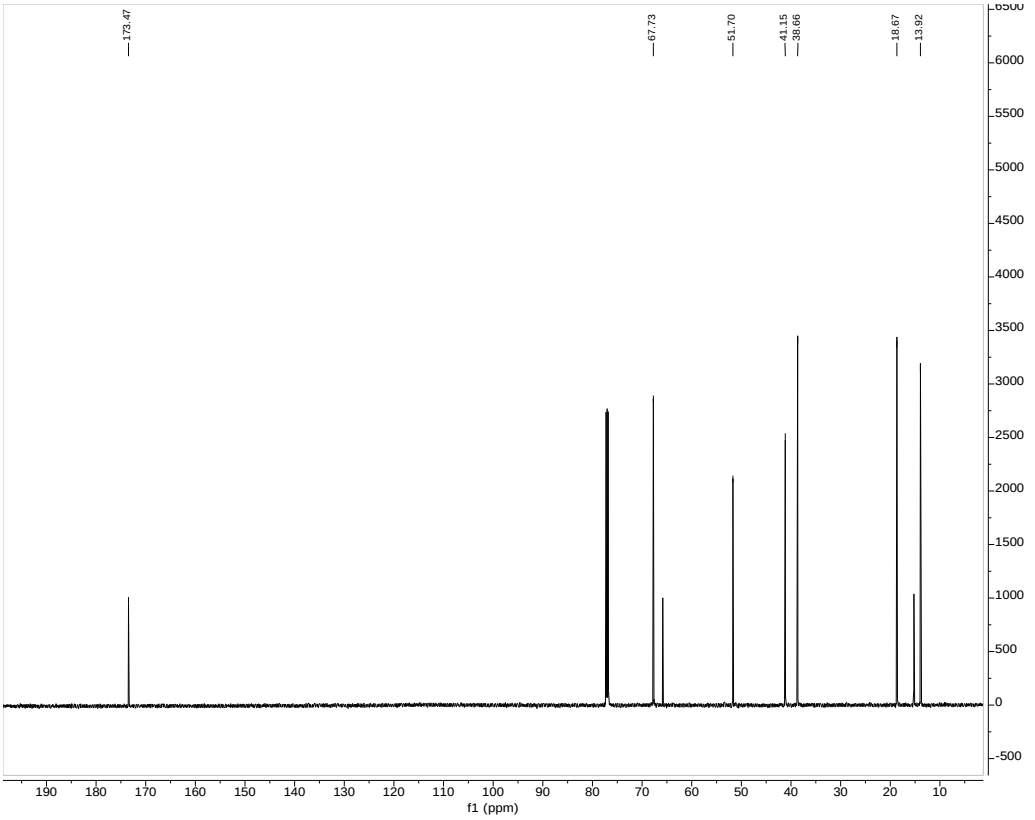


Compound **33**

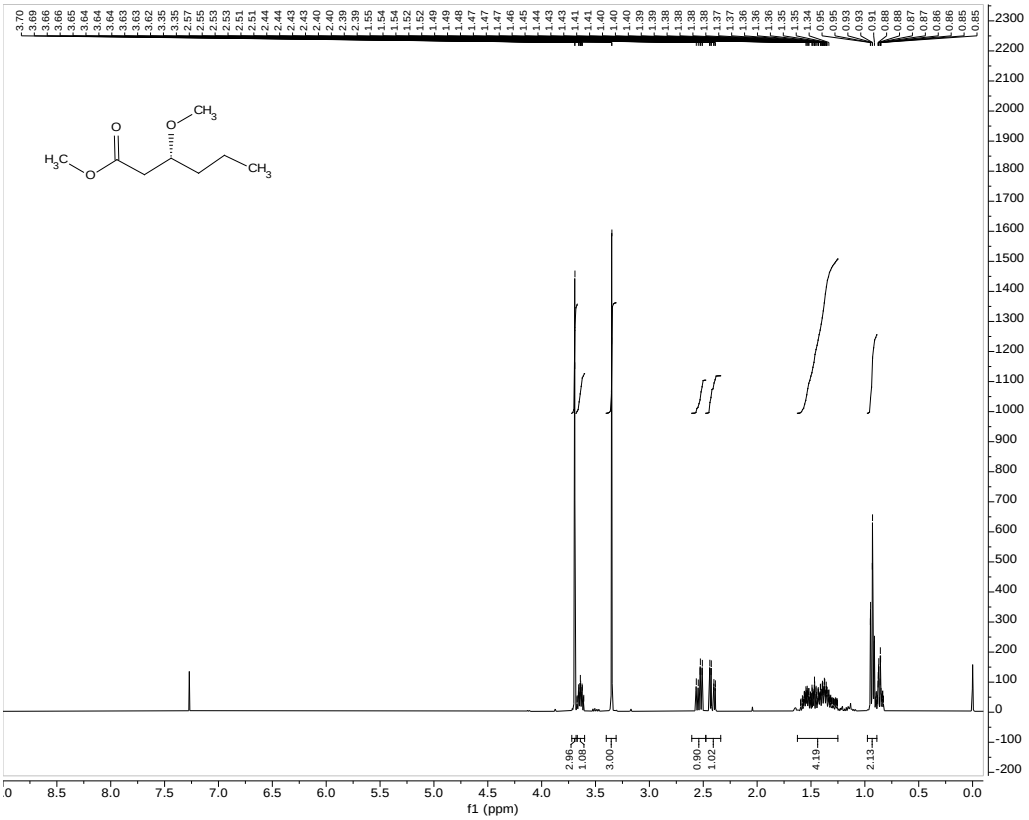


Compound **34**

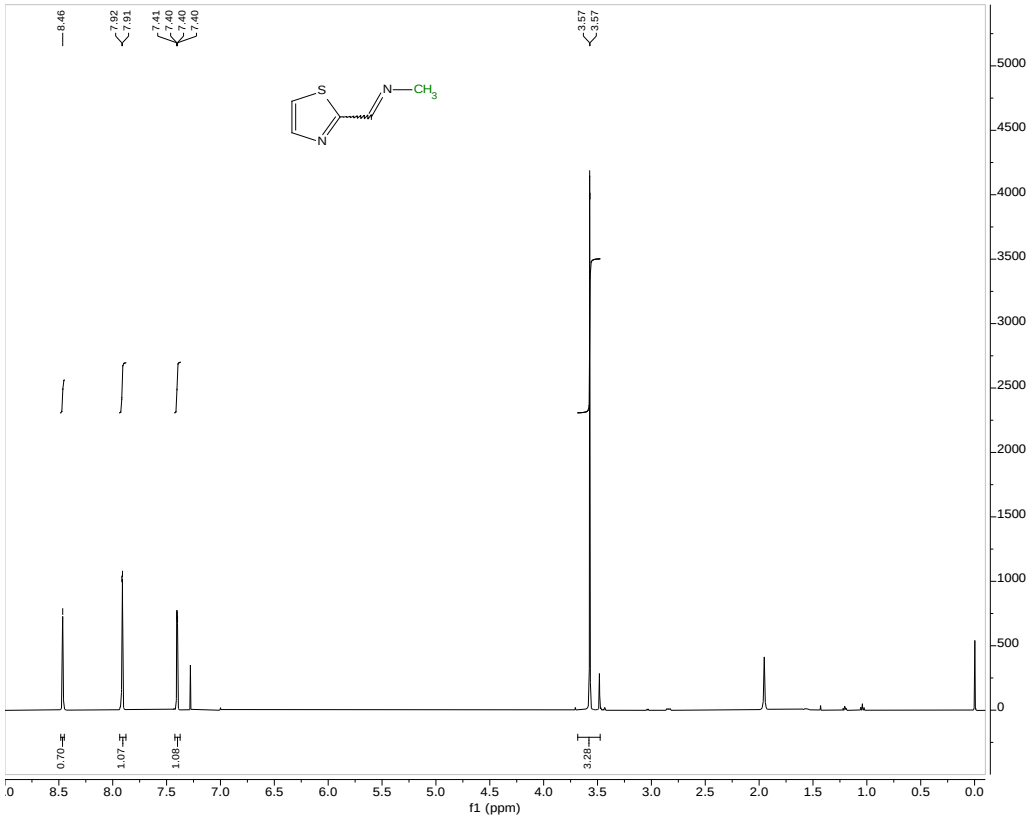

O
OH
O
CH3
CH3
1.00
3.05
0.82
0.94
1.06
1.36
2.67
f1 (ppm)


173.47
67.73
51.70
41.15
38.66
18.67
13.92
f1 (ppm)

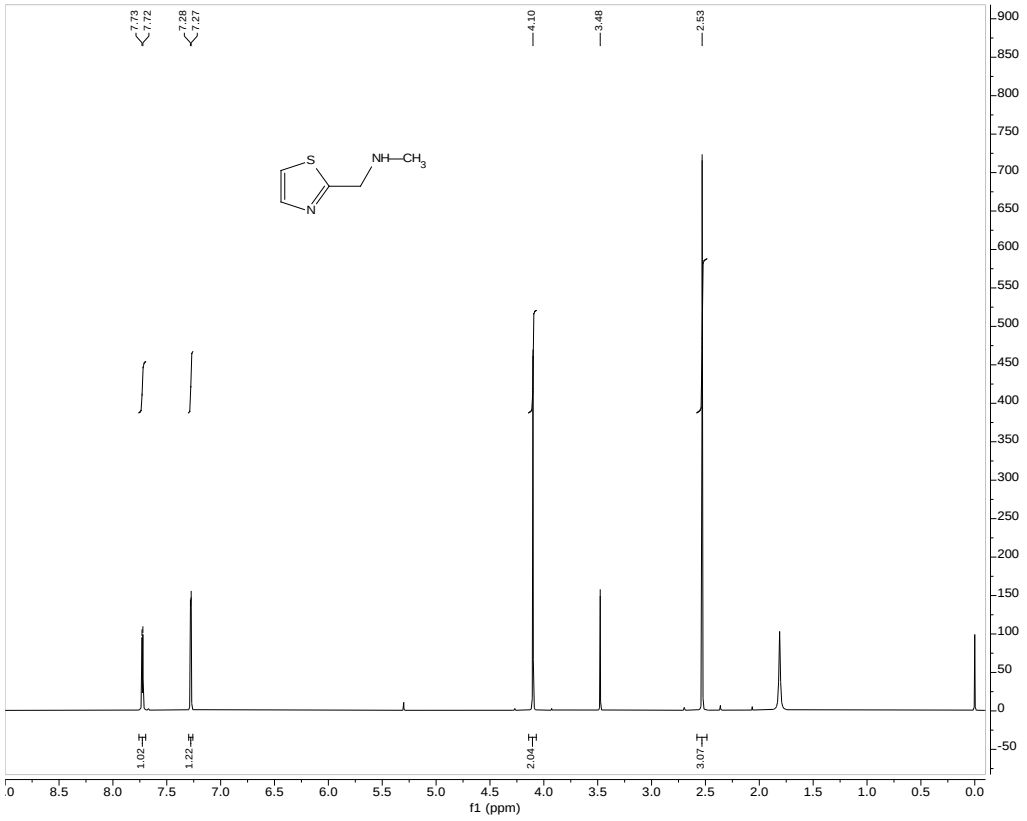
Compound **35**



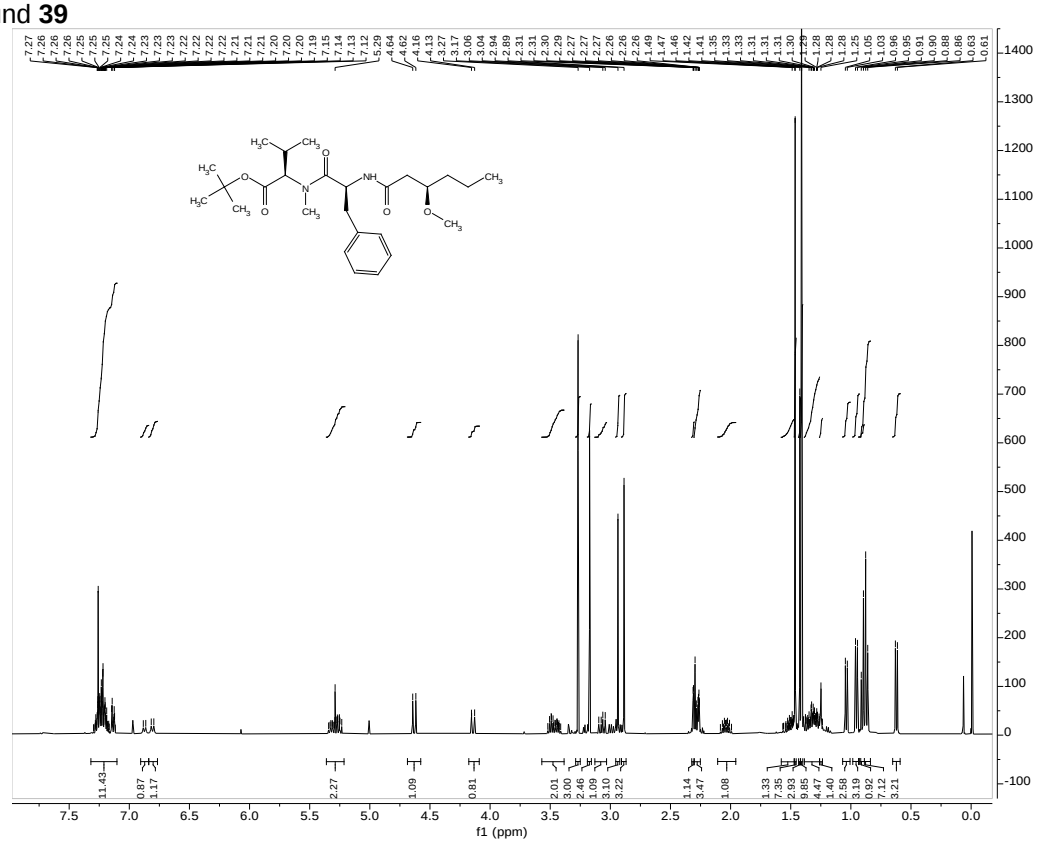
Compound **37**


8.46
7.92
7.91
7.41
7.40
7.40
7.40
3.57
3.57
S
N
N—CH3
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.70
1.07
1.08
3.28
f1 (ppm)

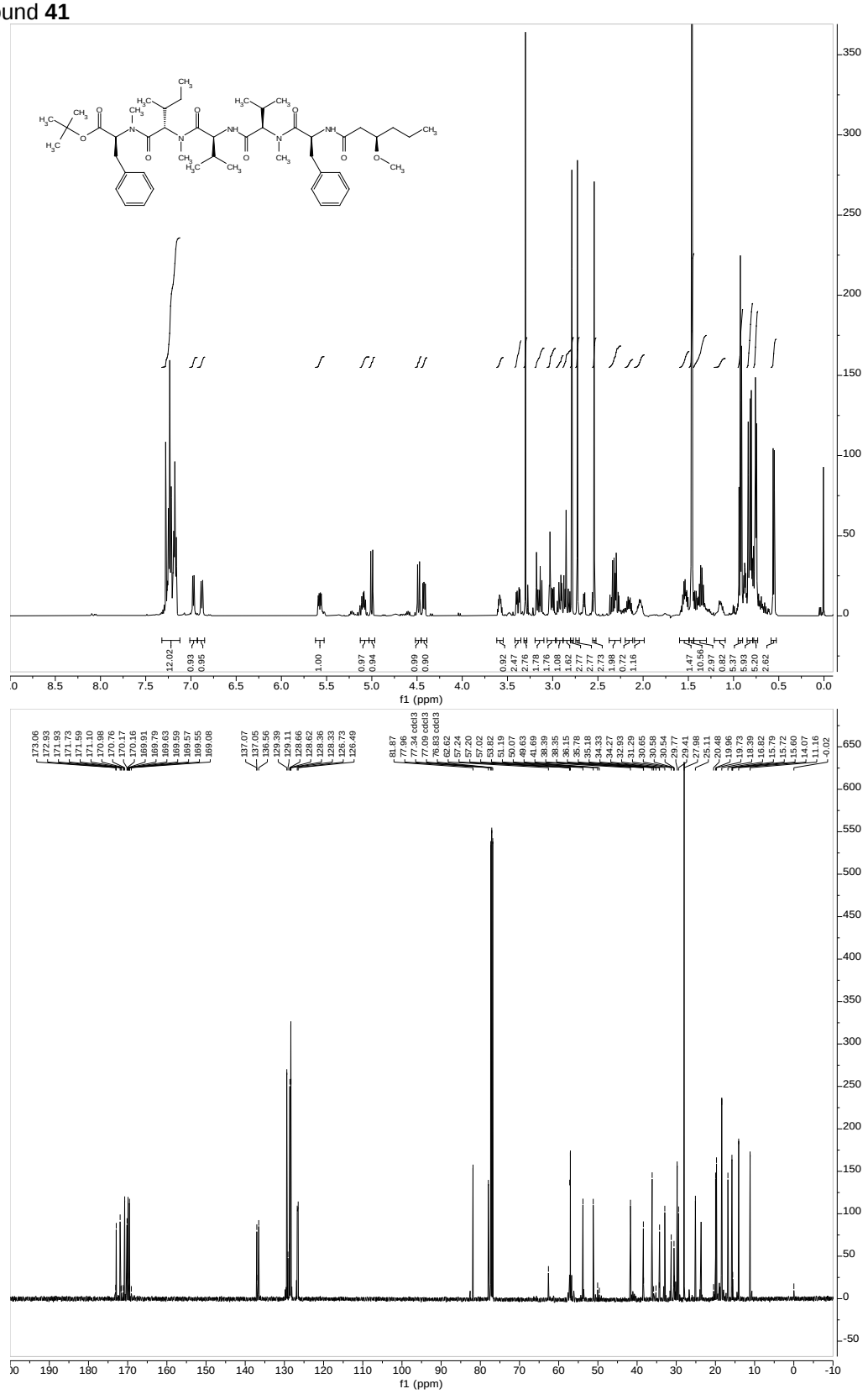
Compound **38**



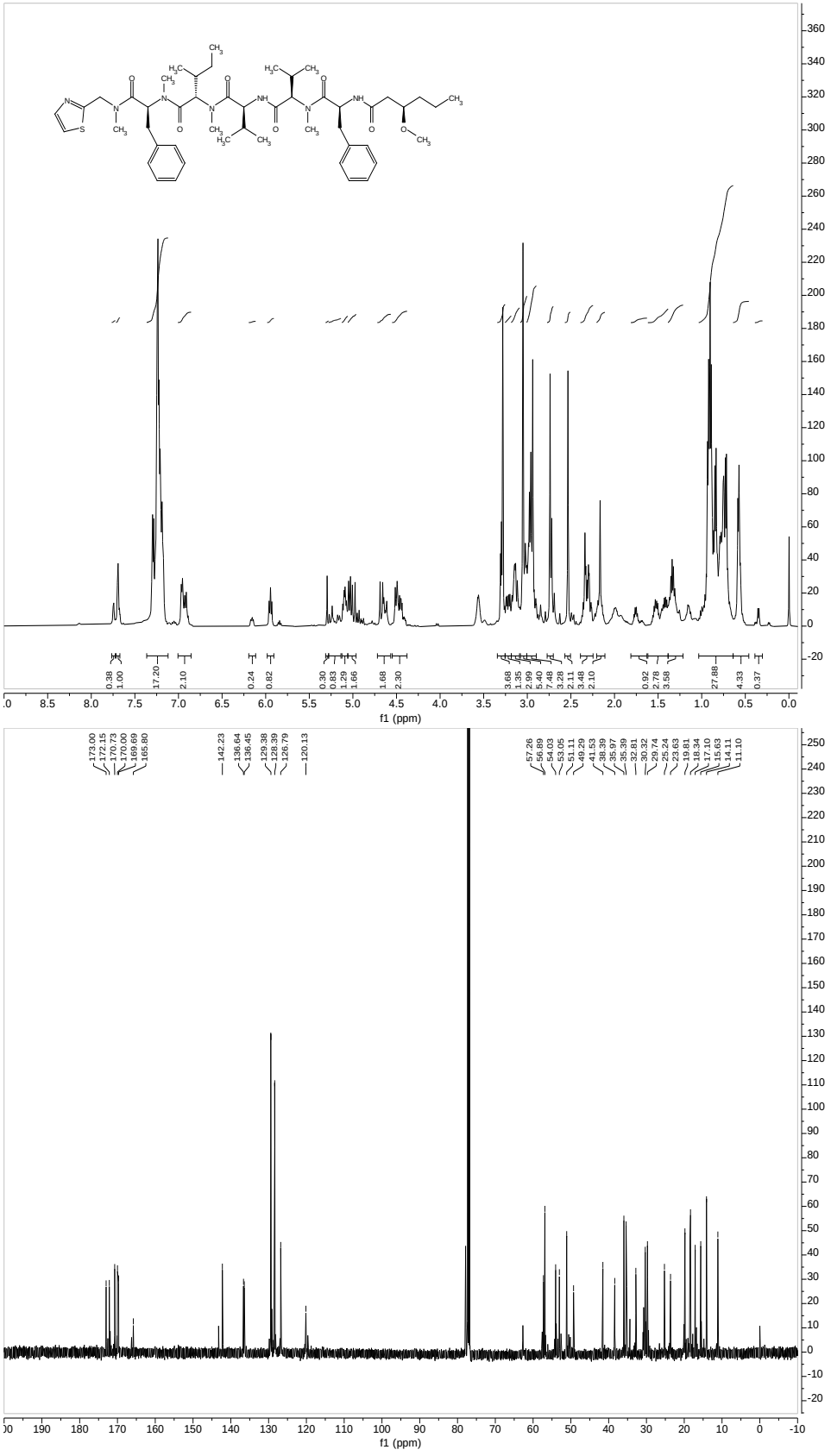
Compund **39**



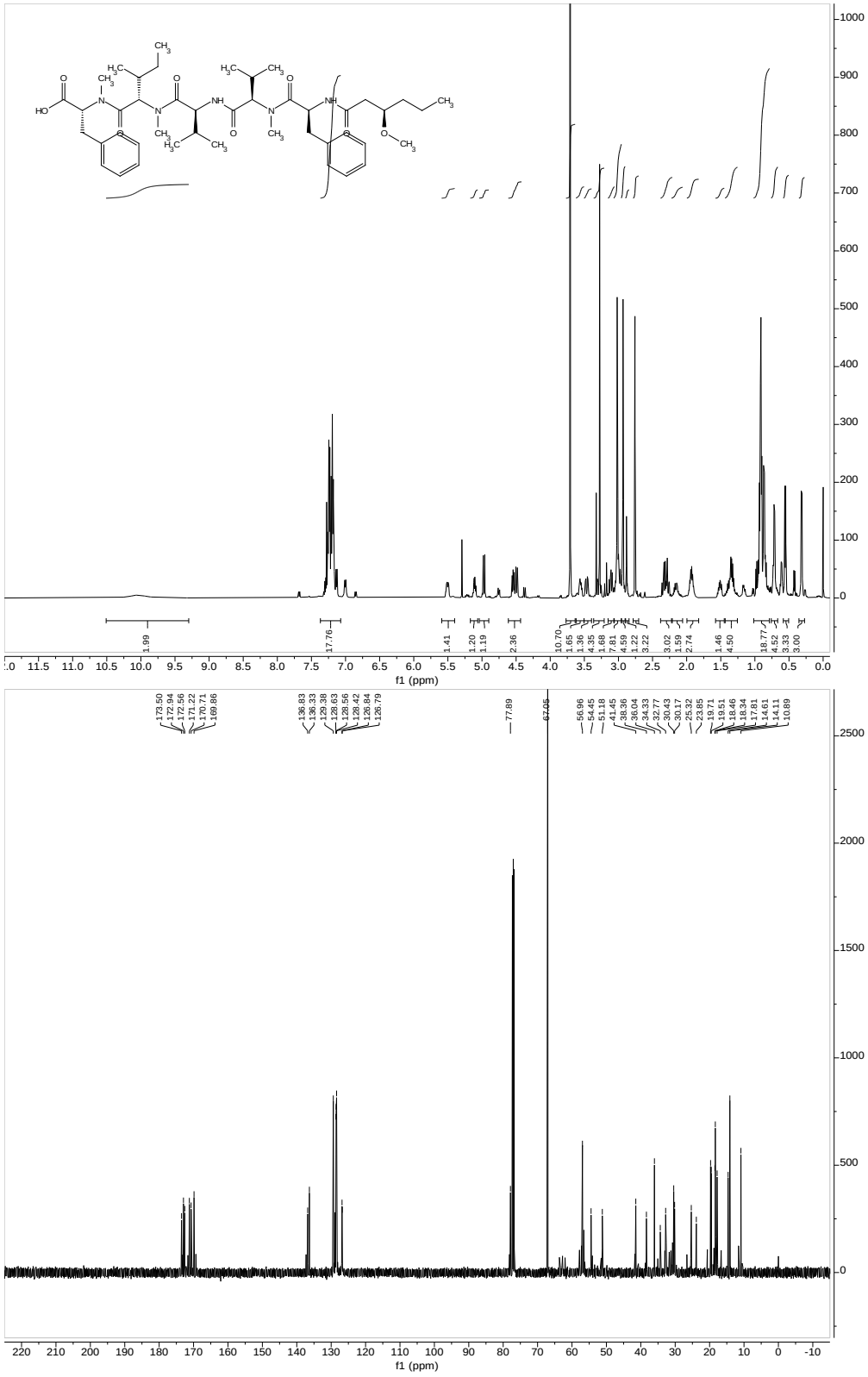
Compound **41**



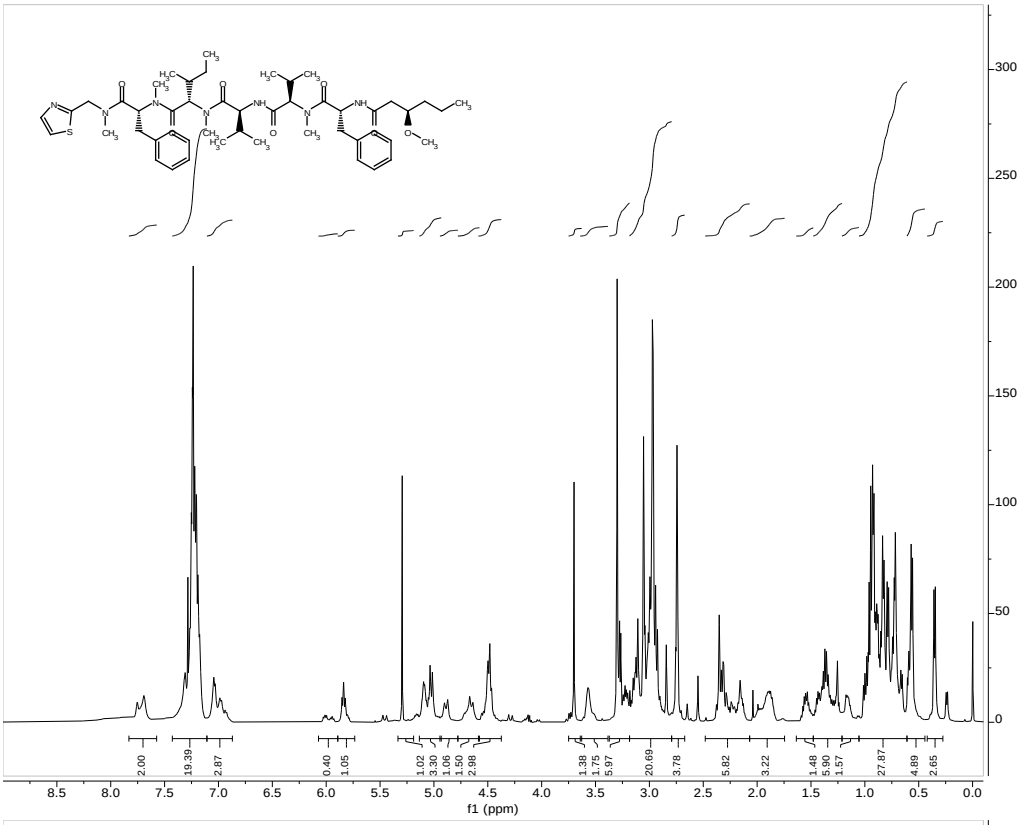
Compound **1**

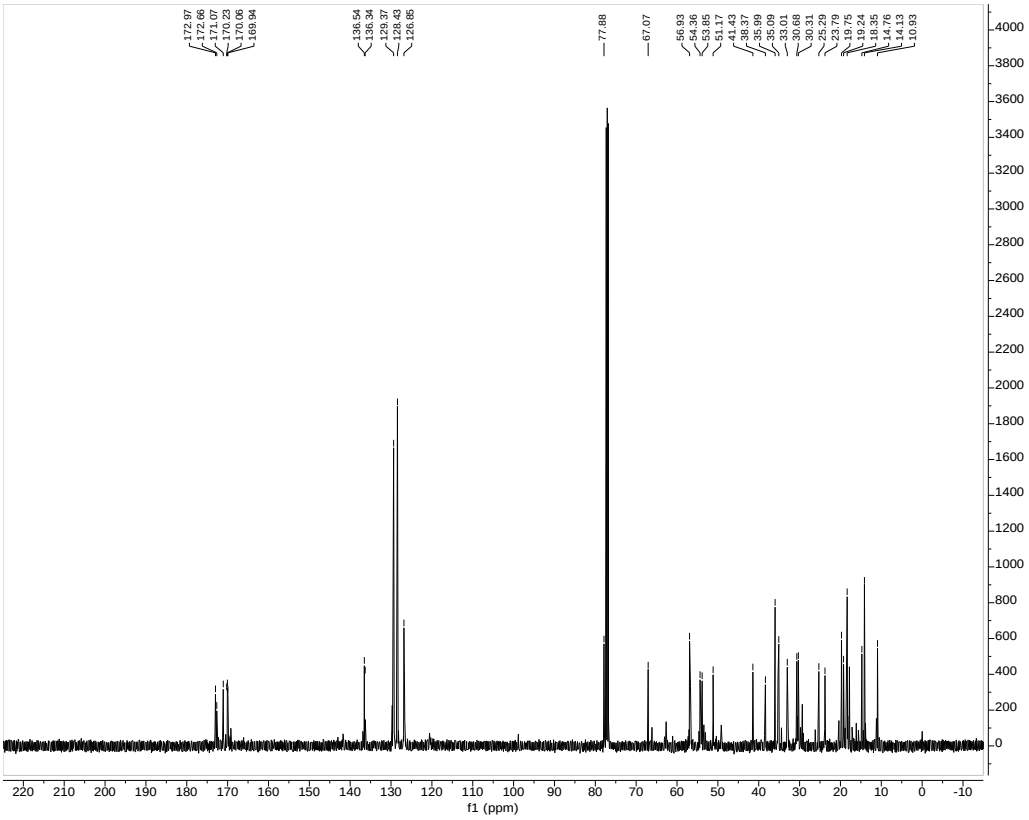

0.38
1.00
17.20
2.10
0.24
0.82
0.30
0.83
1.29
1.66
1.68
2.30
3.68
1.35
2.99
5.40
7.48
3.28
2.11
3.48
2.10
0.92
2.78
3.58
27.88
4.33
0.37
f1 (ppm)
173.00
172.15
171.23
170.00
169.69
165.80
142.23
136.64
136.45
129.38
128.39
126.79
120.13
57.26
56.89
54.03
53.65
51.11
49.29
41.53
38.39
35.97
35.39
32.81
30.32
29.74
25.24
23.63
19.81
18.34
17.10
15.63
14.11
11.10
f1 (ppm)

Compound **43**

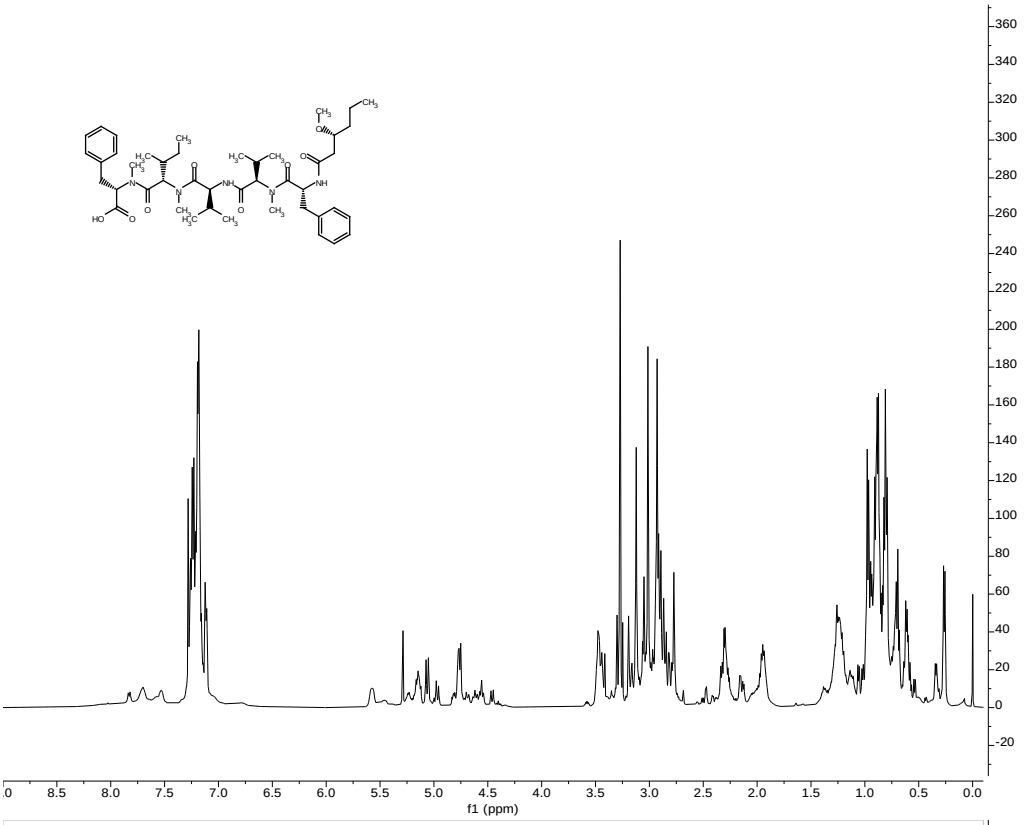

1.99
17.76
1.41
1.20
1.19
2.36
10.70
1.65
1.36
4.35
3.69
7.81
4.59
3.22
1.22
3.02
1.59
2.74
1.46
4.50
18.77
4.52
3.33
3.00
f1 (ppm)
173.50
172.94
172.56
171.22
170.71
169.86
136.83
136.33
129.38
128.63
128.56
128.42
126.84
126.79
77.89
67.05
56.96
54.45
51.18
41.45
38.36
36.04
34.33
32.77
30.43
30.17
25.32
23.85
19.97
19.51
18.46
18.34
17.81
14.61
14.11
10.89
f1 (ppm)

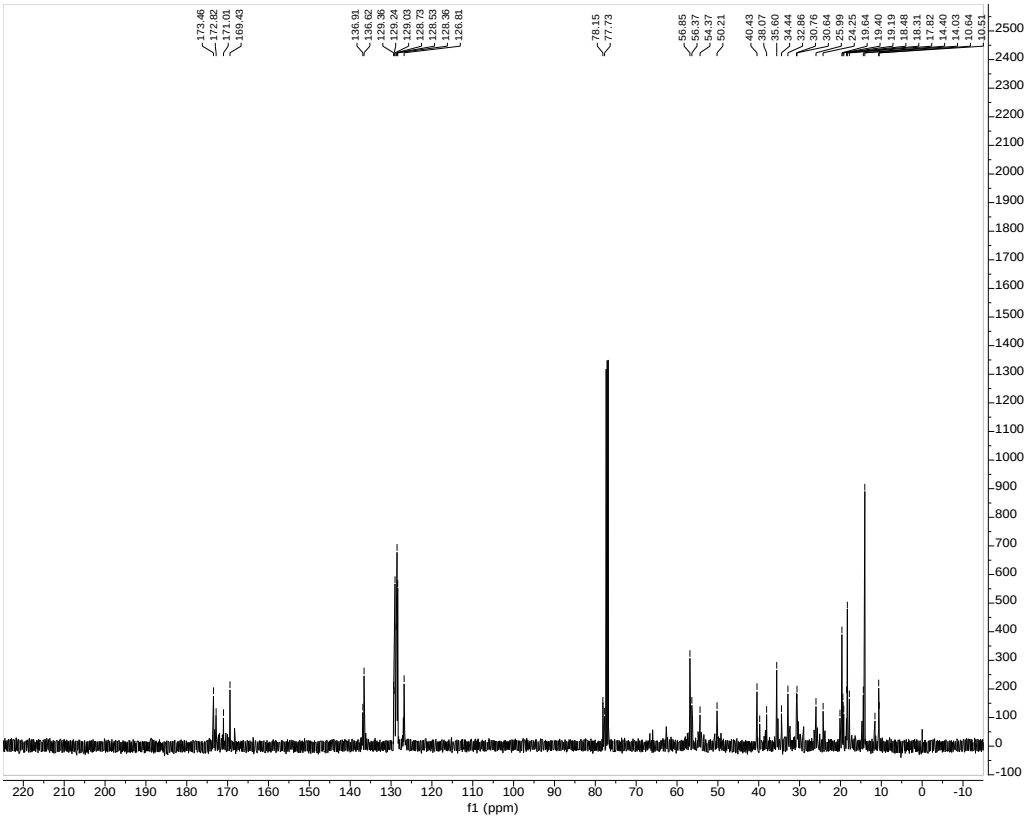
Compound **1a**


2.00
19.39
2.87
0.40
1.05
1.02
3.30
1.06
1.50
2.98
1.38
1.75
5.97
20.69
3.78
5.82
3.22
1.48
5.90
1.57
27.87
4.89
2.65
f1 (ppm)

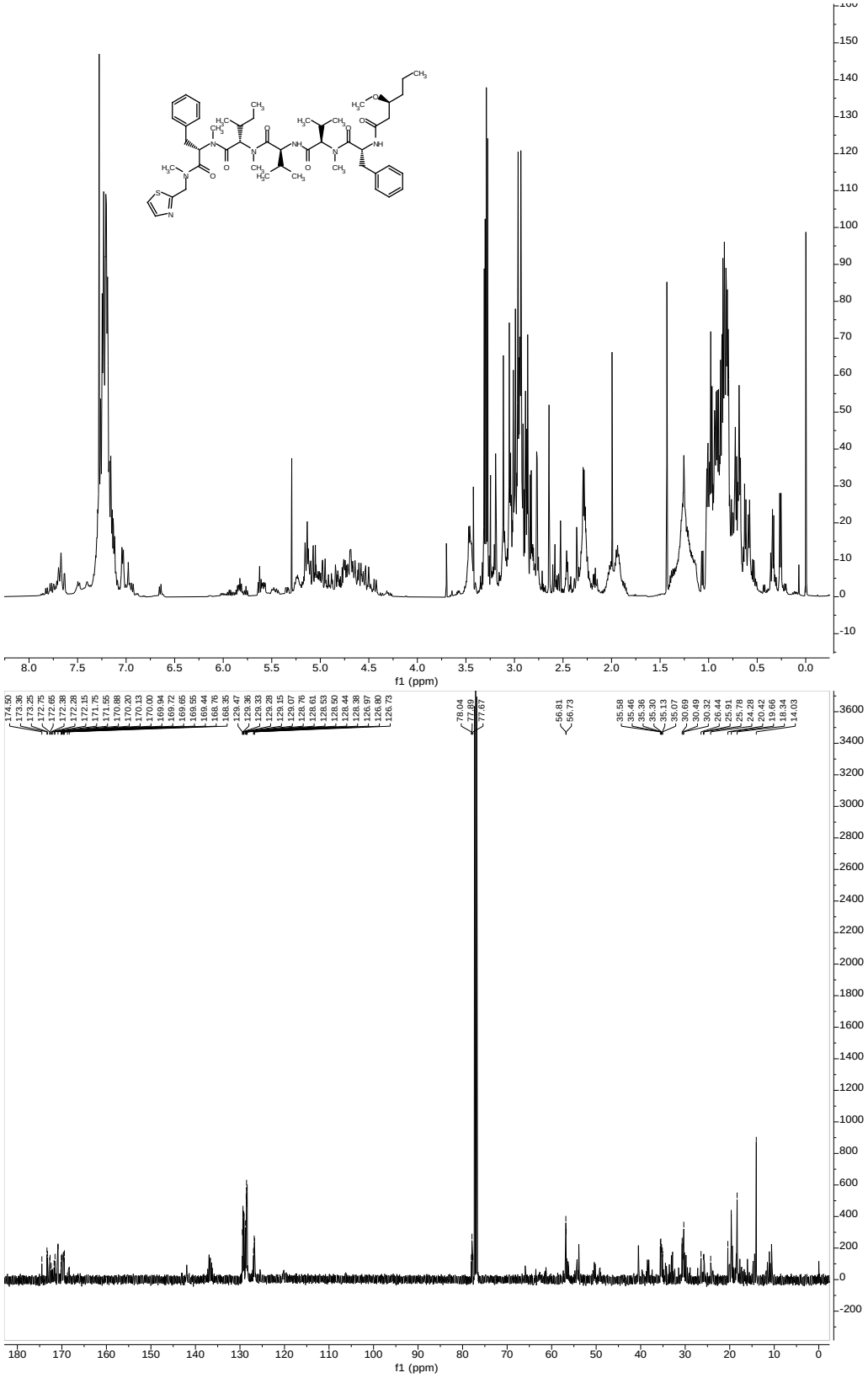

172.97
172.66
171.07
170.23
170.06
169.94
136.54
136.34
129.37
128.43
126.85
77.88
67.07
56.93
54.36
53.85
51.17
41.43
38.37
35.99
35.09
33.01
30.68
30.31
25.29
23.79
19.75
19.24
18.35
14.76
14.13
10.93
f1 (ppm)

Compound **44**

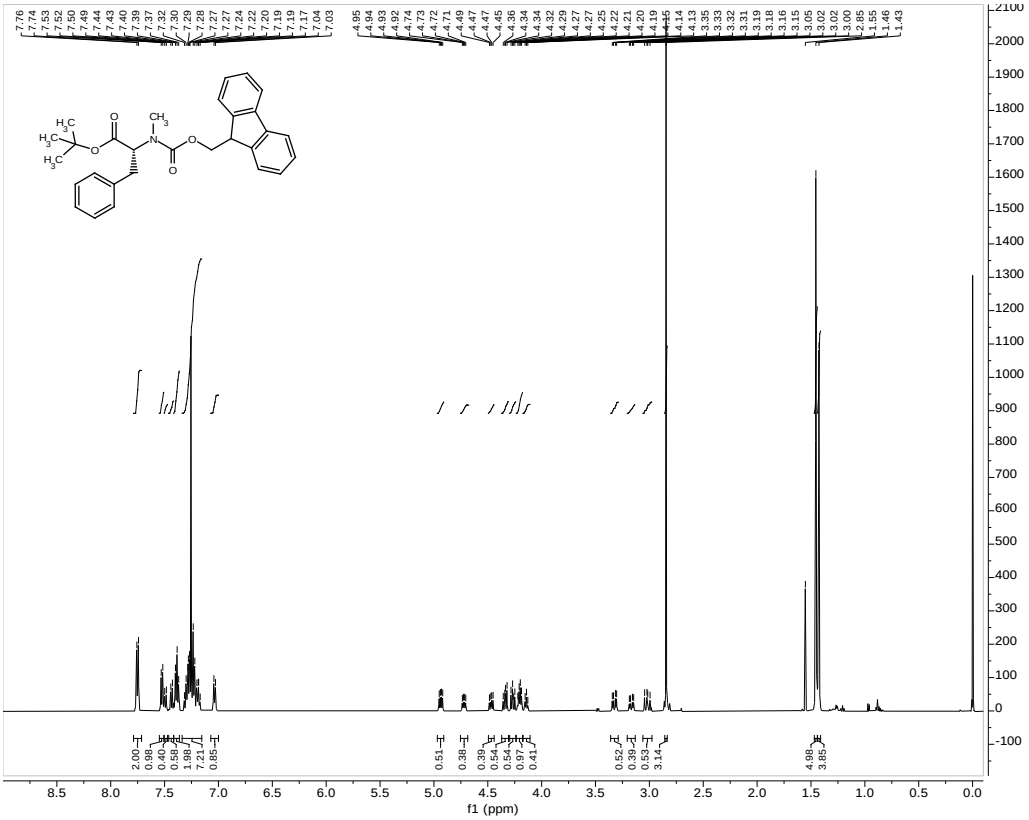

f1 (ppm)


173.46
172.82
171.01
169.43
136.91
136.62
129.36
129.24
129.03
128.73
128.53
128.36
126.81
78.15
77.73
56.85
56.37
54.37
50.21
40.43
38.07
35.60
34.44
32.86
30.76
30.64
25.99
24.25
19.64
19.40
19.19
18.46
18.31
17.82
14.40
14.03
10.64
10.51
f1 (ppm)

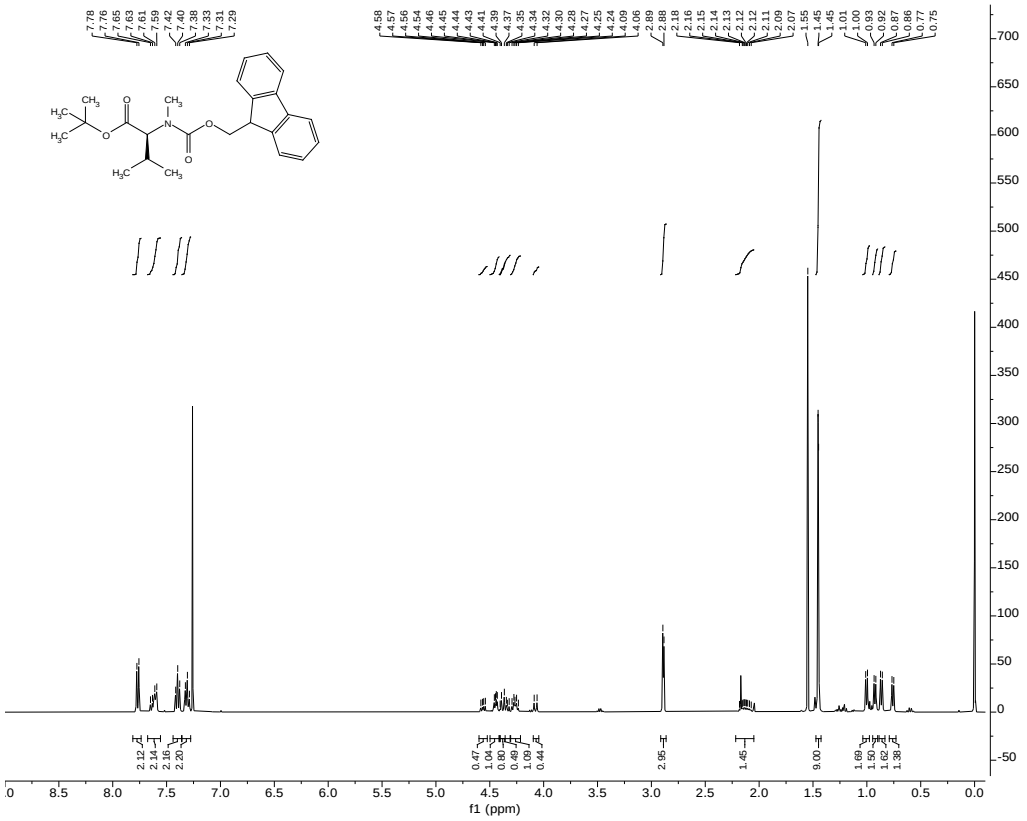
Compound **1b**


f1 (ppm)
174.90
173.36
173.25
172.75
172.65
172.38
172.28
172.15
171.75
171.55
170.88
170.20
170.03
170.00
169.94
169.72
169.65
169.55
169.44
168.76
168.35
129.47
129.36
129.33
129.28
129.15
128.97
128.76
128.61
128.53
128.50
128.44
128.38
126.97
126.80
126.73
78.04
77.89
77.67
56.81
56.73
35.58
35.46
35.36
35.30
35.13
35.07
30.69
30.49
30.32
26.44
25.91
25.78
24.28
20.42
19.66
18.34
14.03
f1 (ppm)

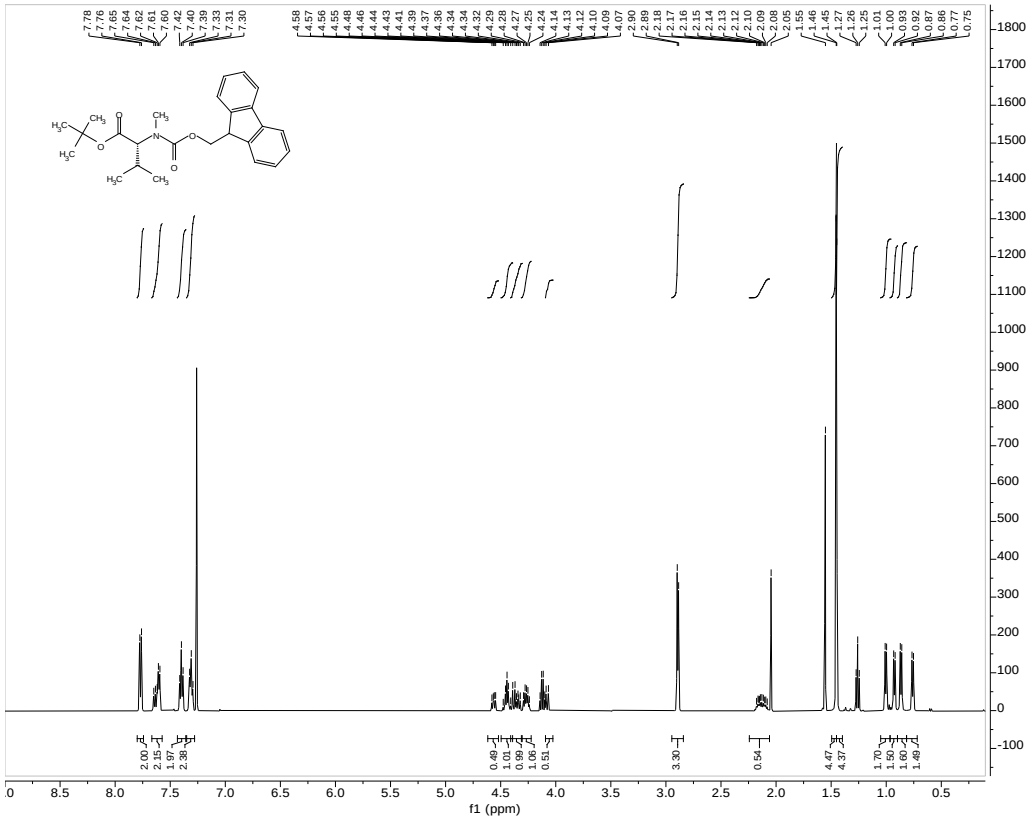
Compound **48**



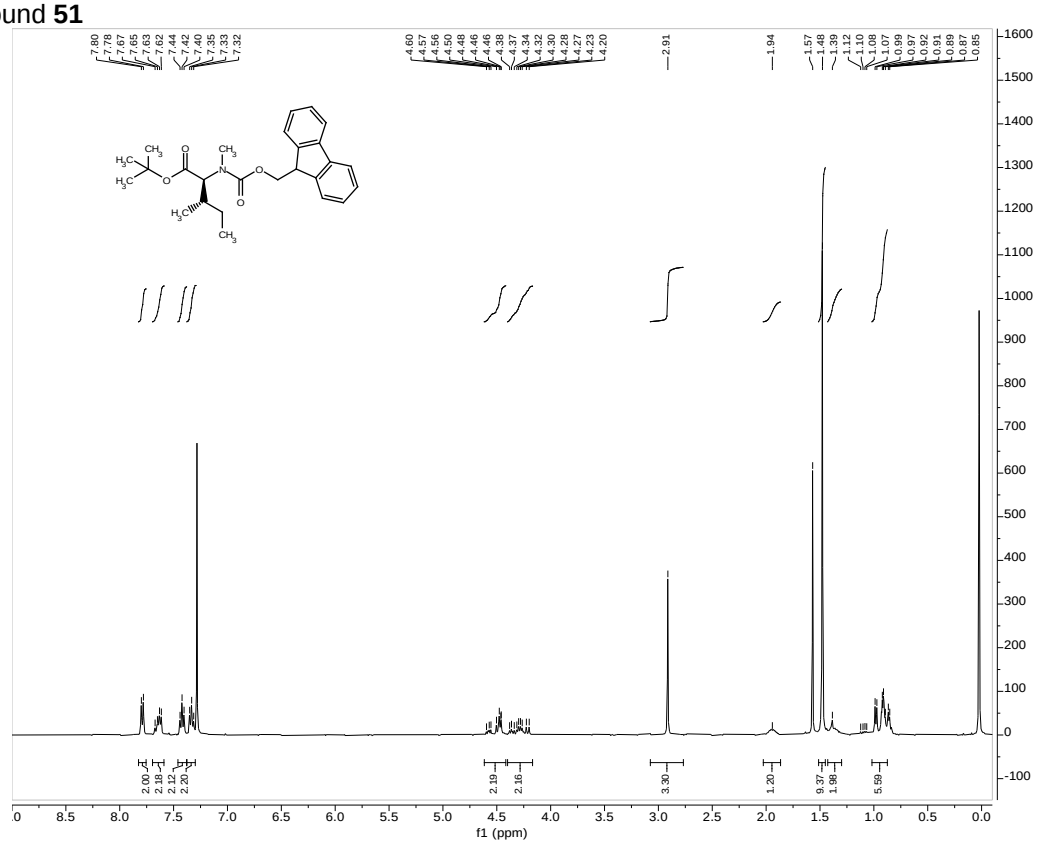
Compound **49**


7.78
7.76
7.65
7.63
7.61
7.59
7.42
7.40
7.38
7.33
7.31
7.29
4.58
4.57
4.56
4.54
4.46
4.45
4.44
4.43
4.41
4.39
4.37
4.35
4.34
4.32
4.30
4.28
4.27
4.25
4.24
4.09
4.06
2.89
2.88
2.18
2.16
2.15
2.14
2.13
2.12
2.12
2.11
2.09
2.07
1.55
1.45
1.45
1.01
1.00
0.93
0.92
0.87
0.86
0.77
0.75
2.12
2.14
2.16
2.20
0.47
1.04
0.98
0.49
1.09
0.44
2.95
1.45
9.00
1.69
1.50
1.62
1.38
f1 (ppm)

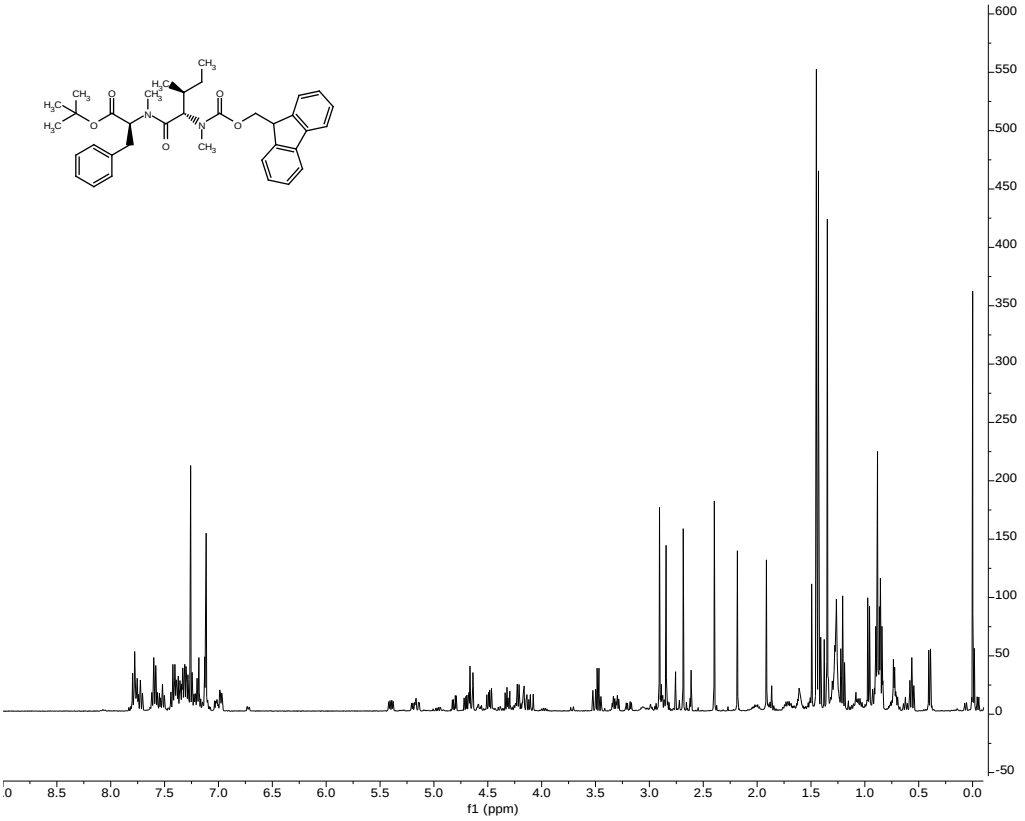
Compound **50**


7.78 7.76 7.65 7.64 7.62 7.61 7.60 7.42 7.40 7.39 7.33 7.31 7.30
4.58 4.57 4.56 4.55 4.48 4.46 4.44 4.43 4.41 4.39 4.37 4.36 4.34 4.34 4.32 4.29 4.28 4.27 4.25 4.24 4.14 4.13 4.12 4.10 4.09 4.07 2.90 2.89 2.18 2.17 2.16 2.15 2.14 2.13 2.12 2.10 2.09 2.08 2.05 1.55 1.46 1.45 1.27 1.26 1.25 1.01 1.00 0.93 0.92 0.87 0.86 0.77 0.75
2.00 2.15 1.97 2.38 0.49 1.01 0.99 1.06 0.51 3.30 0.54 4.47 4.37 1.70 1.50 1.60 1.49
f1 (ppm)

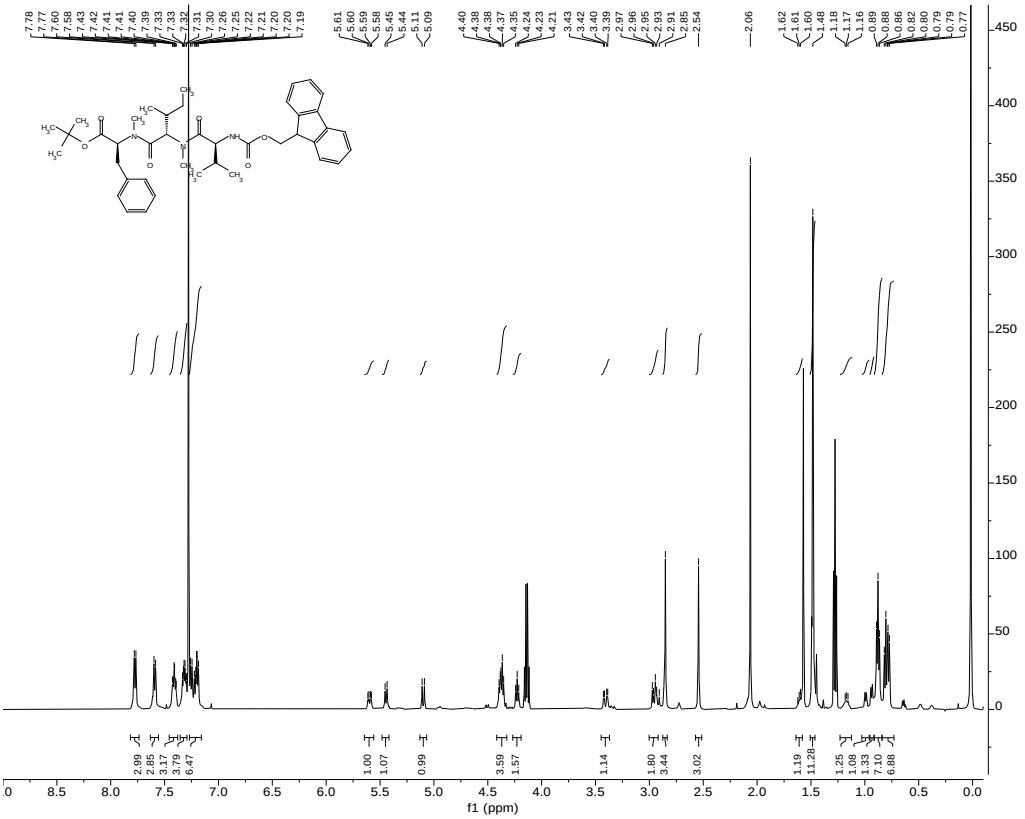
Compound **51**


7.80
7.78
7.67
7.65
7.63
7.62
7.44
7.43
7.40
7.35
7.33
7.32
4.60
4.57
4.56
4.50
4.48
4.46
4.46
4.38
4.37
4.34
4.32
4.30
4.28
4.27
4.23
4.20
2.91
1.94
1.57
1.48
1.39
1.12
1.10
1.08
1.07
0.99
0.97
0.92
0.91
0.89
0.87
0.85
2.00
2.18
2.12
2.20
2.19
2.16
3.30
1.20
9.37
1.98
5.59
f1 (ppm)

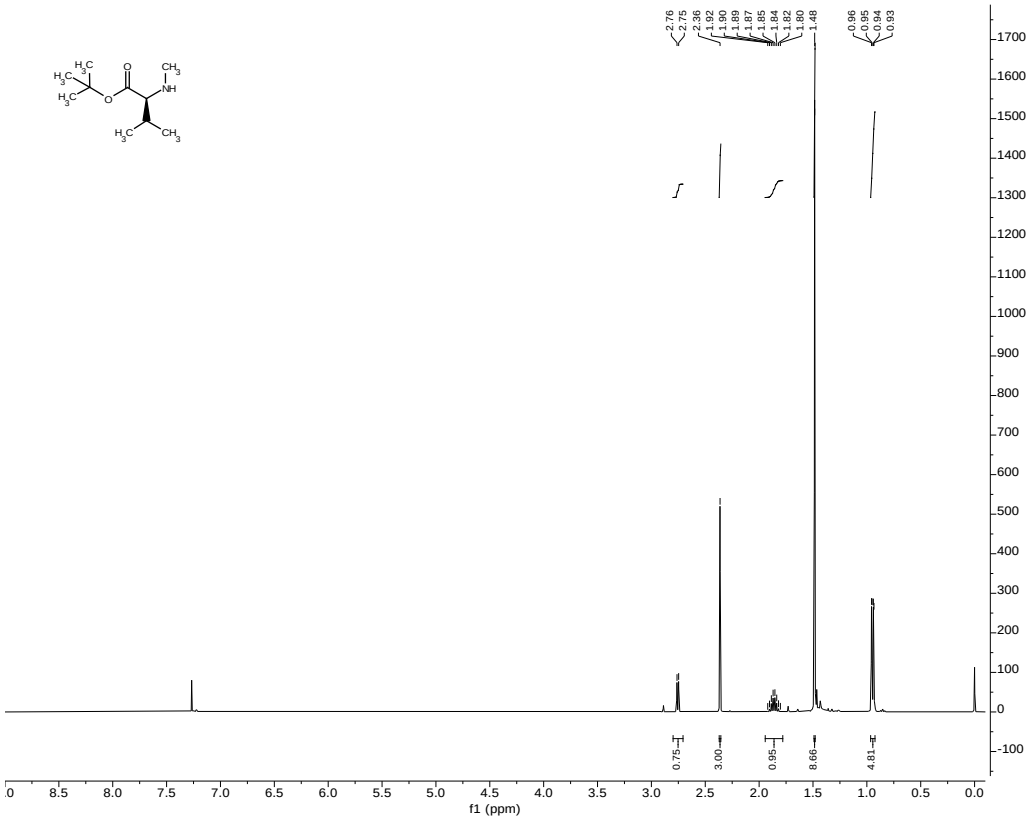
Compound **52**



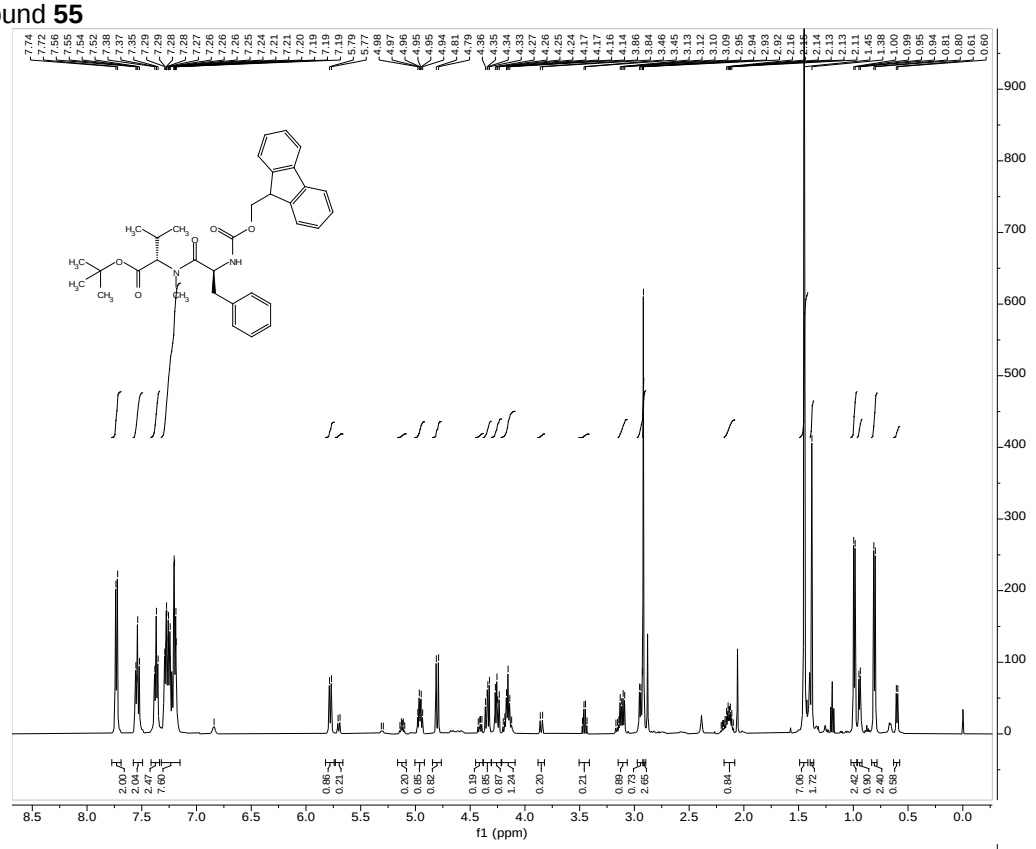
Compound **53**

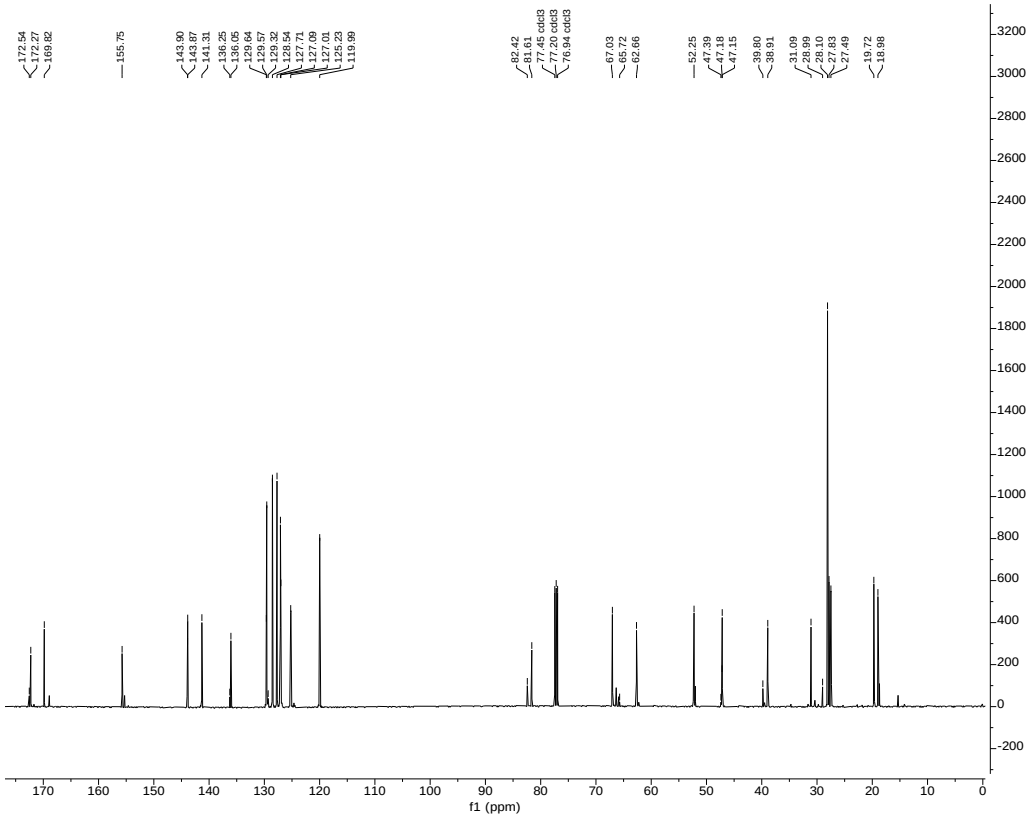

7.78 7.77 7.60 7.58 7.43 7.42 7.41 7.41 7.40 7.39 7.33 7.32 7.31 7.30 7.26 7.25 7.22 7.21 7.20 7.20 7.19
5.61 5.60 5.59 5.58 5.45 5.44 5.11 5.09
4.40 4.38 4.38 4.37 4.35 4.24 4.23 4.21
3.43 3.42 3.40 3.39
2.97 2.96 2.95 2.93 2.91 2.85 2.54
2.06
1.62 1.61 1.60 1.48 1.18 1.17 1.16
0.89 0.88 0.86 0.82 0.80 0.79 0.79 0.77
2.99 2.85 3.17 3.79 6.47
1.00 1.07
0.99
3.59 1.57
1.14
1.80 3.44
3.02
1.19 11.28
1.25 1.08 1.33 7.10 6.88
f1 (ppm)

Compound **54**

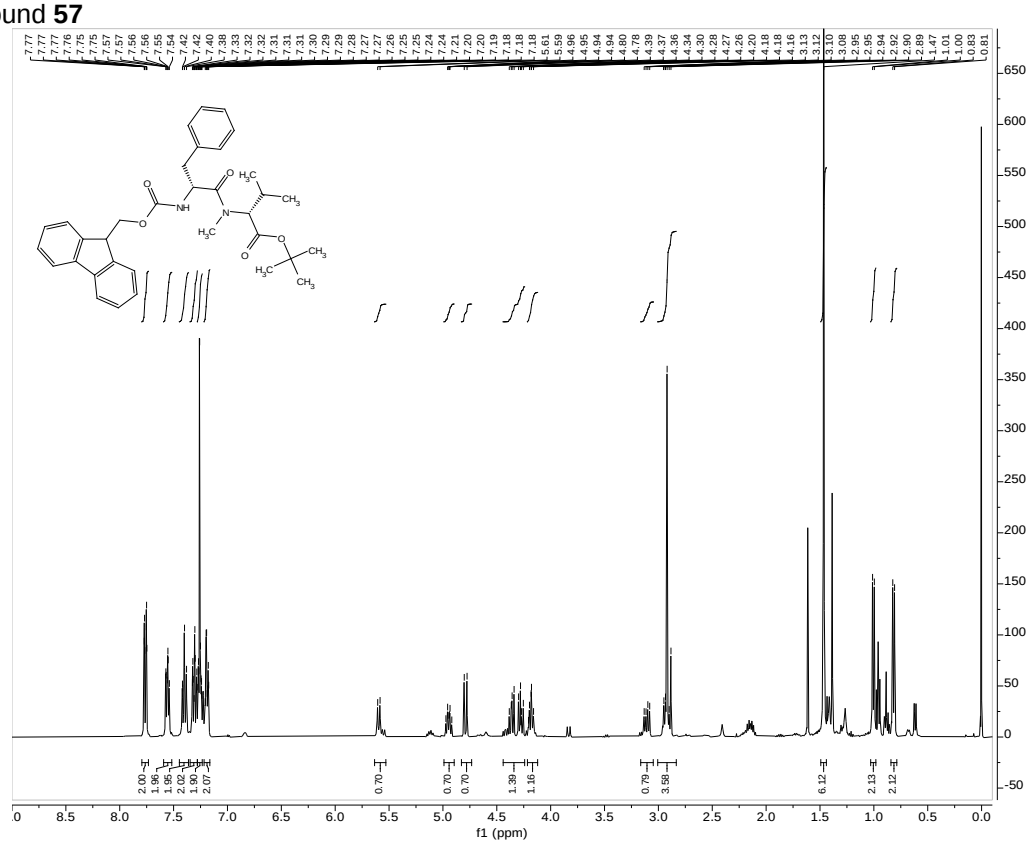

2.76
2.75
2.38
1.92
1.90
1.89
1.87
1.85
1.84
1.82
1.80
1.48
0.96
0.95
0.94
0.93
0.75
3.00
0.95
8.66
4.81
f1 (ppm)

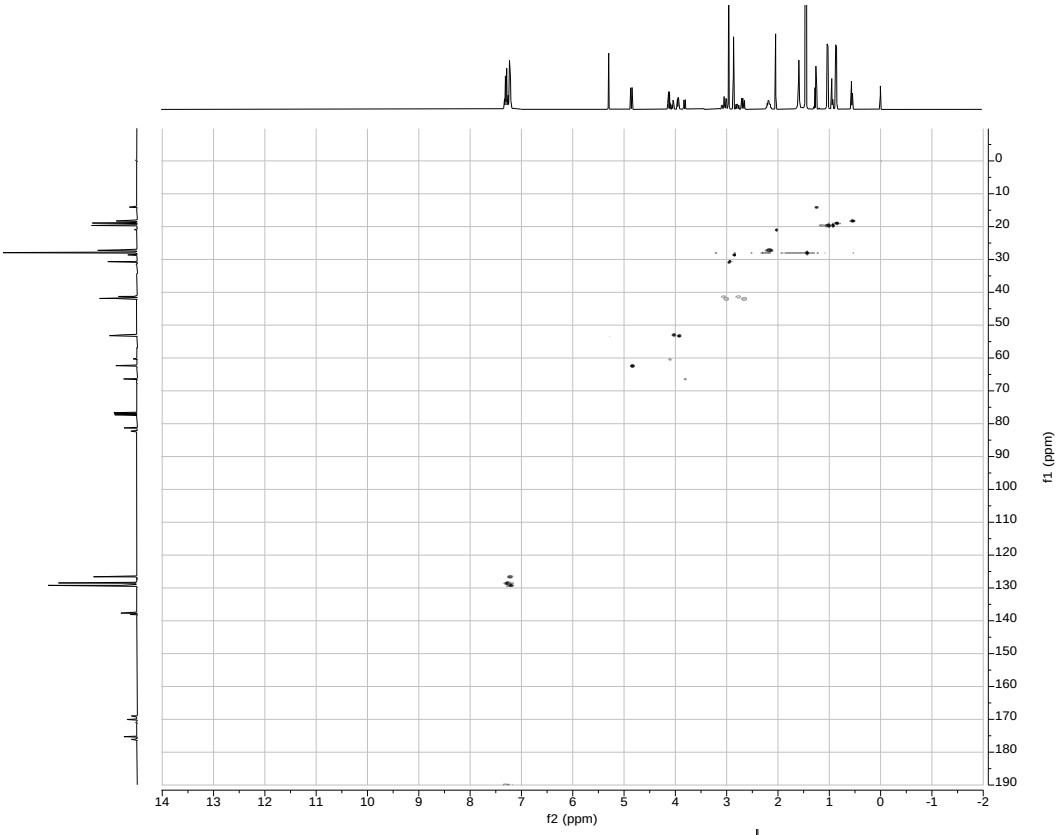
Compound **55**

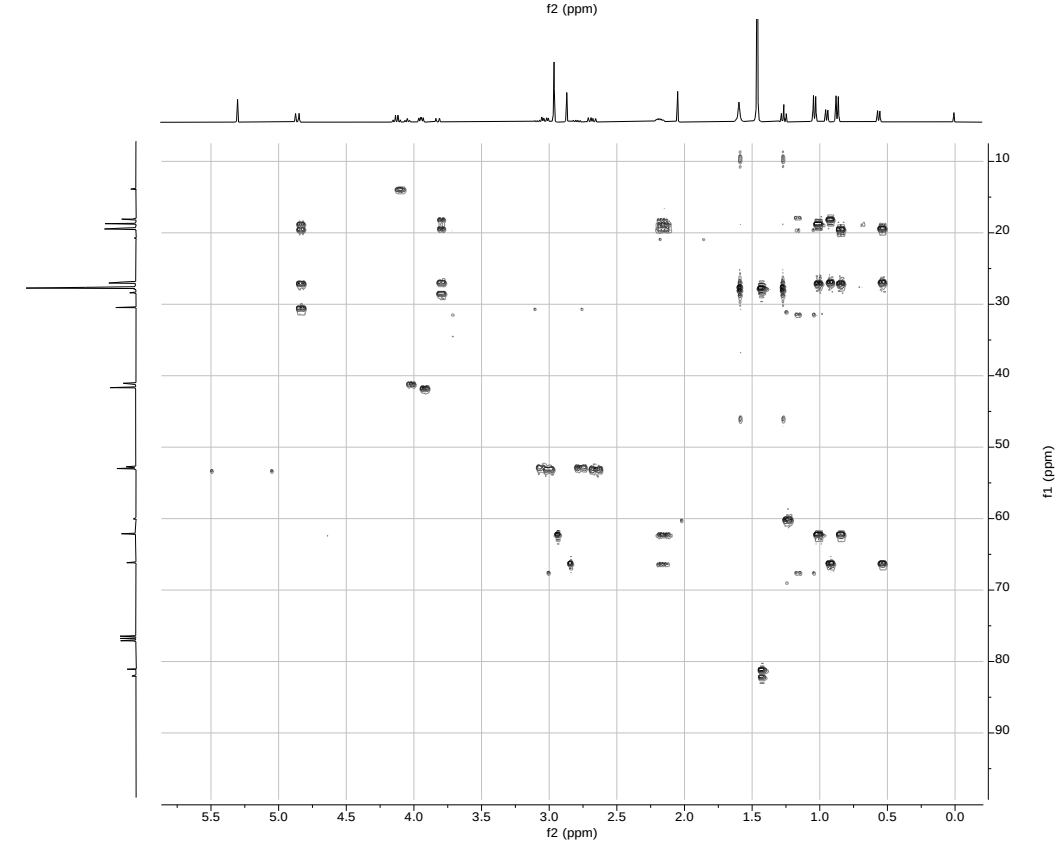




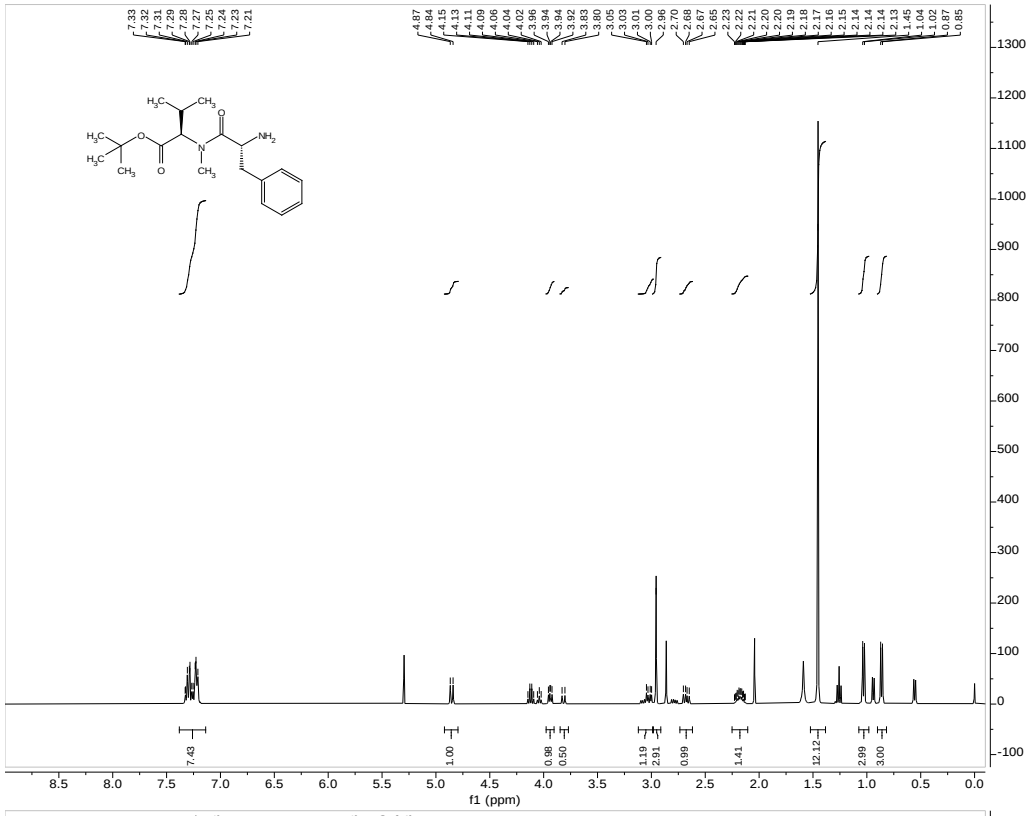
Compound **57**

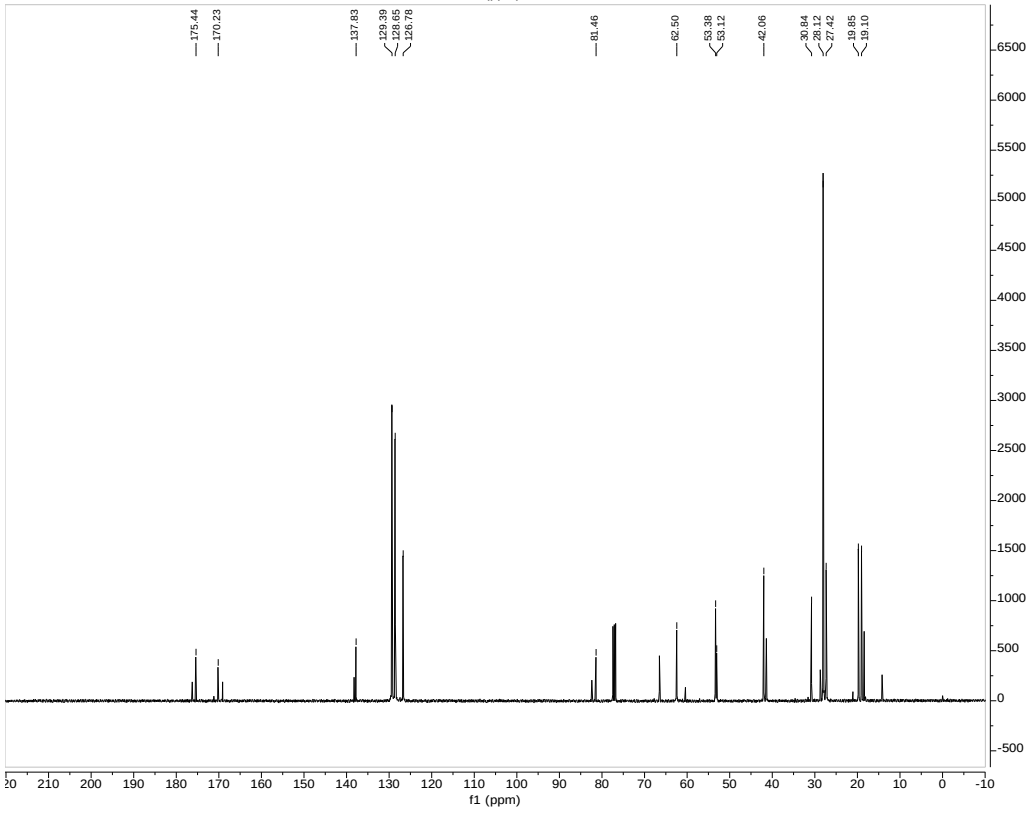



f2 (ppm)
f1 (ppm)

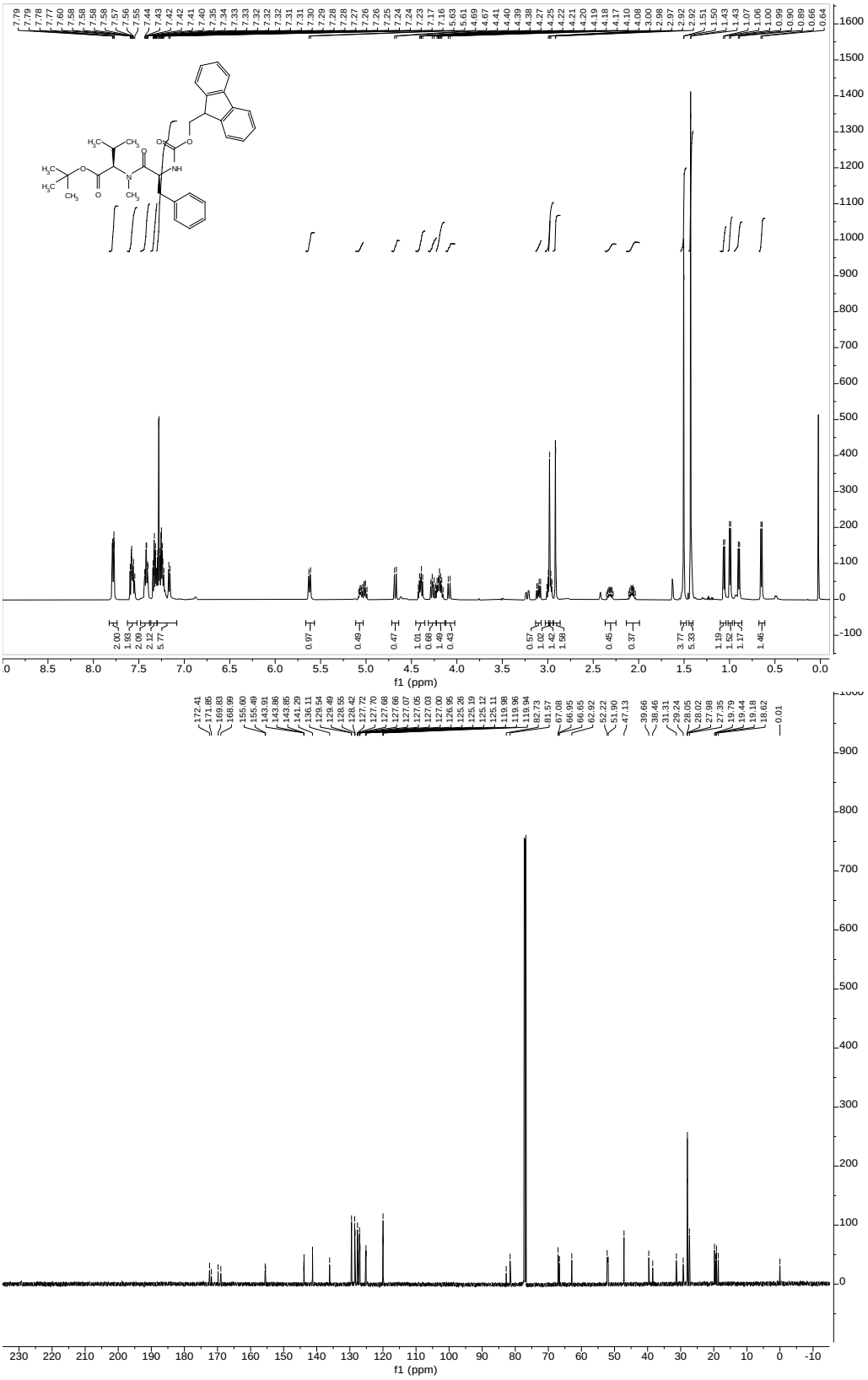

f2 (ppm)
f1 (ppm)

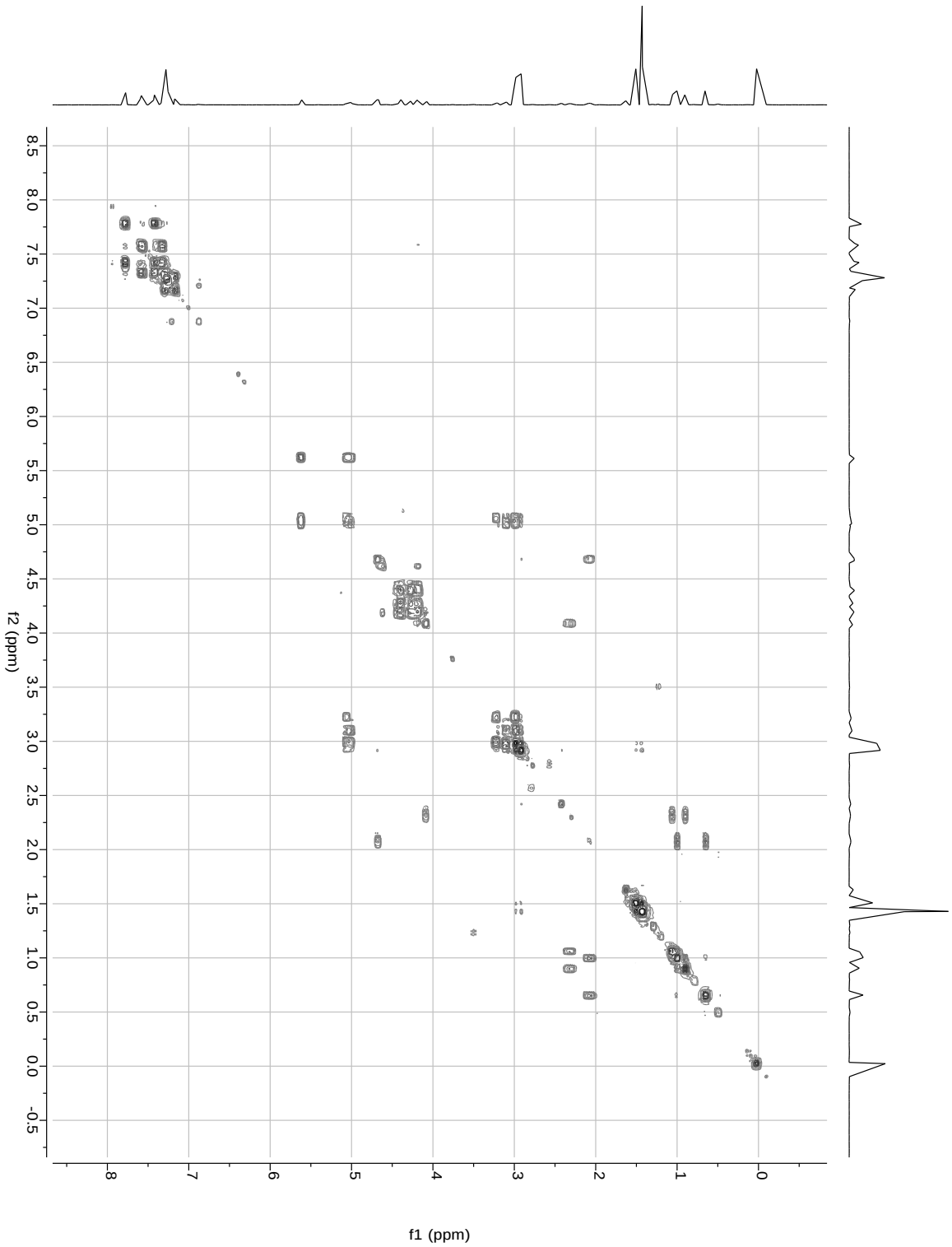
Compound **58**


7.33
7.32
7.31
7.29
7.28
7.27
7.25
7.24
7.23
7.21
4.87
4.84
4.15
4.13
4.11
4.09
4.06
4.04
4.02
3.96
3.94
3.94
3.92
3.85
3.80
3.05
3.03
3.01
3.00
2.96
2.70
2.68
2.67
2.65
2.23
2.22
2.21
2.20
2.20
2.19
2.18
2.17
2.16
2.15
2.14
2.14
2.14
2.13
1.45
1.04
1.02
0.87
0.85
7.43
1.00
0.98
0.50
1.19
2.91
0.99
1.41
12.12
2.99
3.00
f1 (ppm)

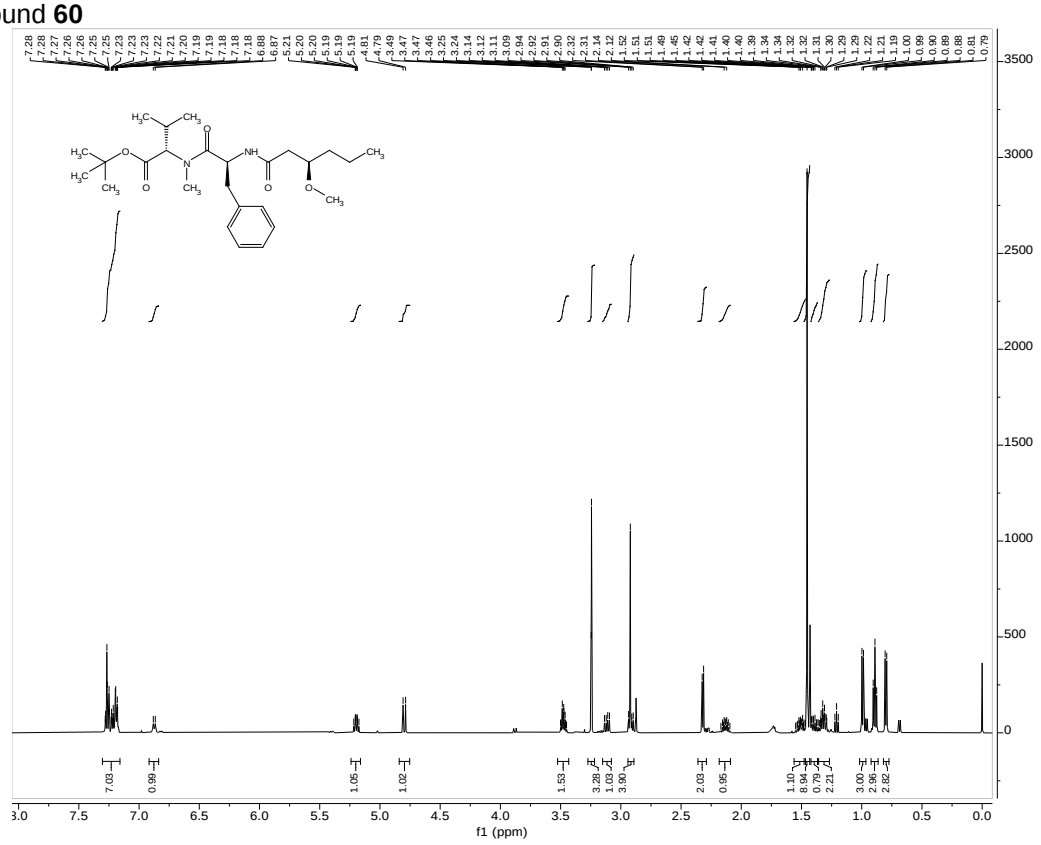

175.44
170.23
137.83
129.39
128.65
126.78
81.46
62.50
53.38
53.12
42.06
30.84
28.12
27.42
19.95
19.10
f1 (ppm)

Compound **59**

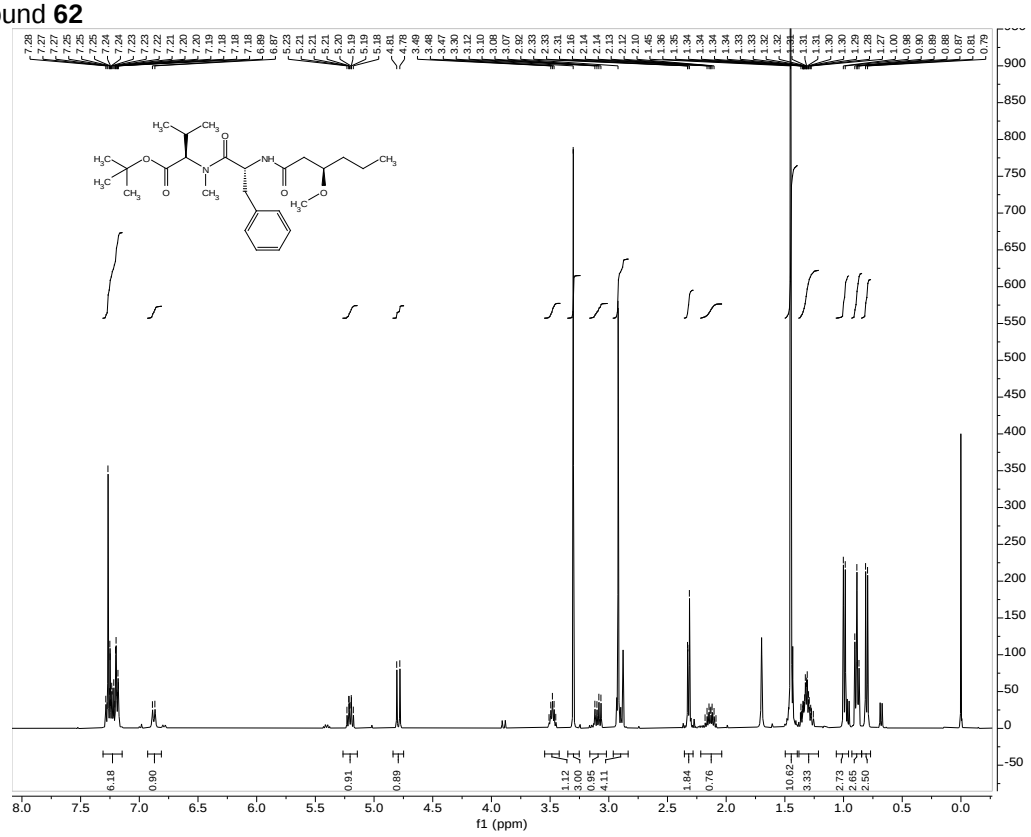



f2 (ppm)
f1 (ppm)

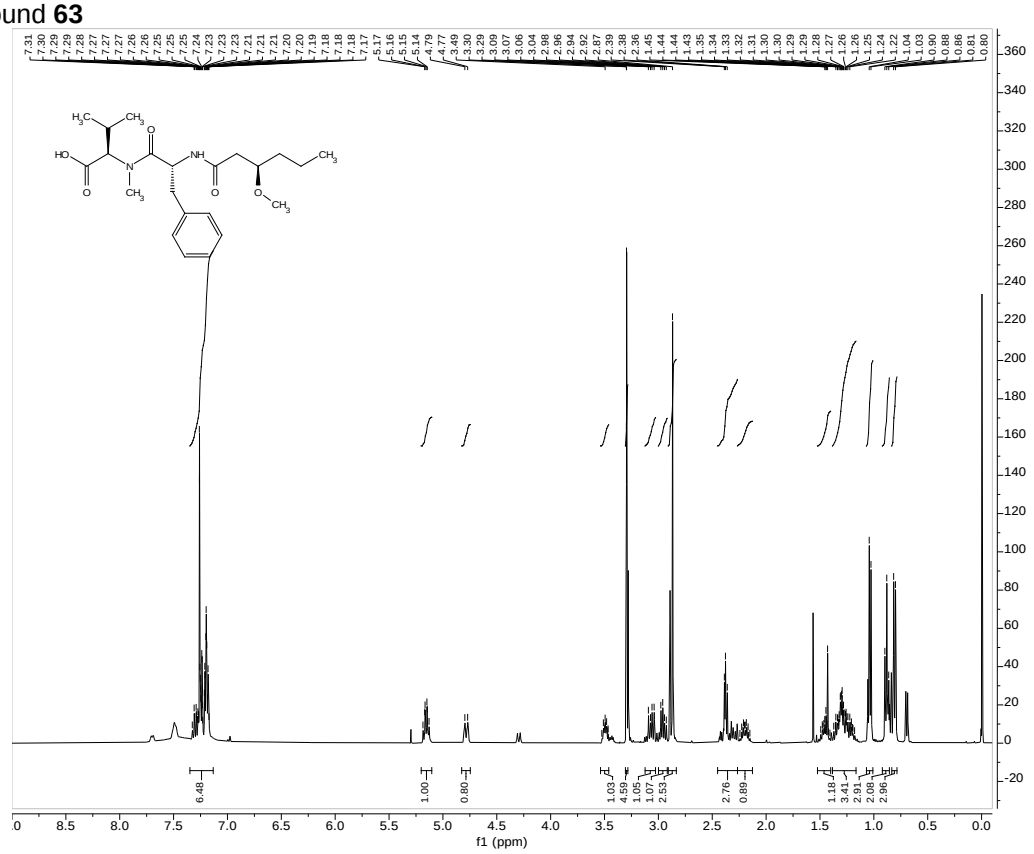
Compound **60**



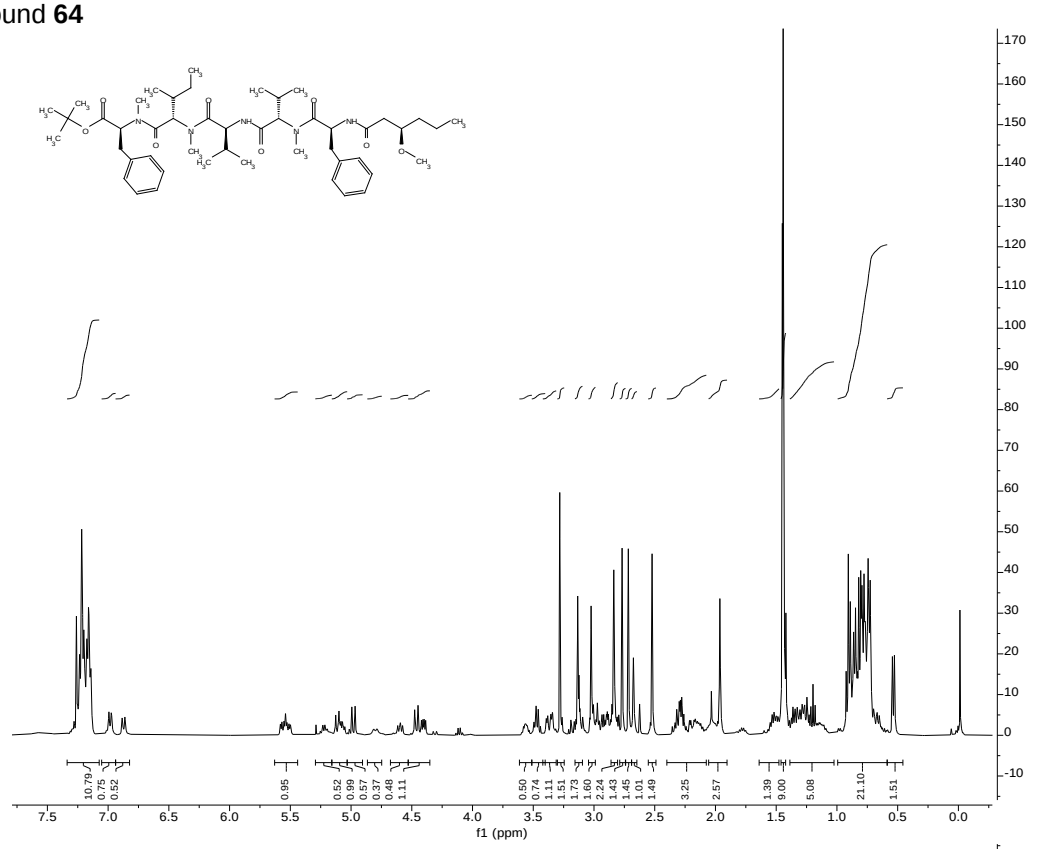
Compound **62**

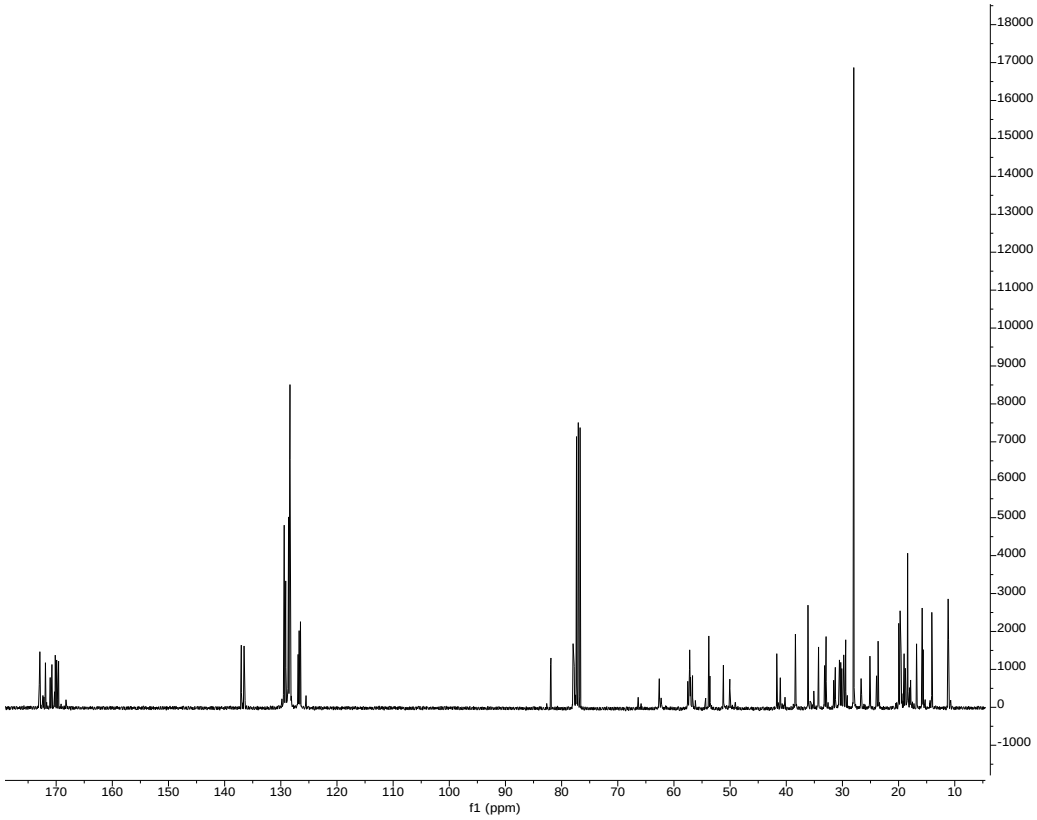


Compound **63**

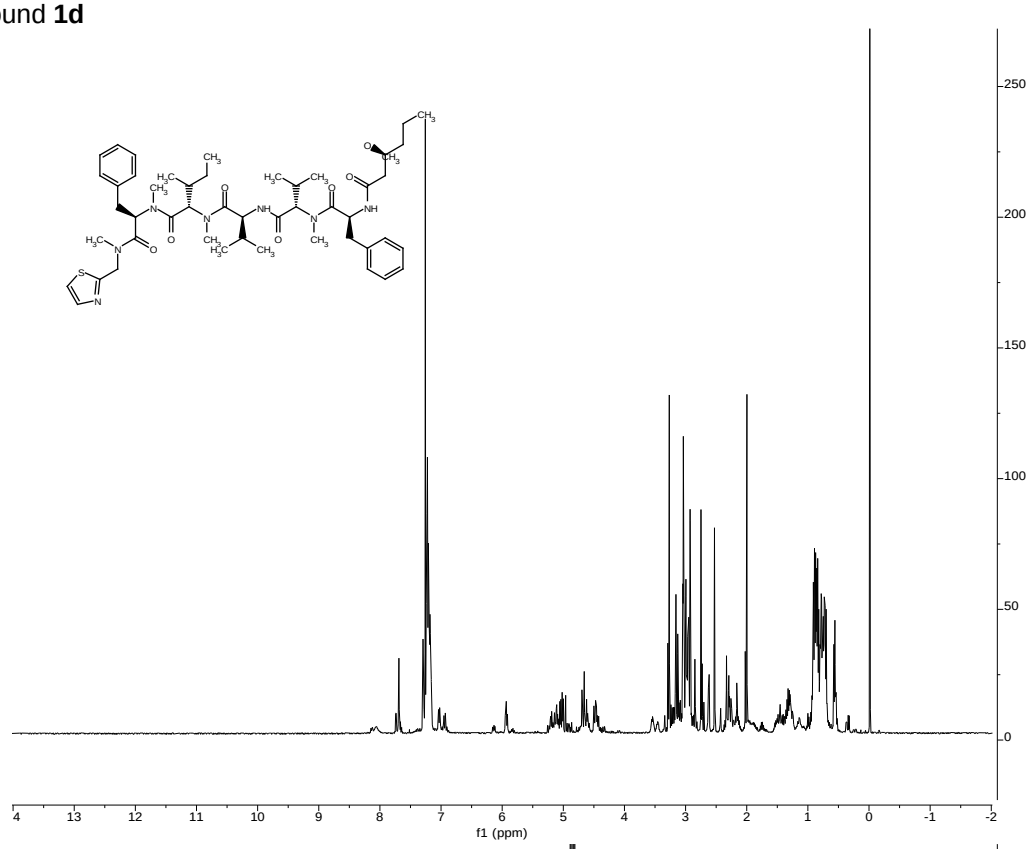


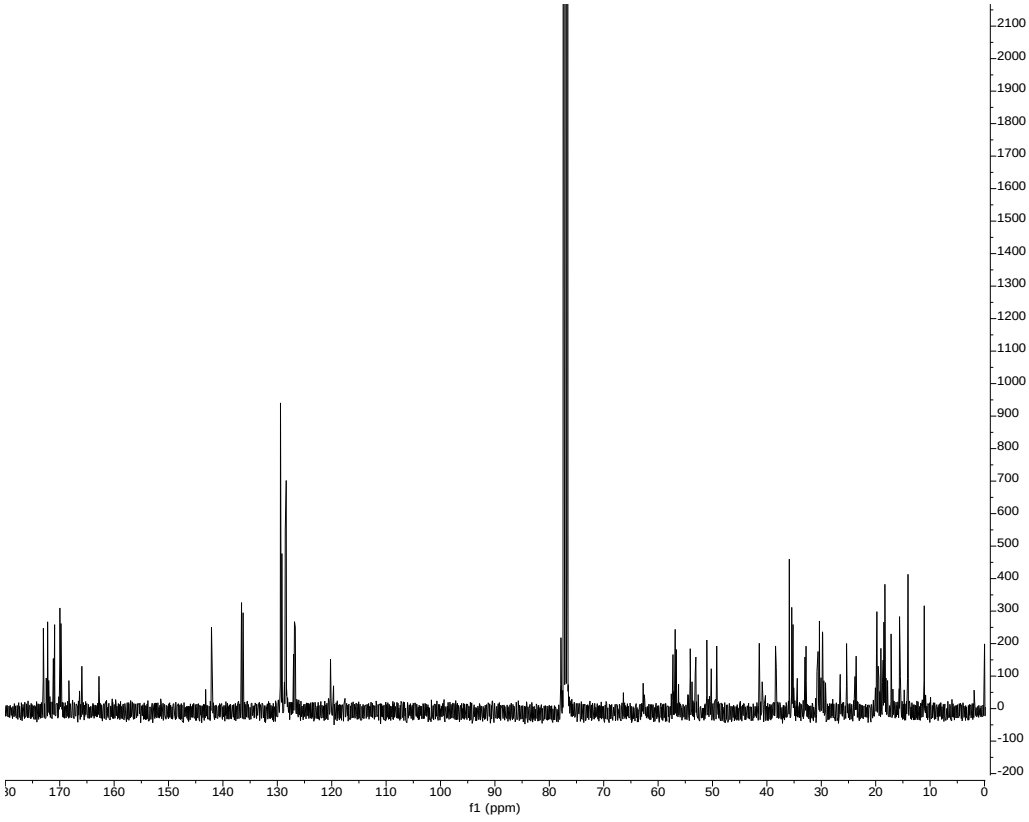
Compound **64**

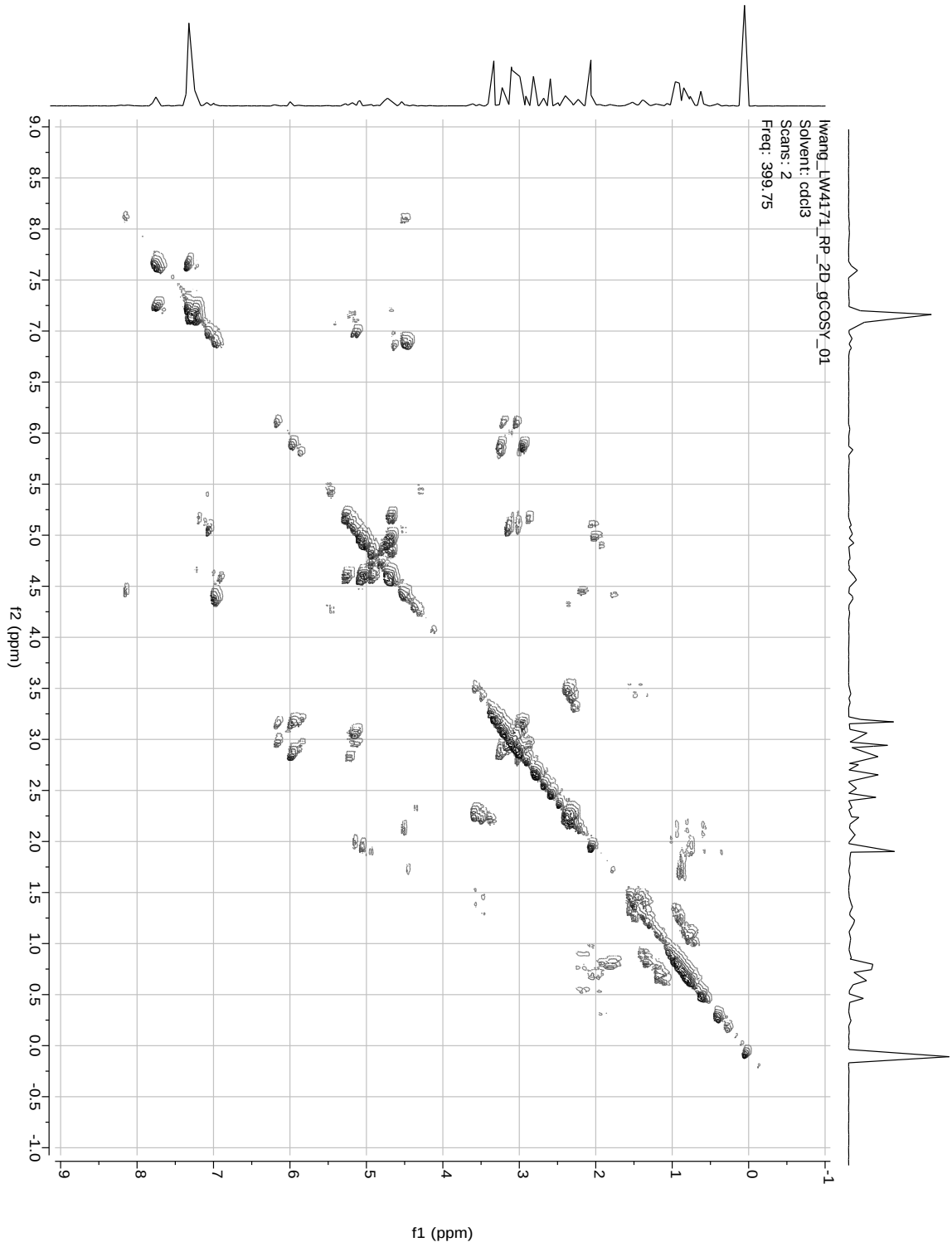

10.79
0.75
0.52
0.95
0.52
0.99
0.57
0.37
0.48
1.11
0.50
0.74
1.11
1.51
1.73
1.60
2.24
1.43
1.45
1.01
1.49
3.25
2.57
1.39
9.00
5.08
21.10
1.51
f1 (ppm)

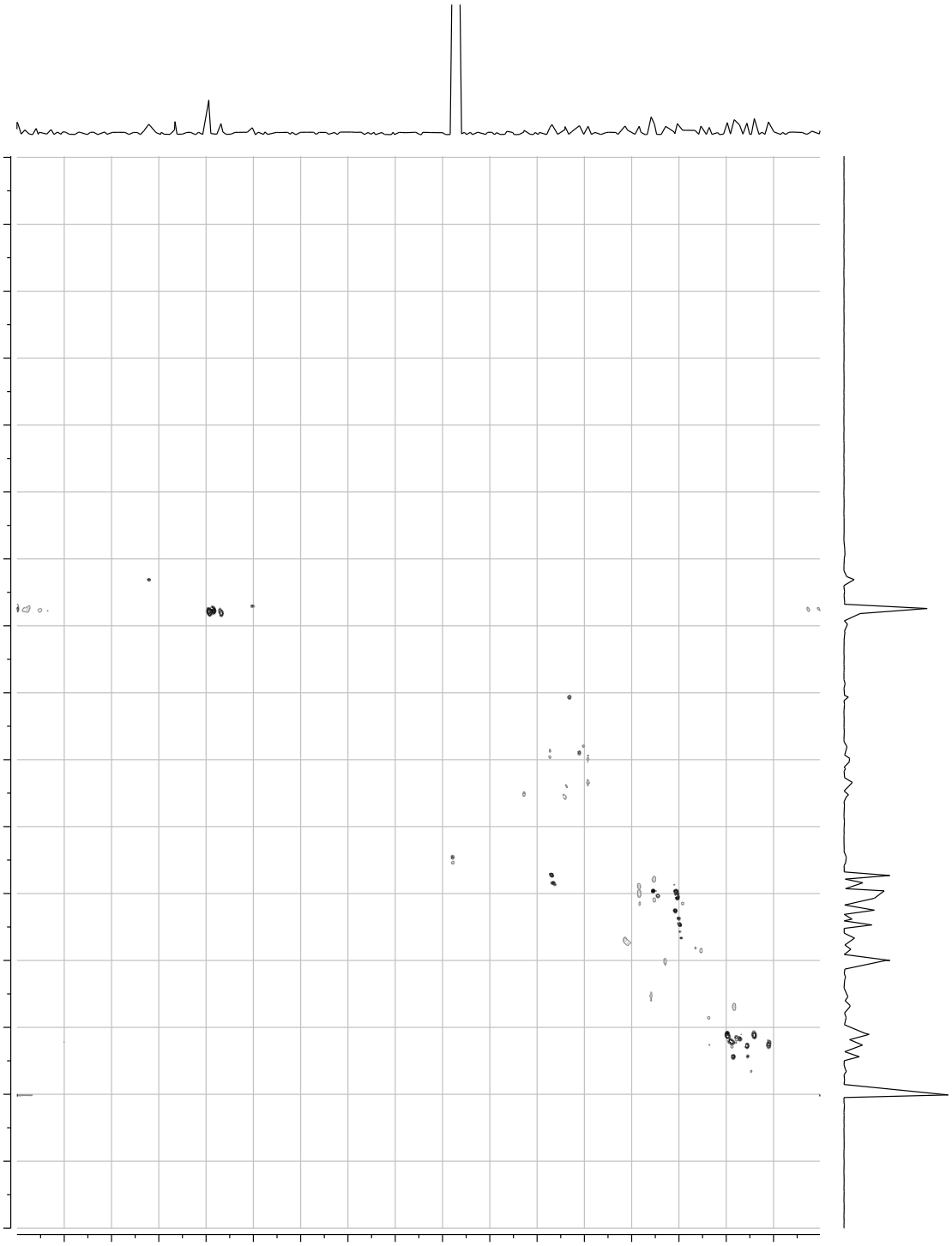

f1 (ppm)

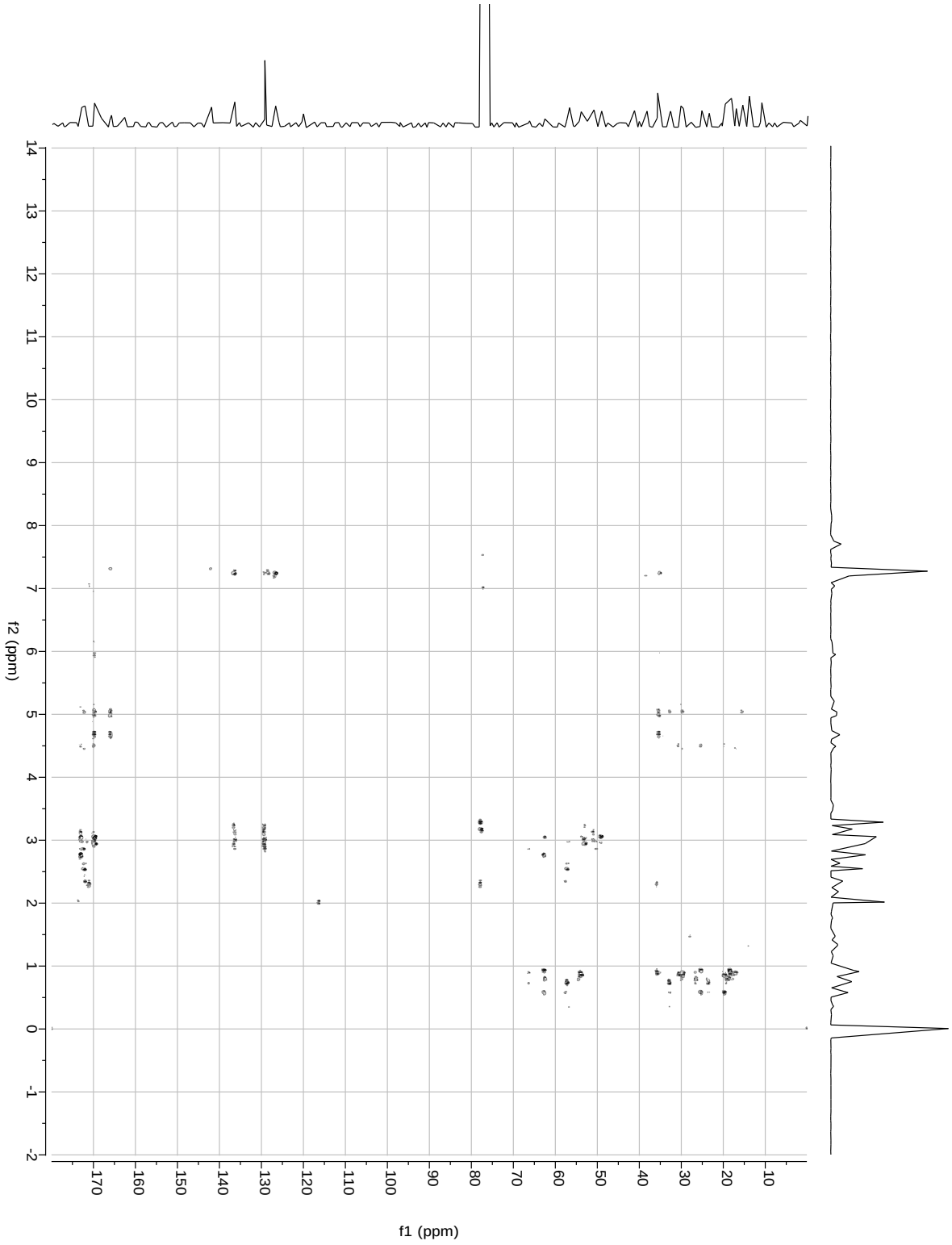
Compound **1d**



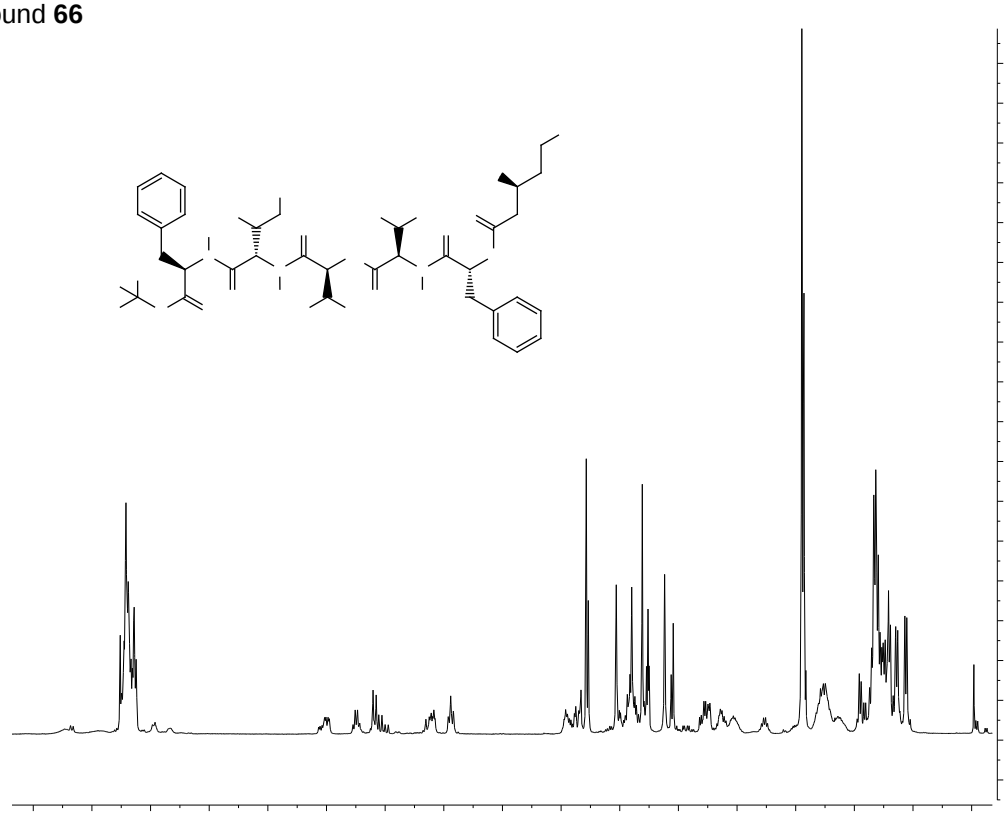



lwang_LW4171_RP_2D_gCOSY_01
Solvent: cdcl3
Scans: 2
Freq: 399.75
f2 (ppm)
f1 (ppm)

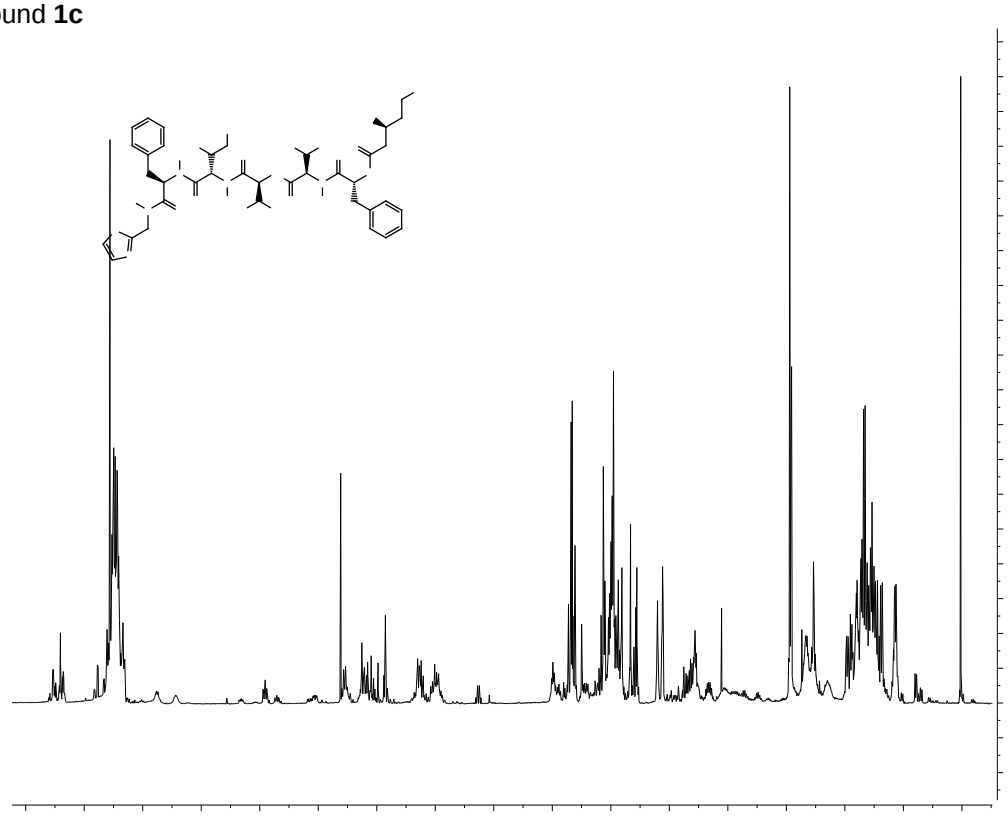



f2 (ppm)
f1 (ppm)

Compound **66**

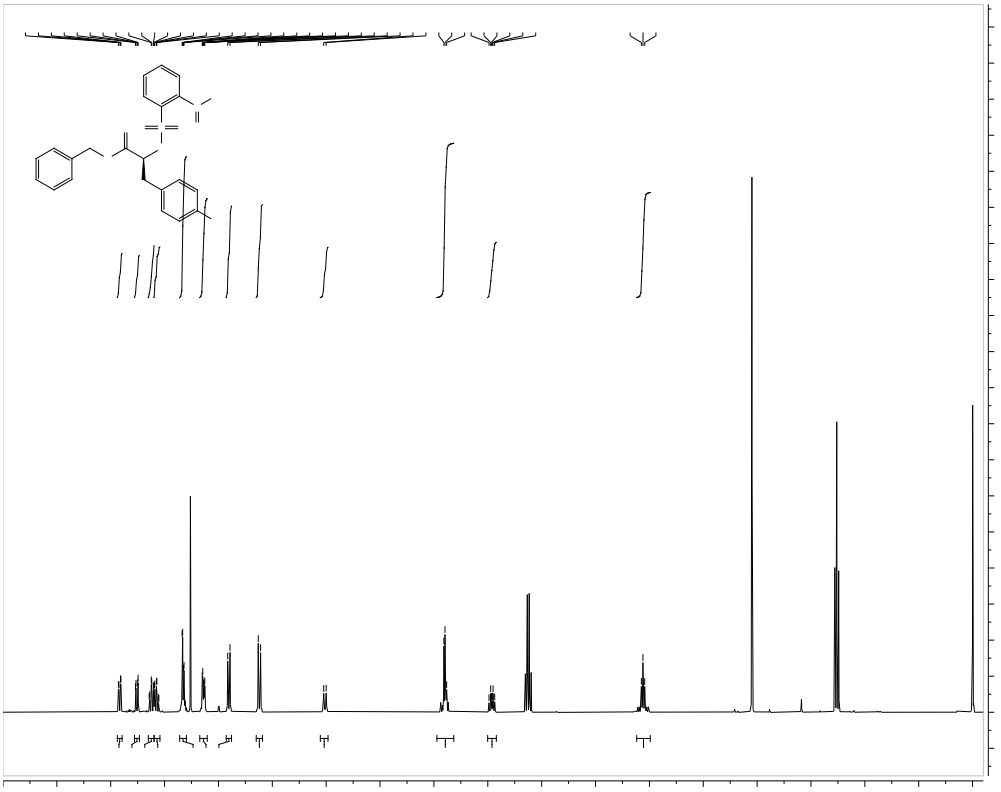


Compound **1c**

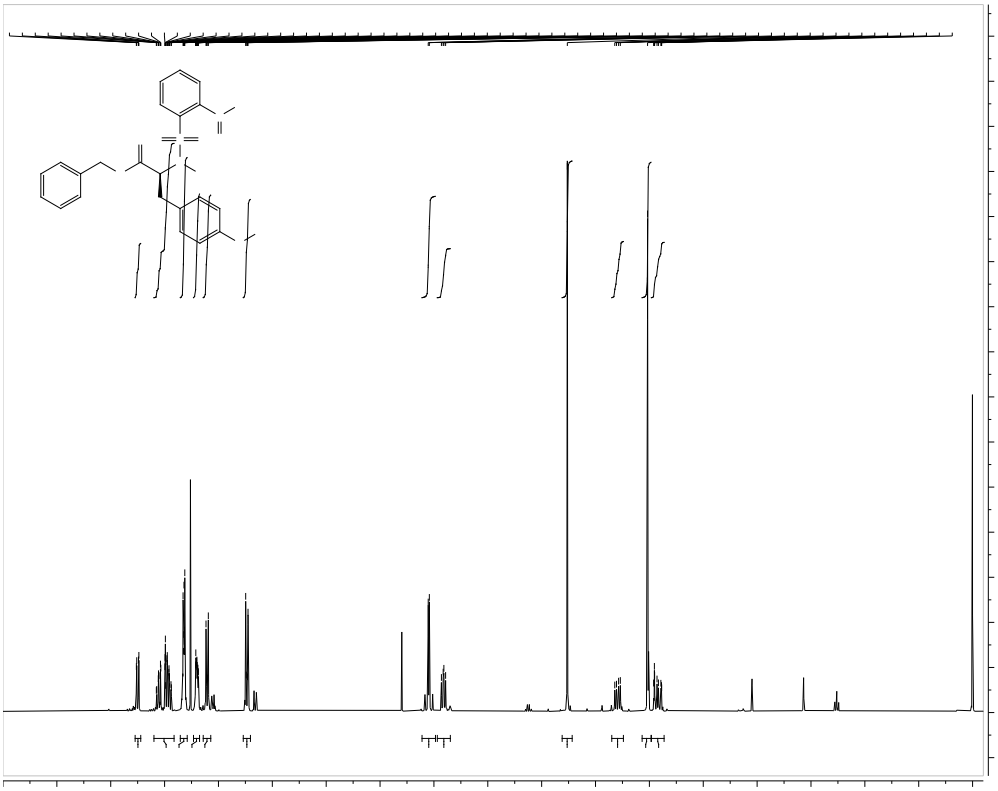


Tyrosine-based Analog

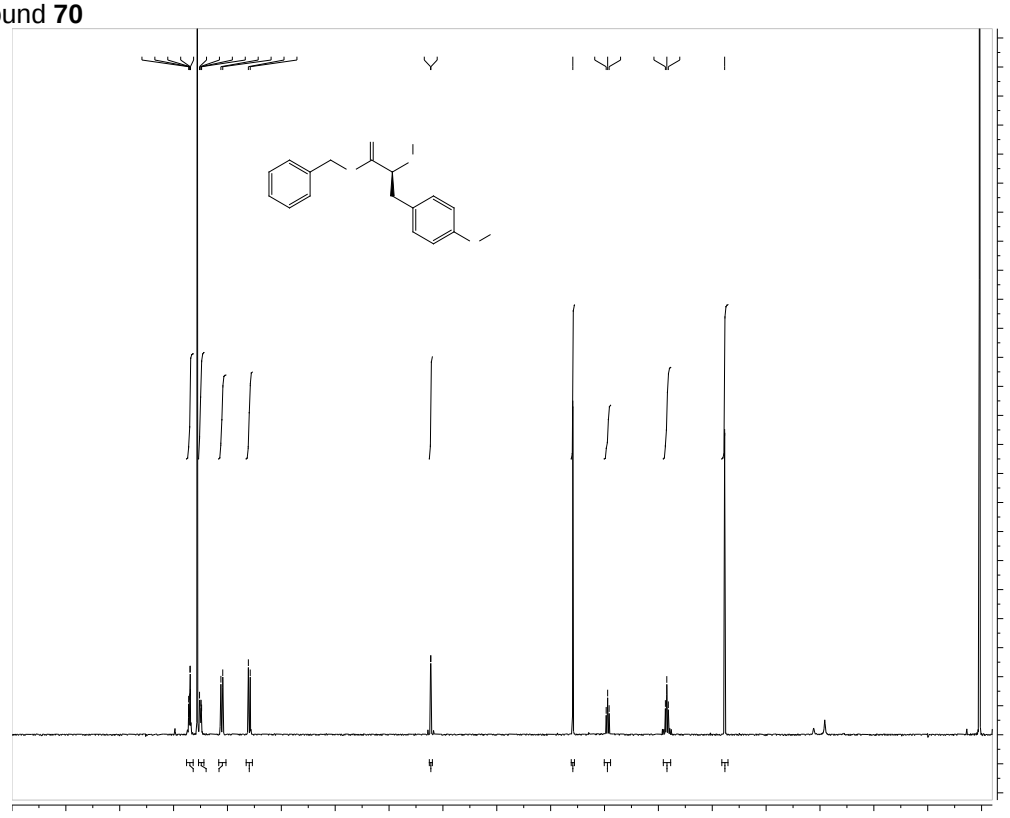
Compound **68**



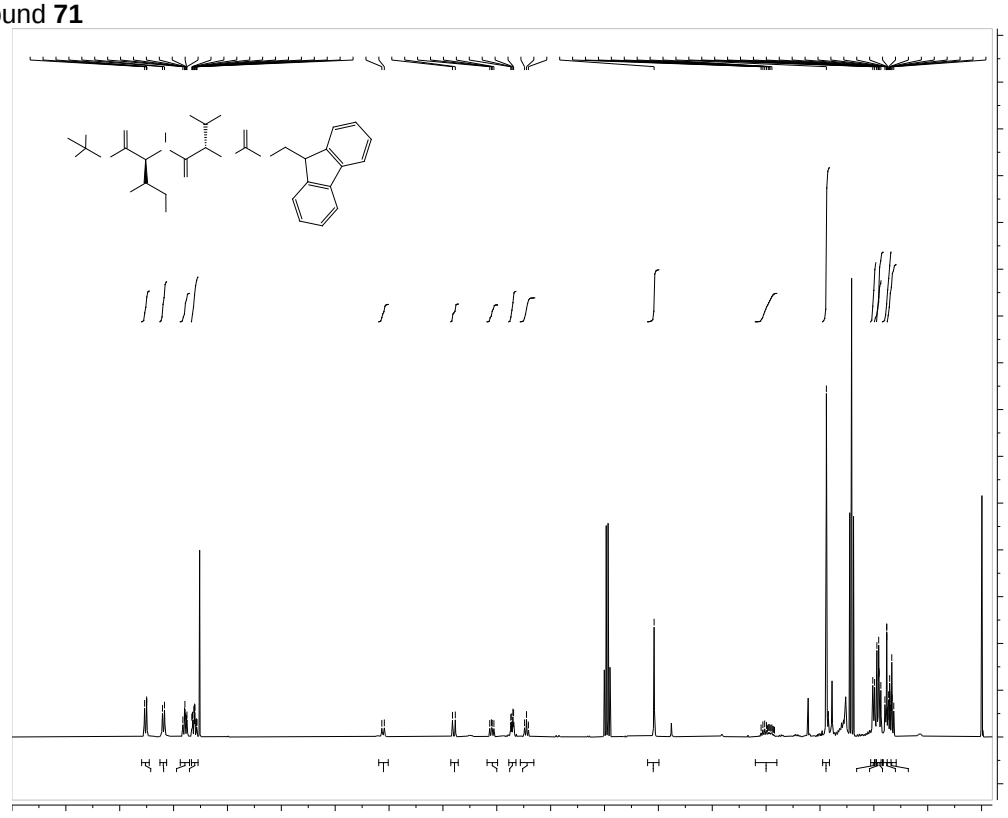
Compound **69**



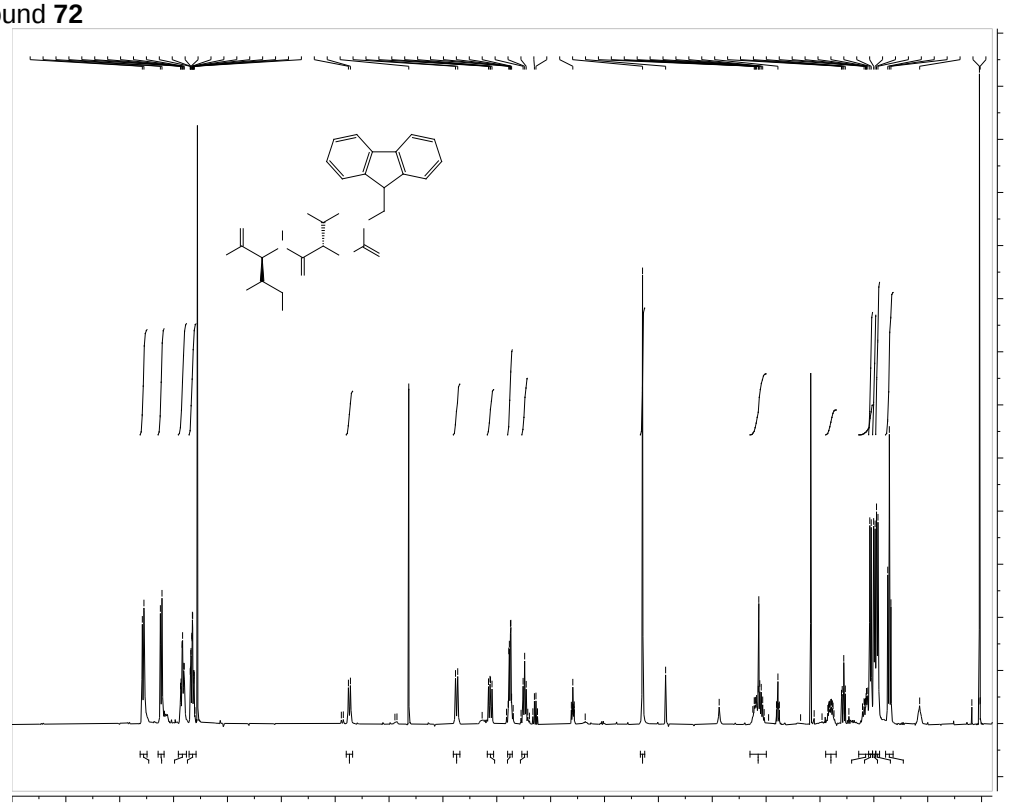
Compound **70**



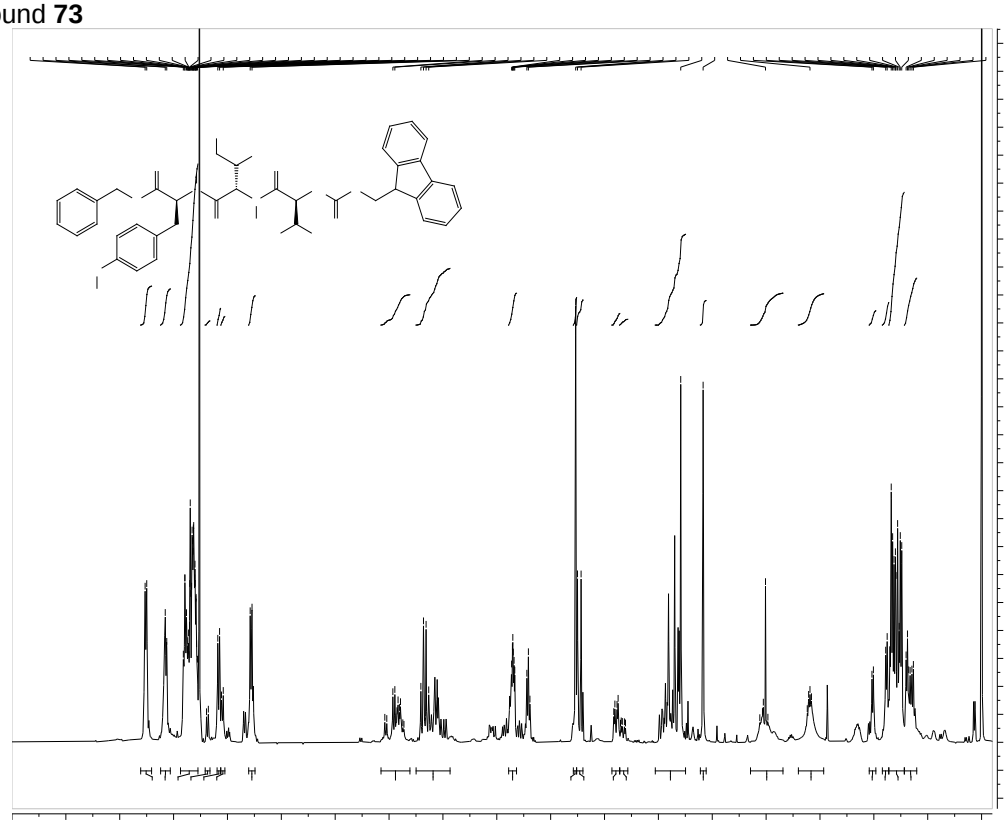
Compound **71**



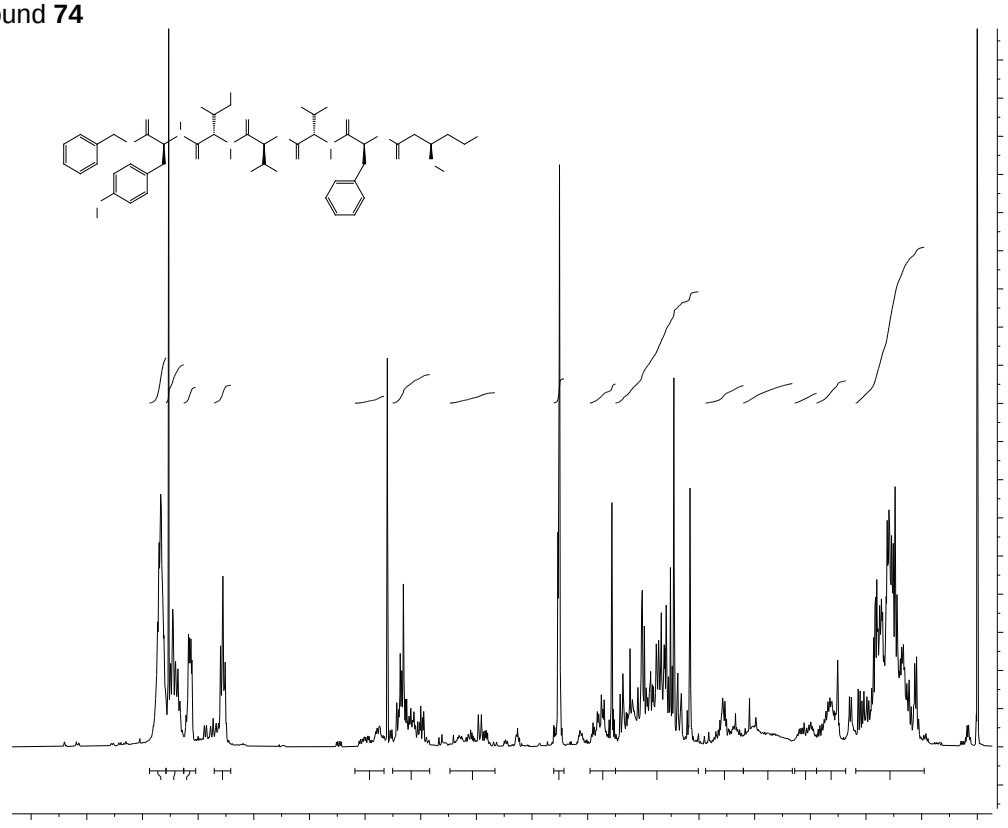
Compound **72**



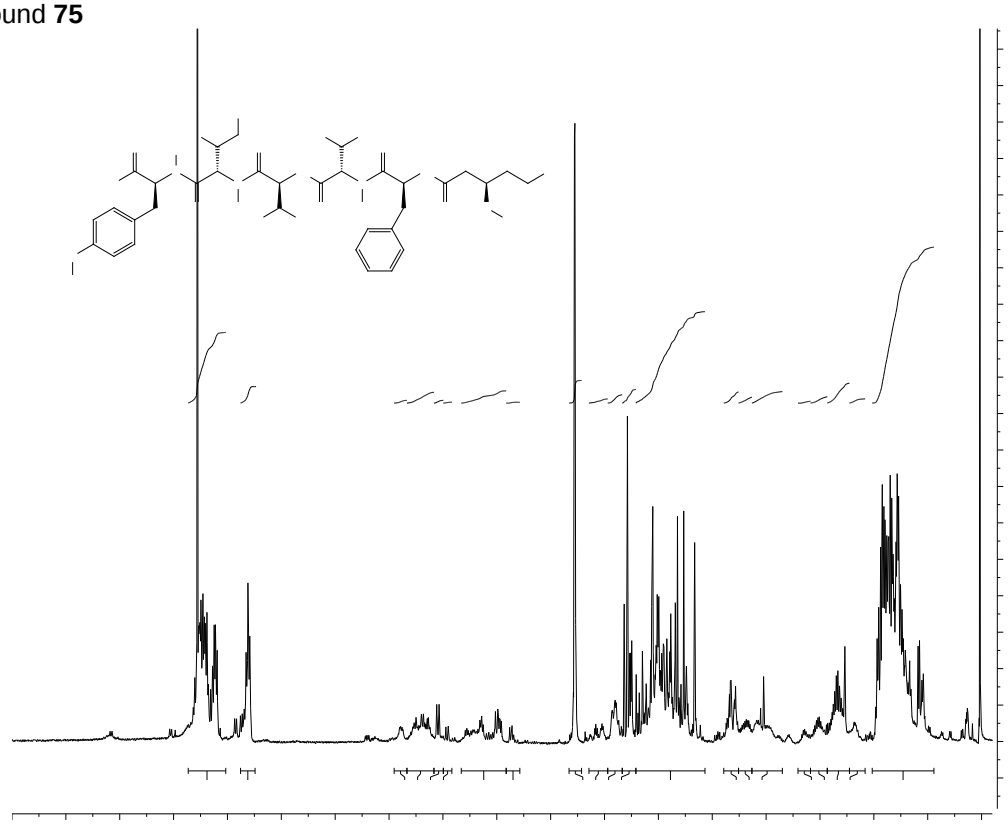
Compound **73**



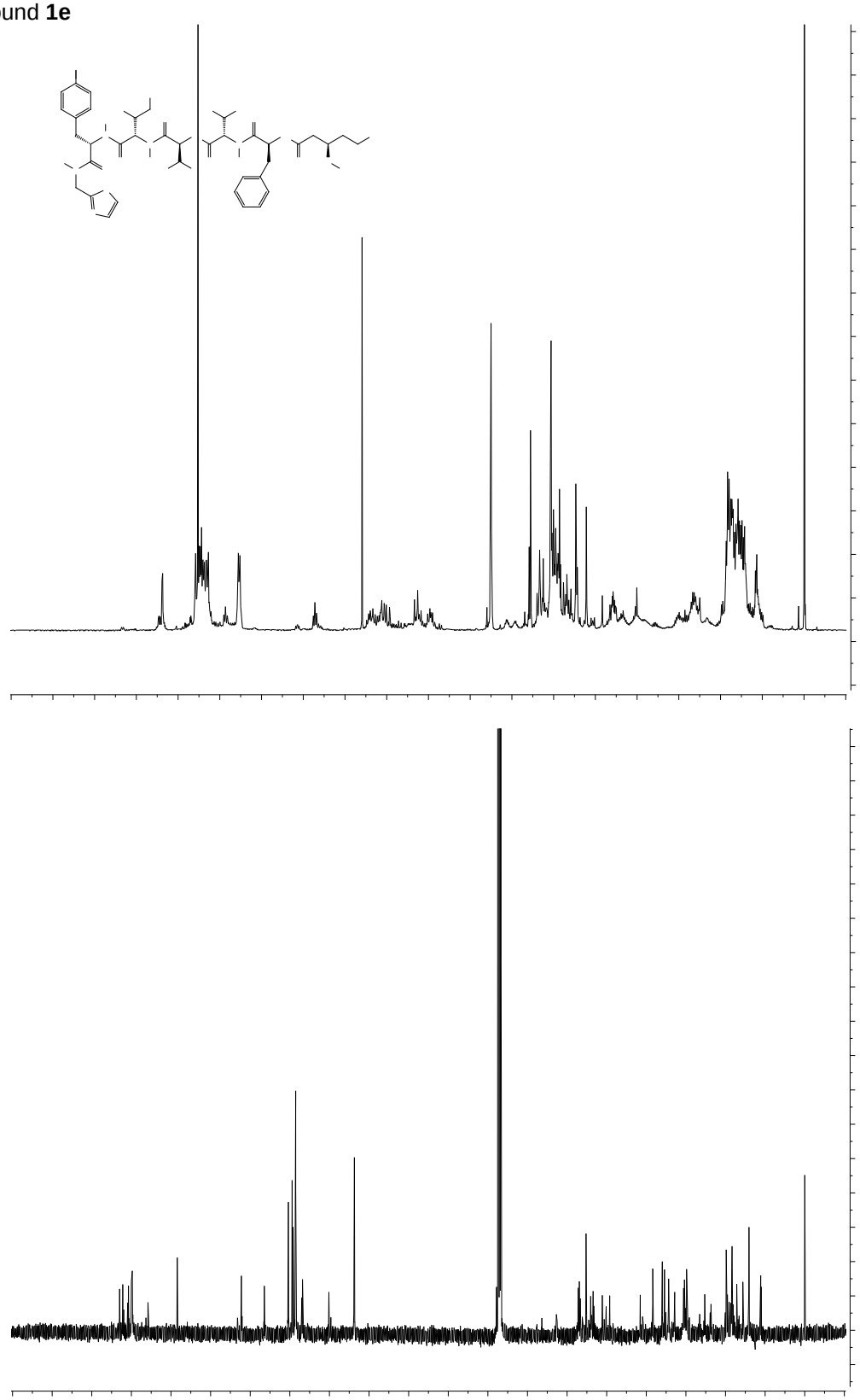
Compound **74**

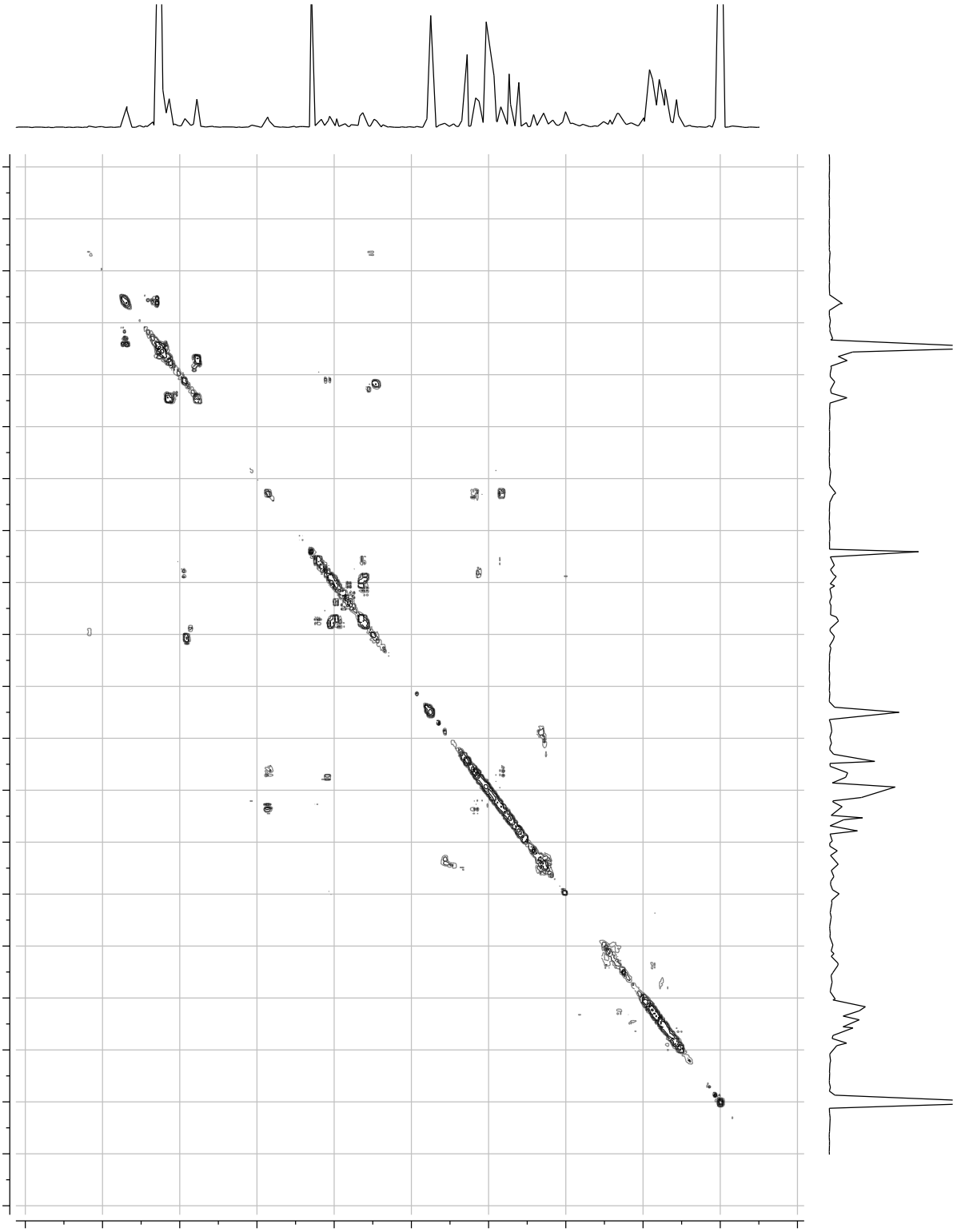


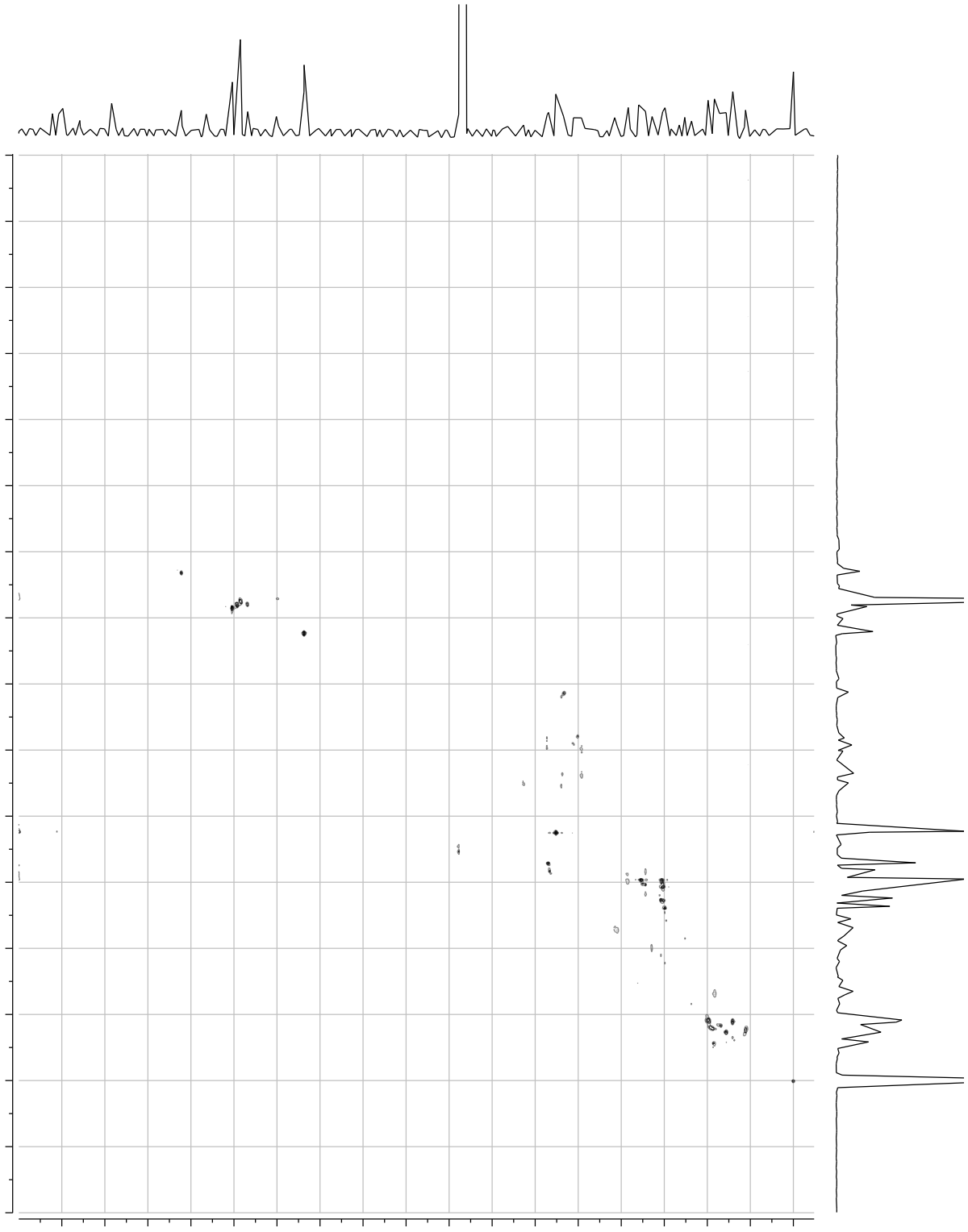
Compound **75**

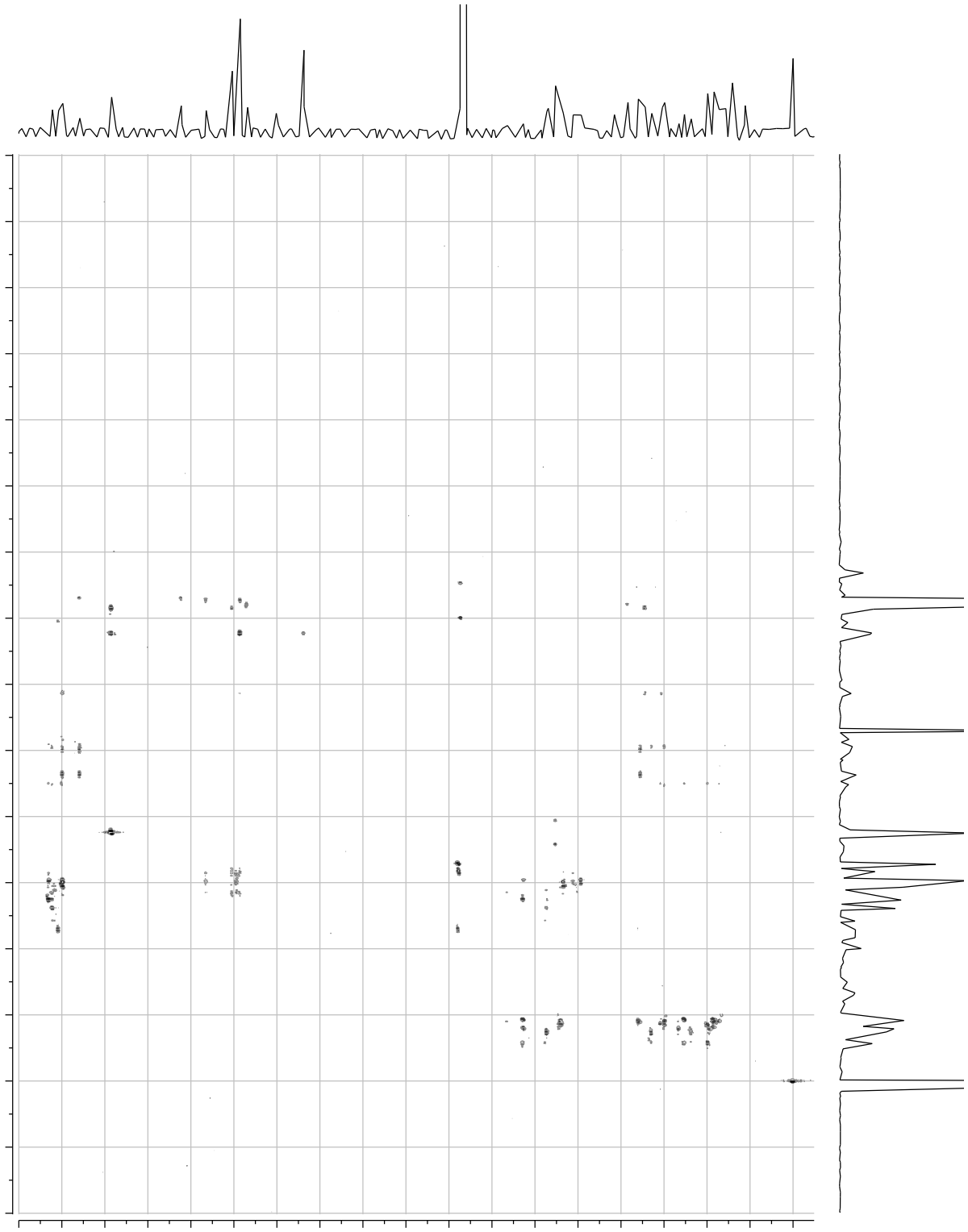


Compound **1e**

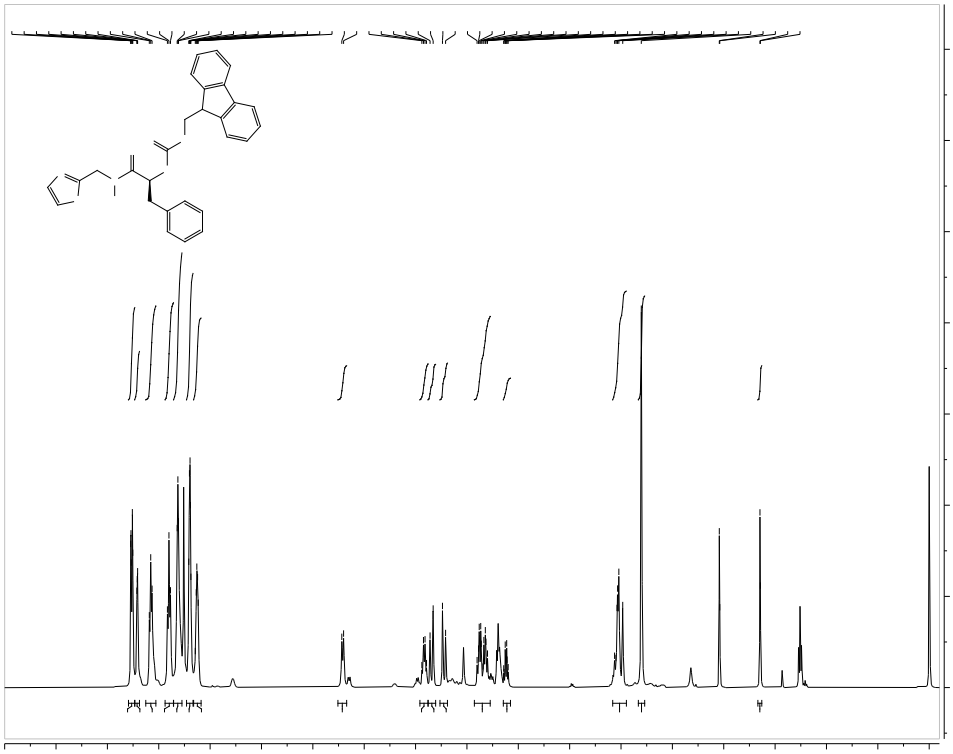


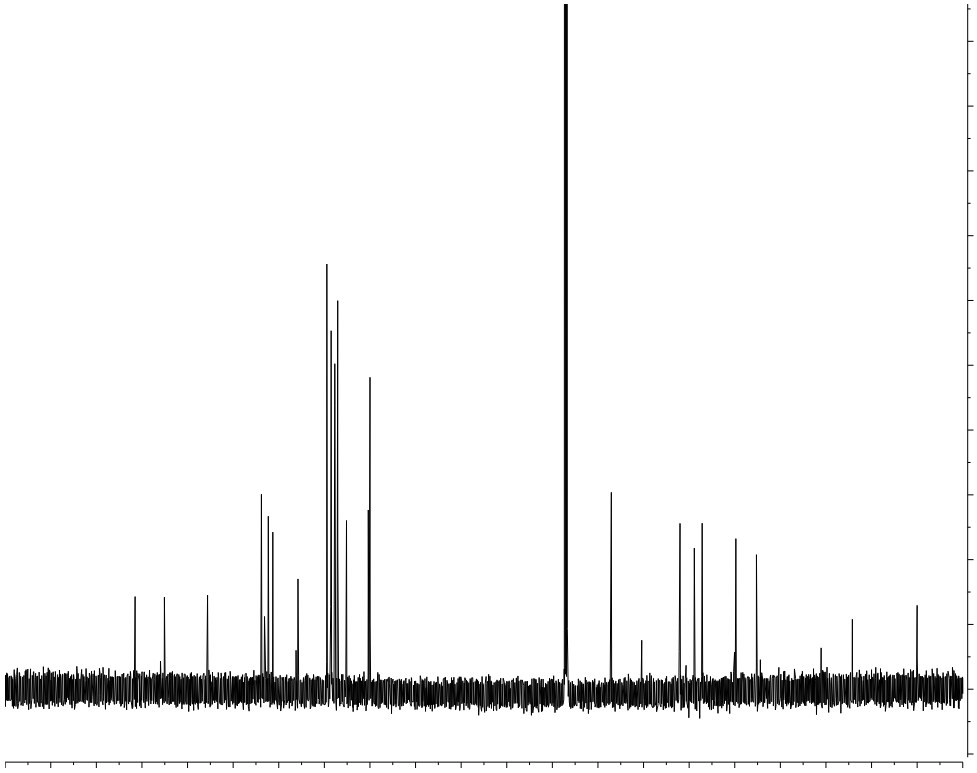




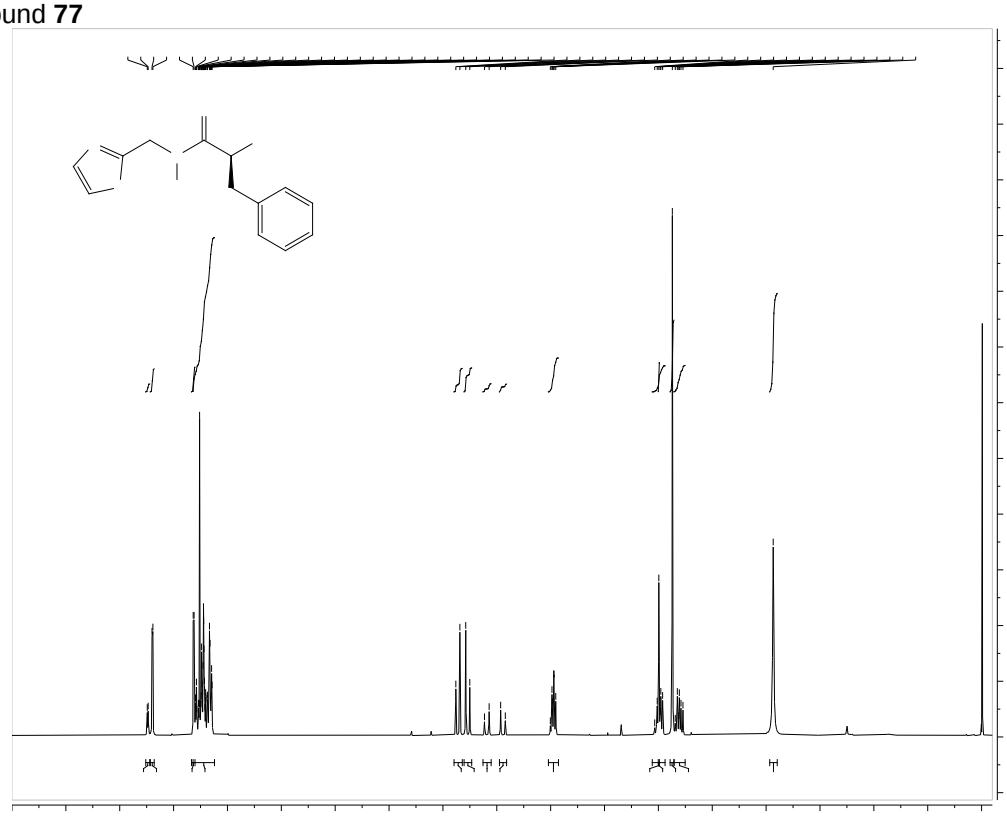


Compound **76**

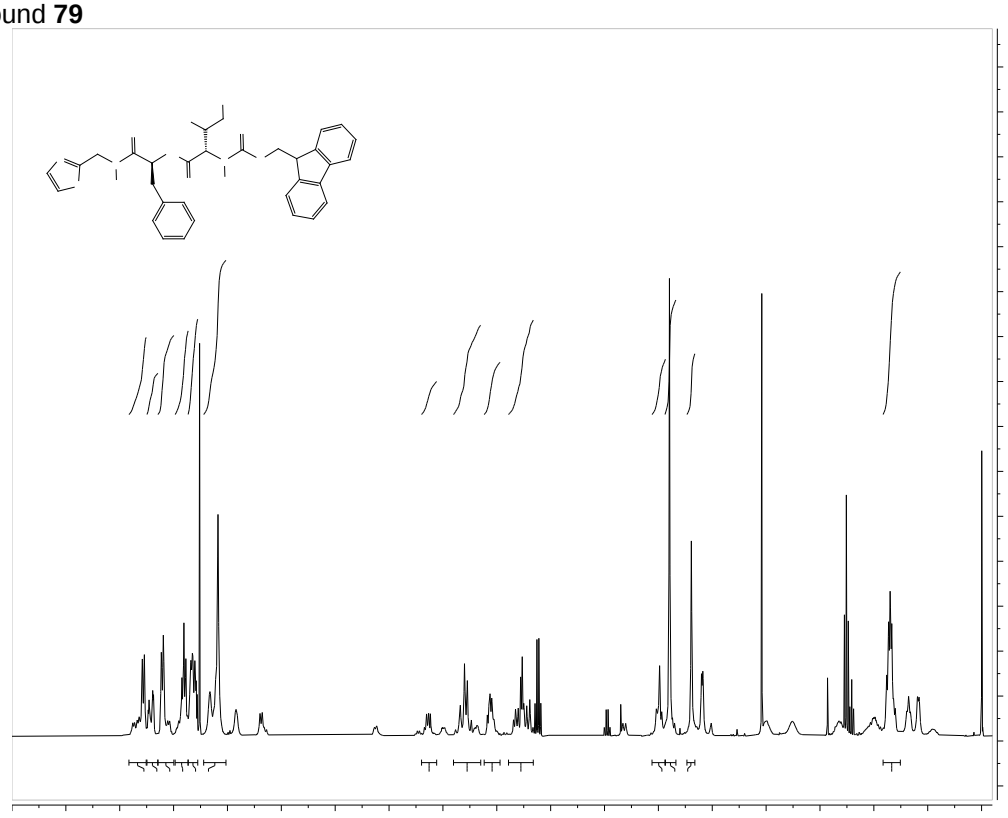




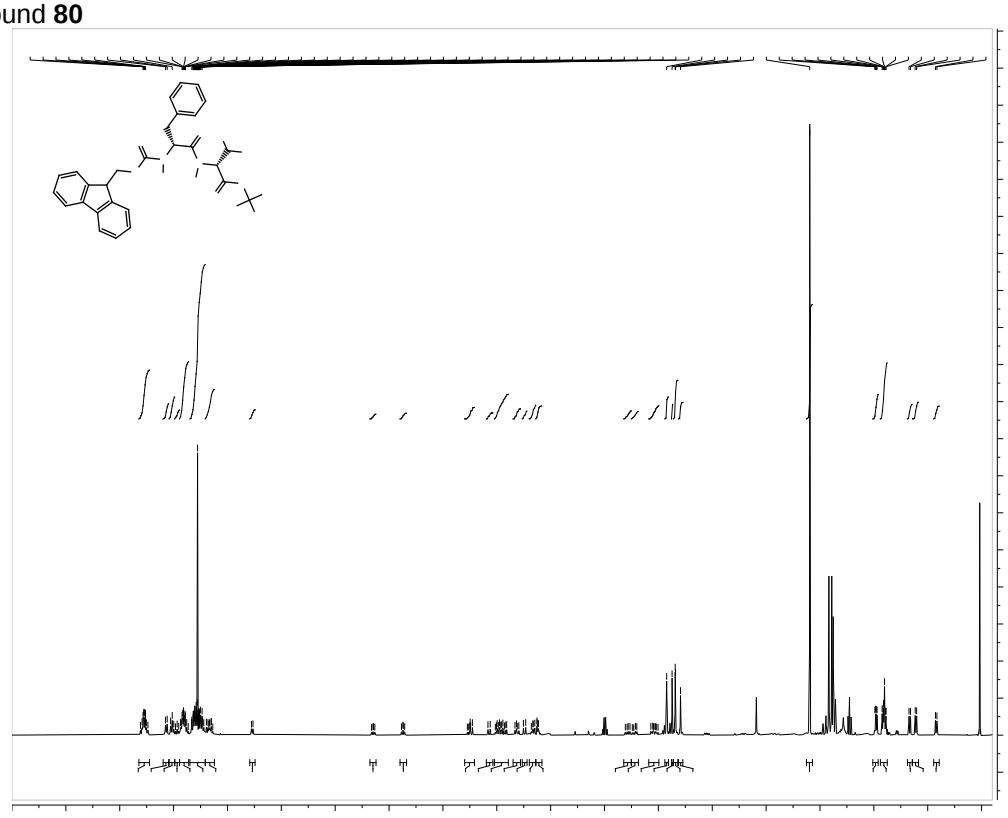
Compound **77**



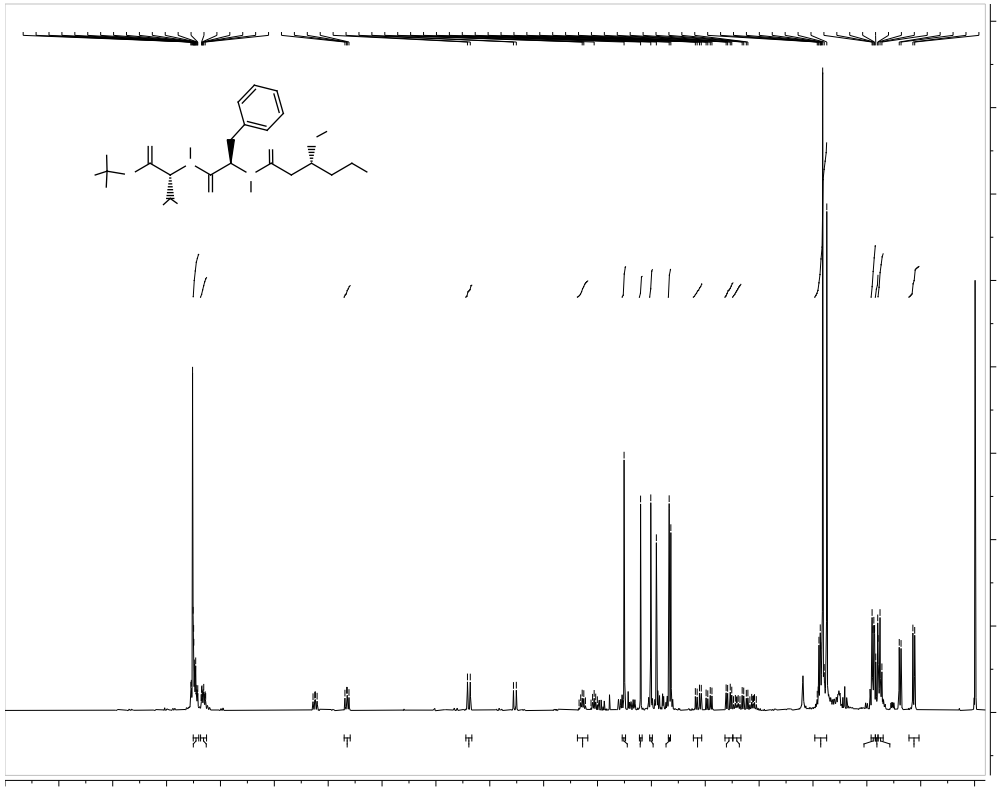
Compound **79**



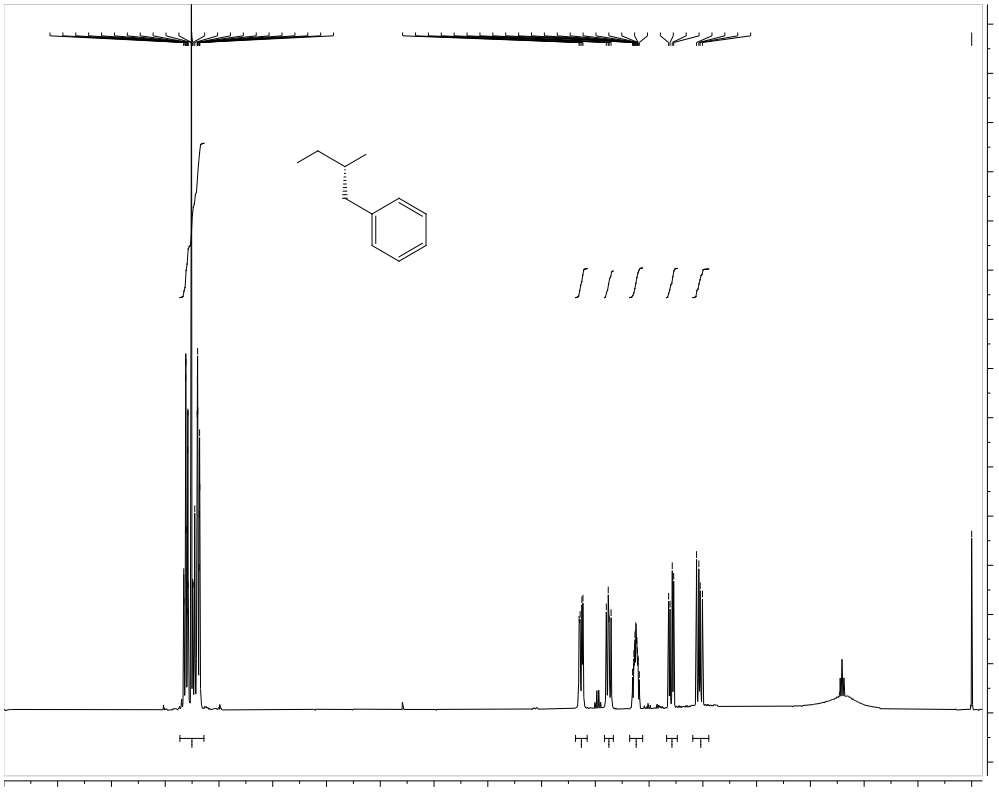
Compound **80**



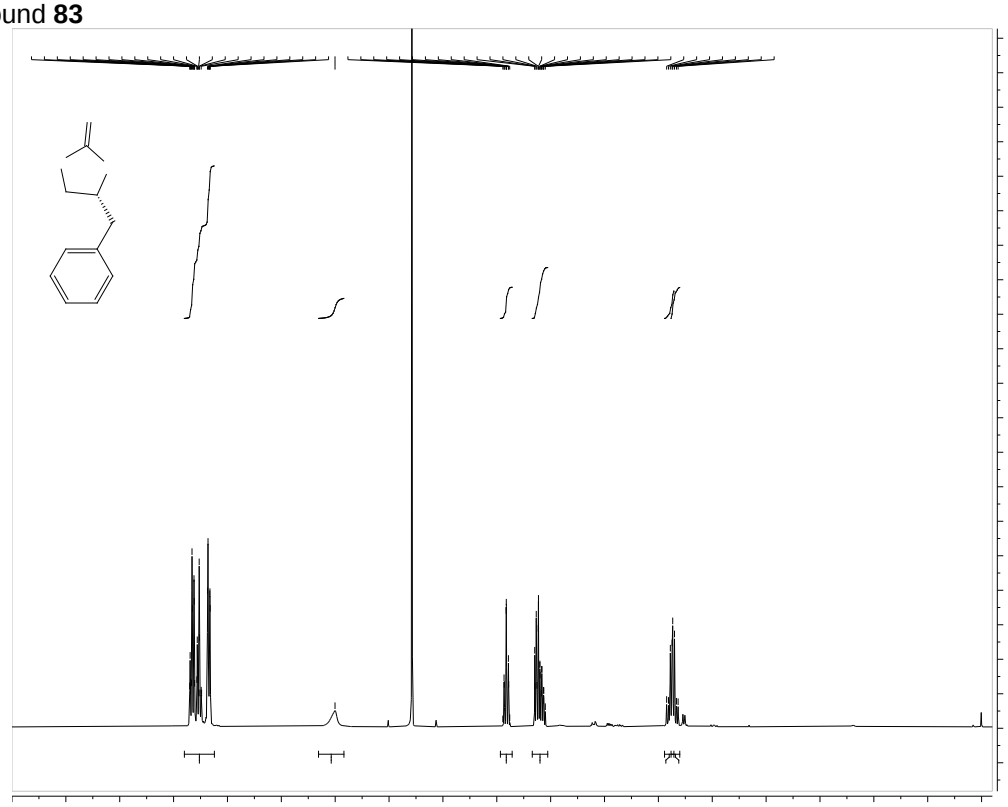
Compound **81**



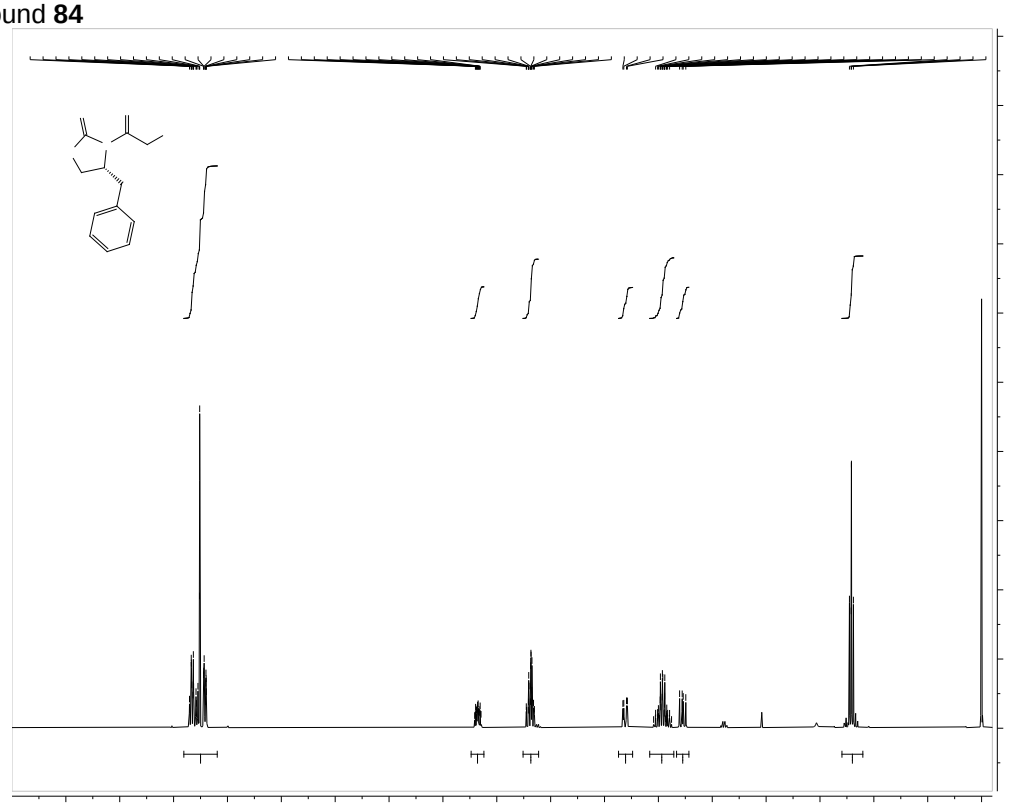
Compound **82**



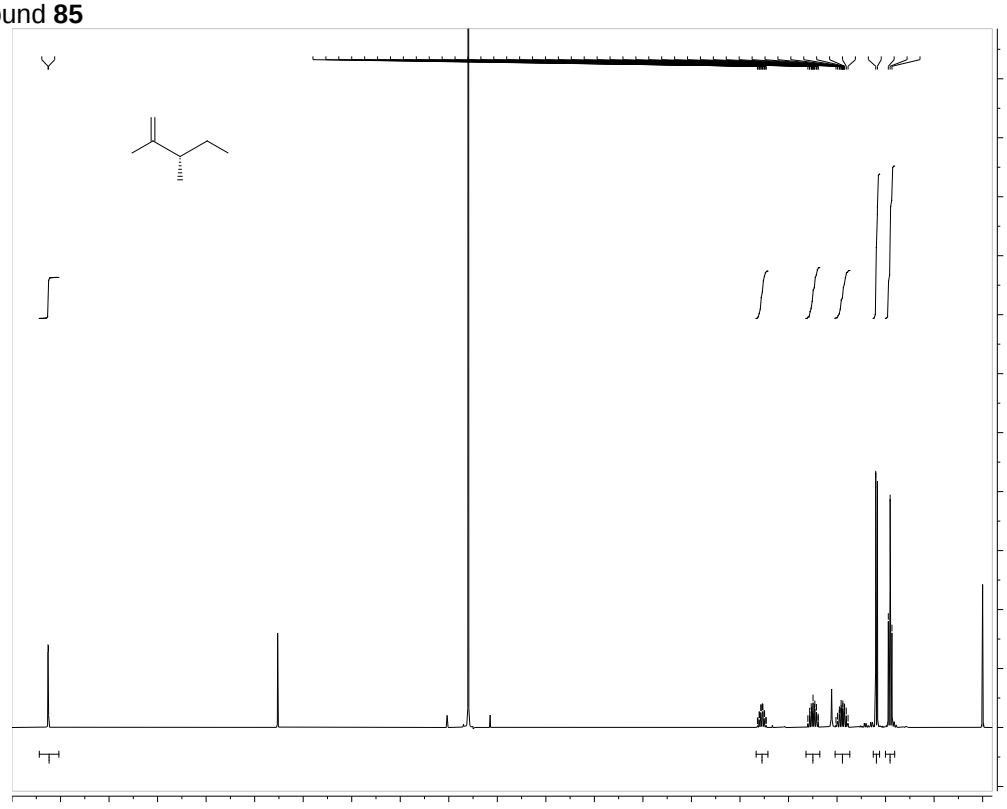
Compound **83**



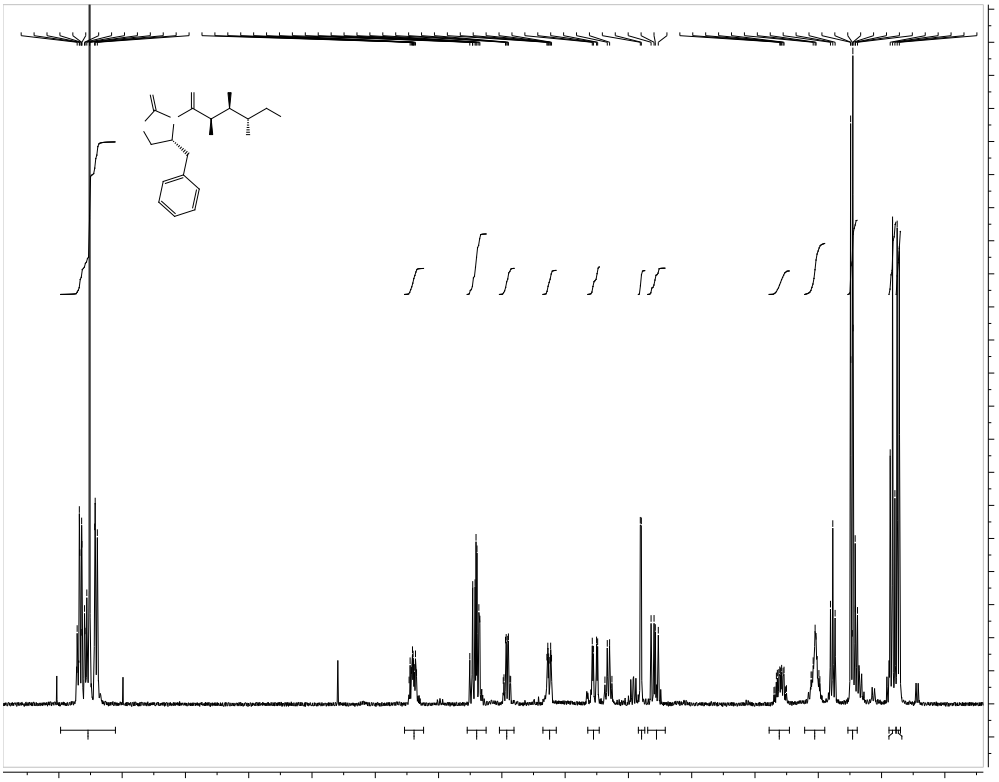
Compound **84**



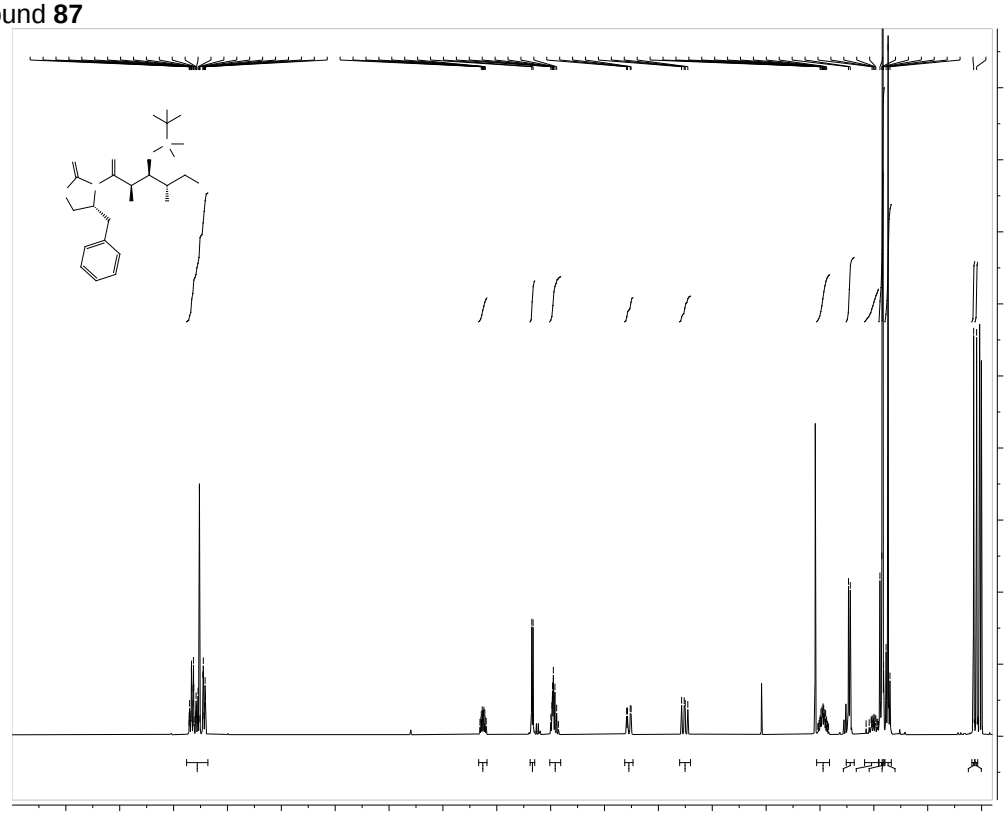
Compound **85**



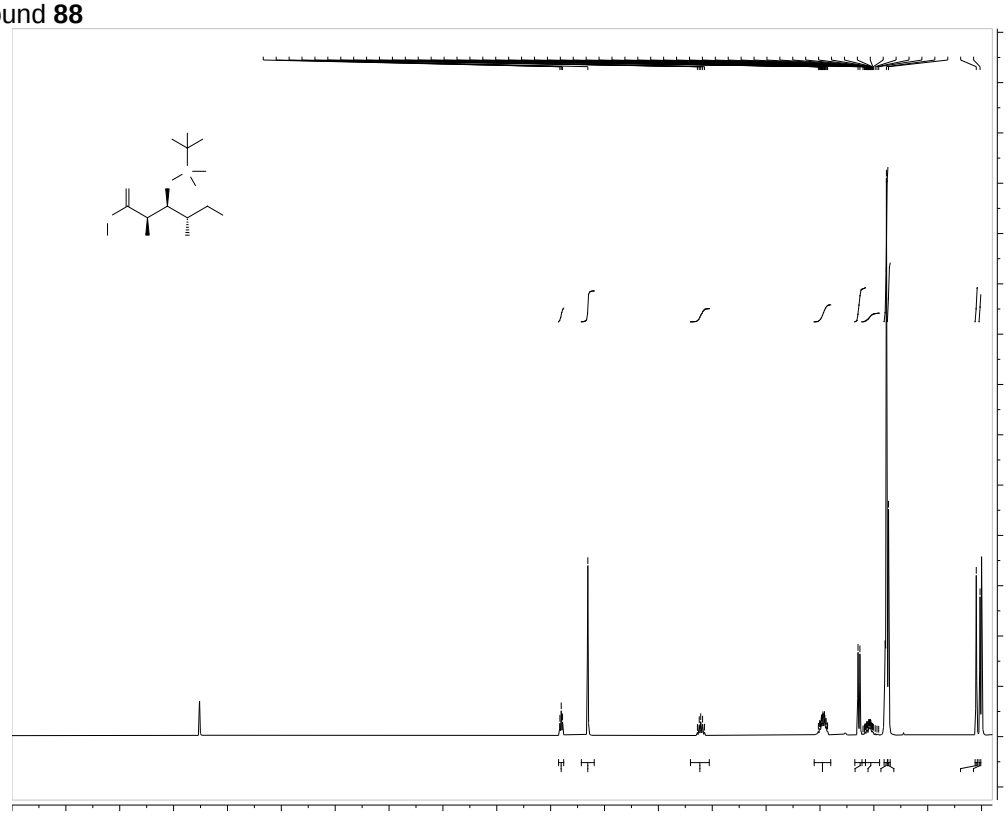
Compound **86**



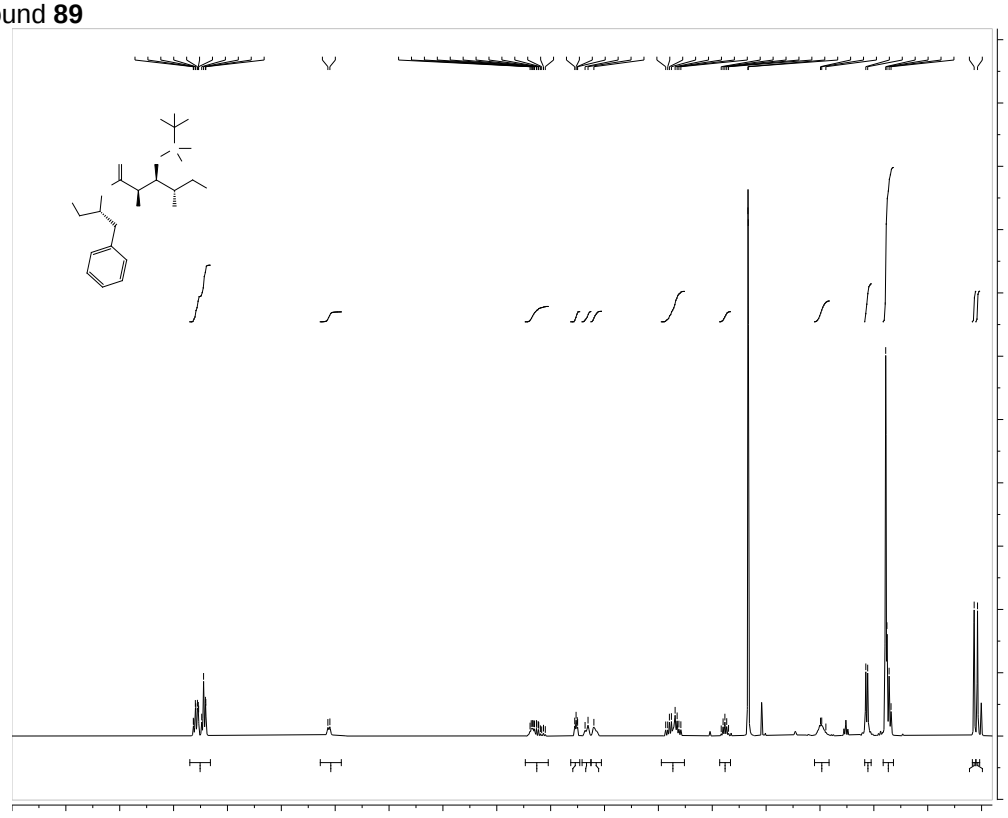
Compound **87**



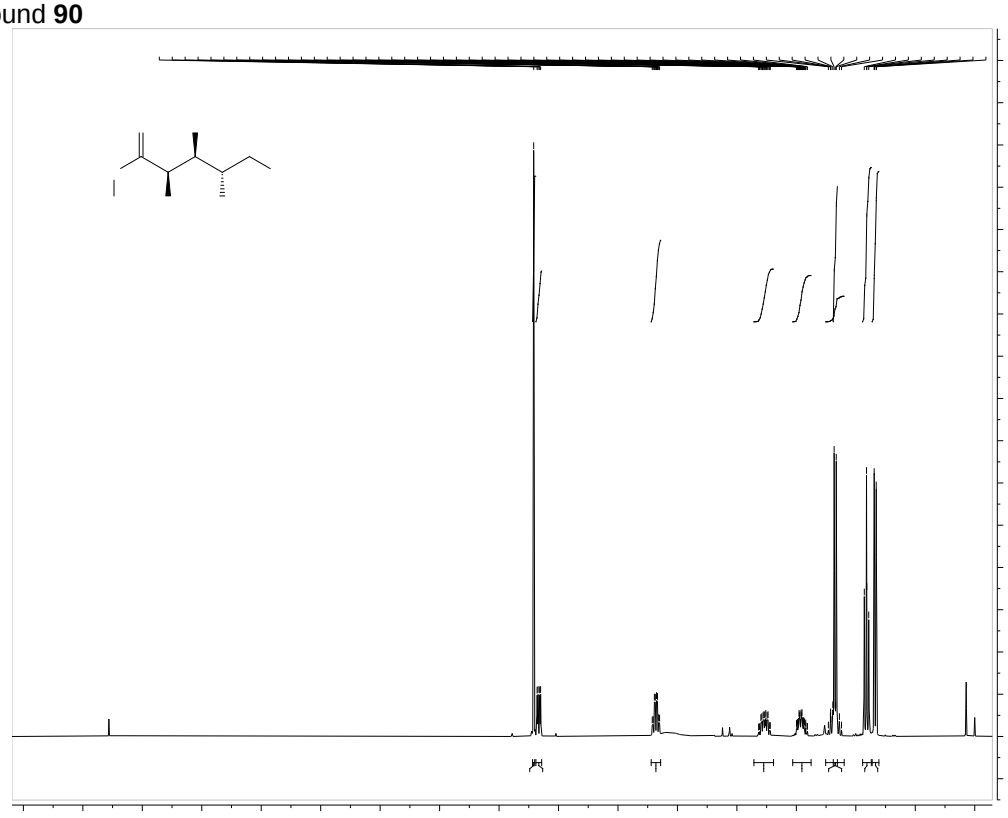
Compound **88**



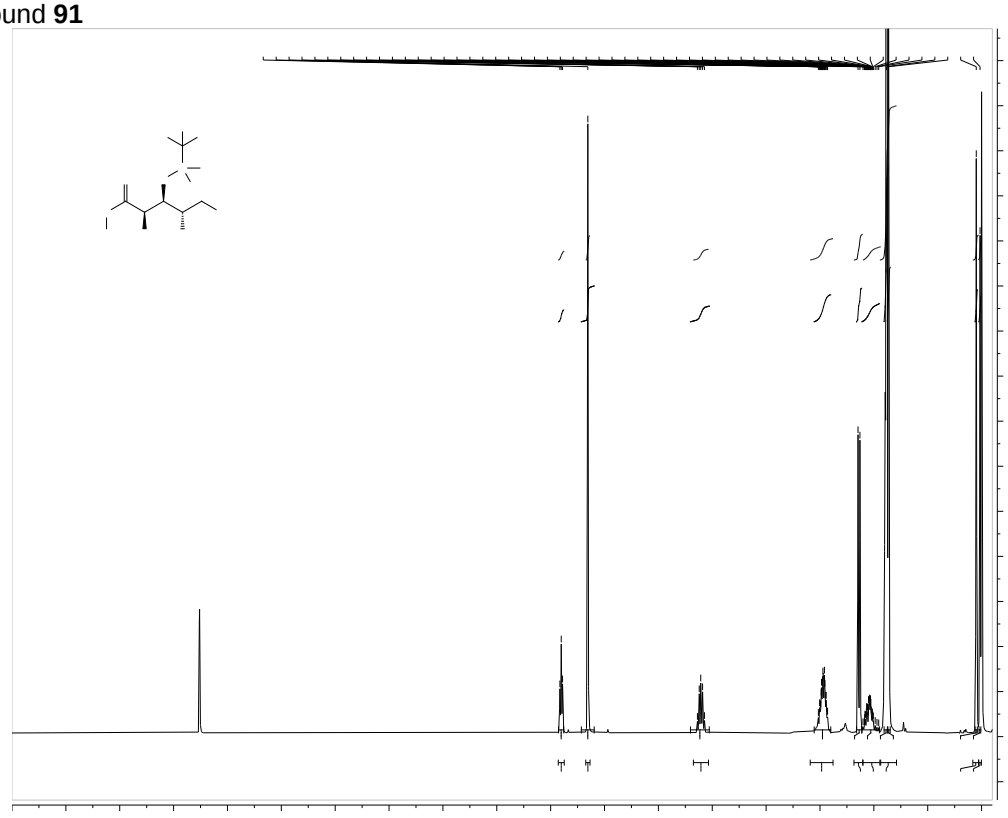
Compound **89**



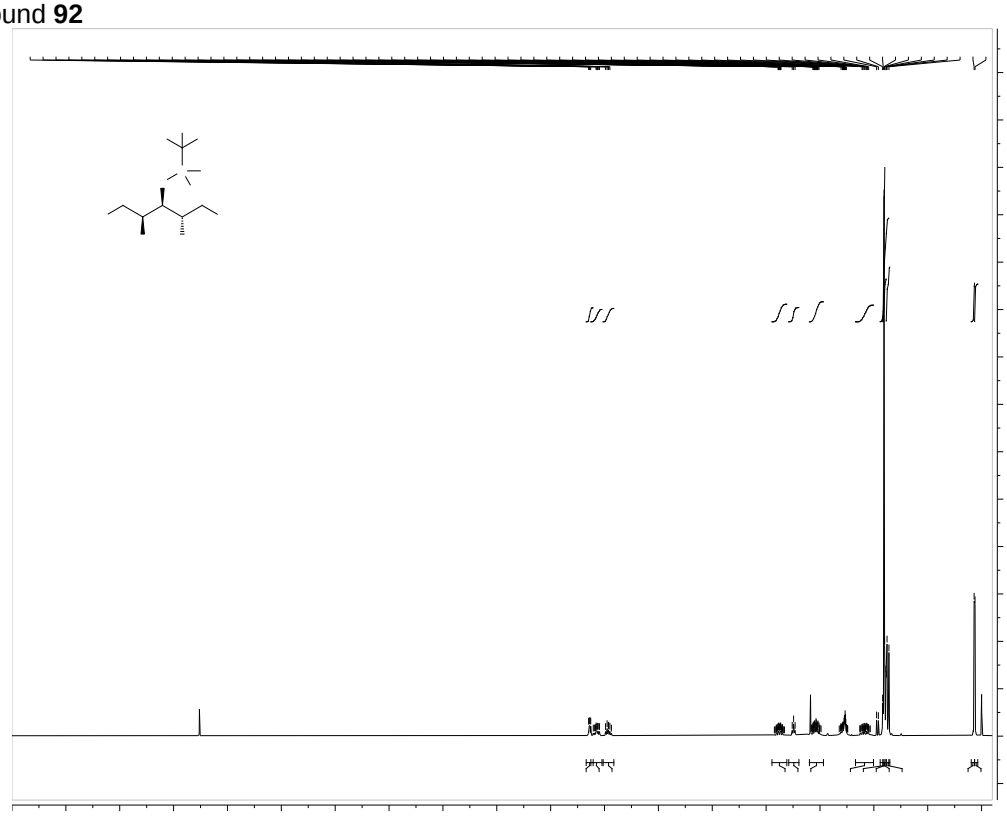
Compound **90**



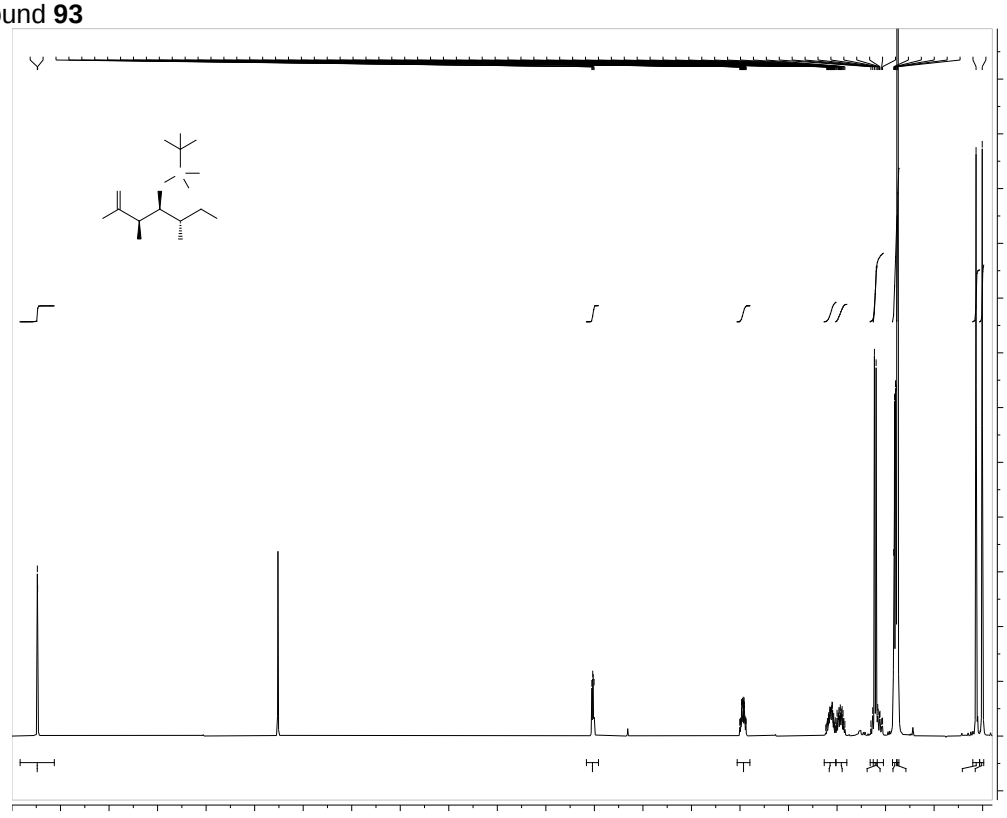
Compound **91**



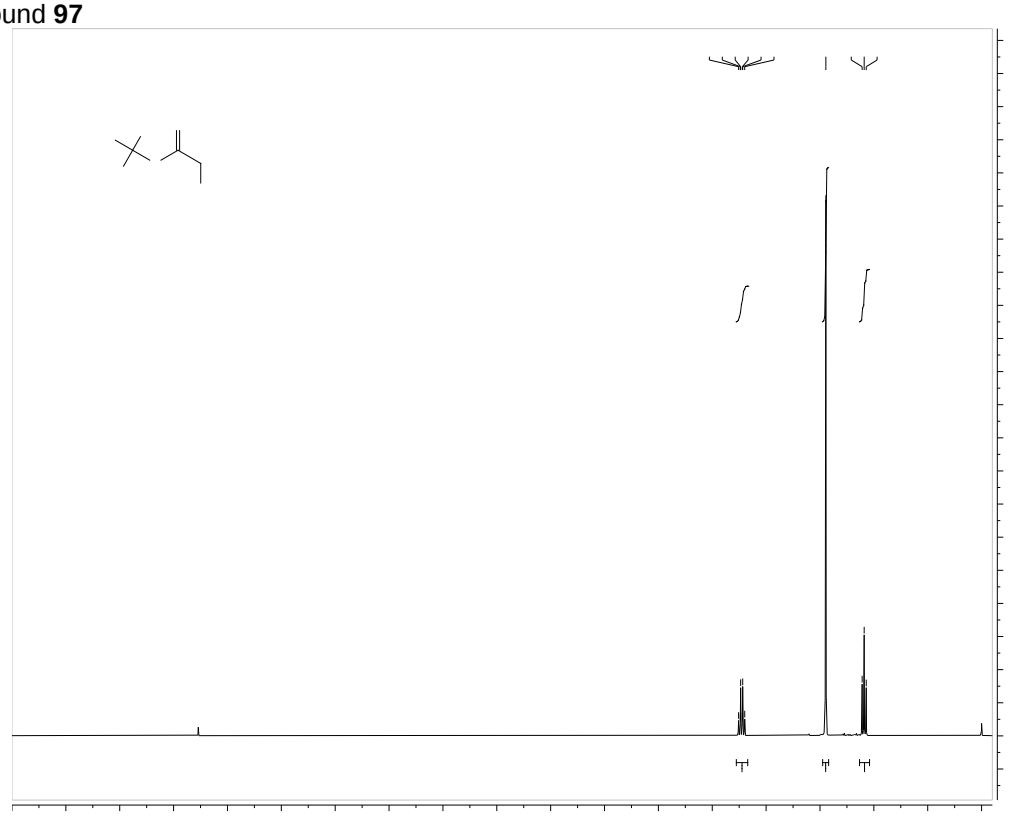
Compound **92**



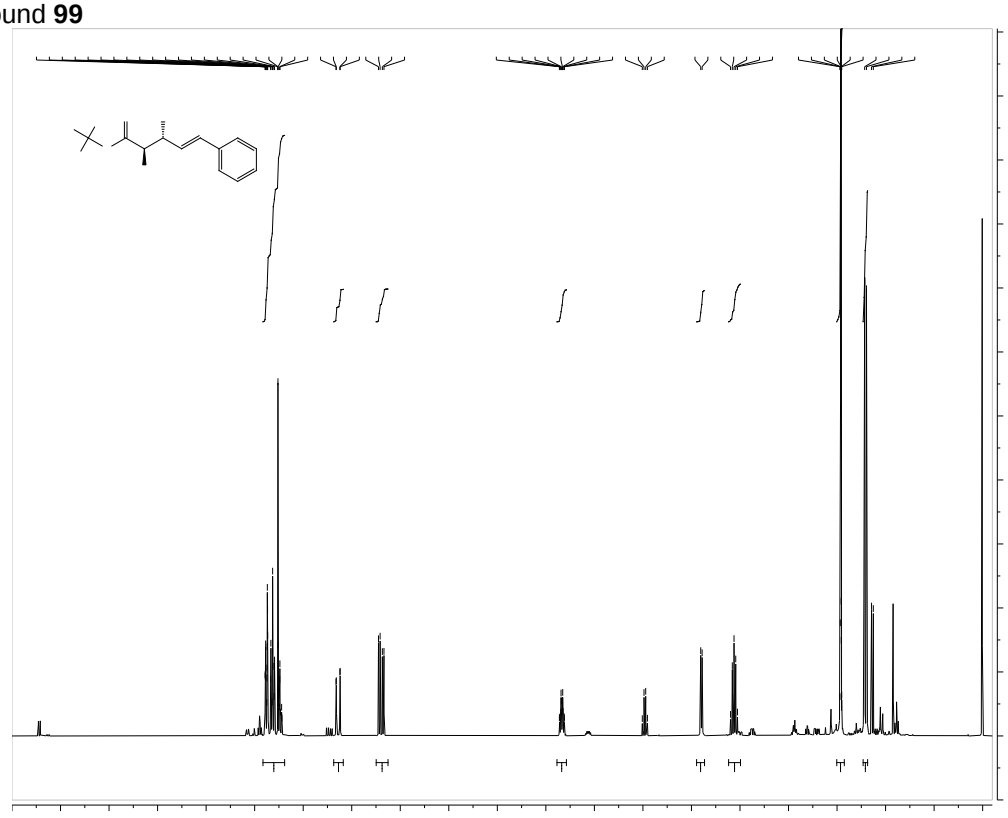
Compound **93**



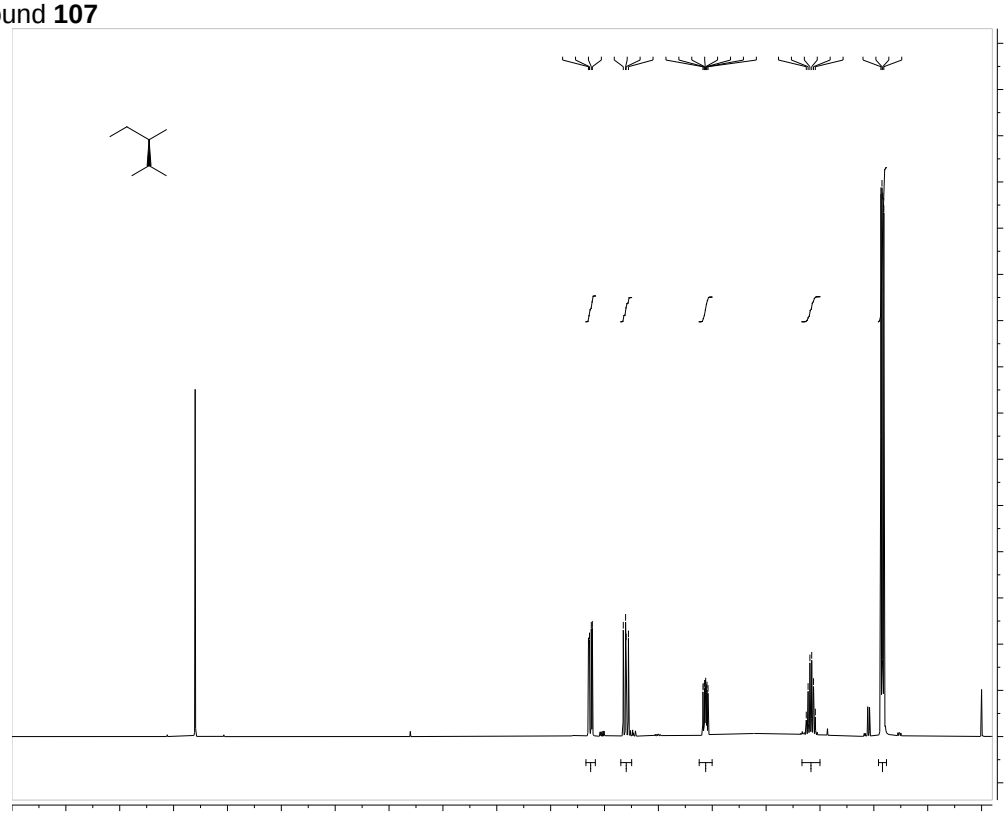
Compound **97**



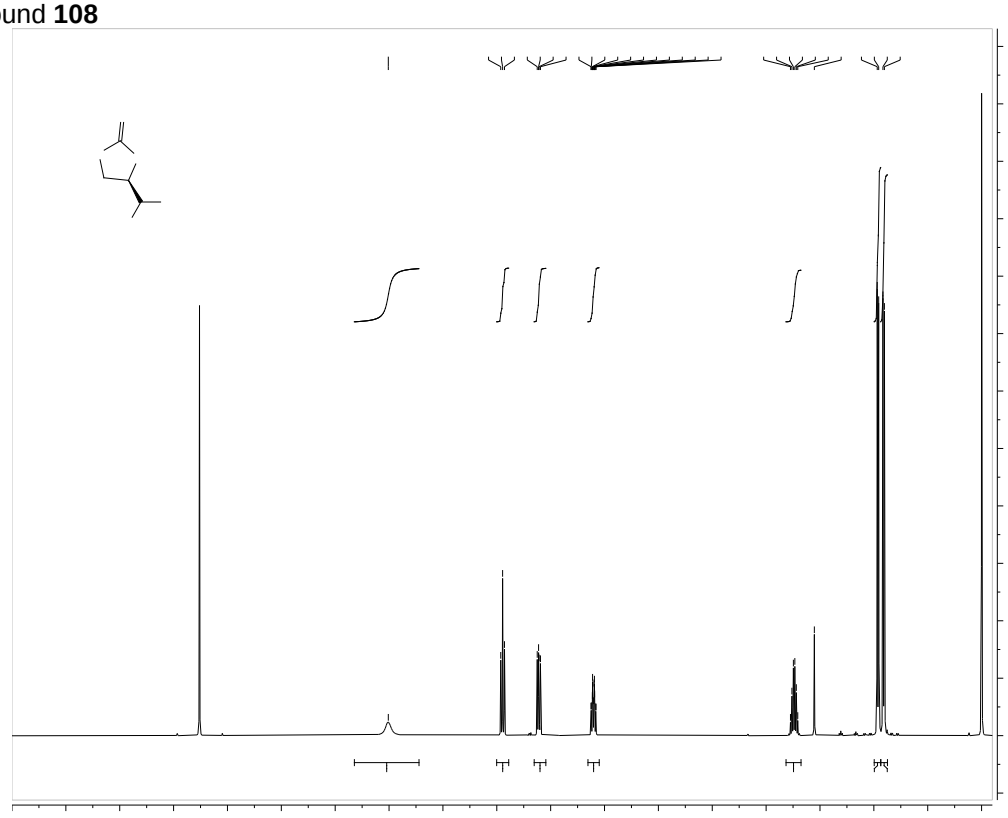
Compound **99**



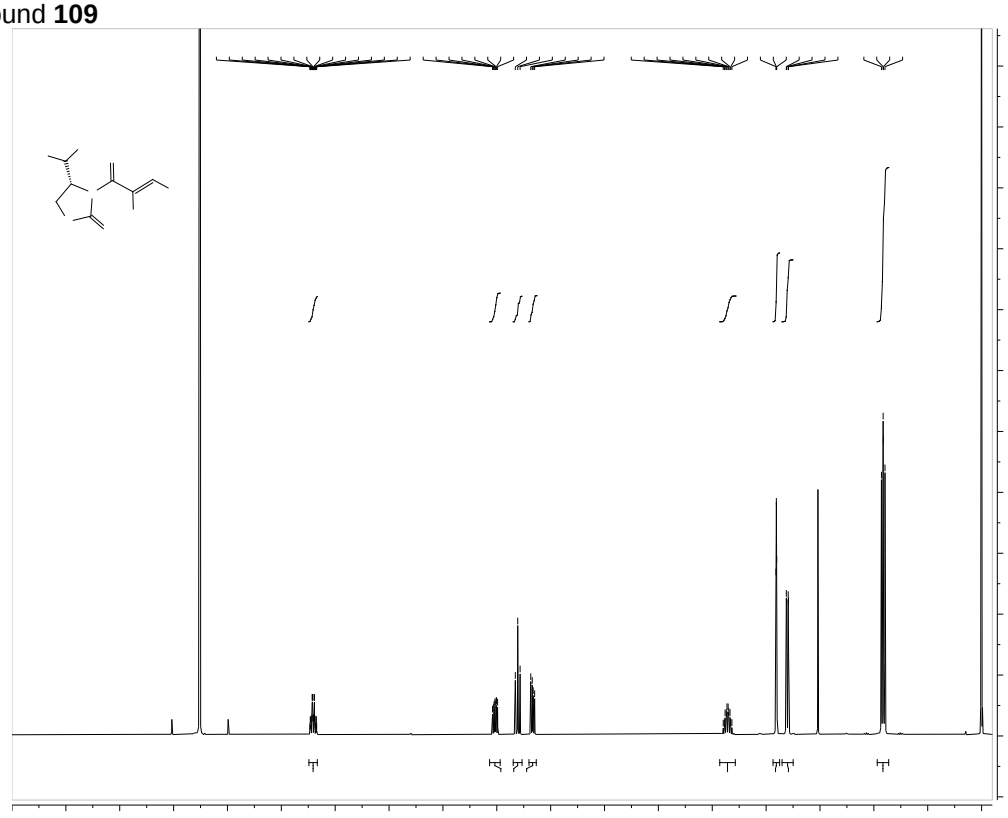
Compound **107**



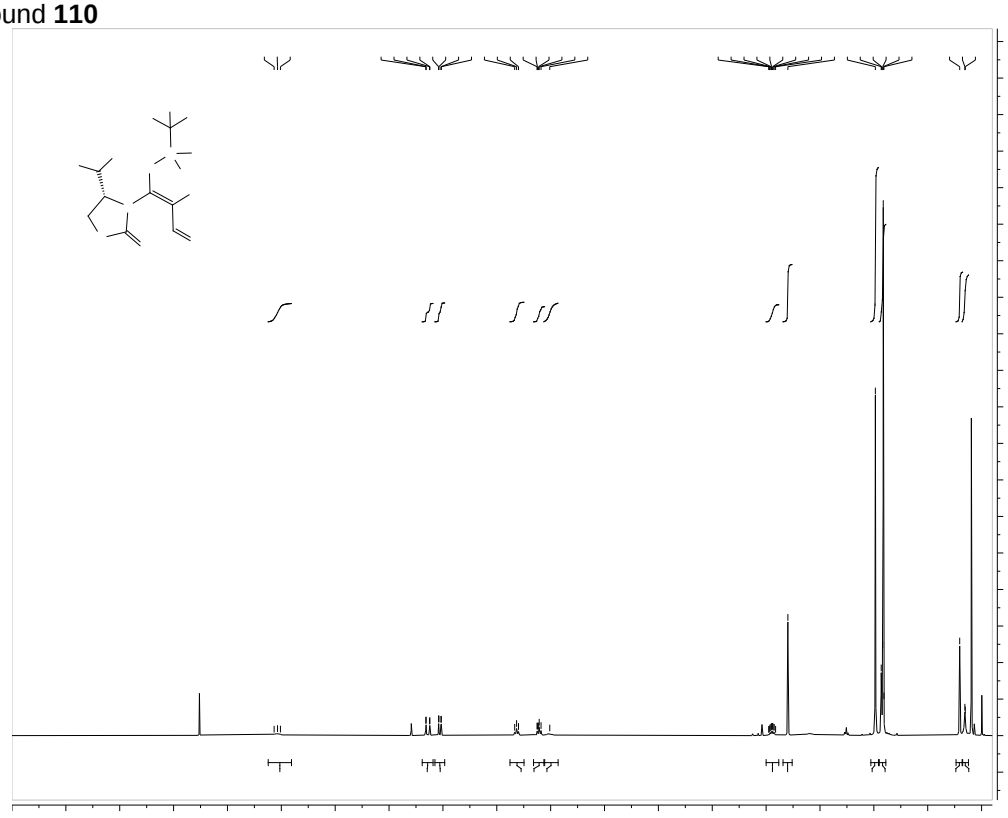
Compound **108**



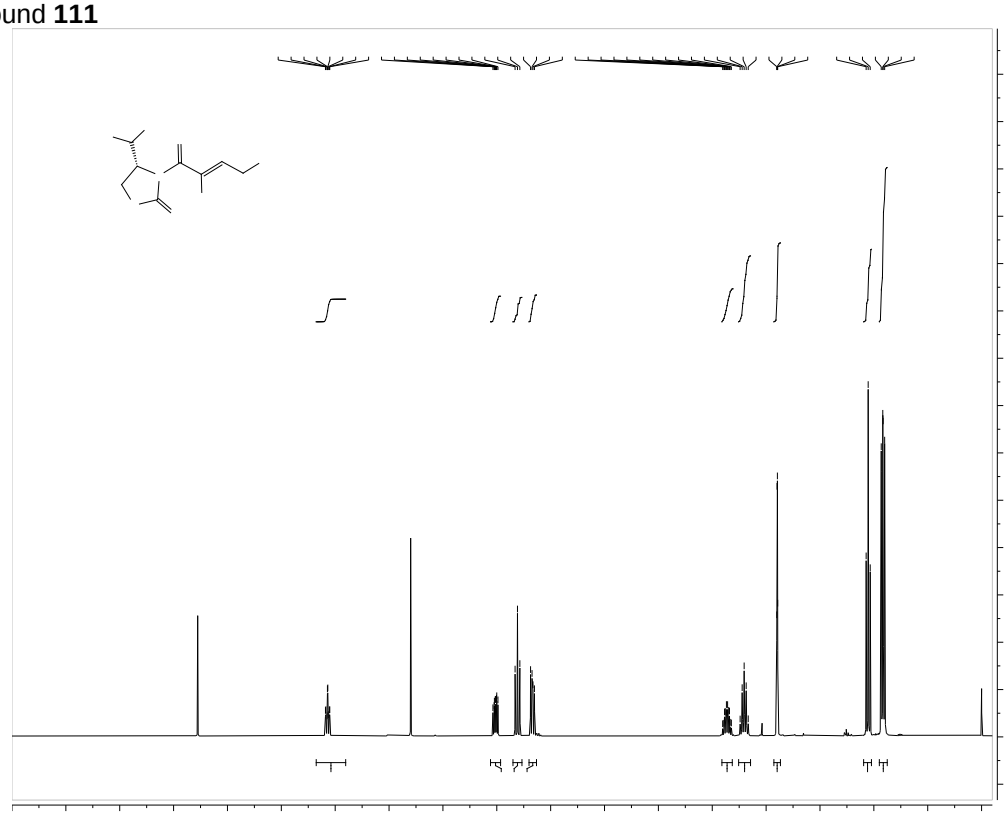
Compound **109**



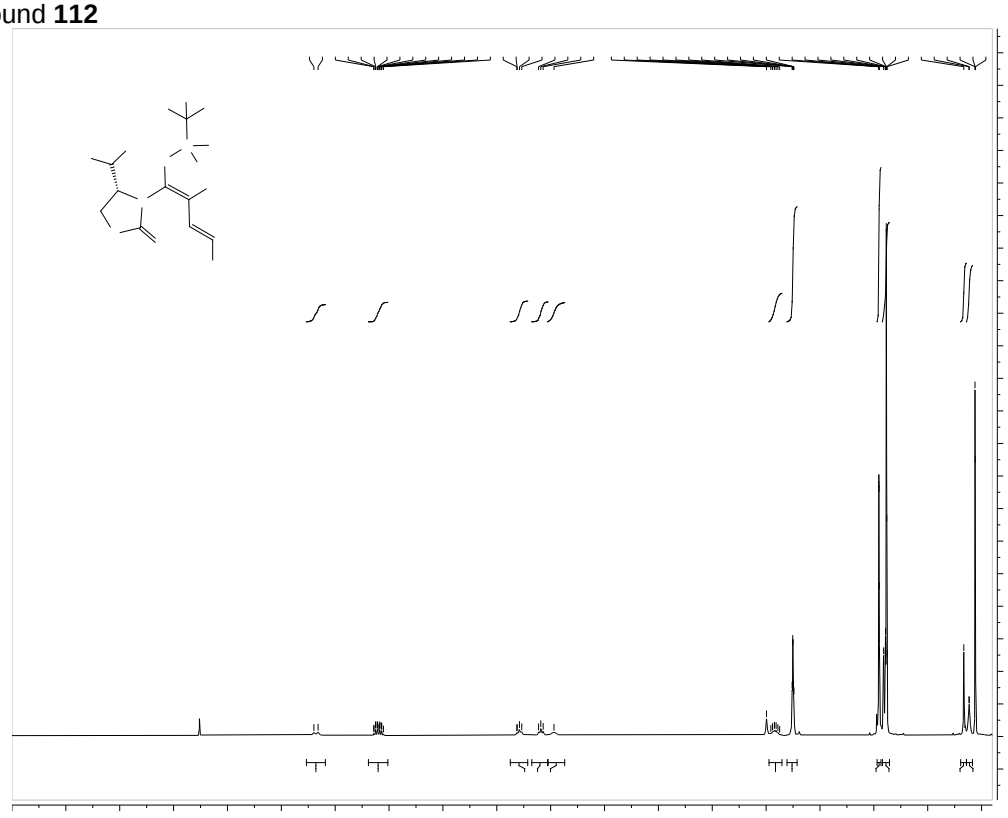
Compound **110**



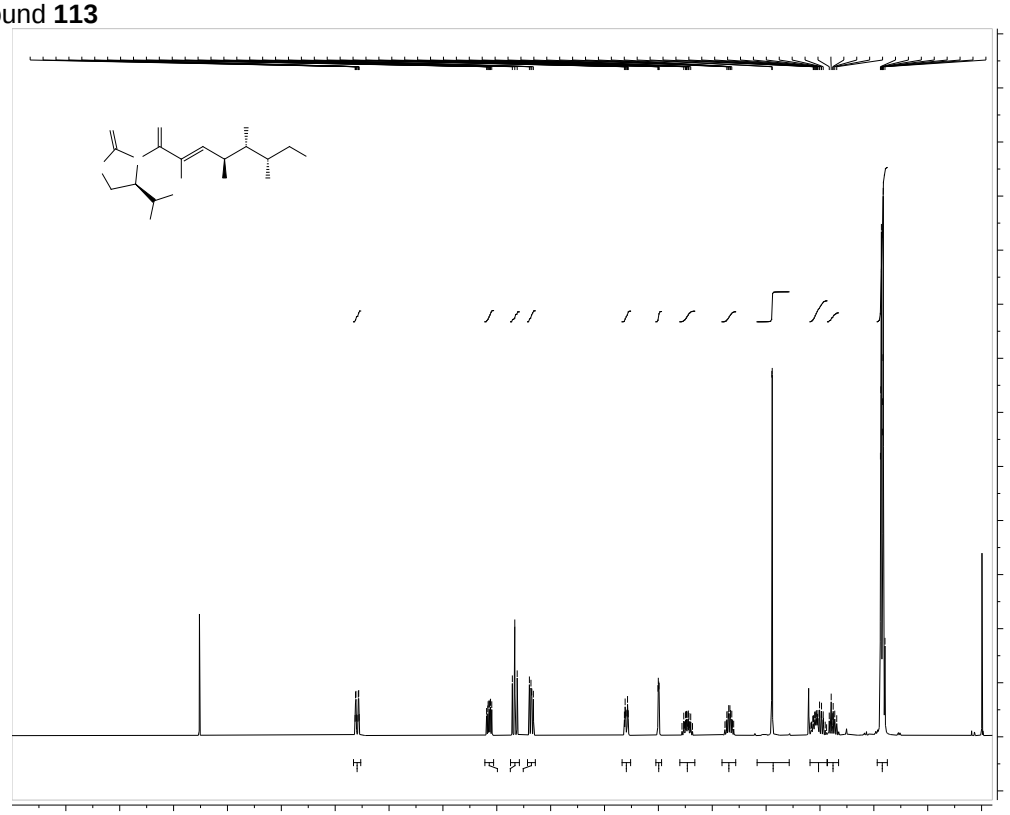
Compound **111**



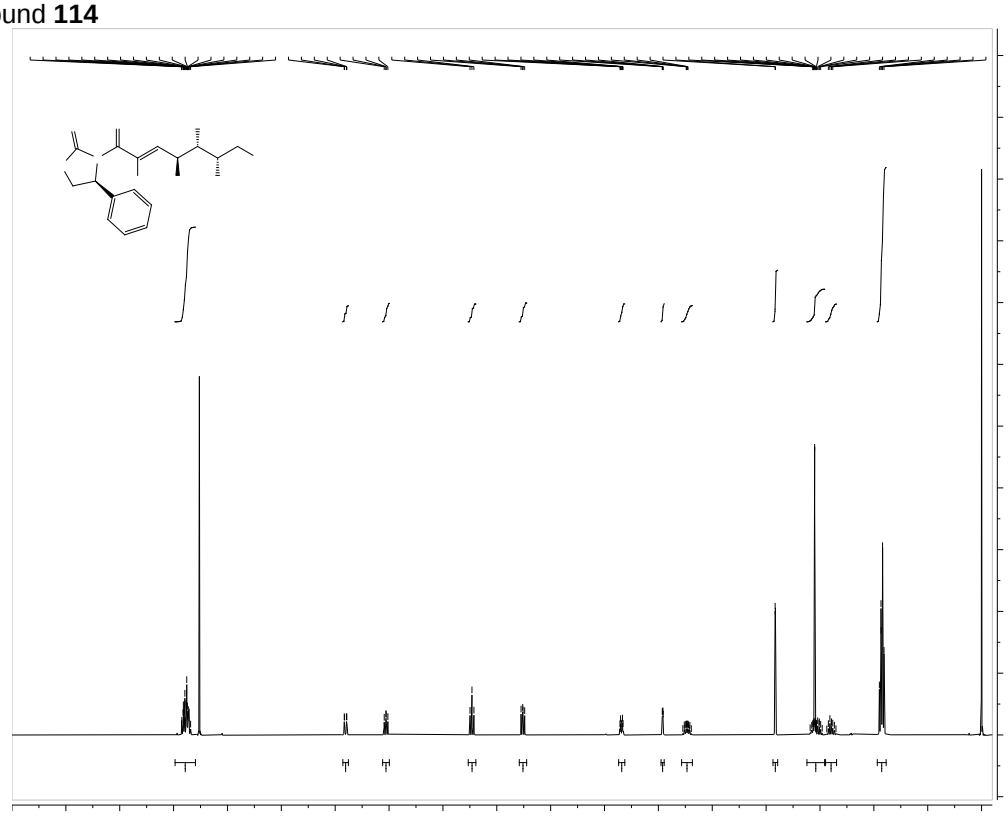
Compound **112**



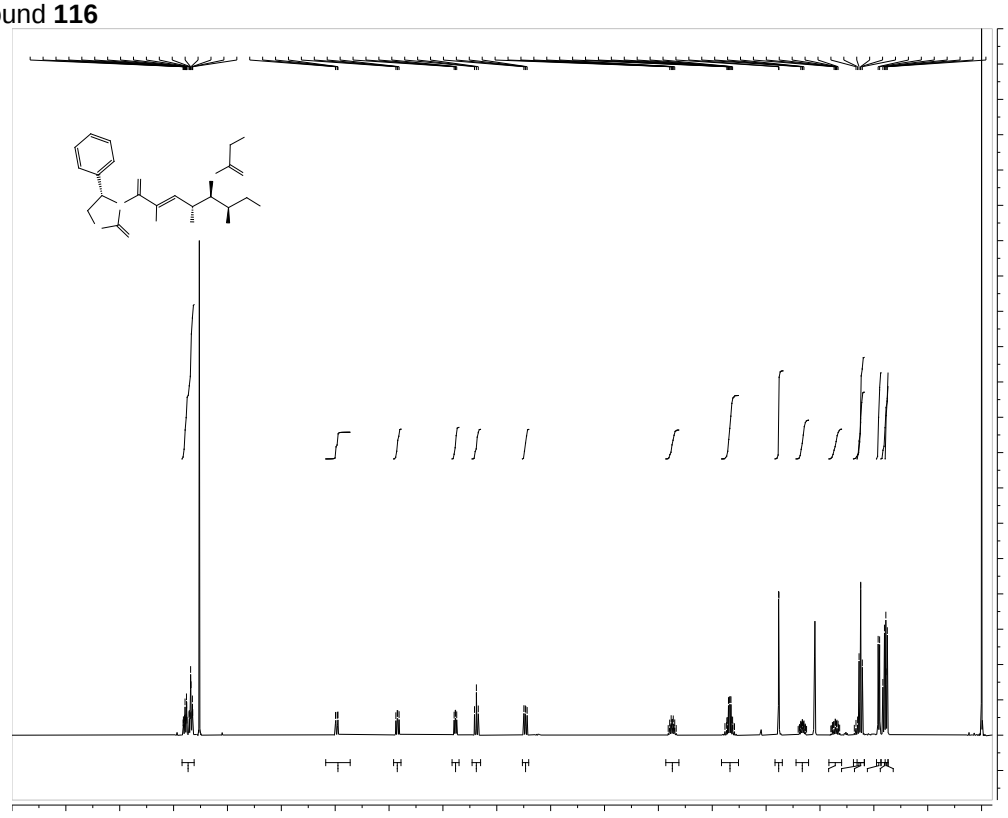
Compound **113**



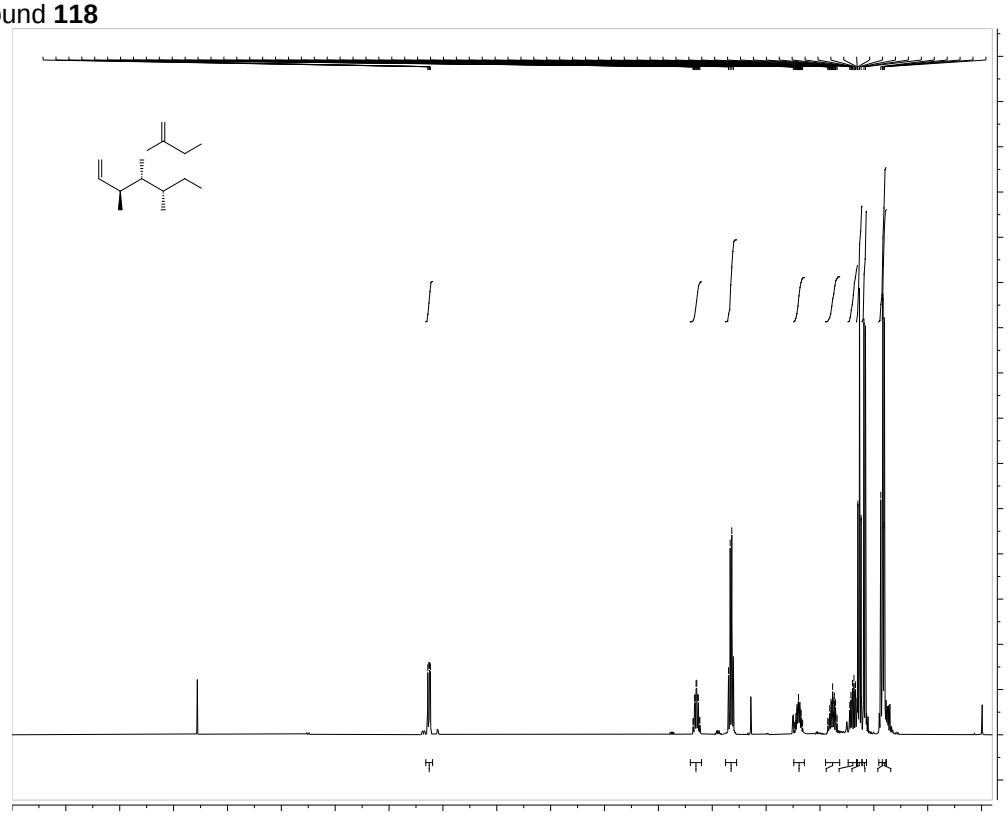
Compound **114**



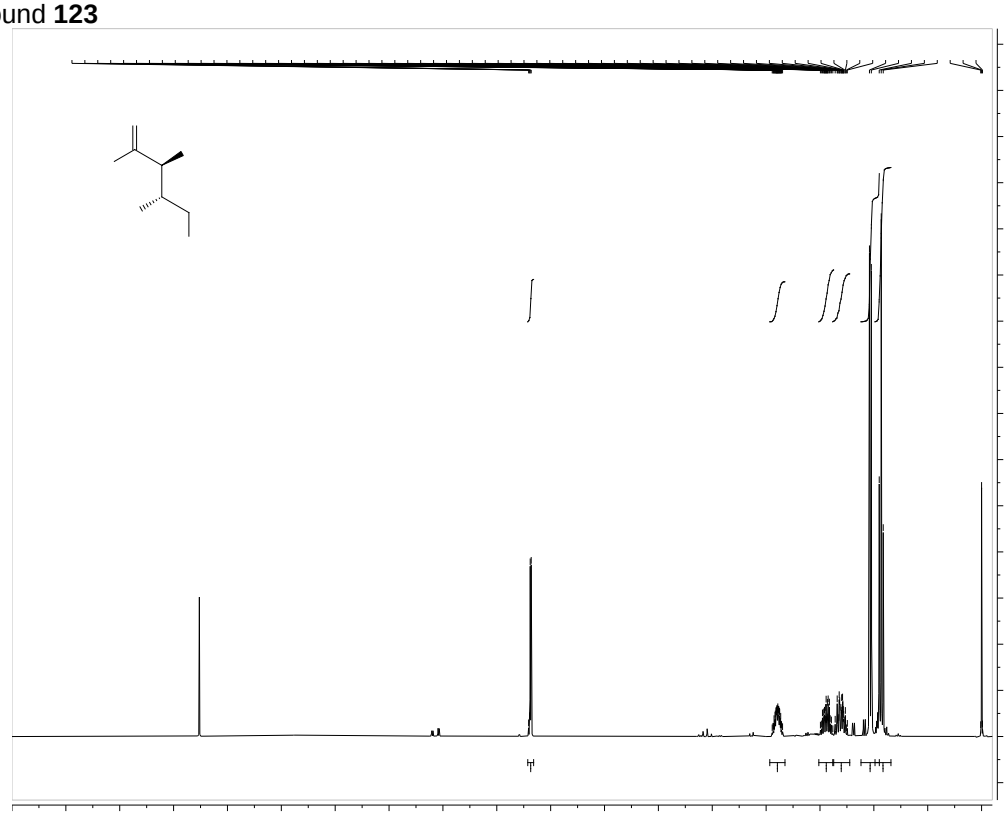
Compound **116**



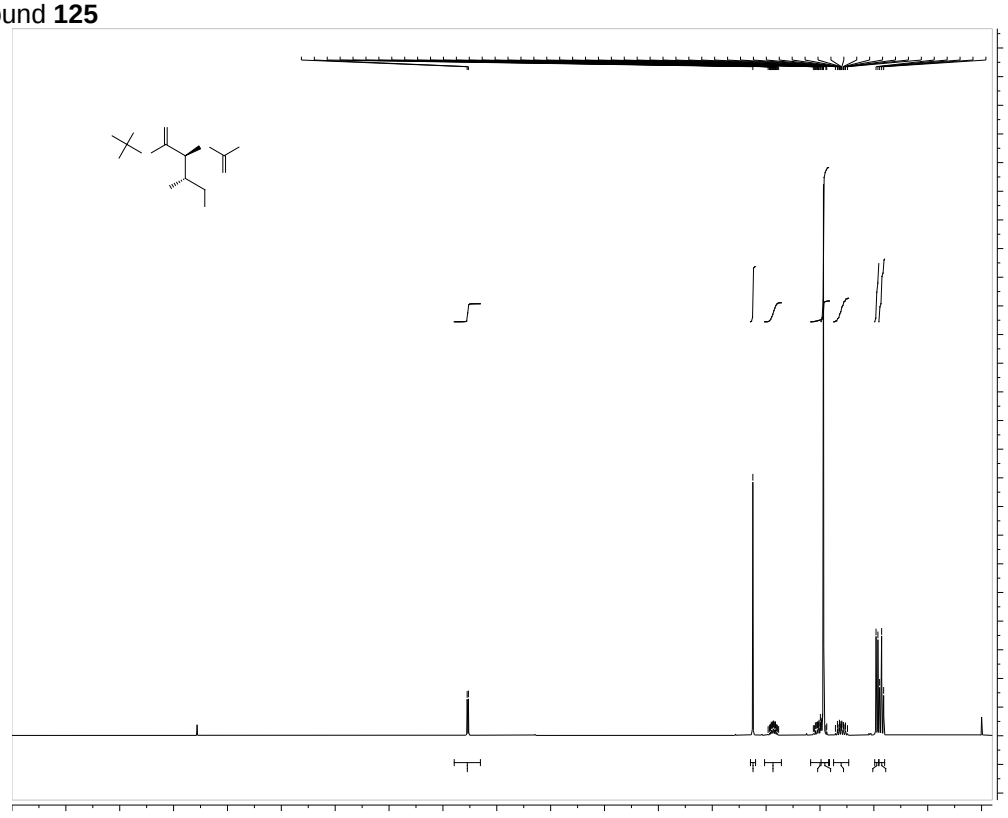
Compound **118**



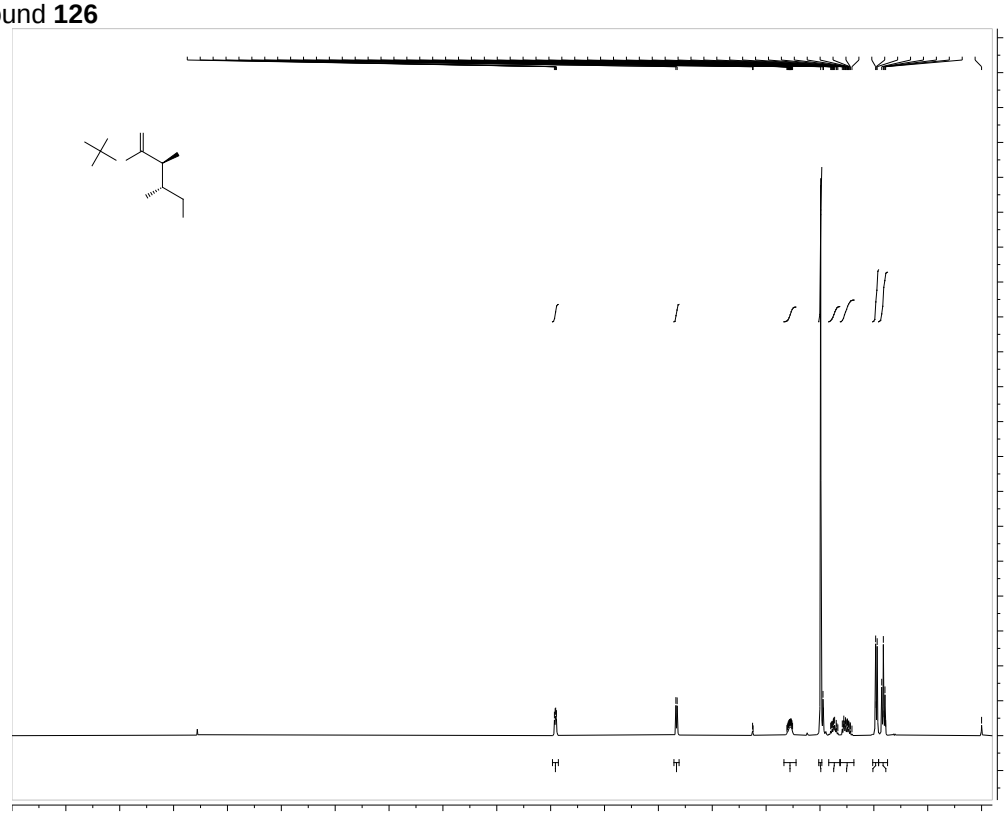
Compound **123**



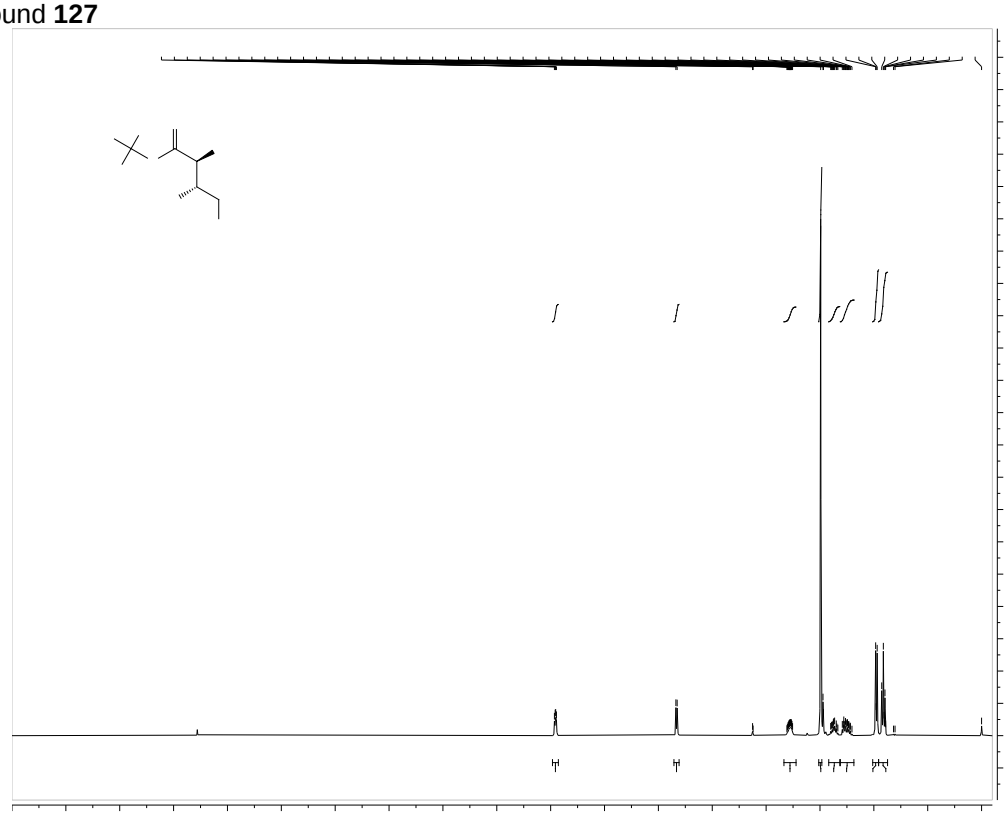
Compound **125**



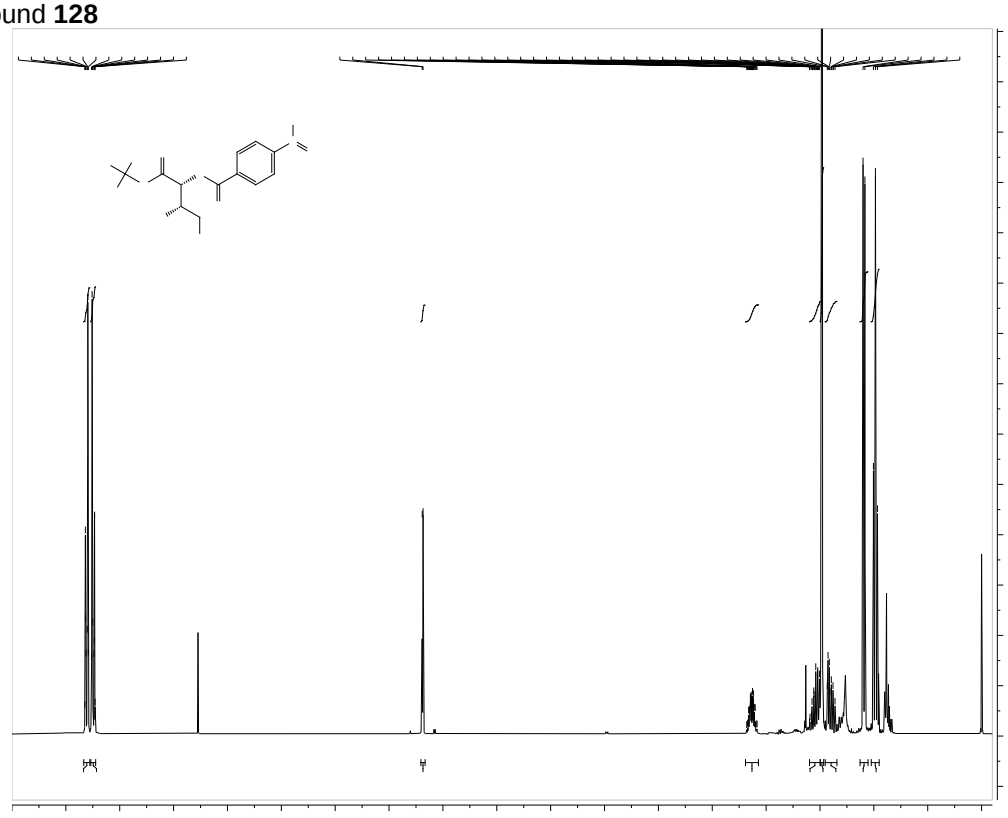
Compound **126**



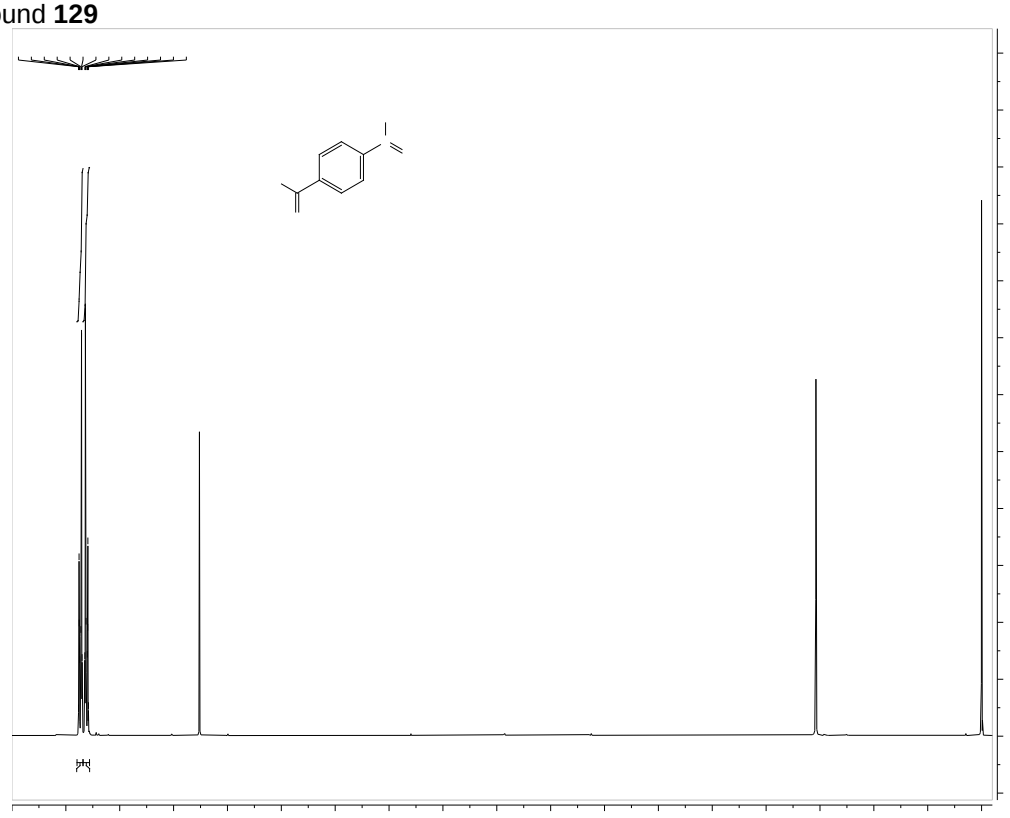
Compound **127**



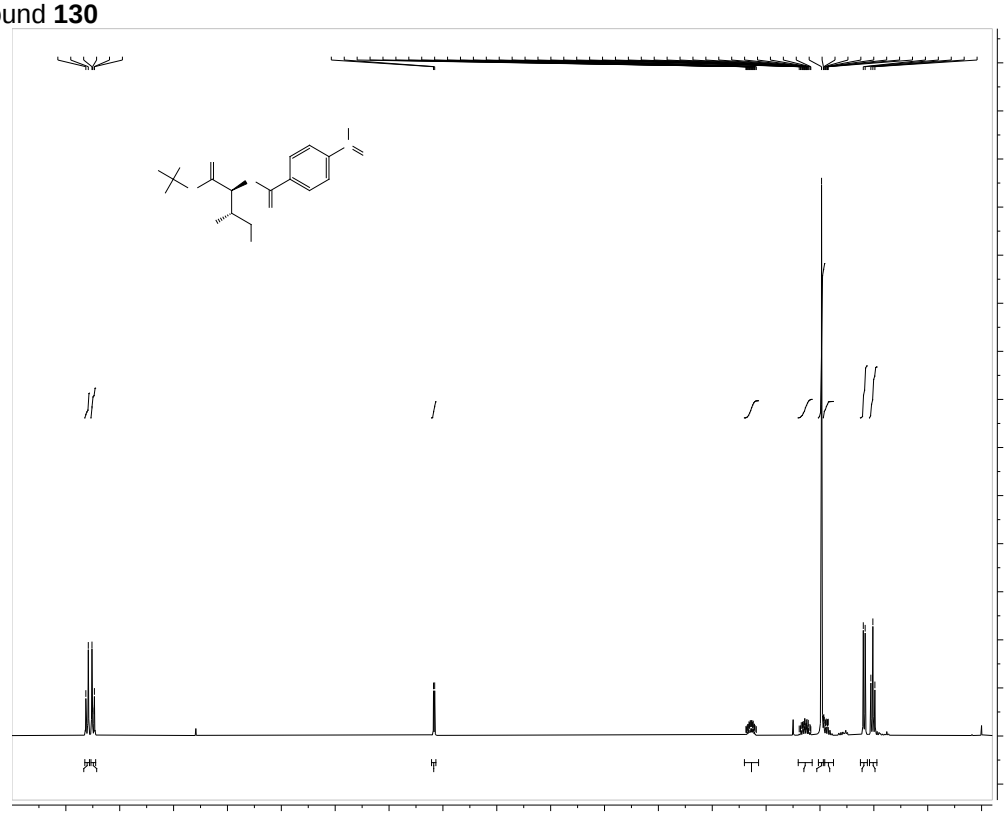
Compound **128**



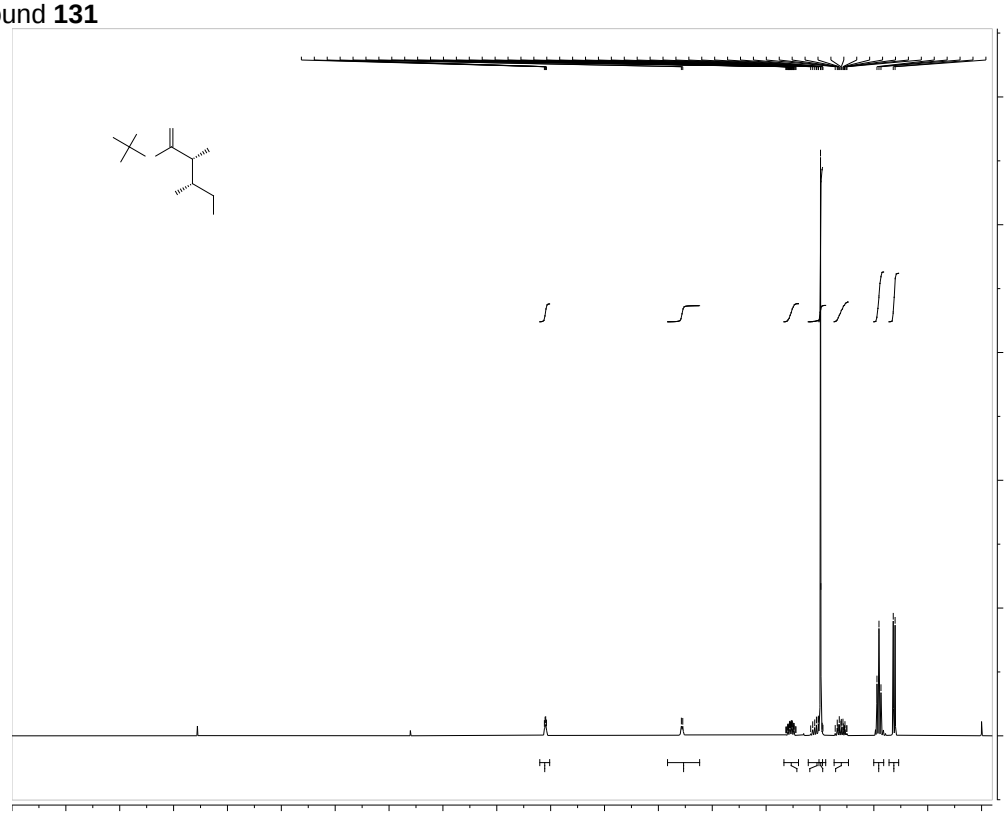
Compound **129**



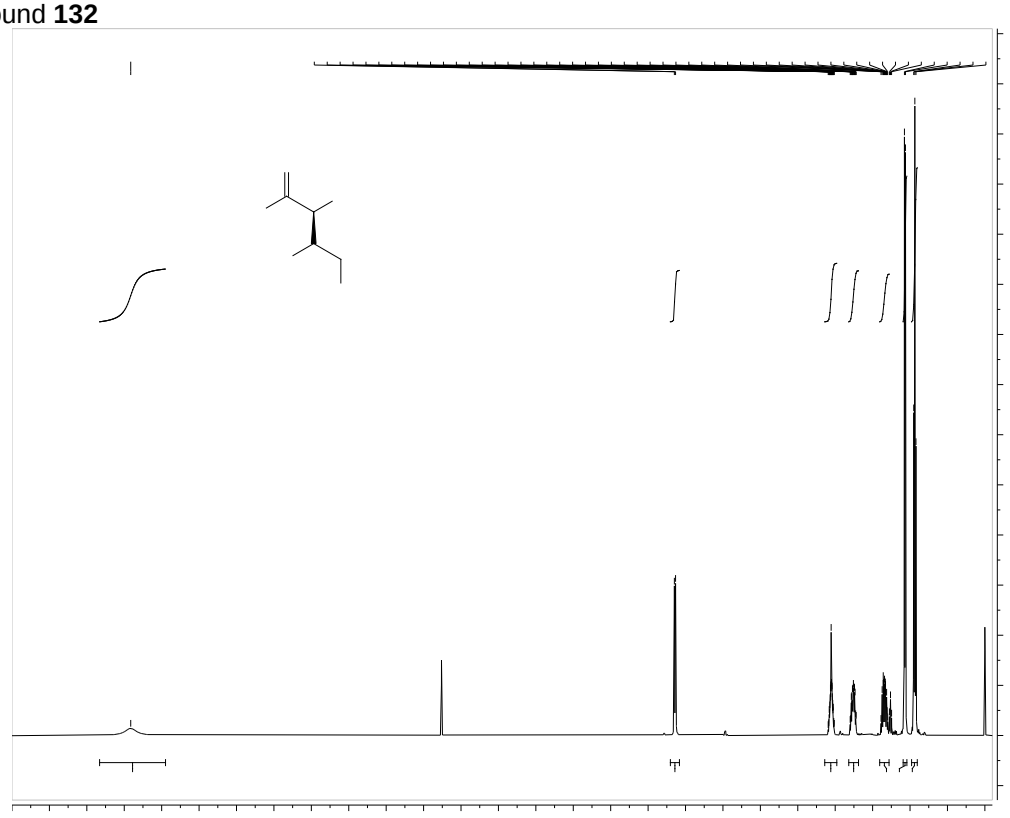
Compound **130**



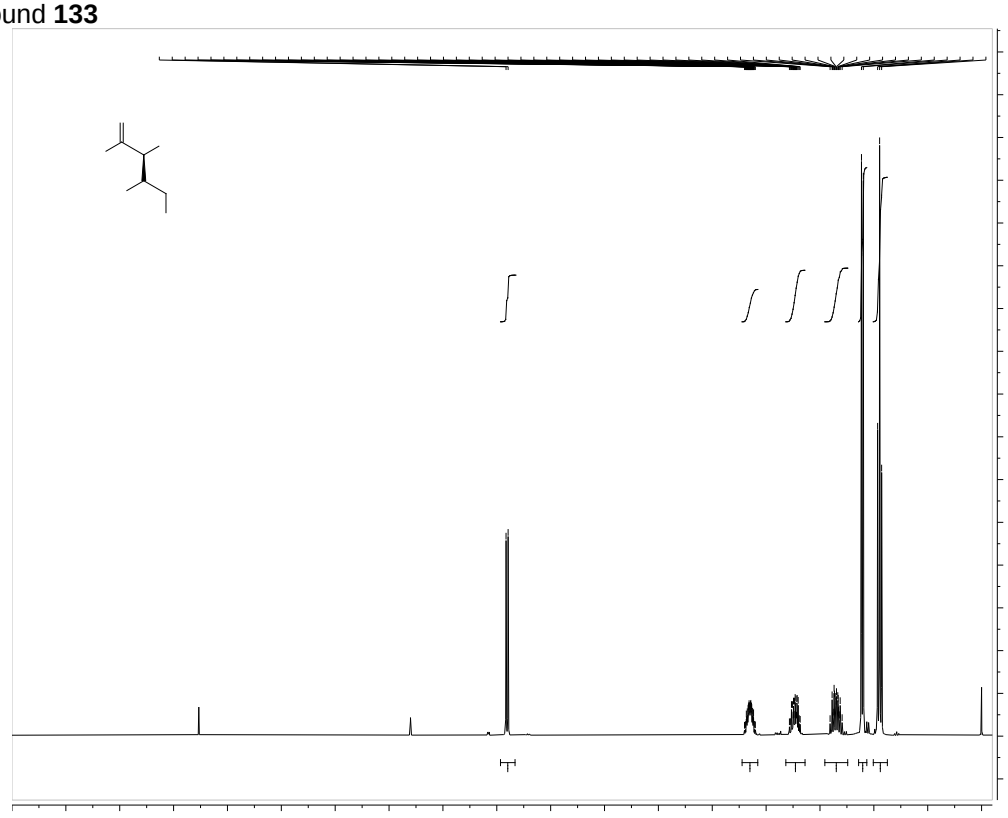
Compound **131**



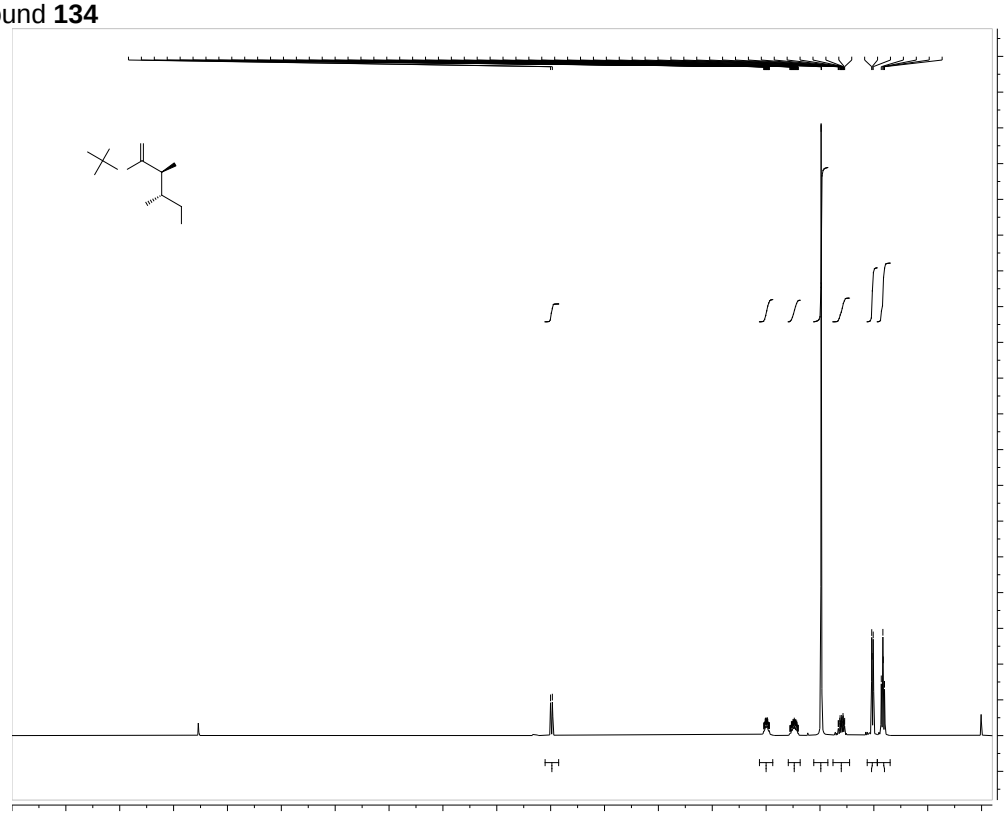
Compound **132**



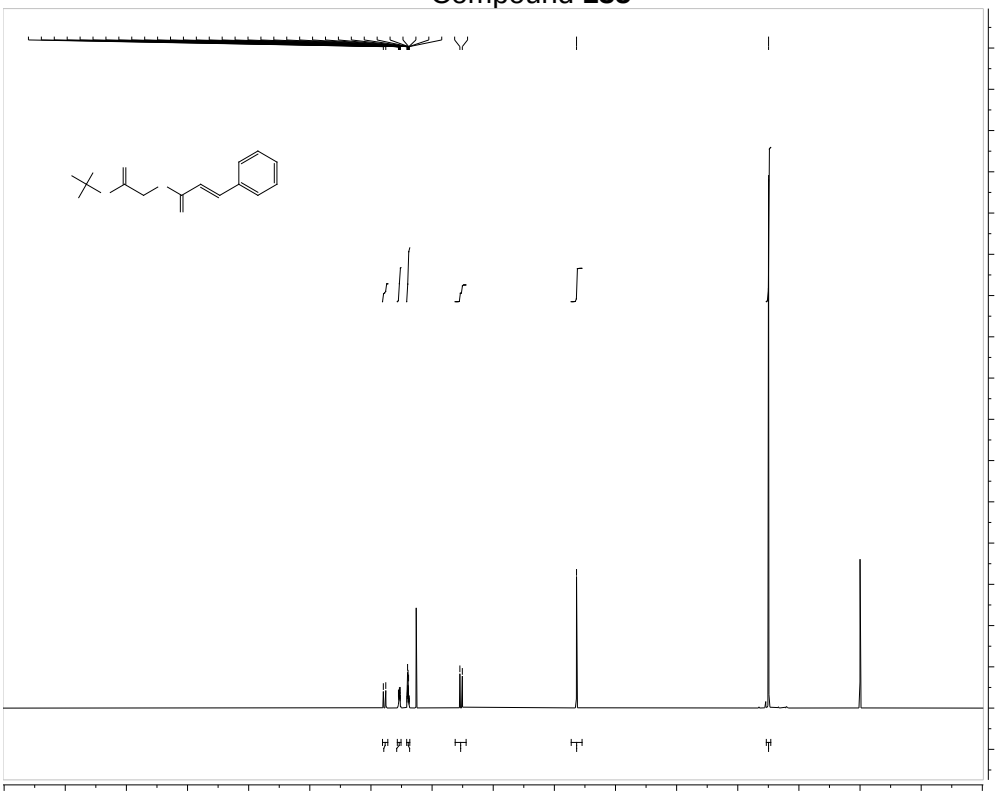
Compound **133**



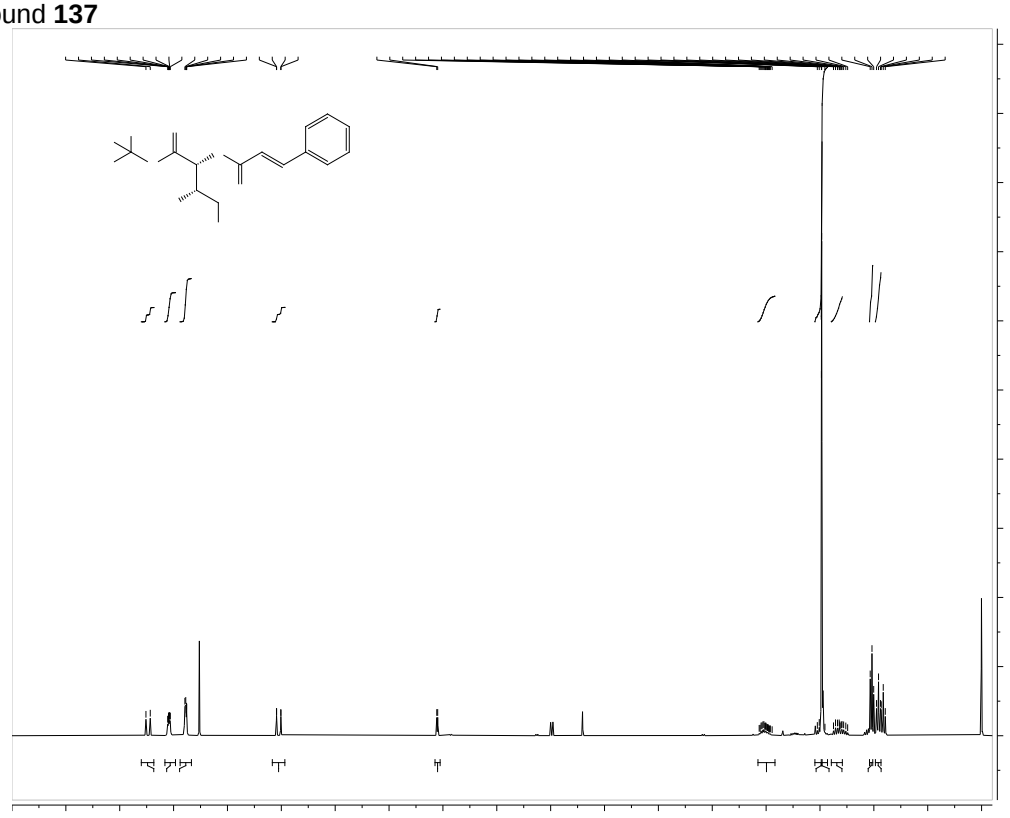
Compound **134**



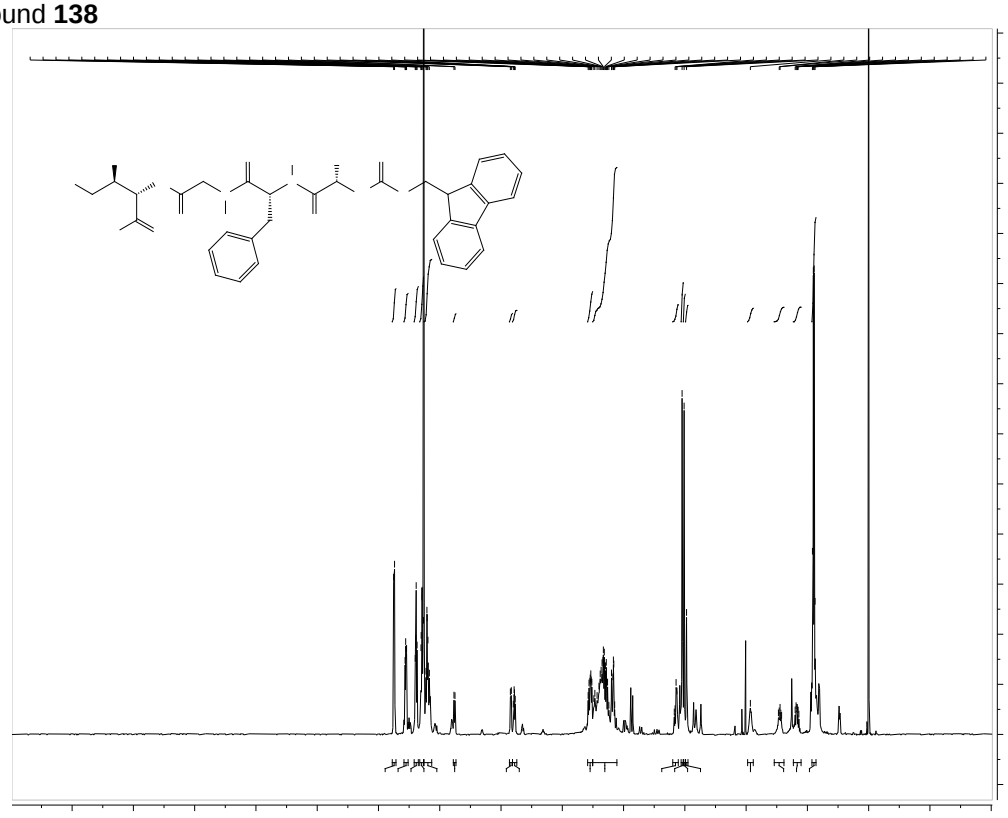
Compound **135**



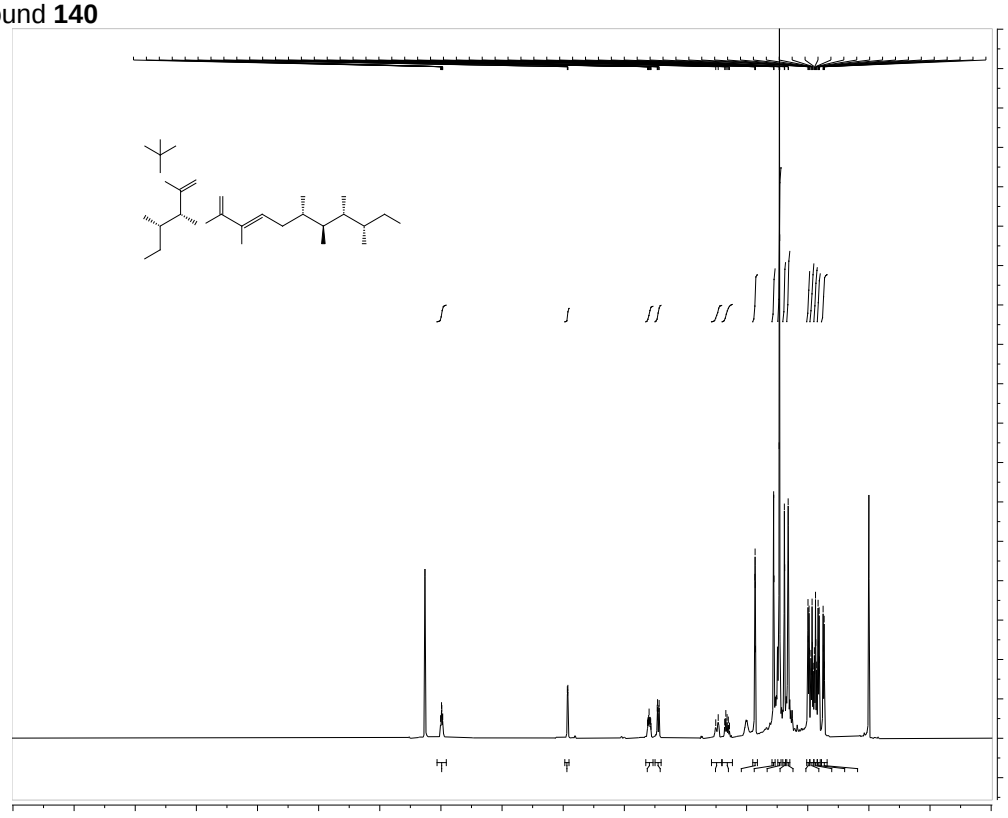
Compound **137**



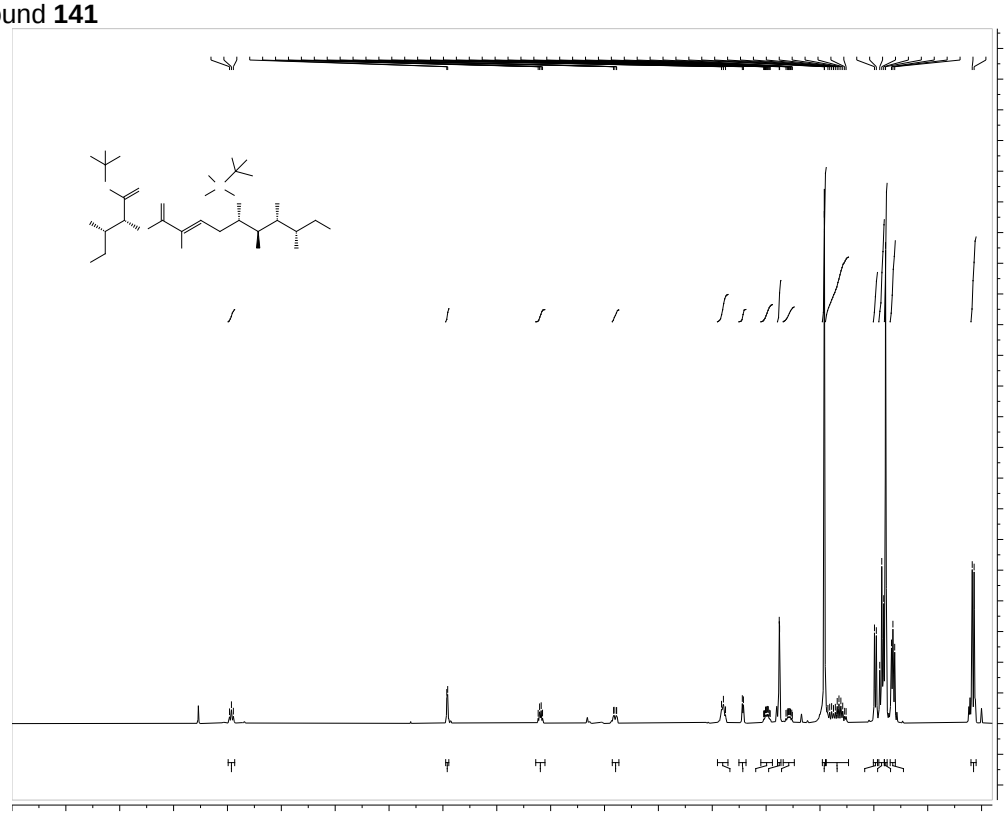
Compound **138**



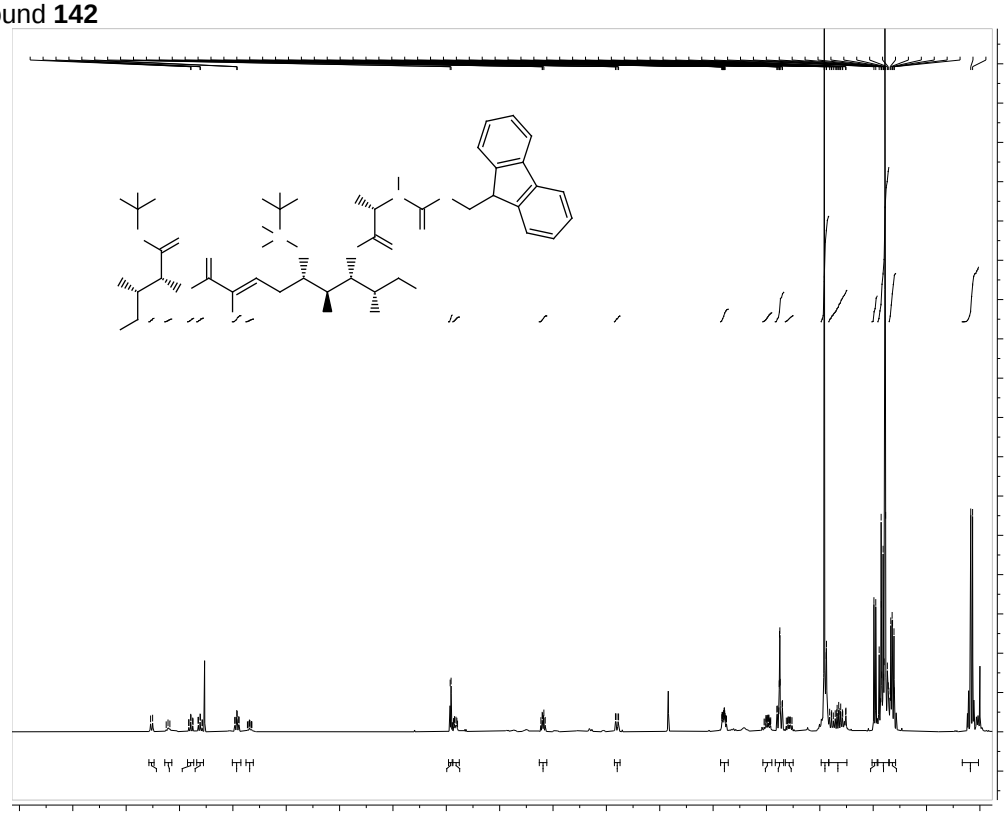
Compound **140**



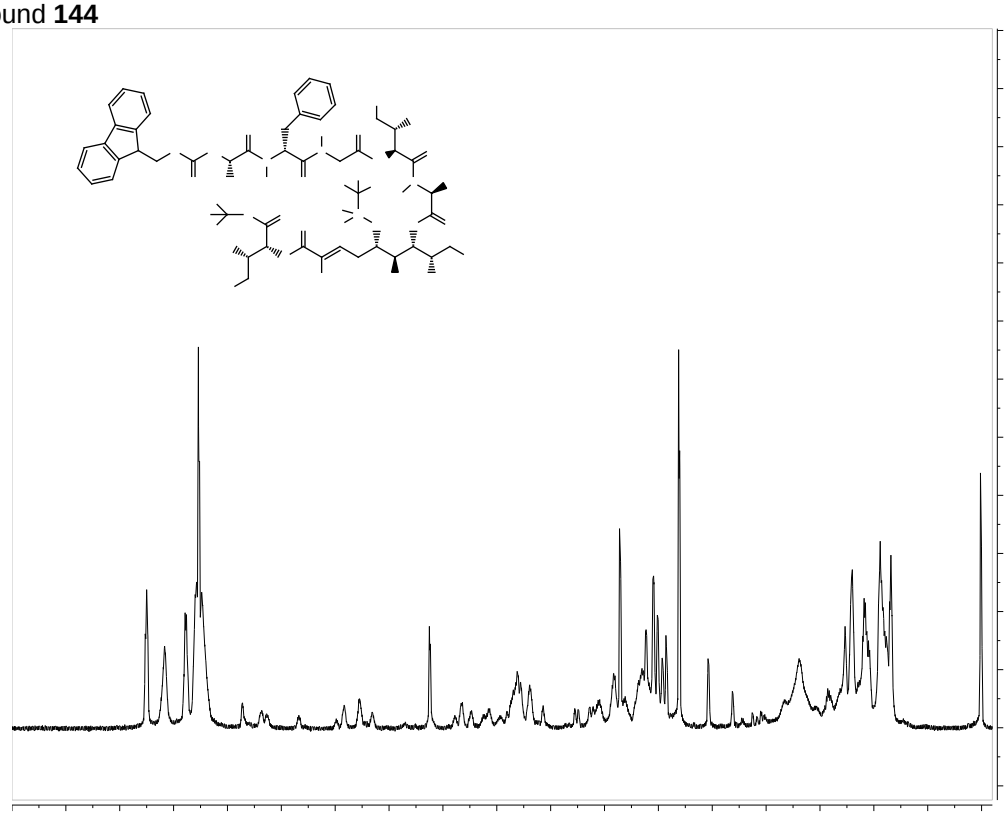
Compound **141**



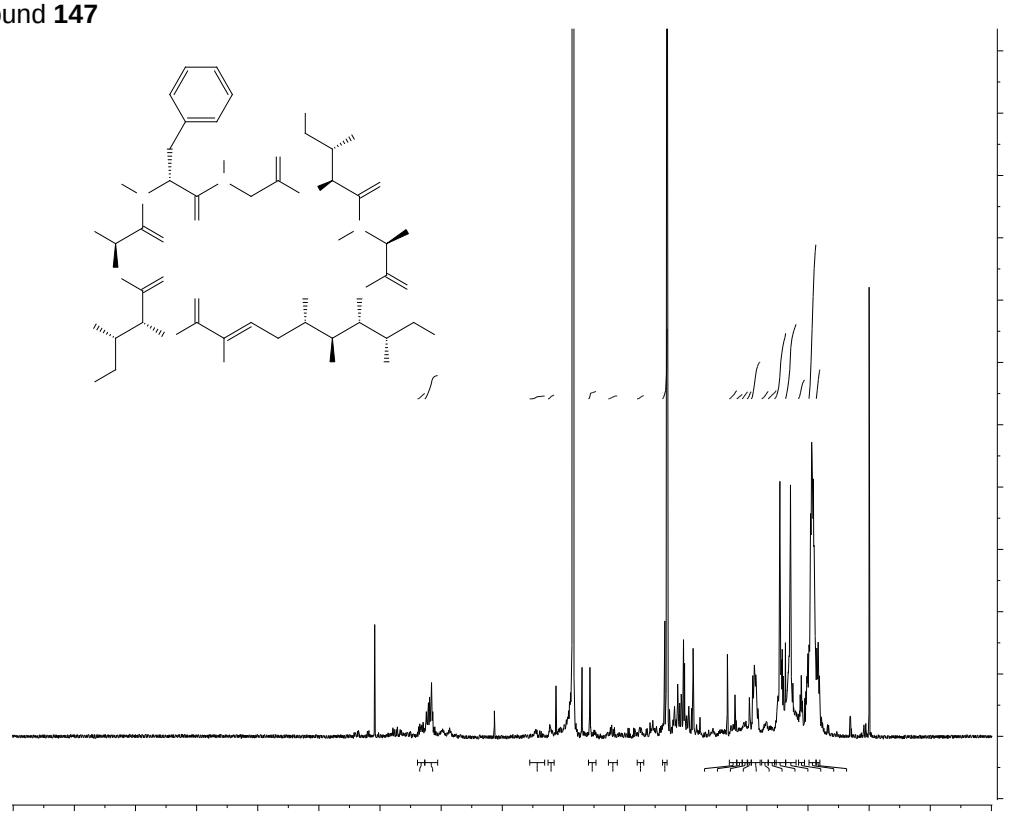
Compound **142**



Compound **144**



Compound **147**



References

(1) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. The Isolation and Structure of a Remarkable Marine Animal Antineoplastic Constituent: Dolastatin 10. *J. Am. Chem. Soc.* **1987**, *109* (22), 6883–6885. https://doi.org/10.1021/ja00256a070.

(2) Williams, P. G.; Yoshida, W. Y.; Quon, M. K.; Moore, R. E.; Paul, V. J. The Structure of Palau'amide, a Potent Cytotoxin from a Species of the Marine Cyanobacterium Lyngbya. *J. Nat. Prod.* **2003**, *66* (12), 1545–1549. https://doi.org/10.1021/np034001r.

(3) Simmons, T. L.; McPhail, K. L.; Ortega-Barría, E.; Mooberry, S. L.; Gerwick, W. H. Belamide A, a New Antimitotic Tetrapeptide from a Panamanian Marine Cyanobacterium. *Tetrahedron Lett.* **2006**, *47* (20), 3387–3390. https://doi.org/10.1016/j.tetlet.2006.03.082.

(4) Al-Awadhi, F. H.; Law, B. K.; Paul, V. J.; Luesch, H. Grassystatins D–F, Potent Aspartic Protease Inhibitors from Marine Cyanobacteria as Potential Antimetastatic Agents Targeting Invasive Breast Cancer. *J. Nat. Prod.* **2017**, *80* (11), 2969–2986. https://doi.org/10.1021/acs.jnatprod.7b00551.

(5) Tripathi, A.; Puddick, J.; Prinsep, M. R.; Rottmann, M.; Tan, L. T. Lagunamides A and B: Cytotoxic and Antimalarial Cyclodepsipeptides from the Marine Cyanobacterium Lyngbya Majuscula. *J. Nat. Prod.* **2010**, *73* (11), 1810–1814. https://doi.org/10.1021/np100442x.

(6) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. Apramides A−G, Novel Lipopeptides from the Marine Cyanobacterium Lyngbya Majuscula. *J. Nat. Prod.* **2000**, *63* (8), 1106–1112. https://doi.org/10.1021/np000078t.

(7) Esquenazi, E.; Jones, A. C.; Byrum, T.; Dorrestein, P. C.; Gerwick, W. H. Temporal Dynamics of Natural Product Biosynthesis in Marine Cyanobacteria. *Proc. Natl. Acad. Sci.* **2011**, *108* (13), 5226–5231. https://doi.org/10.1073/pnas.1012813108.

(8) Williams, P. CHEMICAL INVESTIGATIONS OF MARINE CYANOBACTERIA. THE SEARCH FOR NEW ANTICANCER AGENTS FROM THE SEA, University of Hawai'i, 2003.

(9) Kaiser, K.; Benner, R. Hydrolysis-Induced Racemization of Amino Acids. *Limnol. Oceanogr. Methods* **2005**, *3*, 318–325. https://doi.org/10.4319/lom.2005.3.318.

(10) Dai, L.; Chen, B.; Wang, Z.; Liu, Y.; Lei, H.; Xu, Z.; Ye, T. Total Synthesis and Stereochemical Revision of Lagunamide A. *Chem. Commun. (Camb).* **2012**, *48* (69), 8697–8699. https://doi.org/10.1039/c2cc34187e.

(11) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. Micromide and Guamamide: Cytotoxic Alkaloids from a Species of the Marine Cyanobacterium Symploca. *J. Nat. Prod.* **2004**, *67* (1), 49–53. https://doi.org/10.1021/np030215x.

(12) Luesch, H.; Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. Ulongamides A−F, New β-Amino Acid-Containing Cyclodepsipeptides from Palauan Collections of the

Marine Cyanobacterium Lyngbya Sp. *J. Nat. Prod.* **2002**, *65* (7), 996–1000. https://doi.org/10.1021/np0200461.

(13) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. Symplostatin 2: A Dolastatin 13 Analogue from the Marine Cyanobacterium Symploca Hydnoides. *J. Nat. Prod.* **1999**, *62* (4), 655–658. https://doi.org/10.1021/np980553b.

(14) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. Symplostatin 3, a New Dolastatin 10 Analogue from the Marine Cyanobacterium Symploca Sp. VP452. *J. Nat. Prod.* **2002**, *65* (1), 16–20. https://doi.org/10.1021/np010317s.

(15) Luesch, H.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. Isolation of Dolastatin 10 from the Marine Cyanobacterium Symploca Species VP642 and Total Stereochemistry and Biological Evaluation of Its Analogue Symplostatin 1. *J. Nat. Prod.* **2001**, *64* (7), 907–910. https://doi.org/10.1021/np010049y.

(16) Kavallaris, M.; Verrills, N. M.; Hill, B. T. Anticancer Therapy with Novel Tubulin-Interacting Drugs. *Drug Resist. Updat.* **2001**, *4* (6), 392–401. https://doi.org/10.1054/drup.2002.0230.

(17) Han, J.; Lian, J.; Tian, X.; Zhou, S.; Zhen, X.; Liu, S. Total Synthesis of Micromide: A Marine Natural Product. *European J. Org. Chem.* **2014**, *2014* (32), 7232–7238. https://doi.org/10.1002/ejoc.201402977.

(18) Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. 2,4-Dinitrobenzenesulfonamides: A Simple and Practical Method for the Preparation of a Variety of Secondary Amines and Diamines. *Tetrahedron Lett.* **1997**, *38* (33), 5831–5834. https://doi.org/10.1016/S0040-4039(97)01334-8.

(19) Braun, M.; Devant, R. (R)- and (S)-2-Acetoxy-1,1,2-Triphenylethanol - Effective Synthetic Equivalents of a Chiral Acetate Enolate. *Tetrahedron Lett.* **1984**, *25* (44), 5031–5034. https://doi.org/10.1016/S0040-4039(01)91110-4.

(20) Nevchas, I. Chemical Methodologies Toward the Total Synthesis of Virginiamycin M1 and Micromide Natural Products, Montezuma Publishing, 2009.

(21) Fleming, I.; Henning, R.; Parker, D. C.; Plaut, H. E.; Sanderson, P. E. J. The Phenyldimethylsilyl Group as a Masked Hydroxy Group. *J. Chem. Soc. Perkin Trans. 1* **1995**, *3* (4), 317. https://doi.org/10.1039/p19950000317.

(22) Fleming, I.; Henning, R.; Plaut, H. The Phenyldimethylsilyl Group as a Masked Form of the Hydroxy Group. *J. Chem. Soc. Chem. Commun.* **1984**, No. 1, 29. https://doi.org/10.1039/c39840000029.

(23) Evans, D. A.; Britton, T. C.; Ellman, J. A. Contrasteric Carboximide Hydrolysis with Lithium Hydroperoxide. *Tetrahedron Lett.* **1987**, *28* (49), 6141–6144. https://doi.org/10.1016/S0040-4039(00)61830-0.

(24) Kempf, D. J.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, K. C.; Bryant, P. Symmetry-Based Inhibitors of HIV Protease. Structure-Activity Studies of Acylated 2,4-Diamino-1,5-Diphenyl-3-

Hydroxypentane and 2,5-Diamino-1,6-Diphenylhexane-3,4-Diol. *J. Med. Chem.* **1993**, *36* (3), 320–330. https://doi.org/10.1021/jm00055a003.

(25) Cho, B. T.; Kang, S. K. Direct and Indirect Reductive Amination of Aldehydes and Ketones with Solid Acid-Activated Sodium Borohydride under Solvent-Free Conditions. *Tetrahedron* **2005**, *61* (24), 5725–5734. https://doi.org/10.1016/j.tet.2005.04.039.

(26) El-Faham, A.; Albericio, F. Peptide Coupling Reagents, More than a Letter Soup. *Chem. Rev.* **2011**, *111* (11), 6557–6602. https://doi.org/10.1021/cr100048w.

(27) Albericio, F. Developments in Peptide and Amide Synthesis. *Curr. Opin. Chem. Biol.* **2004**, *8* (3), 211–221. https://doi.org/10.1016/j.cbpa.2004.03.002.

(28) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S. Synthesis of 9-Fluorenylmethyloxycarbonyl-Protected N-Alkyl Amino Acids by Reduction of Oxazolidinones. *J. Org. Chem.* **1983**, *48* (1), 77–81. https://doi.org/10.1021/jo00149a016.

(29) Govender, T.; Arvidsson, P. I. Facile Synthesis of Fmoc-N-Methylated α- and β-Amino Acids. *Tetrahedron Lett.* **2006**, *47* (11), 1691–1694. https://doi.org/10.1016/j.tetlet.2006.01.085.

(30) Hu, D. X.; Grice, P.; Ley, S. V. Rotamers or Diastereomers? An Overlooked NMR Solution. *J. Org. Chem.* **2012**, *77* (11), 5198–5202. https://doi.org/10.1021/jo300734r.

(31) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. Isolation, Structure Determination, and Biological Activity of Dolastatin 12 and Lyngbyastatin 1 from Lyngbya Majuscula/Schizothrix Calcicola Cyanobacterial Assemblages. *J. Nat. Prod.* **1998**, *61* (10), 1221–1225. https://doi.org/10.1021/np9801211.

(32) Penso, M.; Albanese, D.; Landini, D.; Lupi, V.; Tricarico, G. Specific Solvation as a Tool for TheN-Chemoselective Arylsulfonylation of Tyrosine and (4-Hydroxyphenyl)Glycine Methyl Esters. *European J. Org. Chem.* **2003**, *2003* (23), 4513–4517. https://doi.org/10.1002/ejoc.200300366.

(33) Krafts, K.; Hempelmann, E.; Skórska-Stania, A. From Methylene Blue to Chloroquine: A Brief Review of the Development of an Antimalarial Therapy. *Parasitol. Res.* **2012**, *111* (1), 1–6. https://doi.org/10.1007/s00436-012-2886-x.

(34) Tu, Y. The Discovery of Artemisinin (Qinghaosu) and Gifts from Chinese Medicine. *Nat. Med.* **2011**, *17* (10), 1217–1220. https://doi.org/10.1038/nm.2471.

(35) Wang, J.; Zhang, C. J.; Chia, W. N.; Loh, C. C. Y.; Li, Z.; Lee, Y. M.; He, Y.; Yuan, L. X.; Lim, T. K.; Liu, M.; et al. Haem-Activated Promiscuous Targeting of Artemisinin in Plasmodium Falciparum. *Nat. Commun.* **2015**, *6*. https://doi.org/10.1038/ncomms10111.

(36) Tilley, L.; Straimer, J.; Gnädig, N. F.; Ralph, S. A.; Fidock, D. A. Artemisinin Action and Resistance in Plasmodium Falciparum. *Trends Parasitol.* **2016**, *32* (9), 682–696. https://doi.org/10.1016/j.pt.2016.05.010.

(37) Fotie, J. The Potential of Peptides and Depsipeptides from Terrestrial and Marine

Organisms in the Fight against Human Protozoan Diseases. *Bioact. Nat. Prod. Chem. Biol.* **2015**, 279–320. https://doi.org/10.1002/9783527684403.ch10.

(38) Engene, N.; Rottacker, E. C.; Kaštovský, J.; Byrum, T.; Choi, H.; Ellisman, M. H.; Komárek, J.; Gerwick, W. H. Moorea Producens Gen. Nov., Sp. Nov. and Moorea Bouillonii Comb. Nov., Tropical Marine Cyanobacteria Rich in Bioactive Secondary Metabolites. *Int. J. Syst. Evol. Microbiol.* **2012**, *62* (Pt 5), 1171–1178. https://doi.org/10.1099/ijs.0.033761-0.

(39) Mevers, E.; Haeckl, F. P. J.; Boudreau, P. D.; Byrum, T.; Dorrestein, P. C.; Valeriote, F. A.; Gerwick, W. H. Lipopeptides from the Tropical Marine Cyanobacterium *Symploca* Sp. *J. Nat. Prod.* **2014**, *77* (4), 969–975. https://doi.org/10.1021/np401051z.

(40) Evans, D. A.; Weber, A. E. Asymmetric Glycine Enolate Aldol Reactions: Synthesis of Cyclosporine's Unusual Amino Acid, MeBmt1. *J. Am. Chem. Soc.* **1986**, *108* (21), 6757–6761. https://doi.org/10.1021/ja00281a049.

(41) Lucio, P.; Montanari, F.; Quici, S. A GENERAL SYNTHETIC METHOD FOR THE OXIDATION OF PRIMARY ALCOHOLS TO ALDEHYDES: (S)-(+)-2-METHYLBUTANAL. *Org. Synth.* **1990**, *69*, 212. https://doi.org/10.15227/orgsyn.069.0212.

(42) Meyers, A. I.; Spohn, R. F.; Linderman, R. J. Enantioselective Synthesis of the Depsipeptide Unsaturated Acid Portion of Madumycin II. *J. Org. Chem.* **1985**, *50* (19), 3633–3635. https://doi.org/10.1021/jo00219a042.

(43) Davies, S. G.; Fletcher, A. M.; Roberts, P. M.; Thomson, J. E. SuperQuat Chiral Auxiliaries: Design, Synthesis, and Utility. *Org. Biomol. Chem.* **2019**, *17* (6), 1322–1335. https://doi.org/10.1039/C8OB02819B.

(44) Bach, J.; Blachère, C.; Bull, S. D.; Davies, S. G.; Nicholson, R. L.; Price, P. D.; Sanganee, H. J.; Smith, A. D. N-Acyl-5,5-Dimethyloxazolidin-2-Ones as Latent Aldehyde Equivalents. *Org. Biomol. Chem.* **2003**, *1* (11), 2001–2010. https://doi.org/10.1039/B301119D.

(45) Davies, S. G.; Nicholson, R. L.; Smith, A. D. A SuperQuat Glycolate Aldol Approach to the Asymmetric Synthesis of Hexose Monosaccharides. *Org. Biomol. Chem.* **2005**, *3* (2), 348. https://doi.org/10.1039/b415943h.

(46) Brown, H. C.; Desai, M. C.; Jadhav, P. K. Hydroboration. 61. Diisopinocampheylborane of High Optical Purity. Improved Preparation and Asymmetric Hydroboration of Representative Cis-Disubstituted Alkenes. *J. Org. Chem.* **1982**, *47* (26), 5065–5069. https://doi.org/10.1021/jo00147a004.

(47) Jadhav, P. K.; Bhat, K. S.; Perumal, P. T.; Brown, H. C. Chiral Synthesis via Organoboranes. 6. Asymmetric Allylboration via Chiral Allyldialkylboranes. Synthesis of Homoallylic Alcohols with Exceptionally High Enantiomeric Excess. *J. Org. Chem.* **1986**, *51* (4), 432–439. https://doi.org/10.1021/jo00354a003.

(48) Evans, D. A.; Bartroli, J.; Shih, T. L. Enantioselective Aldol Condensations. 2. Erythro-Selective Chiral Aldol Condensations via Boron Enolates. *J. Am. Chem. Soc.* **1981**, *103* (8), 2127–2129. https://doi.org/10.1021/ja00398a058.

(49) Huang, W.; Ren, R. G.; Dong, H. Q.; Wei, B. G.; Lin, G. Q. Diverse Synthesis of Marine Cyclic Depsipeptide Lagunamide A and Its Analogues. *J. Org. Chem.* **2013**, *78* (21), 10747–10762. https://doi.org/10.1021/jo401687s.

(50) Irving, H.; Williams, R. J. P. 637. The Stability of Transition-Metal Complexes. *J. Chem. Soc.* **1953**, No. 3192, 3192. https://doi.org/10.1039/jr9530003192.

(51) Roush, W. R.; Adam, M. A.; Walts, A. E.; Harris, D. J. Stereochemistry of the Reactions of Substituted Allylboronates with Chiral Aldehydes. Factors Influencing Aldehyde Diastereofacial Selectivity. *J. Am. Chem. Soc.* **1986**, *108* (12), 3422–3434. https://doi.org/10.1021/ja00272a043.

(52) Ramachandran, P. V.; Pratihar, D. Asymmetric Aldol Reaction with Diisopinocampheyl Enolborinates of Propionates. *Org. Lett.* **2009**, *11* (7), 1467–1470. https://doi.org/10.1021/ol802850w.

(53) Ghosh, A. K.; Onishi, M. Synthesis of Enantiomerically Pure Anti-Aldols: A Highly Stereoselective Ester-Derived Titanium Enolate Aldol Reaction. *J. Am. Chem. Soc.* **1996**, *118* (10), 2527–2528. https://doi.org/10.1021/ja9539148.

(54) Ghosh, A. K.; Kim, J.-H. H. Highly Diastereoselective Anti -Aldol Reactions Utilizing the Titanium Enolate of Cis -2-Arylsulfonamido-1- Acenaphthenyl Propionate. *Org. Lett.* **2003**, *5* (7), 1063–1066. https://doi.org/10.1021/ol034086n.

(55) Shirokawa, S. I.; Kamiyama, M.; Nakamura, T.; Okada, M.; Nakazaki, A.; Hosokawa, S.; Kobayashi, S. Remote Asymmetric Induction with Vinylketene Silyl n,o-Acetal. *J. Am. Chem. Soc.* **2004**, *126* (42), 13604–13605. https://doi.org/10.1021/ja0465855.

(56) Evans, D. A.; Dart, M. J.; Duffy, J. L.; Rieger, D. L. Double Stereodifferentiating Aldol Reactions. The Documentation of “Partially Matched” Aldol Bond Constructions in the Assemblage of Polypropionate Systems. *J. Am. Chem. Soc.* **1995**, *117* (35), 9073–9074. https://doi.org/10.1021/ja00140a027.

(57) Matsui, R.; Seto, K.; Sato, Y.; Suzuki, T.; Nakazaki, A.; Kobayashi, S. Convergent Total Synthesis of (+)-TMC-151C by a Vinylogous Mukaiyama Aldol Reaction and Ring-Closing Metathesis. *Angew. Chemie Int. Ed.* **2011**, *50* (3), 680–683. https://doi.org/10.1002/anie.201006230.

(58) Hosokawa, S.; Ogura, T.; Togashi, H.; Tatsuta, K. The First Total Synthesis of Trichostatin D. *Tetrahedron Lett.* **2005**, *46* (2), 333–337. https://doi.org/10.1016/j.tetlet.2004.11.004.

(59) Nicolaou, K. C.; Guduru, R.; Sun, Y.-P.; Banerji, B.; Chen, D. Y.-K. Total Synthesis of the Originally Proposed and Revised Structures of Palmerolide A. *Angew. Chemie Int. Ed.* **2007**, *46* (31), 5896–5900. https://doi.org/10.1002/anie.200702243.

(60) Yamaoka, M.; Fukatsu, Y.; Nakazaki, A.; Kobayashi, S. Synthetic Study of Fomitellic Acids: Construction of the AB Ring Moiety. *Tetrahedron Lett.* **2009**, *50* (27), 3849–3852. https://doi.org/10.1016/j.tetlet.2009.04.039.

(61) Hosokawa, S.; Yokota, K.; Imamura, K.; Suzuki, Y.; Kawarasaki, M.; Tatsuta, K. The First

Total Synthesis and Structural Determination of Actinopyrone A. *Tetrahedron Lett.* **2006**, *47* (30), 5415–5418. https://doi.org/10.1016/j.tetlet.2006.05.028.

(62) Tsukada, H.; Mukaeda, Y.; Hosokawa, S. Syn-Selective Kobayashi Aldol Reaction Using Acetals. *Org. Lett.* **2013**, *15* (3), 678–681. https://doi.org/10.1021/ol303519y.

(63) Symkenberg, G.; Kalesse, M. Syn-Selective Vinylogous Kobayashi Aldol Reaction. *Org. Lett.* **2012**, *14* (6), 1608–1611. https://doi.org/10.1021/ol300353w.

(64) Shirokawa, S.; Shinoyama, M.; Ooi, I.; Hosokawa, S.; Nakazaki, A.; Kobayashi, S. Total Synthesis of Khafrefungin Using Highly Stereoselective Vinylogous Mukaiyama Aldol Reaction. *Org. Lett.* **2007**, *9* (5), 849–852. https://doi.org/10.1021/ol0630191.

(65) Ghosh, A. K.; Kim, J.-H. Stereoselective Chloroacetate Aldol Reactions: Syntheses of Acetate Aldol Equivalents and Darzens Glycidic Esters. *Org. Lett.* **2004**, *6* (16), 2725–2728. https://doi.org/10.1021/ol0490835.

(66) Shinoyama, M.; Shirokawa, S.; Nakazaki, A.; Kobayashi, S. A Switch of Facial Selectivities Using Alpha-Heteroatom-Substituted Aldehydes in the Vinylogous Mukaiyama Aldol Reaction. *Org. Lett.* **2009**, *11* (6), 1277–1280. https://doi.org/10.1021/ol9000312.

(67) Mukaeda, Y.; Kato, T.; Hosokawa, S. Syn-Selective Kobayashi Aldol Reaction Using the E,E-Vinylketene Silyl N,O -Acetal. *Org. Lett.* **2012**, *14* (20), 5298–5301. https://doi.org/10.1021/ol3024677.

(68) Chibale, K.; Warren, S. Asymmetric Synthesis of Spirocyclic Pyrrolidines and Tetrahydrofurans by Chiral Aldol Reactions and Phenylthio Migration. *Tetrahedron Lett.* **1991**, *32* (45), 6645–6648. https://doi.org/10.1016/0040-4039(91)80244-Z.

(69) Suenaga, K.; Mutou, T.; Shibata, T.; Itoh, T.; Fujita, T.; Takada, N.; Hayamizu, K.; Takagi, M.; Irifune, T.; Kigoshi, H.; et al. Aurilide, a Cytotoxic Depsipeptide from the Sea Hare Dolabella Auricularia: Isolation, Structure Determination, Synthesis, and Biological Activity. *Tetrahedron* **2004**, *60* (38), 8509–8527. https://doi.org/10.1016/j.tet.2004.06.125.

(70) Hughes, D. L.; Reamer, R. a. The Effect of Acid Strength on the Mitsunobu Esterification Reaction: Carboxyl vs Hydroxyl Reactivity. *J. Org. Chem.* **1996**, *61* (9), 2967–2971. https://doi.org/10.1021/jo952180e.

(71) Parrish, J. P.; Dueno, E. E.; Kim, S.-I.; Jung, K. W. Promoted O-Alkylation of Acids. *Synth. Commun.* **2000**, *30* (15), 2687–2700. https://doi.org/10.1080/00397910008086893.

(72) Evans, D. A.; Ellman, J. A. The Total Syntheses of the Isodityrosine-Derived Cyclic Tripeptides OF4949-III and K-13. Determination of the Absolute Configuration of K-13. *J. Am. Chem. Soc.* **1989**, *111* (3), 1063–1072. https://doi.org/10.1021/ja00185a042.

(73) Andrade, C. K.; Rocha, R. O.; Vercillo, O. E.; Silva, W. A.; Matos, R. A. DCC/DMAP-Mediated Coupling of Carboxylic Acids with Oxazolidinones and Thiazolidinethiones. *Synlett* **2003**, No. 15, 2351–2352. https://doi.org/10.1055/s-2003-42117.

(74) Dondoni, A.; Perrone, D. Total Synthesis of (+)-Galactostatin. An Illustration of the Utility of the Thiazole-Aldehyde Synthesis. *J. Org. Chem.* **1995**, *60* (15), 4749–4754. https://doi.org/10.1021/jo00120a017.

(75) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. Synthetic Preparation of N-Methyl-Alpha-Amino Acids. *Chem. Rev.* **2004**, *104* (12), 5823–5846. https://doi.org/10.1021/cr030024z.

(76) Eissler, S.; Kley, M.; Bächle, D.; Loidl, G.; Meier, T.; Samson, D. Substitution Determination of Fmoc-Substituted Resins at Different Wavelengths. *J. Pept. Sci.* **2017**, *23* (10), 757–762. https://doi.org/10.1002/psc.3021.

(77) Carpino, L. A. 1-Hydroxy-7-Azabenzotriazole. An Efficient Peptide Coupling Additive. *J. Am. Chem. Soc.* **1993**, *115* (10), 4397–4398. https://doi.org/10.1021/ja00063a082.

(78) Baker, R. H.; Bordwell, F. G.; Hauser, C. R.; Hudson, B. E.; Abramovitch, B.; Shivers, J. C. Tert-BUTYL ACETATE. *Org. Synth.* **1944**, *24* (September), 18. https://doi.org/10.15227/orgsyn.024.0018.

(79) Banasik, B. A. Total Synthesis of Lagunamide A via Remote Vinylogous Mukaiyama Aldol Reactions , Chemical Studies Toward the Total Synthesis of Micromide and Preliminary Studies Toward the Total Synthesis of Azaspirene, University of California, San Diego, 2016.

(80) Banasik, B. A.; Wang, L.; Kanner, A.; Bergdahl, B. M. Further Insight into the Asymmetric Vinylogous Mukaiyama Aldol Reaction (VMAR); Application to the Synthesis of the C27–C45 Segment of Lagunamide A. *Tetrahedron* **2016**, *72* (19), 2481–2490. https://doi.org/10.1016/j.tet.2016.03.079.

(81) Petit, Y.; Larchevêque, M. ETHYL GLYCIDATE FROM (S)-SERINE: ETHYL (R)-(+)-2,3-EPOXYPROPANOATE. *Org. Synth.* **1998**, *75* (September), 37. https://doi.org/10.15227/orgsyn.075.0037.