

First total synthesis of cyanobactins balgacyclamide A & B and synthetic efforts towards
balgacyclamide C: Configuration reassignment analyses via β -hydroxyamides dehydrative
cyclization conditions

by

Arnaldo Xavier Torres-Hernandez

B.S., Pontifical Catholic University of Puerto Rico, 2019

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Chemistry
College of Arts and Sciences

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Abstract

Balgacyclamides are a class of natural products, isolated from the freshwater cyanobacterium *Microcystis aeruginosa*, known for their potent antiparasitic activity against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, and *Leishmania donovani*. Previous studies have suggested that balgacyclamides may also exhibit metal chelation properties. Despite their promising biological activities, the total synthesis of these compounds has not been previously reported. This study presents the first total synthesis of the balgacyclamide family, providing insights into their stereochemistry, and exploring the potential for discovering new bioactive compounds through synthetic intermediates.

Chemically, balgacyclamides A & B are macrocyclic hexapeptides that share a common northern fragment, which includes an oxazoline and a thiazole. However, their southern fragments differ; balgacyclamide A contains an additional oxazoline, while balgacyclamide B features a free β -hydroxyamide. The total synthesis of balgacyclamide A, as well as its epimer, was achieved in 21 synthetic steps with overall yields of 5% and 3%, respectively. Key synthetic steps involved a tandem sulfur incorporation, cyclization, and radical oxidation to form the thiazole, along with a late-stage β -hydroxy amide Walden inversion to access the two oxazoline rings. Notably, these oxazolines were derived from the cyclization of *L*-threonine and *L*-valine. Thus, we investigated the effect of β -carbon epimerization at the threonine residue to determine the correct stereochemical assignment. Using reagents such as the Burgess reagent, diethylaminosulfur trifluoride (DAST), and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ catalyst, we compared our synthetic product with the configuration assigned to the isolated natural macrocycle and a previous synthetic study of balgacyclamide A.

Our results led to a reassignment of the configuration from the prior reported synthetic route and confirmed a complete match between our synthetic balgacyclamide A and the isolated natural compound. In addition, balgacyclamide B was synthesized through a 17-step pathway with a 2% overall yield, following a similar balgacyclamide A synthetic strategy and employing standard peptide coupling conditions for macrolactamization. Finally, we explored the impact of molecular physicochemical properties on porin-mediated transport in Gram-negative bacteria (GNB) by synthesizing a library of northern fragment intermediates of balgacyclamides. We observed that altering the position of charged groups within the molecule, while maintaining other physicochemical properties constant, significantly affected penetration across GNB membranes. Notably, charge positioning influenced bacterial penetration, with transport rates ranging from 29% to 79%. This finding suggests that the three-dimensional orientation of charges plays a critical role in porin-mediated transport of small molecules in GNB. Future studies should focus on elucidating the mechanism behind charge orientation in porins and exploring the potential of balgacyclamides for drug delivery and metal chelation applications in greater detail.

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Dr. Ryan J. Rafferty

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I am truly blessed to have all of you in my life.

Dedication

To my parents and sister, who have always believed in me and motivated me to go after my goals. To my grandparents and my aunt Socorro Hernández, for their constant love and support.

Chapter 1 - Cyanobacteria: An Overview of Cyanobacterial Secondary

Metabolites

1.1 Cyanobacteria and their impact

Cyanobacteria are microorganisms that play an important role in the Earth due to their abilities to induce nitrogen fixation, mostly in oceanic and lake waters.¹ However, the combination of climate change, rising the temperature in bodies of water, and eutrophication in multiple ecosystems has been stimulating cyanobacterial blooms uncontrollably. All of which are causing health risk for humans and animals.^{2,3} For example, Lake Erie is one of the five Great Lakes in North America and is considered a valuable natural resource because of the access to potable water for 12 million people, the tourism industry, the fishermen field of work, and the seven billion dollars in revenue acquired annually.⁴ However, every year the lake is plagued with large scale cyanobacterial blooms because of excess of nitrogen and phosphorous present (**Figure 1.1C-D**). This is due to the urban location of the lake in vicinity of multiple industries, and the excess heat during summers.⁵ The algae bloom in this lake represents a potential health concern for the community, since human deaths have been reported in correlation with excess of cyanobacteria growth in freshwaters.^{5,6}

Beyond the human impact, cyanobacterial blooms caused the death of more than 350 elephants and 30,000 flamingos in Botswana's Okavango Delta region and in Lake Bogoria in Kenya, respectively (**Figure 1.1A-B**). Furthermore, cyanobacterial toxins have been found within elephants.^{7,8} This incident is categorized as one of the largest events involving elephants' deaths worldwide. The main reason behind the risks of these blooms is completely correlated with the cyanobactin natural products that are biosynthesized in the cyanobacteria, since excess of concentrations of these cyanotoxins lead to illness and death in humans and animals.⁹ Even though

these cyanobactins have a negative impact when are in excess in waterbodies, researchers as of recent have been highly interested to find potential applications of these molecules as therapeutics. Furthermore, the complex structures of the natural products isolated from them have led to the development of new synthetic methodologies and continue to result in new total synthesis campaigns^{10–15} (**Figure 1.1E-F**).

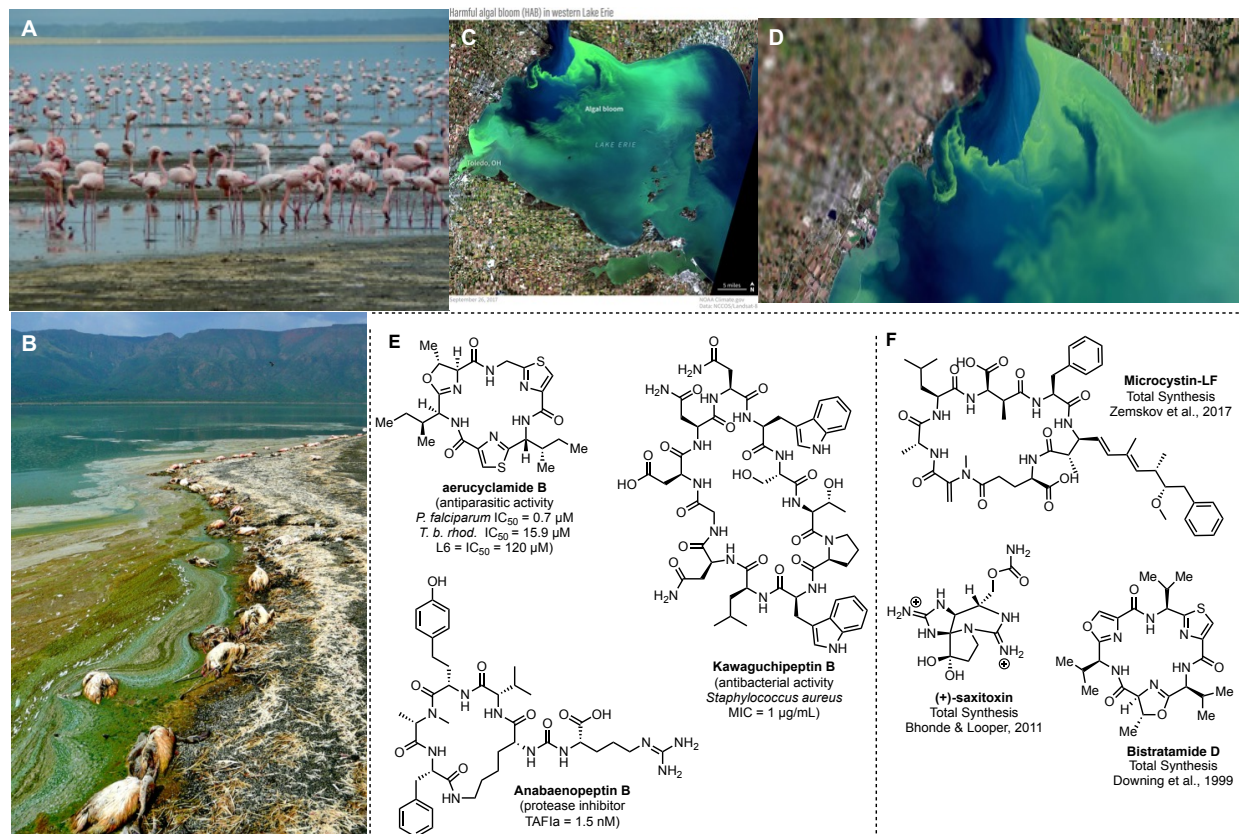


Figure 1.1. The role of cyanobacterial blooms and their secondary metabolites across multiple scientific disciplines: environmental sciences, therapeutics & chemical biology, and organic chemistry in natural product synthesis

A) Lesser flamingos in Lake Nakuro, Kenya where cyanobacterial blooms are a health risk for animals B) Death Lesser flamingos in Lake Bogoria, Kenya in July 2008, in where more than 30,000 flamingos were killed by cyanobacterial blooms in 1999.¹⁶ Images A & B are courtesy of

Dr. Lothar Krienitz, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Germany. C & D) Cyanobacteria blooms at western Lake Erie (September 26, 2017). Graphic by NOAA, satellite imagery from Landsat-8 E) Cyanobacterial secondary metabolites exhibiting potent biological activity F) Total synthesis of three cyanobacterial secondary metabolites, illustrating diversity in their chemical structure.

1.2 Cyanobacterial Secondary Metabolites

Cyanobacterial secondary metabolites, commonly referred to as natural products, are found in cyanobacteria from various environments, including freshwater, thermal springs, Antarctic waters,^{17,18} as well as in the symbiotic systems of terrestrial and marine plants and animals,^{19,20} and oceanic blooms.²¹ Over the past 57 years, more than 3,000 cyanobacterial secondary metabolites have been identified.²² Additionally, cyanobactins belong to both subclass of ribosomally synthesized and post-translationally modified peptides²³ (RiPPs), and non-ribosomally peptide biosynthesized. Because of the multiple differences within their biosynthetic pathway and their chemical structures, these secondary metabolites are categorized into various families. Furthermore, cyanobacterial secondary metabolites have been highly studied because of their impact to the environment, their potential therapeutic applications, and their chemical structure that challenge organic chemists to develop new synthetic methodologies to access the total synthesis of these linear and cyclic peptides (**Figure 1.1A-F**). A brief description of each family will be presented in this chapter, and synthetic methodologies will be further discussed in chapter 2 for selected family and molecules.

1.2.1 Cyanotherapeutics

Since the discovery of cyanobacterial blooms and the isolation of their secondary metabolites, researchers have been investigating the therapeutic potential of these secondary metabolites because of their promising biological activities. These compounds have shown efficacy in fields such as cancer, bacterial infections, tumor growth, HIV, malaria, viral diseases, inflammation, and diabetes, increasing interest in their potential to positively impact human health²⁴ (**Figure 1.2**).

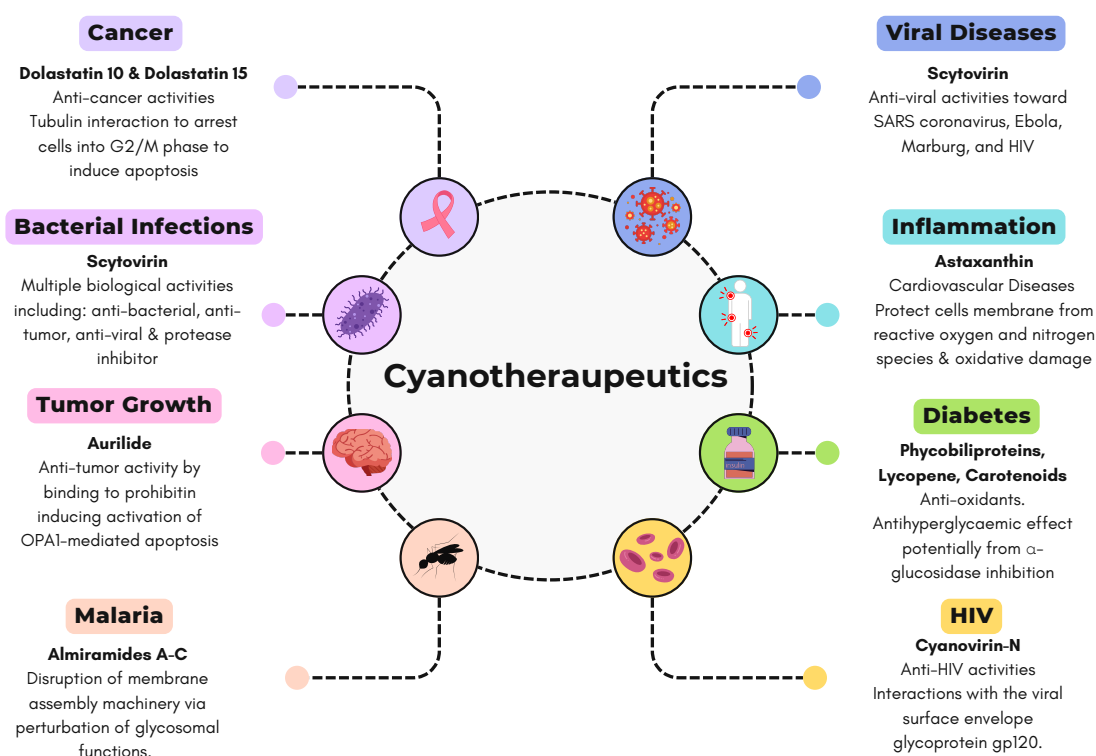


Figure 1.2. Potential therapeutic applications of cyanobacterial secondary metabolites

Examples of selected molecules are presented for each category of therapeutic potential.

In the field of neuroscience and medicinal chemistry, researchers have been working on how to use cyanobactins as a treatment for brain diseases. One such example is that of Largazole, a dithiazole macrocyclic cyanobactin that was found to have blood-brain barrier penetration

abilities, possesses activity against glioblastoma cells, and inhibition towards class I histone deacetylases²⁵ (HDAC) (**Figure 1.3**). Subsequently, organic synthetic chemists established more than sixteen different pathways to access Largazole, developing new synthetic methodologies, increasing the scale, making it eco-friendly, and feasible for industries to access.^{26,27} Furthermore, biochemists and structural biologist have been highly investigating the mechanisms behind the biosynthetic pathway of cyanobacteria to develop new methodologies toward derivatization and bio-inspired syntheses of potent molecules²⁸ and identification of novel drugs.²⁹ For example, in the case of cyclamide family (a type of family from cyanobacterial blooms) the patellamides are the most known and studied molecule by biochemists. Patellamides A-D are macrocyclic peptides containing four heterocycle rings including oxazolines and thiazoles. The identification of multiple clusters, and the enzymes responsible for the amino acids' heterocyclizations, macrocyclizations, cleavages, and oxidations have been identified and characterized for this family and similar cyanobactins.^{30,31} The patellamides have shown multiple biological activities. For example, patellamide B, C, and D have multidrug resistance reversal for the chemotherapy medication Vinblastine, Colchicine for the gout prevention medication, and the antileukemia medication treatment Adriamycin.^{32,33} On the other hand, patellamide D showed cytotoxicity towards fibroblast (MRC5V1) and bladder carcinoma³⁴ (T24).

Additionally, cyanobacterial secondary metabolites have demonstrated significant potential in cancer research, showing multiple pathways to target cancer cells through the induction of apoptosis.^{24,35} As summarized by Tiwari²⁴ and Shahid³⁵, these pathways include cell cycle arrest, mitochondrial dysfunction, oxidative stress, modulation of caspase signaling, alterations in apoptotic regulators, and changes in Na⁺ and K⁺ channel dynamics. Among the most promising cyanobactin families for drug development are the cryptophycins. Isolated from the

cyanobacterium *Nostoc* sp. GSV224, cryptophycin is particularly noted for its antiproliferative effects on KB human nasopharyngeal, LoVo human colorectal cancer cells, and cytotoxic against H-125 lung tumor cells³⁶. The anticancer activity arises when the molecule interacts with microtubule-binding proteins, disturbing tubulin dynamics, and thereby inducing apoptosis in cancer cells. Following the discovery of cryptophycin's potent anticancer activity, various analogs have been synthesized to enhance its efficacy against tumor cells. One such analog is cryptophycin 52 (**Figure 1.3**), which advanced to phase II clinical trials and was used to treat non-small-cell lung cancer patients, although it was ultimately withdrawn due to high toxicity at multiple doses.^{37–39} Other notable compound includes curacin, isolated from *Lyngbya majuscula* and characterized by thiazoline and cyclopropyl moieties, and dolastatin 10, a linear pentapeptide isolated from *Dolabella auricularia*, known for its single thiazole ring.⁴⁰ Both compounds show promise in cancer drug development due to their ability to inhibit microtubule polymerization and induce apoptosis.

Another example of a natural product taken to trials is that of dolastatin 10 a high specific and potent compound, however it failed phase II clinical trials due of lack to avoid toxicity and retain efficacy^{41,42} (**Figure 1.3**). Despite these obstacles, ongoing efforts to synthesize analogs of curacin and dolastatin 10⁴⁰ are being explored for future cancer therapeutic applications.⁴²

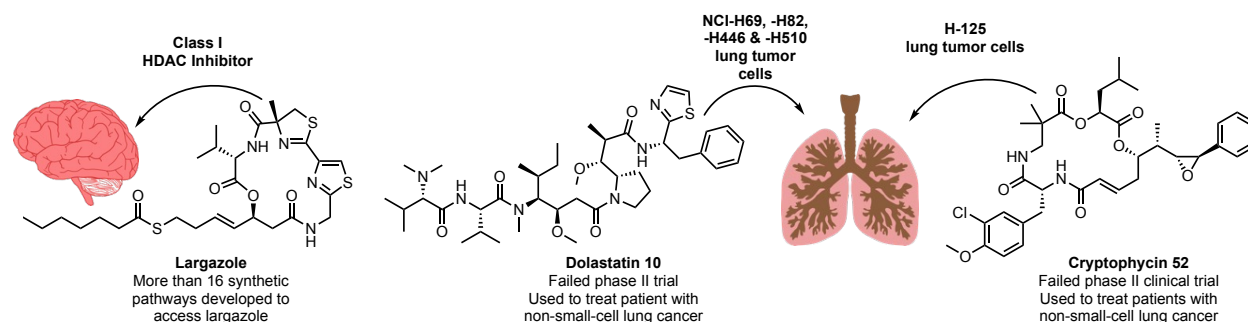


Figure 1.3. Cyanobacterial secondary metabolites with therapeutic potential

From left to right: Largazole, a potent class I histone deacetylase (HDAC) inhibitor; dolastatin 10; and cryptophycin 52, both molecules target lung cancer cell lines.

1.2.2 Cyanobacterial Secondary Metabolites Categories

Cyanobacterial secondary metabolites are categorized into multiple families (**Figure 1.4**), primarily based on their chemical structure and the similarities in their biosynthetic pathway. Janssen and co-workers⁴³ developed a cyanobacterial secondary metabolites data base called CyanoMetDB with more than 3085 compounds. Each compound was classified into a family according to its chemical structure, biosynthesis, and/or cyanobacteria isolation data. This section provides an overview of key cyanobactin families that includes examples of molecules with interesting biological activities, each family main chemical structure, and if a biosynthetic pathway is known.

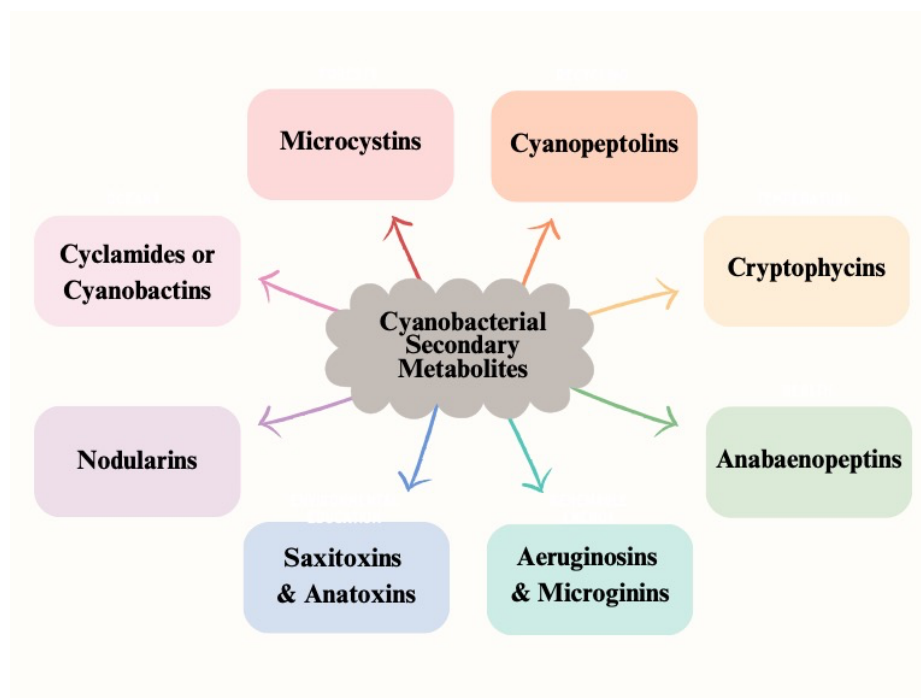


Figure 1.4. List of selected cyanobacterial secondary metabolites

1.2.2.1 Microcystins

Microcystin natural products are among the most extensively studied compounds in environmental science, primarily due to their high concentrations in aquatic cyanobacterial blooms and their potent hepatotoxicity and potential human carcinogenicity, which present significant public health risks. The biosynthetic pathway of this compound family is well established and involves complex mechanisms within the *mcy* gene cluster.⁴⁴ Numerous studies have focused on understanding the role of microcystins in cyanobacteria, linking them to key processes such as carbon and nitrogen metabolism, antioxidation, colony formation, and cell-to-cell communication.⁴⁵ Chemically, microcystins are cyclic heptapeptides that contain non-proteinogenic amino acids, including (all-S,all-E)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA) (**Figure 1.5**, labeled as red) and D-β-methyl-isoaspartate (Figure 6, labeled as blue), which are highly characteristic of this family. The unique chemical structures of microcystins have also attracted the attention of organic synthetic chemists, who have made significant efforts and methodologies toward the ADDA moiety^{46–48} and successful total syntheses of these molecules.¹⁴ These efforts aim to explore additional potential biological activities and develop novel synthetic methodologies to access these natural products. As of today, more than 300 distinct microcystin compounds have been identified.²²

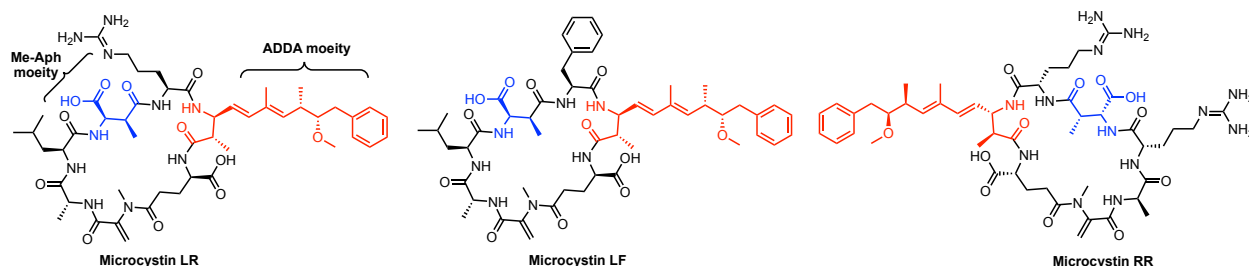


Figure 1.5. Examples of microcystins family

1.2.2.2 Cyanopeptolins

The first cyanopeptolins were isolated and named in 1993 by Martin et al.⁴⁹ Even though their biological activities were not fully elucidated at the time, subsequent research revealed their potential therapeutic applications. For example, micropeptins A-B and micropeptin 90 have demonstrated potent inhibitory effects against human serine proteases, including trypsin and elastase.⁴³ Additionally, microcystilide A has been found to promote cell differentiation in human leukocyte (HL-60) cells and exhibit activity against the HCT116 and HCTV35 cell lines.^{50,51}

The structure of cyanopeptolins is characterized and described as a 19-membered macrocyclic depsipeptide containing a (3*S*)-amino-(6*R*)-hydroxy-2-piperidone (Ahp) moiety (**Figure 1.6**, labeled in red), along with a single *cis*-configuration of an amide-linked amino acid.⁵⁰ This family of natural products are also categorized under the “Ahp-cyclodepsipeptides” molecules⁵² and their biosynthesis is known to be via a non-ribosomal peptide synthetase (NRPS) system.⁵³ To this day, there is a lack of total synthesis achievements toward the family, however, there has been multiple methodologies to access other Ahp-cyclodepsipeptides that can be applied for the total synthesis of cyanopeptolins.⁵²

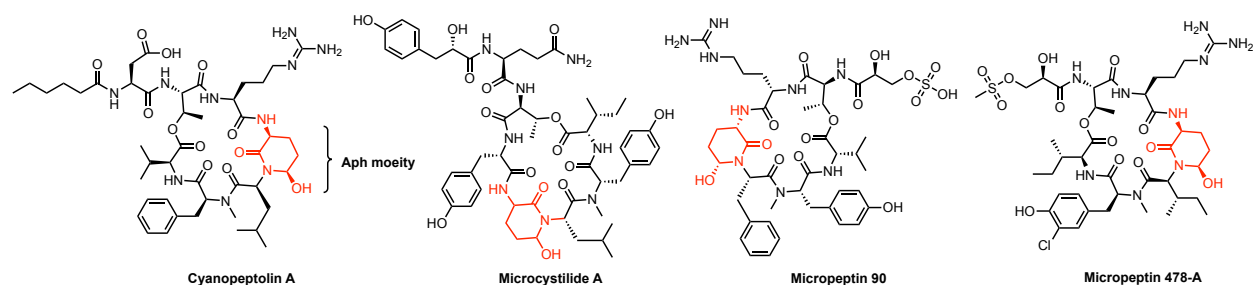


Figure 1.6. Examples of cyanopeptolins family

1.2.2.3 Cryptophycins

The cryptophycins natural products were first discovered and isolated by Schwartz et al.⁵⁴ (1989) from *Nostoc* sp. ATCC 53789. These cyanobacterial secondary metabolites are well known

for their ability to conjugate with drugs and target multi-drug resistant (MDR) cancer cells, due to extensive structure-activity relationship (SAR) studies³⁸ (Weiss et al., 2013). Additionally, as mentioned in section 1.2.1, an anticancer analog of cryptophycin progressed to phase II clinical trials but ultimately failed. The biosynthesis of cryptophycins was elucidated after characterization of metabolic pathway using stable isotope labels, cloning, and sequencing of the gene cluster.⁵⁵ Eight enzymes were also identified throughout the biosynthetic pathway.⁵⁵ Structurally, Weiss and co-workers³⁸ divided the molecule into four main important units based on the retrosynthetic analysis and previous synthetic efforts towards the synthesis of the molecules. Unit A and D consist of hydroxy acids, followed by units B and C containing one amino acid each (**Figure 1.7**). The configuration of the epoxy unit within unit A is worth noting, in that the (R,R) configuration induces a higher bioactivity than the (S,S) configuration.⁵⁶ For this, and other reasons, a lot of synthetic efforts and methodologies toward the synthesis of this unit have been explored.^{56–58} This also includes chemoenzymatic syntheses that allows researchers to access multiple analogs of cryptophycins and to perform a late-stage regio- and stereo-selective epoxy incorporation. For a detailed review of synthetic efforts toward cryptophycins, SAR studies, and biological activities please see Weiss and co-workers review.³⁸

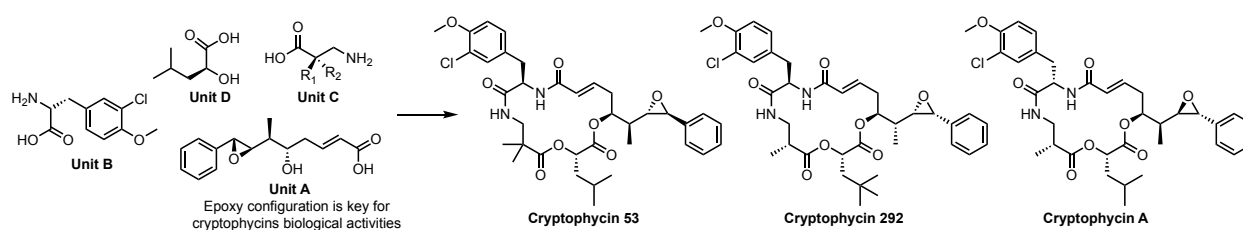


Figure 1.7. Examples of cryptophycins family

1.2.2.4 Anabaenopeptins

Anabaenopeptins are 19-membered ring hexapeptides containing a unique D-lysine moiety (**Figure 1.8**, labeled in red), which is present in all family members, and with additional

proteinogenic and non-proteinogenic amino acids. Furthermore, both anabaenopeptin E and oscillamide C have an exocyclic arginine residue, whereas nodulopeptin 901 has a phenylalanine⁵⁹ (**Figure 1.8**, labeled in blue), of which highlights some of the structural differences in this family. The first anabaenopeptins reported, anabaenopeptins A & B, were isolated by Harada and colleagues⁶⁰, in 1995, and their biosynthesis has been shown to follow the non-ribosomal peptide synthetase pathway. Anabaenopeptins are primarily known for their ability to inhibit multiple proteases, including protein phosphatase 1, trypsin, and leucine aminopeptidase, among others. Due to this protease inhibition, anabaenopeptins have been explored for potential pharmaceutical and biotechnological applications.⁵⁹ However, their protease inhibitory activity is generally less potent than other cyanobacterial secondary metabolites. Synthetic efforts toward anabaenopeptins have been limited, however, recent studies have explored the use of metallaphotoredox catalysis to access the Fmoc-homotyrosine moieties, thus accessing the total synthesis of anabaenopeptins F.¹¹ This study also highlights the potential of metallaphotoredox catalysis to access other variants of anabaenopeptins.

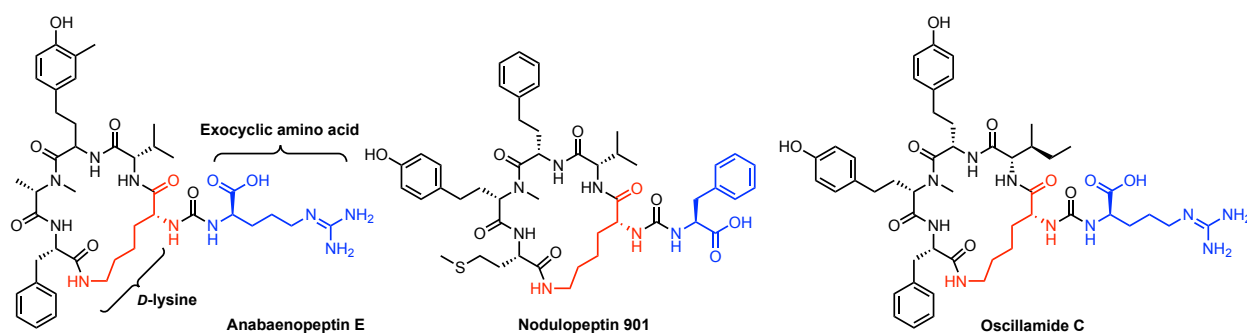


Figure 1.8. Examples of anabaenopeptins family

1.2.2.5 Aeruginosins & Microginins

Linear cyanobacterial secondary metabolites are an important class of natural products within the biosynthetic pathways of cyanobacteria, this includes the aeruginosins and microginins

families. Aeruginosins are known by the presence of the 2-carboxy-6-hydroxy-octahydroindole (Choi) moiety (**Figure 1.9**, labeled as red) in the tetrapeptide core of the molecule. The first aeruginosin isolated was aeruginosins 298A by Mirakami and co-workers⁶¹, in 1994. Since then, numerous distinct aeruginosins have been isolated, and multiple total syntheses reported.^{62–64} Aeruginosins are recognized for their potent inhibition of serine proteases, including thrombin and trypsin.^{65,66} The inhibition mechanism has been elucidated through X-ray crystallography of the aeruginosin-protease complex. For most of the aeruginosins, the biosynthetic pathway is well understood and established.⁶²

On the other hand, microginins are also linear peptides found in cyanobacterial blooms, known for their diverse biological activities. These secondary metabolites are characterized by a core structure of 3-amino-2-hydroxy-decanoic acid (Ahda) (**Figure 1.9**, labeled in blue) at the N-terminus in addition with N-methylations and a 3-6 amino acids peptide chain. Their biosynthesis follows a hybrid pathway that combines non-ribosomal peptide synthetase and polyketide synthase mechanisms.⁶⁷ The biological activities are well known, particularly their ability to inhibit angiotensin-converting enzyme⁶⁷ (ACE) and aminopeptidases such as leucine aminopeptidase N and M.⁶⁸ Due to the inhibitory properties, microginins are being considered as potential therapeutics for hypertension as antihypertensive agents.⁶⁹ Recent studies have also focused on the potential of microginins to target hepatocellular carcinoma.⁷⁰

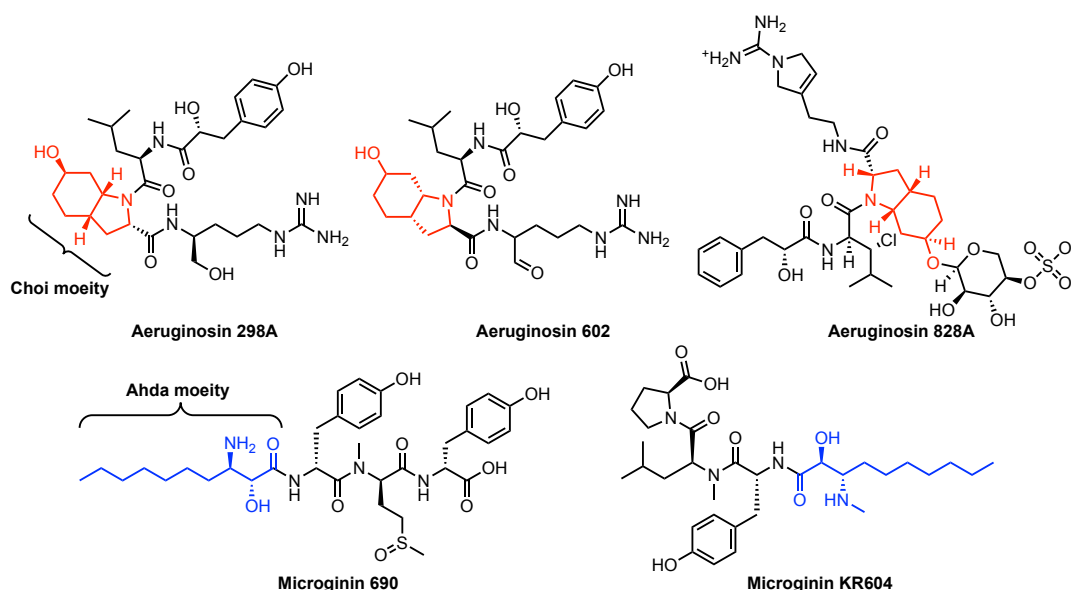


Figure 1.9. Examples of aeruginosins and microginins families

1.2.2.6 Saxitoxins & Anatoxins

Saxitoxins, also known as paralytic shellfish toxins, are a family of cyanobacterial secondary metabolites that have been intensively studied due to their potential to cause severe human poisoning, including numbness, respiratory paralysis, and death.⁷¹ They also present significant environmental risks, and recent reports have shown elevated concentrations of these toxins in freshwaters bodies.⁷¹ Efforts to treat contaminated waters and detect metabolites are ongoing, with the goal of ensuring toxin-free drinking water and controlling the proliferation of these compounds.^{71,72} The potent biological activity of saxitoxins arises from their ability to inhibit voltage-gated sodium channels, thereby inducing neuronal excitation and transmission.⁷³ Structurally, these molecules feature a 5-6-5 tricyclic skeleton that includes two guanidino groups within the rings (**Figure 1.10**). The biosynthetic pathway leading to this core structure has been extensively studied in multiple cyanobacterial species,⁷⁴ bioinspired synthetic approaches to replicate the saxitoxin core have also been successfully developed⁷⁵ and a stereocontrol synthesis have been achieved.^{12,76}

On the other hand, anatoxins are neurotoxins alkaloids also found in cyanobacterial blooms. Anatoxins induce depolarization of the cell membrane via inhibition of the nicotinic acetylcholine receptor, thus leading to neuromuscular dysfunction and asphyxia.⁷⁷ The structure of anatoxins closely resembles the core structure of cocaine, featuring 2-acetyl-9-azabicyclo[4.2.1]non-2-ene (**Figure 1.10**). The synthesis of these molecules has been successfully achieved both through the use of cocaine as starting material and through alternative synthetic pathways.^{78,79} Additionally, the biosynthetic pathway for anatoxins in cyanobacteria is well established and documented.⁷⁷

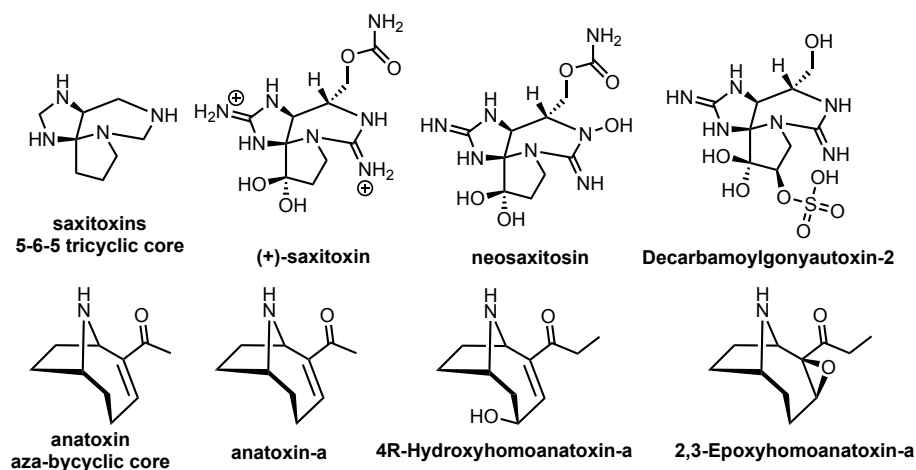


Figure 1.10. Examples of saxitoxins and anatoxins families

1.2.2.7 Cyanobactins: The Cyclamides

The last group of cyanobacterial secondary metabolites are the cyanobactins. This family of natural products are both linear^{80,81} and cyclic peptides isolated mostly from cyanobacteria (fewer from marine animals like sponges, ascidians, and mollusks) that are categorized under one of the sub-classes of the ribosomally synthesized and post-translationally modified peptides⁸² (RiPPs). The categorization of these molecules under the term “cyanobactins” is based on their biosynthetic similarities and not their chemical structures.⁸³ However, multiple common cores and

chemical transformations can be observed within cyanobactins that also make them similar and allow researchers to categorize them even further. Additionally, they can also be classified by genotypes and chemotypes as proposed by Donia & Schmidt.⁸⁴ Cyanobactins contain only proteinogenic *L*-amino acids^{83,85} and rarely *D*-amino acids coming from potential spontaneous epimerization.⁸⁶ Multiple families of cyanobactins could be characterized because of their azolines and azoles moieties that include thiazolines, thiazoles, oxazolines, and oxazoles (**Figure 1.11A**). Amino acids like threonine, cysteine, and serine can undergo heterocyclization via heterocyclases^{87–89} to form thiazolines and oxazolines; however, oxidases^{90,91} can achieve thiazoles and oxazoles. This is true for molecules that contain around six to eleven amino acids; however, cyanobactins' peptide core that lacks heterocycles can be also biosynthesized by cyanobacteria and they tend to be from seven to twenty amino acids in length.⁸³ On the other hand, sporadic prenylation or geranylation can also occur in cyanobactins containing amino acids like tryptophan, tyrosine, threonine, and serine⁹² and *N*- or *O*- methylations can be observed.⁹³

Since the first cyanobactin isolations, ulicyclamide and ulithiacyclamide in 1980 by Ireland & Scheuer⁹⁴, these natural products have been highly investigated due to their bioactivities such as antibacterial, antiparasitic, antifungal, and anticancer activities^{83,95} (**Figure 1.12**). Furthermore, natural and synthetically modified cyanobactins have shown metal chelation with Cu^{+2} , Zn^{+2} , Ca^{+2} , K^{+1} , Ag^{+1} , Fe^{+3} , and Cr^{+3} and multidrug resistance reversing abilities.⁹⁶ The metal chelation, and molecule shape (triangle – westiellamide, square – ascidiacyclamide, type II β -turn – lissoclinamide 7, and figure eight – patellamide D)^{97–102} (**Figure 1.11B**) are highly dependent on the stereocenters of the amino acids present in the molecule. Moreover, stereocenters seem to play an important role in synthetic pathways to perform macrocyclizations to access the total synthesis of these compounds.¹⁰³ Additionally, understanding the absolute configuration of

molecules represents a critical factor for drug discovery and development due the multiple differences in their biological properties depending on the stereocenters configuration.^{104,105} For example, differences in pharmacological, pharmacokinetics, toxicological, and metabolism activities can be observed from one enantiomer to another.¹⁰⁴ Thus, efforts toward understanding cyanobactin conformations, including reassignments of configurations after total syntheses and biosynthetic stereocenter formations, have been exhaustively explored.^{106–108}

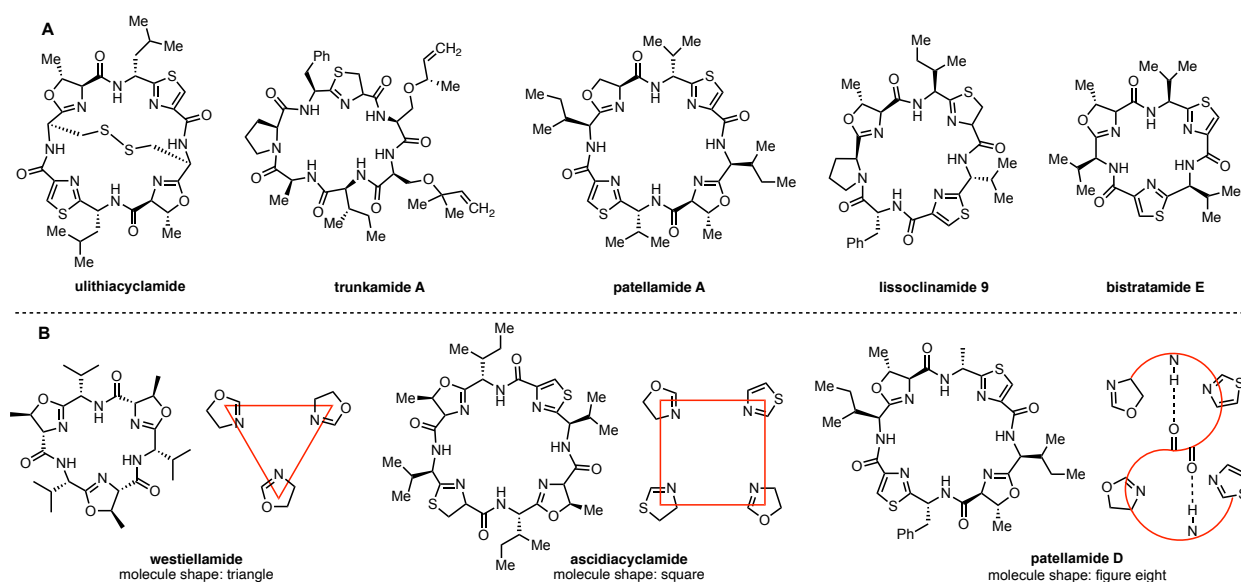


Figure 1.11. Cyanobactin secondary metabolites

(A) Examples of cyclic cyanobactin natural products containing heterocycle rings (ulithiacyclamide, trunkamide A, patellamide A, lissoclinamide 9, and bistratamide E) (B) Conformation of westiellamide, ascidiacyclamide, and patellamide D.

As previously mentioned, certain cyanobactins can be further classified based on their chemical core and/or biosynthetic pathway. This includes the cyclamides subdivision that consist primarily of macrocyclic cyanobactins containing 5-7 proteinogenic amino acids, and one to three combinations of oxazolines, oxazoles, thiazolines, and/or thiazoles (**Figure 1.12**). These compounds may adopt a triangular shape due to their planarity when three heterocycles are

present.^{97,98} Additionally, some of these molecules are depsipeptides. Biologically, cyclamides exhibit the general properties associated with cyanobactins, and multiple biosynthetic pathways have been elucidated^{83,109} (Figure 1.12).

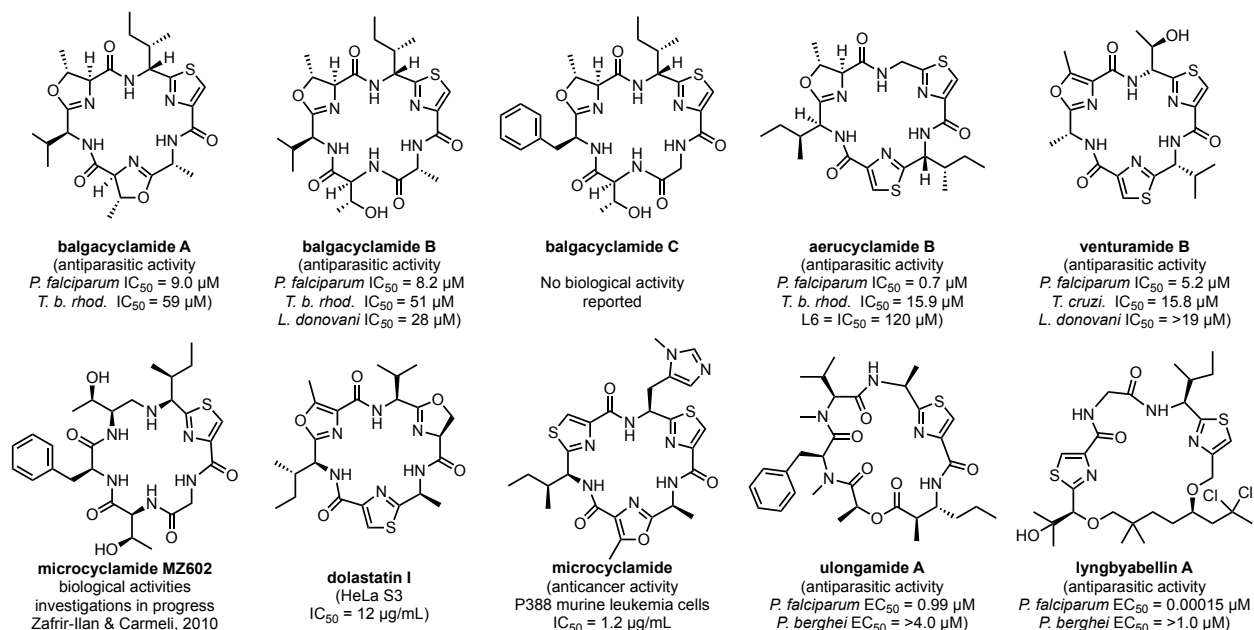


Figure 1.12. Example of cyclamide secondary metabolites

Structure and biological activities of some cyclamides that includes a combination of azolines and azoles, 5-6 amino acids, and polyketide moieties.

In this dissertation, the first total synthesis of the balgacyclamide A & B and synthetic efforts towards balgacyclamide C will be discussed in chapter 3. This will include multiple stereocontrol methodologies for achieving the correct configuration of oxazoline synthesis, as well as thiazole cyclization, oxidation, and macrocyclization efforts.

1.3 Cyclamides: The Balgacyclamides Family

Balgacyclamide family of natural products are macrocyclic hexapeptides isolated from cyanobacteria *Microcystis aeruginosa* EAWAG 251 by Gademann and co-workers¹¹⁰ in 2014. The

family consist of three molecules, balgacyclamide A (1), B (2), and C (3) that share similar chemical structures and are categorized under the cyclamides group of cyanobacterial secondary metabolites (**Figure 1.13**). The northern fragment of the balgacyclamide family shares an oxazoline (ring A) and a thiazole (ring B) (**Figure 1.13**). However, balgacyclamides' A and B northern oxazoline arrives from L-threonine and L-valine, in contrast, balgacyclamide C oxazoline contains L-threonine and L-phenylalanine. On the other hand, the lower fragments from each molecule are different. Balgacyclamide A lower fragment undergoes post-translational cyclization¹¹¹ to access an extra oxazoline (ring C); however, balgacyclamides B and C do not undergo this transformation leaving a free alcohol in the molecule. Finally, balgacyclamide B lower fragment arise from L-threonine and D-alanine (D) but balgacyclamide C from L-threonine and glycine (E). The biological activities of the balgacyclamide family were explored by Gademann and co-workers once they isolated the molecules. Balgacyclamide A and B are active against *Plasmodium falciparum* chloroquine-resistant strain K1 and both show moderate toxicity towards *Trypanosoma brucei rhodesiense* STIB 900. In addition, balgacyclamide B possess toxicity against *Leishmania donovani* MHOM-ET-67/L82. Interestingly, these results are consistent with previous biological activities of cyanobactins with similar balgacyclamide structures like venturamides A and B¹¹² and aerucyclamides A, B, C, and D^{15,113} (**Figure 1.12**). Currently, the biological activity of balgacyclamide C is unknown.

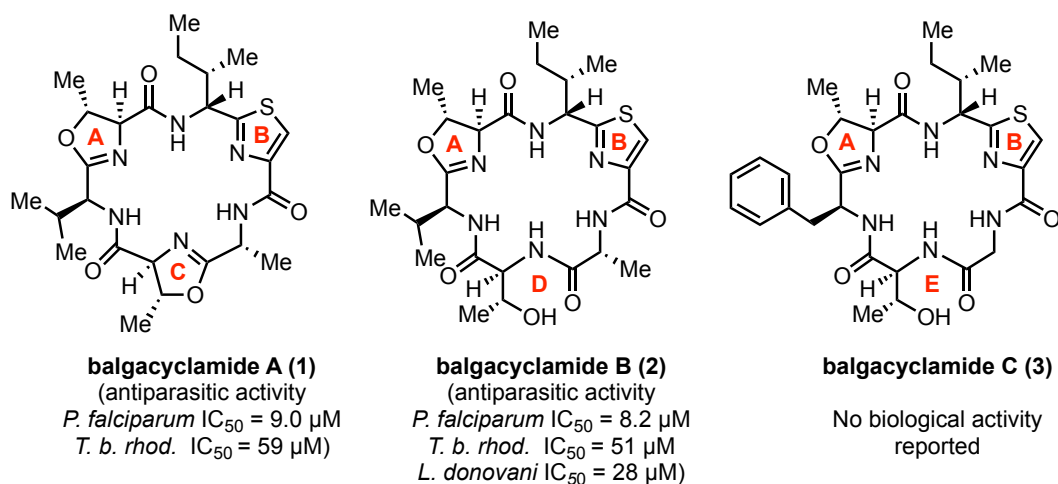


Figure 1.13. Structures and biological activities of the balgacyclamide family

Chapter 2 - Advancements in the Total Synthesis of Hexacyclamides: Aerucyclamides, Bistratamides, Dendroamides, and Dolastatins (1994-2024)

2.1 Introduction

This review provides an overview of several total syntheses of hexacyclamides, including aerucyclamides, bistratamides, dendroamides, and dolastatins. It highlights multiple methodologies for synthesizing azole/azoline moieties, and the conditions applied to macrolactamizations. In the Cyanobacterial Secondary Metabolites Database (CyanoMetDB), Janssen and co-workers²² have classified a total of 86 molecules within the cyclamide class of compounds. Of these, 58 are hexacyclic peptides containing 1 to 3 azole/azoline units; moreover, all are non-depsipeptides. After an extensive database search, it was found that 28 of these 58 molecules have been synthesized. However, due to the broad variation of methodologies employed in the total syntheses of these compounds, 45 syntheses have been reported, including those for balgacyclamide A and B, which will be discussed in chapter 3. A selection criterion was applied to the 45 syntheses to include in this chapter. First, the molecules must be structurally similar to the balgacyclamide family, which is the primary focus of this dissertation, specifically hexacyclic peptides with 2 to 3 azole/azoline units. Second, the molecules must be non-depsipeptides, and third, balgacyclamides are excluded since they will be addressed in chapter 3. As a result, the 45 total syntheses are reduced to 39. Of these, 27 will be discussed in detail.

Synthetically, multiple methodologies to access thiazoles/thiazolines and oxazoles/oxazolines have been applied to develop an accessible synthetic route towards these cyclamides and each one will be discussed under their respective cyclamide synthesis application. These methodologies include,

- A chemo- and enantioselective cyclodehydration of β -hydroxythioamides to access thiazoles through a thiazoline intermediate, thus facilitating the synthesis of both heterocycles.
- Synthesis of oxazoles and thiazoles via dehydro-oligopeptides, eliminating the need for condensing reagents, except in macrocyclization reactions.
- Synthesis of thiazoles through thiolysis of oxazoline intermediates.
- Several modified Hantzsch thiazole syntheses.
- An improved purification process by incorporating fluororous protecting groups into synthetic intermediates, enabling the removal of byproducts and smelling side products via liquid-liquid or solid-liquid extractions.
- A mixture of fluororous-protected enantiomers, allowing for straightforward separation using fluororous HPLC (f-HPLC), providing access to more than eight stereoisomers of any cyclamide and a synthetic pathway with reduced steps.
- A one-pot intramolecular Staudinger-aza-Wittig oxidation reaction.
- Non-controlled and controlled cyclo-oligomerizations utilizing multiple metals and pentafluorophenyl diphenylphosphinate.

The total syntheses are presented in alphabetical order, starting with the aerucyclamides (1 synthesis), followed by bistratamides (16 syntheses), dendroamides (8 syntheses), and dolastatins (2 syntheses) families.

2.2 The Aerucyclamides Family

The aerucyclamide family of natural products was first isolated by Gademann and co-workers from *Microcystis aeruginosa* PCC 7806 in 2008.^{15,113} A total of four molecules were identified, each possessing a chemical structure characterized by a combination of three heterocyclic rings,

including oxazoles/oxazolines and thiazole/thiazolines (**Figure 2.1**). A key feature of the aerucyclamides A-C is the presence of D-*allo*isoleucine amino acids, whereas aerucyclamide D contains a L-methionine residue. To date, only the total synthesis of aerucyclamide B has been successfully achieved by Serra and co-workers.

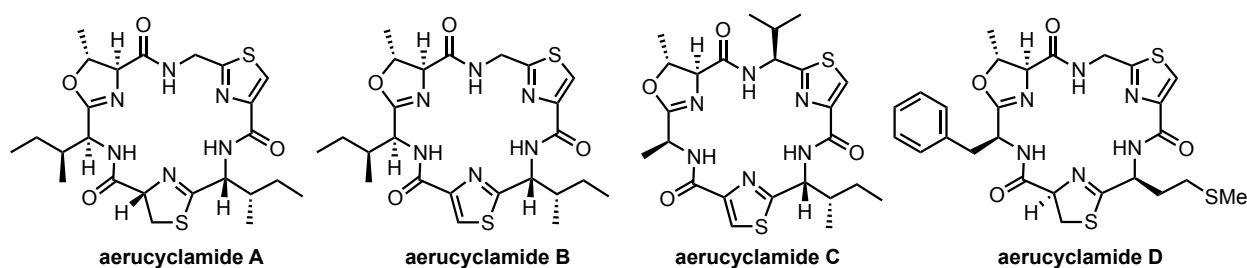


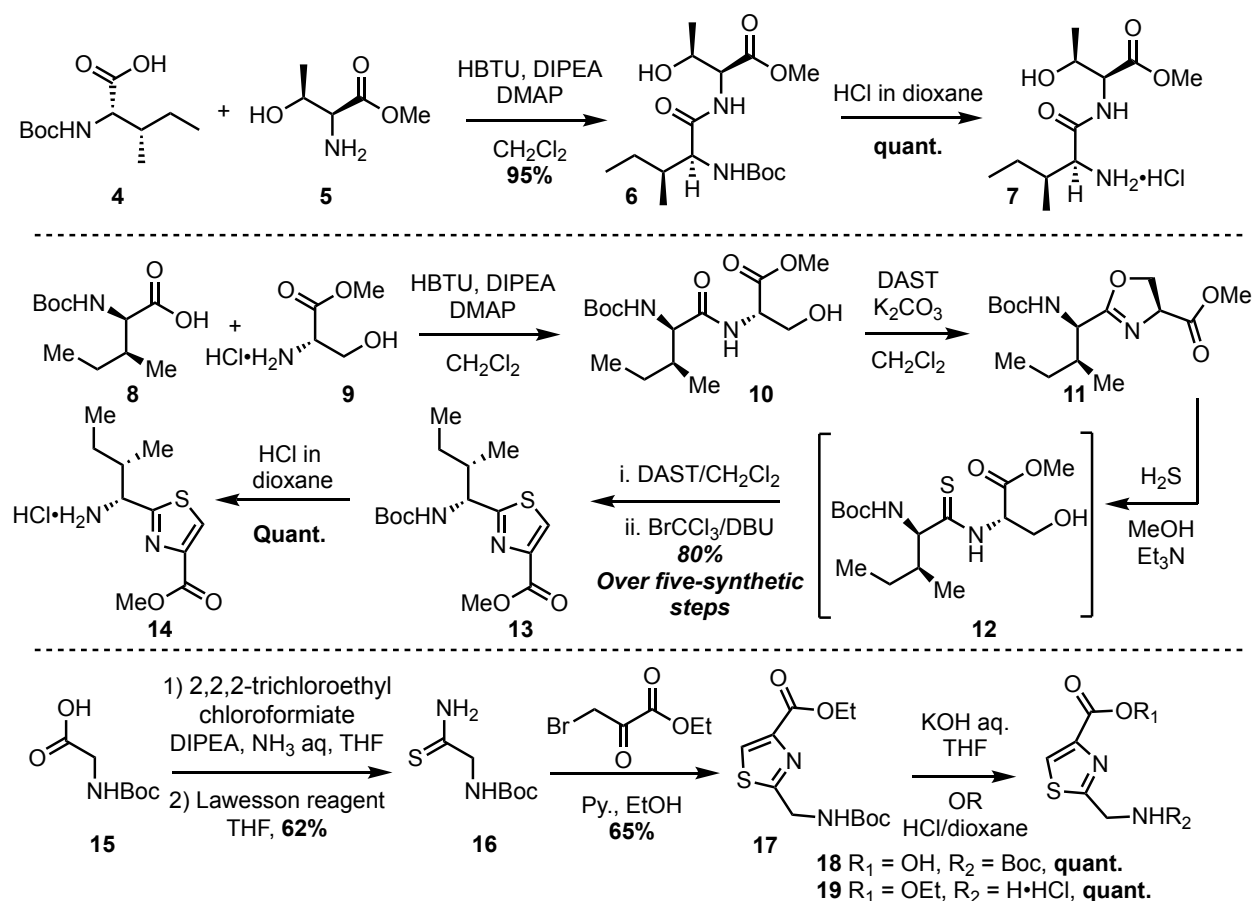
Figure 2.1. Chemical structures of the aerucyclamide family

2.2.1 Serra's Total Synthesis of Aerucyclamide B (2013)

The first total synthesis of aerucyclamide B was achieved by Serra and co-workers in 2013.¹¹⁴ As part of their synthetic efforts toward aerucyclamide B, multiple analogs of the hexacyclic peptide were synthesized by the same group in 2012.^{115,116} The proposed synthetic routes toward aerucyclamide B highlight the significance of the head-to-tail macrolactamization strategy for these cyclic peptides, in which turn-inducing thiazole-constrained peptides play a potential role in enhancing ring closure to form low-energy cyclic products. Additionally, Sierra and co-workers were the first to report a fluorine derivative side product from their attempts to access the oxazoline moiety of aerucyclamide by using deoxy-fluor through β -hydroxyamide cyclodehydration. Furthermore, the synthesis of one thiazole was achieved via thiolysis of an oxazoline intermediate, a methodology developed by Wipf and co-workers,¹¹⁷ which induced the formation of a thioamide, followed by dehydrocyclization and radical oxidation.

The coupling of β -hydroxy amide **6** and bis-thiazole **20** was envisioned as a strategy to access the macrocyclic hexapeptide, followed by a late-stage cyclodehydration to achieve aerucyclamide

B. First, dipeptide **6** was synthesized under standard peptide coupling conditions in 95% yield (**Scheme 2.1**). Next, the synthesis of the (D-*allo*-Iso)thiazole moiety **14** started with a successful coupling of Boc-D-*allo*-isoleucine-OH (**8**) and L-serine methyl ester (**9**), followed by β -hydroxy amide dehydrocyclization to form oxazoline **11**. Thiolytic cyclization of **11** provided the thioamide intermediate **12**, which was further cyclized and oxidized to achieve **13** in 80% overall yield over five steps. Finally, the third fragment was synthesized via amidation, followed by thioamidation, and the Hantzsch reaction to yield (Gly)thiazole **17** in 40% overall yield over three steps.

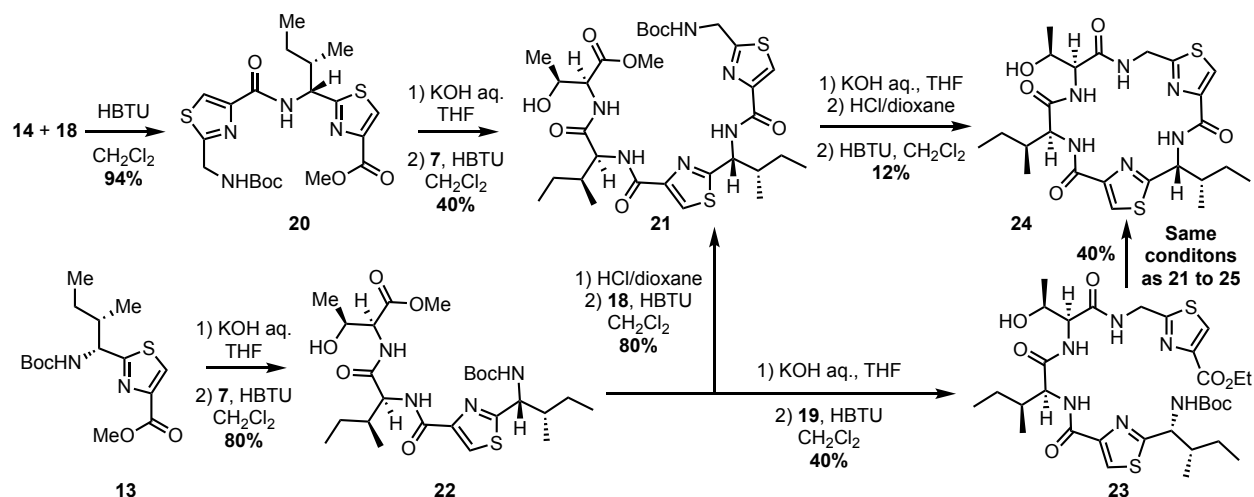


Scheme 2.1. Synthesis towards the three fragments of aerucyclamide B

After obtaining the three main fragments, Serra wanted to explore the significance of head-to-tail macrocyclizations at two distinct points. The assembly of the three fragments began with

the deprotection of the corresponding amine or methyl/ethyl ester to access **7**, **18**, and **19** (**Scheme 2.1**). The thiazoles **14** and **18** were then coupled using HBTU to afford bis-thiazole **20** in 94% yield, which was subsequently saponified and coupled to free amine of dipeptide **7** to furnish hexapeptide **21** in 40% yield (**Scheme 2.2**). It is worth noting that hexapeptide **21** was also synthesized via an alternative pathway, as shown in **scheme 2.2**, where tetrapeptide **23**, accessed from the coupling of the carboxylic acid version of **13** to **14** in 80% yield, was coupled with (Gly)-thiazole (**18**), to accomplish **21** in 80% yield. With hexapeptide **21** in hand investigations for head-to-tail cyclization between *L-allo*-threonine methyl ester and (Gly)thiazole termini could be undertaken. Due to the lack of chirality in glycine (the N-terminus), there were no concerns about epimerization, and steric hindrance obstacles were avoided.

In contrast, the second hexapeptide **23** was assembled by coupling the saponified version of **22** to the (Gly)thiazole free amine (**19**) in 40% yield. With **23** in hand, the team explored the head-to-tail cyclization of (*D-allo*-Ile)-thiazole and the (Gly)thiazole ethyl ester termini, where there was also no potential for epimerization. The hexapeptides **21** and **23** were then subjected to the same series of deprotections (aqueous KOH - HCl in dioxane) and couplings using HBTU. As a result, macrocyclization of **21** and **23** led to the formation of macrocyclic **24** in 12% and 40% yields, respectively. Serra and co-workers concluded that the choice of macrolactamization points is crucial for the synthesis of aerucyclamides, and that a potential turn-inducing thiazole constraint may play a role in enhancing cyclization.

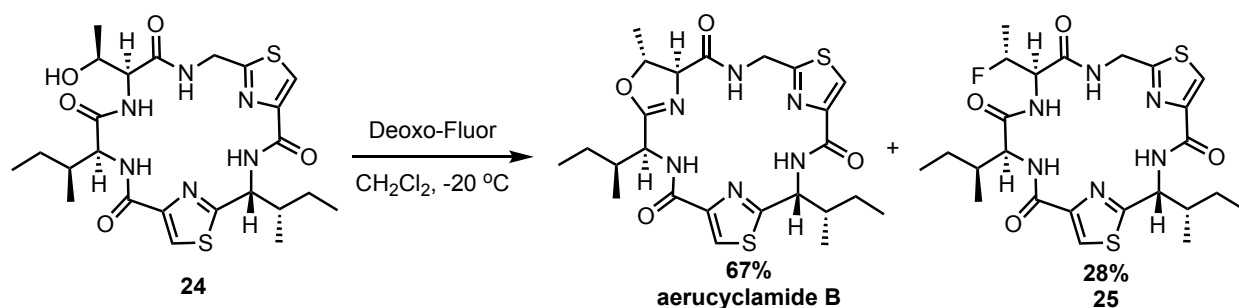


Scheme 2.2. Efforts toward macrolactamization via two hexapeptide precursors

It is important to note that the late-stage formation of the oxazoline was chosen due to the challenges of deprotecting oxazolines under both acidic and basic conditions, as well as the difficulties associated with macrocyclizations when the oxazoline is one of the termini for ring closure. Multiple synthetic researchers have investigated oxazoline deprotections, often facing ring-opening reactions and multiple decomposition products under acidic conditions (as seen in the work of Meyers et al. for the synthesis of bistratamide D)¹³ and under basic conditions (as observed in Serra's synthesis of aerucyclamide B).¹¹⁴ On the other hand, macrocyclizations involving oxazolines as termini have generally resulted in very low yields, likely due to steric hindrance within the oxazoline ring, as in the cases of bistratamide D¹³ and bistratamide F,¹¹⁸ further discussed in this report.

Finally, the late-stage β -hydroxy amide cyclodehydration was employed to access the final oxazoline fragment of aerucyclamide B (**Scheme 2.3**). The use of deoxo-fluor to induce epimerization of L-*allo*-threonine led to the first total synthesis of aerucyclamide B. However, a fluorinated derivative was also obtained **25**. Serra suggested that there might be a potential elimination competition between the fluoride ion and the β -hydroxy amide, because of loss of

nucleophilicity in the amide due to hydrogen bond formation in the conformation of hexapeptide **24** could contribute to this result.



Scheme 2.3. Late-stage oxazoline ring formation via deoxo-fluor induced cyclodehydration

2.3 The Bistratamides Family

The bistratamides A-N, a family of cyanobactins, are macrocyclic hexapeptides composed entirely of L-amino acids and are characterized by the presence of 1 to 3 L-valine substituted heterocycles, except for bistratamides M and N (**Figure 2.2**). Additionally, bistratamide I and J are the only in the family that contains one oxazole, one thiazole, and a β -hydroxyamide moiety. All members of the bistratamide family were isolated from the marine ascidian *Lissoclinum bistratum*, starting with the first isolation of bistratamides A and B by Hawkins and co-workers¹¹⁹ in 1988, followed by Ireland and co-workers,¹²⁰ who isolated bistratamides C and D in 1992. Subsequently, Faulkner and Perez¹²¹ isolated and characterized bistratamides E-J in 2002, and finally, in 2017, Jiménez and co-workers¹²² isolated bistratamides K-N. The first total synthesis of a bistratamide was achieved in 1994 by Meyers and Aguilar,¹²³ and further synthetic efforts were explored from 2005 to 2008, in which most family members were synthetically completed. However, it was not until 2024 that new efforts towards the synthesis of this family were re-explored to apply new and more efficient methodologies to access the thiazole rings. To date, a

total of 16 successful total syntheses of bistratamides have been reported, with the exceptions of bistratamides A, B, and K-N.

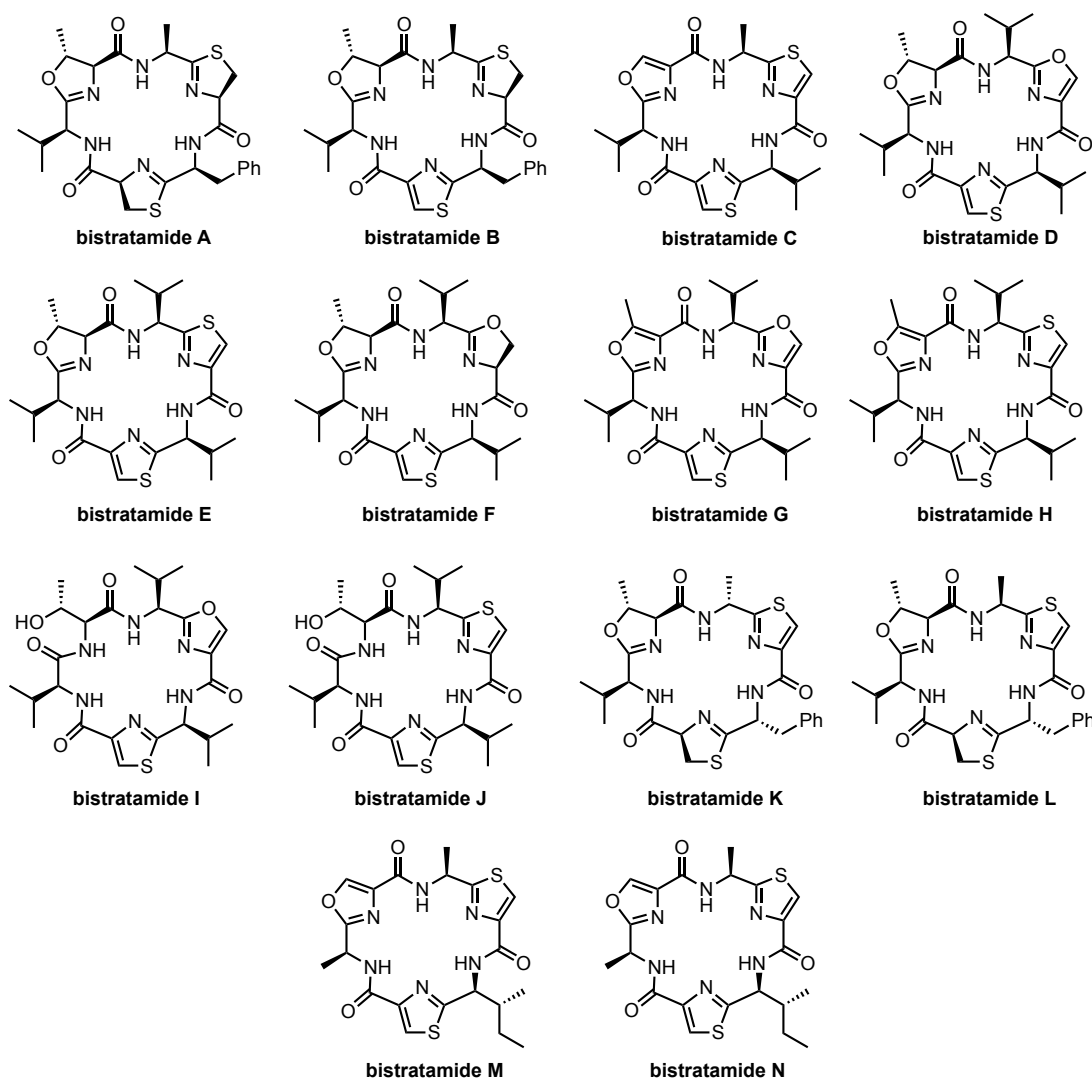
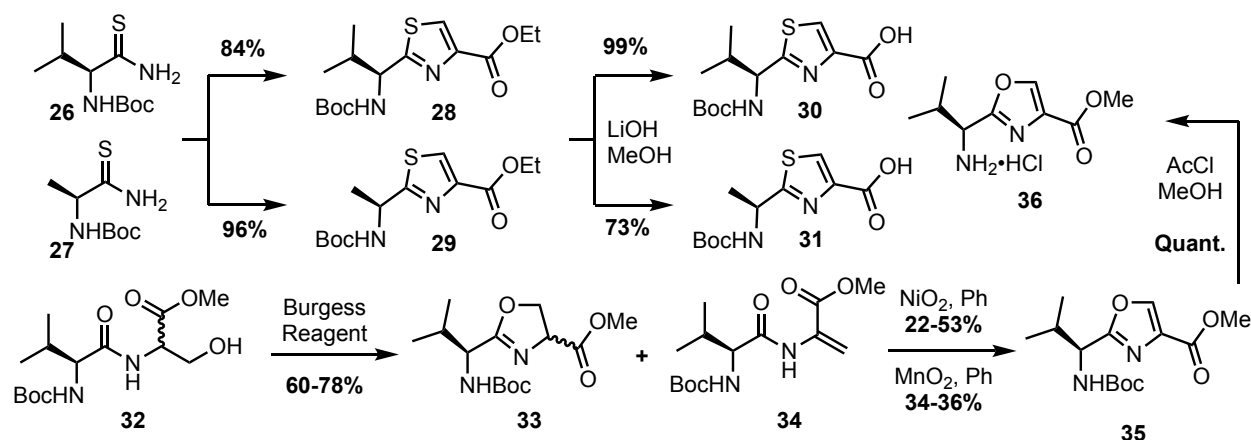


Figure 2.2. Chemical structures of the bistratamide family

2.3.1 Meyers' Total Synthesis of Bistratamide C & D (1994 & 1999)

The total syntheses of bistratamides C and D were successfully achieved by Meyer et al. in 1994¹²³ and 1999¹³, respectively. Bistratamides C and D were the first molecules accessed from this family. Meyers and co-workers began with the synthesis of oxazole **35**, that was successfully formed via β -hydroxy cyclization of dipeptide (**32**) using the Burgess reagent, resulting in a

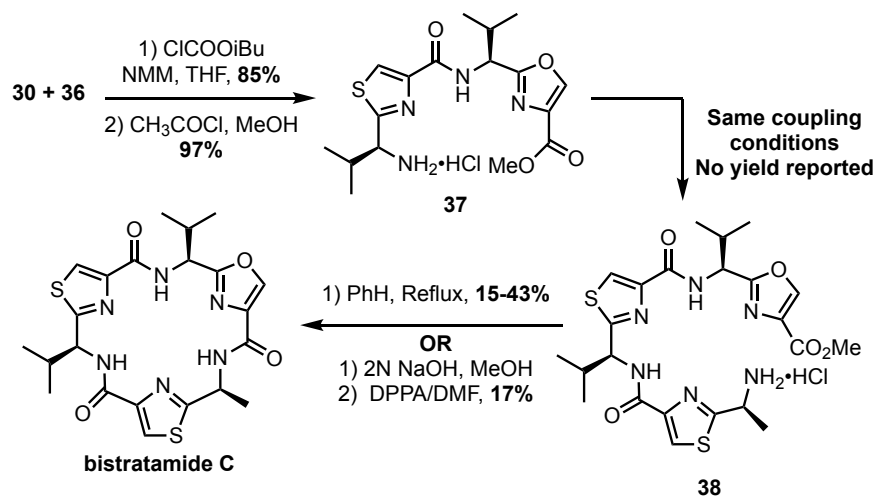
racemic mixture of oxazoline **33** with yields ranging from 60% to 78% (**Scheme 2.4**). However, a small amount of dehydrated dipeptide **34** was also observed. Additionally, an alternative procedure to access **33** was attempted using Vorbruggen's conditions¹²⁴ (not shown), in which a one-pot synthesis of **33** was achieved by directly coupling Boc-valine and serine methyl ester, followed by dehydration and cyclization. However, Vorbruggen's conditions failed to produce **33** in good yields (22%), with enamide **34** obtained in a higher yield (47%). With **33** in hand aromatization to oxazole **35** was accomplished via NiO₂/benzene or MnO₂/benzene in moderate yields ranging from 22-53% and 34-36%, respectively. Construction of the two thiazole units **30** and **31** commenced from their corresponding valine and alanine thioamides, respectively, via Hantzsch conditions in good to superior yields.



Scheme 2.4. Synthesis towards the three fragments of bistratamide C

With the three main core fragments in hand, **30** and **36** were coupled via a mixed anhydride of **30** in 85% yield, after which Boc deprotection was performed under acid conditions to access **37** in 97% yield. Employing the same set of conditions, **37** was coupled to **31** to access hexapeptide **38** in relatively good yields (**Scheme 2.5**). Finally, macrocyclization was performed through aminolysis under toluene reflux for 2 days to successfully achieve bistratamide C in yields between 15% to 43%. An alternative approach for macrocyclization involved the use of

diphenylphosphorylazide (DPPA) to form an acyl azide, followed by the formation of bistratamide C in 17% yield.

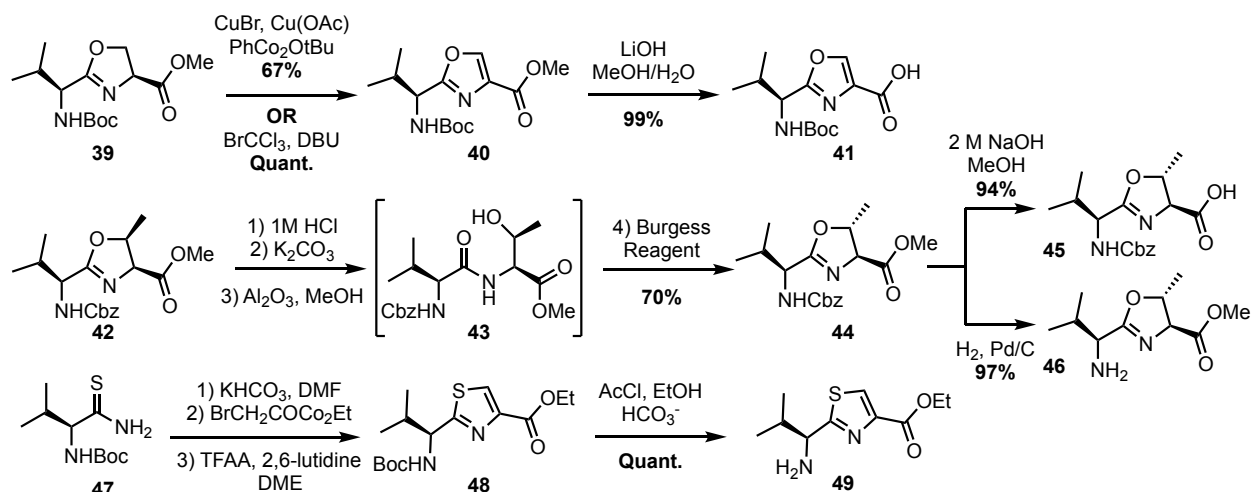


Scheme 2.5. Final steps towards total synthesis of bistratamide C

After the synthesis of bistratamide C, Meyers et al. explored multiple pathways to achieve the first total synthesis of bistratamide D.¹³ Key steps in the synthetic strategies included the synthesis of a trans-4,5-disubstituted oxazoline via a Walden inversion, a new developed and improved radical oxidation step that provided better reproducibility compared to metals such as NiO_2 and MnO_2 , and macrocyclization efforts using standard peptide coupling conditions with HATU.

Synthetic efforts toward bistratamide D commenced with the formation of oxazole **41** (**Scheme 2.6**). Initially, a new methodology developed by Meyers, using CuBr , $\text{Cu}(\text{OAc})_2$, and PhCO_2OtBu in benzene, induced radical oxidation to access **40** from **39** in 67% yield. This approach avoided epimerization of the neighboring valine α -carbon and provided a more reproducible procedure for the synthesis of oxazoles. Alternatively, a procedure reported by William and co-workers,¹²⁵ by incorporating BrCCl_3 and DBU, accessed **41** in quantitative yields. In this way, the use of metals in oxidation reactions for the aromatization of oxazolines was

avoided. Lastly, saponification of **40** with LiOH afforded **41** in 99% yield. The synthesis of Cbz-trans-4,5-disubstituted oxazoline (**44**) was successfully achieved from its epimer **42**. Here, previously reported epimerization methodologies by Wipf were applied,^{126,127} including ring-opening of **42** with 1 M HCl and K₂CO₃, followed by the addition of Al₂O₃ in MeOH to obtain dipeptide **43** with an inverted configuration (L-*allo*-threonine). This dipeptide was then subjected to epimerization at the β-carbon of threonine using the Burgess reagent to accomplish Cbz-trans-oxazoline **44** in 70% yield. This procedure allows synthetic chemists to avoid the use of non-proteinogenic amino acids in synthetic routes. The free amine **46** of trans-oxazoline **44** was obtained through catalytic dehydration in 97% yield. Saponification of **44** afforded **45** in 94% yield. Finally, the synthesis of the third and final fragment, thiazole **48**, was accomplished in quantitative yields via a modified Hantzsch reaction, followed by Boc deprotection under acidic conditions to access **49** in near quantitative yields.

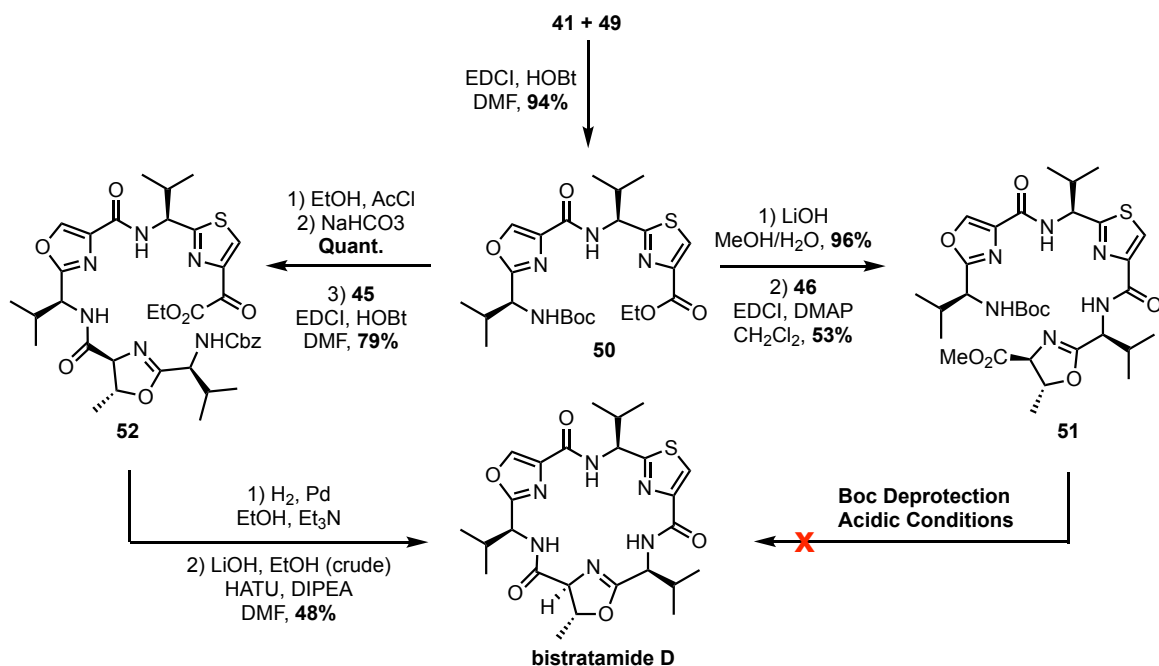


Scheme 2.6. Synthesis towards the three fragments of bistratamide D

With the core components in hand, Meyers et al. attempted to couple these fragments using a mixed acyl carbonate method, similar to those applied in the synthesis of bistratamide C;¹²³ however, the yields ranged from moderate to low. Next, they activated the carboxylic acid of **41**

using EDCI and HOBt, successfully coupled it to **49** giving access to the oxazole-thiazole tetrapeptide **50** with 94% yield (**Scheme 2.7**). To proceed towards the macrolactamization, Meyers explored two potential sites for ring closure. The tetrapeptide **50** was saponified in 96% yield and then coupled with trans-oxazoline **46** under the same conditions accessing **50** to afford hexapeptide **51** in 53% yield via standard peptide coupling conditions. However, when Boc cleavage from **51** was performed under acidic conditions, ring opening of the oxazoline occurred. This ring opening was due to the acid-sensitive nature of the oxazoline ring, once the imine was protonated under acidic conditions, the presence of water potentially induced a nucleophilic attack on the imine carbon, resulting in ring opening and β -hydroxyamide formation. Consequently, the incorporation of the oxazoline moiety was a late-stage step.

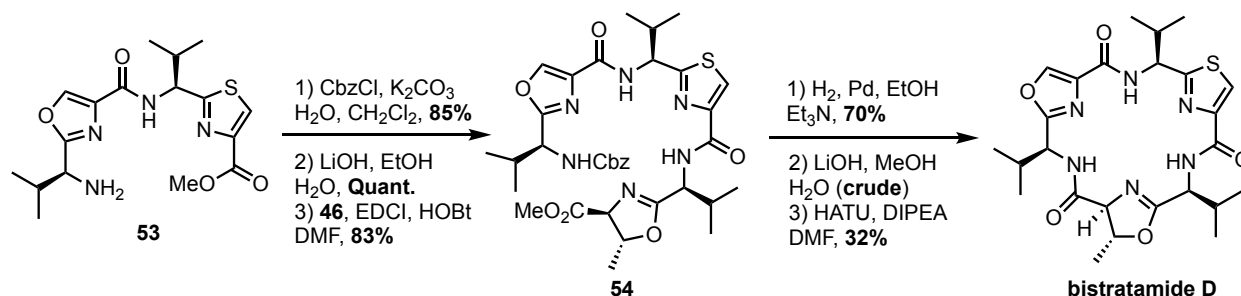
To by-pass the oxazoline ring opening due to acidic conditions for Boc group removal, they decided to Boc deprotect **50** in quantitative yields and couple it to Cbz-trans-oxazoline **45** under the same previously used coupling conditions to access Cbz-hexapeptide **52** in 79% yield. The thought was by accessing **52** possessing a Cbz group, its removal under non-acidic condition would suppress the oxazoline ring opening. Subjecting **52** to Pd/C 10%, for expected Cbz deprotection, did afford the desired free amine but in extremely low yields due to sulfur's ability to poison palladium.¹³ To overcome this issue, a more active catalyst, Pd black, was employed under EtOH/Et₃N and higher pressure (100 psi), resulting in a successful reaction to access the free amine of **52**, which was then saponified to access the free carboxylic acid. The deprotected version of **52** was subjected to macrocyclization conditions using HATU and DIPEA to achieve the first total synthesis of bistratamide D in 48% yield.



Scheme 2.7. Final steps towards total synthesis of bistratamide D and challenges of Boc deprotection under acidic conditions due to presence of oxazoline ring

While Meyers was able to access bistratamide D, in the efforts to increase the yield via the macrocyclization step, a different head-to-tail site was selected. Here the free amine **53** was Cbz protected in 85% yield, then saponified in quantitative yields and finally coupled with EDCI and HOBt to **46** to access **54** in 83% yields (**Scheme 2.8**). After deprotection of the hexapeptides' -N and -C terminus, macrocyclization was performed in 38%, representing a lower yield than macrocyclization at the (L-val)oxazoline -N terminus and the (L-val)thiazole -C terminus. Meyers et al. proposed a higher steric demand from intermediate **54** than from intermediate **52** potentially coming from the bulkiness of the (L-val)oxazole inducing a potential steric hindrance upon macrocyclization between the cis conformation of the oxazoline, making it more challenging to access the cyclic core. On the other hand, **52**, induced a higher yield as a result of a less crowded

macrolactamization upon (L-val)oxazoline N-terminus and a fully flat free acid and activated ester due to the thiazole planarity, thus enhancing the yield of the final product.



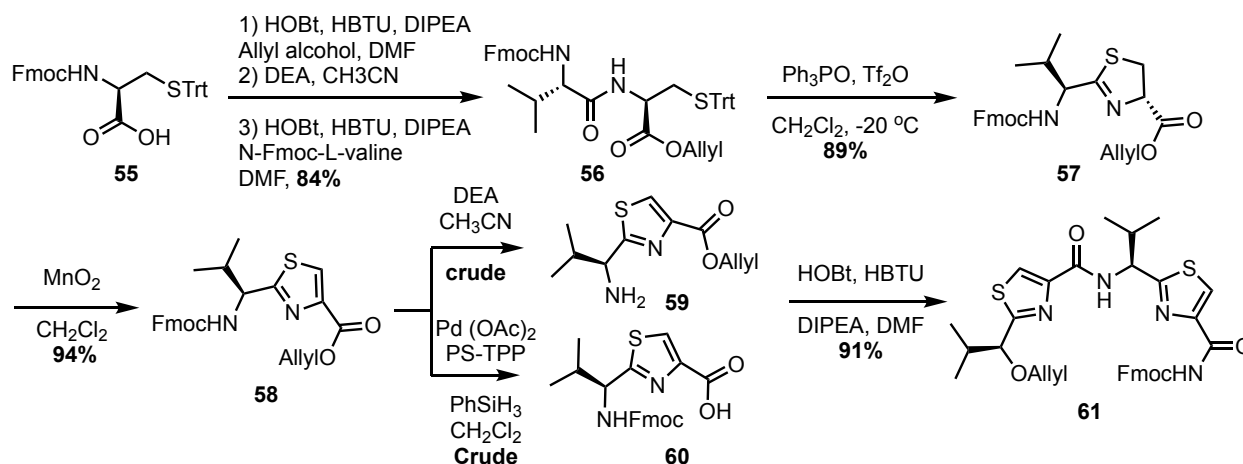
Scheme 2.8. Final steps and macrocyclization towards the total synthesis of bistratamide D

2.3.2 Kelly & You's Total Synthesis of Bistratamide E & J (2004)

Kelly & You were the first one to apply a biomimetic approach in their routes to access bistratamide E & J, via a chemo- and enantioselective cysteine cyclization to access the thiazole moiety through a thiazoline intermediate.¹²⁸ Previous research opted for a direct synthesis of the thiazole via modified Hantzsch reaction, however, by inducing a cyclodehydration of β -hydroxythioamides via a Mitsunobu or Burgess reactions, provides a thiazoline intermediate that can be used to access other thiazoline-containing natural products. Additionally, Fmoc-protected amino acids were explored throughout the synthesis, providing a new protecting group in which oxazolines can resist basic conditions for Fmoc-deprotections without inducing oxazoline ring opening. As expected, the retrosynthetic analysis of bistratamide E & J was proposed from dividing the molecule into three parts, two thiazoles and one trans-oxazoline and two thiazoles and one β -hydroxyamide, respectively.

The total synthesis initiated with the preparation and coupling of the two thiazole moieties (**Scheme 2.9**). Initially, the carboxylic acid of Fmoc- and Trt-protected cysteine (**55**) was protected in the presence of allyl alcohol, HOBt, HBTU, and DIPEA. This was followed by aqueous basic

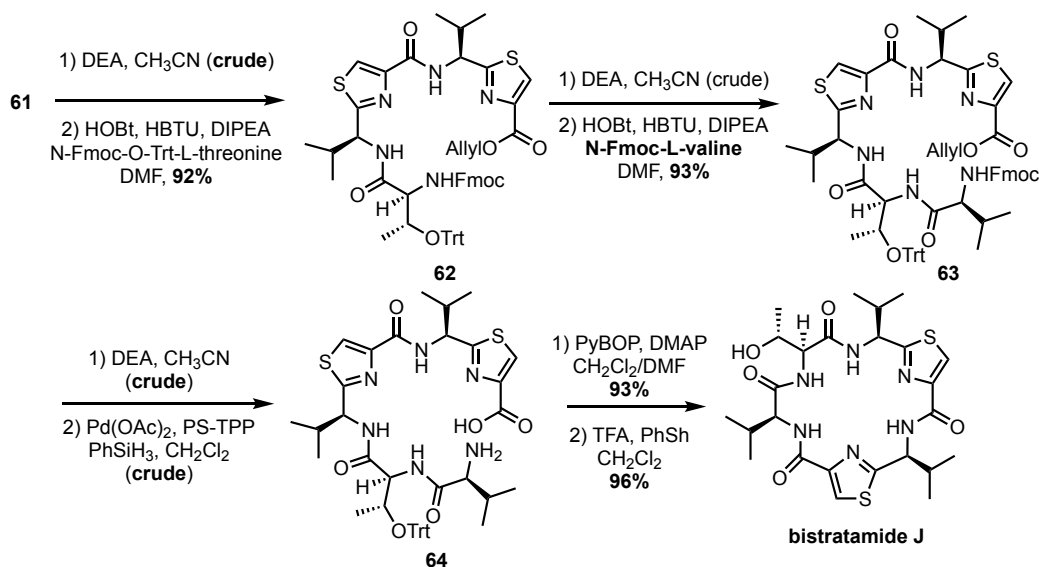
Fmoc deprotection and coupling with N-Fmoc-L-valine, to obtain dipeptide **56** in 84%. After obtaining **56**, thiazoline formation was achieved by adding Ph_3PO and Tf_2O , inducing a nucleophilic attack of the thiol on the activated amide carbonyl in an enantioselective manner, resulting in an 89% yield. The thiazoline (**57**) underwent radical based aromatization with MnO_2 to yield **58** in 94% yield. Since bistratamide E and J contain two identical (L-val)thiazoles (**58**), this fragment was deprotected at both the N- and C-termini to obtain free acid **60** and amine **59**. Finally, activation of **60** was performed using HBTU, HOBt, and DIPEA, followed by nucleophilic attack of **59** to access bis-thiazole **61** in 91% yield over three steps.



Scheme 2.9. Biomimetic approach towards thiazole synthesis and bis-thiazole **61**

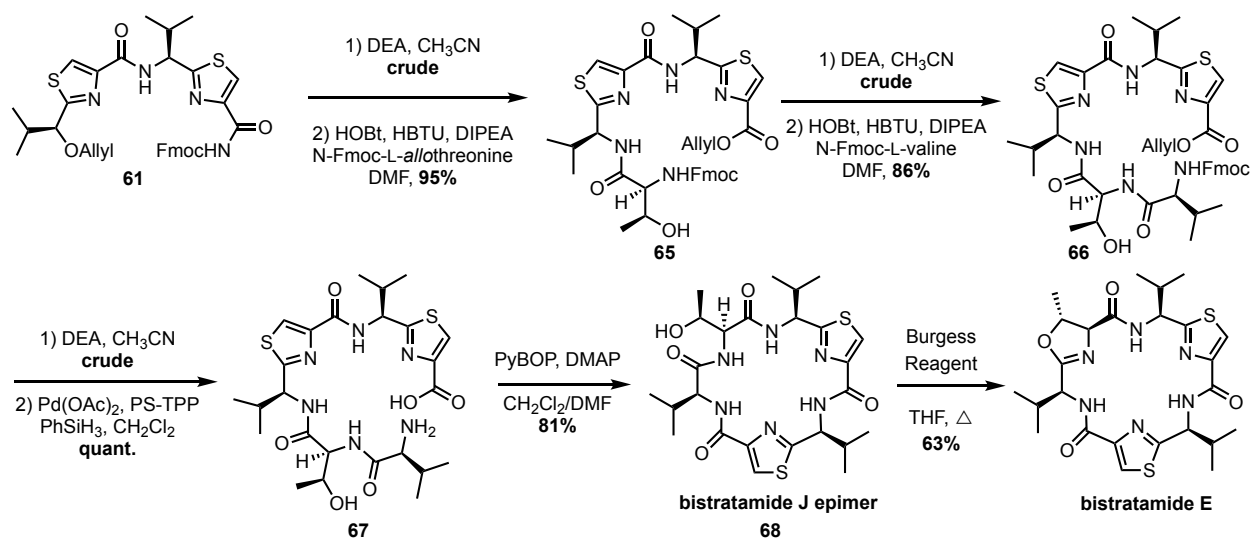
For the coupling of the β -hydroxyamide in the synthesis of bistratamide J and the formation of the oxazoline in bistratamide E, a single coupling amino acid to tetrapeptide **61** was performed (**Scheme 2.9**). In the case of bistratamide J, coupling of N-Fmoc-O-Trt-L-threonine to the free amine of **61** was successfully achieved under standard peptide coupling conditions in 92% yield. This was followed by a second coupling to the free amine of **62** with Fmoc-L-valine to obtain hexapeptide **63** in 93% yield. With **63** in hand, deprotection of both the C- and N-termini was carried out under catalytic and basic conditions, respectively, to yield **64**. Macrocyclization was

then performed using PyBOP and DMAP, leading to the formation of Trt-bistratamide J. Finally, Trt deprotection was performed under acidic conditions in 96% yield to accomplish the first total synthesis of bistratamide J.



Scheme 2.10. Final steps towards the total synthesis of bistratamide J

For bistratamide E, the same pathway was applied; however, the use of L-*allo*threonine instead of L-threonine was implemented since the last step includes a trans-oxazoline formation that under the Burgess reagent epimerization upon β -carbon of L-*allo*threonine achieves the correct oxazoline configuration (**Scheme 2.11**). Thus, providing an epimer of bistratamide J. It is worth to notice that couplings and macrocyclization were performed with a free L-*allo*threonine alcohol, inducing a slight decreased in yields for most steps, potentially coming from hydrogen bonding or nucleophilic behavior of the free alcohol.

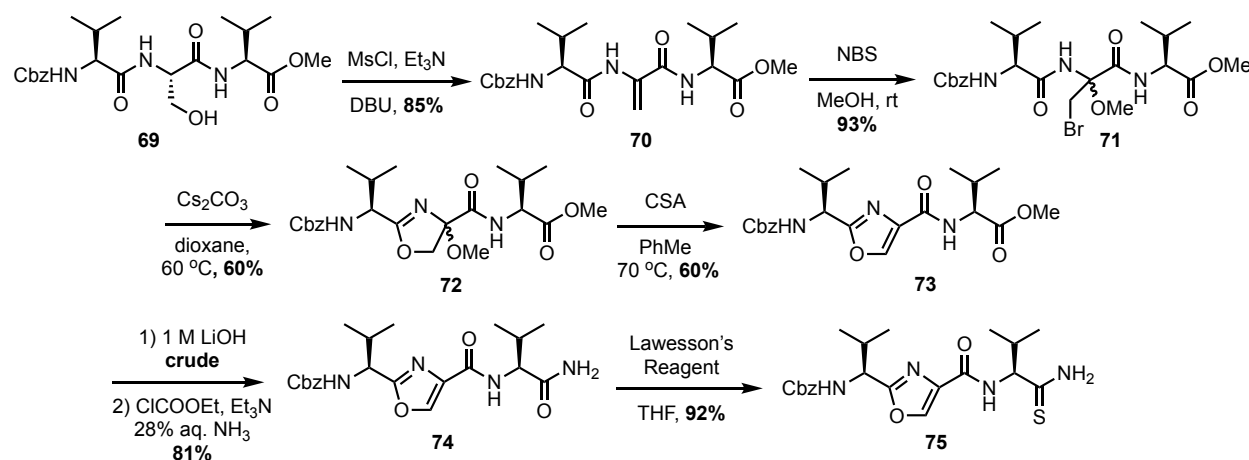


Scheme 2.11. Final steps towards the total synthesis of bistratamide E

2.3.3 Yonezawa and Shin's Total Synthesis of Bistratamide G & H (2004 & 2005)

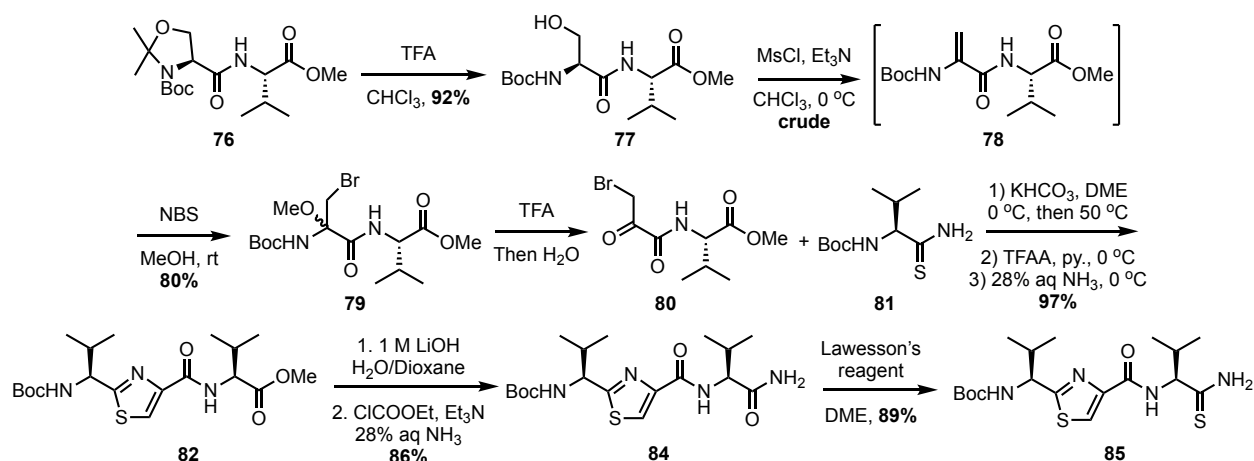
The first total synthesis of bistratamides G and H was reported by Yonezawa et al.¹²⁹ in 2004 and by Shin et al.¹³⁰ in 2005, respectively, using a newly developed methodology involving dehydro-oligopeptides. This approach eliminated the need for condensing reagents, except during the macrocyclization step. Additionally, the synthesis of the oxazole unit is more environmentally friendly, as it avoids the use of metals and carcinogenic reagents commonly employed in previous syntheses. The primary difference between bistratamides G and H lies in a single atom, which forms an oxazoline in bistratamide G and a thiazole in bistratamide H. The synthetic pathway commenced with the synthesis of dehydroalanine **70**, obtained by dehydrating tripeptide **69** (containing serine) under MsCl, Et₃N, and DBU (**Scheme 2.12**). With **70** in hand, elaboration to **71** was performed under bromination with NBS and methoxylation via MeOH in 93% yield. Subjecting **71** with Cs₂CO₃ in dioxane furnished oxazoline 4-methoxyoxazoline **72** in 60% yield. A subsequent β-elimination of the methoxy group was carried out using camphorsulfonic acid (CSA), resulting in the formation of oxazole **73** present within bistratamide G. Saponification of

73 followed by amidation with ClCO_2Et in the presence of Et_3N and aqueous NH_3 led to the formation of carboxamide **74** in 81% yield. Finally, thioamidation was achieved by applying Lawesson's reagent, obtaining the desired thiocarboxamide product **75** in 92% yield, which serves as a key intermediate for the formation of thiazoles in the synthetic pathway.



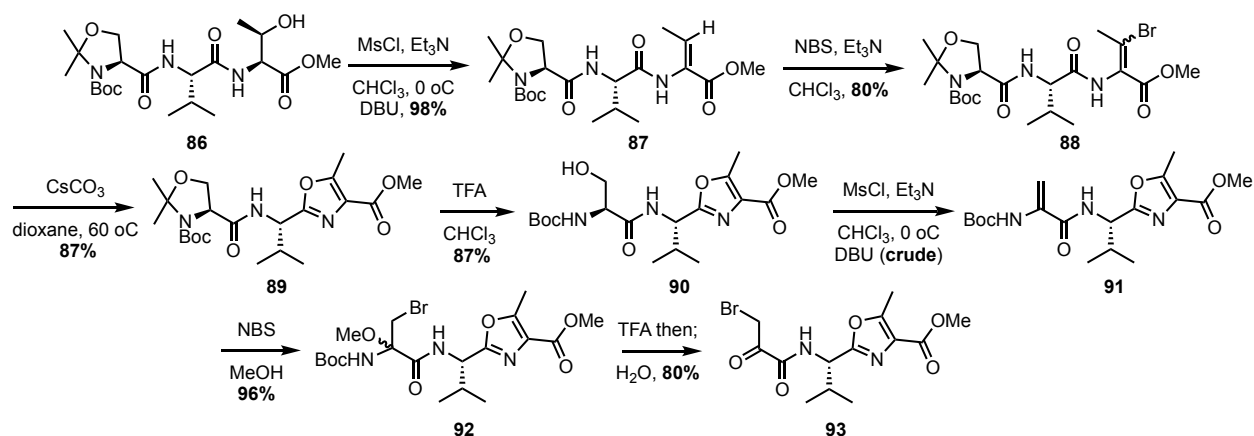
Scheme 2.12. Synthesis of dehydropeptide **70** and oxazole **75**

To access the thiazole **82** within bistratamide H, Yonezawa employed a similar procedure, utilizing two main intermediates, dehydrodipeptide **78** and diketone **80** (Scheme 2.13). First, deprotection of Boc-pseudoproline **76** under acidic conditions produced free alcohol **77**, which was then dehydrated via mesylation to yield dehydroalanine **78**. Methoxy-bromination of **78** resulted in the formation of β -bromo- α -methoxylated dipeptide **79**, which was subjected to deprotection and hydrolysis with TFA and H_2O to yield diketone intermediate **80**. Due to the intermediate's poor stability, the crude material, the material was immediately treated with **84** to access thiazole **82** in 97% yield under Hantzsch reaction conditions. Finally, **82** was treated to saponification, amidation, and thioamidation under basic conditions, carboxylic acid activation and NH_3 addition, and the incorporation of Lawesson's reagent, respectively, to afford thiocarboxamide **85** in 89% yield.



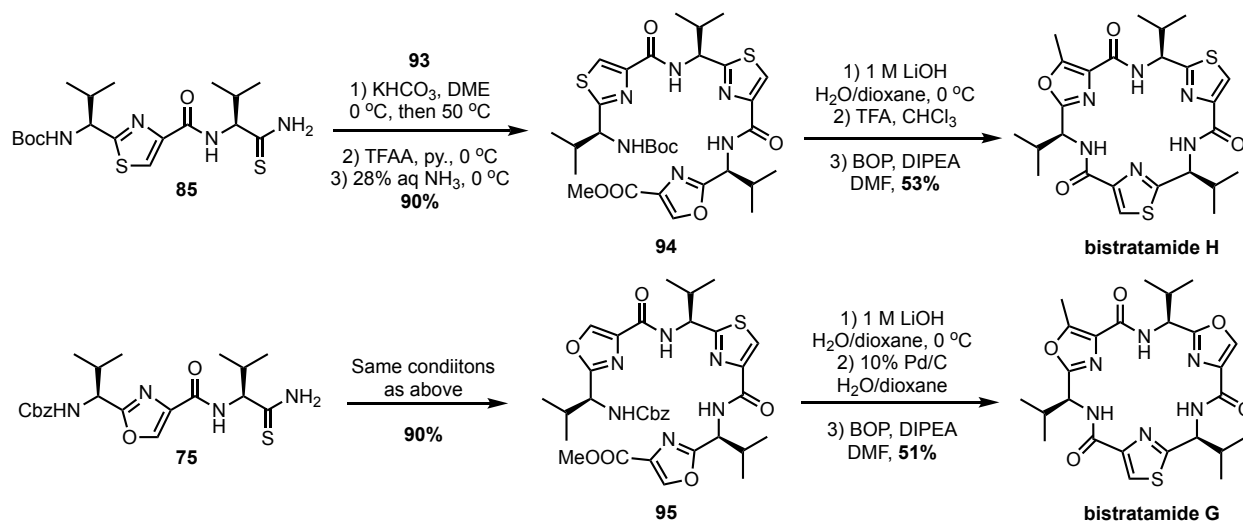
Scheme 2.13. Synthesis of dehydropeptide **78** and thiazole **85**

For the last steps toward the total synthesis of bistratamides **G** and **H**, one of the common fragments between both natural products, oxazoline **89** was also accessed by using the same methodology as previously described. However, the final product in this route contains a reactive side including a β -bromodiketone that promotes the coupling between the previous synthesized fragments to form a thiazole ring, the second and last common fragment between bistratamide **G** and **H** (**Scheme 2.14**). First, β -hydroxyamide **86** was mesylated with subsequent elimination to give **90** in 98% yield, followed by bromination to furnish **88** in 80% yield. Treatment with Cs_2CO_3 afforded 5-methyloxazole **89** in 87% yield. With **89** in hands, selective deprotection was performed under acidic conditions, followed by dehydration to access Boc-dehydroalanine oxazole **91**. Finally, the previous mentioned Boc deprotection and dehydration conditions were applied to access the final reactive diketone fragment **93** in 80% yield.



Scheme 2.14. Synthesis of dehydropeptide **91** and β -bromo diketone **93**

Finally, coupling of each fragment was performed when oxazole-thiocarboxamide (**75**) and thiazole-thiocarboxamide (**85**) were coupled with diketone-oxazole **93** to access hexapeptide **94** and **95**, respectively, both in 90% yield. Interestingly, multiple deprotection were performed for both hexapeptides, including a Cbz deprotection under Pd-C 10% in the presence of thiazoles. As mentioned before, Meyers et al. for the synthesis of bistratamide D,¹³ was not able to access the Cbz deprotection by using standard conditions under Pd-C 10%, instead a more potent catalyst (Pd-black) was used under increased pressure (100 psi). Thus, leading to potential future investigations behind the palladium poisoning under the presence of thiazoles moieties in cyclamides. After synthesis of both hexapeptides, macrocyclizations were performed via condensations reactions by the incorporation of BOP, DIPEA, and DMF to access bistratamide G and H in 51% and 53%, respectively. Both final synthetic natural products' ¹H/¹³CNMR and optical rotation were confirmed to match the isolated natural product.

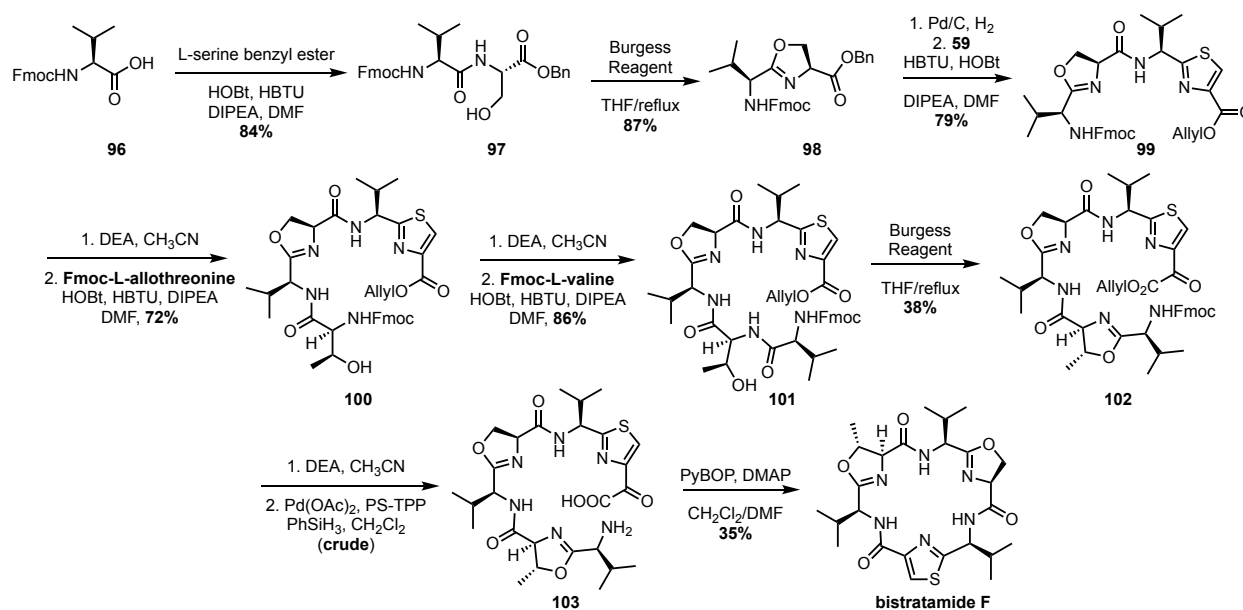


Scheme 2.15. Final synthetic steps towards bistratamide G. Coupling between thioamide **85** and **75** to β -bromo diketone **93**, followed by macrolactamization

2.3.4 Kelly & You's Total Synthesis of Bistratamide F, G, H, and I (2005)

The total syntheses of bistratamides F-G were access by Kelly & You through condensation reactions and a chemo- and enantioselective cysteine cyclization¹¹⁸ to access the thiazole as shown in their previous successful methodologies in the total synthesis of bistratamide E & J.¹²⁸ Bistratamides F-I have a common southern fragment, the (L-val)thiazole (**58**) moiety that, in this case, was also synthesized via the exact same procedure as shown in **Scheme 2.9**. With **58** in hands, the synthetic efforts proceeded through the synthesis of both oxazolines moieties from bistratamide F (**Scheme 2.16**). It is worth to emphasize, that throughout the whole synthetic pathway, the use of standard peptide coupling conditions with HBTU/HOBt and DIPEA were implemented, except for macrocyclizations that employed PyBOP and DMAP as indicated. First, the synthesis of (L-val)oxazoline **98** started from the coupling of Fmoc-L-val-OH with L-serine benzyl ester to access dipeptide **97** in 84% yield, followed by β -hydroxyamide dehydration-cyclization using the Burgess reagent to access **98** in 87% yield. With **98** in hand, benzyl deprotection was performed under standard conditions followed by coupling to the free amine

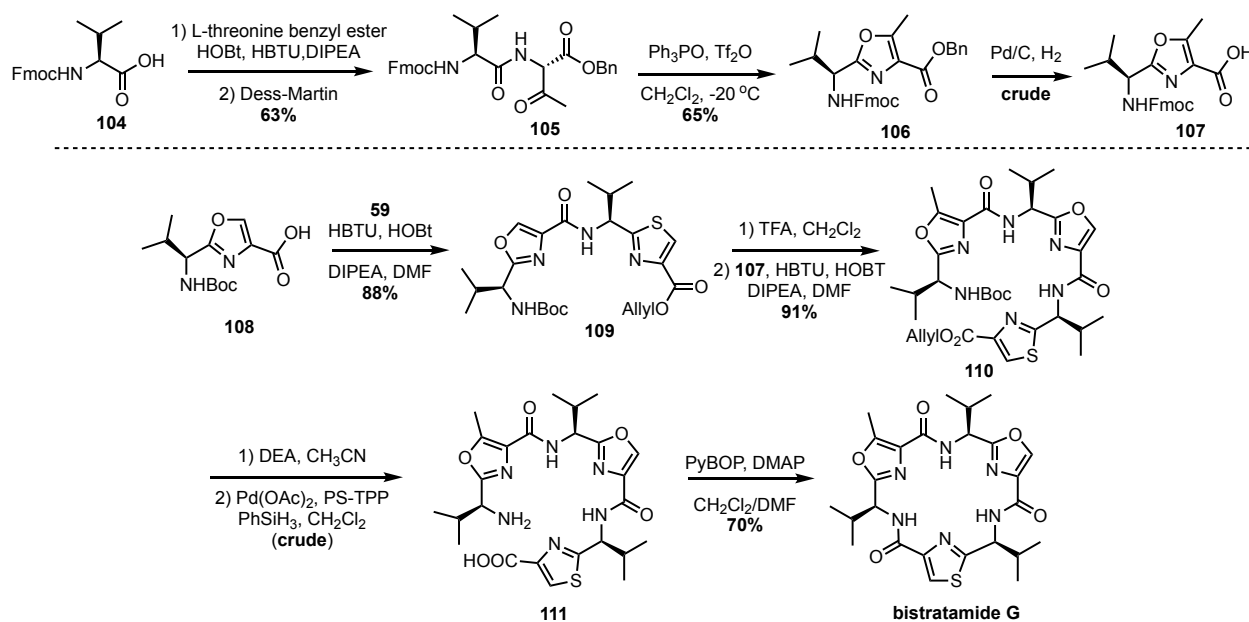
within **59** to access tetrapeptide **99**, in 79% yield. Kelly and You, decided to perform a sequence of single coupling amino acids upon **99** by incorporation of Fmoc-L-*allo*threonine, and Fmoc-L-valine, to access penta- and hexapeptide **100** & **101** in 72% and 86% yields, respectively. Consequently, formation of 5-methyl-transoxazole **102**, was achieved in 38% yield via epimerization upon L-*allo*threonine β -carbon, dehydration, and cyclization induced by the Burgess reagent. Finally, deprotection of both C- and N- termini followed by macrocyclization under PyBOP/DMAP completed the total synthesis of bistratamide F in 35% yield.



Scheme 2.16. Total synthesis of bistratamide F

The total synthesis of bistratamide G was also achieved, this time via condensation reactions, and not through dehydropeptides like Yonezawa's methodology (**Scheme 2.17**). With thiazole **58** in hands, Kelly and You proceeded to synthesize 4-methyloxazole **106** starting from the coupling of Fmoc-L-val-OH and L-threonine benzyl ester, follow by L-threonine β -C oxidation to achieved diketone **105** in 63% yield. Kelly and You were the first ones to explore the application of this methodology for the bistratamides synthesis, in which a β -keto amide is directly converted

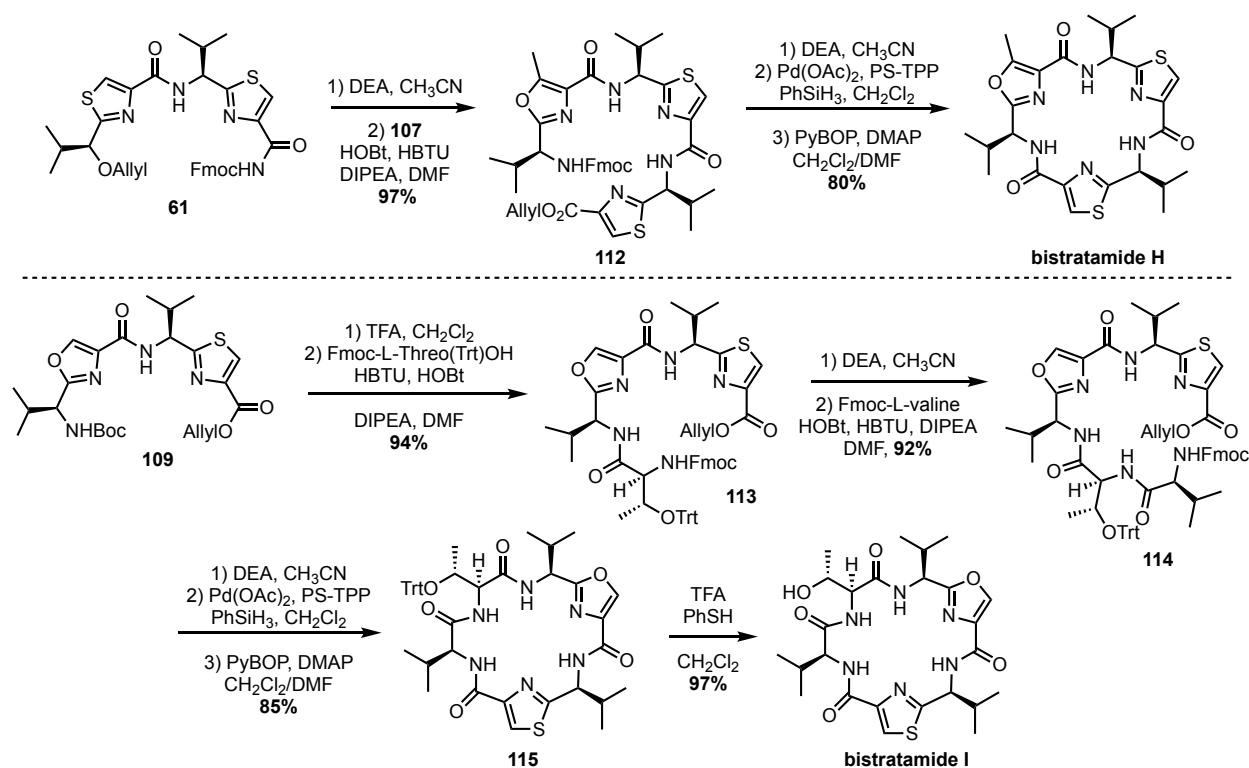
into an oxazole via Ph_3PO and Tf_2O . Oxazole **106** was successfully accessed in 65% yield. Finally, for the preparation of the last (L-val)oxazole fragment, Kelly and You applied the known procedure that Meyer's used to access the fragment for bistratamide D.¹³ Thus, deprotection of **106** under catalytic conditions achieved **107** that was further coupled with **59** to access tetrapeptide **109** in 88% yield. Subsequently, hexapeptide **110** was afforded in 91% yield after Boc-deprotection of **109** followed by coupling of **107** under HBTU/HOBt. Deprotection of each terminus of **110** was performed under basic and catalytic conditions, to induce macrocyclization using PyBOP and DMAP to access bistratamide G in 70% yield. Structure was confirmed by X-ray crystallography.



Scheme 2.17. Total synthesis of bistratamide G

On the other hand, bistratamide H shares two fragments with bistratamide F & G, (L-val)4-methyl oxazole **106** and (L-val)thiazole **58**. The additional fragment includes an extra (L-val)thiazole (**Scheme 2.18**) that can be easily coupled with itself to access tetrapeptide **61** as shown by the same group in **Scheme 2.9**. With **61** in hands, coupling of 4-methyl oxazole **107** was

performed to access hexapeptide **112** in 97% yield. Finally, macrocyclization was successfully achieved to access bistratamide H in 80% yield. For the synthesis of bistratamide I, the coupling of a tetrapeptide **109** to Trt-protected L-threonine and L-valine was achieved in 86% overall yield in 4 steps to furnish **114**. The last steps of the synthesis consisted in macrolactamization of hexapeptide **114** in 85% yield over two-steps, followed by Trt-deprotection to access bistratamide I in 97% yield. Additionally, Kelly and You were able to develop a new methodology to incorporate imidazoles in the synthetic pathway to access analogs of bistratamide. This method consists of an enantiospecific synthesis of imidazole-based amino acids from C-terminal β -amino- α -amino acids in the presence of Ph_3PO .¹³⁰



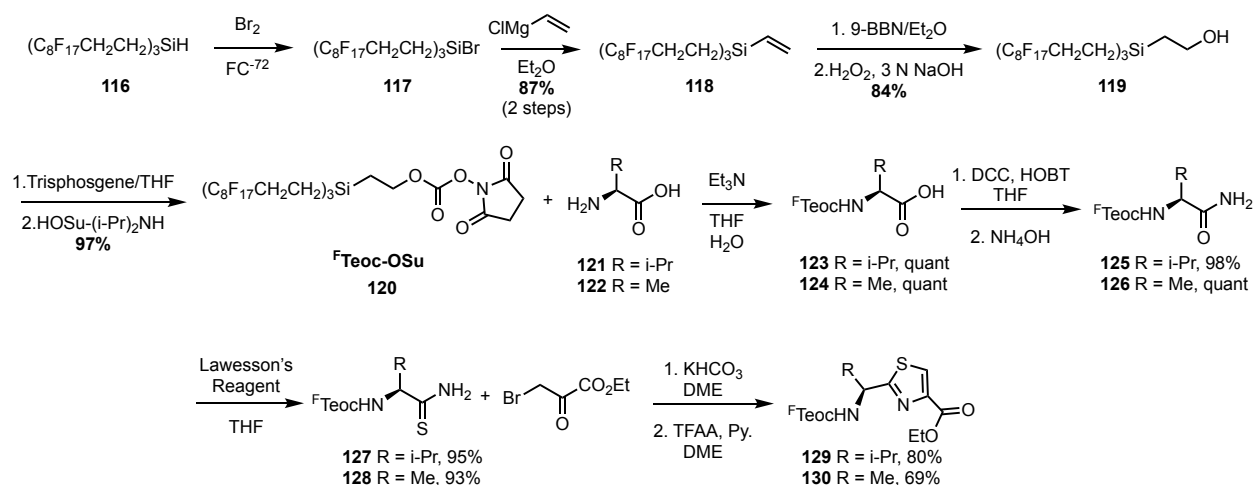
Scheme 2.18. Total synthesis of bistratamide I

2.3.5 Takeuchi & Nakaruma's Total Synthesis of Bistratamide H (2006)

A new methodology to facilitate the purification of intermediates in bistratamide's synthetic pathways was developed by Takeuchi and Nakaruma¹³¹ in 2006. This approach involves the incorporation of fluororous protecting groups during the early synthetic steps, which can be easily separated by liquid-liquid extraction when the fluorine content in the intermediate is sufficiently high (>60 wt.%). This provides an efficient purification process to isolate byproducts and eliminate undesirable, odorous side products. However, if the fluorine content is low (<40 wt.%), purification can be achieved via solid-phase extraction using a fluororous reverse-phase silica gel column. To develop and optimize this methodology, Takeuchi and Nakaruma incorporated the 2-(Tris(perfluoro-decyl)silyl)ethoxycarbonyl (^FTeoc) group into bistratamide intermediates, which have a high fluorine content and can be easily extracted with perfluorohexane (FC-72) and cleaved under tetrabutylammonium fluoride (TBAF). Additionally, the ^FTeoc group can be recycled, allowing for the resynthesis of the reactive ^FTeoc reagent.

To access the reactive ^FTeoc (**120**), a succinimide moiety was incorporated into fluororous hydrosilane **116** (Scheme 2.19). First, **116** was brominated under Br₂ to yield silylbromide **117**, followed by a Grignard reaction with vinyl magnesium chloride to obtain vinylsilane **118** in 87% yield over two-steps. Hydroboration and oxidation of **118** afforded silylethanol **119** in 84% yield over two-steps. Finally, **119** was treated with trisphosgene, followed by the addition of N-hydrosuccinimide to yield the reactive ^FTeoc-OSu (**120**) in 70% overall yield over six-steps from **116**. For subsequent reactions, all intermediates were purified solely by extraction with FC-72, unless otherwise specified, in which purification via SiO₂ or alumina is applied. With the protecting agent **120** in hand, Takeuchi and Nakaruma incorporated the protecting group in an early synthetic step by protecting L-valine and L-alanine under **120**/Et₃N, achieving both protected

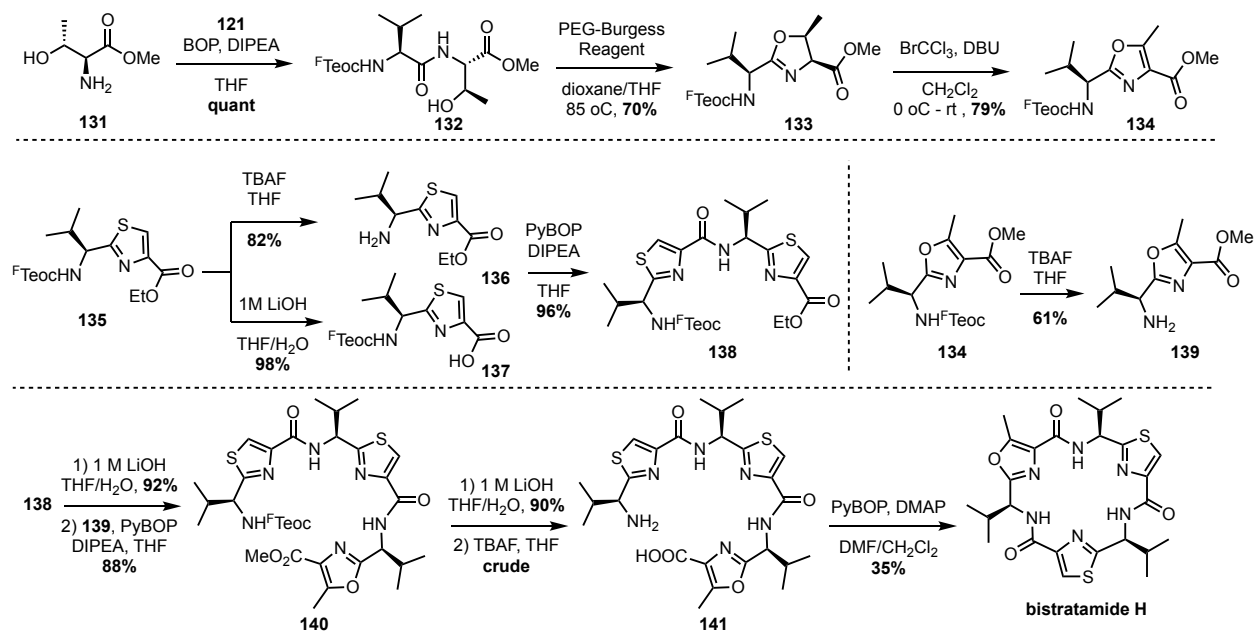
amine in quantitative yields. Amidation of **121** and **122** were performed high yields after activation of the carboxylic acid with DCC and HOBT, followed by the addition of NH_4OH . Sulfur incorporation and thiazole formation were successfully achieved when amide **125** and **126** were treated with Lawesson's reagent, followed by the addition of ethyl bromopyruvate and trifluoroacetic anhydride to yield $^{\text{F}}$ Teoc-protected thiazoles **129** and **130**.



Scheme 2.19. Synthesis of $^{\text{F}}$ Teoc-OSu (**120**) and thiazoles **129** & **130**

To access $^{\text{F}}$ Teoc-oxazole **134**, intermediate **121** was coupled with L-threonine methyl ester **131** to yield $^{\text{F}}$ Teoc-dipeptide **132** in quantitative yields, followed by heterocyclization using the PEG-Burgess reagent to form $^{\text{F}}$ Teoc-trans-oxazoline **133** in 70% yield (**Scheme 2.20**). Finally, radical oxidation of **133** was carried out by applying BrCCl_3 and DBU to yield $^{\text{F}}$ Teoc-oxazole **134** in 55% overall yield through three steps. Additionally, **134** was deprotected under TBAF for future couplings. To access bistratamide H, (L-val)thiazole **135** was saponified to yield free acid **137**, and **135** was also deprotected from the $^{\text{F}}$ Teoc group to obtain the free base **136**. The two thiazoles were coupled under standard peptide coupling conditions to form $^{\text{F}}$ Teoc-bisthiazole **138**, which was further saponified and coupled with oxazoline **139** to obtain hexapeptide **140** in 88% yield. **140**

was deprotected at both the C- and N-termini (**141**) to induce macrocyclization in the presence of PyBOP and DMAP, resulting in the formation of bistratamide H in 35% yield.



Scheme 2.20. Synthesis of oxazole **134** and final steps towards bistratamide H total synthesis

2.3.6 Pattenden's Total Synthesis of Bistratamide H (2007)

Pattenden's group explored the synthesis of multiple tri- and tetramer macrocycles through a one-pot reaction designed to induce cyclooligomerization via the incorporation of pentafluorophenyl diphenylphosphinate (FDPP) and/or diphenyl phosphoryl azide¹³² (DPPA) (**Scheme 2.21**). Due to the symmetrical bis-thiazole fragment in bistratamide H, this methodology was tested for the synthesis of the tetrapeptide **143** fragment of the molecule. The thiazole moieties were synthesized using a modified Hantzsch method, as described in the literature and shown in previous synthesis. Additionally, the free acid/base oxazole **142** was synthesized using methods similar to those employed by Meyer for the total synthesis of bistratamide D. However, the β -hydroxyamide cyclization was performed under deoxo-fluor conditions to yield (L-val)trans-oxazoline **44** in 93% yield, followed by radical oxidation and deprotection to access **142** in 70%

43 $\xrightarrow[\text{DCM, 93\%}]{\text{Deoxo-Fluor}}$ **44** $\xrightarrow[\text{H}_2\text{O/MeOH, 78\%}]{\begin{array}{l} 1) \text{ BrCCl}_3, \text{ DBU} \\ \text{DCM, 0 } ^\circ\text{C, 90\%} \\ 2) \text{ LiOH} \\ 3) \text{ 4 M HCl/dioxane} \end{array}}$ **142**

30 $\xrightarrow[\text{DMF, 70\%}]{\text{DPPA, DIPEA}}$ **143** $\xrightarrow[\text{crude}]{\begin{array}{l} 1) \text{ 4 M HCl in dioxane} \\ 2) \text{ NaOH aq.} \\ \text{MeOH/dioxane} \end{array}}$ **144**

49 $\xrightarrow[\text{DMF, 70\%}]{\text{DPPA, DIPEA}}$ **143**

142, **144** $\xrightarrow[\text{CH}_3\text{CN, 25\%}]{\text{142, FDPP, DIPEA}}$ **bistratamide H**

142, **144** $\xrightarrow[\text{CH}_3\text{CN, 36\%}]{\text{142, FDPP, DIPEA}}$ **bistratamide H**

Alternate pathway via individual fragment oligomerization

2.3.7 Sato's Total Synthesis of Bistratamide J, E, and H (2008)

47

formation after reacting with thiocarboxamides.^{133,134} However, Sato's research focused on the total synthesis of bistratamide H through the intermediates bistratamides E and J, as shown in **figure 2.3**. To access one (L-val)thiazole fragment of the family, Sato followed the same procedure as Yonezawa's synthesis, as illustrated in **scheme 2.13**. In contrast, the synthesis of the second (L-val)thiazole fragment began with the protection of the primary alcohol of dipeptide **146** using TBDPS-Cl to furnish **147** in 93% yield, followed by the deprotection of pseudoproline to yield **148** in 86% yield (**Scheme 2.22**). **148** was then subjected to mesylation and elimination to form dehydroalanine **149**, which was subsequently subjected to bromination and methoxylation to successfully generate β -bromo- α -methoxylated tripeptide **150** in 87% yield. A one-pot deprotection and hydrolysis of **150** induced the formation of β -bromo diketone **151** in 92% yield. With **151** in hand, thiocarboxamide **85** was reacted with β -bromo diketone **151** under K_2CO_3 to form hexapeptide **152** in 90% yield, followed by TBDPS deprotection to yield free alcohol **153**. Finally, deprotection of both chain termini (**154**), followed by head-to-tail macrolactamization, resulted in the total synthesis of bistratamide I in 72% yield.

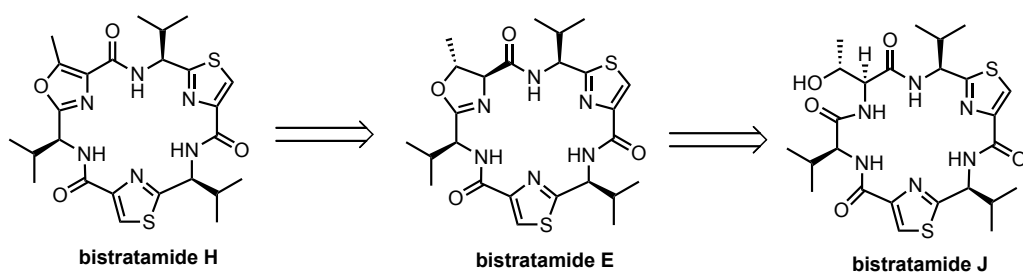
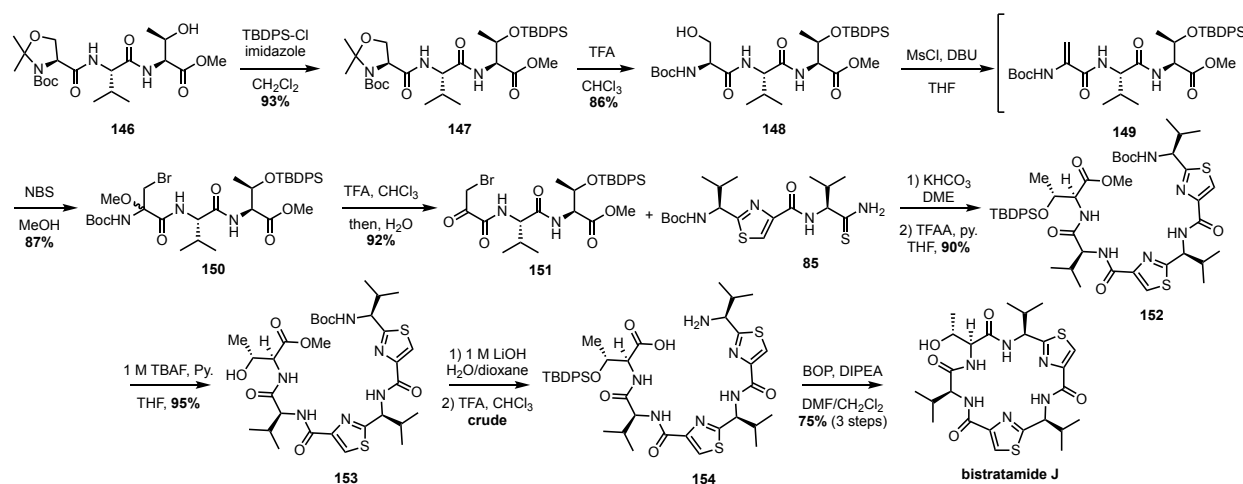
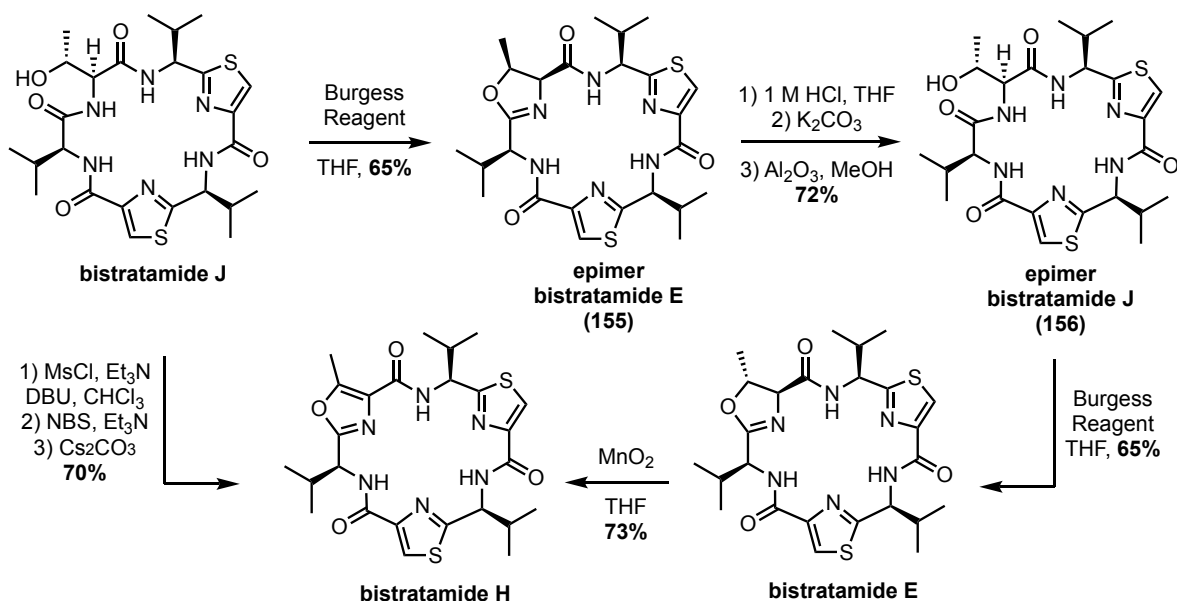


Figure 2.3. Retrosynthetic analysis towards bistratamide H through bistratamide E & J



Scheme 2.22. Total synthesis of bistratamide J via thioamide **85** and diketone **151**

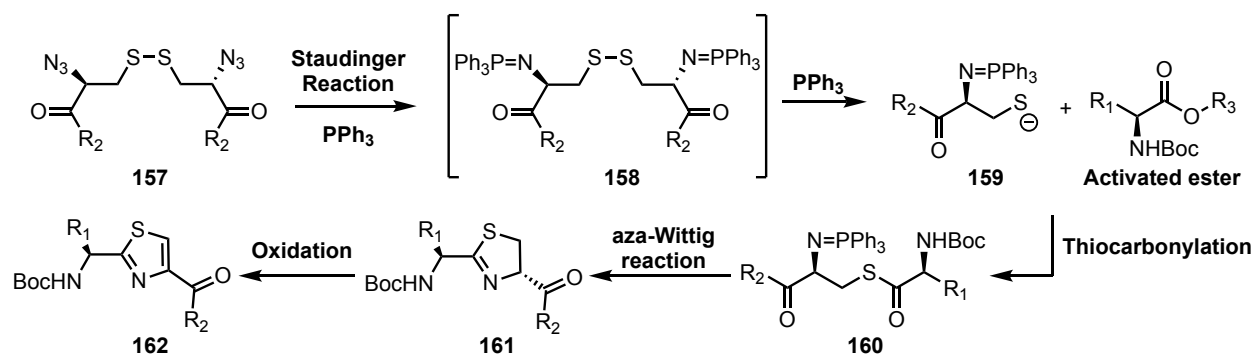
From bistratamide J both bistratamides E and H could be accessed. The free alcohol in bistratamide J underwent dehydration via epimerization at the β -carbon of L-threonine, leading to an epimer of bistratamide E (**155**) in 65% yield (**Scheme 2.23**). This was followed by ring opening under acidic conditions to yield the L-*allo*threonine epimer of bistratamide J (**156**) in 72% yield over two-steps. This methodology allowed the total synthesis of bistratamide E by reacting **156** with the Burgess reagent, which induced epimerization at the β -carbon of L-*allo*threonine to form a trans-oxazoline. Finally, the bistratamide E oxazoline underwent radical oxidation using MnO_2 to yield bistratamide H in 73% yield. Additionally, Sato's work included an alternative pathway for the direct synthesis of bistratamide H from bistratamide J, which involved mesylation and bromination of bistratamide J, followed by cyclization using Cs_2CO_3 , as shown in **scheme 2.23**.



Scheme 2.23. Total synthesis of bistratamide E and H via bistratamide J

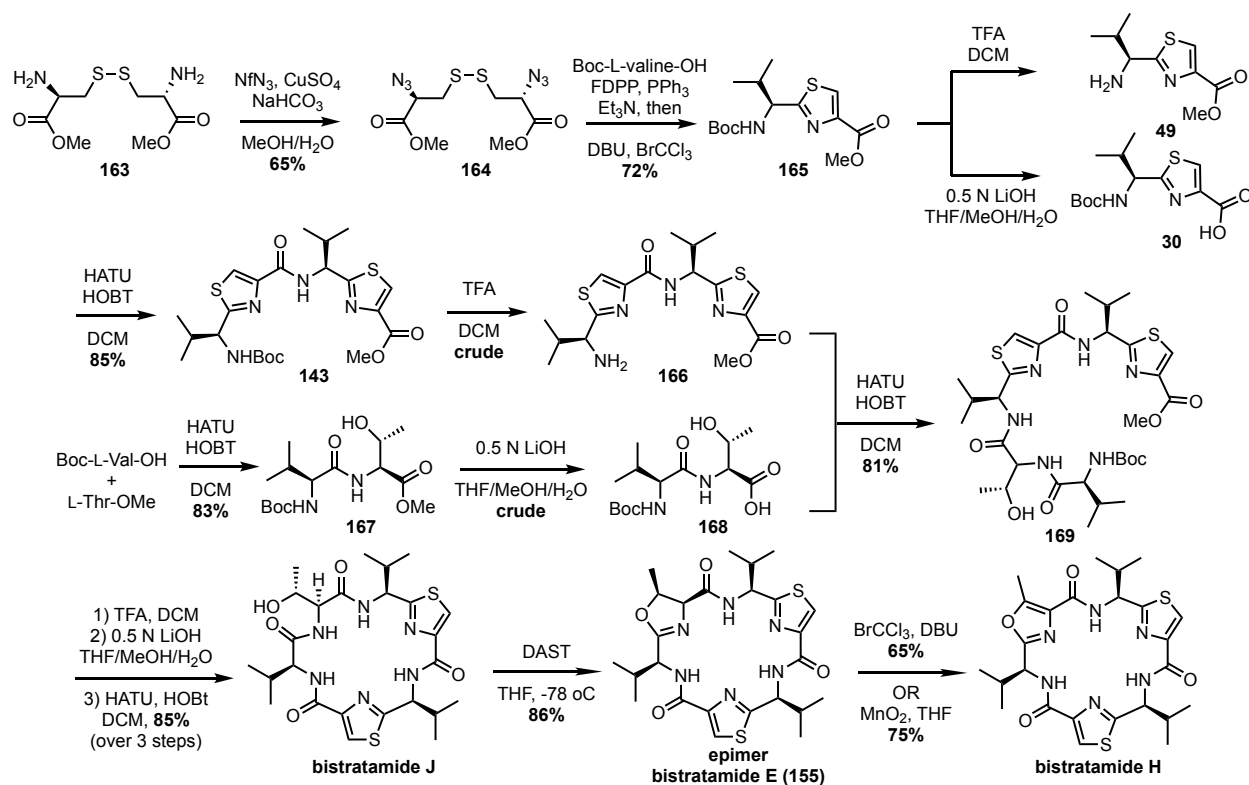
2.3.8 Liu's Total Synthesis of Bistratamide H and J (2024)

The total synthesis of bistratamide H & J were explored by implementing a new methodology to access thiazoles, via a one-pot synthesis using β -azido disulfide esters, that was first developed and established by Liu et al.¹³⁵ for the synthesis of venturamides A & B.¹³⁶ The thiazole synthesis proposed mechanism pathway is shown in **scheme 2.24**. First, β -azido disulfide ester **157** undergoes a Staudinger reaction to form intermediate **158** that will form reactive sulfide ion **159** after disulfide bond breaks. Subsequently, thiocarbonylation is performed by addition of activated carboxylic esters to access thioamide **160**, followed by an aza-Wittig reaction to approach thiazoline **161**. Finally, thiazolines can undergo oxidation via $\text{BrCCl}_3/\text{DBU}$ to achieve thiazole cores (**162**).



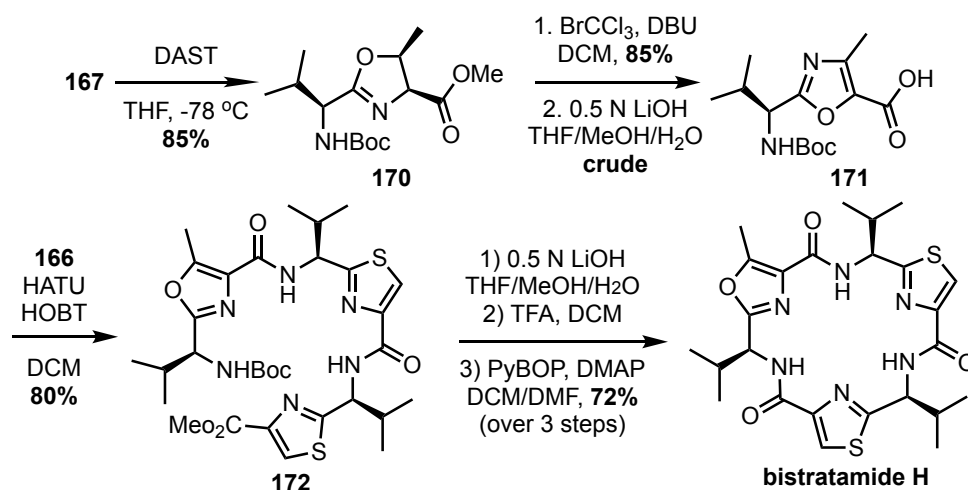
Scheme 2.24. Proposed pathway for thiazole synthesis through one-pot enantioselective Staudinger-aza-Wittig oxidation

For the synthesis of bistratamides, the synthetic pathway began with the formation of thiazole **165** using Liu's methodology (**Scheme 2.25**). First, β -azido disulfide ester **164** was synthesized through a diazo-transfer reaction involving L-cysteine dimethyl ester **163** and nonafluorobutanesulfonyl azide (NfN_3) under basic conditions in 65% yield. Next, in situ activation of Boc-L-val-OH with FDPP, PPh_3 , and Et_3N initiated a cascade reaction, following the proposed route as outlined in **scheme 2.24**, to yield (L-val)thiazole **165** in 72% yield. Due to the symmetry of bis-thiazole **143**, coupling of free acid **30** and free base **49** was performed under standard peptide coupling conditions, resulting in **143** in 85% yield. On the other hand, β -hydroxy amide **167** was synthesized by coupling Boc-L-val-OH with L-Thr-OMe, followed by saponification to obtain **168**. With dipeptide **168** in hand, removal of the bis-thiazole Boc group from bis-thiazole **143** resulted in the formation of free amine **166**, which was then coupled with free acid **168** to synthesize hexapeptide **169** in 81% yield. Saponification, Boc deprotection, and macrolactamization with PyBOP/DMAP in a mixture of DMF and CH_2Cl_2 provided bistratamide J in 75% yield. Finally, bistratamide H was obtained after dehydration and cyclization of bistratamide J, followed by radical oxidation using either $\text{BrCCl}_3/\text{DBU}$ or MnO_2/THF , obtaining the final compound in 65% and 75% yield, respectively.



Scheme 2.25. Total synthesis of bistratamide J and H

Finally, Liu explored another route toward the synthesis of bistratamide H (**Scheme 2.26**) by coupling the already made oxazole **171**, which was accessed from **167** via cyclization with DAST in 85% yield to access **170** and then aromatized and saponified. Oxazole **171** was coupled to bis-thiazole **166** to access hexapeptide **172** in 80% yield. Finally, macrocyclization under PyBOP/DMAP was performed after deprotection of the -C and -N termini to achieved bistratamide H in 72% yield over three synthetic steps.



Scheme 2.26. Alternative route towards bistratamide H synthesis

2.4 The Dendroamides Family

The dendroamide family of cyclamides was first isolated in 1996 from *Stigonema dendroideum* Frey by Smith and co-workers.¹³⁷ To date, three molecules have been identified in this family, including dendroamide A through C (**Figure 2.4**). Structurally, all members share one 4-methyloxazole ring and two thiazoles, however, the substitutions on the heterocyclic rings vary depending on the amino acid composition, in which all of them are D-amino acids. Notably, eight synthetic approaches toward dendroamide A have been explored using different methodologies. Additionally, two research groups have investigated molecular models to determine the optimal points within the molecule for inducing head-to-tail macrolactamization, and they have proposed measurements for the distance between each terminus. To date, no synthetic reports have been published for dendroamides B or C.

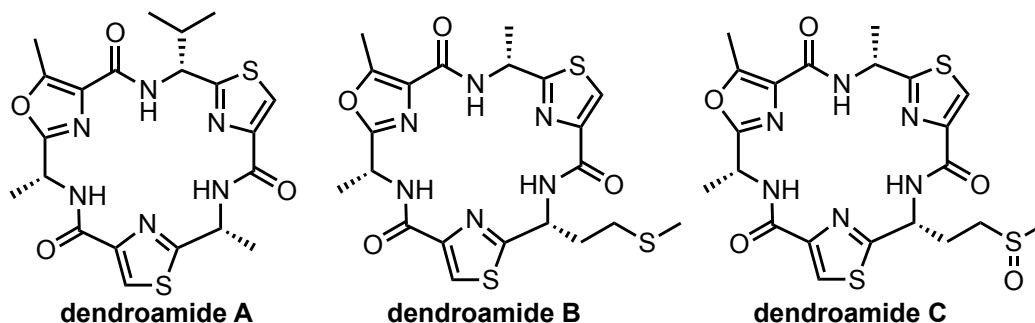
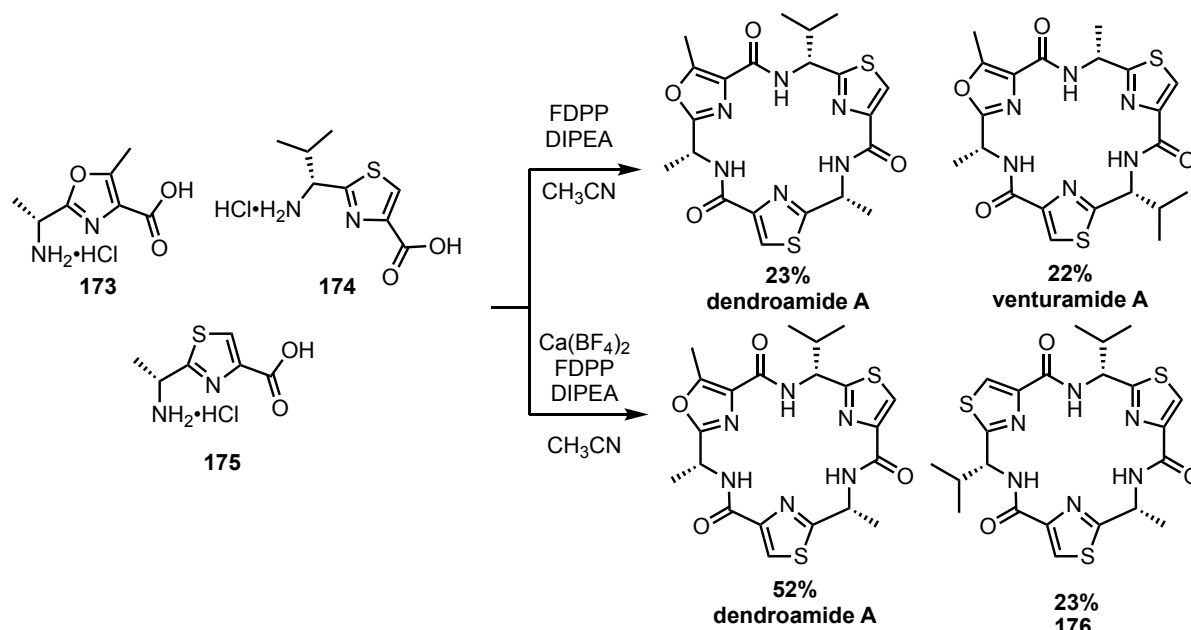


Figure 2.4. Chemical structures of the dendroamide family

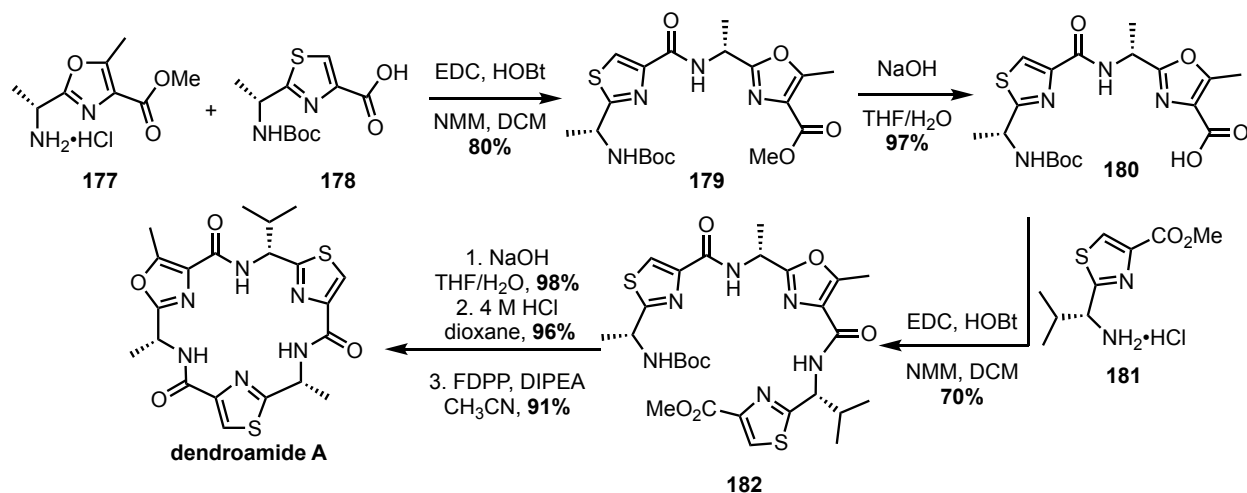
2.4.1 Bertram & Pattenden's Total Synthesis of Dendroamide A & Venturamide A (2000)

Pattenden and Bertram were the first group to achieve the total synthesis of dendroamide A by applying the same cyclooligomerization methodology they used for the synthesis of bistratamide H.¹³⁸ This approach involved the addition of a single-pot mixture of the three thiazole/oxazole fragments of dendroamide A in the presence of pentafluorophenyl diphenylphosphinate (FDPP) (**Scheme 2.27**). To confirm the identity of the synthesized product, a linear synthesis of dendroamide A was also explored. While the synthesis of the three fragments was not detailed, the amino acids used to synthesize each heterocycle were provided. Thiazole **174** was derived from D-valine and cysteine, thiazole **175** from D-alanine and cysteine, and oxazole **173** from D-alanine and threonine. Once these fragments were synthesized and deprotected, a 1:1:1 mixture of the three rings underwent a coupling cascade, including macrocyclization, under acetonitrile with FDPP and DIPEA, producing dendroamide A in 23% yield, along with venturamide A (22%) and two homocouplings from the thiazole moieties. In 2002, Pattenden and Bertram improved the yield from 23% to 52% by adding $\text{Ca}(\text{BF}_4)_2$ to the cyclooligomerization reaction mixture.¹³⁹



Scheme 2.27. Total synthesis of dendroamide A and venturamide A via cyclo-oligomerization reactions

To confirm the structure of the obtained natural product, Pattenden developed a linear synthetic pathway to achieve dendroamide A (**Scheme 2.28**). For all coupling reactions of the rings, except for the macrocyclization step, EDC/HOBt and NMM were used. Saponifications were carried out under standard basic conditions, and Boc deprotections were performed under acidic conditions. Finally, the macrocyclization between the D-alanine- and D-valine-thiazoles termini, using FDPP and DIPEA, induced ring closure to accomplish dendroamide A in 91% yield.

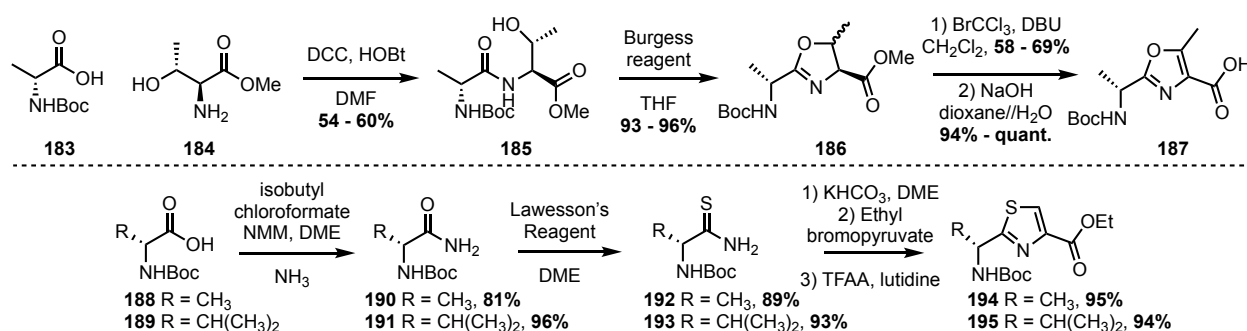


Scheme 2.28. Linear synthetic pathway of dendroamide A

2.4.2 Xia & Smith's Total Synthesis of Dendroamide A (2001)

Xia and Smith, the same group that isolated dendroamide A in 1996, successfully achieved the total synthesis of the molecule by employing a modified Hantzsch reaction to access the thiazole, followed by β -hydroxyamide dehydrocyclization and radical oxidation via BrCCl₃ and DBU to obtain the oxazoline fragment.¹⁴⁰ These methodologies have been applied in various synthetic routes within the bistratamide and aerucyclamide families. Additionally, Smith was able to predict, using molecular models, the optimal hexapeptide for macrolactamization by analyzing the lowest energy conformation of each acyl azide formed after the addition of DPPA and measuring the distance between the C- and N-termini of the molecule. After multiple analyses using MM2 and MOPAC molecular models, they determined that precursor **199** had the closest termini proximity, measuring 3.56 Å (MM2) or 6.14 Å (MOPAC). In contrast, the other hexapeptides exhibited a larger distance between termini, ranging from 8.03 Å to 13.85 Å. With this information in hand, Smith proceeded to develop the synthetic pathway toward dendroamide A and to evaluate the reliability of the molecular models.

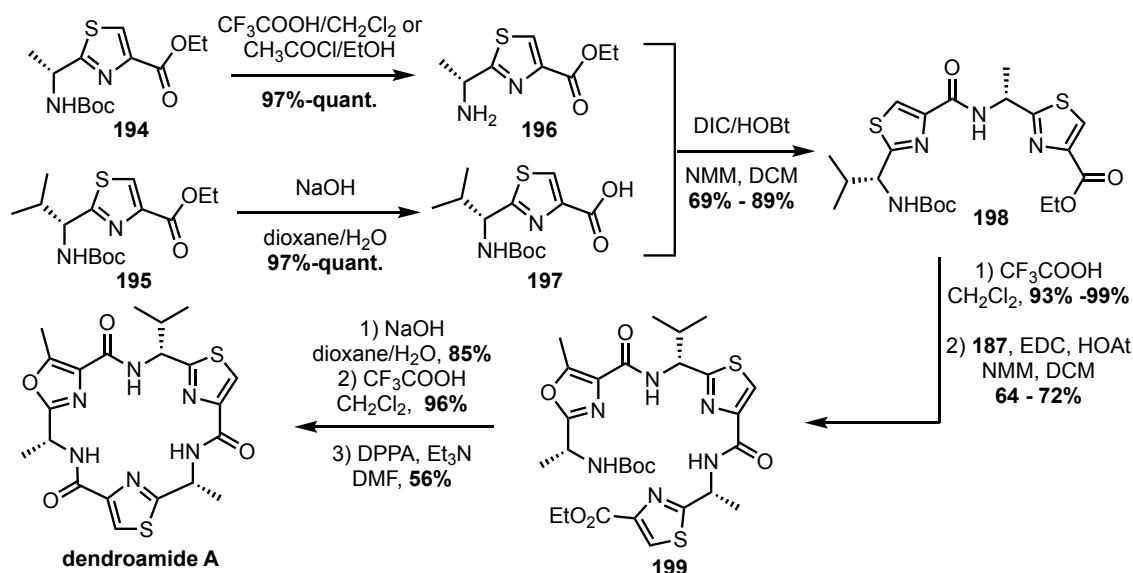
First, D-alanine and L-threonine methyl ester were coupled using DCC/HOBt, followed by dehydrocyclization to form a racemic mixture of oxazolines **186** (Scheme 2.29). Notably, Smith is the only group to have reported a racemic mixture by employing the Burgess reagent to induce ring closure of β -hydroxyamides. This mixture of oxazolines was then converted into the oxazole fragment upon exposure to radical oxidation conditions, accessing the first fragment in 58–69% yield. Furthermore, the oxidation of the oxazoline mixtures was also performed using metal peroxides such as NiO_2 or MnO_2 , but low yields (~30%) were observed. In contrast, the thiazole moieties (**194** and **195**) were both synthesized through a modified Hantzsch reaction. The first step involved the amidation of D-alanine and D-valine using isobutyl chloroformate, NMM, and NH_3 , followed by the formation of both thiazoles using the Lawesson's reagent to afford D-alanine (**192**) and D-valine (**193**) thioamides in 89% and 93% yield, respectively. Finally, the modified Hantzsch reaction was carried out when the thioamides were coupled with ethyl bromopyruvate, followed by dehydration and oxidation, resulting in the formation of both D-alanine (**194**) and D-valine (**195**) thiazoles in 95% and 94% yield, respectively.



Scheme 2.29. Synthesis of dendroamide's A three fragments

With the three fragments in hand, Smith proceeded to deprotect and couple both thiazoles (**194** and **195**) using DIC/HOBt, followed by Boc deprotection of the bis-thiazole **198** in 93–99% yield (Scheme 2.30). After deprotection of oxazoline **186**, coupling was achieved using

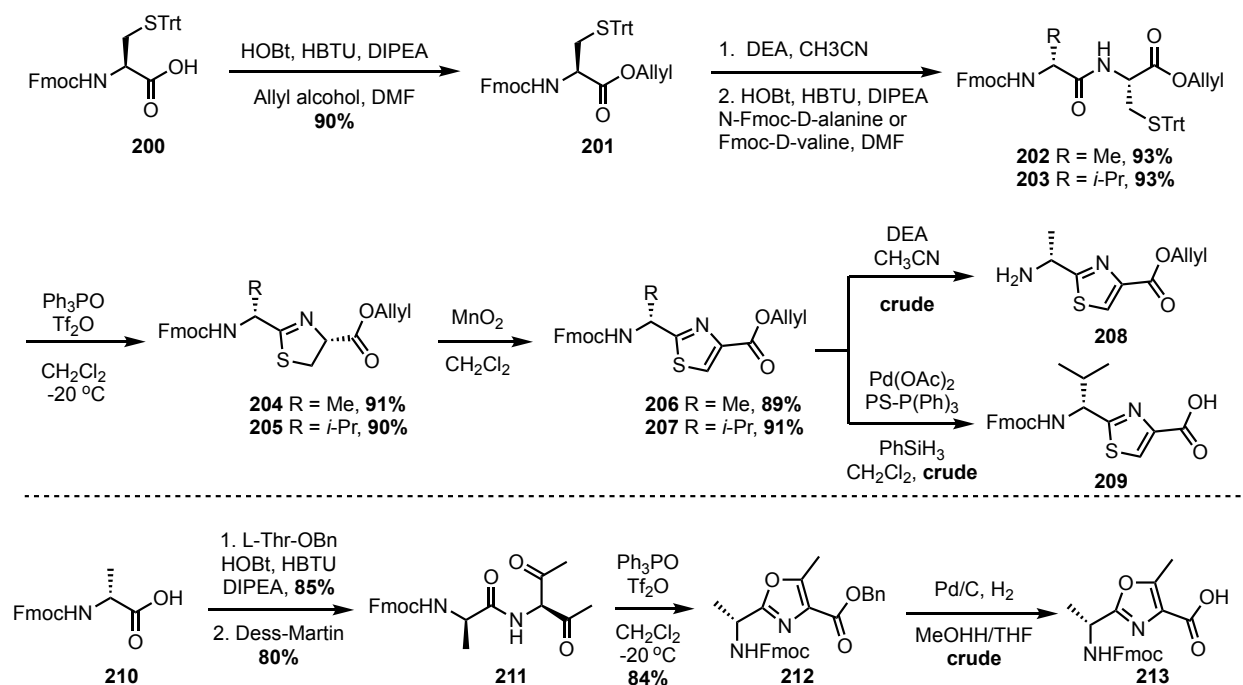
EDC/HOAt, resulting in the formation of hexapeptide **199** in 64–72% yield. Smith also reported a low yield of **199** when attempting the coupling under HOBt instead of HOAt, suggesting that HOAt might be a better choice for cyclizing sterically hindered macrocycles, such as the D-valine N-terminus. This improvement is attributed to HOAt's ability to form an additional hydrogen bond between the hydrogen of the free amine being incorporated into the activated ester and the nitrogen in HOAt's six-membered ring, thus forming a more stable intermediate. With **199** in hand, deprotections, followed by macrocyclization was performed under DPPA to form the acyl-azide intermediate, which Smith had predicted as the lowest energy conformation and with the closest distance between each terminus. After macrocyclization, dendroamide A was obtained in 56% yield, along with a potential isomer (18%). The molecular model predictions were confirmed by the total synthesis of dendroamide A; however, this conclusion holds only when the acid is activated using DPPA. Other peptide coupling reagents have successfully facilitated macrocyclization of dendroamide A through a different C- and N-terminus.



Scheme 2.30. Final synthetic steps towards the total synthesis of dendroamide A

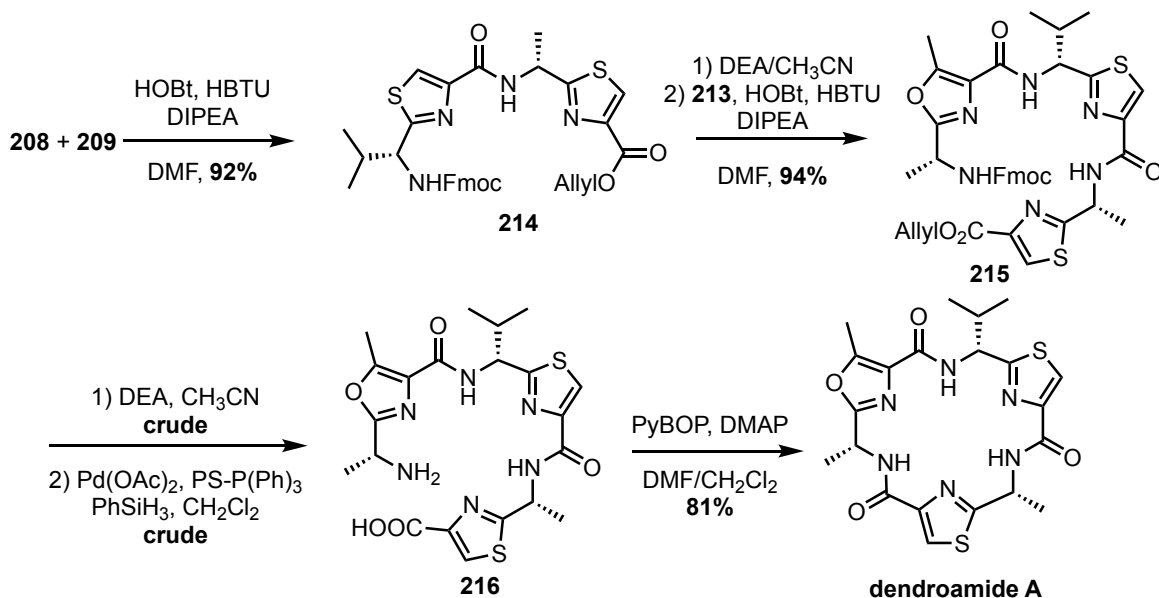
2.4.3 You and Kelly's Total Synthesis of Dendroamide A (2003)

After Kelly and You developed a new biomimetic methodology to access thiazolines via oxodiphosphonium salts¹²⁸ (**Scheme 2.9**), they applied this method to the synthesis of both thiazole fragments of dendroamide A in 2003, and subsequently to the synthesis of bistratamide E through J in 2004 and 2005, as detailed above. The synthesis initiated with the protection of Fmoc-L-tritylcysteine by coupling it with allylic alcohol under HBTU/HOBt conditions, followed by Fmoc deprotection and coupling of Fmoc-D-alanine or Fmoc-D-valine to yield both dipeptides (**202** and **203**) in 93% yield (**Scheme 2.31**). Next, the application of the oxodiphosphonium salt induced cyclization of the dipeptides to form thiazolines **204** and **205**, followed by radical oxidation to yield both thiazoles **206** and **207** in 89% and 91% yield, respectively. The oxazole fragment was successfully synthesized by coupling Fmoc-D-alanine with L-threonine benzyl ester, followed by Dess-Martin oxidation to form diketone **211** in 80% yield. Finally, cyclization was induced by the same oxodiphosphonium conditions, resulting in oxazole **213** in 84% yield.



Scheme 2.31. Synthesis towards dendroamide's A three fragments via cysteine

With the three fragments in hands, Kelly and You coupled each fragment in high yields under standard peptide coupling conditions and multiple basic and catalytic deprotections to access hexapeptide **216** (**Scheme 2.32**). Finally, macrocyclization under PyBOP and DMAP accessed dendroamide A in 81% yield.

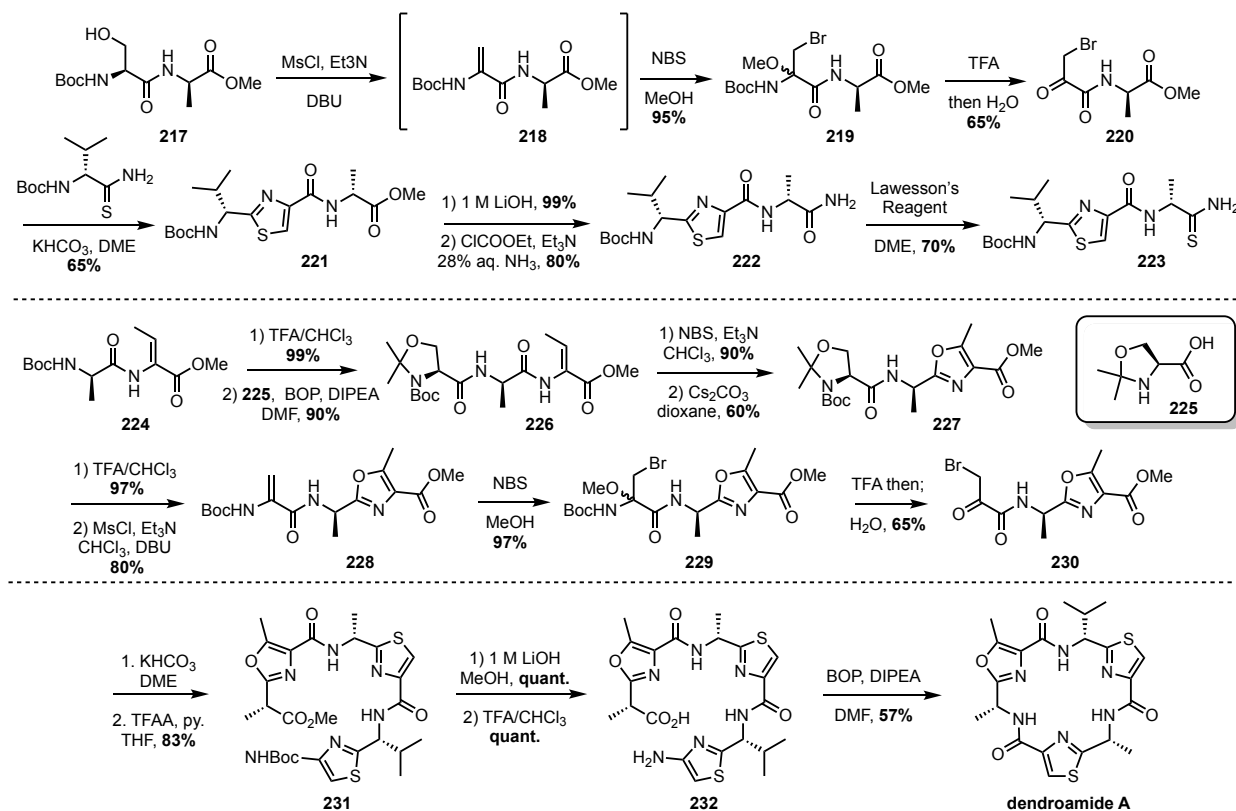


Scheme 2.32. Final steps towards the total synthesis of dendroamide A

2.4.4 Shin's Total Synthesis of Dendroamide A (2005)

Shin and co-workers¹⁴¹ applied Yonezawa's dehydro-oligopeptides methodology of bistratamide H synthesis for the total synthesis of dendroamide A. However, for the synthesis of dendroamide A, all heterocycle substituents arise from D-amino acids including, two D-alanine and one D-valine (**Scheme 2.33**). In contrast, bistratamide H is an all L-valine cyclamide. Additionally, this methodology was also applied to the synthesis of bistratamide G by the same group¹³³ and Bistratamide J, E, & H by Sato and co-workers¹³⁴ in 2008. Thus, the methodology is also applicable to D-amino acids, in which most of the intermediates were achieved from moderate

to excellent yields (65-97%). The point of macrolactamization was performed at (D-valine)thiazole N-terminus and (D-ala)oxazole C-terminus to achieved dendroamide A in 57% yield.

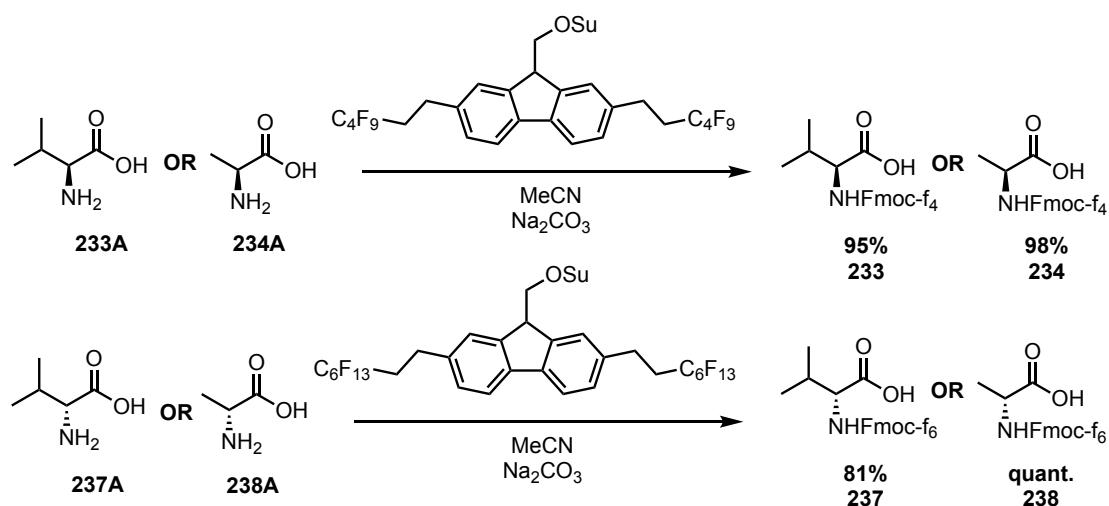


Scheme 2.33. Total synthesis of dendroamide A through dehydropolymers

2.4.5 Matsugi's Total Synthesis of Dendroamide A (2015)

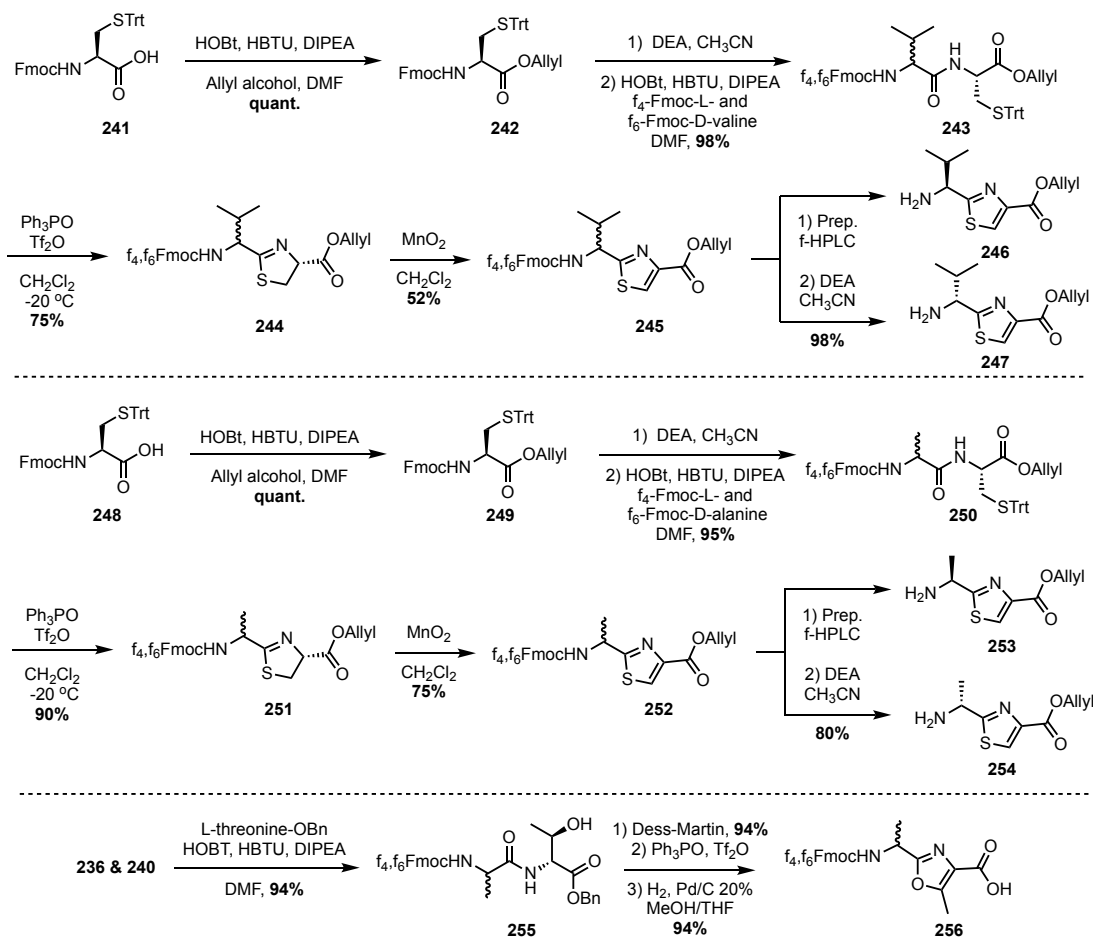
Matsugi's and co-workers accessed dendroamide A and all seven stereoisomers via a liquid-phase fluororous mixture synthesis by incorporating fluororous-Fmoc protecting groups on each intermediate.¹⁴² In contrast with Takeuchi's liquid-liquid extraction¹³¹ by incorporating fluororous boc protecting group to access the total synthesis of bistratamide H, the fluororous tagged molecules can be easily separated from non-fluororous organic molecules via a fluororous solid-phase extraction (FSPE) when using fluororous silica gel, additionally the mixture of stereoisomers can be separated by fluororous HPLC when a mixture of enantiomers encoded with different f-Fmoc tags are present.

Thus, providing a novel methodology to reduce the number of synthetic steps when synthesizing a library of cyclamide's stereoisomers via a mixture of L- and D-amino acids. Additionally, Matsugi first applied this methodology in 2013 for the total synthesis of tenuecyclamide A and its epimer tenuecyclamide B. The first step in the synthesis towards dendroamide A, was to protect the L- and D-amino acids in dioxane with a f-Fmoc reagent containing a C₄F₉ or C₆F₁₃ tags as shown in **Scheme 2.34**. This will give to each enantiomer the proper physical characteristic to be easily extracted by FSPE and separated by f-HPLC.



Scheme 2.34. f₄ & f₆-Fmoc protection of amino acids **233**, **234**, **237** & **238**

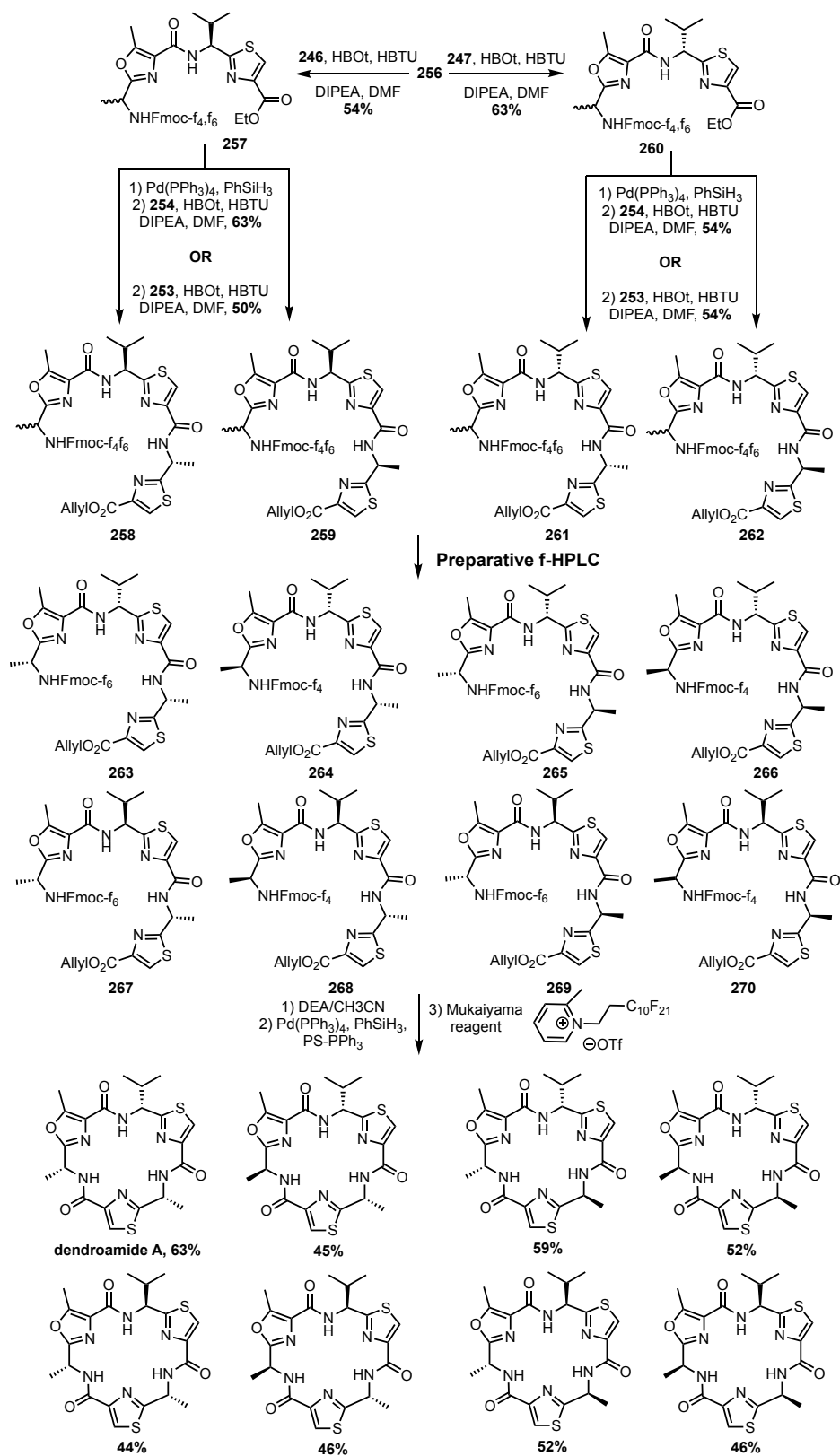
The synthesis towards the thiazole and oxazole moieties was performed by applying Kelly's biomimetic methodology to access thiazolines and oxazolines, in this case, one batch containing a mixture of the L- and D-valines and a second batch containing a mixture of L- and D-alanine was pushed toward the synthesis of the (S) and (R) thiazole's mixtures (**Scheme 2.35**). Throughout the synthetic pathway multiple intermediates were purified by FSPE to separate the non-tagged organic molecules, followed by a preparative f-HPLC purification to successfully achieved enantiomerically pure thiazoles. Subsequently, f-Fmoc deprotection of all four thiazoles accessed free amines **246**, **247**, **253**, & **254**.



Scheme 2.35. Synthesis of optically pure thiazoles **246**, **247**, **253**, **254** and oxazoline **256**

On the other hand, the enantiomeric mixture of the oxazole was coupled with a combination of optically pure L- and D-valine/alanine thiazoles, successfully achieving four hexapeptides (**258**, **259**, **261**, & **262**), each containing an enantiomeric mixture of the ala(oxazole) (**Scheme 2.36**). With the hexapeptides in hand, preparative f-HPLC was employed to separate each enantiomer, followed by deprotection and macrolactamization using a derivative of the Mukaiyama reagent to access dendroamide A and all seven stereoisomers in yields ranging from 63% to 44%. For all fragment couplings, a combination of HOBt and HBTU was used, while f-Fmoc deprotection was

carried out under basic conditions with excess DEA. For allylic deprotection, a catalytic reaction with 20% Pd/C in H₂ or Pd(OAc)₂, PS-PPh₃, and PhSiH₃ was performed.



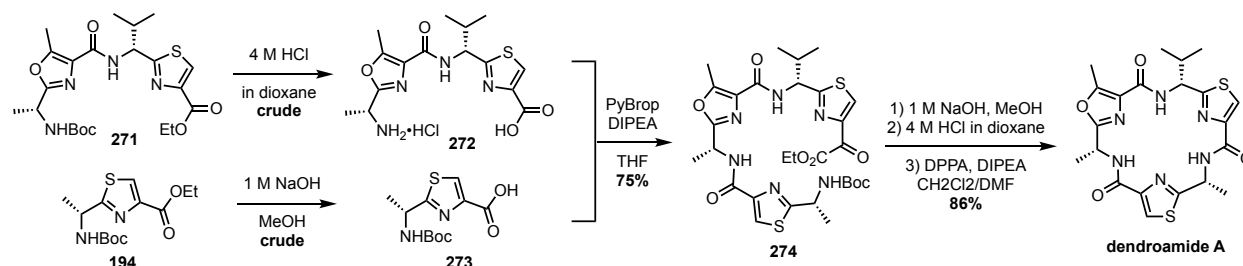
Scheme 2.36. Total synthesis of dendroamide A and its seven stereoisomers

2.4.6 Maezaki's Total Synthesis of Dendroamide A (2021)

The total synthesis of dendroamide A was also achieved by Maezaki and co-workers¹⁴³ in 2021, as part of their efforts towards synthesizing multiple analogs of the molecule. Maezaki aimed to test the macrolactamization hypotheses proposed by Shioiri² in 2007 and Smith¹⁴⁰ in 2001. Smith had suggested that the optimal point for cyclization to access dendroamide A was at the N-terminus of (D-ala)oxazole and the C-terminus of (D-ala)thiazole due to their proximity. He also proposed a potential failure in macrocyclization through the N-terminus of (D-ala)thiazole and the C-terminus of (D-val)thiazole. These hypotheses were derived from multiple analyses using molecular models that measured the distance between the cyclization points of each hexapeptide precursor. Smith and his team confirmed this hypothesis by successfully synthesizing dendroamide A via ring closure at the (D-ala)oxazole and (D-ala)thiazole termini but did not explore other cyclization points. In contrast, Shioiri and co-workers conducted several conformational analyses of linear hexapeptide precursors with three different macrocyclization points. They found that each N- and C-terminal pair was within the 3.43–3.93 Å range, suggesting that dendroamide A could potentially undergo macrolactamization at any of these termini points. Subsequently, Maezaki and co-workers decided to synthesize dendroamide A by performing macrolactamization at the cyclization point proposed by Smith as the potential failure site (**Scheme 2.37**).

For the total synthesis of dendroamide A, the oxazoline and thiazole moieties were synthesized using the same conditions as those employed by Smith (**Scheme 2.29**) and Wipf & Miller for oxazole synthesis, utilizing the Burgess reagent and NiO₂. The coupling of each fragment to form the hexapeptide **274** precursor was successfully achieved through multiple deprotections using NaOH and 4 M HCl in dioxane, followed by condensation reactions with PyBrop in DIPEA, all getting high yields. For the macrocyclization step, Maezaki applied the same

conditions as Smith, using DPPA/DIPEA to generate an acyl azide group, which induced the final cyclization to access dendroamide A in 86% yield. This synthesis confirmed Shioiri's hypothesis, demonstrating that macrocyclization at any of the proposed points successfully led to the total synthesis of dendroamide A.



Scheme 2.37. Macrolactamization of **274** to access dendroamide A

2.5 The Dolastatins Family

Most members of the dolastatin family are linear molecules, however, for the purpose of this review, only the cyclic members were selected based on the proposed guidelines for molecule selection. Two members of the family meet the selection criteria including dolastatin E and dolastatin I, both isolated by Yamada and co-workers^{144,145} in 1995 and 1997, respectively. Both molecules contain an oxazole and a thiazole, but dolastatin E features a thiazoline, while dolastatin I has an oxazoline (**Figure 2.5**). The synthetic efforts for these molecules were among the earliest explored by the same group that isolated the family, with a preference for late-stage incorporation of the azolines.

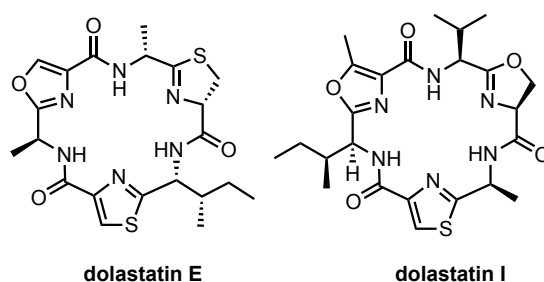
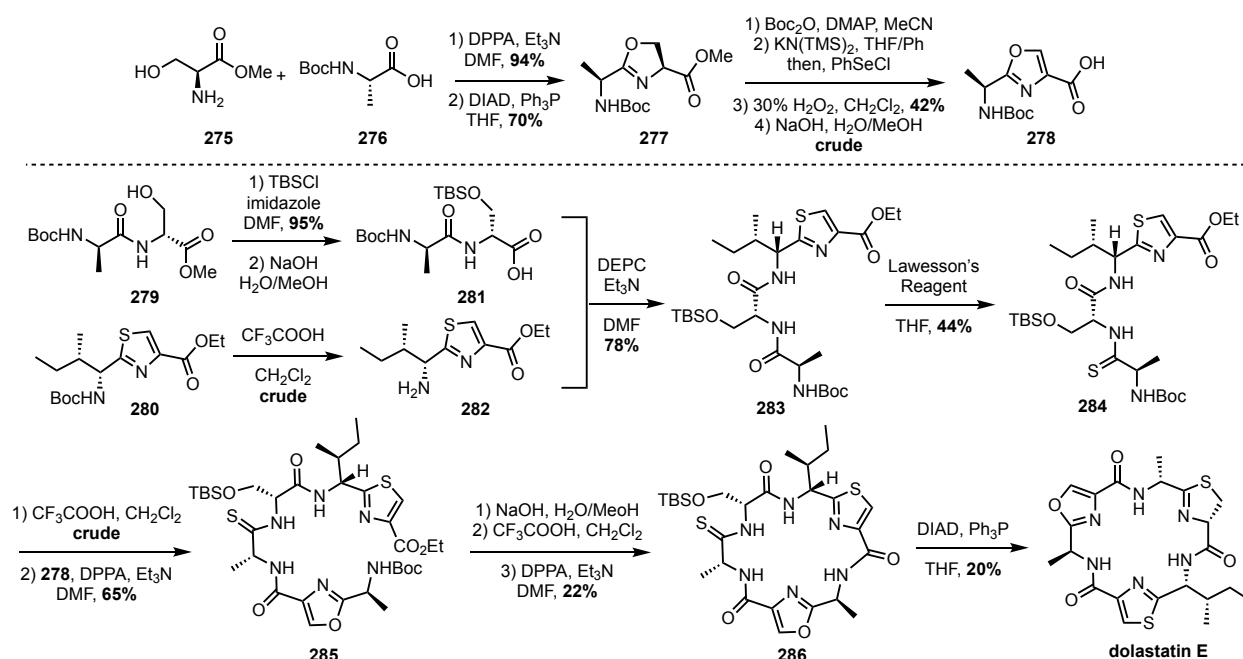


Figure 2.5. Chemical structures of the two hexacyclic dolastatin

2.5.1 Yamada's Total Synthesis of Dolastatin E (1995)

Yamada and co-workers were the first to isolate dolastatin E in 1995 from *Dolabella auricularia*. The stereostructure was confirmed after synthesizing the eight possible stereoisomers of dolastatin E and matching them with the spectroscopic data of the natural product.¹⁴⁶ Yamada proposed a late-stage cyclodehydration of the thioamide to form the thiazoline via a Mitsunobu reaction (**Scheme 2.38**). The synthesis of the (Boc-L-ala)oxazole-OH (**278**) began with the coupling of Boc-L-alanine and L-serine methyl ester, followed by β -hydroxyamide cyclodehydration to form oxazoline **277** in 70% yield. Oxidation of **277** with 30% H_2O_2 in CH_2Cl_2 gave **278** in 42% yield. The thiazole fragment was synthesized in 69% overall yield over four steps by applying Meyer's modified Hantzsch reaction conditions used in bistratamide C synthesis¹²³ (**Scheme 2.6**). Dipeptide **279** was first TBS-protected, followed by saponification and coupling with (D-alloiso)thiazole **282** to form pentapeptide **283** in 78% yield. The Lawesson's reagent successfully formed thioamide **284** in 48% yield. Boc and TBS deprotections of **284** were followed by coupling with (L-ala)oxazole-OH (**278**) and DPPA in Et_3N to achieved hexapeptide **285** in 65% yield. Head-to-tail macrolactamization under DPPA and Et_3N achieved **286** in 22% yield, followed by late-stage cyclodehydration of the thioamide via the Mitsunobu reaction, leading to the first total synthesis of dolastatin E in 20% yield.

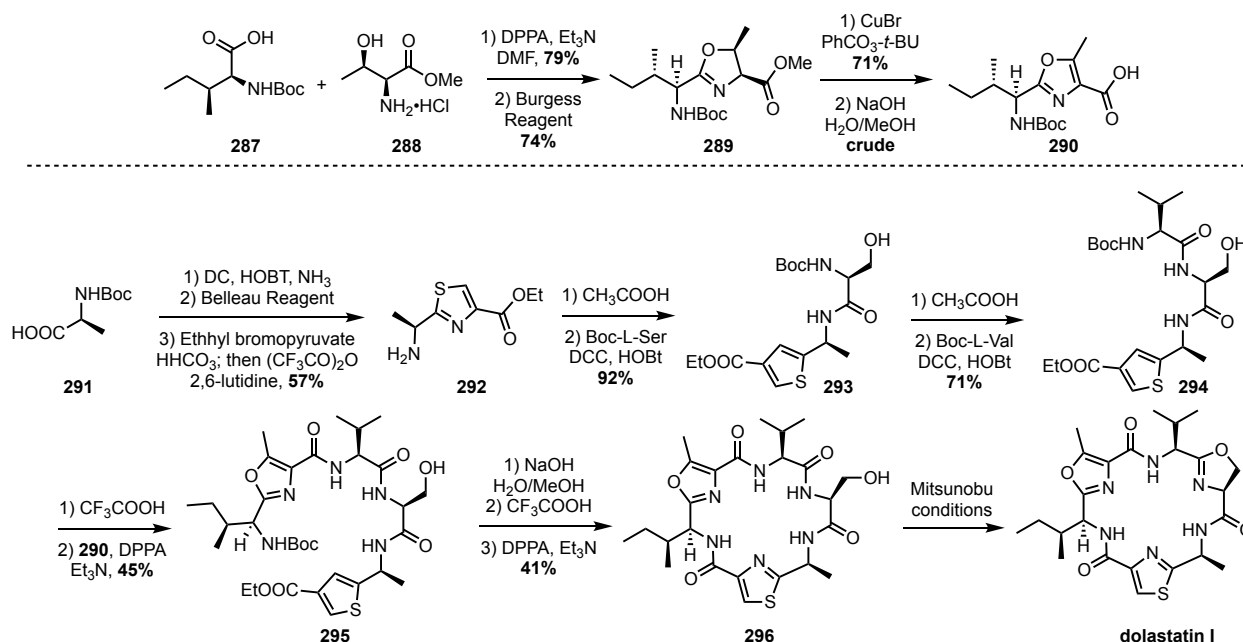


Scheme 2.38. Total synthesis of dolastatin E via late-stage thioamide cyclodehydration

2.5.2 Yamada and Kigoshi's Total Synthesis of Dolastatin I (1999)

In contrast to dolastatin E, the stereostructure of dolastatin I was confirmed after isolation from *Dolabella auricularia* via chiral HPLC analysis, which revealed all L-amino acids. Yamada also reported the first total synthesis of dolastatin I to support the stereochemical structure of the natural product¹⁴⁷ (**Scheme 2.39**). The synthesis of the oxazole **290** fragment began with the coupling of Boc-L-ile and L-thr-OMe, followed by β -hydroxyamide dehydrocyclization and oxidation with CuBr and PhCOOO-t-Bu, achieving the (L-ile)oxazole in 42% overall yield over three steps. The (L-ala)thiazole unit was synthesized through amidation of Boc-L-alanine, followed by thioamide formation using the Belleau reagent and a modified Hantzsch methodology to access thiazole **292** in 57% yield over three steps. A linear synthetic pathway, incorporating single amino acid couplings, produced tetrapeptide **294** after adding Boc-L-serine and Boc-L-valine in 92% and 71% yields, respectively. After Boc deprotection, coupling with oxazoline

formed hexapeptide **295** in 45% yield. Finally, macrocyclization under DPPA/Et₃N and a late-stage β -hydroxyamide dehydration via Mitsunobu conditions gave dolastatin I in 33% overall yield over four steps.



Scheme 2.39. Total synthesis of dolastatin I via late-stage amide cyclodehydration

2.6 Conclusion

The synthetic efforts toward the total synthesis of haxacyclamide natural products have been extensively explored over the past three decades due to the challenges associated with forming each heterocyclic ring and the therapeutic potential they could offer. Various methodologies have been developed to access the azole and azoline rings, providing diverse synthetic pathways for the construction of cyclamides. One approach described is the chemo- and enantioselective cyclodehydration of β -hydroxythioamides, initially explored by Kelly and co-workers. This biomimetic procedure efficiently converts cysteine-derived thiazolines and thiazoles, avoiding sulfur incorporation into amides by other techniques. In addition, Yamazawa and co-workers established a method to avoid the use of condensing reagents for heterocycle coupling by

synthesizing dehydro-oligopeptides, which were then converted into β -bromo diketones. These diketones could be reacted with thioamides, offering a novel synthetic pathway for haxacyclamide syntheses. Pattenden and co-workers also extensively studied macrocyclic peptide formation through cyclo-oligomerization techniques. However, challenges remain in controlling the reaction to prevent the formation of a mixture of macrocyclic peptides, and further research into metal (ion) chemical characteristics is needed to improve the control over heterocycle couplings.

Efforts to enhance the extraction and purification of haxacyclamides have also been explored. Takeuchi and Nakamura (2006), along with Matsugi and co-workers (2015), advanced synthetic steps and purification methods by introducing fluororous protecting groups to the heterocyclic ring, facilitating purification through liquid-liquid extraction with perfluorohexane (FC-72). Matsugi's method also involved the use of fluororous-Fmoc protecting groups, allowing the purification of multiple enantiomeric mixtures via f-HPLC to access the final target molecules and seven additional stereoisomers. This approach has been successfully applied to the total synthesis of all stereoisomers of tenuacyclamide A and dendroamide A. Finally, Liu and co-workers proposed the enantioselective one-pot Staudinger-aza-Wittig oxidation reaction for thiazole synthesis. This method was first used for the synthesis of venturamide A and B in 2018, and more recently for the synthesis of bistratamides H and J in 2024.

Chapter 3 - First Total Synthesis of Balgacyclamide A & B and Synthetic Efforts towards Balgacyclamide C

3.1 Retrosynthetic Analysis

We propose that balgacyclamide A could be accessed from B via a late-stage β -hydroxyamide catalytic cyclodehydration (**Figure 3.1**). This synthetic transformation will require retention of the threonine moiety β -carbon stereocenter; thus, requiring specific cyclization conditions as highlighted in chapter 2 and discussed in this chapter (**Figure 3.2**). The assembly of balgacyclamide B is envisioned to arise from the coupling of three main fragments, i. (D-*allo*-ile)thiazole (**13**) ii. (L-val)oxazoline (**298**), and iii. β -hydroxyamide (**297**). The thiazole can be accessed from L-serine and D-*allo*isoleucine coupling with a subsequent dehydrocyclization, followed by a tandem sulfur incorporation. The oxazoline fragment could arise from coupling of L-*allo*threonine and L-valine, followed by a β -hydroxyamide dehydrocyclization. Lastly, **3** could be access via the coupling of L-threonine and D-alanine.

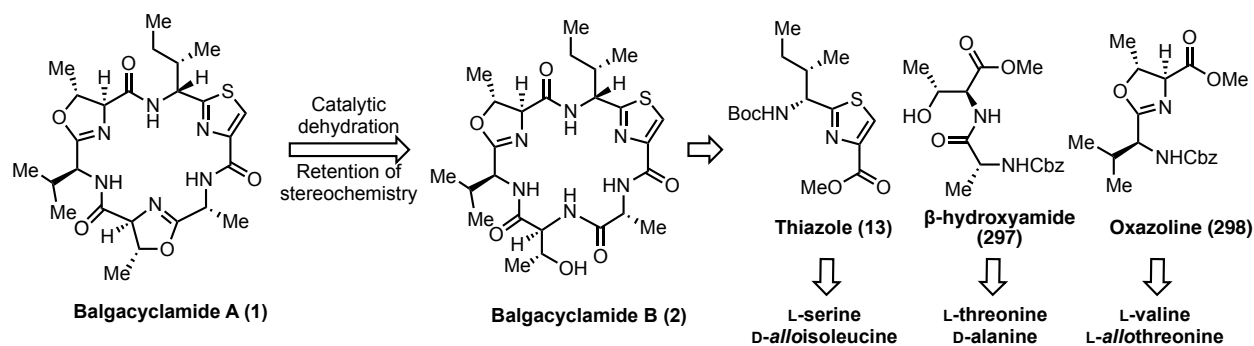


Figure 3.1. Retrosynthetic analysis of balgacyclamide A & B

It is worth noting that balgacyclamide A and B contains two L-threonine amino acids present within (L-val)oxazoline (**298**) and (D-ala)oxazoline rings (balgacyclamide A) and β -

hydroxyamide (**297**) (balgacyclamide B). However, in our retrosynthetic pathway for balgacyclamide B, the application of nonproteinogenic amino acid L-*allo*threonine is needed for the construction of the (L-val)oxazoline (**298**) due to epimerization about β -hydroxy carbon under the employed dehydrocyclization conditions.

3.2 Total Synthesis of Balgacyclamide B

3.2.1 Oxazoline Synthesis

In 1993, Miller and Wipf, confirmed a complete interconversion of L-threonine containing peptides to L-*allo*threonine derivatives.¹²⁶ In this interconversion, the alcohol within L-threonine is activated by the Burgess reagent and is subsequently attacked by the oxygen of the carbonyl to afford the oxazoline ring with the β -carbon of the former threonine epimerized (**Figure 3.2-A**). Treatment of the newly formed oxazoline under acid conditions afforded the corresponding L-*allo*threonine. Thus, further confirming the Walden inversion about this center under Burgess cyclization conditions. In 1995, Lellouche developed an improved methodology towards oxazoline ring formation that was inspired by Jones and co-workers' methodology of inverting the configuration of β -hydroxy amides via dehydrative cyclization with (Diethylamino)sulfur trifluoride (DAST), followed by ring opening with strong acidic conditions (**Figure 3.2-B**).^{148,149} In 2000, Williams and co-workers' brought multiple examples of oxazoline syntheses through β -hydroxy amides using deoxo-fluor or DAST.¹⁵⁰ Finally, in 2005, Ishihara and co-workers reported a first and new methodology where retention of configuration at the threonine β -carbon to form the oxazoline was accessed after exposing the β -hydroxy amides under harsh conditions using molybdenum oxides as a catalyst in toluene (**Figure 3.2-C**).¹⁵¹ These previous methodologies have been used to access multiple NPs, for example, fimsbactin A and B, mycobactin J, (R)-D-ornithine

derivative BE-70016, pseudobactin, lissoclinamide, leupyrrin B1, madurastatin C1, bistratamide D, bistratamide E, bistratamide F, aerucyclamide B, among others.^{13,114,118,128,128,152–156}

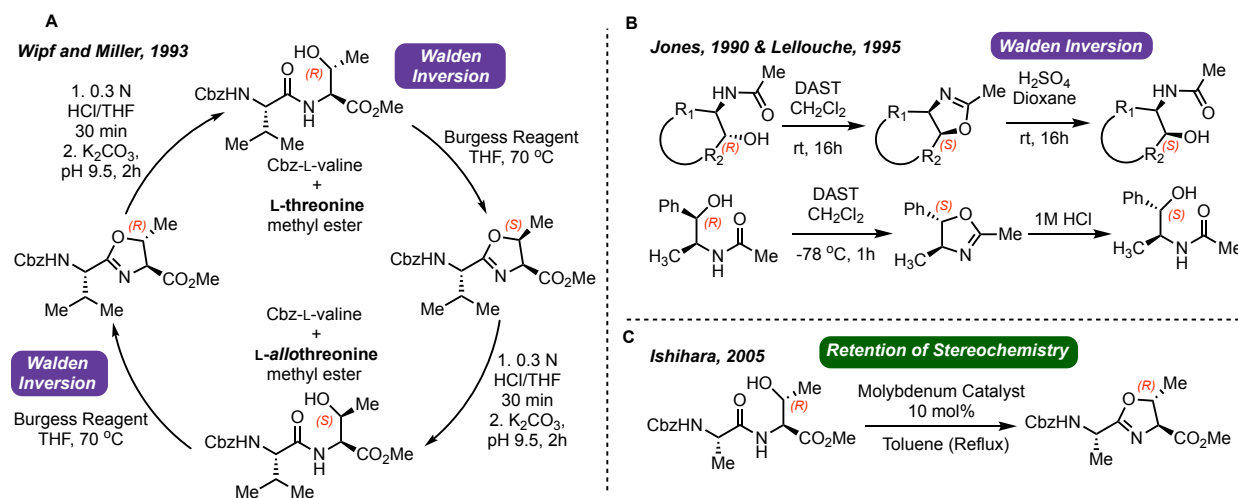
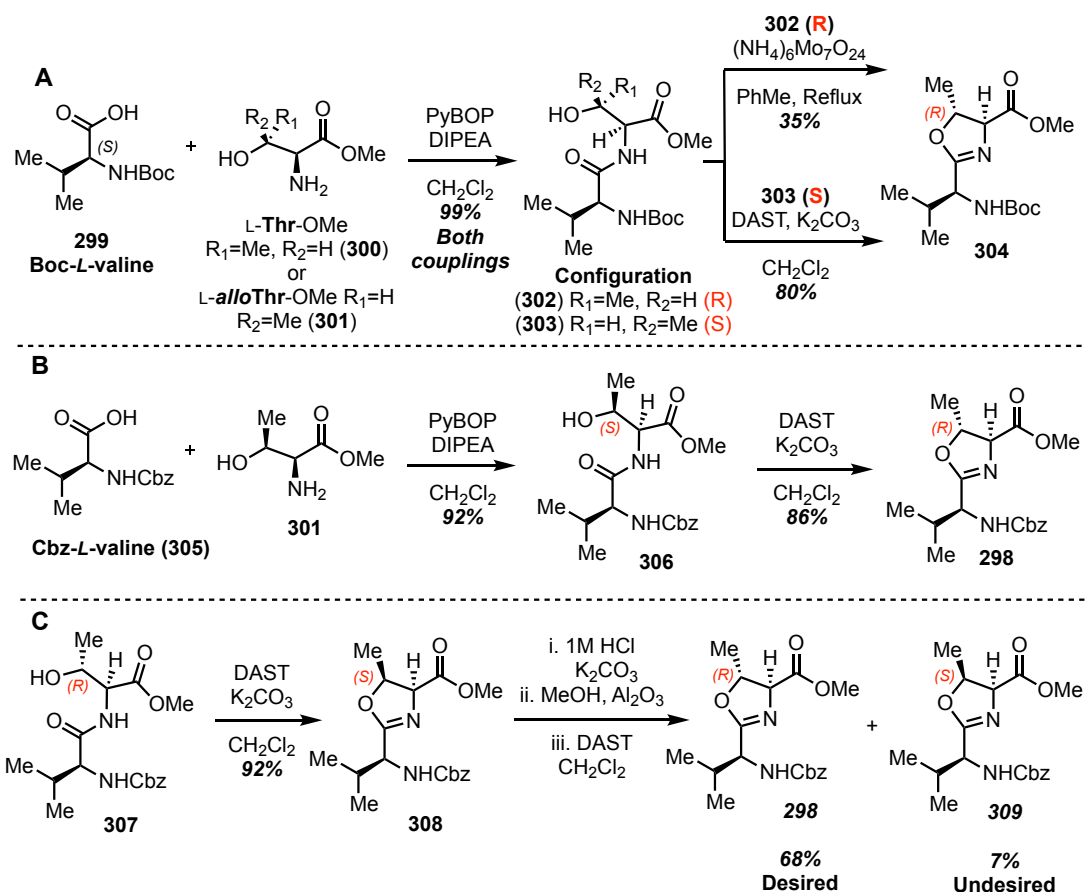


Figure 3.2. State of the art in oxazoline ring construction. Epimerization about the β -carbon of threonine versus retention based on conditions employed

Synthetic efforts started with the northern fragment synthesis of balgacyclamide B, specifically the (L-val)oxazoline synthesis. First, construction **304** was attempted by coupling Boc-L-valine (**299**) and L-threonine methyl ester (**300**) in 99% yields with PyBOP and DIPEA. This was followed by catalytic dehydration via (NH₄)₆Mo₇O₂₄·4H₂O to access (L-val)oxazoline (**304**) in 35% yields with retention of configuration (R) at the threonine β -carbon (**Scheme 3.1-A**). Multiple conditions were explored to optimize the catalytic dehydration reaction (solvent, concentration, equivalence, temperature); unfortunately, none of these conditions improved our yield. For early steps on a synthetic pathway, 35% yield is not a convenient start, thus we decided to synthesize an epimer of β -hydroxyamide **302** to invoke a Walden inversion at the threonine β -carbon center to access **304**. For this, L-allothreonine methyl ester (**301**) and **299** were coupled under PyBOP conditions in 99% yield, followed by dehydration of **303** with DAST and K₂CO₃ at lower temperatures, to access **304** in 80% yields (**Scheme 3.1-A**). Even though our new pathway

was successful with higher yields, when we tried to perform the Boc deprotection of **304**, it underwent ring opening due to strong acidic conditions, and multiple side products were observed. This is speculated to occur under the strong acidic conditions to allow oxazoline ring opening due to protonation of the amine resulting in ring opening. To avoid this, the protecting group was changed to Cbz as they can be deprotected under non-acidic conditions. In this, the reactions performed were the same as in accessing **304** and afforded the Cbz-protected oxazoline **298** in 86% yield. (**Scheme 3.1-B**). To avoid the use of nonproteinogenic amino acid L-*allo*threonine methyl ester, we attempted Miller and Wipf conditions to achieve L-*allo*threonine from L-threonine; however, when oxazoline epimer **308** was exposed to acidic conditions for ring opening, followed by dehydration conditions with DAST, we accessed an inseparable mixture of the desired product **298** in 68% and starting material **309** in 7% by on NMR characterization (**Scheme 3.1-C**). We speculate this could be due to the aluminum oxides employed, in the original paper this reagent was prepared fresh while we employed commercially available material.

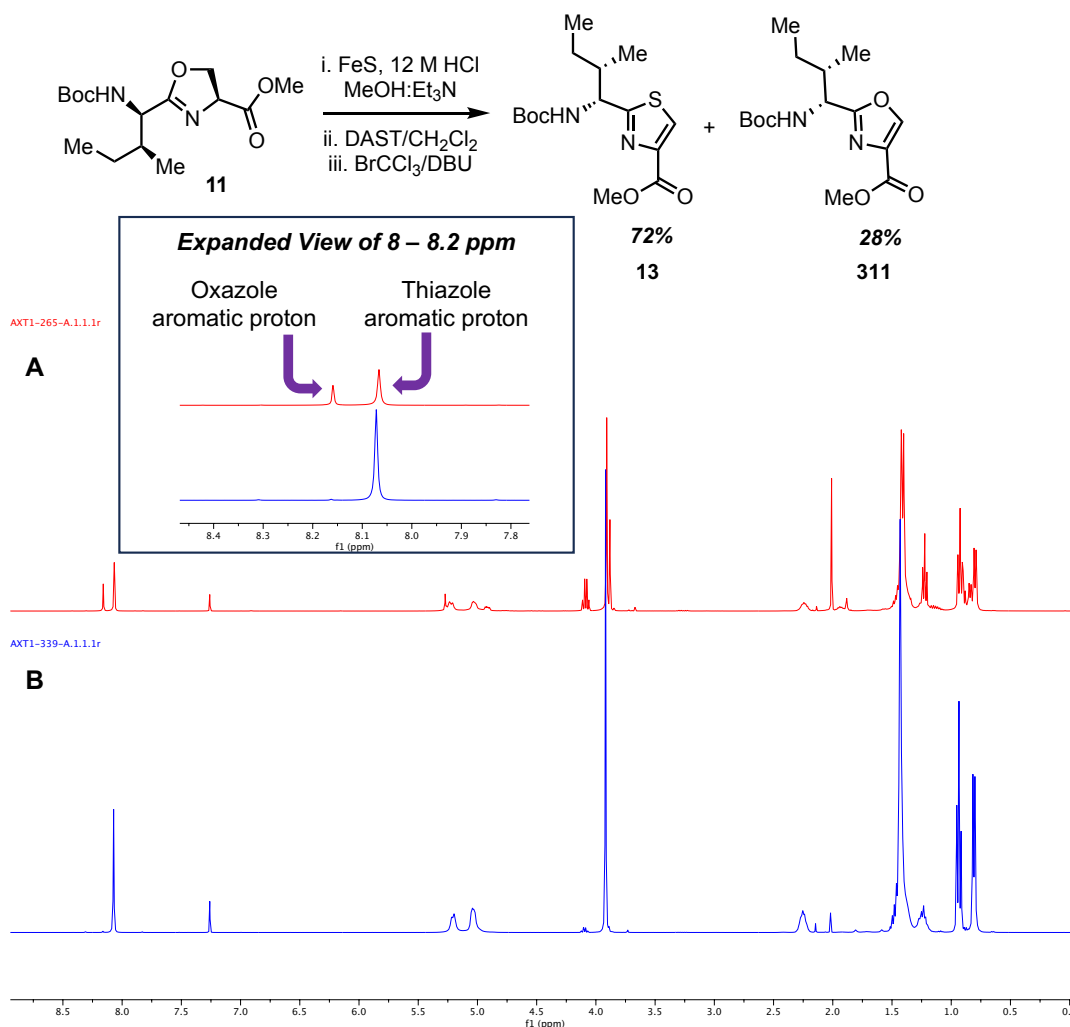


Scheme 3.1. Oxazoline ring synthetic efforts A) Boc protected oxazoline ring (19) synthesis via catalytic and non-catalytic dehydration with retention and inversion of configuration, respectively. B) Cbz protected oxazoline (6) synthesis. C) Miller and Wipf conditions attempt to access 6

3.2.2 Thiazole Synthesis

With **298** in hands, attention towards the construction of thiazole (**13**) was undertaken for the eventual coupling **13** with **298** accessing the upper fragment of balgacyclamide A & B (**Scheme 3.2**). First, commercially available amino acid *D*-alloisoleucine (**310**) was subjected to Boc protection with Boc_2O and NaOH to afford *N*-Boc-*D*-alloisoleucine (**8**) in quantitative yields. The coupling of **8** to L-serine methyl ester (**9**) was accomplished with PyBOP under standard conditions to afford dipeptide **10** in 87% yield. Treating **10** with DAST and K_2CO_3 in CH_2Cl_2 afforded the cyclized oxazoline **11** in 99% yield. Inspired by the total synthesis of aerucyclamide

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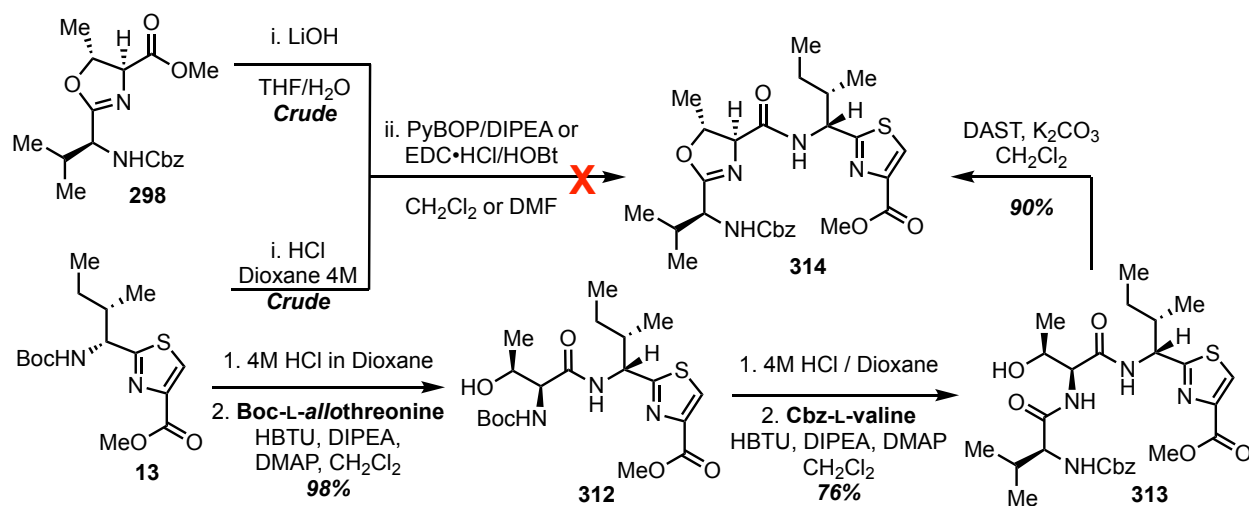


Scheme 3.3. Formation of the thiazole (**13**) from oxazoline (**11**) via *in situ* formation of H₂S and commercially available H₂S. A) Formation of **13** via *in situ* formation of H₂S resulting in the formation of oxazole **311** due to the presence of H₂O. B) Formation of **13** via commercially available anhydrous H₂S

3.2.3 Norther Fragment Synthesis: (L-val)oxazoline-(D-ala)thiazole

Next step was the coupling of **298** with the (D-ala)thiazole ring (**13**) via peptide coupling conditions after respective deprotection of both rings (**Scheme 3.4**). The coupling of free acid (L-

val)oxazoline and free base (D-ala)thiazole under peptide coupling conditions failed to achieve the northern fragment, under a variety of conditions. Given the unsuccessful coupling of the oxazoline and thiazole, we then decide to proceed in the construction of the oxazoline via a single amino acid coupling to **13** to access the upper fragment of balgacyclamide B. The proposed single amino acid coupling pathway has the same number of steps in comparison with the oxazoline and thiazole coupling pathway, which means that it will not affect our route length or sustainability. First, Boc deprotection of the thiazole under acidic conditions were performed followed by coupling of Boc-L-*allo*threonine with HBTU, DIPEA, and DMAP in 98% yield to access free alcohol **312**. With **312** in hand, similar conditions as the previous steps were applied, but the amino acid coupled was Cbz-L-valine to achieved **313** in 76% yield. Rather than using the Boc-L-valine, the Cbz version was chosen as Boc deprotection conditions have been reported to result in the oxazoline ring opening. Dehydration, cyclization, and epimerization of compound **25** were performed using DAST and K₂CO₃ to access **314** in 90% yield.

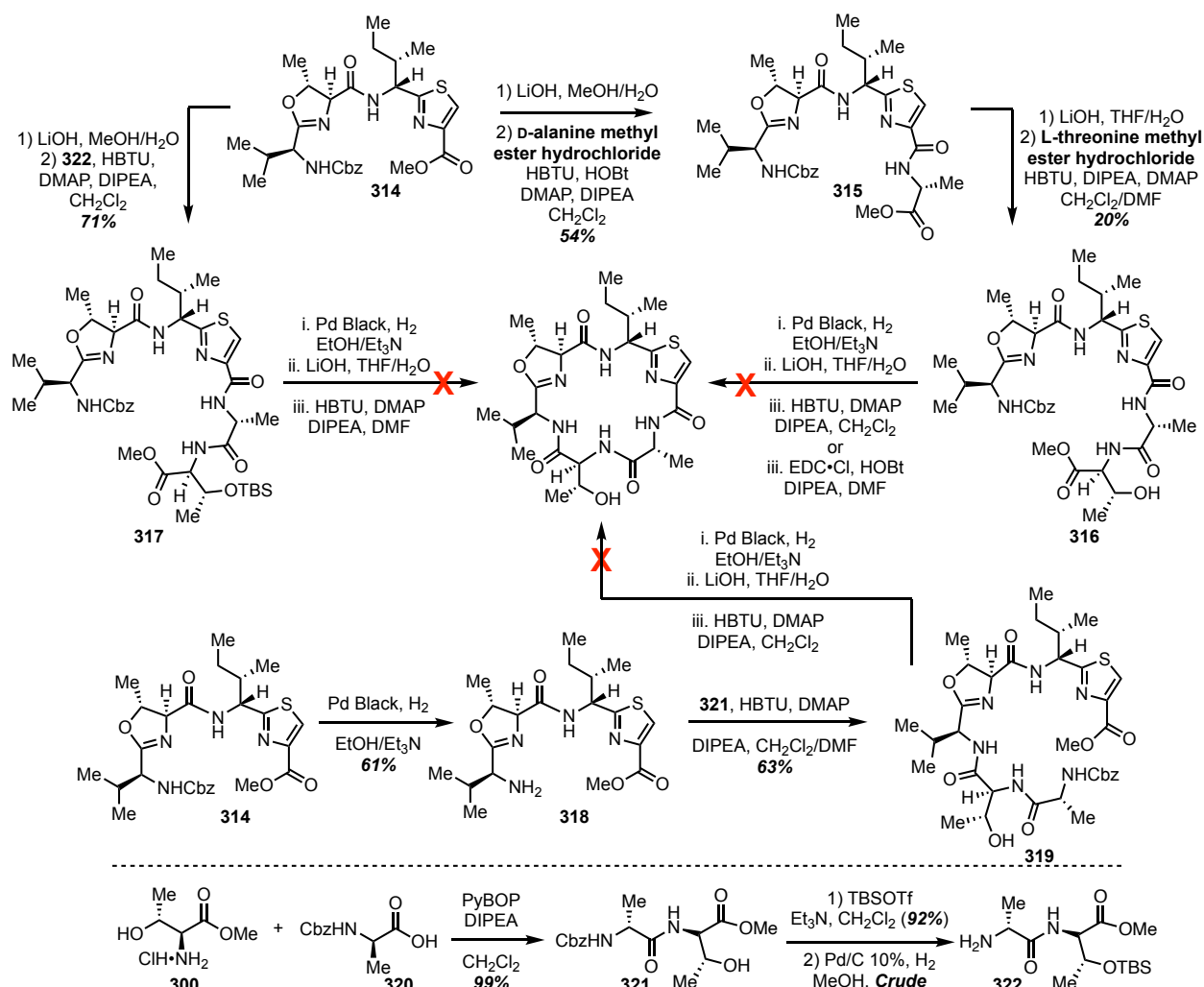


Scheme 3.4. Efforts towards northern fragment synthesis of balgacyclamide A and B via single coupling amino acid and oxazoline-thiazole peptide coupling conditions

3.2.4 Synthesis of Linear Hexapeptide Precursor and Macrolactamization Efforts

Once the upper fragment of balgacyclamide B was synthesized, efforts towards β -hydroxy amide installation and macrocyclization were undertaken. First, we envisioned a coupling of the lower fragment, β -hydroxy amide **321**, to the (D-*allo*-ile)thiazole or the (L-val)oxazoline fragments, followed by macrocyclization conditions to access the first total synthesis of balgacyclamide B (Scheme 3.5). The synthesis started with subjecting intermediate **314** to saponification conditions, LiOH in MeOH/H₂O, to access the free carboxylic acid, followed by coupling D-alanine methyl ester hydrochloride via HBTU, HOBt, DMAP, and DIPEA to access **315** in 54% yield. However, when **315** was saponified and coupled with L-threonine methyl ester hydrochloride, via similar conditions employed, only 20% of hexapeptide **316** was obtained. Conversion of **316** to the corresponding free amine and carboxylic acid via Pd black/H_{2(g)} and LiOH in THF/H₂O, respectively was performed in excellent yields. Unfortunately, the attempting macrocyclization via two different peptide coupling conditions failed to provide balgacyclamide B. Our first thought as to the failed macrocyclization was the presence of the free nucleophilic alcohol, which we thought could directly be interfering in the macrocyclization or via solvent interactions. This then prompted us to TBS protect the alcohol. Noting the poor 20% yield with the coupling of L-threonine methyl ester to the thiazole free acid, it was decided to attempt a direct dipeptide coupling in lieu of the single amino acid couplings. First, β -hydroxyamide **321** was obtained through coupling of **300** and **320** in 99% yield. The secondary alcohol was TBS protected using TBSOTf in 92% yield and subsequently treated with Pd/C 10% and H₂ to access the free base. Saponification of **314** following by the coupling of the free base **322** with HBTU afforded hexapeptide **317** in 71% yield. Unfortunately, subjecting **317** to the same condition upon **316** failed to provide balgacyclamide B. With the failed macrocyclization of the dipeptide free base upon the

oxazoline free amine, we decided to attempt the opposite macrocyclization via the free carboxylic acid of the (D-*allo*-ile)thiazole. Deprotection of the Cbz upon **314** and saponification of **321** afforded the free amine in 61% yield and the free acid as a crude material, respectively. The coupling of these units with HBTU, DMAP, and DIPEA afforded hexapeptide **319** in 63% yield. Macrocyclization of **319** under peptide coupling conditions failed to afford balgacyclamide B, just as the same attempts upon **316**. We hypothesize that conformational flexibility on **316** and **319** are playing a big role in these reactions, where the nucleophile (free amine) is not in close proximity to the activated ester (the former carboxylic acid), thus avoiding macrocyclization.



Scheme 3.5. Di peptide installation to upper fragment and efforts towards macrocyclization

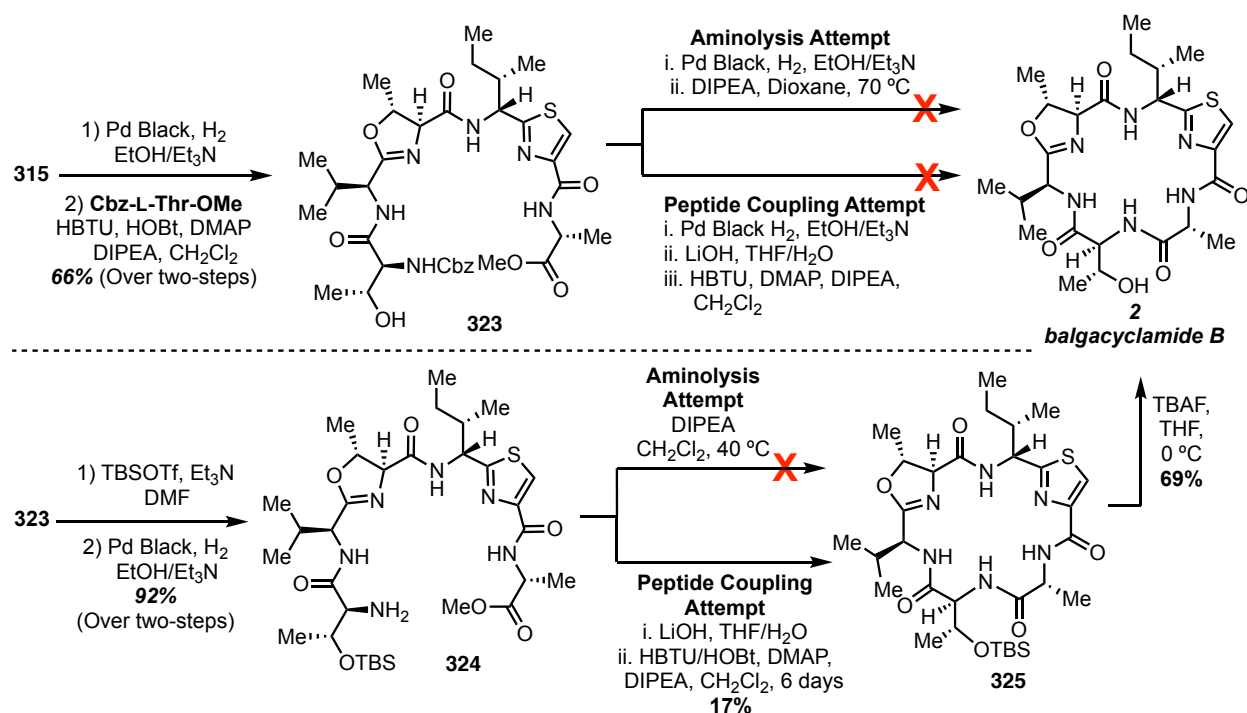
Multiple factors have been investigated into the successful, or failed macrocyclization of linear peptides. Herein, we explore ones of similar size and of amino acid composition. Of these, conformation of the amide bond and the general conformation of the pre-cyclized system is of interest. Microcyclamide MZ602 and MZ568 are two macrocycles that are structural comparable to the balgacyclamide family: possessing a thiazole ring and tetrapeptide unit in their macrocycles. In their efforts towards the total synthesis of these compounds, Du coupled the tetrapeptide unit upon the thiazole and then performed various peptide coupling based macrocyclization conditions, all of which failed to afford the desired product.¹⁵⁷ Noting the *cis/trans* isomerization of peptide bonds,¹⁵⁸ *cis* aiding in the head-to-tail cyclization, they explored incorporating a pseudoproline unit to impart rigidity in the system, as well as the *cis*-conformation. Cyclization with HATU, HOBt in CH₂Cl₂/DMF gave the macrocycle in 55% over three-steps (two deprotections and cyclization). In the synthesis of didmolamide A, containing two planar thiazole units and one oxazoline, Kelly's efforts focused on the construction of the dithiazole unit and the coupling of the dipeptide.¹⁵⁹ Cyclization with PyBOP, DMAP, DIPEA in CH₂Cl₂/DMF afforded the macrocycle in 56% yield (including deprotection steps). In the total synthesis of aerucyclamide B, also possessing two planar thiazole units and a dipeptide unit, Serra attempted the macrocyclization upon the incorporated dipeptide and via single amino acid incorporation on each side.¹¹⁴ The dipeptide macrocyclization afforded the desired cyclic system in 12%, but in the case of single amino acid incorporation on each thiazole a 40% cyclization was observed. In this work, the authors go on to state "that the selection of the point for macrolactam formation is relevant". Selecting the point within a desired macrocycle for its proposed synthesis is key. This is also shown in numerous other works, such as, but not limited to: bistratamide E and J, bistratamide D, and venturamide A and B. In addition to identifying the proper site for a macrocyclization, it has also

been shown that stereoisomers of the same linear peptide have variable cyclization yields. For example, Matsugi and co-workers, in their efforts towards tenuecyclamide A, they synthesized numerous stereoisomers of the same linear peptide and cyclized them at the same point with the Mukaiyama reagent; interestingly, the yields ranged from 50% to no product obtained.¹⁶⁰ All in all, determining the unique point that a linear peptide is macrocyclized is truly a case-by-case basis. With this in mind, and our failed cyclization attempts above, we continued to find the proper point for the macrocyclization of balgacyclamide B.

3.2.5 Final Steps towards Balgacyclamide B

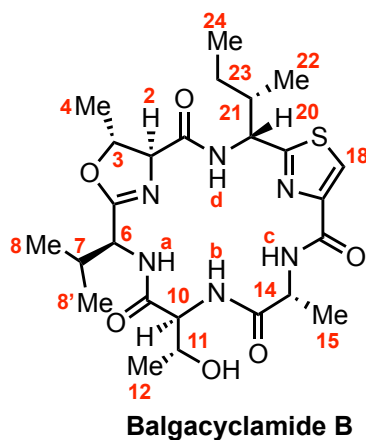
To overcome this assumed conformational constraint and to find the point of macrocyclization, efforts were directed towards installing a single coupling amino acid to each side of **315**. After coupling of Cbz-L-threonine on the oxazoline fragment and D-alanine on the thiazole, it is expected that conformation of the molecule maintains the nucleophile closer to the free carboxylic acid to access macrocyclization. Deprotection of the Cbz upon **315** under standard H₂ conditions afforded the oxazoline free amine in 82% yield, which was then coupled to L-threonine methyl ester with HBTU and HOBT to furnish hexapeptide **35** in 81% yield (**Scheme 3.6**). With **35** in our hands, we attempted aminolysis, as well as peptide coupling conditions, for macrocyclization. However, these conditions did not afford balgacyclamide B. To ensure that the secondary alcohol is not interfering in the macrocyclization, **35** was treated with TBSOTf followed by removal of the Cbz group under standard H₂ conditions to furnish **36** in 92% yield over two steps. The first macrocyclization attempt at accessing balgacyclamide B was via aminolysis of **36**. No desired product was obtained after a variety of temperatures were explored. Finally, macrocyclization via peptide coupling was attempted. Saponification of **36** followed by treatment with HBTU/HOBT, DMAP, and DIPEA afford the desired macrocycle in 17% yield over two

synthetic steps after 6 days. Subsequent TBS deprotection with TBAF was successfully performed in 69% yield giving rise to the first total synthesis of balgacyclamide B in a 17-steps pathway and a 2% overall yield. **Table 3.1** shows the comparison between natural and synthetic balgacyclamide's B ^1H NMR in $\text{DMSO}-d_6$.



Scheme 3.6. Balgacyclamide B final synthetic steps

Table 3.1. Comparison between natural and synthetic balgacyclamide B ¹HNMRs in DMSO-*d*₆.



H no.	Natural Balgacyclamide B [ppm, mult, J (Hz)] 800 MHz	Synthetic Balgacyclamide B [ppm, mult, J(Hz)] 600 MHz	Natural-Synthetic $\Delta\delta$ /ppm
2 (CH)	4.34 (1H, dd, 6.2,1.6)	4.34 (1H, dd, 6.1,1.8)	0.00
3 (CH)	4.84 (1H, dq, 6.2,6.2)	4.84 (1H, dq, 6.3, 6.2)	0.00
4 (CH ₃)	1.29 (3H, d, 6.2)	1.28 (3H, d, 6.2)	0.01
6 (CH)	4.43 (1H, ddd, 8.6,3.7,1.6)	4.43 (1H, ddd, 8.7,3.8,1.8)	0.00
7 (CH)	1.95 (1H, m)	1.95 (1H, m)	0.00
8 (CH ₃)	0.67 (3H, d, 6.9)	0.67 (3H, d, 7.0,1.9)	0.00
8' (CH ₃)	0.53 (3H, d, 6.9)	0.51 (3H, d, 7.0, 2.0)	0.00
NH (a)	6.95 (1H, d, 8.6)	6.95 (1H, d, 8.6)	0.00
10 (CH)	4.23 (1H, dd, 8.7, 3.5)	4.22 (1H, dd, 8.9, 3.5)	0.01
11 (CH)	4.19 (1H, m)	4.19 (1H, m)	0.00
12 (CH ₃)	1.07 (3H, d, 6.3)	1.06 (3H, d, 6.4,2.0)	0.01
OH	4.76 (1H, d, 5.6)	4.82 (1H, d, 5.22)	-0.06
NH (b)	8.47 (1H, d, 8.7)	8.54 (1H, d, 8.8)	-0.07
14 (CH)	4.80 (1H, m)	4.80 (1H, m)	0.00
15 (CH ₃)	1.30 (3H, d, 6.5)	1.29 (3H, d, 6.4)	0.01
NH (c)	8.22 (1H, d, 7.9)	8.22 (1H, d, 8.9)	0.00
18 (CH)	8.22 (1H, s)	8.23 (1H, s)	-0.01
20 (CH)	5.13 (1H, dd, 8.5,5.5)	5.13 (1H, dd, 7.5,5.4,1.9)	0.00
21 (CH)	1.91 (1H, m)	1.90 (1H, m)	0.01
22 (CH ₃)	0.90 (3H, d, 6.8)	0.89 (3H, d, 6.7)	0.01
23 (CH ₂)	1.44 (1H, m)	1.43 (1H, m)	0.01
23' (CH ₂)	1.11 (1H, m)	1.10 (1H, m)	0.01
24 (CH ₃)	0.88 (3H, dd, 7.3,7.3)	0.88 (3H, dd, 7.6, 7.6)	0.00
NH (d)	8.43 (d, 8.5)	8.50 (1H, d, 8.5)	-0.07

3.3 Total Synthesis of Balgacyclamide A

3.3.1 New Proposed Retrosynthetic Analysis

After the total synthesis of balgacyclamide B, our efforts focused to access balgacyclamide A. As noted in session 3.1 (**Figure 3.1**), balgacyclamide A could be access through balgacyclamide B through a molybdenum oxide catalytic β -hydroxy amide dehydrocyclization. It is worth noting that our previous efforts to access the individual oxazoline ring through a catalytic cyclodehydration by incorporation of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ induced a low yielding product of interest. On the other hand, we looked to avoid the use of harsh conditions like refluxing solvents like toluene or benzene. Thus, a new but similar retrosynthetic analysis was established. We propose that balgacyclamide A can be access through a β -hydroxy amide dehydrative cyclization and epimerization of *L*-allothreonine from balgacyclamide B epimer (**326**) (**Figure 3.3**). This transformation will require epimerization via the previous reagents mentioned. Intermediate **323**, could be approach through macrocyclization of hexapeptide **327**. The macrocyclization connection point was suggested based on our previous balgacyclamide B (**2**) total synthesis studies, where the best head-to-tail cyclization was from single coupling amino acid to the oxazoline and thiazole.¹⁶¹ Hexapeptide **327** could be achieved by coupling **314** with *L*-allothreonine and *D*-alanine upon each side of the scaffold. The assembly of the northern scaffold fragment (**314**) could arise through a Walden inversion of free alcohol **313**, to access the oxazoline moiety. Intermediate **313**, is envisioned to arise from the coupling of *L*-allothreonine and *L*-valine to thiazole **13**. Finally, **13** was accessed from the coupling of *D*-alloisoleucine and *L*-serine as reported in the total synthesis of balgacyclamide B.¹⁶¹

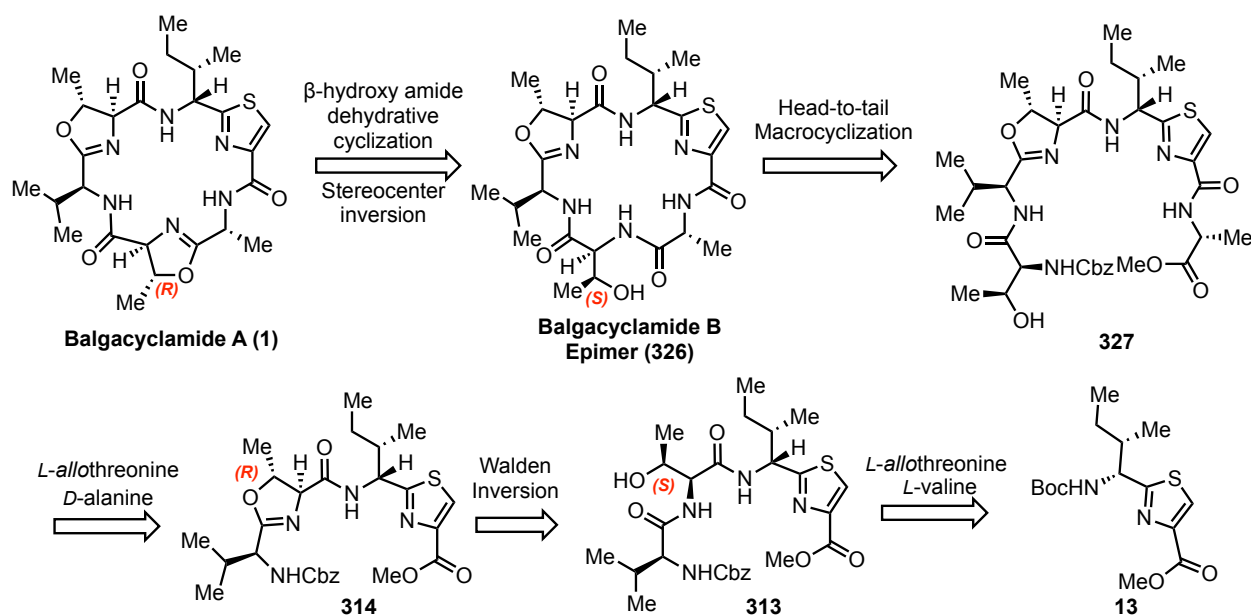


Figure 3.3. Retrosynthetic analysis of balgacyclamide A

3.3.2 Ugale and Gholap Total Synthesis of Balgacyclamide A

Throughout our efforts towards the total synthesis of balgacyclamide A & B, Ugale and Gholap reported the first total synthesis of balgacyclamide A in 2% overall yield via a solution phase fragments condensation pathway.¹⁶² In this pathway, they formed the macrocyclic intermediate **328** (**Figure 3.4-A**) from the coupling of the thiazole unit to the tetrapeptide *L*-Thr-*L*-Val-*L*-Thr-*D*-Ala. From this macrocycle they performed a late-stage β -hydroxyamide dehydrocyclization to access both oxazolines units under three different conditions using i) the Burgess reagent, ii) diethylaminosulfur trifluoride (DAST), and iii) PPh_3 and all conditions successfully achieved the final product in 50%, 32%, and 40% respectively. It is of great importance to note that intermediate **328** consists of two (2*S*,3*R*)-2-amino-3-hydroxybutyric acids (*L*-threonine) and their configuration at the β -carbon is a *R* configuration (**Figure 3.4-A**). Based on previous studies and the extensive review in chapter 2, it is well known that all three mentioned conditions induce epimerization at the β -carbon center of *L*-threonine and derivatives when

accessing oxazolines.¹⁶¹ (**Figure 3.4-B-D**) On the other hand, to avoid epimerization of the β -carbon of *L*-threonine, in oxazolines syntheses, molybdenum oxide catalysts can be employed.^{151,154,163} It is of note to mention the discrepancy between the reported dehydrocyclization and stereochemical outcomes between the work reported in the total synthesis of balgacyclamide A by Ugale and Gholap and the literature precedence by Wipf and Miller, Lellouche, and Galéotti. To this end, we decided to explore oxazoline syntheses via β -hydroxy amide dehydrative cyclization using the Burgess reagent, DAST, and molybdenum oxide catalyst $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ in the context of the balgacyclamides to better understand the reaction outcomes reported by Ugale and Gholap.

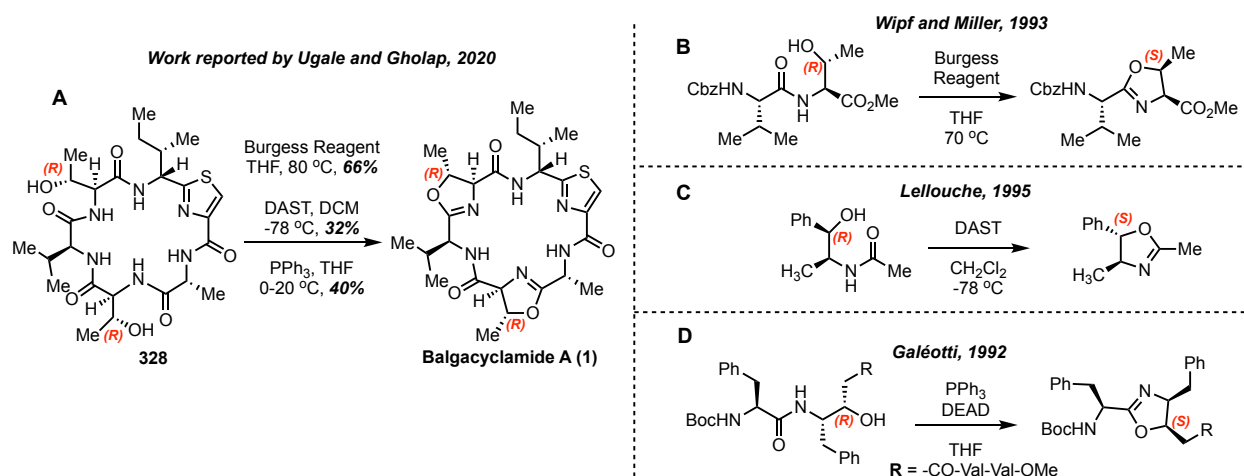


Figure 3.4. (A) Final step of Ugale and Gholap balgacyclamide A total synthesis through intermediate **328** containing two *L*-threonines via dehydrative cyclization with the Burgess reagent, DAST, and PPh_3 . (B) *L*-threonine β -carbon epimerization via the Burgess reagent by Wipf and Miller (C) *L*-threonine β -carbon epimerization via DAST by Lellouche (D) *L*-threonine β -carbon epimerization via PPh_3 by Galéotti.

3.3.3 Oxazoline Formation Analyses via the Burgess Reagent, DAST, and Mo. Oxides

To explore the epimerization effect of multiple dehydration cyclization reagents, the splitting patterns, J coupling values, and chemical shifts of the oxazolines α and β -hydrogen (H_α and H_β) within the scope of the balgacyclamides were evaluated. First, Cbz-*D*-alanine was coupled to *L*-allothreonine methyl ester and *L*-threonine methyl ester via PyBOP, DIPEA in dichloromethane to access β -hydroxy amides **328** and **329**, in 83% and 99% yields, respectively (**Figure 3.5**). Treatment of **328** with DAST and the Burgess reagent afford oxazoline **330** in 79% and 85% yields, respectively. The NMRs of these two transformations accessing **330** were the same, here the DAST NMR is shown, and the Burgess is provided in **Table 3.2**. When looking at the oxazoline Newman projection of **330**, we hypothesize that the H_α and H_β angle is not 180° because of the oxazoline conformation,¹⁶⁴ thus the angle is lower than 180° . Furthermore, both hydrogens are in proximity with the electron density from the methyl and methyl ester groups. Therefore, H_β showed two J couplings of 6.4 & 6.2 Hz, a chemical shift of 4.78 ppm, and a doublet of quartets splitting pattern that highly mimics a pentet since the difference between the two J couplings are very small. When analyzing H_α , a J coupling of 6.9 Hz, a chemical shift of 4.27 ppm, and a doublet splitting pattern was observed. However, when **328** was subjected to $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ catalyst, different patterns were detected. When looking at the Newman projection of **331**, H_α and H_β are now in proximity with an angle close to zero, thus an increased in the J value was observed for both to 10 Hz and 10.1 & 6.3, respectively. For H_β , a more noticeable doublet of quartets splitting pattern is observed since there is a larger difference between the two J couplings. Finally, the lack of electron density from the methyl and methyl ester group induced an increase in chemical shifts for H_α to 4.79 ppm, and H_β to 4.95 ppm. Based on these investigations, and the well-established literature precedence, oxazoline formation via these β -

hydroxyl dehydrations with DAST and the Burgess reagent leads to epimerization and not stereochemical retention as suggested by Ugale and Gholap. To further confirm this, β -hydroxy amide **329** (from *L*-threonine) was subjected to the same oxazoline formation conditions. Subjecting **329** to DAST and the Burgess reagent afforded **332**, which matches **331** via NMR. Likewise, when **329** was treated with the Mo catalyst, oxazoline **333** was assessed, that matched **330** in 69% yield. Together, these experiments confirmed that dehydrative cyclization reagents such as DAST and the Burgess reagents induce epimerization at the threonine β -carbon and that the Mo catalyst retains its configuration. As such, we propose that the synthetic efforts by Ugale and Gholap to their suggested total synthesis of balgacyclamide A is the diastereomer of balgacyclamide A. This claim will be further corroborated below via synthetic and spectroscopic analysis.

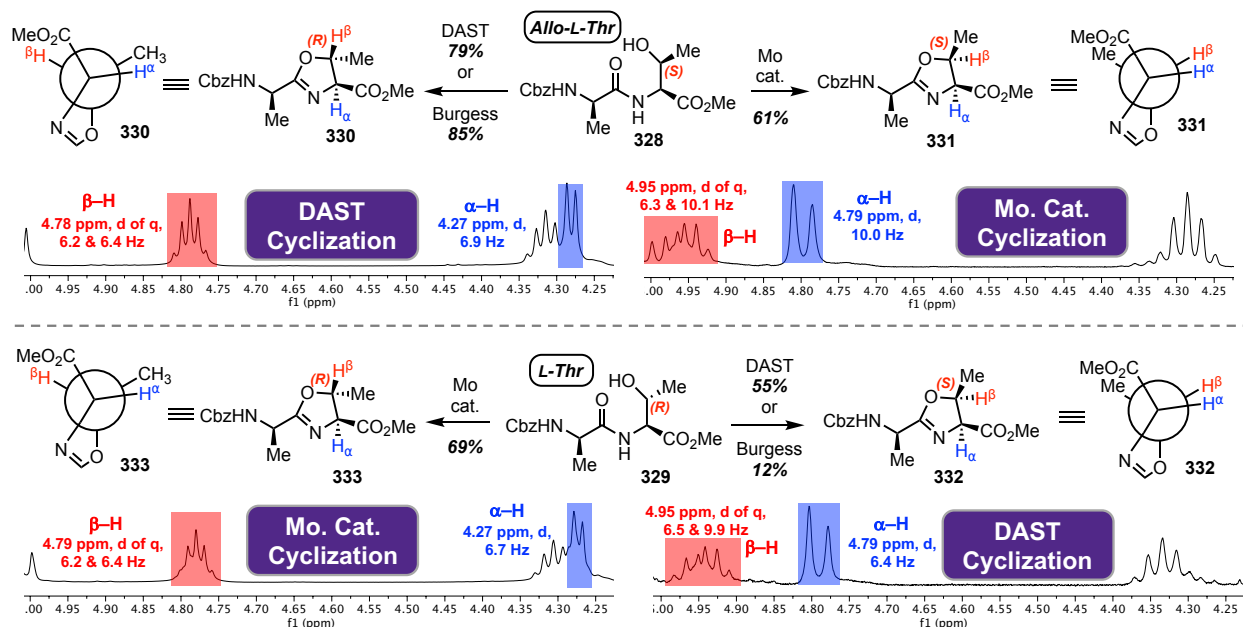
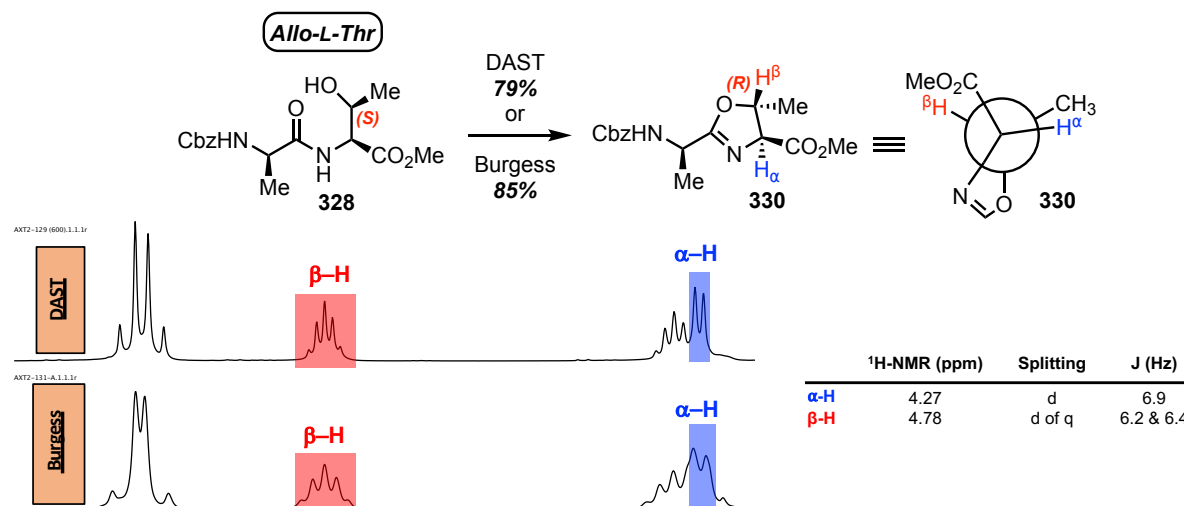


Figure 3.5. Chemical shifts, splitting patterns, and J couplings comparison of oxazoline **330** and **331** after synthesis via β -hydroxy amide dehydrative cyclization conditions of **328** and **329** using the Burgess reagent, DAST, and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$

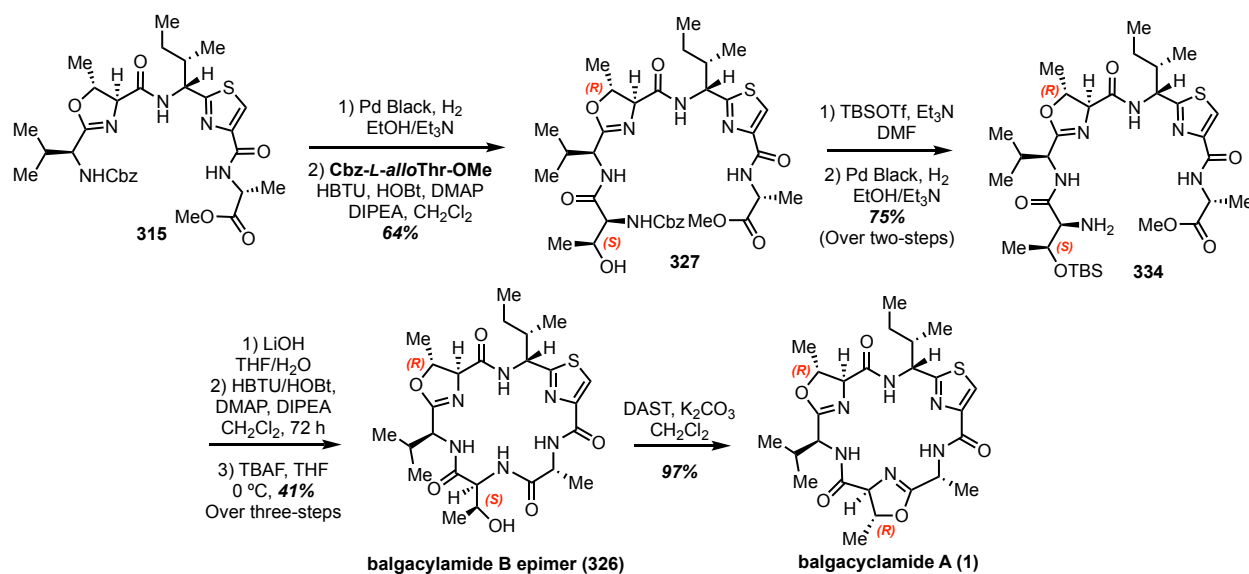
Table 3.2. ^1H -NMR comparison of oxazoline formation via DAST and the Burgess reagent.



3.3.4 Synthesis of Linear Hexapeptide Precursor and Macrolactamization

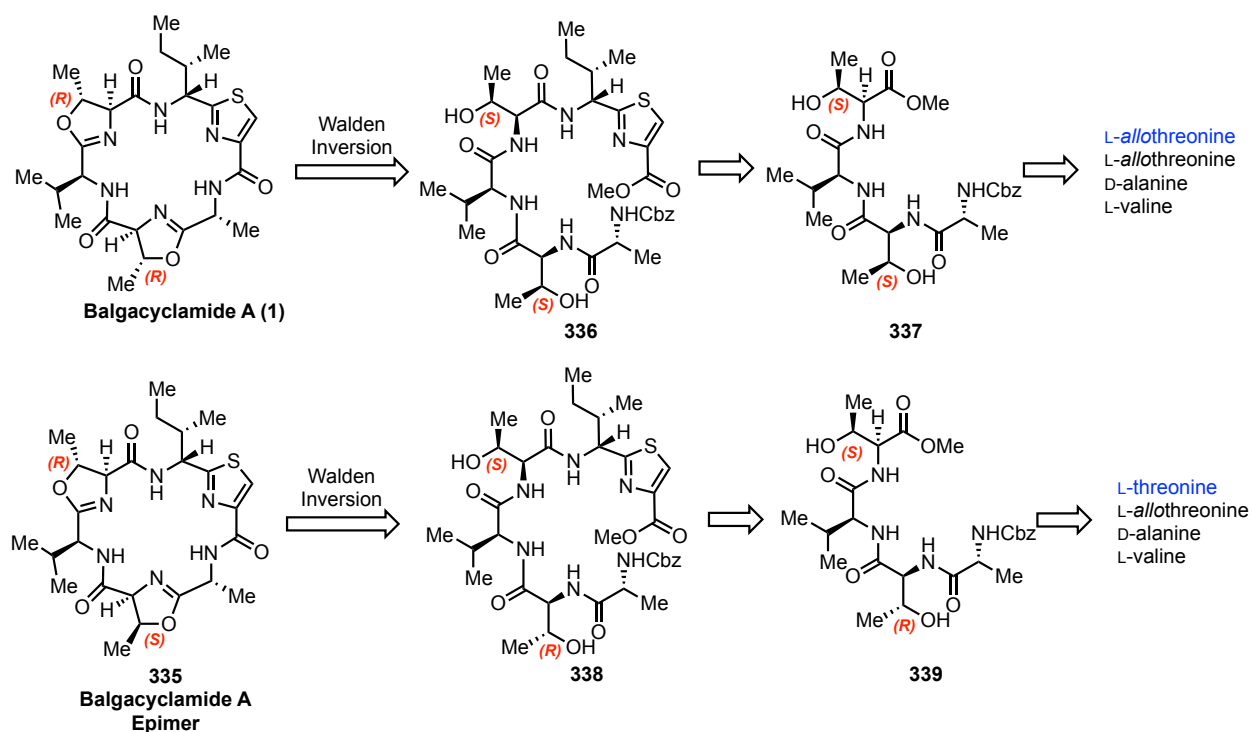
Given the reassignment to the diastereomer of balgacyclamide A, we then proceeded to undertake the first total synthesis of this natural product (**Scheme 3.7**). Mimicking the same synthetic procedure from balgacyclamide B pathway, we access the pentapeptide fragment (**315**) of balgacyclamide A, followed by single coupling of L-allothreonine. First, deprotection of the oxazoline Cbz was performed under standard H_2 conditions in 82% yield, and the free amine was coupled with N-Cbz-L-allothreonine via HBTU, HOBT, DMAP, DIPEA, in dichloromethane in 64% overall yield. TBS protection of the alcohol was performed to avoid potential hydrogen bonding and reduced nucleophilicity of the molecule, as proposed in balgacyclamide B total synthesis, followed by Cbz deprotection under H_2 and Pd black successfully accessed free amine **334** in 75% yield over two steps. Saponification of **334** was performed, followed by macrocyclization via HBTU, HOBT, DMAP, DIPEA, in dichloromethane, the same peptide coupling conditions used in our labs' total synthesis of balgacyclamide B. Lastly, TBS deprotection furnished **326** in 41% yield over three steps. Finally, epimerization upon the β -carbon

of the threonine was performed with DAST to afford the desired final product balgacyclamide A in 21-steps and 5% overall yield.



Scheme 3.7. Synthetic efforts towards the epimer of balgacyclamide B and elaboration onto balgacyclamide A

Our next step was to synthesize balgacyclamide A by mimicking Ugale and Gholap's synthetic pathway but using two *L-allo*threonines instead two *L-threonines* and perform a late-stage formation of both oxazolines via the same conditions used by Gholap (**Scheme 3.8**). Additionally, efforts towards the construction of an epimer of balgacyclamide A, about the south oxazoline unit, was performed to further correlate this to the synthetic natural product, the isolated natural product, and the proposed structure from Ugale and Gholap.



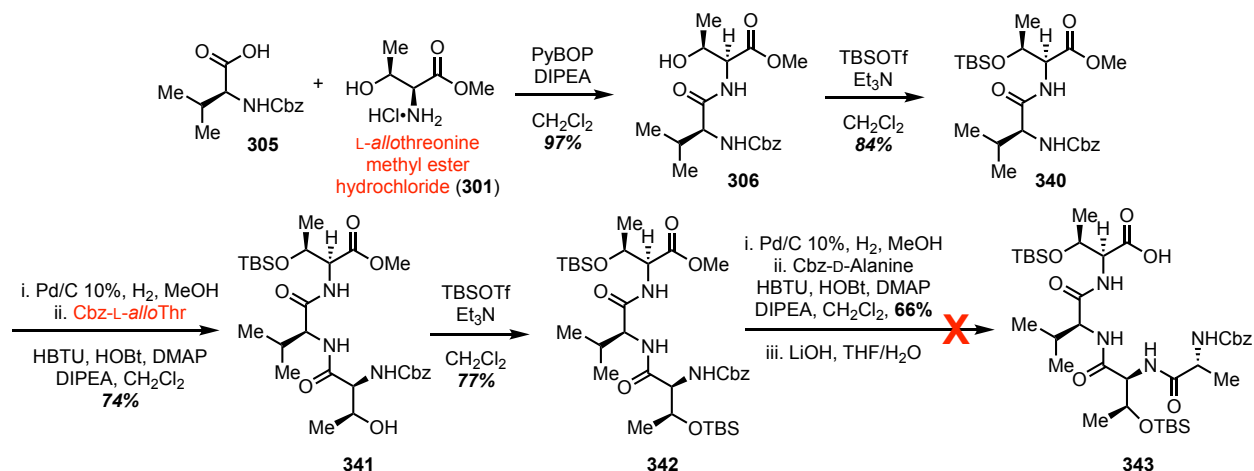
Scheme 3.8. New synthetic approach towards balgacyclamide A and proposed retrosynthetic analysis towards balgacyclamide A epimer

3.3.5 Efforts towards Balgacyclamide A Total Synthesis through a Late-Stage Oxazolines Formation

Formation

The efforts towards the total synthesis of balgacyclamide A through synthesis of tetrapeptide **337** commenced with the coupling of Cbz-L-valine and L-allothreonine methyl ester under standard peptide coupling conditions to accomplish β -hydroxyamide (**306**) in 97% yield (**Scheme 3.9**). Subsequently, protection of free alcohol with TBSOTf, followed by catalytic Cbz-deprotection and coupling of the second Cbz-L-allothreonine furnished tripeptide (**341**) in 74% yield. Finally, a repetitive protection under TBSOTf and coupling of D-alanine successfully accessed methyl ester tetrapeptide in 66% yield. However, the saponification efforts to access **343** were not successful under multiple hydrolysis conditions, including a) LiOH in a 1:1:1 mixture of

THF:MeOH:H₂O, b) trimethyltin hydroxide (Me₃SnOH), c) KOH in a 1:1 mixture of THF/H₂O, d) LiOH in a 1:1 mixture of THF:H₂O. Multiple temperatures, reaction time, and dilutions were also explored but hydrolysis of tetrapeptide was not achieved. Having the multiple challenges to perform the reaction, the next steps towards the synthetic pathway after balgacyclamide A epimer started.



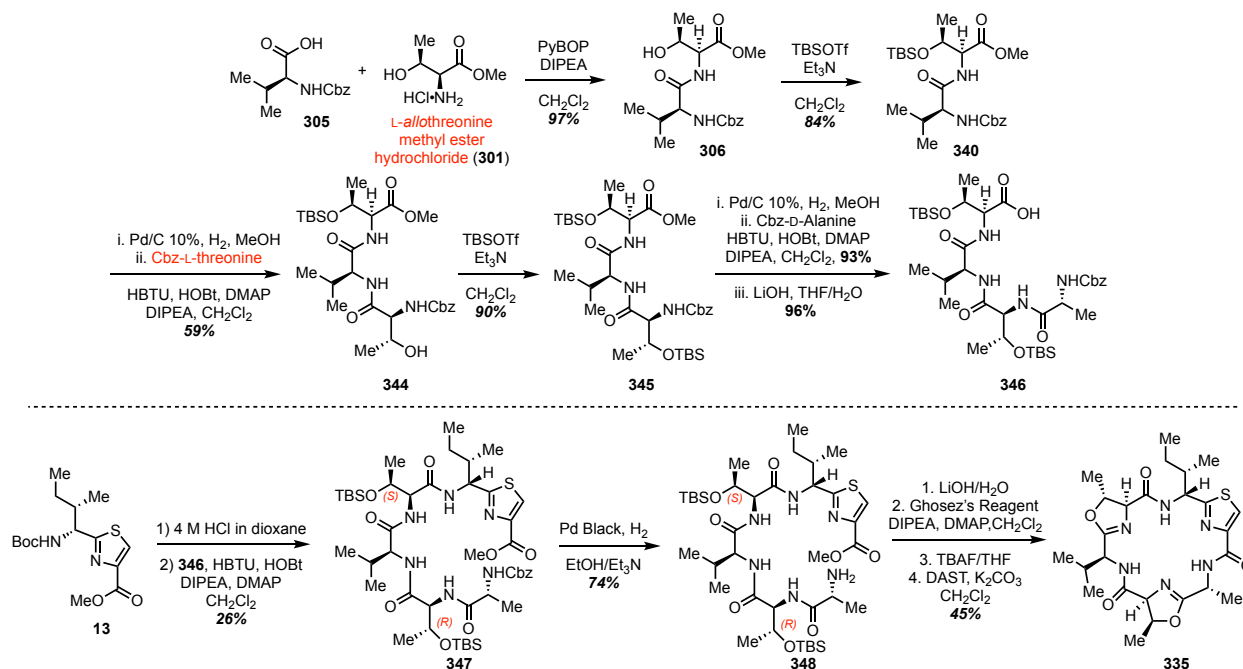
Scheme 3.9. Efforts towards the synthesis of free acid tetrapeptide (343)

3.4 Total Synthesis of Balgacyclamide A Epimer

3.4.1 Tetrapeptide Synthesis and Coupling to Thiazole Pathway

For the total synthesis of balgacyclamide A epimer, one *L-allothreonine* (for the northern oxazoline assembly) and one *L-threonine* (southern oxazoline assembly) were incorporated instead following the same procedure as the linear tetrapeptide (343) synthesis and both free alcohols were protected to facilitate purification and reaction outcomes (**Scheme 3.10**). First, the synthesis of tetrapeptide 346 initiated with the coupling of *L*-valine and *L-allothreonine* methyl ester with PyBOP as a peptide coupling reagent, to achieve the deprotected dipeptide in 97% yields. Protection of the free alcohol was performed with TBSOTf to access the protected β-alcohol within *L-allothreonine* in 88% yield. Quantitative Cbz deprotection was completed under Pd/C 10% and

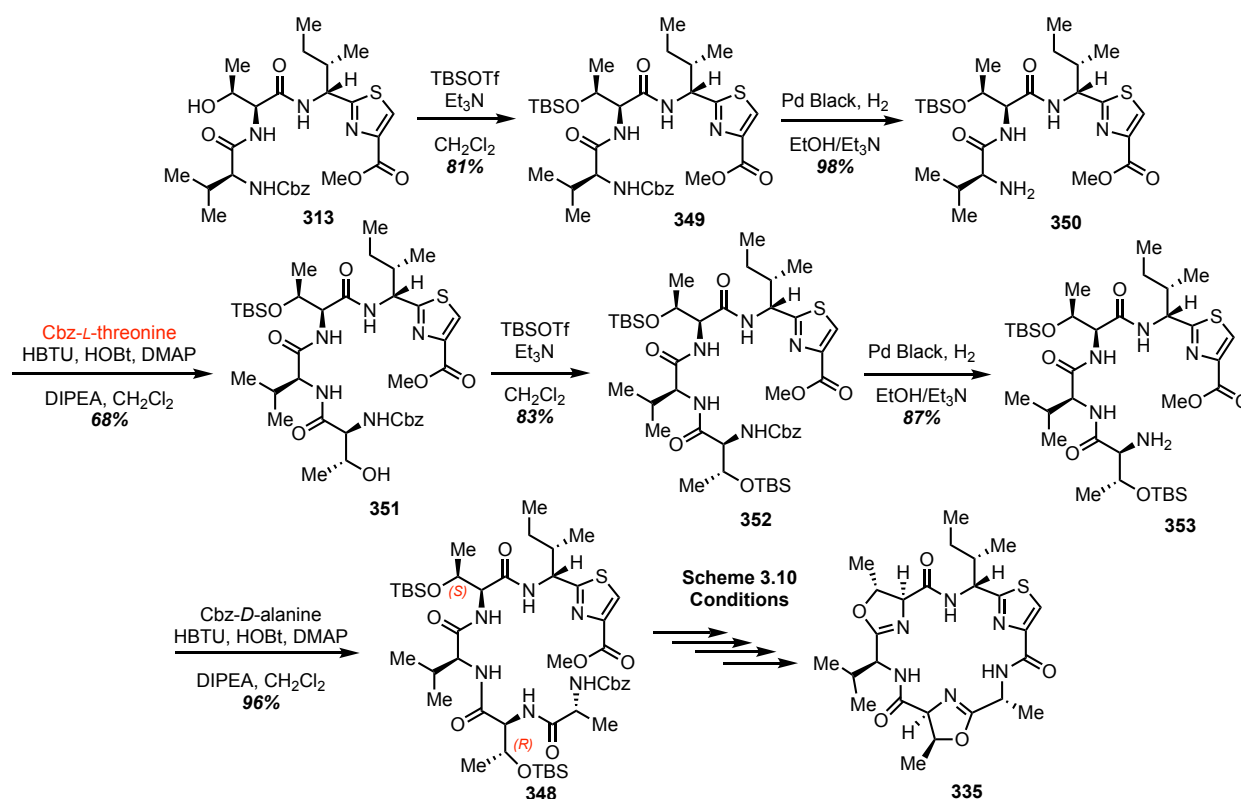
H₂, followed by coupling of Cbz-*L*-threonine to afford the tripeptide in 59% yield. To access tetrapeptide **346**, the same set of conditions were repeated resulting in the Cbz-*D*-alanine incorporation to afford methyl ester tetrapeptide in 84% overall yield over three steps. Saponification of tetrapeptide achieved **346** in 96% yield and then coupled with **13** to access compound **347** in 26% yield. In preparation for the macrocyclization the Cbz group was removed in 86% yield (**348**) followed by saponification in quantitative yields. Transformation of the free acid into its corresponding acid chloride with the Ghose's reagent then induced macrocyclization to furnish the cyclic peptide in 52% yield. Next, both TBS groups were removed with TBAF and then treated with DAST to afford the desired oxazoline rings to achieve balgacyclamide A epimer **335** in 86% over two-steps.



Scheme 3.10. Assembly of the southern epimer of balgacyclamide A via tetrapeptide (**346**) coupling and macrocyclization upon (*D*-alloile)thiazole

3.4.2 Alternative Pathway: Single Coupling Amino Acids to Thiazole

On the other hand, a linear single coupling amino acids pathway to (D-*alloile*)thiazole (**13**) was performed as an alternative route towards the epimer of balgacyclamide A, as shown in **scheme 3.11**. First β -hydroxyamide intermediate (**313**) was TBS protected in 81% yield, followed by Cbz deprotection in 98% to furnish free amine **350**. Coupling of Cbz-L-threonine was successfully achieved in 68% yield and protected under TBSOTf in 83% yield to access **352**. Finally, Cbz deprotection followed by coupling of Cbz-D-alanine under HBUT/HOBt and DMAP, accessed hexapeptide **348** in 96% yield. With **348** in hands, the same conditions from **scheme 3.10** were repeated to access balgacyclamide A epimer (**335**).

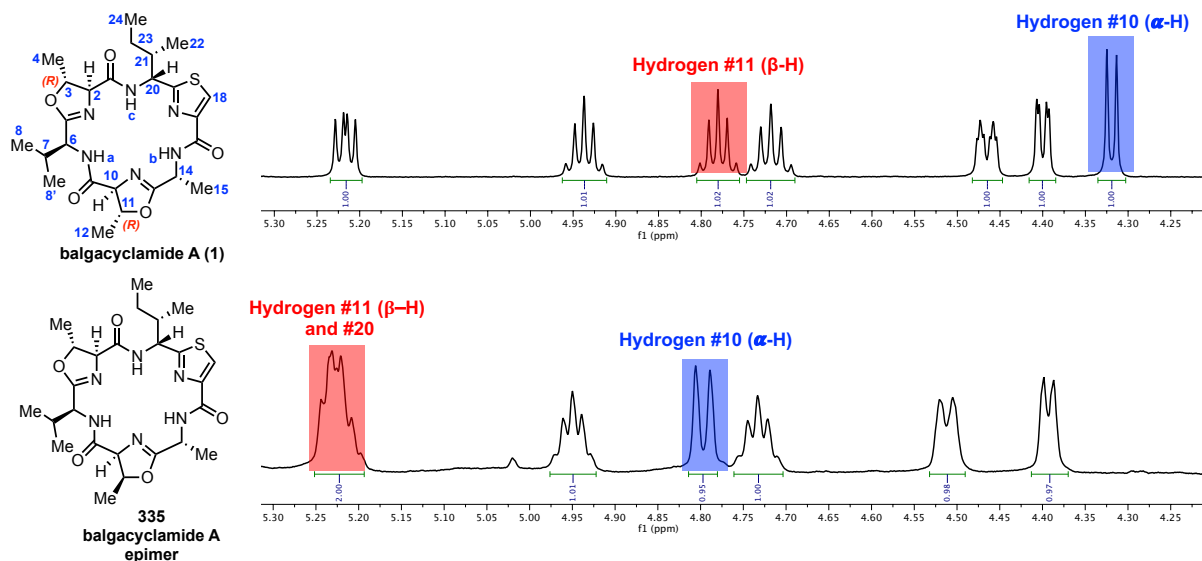


Scheme 3.11. Alternative synthetic pathway to access balgacyclamide A via linear single coupling amino acid to (**313**)

3.4.3 ^1H & ^{13}C -NMR comparison between Balgacyclamide A, its epimer, and isolated natural product

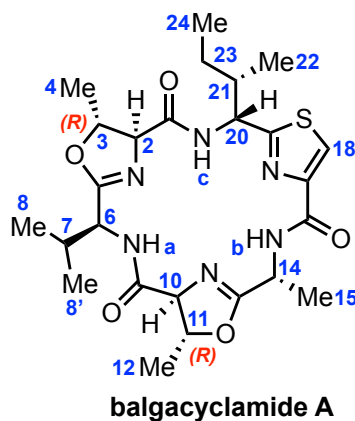
To compare the synthesized balgacyclamide A products with the isolated and Ugale & Gholap synthetic molecule, ^1H -NMR in $\text{DMSO-}d_6$ were performed. The J couplings, chemical shifts, and splitting patterns were analyzed for the lower oxazoline fragment H_α and H_β . As expected, the results showed the same pattern as our oxazoline studies using multiple reagents to understand epimerization upon threonine β -carbon (**Figure 3.5**). All chemical shifts can be found in **Table 3.3** in which the H_α and H_β are highlighted and bolded. Our intermediate **326** was subjected to DAST to perform β -hydroxy amide dehydrative cyclization and epimerization of *L*-allothreonine to get balgacyclamide A (**1**) and the ^1H -NMR matched Gademann spectroscopy data for the natural product¹⁸ (**Table 3.3** - column 2 and 3 and **Table 3.4, 3.5, & 3.6**). H_α showed a doublet (6.8 Hz) and a chemical shift of 4.32 ppm. On the other hand, H_β showed a doublet of quartets (6.5, 6.2 Hz), and a chemical shift of 4.78 ppm. However, when looking at balgacyclamide A epimer (**335**), (**Table 3.3** - column 5 and **Table 3.7**) H_α J coupling increased to 10.2 Hz, followed by a chemical shift to 4.80 ppm. In addition, H_β shifted downfield to 5.23 ppm and overlapped with hydrogen 20. Thus, this confirmed that epimerization of *L*-allothreonine β -carbon is induced by DAST and the same is expected if *L*-threonine is used in the synthetic pathway. When comparing Ugale & Gholap's synthetic balgacyclamide A, the reported chemical shifts and J couplings showed a matched with the natural balgacyclamide A. However, when looking at the splitting pattern of H_α and hydrogen 14 it did not match the natural product splitting pattern (Found in Ugale & Gholap's supplemental information).¹⁶² Additionally, the splitting pattern for H_β and hydrogen 20 seems to be an overlapped multiplet like our reported balgacyclamide A epimer (**335**) ^1H -NMR and not a doublet of doublets as the natural product.

Table 3.3. $^1\text{H-NMR}$ spectroscopy data comparison between natural (500 MHz, $\text{DMSO-}d_6$) and synthetic balgacyclamide A and its epimer (600 MHz, $\text{DMSO-}d_6$)



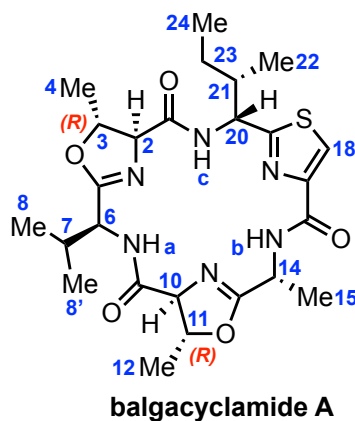
H no.	Natural balgacyclamide A	Synthetic balgacyclamide A	Ugale & Gholap balgacyclamide A	Synthetic balg. A epimer
2	4.41, dd (6.8, 2.0)	4.40, dd (6.8, 2.0)	4.41, dd (6.8, 2.1)	4.39, dd (7.4, 2.1)
3	4.95, dq (6.5, 6.4)	4.94, dq (6.5, 6.4)	4.95, dq (6.5, 6.4)	4.94, dq (6.7, 6.5)
4	1.37, d (6.4)	1.36, d (6.3)	1.37, d (6.5)	1.21, d (6.4)
6	4.48, ddd (9.0, 2.5, 2.0)	4.47, ddd (9.0, 2.5, 2.3)	4.47, ddd (8.9, 2.4, 2.1)	4.51, m
7	1.98, m	1.97, m	1.97, pd (6.9, 2.7)	2.02, m
8	0.62, d (6.9)	0.61, d (6.8)	0.63, d (7.0)	0.73, d (6.9)
8'	0.35, d (6.9)	0.34, d (6.8)	0.36, d (7.0)	0.37, d (7.0)
NH (a)	7.35, d (9.0)	7.35, d (9.0)	7.34, d (9.0)	7.44, d (9.4)
10	4.33, d (6.7)	4.32, d (6.8)	4.38, d (6.7)	4.80, d (10.2)
11	4.79, dq (6.7, 6.3)	4.78, dq (6.5, 6.2)	4.76, dq (6.6, 6.3)	5.23, m*
12	1.43, d (6.3)	1.42, d (6.3)	1.43, d (6.3)	1.38, d (6.3)
14	4.73, dq (7.7, 6.9)	4.72, dq (7.3, 7.0)	4.72, dq (7.5, 7.0)	4.74, dq (7.1, 6.3)
15	1.47, d (6.9)	1.45, d (6.9)	1.48, d (6.9)	1.43, d (6.9)
NH (b)	8.30, d (7.7)	8.29, d (7.6)	8.30, d (7.7)	8.30, d (8.7)
18	8.30, s	8.30, s	8.31, s	8.31, s
20	5.23, dd (8.4, 5.7)	5.22, dd (8.5, 5.7)	5.23, dd (8.4, 5.6)	5.23, m*
21	1.92, m	1.91, m	1.93, m	1.92, m
22	0.93, d (6.8)	0.92, d (6.8)	0.93, d (6.8)	0.91, d (6.9)
23a	1.44, m	1.44, m	1.44, m	1.44, m
23b	1.04, m	1.03, m	1.03, m	1.03, m
24	0.88, dd (7.4, 7.4)	0.88, dd (7.4, 7.3)	0.88, dd (6.6, 6.2)	0.87, dd (7.9, 7.4)
NH (c)	8.21, d (8.4)	8.20, d (8.4)	8.20, d (8.3)	8.29, d (8.3)

Table 3.4. ¹H-NMR spectroscopy data comparison between natural (800 MHz, CDCl₃) and synthetic balgacyclamide A (600 MHz, CDCl₃)



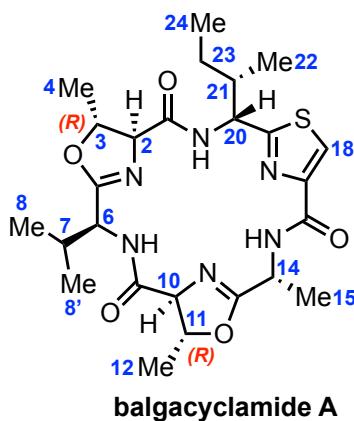
H no.	Natural balgacyclamide A	Synthetic balgacyclamide A	Natural-Synthetic $\Delta\delta$ /ppm
2	4.25, m	4.24, m	0.01
3	4.93, dq (7.3, 6.3)	4.92, dq (6.4, 6.3)	0.01
4	1.53, m	1.53, d (6.3)	0.00
6	4.67, ddd (9.5, 2.7, 2.1)	4.66, ddd (9.4, 2.5, 2.4)	0.01
7	2.11, m	2.10, pd (6.8, 2.9)	0.01
8	0.73, d (6.9)	0.72, d (6.9)	0.01
8'	0.44, d (6.9)	0.43, d (6.9)	0.01
NH (a)	7.41, d (9.5)	7.41, d (9.4)	0.01
10	4.25, m	4.24, m	0.01
11	4.89, dq (6.4, 6.4)	4.89, dq (6.5, 6.4)	0.00
12	1.51, d (6.4)	1.50, d (6.3)	0.01
14	4.84, dq (7.2, 6.6)	4.83, dq (7.1, 6.9)	0.01
15	1.58, m	1.57, d (6.8)	0.01
NH (b)	8.35, d (7.2)	8.35, d (7.5)	0.00
18	8.06, s	8.06, s	0.00
20	5.26, dd (8.3, 5.0)	5.25, dd (8.3, 4.9)	0.01
21	1.95, m	1.94, m	0.01
22	1.01, d (6.9)	1.00, d (6.8)	0.01
23a	1.58, m	1.58, m	0.00
23b	1.01, m	1.01, m	0.00
24	0.95, dd (7.3, 7.3)	0.94, dd (7.3, 7.3)	0.00
NH (c)	7.94, d (8.3)	7.93, d (8.4)	0.01

Table 3.5. $^1\text{H-NMR}$ spectroscopy data comparison between natural (**500 MHz, DMSO- d_6**) and synthetic balgacyclamide A (**600 MHz, DMSO- d_6**)



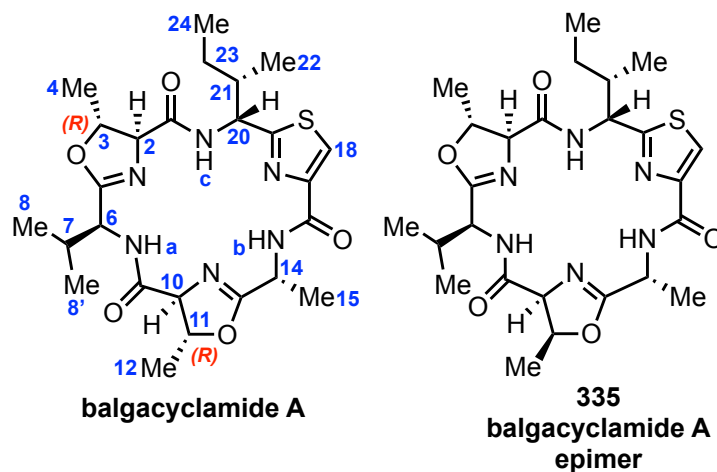
H no.	Natural balgacyclamide A	Synthetic balgacyclamide A	Natural-Synthetic $\Delta\delta/\text{ppm}$
2	4.41, dd (6.8, 2.0)	4.40, dd (6.8, 2.0)	0.01
3	4.95, dq (6.5, 6.4)	4.94, dq (6.5, 6.4)	0.01
4	1.37, d (6.4)	1.36, d (6.3)	0.01
6	4.48, ddd (9.0, 2.5, 2.0)	4.47, ddd (9.0, 2.5, 2.3)	0.01
7	1.98, m	1.97, m	0.01
8	0.62, d (6.9)	0.61, d (6.8)	0.01
8'	0.35, d (6.9)	0.34, d (6.8)	0.01
NH (a)	7.35, d (9.0)	7.34, d (9.0)	0.01
10	4.33, d (6.7)	4.32, d (6.8)	0.01
11	4.79, dq (6.7, 6.3)	4.78, dq (6.5, 6.2)	0.01
12	1.43, d (6.3)	1.42, d (6.3)	0.01
14	4.73, dq (7.7, 6.9)	4.72, dq (7.3, 7.0)	0.02
15	1.47, d (6.9)	1.45, d (6.9)	0.01
NH (b)	8.30, d (7.7)	8.29, d (7.6)	0.00
18	8.30, s	8.30, s	0.01
20	5.23, dd (8.4, 5.7)	5.22, dd (8.5, 5.7)	0.01
21	1.92, m	1.91, m	0.01
22	0.93, d (6.8)	0.92, d (6.8)	0.01
23a	1.44, m	1.44, m	0.00
23b	1.04, m	1.03, m	0.01
24	0.88, dd (7.4, 7.4)	0.88, dd (7.4, 7.3)	0.00
NH (c)	8.21, d (8.4)	8.20, d (8.4)	0.01

Table 3.6. ^{13}C -NMR spectroscopy data comparison between natural (500 MHz, $\text{DMSO}-d_6$) and synthetic balgacyclamide A (600 MHz, $\text{DMSO}-d_6$)



C no.	Natural balgacyclamide A	Synthetic balgacyclamide A	Natural-Synthetic $\Delta\delta/\text{ppm}$
1	168.9	169.8	000.1
2	72.4	72.6	-00.2
3	79.3	79.5	-00.2
4	20.4	20.7	-00.3
5	166.0	166.1	-000.1
6	50.6	50.8	-00.1
7	30.8	31.0	00.8
8	18.1	18.4	-00.3
8'	15.1	15.3	-00.2
9	169.7	169.7	000.0
10	72.7	72.9	-00.2
11	81.1	81.2	-00.1
12	21.0	21.3	-00.3
13	169.7	169.7	000.0
14	42.6	42.9	-00.3
15	19.3	19.7	-00.4
16	159.1	159.2	-000.1
17	148.0	148.1	-000.1
18	124.6	124.8	-000.2
19	169.7	169.1	000.6
20	54.0	54.2	-00.2
21	40.4	40.7	-00.3
22	14.2	14.5	-00.3
23	25.1	25.4	-00.3
24	11.1	11.4	-00.3

Table 3.7. $^1\text{H-NMR}$ spectroscopy data comparison between natural (**500 MHz, DMSO- d_6**) and synthetic balgacyclamide A epimer **28** (**600 MHz, DMSO- d_6**)



H no.	Natural balgacyclamide A	Synthetic balg. A epimer	Natural-Synthetic $\Delta\delta/\text{ppm}$
2	4.41, dd (6.8, 2.0)	4.39, dd (7.4, 2.1)	0.02
3	4.95, dq (6.5, 6.4)	4.94, dq (6.7, 6.5)	0.01
4	1.37, d (6.4)	1.21, d (6.4)	0.16
6	4.48, ddd (9.0, 2.5, 2.0)	4.51, m	-0.03
7	1.98, m	2.02, m	-0.04
8	0.62, d (6.9)	0.73, d (6.9)	-0.11
8'	0.35, d (6.9)	0.37, d (7.0)	-0.02
NH (a)	7.35, d (9.0)	7.44, d (9.4)	-0.09
10	4.33, d (6.7)	4.80, d (10.2)	-0.47
11	4.79, dq (6.7, 6.3)	5.23, m*	-0.44
12	1.43, d (6.3)	1.38, d (6.3)	0.05
14	4.73, dq (7.7, 6.9)	4.74, dq (7.1, 6.3)	-0.01
15	1.47, d (6.9)	1.43, d (6.9)	0.04
NH (b)	8.30, d (7.7)	8.30, m	0.00
18	8.30, s	8.31, s	-0.01
20	5.23, dd (8.4, 5.7)	5.23, m*	0.00
21	1.92, m	1.92, m	0.00
22	0.93, d (6.8)	0.91, d (6.9)	0.02
23a	1.44, m	1.44, m	0.00
23b	1.04, m	1.03, m	0.01
24	0.88, dd (7.4, 7.4)	0.87, dd (7.9, 7.4)	0.01
NH (c)	8.21, d (8.4)	8.29, m	-0.08

3.5 Efforts towards Total Synthesis of Balgacyclamide C

3.5.1 Retrosynthetic Analysis

We anticipated to access the total synthesis of balgacyclamide C by following balgacyclamide's A and B synthetic pathway conditions by substituting the two key amino acids for balgacyclamide's C core: coupling of L-phenylalanine for oxazoline formation (**354**) and glycine for β -hydroxyamide formation (**355**) (**Figure 3.6**).

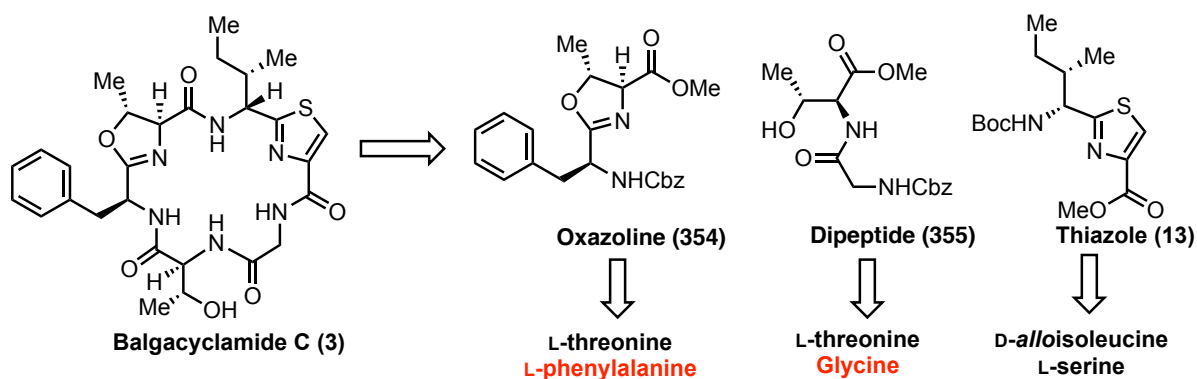
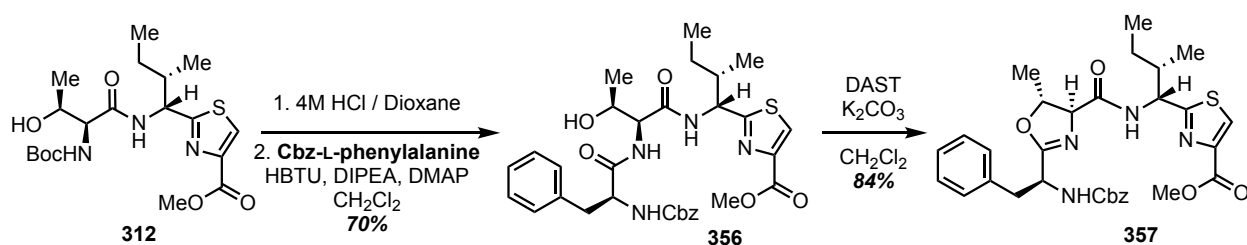


Figure 3.6. Retrosynthetic analysis of balgacyclamide C

3.5.2 Synthesis of Balgacyclamide's C Northern Fragment

The synthesis towards the northern fragment of balgacyclamide C was initiated by coupling of L-phenylalanine with intermediate (**312**) to furnish β -hydroxyamide **356** in 70% yield (**Scheme 3.12**). Followed by Walden inversion upon L-allothreonine β -carbon via dehydrocyclization to successfully achieved the northern fragment (**357**) in 84% yield. Now, efforts towards synthesis completion are on the way.



Scheme 3.12. Synthesis of balgacyclamide's C northern fragment

Chapter 4 - Conclusion and Future Work

In summary, we have accomplished the first total synthesis of balgacyclamide A & B over 21 (5% overall yield) and 17 (2% overall yield) synthetic steps, respectively. Additionally, balgacyclamide's A epimer was synthesized in 21 steps in 3% overall yield and efforts towards balgacyclamide C have been conducted (**Figure 4.1**).

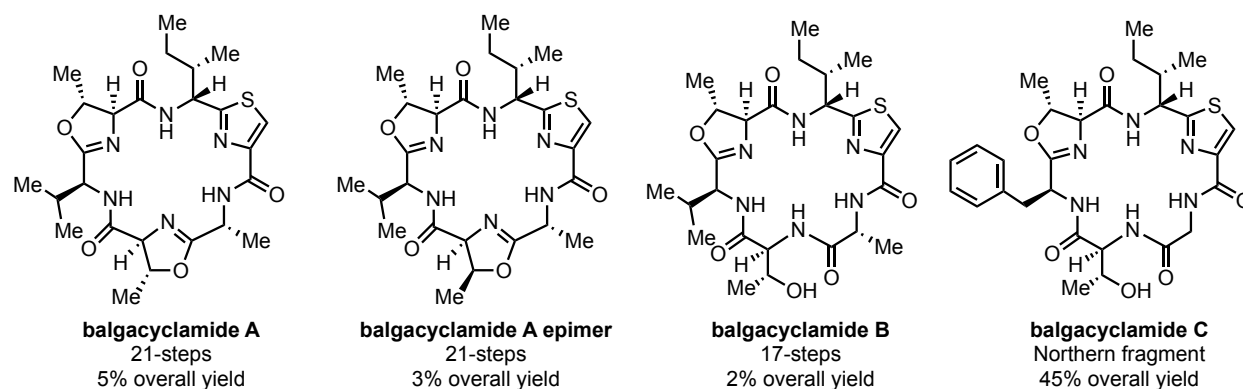


Figure 4.1. Summary of balgacyclamide A-C syntheses

The synthesis of the northern fragment required the incorporation of nonproteinogenic amino acid L-*allo*threonine due to epimerization at the β -hydroxy amide carbon under the selected conditions (**Figure 4.2**). The importance of fully anhydrous conditions for the thiazole synthesis were noticeable after having an oxazole and thiazole inseparable mixture with H_2S formed in situ. To our dismay, a direct coupling of the oxazoline and thiazole moieties for the northern unit failed in our hands due to potential sensitivity of the oxazoline ring under acidic and basic conditions. However, through direct single amino acid coupling upon the thiazole with subsequent dehydrocyclization afforded the desired northern unit in the same number of steps and superior yields. Finally, multiple macrolactamization attempts were performed. The head-tail macrocyclization of balgacyclamide B upon the N-terminus of the oxazoline did not provide the

final ring formation, suggesting that potential conformational and steric effect from the oxazoline plays a role in the reaction success. Lower yields and unsuccessful macrolactamizations were also observed by Meyers' total synthesis of bistratamide D, Kelly's total synthesis of bistratamide F, and our recent synthetic work for balgacyclamide A & B. Thus, the attempts to access oxazoline containing haxacyclamides are preferred to be accomplished in a late stage dehydrocyclization of threonine after macrocyclization is performed. Good examples are, Serra's total synthesis of aerucyclamide B, Kelly's total synthesis of bistratamide E, Sato's total synthesis of bistratamide E, and Yamada's total synthesis of dolastatin E.

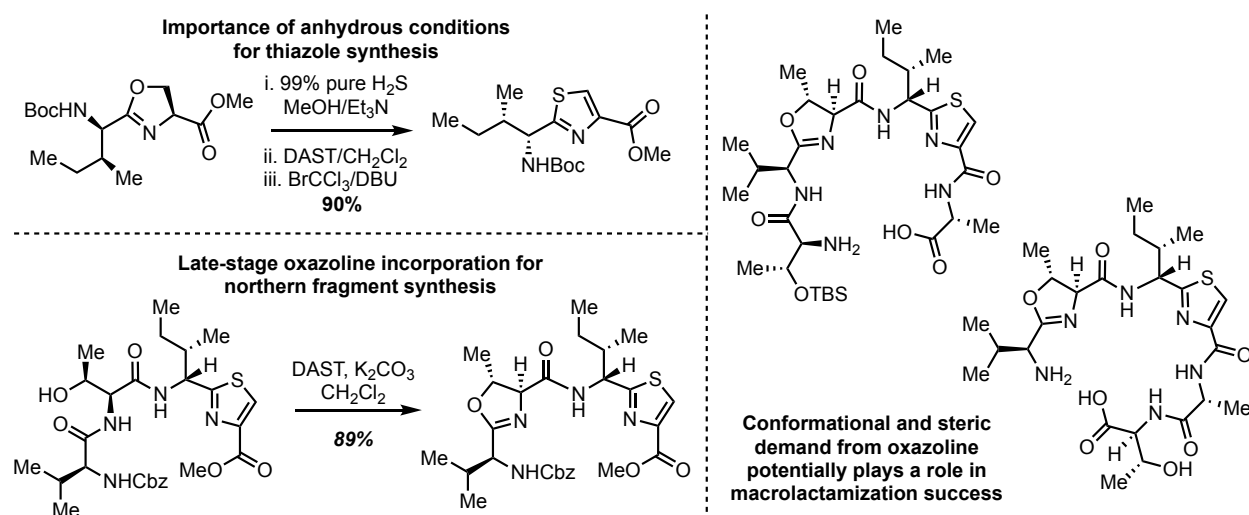


Figure 4.2. Summary of balgacyclamide's B synthetic intermediates outcomes

On the other hand, our studies in β -carbon epimerization of *L*-threonine and *L*-allothreonine via hydroxy amide dehydrative cyclization conditions using DAST and the Burgess reagent supports previous reported literature to access oxazoline syntheses with inversion of configuration (**Figure 4.3**). The oxazolines H_α and H_β displayed important information for the understanding of chemical shifts, splitting patterns, and J couplings in the ^1H -NMR upon configuration in the molecule. After in depth analyses of spectroscopy data from our synthetic balgacyclamide A, the

isolated natural product, and Ugale & Gholap synthetic product, we have found a mismatched in splitting patterns from Ugale's balgacyclamide A synthesis. However, since cyanobactins biological activities could be dependent on the stereocenters present in the molecule, we proposed for future research to explore how these different oxazolines configuration affects their biological activities in parasites like *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* or toward cancer cell lines as shown in our previous studies.^{105,165} On the other hand, two different pathways were developed to access balgacyclamide A and its epimer that represent potential synthetic routes toward balgacyclamide C that our lab is now exploring.

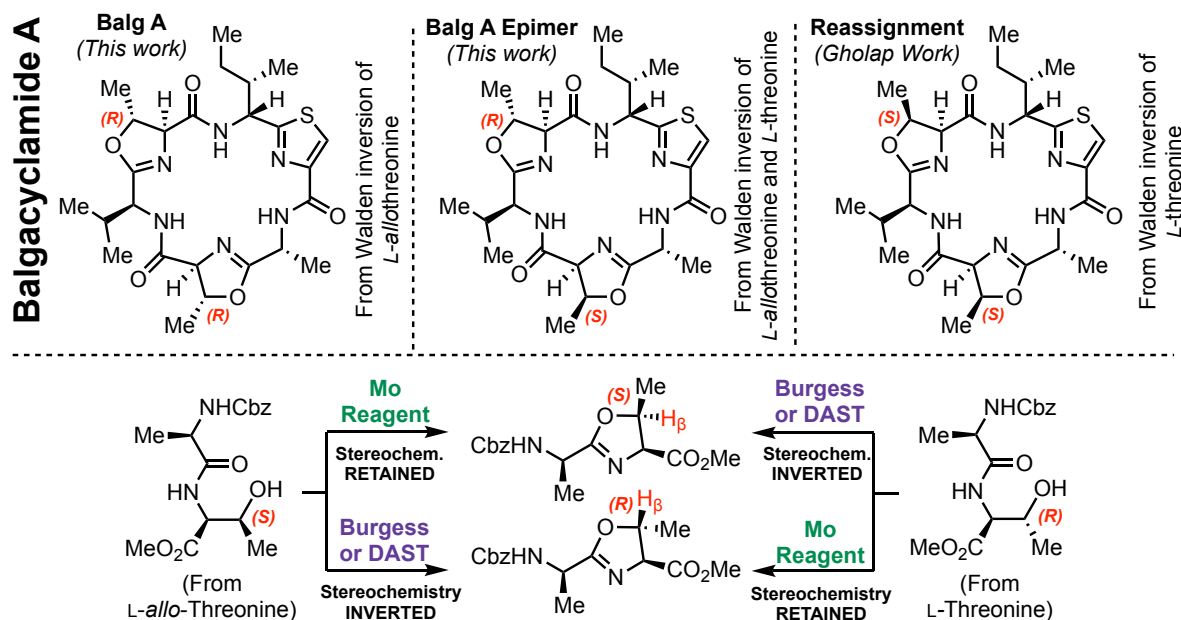


Figure 4.3. Summary of balgacyclamide's A synthetic work

For future research, the synthetic pathways for balgacyclamide A and B could be modified to enable the first total synthesis of balgacyclamide C, as illustrated in **figure 4.4**. This approach would involve the coupling of L-threonine to (L-val)oxazoline and the coupling of glycine to (D-alloile)thiazole to access a hexapeptide linear precursor. Subsequently, a macrolactamization under peptide coupling conditions could be employed to synthesize balgacyclamide C.

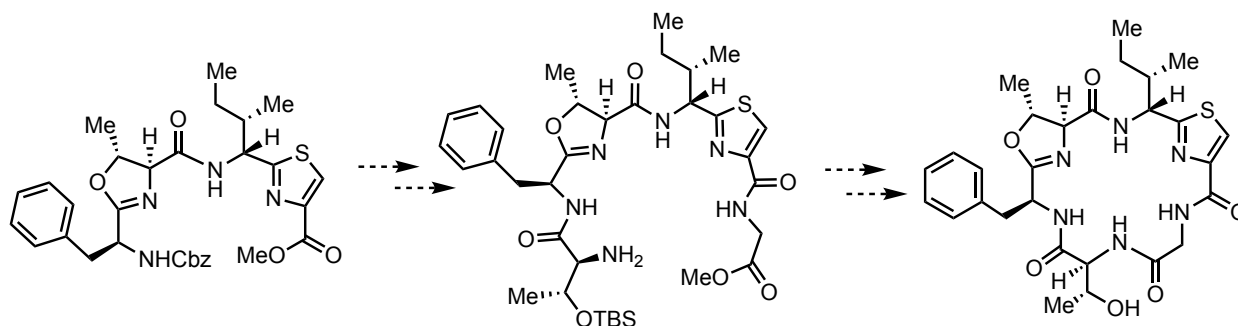


Figure 4.4. Proposed final synthetic steps for balgacyclamide C

By 2050, antimicrobial resistance is projected to cause approximately 10 million deaths annually, comparable to the numbers related with cancer. This highlights the urgency of understanding the mechanisms that control the penetration of small molecules and antibiotics into gram-positive and gram-negative bacteria. In response, our lab aims to expand on this research by constructing a chemical library derived from the northern fragment (**314**) of balgacyclamide A & B. This will allow us to investigate how physicochemical properties, focused on charge orientation, influence antibiotic penetration through porin channels in gram-negative bacteria. Our goal is to gain a deeper understanding of how small molecules and antibiotics interact with these highly charged porins.

Additionally, our lab is exploring strategies to reduce the toxic side effect of the antileukemia drug mercaptopurine. Because its potent adverse effects, we are investigating how incorporating mercaptopurine into balgacyclamide could help mitigate toxicity and enable selective drug delivery. We have already started synthesizing analogs of this compound, and future research will focus on using balgacyclamide as a drug carrier. Further studies are needed to explore the structure-activity relationships and assess the potential of these natural products for metal binding (**Figure 4.5**).

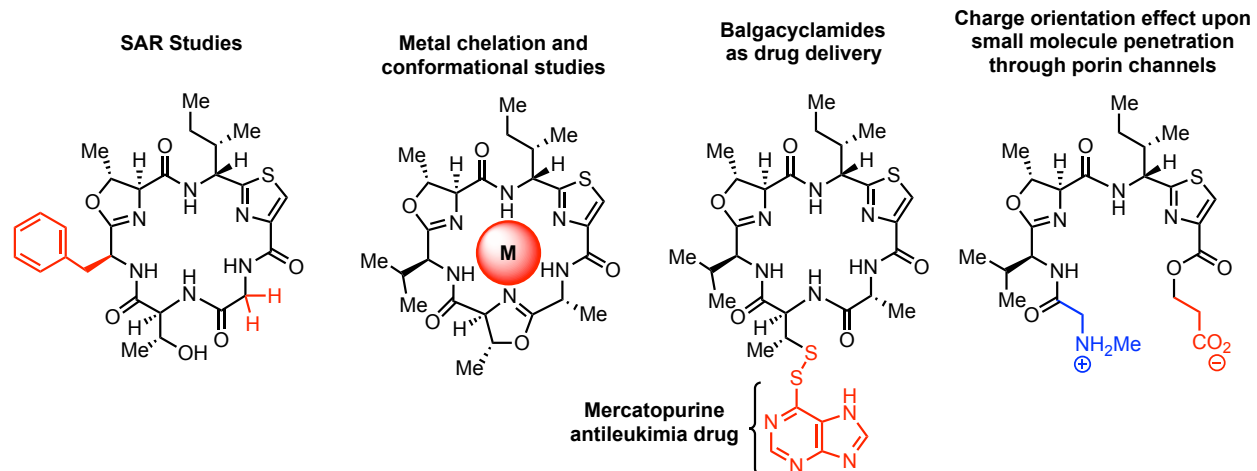


Figure 4.5. Future applications of the balgacyclamide family of natural products

Chapter 5 - References

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Chapter 6 – Experimental Procedures and Spectroscopic Data

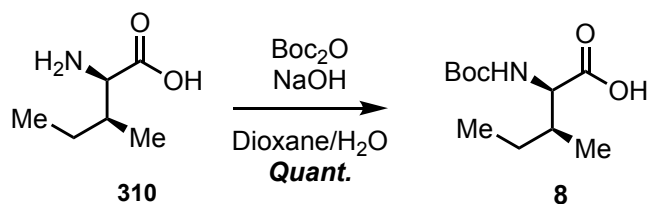
6.1 Material and Methods

Chemical reagents were purchased from commercial sources and used without further purification. All chemicals were purchased commercially and used without further purification. Flash chromatography was performed using silica (230-400 mesh). Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene (PhMe), N,N-dimethylformamide (DMF), acetonitrile (CH_3CN), triethylamine (Et_3N), and methanol (MeOH) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves.

^1H (400, 600 MHz) and ^{13}C (100, 151) spectra were obtained on either a Bruker 400 MHz or 600 MHz spectrometer. Spectra were obtained in the following solvents (reference peaks include ^1H & ^{13}C NMR): CDCl_3 (^1H NMR: 7.26 ppm; ^{13}C NMR: 77.23 ppm) and d_6 -DMSO (^1H NMR: 2.50 ppm; ^{13}C NMR: 39.52 ppm). NMR experiments were performed at room temperature unless otherwise indicated. Chemical shift values are reported in parts per million (ppm) for all ^1H NMR and ^{13}C NMR spectra. ^1H NMR multiplicities are reported as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, etc. Mass spectra were obtained using a high/ low-resolution magnetic sector mass spectrometer.

6.2 Experimental Procedures: Total Synthesis of Balgacyclamide B

Boc-D-alloisoleucine (**8**)



D-alloisoleucine (**310**) (600 mg, 4.57 mmol, 1 eq.) was dissolved in a 2:1 dioxane/water mixture (33.0 mL, 0.14 M) followed by 1M NaOH addition (10.4 mL, 10.4 mmol, 2.27 eq.) and stirring until **310** completely dissolved. Boc-anhydride (1.10 g, 5.03 mmol, 1.1 eq.) was added, and the reaction was allowed to stir at room temperature overnight. The reaction was quenched by bringing the pH to 2 with 5 M HCl, extracted with ethyl acetate (3 x 50 mL), and dried over Na₂SO₄. The organic layer was concentrated under reduced pressure to afford **8** (1.05 g, quant yield) as a colorless oil and was used without further purification.

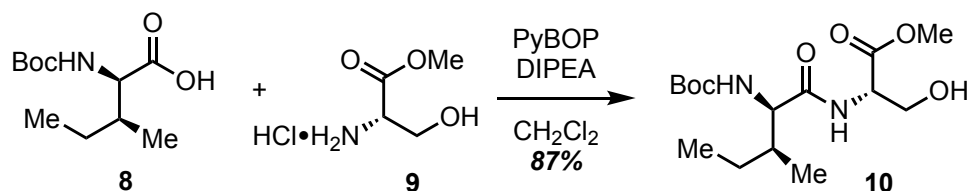
¹H NMR (400 MHz, DMSO-*d*₆) δ 12.45 (brs, 1H), 6.83 (d, *J* = 8.9 Hz, 1H), 3.99 (dd, *J* = 8.9, 5.0 Hz, 1H), 1.82 (hept, *J* = 6.7 Hz, 1H), 1.38 (s, 9H), 1.31 (hept, *J* = 6.9 Hz, 1H), 1.13 (hept, 1H), 0.88 – 0.78 (m, 6H). **¹³C NMR (101 MHz, DMSO)** δ 173.85, 155.90, 77.98, 56.74, 36.00, 28.21, 25.70, 14.81, 11.49. **HRMS(ESI):** *m/z* calc. for C₁₁H₂₁NO₄Na [M+Na]⁺: 254.1363, found: 254.1363

Notebook Entries

Procedure: AXT2-093

¹H NMR and ¹³C NMR – AXT2-093

Methyl (*tert*-butoxycarbonyl)-*D*-alloisoleucyl-*L*-serinate (**10**)



To a RBF containing a stirring solution of **8** (1.04 g, 4.50 mmol, 1.0 eq.) in CH₂Cl₂ (45 mL, 0.1 M) were added PyBOP (2.50 g, 4.72 mmol, 1.05 eq.) and DIPEA (1.20 mL, 6.75 mmol, 1.5 eq.). To a separate RBF L-serine methyl ester hydrochloride (**9**; 909 mg, 5.84 mmol, 1.3 eq.), DIPEA (2.0 mL, 11.7 mmol, 2.6 eq.), and CH₂Cl₂ (1 M) were added. Both reaction mixtures were left to stir for 30 minutes and then were combined and stirred for 1 h. The reaction was quenched with saturated NaHCO₃ (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 30 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **10** (1.30 g, 87% yield) as a white solid.

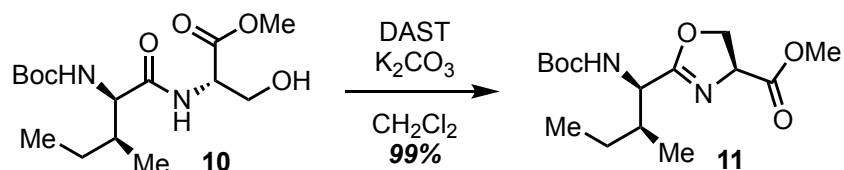
¹H NMR (400 MHz, DMSO-*d*₆) δ 8.02 (d, *J* = 7.7 Hz, 1H), 6.71 (d, *J* = 9.3 Hz, 1H), 5.07 (t, *J* = 5.6 Hz, 1H), 4.34 (dt, *J* = 8.9, 5.0 Hz, 1H), 4.04 (dd, *J* = 9.2, 5.7 Hz, 1H), 3.69 (h, *J* = 11.0, 5.5 Hz, 1H), 3.62 (s, 3H), 3.59 (h, *J* = 10.8, 5.2 Hz, 1H), 1.77 (hept, *J* = 6.9 Hz, 1H), 1.38 (s, 9H), 1.31 (hept, 1H), 1.09 (hept, *J* = 14.1, 7.3 Hz, 1H), 0.84 (t, *J* = 7.3 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H). **¹³C NMR (101 MHz, DMSO)** δ 171.89, 170.94, 155.52, 78.14, 61.24, 57.65, 54.54, 51.80, 36.93, 28.16, 25.69, 14.26, 11.47.

Notebook Entries

Procedure: AXT2-100

¹H NMR and ¹³C NMR – AXT2-028

Methyl (*S*)-2-((1*R*,2*S*)-1-((*tert*-butoxycarbonyl)amino)-2-methylbutyl)-4,5-dihydrooxazole-4-carboxylate (11**)**



To a flamed dried RBF was added compound **10** (1.3 g, 3.79 mmol, 1.0 eq.) and anhydrous CH₂Cl₂ (38.0 mL, 0.10 M), the mixture was then cooled to -78 °C under argon atmosphere. DAST (0.55 mL, 4.17 mmol, 1.10 eq.) was added dropwise to solution and stirred for 1 h, followed by addition of K₂CO₃. The mixture was slowly warmed to RT and then stirred for 6 h. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol equiv.) and the product was extracted with CH₂Cl₂ (3 x 40 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:2 ethyl acetate/hexane) to afford **11** (1.20 g, 99% yield) as a colorless oil.

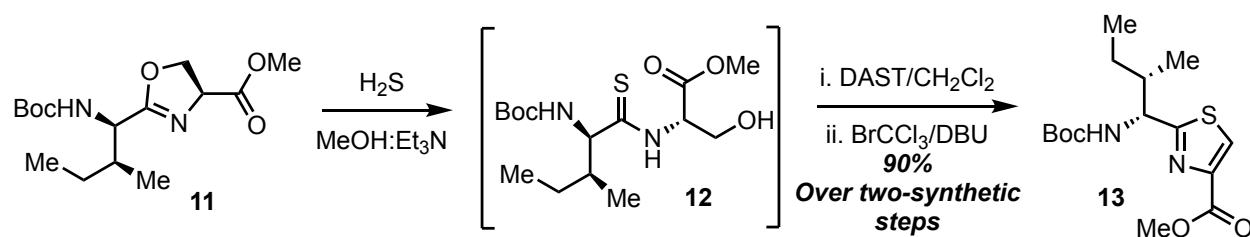
¹H NMR (400 MHz, CDCl₃) δ 5.04 (d, *J* = 9.5 Hz, 1H), 4.75 (dd, *J* = 10.5, 7.8 Hz, 1H), 4.57 (t, *J* = 8.3 Hz, 1H), 4.50 – 4.39 (m, 2H), 3.78 (s, 3H), 1.85 (hept, *J* = 6.4 Hz, 1H), 1.52 – 1.40 (m, 10H), 1.19 (hept, 1H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H). **¹³C NMR (101 MHz, CDCl₃)** δ 171.23, 169.94, 155.54, 79.62, 69.82, 67.82, 52.61, 52.43, 38.20, 28.28, 25.93, 14.29, 11.70.

Notebook Entries

Procedure: AXT1-271

¹H NMR - AXT1-150-A and ¹³C NMR – AXT2-095

Methyl 2-((1*R*,2*S*)-1-((*tert*-butoxycarbonyl)amino)-2-methylbutyl)thiazole-4-carboxylate
(13)



To a flamed dried RBF under an inert Ar atmosphere was added an anhydrous solution of $\text{MeOH}/\text{Et}_3\text{N}$ (25.0 mL, 2:1, 0.12 M) and oxazoline **11** (931 mg, 2.96 mmol, 1 eq.). H_2S (g) was bubbled into the solution for 15 minutes and then stirred for 24 h. The mixture was concentrated under reduced pressure and purified via flash silica gel chromatography (3:2 ethyl acetate/hexane). In a flamed dried RBF thioamide compound (868.4 mg, 2.49 mmol, 1 eq) and anhydrous CH_2Cl_2 (25.0 mL, 0.10 M) were added and then cooled to -78°C under argon atmosphere. While at -78°C DAST (0.72 mL, 5.48 mmol, 2.2 eq.) was added dropwise and stirred for 1.5 h, followed by the addition of K_2CO_3 (520 mg, 3.74 mmol, 1.5 eq.). The mixture was slowly warmed to RT and stirred for 1 h. BrCCl_3 (0.98 mL, 9.97 mmol, 4 eq.) and DBU (1.49 mL, 9.97 mmol, 4 eq) were slowly added to solution, respectively, and stirred for 2.5 h. The reaction was quenched with slow addition of saturated NaHCO_3 (1:1 vol equiv.) and the product was extracted with CH_2Cl_2 (3 x 25 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (2:1 hexane/ethyl acetate) to afford **13** (739 mg, 90% yield) as a yellowish solid.

^1H NMR (400 MHz, CDCl_3) δ 8.07 (s, 1H), 5.21 (d, $J = 9.1$ Hz, 1H), 5.04 (dd, $J = 9.4, 4.6$ Hz, 1H), 3.92 (s, 3H), 2.25 (hept, $J = 6.6$ Hz, 1H), 1.52 – 1.36 (m, 10H), 1.24 (hept, 1H), 0.93 (t, $J =$

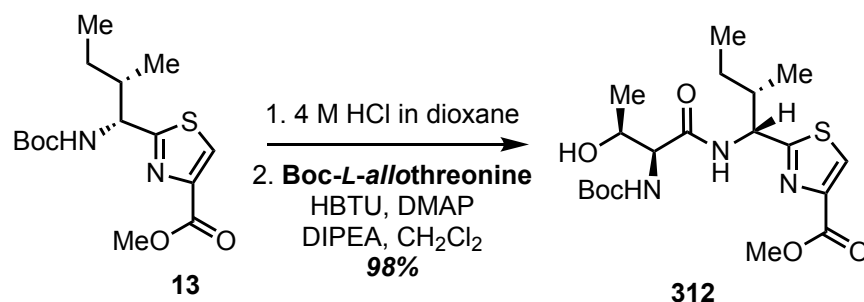
7.4 Hz, 3H), 0.81 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 174.46, 161.72, 155.27, 146.95, 126.94, 79.97, 56.42, 52.25, 39.35, 28.12, 26.40, 13.63, 11.40. HRMS(ESI): m/z calc. for $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_4\text{S}+\text{Na}$ $[\text{M}+\text{Na}]^+$: 351.1349, found: 351.1357

Notebook Entries

Procedure: AXT2-114

^1H NMR - AXT1-339-A and ^{13}C NMR – SGR-038

Methyl 2-((1*R*,2*S*)-1-((2*S*,3*S*)-2-((*tert*-butoxycarbonyl)amino)-3-hydroxybutanamido)-2-methylbutyl)thiazole-4-carboxylate (312**)**



Thiazole **13** (1.12 g, 3.2 mmol, 1.00 eq.) was dissolved in 4 M HCl in dioxane (27.0 mL, 0.12 M) and stirred for 1 h. Solution was concentrated under reduced pressure and crude material carried further without purification. To a stirring solution of crude material in CH_2Cl_2 (28.4 mL, 0.1 M) Boc-*L*-allothreonine (622 mg, 2.84 mmol, 1.0 eq.) was added and DIPEA (1.10 mL, 6.25 mmol, 2.20 eq.) and stirred for 10 minutes, followed by addition of HBTU (1.3 g, 3.41 mmol, 1.2 eq.) and DMAP (69.4 mg, 0.57 mmol, 0.2 eq.). The mixture was left to stir overnight, concentrated under reduced pressure, and purified via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **312** (1.2 g, 98% yield) as a yellowish solid.

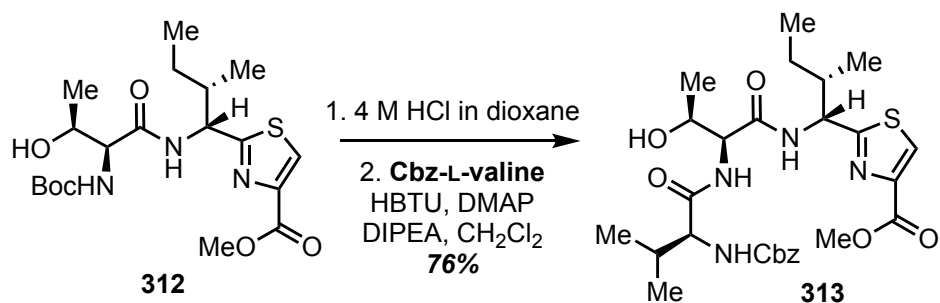
R_f = 0.44 (3:1 ethyl acetate/hexane, UV). [α]_D²⁵ = +15.32 (*c* 0.1, MeOH) **¹H NMR (400 MHz, CDCl₃)** δ 8.07 (s, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 5.63 (d, *J* = 8.0 Hz, 1H), 5.22 (dd, *J* = 8.9, 5.9 Hz, 1H), 4.30 – 4.22 (m, 1H), 4.12 (dd, *J* = 8.0, 2.7 Hz, 1H), 3.95 (d, *J* = 4.3 Hz, 1H), 3.87 (s, 3H), 2.13 (hept, *J* = 10.5, 5.9, 4.8 Hz, 1H), 1.44 – 1.34 (m, 11H), 1.15 – 1.05 (m, 4H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H). **¹³C NMR (151 MHz, CDCl₃)** δ 173.31, 171.39, 161.74, 156.17, 146.72, 127.51, 80.58, 68.88, 59.24, 54.99, 52.54, 39.59, 28.39, 26.80, 19.88, 14.04, 11.69. **FTIR (neat)**: 3325, 3297, 3109, 2969, 2933, 2879, 1715, 1961, 1660, 1523, 1299, 1240, 1168 cm⁻¹. **HRMS(ESI)**: *m/z* calc. for C₁₉H₃₁N₃O₆S+H [M+H]⁺: 430.1967, found: 430.2007

Notebook Entries

Procedure: AXT2-334

¹H NMR – AXT1-129-A and ¹³C NMR – AXT1-402-A

Methyl 2-((5*S*,8*S*,11*R*,12*S*)-8-((*S*)-1-hydroxyethyl)-5-isopropyl-12-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazatetradecan-11-yl)thiazole-4-carboxylate (313**)**



Compound **312** (1.59 g, 3.70 mmol, 1.05 eq.) was dissolved in 4 M HCl in dioxane (15.0 mL, 0.25 M) and stirred for 1 h. The solution was concentrated under reduced pressure and the crude material used carried forward without purification. To a stirring solution of crude material in CH₂Cl₂ (35.0 mL, 0.1 M), Cbz-L-valine (885 mg, 3.52 mmol, 1.0 eq.) and DIPEA (1.35 mL, 7.70 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (1.6 g, 4.22

mmol, 1.2 eq.) and DMAP (86.0 mg, 0.70 mmol, 0.2 eq.). The mixture was stirring overnight, quenched with 1 M HCl (1:1 vol eq.), and extracted with CH₂Cl₂ (3 x 40 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (5% MeOH in CH₂Cl₂) to afford **313** (1.50 g, 76% yield) as a white solid.

R_f = 0.54 (5% MeOH in CH₂Cl₂, UV). **¹H NMR (400 MHz, CDCl₃)** δ 8.07 (s, 1H), 7.36 – 7.27 (m, 5H), 7.21 (d, *J* = 8.3 Hz, 2H), 5.47 – 5.36 (m, 2H), 5.08 (dd, *J* = 11.0 Hz, 2H), 4.42 (dd, *J* = 7.3, 4.5 Hz, 1H), 4.11 (dd, *J* = 7.9, 5.5 Hz, 1H), 4.00 (q, *J* = 6.0 Hz, 1H), 3.91 (s, 3H), 3.79 – 3.62 (m, 1H), 2.20 (hept, 2H), 1.45 (hept, *J* = 13.9, 7.2 Hz, 1H), 1.31 – 1.20 (m, 4H), 0.97 – 0.92 (m, 6H), 0.87 (dd, *J* = 13.8, 6.9 Hz, 6H). **¹³C NMR (101 MHz, cdcl₃)** δ 172.96, 172.41, 170.75, 161.94, 156.76, 146.37, 136.16, 128.70, 128.39, 128.20, 127.74, 68.78, 67.38, 60.69, 58.18, 55.36, 52.63, 39.88, 30.89, 26.71, 19.79, 19.43, 17.73, 14.20, 11.69. **FTIR (neat):** 3299, 2990, 2891, 1718, 1697, 1650, 1525, 1236, 1202 cm⁻¹. **HRMS(ESI):** *m/z* calc. for C₂₇H₃₉N₄O₇S [M+H]⁺: 563.2539, found: 563.2539

Notebook Entries

Procedure: AXT1-093

¹H NMR – AXT1-133-A(2) and ¹³C NMR – PKD2-162-C13-400

Rf = 0.37 (1:1 ethyl acetate/hexane, UV). $[\alpha]_{\text{D}}^{25} = +27.07$ (*c* 0.1, CHCl₃). **¹H NMR (400 MHz, CDCl₃)** δ 7.99 (s, 1H), 7.36 – 7.29 (m, 5H), 5.47 (d, *J* = 8.8 Hz, 1H), 5.33 (dd, *J* = 9.1, 5.0 Hz, 1H), 5.07 (dd, 2H), 4.76 (dq, *J* = 6.4, 6.2 Hz, 1H), 4.39 (dd, *J* = 8.9, 5.4 Hz, 1H), 4.22 (d, *J* = 7.4 Hz, 1H), 3.89 (s, 3H), 2.29 (hept, 1H), 2.09 (hept, *J* = 13.4, 6.6 Hz, 1H), 1.49 – 1.41 (m, 4H), 1.24 (p, *J* = 7.3, 6.7 Hz, 1H), 0.96 – 0.91 (m, 6H), 0.87 (dd, *J* = 10.7, 6.9 Hz, 6H). **¹³C NMR (151 MHz, CDCl₃)** δ 172.69, 171.26, 168.97, 161.62, 155.96, 146.97, 136.11, 128.47, 128.16, 128.03, 126.92, 80.63, 74.29, 66.95, 54.78, 54.71, 52.31, 39.34, 31.22, 26.47, 21.71, 18.70, 17.54, 13.97, 11.46.

FTIR (neat): 3319, 2962, 2932, 2876, 1718, 1653, 1507, 1456, 1326, 1209, 1023, 751, 697 cm⁻¹

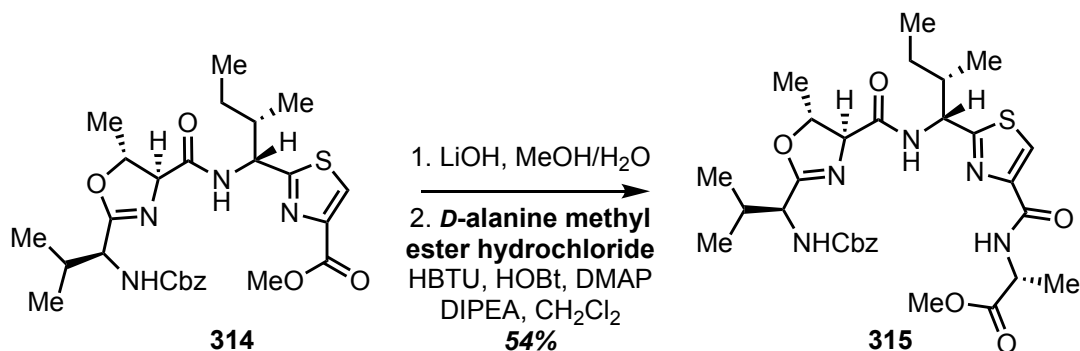
HRMS(ESI): *m/z* calc. for C₂₇H₃₆N₄O₆S+H [M+H]⁺: 545.2389, found: 545.2452

Notebook Entries

Procedure: AXTA-126

¹H NMR - AXT1-041-A and ¹³C NMR – AXT2-031

Methyl (2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-(((benzyloxy)carbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alaninate (315**)**



To a solution of **314** (425 mg, 0.78 mmol, 1 eq) in 1:1 MeOH/H₂O (13.0 mL, 0.06 M), LiOH (94.0 mg, 3.91 mmol, 5 eq) was added and left to stir. After 1 h the solvent was removed under reduce pressure and then dissolved in EtOAc and acidified to a pH of 2 with 1 M HCl. The mixture was extracted with EtOAc (3 x 15 mL), the combined organic layers dried over saturated Na₂SO₄, and concentrated under reduced pressure. The obtained crude material was added to a stirring solution of *D*-alanine methyl ester hydrochloride (148 mg, 1.10 mmol, 1.5 eq.) in CH₂Cl₂ (35.0 mL, 0.1 M), followed by addition of DIPEA (0.27 mL, 1.56 mmol, 2.20 eq.) and stirred for 10 minutes. HBTU (322 mg, 0.85 mmol, 1.2 eq.), HOBT (223 mg, 1.42 mmol, 2 eq.) and DMAP (17.0 mg, 0.14 mmol, 0.2 eq.) were added and stirred for 20 h. The mixture was quenched with 1 M HCl (1:1

vol) and extracted with CH₂Cl₂ (3 x 15 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **315** (376 mg, 54% yield) as a white solid.

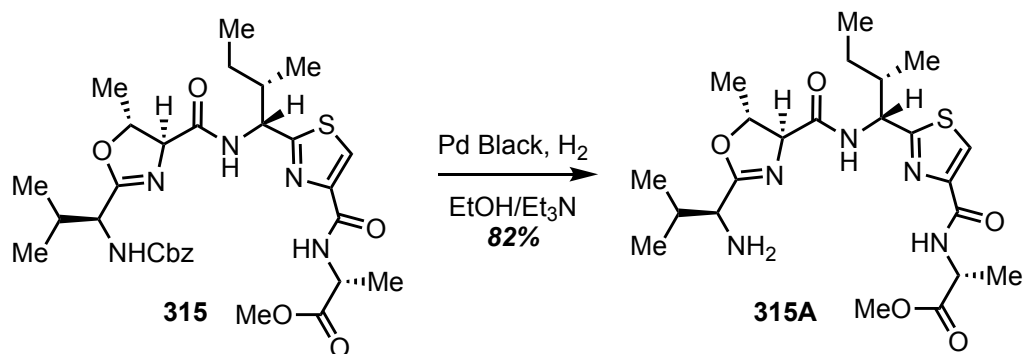
R_f = 0.58 (3:1 ethyl acetate/hexane, UV). [α]_D²⁵ = +45.88 (*c* 0.1, MeOH). **¹H NMR (400 MHz, CDCl₃)** δ 7.93 (s, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.28 (m, 5H), 7.26 (d, *J* = 9.0 Hz, 1H), 5.63 (d, *J* = 8.6 Hz, 1H), 5.27 (dd, *J* = 9.0, 5.3 Hz, 1H), 5.04 (dd, 2H), 4.80 – 4.69 (m, 2H), 4.33 (dd, *J* = 8.6, 5.9 Hz, 1H), 4.23 (d, *J* = 7.2 Hz, 1H), 3.68 (s, 3H), 2.17 (hept, *J* = 6.4 Hz, 1H), 2.06 (hept, *J* = 6.6 Hz, 1H), 1.49 – 1.44 (m, 7H), 1.22 – 1.14 (m, 1H), 0.97 – 0.92 (m, 6H), 0.90 (d, *J* = 7.1 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H). **¹³C NMR (151 MHz, CDCl₃)** δ 173.43, 171.66, 171.33, 169.33, 160.28, 156.18, 149.57, 136.20, 128.56, 128.29, 128.22, 123.06, 80.63, 74.44, 67.04, 55.04, 54.69, 52.54, 47.85, 39.73, 31.04, 26.28, 21.82, 18.79, 18.44, 17.75, 14.49, 11.56. **FTIR (neat)**: 3313, 2963, 2929, 2875, 1718, 1653, 1647, 1507, 1457, 1209, 1157, 1023, 696 cm⁻¹ **HRMS(ESI)**: *m/z* calc. for C₃₀H₄₁N₅O₇S+H [M+H]⁺: 616.2760, found: 616.2817

Notebook Entries

Procedure: AXT1-422

¹H NMR – AXT1-422 (Test tubes fractions) and ¹³C NMR – AXT1-422

Methyl (2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-amino-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alaninate (315A** – step 3)**



To a flamed dried RBF containing a solution of **315** (440 mg, 0.71 mmol, 1 eq) in anhydrous EtOH/Et₃N (15.0 mL, 3:1, 0.05 M) was added Pd Black (760 mg, 7.15 mmol, 10 eq) and stirred for 1 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and purified through amino-functionalized silica gel chromatography (100% ethyl acetate). Product **315A** was accessed (278 mg, 82% yield) as a colorless oil.

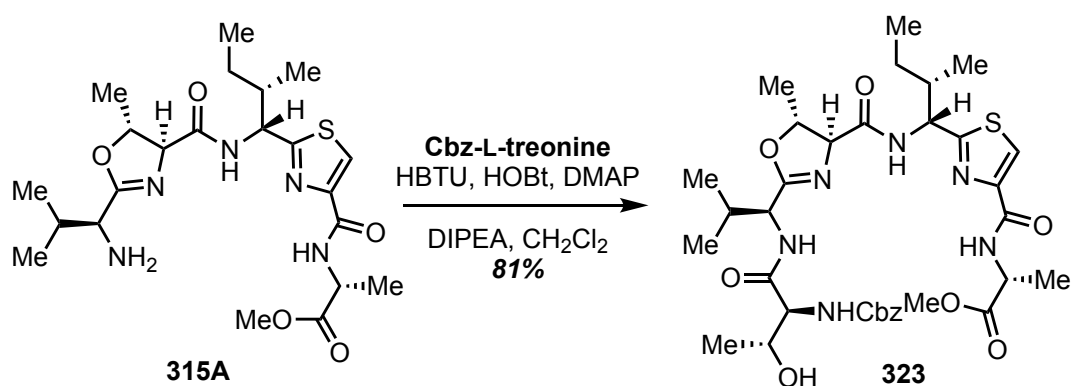
¹H NMR (400 MHz, Chloroform-*d*) δ 7.98 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 5.35 – 5.27 (m, 1H), 4.81 – 4.69 (m, 2H), 4.23 (d, *J* = 7.7 Hz, 1H), 3.78 (s, 3H), 3.40 (d, *J* = 5.4 Hz, 1H), 2.21 (hept, *J* = 6.8 Hz, 1H), 1.97 (hept, *J* = 6.2, 5.6 Hz, 1H), 1.59 (s, 2H), 1.55 – 1.44 (m, 7H), 1.25 (hept, 1H), 1.00 – 0.90 (m, 9H), 0.87 (d, *J* = 6.8 Hz, 3H). **¹³C NMR (101 MHz, CDCl₃)** δ 173.37, 172.83, 172.02, 171.66, 160.47, 149.81, 123.19, 80.22, 74.59, 55.64, 54.56, 52.63, 48.05, 39.84, 32.41, 26.53, 22.00, 19.30, 18.61, 17.36, 14.42, 11.71. **FTIR (neat)**: 3321, 2962, 2932, 2876, 1740, 1654, 1647, 1539, 1534, 1490, 1209, 1158, 729 cm⁻¹. **HRMS(ESI): *m/z*** calc. for C₂₂H₃₅N₅O₅S+H [M+H]⁺: 482.2392, found: 482.2437

Notebook Entries

Procedure: AXT2-076

¹H NMR and ¹³C NMR – AXT2-076

Methyl (2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-((2*S*,3*R*)-2-(((benzyloxy)carbonyl)amino)-3-hydroxybutanamido)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alaninate (323**)**



To a stirring solution of free amine **315A** (265 mg, 0.55 mmol, 1.05 eq.) in CH₂Cl₂ (5.2 mL, 0.1 M) was added Cbz-L-threonine (123 mg, 0.52 mmol, 1eq.) followed by addition of DIPEA (0.20 mL, 1.14 mmol, 2.20 eq.) and stirred for 10 minutes. HBTU (379 mg, 0.63 mmol, 1.2 eq.), HOBT (41 mg, 0.26 mmol, 0.5 eq.) and DMAP (13.0 mg, 0.10 mmol, 0.2 eq.) were added and stirred for 3 h. The mixture was quenched with 1 M HCl (1:1 vol equiv.) and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (100% ethyl acetate) to afford **323** (305 mg, 81% yield) as a white solid.

¹H NMR (400 MHz, CDCl₃) δ 8.21 (d, 1H), 8.02 (s, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.38 – 7.29 (m, 7H), 5.74 (d, *J* = 8.0 Hz, 1H), 5.29 (dd, *J* = 8.9, 5.0 Hz, 1H), 5.10 (s, 2H), 5.04 – 4.93 (m, 2H),

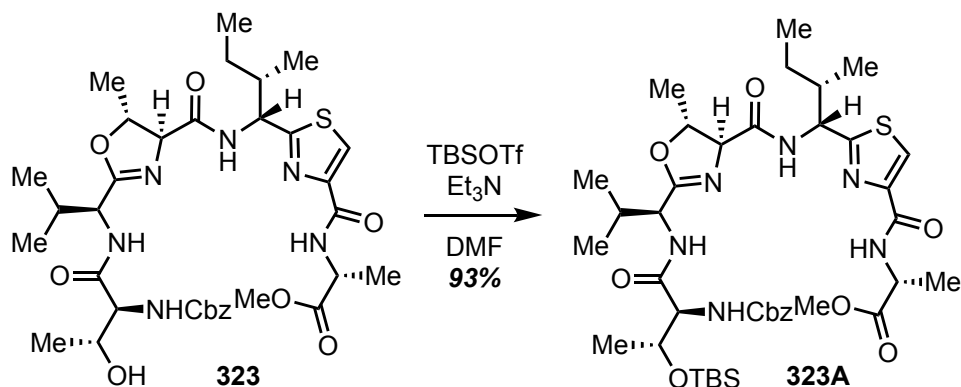
4.87 (p, $J = 6.5$ Hz, 1H), 4.60 (dd, $J = 8.5, 4.3$ Hz, 1H), 4.43 (dd, $J = 8.7, 4.1$ Hz, 1H), 4.27 (t, $J = 7.4$ Hz, 1H), 4.19 (d, $J = 8.0$ Hz, 1H), 3.81 (s, 3H), 2.19 (hept, $J = 12.5, 6.3$ Hz, 1H), 1.99 (hept, $J = 6.6$ Hz, 1H), 1.61 (d, $J = 7.1$ Hz, 3H), 1.54 – 1.49 (m, 4H), 1.20 (d, $J = 6.4$ Hz, 3H), 1.13 (hept, $J = 14.3, 7.2$ Hz, 1H), 0.96 (t, $J = 7.7$ Hz, 9H), 0.70 (d, $J = 6.9$ Hz, 3H). **^{13}C NMR (101 MHz, CDCl_3)** δ 175.08, 170.64, 170.44, 170.16, 169.62, 160.42, 156.13, 149.47, 136.38, 128.70, 128.33, 128.09, 123.57, 81.52, 73.33, 67.98, 67.05, 58.23, 54.59, 53.68, 53.01, 47.57, 41.34, 29.21, 26.13, 21.78, 19.02, 18.81, 17.44, 16.54, 14.58, 11.80. **HRMS(ESI):** m/z calc. for $\text{C}_{34}\text{H}_{48}\text{N}_6\text{O}_9\text{S}+\text{H}$ $[\text{M}+\text{H}]^+$: 717.3237, found: 717.3281. **FTIR (neat):** 3326, 2974, 2924, 1684, 1653, 1636, 1506, 1251, 1157, 1044, 1002, 743, 700 cm^{-1}

Notebook Entries

Procedure: AXT2-079

^1H NMR – AXT2-079 and ^{13}C NMR – AXT2-079-B

Methyl (2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-((2*S*,3*R*)-2-(((benzyloxy)carbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)butanamido)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alaninate (323A**)**



To a flamed dried RBF under an Ar atmosphere was added free alcohol **323** (58 mg, 0.08 mmol, 1 eq) in anhydrous DMF (1.0 mL, 0.08 M) and cooled to 0 °C. Once at temperature, Et_3N (0.04

mL, 0.32 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (0.04 mL, 0.16 mmol, 2 eq) was added, mixture was slowly warmed to RT and stirred for 24 h. The reaction was quenched with H₂O (1:1 vol equiv.) and extracted with ethyl acetate (3 x 2 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 ethyl acetate/hexane) to afford **323A** (62 mg, 93% yield) as a yellowish oil.

¹H NMR (600 MHz, CDCl₃) δ 7.98 (s, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.39 – 7.31 (m, 5H), 7.25 (dd, *J* = 14.1, 8.7 Hz, 2H), 5.85 (d, *J* = 6.1 Hz, 1H), 5.32 (dd, *J* = 9.2, 5.2 Hz, 1H), 5.18 – 5.06 (m, 2H), 4.77 (p, *J* = 7.3 Hz, 1H), 4.72 (p, *J* = 6.6 Hz, 1H), 4.56 (t, *J* = 7.5 Hz, 1H), 4.33 – 4.28 (m, 1H), 4.26 – 4.22 (m, 2H), 3.76 (s, 3H), 2.27 (hept, *J* = 6.6 Hz, 1H), 2.07 (hept, *J* = 13.8, 6.9 Hz, 1H), 1.53 (d, *J* = 7.2 Hz, 3H), 1.51 – 1.44 (m, 4H), 1.28 (hept, *J* = 14.2, 7.6 Hz, 1H), 1.03 (d, *J* = 6.3 Hz, 3H), 1.00 – 0.92 (m, 21H), 0.16 (d, *J* = 28.3 Hz, 6H). **¹³C NMR (151 MHz, CDCl₃)** δ 173.28, 172.31, 171.38, 169.32, 168.43, 160.41, 156.19, 149.81, 136.18, 128.57, 128.23, 128.06, 122.99, 80.42, 74.64, 68.36, 66.97, 59.04, 54.67, 53.46, 52.53, 47.97, 39.30, 31.15, 26.42, 25.76, 21.93, 18.87, 18.56, 18.47, 17.95, 17.65, 14.39, 11.56. **HRMS(ESI):** *m/z* calc. for C₄₀H₆₂N₆O₉SSi+H [M+H]⁺: 831.4102, found: 831.4143. **FTIR (neat):** 2957, 2925, 2849, 1748, 1734, 1700, 1684, 1653, 1558, 1539, 1506, 1026, 419 cm⁻¹

Notebook Entries

Procedure: AXT2-007

¹H NMR and ¹³C NMR - AXT2-007

Chemical reaction scheme showing the reduction of compound **323A** to compound **324**.

Reaction conditions: Pd Black, H₂, EtOH/Et₃N.

Yield: 99%.

¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, *J* = 7.9 Hz, 1H), 7.95 (s, 1H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 9.0 Hz, 1H), 5.32 (dd, *J* = 8.8, 4.9 Hz, 1H), 4.75 (h, *J* = 7.3, 6.6 Hz, 2H), 4.44 – 4.36 (m, 2H), 4.24 (d, *J* = 6.8 Hz, 1H), 3.78 (s, 3H), 3.28 (p, *J* = 7.0 Hz, 1H), 3.01 (s, 1H), 2.29 (hept, *J* = 6.7 Hz, 1H), 2.10 (hept, *J* = 6.6 Hz, 1H), 1.96 (s, 1H), 1.75 (s, 2H), 1.53 (d, *J* = 7.3 Hz, 3H), 1.49 – 1.45 (m, 4H), 1.31 (hept, *J* = 7.4 Hz, 1H), 1.10 (d, *J* = 6.4 Hz, 3H), 1.01 – 0.94 (m, 12H), 0.84 (s, 9H), 0.02 (d, *J* = 18.9 Hz, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 173.25, 173.06, 171.82, 169.36, 160.52, 149.61, 122.97, 80.74, 74.75, 68.07, 60.11, 54.84, 53.66, 52.49, 47.98, 39.05, 30.63, 26.52, 25.77, 21.92, 20.55, 18.91, 18.72, 18.43, 17.91, 14.43, 11.61, -4.45, -4.97.

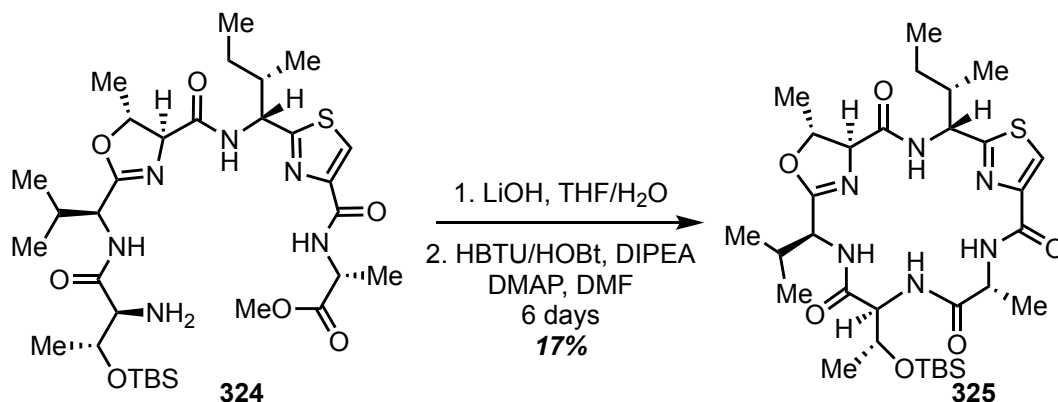
HRMS(ESI): m/z calc. for $C_{32}H_{56}N_6O_7SSi+H$ $[M+H]^+$: 697.3437, found: 697.3774. **FTIR (neat):** 3524, 3400, 2959, 2919, 2850, 1684, 1652, 1620, 1084, 663, 5583 cm^{-1}

Notebook Entries

Procedure: AXT2-086-A

^1H NMR and ^{13}C NMR - AXT2-086-A

Balgacyclamide B (2)



Step 1: To a stirring solution of free amine **324** (197 mg, 0.28 mmol, 1 eq) in THF/H₂O (19.0 mL, 1:1, 0.015 M) was added LiOH (45.0 mg, 1.90 mmol, 6.67 eq) and stirred for 2 h. The mixture was quenched with saturated NH₄Cl (pH = 6) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to access free carboxylic acid. The material was carried on without further purification.

Step 2: The crude material was dissolved in DMF (56.0 mL, 0.005M), DIPEA was added (0.15 mL, 0.85 mmol, 3 eq.) and solution stirred for 10 minutes. HBTU (161 mg, 0.43 mmol, 1.5 eq.), HOBt (67 mg, 0.43 mmol, 1.5 eq.) and DMAP (17.0 mg, 0.14 mmol, 0.5 eq.) were added and allowed to stir for 4 days. The mixture was quenched with saturated NH₄Cl (1:1 vol equiv.) and extracted with ether (5 x 50 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Purification was performed via

[illegible]

¹H NMR (400 MHz, CDCl₃) δ 8.56 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 5.12 (t, *J* = 9.4, 5.5, 2.7 Hz, 1H), 5.05 (q, *J* = 8.5, 7.8 Hz, 1H), 4.92 (q, 1H), 4.54 (td, *J* = 9.0, 3.1 Hz, 3H), 4.28 (d, *J* = 6.3 Hz, 1H), 2.02 – 1.88 (m, 2H), 1.62 – 1.53 (m, 4H), 1.41 (d, *J* = 6.4, 1.6 Hz, 3H), 1.26 – 1.20 (m, 4H), 1.03 (d, *J* = 6.9, 1.5 Hz, 3H), 0.97 – 0.91 (m, 3H), 0.45 (d, *J* = 7.2, 1.7 Hz, 3H), 0.39 (d, *J* = 6.8 Hz, 3H). **¹³C NMR (101 MHz, CDCl₃)** δ 175.38, 171.62, 170.52, 167.99, 166.57, 160.40, 148.74, 123.23, 79.37,

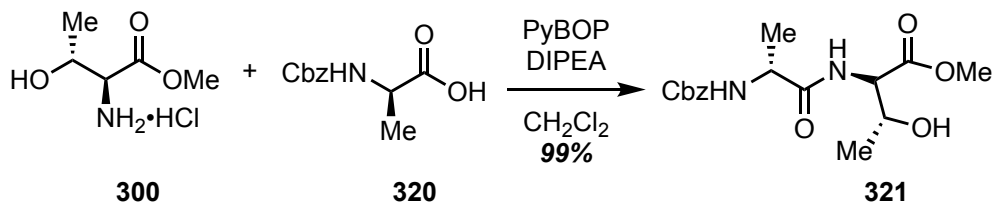
74.49, 65.26, 56.56, 55.54, 52.68, 49.33, 41.33, 31.55, 25.58, 21.72, 19.61, 18.35, 17.51, 17.20, 15.25, 11.70. **HRMS(ESI):** m/z calc. for $C_{25}H_{38}N_6O_6S+H$ $[M+H]^+$: 551.2607, found: 551.2656. **FTIR (neat):** 3311, 2963, 2925, 2875, 1734, 1653, 1646, 1636, 1539, 1507, 1219, 1029 cm^{-1}

Notebook Entries

Procedures: Step 1 – AXT2-087, Step 2 – AXT2-088, Step 3 – AXT2-096

^1H NMR and ^{13}C NMR: Step 1 – no NMR – Step 2 – AXT2-088-A and AXT2-088-B, Step 3 – in chloroform AXT2-096, in DMSO AXT2-0960-DMSO and Balg B DMSO 600

Methyl ((benzyloxy)carbonyl)-*D*-alanyl-*L*-threoninate (**321**)



To a RBF containing **320** (474 g, 2.12 mmol, 1.0 eq.) in CH_2Cl_2 (21.0 mL, 0.1 M) were added PyBOP (1.30 g, 2.54 mmol, 1.20 eq.) and DIPEA (0.44 mL, 2.54 mmol, 1.2 eq.). To a separate RBF L-threonine methyl ester hydrochloride (**300**; 540 mg, 3.18 mmol, 1.5 eq.), DIPEA (1.40 mL, 7.84 mmol, 3.7 eq.), and CH_2Cl_2 (1 M) were added. Both reaction mixtures were left to stir separately. After 30 min the two solutions were combined and stirred overnight. The reaction was quenched with saturated $NaHCO_3$ (1:1 vol equiv.), and the product was extracted with CH_2Cl_2 (3 x 30 mL) and washed with brine (1:1 vol equiv.). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **321** (712 mg, 99% yield) as a white solid.

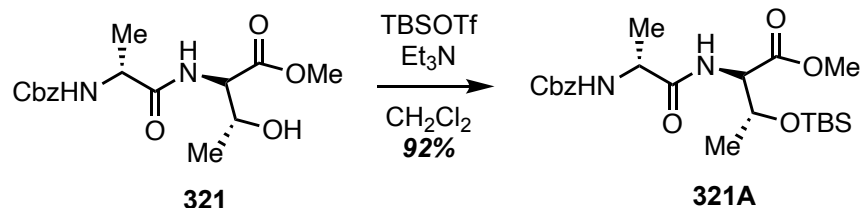
Rf: 0.60 (100% ethyl acetate). **¹H NMR (400 MHz, CDCl₃)** δ 7.35 – 7.25 (m, 5H), 7.21 (d, *J* = 9.0 Hz, 1H), 5.85 (d, *J* = 7.6 Hz, 1H), 5.11 – 5.04 (m, 2H), 4.55 (dd, *J* = 9.0, 2.6 Hz, 1H), 4.39 (p, *J* = 7.0 Hz, 1H), 4.35 – 4.27 (m, 1H), 3.69 (s, 3H), 3.23 (d, *J* = 5.2 Hz, 1H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.15 (d, *J* = 6.4 Hz, 3H). **¹³C NMR (101 MHz, CDCl₃)** δ 173.26, 171.67, 156.23, 128.79, 128.47, 128.38, 68.13, 67.34, 57.44, 52.88, 50.99, 20.10, 19.11. **HRMS(ESI):** *m/z* calc. for C₁₆H₂₂N₂O₆+Na [M+Na]⁺: 361.1370, found: 361.1377

Notebook Entries

Procedure: AXT1-206

¹H NMR - AXT2-122 and ¹³C NMR - AXT2-163

Methyl *N*-(((benzyloxy)carbonyl)-*D*-alanyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-threoninate (321A**)**



To a flamed dried RBF under an Ar atmosphere was added free alcohol **321** (83 mg, 0.25 mmol, 1 eq) in anhydrous CH₂Cl₂ (2.5 mL, 0.1 M) and cooled to 0 °C. Once at temperature Et₃N (0.14 mL, 0.98 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (0.11 mL, 0.49 mmol, 2 eq) was added, mixture was slowly warmed to RT and stirred for 48 h. The reaction was quenched with saturated NH₄Cl (1:1 vol equiv.) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (2:1 hexane / ethyl acetate) to afford **321A** (102 mg, 92% yield) as a colorless oil.

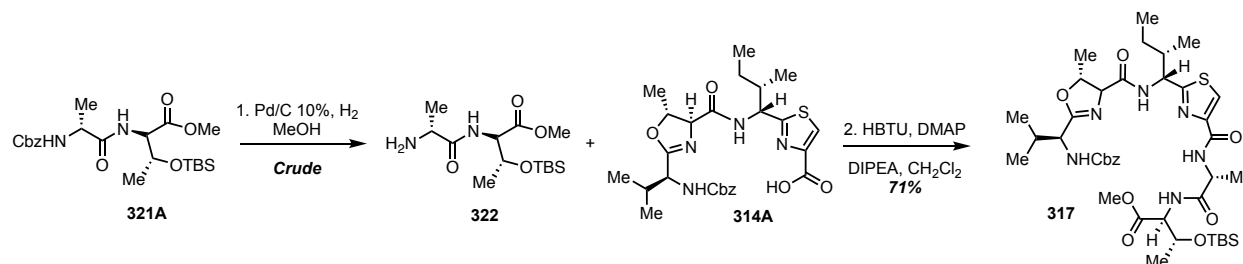
¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.31 (m, 5H), 6.72 (d, *J* = 9.4 Hz, 1H), 5.42 (d, *J* = 7.1 Hz, 1H), 5.21 – 5.04 (m, 2H), 4.54 (dd, *J* = 9.4, 1.7 Hz, 1H), 4.50 – 4.43 (m, 1H), 4.42 – 4.34 (m, 1H), 3.72 (s, 3H), 1.45 (d, *J* = 7.1 Hz, 3H), 1.17 (d, *J* = 6.2 Hz, 3H), 0.87 (s, 9H), 0.06 (s, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 172.60, 170.84, 155.92, 136.18, 128.56, 128.23, 128.17, 68.76, 67.09, 57.73, 52.34, 50.70, 25.61, 20.86, 18.76, 17.80, -4.41, -5.34, -5.62. **HRMS(ESI):** *m/z* calc. for C₂₂H₃₆N₂O₆Si+H [M+H]⁺: 453.2376, found: 453.2419. **FTIR (neat):** 3419, 3302, 2978, 2951, 2926, 2851, 1741, 1719, 1689, 1537, 1518, 1253, 1207, 1069, 694 cm⁻¹

Notebook Entries

Procedure: AXT1-202

¹H NMR and ¹³C NMR : AXT1-202 (CNMR)

Methyl *N*-((2-((1*R*,2*S*)-1-((5*R*)-2-((*S*)-1-(((benzyloxy)carbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alanyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-threoninate (317**)**



To a flamed dried RBF containing a solution of **321A** (128 mg, 0.28 mmol, 1 eq) in anhydrous MeOH (20.0 mL, 0.007 M) was added Pd/C 10% (124 mg, 3 eq) and stirred for 2 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and filtrated through celite to obtain crude free amine of **322**. To a stirring solution of crude material (55 mg, 0.17 mmol, 1.1 eq.) in CH₂Cl₂ (2.0 mL, 0.1 M), free carboxylic acid **314A** (84.0 mg, 0.16 mmol, 1.0 eq.) and DIPEA (0.06 mL, 0.35 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (72.0 mg, 0.19 mmol, 1.2 eq.) and DMAP (4.00 mg, 0.03 mmol, 0.2 eq.). The mixture was stirring overnight, quenched with saturated NH₄Cl (1:1 vol), and extracted with CH₂Cl₂ (3 x 40 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (2:1 ethyl acetate/hexane) to afford **317** (93.0 mg, 71% yield) as a white solid.

¹H NMR (400 MHz, CDCl₃) δ 7.98 (s, 1H), 7.95 (d, *J* = 7.7 Hz, 1H), 7.41 – 7.30 (m, 6H), 6.58 (d, *J* = 9.6 Hz, 1H), 6.00 (d, *J* = 8.7 Hz, 1H), 5.30 (dd, *J* = 9.0, 5.1 Hz, 1H), 5.18 – 5.01 (m, 2H), 4.83 – 4.71 (m, 2H), 4.57 (d, *J* = 9.5 Hz, 1H), 4.32 – 4.23 (m, 3H), 3.65 (s, 3H), 2.16 (hept, *J* = 7.0 Hz, 1H), 2.03 (hept, *J* = 13.4, 6.6 Hz, 1H), 1.55 – 1.44 (m, 7H), 1.20 (hept, *J* = 14.4, 7.1 Hz, 1H),

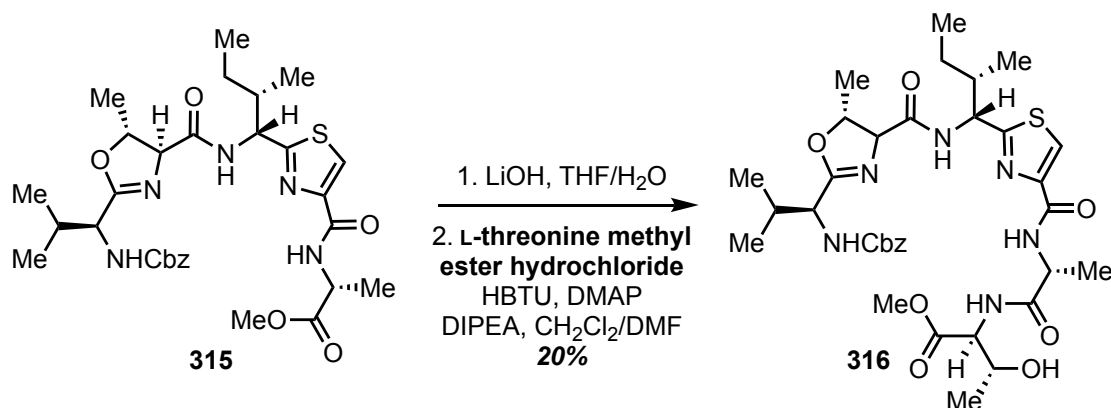
1.03 (d, $J = 6.3$ Hz, 3H), 0.99 – 0.94 (m, 6H), 0.87 – 0.82 (m, 6H), 0.81 (s, 9H), -0.01 (d, $J = 25.6$ Hz, 6H). **^{13}C NMR (101 MHz, CDCl_3)** δ 172.32, 171.36, 170.58, 169.59, 160.35, 156.36, 149.53, 136.32, 128.53, 128.35, 128.23, 122.98, 80.41, 74.50, 68.80, 67.08, 57.70, 55.14, 54.78, 52.25, 48.70, 40.07, 31.04, 26.23, 25.56, 21.80, 20.73, 19.21, 18.73, 17.94, 17.74, 14.48, 11.58, -4.44, -5.35. **HRMS(ESI):** m/z calc. for $\text{C}_{40}\text{H}_{62}\text{N}_6\text{O}_9\text{SSi} + \text{H} [\text{M} + \text{H}]^+$: 831.4102, found: 831.4139. **FTIR (neat):** 3568, 2960, 2930, 2856, 1653, 1628, 1539, 1457, 1250, 1091, 835, 776, 558 cm^{-1}

Notebook Entries

Procedure: AXT1-229

^1H NMR – AXT1-324 (9-11) and ^{13}C NMR – AXT1-324-B

Methyl (2-((1*R*,2*S*)-1-((5*R*)-2-((*S*)-1-(((benzyloxy)carbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alanyl-*L*-threoninate (316)



To a solution of **315** (109 mg, 0.18 mmol, 1 eq.) in THF/ H_2O (3.0 mL, 0.06 M), LiOH (21.0 mg, 0.90 mmol, 5 eq) was added and stirred for 15 minutes. The mixture was quenched with 1M HCl (brought to a pH of 2) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to access free carboxylic acid and

used without further purification. The crude material was added to a stirring solution of L-threonine methyl ester hydrochloride (33 mg, 1.10 mmol, 1.2 eq.) in CH₂Cl₂ (2.0 mL, 0.1 M) and DMF (0.50 mL, 0.36 M) followed by addition of DIPEA (0.07 mL, 0.40 mmol, 2.20 eq.) and stirred for 10 minutes. HBTU (80.0 mg, 0.21 mmol, 1.2 eq.), and DMAP (5.0 mg, 0.04 mmol, 0.2 eq.) were added and stirred overnight. The mixture was quenched with 1 M HCl (1:1 vol) and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (3:2:0.2 ether/CH₂Cl₂/MeOH) to afford **316** (25 mg, 20% yield) as a white solid.

¹H NMR (400 MHz, Methanol-*d*₄) δ 8.11 (d, *J* = 19.1 Hz, 1H), 7.35 – 7.26 (m, 5H), 5.31 – 5.26 (m, 1H), 5.06 – 4.93 (m, 2H), 4.81 – 4.70 (m, 2H), 4.51 (d, *J* = 2.9 Hz, 1H), 4.34 – 4.25 (m, 2H), 4.15 (d, *J* = 7.3 Hz, 1H), 3.71 (s, 3H), 2.31 – 2.17 (m, 1H), 2.06 (h, *J* = 13.5, 6.9 Hz, 1H), 1.51 – 1.43 (m, 6H), 1.42 – 1.40 (m, 1H), 1.28 – 1.21 (m, 1H), 1.15 (dd, 3H), 1.00 – 0.94 (m, 9H), 0.93 – 0.91 (m, 3H). **¹³C NMR (101 MHz, Methanol-*d*₄)** δ 174.93, 173.51, 172.31, 171.64, 162.72, 158.59, 150.33, 138.04, 129.49, 129.06, 128.84, 125.11, 82.15, 75.40, 68.31, 67.72, 59.19, 57.12, 56.29, 52.80, 50.13, 40.62, 31.54, 27.40, 21.76, 20.28, 19.43, 19.36, 19.02, 18.88, 15.07, 11.82.

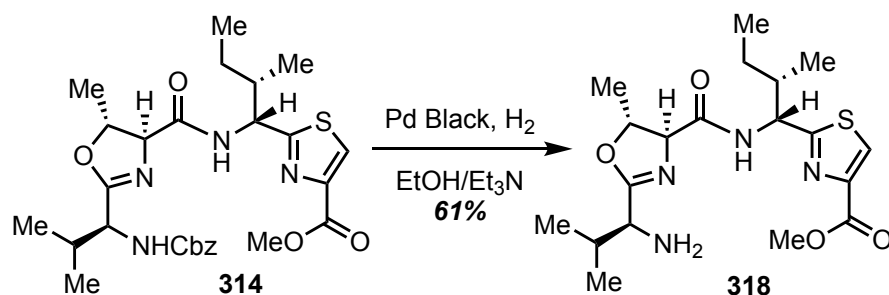
HRMS(ESI): *m/z* calc. for C₃₄H₄₈N₆O₉S+H [M+H]⁺: 717.3237, found: 717.3275

Notebook Entries

Procedure: AXT1-143

¹H NMR and ¹³C NMR – AXT1-143-C (MeOH)

Methyl 2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-amino-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carboxylate (318**)**



To a flamed dried RBF containing a solution of **314** (393 mg, 0.72 mmol, 1 eq) in anhydrous EtOH/Et₃N (72.0 mL, 3:1, 0.01 M) was added Pd Black (763 mg, 7.2 mmol, 10 eq) and stirred for 1.5 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and purified through amino-functionalized silica gel chromatography (100% ethyl acetate). Product **318** was accessed (181 mg, 61% yield) as a white solid.

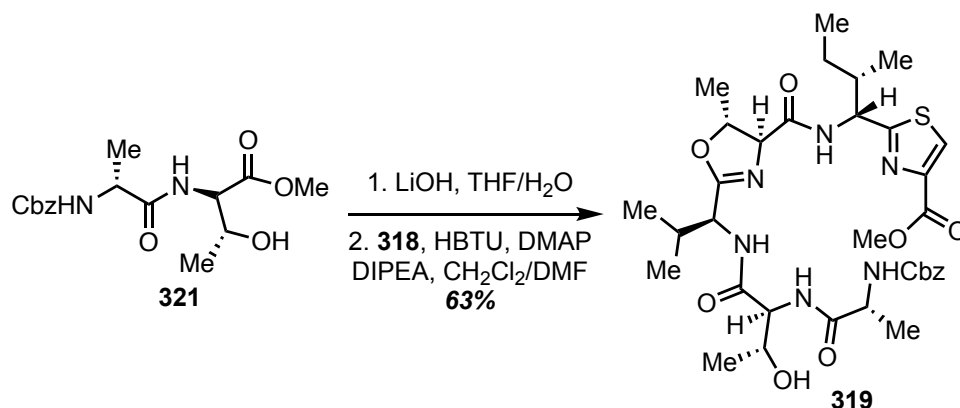
¹H NMR (400 MHz, CDCl₃) δ 8.05 (s, 1H), 7.28 (d, *J* = 9.2 Hz, 1H), 5.34 (dd, *J* = 9.2, 4.9 Hz, 1H), 4.75 – 4.68 (m, 1H), 4.20 (d, *J* = 7.6, 1.1 Hz, 1H), 3.91 (s, 3H), 3.38 (d, *J* = 5.6, 1.0 Hz, 1H), 2.29 (hept, 1H), 2.00 – 1.92 (m, 1H), 1.53 (brs, 2H), 1.47 (d, *J* = 6.2 Hz, 3H), 1.46 – 1.40 (m, 1H), 1.24 (hept, 1H), 0.97 – 0.91 (m, 6H), 0.88 (dd, *J* = 9.0, 6.8 Hz, 6H). **¹³C NMR (151 MHz, CDCl₃)** δ 172.67, 172.64, 171.62, 161.65, 147.04, 127.05, 80.04, 74.40, 55.54, 54.67, 52.39, 39.55, 32.14, 26.54, 21.84, 19.20, 17.37, 14.01, 11.53. **HRMS(ESI):** *m/z* calc. for C₁₉H₃₀N₄O₄S+H [M+H]⁺: 411.2021, found: 411.2069. **FTIR (neat):** 3373, 2961, 2932, 2875, 1723, 1653, 1647, 1507, 1499, 1323, 1239, 1210, 1094, 752 cm⁻¹

Notebook Entries

Procedure: AXT2-037

¹H NMR – AXT2-037 and ¹³C NMR – AXT2-019-A

Methyl 2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((5*R*,8*S*,11*S*)-8-((*R*)-1-hydroxyethyl)-5,12-dimethyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazatridecan-11-yl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carboxylate (319**)**



To a solution of **321** (34.0 mg, 0.10 mmol, 1 eq.) in MeOH/H₂O (1.0 mL, 0.10 M), LiOH (6.0 mg, 0.25 mmol, 2.5 eq) was added and stirred for 3h. Methanol was concentrated, and solution diluted in EtOAc, followed by acidification to pH 2 with 1 M HCl. The mixture was extracted with EtOAc (3 x 5 mL), dried over saturated Na₂SO₄, and concentrated under reduced pressure. Material was pushed to next reaction without further purification. Crude material was added to a stirring solution of free amine **318** (44.0 mg, 0.11 mmol, 1.25 eq.) in CH₂Cl₂/THF (1.0 mL, 1:1, 0.1 M), followed by addition of DIPEA (0.03 mL, 0.19 mmol, 2.20 eq.). HBTU (38.0 mg, 0.10 mmol, 1.2 eq.), and DMAP (2.0 mg, 0.02 mmol, 0.2 eq.) were added and stirred overnight. The mixture was quenched with 1 M HCl (1:1 vol) and extracted with CH₂Cl₂ (3 x 10 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via gravity silica gel chromatography (5% MeOH in DCM) to afford **319** (38 mg, 63% yield).

¹H NMR (400 MHz, CDCl₃) δ 8.08 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.48 (d, *J* = 9.1 Hz, 1H), 7.38 – 7.28 (m, 5H), 7.01 (d, *J* = 8.1 Hz, 1H), 5.48 (d, *J* = 7.3 Hz, 1H), 5.35 (dd, *J* = 8.8, 4.2 Hz,

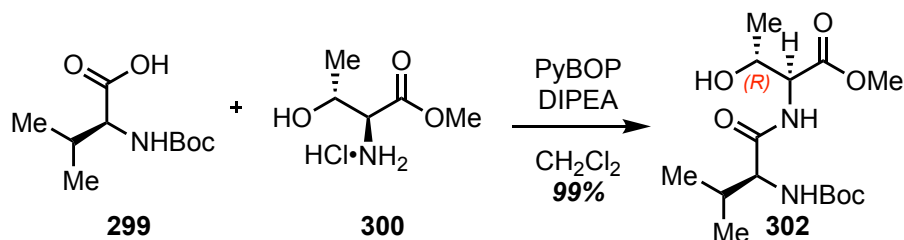
1H), 5.15 – 5.04 (m, 2H), 4.92 (p, $J = 6.3$ Hz, 1H), 4.78 – 4.69 (m, 3H), 4.43 – 4.32 (m, 2H), 4.21 (dd, $J = 6.4, 1.7$ Hz, 1H), 3.94 (s, 3H), 2.32 – 2.20 (m, 1H), 2.08 – 2.00 (m, 1H), 1.57 – 1.50 (m, 1H), 1.48 – 1.43 (m, 6H), 1.18 (hept, 1H), 1.07 (d, $J = 6.2$ Hz, 3H), 1.00 – 0.89 (m, 6H), 0.86 (d, $J = 6.9$ Hz, 3H), 0.66 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.99, 171.51, 171.34, 171.15, 169.88, 155.96, 145.75, 128.69, 128.30, 128.06, 127.26, 81.28, 73.47, 67.65, 67.17, 57.46, 54.86, 53.24, 52.92, 51.02, 41.83, 29.49, 26.46, 21.78, 19.19, 18.12, 16.81, 13.93, 11.88.

Notebook Entries

Procedure: PKD2-380

^1H NMR – PKD2-380-2 and ^{13}C NMR -PKD2-380-2-C13

Methyl (*tert*-butoxycarbonyl)-*L*-valyl-*L*-threoninate (**302**)



To a stirring solution of Boc-L-valine (**299**; 500 mg, 2.39 mmol, 2.3 eq.) in CH_2Cl_2 (23 mL, 0.1 M) were added PyBOP (1.3 g, 2.42 mmol, 1.05 eq.) and DIPEA (0.60 mL, 3.45 mmol, 1.5 eq.). To a separate RBF L-threonine methyl ester hydrochloride (**300**; 753 mg, 4.6 mmol, 2.0 eq.), DIPEA (1.81 mL, 10.4 mmol, 4.5 eq.), and CH_2Cl_2 (5.0 mL, 1M) were added. Both reaction mixtures were left to stir for 30 minutes. Both solutions were combined and stirred overnight. The reaction was quenched with saturated NH_4Cl (1:1 vol) and the product was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under

reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate / hexane) to afford **302** (760 mg, 99% yield) as a white solid.

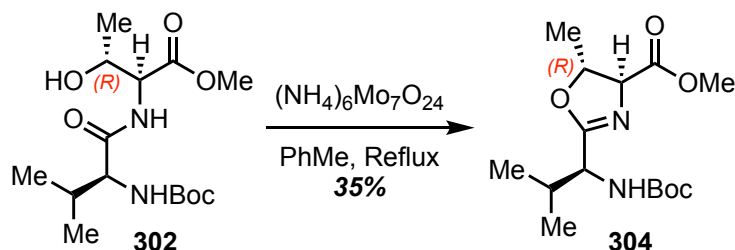
R_f = 0.6 (4:1 ethyl acetate/hexane, PMA). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.08 (d, *J* = 9.2 Hz, 1H), 5.35 (d, *J* = 8.3 Hz, 1H), 4.59 (dd, *J* = 9.0, 2.7 Hz, 1H), 4.33 (qd, *J* = 6.5, 2.8 Hz, 1H), 3.94 (t, *J* = 8.3 Hz, 1H), 3.73 (s, 3H), 2.08 – 1.99 (m, 1H), 1.39 (s, 9H), 1.16 (d, *J* = 6.4 Hz, 3H), 0.96 – 0.91 (m, 6H). **¹³C NMR** (101 MHz, cdcl₃) δ 172.71, 171.39, 156.24, 80.04, 68.09, 60.21, 57.44, 52.55, 30.87, 28.33, 19.81, 19.18, 18.22.

Notebook Entries

Procedure: PDK1-345

¹H NMR – AXT-007-B and ¹³C NMR - AXT-006A Carbon

methyl (4*S*,5*R*)-2-((*S*)-1-((*tert*-butoxycarbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (304**)**



To a flamed dried RBF was added compound **302** (760 mg, 2.29 mmol, 1.0 eq.), anhydrous toluene (45.8 mL, 0.05 M), (NH₄)₆Mo₇O₂₄·4H₂O (284 mg, 0.23 mmol, 0.1 eq.), and Na₂SO₄. The mixture was then heated to 115 °C under argon atmosphere overnight. The reaction was quenched with Na₂SO₄ (1:1 vol equiv.) and the product was extracted with EtOAc (3 x 50 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was

purified via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **304** (252 mg, 35% yield) as a colorless oil.

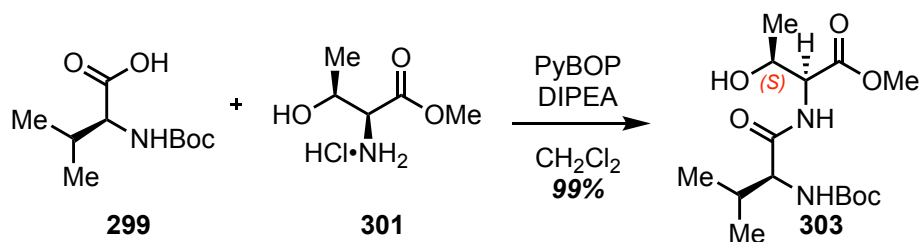
R_f = 0.75 (3:1 ethyl acetate/hexane, PMA). ¹H NMR (400 MHz, Chloroform-*d*) δ 5.19 (d, *J* = 9.3 Hz, 1H), 4.82 (p, *J* = 6.5 Hz, 1H), 4.36 – 4.31 (m, 1H), 4.26 (dd, *J* = 7.0, 1.5 Hz, 1H), 3.76 (s, 3H), 2.10 (td, *J* = 6.9, 4.7 Hz, 1H), 1.44 – 1.41 (m, 12H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H). ¹³C NMR (101 MHz, cdcl₃) δ 171.32, 169.19, 155.47, 79.55, 74.03, 53.70, 52.54, 31.81, 28.26, 20.87, 18.74, 17.13.

Notebook Entries

Procedure: PDK1-349

¹H NMR – AXT-009-A and ¹³C NMR – AXT-015-A-CARBON

Methyl (*tert*-butoxycarbonyl)-*L*-valyl-*L*-allothreoninate (**303**)



To a stirring solution of Boc-L-valine (**299**; 500 mg, 2.39 mmol, 2.3 eq.) in CH₂Cl₂ (23 mL, 0.1 M) were added PyBOP (1.3 g, 2.42 mmol, 1.05 eq.) and DIPEA (0.60 mL, 3.45 mmol, 1.5 eq.). To a separate RBF L-allothreonine methyl ester hydrochloride (**301**; 753 mg, 4.6 mmol, 2.0 eq.), DIPEA (1.81 mL, 10.4 mmol, 4.5 eq.), and CH₂Cl₂ (5.0 mL, 1M) were added. Both reaction mixtures were left to stir for 30 minutes. Both solutions were combined and stirred overnight. The reaction was quenched with saturated NH₄Cl (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under

reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate / hexane) to afford **303** (760 mg, 99% yield) as a white solid.

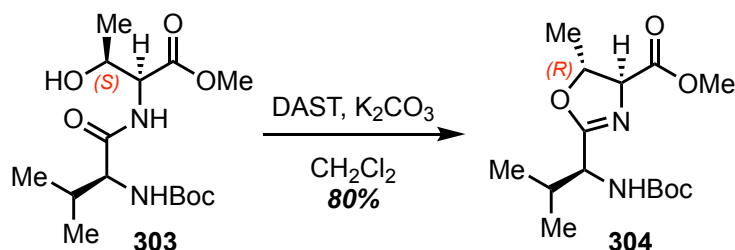
R_f = 0.5 (3:1 ethyl acetate/hexane, PMA). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.35 (d, *J* = 8.0 Hz, 1H), 5.40 (d, *J* = 8.8 Hz, 1H), 4.62 (dd, *J* = 7.9, 3.5 Hz, 1H), 4.09 (qd, *J* = 6.5, 3.6 Hz, 1H), 4.03 – 3.95 (m, 2H), 3.73 (s, 3H), 2.03 (q, *J* = 6.7 Hz, 1H), 1.38 (s, 9H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.94 – 0.88 (m, 6H).

Notebook Entries

Procedure: HH1-29

¹H NMR – HH1-29-400

Methyl (4*S*,5*R*)-2-((*S*)-1-((*tert*-butoxycarbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (304**)**



To a flamed dried RBF was added compound **303** (115 mg, 0.35 mmol, 1.0 eq.) and anhydrous CH₂Cl₂ (2.5 mL, 0.14 M), the mixture was then cooled to -78 °C under argon atmosphere. DAST (0.05 mL, 0.39 mmol, 1.1 eq.) was added dropwise to solution and stirred for 6 h, followed by addition of K₂CO₃. The mixture was slowly warmed to RT and then stirred for 30 minutes. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 5 mL). Organic layer was dried over Na₂SO₄ and concentrated under

reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **304** (90 mg, 80% yield) as a colorless oil.

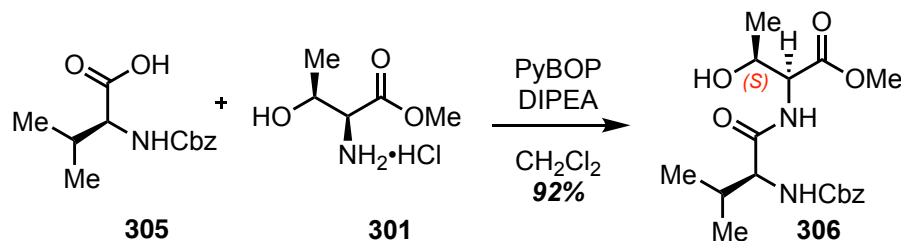
Rf = 0.75 (3:1 ethyl acetate/hexane, PMA). **¹H NMR** (400 MHz, Chloroform-*d*) δ 5.19 (d, *J* = 9.3 Hz, 1H), 4.82 (p, *J* = 6.5 Hz, 1H), 4.36 – 4.31 (m, 1H), 4.26 (dd, *J* = 7.0, 1.5 Hz, 1H), 3.76 (s, 3H), 2.10 (td, *J* = 6.9, 4.7 Hz, 1H), 1.44 – 1.41 (m, 12H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H). **¹³C NMR** (101 MHz, cdcl₃) δ 171.32, 169.19, 155.47, 79.55, 74.03, 53.70, 52.54, 31.81, 28.26, 20.87, 18.74, 17.13.

Notebook Entries

Procedure: PKD2-016

¹H NMR – AXT-009-A and ¹³C NMR – AXT-015-A-CARBON

Methyl ((benzyloxy)carbonyl)-*L*-valyl-*L*-allothreoninate (**306**)



To a stirring solution of Cbz-*L*-valine (**305**; 600 mg, 2.39 mmol, 1.0 eq.) in CH₂Cl₂ (24 mL, 0.1 M) were added PyBOP (1.5 g, 2.87 mmol, 1.2 eq.) and DIPEA (0.50 mL, 2.87 mmol, 1.2 eq.). To a separate RBF *L*-allothreonine methyl ester hydrochloride (**301**; 607 mg, 3.58 mmol, 1.5 eq.), DIPEA (1.53 mL, 8.83 mmol, 3.7 eq.), and CH₂Cl₂ (36 mL, 0.1M) were added. Both reaction mixtures were left to stir for 30 minutes. Both solutions were combined and stirred for 2h. The reaction was quenched with saturated NaHCO₃ (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 80 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 ethyl acetate / hexane) to afford **306** (800 mg, 92% yield) as a white solid.

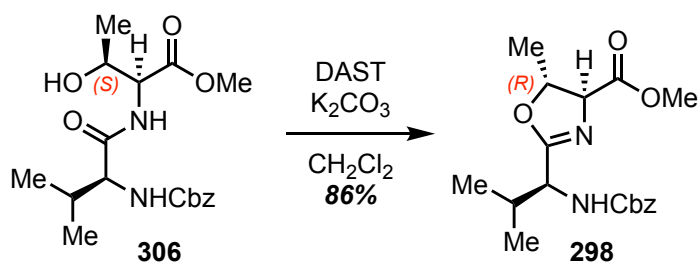
R_f = 0.55 (3:1 ethyl acetate/hexane, PMA). **¹H NMR (600 MHz, DMSO-*d*₆)** δ 8.17 (d, *J* = 7.9 Hz, 1H), 7.38 – 7.33 (m, 5H), 7.29 (d, *J* = 9.1 Hz, 1H), 5.04 – 5.01 (m, 3H), 4.19 (t, *J* = 7.2 Hz, 1H), 3.95 (t, *J* = 8.0 Hz, 1H), 3.87 (q, *J* = 6.3 Hz, 1H), 3.59 (s, 3H), 1.93 (h, *J* = 8.3, 7.5 Hz, 1H), 1.11 (d, *J* = 6.3 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H). **¹³C NMR (151 MHz, DMSO)** δ 171.46, 171.05, 156.09, 137.12, 128.36, 127.79, 127.67, 66.50, 65.36, 59.80, 58.51, 51.54, 30.41, 19.95, 19.06, 18.18.

Notebook Entries

Procedure: AXT1-067

¹H NMR – PKD2-080-400 and ¹³C NMR – PKD2-080-C13-400

Methyl (4*S*,5*R*)-2-((*S*)-1-(((benzyloxy)carbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (298**)**



To a flamed dried RBF was added compound **306** (200 mg, 0.55 mmol, 1.0 eq.) and anhydrous CH₂Cl₂ (4.0 mL, 0.14 M), the mixture was then cooled to -78 °C under argon atmosphere. DAST (0.08 mL, 0.61 mmol, 1.1 eq.) was added dropwise to solution and stirred for 4 h, followed by addition of K₂CO₃. The mixture was slowly warmed to RT and then stirred for 30 minutes. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 10 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 ethyl acetate/hexane) to afford **298** (164 mg, 86% yield) as a colorless oil.

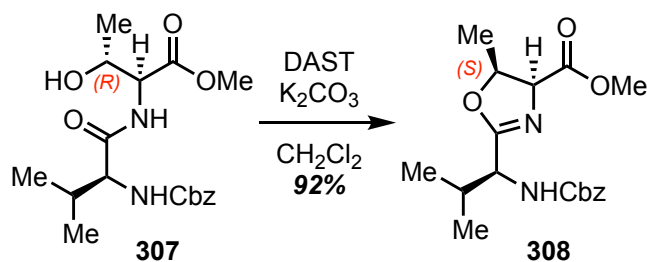
R_f = 0.5 (1:1 ethyl acetate/hexane, PMA). **¹H NMR** (400 MHz, Chloroform-*d*) δ 7.37 – 7.28 (m, 5H), 5.42 (d, *J* = 9.3 Hz, 1H), 5.16 – 5.05 (m, 2H), 4.84 (p, *J* = 6.5 Hz, 1H), 4.42 (ddd, *J* = 9.3, 4.6, 1.4 Hz, 1H), 4.27 (dd, *J* = 7.0, 1.4 Hz, 1H), 3.77 (s, 3H), 2.19 – 2.10 (m, 1H), 1.43 (d, *J* = 6.3 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H). **¹³C NMR** (101 MHz, cdcl₃) δ 171.40, 168.91, 156.24, 136.45, 128.56, 128.17, 128.14, 79.78, 74.20, 67.01, 54.48, 52.66, 31.82, 20.97, 18.86, 17.26.

Notebook Entries

Procedure: AXT1-039

¹H NMR – AXT1-039-A or PKD2-086-400 and ¹³C NMR – PKD2-086-C12-1st-400

Methyl (4*S*,5*S*)-2-((*S*)-1-(((benzyloxy)carbonyl)amino)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (308**)**



To a flamed dried RBF was added compound **307** (207 mg, 0.56 mmol, 1.0 eq.) and anhydrous CH₂Cl₂ (4.0 mL, 0.14 M), the mixture was then cooled to -78 °C under argon atmosphere. DAST (0.08 mL, 0.62 mmol, 1.1 eq.) was added dropwise to solution and stirred for 4 h, followed by addition of K₂CO₃. The mixture was slowly warmed to RT and then stirred for 30 minutes. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 10 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 ethyl acetate/hexane) to afford **308** (195 mg, 92% yield) as a colorless oil.

R_f = 0.5 (1:1 ethyl acetate/hexane, PMA. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.27 (m, 5H), 5.45 (d, *J* = 9.3 Hz, 1H), 5.15 – 5.06 (m, 2H), 4.98 – 4.90 (m, 1H), 4.78 (dd, *J* = 10.2, 1.5 Hz, 1H), 4.40 (dd, *J* = 9.3, 4.7 Hz, 1H), 3.74 (s, 3H), 2.24 – 2.15 (m, 1H), 1.29 (d, *J* = 6.5 Hz, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.9 Hz, 3H).

Notebook Entries

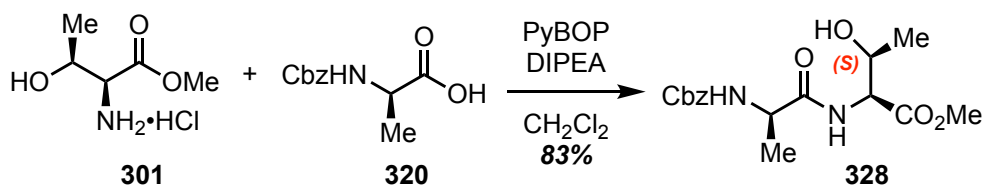
Procedure:

¹H NMR – PKD2-088-400

6.3 Experimental Procedures: Total Synthesis of Balgacyclamide A

6.3.1 Oxazoline Studies

Methyl ((benzyloxy)carbonyl)-*D*-alanyl-*L*-allothreoninate (**328**)



To a RBF containing Cbz-*N*-D-alanine **320** (439 g, 1.97 mmol, 1.0 eq.) in CH₂Cl₂ (20.0 mL, 0.1 M) were added PyBOP (1.20 g, 2.36 mmol, 1.2 eq.) and DIPEA (0.41 mL, 2.36 mmol, 1.2 eq.). To a separate RBF *L*-allothreonine methyl ester hydrochloride **301** (500 mg, 2.95 mmol, 1.5 eq.), DIPEA (1.90 mL, 10.9 mmol, 3.7 eq.), and CH₂Cl₂ (3.0 mL, 1 M) were added. Both reaction mixtures were left to stir separately. After 30 min the two solutions were combined and stirred for 5h. The reaction was quenched with saturated NaHCO₃ (1:1 vol equiv.), and the product was extracted with CH₂Cl₂ (3 x 30 mL) and washed with brine (1:1 vol equiv.). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (100% ethyl acetate) to afford **328** (523 mg, 83% yield) as a white solid.

R_f = 0.42 (5:1 ethyl acetate/hexane, PMA). **¹H NMR** (400 MHz, DMSO-*d*₆) δ 8.12 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.39 – 7.26 (m, 5H), 5.04 (d, *J* = 5.7 Hz, 1H), 5.01 (s, 2H), 4.21 (dd, *J* = 8.5, 6.9 Hz, 1H), 4.13 (p, *J* = 7.2 Hz, 1H), 3.83 (h, *J* = 6.3 Hz, 1H), 3.62 (s, 3H), 1.19 (d, *J* = 7.1 Hz, 3H), 1.06 (d, *J* = 6.3 Hz, 3H). **¹³C NMR** (101 MHz, DMSO) δ 172.60, 171.22, 155.61, 137.04, 128.34, 127.79, 127.71, 66.82, 65.34, 58.26, 51.62, 49.86, 19.98, 18.39. **FTIR (neat):**

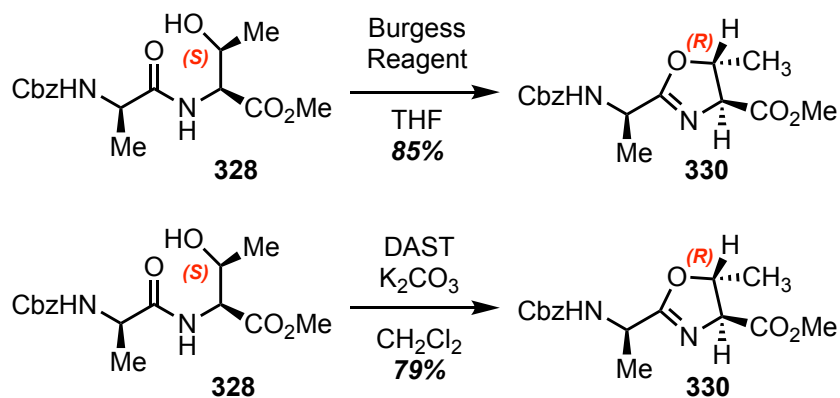
3417, 3352, 3298, 2997, 2876, 1734, 1683, 1539 cm^{-1} . **HRMS(ESI):** m/z calc. for $\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_6$
[$\text{M}+\text{H}$] $^{+}$: 339.1556, found: 339.1556

Notebook Entries

Procedure: AXT2-143

^1H NMR and ^{13}C NMR: AXT2-123-DMSO

Methyl (4*S*,5*R*)-2-((*R*)-1-(((benzyloxy)carbonyl)amino)ethyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (330**)**



Burgess Reagent. To a flamed dried RBF was added compound **328** (150 mg, 0.44 mmol, 1.0 eq.) and anhydrous THF (11.0 mL, 0.04 M), followed by the Burgess reagent (127 mg, 0.53 mmol, 1.2 eq.). The mixture was then heated to 75 °C under argon atmosphere and stirred for 1 h. The reaction was directly concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate/hexane) to afford **330** (121 mg, 85% yield) as a colorless oil.

Rf: 0.56 (4:1 ethyl acetate/hexane, PMA). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.69 (d, $J = 8.2$ Hz, 1H), 7.39 – 7.28 (m, 5H), 5.07 – 4.98 (m, 2H), 4.78 (dq, $J = 6.4, 6.2$ Hz, 1H), 4.32 (q, $J = 7.3$ Hz,

1H), 4.27 (d, J = 6.9 Hz, 1H), 3.63 (s, 3H), 1.28 (dd, J = 6.9, 3.7 Hz, 6H). **¹³C NMR (151 MHz, DMSO-*d*₆)** δ 171.03, 169.02, 155.52, 137.05, 128.36, 127.76, 78.63, 73.68, 65.40, 52.15, 44.62, 20.45, 20.41, 18.20, 18.10. **FTIR (neat):** 3307, 2987, 2943, 1715, 1669, 1510, 1449, 1201 cm⁻¹. **HRMS(ESI):** m/z calc. for C₁₆H₂₁N₂O₅ [M+2H]⁺: 321.1450, found: 321.1451

Notebook Entries

Procedure: AXT2-131

¹H NMR – AXT2-131-A and ¹³C NMR – AXT2-164 (taken from Mo. catalyst reaction)

DAST Reagent. To a flamed dried RBF was added compound **328** (150 mg, 0.44 mmol, 1.0 eq.) and anhydrous CH₂Cl₂ (3.2 mL, 0.14 M), the mixture was then cooled to -78 °C under argon atmosphere. DAST (0.06 mL, 0.48 mmol, 1.1 eq.) was added dropwise to solution and stirred for 6 h, followed by addition of K₂CO₃. The mixture was slowly warmed to RT and then stirred for 1 h. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol equiv.) and the product was extracted with CH₂Cl₂ (3 x 10 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate/hexane) to afford **330** (112 mg, 79% yield) as a colorless oil.

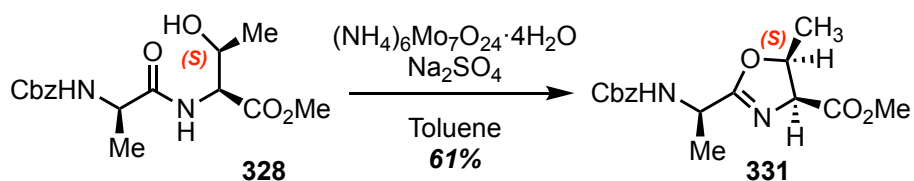
Rf: 0.56 (4:1 ethyl acetate/hexane, PMA). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 7.70 (d, J = 8.2 Hz, 1H), 7.38 – 7.29 (m, 5H), 5.06 – 4.99 (m, 2H), 4.78 (dq, J = 6.4, 6.2 Hz, 1H), 4.31 (q, J = 7.4 Hz, 1H), 4.27 (d, J = 6.9 Hz, 1H), 3.66 (s, 3H), 1.28 (t, J = 6.3 Hz, 6H). **¹³C NMR (151 MHz, DMSO-*d*₆)** δ 171.03, 169.02, 155.52, 137.05, 128.36, 127.76, 78.63, 73.68, 65.40, 52.15, 44.62, 20.45, 20.41, 18.20, 18.10. **FTIR (neat):** 3307, 2987, 2943, 1715, 1669, 1510, 1449, 1201 cm⁻¹. **HRMS(ESI):** m/z calc. for C₁₆H₂₁N₂O₅ [M+2H]⁺: 321.1450, found: 321.1451

Notebook Entries

Procedure: AXT2-129

^1H NMR – AXT2-129 (600) and ^{13}C NMR - AXT2-164 (taken from Mo. catalyst reaction)

Methyl (4*S*,5*S*)-2-((*R*)-1-(((benzyloxy)carbonyl)amino)ethyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (331**)**



To a flamed dried RBF was added compound **328** (150 mg, 0.44 mmol, 1.0 eq.), anhydrous toluene (22.0 mL, 0.02 M), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (55.0 mg, 0.04 mmol, 0.1 eq.), and Na_2SO_4 . The mixture was then heated to 118 °C under argon atmosphere for 7 h. The reaction was quenched with Na_2SO_4 (1:1 vol equiv.) and the product was extracted with CH_2Cl_2 (3 x 25 mL). Organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate/hexane) to afford **331** (87 mg, 61% yield) as a colorless oil.

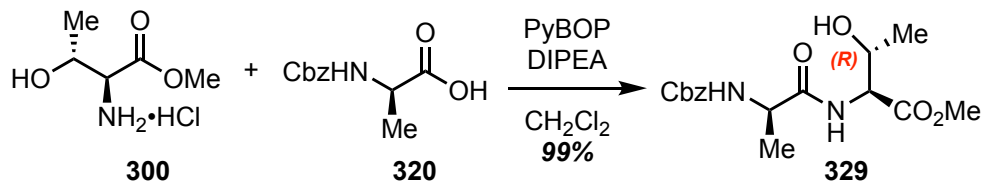
Rf: 0.56 (4:1 ethyl acetate/hexane, PMA). **^1H NMR** (600 MHz, $\text{DMSO}-d_6$) δ 7.75 (d, J = 8.3 Hz, 1H), 7.37 – 7.30 (m, 5H), 5.05 – 4.99 (m, 2H), 4.95 (dq, J = 10.1, 6.3 Hz, 1H), 4.79 (d, J = 10.0 Hz, 1H), 4.28 (d, J = 7.1 Hz, 1H), 3.66 (s, 3H), 1.30 (d, J = 7.2 Hz, 3H), 1.13 (d, J = 6.5 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO) δ 169.90, 169.80, 155.57, 137.03, 128.36, 127.84, 127.78, 77.42, 70.24, 65.40, 51.71, 44.56, 18.03, 15.83. **FTIR (neat)**: 3030, 2997, 2942, 2911, 1716, 1690, 1662, 1543, 1251 cm^{-1} . **HRMS(ESI)**: m/z calc. for $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_5$ $[\text{M}+2\text{H}]^+$: 321.1450, found: 321.1455

Notebook Entries

Procedure: AXT2-155

^1H NMR – AXT2-155 (9-11) and ^{13}C NMR - AXT2-155 (9-11) (600)

Methyl ((benzyloxy)carbonyl)-*D*-alanyl-*L*-threoninate (**329**)



To a RBF containing Cbz-*N*-D-alanine **320** (474 g, 2.12 mmol, 1.0 eq.) in CH₂Cl₂ (21.0 mL, 0.1 M) were added PyBOP (1.30 g, 2.54 mmol, 1.20 eq.) and DIPEA (0.44 mL, 2.54 mmol, 1.2 eq.). To a separate RBF L-threonine methyl ester hydrochloride **300** (540 mg, 3.18 mmol, 1.5 eq.), DIPEA (1.40 mL, 7.84 mmol, 3.7 eq.), and CH₂Cl₂ (1 M) were added. Both reaction mixtures were left to stir separately. After 30 min the two solutions were combined and stirred overnight. The reaction was quenched with saturated NaHCO₃ (1:1 vol equiv.), and the product was extracted with CH₂Cl₂ (3 x 30 mL) and washed with brine (1:1 vol equiv.). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 ethyl acetate/hexane) to afford **329** (712 mg, 99% yield) as a white solid.

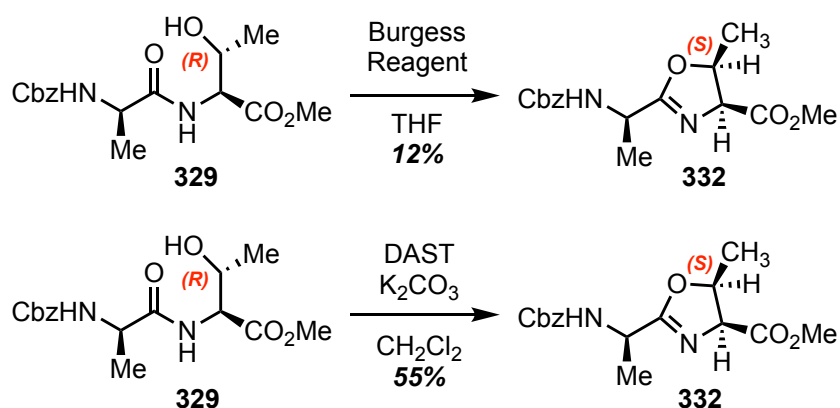
Rf: 0.60 (3:1 ethyl acetate/hexane, PMA). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 7.82 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.38 – 7.29 (m, 5H), 5.06 (d, *J* = 5.5 Hz, 1H), 5.03 (s, 2H), 4.30 (dd, *J* = 8.8, 3.1 Hz, 1H), 4.19 (p, *J* = 7.3 Hz, 1H), 4.15 – 4.09 (m, 1H), 3.63 (s, 3H), 1.24 (d, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 6.4 Hz, 3H). **¹³C NMR** (151 MHz, DMSO) δ 173.15, 171.02, 155.68, 137.04, 128.38, 127.81, 127.72, 66.34, 65.39, 57.45, 51.87, 50.20, 20.04, 18.43. **FTIR (neat)**: 3413, 3298, 3094, 2998, 1716, 1699, 1652, 1536, 1447 cm⁻¹. **HRMS(ESI)**: *m/z* calc. for C₁₆H₂₃N₂O₆ [M+H]⁺: 339.1556, found: 339.1554

Notebook Entries

Procedure: AXT1-206

^1H NMR and ^{13}C NMR - AXT2-163

Methyl (4*S*,5*S*)-2-((*R*)-1-(((benzyloxy)carbonyl)amino)ethyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (332**)**



Burgess Reagent. To a flamed dried RBF was added compound **329** (263 mg, 0.77 mmol, 1.0 eq.) and anhydrous THF (8.0 mL, 0.1 M), followed by the Burgess reagent (222 mg, 0.93 mmol, 1.2 eq.) . The mixture was then heated to 75 °C under argon atmosphere and stirred for 3 h. The reaction was directly concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate/hexane) to afford **332** (29 mg, 12% yield) as a colorless oil.

Rf: 0.56 (4:1 ethyl acetate/hexane, PMA). **^1H NMR** (600 MHz, DMSO-*d*₆) δ 7.75 (d, *J* = 8.2 Hz, 1H), 7.38 – 7.29 (m, 5H), 5.05 – 5.00 (m, 2H), 4.95 (dq, *J* = 9.9, 6.5 Hz, 1H), 4.79 (d, *J* = 10.0 Hz, 1H), 4.27 (d, *J* = 7.2 Hz, 1H), 3.66 (s, 3H), 1.29 (d, *J* = 7.1 Hz, 3H), 1.13 (d, *J* = 6.5 Hz, 3H).

FTIR (neat): 3030, 2997, 2942, 2911, 1716, 1690, 1662, 1543, 1251 cm⁻¹. **HRMS(ESI):** *m/z* calc. for C₁₆H₂₁N₂O₅ [M+2H]⁺: 321.1450, found: 321.1453

Notebook Entries

Procedure: AXT2-130

¹H NMR – AXT2-130 (8-9)

DAST Reagent. To a flamed dried RBF was added compound **329** (150 mg, 0.44 mmol, 1.0 eq.) and anhydrous CH₂Cl₂ (3.2 mL, 0.14 M), the mixture was then cooled to -78 °C under argon atmosphere. DAST (0.06 mL, 0.48 mmol, 1.1 eq.) was added dropwise to solution and stirred for 5.5 h, followed by addition of K₂CO₃. The mixture was slowly warmed to RT and then stirred for 1 h. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol equiv.) and the product was extracted with CH₂Cl₂ (3 x 10 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate/hexane) to afford **332** (79 mg, 55% yield) as a colorless oil.

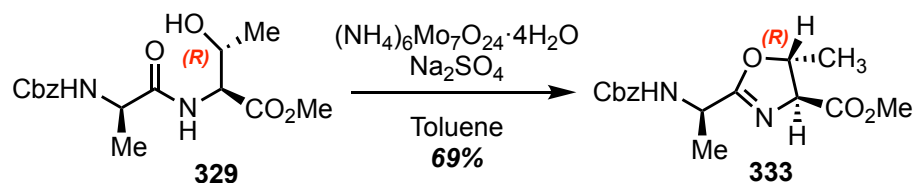
Rf: 0.56 (4:1 ethyl acetate/hexane, PMA). **¹H NMR** (600 MHz, DMSO-*d*₆) δ 7.71 (d, *J* = 8.2 Hz, 1H), 7.37 – 7.30 (m, 6H), 5.03 (s, 2H), 4.94 (dq, *J* = 9.9, 6.5 Hz, 1H), 4.79 (d, *J* = 10.0 Hz, 1H), 4.33 (p, *J* = 7.4 Hz, 1H), 3.65 (s, 3H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.14 (d, *J* = 6.5 Hz, 3H). **FTIR (neat):** 3030, 2997, 2942, 2911, 1716, 1690, 1662, 1543, 1251 cm⁻¹. **HRMS(ESI):** *m/z* calc. for C₁₆H₂₁N₂O₅ [M+2H]⁺: 321.1450, found: 321.1455

Notebook Entries

Procedure: AXT2-128

¹H NMR – AXT2-128 (600)

Methyl (4*S*,5*R*)-2-((*R*)-1-(((benzyloxy)carbonyl)amino)ethyl)-5-methyl-4,5-dihydrooxazole-4-carboxylate (333**)**



To a flamed dried RBF was added compound **329** (150 mg, 0.44 mmol, 1.0 eq.), anhydrous toluene (22.0 mL, 0.02 M), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (55.0 mg, 0.04 mmol, 0.1 eq.), and Na_2SO_4 . The mixture was then heated to 118 °C under argon atmosphere for 7 h. The reaction was quenched with Na_2SO_4 (1:1 vol equiv.) and the product was extracted with CH_2Cl_2 (3 x 25 mL). Organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (4:1 ethyl acetate/hexane) to afford **333** (98 mg, 61% yield) as a colorless oil.

Rf: 0.56 (4:1 ethyl acetate/hexane, PMA). **^1H NMR** (600 MHz, $\text{DMSO}-d_6$) δ 7.70 (d, $J = 8.2$ Hz, 1H), 7.38 – 7.29 (m, 5H), 5.06 – 4.99 (m, 2H), 4.79 (p, $J = 6.4, 6.2$ Hz, 1H), 4.31 (d, $J = 7.5$ Hz, 1H), 4.27 (d, $J = 6.7$ Hz, 1H), 3.66 (s, 3H), 1.32 – 1.26 (m, 6H). **^{13}C NMR** (151 MHz, CDCl_3) δ 171.03, 169.02, 155.52, 137.05, 128.36, 127.76, 78.63, 73.68, 65.40, 52.15, 44.62, 20.45, 20.41, 18.20, 18.10. **FTIR (neat):** 3307, 2987, 2943, 1715, 1669, 1510, 1449, 1201 cm^{-1} . **HRMS(ESI):** m/z calc. for $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_5$ $[\text{M}+2\text{H}]^+$: 321.1450, found: 321.1451

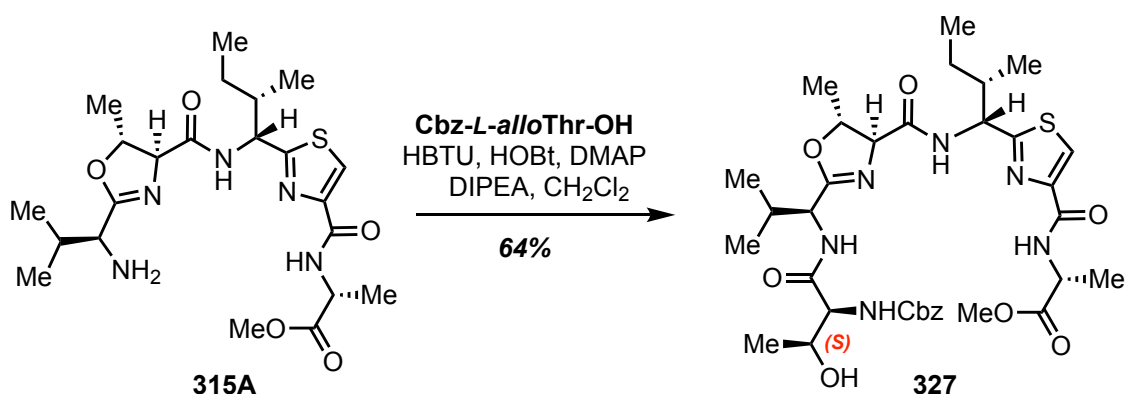
Notebook Entries

Procedure: AXT2-164

^1H NMR – AXT2-164-DMSO and ^{13}C NMR – AXT2-164

6.3.2. Balgacyclamide A Synthesis

Methyl (2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-((2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-3-hydroxybutanamido)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alaninate (327**)**



To a stirring solution of **315A** (180 mg, 0.37 mmol, 1.3 eq.) in CH₂Cl₂ (3.0 mL, 0.1 M) was added Cbz-*L*-threonine (67 mg, 0.29 mmol, 1 eq.) followed by addition of DIPEA (0.11 mL, 0.64 mmol, 2.2 eq.) and stirred for 10 minutes. HBTU (131 mg, 0.34 mmol, 1.2 eq.), HOBT (23 mg, 0.15 mmol, 0.5 eq.) and DMAP (7.1 mg, 0.06 mmol, 0.2 eq.) were added and stirred for 3 h. The mixture was quenched with 1 M HCl (1:1 vol equiv.) and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel gradient chromatography (4:1 ethyl acetate/hexane - 100% ethyl acetate) to afford **327** (131 mg, 64% yield) as a white solid.

R_f = 0.46 (4:1 ethyl acetate/hexane, UV/PMA). [α]_D²⁵ = +38.66 (*c* 0.1, MeOH). **¹H NMR (400 MHz, CDCl₃)** δ 8.10 (d, *J* = 8.9 Hz, 1H), 8.02 (s, 1H), 7.45 – 7.40 (m, 2H), 7.38 – 7.28 (m, 5H), 5.85 (s, 1H), 5.62 (d, *J* = 9.0 Hz, 1H), 5.32 (dd, *J* = 9.0, 4.8 Hz, 1H), 5.10 (d, *J* = 2.1 Hz, 2H), 5.07 – 4.99 (m, 1H), 4.87 (dq, *J* = 6.4, 6.2 Hz, 1H), 4.57 (ddd, *J* = 8.4, 4.3, 1.8 Hz, 1H), 4.23 (d, *J* = 8.0

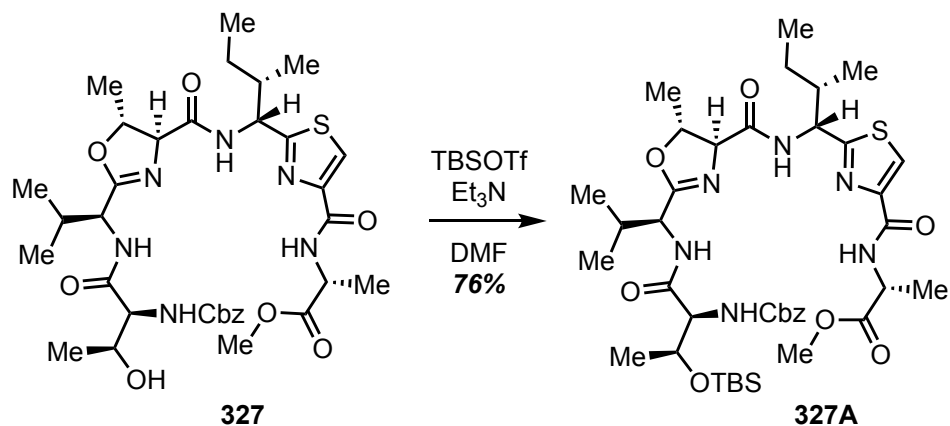
Hz, 1H), 4.17 – 4.10 (m, 1H), 3.86 (s, 3H), 3.79 – 3.70 (m, 1H), 2.25 – 2.18 (m, 1H), 1.99 – 1.91 (m, 1H), 1.61 (d, $J = 7.3$ Hz, 3H), 1.53 (d, $J = 6.2$ Hz, 3H), 1.27 (d, $J = 6.2$ Hz, 3H), 1.17 – 1.06 (m, 2H), 0.98 – 0.92 (m, 9H), 0.73 (d, $J = 7.0$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 174.73, 172.31, 170.68, 170.11, 169.44, 160.38, 156.20, 149.33, 128.67, 128.31, 128.10, 123.53, 81.80, 73.28, 70.06, 67.04, 60.18, 54.44, 54.01, 53.21, 47.59, 41.58, 28.96, 26.08, 21.75, 19.97, 19.16, 19.05, 16.62, 14.43, 11.82. **FTIR (neat):** 3398, 3302, 2968, 2911, 2862, 1715, 1659, 1510, 1451 cm^{-1} **HRMS(ESI):** m/z calc. for $\text{C}_{34}\text{H}_{49}\text{N}_6\text{O}_9\text{S}$ $[\text{M}+\text{H}]^+$: 717.3282, found: 717.3278

Notebook Entries

Procedure: AXT2-356

^1H NMR - AXT2-356(600) and ^{13}C NMR - AXT2-356- CDCl_3

Methyl (2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-((2*S*,3*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*tert*-butyldimethylsilyl)oxy)butanamido)-2-methylpropyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carbonyl)-*D*-alaninate (327A)



To a flamed dried RBF under an Ar atmosphere was added free alcohol **327** (120 mg, 0.17 mmol, 1 eq) in anhydrous DMF (2.0 mL, 0.10 M) and cooled to 0 °C. Once at temperature, Et_3N (0.09 mL, 0.68 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (0.08 mL, 0.33 mmol, 2

eq) was added, mixture was slowly warmed to RT and stirred for 24 h. The reaction was quenched with H₂O (1:1 vol equiv.) and extracted with ethyl acetate (3 x 2 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 ethyl acetate/hexane) to afford **327A** (106 mg, 76% yield) as a white solid.

R_f = 0.40 (1:1 ethyl acetate/hexane, UV). **[α]_D²⁵** = +18.98 (*c* 0.1, CHCl₃). **¹H NMR (600 MHz, Chloroform-*d*)** δ 7.99 (s, 1H), 7.74 (brs, 1H), 7.36 – 7.29 (m, 5H), 7.19 (d, *J* = 9.1 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 5.44 (d, *J* = 8.2 Hz, 1H), 5.31 (dd, *J* = 9.1, 5.3 Hz, 1H), 5.15 – 5.06 (m, 2H), 4.78 (dq, *J* = 7.4, 7.2 Hz, 1H), 4.73 (dq, *J* = 7.8, 6.1 Hz, 1H), 4.60 – 4.56 (m, 1H), 4.22 – 4.19 (m, 1H), 4.18 – 4.14 (m, 1H), 3.78 (s, 3H), 2.26 – 2.19 (m, 1H), 2.13 – 2.07 (m, 1H), 1.62 (s, 3H), 1.52 – 1.43 (m, 7H), 1.29 – 1.24 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H), 0.99 – 0.94 (m, 6H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.84 (s, 9H), 0.05 (s, 6H). **¹³C NMR (151 MHz, CDCl₃)** δ 172.08, 171.31, 169.84, 169.34, 168.76, 160.50, 156.33, 149.86, 136.26, 128.71, 128.41, 128.19, 123.30, 80.84, 74.46, 68.97, 67.28, 54.71, 52.96, 52.79, 48.05, 39.72, 31.41, 26.51, 25.84, 21.91, 18.78, 18.62, 18.07, 15.36, 14.58, 11.69, -4.53. **FTIR (neat)**: 3306, 2974, 2911, 2852, 1713, 1698, 1652, 1545, 1502 cm⁻¹. **HRMS(ESI)**: *m/z* calc. for C₄₀H₆₃N₄O₉SSi [M+H]⁺: 831.4146, found: 831.4144

Notebook Entries

Procedure: AXT2-360

¹H NMR - AXT2-360-B and ¹³C NMR - AXT2-360-A

Rf = 0.61 (100% ethyl acetate, UV) **¹H NMR (600 MHz, Chloroform-*d*)** δ 7.97 (s, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.22 (d, J = 9.0 Hz, 1H), 5.31 (dd, J = 9.0, 5.0 Hz, 1H), 4.79 – 4.74 (m, 2H), 4.55 (dd, J = 8.5, 5.8 Hz, 1H), 4.33 (dq, J = 6.3, 3.8 Hz, 1H), 4.23 (d, J = 7.2 Hz, 1H), 3.78 (s, 3H), 3.47 (d, J = 4.0 Hz, 1H), 2.31 – 2.24 (m, 1H), 2.17 – 2.09 (m, 1H), 1.65 (brs, 4H), 1.53 (d, J = 7.2 Hz, 3H), 1.49 (d, J = 6.2 Hz, 3H), 1.47 – 1.44 (m, 1H), 1.33 – 1.27 (m, 1H), 1.04 (d, J = 6.3 Hz, 3H), 0.98 (t, J = 7.4 Hz, 3H), 0.95 – 0.91 (m, 9H), 0.87 (s, 9H), 0.06 (d, J = 3.5 Hz, 6H). **¹³C NMR (151 MHz, CDCl₃)** δ 173.38, 172.76, 172.68, 171.66, 169.38, 160.54,

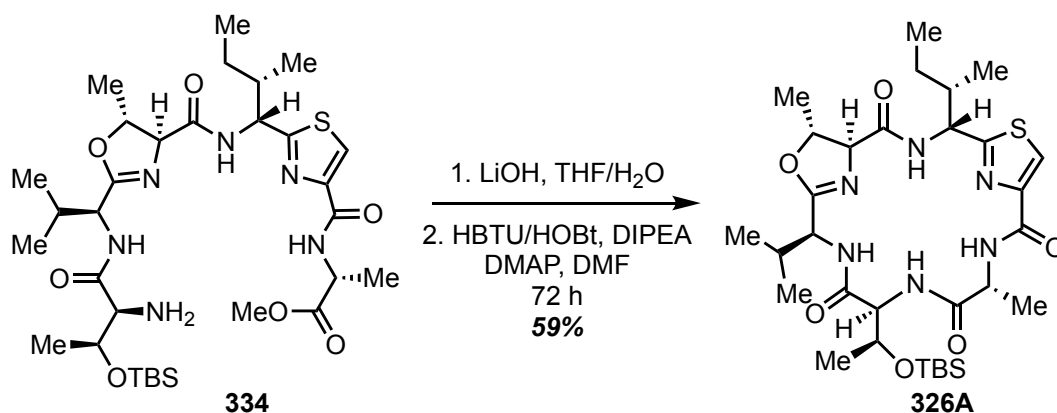
149.87, 123.13, 80.87, 74.65, 68.89, 60.83, 54.81, 52.72, 52.64, 48.08, 39.31, 31.19, 26.63, 25.90, 21.99, 19.12, 18.60, 18.18, 18.14, 17.26, 14.46, 11.71, -4.55, -4.83.

Notebook Entries

Procedure: AXT2-361

¹H NMR and ¹³C NMR - AXT2-361

(1²Z,1⁴S,1⁵R,5²Z,4R,8R,11S,14S)-4-((S)-sec-butyl)-11-((S)-1-((tert-butyl)dimethylsilyl)oxy)ethyl)-14-isopropyl-1⁵,8-dimethyl-1⁴,1⁵-dihydro-3,7,10,13-tetraaza-1(4,2)-oxazola-5(2,4)-thiazolacyclotetradecaphane-2,6,9,12-tetraone (326A)



Step 1: To a stirring solution of free amine **334** (84 mg, 0.12 mmol, 1 eq) in THF/H₂O (8.0 mL, 1:1, 0.015 M) was added LiOH (29.0 mg, 1.21 mmol, 6.67 eq) and stirred for 1 h. The mixture was quenched with saturated NH₄Cl (pH = 5) and extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to access free carboxylic acid. The material was carried on without further purification.

Step 2: The crude material was dissolved in DMF (24 mL, 0.005M), DIPEA was added (0.06 mL, 0.36 mmol, 3 eq.) and solution stirred for 10 minutes. HBTU (50 mg, 0.13 mmol, 1.1 eq.), HOBt

(20.3 mg, 0.13 mmol, 1.1 eq.) and DMAP (7.3 mg, 0.06 mmol, 0.5 eq.) were added and allowed to stir for 72 h. The mixture was quenched with saturated NH_4Cl (1:1 vol equiv.) and extracted with ether (4 x 50 mL), then extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 300 mL), dried over Na_2SO_4 , and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (3:1 ethyl acetate/hexane to afford **326A** (46 mg, 59% yield) as a white solid.

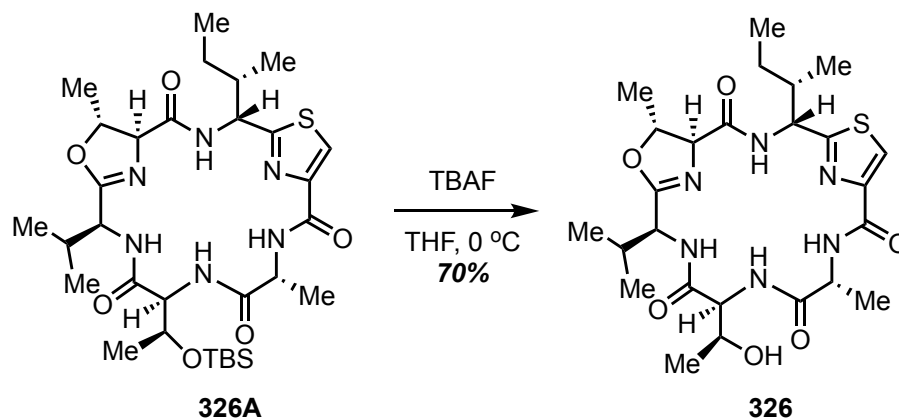
Rf = 0.53 (3:1 ethyl acetate/hexane, UV/PMA) **^1H NMR (600 MHz, Chloroform-*d*)** δ 8.00 (s, 1H), 7.77 (d, J = 9.9 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.25 (d, 1H), 6.59 (d, J = 9.2 Hz, 1H), 5.07 (t, J = 8.7, 7.6 Hz, 1H), 4.93 (dq, J = 10.2, 6.9 Hz, 1H), 4.68 (dd, J = 8.7, 5.7 Hz, 1H), 4.64 – 4.57 (m, 2H), 4.21 (d, J = 7.1 Hz, 1H), 4.18 – 4.13 (m, 1H), 2.10 – 2.02 (m, 2H), 1.50 (d, J = 6.9 Hz, 3H), 1.41 (d, J = 6.2 Hz, 3H), 1.39 – 1.37 (m, 1H), 1.28 (d, J = 6.4 Hz, 3H), 1.09 – 1.06 (m, 1H), 1.04 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H), 0.78 (d, J = 6.8 Hz, 3H), 0.71 (s, 9H), 0.05 (s, 3H), -0.01 (s, 3H). **^{13}C NMR (151 MHz, CDCl_3)** δ 172.23, 170.65, 169.92, 169.12, 166.82, 160.49, 149.12, 123.38, 79.87, 74.52, 68.45, 58.53, 54.95, 53.03, 48.42, 39.88, 31.76, 26.02, 25.29, 21.86, 19.91, 18.58, 18.30, 18.02, 17.77, 15.32, 11.29, -4.40. **FTIR (neat)**: 3302, 2968, 2915, 2849, 1695, 1651, 1526, 1391, 1210 cm^{-1} **HRMS(ESI)**: m/z calc. for $\text{C}_{31}\text{H}_{53}\text{N}_6\text{O}_6\text{SSi}$ $[\text{M}+\text{H}]^+$: 665.3517, found: 665.3513

Notebook Entries

Procedure: Step 1 – AXT2-362, Step 2 – AXT2-363

^1H NMR and ^{13}C NMR – AXT2-363-A

(1²Z,1⁴S,1⁵R,5²Z,4R,8R,11S,14S)-4-((S)-sec-butyl)-11-((S)-1-hydroxyethyl)-14-isopropyl-1⁵,8-dimethyl-1⁴,1⁵-dihydro-3,7,10,13-tetraaza-1(4,2)-oxazola-5(2,4)-thiazolacyclotetradecaphane-2,6,9,12-tetraone (326)



Step 3: To a flamed dried RBF under an Ar atmosphere was added **326A** (38 mg, 0.06 mmol, 1 eq) and anhydrous THF (1.0 mL, 0.1 M) and brought to 0 °C. Once at temperature TBAF (0.02 mL, 0.06 mmol, 1.1 eq.) was added dropwise and stirred for 1h. The reaction was quenched with H₂O (1:1 vol equiv.) and extracted with ethyl acetate (3 x 1 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (100% ethyl acetate to 5% MeOH in EtOAc) to afford **326** (22 mg, 70% yield) as a white solid.

R_f = 0.57 (5 % MeOH in EtOAc, UV) or 0.31 (100% EtOAc, UV). **¹H NMR (600 MHz, Chloroform-*d*)** δ 8.61 (d, *J* = 8.1 Hz, 1H), 8.17 (d, *J* = 8.7 Hz, 1H), 8.05 (s, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 7.5 Hz, 1H), 5.12 (dd, *J* = 8.0, 5.6 Hz, 1H), 4.94 (dq, 6.9, 6.7 Hz, 2H), 4.53 (dd, *J* = 8.7, 4.6 Hz, 1H), 4.47 (dd, *J* = 7.7, 3.4 Hz, 1H), 4.29 (dd, *J* = 6.3, 1.4 Hz, 1H), 4.17 (d, *J* = 7.3 Hz, 1H), 4.08 – 4.01 (m, 1H), 2.01 – 1.92 (m, 2H), 1.61 – 1.54 (m, 4H), 1.41 (dd, *J* = 10.3, 6.4 Hz, 6H), 1.03 (d, *J* = 6.9 Hz, 4H), 0.94 (t, *J* = 7.3 Hz, 3H), 0.49 (d, *J* = 7.0 Hz, 3H), 0.30 (d, *J*

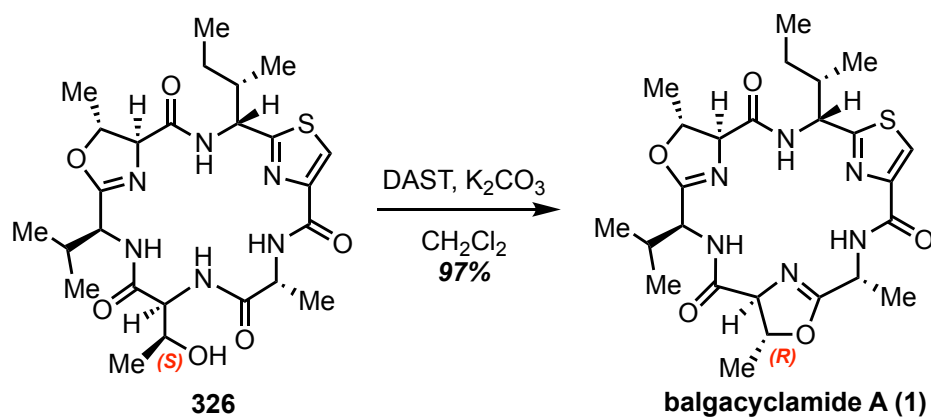
= 6.9 Hz, 3H). **¹³C NMR (151 MHz, CDCl₃)** δ 174.51, 171.45, 170.50, 168.08, 166.44, 160.34, 148.57, 123.19, 79.40, 74.43, 68.49, 55.95, 55.56, 52.81, 49.28, 41.39, 31.25, 25.58, 21.70, 20.50, 19.42, 17.64, 17.05, 15.23, 11.70. **FTIR (neat):** 3309, 2985, 2916, 2876, 1723, 1631, 1536, 1389, 1211 cm⁻¹. **HRMS(ESI):** *m/z* calc. for C₂₅H₃₉N₆O₅S [M+H]⁺: 551.2652, found: 551.2655

Notebook Entries

Procedure: AXT2-364

¹H NMR and ¹³C NMR – AXT2-364(6-9)

(1²Z,1⁴S,1⁵R,5²Z,5⁴S,5⁵R,9²Z,2S,6R,10R)-10-((S)-sec-butyl)-2-isopropyl-1⁵,5⁵,6-trimethyl-1⁴,1⁵,5⁴,5⁵-tetrahydro-3,7,11-triaza-1(2,4),5(4,2)-dioxazola-9(4,2)-thiazolacyclododecaphane-4,8,12-trione (Balgacyclamide A, 1)



To a flamed dried RBF under an Ar atmosphere was added free alcohol **326** (21 mg, 0.04 mmol, 1 eq) and anhydrous CH₂Cl₂ (0.3 mL, 0.14 M). The solution was cooled to -78 °C and then DAST (0.006 mL, 0.042 mmol, 1.1 eq.) was added dropwise. After 1 h K₂CO₃ (8.0 mg, 0.06 mmol, 1.5 eq) was added, the mixture was slowly warmed to RT and stirred for 1 h. The reaction was quenched with slow addition of saturated NaHCO₃ (1:1 vol equiv.) and the product was extracted with CH₂Cl₂ (3 x 1 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (2:1 ethyl acetate/hexane) to afford **1** (20 mg, 97% yield) as a white solid.

R_f = 0.53 (2:1 ethyl acetate/hexane, UV/PMA). ¹H NMR (600 MHz, Chloroform-*d*) δ 8.35 (d, *J* = 7.5 Hz, 1H), 8.06 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 9.4 Hz, 1H), 5.25 (dd, *J* = 8.3, 4.9 Hz, 1H), 4.92 (dq, *J* = 6.4, 6.3 Hz, 1H), 4.89 (dq, *J* = 6.5, 6.4 Hz, 1H), 4.83 (dq, *J* = 7.1, 6.9 Hz, 1H), 4.66 (ddd, *J* = 9.4, 2.5, 2.4 Hz, 1H), 4.24 (m, 2H), 2.10 (pd, *J* = 6.8, 2.9 Hz, 1H), 1.97 – 1.90 (m, 1H), 1.60 – 1.58 (m, 1H), 1.57 (d, *J* = 6.8 Hz, 3H), 1.53 (d, *J* = 6.3 Hz, 3H), 1.50 (d, *J* =

6.3 Hz, 3H), 1.01 (m, 1H), 1.00 (d, $J = 6.8$ Hz, 3H), 0.94 (dd, $J = 7.3, 7.3$ Hz, 3H), 0.72 (d, $J = 6.9$ Hz, 3H), 0.43 (d, $J = 6.9$ Hz, 3H). **^{13}C NMR (151 MHz, CDCl_3)** δ 170.59, 170.51, 170.13, 168.48, 167.87, 159.67, 148.97, 123.36, 81.95, 80.47, 73.88, 73.81, 54.97, 51.52, 43.82, 42.05, 31.50, 25.55, 21.82, 21.68, 19.98, 18.78, 15.81, 14.88, 11.71.

^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.30 (s, 1H), 8.29 (d, $J = 7.6$ Hz, 1H), 8.20 (d, $J = 8.4$ Hz, 1H), 7.34 (d, $J = 9.0$ Hz, 1H), 5.22 (dd, $J = 8.5, 5.7$ Hz, 1H), 4.94 (dq, $J = 6.5, 6.4$ Hz, 1H), 4.78 (dq, $J = 6.5, 6.2$ Hz, 1H), 4.72 (dq, $J = 7.3, 7.0$ Hz, 1H), 4.47 (ddd, $J = 9.2, 2.5, 2.3$ Hz, 1H), 4.40 (dd, $J = 6.8, 2.0$ Hz, 1H), 4.32 (d, $J = 6.8$ Hz, 1H), 2.00 – 1.94 (m, 1H), 1.94 – 1.88 (m, 1H), 1.45 (d, $J = 6.9$ Hz, 3H), 1.44 (m, 1H), 1.42 (d, $J = 6.3$ Hz, 4H), 1.36 (d, $J = 6.3$ Hz, 3H), 1.07 – 0.99 (m, 1H), 0.92 (d, $J = 6.8$ Hz, 3H), 0.88 (dd, $J = 7.4, 7.3$ Hz, 3H), 0.61 (d, $J = 6.8$ Hz, 3H), 0.34 (d, $J = 6.8$ Hz, 3H). **^{13}C NMR (151 MHz, $\text{DMSO}-d_6$)** δ 169.83, 169.70, 169.68, 169.12, 166.09, 159.20, 148.09, 124.77, 81.23, 79.53, 72.94, 72.64, 54.23, 50.76, 42.93, 40.65, 31.03, 25.39, 21.31, 20.71, 19.63, 18.43, 15.34, 14.54, 11.41. **FTIR (neat):** 3395, 2973, 2912, 2872, 1697, 1651, 1546, 1500, 1439 cm^{-1} . **HRMS(ESI):** m/z calc. for $\text{C}_{25}\text{H}_{37}\text{N}_6\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 533.2546, found: 533.2556

Notebook Entries

Procedure: AXT2-365

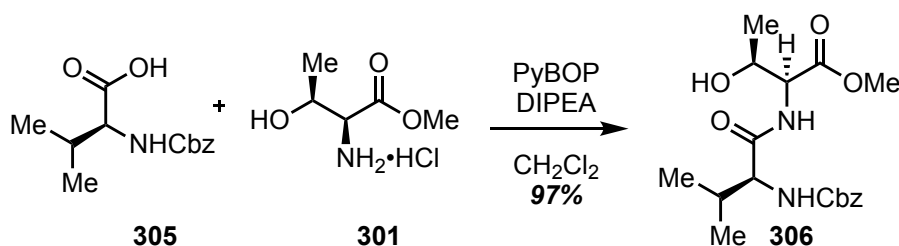
^1H NMR and ^{13}C NMR in CD_3Cl – AXT2-365(6-8)

^1H NMR in $\text{DMSO}-d_6$ - AXT2-365(600)-DMSO-RES

^{13}C NMR in $\text{DMSO}-d_6$ – AXT2-365-DMSO

6.3.3 Balgacyclamide A Tetrapeptide Synthesis

Methyl ((benzyloxy)carbonyl)-*L*-valyl-*L*-allothreoninate (**306**)



To a stirring solution of Cbz-*L*-valine **305** (570 mg, 2.27 mmol, 1.0 eq.) in CH₂Cl₂ (24.0 mL, 0.1 M) were added PyBOP (1.3 g, 2.38 mmol, 1.05 eq.) and DIPEA (0.60 mL, 3.4 mmol, 1.5 eq.). To a separate RBF *L*-allothreonine methyl ester hydrochloride **301** (500 mg, 2.95 mmol, 1.3 eq.), DIPEA (3.0 mL, 0.79 mmol, 2.0 eq.), and CH₂Cl₂ (3.0 mL, 1 M) were added. Both reaction mixtures were left to stir for 30 minutes. Both solutions were combined and stirred for 5 h. The reaction was quenched with saturated NaHCO₃ (1:1 vol) and the product was extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 ethyl acetate / hexane) to afford **306** (802 mg, 97% yield) as a white solid.

Rf: 0.55 (3:1 ethyl acetate/hexane, PMA). **¹H NMR (600 MHz, DMSO-*d*₆)** δ 8.17 (d, *J* = 7.9 Hz, 1H), 7.38 – 7.33 (m, 5H), 7.29 (d, *J* = 9.1 Hz, 1H), 5.04 – 5.01 (m, 3H), 4.19 (t, *J* = 7.2 Hz, 1H), 3.95 (t, *J* = 8.0 Hz, 1H), 3.87 (q, *J* = 6.3 Hz, 1H), 3.59 (s, 3H), 1.93 (h, *J* = 8.3, 7.5 Hz, 1H), 1.11 (d, *J* = 6.3 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H). **¹³C NMR (151 MHz, DMSO)** δ 171.46, 171.05, 156.09, 137.12, 128.36, 127.79, 127.67, 66.50, 65.36, 59.80, 58.51, 51.54, 30.41, 19.95, 19.06, 18.18. **FTIR (neat)**: 3500, 3305, 2998, 2902, 1748, 1702, 1543, 1397, 1211 cm⁻¹. **HRMS(ESI)**: *m/z* calc. for C₁₈H₂₇N₂O₆ [M+H]⁺: 367.1869, found: 367.1862

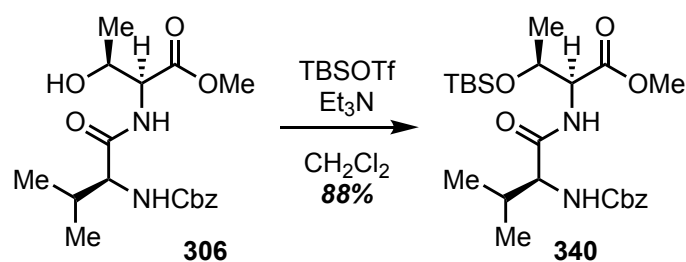
Notebook Entries

Procedure: AXT2-142

^1H NMR and ^{13}C NMR – AXT2-118-DMSO

Methyl *N*-(((benzyloxy)carbonyl)-*L*-valyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate

(340)



To a flamed dried RBF under an Ar atmosphere was added free alcohol **306** (800 mg, 2.18 mmol, 1 eq) in anhydrous CH_2Cl_2 (22.0 mL, 0.1 M) and cooled to 0 °C. Once at temperature Et_3N (1.22 mL, 8.73 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (1.00 mL, 4.37 mmol, 2 eq.) was added, mixture was slowly warmed to RT and stirred overnight. The reaction was quenched with saturated NH_4Cl (1:1 vol) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 hexane / ethyl acetate) to afford **340** (924 mg, 88% yield) as a colorless oil.

R_f = 0.42 (3:1 hexane / ethyl acetate, PMA). ^1H NMR (400 MHz, CDCl_3) δ 7.36 – 7.26 (m, 5H), 6.57 (d, J = 8.1 Hz, 1H), 5.45 (d, J = 8.9 Hz, 1H), 5.13 – 5.06 (m, 2H), 4.51 (dd, J = 8.1, 3.9 Hz, 1H), 4.10 – 4.01 (m, 2H), 3.72 (s, 3H), 2.11 (h, J = 13.4, 6.8 Hz, 1H), 1.23 (d, J = 6.4 Hz, 3H), 0.94 (dd, J = 10.2, 6.8 Hz, 6H), 0.84 (s, 9H), 0.08 – 0.00 (m, 6H). ^{13}C NMR (151 MHz, CDCl_3)

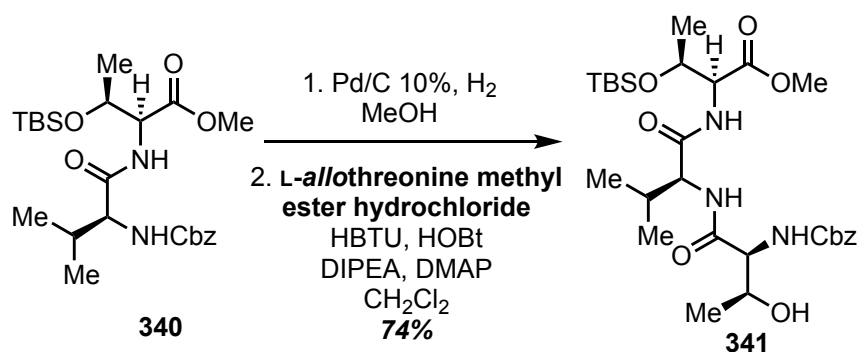
δ 170.81, 170.18, 156.33, 136.41, 128.68, 128.32, 128.22, 69.85, 67.17, 60.49, 58.46, 52.31, 31.46, 25.73, 20.72, 19.16, 18.00, 17.92, -4.32. **FTIR (neat):** 3298, 3243, 3085, 2962, 2921, 2862, 1764, 1702, 1647 cm^{-1} **HRMS(ESI):** m/z calc. for $\text{C}_{24}\text{H}_{41}\text{N}_2\text{O}_6\text{Si}$ $[\text{M}+\text{H}]^+$: 481.2734, found: 481.2740

Notebook Entries

Procedure: AXT2-147

^1H NMR and ^{13}C NMR – AXT2-126

methyl *N*-((benzyloxy)carbonyl)-*L*-allothreonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate (341**)**



To a flamed dried RBF containing a solution of **340** (398 mg, 0.83 mmol, 1.0 eq.) in anhydrous MeOH (42.0 mL, 0.02 M) was added Pd/C 10% (120 mg, 3 eq.) and stirred for 1 h under H_2 (g). The reaction mixture was concentrated under reduced pressure and filtrated through celite to obtain crude free amine of #. To a stirring solution of crude material (287 mg, 0.83 mmol, 1.1 eq.) in CH_2Cl_2 (8.0 mL, 0.1 M), Cbz-*L*-allothreonine # (191 mg, 0.75 mmol, 1.0 eq.) and DIPEA (0.30 mL, 1.65 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (341 mg, 0.83 mmol, 1.2 eq.), HOBT (59.0 mg, 0.38, 0.5 eq.) and DMAP (18.0 mg, 0.15 mmol, 0.2 eq.). The mixture was stirring for 5 h, quenched with saturated NH_4Cl (1:1 vol), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated

under reduced pressure. Purification was performed via a flash silica gel chromatography (1:1 ethyl acetate / hexane) to afford **341** (324 mg, 74% yield) as a white solid.

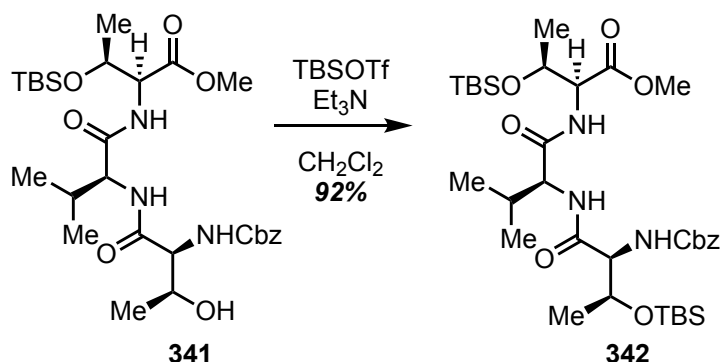
¹H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.32 (m, 5H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.45 (d, *J* = 8.7 Hz, 1H), 5.67 (d, *J* = 8.5 Hz, 1H), 5.12 (d, *J* = 4.0 Hz, 2H), 4.55 (dd, *J* = 8.3, 3.8 Hz, 1H), 4.31 (dd, *J* = 8.7, 5.1 Hz, 1H), 4.08 – 4.01 (m, 3H), 3.92 – 3.83 (m, 1H), 3.74 (s, 3H), 2.31 – 2.20 (m, 1H), 1.31 (d, *J* = 6.3 Hz, 3H), 1.26 – 1.20 (m, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.86 (s, 9H), 0.04 (d, *J* = 1.7 Hz, 6H). **¹³C NMR** (101 MHz, CDCl₃) δ 171.61, 170.86, 170.55, 156.51, 136.22, 128.71, 128.41, 128.25, 70.22, 69.53, 67.39, 59.40, 59.08, 58.58, 52.43, 30.39, 25.76, 20.77, 20.53, 19.37, 18.03, 17.60, -4.35, -4.86.

Notebook Entries

Procedure: AXT2-159

¹H NMR and ¹³C NMR – AXT2-151 (CARBON)

methyl *N*-*N*-((benzyloxy)carbonyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate (342**)**



To a flamed dried RBF under an Ar atmosphere was added free alcohol **341** (830mg, 1.43 mmol, 1 eq.) in anhydrous CH₂Cl₂ (14.0 mL, 0.1 M) and cooled to 0 °C. Once at temperature, Et₃N (0.80

mL, 5.71 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (0.65 mL, 2.86 mmol, 2 eq.) was added, mixture was slowly warmed to RT and stirred for 7 h. The reaction was quenched with saturated NH₄Cl (1:1 vol) and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via a gradient flash silica gel chromatography (5:1 hexane / ethyl acetate to 3:1 hexane / ethyl acetate) to afford **342** (917 mg, 92% yield) as a white solid.

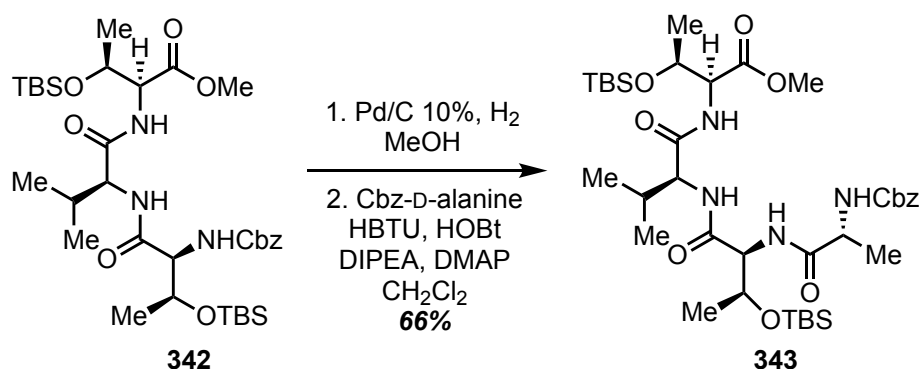
Rf: 0.51 (3:1 ethyl acetate/hexane, PMA). ¹H NMR (600 MHz, Chloroform-*d*) δ 7.37 – 7.30 (m, 5H), 6.81 (d, *J* = 8.3 Hz, 1H), 6.41 (brs, 1H), 5.33 (brs, 1H), 5.15 – 5.08 (m, 2H), 4.49 (dd, *J* = 8.1, 4.1 Hz, 1H), 4.27 (dd, *J* = 8.3, 5.8 Hz, 1H), 4.21 (q, *J* = 5.9 Hz, 1H), 4.19 – 4.15 (m, 1H), 4.10 – 4.05 (m, 1H), 3.73 (s, 3H), 2.15 (dq, *J* = 13.4, 6.7, 6.1 Hz, 1H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.18 (d, *J* = 6.3 Hz, 3H), 0.95 – 0.92 (m, 6H), 0.87 – 0.84 (m, 18H), 0.07 – 0.03 (m, 12H). ¹³C NMR (151 MHz, CDCl₃) δ 170.30, 170.16, 169.37, 156.56, 136.26, 128.70, 128.36, 128.22, 69.71, 68.56, 67.34, 61.36, 58.48, 52.27, 31.43, 25.88, 25.86, 25.75, 20.73, 19.67, 19.02, 18.30, 18.04, -4.31, -4.53.

Notebook Entries

Procedure: AXT2-196

¹H NMR – AXT2-164-A and ¹³C NMR – AXT2-165

methyl *N*-*N*-(((benzyloxy)carbonyl)-*D*-alanyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate (**343**)



To a flamed dried RBF containing a solution of **342** (280 mg, 0.40 mmol, 1.0 eq.) in anhydrous MeOH (20.0 mL, 0.02 M) was added Pd/C 10% (84.0 mg, 3 eq.) and stirred for 1 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and filtrated through celite to obtain crude free amine of **342**. To a stirring solution of crude material (226 mg, 0.40 mmol, 1.1 eq.) in CH₂Cl₂ (4.0 mL, 0.1 M), Cbz-D-alanine (82.0 mg, 0.37 mmol, 1.0 eq.) and DIPEA (0.14 mL, 0.81 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (139 mg, 0.37 mmol, 1.2 eq.), HOBT (57.0 mg, 0.37, 1.2 eq.) and DMAP (9.0 mg, 0.07 mmol, 0.2 eq.). The mixture was stirring for 5 h, quenched with saturated NH₄Cl (1:1 vol), and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via a flash silica gel chromatography (3:2 hexane / ethyl acetate) to afford **343** (186 mg, 66% yield) as a white solid.

¹H NMR (600 MHz, Chloroform-*d*) δ 7.38 – 7.30 (m, 5H), 6.92 (brs, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.67 (brs, 1H), 5.48 (brs, 1H), 5.17 – 5.04 (m, 2H), 4.47 (dd, *J* = 8.1, 4.7 Hz, 1H), 4.38 – 4.31 (m, 3H), 4.20 – 4.14 (m, 1H), 4.07 (t, *J* = 5.7 Hz, 1H), 3.71 (s, 3H), 2.16 – 2.09 (m, 1H), 1.39 (d,

$J = 7.0$ Hz, 3H), 1.23 (d, $J = 6.3$ Hz, 3H), 1.20 (d, $J = 6.3$ Hz, 3H), 0.91 – 0.88 (m, 6H), 0.86 (d, $J = 5.7$ Hz, 18H), 0.07 (s, 12H).

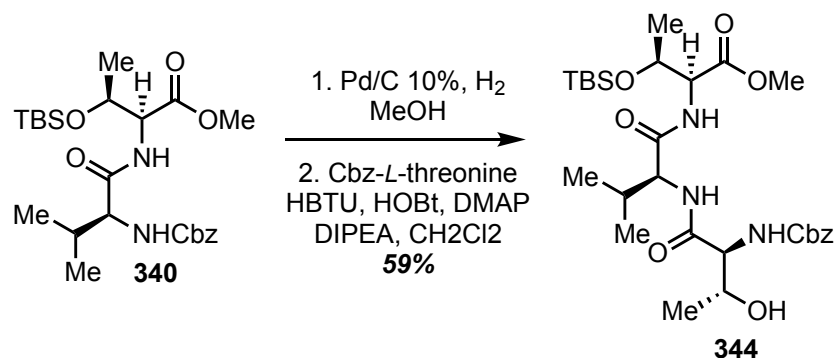
Notebook Entries

Procedure: AXT2-167

^1H NMR – AXT2-167-A and ^{13}C NMR – AXT2-202 (Recrystallization try 1)

6.4 Experimental Procedures: Total Synthesis of Balgacyclamide A Epimer

Methyl *N*-((benzyloxy)carbonyl)-*L*-threonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate (**344**)

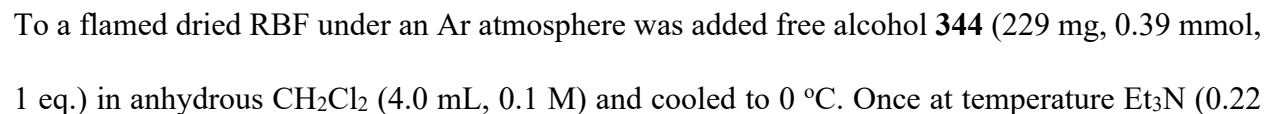


To a flamed dried RBF containing a solution of **340** (356 mg, 0.74 mmol, 1.0 eq.) in anhydrous MeOH (37.0 mL, 0.02 M) was added Pd/C 10% (107 mg, 3 eq.) and stirred for 1 h under H_2 (g). The reaction mixture was concentrated under reduced pressure and filtrated through celite to obtain crude free amine. To a stirring solution of crude material (257 mg, 0.74 mmol, 1.1 eq.) in CH_2Cl_2 (7.0 mL, 0.1 M), Cbz-*L*-threonine (171 mg, 0.67 mmol, 1.0 eq.) and DIPEA (0.26 mL, 1.47 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (307 mg, 0.81 mmol, 1.2 eq.), HOBT (127 mg, 0.81, 1.2 eq.) and DMAP (7.0 mg, 0.13 mmol, 0.2 eq.). The mixture was stirring for 4.5 h, quenched with saturated NH_4Cl (1:1 vol), and extracted with CH_2Cl_2 (3 x

¹H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.27 (m, 5H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 5.97 (d, *J* = 7.8 Hz, 1H), 5.10 (s, 2H), 4.52 (dd, *J* = 8.5, 4.7 Hz, 1H), 4.37 – 4.22 (m, 3H), 4.04 (p, *J* = 6.0 Hz, 1H), 3.69 (s, 3H), 2.19 – 2.08 (m, 1H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.14 (d, *J* = 6.3 Hz, 3H), 0.89 (t, *J* = 6.6 Hz, 6H), 0.84 (s, 9H), 0.03 (s, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 171.03, 170.94, 156.77, 136.27, 128.51, 128.14, 128.01, 69.62, 67.43, 67.13, 58.90, 58.81, 58.53, 52.14, 30.90, 25.62, 20.58, 19.05, 18.29, 18.18, 17.86, -4.44, -5.09.

Procedure: AXT2-041

Methyl *N*-*N*-((benzyloxy)carbonyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-threonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate (345)



mL, 1.58 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (0.18 mL, 0.79 mmol, 2 eq.) was added, mixture was slowly warmed to RT and stirred for 7 h. The reaction was quenched with saturated NH_4Cl (1:1 vol) and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (5:1 hexane / ethyl acetate) to afford **345** (247 mg, 90% yield) as a colorless oil.

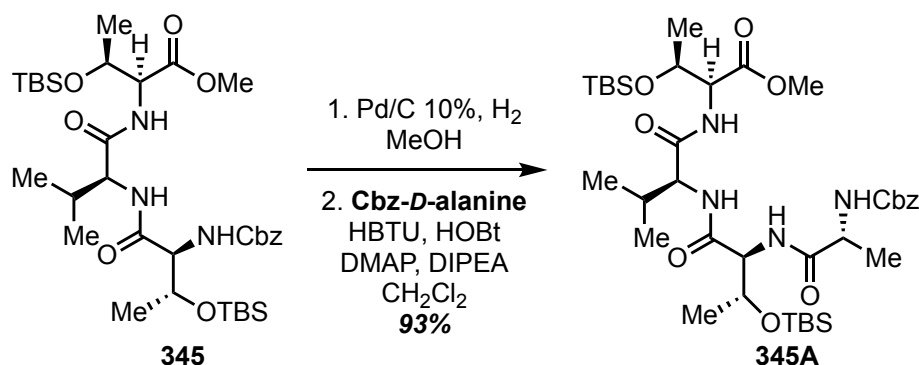
^1H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.30 (m, 6H), 6.37 (d, J = 8.2 Hz, 1H), 5.82 (d, J = 6.0 Hz, 1H), 5.18 – 5.08 (m, 2H), 4.52 (dd, J = 8.2, 4.2 Hz, 1H), 4.41 – 4.34 (m, 1H), 4.25 – 4.16 (m, 2H), 4.09 – 4.02 (m, 1H), 3.73 (s, 3H), 2.15 – 2.04 (m, 1H), 1.23 (d, J = 6.4 Hz, 3H), 1.10 (d, J = 6.4 Hz, 3H), 0.96 – 0.92 (m, 15H), 0.86 (s, 9H), 0.15 (d, J = 8.5 Hz, 6H), 0.04 (d, J = 2.3 Hz, 6H). **^{13}C NMR (101 MHz, CDCl_3)** δ 170.47, 170.14, 169.52, 156.42, 136.31, 128.71, 128.37, 128.24, 69.90, 68.35, 67.18, 59.40, 59.11, 58.43, 52.24, 31.14, 25.94, 25.76, 20.70, 19.18, 18.48, 18.10, 18.02, -4.34, -4.57, -4.88.

Notebook Entries

Procedure: AXT2-051

^1H NMR and ^{13}C NMR - AXT2-051

Methyl *N*-*N*-(((benzyloxy)carbonyl)-*D*-alanyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-threonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreoninate (345A**)**



To a flamed dried RBF containing a solution of **345** (247 mg, 0.36 mmol, 1.0 eq.) in anhydrous MeOH (18.0 mL, 0.02 M) was added Pd/C 10% (74.0 mg, 3 eq.) and stirred for 2 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and filtrated through celite to obtain crude free amine. To a stirring solution of crude material (200 mg, 0.35 mmol, 1.1 eq.) in CH₂Cl₂ (3.2 mL, 0.1 M), Cbz-*D*-alanine (72.0 mg, 0.32 mmol, 1.0 eq.) and DIPEA (0.12 mL, 0.70 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (147 mg, 0.39 mmol, 1.2 eq.), HOBT (61.0 mg, 0.39, 1.2 eq.) and DMAP (8.0 mg, 0.06 mmol, 0.2 eq.). The mixture was stirring for 5.5 h, quenched with saturated NH₄Cl (1:1 vol), and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via a flash silica gel chromatography (3:2 hexane / ethyl acetate) to afford **345A** (230 mg, 93% yield) as a white solid.

¹H NMR (400 MHz, Chloroform-*d*) δ 7.47 (d, *J* = 7.9 Hz, 1H), 7.36 – 7.27 (m, 5H), 7.20 (brs, 1H), 6.90 (brs, 1H), 5.56 (brs, 1H), 5.09 (q, *J* = 12.2 Hz, 2H), 4.58 (dd, *J* = 8.6, 5.2 Hz, 1H), 4.47 – 4.27 (m, 4H), 4.03 (p, *J* = 6.0 Hz, 1H), 3.71 (s, 3H), 2.13 – 1.99 (m, 1H), 1.39 (d, *J* = 7.0 Hz, 3H), 1.19 (d, *J* = 6.7 Hz, 3H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 10.4 Hz, 15H), 0.84 (s, 9H),

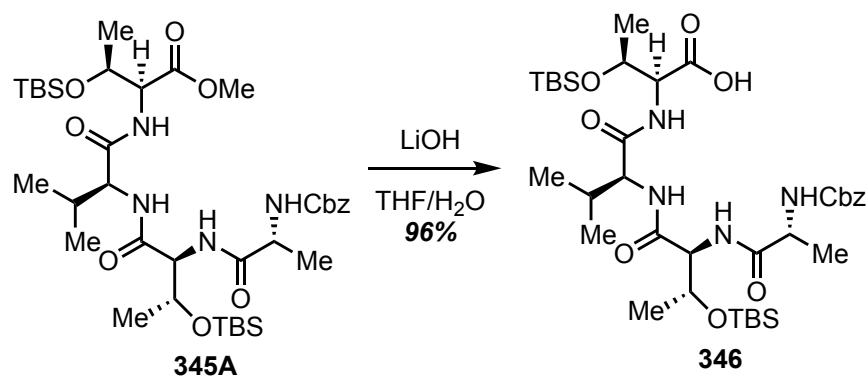
0.15 (d, $J = 11.2$ Hz, 6H), 0.03 (s, 6H). **^{13}C NMR (101 MHz, CDCl_3)** δ 172.65, 171.18, 170.33, 169.33, 155.99, 136.40, 128.62, 128.24, 128.13, 69.85, 67.62, 67.07, 58.76, 58.33, 58.10, 52.28, 50.67, 31.40, 29.81, 25.90, 25.73, 20.57, 19.31, 19.14, 18.51, 18.32, 18.06, 17.99, -4.35, -4.63, -4.92, -5.21.

Notebook Entries

Procedure: AXT2-057

^1H NMR and ^{13}C NMR - AXT2-057

***N*-*N*-(((benzyloxy)carbonyl)-*D*-alanyl)-*O*-(*tert*-butyldimethylsilyl)-*L*-threonyl-*L*-valyl-*O*-(*tert*-butyldimethylsilyl)-*L*-allothreonine (**346**)**



To a solution of **345A** (42 mg, 0.05 mmol, 1 eq) in THF/H₂O (3.70 mL, 1:1, 0.015 M), LiOH (8.0 mg, 0.17 mmol, 6.67 eq) was added and stirred for 21 h. The mixture was quenched with saturated NH₄Cl (1:1 vol) and extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to access free carboxylic acid **346**. Material was pushed to next reaction without further purification.

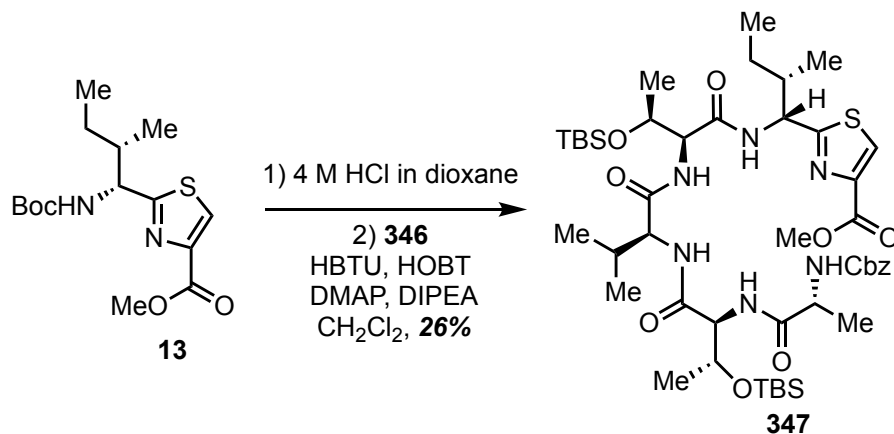
¹H NMR (400 MHz, Chloroform-*d*) δ 7.69 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 7.3 Hz, 1H), 7.38 – 7.30 (m, 5H), 5.82 (d, *J* = 7.2 Hz, 1H), 5.15 – 5.03 (m, 2H), 4.68 – 4.58 (m, 2H), 4.49 (brs, 1H), 4.42 – 4.34 (m, 1H), 4.31 (brs, *J* = 7.6 Hz, 1H), 4.18 – 4.09 (m, 1H), 2.10 – 1.99 (m, 1H), 1.38 (d, *J* = 7.0 Hz, 3H), 1.28 (brs, 1H), 1.22 (d, *J* = 6.3 Hz, 4H), 1.09 (d, *J* = 6.3 Hz, 3H), 0.97 (s, 9H), 0.95 – 0.94 (m, 1H), 0.87 (s, 9H), 0.21 – 0.15 (m, 6H), 0.08 – 0.02 (m, 6H).

Notebook Entries

Procedure: AXT2-063

¹H NMR - AXT2-063

Methyl 2-((5*R*,8*S*,11*S*,14*S*,17*R*,18*S*)-8-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-14-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-11-isopropyl-5,18-dimethyl-3,6,9,12,15-pentaoxo-1-phenyl-2-oxa-4,7,10,13,16-pentaazaicosan-17-yl)thiazole-4-carboxylate (347**)**



Thiazole **13** (120 mg, 0.34 mmol, 1.00 eq.) was dissolved in 4 M HCl in dioxane (3.0 mL, 0.12 M) and stirred for 1 h. Solution was concentrated under reduced pressure and crude material carried further without purification. Free carboxylic acid **346** (173 mg, 0.23 mmol, 1 eq.) and crude free amine (88.0 mg, 0.25 mmol, 1.1 eq) were dissolved in CH₂Cl₂ (2.3 mL, 0.1 M) and DIPEA was added (0.10 mL, 0.51 mmol, 2.2 eq.) and stirred for 10 minutes. HBTU (104 mg, 0.28 mmol, 1.2 eq.), HOBt (45.0 mg, 0.28, 1.2 eq.) and DMAP (6.0 mg, 0.05 mmol, 0.2 eq.) were added to solution and stirred for 5 h. Reaction mixture was quenched with saturated NH₄Cl (1:1 vol) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via a flash silica gel chromatography (1:1 ethyl acetate / hexane) to afford **347** (57 mg, 26% yield) as a white solid.

Rf: 0.32 (3:1 ethyl acetate/hexane, UV/PMA). **¹H NMR (400 MHz, Chloroform-*d*)** δ 8.03 (s, 1H), 7.38 – 7.27 (m, 6H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.00 (d, *J* = 6.7 Hz, 1H), 6.54 (d, *J* = 7.3 Hz, 1H), 5.40 – 5.31 (m, 2H), 5.13 – 5.00 (m, 2H), 4.55 (t, *J* = 6.3 Hz, 1H), 4.48 – 4.39 (m, 2H), 4.28

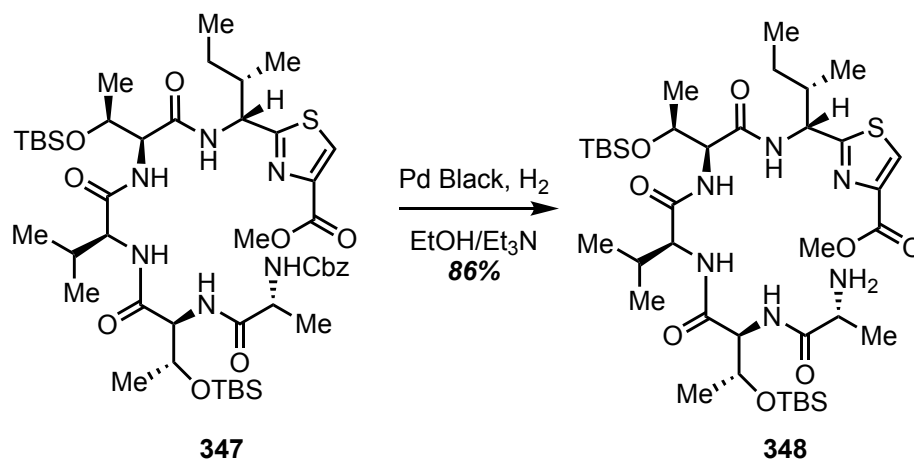
(d, $J = 6.6$ Hz, 1H), 4.17 (q, $J = 6.7$ Hz, 1H), 4.11 (t, $J = 5.7$ Hz, 1H), 3.91 (s, 3H), 2.38 – 2.29 (m, 1H), 2.22 (dt, $J = 13.5, 7.8$ Hz, 1H), 1.44 (d, $J = 7.1$ Hz, 4H), 1.28 – 1.21 (m, 1H), 1.17 (d, $J = 6.4$ Hz, 3H), 1.12 (d, $J = 6.4$ Hz, 3H), 0.99 (d, $J = 6.8$ Hz, 6H), 0.96 – 0.92 (m, 6H), 0.90 (s, 9H), 0.84 (s, 9H), 0.10 – 0.03 (m, 12H). **^{13}C NMR (101 MHz, CDCl_3)** δ 174.08, 172.90, 171.23, 170.96, 169.87, 162.08, 156.34, 147.05, 135.93, 128.75, 128.54, 128.28, 127.04, 67.92, 67.49, 67.37, 60.95, 59.47, 55.68, 52.49, 51.33, 39.32, 29.96, 26.54, 25.91, 20.34, 19.48, 19.06, 18.44, 18.13, 18.05, 17.88, 14.42, 11.73, -4.57, -4.61, -4.69, -4.96. **FTIR (neat):** 3290, 2950, 2932, 2850, 1626, 1510, 1398, 1255, 1201 cm^{-1} . **HRMS(ESI):** m/z calc. for $\text{C}_{46}\text{H}_{79}\text{N}_6\text{O}_{10}\text{SSi}_2$ $[\text{M}+\text{H}]^+$: 963.5117, found: 963.5117

Notebook Entries

Procedure: AXT2-069

^1H NMR and ^{13}C NMR – AXT2-053

Methyl 2-((5*R*,6*S*,9*S*,12*S*,15*R*,16*S*)-6-((*R*)-2-aminopropanamido)-12-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-9-isopropyl-2,2,3,3,5,16-hexamethyl-7,10,13-trioxo-4-oxa-8,11,14-triaza-3-silaoctadecan-15-yl)thiazole-4-carboxylate (348**)**



To a flamed dried RBF containing a solution of **347** (53 mg, 0.06 mmol, 1 eq) in anhydrous EtOH/Et₃N (3.0 mL, 3:1, 0.02 M) was added Pd Black (58 mg, 0.55 mmol, 10 eq) and stirred for 1 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and purified through amino-functionalized silica gel chromatography (3:1 ethyl acetate / hexane). Product **348** was accessed (39 mg, 86% yield) as a white solid.

¹H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, *J* = 6.5 Hz, 1H), 8.04 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 6.99 (d, *J* = 6.1 Hz, 1H), 6.46 (d, *J* = 7.6 Hz, 1H), 5.33 (dd, *J* = 8.5, 5.7 Hz, 1H), 4.56 (dd, *J* = 7.6, 5.0 Hz, 1H), 4.46 – 4.38 (m, 2H), 4.30 (dd, *J* = 6.5, 3.0 Hz, 1H), 4.15 – 4.10 (m, 1H), 3.92 (s, 3H), 3.59 (q, *J* = 7.0 Hz, 1H), 2.40 – 2.32 (m, 1H), 2.27 – 2.18 (m, 1H), 1.77 (brs, 2H), 1.49 – 1.41 (m, 1H), 1.38 (d, *J* = 7.0 Hz, 3H), 1.22 – 1.19 (m, 1H), 1.14 (d, *J* = 6.4 Hz, 3H), 1.10 (d, *J* = 6.4 Hz, 3H), 0.99 – 0.92 (m, 12H), 0.91 (s, 9H), 0.85 (s, 9H), 0.11 (d, *J* = 7.8 Hz, 6H), 0.06 (d, *J* = 3.2 Hz, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 176.89, 173.77, 171.10, 169.71,

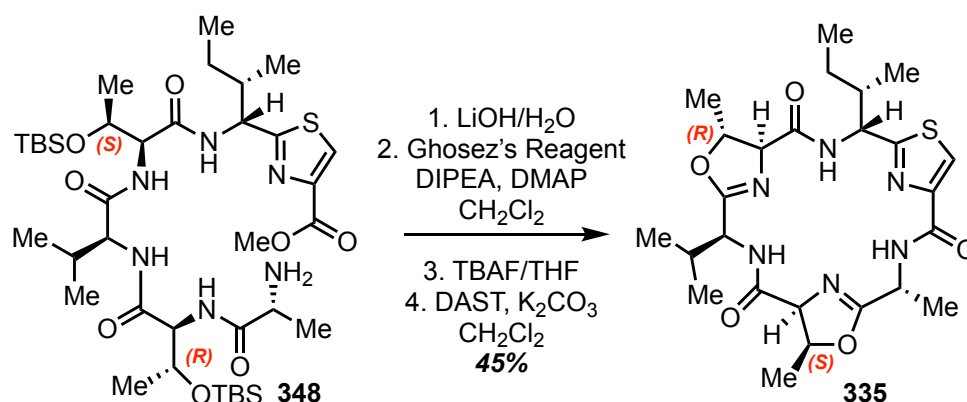
162.04, 147.01, 127.14, 67.91, 67.43, 60.44, 59.38, 58.71, 55.68, 52.50, 50.90, 39.12, 30.02, 29.84, 26.56, 25.91, 21.83, 19.88, 19.64, 19.23, 18.12, 18.03, 14.47, 11.69, -4.54, -4.61, -4.71, -4.87.

Notebook Entries

Procedure: AXT2-085

^1H NMR and ^{13}C NMR – AXT2-058

Balgacyclamide A Epimer (335)



Step 1: To a solution of free amine **348** (35 mg, 0.04 mmol, 1 eq) in THF/H₂O (2.67 mL, 1:1, 0.015 M), LiOH (7.0 mg, 0.28 mmol, 6.67 eq) was added and stirred for 1 h. The mixture was quenched with saturated NH₄Cl and extracted with EtOAc (4 x 5 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to access free carboxylic acid. Material was pushed to next reaction without further purification.

Step 2: To a 0 °C solution of crude material in dry CH₂Cl₂ (12.0 mL, 0.003M) under argon atmosphere, the Ghosez's reagent was added (0.01 mL, 0.05 mmol, 1.2 eq.) and solution stirred for 15 minutes. DIPEA (0.01 mL, 0.12 mmol, 3 eq.) and DMAP (2.40 mg, 0.02 mmol, 0.50 eq.)

were added and stirred for 19 h. The mixture was quenched with saturated NH_4Cl and extracted with CH_2Cl_2 (3 x 1 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification was performed via preparative thin layer chromatography (1:1 ethyl acetate / hexane), followed by extraction with ethyl acetate to afford di-TBS protected macrocycle (17 mg, 52% yield).

Step 3: To a 0 °C stirring solution of di-TBS protected macrocycle (17 mg, 0.02 mmol, 1 eq) in anhydrous THF (0.5 mL, 0.04 M) under argon atmosphere, TBAF (0.02 mL, 0.05 mmol, 2.5 eq.) was added and stirred for 2 h. The reaction was quenched with H_2O (1:1 vol) and extracted with ethyl acetate (4 x 1 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Material was pushed to next reaction without further purification.

Step 4: To a -78 °C stirring solution of diols (12.0 mg, 0.02 mmol, 1 eq) in anhydrous CH_2Cl_2 (0.5 mL, 0.04 M) under argon atmosphere, DAST (0.01 mL, 0.06 mmol, 3.0 eq.) was added dropwise and stirred for 1 h. Then, K_2CO_3 (5.0 mg, 0.03 mmol, 1.5 eq) was added, mixture was slowly warmed to RT and stirred for 2 h. The reaction was quenched with slow addition of saturated NaHCO_3 (1:1 vol) and the product was extracted with CH_2Cl_2 (3 x 1 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification was performed via gradient preparative thin layer chromatography (3:1 to 4:1 ethyl acetate / hexane), followed by extraction with ethyl acetate to afford Balgacyclamide A epimer **335** (10 mg, 45% yield over four steps) as a white solid.

Rf: 0.80 (9:1 ethyl acetate/hexane, UV/PMA) or 0.29 (1:1 ethyl acetate/hexane) **¹H NMR (600 MHz, Chloroform-*d*)** δ 8.41 (d, J = 7.6 Hz, 1H), 8.07 (s, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 9.8 Hz, 1H), 5.25 (dd, J = 8.2, 4.7 Hz, 1H), 5.21 (dq, J = 13.0, 6.5 Hz, 1H), 4.93 (dq, J = 6.0, 5.5 Hz, 1H), 4.88 (dq, J = 7.0, 6.9 Hz, 1H), 4.84 (d, J = 10.2 Hz, 1H), 4.74 (dd, J = 9.8, 3.3 Hz, 1H), 4.26 (dd, J = 8.1, 1.8 Hz, 1H), 2.19 – 2.13 (m, 1H), 1.99 – 1.94 (m, 1H), 1.55 (d, J = 6.8 Hz, 3H), 1.51 (d, J = 6.2 Hz, 3H), 1.38 (d, J = 1.3 Hz, 2H), 1.35 (d, J = 6.5 Hz, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 5.5 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.47 (d, J = 6.8 Hz, 3H). **¹³C NMR (151 MHz, CDCl₃)** δ 170.83, 170.19, 168.70, 168.46, 168.04, 159.83, 149.00, 123.47, 80.70, 80.30, 74.28, 70.83, 55.39, 51.54, 44.05, 42.04, 30.99, 25.50, 21.71, 20.00, 19.28, 16.92, 15.84, 15.08, 11.87. **FTIR (neat):** 3395, 3298, 2963, 2915, 2850, 1648, 1502, 1395, 1203 cm⁻¹ **HRMS(ESI):** m/z calc. for C₂₅H₃₇N₆O₅S [M+H]⁺: 533.2546, found: 533.2546

¹H NMR (600 MHz, DMSO-*d*₆) δ 8.32 – 8.26 (m, 2H), 8.31 (s, 1H), 7.44 (d, J = 9.4 Hz, 1H), 5.25 – 5.19 (m, 2H), 4.94 (dq, J = 6.7, 6.5 Hz, 1H), 4.80 (d, J = 10.2 Hz, 1H), 4.74 (dq, J = 7.1, 6.3 Hz, 1H), 4.53 – 4.49 (m, 1H), 4.39 (dd, J = 7.4, 2.1 Hz, 1H), 2.04 – 1.99 (m, 1H), 1.96 – 1.90 (m, 1H), 1.43 (d, J = 6.9 Hz, 3H), 1.38 (d, J = 6.3 Hz, 3H), 1.21 (d, J = 6.4 Hz, 3H), 1.05 – 1.01 (m, 1H), 0.91 (d, J = 6.9 Hz, 3H), 0.87 (t, J = 7.9, 7.4 Hz, 4H), 0.73 (d, J = 6.9 Hz, 3H), 0.37 (d, J = 7.0 Hz, 3H).

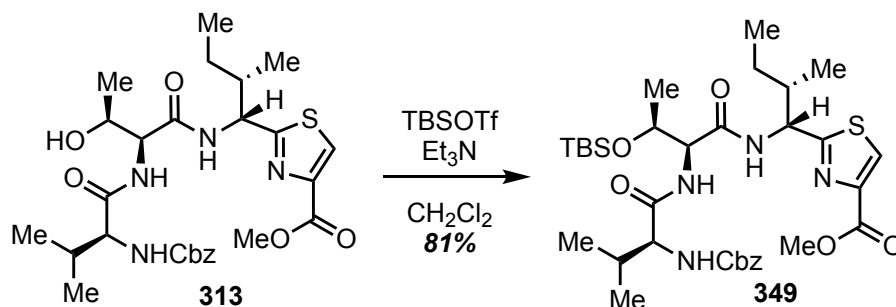
Notebook Entries

Procedure: Step 1 – AXT2-090, Step 2 – AXT2-091, Step 3 - AXT2-112, Step 4 – AXT2-113

¹H NMR in DMSO-*d*₆ – AXT2-113-DMSO (600)

¹H NMR and ¹³C NMR in CDCl₃ – AXT2-113 (600)

methyl 2-((5*S*,8*S*,11*R*,12*S*)-8-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-5-isopropyl-12-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazatetradecan-11-yl)thiazole-4-carboxylate
(349)



To a flamed dried RBF under an Ar atmosphere was added free alcohol **313** (250 mg, 0.44 mmol, 1 eq) in anhydrous CH₂Cl₂ (4.4 mL, 0.1 M) and cooled to 0 °C. Once at temperature Et₃N (0.25 mL, 1.78 mmol, 4 eq.) was added and stirred. After 10 minutes TBSOTf (0.20 mL, 0.89 mmol, 2 eq) was added, mixture was slowly warmed to RT and stirred for 24 h. The reaction was quenched with saturated NH₄Cl (1:1 vol) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (2:1 hexane / ethyl acetate) to afford **349** (243 mg, 81% yield) as a colorless oil.

¹H NMR (600 MHz, CDCl₃) δ 7.96 (s, 1H), 7.32 – 7.22 (m, 6H), 7.01 (d, *J* = 8.0 Hz, 1H), 5.80 (d, *J* = 7.3 Hz, 1H), 5.28 (t, *J* = 8.9, 8.1 Hz, 1H), 5.10 – 4.96 (m, 2H), 4.51 (t, *J* = 7.0 Hz, 1H), 4.22 (p, *J* = 6.4 Hz, 1H), 4.06 (t, *J* = 6.8 Hz, 1H), 3.83 (s, 3H), 2.27 (hept, *J* = 6.7 Hz, 1H), 2.11 (hept, *J* = 6.9 Hz, 1H), 1.39 (hept, *J* = 13.7, 7.0 Hz, 1H), 1.18 (hept, 1H), 1.15 (d, *J* = 6.4 Hz, 3H), 0.90 – 0.85 (m, 12H), 0.83 (s, 9H), 0.05 (d, 6H). **¹³C NMR (151 MHz, CDCl₃)** δ 173.06, 171.47, – 0.85 (m, 12H), 0.83 (s, 9H), 0.05 (d, 6H).

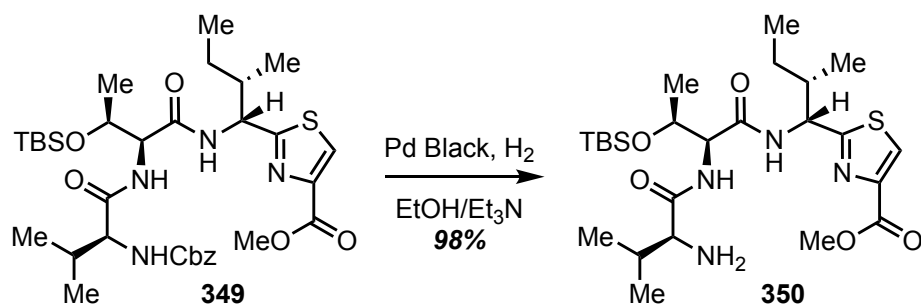
169.65, 161.73, 156.71, 146.73, 136.14, 128.44, 128.08, 127.81, 127.06, 68.44, 66.98, 61.09, 59.13, 55.50, 52.22, 38.91, 30.64, 26.30, 25.71, 19.86, 19.20, 17.89, 14.40, 11.45, -4.62, -4.82.

Notebook Entries

Procedure: AXT2-024

¹H NMR and ¹³C NMR - AXT2-024

Methyl 2-((1*R*,2*S*)-1-((2*S*,3*S*)-2-((*S*)-2-amino-3-methylbutanamido)-3-((*tert*-butyldimethylsilyl)oxy)butanamido)-2-methylbutyl)thiazole-4-carboxylate (350**)**



To a flamed dried RBF containing a solution of **349** (100 mg, 0.15 mmol, 1 eq) in anhydrous EtOH/Et₃N (7.5 mL, 3:1, 0.02 M) was added Pd Black (157 mg, 1.5 mmol, 10 eq) and stirred for 1 h under H₂ (g). The reaction mixture was concentrated under reduced pressure and purified through amino-functionalized silica gel chromatography (1:1 ethyl acetate / hexane). Product **350** was accessed (79 mg, 98% yield) as a white solid.

¹H NMR (400 MHz, CDCl₃) δ 8.10 (d, *J* = 8.2 Hz, 1H), 8.00 (s, 1H), 7.08 (d, *J* = 8.7 Hz, 1H), 5.30 (t, *J* = 6.7, 3.3 Hz, 1H), 4.46 (t, 1H), 4.24 (p, *J* = 6.1 Hz, 1H), 3.88 (s, 3H), 3.31 – 3.27 (m, 1H), 2.33 (hept, *J* = 9.1, 5.3 Hz, 1H), 2.24 (hept, *J* = 6.9 Hz, 1H), 1.43 (brs, 2H), 1.39 (hept, 1H), 1.21 (d, 3H), 1.16 (hept, *J* = 7.1 Hz, 1H), 0.97 (d, *J* = 7.1, 1.9 Hz, 3H), 0.92 – 0.86 (m, 3H), 0.84 – 0.78 (m, 15H), 0.04 (s, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 175.30, 173.25, 169.84, 161.80, – 0.78 (m, 15H), 0.04 (s, 6H).

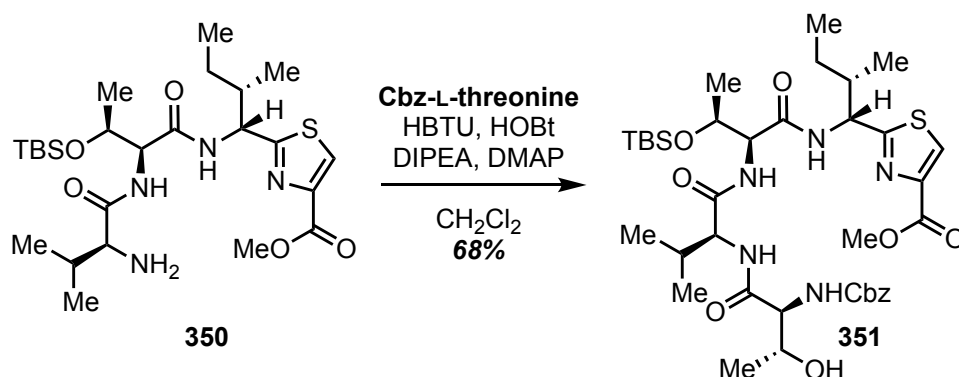
146.93, 127.17, 68.42, 60.14, 58.91, 55.05, 52.40, 39.44, 30.65, 26.60, 25.81, 19.89, 19.79, 17.98, 16.01, 14.26, 11.65, -4.42, -4.83.

Notebook Entries

Procedure: AXT2-025

¹H NMR and ¹³C NMR – AXT2-025

Methyl 2-((5*S*,8*S*,11*S*,14*R*,15*S*)-11-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-5-((*R*)-1-hydroxyethyl)-8-isopropyl-15-methyl-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazaheptadecan-14-yl)thiazole-4-carboxylate (351**)**



Cbz-L-threonine **351** (67 mg, 0.26 mmol, 1 eq.) was added to a solution of free amine **350** (152 mg, 0.29 mmol, 1.10 eq.) in CH₂Cl₂ (3.0 mL, 0.1 M), followed by addition of DIPEA (0.10 mL, 0.58 mmol, 2.20 eq.) and stirred for 10 minutes. HBTU (120 mg, 0.32 mmol, 1.2 eq.), HOBT (50 mg, 0.32 mmol, 1.2 eq.) and DMAP (6.4 mg, 0.10 mmol, 0.2 eq.) were added and stirred for 3h. The mixture was quenched with saturated NH₄Cl (1:1 vol) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic layer were dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (2:1 ethyl acetate / hexane) to afford **351** (134 mg, 68% yield) as a white solid.

eq) was added, mixture was slowly warmed to RT and stirred for 24 h. The reaction was quenched with saturated NH_4Cl (1:1 vol) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (2:1 hexane / ethyl acetate) to afford **352** (131 mg, 83% yield) as a colorless oil.

^1H NMR (400 MHz, CDCl_3) δ 8.01 (s, 1H), 7.38 – 7.32 (m, 5H), 7.12 – 7.06 (m, 2H), 6.40 (d, J = 7.4 Hz, 1H), 5.80 (d, J = 6.0 Hz, 1H), 5.35 (dd, J = 8.6, 5.4 Hz, 1H), 5.18 – 5.07 (m, 2H), 4.53 (t, J = 7.5, 4.9 Hz, 1H), 4.43 – 4.36 (m, 2H), 4.19 (dd, J = 6.1, 3.1 Hz, 1H), 4.10 (t, J = 5.8 Hz, 1H), 3.90 (s, 3H), 2.30 (hept, J = 12.1, 5.7 Hz, 1H), 2.19 (hept, 1H), 1.43 (hept, J = 12.8, 7.3, 6.5 Hz, 1H), 1.25 – 1.18 (m, 1H), 1.15 (d, J = 6.3 Hz, 6H), 0.97 – 0.92 (m, 12H), 0.91 (s, 9H), 0.85 (s, 9H), 0.11 (d, J = 5.7 Hz, 6H), 0.06 (d, J = 4.5 Hz, 6H). **^{13}C NMR (101 MHz, CDCl_3)** δ 173.47, 170.88, 170.77, 169.62, 161.94, 156.60, 147.03, 136.07, 128.76, 128.52, 128.31, 127.04, 68.10, 68.01, 67.41, 60.35, 60.17, 59.42, 55.62, 52.44, 39.46, 30.14, 26.53, 25.91, 19.57, 19.31, 18.11, 14.42, 11.70, -4.56, -4.60, -4.68, -4.90.

Notebook Entries

Procedure: AXT2-049

^1H NMR – AXT2-049-C and ^{13}C NMR – AXT2-049

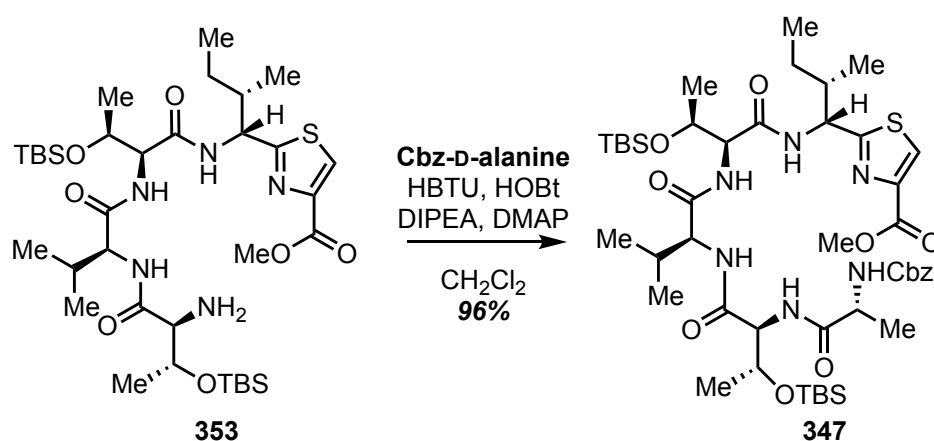
¹H NMR (400 MHz, CDCl₃) δ 8.26 (d, *J* = 5.6 Hz, 1H), 8.06 (s, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 6.50 (d, *J* = 7.7 Hz, 1H), 5.37 (dd, *J* = 8.7, 5.5 Hz, 1H), 4.56 – 4.50 (m, 2H), 4.37 (qd, *J* = 6.3, 4.3 Hz, 1H), 4.02 (t, *J* = 5.5 Hz, 1H), 3.94 (s, 3H), 3.20 (d, *J* = 1.8 Hz, 1H), 2.37 – 2.27 (m, 2H), 1.77 (brs, 2H), 1.46 (hept, *J* = 13.3, 7.6, 5.7 Hz, 1H), 1.30 – 1.23 (m, 1H), 1.17 (d, *J* = 6.3 Hz, 3H), 1.14 (d, *J* = 6.4 Hz, 3H), 1.05 – 0.99 (m, 6H), 0.97 – 0.92 (m, 6H), 0.86 (s, 9H), 0.84 (s, 9H), 0.07 (s, 6H), 0.01 (d, *J* = 30.5 Hz, 6H). **¹³C NMR (101 MHz, CDCl₃)** δ 174.97, 173.75, 171.13, 169.53, 161.86, 146.79, 126.87, 68.16, 67.52, 60.17, 58.92, 55.60, 52.33, 39.36, 29.71, 29.39, 26.40, 25.77, 25.74, 20.84, 19.71, 19.28, 17.98, 17.90, 14.37, 11.63, -4.52, -4.67, -4.87, -4.99.

Notebook Entries

Procedure: AXT2-052

¹H NMR and ¹³C NMR – AXT2-052

Methyl 2-((5*R*,8*S*,11*S*,14*S*,17*R*,18*S*)-8-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-14-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-11-isopropyl-5,18-dimethyl-3,6,9,12,15-pentaoxo-1-phenyl-2-oxa-4,7,10,13,16-pentaazaicosan-17-yl)thiazole-4-carboxylate (347**)**



Cbz-D-alanine (23 mg, 0.11 mmol, 1 eq.) was added to a solution of free amine **353** (95.0 mg, 0.12 mmol, 1.10 eq.) in CH₂Cl₂ (1.1 mL, 0.1 M), followed by addition of DIPEA (0.04 mL, 0.24 mmol, 2.20 eq.) and stirred for 10 minutes. HBTU (52.0 mg, 0.14 mmol, 1.2 eq.), HOBT (22.0 mg, 0.14 mmol, 1.2 eq.) and DMAP (2.70 mg, 0.10 mmol, 0.2 eq.) were added and stirred for 5.5h. The mixture was quenched with saturated NH₄Cl (1:1 vol) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (1:1 ethyl acetate / hexane) to afford **347** (105 mg, 96% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.03 (s, 1H), 7.38 – 7.30 (m, 5H), 7.28 (d, *J* = 5.5 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.00 (d, *J* = 6.7 Hz, 1H), 6.54 (d, *J* = 7.3 Hz, 1H), 5.40 – 5.31 (m, 2H), 5.13 – 5.00 (m, 2H), 4.55 (t, *J* = 6.3 Hz, 1H), 4.48 –

4.39 (m, 2H), 4.28 (d, $J = 6.6$ Hz, 1H), 4.17 (q, $J = 6.7$ Hz, 1H), 4.11 (t, $J = 5.7$ Hz, 1H), 3.91 (s, 3H), 2.34 (hept, 1H), 2.22 (hept, $J = 13.5, 7.8$ Hz, 1H), 1.48 (hept, $J = 7.0$ Hz, 1H), 1.44 (d, $J = 7.1$ Hz, 3H), 1.27 – 1.21 (m, 1H), 1.17 (d, $J = 6.4$ Hz, 3H), 1.12 (d, $J = 6.4$ Hz, 3H), 0.99 (d, $J = 6.8$ Hz, 6H), 0.93 (d, 6H), 0.90 (s, 9H), 0.84 (s, 9H), 0.10 – 0.03 (m, 12H). **^{13}C NMR (101 MHz, CDCl_3)** δ 174.07, 172.89, 171.22, 169.86, 162.08, 156.33, 147.04, 135.92, 128.74, 128.54, 128.28, 127.04, 67.92, 67.49, 67.37, 60.95, 59.47, 55.68, 52.49, 51.33, 39.32, 29.96, 26.55, 25.92, 20.35, 19.48, 19.06, 18.45, 18.13, 18.05, 17.88, 14.42, 11.74, -4.56, -4.61, -4.68, -4.95.

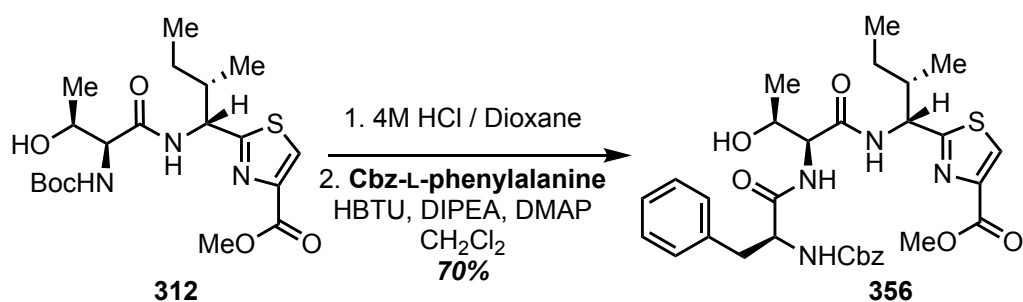
Notebook Entries

Procedure: AXT2-053

^1H NMR and ^{13}C NMR – AXT2-053

6.5 Experimental Procedures: Efforts towards Total Synthesis of Balgacyclamide C

methyl 2-((5*S*,8*S*,11*R*,12*S*)-5-benzyl-8-((*S*)-1-hydroxyethyl)-12-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazatetradecan-11-yl)thiazole-4-carboxylate (**356**)



Compound **312** (125 Mg, 3.70 mmol, 1.05 eq.) was dissolved in 4 M HCl in dioxane (1.2 mL, 0.25 M) and stirred for 1 h. The solution was concentrated under reduced pressure and the crude material used carried forward without purification. To a stirring solution of crude material in CH₂Cl₂ (3.0 mL, 0.1 M), Cbz-L-phenylalanine (106 mg, 0.29mmol, 1.05 eq.) and DIPEA (0.11 mL, 0.62 mmol, 2.20 eq.) were added and stirred for 10 minutes, followed by addition of HBTU (127 mg, 0.34 mmol, 1.2 eq.) and DMAP (7.0 mg, 0.06 mmol, 0.2 eq.). The mixture was stirring overnight, quenched with 1 M HCl (1:1 vol eq.), and extracted with CH₂Cl₂ (3 x 5 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated under reduced pressure. Purification was performed via flash silica gel chromatography (5% MeOH in CH₂Cl₂) to afford **356** (118 mg, 70% yield) as a white solid.

R_f = 0.28 (5% MeOH in CH₂Cl₂, UV). **¹H NMR (600 MHz, CDCl₃)** δ 8.02 (s, 1H), 7.64 (d, 2H), 7.30 – 7.25 (m, 3H), 7.21 – 7.17 (m, 4H), 7.15 – 7.08 (m, 3H), 5.84 (d, *J* = 7.9 Hz, 1H), 5.41 (dd, *J* = 9.1, 4.8 Hz, 1H), 5.05 – 4.92 (m, 2H), 4.68 – 4.63 (m, 1H), 4.58 (dd, *J* = 7.6, 4.6 Hz, 1H), 4.32 (d, *J* = 7.7 Hz, 1H), 4.05 – 3.99 (m, 1H), 3.88 (s, 3H), 3.05 (ddd, *J* = 64.1, 14.1, 6.8 Hz, 2H), 2.25

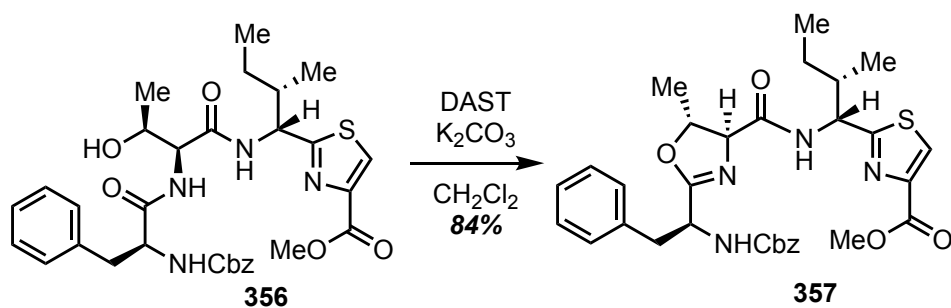
(dq, $J = 13.2, 6.6$ Hz, 1H), 1.47 – 1.40 (m, 1H), 1.28 – 1.23 (m, 1H), 1.21 (d, $J = 6.5$ Hz, 3H), 0.92 (t, $J = 7.4$ Hz, 3H), 0.85 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 173.13, 172.38, 170.43, 165.75, 161.71, 156.24, 146.63, 136.22, 136.09, 129.25, 128.64, 128.48, 128.10, 127.87, 127.51, 127.00, 68.66, 66.98, 58.38, 56.15, 55.26, 52.39, 39.42, 38.64, 38.32, 26.60, 19.43, 14.07, 11.61.

Notebook Entries

Procedure: AXT1-425

^1H NMR and ^{13}C NMR – AXT1-425

methyl 2-((1*R*,2*S*)-1-((4*S*,5*R*)-2-((*S*)-1-(((benzyloxy)carbonyl)amino)-2-phenylethyl)-5-methyl-4,5-dihydrooxazole-4-carboxamido)-2-methylbutyl)thiazole-4-carboxylate (357**)**



To a flamed dried RBF under an Ar atmosphere was added free alcohol **356** (592 mg, 0.97 mmol, 1 eq) and anhydrous CH_2Cl_2 (7.0 mL, 0.1 M). The solution was cooled to -78°C and then DAST (0.14 mL, 1.07 mmol, 1.1 eq.) was added dropwise. After 1 h K_2CO_3 (200 mg, 1.46 mmol, 1.5 eq) was added, the mixture was slowly warmed to RT and stirred for 1 h. The reaction was quenched with slow addition of saturated NaHCO_3 (1:1 vol equiv.) and the product was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under

reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 ethyl acetate/hexane) to afford **357** (480 mg, 84% yield) as a colorless oil.

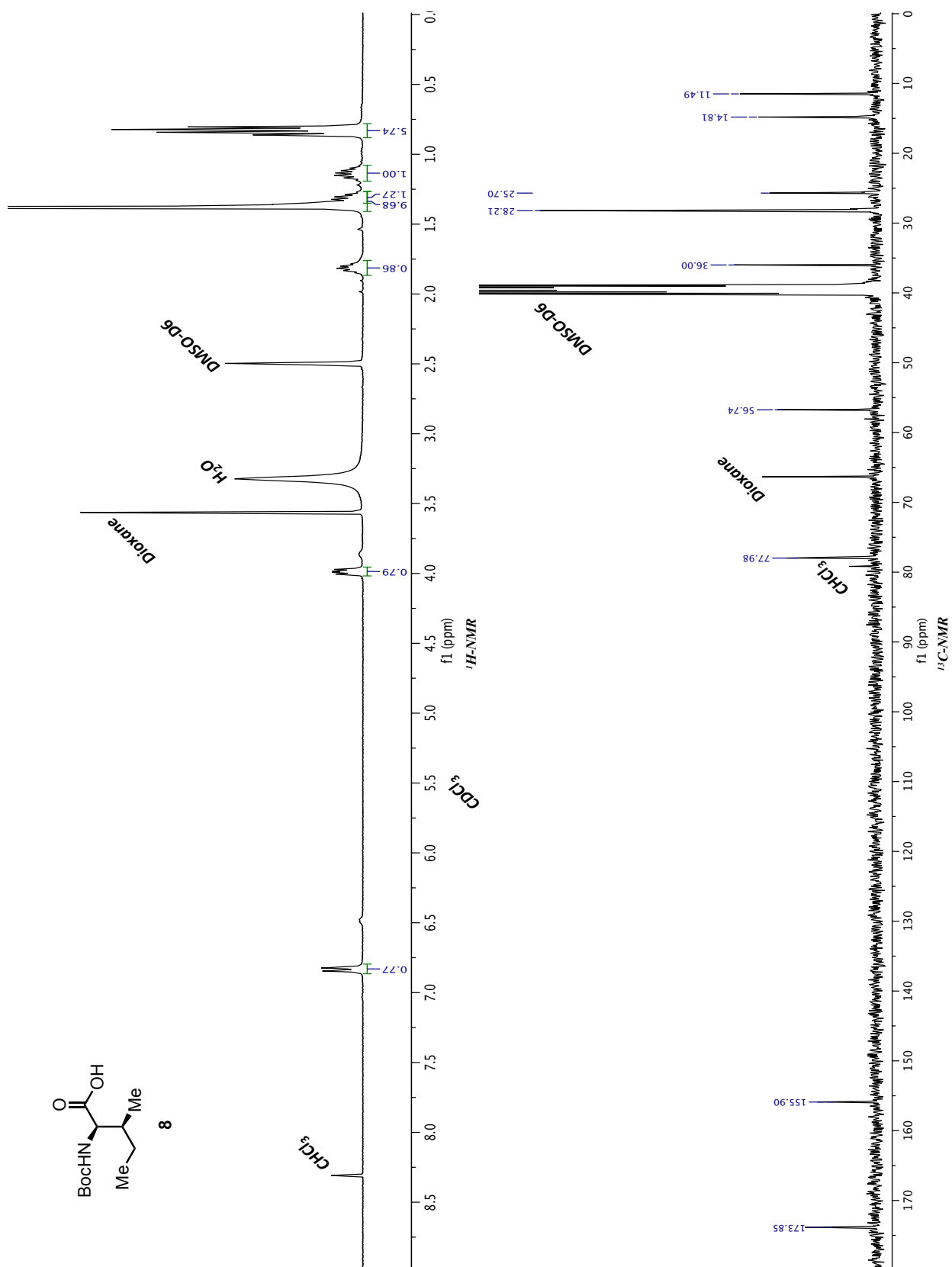
Rf = 0.42 (1:1 ethyl acetate/hexane). **¹H NMR (600 MHz, CDCl₃)** δ 8.05 (s, 1H), 7.39 – 7.34 (m, 5H), 7.20 – 7.16 (m, 3H), 7.15 – 7.12 (m, 3H), 5.40 (d, J = 8.1 Hz, 1H), 5.36 – 5.32 (m, 1H), 5.15 – 5.06 (m, 2H), 4.84 – 4.76 (m, 2H), 4.21 (d, J = 7.8 Hz, 1H), 3.90 (s, 3H), 3.18 – 3.08 (m, 2H), 2.34 – 2.27 (m, 1H), 1.51 (d, J = 6.2 Hz, 3H), 1.48 – 1.44 (m, 1H), 1.26 – 1.21 (m, 1H), 0.96 (t, J = 7.4 Hz, 3H), 0.87 (d, J = 6.9 Hz, 3H). **¹³C NMR (151 MHz, CDCl₃)** δ 172.64, 171.11, 168.53, 161.76, 155.60, 146.98, 136.22, 135.67, 129.37, 128.57, 128.51, 128.26, 128.10, 127.18, 127.04, 80.87, 74.61, 67.01, 60.43, 54.93, 52.42, 50.68, 39.49, 38.44, 26.52, 21.61, 21.09, 14.23, 14.18, 11.60.

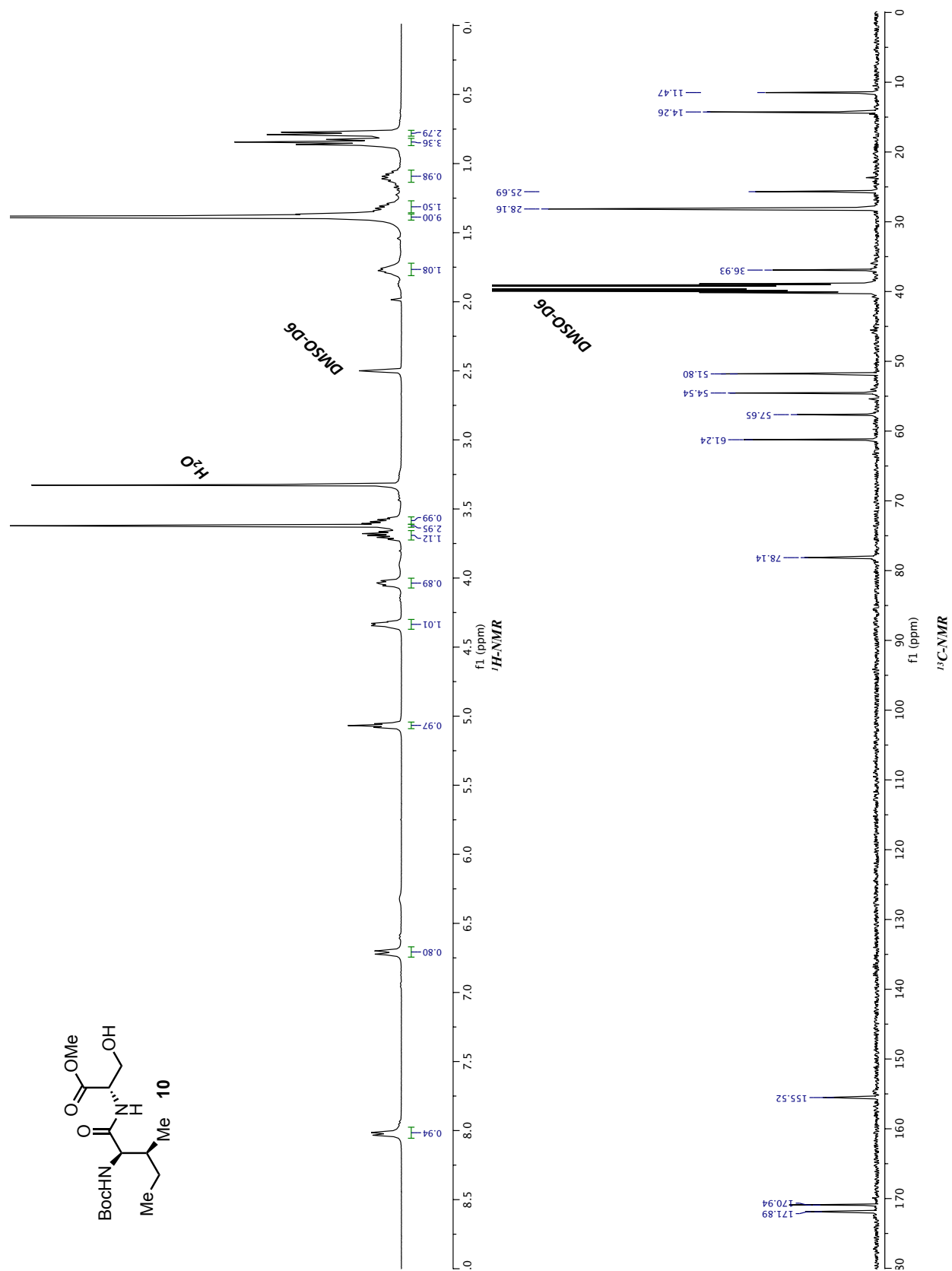
Notebook Entries

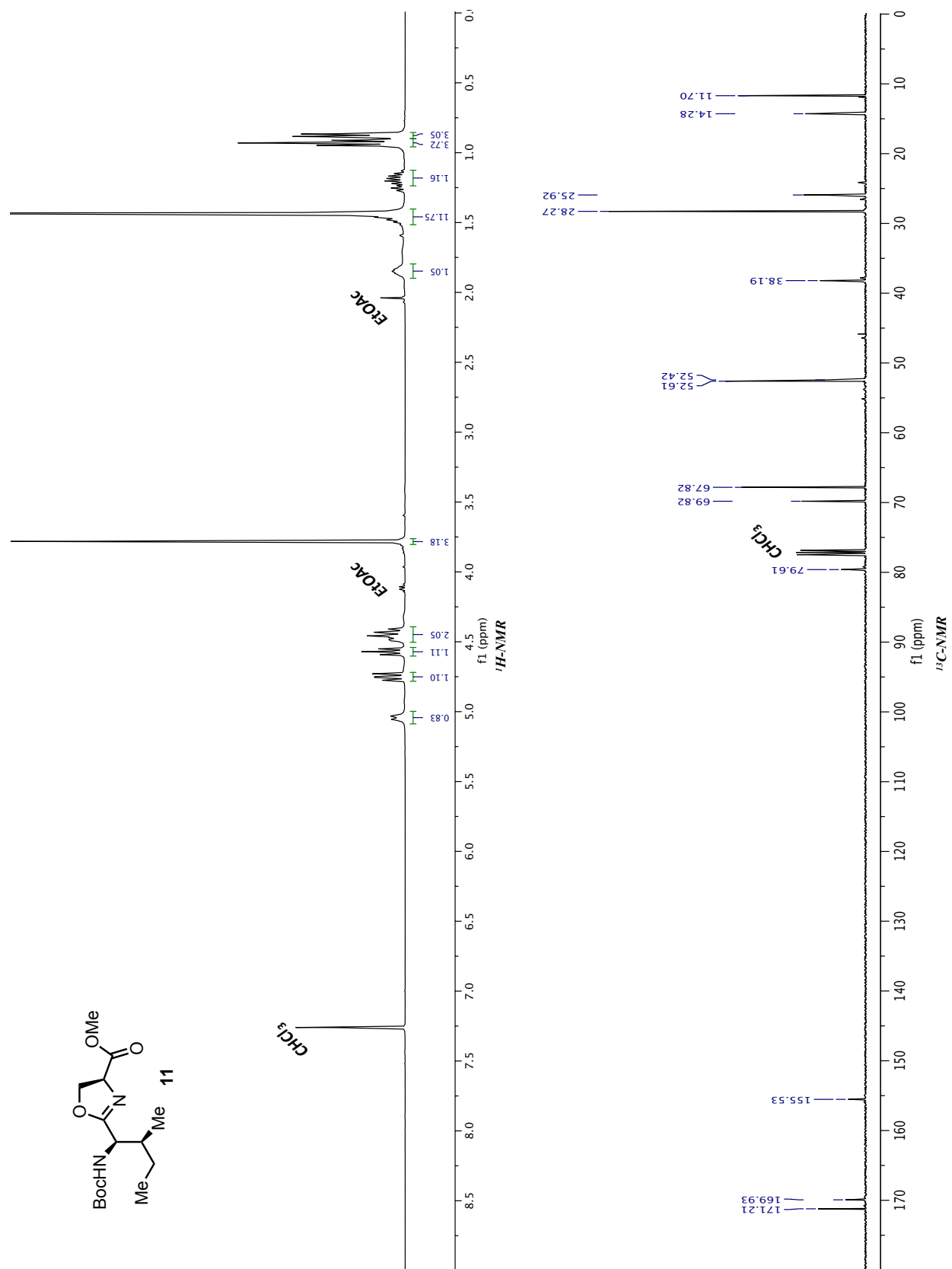
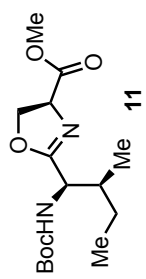
Procedure: AXT2-153

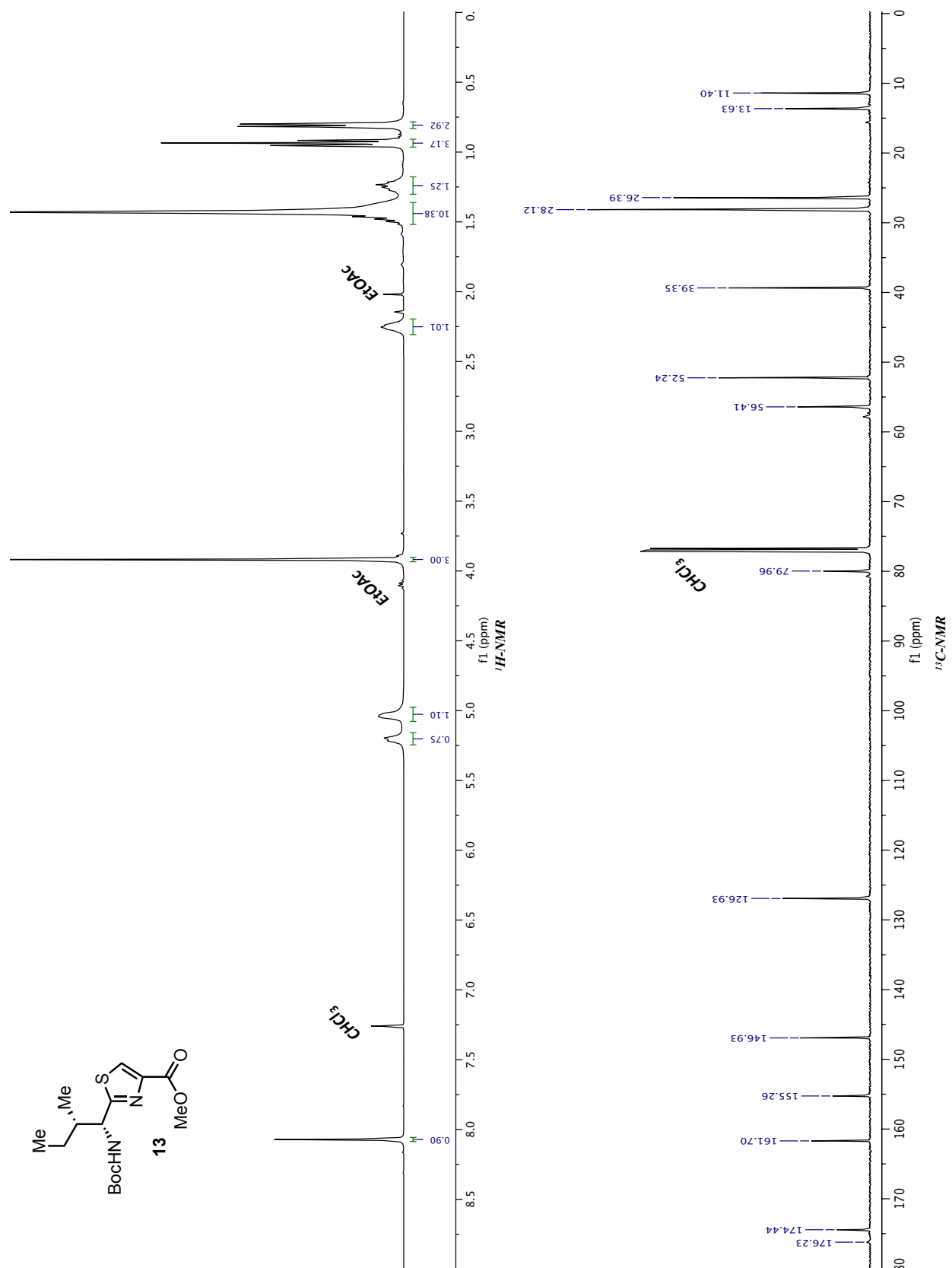
¹H NMR and ¹³C NMR – AXT2-153 (600)

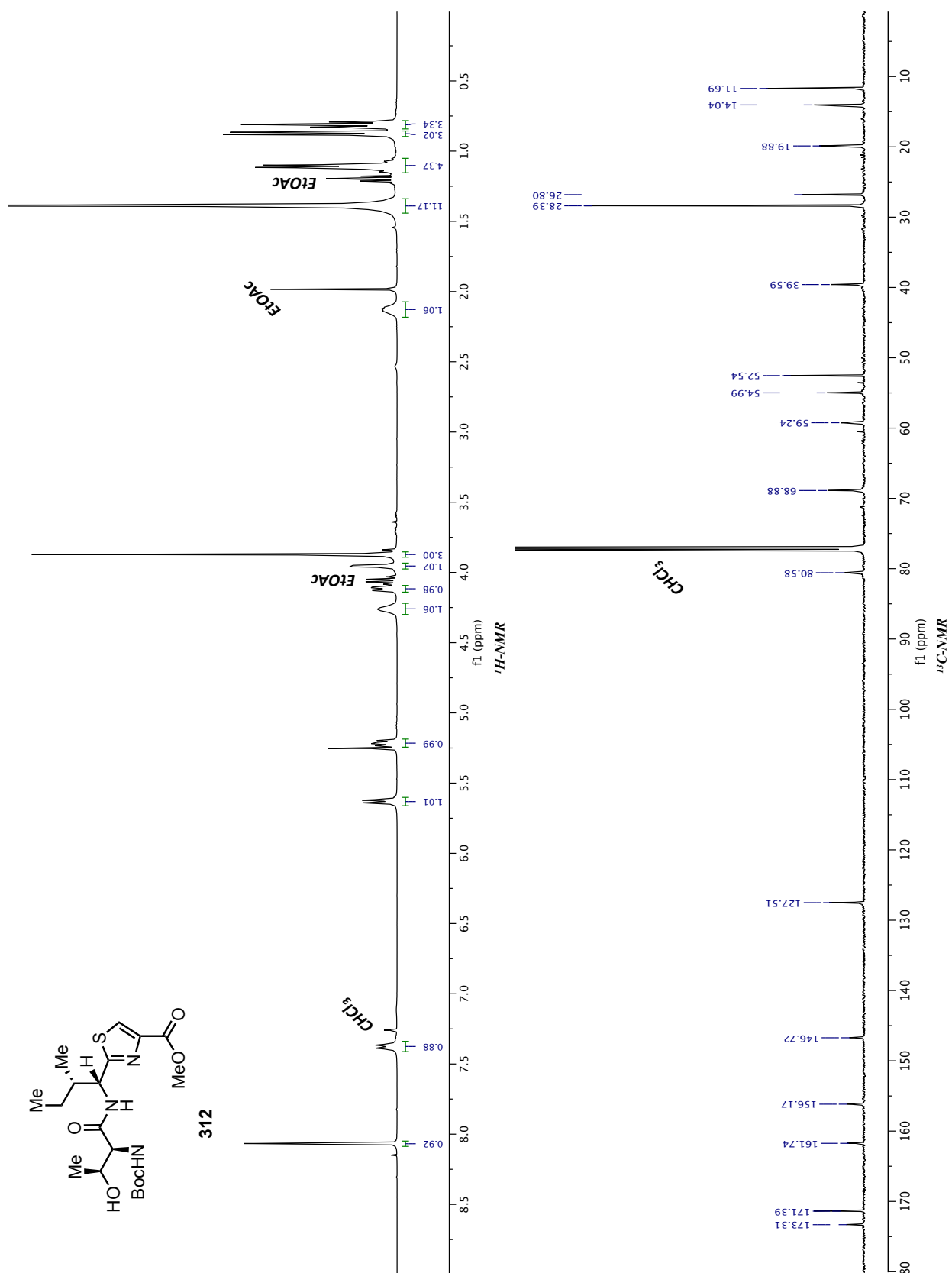
6.6 ^1H -NMR, ^{13}C -NMR & COSY

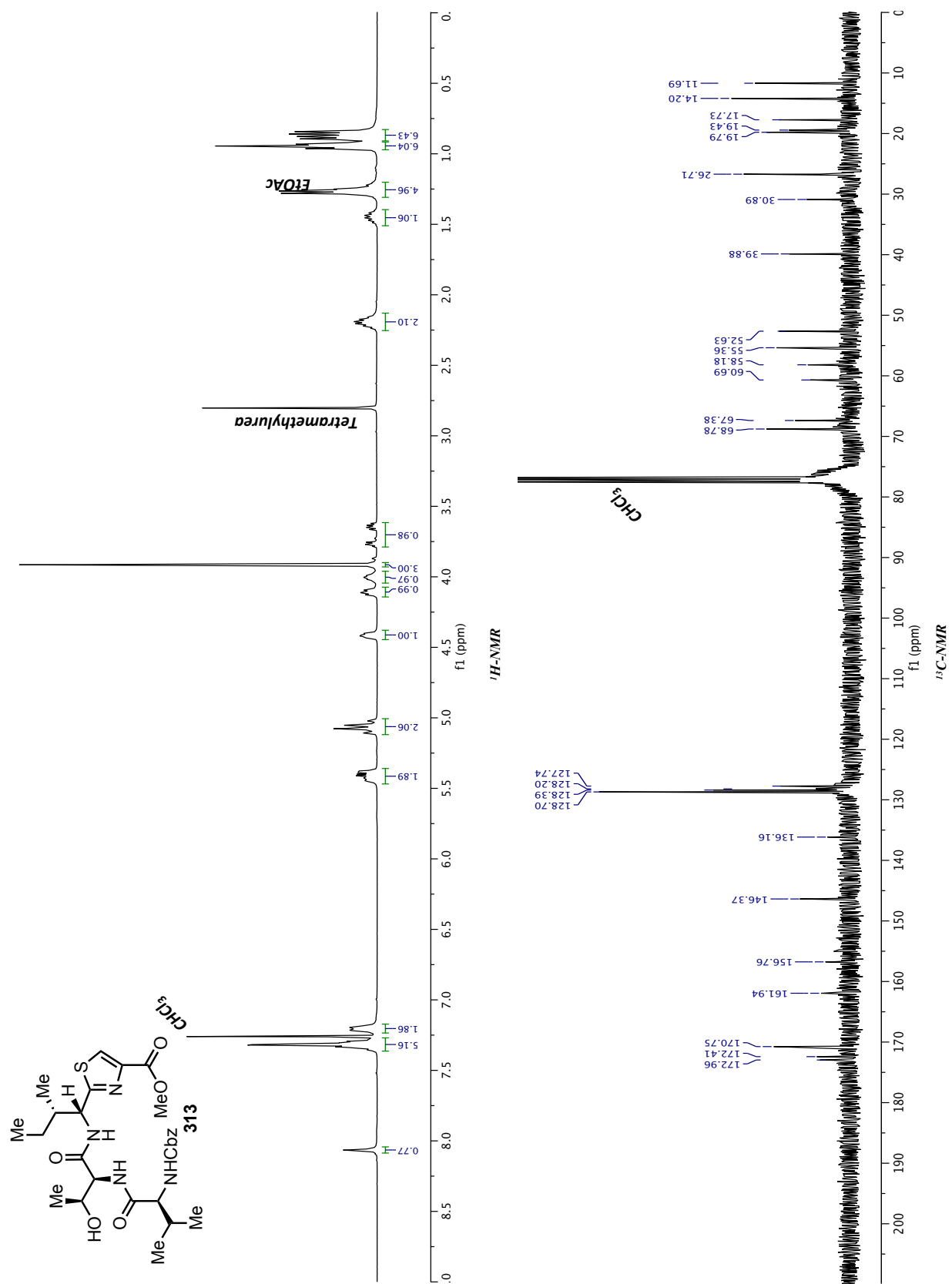


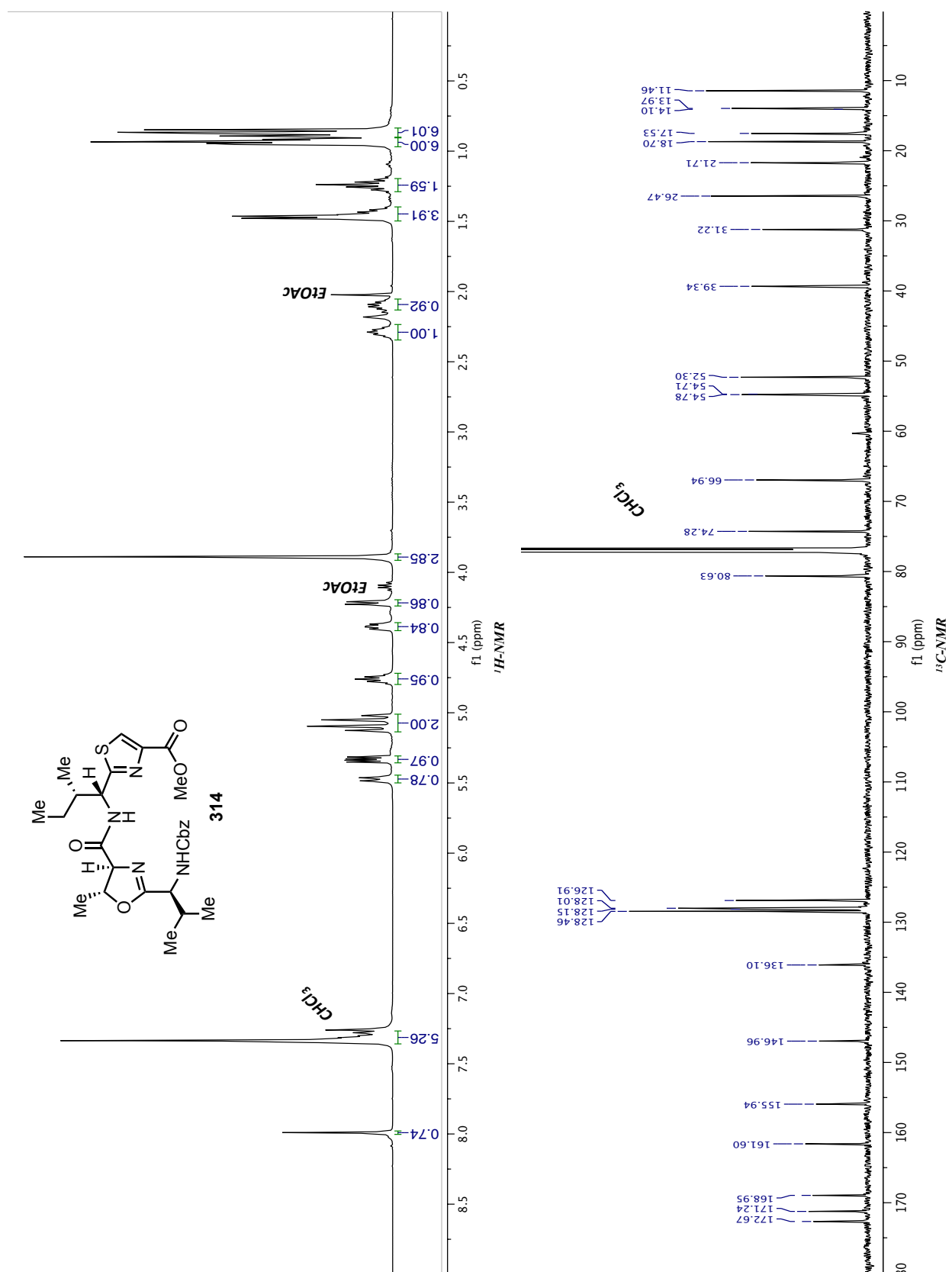


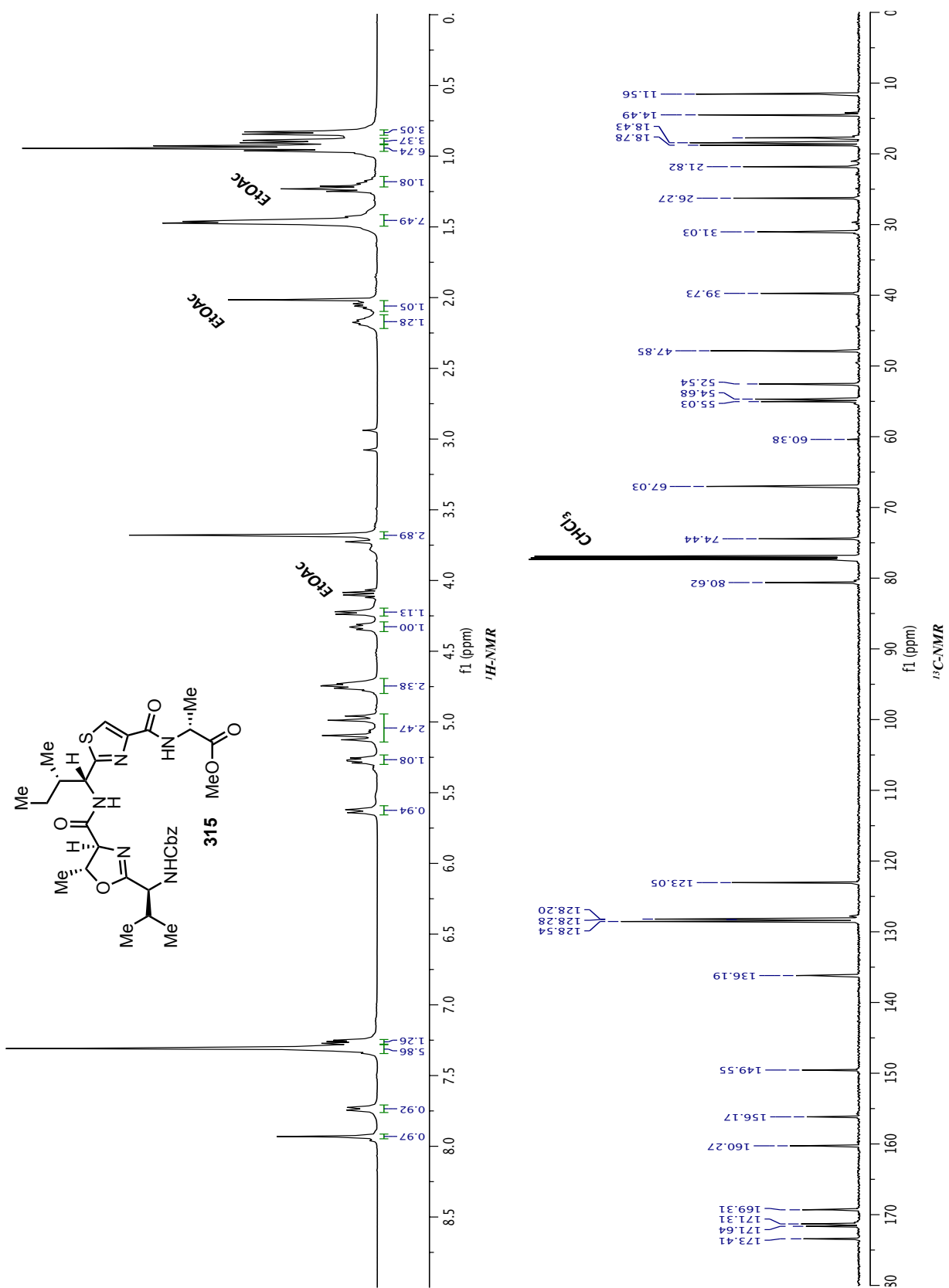


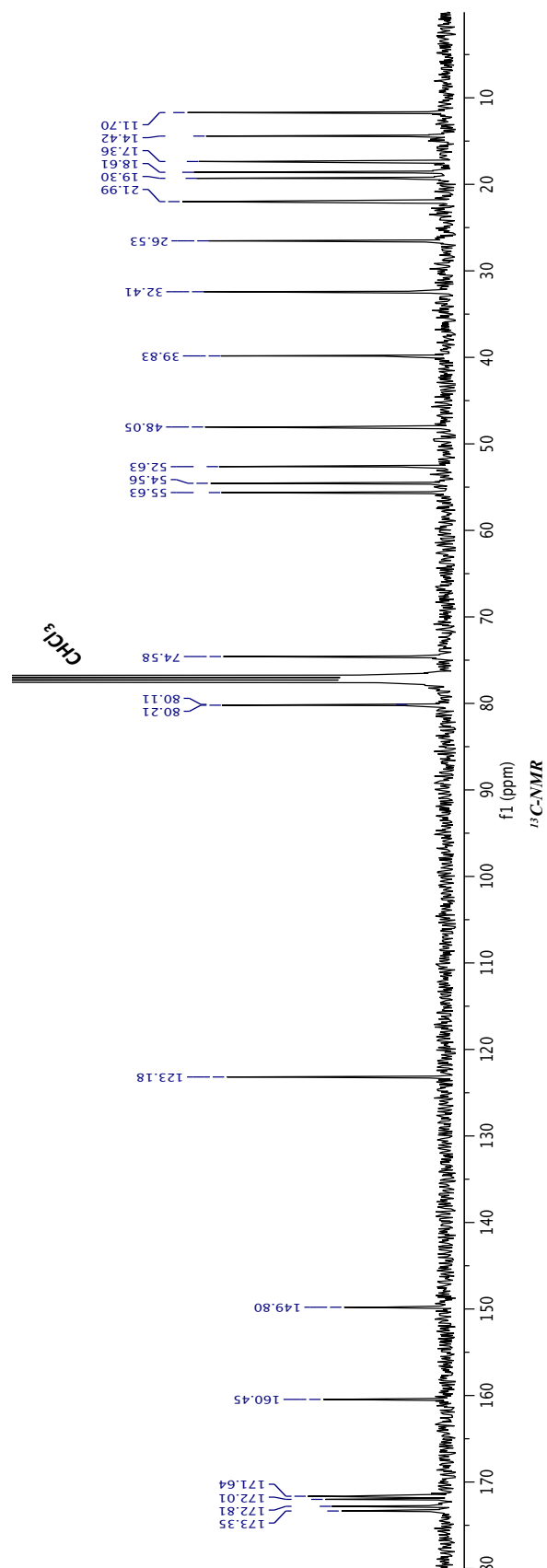
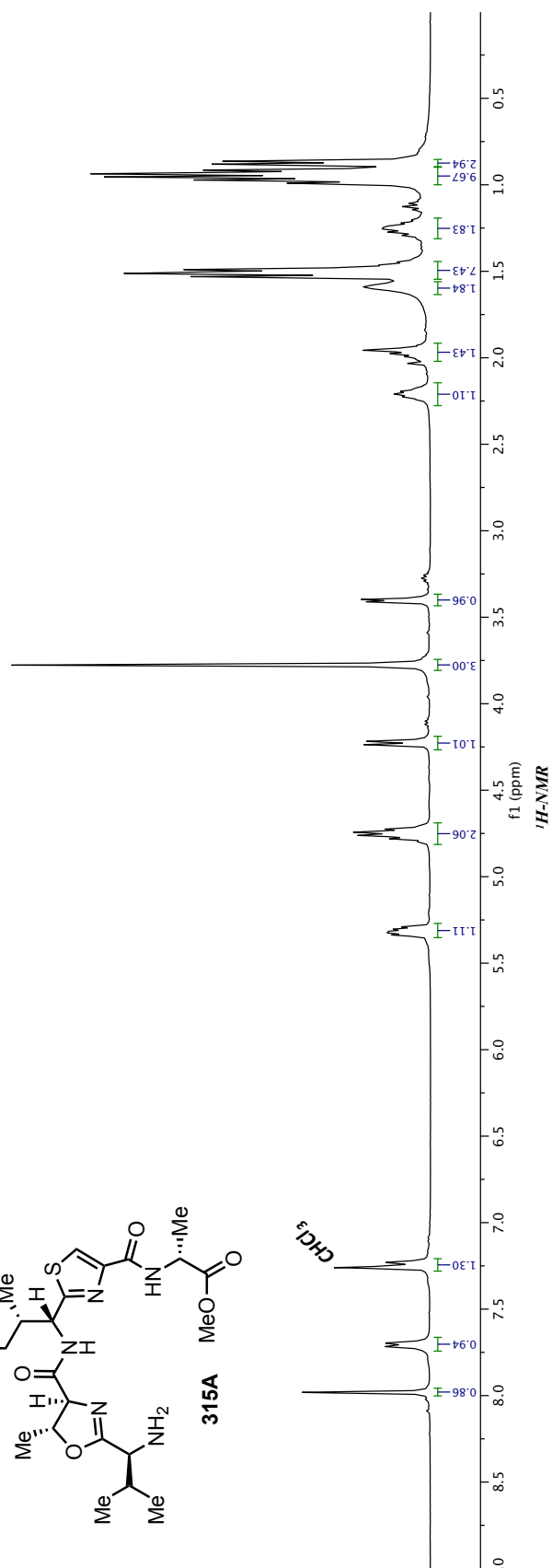
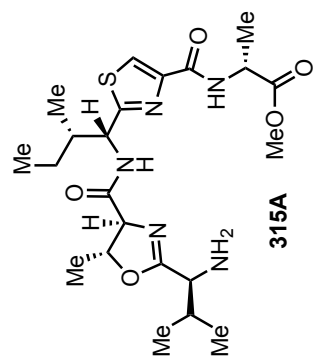






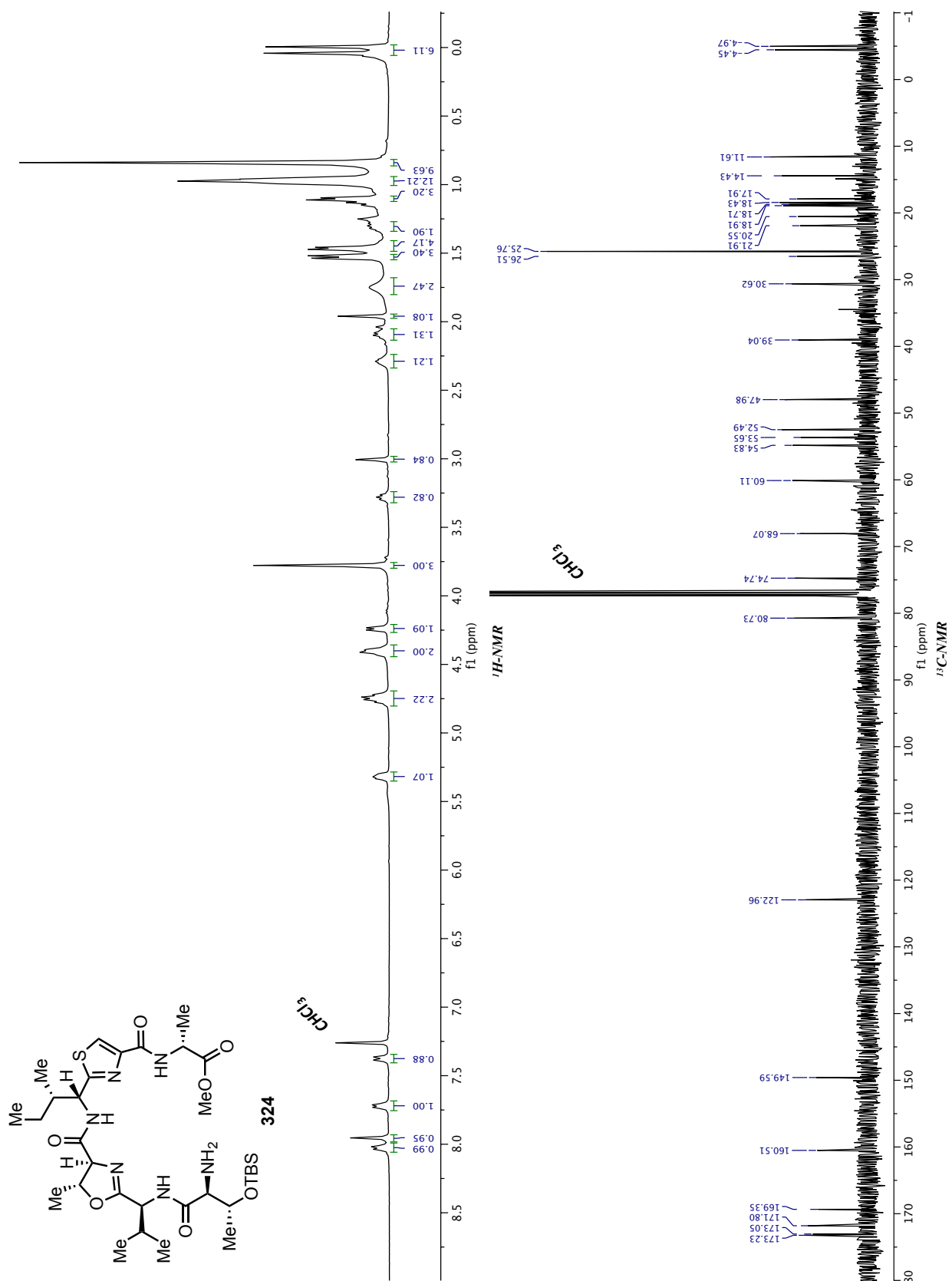




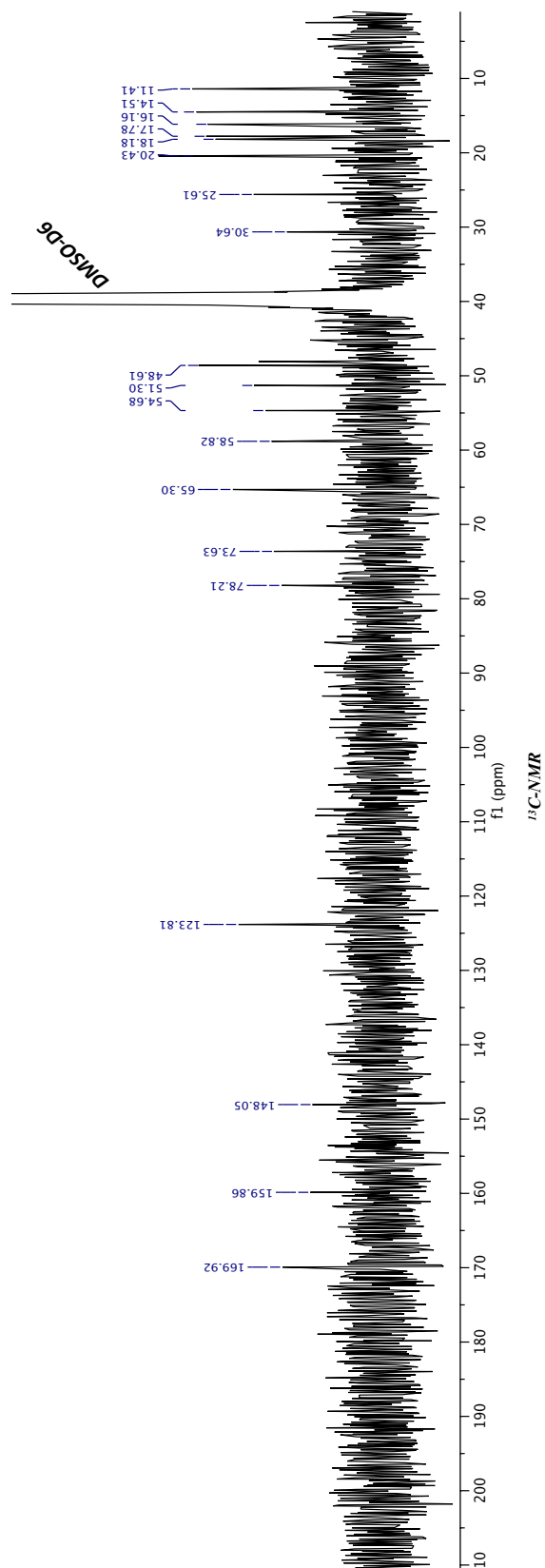
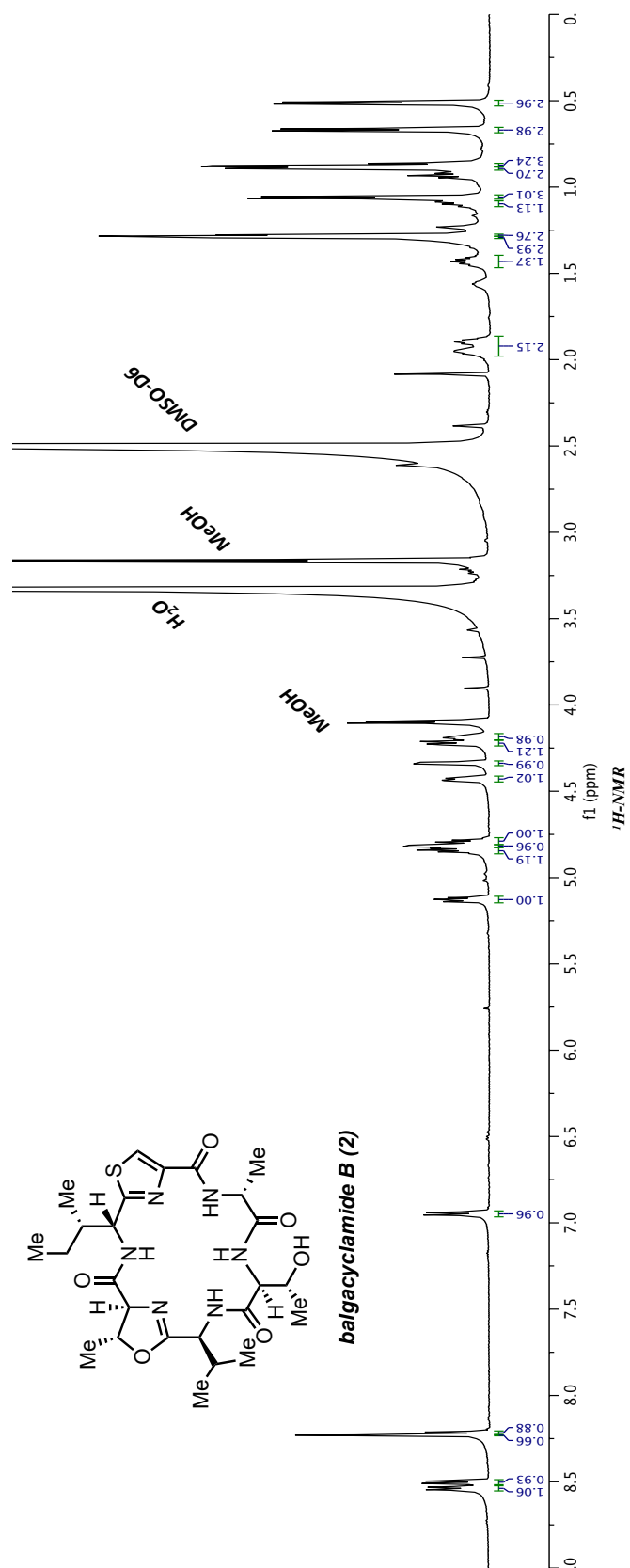


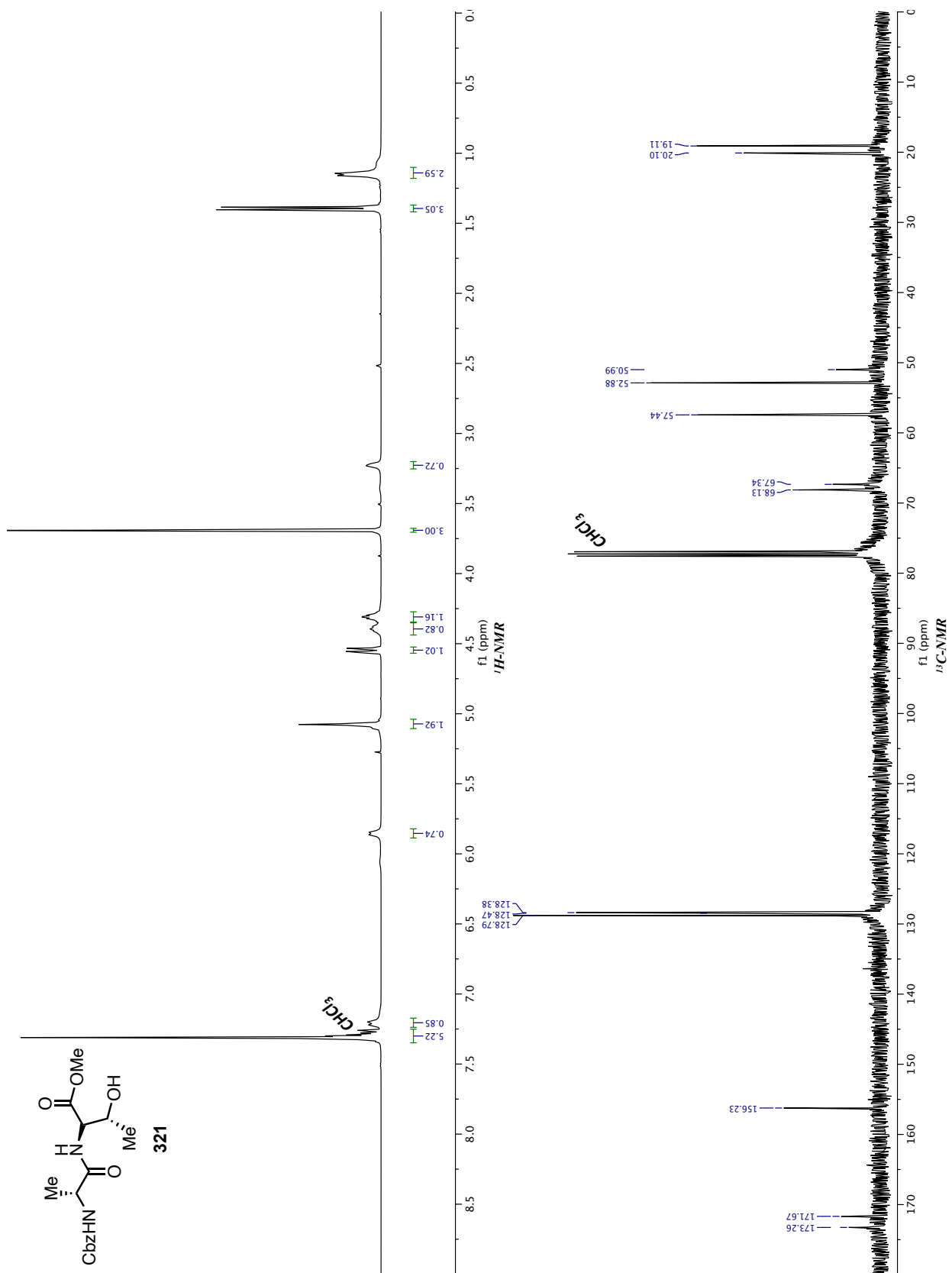


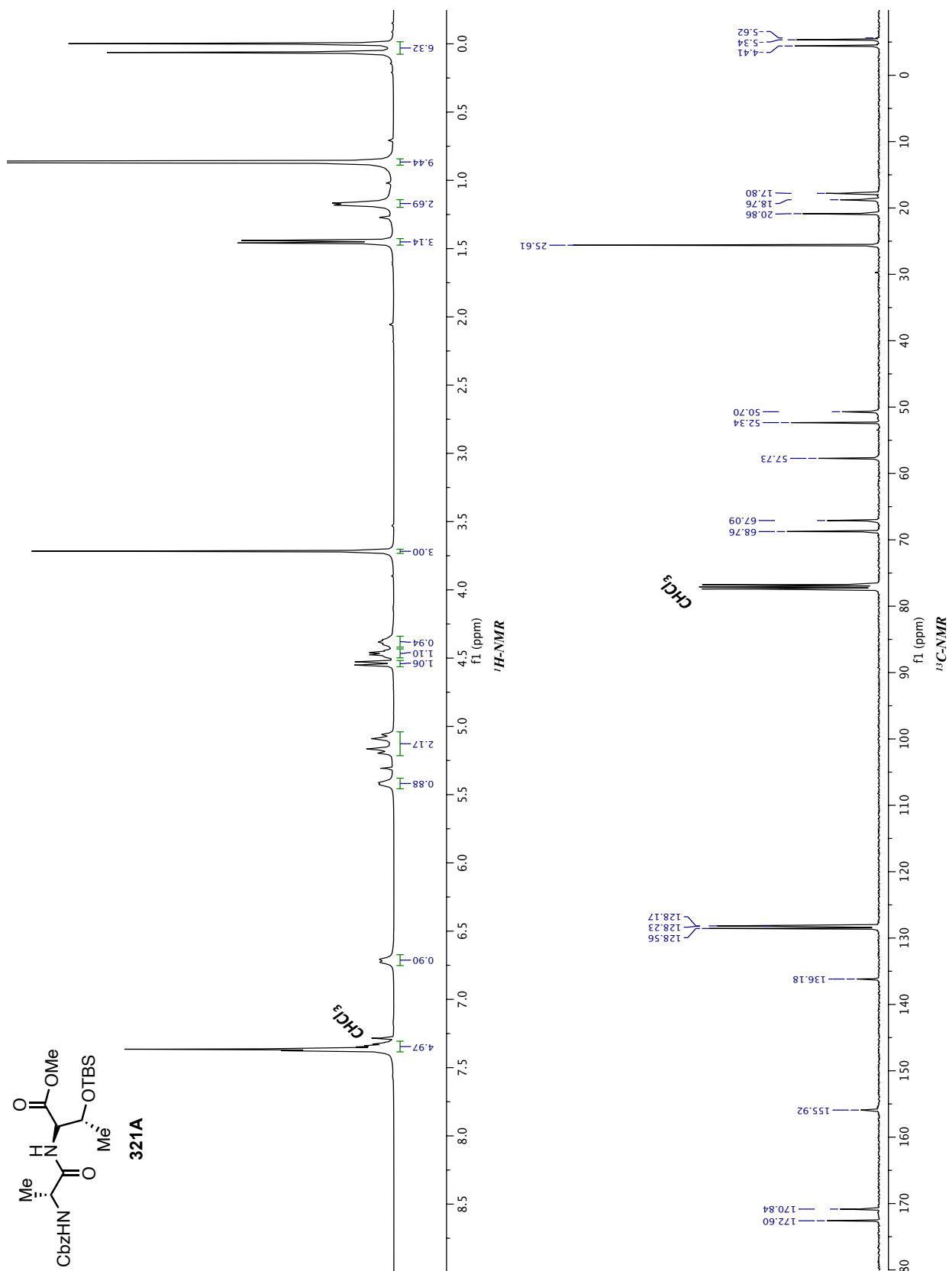


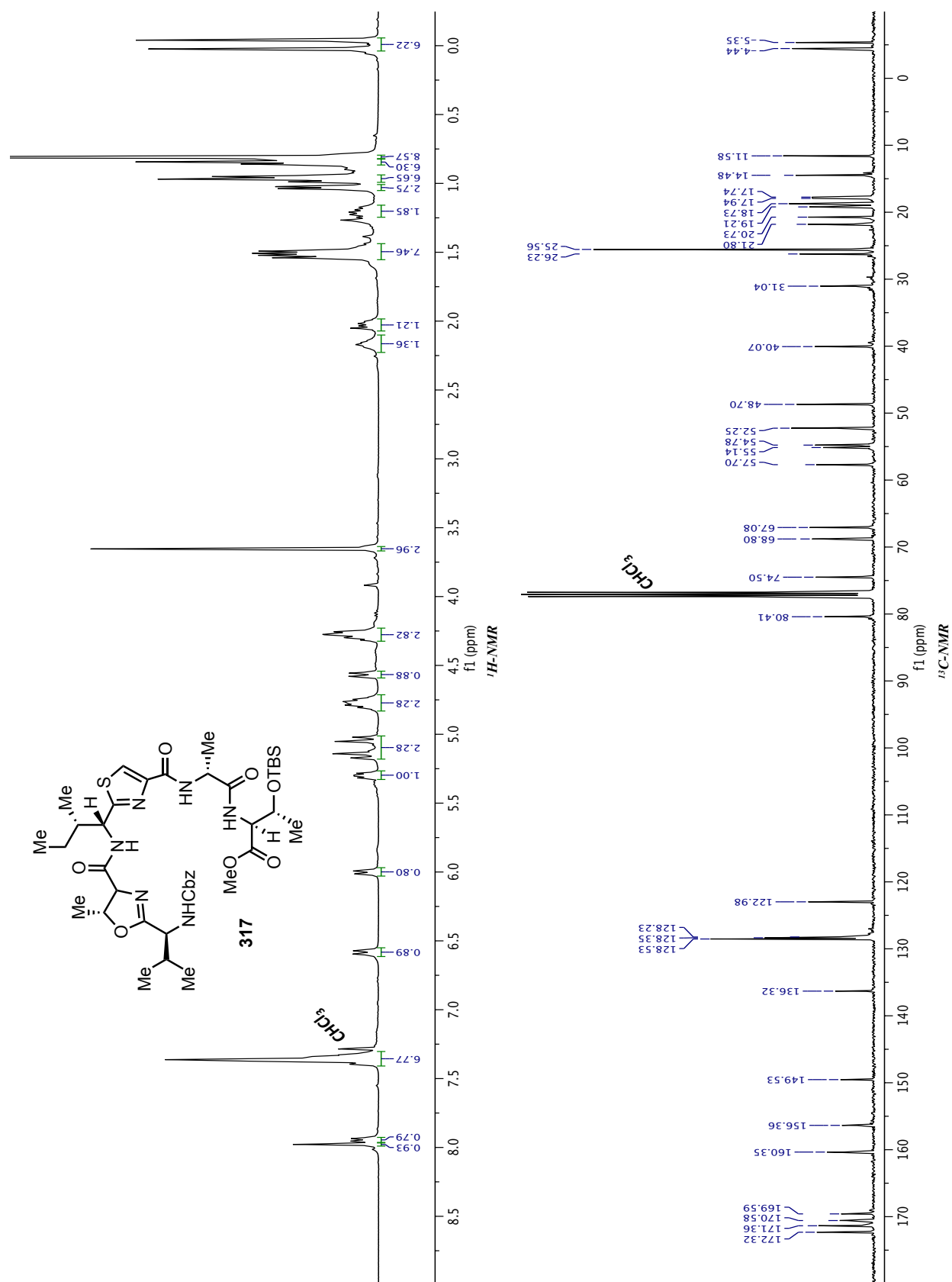


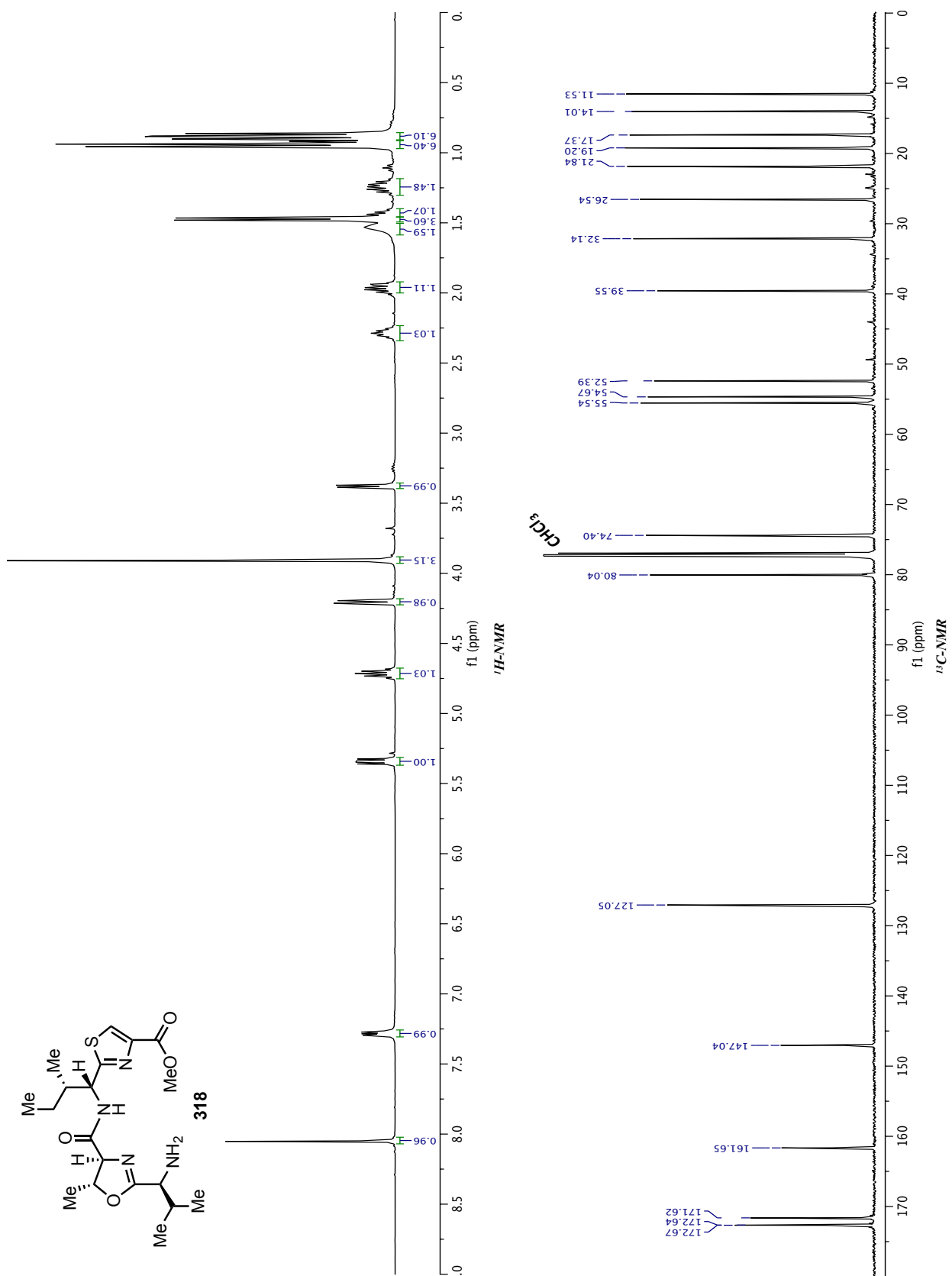


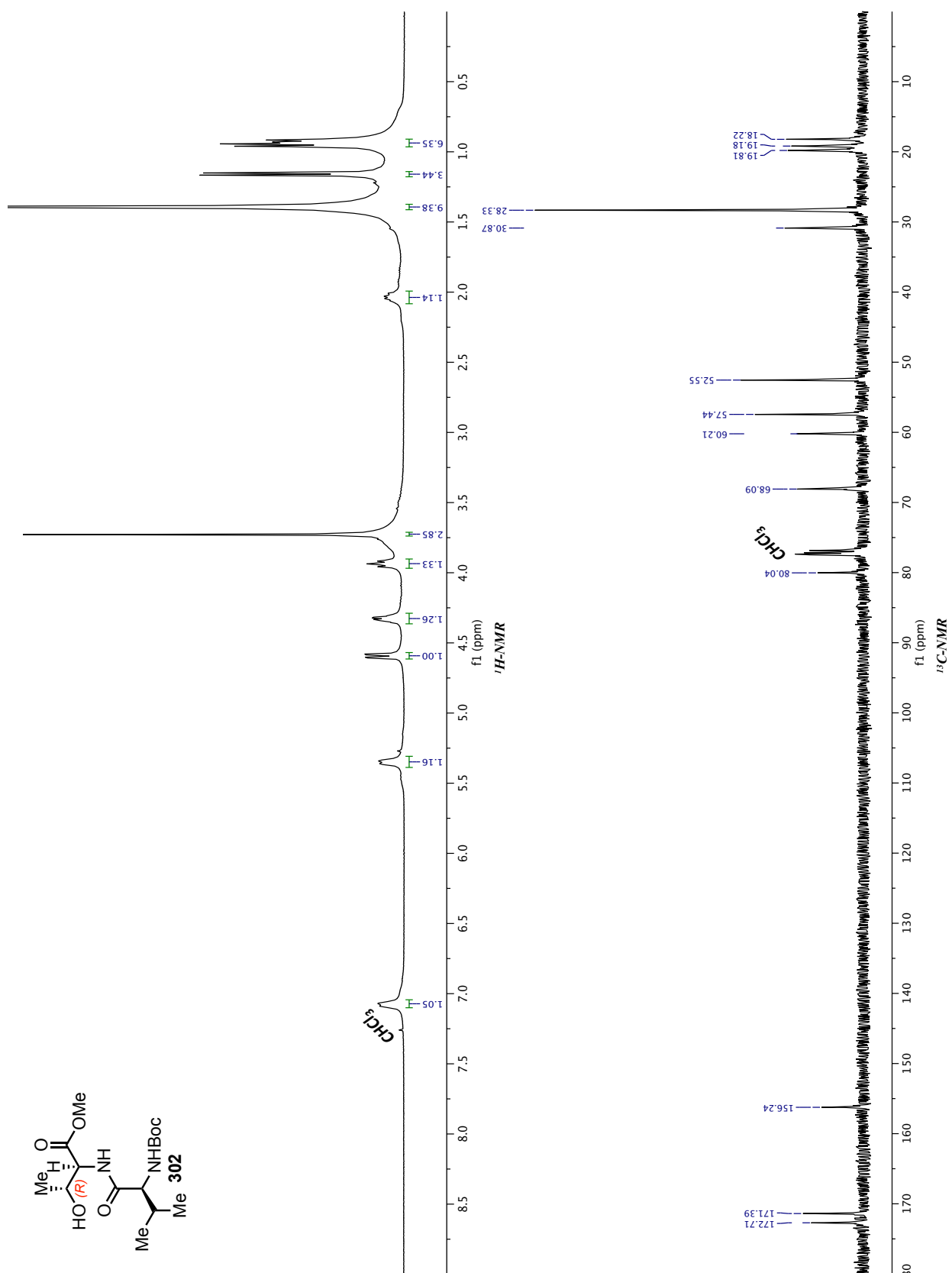


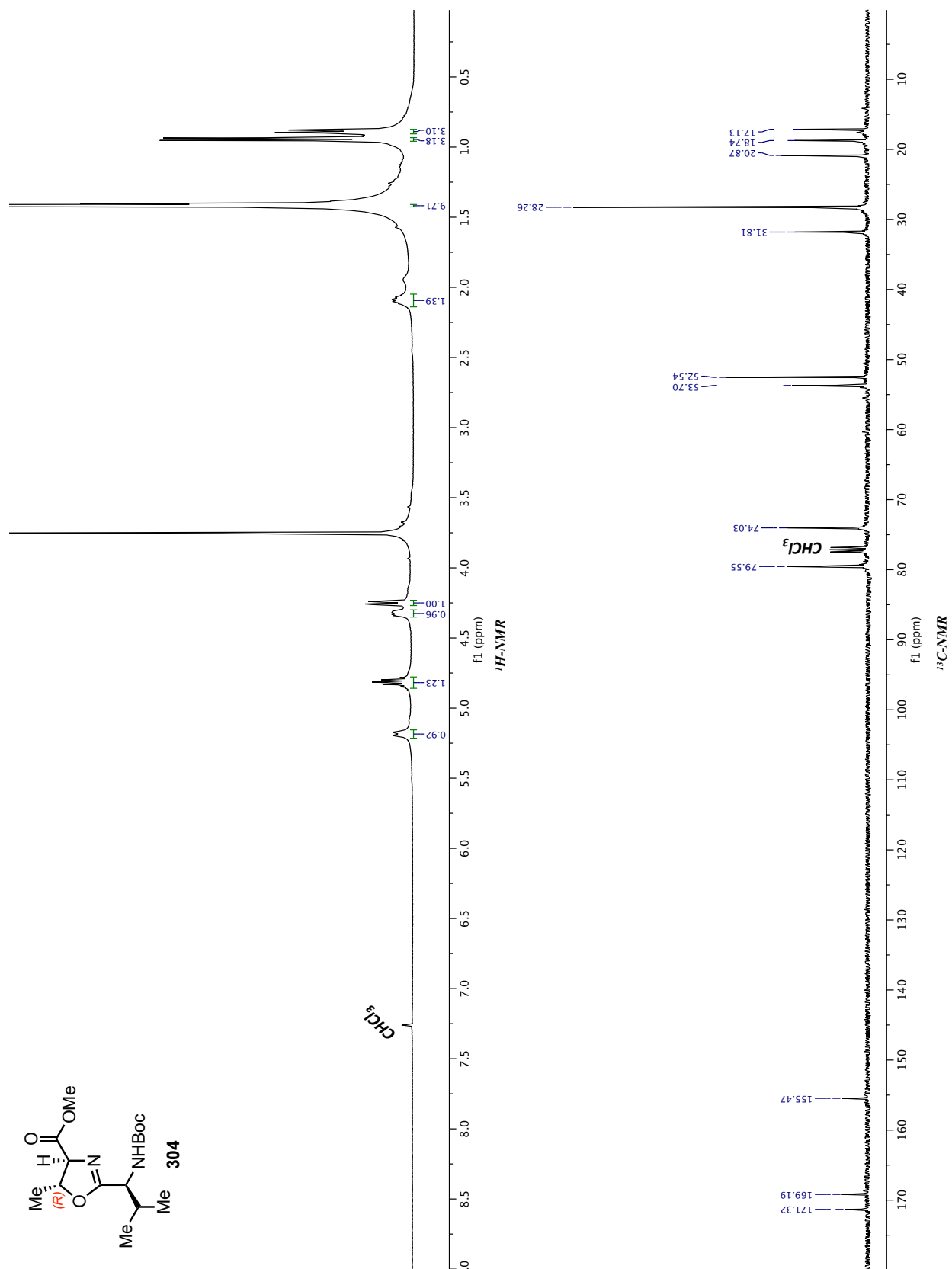
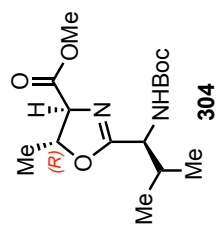


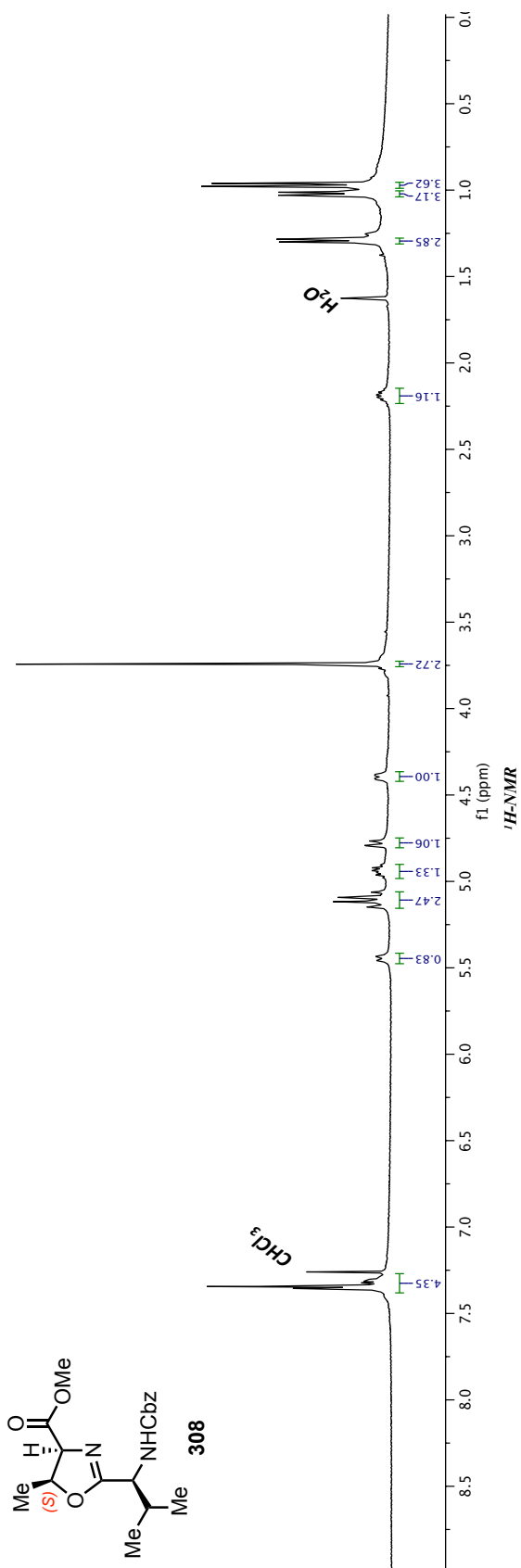
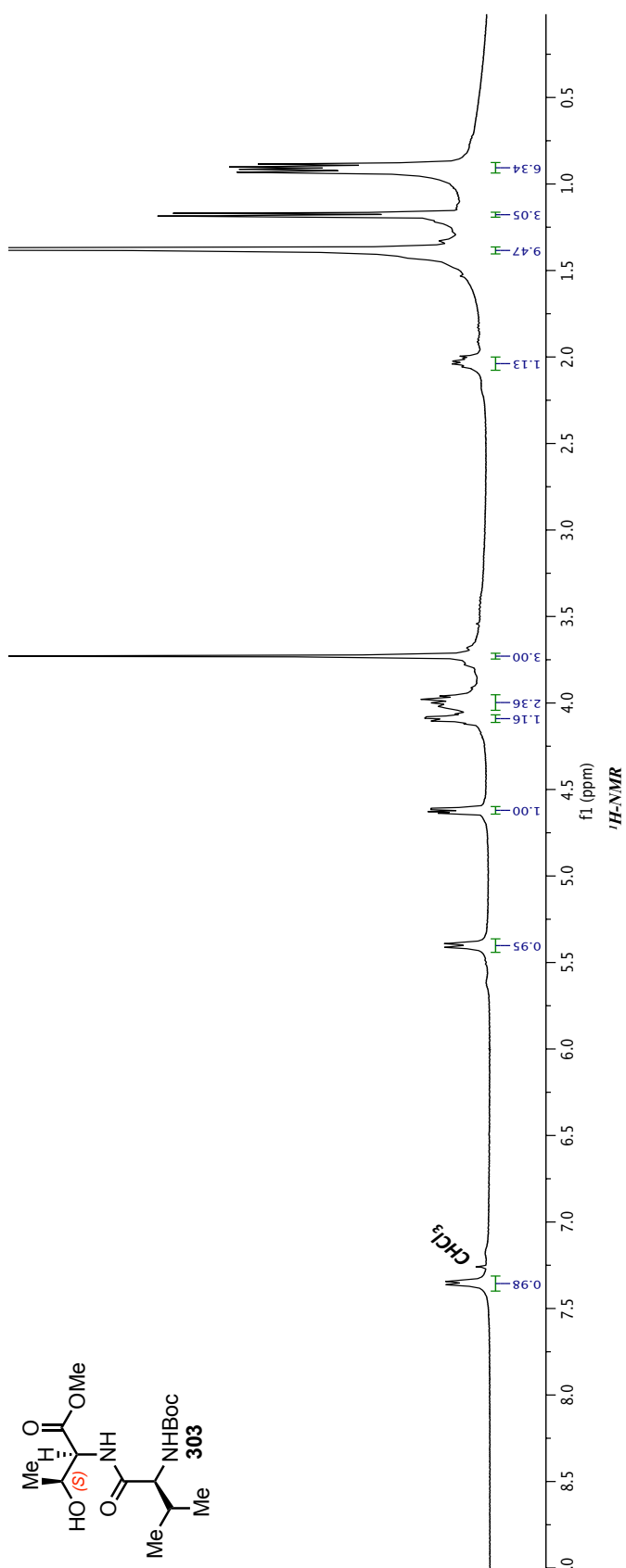


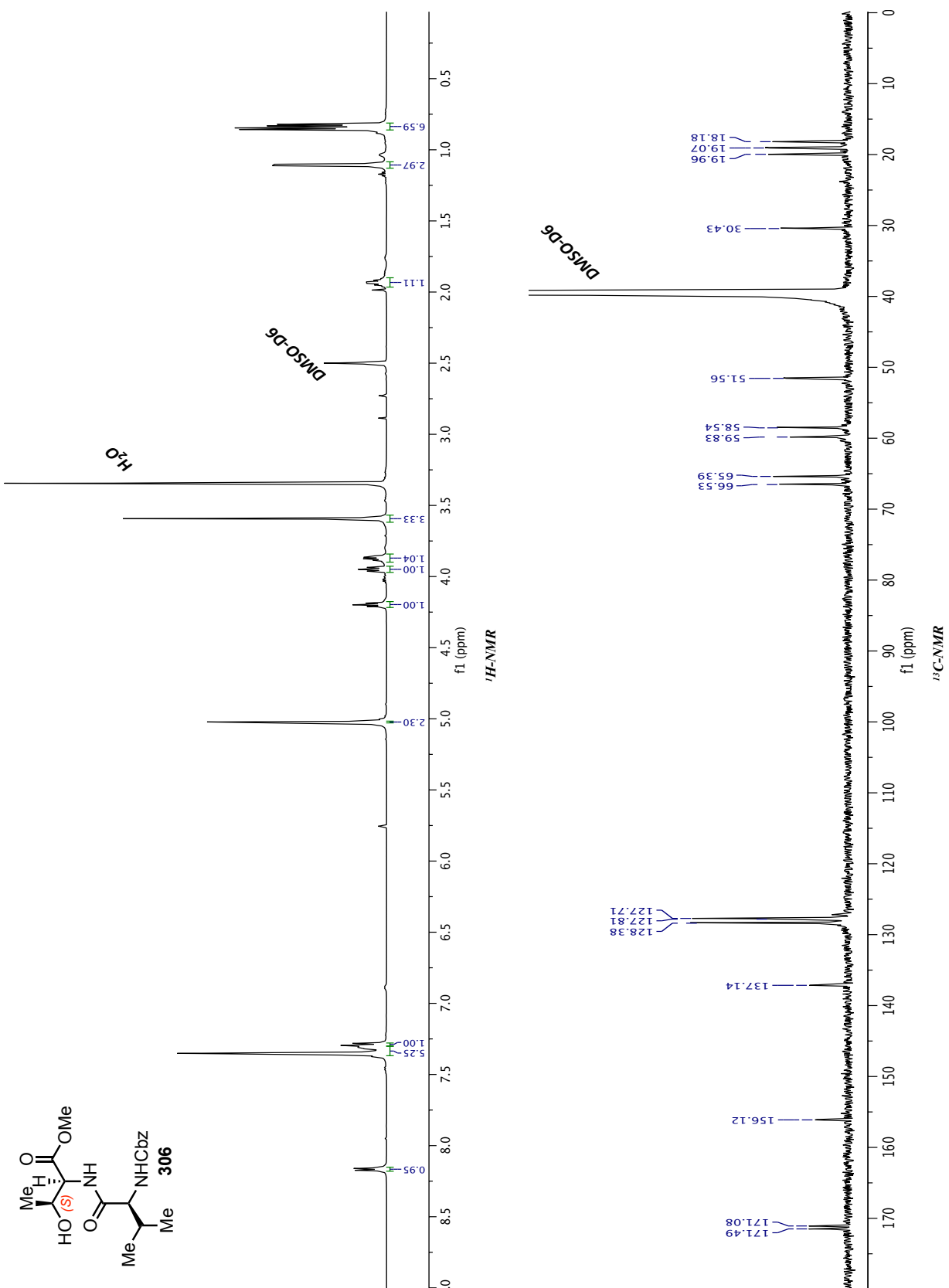


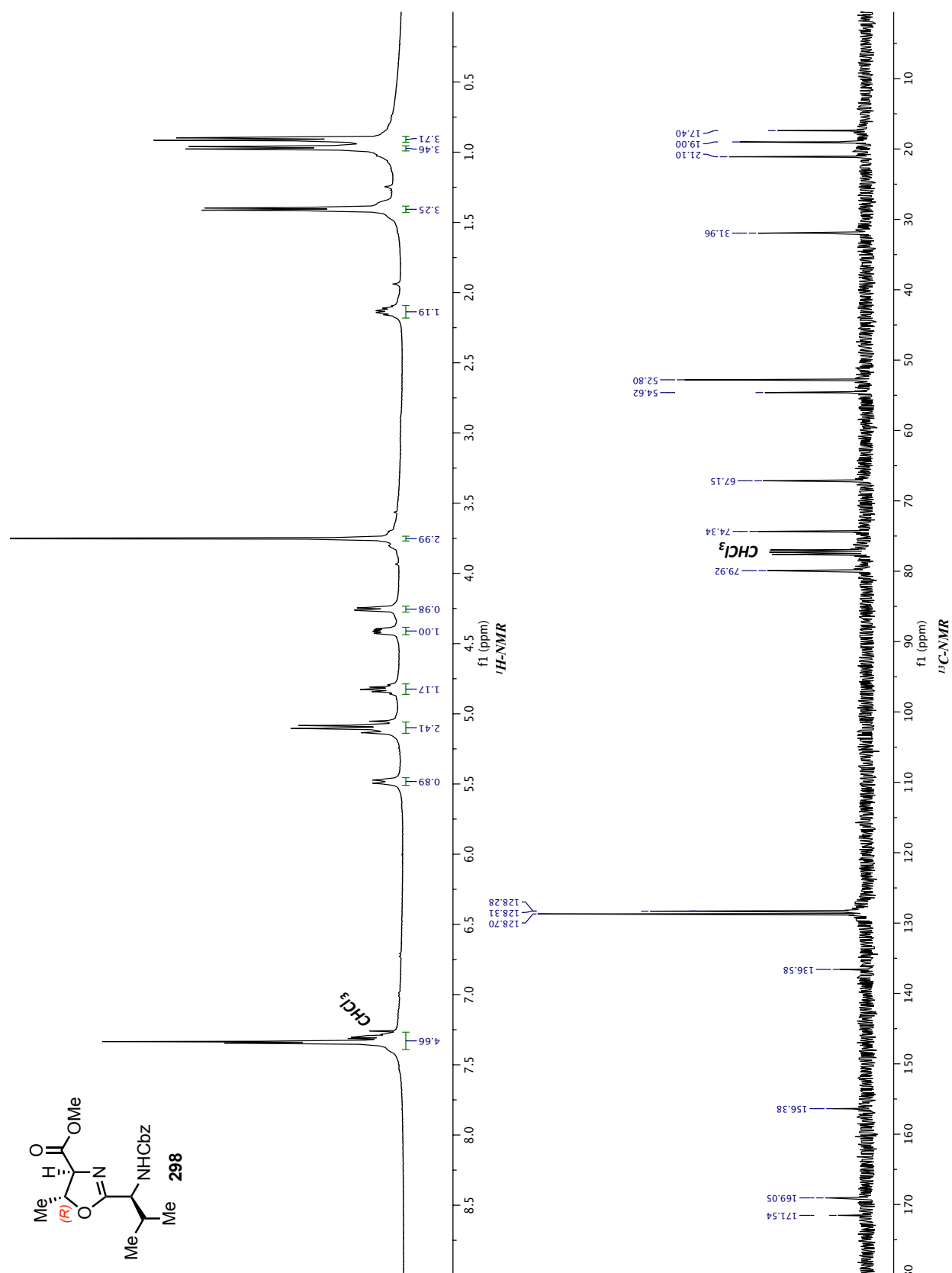


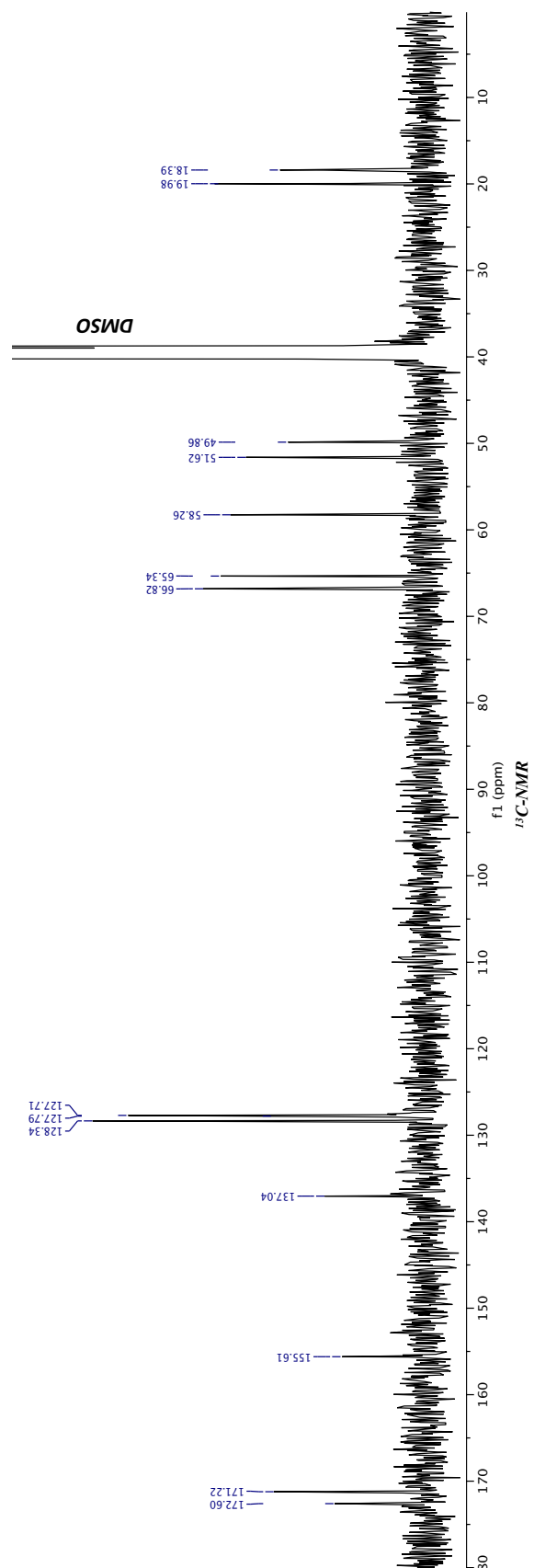
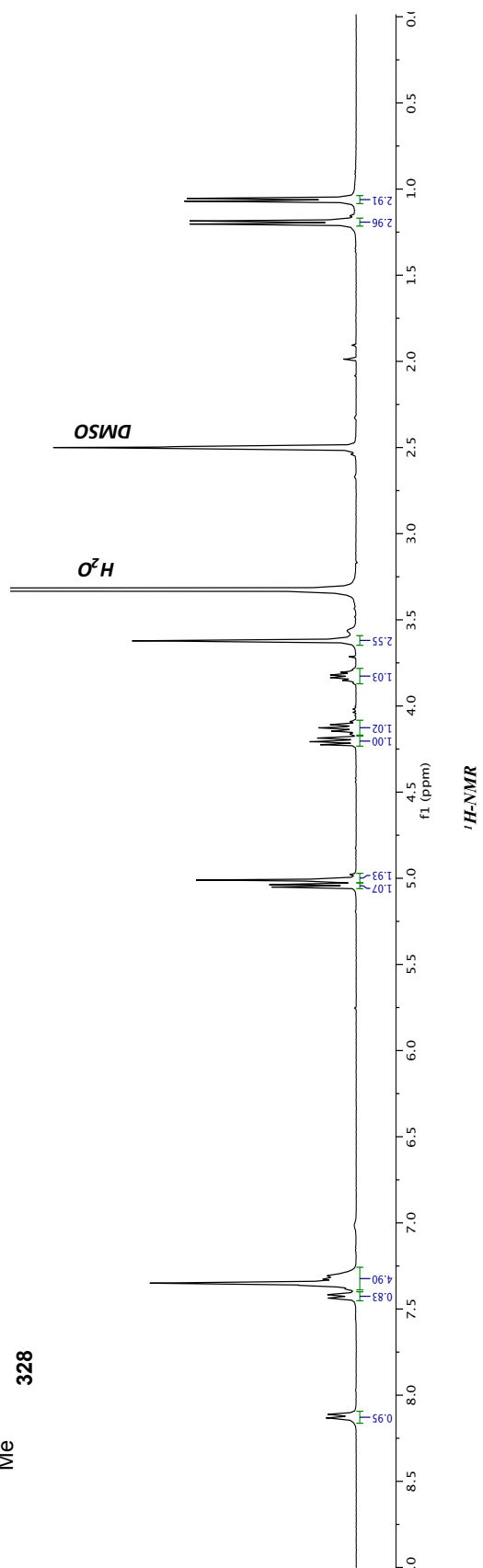
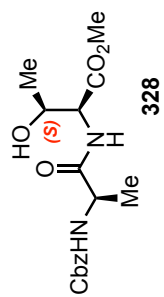


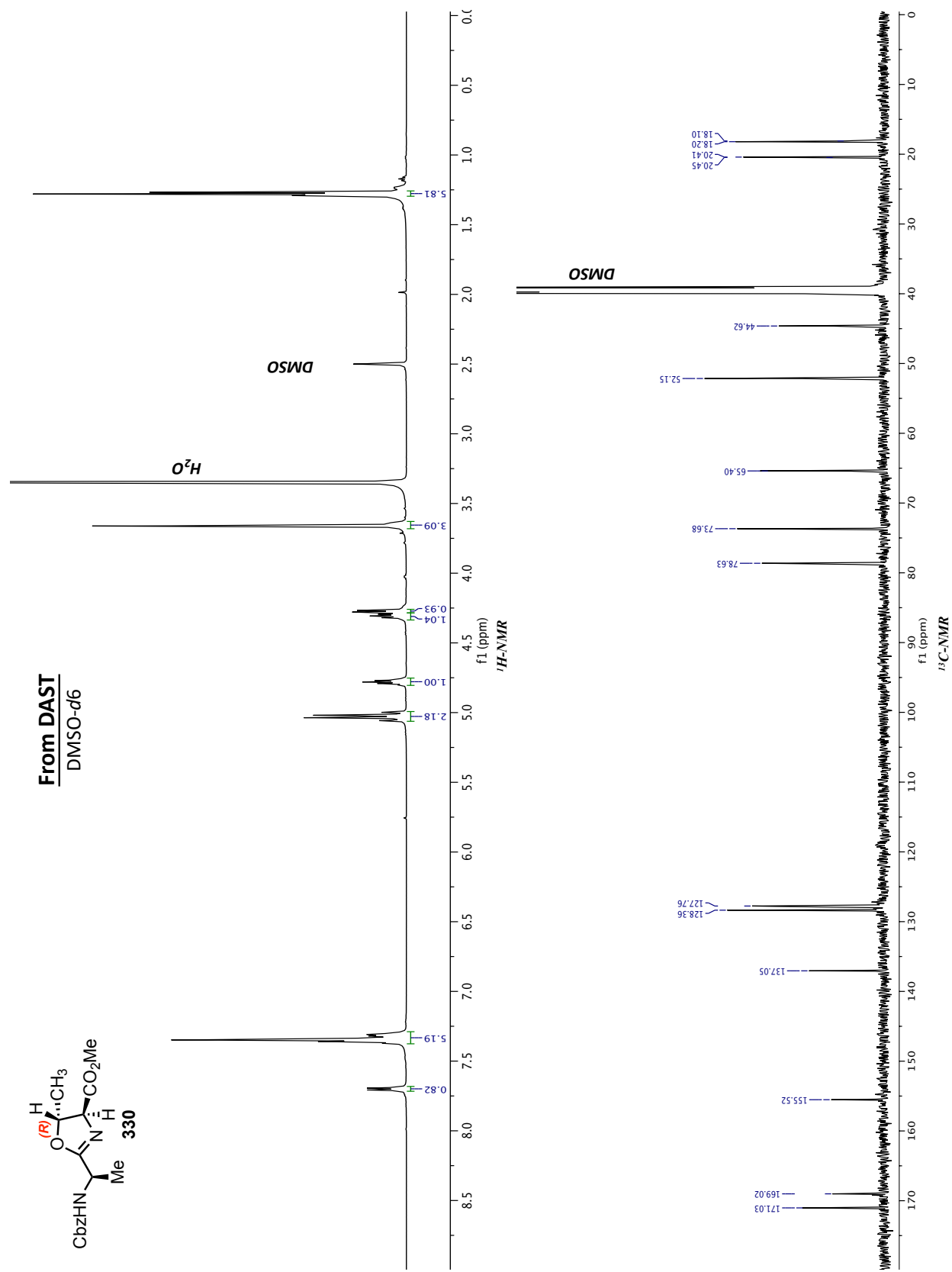


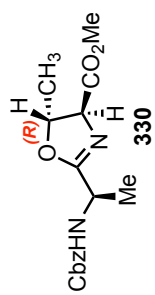






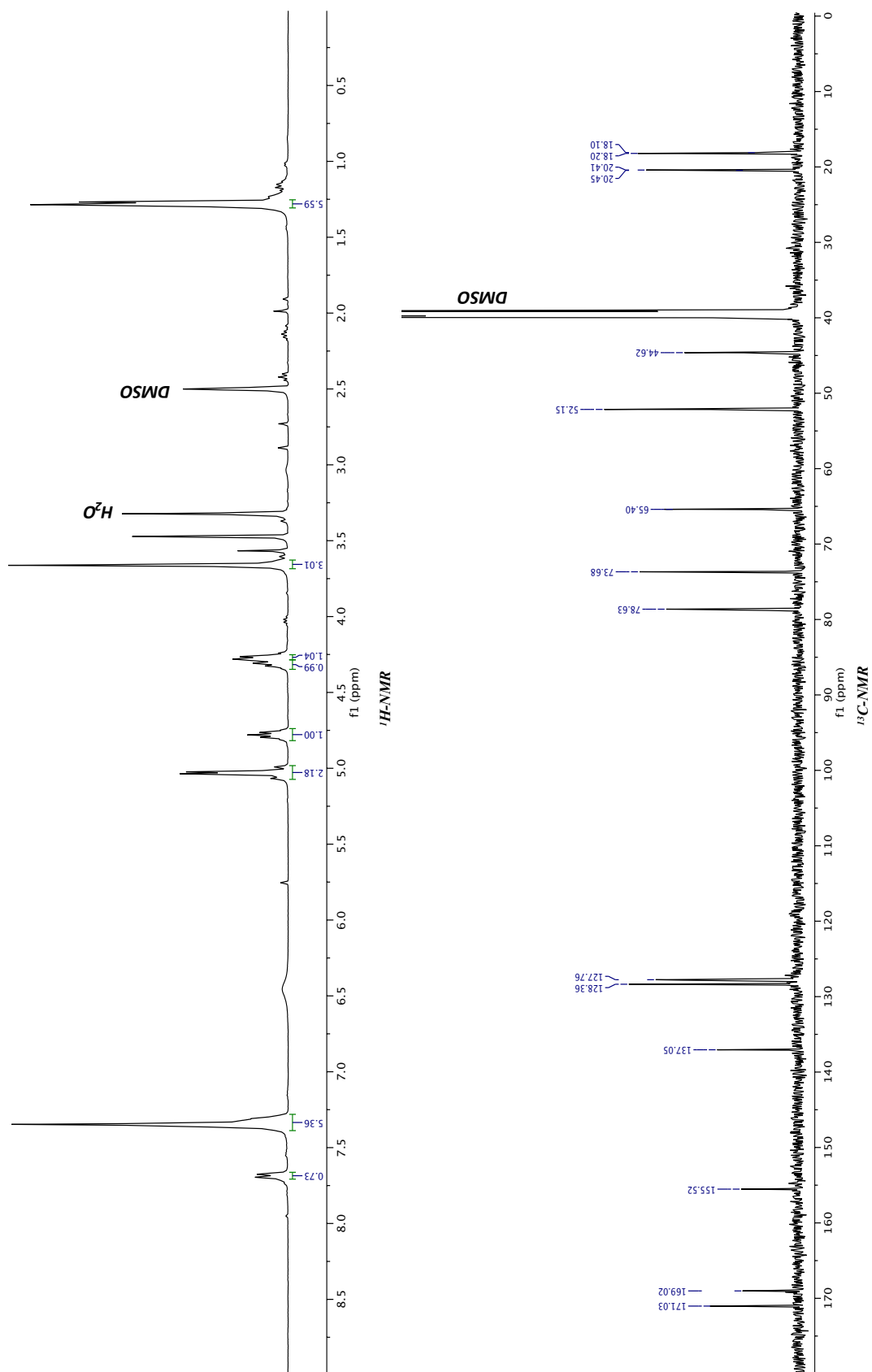


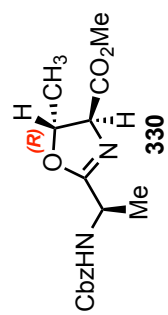




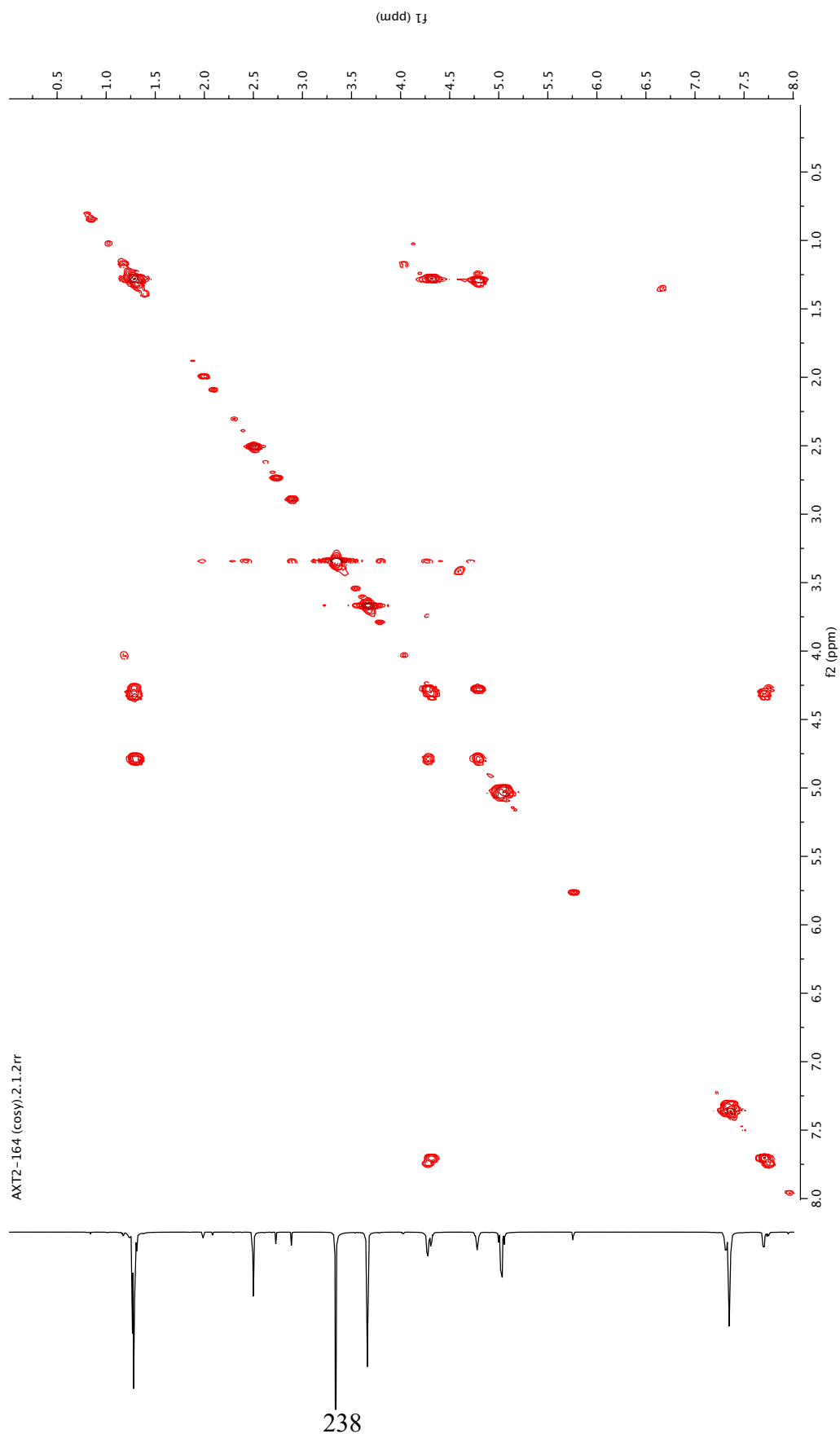
From the Burgess reagent

DMSO-*d*₆

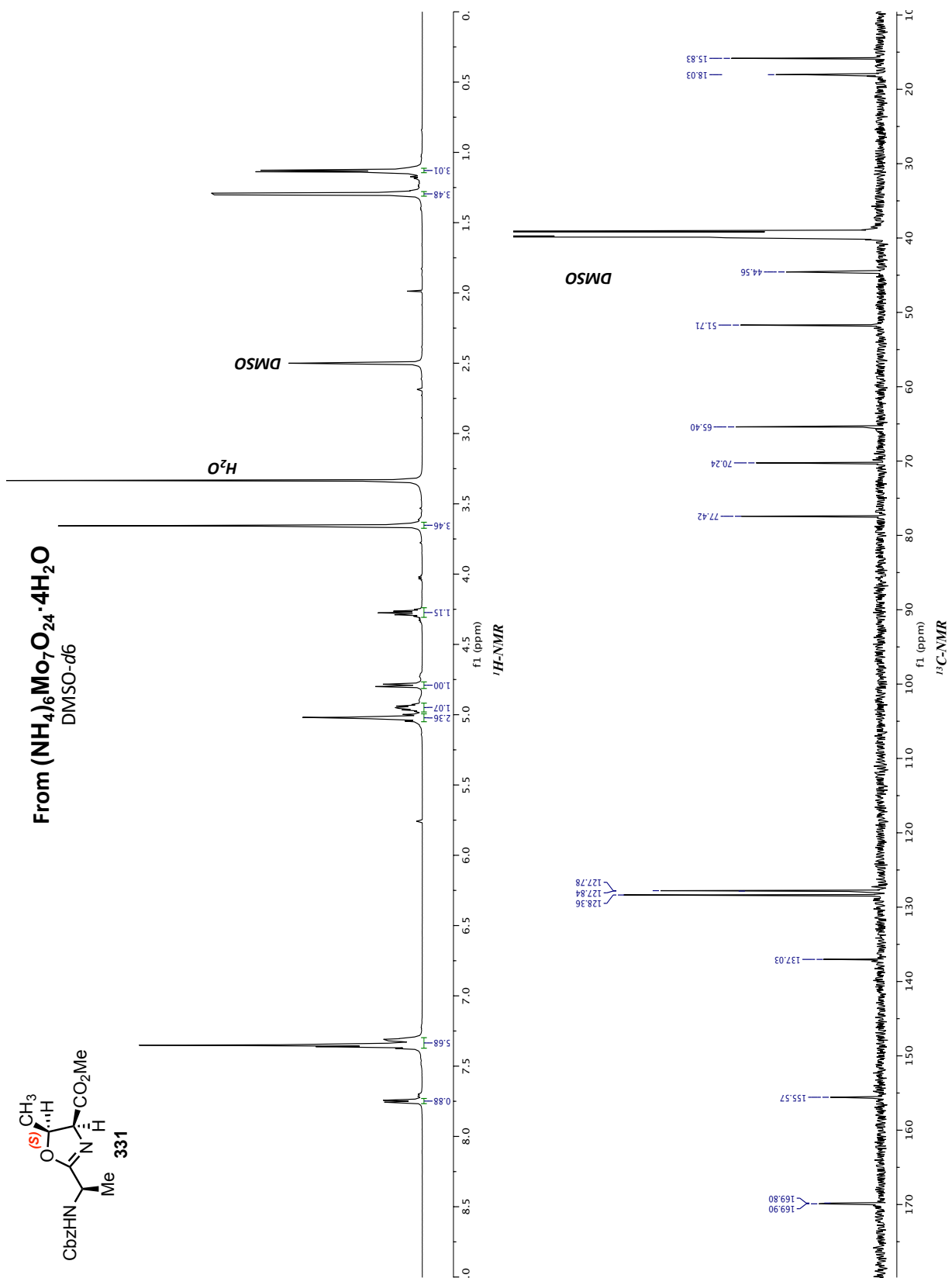


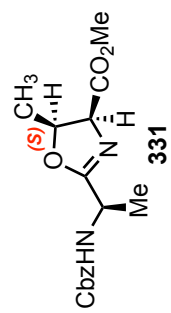


AXT2-164 (cosy).2.1.2rr

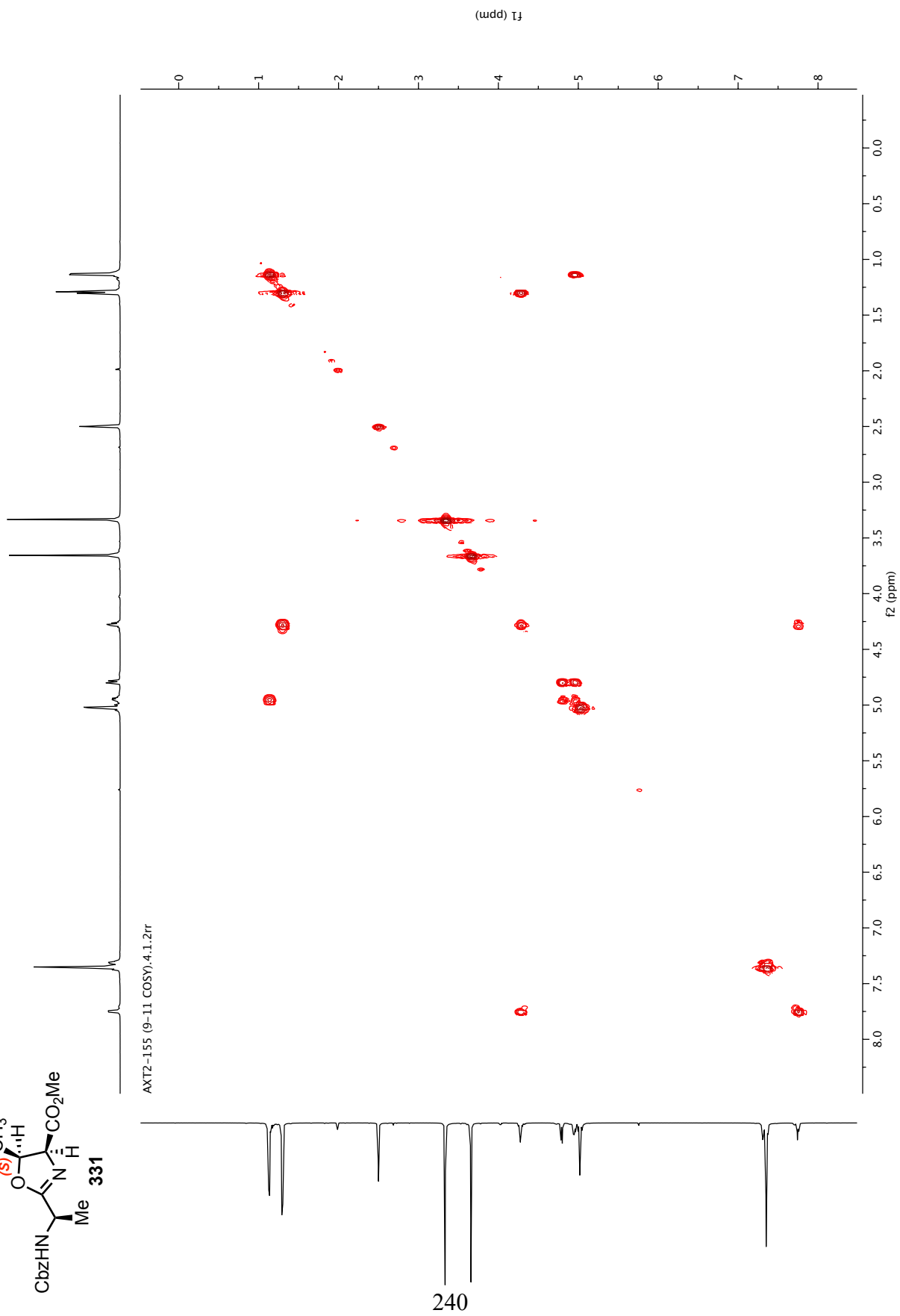


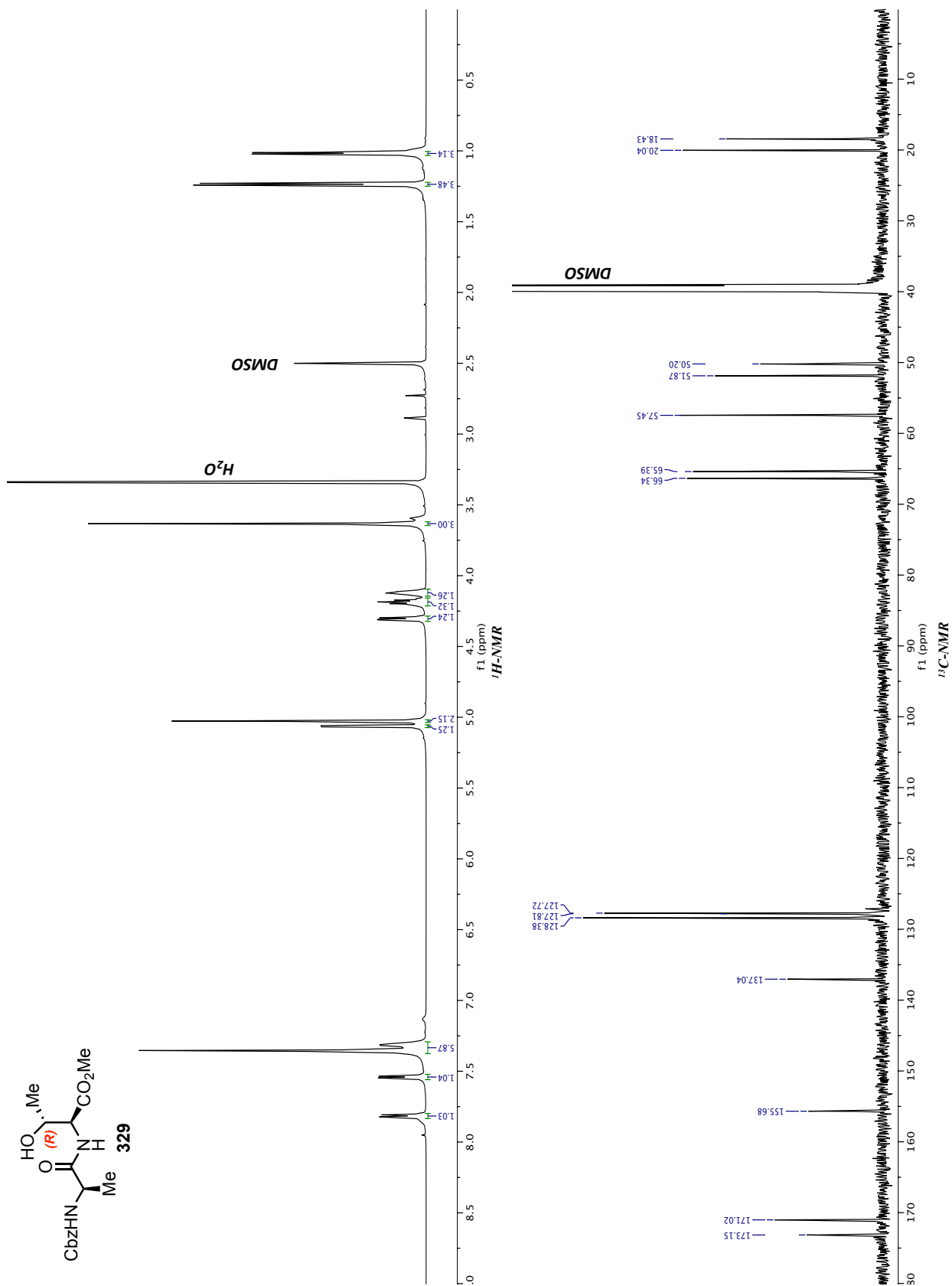
238

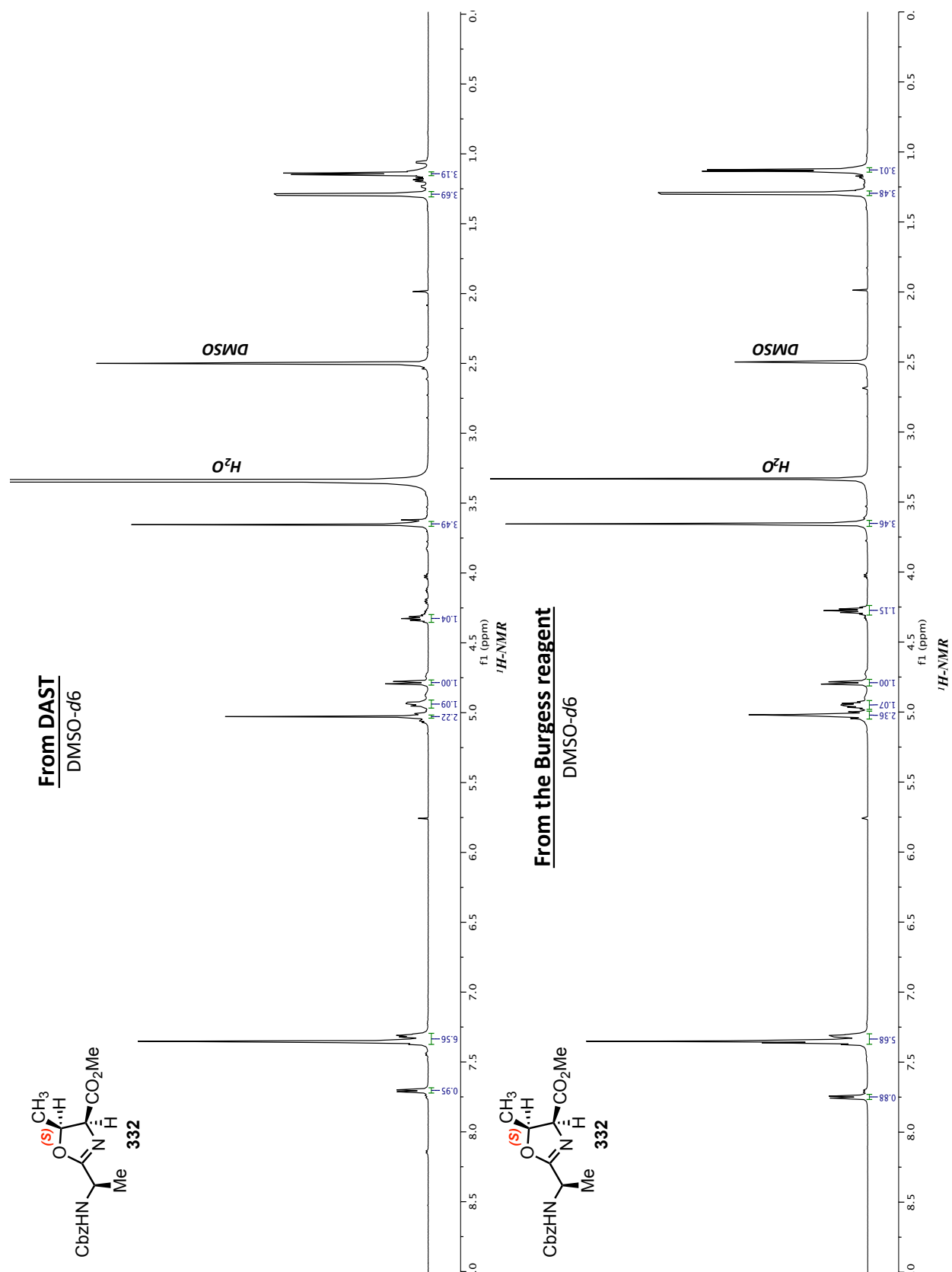


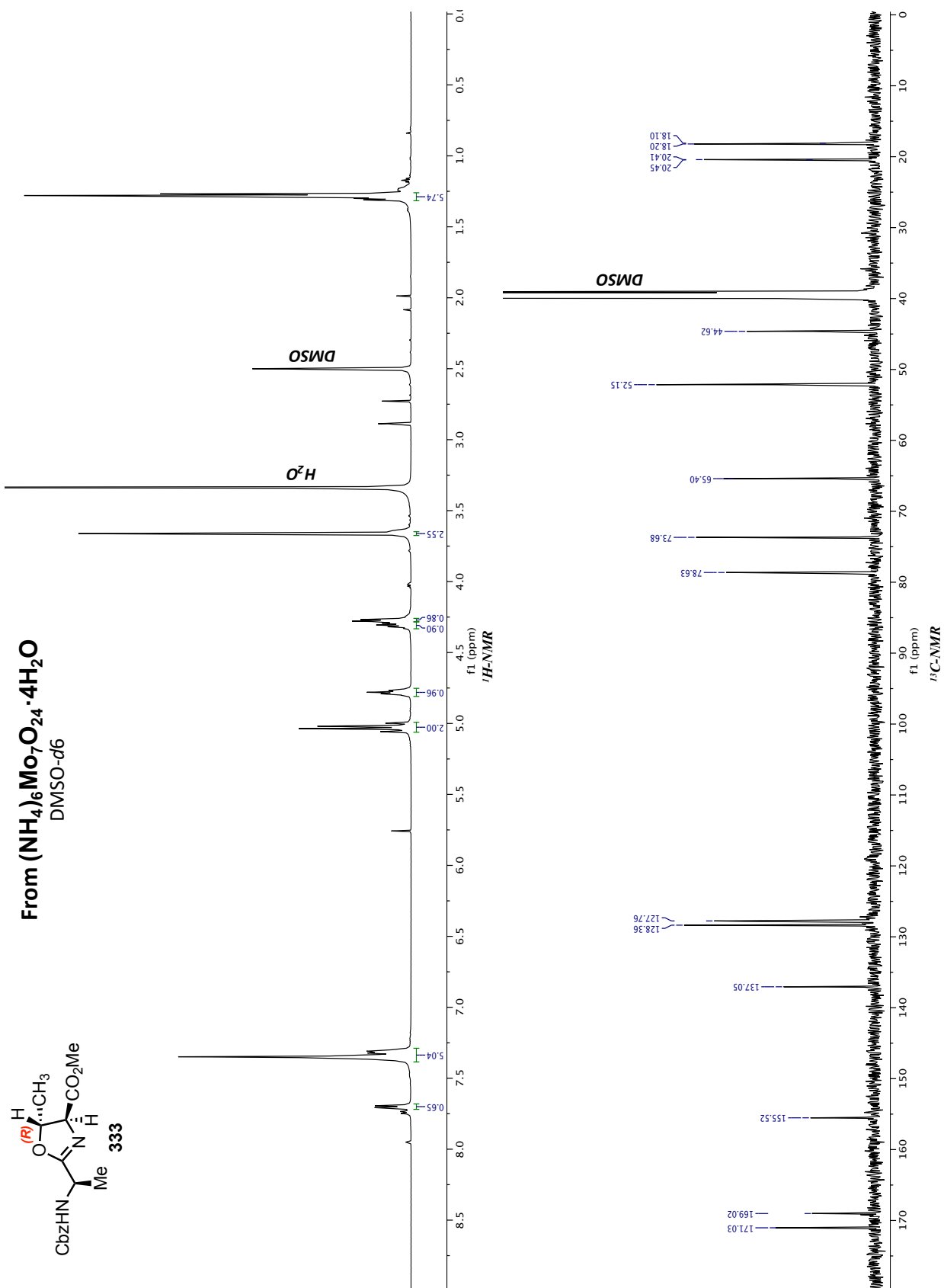


AXT2-155 (9-11 COSY). 4.1.2tr



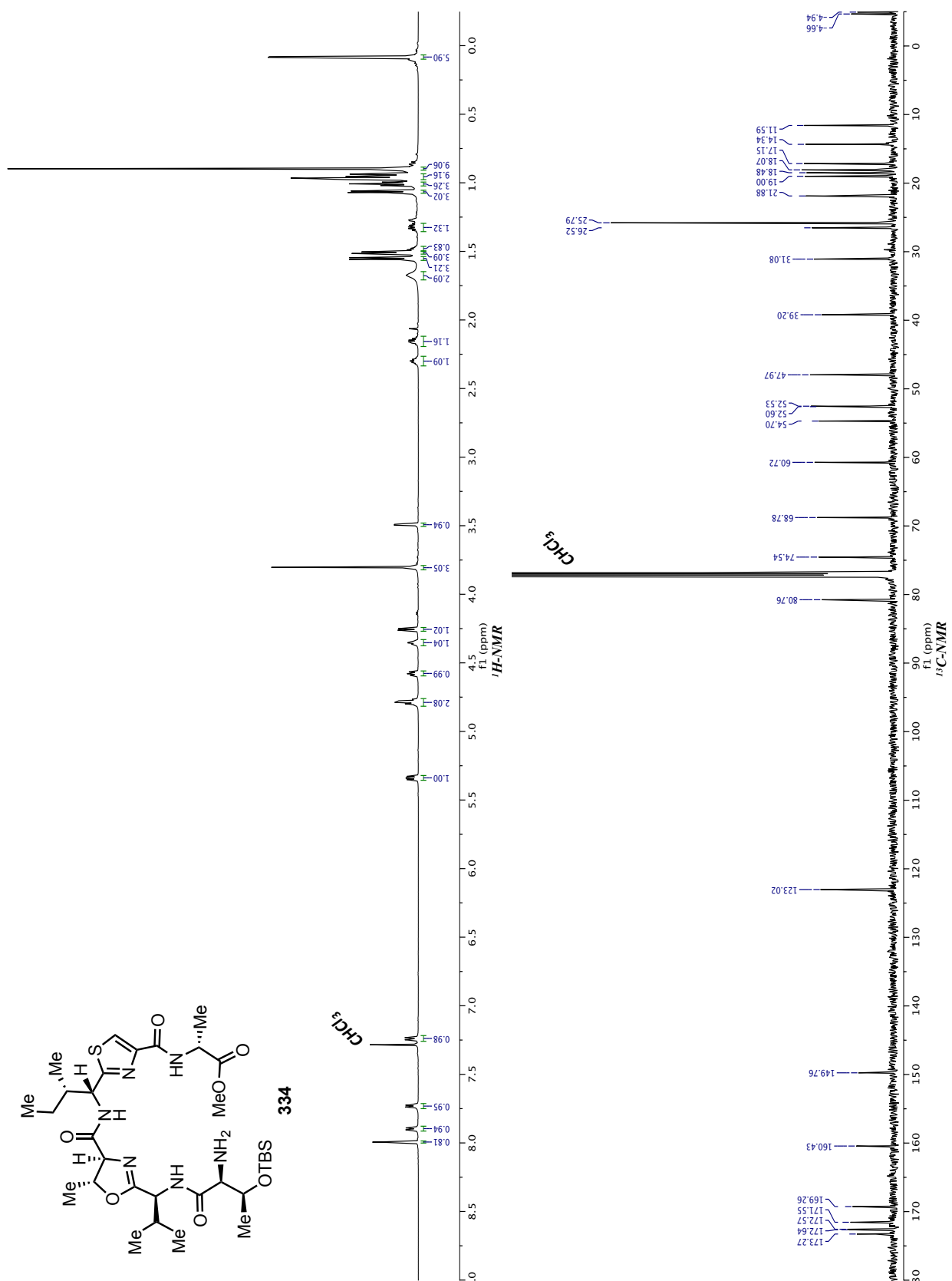








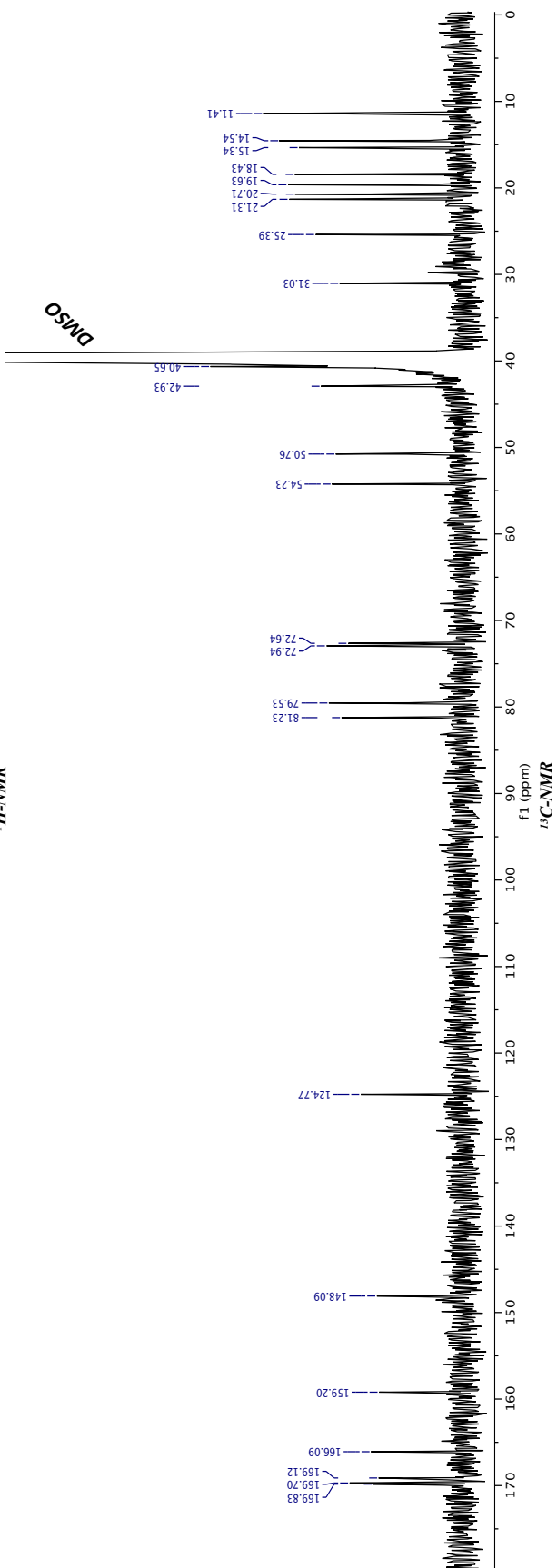
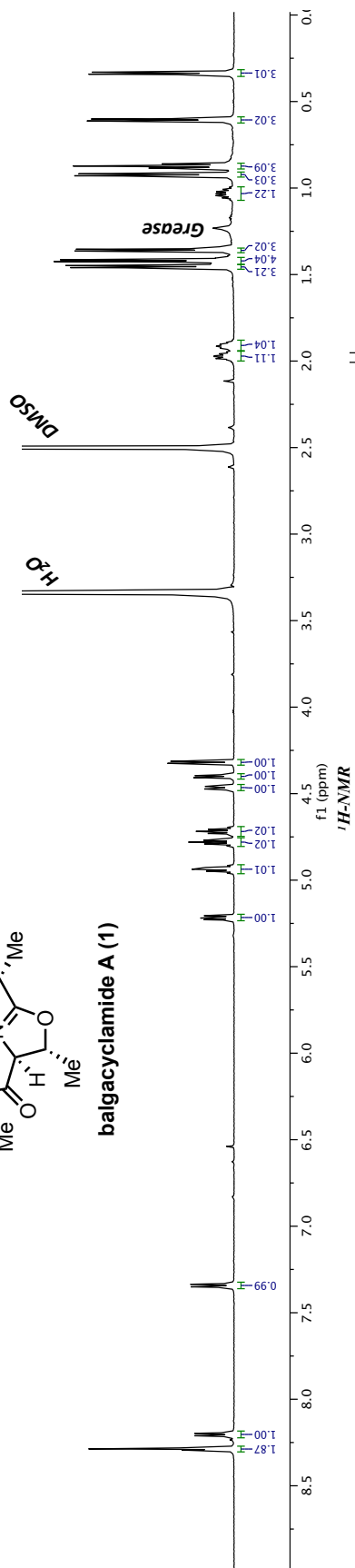
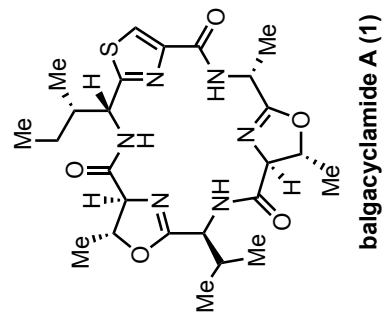


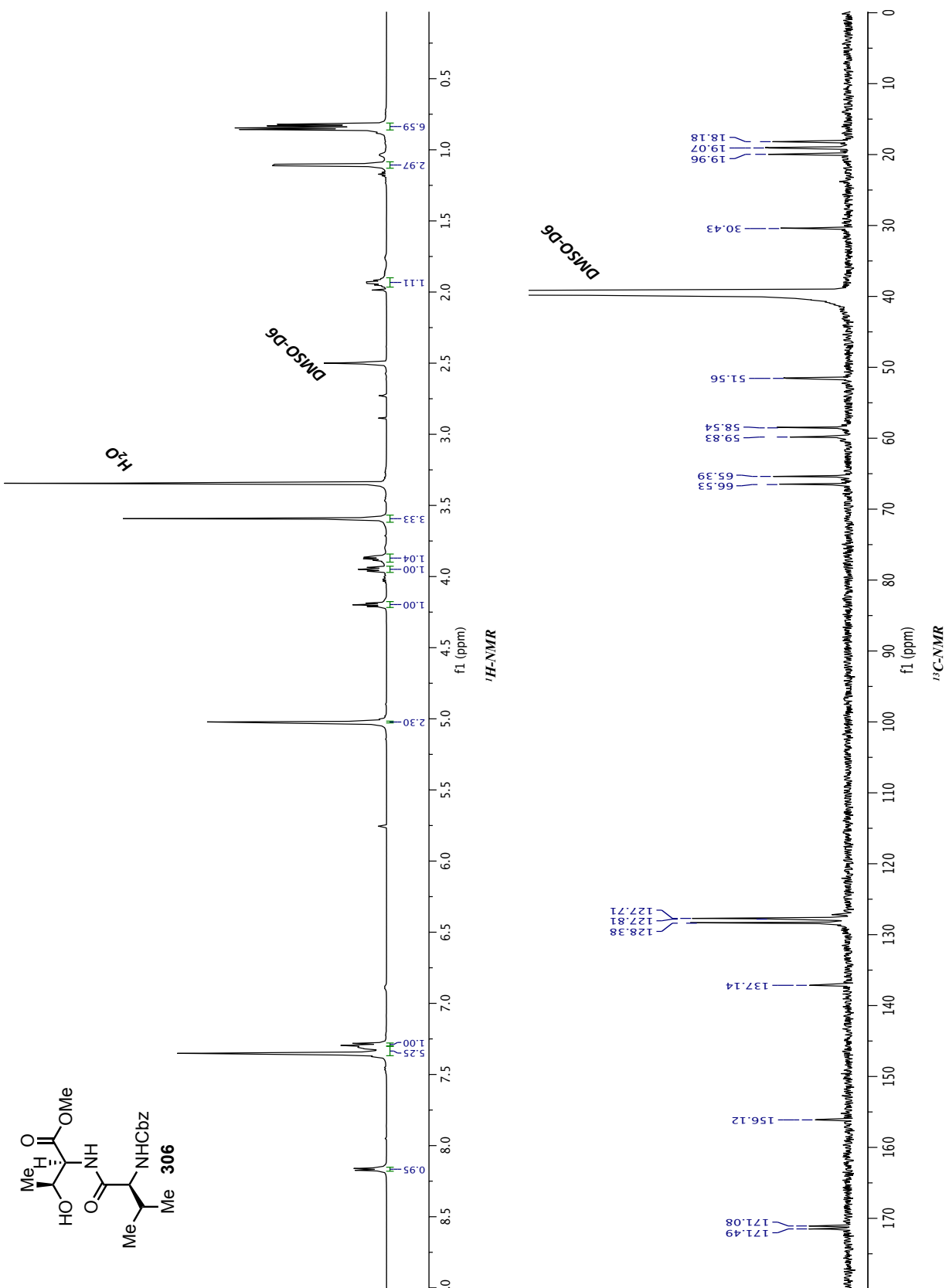


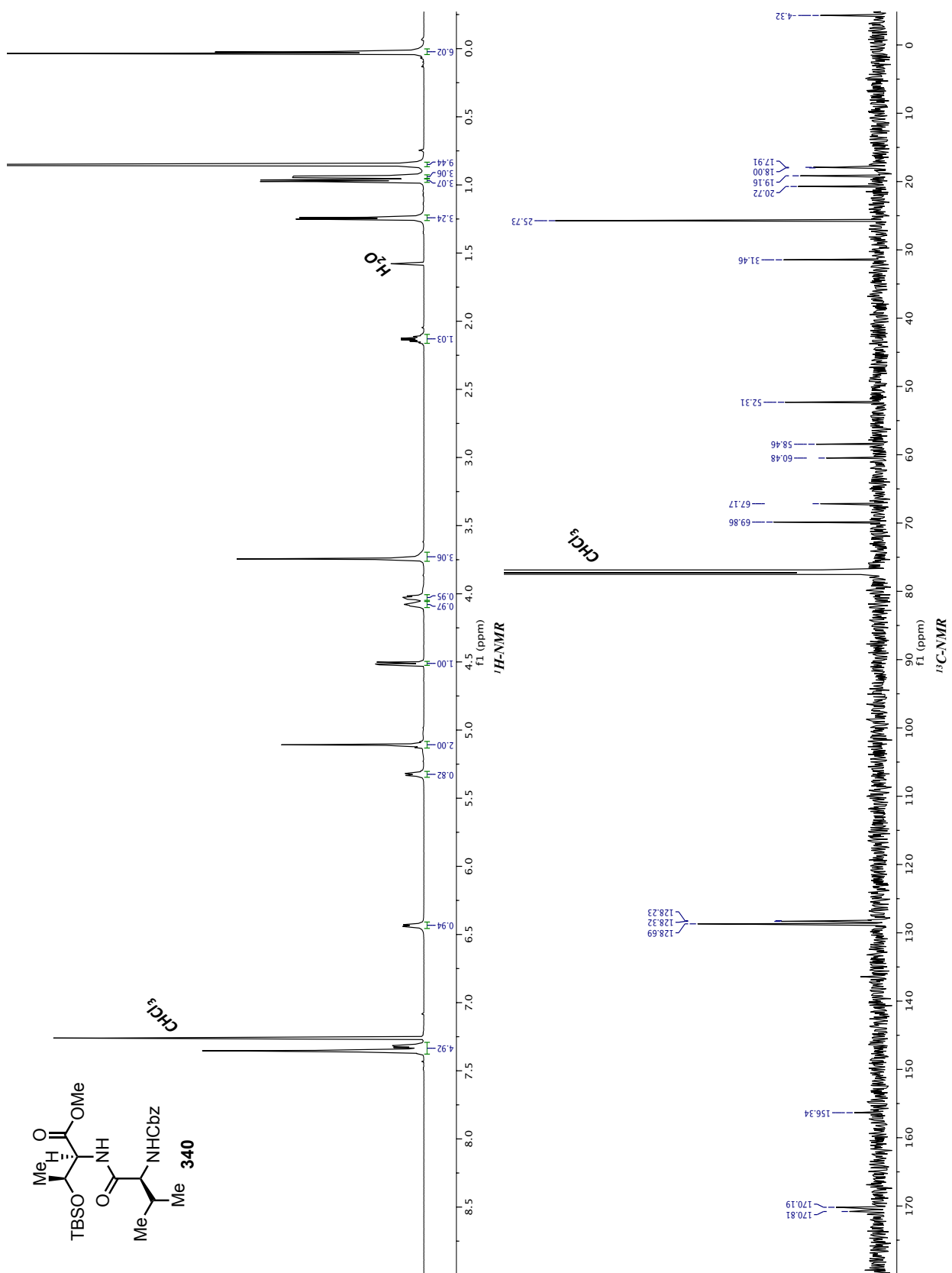






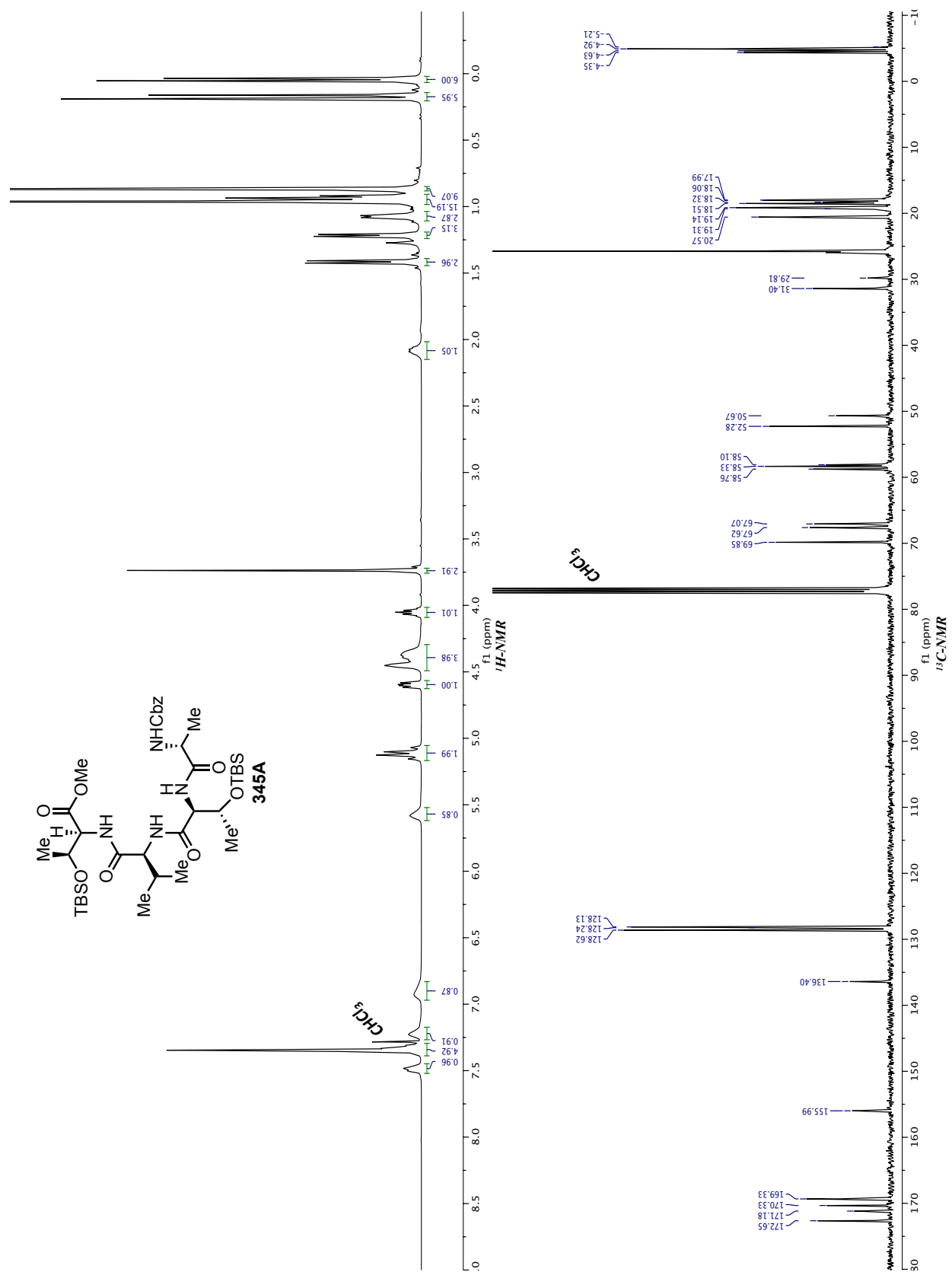




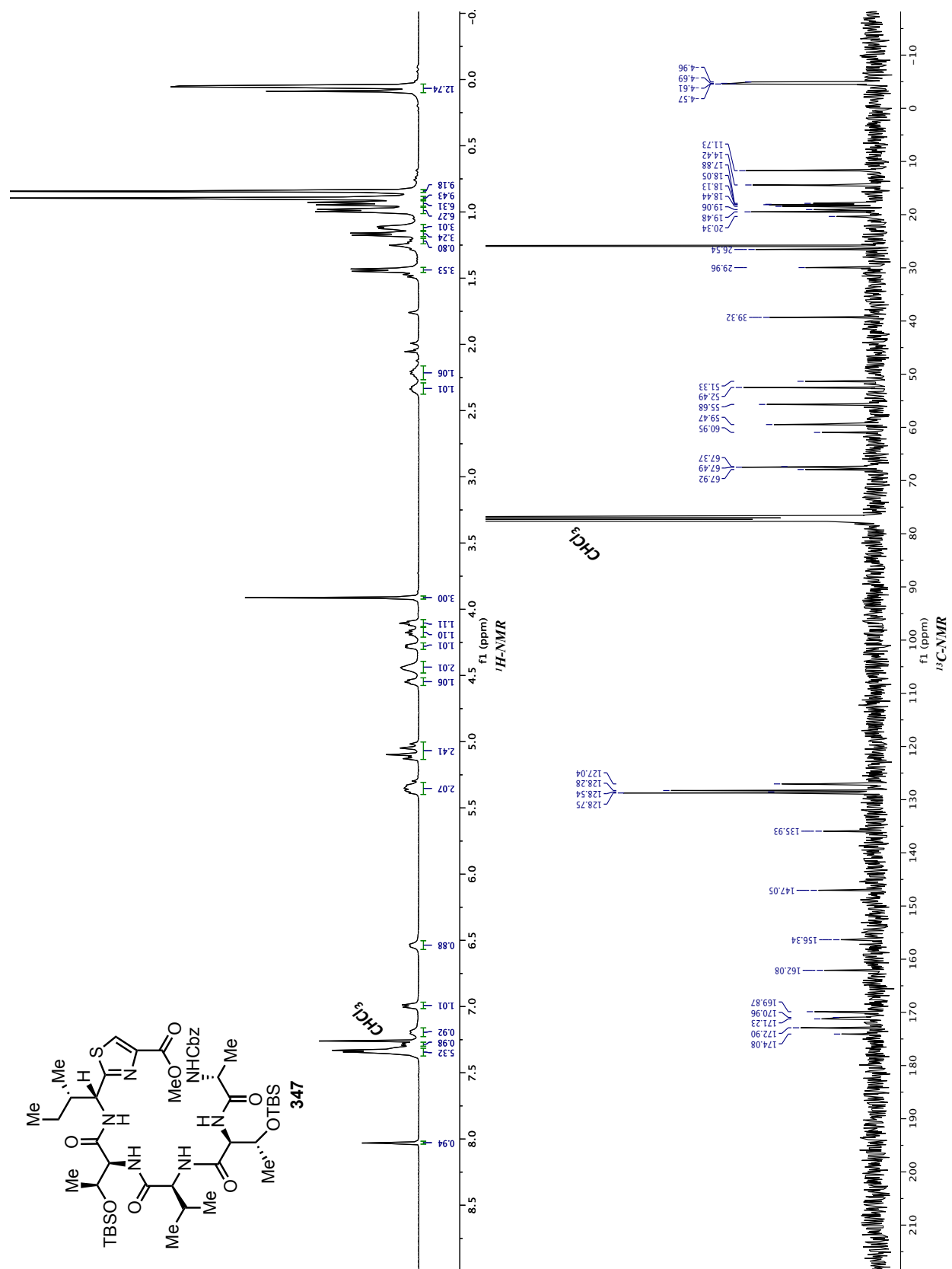


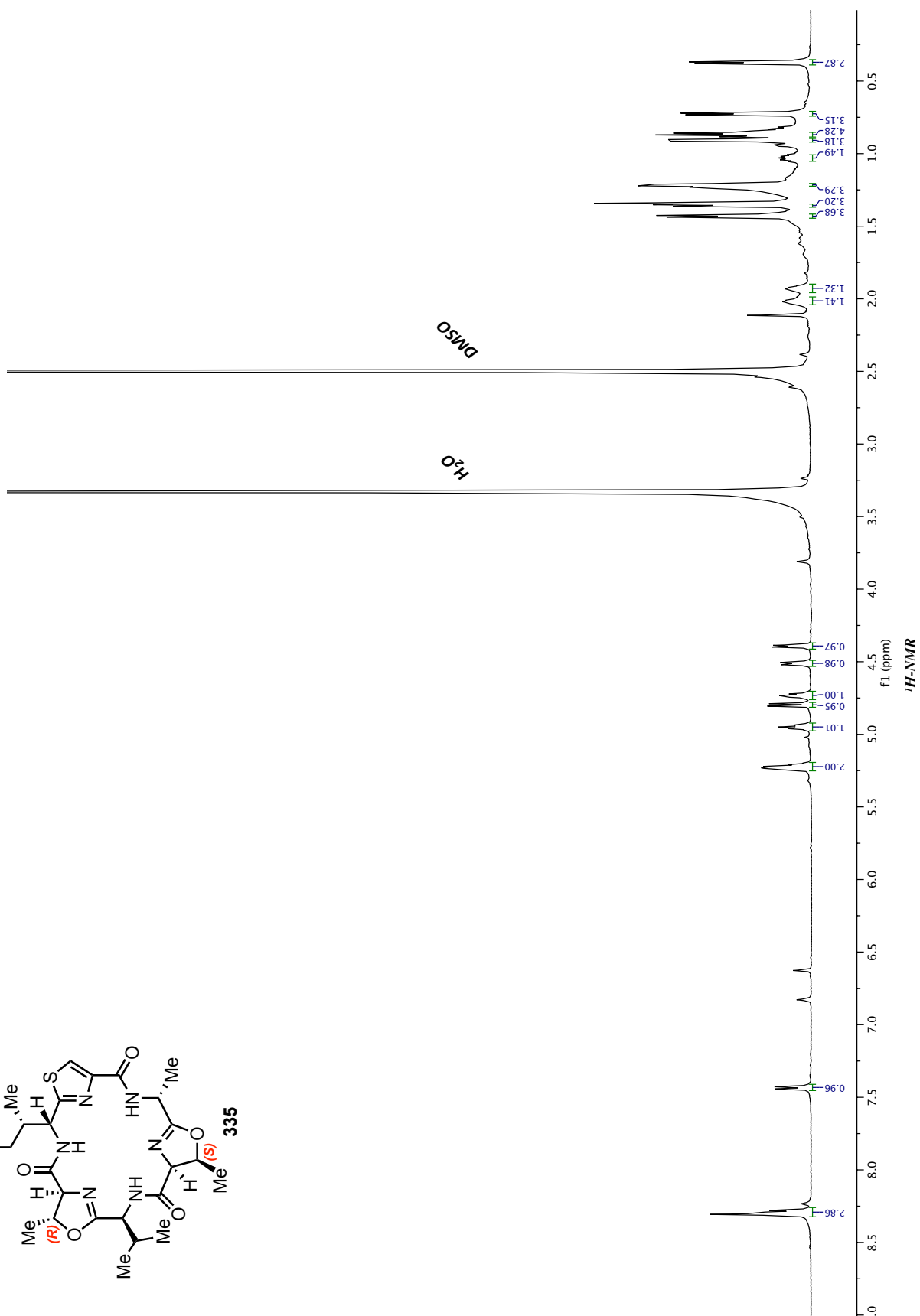
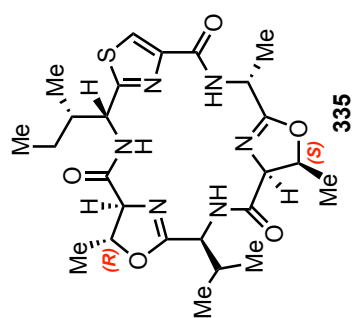


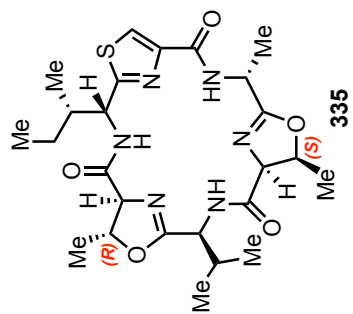






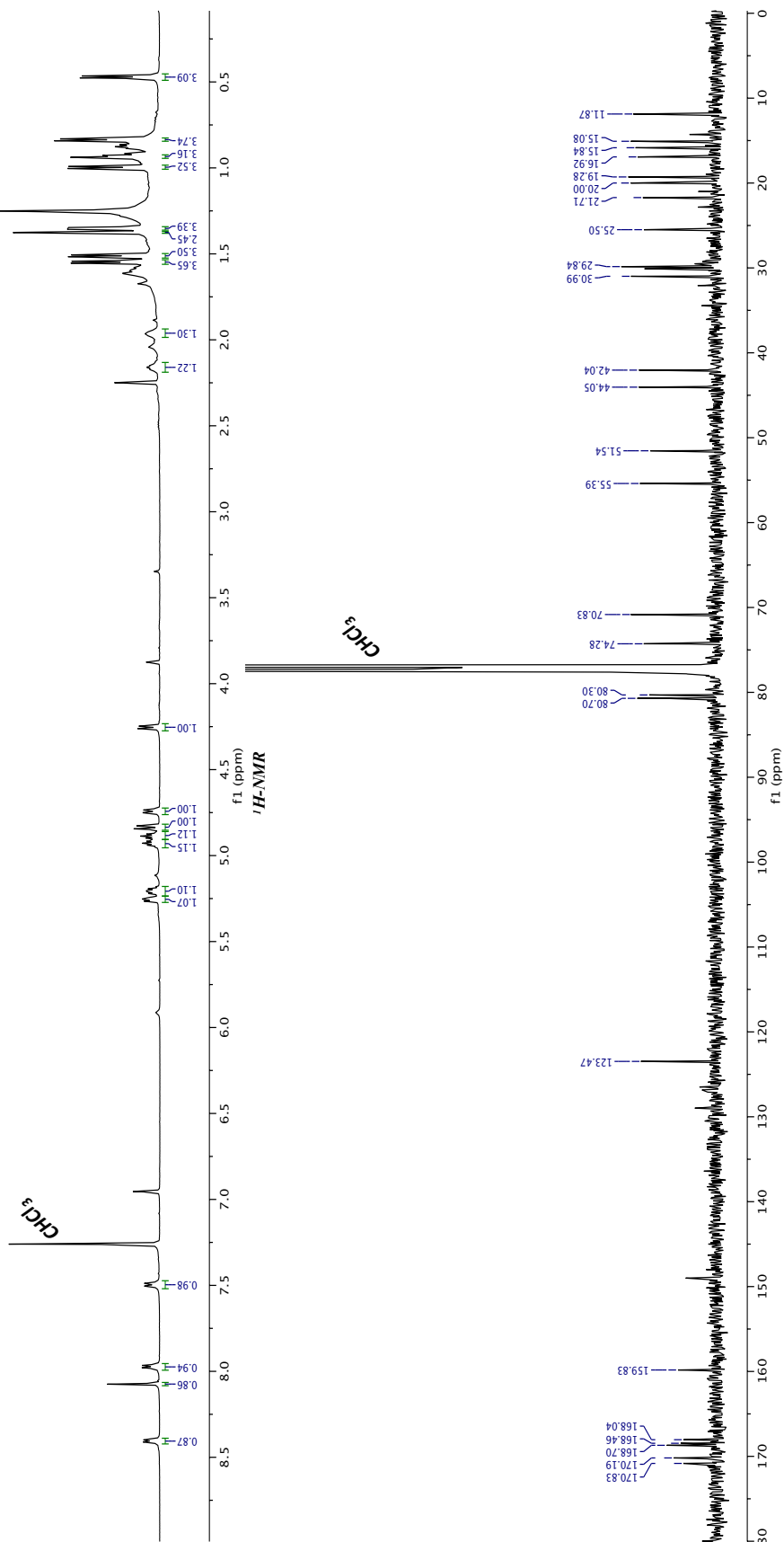


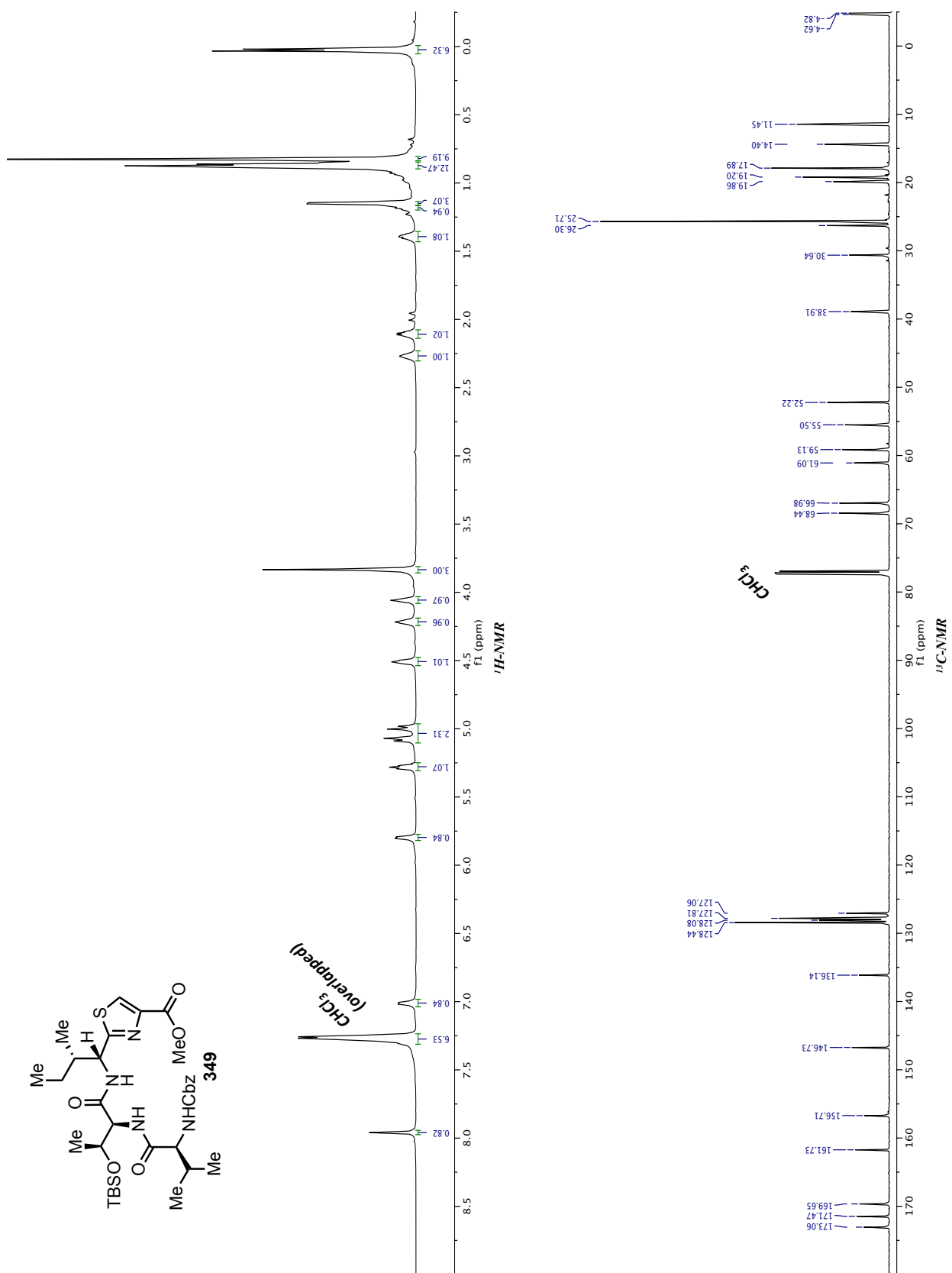




Grease

CHCl₃

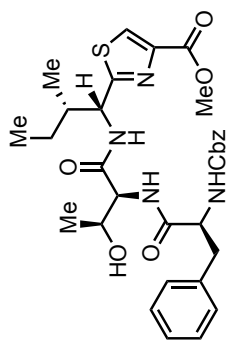




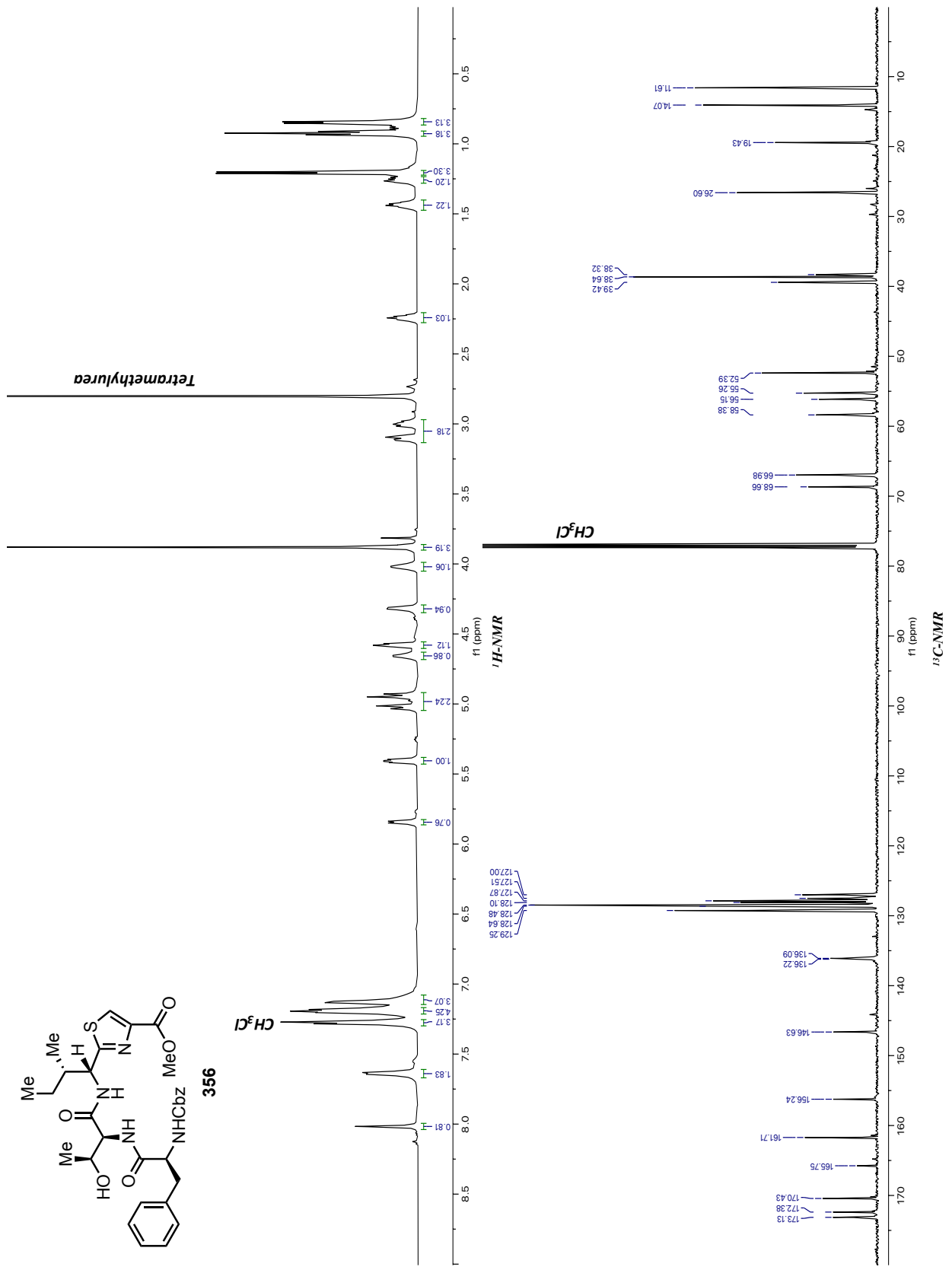


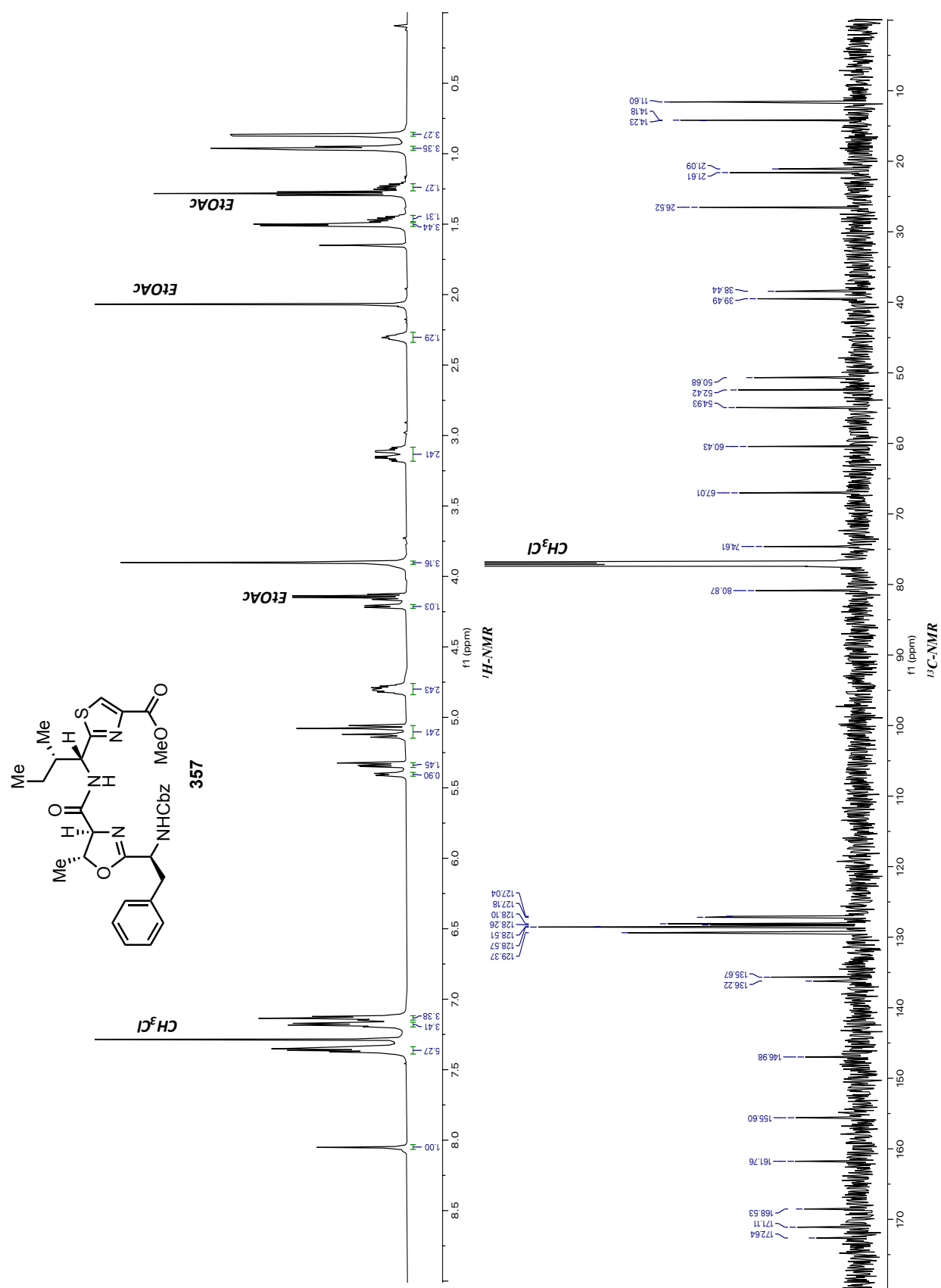






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Appendix A - Torres Hernández CV

Arnaldo X. Torres Hernández

1212 Mid-Campus Dr. North, 341 CBC Building, Chemistry Department, Kansas State University
Manhattan, KS 66502 | Email: axtorres@ksu.edu

PROFILE

PhD Candidate in Organic Chemistry at Kansas State University with nine years of graduate and undergraduate research and four years of teaching experience, including mentoring undergraduate researchers. Expertise in total synthesis of macrocyclic peptides, in which multiple synthetic methodologies and strategies to access complex cores are applied. Skilled in manuscript writing and developing research proposals to secure funding through multiple research programs. Looking for a postdoc position to expand my knowledge in catalytic organic synthesis or chemistry education with an emphasis in professional development in qualitative and quantitative research methodologies. As a long-term goal, I'm interested in integrating sign language and/or gestures in organic chemistry to improve learning outcomes in Deaf and Hard of Hearing students.

EDUCATION

PhD in Organic Chemistry, Kansas State University | GPA 3.89/4.00

Advisor: Dr. Ryan J. Rafferty

Research: *First total synthesis of cyanobactins balgacyclamide A & B: Configuration reassignment analyses via β -hydroxy amides dehydrative cyclization conditions*

2019 – Present
Graduation
May 2025

Side research project: *Redesigning antileukemic drug 6-thiopurine: Decreasing toxic side effects while maintaining efficacy*

B.Sc. in Chemistry & Minor in Criminology, Pontifical Catholic University of Puerto Rico | GPA 3.90/4.00, Advisor: Dr. Batista Parra

2014 - 2019

Project: *Synthesis of catechol and hydroquinone analogs for self-assembled monolayers application*

RESEARCH EXPERIENCE

Pediatric Oncology Education (POE) Program Chemical Biology and Therapeutics Department, St. Jude Children's Research Hospital, Memphis, TN
Advisor: Dr. Daniel Blair

Summer 2024

Research: *Continuous collective analysis of chemical reactions*

Puerto Rico Louis Stokes Alliance for Minority Participation (PRLSAMP)
Department of Chemistry, University of PR, RP and PCUPR Ponce
Advisor: Dr. Batista Parra

2016 - 2019

Research: *Synthesis of catechol and hydroquinone analogs for their future applications in self assembled monolayers*

Research Experience for Undergraduates (REU) Chemistry Program
 Department of Chemistry, KSU, Manhattan, KS
 Advisor: Dr. Ryan J. Rafferty
Research: *Investigations into the re-design of the antileukemic drug 6-thiopurine: Analogs synthesis and biological evaluation* **Summer 2018**

Summer Undergraduate Research Opportunity Program (SUROP)
 Department of Chemistry, KSU, Manhattan, KS
 Advisor: Dr. Ryan J. Rafferty
Research: *Synthetic efforts towards balgacyclamide A for biological investigation into selective cellular delivery* **Summer 2017**

APPOINTMENTS & POSITIONS

Graduate Research Assistance
 Department of Chemistry, Kansas State University, Manhattan, KS **2022 - Present**

Graduate Research Assistance
 Chemical Biology and Therapeutics Department, St. Jude Children's Research Hospital, Memphis, TN **Summer 2024**

Graduate Teaching Assistance
 Department of Chemistry, Kansas State University, Manhattan, KS **2019 - 2022**

CURRENT SUPPORT INFORMATION

Ford Foundation Predoctoral Fellowship (NASEMFordFellow)
 Graduate Research Assistance: **\$81,000** **2022 - Present**

GRANTS UNDER REVIEW

PI/Co-PI

Torres-Hernandez, A. X.; Goudreau Collison, C.; Michel, L. (Submitted 12/03/2024). Sign Language Incorporation in Chemistry Education: Assessing the Learning Experience Impact of Signs for Multilingual Students. NSF. (**\$170,000**). Roles: **Torres-Hernandez**, PI. Goudreau Collison, Sponsoring Researcher, & Michel, advisor.

PUBLICATIONS

- Hu, M., Yang, L.; Twarog, N.; Ochoada, J.; Li, Y.; Vrettos, E. I.; **Torres-Hernandez, A. X.**; Martinez, J. B.; Bhatia, J.; Young, B. M.; Price, J.; McGowan, K.; Nguyen, T. H.; Shi, Z.; Anyanwu, M.; Rimmer, M. A.; Mercer, S.; Rankovic, Z.; Shelat, A. A.; Blair, D. J. Continuous collective analysis of chemical reactions. *Nature*. **2024**, 636(8042), 374–379. <https://doi.org/10.1038/s41586-024-08211-4>
- **Torres-Hernandez, A. X.**; Desman, P.; Nguyen, T.; Hoang, V.; Zhang, Y.; Bartels, A.; Rafferty, R. J. Total synthesis of cyanobactin natural product balgacyclamide B. *Chemistry - a European Journal*. **2023**, 30(3). <https://doi.org/10.1002/chem.202303316>

- Fernando, A., Khadka, S., **Torres-Hernandez, A. X.**, Wojcinski, L., Rafferty, R., Sinha, A. Organic Chemistry Laboratory Manual. *Hayden-Mcneil Lab Solutions*. **2020**
- **Torres-Hernandez, A. X.**; Weeramange, C. J.; Desman, P.; Fatino, A.; Haney, O.; Rafferty, R. J. Efforts in Redesigning the Antileukemic Drug 6-Thiopurine: Decreasing Toxic Side Effects While Maintaining Efficacy. *MedChemComm*. **2018**, *10* (1), 169–179.
<https://doi.org/10.1039/c8md00463c>.

Submitted and Under Peer-Review

- **Torres-Hernandez, A. X.**, Desman, P., & Rafferty, R. J. - Balgacyclamide A: Structural revision of the first reported total synthesis and evaluation of oxazoline synthesis via β -hydroxy amides dehydrative cyclization conditions. Submitted to *Organic Letters*.

In preparation

- Torres-Hernandez, A. X., Desman, P., & Rafferty, R. J. - Synthetic pathways evaluation toward balgacyclamide A natural product. Expected submission for peer review April 2025
- Torres-Hernandez, A. X. & Rafferty, R. J. - Previous Advances in Cyclamides' Total Syntheses: A Review. Expected submission for peer review Summer 2025

TEACHING & MENTORING EXPERIENCE

Graduate teaching assistance: Courses

- Organic Chemistry I and II (CHEM 201-202)
- General Chemistry I and II (CHEM 105-106)
- Advance Organic Chemistry Lab (CHM 551)
- General Organic Chemistry Lecture (CHM 350)
- General Chemistry I Lab (CHM 210)
- Organic Chemistry I Lab (CHM 532)

Campus

PUCPR
PUCPR
KSU
KSU
KSU

Undergraduate student mentoring

- Jacque Moon Yee (REU)
- Silvia Rivera (REU)
- Ashley Bartels (Rafferty Lab)
- Chahat Sehgal (Rafferty Lab)
- Maggie Banhart (Rafferty Lab)
- Hongjib Jang (Rafferty Lab)
- Taysear Altarb (POE)

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KSU
KSU
KSU
KSU
St. Jude Child.
Hospt.

HONORS & AWARDS

Bill Miller & Debbie Leckron Miller K-State First Book Scholarship. \$1,000
Ford Foundation Predoctoral Fellowship (NASEMFordFellow). \$81,000
Graduate Cancer Research Award by Johnson Cancer Research Center
Berschied and Destadt Summer Fellowship. \$7,500
Honorable Mention NSF-GRFP
ACS Bridge Travel & Career/Professional Development Award. \$2,500
NSF-GRFP Honorable Mention

March 2025
March 2022
April 2021
April 2021
March 2021
Feb. 2020

Sor Isolina Ferré Award	April 2019
Luis Torres Nadal Award	May 2019
Travel Award by PRLSAMP ACS National Meeting & Expo. \$700.00	May 2019
Travel Award Emerging Researchers National (ERN) Conference in STEM	April 2019
President Award at PCUPR 100 highest GPA	Feb. 2019
Assembly of Honor by the College of Sciences at PCUPR	2015-2019
Luis Torres Nadal Scholarship at PCUPR	2015-2019
Travel Award Annual Biomedical Research Conference for Minority Students	2015-2019
	Nov. 2018

MEDIA

- Geering M, Hohenbary J. Doctoral student awarded prestigious Ford Foundation fellowship. [internet]. Manhattan, KS: K-State News; 2022 September. Available from: <https://www.k-state.edu/media/newsreleases/2022-09/ford-foundation-fellow9922.html>
- Bohn B. Doctoral student in chemistry named honorable mention for NSF Graduate Research Fellowship. [internet]. Manhattan, Kansas: K-State Today; 2021 March. Available from: <https://www.k-state.edu/today/announcement/?id=74017>.
- Torres C. ¡Teatrero, científico e investigador!. [internet]. Ponce, PR: Huellas del Futuro Informa; 2019 May. Available from: <https://huellas.pucpr.edu/teatrero-cientifico-e-investigador/>

PRESENTATIONS | CONFERENCES

Torres-Hernandez AX, Vrettos E, Hu M, Blair D. High-Throughput Synthesis Direct to Biology: Clarithromycin Derivative Synthesis. POE Lunch & Learn Series. 2024 July; Memphis, TN, United States.

Torres Hernandez AX, Desman P, Nguyen T, Hoang V, Zhang Y, Bartels A. Total synthesis of cyanobactin natural product Balgacyclamide B and synthetic efforts toward Balgacyclamide A. American Chemical Society Meeting & Expositions. 2024 March; New Orleans, LA, United States.

Torres-Hernandez AX, Desman P, Nguyen T, Rivera S, Rafferty RJ. Enhancing Small Molecule Penetration into Bacteria. Future Fellow Research Conference (FFRC). 2023 March; Memphis, TN, United States.

Torres-Hernandez AX, Desman P, Nguyen T, Bartels A, Rafferty RJ. Efforts towards the Total Synthesis of Balgacyclamide A and B. Gordon Research Conference: Natural Products and Bioactive Compounds. 2022 August; Andover, NH, United States.

Torres-Hernandez AX, Weeramange CJ, Desman P, Fatino A, Haney O, Rafferty RJ. Efforts in Redesigning the Antileukemic Drug 6-Thiopurine: Decreasing Toxic Side Effects while Maintaining Efficacy. Designing Molecules Workshop and Conference: Sponsored by Japan Society for Promotion of Science. 2019 August; Manhattan, KS, United States.

Torres-Hernandez AX, Weeramange CJ, Desman P, Fatino A, Haney O, Rafferty RJ. Efforts in Redesigning the Antileukemic Drug 6-Thiopurine: Decreasing Toxic Side Effects while Maintaining Efficacy. American Chemical Society Meeting & Expositions. 2019 April; Orlando, FL, United States.

Torres-Hernandez AX, Padilla A, Torres N, Batista-Parra A. Synthesis of Hydroquinone and Catechol Analogs for their Applications in Self-Assembled Monolayers. XVII Symposium of Scientific Research (STEM). 2019 April; Ponce, PR, Puerto Rico.

Torres-Hernandez AX, Padilla A, Batista-Parra A. Synthesis of Catechol and Hydroquinone Analogs for Their Applications in Self-Assembled Monolayers. Emerging Researchers National (ERN) Conference in STEM. 2019 February; Washington, DC, United States.

Torres-Hernandez AX, Weeramange CJ, Desman P, Fatino A, Hull O, Rafferty RJ. Re-engineering 6-Thiopurine: Synthesis and Biological Evaluation of Analogs. Annual Biomedical Research Conference (ABRCMS). 2018 November; Indianapolis, IN.

Torres-Hernandez AX, Padilla A, Batista-Parra A. Re-engineering 6-Thiopurine: Synthesis and Biological Applications. Puerto Rico Louis Stokes Alliance for Minority Participation (PRLSAMP) Advisory Board Meeting. 2018 September; Rio Piedras, PR, Puerto Rico.

Torres-Hernandez AX, Weeramange CJ, Desman P, Fatino A, Haney O, Rafferty RJ. Re-engineering 6-Thiopurine: Synthesis and Biological Applications. 7th Annual Chemistry NSF REU Symposium. 2018 August; Manhattan, KS, United States.

Torres-Hernandez AX, Padilla A, Echevarria C, Batista-Parra A. Synthesis of Hydroquinone Analogs for their Applications in Self-Assembled Monolayers. XVI Symposium of Scientific Research (STEM). 2018 April; Ponce, PR, Puerto Rico

Torres-Hernandez AX, Padilla A, Echevarria C, Batista-Parra A. Synthesis of Hydroquinone Analogs for their Applications in Self-Assembled Monolayers. Junior Technical Meeting and PR Interdisciplinary Meeting (JTM/PRIMS). 2018 April; Gurabo, PR, Puerto Rico.

Torres-Hernandez AX, Desman P, Rafferty RJ. Synthetic Efforts towards Balgacyclamide A for Biological Investigations into Selective Cellular Delivery. Summer Undergraduate Research Opportunity Program (SUROP) Symposium. 2017 July; Manhattan, KS, United States.

Torres-Hernandez AX, Padilla A, Echevarria C, Batista-Parra A. Synthesis of Catechol and Hydroquinone Analogs. XV Symposium of Scientific Research (STEM). 2017 April; Ponce, PR, Puerto Rico.

SIGN LANGUAGE, OUTREACH, & LEADERSHIP

Sign Language courses: Sign Language Basic (ELED-385), Manual Communication I (FSHS-415), & Manual Communication II (FSHS-615)

Sign Language Club: Member (2021-2025), External Programming (2022-2024), Vice-president (2024-2025)

- Coordinator of American Sign Language Panel Event: Deaf, CODA, and interpreters
- Coordinator of monthly meetings to raise awareness of the Deaf community in Kansas State University students
- Bring resources to students to learn sign language

- Participate in university events to talk about the Sign Language Club and the needs of Deaf people within the community

Outreach & Extracurricular (K-12, Chemistry Department, KSU & PCUPR)

- Assistance professor search committee: Graduate student representation (August-December 2022)
- Panelist at “2023 & 2021 Mental Health Panel” event at KSU
- Coordinator of “2022 Mental Health and Well-being Resource Panel” event at KSU
- Marlatt’s Elementary School International Week to K-5 students | Presentation: Puerto Rico
- Bonifacio Alvarado Elementary and Middle School K-9 students | Presentation: My Journey as a Scientist
- Panelist at “Graduate Student Panel” event at KSU & PCUPR | Motivating undergrads to pursue graduate studies
- Scientific Role Model Seminar at PCUPR | Presentation: My Journey as a Scientist
- High School STEM Workshop, Montaña en La Costa LSAMP | Presentation: Slime Chemistry
- ACS Festival at Paseo de la Princesa, SJ | Chemistry in Daily Life
- ACS Chemistry Demonstrations at Plaza del Caribe Ponce Mall
- Angeles de Esperanza Workshop | Presentation: Suicide Prevention

Organizations/Leadership

- Sign Language Club | Vice-president 2024 - 2025
- Phi Lambda Upsilon Chemistry Organization (K-State) | Alumni Secretary 2021-2022
- Angeles de Esperanza Suicide Prevention Club | President 2015-2018
- Campus Verde Pionero | Sub-treasurer 2015-2017
- American Chemical Society | Member 2016 – Present

LANGUAGES

Spanish – Native language

English – Full professional proficiency

Puerto Rico & American Sign Language – Intermediate working proficiency

REFERENCES

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Appendix B - Publications

<https://doi.org/10.1002/chem.202303316>

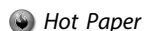


Chemistry—A European Journal

Research Article
doi.org/10.1002/chem.202303316



www.chemeurj.org



Total Synthesis of Cyanobactin Natural Product Balgacyclamide B

Arnaldo X. Torres-Hernandez^{+, [a]}, Prathibha Desman^{+, [a]}, Thi Nguyen,^[a] Vinh Hoang,^[a] Yichao Zhang,^[a] Ashley Bartels,^[a] and Ryan J. Rafferty^{*, [a]}

Balgacyclamide A–C are a family of cyanobactin natural products isolated from freshwater cyanobacteria *Microcystis aeruginosa*. These macrocyclic peptides are characterized by their oxazoline-thiazole core, their 7 or 8 stereocenters, and their antiparasitic activities. Balgacyclamide B is known for its activity towards *Plasmodium falciparum* chloroquine-resistant strain K1, *Trypanosoma brucei rhodesiense*, and *Leishmania donovani*. In this report, the first total

synthesis of Balgacyclamide B is described in a 17-steps pathway and a 2% overall yield. The synthetic pathway toward balgacyclamide B can be adapted for the future syntheses of balgacyclamide A and C. In addition, a brief history background of oxazolines syntheses is shown to emphasize the importance of the cyclization conditions used to interconvert or retain configuration of β -hydroxy amides via dehydrative cyclization.

Introduction

The cyanobactin family of natural products (NPs) consists of cyclic peptides isolated from multiple cyanobacteria that are biosynthetically formed at the ribosome and post-translationally modified by enzymes.^[1] These NPs are well known to exhibit antiparasitic, antibacterial and anticancer properties, in addition to metal chelation and multidrug resistance reversing abilities.^[2] Multiple cyanobactins possess strong similarities in their core skeletons, containing most notably heterocycles such as oxazolines, oxazoles, thiazolines, and thiazoles. However, these NPs can also contain isoprenoid amino acid derivatives or cyclic peptides only consisting in proteinogenic amino acids.^[3] Cyanobactins' total syntheses have been an important contribution to their studies since many of their original reported isolation structures have been revised after the first total synthesis is accessed.^[4] On the other hand, their natural role is unknown; thus, accessing these NPs can open future lines of research towards their role in nature. Multiple cyanobactins have been synthesized, for example, bistratamide D,^[5] trunkamide A,^[6] aerucyclamide B,^[7] patellamide A and its derivatives,^[4,8] among others, however, several cyanobactins have not been accessed synthetically yet (Figure 1A). Herein, we report the first total synthesis of balgacyclamide B cyanobactin.

The balgacyclamide NPs were isolated from freshwater cyanobacteria *Microcystis aeruginosa* EAWAG 251 by Gademann and co-workers in 2014.^[9] Balgacyclamide A, B, and C are macrocyclic peptides characterized by their thiazole and

oxazoline moieties (Figure 1B). The northern thiazole (ring A) and oxazoline (ring B) of balgacyclamide A and B are identical, however, they differ in the southern portion. Both balgacyclamide A and B's southern portions are composed of a L-threonine and D-alanine dipeptide, they differ in A's undergoing a post-translational cyclization to an oxazoline whereas B does not undergo this modification. In contrast, balgacyclamide C southern dipeptide includes L-threonine and glycine (E) and its northern oxazoline (ring B) differs by a single amino acid; balgacyclamide C oxazoline includes L-phenyl alanine and balgacyclamide A and B L-valine. In terms of their biological properties, balgacyclamide A and B show antiparasitic activity against *Plasmodium falciparum* chloroquine-resistant strain K1 and moderate toxicity towards *Trypanosoma brucei rhodesiense* (Figure 1B). Balgacyclamide B also has been reported to possess toxicity against *Leishmania donovani*. Meanwhile, balgacyclamide C has not been evaluated to have antiparasitic properties, and its biological activity is unknown.

Results and Discussion

While the scope of this work is for the development of a synthetic route to balgacyclamide B, we propose that balgacyclamide A could be accessed from B via a catalytic dehydration cyclization (Scheme 1). This synthetic transformation will require retention of the threonine moiety β -carbon stereocenter; thus, requiring specific cyclization conditions (vide infra). The assembly of balgacyclamide B is envisioned to arise from the coupling of three main fragments, *i.* thiazole (4) *ii.* oxazoline (6), and *iii.* dipeptide (5). The thiazole can be accessed from L-serine and D-alloisoleucine coupling with subsequent cyclization with tandem sulfur incorporation. The oxazoline fragment could arise from coupling of L-alloisoleucine and L-valine and cyclization. Lastly, 5 could be accessed via the coupling of L-threonine and D-alanine.

[a] A. X. Torres-Hernandez,⁺ Dr. P. Desman,⁺ Dr. T. Nguyen, V. Hoang, Y. Zhang, A. Bartels, Prof. Dr. R. J. Rafferty
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[⁺] These authors contributed equally to this work.

Supporting information for this article is available on the WWW under <https://doi.org/10.1002/chem.202303316>



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2019, 10, 169

Efforts in redesigning the antileukemic drug 6-thiopurine: decreasing toxic side effects while maintaining efficacy†

Arnaldo X. Torres Hernandez,^{‡,ab} Chamitha J. Weeramange,^{‡,b} Prathibha Desman,^b Anthony Fatino,^b Olivia Haney^b and Ryan J. Rafferty^{id}*,^b

6-Thiopurine (6TP) is a currently prescribed drug in the treatment of diseases ranging from Crohn's disease to acute lymphocytic leukemia. While its potent mode of action is through incorporation into DNA as a thiol mimic of deoxyguanosine, severe toxicities are associated with its administration which hinder the potential therapeutic application. We have previously reported *in vitro* that the oxidative metabolites of 6TP, specifically 6-thiouric acid (6TU, K_i 7 μM), are potent inhibitors of UDP-glucose dehydrogenase (UDPGDH), an enzyme that is responsible for the formation of UDP-glucuronic acid (UDPGA), an essential substrate that is used in detoxification processes in the liver. An *in vivo* investigation was undertaken to probe if 6TU inhibits UDPGDH in rat hepatocytes, and it was observed that 6TU does greatly suppress the conjugation of bilirubin with UDPGA. The failed excretion of bilirubin is linked to a majority of the reported toxicities associated with 6TP administration. Efforts were undertaken for the construction of 6TP analogs, substituted at the C8 position, to reduce inhibition of UDPGDH while retaining therapeutic efficacy. Three new 6TP analogs bearing a halogen (Br, Cl, and F) at the C8 position have been achieved over five-synthetic steps in overall yields of 16 to 32%. Each of these analogs were shown to have reduced inhibition towards UDPGDH, with K_i values of 192, 163, 215 μM , respectively. In addition, the bromine, chlorine, and fluorine analogs were shown to possess cytotoxicity towards the REH cell line (acute lymphocytic leukemia) having IC_{50} values of 9.54 μM (± 0.97), 3.95 μM (± 1.94), and 4.71 μM (± 1.40), respectively. These three new 6TP analogs represent the first steps in the redesign of this potent anticancer agent into a better drug that possesses reduced toxic side effects while retaining therapeutic potency.

Received 14th September 2018,
Accepted 15th December 2018

DOI: 10.1039/c8md00463c

rsc.li/medchemcomm

1. Introduction

6-Thiopurine (6TP, 1) has been a continuously prescribed therapeutic since its FDA approval in 1952, shortly after its discovery in 1950 by Gertrude Elion and George Hitchings at Burroughs Wellcome.¹ This potent therapeutic serves as a treatment option for numerous diseases, such as, but not limited to: acute lymphocytic leukemia (ALL),^{2,3} non-Hodgkin's leukemia,^{4,5} Crohn's disease,^{6–8} and inflammatory bowel disease.^{9,10} 6TP has no therapeutic properties itself, however, it is metabolized into a therapeutic form, a deoxyguanosine nucleotide mimic, commencing with the phosphoribosylation by hypoxanthine-guanine phosphoribosyltransferase (HGPRT) forming the nucleotide mimic 6-thioinosinic acid mono-

phosphate (TIMP, 2). HGPRT is an enzyme that usually catalyzes phosphoribosylation of hypoxanthine (C6 hydroxyl) to inosinic acid (IMP).¹¹ Oxidation of the C2 position of 6TP is accomplished by inosine-5'-monophosphate dehydrogenase (IMPD), an enzyme that is overexpressed in the presence of 6TP, followed by C2 amine installation by guanine monophosphate synthase (GMPS) giving rise to either thio-guanosine mono phosphate (TGMP or dTGMP, 3).^{11–13} Multiple kinase transformations then afford dTGTP (4), which gets incorporated into DNA in place of dGTP. However, incorporation of dTGTP does not immediately result in apoptosis, *via* base pair mismatching, but requires multiple passages through the S-phase allowing for sufficient dTGTP incorporation into DNA.¹⁴ It has also been shown that 6-thioguanine (6TG, 5) can be used in conjunction with 6TP and is acted upon by HGPRT accessing the monophosphate nucleotide formed *via* 6TP metabolism.

Checkpoint activation is required for recognition of the mismatched base pairing within DNA post 4 incorporation; a process commonly suppressed within cancer cells.^{15–17} Mutually 6TP and 6TG can be methylated to form the

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8md00463c

‡ These authors contributed equally to the work.

Article

Continuous collective analysis of chemical reactions

<https://doi.org/10.1038/s41586-024-08211-4>

Received: 8 May 2024

Accepted: 14 October 2024

Published online: 11 December 2024

 Check for updates

Maowei Hu¹, Lei Yang², Nathaniel Twarog³, Jason Ochoada³, Yong Li², Eirinaios I. Vrettos¹, Arnaldo X. Torres-Hernandez¹, James B. Martinez¹, Jiya Bhatia¹, Brandon M. Young⁴, Jeanine Price⁴, Kevin McGowan⁴, Theresa H. Nguyen⁴, Zhe Shi⁴, Matthew Anyanwu³, Mary Ashley Rimmer², Shea Mercer⁵, Zoran Rankovic^{2,4}, Anang A. Shelat³ & Daniel J. Blair^{1✉}

The automated synthesis of small organic molecules from modular building blocks has the potential to transform our capacity to create medicines and materials^{1–3}. Disruptive acceleration of this molecule-building strategy broadly unlocks its functional potential and requires the integration of many new assembly chemistries. Although recent advances in high-throughput chemistry^{4–6} can speed up the development of appropriate synthetic methods, for example, in selecting appropriate chemical reaction conditions from the vast range of potential options, equivalent high-throughput analytical methods are needed. Here we report a streamlined approach for the rapid, quantitative analysis of chemical reactions by mass spectrometry. The intrinsic fragmentation features of chemical building blocks generalize the analyses of chemical reactions, allowing sub-second readouts of reaction outcomes. Central to this advance was identifying that starting material fragmentation patterns function as universal barcodes for downstream product analysis by mass spectrometry. Combining these features with acoustic droplet ejection mass spectrometry^{7,8} we could eliminate slow chromatographic steps and continuously evaluate chemical reactions in multiplexed formats. This enabled the assignment of reaction conditions to molecules derived from ultrahigh-throughput chemical synthesis experiments. More generally, these results indicate that fragmentation features inherent to chemical synthesis can empower rapid data-rich experimentation.

Increasingly optimized methods for automated iterative chemical synthesis continue to advance the important goal of finding new molecular functions^{1,2} (Fig. 1a). Automated synthesis of small organic molecules has recently reached a level of sophistication that provides on-demand access to natural products, pharmaceuticals and functional materials^{9,10}. However, although the cycle time of these processes is shrinking¹¹, it fails to meet the requirements for efficient exploration of chemical space. Radical advancements in many other fields^{12–14} have arisen from combining modular platforms with high-throughput analytics—thereby contracting the time to data output and process optimization. Yet for small molecules, asynchronous analytical processes limit progress towards this goal (Fig. 1b), so a marked increase in pace must be realized.

Generalized access to chemical space stems from the incorporation of a wide range of chemical processes into modularized automated formats. However, the inherent complexity of forming chemical unions suggests that the concept of generalized conditions for many reactions is a foregone impossibility because of the vast range of potential coupling partners¹⁵. Accordingly, automated high-throughput reaction scouting⁴ has become the method of choice for obtaining a molecule

of interest, as this allows multiple reaction conditions to be actioned in parallel. Despite these benefits, high-content chemical synthesis is analytically cumbersome on even modest scales (that is, 2–3 min per sample by liquid chromatography = 2 days for about 1,000 reactions)^{4–6}. Moreover, although leading solutions for high-throughput analysis have broken the 1 s per sample barrier¹⁶, they offer limited resolution providing global rather than local trends¹⁷. With predictive models becoming progressively more effective for structurally aware condition prioritization, there is a present and growing need for accurate high-content chemical reaction data^{15,18–20}.

A common strategy for fast target-independent analysis can be found in proteomics²¹, in which the loss of common chemical pieces permits rapid readouts by tandem mass spectrometry. These methods forgo a priori information about analytes except that they contain fragmentation-compatible analytics-enabling chemical pieces. A powerful aspect of these techniques is that common lost chemical pieces provide a direct means to select the desired analyte from among complex mixtures. Other advances in tandem mass spectrometry have increased the pace of reaction mixture analysis^{4,7,8}. However, progress in this space has not yielded generalizable solutions because of the

¹Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA. ²Analytical Technologies Center, Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA. ³Lead Discovery Informatics Center, Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA. ⁴Medicinal Chemistry Center, Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA. ⁵Program Management, Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN, USA. ✉e-mail: Daniel.Blair@stjude.org

Appendix C - Research Proposition Oral Examination

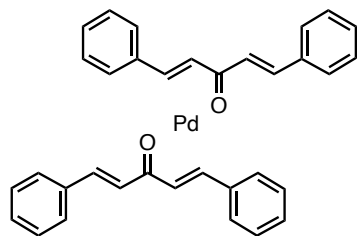
**Akuammiline Indole Alkaloids: Total Synthesis of Skeletal System
of Alstiphyllanine E and Rhazimal Photoaffinity Labeled Analogs**

**Arnaldo X. Torres Hernandez
Research Proposal
Rafferty Lab
Kansas State University, Chemistry Department
05/18/2022**

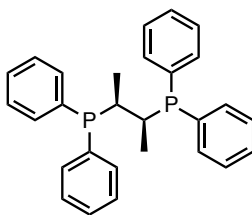
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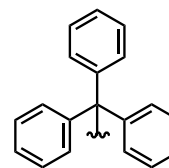
Abbreviations List



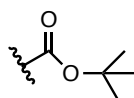
Pd(dba)₂
Bis(dibenzylideneacetone)palladium(0)



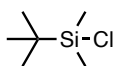
CHIRAPHOS
(2S,3S)-(-)-Bis(diphenylphosphino)butane



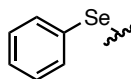
Trt
Triphenylmethane group



Boc
tert-Butylcarbonyl group



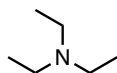
TBSCl
tert-butylchlorodimethylsilane



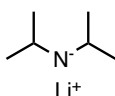
PhSe
Phenyl selenide group



POCl₃
Phosphorus(V) oxychloride



Et₃N
Triethylamine



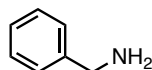
LDA
Lithium diisopropylamide



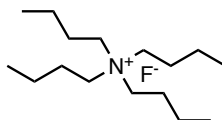
THF
Tetrahydrofuran



DMF
Dimethylformamide



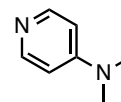
BnNH₂
benzylamine



TBAF
Tetra-n-butylammonium fluoride



DCM or CH₂Cl₂
dichloromethane



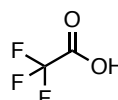
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4-(Dimethylamino)pyridine



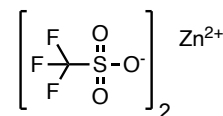
NaO₂
Sodium Nitrite



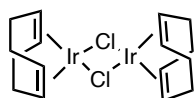
HSiCl₃
Trichlorosilane



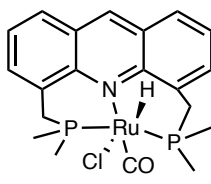
TFA
Trifluoroacetic acid



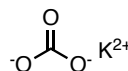
Zn(OTf)₂
Zinc trifluoromethanesulfonate



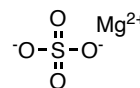
[Ir(cod)Cl]₂
Bis(1,5-cyclooctadiene)diiridium(I) dichloride



(AcrPNP)RuH(CO)Cl



K₂CO₃
Potassium Carbonate



MgSO₄
Magnesium sulfate



CH₃I
Iodomethane



NaI
Sodium Iodide



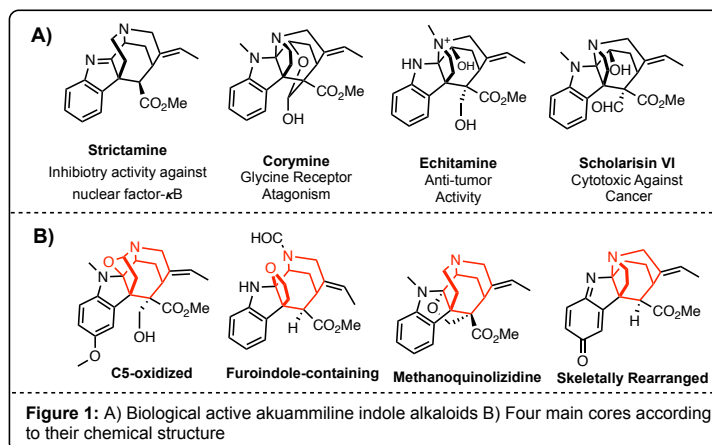
Na⁺ BH₄⁻
Sodium borohydride

Abstract / Project Summary

Akuammiline indole alkaloids are molecules biosynthetically accessed from *Alstonia scholaris* trees, which contain a complex chemical cage-framework and multiple biological activities. Synthetic efforts of these indoles monoterpenoids synthesis have contributed new synthetic methodologies and total syntheses. However, their biosynthetic pathway is not fully known, and several isolated akuammiline alkaloids have not been synthesized yet. The major two aims of this research proposal are the following: to access the first skeletal synthesis of biological active akuammiline indole alkaloid alstiphyllanine E and to clarify the biosynthetic pathway of akuammiline indoles alkaloids by identification of substrate-enzymes interaction. We hypothesize that by a Tsuji Trost and iridium catalyzed indole cyclization, an alstiphyllanine E cage-framework can be achieved. On the other hand, it is expected that an intermediate from the biosynthetic pathway will serve as a probe for enzyme identification. Alstiphyllanine E synthesis will lead to potential new synthetic pathways to access the whole family of alstiphyllanine A-O and other akuammiline indoles alkaloids. Likewise, it will fill a gap of knowledge on their biosynthetic pathway that can lead to new bio-inspired synthetic methodologies and biosynthetic mechanism studies.

Aim 1 Introduction

Akuammiline indoles alkaloids are well known for their complex chemical structure and their biological activity. Chemically, akuammilines are characterized by a compact cage-like structure possessing bridge head nitrogens, and in some cases ethers and hemiaminals, which makes their



synthesis highly challenging. This class of caged systems have been categorized into four main groups according to their skeletal structure: 1) *furoindoline-containing akuammilines*, 2) *C5-oxidized akuammilines*, 3) *skeletally rearranged akuammiline* and, 4) *methanoquinolizidine akuammilines* (Figure 1).¹ Synthetic efforts have been made mainly to develop new methodologies and strategies to build akuammiline indoles alkaloid frameworks and to access their first total synthesis. On the other hand, extensive investigations on this family have revealed that these compounds possess a wide range of biological activities. For example, anti-inflammatory activity, cytotoxicity towards cancer cells, antitumor and antimalarial activity, glycine receptor antagonisms, inhibition towards transcription factor NF- κ B, among others.²⁻⁶

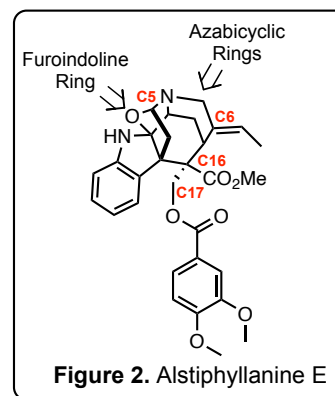
Alstiphyllanines are molecules that have been classified under akuammiline indoles alkaloids with a complex chemical structure and multiple biological activities.⁷ In 2009 and 2010, Morita and co-workers reported alstiphyllanine A-H first isolations from *Alstonia macrophylla* leaves and described their skeletal structures under C5-oxidized akuammiline core. Biologically, alstiphyllanine E and F, show Na⁺-glucose cotransporter (SGLT) inhibitory activity. SGLT helps for glucose re-absorption in the kidney; hence, inhibiting SGLTs might have a direct effect in lowering blood glucose levels by decreasing glucose re-absorption and increasing the urinary sugar excretion.⁸ On the other hand, alstiphyllanine A-D showed antiplasmodial and vasorelaxant activity.⁷

Synthetic efforts towards alstiphyllanines have not been explored. Aim 1 proposes to access the first skeletal synthesis of alstiphyllanine E. Aim 1 is focused on the skeletal framework synthesis of alstiphyllanine E through a Tsuji-Trost reaction and iridium catalyzed indole cyclization.

Aim 1: Skeletal Framework Synthesis of Alstiphyllanine E through a Tsuji-Trost and Iridium Catalyzed Indole Cyclization

Background

Alstiphyllanines are composed of multiple fused rings, such as an indoline moiety and an azabicyclic core linked through carbon five to a furoindoline ring (**Figure 2**). Alstiphyllanines cores are also highly functionalized; however, common functional groups that are present within this family are a methyl ester (**C16**), di or tri methoxybenzoate (**C17**), and a trisubstituted vinyl group (**C6**). We *hypothesize* that by applying a Tsuji-Trost reaction and iridium catalyzed indole cyclization the alstiphyllanines main core can be accessed followed by alstiphyllanine E skeletal framework synthesis.



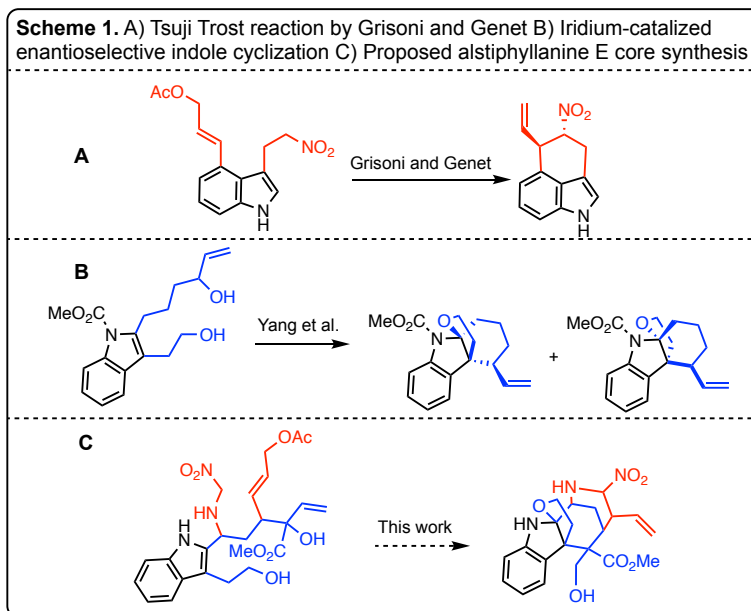
The Tsuji-Trost reaction was first introduced in 1965 when allylacetoacetate and allylmalonate were synthesized by exposing π -allylpalladium chloride with a soft carbon nucleophile.⁹ The π -allylpalladium allylation catalytic pathway was elucidated in 1970 upon investigations of various allylic esters and allyl phenyl esters. After its discovery, the reaction has been used to synthesize multiple natural products and to explore different methodologies by modifying substrates and conditions.^{10, 11} For example, Grisoni and Genet were able to successfully access tricyclic nitro ergoline alkaloids via enantioselective Pd (0) intramolecular catalyzed cyclization (**Scheme 1A**).¹² Subjecting the starting material with Pd(dba)₃/CHIRAPHOS formed the π -allylpalladium intermediate from the allyl acetate. Subsequent deprotonation of the acidic alpha hydrogen of the nitro-group allows for Pd coordination to form the C-C bond followed by β -hydride elimination of the Pd to afford the vinyl group. Through the Tsuji-Trost cyclization it is anticipated that the azacyclic ring (**3**, **Scheme 2**) will be accessed. Further elaboration onto the furoindoline moiety is

envisioned to arise from an iridium-catalyzed cyclization, based upon precedence of Yang and co-workers in 2016. Yang developed an iridium-catalyzed enantioselective indole cyclization that allowed for the synthesis of a scarcely functionalized carbazole and furoindoline core (**Scheme 1B**)¹³, similar to akuammiline indoles alkaloids structure. In this work,

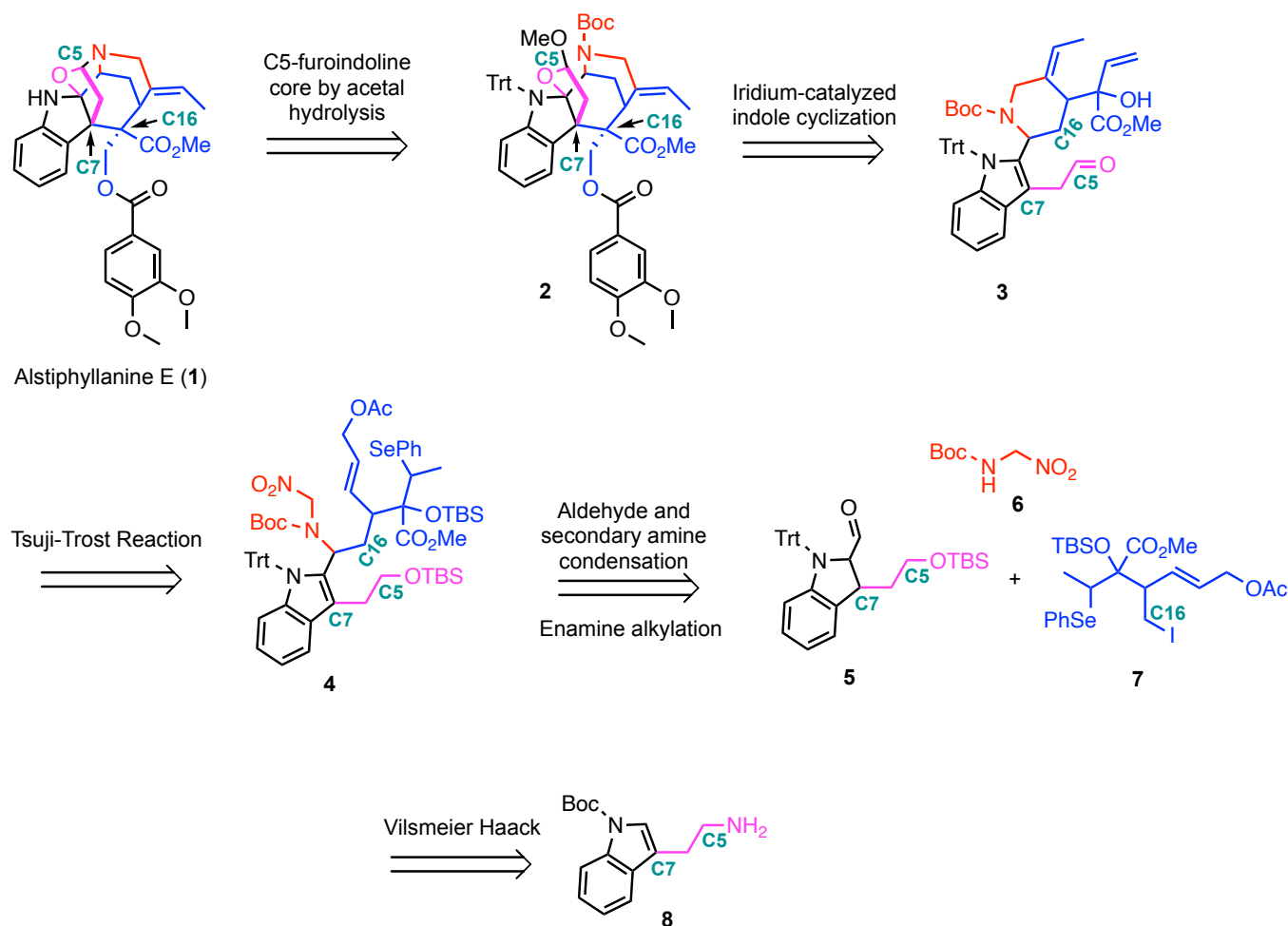
they were able to take an indole bearing a primary alcohol and a secondary allylic alcohol and expose it to an iridium catalyst and Lewis acid to induce a cyclization cascade through an enantioselective allylic alkylation and iminium ion. Inspired by these works, it is proposed that the Grisoni and Yang strategy can be applied to a highly functionalized disubstituted indole to undergo intramolecular cyclization to form an azacyclic ring and an indole cyclization using a tertiary allylic alcohol (**Scheme 1C**). Slightly modifications in the synthesized core can lead to the first skeletal synthesis of alstiphyllanine E.

Retrosynthetic analysis

Alstiphyllanine E (**1**) is envisioned to be accessed via an acetal hydrolysis followed by in situ Boc and Trt deprotection of (**2**, **Scheme 2**). Furoindoline and azabicyclic core of Alstiphyllanine E are expected to be accessed in one step reaction through an iminium-catalyzed indole cyclization from tertiary allylic alcohol **3**. To achieve an azacyclic ring of **3**, a Tsuji Trost reaction will be performed on **4** to induce an intramolecular cyclization. Compound **4** can be synthesized by an aldehyde/amine condensation between **5** and **6**, followed by an enamine alkylation with highly functionalized carbon chain **7**. Finally, indoline **5** can be accessed through a Vilsmeier Haack reaction on commercially available Boc protected tryptamine (**8**).



Scheme 2. Alstiphyllanine E retrosynthetic analysis

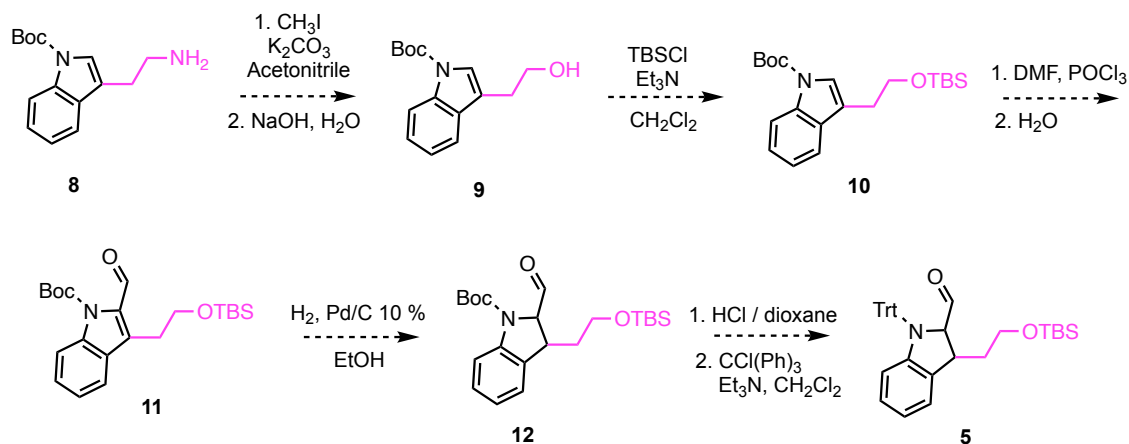


Proposed Synthetic Pathway

Deamination of commercially available Boc protected tryptamine (8) can be performed by trimethylation of the primary amine with excess CH_3I in K_2CO_3 followed by an addition of sodium hydroxide to access 9 (Scheme 3). However, multiple synthetic approaches towards 9 can be taken into consideration. For example, Milstein and co-workers were able to induce a direct deamination with $(\text{AcrPNP})\text{RuH}(\text{CO})\text{Cl}$ catalyst in H_2O and dioxane.¹⁴ On the other hand, a Hoffmann elimination could be applied followed by hydroboration of the alkene. Once 9 is in hand, it can be exposed to TBSCl in presence of Et_3N to protect the alcohol and access 10. Exposing 10 into a Vilsmeier-Haack reaction will install an aldehyde functionality upon indole C2 furnishing 11. Hydrogenation of 11 with H_2 , Pd/C 10 % will give 12. Due to anticipated late-stage chemistry, the

Boc group will be removed under acidic conditions and replaced with a more robust trityl protecting group, which will be installed with trityl chloride and Et₃N to access **5**.

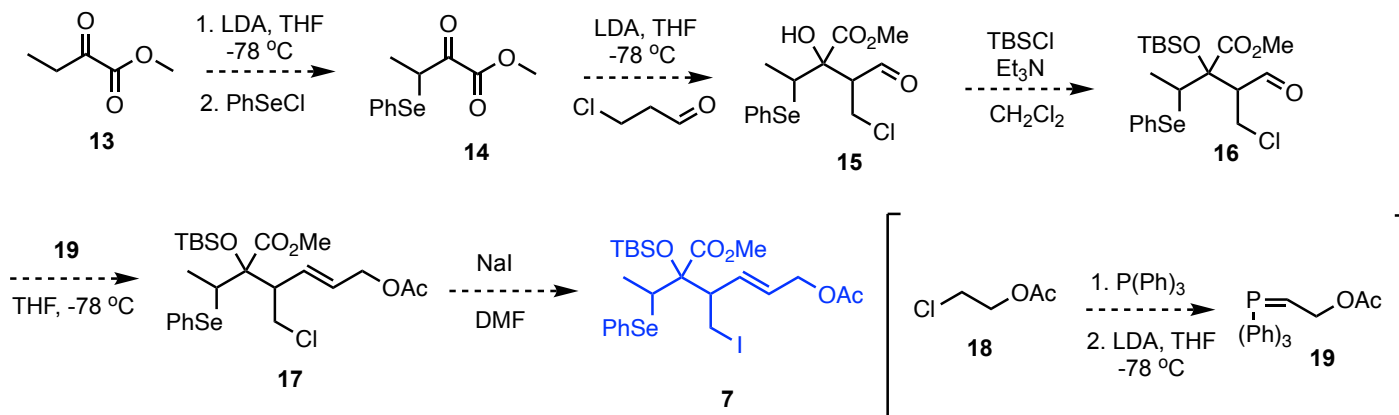
Scheme 3. Proposed synthesis of indoline **5**



Allyl Acetate **7** Synthesis and Ylide Formation

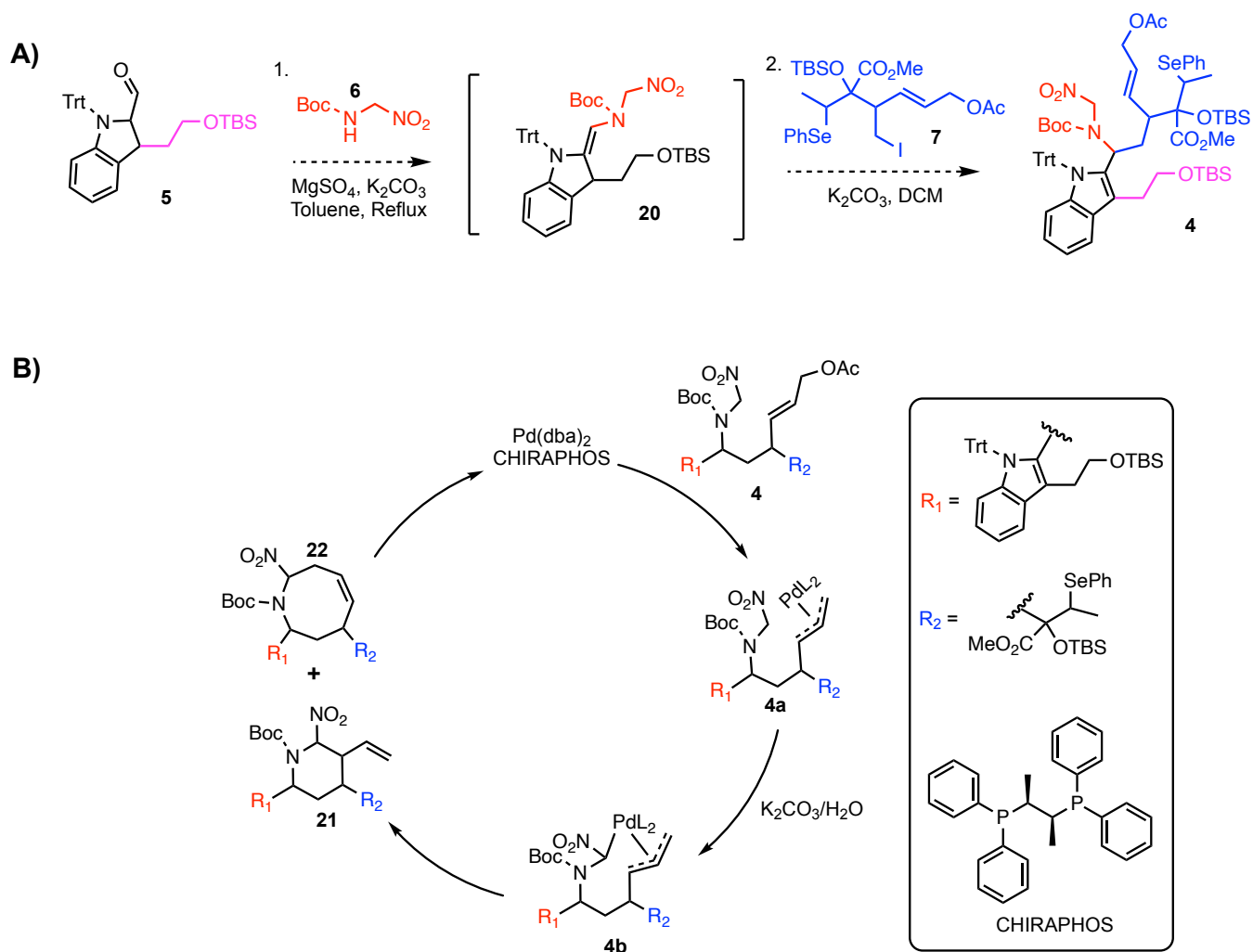
Enolization of methyl 2-oxobutanoate (**13**) with LDA in THF followed by treatment with phenylselenium chloride will give access to **14** (Scheme 4). Accessing **15** will commence with the enolization of 3-chloropropanal via LDA which will then be added to **14** for the subsequent aldol addition. Protection of the alcohol within **15** will be done via TBSCl and Et₃N to furnish **16**. The synthesis of **17** will be accomplished via a Wittig reaction upon **16**. Preparation of Wittig reagent can be done with triphenylphosphine in presence of alkyl halide **16**, followed by deprotonation with LDA in THF at -78 °C. Lastly, the exchange of Cl with I will be performed by a Finkelstein reaction with NaI in DMF to afford **7**.

Scheme 4. Proposed synthesis of allyl acetate **7** and ylide formation



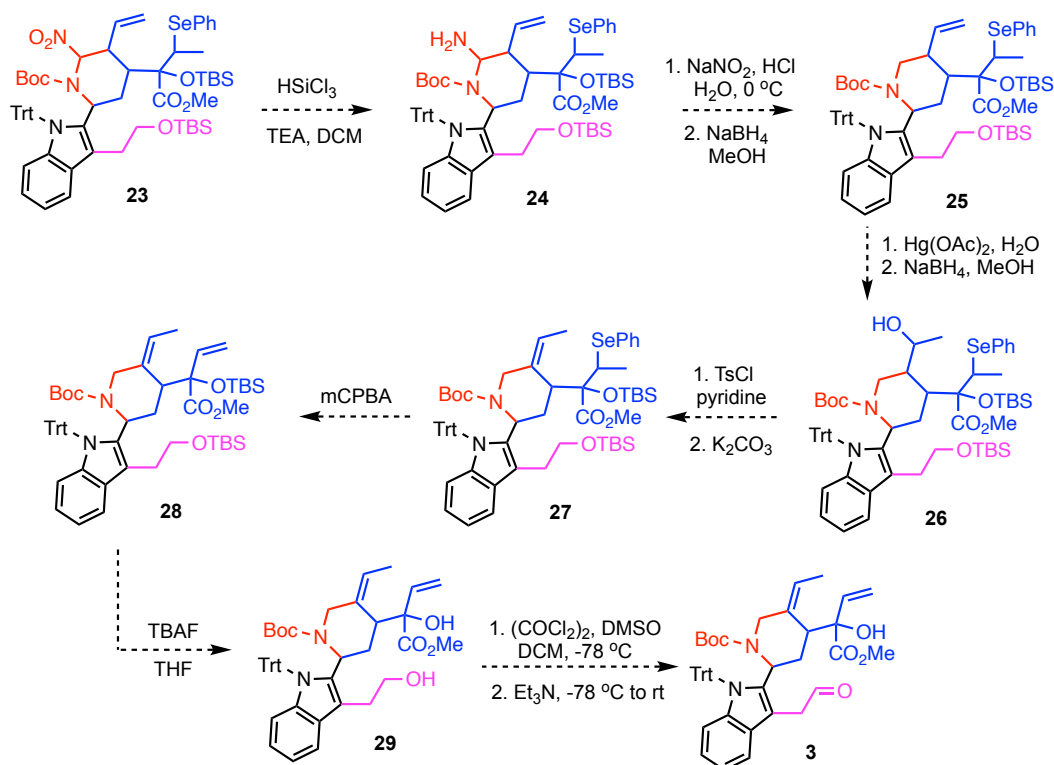
In 2004, Umani-Ronchi and co-workers accessed a secondary amine by refluxing an indole carboxaldehyde with toluene, BnNH₂ and MgSO₄ followed by a reduction of iminium ion with NaBH₄ in MeOH.¹⁵ Subjecting indoline carboxaldehyde **5** and Boc protected amine **6** to similar conditions stated above it expected to afford intermediate enamine **20** in situ (**Scheme 5A**). Compound **20** will undergo enamine alkylation with allyl acetate **7** to form **4**. Similar methodology from Grisoni and Genet can be useful for an intramolecular cyclization on **21** to form the azacyclic core. Disubstituted indole **4** can undergoes a Tsuji-Trost reaction with Pd(dba)₂, K₂CO₃ in H₂O (**Scheme 5B**). As previously discussed, the catalytic cycle starts with Pd(dba)₂ coordination to the allyl acetate (**4**) forming the π -allylpalladium intermediate (**4a**). Deprotonation of the acidic alpha hydrogen of the nitro-group followed by insertion into the palladium gives **4b**, which undergoes C-C bond formation and β -hydride elimination of the Pd to afford the azacycle **21** (six-membered ring) and the internal olefin variant azacycle **22** (eight-membered ring).

Scheme 5. Proposed synthesis of **21** and Tsuji Trost reaction cyclization to access **22**



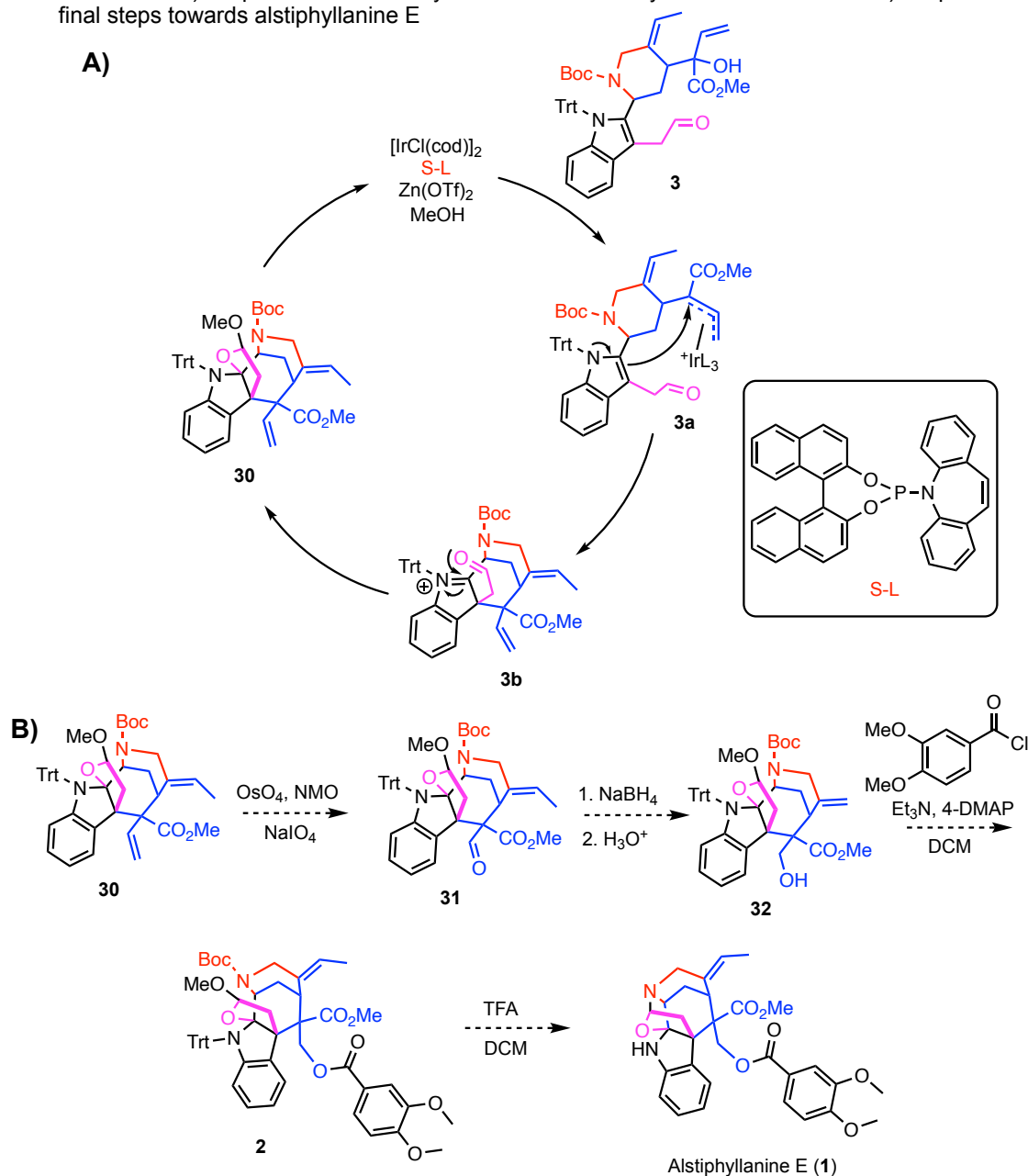
Reduction of aliphatic nitro will be done by exposing **23** with HSiCl_3 , Et_3N and DCM to access free amine **24** (Scheme 6).¹⁶ Subjecting primary amine **24** to diazotization with nitrous acid with NaNO_2 and HCl, followed by reduction with NaBH_4 will form **25**. Trisubstituted alkene can be synthesized through two steps reaction a) oxymercuration reaction to access secondary alcohol **26** b) tosyl protection of the alcohol followed by K_2CO_3 addition to form alkene **27**. Selenoxide elimination will be performed by addition of mCPBA into **27** to form monosubstituted alkene **28**. To access diol **29**, a TBS deprotection will be performed upon **28** using TBAF. Oxidation of the primary alcohol within **29** will undergo a Swern oxidation to access free aldehyde **3**.

Scheme 6. Proposed synthesis towards free aldehyde **3**



Compound **3** can undergo an iridium catalyzed indole cyclization once it is submitted to $[\{\text{Ir}(\text{cod})\text{Cl}\}_2]$ catalyst (the Carreira ligand), $\text{Zn}(\text{OTf})_2$ as an activator, and dichloroethane to achieve compound **30**.¹³ Monosubstituted alkene **30** can be transform into aldehyde **31** by a Lemieux-Johnson oxidation, followed by a reduction with NaBH_4 under mild acidic conditions to form primary alcohol **32**. Esterification of **32** can be perform with 3,4-dimethoxybenzoyl chloride in Et_3N , 4-DMAP, and DCM to form **2**. Finally, to access the first skeletal synthesis of alstiphyllanine E (**1**), Boc and Trt deprotection need to be completed under acidic conditions with TFA in DCM.

Scheme 7. A) Proposed iridium catalyzed intramolecular cyclization mechanism B) Proposed final steps towards alstiphyllanine E



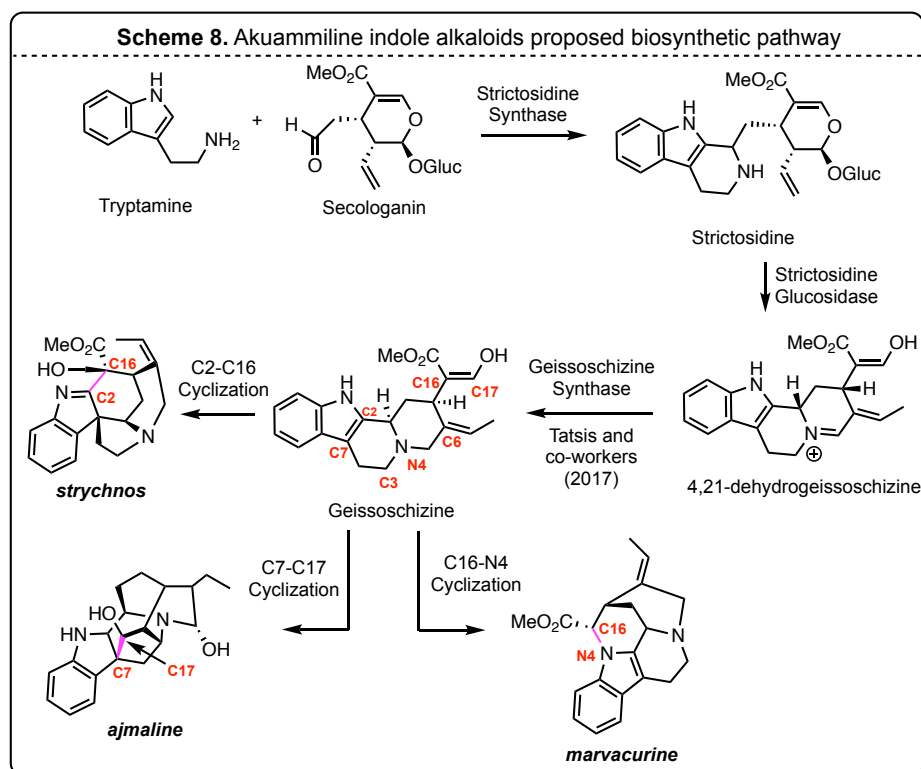
Significance

This aim will provide the first total synthesis of Alstiphyllanine E skeletal system. Alstiphyllanine E synthetic pathway can be modified to access multiple alstiphyllanines and other natural products with similar core structures. Furoindoline containing akuammiline cores can also be access through this synthetic pathway, which will lead to synthesis of other akuammiline indoles alkaloids. This

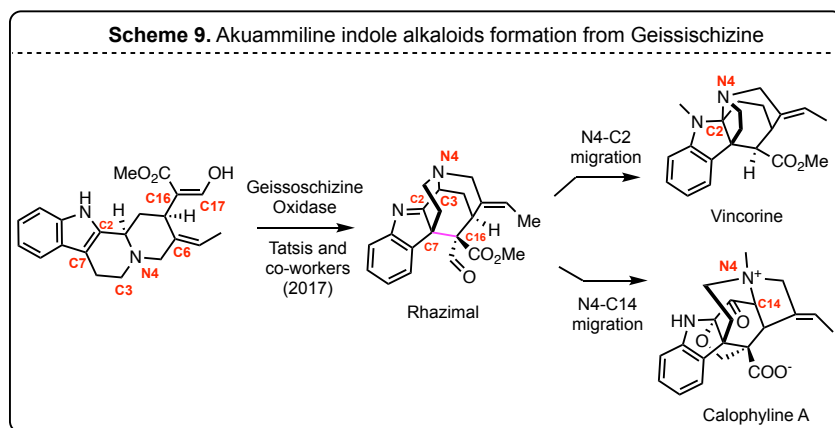
project will provide a synthetic pathway that can be study to bring new efforts towards the asymmetric total synthesis of Alstiphyllanine E. On the other hand, potential Alstiphyllanine E biological activities can be explored such as: drug delivery, cytotoxicity, anti-parasitic, anti-inflammatory, anti-fungal activities, etc.

Aim 2 Introduction

Biosynthetically, the synthesis of the akuammiline indoles alkaloids start with the condensation of tryptamine and secologanin via strictosidine synthase.¹⁷ Upon formation of this Shift-base a Pictet-Spengler reaction will commence to form strictosidine (**Scheme 8**). Strictosidine glucosidase then removes the glucose upon strictosidine giving rise to 4,21-dehydrogeissoschizine. Reduction of the iminium ion with NADPH by geissoschizine synthase affords geissoschizine. Multiple natural products can then be formed via geissoschizine by various C-C and C-N cyclizations as shown in Scheme 8.



In addition to the cyclization reactions upon geissoschizine, it can be biosynthetically transformed into rhazimal by geissoschizine oxidase via a C7-C16 cyclization.¹⁸ Rhazimal serves as starting point for the formation of multiple akuammiline indole alkaloids by the carbon migration from N4 to C2 or C14 followed by various oxidations, reductions, acetylations, and other transformations (Scheme 9).¹⁹ The process of how this N4 migration to C2/C14 has yet to be understood biosynthetically. The second aim of this proposed focuses on employing photoaffinity labeling techniques upon rhazimal to identify the enzymes involved in this intriguing transformation.



Aim 2: Synthesis of Rhazimal photoaffinity labels and future approaches

Background

Photoaffinity labeling (PAL) is a technique used to target specific proteins/enzymes with its substrates. When working with PAL techniques, multiple areas need to be taken into consideration such as: photoaffinity probe design, photoaffinity groups, reporter tags, and molecular target identification.²¹ Probes design involves two main parts, a) substrate with a photoreactive diazirine and alkyne moiety and b) reporter tag with azide moiety. The purpose of synthesizing a probe with a photoreactive diazirine is to induce a covalent crosslinking bond to ‘trap’ the protein of interest. Once the protein of interest is ‘trap’, the reporter tag diazirine can be added to perform an azide-alkyne cycloaddition (click chemistry) to later facilitate protein of interest isolation (Figure 3).

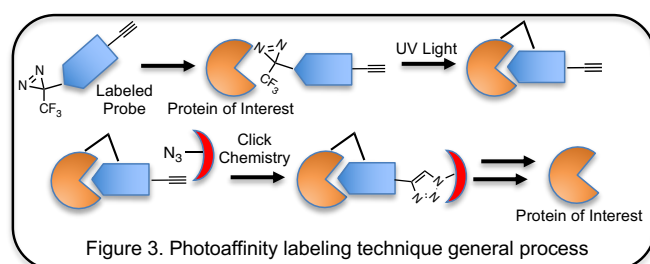
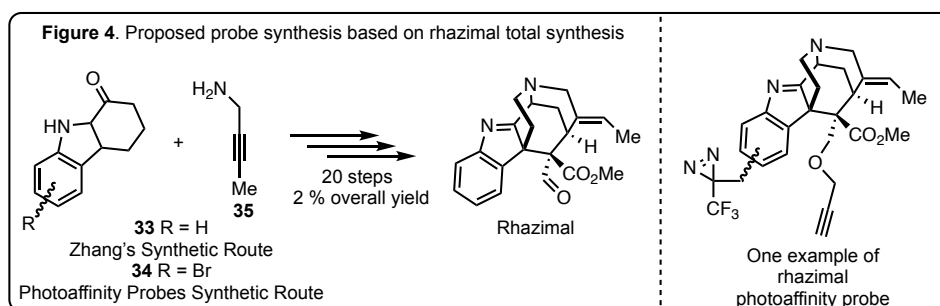


Figure 3. Photoaffinity labeling technique general process

Probes design involves two main parts, a) substrate with a photoreactive diazirine and alkyne moiety and b) reporter tag with azide moiety. The purpose of synthesizing a probe with a photoreactive diazirine is to induce a covalent crosslinking bond to ‘trap’ the protein of interest. Once the protein of interest is ‘trap’, the reporter tag diazirine can be added to perform an azide-alkyne cycloaddition (click chemistry) to later facilitate protein of interest isolation (Figure 3). Aim 2 is only focused on PAL probe design using rhazimal as a substrate. We *hypothesize* that rhazimal photoaffinity label analogs can bind to the enzyme(s) responsible for N4 migration to C2 or C14.

Proposed Probe Synthesis

First total synthesis of rhazimal was first established by Zhang and co-workers in 2019.²¹ Rhazimal was accessed in 20 synthetic steps starting from functionalized indole (**33**) and alkyne (**35**) shown in **Figure 4**. Possible handles to be used for the incorporation of both the diazirine and alkyne can be installed upon the starting indole (**34**) or could also be incorporated at later stages via known arene chemistry.²² While the diazirine and alkyne are shown to be installed upon distance positions within rhazimal, they could also be present upon an alkane chain that is tethered upon the arene in case further distance of both groups is needed for substrate recognition by the appropriate enzyme(s).



Future Approach

After synthesis of multiple potential photoaffinity probes, further steps are needed to explore protein-substrate binding. First, treatment of cells with probes will lead to target protein trap after UV light irradiation. UV light will induce a covalent crosslinking between the probe and the protein. Second, addition of reporter tag with azide moiety that will undergo click chemistry with the substrate. Third, proteins can be isolated by chromatography techniques or SDS-PAGE Western Blotting. Finally, to characterize proteins, LC-MS/MS analysis can be performed. Since aim 2 focus is the synthesis of rhazimal photoaffinity probes, further steps towards capture, isolation, and identification of proteins will be done in collaboration.

Significance

This aim will provide new synthetic probes for future protein identification through photoaffinity label techniques. Isolation of proteins who are involved in biosynthetic pathways of natural products plays an important role for future investigations. For example, proteins who interact with rhazimal probes need to be fully characterized and to be studied to explore how N4-C2 or N4-C14 migration is mechanistically happening in the active site. Understanding mechanisms in biosynthetic pathways can lead to new biomimetic reactions that can be applied to future total syntheses of akuammiline indole alkaloids.

General Methodology

Air and moisture sensitive reactions will be performed in flame dry glassware and under an inert atmosphere. Reactions will be monitored by thin-layer chromatography (TLC) or via real-time $^1\text{H}/^{13}\text{C}$ Nuclear Magnetic Spectroscopy (NMR). Purification of synthesized compounds will be accomplished by flash silica gel chromatography. Full characterization of each product will be performed by spectroscopy techniques such as $^1\text{H}/^{13}\text{C}$ -NMR, Infrared Spectroscopy (IR), and Mass Spectroscopy. More advanced characterization can be undertaken for the structural elucidation of compounds using such 2D NMR techniques as: COSY, HMBC, HSQC, and NOESY. X-Ray Crystallography will be undertaken on crystalline compounds for full stereochemical assignments.

Conclusion

Aim 1 and 2 of this research proposal will serve as a pathway for the first total synthesis of alstiphyllanine E skeletal carbon framework and as a starting point for future studies in akuammiline indoles alkaloids biosynthetic pathway. Aim 1 applies two main key steps for the construction of furoindoline-containing akuammiline core, a Tsuji-Trost reaction and an iridium catalyzed indole cyclization. This new proposed synthetic pathway will lead to potential intermediates that can be used to access different akuammiline indoles alkaloids or natural products with similar core structures. However, studies towards the asymmetric total synthesis of alstiphyllanine E is needed. Potential challenges in aim 1 can arise from catalytic cycles, where optimization with different solvents, ligands, catalysts, and activators will be taken into consideration. On the other hand, aim 2 provides the synthesis of rhazimal photoaffinity label probes that will be used to explore protein-substrate binding. Pull-out proteins that interact with rhazimal will lead to further investigations of protein characterization and rhazimal transformation into other types of akuammilines. Incorporation of diazirine and alkyne moieties can represent a challenge if they are incorporated in early steps of rhazimal synthetic pathway. However, adding these functionalities at any intermediates or late synthetic steps can avoid any diazirine or alkyne side reactions. Finally, last protocols towards crosslinking, click chemistry, isolation, and characterization of proteins will be done in collaboration.

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