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TOWARD THE TOTAL SYNTHESIS OF DRAGONAMIDE E AND ITS POTENTIAL MEDICINAL APPLICATION AGAINST LEISHMANIASIS

Nathaniel M. Smith
University of Texas at Tyler, Nsmith30@patriots.uttyler.edu

TOWARD THE TOTAL SYNTHESIS OF DRAGONAMIDE E AND ITS POTENTIAL MEDICINAL APPLICATION AGAINST LEISHMANIASIS

by

NATHANIEL M. SMITH

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NATHANIEL M. SMITH

has been approved for the thesis requirement on
July 14, 2021
for the Master of Science degree

Approvals:

Thesis Chair: Sean C. Butler, Ph.D.

Member: Jiyong Lee, Ph.D.

Member: Rachel N. Mason, Ph.D.

Chair, Department of Chemistry and Biochemistry

Dean, College of Arts and Sciences

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LIST OF ABBREVIATIONS

α	alpha
Å	angstrom(s)
Ac	acetyl
app	apparent (NMR)
APCIMS	atmospheric pressure chemical ionization mass spectrometry
β	beta
n-Bu	*normal*-butyl
t-Bu	*tert*-butyl
^{13}C	carbon-13
°C	degrees Celsius
CAM	cerium ammonium molybdate
CL	cutaneous leishmaniasis
cm^{-1}	wavenumbers (IR)
δ	delta; chemical shift in ppm downfield from tetramethylsilane
d	day(s); doublet (NMR)
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMF	*N*,*N*-dimethylformamide

DMP	Dess–Martin periodinane (1,1,1-Tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one)
DMSO	dimethylsulfoxide
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
Fmoc	fluorenylmethyloxycarbonyl
Fmoc-OSu	*N*-(9-Fluorenylmethoxycarbonyloxy)succinimide
g	gram(s)
h	hour(s)
^1H	proton
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HBTU	*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HOBt	hydroxybenzotriazole
HWE	Horner–Wadsworth–Emmons
Hz	hertz (NMR)
IBX	2-iodoxybenzoic acid
IC_{50}	half-maximal inhibitory concentration
IR	infrared
i-Pr	isopropyl
J	coupling constant in hertz (NMR)
L	liter(s)
µ	mu; micro

m	meter(s); milli; multiplet (NMR)
M	mega; moles per liter
$[M+H]^+$	molecular ion plus hydrogen (mass spectrometry)
m/z	mass to charge ratio
Me	methyl
min	minute(s)
mol	mole(s)
ML	mucocutaneous leishmaniasis
NMR	nuclear magnetic resonance
p	*para*
PAA	*para*-anisaldehyde
Ph	phenyl
pH	potential of hydrogen
ppm	parts per million
q	quartet (NMR)
quint	quintet (NMR)
R_f	retention factor
RP-HPLC	reverse phase – high performance liquid chromatography
RP-SPE	reverse phase – solid phase extraction
rt	room temperature
s	second(s); singlet (NMR)
SAR	structure-activity relationship
SPPS	solid phase peptide synthesis

t	triplet (NMR)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
p-TsOH	*para*-toluenesulfonic acid
v/v	volume to volume
VL	visceral leishmaniasis
WHO	World Health Organization

ABSTRACT

TOWARD THE TOTAL SYNTHESIS OF DRAGONAMIDE E AND ITS POTENTIAL MEDICINAL APPLICATION AGAINST LEISHMANIASIS

Nathaniel M. Smith

Thesis Chair: Sean C. Butler, Ph.D.

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July 2021

Leishmaniasis is one of the world's most neglected tropical diseases, with hundreds of thousands of cases occurring worldwide annually. The disease originates from being infected by protozoa of the *Leishmania* genus, which are parasites that destroy mammalian cells as part of their life cycle. Currently utilized treatment strategies for leishmaniasis have many disadvantages, warranting the search for a new leishmaniasis treatment.

Cyanobacteria have been discovered to synthesize a wide variety of cytotoxic chemicals as part of their own defense mechanism. One strain of cyanobacteria, *Lyngbya majuscula*, produces several families of compounds, one of which is known as the "dragonamides". Several of the compounds in this class have activity against the leishmania parasite, with the most efficacious one being dragonamide E.

To further understand the antileishmanial mechanism of dragonamide E, a total synthesis is proposed so that researchers can produce dragonamide E in the lab without having to extract it from *Lyngbya majuscula*. The greater availability of dragonamide E

due to a published synthesis route will increase the ease with which research can be done into the antileishmanial properties of dragonamide E, promoting the development of new and more efficacious leishmaniasis treatments.

CHAPTER 1

A BRIEF OVERVIEW OF LEISHMANIASIS AND ITS SIGNIFICANCE

1.1 An Introduction to Leishmaniasis

Leishmaniasis is one of the world's most neglected and devastating tropical diseases, with an estimated 700,000 to 1 million new cases occurring annually.[1,2] The disease is onset by the bite of an infected female phlebotomine sandfly, which can transmit parasitic protozoans of the *Leishmania* genus, hence the disease's name.[3] Once the protozoan enters the human body, the body's phagocytic cells encapsulate the foreign material in an attempt to destroy it.[4] However, the environment inside the phagocytic cells is what *Leishmania* protozoa require to replicate.[4] After the protozoan multiplies by simple division, the phagocytic cell is lysed and releases more of the parasite into the body, continuing the cycle.[4]

There are three main types of leishmaniasis: visceral, cutaneous, and mucocutaneous.[2] Visceral leishmaniasis (VL) occurs when the parasite infects vital organs, and is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anemia. If left untreated, VL is the most fatal of the leishmaniasis variants.[1–3] Cutaneous leishmaniasis (CL) is the most common form of the disease, which occurs when the parasite only infects the tissue surrounding the sandfly bite. CL is characterized by principally ulcerated skin lesions on uncovered parts of the body, thereby leaving

noticeable lifelong scars and serious disability.[1–3] Mucocutaneous leishmaniasis (ML) is the rarest form of the disease and is a variant of CL, where the parasite disseminates from the original lesion via hematogenous or lymphatic pathways, resulting in partial or total destruction of the nose, mouth, and/or throat mucous membranes.[1–3]

1.1.1 Worldwide Effects of Leishmaniasis

Leishmaniasis is caused by any of the 20+ known parasitic protozoans of the *Leishmania* genus, so the specific strain of leishmaniasis differs by region (Figure 1.1). VL is most common in Brazil, East Africa, and India, with an estimated 50,000–90,000 new cases occurring annually. In 2018, more than 95% of new VL cases reported to the World Health Organization (WHO) occurred in ten countries: Brazil, China, Ethiopia, India, Iraq, Kenya, Nepal, Somalia, South Sudan, and Sudan.[2] About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia, with an estimated 600,000 – 1 million new cases occurring annually. In 2018, over 85% of new CL cases occurred in ten countries: Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iran (Islamic Republic of), Iraq, Pakistan, the Syrian Arab Republic, and Tunisia.[2] Finally, ML, the rarest form of leishmaniasis, occurs mostly in Bolivia (the Plurinational State of), Brazil, Ethiopia, and Peru.[2]

1.1.2 Current Leishmaniasis Treatments

Since each of the three leishmaniasis variants originate from infection via *Leishmania* protozoans, the main goal of treatment is to eradicate the parasites while not harming the host. The drugs typically used first when treating leishmaniasis are

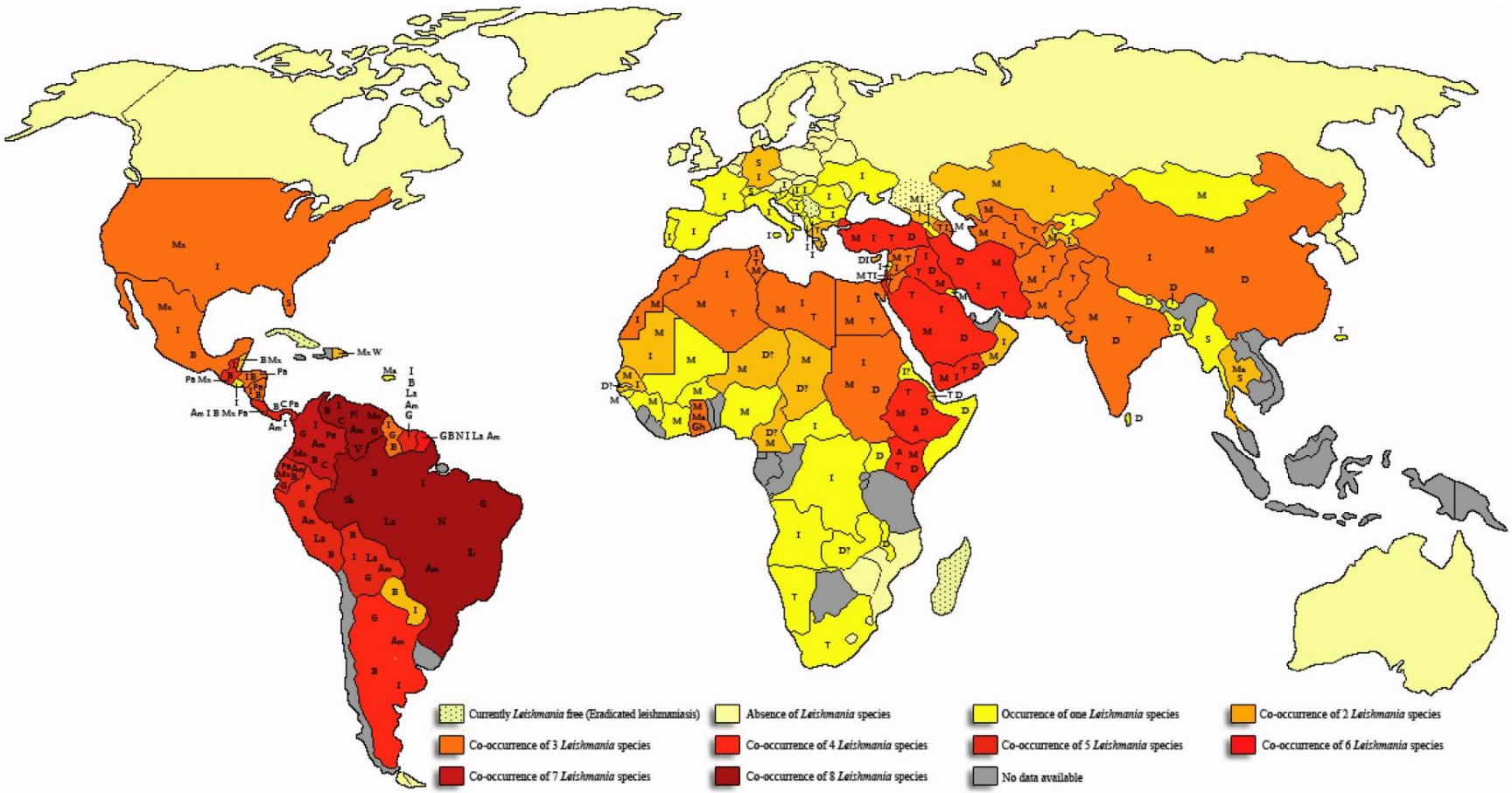


Figure 1.1. Heat map showing the worldwide presence of various *Leishmania* species.[5]

intravenous, intralesional, or intramuscular administration of pentavalent antimonials such as sodium stibogluconate (**1.1**).[3] If pentavalent antimonials are not available, then intravenous administration of amphotericin B (**1.2**) is used, which is a polyenic antibody with high antileishmanial activity.[3] However, there are unfavorable characteristics regarding both of these treatment methods. Pentavalent antimonials and amphotericin B can be cardiotoxic and/or nephrotoxic, and both treatments come with a variety of negative side effects ranging from muscle pain to vomiting (Table 1.1).[3]

More recent advances in leishmaniasis treatment have provided alternative methods to the ones previously mentioned. Pentamidine (**1.3**), an aromatic diamine, is administered intravenously or intramuscularly and has a treatment period of just one week, though potential side effects include hypoglycemia and hyperglycemia.[3] Miltefosine (**1.4**), a choline, was originally an anticancer drug but was discovered to have antileishmanial activity as well, with the added bonus of being able to be administered orally.[3] However, *Leishmania* can quickly become resistant to miltefosine.[3] Paromomycin (**1.5**) is an interesting potential candidate for leishmanial treatment because it is administered topically, unlike many of the other treatments. Unfortunately, this makes paromomycin only applicable toward cutaneous leishmaniasis. Even against cutaneous leishmaniasis, paromomycin's large molecular weight and hydrophilic nature prevents it from permeating human skin quickly.[3] Another leishmaniasis-specific drug is sitamaquine (**1.6**), which exclusively targets visceral leishmaniasis.[3] Sitamaquine is a very recent drug and is still undergoing clinical trials for oral use, although there is a lack of knowledge regarding the toxicity of its metabolic.[3] Nearly all current treatments for leishmaniasis have drawbacks that prevent any one treatment from being the most efficient. It is to this end that either

new treatment strategies need to be developed or new antileishmanial drugs need to be discovered. A larger selection of unique leishmanial drugs would provide greater versatility in leishmaniasis treatment.

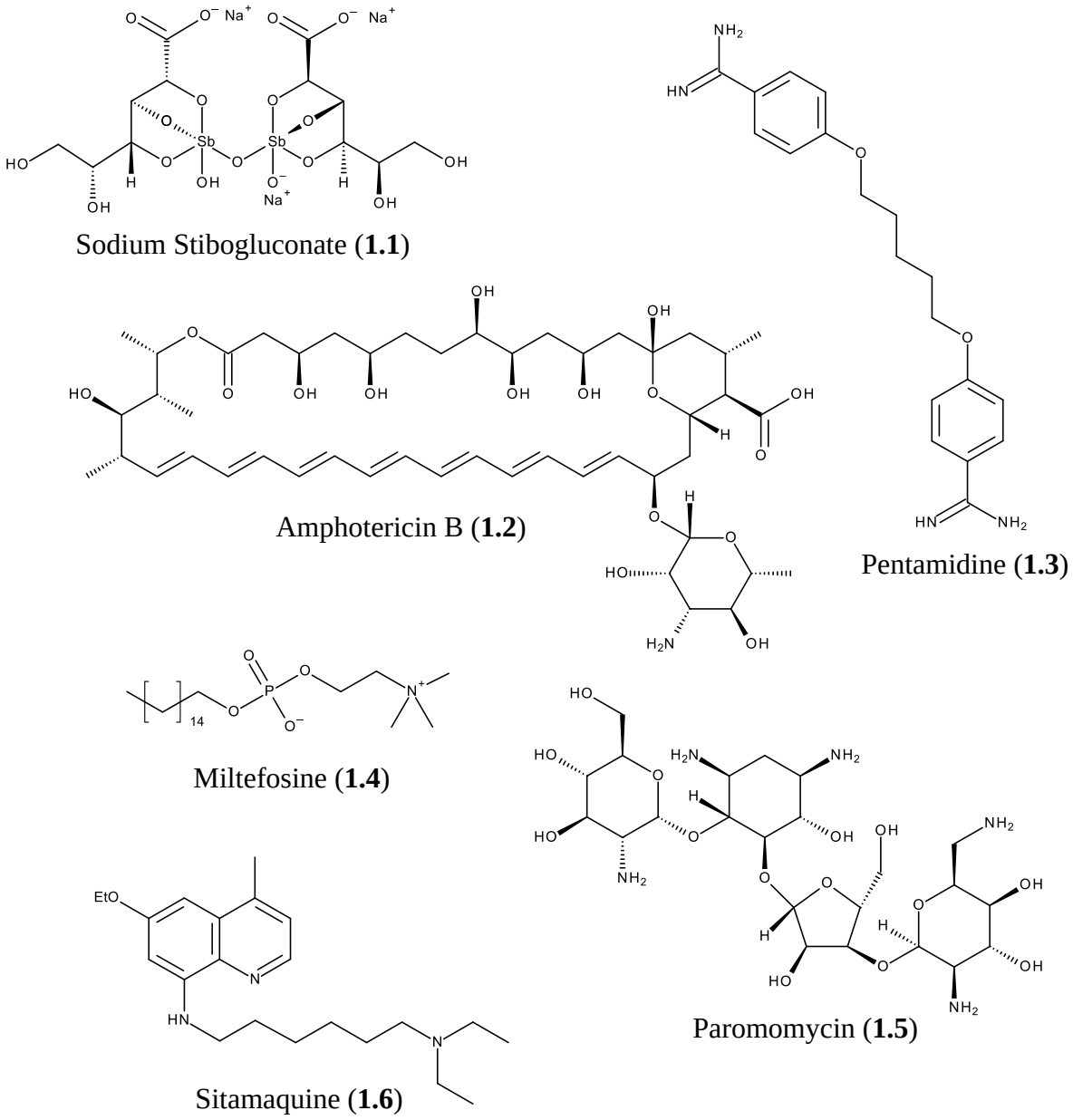


Figure 1.2. Structures of various leishmaniasis treatments.

Drugs	Amphotericin B deoxycholate	Liposomal amphotericin B	Miltefosine	Paromomycin	Pentamidine	Pentavalent antimonials	Sitamaquine
Administration Routes	Intravenous	Intravenous	Oral	Intramuscular, intravenous or topic	Intramuscular	Intramuscular, intravenous or intralesional	Oral
Dosage	0.75-1 mg/kg (15 or 20 days daily or alternatively)	3-5 mg/kg single dose or 10-30 mg/kg total dose	100-150 mg/day for 28 days	15 mg/day for 21 days or 20 mg/kg for 17 days	3 mg/kg/day every other day for 4 injections	20 mg/kg/day for 28-30 days	2 mg/kg/day for 21 days
Advantages	Primary resistance is unknown	Highly effective and low toxicity	Effective and safe	Low cost	Short treatment	Ease of availablity and low cost	Effective
Disadvantages	Need slow intravenous infusion, toxicity, unstable in high temperatures	Need slow intravenous infusion, high cost, unstable in high temperatures	Cost, poor patient compliance, cannot be used in pregnant patients	Efficacy varies between and within regions	Efficacy varies between *Leishmania* species	Length of treatment, painful injection and toxicity	Toxicity
Resistance	Laboratory strains	Not documented	Laboratory strains, some cases reported in India	Laboratory strains	Not documented	Common	Not documented

Table 1.1. Various treatments of leishmaniasis and their relative advantages and disadvantages.[3]

1.2 *Lyngbya majuscula*

Development and discovery of medicinal treatments for most diseases is based on combinatorial chemistry, which is the process of trying slightly varying chemicals on a disease to see if any of the chemicals treat the disease exceptionally well.[6] This technique works best when an initial chemical is discovered that has strong activity against the disease, upon which further research can be done into optimizing that chemical's activity against the disease. Until that initial chemical is discovered however, combinatorial chemistry can be difficult to properly utilize. Another approach to discovering disease treatments is to isolate biological natural products from various organisms.[6] This can either lead to compounds that immediately exhibit strong activity against a disease or can provide

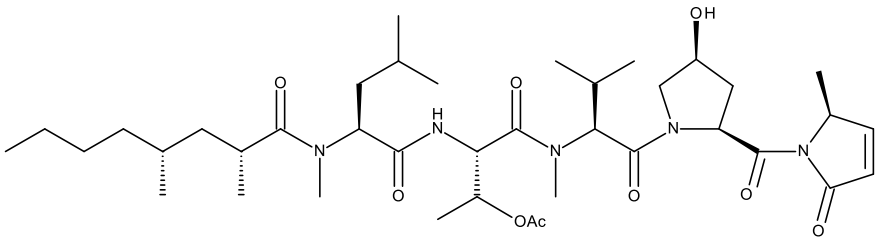
the starting chemical for combinatorial chemistry. Marine organisms must protect themselves from the tremendous variety of environments and dangers they encounter, and they do so by producing many cytotoxic chemicals as a makeshift immune system. These cytotoxic chemicals are promising candidates for compound isolation.[6] For example, approximately half of anti-cancer discovery efforts, as of 2004, were based on marine organisms.[6] One of these marine organisms is called *Lyngbya majuscula*, which is a tropical, filamentous cyanobacteria that has had over 110 metabolites identified as of 2004, with 75% of them being biologically active in some way.[6]

1.2.1 Classes of Compounds Isolated from *Lyngbya majuscula*

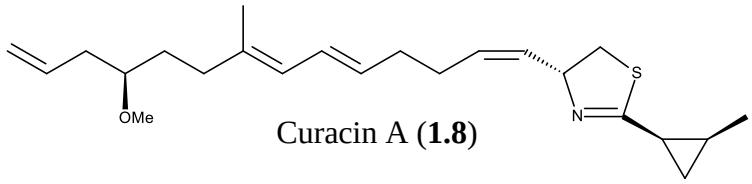
Lyngbya majuscula synthesizes many different metabolites, and each of these metabolites can be sorted into general classes based on the metabolites' repeating units or active regions. These classes include alkaloids, amides, amines, fatty acids, imidazoles, lactones, lipopeptides, and malyngolides.[7]

1.2.2 Biologically Important Compounds from *Lyngbya majuscula*

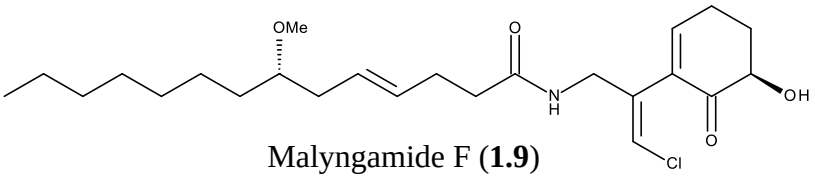
Several biologically relevant compounds have been isolated from *Lyngbya majuscula*, each with differing purposes and efficacies for said purpose. Examples of such compounds are microcolin A (**1.7**), curacin A (**1.8**), and malyngamide F (**1.9**).[7] Microcolin A was found to have not only immunosuppressive activity, but also the ability to mediate thymocyte apoptosis via a novel mechanism.[7] Research also suggests that microcolin A could be used as antineoplastic agents.[7] Initial research into curacin A suggested that it had substantial activity against proliferative cells, and further studies were done to examine



Microcolin A (**1.7**)



Curacin A (**1.8**)



Malyngamide F (**1.9**)

Figure 1.3. Structures of various compounds isolated from *Lyngbya majuscula.*

this quality in both curacin A and its analogs.[7] The malyngamide class of compounds are generally not bio-active, yet their presence in a wide variety of cyanobacteria strains around the world suggests that their purpose has just not yet been identified.[7]

Another type of compound isolated from *Lyngbya majuscula* are lipopeptides, which are short peptide sequences that have a carbon chain at one end of the molecule. These compounds can be cyclic or acyclic and are generally extremely bioactive.[7] Lipopeptides have an affinity for liposomes and cell membranes as well as a low molecular weight, which explains the extreme cytotoxicity of lipopeptides.[7] One such example of a lipopeptide found in *Lyngbya majuscula* is called dragonamide E, which has been discovered to have antileishmanial activity.[8]

CHAPTER 2

DRAGONAMIDE E AND RELATED COMPOUNDS

2.1 Dragonamide E

Dragonamide E (**2.1**) is a straight chain lipopeptide consisting of one phenylalanine residue, three valine residues, and a carbon chain that contains an alkene and a terminal alkyne. Additionally, each of the amino acid residues is N-methylated. Biologically produced samples do not often contain terminal alkynes nor N-methylated amino acid residues, so the presence of both functionalities in **2.1** is quite interesting.

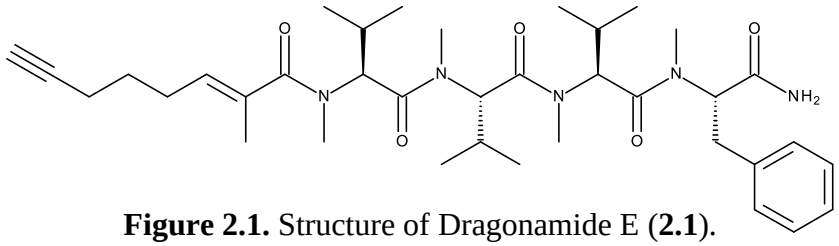


Figure 2.1. Structure of Dragonamide E (**2.1**).

2.1.1 Isolation

In a research article presented in 2010 by Gerwick and coworkers, *Lyngbya majuscula* was collected and processed via flash-phase chromatography to reveal that certain portions of the elution had strong antileishmanial activity.[8] After purification via RP-SPE column chromatography and RP-HPLC, the resulting fraction contained two compounds. Gerwick's group determined that the major component of the fraction was

dragonamide A (**2.2**), and stated,

> ^{1}H and ^{13}C NMR signals [were] indicative of phenylalanyl and valinyl residues, along with a fatty acyl chain. Combined with a prominent $[M+H]^{+}$ peak by APCIMS at m/z 654, [this] data [was] fully consistent with literature values for the known metabolite dragonamide A.[8]

The minor component of the fraction was a unique compound that needed to be spectroscopically identified as well.

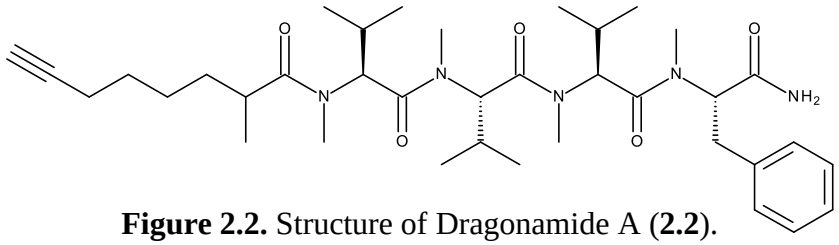


Figure 2.2. Structure of Dragonamide A (**2.2**).

2.1.2 Structure Elucidation

Initial investigation into this second metabolite's structure revealed that it was unprecedented in marine literature and that it was incredibly similar to dragonamide A, including ^{1}H NMR signals for a terminal NH_2, an *N*-methyl phenylalanyl, and three *N*-methyl valinyl residues, and ^{13}C NMR signals for a terminal alkyne.[8] However, dragonamide A possessed two more protons and one less degree of unsaturation than this new compound. Analysis of the ^{1}H NMR and ^{13}C NMR signals revealed the presence of an α, β unsaturated double bond between carbons C35 and C36 (Figure 2.3), providing the sole distinction between dragonamide A and the new metabolite.[8] This metabolite was named "dragonamide E" (**2.1**) as it was the fifth compound in the dragonamide family to have been isolated from *Lyngbya* cyanobacteria.[8] The discovery of **2.1** was novel due to how all of the other dragonamide compounds have a secondary methyl group with the (*S*) absolute configuration at C35 or the equivalent position.[8] The structure of dragonamide E

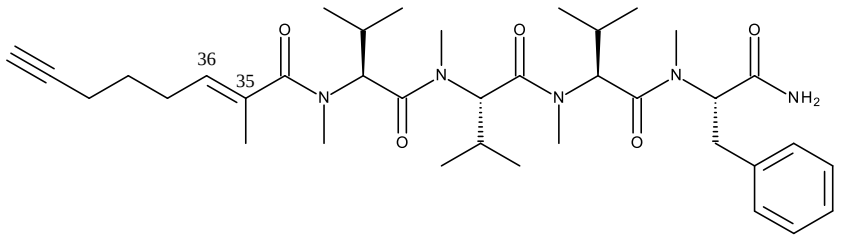


Figure 2.3. Structure of Dragonamide E (**2.1**) with C35 and C36 labeled.

suggests that it could be a precursor to other compounds in the dragonamide family, such as dragonamide A (**2.2**).[8]

2.1.3 Biological Activity and Significance

After having isolated and characterized dragonamide E, Gerwick and coworkers set out to determine the efficacy of **2.1** against various diseases. Dragonamide E was tested against *Plasmodium falciparum* (malaria), *Leishmania donovani* (leishmaniasis), and *Trypanosoma cruzi* (Chagas' disease), with *in vitro* antileishmanial activity being the most prominent with an IC_{50} = 5.1 μM.[8] Ultimately, it is the high antileishmanial activity of **2.1** that makes it attractive as a potential leishmaniasis treatment and, thus, why it was pursued in this study.

2.2 Similar Compounds to Dragonamide E

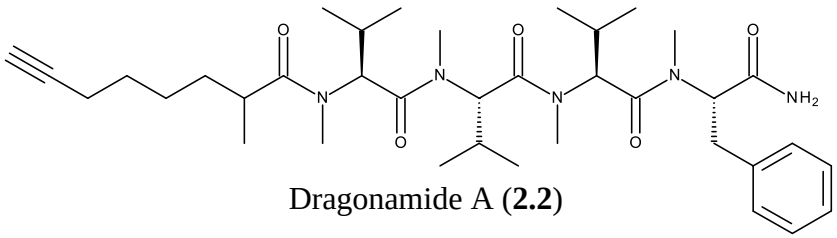
Many types of lipopeptides have been isolated from *Lyngbya majuscula*, each of them having different efficacies against a variety of diseases.[7] Observing the similarities and differences between these and other lipopeptides could allow for insight as to which parts of lipopeptides are crucial for cytotoxic activity in general, and which parts specialize each lipopeptide for individual disease treatments.

2.2.1 Dragonamides

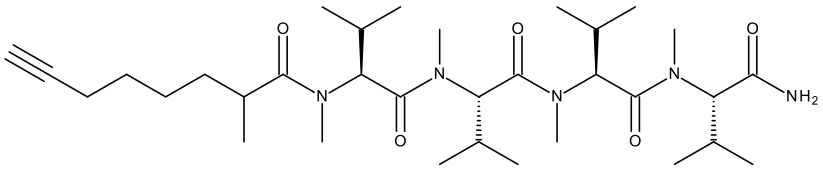
The dragonamide class of lipopeptides are characterized by a terminal alkyne moiety attached to a tetrapeptide containing many valines. Each of the amino acids in the tetrapeptide are N-methylated as well. Included in this class are dragonamides A–E (**2.1–2.5**). The key factor in differentiating these compounds is the exact structure of the alkyne moiety and the tetrapeptide sequence. Dragonamides A and E have a phenylalanine residue at the C-terminus, whereas dragonamides B, C, and D have a fourth valine residue at that position. This difference in amino acid sequence is crucial to the cytotoxic activity of the compound. For instance, dragonamide A has good antimalarial and antileishmanial activity (IC_{50} = 7.7 μM and 6.5 μM, respectively), but dragonamide B has no activity against either disease, suggesting that the terminal phenylalanine residue is vital to the cytotoxic activity.[9] Of the dragonamide compounds, dragonamide E has the best antileishmanial activity with an IC_{50} of 5.1 μM.[8]

2.2.2 Dragomabin

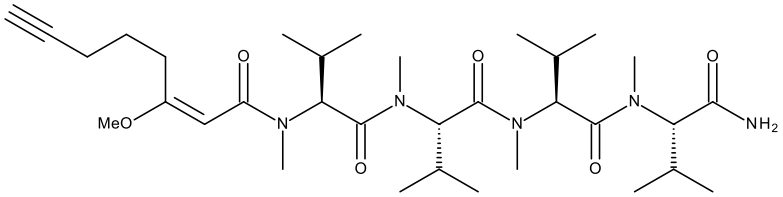
Whereas there are multiple dragonamides, dragomabin (**2.6**) is the only compound in its class. However, it is still a linear lipopeptide with an alkyne moiety attached to a tetrapeptide. The key difference in dragomabin compared to the dragonamides is the difference in amino acid sequence. Dragomabin also lacks the *N*-methylation on one of its amino acid. While no antileishmanial IC_{50} has been reported for dragomabin, dragomabin's antimalarial IC_{50} is 6.0 μM.[9] Additionally, the IC_{50} against Vero cells is quite large (IC_{50} = 182.3 μM) implying that dragomabin is highly selective against malaria over mammalian cells, making dragomabin a good malaria treatment candidate.[9]



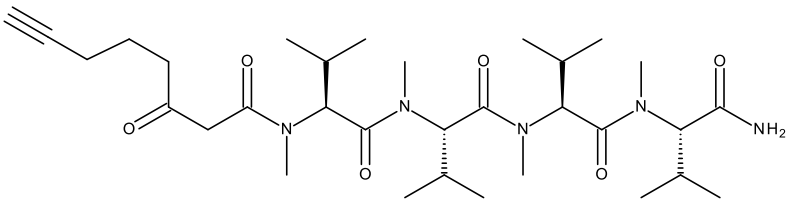
Dragonamide A (**2.2**)



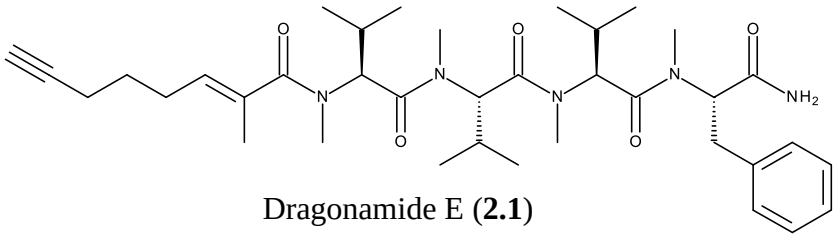
Dragonamide B (**2.3**)



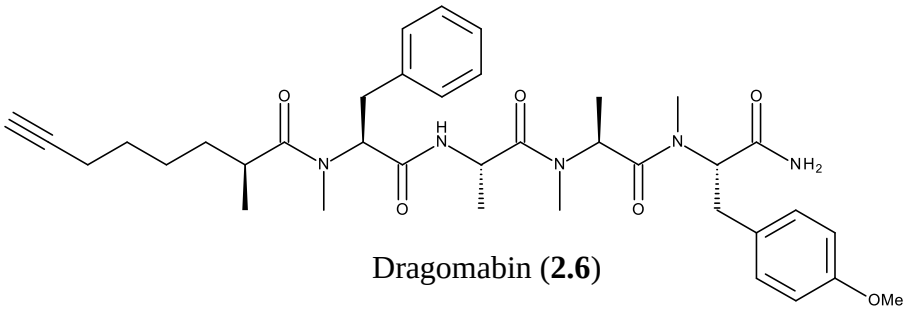
Dragonamide C (**2.4**)



Dragonamide D (**2.5**)



Dragonamide E (**2.1**)

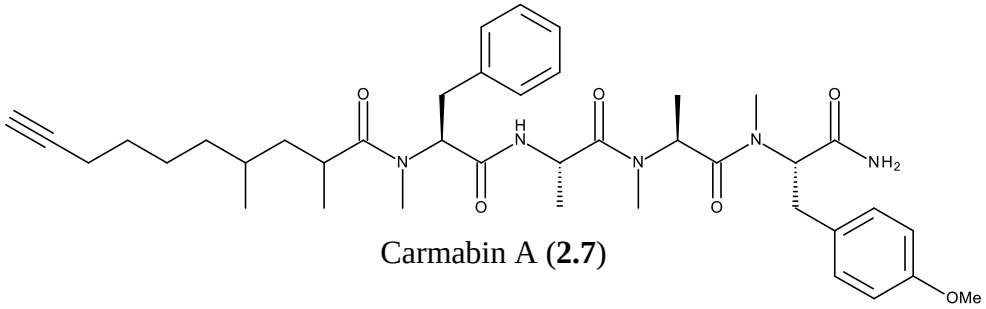


Dragomabin (**2.6**)

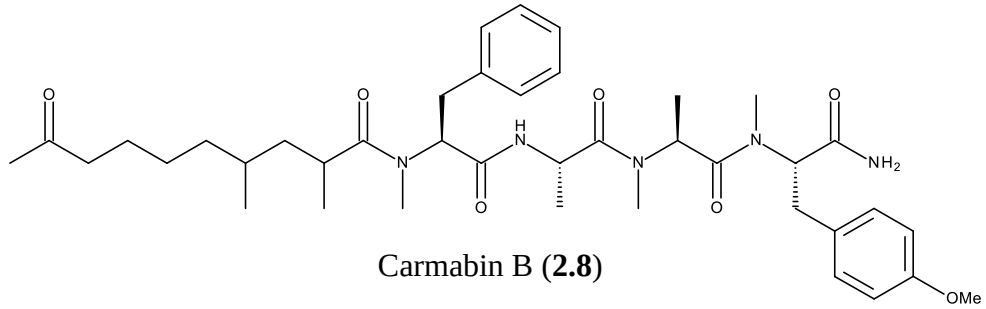
Figure 2.4. Structures of Dragonamides A-E (**2.1**–**2.5**) and Dragomabin (**2.6**).

2.2.3 Carmabins

The carmabins (**2.7–2.8**) are extremely similar to dragomabin, with the only difference being the exact structure of the hydrophobic chain on the left side of the molecule, herein referred to as the "hydrophobic tail". Carmabin A in particular has similar antimalarial activity as dragomabin (IC_{50} = 4.3 µM), but its IC_{50} for Vero cells is 9.8 µM, indicating its cytotoxic properties are much stronger and less specific than dragomabin.[9] This is presumably due to the longer and more branched alkyne moiety of the carmabins.[9]



Carmabin A (**2.7**)

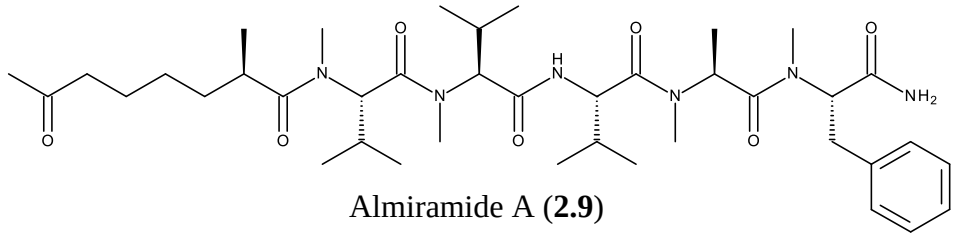


Carmabin B (**2.8**)

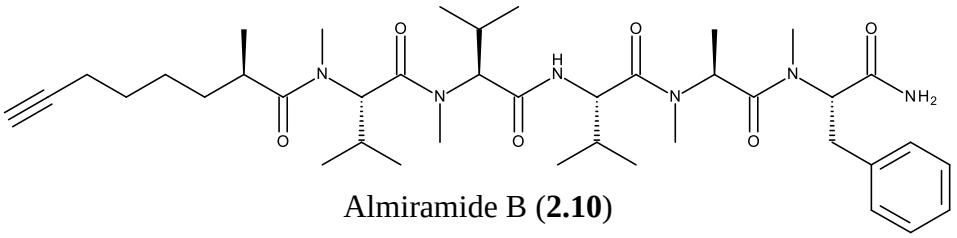
Figure 2.5. Structures of Carmabins (**2.7**–**2.8**).

2.2.4 Almiramides

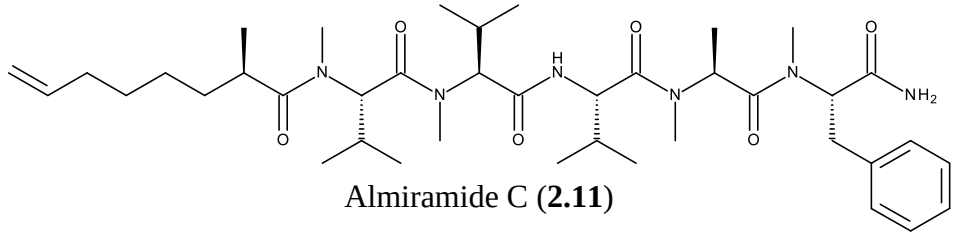
The almiramides (**2.9**–**2.11**) resemble the dragonamides the least of the related compounds discussed so far, yet their antileishmanial activity rivals and even exceeds that of the dragonamides.[10] Almiramides are linear lipopeptides that feature five amino



Almiramide A (**2.9**)



Almiramide B (**2.10**)



Almiramide C (**2.11**)

Figure 2.6. Structures of Almiramides (**2.9**–**2.11**).

acid residues instead of the four seen previously. The additional amino acid is an alanine found between the phenylalanine and valine residues; otherwise, the amino acid sequence is the same as the dragonamides. The other notable distinctions are that the third amino acid is not *N*-methylated and that the exact structure of the hydrophobic tail is slightly different.

While almiramide A has no activity against *Leishmania donovani*, almiramide B and C have an IC_{50} of 2.4 μM and 1.9 μM against *Leishmania donovani* respectively, exceeding that of even dragonamide E (5.1 μM).[8,10] This implies that there must be unsaturation of some type at the end of the hydrophobic moiety for there to be antileishmanial activity.[10] In an almiramide discovery and development paper, Linington

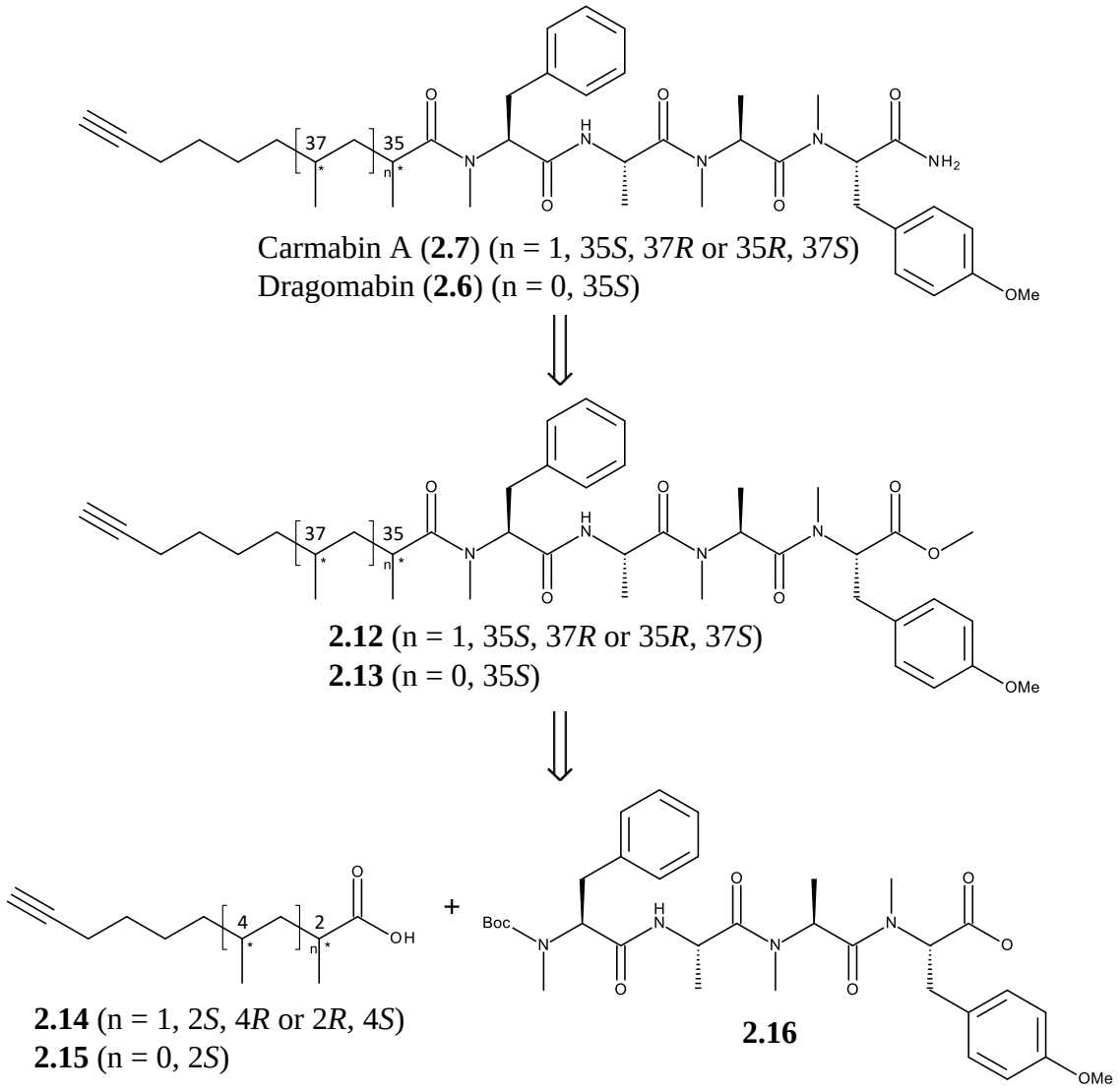
and coworkers made various analogues to the almiramides in an effort to determine what regions of the almiramides were crucial to their antileishmanial activity while also keeping cytotoxicity toward mammalian cells low.[10] The main takeaways from the testing were that some type of unsaturation is needed at the edge of the hydrophobic moiety, all of the amino acids should have their nitrogens methylated (unlike the standard almiramides), and that the C-terminus of the peptide sequence should be capped by either an NH_2 or an NMe_2 group.[10] These characteristics are already present in dragonamide E, making further research into its antileishmanial activity all the more promising.

2.3 The Call for the Synthesis of Dragonamide E

While the research done by Gerwick and coworkers provided the basis for dragonamide E and its potential use in the treatment of leishmaniasis, little has been accomplished to that end since the initial publication in 2010.[8] For this reason, research was done into dragonamide E in hopes of developing a proper synthesis, as commercialized isolation of dragonamide E from *Lyngbya majuscula* would be far too costly both in economical and environment terms.[6] This new synthesis route would make dragonamide E much more available to researchers, providing a higher likelihood of researchers discovering its antileishmanial mechanism.

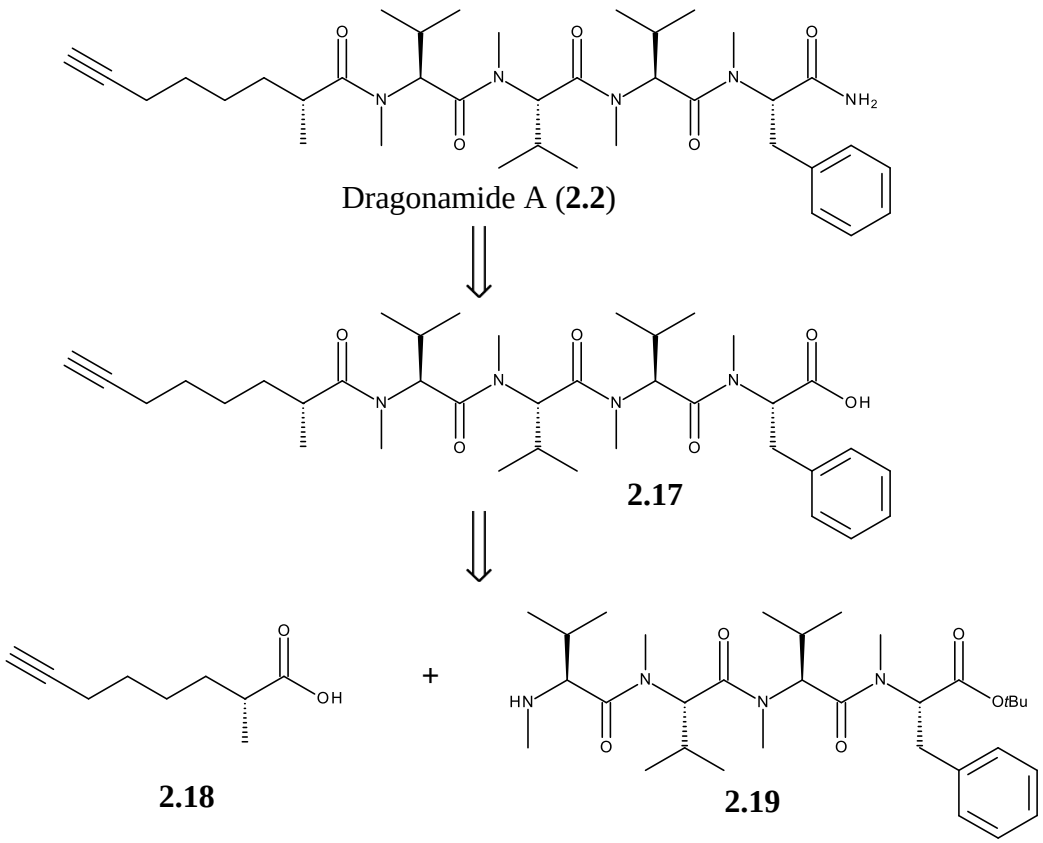
2.3.1 Previous Syntheses of Dragonamides and Related Compounds

While there have not been any published syntheses of dragonamide E specifically, there have been reports published for the synthesis of similar compounds. Carmabin A and dragomabin were synthesized by Baijun Ye and coworkers in 2018 (Scheme 2.1), and



Scheme 2.1. Retrosynthesis of carmabin A (**2.7**) and dragomabin (**2.6**).[11]

dragonamide A (known only as dragonamide at the time of the publication) was synthesized by Chen and coworkers (Scheme 2.2).[11–12] Each of these approaches focuses on synthesizing the hydrophobic tail with a carboxylic acid group at the end that would connect to the peptide sequence, then coupling this moiety to the modified amino acids with a peptide bond.[11–12] The synthesis for dragonamide E will bear a resemblance to this general method.

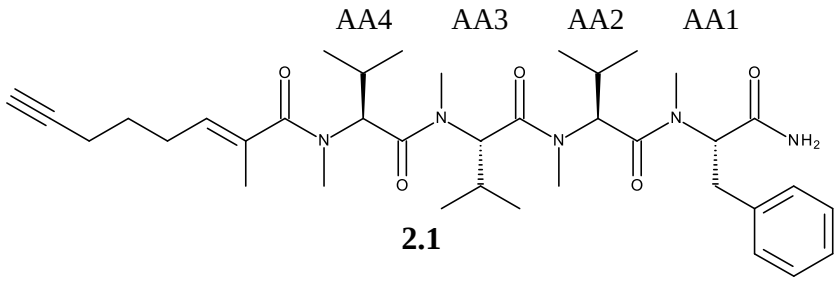


Scheme 2.2. Retrosynthesis of dragonamide A (**2.2**).[12]

2.3.2 Dragonamide E Synthesis Goals

With the knowledge that dragonamide E has both prominent antileishmanial activity and has not been explored as a potential treatment for leishmaniasis, research into dragonamide E should provide invaluable results to leishmaniasis drug development. The research accomplished with dragonamide E in this work were based on two goals. The first was to develop a working synthesis for dragonamide E from relatively low-cost materials, and the second was to use this synthesis to create a wide variety of dragonamide E analogues (Table 2.1). While dragonamide E contained *N*-methylphenylalanine and *N*-methylvaline residues, the analogues were planned to have various permutations of N-

methylated amino acid residues and unmethylated residues to utilize in Structure-Activity relationship (SAR) studies. The reason for planning to synthesize these analogues was to discover if the N-methylation was beneficial to the antileishmanial activity. If any of the analogues had significantly better or worse antileishmanial activity than the original dragonamide E, then the antileishmanial mechanism of dragonamide E could potentially be discovered by identifying the parts of the dragonamide E that are crucial to antileishmanial activity and the parts that are not.



2.1

	Dragonamide E	Analogue 1	Analogue 2	Analogue 3	Analogue 4	Analogue 5	Analogue 6	Analogue 7
Amino Acid 1:	**N-Me-L-Phe**	N-Me-L-Phe	N-Me-L-Phe	N-Me-L-Phe	N-Me-L-Phe	N-Me-L-Phe	N-Me-L-Phe	N-Me-L-Phe
Amino Acid 2:	**N-Me-L-Val**	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val	L-Val
Amino Acid 3:	**N-Me-L-Val**	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val
Amino Acid 4:	**N-Me-L-Val**	L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	L-Val	N-Me-L-Val	L-Val

	Analogue 8	Analogue 9	Analogue 10	Analogue 11	Analogue 12	Analogue 13	Analogue 14	Analogue 15
Amino Acid 1:	L-Phe	L-Phe	L-Phe	L-Phe	L-Phe	L-Phe	L-Phe	L-Phe
Amino Acid 2:	N-Me-L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val	L-Val
Amino Acid 3:	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val
Amino Acid 4:	N-Me-L-Val	L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	L-Val	N-Me-L-Val	L-Val

	Analogue 16	Analogue 17	Analogue 18	Analogue 19	Analogue 20	Analogue 21	Analogue 22	Analogue 23
Amino Acid 1:	N-Me-L-Tyr	N-Me-L-Tyr	N-Me-L-Tyr	N-Me-L-Tyr	N-Me-L-Tyr	N-Me-L-Tyr	N-Me-L-Tyr	N-Me-L-Tyr
Amino Acid 2:	N-Me-L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val	L-Val
Amino Acid 3:	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val
Amino Acid 4:	N-Me-L-Val	L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	L-Val	N-Me-L-Val	L-Val

	Analogue 24	Analogue 25	Analogue 26	Analogue 27	Analogue 28	Analogue 29	Analogue 30	Analogue 31
Amino Acid 1:	L-Tyr	L-Tyr	L-Tyr	L-Tyr	L-Tyr	L-Tyr	L-Tyr	L-Tyr
Amino Acid 2:	N-Me-L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val	L-Val
Amino Acid 3:	N-Me-L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	N-Me-L-Val	L-Val	L-Val
Amino Acid 4:	N-Me-L-Val	L-Val	N-Me-L-Val	N-Me-L-Val	L-Val	L-Val	N-Me-L-Val	L-Val

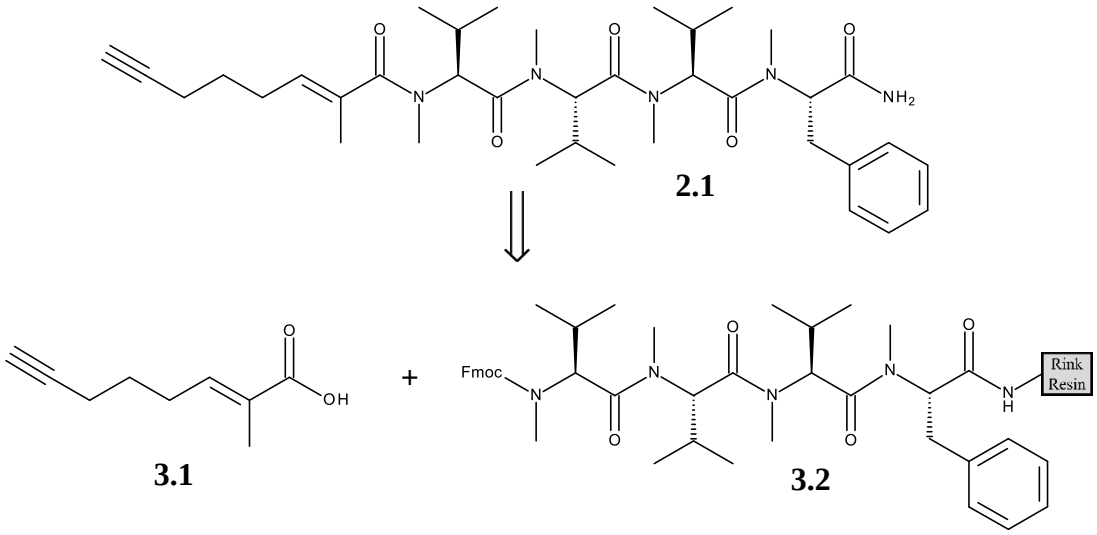
Table 2.1. Planned analogues of dragonamide E (**2.1**).

CHAPTER 3

TOWARD THE TOTAL SYNTHESIS OF DRAGONAMIDE E

3.1 Planned Synthetic Route for Dragonamide E

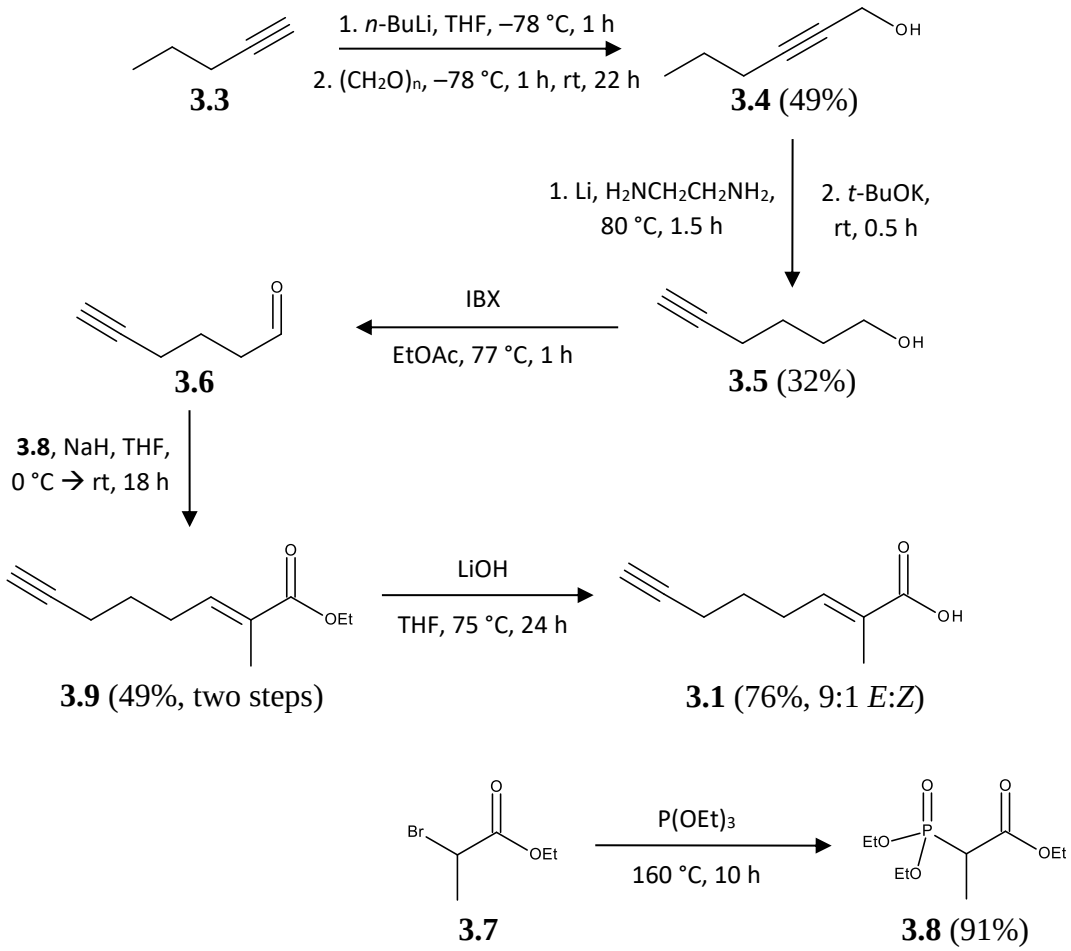
In the retrosynthetic analysis, dragonamide E (**2.1**) was treated as two separate compounds: hydrophobic tail **3.1** and tetra amino acid head **3.2** (Scheme 3.1). Whereas **3.2** could be made via solid phase peptide synthesis (SPPS) using amino acid coupling chemistry, synthesizing **3.1** would require a more involved approach.



Scheme 3.1. Retrosynthesis of dragonamide E (**2.1**).

3.1.1 Synthesis of Carboxylic Acid 3.1

The starting material for the synthesis of **3.1** was 1-pentyne (**3.3**). The alkyne was



Scheme 3.2. Hydrophobic tail synthesis.

first deprotonated using *n*-butyllithium (*n*-BuLi) in tetrahydrofuran (THF) at –78 °C, then underwent an alkynylation using paraformaldehyde to give primary alcohol **3.4** (Scheme 3.2).[13] The alkynylation of **3.3** into **3.4** was easily the most cumbersome reaction of this entire work. Prior to using the *n*-BuLi, it was titrated to determine its concentration in solution. This ensured the correct amount of *n*-BuLi was used in the deprotonation of the alkyne, as an excess amount of *n*-BuLi promotes side reactions. The titration techniques used often suggested that the concentration of *n*-BuLi was far less concentrated than expected, yet experimental observations implied that an excess of *n*-BuLi was being used.

Considering that the highest yield for this reaction was a mere 49% with a notable presence of 1-pentanol, the primary byproduct in this reaction, via TLC analysis, this reaction stands to be improved the most. Ideally, a different set of reagents could be used for the alkynylation which would remove the need for a supplementary titration to be performed and thus improve the yield of this reaction.

Lithium metal was dissolved in ethylene-1,2-diamine at 80 °C, then treated with potassium *tert*-butoxide (*t*-BuOK) in preparation for an alkyne zipper reaction. Alkynol **3.4** was added to the solution to result in primary alcohol **3.5**.[13] The alkyne zipper reaction of **3.4** into **3.5** was initially performed using NaH in ethylene-1,2-diamine, but the reaction required significant temperature moderating and an extended reaction time.[14] The lithium metal and potassium *tert*-butoxide variant of the alkyne zipper reaction required much less time investment due to performing the reaction at a constant elevated temperature with no need for intervention. Unfortunately, this reaction was only performed on impure samples of **3.4**, so the percent yields may not properly reflect the efficiency of the reaction. Using this method, the highest percent yield of **3.5** after purification was 32%, which was likely influenced by the inaccurate mass determination of **3.4** prior to starting the reaction. Performing this reaction with a pure sample of **3.4** would immediately provide better insight into the efficiency of the reaction.

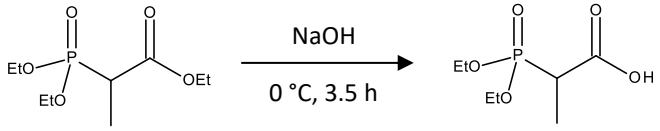
Alcohol **3.5** was then stirred in ethyl acetate (EtOAc) alongside solid 2-iodoxybenzoic acid (IBX) under reflux to oxidize the alcohol into aldehyde **3.6**.[15] The oxidation of **3.5** into **3.6** required testing multiple different reaction conditions with varying levels of success. Initially, Dess–Martin periodinane (DMP) was used to attempt the oxidation, but the reaction never fully progressed.[16] The second oxidation method tested

utilized the Swern oxidation with oxalyl chloride, triethylamine, and dimethylsulfoxide (DMSO).[17] While this reaction produced aldehyde **3.6** in good crude yields (80%), the need of dry ice/acetone bath temperatures and multiple washes and extractions along with the presence of malodorous dimethyl sulfide as a side product reduced the desirability of the method. The last reaction tested utilized solid IBX suspended in ethyl acetate heated under reflux. Not only did this produce the aldehyde in similar crude yields as the Swern oxidation, but the workup steps were incredibly simple. Any solid byproducts and excess IBX were filtered away and the ethyl acetate was removed *in vacuo* to yield crude **3.6**. Ultimately, the use of IBX was the clear choice for the oxidation of alcohol **3.5** into aldehyde **3.6**.

Triethyl-2-phosphonopropionate (**3.8**) was prepared via a Michaelis–Arbuzov reaction by heating ethyl-2-bromopropionate (**3.7**) and triethylphosphite at 160 °C, while distilling the bromoethane byproduct.[18] The Michaelis–Arbuzov reaction to produce phosphonate **3.8** was easily the most successful reaction of this entire work. Ethyl-2-bromopropionate (**3.7**) and triethylphosphite reacted together with no need for a solvent, and the reaction flask was connected to a distillation apparatus to remove bromoethane as it was being produced in the reaction. A separate distillation to purify phosphonate **3.8** gave a respectable 91% yield upon its completion.

Phosphonate **3.8** was used in a Horner–Wadsworth–Emmons (HWE) reaction with aldehyde **3.6** in THF to give ester **3.9**.[19] When performing the HWE reaction between aldehyde **3.6** and phosphonate **3.8**, **3.6** was used in its crude state without further purification. The actual reaction process was quite simple and gave **3.9** in a fair yield (49% over two steps).

While the HWE reaction was one of the pivotal points for synthesizing **3.1**, there was initial uncertainty if phosphonate **3.8** should undergo hydrolysis to give a carboxylic acid (**3.10**) prior to the HWE reaction, and then give **3.1** immediately after the HWE reaction upon workup (Scheme 3.3).[20] While the synthesis of **3.10** went smoothly, its viscosity was such that it was incredibly cumbersome to work with, especially when using syringes. This led to the ester hydrolysis step being performed after the HWE reaction with phosphonate **3.8** instead of before it.

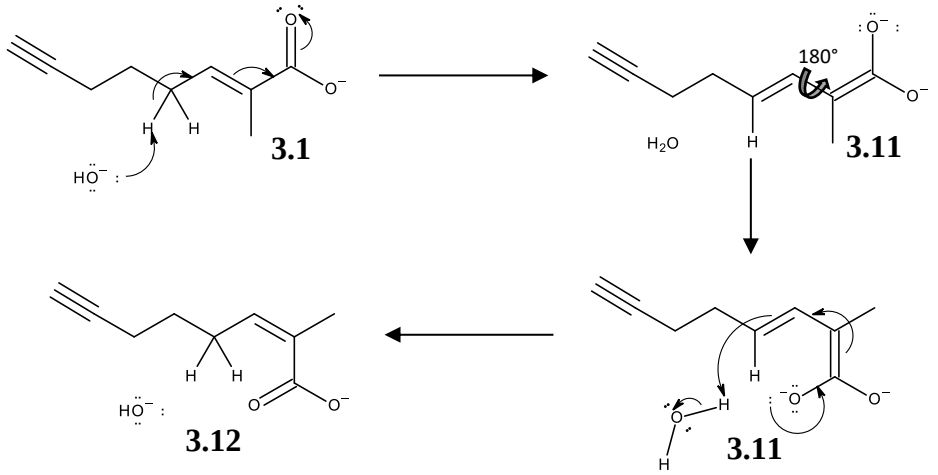


3.8 **3.10** (87%)

Scheme 3.3. Synthesis of carboxylic acid functional group prior to HWE olefination.

Finally, hydrolysis of **3.9** with lithium hydroxide (LiOH) under reflux in aqueous THF gave carboxylic acid **3.1**.[21] The hydrolysis of **3.9** into **3.1** initially appeared to be a simple reaction but had an unforeseen result. While the literature suggested reacting **3.9** at 0 °C, it was instead observed that refluxing the reaction greatly improved reaction times as verified by TLC analysis.[21] However, a surprising result was that ^{1}H NMR and ^{13}C NMR analysis of **3.1** showed an isomerization of the alkene had occurred with a 9:1 *E*:*Z* ratio in all experimental results. This reaction was performed separately at both ambient and reflux temperatures with identical ^{1}H NMR and ^{13}C NMR patterns, so it was concluded that the temperature did not cause the isomerization. Instead, it is hypothesized that the basic conditions of the reaction promoted the isomerization by removing a particularly acidic hydrogen from **3.8**, causing a shift in electrons to allow the previously double-bonded

carbons to freely rotate (Scheme 3.4). A potential workaround for this issue would be to use an acidic environment for the hydrolysis instead, as that would prevent any base-mediated isomerization while still promoting the primary reaction.

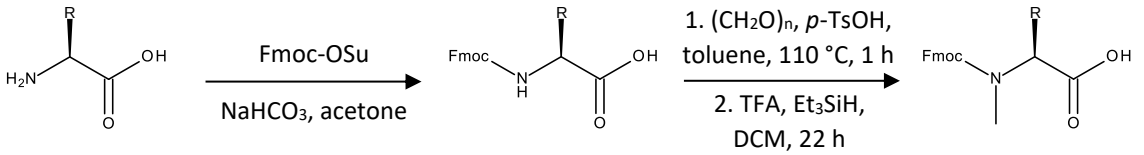


Scheme 3.4. Proposed mechanism for the *E/Z* isomerization of **3.1** to **3.12**.

3.1.2 Synthesis of Modified Amino Acids 3.12–3.15

Modification of both L-valine (**3.13**) and L-phenylalanine (**3.14**) followed the same general procedures. For the first step, each of the amino acids were introduced to the fluorenylmethyloxycarbonyl (Fmoc) protecting group, specifically with the use of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu), which would eventually be used to protect the amine group during the coupling reactions. Each amino acid and Fmoc-OSu were dissolved in acetone in a basic environment to produce amino acids **3.15** and **3.16** (Scheme 3.5).[22] Adding the Fmoc protecting group to both L-valine and L-phenylalanine went smoothly with yields being 67% and 61%, respectively.

Since some of the planned dragonamide E analogues contained unmethylated



3.13 (R = *i*-Pr)
3.14 (R = CH_2Ph)

3.15 (R = *i*-Pr)
3.16 (R = CH_2Ph)

3.17 (R = *i*-Pr)
3.18 (R = CH_2Ph)

Scheme 3.5. Synthesis of the N-methylated amino acids.

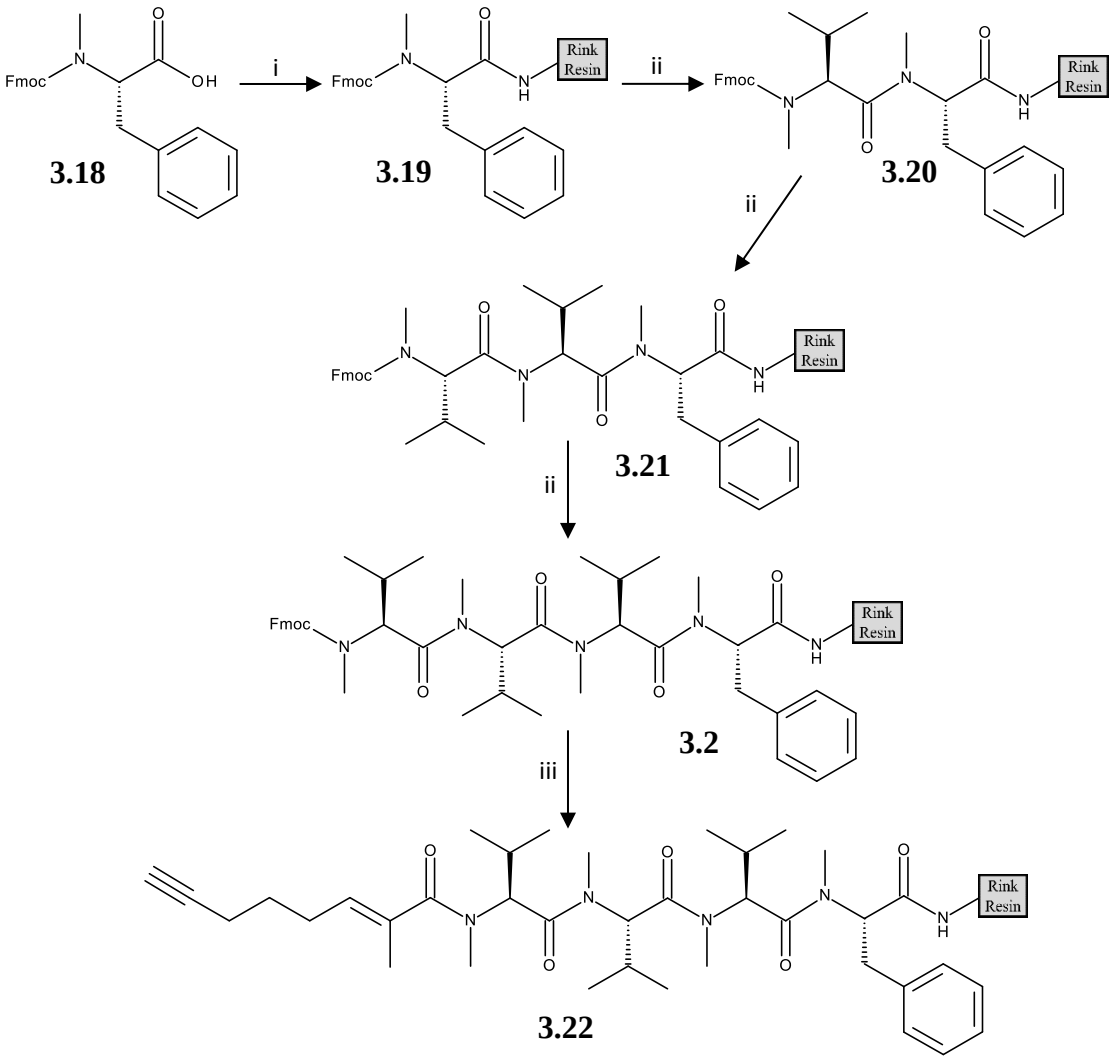
amino acids, half of the synthesized **3.15** and **3.16** was stored for later use, while the other half was used in the next steps. The next reaction was to treat the Fmoc-protected amino acid with paraformaldehyde and a catalytic amount of *para*-toluenesulfonic acid (*p*-TsOH) in toluene under reflux to generate an oxazolidinone. This functional group was then cleaved using trifluoroacetic acid (TFA) and triethylsilane (Et_3SiH) in dichloromethane (DCM) to generate N-methylated Fmoc-protected amino acids **3.17** and **3.18**.[23] The N-methylation step for the amino acids was more troublesome. Regarding valine, while the reaction overall was a success, the yield was low (28%). Phenylalanine proved to be the bigger problem however, as toward the end of the workup steps, most of the solvated **3.18** would turn into a viscous, sticky oil that was cumbersome to work with upon rotary evaporation. Eventually some of **3.18** was obtained as a white powder (37%), but not without additional efforts to separate the remaining solvated **3.18** from the portion that turned into the viscous oil.

3.1.3 Initial Plan to Combine 3.1 and 3.2

Once ample amounts of **3.1**, **3.17**, and **3.18** were synthesized, the hydrophobic tail and tetra amino acid head could be linked to form the final dragonamide E product. However, the coupling process did not go according to plan and required modification. The

initial procedure was to couple amino acid **3.18** to Rink amide resin in a two-step process. First, piperidine would be used to deprotect the resin, then the coupling would be accomplished using hydroxybenzotriazole (HOBt), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU), and *N*,*N*-diisopropylethylamine (DIPEA) to produce the intermediate amino acid **3.19** (Scheme 3.6).[24] Amino acid **3.17** could be coupled three sequential times using the same reaction conditions (Scheme 3.6, ii), using five equivalents of each of the reagents instead of the previous three equivalents as well as the utilization of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) instead of HBTU (**3.20**, **3.21**, **3.2**).[24] Finally, **3.1** could be added using the same conditions as the **3.17** additions (Scheme 3.6, iii) to give dragonamide E tethered to the Rink amide resin (**3.22**). Dragonamide E could then be cleaved off the resin.[24]

The coupling of the N-methylated amino acids revealed a flaw with the initial retrosynthesis reasoning. Amino acid **3.18** was successfully linked to the Rink amide resin as per the initial procedure and was verified with a Kaiser test. The problems arose when **3.17** was introduced to the resin. Because all the amino acids in dragonamide E are N-methylated, each of the amino acids used in the coupling must also be N-methylated. Thus, the amines are secondary rather than primary, which drastically reduces the coupling reactivity of the amino acids. The first addition of **3.17** took six hours to complete, but the second addition was not complete even fourteen hours after the start of the reaction, as the chloranil test used for reaction verification always gave a positive result, indicating that the reaction was not complete. This ultimately brought the synthesis of dragonamide E to a halt. However, there may be a simple solution to this situation. The Fmoc-*N*-methyl amino



(i) 1. Rink Amide resin (100-200 mesh), piperidine, DMF, 40 min; 2. HOBt, HBTU, DIPEA, 2 h; (ii) 1. piperidine, DMF, 40 min; 2. HOBt, HATU, DIPEA, **3.17**, 6 h; (iii) 1. piperidine, DMF, 40 min; 2. HOBt, HATU, DIPEA, **3.1**, 6 h

Scheme 3.6. Planned amino acid coupling reactions and conditions.

acids could have their carboxylic acids converted into the much more reactive acyl chlorides. This would require one more step in the synthesis, with no added purification, but the increased reactivity from the acyl chlorides would greatly reduce the coupling reaction times.

3.2 Summary and Future Advancements

While neither dragonamide E nor any of its analogues were directly synthesized in this work, many important steps have been made toward accomplishing this goal. With the main synthesis route now established, optimizing each reaction individually will culminate into a reliable synthesis of dragonamide E. The synthesis framework could then potentially branch out to create other lipopeptides generated by *Lyngbya majuscula*.

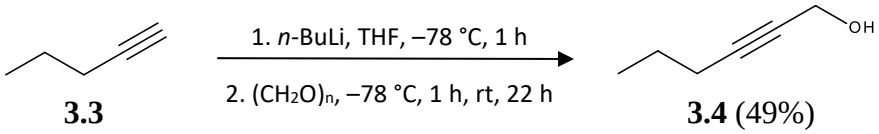
The most obvious plan for future endeavors in this synthetic field is to put the previously mentioned changes into effect and observe any new developments that arise. Optimizing existing reactions such as the alkynylation and ester hydrolysis would lead to a more confident total synthesis. Additionally, once the dragonamide E synthesis route is realized, the SAR studies into unmethylated amino acids in dragonamide E can commence. This will provide insight into whether dragonamide E may potentially become one of the newest drugs for leishmaniasis treatment.

CHAPTER 4

EXPERIMENTAL DETAILS

General Methods: Unless otherwise stated, all oxygen and moisture-sensitive reactions were performed under anhydrous conditions by use of argon-charged glassware. Solutions and solvents sensitive to moisture were transferred using standard syringe techniques. All commercial reagents were purchased as reagent grade and used without further purification. All organic solvents were used dry: tetrahydrofuran (THF), diethyl ether (Et_2O), dichloromethane (CH_2Cl_2), and toluene were purified via a Pure Solv MD-7 Solvent Purification System. Thin-layer chromatography was performed using Silicycle Glass Backed TLC Extra Hard 60 Å, 250 μm F-254 TLC plates that were visualized by *p*-anisaldehyde (PAA), cerium ammonium molybdate (CAM), and potassium permanganate ($KMnO_4$) staining. Column chromatography was performed using Silicycle SiliaFlash P60 Silica gel. Nuclear Magnetic Resonance (NMR) spectra were obtained for proton (^1H) and carbon (^{13}C) nuclei using a Varian AS400 NMR spectrometer; residual solvent peak signals for $CDCl_3$ were set at 7.26 and 77.16 ppm in the ^1H and ^{13}C spectra, respectively. A Nicolet iS10 smart iTR spectrometer was used to record infrared spectra and absorptions are reported in reciprocal centimeters.

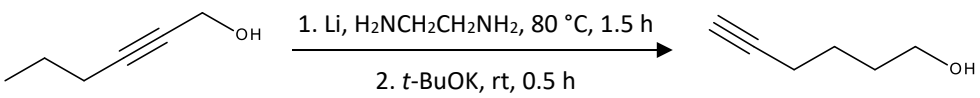
Alcohol 3.4



3.3 **3.4** (49%)

1-Pentyne (**3.3**, 50.0 mL, 0.507 mol) and THF (500 mL) were added to an argon-charged 2-L round bottomed flask. The flask was cooled to –78 °C with a dry ice/acetone bath, and the *n*-BuLi (270 mL, 0.512 mol) was added via syringe at a rate of 11 mL/min. The solution was stirred for 1 h. The paraformaldehyde (16.74 g, 0.557 mol) was added and the mixture continued to stir for 22 h. Ammonium chloride (saturated, 250 mL) was added to quench the reaction. Et_2O (1,600 mL) and H_2O (800 mL) were added to the solution in four portions. Each portion was put into a 2-L separatory funnel and the aqueous and organic layers were separated. The combined aqueous layer was extracted with Et_2O (3 x 375 mL). The combined organic layer was washed with brine (750 mL), dried over $MgSO_4$, filtered, and concentrated via rotary evaporation. The resulting oil was distilled under reduced pressure to give **3.4** (24.44 g, 49.11%) as a colorless oil; R_f = 0.43 (4:1 hexanes:ethyl acetate, v/v); IR (neat) 3311, 2961, 2933, 2873, 2289, 2227, 1726, 1457, 1379, 1338, 1277, 1239, 1137, 1033, 1005, 867, 633 cm^{-1}. Other spectroscopic data matched the literature.[13]

Alcohol 3.5

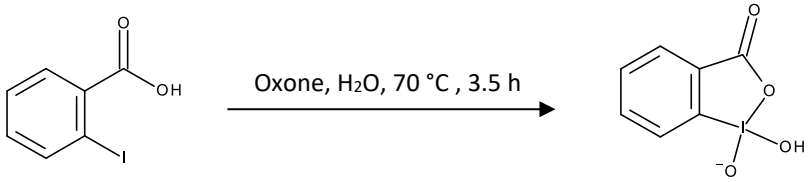


3.4 **3.5** (32%)

A lithium rod (0.44 g, 63.0 mmol) and ethylene-1,2-diamine (40 mL) were placed

in an argon-charged 100-mL three neck flask at 80 °C and stirred for 1.5 h. The solution was cooled to rt naturally and *t*-BuOK (4.52 g, 40.3 mmol) was added and stirred for 30 min. 2-hexyn-1-ol (**3.4**, 1.01 g, 10.32 mmol) was syringed into the flask over 5 min and stirred for 2.5 h. The purple solution was poured into a 250-mL separatory funnel along with ice water (40 mL), then Et_2O (60 mL) once the heat dissipated. The layers were separated and the aqueous layer was extracted with Et_2O (3 x 20 mL). The combined organic layer was washed with 1 M HCl (2 x 16 mL), saturated $NaHCO_3$ (16 mL), and brine (16 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated via rotary evaporation. The resulting oil was purified via column chromatography with a 4:1 hexanes:ethyl acetate solvent. **3.5** (0.32 g, 31.93%) was obtained as a colorless oil; R_f = 0.21 (4:1 hexanes:ethyl acetate, v/v); IR (neat) 3294, 2940, 2867, 2116, 1455, 1434, 1329, 1161, 1059, 989, 937, 629 cm^{-1}. Other spectroscopic data matched the literature.[13]

2-Iodoxybenzoic Acid (IBX) 4.2

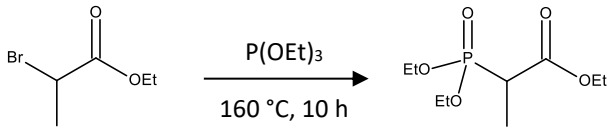


4.1 **4.2** (94%)

2-Iodobenzoic acid (50.21 g, 0.202 mol), oxone (166.54 g, 0.267 mol), and H_2O (650 mL) were added to a 2-L round bottomed flask with a reflux condenser attached. The flask was stirred and heated to 70 °C over 20 min, then stirred for 3 h. The flask was removed from the oil bath, cooled to rt, then put in a cold-water bath and stirred for 1.5 h. The flask's contents were poured over a medium-grade fritted funnel and the solid was

washed with H_2O (6 x 100 mL) and acetone (2 x 100 mL). The solid (**4.2**, 53.08 g, 93.84%) was dried overnight and obtained as a white powder. It was used immediately.

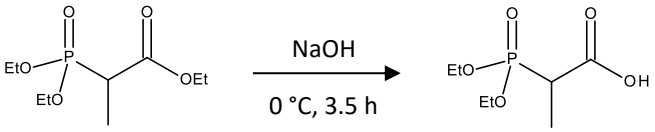
Phosphonate 3.8



3.7 **3.8** (91%)

Ethyl-2-bromopropionate (**3.7**, 65.0 mL, 0.500 mol) and $P(OEt)_3$ (86.0 mL, 0.506 mol) were put into a 250-mL round bottomed flask with a distillation head attached. The reaction was heated at 160 °C for 10 h, during which time the bromoethane was distilled. After the 10 h, the flask was connected to a distillation apparatus under reduced pressure to purify triethyl-2-phosphonopropionate (**3.8**, 108.03 g, 90.70%) as a fragrant colorless oil; R_f = 0.15 (4:1 hexanes:ethyl acetate v/v); IR (neat) 2983, 2942, 2909, 1732, 1457, 1392, 1368, 1314, 1252, 1179, 1162, 1094, 1017, 958, 904, 860, 803 cm^{-1}. Other spectroscopic data matched the literature.[18]

Phosphonic Acid 3.10

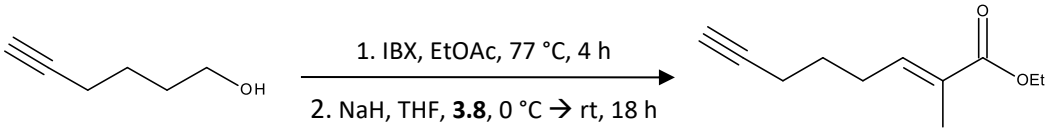


3.8 **3.10** (87%)

Triethyl-2-phosphonopropionate (**3.8**, 18.41 g, 77 mmol) and H_2O (30 mL) were added to a 200-mL round bottomed flask. The solution was stirred and cooled to 0 °C, then 10 M NaOH (8.00 mL, 80 mmol) was added and stirred for 1 h. The reaction was warmed

to rt and stirred another 2.5 h. The reaction was cooled to 0 °C again and acidified with HCl (concentrated) until pH ≤ 1. The reaction was brought to rt and saturated with NaCl. The mixture was extracted with DCM (3 x 25 mL), dried over $MgSO_4$, filtered, and concentrated via rotary evaporation to give **3.10** (14.21 g, 87.45%) as a clear yellow-tinted oil. IR (neat) 2984, 2942, 2572, 1732, 1458, 1393, 1294, 1176, 1016, 964, 856, 817, 735, 693 cm^{-1}. Other spectroscopic data matched the literature.[20]

Ester 3.9



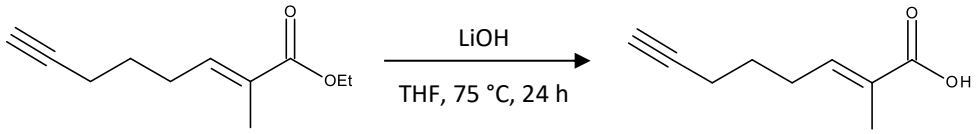
3.5 **3.9** (49% over two steps)

5-hexyn-1-ol (**3.5**, 9.00 g, 91.7 mmol) and EtOAc (650 mL) were added to a 1-L round bottomed flask with a reflux condenser. After having dissolved, IBX (77.60 g, 275.2 mmol) was added and heated to 77 °C and stirred for 5 h with TLC verification. The mixture was then cooled to rt and filtered through a medium-fritted funnel. The solid was washed with EtOAc (3 x 100 mL). The combined filtrate was concentrated via rotary evaporation to give crude 5-hexynal (**3.6**), which was used immediately.

A separate 1-L round bottomed flask was charged with argon, NaH (4.40 g, 110 mmol), and THF (375 mL) at 0 °C. Phosphonate **3.8** (28.38 g, 119 mmol) was then added and stirred for 18 h, during which the reaction warmed to rt. Crude aldehyde **3.6** (supposed 8.82 g, 92 mmol) was syringed into the flask and stirred at rt for 2 h. The solution was put into a 1-L separatory funnel, followed by H_2O (100 mL), then Et_2O (100 mL). The aqueous phase was removed and the organic phase was washed with H_2O (3 x 60 mL). The

combined aqueous phase was extracted with DCM (2 x 60 mL). The combined organic phase was washed with brine (120 mL), dried over $MgSO_4$, filtered, and concentrated via rotary evaporation. The resulting oil was purified over a column chromatography with 19:1 hexanes:ethyl acetate solvent. **3.9** (8.09 g, 48.95% over two steps) was obtained as a fragrant colorless oil; R_f = 0.64 (4:1 hexanes:ethyl acetate v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (m, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 2.23 (app q, *J* = 7.2 Hz, 2H), 2.15 (td, *J* = 6.8, 2.4 Hz, 2H), 1.91 (t, *J* = 2.8 Hz, 1H), 1.78 (s, 3H), 1.60 (quint, *J* = 7.2 Hz, 2H), 1.22 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 140.7, 128.7, 83.7, 68.9, 60.4, 27.4, 27.3, 18.0, 14.2, 12.3; IR (neat) 3299, 2981, 2935, 2867, 2118, 1705, 1651, 1446, 1389, 1367, 1256, 1210, 1175, 1118, 1082, 1037, 869, 743, 631 cm^{-1}.

Carboxylic Acid 3.1

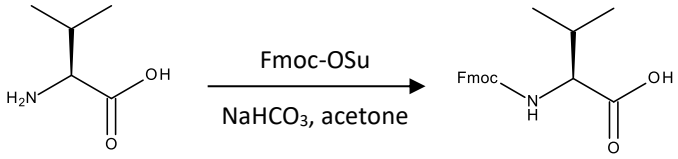


3.9 **3.1** (76% 9:1 *E*:*Z*)

Ethyl-(2E)-2-methyl-2-octen-7-ynoate (**3.9**, 8.09 g, 44.9 mmol) and THF (45 mL) were put into a 1-L round bottomed flask. 2 M LiOH was added (45.0 mL, 90 mmol) and the contents were heated under reflux at 75 °C for 24 h with TLC verification. The solution was put into a 500-mL separatory funnel, diluted with H_2O (90 mL), then washed with DCM (2 x 135 mL). After acidifying the aqueous layer with 1 M HCl until pH ≤ 2, the aqueous layer was extracted with EtOAc (3 x 135 mL). The combined EtOAc was washed with brine, dried over $MgSO_4$, filtered, and concentrated via rotary evaporation. The slightly yellow oil was purified over a column chromatography with 7:1 hexanes:ethyl

acetate to give carboxylic acids **3.1** (5.22 g, 76.41%) in a 9:1 *E/Z* ratio; R_f = 0.36 (4:1 hexanes:ethyl acetate v/v); ^{1}H NMR (400 MHz, $CDCl_3$) for *E* isomer (**3.1**, major): δ 11.96 (s, 1H), 6.86 (m, 1H), 2.31 (app q, *J* = 7.6 Hz, 2H), 2.19 (td, *J* = 6.8, 2.4 Hz, 2H), 1.96 (t, *J* = 2.8 Hz, 1H), 1.82 (d, *J* = 0.8 Hz, 3H), 1.66 (quint, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) for *E* isomer (**3.1**, major): δ 173.9, 143.9, 128.1, 83.6, 69.1, 27.7, 27.2, 18.1, 12.0; ^{13}C NMR (100 MHz, $CDCl_3$) for *Z* isomer (minor): δ 173.9, 145.2, 127.2, 84.1, 68.7, 28.9, 28.3, 20.5, 18.2; IR (neat) 3299, 2934, 2665, 2118, 1682, 1641, 1419, 1388, 1277, 1184, 1130, 1087, 933, 798, 744, 632 cm^{-1}.

Fmoc-protected L-Valine (3.15)

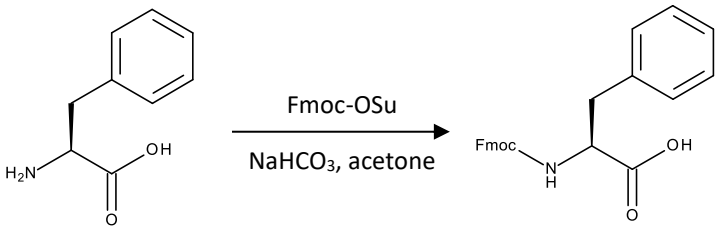


3.13 **3.15** (67%)

L-valine (**3.13**, 29.29 g, 0.250 mol) was dissolved in H_2O (940 mL) in a 3-L round bottomed flask. Fmoc-OSu (85.83 g, 0.254 mol), $NaHCO_3$ (22.08 g, 0.263 mol), and acetone (940 mL) were all added and stirred at rt until clear. The solution was then acidified with 10% $NaHSO_4$ solution (1,640 mL), which caused the product to precipitate. The solid was filtered, washed with H_2O (3 x 100 mL), and dried. The clumpy solid was recrystallized by dissolving it in a minimal amount of boiling EtOAc, then adding enough hexanes to begin recrystallization. The heat was removed and the flask was brought to rt with no stirring. The flask was put into an ice bath for 10 min. Filtration and drying of the product gave **3.15** (57.25 g, 67.46%) as a white powder. Spectroscopic data was consistent

with that in the literature.[25]

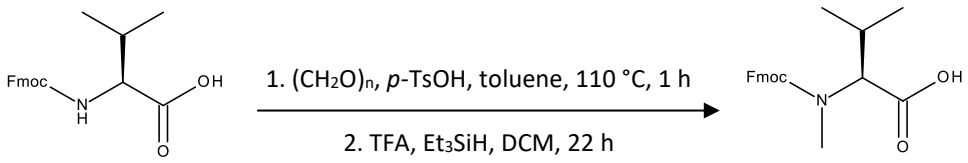
Fmoc-protected L-Phenylalanine (3.16)



3.14 **3.16** (61%)

L-phenylalanine (**3.14**, 41.32 g, 0.250 mol) was dissolved in H_2O (940 mL) in a 3-L round bottomed flask. Fmoc-OSu (85.95 g, 0.255 mol), $NaHCO_3$ (21.74 g, 0.259 mol), and acetone (940 mL) were all added and stirred at rt until clear. The solution was then acidified with 10% $NaHSO_4$ solution (1,640 mL), which caused the product to precipitate. The solid was filtered, washed with H_2O (3 x 100 mL), and dried. The clumpy solid was recrystallized by dissolving it in a minimal amount of boiling EtOAc, then adding enough hexanes to begin recrystallization. The heat was removed and the flask was brought to rt with no stirring. The flask was put into an ice bath for 10 min. Filtration and drying of the product gave **3.16** (59.07 g, 60.99%) as a white powder. Spectroscopic data was consistent with that in the literature.[22]

Fmoc-*N*-methylvaline (3.17)

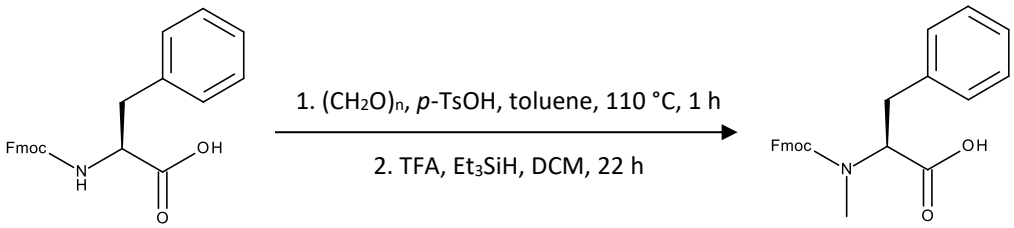


3.15 **3.17** (28%)

Fmoc-L-valine (**3.15**, 29.01 g, 80 mmol) was put into a 2-L round bottomed flask and suspended in toluene (300 mL). Paraformaldehyde (16.75 g, 558 mmol) and a catalytic amount of *p*-TsOH (1.29 g, 6.80 mmol) were added and heated under reflux with a Dean–Stark trap attached for azeotropic water removal for 1 h. The solution was cooled to rt, washed with 1 M $NaHCO_3$ (3 x 200 mL), dried with $MgSO_4$ filtered, and concentrated via rotary evaporation as a viscous golden oil.

The oil was then dissolved in DCM (22 mL) in an argon-charged flask. Trifluoroacetic acid (22 mL, 297 mmol) and Et_3SiH (5.32 mL, 33.4 mmol) were added and stirred at rt for 22 h. Compressed air was blown over the flask to evaporate the TFA and Et_3SiH. Once concentrated, the golden oil was dissolved in toluene (75 mL) and subsequently removed by rotary evaporation. This was done three times. The solid was then recrystallized in a solution of 1:1 Et_2O:EtOAc to give **3.17** (8.56 g, 28.40%) as a white powder. Spectroscopic data was consistent with that in the literature.[23]

Fmoc-*N*-methylphenylalanine (3.18)



3.16 **3.18** (37%)

Fmoc-L-phenylalanine (**3.16**, 19.40 g, 50.06 mmol) was put into a 2-L round bottomed flask and suspended in toluene (1,000 mL). Paraformaldehyde (10.07 g, 335 mmol) and a catalytic amount of *p*-TsOH (0.54 g, 2.83 mmol) were added and heated under

reflux with a Dean Stark trap attached for azeotropic water removal for 1 h. The solution was cooled to rt, washed with 1 M $NaHCO_3$ (3 x 200 mL), dried with $MgSO_4$ filtered, and concentrated via rotary evaporation as a viscous golden oil.

The oil was then dissolved in DCM (150 mL) in an argon-charged flask. Trifluoroacetic acid (150 mL) and Et_3SiH (24.0 mL, 151 mmol) were added and stirred at rt for 22 h. Compressed air was blown over the flask to evaporate the TFA and Et_3SiH. Once concentrated, the golden oil was dissolved in Et_2O (50 mL) and subsequently removed by rotary evaporation. This was done three times. The solid was precipitated with hexanes (400 mL). A white solid formed at first, followed by a viscous, sticky oil. The liquid was decanted off and concentrated via rotary evaporation, then dried under reduced pressure to give **3.18** (7.45 g, 36.70%) as a white powder. Spectroscopic data was consistent with that in the literature.[23]

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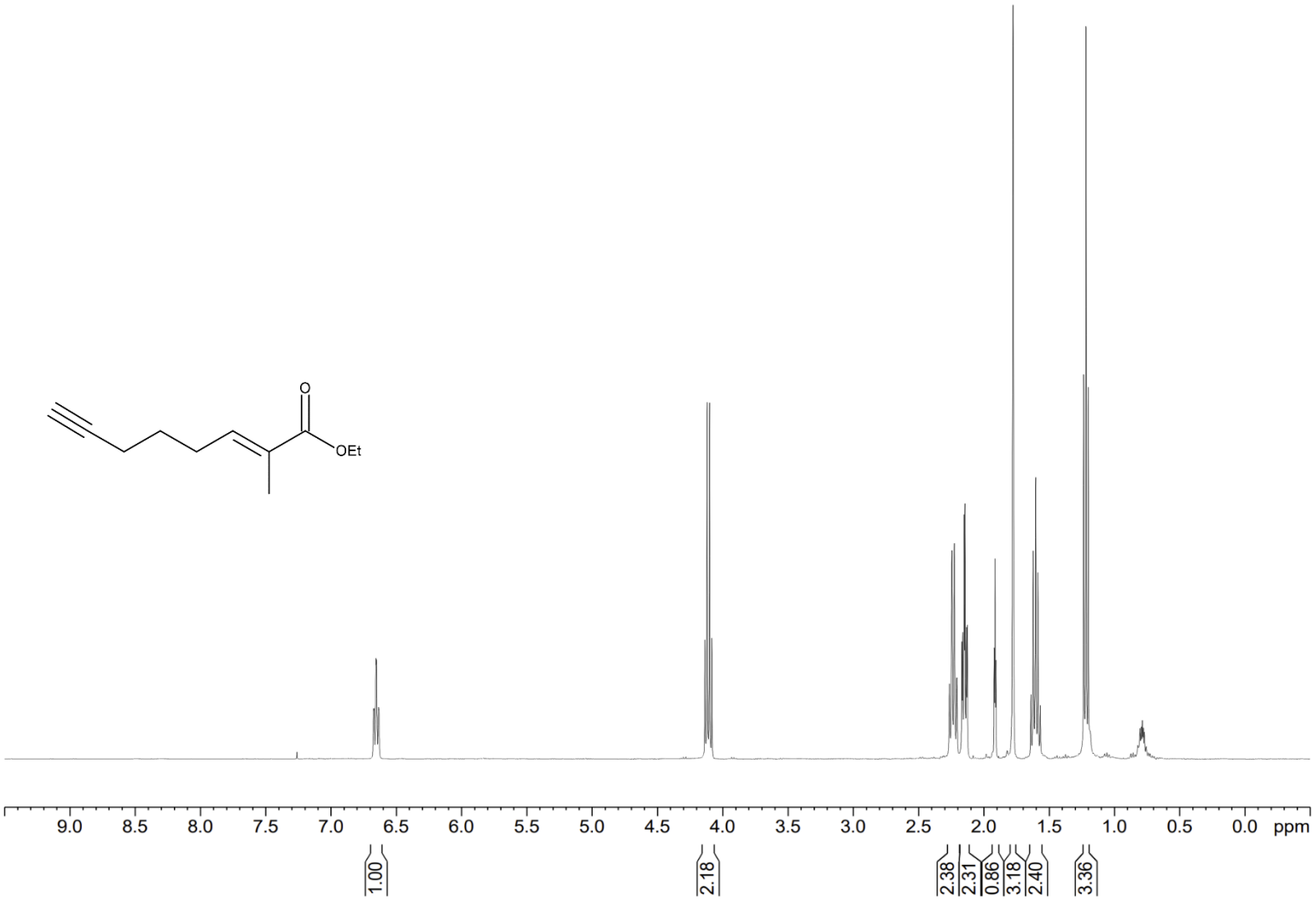
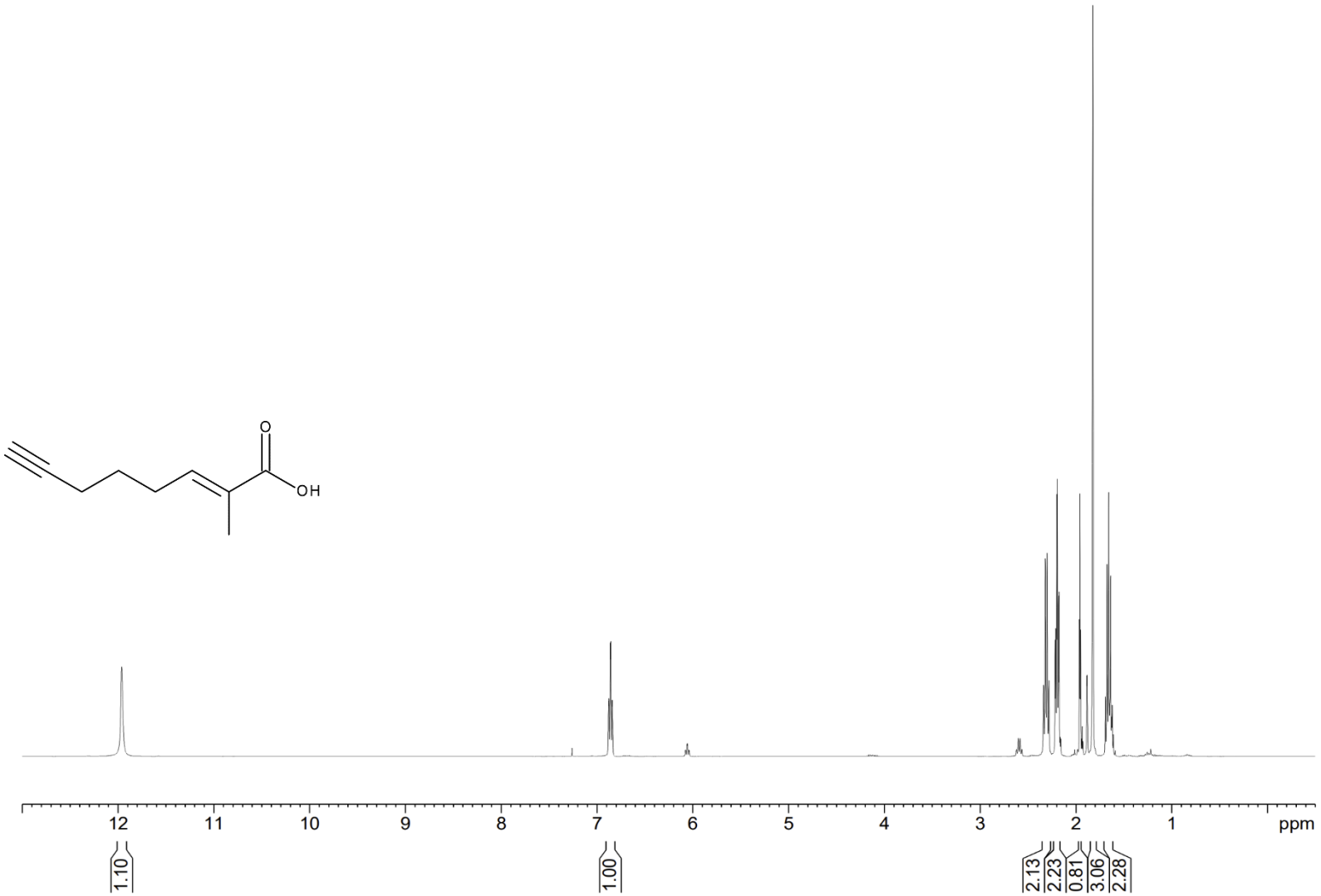
APPENDIX A:

^{1}H NMR SPECTRA



^{1}H NMR Spectrum of **3.9** (400 MHz, $CDCl_3$)

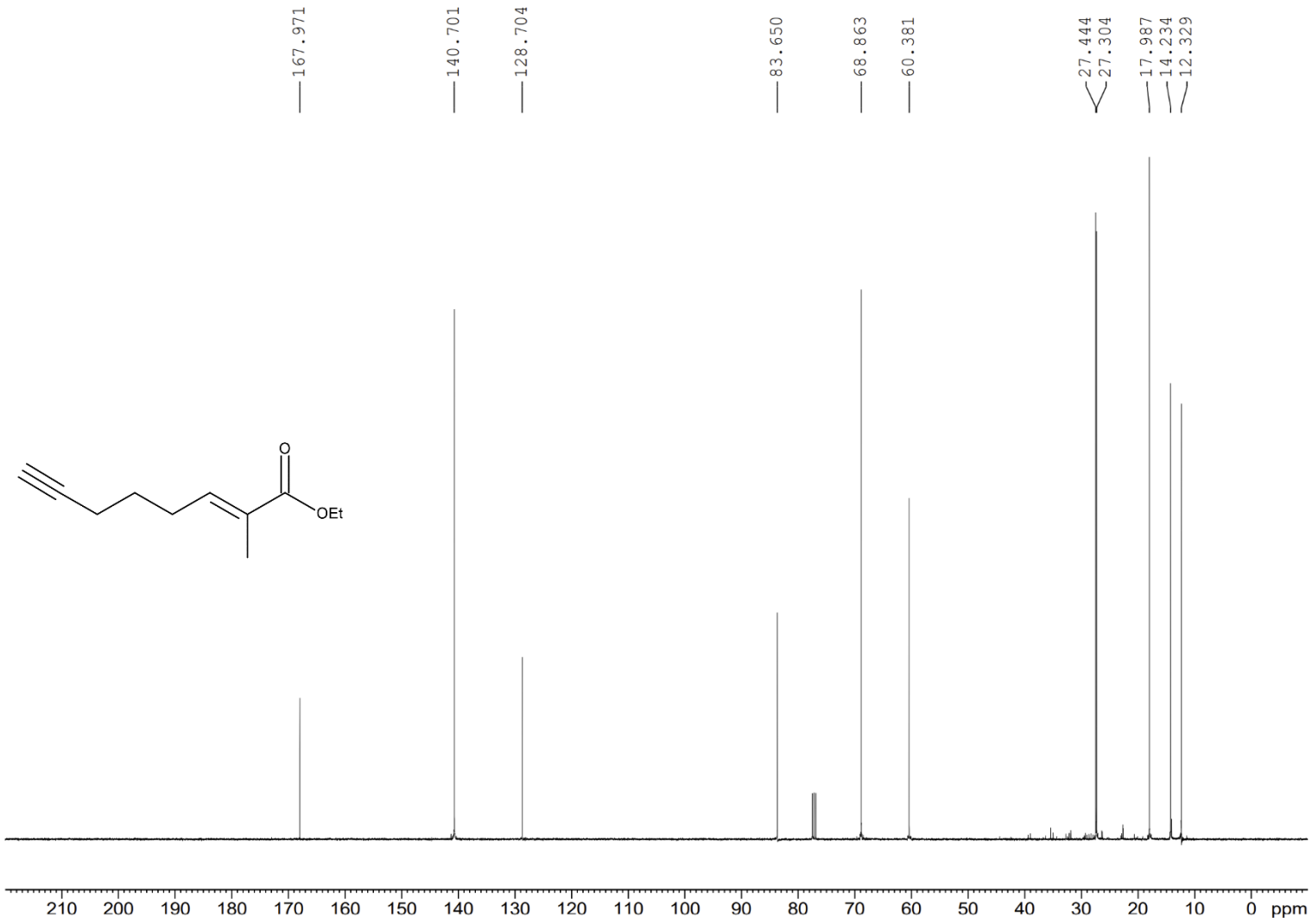


^{1}H NMR Spectrum of **3.1** (400 MHz, $CDCl_3$)

Zoomed in ^{1}H NMR Spectrum of **3.1** to show *E/Z* isomeric ratio (400 MHz, $CDCl_3$)

APPENDIX B:

^{13}C NMR SPECTRA



^{13}C NMR Spectrum of **3.9** (100 MHz, $CDCl_3$)

^{13}C NMR Spectrum of **3.9** (100 MHz, $CDCl_3$, mixture of *E/Z* isomers)