ABSTRACT

SHI, YUNLONG. Chemistry and Biology Inspired by the Marine Natural Product Monanchocidin A. (Under the direction of Dr. Joshua G. Pierce).

This dissertation describes the chemical and biological approaches toward the study of the marine natural product monanchocidin A and related natural products. Four directions of our efforts include 1) synthetic method development, 2) total synthesis, 3) structure activity relationship, and 4) mechanisms of action. The first chapter of this dissertation describes the synthesis of the dihydroxymorpholinone fragment of monanchocidin A, a highly oxygenated heterocycle that is unique to the monanchocidin family. An acid-promoted cyclization reaction with exclusive regioselectivity and high diastereoselectivity was developed. We also found a mild transformation sequence from a carboxylic acid with one less carbon to the key α-ketoaldehyde substrate. This sequence was applied to the total synthesis of (+)-Plagiogyrin A, which is featured in the second chapter. A stereocontrolled aldol reaction and a hemiacetal formation/rearrangement sequence were responsible for all five chiral centers in this molecule.

In the third section of this dissertation, we report a practical synthesis of a biologically active analog of pentacyclic guanidinium alkaloids. Preliminary results regarding the structure activity relationship were obtained on the basis of this analog. We also provide our initial results on the mechanisms of action of the spermidine motif in pentacyclic guanidinium alkaloids.
Chemistry and Biology Inspired by the Marine Natural Product Monanchocidin A

by

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BIOGRAPHY

Yunlong Shi was born on August 8, 1988 in the city of Zhengzhou, China. He is the only child of Jinsheng Shi and Linghua Feng, who both worked in the field of education. At a young age, he first interacted with the knowledge of chemistry by skimming his mother’s old textbook, discovering the Tollen’s Test (the "silver mirror" reaction). He then gradually became fascinated by this subject and received multiple awards in high school Chemistry Olympiad, which granted his entry to study chemistry in Zhejiang University. While maintaining a top-10% GPA, he participated in research in Professor Shengming Ma and Professor Zizhang Zhang’s labs. In 2011, he traveled to Raleigh, United States and began the Ph.D. study in chemistry at North Carolina State University. Under the supervision of Professor Joshua Pierce, he gained expertise in synthetic organic chemistry and mastered biological techniques in order to test potentially active molecules in the lab. Yunlong will continue his research career as a postdoctoral associate in Professor Kate Carroll’s lab in the Scripps Research Institute in 2017.
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My undergraduate mentor told me that chemistry should be interpreted as “chem-istry”. While there’s trial, there’s error. Having to deal with problems and difficulties almost every day, I can’t imagine completing my Ph.D. study without the support, assistance or guidance of the following people:

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LIST OF ABBREVIATIONS

Ac: Acetate
ATCC: American type culture collection
Bn: Benzyl
Bz: Benzoyl
Boc: tert-Butyloxycarbonyl
BOP: (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
brsm: Based on recovered starting materials
Cbz: Carboxybenzyl
CHO: Chinese hamster ovary
CSA: Camphorsulfonic acid
DNA: Deoxyribonucleic acid
DBU: 1,8-Diazabicycloundec-7-ene
DCM: Dichloromethane
DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIAD: Diisopropyl azodicarboxylate
DIEPA: N,N'-Diisopropylethylamine
DFMO: Difluoromethylornithine
DHS: Deoxyhypusine synthase
DMAP: 4-Dimethylaminopyridine
DMF: Dimethylformamide
DMP: Dess-Martin periodinane
DMDO: Dimethyldioxirane
DMSO: Dimethylsulfoxide
DOHH: Deoxyhypusine hydroxylase
dr: diastereomeric ratio
Δ: Heat
E: Electrophile
EDC/EDCI: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDG: Electron donating group
eIF5A: Eukaryotic Initiation factor 5A
EWG: Electron withdrawing group
HMBC: Heteronuclear multiple bond correlation
hv: Light
HPLC: High pressure liquid chromatography
HTS: High throughput screening
IBX: 2-Iodoxybenzoic acid
KHMDS: Potassium hexamethyldisilazide
LC: Liquid chromatography
LC-MS: Mass spectrometer coupled liquid chromatography
LDA: Lithium diisopropylamide
LHMDS: Lithium hexamethyldisilazide
NAD: Nicotinamide adenine dinucleotide
NBS: N-Bromosuccinimide
NMO: N-methylmorpholine
NMR: Nuclear magnetic resonance
NOE: Nuclear overhauser effect
Nu: Nucleophile
NMWL: Nominal molecular weight limit
ODC: Ornithine decarboxylase
PAO: Polyamine oxidase
PG: Protecting group
PGA: Pentacyclic guanidinium alkaloids
PPTS: Pyridinium p-toluenesulfonate
PTM: Post-translational modification
PTS: Polyamine transport system
p-TsOH: para-Toluene sulfonic acid
rt: Room temperature
RNA: Ribonucleic acid
SAR: Structure-activity relationship
SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SM: Starting material
SSAT: Spermine/spermidine acetyltransferase
TBAF: Tetrabutylammonium fluoride
TBAI: Tetrabutylammonium iodide
TBAT: Tetrabutylammonium difluorotriphenylsilicate
TBS: tert-Butyldimethylsilyl
TBDPS: tert-Butyldiphenylsilyl
TFA: Trifluoroacetate
THF: Tetrahydrofuran
TLC: Thin layer chromatography
TMS: Trimethylsilyl
TMS-EBX: Trimethylsilyl ethynyl benziodoxolone
UV: Ultraviolet
xs: excess
CHAPTER 1

General Introduction
“The chemical synthesis of natural products, that is, of substances produced by living organisms, marked the beginning of organic chemistry.”

-- Dr. Reinhard W. Hoffmann

1.1 The Past and Present of Natural Products and Marine Derived Pharmaceuticals

Nature has long been recognized as a source of medicine. Although humans have used plants and plant substances to treat diseases and medical conditions since prehistoric times, two discoveries at the beginning of the 19th century initiated the transformation of studies in natural products from a state of alchemy to an acknowledged branch of science.

The first was the discovery of morphine. In 1804, Friedrich Sertürner, a 21-year-old pharmacist's apprentice in Paderborn, isolated morphine from the opium poppy plant (Papaver somniferum). Later, morphine was distributed by him and then commercially sold by Merck as an analgesic drug. It is generally believed to be the first single-substance drug derived from nature. Because it is chemically pure, morphine can be administrated in precise dosages, which is not affected by the age or source of the plant.

The second historically significant discovery was the synthesis of urea. Friedrich Wöhler successfully converted ammonium cyanate into urea in 1828. The production of an organic compound from an inorganic reactant disproved the old theory which stated organic compounds possess “vital forces” and are fundamentally different from inorganic compounds. Wöhler’s synthesis is considered the birth of modern organic chemistry.
The two discoveries marked the beginning of a new era wherein natural products, especially those with therapeutic effects, are isolated from living organisms, structurally identified and chemically synthesized to provide the same active chemical compounds that exist in nature. Driven by substantial method developments in organic chemistry, analytical chemistry, biochemistry and many other related fields, new natural products are constantly discovered and explored. By 1990, ~80% of drugs were either natural products or analogues inspired by them. Of the 20 best-selling non-protein drugs in 1999, nine were either derived from or developed as the result of leads generated by natural products, with combined annual sales of more than 16 billion US dollars. Human beings have benefited greatly from the drugs of natural origins: life expectancy has increased from ~40 years (for newborns, 60 years for 20-year-olds) in the 1850s to near 80 years in the United States today, where the contribution of natural products can not be overlooked.

However, despite the historical success of natural products in drug discovery, industrial research into natural products has declined since the mid-1990s. The paradigm shift of drug discovery is mainly caused by the development of high-throughput screening (HTS) technologies, which have the ability to test a large library of chemical entities rapidly. Natural products, as defined molecular targets, are less amenable to HTS than synthetic molecules. The latter are simpler in structure, more accessible, and supplied by combinational chemistry. As a consequence, many pharmaceutical companies have shut down their natural product research; therefore, the number of natural product based drugs in clinical studies suffered a ~30% drop between 2001 and 2008.
Nevertheless, the research of natural products has not come to an end. Modern tools in smart screening, robotic separation/analysis, metabolism engineering and synthetic biology are being developed to keep natural products in the pipeline.\(^3\) Additionally, synthetic compound libraries have their limitations and have failed to deliver as many new chemical entities as expected.\(^8\) The hit rate of synthetic libraries is much lower than that of natural products. For example, 20 commercial drugs were developed from ~7000 polyketide natural products with a hit rate of 0.3%, which is much better than the <0.001\% hit rate of synthetic compounds.\(^9\) There are three unique features of natural products that synthetic libraries cannot offer: 1) Natural products usually possess well-defined 3D structures, which are the outcome of evolving from a chiral biological environment. On the other hand, combinational libraries are either intentionally created with high randomness, or are flat and contain a high percentage of \(sp^2\) carbons. 2) Structural complexity is a major advantage for natural products to be ligands of complicated interactions, such as protein-protein interactions, which form the majority of targets in signal transduction pathways.\(^10\) 3) Nature is a vast source of biodiversity, where novel chemical structures are continuously discovered. This is particularly important in the search for lead molecules for newly discovered targets without known leads\(^5\).

Natural products are isolated from three main sources: terrestrial plants, microorganisms and marine species. Terrestrial plants have long been exploited. It is estimated that ~10\% of the ~250,000 species of plants have been submitted to biological tests\(^11\). In comparison, the sea covers more than 70\% of the earth’s surface and hosts more than ten million species (estimated)\(^12\), but only a limited number of marine species have been used in
drug discovery. The major problem is the inaccessibility of those species. In the drug discovery process, significant quantities of compound are required. Typically, putting a lead on the development track requires ~100 grams of the pure compound. For valuable compounds produced by unculturable marine species that must be hand collected by scuba diving, acquiring such quantities is not feasible. With the limited source of supply, marine natural product research began slowly in the mid-20th century. Until now, only dozens of compounds were approved or in different phases of clinical trials; however, marine natural products have attracted researchers with their unusual chemistry, and have become a productive research area. Five of seven marine-derived commercial drugs were approved by the FDA in the past fifteen years. In addition to the technical advances in aquaculture and assay developments, plenty of preparative-scale total syntheses that produce sufficient quantities for biological studies have contributed to the recent success of marine pharmaceuticals. Synthetic analogues of marine natural products also provide exciting molecules and it is certain that organic synthesis will continue to play an important role in the studies of marine natural products.
1.2 References


http://marinepharmacology.midwestern.edu/clinPipeline.htm (accessed Dec 29, 2016)

CHAPTER 2

Synthesis of the 5,6-Dihydroxymorpholin-3-one Fragment of Monanchocidin A

Portions of this chapter were published in Organic Letters
Department of Chemistry, North Carolina State University;
Raleigh, North Carolina, 27695-8204.
2.1 Abstract

Monanchocidin A is a recently isolated pentacyclic guanidinium alkaloid that contains an unusual highly oxidized morpholinone fragment. Herein we report a rapid synthesis of this heterocyclic scaffold and confirm its structure. The key reaction involves an acid promoted hemiketalization/hemiaminalization of an α-hydroxyamide and α-ketoaldehyde that proceeds with exclusive regioselectivity and high diastereoselectivity to form the natural scaffold in moderate to high yield.

2.2 Introduction and Background

2.2.1 Polycyclic Guanidinium Alkaloids

Guanidine units are abundant in nature and can be found in the amino acid arginine (1) and the nucleobase guanine (2). Because of the basicity of guanidine (pKₐ = 13.6), its conjugate acid, guanidinium cation (3) binds with anionic substrates such as carboxylate or phosphate. This property most likely gives rise to the interesting bioactivities of guanine-containing natural products.¹⁻³ Among these, pentacyclic guanidinium alkaloids are a class of marine natural products that bear a (5,6,8b)-triazaperhydroacenaphthalene skeleton (4) and two hemiaminal rings. The first member of this class, ptilomycalin A (6) was isolated from the Caribbean sponge *Ptilocaulis spiculifer* and from a red *Hemimycale* sp. of the Red Sea in 1989.⁴ Since then, several families of pentacyclic guanidinium alkaloids have been discovered, namely, ptilomycalins,⁴,⁵ crambescidins,⁶⁻¹¹ fromiamycalin,⁸ celerimycalin,⁸ monanchocidins,¹²,¹³ monanchomycalins¹⁴,¹⁵ and normonanchocidins¹⁶. Most members of the pentacyclic guanidinium alkaloids share similar structural features (Figure 2.1): a pentacyclic
guanidinium core (5, 9 or 10) that connects a long hydrocarbon chain by an ester linkage on C-14, with an optional spermidine or spermidine-derived moiety on the other end of the long chain. Most members possess the same pentacyclic core 5, with a few exceptions: 13,14,15-isocrambescidin 800 (11) is the diastereomer of crambescidin 800 (7) with three different chiral centers in its pentacyclic core (9). Monanchocidins A, D and E feature one different spiro-ring in the pentacyclic core (10), but the stereochemistry is identical to 5.
Figure 2.1: Structures of pentacyclic guanidinium alkaloids
2.2.2 Synthesis and Application of Morpholine Derivatives

Morpholine is frequently employed in organic synthesis; most commonly functioning as a secondary amine in various transformations.\textsuperscript{17} For example, morpholine is a typical catalyst in Knoevenagel reactions where it readily condenses with an aldehyde to give an iminium salt intermediate, then a nucleophile attacks the activated C=N bond, followed by the elimination of morpholine from the adduct (Scheme 2.1).\textsuperscript{18}

\begin{center}
\textbf{Scheme 2.1}: The morpholine catalyzed Knoevenagel reaction
\end{center}

Morpholine derivatives find use as chiral auxiliaries in organic synthesis. For example, Williams and coworkers developed morpholines 12a and 12b, which are used in the asymmetric synthesis of amino acids (Scheme 2.2).\textsuperscript{17}
The C-substituted morpholine unit is present in a variety of biologically active compounds (Figure 2.2). For instance, the antidepressant drug reboxetine (13), the appetite suppressant drug phendimetrazine (14), the antifungal compound fenpropimorph (15), antibacterial compound 16, camptothecin analogue 17, and the α1-agonist 18. Bioactive morpholine derivatives are isolated from nature as well. Chenolin A (19), isolated from the marine sponge *Chelonaplysilla* sp., has antimicrobial activity against *Bacillus subtilis* and presented *in vivo* anti-inflammatory activity. The cytotoxic compound 20 was isolated from
the mussel *Mytilus galloprovincialis*.\textsuperscript{20} The antitumor compound C-1027 (21) was isolated from *Streptomyces globisporus* and it was believed to cause DNA double-strand scission.\textsuperscript{21}

**Figure 2.2.** Examples of biologically active C-substituted morpholine derivatives

Due to their applications and biological relevance, C-substituted morpholine derivatives have attracted significant synthetic interest. The synthesis of C-substituted morpholines has been achieved by employing various starting materials including amino alcohols, epoxides, organometallic reagents and others.
It is straightforward to consider an amino alcohol as a precursor to morpholine. Reaction of a 2-amino-ethanol derivative and a 2-chloro or 2-bromo acyl chloride or ester results in the formation of a morpholin-2-one or a morpholin-3-one, depending on the reactant or reaction condition. Certain 2-hydroxymorpholines can also be prepared from an amino alcohol and a 1-bromoketone (Scheme 2.3). Keto-aldehydes or keto-acids also react with amino alcohols to form the morpholine ring; and two routes were reported by the Merck laboratories for the synthesis of aprepitant (22), a human neurokinin-1 receptor antagonist, via this approach (Scheme 2.4). In the first route, keto-aldehyde 23 reacted with amino alcohol 24 to give hemiacetal 25, followed by imine formation and tautomerism, generating the morpholine 26. In the second route, amino alcohol 27 reacted with glyoxylic acid 28 to afford five-membered ring aminal 29, which readily rearranged to provide morpholine 30.

Scheme 2.3: C-substituted morpholine syntheses from amino alcohols
Scheme 2.4: Two different approaches to constructing the morpholine ring in apremitant (22)

Similarly, ring opening of an epoxide with an amine also gives an intermediate that undergoes intermolecular nucleophilic substitution to yield a morpholine. For example, (R)-2-benzyl-morpholine (32) was prepared from chiral epoxide 31 (Scheme 2.5). The synthesis of morpholine derivatives can also be accomplished from olefins or carboxylic acid derivatives, and these approaches are fundamentally similar to the approaches outlined above.
Scheme 2.5. A chemoenzymatic synthesis of (R)-2-benzyl-morpholine (32)

A palladium(0)-catalyzed tandem reaction of a π-allylpalladium complex was reported by Uozumi and coworkers. Chiral ligands on the palladium catalyst only led to poor to moderate yield and enantioselection of 2-vinyl-morpholine (33) (Scheme 2.6). The Trost research group also developed a two-step highly diastereoselective synthesis of morpholines 34a and 34b, with their route featuring a similar π-allylpalladium intermediate. The ratio of 34a:34b was influenced by the chiral ligands 35-37 on the catalyst and the nature of the nucleophile (the chiral amino ester), ranging from 1:14.4 (matched) to 1:2 (mismatched) to 22.7:1 (matched) (Scheme 2.7).
Scheme 2.6: Palladium(0)-catalyzed synthesis of 2-vinyl-morpholine (33)

Scheme 2.7: A two-step morpholine synthesis using chiral palladium catalyst

The Petasis reaction can also be utilized in C-substituted morpholine synthesis. This three-component reaction features an imine formation step, a ring closure step and an
alkylation step. Both the Berree group and the Xu group used the Petasis reaction in their morpholine syntheses (Scheme 2.8).  

Scheme 2.8: Applications of the Petasis reaction in morpholine syntheses

Morpholine ring formation via other organometallic intermediates, such as chromium-coordinated ketene and copper(II)-palladium(II) complexes were also reported.

2.3 The Morpholinone Fragment of the Marine Natural Product Monanchocidins

Pentacyclic guanidinium alkaloids are a family of marine natural products isolated from several species of marine sponges. These complex molecules have received significant interest from the scientific community due to their complex structures and diverse biological activity (details will be discussed in Chapter 4). In 2010 and 2011, a new pentacyclic guanidine family,
the monanchocidins, was isolated from the sponge *Monanhora pulchra* (Figure 2.2). Monanchocidins possess a spermidine-embeded and heavily oxidized morpholinone fragment, which is the most complex “anchor domain” observed in the pentacyclic guanidinium alkaloids to date and raises interesting questions regarding the potential role of this scaffold in the biological activity of the monanchocidins. In this chapter we report our synthetic approaches toward this morpholinone scaffold.

![Figure 2.3: The structures of monanchocidins A-E](image)

### 2.4 Our First Synthetic Plan towards the Target Molecule

In our first retrosynthetic plan (Figure 2.3), we proposed installing the aliphatic chain in the last step, because the molecule with the non-polar hydrocarbon chain and the morpholinone moiety with multiple polar functional groups would likely add additional complexity to initial reaction development. Considering this, our target molecule became morpholinone 38 with an allyl group at C-2, which would be converted to a long chain via olefin cross metathesis and hydrogenation, or other metal mediated C-C bond forming reactions. The hydroxyl group at C-5 would come from the regio- and stereoselective reduction
of a carbonyl group in compound 39, directed by the hydroxyl group at C-6. The morpholinone ring would be closed by hemiketal formation upon deprotection of keto alcohol (40). The A-values (-OH: 0.87 kcal/mol, -CH2CH3: 1.75 kcal/mol) indicated the C-6 hydroxyl group preferred to sit in the axial position. Thus, morpholinone 39 would be the thermodynamically preferred product (as observed in the natural product). Imide 40 would be formed from the reaction of acyl chloride 41 and amide 42, the latter arising from peptide coupling of diamine 43 and the alpha-keto acid 44.

Figure 2.4: Our first generation synthetic plan
According to this synthetic plan, we began a model study with the C-6 and C-2 substituents being an ethyl and a methyl group, respectively. TBS protection and hydrolysis\(^{35}\) of (-)-ethyl L-lactate (45) yielded carboxylic acid 46. The mono protected diamine 48 was prepared from Boc anhydride and excess 1,3-diaminopropane (47).\(^{36}\) Peptide coupling of the amine 48 and the commercially available 2-ketobutyric acid (49) with EDCI\(^{37}\) gave amide 50 in 62\% yield (Scheme 2.9).

![Scheme 2.9: Starting material synthesis for our first generation plan](image)

The carboxylic acid 46 was treated with oxalyl chloride and converted to the acyl chloride 51; however, reaction of acyl chloride 51 with amide 50 under basic conditions was not successful. We tested a number of bases and reaction conditions, but most of them only provided trace amount of imide 52 (Table 2.1) together with multiple side products even when the reaction was cooled to -78 °C. The best base was identified to be KHMDS, although we
were unable to isolate the product in high purity, possibly because imide 52 was unstable at room temperature or sensitive to silica gel.

**Table 2.1:** The reaction of amide 50 and acyl chloride 51 in the presence of base

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base used</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1 eq. LiHMDS</td>
<td>1 eq. 46, 1.1 eq. (COCl)$_2$, 0 °C - rt</td>
<td>Trace of 52 + side products</td>
</tr>
<tr>
<td>2</td>
<td>1.1 eq. LiHMDS</td>
<td>1 eq. 46, 1.1 eq. (COCl)$_2$, -78 °C - rt</td>
<td>Trace of 52 + side products</td>
</tr>
<tr>
<td>3</td>
<td>1.1 eq. LiHMDS</td>
<td>1 eq. 46, 1.1 eq. (COCl)$_2$, -78 °C, quench by HCl</td>
<td>Low yield of 52 (decomposed after quench)</td>
</tr>
<tr>
<td>4</td>
<td>1.1 eq. LiHMDS</td>
<td>1 eq. 46, 1.1 eq. (COCl)$_2$, -78 °C, quench by AcOH</td>
<td>Low yield of 52 + side products</td>
</tr>
<tr>
<td>5</td>
<td>1.1 eq. LiHMDS</td>
<td>3 eq. 46, 3.3 eq. (COCl)$_2$, -78 °C, quench by AcOH</td>
<td>Trace yield of 52 + side products</td>
</tr>
<tr>
<td>6</td>
<td>2.2 eq. LiHMDS</td>
<td>1 eq. 46, 1.1 eq. (COCl)$_2$, -78 °C, quench by AcOH</td>
<td>Trace yield of 52 + side products</td>
</tr>
<tr>
<td>7</td>
<td>2.2 eq. LiHMDS</td>
<td>1 eq. 46, 3 eq. (COCl)$_2$, -78 °C, quench by AcOH</td>
<td>Trace yield of 52 + side products</td>
</tr>
<tr>
<td>8</td>
<td>1.2 eq. NaHMDS</td>
<td>1.1 eq. 46, 1.2 eq. (COCl)$_2$, -78 °C,</td>
<td>Trace yield of 52 + side products</td>
</tr>
<tr>
<td>9</td>
<td>1.2 eq. KHMDS</td>
<td>1.1 eq. 46, 1.2 eq. (COCl)$_2$, -78 °C,</td>
<td>13% isolation yield of 52$^a$ + side products</td>
</tr>
<tr>
<td>10</td>
<td>1.5 eq. KHMDS</td>
<td>1.1 eq. 46, 1.2 eq. (COCl)$_2$, -15 °C,</td>
<td>Trace yield of 52$^a$ + side products</td>
</tr>
<tr>
<td>11</td>
<td>1.5 eq. LDA</td>
<td>1.1 eq. 46, 1.2 eq. (COCl)$_2$, -78 °C,</td>
<td>Trace yield of 52$^a$ + side products</td>
</tr>
<tr>
<td>12</td>
<td>1.8 eq. Et$_3$N</td>
<td>1.1 eq. 46, 1.2 eq. (COCl)$_2$, 5 mol% DMAP, rt</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

$^a$ Low purity. It may decompose during or after separation by silica gel flash chromatography
2.5 Our Second Generation Synthetic Plan

2.5.1 The Keto-imine Key Intermediate

The failure in preparing imide 52 forced us to rethink our synthetic strategy. Inspired by the imine addition reaction in Danishefsky’s synthesis of benzazepine derivatives (Scheme 2.10),\textsuperscript{38} we envisioned keto-imine intermediate 55 for the construction of the tertiary nitrogen center in our target molecule. The keto-imine 55, prepared from the condensation of keto-aldehyde 53 and primary amine 54, would add to acyl chloride 51, and the resulting iminium ion would be intercepted by a hydroxide group to form a hemiaminal 56 upon quenching with an aqueous base. Subsequent deprotection-intramolecular hemiketal formation would close the morpholinone ring 57 (Scheme 2.11).

Scheme 2.10: Imine addition in Danishefsky’s synthesis of benzazepine derivatives

Scheme 2.11: Our second generation synthetic plan featuring a keto-imine intermediate
Van Koten and coworkers reported that the reaction of simple primary amines and pyruvaldehyde (57) didn’t stop at the keto-imine (58) stage. Instead, the keto-imine reacted further to form diimines (59), with exceptions when the amine was bulky, such as tert-butyl amine or 1,1-dimethylpropyl amine (Scheme 2.12). There was no literature precedent for the condensation of more complicated keto-aldehydes (such as 53) and primary amines.

Scheme 2.12: Van Koten’s study on the diimine or keto-imine formation reactions

2.5.2 Synthetic Attempts toward Keto-aldehydes by Oxidation of Diols

To the best of our knowledge, there was no existing synthesis of 4-substituted-2-keto-butyraldehyde. In fact, only a limited number of methods were reported for the preparation of aliphatic keto-aldehydes. As the go-to method for preparing aldehydes, the oxidation of alcohols was first explored in our attempt to make keto-aldehydes. Initially, 1,2-butanediol (60) was subjected to Swern oxidation conditions. A new compound was observed by TLC but it became a mixture of byproducts after work-up. Treatment of the crude reaction with amine 58 didn’t yield any keto-imine or diimine that could be observed by LC-MS (Scheme 2.13).
Scheme 2.13: The Swern oxidation of 1,2-butanediol

The Swern oxidation of 4-phthalimido-1,2-butanediol (61) was also investigated (Scheme 2.14). Diol 61 was prepared from Mitsunobu reaction\(^\text{41}\) of phthalimide (62) and 3-buten-1-ol (63), followed by Upjohn dihydroxylation\(^\text{42}\) of alkene 64. To our surprise, only the secondary alcohol was oxidized when diol 61 was subjected to Swern oxidation with 2.2 eq. of oxalyl chloride, giving keto-alcohol 65 as the product. Increasing the amount of oxalyl chloride to 4.0 eq. led to intractable mixtures. These observations suggested that keto-aldehydes might be sensitive to Swern oxidation conditions. Side reactions were likely to occur upon addition of triethylamine and warming up the reaction to room temperature.

Dess-Martin periodinane\(^\text{43}\) (DMP, 66) was also employed in the oxidation of diol 60. The volatile products were believed to come from diol cleavage, a well-known process in which oxidants such as DMP (66) and PCC (67) cleave the C-C bond of a 1,2-diol though a cyclic intermediate.\(^\text{44}\) The precursor of DMP (66), 2-iodoxybenzoic acid (IBX, 68), was known for oxidizing vicinal diols without cleavage of the C-C bond.\(^\text{44}\) However, IBX also failed to
produce the keto-aldehyde from 1,2-butanediol (60) or 4-phthalimido-1,2-butanediol (61) (Scheme 2.15).

Scheme 2.14: The Swern oxidation of 4-phthalimido-1,2-butanediol (61)

Scheme 2.15: Oxidations of 1,2-diols by IBX and DMP
We protected diol 61 with TBS then selectively removed one TBS group in acetic acid buffered TBAP\textsuperscript{45} to reveal primary alcohol 69. Swern oxidation of alcohol 69 successfully produced aldehyde 70. Only trace amount of imine 71 was found by LC-MS when reacting aldehyde 71 with amine 48 over 3 Å molecular sieves (Scheme 2.16). We believe that the phthalimide protecting group may not be appropriate because it is electrophilic and sensitive to primary amines.\textsuperscript{46}

![Reaction Scheme](image)

Scheme 2.16: Imine formation of aldehyde 70 bearing a protected secondary alcohol

### 2.5.3 Synthetic Attempts to Prepare Keto-aldehydes by Riley Oxidation

In 1932, Riley prepared pyruvaldehyde (57) from selenium dioxide in refluxing acetone.\textsuperscript{47} This type of reaction that converts a methyl or methylene group activated by an adjacent double bond or carbonyl into a carbonyl or alkoxyl group using selenium (IV) dioxide is generally referred to as the Riley oxidation.\textsuperscript{48} Despite its long history, Riley oxidation has been applied in the preparation of dicarbonyl compounds in total synthesis for only a few times (Scheme 2.17), such as Gribble’s synthesis of sempervirine (72)\textsuperscript{49} and the syntheses of hamigeran B (73) by Trost\textsuperscript{50} and Mehta\textsuperscript{51}.  

---

27
We selected a number of methyl ketones and aldehydes and subjected them to Riley oxidations (Scheme 2.18), but none of them gave satisfactory results (Table 2.2). In some cases (entry 1, 2, 3, 6 and 7), we had evidence for the formation of keto-aldehyde products, including crude $^1$H NMR with a characteristic aldehyde peak at ~9 ppm (in CDCl$_3$), mass of the product or mono-hydrate of the product observed by LC-MS, and the observation of mass of the keto-imine when adding amine 48 to the crude Riley oxidation mixture; however, we were unable to isolate the keto-aldehyde in high purity, either by distillation or silica gel flash chromatography. The product underwent an unknown side reaction that degraded ~90% of the keto-aldehyde (entry 3, calculated by the $^1$H NMR integrations of the phthalimide hydrogens and the aldehyde hydrogen). It is noteworthy that the diimine was never found when reacting
the keto-aldehydes (entry 1, 2, 3, 6 and 7) with amine 48. Crude $^1$H NMR spectra (absence of the imine hydrogen peak at ~8 ppm by $^1$H NMR in CDCl$_3$) suggested the formation of cyclized aminal 74, which introduced considerable steric hindrance to potentially disfavor diimine formation (Scheme 2.19).

Scheme 2.18: Synthesis of the substrates for Riley oxidation
Table 2.2. Riley oxidation of methyl ketones or aldehydes

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et</td>
<td>1 eq. SeO$_2$, dioxane-H$_2$O, reflux</td>
<td>Crude 1H NMR showed an aldehyde peak, but the product was hard to isolate (by distillation or chromatography)</td>
</tr>
<tr>
<td>2</td>
<td>Et</td>
<td>5.5-6.5:1 ketone:SeO$_2$ (molar ratio), neat, reflux</td>
<td>Similar with entry #1 with slightly better yield</td>
</tr>
<tr>
<td>3</td>
<td>See below</td>
<td>1 eq. SeO$_2$, dioxane-H$_2$O, with or without AcOH (0.15 eq.), 90 °C or reflux</td>
<td>Product was formed, but chromatography resulted in decomposition</td>
</tr>
<tr>
<td>4</td>
<td>See below</td>
<td>1 eq. SeO$_2$, dioxane-H$_2$O, AcOH (0.15 eq.), 90 °C</td>
<td>Mixtures, product was not found</td>
</tr>
<tr>
<td>5</td>
<td>CH$_2$CH$_2$</td>
<td>1 eq. SeO$_2$, dioxane-H$_2$O, AcOH (0.15 eq.), 90 °C</td>
<td>Mixtures, product was not found</td>
</tr>
<tr>
<td>6</td>
<td>CH$_2$CH$_2$OTBDPS</td>
<td>1 eq. SeO$_2$, dioxane-H$_2$O, AcOH (0.15 eq.), 90 °C</td>
<td>Product was formed, but chromatography resulted in decomposition</td>
</tr>
<tr>
<td>7</td>
<td>CH$_3$CH$_2$NHBOc</td>
<td>1 eq. SeO$_2$, dioxane-H$_2$O, AcOH (0.15 eq.), 90 °C</td>
<td>Product was formed, but chromatography resulted in decomposition</td>
</tr>
<tr>
<td>8</td>
<td>CH$_3$CH$_2$N$_3$</td>
<td>1 or 2 eq. SeO$_2$, dioxane-H$_2$O, with or without 1.25 eq. AcOH, 50 °C or 80 °C</td>
<td>Mixtures, product was not found</td>
</tr>
</tbody>
</table>

**Scheme 2.19:** Possible intramolecular aminal formation
2.5.4 Other Methods to Prepare Keto-aldehydes

Hayashi and coworkers reported the preparation of 2-keto-butyraldehyde (77) using modified procedures, originally developed by Mikol and Russell. In this method, the deprotonated dimethyl sulfoxide (DMSO) attacked ethyl propionate (75), and the product, a hemimercaptal 76, subsequently underwent hydrolysis to provide 2-keto-butyraldehyde (77) (Scheme 2.20).

Scheme 2.20: Hayashi’s preparation of 2-keto-butyraldehyde

We attempted to apply this method in our synthesis of 4-substituted-2-keto-butyraldehyde. We chose three β-substituted ethyl propionates (78-80) as the substrates for this method (Scheme 2.21). Our investigation began with ethyl 3-(( tert-butoxycarbonyl)amino)propanoate (78), which was prepared from β-alanine (81) in two steps. Upon addition of sodium hydride in DMSO, the ester was converted to a mixture of compounds, but never formed the hemimercaptal. We thought the problem was the acidic hydrogen of the carbamate, so we tested the other two β-substituted ethyl propionates (69 and 80). Unfortunately, reaction of these two esters with sodium hydride in DMSO didn’t yield the desired hemimercaptal. Since the bromo and azido groups are good β-leaving groups, we suspected they underwent elimination reactions under basic conditions. Absence of the bromine characteristic mass patterns (1:1, [M]:[M+2]) in the crude reaction of ester 79 supported this hypothesis.
Scheme 2.21: Our attempts to prepare 4-substituted-2-keto-butyraldehyde from esters

Knochel and coworkers utilized Kornblum oxidation\(^\text{54}\) to make keto-aldehydes in their synthesis of coelenterazine\(^\text{55}\) (Scheme 2.22). To investigate if we could use this method in our synthesis of 4-substituted-2-keto-butyraldehyde, we prepared two nitrate esters (82 and 83) from methyl vinyl ketone in a few steps and subject them to Kornblum oxidation. In both reactions, no organic compounds were extracted (5x ether and 5x ethyl acetate) from the aqueous phase after work-up, and crude \(^1\)H NMR also indicated no aldehyde formation. However, treatment of nitrate esters 82 and 83 with NaOAc in CH\(_2\)Cl\(_2\)-DMSO (10:1) or THF-H\(_2\)O both resulted in aldehyde formation (aldehyde hydrogen observed by crude \(^1\)H NMR). This suggested that unlike typical Kornblum oxidation, DMSO was not the oxidant in this case. Instead, the aldehyde was formed by the base-catalyzed elimination of nitrate esters.\(^\text{56}\) We were unable to determine if the aldehyde was the desired keto-aldehyde, as purification by silica gel flash chromatography led to product decomposition, which was similar with the case of Riley
oxidation. Taking the crude of Kornblum oxidation directly to the next imine formation step gave a more complicated reaction mixture, wherein the mass of the keto-imine was not found by LC-MS (Scheme 2.23).

Scheme 2.22: Kornblum oxidation used in Knochel’s synthesis of coelenterazine

Scheme 2.23: Our synthetic attempts to convert nitrate esters to keto-aldehydes
2.6 α,β-Unsaturated Aldehydes as a Synthetic Equivalent of Keto-Aldehydes

To this point we had encountered problems in the preparation of keto-aldehydes. It became clear that the sensitive keto-aldehyde compound needed to be protected, i.e. the ketone carbonyl needed to remain latent. We chose α,β-unsaturated aldehydes as the synthetic equivalent of keto-aldehydes, as the methylene group can be cleaved by ozonolysis or Lemieux-Johnson oxidation in later steps to reveal the ketone carbonyl.

In our model study, methacrolein (84) was condensed with 3-azidopropan-1-amine (85) to give imine 86. The best yield was obtained in THF with 4 Å molecular sieves as the dehydrating agent. Because imine 86 didn’t behave well on silica gel, it was purified by Kugelrohr distillation (b.p. 75-80 °C, <1 mbar). Acyl chloride 87, prepared from glycolic acid, was reacted with imine 86. Quenching with aqueous base resulted in fast decomposition of the hemiaminal to the amide. To deal with the stability issue of hemiaminal 87, we quenched the reaction with methanol in the presence of triethylamine. The product, hemiaminal ether 88 was relatively stable and could be chromatographed on silica gel with a 51% isolated yield. Lemieux-Johnson oxidation successfully cleaved the terminal alkene moiety, giving compound 89 in high yield (Scheme 2.24).
Scheme 2.24: Our model study featuring an α,β-unsaturated imine and acyl chloride reaction

With the success in our model study, we decided to apply this method in the synthesis of our target structure (Scheme 2.25). 4-Azidobutanal (90) was prepared from THF in four steps according to Bate’s procedure. Mannich reaction of 4-azidobutanal (90), formaldehyde and pyrrolidine yielded the α,β-unsaturated aldehyde 91, which was condensed with 3-azidopropan-1-amine (85) under previously developed conditions (4 Å molecular sieves, THF) to form imine 92. Because of the high boiling point of imine 92, it cannot be purified by Kugelrohr distillation (decomposed ~100 °C). Imine 92 was partially converted back to aldehyde 91 on silica gel; however, to our satisfaction, flash chromatography on base washed
(10:1 hexanes:triethylamine, by volume), vacuum dried (120 °C, <1 mbar, 1 h) silica gel completely suppressed the hydrolysis.

Reaction of imine 92 and simple α-hydroxy acyl chloride 87 followed by methanol-triethylamine quench gave hemiaminal ether 93 in moderate yield. Lemieux-Johnson oxidation of compound 93 yielded the corresponding ketone 94. Ketone 94 was treated with TBAF in order to remove the silyl ether protecting group. The sole product showed terminal alkene characteristic peaks by 1H NMR. Since most commercial TBAF solutions are basic, we thought it might cause elimination of the azido group at the β-position, forming a terminal alkene 95. With this in mind, we buffered the TBAF solution with acetic acid and added it to compound 94. The rate of silyl ether removal slowed down but the same eliminated product was the major observed product. We also applied Phillips’ silyl ether deprotection method using a catalytic amount of TBAF in neutral K2HPO4 buffer58, but still obtained the same product.
While there are plenty of non-basic reagents for silyl ether deprotection, such as cesium fluoride (CsF), tetrabutylammonium difluorotriphenylsilicate (TBAT) or acids such as camphorsulfonic acid (CSA), we ceased our search for deprotection reagents because we discovered that the imine-acyl chloride reaction became problematic when the acyl chloride was bulky (Scheme 2.26). We prepared acyl chloride 97 from butyraldehyde in a few steps.
The reaction of acyl chloride 97 and imine 92 proceeded slowly, and eventually resulted in the decomposition of starting materials. Switching the solvent from dichloromethane to the more polar solvent acetonitrile didn’t help improve the yield. Considering that the TBDPS protecting group is particularly bulky in size, we prepared another acyl chloride 99, bearing a methyl group and a benzyl protected alcohol on its α-carbon. Acyl chloride 99 reacted with imine 92 and gave hemiaminal ether 100 in only 28% yield. To prepare our natural product, an allyl group was originally designed to be on the α-carbon. In addition, the protecting group on the alcohol was preferred to be a silyl ether (so it could be removed under mild conditions in the presence of the sensitive hemiaminal ether moiety), is sterically large. These restrictions forced the acyl chloride to be bulky, and hence, the acyl chloride-imine reaction was expected to be inefficient in the final synthetic route to the monanchocidins.
Scheme 2.26: Problematic reactions of imines with bulky acyl chlorides

2.7 Our Fourth Synthetic Plan – One Step Cyclization of Morpholinone Ring

From the outset of this project we analyzed the morpholinone fragment of the monanchocidins and came up with a straightforward retrosynthesis of this molecule. Breakage of the adjacent hemiaminal and hemiketal bonds would give the α-hydroxyamide 101 and keto-aldehyde 102. One question remained: if we reverse the process, reacting an α-hydroxyamide with a keto-aldehyde to form a hemiaminal bond and a hemiketal bond in one step, will it produce the correct regioisomer, or even the desired stereoisomer (Figure 2.4)?
Our investigations began with the model system where the commercially available pyruvaldehyde 57 was used. Amide 105 was prepared from the chiral amino acid pool. We selected L-phenylalanine (103) as our starting material (R = benzyl) because its A-value would be similar with the long hydrocarbon chain in the natural product, and its UV-active phenyl group would provide visualization for us to track the reaction progress. L-phenylalanine (103) was treated with nitrous acid then underwent hydrolysis to give (S)-2-hydroxy-3-phenylpropanoic acid (104). After protection of the hydroxyl group, the acid was coupled with 3-azidopropan-1-amine (85). Deprotection of silyl ether 96 provided chiral amide 95.
Reaction of amide 105 and pyruvaldehyde (57) was carried out under acidic conditions (Scheme 2.27). To our delight, our first condition, 0.5 M L-camphorsulfonic acid (L-CSA) in CH₂Cl₂ at room temperature, led to the formation of a morpholinone product. The reaction progressed slowly and after 2 days, a ~1:1 mixture of starting material 105 and morpholinone product 107 was obtained (based on the intensity of UV peaks on LC). The enantiomer of the acid, D-CSA, was used and gave the same result. Apparently, an equilibrium was established as no evident change was observed if the reaction was continued for one extra day. In addition, subjecting the isolated product 107 to the original reaction condition resulted in partial conversion back to the starting material 105; however, increasing the acid concentration to 2.0 M or increasing the amount of pyruvaldehyde to 5 eq. did not yield more product, which suggested this reaction did not reach a simple equilibrium. Switching the solvent to toluene and heating at 70 °C resulted in product decomposition. Our optimized condition (Table 2.3) was 3 eq. pyruvaldehyde and 1.0 M L-CSA, which provided morpholinone 107 in 29% isolated yield (45% yield based on recovered starting materials). Four different acids, TFA, HCl, p-TsOH and PPTS were mostly ineffective for the reaction. Screening of solvents revealed that toluene, acetonitrile, dioxane, and chloroform provided slightly better yield than dichloromethane, but only trace amount of product was formed in polar aprotic solvents such as DMF and no reaction was observed in polar protic solvents such as methanol. (Table 2.4)
Scheme 2.27: One-step morpholinone ring cyclization with two bonds formed

Table 2.3: Conditions used to form the morpholinone ring

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 eq. 57, 0.5 M CSA (5 eq.), CH₂Cl₂, rt, 2 d</td>
<td>About 1:1 ratio of 105 and 107</td>
</tr>
<tr>
<td>2</td>
<td>1 eq. 57, 1.0 M CSA (5 eq.), CH₂Cl₂, rt, 2 d</td>
<td>About 1:1 ratio of 105 and 107</td>
</tr>
<tr>
<td>3</td>
<td>3 eq. 57, 0.5 M CSA (5 eq.), CH₂Cl₂, rt, 2 d</td>
<td>About 1:1 ratio of 105 and 107</td>
</tr>
<tr>
<td>4</td>
<td>1 eq. 57, 0.5 M CSA (5 eq.), toluene, 70 °C, 2 d</td>
<td>Product decomposed</td>
</tr>
<tr>
<td>5</td>
<td>1 eq. 57, 0.5 M p-TsOH (5 eq.), CH₂Cl₂, rt, 2 d</td>
<td>Trace amount of 107</td>
</tr>
<tr>
<td>6</td>
<td>1 eq. 57, 10 mol% TFA, toluene, rt, 2 d</td>
<td>Trace amount of 107</td>
</tr>
</tbody>
</table>
Table 2.4: Acid and solvent screen of the cyclization reaction to form the morpholinone ring.

Extensive NMR studies were conducted to determine the structure of the morpholinone product 107. $^1$H NMR and $^{13}$C NMR showed only one set of peaks, indicating only one diastereomer was isolated. One-dimensional $^1$H NMR, $^{13}$C NMR and $^{13}$C DEPT data were consistent with the morpholinone structure 107 shown below. The chemical shifts were not identical with the natural product due to several structural differences, but they were reasonably close (Table 2.5). 2D NMR techniques were also employed. COSY and HMBC
experiments further confirmed the product was the correct regioisomer 107. Several HMBCs were able to rule out the possibility of the other regioisomer 108 (Figure 2.5).

**Table 2.5:** NMR data of morpholinone 107 and the morpholinone fragment of monanchocidin A.

<table>
<thead>
<tr>
<th>Atom #</th>
<th>¹H NMR</th>
<th>¹³C NMR</th>
<th>Atom #</th>
<th>¹H NMR</th>
<th>¹³C NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>126.0</td>
<td></td>
<td>5'</td>
<td>2 H, 1.73 (m)</td>
<td>31.9</td>
</tr>
<tr>
<td>2</td>
<td>5 H, 7.29-7.16 (m)</td>
<td>129.3</td>
<td>6'</td>
<td>1 H, 4.08 (dd, J = 3.6, 7.8 Hz)</td>
<td>70.8</td>
</tr>
<tr>
<td>3</td>
<td>128.0</td>
<td></td>
<td>7'</td>
<td>-</td>
<td>169.2</td>
</tr>
<tr>
<td>4</td>
<td>138.8</td>
<td></td>
<td>8'</td>
<td>1 H, 3.45 (m)</td>
<td>41.7</td>
</tr>
<tr>
<td>5</td>
<td>1 H, 3.16 (dd, J = 14.1, 2.8 Hz)</td>
<td>38.0</td>
<td>9'</td>
<td>2 H, 1.80 (m)</td>
<td>25.5</td>
</tr>
<tr>
<td>6</td>
<td>1 H, 2.88 (dd, J = 14.1, 9.9 Hz)</td>
<td></td>
<td>10'</td>
<td>2 H, 2.77 (m)</td>
<td>36.7</td>
</tr>
<tr>
<td>7</td>
<td>168.2</td>
<td></td>
<td>11'</td>
<td>1 H, 4.41 (d, J = 6.4 Hz)</td>
<td>80.9</td>
</tr>
<tr>
<td>8</td>
<td>1 H, 3.49 (dt, J = 13.8, 7.0 Hz)</td>
<td>42.1</td>
<td>11'-OH</td>
<td>1 H, 6.52 (d, J = 6.9 Hz)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1 H, 3.21 (dt, J = 13.7, 6.9 Hz)</td>
<td></td>
<td>12'-OH</td>
<td>1 H, 6.61 (s)</td>
<td>94.4</td>
</tr>
<tr>
<td>10</td>
<td>2 H, 3.33 (m)</td>
<td>48.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1 H, 4.32 (d, J = 8.2 Hz)</td>
<td>82.1</td>
<td>13'</td>
<td>1 H, 2.04 (m)</td>
<td>34.7</td>
</tr>
<tr>
<td>11-OH</td>
<td>1 H, 5.98 (d, J = 8.2 Hz)</td>
<td></td>
<td>14'</td>
<td>1 H, 2.93 (m)</td>
<td>34.3</td>
</tr>
<tr>
<td>12-OH</td>
<td>1 H, 6.42 (s)</td>
<td>94.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>3 H, 1.29 (s)</td>
<td>24.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Solvent: DMSO-d₆, ¹H NMR spectra are tabulated as follows: number of protons, chemical shifts, multiplicity and coupling constant(s) in Hz.
Figure 2.6: Key HMBCs ruled out the possible regioisomer 108.

The stereochemistry of morpholinone product 107 was determined by a NOESY experiment (Figure 2.6). First, the stereochemistry of C-2 was already known because it was originated from the chiral amino acid (L-phenylalanine). Using C-2 as the reference, we derived the stereochemistry of C-6. Correlation between C-2 H and C-6 OH was observed (arrow a) and correlation between C-2 H and C-6 CH₃ was absent. Also, C-6 CH₃ correlated with both C-5 OH and C-5 H (arrow b and c), indicating C-2 CH₃ must be sitting in the gauche conformation. The stereochemistry of C-5 was determined by the presence of C-6 OH and C-5 H correlation (arrow d). The C-5 epimer would not have such correlation because C-6 OH and C-5 H were sitting in anti conformation (arrow e).

Figure 2.7: Determination of stereochemistry of the morpholinone product 107.
With the success in constructing morpholinone ring 107 from amide 105 and pyruvaldehyde (57), our goal became the preparation of 4-substituted-2-keto-butyraldehyde again. We repeated the Riley oxidation of ketone 109 at two different temperatures with or without the addition of acetic acid. The four crude reaction mixtures were treated with amide 105 in 1.0 M L-CSA, separately. None of them yielded the desired product, morpholinone 110 (Scheme 2.28).

Scheme 2.28: Crude Riley oxidation product and 105 failed to give morpholinone 110.

We came up with some potential explanations that could explain the previous failure of preparing the 4-substituted-2-keto-butyraldehyde. The group on the β-carbon was likely to undergo elimination under harsh conditions, such as high temperature or strong base. The keto-aldehydes might be sensitive to silica gel so chromatography separation should be avoided. The product might be very hydrophilic as well, causing extraction problems when aqueous work-up was applied. We did a thorough search for keto-aldehyde preparation methods and found that the dimethyldioxirane (DMDO) oxidation of a diazo compound might be a viable way to circumvent all the issues above. This reaction proceeds under particularly mild conditions, and its sole byproduct, acetone, is volatile and easy to be removed from the system, so work-up is not necessary.
To begin our synthesis, 3-azidopropanoic acid (112) was prepared from 3-bromopropanoic acid (111), followed by conversion to its acyl chloride 113. After removing volatiles (including excess oxalyl chloride reagent), crude 113 was redissolved in acetonitrile and a diethyl ether solution of (trimethylsilyl)diazomethane was added. Concerning the sensitive nature of β-azido diazoketone 114, the reaction was kept cold at 0 °C. Moreover, after a quick evaporation of the solvents, the residue was cooled to -20 °C before loading on a silica gel cartridge, which was also cooled to -20 °C. The purified diazoketone 114 was stable enough for a few hours, allowing us to characterize this compound. Low temperature (-30 °C freezer) was required for its prolonged storage.

We also prepared an acetone solution of DMDO according to Adam’s procedure, and used it in the oxidation of diazo compound 114. The reaction was fast and efficient. After TLC indicated completion, the mixture was concentrated and immediately treated with amide 105 and CSA. Previously optimized conditions were employed (3 eq. 105, 1.0 M CSA in acetonitrile). The cyclization reaction proceeded analogous to the model system, providing the desired morpholinone product 110 in 41% yield (Scheme 2.29) along with a minor diastereomer (d.r. = 9:1 as determined by integrations of 1H NMR) and recovered starting material (69% brsm). 1D (1H, 13C) and 2D (COSY, HMBC, NOESY) NMR experiments confirmed that the major product 110 was the correct regioisomer and stereoisomer. Key HMBC and NOESY correlations that were used in the structural elucidation of compound 107 were also observed in compound 110 (Figure 2.7).
Scheme 2.29: Cyclization reaction of amide 105 and 4-azido-2-oxobutanal

Figure 2.8: Key HMBC and NOESY correlations of morpholinone 110

Analogous to the preparation of morpholinone 110, an allyl-functionalized morpholinone 115 was prepared (Scheme 2.30). The allyl moiety serves as a handle for future incorporation into the natural product. In the CSA-promoted cyclization step, chloroform turned out to be a better solvent than acetonitrile possibly due to solubility. A drop in diastereoselectivity was observed (allyl side chain dr = 4:1, benzyl side chain dr = 9:1), which can be attributed to the smaller size of an allyl group comparing to a benzyl group. We also revised our synthesis of the hydroxyamide without the protection of the hydroxyl group. By directly coupling the hydroxyacid with the primary amine, less steps and better overall reaction yield (46% vs. previously 34%, benzyl side chain) was achieved.
Scheme 2.30: Revision of the hydroxyamide synthesis and the preparation of an allyl-functionalized morpholinone 115.

At last, we needed to reduce the azido groups to primary amines. We first attempted the Staudinger reduction, but PPh₃ or polymer-supported PPh₃ both led to the degradation of the morpholinone structure, likely due to the basicity of the organophosphine reagents. Reduction of the bis-azide was accomplished via hydrogenation (1 atm) over Pd/C (10 mol%). In the case where an allyl side chain was present, more palladium catalyst (20 mol%) was required to reduce the alkene (Scheme 2.31).
**Scheme 2.31.** Reduction of the bis-azide and the allyl side chain.

Morpholinone 117 with an alkyl side chain shares many structural similarity with the morpholione fragment of monanchocidin A, and its $^1$H and $^{13}$C NMR data matched well with the literature (Table 2.6). This further confirmed the success of our synthesis.
Table 2.6: NMR data comparison of morpholinone 117 and the morpholinone fragment of monanchocidin A

![Diagram of 117 and morpholine fragment of monanchocidin A]

<table>
<thead>
<tr>
<th>Atom #</th>
<th>(^1)H NMR</th>
<th>(^{13})C NMR</th>
<th>Atom #</th>
<th>(^1)H NMR</th>
<th>(^{13})C NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 H, 0.87 (t, (J = 7.4) Hz)</td>
<td>14.7</td>
<td>1'</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>2 H, 1.38 (m)</td>
<td>19.0</td>
<td>2'</td>
<td>2 H, 1.22 (m)</td>
<td>28.9</td>
</tr>
<tr>
<td>3</td>
<td>2 H, 1.69 (m)</td>
<td>34.9</td>
<td>3'</td>
<td>2 H, 1.73 (m)</td>
<td>31.9</td>
</tr>
<tr>
<td>4</td>
<td>1 H, 4.10 (dd, (J = 7.4, 4.0) Hz)</td>
<td>71.5</td>
<td>4'</td>
<td>1 H, 4.08 (dd, (J = 7.8, 3.6) Hz)</td>
<td>70.8</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>170.1</td>
<td>5'</td>
<td>--</td>
<td>169.2</td>
</tr>
<tr>
<td>6</td>
<td>1 H, 3.28 (m)</td>
<td>42.6</td>
<td>6'</td>
<td>1 H, 3.25 (m)</td>
<td>41.7</td>
</tr>
<tr>
<td>7</td>
<td>1 H, 3.44 (m)</td>
<td>26.5</td>
<td>7'</td>
<td>2 H, 1.80 (m)</td>
<td>25.6</td>
</tr>
<tr>
<td>8</td>
<td>1 H, 2.77 (m)</td>
<td>37.6</td>
<td>8'</td>
<td>2 H, 2.77 (m)</td>
<td>36.7</td>
</tr>
<tr>
<td>9</td>
<td>1 H, 4.41 (s)(^b)</td>
<td>81.8</td>
<td>9'</td>
<td>1 H, 4.41 (d, (J = 6.4) Hz)</td>
<td>80.9</td>
</tr>
<tr>
<td>9-OH</td>
<td>Broad peak due to proton exchange</td>
<td>--</td>
<td>9'-OH</td>
<td>1 H, 6.52 (d, (J = 6.9) Hz)</td>
<td>--</td>
</tr>
<tr>
<td>10-OH</td>
<td>Broad peak due to proton exchange</td>
<td>95.4</td>
<td>10'-OH</td>
<td>1 H, 6.61 (s)</td>
<td>94.4</td>
</tr>
<tr>
<td>11</td>
<td>1 H, 1.92 (m)</td>
<td>35.6</td>
<td>11'</td>
<td>1 H, 1.94 (m)</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>1 H, 2.04 (m)</td>
<td>35.2</td>
<td></td>
<td>1 H, 2.04 (m)</td>
<td>34.7</td>
</tr>
<tr>
<td>12</td>
<td>2 H, 2.93 (m)</td>
<td>35.2</td>
<td>12'</td>
<td>2 H, 2.93 (m)</td>
<td>34.3</td>
</tr>
</tbody>
</table>

\(^a\)Solvent: DMSO-\(d_6\), \(^1\)H NMR spectra are tabulated as follows: number of protons, chemical shifts, multiplicity and coupling constants in Hz. \(^b\)A singlet was observed instead of a doublet due to proton-deuterium exchange of 9-OH. \(^c\)Both compounds are present as the bis(trifluoroacetate) salt of the structures shown above.
2.8 Outlook

2.8.1 Connecting the Morpholinone Fragment to the Guanidinium Core

We have prepared the morpholinone fragment with two different side chains – benzyl and allyl (reduced to n-propyl at the last step). Theoretically, our synthetic sequence can be applied with other amino acids as the starting material. The challenge becomes how to connect the morpholinone and the pentacyclic guanidinium part together. In general, we would like to make the connection before the azido groups are reduced. Because we observed that reduction of an alkene was slower than azides when performing the hydrogenation of 115, selective reduction of the azido groups while retaining the alkene moiety in the guanidinium core is possible with careful control (Scheme 2.32).

![Scheme 2.32: Proposed final steps of monanchocidin A synthesis](image)

2.8.2 Connection by Esterification

Due to a high degree of steric congestion around the carboxylate carbon, traditional peptide coupling reactions (carbodiimides, BOP reagents, T3P® etc.) which rely on the activation of the carboxylic acids are not favored. Overman and coworkers tackled this problem by using the carboxylate as a nucleophile (Scheme 2.33). They first examined a
Mitsunobu reaction and prepared the ester 118 in low yield. Later, they used primary alkyl iodides as electrophiles with base and silver nitrate as additives, furnishing the ester in good yields.

\[ \text{Scheme 2.33: Esterification using the carboxylate as a nucleophile} \]

The previous results in the Staudinger reaction indicated that bases can decompose the morpholinone ring; therefore, weakly basic conditions and low temperature should be considered for the esterification of the morpholinone.

### 2.8.3 Connection by Olefin Metathesis

Olefin metathesis reactions are widely employed when joining two complex fragments in organic synthesis;\textsuperscript{63} however, functional group compatibility, selectivity and reduction of the olefin product pose difficulties for application to the target natural product. No reaction was observed between an allyl-substituted morpholinone 119 and 1-tridecene (120) under the catalysis of several common Grubbs catalysts, while a hydroxylamine 121 was a suitable substrate (Scheme 2.34). This indicated the hemiacetal/hemiaminal scaffolds are likely to cause problems and need to be protected. Since terminal olefins undergo fast
homodimerization,\textsuperscript{64} one olefin partner must be in uneconomical excess for statistical cross metathesis to be successful. In addition, reducing the newly formed alkene in presence of the other alkene on the pentacycle will be hard to accomplish. Considering these factors, the metathesis reaction might only find applications in the synthesis of simplified monanchocidin analogs.

Scheme 2.34: Olefin cross metathesis reaction to install a linker group to 119 and 121

2.8.4 Connection by Alkyl-alkyl Cross Coupling

Despite the high demand for general methods of making $C(sp^3)-C(sp^3)$ bonds, alkyl-alkyl cross coupling reactions are underdeveloped as a result of 1) $C(sp^3)$-X bonds are more electron-rich than $C(sp^2)$-X bonds therefore they’re slower in the oxidative addition step. 2) In contrast, $\beta$-hydride elimination of $C(sp^3)$-M organometallic compounds is relatively fast.\textsuperscript{65} Suzuki and coworkers reported cross coupling reactions between primary alkyl iodides and alkyl 9-BBN boranes with Pd($\text{PPh}_3$)$_4$ as the catalyst.\textsuperscript{66} With the screening of different phosphine ligands, Fu expanded the substrate scope to alkyl bromides,\textsuperscript{67} chlorides\textsuperscript{68} and tosylates\textsuperscript{69}, tolerating functional groups including nitriles, esters, ketones, alcohols and amides
The Fu group also developed the alkyl-alkyl Suzuki coupling of boronic acids,\textsuperscript{70} nickel(II)-catalyzed alkyl-alkyl Suzuki coupling\textsuperscript{71} and alkyl-alkyl Negishi coupling reactions\textsuperscript{72}.

\textbf{Scheme 2.35:} Alkyl-alkyl coupling reactions pioneered by Suzuki and Fu.

It is desirable to engineer an alkyl-alkyl coupling reaction to connect the guanidine and the morpholinone fragments, since there would not be the problem of the selective reduction of an alkene as in the cross metathesis or alkyl-alkenyl cross coupling reactions. However, mild conditions must be employed due to the base and temperature sensitivity of the morpholinone. There have been reports of room temperature and/or mildly basic alkyl-alkyl cross coupling reactions,\textsuperscript{73} and it is straightforward to prepare non-basic organoborane species from morpholinone \textbf{115} which we already have access to (Scheme 2.36). Therefore, alkyl-alkyl coupling reactions are worth investigating for the side-chain functionalization of the allyl-substituted morpholinone.
2.9 Conclusion

In conclusion, we have developed a synthetic approach to the unusual and heavily oxidized morpholinone heterocycle contained in monanchocidin A. These initial studies have revealed key insights into the chemical properties and stability of these heterocycles and pave the way for their further study. Exploration into the role of these scaffolds in biology, particularly as probes to explore polyamine signaling pathways, is ongoing and will be reported in due course.

2.10 Supporting Information

General. All reactions were performed under an N$_2$ atmosphere and all glassware was dried in an oven at 125 °C for 2 h prior to use, unless otherwise noted. Reactions carried out at -78 °C employed a CO$_2$ / acetone bath. THF and CH$_2$Cl$_2$ were purified using an alumina filtration system.

Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F$_{254}$ plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), $p$-
anisaldehyde solution (2.5 mL of p-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H$_2$SO$_4$ in 100 mL of 95% EtOH), Bromocresol green solution (0.04 g of bromocresol green in 100 mL of absolute EtOH. Slowly drip in a 0.1 M solution of NaOH until the solution turned pale blue) or a KMnO$_4$ solution (1.5 g of KMnO$_4$, 10 g of K$_2$CO$_3$, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reaction were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 µm C18 50 x 2.10 mm). Flash chromatography on SiO$_2$ was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients.

Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Infrared spectra were determined on a Jasco FT/IR-4100 spectrometer. $^1$H and $^{13}$C NMR spectra were obtained on a Varian Mercury-VX 300 or a Varian Mercury-VX 400 instrument in CDCl$_3$ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard (CDCl$_3$ = 7.26 ppm). $^1$H NMR spectra were run at 300, 400 or 700 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, brs = broad singlet), number of protons, and coupling constant(s). $^{13}$C NMR spectra were run at 100 or 175 MHz using a proton-decoupled pulse sequence with a d$_1$ of 0 second unless otherwise noted, and are tabulated by observed peak. High-resolution mass spectra were obtained on a Thermo Fisher Scientific, Exactive Plus mass spectrometer using Heated Electrospray Ionization (HESI). Reactions “tracked by LC-MS” (Shimadzu LC-MS 2020 with Kinetex 2.6 µm C18 50 x 2.10 mm) indicate that we predicted the outcome of the reaction by analyzing the mass of products.
Due to the small scale we used in the development of synthetic routes, this method enables us to rapidly determine optimal reaction conditions.

\[
\begin{align*}
\text{(S)-2-Hydroxy-3-phenylproanoic acid (104).} & \quad \text{L-Phenylalanine (25.0 mmol, 4.13 g) was dissolved in 50 mL 1 M H}_2\text{SO}_4. \text{ The solution was cooled to 0 °C in an ice-water bath and an aqueous solution (30 mL) of sodium nitrite (150.0 mmol, 10.35 g) was slowly added over 3 h. After addition, the mixture was warmed to room temperature and stirred for 24 h. The mixture was saturated with NaCl and extracted with ether (2 x 30 mL). The organic layer was washed with water (2 x 30 mL) and brine (1 x 30 mL), concentrated in vacuo and the crude residue was recrystallized in ether-hexanes to afford the title compound as a white crystalline solid (2.95 g, 71%): ¹H NMR (400 MHz, CDCl}_3) & \delta 7.38 - 7.22 (m, 5 H), 4.53 (dd, 1 H, J = 7.2, 4.3 Hz), 3.22 (dd, 1 H, J = 14.0, 4.3 Hz), 3.01 (dd, 1 H, J = 14.0, 7.2 Hz). MS (ESI, [M-H\textsuperscript{+}]): 165.
\end{align*}
\]

\[
\begin{align*}
\text{(S)-2-Hydroxypent-4-enoic acid (122).} & \quad \text{L-Allylglycine (8.51 mmol, 1.00 g) was dissolved in 70 mL H}_2\text{O and 17.5 mL AcOH (4:1 v/v). The solution was cooled to 0 °C in an ice-water bath and an aqueous solution (12 mL) of sodium nitrite (17.02 mmol, 1.17 g) was added at a rate of 4 mL/h via a syringe injector. After addition, the reaction was quenched by addition of 8.7 mL methylamine (2.0 M in THF). The solution was then acidified to pH 2 by adding 6 M HCl, and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried (Na}_2\text{SO}_4) and concentrated in vacuo. The residue was purified by flash chromatography (89:10:1 CHCl}_3:MeOH:AcOH) to afford the title compound}
\end{align*}
\]
as a yellow oil (0.67 g, 68%): \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 5.91 - 5.75 (m, 1 H), 5.26 - 5.17 (m, 2 H), 4.37 (dd, 1 H, \textit{J} = 6.2, 4.4 Hz), 2.71 - 2.60 (m, 1 H), 2.56 - 2.45 (m, 1 H). MS (ESI, [M-H\textsuperscript{+}]): 115.

\textsuperscript{3}H₂N\textsuperscript{N₃} 3-Azidopropan-1-amine (85). 3-Bromopropylamine hydrobromide (3.2 g, 15 mmol) was dissolved in 10 mL water. An aqueous solution (15 mL) of sodium azide (3.2 g, 50 mmol) was added and the mixture was heated to reflux and stirred for 16 h. Ether (50 mL) was added and the mixture was cooled in an ice-water bath. KOH pellets (4 g) were slowly added while keeping the temperature below 10 °C. The organic layer was separated and the aqueous phase was extracted with ether (2 x 30 mL). The combined organic layers were dried (K₂CO₃) and concentrated to give the title compound as a yellow oil (1.44 g, 96%): \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 3.37 (t, 2 H, \textit{J} = 6.7 Hz), 2.80 (t, 2 H, \textit{J} = 6.8 Hz), 1.73 (p, 2 H, \textit{J} = 6.7 Hz), 1.45 (brs, 2 H). MS (ESI, [M+H\textsuperscript{+}]): 101.

\begin{center}
\textbf{(S)-N-(3-Azidopropyl)-2-hydroxy-3-phenylpropanamide} (105). 3-Azidopropan-1-amine (1.200 g, 12.00 mmol) was dissolved in 25 mL dichloromethane and cooled to 0 °C in an ice-water bath. (S)-2-Hydroxy-3-phenylpropanoic acid, 1.826 g, 11.00 mmol), 4-dimethylaminopyridine (0.670 g, 5.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (2.300 g, 12 mmol) was successively added. The mixture was stirred at 0 °C for 2 h, then it was warmed to room temperature and stirred for 16 h. The solvent was removed \textit{in vacuo} and the residue was dissolved in ethyl
acetate (60 mL) and water (12 mL). The organic phase was washed with saturated NaHCO$_3$ (2 x 30 mL) and water (2 x 30 mL), dried (Na$_2$SO$_4$) and concentrated in vacuo. The residue was purified by flash chromatography (17-85% ethyl acetate in hexanes gradient) to afford the title compound (1.265 g, 46%) as a colorless oil: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.38 - 7.23 (m, 5 H), 6.73 (brs, 1 H), 4.32 - 4.28 (m, 1 H), 3.40 - 3.18 (m, 5 H), 3.00 (d, 1 H, $J$ = 4.7 Hz), 2.90 (dd, 1 H, $J$ = 13.9, 8.1 Hz), 1.73 (p, 2 H, $J$ = 6.7 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 173.2, 137.1, 129.9, 129.0, 127.3, 73.1, 49.3, 41.1, 36.8, 29.0. IR (KBr): 3389, 2930, 2877, 2098, 1652, 1540, 1454, 1264, 1086, 745, 701 cm$^{-1}$. [α]$^D_{23}$ = -69.1 (c = 0.6, CH$_2$Cl$_2$). HRMS (ESI): calculated for C$_{12}$H$_{16}$N$_4$O$_2$Na [M+Na]$^+$, 271.1166; found 271.1161.

(S)-N-(3-Azidopropyl)-2-hydroxypent-4-enamide (123).

3-Azidopropan-1-amine (0.635 g, 6.34 mmol) was dissolved in 10 mL dichloromethane and cooled to 0 °C in an ice-water bath. (S)-2-Hydroxypent-4-enoic acid (0.670 g, 5.77 mmol), 4-dimethylaminopyridine (0.356 g, 2.88 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carboidiimide (1.228 g, 6.34 mmol) was successively added. The mixture was stirred at 0 °C for 2 h, then it was warmed to room temperature and stirred for 16 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (40 mL) and water (5 mL). The organic phase was washed with saturated NaHCO$_3$ (2 x 30 mL) and water (2 x 30 mL), dried (Na$_2$SO$_4$) and concentrated. The residue was purified by flash chromatography (17-85% ethyl acetate in hexanes gradient) to afford the title compound (0.493 g, 43%) as a colorless oil: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.78 (brs, 1 H), 5.86 - 5.74 (m, 1 H), 5.24 - 5.18 (m, 2 H), 4.14 (dd, 1 H, $J$ = 7.8, 4.0 Hz), 3.41 - 3.33 (m, 4 H), 2.72 - 2.65 (m, 1
H), 2.46 - 2.36 (m, 1 H), 1.81 (p, 2 H, \( J = 6.7 \) Hz). \(^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)) \( \delta \) 174.0, 133.5, 119.1, 71.2, 49.3, 39.3, 36.7, 28.9. \( \text{IR (KBr): 3352, 3080, 2938, 2099, 1652, 1539, 1438, 1262, 1071, 993, 919, 626 cm}^{-1}. \) \( [\alpha]_D^{21} = -41.4 \) (c = 0.88, CH\(_2\)Cl\(_2\)). \( \text{HRMS (ESI): calculated for C}_8\text{H}_{15}\text{O}_2\text{N}_4 [M+H]^+, 199.1190; found 199.1189.}

\[
(2S,5S,6R)-4-(3-Azidopropyl)-2-benzyl-5,6-dihydroxy-6-methylmorpholin-3-one \ (107).
\]

Amide 105 (120.5 mg, 0.4858 mmol) was dissolved in dichloromethane (2.5 mL), and a pyruvaldehyde (57) solution (40 wt. % in water, 1.46 mmol, 225 \( \mu\)L) and camphorsulfonic acid (564.2 mg, 2.429 mmol) were added, and stirred at room temperature for 48 h. The reaction was partitioned between brine (10 mL) and dichloromethane (10 mL), extracted with dichloromethane (2 x 10 mL) and the combined organic layers were dried (MgSO\(_4\)), concentrated \textit{in vacuo}, and purified by flash chromatography (17-85% ethyl acetate in hexanes gradient) to afford the title compound (93.7 mg, 29%, 45% brsm) as a colorless oil: \(^1\text{H NMR} \) (700 MHz, DMSO-\( d_6 \)) \( \delta \) 7.29 - 7.16 (m, 5 H), 6.42 (s, 1 H), 5.98 (d, 1 H, \( J = 8.2 \) Hz), 4.32 (d, 1 H, \( J = 8.2 \) Hz), 4.29 (dd, 1 H, \( J = 9.9, 2.7 \) Hz), 3.49 (dt, 1 H, \( J = 13.8, 7.0 \) Hz), 3.36 - 3.30 (m, 2 H), 3.21 (dt, 1 H, \( J = 13.7, 6.9 \) Hz), 3.16 (dd, 1H, \( J = 14.1, 2.7 \) Hz), 2.88 (dd, 1 H, \( J = 14.1, 9.9 \) Hz). 1.81 - 1.71 (m, 2 H), 1.29 (s, 3 H). \(^{13}\text{C NMR} \) (175 MHz, DMSO-\( d_6 \)) \( \delta \) 168.2, 138.8, 129.4, 128.0, 126.1, 94.4, 82.1, 72.1, 48.3, 42.1, 38.0, 26.8, 24.5. \( \text{IR (KBr): 3512, 3408, 3284, 3030, 2933, 2101, 1733, 1650, 1635, 1490, 1454, 1381, 1259, 1092, 939, 749, 703, 612, 543 cm}^{-1}. \) \( [\alpha]_D^{23} = -121.9 \) (c = 1.4, CH\(_2\)Cl\(_2\)). \( \text{HRMS (ESI): calculated for C}_{15}\text{H}_{20}\text{N}_4\text{O}_4\text{Na} [M+Na]^+, 343.1377; found 343.1374. \)
3-Azidopropionic acid (112). 3-Bromopropionic acid (3.82 g, 25.0 mmol) was dissolved in acetonitrile (40 mL), sodium azide (3.25 g, 50 mmol) was added to the solution, and the mixture was heated to reflux and stirred for 4 h. Acetonitrile was removed *in vacuo* and the resulting yellow residue was suspended in ethyl acetate (30 mL) and washed with 0.1 M HCl (50 mL). The aqueous phase was extracted with ethyl acetate (2 x 30 mL), the combined organic layer was washed with water (40 mL) and brine (40 mL), dried (Na$_2$SO$_4$), and concentrated *in vacuo* to afford the title compound (1.38 g, 48%) as a yellow liquid: $^1$H NMR (400 MHz, CDCl$_3$) δ 3.59 (t, $J_1$ = 6.4 Hz, 2 H), 2.63 (t, $J_1$ = 6.4 Hz, 2 H).

4-Azido-1-diazobutan-2-one (114). 3-Azidopropionic acid (112) (460.1 mg, 4.000 mmol) was dissolved in 5 mL dichloromethane with a catalytic amount of DMF (one drop). Oxalyl chloride (6.000 mmol, 761.6 mg, 515 µL) was slowly added to the solution and the mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo*, the residue was dissolved in acetonitrile (25 mL), cooled to 0 °C in an ice-water bath, and trimethylsilyldiazomethane (2 mL, 2 M in ether) was slowly added and the mixture was stirred at 0 °C for 1 h. The solvent was evaporated *in vacuo* and the residue was cooled to -20 °C before loading onto a silica gel cartridge, which was also cooled to -20 °C. Flash chromatography was performed at room temperature (17-55% ethyl acetate in hexane gradient) to provide the title compound (394.9 mg, 71%) as a yellow oil: $^1$H NMR (400 MHz, CDCl$_3$) δ 5.33 (brs, 1 H), 3.65 - 3.55 (m, 2 H), 2.61 - 2.52 (m, 2 H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 191.7, 55.7, 46.9, 39.8. IR (KBr): 3095, 2939, 2104, 1736, 1638, 1445, 1384, 1325,
HRMS (ESI): calculated for C₄H₆ON₅ [M+H⁺], 140.0567; found 140.0566.

4-Azido-2-oxobutanal (124). The acetone solution of dimethyldioxirane (DMDO) was prepared according to Adam’s procedure (concentration was determined by thioanisole assay, and was found to be 62 μmol/mL). The DMDO-acetone solution (30.3 mL, 1.88 mmol) was added to diazo compound 114 (1.88 mmol, 55.6 mg) in several portions. Gas evolution was observed. After the reaction was complete (monitored by TLC), it was concentrated in vacuo to afford the crude title compound which was used immediately in the next step.

(2S,5S,6R)-6-(2-Azidoethyl)-4-(3-azidopropyl)-2-benzyl-5,6-dihydroxymorpholin-3-one (110). Amide 105 (99.2 mg, 0.400 mmol) was dissolved in acetonitrile (2 mL), 4-azido-2-oxobutanal (124) (1.2 mmol) and camphorsulfonic acid (464.6 mg, 2.000 mmol) were added. The mixture was stirred at room temperature for 48 h, partitioned between brine (10 mL) and dichloromethane (10 mL), and extracted with dichloromethane (2 x 10 mL). The combined organic layers were dried (MgSO₄), concentrated in vacuo and the residue was purified by flash chromatography (17-85% ethyl acetate in hexanes gradient) to afford the title compound (61.3 mg, 41%, 69% brsm) as a light yellow oil: ¹H NMR (700MHz, DMSO-d₆) δ 7.28 - 7.17 (m, 5 H), 6.52 (s, 1 H), 6.20 (d, 1 H, J = 7.8 Hz), 4.39 (d, 1 H, J = 7.8 Hz), 4.28
(dd, 1 H, \( J = 9.9, 2.8 \) Hz), 3.51 - 3.45 (m, 1 H), 3.36 - 3.31 (m, 4 H), 3.25 - 3.20 (m, 1 H), 3.15 (dd, 1 H, \( J = 14.2, 2.8 \) Hz), 2.90 (dd, 1 H, \( J = 14.2, 9.9 \) Hz), 2.04 - 1.99 (m, 1 H), 1.91 - 1.84 (m, 1 H), 1.80 - 1.72 (m, 2 H). \(^{13}\)C NMR (175 MHz, DMSO-d\(_6\)) \( \delta \) 168.0, 138.6, 129.4, 128.0, 126.1, 94.6, 80.9, 72.0, 48.3, 45.8, 42.3, 37.9, 35.6, 26.8. IR (KBr): 3508, 3350, 2936, 2100, 1732, 1637, 1491, 1454, 1259, 1088, 749, 703 cm\(^{-1}\). \([\alpha]D^{21} = -74.7 \) (c = 1.2, CH\(_2\)Cl\(_2\)). HRMS (ESI): calculated for C\(_{16}\)H\(_{21}\)N\(_7\)O\(_4\)Na \([M+Na]^+\), 398.1547; found 398.1544.

\((2S,5S,6R)-6-(2\text{-Aminoethyl})-4-(3\text{-aminopropyl})-2\text{-benzyl}-5,6\text{-dihydroxymorpholin-3-one} \quad (116)\).

Bisazide 115 (37.5 mg, 0.100 mmol) was dissolved in 2 mL methanol. Palladium (10 wt. % on carbon, 10.6 mg, 0.0100 mmol) was added and the mixture was stirred under H\(_2\) (1 atm) at room temperature for 1 h. The reaction mixture was filtered, the filtrate was concentrated \textit{in vacuo} and subjected to reversed-phase chromatography (100-85% water in acetonitrile, with 0.1 % TFA additive). Fractions containing the bis(trifluoroacetate) salt of 116 was lyophilized to give a pale yellow oil (27.2 mg, 58%): \(^1\)H NMR (400MHz, DMSO-d\(_6\)) \( \delta \) 7.82 - 7.62 (m, 6 H), 7.32 - 7.17 (m, 5 H), 4.41 (s, 1 H), 4.31 (dd, 1 H, \( J = 9.9, 2.9 \) Hz), 3.53 - 3.44 (m, 1 H), 3.33 - 3.24 (m, 1 H), 3.18 (dd, 1 H, \( J = 14.2, 2.9 \) Hz), 2.93 (dd, 1 H, \( J = 14.1, 9.9 \) Hz), 2.90 - 2.70 (m, 4 H), 2.04 - 1.87 (m, 2 H), 1.86 - 1.77 (m, 2 H). \(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) \( \delta \) 13C NMR (100 MHz, DMSO) \( \delta \) 169.4, 160.1, 159.7, 159.4, 159.0, 139.4, 130.3, 129.1, 127.2, 121.3, 118.4, 115.5, 64
112.6, 95.5, 81.9, 72.8, 42.9, 38.8, 37.6, 35.6, 35.2, 26.5. \textbf{IR} (KBr): 3411, 3265, 2920, 2854, 2359, 2341, 1682, 1636, 1436, 1204, 1133, 1028, 839, 800, 723, 668 cm\(^{-1}\). [\(\alpha\)]\(D\)^{21} = -25.2 (c = 0.17, CH\(_3\)OH). HRMS (ESI): calculated for C\(_{16}\)H\(_{25}\)N\(_3\)O\(_4\) [M+H\(^+\)], 324.1918; found 323.1922.

\((2S,5S,6R)-2\text{-Allyl-6-(2-azidoethyl)-4-(3-azidopropyl)-5,6-dihydroxymorpholin-3-one} \ (115)\). Amide \textbf{123} (125.0 mg, 0.628 mmol) was dissolved in chloroform (3 mL), 4-azido-2-oxobutanal (124) (1.884 mmol) and camphorsulfonic acid (696.9 mg, 3.000 mmol) were added. The mixture was stirred at room temperature for 48 h, partitioned between brine (10 mL) and dichloromethane (15 mL), and extracted with dichloromethane (2 x 15 mL). Excessive keto-aldehyde was removed by adding aldehyde scavenger \textit{N}-benzylethylenediamine (40 \(\mu\)L) to the combined organic layers. The resulting solution were dried (MgSO\(_4\)), concentrated \textit{in vacuo} and the residue was purified by flash chromatography (17-75\% ethyl acetate in hexanes gradient) to afford \textbf{115} (67.1 mg, 33\%, 58\% brsm) as a light yellow oil: \textbf{\(\text{\textit{H NMR}}\)} (400MHz, DMSO-d\(_6\)) \(\delta\) 6.51 (s, 1 H), 6.32 (d, 1 H, \(J = 7.4\) Hz), 5.94 - 5.79 (m, 1 H), 5.12 - 4.97 (m, 2 H), 4.39 (d, 1 H, \(J = 7.5\) Hz), 4.14 (dd, 1 H, \(J = 7.8, 3.8\) Hz), 3.49 - 3.30 (m, 3 H), 3.25 - 3.17 (m, 1 H), 2.56 - 2.37 (m, 2 H), 3.15 (dd, 1 H, \(J = 14.2, 2.8\) Hz), 2.90 (dd, 1 H, \(J = 14.2, 9.9\) Hz), 2.07 - 1.98 (m, 1 H), 1.97 - 1.88 (m, 1 H), 1.80 - 1.71 (m, 2 H). \textbf{\(\text{\textit{C NMR}}\)} (100 MHz, DMSO-d\(_6\)) \(\delta\) 168.9, 135.8, 117.7, 95.5, 81.8, 71.6, 49.3, 46.7, 43.1, 37.0, 36.5, 27.7. \textbf{IR} (KBr): 3337, 3082, 2938, 2879, 2100, 1638, 1557, 1488, 1290, 1082, 921,
654, 557 cm\(^{-1}\).  \([\alpha]D^{21} = -45.3 \text{ (c = 1.36, CH}_2\text{Cl}_2\). \textbf{HRMS} (ESI): calculated for C\(_{12}\)H\(_{20}\)O\(_4\)N\(_7\) [M+H\(^+\)], 326.1571; found 326.1567.

(2S,5S,6R)-6-(2-Aminoethyl)-4-(3-aminopropyl)-5,6-dihydroxy-2-propylmorpholin-3-one (117). Bisazide 115 (32.5 mg, 0.100 mmol) was dissolved in 2 mL methanol. Palladium (10 wt. % on carbon, 10.6 mg, 0.0100 mmol) was added and the mixture was stirred under H\(_2\) (1 atm) at room temperature for 1 h. The reaction mixture was filtered, the filtrate was concentrated \textit{in vacuo} and subjected to reversed-phase chromatography (100-85% water in acetonitrile, with 0.1 % TFA additive). Fractions containing the bis(trifluoroacetate) salt of 117 was lyophilized to give a pale yellow oil (36.5 mg, 73\%): \textbf{\(^1\)H NMR} (400 MHz, DMSO-\textit{d}_6) \(\delta\) 7.81 - 7.64 (m, 6 H), 4.41 (s, 1 H), 4.10 (dd, 1 H, \(J = 7.4, 4.0\) Hz), 3.49 - 3.39 (m, 1 H), 3.33 - 3.23 (m, 1 H), 2.99 - 2.87 (m, 2 H), 2.83 - 2.70 (m, 2 H), 2.09 - 1.98 (m, 1 H), 1.98 - 1.87 (m, 1 H), 1.86 - 1.76 (m, 2 H), 1.74 - 1.64 (m, 2 H), 1.48 - 1.28 (m, 2 H), 0.87 (t, 3 H, \(J = 7.4\) Hz). \textbf{\(^{13}\)C NMR} (100 MHz, DMSO-\textit{d}_6) \(\delta\) 170.1, 95.4, 81.8, 71.5, 42.6, 37.6, 35.6, 35.2, 34.9, 26.5, 19.0, 14.7. \textbf{IR} (KBr): 3067, 2967, 2359, 2341, 1678, 1643, 1490, 1435, 1202, 1137, 1085, 944, 841, 800, 723 cm\(^{-1}\).  \([\alpha]D^{22} = -22.6 \text{ (c = 0.34, CH}_3\text{OH)}. \textbf{HRMS} (ESI): calculated for C\(_{12}\)H\(_{26}\)O\(_4\)N\(_5\) [M+H\(^+\)], 276.1918; found 276.1916.
2.11 References


CHAPTER 3

Stereocontrolled Synthesis of (+)-Plagiogyrin A

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Raleigh, North Carolina, 27695-8204.
3.1 The Two Faces of Reactive Oxygen Species

Reactive oxygen species (ROS) such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (·OH) are byproducts of aerobic metabolism from the incomplete reduction of oxygen. Due to their high reactivity, ROS can intrinsically damage macromolecules including lipids, proteins and DNA. In the 1950s, Denham Harman put forth the free-radical theory of aging, which states that aging is caused by accumulation of free radical damages (later, “free radicals” has been expanded to include other ROS such as H$_2$O$_2$). Although recently this theory has been challenged, evidence suggests that ROS are tightly related with many age-related diseases such as neurodegenerative diseases, cardiovascular diseases and cancer.

On the other hand, other than inducing pathology, ROS are also capable of regulating cellular processes by serving as signaling molecules (Figure 3.1). The cysteine residue is the archetypal redox-regulator because 1) It can cycle between different stable redox states (from/to sulfenic acids (-SOH), disulfides (-S-S-), sulfenamides (-SNR$_2$), etc), and 2) Its chemical reactivity is dependent on the protein environment, allowing specific oxidation of cysteine residues in vivo. During cysteine-based redox signaling, the thiol/thiolate residue (Cys-SH) is oxidized to sulfenic acid (Cys-SOH) by H$_2$O$_2$, causing conformational change which leads to allosteric regulation of protein function. The sulfenic form can be reduced by the disulfidereductases thioredoxin (Trx) and glutaredoxin (Grx) to its original thiol form. It appears that nature has used redox signaling to adapt changes in environmental nutrients and the oxidative environment.
3.2 Antioxidant Effects of Natural Phenolic Compounds

Terrestrial plants are a rich source of phenol containing secondary metabolites, where a significant diversity of phenolic compounds, such as phenolic acids, flavonoids, quinones, coumarins, lignans, tannins and stilbenes, can be found (Figure 3.2b). Biosynthetically, the common precursor to most simple phenols is \( p \)-coumaric acid, which is the amino elimination and hydroxylation product of phenylalanine (Figure 3.2a).\(^6\) Phenolic compounds can play a role in the defense system of plants. Some phenolic compounds display toxic activities against fungal and bacterial pathogens, as well as other invading organisms.\(^7\) Some phenolic compounds serve as antioxidants and protect the host from oxidative stress. While they can naturally react and scavenge ROS, phenolic antioxidants can also complex with low valent metal ions like \( \text{Fe}^{2+} \) and \( \text{Cu}^+ \), which are the substrates for the Fenton’s reaction (\( \text{Cu}^+/\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+}/\text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^- \)), therefore inhibit the generation of the more harmful hydroxyl...
radical. Some evidence suggested that consumption of food with rich phenolic antioxidants is linked with reduced risk of cancer, cardiovascular disease, diabetes and other age-related diseases. While the direct beneficial effect of phenolic contents is under debate, numerous natural phenolic compounds are isolated and found to possess anti-inflammatory, antitumor, antibacterial or antiviral activities. Efforts involving synthetic chemistry are required to explore their potential therapeutic use.

**Figure 3.2:** a) Biosynthetic origin of simple phenols; b) Examples of terrestrial phenols (their corresponding classes are shown in parentheses)
3.3 Introduction to Plagiogyrin A

Ferns of the genus Plagiogyria are mainly found in Asia and select Plagiogyria species have been employed in traditional Chinese medicine to treat flu symptoms. Studies have shown that Plagiogyria maxima and Plagiogyria distinctissima are rich sources of phenolic contents (0.62 and 0.95 weight percent) and they display moderate radical scavenging ability (IC\textsubscript{50} = 22.1 and 57.4 μg/mL, respectively). Phenol-containing natural products plagiogyrin A (1), plagiogyrin B (2) and astragalin (3) were isolated from the fronds of Plagiogyria matsumureana by Murakami in 1983 (Figure 3.3). Biosynthetically, 1 is proposed to derive from the hemiacetal rearrangement of plagiogyrin B (Figure 3.3), however, to date no biosynthetic or synthetic studies toward 1 have been reported. Building on our interest in heavily oxidized heterocycles, we targeted an expedient and stereoselective synthesis of 1 and our successful efforts towards this goal are reported in this chapter.

Figure 3.3: Structures of phenol-containing natural products from Plagiogyria matsumureana.
3.4 Retrosynthetic Analysis

The 1,4-dioxane ring of plagiogyrin A features two hemiacetals, retrosynthetically arising from an α-ketoaldehyde and a diol (4, Figure 3.4a). Due to the sensitive nature of α-ketoaldehydes, methods to prepare these intermediates from 1,2-diols prove to be difficult on functionalized substrates. In the previous chapter, we examined methods such as the Riley (selenium dioxide) oxidation and oxidations of α-keto hemimercaptals or nitrate esters to prepare α-ketoaldehydes (Figure 3.4b); however, these methods also tend to employ harsh conditions that can interfere with other functional groups and can provide over-oxidized products in some cases. In our previous synthetic efforts toward the morpholinone fragment of monanchocidin A, we found the dimethyldioxirane (DMDO) oxidation of an α-diazoketone offered a mild and efficient way to prepare α-ketoaldehydes in complex settings; therefore, we envisioned this approach for our synthesis of 1.

Figure 3.4: a) Retrosynthesis of the cyclic bis-hemiacetal of 1; b) Common methods to prepare α-ketoaldehydes.
Retrosynthetically, this approach would require carboxylic acid 5 that we proposed could arise from lactonization of compound 6, itself derived from a stereocontrolled aldol reaction of ester 7 and aldehyde 8 (Figure 3.5).

![Figure 3.5: Retrosynthetic approach to diol 4.](image)

### 3.5 Stobbe Condensation

To install the benzylidene moiety in 7, we employed the Stobbe condensation because it is expected to give the desired E-alkene and it differentiates the two carboxyl groups, allowing selective manipulation. The mechanism of the Stobbe condensation features a γ-butyrolactone intermediate. Due to steric effect, formation of an E-alkene is favored. The ester adjacent to the alkene is retained while the other ester is hydrolyzed (Figure 3.6).
Our synthesis began with the Stobbe condensation of dimethyl succinate (9) and 4-anisaldehyde (10). A mixture of both reactants was added in a solution of 'BuOK in 'BuOH at room temperature, then it was heated at reflux for 1 h; however, mass analysis of the product indicated that the condensation happened twice, and yielded 11 as the major product (Entry 1, Table 3.1). Solvent-free conditions,\textsuperscript{22} with portion-wise addition of base or adding base in one portion, were used but gave the same overreacted product 11 (Entry 2 and 3, Table 3.1). Other bases such as NaOMe in MeOH or 'BuONa in 'BuOH resulted in no reaction (Entry 4 and 5, Table 3.1). Finally, addition of both reactants slowly into a solution of 'BuOK in 'BuOH at elevated temperature successfully produced the desired product 12 (Entry 6, Table 3.1). It is
important to note that the reaction should be kept free from moisture otherwise some of the product 12 would be hydrolyzed to bis-acid 13.

**Table 3.1:** Conditions for the Stobbe condensation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Condition</th>
<th>Result*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>tBuOK in tBuOH</td>
<td>add both reactants to base at rt, then heat to reflux for 1h</td>
<td>major product is 11</td>
</tr>
<tr>
<td>2</td>
<td>tBuOK in tBuOH</td>
<td>grind at room temperature for 10 min (solvent-free), base added in one portion</td>
<td>major product is 11</td>
</tr>
<tr>
<td>3</td>
<td>tBuOK in tBuOH</td>
<td>grind at room temperature for 10 min (solvent-free), base added portion-wise</td>
<td>major product is 11</td>
</tr>
<tr>
<td>4</td>
<td>NaOMe in MeOH</td>
<td>add both reactants to base at rt, then heat to reflux for 1h</td>
<td>no extensive reaction</td>
</tr>
<tr>
<td>5</td>
<td>tBuONa in tBuOH</td>
<td>add both reactants to base at rt, then heat to reflux for 1h</td>
<td>no extensive reaction</td>
</tr>
<tr>
<td>6</td>
<td>tBuOK in tBuOH</td>
<td>add both reactants slowly (30 min) to base at 100 °C, then heat to reflux for 0.5h</td>
<td>12b, 88% isolated yield</td>
</tr>
</tbody>
</table>

* determined by LCMS unless otherwise noted  
  b product 12 is partially hydrolyzed to 13 if tBuOH is not dry

### 3.6 Aldol Reaction

With the Stobbe condensation product 12 in hand, we attempted the aldol reaction of 12 and aldehyde 14 with 2.2 eq. of LDA to deprotonate the carboxylic acid and the enol; however, only a small amount of the aldol product was observed, along with two side reactions: ester hydrolysis of starting material 12 and elimination of aldol product 15. We explored a variety of bases and aldehydes but could not prevent these side reactions (Table 3.2). The
proximity of the carboxylate anion in our enolate substrate is likely responsible for the complex reaction mixtures.

**Table 3.2: Conditions for the aldol condensation between 12 and aldehydes.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>R-CHO</th>
<th>Result¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LDA (2.2 eq.)</td>
<td>(14)</td>
<td>small amount of desired product 15 and two byproducts 13 and 16</td>
</tr>
<tr>
<td>2</td>
<td>LHMDS (2.1 eq.)</td>
<td>PhenylCHO</td>
<td>starting material and some hydrolyzed product 13</td>
</tr>
<tr>
<td>3</td>
<td>KHMD (2.4 eq.)</td>
<td>PhenylCHO</td>
<td>small amount of desired product 15 and two byproducts 13 and 16</td>
</tr>
<tr>
<td>4</td>
<td>tBuOK (2.4 eq.)</td>
<td>PhenylCHO</td>
<td>small amount of desired product 15 and two byproducts 13 and 16</td>
</tr>
<tr>
<td>5</td>
<td>NaN₃ (2.2 eq.)</td>
<td>PhenylCHO</td>
<td>mostly hydrolyzed product 13</td>
</tr>
</tbody>
</table>

* unless otherwise noted  
¹ determined by LCMS

To avoid the formation of dianions, we protected 12 as a TBDPS ester. Aldol reaction of the bis-ester 17 proceeded with improved, but still poor, yield. Removal of the TBDPS protecting group with TBAF was rapid, but also resulted in hydroxyl elimination of the aldol product 18, potentially due to the basicity of this reagent. Acid-promoted deprotection (such as p-TsOH) was not ideal in the presence of the acid-labile acetonide group. Considering that, bis-ethyl ester 23 was prepared from Stobbe condensation and esterification of diethyl
succinate 21. Treatment of 23 and 14 with LHMDS yielded an inseparable mixture of at least two diastereomers of the aldol product 24 in good yield. Two most abundant diastereomers were found to be in 2.65:1 ratio based on $^1$H-NMR integrations (Scheme 3.1). At this stage it was difficult to determine which isomer was the desired product; therefore ester 24 was carried forward and its stereochemistry would be analyzed later.

Scheme 3.1: Successful aldol reactions of bis-esters 17 and 23.
When bisethyl ester 24 was subjected to various inorganic bases, one ethyl group was hydrolyzed, together with an unexpected loss of water (observed by LC-MS) even under carefully controlled conditions. Crude $^1$H-NMR data suggested the hydroxyl group underwent elimination instead of lactonization. Further treatment of 25 with excessive amount of base hydrolyzed the second ethyl ester (Figure 3.7a). We proposed the mechanism of the first step resembles the Stobbe condensation (Figure 3.7b) which involves a lactone intermediate.

![Possible mechanism of hydrolysis and lactonization](image)

**Figure 3.7:** a) Hydrolysis of ester 24; b) Possible mechanism of formation of alkene 25

We also tried to lactonize the bisethyl ester 24 by adding base (NaH) but obtained complex mixtures (Scheme 3.2).
Facing difficulties when hydrolyzing the bisethyl ester, a two-step hydrolysis strategy (Figure 3.8) was explored. By installing an orthogonal protecting group (ester) on the Stobbe condensation product 12 and removing it after the aldol reaction, the acidity of the α-proton will be greatly reduced because of the adjacent anion and the undesired elimination will be prevented in the base-promoted hydrolysis step. An allyl protecting group was employed, and it was efficiently installed by the Fisher esterification with allyl alcohol and removed with Pd(0) after the aldol reaction. To our satisfaction, the mono ester 30 was successfully hydrolyzed to give the bis-acid 27 which is ready for lactonization.
3.7 Cyclization of the Bis-acid 27

We envisioned that bis-acid 27 would readily cyclize to form a 5-membered lactone 31. However, unlike previous literature suggested, 27 didn’t cyclize upon standing or treatment with 1 M citric acid in chloroform. EDCI was added to activate the carboxylic acids, leading to a fast conversion to cyclized products, which contained diastereomers separable by flash chromatography. Since LC-MS indicated a loss of water (M-18) and 1H-NMR suggested no elimination of the hydroxyl group, we proposed this product was our desired lactone 31. Next, we tried to convert the carboxylic acid moiety of 31 to acyl chloride by adding oxalyl chloride and catalytic amount of DMF, but the reaction didn’t proceed as expected because no
gas release (CO and CO$_2$) was observed. Further attempts to convert this mixture to a methyl ester or diazoketone also failed. Questioning the true identity of the cyclized product, we isolated the major diastereomer and obtained its X-ray structure by recrystallizing it from pentane-ethyl acetate. Surprisingly, the hydroxyl group in compound 27 didn’t participate in the EDC-mediated cyclization and 32 turned out to be a mixed anhydride (Scheme 3.3).

**Scheme 3.3**: EDCI-promoted cyclization of bis-acid 27

Although this result was disappointing, 32 possessed all three desired stereocenters found in plagiogyrin A (1). To explain the observed stereoselectivity, we modeled the geometry of the enolate generated from ester 28. The hindered rotation of this conjugated system results in a preferred $E$-enolate to minimize repulsion (Figure 3.9). The $E$-enolate, together with a polar Felkin addition model,$^{26}$ would result in the 2,3-$anti$, 3,4-$anti$ product, which is what is observed.
3.8 Revised Synthesis of Lactone 35

Due to the unexpected formation of the mixed anhydride 32, we revised our synthesis in which the carboxyl and hydroxyl group are exposed for lactonization and the other carboxylic acid is protected. However, the two carboxyl groups of the Stobbe condensation product are protected (esterified) in the opposite way. Thus, a hydrolysis/selective esterification sequence is required to inverse their deprotection sequence (Figure 3.10).

To begin, 12 was hydrolyzed and subjected to a regioselective Fischer esterification. The unsaturated carboxylic acid is less electrophilic than the other carboxylic acid and
therefore is not esterified. The aldol reaction of 33 proceed smoothly because the two anions of its enolate are separated by a larger distance. Next, the aldol product underwent EDCI-mediated cyclization in high yield to provide lactone 34. We found that diastereomers are more efficiently separated at this stage rather than later in the synthesis. Pd(0)-catalyzed deallylation of the major isomer 34 revealed the carboxylic acid 35, whose crystal structure confirmed the structure of the lactone backbone of plagiogyrin A (1) with all three required stereocenters.

**Figure 3.10:** Retrosynthetic analysis and forward synthesis of lactone 35

### 3.9 α-Diazoketone Synthesis

With 35 in hand it was now time to employ our proposed α-ketoaldehyde synthesis. To this end, attempts to convert carboxylic acid 35 to an acyl chloride using oxalyl chloride were unsuccessful, most likely due to the generation of acidic byproducts that reacted with the
acetonide protecting group. Other than acyl chloride, mixed anhydride is also reported in diazoketone preparation (as a part of the Arndt-Eistert synthesis). We chose methyl chloroformate to react with acid 35, trapped the generated hydrochloric acid with triethylamine and successfully prepared the mixed anhydride 36. Unfortunately, 36 failed to react with trimethylsilyl diazomethane, even with excess reagent, elevated temperature and prolonged reaction time, potentially as a result of the congestion around the mixed anhydride against this relatively bulky nucleophile. Although trimethylsilyl diazomethane can react with protic solvents to generate diazomethane in situ, this process is acid-catalyzed and not favored in basic environments. For that reason, we needed to prepare diazomethane and directly react it with our mixed anhydride 36. Diazald® was reacted with a 37% aqueous solution of KOH to release diazomethane gas into the mixed anhydride solution, producing our wanted α-diazoketone 37 in mediocre yield. This reaction had to be kept at 0 °C to avoid decomposition but further lowering the temperature would significantly extend the reaction time. Since a minor esterified product 38 was also observed, it indicated that the mixed anhydride could be hydrolyzed back to the acid by the moisture in the system, and subsequently esterified by diazomethane; however, isopropyl chloroformate, which is anticipated to form more stable mixed anhydrides than methyl chloroformate, didn’t offer better results (Table 3.3).
Table 3.3: Preparation of α-diazoketone 37 from acid 35

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cl</td>
<td>TMSCH₂N₂, MeCN, 0 °C</td>
<td>Failed to prepare the acyl chloride</td>
</tr>
<tr>
<td>2</td>
<td>OCO₂Me</td>
<td>Up to 2.2 eq. TMSCHN₂, Up to 45 °C and 30 h</td>
<td>No reaction before decomposition</td>
</tr>
<tr>
<td>3</td>
<td>OCO₂Me</td>
<td>CH₂N₂ (xs.), Et₂O, 0 °C, 3 h</td>
<td>28% yield of 37 and a small amount of 38</td>
</tr>
<tr>
<td>4</td>
<td>OCO₂Me</td>
<td>CH₂N₂ (xs.), Et₂O, 0 °C-rt</td>
<td>Decomposition as temperature was raised</td>
</tr>
<tr>
<td>5</td>
<td>OCO₂Me</td>
<td>CH₂N₂ (xs.), Et₂O, -30 °C</td>
<td>Reaction progressed slowly, lasting &gt; 30 h</td>
</tr>
<tr>
<td>6</td>
<td>OCO₂Pr</td>
<td>CH₂N₂ (xs.), Et₂O, 0 °C, 3 h</td>
<td>Similar to entry 3, but with more byproducts</td>
</tr>
</tbody>
</table>

3.10 Deprotection and Cyclization of the α-Ketoaldehyde

The α-diazoketone 37 was then readily oxidized by DMDO to provide our key α-ketoaldehyde 39. To pursue the natural product, we need to remove the protecting group on the diol (acetonide) and the phenol (methyl ether), as well as form the required hemiacetal/ketal between the diol and α-ketoaldehyde. Conceptually, all these transformations can be accomplished under acidic conditions. To this end, we subjected the freshly prepared α-ketoaldehyde 39 to various Lewis and Brønsted acids. Most of these conditions led to an efficient release of the diol, but didn’t cleave the phenolic ether. Interestingly, when HCl (0.5
M in THF-H$_2$O (1:1)) was added, LC-MS analysis showed two peaks with the same mass corresponding to the loss of the acetonide group. This could mean the released diol was partially closed to a hemiacetal/ketal under aqueous conditions. On the other hand, the α-ketoaldehyde moiety tended to undergo C-C bond cleavage to form carboxylic acid 41, adding extra problems when attempting to use more vigorous conditions. (Table 3.4).

**Table 3.4:** Acid-promoted deprotection of the acetonide and phenolic ether group

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AlCl$_3$, rt, 16 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>2</td>
<td>AlCl$_3$, EtSH, rt, 6 h</td>
<td>Acetonide was removed, the phenolic ether remained intact.</td>
</tr>
<tr>
<td>3</td>
<td>HCl (0.5 M) H$_2$O-THF (1:1)</td>
<td>Acetonide was efficiently removed, yielding at least two isomers. The phenolic ether remained intact.</td>
</tr>
<tr>
<td>4</td>
<td>After entry 3, add BBr$_3$, rt-40 °C, 2 d</td>
<td>No deprotection of the phenolic ether before decomposition took place.</td>
</tr>
<tr>
<td>5</td>
<td>After entry 3, add BBr$_3$+EtSH, rt, 16 h</td>
<td>No deprotection of the phenolic ether before decomposition took place.</td>
</tr>
<tr>
<td>6</td>
<td>After entry 3, add AlBr$_3$+EtSH, rt, 16 h</td>
<td>No deprotection of the phenolic ether before decomposition took place.</td>
</tr>
<tr>
<td>7</td>
<td>After entry 3, add TMSI, rt, on</td>
<td>Fast decomposition took place.</td>
</tr>
</tbody>
</table>
3.11 Revised Synthesis with a More Labile Phenol Protecting Group

Given the oxygen-rich nature of 40, along with the sensitivity of the α-ketoaldehyde moiety, we required a more labile phenol protecting group at this late stage. Because the phenolic ether was present all the way through our previous synthesis, it is impractical to have a fragile protecting group at the beginning. Thus, we decided to switch the phenolic ether in the middle of our synthesis. Having careful thoughts about the conditions we have used, we set lactone 44 as the juncture for protecting group transformation. We started with an allyl protected phenol, 4-(allyloxy)benzaldehyde (42), so that two allyl groups can be removed at the same time. The synthesis continued as before without incident. Moreover, the diastereomeric ratio was determined after the EDC-mediated cyclization step. 1H-NMR revealed three detectable isomers which had a ratio of 82:12:6. The better selectivity (compared to the formation of 24 with a 2.65:1 d.r.) can be attributed to the more disfavored dianion interaction in the Z-enolate 43Z (see also Figure 3.9). It is also important to note that the previous dealylation condition (Pd(PPh₃)₄ and morpholine) only removed the allyl ester. A stronger allyl scavenging reagent, NaBH₄, was employed and successfully deprotected the allyl ester and phenolic ether (Scheme 3.4).
Scheme 3.4: Synthesis of phenol 45

The new phenol protecting group for compound 45 should meet two criteria: 1) It would be removed under mild conditions at end, and 2) It could be installed in the presence of a carboxylic acid. Therefore, a TBS group was installed on the phenol followed by conversion of the carboxylic acid side chain to α-ketoaldehyde 47 using previously described methods. Surprisingly, the TBS group showed unusual stability under aqueous acidic conditions, but was eventually cleaved with 1 M HCl at 55 °C (Scheme 3.5).
With all protecting groups removed, we envisioned the product to rapidly undergo hemiacetal formation to afford 1; however, crude $^1$H-NMR of our material did not match that reported for the natural product,\textsuperscript{13} most likely due to the formation of a regioisomer or partially cyclized material. Based on crude $^1$H-NMR data, we hypothesized that our product was most likely a six-membered hemiacetal and a free primary alcohol (49). Further manipulation on this product was required to approach our final target.

3.12 Rearrangement of the Hemiacetal

The acid and base sensitivity of compound 47 limited our study on the interconversion of these materials and we therefore sought alternate deprotection/cyclization conditions. It has been reported that subjection of tetra-acetate 50 to 3\% HCl in dioxane under reflux provides plagiogyrin A in low yield (Scheme 3.6).\textsuperscript{14} The proposed mechanism involved a stereospecific
nucleophilic attack of the hemiacetal by the primary alcohol, and subsequent formation of a hemiketal. Inspired by this report, we treated compound 47 with 3% HCl in dioxane at room temperature. The acetonide group was cleaved immediately, although the TBS group remained intact, even upon heating. LC-MS analysis revealed product 50 had an identical mass with 48 (Table 3.5) but was less polar, likely revealing an alternate hemiacetal formation. Brønsted acids again proved ineffective at removing the TBS group. Various fluoride sources were effective, but also caused the α-ketoaldehyde moiety to decompose over time. Finally, TASF, known for its mild and anhydrous properties, removed the TBS ether without significant byproducts. The fully deprotected and cyclized material showed high polarity thus needed to be purified by reversed-phase chromatography. Lyophilization of the collected fractions produced a white powder which we were gratified to find that the $^1$H and $^{13}$C NMR data matched that reported for 1.

![Scheme 3.6: Reported conversion from tetraacetate 50 to plagiogyrin A (1)](image-url)
Table 3.5: Final steps of plagiogyrin A (1) synthesis

![Diagram of chemical synthesis]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Same pot, heated to 55 °C</td>
<td>TBS group was not removed.</td>
</tr>
<tr>
<td>2</td>
<td>AcOH, up to 55 °C, 5 d</td>
<td>TBS group was not removed.</td>
</tr>
<tr>
<td>3</td>
<td>AcOH, H₂O, THF (3:1:1)</td>
<td>TBS group was not removed.</td>
</tr>
<tr>
<td>4</td>
<td>p-TsOH (1.1 eq.)</td>
<td>TBS group was not removed.</td>
</tr>
<tr>
<td>5</td>
<td>TBAF (1.1 eq.), THF</td>
<td>TBS was cleaved + side reactions</td>
</tr>
<tr>
<td>6</td>
<td>TBAF(1.1 eq., 1 M in THF)-AcOH (1:1 v/v)</td>
<td>TBS was cleaved + side reactions</td>
</tr>
<tr>
<td>7</td>
<td>CsF (0.5-1.1 eq.)</td>
<td>Unknown byproduct</td>
</tr>
<tr>
<td>8</td>
<td>HF-Pyridine (1.1 eq.)</td>
<td>TBS was cleaved + side reactions</td>
</tr>
<tr>
<td>9</td>
<td>TASF (1.05 eq.), DMF</td>
<td><strong>1, 49% yield (3 steps from 46)</strong></td>
</tr>
</tbody>
</table>

* other than entry #1, crude 51 was extracted (EtOAc) and concentrated then subjected to the listed conditions

3.13 Conclusions

In conclusion, we have developed a stereocontrolled synthesis of the natural product plagiogyrin A (1) in 12 steps and 3.9% overall yield. Utilizing an intermediate poised to provide our required E-enolate, the key aldol reaction set two new stereocenters with good selectivity. Subsequent conversion of a carboxylic acid to an α-ketoaldehyde provided the
required oxidation state of the natural product under mild conditions, and a final anhydrous acid-promoted hemiacetal formation concluded the synthesis.

3.14 Supporting Information

Part 1. General

All reactions were performed under an N₂ atmosphere and all glassware was dried in an oven at 140 °C for 2 h prior to use, unless otherwise noted. Reactions carried out at −78 °C employed a CO₂/acetone bath. THF and CH₂Cl₂ were purified using an alumina filtration system.

Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F₂₅₄ plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), p-anisaldehyde solution (2.5 mL of p-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H₂SO₄ in 100 mL of 95% EtOH), Bromocresol green solution (0.04 g of bromocresol green in 100 mL of absolute EtOH. Slowly drip in a 0.1 M solution of NaOH until the solution turned pale blue) or a KMnO₄ solution (1.5 g of KMnO₄, 10 g of K₂CO₃, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reaction were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 µm C18 50 x 2.10 mm). Flash chromatography on SiO₂ was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients.

Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Infrared spectra were determined on a Jasco FT/IR-4100 spectrometer. Optical rotation was determined on a Jasco P-2000 Digital Polarimeter. ^1H and ^13C NMR spectra were obtained on
a Varian Mercury-VX 400 or a Bruker DRX-500 NMR spectrometer. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. $^1$H NMR spectra were run at 400 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, brs = broad singlet), coupling constant(s) and number of protons. $^{13}$C NMR spectra were run at 100 or 125 MHz using a proton-decoupled pulse sequence with a $\tau_1$ of 0 second unless otherwise noted, and are tabulated by observed peak. High-resolution mass spectra were obtained on a Thermo Fisher Scientific, Exactive Plus mass spectrometer using Heated Electrospray Ionization (HESI).

Reactions “tracked by LC-MS” (Shimadzu LC-MS 2020 with Kinetex 2.6 µm C18 50 x 2.10 mm) indicate that we predicted the outcome of the reaction by analyzing the mass of products. Due to the small scale we used in the development of synthetic routes, this method enables us to rapidly determine optimal reaction conditions.

NMR reference points:

CDCl$_3$ $^1$H: 7.26 ppm $^{13}$C: 77.16 ppm

CD$_3$OD $^1$H: 3.31 ppm $^{13}$C: 49.00 ppm

(CD$_3$)$_2$CO $^1$H: 2.05 ppm $^{13}$C: 29.84 ppm

Pyridine-d$_5$ $^1$H: 8.74 ppm $^{13}$C: 150.35 ppm
Part 2. Synthetic procedures to plagiogyrin A (1)

\[
\text{(E)-4-(allyloxy)-2-(4-(allyloxy)benzylidene)-4-oxobutanoic acid}
\]

Step 1: Stobbe condensation (General Procedure A)

Potassium tert-butoxide (1.01 g, 9.00 mmol) was dissolved in 30 mL tert-butanol. The solution was heated to reflux then a solution containing 4-(allyloxy)benzaldehyde (1.35 g, 8.33 mmol) and dimethyl succinate (1.32 g, 9.00 mmol) in tert-butanol (10 mL) was slowly added via a syringe injector over 30 min. After addition, the resulting orange solution was refluxed for another 20 min, cooled and concentrated in vacuo.

Step 2: Hydrolysis (General Procedure B)

The residue above was dissolved in 30 mL THF, an aqueous solution of LiOH was added (1.0 M, 30 mL, 30 mmol). The solution was stirred at room temperature overnight (16 h) and washed with DCM (30 mL x 2). The aqueous layer was acidified (pH < 2) by slow addition of
concentrated HCl solution, and extracted with ethyl acetate (20 mL x 3). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated \textit{in vacuo}.

Step 3: Fischer esterification (General Procedure C)

The residue above was dissolved in 30 mL dry CHCl$_3$, allyl alcohol (261 mg, 4.50 mmol) and p-toluenesulfonic acid monohydrate (86.0 mg, 0.45 mmol) were added. The mixture was heated to reflux under a Dean-Stark apparatus filled with 4 Å molecular sieves. After 16 h, more allyl alcohol (261 mg, 4.50 mmol) and p-toluenesulfonic acid monohydrate (86.0 mg, 0.45 mmol) were added, and the reflux was continued for 6 h. The residue was concentrated \textit{in vacuo} and purified by flash chromatography (10-55% ethyl acetate in hexanes, gradient) to afford the title compound 43 (1.32 g, 52% yield in three steps) as a yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.97 (s, 1 H), 7.37 – 7.34 (m, 2 H), 6.97 – 6.92 (m, 2 H), 6.10 – 6.00 (m, 1 H), 5.98 – 5.88 (m, 1 H), 5.45 – 5.22 (m, 4 H), 4.68 – 4.64 (m, 2 H), 4.58 – 4.55 (m, 2 H), 3.62 (s, 2 H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 173.5, 170.9, 159.7, 144.1, 132.8, 132.0, 131.3, 127.3, 122.9, 118.4, 118.1, 115.0, 68.9, 65.7, 33.5. Melting point 79 °C. IR (KBr, cm$^{-1}$) 2947, 1728, 1675, 1601, 1509, 1174. HRMS (ESI): calculated for C$_{17}$H$_{17}$O$_5$ [M-H]$^-$, 301.1082; found 301.1083.
allyl (2S,3R)-4-((E)-4-(allyloxy)benzylidene)-2-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-5-oxotetrahydrofuran-3-carboxylate

Step 1: Aldol reaction (General Procedure D)

Compound 43 (453 mg, 1.50 mmol) was dissolved in dry THF (20 mL), the solution was stirred at -78 °C (dry ice-acetone bath) and LHMDS (6.00 mL, 1.0 M, 6.00 mmol) was added dropwise. After stirring at -78 °C for 30 min, a solution of (R)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (781 mg, 6.00 mmol) in THF (5 mL) was added dropwise. Stirring was continued at -78 °C for 30 min, then the solution was warmed to room temperature and 1.0 M HCl (20 mL) was added. The solution was extracted with diethyl ether (20 mL x 3) and the combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The crude mixture was purified by flash chromatography (10-55% ethyl acetate in hexanes, gradient) to afford a mixture of diastereomers of the aldol product.

Step 2: EDCI-mediated lactonization (General Procedure E)

The mixture of diastereomers above was dissolved in dry DCM (20 mL), EDCI·HCl (288 mg, 1.50 mmol) was added and the resulting solution was stirred at room temperature for 30 min
then concentrated in vacuo. The crude mixture (dr =82:12:6, as determined by $^1$H-NMR integration of aromatic protons) was purified by flash chromatography (10-35% ethyl acetate in hexanes, gradient). The major diastereomer 44 was isolated as a yellow oil (394 mg, 63% yield in two steps). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.61 (d, 1 H, $J$ = 2.0 Hz), 7.56 – 7.51 (m, 2 H), 6.97 – 6.92 (m, 2 H), 6.09 – 5.98 (m, 1 H), 5.89 – 5.78 (m, 1 H), 5.45 – 5.17 (m, 4 H), 4.61 – 4.56 (m, 4 H), 4.46 (dd, 1 H, $J$ = 9.1, 7.4 Hz), 4.38 (dd, 1 H, $J$ = 7.4, 2.0 Hz), 4.31 (ddd, 1 H, $J$ = 9.0, 5.9, 4.2 Hz, 4.17 (dd, 1 H, $J$ = 9.0, 5.9 Hz), 4.04 (dd, 1 H, $J$ = 9.0, 4.3 Hz), 1.43 (s, 3 H), 1.32 (s, 3 H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.8, 168.7, 160.7, 139.9, 132.6, 132.6, 131.4, 126.1, 120.2, 119.2, 118.3, 115.3, 110.2, 78.2, 74.1, 69.0, 67.8, 66.3, 47.5, 27.1, 25.2. IR (KBr, cm$^{-1}$) 2984, 1754, 1600, 1509, 1161, 829. $[\alpha]D^{20} = +133^\circ$ (c = 1.0, MeOH). HRMS (ESI): calculated for C$_{23}$H$_{26}$O$_7$Na [M+Na]$^+$, 437.1571; found 437.1562.

(2S,3R)-2-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-((E)-4-hydroxybenzylidene)-5-oxotetrahydrofuran-3-carboxylic acid
Compound 44 (394.0 mg, 0.952 mmol) was dissolved in dry THF (20 mL). Pd(PPh₃)₄ (55.0 mg, 0.0476 mmol) and NaBH₄ (86.4 mg, 2.28 mmol) were added. The resulting solution was stirred at room temperature for 2 h, quenched by addition of water (25 mL) and washed with DCM (25 mL x 2). The aqueous layer was separated, acidified using 1.0 M aqueous HCl solution (10 mL) and extracted with diethyl ether (20 mL x 3). The combined organic layers were dried (Na₂SO₄), concentrated in vacuo and purified by flash chromatography (10-65% ethyl acetate in hexanes, gradient) to afford the title compound 45 as a white solid (282.7 mg, 89% yield). ¹H NMR (400 MHz, CD₃OD) δ 7.58 – 7.54 (m, 2 H), 7.51 (d, 1 H, J = 1.8 Hz), 6.88 – 6.84 (m, 2 H), 4.56 (dd, 1 H, J = 8.4, 7.2 Hz), 4.43 (ddd, 1 H, J = 8.3, 6.0, 4.5 Hz), 4.33 (dd, 1 H, J = 7.1, 1.9 Hz), 4.17 (dd, 1 H, J = 8.8, 6.0 Hz), 4.01 (dd, 1 H, J = 8.8, 4.6 Hz), 1.44 (s, 3 H), 1.33 (s, 3 H). ¹³C NMR (100 MHz, CD₃OD) δ 173.3, 172.6, 161.5, 140.4, 134.0, 126.1, 121.5, 116.9, 111.1, 79.9, 75.6, 68.1, 48.8, 27.1, 25.4. Melting point 83 °C. IR (KBr, cm⁻¹) 3369, 1734, 1598, 1518, 1168, 841. [α]D²⁰ = +174° (c = 1.0, MeOH). HRMS (ESI): calculated for C₁₇H₁₇O₇ [M-H]-, 333.0980; found 333.0980.

\[ (4R,5S)-3-((E)-4-((\text{tert-butyldimethylsilyl})\text{oxy})\text{benzylidene})-4-(2\text{-diazooacetyl})-5-((R)-2,2\text{-dimethyl}-1,3\text{-dioxolan-4-yl})\text{dihydrofuran-2(3H)-one} \]
Compound 45 (59.1 mg, 0.177 mmol) was dissolved in DMF (2 mL), TBSCI (66.7 mg, 0.442 mmol) and imidazole (60.2 mg, 0.885 mmol) were added at room temperature. The resulting solution was stirred at room temperature for 16 h then water (10 mL) was added. The mixture was stirred for 15 min, acidified with 5% aqueous acetic acid (15 mL), and extracted with ethyl acetate (15 mL x 3). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried (Na$_2$SO$_4$) and concentrated in vacuo. The crude product was purified by flash chromatography (10-55% ethyl acetate in hexanes, gradient) to afford the TBS ether (51.7 mg, 65% yield).

The TBS ether above (51.7 mg, 0.115 mmol) was dissolved in diethyl ether (4 mL), triethylamine (12.8 mg, 0.127 mmol) and methyl chloroformate (13.1 mg, 0.138 mmol) were added. After stirring at room temperature for 1 h, the mixture was filtered and the ethereal solution was transferred to an Aldrich® diazomethane-generator (Z411736) and cooled to 0 °C. Diazald® (321 mg, 1.50 mmol) in carbitol (1 mL) and KOH (37% aqueous solution, 1.5 mL) were reacted to generate diazomethane to the ethereal solution. After stirring at 0 °C for 3 h. Glacial acetic acid (10 drops) was added to the ethereal solution while keeping the temperature at 0 °C. The solution was then concentrated in vacuo and purified by flash chromatography (10-35% ethyl acetate in hexanes, gradient) to afford the title compound 46 as a light yellow oil (22.6 mg, 42% yield, 27% yield from compound 45).

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.70 (d, 1 H, $J$ = 2.0 Hz), 7.45 – 7.41 (m, 2 H), 6.92 – 6.88 (m, 2 H), 5.42 (s, 1 H), 4.58 – 4.55 (m, 1 H), 4.20 – 4.07 (m, 4 H), 1.34 (s, 3 H), 1.31 (s, 3 H), 0.98 (s, 9 H), 0.23 (s, 6 H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ 191.1, 171.2, 158.8, 140.9, 132.6, 126.4, 121.0, 119.0, 110.5, 80.5, 76.1, 66.0, 54.9, 51.6, 26.5, 25.7, 24.7, 18.4, -4.2. IR (KBr, cm$^{-1}$) 2930, 2112,
1757, 1598, 1510, 1169, 910, 840. [\alpha]D^{19} = -103^\circ (c = 1.0, \text{MeOH}). \textbf{HRMS} (ESI): calculated for C_{24}H_{33}N_{2}O_{6}Si [M+H]^+, 473.2102; found 473.2097.

![Chemical structure](image)

(3aR,4R,5S,8R,8aS)-4,5-dihydroxy-3-((E)-4-hydroxybenzylidene)hexahydro-4,8-epoxyfuro[2,3-d]oxepin-2(3H)-one

Dimethyldioxirane (DMDO) was prepared and titrated according to Adam’s method.\textsuperscript{35,36} DMDO in acetone (1.00 mL, 0.0600 mmol/mL, 0.0600 mmol) was slowly added to compound 46 (22.6 mg, 0.0479 mmol). After stirring at room temperature for 10 min, the solvent was removed \textit{in vacuo}. The residue was dissolved in 3 mL anhydrous dioxane with 3\% HCl and stirred at room temperature for 2 h, then 15 mL saturated brine solution was added. The solution was extracted with ethyl acetate (15 ml x 3), dried (Na\textsubscript{2}SO\textsubscript{4}) and concentrated \textit{in vacuo}. The residue was dissolved in acetonitrile (2.0 mL), cooled to 0 \textdegree C and TASF reagent (0.10 M in DMF, 0.50 mL, 0.050 mmol) was added. The brown colored solution was stirred at 0 \textdegree C for 10 min, concentrated \textit{in vacuo} and separated by reversed phase chromatography (Biotage\textsuperscript{\textregistered} SNAP Ultra C18 12 g, 10-25\% acetonitrile in water (with 0.1\% TFA), gradient). Fractions containing the title compound 1 were lyophilized to give a white solid (7.2 mg, 49\% yield). \textbf{\textsuperscript{1}H NMR} (400 MHz, pyridine-d\textsubscript{5}) \(\delta\) 8.39 (d, \(J = 8.6\) Hz, 2 H), 8.06 (d, \(J = 2.1\) Hz, 1 H), 7.30 (d, \(J = 8.6\) Hz, 2 H), 5.51 (s, 1 H), 5.34 (d, \(J = 6.5\) Hz, 1 H), 4.83 (dd, \(J = 6.5, 2.1\) Hz, 1 H).
H), 4.50 (s, 1 H), 4.02 (s, 2 H). $^{13}$C NMR (125 MHz, pyridine-d$_5$) δ 173.6, 161.9, 142.0, 135.1, 127.0, 120.5, 117.0, 107.3, 97.8, 84.2, 79.2, 67.5, 45.9. Melting point decomposed to a black tar at 185 °C. IR (KBr, cm$^{-1}$) 3416, 1735, 1649, 1602, 1516, 835. $[\alpha]_D^{23} = +390^\circ$ (c = 0.4, MeOH). HRMS (ESI) calculated for C$_{15}$H$_{15}$O$_7$ [M+H]$^+$, 307.0812; found 307.0810.

**Literature**$^{13}$ reported characterization of the natural product plagiogyrin A (1)

Plagiogyrin A: $^1$H NMR (100 MHz, pyridine-d$_5$) δ 8.32 (d, $J = 9$ Hz, 2 H), 7.98 (d, $J = 2$ Hz, 1 H), 7.22 (d, $J = 9$ Hz, 2 H), 5.45 (s, 1 H), 5.32 (d, $J = 7$ Hz, 1 H), 4.78 (dd, $J = 7$, 2 Hz, 1 H), 4.48 (brs, 1 H), 4.02 (brs, 2 H). $^{13}$C NMR (25 MHz, pyridine-d$_5$) δ 173.0, 161.2, 141.4, 134.5, 126.5, 119.9, 116.4, 106.7, 97.2, 83.6, 78.7, 67.1, 45.3. Melting point 223-225 °C. IR (KBr, cm$^{-1}$) 3470, 1745, 1645, 1595, 1520, 830. $[\alpha]_D^{25} = +438^\circ$ (c = 1.0, MeOH). HRMS calculated for C$_{15}$H$_{14}$O$_7$ (M$^+$), 306.0740; found 306.0727.

**Notes:**

1. The NMR reference point of pyridine-d$_5$ was not reported in the literature. We used 8.74 ppm for $^1$H and 150.35 ppm for $^{13}$C, and observed reasonable deviations with the literature data. (Also see Table S1 for $^{13}$C NMR comparison).

2. Plagiogyrin A (1) appeared to be unstable in basic solvents like pyridine. When we acquired the $^{13}$C NMR in an overnight experiment at room temperature, we found several unidentified peaks with low intensity. Re-purification of this mixture and LC-MS analysis suggested the impurity was likely a diastereomer of 1.
Part 3. Synthetic procedures to compound 35

(\(E\))-4-(allyloxy)-2-(4-methoxybenzylidene)-4-oxobutanoic acid

Compound 12 ((\(E\))-3-(methoxycarbonyl)-4-(4-methoxyphenyl)but-3-enoic acid) was prepared as described in the literature in 70% yield.\(^{37}\) Compound 12 (500 mg, 2.00 mmol) was hydrolyzed according to general procedure B and selectively esterified according to general procedure C to give compound 33 (336 mg, 61% yield in two steps). \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.98 (s, 1 H), 7.38 – 7.35 (m, 2 H), 6.95 – 6.92 (m, 2 H), 5.93 (dd, \(J = 17.2, 10.5\) Hz, 1 H), 5.34 (dd, \(J = 17.2, 1.4\) Hz, 1 H), 5.24 (dd, \(J = 10.5, 1.4\) Hz, 1 H), 4.66 (dt, \(J = 5.6, 1.4\) Hz, 2 H), 3.84 (s, 3 H), 3.62 (s, 2 H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 173.4, 170.9, 160.7, 144.1, 132.0, 131.4, 127.2, 122.9, 118.4, 114.3, 65.8, 55.5, 33.5. Melting point 102 °C. IR (KBr, cm\(^{-1}\)) 2948, 1728, 1670, 1610, 1512, 1432, 1251, 1181, 1027. HRMS (ESI): calculated for C\(_{15}\)H\(_{15}\)O\(_5\) [M-H]\(^{-}\), 275.0925; found 275.0926.
Compound 33 (210 mg, 0.761 mmol) underwent aldol reaction (see general procedure D) then lactonized with EDCI (see general procedure E) to give allyl ester 34 (170 mg, 0.438 mmol, 58% yield in two steps). Deallylation of compound 34 was conducted as follows (general procedure F):

Compound 34 (170 mg, 0.438 mmol) was dissolved in dry THF (15 mL). Pd(PPh₃)₄ (15.2 mg, 0.0131 mmol) and morpholine (191 mg, 2.19 mmol) were added. The resulting solution was stirred at room temperature for 3 h, then water (15 mL) and saturated NaHCO₃ (5 mL) were added. The aqueous solution was washed with DCM (25 mL x 2), acidified using 1.0 M aqueous HCl solution (10 mL) and extracted with diethyl ether (20 mL x 3). The combined ether layers were dried (Na₂SO₄), concentrated in vacuo and purified by flash chromatography (10-65% ethyl acetate in hexanes, gradient) to afford the title compound 35 (80.7 mg, 0.232 mmol, 53% yield) as a white, fluffy semi-solid. ¹H NMR (400 MHz, CD₃OD) δ 7.67 – 7.64 (m, 2 H), 7.54 (d, J = 1.8 Hz, 1 H), 7.03 – 6.99 (m, 2 H), 4.57 (dd, J = 8.4, 7.1 Hz, 1 H), 4.46 – 4.41 (m, 1 H), 4.36 (dd, J = 7.1, 1.9 Hz, 1 H), 4.18 (dd, J = 8.8, 6.0 Hz, 1 H), 4.02 (dd, J = 8.8, 4.5 Hz, 1 H), 3.85 (s, 3 H), 1.44 (s, 3 H), 1.34 (s, 3 H). ¹³C NMR (100 MHz, CD₃OD) δ
173.0, 172.5, 163.2, 139.9, 133.8, 127.3, 122.6, 115.5, 111.1, 79.9, 75.6, 68.1, 56.0, 48.8, 27.1, 25.3. \textbf{IR} (KBr, cm$^{-1}$) 2363, 1744, 1602, 1516, 1259, 1173, 1031, 834. $[\alpha]_D^{23} = +194^\circ$ (c = 0.84, MeOH) \textbf{HRMS} (ESI): calculated for C$_{18}$H$_{19}$O$_7$ [M-H]$^-$, 347.1136; found 347.1135.

X-ray structural determination of 35

A colorless block-like specimen of C$_{18}$H$_{20}$O$_7$, approximate dimensions 0.117 mm x 0.171 mm x 0.393 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured.

The integration of the data using a triclinic unit cell yielded a total of 5034 reflections to a maximum $\theta$ angle of 30.63$^\circ$ (0.70 Å resolution), of which 5034 were independent (average redundancy 1.000, completeness = 99.6%, $R_{\text{sig}} = 3.01\%$) and 4395 (87.31\%) were greater than 2$\sigma$(F$^2$). The final cell constants of $a = 8.8256(3)$ Å, $b = 9.3741(4)$ Å, $c = 10.7509(4)$ Å, $\alpha = 69.044(2)^\circ$, $\beta = 81.513(2)^\circ$, $\gamma = 81.953(2)^\circ$, volume = 817.85(6) Å$^3$, are based upon the refinement of the XYZ-centroids of reflections above 20 $\sigma$(I).

The final anisotropic full-matrix least-squares refinement on F$^2$ with 233 variables converged at $R1 = 3.70\%$, for the observed data and $wR2 = 9.48\%$ for all data. The goodness-of-fit was 1.045. The largest peak in the final difference electron density synthesis was 0.472 e/Å$^3$ and the largest hole was -0.215 e/Å$^3$ with an RMS deviation of 0.052 e/Å$^3$. On the basis of the final model, the calculated density was 1.415 g/cm$^3$ and F(000), 368 e$^-$. 

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Figure 3.11: ORTEP drawing of compound 35

Part 4. Synthetic procedures to compound 32

(R)-3-((S)-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy)methyl)-4-((E)-4-methoxybenzylidene)dihydrofuran-2,5-dione
Compound 12 ((E)-3-(methoxycarbonyl)-4-(4-methoxyphenyl)but-3-enoic acid) was prepared as described in the literature in 70% yield. Compound 12 (0.500 g, 2.00 mmol) underwent esterification (see general procedure C), followed by aldol reaction (see general procedure D), then deallylation (see general procedure F) and hydrolysis (see general procedure B), at last cyclized with EDCI (see general procedure E) to give compound 32 (48.5 mg, 7% yield over five steps). $^1$H NMR (400 MHz, (CD$_3$)$_2$CO) $\delta$ 7.73 – 7.67 (m, 3 H), 7.08 – 7.04 (m, 2 H), 5.35 (d, $J$ = 5.8 Hz, 1 H), 4.59 (brs, 1 H), 4.58 – 4.52 (m, 1 H), 4.16 (dd, 1 H, $J$ = 8.8, 6.6 Hz), 4.02 (ddd, 1 H, $J$ = 9.4, 5.8, 1.4 Hz), 3.89 (s, 3 H), 3.84 – 3.79 (m, 1 H), 1.53 (s, 3 H), 1.40 (s, 3 H). $^{13}$C NMR (100 MHz, (CD$_3$)$_2$CO) 170.9, 167.9, 162.9, 139.0, 134.0, 126.4, 122.7, 115.5, 110.3, 75.9, 72.7, 68.0, 55.9, 48.7, 27.2, 25.2. Melting point 162 °C. [α]$_{D}^{23}$ = -5.7° (c = 1.0, acetone) IR (KBr, cm$^{-1}$) 2359, 2340, 1827, 1761, 1603, 1516, 1259. HRMS (ESI): calculated for C$_{18}$H$_{19}$O$_7$ [M-H], 347.1136; found 347.1134.

X-ray structural determination of 32

A colorless block-like specimen of C$_{18}$H$_{20}$O$_7$, approximate dimensions 0.202 mm x 0.257 mm x 0.341 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured.

The total exposure time was 10.00 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 24547 reflections to a maximum $\theta$ angle of 33.23° (0.65 Å resolution), of which 6413 were independent (average redundancy 3.828, completeness =
100.0%, R_int = 2.87%, R_sig = 3.23%) and 5825 (90.83%) were greater than 2σ(F^2). The final cell constants of a = 6.7535(2) Å, b = 10.0644(2) Å, c = 12.3488(3) Å, β = 91.2050(10)°, volume = 839.16(4) Å^3, are based upon the refinement of the XYZ-centroids of 257 reflections above 20 σ(I) with 7.250° < 2θ < 64.48°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.956. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9650 and 0.9790.

The final anisotropic full-matrix least-squares refinement on F^2 with 233 variables converged at R1 = 3.62%, for the observed data and wR2 = 9.09% for all data. The goodness-of-fit was 1.020. The largest peak in the final difference electron density synthesis was 0.348 e/Å^3 and the largest hole was -0.185 e/Å^3 with an RMS deviation of 0.043 e/Å^3. On the basis of the final model, the calculated density was 1.379 g/cm^3 and F(000), 368 e^−.
Figure 3.12: ORTEP drawing of compound 32
3.15 References


CHAPTER 4

Synthesis and Biology of Pentacyclic Guanidinium Alkaloids
4.1 Biological Activities of Pentacyclic Guanidinium Alkaloids


4.2 Synthesis of Pentacyclic Guanidinium Alkaloids

4.2.1 Synthetic Contribution from the Snider group

The Snider group was first to perform a biomimetic synthesis of the pentacyclic nucleus of ptilomycalin A (7) in 1994.\(^1\) In this 14-step convergent route, Knoevenagel condensation of β-ketoester 1 and aldehyde 2 furnished the bis enone 3. As previously demonstrated in their model studies,\(^2\) the bis enone can be converted to a tricyclic guanidine in two steps. First, the 1:1 mixture of E/Z isomers 3a and 3b underwent a double Michael addition with O-methylisourea to yield a 4:1 mixture of two cis diastereomers 4a and two trans diastereomers 4b. Second, 4a and 4b were both converted to a 1:1 mixture of two cis diastereomers 5a and 5b by ammonolysis. With the tricyclic guanidine 5a and 5b in hand, deprotection with fluoride followed by treatment with triethylamine in MeOH gave the desired pentacyclic guanidinium methyl ester 6a and its epimer 6b with an equatorial methyl ester in 1.3:1 ratio. Flash chromatography purification of the crude mixture separated 6a and 6b in 80-85% purity. Therefore, the 1.3:1 mixture of 6a and 6b was treated with triethylamine in 1:1 MeOH-H\(_2\)O to give tetracyclic alcohol 7, which was purified and cyclized again to yield a 1.3:1 mixture of 6a
and 6b. Separation of this mixture was successful. The pure compound 6a, the methyl ester of the pentacyclic core in ptilomycalin A, was obtained (Scheme 4.1).

Scheme 4.1: Snider’s biomimetic synthesis of the methyl ester of the guanidine nucleus
4.2.2 Synthetic Contribution from the Overman Group

The Overman group has been very active in the total synthesis, analog development and structure-activity relationship studies of polycyclic guanidine alkaloids for the past 20 years. They reported the first enantioselective total synthesis of ptilomycalin A in 1995 (Scheme 4.2).³ As suggested in their preliminary studies,⁴ a fused bicyclic dihydropyrimidinone 11 prepared by a tethered Biginelli condensation was the key intermediate in this route. Amino alcohol 9 was treated with KOCN followed by ozonolysis to yield the Biginelli precursor 10, which condensed with β-ketoester 8 to give the Biginelli product 11 with good diastereoselectivity (dr = 7.5:1). The bicyclic compound 11 was further cyclized, generating one of the spiroaminal rings. A side chain was installed on the tricyclic compound 12 to give 13. Deprotection and cyclization of 13 provided the pentacyclic guanidine skeleton 14. The spermidine unit was placed on the hydrocarbon chain, furnishing compound 15. However, the stereochemistry of the ester group on C-14 was different from the natural product 17. Therefore, it had to be epimerized with triethylamine in MeOH. Three cycles of epimerization gave guanidine 16 in 50% yield. Removal of the Boc protecting group yielded the natural product, (-)-ptilomycalin A (17).
Scheme 4.2: The first enantioselective synthesis of (-)-ptilomycin A

In 2000, the Overman group reported a second-generation synthesis of ptilomycin A and several related natural products with the identical guanidine core. The second-generation synthesis was more convergent than the first generation. Instead of putting on fragments one
by one in a linear fashion, all carbons were put together before the critical Biginelli reaction. The synthesis went as follows: organolithium reagent was prepared from iodo-compound 18, and was reacted with Weinreb amide 19 to give ketone 20. The ketone was protected, and a urea group was introduced at the β-position. The tri-substituted alkene in compound 21 was selectively dihydroxylated and cleaved by Pb(IV) to generate an aldehyde, which reacted with morpholinium acetate and cyclized to produce compound 22 for the Biginelli reaction. Analogous to the first-generation synthesis, the β-ketoester 23 approached from the less hindered face, and yielded the major product 24a and the less favored product 24b in a 6-7:1 ratio. The bicyclic compound 24a was successively cyclized in similar steps with the first-generation synthesis. The pentacyclic product 25a again needed to be epimerized at C-14 to provide the required stereochemistry for the natural products. Optimized conditions gave 52% yield of the pentacyclic guanidinium core 25b after two cycles (Scheme 4.3).

4.2.3 Synthetic Contribution from the Murphy Group

Murphy and coworkers reported a double conjugate addition that allowed quick access to a simple pentacyclic guanidine, crambscidin 359 (26). Wittig reaction of ylide 27 and aldehyde 28 yielded bis enone 29, which resembled bis enone 3 in Snider’s synthesis. Guanidine was used for the double aza-Michael addition of bis enone 29, however, the addition was not diastereoselective. Thus, half of the tricyclic guanidine didn’t undergo cyclization upon removal of the silyl ether, which resulted in a low 18% yield of crambscidin 359 (26, Scheme 4.4).
Scheme 4.3: The second-generation synthesis of the pentacyclic core of ptilomycalin A (17)
Scheme 4.4: Murphy’s synthesis of crambescidin 359 (26)

4.2.4 Synthetic Contribution from the Nagasawa Group

The Nagasawa research group utilized two successive 1,3-dipolar cycloadditions in their total synthesis of crambescidin 359 (26, Scheme 4.5). Starting from nitrone 30, the 1,3-dipolar cycloaddition with alkene 31 stereoselectively gave isoxazolidine 32. The hydroxyl group that directed the stereochemistry was removed under Barton–McCombie deoxygenation conditions to yield compound 33. The nitrone moiety was regenerated by treating compound 33 with m-CPBA. A second 1,3-dipolar cycloaddition took place in a regioselective and stereoselective fashion, as the terminal alkene moiety prefers to approach the 1,3 dipole from the less hindered exo-face. Oxidation by m-CPBA generated nitrone 34. The following reduction with NaBH4 gave a 7:1 mixture of cis:trans isomer. The major cis isomer was isolated, and subjected to deoxygenation with the reducing agent Mo(CO)6 to give the 2,5-cis-pyrrolidine 35. Boc protected guanidine 36 was generated by reacting compound 35 with bis-
$N$-Boc-thiourea and HgCl$_2$. Oxidation of the bis secondary alcohol gave the diketone 37, which readily reacted with the guanidine and cyclized to give the pentacyclic guanidinium product upon treatment with CSA at 100 °C. Subsequent anion exchange with saturated NaBF$_4$ and NH$_4$Cl yielded crambescidin 359 (26). Interestingly, treating diketone 37 with HCl gave the rearranged cyclization product 38, which was found to be the pentacyclic core of the monanchocidins A, D and E.

Scheme 4.5: Nagasawa’s total synthesis of crambescidin 359 (26)
4.2.5 Synthetic Contribution from the Herzon Group

Recently, the Herzon group reported an enantioselective synthesis of (+)-batzelladine B (39) (Scheme 4.6). Although 39 is a tricyclic guanidinium alkaloid, this synthesis offers a concise and novel way of building a functionalized cis-pyrrolidine ring therefore it is worth discussing here. Starting from a simple pyrrole 40, formal [4+3] cycloaddition with α-diazoester 41 with a chiral dirhodium(II) catalyst provided a bicyclic product 42 with high yield and stereoselectivity. After selective hydrogenation with Wilkinson’s catalyst, 43 underwent electrophilic alkynylation with TMS-EBX (44) to give 45 as a single diastereomer. Bicyclic guanidine 46 was obtained upon treatment of 45 with 1 eq. of base and 1.8 eq. of lithium benzyl octanoate. This step was thought to proceed by a 1,2-addition of the β-ketoester and retro-aldol ring opening to provide intermediate 47, followed by isomerization and intramolecular Michael addition to access 46. Following the cleavage of the benzyl ester and removal of the chiral auxiliary, acid 48 was converted to the natural product 39. Comparing with previously described syntheses, this approach requires relatively less redox manipulations because it utilizes carbon-carbon bond-forming reactions deriving from pyrrole-based starting materials to achieve complexity. However, the stereochemistry of the carboxyl-linking carbon in 48 differs that of the pentacyclic guanidinium alkaloids.
Scheme 4.6: Herzon’s synthesis of (+)-batzelladine B (39)

4.3 Eukaryotic Initiation Factor 5A

4.3.1 Post-translational Modification of Proteins

Proteins are macromolecules that perform a vast variety of functions within organisms. They contain primary sequences of amino acid residues that direct the folding into three-dimensional structures. The nucleotide sequence of genes is passed into proteins by the form
of triplet codes. However, it is estimated that there are 30,000 human genes but as many as 300,000-3,000,000 distinct protein forms. With only 20 standard encoded amino acids, nature needs to build mechanisms to expand its protein inventory.\(^9\)

Proteome diversification can happen at the mRNA level or protein level. By using an alternative starting point (promoter) or splicing (for example, exon skipping), one primary mRNA sequence can translate into different protein variants (Figure 4.1). Alternative splicing occurred in ~95% of human genes with multiple exons,\(^10\) and tissue-specific splicing leads to tissue-specific variants of proteins.\(^11\)

**Figure 4.1:** Examples of alternative transcript initiation and splicing.

Proteins can also be covalently modified after translation, termed as post-translational modifications (PTM). PTM can be divided into two board types: hydrolytic cleavage and covalent addition, both of which are significant in eukaryotic organisms due to their complex signaling pathways and high demand for protein complexity.\(^9\) About 500 proteases, which are
enzymes that specialize in hydrolytic cleavage of peptide bonds, are encoded in the human genome. Moreover, enzymes that perform covalent modifications, such as alkylation, acetylation, glycosylation and phosphorylation, expand the chemical groups available to proteins. It is estimated that 5% of the human proteome contains enzymes that perform more than 200 types of post-translational modifications (Figure 4.2).  

**Figure 4.2**: Examples of protein post-translational modifications.

PTMs modulate both the structure and function of proteins. For example, phosphorylation, performed by protein kinases, is a highly evolved (~500 human kinases) and widely distributed (~30% of all human proteins may be phosphorylated) biological process, which serves as a functional activation mechanism in the regulation of cellular pathways. Glycosylation enzymes add bulky, hydrophilic glycans to proteins, and therefore affect their physical properties (e.g., solubility, stability and folding) and biological functions (e.g.,
immune response, cell adhesion events, signaling).\textsuperscript{13} Even the addition of small groups, such as alkyl or acyl groups, has implications on protein-protein interactions. For instance, arginine methylation affects various cellular processes including protein trafficking, signal transduction and transcriptional regulation.\textsuperscript{14} Furthermore, protein PTM are not only associated with normal cellular functions, but also linked with diseases such as cancer, metabolic disorders, pathogenic infections, diabetes, inflammation and neurodegenerative disorders. Hence, PTM are tracked as disease markers or used as molecular targets for drug development.\textsuperscript{15}

4.3.2 Post-translational Modification of eIF5A

The eukaryotic initiation factor 5A (eIF5A), formerly called eIF4D, was originally identified to stimulate the yield of methionyl-puromycin synthesis, which is routinely used as an assay for initiation of eukaryotic protein translation (Figure 4.3a).\textsuperscript{16} However, eIF5A does not affect the translation of natural mRNA or any of the initiation reactions. While the precise role of eIF5A in protein translation is unknown, recent evidence suggests that it involves in translation elongation, where eIF5A functions together with eEF2 to promote ribosomal translocation (Figure 4.3b).\textsuperscript{17}
Protein eIF5A is a single polypeptide chain with a unique unnatural amino acid residue, hypusine (49, whose name is derived from the combination of hydroxyputrescine and lysine). The hypusine residue of eIF5A is installed via two post-translational modification steps on the eIF5A precursor: first, deoxyhypusine synthase (DHS) functionalizes the Lys50 residue with
a butylamino group from spermidine, then deoxyhypusine hydroxylase (DOHH) installs a hydroxyl group (Figure 4.4a).\(^\text{18}\) DHS, an NAD-dependent dehydrogenase, catalyzes the rate-limiting step of eIF5A synthesis: at first, spermidine (50) is oxidized to an imine (51) by NAD, then transferred to DHS to form the enzyme-imine intermediate (Enz-Lys-N=CH(CH\(_2\)_3NH\(_2\)), followed by another transfer of the butylamino group to the eIF5A precursor. At last, the imine is reduced by NADH and the modified eIF5A is released (Figure 4.4b).\(^\text{19}\)

**Figure 4.4:** a) The post-translational modification of eIF5A b) The proposed catalytic cycle of DHS.
4.3.3 Two Isoforms of eIF5A: eIF5A-1 and eIF5A-2

In human, there are two genes encoding two isoforms of eIF5A. Although they’re 84% identical, their distribution and biological function are significantly different. Two eIF5A isoforms are differentially expressed in human cells. The first isoform, eIF5A-1, is universally expressed and especially abundant in proliferating cells; while the other isoform, eIF5A-2, is only detectable in certain tissues and cancer cell lines. In terms of function, it is well known that eIF5A-1 is essential for cell proliferation and survival. It is involved in protein translation (elongation), actin dynamics, cell cycle progression, mRNA turnover and mRNA decay. Moreover, eIF5A regulates apoptosis via various pathways (IFN-α, TNF-α, p53). The mechanism of apoptosis induction by eIF5A is unclear, but it is interesting to note that not only reducing the level of mature (hypusinated) eIF5A-1, but also overproducing the eIF5A-1 precursor (unhypusinated) could lead to apoptosis. This suggests that the eIF5A-1 precursor may induce apoptosis by its own pathways. On the other hand, eIF5A-2 is often found in various cancer cells. In the clinic, overexpression of eIF5A-2 is associated with poor prognosis (more malignant and invasive) of some types of cancer therefore is considered as an oncogenic marker. Considering their close relationship with human cancer, both eIF5A isoforms can be explored in novel cancer therapy. The specific function of DHS and DOHH in the post-translational modification of eIF5A highlights their potential as drug targets (Figure 4.5).
4.3.4 Inhibition of eIF5A

In the first and rate-limiting step of eIF5A post-translational modification, DHS converts the lysine residue to deoxyhypusine by a NAD-dependent transfer of a butylamino moiety. DHS is present as a homotetramer in its crystal structure, but the fundamental catalytic unit is a dimer. The active sites of the DHS tetramer are deep tunnels formed between dimeric subunits. Each dimeric subunit (A1 and B1, A2 and B2) provides two antiparallel tunnels, making a total of four active sites in the DHS tetramer. The NAD binding sites are located next to the active sites. (Figure 4.6a) Interestingly, the DHS tetramer only bind to one eIF5A monomer, indicating a potential asymmetric conformational change that prevents successive binding.\textsuperscript{22}
The active site tunnel of DHS is 17 Å in depth. Its entrance has a funnel-like shape that is 7 Å deep, making the bottom of the funnel 24 Å from the protein surface. Electron density calculation reveals that both the tunnel entrance and bottom are negatively charged and able to interact with positively charged ammonium groups (Figure 4.6b). This explains the fact that the optimal pH of DHS is 9.0-9.5 ($pK_a$'s of spermidine are 9.94 ($N$-1), 8.40 ($N$-4) and 10.81 ($N$-8). At pH 9.0-9.5, spermidine is expected to be di-protonated at the terminal amino groups). Jakus and coworkers synthesized series of spermidine derivatives and tested their activity as competitive inhibitors of DHS (Figure 4.7). Their findings can be summarized as follows: 1) Replacing of the terminal amino groups of spermidine with other uncharged groups results in
complete loss of activity. 2) Modifying the chain length of spermidine decreases its activity.
3) Any substitution on the carbon chain or N-4 atom leads to significantly diminished activity.

These results correspond well with the shape of the DHS active site, which, as described before, is a deep narrow tunnel with both ends negatively charged. One of their spermidine derivative, named GC7, showed extraordinary activity in DHS inhibition (IC$_{50}$ = 17 nM). By structure, GC7 and spermidine have similar length, but GC7 has an all-carbon chain with an amino group on one end and a guanyl group on the other. The crystal structure of GC7-DHS complex indicates that the bottom of the tunnel is optimized to host two positively charged groups like two amino groups in a guanidine moiety.$^{22}$ As a result, GC7 outcompetes other spermidine derivatives and remains to be the best inhibitor of DHS to date. GC7 has shown to induce cell cycle arrest and apoptosis in various tumor cell lines; however, due to off-target binding, GC7 exhibits systemic toxicity that limits its application in vivo.$^{24}$

![Figure 4.7: DHS inhibition activities of representative spermidine derivatives (IC$_{50}$, µM).](image-url)
Other than spermidine derivatives, there are very few reports of small molecule inhibition of DHS. More recently, Meier and coworkers performed an *in silico* design, chemical synthesis and biological screening of DHS inhibitors.\(^{25}\) Candidates were generated from the mix-and-match of core and anchor fragments in commercial databases, followed by docking and scoring. Ten molecules of interest were purchased or synthesized. Among these, only compound \(52\), synthesized from iodoaniline derivative \(53\) via Sonogashira coupling and indole formation (Figure 4.8), displayed moderate activity of DHS inhibition (IC\(_{50}\) ~12 \(\mu\)M). Compound \(52\) at a concentration of 2 \(\mu\)M also showed a 14% inhibition on HIV-1 replication in PM1 cells while no drug-induced cytotoxicity were detected at this concentration.

\[\text{Scheme 4.7: Synthesis of a DHS inhibitor 52.}\]

DOHH catalyzes the second step of eIF5A post-translational modification. It is a non-heme diiron enzyme, which, when oxidized by molecular oxygen, forms a peroxo-diiron(III) intermediate. In 2015, a crystal structure of this intermediate was first obtained (Figure 4.9a).\(^{26}\)
Comparing to DHS, inhibition studies of DOHH is limited. Several iron-binding compounds, such as commercial drugs ciclopirox (topical antifungal) and deferiprone (treatment of iron overload), were shown to block DOHH activity (Figure 4.9b). They were both found to inhibit the proliferation of cancer cells and HIV-1 gene expression, likely as a consequence of interfering with the hypusination of eIF5A.

Figure 4.8: a) Crystal structure of DOHH showing a peroxo bridge. b) Structures of DOHH inhibitors.

4.4 Polyamine Transport System

4.4.1 Introduction to Polyamines

Polyamines are small organic molecules with two or more primary amino groups. Since the original discovery of spermine by Leuwenhoek in 1678, they have been found in all eukaryotes and most prokaryotes. Putrescine, spermidine and spermine are the most widely
distributed cellular polyamines in eukaryotic cells, with a total concentration in the millimolar range. However, due to the basicity of their amino groups, they usually exists as polycations that bind to various anionic biomolecules such as DNA, RNA, proteins and phospholipids, so levels of free polyamines are significantly low. Despite their long history of discovery, research on the biological functions of polyamines are complicated by their promiscuous and reversible interactions with other molecules. It is known that polyamines have supportive roles in cell growth and survival. To be more specific, they are in association with stabilization of nucleic acids, maintenance of chromatin conformation, regulation of specific gene expression, ion-channel regulation, maintenance of membrane stability, provision of a precursor in the synthesis of eukaryotic translation initiation factor 5A (see also chapter 4.3) and free-radical scavenging.

4.4.2 Regulation of Polyamines

Cellular polyamine levels are regulated by synthesis, catabolism and transport. Polyamines can be synthesized from amino acids arginine, ornithine and methionine. At first, ornithine decarboxylase (ODC) catalyzes the rate-limiting step in polyamine biosynthesis by converting ornithine to putrescine. It is regulated at the transcriptional and post-translational stage. ODC has a very short half-life (10-30 minutes in mammalian systems) and is destroyed by the 26S proteasome when binds to the regulatory enzyme ODC antizyme. Next, spermidine synthase and spermine synthase sequentially transfer aminopropyl groups to putrescine, producing spermidine and spermine. Higher order polyamines can be converted back to simpler ones via acetylation by spermine/spermidine acetyltransferase (SSAT),
followed by oxidative cleavage by polyamine oxidase (PAO) at the peroxisome (Figure 4.10).\textsuperscript{31}

\textbf{Figure 4.9:} Biosynthesis and catabolism of polyamines.

Polyamines can also be sourced from extracellular uptake. Because they are positively charged at physiological pH, polyamines are hydrophilic and not permeable through hydrophobic cell membranes. Therefore, a polyamine transport system (PTS) is required for the import and export of polyamines.\textsuperscript{35} In bacteria such as \textit{E. coli}. and yeasts such as \textit{S. cerevisiae}, their PTSs are well characterized.\textsuperscript{36} However, the PTS in mammalian cells is
different and has yet to be described at a molecular level. Poulin and coworkers proposed that polyamines are transported into the cell by an electronegative membrane potential, then accumulated as vesicles.\textsuperscript{37} In another model by Belting and coworkers, they suggested that polyamines bind to heparan sulphate (a polysaccharide attached to the cell surface) in glypican-1, before being transported into the cell by receptor-mediated endocytosis.\textsuperscript{38}

### 4.4.3 Utilizing the Polyamine Transport System in Cancer Therapy

Since transformed cells usually require higher levels of polyamines than normal cells, polyamines are considered as a potential target for cancer management. However, despite numerous successful efforts into the development of inhibitors that selectively block key enzymes in polyamine synthesis, most of them exhibit limited anticancer activity.\textsuperscript{39,40} This is because when polyamine biosynthesis is impeded, PTS actively compensates the deficiency by increasing the polyamine uptake from external sources, such as diet and microbial activity in the gut. Hence, depleting the polyamine pool by targeting only one of these two pathways would be ineffective. Next generation approaches utilize several properties of PTS to provide a novel anticancer strategy:\textsuperscript{40} 1) Fast-proliferating cells like cancer cells accumulate polyamines at an enhanced rate. To meet this need, polyamine biosynthetic enzymes such as ODC and AdoMet are upregulated, and PTS uptake becomes more active than normal cells. 2) PTS regulates polyamine homeostasis, i.e. PTS uptake is inversely correlated with intracellular polyamine levels; 3) The recognition of polyamines by PTS is structurally promiscuous, allowing polyamine-like molecules to be successfully delivered inside. Moreover, for drugs that act in the nucleus, attaching a polyamine vector with DNA-binding ability may help
improve its activity. For these reasons, many recent efforts have focused on utilizing synthetic
drug-polyamine conjugates in hopes of achieving higher therapeutic indexes for highly
cytotoxic molecules (Figure 4.11).\textsuperscript{41,42} 

\textbf{Figure 4.10:} Concept of polyamine-drug conjugates.

The first notable example of drug-polyamine conjugates is the chlorambucil-spermidine conjugate developed by Cohen and coworkers.\textsuperscript{43} Chlorambucil (54), a nitrogen mustard alkylating agent used in the treatment of leukemia and lymphoma, is associated with severe side effects such as bone marrow suppression, which limits its maximum therapeutic dose. By installing a spermidine anchor via an \textit{N}-4 alkyl amide linkage to chlorambucil, the conjugate 55 displayed better anticancer activity against ADJ/PC6 murine tumor than the drug itself. 55 is 4-fold more effective (ED\textsubscript{50} \textit{in vivo} and 7-fold (72 h) to 35-fold (1 h) more toxic (IC\textsubscript{50} \textit{in vitro}. In polyamine-depleted ADJ/PC6 cells which were pretreated with DFMO (an irreversible polyamine biosynthesis inhibitor that shuts down ODC activity), the drug conjugate 55 showed a striking 225-fold increase of cytotoxicity. The enhanced performance
of chlorambucil-spermidine conjugate could be attributed to elevated polyamine uptake in tumor cells, especially when polyamines are depleted. Cullis and coworkers synthesized a fluorescent spermidine-MANT conjugate (56) with a similar linker, and found it accumulated in granular structures in the cytoplasm of A549 and CHO cells. Interestingly, fluorescence was not detected in the nucleus in spite of the DNA-affinity of spermidine (Figure 4.12, top).44

Callery and coworkers synthesized N-1 and N-8 aziridinyl analogs of spermidine (57 and 58). Both reagents were potentiated in DFMO pretreated L1210 murine leukemic cells. In contrast, coincubation with spermidine protected the cells from these two alkylating reagents. HPLC analysis directly proved that DMFO pretreatment increased the cellular accumulation of both aziridinyl compounds. In addition, aziridinyl analogs of spermidine caused dose-dependent inhibition of the uptake of spermidine (Figure 4.12, middle).45

The successful design of an antitumor agent F14512 also highlights PTS as an attractive target. F14512 possesses a spermine-linked epipodophyllotoxin core, which targets topoisomerase II. The spermine moiety is expected to function as a drug delivery vector, facilitates solubilization and reinforces the topoisomerase inhibition. In CHO cells, F14512 was 73-fold more cytotoxic compared to the PTS-defective mutant CHO-MG cells (Figure 4.12, bottom). It also induced in vivo tumor regression in MX1 breast tumor models.46,47

Due to the lack of molecular identification of mammalian PTS, the DFMO/spermidine and CHO/CHO-MG systems have been widely-used as indirect evidence of PTS recognition.
Other synthetic drug-polyamine conjugates may also use PTS to gain entry to the cells.\textsuperscript{41} Moreover, biologically active natural polyamine derivatives are of particular interest, because revealing their potential relationship with PTS will help elucidate their modes of action and guide the design of other polyamine-based drug vectors.

![Chemical structures](image)

<table>
<thead>
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<th></th>
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<td></td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>IC\textsubscript{50} (µM), L1210 cells</th>
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<th>58</th>
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</thead>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                | IC\textsubscript{50} (µM), L1210 cells pretreated with 100 µM DFMO | 0.05 | 0.15 |
|                | IC\textsubscript{50} (µM), L1210 cells coincubated with 3.7 µM spermidine | 1.1 | 2.4 |

<table>
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<th>CHO cells</th>
<th>CHO-MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC\textsubscript{50} (µM), 59</td>
<td>0.12</td>
<td>8.7</td>
</tr>
</tbody>
</table>

**Figure 4.11**: Examples of drug-polyamine conjugates that may be taken up by PTS.
4.5 Monanchocidin A’s Morpholinone Fragment as a Potential Inhibitor of DHS

4.5.1 General Description of the DHS Inhibition Assay

Structures of pentacyclic guanidinium alkaloids clearly show a pattern of guanidinium core-linker-spermidine tail. Possible modes of action about the guanidinium core have been discussed in chapter 4.1. In comparison, very little is known about the spermidine-derived tail, which likely possesses distinctive functions, or work synergistically with the guanidinium core. Spermidine-related cellular mechanisms described in chapter 4.3 and 4.4 provide two hypotheses about this tail: it may inhibit the post-translational modification of eIF5A, or utilize the polyamine transport system to gain entry into the cells. Our initial investigations on these hypotheses will be discussed.

To begin with, we tested if monanchocidin A’s morpholinone fragment could inhibit DHS. DHS inhibition assays using radiolabeled materials were described previously. In general, radiolabeled spermidine, the substrate of DHS, react with the unhypusinated eIF5A precursor protein, in the presence of DHS, NAD and other additives which preserve enzymatic activity. After a set period of time, the radiolabeled protein product was isolated from the mixture containing excess radiolabeled spermidine. An inhibitor of DHS would cause a decrease in radioactivity in the protein product (Figure 4.13).
4.5.2 Assay Development

Several literature reported protocols of the DHS radiolabeling assay are inconsistent regarding to some key details. Therefore, we need to develop an optimal standard protocol that fits the settings in our laboratory.

**Choice of radioactive isotope.** Although the use of spermidine [1,4-$^{14}$C] trihydrochloride was reported in a DHS assay,$^{48,50}$ it is not sensitive enough for quantitative analysis in our liquid scintillation counter (model: Packard Tri-Carb Model 2900 TR), as the commercially available $^{14}$C-labelled spermidine has a specific radioactivity of 0.1 Ci/mmol, about 400 times less than the $^3$H-labelled one (43.3 Ci/mmol). Therefore, [terminal methylenes-$^3$H(N)]-spermidine trihydrochloride was used in our assay.
Protein preparation. Due to the lack of post-translational modification enzymes (DHS and DOHH) in bacteria, the eIF5A precursor protein (unhypusined form) can be synthesized by the overexpression of \(\text{EIF5A}\) gene in \(\text{E. coli}\). We prepared the GST-tagged eIF5A precursor protein using the pGEX-eIF5A DNA plasmid according to a known protocol, followed by a batch purification using glutathione agarose resin (Thermo Scientific catalog # PI-16000) according to the manufacture’s protocol. Human deoxyhypusine synthase (DHS) was purchased from Reagent Proteins (CMB-333).

Separation techniques. Since an excessive amount of radiolabeled spermidine is used in this enzymatic reaction, the radiolabeled protein product must be carefully separated and recovered for analysis. According to several literature protocols, we attempted different separation methods including: 1) precipitate the protein samples using trichloroacetic acid (TCA), then repeatedly wash with acetone; 2) a filter-binding assay using nitrocellulose membranes, which quantitatively bind to protein samples; 3) subject the reaction mixture to gel electrophoresis (SDS-PAGE), then cut the band corresponding to eIF5A, followed by \(\text{H}_2\text{O}_2\) digestion. However, these methods generated high levels of systematic errors and/or background signals. Finally, using a centrifugal filter unit with a nominal molecular weight limit (NMWL) of 10 kDa (eIF5A has a molar weight of \(\sim\)16.8 kDa), followed by repeated wash with PBS buffer, we successfully isolated the protein samples from other small molecules with consistent retention and low background signal.
Reaction parameters. The reactants used in this assay are listed below (Table 4.1)

Table 4.1: List of reactants in the DHS inhibition assay.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Volume (μL)</th>
<th>Amount or final concentration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction buffer (0.3 M Glycine-NaOH, pH 9)</td>
<td>185.8</td>
<td>-</td>
<td>low yield at pH 8</td>
</tr>
<tr>
<td>DTT (0.1 M)</td>
<td>2</td>
<td>1 mM</td>
<td>stabilizes proteins by preventing oxidation</td>
</tr>
<tr>
<td>NAD+ (0.1 M)</td>
<td>2</td>
<td>1 mM</td>
<td>coenzyme for DHS</td>
</tr>
<tr>
<td>BSA (5 mg/mL)</td>
<td>2</td>
<td>50 μg/mL</td>
<td>minimizes protein adhesion to plastic surfaces</td>
</tr>
<tr>
<td>eIF5A (2.5 mg/mL)</td>
<td>4</td>
<td>10 μg</td>
<td>limiting reagent</td>
</tr>
<tr>
<td>DHS (1 mg/mL)</td>
<td>3</td>
<td>3 μg</td>
<td>catalyst</td>
</tr>
<tr>
<td>(^3)H-spermidine, 1 μCi/μL, 43.3 Ci/mmol</td>
<td>0.2</td>
<td>0.2 μCi</td>
<td>in excess</td>
</tr>
<tr>
<td>Test compounds (various concentrations)</td>
<td>1</td>
<td>-</td>
<td>dissolved in DMSO</td>
</tr>
<tr>
<td>Total Volume</td>
<td>200</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

In this reaction, eIF5A is the limiting reagent therefore the radioactivity in protein products is positively correlated with the amount of eIF5A. The reaction rate appeared to be independent upon the amount of the DHS enzyme (0.5-3 μg). At the optimal pH (9.0) of DHS, a linear relationship between protein radioactivity and time was reported within 10 h,\(^{53}\) therefore the reaction time was set at 2 h.

Accuracy control. To minimize the errors from pipet transfer of reagents, a “master solution” containing all reactants except for the test compounds and DHS enzyme was prepared. The master solution had a volume of 196*\(n\) μL (\(n\) = the number of tests), and was
transferred into *n* Eppendorf tubes, followed by the addition of test compounds (1 μL). At last, 3 μL of DHS was added to each tube to start the reaction, which was kept at a 23 °C shaking incubator for 2 h.

**4.5.3 Results and Discussion**

We tested two morpholinone compounds 60 and 61 which represent the anchor part of monanchocidin A (see also scheme 2.31), together with GC7 as a positive control (literature reported IC$_{50}$ = 17 nM$^{23}$), DMSO as a vehicle control, and a background control without the addition of DHS. Our results indicated no inhibition of DHS by these morpholinones at a 100 μM concentration, while GC7 exerted strong inhibition at both 0.1 and 1.5 μM concentration. This result agrees with the previous report by Jakus et al.$^{23}$ which suggested any N-4 and alkyl chain substitution of spermidine would diminish its binding activity with DHS. However, a proteomic study and Western-blot analysis from the Dyshlovoy group indicated that monanchocidin A inhibited the hypusination (post-translational modification) of eIF5A, but did not affect the expression of this protein. Therefore, three modes of hypusination inhibition are possible: 1) the guanidinium part of monanchocidin A could inhibit DHS; 2) monanchocidin A could inhibit DOHH; and 3) monanchocidin A could affect the cellular polyamine pathways and deplete cellular spermidine which is required for hypusination. Considering the structures of known inhibitors of DHS (linear polyamines) and DOHH (metal coordinators)$^{29}$, the last hypothesis is more likely to be the cause of hypusination inhibition by monanchocidin A. Future work should be primarily focused on how monanchocidin A affects
the synthesis, catabolism or transport of cellular polyamines, and these studies are underway in our laboratory.

![Figure 4.13](image)

**Figure 4.13:** Measured radioactivity from the DHS inhibition assay. 1) DMSO, 2) 100 µM compound 60, 3) 100 µM compound 61, 4) 0.1 µM GC7, 5) 1.5 µM GC7. Background signals were subtracted from the data shown above.

### 4.6 A Rapid Synthesis of an Analog of Ptilomycalin A

**4.6.1 Purpose of this Study**

The difficulties of obtaining enough materials has impeded the progress of biological research in pentacyclic guanidinium alkaloids (PGAs). For example, the isolation of monanchocidin A, one of the relatively abundant PGA, only offers a 0.02% dry weight yield. Despite significant efforts in total synthesis of PGAs (detailed in chapter 4.2), only the Overman group has successfully performed the total synthesis of several PGAs with 16 or
more linear steps. Simplified PGA analogs designed and synthesized by the Murphy and Nagasawa group showed interesting biological activities, but the structures of these analogs do not reflect the ester linkage in the natural products. Therefore, an efficient synthetic route to active PGA analogs is desired in order to tackle the supply problem of PGAs for biological studies.

4.6.2 Design of the Analog and Retrosynthetic Analysis

The two spiro-aminal rings of ptilomycalin A and other PGAs possess stereocenters, unsaturation, branching and different ring sizes. However, previous cytotoxic data suggested the spiro-rings had little impact on the biological activities of PGAs. Considering that, we simplified the spiro-rings to two tetrahydropyran moieties and maintained the guanidinium core structure and ester linkage.

To synthesize this proposed analog 62, we chose to install the spermidine unit on 63 via an amide linkage, avoiding esterification at a congested environment (see also chapter 2.8.2). We planned to use the biomimetic route featuring a double-aza-Michael addition of guanidine on a bis-α,β-unsaturated ketone 64, which would be synthesized from a Knoevenagel reaction between 65 and 66. Transesterification of readily-accessible compounds 67 and 68 would form the Knoevenagel substrate 66.
Scheme 4.8: Design of the PGA analog 62 and retrosynthetic analysis of 62.
4.6.3 Progress towards the Synthesis of a PGA Analog

To begin with, dicarbonyl compound 71 was synthesized from compound 69 and 70 according to a known procedure. Macrolactone opening of 72 with 'BuOK also successfully generated the 16-hydroxyl ester 73. A butyl group was selected to protect the ester so that it would be cleaved together with the acid-promoted cyclization in a later step. Refluxing alcohol 73 and ester 71 in toluene and trapping the ethanol product with 4 Å molecular sieves gave the transesterification product 74 in good yield. On the other hand, stabilized Wittig reagent 76 and excessive succinaldehyde (75) yielded the aldehyde 77 which was ready for the Knoevenagel reaction (Scheme 4.8).

Scheme 4.9: Synthesis of the two substrates for the Knoevenagel reaction.

The Murphy group encountered problems when attempting a similar Knoevenagel reaction using typical base catalysts like piperidine or morpholine. They observed a Baylis-Hillman type reaction which gave 79 as the major product. We obtained similar results with piperidine: the reaction progressed slowly at -30 °C but if warmed to 0 °C, the Baylis-Hillman
side reaction took over. Switching the catalyst to piperidine acetate or Lewis acids such as CuCl$_2$ didn’t improve the yield. Our best result was obtained with proline as the catalyst. Although the major product is the desired one, the reaction didn’t go to completion despite the addition of extra catalyst or anhydrous MgSO$_4$ (to remove water and push the equilibrium forward). Two alkene isomers 78E and 78Z were individually isolated in an approximately 1:1 ratio.

**Table 4.2**: Conditions used for the Knoevenagel reaction between 74 and 77

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Result</th>
</tr>
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<tbody>
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<td>piperidine (20 mol%), -30 °C - 0 °C, 3 d</td>
<td>slow progress at -30 °C for 3 d byprod formed upon warming to 0 °C</td>
</tr>
<tr>
<td>2</td>
<td>piperidine acetate (20 mol%) -30 °C - 0 °C, 3 d</td>
<td>similar to #1</td>
</tr>
<tr>
<td>3</td>
<td>CuCl$_2$ (20 mol%), THF, rt</td>
<td>complex mixture</td>
</tr>
<tr>
<td>4</td>
<td>proline (20 mol%), DMSO, rt, 6 h</td>
<td>35% (1.17:1 ratio of E:Z), 69% brsm</td>
</tr>
<tr>
<td>5</td>
<td>proline (50 mol%), DMSO, MgSO$_4$, rt, 16 h</td>
<td>similar to #4</td>
</tr>
</tbody>
</table>

With compound 78 in hand, it was time to employ the “biomimetic sequence”. The double aza-Michael addition of guanidine was performed at room temperature with DMF as the solvent. Sodium bicarbonate was also added to free-base the hydrochloride salt of guanidine. After the consumption of the starting material 78, the DMF solution was acidified in order to form the spiro aminal rings in the pentacycle. When HCl (4 M in dioxane) or TFA
(1:1 v/v in DCM) was added, a rapid cyclization to the pentacycle was observed, while the tert-butyl ester remained intact. The DMF in the mixture was found to be the cause of the problem, as removing DMF prior to the addition of TFA (1:1 v/v in DCM) led to the cleavage of the tert-butyl group.

Difficulties were encountered when purifying the product 79 by flash chromatography on silica-gel with various solvent systems (DCM, acetone, EtOAc, MeOH). Mixtures of diastereomers along with some unknown products were collected. Fortunately, amide coupling of the impure sample of 79 with bis-Boc-protected spermidine (82) proceeded without issue. Two diastereomers of the amide product (80a and 80b) were distinguishable on a TLC plate developed with 10% MeOH in EtOAc. We performed a preparative TLC (PTLC) and separated the diastereomers. 3J coupling constant (4.6 Hz) in 1H NMR suggested the less-polar diastereomer 80a had the desired cis-configuration between the hydrogen atom Hα connected to the ester linkage and its adjacent proton Hβ on the pyrrolidine ring (Scheme 4.9). The other diastereomer 80b (J = 11.3 Hz) has a trans-configuration. It is worth noting that the alkene geometry of 78E/78Z did not affect the diastereomeric ratio of 80a:80b. At last, HCl in dioxane removed the Boc protecting groups of 80a, furnishing the desired PGA analog 81a.
Scheme 4.10: Cyclization reaction to form 79 and installation of the spermidine unit.

4.6.4 Preliminary Biological Results of PGA Analog 81a

Due to its limited quantity, we have yet to fully characterize compound 81a in order to confirm that it has a ptilomycalin A-like structure. However, 81a showed comparable cytotoxicity to ptilomycalin A (17). Interestingly, a sample of 79 containing a mixture of diastereomers, showed significantly reduced activity. Moreover, the long chain-tethered spermidine derivative 82, which represents the structure of ptilomycalin A (17) without the guanidinium core, displayed moderate activity, while spermidine alone is non-toxic (Table 4.3).
Table 4.3: Preliminary cytotoxicity data of synthetic PGA-related compounds.

<table>
<thead>
<tr>
<th>Compound, cell line</th>
<th>LD₅₀ (µM)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>81a, HeLa</td>
<td>5.0</td>
</tr>
<tr>
<td>81a, A549</td>
<td>2.5</td>
</tr>
<tr>
<td>81a, HL-60</td>
<td>0.6</td>
</tr>
<tr>
<td>ptlomycin A (17), HeLa</td>
<td>3.2ᵇ</td>
</tr>
<tr>
<td>ptlomycin A (17), A549</td>
<td>9.8ᵇ</td>
</tr>
<tr>
<td>79 (as mixtures)</td>
<td>20</td>
</tr>
<tr>
<td>HeLa</td>
<td></td>
</tr>
<tr>
<td>82, A549</td>
<td>5.0</td>
</tr>
<tr>
<td>spermidine, A549</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

ᵃ Concentrations at which causes 50% cell death after 48 h incubation. Determined by trypan blue exclusion assay.ᵇ Reported value from ref. [65]

4.7 Conclusion

To conclude, we established a 7-step synthetic route to compound 81a, which likely represents the key structure features of ptlomycin A (17), but awaits further confirmation. Our initial results indicate that simplification of the spiro aminal rings of a PGA has little impact on its cytotoxicity. Additionally, the presence of all three structural units (guanidinium core, long chain linker and spermidine tail) is necessary for an active PGA. In particular, this finding raises our curiosity on the exact function of the mysterious spermidine tail. As discussed in Chapter 4.3 and 4.4, future work will be centered on its potential roles in eIF5A post-translational synthesis and cellular polyamine transport. With the rapid access to PGA analogs, a clearer view of the SAR profile of PGAs will also be reported in due course.
4.8 Supporting Information

Part 1. General

All reactions were performed under an N\textsubscript{2} atmosphere and all glassware was dried in an oven at 140 °C for 2 h prior to use, unless otherwise noted. Reactions carried out at –78 °C employed a CO\textsubscript{2}/acetone bath. THF and CH\textsubscript{2}Cl\textsubscript{2} were purified using an alumina filtration system.

Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F\textsubscript{254} plates, 250 µm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), p-anisaldehyde solution (2.5 mL of p-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H\textsubscript{2}SO\textsubscript{4} in 100 mL of 95% EtOH), Bromocresol green solution (0.04 g of bromocresol green in 100 mL of absolute EtOH. Slowly drip in a 0.1 M solution of NaOH until the solution turned pale blue) or a KMnO\textsubscript{4} solution (1.5 g of KMnO\textsubscript{4}, 10 g of K\textsubscript{2}CO\textsubscript{3}, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reaction were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 µm C18 50 x 2.10 mm). Flash chromatography on SiO\textsubscript{2} was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients.

Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Infrared spectra were determined on a Jasco FT/IR-4100 spectrometer. Optical rotation was determined on a Jasco P-2000 Digital Polarimeter. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were obtained on a Varian Mercury-VX 400 or a Bruker DRX-500 NMR spectrometer. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. \textsuperscript{1}H NMR spectra were run at 400 MHz and are tabulated as follows: chemical shift, multiplicity.
(s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, brs = broad singlet),
coupling constant(s) and number of protons. $^{13}$C NMR spectra were run at 100 or 125 MHz
using a proton-decoupled pulse sequence with a $d_1$ of 0 second unless otherwise noted, and are
tabulated by observed peak. High-resolution mass spectra were obtained on a Thermo Fisher
Scientific, Exactive Plus mass spectrometer using Heated Electrospray Ionization (HESI).

NMR reference points:

CDCl$_3$ $^1$H: 7.26 ppm $^{13}$C: 77.16 ppm

CD$_3$OD $^1$H: 3.31 ppm $^{13}$C: 49.00 ppm

**Part 2. Synthetic procedures toward compound 81a**

![ tert-butyl 16-hydroxyhexadecanoate (73) ]

16-Hexadecanolide (1.02 g, 4.00 mmol) was dissolved in 20 mL dry tert-butanol. Potassium
tert-butyl oxide (0.56 g, 5.00 mmol) was added and the mixture was heated to reflux. After 2.5
h, the reaction was quenched with 30 mL 0.2 M aqueous HCl, extracted with DCM (30 mL x 3). The combined organic layers was washed with brine, dried (Na$_2$SO$_4$) and concentrated in
vacuo. The crude mixture was purified by flash chromatography (5-20% ethyl acetate in
hexanes, gradient) to afford the title compound 73 (0.88 g, 67% yield) as a white waxy solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 3.60 (t, $J = 6.7$ Hz, 2H), 2.17 (t, $J = 7.5$ Hz, 2H), 1.60 – 1.48
(m, 4H), 1.41 (s, 9H), 1.34 – 1.21 (m, 22H) $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.5, 80.0, 63.1,
35.7, 32.9, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 28.2, 25.9, 25.2. (Contains overlapped peaks) **Melting point** 36 °C. **IR** (KBr, cm⁻¹) 3376, 2918, 1725, 1469, 1365. **HRMS** (ESI): calculated for C₂₀H₄₀O₃Na [M+Na]⁺, 351.2870; found 351.2865.

![Chemical Structure](image)

Ethyl 7-((tert-butyldimethylsilyloxy)-3-oxoheptanoate (71) was prepared according to a literature procedure in 72% yield.⁶⁰ 71 (605.0 mg, 2.000 mmol) and 73 (820.0 mg, 2.500 mmol) was dissolved in 30 mL dry toluene. The mixture was refluxed under a Dean-Stark trap filled with 4 Å molecular sieves (activated). After 16 h, the mixture was concentrated and purified by flash chromatography (3-12% ethyl acetate in hexanes, gradient) to afford the title compound 74 (920 mg, 79% yield) as a colorless liquid. **¹H NMR** (400 MHz, CDCl₃) δ 4.07 (t, J = 6.8 Hz, 2H), 3.56 (t, J = 6.2 Hz, 2H), 3.38 (s, 2H), 2.53 (t, J = 7.3 Hz, 2H), 2.15 (t, J = 7.5 Hz, 2H), 1.65 –1.42 (m, 8H), 1.39 (s, 9H), 1.31 – 1.18 (m, 22H), 0.84 (s, 9H), -0.01 (s, 6H). **¹³C NMR** (100 MHz, CDCl₃) δ 202.6, 173.3, 167.3, 79.8, 65.5, 62.7, 49.3, 42.8, 35.7, 32.1, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 28.2, 26.0, 25.9, 25.2, 20.1, 18.4, -5.3. (Contains overlapped peaks) **IR** (KBr, cm⁻¹) 2921, 1728, 1150, 835. **HRMS** (ESI): calculated for C₃₃H₆₄O₆SiNa [M+Na]⁺, 607.4364; found 607.4356.
(E)-10-((tert-butyldimethylsilyl)oxy)-6-oxodec-4-enal (77) was prepared according to a literature procedure in 40% yield.\textsuperscript{62} Compound 74 (400.0 mg, 0.683 mmol) was dissolved in 5 mL dry DMSO, L-proline (31.4 mg, 0.273 mmol) was added. After stirring at room temperature for 5 min, aldehyde 77 (298.4 mg, 1.000 mmol) was added. The mixture was kept stirring at room temperature for 6 h, diluted with water (30 mL), extracted with EtOAc (30 mLx3), washed with brine (30 mL), dried with Na\textsubscript{2}SO\textsubscript{4} and concentrated \textit{in vacuo}. The crude mixture was purified by flash chromatography (3-18% ethyl acetate in hexanes, gradient) to afford the title compound 78\textit{E} and 78\textit{Z} (yellow liquids, 1.17:1 \textit{E}:\textit{Z}, 206.6 mg, 35\%) along with a recovery of starting material 74 (197.8 mg, 69\% yield brsm).

Characterization of 78\textit{E}:

\textbf{\textsuperscript{1}H NMR} (400 MHz, CDCl\textsubscript{3}) \(\delta\) 6.90 – 6.81 (m, 1H), 6.81 – 6.72 (m, 1H), 6.11 (d, \(J = 15.8\) Hz, 1H), 4.15 (t, \(J = 6.8\) Hz, 2H), 3.60 (t, \(J = 6.3\) Hz, 4H) 2.64 (t, \(J = 7.3\) Hz, 2H), 2.55 (t, \(J = 7.3\) Hz, 2H), 2.40 – 2.33 (m, 4H), 2.18 (t, \(J = 7.2\) Hz, 2H), 1.70 – 1.60 (m, 6 H), 1.58 – 1.48 (m, 6 H), 1.43 (s, 9H), 1.30 – 1.22 (m, 22 H), 0.87 (s, 18H), 0.03 (s, 12H). \textbf{\textsuperscript{13}C NMR} (100 MHz, CDCl\textsubscript{3}) \(\delta\) 203.2, 200.1, 173.3, 164.3, 145.9, 144.1, 136.6, 131.0, 79.8, 65.4, 62.8, 62.7, 52.9,
43.1, 40.0, 35.6, 32.3, 32.2, 31.2, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.5, 28.1, 27.8, 25.9, 25.9, 25.1, 20.5, 20.1, 18.3,-5.3. (Contains overlapped peaks) IR (KBr, cm⁻¹) 2927, 2855, 1730, 1103. HRMS (ESI): calculated for C₄₀H₉₁O₈Si₂ [M-H]⁻, 863.6258; found 863.6257.

Characterization of 78\text{Z}:

$^1$H NMR (400 MHz, CDCl₃) δ 6.85 – 6.72 (m, 2H), 6.11 (dt, $J = 15.9, 1.5$ Hz, 1H), 4.22 (t, $J = 6.8$ Hz, 2H), 3.60 (m, 4H), 2.63 (t, $J = 7.3$ Hz, 2H), 2.54 (t, $J = 7.3$ Hz, 2H), 2.52 – 2.44 (m, 2H), 2.43 – 2.34 (m, 2H), 2.18 (t, $J = 7.5$ Hz, 2H), 1.70 – 1.60 (m, 6H), 1.56 – 1.46 (m, 6H), 1.43 (s, 9H), 1.29 – 1.22 (m, 22 H), 0.87 (s, 9H), 0.87 (s, 9H), 0.03 (s, 6H), 0.02 (s, 6H). $^{13}$C NMR (100 MHz, CDCl₃) δ 200.2, 197.5, 173.5, 166.5, 145.3, 144.3, 137.6, 131.1, 80.0, 65.7, 63.0, 62.9, 40.2, 39.4, 35.7, 32.4, 32.3, 31.1, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.4, 29.4, 29.2, 28.7, 28.4, 28.2, 26.1, 25.2, 20.7, 20.6, 18.5, -5.2. (Contains overlapped peaks) IR (KBr, cm⁻¹) 2927, 2855, 1731, 1104. HRMS (ESI): calculated for C₄₀H₉₁O₈Si₂ [M-H]⁻, 863.6258; found 863.6263.
Compound **78E** and **78Z** (70.0 mg, 0.0810 mmol) were dissolved in DMF (2 mL), guanidine hydrochloride (23.2 mg, 0.243 mmol) and NaHCO$_3$ (34.0 mg, 0.405 mmol) were added. The mixture was stirred at room temperature for 2 h, then DMF was removed *in vacuo*, followed by the addition of TFA-DCM (4 mL, 1:1 v/v). After stirring at room temperature for 1 h, water (10 mL) was added. The solution was extracted with DCM (10 mLx3), dried with Na$_2$SO$_4$ and concentrated *in vacuo*. Flash chromatography (2-10% MeOH in DCM) eluted a mixture of diastereomers of acid **79** along with small amount of unknown products.

The impure sample of **79** above was dissolved in DCM (5 mL), Boc-protected spermidine (**82**) (33.6 mg, 0.0972 mmol), EDCI (18.6 mg, 0.0972 mmol) and DMAP (4.9 mg, 0.041 mmol) were sequentially added at room temperature. The solution was stirred at room temperature for 16 h then concentrated *in vacuo*. Flash chromatography (2-10% MeOH in EtOAc) yielded a mixture of **80a** and **80b**. The mixture (17.0 mg) was further subjected to PTLC separation (0.5 mm silica-gel plate, eluent: 10% MeOH in EtOAc). The less polar (top) band was found to be the title compound **80a** and the other (bottom) band was **80b**. Silica gel from the top band was cut from the plate, extracted by MeOH (10 mLx5), filtered and concentrated *in vacuo* to give **80a** as a colorless liquid (2.9 mg, 3.4% yield from **78E/Z**). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.25 – 4.16 (m, 1H), 4.10 – 4.00 (m, 3H), 3.99 – 3.86 (m, 2H), 3.73 – 3.63 (m, 2H), 3.42 – 3.34 (m, 2H), 3.33 – 3.25 (m, 1H), 3.25 – 3.20 (m, 1H), 3.17 – 3.10 (m, 2H), 3.07 – 3.01 (m, 2H), 2.92 (d, $J = 4.6$ Hz, 1H), 2.40 – 2.12 (m, 10H), 1.94 – 1.52 (m, 16H), 1.44 (s, 9H), 1.42 (s, 9H), 1.37 – 1.22 (m, 26H). HRMS (ESI): calculated for C$_{51}$H$_{91}$N$_6$O$_9^+$ [M]$^+$, 931.6842; found 931.6852.
HCl in dioxane (1 mL, 4.0 M) was added to compound 80a. After stirring at room temperature for 1 h, solvent was removed in vacuo to yield compound 81a as a colorless liquid. ¹H NMR (400 MHz, CDCl₃) δ 4.25 – 4.16 (m, 1H), 4.10 – 4.00 (m, 3H), 3.95 – 3.84 (m, 2H), 3.80 – 3.75 (m, 1H), 3.67 – 3.62 (m, 1H), 3.42 – 3.34 (m, 2H), 3.33 – 3.25 (m, 1H), 3.25 – 3.20 (m, 1H), 3.17 – 3.10 (m, 2H), 3.07 – 3.01 (m, 2H), 2.93 (d, J = 4.4 Hz, 1H), 2.38 – 2.10 (m, 10H), 1.90 – 1.48 (m, 16H), 1.37 – 1.20 (m, 26H). HRMS (ESI): calculated for C₄₁H₇₅N₆O₅⁺ [M]⁺, 731.5794; found 731.5776.

Part 3. DHS inhibition assay

Material

Human deoxyhypusine synthase (DHS) was purchased from Reagent Proteins (CMB-333). ³H-spermidine was purchased from American Radiolabeled Chemicals, Inc (ART 1749). Other reagents were purchased from Thermo/Fisher. The pGEX-eIF5A DNA plasmid was constructed by the Hauber group.⁶⁶
Expression and purification of eIF5A(lys)

eIF5A was expressed as a glutathione S transferase (GST) fusion protein in *E. coli* TOP10. A 2.5 L culture of LB-ampicillin medium was inoculated with a 250 mL overnight culture of *E. coli* TOP10 transformed with pGEX-eIF5A and grown at 37 °C. When OD600 of 0.8 was reached, expression was induced by the addition of 0.5 mM IPTG, and the cultures were incubated for a further 4 h at 37 °C. Bacteria were harvested by centrifugation (10 min, 5000 g, 4 °C) and stored at -80 °C.

Cell pellets were resuspended in 100 ml PBS, 0.1 mM PMSF, leupeptin (2 μg/ml), aprotinin (2 μg/ml), and DNaseI (0.5 μg/ml). Small amounts of lysozyme, 2.6 mM MnCl2, and 26 mM MgCl2 were added, and the lysate was incubated for 15 min on ice. Subsequently the lysate was sonicated (3 × 10 sec and 2 × 30 sec); 0.5 M NaCl and 1% Triton X-100 were added and the lysate was again incubated on ice for 10 min. Insoluble cell debris was removed by centrifugation (30 min at 4 °C with 30,000 g). Small-scale purification was performed in batch using glutathione agarose resin (Thermo Scientific catalog # PI-16000) according to the manufacture’s protocol. About 500 μg eIF5A(lys) protein was recovered (11.2 μg/μL, determined by BCA Protein Assay Kit, Thermo Scientific catalog # 23227), which was diluted to 2.5 μg/μL with PBS buffer (pH 7.4) and stored at -80 °C in aliquots.

**DHS inhibition assay**

The assay protocol was based on the report by Steinkasserer52 with modifications. At 4 °C, 10 μg eIF5A (lys) and 0.2 μCi ³H-spermidine (1 μCi/μL, 43.3 Ci/mmol) to a 0.3 M glycine-NaOH buffer (pH 9.0) containing 1 mM NAD, 1 mM DTT and 50 μg/mL BSA (total volume = 200 μL). (A master solution containing the reactants above was prepared to ensure accuracy of this
assay, see Chapter 4.5.2.) Test compound or DMSO was added. The enzymatic reaction was started by adding DHS (3 µg) to the mixture. After 2 h incubation at 23 °C, the reaction was stopped by adding 100 µL non-radiolabeled spermidine (20 mM in PBS buffer (pH 7.4)). To remove the excessive radiolabeled spermidine from the protein, the mixture was transferred to an EMD Millipore Amicon™ ultra-0.5 centrifugal filter unit (NWDL = 10 kDa, EMD Millipore Catalog # UFC501096) and spun at 13,300 × g for 20 min. The filter was then washed by adding 300 µL PBS buffer followed by spinning at 13,300 × g for 20 min. The washing process was repeated for a total of three times. After washing, the concentrated protein solution was taken up by 150 µL PBS buffer and recovered by reverse spin (1000 × g, 2 min) of the filter device. The recovered solution was dissolved in 4 mL liquid scintillation cocktail (Fisher catalog # 50-899-90173) and the radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb Model 2900 TR). Measurements were repeated in triplicates for each test, and background signal (no DHS control) was subtracted from the data reported in Figure 4.14.

**Part 4. Cell culture and cytotoxicity assay**

HeLa (human cervix cancer), A549 (human non-small cell lung cancer) and HL-60 (human leukemia) cell lines were obtained from American Type Culture Collection (ATCC) and cultured according to the ATCC protocol. In general, cells were incubated in ATCC recommended media with 10%-20% fetal bovine serum (FBS) and 1x penicillin streptomycin in a 37 °C incubator with 5% CO₂ and water (95% relative humidity). Cells were subcultured before reaching ~80% confluency.
For a rapid analysis of cytotoxicity, a trypan blue exclusion assay was used. Cells (30,000 per mL) were seeded in a 12-well plate (1 mL per well), after incubation for 16 h, culture media were removed and replaced by fresh media containing test compounds in DMSO (final DMSO concentration must be < 0.5%). For non-adhesive cell lines, test compounds in DMSO were directly added. After 48 h incubation, trypan blue (2x, 1 mL) was added in each well and mixed. After 1 min, the media was decanted and the plate was observed under a bifocal microscopy. LD$_{50}$ were determined when half of the cells were stained (dead).
4.9 References


APPENDIX I

Synthesis and Biological Properties of the Pentacyclic Guanidinium Alkaloids.

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Structure, Synthesis and Biological Properties of the Pentacyclic Guanidinium Alkalds

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ABSTRACT

The pentacyclic guanidinium alkaloids (PGAs) are a family of marine natural products that possess a polycyclic guanidine-containing core and a long alkyl chain tethered spermidine-derived tail that is rarely observed in other natural products. These natural products exhibit potent activities on a wide range of organisms and therefore have attracted the attention of many synthetic chemists; however, the structure-activity relationships and mechanisms of action of PGAs remain largely elusive. Herein we summarize the structure, synthesis, toxicity and mechanisms of action of PGAs and highlight their potential as chemical probes and/or therapeutic leads.

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1. Introduction

For many years the diverse molecular architectures of natural products have been a major source of inspiration for both novel reaction development and therapeutic lead molecules. The majority of the natural products utilized as leads in drug discovery and development research are of terrestrial origin; however, over the past few decades the marine environment has been demonstrated to be a prolific source of chemical and biological diversity.¹

Despite the enormous diversity offered by marine life, this source of novel small molecules remains largely unexplored, partially due to the lack of necessary technologies required for sample collection and the difficulties in the isolation and purification of the many metabolites obtained in a single sample.² Nevertheless, in a matter of only a few decades of exploration, seven marine natural products, cyaarabine (Cytosar-U³⁵), Depoet³⁶, vidarabine (Vira-A³⁷), ziconotide (Prialt³¹), omega-3-acid ethyl esters (Lovaza³⁸), eribulin mesylate (Halaven³⁹), brentuximab vedotin (Adcetris³⁰) and trabectedin (Yondelis³¹) have become approved by the Food and Drug Administration (FDA) in the United States, along with a plethora of other molecules in different phases of preclinical and clinical development.³ Through the emergence of recent technological advancements such as robotic collection, aquaculture, “smart screening” along with advances in synthetic chemistry and biology,⁴ more attention has been directed to exploit the biodiversity that is offered by the marine environment.⁵

The guanidine motif is abundant in nature and is observed in the amino acid arginine (1) and the nucleobase guanine (2) (Fig. 1). Because of the basicity of guanidine (pKb = 13.6), its conjugate acid, guaninium cation (3), binds with anionic substrates such as carboxylates or phosphates.⁶ Marine natural products containing the guanidine functionality exhibit a broad spectrum of biological activities, attributed to the multiple ways that the guanidinium cation engages in non-covalent interactions.⁷

![Figure 1. Natural occurrences of the guanidine motif.](image)

Among the guanidine containing scaffolds, pentacyclic guanidinium alkaloids (PGAs) are a class of marine natural products that bear a (5,6,8b)-triazaphenoxathiaphenalene skeleton (4) and two hemiaminal rings as their core structure ⁸⁻¹⁰ The first member of this class to be reported, pilomycin A (6, Fig. 2), was isolated from the Caribbean sponge Pilocaulis spiculifer and from the Red Sea sponge Hemimycella sp. in 1989 by Kashman and co-workers.¹¹ In the following years, several PGA families, namely the crambeascidins¹²⁻¹⁴, neoflortispatines¹⁵, fromiamycins¹⁶, celeromyacin¹⁷, monanchocidins¹⁸⁻¹⁹, monanchomyacin²⁰,²¹ and normonanchocidins²² have been isolated, primarily from marine sponges and other aquatic organisms.
Most members of the PGAs possess similar structural features, including a densely functionalized and rigid pentacyclic guanidinium core (the “vessel unit”, such as 5, 19 or 23) tethered via a long hydrocarbon fatty acid chain at the C-14 ester linkage to a spermidine or a spermidine-derived moiety (the “anchor unit”).

The guanidinium core 5 is shared amongst the majority of the members of this class with a few exceptions.

Figure 2. Representative structures of pentacyclic guanidinium alkaloids (PGAs).

13,14,15-isocrambesicid 800 (20) possessing a trans-ring juncture is the diastereomer of crambesicid 800 (13) with three alternate stereocenters in its pentacyclic core. The variation of stereochemical configuration in PGAs is generally limited to the pentacyclic guanidinium core, specifically in the pyrrolidine subunit. In addition to the observed stereochemical diversity, members such as monanchocid A (24), D (25) and E (26) and monanchocymalin A (21) feature a 5-membered spiro-ring versus the more frequently observed 7-membered spiro-ring.

As highlighted in Fig. 2, the majority of the structural diversity in this class of natural products is contained in the pendent diversity and the anchor unit. Members of this class feature ester linkages and anchor units of varying chain length, substitution pattern, and oxidation states. Other structural variations include the unusual and heavily oxygenated morpholine fragment, which is a unique feature only observed in the monanchocid family, and to date is
the most complex “anchor unit” observed in this class of natural products.\textsuperscript{13,19} Moreover, crambeosin 359 (11) isolated from the marine sponge \textit{Monanchom unguiculata} in 2000, is the first member of this class devoid of the C-14 ester linkage.\textsuperscript{24}

Overall, the pentacyclic guanidinium natural products represent some of the most complex guanidinium alkaloids isolated to date. The intricacy and novelty observed in the structure of the PGAs, coupled with the wide range of biological activities exhibited by these molecules have attracted significant attention from the scientific community. Indeed, PGAs have been the subject of intense study by numerous research groups, and significant synthetic contributions have been made in this area. A brief summary of those synthetic efforts is highlighted in the next section. Despite the elegant approaches to these natural products, surprisingly little information regarding the true interaction of these molecules with biological targets is known. Herein, we offer a summary of the important biological activities exhibited by the vessel and the anchor units of the PGAs, and highlight a few of the many unanswered questions that remain.

2. **An overview of synthetic approaches**

As mentioned earlier, the structural complexity, low natural abundance, and impressive biological activities exhibited by the PGAs have made these natural products exciting targets for total synthesis. Several members of the batzelladine, crambeosidin, and ptilomyacin families have been accessed through total synthesis.\textsuperscript{8,10} The challenges faced in all of the approaches to this class of natural products have highlighted the many difficulties associated with the synthesis of charged and densely functionalized structures in an asymmetric fashion. These efforts have provided the first synthetic access to this class of natural products and in some limited studies the material obtained through synthesis has provided insight into the biological potential offered by these alkaloids.

The Snider group developed a synthetic route to the methyl ester of the guanidinium core of ptilomyacin A (6), based on a cascade approach (Scheme 1).\textsuperscript{27} The use of a cascade approach was of particular interest to them based on the hypothesis that the biogenesis of the natural product follows a similar path. Their synthesis relied on the construction of bis-enone intermediate 31, followed by the Michael addition of D-methylisourea 32 to generate intermediate 33 as a mixture of diastereomers. Ammonolysis of intermediate 33, followed by removal of silyl ether protecting groups, and the subsequent imine and hemiaminal formation resulted in the desired pentacyclic guanidinium core and the corresponding diastereomer. Overall, this synthesis was accomplished in 14 steps with a 2.7% yield starting from commercially available materials. Most notably, their synthesis demonstrated the feasibility of a cascade approach in complex guanidinium natural product synthesis. This approach was also utilized for the construction of the tricyclic guanidinium core of other structurally related natural products such as the batzelladine family.\textsuperscript{29} A conceptually similar strategy, also utilizing a linear precursor cyclization cascade approach was employed by the Murphy group in the synthesis of crambeosidin 359 (11), and the core of the tricyclic guanidinium alkaloids such as the batzelladine family.\textsuperscript{10,27-29} Their synthetic approach utilized guanidine directly

![Figure 3. Representative synthetic approaches toward pentacyclic guanidinium alkaloids (PGAs).](image)
in place of a guanidine surrogate in the double Michael addition to the requisite bis-enone intermediate.

The first significant step towards the asymmetric total synthesis of the PGAs was achieved through the work of the Overman group, who has been one of the pioneers in this research area. The first total synthesis of pilomycalin A (6) was accomplished by the Overman group by employing a tethered Biginelli reaction. To this end, β-keto ester 34 and compound 35 served as the urea and the masked aldehyde units of this reaction to furnish intermediate 36. Silyl deprotection of 36, and further functional group manipulations resulted in formation of a single penta cyclic guanidinium core in 7% yield over 13 linear steps. Overall, this synthesis was a landmark achievement which allowed for the first enantioselective preparation of this core. The versatility of the tethered Biginelli condensation was further extended to the synthesis of several other tricyclic guanidinium alkaloids as well. More notably, their synthetic strategy not only allowed for the first enantioselective total synthesis of a penta cyclic guanidinium natural product, but the sequence also controlled the stereochemical configuration of either cis or trans pyrrolidine via the proper choice of reaction conditions, which ultimately allowed for the synthesis of other polycyclic guanidinium alkaloids such as isocrambescidin 800 (20).56-58

The Nagasawa group has also reported a total synthesis of crambescidin 359 (11), the PGA lacking the ester linkage at the C-14 position.59 Their synthesis was accomplished using a completely distinct synthetic strategy towards the guanidinium core, relying on successive 1,3-dipolar cycloaddition reactions of an optically active nitroine 37 and an olefin 38 to generate the isoxazolidine 39. Notably, both cycloaddition reactions proceeded with complete regio- and stereocontrol. Subsequent cleavage of the isoxazolidine 39 using m-CPBA, followed by reduction of the corresponding nitroene resulted in the targeted 2,5-cis pyrrolidine intermediate, which after further functionalization through oxidation, installation of the guanidine moiety, and acid-mediated cyclization resulted in the natural product. This synthetic route was initially developed for the synthesis of crambescidin 359 (11), but was later extended to the synthesis of the tricyclic guanidinium core of batzelladine A, which possess the anti-configuration of the pyrrolidine ring and the ester linkage at C-14. Using their synthetic strategy, they also designed and synthesized a small collection of C3-symmetric penta cyclic guanidinium derivatives for their application as phase-transfer catalysts in asymmetric alkylation of N-glycine Schiff’s base using various alkylating reagents.5 This design was based on the charged and cage-like structure of the PGAs, which could serve as a chiral cavity to interact with a guest anion molecule through ionic and hydrogen binding interactions.

As part of efforts to further investigate the chemistry and biology of PGAs, the Pierce group developed a rapid synthesis of the unusual and heavily oxygenated morpholinone fragment (43) of the monanchocidins.60 The key reaction of this approach features an acid promoted hemiketalization/hemiamination of α-hydroxyamide 40 and α-ketoaldehyde 41 that proceeds with exclusive regioselectivity and high diastereoselectivity (up to 9:1 dr) to form the morpholinone 42 in moderate yield.

### 3. Toxicity and general SAR picture

Cytotoxic, antibacterial, antiviral, antifungal and antiprotozoal activities of PGAs have been reported (Table 1).

<table>
<thead>
<tr>
<th>Natural Product</th>
<th>Cytotoxic</th>
<th>Antibacterial</th>
<th>Antiviral</th>
<th>Antifungal</th>
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</table>

*Only including toxicity data of naturally occurring PGAs. Synthetic and natural product analogs are not listed.

*Including those with potency against HIV-infected cells.

*These compounds lack the spermidine-derived tail and possess reduced cytotoxicity relative to other reported compounds.
In general, these toxic effects appear to be non-selective for various organisms. For example, ptilomycin A (6) displays anti-HIV-1 activity in human PBM cells with EC<sub>50</sub> and EC<sub>90</sub> of 11 mM and 46 mM, respectively, antibacterial activity against both S. aureus and methicillin-resistant S. aureus with an MIC of 0.63 μg/mL activity against protozoan parasites P. falciparum D6 and W2 (IC<sub>50</sub> = 0.11-0.12 μg/mL), and cytotoxicity against multiple human cancer cell lines with GI<sub>50</sub> values ranging from 0.03 to 0.08 μg/mL. However, Dyshlovey et al. reported that monachocin A (24) was equally active in cisplatin-sensitive and -resistant germ cell tumor (GCT) cell lines. Strong synergistic effects of combined treatment of monachocin A (24) and cisplatin were observed as well. 46

Although there are no systematic studies on the structure-activity relationship (SAR) of PGAs across organisms or therapeutic areas, some generalizations can be made based on previous reports (Fig. 4). Most biologically active PGAs are fully functionalized with all three structural units (guanidinium core, aliphatic linker and spermidine tail), and the absence of the spermidine unit and/or the linker (for example: crambesic acid (14) 36 and crambesic acid 359 (11) 36) results in significant loss of activity.

There are five stereocenters in the guanidinium-containing tricyclic frame which the skeleton of the guanidinium core. A diastereomer of crambesic acid 800 (13), 13,14,15-t iso-crambesic acid 800 (20) which possesses a completely different conformation has been shown to have diminished antiviral and cytotoxic activities. 36 On the other hand, the structure of the aminal spiro-rings appears to have little impact on the activity of PGAs, as two types of spiro-rings (6 to 16 carbons, the natural product contains 16 carbons) 31. Interestingly, replacing the linker and spermidine unit with non-polar groups such as in the cinnamyl analog 46 (Fig. 6), also generated a potent cytotoxic compound, which further complicates the side chain requirement for an active PGA. 31

The spermidine unit in PGAs are often oxidized to varying degrees, from a hydroxylspermidine (e.g. crambesic acid 800), to a heavily oxidized morpholinone ring (e.g. monachocinids). Monochocinid A, B and D (27-29) lose an aminobutyl group in their spermidine unit. PGAs with a truncated or modified spermidine unit appear to be slightly less active; 42 however, the SAR profile of the spermidine unit has yet to be directly studied.

4. Mechanism of action

Although there has been significant synthetic study and biological screening of PGAs since their first isolation in 1989, the mechanisms behind their striking biological activities are less well studied. In the past decade, the isolation of several potent PGA families from the marine sponge Monanchora pulchra has rekindled the desire to explore the treasure trove of biology surrounding these exciting natural products. 16-22 In particular, cellular mechanisms regarding how PGAs affect cancer cells have been the subject of study recently due to their potential use in antitumor therapy; however, the detailed function at a molecular level remains largely unknown, with several hypotheses and limited model studies. Herein, we wish to summarize the previous studies on the mechanism of action of PGAs.

4.1. PGAs as potential anion hosts

Structurally, the pentacyclic guanidinium core is a shell-like molecule with a well-defined 3-dimensional architecture. Electronically, the positively-charged guanidinium moiety is known for its ability to engage in anion binding. 3,22 Together, these properties make this core a promising candidate as an anion host molecule (Fig. 5, left). Several observations support the anion hosting properties of the PGAs: 1) 13,14,15-t iso-crambesic acid 800 (20), an isomer which lacks the cage-like shape, displayed reduced biological activity compared to crambesic acid 800 (13). 2) A crystal structure highlighted bidentate hydrogen bonding between synthetic analogs of a pentacyclic guanidinium core and tetrafluoroanlate anion. 23-3) Ptilomycin A (6) is a relatively non-polar compound which is readily dissolved in chloroform, indicating its polar functional groups, such as the guanidine and spermidine moiety, are likely buried inside the pockets. 4) A constant difference in the 'H NMR chemical shift of two methyl groups was observed when mixing 2-methylpropionate anion (Me₂CHCO₂⁻) and a ptilomycin A-TFA derivative at various concentrations (0.3-6.0 mM), indicating that the anion is tightly bound in a chiral environment. 4-5) A similar guanidine-containing structure that enantioselectively recognizes carboxylate anions has been reported. 17 Although the guest molecule which gives rise to the biological activities of PGAs is yet to be identified, a derivative of ptilomycin A (6) showed selective binding capability toward different N-acetylamino carboxylates. 34

![Figure 5. Potential ionic and covalent interactions of PGAs](image)

4.2. Acceptor for biological nucleophiles

Covalent modification of protein targets may also be responsible for the demonstrated biological activities of PGAs. A study regarding crambesic acid 359 (11) has revealed a base-promoted spiro-ring opening to generate an imine electrophile (44).
which could covalently bind to protein targets (Fig. 5, right); however, this mechanism was only observed when employing ethanethiol as a nucleophile and has not been observed in a biological setting to date.17 The lack of detailed structure-function studies of the guanidine core presents challenges in evaluating the true potential of this scaffold as a selective chemical probe. It therefore remains an open question as to whether or not these reactive chemical entities can be useful as therapeutic lead molecules, but is certain that their unique properties and potent biological activities warrant further investigation.

4.3. Ability to block Na⁺, K⁺ and Ca²⁺ transport

Some guanidine-containing natural toxins (such as saxitoxin and tetrodotoxin) block the voltage-gated ion channels and as a result interfere with normal cellular function.26 Bremecridine 816 (8) was found to exhibit a strong but reversible Ca²⁺ antagonist activity (IC₅₀ = 0.15 nM) in neuroblastoma hybrid NG 108-15 cells, as well as inhibition of the acetylcholine-induced contraction of guinea pig ileum at very low concentrations.27 The Botana group also reported that bremecridine 816 (8) partially blocked Na⁺ and Ca²⁺, but not K⁺ current in cortical neurons from embryonic mice. They further identified the L-type calcium channels as the main targets.28

Pitlymocin A (6) inhibited brain Na⁺, K⁺-ATPase and Ca²⁺-ATPase from skeletal sarcoplasmic reticulum in a dose-dependent manner (IC₅₀ = 2 μM and 10 μM, respectively). Kinetic studies suggested it acted on the ATP binding site in a competitive manner.29 The Nagasawa group synthesized two analogs of pitlymocin A (47 and 48), with one analog (47) featuring a “twisted” guanidine core similar to iso-bremecridine 810 (18). Both analogs (47 and 48) displayed a strong inhibitory effect on Ca²⁺-ATPase (Fig. 6, IC₅₀ = 1-3 μM). Interestingly, the guanidine core alone did not show such activity.30 It is unclear if the blocking of ATPases is linked to toxicity, since cytotoxicity data was not reported on these analogs.

Figure 6. PGA analogs with potent activities.

Nicotinic acetylcholine receptors (nAChRs) are ligand-based, non-selective ion channels which upon acetylcholine binding become permeable to Na⁺, K⁺ and sometimes Ca²⁺.31 The Kashkevov group conducted an in-silico docking-guided screening of 13 marine natural products as nAChR inhibitors. Among these candidates, PGAs bremecridine 359 (29) and monochocin A (22) displayed moderate affinity with T. californica and human α7 nAChR with K₅ values ranging from 8.0-310 μM. At 10 μM concentration, they also efficiently blocked murine muscle-type and human α7 nAChR expressed in Xenopus laevis oocytes.32

4.4. Mode of anti-HIV action

Approved anti-HIV drugs exert their effects on various targets by blocking viral entry or fusion, or by inhibiting key enzymes such as integrase, reverse transcriptase or protease.33 Novel anti-HIV agents are in constant demand due to the emergence of drug resistance and side effects associated with long-term treatment. Several reports suggested that members of the PGA family are promising as anti-HIV lead compounds. For instance, pitlymocin A (6) and bremecridine 800 (13) were highly effective in HIV-1 infected human PBMC cells (EC₅₀ = 11 nM and 40 nM, EC₉₀ = 46 nM and 120 nM, respectively).15 It appears PGAs battle HIV-1 via multiple modes of action: bremecridine 800 (13), 826 (12) and fromiamycin (17) all efficiently inhibited HIV-1 envelope-mediated fusion (IC₅₀ = 1-3 μM) against a T-cell tropic strain and a macrophage tropic strain.15 In another study, The Overman group focused on the HIV-1 Nef protein, which is required for the replication of HIV-1 and the progression to AIDS. Several tricyclic (batzelladine-based) and pentacyclic (bremecridine-based) guanidinium alkaloid analogs inhibited Nef interactions with ligands including p53, actin, and p5634. The most potent compounds possessed IC₅₀ values in the low micromolar range; however, those compounds possessed high levels of cytotoxicity, preventing analysis in cell culture.35 The Murphy group reported that pitlymocin A (6) and two simplified analogs inhibited HIV-1 reverse transcriptase (HIV-1 RT) by 55-64% at 10 μM concentration.36

4.5. Cellular mechanisms of cytotoxic action

The Kobayashi group reported that bremecridine 800 (13) caused cell cycle arrest of K562 chronic myelogenous leukemia cells in S-phase. Additionally, an increased expression of p21 was observed, often indicating the induction of differentiation. Meanwhile, treatment with bremecridine 800 (13) led to changes in Neuro 2A cell morphology at bipolar orientation.37

The Botana group reported that bremecridine 816 (8) was cytotoxic against hepatocellular carcinoma HepG2 cells and several other cancer cell lines at sub-micromolar concentrations. Microarray results revealed approximately 5% altered gene targets upon short treatment at low concentration (6 h, 150 nM). Among the down-regulated targets, genes involving cell migration, cell-cell/matrix adhesion and regulation of cell cycle were found. These results were further validated: cell cycle analysis showed an arrest at the G0/G1 phase of HepG2 cells; Western blot analysis and microscopic images provided evidence on the disruption of cell-cell and cell-matrix adhesion; finally, a dose-dependent inhibition of cell migration was directly observed in a wound healing assay.38

In a follow-up study, the Botana group investigated three bremecridine-type natural products (bremecridine 800 (13), 816 (8), 830 (9)). They displayed different potency levels against several tumor cell lines. Generally, the order of cytotoxicity was bremecridine 816 (8) > 830 (9) > 800 (13). They also decreased cell-cell and cell-matrix adhesion, halted the cell cycle in the G0/G1 phase and induced p53-dependent apoptosis. Again, bremecridine 800 (13) was the least potent compound which required relatively higher concentrations to exhibit the observed activities. The authors suggested a potential dehydroxylation of the activated C-13 hydroxyl group in bremecridine 816 (8) and 830 (9), revealing an electrophilic imine for covalent binding that contributes to their biological activity. Furthermore, bremecridine
816 (8) was tested in a zebrafish xenograft model, showing in vivo activity against human colorectal carcinoma HCT-116 cells.48

4.6 Novel targets and mechanisms

A study by the Dyshlovoy group revealed a dual mode of cytotoxic action by monocholinoid A (24); in addition to traditional apoptosis hallmarks including the cleavage of PARP and caspase-3, at low concentration (< 2 μM), monocholinoid A (24) was observed to induce autophagy and cell cycle arrest at the G2-phase; at high concentration (> 2 μM), it caused permeabilization of the lysosomal membranes and cell-cycle arrest at the G1-phase. These mechanisms eventually led to the non-classical cell death of cisplatin-resistant NCCIT-R cells.46

The Dyshlovoy group also reported four PGAs (pitlyminyl A (6), monocholinoid C (22), monocholinoid A (24) and B (25)) that inhibit the tumor promoter EGFR-induced colony formation of murine epithelial J66 P’ C141 cells at non-toxic concentrations. Evidence suggested that these compounds did not use p53-dependent pathways, and instead activation of the MAPK/ERK1 signaling pathway was likely the cause of apoptosis. These compounds and two other PGAs (monocholinoid B (30) and normonocholinoid D (29)) displayed similar activities against human cervix caner HeLa cells (IC50 = 0.58-2.1 μM). At their corresponding IC50 values, all compounds induced cell cycle arrest at S-phase, DNA fragmentation, and elevated caspase-3/7 activity; however, comparing to traditional antitumor agent cisplatin, significantly weaker activations of caspase-3/7 were observed for these PGAs, indicating that underlying non-apoptotic mechanisms may exist.42

To determine the molecular targets of monocholinoid A (24), a proteomics study was conducted to identify altered proteins.43 When NCCIT-R cells were treated with monocholinoid A (24) for 48 h, 11 (0.5 μM) and 1 (1 μM) instances of differential protein expression with greater than 2-fold changes were observed. These targets are associated with cell migration, growth and proliferation, cell death and survival, metastasis formation and cell cycle progression. Three of them (vimentin, apoE and eIF5A) were further analyzed. Vimentin is an intermediate filament protein and one of the major components that forms the cytoskeleton. Overexpression of vimentin in cancer cells is associated with enhanced growth rate and aggressiveness.43 Monocholinoid A (24) did not affect the total level of vimentin, but instead regulated the distribution of its isoforms which likely have different phosphorylation states. Consequently, the altered filament structure could give rise to the observed activities of monocholinoid A (24) in cell migration and colony formation assays. Monocholinoid A (24) caused an up-regulation of apoE, an apolipoprotein protein with anti-adhesive activity.47 This event could also contribute to its anti-migratory activity. The third protein of interest, eIF5A, is the only known protein with a hypusine residue. This unnatural amino acid residue is installed via a 2-step post-translational modification on the eIF5A precursor protein: first, deoxyhypusine synthase (DHS) functionalizes the Lys50 residue by transferring an aminobutyl moiety from spermidine, followed by installation of a hydroxy group by deoxyhypusine hydroxylase (DOHH) (Fig. 7). Due to its involvement in many key cellular processes, eIF5A is critical to cell viability and proliferation.49 The predominant isoform, eIF5A-1, plays important roles in regulating apoptosis.50 Interestingly, accumulation of the eIF5A-1 precursor (lacking the hypusine residue) leads to apoptotic cell death.51 Upon treatment with 1 μM monocholinoid A (24), the hypusine-containing form was suppressed by 50%, while the total eIF5A level was not affected. Two questions remain to be addressed: 1) is the shift towards eIF5A precursor the cause of apoptosis induced by monocholinoid A (24), and 2) how does monocholinoid A (24) affect the hypusine synthesis? The morpholine unit in monocholinoid A (24) has been suggested as a potential inhibitor of DHS, as it resembles the enzyme substrate (spermidine); however, this hypothesis has been challenged because PGAs with different spermidine-derived units did not exhibit significant differences in cytotoxic activity.42

Figure 7. Post-translational modification of eIF5A.

5. Conclusions

In this review, we summarized the structures, syntheses, biological activities and mechanisms of action of PGAs. Many elegant synthetic approaches toward the guanidinium alkaloids have been reported, while more recent studies on PGAs have been aimed at unravelling their biological mechanisms (Fig. 8). Given the broad range of biological activity displayed by PGAs and the lack of understanding of their mechanism of action at a molecular level, many questions remain regarding the potential of these scaffolds as chemical probes and/or therapeutic leads. The application of modern chemical biology approaches, in combination with chemical synthesis efforts, as well as advances in molecular biology and genetics, will no doubt reveal important insights into PGAs mechanism and therapeutic potential.

Figure 8. Summary of recent mechanistic studies of PGAs (note: not all PGAs exert these functions, and some are evidence-supported hypotheses)

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References and notes
